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(19) **United States**(12) **Patent Application Publication**
Barbieri et al.(10) **Pub. No.: US 2021/0300970 A1**(43) **Pub. Date: Sep. 30, 2021**(54) **COVID-19 VACCINE****Publication Classification**(71) Applicants: **The Medical College of Wisconsin, Inc.**, Milwaukee, WI (US); **Wisconsin Alumni Research Foundation**, Madison, WI (US)(51) **Int. Cl.****C07K 14/005** (2006.01)**C07K 14/33** (2006.01)**A61K 39/215** (2006.01)**A61K 39/08** (2006.01)(72) Inventors: **Joseph T. Barbieri**, New Berlin, WI (US); **Amanda Przedpelski**, Milwaukee, WI (US); **Eric A. Johnson**, Madison, WI (US); **Sabine Pellett**, Madison, WI (US); **William H. Tepp**, Stoughton, WI (US)(52) **U.S. Cl.**CPC **C07K 14/005** (2013.01); **A61K 39/08** (2013.01); **A61K 39/215** (2013.01); **C07K 14/33** (2013.01)(21) Appl. No.: **17/211,379**(22) Filed: **Mar. 24, 2021****Related U.S. Application Data**

(60) Provisional application No. 62/994,081, filed on Mar. 24, 2020, provisional application No. 63/104,360, filed on Oct. 22, 2020.

(57)

ABSTRACT

Provided herein are antigenic peptides comprising the SARS-CoV-2 spike protein receptor binding domain (CRBD) polypeptide or portions thereof, linked to a non-catalytic, non-toxic tetanus toxin variant (i.e., a modified tetanus toxin or "MTT") and vaccine compositions comprising the same. In addition, provided herein are methods for making and using CRBD-MTT fusion proteins as immunogenic agents.

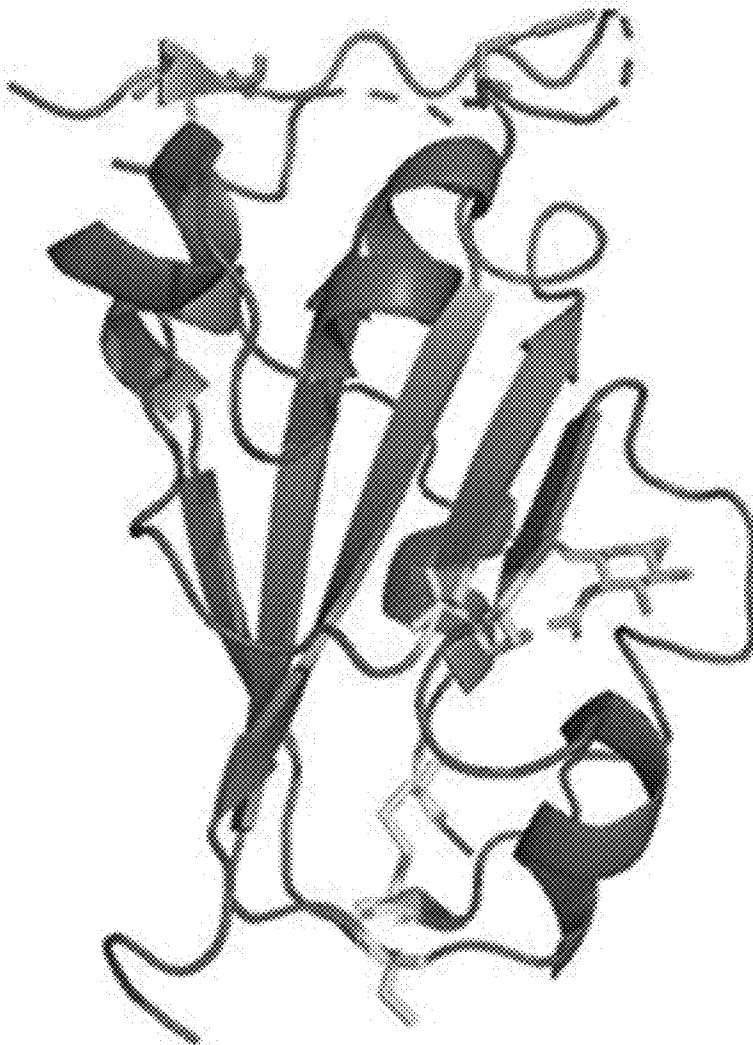
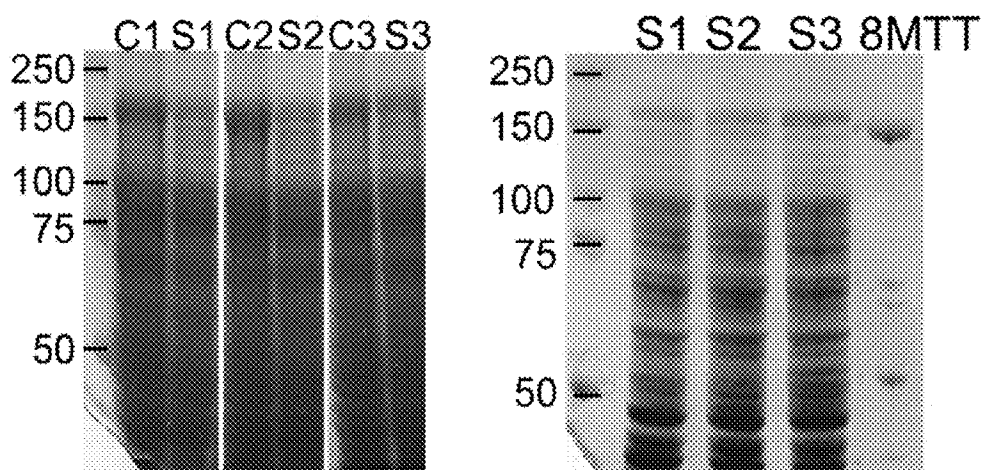
Specification includes a Sequence Listing.

FIG. 1



FIG. 2



C1/S1 - Crude(C1) and Soluble(S1) Lysate in 10mM PO⁴

C2/S2 - Crude(C2) and Soluble(S2) Lysate in 10mM PO⁴ and 2% Triton-X100

C3/S3 - Crude(C3) and Soluble(S3) Lysate in 10mM PO⁴ and 2% Triton-X100 and 4M urea

FIG. 3

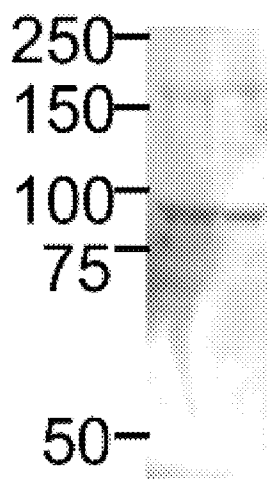


FIG. 4

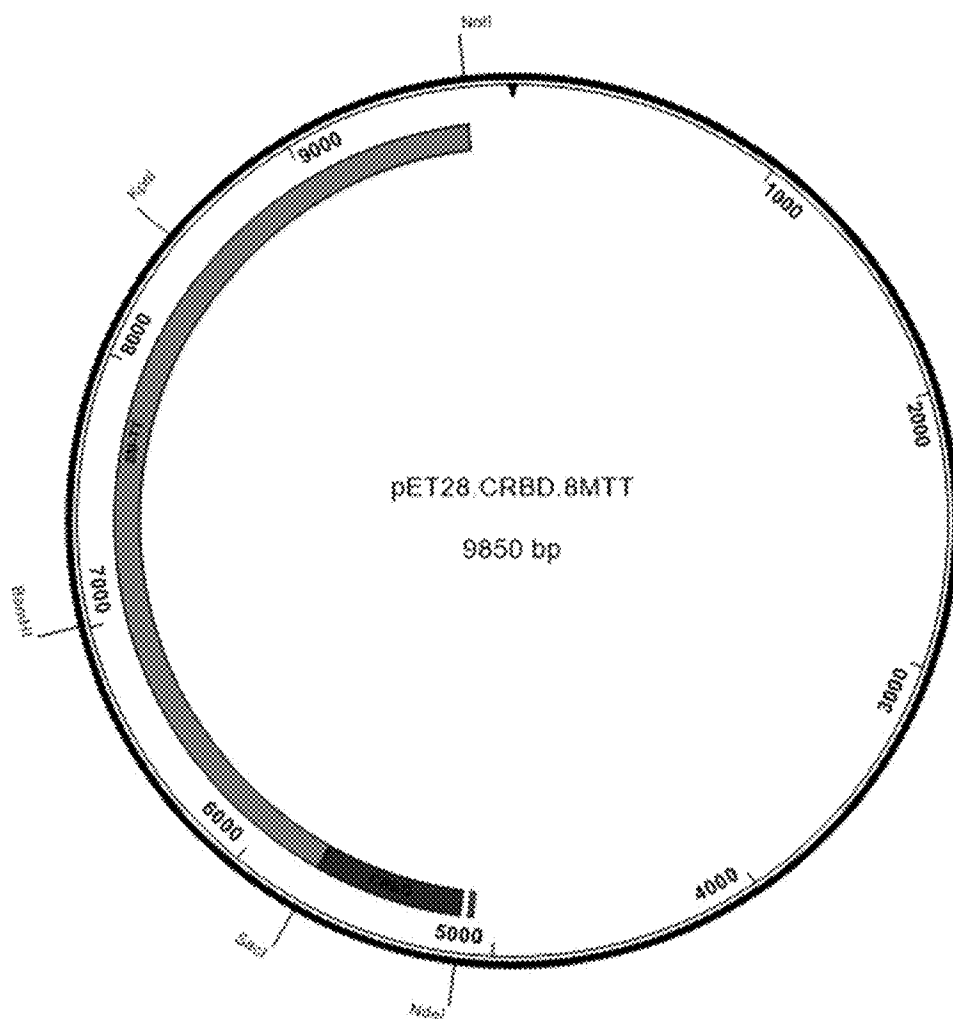


FIG. 5

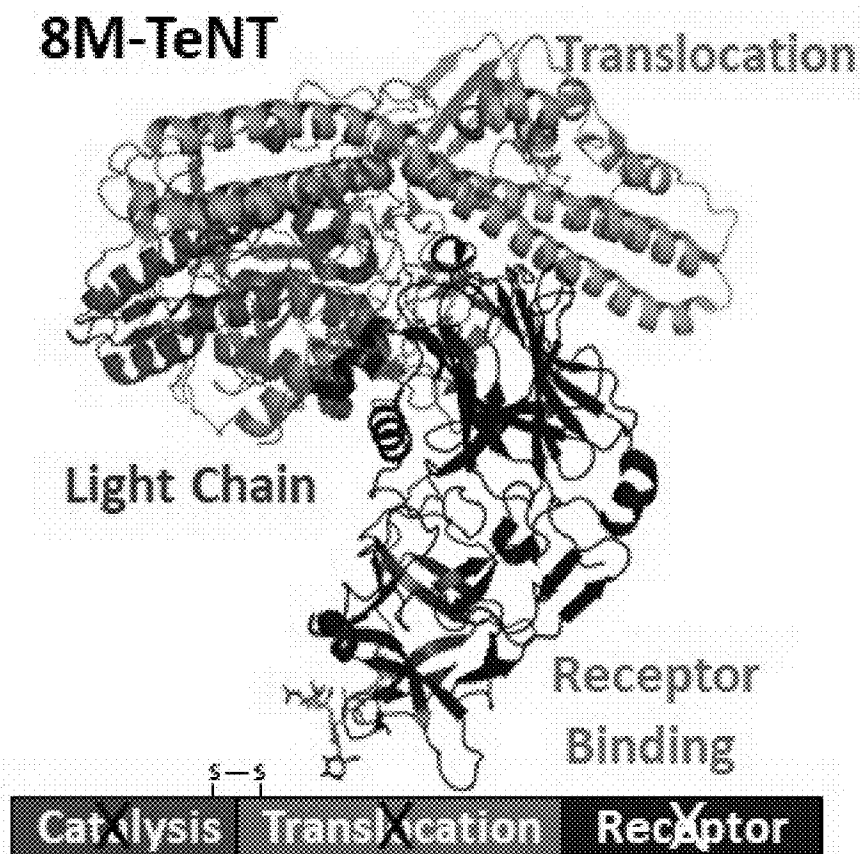


FIG. 6

2M-TT (TeNTRY) (GenBank X06214.1. modified for optimized expression in *Escherichia coli*)

MPITINNFRYSDPVNNDTIIMMEPPYCKGLDIYYKAFKITDRIWIVPERYEFGTKPEDFNPPSSLIEGASEYYDPNYLRTDS
DKDRFLQTMVKLFNRIKNNVAGEALLDKIINAIPYLGNSYSLLDKFDTNSNSVSFNLEQDPSGATTKSAMLTNLIIFGPG
PVLNKNEVRGIVLRVDNKNYFPCRDGFGSIMQMAFCPEYVPTFDNVNIENITSLTIGKSKYFQDPALLMHLEIHLVHLGLY
GMQVSSHEIIPSKQEIYMQHTYPISAEELFTFGGQDANLISIDIKNDLYEKTLDYKAIANKLSQVTSNDPNIDIDSYKQIY
QQKYQFDKDSNGQYIVNEDKFQILYNSIMYGFTIELGKKFNIKTALSFMSMNHDPVKIPNLLDDTIYNDTEGFNIESKDL
KSEYKGQNMVRVNTNAFRNVDGSGLVSKLIGLCKKIIPTNIRENLYNRTASLTDLGELCIKIKNEDLTFAEKNSFSEEPF
QDEIVSYNTKNKPLNFNYSLDKIIVDYNLQSKITLPNDRTTPVTKGIPYAPEYKSNAASTIEIHNIDDNTIYQYLYAQKSPTTL
QRITMTNSVDDALINSTKIYSYFSPVISKVNQGAQGILFLQWVRDIIDDFTNESSQKTTIDKISDVSTIVPYIGPALNIVKQG
YEGNFIGALETGGVLLLEIPEITLPVIAALSIAESSTQKEKIIKTIDNFKRYEKWIEVYKLVKAKWLGTVNTQFQKRSYQ
MYRSLEYQVDAIKKIIDEYKIYSGPDKEQIADENNLKNKLEEKANKAMININIFMRESSRSFLVNQMINEAKKQLEFDT
QSKNLMQYIKANSKFIGITELKKLESKINKVFSTPIPFYSKNLDCWVDNEEDIDVILKKSTILNLDINNDIISDISGFNSSVIT
YPDAQLVPGINGKAIHLVNNESEVIVHKAMDIEYNDMFNNFTVSFWLRVPKVSASHLEQYGTNEYSIISMKKHSLSIG
SGWSVSLKGNNLIWTLKDSAGEVRQITFRDLPDKFNAYLANKWVFITITNDRSSANLYINGVLMGSAEITGLGAIREDN
NITLKLDRCNNNNQYVSIDKFRIFCKALNPKEIEKLYTSYLSITFLRDFWGNPLRYDTEYYLIPVASSSKDVQLKNITDYMIL
TNAPSYTNGKLNIIYRRLYNGLKFIKRYTPNNEIDSFVKSQDFIKLYVSYNNEHIVGYPKDGNAFNNDRLRVGYNAP
GIPLYKKMEAVKLRLDKTYSVQLKLYDDKNASGLVGTHTNGQIGNDPNRDILIASNWFNHLKDKILGCDWYFVPTDEG
WTND

FIG. 7

5MTT

MPITINNFRYSDPVNNDTIIMMEPPYCKGLDIYYKAFKITDRIWIVPERYEFGTKPEDFNPPSSLIEGASEYYDPNYLRTDS
DKDRFLQTMVKLFNRIKNNVAGEALLDKIINAIPYLGNSYSLLDKFDTNSNSVSFNLEQDPSGATTKSAMLTNLIIFGPG
PVLNKNEVRGIVLRVDNKNYFPCRDGFGSIMQMAFCPEYVPTFDNVNIENITSLTIGKSKYFQDPALLMHQLIHLVHLGLY
GMQVSSHEIIPSKQEIYMQHTYPISAEELFTFGGQDANLISIDIKNDLYEKTLDYKAIANKLSQVTSNDPNIDIDSYKQIY
QQKYQFDKDSNGQYIVNEDKFQILYNSIMYGFTIELGKKFNIKTALSFMSMNHDPVKIPNLLDDTIYNDTEGFNIESKDL
KSEYKGQNMVRVNTNAFRNVDGSGLVSKLIGLCKKIIPTNIRENLYNRTASLTDLGELCIKIKNEDLTFAEKNSFSEEPF
QDEIVSYNTKNKPLNFNYSLDKIIVDYNLQSKITLPNDRTTPVTKGIPYAPEYKSNAASTIEIHNIDDNTIYQYLYAQKSPTTL
QRITMTNSVDDALINSTKIYSYFSPVISKVNQGAQGILFLQWVRDIIDDFTNESSQKTTIDKISDVSTIVPYIGPALNIVKQG
YEGNFIGALETGGVLLLEIPEITLPVIAALSIAESSTQKEKIIKTIDNFKRYEKWIEVYKLVKAKWLGTVNTQFQKRSYQ
MYRSLEYQVDAIKKIIDEYKIYSGPDKEQIADENNLKNKLEEKANKAMININIFMRESSRSFLVNQMINEAKKQLEFDT
QSKNLMQYIKANSKFIGITELKKLESKINKVFSTPIPFYSKNLDCWVDNEEDIDVILKKSTILNLDINNDIISDISGFNSSVIT
YPDAQLVPGINGKAIHLVNNESEVIVHKAMDIEYNDMFNNFTVSFWLRVPKVSASHLEQYGTNEYSIISMKKHSLSIG
SGWSVSLKGNNLIWTLKDSAGEVRQITFRDLPDKFNAYLANKWVFITITNDRSSANLYINGVLMGSAEITGLGAIREDN
NITLKLDRCNNNNQYVSIDKFRIFCKALNPKEIEKLYTSYLSITFLRDFWGNPLRYDTEYYLIPVASSSKDVQLKNITDYMIL
TNAPSYTNGKLNIIYRRLYNGLKFIKRYTPNNEIDSFVKSQDFIKLYVSYNNEHIVGYPKDGNAFNNDRLRVGYNAPG
IPLYKKMEAVKLRLDKTYSVQLKLYDDKNASGLVGTHTNGQIGNDPNRDILIASNWFNHLKDKILGCDWYFVPTDEGW
TND

