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(54) Title: MINI-PROTEIN IMMUNOGENS DISPLAYNG NEUTRALIZATION EPITOPES FOR RESPIRATORY SYNCYTIAL VIRUS (RSV)

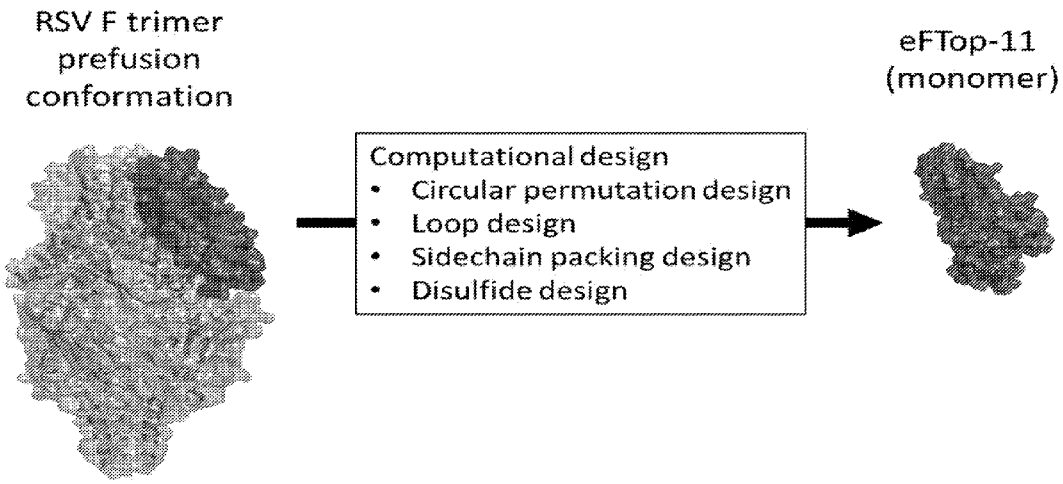

Fig. 1

(57) Abstract: Polypeptides and their use for treating or limiting a respiratory syncytial virus infection are provided.

[Continued on next page]

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Mini-protein immunogens displaying neutralization epitopes for respiratory syncytial virus (RSV)

Cross Reference

This application claims priority to U.S. Provisional Patent Application Serial Number 62/474947 filed March 22, 2017, incorporated by reference herein in its entirety.

Background

Respiratory Syncytial Virus (RSV) is the leading cause of viral death in infants worldwide and also causes disease in the elderly and immune-compromised. The RSV-F protein is a trimeric glycoprotein that contains both neutralizing and non-neutralizing epitopes. The development of a stable, minimal, monomeric domain containing potent neutralizing epitopes and minimizing non-neutralizing epitopes would offer an attractive alternative to the pre-fusion RSV F protein trimer that is currently being pursued by most in the field.

Summary

In one aspect are provided polypeptides comprising a first domain, wherein the first domain comprises the amino acid sequence of SEQ ID NO:1. In one embodiment, first domain comprises the amino acid sequence of SEQ ID NOS: 2-8. In another embodiment, the first domain is present in two or more copies. In a further embodiment, the polypeptide further

further comprises a multimerization domain. In various embodiments, the multimerization domain comprises the amino acid sequence selected from the group consisting of SEQ ID NOS:9-28.

In another aspect are provided polypeptides comprising:

(a) a multimerization domain comprising the amino acid sequence selected from the group consisting of SEQ ID NO:10, 14, and 15-28; and

(b) one or more copies of a respiratory syncytial virus (RSV) antigen.

In one embodiment, the multimerization domain comprises the amino acid sequence selected from the group consisting of SEQ ID NO: 10 and 15-28. In another embodiment, the polypeptide further comprises an amino acid linker between the first domain and the multimerization domain, or between the multimerization domain and the RSV antigen. In

another embodiment, the polypeptide comprises the amino acid sequence selected from the group consisting of SEQ ID NOS: 29-41.

In another embodiment are provided multimers of the polypeptides of the disclosure. In various embodiments, the multimer comprises 2, 3, 4, 5, 6, 7, 8, or more copies of the polypeptides of the disclosure.

In one aspect nucleic acids are provided encoding the polypeptide of any embodiment of the disclosure. In one embodiment, the nucleic acid comprises the sequence selected from the group consisting of SEQ ID NOS: 44-47. In another aspect recombinant expression vectors comprising the nucleic acids of the disclosure operatively linked to a suitable control sequence are provided. In a further aspect recombinant host cells comprising the recombinant expression vectors of the disclosure are provided.

In another aspect pharmaceutical compositions are provided that comprise

(a) the polypeptide, the multimer, the nucleic acid, the recombinant expression vector, or the recombinant host cell of any embodiment of the disclosure; and

(b) a pharmaceutically acceptable carrier.

In a further aspect, methods for treating a respiratory syncytial virus (RSV) infection are provided, comprising administering to a subject infected with RSV an amount effective to treat the infection of the polypeptide, the multimer, the nucleic acid, the recombinant expression vector, the recombinant host cell, or the pharmaceutical composition, of any embodiment of the disclosure.

In another aspect, methods for limiting development of an RSV infection are provided, comprising administering to a subject at risk of RSV infection an amount effective to limit development of an RSV infection of the polypeptide, the multimer, the nucleic acid, the recombinant expression vector, the recombinant host cell, or the pharmaceutical composition, of any embodiment of the disclosure.

In one aspect, methods for generating an immune response in a subject are provided, comprising administering to the subject an amount effective to generate an immune response of the polypeptide, the multimer, the nucleic acid, the recombinant expression vector, the recombinant host cell, or the pharmaceutical composition, of any embodiment of the disclosure.

In a further aspect, methods for monitoring an RSV-induced disease in a subject and/or monitoring response of the subject to immunization by an RSV vaccine are provided, comprising contacting of the polypeptide, the multimer, the nucleic acid, the recombinant expression vector, the recombinant host cell, or the pharmaceutical composition, of any embodiment of the disclosure, with a bodily fluid from the subject and detecting RSV-

binding antibodies in the bodily fluid of the subject. In one embodiment, the bodily fluid comprises serum or whole blood.

In another aspect, methods for detecting RSV binding antibodies are provided, comprising

(a) contacting of the polypeptide, the multimer, the nucleic acid, the recombinant expression vector, the recombinant host cell, or the pharmaceutical composition, of any embodiment of the disclosure with a composition comprising a candidate RSV binding antibody under conditions suitable for binding of RSV antibodies to the polypeptide, multimer, or composition; and

(b) detecting RSV antibody complexes with the polypeptide, multimer, or composition.

In one embodiment, the method further comprises isolating the RSV antibodies.

In another aspect methods for producing RSV antibodies are provided, comprising

(a) administering to a subject an amount effective to generate an antibody response of the polypeptide, the multimer, the nucleic acid, the recombinant expression vector, the recombinant host cell, or the pharmaceutical composition, of any embodiment of the disclosure; and

(b) isolating antibodies produced by the subject.

Description of the Figures

Figure 1. Model of the RSV F glycoprotein and the engineered domain from the "top" of RSV-F (eFTop).

Figure 2. Model of eFTop 11 bound to two potent RSV neutralizing antibodies (D25 and Mota)

Figure 3A-C. Biophysical characterization of eFTop. (A) Size Exclusion Chromatography with Multi-Angle Light Scattering (SEC-MALS) data demonstrating that eFTop is a monomer in solution. (B) Differential Scanning Calorimetry (DSC) data showing that the melting temperature of eFTop is 75°C. (C) Circular Dichroism (CD) data indicating that eFTop has and maintains secondary structure up to 75°C.

Detailed Description

All references cited are herein incorporated by reference in their entirety. As used herein, the singular forms "a", "an" and "the" include plural referents unless the context clearly dictates otherwise. “And” as used herein is interchangeably used with “or” unless expressly stated otherwise.

All embodiments of any aspect of the disclosure can be used in combination, unless the context clearly dictates otherwise.

