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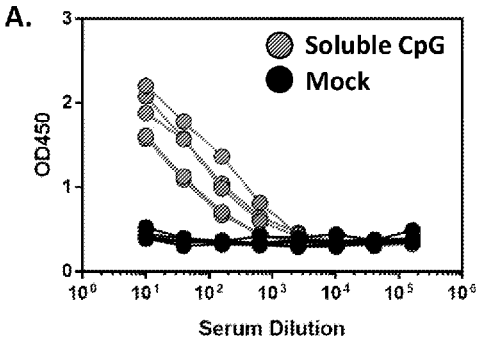
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(54) Title: COMPOSITIONS AND METHODS FOR INDUCING AN IMMUNE RESPONSE AGAINST CORONAVIRUS

FIG. 1A



(57) Abstract: Disclosed herein are CpG-amphiphiles and corona virus antigens (e.g., a coronavirus spike protein, a peptide thereof, or a nucleic acid sequence encoding the same) for use in inducing an immune response in a subject, and methods of administering CpG-amphiphiles and coronavirus antigens (e.g., a coronavirus spike protein, a peptide thereof, a coronavirus nucleocapsid protein, a peptide thereof, or a nucleic acid sequence encoding the same) to induce an immune response in a subject.

WO 2021/263131 A1

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COMPOSITIONS AND METHODS FOR INDUCING AN IMMUNE RESPONSE AGAINST CORONAVIRUS

PRIORITY CLAIM

The present application claims benefit of the filing dates of U.S. Provisional Application No. 63/044,773, filed June 26, 2020, U.S. Provisional Application No. 63/064,836, filed August 12, 2020, U.S. Provisional Application No. 63/124,200, filed December 11, 2020, and U.S. Provisional Application No. 63/145,200, filed February 3, 2021, each of which is hereby incorporated herein by reference in its entirety.

BACKGROUND OF THE INVENTION

Coronaviruses are a large family of viruses capable of infecting mammals and birds. The coronavirus family includes four genera: alpha-, beta-, gamma-, and deltacoronavirus. Coronavirus infections in humans usually cause mild to moderate upper-respiratory tract illnesses, like the common cold. Recently, coronavirus outbreaks, which have emerged from zoonotic spillover, are causing severe disease and global transmission concerns.

Up until 2019, six human coronaviruses were known, including the alphacoronaviruses (e.g., human coronavirus 229E (HCoV-229E) and human coronavirus NL63 (HCoV-NL63)) and the betacoronaviruses (e.g., human coronavirus OC43 (HCoV-OC43), human coronavirus-HKU1 (HCoV-HKU1), severe acute respiratory syndrome (SARS) associated coronavirus (SARS-CoV), and Middle East Respiratory Syndrome (MERS-CoV)). The 2019 novel betacoronavirus (SARS-CoV-2), which is the cause of the highly infectious disease known as COVID-19, emerged recently in China and has quickly spread worldwide, resulting in >7,690,708 confirmed cases and >427,630 deaths as of June 14, 2020.

Based on hospitalized patient data, the majority of COVID-19 cases (about 80%) present with asymptomatic or mild symptoms, while the remainder are severe or critical (Huang et al., Lancet 395:497 (2020); Chan et al., Lancet 395:514 (2020)). Although the vast majority of patients experience only a mild form of the illness, approximately 15% of the patients experience a severe for of the illness that often requires assisted ventilation and oxygenation. Currently, the severity and fatality rate of COVID-19 is milder than that of SARS-CoV-1 and MERS but shows great efficiency with respect to infectivity. With similar clinical presentations as SARS-CoV-1 and MERS, the most common symptoms of COVID-19 are fever, fatigue, and respiratory symptoms, including cough, sore throat, and shortness of breath. A study of 41 hospitalized patients showed that high-levels of proinflammatory cytokines were observed in the COVID-19 severe cases (Huang et al., Lancet 395:497 (2020)). These findings are in line with SARS and MERS in that the presence of lymphopenia and “cytokine storm” likely plays a major role in the pathogenesis of COVID-19 (see, e.g., Nicholls et al., Lancet 361(9371):1773 (2003); Mahallawi et al., Cytokine 104:8 (2018); and Wong et al., Clin Exp Immunol.136(1):95 (2004)). This so-called “cytokine storm” can initiate viral sepsis and inflammatory-induced lung injury, which can lead to other complications, including pneumonia, acute respiratory distress syndrome (ARDS), respiratory failure, septic shock, organ

failure, and death. As a result, there is an urgent need for safe and effective methods of producing an immune response against coronavirus infections, such SARS-CoV-2 and related viruses.

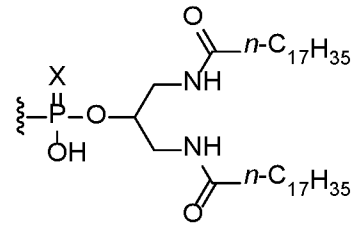
SUMMARY OF THE INVENTION

Disclosed herein are CpG-amphiphiles and coronavirus antigens (e.g., a coronavirus spike protein or a peptide thereof, and/or a coronavirus nucleocapsid protein or a peptide thereof, or a nucleic acid sequence encoding the same) for use in inducing an immune response in a subject. Also, disclosed are methods of administering CpG-amphiphiles and coronavirus antigens (e.g., a coronavirus spike protein or a peptide thereof, and/or a coronavirus nucleocapsid protein or a peptide thereof, or a nucleic acid sequence encoding the same) to induce an immune response in a subject.

In an aspect, the disclosure provides a method of inducing an immune response against a coronavirus antigen in a subject including administering (1) a CpG-amphiphile and (2) a coronavirus antigen or a nucleic acid sequence encoding the coronavirus antigen to the subject. Corresponding compositions and kits are also provided.

In some embodiments, the coronavirus antigen is a coronavirus spike protein or a peptide thereof or a nucleic acid sequence encoding the coronavirus spike protein or peptide. In some embodiments, the CpG-amphiphile includes a CpG sequence bonded to a lipid. In some embodiments, the CpG-amphiphile includes a CpG sequence linked to a lipid by a linker. In some embodiments, the linker includes a polymer, a string of amino acids, a string of nucleic acids, a polysaccharide, or a combination thereof. In some embodiments, the linker includes a string of nucleic acids. In some embodiments, the string of nucleic acids includes between 1 and 50 (e.g., 2, 3, 4, 5, 10, 15, 20, 25, 30, 35, 40, 45, or 50) nucleic acid residues. In some embodiments, the string of nucleic acids includes between 5 and 30 (e.g., 6, 7, 8, 9, 10, 15, 20, 25, 26, 27, 28, 29, or 30) nucleic acid residues. In some embodiments, the string of nucleic acids includes “N” guanines, where N is 1-10 (e.g., 2, 3, 4, 5, 6, 7, 8, or 9). In some embodiments, the linker includes consecutive polyethylene glycol units. In some embodiments, the linker includes “N” consecutive polyethylene glycol units, where N is between 20 and 80 (e.g., 22, 23, 24, 25, 26, 27, 28, 29, 29, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, or 80). In some embodiments, the linker includes “N” consecutive polyethylene glycol units, where N is between 30 and 70 (e.g., 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 45, 50, 55, 60, 65, or 70). In some embodiments, the linker includes “N” consecutive polyethylene glycol units, where N is between 40 and 60 (e.g., 41, 42, 43, 44, 45, 50, 55, or 60). In some embodiments, the linker includes “N” consecutive polyethylene glycol units, where N is between 45 and 55 (e.g., 46, 47, 48, 49, 50, 51, 52, 53, 54, or 55). In some embodiments, the linker includes 48 consecutive polyethylene glycol units.

In some embodiments, the lipid is a diacyl lipid. In some embodiments, the diacyl lipid has the following structure:



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or a salt thereof, wherein X is O or S. In some embodiments, the CpG sequence includes the nucleotide sequence 5'-TCGTCGTTTTGTCGTTTTGTCGTT-3' (SEQ ID NO:1). In some embodiments, the CpG sequence includes the nucleotide sequence of 5'-TCCATGACGTTCCTGACGTT-3' (SEQ ID NO: 2). In some embodiments, all internucleoside groups connecting the nucleosides in the CpG sequence are phosphorothioates. In some embodiments, the coronavirus spike protein or peptide thereof is a SARS-CoV-2 spike protein or peptide thereof. In some embodiments, the peptide of the coronavirus spike protein is a receptor binding domain the specifically binds angiotensin-converting enzyme 2 (ACE2). In some embodiments, the peptide of the coronavirus spike protein including a polypeptide sequence having at least 90% (e.g., 91% 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%) sequence identity to:
RVQPTESIVRFPNITNLCPFGEVFNATRFASVYAWNRKRISNCVADYSVLYNSASFSTFKCYGVSPTK
LNDLCFTNVYADSFVIRGDEVRQIAPGQTGKIADYNYKLPDDFTGCVIAWNSNNLDSKVGGNYNYLY
RLFRKSNLKPFERDISTEIYQAGSTPCNGVEGFNCYFPLQSYGFQPTNGVGYQPYRVVVLSFELLHA
PATVCGPKKSTNLVKNKCVNFNFNGLTGTGVLTESNKKFLPFQQFGRDIADTTDAVRDPQTLEILDITP
CS (SEQ ID NO: 3). In some embodiments, the peptide of the coronavirus spike protein includes the polypeptide sequence of:
RVQPTESIVRFPNITNLCPFGEVFNATRFASVYAWNRKRISNCVADYSVLYNSASFSTFKCYGVSPTK
LNDLCFTNVYADSFVIRGDEVRQIAPGQTGKIADYNYKLPDDFTGCVIAWNSNNLDSKVGGNYNYLY
RLFRKSNLKPFERDISTEIYQAGSTPCNGVEGFNCYFPLQSYGFQPTNGVGYQPYRVVVLSFELLHA
PATVCGPKKSTNLVKNKCVNFNFNGLTGTGVLTESNKKFLPFQQFGRDIADTTDAVRDPQTLEILDITP
CS (SEQ ID NO: 3).

In some embodiments, the coronavirus antigen is a coronavirus nucleocapsid protein or a peptide thereof.

In some embodiments, the coronavirus nucleocapsid protein includes a polypeptide sequence having at least 90% (e.g., 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%) sequence identity to:
MSDNGPQNQRNAPRITFGGPSDSTGSNQNGERSGARSKQRRPQGLPNNTASWFTALTQHGKEDL
KFPRGQGVPINTNSSPDDQIGYYRRATRRIRGGDGKMKDLSPRWYFYYLGTGPEAGLPYGANKDGII
WVATEGALNTPKDHIGTRNPANNAAIVLQLPQGTTLPKGFYAEGSRGGSQASSRSSSRSRNSSRNS
TPGSSRGTSPARMAGNGGDAALALLLLDRLNQLESKMSGKGQQQQGQTVTKKSAAEASKKPRQKR
TATKAYNVTQAFGRRGPEQTQGNFGDQELIRQGTDYKHWPQIAQFAPSASAFFGMSRIGMEVTPSG
TWLTYTGAIKLDDKDPNFKDQVILLNKHIDAYKTFPPTEPKKDKKKKADETQALPQRQKKQQTVTLLP
AADLDDFSKQLQQSMSSADSTQA (SEQ ID NO:68).

In some embodiments, the coronavirus nucleocapsid protein includes the sequence of SEQ ID NO:68.

In some embodiments, the coronavirus nucleocapsid protein includes the polypeptide sequence of:

MSDNGPQNQRNAPRITFGGPSDSTGSNQNGERSGARSKQRRPQGLPNNTASWFTALTQHGKEDL
KFPRGQGVPINTNSSPDDQIGYYRRATRRIRGGDGKMKDLSPRWYFYYLGTGPEAGLPYGANKDGII
WVATEGALNTPKDHIGTRNPANNAAIVLQLPQGTTLPKGFYAEGSRGGSQASSRSSSRSRNSSRNS
TPGSSRGTSPARMAGNGGDAALALLLLDRLNQLESKMSGKGQQQQGQTVTKKSAAEASKKPRQKR
TATKAYNVTQAFGRRGPEQTQGNFGDQELIRQGTDYKHWPQIAQFAPSASAFFGMSRIGMEVTPSG
TWLTYTGAIKLDDKDPNFKDQVILLNKHIDAYKTFPPTEPKKDKKKKADETQALPQRQKKQQTVTLLP
AADLDDFSKQLQQSMSSADSTQAENLYFQGHHHHHH (SEQ ID NO:63).

In some embodiments, the coronavirus antigen includes one or more tags. In some embodiments, the tag is an Avi tag. In some embodiments, the tag is a histidine tag. In some embodiments, the coronavirus antigen includes an Avi tag and a histidine tag. In some embodiments, the coronavirus antigen includes a linker between the polypeptide sequence and the one or more tags. In some embodiments, the coronavirus antigen includes a protease cleavage site between the polypeptide sequence and the one or more tags. In some embodiments, the protease cleavage site is a cleavage site for a tobacco etch virus (TEV) protease (e.g., one having the sequence of ENLYFQG; SEQ ID NO:64).

In some embodiments, the coronavirus spike protein is administered. In some embodiments, a trimer of the coronavirus spike protein is administered. In some embodiments, the trimer is a trimer of a protein construct comprising a polypeptide sequence having at least 90% (e.g., 91%, 92%, 93%,94%, 95%, 96%, 97%, 98%, or 99%) sequence identity to:

VNLTTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHSTQDLFLPFFSNVTWFHAIHVSGTNGTKRFDNP
VLPFNDGVYFASTEKSNIIRGWIFGTTLDSKTQSLLIVNNATNVVIKVCEFQFCNDPFLGVYYHKNNKS
WMESEFRVYSSANNCTFEYVSQPFLMDLEGKQGNFKNLREFVFKNIDGYFKIYSKHTPINLVRDLPQ
GFSALEPLVDLPIGINITRFQTLLALHRSYLTPGDSSSGWTAGAAAYYVGYLQPRTFLLKYNENGTITD
AVDCALDPLSETKCTLKSFTVEKGIYQTSNFRVQPTESIVRFPNITNLCPFGEVFNATRFASVYAWNR
KRISNCVADYSVLYNSASFSTFKCYGVSPTKLNDLCFTNVYADSFVIRGDEVRQIAPGQTGKIADYNY
KLPDDFTGCVIAWNSNNLDSKVGGNYNYLYRLFRKSNLKPFERDISTEIYQAGSTPCNGVEGFNCYF
PLQSYGFQPTNGVGYQPYRVVVLSFELLHAPATVCGPKKSTNLVKNKCVNFNFNGLTGTGVLTESNK
KFLPFQQFGRDIADTTDAVRDPQTLEILDITPCSFGGVSVITPGTNTSNQVAVLYQDVNCTEVPVAIHA
DQLTPTWRVYSTGSNVFQTRAGCLIGAEHVNNSYECDIPIGAGICASYQTQTNSPRAAASVASQSIIA
YTMSLGAENSVAYSNNSIAIPTNFTISVTTEILPVSMTKTSVDCTMYICGDSTECSNLLLQYGSFCTQLN
RALTGIAVEQDKNTQEVFAQVKQIYKTPPIKDFGGFNFSQILPDPSKPSKRSFIEDLLFNKVTLADAGFI
KQYGDCLGDIAARDLICAQKFNGLTVLPPLLTDEMIAQYTSALLAGTITSGWTFGAGAALQIPFAMQMA
YRFNGIGVTQNVLYENQKLIANQFNSAIGKIQDSLSSTASALGKLQDVVNQNAQALNTLVKQLSSNFG
AISSVLNDILSRLDKVEAEVQIDRLITGRLQSLQTYVTQQLIRAAEIRASANLAATKMSECVLGQSKRVD
FCGKGYHLMSFPQSAPHGVVFLHVTYVPAQEKNFTTAPAICHDGKAHFPREGVFVSNGTHWFVTQR
NFYEPQIITTDNTFVSGNCDVVIGIVNNTVYDPLQPELDSFKEELDKYFKNHTSPDVDLGDISGINASVV
NIQKEIDRLNEVAKNLNESLIDLQELGKYEQYIKWPGGGSGGGSHHHHHHHHHH (SEQ ID NO:66).

In some embodiments, the trimer includes the sequence of SEQ ID NO:66.

In some embodiments, a coronavirus spike protein, or a peptide thereof, and a coronavirus nucleocapsid protein, or a peptide thereof, are administered. In some embodiments, a trimer of a coronavirus spike protein construct comprising a polypeptide sequence having at least 90% (e.g., 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%) sequence identity to:

VNLTTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHSTQDLFLPFFSNVTWFHAIHVSGTNGTKRFDNP
VLPFNDGVYFASTEKSNIIRGWIFGTTLDSKTQSLLIVNNATNVVIKVCEFQFCNDPFLGVYYHKNNKS
WMESEFRVYSSANNCTFEYVSQPFLMDLEGKQGNFKNLREFVFKNIDGYFKIYSKHTPINLVRDLPQ
GFSALEPLVDLPIGINITRFQTLLALHRSYLTPGDSSSGWTAGAAAYYVGYLQPRTFLLKYNENGTITD
AVDCALDPLSETKCTLKSFTVEKGIYQTSNFRVQPTESIVRFPNITNLCPFGEVFNATRFASVYAWNR
KRISNCVADYSVLYNSASFSTFKCYGVSPTKLNDLCFTNVYADSFVIRGDEVRQIAPGQTGKIADYNY
KLPDDFTGCVIAWNSNNLDSKVGGNYNYLYRLFRKSNLKPFERDISTEIYQAGSTPCNGVEGFNCYF
PLQSYGFQPTNGVGYQPYRVVVLSFELLHAPATVCGPKKSTNLVKNKCVNFNFNGLTGTGVLTESNK
KFLPFQQFGRDIADTTDAVRDPQTLEILDITPCSFGGVSVITPGTNTSNQVAVLYQDVNCTEVPVAIHA
DQLTPTWRVYSTGSNVFQTRAGCLIGAEHVNNSYECDIPIGAGICASYQTQTNSPRAAASVASQSIIA
YTMSLGAENSVAYSNNSIAIPTNFTISVTTEILPVSMTKTSVDCTMYICGDSTECSNLLLQYGSFCTQLN
RALTGIAVEQDKNTQEVFAQVKQIYKTPPIKDFGGFNFSQILPDPSKPSKRSFIEDLLFNKVTLADAGFI
KQYGDCLGDIAARDLICAQKFNGLTVLPPLLTDEMIAQYTSALLAGTITSGWTFGAGAALQIPFAMQMA
YRFNGIGVTQNVLYENQKLIANQFNSAIGKIQDSLSSTASALGKLQDVVNQNAQALNTLVKQLSSNFG
AISSVLNDILSRLDKVEAEVQIDRLITGRLQSLQTYVTQQLIRAAEIRASANLAATKMSECVLGQSKRVD
FCGKGYHLMSFPQSAPHGVVFLHVTYVPAQEKNFTTAPAICHDGKAHFPREGVFVSNGTHWFVTQR
NFYEPQIITTDNTFVSGNCDVVIGIVNNTVYDPLQPELDSFKEELDKYFKNHTSPDVDLGDISGINASVV
NIQKEIDRLNEVAKNLNESLIDLQELGKYEQYIKWPGGGSGGGSHHHHHHHHHH (SEQ ID NO:66),

and a coronavirus nucleocapsid protein construct comprising a polypeptide sequence having at least 90% (e.g., 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%) sequence identity to:

MSDNGPQNQRNAPRITFGGPSDSTGSNQNGERSGARSKQRRPQGLPNNTASWFTALTQHGKEDL
KFPRGQGVPINTNSSPDDQIGYYRRATRRIRGGDGKMKDLSPRWYFYYLGTGPEAGLPYGANKDGII
WVATEGALNTPKDHIGTRNPANNAAIVLQLPQGTTLPKGFYAEGSRGGSQASSRSSSRSRNSSRNS
TPGSSRGTSPARMAGNGGDAALALLLLDRLNQLESKMSGKGQQQQGQTVTKKSAAEASKKPRQKR
TATKAYNVTQAFGRRGPEQTQGNFGDQELIRQGTDYKHWPQIAQFAPSASAFFGMSRIGMEVTPSG
TWLTYTGAIKLDDKDPNFKDQVILLNKHIDAYKTFPPTEPKKDKKKKADETQALPQRQKKQQTVTLLP
AADLDDFSKQLQQSMSSADSTQAENLYFQGHHHHHH (SEQ ID NO:63) are administered.

In some embodiments, a trimer of a coronavirus spike protein construct comprising the polypeptide sequence of SEQ ID NO:66 and a coronavirus nucleocapsid protein construct comprising the polypeptide sequence of SEQ ID NO:63 are administered.

In some embodiments, an mRNA encoding the coronavirus antigen is administered. In some embodiments, the CpG-amphiphile and the coronavirus antigen or nucleic acid encoding the same are administered concurrently. In some embodiments, the CpG-amphiphile and the coronavirus antigen or nucleic acid encoding the same are administered sequentially. In some embodiments, the

CpG-amphiphile is administered first, followed by administering of the coronavirus antigen or nucleic acid encoding the same. In some embodiments, the coronavirus antigen or nucleic acid encoding the same is administered first, followed by administering of CpG-amphiphile.

In some embodiments, the method comprises administering a second adjuvant to the subject.

In some embodiments the method comprises administering a coronavirus vaccine to the subject as a prime or a boost.

In some embodiments, the CpG-amphiphile is administered subcutaneously, intranasally, intratracheally, or by inhalation during mechanical ventilation. In one embodiment, the CpG-amphiphile is administered subcutaneously. In some embodiments, the coronavirus antigen (e.g., a spike protein, peptide thereof, nucleocapsid protein, or nucleic acid encoding the same) is administered subcutaneously, intranasally, intratracheally, or by inhalation during mechanical ventilation. In some embodiments, the subject is a mammal. In some embodiments, the subject is a human.

In other aspects, the disclosure provides compositions and kits that employ the components described for the above methods.

In another aspect, the disclosure provides a pharmaceutical composition comprising a CpG-amphiphile and a coronavirus antigen, or a nucleic acid sequence encoding the coronavirus antigen, and a pharmaceutically acceptable carrier. In some embodiments, the coronavirus antigen is a coronavirus spike protein or a peptide thereof. In some embodiments, the coronavirus antigen is a coronavirus nucleocapsid protein or a peptide thereof. In some embodiments, the coronavirus antigen is a combination of a coronavirus spike protein or a peptide thereof, and a coronavirus nucleocapsid protein or a peptide thereof. In some embodiments, the CpG-amphiphile is as more specifically described in the embodiments provided above and elsewhere herein and/or the coronavirus antigen is as more specifically described in the embodiments provided above and elsewhere herein.

In some embodiments, the subject is administered a dosage of about 10 μg to about 1.0 mg of the coronavirus antigen (e.g., a coronavirus spike protein or a peptide thereof, and/or a coronavirus nucleocapsid protein or peptide thereof, or a nucleic acid sequence encoding the same). In other embodiments, the dosage of the coronavirus antigen administered is about 10 μg to 1 mg, 40 μg to 60 μg, is about 50 μg to 70 μg, is about 50 μg to 150 μg, is about 70 μg to 150 μg, is about 100 μg to 150 μg, is about 100 μg to 200 μg, is about 140 μg to 250 μg, is about 200 μg to 300 μg, is about 250 μg to 500 μg, is about 300 μg to 600 μg, or is about 500 μg to 1.0 mg. In other embodiments, the dosage of the coronavirus antigen administered to the subject is about 10 μg, 20 μg, 30 μg, 40 μg, 50 μg, 60, μg, 70 μg, 80 μg, 90 μg, 100 μg, 110 μg, 120 μg, 130 μg, 140 μg, 150 μg, 200 μg, 250 μg, 300 μg, 400 μg, 500 μg, 600 μg, 700 μg, 800 μg, 900 μg, or 1.0 mg.

In some embodiments, the subject is administered a dosage of the CpG amphiphile of about 0.1 mg to 20 mg. In other embodiments, the dosage of the CpG amphiphile administered is about 0.1 mg to 1.0 mg, is about 0.5 mg to 3.0 mg, is about 1.0 mg to about 5.0 mg, is about 2.0 to 5.0 mg, is about 3.0 to 5.0 mg, is about 3.0 mg to about 10.0 mg, is about 4.0 mg to 12.0 mg, is about 5.0 mg to 15.0 mg, or is about 50 mg to 20.0 mg. The other embodiments, the dosage of the CpG amphiphile

administered to the subject is about 0.1 mg, 0.2 mg, 0.3 mg, 0.4 mg, 0.5 mg, 1.0 mg, 2.0 mg, 3.0 mg, 4.0 mg, 5.0 mg, 6.0 mg, 7.0 mg, 8.0 mg, 9.0 mg, 10.0 mg, 11.0 mg, 12.0 mg, 13.0 mg, 14.0 mg, 15.0 mg, 16.0 mg, 17,0 mg, 18.0 mg, 19.0 mg, or 20.0 mg.

In another aspect, the disclosure provides a kit comprising a CpG-amphiphile and a coronavirus antigen or a nucleic acid sequence encoding the coronavirus antigen. In some embodiments, the coronavirus antigen is a coronavirus spike protein or a peptide thereof. In some embodiments, the coronavirus antigen is a coronavirus nucleocapsid protein or a peptide thereof. In some embodiments, the coronavirus antigen is a combination of a coronavirus spike protein or a peptide thereof, and a coronavirus nucleocapsid protein or a peptide thereof.

BRIEF DESCRIPITON OF THE DRAWINGS

FIG. 1A-FIG. 1C are graphs showing the amount of serum IgG/IgM antibodies measured by an enzyme-linked immunosorbent assay (ELISA) assay for C57Bl6 mice which were administered two doses of 10 µg of a coronavirus spike protein (SEQ ID NO: 3) and 8 µg of either soluble CpG (Fig. 1A) or a CpG-amphiphile (FIG. 1B) over a range of dilutions for (from left to right) mice that were administered PBS (Mock), coronavirus spike protein with soluble CpG (Soluble CpG), or coronavirus spike protein with AMP-CpG (AMP-CpG) (FIG. 1C).

FIG. 2A-FIG. 2C are graphs showing the amount of serum IgG/IgM antibodies measured by an ELISA assay for C57Bl6 mice which were administered three doses of 10 µg of a coronavirus spike protein (SEQ ID NO: 3) and 8 µg of either soluble CpG or a CpG-amphiphile. FIG. 2A is a graph showing the OD450 for serum from mice who were administered the soluble CpG; FIG. 2B is a graph showing the OD450 for serum from mice who were administered the CpG-amphiphile; and FIG. 2C is a graph showing the amount of IgG/M titer for mice that were administered (from left to right) PBS as a control (Mock), coronavirus spike protein with soluble CpG (Soluble CpG), or coronavirus spike protein with AMP-CpG (AMP-CpG).

FIG. 3A-FIG. 3D are graphs showing the concentration of neutralizing antibodies produced that block the ability of the coronavirus spike protein to interact with the angiotensin-converting enzyme 2 (ACE2) receptor for C57Bl6 mice that were administered three doses of 10 µg of a coronavirus spike protein (SEQ ID NO: 3) and 8 µg of either soluble CpG (FIG. 3A) or a CpG-amphiphile (FIG. 3B) in comparison to human convalescent serum (FIG. 3C). FIG. 3D shows the amount of neutralization antibodies produced for (from left to right) mice that were administered PBS as a control (Mock), coronavirus spike protein with soluble CpG (Soluble CpG), or coronavirus spike protein with AMP-CpG (AMP-CpG), compared to human convalescent serum.

FIG. 4A-FIG. 4C are graphs showing the amount of IFNγ (also referred to as IFNg) (FIG. 4A), TNFa (also referred to as TNFα) (FIG. 4B), and IL6 (FIG. 4C) produced by C57Bl6 mice who were administered three doses of (from left to right) PBS as a control (Mock), 10 µg of a coronavirus spike protein (SEQ ID NO: 3) and 8 µg of soluble CpG (Soluble CpG), or 10 µg of a coronavirus spike protein (SEQ ID NO: 3) and 8 µg of CpG-amphiphile (AMP-CpG).

FIG. 5A and FIG. 5B are graphs showing the concentration of IFNg produced in C57Bl6 mice (FIG. 5A) and Balb/C mice (FIG. 5B) that were administered three doses of (from left to right) PBS as

a control (Mock), 10 µg of a coronavirus spike protein (SEQ ID NO: 3) and 8 µg of soluble CpG (Soluble CpG), 10 µg of a coronavirus spike protein (SEQ ID NO: 3) and 8 µg of CpG-amphiphile (AMP-CpG).

FIG. 6A-FIG. 6C are graphs showing the amount of serum IgG/IgM antibodies measured by an ELISA assay for Balb/C mice which were administered two doses of 10 µg of a coronavirus spike protein (SEQ ID NO: 3) and 8 µg of either soluble CpG (Fig. 6A) or a CpG-amphiphile (FIG. 6B) over a range of dilutions for mice that were administered (from left to right) a PBS control (Mock), coronavirus spike protein with soluble CpG (Soluble CpG), or coronavirus spike protein with AMP-CpG (AMP-CpG) (FIG. 6C).

FIG. 7A-FIG. 7C are graphs showing the amount of serum IgG/IgM antibodies measured by an ELISA assay for Balb/C mice which were administered three doses of 10 µg of a coronavirus spike protein (SEQ ID NO: 3) and 8 µg of either soluble CpG or a CpG-amphiphile. FIG. 7A is a graph showing the OD450 for serum from mice who were administered the soluble CpG; FIG. 7B is a graph showing the OD450 for serum from mice who were administered the CpG-amphiphile; and FIG. 7C is a graph showing the amount of IgG/M titer for mice that were administered (from left to right) a PBS control (Mock), coronavirus spike protein with soluble CpG (Soluble CpG), or coronavirus spike protein with AMP-CpG (AMP-CpG).

FIG. 8A-FIG. 8D are graphs showing the concentration of neutralizing antibodies produced that block the ability of the coronavirus spike protein to interact with the ACE2 receptor for Balb/C mice that were administered three doses of 10 µg of a coronavirus spike protein (SEQ ID NO: 3) and 8 µg of either soluble CpG (FIG. 8A) or a CpG-amphiphile (FIG. 8B) in comparison to human convalescent serum (FIG. 8C). FIG. 8D shows the amount of neutralization antibodies produced for mice that were administered (from left to right) a PBS control (Mock), coronavirus spike protein with soluble CpG (Soluble CpG), or coronavirus spike protein with AMP-CpG (AMP-CpG), in comparison to human convalescent serum.

FIG. 9A-FIG. 9C are graphs showing the amount of IFNγ (FIG. 9A), TNFa (FIG. 9B), and IL6 (FIG.9C) produced by Balb/C mice which were administered three doses (from left to right) a PBS control (Mock), 10 µg of a coronavirus spike protein (SEQ ID NO: 3) and 8 µg of soluble CpG (Soluble CpG), or 10 µg of a coronavirus spike protein (SEQ ID NO: 3) and 8 µg of CpG-amphiphile (AMP-CpG).

FIG. 10 is a graph showing the amount of (from left to right for each column) TNFa, IFNg, IL-6, IL-2, and IL-4 produced in mice which were administered two doses of 10 µg of a coronavirus spike protein (SEQ ID NO: 3) and 8 µg of either soluble CpG or a CpG-amphiphile in comparison to mice that were administered alum, IFA, or a control.

FIG. 11 is a graph showing the splenocyte IFNγ co-culture ELISpot responses of C57Bl6 mice and Balb/C mice that were administered four doses of 10 µg of a coronavirus spike protein (SEQ ID NO: 3) and 8 µg of either CpG-amphiphile, soluble CpG, or a Mock Tx in comparison to a positive or negative control.

FIG. 12A-FIG. 12D are graphs showing the amount of IgG1 (FIG. 12A), IgG2bc (FIG. 12B), IgG3 (FIG. 12C), and the IgG2bc:IgG1 ratio (FIG 12D) for C57Bl6 mice administered three doses of

(from left to right) a PBS control (Mock), Alum, IFA, 10 µg of a coronavirus spike protein (SEQ ID NO: 3) and 8 µg soluble CpG (Soluble CpG), or 10 µg of a coronavirus spike protein (SEQ ID NO: 3) and 8 µg CpG-amphiphile (AMP-CpG). The ratio of IgG2bc:IgG1 in FIG. 12D shows that for, Amp-CpG, the immune response skews strongly to Th1 and not Th2. A Th2 response can be detrimental for SARS-CoV-2.

FIG. 13A-FIG. 13D are graphs showing the amount of IFNγ (FIG. 13A), TNFa (FIG. 13B), IL-2 (FIG. 13C), and IL-6 (FIG. 13D produced by mice which were administered two doses of 10 µg of a coronavirus spike protein (SEQ ID NO: 3) and 8 µg of either CpG-amphiphile, soluble CpG, Alhydrogel, IFA, or Mock Tx in comparison to a positive or negative control.

FIG. 14A-FIG. 14D are graphs showing the amount of IFNγ (FIG. 14A), TNFa (FIG. 14B), IL-2 (FIG. 14C), and IL-6 (FIG. 14D) produced by mice which were administered three doses of 10 µg of a coronavirus spike protein (SEQ ID NO: 3) and 8 µg of either CpG-amphiphile, soluble CpG, Alhydrogel, IFA, or Mock Tx in comparison to a positive or negative control.

FIG. 15 is a graph showing the percent of (from top to bottom in each column) both IFNγ and TNFα, only TNFα, and only IFNγ in CD8 T-cells in mice that were administered three doses of 10 µg of a coronavirus spike protein (SEQ ID NO: 3) and 8 µg of either CpG-amphiphile, soluble CpG, Alhydrogel, IFA, or Mock Tx in comparison to a positive or negative control.

FIG. 16A-FIG. 16B are graphs showing the amount of pseudovirus neutralization titer at half maximal inhibitory dilution ($pVNT_{50}$) in C57Bl/6J mice (FIG.16A) and BALB/c mice (FIG. 16B) (n= 5 per group) that were administered four doses of 10 µg of a coronavirus spike protein (SEQ ID NO: 3) in combination 1 nmol soluble CpG or AMP-CpG compared to convalescent serum. Values depicted are mean ± standard deviation. Not detected values are shown on the baseline; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$, ns= not significant by two-sided Mann-Whitney test. Pseudovirus LOD (indicated by the dotted line) was determined as mean + 90% CI calculated for mock treatment.

FIG. 16C-FIG. 16D are graphs showing the amount of IFNγ produced by either C57Bl/6J mice (FIG. 16C) or BALB/c mice (FIG. 16D) (n = 5 per group) that had been administered four doses of 10 µg of a coronavirus spike protein (SEQ ID NO: 3) in combination with 1 nmol soluble CpG or AMP-CpG. Values depicted are mean ± standard deviation. Not detected values are shown on the baseline; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$; ns = not significant, by two-sided Mann-Whitney test. Pseudovirus LOD (indicated by the dotted line) was determined as mean + 90% CI calculated for mock treatment.

FIG. 17A: is a graph showing the number of IFNγ spot forming cells per $1x10^6$ splenocytes that were restimulated with overlapping coronavirus spike peptides in C57BL/6J mice (n=10 per group) that received three doses of 10 µg of a coronavirus spike protein (SEQ ID NO: 3) in combination with 100 µg Alum, 1 nmol soluble CpG, or 1 nmol AMP-CpG. Values depicted are mean ± standard deviation. *** $P < 0.001$; **** $P < 0.0001$, by two-sided Mann-Whitney test applied to cytokine$^+$ T cell frequencies.

FIG. 17B-FIG. 17C are graphs showing the frequency of intracellular cytokine production, including, from top to bottom in each column, IFNγ and TNFα, only TNFα, and only IFNγ, in $CD8^+$ T cells (FIG. 17B) or $CD4^+$ T cells (FIG. 17C) isolated from peripheral blood cells that were restimulated

with overlapping coronavirus spike peptides in C57BL/6J mice (n=10 per group) that were administered three doses of 10 µg of a coronavirus spike protein (SEQ ID NO: 3) in combination with 100 µg Alum, 1 nmol soluble CpG, or 1 nmol AMP-CpG. Values depicted are mean ± standard deviation. * P < 0.05; ** P < 0.01; *** P < 0.001; **** P < 0.0001; ns= not significant, by two-sided Mann-Whitney test applied to cytokine$^+$ T cell frequencies.

FIG. 18A-FIG. 18B are graphs showing the frequency of intracellular cytokine production, including, from top to bottom in each column, IFNγ and TNFα, only TNFα, and only IFNγ, in CD8$^+$ T cells (FIG. 18A) or CD4$^+$ T cells (FIG. 18B) isolated from perfuse lung tissue that was restimulated with overlapping coronavirus spike peptides in C57BL/6J mice (n = 10 per group) that were administered three doses of 10 µg of a coronavirus spike protein (SEQ ID NO: 3) in combination with 100 µg Alum, 1 nmol soluble CpG, or 1 nmol AMP-CpG. Values depicted are mean ± standard deviation. * P < 0.05; ** P < 0.01; *** P < 0.001; **** P < 0.0001, ns= not significant, by two-sided Mann-Whitney test applied to cytokine$^+$ T cell frequencies or cytokine concentrations.

FIG. 18C-FIG. 18D are graphs showing the cytokine concentration, including IFNγ (FIG. 18C), TFNα, IL-6, IL-4, IL-10, and IL17 (FIG. 18D), found in the supernatants of perfuse lung tissue that was restimulated with overlapping coronavirus spike peptides in C57BL/6J mice (n= 10 per group) that were administered three doses of 10 µg of a coronavirus spike protein (SEQ ID NO: 3) in combination with 100 µg Alum, 1 nmol soluble CpG, or 1 nmol AMP-CpG. Values depicted are mean ± standard deviation. * P < 0.05; ** P < 0.01; *** P < 0.001; **** P < 0.0001, ns= not significant, by two-sided Mann-Whitney test applied to cytokine$^+$ T cell frequencies or cytokine concentrations.

FIG. 19A-FIG. 19F are graphs showing the CD8$^+$ (FIG. 19A) and the CD4$^+$ (FIG. 19D) T cell count, the percentage of naive CD8$^+$ (FIG. 19B) and naive CD4$^+$ (FIG. 19E) T-cells, and the percent of effector memory CD8$^+$ (FIG. 19C) and CD4$^+$ (FIG. 19F) T-cells in cells collected from bronchoalveolar lavage in C57BL/6J mice (n= 10 per group) that were administered three doses of 10 µg of a coronavirus spike protein (SEQ ID NO: 3) in combination with (from left to right) 100 µg Alum, 1 nmol soluble CpG, or 1 nmol AMP-CpG. Values depicted are mean ± standard deviation. * P < 0.05; ** P < 0.01; *** P < 0.001; **** P < 0.0001; ns= not significant, by two-sided Mann-Whitney test applied to T cell frequencies.

FIG. 20A-FIG. 20G are graphs showing the humoral responses of C57B1/6J mice (n= 10 per group) that were administered three doses of 10 µg of a coronavirus spike protein (SEQ ID NO: 3) in combination with 100 µg Alum, 1 nmol soluble CpG, or 1 nmol AMP-CpG. The humoral response was assessed in serum for neutralization titer in comparison to convalescent serum (FIG. 20A), IgM (FIG. 20B), IgG (FIG. 20C), IgG1 (FIG. 20D), IgG2bc (FIG. 20E), the ratio of IgG2bc to IgG19 (FIG. 20F), and IgG3 (FIG. 20G) using either a pseudovirus neutralization assay or ELISA assay. Values depicted are mean ± standard deviation. Not detected values are shown on the baseline; * P < 0.05; ** P < 0.01; *** P < 0.001; **** P < 0.0001, ns= not significant, by two-sided Mann-Whitney test.

FIG. 21A is a graph showing frequency of IFNγ spot forming cells per 1x10^6 splenocytes in splenocytes that were restimulated with overlapping coronavirus spike peptides from C57BL/6J mice (n= 10 per group) that were administered three doses of only 100 µg Alum, only 1 nmol soluble CpG, only 1 nmol AMP-CpG, 100 µg Alum and 10 µg of a coronavirus spike protein (SEQ ID NO: 3), 1 nmol

soluble CpG and 10 µg of a coronavirus spike protein (SEQ ID NO: 3), 1 nmol AMP-CpG and 10 µg of a coronavirus spike protein (SEQ ID NO: 3), 1 nmol AMP-CpG and 5 µg of a coronavirus spike protein (SEQ ID NO: 3), and 1 nmol AMP-CpG and 1 µg of a coronavirus spike protein (SEQ ID NO: 3). Values depicted are mean ± standard deviation. Not detected values are shown on the baseline; * P < 0.05; ** P < 0.01; *** P < 0.001; **** P < 0.0001; ns= not significant, by two-sided Mann-Whitney test applied to cytokine$^+$ T cell frequencies.

FIG. 21B-FIG. 21C are graphs showing frequency cytokines, including (from top to bottom in each column) IFNγ and TNFα, only TNFα, and only IFNγ, of CD8$^+$ T-cells (FIG. 21B) and CD4$^+$ T-cells (FIG. 21C) found in peripheral blood cells collected from C57BL/6J mice (n=10 per group) that were administered three doses of only 100 µg Alum, only 1 nmol soluble CpG, only 1 nmol AMP-CpG, 100 µg Alum and 10 µg of a coronavirus spike protein (SEQ ID NO: 3), 1 nmol soluble CpG and 10 µg of a coronavirus spike protein (SEQ ID NO: 3), 1 nmol AMP-CpG and 10 µg of a coronavirus spike protein (SEQ ID NO: 3), 1 nmol AMP-CpG and 5 µg of a coronavirus spike protein (SEQ ID NO: 3), and 1 nmol AMP-CpG and 1 µg of a coronavirus spike protein (SEQ ID NO: 3). Values depicted are mean ± standard deviation. Not detected values are shown on the baseline; * P < 0.05; ** P < 0.01; *** P < 0.001; **** P < 0.0001; ns= not significant, by two-sided Mann-Whitney test applied to cytokine$^+$ T cell frequencies.

FIG. 21D-FIG. 21E are graphs showing frequency of cytokines, including (from top to bottom in each column) IFNγ and TNFα, only TNFα, and only IFNγ, of CD8$^+$ T-cells (FIG. 21D) and CD4$^+$ (FIG. 21E) found in perfused lung tissue cells, restimulated with overlapping coronavirus spike peptides, collected from C57BL/6J mice (n= 10 per group) that were administered three doses of only 100 µg Alum, only 1 nmol soluble CpG, only 1 nmol AMP-CpG, 100 µg Alum and 10 µg of a coronavirus spike protein (SEQ ID NO: 3), 1 nmol soluble CpG and 10 µg of a coronavirus spike protein (SEQ ID NO: 3), 1 nmol AMP-CpG and 10 µg of a coronavirus spike protein (SEQ ID NO: 3), 1 nmol AMP-CpG and 5 µg of a coronavirus spike protein (SEQ ID NO: 3), and 1 nmol AMP-CpG and 1 µg of a coronavirus spike protein (SEQ ID NO: 3). Values depicted are mean ± standard deviation. Not detected values are shown on the baseline; * P < 0.05; ** P < 0.01; *** P < 0.001; **** P < 0.0001; ns= not significant, by two-sided Mann-Whitney test applied to cytokine$^+$ T cell frequencies.

FIG. 22A-FIG. 22G are graphs showing the humoral responses assessed in serum for neutralization titer in comparison to convalescent serum (FIG. 22A), IgM (FIG. 22B), IgG (FIG. 22C), IgG1 (FIG. 22D), IgG2bc (FIG. 22E), the ratio of IgG2bc to IgG19 (FIG. 22F), and IgG3 (FIG. 22G) using either a pseudovirus neutralization assay or ELISA assay for C57B1/6J mice (n= 10 per group)that were administered three doses of only 10 µg of a coronavirus spike protein (SEQ ID NO: 3) in combination with only 100 µg Alum, only 1 nmol soluble CpG, only 1 nmol AMP-CpG, 100 µg Alum and 10 µg of a coronavirus spike protein (SEQ ID NO: 3), 1 nmol soluble CpG and 10 µg of a coronavirus spike protein (SEQ ID NO: 3), 1 nmol AMP-CpG and 10 µg of a coronavirus spike protein (SEQ ID NO: 3), 1 nmol AMP-CpG and 5 µg of a coronavirus spike protein (SEQ ID NO: 3), and 1 nmol AMP-CpG and 1 µg of a coronavirus spike protein (SEQ ID NO: 3). Values depicted are mean ± standard deviation. Not detected values are shown on the baseline; * P < 0.05; ** P < 0.01; *** P < 0.001; **** P < 0.0001 by two-sided Mann-Whitney test.

FIG. 23A-FIG. 23B are graphs showing the frequency of cytokines, including (from top to bottom in each column) IFNγ and TNFα, only TNFα, and only IFNγ, found in peripheral blood cells collected from 37 week old C57BL/6J mice (n= 10 per group) that were administered three doses of only 100 µg Alum, only 1 nmol soluble CpG, only 1 nmol AMP-CpG, 100 µg Alum and 10 µg of a coronavirus spike protein (SEQ ID NO: 3), 1 nmol soluble CpG and 10 µg of a coronavirus spike protein (SEQ ID NO: 3), and 1 nmol AMP-CpG and 10 µg of a coronavirus spike protein (SEQ ID NO: 3) (FIG. 23A); and in C57BL/6J mice that were administered three doses of (from left to right) 100 µg Alum and 10 µg of a coronavirus spike protein (SEQ ID NO: 3), 1 nmol soluble CpG and 10 µg of a coronavirus spike protein (SEQ ID NO: 3), 1 nmol AMP-CpG and 10 µg of a coronavirus spike protein (SEQ ID NO: 3), 1 nmol AMP-CpG and 5 µg of a coronavirus spike protein (SEQ ID NO: 3), and 1 nmol AMP-CpG and 1 µg of a coronavirus spike protein (SEQ ID NO: 3) (FIG. 23B). Values depicted are mean ± standard deviation. * $P < 0.05$;
** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$; ns= not significant by two-sided Mann-Whitney test applied to cytokine$^+$ T cell frequencies.

FIG. 23C-FIG. 23D are graphs showing the frequency of cytokines, including (from top to bottom in each column) IFNγ and TNFα, only TNFα, and only IFNγ, found in perfused lung tissue cells collected from 37 week old C57BL/6J mice (n= 10 per group) that were administered three doses of (from left to right) only 100 µg Alum, only 1 nmol soluble CpG, only 1 nmol AMP-CpG, 100 µg Alum and 10 µg of a coronavirus spike protein (SEQ ID NO: 3), 1 nmol soluble CpG and 10 µg of a coronavirus spike protein (SEQ ID NO: 3), and 1 nmol AMP-CpG and 10 µg of a coronavirus spike protein (SEQ ID NO: 3) (FIG. 23C); and in C57BL/6J mice that were administered three doses of (from left to right) 100 µg Alum and 10 µg of a coronavirus spike protein (SEQ ID NO: 3), 1 nmol soluble CpG and 10 µg of a coronavirus spike protein (SEQ ID NO: 3), 1 nmol AMP-CpG and 10 µg of a coronavirus spike protein (SEQ ID NO: 3), 1 nmol AMP-CpG and 5 µg of a coronavirus spike protein (SEQ ID NO: 3), and 1 nmol AMP-CpG and 1 µg of a coronavirus spike protein (SEQ ID NO: 3) (FIG. 23D). Values depicted are mean ± standard deviation. ** $P < 0.01$; **** $P < 0.0001$; ns= not significant by two-sided Mann-Whitney test applied to cytokine$^+$ T cell frequencies.

FIG. 23E-FIG. 23F are graphs showing the frequency of cytokines, including (from top to bottom) IFNγ and TNFα, only TNFα, and only IFNγ, found in perfused lung tissue cells, restimulated with overlapping coronavirus spike peptides, that were collected from 37 week old C57BL/6J mice (n= 10 per group) that were administered three doses of (from left to right) only 100 µg Alum, only 1 nmol soluble CpG, only 1 nmol AMP-CpG, 100 µg Alum and 10 µg of a coronavirus spike protein (SEQ ID NO: 3), 1 nmol soluble CpG and 10 µg of a coronavirus spike protein (SEQ ID NO: 3), and 1 nmol AMP-CpG and 10 µg of a coronavirus spike protein (SEQ ID NO: 3) (FIG. 23A); and in C57BL/6J mice that were administered three doses of (from left to right) 100 µg Alum and 10 µg of a coronavirus spike protein (SEQ ID NO: 3), 1 nmol soluble CpG and 10 µg of a coronavirus spike protein (SEQ ID NO: 3), 1 nmol AMP-CpG and 10 µg of a coronavirus spike protein (SEQ ID NO: 3), 1 nmol AMP-CpG and 5 µg of a coronavirus spike protein (SEQ ID NO: 3), and 1 nmol AMP-CpG and 1 µg of a coronavirus spike protein (SEQ ID NO: 3) (FIG. 23F). Values depicted are mean ± standard deviation. * $P < 0.05$;

** P < 0.01; *** P < 0.001; **** P < 0.0001; ns= not significant by two-sided Mann-Whitney test applied to cytokine$^+$ T cell frequencies.

FIG. 24A is a graph showing the amount of pseudovirus neutralization titer at half maximal inhibitory dilution ($pVNT_{50}$) in 37 week old C57Bl/6J mice (n= 10 per group) that were administered three doses of only 100 µg Alum, only 1 nmol soluble CpG, only 1 nmol AMP-CpG, 100 µg Alum and 10 µg of a coronavirus spike protein (SEQ ID NO: 3), 1 nmol soluble CpG and 10 µg of a coronavirus spike protein (SEQ ID NO: 3), and 1 nmol AMP-CpG and 10 µg of a coronavirus spike protein (SEQ ID NO: 3), compared to convalescent serum. Values depicted are mean ± standard deviation. Not detected values are shown on the baseline; * P < 0.05; ** P < 0.01; *** P < 0.001 by two-sided Mann-Whitney test.

FIG. 24B is a graph showing the amount of pseudovirus neutralization titer at half maximal inhibitory dilution ($pVNT_{50}$) in 37 week old C57Bl/6J mice (n= 10 per group) that were administered three doses of 100 µg Alum and 10 µg of a coronavirus spike protein (SEQ ID NO: 3), 1 nmol soluble CpG and 10 µg of a coronavirus spike protein (SEQ ID NO: 3), and 1 nmol AMP-CpG and 10 µg of a coronavirus spike protein (SEQ ID NO: 3), 1 nmol AMP-CpG and 5 µg of a coronavirus spike protein (SEQ ID NO: 3), and 1 nmol AMP-CpG and 1 µg of a coronavirus spike protein (SEQ ID NO: 3), compared to convalescent serum. Values depicted are mean ± standard deviation. Not detected values are shown on the baseline; * P < 0.05; ** P < 0.01; ns= not significant by two-sided Mann-Whitney test.

FIG. 24C- FIG. 24G are graphs showing the humoral responses of 37 week old C57B1/6J mice (n= 10 per group) that were administered three doses of only 100 µg Alum, only 1 nmol soluble CpG, only 1 nmol AMP-CpG, 100 µg Alum and 10 µg of a coronavirus spike protein (SEQ ID NO: 3), 1 nmol soluble CpG and 10 µg of a coronavirus spike protein (SEQ ID NO: 3), and 1 nmol AMP-CpG and 10 µg of a coronavirus spike protein (SEQ ID NO: 3), 1 nmol AMP-CpG and 5 µg of a coronavirus spike protein (SEQ ID NO: 3), and 1 nmol AMP-CpG and 1 µg of a coronavirus spike protein (SEQ ID NO: 3). The humoral response was assessed in serum for IgG (FIG. 24 C), IgG1 (FIG. 24D), IgG2bc (FIG. 24E), the ratio of IgG2bc to IgG19 (FIG. 24F), and IgG3 (FIG. 24G) using either a pseudovirus neutralization assay or ELISA assay. Values depicted are mean ± standard deviation. Not detected values are shown on the baseline; * P < 0.05; ** P < 0.01; *** P < 0.001; **** P < 0.0001, ns= not significant by two-sided Mann-Whitney test.

FIG. 25A - FIG. 25D are a series of graphs showing that vaccination with AMP-CpG in aged mice enables durable Spike RBD-specific T cells in blood, spleen, and lung tissue. 37 week old C57Bl/6 mice (n = 5-10 per group) were immunized on day 0, 14, and 28 with 10 ug Spike RBD protein admixed with 100 ug Alum or 1 nmol soluble CpG, or AMP-CpG. Adjuvant control animals were dosed with AMP-CpG adjuvant alone. Humoral responses specific to Spike RBD were assessed in serum from immunized animals by ELISA on day 35, 49, and 70. Shown are endpoint titers determined for IgG (FIG. 25A). T cell responses were analyzed on day 21, 35, 49, and 70. Cells were collected from peripheral blood on day 21, 35, 49, and 70 (FIG. 25B) and were restimulated with overlapping Spike RBD peptides and assayed for intracellular cytokine production to detect antigen-specific T cell responses. Shown are frequencies of IFNγ-positive cells among

peripheral blood $CD8^+$ T cells (FIG. 25A), and cells were collected from spleen (FIG. 25C) and lungs (FIG. 25D), and were restimulated with overlapping Spike RBD peptides and assayed for IFNγ production by ELISPOT assay. Shown is the frequency of IFNγ spot forming cells (SFC) per $1x10^6$ cells (n = 5 mice per group). Values depicted are mean ± standard deviation. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$ by two-sided Mann-Whitney test applied to cytokine+ T cell frequencies. In FIG. 25A, the data for day 35, 49, and 70 are shown left to right for (1) alum, (2) soluble CpG, and (3) AMP-CpG. In FIG. 25 B, the data in the graph are shown bottom to top, (1) alum, (2) soluble CpG, and (3) AMP-CpG. In FIG. 25C and FIG. 25D, the data are shown left to right, (1) adjuvant control, (2) alum, (3) soluble CpG, and (4) AMP-CpG.

FIG. 26A - FIG. 26E are a series of graphs showing that two-dose vaccination with AMP-CpG-7909 elicits potent Spike RBD-specific cellular immunity in blood and lung, and humoral immunity in blood. C57Bl/6 mice (n = 5 per group) were immunized on day 0 and 14 with 0.5, 1.0, or 5.0 ug Spike RBD protein admixed with 1.0, 2.5, or 5.0 nmol AMP-CpG, and T cell and IgG responses analyzed on day 21. Peripheral blood cells (FIG. 26A and FIG. 26B) or cells collected from perfused lungs (FIG. 26C and FIG. 26D) were restimulated with overlapping Spike RBD peptides and assayed by flow cytometry for intracellular cytokine production to detect antigen-specific T cell responses. Shown are frequencies of IFNγ, TNFα, and double-positive T cells among $CD8^+$ (FIG. 26A and FIG. 26C) and $CD4^+$ (FIG. 26B and FIG. 26D) T cells. Humoral responses specific to Spike RBD were assessed in serum from immunized animals by ELISA. Shown are endpoint titers for IgG on day 35 (n = 5 mice per group; FIG. 26E). Values depicted are mean ± standard deviation. In FIG. 26A – Fig. 26D, for each bar, $INFγ^+$ and $TNFα^+$ are at the top of the bar, $TNFα^+$ is at the middle of the bar, and $INFγ^+$ is at the bottom of the bar.

FIG. 27 is a series of graphs showing that AMP-CpG induces a potent polyfunctional CD8 T cell response targeting SARS CoV-2 spike protein. A mock vaccine, or a vaccine containing 10 µg coronavirus spike protein, 10 µg coronavirus nucleocapsid protein and (1) 100 µg alum, (2) 6 µg soluble CpG, or (3) 6 µg AMP-CpG was administered. The percent cytokine positive cells observed were: mock (0%), alum (0%), soluble CpG (5%), and AMP-CpG (34%). In the bar graph showing percent cytokine positive of $CD8^+$ T cells, the top of each bar shows $IFNγ^+$ and $TNFα^+$, the middle of the bar shows TNF $α^+$, and the bottom of the bar shows $IFNγ^+$ cells.

FIG. 28 is a series of graphs showing that AMP-CpG induces a potent polyfunctional CD4 T cell response targeting SARS CoV-2 spike protein. A mock vaccine, or a vaccine containing 10 µg coronavirus spike protein, 10 µg coronavirus nucleocapsid protein and (1) 100 µg alum, (2) 6 µg soluble CpG, or (3) 6 µg AMP-CpG was administered. The percent cytokine positive cells observed were: mock (0.2%), alum (0.5%), soluble CpG (0.5%), and AMP-CpG (12%). In the bar graph showing percent cytokine positive of $CD4^+$ T cells, the top of each bar shows $IFNγ^+$ and $TNFα^+$, the middle of the bar shows TNF $α^+$, and the bottom of the bar shows $IFNγ^+$ cells.

FIG. 29 is a graph showing the number of IFNγ spot forming cells per $1x10^6$ splenocytes that were restimulated with overlapping coronavirus spike peptides in C57BL/6J mice (n=10 per group) that had received a mock vaccine or 10 µg of a full-length coronavirus spike protein construct (SEQ ID NO: 66) in combination with 10 µg of a coronavirus nucleocapsid protein construct (SEQ ID NO:63)

and (1) 100 µg alum, (2) 6 µg soluble CpG, or (3) 6 µg AMP-CpG. Values depicted are mean ± standard deviation. This graph shows that AMP-CpG induces a potent T cell response targeting SARS CoV-2 spike protein.

FIG. 30 is a series of graphs showing that AMP-CpG induces a potent lung-resident polyfunctional $CD8^+$ T cell response targeting SARS CoV-2 spike protein. A mock vaccine, or a vaccine containing 10 µg coronavirus spike protein, 10 µg coronavirus nucleocapsid protein and (1) 100 µg alum, (2) 6 µg soluble CpG, or (3) 6 µg AMP-CpG was administered. The percent cytokine positive cells observed were: mock (0%), alum (0%), soluble CpG (3%), and AMP-CpG (26%). In the bar graph showing percent cytokine positive of $CD8^+$ T cells, the top of each bar shows $IFN\gamma^+$ and $TNF\alpha^+$, the middle of the bar shows $TNF\alpha^+$, and the bottom of the bar shows $IFN\gamma^+$ cells.

FIG. 31 is a series of graphs showing that AMP-CpG induces a potent lung-resident polyfunctional $CD4^+$ T cell response targeting SARS CoV-2 spike protein. A mock vaccine, or a vaccine containing 10 µg coronavirus spike protein, 10 µg coronavirus nucleocapsid protein and (1) 100 µg alum, (2) 6 µg soluble CpG, or (3) 6 µg AMP-CpG was administered. The percent cytokine positive cells observed were: mock (0.2%), alum (0.2%), soluble CpG (1%), and AMP-CpG (7%). In the bar graph showing percent cytokine positive of $CD4^+$ T cells, the top of each bar shows $IFN\gamma^+$ and $TNF\alpha^+$, the middle of the bar shows $TNF\alpha^+$, and the bottom of the bar shows $IFN\gamma^+$ cells.

FIG. 32 is a series of graphs showing that AMP-CpG induces a potent peripheral blood polyfunctional $CD8^+$ and $CD4^+$ T cell response targeting SARS CoV-2 nucleocapsid protein. A mock vaccine, or a vaccine containing 10 µg coronavirus spike protein, 10 µg coronavirus nucleocapsid protein and (1) 100 µg alum, (2) 6 µg soluble CpG, or (3) 6 µg AMP-CpG was administered. In the bar graphs showing percent cytokine positive of $CD8^+$ T cells or percent cytokine positive of $CD4^+$ T cells, the top of each bar shows $IFN\gamma^+$ and $TNF\alpha^+$, the middle of the bar shows TNF α^+, and the bottom of the bar shows $IFN\gamma^+$ cells.