FIG. 8

6MTT

MPITINNFRYSDPVNNDTIIMMEPPYCKGLDIYYKAFKITDRIWIVPERYEFGTKPEDFNPPSSLIEGASEYYDPNYLRTDS
DKDRFLQTMVKLFNRIKNNVAGEALLDKIINAIPYLGNSYSLLDKFDTNSNSVSFNLEQDPSGATTKSAMLTNLIIFGPG
PVLNKNEVRGIVLRVDNKNYFPCRDGFGSIMQMAFCPEYVPTFDNVIENTSLTIGKSKYFQDPALLMHQLIHVLHGLY
GMQVSSHEIIPSKQEIYMQHTYPISAEELFTFGGQDANLISIDIKNDLYEKTLDYKAIANKLSQVTSNDPNIDIDSYKQIY
QQKYQFDKDSNGQYIVNEDKFQILYNSIMYGFTIELGKKFNIKTALSFMSMNHDPVKIPNLLDDTIYNDTEGFNIESKDL
KSEYKGQNMVRVNTNAFRNVDGSGLVSKLIGLCKKIIPTNIRENLYNRTASLTDLGELCIKIKNEDLTFAEKNSFSEEPF
QDEIVSYNTKNKPLNFNYSLDKIIDYNLQSKITLPNDRTTPVTKGIPYAPEYKSNAASTIEIHNIDDNTIYQYLYAQKSPTTL
QRITMTNSVDDALINSTKIYSYFPSVISKVNQGAQGILFLQWVRDIIDDFTNESSQKTTIDKISDVSTIVPYIGPALNIVKQG
YEGNFIGALETGGVLLLEIPEITLPVIAALSIAESSTQKEKIIKTIDNLEKRYEKWIEVYKLVKAKWLGTVNTQFQKRSYQ
MYRSLEYQVDAIKKIIDYEYKIYSGPDAEQIADEINNKNKLEEKANKAMININIFMRESSRSFLVNQMINEAKKQLEFDT
QSKNILMQYIKANSKFIGITELKKLESKINKVFSTPIPFYSKNLDCWVDNEEDIDVILKKSTILNLDINNDIISDISGFNSSVIT
YPDAQLVPGINGKAIHLVNNESEVIVHKAMDIEYNDMFNNFTVSFWLRVPKVSASHLEQYGTNEYSIISMKKHLSISIG
SGWSVSLKGNNLIWTLKDSAGEVRQITFRDLPDKFNAYLANKWVFITITNDRLSSANLYINGVLMGSAEITGLGAIREDN
NITLKLDRCNNNNQYVSIDKFRIFCKALNPKEIEKLYTSYLSITFLRDFWGNPLRYDTEYYLIPVASSSKDVQLKNITDYMYL
TNAPSYTNGKLNIIYRRLYNGLKFIKRYTPNNEIDSFVKSGDFIKLYVSYNNNEHIVGYPKDGNAFNLLDRILLVGYNAPG
IPLYKKMEAVKLRLDKTYSVQLKLYDDKNASGLVGTHNGQIGNDPNRDILIASNAYFNHLKDKILGCDWYFVPTDEGW
TND

FIG. 9

7MTT

MPITINNFRYSDPVNNDTIIMMEPPACKGLDIYYKAFKITDRIWIVPERYEFGTKPEDFNPPSSLIEGASEYYDPNYLRTDS
DKDRFLQTMVKLFNRIKNNVAGEALLDKIINAIPYLGNSYSLLDKFDTNSNSVSFNLEQDPSGATTKSAMLTNLIIFGPG
PVLNKNEVRGIVLRVDNKNYFPCRDGFGSIMQMAFCPEYVPTFDNVIENTSLTIGKSKYFQDPALLMHQLIHVLHGLY
GMQVSSHEIIPSKQEIYMQHTYPISAEELFTFGGQDANLISIDIKNDLYEKTLDYKAIANKLSQVTSNDPNIDIDSYKQIY
QQKYQFDKDSNGQYIVNEDKFQILYNSIMYGFTIELGKKFNIKTALSFMSMNHDPVKIPNLLDDTIYNDTEGFNIESKDL
KSEYKGQNMVRVNTNAFRNVDGSGLVSKLIGLCKKIIPTNIRENLYNRTASLTDLGELCIKIKNEDLTFAEKNSFSEEPF
QDEIVSYNTKNKPLNFNYSLDKIIDYNLQSKITLPNDRTTPVTKGIPYAPEYKSNAASTIEIHNIDDNTIYQYLYAQKSPTTL
QRITMTNSVDDALINSTKIYSYFPSVISKVNQGAQGILFLQWVRDIIDDFTNESSQKTTIDKISDVSTIVPYIGPALNIVKQG
YEGNFIGALETGGVLLLEIPEITLPVIAALSIAESSTQKEKIIKTIDNLEKRYEKWIEVYKLVKAKWLGTVNTQFQKRSYQ
MYRSLEYQVDAIKKIIDYEYKIYSGPDAEQIADEINNKNKLEEKANKAMININIFMRESSRSFLVNQMINEAKKQLEFDT
QSKNILMQYIKANSKFIGITELKKLESKINKVFSTPIPFYSKNLDCWVDNEEDIDVILKKSTILNLDINNDIISDISGFNSSVIT
YPDAQLVPGINGKAIHLVNNESEVIVHKAMDIEYNDMFNNFTVSFWLRVPKVSASHLEQYGTNEYSIISMKKHLSISIG
SGWSVSLKGNNLIWTLKDSAGEVRQITFRDLPDKFNAYLANKWVFITITNDRLSSANLYINGVLMGSAEITGLGAIREDN
NITLKLDRCNNNNQYVSIDKFRIFCKALNPKEIEKLYTSYLSITFLRDFWGNPLRYDTEYYLIPVASSSKDVQLKNITDYMYL
TNAPSYTNGKLNIIYRRLYNGLKFIKRYTPNNEIDSFVKSGDFIKLYVSYNNNEHIVGYPKDGNAFNLLDRILLVGYNAPG
IPLYKKMEAVKLRLDKTYSVQLKLYDDKNASGLVGTHNGQIGNDPNRDILIASNAYFNHLKDKILGCDWYFVPTDEGW
TND

FIG. 10

8MTT

MPITINNFRYSDPVNNDTIIMMEPPACKGLDIYYKAFKITDRIWIVPERYEFGTKPEDFNPPSSLIEGASEYYDPNYLRTDS
DKDRFLQTMVKLFNRIKNNVAGEALLDKIINAIPYLGNSYSLLDKFDTNSNSVSFNLLEQDPSGATTKSAMLTNLIIFGPG
PVLNKNNEVRGIVLRVDNKNYFPCRDGFGSIMQMAFCPEYVPTFDNVIENTSLTIGKSKYFQDPALLKMHQLIHVLHGLY
GMQVSSHEIIPSKQEIYMQHTYPISAEELFTFGGQDANLISIDIKNDLYEKTLDYKAJANKLSQVTSNDPNIDIDSYKQIY
QQKYQFDKDSNGQYIVNEDKFQILYNSIMYGFEIELGKKFNIKTALSFFSMNHDPVKIPNLLDDTIYNDTEGFNIESKDL
KSEYKGQNM RVNTNAFRNVDGSGLVSKLIGLCKKIIPPTNIRENLNRTASLTDLGELCIKKNEDLTFAEKNSFSEEPF
QDEIVSYNTKNKPLNFNYSLDKIIVDYNLQSKITLPNDRTTPVTKGIPYAPEYKSNAASTIEIHNIDDNTIYQYLYAQKSP TTL
QRITMTNSVDDALINSTKIYSYFSPVISKVNQGAQGILFLQWVRDIIDDFTNESSQKTTIDKISDVSTIVPYIGPALNIVKQG
YEGNFIGALETTGVVLLLEYIPEITLPVIAALSIAESSTQKEKIIKTIDNFLEKRYEKWIEVYKLVKAKWLGTVNTQFQKRSYQ
MYRSLEYQVDAIKKIIDYEQIYSGPDAEQIADEINN LKNKLEEKANKAMININIFMRESSRSFLVNQMINEAKKQLLEFDT
QSKNILMQYIKANSKFIGITELKKLESKINKVFSTPIPSYSKNLDCWVDNEEDIDVILKKSTILNLDINNDIISDISGFNSSVIT
YPDAQLVPGINGKAIHLVNNESEVIVHKAMDIEYNDMFNNFTVSFWLRVPKVSASHLEQYGTNEYSIISSMKKHLSIG
SGWSVSLKGNNLIWTLKDSAGEVRQITFRDLPDKFNAYLAN KWVFITITNDR LSSANLYINGVLMGSAEITGLGAIEDN
NITLKLDRCNNNNQYVSIDKFRIFCKALNPKEIEKLYTSYLSITFLRDFWGNPLRYDTEYYLIPVASSSKDVQLKNITDYMYL
TNAPSYTNGKLNIIYRRLYNGLKFIKRYTPNNEIDSFVKS GDFIKLYVSYNNNEHIVGYPKDGNAFNNLDRILLVGYNAPG
IPLYKKMEAVKLRLDKTYSVQLKLYDDKNASGLVGTHNGQIGNDPNRDILIASNAYFNHLKDKILGCDWYFVPTDEGW
TND

FIG. 11

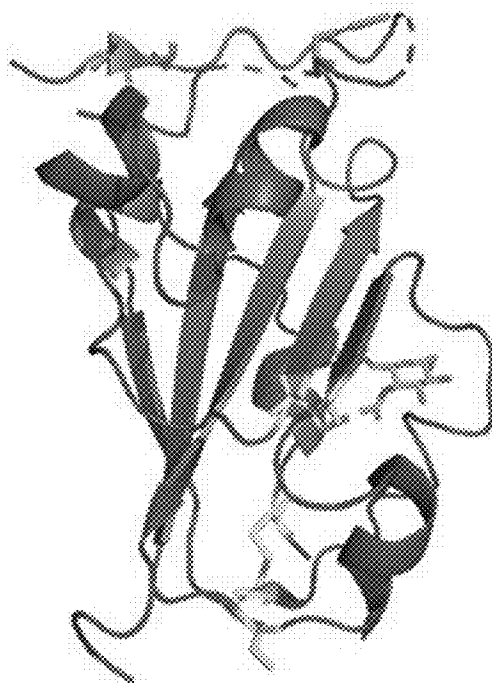
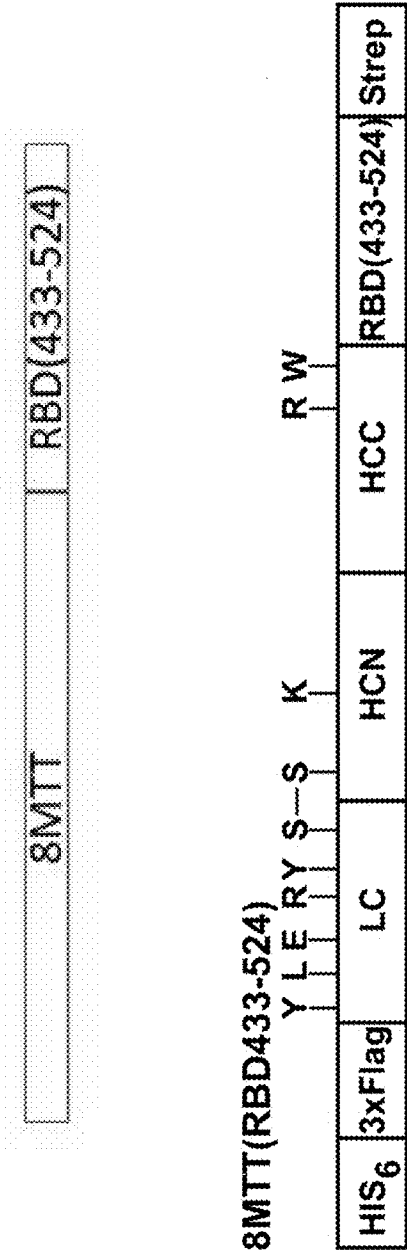


FIG. 12



COVID-19 VACCINE**CROSS-REFERENCE TO RELATED APPLICATIONS**

[0001] This application claims priority to U.S. Provisional Applications No. 62/994,081 filed on Mar. 24, 2020 and 63/104,360 filed on Oct. 22, 2020, the contents of which are incorporated by reference in their entireties.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH

[0002] Not applicable.

REFERENCE TO A SEQUENCE LISTING SUBMITTED VIA EFS-WEB

[0003] A Sequence Listing accompanies this application and is submitted as an ASCII text file of the sequence listing named "650053_00784_ST25.txt" which is 99.1 KB in size and was created on Mar. 23, 2021. The sequence listing is electronically submitted via EFS-Web with the application and is incorporated herein by reference in its entirety.

BACKGROUND

[0004] Coronavirus disease 2019 (COVID-19) is a contagious disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), a β -Coronavirus, in the family with Severe Acute Respiratory Syndrome (SARS) and Middle East Respiratory Syndrome (MERS) viruses. Studies from the SARS coronavirus identified the role for the spike (S) protein and, more specifically, the receptor binding domain (RBD) in the viral protein response for host cell binding. Recently, the cryo-EM of the spike protein was solved revealing the structure of the RBD. Aligning the amino acid sequence of the spike protein with the structure showed that amino acids 330-521 comprise the RBD (CRBD) of SARS-CoV-2.

[0005] As the pandemic threat of COVID-19 grows, a need exists for improved COVID-19 vaccination platforms.

SUMMARY OF THE INVENTION

[0006] In a first aspect, provided herein is a fusion protein comprising (i) a modified tetanus toxin (MTT) polypeptide comprising a sequence having at least 95% identity to SEQ ID NO:1, having a mutation at each of positions R372 and Y375, and having a mutation at two or more positions selected from E234, K768, R1226, and W1289, wherein each position is numbered relative to SEQ ID NO:1, the MTT polypeptide having reduced toxicity and receptor binding compared to the toxicity and receptor binding of SEQ ID NO:1; and (ii) a SARS-CoV-2 spike protein receptor binding domain (CRBD) polypeptide comprising a sequence having at least 95% identity to SEQ ID NO:3.

[0007] In some embodiments, the MTT polypeptide and the CRBD polypeptide are connected by a linker polypeptide. In some embodiments, the linker polypeptide is a poly-glycine sequence.

[0008] In some embodiments, the MTT polypeptide and the CRBD polypeptide are chemically cross-linked.

[0009] In some embodiments, in the MTT polypeptide amino acid R at position R372 is replaced with amino acid A, and wherein amino acid Y at position Y375 is replaced with amino acid F. In some embodiments, the MTT poly-

peptide mutations comprise R372A, Y375F, E234Q, R1226L, and W1289A. In some embodiments, the MTT polypeptide comprises SEQ ID NO:5. In some embodiments, the MTT polypeptide mutations comprise R372A, Y375F, E234Q, K768A, R1226L, and W1289A. In some embodiments, the MTT polypeptide comprises SEQ ID NO:6. In some embodiments, the MTT polypeptide further comprises a mutation at one or both of positions L230 and Y26, wherein each position is numbered relative to SEQ ID NO:1. In some embodiments, the mutations at one or both of positions L230 and Y26 comprise L230K and Y26A. In some embodiments, the MTT polypeptide comprises SEQ ID NO:7 or SEQ ID NO:8. In some embodiments, the fusion protein comprises a sequence at least 95% identical to SEQ ID NO:10.

[0010] In a second aspect, provided herein is a polynucleotide encoding a CRBD-MTT fusion protein as described herein. In some embodiments, the polynucleotide comprises a sequence at least 95% identical to SEQ ID NO:9.

[0011] In a third aspect, provided herein is a vector comprising the polynucleotide of claim 14.

[0012] In a fourth aspect, provided herein is a method for producing a CRBD-MTT fusion protein comprising expressing in a cell a polynucleotide encoding a CRBD-MTT fusion protein as described herein and isolating the CRBD-MTT fusion protein from the cell, whereby the CRBD-MTT fusion protein is produced.

[0013] In a fifth aspect, provided herein is a composition comprising a CRBD-MTT fusion protein as described herein and a pharmaceutically acceptable carrier.

[0014] In a sixth aspect, provided herein is a method of reducing the risk of a subject developing COVID-19 by inducing an immune response through administering to the subject a therapeutically effective amount of a CRBD-MTT fusion protein as described herein.

[0015] In a seventh aspect, provided herein is a use of a CRBD-MTT fusion protein as described herein as a vaccine.

[0016] In an eighth aspect, provided herein is a method for producing a CRBD-MTT chemically cross-linked fusion protein comprising obtaining a polypeptide composition comprising (i) a modified tetanus toxin (MTT) polypeptide comprising a sequence having at least 95% identity to SEQ ID NO:1, having a mutation at each of positions R372 and Y375, and having a mutation at two or more positions selected from E234, K768, R1226, and W1289, wherein each position is numbered relative to SEQ ID NO:1, and (ii) a SARS-CoV-2 spike protein receptor binding domain (CRBD) polypeptide comprising a sequence having at least 95% identity to SEQ ID NO:3 and contacting the polypeptide composition with a crosslinking agent for a time and under conditions sufficient to chemically crosslink the CRBD polypeptide and the MTT polypeptide; whereby a CRBD-MTT chemically crosslinked fusion protein is produced. In some embodiments, the crosslinking agent is selected from the group consisting of formaldehyde, disuccinimidyl suberate (DSS), succinimidyl-4-(N-maleimidomethyl)cyclohexane-1-carboxylate (SMCC), ethylene glycol bis(sulfosuccinimidylsuccinate) (Sulfo-EGS), bis(sulfosuccinimidyl) suberate (BS3), and dithiobis(succinimidylpropionate) (DSP).

BRIEF DESCRIPTION OF DRAWINGS

[0017] The patent or patent application file contains at least one drawing in color. Copies of this patent or patent

application publication with color drawings will be provided by the Office upon request and payment of the necessary fee.

[0018] FIG. 1 shows the schematic of the CRBD-8MTT polypeptide.

[0019] FIG. 2 shows an SDS-PAGE gel of various buffer conditions used to harvest CRBD-8MTT.

[0020] FIG. 3 shows a Western blot using anti-8MTT IgG sera.

[0021] FIG. 4 shows the vector map for the pET28.CRBD.8MTT expression vector.

[0022] FIG. 5 shows a crystal structure of TeNTRY PDB:5n0b. Four TT functions were inactivated: Light Chain E234Q, R373A, Y376F (Zn⁺⁺ binding), L230K (VAMP-2 cleavage) and Y26A (VAMP-2 binding), K768A (LC translocation), and R1226L and W1289A (receptor binding).

[0023] FIG. 6 is the amino acid sequence of 2MTT (SEQ ID NO:4).

[0024] FIG. 7 is the amino acid sequence of SMTT (SEQ ID NO:5).

[0025] FIG. 8 is the amino acid sequence of 6MTT (SEQ ID NO:6).

[0026] FIG. 9 is the amino acid sequence of 7MTT (SEQ ID NO:7).

[0027] FIG. 10 is the amino acid sequence of 8MTT (SEQ ID NO:8).

[0028] FIG. 11 shows the structure of the SARS-CoV-2 RBD ("CRBD"), corresponding to amino acids 330-525 of the SARS-CoV-2 spike protein (see SEQ ID NO:2). Amino acids indicated in green are the ACE2 binding motif shared with the related SARS virus spike protein.

[0029] FIG. 12 presents schematic illustrations of fusion proteins comprising 8MTT and RBD(433-524), which is a portion of the SARS-CoV-2 spike protein's receptor binding domain (corresponding to amino acids 330-525 of SEQ ID NO:2).

[0030] INCORPORATION BY REFERENCE

[0031] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, and patent application was specifically and individually indicated to be incorporated by reference.

DETAILED DESCRIPTION OF THE INVENTION

[0032] The present invention has been described in terms of one or more preferred embodiments, and it should be appreciated that many equivalents, alternatives, variations, and modifications, aside from those expressly stated, are possible and within the scope of the invention.

[0033] SARS-CoV-2 antigenic polypeptides of the present disclosure comprise the SARS-COV-2 spike protein receptor binding domain (RBD) polypeptide or a portion thereof and a genetically modified tetanus toxin comprising genetic modifications at multiple targets relative to a wild-type toxin, where such modifications eliminate residual toxicity and enhance safety, which will be expanded upon below. Inventor's previous work determined that immune cells take up a non-catalytic, non-receptor binding form of tetanus toxin and mount a similar neutralizing immune response as observed with catalytic tetanus toxin. See WO2019118974, which is incorporated herein by reference in its entirety. Described herein is use of the inactivated tetanus toxin conjugate vaccine platform to produce tetanus toxin-CRBD

fusion proteins. The tetanus toxin-CRBD fusion proteins can be used for eliciting an immune response to SARS-Cov-2 spike.