Unless the context clearly requires otherwise, throughout the description and the claims, the words 'comprise', 'comprising', and the like are to be construed in an inclusive sense as opposed to an exclusive or exhaustive sense; that is to say, in the sense of "including, but not limited to". Words using the singular or plural number also include the plural and singular number, respectively. Additionally, the words "herein," "above," and "below" and words of similar import, when used in this application, shall refer to this application as a whole and not to any particular portions of the application.

As used throughout the present application, the term "protein" or "polypeptide" are used in their broadest sense to refer to a sequence of subunit amino acids. The proteins or polypeptides of the disclosure may comprise L-amino acids, D-amino acids (which are resistant to L-amino acid-specific proteases in vivo), or a combination of D- and L-amino acids. The proteins or polypeptides described herein may be chemically synthesized or recombinantly expressed.

The description of embodiments of the disclosure is not intended to be exhaustive or to limit the disclosure to the precise form disclosed. While the specific embodiments of, and examples for, the disclosure are described herein for illustrative purposes, various equivalent modifications are possible within the scope of the disclosure, as those skilled in the relevant art will recognize.

The development of a stable, minimal, monomeric domain containing potent neutralizing epitopes and minimizing non-neutralizing epitopes would offer an attractive alternative to the pre-fusion Respiratory Syncytial Virus (RSV) F protein trimer that is currently being pursued by most in the field. Toward that end, we engineered a domain from the "top" of RSV-F (eFTop) by computational design (Figure 1). The design interventions to stabilize this domain in the absence of the rest of the RSV F glycoprotein included circular permutation, loop design, repacking and disulfide stapling

In a first aspect, the disclosure provides polypeptides comprising a first domain, wherein the first domain comprises the amino acid sequence of SEQ ID NO:1. Polypeptides falling within the scope of SEQ ID NO:1 include efTop mutations disclosed in the examples that follow, which can be used, for example, in fusion polypeptides of the disclosure, and are more effective candidates for treating RSV infection and generating a neutralizing anti-RSV immune response than currently used stabilized prefusion trimers.

eFTop genus
AIAS(C/G)EAV(S/C)KVLHLEGEVRKIKSALKSTNKAVVSLSNGVSVLT(S/F)KVLDLKNYIDK

QLLPI(V/L/A)NKQSCSISNP(E/N)T(V/L/T)KEFQQK(N/L)NR(L/F)L(E/N)I(T/A)REFS(V/N)N(A/S)GVTTPVSTYMLTNSELLSLINDMPITNDQKKLMSNNVQIVR(Q/N)QSYS(V/I)M(S/C)IIKEEVLAYVV(C/Q)LPLPGHGGWYTSVITIELSNIKENKCNGTDAKVKL(I/F)KQE(L/A)D(K/N)Y(K/S)NA(V/M)TELQNLM (SEQ ID NO:1)

As used herein, the amino acid residues are abbreviated as follows: alanine (Ala; A), asparagine (Asn; N), aspartic acid (Asp; D), arginine (Arg; R), cysteine (Cys; C), glutamic acid (Glu; E), glutamine (Gln; Q), glycine (Gly; G), histidine (His; H), isoleucine (Ile; I), leucine (Leu; L), lysine (Lys; K), methionine (Met; M), phenylalanine (Phe; F), proline (Pro; P), serine (Ser; S), threonine (Thr; T), tryptophan (Trp; W), tyrosine (Tyr; Y), and valine (Val; V).

Parentheses represent variable positions in the polypeptide, with the recited amino acid residues as alternatives in these positions.

In one embodiment, the first domain comprises the amino acid sequence of SEQ ID NOS: 1-8. The polypeptides of SEQ ID NOS:2-8 are described in the examples that follow.

eFTop-10
AIASCEAVSKVLHLEGEVRKIKSALKSTNKAVVSLSNGVSVLTSKVLDLKNYIDKQLLPIVNK
QSCSISNPETVKEFQQKNNRLLEITREFSVNAGVTTPVSTYMLTNSELLSLINDMPITNDQKKL
MSNNVQIVRQQSYSVMSIIKEEVLAYVVCLPLPGHGGWYTSVITIELSNIKENKCNGTDAKV
KLIKQELDKYKNAVTELQNLM (SEQ ID NO:2)

eFTop-11
AIASGEAVCKVLHLEGEVRKIKSALKSTNKAVVSLSNGVSVLTSKVLDLKNYIDKQLLPIVN
KQSCSISNPETVKEFQQKNNRLLEITREFSVNAGVTTPVSTYMLTNSELLSLINDMPITNDQKK
LMSNNVQIVRQQSYSIMCIIKEEVLAYVVQLPLPGHGGWYTSVITIELSNIKENKCNGTDAKV
KLIKQELDKYKNAVTELQNLM (SEQ ID NO:3)

eFtop-11.1 mC
AIASGEAVCKVLHLEGEVRKIKSALKSTNKAVVSLSNGVSVLT**F**KVLDLKNYIDKQLLPI**L**NK
QSCSISNPETVKEFQQKNNRLLEITREFSVNAGVTTPVSTYMLTNSELLSLINDMPITNDQKKL
MSNNVQIVRQQSYSIMCIIKEEVLAYVVQLPLPGHGGWYTSVITIELSNIKENKCNGTDAKVK
LIKQELDKYKNAVTELQNLM (SEQ ID NO:4)

eFtop-11.2
AIASGEAVCKVLHLEGEVRKIKSALKSTNKAVVSLSNGVSVLTSKVLDLKNYIDKQLLPI**A**NK
QSCSISNPET**L**KEFQQK**L**NR**F**LEI**A**REFSVNAGVTTPVSTYMLTNSELLSLINDMPITNDQKKL
MSNNVQIVRQQSYSIMCIIKEEVLAYVVQLPLPGHGGWYTSVITIELSNIKENKCNGTDAKVK
L**F**KQE**A**DKYKNA**M**TELQNLM (SEQ ID NO:5)

eFtop11_g3
AIASGEAVCKVLHLEGEVRKIKSALKSTNKAVVSLSNGVSVLTSKVLDLKNYIDKQLLPIVNK
QSCSISNPNTTKEFQQKNNRLLEITREFSNNSGVTTPVSTYMLTNSELLSLINDMPITNDQKKL
MSNNVQIVRNQSYSIMCIIKEEVLAYVVQLPLPGHGGWYTSVITIELSNIKENKCNGTDAKVK
LIKQELDKYKNAVTELQNLM (SEQ ID NO:6)

eFtop11_g4a
AIASGEAVCKVLHLEGEVRKIKSALKSTNKAVVSLSNGVSVLTSKVLDLKNYIDKQLLPIVNK
QSCSISNPNTTKEFQQKNNRLLEITREFSNNSGVTTPVSTYMLTNSELLSLINDMPITNDQKKL

MSNNVQIVRNQSYSIMCIIKEEVLAYVVQLPLPGHGGWYTSVITIELSNIKENKCNGTDAKVK LIKQELDNYSNAVTELQNLM (SEQ ID NO:7)

eFtop11_g4b
AIASGEAVCKVLHLEGEVRKIKSALKSTNKAVVSLSNGVSVLTSKVLDLKNYIDKQLLPIVNK QSCSISNPNTTKEFQQKNNRLLNITREFSNNSGVTTPVSTYMLTNSELLSLINDMPITNDQKKL MSNNVQIVRNQSYSIMCIIKEEVLAYVVQLPLPGHGGWYTSVITIELSNIKENKCNGTDAKVK LIKQELDKYKNAVTELQNLM (SEQ ID NO:8)

In a further embodiment, the polypeptides of the disclosure may comprise two or more (i.e.: 2, 3, 4, 5, or more) copies of the first domain.

In one non-limiting embodiment, the polypeptides of the disclosure may further comprise a multimerization domain. Any suitable multimerization domain may be used that can result in a polypeptide multimer that can present multiple copies of the polypeptides of the disclosure to, for example, the immune system of a subject to which the polypeptides are administered. In various non-limiting embodiments, the multimerization domain comprises the amino acid sequence selected from the group consisting of SEQ ID NOS:9-12.