FIG. 33 is a graph showing the number of IFNγ spot forming cells per $1x10^6$ splenocytes that were restimulated with overlapping coronavirus nucleocapsid peptides in C57BL/6J mice (n=10 per group) that received a mock vaccine or 10 µg of a full-length coronavirus spike protein construct (SEQ ID NO: 66) in combination with 10 µg of a coronavirus nucleocapsid protein construct (SEQ ID NO:63) and (1) 100 µg alum, (2) 6 µg soluble CpG, or (3) 6 µg AMP-CpG. Values depicted are mean ± standard deviation. This graph shows that AMP-CpG induces a potent T cell response targeting SARS CoV-2 nucleocapsid protein.

FIG. 34 is a graph showing that the reformulated AMP-CpG vaccine induced a robust antibody response to Genscript RBD in non-human primates. The dotted line indicates the assay limit of detection (LOD).

FIG. 35 is a graph showing that the reformulated AMP-CpG vaccine induces IgG antibodies to the UK SARS-CoV-2 variant (right column). Wild-type SARS-CoV-2 is shown in the left column. The dotted line indicates the assay LOD.

FIG. 36A and FIG. 36B are graphs showing that the reformulated AMP-CPG vaccine induces $CD8^+$ T-cell responses to spike RBD.

FIG. 37A and FIG. 37B are graphs showing that the reformulated AMP-CPG vaccine induces $CD4^+$ and $CD8^+$ T-cell responses to spike RBD. In each column, %TNFα is shown at the top, %IL2 is shown in the middle, and %IFNγ is shown at the bottom.

FIG. 38 is a graph showing results of a tetramer analysis for C57BL/6J mice administered two doses of adjuvant control (Adj only), reformulated AMP-CPG dual WT RBD and B.1.351 RBD vaccine having 5mg per 100 µL injection of each WT RBD and B.1.351 RBD antigens (Dual Vax), or reformulated AMP-CPG B.1.351 RBD vaccine having 5mg per 100 µL injection of B.1.351 RBD antigen (Amp Vax).

FIG. 39A, FIG. 39B, and FIG, 39C are graphs showing results of an Intracellular Stain (ICS) analysis for C57BL/6J mice administered two doses of adjuvant control (Adj only), reformulated AMP-CPG dual WT RBD and B.1.351 RBD vaccine having 5mg per 100 µL injection of each WT RBD and B.1.351 RBD antigens (Dual Vax), or reformulated AMP-CPG B.1.351 vaccine having 5mg per 100 µL injection of B.1.351 antigen (B.1.351). FIG. 39A shows that the reformulated AMP-CPG dual WT RBD and B.1.351 RBD vaccine and the reformulated AMP-CPG B.1.351 vaccine induces $CD8^+$ lung cells to secrete more cytokines IFNγ and TNFα as compared to the adjuvant only vaccine following dose 2. FIG. 39B shows that the reformulated AMP-CPG dual WT RBD and B.1.351 RBD vaccine and the reformulated AMP-CPG B.1.351 vaccine induces $CD4^+$ lung cells to secrete more cytokines IFNγ and TNFα as compared to the adjuvant only vaccine following dose 2. FIG. 39C shows that the reformulated AMP-CPG dual WT RBD and B.1.351 RBD vaccine and the reformulated AMP-CPG B.1.351 vaccine induces $CD8^+$ blood cells to secrete more cytokines IFNγ and TNFα as compared to the adjuvant only vaccine following dose 2. In each column %IFNγ+TNFα is shown at the top, %TNFα is shown in the middle, and %IFNγ is shown at the bottom.

FIG. 40 is a graph showing results of an ELISpot analysis for C57BL/6J mice administered two doses of adjuvant control (Adj only), reformulated AMP-CPG dual WT RBD and B.1.351 RBD vaccine having 5mg per 100 µL injection of each WT RBD and B.1.351 RBD antigens (Dual Vax), or reformulated AMP-CPG B.1.351 vaccine having 5mg per 100 µL injection of B.1.351 RBD antigen (B.1.351).

FIG. 41 is a graph showing the amount of antibody serum measured by ELISA analysis for C57BL/6J mice administered two doses of adjuvant control (Adj only), reformulated AMP-CPG dual WT RBD and B.1.351 RBD vaccine having 5mg per 100 µL injection of each WT RBD and B.1.351 RBD antigens (Dual Vax), or reformulated AMP-CPG B.1.351 vaccine having 5mg per 100 µL injection of B.1.351 RBD antigen (B.1.351).

Definitions

Terms used in the claims and specification are defined as set forth below unless otherwise specified.

It must be noted that, as used in the specification and the appended claims, the singular forms "a," "an," and "the" include plural referents unless the context clearly dictates otherwise.

As used herein, the term “about” refers to a value that is within 10% above or below the value being described.

As used herein, the term "adjuvant" refers to a compound that, with a specific immunogen or antigen, will augment or otherwise alter or modify the resultant immune response. Modification of the immune response includes intensification or broadening the specificity of either or both antibody and cellular immune responses. Modification of the immune response can also mean decreasing or suppressing certain antigen-specific immune responses. In certain embodiments, the adjuvant is a cyclic dinucleotide.

As used herein, the term "amino acid" refers to naturally occurring and synthetic amino acids, as well as amino acid analogs and amino acid mimetics that function in a manner similar to the naturally occurring amino acids. Naturally occurring amino acids are those encoded by the genetic code, as well as those amino acids that are later modified, e.g., hydroxyproline, γ-carboxyglutamate, and O-phosphoserine. The term “amino acid analogs” refers to compounds that have the same basic chemical structure as a naturally occurring amino acid, i.e., an α carbon that is bound to a hydrogen, a carboxyl group, an amino group, and an R group, e.g., homoserine, norleucine, methionine sulfoxide, methionine methyl sulfonium. Such analogs have modified R groups (e.g., norleucine) or modified peptide backbones, but retain the same basic chemical structure as a naturally occurring amino acid. The term “amino acid mimetics” refers to chemical compounds that have a structure that is different from the general chemical structure of an amino acid, but that function in a manner similar to a naturally occurring amino acid. Amino acids can be referred to herein by either their commonly known three letter symbols or by the one-letter symbols recommended by the IUPAC-IUB Biochemical Nomenclature Commission. Nucleotides, likewise, can be referred to by their commonly accepted single-letter codes.

As used herein, the terms “amphiphile” and “amphiphilic” refer to a conjugate comprising a hydrophilic head group and a hydrophobic tail, thereby forming an amphiphilic conjugate. In some embodiments, an amphiphile conjugate comprises a CpG oligodeoxynucleotide (ODN) and one or more hydrophobic lipid tails, referred to herein as a “CpG-amphiphile.”

As used herein, “conjugated” refers to covalent attachment or crosslink of the CpG-amphiphile to a lipid. The CpG-amphiphile may be bonded to the lipid through a covalent attachment by reaction of complementary reactive groups on the CpG-amphiphile and the lipid.

As used herein, the terms “CpG oligodeoxynucleotide” and “CpG motif” refer to a short single-stranded DNA molecule which includes a 5' C nucleotide connected to a 3' G nucleotide through a phosphodiester internucleotide linkage or a phosphodiester derivative internucleotide linkage. In some embodiments, a CpG motif includes a phosphodiester internucleotide linkage. In some embodiments, a CpG motif includes a phosphodiester derivative internucleotide linkage.

As used herein, the terms “coronavirus spike protein” and “coronavirus spike peptide” refer to a full-length or fragment of a large, type 1 transmembrane protein, sometimes referred to as an “S protein,” which includes an S1 and S2 domain. Coronavirus spike proteins are highly glycosylated and assemble in trimers on the virion surface, such as the surface of the SAR-CoV-2 virion. In the case of SARS-CoV-2, the spike protein binds with a human angiotensin-converting enzyme 2 (ACE2) receptor to infect human cells, e.g., respiratory epithelial cells (e.g., type II alveolar cells), as well as cells (e.g., epithelial cells, endothelial cells, neurons, glial cells, smooth muscle cells, and enterocytes)

in many other tissues and organs including, e.g., the heart, blood vessels, kidney, liver, gastrointestinal tract, and the nervous system (e.g., the brain and the peripheral nervous system). In some embodiments, the spike protein peptide may have an amino acid sequence having at least 90% (e.g., at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%) sequence identity to SEQ ID NO: 3. In some embodiments, the coronavirus spike protein peptide has an amino acid sequence of SEQ ID NO: 3.

As used herein, "immune response" refers to a response made by the immune system of an organism to a substance, which includes but is not limited to foreign or self proteins. Three general types of "immune response" include mucosal, humoral, and cellular immune responses. An immune response may include at least one of the following: antibody production, inflammation, developing immunity, developing hypersensitivity to an antigen, the response of antigen-specific lymphocytes to antigen, and transplant or graft rejection.

As used herein, "immunogenic" refers to the ability of an agent (e.g., a CpG-amphiphile and a coronavirus spike protein or peptide), to trigger an immune response, e.g., as measured by antibody titer.

As used herein, the term "immunogenic amount" refers to an amount of a CpG-amphiphile and a coronavirus spike protein or peptide that induces an immune response in a subject (e.g., reflected by an increase in antibody titer in the subject as determined by conventional techniques, such as enzyme-linked immunosorbent assay (ELISA)).

The term "infectious agent," as used herein, refers to agents that cause an infection and/or a disease. Infectious agents include viruses, bacteria, fungi, and parasites. In some embodiments, the infectious agent is a virus (e.g., a coronavirus, e.g., SARS-CoV-2).

"Nucleic acid" refers to deoxyribonucleotides or ribonucleotides and polymers thereof in either single- or double-stranded form. Unless specifically limited, the term encompasses nucleic acids containing known analogues of natural nucleotides that have similar binding properties as the reference nucleic acid and are metabolized in a manner similar to naturally occurring nucleotides. Unless otherwise indicated, a particular nucleic acid sequence also implicitly encompasses conservatively modified variants thereof (e.g., degenerate codon substitutions) and complementary sequences and as well as the sequence explicitly indicated. Specifically, degenerate codon substitutions can be achieved by generating sequences in which the third position of one or more selected (or all) codons is substituted with mixed-base and/or deoxyinosine residues (Batzer *et al., Nucleic Acid Res.* 19:5081, 1991; Ohtsuka *et al., J. Biol. Chem.* 260:2605-2608, 1985); and Cassol *et al.,* 1992; Rossolini *et al., Mol. Cell. Probes* 8:91-98, 1994). For arginine and leucine, modifications at the second base can also be conservative. The term nucleic acid is used interchangeably with gene, cDNA, and mRNA encoded by a gene.

"Percent (%) sequence identity" with respect to a reference polynucleotide or polypeptide sequence is defined as the percentage of nucleic acids or amino acids in a candidate sequence that are identical to the nucleic acids or amino acids in the reference polynucleotide or polypeptide sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity. Alignment for purposes of determining percent nucleic acid or amino acid

sequence identity can be achieved in various ways that are within the capabilities of one of skill in the art, for example, using publicly available computer software such as BLAST, BLAST-2, or Megalign software. Those skilled in the art can determine appropriate parameters for aligning sequences, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared. For example, percent sequence identity values may be generated using the sequence comparison computer program BLAST. As an illustration, the percent sequence identity of a given nucleic acid or amino acid sequence, A, to, with, or against a given nucleic acid or amino acid sequence, B, (which can alternatively be phrased as a given nucleic acid or amino acid sequence, A that has a certain percent sequence identity to, with, or against a given nucleic acid or amino acid sequence, B) is calculated as follows:

100 multiplied by (the fraction X/Y)

where X is the number of nucleotides or amino acids scored as identical matches by a sequence alignment program (e.g., BLAST) in that program's alignment of A and B, and where Y is the total number of nucleic acids in B. It will be appreciated that where the length of nucleic acid or amino acid sequence A is not equal to the length of nucleic acid or amino acid.

As generally used herein, “pharmaceutically acceptable” refers to those compounds, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues, organs, and/or bodily fluids of human beings and animals without excessive toxicity, irritation, allergic response, or other problems or complications commensurate with a reasonable benefit/risk ratio.

A “pharmaceutically acceptable carrier,” as used herein, refers to a vehicle capable of suspending or dissolving the active compound, and having the properties of being nontoxic and non-inflammatory in a patient. Moreover, a pharmaceutically acceptable carrier may include a pharmaceutically acceptable additive, such as a preservative, antioxidant, fragrance, emulsifier, dye, or excipient known or used in the field of drug formulation and that does not significantly interfere with the therapeutic effectiveness of the biological activity of the active agent, and that is non-toxic to the patient.

The term “pharmaceutically acceptable excipient,” as used herein, refers to any inactive ingredient having the properties of being nontoxic and non-inflammatory in a subject. Typical excipients include, for example: carriers, binders, fillers, lubricants, emulsifiers, suspending agents, sweeteners, flavorings, preservatives, buffers, wetting agents, disintegrants, effervescent agents, and other conventional excipients and additives and/or other additives that may enhance stability, delivery, absorption, half-life, efficacy, pharmacokinetics, and/or pharmacodynamics, reduce adverse side effects, or provide other advantages for pharmaceutical use.

Polynucleotides of the present invention can be composed of any polyribonucleotide or polydeoxribonucleotide, which can be unmodified RNA or DNA or modified RNA or DNA. For example, polynucleotides can be composed of single- and double-stranded DNA, DNA that is a mixture of single- and double-stranded regions, single- and double-stranded RNA, and RNA that is mixture of single- and double-stranded regions, hybrid molecules comprising DNA and RNA that can

be single-stranded or, more typically, double-stranded or a mixture of single- and double-stranded regions. In addition, the polynucleotide can be composed of triple-stranded regions comprising RNA or DNA or both RNA and DNA. A polynucleotide can also contain one or more modified bases or DNA or RNA backbones modified for stability or for other reasons. “Modified” bases include, for example, tritylated bases and unusual bases such as inosine. A variety of modifications can be made to DNA and RNA; thus, "polynucleotide" embraces chemically, enzymatically, or metabolically modified forms.

The term “pharmaceutically acceptable salt,” as used herein, means any pharmaceutically acceptable salt of a conjugate, oligonucleotide, or peptide disclosed herein. Pharmaceutically acceptable salts of any of the compounds described herein may include those that are within the scope of sound medical judgment, suitable for use in contact with the tissues of humans and animals without undue toxicity, irritation, allergic response and are commensurate with a reasonable benefit/risk ratio. Pharmaceutically acceptable salts are well known in the art. For example, pharmaceutically acceptable salts are described in: Berge et al., J. Pharmaceutical Sciences 66:1-19, 1977 and in Pharmaceutical Salts: Properties, Selection, and Use (Eds. P.H. Stahl and C.G. Wermuth), Wiley-VCH, 2008. The salts can be prepared in situ during the final isolation and purification of the compounds described herein or separately by reacting a free base group with a suitable acid. Representative acid addition salts include acetate, adipate, alginate, ascorbate, aspartate, benzenesulfonate, benzoate, bisulfate, borate, butyrate, camphorate, camphorsulfonate, citrate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, fumarate, glucoheptonate, glycerophosphate, hemisulfate, heptonate, hexanoate, hydrobromide, hydrochloride, hydroiodide, 2-hydroxy-ethanesulfonate, lactobionate, lactate, laurate, lauryl sulfate, malate, maleate, malonate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, nitrate, oleate, oxalate, palmitate, pamoate, pectinate, persulfate, 3-phenylpropionate, phosphate, picrate, pivalate, propionate, stearate, succinate, sulfate, tartrate, thiocyanate, toluenesulfonate, undecanoate, valerate salts, and the like. Representative alkali or alkaline earth metal salts include sodium, lithium, potassium, calcium, magnesium, and the like, as well as nontoxic ammonium, quaternary ammonium, and amine cations, including, but not limited to ammonium, tetramethylammonium, tetraethylammonium, methylamine, dimethylamine, trimethylamine, triethylamine, ethylamine, and the like. References to the conjugates, oligonucleotides, or peptides include pharmaceutically acceptable salts thereof unless otherwise indicated or not applicable.

“Polypeptide,” “peptide,” and “protein” are used interchangeably herein to refer to a polymer of amino acid residues. The terms apply to amino acid polymers in which one or more amino acid residue is an artificial chemical mimetic of a corresponding naturally occurring amino acid, as well as to naturally occurring amino acid polymers and non-naturally occurring amino acid polymer.

As used herein, the term “preventing” or “reducing the risk of acquiring” means decreasing the risk of (e.g., by 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 97%, 99%, or about 100%) contracting an infectious disease, e.g., a viral infection, e.g., an infection by a beta-coronavirus such as SARS-CoV-2, or a related virus. To determine whether the prevention is effective, a comparison can be made between the subject who received a

composition of the invention and a similarly-situated subject (e.g., one at risk of a viral infection, such as a SARS-CoV-2 infection, or an infection by a related virus) who did not receive the composition. A comparison can also be made between the subject who received the composition and a control, a baseline, or a known level of measurement.

As used here, the term "subject" or "mammal" or "patient" as used herein includes both humans and non-humans and includes, but is not limited to, humans, non-human primates, canines, felines, murines, bovines, equines, and porcines.

As used herein, the term "therapeutically effective amount" is an amount that is effective to ameliorate a symptom of a disease. A therapeutically effective amount can be a "prophylactically effective amount" as prophylaxis can be considered therapy.

The terms "treat," "treatment," and "treating" refer to therapeutic approaches in which the goal is to reverse, alleviate, ameliorate, inhibit, slow down, or stop the progression or severity of a condition associated with a disease or disorder, e.g., COVID-19. These terms include reducing or alleviating at least one adverse effect or symptom of a condition, disease, or disorder. Treatment is generally "effective" if one or more symptoms or clinical markers are reduced, or if a desired response (e.g., a specific immune response) is induced. Alternatively, treatment is "effective" if the progression of a disease is reduced or halted.

As used herein, the term "vaccine" or "immunogenic composition" refers to a formulation which contains a CpG-amphiphile and/or a coronavirus antigen (e.g., a coronavirus spike protein, a peptide thereof, or a nucleic acid sequence encoding the same) as described herein, optionally combined with an adjuvant, which is in a form that is capable of being administered to a vertebrate and which induces a protective or therapeutic immune response sufficient to induce immunity to prevent and/or ameliorate an infection or disease and/or to reduce at least one symptom of an infection or disease. Typically, the vaccine or immunogenic composition comprises a conventional saline or buffered aqueous solution medium in which a composition as described herein is suspended or dissolved. In this form, a composition as described herein is used to prevent, ameliorate, or otherwise treat an infection or disease. Upon introduction into a host, the vaccine or immunogenic composition provokes an immune response including, but not limited to, the production of antibodies and/or cytokines and/or the activation of cytotoxic T cells, antigen presenting cells, helper T cells, dendritic cells and/or other cellular responses.

Other features and advantages of the invention will be apparent from the following detailed description, the drawings, and the claims.

DETAILED DESCRIPTION OF THE INVENTION

The invention provides compositions that can be used in inducing an immune response in a subject. The compositions include CpG oligodeoxynucleotides (ODNs) linked to a lipid by way of a linker or without the use of linker (i.e., bonded directly) forming an CpG-amphiphile, and coronavirus antigen (e.g., a coronavirus spike protein or a peptide thereof, and/or a coronavirus nucleocapsid protein or a peptide thereof, or a nucleic acid sequence encoding the same). Together the compounds described herein induce an immune response in a subject, such as a human subject,

when administered concurrently or separately. The CpG-amphiphile can function as an adjuvant to elicit an immune response in a subject, such as an immune response against a coronavirus antigen (e.g., a SARS-CoV-2 antigen, e.g., a SARS-CoV-2 spike protein or peptide thereof or a SARS-CoV-2 nucleocapsid protein or a peptide thereof).

CpG

CpG oligodeoxynucleotides (ODNs) are short synthetic single-stranded DNA molecules containing unmethylated CpG dinucleotides in particular sequence contexts. CpG ODNs possess a partially or completely phosphorothioated (PS) backbone, as opposed to the natural phosphodiester (PO) backbone in DNA molecules. Three major classes of stimulatory CpG ODNs have been identified based on structural characteristics and activity on human peripheral blood mononuclear cells (PBMCs), in particular B cells and plasmacytoid dendritic cells (pDCs). These three classes are Class A (Type D), Class B (Type K), and Class C.

In some embodiments, the CpG ODN may be a Class A ODN. For example, the Class A ODN may be selected from the group including CpG 1585, having an amino acid sequence of GGGGTCAACGTTGAGGGGGG (SEQ ID NO: 5); CpG 2216, having an amino acid sequence of GGGGGACGATCGTCGGGGGG (SEQ ID NO: 6); and CpG 2336, having the amino acid sequence of GGGGACGACGTCGTGGGGGGG (SEQ ID NO: 7).

In some embodiments, the CpG ODN may be a Class B ODN. Class B CpG ODNs contain a full PS backbone with one or more CpG dinucleotides. They strongly activate B cells and TLR9-dependent NF-κB signaling but weakly stimulate IFN-α secretion. For example, the Class B ODN may be selected from the group including CpG 1668, having the amino acid sequence of TCCATGACGTTCCTGATGCT (SEQ ID NO:71); CpG 7909, also known as CpG 2006, having the amino acid sequence of TCGTCGTTTTGTCGTTTTGTCGTT (SEQ ID NO: 1); CpG 2007, having the amino acid sequence of TCGTCGTTGTCGTTTTGTCGTT (SEQ ID NO: 8); CpG BW006, having the amino acid sequence of TCGACGTTCGTCGTTCGTCGTTC (SEQ ID NO: 9); CpG D-SL01, having the amino acid sequence of TCGCGACGTTCGCCCGACGTTCGGTA (SEQ ID NO: 10); CpG 1018, having the amino acid sequence of TGACTGTGAACGTTCGAGATGA (SEQ ID NO: 15),and CpG 1826, having an amino acid sequence of TCCATGACGTTCCTGACGTT (SEQ ID NO: 2).). In some embodiments, the CpG ODN is CpG 7909 (SEQ ID NO: 1). In some embodiments, the CpG ODN is CpG 1826 (SEQ ID NO: 2).

In some embodiments, the CpG ODN may be a Class C ODN. For example, the Class C ODN may be selected from the group including CpG 2395, having the amino acid sequence of TCGTCGTTTTCGGCGCGCGCCG (SEQ ID NO: 11); CpG M362, having the amino acid sequence of TCGTCGTCGTTCGAACGACGTTGAT (SEQ ID NO: 12); and CpG D-SL03, having the amino acid sequence of TCGCGAACGTTCGCCGCGTTCGAACGCGG (SEQ ID NO: 13).

In some embodiments, all the internucleoside groups connecting the nucleosides in the CpG sequence are phosphorothionates

In some embodiments, an immunogenic composition includes an amphiphilic conjugate. An amphiphilic conjugate refers to a conjugate that includes a CpG ODN covalently linked to an albumin-

binding domain (e.g., a lipid). In some embodiments, an amphiphilic conjugate includes a CpG ODN that is covalently linked to an albumin-binding domain (e.g., a lipid) directly. In some embodiments, an amphiphilic conjugate includes a CpG ODN that is covalently linked to an albumin-binding domain (e.g., a lipid) through a linker. For amphiphilic conjugates that include CpG ODN conjugated to an albumin-binding domain either directly or through a linker, the albumin binding domain binds to endogenous albumin, which prevents the CpG-amphiphile from rapidly flushing into the bloodstream and instead re-targets them to lymphatics and draining lymph nodes where they accumulate due to filtering of albumin by antigen presenting cells.

CpG ODNs may be bonded directly or linked by way of a linker to a lipid to a form an CpG amphiphile. These compounds may be produced using the ordinary phosphoramidite chemistry known in the art. In some examples, the CpG ODN or CpG ODN-GG may be reacted with the following compound: to produce an intermediate, which upon oxidation with (e.g., phosphite oxidation methods known in the art, e.g., a sulfurizing agent, such as 3-((N,N-dimethylaminomethylidene)amino)-3H-1,2,4-dithiazole-5-thione) and hydrolysis of the cyanoethyl group may produce a compound of the invention.

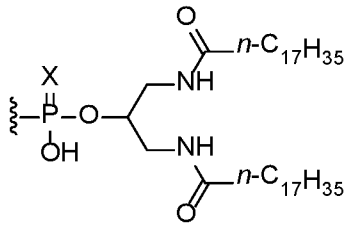
Reference to CpG molecules herein, as well as amphiphiles including a CpG molecule, is to be understood as including pharmaceutically acceptable salts thereof.

Lipid

The CpG-amphiphiles disclosed herein include a hydrophobic lipid, which may be an albumin binding domain. The lipid can be linear, branched, or cyclic. The lipid is preferably at least 17 to 18 carbons in length but may be shorter if it shows good albumin binding and adequate targeting to the lymph nodes. In some embodiments, the activity relies, in-part, on the ability of the CpG-amphiphile to associate with albumin in the blood of the subject. Therefore, lymph node-targeted CpG-amphiphiles typically include a lipid that can bind to albumin under physiological conditions. Lipids suitable for targeting the lymph node can be selected based on the ability of the lipid or a lipid conjugated to a CpG ODN to bind to albumin. Suitable methods for testing the ability of the lipid or lipid conjugated to a CpG ODN to bind to albumin are known in the art.

Examples of preferred lipids for use in lymph node targeting with CpG-amphiphiles include, but are not limited to fatty acids with aliphatic tails of 8-30 carbons including, but not limited to, linear and unsaturated saturated fatty acids, branched saturated and unsaturated fatty acids, and fatty acids derivatives, such as fatty acid esters, fatty acid amides, and fatty acid thioesters, diacyl lipids, cholesterol, cholesterol derivatives, and steroid acids such as bile acids; Lipid A or combinations thereof.

In some embodiments, the lipid is a diacyl lipid or two-tailed lipid. In some embodiments, the tails in the diacyl lipid contain from about 8 to about 30 carbons and can be saturated, unsaturated, or combinations thereof. In some embodiments, the diacyl lipid has the following structure:



or a salt thereof, wherein X is O or S. The tails of a lipid can be coupled to the head group via ester bond linkages, amide bond linkages, thioester bond linkages, or combinations thereof. In a particular embodiment, the diacyl lipids are phosphate lipids, glycolipids, sphingolipids, or combinations thereof.

Lymph node-targeting conjugates typically include a lipid that is 8 or more carbon units in length. Increasing the number of lipid units can reduce insertion of the lipid into plasma membrane of cells, allowing the lipid conjugate to remain free to bind albumin and traffic to the lymph node. For example, the lipid can be a diacyl lipid composed of two C18 hydrocarbon tails. In some embodiments, the lipid for use in preparing lymph node targeting lipid conjugates is not a single chain hydro-carbon (e.g., C18), or cholesterol. Cholesterol conjugation has been explored to enhance the immunomodulation of molecular adjuvants such as CpG and immunogenicity of peptides.

Reference to lipids herein, as well as amphiphiles including the lipid, is to be understood as including pharmaceutically acceptable salts thereof.

Linkers

For the CpG-amphiphile to be trafficked efficiently to the lymph node, the CpG ODN should remain soluble. Therefore, a polar block linker can be included between the CpG ODN and the lipid to which it is conjugated to increase solubility of the CpG ODN. In some embodiments, the CpG-amphiphile includes a CpG sequence linked to a lipid by a linker. The linker may reduce or prevent the ability of the lipid to insert into the plasma membrane of cells, such as cells in the tissue adjacent to the injection site. The linker can also reduce or prevent the ability of the CpG ODN from non-specifically associating with extracellular matrix proteins at the site of administration. The linker may increase the solubility of the CpG ODN without preventing its ability to bind to albumin. This combination of characteristics can allow the CpG ODN to bind to albumin present in the serum or interstitial fluid and remain in circulation until the albumin is trafficked to and retained in a lymph node.

The length and composition of the linker can be adjusted based on the lipid and CpG ODN selected. For example, for some CpG ODNs, the oligonucleotide itself may be polar enough to ensure solubility; for example, oligonucleotides that are 10, 15, 20 or more nucleotides in length. Therefore, in some embodiments, no additional linker is required. However, depending on the amino acid sequence, some lipidated peptides can be essentially insoluble. In these cases, it can be desirable to include a linker that mimics the effect of a polar oligonucleotide. A linker can be used as part of any of lipid conjugates described herein, for example, lipid-oligonucleotide conjugates and lipid-peptide conjugates, which reduce cell membrane insertion/preferential portioning onto albumin.

Suitable linkers include, but are not limited to, oligonucleotides such as those discussed above, including a string of nucleic acids, a hydrophilic polymer including but not limited to poly(ethylene glycol) (MW: 500 Da to 20,000 Da), polyacrylamide (MW: 500 Da to 20,000 Da),

polyacrylic acid; a string of hydrophilic amino acids such as serine, threonine, cysteine, tyrosine, asparagine, glutamine, aspartic acid, glutamic acid, lysine, arginine, histidine, or combinations thereof; polysaccharides, including but not limited to, dextran (MW: 1,000 Da to 2,000,000 Da), or combinations thereof. The hydrophobic lipid and the linker/CpG ODN are covalently linked. The covalent bond may be a non-cleavable linkage or a cleavable linkage. The non-cleavable linkage can include an amide bond or phosphate bond, and the cleavable linkage can include a disulfide bond, acid-cleavable linkage, ester bond, anhydride bond, biodegradable bond, or enzyme-cleavable linkage.

In some embodiments, the linker is one or more ethylene glycol (EG) units, more preferably two or more EG units (i.e., polyethylene glycol (PEG)). For example, in some embodiments, the CpG-amphiphile includes a CpG and a hydrophobic lipid linked by a polyethylene glycol (PEG) molecule or a derivative or analog thereof.

In some embodiments, CpG-amphiphiles described herein contain a CpG ODN linked to PEG which is in turn linked to a hydrophobic lipid, or lipid-Gn-ON conjugates, either covalently or via formation of protein-oligo conjugates that hybridize to oligo micelles. The precise number of PEG units depends on the lipid and the cargo, however, typically, a linker can have between about 1 and about 100, between about 20 and about 80, between about 30 and about 70, or between about 40 and about 60 PEG units. In some embodiments, the linker has between about 45 and 55 PEG, units. For example, in some embodiments, the linker has 48 PEG units.

As discussed above, in some embodiments, the linker is an oligonucleotide which includes a string of nucleic acids. In some embodiments, the CpG amphiphiles described herein include a CpG ODN linked to a string of nucleic acids, which is in turn linked to a hydrophobic lipid. The linker can have any sequence, for example, the sequence of the oligonucleotide can be a random sequence, or a sequence specifically chosen for its molecular or biochemical properties (e.g., highly polar). In some embodiments, the linker includes 20 one or more series of consecutive adenine (A), cytosine (C), guanine (G), thymine (T), uracil (U), or analog thereof. In some embodiments, the linker consists of a series of consecutive adenine (A), cytosine (C), guanine (G), thymine (T), uracil (U), or analog thereof.

In some embodiments, the string of nucleic acids includes between 1 and 50 nucleic acid residues. In some embodiments, the string of nucleic acids includes between 5 and 30 nucleic acid residues. In some embodiments, the linker includes one or more guanines, for example between 1-10 guanines. It has been discovered that altering the number of guanines between a CpG ODN and a lipid tail controls micelle stability in the presence of serum proteins. Therefore, the number of guanines in the linker can be selected based on the desired affinity of the CpG ODN for serum proteins such as albumin.

In some embodiments, the linker is an oligonucleotide that includes a string of amino acids. In some embodiments, the CpG amphiphiles include a CpG ODN linked to string of amino acids, which is in turn linked to a hydrophobic lipid. The linker can have any amino acid sequence, for example, the sequence of the oligonucleotide can be a random sequence, or a sequence chosen for its molecular or biochemical properties (e.g., high flexibility). In some embodiments, the linker

includes a series of glycine residue to form a polyglycine linker. In some embodiments, the linker includes an amino acid sequence of $(Gly)_n$, wherein n may be between 2 and 20 residues. Examples of polyglycine linkers include but are not limited to GGG, GGGA (SEQ ID NO:18), GGGG (SEQ ID NO:19), GGGAG (SEQ ID NO:20), GGGAGG (SEQ ID NO:21), GGGAGGG (SEQ ID NO:22), GGAG (SEQ ID NO:23),GGSG (SEQ ID NO:24), AGGG (SEQ ID NO:25), SGGG (SEQ ID NO:26), GGAGGA (SEQ ID NO:27), GGSGGS (SEQ ID NO:28), GGAGGAGGA (SEQ ID NO:29), GGSGGSGGS (SEQ ID NO:30), GGAGGAGGAGGA (SEQ ID NO:31), GGSGGSGGSGGS (SEQ ID NO:32), GGAGGGAG (SEQ ID NO:33), GGSGGGSG (SEQ ID NO:34), GGAGGGAGGGAG (SEQ ID NO:35), GGSGGGSGGGSG (SEQ ID NO:36), GGGGAGGGGAGGGGA (SEQ ID NO:37), GGGGSGGGGSGGGGS (SEQ ID NO:38), and GGGSGGGS (SEQ ID NO:62).

Linkers described herein (e.g., polyglycine linkers) can also be used to link a polypeptide sequence (e.g., a coronavirus spike protein or peptide thereof or a coronavirus nucleocapsid protein or a peptide thereof) to a tag (e.g., a histidine tag and/or an Avi tag).

Coronavirus Antigen

In an aspect, the disclosure provides a full-length or fragment of a SARS-CoV-2 spike glycoprotein, which has been identified as immunogenic or a multimer (e.g., a trimer) of this spike protein (Grifoni et al. *Cell Host Microbe*. 2020; 27(4): 671-80; Ou et al. *Nat Commun*. 2020, 11(1): 1620; Walls et al. *Cell*. 2020; 181(2): 281-92). In addition, the antigen may correspond to SARS-CoV-2 nucleocapsid protein, membrane protein, etc., or a peptide thereof. The antigen may also correspond to a specific functional region of a coronavirus spike protein (i.e., protein subunit). For example, the antigen may correspond to or comprise the S1, S2, or receptor-binding domain (RBD) region of the SARS-CoV-2 spike glycoprotein, or S protein.

The antigen(s) may also be a peptide (or several peptides) that correspond to immunogenic sequences in the infectious agent of interest. The peptides behave as epitopes that can elicit various immune responses. For example, the peptides may represent various positions of the SARS-CoV-2 spike glycoprotein which are predicted in both cellular and humoral immunogenicity (Fast et al. *bioRxiv*. 2020: 2020.02.19.955484). Regarding antigens made up of several peptides, the antigen(s) may be a cocktail of overlapping peptides that encompass a whole protein or a functional region thereof, or it may be a mixture of peptides that correspond to immunogenic regions of different proteins. For example, the antigen(s) may be a mix of peptides that includes SARS-CoV-2 spike protein, nucleocapsid protein, and membrane protein The SARS-CoV-2 spike protein may have the amino acid sequence of

MFIFLLFLTLTSGSDLDRCTTFDDVQAPNYTQHTSSMRGVYYPDEIFRSDTLYLTQDLFLPFYSNVTGF
HTINHTFGNPVIPFKDGIYFAATEKSNVVRGWVFGSTMNNKSQSVIIINNSTNVVIRACNFELCDNPFF
AVSKPMGTQTHTMIFDNAFNCTFEYISDAFSLDVSEKSGNFKHLREFVFKNKDGFLYVYKGYQPIDVV
RDLPSGFNTLKPIFKLPLGINITNFRAILTAFSPAQDIWGTSAAAYFVGYLKPTTFMLKYDENGTITDAV
DCSQNPLAELKCSVKSFEIDKGIYQTSNFRVVPSGDVVRFPNITNLCPFGEVFNATKFPSVYAWERKK
ISNCVADYSVLYNSTFFSTFKCYGVSATKLNDLCFSNVYADSFVVKGDDVRQIAPGQTGVIADYNYKL
PDDFMGCVLAWNTRNIDATSTGNYNYKYRYLRHGKLRPFERDISNVPFSPDGKPCTPPALNCYWPL

NDYGFYTTTGIGYQPYRVVVLSFELLNAPATVCGPKLSTDLIKNQCVNFNFNGLTGTGVLTPSSKRFQ
PFQQFGRDVSDFTDSVRDPKTSEILDISPCSFGGVSVITPGTNASSEVAVLYQDVNCTDVSTAIHADQ
LTPAWRIYSTGNNVFQTQAGCLIGAEHVDTSYECDIPIGAGICASYHTVSLLRSTSQKSIVAYTMSLGA
DSSIAYSNNTIAIPTNFSISITTEVMPVSMAKTSVDCNMYICGDSTECANLLLQYGSFCTQLNRALSGIA
AEQDRNTREVFAQVKQMYKTPTLKYFGGFNFSQILPDPLKPTKRSFIEDLLFNKVTLADAGFMKQYGE
CLGDINARDLICAQKFNGLTVLPPLLTDDMIAAYTAALVSGTATAGWTFGAGAALQIPFAMQMAYRFN
GIGVTQNVLYENQKQIANQFNKAISQIQESLTTTSTALGKLQDVVNQNAQALNTLVKQLSSNFGAISSV
LNDILSRLDKVEAEVQIDRLITGRLQSLQTYVTQQLIRAAEIRASANLAATKMSECVLGQSKRVDFCGK
GYHLMSFPQAAPHGVVFLHVTYVPSQERNFTTAPAICHEGKAYFPREGVFVFNGTSWFITQRNFFSP
QIITTDNTFVSGNCDVVIGIINNTVYDPLQPELDSFKEELDKYFKNHTSPDVDLGDISGINASVVNIQKEI
DRLNEVAKNLNESLIDLQELGKYEQYIKWPWYVWLGFIAGLIAIVMVTILLCCMTSCCSCLKGACSCGS
CCKFDEDDSEPVLKGVKLHYT (SEQ ID NO: 14). In some embodiments, the coronavirus spike protein may have the amino acid sequence of

MFIFLLFLTLTSGSDLDRCTTFDDVQAPNYTQHTSSMRGVYYPDEIFRSDTLYLTQDLFLPFYSNVTGF
HTINHTFGNPVIPFKDGIYFAATEKSNVVRGWVFGSTMNNKSQSVIIINNSTNVVIRACNFELCDNPFF
AVSKPMGTQTHTMIFDNAFNCTFEYISDAFSLDVSEKSGNFKHLREFVFKNKDGFLYVYKGYQPIDVV
RDLPSGFNTLKPIFKLPLGINITNFRAILTAFSPAQDIWGTSAAAYFVGYLKPTTFMLKYDENGTITDAV
DCSQNPLAELKCSVKSFEIDKGIYQTSNFRVVPSGDVVRFPNITNLCPFGEVFNATKFPSVYAWERKK
ISNCVADYSVLYNSTFFSTFKCYGVSATKLNDLCFSNVYADSFVVKGDDVRQIAPGQTGVIADYNYKL
PDDFMGCVLAWNTRNIDATSTGNYNYKYRYLRHGKLRPFERDISNVPFSPDGKPCTPPALNCYWPL
NDYGFYTTTGIGYQPYRVVVLSFELLNAPATVCGPKLSTDLIKNQCVNFNFNGLTGTGVLTPSSKRFQ
PFQQFGRDVSDFTDSVRDPKTSEILDISPCSFGGVSVITPGTNASSEVAVLYQDVNCTDVSTAIHA**G**Q
LTPAWRIYSTGNNVFQTQAGCLIGAEHVDTSYECDIPIGAGICASYHTVSLLRSTSQKSIVAYTMSLGA
DSSIAYSNNTIAIPTNFSISITTEVMPVSMAKTSVDCNMYICGDSTECANLLLQYGSFCTQLNRALSGIA
AEQDRNTREVFAQVKQMYKTPTLKYFGGFNFSQILPDPLKPTKRSFIEDLLFNKVTLADAGFMKQYGE
CLGDINARDLICAQKFNGLTVLPPLLTDDMIAAYTAALVSGTATAGWTFGAGAALQIPFAMQMAYRFN
GIGVTQNVLYENQKQIANQFNKAISQIQESLTTTSTALGKLQDVVNQNAQALNTLVKQLSSNFGAISSV
LNDILSRLDKVEAEVQIDRLITGRLQSLQTYVTQQLIRAAEIRASANLAATKMSECVLGQSKRVDFCGK
GYHLMSFPQAAPHGVVFLHVTYVPSQERNFTTAPAICHEGKAYFPREGVFVFNGTSWFITQRNFFSP
QIITTDNTFVSGNCDVVIGIINNTVYDPLQPELDSFKEELDKYFKNHTSPDVDLGDISGINASVVNIQKEI
DRLNEVAKNLNESLIDLQELGKYEQYIKWPWYVWLGFIAGLIAIVMVTILLCCMTSCCSCLKGACSCGS
CCKFDEDDSEPVLKGVKLHYT (SEQ ID NO: 16).

In some embodiments, a coronavirus spike protein construct includes a sequence that is at least 90% (e.g., at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%) identical to the following sequence:

VNLTTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHSTQDLFLPFFSNVTWFHAIHVSGTNGTKRFDNP
VLPFNDGVYFASTEKSNIIRGWIFGTTLDSKTQSLLIVNNATNVVIKVCEFQFCNDPFLGVYYHKNNKS
WMESEFRVYSSANNCTFEYVSQPFLMDLEGKQGNFKNLREFVFKNIDGYFKIYSKHTPINLVRDLPQ
GFSALEPLVDLPIGINITRFQTLLALHRSYLTPGDSSSGWTAGAAAYYVGYLQPRTFLLKYNENGTITD
AVDCALDPLSETKCTLKSFTVEKGIYQTSNFRVQPTESIVRFPNITNLCPFGEVFNATRFASVYAWNR

KRISNCVADYSVLYNSASFSTFKCYGVSPTKLNDLCFTNVYADSFVIRGDEVRQIAPGQTGKIADYNY
KLPDDFTGCVIAWNSNNLDSKVGGNYNYLYRLFRKSNLKPFERDISTEIYQAGSTPCNGVEGFNCYF
PLQSYGFQPTNGVGYQPYRVVVLSFELLHAPATVCGPKKSTNLVKNKCVNFNFNGLTGTGVLTESNK
KFLPFQQFGRDIADTTDAVRDPQTLEILDITPCSFGGVSVITPGTNTSNQVAVLYQDVNCTEVPVAIHA
DQLTPTWRVYSTGSNVFQTRAGCLIGAEHVNNSYECDIPIGAGICASYQTQTNSPRAAASVASQSIIA
YTMSLGAENSVAYSNNSIAIPTNFTISVTTEILPVSMTKTSVDCTMYICGDSTECSNLLLQYGSFCTQLN
RALTGIAVEQDKNTQEVFAQVKQIYKTPPIKDFGGFNFSQILPDPSKPSKRSFIEDLLFNKVTLADAGFI
KQYGDCLGDIAARDLICAQKFNGLTVLPPLLTDEMIAQYTSALLAGTITSGWTFGAGAALQIPFAMQMA
YRFNGIGVTQNVLYENQKLIANQFNSAIGKIQDSLSSTASALGKLQDVVNQNAQALNTLVKQLSSNFG
AISSVLNDILSRLDKVEAEVQIDRLITGRLQSLQTYVTQQLIRAAEIRASANLAATKMSECVLGQSKRVD
FCGKGYHLMSFPQSAPHGVVFLHVTYVPAQEKNFTTAPAICHDGKAHFPREGVFVSNGTHWFVTQR
NFYEPQIITTDNTFVSGNCDVVIGIVNNTVYDPLQPELDSFKEELDKYFKNHTSPDVDLGDISGINASVV
NIQKEIDRLNEVAKNLNESLIDLQELGKYEQYIKWPGGGSGGGSHHHHHHHHHH (SEQ ID NO:66).

In some embodiments, a coronavirus spike protein construct includes the following sequence:

VNLTTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHSTQDLFLPFFSNVTWFHAIHVSGTNGTKRFDNP
VLPFNDGVYFASTEKSNIIRGWIFGTTLDSKTQSLLIVNNATNVVIKVCEFQFCNDPFLGVYYHKNNKS
WMESEFRVYSSANNCTFEYVSQPFLMDLEGKQGNFKNLREFVFKNIDGYFKIYSKHTPINLVRDLPQ
GFSALEPLVDLPIGINITRFQTLLALHRSYLTPGDSSSGWTAGAAAYYVGYLQPRTFLLKYNENGTITD
AVDCALDPLSETKCTLKSFTVEKGIYQTSNFRVQPTESIVRFPNITNLCPFGEVFNATRFASVYAWNR
KRISNCVADYSVLYNSASFSTFKCYGVSPTKLNDLCFTNVYADSFVIRGDEVRQIAPGQTGKIADYNY
KLPDDFTGCVIAWNSNNLDSKVGGNYNYLYRLFRKSNLKPFERDISTEIYQAGSTPCNGVEGFNCYF
PLQSYGFQPTNGVGYQPYRVVVLSFELLHAPATVCGPKKSTNLVKNKCVNFNFNGLTGTGVLTESNK
KFLPFQQFGRDIADTTDAVRDPQTLEILDITPCSFGGVSVITPGTNTSNQVAVLYQDVNCTEVPVAIHA
DQLTPTWRVYSTGSNVFQTRAGCLIGAEHVNNSYECDIPIGAGICASYQTQTNSPRAAASVASQSIIA
YTMSLGAENSVAYSNNSIAIPTNFTISVTTEILPVSMTKTSVDCTMYICGDSTECSNLLLQYGSFCTQLN
RALTGIAVEQDKNTQEVFAQVKQIYKTPPIKDFGGFNFSQILPDPSKPSKRSFIEDLLFNKVTLADAGFI
KQYGDCLGDIAARDLICAQKFNGLTVLPPLLTDEMIAQYTSALLAGTITSGWTFGAGAALQIPFAMQMA
YRFNGIGVTQNVLYENQKLIANQFNSAIGKIQDSLSSTASALGKLQDVVNQNAQALNTLVKQLSSNFG
AISSVLNDILSRLDKVEAEVQIDRLITGRLQSLQTYVTQQLIRAAEIRASANLAATKMSECVLGQSKRVD
FCGKGYHLMSFPQSAPHGVVFLHVTYVPAQEKNFTTAPAICHDGKAHFPREGVFVSNGTHWFVTQR
NFYEPQIITTDNTFVSGNCDVVIGIVNNTVYDPLQPELDSFKEELDKYFKNHTSPDVDLGDISGINASVV
NIQKEIDRLNEVAKNLNESLIDLQELGKYEQYIKWPGGGSGGGSHHHHHHHHHH (SEQ ID NO:66).
This protein construct includes a ten-histidine tag (HHHHHHHHHH; SEQ ID NO:67) linked to the spike protein sequence with a GGGSGGGS (SEQ ID NO:62) linker. The spike protein has the following mutations to stabilize the trimer: R683A, R685A. This construct is available from ACROBiosystems under product number SPN-C52H2.

In some embodiments, the peptide of the coronavirus spike protein corresponds to a receptor binding domain of the coronavirus spike protein that specifically binds angiotensin-converting enzyme 2 (ACE2). The region of the SARS-CoV-2 spike protein, which is known to interact with the ACE2

receptor, corresponds to amino acids 323-502 on the 1255 amino acid protein (SEQ ID NO: 14) having the amino acid sequence of sequence of CPFGEVFNATKFPSVYAWERKKISNCVADYSVLYNSTFFSTFKCYGVSATKLNDLCFSNVYADSFVV KGDDVRQIAPGQTGVIADYNYKLPDDFMGCVLAWNTRNIDATSTGNYNYKYRYLRHGKLRPFERDIS NVPFSPDGKPCTPPALNCYWPLNDYGFYTTTGIGYQPYRVVVLSFE (SEQ ID NO:17), and thus acts as a region-binding domain (RBD).

In some embodiments, the coronavirus spike protein or peptide of the invention described herein has an amino acid sequence that is identical to a fragment of the SARS-CoV-2 spike protein RBD. In some embodiments, the coronavirus spike protein or peptide of the invention described herein has an amino acid sequence that is at least 90% (e.g., at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%) identical to RVQPTESIVRFPNITNLCPFGEVFNATRFASVYAWNRKRISNCVADYSVLYNSASFSTFK CYGVSPTKLNDLCFTNVYADSFVIRGDEVRQIAPGQTGKIADYNYKLPDDFTGCVIAWNSNNLDSKVG GNYNYLYRLFRKSNLKPFERDISTEIYQAGSTPCNGVEGFNCYFPLQSYGFQPTNGVGYQPYRVVVL SFELLHAPATVCGPKKSTNLVKNKCVNFNFNGLTGTGVLTESNKKFLPFQQFGRDIADTTDAVRDPQ TLEILDITPCS (SEQ ID NO: 3). In some embodiments, the coronavirus spike protein peptide has an amino acid sequence of SEQ ID NO: 3.

In some embodiments, the coronavirus spike protein or spike RBD contains one or more mutations. A mutation may be the N501Y mutation detected in a variant in the United Kingdom (202012/01), the A67V, 69del, 70del, 144del, E484K, D614G, Q677H, and F888L mutations detected in a variant in the United Kingdom and Nigeria (the 20A/S:484K variant), the 69del, 70del, 144del, N501Y, A570D, D614G, P681H, T716I, S982A, and D1118H mutations detected in a variant in the United Kingdom (the B.1.1.7 variant, also known as the Alpha variant, or 20I/501Y.V1 variant), the T95I, D253G and D614G mutations detected in a variant in the United States, D80G, 144del, F157S, L452R, D614G, and D950H mutations detected in a variant in the United States (the 20C variant), the L452R and D614G mutations found in the United States (the B.1.472 variant also known as the 20C/S:452R variant), the S13I, W152C, L452R, D614G mutations detected in a variant in the United States (the B.1.429 variant also known as the 20C/S:452R variant), the L18F, T20N, P26S, D138Y, R190S, K417T, E484K, N501Y, D614G, H655Y, and T1027Imutations detected in a variant in Brazil (the P.1 variant, also known as the Gamma variant, or the 20J/501Y.V3 variant), the E484K, D614G, and V1176F mutations detected in a variant in Brazil (the 20J variant), the L18F, the L452R, E484Q, and D614G mutations found in the variant in India (the 20A variant), the G142D, E154K, L452R, E484Q, D614G, P681R, and Q1071H mutations found in a variant in India (the 20A/S:154K variant), the T19R, G142D, L452R, E484Q, D614G, P681R, and D950N mutations found in a variant in India (the B.1.617.2 variant also known as the Delta variant, the 20A/S:478K variant, or the 20J variant), or the combination of N501&, K417N, and E484K mutations (with or without the D80A, D215G, 241del, 242del, 243del, D614G, and A701V mutations)detected in a variant in South Africa (the B.1.351 variant also known as the Beta variant, 501.V2 variant, or 501.V2, 20H/501Y.V2). The numbering of the variant mutations is relative to the full-length spike protein.

The spike RBD may contain any of the SARS-CoV2 variant mutations. In some embodiments, spike RBD has at least 90% (e.g., 91% 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%) sequence identity to the sequence of any of the forementioned SARS-CoV2 variants. In one embodiment, the spike RBD has at least 90% (e.g., 91% 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%) sequence identity to the sequence of the B.1.351 variant.

In some embodiments, the spike RBD contains the N501Y mutation and includes the sequence shown below:

RVQPTESIVRFPNITNLCPFGEVFNATRFASVYAWNRKRISNCVADYSVLYNSASFSTFKCYGVSPTK
LNDLCFTNVYADSFVIRGDEVRQIAPGQTGKIADYNYKLPDDFTGCVIAWNSNNLDSKVGGNYNYLY
RLFRKSNLKPFERDISTEIYQAGSTPCNGVEGFNCYFPLQSYGFQPTYGVGYQPYRVVVLSFELLHAP
ATVCGPKKSTNLVKNKCVNF (SEQ ID NO:69).

In some embodiments, a histidine-tag is added to the C-terminus of the sequence of SEQ ID NO:69. In some embodiments, the histidine-tag sequence is: AHHHHHHHHHH (SEQ ID NO:70).

In some embodiments, the coronavirus antigen is a coronavirus nucleocapsid protein or a peptide thereof. In some embodiments the coronavirus nucleocapsid protein includes a sequence that is at least 90% (e.g., at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%) identical to the following amino acid sequence:

MSDNGPQNQRNAPRITFGGPSDSTGSNQNGERSGARSKQRRPQGLPNNTASWFTALTQHGKEDL
KFPRGQGVPINTNSSPDDQIGYYRRATRRIRGGDGKMKDLSPRWYFYYLGTGPEAGLPYGANKDGII
WVATEGALNTPKDHIGTRNPANNAAIVLQLPQGTTLPKGFYAEGSRGGSQASSRSSSRSRNSSRNS
TPGSSRGTSPARMAGNGGDAALALLLLDRLNQLESKMSGKGQQQQGQTVTKKSAAEASKKPRQKR
TATKAYNVTQAFGRRGPEQTQGNFGDQELIRQGTDYKHWPQIAQFAPSASAFFGMSRIGMEVTPSG
TWLTYTGAIKLDDKDPNFKDQVILLNKHIDAYKTFPPTEPKKDKKKKADETQALPQRQKKQQTVTLLP
AADLDDFSKQLQQSMSSADSTQA (SEQ ID NO:68).

In some embodiments, the coronavirus nucleocapsid protein includes the amino acid sequence of SEQ ID NO:68.

In some embodiments, a coronavirus nucleocapsid protein construct includes the following sequence:

MSDNGPQNQRNAPRITFGGPSDSTGSNQNGERSGARSKQRRPQGLPNNTASWFTALTQHGKEDL
KFPRGQGVPINTNSSPDDQIGYYRRATRRIRGGDGKMKDLSPRWYFYYLGTGPEAGLPYGANKDGII
WVATEGALNTPKDHIGTRNPANNAAIVLQLPQGTTLPKGFYAEGSRGGSQASSRSSSRSRNSSRNS
TPGSSRGTSPARMAGNGGDAALALLLLDRLNQLESKMSGKGQQQQGQTVTKKSAAEASKKPRQKR
TATKAYNVTQAFGRRGPEQTQGNFGDQELIRQGTDYKHWPQIAQFAPSASAFFGMSRIGMEVTPSG
TWLTYTGAIKLDDKDPNFKDQVILLNKHIDAYKTFPPTEPKKDKKKKADETQALPQRQKKQQTVTLLP
AADLDDFSKQLQQSMSSADSTQAENLYFQGHHHHHH (SEQ ID NO:63).

This construct includes a cleavage site for a tobacco etch virus (TEV) protease (ENLYFQG; SEQ ID NO:64) between the nucleocapsid protein sequence and the six-histidine tag (HHHHHH; SEQ ID NO:65), and is available from ACROBiosystems under product number NUN-C5227.

In some embodiments, the coronavirus spike protein or peptide thereof or the coronavirus nucleocapsid protein or peptide thereof includes one or more tags (e.g., a histidine tag or an Avi tag).

A tag may be used for, for example, protein purification (e.g., affinity tags), to increase the solubility of a protein, to alter chromatographic properties, to give a fluorescent read out, or another purpose. Protein tags include but are not limited to a chitin binding protein (CBP) tag, a maltose binding protein (MBP) tag, a Strep-tag, a glutathione-S-transferase (GST) tag, a histidine tag, an AviTag, a C-tag, a calmodulin tag, an E-tag, a FLAG tag, a human influenza hemagglutinin (HA) tag, a Myc, an S-tag, and an NE-tag. In some embodiments, the coronavirus spike protein or peptide has a histidine tag. In some embodiments, the coronavirus spike protein has an Avi tag. In other embodiments, the coronavirus spike protein or peptide has both a histidine and an Avi tag.

In some embodiments, the coronavirus spike protein or peptide thereof or the coronavirus nucleocapsid protein or peptide thereof includes a protease cleavage site. In some embodiments, the protease cleavage site is between coronavirus spike protein, coronavirus nucleocapsid protein, or peptide sequence and a tag. In some embodiments the protease cleavage site is for TEV. In some embodiments, the TEV cleavage site has the amino acids sequence ENLYFQG (SEQ ID NO:64).

Other examples for predicted immunogenic epitopes that may give rise to an immune response can be found throughout literature (Grifoni et al. *Cell Host Microbe*. 2020; 27(4): 671-80; Prachar et al. *bioRxiv*. 2020: 2020.03.20.000794; Chour et al. *medRxiv*. 2020; 2020.05.04.20085779) and SARS-CoV-2 antigens' vendors' websites (e.g., Sino Biological, Creative Diagnostics, Sengenics, ABclonal Technology). Prediction tools for identifying immunogenic regions based on MHC binding ability are also widely available.

Alternatively, nucleic acids, such as messenger RNA (mRNA), that encode the coronavirus antigen (e.g., a coronavirus spike protein or peptide thereof or a coronavirus nucleocapsid protein or peptide thereof) may be administered. Once injected, the mRNA enters the cell's cytoplasm where it is translated into the desired protein or peptide, that can ultimately activate cellular and humoral immune response. For effective expression of the coronavirus spike protein or peptide, the mRNA will be synthesized to comprise the following: 5' cap – 5' untranslated region (UTR) – antigen-encoding sequence – 3' untranslated region (UTR) – poly A tail. The design of the 5' UTR and 3' UTR are important for mRNA stability, translation, protein production, and structure; there are several online tools that optimize the design of 5' UTR and 3' UTR based on mRNA of interest. The coronavirus spike protein or peptide-encoding sequence can be any mRNA sequence that codes for a specific protein or protein subunit; for example, mRNA that encodes SARS-CoV-2 spike protein, spike RBD domain, spike S1 domain, etc. The mRNA may also be non-modified, nucleoside-modified, or self-amplifying. To increase potency, stability, and protein yield, the mRNA may be subject to codon optimization and use of modified nucleosides. For example, incorporation of modified uridines or modified cytidine may be done to avoid premature recognition by innate immune molecules and improve efficiency of translation.

In some embodiments, the mRNA that encodes the coronavirus antigen (e.g., a coronavirus spike protein or peptide thereof or a coronavirus nucleocapsid protein or peptide thereof) is formulated in a lipid nanoparticle (LNP). Lipid nanoparticles typically comprise ionizable cationic lipid,

non-cationic lipid, sterol, and PEG lipid components along with the mRNA of interest (e.g., an mRNA encoding a coronavirus antigen such as a coronavirus spike protein or peptide thereof). The lipid nanoparticles can be generated using components, compositions, and methods as are generally known in the art, see for example PCT/US2016/052352; PCT/US2016/068300; PCT/US2017/037551; PCT/US2015/027400; PCT/US2016/047406; PCT/US2016000129; PCT/US2016/014280; PCT/US2016/014280; PCT/US2017/038426; PCT/US2014/027077; PCT/US2014/055394; PCT/US2016/52117; PCT/US2012/069610; PCT/US2017/027492; PCT/US2016/059575; and PCT/US2016/069491; all of which are incorporated by reference herein in their entirety. The mRNA that encodes the coronavirus antigen may be formulated in a lipid nanoparticle. In some embodiments, the lipid nanoparticle includes at least one ionizable cationic lipid, at least one non-cationic lipid, at least one sterol, and/or at least one polyethylene glycol (PEG)-modified lipid.