[0034] The SARS-COV-2 spike protein sequence is provided herein as SEQ ID NO:2. The SARS-COV-2 receptor binding domain (CRBD) is residues 330-521 of the spike protein and is provided herein as SEQ ID NO:3.

[0035] Provided herein are SARS-COV-2 antigenic fusion proteins including the CRBD polypeptide, or a polypeptide with a sequence at least 90%, 95%, 98%, or 99% sequence identity thereto, and a modified tetanus toxin (MTT) with reduced toxicity.

[0036] In some cases, a SARS-COV-2 antigenic fusion protein comprises RBD(376-525) or RBD(433-524), which refer to portions of the receptor binding domain (amino acids 330-525) of the SARS-CoV-2 spike protein. In some cases, the SARS-COV-2 antigenic fusion protein can comprise RBD(433-524) and a genetically modified tetanus toxin comprising genetic modifications at multiple targets relative to a wild-type toxin. For example, in some cases, the SARS-COV-2 antigenic fusion protein is 8MTT(RBD376-525). In other cases, the SARS-COV-2 antigenic fusion protein can comprise RBD(433-524) and a genetically modified tetanus toxin comprising genetic modifications at multiple targets relative to a wild-type toxin. For example, in other cases, the SARS-COV-2 antigenic fusion protein is 8MTT(RBD433-524) (see FIG. 12). RBD(433-524) contains the binding sites for several monoclonal antibodies (REGN10987 and REGN10933) that complementally neutralize SARS-CoV-2 infections in cultured cells. As described herein, 8MTT(RBD433-524) reacts with antisera to the RBD of the SARS-CoV-2 spike protein, and is a soluble protein when produced in *E. coli*, which suggests it is particularly well suited for use as a vaccine.

[0037] In some embodiments, in the CRBD-MTT fusion protein, the CRBD polypeptide is linked to the MTT using a spacer moiety employed as spacer arm bridge between the MTT and CRBD polypeptide. The spacer moiety can be any of a wide variety of molecular structures including, without limitation, dextran, polyglutamic acid, and oligopeptides.

[0038] In some embodiments, the spacer is a linker polypeptide. Suitable linker polypeptide sequences are known in the art. In some embodiments, the linker polypeptide is a poly-glycine sequence. In some embodiments, the linker polypeptide is a poly-alanine sequence. The linker polypeptide sequence can be from about 2-20 amino acids, preferably glycines, alanines, or combinations thereof. Other amino acids are contemplated and can be used for the linker sequences.

[0039] In some embodiments, the CRBD-MTT fusion proteins, the CRBD polypeptide and the MTT polypeptide are linked using chemical crosslinking. Suitable chemical crossing reactions and methods are known and described in the art. The chemical crosslinking reaction may target amine, sulfhydryl, carboxyl, carbonyl, hydroxyl functional groups, or combinations thereof, within the CRBD and MTT polypeptides. In some embodiments, the CRBD and the MTT polypeptides are crosslinked by reacting with a cross-linking agent. As used herein, "crosslinking agent" refers to a compound or group of compounds that when reacted under suitable conditions chemically crosslink functional groups of a polypeptide. Suitable crosslinking agents include, without limitation, formaldehyde, disuccinimidyl suberate (DSS), succinimidyl-4-(N-maleimidomethyl)cyclohexane-

1-carboxylate (SMCC), ethylene glycol bis(sulfosuccinimidylsuccinate) (Sulfo-EGS), bis(sulfosuccinimidyl) suberate (BS3), and dithiobis(succinimidylpropionate) (DSP).

[0040] As used herein, “toxin” refers to a noxious or poisonous substance (e.g., a cytotoxin) that is formed or elaborated either as an integral part of a cell or tissue (endotoxin), as an intracellular or extracellular product (exotoxin), or as a combination thereof, during the metabolism and growth of certain microorganisms. As used herein, the term “modified toxin” refers to a non-catalytic, non-toxic variant form of a toxin, wherein the toxin is rendered non-catalytic and non-toxic by genetically engineered (e.g., non-naturally occurring, man-made) modifications to the amino acid sequence of the polypeptide toxin. In exemplary embodiments, modified toxins are genetically engineered or otherwise modified variants of a toxin produced by a bacterium of the genus *Clostridium* (e.g., *C. difficile*, *C. novyi*, *C. sordellii*, *C. perfringens*, *C. tetani*, and *C. botulinum*). The toxins may be recombinant, synthetic, part of a fusion protein (which includes, e.g., an antigen, or a polypeptide (e.g., His₆) which facilitates purification of the fusion protein), covalently conjugated to an antigen, and/or chemically cross-linked to an antigen. In some cases, non-catalytic, non-toxic forms of toxins are referred to as toxoids. Toxoids lack toxicity but retain their antigenicity and their immunizing capacity.

protein active ingredient can be administered to a mammal at a dose level which is the same or greater than what is a fatal for the first composition but without death resulting to the mammal. Reduced toxicity encompasses partially or completely eliminated toxicity as detectable by methods known to those who practice in the art. In addition, reduced toxicity encompasses reduced systemic toxicity (i.e., upon intravenous administration) or reduced toxicity upon intramuscular administration.

[0043] In some embodiments, the MTT has mutations at amino acid residues 372 and 375, and further having a mutation at one or more of residues 234, 1226, and 1289, where the residue positions are numbered relative to the full-length wild-type tetanus neurotoxin (*Clostridium tetani* CN3911; GenBank accession no. X06214) set forth as SEQ ID NO:1. In certain embodiments, the amino acid mutations at residues 372 and 375 are R372A and Y375F, and the modified toxin further comprises at least one mutation selected from E234Q, R1226L, and W1289A. In some cases, the modified toxin comprises five mutations (R372A, Y375F, E234Q, R1226L, and W1289A) numbered relative to SEQ ID NO:1 and is referred to herein as “5M-TeNT” or “SMTT.” See Table 1. In some embodiments, 5M-TeNT comprises the amino acid sequence set forth as SEQ ID NO:5.

TABLE 1

Exemplary mutations for inactivating multiple independent TT functions					
		Function			
		Zn++ binding	Substrate Binding & catalysis	Light Chain translocation	Ganglioside receptor binding
Function relevant mutations	2MTT	E234Q, R372A, Y375F (TT(R372A, Y375F. 2MTT) is 125,000-fold less toxic than native TT ⁴²)	Y26A, L230K	K768A Inhibits LC translocation (preliminary data)	R1226L, W1289A This mutation is ~800-fold less toxic than TT WT
	5MTT	R372A, Y375F			R1226L, W1289A
	6MTT	E234Q, R372A, Y375F		K768A (or D767A or E769A)	R1226L, W1289A
	7MTT	E234Q, R372A, Y375F	L230K	K768A (or D767A or E769A)	R1226L, W1289A
	8MTT	E234Q, R372A, Y375F	Y26A, L230K	K768A (or D767A or E769A)	R1226L, W1289A

[0041] A tetanus toxin modified as described herein exhibits one or more altered properties as compared to the wild-type tetanus toxin polypeptide shown in SEQ ID NO:1, for example, significantly decreased catalytic activity and receptor binding activity. In some embodiments, the modified tetanus toxins described herein are at least 1,000,000 times less toxic than wild-type tetanus toxin.

[0042] As used herein, the term “reduced toxicity” means that, relative to a first composition comprising a particular protein active ingredient (e.g., wild-type TT), a second composition comprising a modified version of a particular

[0044] Wild-type *Clostridium tetani* CN3911 tetanus toxin is shown in SEQ ID NO:1.

[0045] In some embodiments, the tetanus toxin has a modified translocation domain. For example, the lysine (K) residue at position 768 is located within a loop that connects two long alpha helices. Mutation of this single amino acid to an alanine (A) inactivates or blocks light chain translocation. In some cases, the K768A mutation is added to 5MTT modified toxin to produce 6MTT, whereby the resulting modified tetanus toxins comprises independent mutations at six positions (see Tables 2 and 3). In some cases, the

modified toxin comprises six mutations (R372A, Y375F, E234Q, K768A, R1226L, and W1289A) numbered relative to SEQ ID NO:1 and is referred to herein as “6M-TeNT” or “6MTT.” In some cases, 6M-TeNT comprises the amino acid sequence set forth as SEQ ID NO:6. Without being bound by any particular mechanism or theory, vaccine potency of 6MTT is expected to be higher than 5MTT but should have a lower rate of reversion than 5MTT. The addition of a mutation at one or more of D767, K768, or E769A to 5MTT will yield more complete inactivation of the genetically engineered vaccine by inactivating a function of the translocation domain in addition to disrupted functionality of the catalytic and receptor binding domains.

[0046] In some embodiments, the tetanus toxin has been modified to inhibit VAMP-2 cleavage. For example, mutation of the leucine residue at position 230 (for example, mutation of the leucine to a lysine (K)) inactivates the toxin’s catalytic activity for VAMP-2 cleavage. In some cases, the L230K mutation is added to 6MTT modified toxin to produce 7MTT, whereby the resulting modified tetanus toxins comprises independent mutations at seven positions (Table 3). In some cases, the modified toxin comprises eight independent mutations (R372A, Y375F, E234Q, R1226L, W1289A, K768A, and L230K) numbered relative to SEQ ID NO:1 and is referred to herein as “7M-TeNT” or “7MTT.” In some cases, 7M-TeNT comprises the amino acid sequence set forth as SEQ ID NO:7.

[0047] In some embodiments, the tetanus toxin has been modified to inhibit VAMP-2 binding. For example, mutation of the tyrosine (Y) residue at position 26 (for example, mutation of the tyrosine to an alanine (A)) inactivates VAMP-2 binding capacity of the toxin. In some cases, the Y26A mutation is added to 7MTT modified toxin to produce 8MTT, whereby the resulting modified tetanus toxins comprises independent mutations at eight positions (Table 3). In some cases, the modified toxin comprises eight independent mutations (R372A, Y375F, E234Q, R1226L, W1289A, K768A, L230K, and Y26A) numbered relative to SEQ ID NO:1 and is referred to herein as “8M-TeNT” or “8MTT.” In some embodiments, 8M-TeNT comprises the amino acid sequence set forth as SEQ ID NO:8.

[0048] Advantageously, modified tetanus toxins of this disclosure do not require detoxification with formalin for use as a vaccine or adjuvant. In some cases, small quantities of formalin (~0.04%) or another fixative or stabilizing reagent (e.g., formalin, glutaraldehyde, β -propiolactone and the like) are added to the modified tetanus toxin as a stabilizing agent, but such quantities are smaller (e.g., smaller by an order of magnitude) than those generally used (~0.4%) to detoxify wild-type tetanus toxin (or tetanus toxin not modified as described herein) to form “tetanus toxoid.”

[0049] In some cases, the modified tetanus toxin comprises other amino acid substitutions at residue positions 372, 375, 234, 768, 1226, 1289, 230, and/or 26. For example, amino acids that may substitute for the listed amino acids include substitutions that reverse the charge or hydrophobicity reversal of the original residue, conservative amino acid substitutions, and substitutions that delete the original residue.

[0050] As is well known to those skilled in the art, altering the primary structure of a polypeptide by a conservative amino acid substitution may not significantly alter the activity of that polypeptide because the side-chain of the amino acid which is inserted into the sequence may be able to form

similar bonds and contacts as the side chain of the amino acid which has been substituted out. This is so even when the substitution is in a region critical in determining the polypeptide’s conformation.

[0051] Conservative amino acid substitutions are art recognized substitutions of one amino acid for another amino acid having similar characteristics. Conservative amino acid substitutions may be achieved by modifying a nucleotide sequence to introduce a nucleotide change that will encode the conservative substitution. For example, each amino acid may be described as having one or more of the following characteristics: electropositive, electronegative, aliphatic, aromatic, polar, hydrophobic and hydrophilic. Conservative substitutions include substitution among amino acids within each group. Acidic amino acids include aspartate, glutamate. Basic amino acids include histidine, lysine, arginine; aliphatic amino acids include isoleucine, leucine and valine. Aromatic amino acids include phenylalanine, glycine, tyrosine and tryptophan. Polar amino acids include aspartate, glutamate, histidine, lysine, asparagine, glutamine, arginine, serine, threonine and tyrosine. Hydrophobic amino acids include alanine, cysteine, phenylalanine, glycine, isoleucine, leucine, methionine, proline, valine and tryptophan. Amino acids may also be described in terms of relative size, where alanine, cysteine, aspartate, glycine, asparagine, proline, threonine, serine, valine, are considered to be small.

[0052] In some cases, non-conservative substitutions are possibly provided if these substitutions do not disrupt the tertiary structure of an epitope within the polypeptide, for example, which do not interrupt the immunogenicity (for example, the antigenicity) of the polypeptide and do not restore toxicity.

[0053] The terms “polypeptide,” “peptide,” and “protein,” as used herein, refer to a polymer comprising amino acid residues predominantly bound together by covalent amide bonds. By the term “protein,” we mean to encompass all the above definitions. The terms apply to amino acid polymers in which one or more amino acid residue may be an artificial chemical mimetic of a naturally occurring amino acid, as well as to naturally occurring amino acid polymers and non-naturally occurring amino acid polymers. As used herein, the terms may encompass amino acid chains of any length, including full length proteins, wherein the amino acids are linked by covalent peptide bonds. The protein or peptide may be isolated from a native organism, produced by recombinant techniques, or produced by synthetic production techniques known to one skilled in the art.

[0054] Sequence identity between amino acid sequences can be determined by comparing an alignment of the sequences. When an equivalent position in the compared sequences is occupied by the same amino acid, then the molecules are identical at that position. Scoring an alignment as a percentage of identity is a function of the number of identical amino acids at positions shared by the compared sequences. When comparing sequences, optimal alignments may require gaps to be introduced into one or more of the sequences, to take into consideration possible insertions and deletions in the sequences. Sequence comparison methods may employ gap penalties so that, for the same number of identical molecules in sequences being compared, a sequence alignment with as few gaps as possible, reflecting higher relatedness between the two compared sequences, will achieve a higher score than one with many gaps. Calculation of maximum percent identity involves the pro-

duction of an optimal alignment, taking into consideration gap penalties. As mentioned above, the percentage sequence identity may be determined using the Needleman-Wunsch Global Sequence Alignment tool, publicly available at blast.ncbi.nlm.nih.gov/Blast.cgi, using default parameter settings. The Needleman-Wunsch algorithm was published in *J. Mol. Biol.* (1970) vol. 48:443-53.

[0055] Polypeptides and nucleic acids of the invention may be prepared synthetically using conventional synthesizers. Alternatively, they may be produced using recombinant DNA technology and may be incorporated into suitable expression vector, which is then used to transform a suitable host cell, such as a prokaryotic cell such as *E. coli*. The transformed host cells are cultured and the polypeptide isolated therefrom.

[0056] Provided herein are nucleic acid sequences which code for the CRBD-MTT fusion proteins and other nucleic acid sequence which hybridize to a nucleic molecule consisting of the above-described nucleotide sequences under high stringency conditions. In a particular embodiment provided herein are DNA sequences encoding CRBD-MTT fusion proteins with the modified tetanus toxin having mutations at amino acid residues 372 and 375, and further comprises a mutation at one or more of residues 234, 1226, and 1289 or the modified catalytic domain described herein.

[0057] The term “stringent conditions” as used herein refers to parameters with which the art is familiar. For example, nucleic acid hybridization parameters may be found in references which compile such methods, e.g., *Molecular Cloning: A Laboratory Manual*, J. Sambrook, et al., eds., Second Edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989, or *Current Protocols in Molecular Biology*, F. M. Ausubel, et al., eds., John Wiley & Sons, Inc., New York. More specifically, high stringency conditions as used herein, refers to hybridization at 65° C. in hybridization buffer (3.5×SSC, 0.02% Ficoll, 0.02% Polyvinyl pyrrolidone, 0.02% Bovine Serum Albumin, 25 mM NaH₂PO₄ (pH 7), 0.5% SDS, 2 mM EDTA). SSC is 0.15M Sodium Chloride/0.015M Sodium Citrate, pH 7; SDS is Sodium Dodecyl Sulphate; and EDTA is Ethylene diaminetetraacetic acid. After hybridization, the membrane upon which the DNA is transferred is washed at 2×SSC at room temperature and then at 0.1-0.5×SSC/0.1×SDS at temperatures up to 68° C., e.g., 55° C., 60° C., 65° C. or 68° C. Alternatively, high stringency hybridization may be performed using a commercially available hybridization buffer, such as ExpressHyb™ buffer (Clontech) using hybridization and washing conditions described by the manufacturer.

[0058] It will also be understood that the invention embraces the use of the sequences in expression vectors, as well as to transfect host cells and cell lines, be these prokaryotic (e.g. *E. coli*), or eukaryotic (e.g., dendritic cells, CHO cells, COS cells, yeast expression systems, recombinant baculovirus expression in insect cells). The expression vectors require that the pertinent sequence, i.e., those described supra, be operably linked to a promoter. In one embodiment, the invention provides a host cell capable of producing the fusion protein.

[0059] The term “vector,” or “recombinant vector” as used herein, refers to a nucleic acid molecule capable of propagating another nucleic acid to which it is linked. The term includes the vector as a self-replicating nucleic acid structure as well as the vector incorporated into the genome of a host cell into which it has been introduced. Certain vectors

are capable of directing the expression of nucleic acids to which they are operatively linked. Such vectors are referred to herein as “expression vectors”. Vectors, including expression vectors, comprise the nucleotide sequence encoding the antibodies or antigen-binding fragments described herein and a heterogeneous sequence necessary for proper propagation of the vector and expression of the encoded polypeptide. The heterogeneous sequence (i.e., sequence from a difference species than the polypeptide) can comprise a heterologous promoter or heterologous transcriptional regulatory region that allows for expression of the polypeptide. As used herein, the terms “heterologous promoter,” “promoter,” “promoter region,” or “promoter sequence” refer generally to transcriptional regulatory regions of a gene, which may be found at the 5' or 3' side of the polynucleotides described herein, or within the coding region of the polynucleotides, or within introns in the polynucleotides. Typically, a promoter is a DNA regulatory region capable of binding RNA polymerase in a cell and initiating transcription of a downstream (3' direction) coding sequence. The typical 5' promoter sequence is bounded at its 3' terminus by the transcription initiation site and extends upstream (5' direction) to include the minimum number of bases or elements necessary to initiate transcription at levels detectable above background. Within the promoter sequence is a transcription initiation site, as well as protein binding domains (consensus sequences) responsible for the binding of RNA polymerase.

[0060] In another aspect, provided herein is an immunogenic composition including the CRBD-MTT fusion proteins as described herein that, upon introduction into a host, will confer immunity to that host, in the event the host is subsequently challenged by a SARS-COV-2 virus. In preferred embodiments, the immunogenic composition is a vaccine comprising the CRBD-MTT fusion proteins as described herein and further comprising an excipient and/or diluent appropriate where the composition is to be administered to a subject in need of vaccination against SARS-COV-2.

[0061] The term “vaccine,” as used herein, refers to a composition that includes an antigen. Vaccine may also include a biological preparation that improves immunity to a particular disease. A vaccine may typically contain an agent, referred to as an antigen, that resembles a disease-causing microorganism, and the agent may often be made from weakened or killed forms of the microbe, its toxins or one of its surface proteins. The antigen may stimulate the body's immune system to recognize the agent as foreign, destroy it, and “remember” it, so that the immune system can more easily recognize and destroy any of these microorganisms that it later encounters. Similarly, the modified toxin preparations, combined with vaccines against other pathogens, could “boost” the immune responses to the pathogen of interest, by acting themselves as vaccine adjuvants. Adjuvants can be classified according to their physiochemical properties or mechanisms of action. The two major classes of adjuvants include compounds that directly act on the immune system such as bacterial toxins that stimulate immune responses, and molecules able to facilitate the presentation of antigens in a controlled manner and behaving as a carrier.