MQIY(E/C)GK(L/C)(T/G)AEGLRFGIVASR(F/A)NHALVDRLVEGAIDAIV(R/C)(H/F/M)GGREEDITLV(R/C)V(P/C)GSWEIP(V/C)AAGELARKEDIDAVIAIGVL(I/C)RG A(T/C)(P/G)(H/S)FDYIASEVSKGLADLS(L/C)ELRKPITFGVITACTLEQAIE(R/A)AGT(K/C)HGNKGWEAALSAIEMANLFKSLR (SEQ ID NO:9, which is a genus of lumazine synthase (LS), including a series of LS mutants described herein.

MQIY(E/C)GK(L/C)(T/G)AEGLRFGIVASRFNHALVDRLVEGAIDAIV(R/C)(H/F /M)GGREEDITLV(R/C)V(P/C)GSWEIP(V/C)AAGELARKEDIDAVIAIGVLIRGA(T/C)(P /G)HFDYIASEVSKGLADLS(L/C)ELRKPITFGVITACTLEQAIERAGTKHGNKGWEAA LSAIEMANLFKSLR (SEQ ID NO:10) which is a genus of LS mutants described herein.

RMKQIEDKIEEILSKIYHIENEIARIKKLIGER (SEQ ID NO:11), which is a coiled coil trimerization motif.

MKVKQLEDVVEELLSVNYHLENVVARLKKLVGER (SEQ ID NO:12), which is a tetramerization motif having 4 helices curling around each other in helical manner. For example, a fusion with this multimerization domain may comprise fusing one copy of an EF-TOP polypeptide of the disclosure to the N- terminus of SEQ ID NO:12 and a second copy of an EF-TOP polypeptide of the disclosure to the C- terminus of SEQ ID NO:12.

In one embodiment, the multimerization domain comprises the amino acid sequence selected from the group consisting of SEQ ID NOS:13-28.

MQIYEGKLTAEGLRFGIVASRFNHALVDRLVEGAIDAIVRHGGREEDITLVRVPGSWEIPVAA GELARKEDIDAVIAIGVLIRGATPHFDYIASEVSKGLADLSLELRKPITFGVITADTLEQAIERA GTKHGNKGWEAALSAIEMANLFKSLR (SEQ ID NO:13) (LS)

MQIYEGKLTAEGLRFGIVASRANHALVDRLVEGAIDAIVRHGGREEDITLVRVCGSWEIPVA
AGELARKEDIDAVIAIGVLCRGATPSFDYIASEVSKGLADLSLELRKPITFGVITADTLEQAIEA
AGTCHGNKGWEAALCAIEMANLFKSLR (SEQ ID NO:14) (d41m3)

MQIYEGKLTAEGLRFGIVASRFNHALVDRLVEGAIDAIVR[X]GGREEDITLVRVPGSWEIPVAA
GELARKEDIDAVIAIGVLIRGATPHFDYIASEVSKGLADLSLELRKPITFGVITADTLEQAIERA
GTKHGNKGWEAALSAIEMANLFKSLR (SEQ ID NO:15)

MQIYEGKLTAEGLRFGIVASRFNHALVDRLVEGAIDAIVR[X]GGREEDITLVRVPGSWEIPVAA
GELARKEDIDAVIAIGVLIRGATPHFDYIASEVSKGLADLSLELRKPITFGVITADTLEQAIERA
GTKHGNKGWEAALSAIEMANLFKSLR (SEQ ID NO:16)

MQIYEG[X]GTAEGLRFGIVASRFNHALVDRLVEGAIDAIV[X]HGGREEDITLVRVPGSWEIPVAA
GELARKEDIDAVIAIGVLIRGATPHFDYIASEVSKGLADLSLELRKPITFGVITADTLEQAIERA
GTKHGNKGWEAALSAIEMANLFKSLR (SEQ ID NO:17)

MQIYEGK[X]TAEGLRFGIVASRFNHALVDRLVEGAIDAIV[X]HGGREEDITLVRVPGSWEIPVAA
GELARKEDIDAVIAIGVLIRGATPHFDYIASEVSKGLADLSLELRKPITFGVITADTLEQAIERA
GTKHGNKGWEAALSAIEMANLFKSLR (SEQ ID NO:18)

MQIYEGK[X]TAEGLRFGIVASRFNHALVDRLVEGAIDAIVR[X]GGREEDITLVRVPGSWEIPVAA
GELARKEDIDAVIAIGVLIRGATPHFDYIASEVSKGLADLSLELRKPITFGVITADTLEQAIERA
GTKHGNKGWEAALSAIEMANLFKSLR (SEQ ID NO:19)

MQIYEGKLTAEGLRFGIVASRFNHALVDRLVEGAIDAIVRHGGREEDITLVRVPGSWEIPVAA
GELARKEDIDAVIAIGVL[X]RGATPHFDYIASEVSKGLADLSLELRKPITFGVITADTLEQAIERA
GT[X]HGNKGWEAALSAIEMANLFKSLR (SEQ ID NO:20)

MQIYEGKLTAEGLRFGIVASRFNHALVDRLVEGAIDAIVRHGGREEDITLVRVPGSWEIPVAAG
ELARKEDIDAVIAIGVL[X]RGATPHFDYIASEVSKGLADLSLELRKPITFGVITADTLEQAIERAG
T[X]HGNKGWEAALSAIEMANLFKSLR (SEQ ID NO:21)

MQIYEGKLTAEGLRFGIVASRFNHALVDRLVEGAIDAIVRHGGREEDITLVRV[X]GSWEIPVAA
GELARKEDIDAVIAIGVL[X]RGATPHFDYIASEVSKGLADLSLELRKPITFGVITADTLEQAIERA
GT[X]HGNKGWEAAL[X]AIEMANLFKSLR (SEQ ID NO:22)

MQIYEGKLTAEGLRFGIVASRFNHALVDRLVEGAIDAIVRHGGREEDITLVRVPGSWEIPVAA
GELARKEDIDAVIAIGVL[X]RGA[X]GHFDYIASEVSKGLADLSLELRKPITFGVITA[X]TLEQAIER
AGT[X]HGNKGWEAALSAIEMANLFKSLR (SEQ ID NO:23)

MQIYEGKLTAEGLRFGIVASRFNHALVDRLVEGAIDAIVRHGGREEDITLVRVPGSWEIP[X]AA
GELARKEDIDAVIAIGVL[X]RGATPHFDYIASEVSKGLADLS[X]ELRKPITFGVITADTLEQAIER
AGT[X]HGNKGWEAALSAIEMANLFKSLR (SEQ ID NO:24)

MQIY[X]GKLTAEGLRFGIVASRFNHALVDRLVEGAIDAIVRHGGREEDITLV[X]VPGSWEIPVAA
GELARKEDIDAVIAIGVL[X]RGATPHFDYIASEVSKGLADLSLELRKPITFGVITADTLEQAIERA
GT[X]HGNKGWEAALSAIEMANLFKSLR (SEQ ID NO:25)

MQIYEGKLTAEGLRFGIVASRFNHALVDRLVEGAIDAIVRHGGREEDITLVRV[X]GSWEIPVAA
GELARKEDIDAVIAIGVLCRGATPHFDYIASEVSKGLADLSLELRKPITFGVITADTLEQAIERA
GTCHGNKGWEAAL[X]AIEMANLFKSLR (SEQ ID NO:26)

MQIYEGKLTAEGLRFGIVASRFNHALVDRLVEGAIDAIVRHGGREEDITLVRVCGSWEIPVAA
GELARKEDIDAVIAIGVLCRGATPHFDYIASEVSKGLADLSLELRKPITFGVITADTLEQAIE[X]A
GTCHGNKGWEAALCAIEMANLFKSLR (SEQ ID NO:27)

MQIYEGKLTAEGLRFGIVASRFNHALVDRLVEGAIDAIVRHGGREEDITLVRVCGSWEIPVAA
GELARKEDIDAVIAIGVLCRGATPSFDYIASEVSKGLADLSLELRKPITFGVITADTLEQAIEAA
GTCHGNKGWEAALCAIEMANLFKSLR (SEQ ID NO:28)

In another aspect, the disclosure provides polypeptides comprising

(a) a multimerization domain comprising the amino acid sequence selected from the group consisting of SEQ ID NO:10, 14, and 15-28; and

(b) one or more copies of a respiratory syncytial virus (RSV) antigen.