The lipid composition of the lipid nanoparticle composition in which the mRNA encoding the coronavirus antigen is formulated can include one or more phospholipids, for example, one or more saturated or (poly)unsaturated phospholipids or a combination thereof. In general, phospholipids include a phospholipid moiety and one or more fatty acid moieties.

A phospholipid moiety can be selected, for example, from the non-limiting group consisting of phosphatidyl choline, phosphatidyl ethanolamine, phosphatidyl glycerol, phosphatidyl serine, phosphatidic acid, 2-lysophosphatidyl choline, and a sphingomyelin.

The lipid composition in which the mRNA encoding the coronavirus antigen is formulated can comprise one or more structural lipids. As used herein, the term structural lipid refers to sterols and also to lipids containing sterol moieties.

Incorporation of structural lipids in the lipid nanoparticle may help mitigate aggregation of other lipids in the particle. Structural lipids can be selected from the group including but not limited to, cholesterol, fecosterol, sitosterol, ergosterol, campesterol, stigmasterol, brassicasterol, tomatidine, tomatine, ursolic acid, alpha-tocopherol, hopanoids, phytosterols, steroids, and mixtures thereof. In some embodiments, the structural lipid is a sterol.

The lipid composition in which the mRNA encoding the coronavirus antigen is formulated can include one or more a polyethylene glycol (PEG) lipid. In some embodiments, the PEG-lipid includes, but is not limited to 1,2-dimyristoyl-sn-glycerol methoxypolyethylene glycol (PEG-DMG), 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[amino(polyethylene glycol)] (PEG-DSPE), PEG-disteryl glycerol (PEG-DSG), PEG-dipalmetoleyl, PEG-dioleyl, PEG-distearyl, PEG-diacylglycamide (PEG-DAG), PEG-dipalmitoyl phosphatidylethanolamine (PEG-DPPE), or PEG-l,2-dimyristyloxlpropyl-3-amine (PEG-c-DMA).

The mRNA that encodes the coronavirus antigen may be encoded within a recombinant vector. The vectors can be used to deliver the mRNA that encodes the coronavirus antigen. The vector may be a mammalian, a viral, or a bacterial expression vector.

The vectors may be, for example, a plasmid, an artificial chromosome (e.g., a BAG, PAC, or YAC), or a virus or phage vector, and may optionally include a promoter, enhancer, or regulator for the expression of the polynucleotide. The vector may also contain one or more selectable marker genes, for example an ampicillin, neomycin, and/or kanamycin resistance gene in the case of a

bacterial plasmid or a resistance gene for a fungal vector. Vectors may be used *in vitro*, for example, for the production of DNA or RNA or used to transfect or transform a host cell, for example, a mammalian host cell, e.g., for the production of protein encoded by the vector. The vectors may also be adapted to be used *in vivo*, for example in a method of DNA vaccination, RNA vaccination, or gene therapy.

Viral genomes provide a rich source of vectors that can be used for the efficient delivery of the mRNA encoding the coronavirus antigen into the genome of a cell (e.g., a eukaryotic or prokaryotic cell). Viral genomes are particularly useful vectors for gene delivery because the polynucleotides contained within such genomes are typically incorporated into the genome of a target cell by generalized or specialized transduction. These processes occur as part of the natural viral replication cycle, and do not require added proteins or reagents in order to induce gene integration. Examples of viral vectors that can be used to deliver the mRNA encoding the coronavirus antigen include a retrovirus, adenovirus (e.g., Ad2, Ad5, Ad11, Ad12, Ad24, Ad26, Ad34, Ad35, Ad40, Ad48, Ad49, Ad50, Ad52 (e.g., a RhAd52), Ad59 (e.g., a RhAd59), and Pan9 (also known as AdC68)), parvovirus (e.g., adeno-associated viruses), coronavirus, negative strand RNA viruses such as orthomyxovirus (e.g., influenza virus), rhabdovirus (e.g., rabies and vesicular stomatitis virus), paramyxovirus (e.g. measles and Sendai), positive strand RNA viruses, such as picornavirus and alphavirus, and double stranded DNA viruses including adenovirus, herpesvirus (e.g., Herpes Simplex virus types 1 and 2, Epstein-Barr virus, cytomegalovirus), and poxvirus (e.g., vaccinia, modified vaccinia Ankara (MVA), fowlpox and canarypox). Other viruses useful for delivering polynucleotides encoding immunogens (e.g., polypeptides) include Norwalk virus, togavirus, coronavirus, reoviruses, papovavirus, hepadnavirus, and hepatitis virus, for example. Examples of retroviruses include: avian leukosis-sarcoma, mammalian C-type, B-type viruses, D-type viruses, HTLV-BLV group, lentivirus, spumavirus (Coffin, J. M., Retroviridae: The viruses and their replication, *In Fundamental Virology*, Third Edition, B. N. Fields, et al., Eds., Lippincott-Raven Publishers, Philadelphia, 1996). These adenovirus vectors can be derived from, for example, human, chimpanzee, or rhesus adenoviruses. Other examples include murine leukemia viruses, murine sarcoma viruses, mouse mammary tumor virus, bovine leukemia virus, feline leukemia virus, feline sarcoma virus, avian leukemia virus, human T-cell leukemia virus, baboon endogenous virus, Gibbon ape leukemia virus, Mason Pfizer monkey virus, simian immunodeficiency virus, simian sarcoma virus, Rous sarcoma virus and lentiviruses. Other examples of vectors are described, for example, in McVey et al., (U.S. Patent. No. 5,801,030); incorporated herein in its entirety by reference. The nucleic acid material (e.g., including a nucleic acid molecule) of the viral vector may be encapsulated, e.g., in a lipid membrane or by structural proteins (e.g., capsid proteins), that may include one or more viral polypeptides (e.g., a glycoprotein). The viral vector can be used to infect cells of a subject, which, in turn, promotes the translation of the heterologous gene(s) of the viral vector into the immunogens.

Adenoviral vectors disclosed in International Patent Application Publications WO 2006/040330 and WO 2007/104792, each incorporated by reference herein, are particularly useful as vectors. These adenoviral vectors can encode and/or deliver one or more of the immunogens (e.g., SARS-CoV-2 polypeptides) to treat a subject having a pathological condition associated with a viral

infection (e.g., a SARS-CoV-2 infection). In some embodiments, one or more recombinant adenovirus vectors can be administered to the subject in order to express more than one type of immunogen (e.g., SARS-CoV-2 polypeptide). Besides adenoviral vectors, other viral vectors and techniques are known in the art that can be used to facilitate delivery and/or expression of one or more of the immunogens in a subject (e.g., a human). These viruses include poxviruses (e.g., vaccinia virus and modified vaccinia virus Ankara (MVA); see, e.g., U.S. Patent Nos. 4,603,112 and 5,762,938, each incorporated by reference herein), herpesviruses, togaviruses (e.g., Venezuelan Equine Encephalitis virus; see, e.g., U.S. Patent No. 5,643,576, incorporated by reference herein), picornaviruses (e.g., poliovirus; see, e.g., U.S. Patent No. 5,639,649, incorporated by reference herein), baculoviruses, and others described by Wattanapitayakul and Bauer (*Biomed. Pharmacother.* 54:487 (2000), incorporated by reference herein).

In some embodiments, the mRNA encoding a coronavirus antigen is incorporated into a recombinant AAV (rAAV) vectors and/or virions in order to facilitate their introduction into a cell. rAAV vectors useful in the compositions and methods described herein are recombinant polynucleotide constructs that include (1) a heterologous sequence to be expressed (e.g., a polynucleotide encoding a coronavirus antigen to be expressed) and (2) viral sequences that facilitate stability and expression of the heterologous genes. The viral sequences may include those sequences of AAV that are required in cis for replication and packaging (e.g., functional ITRs) of the DNA into a virion. Such rAAV vectors may also contain marker or reporter genes. Useful rAAV vectors have one or more of the AAV WT genes deleted in whole or in part but retain functional flanking ITR sequences. The AAV ITRs may be of any serotype suitable for a particular application. Methods for using rAAV vectors are described, for example, in Tal et al., J. Biomed. Sci. 7:279 (2000), and Monahan and Samulski, Gene Delivery 7:24 (2000), the disclosures of each of which are incorporated herein by reference as they pertain to AAV vectors for gene delivery.

The mRNA encoding a coronavirus antigen can be incorporated into a rAAV virion in order to facilitate introduction of the mRNA encoding a coronavirus antigen into a cell. The capsid proteins of an AAV compose the exterior, non-nucleic acid portion of the virion and are encoded by the AAV cap gene. The cap gene encodes three viral coat proteins, VP1, VP2 and VP3, which are required for virion assembly. The construction of rAAV virions has been described, for instance, in US 5,173,414; US 5,139,941; US 5,863,541; US 5,869,305; US 6,057,152; and US 6,376,237; as well as in Rabinowitz et al., J. Virol. 76:791 (2002) and Bowles et al., J. Virol. 77:423 (2003), the disclosures of each of which are incorporated herein by reference as they pertain to AAV vectors for gene delivery.

Useful rAAV virions include those derived from a variety of AAV serotypes including AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12 rh10, rh39, rh43, rh74, and Anc80. Construction and use of AAV vectors and AAV proteins of different serotypes are described, for instance, in Chao et al., Mol. Ther. 2:619 (2000); Davidson et al., Proc. Natl. Acad. Sci. USA 97:3428 (2000); Xiao et al., J. Virol. 72:2224 (1998); Halbert et al., J. Virol. 74:1524 (2000); Halbert et al., J. Virol. 75:6615 (2001); and Auricchio et al., Hum. Molec. Genet. 10:3075 (2001), the disclosures of each of which are incorporated herein by reference as they pertain to AAV vectors for gene delivery.

AAV vectors may be pseudotyped vectors. Pseudotyped vectors include AAV vectors of a given serotype (e.g., AAV9) pseudotyped with a capsid gene derived from a serotype other than the given serotype (e.g., AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, etc.). Techniques involving the construction and use of pseudotyped rAAV virions are known in the art and are described, for instance, in Duan et al., J. Virol. 75:7662 (2001); Halbert et al., J. Virol. 74:1524 (2000); Zolotukhin et al., Methods, 28:158 (2002); and Auricchio et al., Hum. Molec. Genet. 10:3075 (2001).

AAV virions that have mutations within the virion capsid may be used to infect particular cell types more effectively than non-mutated capsid virions. For example, suitable AAV mutants may have ligand insertion mutations for the facilitation of targeting an AAV to specific cell types. The construction and characterization of AAV capsid mutants including insertion mutants, alanine screening mutants, and epitope tag mutants is described in Wu et al., J. Virol. 74:8635 (2000). Other rAAV virions that can be used in methods described herein include those capsid hybrids that are generated by molecular breeding of viruses as well as by exon shuffling. See, e.g., Soong et al., Nat. Genet., 25:436 (2000) and Kolman and Stemmer, Nat. Biotechnol. 19:423 (2001).

Gene transfer techniques using these viruses are known to those skilled in the art. Retrovirus vectors for example may be used to stably integrate the polynucleotide into the host genome, although such recombination is not preferred. Replication-defective adenovirus vectors by contrast remain episomal and therefore allow transient expression.

Vectors capable of driving expression in insect cells (for example baculovirus vectors), in human cells, in yeast or in bacteria may be employed in order to produce quantities of coronavirus antigen encoded by the mRNA, for example, for use as subunit vaccines or in immunoassays.

Adjuvants

In some embodiments, an immunogenic composition described herein may include one or more adjuvants. An adjuvant refers to a substance that cause stimulation of the immune system. In this context, an adjuvant is used to enhance an immune response to one or more antigens (e.g., a coronavirus antigen (e.g., a coronavirus spike protein or a peptide thereof, and/or a coronavirus nucleocapsid protein or peptide thereof, or a nucleic acid sequence encoding the same)). An adjuvant may be administered to a subject before, in combination with, or after administration of the antigens (e.g., a coronavirus antigen (e.g., a coronavirus spike protein or a peptide thereof, and/or a coronavirus nucleocapsid protein or peptide thereof, or a nucleic acid sequence encoding the same)). In some embodiments, an additional adjuvant is administered to the subject in combination with the CpG-amphiphile and the coronavirus antigen (e.g., a coronavirus spike protein or a peptide thereof, and/or a coronavirus nucleocapsid protein or peptide thereof, or a nucleic acid sequence encoding the same) described herein. In some embodiments, an adjuvant may be conjugated to a lipid. The adjuvant may be without limitation lipids (e.g., monophosphoryl lipid A (MPLA)), alum (e.g., aluminum hydroxide, aluminum phosphate); Freund's adjuvant; saponins purified from the bark of the Q. saponaria tree such as QS21 (a glycolipid that elutes in the 21st peak with HPLC fractionation; Antigenics, Inc., Worcester, Mass.); poly[di(carboxylatophenoxy)phosphazene (PCPP polymer; Virus

Research Institute, USA), Flt3 ligand, Leishmania elongation factor (a purified Leishmania protein; Corixa Corporation, Seattle, Wash.), ISCOMS (immunostimulating complexes which contain mixed saponins, lipids and form virus-sized particles with pores that can hold antigen; CSL, Melbourne, Australia), Pam3Cys, SB-AS4 (SmithKline Beecham adjuvant system #4 which contains alum and MPL; SBB, Belgium), non-ionic block copolymers that form micelles such as CRL 1005 (these contain a linear chain of hydrophobic polyoxypropylene flanked by chains of polyoxyethylene, Vaxcel, Inc., Norcross, Ga.), and Montanide IMS (e.g., IMS1312, water-based nanoparticles combined with a soluble immunostimulant, Seppic), and CDNs (cyclic di-nucleotides).

Adjuvants may be toll-like receptor (TLR) ligands. Adjuvants that act through TLR3 include without limitation double-stranded RNA. Adjuvants that act through TLR4 include without limitation derivatives of lipopolysaccharides such as monophosphoryl lipid A (MPLA; Ribi ImmunoChem Research, Inc., Hamilton, Mont.) and muramyl dipeptide (MDP; Ribi) andthreonyl-muramyl dipeptide (t-MDP; Ribi); OM-174 (a glucosamine disaccharide related to lipid A; OM Pharma SA, Meyrin, Switzerland). Adjuvants that act through TLR5 include without limitation flagellin. Adjuvants that act through TLR7 and/or TLR8 include single-stranded RNA, oligoribonucleotides (ORN), synthetic low molecular weight compounds such as imidazoquinolinamines (e.g., imiquimod (R-837), resiquimod (R-848)). Adjuvants 5 acting through TLR9 include DNA of viral or bacterial origin, or synthetic oligodeoxynucleotides (ODN), such as CpG ODN. Another adjuvant class is phosphorothioate containing molecules such as phosphorothioate nucleotide analogs and nucleic acids containing phosphorothioate backbone linkages.

Pharmaceutical Compositions and Preparations

Described herein are pharmaceutical compositions of the invention including a CpG-amphiphile and a coronavirus antigen (e.g., a coronavirus spike protein or a peptide thereof, and/or a coronavirus nucleocapsid protein or peptide thereof, or a nucleic acid sequence encoding the same). In addition to a therapeutic amount of the CpG-amphiphile and a coronavirus antigen (e.g., a coronavirus spike protein or a peptide thereof, and/or a coronavirus nucleocapsid protein or peptide thereof, or a nucleic acid sequence encoding the same), the pharmaceutical compositions may contain a pharmaceutically acceptable carrier or excipient, which can be formulated by methods known to those skilled in the art. In other embodiments, pharmaceutical compositions of the invention may contain nucleic acid molecules encoding one or more coronavirus antigens (e.g., coronavirus spike protein or a peptide thereof, and/or a coronavirus nucleocapsid protein or peptide thereof) described herein (e.g., in a vector, such as a viral vector). The nucleic acid molecule encoding the coronavirus antigen (e.g., a coronavirus spike protein or a peptide thereof, and/or a coronavirus nucleocapsid protein or peptide thereof) thereof described herein may be cloned into an appropriate expression vector, which may be delivered via well-known methods in gene therapy.

Acceptable carriers and excipients in the pharmaceutical compositions of the CpG-amphiphile and the coronavirus antigen (e.g., a coronavirus spike protein or a peptide thereof, and/or a coronavirus nucleocapsid protein or peptide thereof, or a nucleic acid sequence encoding the same) are nontoxic to recipients at the dosages and concentrations employed. In certain embodiments, the

formulation material(s) are for subcutaneous (s.c.) and/or intravenous (i.v.) administration. In some embodiments, administration is by inhalation or intranasal administration. In some embodiments, the formulation material(s) are for intratracheal administration. In some embodiments, the formulation material(s) are for administration by inhalation during mechanical ventilation. In some embodiments, the pharmaceutical composition can contain formulation materials for modifying, maintaining, or preserving, for example, the pH, osmolality, viscosity, clarity, color, isotonicity, odor, sterility, stability, rate of dissolution or release, adsorption, or penetration of the composition. In some embodiments, suitable formulation materials include, but are not limited to, amino acids (such as glycine, glutamine, asparagine, arginine or lysine); antimicrobials; antioxidants (such as ascorbic acid, methionine, sodium sulfite or sodium hydrogen- sulfite); buffers (such as borate, bicarbonate, Tris-HCl, citrates, HEPES, TAE, phosphates or other organic acids); bulking agents (such as mannitol or glycine); chelating agents (such as ethylenediamine tetraacetic acid (EDTA)); complexing agents (such as caffeine, polyvinylpyrrolidone, beta-cyclodextrin or hydroxypropyl-beta- cyclodextrin); fillers; monosaccharides; disaccharides; and other carbohydrates (such as glucose, sucrosemannose or dextran); proteins (such as human serum albumin, gelatin, dextran, and immunoglobulins); coloring, flavoring and diluting agents; emulsifying agents; hydrophilic polymers (such as polyvinylpyrrolidone); low molecular weight polypeptides; salt-forming counterions (such as sodium); preservatives (such as hexamethonium chloride, octadecyldimethylbenzyl ammonium chloride, resorcinol, and benzalkonium chloride, benzoic acid, salicylic acid, thimerosal, phenethyl alcohol, methylparaben, propylparaben, chlorhexidine, sorbic acid or hydrogen peroxide); solvents (such as glycerin, propylene glycol or polyethylene glycol); sugar alcohols (such as mannitol or sorbitol); suspending agents; surfactants or wetting agents (such as pluronics, PEG, sorbitan esters, polysorbates such as polysorbate 20, polysorbate 80, triton, tromethamine, lecithin, cholesterol, tyloxapal); stability enhancing agents (such as sucrose or sorbitol); tonicity enhancing agents (such as alkali metal halides, preferably sodium or potassium chloride, mannitol sorbitol); delivery vehicles; diluents; excipients and/or pharmaceutical adjuvants. (Remington's Pharmaceutical Sciences, 18th Edition, A. R. Gennaro, ed., Mack Publishing Company (1995). In some embodiments, the optimal pharmaceutical composition will be determined by one skilled in the art depending upon, for example, the intended route of administration, delivery format and desired dosage. See, for example, Remington's Pharmaceutical Sciences, *supra*. In some embodiments, such compositions may influence the physical state, stability, rate of in vivo release and rate of in vivo clearance of the amphiphilic conjugate.

In some embodiments, the primary vehicle or carrier in a pharmaceutical composition, including a CpG-amphiphile and a coronavirus antigen (e.g., a coronavirus spike protein or a peptide thereof, and/or a coronavirus nucleocapsid protein or peptide thereof, or a nucleic acid sequence encoding the same), can be either aqueous or non-aqueous in nature. For example, in some embodiments, a suitable vehicle or carrier can be water for injection, physiological saline solution, or artificial cerebrospinal fluid, possibly supplemented with other materials common in compositions for parenteral administration. In some embodiments, the saline includes isotonic phosphate-buffered saline. In certain embodiments, neutral buffered saline or saline mixed with serum albumin are further exemplary vehicles. In some embodiments, pharmaceutical compositions include Tris buffer of about

pH 7.0-8.5, or acetate buffer of about pH 4.0-5.5, which can further include sorbitol or a suitable substitute therefor. In some embodiments, a composition including a CpG-amphiphile or a coronavirus antigen (e.g., a coronavirus spike protein or a peptide thereof, and/or a coronavirus nucleocapsid protein or peptide thereof, or a nucleic acid sequence encoding the same) can be prepared for storage by mixing the selected composition having the desired degree of purity with optional formulation agents (Remington's Pharmaceutical Sciences, *supra*) in the form of a lyophilized cake or an aqueous solution. Further, in some embodiments, a composition including a CpG-amphiphile or a coronavirus antigen (e.g., a coronavirus spike protein or a peptide thereof, and/or a coronavirus nucleocapsid protein or peptide thereof, or a nucleic acid sequence encoding the same), can be formulated as a lyophilizate using appropriate excipients such as sucrose.

In some embodiments, the pharmaceutical composition may be selected for parenteral delivery. The preparation of such pharmaceutically acceptable compositions is within the ability of one skilled in the art.

In some embodiments, the formulation components are present in concentrations that are acceptable to the site of administration. In some embodiments, buffers are used to maintain the composition at physiological pH or at a slightly lower pH, typically within a pH range of from about 5 to about 8.

In some embodiments, when parenteral administration is contemplated, a therapeutic composition can be in the form of a pyrogen-free, parenterally acceptable aqueous solution including a CpG-amphiphile and a coronavirus antigen (e.g., a coronavirus spike protein or a peptide thereof, and/or a coronavirus nucleocapsid protein or peptide thereof, or a nucleic acid sequence encoding the same), in a pharmaceutically acceptable vehicle. In some embodiments, a vehicle for parenteral injection is sterile distilled water in which a CpG-amphiphile or a coronavirus antigen (e.g., a coronavirus spike protein or a peptide thereof, and/or a coronavirus nucleocapsid protein or peptide thereof, or a nucleic acid sequence encoding the same) is formulated as a sterile, isotonic solution, properly preserved. In some embodiments, the preparation can involve the formulation of the desired molecule with an agent, such as injectable microspheres, bio-erodible particles, polymeric compounds (such as polylactic acid or polyglycolic acid), beads or liposomes, that can provide for the controlled or sustained release of the product which can then be delivered via a depot injection. In some embodiments, hyaluronic acid can also be used, and can have the effect of promoting sustained duration in the circulation. In some embodiments, implantable drug delivery devices can be used to introduce the desired molecule.

The pharmaceutical composition may be administered in therapeutically effective amount such as to induce an immune response. The therapeutically effective amount of the CpG-amphiphile and the coronavirus protein or peptide, included in the pharmaceutical preparations may be determined by one of skill in art, such that the dosage (e.g., a dose within the range of 0.01-100 mg/kg of body weight) induces an immune response in the subject.

Vectors may be used as in vivo nucleic acid delivery vehicle include, but are not limited to, retroviral vectors, adenoviral vectors, poxviral vectors (e.g., vaccinia viral vectors, such as Modified Vaccinia Ankara (MVA)), adeno-associated viral vectors, and alphaviral vectors. In some

embodiments, a vector can include internal ribosome entry site (IRES) that allows the expression of multiple coronavirus antigens (e.g., a coronavirus spike protein, a peptide thereof, or a nucleic acid sequence encoding the same) described herein. Other vehicles and methods for nucleic acid delivery are described in US Patent Nos. 5,972,707, 5,697,901, and 6,261,554, each of which is incorporated by reference herein in its entirety. Other methods of producing pharmaceutical compositions are described in, e.g., US Patent Nos. 5,478,925, 8,603,778, 7,662,367, and 7,892,558, all of which are incorporated by reference herein in their entireties.

Routes, Dosage, and Timing of Administration

Pharmaceutical compositions of the invention that contain a CpG-amphiphile and a coronavirus antigen (e.g., a coronavirus spike protein or a peptide thereof, and/or a coronavirus nucleocapsid protein or a peptide thereof, or a nucleic acid sequence encoding the same) described herein as the therapeutic agents may be formulated for parenteral administration, subcutaneous administration, intravenous administration, intramuscular administration, intranasal administration, inhalation, intratracheal administration, or administration by inhalation during mechanical ventilation. Methods of administering therapeutic proteins are known in the art. See, for example, US Patent Nos. 6,174,529, 6,613,332, 8,518,869, 7,402,155, and 6,591,129, and US Patent Application Publication Nos. US20140051634, WO1993000077, and US20110184145, the disclosures of which are incorporated by reference in their entireties.

One or more of these methods may be used to administer a pharmaceutical composition of the invention that contains a CpG-amphiphile and a coronavirus antigen (e.g., a coronavirus spike protein or a peptide thereof, and/or a coronavirus nucleocapsid protein or peptide thereof, or a nucleic acid sequence encoding the same) described herein. For injectable formulations, various effective pharmaceutical carriers are known in the art. See, e.g., Pharmaceutics and Pharmacy Practice, J. B. Lippincott Company, Philadelphia, Pa., Banker and Chalmers, eds., pages 238-250 (1982), and ASHP Handbook on Injectable Drugs, Toissel, 4th ed., pages 622-630 (1986). The dosage of the pharmaceutical compositions of the invention depends on factors including the route of administration and the physical characteristics, e.g., age, weight, general health, of the subject. Typically, the amount of a CpG-amphiphile and a coronavirus antigen (e.g., a coronavirus spike protein or a peptide thereof, and/or a coronavirus nucleocapsid protein or peptide thereof, or a nucleic acid sequence encoding the same) described herein contained within a single dose may be an amount that effectively induces an immune response in the subject without inducing significant toxicity. A pharmaceutical composition of the invention may include a dosage of a CpG-amphiphile and a coronavirus antigen (e.g., a coronavirus spike protein or a peptide thereof, and/or a coronavirus nucleocapsid protein or peptide thereof, or a nucleic acid sequence encoding the same) described herein ranging from 0.001 to 500 mg (e.g., 0.01, 0.05, 0.1, 0.2, 0.3, 0.5, 0.7, 0.8, 1 mg, 2 mg, 3 mg, 4 mg, 5 mg, 10 mg, 15 mg, 20 mg, 30 mg, 50 mg, 100 mg, 250 mg, or 500 mg) and, in a more specific embodiment, about 0.1 to about 100 mg. The dosage may be adapted by the clinician in accordance with the different parameters of the subject.

In particular embodiments, the subject receives a dosage of about 10 μg to about 1.0 mg of the coronavirus antigen (e.g., a coronavirus spike protein or a peptide thereof, and/or a coronavirus nucleocapsid protein or peptide thereof, or a nucleic acid sequence encoding the same). In particular, the dosage of the coronavirus antigen administered is about 40 μg to 60 μg, is about 50 μg to 70 μg, is about 50 μg to 150 μg, is about 70 μg to 150 μg, is about 100 μg to 150 μg, is about 100 μg to 200 μg, is about 140 μg to 250 μg, is about 200 μg to 300 μg, is about 250 μg to 500 μg, is about 300 μg to 600 μg, or is about 500 μg to 1.0 mg. In particular, the dosage of the coronavirus antigen administered to the subject may be about 10 μg, 20 μg, 30 μg, 40 μg, 50 μg, 60, μg, 70 μg, 80 μg, 90 μg, 100 μg, 110 μg, 120 μg, 130 μg, 140 μg, 150 μg, 200 μg, 250 μg, 300 μg, 400 μg, 500 μg, 600 μg, 700 μg, 800 μg, 900 μg, or 1 mg. The subject also may receive a dosage in a range between any two of these particular dosages of the coronavirus antigen.

In particular embodiments, the dosage of the CpG amphiphile is about 0.1 mg to 20 mg. In particular, the dosage of the CpG amphiphile administered is about 0.1 mg to 1.0 mg, is about 0.5 mg to 3.0 mg, is about 1.0 mg to 5.0 mg, is about 2.0 to 5.0 mg, is about 3.0 to 5.0 mg, is about 3.0 mg to 10.0 mg, is about 4.0 mg to 12.0 mg, is about 5.0 mg to 15.0 mg or is about 5.0 mg to 20 mg. The particular dosage of the CpG amphiphile administered to the subject may be about 0.1 mg, 0.2 mg, 0.3 mg, 0.4 mg, 0.5 mg, 1.0 mg, 2.0 mg, 3.0 mg, 4.0 mg, 5.0 mg, 6.0 mg, 7.0 mg, 8.0 mg, 9.0 mg, 10.0 mg, 11.0 mg, 12.0 mg, 13.0 mg, 14.0 mg, 15.0 mg, 16.0 mg, 17.0 mg, 18.0 mg, 19.0 mg, or 20.0 mg. The subject also may receive a dosage in a range between any two of these particular dosages of the CpG amphiphile.

Pharmaceutical compositions of the invention that contain a CpG-amphiphile and a coronavirus antigen (e.g., a coronavirus spike protein or a peptide thereof, and/or a coronavirus nucleocapsid protein or peptide thereof, or a nucleic acid sequence encoding the same) described herein may be administered to a subject in need thereof, for example, one or more times (e.g., 1-10 times or more) daily, weekly, monthly, biannually, annually, or as medically necessary.

In some embodiments, a trimer of the coronavirus spike protein or peptide is administered to the subject. In some embodiments, an mRNA encoding a coronavirus antigen (e.g., a coronavirus spike protein or a peptide thereof, and/or a coronavirus nucleocapsid protein or peptide thereof, or a nucleic acid sequence encoding the same) is administered to the subject. In some embodiments, the coronavirus antigen (e.g., a coronavirus spike protein or a peptide thereof, and/or a coronavirus nucleocapsid protein or peptide thereof, or a nucleic acid sequence encoding the same) and the CpG-amphiphile are administered concurrently or essentially at the same time to the subject. The CpG-amphiphile and the coronavirus antigen (e.g., a coronavirus spike protein or a peptide thereof, and/or a coronavirus nucleocapsid protein or peptide thereof, or a nucleic acid sequence encoding the same) may be co-formulated, or they may be administered as two separate formulations. In some embodiments, the CpG-amphiphile and the coronavirus antigen (e.g., a coronavirus spike protein or a peptide thereof, and/or a coronavirus nucleocapsid protein or peptide thereof, or a nucleic acid sequence encoding the same) administered sequentially. For example, the CpG-amphiphile may be administered first and the coronavirus antigen (e.g., a coronavirus spike protein or a peptide thereof, and/or a coronavirus nucleocapsid protein or peptide thereof, or a nucleic acid sequence encoding the

same) may be administered second, or, in some embodiments, the coronavirus antigen (e.g., a coronavirus spike protein or a peptide thereof, and/or a coronavirus nucleocapsid protein or peptide thereof, or a nucleic acid sequence encoding the same) may be administered first and the CpG-amphiphile is administered second. In some embodiments, the CpG-amphiphile and the coronavirus antigen (e.g., a coronavirus spike protein or a peptide thereof, and/or a coronavirus nucleocapsid protein or peptide thereof, or a nucleic acid sequence encoding the same) are administered with a second adjuvant.

Methods of Inducing an Immune Response

The invention provides methods of inducing an immune response in a subject by administering a CpG-amphiphile and a coronavirus antigen (e.g., a coronavirus spike protein or a peptide thereof, and/or a coronavirus nucleocapsid protein or peptide thereof, or a nucleic acid sequence encoding the same) to a subject. The subject may be a mammal (e.g., a human, a dog, or a cat). In some embodiments, the subject is a human subject. The immune response is induced in the subject by administering to the subject a therapeutically effective amount of an immunogenic composition or pharmaceutical composition described herein. The immunogenic composition or pharmaceutical composition includes a CpG-amphiphile and a coronavirus antigen (e.g., a coronavirus spike protein or a peptide thereof, and/or a coronavirus nucleocapsid protein or peptide thereof, or a nucleic acid sequence encoding the same) described herein. In some embodiments, the CpG-amphiphile and the coronavirus antigen (e.g., a coronavirus spike protein or a peptide thereof, and/or a coronavirus nucleocapsid protein or peptide thereof, or a nucleic acid sequence encoding the same) may be administered with one or more additional adjuvants. In some embodiments, the CpG-amphiphile and the coronavirus antigen (e.g., a coronavirus spike protein or a peptide thereof, and/or a coronavirus nucleocapsid protein or peptide thereof, or a nucleic acid sequence encoding the same) may be administered without one or more additional adjuvants. In some embodiments, the method includes administering to the subject 1) a therapeutically effective amount of a CpG-amphiphile described herein, and 2) a coronavirus antigen (e.g., a coronavirus spike protein or a peptide thereof, and/or a coronavirus nucleocapsid protein or peptide thereof, or a nucleic acid sequence encoding the same). In some embodiments, the CpG-amphiphile and the coronavirus antigen (e.g., a coronavirus spike protein or a peptide thereof, and/or a coronavirus nucleocapsid protein or peptide thereof, or a nucleic acid sequence encoding the same) are administered substantially simultaneously. In some embodiments, the CpG-amphiphile and the coronavirus antigen (e.g., a coronavirus spike protein or a peptide thereof, and/or a coronavirus nucleocapsid protein or peptide thereof, or a nucleic acid sequence encoding the same) are administered separately. In some embodiments, the CpG-amphiphile is administered first, followed by administering of the coronavirus spike protein or peptide. In some embodiments, the coronavirus antigen (e.g., a coronavirus spike protein or a peptide thereof, and/or a coronavirus nucleocapsid protein or peptide thereof, or a nucleic acid sequence encoding the same) is administered first, followed by administering of the CpG-amphiphile.

In some embodiments, the immune response is protective against SARS-CoV-2 infection.

In some embodiments, the immune response is protective against Covid-19 disease.

In some embodiments, the immune response is protective against severe Covid-19 disease with requirement of assisted ventilation and oxygenation. These patients suffer from acute respiratory distress syndrome. Some patients develop severe cardiovascular damage. Other complications include, e.g., acute cardiac injury, acute kidney injury, septic shock, multi-organ failure, and increased risk of death.

In some embodiments, the immune response is protective against the development of one or more COVID-19 disease symptoms selected from the group consisting of fever, sore throat, runny nose, sneezing, nasal congestion, snoring, coughing, dry cough, shortness of breath, difficulty breathing, persistent pain or pressure in the chest, dyspnea, pneumonia, acute respiratory syndrome, cyanosis, myalgia, headache, encephalopathy, myocardial injury, heart failure, arrhythmia, coagulation dysfunction, acute kidney injury, confusion or inability to arouse, fatigue, and gastrointestinal symptoms.

In some embodiments, the immune response reduces the incidence of one or more COVID-19 disease symptoms selected from the group consisting of fever, sore throat, runny nose, sneezing, nasal congestion, snoring, coughing, dry cough, shortness of breath, difficulty breathing, persistent pain or pressure in the chest, dyspnea, pneumonia, acute respiratory syndrome, cyanosis, myalgia, headache, encephalopathy, myocardial injury, heart failure, arrhythmia, coagulation dysfunction, acute kidney injury, confusion or inability to arouse, fatigue, and gastrointestinal symptoms.

In some embodiments, the immune response is therapeutic against one or more COVID-19 disease symptoms selected from the group consisting of fever, sore throat, runny nose, sneezing, nasal congestion, snoring, coughing, dry cough, shortness of breath, difficulty breathing, persistent pain or pressure in the chest, dyspnea, pneumonia, acute respiratory syndrome, cyanosis, myalgia, headache, encephalopathy, myocardial injury, heart failure, arrhythmia, coagulation dysfunction, acute kidney injury, confusion or inability to arouse, fatigue, and gastrointestinal symptoms. The immune response is therapeutic if it reverses, alleviates, ameliorates, inhibits, slows down, or stops the progression or severity of a COVID-19 disease symptom.

In some embodiments, the immune response reduces the likelihood of COVID-19 recurrence or SARS-CoV-2 reinfection.

In some embodiments, the immune response reduces the likelihood of transmission of SARS-CoV-2.

In some embodiments, the subject is an asymptomatic carrier of SARS-CoV-2.

In some embodiments, the subject has one or more symptoms of COVID-19 selected from the group consisting of fever, sore throat, runny nose, sneezing, nasal congestion, snoring, coughing, dry cough, shortness of breath, difficulty breathing, persistent pain or pressure in the chest, dyspnea, pneumonia, acute respiratory syndrome, cyanosis, myalgia, headache, encephalopathy, myocardial injury, heart failure, arrhythmia, coagulation dysfunction, acute kidney injury, confusion or inability to arouse, fatigue, and gastrointestinal symptoms.

In some embodiments, the subject has been diagnosed with SARS-CoV-2 infection.

In some embodiments, the subject is at high risk of SARS-CoV-2 infection (e.g., medical personnel and/or first responders).

In some embodiments, an immunogenic composition or pharmaceutical composition described herein is administered to a subject who has been in contact with someone who has been diagnosed with a coronavirus infection (e.g., COVID-19) or who has recently travelled or is planning to travel to an area experiencing an outbreak of COVID-19 or other coronavirus infection.

In some embodiments, the spike protein or peptide thereof is comprised within a preparation of an inactivated or killed virus vaccine.

In some embodiments, the spike protein or fragment thereof is in subunit form.

In some embodiments, the nucleic acid encoding the coronavirus spike protein encodes a prefusion stabilized form of the spike protein.

In some embodiments, the nucleic acid encoding the coronavirus spike protein or peptide thereof is comprised within an adenovirus vector.

Combination Therapies

The invention described herein also provides methods of inducing an immune response in a subject by administering a CpG-amphiphile and a coronavirus antigen in combination with one or more additional therapeutics. The particular combination of therapeutics that can be employed in a combination regimen will take into account compatibility of the desired therapeutics and/or procedures and the desired therapeutic effect to be achieved. It will also be appreciated that the therapies employed may achieve a desired effect for the same disorder, or they may achieve different effects (e.g., control of one or more adverse effects). The CpG-amphiphile and coronavirus antigen may be administered in combination with an antiviral agent (e.g., remdesivir), an antiviral vaccine (e.g., a coronavirus vaccine such as a vaccine against SARS-CoV-2), an antibiotic agent, an antifungal agent, an anti-inflammatory agent, an antiparasitic agent, and an immunotherapy agent.

In some embodiments, the antiviral agent may be remdesivir, chloroquine, hydroxychloroquine, baricitinib, lopinavir/ritonavir, interferon beta, umifenovir, favipiravir, tocilizumab, ribavirin or other drugs. In some embodiments, the antiviral agent is remdesivir.

In some embodiments, the antiviral vaccine includes any composition that elicits an immune response in a subject directed against a coronavirus, such as a HCoV-NL3 vaccine, a SARS-CoV-1 vaccine, a SARS-CoV-2 vaccine, or a MERS vaccine. In some embodiments, the antiviral vaccine includes an inactivated or killed virus, such as an HCoV-NL3 virus, a SARS-CoV-1 virus, a SARS-CoV-2 virus, or a MERS virus. In some embodiments, the antiviral vaccine is administered as a heterologous prime or boost or in combination with the immunogenic composition including a CpG-amphiphile described herein.

In some embodiments, the antibiotic agent may be elected from amikacin, gentamicin, kanamycin, neomycin, netilmicin, tobramycin, paromomycin, streptomycin, spectinomycin, geldanamycin, herbimycin, rifaximin, loracarbef, ertapenem, doripenem, imipenem/cilastatin, meropenem, cefadroxil, cefazolin, cefalotin, cefalexin, cefaclor, cefamandole, cefoxitin, cefprozil, cefuroxime, cefixime, cefdinir, cefditoren, cefoperazone, cefotaxime, cefpodoxime, ceftazidime, ceftibuten, ceftizoxime, ceftriaxone, cefepime, ceftaroline fosamil, ceftobiprole, teicoplanin, vancomycin, telavancin, dalbavancin, oritavancin, clindamycin, lincomycin, daptomycin, azithromycin,

clarithromycin, dirithromycin, erythromycin, roxithromycin, troleandomycin, telithromycin, spiramycin, aztreonam, furazolidone, nitrofurantoin, linezolid, posizolid, radezolid, torezolid, amoxicillin, ampicillin, azlocillin, carbenicillin, cloxacillin, dicloxacillin, flucloxacillin, mezlocillin, methicillin, nafcillin, oxacillin, penicillin g, penicillin v, piperacillin, temocillin, ticarcillin, amoxicillin clavulanate, ampicillin/sulbactam, piperacillin/tazobactam, ticarcillin/clavulanate, bacitracin, colistin, polymyxin b, ciprofloxacin, enoxacin, gatifloxacin, gemifloxacin, levofloxacin, lomefloxacin, moxifloxacin, nalidixic acid, norfloxacin, ofloxacin, trovafloxacin, grepafloxacin, sparfloxacin, temafloxacin, mafenide, sulfacetamide, sulfadiazine, silver sulfadiazine, sulfadimethoxine, sulfamethizole, sulfamethoxazole, sulfanilimide, sulfasalazine, sulfisoxazole, trimethoprim-sulfamethoxazole (tmp-smx), sulfonamidochrysoidine, demeclocycline, doxycycline, minocycline, oxytetracycline, tetracycline, clofazimine, dapsone, capreomycin, cycloserine, ethambutol(bs), ethionamide, isoniazid, pyrazinamide, rifampicin, rifabutin, rifapentine, streptomycin, arsphenamine, chloramphenicol, fosfomycin, fusidic acid, metronidazole, mupirocin, platensimycin, quinupristin/dalfopristin, thiamphenicol, tigecycline, tinidazole, and trimethoprim.

In some embodiments, the antifungal agent may be selected from amphotericin B, candicidin, filipin, hamycin, natamycin, nystatin, rimocidin,, bifonazole, butoconazole, clotrimazole, econazole, fenticonazole, isoconazole, ketoconazole, luliconazole, miconazole, omoconazole, oxiconazole, sertaconazole, sulconazole, tioconazole, triazoles, albaconazole, efinaconazole, epoxiconazole, fluconazole, isavuconazole, itraconazole, posaconazole, propiconazole, ravuconazole, terconazole, voriconazole, thiazoles, abafungin, amorolfin, butenafine, naftifine, terbinafine, anidulafungin, caspofungin, micafungin, ciclopirox, flucytosine, griseofulvin, tolnaftate, and undecylenic acid. In some embodiments, the antiparasitic agent may be chloroquine or hydroxychloroquine.

In some embodiments, the anti-inflammatory agent may be dexamethasone. In some embodiments the anti-inflammatory agent may be selected from celecoxib, diclofenac, difunisal, etodolac, ibuprofen, indomethacin, ketoprofen, ketorolac, nabumetone, naproxen, oxaprozin, prednisone, prednisolone, methylprednisolone, metformin, and dexamethasone.

In some embodiments, the immunotherapy agent may be selected from Targretin, Interferon-alpha, Interferon-beta, clobestasol, Peg Interferon (e.g., PEGASYS®), prednisone, Romidepsin, Bexarotene, methotrexate, Trimcinolone cream, anti-chemokines, Vorinostat, gabapentin, antibodies to lymphoid cell surface receptors and/or lymphokines, antibodies to surface cancer proteins, and/or small molecular therapies like Vorinostat. In some embodiments, the immunotherapy agent is interferon-beta, tocilizumab, or baricitinib. In some embodiments, the immunotherapy agent may include an antibody. In some embodiments, the immunotherapy agent may be convalescent plasma (e.g., human convalescent plasma).

The CpG-amphiphile and the coronavirus antigen and the one or more additional therapeutics may be administered sequentially (e.g., 1 day apart, 2 days apart, 3 days apart, 1 week apart, 1 month apart, 6 months apart, or more) or substantially simultaneously (e.g., within 1 day). The CpG-amphiphile and the coronavirus antigen and the one or more additional therapeutics may be formulated in a single pharmaceutical composition or may be administered as separate pharmaceutical compositions. The CpG-amphiphile and the coronavirus antigen and the one or more

additional therapeutics may be administered by the same route of administration or different routes of administration. The two or more agents may be administered at the same frequency or different frequencies.

The additional therapeutic agent may be administered orally, topically, intravenously, intramuscularly, transdermally, intradermally, intra-arterially, intracranially, subcutaneously, intraorbitally, intraventricularly, intraspinally, intraperitoneally, intranasally, intratracheally, or by inhalation during mechanical ventilation. In particular embodiments, the additional therapeutic is administered intravenously or the additional therapeutic agent may be administered in its regulatory approved form. The additional therapeutic agent, or a pharmaceutically acceptable salt thereof, can be administered in a pharmaceutical composition that includes one or more pharmaceutically acceptable carriers, excipients, or diluents. Examples of suitable carriers, excipients, or diluents include, e.g., saline, sterile water, polyalkylene glycols, oils of vegetable origin, hydrogenated napthalenes, suitable buffer, 1,3-butanediol, Ringer's solution and/or sodium chloride solution. Exemplary formulations for parenteral administration can include solutions prepared in water suitably mixed with a surfactant, e.g., hydroxypropylcellulose. Dispersions can also be prepared in glycerol, liquid polyethylene glycols, DMSO and mixtures thereof with or without alcohol, and in oils. Under ordinary conditions of storage and use, these preparations may contain a preservative to prevent the growth of microorganisms. Other exemplary carriers, excipients, or diluents are described in the Handbook of Pharmaceutical Excipients, 6th Edition, Rowe et al., Eds., Pharmaceutical Press (2009), hereby incorporated by reference in its entirety. The additional therapeutic agent may be administered in a pharmaceutical composition useful in the methods of the invention and can take the form of tablets, gelcaps, capsules, pills, powders, granulates, suspensions, emulsions, a sterile solution or suspension, and/or a sustained-release formulation.

Kits

A kit can include a CpG-amphiphile and a coronavirus antigen (e.g., a coronavirus spike protein or a peptide thereof, and/or a coronavirus nucleocapsid protein or a peptide thereof, or a nucleic acid sequence encoding the same), as disclosed herein, and instructions for use. The kits may include, in one or more suitable containers, a CpG-amphiphile and coronavirus antigen (e.g., a coronavirus spike protein or a peptide thereof, and/or a coronavirus nucleocapsid protein or a peptide thereof, or a nucleic acid sequence encoding the same), one or more controls, and various buffers, reagents, enzymes and other standard ingredients well known in the art. In some embodiments, the kits further include an adjuvant.

The container can include one or more vials, wells, test tubes, flasks, bottles, syringes, or other container means, into which the CpG-amphiphile or the coronavirus antigen (e.g., a coronavirus spike protein or a peptide thereof, and/or a coronavirus nucleocapsid protein or a peptide thereof, or a nucleic acid sequence encoding the same) may be placed, and in some instances, suitably aliquoted. When an additional component is provided, the kit can contain additional containers into which this compound may be placed. The kits can also include a means for containing the CpG-amphiphile and the coronavirus antigen (e.g., a coronavirus spike protein or a peptide thereof, and/or a coronavirus

nucleocapsid protein or a peptide thereof, or a nucleic acid sequence encoding the same), and any other reagent containers in close confinement for commercial sale. Such containers may include injection or blow-molded plastic containers into which the desired vials are retained. Containers and/or kits can include labeling with instructions for use and/or warnings.

In some embodiments, the disclosure provides a kit including one or more containers including a composition including a CpG-amphiphile and a composition including a coronavirus antigen (e.g., a coronavirus spike protein or a peptide thereof, and/or a coronavirus nucleocapsid protein or a peptide thereof, or a nucleic acid sequence encoding the same), an optional pharmaceutically acceptable carrier, and a package insert including instructions for administration of the composition for inducing an immune response. In some embodiments, the kit further includes an additional adjuvant and instructions for administration of the adjuvant.

In some embodiments, the disclosure provides a kit including a medicament including a composition including a CpG-amphiphile and a coronavirus antigen (e.g., a coronavirus spike protein or a peptide thereof, and/or a coronavirus nucleocapsid protein or a peptide thereof, or a nucleic acid sequence encoding the same), an optional pharmaceutically acceptable carrier, and a package insert including instructions for administration of the medicament alone or in combination with a composition including an additional adjuvant and an optional pharmaceutically acceptable carrier, for inducing an immune response.

In some embodiments, the disclosure provides a kit including a container including a composition including a CpG-amphiphile and a composition including a coronavirus antigen (e.g., a coronavirus spike protein or a peptide thereof, and/or a coronavirus nucleocapsid protein or a peptide thereof, or a nucleic acid sequence encoding the same), an optional pharmaceutically acceptable carrier, and a package insert including instructions for administration of a composition vaccine for inducing an immune response in a subject. In some embodiments, the kit further includes an additional adjuvant and instructions for administration of the adjuvant for inducing an immune response in a subject.

In addition to the compositions described herein, the kit can include other components or ingredients, such as a container(s) of a solvent or buffer, a stabilizer, a preservative, a flavoring agent (e.g., a bitter antagonist or a sweetener), a fragrance, a dye or coloring agent, for example, to tint or color one or more components in the kit. The kit can also include a second agent for treating a condition or disorder described herein (e.g., a coronavirus infection). Alternatively, other component(s) can be included in the kit, but in different compositions or containers distinct from the composition the CpG-amphiphile and the coronavirus antigen (e.g., a coronavirus spike protein or a peptide thereof, and/or a coronavirus nucleocapsid protein or a peptide thereof, or a nucleic acid sequence encoding the same). In such embodiments, the kit can include instructions for admixing a compound described herein and the other component(s), or for using a compound described herein (e.g., the CpG-amphiphile and the coronavirus antigen (e.g., a coronavirus spike protein or a peptide thereof, and/or a coronavirus nucleocapsid protein or a peptide thereof, or a nucleic acid sequence encoding the same)) together with the other component(s).

A composition described herein can be provided in any form, e.g., liquid, dried or lyophilized

form. It is preferred that a compound described herein be substantially pure and/or sterile. When a compound described herein is provided in a liquid solution, the liquid solution preferably is an aqueous solution, with a sterile aqueous solution being preferred. When a compound described herein is provided as a dried form, reconstitution generally is by the addition of a suitable solvent. The solvent, e.g., sterile water or buffer, can optionally be provided in the kit.

The containers of the kits can be airtight, waterproof (e.g., impermeable to changes in moisture or evaporation), and/or light-tight.

The kit optionally includes a device suitable for delivery of the composition, e.g., a syringe.

Numbered Embodiments

Some embodiments of the technology described herein can be defined according to any of the following numbered embodiments. Also encompassed are compositions and kits that include the components used in the methods described herein.

1. A method of inducing an immune response against a coronavirus antigen in a subject, the method comprising administering (1) a CpG-amphiphile and (2) a coronavirus antigen or a nucleic acid sequence encoding the coronavirus antigen to the subject.

2. A CpG-amphiphile and a coronavirus antigen or a nucleic acid sequence encoding the coronavirus antigen for use in inducing an immune response against a coronavirus antigen in a subject, wherein the CpG-amphiphile and the coronavirus antigen or a nucleic acid sequence encoding the coronavirus antigen are formulated for administration to the subject.

3. The method of embodiment 1, wherein the coronavirus antigen is a coronavirus spike protein or a peptide thereof or a nucleic acid sequence encoding the coronavirus spike protein or peptide.

4. The method of embodiment 1 or 3, wherein the CpG-amphiphile comprises a CpG sequence bonded to a lipid.

5. The method of embodiment 1 or 3, the CpG-amphiphile comprises a CpG sequence linked to a lipid by a linker.

6. The method of embodiment 5, wherein the linker comprises a polymer, a string of amino acids, a string of nucleic acids, a polysaccharide, or a combination thereof.

7. The method of embodiment 6, wherein the linker comprises a string of nucleic acids.

8. The method of embodiment 7, wherein the string of nucleic acids comprises between 1 and 50 nucleic acid residues.

9. The method of embodiment 8, wherein the string of nucleic acids comprises between 5 and 30 nucleic acid residues.

10. The method of any one of embodiments 5-9, wherein the string of nucleic acids comprises "N" guanines, wherein N is 1-10.

11. The method of embodiment 6, wherein the linker comprises consecutive polyethylene glycol units.

12. The method of embodiment 11, wherein the linker comprises "N" consecutive polyethylene glycol units, wherein N is between 20 and 80.

13. The method of embodiment 12, wherein the linker comprises "N" consecutive polyethylene glycol units, wherein N is between 30 and 70.

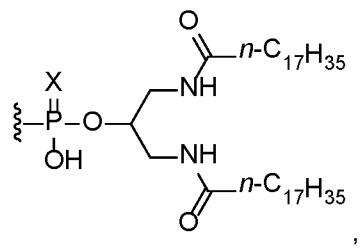
14. The method of embodiment 13, wherein the linker comprises "N" consecutive polyethylene glycol units, wherein N is between 40 and 60.

15. The method of embodiment 14, wherein the linker comprises "N" consecutive polyethylene glycol units, wherein N is between 45 and 55.

16. The method of embodiment 15, wherein the linker comprises 48 consecutive polyethylene glycol units.

17. The method of any one of embodiments 1 and 3-16, wherein the lipid is a diacyl lipid.

18. The method of embodiment 17, wherein the diacyl lipid has the following structure:



,

or a salt thereof,

wherein X is O or S.

19. The method of any one of embodiments 1 and 3-18 wherein the CpG sequence comprises the nucleotide sequence 5'-TCGTCGTTTTGTCGTTTTGTCGTT-3' (SEQ ID NO:1).

20. The method of any one of embodiments 1 and 3-18, wherein the CpG sequence comprises the nucleotide sequence of 5'-TCCATGACGTTCCTGACGTT-3' (SEQ ID NO: 2).

21. The method embodiment 19 or embodiment 20, wherein all internucleoside groups connecting the nucleosides in the CpG sequence are phosphorothioates.

22. The method of any one of embodiments 1 and 3-21, wherein the coronavirus spike protein or peptide thereof is a SARS-CoV-2 spike protein or peptide thereof.

23. The method of any one of embodiments 1 and 3-22, wherein the peptide of the coronavirus spike protein is a receptor binding domain that specifically binds angiotensin-converting enzyme 2 (ACE2).

24. The method of any one of embodiments 1 and 3-23, wherein the peptide of the coronavirus spike protein comprises a polypeptide sequence having at least 90% sequence identity to: RVQPTESIVRFPNITNLCPFGEVFNATRFASVYAWNRKRISNCVADYSVLYNSASFSTFKCYGVSPTK LNDLCFTNVYADSFVIRGDEVRQIAPGQTGKIADYNYKLPDDFTGCVIAWNSNNLDSKVGGNYNYLY RLFRKSNLKPFERDISTEIYQAGSTPCNGVEGFNCYFPLQSYGFQPTNGVGYQPYRVVVLSFELLHA PATVCGPKKSTNLVKNKCVNFNFNGLTGTGVLTESNKKFLPFQQFGRDIADTTDAVRDPQTLEILDITP CS (SEQ ID NO: 3).

25. The method of embodiment 24, wherein the peptide of the coronavirus spike protein comprises the polypeptide sequence of: RVQPTESIVRFPNITNLCPFGEVFNATRFASVYAWNRKRISNCVADYSVLYNSASFSTFKCYGVSPTK LNDLCFTNVYADSFVIRGDEVRQIAPGQTGKIADYNYKLPDDFTGCVIAWNSNNLDSKVGGNYNYLY RLFRKSNLKPFERDISTEIYQAGSTPCNGVEGFNCYFPLQSYGFQPTNGVGYQPYRVVVLSFELLHA PATVCGPKKSTNLVKNKCVNFNFNGLTGTGVLTESNKKFLPFQQFGRDIADTTDAVRDPQTLEILDITP CS (SEQ ID NO: 3).

26. The method of any one of embodiments 1 and 4-22, wherein the coronavirus antigen is a coronavirus nucleocapsid protein or a peptide thereof.

27. The method of embodiment 26, wherein the coronavirus nucleocapsid protein antigen comprises a polypeptide sequence having at least 90% sequence identity to: MSDNGPQNQRNAPRITFGGPSDSTGSNQNGERSGARSKQRRPQGLPNNTASWFTALTQHGKEDL KFPRGQGVPINTNSSPDDQIGYYRRATRRIRGGDGKMKDLSPRWYFYYLGTGPEAGLPYGANKDGII WVATEGALNTPKDHIGTRNPANNAAIVLQLPQGTTLPKGFYAEGSRGGSQASSRSSSRSRNSSRNS TPGSSRGTSPARMAGNGGDAALALLLLDRLNQLESKMSGKGQQQQGQTVTKKSAAEASKKPRQKR TATKAYNVTQAFGRRGPEQTQGNFGDQELIRQGTDYKHWPQIAQFAPSASAFFGMSRIGMEVTPSG TWLTYTGAIKLDDKDPNFKDQVILLNKHIDAYKTFPPTEPKKDKKKKADETQALPQRQKKQQTVTLLP AADLDDFSKQLQQSMSSADSTQA (SEQ ID NO:68).

28. The method of embodiment 26, wherein the coronavirus nucleocapsid protein antigen comprises the polypeptide sequence of:
MSDNGPQNQRNAPRITFGGPSDSTGSNQNGERSGARSKQRRPQGLPNNTASWFTALTQHGKEDL
KFPRGQGVPINTNSSPDDQIGYYRRATRRIRGGDGKMKDLSPRWYFYYLGTGPEAGLPYGANKDGII
WVATEGALNTPKDHIGTRNPANNAAIVLQLPQGTTLPKGFYAEGSRGGSQASSRSSSRSRNSSRNS
TPGSSRGTSPARMAGNGGDAALALLLLDRLNQLESKMSGKGQQQQGQTVTKKSAAEASKKPRQKR
TATKAYNVTQAFGRRGPEQTQGNFGDQELIRQGTDYKHWPQIAQFAPSASAFFGMSRIGMEVTPSG
TWLTYTGAIKLDDKDPNFKDQVILLNKHIDAYKTFPPTEPKKDKKKKADETQALPQRQKKQQTVTLLP
AADLDDFSKQLQQSMSSADSTQAENLYFQGHHHHHH (SEQ ID NO:63).

29. The method of any one of embodiments 1 and 3-28, wherein the coronavirus antigen comprises one or more tags.

30. The method of embodiment 29, wherein the tag is an Avi tag.

31. The method of embodiment 29, wherein the tag is a histidine tag.

32. The method of any one of embodiments 29-31, wherein the coronavirus antigen comprises an Avi tag and a histidine tag.

33. The method of any one of embodiments 29-32, wherein the coronavirus antigen comprises a linker between the polypeptide sequence and the one or more tags.