[0062] Selection of appropriate vaccine components is within the routine capability of the skilled person. For example, the vaccine composition of the invention may

conveniently be formulated using a pharmaceutically acceptable excipient or diluent, such as, for example, an aqueous solvent, non-aqueous solvent, non-toxic-excipient, such as a salt, preservative, buffer and the like. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oil and injectable organic esters such as ethyloleate. Aqueous solvents include water, alcoholic/aqueous solutions, saline solutions, parenteral vehicles such as sodium chloride, Ringer's dextrose, etc. Preservatives include antimicrobial, anti-oxidants, chelating agents and inert gases. The pH and exact concentration of the various components the vaccine composition are adjusted according to routine skills.

[0063] In some cases, the preparation described herein may include purified modified toxins, including preparation comprising partial toxin complexes. In some embodiments, the preparations may further include stabilizers that are known to stabilize the CRBD and tetanus toxin proteins. Suitable stabilizers are known in the art, and include, but are not limited to, for example, human or bovine serum albumin, gelatin, recombinant albumin as described in US Publication US2005/0238663 (the contents of which are incorporated by reference in its entirety) among others.

[0064] The terms "subject" and "patient" are used interchangeably and refer to any animal (e.g., a mammal), including, but not limited to, humans, non-human primates, rodents, and the like, which is to be the recipient of a particular treatment. Typically, the terms "subject" and "patient" are used interchangeably herein in reference to a human subject.

[0065] A preparation of the present invention can be administered in a therapeutically effective amount. The terms "effective amount" or "therapeutically effective amount" refer to an amount of an antigen or vaccine that would induce an immune response in a subject receiving the antigen or vaccine which is adequate to prevent signs or symptoms of disease, including adverse health effects or complications thereof, caused by infection with a pathogen, such as a virus or a bacterium. Humoral immunity or cell mediated immunity or both humoral and cell mediated immunity may be induced. The immunogenic response of an animal to a vaccine or composition may be evaluated, e.g., indirectly through measurement of antibody titers, lymphocyte proliferation assays, or directly through monitoring signs and symptoms after challenge with wild-type strain. The protective immunity conferred by a vaccine or composition may be evaluated by measuring, e.g., reduction in clinical signs such as mortality, morbidity, temperature number, overall physical condition, and overall health and performance of the subject. The amount of a vaccine that is therapeutically effective may vary depending on the particular preparation used, or the condition of the subject, and may be determined by a physician.

[0066] A preparation of the present invention can be administered in a therapeutically effective amount depending on the type of treatment necessary. Methods of determining suitable dosage or dosage ranges for individual treatment are known to those in the art. For methods provided herein, a preparation of the present invention can be administered by any means that achieves the intended purpose or is deemed appropriate by those skilled in the art. In an exemplary embodiment, a modified toxin preparation is administered either as a single dose or, when appropriate, as continuous administration using, for instance, a mini

pump system. In some cases, a CRBD-MTT fusion proteins as described herein is provided as a liquid dosages form or as a lyophilized dosages form that is, for example, reconstituted prior to administration.

[0067] The fusion peptide or compositions comprising the same can be used to elicit an immune response against the fusion peptide, particularly the SARS-CoV-2 peptide. A suitable immune response to the fusion protein or composition is the development in a subject of a humoral and/or a cellular immune response to the SARS-CoV-2 antigen present in the composition of interest. For purposes of the present invention, a "humoral immune response" refers to an immune response mediated by antibody molecules, including secretory (IgA) or IgG molecules, while a "cellular immune response" is one mediated by T-lymphocytes and/or other white blood cells. One important aspect of cellular immunity involves an antigen-specific response by cytolytic T-cells ("CTL"s). CTLs have specificity for peptide antigens that are presented in association with proteins encoded by the major histocompatibility complex (MHC) and expressed on the surfaces of cells. CTLs help induce and promote the destruction of intracellular pathogens, or the lysis of cells infected with such pathogens. The fusion peptide or compositions described herein can be used to elicit an immune response that reduces COVID-19 disease progression or can reduce COVID-19. In some embodiments, the fusion peptide or compositions described herein reduce or eliminate moderate or severe COVID-19 disease, by reducing or inhibiting one or more symptoms of COVID-19. Further, the immune response in some embodiments may reduce the viral titer load of an infected individual.

[0068] The term "protected," as used herein, refers to immunization of a patient against a disease or condition. The immunization may be caused by administering a vaccine comprising an antigen. Specifically, in the present invention, the immunized patient is protected from COVID-19.

[0069] In one embodiment, a suitable dosage is from about 1 μ g to 25 μ g.

[0070] Suitable routes of administration for the preparations of the CRBD-MTT fusion proteins described herein include, but are not limited to, direct injection. In certain embodiments, each dose is administered intramuscularly.

[0071] Dosage, toxicity, and therapeutic efficacy of the agents of the present technology can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., for determining the LD₅₀ (the dose lethal to 50% of the population) and the ED₅₀ (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD₅₀/ED₅₀.

[0072] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the invention pertains. All definitions, as defined and used herein, should be understood to control over dictionary definitions, definitions in documents incorporated by reference, and/or ordinary meanings of the defined terms.

[0073] All references, patents and patent applications disclosed herein are incorporated by reference with respect to the subject matter for which each is cited, which in some cases may encompass the entirety of the document.

[0074] The indefinite articles “a” and “an,” as used herein in the specification and in the claims, unless clearly indicated to the contrary, should be understood to mean “at least one.”

[0075] The phrase “and/or,” as used herein in the specification and in the claims, should be understood to mean “either or both” of the elements so conjoined, i.e., elements that are conjunctively present in some cases and disjunctively present in other cases. Multiple elements listed with “and/or” should be construed in the same fashion, i.e., “one or more” of the elements so conjoined. Other elements may optionally be present other than the elements specifically identified by the “and/or” clause, whether related or unrelated to those elements specifically identified. Thus, as a non-limiting example, a reference to “A and/or B”, when used in conjunction with open-ended language such as “comprising” can refer, in one embodiment, to A only (optionally including elements other than B); in another embodiment, to B only (optionally including elements other than A); in yet another embodiment, to both A and B (optionally including other elements); etc.

[0076] As used herein in the specification and in the claims, “or” should be understood to have the same meaning as “and/or” as defined above. For example, when separating items in a list, “or” or “and/or” shall be interpreted as being inclusive, i.e., the inclusion of at least one, but also including more than one, of a number or list of elements, and, optionally, additional unlisted items. Only terms clearly indicated to the contrary, such as “only one of” or “exactly one of,” or, when used in the claims, “consisting of,” will refer to the inclusion of exactly one element of a number or list of elements. In general, the term “or” as used herein shall only be interpreted as indicating exclusive alternatives (i.e., “one or the other but not both”) when preceded by terms of exclusivity, such as “either,” “one of” “only one of” or “exactly one of” “Consisting essentially of” when used in the claims, shall have its ordinary meaning as used in the field of patent law.

[0077] As used herein, the terms “approximately” or “about” in reference to a number are generally taken to include numbers that fall within a range of 5% in either direction (greater than or less than) the number unless otherwise stated or otherwise evident from the context (except where such number would exceed 100% of a possible value). Where ranges are stated, the endpoints are included within the range unless otherwise stated or otherwise evident from the context.

[0078] The present invention has been described in terms of one or more preferred embodiments, and it should be appreciated that many equivalents, alternatives, variations, and modifications, aside from those expressly stated, are possible and within the scope of the invention.

EXAMPLES

Example 1

[0079] This section describes an embodiment of the SARS-CoV-2 inactivated tetanus toxin vaccines provided in this disclosure.

[0080] DNA encoding CRBD commercially synthesized for optimal protein expression in *E. coli*. DNA encoding CRBD was subcloned 5' to the gene encoding genetically inactivated tetanus toxin (8MTT) for expression in *E. coli*, using a pET28 vector for expression (FIGS. 1 and 4). The entire open reading frame of CRBD-8MTT was sequenced to confirm the chimera and that there were no secondary mutations in the clone. Using a standard protein purification protocol, CRBD-8MTT has been produced as a soluble protein. CRBD-8MTT solubility is approximately 40% based on total CRBD-8MTT protein made. By SDS-PAGE, a band that reacts with anti-8MTT IgG was observed to migrate at a higher molecular weight than 8MTT consistent with the production of CRBD-8MTT (FIGS. 2 and 3).

[0081] A fusion protein was engineered to contain the receptor binding domain of SARS-COV-2 encoding residues 330-521 (CRBD) of the spike protein of the SARS-COV-2¹ fused to 8MTT. The resulting product is termed CRBD-8MTT. The CRBD is the interaction site of the spike protein with ACE2. ACE2 is the host protein receptor for SARS coronavirus and SARS-COV-2. The RBD is also the binding site for several neutralizing antibodies of the related SARS coronavirus^{1,2}.

[0082] The CRBD-8MTT construct includes the 192 amino acid Receptor Binding domain of the SARS-COV-2 spike protein. Within the SARS-COV-2 spike protein, the RBD is residues 330-521. The CRBD-8MTT construct also includes the 1315 amino acids of the inactivated tetanus toxin. The inactivated tetanus toxin includes 8 point mutations at positions 26, 230, 234, 372, 375, 768, 1226, and 1289 that inactivate the catalytic, LC translocation, and receptor binding domains of the tetanus toxin.

[0083] Using recombinant DNA technology, DNA encoding the CRBD was subcloned 5' of the gene encoding 8MTT, producing CRBD-8MTT. The CRBD-8MTT construct includes a first restriction site (amino acids HM, indicated in underline lower case letters), the SARS-COV-2 receptor binding domain (CRBD, indicated in bold text), a GGGGG-penta glycine bridge (indicated in bold underlined text), a second restriction site (amino acids EL, indicated in underlined UPPER CASE TEXT), and the 8MTT inactivated tetanus toxin (indicated in italics), including the 8 point mutations (indicated in underlined italics). DNA and the encoded protein comprising CRBD-8MTT follow.

[0084] DNA sequence of SARS-COV-2(RBD)-8MTT (SEQ ID NO:9):

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catatgCCGAACATTACCAACCTGTGCCCGTTTGGCGAAGTGTTTAACGCGACCCG
TTTTCGAGCGTGTATGCGTGGAACCGTAAACGTATTAGCAACTGCGTGGCGG
ATTATAGCGTGTGTATAACAGCGGAGCTTTAGCACCTTTAAATGCTATGGCG
TGAGCCCGACCAAACTGAACGATCTGTGCTTTACCAACGTGTATGCGGATAGC
TTTGTGATTCTGTGGCGATGAAGTGCGTCAGATTGCGCCGGGCCAGACCGGCAA
AATTGCGGATTATAACTATAAACTGCCGGATGATTTTACCGGCTGCGTGATTGC
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- continued

GTGGAAACAGCAACAACCTGGATAGCAAAGTGGGCGGCAACTATAACTATCTGT
 ATCGTCTGTTTCGTAAAAGCAACCTGAAACCGTTTGAACGTGATATTAGCACCG
 AAATTTATCAGGCGGGCAGCACCCCGTGCAACGGCGTGGAAGGCTTTAACTGC
 TATTTTCCGCTGCAGAGCTATGGCTTTCAGCCGACCAACGGCGTGGGCTATCA
 GCGGTATCGTGTGGTGGTCTGAGCTTTGAACTGCTGCATGCGCCGGGCGGtG
GCGGCGGtGAGCTCatgcccATTACCATTAACAACCTTCGTTATAGCGATCCGGTGAACAAC
 GATACCATTATTATGATGGAAACCGCCGgCGTGCAAAGGCCTGGATATTATTATAAAGCGTT
 TAAAATTACCGATCGTATTGTGGATTGTGCCGGAACGTTATGAATTGGCACAAACCGGAA
 GATTTCAACCCGCCGAGCAGCCTGATTGAAGGCGCGAGCGAATATTATGATCCGAACTAT
 CTGCGTACCGATAGCGATAAAGATCGTTTCCTGCAGACCATGGTGAAACTGTTTAAACGTA
 TTAAGAACAACGTGGCGGGCGAAGCGCTGCTGGATAAAATTATTAACGCGATTCCGTATCT
 GGGCAACAGCTATAGCCTGCTGGATAAATTTGATACCAACAGCAACAGCGTGAGCTTTAAC
 CTGCTGGAACAAGATCCGAGCGGCGGACCAACAAAAGCGCGATGCTGACCAACCTGAT
 TATTTTCGGCCCGGGCCCGGTGCTGAACAAAACGAAGTGCCTGGCATTGTGCTGCGTGT
 GGATAACAAGAACTATTTCCTGCGCGTGATGGCTTTGGCAGCATTATGAGATGGCGTTT
 TGCCCGGAATATGTGCCGACCTTTGATAACGTGATTGAAAACATTACCAGCCTGACCATTG
 GCAAAGCAAATATTTCGAAGATCCGCGCTGaaactGATGCATcaaCTGATTCATGTGCTG
 CATGGCCTGTATGGCATGCAGGTGAGCAGCATGAAATTATTCGAGCAAAACAGGAAATTT
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Protein sequence of SARS-COV-2 (RBD) -8MTT (SEQ ID NO: 10):

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QPTNGVGYQPYRVVLSFELLHAPGGGGELMPITINNFRYSDPVNNDTIIMMEPPACK

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VSYNNNEHIVGYPKDGNAFNLDRI LLVGYNAPGIPLYKMEAVKLRDLKTYSVQLKLYDDKN
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REFERENCES

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- [0086] 2. Bisht, H.; Roberts, A.; Vogel, L.; Subbarao, K.; Moss, B., Neutralizing antibody and protective immunity to SARS coronavirus infection of mice induced by a soluble recombinant polypeptide containing an N-terminal segment of the spike glycoprotein. *Virology* 2005, 334 (2), 160-5.
- [0087] 3. Graham, R. L.; Donaldson, E. F.; Baric, R. S., A decade after SARS: strategies for controlling emerging coronaviruses. *Nat Rev Microbiol* 2013, 11 (12), 836-48.

Example 2

[0088] This section describes additional embodiments of SARS-COV-2 inactivated tetanus toxin antigenic polypeptides of this disclosure. It was determined that fusion of various portions of the SARS-COV-2 spike protein RBD to the N-terminus of 8MTT produced chimeras that were not soluble or poorly soluble. To address this problem, additional fusion proteins were engineered in which portions of the RBD were fused to the C-terminus of 8MTT, thus

constructing; 8MTT(RBD330-525), 8MTT(RBD376-525) and 8MTT(RBD433-524). The three chimeras were expressed in *E. coli*, but only 8MTT(RBD376-525) and 8MTT(RBD433-524) were soluble. 8MTT(RBD433-524) was determined to be more manageable to work with than 8MTTRBD(376-525), so further experiments were performed with 8MTT(RBD433-524).

[0089] FIG. 12 illustrates exemplary “8MTT(RBD433-524)” fusion protein embodiments. RBD(433-524) refers to a portion of the receptor binding domain (amino acids 330-525) of the SARS-CoV-2 spike protein. RBD(433-524) contains the binding sites for several monoclonal antibodies (REGN10987 and REGN10933) that complementally neutralize SARS-CoV-2 infections in cultured cells (Ref 1). Accordingly, experiments were designed to test the ability of RBD(433-524) to stimulate the production of antibodies that will neutralize SARS-CoV-2 by redundant mechanisms. It was determined that 8MTT(RBD433-524) reacts with antisera to the RBD of the SARS-CoV-2 spike protein. Importantly, 8MTT(RBD433-524) is a soluble protein when produced in *E. coli*, which suggests it is well suited for use as a vaccine. Experiments are underway to purify 8MTT(RBD433-524) and to test the purified protein via vaccination into mice. Antisera to 8MTT(RBD433-524) will be tested to determine whether the antigen raises antibodies to the RBD433-524 domain and whether those antibodies block RBD-ACE2 interactions.

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<212> TYPE: PRT

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<220> FEATURE:

<221> NAME/KEY: MISC_FEATURE

<222> LOCATION: (1)..(1315)

<223> OTHER INFORMATION: Wild-type tetanus toxin

<400> SEQUENCE: 1

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Tyr Tyr Lys Ala Phe Lys Ile Thr Asp Arg Ile Trp Ile Val Pro Glu
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Arg Tyr Glu Phe Gly Thr Lys Pro Glu Asp Phe Asn Pro Pro Ser Ser
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Leu Ile Glu Gly Ala Ser Glu Tyr Tyr Asp Pro Asn Tyr Leu Arg Thr
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Asp Ser Asp Lys Asp Arg Phe Leu Gln Thr Met Val Lys Leu Phe Asn
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Asp Thr Asn Ser Asn Ser Val Ser Phe Asn Leu Leu Glu Gln Asp Pro
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Gly Pro Gly Pro Val Leu Asn Lys Asn Glu Val Arg Gly Ile Val Leu
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Arg Val Asp Asn Lys Asn Tyr Phe Pro Cys Arg Asp Gly Phe Gly Ser
      180            185            190

Ile Met Gln Met Ala Phe Cys Pro Glu Tyr Val Pro Thr Phe Asp Asn
      195            200            205

Val Ile Glu Asn Ile Thr Ser Leu Thr Ile Gly Lys Ser Lys Tyr Phe
      210            215            220

Gln Asp Pro Ala Leu Leu Leu Met His Glu Leu Ile His Val Leu His
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Gly Leu Tyr Gly Met Gln Val Ser Ser His Glu Ile Ile Pro Ser Lys
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Gln Glu Ile Tyr Met Gln His Thr Tyr Pro Ile Ser Ala Glu Glu Leu
      260            265            270

Phe Thr Phe Gly Gly Gln Asp Ala Asn Leu Ile Ser Ile Asp Ile Lys
      275            280            285

Asn Asp Leu Tyr Glu Lys Thr Leu Asn Asp Tyr Lys Ala Ile Ala Asn
      290            295            300

Lys Leu Ser Gln Val Thr Ser Cys Asn Asp Pro Asn Ile Asp Ile Asp
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Ser Tyr Lys Gln Ile Tyr Gln Gln Lys Tyr Gln Phe Asp Lys Asp Ser
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Ile	Lys	Thr	Arg	Leu	Ser	Tyr	Phe	Ser	Met	Asn	His	Asp	Pro	Val	Lys	
			370						375			380				
Ile	Pro	Asn	Leu	Leu	Asp	Asp	Thr	Ile	Tyr	Asn	Asp	Thr	Glu	Gly	Phe	
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Lys	Asn	Ser	Phe	Ser	Glu	Glu	Pro	Phe	Gln	Asp	Glu	Ile	Val	Ser	Tyr	
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Asn	Thr	Lys	Asn	Lys	Pro	Leu	Asn	Phe	Asn	Tyr	Ser	Leu	Asp	Lys	Ile	
			500						505			510				
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Thr	Thr	Pro	Val	Thr	Lys	Gly	Ile	Pro	Tyr	Ala	Pro	Glu	Tyr	Lys	Ser	
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Phe His Ala Ile His Val Ser Gly Thr Asn Gly Thr Lys Arg Phe Asp
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85 90 95
Lys Ser Asn Ile Ile Arg Gly Trp Ile Phe Gly Thr Thr Leu Asp Ser
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Lys Thr Gln Ser Leu Leu Ile Val Asn Asn Ala Thr Asn Val Val Ile
115 120 125
Lys Val Cys Glu Phe Gln Phe Cys Asn Asp Pro Phe Leu Gly Val Tyr
130 135 140