The polypeptides of this aspect of the disclosure are fusion proteins that comprise a lumazine synthase mutation of the disclosure fused to an RSV antigen. The polypeptides of this aspect of the disclosure can be used, for example, in the methods of the disclosure. The RSV antigen may be any suitable RSV antigen, including but not limited to the RSV F protein, or an antigenic portion thereof.

In one embodiment, the multimerization domain comprises the amino acid sequence selected from the group consisting of SEQ ID NO: 10 and 15-28.

The polypeptides of the disclosure may further comprise a linker between different domains within the polypeptide. For example, the polypeptides may further comprise an amino acid linker between the first domain and the multimerization domain, or between the multimerization domain and the one or more copies of the RSV antigen.

In various non-limiting embodiments, the polypeptides of the disclosure may comprise the amino acid sequence selected from the group consisting of SEQ ID NOS: 29-41.

8mer
eFtop11_8mer_mC
AIASGEAVCKVLHLEGEVRKIKSALKSTNKAVVSLSNGVSVLTSKVLDLKNYIDKQLLPIVNK
QSCSISNPETVKEFQQKNNRLLEITREFSVNAGVTTPVSTYMLTNSELLSLINDMPITNDQKKL
MSNNVQIVRQQSYSIMCIIKEEVLAYVVQLPLPGHGGWYTSVITIELSNIKENKCNGTDAKVK
LIKQELDKYKNAVTELQNLMGGSGGSGGSGGG*MKVKQLEDVVEELLSVNYHLENVVARLKKLV*
*GER*GGSGGSGGSGGGAIASGEAVCKVLHLEGEVRKIKSALKSTNKAVVSLSNGVSVLTSKVL
DLKNYIDKQLLPIVNKQSCSISNPETVKEFQQKNNRLLEITREFSVNAGVTTPVSTYMLTNSEL
LSLINDMPITNDQKKLMSNNVQIVRQQSYSIMCIIKEEVLAYVVQLPLPGHGGWYTSVITIELS
NIKENKCNGTDAKVKLIKQELDKYKNAVTELQNLM (SEQ ID NO:29)

60 mer
eFtop11.1_g4b_d41m3_Nt_60mer
AIASGEAVCKVLHLEGEVRKIKSALKSTNKAVVSLSNGVSVLTFKVLDLKNYIDKQLLPILNK
QSCSISNPNTTKEFQQKNNRLLNITREFSNNSGVTTPVSTYMLTNSELLSLINDMPITNDQKKL
MSNNVQIVRNQSYSIMCIIKEEVLAYVVQLPLPGHGGWYTSVITIELSNIKENKCNGTDAKVK
LIKQELDKYKNAVTELQNLMGGSGGSGGSGGSGGGMQIYEGKLTAEGLRFGIVASRANHAL
VDRLVEGAIDAIVRHGGREEDITLVRVCGSWEIPVAAGELARKEDIDAVIAIGVLCRGATPSF
DYIASEVSKGLADLSLELRKPITFGVITADTLEQAIEAAGTCHGNKGWEAALCAIEMANLFKS
LR (SEQ ID NO:30)

8mer

eFtop11.1_8mer_mC
AIASGEAVCKVLHLEGEVRKIKSALKSTNKAVVSLSNGVSVLTEKVLDLKNYIDKQLLPILNK
QSCSISNPETVKEFQQKNNRLLEITREFSVNAGVTTPVSTYMLTNSELLSLINDMPITNDQKKL
MSNNVQIVRQQSYSIMCIIKEEVLAYVVQLPLPGHGGWYTSVITIELSNIKENKCNGTDAKVK
LIKQELDKYKNAVTELQNLMGGSGGSGGSGGG*MKVKQLEDVVEELLSVNYHLENVVARLKKLV*
*GER*GGSGGSGGSGGGAIASGEAVCKVLHLEGEVRKIKSALKSTNKAVVSLSNGVSVLTEKVL
DLKNYIDKQLLPILNKQSCSISNPETVKEFQQKNNRLLEITREFSVNAGVTTPVSTYMLTNSEL
LSLINDMPITNDQKKLMSNNVQIVRQQSYSIMCIIKEEVLAYVVQLPLPGHGGWYTSVITIELS
NIKENKCNGTDAKVKLIKQELDKYKNAVTELQNLM (SEQ ID NO:31)

eFtop11_g4b_Ntf_60mer_m
MQIYEGKLTAEGLRFGIVASRFNHALVDRLVEGAIDAIVRHGGREEDITLVRVPGSWEIPVAA
GELARKEDIDAVIAIGVLIRGATPHFDYIASEVSKGLADLSLELRKPITFGVITADTLEQAIERA
GTKHGNKGWEAALSAIEMANLFKSLRGGSGGSGGSGGSGGSGGSGGGAIASGEAVCKVLHL
EGEVRKIKSALKSTNKAVVSLSNGVSVLTSKVLDLKNYIDKQLLPIVNKQSCSISNPNTTKEF
QQKNNRLLNITREFSNNSGVTTPVSTYMLTNSELLSLINDMPITNDQKKLMSNNVQIVRNQSY
SIMCIIKEEVLAYVVQLPLPGHGGWYTSVITIELSNIKENKCNGTDAKVKLIKQELDKYKNAV
TELQNLM (SEQ ID NO:32)

eFTop-11 3-mer
eFtop11_3mer-1gcm
AIASGEAV**C**KVLHLEGEVRKIKSALKSTNKAVVSLSNGVSVLTSKVLDLKNYIDKQLLPIVNK
QSCSISNPETVKEFQQKNNRLLEITREFSVNAGVTTPVSTYMLTNSELLSLINDMPITNDQKKL
MSNNVQIVRQQSYSIM**C**IIKEEVLAYVVQLPLPGHGGWYTSVITIELSNIKENKCNGTDAKVK
LIKQELDKYKNAVTELQNLMGGSGGSGGSGGG*RMKQIEDKIEEILSKIYHIENEIARIKKLIGER*
(SEQ ID NO:33)

eFTop-11 4-mer
#uses 2b22
eFtop11_4mer
AIASGEAV**C**KVLHLEGEVRKIKSALKSTNKAVVSLSNGVSVLTSKVLDLKNYIDKQLLPIVNK
QSCSISNPETVKEFQQKNNRLLEITREFSVNAGVTTPVSTYMLTNSELLSLINDMPITNDQKKL
MSNNVQIVRQQSYSIM**C**IIKEEVLAYVVQLPLPGHGGWYTSVITIELSNIKENKCNGTDAKVK
LIKQELDKYKNAVTELQNLMGGSGGSGGSGGG*MKVKQLEDVVEELLSVNYHLENVVARLKKLV*
GER (SEQ ID NO:34)

eFTop-11 8-mer
#uses 2b22
eFtop11_8mer
AIASGEAV**C**KVLHLEGEVRKIKSALKSTNKAVVSLSNGVSVLTSKVLDLKNYIDKQLLPIVNK
QSCSISNPETVKEFQQKNNRLLEITREFSVNAGVTTPVSTYMLTNSELLSLINDMPITNDQKKL
MSNNVQIVRQQSYSIM**C**IIKEEVLAYVVQLPLPGHGGWYTSVITIELSNIKENKCNGTDAKVK
LIKQELDKYKNAVTELQNLMGGSGGSGGSGGG*MKVKQLEDVVEELLSVNYHLENVVARLKKLV*
*GER*GGSGGSGGSGGGAIASGEAV**C**KVLHLEGEVRKIKSALKSTNKAVVSLSNGVSVLTSKVL
DLKNYIDKQLLPIVNKQSCSISNPETVKEFQQKNNRLLEITREFSVNAGVTTPVSTYMLTNSEL
LSLINDMPITNDQKKLMSNNVQIVRQQSYSIM**C**IIKEEVLAYVVQLPLPGHGGWYTSVITIELS
NIKENKCNGTDAKVKLIKQELDKYKNAVTELQNLM (SEQ ID NO:35)

eFtop11_g3_Ntf_60mer_m
MQIYEGKLTAEGLRFGIVASRFNHALVDRLVEGAIDAIVRHGGREEDITLVRVPGSWEIPVAA
GELARKEDIDAVIAIGVLIRGATPHFDYIASEVSKGLADLSLELRKPITFGVITADTLEQAIERA
GTKHGNKGWEAALSAIEMANLFKSLRGGSGGSGGSGGSGGSGGSGGGAIASGEAVCKVLHL
EGEVRKIKSALKSTNKAVVSLSNGVSVLTSKVLDLKNYIDKQLLPIVNKQSCSISNPNTTKEF
QQKNNRLLEITREFSNNSGVTTPVSTYMLTNSELLSLINDMPITNDQKKLMSNNVQIVRNQSY