34. The method of any one of embodiments 1, 3-25, and 29-32, wherein the coronavirus spike protein is administered.

35. The method of embodiment 34, wherein a trimer of the coronavirus spike protein is administered.

36. The method of embodiment 35, wherein the trimer is a trimer of a protein construct comprising the sequence:
VNLTTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHSTQDLFLPFFSNVTWFHAIHVSGTNGTKRFDNP
VLPFNDGVYFASTEKSNIIRGWIFGTTLDSKTQSLLIVNNATNVVIKVCEFQFCNDPFLGVYYHKNNKS
WMESEFRVYSSANNCTFEYVSQPFLMDLEGKQGNFKNLREFVFKNIDGYFKIYSKHTPINLVRDLPQ
GFSALEPLVDLPIGINITRFQTLLALHRSYLTPGDSSSGWTAGAAAYYVGYLQPRTFLLKYNENGTITD
AVDCALDPLSETKCTLKSFTVEKGIYQTSNFRVQPTESIVRFPNITNLCPFGEVFNATRFASVYAWNR
KRISNCVADYSVLYNSASFSTFKCYGVSPTKLNDLCFTNVYADSFVIRGDEVRQIAPGQTGKIADYNY
KLPDDFTGCVIAWNSNNLDSKVGGNYNYLYRLFRKSNLKPFERDISTEIYQAGSTPCNGVEGFNCYF
PLQSYGFQPTNGVGYQPYRVVVLSFELLHAPATVCGPKKSTNLVKNKCVNFNFNGLTGTGVLTESNK
KFLPFQQFGRDIADTTDAVRDPQTLEILDITPCSFGGVSVITPGTNTSNQVAVLYQDVNCTEVPVAIHA
DQLTPTWRVYSTGSNVFQTRAGCLIGAEHVNNSYECDIPIGAGICASYQTQTNSPRAAASVASQSIIA

YTMSLGAENSVAYSNNSIAIPTNFTISVTTEILPVSMTKTSVDCTMYICGDSTECSNLLLQYGSFCTQLN
RALTGIAVEQDKNTQEVFAQVKQIYKTPPIKDFGGFNFSQILPDPSKPSKRSFIEDLLFNKVTLADAGFI
KQYGDCLGDIAARDLICAQKFNGLTVLPPLLTDEMIAQYTSALLAGTITSGWTFGAGAALQIPFAMQMA
YRFNGIGVTQNVLYENQKLIANQFNSAIGKIQDSLSSTASALGKLQDVVNQNAQALNTLVKQLSSNFG
AISSVLNDILSRLDKVEAEVQIDRLITGRLQSLQTYVTQQLIRAAEIRASANLAATKMSECVLGQSKRVD
FCGKGYHLMSFPQSAPHGVVFLHVTYVPAQEKNFTTAPAICHDGKAHFPREGVFVSNGTHWFVTQR
NFYEPQIITTDNTFVSGNCDVVIGIVNNTVYDPLQPELDSFKEELDKYFKNHTSPDVDLGDISGINASVV
NIQKEIDRLNEVAKNLNESLIDLQELGKYEQYIKWPGGGSGGGSHHHHHHHHHH (SEQ ID NO:66).

37. The method of any one of embodiments 1 and 3-36, wherein a coronavirus spike protein, or a peptide thereof, and a coronavirus nucleocapsid protein, or a peptide thereof, are administered.

38. The method of embodiment 37, wherein a trimer of a coronavirus spike protein construct comprising the sequence:
VNLTTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHSTQDLFLPFFSNVTWFHAIHVSGTNGTKRFDNP
VLPFNDGVYFASTEKSNIIRGWIFGTTLDSKTQSLLIVNNATNVVIKVCEFQFCNDPFLGVYYHKNNKS
WMESEFRVYSSANNCTFEYVSQPFLMDLEGKQGNFKNLREFVFKNIDGYFKIYSKHTPINLVRDLPQ
GFSALEPLVDLPIGINITRFQTLLALHRSYLTPGDSSSGWTAGAAAYYVGYLQPRTFLLKYNENGTITD
AVDCALDPLSETKCTLKSFTVEKGIYQTSNFRVQPTESIVRFPNITNLCPFGEVFNATRFASVYAWNR
KRISNCVADYSVLYNSASFSTFKCYGVSPTKLNDLCFTNVYADSFVIRGDEVRQIAPGQTGKIADYNY
KLPDDFTGCVIAWNSNNLDSKVGGNYNYLYRLFRKSNLKPFERDISTEIYQAGSTPCNGVEGFNCYF
PLQSYGFQPTNGVGYQPYRVVVLSFELLHAPATVCGPKKSTNLVKNKCVNFNFNGLTGTGVLTESNK
KFLPFQQFGRDIADTTDAVRDPQTLEILDITPCSFGGVSVITPGTNTSNQVAVLYQDVNCTEVPVAIHA
DQLTPTWRVYSTGSNVFQTRAGCLIGAEHVNNSYECDIPIGAGICASYQTQTNSPRAAASVASQSIIA
YTMSLGAENSVAYSNNSIAIPTNFTISVTTEILPVSMTKTSVDCTMYICGDSTECSNLLLQYGSFCTQLN
RALTGIAVEQDKNTQEVFAQVKQIYKTPPIKDFGGFNFSQILPDPSKPSKRSFIEDLLFNKVTLADAGFI
KQYGDCLGDIAARDLICAQKFNGLTVLPPLLTDEMIAQYTSALLAGTITSGWTFGAGAALQIPFAMQMA
YRFNGIGVTQNVLYENQKLIANQFNSAIGKIQDSLSSTASALGKLQDVVNQNAQALNTLVKQLSSNFG
AISSVLNDILSRLDKVEAEVQIDRLITGRLQSLQTYVTQQLIRAAEIRASANLAATKMSECVLGQSKRVD
FCGKGYHLMSFPQSAPHGVVFLHVTYVPAQEKNFTTAPAICHDGKAHFPREGVFVSNGTHWFVTQR
NFYEPQIITTDNTFVSGNCDVVIGIVNNTVYDPLQPELDSFKEELDKYFKNHTSPDVDLGDISGINASVV
NIQKEIDRLNEVAKNLNESLIDLQELGKYEQYIKWPGGGSGGGSHHHHHHHHHH (SEQ ID NO:66),
and a coronavirus nucleocapsid protein construct having the polypeptide sequence of:
MSDNGPQNQRNAPRITFGGPSDSTGSNQNGERSGARSKQRRPQGLPNNTASWFTALTQHGKEDL
KFPRGQGVPINTNSSPDDQIGYYRRATRRIRGGDGKMKDLSPRWYFYYLGTGPEAGLPYGANKDGII
WVATEGALNTPKDHIGTRNPANNAAIVLQLPQGTTLPKGFYAEGSRGGSQASSRSSSRSRNSSRNS
TPGSSRGTSPARMAGNGGDAALALLLLDRLNQLESKMSGKGQQQQGQTVTKKSAAEASKKPRQKR
TATKAYNVTQAFGRRGPEQTQGNFGDQELIRQGTDYKHWPQIAQFAPSASAFFGMSRIGMEVTPSG
TWLTYTGAIKLDDKDPNFKDQVILLNKHIDAYKTFPPTEPKKDKKKKADETQALPQRQKKQQTVTLLP
AADLDDFSKQLQQSMSSADSTQAENLYFQGHHHHHH (SEQ ID NO:63) are administered.

39. The method of any one of embodiments 1, 3-26, 29-32, and 37, wherein an mRNA encoding the coronavirus antigen is administered.

40. The method of any one of embodiments 1 and 3-39, wherein the CpG-amphiphile and the coronavirus antigen or nucleic acid sequence encoding the same are administered concurrently.

41. The method of any one of embodiments 1 and 3-39, wherein the CpG-amphiphile and the coronavirus antigen, or nucleic acid sequence enclosing the same are administered sequentially.

42. The method of embodiment 41, wherein the CpG-amphiphile is administered first, followed by administering of the coronavirus antigen or nucleic acid sequence encoding the same.

43. The method of embodiment 41, wherein said the coronavirus antigen or nucleic acid sequence encoding the same is administered first, followed by administering of CpG-amphiphile.

44. The method of any one of embodiments 1 and 3-43, wherein the method comprises administering a second adjuvant to the subject.

45. The method of any one of embodiments 1 and 3-44, wherein the method comprises administering a coronavirus vaccine to the subject as a prime or a boost.

46. The method of any one of embodiments 1 and 3-45, wherein the CpG-amphiphile is administered subcutaneously, intranasally, intratracheally, or by inhalation during mechanical ventilation.

47. The method of embodiment 46, wherein the CpG-amphiphile is administered subcutaneously.

48. The method of any one of embodiments 1 and 3-47, wherein the coronavirus antigen is administered subcutaneously, intranasally, intratracheally, or by inhalation during mechanical ventilation.

49. The method of any one of embodiments 1 and 3-48, wherein the subject is a mammal.

50. The method of embodiment 49, wherein the subject is a human.

51. A pharmaceutical composition comprising a CpG-amphiphile and a coronavirus antigen, or a nucleic acid sequence encoding the coronavirus antigen, and a pharmaceutically acceptable carrier.

52. The pharmaceutical composition of embodiment 51, wherein the coronavirus antigen is a coronavirus spike protein or a peptide thereof.

53. The pharmaceutical composition of embodiment 51, wherein the coronavirus antigen is a coronavirus nucleocapsid protein or a peptide thereof.

54. The pharmaceutical composition of embodiment 51, wherein the coronavirus antigen comprises a coronavirus spike protein or a peptide thereof and a coronavirus nucleocapsid protein or a peptide thereof.

55. A kit comprising a CpG-amphiphile and a coronavirus antigen or a nucleic acid sequence encoding the coronavirus antigen.

56. The kit of embodiment 55, wherein the coronavirus antigen is a coronavirus spike protein or a peptide thereof.

57. The kit of embodiment 55, wherein the coronavirus antigen is a coronavirus nucleocapsid protein or a peptide thereof.

58. The kit of embodiment 55, wherein the coronavirus antigen comprises a coronavirus spike protein or a peptide thereof and a coronavirus nucleocapsid protein or a peptide thereof.

59. The CpG-amphiphile and a coronavirus antigen, or a nucleic acid sequence encoding the coronavirus antigen for use according to embodiment 2, wherein the coronavirus antigen is a coronavirus spike protein or a peptide thereof or a nucleic acid sequence encoding the coronavirus spike protein or peptide.

60. The CpG-amphiphile and a coronavirus antigen, or a nucleic acid sequence encoding the coronavirus antigen for use according to embodiment 2 or 59, wherein the CpG-amphiphile comprises a CpG sequence bonded to a lipid.

61. The CpG-amphiphile and a coronavirus antigen, or a nucleic acid sequence encoding the coronavirus antigen for use according to embodiment 2 or 59, the CpG-amphiphile comprises a CpG sequence linked to a lipid by a linker.

62. The CpG-amphiphile and a coronavirus antigen, or a nucleic acid sequence encoding the coronavirus antigen for use according to embodiment 61, wherein the linker comprises a polymer, a string of amino acids, a string of nucleic acids, a polysaccharide, or a combination thereof.

63. The CpG-amphiphile and a coronavirus antigen, or a nucleic acid sequence encoding the coronavirus antigen for use according to embodiment 62, wherein the linker comprises a string of nucleic acids.

64. The CpG-amphiphile and a coronavirus antigen, or a nucleic acid sequence encoding the coronavirus antigen for use according to embodiment 63, wherein the string of nucleic acids comprises between 1 and 50 nucleic acid residues.

65. The CpG-amphiphile and a coronavirus antigen, or a nucleic acid sequence encoding the coronavirus antigen for use according to embodiment 64, wherein the string of nucleic acids comprises between 5 and 30 nucleic acid residues.

66. The CpG-amphiphile and a coronavirus antigen, or a nucleic acid sequence encoding the coronavirus antigen for use according to any one of embodiments 61-65, wherein the string of nucleic acids comprises “N” guanines, wherein N is 1-10.

67. The CpG-amphiphile and a coronavirus antigen, or a nucleic acid sequence encoding the coronavirus antigen for use according to embodiment 63, wherein the linker comprises consecutive polyethylene glycol units.

68. The CpG-amphiphile and a coronavirus antigen, or a nucleic acid sequence encoding the coronavirus antigen for use according to embodiment 67, wherein the linker comprises “N” consecutive polyethylene glycol units, wherein N is between 20 and 80.

69. The CpG-amphiphile and a coronavirus antigen, or a nucleic acid sequence encoding the coronavirus antigen for use according to embodiment 68, wherein the linker comprises “N” consecutive polyethylene glycol units, wherein N is between 30 and 70.

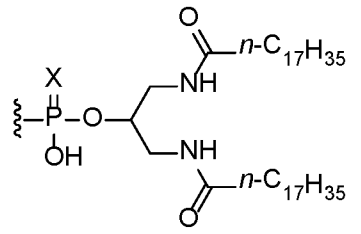
70. The CpG-amphiphile and a coronavirus antigen, or a nucleic acid sequence encoding the coronavirus antigen for use according to embodiment 69, wherein the linker comprises “N” consecutive polyethylene glycol units, wherein N is between 40 and 60.

71. The CpG-amphiphile and a coronavirus antigen, or a nucleic acid sequence encoding the coronavirus antigen for use according to embodiment 70, wherein the linker comprises “N” consecutive polyethylene glycol units, wherein N is between 45 and 55.

72. The CpG-amphiphile and a coronavirus antigen, or a nucleic acid sequence encoding the coronavirus antigen for use according to embodiment 71, wherein the linker comprises 48 consecutive polyethylene glycol units.

73. The CpG-amphiphile and a coronavirus antigen, or a nucleic acid sequence encoding the coronavirus antigen for use according to any one of embodiments 2 or 59-72, wherein the lipid is a diacyl lipid.

74. The CpG-amphiphile and a coronavirus antigen, or a nucleic acid sequence encoding the coronavirus antigen for use according to embodiment 73, wherein the diacyl lipid has the following structure:



,

or a salt thereof,
wherein X is O or S.

75. The CpG-amphiphile and a coronavirus antigen, or a nucleic acid sequence encoding the coronavirus antigen for use according to any one of embodiments 2 or 59-74 wherein the CpG sequence comprises the nucleotide sequence 5'-TCGTCGTTTTGTCGTTTTGTCGTT-3' (SEQ ID NO:1).

76. The CpG-amphiphile and a coronavirus antigen, or a nucleic acid sequence encoding the coronavirus antigen for use according to any one of embodiments 2 or 59-75, wherein the CpG sequence comprises the nucleotide sequence of 5'-TCCATGACGTTCCTGACGTT-3' (SEQ ID NO: 2).

77. The CpG-amphiphile and a coronavirus antigen, or a nucleic acid sequence encoding the coronavirus antigen for use according to embodiment 75 or embodiment 76, wherein all internucleoside groups connecting the nucleosides in the CpG sequence are phosphorothioates.

78. The CpG-amphiphile and a coronavirus antigen, or a nucleic acid sequence encoding the coronavirus antigen for use according to any one of embodiments 2 or 59-77, wherein the coronavirus spike protein or peptide thereof is a SARS-CoV-2 spike protein or peptide thereof.

79. The CpG-amphiphile and a coronavirus antigen, or a nucleic acid sequence encoding the coronavirus antigen for use according to any one of embodiments 2 or 59-78, wherein the peptide of the coronavirus spike protein is a receptor binding domain that specifically binds angiotensin-converting enzyme 2 (ACE2).

80. The CpG-amphiphile and a coronavirus antigen, or a nucleic acid sequence encoding the coronavirus antigen for use according to any one of embodiments 2 or 59-79, wherein the peptide of the coronavirus spike protein comprises a polypeptide sequence having at least 90% sequence identity to:
RVQPTESIVRFPNITNLCPFGEVFNATRFASVYAWNRKRISNCVADYSVLYNSASFSTFKCYGVSPTK
LNDLCFTNVYADSFVIRGDEVRQIAPGQTGKIADYNYKLPDDFTGCVIAWNSNNLDSKVGGNYNYLY

RLFRKSNLKPFERDISTEIYQAGSTPCNGVEGFNCYFPLQSYGFQPTNGVGYQPYRVVVLSFELLHA
PATVCGPKKSTNLVKNKCVNFNFNGLTGTGVLTESNKKFLPFQQFGRDIADTTDAVRDPQTLEILDITP
CS (SEQ ID NO: 3).

81. The CpG-amphiphile and a coronavirus antigen, or a nucleic acid sequence encoding the coronavirus antigen for use according to embodiment 80, wherein the peptide of the coronavirus spike protein comprises the polypeptide sequence of:
RVQPTESIVRFPNITNLCPFGEVFNATRFASVYAWNRKRISNCVADYSVLYNSASFSTFKCYGVSPTK
LNDLCFTNVYADSFVIRGDEVRQIAPGQTGKIADYNYKLPDDFTGCVIAWNSNNLDSKVGGNYNYLY
RLFRKSNLKPFERDISTEIYQAGSTPCNGVEGFNCYFPLQSYGFQPTNGVGYQPYRVVVLSFELLHA
PATVCGPKKSTNLVKNKCVNFNFNGLTGTGVLTESNKKFLPFQQFGRDIADTTDAVRDPQTLEILDITP
CS (SEQ ID NO: 3).

82. The CpG-amphiphile and a coronavirus antigen, or a nucleic acid sequence encoding the coronavirus antigen for use according to any one of embodiments 2 or 61-79, wherein the coronavirus antigen is a coronavirus nucleocapsid protein or a peptide thereof.

83. The CpG-amphiphile and a coronavirus antigen, or a nucleic acid sequence encoding the coronavirus antigen for use according to embodiment 82, wherein the coronavirus nucleocapsid protein antigen comprises a polypeptide sequence having at least 90% sequence identity to:
MSDNGPQNQRNAPRITFGGPSDSTGSNQNGERSGARSKQRRPQGLPNNTASWFTALTQHGKEDL
KFPRGQGVPINTNSSPDDQIGYYRRATRRIRGGDGKMKDLSPRWYFYYLGTGPEAGLPYGANKDGII
WVATEGALNTPKDHIGTRNPANNAAIVLQLPQGTTLPKGFYAEGSRGGSQASSRSSSRSRNSSRNS
TPGSSRGTSPARMAGNGGDAALALLLLDRLNQLESKMSGKGQQQQGQTVTKKSAAEASKKPRQKR
TATKAYNVTQAFGRRGPEQTQGNFGDQELIRQGTDYKHWPQIAQFAPSASAFFGMSRIGMEVTPSG
TWLTYTGAIKLDDKDPNFKDQVILLNKHIDAYKTFPPTEPKKDKKKKADETQALPQRQKKQQTVTLLP
AADLDDFSKQLQQSMSSADSTQA (SEQ ID NO:68).

84. The CpG-amphiphile and a coronavirus antigen, or a nucleic acid sequence encoding the coronavirus antigen for use according to embodiment 82, wherein the coronavirus nucleocapsid protein antigen comprises the polypeptide sequence of:
MSDNGPQNQRNAPRITFGGPSDSTGSNQNGERSGARSKQRRPQGLPNNTASWFTALTQHGKEDL
KFPRGQGVPINTNSSPDDQIGYYRRATRRIRGGDGKMKDLSPRWYFYYLGTGPEAGLPYGANKDGII
WVATEGALNTPKDHIGTRNPANNAAIVLQLPQGTTLPKGFYAEGSRGGSQASSRSSSRSRNSSRNS
TPGSSRGTSPARMAGNGGDAALALLLLDRLNQLESKMSGKGQQQQGQTVTKKSAAEASKKPRQKR
TATKAYNVTQAFGRRGPEQTQGNFGDQELIRQGTDYKHWPQIAQFAPSASAFFGMSRIGMEVTPSG
TWLTYTGAIKLDDKDPNFKDQVILLNKHIDAYKTFPPTEPKKDKKKKADETQALPQRQKKQQTVTLLP
AADLDDFSKQLQQSMSSADSTQAENLYFQGHHHHHH (SEQ ID NO:63).

85. The CpG-amphiphile and a coronavirus antigen, or a nucleic acid sequence encoding the coronavirus antigen for use according to any one of embodiments 2 or 59-83, wherein the coronavirus antigen comprises one or more tags.

86. The CpG-amphiphile and a coronavirus antigen, or a nucleic acid sequence encoding the coronavirus antigen for use according to embodiment 85, wherein the tag is an Avi tag.

87. The CpG-amphiphile and a coronavirus antigen, or a nucleic acid sequence encoding the coronavirus antigen for use according to embodiment 85, wherein the tag is a histidine tag.

88. The CpG-amphiphile and a coronavirus antigen, or a nucleic acid sequence encoding the coronavirus antigen for use according to any one of embodiments 85-86, wherein the coronavirus antigen comprises an Avi tag and a histidine tag.

89. The CpG-amphiphile and a coronavirus antigen, or a nucleic acid sequence encoding the coronavirus antigen for use according to any one of embodiments 85-88, wherein the coronavirus antigen comprises a linker between the polypeptide sequence and the one or more tags.

90. The CpG-amphiphile and a coronavirus antigen, or a nucleic acid sequence encoding the coronavirus antigen for use according to any one of embodiments 2 and 59-89, wherein the coronavirus spike protein is to be administered.

91. The CpG-amphiphile and a coronavirus antigen, or a nucleic acid sequence encoding the coronavirus antigen for use according to embodiment 90, wherein a trimer of the coronavirus spike protein is to be administered.

92. The CpG-amphiphile and a coronavirus antigen, or a nucleic acid sequence encoding the coronavirus antigen for use according to embodiment 91, wherein the trimer is a trimer of a protein construct comprising the sequence:

VNLTTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHSTQDLFLPFFSNVTWFHAIHVSGTNGTKRFDNP
VLPFNDGVYFASTEKSNIIRGWIFGTTLDSKTQSLLIVNNATNVVIKVCEFQFCNDPFLGVYYHKNNKS
WMESEFRVYSSANNCTFEYVSQPFLMDLEGKQGNFKNLREFVFKNIDGYFKIYSKHTPINLVRDLPQ
GFSALEPLVDLPIGINITRFQTLLALHRSYLTPGDSSSGWTAGAAAYYVGYLQPRTFLLKYNENGTITD
AVDCALDPLSETKCTLKSFTVEKGIYQTSNFRVQPTESIVRFPNITNLCPFGEVFNATRFASVYAWNR
KRISNCVADYSVLYNSASFSTFKCYGVSPTKLNDLCFTNVYADSFVIRGDEVRQIAPGQTGKIADYNY
KLPDDFTGCVIAWNSNNLDSKVGGNYNYLYRLFRKSNLKPFERDISTEIYQAGSTPCNGVEGFNCYF
PLQSYGFQPTNGVGYQPYRVVVLSFELLHAPATVCGPKKSTNLVKNKCVNFNFNGLTGTGVLTESNK
KFLPFQQFGRDIADTTDAVRDPQTLEILDITPCSFGGVSVITPGTNTSNQVAVLYQDVNCTEVPVAIHA
DQLTPTWRVYSTGSNVFQTRAGCLIGAEHVNNSYECDIPIGAGICASYQTQTNSPRAAASVASQSIIA
YTMSLGAENSVAYSNNSIAIPTNFTISVTTEILPVSMTKTSVDCTMYICGDSTECSNLLLQYGSFCTQLN

RALTGIAVEQDKNTQEVFAQVKQIYKTPPIKDFGGFNFSQILPDPSKPSKRSFIEDLLFNKVTLADAGFI
KQYGDCLGDIAARDLICAQKFNGLTVLPPLLTDEMIAQYTSALLAGTITSGWTFGAGAALQIPFAMQMA
YRFNGIGVTQNVLYENQKLIANQFNSAIGKIQDSLSSTASALGKLQDVVNQNAQALNTLVKQLSSNFG
AISSVLNDILSRLDKVEAEVQIDRLITGRLQSLQTYVTQQLIRAAEIRASANLAATKMSECVLGQSKRVD
FCGKGYHLMSFPQSAPHGVVFLHVTYVPAQEKNFTTAPAICHDGKAHFPREGVFVSNGTHWFVTQR
NFYEPQIITTDNTFVSGNCDVVIGIVNNTVYDPLQPELDSFKEELDKYFKNHTSPDVDLGDISGINASVV
NIQKEIDRLNEVAKNLNESLIDLQELGKYEQYIKWPGGGSGGGSHHHHHHHHHH (SEQ ID NO:66).

93. The CpG-amphiphile and a coronavirus antigen, or a nucleic acid sequence encoding the coronavirus antigen for use according to any one of embodiments 2 and 59-92, wherein a coronavirus spike protein, or a peptide thereof, and a coronavirus nucleocapsid protein, or a peptide thereof, are to be administered.

94. The CpG-amphiphile and a coronavirus antigen, or a nucleic acid sequence encoding the coronavirus antigen for use according to embodiment 93, wherein a trimer of a coronavirus spike protein construct comprising the sequence:
VNLTTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHSTQDLFLPFFSNVTWFHAIHVSGTNGTKRFDNP
VLPFNDGVYFASTEKSNIIRGWIFGTTLDSKTQSLLIVNNATNVVIKVCEFQFCNDPFLGVYYHKNNKS
WMESEFRVYSSANNCTFEYVSQPFLMDLEGKQGNFKNLREFVFKNIDGYFKIYSKHTPINLVRDLPQ
GFSALEPLVDLPIGINITRFQTLLALHRSYLTPGDSSSGWTAGAAAYYVGYLQPRTFLLKYNENGTITD
AVDCALDPLSETKCTLKSFTVEKGIYQTSNFRVQPTESIVRFPNITNLCPFGEVFNATRFASVYAWNR
KRISNCVADYSVLYNSASFSTFKCYGVSPTKLNDLCFTNVYADSFVIRGDEVRQIAPGQTGKIADYNY
KLPDDFTGCVIAWNSNNLDSKVGGNYNYLYRLFRKSNLKPFERDISTEIYQAGSTPCNGVEGFNCYF
PLQSYGFQPTNGVGYQPYRVVVLSFELLHAPATVCGPKKSTNLVKNKCVNFNFNGLTGTGVLTESNK
KFLPFQQFGRDIADTTDAVRDPQTLEILDITPCSFGGVSVITPGTNTSNQVAVLYQDVNCTEVPVAIHA
DQLTPTWRVYSTGSNVFQTRAGCLIGAEHVNNSYECDIPIGAGICASYQTQTNSPRAAASVASQSIIA
YTMSLGAENSVAYSNNSIAIPTNFTISVTTEILPVSMTKTSVDCTMYICGDSTECSNLLLQYGSFCTQLN
RALTGIAVEQDKNTQEVFAQVKQIYKTPPIKDFGGFNFSQILPDPSKPSKRSFIEDLLFNKVTLADAGFI
KQYGDCLGDIAARDLICAQKFNGLTVLPPLLTDEMIAQYTSALLAGTITSGWTFGAGAALQIPFAMQMA
YRFNGIGVTQNVLYENQKLIANQFNSAIGKIQDSLSSTASALGKLQDVVNQNAQALNTLVKQLSSNFG
AISSVLNDILSRLDKVEAEVQIDRLITGRLQSLQTYVTQQLIRAAEIRASANLAATKMSECVLGQSKRVD
FCGKGYHLMSFPQSAPHGVVFLHVTYVPAQEKNFTTAPAICHDGKAHFPREGVFVSNGTHWFVTQR
NFYEPQIITTDNTFVSGNCDVVIGIVNNTVYDPLQPELDSFKEELDKYFKNHTSPDVDLGDISGINASVV
NIQKEIDRLNEVAKNLNESLIDLQELGKYEQYIKWPGGGSGGGSHHHHHHHHHH (SEQ ID NO:66),
and a coronavirus nucleocapsid protein construct having the polypeptide sequence of:
MSDNGPQNQRNAPRITFGGPSDSTGSNQNGERSGARSKQRRPQGLPNNTASWFTALTQHGKEDL
KFPRGQGVPINTNSSPDDQIGYYRRATRRIRGGDGKMKDLSPRWYFYYLGTGPEAGLPYGANKDGII
WVATEGALNTPKDHIGTRNPANNAAIVLQLPQGTTLPKGFYAEGSRGGSQASSRSSSRSRNSSRNS
TPGSSRGTSPARMAGNGGDAALALLLLDRLNQLESKMSGKGQQQQGQTVTKKSAAEASKKPRQKR
TATKAYNVTQAFGRRGPEQTQGNFGDQELIRQGTDYKHWPQIAQFAPSASAFFGMSRIGMEVTPSG

TWLTYTGAIKLDDKDPNFKDQVILLNKHIDAYKTFPPTEPKKDKKKKADETQALPQRQKKQQTVTLLP AADLDDFSKQLQQSMSSADSTQAENLYFQGHHHHHH (SEQ ID NO:63) are to be administered.

95. The CpG-amphiphile and a coronavirus antigen, or a nucleic acid sequence encoding the coronavirus antigen for use according to any one of embodiments 2 and 59-94, wherein an mRNA encoding the coronavirus antigen is administered.

96. The CpG-amphiphile and a coronavirus antigen, or a nucleic acid sequence encoding the coronavirus antigen for use according to any one of embodiments 2 and 59-95, wherein the CpG-amphiphile and the coronavirus antigen or nucleic acid sequence encoding the same are to be administered concurrently.

97. The CpG-amphiphile and a coronavirus antigen, or a nucleic acid sequence encoding the coronavirus antigen for use according to any one of embodiments 2 and 59-96, wherein the CpG-amphiphile and the coronavirus antigen, or nucleic acid sequence enclosing the same are to be administered sequentially.

98. The CpG-amphiphile and a coronavirus antigen, or a nucleic acid sequence encoding the coronavirus antigen for use according to embodiment 97, wherein the CpG-amphiphile is to be administered first, followed by administering of the coronavirus antigen or nucleic acid sequence encoding the same.

98. The CpG-amphiphile and a coronavirus antigen, or a nucleic acid sequence encoding the coronavirus antigen for use according to embodiment 97, wherein said the coronavirus antigen or nucleic acid sequence encoding the same is to be administered first, followed by administering of CpG-amphiphile.

99. The CpG-amphiphile and a coronavirus antigen, or a nucleic acid sequence encoding the coronavirus antigen for use according to any one of embodiments 2 and 59-99, wherein a second adjuvant is to be administered to the subject.

100. The CpG-amphiphile and a coronavirus antigen, or a nucleic acid sequence encoding the coronavirus antigen for use according to any one of embodiments 2 and 59-99, wherein a coronavirus vaccine is to be administered to the subject as a prime or a boost.

101. The CpG-amphiphile and a coronavirus antigen, or a nucleic acid sequence encoding the coronavirus antigen for use according to any one of embodiments 2 and 59-100, wherein the CpG-amphiphile is to be administered subcutaneously, intranasally, intratracheally, or by inhalation during mechanical ventilation.

102. The CpG-amphiphile and a coronavirus antigen, or a nucleic acid sequence encoding the coronavirus antigen for use according to embodiment 101, wherein the CpG-amphiphile is to be administered subcutaneously.

103. The CpG-amphiphile and a coronavirus antigen, or a nucleic acid sequence encoding the coronavirus antigen for use according to any one of embodiments 2 and 59-102, wherein the coronavirus antigen is to be administered subcutaneously, intranasally, intratracheally, or by inhalation during mechanical ventilation.

104. The CpG-amphiphile and a coronavirus antigen, or a nucleic acid sequence encoding the coronavirus antigen for use according to any one of embodiments 2 and 59-103, wherein the subject is a mammal.

105. The CpG-amphiphile and a coronavirus antigen, or a nucleic acid sequence encoding the coronavirus antigen for use according to embodiment 104, wherein the subject is a human.

EXAMPLES

The following examples, which are intended to illustrate, rather than limit, the disclosure, are put forth to provide those of ordinary skill in the art with a description of how the compositions and methods described herein may be used, made, and evaluated. The examples are intended to be purely exemplary of the disclosure and are not intended to limit the scope of what the inventors regard as their invention.

Example 1: Inducing an immune response in mice

Stock solutions of the free CpG (CpG 1826), CpG-amphiphile (aCpG 1826) were made by resuspending the CpG-amphiphile in limulus amebocyte lysate (LAL) H_2O. Final injections were diluted with 1x phosphate buffered saline (PBS) such that the final concentration of CpG was 1 nmol/100 µL injection. The SARS-CoV2 Spike S1 RBD protein (SEQ ID NO: 3) (Table 1) stock solutions were dissolved in PBS at a concentration of 1.2 mg/ml. Final injections were diluted with 1x PBS to 10 µg/100 µL injection.

IFA (Incomplete Freund's Adjuvant) solutions were made using a 1:1 mix of 50 µL antigen suspended in PBS and 50 µL IFA, followed by pipetting up and down vigorously for 30 seconds. Alhydrogel 2% (10mg/ml) solutions were made using a 1:9 mix of 10 µg antigen suspended in PBS and 100 µg alum, which is equivalent 10 µL. To this solution the antigen was added made up with 100 µL with PBS. The solution was mixed by pipetting vigorously for 5min.

Immunizations were administered subcutaneously (SC) into the tail base of female C57Bl6 mice bilaterally with 50 µL per side. Booster doses was given at roughly 2-week intervals.

SC injections ensured that the vaccine was optimally delivered into lymph nodes via natural lymph drainage. Bi-weekly injections were determined to be optimal in immune response generation in previous mouse studies.

Table 1: Vaccine Components

Vaccine Components	Sequence or Cat#	Source	Lot #
SARS-CoV2 RBD, His	40592-V08H	SinoBio	MA14MA1904
CpG 1826	5'-ggt cca tga cgt tcc tga cgt t-3' (SEQ ID NO:2)	InvivoGen	4103-24T
aCpG 1826	5'-(Diacyl lipid)ggt cca tga cgt tcc tga cgt t-3' (SEQ ID NO:2)	Avecia	S17-048-S3-B1
Alhydrogel	vac-alu-250	InvivoGen	1614532
IFA	vac-ifa-10	InvivoGen	IFA-41-01

Intracellular Stain (ICS) assays for TNFα and IFNγ were performed on peripheral blood mononuclear cells (PBMCs) 7 days after dosing. Cells were surface stained for CD4 and CD8 and sometimes CD3. For ICS#1, after the first dose, and ICS#2, after the second dose, PBMCs were activated for 5 hours (4 hours in the presence of Brefeldin A) with 1µg/well of peptide (Table 2). C57Bl6 cells were re-stimulated with peptides that have a calculated affinity to Db/Kb. Balb/c cells were re-stimulated with peptides that have a calculated affinity to Dd/Kd

Table 2: Re-Stimulation Peptides

Re-stimulation Peptides	Sequence	Source	Lot #
H-2-Db1	YSVLYNSASF (SEQ ID NO:39)	GenScript	U956FFC260-1-PE8358
H-2-Db4/Dd4	YQPYRVVVL (SEQ ID NO:40)	GenScript	U956FFC260-10-PE8364
H-2-Db5/Kb8/Dd5/Kd5	VRFPNITNL (SEQ ID NO:41)	GenScript	U956FFC260-13-PE8366
H-2-Kb2	FNATRFASV (SEQ ID NO:42)	GenScript	U956FFC260-19-PE8370
H-2-Kb3	KIADYNYKL (SEQ ID NO:43)	GenScript	U956FFC260-22-PE8372
H-2-Kb7/Dd7	VSPTKLNDL (SEQ ID NO:44)	GenScript	U956FFC260-34-PE8380
H-2-Dd1	VCGPKKSTNL (SEQ ID NO:45)	GenScript	U956FFC260-37-PE8382
H-2-Kd2	SYGFQPTNGV (SEQ ID NO:46)	GenScript	U956FFC260-49-PE8390
H-2-Kd3	VYAWNRKRI (SEQ ID NO:47)	GenScript	U956FFC260-52-PE8392
H-2-Kd4	SFVIRGDEV (SEQ ID NO:48)	GenScript	U956FFC260-55-PE8394

Cytometric Bead Array (CBA) analysis was performed for IL2, IL4, IL6, IL10, IL17, TNFα and IFNγ was performed on splenocytes 7 days after dose administration. For CBA#1, after dose one, PBMCs were activated overnight with 5µg/well of peptide (Table 2). For CBA#2, after the second dose, PBMCs were activated overnight with 0.42µg/well of PepMix™ SARS-CoV-2 Spike Glycoprotein (315 peptides each at 0. 42µg/well) (Table 3). For CBA#3, after dose three, PBMCs were activated overnight with 1µg/well of PepMix™ SARS-CoV-2 Spike Glycoprotein (315 peptides each at 1µg/well) (Table 3).

Table 3: Re-Stimulation PepMix

Re-stimulation Peptides	Sequence	Source	Lot #
PepMix™ SARS-CoV-2 Spike Glycoprotein	315 15mers spanning Spike Protein Sequence, overlap 11aa	JPT	42669FRa-1 (158 peptides) 42669FRa-2 (157 peptides)

SARS-CoV2 specific serum antibody enzyme-linked immunosorbent assays (ELISA) were performed on mouse serum 7 days after each dose, to detect any RBD-specific antibody response. Whole blood was spun down using Ser-gel tubes (NC9436363, Fisher Scientific). Serum was either used fresh or stored at -80°C until used. 96-well plates were coated with 200 ng/100 µl (2 µg/ml) of CoV2 RBD protein (Z03483, GenScript) overnight at 4°C. Then plates were pre-blocked with PBS+ 2% BSA for 2h at room temperature. Mouse serum was diluted 1:10 and then serially diluted (1:4 → 8 concentrations) in a dummy plate. Samples were transferred to the ELISA plate and incubated for 2h at room temperature. As positive control, two antibodies were used: Creative Diagnostics (CABT-CS035; clone 211184): mouse αRBD Mab and MyBioSource (MBS434247): mouse αRBD Mab. Plates were washed 4 times with washing buffer (BioLegend 4211601). As secondary detection Abs, the following were used at 1:2000 in PBS+ and incubated for 1h at room temperature (RT): In initial experiments, AffiniPure Rabbit Anti-Mouse IgG + IgM (H+L) HRP (315-035-048, Jackson ImmunoResearch) was used, but in subsequent experiments, AffiniPure Rabbit Anti-Mouse IgM (µ chain) HRP (315-035-049, Jackson ImmunoResearch) and AffiniPure Rabbit Anti-Mouse IgG (Fcγ) HRP (315-035-046, Jackson ImmunoResearch) were used. Plates were washed 4 times with washing buffer. The reaction was visualized by addition of substrate 3,3',5,5'-Tetramethylbenzidine (TMB) for 10min at RT and stopped by H_2SO_4 (1 N). The absorbance at 450 nm was measured by an ELISA plate reader.

Anti-His tag ELISA assays were performed to determine if some of the immune response generated upon vaccination with the RBD-His protein construct is directed against the His-tag rather than the Spike RBD itself. Plates were coated with irrelevant protein, which was His-tagged (PDL1-His). Sera were only tested at undiluted concentrations. As secondary antibody, Rabbit Anti-Mouse IgG (Fcγ) HRP (315-035-046, was used. As positive control, THE His Tag Antibody [HRP] (A00612, GenScript) was used. Otherwise, ELISAs were performed as described above.

Neutralizing Antibody Assays were performed using the SARS-CoV-2 Surrogate Virus Neutralization Test Kit (Cat# L00847) from GenScript. The horseradish peroxidase (HRP)-RBD was prepared by conjugated RBD 1 in 1000 with HRP dilution buffer. For preparation of a whole plate, 5994µl buffer + 6µl HRP-conjugated RBD was used. 55µl of diluted HRP-RBD was transferred to a fresh plate, referred to as the "serum incubation plate". Serially diluted sample sera were placed in a separate V-bottom plate, referred to as "serial dilution plate." To the first row, 20µl of undiluted serum was added. 8µl of undiluted serum was then transferred to the subsequent wells, which contain 24µl of PBS (1 in 4 serial dilution). Dilute sample sera (as well as positive and negative controls) were diluted 1 in 10 with sample dilution buffer. This was done by adding 54µl buffer to a fresh plate, referred to as "final serum dilution plate" and transferring 6µl of the serially diluted serum to that plate. 55 µL of finally diluted serum (and controls) were transferred to the serum incubation plate, which already contained 55 µl of HRP-conjugated RBD, which resulted in a 1:1 mix of RBD and serum, for a final dilution of 1 in 20, with subsequent 1:4 serial dilution.

The serum incubation plate was incubated at 37°C for 30 min. 100µL of the incubated serum-RBD mixture was transferred to the ACE2-precoated assay plates. The plates were covered with the

provided sealer and incubated at 37°C for 15 min. The plates were then washed 4 times with 200µl of 1x Wash Solution, which consisted of 20x Wash Buffer diluted with deionized water. 100µl of TMB Solution was added to each well and incubated at room temperature for 10 min. To quench the reaction, 50µl of Stop Solution was added to each well. Absorbance was read immediately at 450 nm.

ELI-spot analysis for IFNγ was performed on splenocytes 9 days after administration of the fourth dose. Splenocytes ($0.5x10^6$ cells/well) were activated with either 5 µg/well Peptides (Table 2 or Table 4) or 0.84 µg/well PepMix (Table 3). IFNy plates were stimulated overnight.

Table 4: Re-Stimulation Peptides (2nd Batch)

Re-stimulation Peptides	Sequence	Source	Lot #
CoV2 #1	VNFNFNGL (SEQ ID NO:49)	GenScript	U842NFE140-0/PE2815
CoV2 #2	KCYGVSPTKL (SEQ ID NO:50)	GenScript	U842NFE140-4/PE2818
CoV2 #3	CYGVSPTKL (SEQ ID NO:51)	GenScript	U842NFE140-7/PE2821
CoV2 #4	CYGVSATKL (SEQ ID NO:52)	GenScript	U842NFE140-10/PE2824
CoV2 #5	YGVSPTKL (SEQ ID NO:53)	GenScript	U842NFE140-13/PE2827
CoV-Db2	SKVGGNYNYL (SEQ ID NO:54)	GenScript	U842NFE140-16/PE4296
CoV-Db3	VIAWNSNNL (SEQ ID NO:55)	GenScript	U842NFE140-37/PE4338
CoV-Kb1	ESIVRFPNI (SEQ ID NO:56)	GenScript	U842NFE140-22/PE4323
CoV-Kb4	VVVLSFELL (SEQ ID NO:57)	GenScript	U842NFE140-25/PE4326
CoV-Kb5	GNYNYLYRL (SEQ ID NO:58)	GenScript	U842NFE140-28/PE4329
CoV-Kb6/CoV-Dd6	VGYQPYRVV (SEQ ID NO:59)	GenScript	U842NFE140-31/PE4332
CoV-Dd2	YNSASFSTF (SEQ ID NO:60)	GenScript	U842NFE140-34/PE4335
CoV-Dd3	IAPGQTGKI (SEQ ID NO:61)	GenScript	U842NFE140-19/PE4320

Pseudovirus Neutralization Assays were performed by GenScript according to their procedures and protocols (SC1993-8). All 60 mouse samples of post dose 4 serum were sent to GenScript on dry ice, along with 22 human samples.

In order to induce an immune response, either C57Bl6 or Balb/C mice were administered a pharmaceutical composition formulated for a vaccine including 10 µg of a coronavirus spike protein peptide (SEQ ID NO: 3) and 8 µg equivalent of either a soluble CpG or a CpG -amphiphile. The mice were administered a first dose on day 0 and a second dose on day 14. On day 21, the amount of serum IgG/IgM antibodies was measured using a serum ELISA assay (FIG. 1A-FIG. 1C and FIG. 6A-FIG. 6C). Another dose of the CpG-amphiphile and the coronavirus spike protein peptide was administered on day 28. The amount of IgG/IgM antibodies for the mice that were administered the soluble CpG or the CpG-amphiphile was measured after 35 days using a serum ELISA assay (FIG. 2A-FIG. 2C and FIG. 7A-FIG. 7C) in comparison to a control. Also, after 35 days and three doses a peripheral blood mononuclear cell cytokine assay was performed to identify the amount of neutralizing antibodies present which block the interaction between the coronavirus spike protein and the ACE2 receptor for C57Bl6 mice (FIG. 3A and FIG. 3B) and Balb/C mice (FIG. 8A and FIG. 8B) in comparison to the concentration of neutralizing antibodies in human convalescent serum, from a

patient having had a COVID-19 infection (FIG. 3C and FIG. 3D and FIG: 8C and FIG. 8D). Additionally, on day 35, the polyfunctional cytokine secreting T-cell response was measured for IFNγ, TNFa, and IL6 for C57Bl6 mice (FIG. 4A-FIG. 4C) and Balb/C mice (FIG. 9A-FIG. 9C). Also, on day 35 the C57Bl6 and Balb/C mice were evaluated for the amount IFNg present after receiving three doses of the coronavirus spike protein and the CpG (FIG. 5A and FIG. 5B). The concentration of TNFa, IFNg, IL-6, IL-2, and IL-4 present after 21 days and after receiving two doses is summarized in FIG. 10. ELISpot assays were performed on C57Bl6 and Balb/C mice after being administered four doses in order to assess the amount of splenocyte IFNγ, with the highest amount in those dosed with a CpG-amphiphile as shown in FIG. 11. The amount of IgG1 (FIG. 12A), IgG2bc (FIG. 12B), IgG3 (FIG. 12C), and the IgG2bc:IgG1 ratio (FIG 12D) for C57Bl6 mice administered three doses was analyzed to understand the amount of Th1 and Th2 response. The ratio of IgG2bc:IgG1 in FIG. 12D shows that for, mice administered the CpG-amphiphile, the immune response skews strongly to Th1 and not Th2. This is advantageous because a Th2 response can be detrimental for SARS-CoV-2.

To compare how the CpG-amphiphile compares to other adjuvants, the amount of IFNγ, TNFa, IL-2, and IL-6 produced by mice which were administered two doses (FIGS. 13A-FIG. 13D) or three doses (FIG. 14A-FIG. 14D) of 10 μg of a coronavirus spike protein and 8 μg of either CpG-amphiphile, soluble CpG, Alhydrogel, IFA, or Mock Tx in comparison to a positive or negative control. The results showed the CpG-amphiphile yielded a superior immune response relative to the other tested adjuvants (FIG.15).

Further, female, 6 to 8-week-old C57BL/6J and BALB/c mice purchased from Jackson Laboratory (Bar Harbor, ME) were injected with 1 nmol CpG (soluble CpG), 1 nmol lipid-conjugated CpG (AMP-CpG), or 100 μg Alum admixed with phosphate-buffered saline (PBS) only (adjuvant controls), or 1-10 μg of coronavirus spike protein (SEQ ID NO: 3) (Sino Biological, Cat: 40592-V08H or GenScript, Cat: Z03483). “Mock” groups received PBS alone. Injections (100 μL) were administered subcutaneously at the base of the tail (50 μL bilaterally) on days 0, 14, and 28 of the experiment. Blood samples were collected on days 7, 21, and 35. Mice were sacrificed on day 35 for lung harvest and collection of bronchoalveolar lavage (BAL) fluid. Only the set of mice (FIG. 16A-FIG. 16D) received a fourth dose on day 42 and samples were collected on day 49.

A pseudovirus neutralization assay was performed using the ACE2-HEK293 recombinant cell line (BPS Bioscience, Cat: 79951) or the control HEK293 cell line (ATCC) and the SARS-CoV2 Spike Pseudotyped Lentivirus (BPS Bioscience, Cat: 79942) containing the luciferase reporter gene and the SARS-CoV2 Spike envelope glycoproteins, thus specifically transducing ACE2-expressing cells. Mouse or human sera dilutions were performed in the Thaw Medium 1 (BPS Bioscience, Cat: 60187) in a 96-well white clear-bottom luminescence plate (Corning, Cat: 3610) and then pre-incubated with 10 μL of virus for 30 minutes at RT. ACE2-HEK293 or control HEK293 cells (40 μL), containing 10,000 cells, were then added to the wells and incubated at 37°C for 48 h. Control wells included ACE2-HEK293 cells or control HEK293 cells with the virus, but no sera, and provided the maximum transduction level and the background, respectively. Luciferase activity was detected by adding 70 μL of freshly prepared ONE-Step Luciferase reagent (BPS Bioscience, Cat: 60690) for 15 minutes at RT and luminescence was measured with a Synergy H1 Hybrid reader (BioTek). Pseudovirus

neutralization data for the experiment was performed by GenScript (Nanjing, China) following the same protocol, but using in-house ACE2-HEK293 cells and Spike RBD-HRP recombinant protein. Pseudovirus neutralization titers at the half-maximal inhibitory dilution ($pVNT_{50}$) were calculated as the serum dilution at which RLU were reduced by 50% compared to RLU in virus control wells for C57Bl/6J mice (FIG.16A) and BALB/c mice (FIG. 16C) that had been administered four doses of 10 µg of a coronavirus spike protein (SEQ ID NO: 3) in combination with 1 nmol soluble CpG or AMP-CpG compared to convalescent serum. The convalescent serum samples (n=7) and plasma samples (n=15) were obtained from patients who had recovered from SARS-CoV-2 infection (COVID-19) and were obtained from US Biolab (Rockville, MD) and ALLCELLS (Alameda, CA), respectively.

Additionally, the amount of IFNγ produced by either C57Bl/6J mice (FIG. 16C) or BALB/c mice (FIG. 16D) was analyzed by individually collecting the mice spleens in RPMI 1640 media supplemented with 10% FBS and penicillin, streptomycin, nonessential amino acids, sodium pyruvate, and beta-mercaptoethanol (complete media) then processing into single cell suspensions and passing through a 70 µm nylon filter. Cell pellets were re-suspended in 3 mL of ACK lysis buffer (Quality Biological, Inc., Cat: 118156101) for 5 min on ice; then PBS was added to stop the reaction. The samples were centrifuged at 400×g for 5 min at 4°C and cell pellets were re-suspended in complete media. ELISpot assays were performed using the Mouse IFN-γ ELISpot Set (BD, Cat: BD551083). 96-well ELISpot plates precoated with capture antibody overnight at 4°C were blocked with complete media for 2 h at RT. 500,000 mouse splenocytes were plated into each well and stimulated overnight with 1 µg/peptide per well of Spike-derived overlapping peptides. The spots were developed based on manufacturer's instructions. PMA (50 ng/mL) and ionomycin (1 µM) were used as positive controls, and complete medium only as the negative control. Spots were scanned and quantified by an ImmunoSpot CTL reader. Initial assessments in C57BL/6J and BALB/C mice receiving immunization containing AMP-CpG produced a 10- to 30-fold higher pseudovirus neutralizing titer than natural antibody responses present in human convalescent serum (obtained from recovered COVID-19 patients; FIG. 16A and FIG. 16C), indicating the potential for AMP-CpG to produce neutralizing antibody responses more potent than natural immunity. By comparison, animals immunized with a dose-matched regimen containing unmodified (soluble) CpG produced neutralizing titers comparable to those observed in human convalescing patients. The results of splenocyte ELISpot assays showed that compared with soluble CpG, mice immunized with the coronavirus spike protein admixed with AMP-CpG elicited approximately 4-fold greater frequencies of antigen-specific functional T cells, producing IFNγ upon restimulation with coronavirus spike protein derived overlapping peptides (FIG. 16B and FIG. 16D).

In the same manner, cytokine-producing cells in splenocytes and peripheral blood from C57BL/6J mice were determined. The number of IFNγ spot forming cells per $1x10^6$ splenocytes that were restimulated with overlapping coronavirus spike peptides were analyzed from C57BL/6J mice (n=10 per group) that received three doses of 10 µg of a coronavirus spike protein (SEQ ID NO: 3) in combination with 100 µg Alum, 1 nmol soluble CpG, or 1 nmol AMP-CpG (FIG. 17A). Mice immunized with the coronavirus spike protein in combination with AMP-CpG had substantially higher

IFNγ spot forming cells than mice dosed coronavirus spike protein in combination with soluble CpG, alum, or mock (PBS).

Example 2: Inducing a humoral immune response in mice

The humoral immune response induced in mice was determined for C57B1/6J mice that were administered three doses of 10 µg of a coronavirus spike protein (SEQ ID NO: 3) in combination with 100 µg Alum, 1 nmol soluble CpG, or 1 nmol AMP-CpG. The humoral response was evaluated in terms of neutralization titer in comparison to convalescence serum (FIG. 20A), IgM (FIG. 20B), IgG (FIG. 20C), IgG1 (FIG. 20D), IgG2bc (FIG. 20E), the ratio of IgG2bc to IgG19 (FIG. 20F), and IgG3 (FIG. 20G) using either a pseudovirus neutralization assay or ELISA assay.

Neutralizing antibody responses to the coronavirus spike protein were measured through the inhibition of the coronavirus spike protein-ACE2 interaction in an ELISA-based surrogate assay. Results for serum collected on day 35 for cohorts of immunized C57BL/6J mice are shown in FIG. 20A. Comparable levels of neutralizing activity were induced in animals immunized with AMP-CpG, soluble CpG, and alum. Comparison with samples obtained from a cohort of convalescent humans showed that the vaccine-induced responses were significantly higher than those generated through response to natural infection.

Seven days after the initial immunization, all cohorts, except the control receiving mock immunization, showed robust coronavirus spike protein specific IgM responses (FIG. 20B); which underwent isotype switching to produce IgG responses with similar titer following subsequent boosting immunizations (FIG. 20C).

To assess Th1/Th2-bias in the coronavirus spike protein specific IgG response elicited through immunization, the IgG subclasses present were evaluated and showed that mice immunized with AMP-CpG or soluble CpG had significantly lower Th2 associated IgG1 titers (approximately 10-fold) than mice immunized with alum (FIG. 20D). The reverse was true for Th1 associated IgG2bc: titers were significantly higher (approximately 50-fold) for mice immunized with AMP-CpG or soluble CpG (FIG. 20E). The ratio of IgG2bc:IgG1 titer indicated a strong bias towards Th1 for AMP-CpG immunized animals, while soluble CpG and alum produced a balanced Th1/Th2 profile or Th2-dominant response, respectively (FIG. 20F). Further analysis showed AMP-CpG immunized animals produced significantly higher IgG3 titers than either soluble CpG (approximately 3-fold) or alum (>800-fold) treatment groups, which is consistent with the observed Th1-bias resulting from AMP-CpG immunization (FIG. 20G).

Additionally, the humoral response was assessed in serum for neutralization titer in comparison to convalescent serum (FIG. 22A), IgM (FIG. 22B), IgG (FIG. 22C), IgG1 (FIG. 22D), IgG2bc (FIG. 22E), the ratio of IgG2bc to IgG19 (FIG. 22F), and IgG3 (FIG. 22G) using either a pseudovirus neutralization assay or ELISA assay for C57B1/6J mice that were administered three doses of only 10 µg of a coronavirus spike protein (SEQ ID NO: 3) in combination with only 100 µg Alum, only 1 nmol soluble CpG, only 1 nmol AMP-CpG, 100 µg Alum and 10 µg of a coronavirus spike protein (SEQ ID NO: 3), 1 nmol soluble CpG and 10 µg of a coronavirus spike protein (SEQ ID

NO: 3), 1 nmol AMP-CpG and 10 µg of a coronavirus spike protein (SEQ ID NO: 3), 1 nmol AMP-CpG and 5 µg of a coronavirus spike protein (SEQ ID NO: 3), and 1 nmol AMP-CpG and 1 µg of a coronavirus spike protein (SEQ ID NO: 3). On day 35 following repeat dose immunization, the induction of coronavirus-specific antibody responses among the AMP-CpG immunized animals at each specified coronavirus spike protein dose level was assessed and compared to responses generated by immunization with either soluble CpG or alum at the 10 µg dose. Neutralizing activity was assessed through measurement of pseudovirus neutralization titers at the half-maximal inhibitory dilution ($pVNT_{50}$) as described in Example 1. Similar levels of pseudovirus neutralization titers were observed for all treatment groups, at levels that were 265, 230, or 94-fold greater than those observed in convalescent human samples, for AMP-CpG, soluble CpG, and alum immunized mice, respectively (FIG. 22A). These levels were maintained in animals immunized with AMP-CpG at lower coronavirus spike protein dose levels with mean $pVNT_{50}$ at least 115-fold greater than those measured in recovering COVID-19 patients (FIG. 22B).

Total IgG titers were similar among the groups administered AMP-CpG, and these were reduced approximately 2-fold in comparison to titers measured among groups dosed with either soluble CpG or alum adjuvanted vaccines (FIG. 22C). Isotype analysis demonstrated similar trends to those initially observed in comparison at the 10 µg dose level (FIG. 22C). Alum and soluble CpG immunization produced significantly higher Th2-associated IgG1 titers (approximately 100-fold) compared to all coronavirus spike protein dose levels admixed with AMP-CpG (FIG. 22D). Th1-associated IgG2bc levels were elevated approximately 20-fold in all AMP-CpG immunized animals compared with soluble CpG and alum immunized groups, with no significant difference observed with reduced coronavirus spike protein dose level. These trends were further evident in the comparison of IgG2bc:IgG1 titer ratio (FIG. 22F), where AMP-CpG containing regimens induced highly Th1-dominant isotype profile (IgG2bc:IgG1 >10) , compared with more balanced and Th2-skewed responses in soluble CpG (IgG2bc:IgG1 approximately 2) and alum (IgG2bc:IgG1 <1) vaccinated animals respectively. Finally, only animals immunized with AMP-CpG showed evidence of coronavirus spike protein specific IgG3 titers, with comparable levels detected among all coronavirus spike protein dose levels (approximately 500-fold over background). Further analysis showed AMP-CpG immunized animals produced significantly higher IgG3 titers than either soluble CpG (approximately 40-fold) or alum (>20-fold) treatment groups consistent with the observed Th1-bias resulting from AMP-CpG immunization (FIG 22G). Together these data support AMP-CpG - enabling at least 10-fold dose sparing of coronavirus spike protein antigen for induction of neutralizing, high titer, and optimal Th1 profile antibody responses against coronavirus spike protein. While soluble CpG and alum induced marginally higher total IgG responses, these did not result in significant differences in neutralizing activity compared to AMP-CpG immunization. Alum and, to a lesser degree, soluble CpG responses were dominated by the Th2-associated IgG1 isotype raising the potential for a risk of toxicity in human translation based on prior outcomes in SARS and MERS vaccine development.

Example 3: Inducing a cellular immune response in mice

Intracellular cytokine staining experiments were performed to assess the cellular immune response in mice administered a coronavirus spike protein in combination with an adjuvant, including alum, soluble CpG, and AMP-CpG. Peripheral blood cells that were collected 7 days after each booster dose and lung-resident leukocytes that were collected after the final booster dose were stimulated overnight with 1 µg of overlapping coronavirus spike peptide per well at 37°C, 5% CO_2 in the presence of brefeldin A (Invitrogen, Cat: 00-4506-15) and monensin (BioLegend, Cat: 420701) as described in Example 1. Cells were stained with the following antibodies: PE anti-mouse IFNγ (BD, Cat: 554412), FITC anti-mouse TNFα (BD, Cat: 554418), APC-Cy™7 anti-mouse CD3 (BD, Cat: 560590), PE-Cy7 anti-mouse $CD4^+$ (Invitrogen, Cat: 25-0041-82), and APC anti-mouse CD8a (eBioscience, Cat: 17-0081-83). PMA (50 ng/mL) and ionomycin (1 µM) were used as positive controls, and complete medium only as the negative control. Cells were permeabilized and fixed (Invitrogen, Cat: 00-5523-00). A LIVE/DEAD fixable (aqua) dead cell stain kit (Invitrogen, Cat: L34966) was used to evaluate viability of the cells during flow cytometry. Sample acquisition was performed on FACSCanto II (BD) and data analyzed with FlowJo V10 software (TreeStar).

The frequency of both IFNγ and TNFα (double-positive T-cells), only TNFα, and only IFNγ, in $CD8^+$ T cells (FIG. 17B) or $CD4^+$ T cells (FIG. 17C) were analyzed in peripheral blood cells from C57BL/6J mice that were administered three doses of 10 µg of a coronavirus spike protein (SEQ ID NO: 3) in combination with 100 µg Alum, 1 nmol soluble CpG, or 1 nmol AMP-CpG. Approximately 43% of $CD8^+$ T cells derived from peripheral blood in AMP-CpG immunized mice were cytokine producing (IFNγ, TNFα, or double-positive T cells); in comparison, approximately 13% and <2% of $CD8^+$ T cells were cytokine-producing for soluble CpG-immunized mice and alum-immunized mice, respectively (FIG. 17B). A similar trend was observed for $CD4^+$ T cells, though percentages were relatively smaller: approximately 1.5% of T cells in peripheral blood from AMP-CpG immunized mice were cytokine-producing compared with <1% in CpG-immunized mice and <0.5% for alum-immunized mice and mock-immunized mice (FIG. 17C).