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Met	Asp	Leu	Glu	Gly	Lys	Gln	Gly	Asn	Phe	Lys	Asn	Leu	Arg	Glu	Phe	180	185	190	
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Pro	Ile	Asn	Leu	Val	Arg	Asp	Leu	Pro	Gln	Gly	Phe	Ser	Ala	Leu	Glu	210	215	220	
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Val	Asp	Cys	Ala	Leu	Asp	Pro	Leu	Ser	Glu	Thr	Lys	Cys	Thr	Leu	Lys	290	295	300	
Ser	Phe	Thr	Val	Glu	Lys	Gly	Ile	Tyr	Gln	Thr	Ser	Asn	Phe	Arg	Val	305	310	315	320
Gln	Pro	Thr	Glu	Ser	Ile	Val	Arg	Phe	Pro	Asn	Ile	Thr	Asn	Leu	Cys	325	330	335	
Pro	Phe	Gly	Glu	Val	Phe	Asn	Ala	Thr	Arg	Phe	Ala	Ser	Val	Tyr	Ala	340	345	350	
Trp	Asn	Arg	Lys	Arg	Ile	Ser	Asn	Cys	Val	Ala	Asp	Tyr	Ser	Val	Leu	355	360	365	
Tyr	Asn	Ser	Ala	Ser	Phe	Ser	Thr	Phe	Lys	Cys	Tyr	Gly	Val	Ser	Pro	370	375	380	
Thr	Lys	Leu	Asn	Asp	Leu	Cys	Phe	Thr	Asn	Val	Tyr	Ala	Asp	Ser	Phe	385	390	395	400
Val	Ile	Arg	Gly	Asp	Glu	Val	Arg	Gln	Ile	Ala	Pro	Gly	Gln	Thr	Gly	405	410	415	
Lys	Ile	Ala	Asp	Tyr	Asn	Tyr	Lys	Leu	Pro	Asp	Asp	Phe	Thr	Gly	Cys	420	425	430	
Val	Ile	Ala	Trp	Asn	Ser	Asn	Asn	Leu	Asp	Ser	Lys	Val	Gly	Gly	Asn	435	440	445	
Tyr	Asn	Tyr	Leu	Tyr	Arg	Leu	Phe	Arg	Lys	Ser	Asn	Leu	Lys	Pro	Phe	450	455	460	
Glu	Arg	Asp	Ile	Ser	Thr	Glu	Ile	Tyr	Gln	Ala	Gly	Ser	Thr	Pro	Cys	465	470	475	480
Asn	Gly	Val	Glu	Gly	Phe	Asn	Cys	Tyr	Phe	Pro	Leu	Gln	Ser	Tyr	Gly	485	490	495	
Phe	Gln	Pro	Thr	Asn	Gly	Val	Gly	Tyr	Gln	Pro	Tyr	Arg	Val	Val	Val	500	505	510	
Leu	Ser	Phe	Glu	Leu	Leu	His	Ala	Pro	Ala	Thr	Val	Cys	Gly	Pro	Lys	515	520	525	
Lys	Ser	Thr	Asn	Leu	Val	Lys	Asn	Lys	Cys	Val	Asn	Phe	Asn	Phe	Asn	530	535	540	

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Gly	Leu	Thr	Gly	Thr	Gly	Val	Leu	Thr	Glu	Ser	Asn	Lys	Lys	Phe	Leu
545					550					555					560
Pro	Phe	Gln	Gln	Phe	Gly	Arg	Asp	Ile	Ala	Asp	Thr	Thr	Asp	Ala	Val
				565					570					575	
Arg	Asp	Pro	Gln	Thr	Leu	Glu	Ile	Leu	Asp	Ile	Thr	Pro	Cys	Ser	Phe
			580					585					590		
Gly	Gly	Val	Ser	Val	Ile	Thr	Pro	Gly	Thr	Asn	Thr	Ser	Asn	Gln	Val
		595					600					605			
Ala	Val	Leu	Tyr	Gln	Asp	Val	Asn	Cys	Thr	Glu	Val	Pro	Val	Ala	Ile
	610					615					620				
His	Ala	Asp	Gln	Leu	Thr	Pro	Thr	Trp	Arg	Val	Tyr	Ser	Thr	Gly	Ser
625					630					635					640
Asn	Val	Phe	Gln	Thr	Arg	Ala	Gly	Cys	Leu	Ile	Gly	Ala	Glu	His	Val
			645						650					655	
Asn	Asn	Ser	Tyr	Glu	Cys	Asp	Ile	Pro	Ile	Gly	Ala	Gly	Ile	Cys	Ala
			660					665					670		
Ser	Tyr	Gln	Thr	Gln	Thr	Asn	Ser	Pro	Arg	Arg	Ala	Arg	Ser	Val	Ala
		675					680					685			
Ser	Gln	Ser	Ile	Ile	Ala	Tyr	Thr	Met	Ser	Leu	Gly	Ala	Glu	Asn	Ser
	690					695					700				
Val	Ala	Tyr	Ser	Asn	Asn	Ser	Ile	Ala	Ile	Pro	Thr	Asn	Phe	Thr	Ile
705					710					715					720
Ser	Val	Thr	Thr	Glu	Ile	Leu	Pro	Val	Ser	Met	Thr	Lys	Thr	Ser	Val
				725					730					735	
Asp	Cys	Thr	Met	Tyr	Ile	Cys	Gly	Asp	Ser	Thr	Glu	Cys	Ser	Asn	Leu
			740					745					750		
Leu	Leu	Gln	Tyr	Gly	Ser	Phe	Cys	Thr	Gln	Leu	Asn	Arg	Ala	Leu	Thr
		755					760					765			
Gly	Ile	Ala	Val	Glu	Gln	Asp	Lys	Asn	Thr	Gln	Glu	Val	Phe	Ala	Gln
	770					775					780				
Val	Lys	Gln	Ile	Tyr	Lys	Thr	Pro	Pro	Ile	Lys	Asp	Phe	Gly	Gly	Phe
785					790					795					800
Asn	Phe	Ser	Gln	Ile	Leu	Pro	Asp	Pro	Ser	Lys	Pro	Ser	Lys	Arg	Ser
			805					810						815	
Phe	Ile	Glu	Asp	Leu	Leu	Phe	Asn	Lys	Val	Thr	Leu	Ala	Asp	Ala	Gly
			820					825					830		
Phe	Ile	Lys	Gln	Tyr	Gly	Asp	Cys	Leu	Gly	Asp	Ile	Ala	Ala	Arg	Asp
		835					840					845			
Leu	Ile	Cys	Ala	Gln	Lys	Phe	Asn	Gly	Leu	Thr	Val	Leu	Pro	Pro	Leu
	850					855					860				
Leu	Thr	Asp	Glu	Met	Ile	Ala	Gln	Tyr	Thr	Ser	Ala	Leu	Leu	Ala	Gly
865					870					875					880
Thr	Ile	Thr	Ser	Gly	Trp	Thr	Phe	Gly	Ala	Gly	Ala	Ala	Leu	Gln	Ile
			885					890						895	
Pro	Phe	Ala	Met	Gln	Met	Ala	Tyr	Arg	Phe	Asn	Gly	Ile	Gly	Val	Thr
			900					905					910		
Gln	Asn	Val	Leu	Tyr	Glu	Asn	Gln	Lys	Leu	Ile	Ala	Asn	Gln	Phe	Asn
		915					920					925			
Ser	Ala	Ile	Gly	Lys	Ile	Gln	Asp	Ser	Leu	Ser	Ser	Thr	Ala	Ser	Ala
	930					935					940				
Leu	Gly	Lys	Leu	Gln	Asp	Val	Val	Asn	Gln	Asn	Ala	Gln	Ala	Leu	Asn

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945	950	955	960
Thr Leu Val Lys Gln Leu Ser Ser Asn Phe Gly Ala Ile Ser Ser Val			
	965	970	975
Leu Asn Asp Ile Leu Ser Arg Leu Asp Lys Val Glu Ala Glu Val Gln			
	980	985	990
Ile Asp Arg Leu Ile Thr Gly Arg Leu Gln Ser Leu Gln Thr Tyr Val			
	995	1000	1005
Thr Gln Gln Leu Ile Arg Ala Ala Glu Ile Arg Ala Ser Ala Asn			
	1010	1015	1020
Leu Ala Ala Thr Lys Met Ser Glu Cys Val Leu Gly Gln Ser Lys			
	1025	1030	1035
Arg Val Asp Phe Cys Gly Lys Gly Tyr His Leu Met Ser Phe Pro			
	1040	1045	1050
Gln Ser Ala Pro His Gly Val Val Phe Leu His Val Thr Tyr Val			
	1055	1060	1065
Pro Ala Gln Glu Lys Asn Phe Thr Thr Ala Pro Ala Ile Cys His			
	1070	1075	1080
Asp Gly Lys Ala His Phe Pro Arg Glu Gly Val Phe Val Ser Asn			
	1085	1090	1095
Gly Thr His Trp Phe Val Thr Gln Arg Asn Phe Tyr Glu Pro Gln			
	1100	1105	1110
Ile Ile Thr Thr Asp Asn Thr Phe Val Ser Gly Asn Cys Asp Val			
	1115	1120	1125
Val Ile Gly Ile Val Asn Asn Thr Val Tyr Asp Pro Leu Gln Pro			
	1130	1135	1140
Glu Leu Asp Ser Phe Lys Glu Glu Leu Asp Lys Tyr Phe Lys Asn			
	1145	1150	1155
His Thr Ser Pro Asp Val Asp Leu Gly Asp Ile Ser Gly Ile Asn			
	1160	1165	1170
Ala Ser Val Val Asn Ile Gln Lys Glu Ile Asp Arg Leu Asn Glu			
	1175	1180	1185
Val Ala Lys Asn Leu Asn Glu Ser Leu Ile Asp Leu Gln Glu Leu			
	1190	1195	1200
Gly Lys Tyr Glu Gln Tyr Ile Lys Trp Pro Trp Tyr Ile Trp Leu			
	1205	1210	1215
Gly Phe Ile Ala Gly Leu Ile Ala Ile Val Met Val Thr Ile Met			
	1220	1225	1230
Leu Cys Cys Met Thr Ser Cys Cys Ser Cys Leu Lys Gly Cys Cys			
	1235	1240	1245
Ser Cys Gly Ser Cys Cys Lys Phe Asp Glu Asp Asp Ser Glu Pro			
	1250	1255	1260
Val Leu Lys Gly Val Lys Leu His Tyr Thr			
	1265	1270	

<210> SEQ ID NO 3

<211> LENGTH: 196

<212> TYPE: PRT

<213> ORGANISM: SARS-COV-2

<220> FEATURE:

<221> NAME/KEY: MISC_FEATURE

<222> LOCATION: (1)..(196)

<223> OTHER INFORMATION: Receptor binding domain (CRBD; residues 330-521 of the spike protein)

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<400> SEQUENCE: 3

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Pro Asn Ile Thr Asn Leu Cys Pro Phe Gly Glu Val Phe Asn Ala Thr
1      5      10      15
Arg Phe Ala Ser Val Tyr Ala Trp Asn Arg Lys Arg Ile Ser Asn Cys
20     25     30
Val Ala Asp Tyr Ser Val Leu Tyr Asn Ser Ala Ser Phe Ser Thr Phe
35     40     45
Lys Cys Tyr Gly Val Ser Pro Thr Lys Leu Asn Asp Leu Cys Phe Thr
50     55     60
Asn Val Tyr Ala Asp Ser Phe Val Ile Arg Gly Asp Glu Val Arg Gln
65     70     75     80
Ile Ala Pro Gly Gln Thr Gly Lys Ile Ala Asp Tyr Asn Tyr Lys Leu
85     90     95
Pro Asp Asp Phe Thr Gly Cys Val Ile Ala Trp Asn Ser Asn Asn Leu
100    105    110
Asp Ser Lys Val Gly Gly Asn Tyr Asn Tyr Leu Tyr Arg Leu Phe Arg
115    120    125
Lys Ser Asn Leu Lys Pro Phe Glu Arg Asp Ile Ser Thr Glu Ile Tyr
130    135    140
Gln Ala Gly Ser Thr Pro Cys Asn Gly Val Glu Gly Phe Asn Cys Tyr
145    150    155    160
Phe Pro Leu Gln Ser Tyr Gly Phe Gln Pro Thr Asn Gly Val Gly Tyr
165    170    175
Gln Pro Tyr Arg Val Val Val Leu Ser Phe Glu Leu Leu His Ala Pro
180    185    190
Ala Thr Val Cys
195

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<210> SEQ ID NO 4

<211> LENGTH: 1315

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic - Modified tetanus toxin (MTT)
polypeptide "2MTT"

<400> SEQUENCE: 4

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

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Asp	Thr	Asn	Ser	Asn	Ser	Val	Ser	Phe	Asn	Leu	Leu	Glu	Gln	Asp	Pro
130						135					140				
Ser	Gly	Ala	Thr	Thr	Lys	Ser	Ala	Met	Leu	Thr	Asn	Leu	Ile	Ile	Phe
145					150					155					160
Gly	Pro	Gly	Pro	Val	Leu	Asn	Lys	Asn	Glu	Val	Arg	Gly	Ile	Val	Leu
				165					170					175	
Arg	Val	Asp	Asn	Lys	Asn	Tyr	Phe	Pro	Cys	Arg	Asp	Gly	Phe	Gly	Ser
			180					185					190		
Ile	Met	Gln	Met	Ala	Phe	Cys	Pro	Glu	Tyr	Val	Pro	Thr	Phe	Asp	Asn
		195					200					205			
Val	Ile	Glu	Asn	Ile	Thr	Ser	Leu	Thr	Ile	Gly	Lys	Ser	Lys	Tyr	Phe
	210					215					220				
Gln	Asp	Pro	Ala	Leu	Leu	Leu	Met	His	Glu	Leu	Ile	His	Val	Leu	His
225					230					235					240
Gly	Leu	Tyr	Gly	Met	Gln	Val	Ser	Ser	His	Glu	Ile	Ile	Pro	Ser	Lys
				245					250					255	
Gln	Glu	Ile	Tyr	Met	Gln	His	Thr	Tyr	Pro	Ile	Ser	Ala	Glu	Glu	Leu
			260					265					270		
Phe	Thr	Phe	Gly	Gly	Gln	Asp	Ala	Asn	Leu	Ile	Ser	Ile	Asp	Ile	Lys
		275					280					285			
Asn	Asp	Leu	Tyr	Glu	Lys	Thr	Leu	Asn	Asp	Tyr	Lys	Ala	Ile	Ala	Asn
	290					295					300				
Lys	Leu	Ser	Gln	Val	Thr	Ser	Cys	Asn	Asp	Pro	Asn	Ile	Asp	Ile	Asp
305					310					315					320
Ser	Tyr	Lys	Gln	Ile	Tyr	Gln	Gln	Lys	Tyr	Gln	Phe	Asp	Lys	Asp	Ser
			325						330					335	
Asn	Gly	Gln	Tyr	Ile	Val	Asn	Glu	Asp	Lys	Phe	Gln	Ile	Leu	Tyr	Asn
			340					345					350		
Ser	Ile	Met	Tyr	Gly	Phe	Thr	Glu	Ile	Glu	Leu	Gly	Lys	Lys	Phe	Asn
		355					360					365			
Ile	Lys	Thr	Ala	Leu	Ser	Phe	Phe	Ser	Met	Asn	His	Asp	Pro	Val	Lys
	370					375					380				
Ile	Pro	Asn	Leu	Leu	Asp	Asp	Thr	Ile	Tyr	Asn	Asp	Thr	Glu	Gly	Phe
385					390					395					400
Asn	Ile	Glu	Ser	Lys	Asp	Leu	Lys	Ser	Glu	Tyr	Lys	Gly	Gln	Asn	Met
				405					410					415	
Arg	Val	Asn	Thr	Asn	Ala	Phe	Arg	Asn	Val	Asp	Gly	Ser	Gly	Leu	Val
			420					425					430		
Ser	Lys	Leu	Ile	Gly	Leu	Cys	Lys	Lys	Ile	Ile	Pro	Pro	Thr	Asn	Ile
		435					440					445			
Arg	Glu	Asn	Leu	Tyr	Asn	Arg	Thr	Ala	Ser	Leu	Thr	Asp	Leu	Gly	Gly
	450					455					460				
Glu	Leu	Cys	Ile	Lys	Ile	Lys	Asn	Glu	Asp	Leu	Thr	Phe	Ile	Ala	Glu
465					470					475					480
Lys	Asn	Ser	Phe	Ser	Glu	Glu	Pro	Phe	Gln	Asp	Glu	Ile	Val	Ser	Tyr
				485					490					495	
Asn	Thr	Lys	Asn	Lys	Pro	Leu	Asn	Phe	Asn	Tyr	Ser	Leu	Asp	Lys	Ile
				500				505					510		
Ile	Val	Asp	Tyr	Asn	Leu	Gln	Ser	Lys	Ile	Thr	Leu	Pro	Asn	Asp	Arg
		515					520					525			
Thr	Thr	Pro	Val	Thr	Lys	Gly	Ile	Pro	Tyr	Ala	Pro	Glu	Tyr	Lys	Ser

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530	535	540
Asn Ala Ala Ser Thr Ile Glu Ile His Asn Ile Asp Asp Asn Thr Ile		
545	550	555 560
Tyr Gln Tyr Leu Tyr Ala Gln Lys Ser Pro Thr Thr Leu Gln Arg Ile		
	565	570 575
Thr Met Thr Asn Ser Val Asp Asp Ala Leu Ile Asn Ser Thr Lys Ile		
	580	585 590
Tyr Ser Tyr Phe Pro Ser Val Ile Ser Lys Val Asn Gln Gly Ala Gln		
	595	600 605
Gly Ile Leu Phe Leu Gln Trp Val Arg Asp Ile Ile Asp Asp Phe Thr		
610	615	620
Asn Glu Ser Ser Gln Lys Thr Thr Ile Asp Lys Ile Ser Asp Val Ser		
625	630	635 640
Thr Ile Val Pro Tyr Ile Gly Pro Ala Leu Asn Ile Val Lys Gln Gly		
	645	650 655
Tyr Glu Gly Asn Phe Ile Gly Ala Leu Glu Thr Thr Gly Val Val Leu		
	660	665 670
Leu Leu Glu Tyr Ile Pro Glu Ile Thr Leu Pro Val Ile Ala Ala Leu		
	675	680 685
Ser Ile Ala Glu Ser Ser Thr Gln Lys Glu Lys Ile Ile Lys Thr Ile		
690	695	700
Asp Asn Phe Leu Glu Lys Arg Tyr Glu Lys Trp Ile Glu Val Tyr Lys		
705	710	715 720
Leu Val Lys Ala Lys Trp Leu Gly Thr Val Asn Thr Gln Phe Gln Lys		
	725	730 735
Arg Ser Tyr Gln Met Tyr Arg Ser Leu Glu Tyr Gln Val Asp Ala Ile		
	740	745 750
Lys Lys Ile Ile Asp Tyr Glu Tyr Lys Ile Tyr Ser Gly Pro Asp Lys		
	755	760 765
Glu Gln Ile Ala Asp Glu Ile Asn Asn Leu Lys Asn Lys Leu Glu Glu		
770	775	780
Lys Ala Asn Lys Ala Met Ile Asn Ile Asn Ile Phe Met Arg Glu Ser		
785	790	795 800
Ser Arg Ser Phe Leu Val Asn Gln Met Ile Asn Glu Ala Lys Lys Gln		
	805	810 815
Leu Leu Glu Phe Asp Thr Gln Ser Lys Asn Ile Leu Met Gln Tyr Ile		
	820	825 830
Lys Ala Asn Ser Lys Phe Ile Gly Ile Thr Glu Leu Lys Lys Leu Glu		
	835	840 845
Ser Lys Ile Asn Lys Val Phe Ser Thr Pro Ile Pro Phe Ser Tyr Ser		
850	855	860
Lys Asn Leu Asp Cys Trp Val Asp Asn Glu Glu Asp Ile Asp Val Ile		
865	870	875 880
Leu Lys Lys Ser Thr Ile Leu Asn Leu Asp Ile Asn Asn Asp Ile Ile		
	885	890 895
Ser Asp Ile Ser Gly Phe Asn Ser Ser Val Ile Thr Tyr Pro Asp Ala		
	900	905 910
Gln Leu Val Pro Gly Ile Asn Gly Lys Ala Ile His Leu Val Asn Asn		
	915	920 925
Glu Ser Ser Glu Val Ile Val His Lys Ala Met Asp Ile Glu Tyr Asn		
930	935	940