SIMCIIKEEVLAYVVQLPLPGHGGWYTSVITIELSNIKENKCNGTDAKVKLIKQELDKYKNAV
TELQNLM (SEQ ID NO:36)

eFtop11_g4a_Ntf_60mer_m
MQIYEGKLTAEGLRFGIVASRFNHALVDRLVEGAIDAIVRHGGREEDITLVRVPGSWEIPVAA
GELARKEDIDAVIAIGVLIRGATPHFDYIASEVSKGLADLSLELRKPITFGVITADTLEQAIERA
GTKHGNKGWEAALSAIEMANLFKSLRGGSGGSGGSGGSGGSGGSGGGAIASGEAVCKVLHL
EGEVRKIKSALKSTNKAVVSLSNGVSVLTSKVLDLKNYIDKQLLPIVNKQSCSISNPNTTKEF
QQKNNRLLEITREFSNNSGVTTPVSTYMLTNSELLSLINDMPITNDQKKLMSNNVQIVRNQSY
SIMCIIKEEVLAYVVQLPLPGHGGWYTSVITIELSNIKENKCNGTDAKVKLIKQELDNYSNAV
TELQNLM** (SEQ ID NO:37)

eFtop11_g4b_Ntf_60mer_m
MQIYEGKLTAEGLRFGIVASRFNHALVDRLVEGAIDAIVRHGGREEDITLVRVPGSWEIPVAA
GELARKEDIDAVIAIGVLIRGATPHFDYIASEVSKGLADLSLELRKPITFGVITADTLEQAIERA
GTKHGNKGWEAALSAIEMANLFKSLRGGSGGSGGSGGSGGSGGSGGGAIASGEAVCKVLHL
EGEVRKIKSALKSTNKAVVSLSNGVSVLTSKVLDLKNYIDKQLLPIVNKQSCSISNPNTTKEF
QQKNNRLLNITREFSNNSGVTTPVSTYMLTNSELLSLINDMPITNDQKKLMSNNVQIVRNQSY
SIMCIIKEEVLAYVVQLPLPGHGGWYTSVITIELSNIKENKCNGTDAKVKLIKQELDKYKNAV
TELQNLM (SEQ ID NO:38)

Connect C-term of d41m3 to N-term of eFTop-11.1_g4a
eFTop11.1_g4a_d41m3_Ct_60mer
MQIYEGKLTAEGLRFGIVASRANHALVDRLVEGAIDAIVRHGGREEDITLVRVCGSWEIPVA
AGELARKEDIDAVIAIGVLCRGATPSFDYIASEVSKGLADLSLELRKPITFGVITADTLEQAIEA
AGTCHGNKGWEAALCAIEMANLFKSLRGGSGGSGGSGGSGGGAIASGEAVCKVLHLEGEVR
KIKSALKSTNKAVVSLSNGVSVLTFKVLDLKNYIDKQLLPILNKQSCSISNPNTTKEFQQKNN
RLLEITREFSNNSGVTTPVSTYMLTNSELLSLINDMPITNDQKKLMSNNVQIVRNQSYSIMCII
KEEVLAYVVQLPLPGHGGWYTSVITIELSNIKENKCNGTDAKVKLIKQELDNYSNAVTELQN
LM (SEQ ID NO:39)

Connect C-term of eFTop-11.1_g4a to N-term of d41m3
eFTop11.1_g4a_d41m3_Nt_60mer
AIASGEAVCKVLHLEGEVRKIKSALKSTNKAVVSLSNGVSVLTFKVLDLKNYIDKQLLPILNK
QSCSISNPNTTKEFQQKNNRLLEITREFSNNSGVTTPVSTYMLTNSELLSLINDMPITNDQKKL
MSNNVQIVRNQSYSIMCIIKEEVLAYVVQLPLPGHGGWYTSVITIELSNIKENKCNGTDAKVK
LIKQELDNYSNAVTELQNLMGGSGGSGGSGGSGGGMQIYEGKLTAEGLRFGIVASRANHAL
VDRLVEGAIDAIVRHGGREEDITLVRVCGSWEIPVAAGELARKEDIDAVIAIGVLCRGATPSF
DYIASEVSKGLADLSLELRKPITFGVITADTLEQAIEAAGTCHGNKGWEAALCAIEMANLFKS
LR** (SEQ ID NO:40)

Connect C-term of d41m3 to N-term of eFtop11.1_g4b
eFtop11.1_g4b_d41m3_Ct_60mer
MQIYEGKLTAEGLRFGIVASRANHALVDRLVEGAIDAIVRHGGREEDITLVRVCGSWEIPVA
AGELARKEDIDAVIAIGVLCRGATPSFDYIASEVSKGLADLSLELRKPITFGVITADTLEQAIEA
AGTCHGNKGWEAALCAIEMANLFKSLRGGSGGSGGSGGSGGGAIASGEAVCKVLHLEGEVR
KIKSALKSTNKAVVSLSNGVSVLTFKVLDLKNYIDKQLLPILNKQSCSISNPNTTKEFQQKNN
RLLNITREFSNNSGVTTPVSTYMLTNSELLSLINDMPITNDQKKLMSNNVQIVRNQSYSIMCII
KEEVLAYVVQLPLPGHGGWYTSVITIELSNIKENKCNGTDAKVKLIKQELDKYKNAVTELQN
LM (SEQ ID NO:41)

In specific embodiments, the polypeptides may comprise the amino acid sequence selected from the group consisting of SEQ ID NOS: 29-31.

In a further embodiment, the disclosure provides multimers, comprising two or more copies (2, 3, 4, 5, 6, 7, 8, 10, 20, 30, 40, 50, 60, or more copies) of the polypeptides of the disclosure that include a multimerization domain. The multimer may be a self-assembling multimer and/or may be present on a surface, including but not limited to a particle or bead.

In one specific embodiment, the multimer comprises eight or more copies of the polypeptide; in another specific embodiment, the multimer comprises 60 or more copies of the polypeptide.

In another aspect, the present disclosure provides isolated nucleic acids encoding a polypeptide of the present disclosure. The isolated nucleic acid sequence may comprise RNA or DNA. As used herein, "isolated nucleic acids" are those that have been removed from their normal surrounding nucleic acid sequences in the genome or in cDNA sequences. Such isolated nucleic acid sequences may comprise additional sequences useful for promoting expression and/or purification of the encoded protein, including but not limited to polyA sequences, modified Kozak sequences, and sequences encoding epitope tags, export signals, and secretory signals, nuclear localization signals, and plasma membrane localization signals. It will be apparent to those of skill in the art, based on the teachings herein, what nucleic acid sequences will encode the polypeptides of the disclosure. In various non-limiting embodiments, the nucleic acid comprises the sequence selected from the group consisting of SEQ ID NOS: 44-47, which show improved expression compared to other encoding nucleic acid sequences.