Likewise, to determine whether immunization could induce tissue resident T cell responses at a site of likely SARS-CoV-2 exposure the frequency of IFNγ and TNFα, only TNFα, and only IFNγ found in $CD8^+$ T cells (FIG. 18A) or $CD4^+$ T cells (FIG. 18B) perfuse lung tissue that was restimulated with overlapping coronavirus spike peptides in C57BL/6J mice that were administered three doses of 10 µg of a coronavirus spike protein (SEQ ID NO: 3) in combination with 100 µg Alum, 1 nmol soluble CpG, or 1 nmol AMP-CpG was analyzed. T cells in the lung tissue had a greater proportion of the cytokine-producing cells than observed in peripheral blood. Observations in AMP-CpG immunized mice showed that approximately 73% of $CD8^+$ T cells from perfused lung tissues were cytokine-producing, with approximately 40% exhibited polyfunctional secretion of both Th1 cytokines IFNγ and TNFα. By comparison, immunization with soluble CpG or alum induced >5-fold and >25-fold lower responses, respectively. Similar assessment of $CD4^+$ T cells showed that only AMP-CpG immunized animals generated responses above background, with approximately 6% of $CD4^+$ T cells producing IFNγ and/or TNFα, again exhibiting strong polyfunctional effector functionality, with the majority of

these cells able to produce both IFNγ and TNFα upon antigen restimulation. These results support that the more potent lymph node action of AMP-CpG induces enhanced expansion of antigen-specific T cells with potentially beneficial tissue homing properties, establishing protective tissue resident cells at a primary site of initial viral exposure.

To more comprehensively understand the Th1/Th2/Th17 profile of the elicited T cell responses, a multiplexed cytokine assay was used to assess various cytokine concentrations from supernatants of cells collected from perfused lungs following restimulation with coronavirus spike overlapping peptides. Specifically, cytometric bead array flow cytometry was performed to determine cytokine production, including IFNγ (FIG. 18C), TFNα, IL-6, IL-4, IL-10, and IL17 (FIG. 18D), for C57BL/6J mice that were administered three doses of 10 µg of a coronavirus spike protein (SEQ ID NO: 3) in combination with 100 µg Alum, 1 nmol soluble CpG, or 1 nmol AMP-CpG. Lung-resident leukocytes (collected after the final booster dose) were activated overnight with overlapping coronavirus spike peptides at 1 µg/peptide per well (consisting of 315 peptides, derived from a peptide scan resulting in 15-mers with 11 amino acid overlap) (JPT, Cat: PM-WCPV-5 or GenScript, Cat: RP30020). Phorbol Myristate Acetate (PMA, 50 ng/mL) and ionomycin (1 µM) were used as positive controls, and complete medium only as the negative control. Culture supernatants were harvested and Th1/Th2 cytokine production was measured (CBA Mouse Th1/Th2/Th17 Cytokine Kit: BD, Cat: BDB560485). Briefly, bead populations with distinct fluorescence intensities that are coated with capture antibodies specific for various cytokines including IFNγ, TNFα, IL-4, IL-6, IL-10, and IL-17 were incubated with culture supernatants. The different cytokines in the sample were captured by their corresponding beads. The cytokine-captured beads were then mixed with phycoerythrin (PE)-conjugated detection antibodies. Following incubation, samples were washed, and fluorescent intensity of PE on the beads were measured and analyzed by flow cytometry (BD FACSCanto II). Mean fluorescent intensities (MFI) were calculated using FACSDiva software (BD) and protein concentrations were extrapolated using Microsoft Excel. AMP-CpG immunized mice exhibited a Th1 effector profile consistent with prior assessment by flow cytometry with IFNγ and TNFα concentrations that were significantly higher than cohorts immunized with the other adjuvants such as soluble CpG and alum or mock. The IFNγ concentration was at least 200-fold higher than observed with the other adjuvants or mock, and the TNFα concentration was at least 7-fold higher than the other adjuvants or mock (FIG. 18C). Concentrations of common Th2 or Th17 associated cytokines IL-4, IL-6, IL-10, and IL-17 were undetectable for all cohorts (FIG. 18D). These results further demonstrate the greatly enhanced potency and Th1-bias in T cells elicited through immunization with AMP-CpG immunization compared with either soluble CpG or alum.

To further evaluate whether lung -resident T cell responses induced by immunization could localize into lung secretions, bronchoalveolar lavage (BAL) fluid was collected from C57BL/6J mice that were administered three doses of 10 µg of a coronavirus spike protein (SEQ ID NO: 3) in combination with 100 µg Alum, 1 nmol soluble CpG, or 1 nmol AMP-CpG. $CD8^+$ (FIG. 19A) and $CD4^+$ (FIG. 19D) T cell count, along with the percentage of naïve $CD8^+$ (FIG. 19B) and naïve $CD4^+$ (FIG. 19E) T-cells, and the percent of effector memory $CD8^+$ (FIG. 19C) and $CD4^+$ (FIG. 19F) T-cells were determined. Significantly more $CD8^+$ T cells were found in BAL fluid of AMP-CpG immunized mice

than other treatment groups (FIG. 19A). In addition, a significantly lower proportion of cells detected in the BAL collected from AMP-CpG immunized animals exhibited a naïve phenotype ($CD44^-$, $CD62L^+$; FIG. 19B) with a corresponding increase in the frequency of effector memory phenotype (T_{EM}; $CD44^+$, CD62L-; FIG. 19C). The $CD4^+$ T cell count was enhanced relative to mock treatment and generally similar across all treatment groups (FIG. 19D), but the AMP-CpG cohort showed evidence that a significantly greater proportion of the BAL-resident $CD4^+$ T cells had differentiated from naïve to T_{EM} phenotype than in the other treatment groups (FIG. 19F). The improved numbers and phenotype of BAL-resident T cells present in AMP-CpG immunized animals demonstrate a greater potential for early immunological detection and control at the point of viral exposure.

The T cell responses on day 35 in spleen, peripheral blood, and lung tissues were evaluated. The results showed that the number of IFNγ-producing cells in splenocytes collected from AMP-CpG immunized C57BL/6J mice tended to increase with antigen concentration, but, even at the lowest antigen dose admixed with AMP-CpG, the number of IFNγ-producing cells was significantly higher than observed in cohorts that received the highest antigen dose (10 µg) with either soluble CpG (approximately 4-fold) or alum (>30-fold) (FIG. 21A). Additionally, the frequency of cytokines, including IFNγ and TNFα, only TNFα, and only IFNγ, from $CD8^+$ T-cells (FIG. 21B) and $CD4^+$ T-cells (FIG. 21C) found in peripheral blood cells collected from C57BL/6J mice that were administered three doses of (from left to right) only 100 µg Alum, only 1 nmol soluble CpG, only 1 nmol AMP-CpG, 100 µg Alum and 10 µg of a coronavirus spike protein (SEQ ID NO: 3), 1 nmol soluble CpG and 10 µg of a coronavirus spike protein (SEQ ID NO: 3), 1 nmol AMP-CpG and 10 µg of a coronavirus spike protein (SEQ ID NO: 3), 1 nmol AMP-CpG and 5 µg of a coronavirus spike protein (SEQ ID NO: 3), and 1 nmol AMP-CpG and 1 µg of a coronavirus spike protein (SEQ ID NO: 3) was determined. Likewise, the frequency of IFNγ and TNFα, only TNFα, and only IFNγ, of $CD8^+$ T-cells (FIG. 21D) and $CD4^+$ T-cells (FIG. 21E) found in perfused lung tissue cells, restimulated with overlapping coronavirus spike peptides, collected from C57BL/6J mice that were administered three doses of (from left to right) only 100 µg Alum, only 1 nmol soluble CpG, only 1 nmol AMP-CpG, 100 µg Alum and 10 µg of a coronavirus spike protein (SEQ ID NO: 3), 1 nmol soluble CpG and 10 µg of a coronavirus spike protein (SEQ ID NO: 3), 1 nmol AMP-CpG and 10 µg of a coronavirus spike protein (SEQ ID NO: 3), 1 nmol AMP-CpG and 5 µg of a coronavirus spike protein (SEQ ID NO: 3), and 1 nmol AMP-CpG and 1 µg of a coronavirus spike protein (SEQ ID NO: 3) were determined. In both peripheral blood (FIG. 21B and FIG. 21C) and lung tissue (FIG. 21D and FIG. 21E), the percent of $CD8^+$ and $CD4^+$ T cells producing cytokine was significantly higher for AMP-CpG treated mice at any concentration of antigen compared with the other adjuvants tested. Notably, no significant decrease in the frequency of cytokine-producing $CD8^+$ or $CD4^+$ T cells was observed in the peripheral blood of animals immunized with AMP-CpG admixed with antigen at 10, 5, or 1 µg dose levels as these were maintained at approximately 40-50% of $CD8^+$ and 2-4% of $CD4^+$ T cells. While a decreasing trend was observed in the frequency of lung-resident cytokine-producing $CD8^+$ T cells in AMP-CpG immunized animals, even the 1 µg dose level produced frequencies >3-fold or >18-fold higher than animals immunized with soluble CpG or alum, respectively. This supports AMP-CpG enabling at least 10-fold dose sparing of the coronavirus spike protein.

Two-dose vaccination with AMP-CpG-7909 elicits potent Spike RBD-specific cellular immunity in blood and lung, and humoral immunity in blood. C57Bl/6 mice (n = 5 per group) were immunized on day 0 and 14 with 0.5, 1.0, or 5.0 ug Spike RBD protein admixed with 1.0, 2.5, or 5.0 nmol AMP-CpG, and T cell and IgG responses analyzed on day 21. Peripheral blood cells (FIG. 26A and FIG. 26B) or cells collected from perfused lungs (FIG. 26C and FIG. 26D) were restimulated with overlapping Spike RBD peptides and assayed by flow cytometry for intracellular cytokine production to detect antigen-specific T cell responses. Shown are frequencies of IFNγ, TNFα, and double-positive T cells among $CD8^+$ (FIG. 26A and FIG. 26C) and $CD4^+$ (FIG. 26B and FIG. 26D) T cells. Humoral responses specific to Spike RBD were assessed in serum from immunized animals by ELISA. Shown are endpoint titers for IgG on day 35 (FIG. 26E; n = 5 mice per group). Values depicted are mean ± standard deviation.

These results show that a two-dose regimen with AMP-CpG induces potent polyfunctional CD8 and CD4 T cell responses in blood and in the lungs.

Example 4: Inducing an immune response in aged mice

T cell responses in 37 week old C57BL/6J aged mice that were administered three doses of only 100 µg Alum, only 1 nmol soluble CpG, only 1 nmol AMP-CpG, 100 µg Alum and 10 µg of a coronavirus spike protein (SEQ ID NO: 3), 1 nmol soluble CpG and 10 µg of a coronavirus spike protein (SEQ ID NO: 3), 1 nmol AMP-CpG and 10 µg of a coronavirus spike protein (SEQ ID NO: 3), 1 nmol AMP-CpG and 5 µg of a coronavirus spike protein (SEQ ID NO: 3), or 1 nmol AMP-CpG and 1 µg of a coronavirus spike protein (SEQ ID NO: 3) mice were evaluated on days 21 and 35. Assessment on day 21 of cytokine-producing $CD8^+$ T cells in peripheral blood following Spike-derived overlapping peptide restimulation showed that AMP-CpG induced potent responses (approximately 15% of $CD8^+$ T cells), greatly outperforming soluble CpG (approximately 2.5% of $CD8^+$ T cells), and alum (<0.5% of $CD8^+$ T cells) (FIG. 23A). Although these responses were reduced approximately 2-fold compared to those observed in young healthy mice, they nonetheless exceeded those generated by the soluble CpG and alum comparators by 7- and 30-fold, respectively. AMP-CpG immunization further enabled comparable responses at 10 µg and 5 µg coronavirus spike protein doses, and although responses at 1 µg were decreased, these still exceeded the response observed for alum (20-fold) and were similar to those generated through immunization with soluble CpG at the 10 µg dose level (FIG. 23B).

Analysis on day 35 of $CD8^+$ and $CD4^+$ T cells in lung tissue of aged mice showed a similar trend, with AMP-CpG immunized animals producing high frequencies of cytokine producing $CD8^+$ and $CD4^+$ T cells. Specifically, AMP-CpG immunization elicited Th1 cytokine production in approximately 60% of lung resident $CD8^+$ T cells, approximately 7-fold and >120-fold higher than soluble CpG and alum immunization (FIG. 23C). As observed in prior studies, the elicited T cells were highly polyfunctional with more than half of the induced cells exhibiting simultaneous production of IFNγ and TNFα. Unlike the responses in peripheral blood, lung-resident cytokine producing $CD8^+$ T cell frequencies did not decline in aged mice following AMP-CpG immunization relative to responses in young healthy animals and were maintained at statistically comparable levels in the 5 µg and 1 µg

coronavirus spike protein dosed groups (FIG. 23D). Lung resident $CD4^+$ T cell responses exhibited a similar pattern with AMP-CpG inducing higher frequencies of Th1 cytokine producing cells (approximately 10% of $CD4^+$ T cells) compared to soluble CpG (approximately 0.6% of $CD4^+$ T cells) and alum (<0.5% of $CD4^+$ T cells) (FIG. 23E). Again, these response levels were comparable to those observed in young healthy mice showing that AMP-CpG immunization can raise comparable lung-resident T cell responses in young and aged mice. Finally, the lung-resident $CD4^+$ T cell responses were maintained at comparable levels among all coronavirus spike protein dose levels tested, and AMP-CpG immunization at the lowest concentration of coronavirus spike protein (1 μg) outperformed both soluble CpG and alum at a 10-fold higher antigen dose (10 μg) (FIG. 23F).

Coronavirus spike protein-specific antibody responses were evaluated on day 35 after repeat dose immunization with comparator vaccines in aged mice. Pseudovirus neutralization showed that AMP-CpG immunization at the 10 μg antigen dose level elicited enhanced neutralizing titers, at least 5-fold greater than those observed for soluble CpG and alum comparators, and >50-fold greater than observed in human convalescent sera/plasma (FIG. 24A). Reduced doses of coronavirus spike protein with AMP-CpG gave lower neutralizing titers which were comparable to soluble CpG and alum (FIG. 24B). Of particular interest was the equivalency of titers from animals immunized with 10 μg coronavirus spike protein with soluble CpG or alum relative to those receiving the lower 1 μg coronavirus spike protein dose with AMP-CpG. Assessment of total IgG showed AMP-CpG and alum produced comparable coronavirus spike protein-specific IgG titers, both in excess of that generated in soluble CpG immunized animals (FIG. 24C). Although a significant decline was observed in IgG titer with decreasing coronavirus spike protein dose in AMP-CpG immunized animals, there was no statistical difference between AMP-CpG and alum given with 10 μg coronavirus spike protein. Isotype analysis yielded similar observations to those made in young healthy mice, with AMP-CpG driving more Th1, IgG2bc-dominant responses compared with soluble CpG or alum, which yielded more balanced or Th1, IgG1-biased profiles (FIG. 24D- FIG. 24G). No significant difference was observed among AMP-CpG immunized animals at the varying dose levels of coronavirus spike protein (FIG. 24F), although the strength of Th1-bias observed for AMP-CpG immunized mice was reduced in aged mice relative to young healthy mice. As previously observed in young healthy mice, IgG3 titers were enhanced in AMP-CpG immunized animals compared with soluble CpG or alum (FIG. 24G). Together, these results support AMP-CpG being able to elicit potent and functional coronavirus spike protein-specific humoral immunity in aged mice beyond what was observed for soluble CpG or alum vaccine comparators while producing an optimal Th1-biased isotype profile and enabling at least 10-fold dose sparing of antigen.

Vaccination with AMP-CpG in aged mice enables durable Spike RBD-specific T cells in blood, spleen, and lung tissue. 37 week old C57Bl/6 mice (n = 5-10 per group) were immunized on day 0, 14, and 28 with 10 ug Spike RBD protein admixed with 100 ug Alum or 1 nmol soluble-, or AMP-CpG. Adjuvant control animals were dosed with AMP-CpG adjuvant alone. Humoral responses specific to Spike RBD were assessed in serum from immunized animals by ELISA on day 35, 49, and 70. Shown are endpoint titers determined for IgG (FIG. 25A). T cell responses were analyzed on day 21, 35, 49, and 70 . Cells were collected from peripheral blood on day 21, 35, 49, and 70 (FIG. 25B) and

were restimulated with overlapping Spike RBD peptides and assayed for intracellular cytokine production to detect antigen-specific T cell responses. Shown are frequencies of IFNγ-positive cells among peripheral blood $CD8^+$ T cells (FIG. 25A), and cells were collected from spleen (FIG. 25C) and lungs (FIG. 25D) and were restimulated with overlapping Spike RBD peptides and assayed for IFNγ production by ELISPOT assay. Shown are the frequency of IFNγ spot forming cells (SFC) per $1x10^6$ cells (n = 5 mice per group). Values depicted are mean ± standard deviation. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$ by two-sided Mann-Whitney test applied to cytokine+ T cell frequencies.

These results show that, in aged mice, AMP-CpG induces high frequency T cell responses that persist for months after dosing.

Example 5: Inducing an immune response using a full-length spike protein antigen and a nucleocapsid protein antigen

The SARS-CoV-2 spike protein has a molecular weight of approximately 138 kDa and the SARS-CoV-2 nucleocapsid protein has a molecular weight of approximately 50 kDa. Based on these sizes, both the spike protein and the nucleocapsid protein are predicted to be suitable for lymph node targeting.

The following nucleocapsid protein construct was used to generate the data shown in FIG. 27 – FIG. 33:

MSDNGPQNQRNAPRITFGGPSDSTGSNQNGERSGARSKQRRPQGLPNNTASWFTALTQHGKEDL
KFPRGQGVPINTNSSPDDQIGYYRRATRRIRGGDGKMKDLSPRWYFYYLGTGPEAGLPYGANKDGII
WVATEGALNTPKDHIGTRNPANNAAIVLQLPQGTTLPKGFYAEGSRGGSQASSRSSSRSRNSSRNS
TPGSSRGTSPARMAGNGGDAALALLLLDRLNQLESKMSGKGQQQQGQTVTKKSAAEASKKPRQKR
TATKAYNVTQAFGRRGPEQTQGNFGDQELIRQGTDYKHWPQIAQFAPSASAFFGMSRIGMEVTPSG
TWLTYTGAIKLDDKDPNFKDQVILLNKHIDAYKTFPPTEPKKDKKKKADETQALPQRQKKQQTVTLLP
AADLDDFSKQLQQSMSSADSTQAENLYFQGHHHHHH (SEQ ID NO:63).

This protein is available from ACROBiosystems under product number NUN-C5227. It includes a cleavage site for a tobacco etch virus (TEV) protease (ENLYFQG; SEQ ID NO:64) between the nucleocapsid protein sequence and the six-histidine tag (HHHHHH; SEQ ID NO:65).

The following full-length spike protein construct was used to generate the data shown in FIG. 27- FIG. 33:

VNLTTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHSTQDLFLPFFSNVTWFHAIHVSGTNGTKRFDNP
VLPFNDGVYFASTEKSNIIRGWIFGTTLDSKTQSLLIVNNATNVVIKVCEFQFCNDPFLGVYYHKNNKS
WMESEFRVYSSANNCTFEYVSQPFLMDLEGKQGNFKNLREFVFKNIDGYFKIYSKHTPINLVRDLPQ
GFSALEPLVDLPIGINITRFQTLLALHRSYLTPGDSSSGWTAGAAAYYVGYLQPRTFLLKYNENGTITD
AVDCALDPLSETKCTLKSFTVEKGIYQTSNFRVQPTESIVRFPNITNLCPFGEVFNATRFASVYAWNR
KRISNCVADYSVLYNSASFSTFKCYGVSPTKLNDLCFTNVYADSFVIRGDEVRQIAPGQTGKIADYNY
KLPDDFTGCVIAWNSNNLDSKVGGNYNYLYRLFRKSNLKPFERDISTEIYQAGSTPCNGVEGFNCYF
PLQSYGFQPTNGVGYQPYRVVVLSFELLHAPATVCGPKKSTNLVKNKCVNFNFNGLTGTGVLTESNK
KFLPFQQFGRDIADTTDAVRDPQTLEILDITPCSFGGVSVITPGTNTSNQVAVLYQDVNCTEVPVAIHA
DQLTPTWRVYSTGSNVFQTRAGCLIGAEHVNNSYECDIPIGAGICASYQTQTNSPRAAASVASQSIIA
YTMSLGAENSVAYSNNSIAIPTNFTISVTTEILPVSMTKTSVDCTMYICGDSTECSNLLLQYGSFCTQLN

RALTGIAVEQDKNTQEVFAQVKQIYKTPPIKDFGGFNFSQILPDPSKPSKRSFIEDLLFNKVTLADAGFI
KQYGDCLGDIAARDLICAQKFNGLTVLPPLLTDEMIAQYTSALLAGTITSGWTFGAGAALQIPFAMQMA
YRFNGIGVTQNVLYENQKLIANQFNSAIGKIQDSLSSTASALGKLQDVVNQNAQALNTLVKQLSSNFG
AISSVLNDILSRLDKVEAEVQIDRLITGRLQSLQTYVTQQLIRAAEIRASANLAATKMSECVLGQSKRVD
FCGKGYHLMSFPQSAPHGVVFLHVTYVPAQEKNFTTAPAICHDGKAHFPREGVFVSNGTHWFVTQR
NFYEPQIITTDNTFVSGNCDVVIGIVNNTVYDPLQPELDSFKEELDKYFKNHTSPDVDLGDISGINASVV
NIQKEIDRLNEVAKNLNESLIDLQELGKYEQYIKWPGGGSGGGSHHHHHHHHHH (SEQ ID NO:66).
This protein is available from ACROBiosystems under product number SPN-C52H2. A ten-histidine tag (HHHHHHHHHH; SEQ ID NO:67) is linked to the spike protein sequence with a GGGSGGGS (SEQ ID NO:62) linker. The spike protein has the following mutations to stabilize the trimer: R683A, R685A.

As shown in FIG. 27, AMP-CpG induces a potent polyfunctional CD8 T cell response targeting SARS CoV-2 spike protein. A mock vaccine, or a vaccine containing 10 μg coronavirus spike protein, 10 μg coronavirus nucleocapsid protein and (1) 100 μg alum, (2) 6 μg soluble CpG, or (3) 6 μg AMP-CpG was administered. The percent cytokine positive cells observed were: mock (0%), alum (0%), soluble CpG (5%), and AMP-CpG (34%).

As shown in FIG. 28, AMP-CpG also induces a potent polyfunctional CD4 T cell response targeting SARS CoV-2 spike protein. A mock vaccine, or a vaccine containing 10 μg coronavirus spike protein, 10 μg coronavirus nucleocapsid protein and (1) 100 μg alum, (2) 6 μg soluble CpG, or (3) 6 μg AMP-CpG was administered. The percent cytokine positive cells observed were: mock (0.2%), alum (0.5%), soluble CpG (0.5%), and AMP-CpG (12%).

Restimulating mice (C57BL/6J mice; n=10 per group) that had received 10 μg of a full-length coronavirus spike protein construct (SEQ ID NO: 66) in combination with 10 μg of a coronavirus nucleocapsid protein construct (SEQ ID NO:63) and (1) 100 μg alum, (2) 6 μg soluble CpG, or (3) 6 μg AMP-CpG with overlapping coronavirus spike peptides resulted in a potent T cell response against SARS CoV-2 spike protein (FIG. 29).

AMP-CpG induces a potent lung-resident polyfunctional $CD8^+$ T cell response targeting SARS CoV-2 spike protein (FIG. 30). A mock vaccine, or a vaccine containing 10 μg coronavirus spike protein, 10 μg coronavirus nucleocapsid protein and (1) 100 μg alum, (2) 6 μg soluble CpG, or (3) 6 μg AMP-CpG was administered. The percent cytokine positive cells observed were: mock (0%), alum (0%), soluble CpG (3%), and AMP-CpG (26%).

AMP-CpG also induces a potent lung-resident polyfunctional $CD4^+$ T cell response targeting SARS CoV-2 spike protein (FIG. 31). A mock vaccine, or a vaccine containing 10 μg coronavirus spike protein, 10 μg coronavirus nucleocapsid protein and (1) 100 μg alum, (2) 6 μg soluble CpG, or (3) 6 μg AMP-CpG was administered. The percent cytokine positive cells observed were: mock (0.2%), alum (0.2%), soluble CpG (1%), and AMP-CpG (7%).

AMP-CpG induces a potent peripheral blood polyfunctional $CD8^+$ and $CD4^+$ T cell response targeting SARS CoV-2 nucleocapsid protein (FIG. 32). A mock vaccine, or a vaccine containing 10 μg coronavirus spike protein, 10 μg coronavirus nucleocapsid protein and (1) 100 μg alum, (2) 6 μg soluble CpG, or (3) 6 μg AMP-CpG was administered.

Restimulating mice (C57BL/6J mice; n=10 per group) that had received 10 µg of a full-length coronavirus spike protein construct (SEQ ID NO: 66) in combination with 10 µg of a coronavirus nucleocapsid protein construct (SEQ ID NO:63) and (1) 100 µg alum, (2) 6 µg soluble CpG, or (3) 6 µg AMP-CpG with overlapping coronavirus nucleocapsid peptides induced a potent T cell response targeting SARS CoV-2 nucleocapsid protein (FIG. 33).

Example 6: Inducing an immune response in non-human primates

A study was initiated in non-human primates (NHP) to test spike RBD and AMP-CpG in a vaccine. Use of RBD + Alum in the vaccine was compared to RBD + AMP-CpG. An initial dose of 500 µg of AMP-CpG was tested in a two-dose schedule (week 0 and week 4) immunized subcutaneously. Assessments included weekly clinical examination post each dose, CBC (complete blood count) panel, and collection of blood and sera for immunogenicity. In these tests, AMP-CpG did not induce an antibody or T-cell response to BioE spike RBD. As no response was seen to AMP-CpG after 2 doses, the same animals were immunized with a new vaccine formulation. Here 3,000 µg of AMP-CpG were used and 140 µg Genscript RBD were used. (A different lot and higher concentration of AMP-CpG and a new source and higher concentration of RBD.) The comparison group remained the same (1.5 mg Alum + 70 µg BioE RBD).

The reformulated AMP-CpG vaccine induced a robust antibody response to Genscript RBD (FIG. 34.) The reformulated AMP-CpG vaccine also induces IgG antibodies to the UK SARS-CoV-2 variant having the N501Y mutation (SEQ ID NO:69) (FIG. 35). Further, the reformulated AMP-CPG vaccine induces $CD8^+$ T-cell responses to spike RBD (FIG. 36A and FIG. 36B), and $CD4^+$ and $CD8^+$ T-cell responses to spike RBD (FIG. 37A and FIG. 37B).

No adverse safety signals (temperature, reactogenicity, chemistry, and hematology) were observed for the reformulated RBD and AMP-CpG vaccine.

Example 7: Inducing an immune response to B.1.351 variant

The B.1.351, or South Africa, variant of COVID is a strain of SARS-CoV2. A study was initiated in mice to determine the immunogenicity of the spike RBD and AMP-CpG in a vaccine if the antigen is changed to the B.1.351 RBD variant or used in conjunction with the WT RBD antigen. The cross-reactivity of the immune response towards the different variants was also determined for the reformulated AMP-CPG dual RBG and B.1.351 vaccine and the reformulated AMP-CPG B.1.351 vaccine.

Control, WT RBG, B.1.351 RGB, and dual WT RBG and B.1.351 stock solutions were prepared. Control adjuvant stock solutions were resuspended in limulus amebocyte lysate (LAL) water. Final injections were diluted 1x Phosphate-buffered saline (PBS). SARS-CoV2 Spike S1 RBD protein stock solutions comprising the WT and B.1.351 antigens were dissolved in PBS at a concentration of 0.88 and 0.95 mg/ml, respectively, having 5 µg per 100 µl injection. Final injections

were diluted with 1x PBS. A dual WT and B.1.351 SARS-CoV2 Spike S1 RBD protein stock solution was also prepared with 5 µg of each antigen per 100 µl injection.

Table 5: Experimental Design

Group	Treatment Name	Vaccine Components		Dosing and Sample Collection			
				Day 1	Day 13	Day 14	Day 21
		Antigen (5ug)	Adjuvant (1nmol)	Dose 1	Read-out	Dose 2	Read-out
1	AMP Vax (B.1.351)	B.1.351 RBD	DSPE-PEG-CpG7909	x	PBMCs ICS ----- Serum Ab ELISA	x	PBMC/ Lung ICS ----- Serum ----- Spleen ELISpot
2	AMP Dual Vax	B.1.351 RBD + WT RBD	DSPE-PEG-CpG7909	x		x	
3	AMP Vax (WT)	WT RBD	DSPE-PEG-CpG7909	x		x	
4	Sol Vax (B.1.351)	---	CpG7909	x		x	
4	Adj Ctrl	---	DSPE-PEG-CpG7909	x		x	

5 groups of 5 C57BL/6J mice each were used. Immunizations were administered subcutaneously (SC) into the tail base of female B6 mice, bilaterally, 50 µl per side. Booster doses were given at roughly 2-week intervals. SC injections may aid in delivering the vaccine into the lymph nodes via natural lymph drainage. Bi-weekly injections may aid in optimal response generation in mice based on previous mouse studies.

Table 6: Vaccine Components

Vaccine Components	Sequence or Cat#	Source	Lot #
SARS-CoV2 RBD, His (WT)	Z03483	GenScript	P50142007
SARS-CoV2 RBD, His (B.1.351)	Z03537	GenScript	B2101019
aCpG 7909	5'-(Diacyl lipid)tcg tcg ttt tgt cgt ttt gtc gtt-3' (SEQ ID NO:1)	Avecia	S18-079-S3-B1

Tetramer analysis was conducted, and results are shown in FIG. 38. Intracellular Stain (ICS) Assay for TNFα and IFNγ was performed on PBMCs 7 days after dosing. ICS was also performed on lung samples 7 days post dose 2. Cells were surface stained for CD4, CD8, and CD3. See Table 7 for antibody information. ICS samples were activated overnight (in the presence of Brefeldin A and Monensin) with 1 mg per well of SARS-CoV-2 Spike Glycoprotein Peptide Pool Mix (315 peptides each at 1 mg per well). Results are shown in FIG. 39A, FIG. 39B, and FIG. 39C for $CD8^+$ lung cells,

$CD4^+$ lung cells, and $CD8^+$ lung cells respectively following dose 2. See Table 8 for peptide information.

Table 7: Antibodies Used for ICS

Antigen	Color	Source	Product #	Lot #
TNFα	FITC	BD	554418	9123915
IFNγ	PE	BD	554412	9154769
CD8a	APC	eBioscience	17-0081-83	4321418
CD4	PE-Cy7	Invitrogen	25-0041-82	2123767
CD3	APC-Cy7	BD	560590	9179637
LiveDead	Aqua	Invitrogen	L34966	1832692
Brefeldin A	---	Invitrogen	00-4506-51	1915300
Monensin	---	BioLegend	420701	B297750

Table 8: Re-Stimulation Peptides

Re-stimulation Peptides	Sequence	Source	Lot #
SARS-CoV-2 Spike Glycoprotein Peptide Pool Mix	315 15mers spanning Spike Protein Sequence, overlap 11aa	GenScript	custom

ELISpot analysis for IFNγ was performed on splenocytes after dose 3 administration. Splenocytes (0.2x106 cells/well) were activated with 1 µg per well PepMix. See Table 8 for peptide information. IFNγ plates were stimulated overnight. Results are shown in FIG. 40.

SARS-CoV2 specific serum ELISA (enzyme-linked immunosorbent assay) was performed on mouse serum 7 days after each dose to detect any RBD-specific antibody response. Whole blood was centrifuged using Ser-gel tubes (NC9436363, Fisher Scientific). Serum was either used fresh or stored at -80°C until used. 96-well plates were coated with 200 ng/100 µl (2 µg/ml) of CoV2 RBD protein (WT, B.1.351 and B.1.1.7) overnight at 4°C. Then plates were pre-blocked with 2% BSA for 2h at RT. Mouse serum was diluted 1:20 and serially diluted (1:5 to 8 concentrations) in a dummy plate. ELISA plates were washed once with ELISA washing buffer (BioLegend 4211601). Samples were transferred to the ELISA plate and incubated for 2h at RT. Plates were washed 4 times with washing buffer. For serum antibody detection the secondary HRP-conjugated antibodies in Table 9 were used at 1:2000 in PBS+ and incubated for 1h at room temperature. Plates were washed 4 times with washing buffer. The reaction was visualized by addition of substrate 3,3',5,5'-Tetramethylbenzidine (TMB) for 10min at RT and stopped by H_2SO_4 (1 N). The absorbance at 450 nm was measured by an ELISA plate reader. Results are shown in FIG. 41.

Table 9: Secondary HRP-Conjugated Antibodies

Antigen	Source	Product #	Lot #
IgG	Jackson Immunoresearch	315-035-046	147406

The reformulated AMP-CPG dual WT RBD and B.1.351 RBD vaccine and the reformulated AMP-CPG B.1.351 RBD vaccine elicits similar immunological responses to the vaccine that uses solely the WT RBD antigen seen in previous experiments. The T-cell as well as antibody responses are equally cross-reactive against all tested variants of SARS-CoV2 RBD.

Example 8: Inducing an immune response in human subjects

According to the methods disclosed herein, a subject, such as a human subject, can be administered a CpG amphiphile and a coronavirus antigen (e.g., a coronavirus spike protein or a peptide thereof, e.g., a RBD peptide, and/or a coronavirus nucleocapsid protein or a peptide thereof, or a nucleic acid sequence encoding the same) to induce an immune response in the subject. To this end, the patient is administered a CpG amphiphile and the coronavirus antigen (e.g., a coronavirus spike protein or a peptide thereof, e.g., a RBD peptide, and/or a coronavirus nucleocapsid protein or a peptide thereof, or a nucleic acid sequence encoding the same). The CpG amphiphile or a pharmaceutical composition thereof is administered to the subject subcutaneously in the form of a vaccine. The CpG amphiphile or a pharmaceutical composition thereof may also be administered intranasally, intratracheally, or by inhalation during mechanical ventilation. The subject is also administered a coronavirus antigen (e.g., a coronavirus spike protein or a peptide thereof, e.g., a RBD peptide, and/or a coronavirus nucleocapsid protein or a peptide thereof, or a nucleic acid sequence encoding the same) or a pharmaceutical composition thereof subcutaneously in the form of a vaccine. The coronavirus antigen or a pharmaceutical composition thereof may also be administered intranasally, intratracheally, or by inhalation during mechanical ventilation. Both the CpG amphiphile and the coronavirus antigen (e.g., a coronavirus spike protein or a peptide thereof, e.g., a RBD peptide, and/or a coronavirus nucleocapsid protein or a peptide thereof, or a nucleic acid sequence encoding the same) may be administered bilaterally on the inner thigh. The CpG amphiphile and the (e.g., a coronavirus spike protein or a peptide thereof, e.g., a RBD peptide, and/or a coronavirus nucleocapsid protein or a peptide thereof, or a nucleic acid sequence encoding the same) may be administered separately or concurrently to the subject. The subject may receive a dosage of the CpG amphiphile and the (e.g., a coronavirus spike protein or a peptide thereof, e.g., a RBD peptide, and/or a coronavirus nucleocapsid protein or a peptide thereof, or a nucleic acid sequence encoding the same) at week 0, week 4, and week 10 or at week 0 and week 4. The subject may receive a dosage of about 0.1 mg to 20.0 mg. In particular, the dosage administered may be in the range of about 0.1 mg to 1.0 mg, of about 0.5 mg to 3.0 mg, of about 1.0 mg to 5.0 mg, of about 2.0 to 5.0 mg, of about 3.0 to 5.0 mg, of about 3.0 mg to 10.0 mg, of about 4.0 mg to 12.0 mg, of about 5.0 mg to 15.0 mg, or of about 5.0 to 20.0 mg. The particular dosage administered to the subject may be about 0.1 mg, 0.2 mg, 0.3 mg, 0.4 mg, 0.5 mg, 1.0 mg, 2.0 mg, 3.0 mg, 4.0 mg, 5.0 mg, 6.0 mg, 7.0 mg, 8.0 mg, 9.0 mg,

10.0 mg, 11.0 mg, 12.0 mg, 13.0 mg, 14.0 mg, 15.0 mg, 16.0 mg, 17.0 mg, 18.0 mg, 19.0 mg, or 20.0 mg of the CpG amphiphile. The subject also may receive a dosage in a range between any two of these particular dosages of the CpG amphiphile. The subject may receive a dosage of about 10 µg to about 1.0 mg of the coronavirus antigen. In particular, the subject may receive a dosage of about 40 µg to 60 µg, of about 50 µg to 70 µg, of about 50 µg to 150 µg, of about 70 µg to 150 µg, of about 100 µg to 150 µg, of about 100 µg to 200 µg, of about 140 µg to 250 µg, of about 200 µg to 300 µg, of about 250 µg to 500 µg, of about 300 µg to 600 µg, or of about 500 µg to 1.0 mg of the corona virus antigen. In particular, the dosage administered to the subject may be about 10 µg, 20 µg, 30 µg, 40 µg, 50 µg, 60, µg, 70 µg, 80 µg, 90 µg, 100 µg, 110 µg, 120 µg, 130 µg, 140 µg, 150 µg, 200 µg, 250 µg, 300 µg, 400 µg, 500 µg, 600 µg, 700 µg, 800 µg, 900 µg, or 1.0 mg of the coronavirus antigen (e.g., spike protein or spike protein RBD). The subject also may receive a dosage in a range between any two of these particular dosages of the coronavirus antigen.

OTHER EMBODIMENTS

Various modifications and variations of the described compositions, methods, and uses of the invention will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with specific embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the invention that are obvious to those skilled in the art are intended to be within the scope of the invention.

All publications, patents, and patent applications are herein incorporated by reference in their entirety to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference in its entirety.

What is claimed is:

CLAIMS

1. A method of inducing an immune response against a coronavirus antigen in a subject, the method comprising administering (1) a CpG-amphiphile and (2) a coronavirus antigen or a nucleic acid sequence encoding the coronavirus antigen to the subject.

2. The method of claim 1, wherein the coronavirus antigen is a coronavirus spike protein or a peptide thereof or a nucleic acid sequence encoding the coronavirus spike protein or peptide.

3. The method of claim 1 or 2, wherein the CpG-amphiphile comprises a CpG sequence bonded to a lipid.

4. The method of claim 1 or 2, the CpG-amphiphile comprises a CpG sequence linked to a lipid by a linker.

5. The method of claim 4, wherein the linker comprises a polymer, a string of amino acids, a string of nucleic acids, a polysaccharide, or a combination thereof.

6. The method of claim 5, wherein the linker comprises a string of nucleic acids.

7. The method of claim 6, wherein the string of nucleic acids comprises between 1 and 50 nucleic acid residues.

8. The method of claim 7, wherein the string of nucleic acids comprises between 5 and 30 nucleic acid residues.

9. The method of any one of claims 5-8, wherein the string of nucleic acids comprises “N” guanines, wherein N is 1-10.

10. The method of claim 5, wherein the linker comprises consecutive polyethylene glycol units.

11. The method of claim 10, wherein the linker comprises “N” consecutive polyethylene glycol units, wherein N is between 20 and 80.

12. The method of claim 11, wherein the linker comprises “N” consecutive polyethylene glycol units, wherein N is between 30 and 70.

13. The method of claim 12, wherein the linker comprises “N” consecutive polyethylene glycol units, wherein N is between 40 and 60.

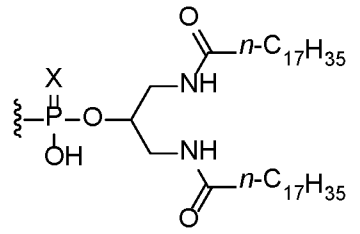
14. The method of claim 13, wherein the linker comprises “N” consecutive polyethylene

glycol units, wherein N is between 45 and 55.

15. The method of claim 14, wherein the linker comprises 48 consecutive polyethylene glycol units.

16. The method of any one of claims 1-15, wherein the lipid is a diacyl lipid.

17. The method of claim 16, wherein the diacyl lipid has the following structure:



,

or a salt thereof,
wherein X is O or S.

18. The method of any one of claims 1-17 wherein the CpG sequence comprises the nucleotide sequence 5'-TCGTCGTTTTGTCGTTTTGTCGTT-3' (SEQ ID NO:1).

19. The method of any one of claims 1-17, wherein the CpG sequence comprises the nucleotide sequence of 5'-TCCATGACGTTCCTGACGTT-3' (SEQ ID NO: 2).

20. The method claim 18 or claim 19, wherein all internucleoside groups connecting the nucleosides in the CpG sequence are phosphorothioates.

21. The method of any one of claims 1-20, wherein the coronavirus spike protein or peptide thereof is a SARS-CoV-2 spike protein or peptide thereof.

22. The method of any one of claims 1-21, wherein the peptide of the coronavirus spike protein is a receptor binding domain that specifically binds angiotensin-converting enzyme 2 (ACE2).

23. The method of any one of claims 1-22, wherein the peptide of the coronavirus spike protein comprises a polypeptide sequence having at least 90% sequence identity to: RVQPTESIVRFPNITNLCPFGEVFNATRFASVYAWNRKRISNCVADYSVLYNSASFSTFKCYGVSPTKLN DLCFTNVYADSFVIRGDEVRQIAPGQTGKIADYNYKLPDDFTGCVIAWNSNNLDSKVGGNYNYLYRLFRK SNLKPFERDISTEIYQAGSTPCNGVEGFNCYFPLQSYGFQPTNGVGYQPYRVVVLSFELLHAPATVCGPK KSTNLVKNKCVNFNFNGLTGTGVLTESNKKFLPFQQFGRDIADTTDAVRDPQTLEILDITPCS (SEQ ID NO: 3).

24. The method of claim 23, wherein the peptide of the coronavirus spike protein comprises the polypeptide sequence of:
RVQPTESIVRFPNITNLCPFGEVFNATRFASVYAWNRKRISNCVADYSVLYNSASFSTFKCYGVSPTKLN
DLCFTNVYADSFVIRGDEVRQIAPGQTGKIADYNYKLPDDFTGCVIAWNSNNLDSKVGGNYNYLYRLFRK
SNLKPFERDISTEIYQAGSTPCNGVEGFNCYFPLQSYGFQPTNGVGYQPYRVVVLSFELLHAPATVCGPK
KSTNLVKNKCVNFNFNGLTGTGVLTESNKKFLPFQQFGRDIADTTDAVRDPQTLEILDITPCS (SEQ ID NO: 3).

25. The method of any one of claims 1 and 3-21, wherein the coronavirus antigen is a coronavirus nucleocapsid protein or a peptide thereof.

26. The method of claim 25, wherein the coronavirus nucleocapsid protein antigen comprises a polypeptide sequence having at least 90% sequence identity to:
MSDNGPQNQRNAPRITFGGPSDSTGSNQNGERSGARSKQRRPQGLPNNTASWFTALTQHGKEDLKFP
RGQGVPINTNSSPDDQIGYYRRATRRIRGGDGKMKDLSPRWYFYYLGTGPEAGLPYGANKDGIIWVATE
GALNTPKDHIGTRNPANNAAIVLQLPQGTTLPKGFYAEGSRGGSQASSRSSSRSRNSSRNSTPGSSRGT
SPARMAGNGGDAALALLLLDRLNQLESKMSGKGQQQQGQTVTKKSAAEASKKPRQKRTATKAYNVTQA
FGRRGPEQTQGNFGDQELIRQGTDYKHWPQIAQFAPSASAFFGMSRIGMEVTPSGTWLTYTGAIKLDDK
DPNFKDQVILLNKHIDAYKTFPPTEPKKDKKKKADETQALPQRQKKQQTVTLLPAADLDDFSKQLQQSMS
SADSTQA (SEQ ID NO:68).

27. The method of claim 25, wherein the coronavirus nucleocapsid protein antigen comprises the polypeptide sequence of:
MSDNGPQNQRNAPRITFGGPSDSTGSNQNGERSGARSKQRRPQGLPNNTASWFTALTQHGKEDLKFP
RGQGVPINTNSSPDDQIGYYRRATRRIRGGDGKMKDLSPRWYFYYLGTGPEAGLPYGANKDGIIWVATE
GALNTPKDHIGTRNPANNAAIVLQLPQGTTLPKGFYAEGSRGGSQASSRSSSRSRNSSRNSTPGSSRGT
SPARMAGNGGDAALALLLLDRLNQLESKMSGKGQQQQGQTVTKKSAAEASKKPRQKRTATKAYNVTQA
FGRRGPEQTQGNFGDQELIRQGTDYKHWPQIAQFAPSASAFFGMSRIGMEVTPSGTWLTYTGAIKLDDK
DPNFKDQVILLNKHIDAYKTFPPTEPKKDKKKKADETQALPQRQKKQQTVTLLPAADLDDFSKQLQQSMS
SADSTQAENLYFQGHHHHHH (SEQ ID NO:63).

28. The method of any one of claims 1-26, wherein the coronavirus antigen comprises one or more tags.

29. The method of claim 28, wherein the tag is an Avi tag.

30. The method of claim 28, wherein the tag is a histidine tag.

31. The method of any one of claims 28-30, wherein the coronavirus antigen comprises an Avi tag and a histidine tag.

32. The method of any one of claims 28-31, wherein the coronavirus antigen comprises a linker between the polypeptide sequence and the one or more tags.

33. The method of any one of claims 1-24 and 28-31, wherein the coronavirus spike protein is administered.

34. The method of claim 33, wherein a trimer of the coronavirus spike protein is administered.

35. The method of claim 34, wherein the trimer is a trimer of a protein construct comprising the sequence:
VNLTTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHSTQDLFLPFFSNVTWFHAIHVSGTNGTKRFDNPVLP
FNDGVYFASTEKSNIIRGWIFGTTLDSKTQSLLIVNNATNVVIKVCEFQFCNDPFLGVYYHKNNKSWMESE
FRVYSSANNCTFEYVSQPFLMDLEGKQGNFKNLREFVFKNIDGYFKIYSKHTPINLVRDLPQGFSALEPLV
DLPIGINITRFQTLLALHRSYLTPGDSSSGWTAGAAAYYVGYLQPRTFLLKYNENGTITDAVDCALDPLSET
KCTLKSFTVEKGIYQTSNFRVQPTESIVRFPNITNLCPFGEVFNATRFASVYAWNRKRISNCVADYSVLYN
SASFSTFKCYGVSPTKLNDLCFTNVYADSFVIRGDEVRQIAPGQTGKIADYNYKLPDDFTGCVIAWNSNN
LDSKVGGNYNYLYRLFRKSNLKPFERDISTEIYQAGSTPCNGVEGFNCYFPLQSYGFQPTNGVGYQPYR
VVVLSFELLHAPATVCGPKKSTNLVKNKCVNFNFNGLTGTGVLTESNKKFLPFQQFGRDIADTTDAVRDP
QTLEILDITPCSFGGVSVITPGTNTSNQVAVLYQDVNCTEVPVAIHADQLTPTWRVYSTGSNVFQTRAGCL
IGAEHVNNSYECDIPIGAGICASYQTQTNSPRAAASVASQSIIAYTMSLGAENSVAYSNNSIAIPTNFTISVT
TEILPVSMTKTSVDCTMYICGDSTECSNLLLQYGSFCTQLNRALTGIAVEQDKNTQEVFAQVKQIYKTPPIK
DFGGFNFSQILPDPSKPSKRSFIEDLLFNKVTLADAGFIKQYGDCLGDIAARDLICAQKFNGLTVLPPLLTD
EMIAQYTSALLAGTITSGWTFGAGAALQIPFAMQMAYRFNGIGVTQNVLYENQKLIANQFNSAIGKIQDSL
SSTASALGKLQDVVNQNAQALNTLVKQLSSNFGAISSVLNDILSRLDKVEAEVQIDRLITGRLQSLQTYVT
QQLIRAAEIRASANLAATKMSECVLGQSKRVDFCGKGYHLMSFPQSAPHGVVFLHVTYVPAQEKNFTTA
PAICHDGKAHFPREGVFVSNGTHWFVTQRNFYEPQIITTDNTFVSGNCDVVIGIVNNTVYDPLQPELDSFK
EELDKYFKNHTSPDVDLGDISGINASVVNIQKEIDRLNEVAKNLNESLIDLQELGKYEQYIKWPGGGSGGG
SHHHHHHHHHH (SEQ ID NO:66).

36. The method of claim one of claims 1-35, wherein a coronavirus spike protein, or a peptide thereof, and a coronavirus nucleocapsid protein, or a peptide thereof, are administered.

37. The method of claim 36, wherein a trimer of a coronavirus spike protein construct comprising the sequence:
VNLTTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHSTQDLFLPFFSNVTWFHAIHVSGTNGTKRFDNPVLP
FNDGVYFASTEKSNIIRGWIFGTTLDSKTQSLLIVNNATNVVIKVCEFQFCNDPFLGVYYHKNNKSWMESE
FRVYSSANNCTFEYVSQPFLMDLEGKQGNFKNLREFVFKNIDGYFKIYSKHTPINLVRDLPQGFSALEPLV
DLPIGINITRFQTLLALHRSYLTPGDSSSGWTAGAAAYYVGYLQPRTFLLKYNENGTITDAVDCALDPLSET
KCTLKSFTVEKGIYQTSNFRVQPTESIVRFPNITNLCPFGEVFNATRFASVYAWNRKRISNCVADYSVLYN
SASFSTFKCYGVSPTKLNDLCFTNVYADSFVIRGDEVRQIAPGQTGKIADYNYKLPDDFTGCVIAWNSNN
LDSKVGGNYNYLYRLFRKSNLKPFERDISTEIYQAGSTPCNGVEGFNCYFPLQSYGFQPTNGVGYQPYR

VVVLSFELLHAPATVCGPKKSTNLVKNKCVNFNFNGLTGTGVLTESNKKFLPFQQFGRDIADTTDAVRDP
QTLEILDITPCSFGGVSVITPGTNTSNQVAVLYQDVNCTEVPVAIHADQLTPTWRVYSTGSNVFQTRAGCL
IGAEHVNNSYECDIPIGAGICASYQTQTNSPRAAASVASQSIIAYTMSLGAENSVAYSNNSIAIPTNFTISVT
TEILPVSMTKTSVDCTMYICGDSTECSNLLLQYGSFCTQLNRALTGIAVEQDKNTQEVFAQVKQIYKTPPIK
DFGGFNFSQILPDPSKPSKRSFIEDLLFNKVTLADAGFIKQYGDCLGDIAARDLICAQKFNGLTVLPPLLTD
EMIAQYTSALLAGTITSGWTFGAGAALQIPFAMQMAYRFNGIGVTQNVLYENQKLIANQFNSAIGKIQDSL
SSTASALGKLQDVVNQNAQALNTLVKQLSSNFGAISSVLNDILSRLDKVEAEVQIDRLITGRLQSLQTYVT
QQLIRAAEIRASANLAATKMSECVLGQSKRVDFCGKGYHLMSFPQSAPHGVVFLHVTYVPAQEKNFTTA
PAICHDGKAHFPREGVFVSNGTHWFVTQRNFYEPQIITTDNTFVSGNCDVVIGIVNNTVYDPLQPELDSFK
EELDKYFKNHTSPDVDLGDISGINASVVNIQKEIDRLNEVAKNLNESLIDLQELGKYEQYIKWPGGGSGGG
SHHHHHHHHHH (SEQ ID NO:66), and a coronavirus nucleocapsid protein construct having the polypeptide sequence of:
MSDNGPQNQRNAPRITFGGPSDSTGSNQNGERSGARSKQRRPQGLPNNTASWFTALTQHGKEDLKFP
RGQGVPINTNSSPDDQIGYYRRATRRIRGGDGKMKDLSPRWYFYYLGTGPEAGLPYGANKDGIIWVATE
GALNTPKDHIGTRNPANNAAIVLQLPQGTTLPKGFYAEGSRGGSQASSRSSSRSRNSSRNSTPGSSRGT
SPARMAGNGGDAALALLLLDRLNQLESKMSGKGQQQQGQTVTKKSAAEASKKPRQKRTATKAYNVTQA
FGRRGPEQTQGNFGDQELIRQGTDYKHWPQIAQFAPSASAFFGMSRIGMEVTPSGTWLTYTGAIKLDDK
DPNFKDQVILLNKHIDAYKTFPPTEPKKDKKKKADETQALPQRQKKQQTVTLLPAADLDDFSKQLQQSMS
SADSTQAENLYFQGHHHHHH (SEQ ID NO:63) are administered.

38. The method of any one of claims 1-25, 28-31, and 36, wherein an mRNA encoding the coronavirus antigen is administered.

39. The method of any one of claims 1-38, wherein the CpG-amphiphile and the coronavirus antigen or nucleic acid sequence encoding the same are administered concurrently.

40. The method of any one of claims 1-38, wherein the CpG-amphiphile and the coronavirus antigen, or nucleic acid sequence enclosing the same are administered sequentially.

41. The method of claim 40, wherein the CpG-amphiphile is administered first, followed by administering of the coronavirus antigen or nucleic acid sequence encoding the same.

42. The method of claim 40, wherein said the coronavirus antigen or nucleic acid sequence encoding the same is administered first, followed by administering of CpG-amphiphile.

43. The method of any one of claims 1-42, wherein the method comprises administering a second adjuvant to the subject.

44. The method of any one of claims 1-43, wherein the method comprises administering a coronavirus vaccine to the subject as a prime or a boost.

45. The method of any one of claims 1-44, wherein the CpG-amphiphile is administered subcutaneously, intranasally, intratracheally, or by inhalation during mechanical ventilation.

46. The method of claim 45, wherein the CpG-amphiphile is administered subcutaneously.

47. The method of any one of claims 1-46, wherein the coronavirus antigen is administered subcutaneously, intranasally, intratracheally, or by inhalation during mechanical ventilation.

48. The method of any one of claims 1-47, wherein the subject is a mammal.

49. The method of claim 48, wherein the subject is a human.

50. A pharmaceutical composition comprising a CpG-amphiphile and a coronavirus antigen, or a nucleic acid sequence encoding the coronavirus antigen, and a pharmaceutically acceptable carrier.

51. The pharmaceutical composition of claim 50, wherein the coronavirus antigen is a coronavirus spike protein or a peptide thereof.

52. The pharmaceutical composition of claim 50, wherein the coronavirus antigen is a coronavirus nucleocapsid protein or a peptide thereof.

53. The pharmaceutical composition of claim 50, wherein the coronavirus antigen comprises a coronavirus spike protein or a peptide thereof and a coronavirus nucleocapsid protein or a peptide thereof.

54. A kit comprising a CpG-amphiphile and a coronavirus antigen or a nucleic acid sequence encoding the coronavirus antigen.

55. The kit of claim 54, wherein the coronavirus antigen is a coronavirus spike protein or a peptide thereof.

56. The kit of claim 54, wherein the coronavirus antigen is a coronavirus nucleocapsid protein or a peptide thereof.

57. The kit of claim 54, wherein the coronavirus antigen comprises a coronavirus spike protein or a peptide thereof and a coronavirus nucleocapsid protein or a peptide thereof.