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Asp Met Phe Asn Asn Phe Thr Val Ser Phe Trp Leu Arg Val Pro Lys	
945	950 955 960
Val Ser Ala Ser His Leu Glu Gln Tyr Gly Thr Asn Glu Tyr Ser Ile	
	965 970 975
Ile Ser Ser Met Lys Lys His Ser Leu Ser Ile Gly Ser Gly Trp Ser	
	980 985 990
Val Ser Leu Lys Gly Asn Asn Leu Ile Trp Thr Leu Lys Asp Ser Ala	
	995 1000 1005
Gly Glu Val Arg Gln Ile Thr Phe Arg Asp Leu Pro Asp Lys Phe	
1010	1015 1020
Asn Ala Tyr Leu Ala Asn Lys Trp Val Phe Ile Thr Ile Thr Asn	
1025	1030 1035
Asp Arg Leu Ser Ser Ala Asn Leu Tyr Ile Asn Gly Val Leu Met	
1040	1045 1050
Gly Ser Ala Glu Ile Thr Gly Leu Gly Ala Ile Arg Glu Asp Asn	
1055	1060 1065
Asn Ile Thr Leu Lys Leu Asp Arg Cys Asn Asn Asn Asn Gln Tyr	
1070	1075 1080
Val Ser Ile Asp Lys Phe Arg Ile Phe Cys Lys Ala Leu Asn Pro	
1085	1090 1095
Lys Glu Ile Glu Lys Leu Tyr Thr Ser Tyr Leu Ser Ile Thr Phe	
1100	1105 1110
Leu Arg Asp Phe Trp Gly Asn Pro Leu Arg Tyr Asp Thr Glu Tyr	
1115	1120 1125
Tyr Leu Ile Pro Val Ala Ser Ser Ser Lys Asp Val Gln Leu Lys	
1130	1135 1140
Asn Ile Thr Asp Tyr Met Tyr Leu Thr Asn Ala Pro Ser Tyr Thr	
1145	1150 1155
Asn Gly Lys Leu Asn Ile Tyr Tyr Arg Arg Leu Tyr Asn Gly Leu	
1160	1165 1170
Lys Phe Ile Ile Lys Arg Tyr Thr Pro Asn Asn Glu Ile Asp Ser	
1175	1180 1185
Phe Val Lys Ser Gly Asp Phe Ile Lys Leu Tyr Val Ser Tyr Asn	
1190	1195 1200
Asn Asn Glu His Ile Val Gly Tyr Pro Lys Asp Gly Asn Ala Phe	
1205	1210 1215
Asn Asn Leu Asp Arg Ile Leu Arg Val Gly Tyr Asn Ala Pro Gly	
1220	1225 1230
Ile Pro Leu Tyr Lys Lys Met Glu Ala Val Lys Leu Arg Asp Leu	
1235	1240 1245
Lys Thr Tyr Ser Val Gln Leu Lys Leu Tyr Asp Asp Lys Asn Ala	
1250	1255 1260
Ser Leu Gly Leu Val Gly Thr His Asn Gly Gln Ile Gly Asn Asp	
1265	1270 1275
Pro Asn Arg Asp Ile Leu Ile Ala Ser Asn Trp Tyr Phe Asn His	
1280	1285 1290
Leu Lys Asp Lys Ile Leu Gly Cys Asp Trp Tyr Phe Val Pro Thr	
1295	1300 1305
Asp Glu Gly Trp Thr Asn Asp	
1310	1315

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<210> SEQ ID NO 5
<211> LENGTH: 1315
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic - Modified tetanus toxin (MTT)
polypeptide "5MTT"

<400> SEQUENCE: 5

Met	Pro	Ile	Thr	Ile	Asn	Asn	Phe	Arg	Tyr	Ser	Asp	Pro	Val	Asn	Asn	
1				5					10					15		
Asp	Thr	Ile	Ile	Met	Met	Glu	Pro	Pro	Tyr	Cys	Lys	Gly	Leu	Asp	Ile	
		20						25					30			
Tyr	Tyr	Lys	Ala	Phe	Lys	Ile	Thr	Asp	Arg	Ile	Trp	Ile	Val	Pro	Glu	
		35				40						45				
Arg	Tyr	Glu	Phe	Gly	Thr	Lys	Pro	Glu	Asp	Phe	Asn	Pro	Pro	Ser	Ser	
	50					55					60					
Leu	Ile	Glu	Gly	Ala	Ser	Glu	Tyr	Tyr	Asp	Pro	Asn	Tyr	Leu	Arg	Thr	
65					70				75					80		
Asp	Ser	Asp	Lys	Asp	Arg	Phe	Leu	Gln	Thr	Met	Val	Lys	Leu	Phe	Asn	
			85						90					95		
Arg	Ile	Lys	Asn	Asn	Val	Ala	Gly	Glu	Ala	Leu	Leu	Asp	Lys	Ile	Ile	
			100					105					110			
Asn	Ala	Ile	Pro	Tyr	Leu	Gly	Asn	Ser	Tyr	Ser	Leu	Leu	Asp	Lys	Phe	
		115					120					125				
Asp	Thr	Asn	Ser	Asn	Ser	Val	Ser	Phe	Asn	Leu	Leu	Glu	Gln	Asp	Pro	
	130					135						140				
Ser	Gly	Ala	Thr	Thr	Lys	Ser	Ala	Met	Leu	Thr	Asn	Leu	Ile	Ile	Phe	
145					150					155					160	
Gly	Pro	Gly	Pro	Val	Leu	Asn	Lys	Asn	Glu	Val	Arg	Gly	Ile	Val	Leu	
			165						170					175		
Arg	Val	Asp	Asn	Lys	Asn	Tyr	Phe	Pro	Cys	Arg	Asp	Gly	Phe	Gly	Ser	
			180					185					190			
Ile	Met	Gln	Met	Ala	Phe	Cys	Pro	Glu	Tyr	Val	Pro	Thr	Phe	Asp	Asn	
		195					200						205			
Val	Ile	Glu	Asn	Ile	Thr	Ser	Leu	Thr	Ile	Gly	Lys	Ser	Lys	Tyr	Phe	
	210					215					220					
Gln	Asp	Pro	Ala	Leu	Leu	Leu	Met	His	Gln	Leu	Ile	His	Val	Leu	His	
225				230						235					240	
Gly	Leu	Tyr	Gly	Met	Gln	Val	Ser	Ser	His	Glu	Ile	Ile	Pro	Ser	Lys	
			245						250					255		
Gln	Glu	Ile	Tyr	Met	Gln	His	Thr	Tyr	Pro	Ile	Ser	Ala	Glu	Glu	Leu	
		260						265					270			
Phe	Thr	Phe	Gly	Gly	Gln	Asp	Ala	Asn	Leu	Ile	Ser	Ile	Asp	Ile	Lys	
		275					280						285			
Asn	Asp	Leu	Tyr	Glu	Lys	Thr	Leu	Asn	Asp	Tyr	Lys	Ala	Ile	Ala	Asn	
	290					295					300					
Lys	Leu	Ser	Gln	Val	Thr	Ser	Cys	Asn	Asp	Pro	Asn	Ile	Asp	Ile	Asp	
305				310						315				320		
Ser	Tyr	Lys	Gln	Ile	Tyr	Gln	Gln	Lys	Tyr	Gln	Phe	Asp	Lys	Asp	Ser	
			325						330					335		
Asn	Gly	Gln	Tyr	Ile	Val	Asn	Glu	Asp	Lys	Phe	Gln	Ile	Leu	Tyr	Asn	
		340						345					350			

Ser	Ile	Met	Tyr	Gly	Phe	Thr	Glu	Ile	Glu	Leu	Gly	Lys	Lys	Phe	Asn
355							360					365			
Ile	Lys	Thr	Ala	Leu	Ser	Phe	Phe	Ser	Met	Asn	His	Asp	Pro	Val	Lys
370						375					380				
Ile	Pro	Asn	Leu	Leu	Asp	Asp	Thr	Ile	Tyr	Asn	Asp	Thr	Glu	Gly	Phe
385					390					395					400
Asn	Ile	Glu	Ser	Lys	Asp	Leu	Lys	Ser	Glu	Tyr	Lys	Gly	Gln	Asn	Met
				405					410					415	
Arg	Val	Asn	Thr	Asn	Ala	Phe	Arg	Asn	Val	Asp	Gly	Ser	Gly	Leu	Val
			420					425					430		
Ser	Lys	Leu	Ile	Gly	Leu	Cys	Lys	Lys	Ile	Ile	Pro	Pro	Thr	Asn	Ile
435							440					445			
Arg	Glu	Asn	Leu	Tyr	Asn	Arg	Thr	Ala	Ser	Leu	Thr	Asp	Leu	Gly	Gly
450						455					460				
Glu	Leu	Cys	Ile	Lys	Ile	Lys	Asn	Glu	Asp	Leu	Thr	Phe	Ile	Ala	Glu
465					470					475					480
Lys	Asn	Ser	Phe	Ser	Glu	Glu	Pro	Phe	Gln	Asp	Glu	Ile	Val	Ser	Tyr
				485					490					495	
Asn	Thr	Lys	Asn	Lys	Pro	Leu	Asn	Phe	Asn	Tyr	Ser	Leu	Asp	Lys	Ile
			500					505					510		
Ile	Val	Asp	Tyr	Asn	Leu	Gln	Ser	Lys	Ile	Thr	Leu	Pro	Asn	Asp	Arg
515							520					525			
Thr	Thr	Pro	Val	Thr	Lys	Gly	Ile	Pro	Tyr	Ala	Pro	Glu	Tyr	Lys	Ser
530					535						540				
Asn	Ala	Ala	Ser	Thr	Ile	Glu	Ile	His	Asn	Ile	Asp	Asp	Asn	Thr	Ile
545					550					555					560
Tyr	Gln	Tyr	Leu	Tyr	Ala	Gln	Lys	Ser	Pro	Thr	Thr	Leu	Gln	Arg	Ile
				565					570					575	
Thr	Met	Thr	Asn	Ser	Val	Asp	Asp	Ala	Leu	Ile	Asn	Ser	Thr	Lys	Ile
			580					585					590		
Tyr	Ser	Tyr	Phe	Pro	Ser	Val	Ile	Ser	Lys	Val	Asn	Gln	Gly	Ala	Gln
595						600						605			
Gly	Ile	Leu	Phe	Leu	Gln	Trp	Val	Arg	Asp	Ile	Ile	Asp	Asp	Phe	Thr
610					615					620					
Asn	Glu	Ser	Ser	Gln	Lys	Thr	Thr	Ile	Asp	Lys	Ile	Ser	Asp	Val	Ser
625					630					635					640
Thr	Ile	Val	Pro	Tyr	Ile	Gly	Pro	Ala	Leu	Asn	Ile	Val	Lys	Gln	Gly
			645					650					655		
Tyr	Glu	Gly	Asn	Phe	Ile	Gly	Ala	Leu	Glu	Thr	Thr	Gly	Val	Val	Leu
			660				665					670			
Leu	Leu	Glu	Tyr	Ile	Pro	Glu	Ile	Thr	Leu	Pro	Val	Ile	Ala	Ala	Leu
			675			680						685			
Ser	Ile	Ala	Glu	Ser	Ser	Thr	Gln	Lys	Glu	Lys	Ile	Ile	Lys	Thr	Ile
690					695						700				
Asp	Asn	Phe	Leu	Glu	Lys	Arg	Tyr	Glu	Lys	Trp	Ile	Glu	Val	Tyr	Lys
705					710										

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Lys	Lys	Ile	Ile	Asp	Tyr	Glu	Tyr	Lys	Ile	Tyr	Ser	Gly	Pro	Asp	Lys
		755						760				765			
Glu	Gln	Ile	Ala	Asp	Glu	Ile	Asn	Asn	Leu	Lys	Asn	Lys	Leu	Glu	Glu
	770					775					780				
Lys	Ala	Asn	Lys	Ala	Met	Ile	Asn	Ile	Asn	Ile	Phe	Met	Arg	Glu	Ser
	785				790					795					800
Ser	Arg	Ser	Phe	Leu	Val	Asn	Gln	Met	Ile	Asn	Glu	Ala	Lys	Lys	Gln
			805						810					815	
Leu	Leu	Glu	Phe	Asp	Thr	Gln	Ser	Lys	Asn	Ile	Leu	Met	Gln	Tyr	Ile
			820					825					830		
Lys	Ala	Asn	Ser	Lys	Phe	Ile	Gly	Ile	Thr	Glu	Leu	Lys	Lys	Leu	Glu
		835					840					845			
Ser	Lys	Ile	Asn	Lys	Val	Phe	Ser	Thr	Pro	Ile	Pro	Phe	Ser	Tyr	Ser
	850					855					860				
Lys	Asn	Leu	Asp	Cys	Trp	Val	Asp	Asn	Glu	Glu	Asp	Ile	Asp	Val	Ile
	865				870					875					880
Leu	Lys	Lys	Ser	Thr	Ile	Leu	Asn	Leu	Asp	Ile	Asn	Asn	Asp	Ile	Ile
			885						890					895	
Ser	Asp	Ile	Ser	Gly	Phe	Asn	Ser	Ser	Val	Ile	Thr	Tyr	Pro	Asp	Ala
			900					905					910		
Gln	Leu	Val	Pro	Gly	Ile	Asn	Gly	Lys	Ala	Ile	His	Leu	Val	Asn	Asn
		915					920					925			
Glu	Ser	Ser	Glu	Val	Ile	Val	His	Lys	Ala	Met	Asp	Ile	Glu	Tyr	Asn
	930					935					940				
Asp	Met	Phe	Asn	Asn	Phe	Thr	Val	Ser	Phe	Trp	Leu	Arg	Val	Pro	Lys
	945				950					955					960
Val	Ser	Ala	Ser	His	Leu	Glu	Gln	Tyr	Gly	Thr	Asn	Glu	Tyr	Ser	Ile
			965					970						975	
Ile	Ser	Ser	Met	Lys	Lys	His	Ser	Leu	Ser	Ile	Gly	Ser	Gly	Trp	Ser
			980					985					990		
Val	Ser	Leu	Lys	Gly	Asn	Asn	Leu	Ile	Trp	Thr	Leu	Lys	Asp	Ser	Ala
		995					1000					1005			
Gly	Glu	Val	Arg	Gln	Ile	Thr	Phe	Arg	Asp	Leu	Pro	Asp	Lys	Phe	
	1010					1015					1020				
Asn	Ala	Tyr	Leu	Ala	Asn	Lys	Trp	Val	Phe	Ile	Thr	Ile	Thr	Asn	
	1025					1030					1035				
Asp	Arg	Leu	Ser	Ser	Ala	Asn	Leu	Tyr	Ile	Asn	Gly	Val	Leu	Met	
	1040					1045					1050				
Gly	Ser	Ala	Glu	Ile	Thr	Gly	Leu	Gly	Ala	Ile	Arg	Glu	Asp	Asn	
	1055					1060					1065				
Asn	Ile	Thr	Leu	Lys	Leu	Asp	Arg	Cys	Asn	Asn	Asn	Asn	Gln	Tyr	
	1070					1075					1080				
Val	Ser	Ile	Asp	Lys	Phe	Arg	Ile	Phe	Cys	Lys	Ala	Leu	Asn	Pro	
	1085					1090					1095				
Lys	Glu	Ile	Glu	Lys	Leu	Tyr	Thr	Ser	Tyr	Leu	Ser	Ile	Thr	Phe	
	1100					1105					1110				
Leu	Arg	Asp	Phe	Trp	Gly	Asn	Pro	Leu	Arg	Tyr	Asp	Thr	Glu	Tyr	
	1115					1120					1125				
Tyr	Leu	Ile	Pro	Val	Ala	Ser	Ser	Ser	Lys	Asp	Val	Gln	Leu	Lys	
	1130					1135					1140				
Asn	Ile	Thr	Asp	Tyr	Met	Tyr	Leu	Thr	Asn	Ala	Pro	Ser	Tyr	Thr	

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1145	1150	1155
Asn Gly Lys Leu Asn Ile Tyr Tyr Arg Arg Leu Tyr Asn Gly Leu		
1160	1165	1170
Lys Phe Ile Ile Lys Arg Tyr Thr Pro Asn Asn Glu Ile Asp Ser		
1175	1180	1185
Phe Val Lys Ser Gly Asp Phe Ile Lys Leu Tyr Val Ser Tyr Asn		
1190	1195	1200
Asn Asn Glu His Ile Val Gly Tyr Pro Lys Asp Gly Asn Ala Phe		
1205	1210	1215
Asn Asn Leu Asp Arg Ile Leu Leu Val Gly Tyr Asn Ala Pro Gly		
1220	1225	1230
Ile Pro Leu Tyr Lys Lys Met Glu Ala Val Lys Leu Arg Asp Leu		
1235	1240	1245
Lys Thr Tyr Ser Val Gln Leu Lys Leu Tyr Asp Asp Lys Asn Ala		
1250	1255	1260
Ser Leu Gly Leu Val Gly Thr His Asn Gly Gln Ile Gly Asn Asp		
1265	1270	1275
Pro Asn Arg Asp Ile Leu Ile Ala Ser Asn Ala Tyr Phe Asn His		
1280	1285	1290
Leu Lys Asp Lys Ile Leu Gly Cys Asp Trp Tyr Phe Val Pro Thr		
1295	1300	1305
Asp Glu Gly Trp Thr Asn Asp		
1310	1315	

<210> SEQ ID NO 6
 <211> LENGTH: 1315
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Synthetic - Modified tetanus toxin (MTT)
 polypeptide "6MTT"

<400> SEQUENCE: 6

Met Pro Ile Thr Ile Asn Asn Phe Arg Tyr Ser Asp Pro Val Asn Asn		
1	5	10 15
Asp Thr Ile Ile Met Met Glu Pro Pro Tyr Cys Lys Gly Leu Asp Ile		
20	25	30
Tyr Tyr Lys Ala Phe Lys Ile Thr Asp Arg Ile Trp Ile Val Pro Glu		
35	40	45
Arg Tyr Glu Phe Gly Thr Lys Pro Glu Asp Phe Asn Pro Pro Ser Ser		
50	55	60
Leu Ile Glu Gly Ala Ser Glu Tyr Tyr Asp Pro Asn Tyr Leu Arg Thr		
65	70	75 80
Asp Ser Asp Lys Asp Arg Phe Leu Gln Thr Met Val Lys Leu Phe Asn		
85	90	95
Arg Ile Lys Asn Asn Val Ala Gly Glu Ala Leu Leu Asp Lys Ile Ile		
100	105	110
Asn Ala Ile Pro Tyr Leu Gly Asn Ser Tyr Ser Leu Leu Asp Lys Phe		
115	120	125
Asp Thr Asn Ser Asn Ser Val Ser Phe Asn Leu Leu Glu Gln Asp Pro		
130	135	140
Ser Gly Ala Thr Thr Lys Ser Ala Met Leu Thr Asn Leu Ile Ile Phe		
145	150	155 160