Connect C-term of d41m3 to N-term of eFTop-11.1_g4a

eFTop11.1_g4a_d41m3_Ct_60mer

ATGCAGATCTACGAAGGAAAACTGACCGCTGAGGGACTGAGGTTCGGAATTGTCGCAAG
CCGCGCGAATCACGCACTGGTGGATAGGCTGGTGGAAGGCGCTATCGACGCAATTGTCC
GGCACGGCGGGAGAGAGGAAGACATCACACTGGTGAGAGTCTGCGGCAGCTGGGAGAT
TCCCGTGGCAGCTGGAGAACTGGCTCGAAAGGAGGACATCGATGCCGTGATCGCTATTG
GGGTCCTGTGCCGAGGAGCAACTCCCAGCTTCGACTACATCGCCTCAGAAGTGAGCAAG
GGGCTGGCTGATCTGTCCCTGGAGCTGAGGAAACCTATCACTTTTGGCGTGATTACTGCC
GACACCCTGGAACAGGCAATCGAGGCGGCCGGCACCTGCCATGGAAACAAAGGCTGGG
AAGCAGCCCTGTGCGCTATTGAGATGGCAAATCTGTTCAAATCTCTGCGA*GGAGGCTCCG*
*GAGGATCTGGAGGGAGTGGAGGCTCAGGAGGAGGC*GCCATCGCTAGCGGAGAGGCCGTGT
GCAAGGTCCTGCACCTGGAGGGCGAAGTGAGGAAGATCAAGTCTGCACTGAAGAGTACC
AACAAAGCCGTGGTCAGCCTGTCCAATGGCGTGTCCGTCCTGACATTCAAGGTGCTGGAC
CTGAAAAACTATATCGATAAGCAGCTGCTGCCAATTCTGAACAAGCAGTCTTGTAGTATC
TCAAATCCCAATACTACAAAAGAGTTCCAGCAGAAGAACAATCGGCTGCTGGAGATCAC
CAGAGAGTTCAGCAACAACTCTGGAGTCACCACCCCCGTGAGCACCTACATGCTGACCA
ATTCAGAGCTGCTGAGCCTGATCAACGACATGCCCATTACCAATGATCAGAAGAAACTG
ATGAGCAACAATGTGCAGATCGTCCGGAATCAGTCTTACTCCATTATGTGCATCATCAAG
GAGGAAGTGCTGGCTTATGTGGTCCAGCTGCCACTGCCTGGGCATGGCGGATGGTACAC

ATCCGTGATCACTATTGAGCTGTCTAACATCAAGGAAAACAAATGTAACGGAACAGACG
CTAAGGTCAAACTGATTAAGCAGGAGCTGGATAACTATAGCAACGCAGTGACTGAACTG
CAGAATCTGATG**TGATAA (SEQ ID NO:44)**

Connect C-term of eFTop-11.1_g4a to N-term of d41m3
eFTop11.1_g4a_d41m3_Nt_60mer
GCCATCGCTAGCGGAGAGGCCGTGTGCAAGGTCCTGCACCTGGAGGGCGAAGTGAGGAA
GATCAAGTCTGCACTGAAGAGTACCAACAAAGCCGTGGTCAGCCTGTCCAATGGCGTGT
CCGTCCTGACATTCAAGGTGCTGGACCTGAAAAACTATATCGATAAGCAGCTGCTGCCAA
TTCTGAACAAGCAGTCTTGTAGTATCTCAAATCCCAATACTACAAAAGAGTTCCAGCAGA
AGAACAATCGGCTGCTGGAGATCACCAGAGAGTTCAGCAACAACTCTGGAGTCACCACC
CCCGTGAGCACCTACATGCTGACCAATTCAGAGCTGCTGAGCCTGATCAACGACATGCCC
ATTACCAATGATCAGAAGAAACTGATGAGCAACAATGTGCAGATCGTCCGGAATCAGTC
TTACTCCATTATGTGCATCATCAAGGAGGAAGTGCTGGCTTATGTGGTCCAGCTGCCACT
GCCTGGGCATGGCGGATGGTACACATCCGTGATCACTATTGAGCTGTCTAACATCAAGGA
AAACAAATGTAACGGAACAGACGCTAAGGTCAAACTGATTAAGCAGGAGCTGGATAACT
ATAGCAACGCAGTGACTGAACTGCAGAATCTGATG*GGAGGCTCCGGAGGATCTGGAGGGA*
*GTGGAGGCTCAGGAGGAGGC*ATGCAGATCTACGAAGGAAAACTGACCGCTGAGGGACTG
AGGTTCGGAATTGTCGCAAGCCGCGCGAATCACGCACTGGTGGATAGGCTGGTGGAAGG
CGCTATCGACGCAATTGTCCGGCACGGCGGGAGAGAGGAAGACATCACACTGGTGAGAG
TCTGCGGCAGCTGGGAGATTCCCGTGGCAGCTGGAGAACTGGCTCGAAAGGAGGACATC
GATGCCGTGATCGCTATTGGGGTCCTGTGCCGAGGAGCAACTCCCAGCTTCGACTACATC
GCCTCAGAAGTGAGCAAGGGGCTGGCTGATCTGTCCCTGGAGCTGAGGAAACCTATCAC
TTTTGGCGTGATTACTGCCGACACCCTGGAACAGGCAATCGAGGCGGCCGGCACCTGCC
ATGGAAACAAAGGCTGGGAAGCAGCCCTGTGCGCTATTGAGATGGCAAATCTGTTCAAA
TCTCTGCGA**TGATAA (SEQ ID NO:45)**

Connect C-term of d41m3 to N-term of eFtop11.1_g4b
eFtop11.1_g4b_d41m3_Ct_60mer
ATGCAGATCTACGAAGGAAAACTGACCGCTGAGGGACTGAGGTTCGGAATTGTCGCAAG
CCGCGCGAATCACGCACTGGTGGATAGGCTGGTGGAAGGCGCTATCGACGCAATTGTCC
GGCACGGCGGGAGAGAGGAAGACATCACACTGGTGAGAGTCTGCGGCAGCTGGGAGAT
TCCCGTGGCAGCTGGAGAACTGGCTCGAAAGGAGGACATCGATGCCGTGATCGCTATTG
GGGTCCTGTGCCGAGGAGCAACTCCCAGCTTCGACTACATCGCCTCAGAAGTGAGCAAG
GGGCTGGCTGATCTGTCCCTGGAGCTGAGGAAACCTATCACTTTTGGCGTGATTACTGCC
GACACCCTGGAACAGGCAATCGAGGCGGCCGGCACCTGCCATGGAAACAAAGGCTGGG
AAGCAGCCCTGTGCGCTATTGAGATGGCAAATCTGTTCAAATCTCTGCGA*GGAGGCTCCG*
*GAGGATCTGGAGGGAGTGGAGGCTCAGGAGGAGGC*GCAATCGCATCCGGAGAGGCCGTGT
GCAAGGTGCTGCACCTGGAGGGCGAGGTGAGGAAGATCAAGAGCGCCCTGAAGTCCACC
AACAAGGCCGTGGTGAGCCTGTCCAATGGCGTGTCTGTGCTGACATTCAAGGTGCTGGAC
CTGAAGAACTATATCGATAAGCAGCTGCTGCCAATCCTGAATAAGCAGTCTTGTAGCATC
TCCAACCCCAATACCACAAAGGAGTTCCAGCAGAAGAACAATCGGCTGCTGAACATCAC
CAGAGAGTTTTCCAACAATTCTGGCGTGACCACCCCCGTGAGCACCTACATGCTGACAAA
TTCCGAGCTGCTGTCTCTGATCAACGACATGCCCATCACAAATGATCAGAAGAAGCTGAT
GAGCAACAATGTGCAGATCGTGCGGAACCAGTCTTACAGCATCATGTGCATCATCAAGG
AGGAGGTGCTGGCCTATGTGGTGCAGCTGCCACTGCCTGGCCACGGCGGCTGGTACACC
AGCGTGATCACAATCGAGCTGTCCAATATCAAGGAGAACAAGTGTAATGGCACCGACGC
CAAGGTGAAGCTGATCAAGCAGGAGCTGGATAAGTATAAGAACGCCGTGACAGAGCTGC
AGAATCTGATG**TGATAA (SEQ ID NO:46)**