FIG. 1A-FIG. 1C

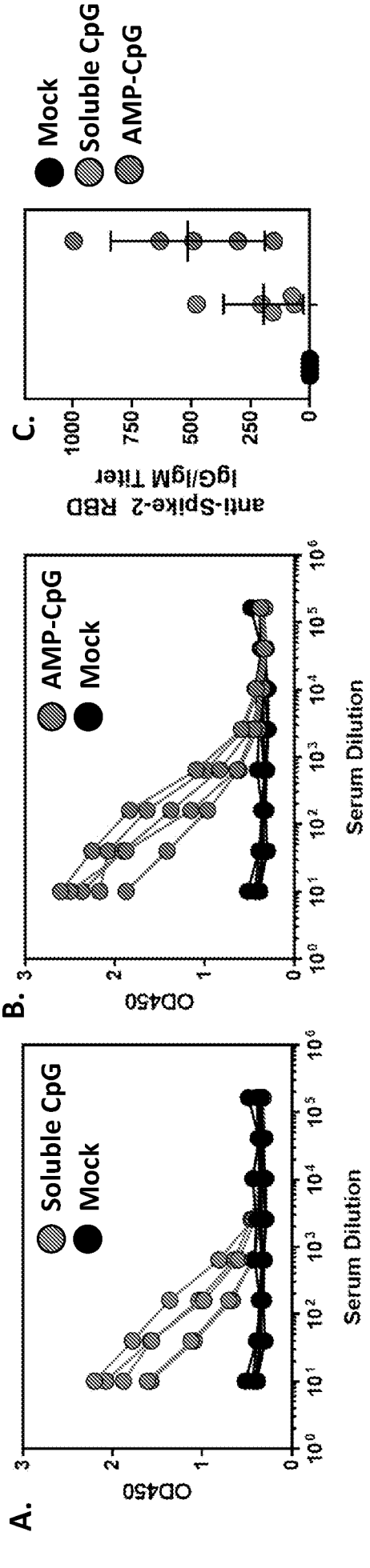

A.
OD450
Soluble CpG
Mock
Serum Dilution
B.
OD450
AMP-CpG
Mock
Serum Dilution
C.
anti-Spike-2 RBD
IgG/IgM Titer
Mock
Soluble CpG
AMP-CpG

FIG. 2A-FIG. 2C

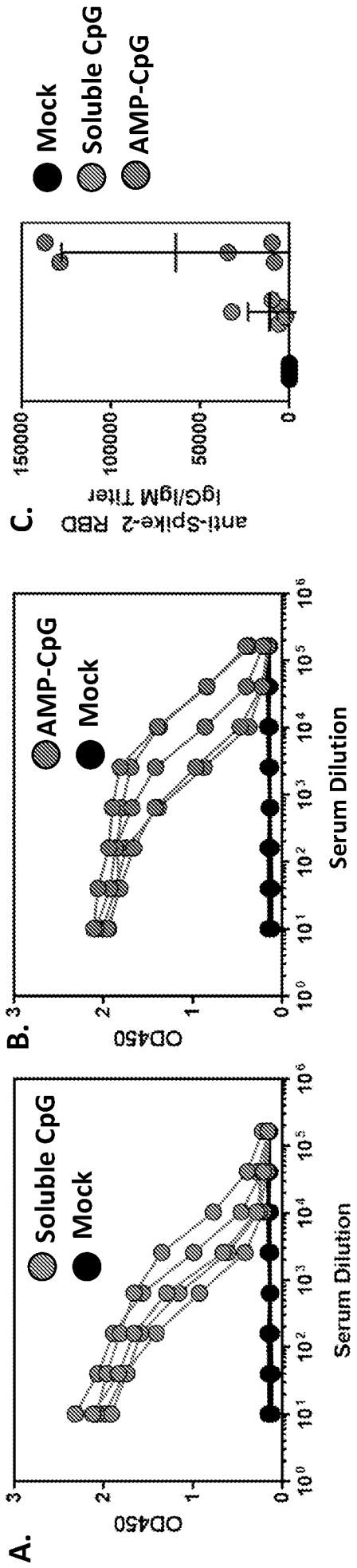

A.
OD450
Soluble CpG
Mock
Serum Dilution
B.
OD450
AMP-CpG
Mock
Serum Dilution
C.
anti-Spike-2 RBD IgG/IgM Titer
Mock
Soluble CpG
AMP-CpG

FIG. 3A-FIG. 3D

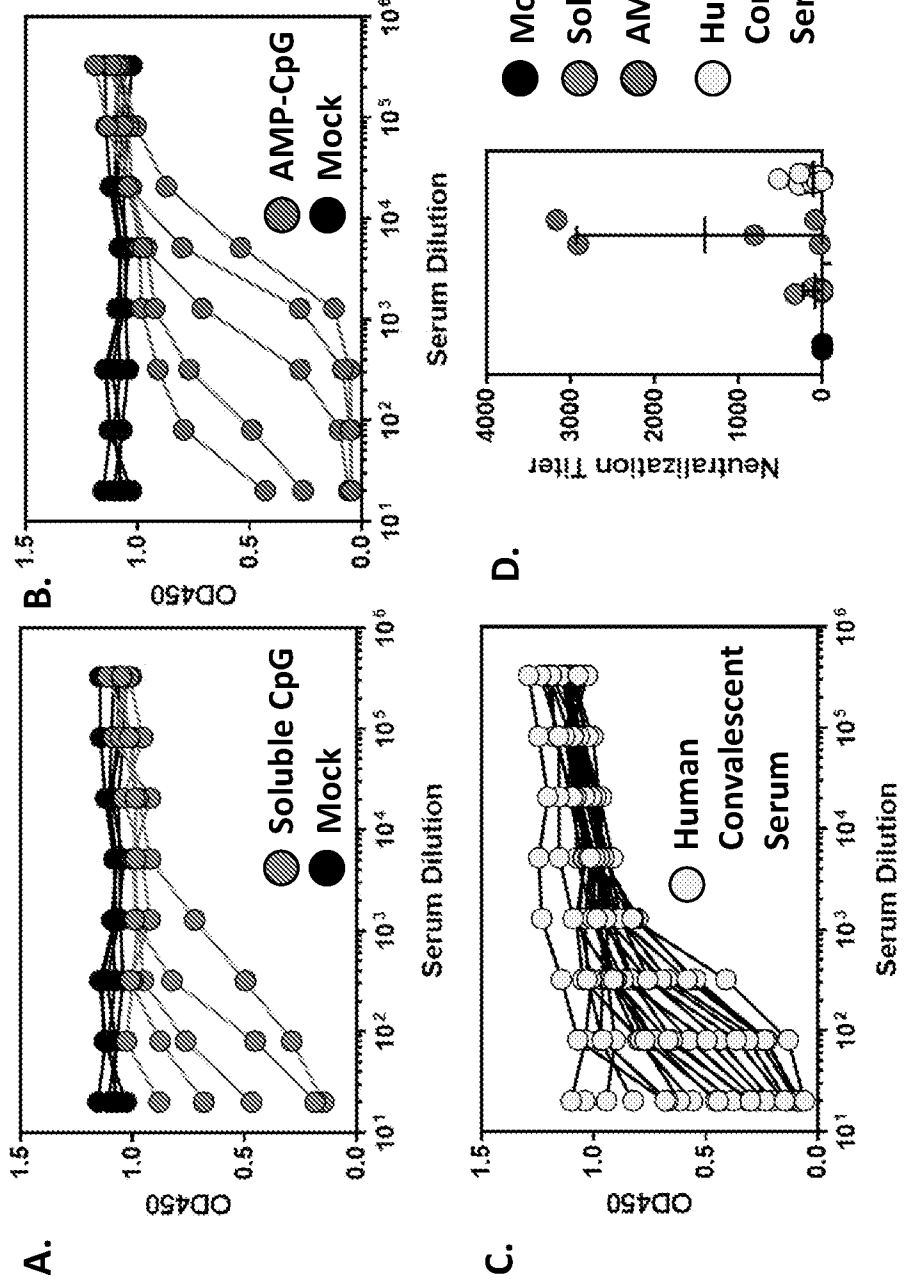

A.
B.
C.
D.
OD450
Serum Dilution
Soluble CpG
Mock
AMP-CpG
Human Convalescent Serum
Neutralization Titer

FIG. 4A-FIG. 4C

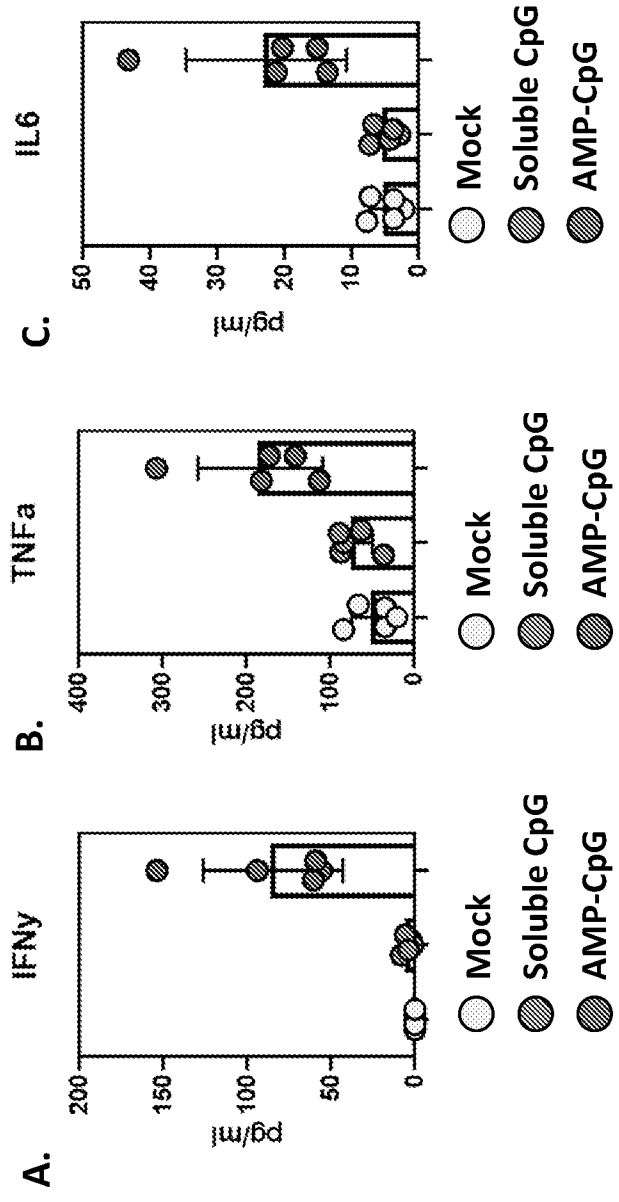

A.
IFNy
pg/ml
200
150
100
50
0
B.
TNFa
pg/ml
400
300
200
100
0
C.
IL6
pg/ml
50
40
30
20
10
0
Mock
Soluble CpG
AMP-CpG

FIG. 5A-FIG. 5B

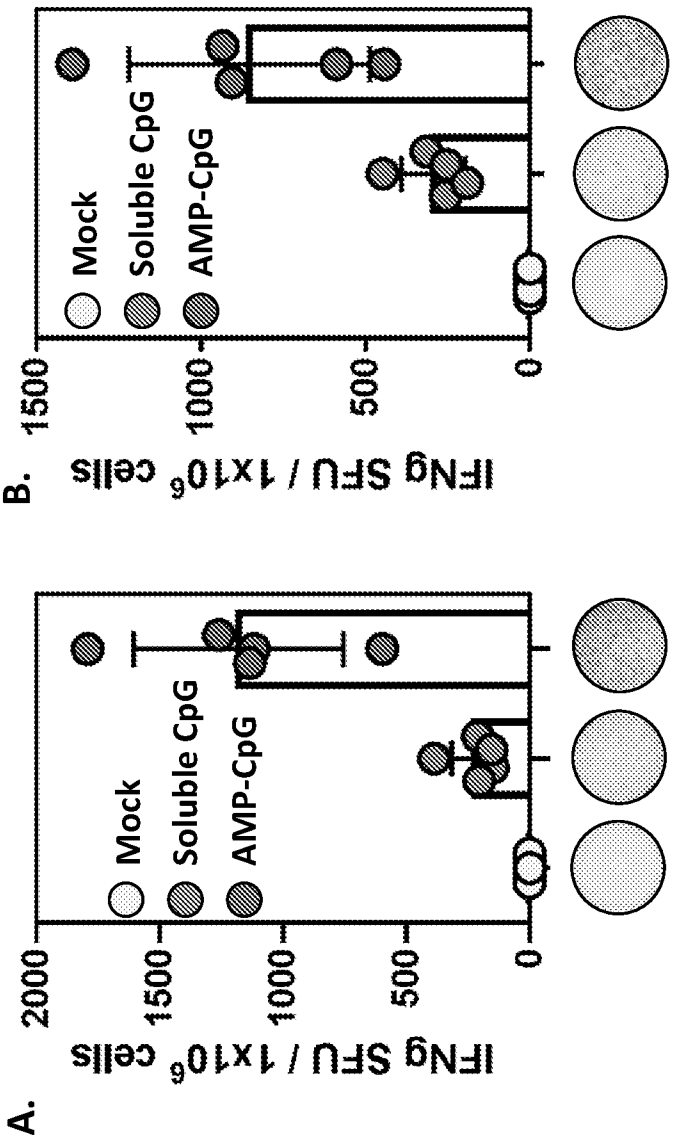

A.
IFNg SFU / 1x10^6 cells
2000
1500
1000
500
0
Mock
Soluble CpG
AMP-CpG
B.
IFNg SFU / 1x10^6 cells
1500
1000
500
0
Mock
Soluble CpG
AMP-CpG

FIG. 6A-FIG. 6C

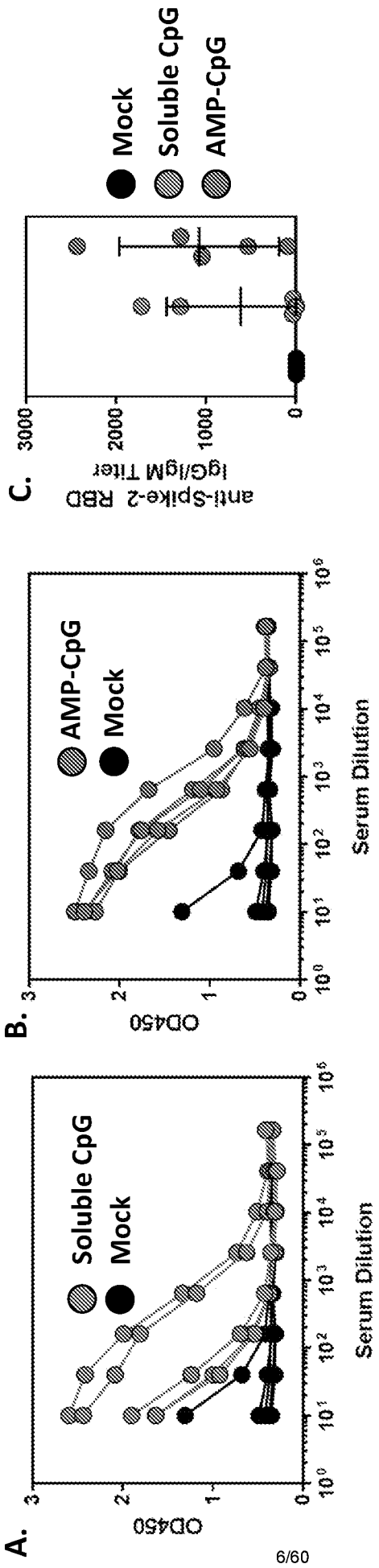

A.
OD450
Soluble CpG
Mock
Serum Dilution
B.
OD450
AMP-CpG
Mock
Serum Dilution
C.
anti-Spike-2 RBD
IgG/IgM Titer
Mock
Soluble CpG
AMP-CpG

FIG. 7A-FIG. 7C

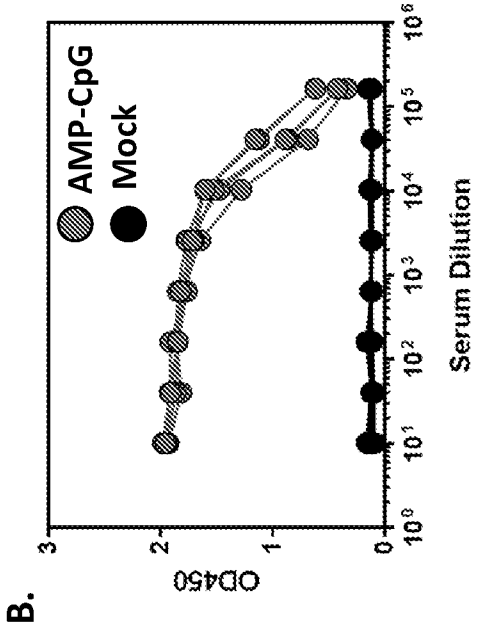

A.
OD450
Soluble CpG
Mock
Serum Dilution
B.
OD450
AMP-CpG
Mock
Serum Dilution
C.
anti-Spike-2 RBD
IgG/IgM Titer
Mock
Soluble CpG
AMP-CpG

FIG. 8A-FIG. 8D

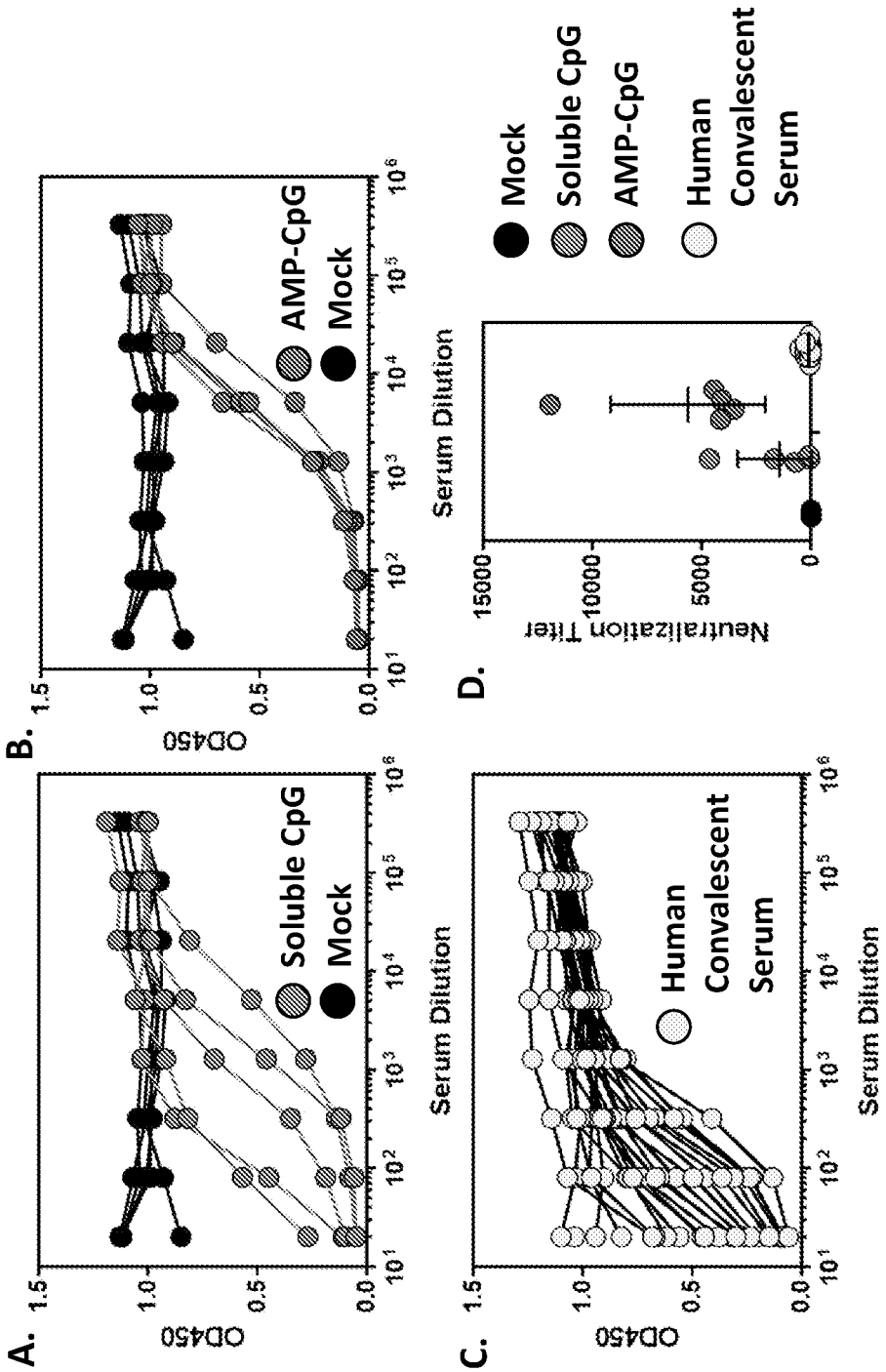
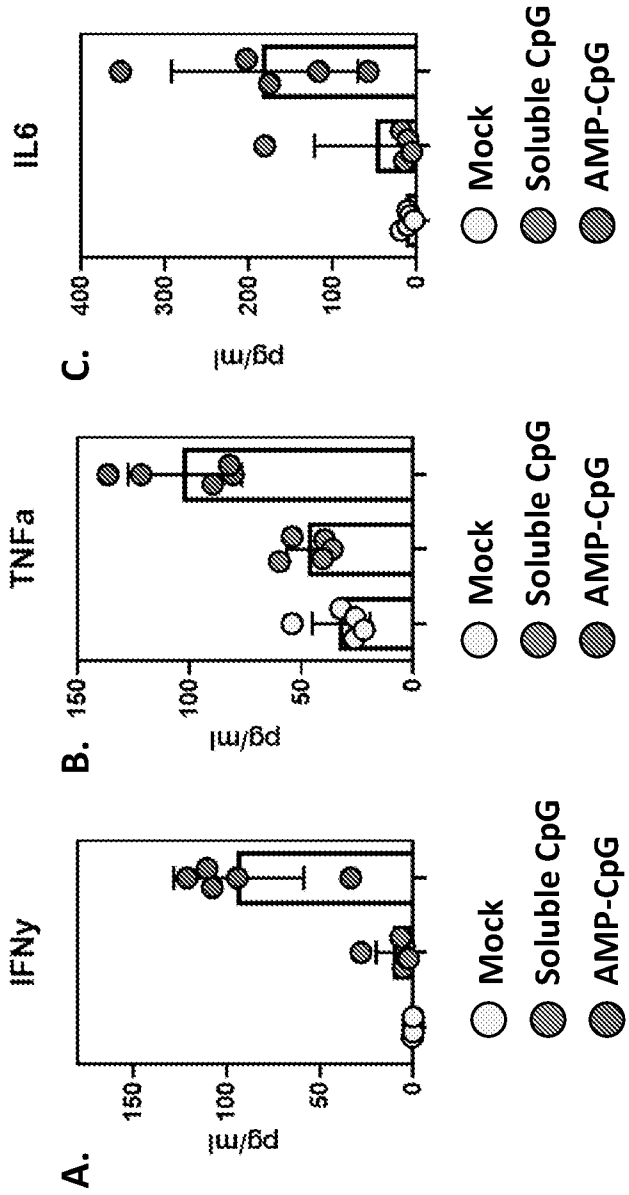

A.
B.
C.
D.
OD450
Serum Dilution
Soluble CpG
Mock
AMP-CpG
Human Convalescent Serum
Neutralization Titer
15000
10000
5000
0

FIG. 9A-FIG. 9C


A.
IFNy
pg/ml
150
100
50
0
B.
TNFa
pg/ml
150
100
50
0
C.
IL6
pg/ml
400
300
200
100
0
Mock
Soluble CpG
AMP-CpG

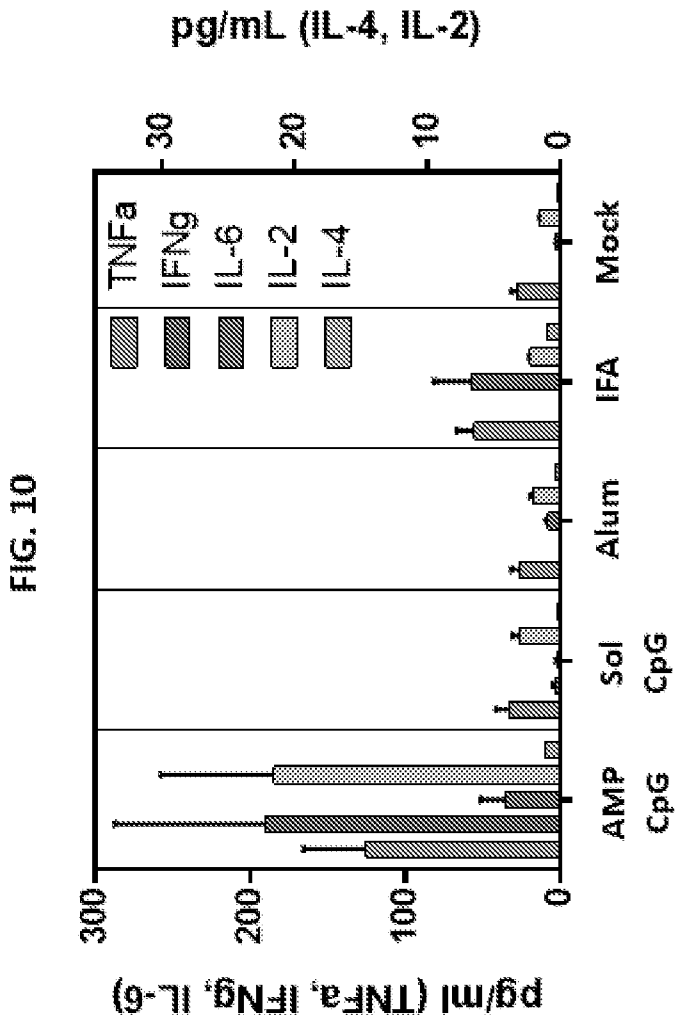

pg/mL (IL-4, IL-2)
30
20
10
0
TNFa
IFNg
IL-6
IL-2
IL-4
Mock
IFA
Alum
Sol
CpG
AMP
CpG
300
200
100
0
pg/ml (TNFa, IFNg, IL-6)

FIG. 10

FIG.11

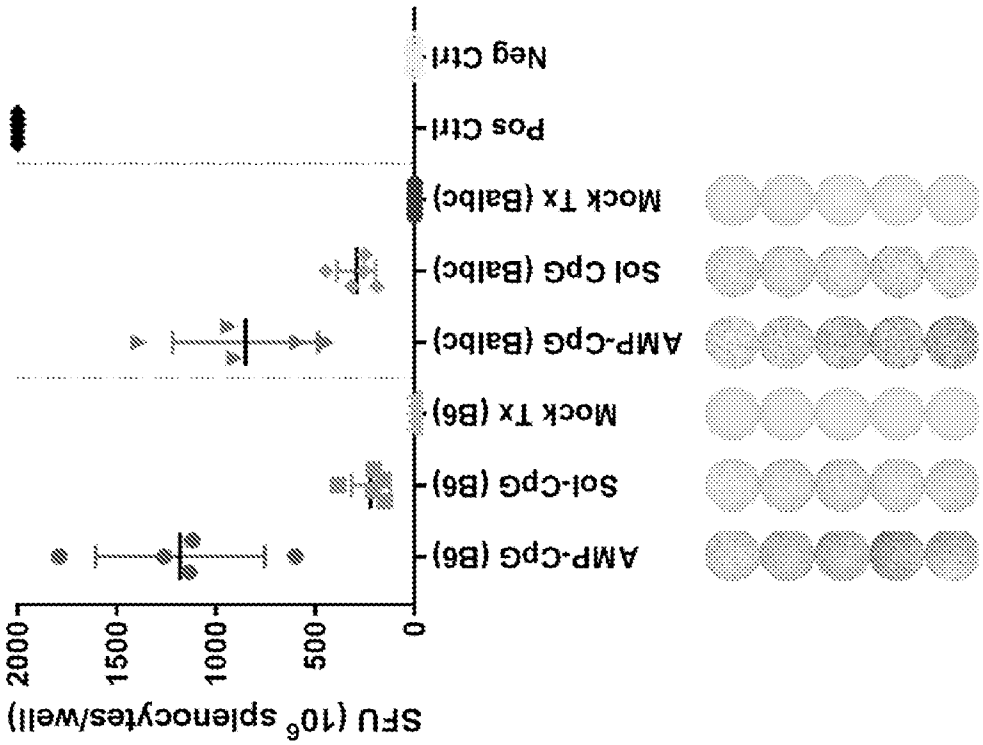
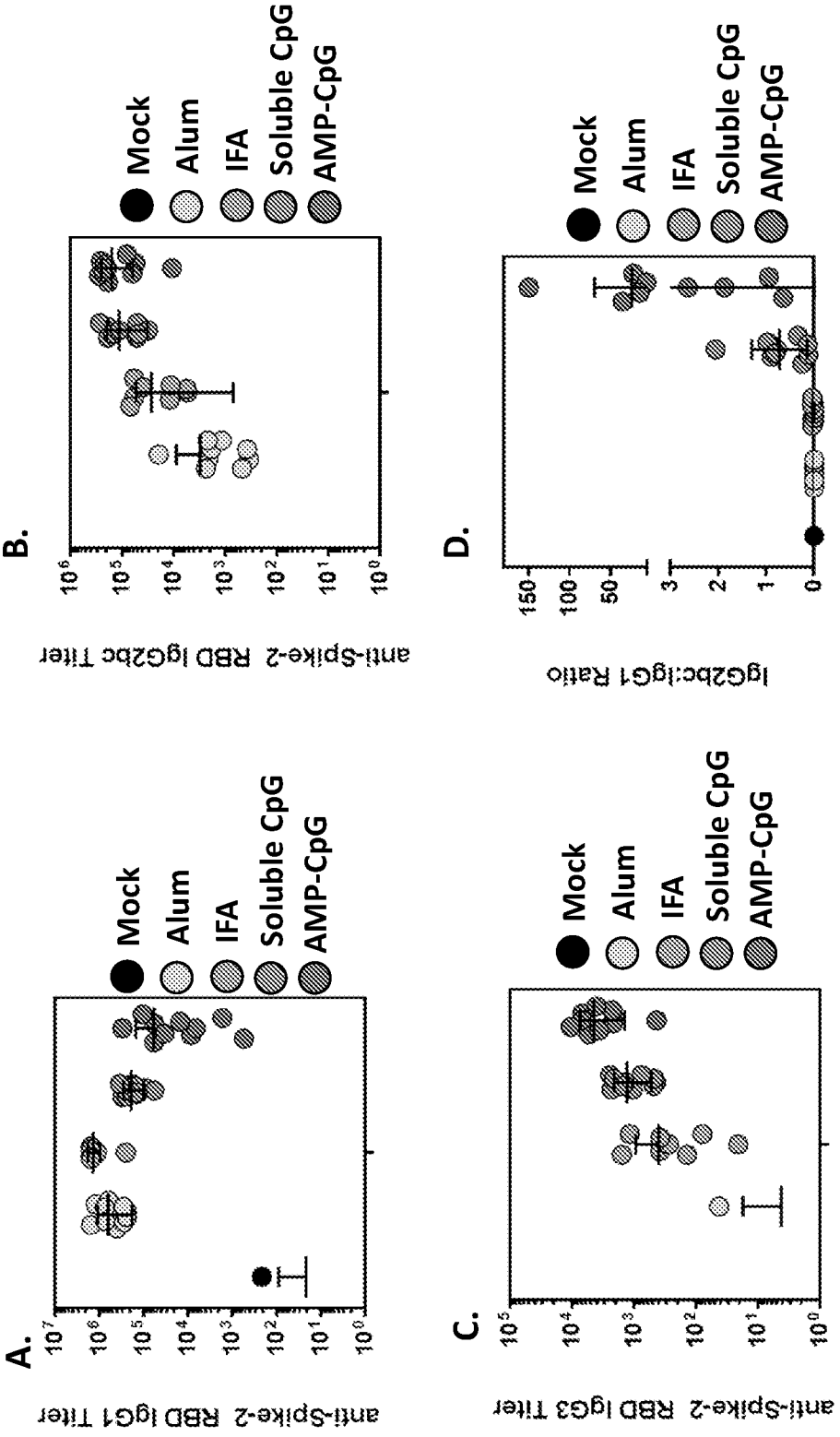

SFU (10^6 splenocytes/well)
2000
1500
1000
500
0
AMP-CpG (B6)
Sol-CpG (B6)
Mock Tx (B6)
AMP-CpG (Balbc)
Sol CpG (Balbc)
Mock Tx (Balbc)
Pos Ctrl
Neg Ctrl

FIG.12A-FIG. 12D



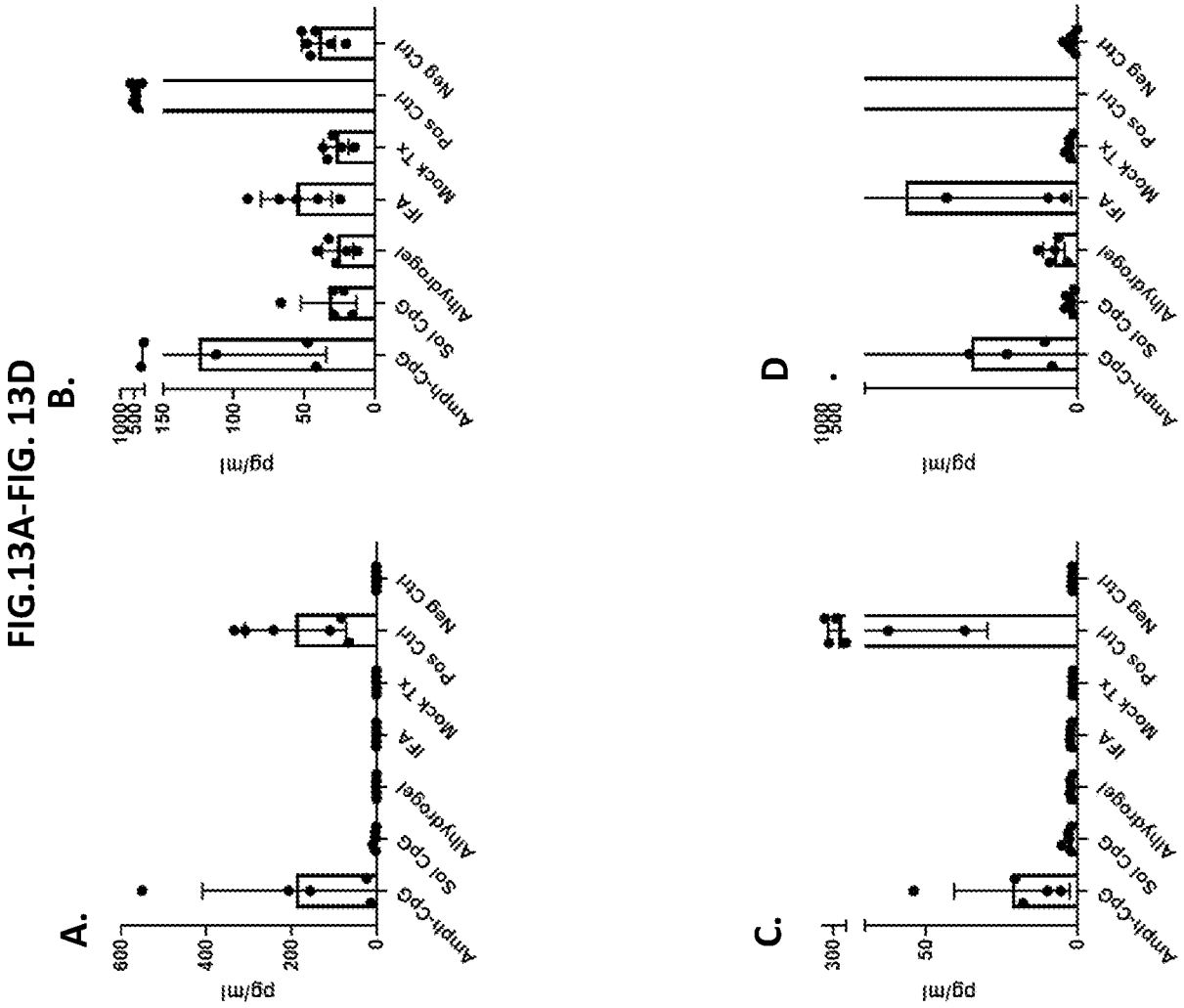


FIG.13A-FIG. 13D

FIG.14A-FIG. 14D

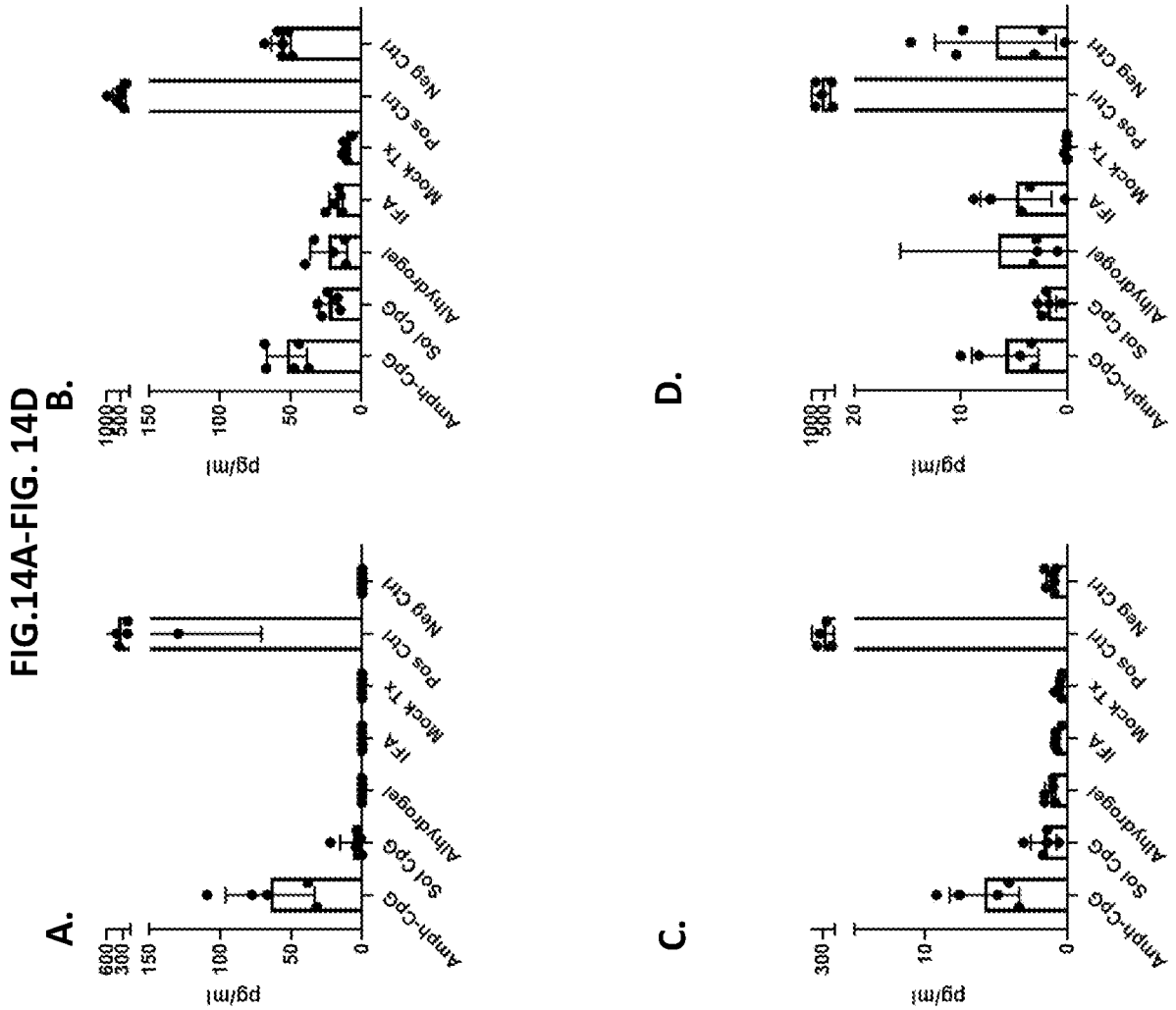


FIG.15

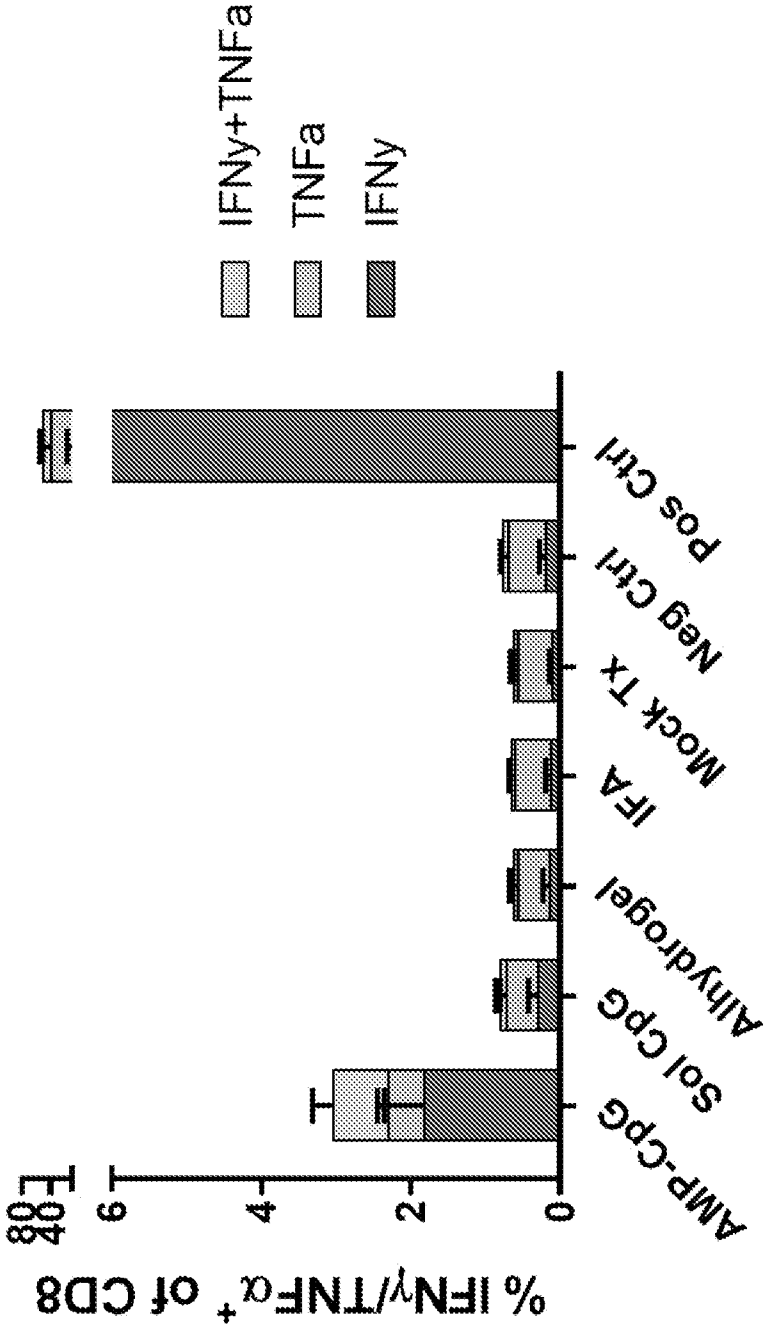

IFNy+TNFa
TNFa
IFNy
% IFNγ/TNFα+ of CD8
80
40
6
4
2
0
AMP-CPG
Sol CPG
Alhydrogel
IFA
Mock Tx
Neg Ctrl
Pos Ctrl

FIG. 16A - FIG. 16D

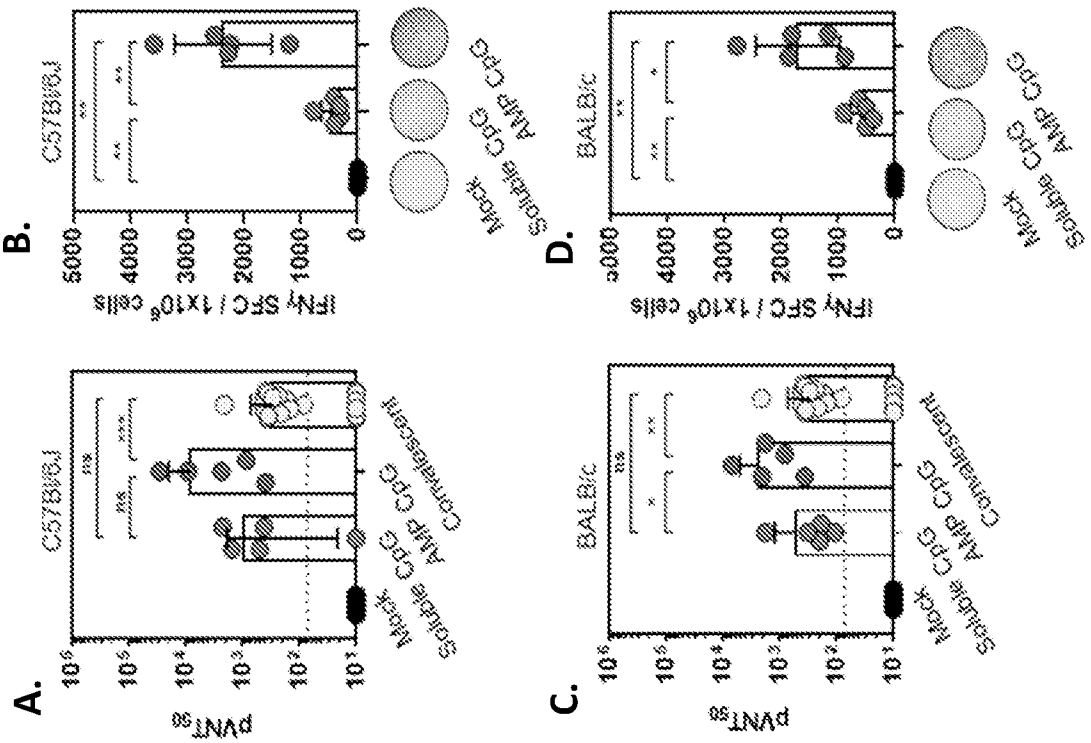


FIG. 17A – FIG. 17C

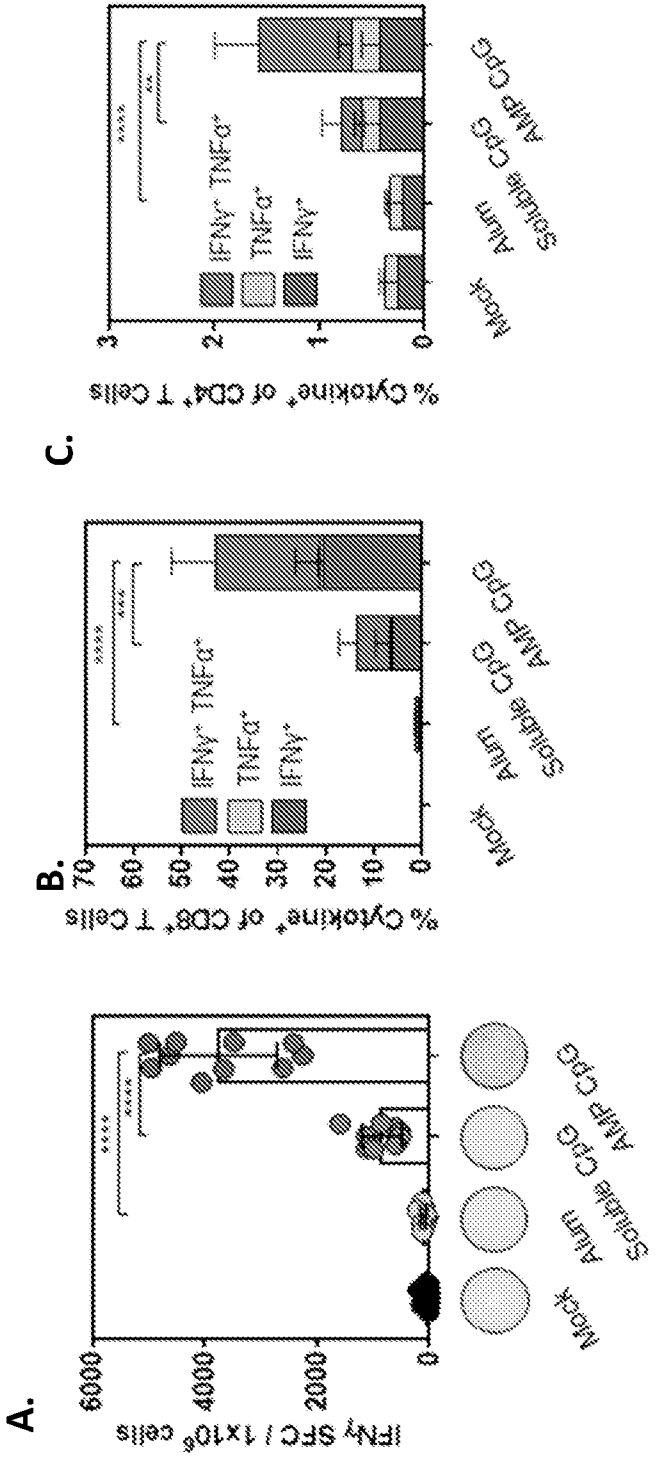


FIG. 18A - FIG. 18B

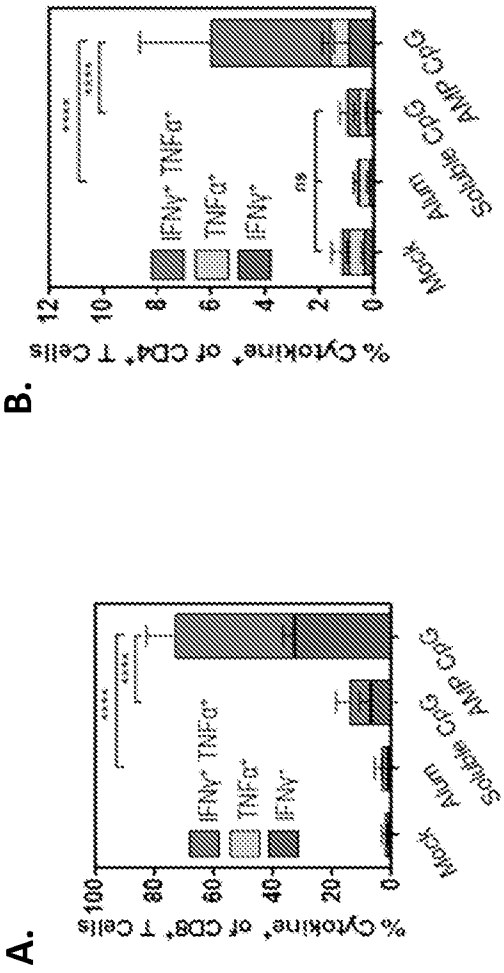


FIG. 18C - FIG. 18D

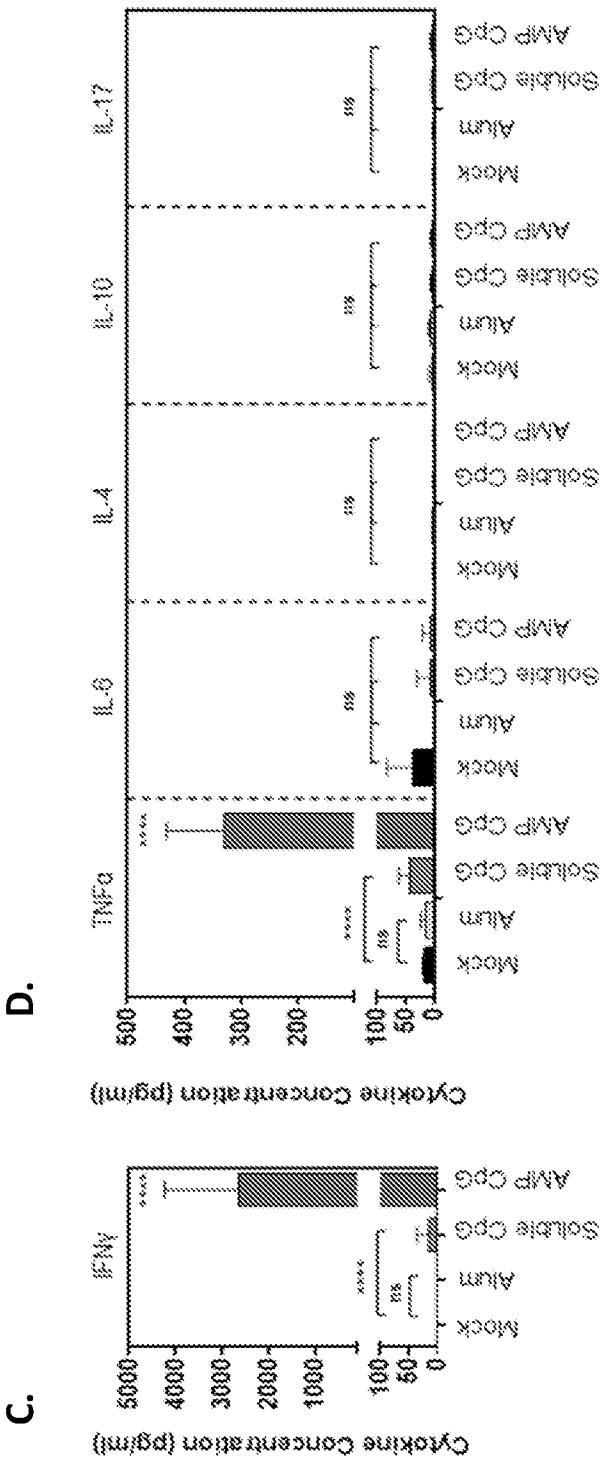


FIG. 19A - FIG. 19B

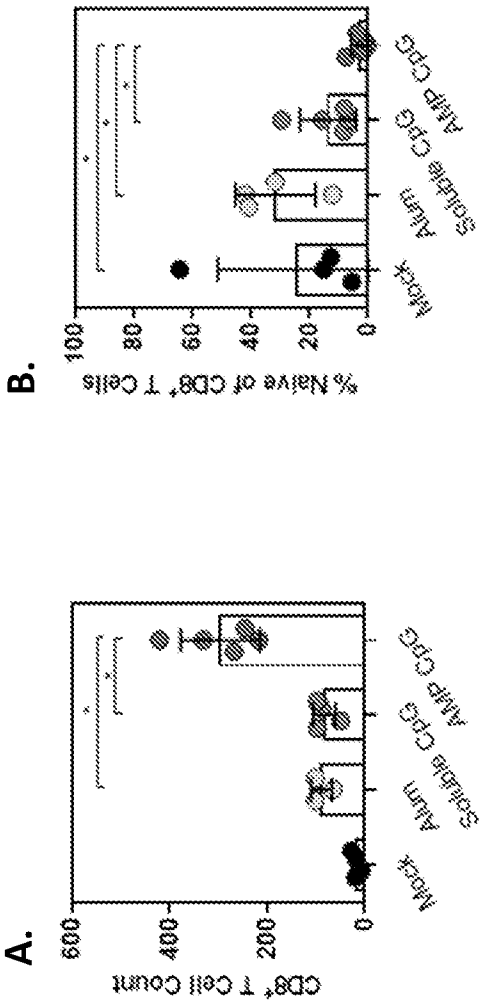


FIG. 19C - FIG. 19D

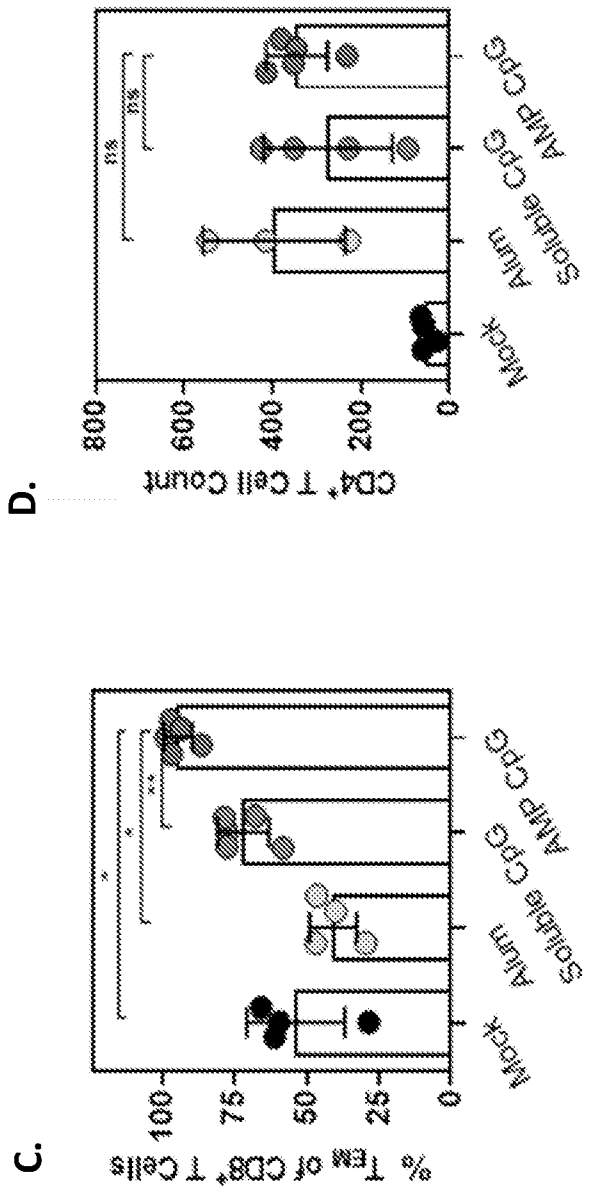


FIG. 19E - FIG. 19F

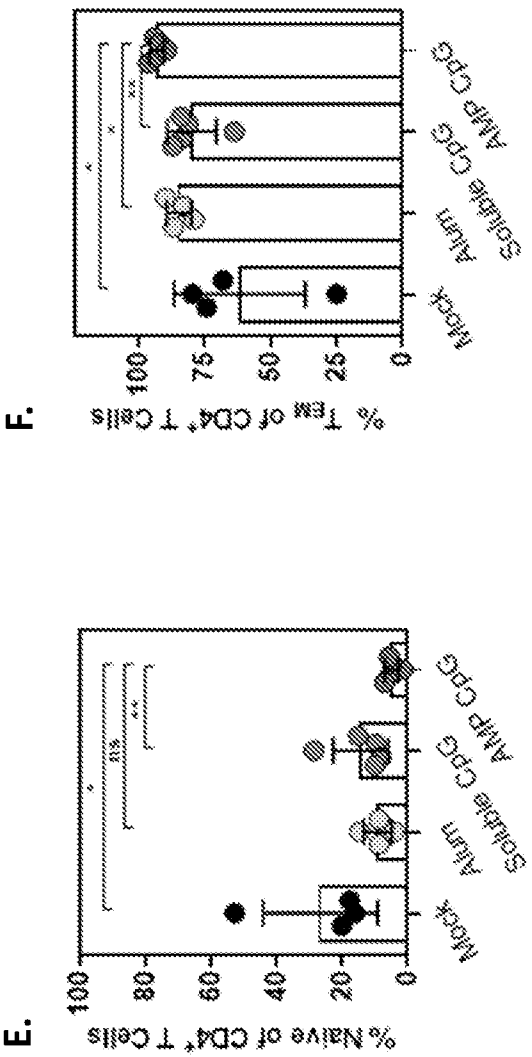


FIG. 20A - FIG. 20B

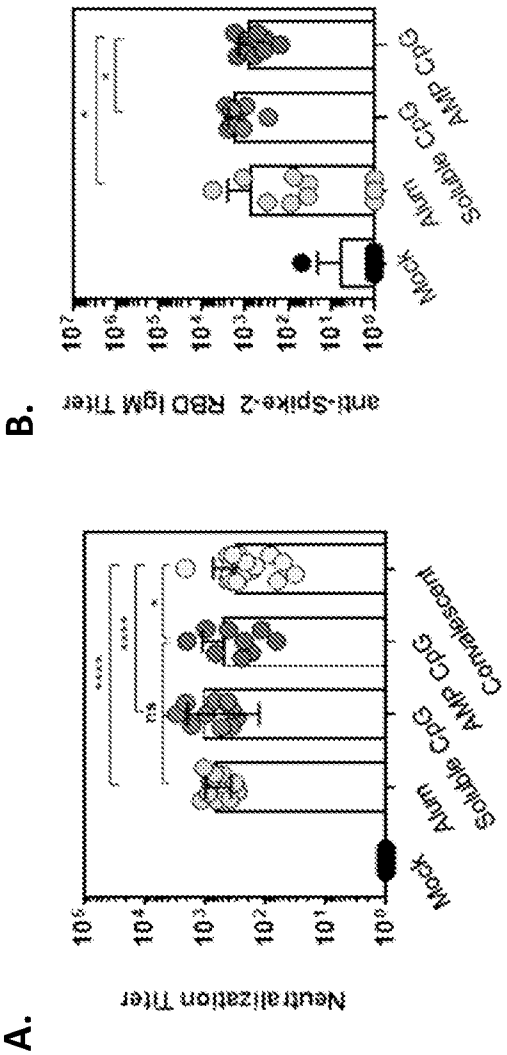
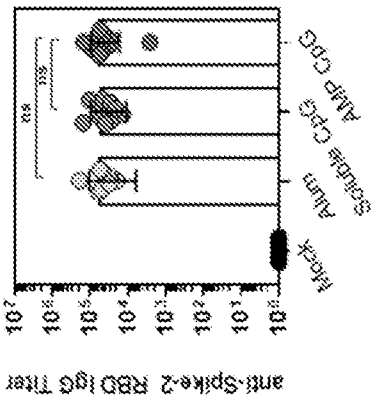


FIG. 20C - FIG. 20D

C.



D.