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Gly	Pro	Gly	Pro	Val	Leu	Asn	Lys	Asn	Glu	Val	Arg	Gly	Ile	Val	Leu
				165					170					175	
Arg	Val	Asp	Asn	Lys	Asn	Tyr	Phe	Pro	Cys	Arg	Asp	Gly	Phe	Gly	Ser
			180					185					190		
Ile	Met	Gln	Met	Ala	Phe	Cys	Pro	Glu	Tyr	Val	Pro	Thr	Phe	Asp	Asn
		195					200					205			
Val	Ile	Glu	Asn	Ile	Thr	Ser	Leu	Thr	Ile	Gly	Lys	Ser	Lys	Tyr	Phe
	210					215					220				
Gln	Asp	Pro	Ala	Leu	Leu	Leu	Met	His	Gln	Leu	Ile	His	Val	Leu	His
	225				230					235					240
Gly	Leu	Tyr	Gly	Met	Gln	Val	Ser	Ser	His	Glu	Ile	Ile	Pro	Ser	Lys
				245					250					255	
Gln	Glu	Ile	Tyr	Met	Gln	His	Thr	Tyr	Pro	Ile	Ser	Ala	Glu	Glu	Leu
			260					265					270		
Phe	Thr	Phe	Gly	Gly	Gln	Asp	Ala	Asn	Leu	Ile	Ser	Ile	Asp	Ile	Lys
		275					280					285			
Asn	Asp	Leu	Tyr	Glu	Lys	Thr	Leu	Asn	Asp	Tyr	Lys	Ala	Ile	Ala	Asn
	290					295					300				
Lys	Leu	Ser	Gln	Val	Thr	Ser	Cys	Asn	Asp	Pro	Asn	Ile	Asp	Ile	Asp
	305				310					315					320
Ser	Tyr	Lys	Gln	Ile	Tyr	Gln	Gln	Lys	Tyr	Gln	Phe	Asp	Lys	Asp	Ser
			325						330					335	
Asn	Gly	Gln	Tyr	Ile	Val	Asn	Glu	Asp	Lys	Phe	Gln	Ile	Leu	Tyr	Asn
			340					345					350		
Ser	Ile	Met	Tyr	Gly	Phe	Thr	Glu	Ile	Glu	Leu	Gly	Lys	Lys	Phe	Asn
		355					360					365			
Ile	Lys	Thr	Ala	Leu	Ser	Phe	Phe	Ser	Met	Asn	His	Asp	Pro	Val	Lys
	370					375					380				
Ile	Pro	Asn	Leu	Leu	Asp	Asp	Thr	Ile	Tyr	Asn	Asp	Thr	Glu	Gly	Phe
	385				390					395					400
Asn	Ile	Glu	Ser	Lys	Asp	Leu	Lys	Ser	Glu	Tyr	Lys	Gly	Gln	Asn	Met
				405					410					415	
Arg	Val	Asn	Thr	Asn	Ala	Phe	Arg	Asn	Val	Asp	Gly	Ser	Gly	Leu	Val
			420					425					430		
Ser	Lys	Leu	Ile	Gly	Leu	Cys	Lys	Lys	Ile	Ile	Pro	Pro	Thr	Asn	Ile
		435					440					445			
Arg	Glu	Asn	Leu	Tyr	Asn	Arg	Thr	Ala	Ser	Leu	Thr	Asp	Leu	Gly	Gly
	450					455					460				
Glu	Leu	Cys	Ile	Lys	Ile	Lys	Asn	Glu	Asp	Leu	Thr	Phe	Ile	Ala	Glu
	465				470					475					480
Lys	Asn	Ser	Phe	Ser	Glu	Glu	Pro	Phe	Gln	Asp	Glu	Ile	Val	Ser	Tyr
				485					490					495	
Asn	Thr	Lys	Asn	Lys	Pro	Leu	Asn	Phe	Asn	Tyr	Ser	Leu	Asp	Lys	Ile
			500					505					510		
Ile	Val	Asp	Tyr	Asn	Leu	Gln	Ser	Lys	Ile	Thr	Leu	Pro	Asn	Asp	Arg
		515					520					525			
Thr	Thr	Pro	Val	Thr	Lys	Gly	Ile	Pro	Tyr	Ala	Pro	Glu	Tyr	Lys	Ser
	530					535					540				
Asn	Ala	Ala	Ser	Thr	Ile	Glu	Ile	His	Asn	Ile	Asp	Asp	Asn	Thr	Ile
	545				550					555					560
Tyr	Gln	Tyr	Leu	Tyr	Ala	Gln	Lys	Ser	Pro	Thr	Thr	Leu	Gln	Arg	Ile

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565								570					575				
Thr	Met	Thr	Asn	Ser	Val	Asp	Asp	Ala	Leu	Ile	Asn	Ser	Thr	Lys	Ile		
			580					585					590				
Tyr	Ser	Tyr	Phe	Pro	Ser	Val	Ile	Ser	Lys	Val	Asn	Gln	Gly	Ala	Gln		
		595					600					605					
Gly	Ile	Leu	Phe	Leu	Gln	Trp	Val	Arg	Asp	Ile	Ile	Asp	Asp	Phe	Thr		
610						615					620						
Asn	Glu	Ser	Ser	Gln	Lys	Thr	Thr	Ile	Asp	Lys	Ile	Ser	Asp	Val	Ser		
625					630					635					640		
Thr	Ile	Val	Pro	Tyr	Ile	Gly	Pro	Ala	Leu	Asn	Ile	Val	Lys	Gln	Gly		
				645					650					655			
Tyr	Glu	Gly	Asn	Phe	Ile	Gly	Ala	Leu	Glu	Thr	Thr	Gly	Val	Val	Leu		
			660					665					670				
Leu	Leu	Glu	Tyr	Ile	Pro	Glu	Ile	Thr	Leu	Pro	Val	Ile	Ala	Ala	Leu		
		675					680					685					
Ser	Ile	Ala	Glu	Ser	Ser	Thr	Gln	Lys	Glu	Lys	Ile	Ile	Lys	Thr	Ile		
690						695					700						
Asp	Asn	Phe	Leu	Glu	Lys	Arg	Tyr	Glu	Lys	Trp	Ile	Glu	Val	Tyr	Lys		
705					710					715					720		
Leu	Val	Lys	Ala	Lys	Trp	Leu	Gly	Thr	Val	Asn	Thr	Gln	Phe	Gln	Lys		
				725					730					735			
Arg	Ser	Tyr	Gln	Met	Tyr	Arg	Ser	Leu	Glu	Tyr	Gln	Val	Asp	Ala	Ile		
			740					745					750				
Lys	Lys	Ile	Ile	Asp	Tyr	Glu	Tyr	Lys	Ile	Tyr	Ser	Gly	Pro	Asp	Ala		
		755					760					765					
Glu	Gln	Ile	Ala	Asp	Glu	Ile	Asn	Asn	Leu	Lys	Asn	Lys	Leu	Glu	Glu		
770						775					780						
Lys	Ala	Asn	Lys	Ala	Met	Ile	Asn	Ile	Asn	Ile	Phe	Met	Arg	Glu	Ser		
785					790					795					800		
Ser	Arg	Ser	Phe	Leu	Val	Asn	Gln	Met	Ile	Asn	Glu	Ala	Lys	Lys	Gln		
				805					810					815			
Leu	Leu	Glu	Phe	Asp	Thr	Gln	Ser	Lys	Asn	Ile	Leu	Met	Gln	Tyr	Ile		
			820					825					830				
Lys	Ala	Asn	Ser	Lys	Phe	Ile	Gly	Ile	Thr	Glu	Leu	Lys	Lys	Leu	Glu		
		835					840					845					
Ser	Lys	Ile	Asn	Lys	Val	Phe	Ser	Thr	Pro	Ile	Pro	Phe	Ser	Tyr	Ser		
850						855					860						
Lys	Asn	Leu	Asp	Cys	Trp	Val	Asp	Asn	Glu	Glu	Asp	Ile	Asp	Val	Ile		
865				870						875					880		
Leu	Lys	Lys	Ser	Thr	Ile	Leu	Asn	Leu	Asp	Ile	Asn	Asn	Asp	Ile	Ile		
				885					890					895			
Ser	Asp	Ile	Ser	Gly	Phe	Asn	Ser	Ser	Val	Ile	Thr	Tyr	Pro	Asp	Ala		
			900						905					910			
Gln	Leu	Val	Pro	Gly	Ile	Asn	Gly	Lys	Ala	Ile	His	Leu	Val	Asn	Asn		
		915					920						925				
Glu	Ser	Ser	Glu	Val	Ile	Val	His	Lys	Ala	Met	Asp	Ile	Glu	Tyr	Asn		
930						935					940						
Asp	Met	Phe	Asn	Asn	Phe	Thr	Val	Ser	Phe	Trp	Leu	Arg	Val	Pro	Lys		
945					950					955					960		
Val	Ser	Ala	Ser	His	Leu	Glu	Gln	Tyr	Gly	Thr	Asn	Glu	Tyr	Ser	Ile		
				965					970					975			

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Ile Ser Ser Met Lys Lys His Ser Leu Ser Ile Gly Ser Gly Trp Ser
      980                      985                      990

Val Ser Leu Lys Gly Asn Asn Leu Ile Trp Thr Leu Lys Asp Ser Ala
      995                      1000                      1005

Gly Glu Val Arg Gln Ile Thr Phe Arg Asp Leu Pro Asp Lys Phe
  1010                      1015                      1020

Asn Ala Tyr Leu Ala Asn Lys Trp Val Phe Ile Thr Ile Thr Asn
  1025                      1030                      1035

Asp Arg Leu Ser Ser Ala Asn Leu Tyr Ile Asn Gly Val Leu Met
  1040                      1045                      1050

Gly Ser Ala Glu Ile Thr Gly Leu Gly Ala Ile Arg Glu Asp Asn
  1055                      1060                      1065

Asn Ile Thr Leu Lys Leu Asp Arg Cys Asn Asn Asn Asn Gln Tyr
  1070                      1075                      1080

Val Ser Ile Asp Lys Phe Arg Ile Phe Cys Lys Ala Leu Asn Pro
  1085                      1090                      1095

Lys Glu Ile Glu Lys Leu Tyr Thr Ser Tyr Leu Ser Ile Thr Phe
  1100                      1105                      1110

Leu Arg Asp Phe Trp Gly Asn Pro Leu Arg Tyr Asp Thr Glu Tyr
  1115                      1120                      1125

Tyr Leu Ile Pro Val Ala Ser Ser Ser Lys Asp Val Gln Leu Lys
  1130                      1135                      1140

Asn Ile Thr Asp Tyr Met Tyr Leu Thr Asn Ala Pro Ser Tyr Thr
  1145                      1150                      1155

Asn Gly Lys Leu Asn Ile Tyr Tyr Arg Arg Leu Tyr Asn Gly Leu
  1160                      1165                      1170

Lys Phe Ile Ile Lys Arg Tyr Thr Pro Asn Asn Glu Ile Asp Ser
  1175                      1180                      1185

Phe Val Lys Ser Gly Asp Phe Ile Lys Leu Tyr Val Ser Tyr Asn
  1190                      1195                      1200

Asn Asn Glu His Ile Val Gly Tyr Pro Lys Asp Gly Asn Ala Phe
  1205                      1210                      1215

Asn Asn Leu Asp Arg Ile Leu Leu Val Gly Tyr Asn Ala Pro Gly
  1220                      1225                      1230

Ile Pro Leu Tyr Lys Lys Met Glu Ala Val Lys Leu Arg Asp Leu
  1235                      1240                      1245

Lys Thr Tyr Ser Val Gln Leu Lys Leu Tyr Asp Asp Lys Asn Ala
  1250                      1255                      1260

Ser Leu Gly Leu Val Gly Thr His Asn Gly Gln Ile Gly Asn Asp
  1265                      1270                      1275

Pro Asn Arg Asp Ile Leu Ile Ala Ser Asn Ala Tyr Phe Asn His
  1280                      1285                      1290

Leu Lys Asp Lys Ile Leu Gly Cys Asp Trp Tyr Phe Val Pro Thr
  1295                      1300                      1305

Asp Glu Gly Trp Thr Asn Asp
  1310                      1315

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<210> SEQ ID NO 7

<211> LENGTH: 1315

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

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<223> OTHER INFORMATION: Synthetic - Modified tetanus toxin (MTT)
polypeptide "7MTT"

<400> SEQUENCE: 7

Met	Pro	Ile	Thr	Ile	Asn	Asn	Phe	Arg	Tyr	Ser	Asp	Pro	Val	Asn	Asn	1	5	10	15
Asp	Thr	Ile	Ile	Met	Met	Glu	Pro	Pro	Ala	Cys	Lys	Gly	Leu	Asp	Ile	20	25	30	
Tyr	Tyr	Lys	Ala	Phe	Lys	Ile	Thr	Asp	Arg	Ile	Trp	Ile	Val	Pro	Glu	35	40	45	
Arg	Tyr	Glu	Phe	Gly	Thr	Lys	Pro	Glu	Asp	Phe	Asn	Pro	Pro	Ser	Ser	50	55	60	
Leu	Ile	Glu	Gly	Ala	Ser	Glu	Tyr	Tyr	Asp	Pro	Asn	Tyr	Leu	Arg	Thr	65	70	75	80
Asp	Ser	Asp	Lys	Asp	Arg	Phe	Leu	Gln	Thr	Met	Val	Lys	Leu	Phe	Asn	85	90	95	
Arg	Ile	Lys	Asn	Asn	Val	Ala	Gly	Glu	Ala	Leu	Leu	Asp	Lys	Ile	Ile	100	105	110	
Asn	Ala	Ile	Pro	Tyr	Leu	Gly	Asn	Ser	Tyr	Ser	Leu	Leu	Asp	Lys	Phe	115	120	125	
Asp	Thr	Asn	Ser	Asn	Ser	Val	Ser	Phe	Asn	Leu	Leu	Glu	Gln	Asp	Pro	130	135	140	
Ser	Gly	Ala	Thr	Thr	Lys	Ser	Ala	Met	Leu	Thr	Asn	Leu	Ile	Ile	Phe	145	150	155	160
Gly	Pro	Gly	Pro	Val	Leu	Asn	Lys	Asn	Glu	Val	Arg	Gly	Ile	Val	Leu	165	170	175	
Arg	Val	Asp	Asn	Lys	Asn	Tyr	Phe	Pro	Cys	Arg	Asp	Gly	Phe	Gly	Ser	180	185	190	
Ile	Met	Gln	Met	Ala	Phe	Cys	Pro	Glu	Tyr	Val	Pro	Thr	Phe	Asp	Asn	195	200	205	
Val	Ile	Glu	Asn	Ile	Thr	Ser	Leu	Thr	Ile	Gly	Lys	Ser	Lys	Tyr	Phe	210	215	220	
Gln	Asp	Pro	Ala	Leu	Leu	Leu	Met	His	Gln	Leu	Ile	His	Val	Leu	His	225	230	235	240
Gly	Leu	Tyr	Gly	Met	Gln	Val	Ser	Ser	His	Glu	Ile	Ile	Pro	Ser	Lys	245	250	255	
Gln	Glu	Ile	Tyr	Met	Gln	His	Thr	Tyr	Pro	Ile	Ser	Ala	Glu	Glu	Leu	260	265	270	
Phe	Thr	Phe	Gly	Gly	Gln	Asp	Ala	Asn	Leu	Ile	Ser	Ile	Asp	Ile	Lys	275	280	285	
Asn	Asp	Leu	Tyr	Glu	Lys	Thr	Leu	Asn	Asp	Tyr	Lys	Ala	Ile	Ala	Asn	290	295	300	
Lys	Leu	Ser	Gln	Val	Thr	Ser	Cys	Asn	Asp	Pro	Asn	Ile	Asp	Ile	Asp	305	310	315	320
Ser	Tyr	Lys	Gln	Ile	Tyr	Gln	Gln	Lys	Tyr	Gln	Phe	Asp	Lys	Asp	Ser	325	330	335	
Asn	Gly	Gln	Tyr	Ile	Val	Asn	Glu	Asp	Lys	Phe	Gln	Ile	Leu	Tyr	Asn	340	345	350	
Ser	Ile	Met	Tyr	Gly	Phe	Thr	Glu	Ile	Glu	Leu	Gly	Lys	Lys	Phe	Asn	355	360	365	
Ile	Lys	Thr	Ala	Leu	Ser	Phe	Phe	Ser	Met	Asn	His	Asp	Pro	Val	Lys	370	375	380	

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

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Lys	Ala	Asn	Lys	Ala	Met	Ile	Asn	Ile	Asn	Ile	Phe	Met	Arg	Glu	Ser	785	790	795	800
Ser	Arg	Ser	Phe	Leu	Val	Asn	Gln	Met	Ile	Asn	Glu	Ala	Lys	Lys	Gln	805	810	815	
Leu	Leu	Glu	Phe	Asp	Thr	Gln	Ser	Lys	Asn	Ile	Leu	Met	Gln	Tyr	Ile	820	825	830	
Lys	Ala	Asn	Ser	Lys	Phe	Ile	Gly	Ile	Thr	Glu	Leu	Lys	Lys	Leu	Glu	835	840	845	
Ser	Lys	Ile	Asn	Lys	Val	Phe	Ser	Thr	Pro	Ile	Pro	Phe	Ser	Tyr	Ser	850	855	860	
Lys	Asn	Leu	Asp	Cys	Trp	Val	Asp	Asn	Glu	Glu	Asp	Ile	Asp	Val	Ile	865	870	875	880
Leu	Lys	Lys	Ser	Thr	Ile	Leu	Asn	Leu	Asp	Ile	Asn	Asn	Asp	Ile	Ile	885	890	895	
Ser	Asp	Ile	Ser	Gly	Phe	Asn	Ser	Ser	Val	Ile	Thr	Tyr	Pro	Asp	Ala	900	905	910	
Gln	Leu	Val	Pro	Gly	Ile	Asn	Gly	Lys	Ala	Ile	His	Leu	Val	Asn	Asn	915	920	925	
Glu	Ser	Ser	Glu	Val	Ile	Val	His	Lys	Ala	Met	Asp	Ile	Glu	Tyr	Asn	930	935	940	
Asp	Met	Phe	Asn	Asn	Phe	Thr	Val	Ser	Phe	Trp	Leu	Arg	Val	Pro	Lys	945	950	955	960
Val	Ser	Ala	Ser	His	Leu	Glu	Gln	Tyr	Gly	Thr	Asn	Glu	Tyr	Ser	Ile	965	970	975	
Ile	Ser	Ser	Met	Lys	Lys	His	Ser	Leu	Ser	Ile	Gly	Ser	Gly	Trp	Ser	980	985	990	
Val	Ser	Leu	Lys	Gly	Asn	Asn	Leu	Ile	Trp	Thr	Leu	Lys	Asp	Ser	Ala	995	1000	1005	
Gly	Glu	Val	Arg	Gln	Ile	Thr	Phe	Arg	Asp	Leu	Pro	Asp	Lys	Phe		1010	1015	1020	
Asn	Ala	Tyr	Leu	Ala	Asn	Lys	Trp	Val	Phe	Ile	Thr	Ile	Thr	Asn		1025	1030	1035	
Asp	Arg	Leu	Ser	Ser	Ala	Asn	Leu	Tyr	Ile	Asn	Gly	Val	Leu	Met		1040	1045	1050	
Gly	Ser	Ala	Glu	Ile	Thr	Gly	Leu	Gly	Ala	Ile	Arg	Glu	Asp	Asn		1055	1060	1065	
Asn	Ile	Thr	Leu	Lys	Leu	Asp	Arg	Cys	Asn	Asn	Asn	Asn	Gln	Tyr		1070	1075	1080	
Val	Ser	Ile	Asp	Lys	Phe	Arg	Ile	Phe	Cys	Lys	Ala	Leu	Asn	Pro		1085	1090	1095	
Lys	Glu	Ile	Glu	Lys	Leu	Tyr	Thr	Ser	Tyr	Leu	Ser	Ile	Thr	Phe		1100	1105	1110	
Leu	Arg	Asp	Phe	Trp	Gly	Asn	Pro	Leu	Arg	Tyr	Asp	Thr	Glu	Tyr		1115	1120	1125	
Tyr	Leu	Ile	Pro	Val	Ala	Ser	Ser	Ser	Lys	Asp	Val	Gln	Leu	Lys		1130	1135	1140	
Asn	Ile	Thr	Asp	Tyr	Met	Tyr	Leu	Thr	Asn	Ala	Pro	Ser	Tyr	Thr		1145	1150	1155	
Asn	Gly	Lys	Leu	Asn	Ile	Tyr	Tyr	Arg	Arg	Leu	Tyr	Asn	Gly	Leu		1160	1165	1170	
Lys	Phe	Ile	Ile	Lys	Arg	Tyr	Thr	Pro	Asn	Asn	Glu	Ile	Asp	Ser					