Connect C-term of eFtop11.1_g4b to N-term of d41m3
eFtop11.1_g4b_d41m3_Nt_60mer
GCAATCGCATCCGGAGAGGCCGTGTGCAAGGTGCTGCACCTGGAGGGCGAGGTGAGGAA
GATCAAGAGCGCCCTGAAGTCCACCAACAAGGCCGTGGTGAGCCTGTCCAATGGCGTGT
CTGTGCTGACATTCAAGGTGCTGGACCTGAAGAACTATATCGATAAGCAGCTGCTGCCAA

TCCTGAATAAGCAGTCTTGTAGCATCTCCAACCCCAATACCACAAAGGAGTTCCAGCAGA
AGAACAATCGGCTGCTGAACATCACCAGAGAGTTTTCCAACAATTCTGGCGTGACCACCC
CCGTGAGCACCTACATGCTGACAAATTCCGAGCTGCTGTCTCTGATCAACGACATGCCCA
TCACAAATGATCAGAAGAAGCTGATGAGCAACAATGTGCAGATCGTGCGGAACCAGTCT
TACAGCATCATGTGCATCATCAAGGAGGAGGTGCTGGCCTATGTGGTGCAGCTGCCACTG
CCTGGCCACGGCGGCTGGTACACCAGCGTGATCACAATCGAGCTGTCCAATATCAAGGA
GAACAAGTGTAATGGCACCGACGCCAAGGTGAAGCTGATCAAGCAGGAGCTGGATAAGT
ATAAGAACGCCGTGACAGAGCTGCAGAATCTGATG*GGAGGCTCCGGAGGATCTGGAGGGA*
*GTGGAGGCTCAGGAGGAGGC*ATGCAGATCTACGAAGGAAAACTGACCGCTGAGGGACTG
AGGTTCGGAATTGTCGCAAGCCGCGCGAATCACGCACTGGTGGATAGGCTGGTGGAAGG
CGCTATCGACGCAATTGTCCGGCACGGCGGGAGAGAGGAAGACATCACACTGGTGAGAG
TCTGCGGCAGCTGGGAGATTCCCGTGGCAGCTGGAGAACTGGCTCGAAAGGAGGACATC
GATGCCGTGATCGCTATTGGGGTCCTGTGCCGAGGAGCAACTCCCAGCTTCGACTACATC
GCCTCAGAAGTGAGCAAGGGGCTGGCTGATCTGTCCCTGGAGCTGAGGAAACCTATCAC
TTTTGGCGTGATTACTGCCGACACCCTGGAACAGGCAATCGAGGCGGCCGGCACCTGCC
ATGGAAACAAAGGCTGGGAAGCAGCCCTGTGCGCTATTGAGATGGCAAATCTGTTCAAA
TCTCTGCGA**TGATAA (SEQ ID NO:47)**

In a further aspect, the present disclosure provides recombinant expression vectors comprising the isolated nucleic acid of any aspect of the disclosure operatively linked to a suitable control sequence. "Recombinant expression vector" includes vectors that operatively link a nucleic acid coding region or gene to any control sequences capable of effecting expression of the gene product. "Control sequences" operably linked to the nucleic acid sequences of the disclosure are nucleic acid sequences capable of effecting the expression of the nucleic acid molecules. The control sequences need not be contiguous with the nucleic acid sequences, so long as they function to direct the expression thereof. Thus, for example, intervening untranslated yet transcribed sequences can be present between a promoter sequence and the nucleic acid sequences and the promoter sequence can still be considered "operably linked" to the coding sequence. Other such control sequences include, but are not limited to, polyadenylation signals, termination signals, and ribosome binding sites. Such expression vectors can be of any type known in the art, including but not limited plasmid and viral-based expression vectors.

In another aspect, the present disclosure provides host cells that have been transfected with the recombinant expression vectors disclosed herein, wherein the host cells can be either prokaryotic or eukaryotic. The cells can be transiently or stably transfected. Such transfection of expression vectors into prokaryotic and eukaryotic cells can be accomplished via any technique known in the art, including but not limited to standard bacterial transformations, calcium phosphate co-precipitation, electroporation, or liposome mediated-, DEAE dextran mediated-, polycationic mediated-, or viral mediated transfection. (See, for example, *Molecular Cloning: A Laboratory Manual* (Sambrook, et al., 1989, Cold Spring Harbor Laboratory Press; *Culture of Animal Cells: A Manual of Basic Technique, 2nd Ed.* (R.I. Freshney. 1987. Liss, Inc. New York, NY). A method of producing a polypeptide

according to the disclosure is an additional part of the disclosure. The method comprises the steps of (a) culturing a host according to this aspect of the disclosure under conditions conducive to the expression of the polypeptide, and (b) optionally, recovering the expressed polypeptide. The expressed polypeptide can be recovered from the cell free extract, but preferably they are recovered from the culture medium. Methods to recover polypeptide from cell free extracts or culture medium are well known to the man skilled in the art.

In another aspect, the present disclosure provides pharmaceutical compositions (such as a vaccine), comprising one or more polypeptides, multimers, nucleic acids, recombinant expression vectors, or host cells of the disclosure and a pharmaceutically acceptable carrier. The pharmaceutical compositions of the disclosure can be used, for example, in the methods of the disclosure described below. The polypeptides may be the sole active agent in the pharmaceutical composition, or the composition may further comprise one or more other agents suitable for an intended use, including but not limited to adjuvants to stimulate the immune system generally and improve immune responses overall. Any suitable adjuvant can be used.

In a further aspect, the present disclosure provides methods for treating and/or limiting an RSV infection, comprising administering to a subject in need thereof a therapeutically effective amount of one or more polypeptides of the disclosure, salts thereof, conjugates thereof, multimers thereof, nucleic acids of the disclosure (such as RNA), host cells or pharmaceutical compositions thereof, to treat and/or limit the RSV infection. In another embodiment, the method comprises eliciting an immune response in an individual having or at risk of an RSV infection, comprising administering to a subject in need thereof a therapeutically effective amount of one or more polypeptides of the disclosure, salts thereof, conjugates thereof, multimers thereof, nucleic acids of the disclosure (such as RNA), host cells or pharmaceutical compositions thereof, to generate an immune response.

RSV is a negative-sense, single-stranded RNA virus of the family Paramyxoviridae that causes a respiratory disease, especially in children. For treating an RSV infection, the therapeutic is administered to a subject already infected with the RSV, and/or who is suffering from symptoms (including but not limited to lower respiratory tract infections, upper respiratory tract infections, bronchiolitis, pneumonia, fever, listlessness, diminished appetite, recurrent wheezing, and asthma) indicating that the subject is likely to have been infected with the RSV. As used herein, "treat" or "treating" means accomplishing one or more of the following: (a) reducing RSV titer in the subject; (b) limiting any increase of RSV titer in the subject; (c) reducing the severity

of RSV symptoms; (d) limiting or preventing development of RSV symptoms after infection; (e) inhibiting worsening of RSV symptoms; (f) limiting or preventing recurrence of RSV symptoms in subjects that were previously symptomatic for RSV infection. In one embodiment method, the therapeutic is used as a "therapeutic vaccines" to ameliorate the existing infection and/or provide prophylaxis against infection with additional RSV virus.

The therapeutic can also be administered prophylactically to a subject at risk of RSV infection to limit development of an RSV infection. Groups at particularly high risk include children under age 18 (particularly infants 3 years or younger), adults over the age of 65, and individuals suffering from any type of immunodeficiency.

A "therapeutically effective amount" is an amount of the therapeutic effective for treating and/or limiting RSV infection. A suitable dosage range may, for instance, be 0.1 ug/kg-100 mg/kg body weight; alternatively, it may be 0.5 ug/kg to 50 mg/kg; 1 ug/kg to 25 mg/kg, or 5 ug/kg to 10 mg/kg body weight. The therapeutic can be delivered in a single bolus, or may be administered more than once (e.g., 2, 3, 4, 5, or more times) as determined by an attending physician.