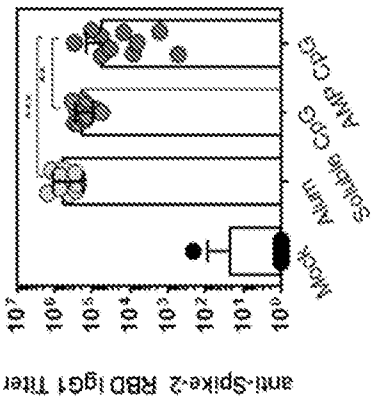
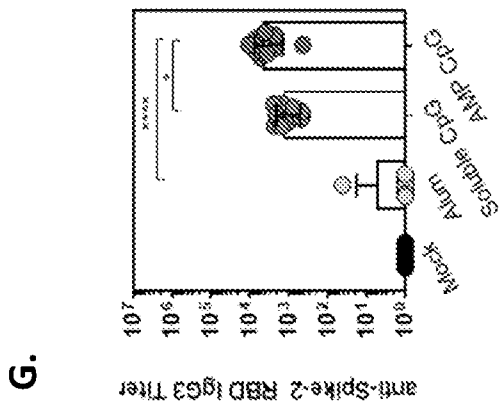
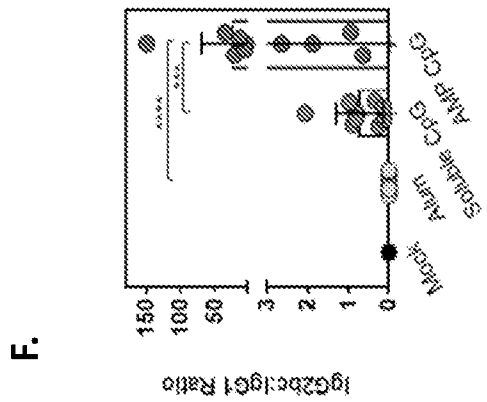


FIG. 20E - FIG. 20G





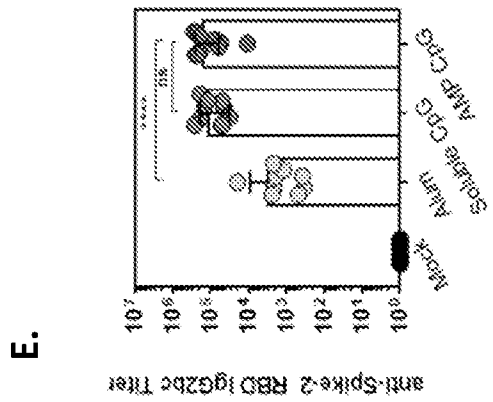


FIG. 21A

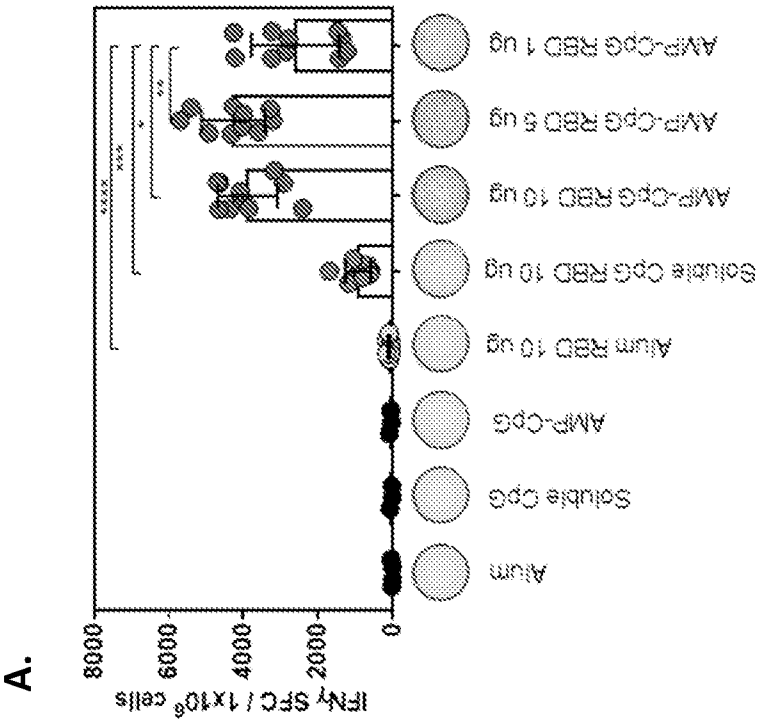

A.
IFNγ SFC / 1x10^6 cells
8000
6000
4000
2000
0
Alum
Soluble CpG
AMP-CpG
Alum RBD 10 ug
Soluble CpG RBD 10 ug
AMP-CpG RBD 10 ug
AMP-CpG RBD 5 ug
AMP-CpG RBD 1 ug
****
***
*
**

FIG. 21B - FIG. 21C

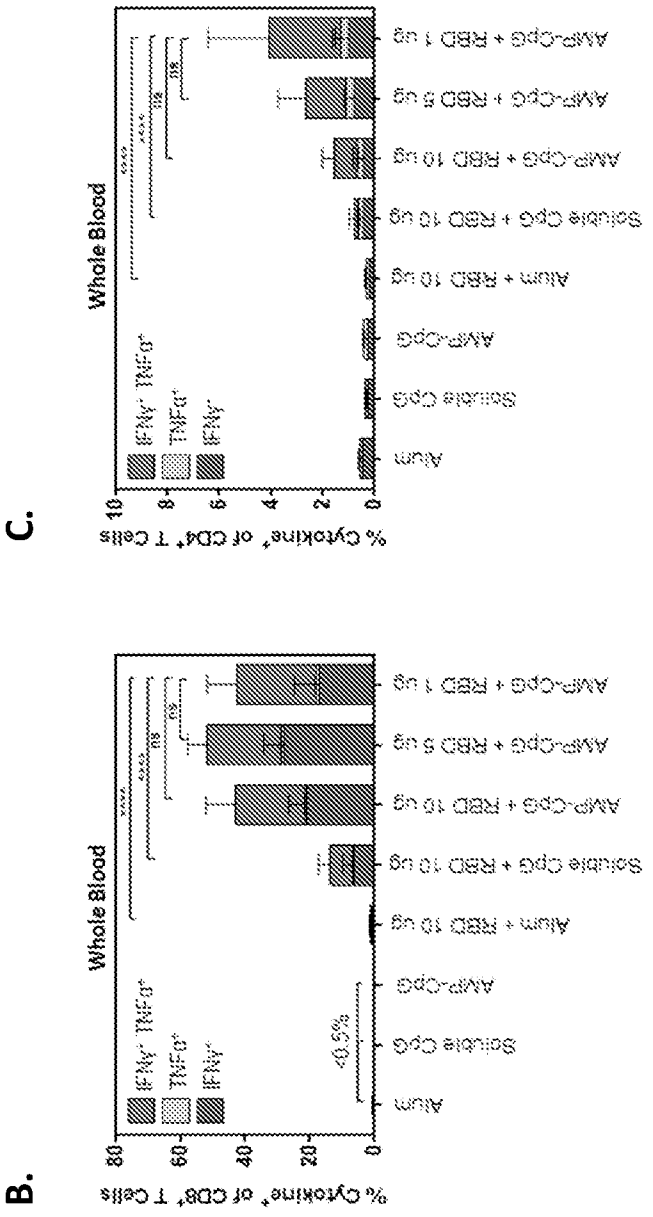


FIG. 21D - FIG. 21E

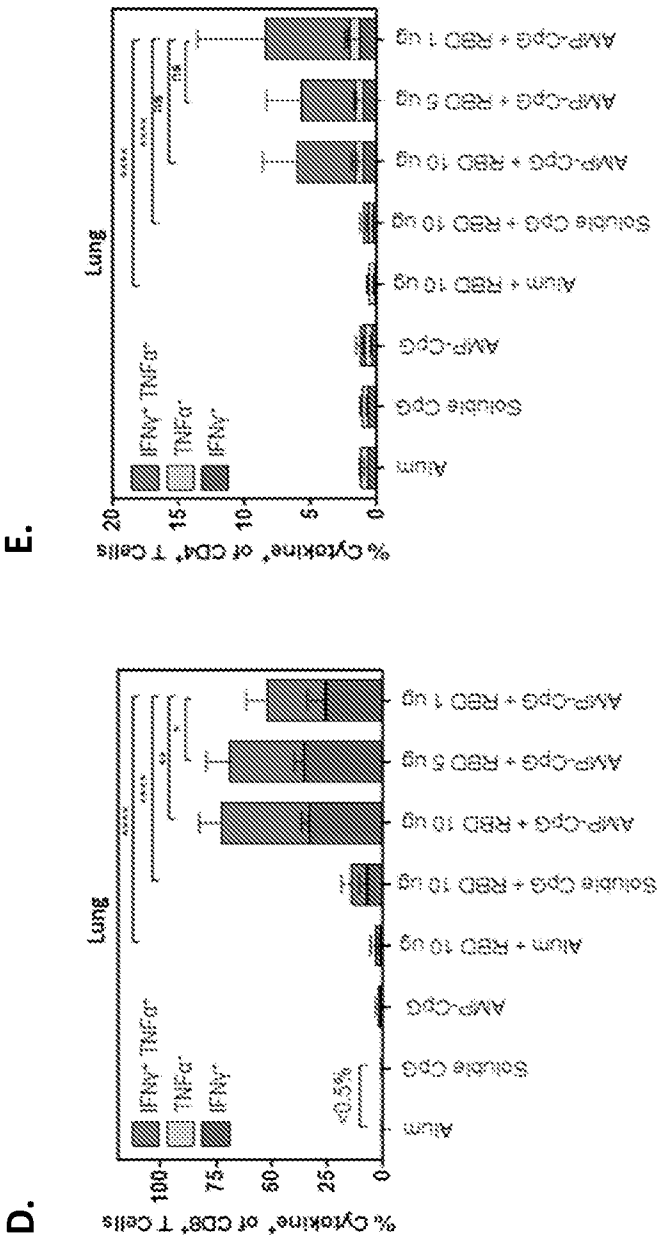


FIG. 22A - FIG. 22B

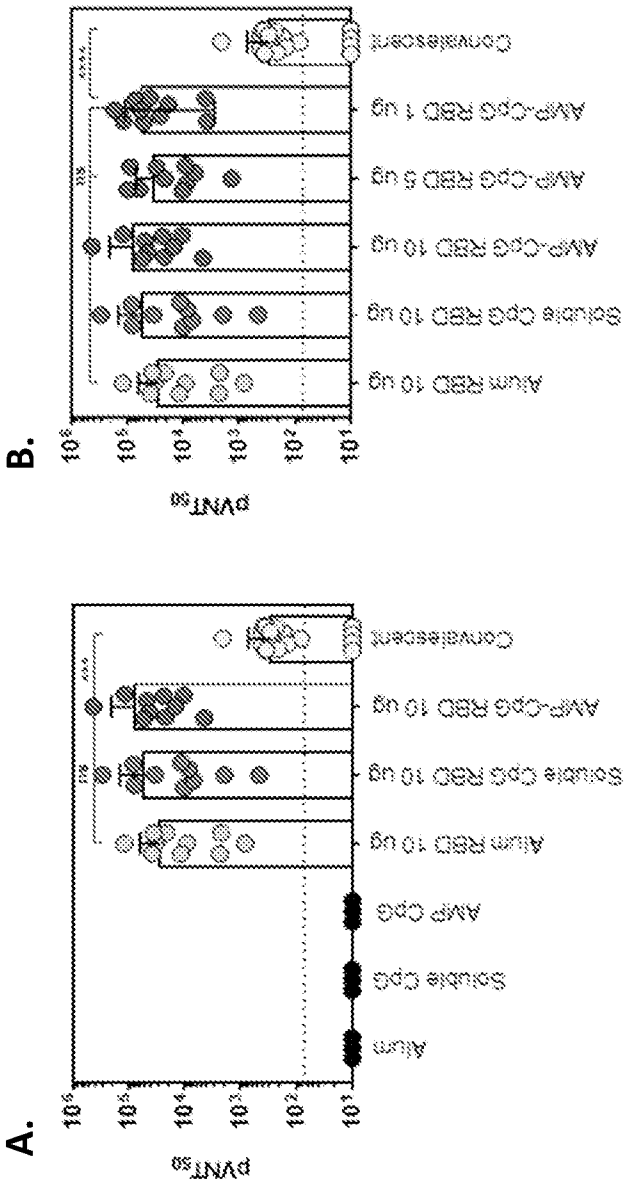
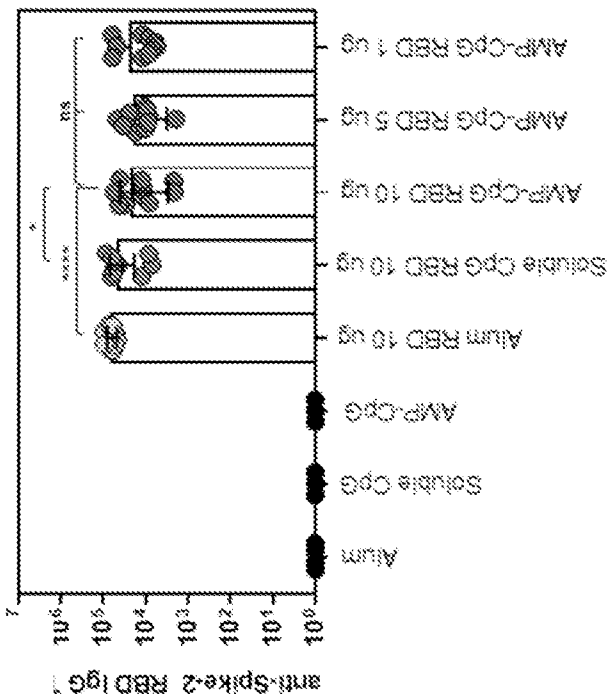


FIG. 22C - FIG. 22D



D.

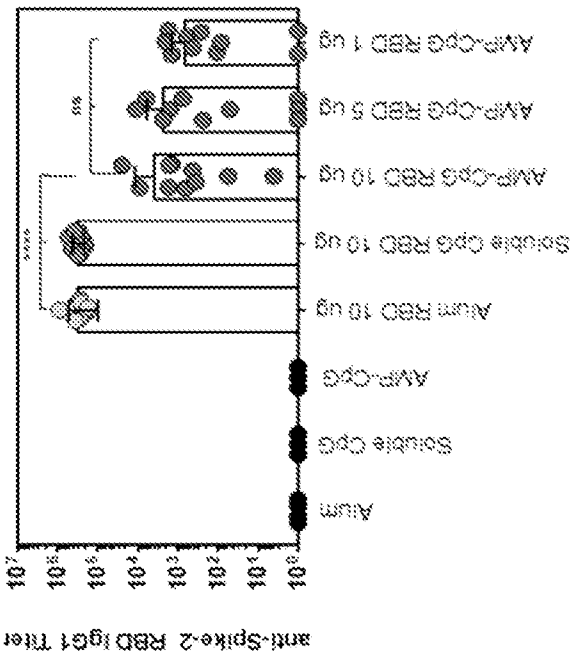
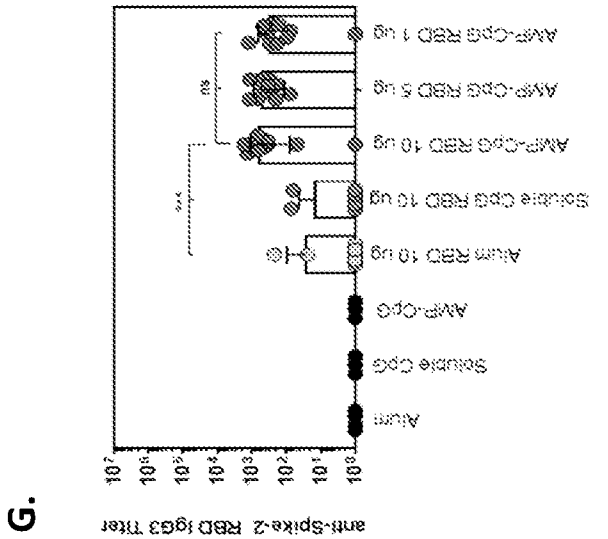
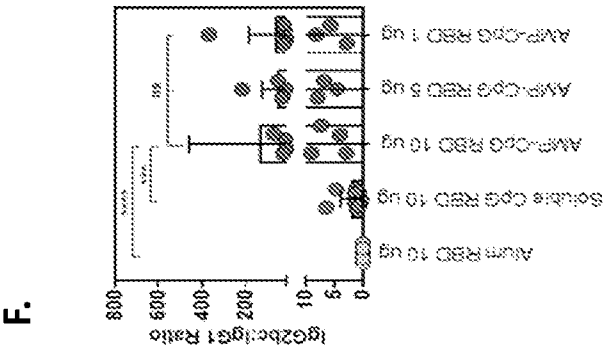


FIG. 22E - FIG. 22G





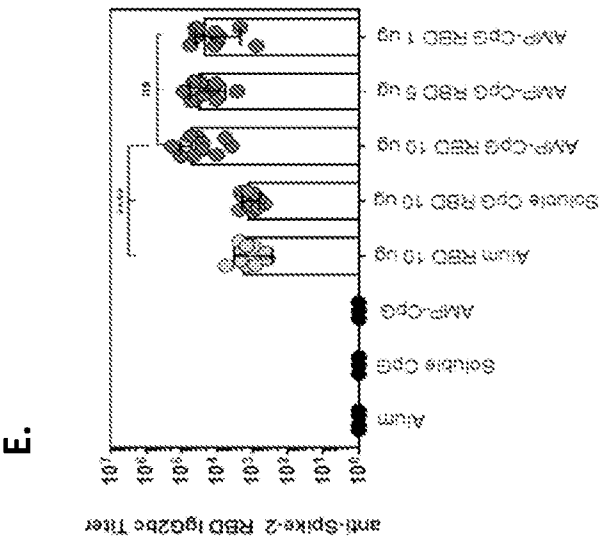


FIG. 23A - FIG. 23B

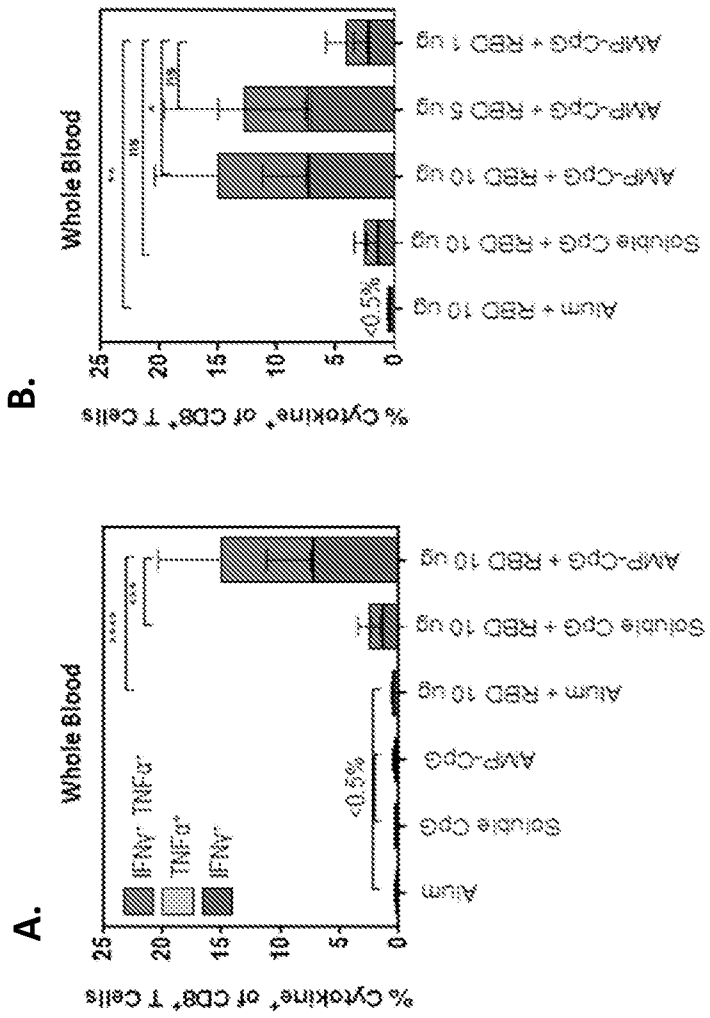


FIG. 23C - FIG. 23D

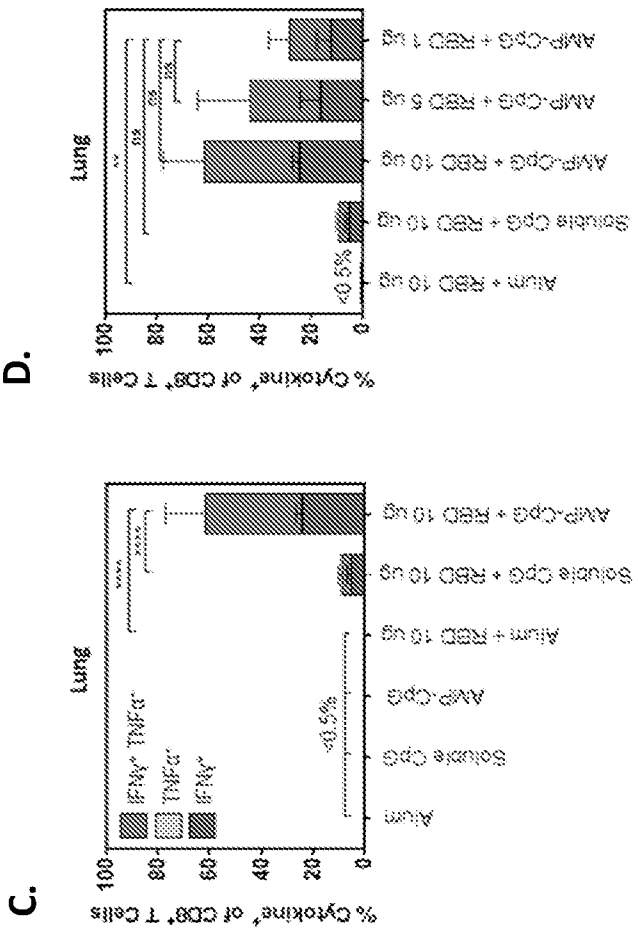


FIG. 23E - FIG. 23F

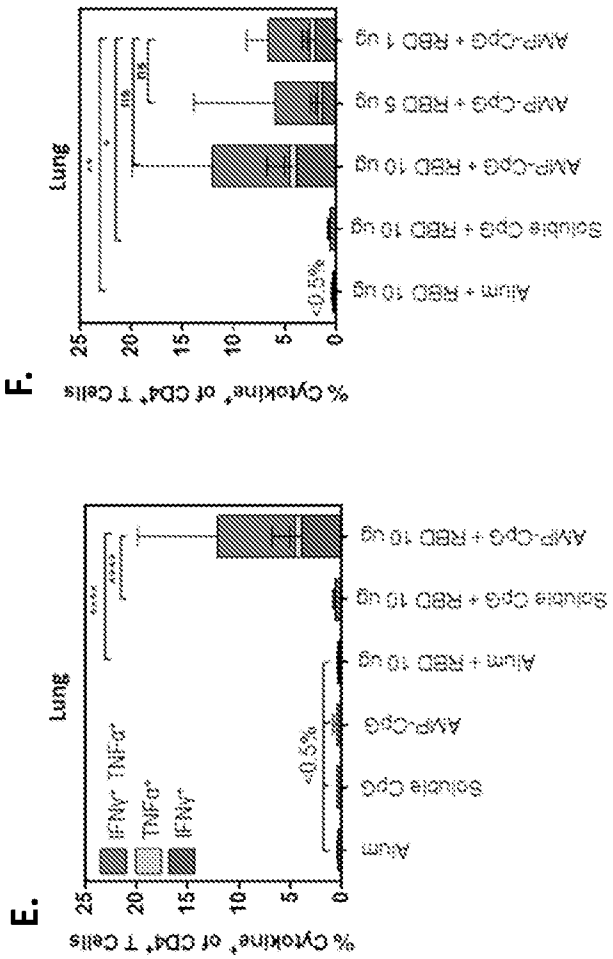


FIG. 24A - FIG. 24B

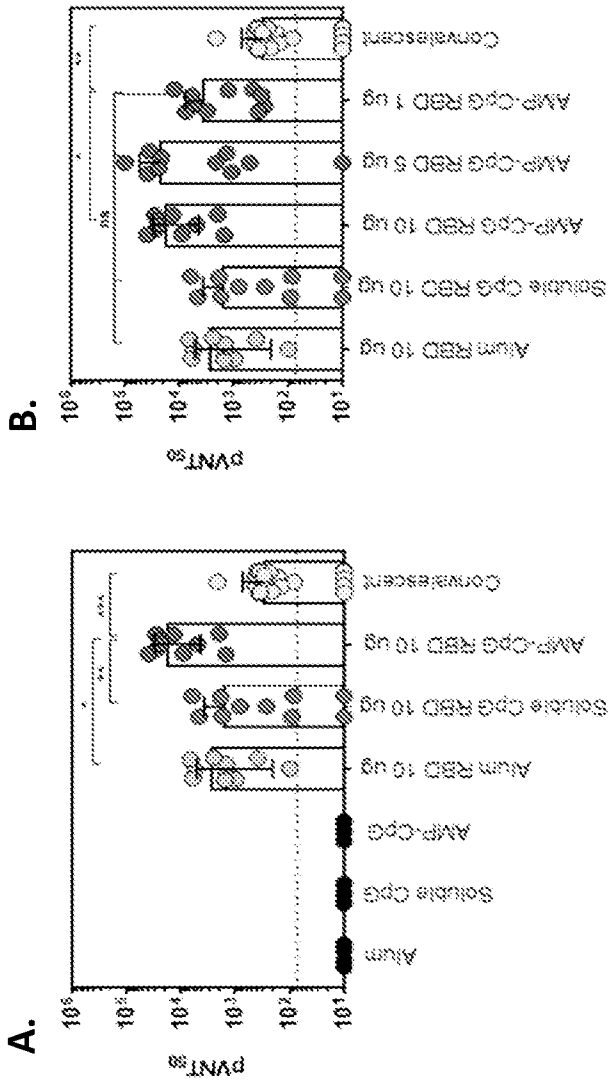


FIG. 24C - FIG. 24D

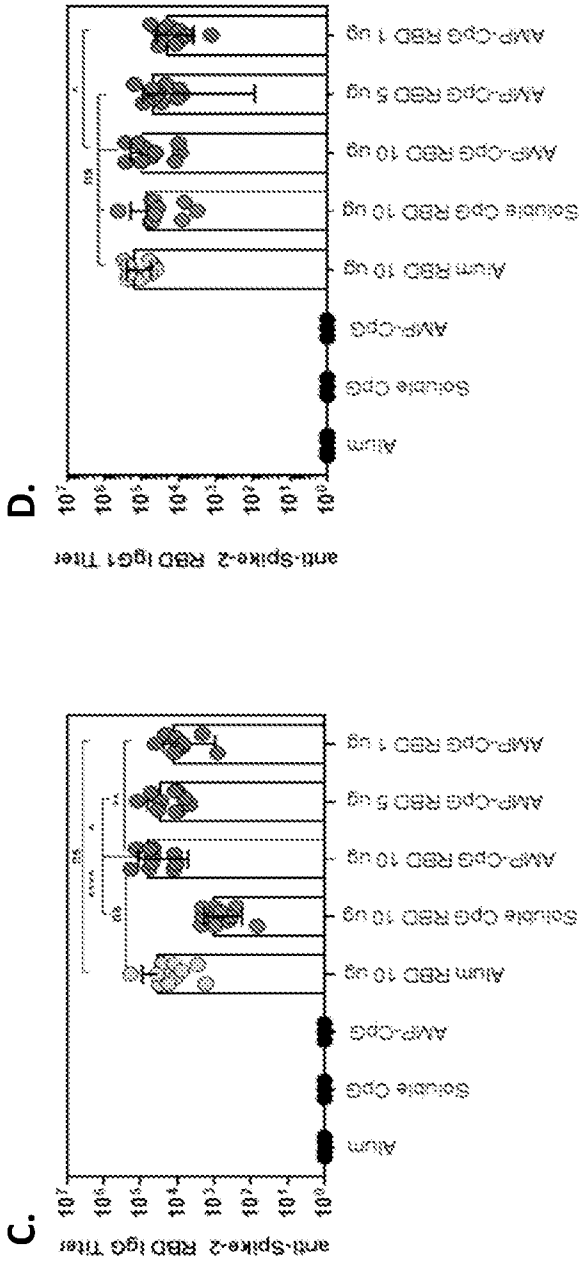


FIG. 24E - FIG. 24G

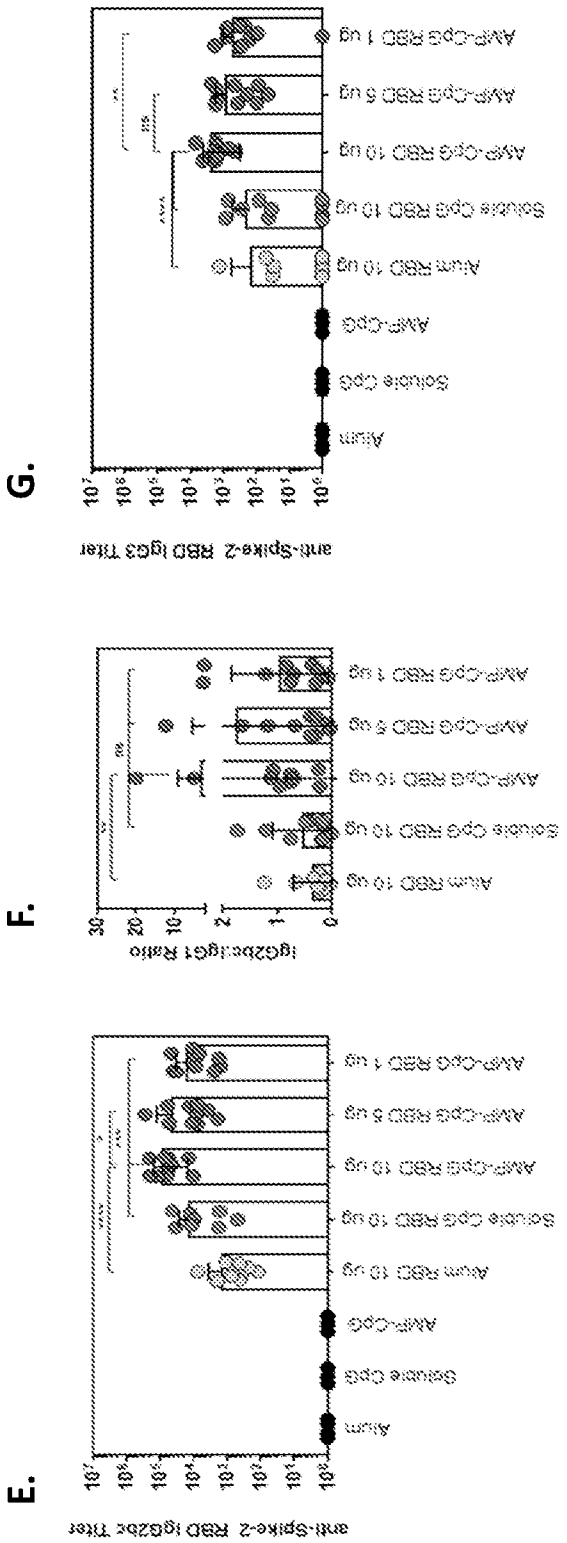
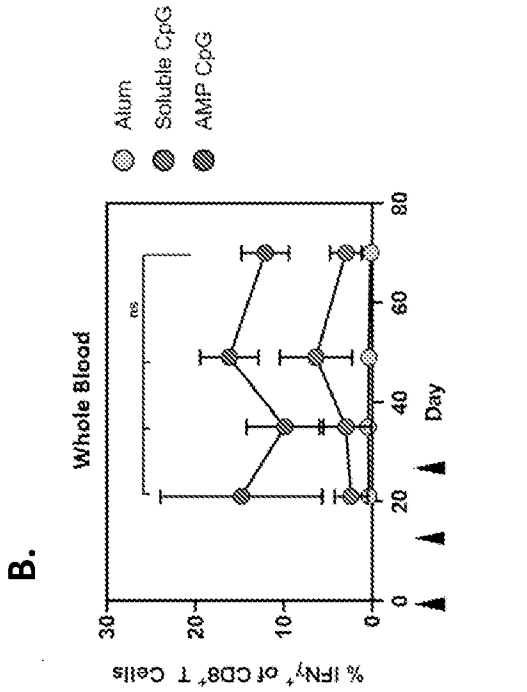


FIG. 25A - FIG. 25B



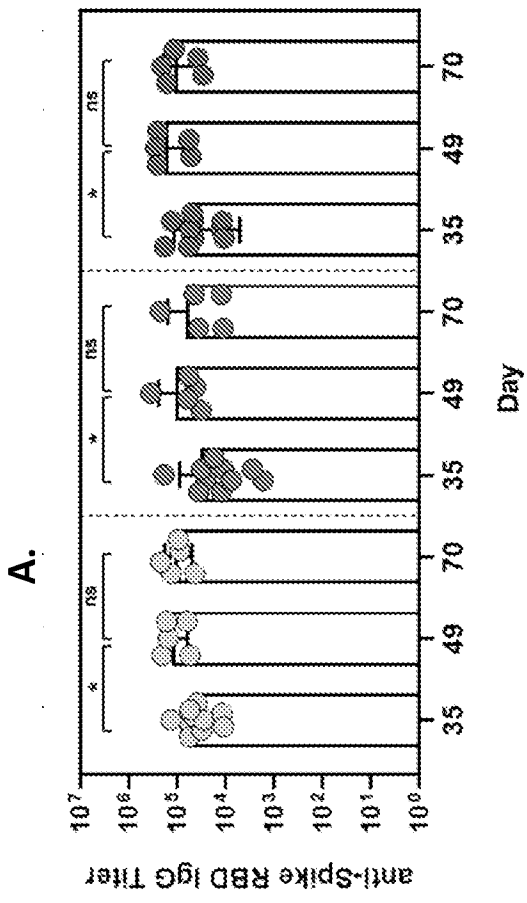
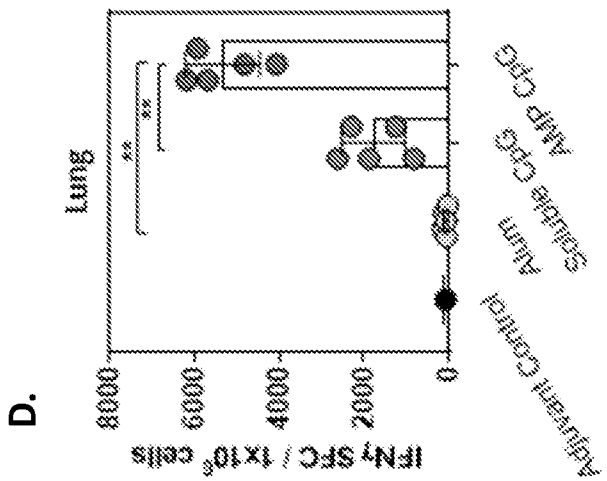


FIG. 25C - FIG. 25D



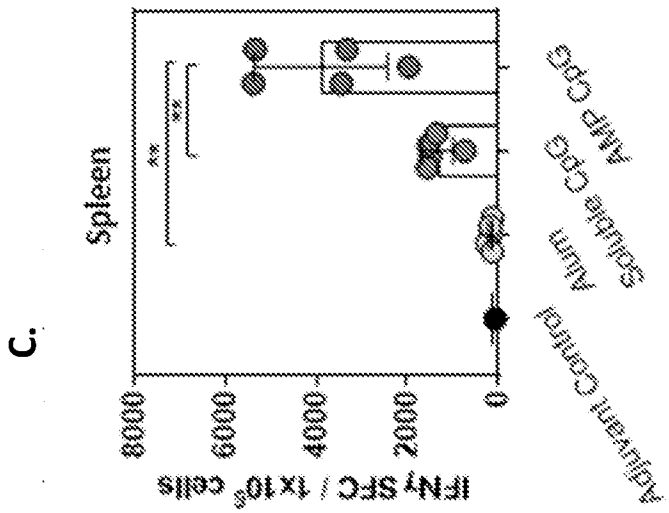
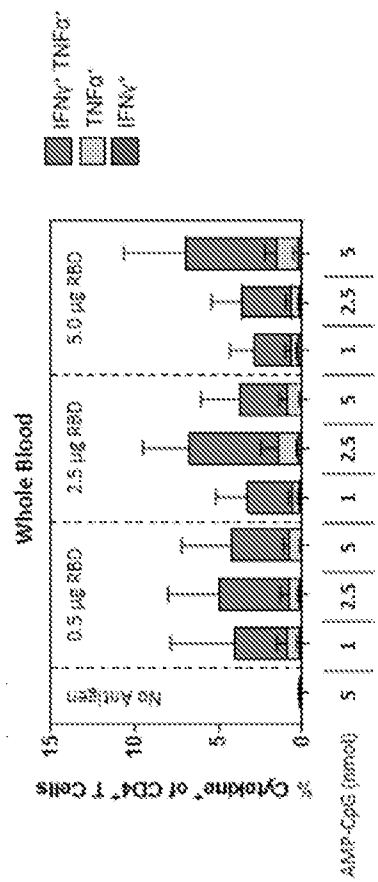


FIG. 26A - FIG. 26B



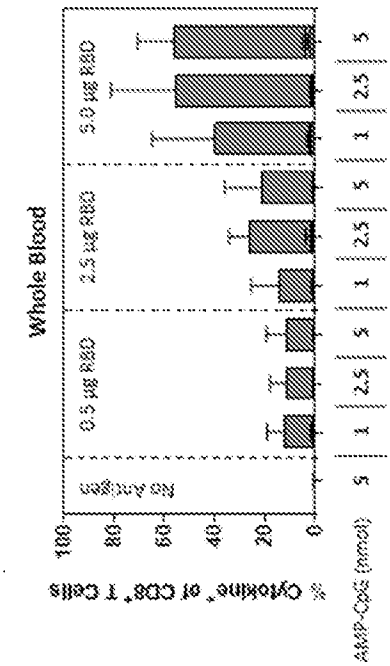


FIG. 26C - FIG. 26E

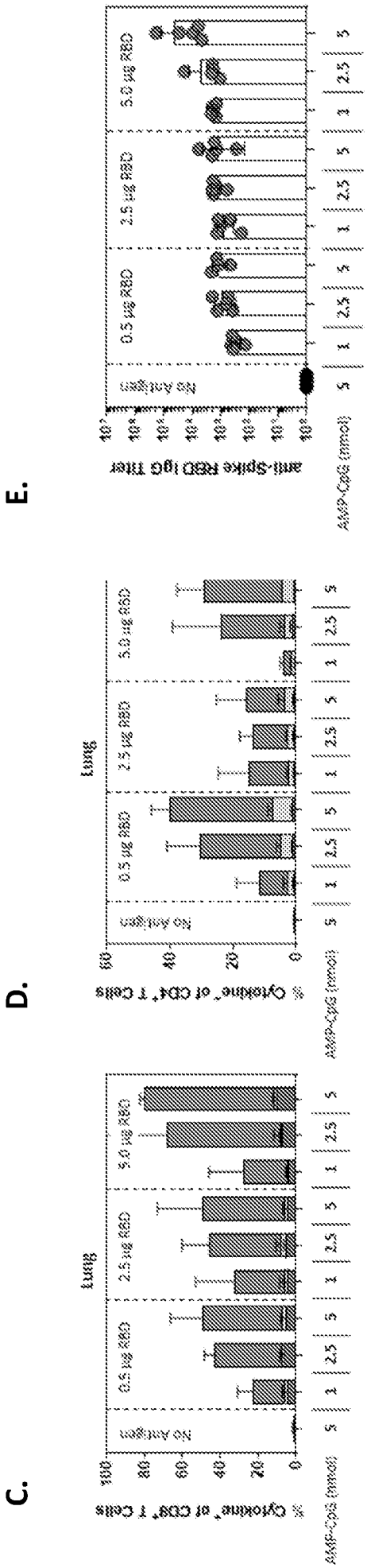


FIG. 27

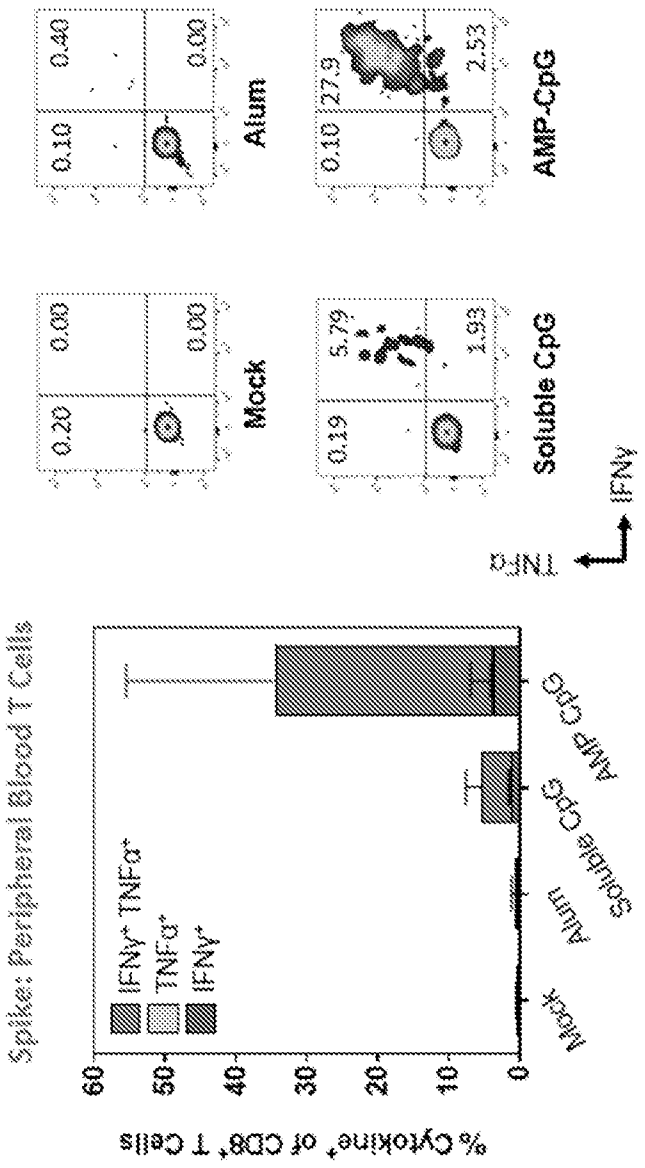

Spike: Peripheral Blood T Cells
% Cytokine+ of CD8+ T Cells
60
50
40
30
20
10
0
IFNγ+ TNFα+
TNFα+
IFNγ+
Mock
Alum
Soluble CpG
AMP-CpG
TNFα
IFNγ
0.20
0.00
0.00
Mock
0.10
0.40
0.00
Alum
0.19
5.79
1.93
Soluble CpG
0.10
27.9
2.53
AMP-CpG

FIG. 28

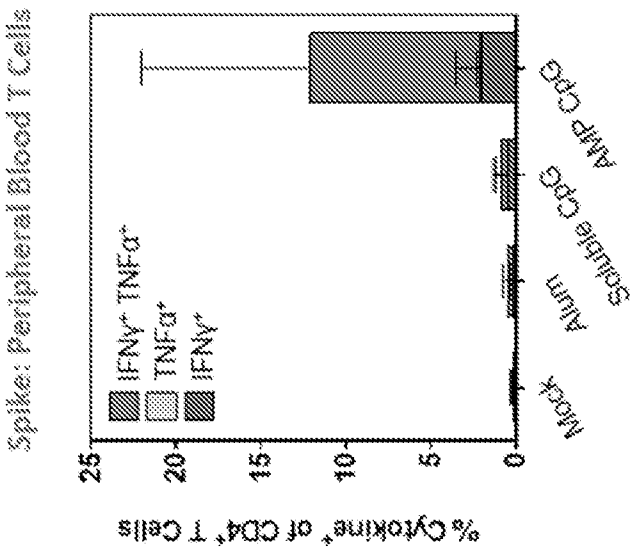

Spike: Peripheral Blood T Cells
% Cytokine+ of CD4+ T cells
25
20
15
10
5
0
IFNγ+ TNFα+
TNFα+
IFNγ+
Mock
Alum
Soluble CpG
AMP CpG
TNFα
IFNγ
0.15
0.00
0.00
Mock
0.33
0.44
0.11
Alum
0.00
0.42
0.21
Soluble CpG
0.11
10.5
3.84
AMP-CpG

FIG. 29

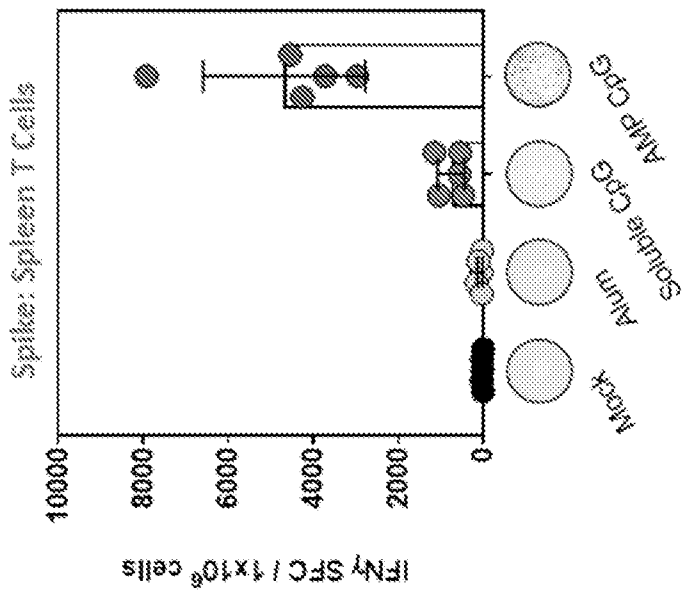

Spike: Spleen T Cells
IFNγ SFC / 1x10^6 cells
10000
8000
6000
4000
2000
0
Mock
Alum
Soluble CpG
Alum CpG

FIG. 30

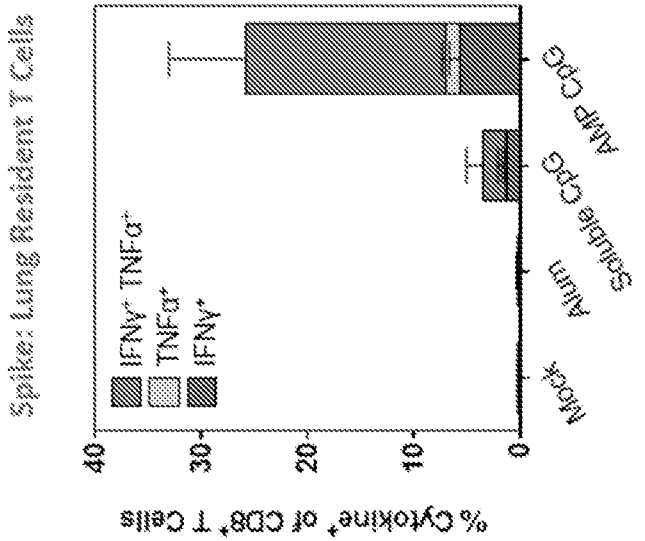

Spike: Lung Resident T Cells
IFNγ+ TNFα+
TNFα+
IFNγ+
% Cytokine+ of CD8+ T Cells
0
10
20
30
40
Mock
Alum
Soluble CpG
AMP CpG
TNFα
IFNγ
0.08
0.2
0.06
0.10
2.54
1.52
0.05
0.05
0.00
1.21
19.3
5.60
Mock
Soluble CpG
Alum
AMP-CpG

FIG. 31

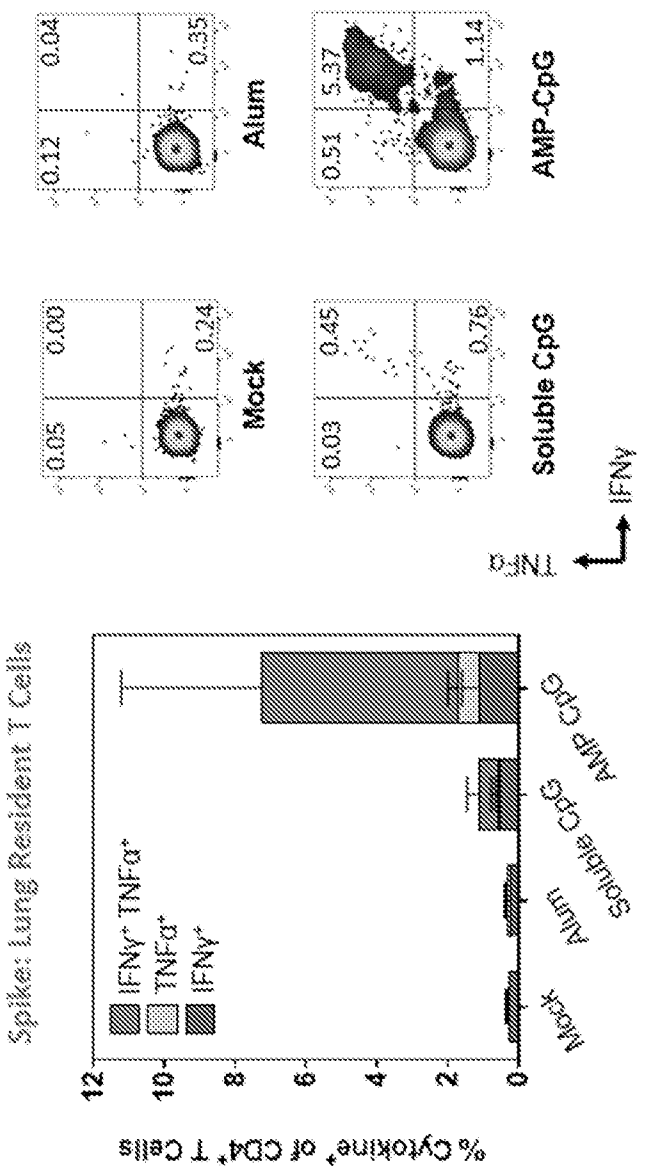

Spike: Lung Resident T Cells
% Cytokine+ of CD4+ T Cells
IFNγ+ TNFα+
TNFα+
IFNγ+
Mock
Alum
Soluble CpG
AMP CpG
TNFα
IFNγ
0.05
0.00
0.24
0.12
0.04
0.35
0.03
0.45
0.76
0.51
5.37
1.14
Mock
Soluble CpG
Alum
AMP-CpG

FIG. 32

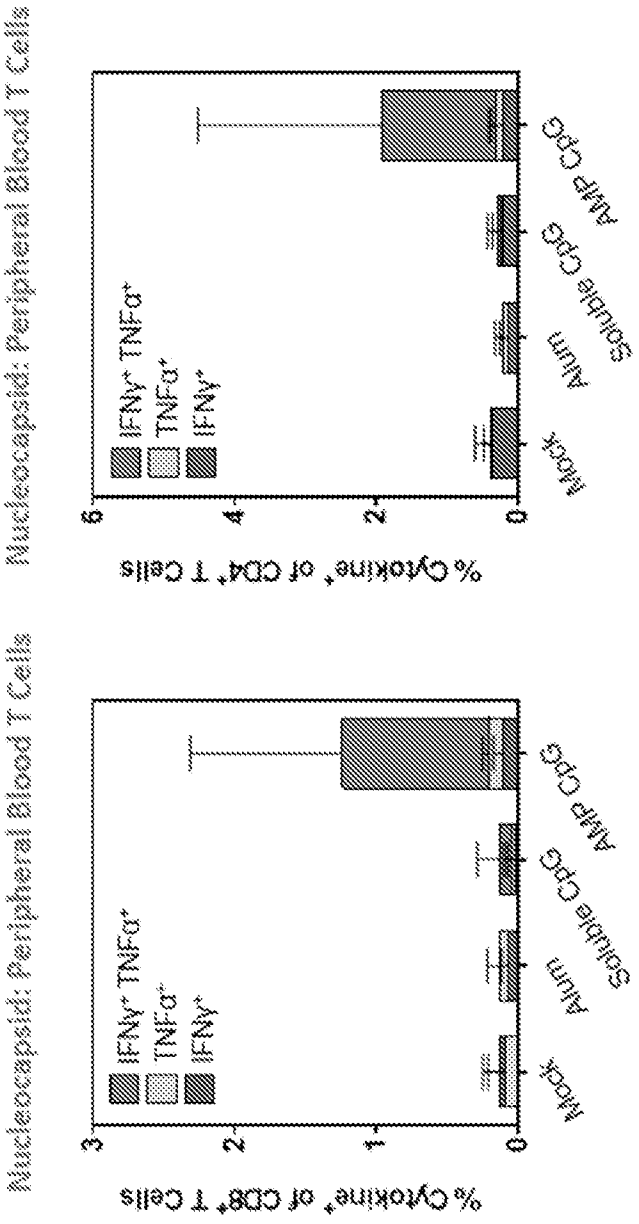

Nucleocapsid: Peripheral Blood T Cells
% Cytokine+ of CD8+ T cells
IFNγ+ TNFα+
TNFα+
IFNγ+
Mock
Alum
Soluble cpG
Alhp cpG
Nucleocapsid: Peripheral Blood T Cells
% Cytokine+ of CD4+ T cells
IFNγ+ TNFα+
TNFα+
IFNγ+
Mock
Alum
Soluble cpG
Alhp cpG

FIG. 33

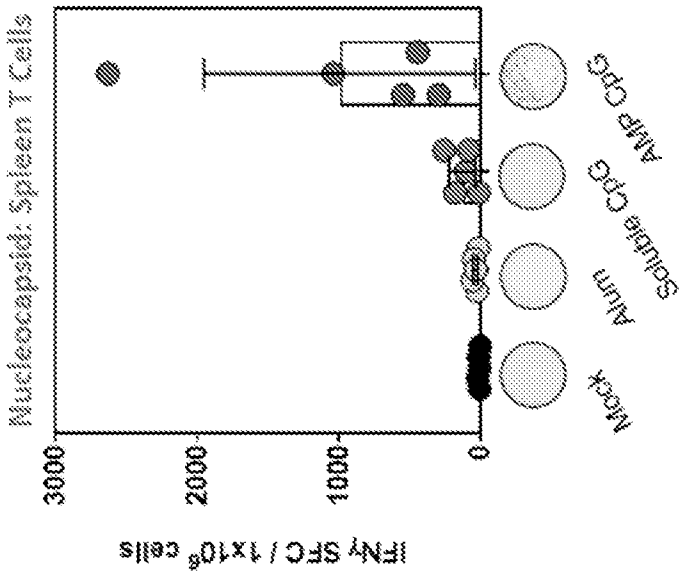

Nucleocapsid: Spleen T Cells
IFNγ SFC / 1x10^6 cells
3000
2000
1000
0
Mock
Alum
Soluble CpG
Alum CpG

FIG. 34

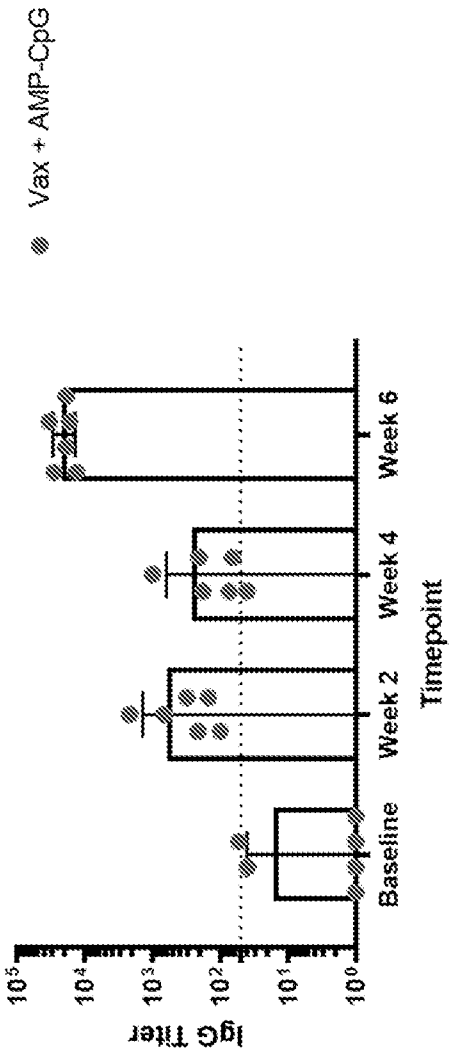

Vax + AMP-CpG
IgG Titer
10^5
10^4
10^3
10^2
10^1
10^0
Baseline
Week 2
Week 4
Week 6
Timepoint

FIG. 35

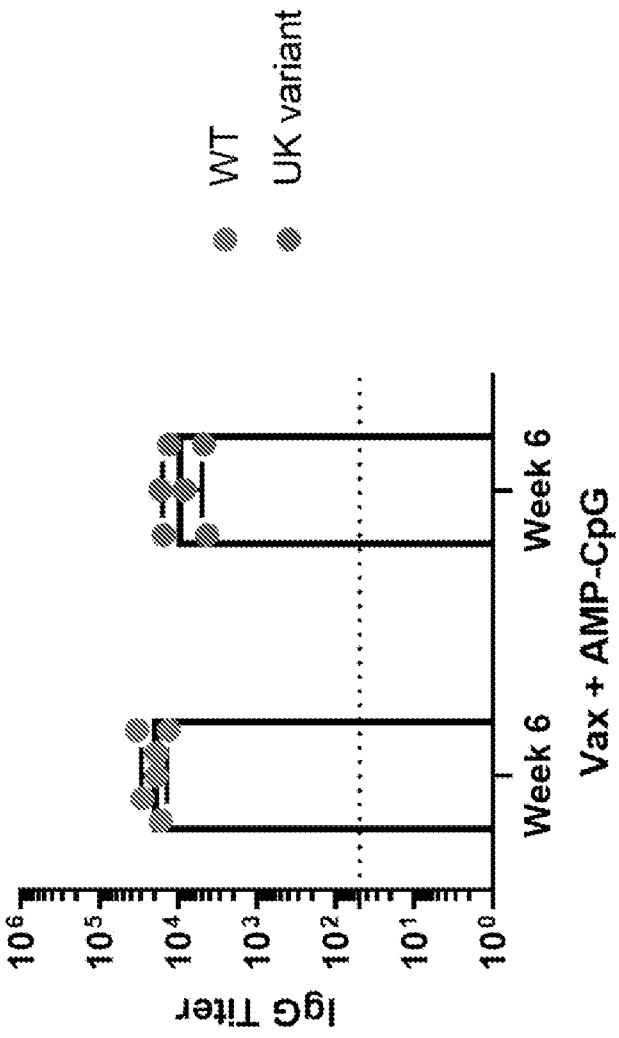

WT
UK variant
IgG Titer
10^6
10^5
10^4
10^3
10^2
10^1
10^0
Week 6
Week 6
Vax + AMP-CpG

FIG. 36A

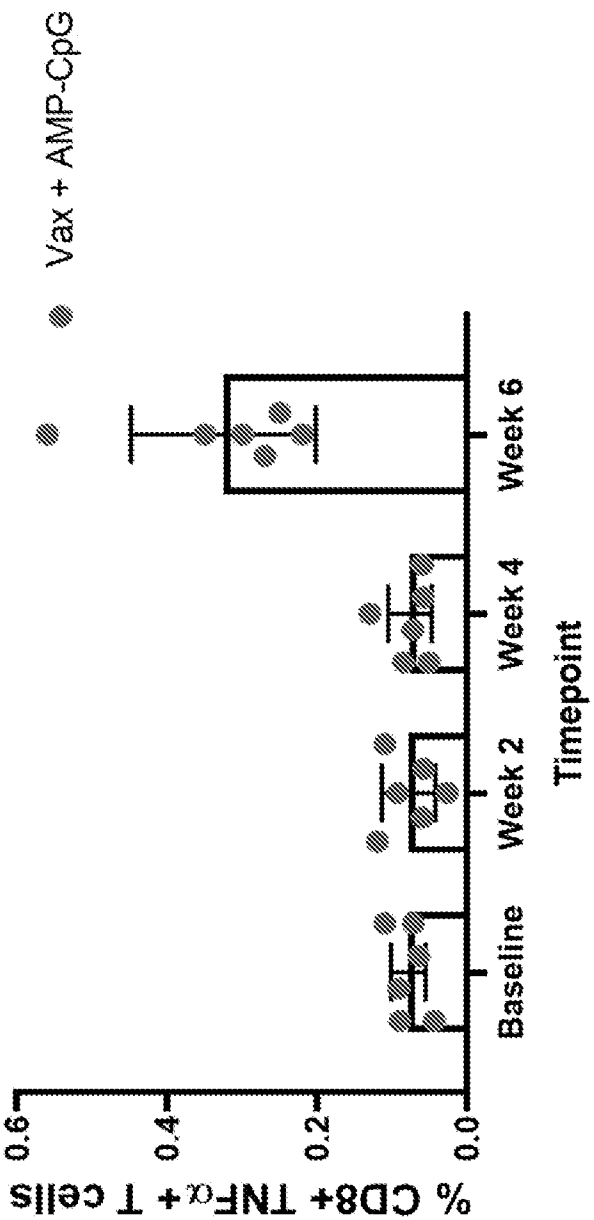

Vax + AMP-CpG
% CD8+ TNFα+ T cells
0.0
0.2
0.4
0.6
Baseline
Week 2
Week 4
Week 6
Timepoint