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1175	1180	1185
Phe Val Lys Ser Gly Asp	Phe Ile Lys Leu Tyr	Val Ser Tyr Asn
1190	1195	1200
Asn Asn Glu His Ile Val	Gly Tyr Pro Lys Asp	Gly Asn Ala Phe
1205	1210	1215
Asn Asn Leu Asp Arg Ile	Leu Leu Val Gly Tyr	Asn Ala Pro Gly
1220	1225	1230
Ile Pro Leu Tyr Lys Lys	Met Glu Ala Val Lys	Leu Arg Asp Leu
1235	1240	1245
Lys Thr Tyr Ser Val Gln	Leu Lys Leu Tyr Asp	Asp Lys Asn Ala
1250	1255	1260
Ser Leu Gly Leu Val Gly	Thr His Asn Gly Gln	Ile Gly Asn Asp
1265	1270	1275
Pro Asn Arg Asp Ile Leu	Ile Ala Ser Asn Ala	Tyr Phe Asn His
1280	1285	1290
Leu Lys Asp Lys Ile Leu	Gly Cys Asp Trp Tyr	Phe Val Pro Thr
1295	1300	1305
Asp Glu Gly Trp Thr Asn	Asp	
1310	1315	
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<211> LENGTH: 1315		
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<213> ORGANISM: Artificial Sequence		
<220> FEATURE:		
<223> OTHER INFORMATION: Synthetic - Modified tetanus toxin (MTT)		
polypeptide "8MTT"		
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Asp Thr Ile Ile Met Met Glu Pro Pro Ala Cys Lys Gly Leu Asp Ile		
20 25 30		
Tyr Tyr Lys Ala Phe Lys Ile Thr Asp Arg Ile Trp Ile Val Pro Glu		
35 40 45		
Arg Tyr Glu Phe Gly Thr Lys Pro Glu Asp Phe Asn Pro Pro Ser Ser		
50 55 60		
Leu Ile Glu Gly Ala Ser Glu Tyr Tyr Asp Pro Asn Tyr Leu Arg Thr		
65 70 75 80		
Asp Ser Asp Lys Asp Arg Phe Leu Gln Thr Met Val Lys Leu Phe Asn		
85 90 95		
Arg Ile Lys Asn Asn Val Ala Gly Glu Ala Leu Leu Asp Lys Ile Ile		
100 105 110		
Asn Ala Ile Pro Tyr Leu Gly Asn Ser Tyr Ser Leu Leu Asp Lys Phe		
115 120 125		
Asp Thr Asn Ser Asn Ser Val Ser Phe Asn Leu Leu Glu Gln Asp Pro		
130 135 140		
Ser Gly Ala Thr Thr Lys Ser Ala Met Leu Thr Asn Leu Ile Ile Phe		
145 150 155 160		
Gly Pro Gly Pro Val Leu Asn Lys Asn Glu Val Arg Gly Ile Val Leu		
165 170 175		
Arg Val Asp Asn Lys Asn Tyr Phe Pro Cys Arg Asp Gly Phe Gly Ser		
180 185 190		

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Ile	Met	Gln	Met	Ala	Phe	Cys	Pro	Glu	Tyr	Val	Pro	Thr	Phe	Asp	Asn
	195						200					205			
Val	Ile	Glu	Asn	Ile	Thr	Ser	Leu	Thr	Ile	Gly	Lys	Ser	Lys	Tyr	Phe
	210					215					220				
Gln	Asp	Pro	Ala	Leu	Leu	Lys	Met	His	Gln	Leu	Ile	His	Val	Leu	His
	225				230					235					240
Gly	Leu	Tyr	Gly	Met	Gln	Val	Ser	Ser	His	Glu	Ile	Ile	Pro	Ser	Lys
				245					250					255	
Gln	Glu	Ile	Tyr	Met	Gln	His	Thr	Tyr	Pro	Ile	Ser	Ala	Glu	Glu	Leu
			260					265					270		
Phe	Thr	Phe	Gly	Gly	Gln	Asp	Ala	Asn	Leu	Ile	Ser	Ile	Asp	Ile	Lys
		275					280					285			
Asn	Asp	Leu	Tyr	Glu	Lys	Thr	Leu	Asn	Asp	Tyr	Lys	Ala	Ile	Ala	Asn
	290					295					300				
Lys	Leu	Ser	Gln	Val	Thr	Ser	Cys	Asn	Asp	Pro	Asn	Ile	Asp	Ile	Asp
	305				310					315					320
Ser	Tyr	Lys	Gln	Ile	Tyr	Gln	Gln	Lys	Tyr	Gln	Phe	Asp	Lys	Asp	Ser
			325						330					335	
Asn	Gly	Gln	Tyr	Ile	Val	Asn	Glu	Asp	Lys	Phe	Gln	Ile	Leu	Tyr	Asn
			340					345					350		
Ser	Ile	Met	Tyr	Gly	Phe	Thr	Glu	Ile	Glu	Leu	Gly	Lys	Lys	Phe	Asn
		355					360					365			
Ile	Lys	Thr	Ala	Leu	Ser	Phe	Phe	Ser	Met	Asn	His	Asp	Pro	Val	Lys
	370					375					380				
Ile	Pro	Asn	Leu	Leu	Asp	Asp	Thr	Ile	Tyr	Asn	Asp	Thr	Glu	Gly	Phe
	385				390					395					400
Asn	Ile	Glu	Ser	Lys	Asp	Leu	Lys	Ser	Glu	Tyr	Lys	Gly	Gln	Asn	Met
				405					410					415	
Arg	Val	Asn	Thr	Asn	Ala	Phe	Arg	Asn	Val	Asp	Gly	Ser	Gly	Leu	Val
			420					425					430		
Ser	Lys	Leu	Ile	Gly	Leu	Cys	Lys	Lys	Ile	Ile	Pro	Pro	Thr	Asn	Ile
		435					440					445			
Arg	Glu	Asn	Leu	Tyr	Asn	Arg	Thr	Ala	Ser	Leu	Thr	Asp	Leu	Gly	Gly
	450					455					460				
Glu	Leu	Cys	Ile	Lys	Ile	Lys	Asn	Glu	Asp	Leu	Thr	Phe	Ile	Ala	Glu
	465				470					475					480
Lys	Asn	Ser	Phe	Ser	Glu	Glu	Pro	Phe	Gln	Asp	Glu	Ile	Val	Ser	Tyr
				485					490					495	
Asn	Thr	Lys	Asn	Lys	Pro	Leu	Asn	Phe	Asn	Tyr	Ser	Leu	Asp	Lys	Ile
			500					505					510		
Ile	Val	Asp	Tyr	Asn	Leu	Gln	Ser	Lys	Ile	Thr	Leu	Pro	Asn	Asp	Arg
		515					520					525			
Thr	Thr	Pro	Val	Thr	Lys	Gly	Ile	Pro	Tyr	Ala	Pro	Glu	Tyr	Lys	Ser
	530					535					540				
Asn	Ala	Ala	Ser	Thr	Ile	Glu	Ile	His	Asn	Ile	Asp	Asp	Asn	Thr	Ile
	545				550					555					560
Tyr	Gln	Tyr	Leu	Tyr	Ala	Gln	Lys	Ser	Pro	Thr	Thr	Leu	Gln	Arg	Ile
			565						570					575	
Thr	Met	Thr	Asn	Ser	Val	Asp	Asp	Ala	Leu	Ile	Asn	Ser	Thr	Lys	Ile
			580					585					590		
Tyr	Ser	Tyr	Phe	Pro	Ser	Val	Ile	Ser	Lys	Val	Asn	Gln	Gly	Ala	Gln

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595					600					605					
Gly 610	Ile	Leu	Phe	Leu	Gln	Trp 615	Val	Arg	Asp	Ile	Ile 620	Asp	Asp	Phe	Thr
Asn 625	Glu	Ser	Ser	Gln	Lys 630	Thr	Thr	Ile	Asp	Lys 635	Ile	Ser	Asp	Val	Ser 640
Thr	Ile	Val	Pro	Tyr 645	Ile	Gly	Pro	Ala	Leu 650	Asn	Ile	Val	Lys	Gln	Gly 655
Tyr	Glu	Gly	Asn	Phe 660	Ile	Gly	Ala	Leu 665	Glu	Thr	Thr	Gly	Val 670	Val	Leu
Leu	Leu	Glu 675	Tyr	Ile	Pro	Glu	Ile 680	Thr	Leu	Pro	Val	Ile 685	Ala	Ala	Leu
Ser 690	Ile	Ala	Glu	Ser	Ser	Thr 695	Gln	Lys	Glu	Lys	Ile 700	Ile	Lys	Thr	Ile
Asp 705	Asn	Phe	Leu	Glu	Lys 710	Arg	Tyr	Glu	Lys	Trp 715	Ile	Glu	Val	Tyr	Lys 720
Leu	Val	Lys	Ala	Lys 725	Trp	Leu	Gly	Thr	Val 730	Asn	Thr	Gln	Phe	Gln	Lys 735
Arg	Ser	Tyr	Gln	Met 740	Tyr	Arg	Ser	Leu 745	Glu	Tyr	Gln	Val	Asp 750	Ala	Ile
Lys	Lys	Ile 755	Ile	Asp	Tyr	Glu	Tyr 760	Lys	Ile	Tyr	Ser	Gly 765	Pro	Asp	Ala
Glu 770	Gln	Ile	Ala	Asp	Glu	Ile 775	Asn	Asn	Leu	Lys	Asn 780	Lys	Leu	Glu	Glu
Lys 785	Ala	Asn	Lys	Ala	Met 790	Ile	Asn	Ile	Asn	Ile 795	Phe	Met	Arg	Glu	Ser 800
Ser	Arg	Ser	Phe	Leu 805	Val	Asn	Gln	Met	Ile 810	Asn	Glu	Ala	Lys	Lys	Gln 815
Leu	Leu	Glu	Phe 820	Asp	Thr	Gln	Ser	Lys 825	Asn	Ile	Leu	Met	Gln 830	Tyr	Ile
Lys	Ala	Asn 835	Ser	Lys	Phe	Ile	Gly 840	Ile	Thr	Glu	Leu	Lys 845	Lys	Leu	Glu
Ser 850	Lys	Ile	Asn	Lys	Val	Phe 855	Ser	Thr	Pro	Ile	Pro 860	Phe	Ser	Tyr	Ser
Lys 865	Asn	Leu	Asp	Cys	Trp 870	Val	Asp	Asn	Glu	Glu 875	Asp	Ile	Asp	Val	Ile 880
Leu	Lys	Lys	Ser	Thr 885	Ile	Leu	Asn	Leu	Asp 890	Ile	Asn	Asn	Asp	Ile	Ile 895
Ser	Asp	Ile	Ser 900	Gly	Phe	Asn	Ser	Ser 905	Val	Ile	Thr	Tyr	Pro 910	Asp	Ala
Gln 915	Leu	Val	Pro	Gly	Ile	Asn	Gly 920	Lys	Ala	Ile	His 925	Leu	Val	Asn	Asn
Glu 930	Ser	Ser	Glu	Val	Ile	Val 935	His	Lys	Ala	Met	Asp 940	Ile	Glu	Tyr	Asn
Asp 945	Met	Phe	Asn	Asn	Phe 950	Thr	Val	Ser	Phe	Trp 955	Leu	Arg	Val	Pro	Lys 960
Val	Ser	Ala	Ser	His 965	Leu	Glu	Gln	Tyr	Gly 970	Thr	Asn	Glu	Tyr	Ser	Ile 975
Ile	Ser	Ser	Met 980	Lys	Lys	His	Ser	Leu 985	Ser	Ile	Gly	Ser	Gly 990	Trp	Ser
Val	Ser	Leu 995	Lys	Gly	Asn	Asn	Leu	Ile 1000	Trp	Thr	Leu	Lys	Asp 1005	Ser	Ala

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

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<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic - DNA encoding SARS-COV-2 (RBD)-8MTT

<400> SEQUENCE: 9

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ctgtataaca gcgcgagctt tagcaccttt aaatgctatg gcgtgagccc gaccaaactg	180
aacgatctgt gctttacca cgtgtatgcg gatagctttg tgattcgtgg cgatgaagtg	240
cgtcagattg cgccgggcca gaccggcaaa attgcggatt ataactataa actgccggat	300
gattttaccg gctgcgtgat tgcgtggaac agcaacaacc tggatagcaa agtgggcggc	360
aactataact atctgtatcg tctgtttcgt aaaagcaacc tgaaccgtt tgaacgtgat	420
attagcaccg aaatttatca ggcgggcagc accccgtgca acggcgtgga aggetttaac	480
tgctattttc cgctgcagag ctatggcttt cagccgacca acggcgtggg ctatcagccg	540
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accgatcgta tttggattgt gccggaacgt tatgaatttg gcaccaaacc ggaagatttc	780
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ccggaatatg tgccgacctt tgataacgtg attgaaaaca ttaccagcct gaccattggc	1260
aaaagcaaat atttccaaga tccggcgtg aaactgatgc atcaactgat tcatgtgctg	1320
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1505	1510	1515

We claim:

1. A fusion protein comprising
 - (i) a modified tetanus toxin (MTT) polypeptide comprising a sequence having at least 95% identity to SEQ ID

NO:1, having a mutation at each of positions R372 and Y375, and having a mutation at two or more positions selected from E234, K768, R1226, and W1289, wherein each position is numbered relative to SEQ ID

- NO:1, the MTT polypeptide having reduced toxicity and receptor binding compared to the toxicity and receptor binding of SEQ ID NO:1; and
- (ii) a SARS-CoV-2 spike protein receptor binding domain (CRBD) polypeptide or a portion thereof.
2. The fusion protein of claim 1, wherein the CRBD polypeptide or a portion thereof comprises at least a portion of SEQ ID NO:3.
3. The fusion protein of claim 1, wherein the CRBD polypeptide or a portion thereof comprises a sequence having at least 95% identity to SEQ ID NO:3.
4. The fusion protein of claim 1, wherein the CRBD polypeptide or a portion thereof comprises an amino acid sequence corresponding to residues 376-525 or 433-524 of SEQ ID NO:2.
5. The fusion protein of claim 1, wherein the MTT polypeptide and the CRBD polypeptide are connected by a linker polypeptide.
6. The fusion protein of claim 5, wherein the linker polypeptide is a poly-glycine sequence.
7. The fusion protein of claim 1, wherein the MTT polypeptide and the CRBD polypeptide are chemically cross-linked.
8. The fusion protein of claim 1, wherein in the MTT polypeptide amino acid Rat position R372 is replaced with amino acid A, and wherein amino acid Y at position Y375 is replaced with amino acid F.
9. The fusion protein of claim 1, wherein the MTT polypeptide mutations comprise R372A, Y375F, E234Q, R1226L, and W1289A.
10. The fusion protein of claim 9, wherein the MTT polypeptide comprises SEQ ID NO:5.
11. The fusion protein of claim 1, wherein the MTT polypeptide mutations comprise R372A, Y375F, E234Q, K768A, R1226L, and W1289A.
12. The fusion protein of claim 11, wherein the MTT polypeptide comprises SEQ ID NO:6.
13. The fusion protein of claim 1, wherein the MTT polypeptide further comprises a mutation at one or both of positions L230 and Y26, wherein each position is numbered relative to SEQ ID NO:1.
14. The fusion protein of claim 13, wherein the mutations at one or both of positions L230 and Y26 comprise L230K and Y26A.
15. The fusion protein of claim 14, wherein the MTT polypeptide comprises SEQ ID NO:7 or SEQ ID NO:8.
16. The fusion protein of claim 1, wherein the fusion protein comprises a sequence at least 95% identical to SEQ ID NO:10.
17. A polynucleotide encoding the fusion protein of claim 1.

18. The polynucleotide of claim 17, wherein the polynucleotide comprises a sequence at least 95% identical to SEQ ID NO:9.

19. A vector comprising the polynucleotide of claim 18.

20. A composition comprising the fusion protein of claim 1 and a pharmaceutically acceptable carrier.

21. A method of reducing the risk of a subject developing COVID-19 by inducing an immune response through administering to the subject a therapeutically effective amount of the fusion protein of claim 1.

22. A method for producing a CRBD-MTT chemically cross-linked fusion protein comprising:

obtaining a polypeptide composition comprising (i) a modified tetanus toxin (MTT) polypeptide comprising a sequence having at least 95% identity to SEQ ID NO:1, having a mutation at each of positions R372 and Y375, and having a mutation at two or more positions selected from E234, K768, R1226, and W1289, wherein each position is numbered relative to SEQ ID NO:1, and (ii) a SARS-CoV-2 spike protein receptor binding domain (CRBD) polypeptide or a portion thereof; and

contacting the polypeptide composition with a crosslinking agent for a time and under conditions sufficient to chemically crosslink the CRBD polypeptide and the MTT polypeptide; whereby a CRBD-MTT chemically cross-linked fusion protein is produced.

23. The method of claim 22, wherein the crosslinking agent is selected from the group consisting of formaldehyde, disuccinimidyl suberate (DSS), succinimidyl-4-(N-maleimidomethyl)cyclohexane-1-carboxylate (SMCC), ethylene glycol bis(sulfosuccinimidylsuccinate) (Sulfo-EGS), bis(sulfosuccinimidyl) suberate (BS3), and dithiobis(succinimidylpropionate) (DSP).

24. The method of claim 22, wherein the CRBD polypeptide comprises a sequence having at least 95% identity to SEQ ID NO:3.

25. The method of claim 22, wherein the CRBD polypeptide or a portion thereof comprises an amino acid sequence corresponding to residues 433-524 of SEQ ID NO:2.

26. The method of claim 22, wherein the CRBD polypeptide or a portion thereof comprises an amino acid sequence corresponding to residues 376-525 of SEQ ID NO:2.

* * * * *