FIG. 36B

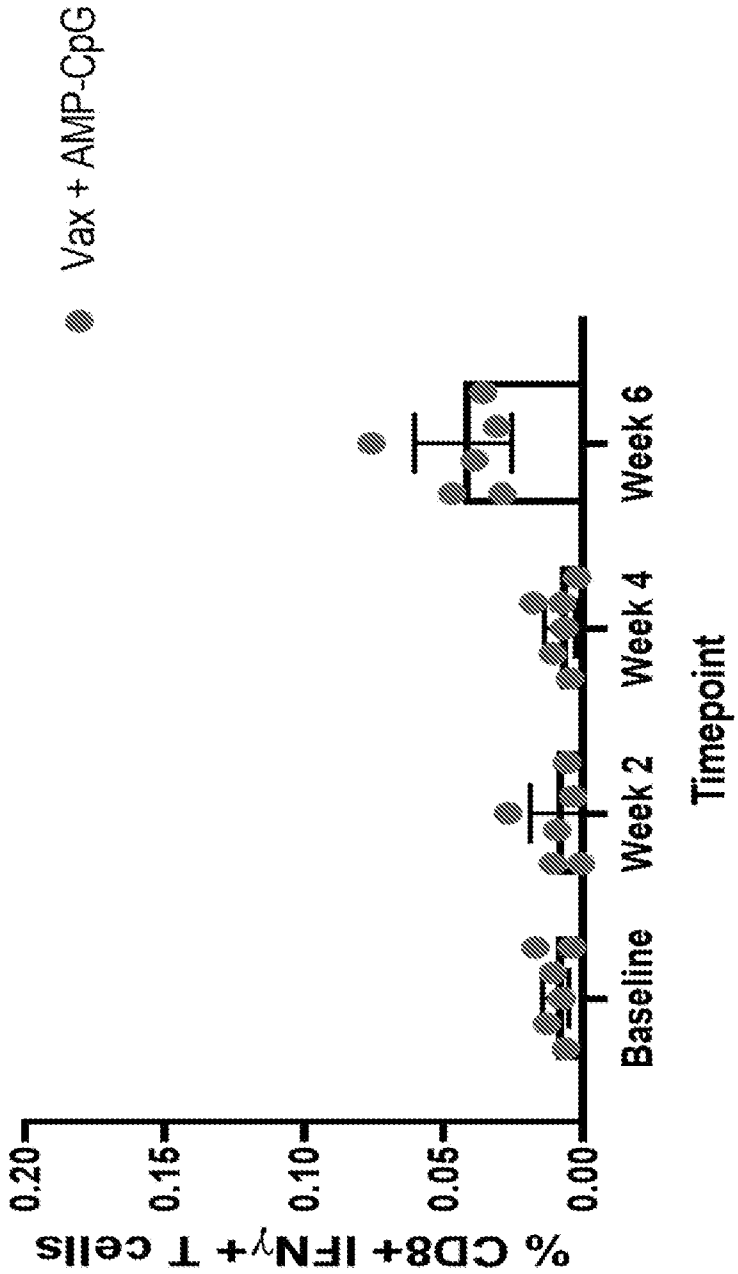
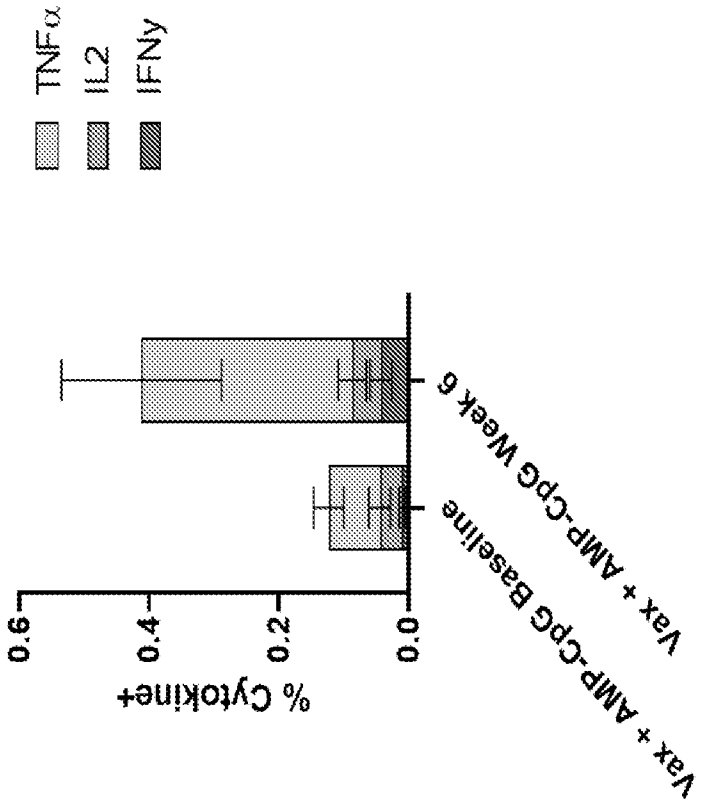

Vax + AMP-CpG
% CD8+ IFNγ+ T cells
0.20
0.15
0.10
0.05
0.00
Baseline
Week 2
Week 4
Week 6
Timepoint

FIG. 37A


TNFα
IL2
IFNγ
% Cytokine+
0.0
0.2
0.4
0.6
Vax + AMP-CpG Baseline
Vax + AMP-CpG Week 6

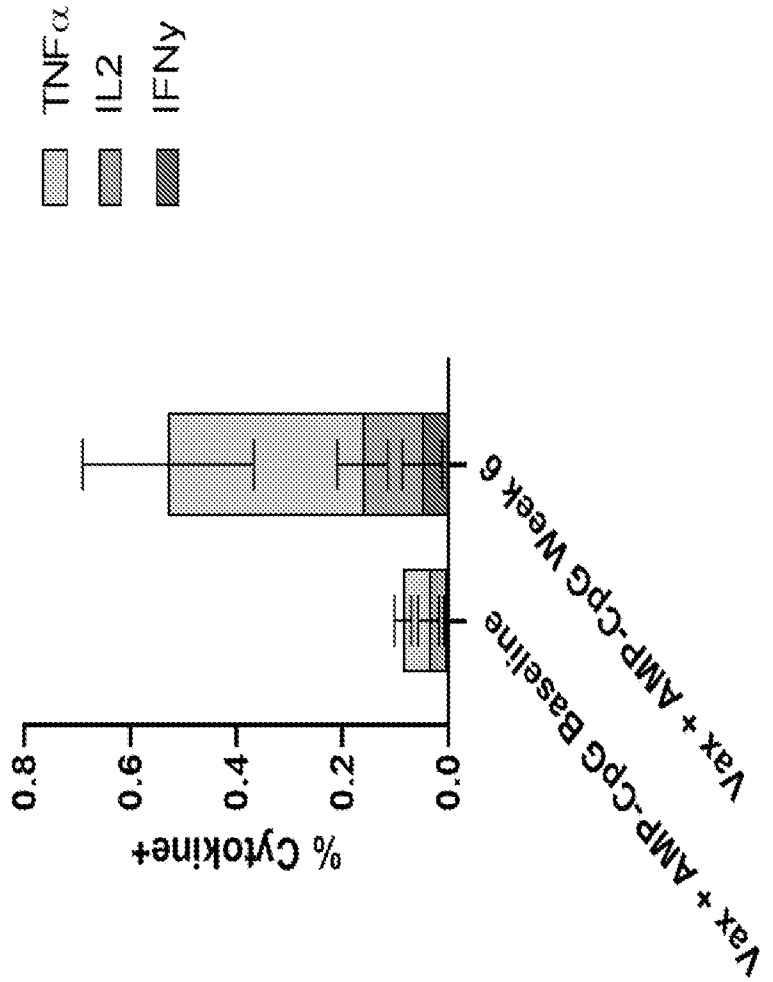

TNFα
IL2
IFNγ
% Cytokine+
0.0
0.2
0.4
0.6
0.8
Vax + AMP-CpG Baseline
Vax + AMP-CpG Week 6

FIG. 37B

FIG. 38

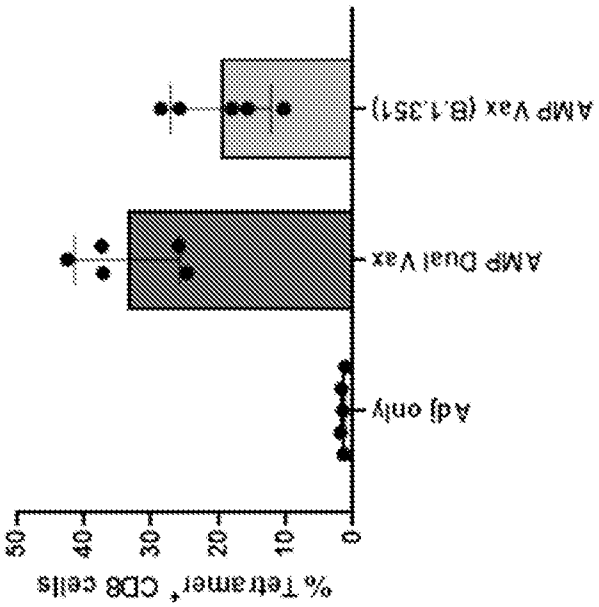

% Tetramer+ CD8 cells
0
10
20
30
40
50
Ad only
AMP Dual Vax
AMP Vax (B.1.351)

FIG. 39A

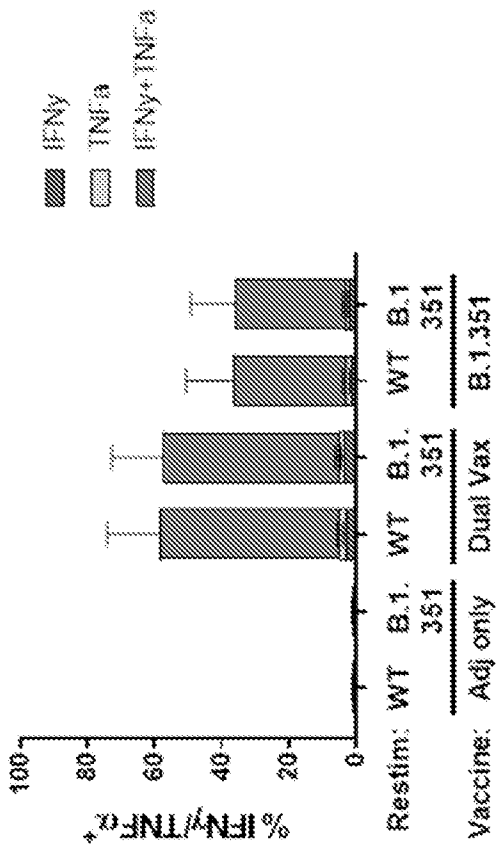

IFNγ
TNFα
IFNγ+TNFα
% IFNγ/TNFα+
100
80
60
40
20
0
Restim: WT B.1.351 WT B.1.351 WT B.1.351
Vaccine: Adj only Dual Vax B.1.351

FIG. 39B

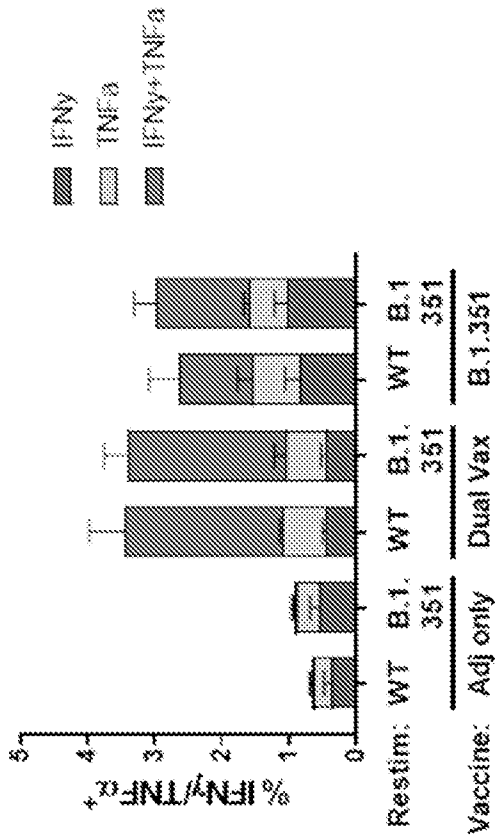

IFNγ
TNFα
IFNγ+TNFα
% IFNγ/TNFα+
0
1
2
3
4
5
Restim: WT B.1.351 WT B.1.351 WT B.1.351
Vaccine: Adj only Dual Vax B.1.351

FIG. 39C

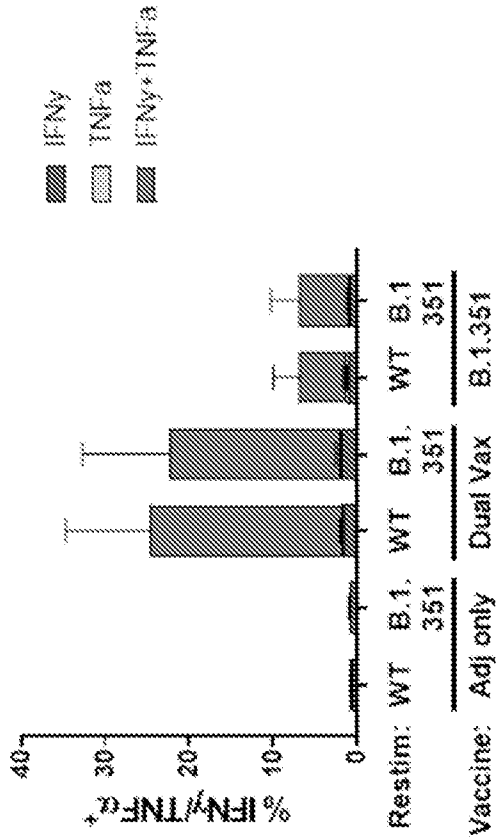

IFNγ
TNFα
IFNγ+TNFα
% IFNγ/TNFα+
40
30
20
10
0
Restim: WT B.1.351 WT B.1.351 WT B.1.351
Vaccine: Adj only Dual Vax B.1.351

FIG. 40

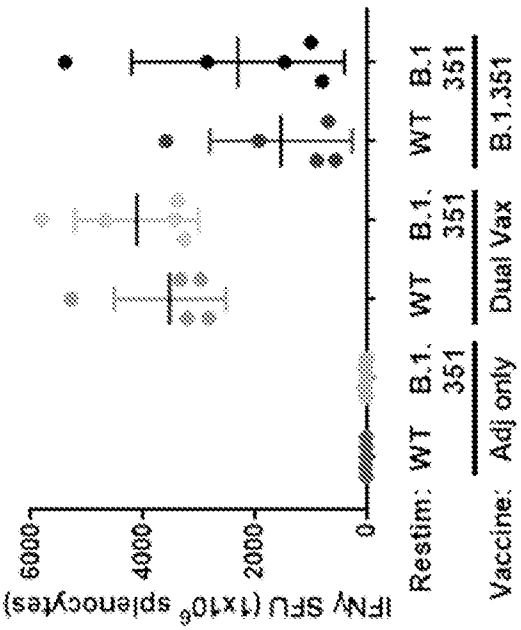
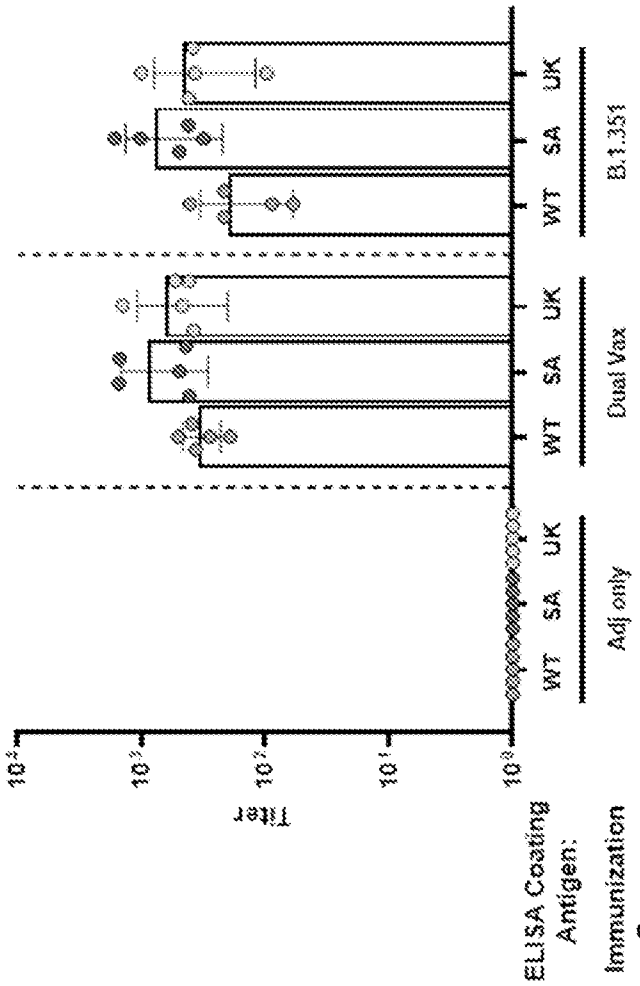

IFNγ SFU (1x10^6 splenocytes)
6000
4000
2000
0
Restim: WT B.1.351 WT B.1.351 WT B.1.351
Vaccine: Adj only Dual Vax B.1.351

FIG. 41


Post Dose 2
Titer
10^4
10^3
10^2
10^1
10^0
ELISA Coating Antigen:
WT SA UK WT SA UK WT SA UK
Immunization Groups:
Adj only
Dual Vax
B.1.351

SEQUENCE LISTING

<110> Elicio Therapeutics, Inc.

<120> COMPOSITIONS AND METHODS FOR INDUCING AN IMMUNE RESPONSE AGAINST CORONAVIRUS

<130> 51026-039WO5

<140> PCT/US2021/039134
<141> 2021-06-25

<150> US 63/145,200
<151> 2021-02-03

<150> US 63/124,200
<151> 2020-12-11

<150> US 63/064,836
<151> 2020-08-12

<150> US 63/044,773
<151> 2020-06-26

<160> 71

<170> PatentIn version 3.5

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<220>
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tcgtcgtttt gtcgttttgt cgtt 24

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<211> 20
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<213> Artificial Sequence

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<400> 2
tccatgacgt tcctgacgtt 20

<210> 3

<211> 273
<212> PRT
<213> Severe acute respiratory syndrome coronavirus 2

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Arg Val Gln Pro Thr Glu Ser Ile Val Arg Phe Pro Asn Ile Thr Asn
1 5 10 15

Leu Cys Pro Phe Gly Glu Val Phe Asn Ala Thr Arg Phe Ala Ser Val
20 25 30

Tyr Ala Trp Asn Arg Lys Arg Ile Ser Asn Cys Val Ala Asp Tyr Ser
35 40 45

Val Leu Tyr Asn Ser Ala Ser Phe Ser Thr Phe Lys Cys Tyr Gly Val
50 55 60

Ser Pro Thr Lys Leu Asn Asp Leu Cys Phe Thr Asn Val Tyr Ala Asp
65 70 75 80

Ser Phe Val Ile Arg Gly Asp Glu Val Arg Gln Ile Ala Pro Gly Gln
85 90 95

Thr Gly Lys Ile Ala Asp Tyr Asn Tyr Lys Leu Pro Asp Asp Phe Thr
100 105 110

Gly Cys Val Ile Ala Trp Asn Ser Asn Asn Leu Asp Ser Lys Val Gly
115 120 125

Gly Asn Tyr Asn Tyr Leu Tyr Arg Leu Phe Arg Lys Ser Asn Leu Lys
130 135 140

Pro Phe Glu Arg Asp Ile Ser Thr Glu Ile Tyr Gln Ala Gly Ser Thr
145 150 155 160

Pro Cys Asn Gly Val Glu Gly Phe Asn Cys Tyr Phe Pro Leu Gln Ser
165 170 175

Tyr Gly Phe Gln Pro Thr Asn Gly Val Gly Tyr Gln Pro Tyr Arg Val
180 185 190

Val Val Leu Ser Phe Glu Leu Leu His Ala Pro Ala Thr Val Cys Gly
195 200 205

Pro Lys Lys Ser Thr Asn Leu Val Lys Asn Lys Cys Val Asn Phe Asn
210 215 220

Phe Asn Gly Leu Thr Gly Thr Gly Val Leu Thr Glu Ser Asn Lys Lys
225 230 235 240

Phe Leu Pro Phe Gln Gln Phe Gly Arg Asp Ile Ala Asp Thr Thr Asp
245 250 255

Ala Val Arg Asp Pro Gln Thr Leu Glu Ile Leu Asp Ile Thr Pro Cys
260 265 270

Ser

<210> 4

<400> 4
000

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ggggtcaacg ttgagggggg 20

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<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 6
gggggacgat cgtcgggggg 20

<210> 7
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 7
ggggacgacg tcgtgggggg g 21

<210> 8
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 8
tcgtcgttgt cgttttgtcg tt 22

<210> 9
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 9
tcgacgttcg tcgttcgtcg ttc 23

<210> 10
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<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 10
tcgcgacgtt cgcccgacgt tcggta 26

<210> 11
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 11
tcgtcgtttt cggcgcgcgc cg 22

<210> 12
<211> 25
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 12
tcgtcgtcgt tcgaacgacg ttgat 25

<210> 13
<211> 29
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 13
tcgcgaacgt tcgccgcgtt cgaacgcgg 29

<210> 14
<211> 1255
<212> PRT
<213> Severe acute respiratory syndrome coronavirus 2

<400> 14

Met Phe Ile Phe Leu Leu Phe Leu Thr Leu Thr Ser Gly Ser Asp Leu
1 5 10 15

Asp Arg Cys Thr Thr Phe Asp Asp Val Gln Ala Pro Asn Tyr Thr Gln
20 25 30

His Thr Ser Ser Met Arg Gly Val Tyr Tyr Pro Asp Glu Ile Phe Arg
35 40 45

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

```
Asn Val Thr Gly Phe His Thr Ile Asn His Thr Phe Gly Asn Pro Val
65                  70                  75                  80

Ile Pro Phe Lys Asp Gly Ile Tyr Phe Ala Ala Thr Glu Lys Ser Asn
                85                  90                  95

Val Val Arg Gly Trp Val Phe Gly Ser Thr Met Asn Asn Lys Ser Gln
            100                 105                 110

Ser Val Ile Ile Ile Asn Asn Ser Thr Asn Val Val Ile Arg Ala Cys
        115                 120                 125

Asn Phe Glu Leu Cys Asp Asn Pro Phe Phe Ala Val Ser Lys Pro Met
    130                 135                 140

Gly Thr Gln Thr His Thr Met Ile Phe Asp Asn Ala Phe Asn Cys Thr
145                 150                 155                 160

Phe Glu Tyr Ile Ser Asp Ala Phe Ser Leu Asp Val Ser Glu Lys Ser
                165                 170                 175

Gly Asn Phe Lys His Leu Arg Glu Phe Val Phe Lys Asn Lys Asp Gly
            180                 185                 190

Phe Leu Tyr Val Tyr Lys Gly Tyr Gln Pro Ile Asp Val Val Arg Asp
        195                 200                 205

Leu Pro Ser Gly Phe Asn Thr Leu Lys Pro Ile Phe Lys Leu Pro Leu
    210                 215                 220

Gly Ile Asn Ile Thr Asn Phe Arg Ala Ile Leu Thr Ala Phe Ser Pro
225                 230                 235                 240

Ala Gln Asp Ile Trp Gly Thr Ser Ala Ala Ala Tyr Phe Val Gly Tyr
                245                 250                 255

Leu Lys Pro Thr Thr Phe Met Leu Lys Tyr Asp Glu Asn Gly Thr Ile
            260                 265                 270
```

```
Thr Asp Ala Val Asp Cys Ser Gln Asn Pro Leu Ala Glu Leu Lys Cys
        275                 280                 285

Ser Val Lys Ser Phe Glu Ile Asp Lys Gly Ile Tyr Gln Thr Ser Asn
    290                 295                 300

Phe Arg Val Val Pro Ser Gly Asp Val Val Arg Phe Pro Asn Ile Thr
305                 310                 315                 320

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

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

Ser Val Leu Tyr Asn Ser Thr Phe Phe Ser Thr Phe Lys Cys Tyr Gly
        355                 360                 365

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

Asp Ser Phe Val Val Lys Gly Asp Asp Val Arg Gln Ile Ala Pro Gly
385                 390                 395                 400

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

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

Thr Gly Asn Tyr Asn Tyr Lys Tyr Arg Tyr Leu Arg His Gly Lys Leu
        435                 440                 445

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

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

```
Tyr Gly Phe Tyr Thr Thr Thr Gly Ile Gly Tyr Gln Pro Tyr Arg Val
                485                 490                 495

Val Val Leu Ser Phe Glu Leu Leu Asn Ala Pro Ala Thr Val Cys Gly
            500                 505                 510

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

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

Phe Gln Pro Phe Gln Gln Phe Gly Arg Asp Val Ser Asp Phe Thr Asp
545                 550                 555                 560

Ser Val Arg Asp Pro Lys Thr Ser Glu Ile Leu Asp Ile Ser Pro Cys
                565                 570                 575

Ser Phe Gly Gly Val Ser Val Ile Thr Pro Gly Thr Asn Ala Ser Ser
            580                 585                 590

Glu Val Ala Val Leu Tyr Gln Asp Val Asn Cys Thr Asp Val Ser Thr
        595                 600                 605

Ala Ile His Ala Asp Gln Leu Thr Pro Ala Trp Arg Ile Tyr Ser Thr
    610                 615                 620

Gly Asn Asn Val Phe Gln Thr Gln Ala Gly Cys Leu Ile Gly Ala Glu
625                 630                 635                 640

His Val Asp Thr Ser Tyr Glu Cys Asp Ile Pro Ile Gly Ala Gly Ile
                645                 650                 655

Cys Ala Ser Tyr His Thr Val Ser Leu Leu Arg Ser Thr Ser Gln Lys
            660                 665                 670

Ser Ile Val Ala Tyr Thr Met Ser Leu Gly Ala Asp Ser Ser Ile Ala
        675                 680                 685

Tyr Ser Asn Asn Thr Ile Ala Ile Pro Thr Asn Phe Ser Ile Ser Ile
```

690 695 700

Thr Thr Glu Val Met Pro Val Ser Met Ala Lys Thr Ser Val Asp Cys
705 710 715 720

Asn Met Tyr Ile Cys Gly Asp Ser Thr Glu Cys Ala Asn Leu Leu Leu
725 730 735

Gln Tyr Gly Ser Phe Cys Thr Gln Leu Asn Arg Ala Leu Ser Gly Ile
740 745 750

Ala Ala Glu Gln Asp Arg Asn Thr Arg Glu Val Phe Ala Gln Val Lys
755 760 765

Gln Met Tyr Lys Thr Pro Thr Leu Lys Tyr Phe Gly Gly Phe Asn Phe
770 775 780

Ser Gln Ile Leu Pro Asp Pro Leu Lys Pro Thr Lys Arg Ser Phe Ile
785 790 795 800

Glu Asp Leu Leu Phe Asn Lys Val Thr Leu Ala Asp Ala Gly Phe Met
805 810 815

Lys Gln Tyr Gly Glu Cys Leu Gly Asp Ile Asn Ala Arg Asp Leu Ile
820 825 830

Cys Ala Gln Lys Phe Asn Gly Leu Thr Val Leu Pro Pro Leu Leu Thr
835 840 845

Asp Asp Met Ile Ala Ala Tyr Thr Ala Ala Leu Val Ser Gly Thr Ala
850 855 860

Thr Ala Gly Trp Thr Phe Gly Ala Gly Ala Ala Leu Gln Ile Pro Phe
865 870 875 880

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

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

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


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


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


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


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


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


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


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


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


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


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


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


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

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

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

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

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

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

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

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

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

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

Gly Val  Lys Leu His Tyr Thr
    1250                1255


<210>  15
<211>  22
<212>  DNA
<213>  Artificial Sequence

<220>
<223>  Synthetic Construct

<400>  15
tgactgtgaa cgttcgagat ga                                              22
```

<210> 16
<211> 1255
<212> PRT
<213> Severe acute respiratory syndrome coronavirus 2

<400> 16

Met Phe Ile Phe Leu Leu Phe Leu Thr Leu Thr Ser Gly Ser Asp Leu
1 5 10 15

Asp Arg Cys Thr Thr Phe Asp Asp Val Gln Ala Pro Asn Tyr Thr Gln
20 25 30

His Thr Ser Ser Met Arg Gly Val Tyr Tyr Pro Asp Glu Ile Phe Arg
35 40 45

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

Asn Val Thr Gly Phe His Thr Ile Asn His Thr Phe Gly Asn Pro Val
65 70 75 80

Ile Pro Phe Lys Asp Gly Ile Tyr Phe Ala Ala Thr Glu Lys Ser Asn
85 90 95

Val Val Arg Gly Trp Val Phe Gly Ser Thr Met Asn Asn Lys Ser Gln
100 105 110

Ser Val Ile Ile Ile Asn Asn Ser Thr Asn Val Val Ile Arg Ala Cys
115 120 125

Asn Phe Glu Leu Cys Asp Asn Pro Phe Phe Ala Val Ser Lys Pro Met
130 135 140

Gly Thr Gln Thr His Thr Met Ile Phe Asp Asn Ala Phe Asn Cys Thr
145 150 155 160

Phe Glu Tyr Ile Ser Asp Ala Phe Ser Leu Asp Val Ser Glu Lys Ser
165 170 175

Gly Asn Phe Lys His Leu Arg Glu Phe Val Phe Lys Asn Lys Asp Gly
180 185 190

```
Phe Leu Tyr Val Tyr Lys Gly Tyr Gln Pro Ile Asp Val Val Arg Asp
        195                 200                 205

Leu Pro Ser Gly Phe Asn Thr Leu Lys Pro Ile Phe Lys Leu Pro Leu
    210                 215                 220

Gly Ile Asn Ile Thr Asn Phe Arg Ala Ile Leu Thr Ala Phe Ser Pro
225                 230                 235                 240

Ala Gln Asp Ile Trp Gly Thr Ser Ala Ala Ala Tyr Phe Val Gly Tyr
                245                 250                 255

Leu Lys Pro Thr Thr Phe Met Leu Lys Tyr Asp Glu Asn Gly Thr Ile
            260                 265                 270

Thr Asp Ala Val Asp Cys Ser Gln Asn Pro Leu Ala Glu Leu Lys Cys
        275                 280                 285

Ser Val Lys Ser Phe Glu Ile Asp Lys Gly Ile Tyr Gln Thr Ser Asn
    290                 295                 300

Phe Arg Val Val Pro Ser Gly Asp Val Val Arg Phe Pro Asn Ile Thr
305                 310                 315                 320

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

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

Ser Val Leu Tyr Asn Ser Thr Phe Phe Ser Thr Phe Lys Cys Tyr Gly
        355                 360                 365

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

Asp Ser Phe Val Val Lys Gly Asp Asp Val Arg Gln Ile Ala Pro Gly
385                 390                 395                 400
```

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

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

Thr Gly Asn Tyr Asn Tyr Lys Tyr Arg Tyr Leu Arg His Gly Lys Leu
        435                 440                 445

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

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

Tyr Gly Phe Tyr Thr Thr Thr Gly Ile Gly Tyr Gln Pro Tyr Arg Val
               485                 490                 495

Val Val Leu Ser Phe Glu Leu Leu Asn Ala Pro Ala Thr Val Cys Gly
            500                 505                 510

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

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

Phe Gln Pro Phe Gln Gln Phe Gly Arg Asp Val Ser Asp Phe Thr Asp
545                 550                 555                 560

Ser Val Arg Asp Pro Lys Thr Ser Glu Ile Leu Asp Ile Ser Pro Cys
               565                 570                 575

Ser Phe Gly Gly Val Ser Val Ile Thr Pro Gly Thr Asn Ala Ser Ser
            580                 585                 590

Glu Val Ala Val Leu Tyr Gln Asp Val Asn Cys Thr Asp Val Ser Thr
        595                 600                 605
```

```
Ala Ile His Ala Gly Gln Leu Thr Pro Ala Trp Arg Ile Tyr Ser Thr
    610                 615                 620

Gly Asn Asn Val Phe Gln Thr Gln Ala Gly Cys Leu Ile Gly Ala Glu
625                 630                 635                 640

His Val Asp Thr Ser Tyr Glu Cys Asp Ile Pro Ile Gly Ala Gly Ile
                645                 650                 655

Cys Ala Ser Tyr His Thr Val Ser Leu Leu Arg Ser Thr Ser Gln Lys
            660                 665                 670

Ser Ile Val Ala Tyr Thr Met Ser Leu Gly Ala Asp Ser Ser Ile Ala
        675                 680                 685

Tyr Ser Asn Asn Thr Ile Ala Ile Pro Thr Asn Phe Ser Ile Ser Ile
    690                 695                 700

Thr Thr Glu Val Met Pro Val Ser Met Ala Lys Thr Ser Val Asp Cys
705                 710                 715                 720

Asn Met Tyr Ile Cys Gly Asp Ser Thr Glu Cys Ala Asn Leu Leu Leu
                725                 730                 735

Gln Tyr Gly Ser Phe Cys Thr Gln Leu Asn Arg Ala Leu Ser Gly Ile
            740                 745                 750

Ala Ala Glu Gln Asp Arg Asn Thr Arg Glu Val Phe Ala Gln Val Lys
        755                 760                 765

Gln Met Tyr Lys Thr Pro Thr Leu Lys Tyr Phe Gly Gly Phe Asn Phe
    770                 775                 780

Ser Gln Ile Leu Pro Asp Pro Leu Lys Pro Thr Lys Arg Ser Phe Ile
785                 790                 795                 800

Glu Asp Leu Leu Phe Asn Lys Val Thr Leu Ala Asp Ala Gly Phe Met
                805                 810                 815

Lys Gln Tyr Gly Glu Cys Leu Gly Asp Ile Asn Ala Arg Asp Leu Ile
```

```
            820                 825                 830

Cys Ala Gln Lys Phe Asn Gly Leu Thr Val Leu Pro Pro Leu Leu Thr
        835                 840                 845

Asp Asp Met Ile Ala Ala Tyr Thr Ala Ala Leu Val Ser Gly Thr Ala
    850                 855                 860

Thr Ala Gly Trp Thr Phe Gly Ala Gly Ala Ala Leu Gln Ile Pro Phe
865                 870                 875                 880

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Gly Val Lys Leu His Tyr Thr
    1250   1255

<210> 17
<211> 180
<212> PRT
<213> Severe acute respiratory syndrome coronavirus 2

<400> 17

Cys Pro Phe Gly Glu Val Phe Asn Ala Thr Lys Phe Pro Ser Val Tyr
1   5   10   15

Ala Trp Glu Arg Lys Lys Ile Ser Asn Cys Val Ala Asp Tyr Ser Val
            20   25   30

Leu Tyr Asn Ser Thr Phe Phe Ser Thr Phe Lys Cys Tyr Gly Val Ser
        35   40   45

Ala Thr Lys Leu Asn Asp Leu Cys Phe Ser Asn Val Tyr Ala Asp Ser
    50   55   60

Phe Val Val Lys Gly Asp Asp Val Arg Gln Ile Ala Pro Gly Gln Thr
65   70   75   80

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

Cys Val Leu Ala Trp Asn Thr Arg Asn Ile Asp Ala Thr Ser Thr Gly
            100   105   110

Asn Tyr Asn Tyr Lys Tyr Arg Tyr Leu Arg His Gly Lys Leu Arg Pro
        115   120   125

Phe Glu Arg Asp Ile Ser Asn Val Pro Phe Ser Pro Asp Gly Lys Pro
    130   135   140

Cys Thr Pro Pro Ala Leu Asn Cys Tyr Trp Pro Leu Asn Asp Tyr Gly

145 150 155 160

Phe Tyr Thr Thr Thr Gly Ile Gly Tyr Gln Pro Tyr Arg Val Val Val
165 170 175

Leu Ser Phe Glu
180

<210> 18
<211> 4
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 18

Gly Gly Gly Ala
1

<210> 19
<211> 4
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 19

Gly Gly Gly Gly
1

<210> 20
<211> 5
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 20

Gly Gly Gly Ala Gly
1 5

<210> 21
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 21

Gly Gly Gly Ala Gly Gly
1 5

<210> 22
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 22

Gly Gly Gly Ala Gly Gly Gly
1 5

<210> 23
<211> 4
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 23

Gly Gly Ala Gly
1

<210> 24
<211> 4
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 24

Gly Gly Ser Gly

1

<210> 25
<211> 4
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 25

Ala Gly Gly Gly
1

<210> 26
<211> 4
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 26

Ser Gly Gly Gly
1

<210> 27
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 27

Gly Gly Ala Gly Gly Ala
1 5

<210> 28
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 28

Gly Gly Ser Gly Gly Ser
1 5

<210> 29
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 29

Gly Gly Ala Gly Gly Ala Gly Gly Ala
1 5

<210> 30
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 30

Gly Gly Ser Gly Gly Ser Gly Gly Ser
1 5

<210> 31
<211> 12
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 31

Gly Gly Ala Gly Gly Ala Gly Gly Ala Gly Gly Ala
1 5 10

<210> 32
<211> 12
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 32

Gly Gly Ser Gly Gly Ser Gly Gly Ser Gly Gly Ser
1   5   10

<210> 33
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 33

Gly Gly Ala Gly Gly Gly Ala Gly
1   5

<210> 34
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 34

Gly Gly Ser Gly Gly Gly Ser Gly
1   5

<210> 35
<211> 12
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 35

Gly Gly Ala Gly Gly Gly Ala Gly Gly Gly Ala Gly
1   5   10

<210> 36
<211> 12

<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 36

Gly Gly Ser Gly Gly Gly Ser Gly Gly Gly Ser Gly
1 5 10

<210> 37
<211> 15
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 37

Gly Gly Gly Gly Ala Gly Gly Gly Gly Ala Gly Gly Gly Gly Ala
1 5 10 15

<210> 38
<211> 15
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 38

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
1 5 10 15

<210> 39
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 39

Tyr Ser Val Leu Tyr Asn Ser Ala Ser Phe
1 5 10

<210> 40
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 40

Tyr Gln Pro Tyr Arg Val Val Val Leu
1 5

<210> 41
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 41

Val Arg Phe Pro Asn Ile Thr Asn Leu
1 5

<210> 42
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 42

Phe Asn Ala Thr Arg Phe Ala Ser Val
1 5

<210> 43
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 43

Lys Ile Ala Asp Tyr Asn Tyr Lys Leu
1 5

<210> 44
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 44

Val Ser Pro Thr Lys Leu Asn Asp Leu
1 5

<210> 45
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 45

Val Cys Gly Pro Lys Lys Ser Thr Asn Leu
1 5 10

<210> 46
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 46

Ser Tyr Gly Phe Gln Pro Thr Asn Gly Val
1 5 10

<210> 47
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 47

Val Tyr Ala Trp Asn Arg Lys Arg Ile
1 5

<210> 48
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 48

Ser Phe Val Ile Arg Gly Asp Glu Val
1 5

<210> 49
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 49

Val Asn Phe Asn Phe Asn Gly Leu
1 5

<210> 50
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 50

Lys Cys Tyr Gly Val Ser Pro Thr Lys Leu
1 5 10

<210> 51
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 51

Cys Tyr Gly Val Ser Pro Thr Lys Leu
1   5

<210> 52
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 52

Cys Tyr Gly Val Ser Ala Thr Lys Leu
1   5

<210> 53
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 53

Tyr Gly Val Ser Pro Thr Lys Leu
1   5

<210> 54
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 54

Ser Lys Val Gly Gly Asn Tyr Asn Tyr Leu
1   5   10

<210> 55

<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 55

Val Ile Ala Trp Asn Ser Asn Asn Leu
1 5

<210> 56
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 56

Glu Ser Ile Val Arg Phe Pro Asn Ile
1 5

<210> 57
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 57

Val Val Val Leu Ser Phe Glu Leu Leu
1 5

<210> 58
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 58

Gly Asn Tyr Asn Tyr Leu Tyr Arg Leu
1 5

<210> 59
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 59

Val Gly Tyr Gln Pro Tyr Arg Val Val
1 5

<210> 60
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 60

Tyr Asn Ser Ala Ser Phe Ser Thr Phe
1 5

<210> 61
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 61

Ile Ala Pro Gly Gln Thr Gly Lys Ile
1 5

<210> 62
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 62

Gly Gly Gly Ser Gly Gly Gly Ser
1 5

<210> 63
<211> 432
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 63

Met Ser Asp Asn Gly Pro Gln Asn Gln Arg Asn Ala Pro Arg Ile Thr
1 5 10 15

Phe Gly Gly Pro Ser Asp Ser Thr Gly Ser Asn Gln Asn Gly Glu Arg
20 25 30

Ser Gly Ala Arg Ser Lys Gln Arg Arg Pro Gln Gly Leu Pro Asn Asn
35 40 45

Thr Ala Ser Trp Phe Thr Ala Leu Thr Gln His Gly Lys Glu Asp Leu
50 55 60

Lys Phe Pro Arg Gly Gln Gly Val Pro Ile Asn Thr Asn Ser Ser Pro
65 70 75 80

Asp Asp Gln Ile Gly Tyr Tyr Arg Arg Ala Thr Arg Arg Ile Arg Gly
85 90 95

Gly Asp Gly Lys Met Lys Asp Leu Ser Pro Arg Trp Tyr Phe Tyr Tyr
100 105 110

Leu Gly Thr Gly Pro Glu Ala Gly Leu Pro Tyr Gly Ala Asn Lys Asp
115 120 125

Gly Ile Ile Trp Val Ala Thr Glu Gly Ala Leu Asn Thr Pro Lys Asp
130 135 140

His Ile Gly Thr Arg Asn Pro Ala Asn Asn Ala Ala Ile Val Leu Gln
145 150 155 160

```
Leu Pro Gln Gly Thr Thr Leu Pro Lys Gly Phe Tyr Ala Glu Gly Ser
                165                 170                 175

Arg Gly Gly Ser Gln Ala Ser Ser Arg Ser Ser Ser Arg Ser Arg Asn
            180                 185                 190

Ser Ser Arg Asn Ser Thr Pro Gly Ser Ser Arg Gly Thr Ser Pro Ala
        195                 200                 205

Arg Met Ala Gly Asn Gly Gly Asp Ala Ala Leu Ala Leu Leu Leu Leu
    210                 215                 220

Asp Arg Leu Asn Gln Leu Glu Ser Lys Met Ser Gly Lys Gly Gln Gln
225                 230                 235                 240

Gln Gln Gly Gln Thr Val Thr Lys Lys Ser Ala Ala Glu Ala Ser Lys
                245                 250                 255

Lys Pro Arg Gln Lys Arg Thr Ala Thr Lys Ala Tyr Asn Val Thr Gln
            260                 265                 270

Ala Phe Gly Arg Arg Gly Pro Glu Gln Thr Gln Gly Asn Phe Gly Asp
        275                 280                 285

Gln Glu Leu Ile Arg Gln Gly Thr Asp Tyr Lys His Trp Pro Gln Ile
    290                 295                 300

Ala Gln Phe Ala Pro Ser Ala Ser Ala Phe Phe Gly Met Ser Arg Ile
305                 310                 315                 320

Gly Met Glu Val Thr Pro Ser Gly Thr Trp Leu Thr Tyr Thr Gly Ala
                325                 330                 335

Ile Lys Leu Asp Asp Lys Asp Pro Asn Phe Lys Asp Gln Val Ile Leu
            340                 345                 350

Leu Asn Lys His Ile Asp Ala Tyr Lys Thr Phe Pro Pro Thr Glu Pro
        355                 360                 365
```

```
Lys Lys Asp Lys Lys Lys Lys Ala Asp Glu Thr Gln Ala Leu Pro Gln
    370                 375                 380

Arg Gln Lys Lys Gln Gln Thr Val Thr Leu Leu Pro Ala Ala Asp Leu
385                 390                 395                 400

Asp Asp Phe Ser Lys Gln Leu Gln Gln Ser Met Ser Ser Ala Asp Ser
                405                 410                 415

Thr Gln Ala Glu Asn Leu Tyr Phe Gln Gly His His His His His His
            420                 425                 430
```

<210> 64
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 64

```
Glu Asn Leu Tyr Phe Gln Gly
1               5
```

<210> 65
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 65

```
His His His His His His
1               5
```

<210> 66
<211> 1216
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 66

```
Val Asn Leu Thr Thr Arg Thr Gln Leu Pro Pro Ala Tyr Thr Asn Ser
1               5                   10                  15

Phe Thr Arg Gly Val Tyr Tyr Pro Asp Lys Val Phe Arg Ser Ser Val
            20                  25                  30

Leu His Ser Thr Gln Asp Leu Phe Leu Pro Phe Phe Ser Asn Val Thr
        35                  40                  45

Trp Phe His Ala Ile His Val Ser Gly Thr Asn Gly Thr Lys Arg Phe
    50                  55                  60

Asp Asn Pro Val Leu Pro Phe Asn Asp Gly Val Tyr Phe Ala Ser Thr
65                  70                  75                  80

Glu Lys Ser Asn Ile Ile Arg Gly Trp Ile Phe Gly Thr Thr Leu Asp
                85                  90                  95

Ser Lys Thr Gln Ser Leu Leu Ile Val Asn Asn Ala Thr Asn Val Val
            100                 105                 110

Ile Lys Val Cys Glu Phe Gln Phe Cys Asn Asp Pro Phe Leu Gly Val
        115                 120                 125

Tyr Tyr His Lys Asn Asn Lys Ser Trp Met Glu Ser Glu Phe Arg Val
    130                 135                 140

Tyr Ser Ser Ala Asn Asn Cys Thr Phe Glu Tyr Val Ser Gln Pro Phe
145                 150                 155                 160

Leu Met Asp Leu Glu Gly Lys Gln Gly Asn Phe Lys Asn Leu Arg Glu
                165                 170                 175

Phe Val Phe Lys Asn Ile Asp Gly Tyr Phe Lys Ile Tyr Ser Lys His
            180                 185                 190

Thr Pro Ile Asn Leu Val Arg Asp Leu Pro Gln Gly Phe Ser Ala Leu
        195                 200                 205
```

```
Glu Pro Leu Val Asp Leu Pro Ile Gly Ile Asn Ile Thr Arg Phe Gln
    210                 215                 220

Thr Leu Leu Ala Leu His Arg Ser Tyr Leu Thr Pro Gly Asp Ser Ser
225                 230                 235                 240

Ser Gly Trp Thr Ala Gly Ala Ala Ala Tyr Tyr Val Gly Tyr Leu Gln
                245                 250                 255

Pro Arg Thr Phe Leu Leu Lys Tyr Asn Glu Asn Gly Thr Ile Thr Asp
            260                 265                 270

Ala Val Asp Cys Ala Leu Asp Pro Leu Ser Glu Thr Lys Cys Thr Leu
        275                 280                 285

Lys Ser Phe Thr Val Glu Lys Gly Ile Tyr Gln Thr Ser Asn Phe Arg
    290                 295                 300

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

Cys Pro Phe Gly Glu Val Phe Asn Ala Thr Arg Phe Ala Ser Val Tyr
                325                 330                 335

Ala Trp Asn Arg Lys Arg Ile Ser Asn Cys Val Ala Asp Tyr Ser Val
            340                 345                 350

Leu Tyr Asn Ser Ala Ser Phe Ser Thr Phe Lys Cys Tyr Gly Val Ser
        355                 360                 365

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

Phe Val Ile Arg Gly Asp Glu Val Arg Gln Ile Ala Pro Gly Gln Thr
385                 390                 395                 400

Gly Lys Ile Ala Asp Tyr Asn Tyr Lys Leu Pro Asp Asp Phe Thr Gly
                405                 410                 415
```

```
Cys Val Ile Ala Trp Asn Ser Asn Asn Leu Asp Ser Lys Val Gly Gly
            420                 425                 430

Asn Tyr Asn Tyr Leu Tyr Arg Leu Phe Arg Lys Ser Asn Leu Lys Pro
        435                 440                 445

Phe Glu Arg Asp Ile Ser Thr Glu Ile Tyr Gln Ala Gly Ser Thr Pro
    450                 455                 460

Cys Asn Gly Val Glu Gly Phe Asn Cys Tyr Phe Pro Leu Gln Ser Tyr
465                 470                 475                 480

Gly Phe Gln Pro Thr Asn Gly Val Gly Tyr Gln Pro Tyr Arg Val Val
                485                 490                 495

Val Leu Ser Phe Glu Leu Leu His Ala Pro Ala Thr Val Cys Gly Pro
            500                 505                 510

Lys Lys Ser Thr Asn Leu Val Lys Asn Lys Cys Val Asn Phe Asn Phe
        515                 520                 525

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

Leu Pro Phe Gln Gln Phe Gly Arg Asp Ile Ala Asp Thr Thr Asp Ala
545                 550                 555                 560

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

Phe Gly Gly Val Ser Val Ile Thr Pro Gly Thr Asn Thr Ser Asn Gln
            580                 585                 590

Val Ala Val Leu Tyr Gln Asp Val Asn Cys Thr Glu Val Pro Val Ala
        595                 600                 605

Ile His Ala Asp Gln Leu Thr Pro Thr Trp Arg Val Tyr Ser Thr Gly
    610                 615                 620

Ser Asn Val Phe Gln Thr Arg Ala Gly Cys Leu Ile Gly Ala Glu His
```

625 630 635 640

Val Asn Asn Ser Tyr Glu Cys Asp Ile Pro Ile Gly Ala Gly Ile Cys
645 650 655

Ala Ser Tyr Gln Thr Gln Thr Asn Ser Pro Arg Ala Ala Ala Ser Val
660 665 670

Ala Ser Gln Ser Ile Ile Ala Tyr Thr Met Ser Leu Gly Ala Glu Asn
675 680 685

Ser Val Ala Tyr Ser Asn Asn Ser Ile Ala Ile Pro Thr Asn Phe Thr
690 695 700

Ile Ser Val Thr Thr Glu Ile Leu Pro Val Ser Met Thr Lys Thr Ser
705 710 715 720

Val Asp Cys Thr Met Tyr Ile Cys Gly Asp Ser Thr Glu Cys Ser Asn
725 730 735

Leu Leu Leu Gln Tyr Gly Ser Phe Cys Thr Gln Leu Asn Arg Ala Leu
740 745 750

Thr Gly Ile Ala Val Glu Gln Asp Lys Asn Thr Gln Glu Val Phe Ala
755 760 765

Gln Val Lys Gln Ile Tyr Lys Thr Pro Pro Ile Lys Asp Phe Gly Gly
770 775 780

Phe Asn Phe Ser Gln Ile Leu Pro Asp Pro Ser Lys Pro Ser Lys Arg
785 790 795 800

Ser Phe Ile Glu Asp Leu Leu Phe Asn Lys Val Thr Leu Ala Asp Ala
805 810 815

Gly Phe Ile Lys Gln Tyr Gly Asp Cys Leu Gly Asp Ile Ala Ala Arg
820 825 830

Asp Leu Ile Cys Ala Gln Lys Phe Asn Gly Leu Thr Val Leu Pro Pro
835 840 845

```
Leu Leu Thr Asp Glu Met Ile Ala Gln Tyr Thr Ser Ala Leu Leu Ala
    850                 855                 860

Gly Thr Ile Thr Ser Gly Trp Thr Phe Gly Ala Gly Ala Ala Leu Gln
865                 870                 875                 880

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

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

Asn Ser Ala Ile Gly Lys Ile Gln Asp Ser Leu Ser Ser Thr Ala Ser
        915                 920                 925

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

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

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

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

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

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

Arg Val  Asp Phe Cys Gly Lys  Gly Tyr His Leu Met  Ser Phe Pro
    1025                1030                1035

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

```
Pro Ala  Gln Glu Lys Asn Phe  Thr Thr Ala Pro Ala  Ile Cys His
    1055                1060                1065

Asp Gly  Lys Ala His Phe Pro  Arg Glu Gly Val Phe  Val Ser Asn
    1070                1075                1080

Gly Thr  His Trp Phe Val Thr  Gln Arg Asn Phe Tyr  Glu Pro Gln
    1085                1090                1095

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

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

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

His Thr  Ser Pro Asp Val Asp  Leu Gly Asp Ile Ser  Gly Ile Asn
    1145                1150                1155

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

Val Ala  Lys Asn Leu Asn Glu  Ser Leu Ile Asp Leu  Gln Glu Leu
    1175                1180                1185

Gly Lys  Tyr Glu Gln Tyr Ile  Lys Trp Pro Gly Gly  Gly Ser Gly
    1190                1195                1200

Gly Gly  Ser His His His His  His His His His His  His
    1205                1210                1215
```

<210> 67
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Construct

<400> 67

His His His His His His His His His His
1 5 10

<210> 68
<211> 419
<212> PRT
<213> Severe acute respiratory syndrome coronavirus 2

<400> 68

Met Ser Asp Asn Gly Pro Gln Asn Gln Arg Asn Ala Pro Arg Ile Thr
1 5 10 15

Phe Gly Gly Pro Ser Asp Ser Thr Gly Ser Asn Gln Asn Gly Glu Arg
20 25 30

Ser Gly Ala Arg Ser Lys Gln Arg Arg Pro Gln Gly Leu Pro Asn Asn
35 40 45

Thr Ala Ser Trp Phe Thr Ala Leu Thr Gln His Gly Lys Glu Asp Leu
50 55 60

Lys Phe Pro Arg Gly Gln Gly Val Pro Ile Asn Thr Asn Ser Ser Pro
65 70 75 80

Asp Asp Gln Ile Gly Tyr Tyr Arg Arg Ala Thr Arg Arg Ile Arg Gly
85 90 95

Gly Asp Gly Lys Met Lys Asp Leu Ser Pro Arg Trp Tyr Phe Tyr Tyr
100 105 110

Leu Gly Thr Gly Pro Glu Ala Gly Leu Pro Tyr Gly Ala Asn Lys Asp
115 120 125

Gly Ile Ile Trp Val Ala Thr Glu Gly Ala Leu Asn Thr Pro Lys Asp
130 135 140

His Ile Gly Thr Arg Asn Pro Ala Asn Asn Ala Ala Ile Val Leu Gln
145 150 155 160

```
Leu Pro Gln Gly Thr Thr Leu Pro Lys Gly Phe Tyr Ala Glu Gly Ser
                165                 170                 175

Arg Gly Gly Ser Gln Ala Ser Ser Arg Ser Ser Ser Arg Ser Arg Asn
            180                 185                 190

Ser Ser Arg Asn Ser Thr Pro Gly Ser Ser Arg Gly Thr Ser Pro Ala
        195                 200                 205

Arg Met Ala Gly Asn Gly Gly Asp Ala Ala Leu Ala Leu Leu Leu Leu
    210                 215                 220

Asp Arg Leu Asn Gln Leu Glu Ser Lys Met Ser Gly Lys Gly Gln Gln
225                 230                 235                 240

Gln Gln Gly Gln Thr Val Thr Lys Lys Ser Ala Ala Glu Ala Ser Lys
                245                 250                 255

Lys Pro Arg Gln Lys Arg Thr Ala Thr Lys Ala Tyr Asn Val Thr Gln
            260                 265                 270

Ala Phe Gly Arg Arg Gly Pro Glu Gln Thr Gln Gly Asn Phe Gly Asp
        275                 280                 285

Gln Glu Leu Ile Arg Gln Gly Thr Asp Tyr Lys His Trp Pro Gln Ile
    290                 295                 300

Ala Gln Phe Ala Pro Ser Ala Ser Ala Phe Phe Gly Met Ser Arg Ile
305                 310                 315                 320

Gly Met Glu Val Thr Pro Ser Gly Thr Trp Leu Thr Tyr Thr Gly Ala
                325                 330                 335

Ile Lys Leu Asp Asp Lys Asp Pro Asn Phe Lys Asp Gln Val Ile Leu
            340                 345                 350

Leu Asn Lys His Ile Asp Ala Tyr Lys Thr Phe Pro Pro Thr Glu Pro
        355                 360                 365

Lys Lys Asp Lys Lys Lys Lys Ala Asp Glu Thr Gln Ala Leu Pro Gln
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370 375 380

Arg Gln Lys Lys Gln Gln Thr Val Thr Leu Leu Pro Ala Ala Asp Leu
385 390 395 400

Asp Asp Phe Ser Lys Gln Leu Gln Gln Ser Met Ser Ser Ala Asp Ser
405 410 415

Thr Gln Ala

<210> 69
<211> 223
<212> PRT
<213> Severe acute respiratory syndrome coronavirus 2

<400> 69

Arg Val Gln Pro Thr Glu Ser Ile Val Arg Phe Pro Asn Ile Thr Asn
1 5 10 15

Leu Cys Pro Phe Gly Glu Val Phe Asn Ala Thr Arg Phe Ala Ser Val
20 25 30

Tyr Ala Trp Asn Arg Lys Arg Ile Ser Asn Cys Val Ala Asp Tyr Ser
35 40 45

Val Leu Tyr Asn Ser Ala Ser Phe Ser Thr Phe Lys Cys Tyr Gly Val
50 55 60

Ser Pro Thr Lys Leu Asn Asp Leu Cys Phe Thr Asn Val Tyr Ala Asp
65 70 75 80

Ser Phe Val Ile Arg Gly Asp Glu Val Arg Gln Ile Ala Pro Gly Gln
85 90 95

Thr Gly Lys Ile Ala Asp Tyr Asn Tyr Lys Leu Pro Asp Asp Phe Thr
100 105 110

Gly Cys Val Ile Ala Trp Asn Ser Asn Asn Leu Asp Ser Lys Val Gly
115 120 125

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Gly Asn Tyr Asn Tyr Leu Tyr Arg Leu Phe Arg Lys Ser Asn Leu Lys
    130                 135                 140

Pro Phe Glu Arg Asp Ile Ser Thr Glu Ile Tyr Gln Ala Gly Ser Thr
145                 150                 155                 160

Pro Cys Asn Gly Val Glu Gly Phe Asn Cys Tyr Phe Pro Leu Gln Ser
                165                 170                 175

Tyr Gly Phe Gln Pro Thr Tyr Gly Val Gly Tyr Gln Pro Tyr Arg Val
            180                 185                 190

Val Val Leu Ser Phe Glu Leu Leu His Ala Pro Ala Thr Val Cys Gly
        195                 200                 205

Pro Lys Lys Ser Thr Asn Leu Val Lys Asn Lys Cys Val Asn Phe
    210                 215                 220
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Ala His His His His His His His His His His
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<400> 71
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