In a further aspect, the present disclosure provides methods for monitoring an RSV-induced disease in a subject and/or monitoring response of the subject to immunization by an RSV vaccine, comprising contacting a polypeptide, multimer, recombinant host cell, or pharmaceutical composition of the disclosure with a bodily fluid from the subject and detecting RSV-binding antibodies in the bodily fluid of the subject. By "RSV-induced disease" is intended any disease caused, directly or indirectly, by RSV. The method comprises contacting a polypeptide, multimer, recombinant host cell, or pharmaceutical composition of the disclosure with an amount of bodily fluid (such as serum, whole blood, etc.) from the subject; and detecting RSV-binding antibodies in the bodily fluid of the subject. The detection of the RSV binding antibodies allows the RSV disease in the subject to be monitored. In addition, the detection of RSV binding antibody also allows the response of the subject to immunization by an RSV vaccine to be monitored. Any suitable detection assay can be used, including but not limited to homogeneous and heterogeneous binding immunoassays, such as radioimmunoassays (RIA), ELISA, immunofluorescence, immunohistochemistry, FACS, BIACORE and Western blot analyses. The methods may be carried out in solution, or the polypeptide(s) of the disclosure may be bound or attached to a carrier or substrate, such as microtiter plates (ex: for ELISA), membranes

and beads, etc. The polypeptides of the disclosure for use in this aspect may be conjugated to a detectable tag, to facilitate detection technique.

In a still further aspect, the present disclosure provides methods for detecting RSV binding antibodies, comprising contacting a polypeptide, multimer, recombinant host cell, or pharmaceutical composition of the disclosure with a composition comprising a candidate RSV binding antibody under conditions suitable for binding of RSV antibodies to the polypeptide, VLP, or composition; and

(b) detecting RSV antibody complexes with the polypeptide, multimer, recombinant host cell, or pharmaceutical composition of the disclosure.

In this aspect, the methods are performed to determine if a candidate RSV binding antibody recognizes the RSV F epitope present in the polypeptides of the disclosure. Any suitable composition may be used, including but not limited to bodily fluid samples (such as serum, whole blood, etc.) from a suitable subject (such as one who has been infected with RSV), naive libraries, modified libraries, and libraries produced directly from human donors exhibiting an RSV-specific immune response. The assays are performed under conditions suitable for promoting binding of antibodies against the polypeptide, multimer, recombinant host cell, or pharmaceutical composition of the disclosure; such conditions can be determined by those of skill in the art based on the teachings herein. Any suitable detection assay can be used, such as those described above. The polypeptides of the disclosure for use in this aspect may comprise a conjugate as disclosed above, to provide a tag useful for any detection technique suitable for a given assay. In a further embodiment, the RSV F-binding antibodies are isolated using standard procedures.

In another aspect, the present disclosure provides methods for producing RSV antibodies, comprising

(a) administering to a subject an amount effective to generate an antibody response of the polypeptide, multimer, or pharmaceutical composition of the disclosure of the disclosure; and

(b) isolating antibodies produced by the subject.

The antibodies can be used, for example, in RSV research. The subject is preferably an animal typically used for antibody production, including but not limited to rodents, rabbits, goats, sheep, etc. The antibodies can be either polyclonal or monoclonal antibodies.

As used herein, the singular forms "a", "an" and "the" include plural referents unless the context clearly dictates otherwise. “And” as used herein is interchangeably used with “or” unless expressly stated otherwise.

All embodiments of any aspect of the disclosure can be used in combination, unless the context clearly dictates otherwise.

Examples

The RSV-F is a trimeric glycoprotein that contains both neutralizing and non-neutralizing epitopes. The development of a stable, minimal, monomeric domain containing potent neutralizing epitopes and minimizing non-neutralizing epitopes would offer an attractive alternative to the pre-fusion RSV F protein trimer that is currently being pursued by most in the field. Toward that end, we engineered a domain from the "top" of RSV-F (eFTop) by computational design (Figure 1). The design interventions to stabilize this domain in the absence of the rest of the RSV F glycoprotein included circular permutation, loop design, repacking and disulfide stapling.

A series of 18 molecules were prepared that included variations and combinations in the design parameters that ranged from the smallest foldable immunogen we could construct which only contained the D25 epitope to one containing both the D25 and Mota epitopes. A subset (four) of the molecules expressed to usable yields in HEK293F cells. Two were of the smallest foldable variety and contained only the D25 epitope. Two were larger and contained both D25 and Mota epitopes. The two larger variants, named eFTop-10 and eFTop-11, respectively, may be more desirable as immunogens because they contain both epitopes (Figure 2).

eFTop-10 and eFTop-11 each contain 210 amino acids (not including an optional histag for purification), considerably smaller than the 492 amino acids in a single protomer of the RSV F glycoprotein trimer. eFTop-10 and eFTop-11 only differ in the design of an additional disulfide.

eFTop-11 is a monomer in solution as determined by SEC-MALS (Figure 3A). eFTop-11 has a melting temperature of 75 degrees C according to Differential Scanning Calorimetry (DSC) (Figure 3B) and a slightly higher melting temperature according to Circular Dichroism (CD) (Figure 3C, left). CD also confirmed that eFTop-11 maintains secondary structure to 75 degrees C (Figure 3C, right). The thermal stability of eFTop-11 is a marked improvement from the published stabilized trimer known as RSVF-DSCAV1, for which we measure a melting temperature of 52 degrees C.

eFTop-10 is also a monomer in solution according to SEC-MALS. The affinity of eFTop-10 for D25 was measured by SPR as 181 pM (data not shown). In this measurement, D25 IgG was captured on the sensor chip and monomeric eFTop-10 was analyte.

The affinity of eFTop-11 for D25 and Mota was measured by SPR, also with IgG

captured as ligand and eFTop-11 as analyte. The affinities were too high to be properly measured by SPR, as our SPR instrument has a sensitivity limit of 16 pM. By SPR, eFTop-11 binds to either D25 or Mota with K_D < 16 pM (data not shown).

The affinity of eFTop-11 for D25 was also measured using the solution-based method KinExa. KinExa is optimized for extremely high affinities and slow off-rates. With this method, we determined that eFTop-11 binds to D25 with extremely high affinity, with K_D = 2.33 pM (data not shown).

2. Variants of eFTop with further improved thermal and/or conformational stability.

Multiple variants of eFTop-11 were designed using RosettaFixbb™ to remove void volumes within the core of eFTop-11. Two such stabilized variants, eFTop-11.1 and eFTop-11.2, were discovered to be more stable than eFTop-11 while retaining similar antigenic profiles to eFTop-11. The melting temperature of eFTop-11.1 was measured by DSC to be 87 degrees C, while the melting temperature of eFTop-11.2 was 78 degrees C.

3. Glycan masking variants.

We designed variants of eFTop-11 containing additional N-glycosylation sites to potentially reduce immune responses to epitopes that do not mimic the RSV trimer. Three glycosylated variants were designed: eFTop-11_g3, eFTop-11_g4a, eFTop-11_g4b.

4. Self-assembling multimers and nanoparticles displaying multiple copies of eFTop for improved immune responses.

Multimerization and nanoparticle display been shown to improve immune responses. The eFTop-11, originally designed as a monomer, was engineered via computational design and genetic fusion to create 3-mer, 4-mer and 8-mer multimers. These constructs were expressed in 293F cells and purified using standard Nickel and Size Exclusion methods. By SEC-MALS, each construct was shown to have the correct molecular weight according to its intended multimeric state. The antigenic profiles of the constructs were tested and the results showed binding to D25, Motavizumab and Palivizumab.

To create a nanoparticle platform for eFTop, we investigated a number of different platforms, including but not limited to the lumazine synthase 60-mer used in Jardine, Julien, Menis et al. Science 2013 and Jardine, Ota, Sok et al. Science 2015. Multiple attempts at creating eFTop genetic fusions to particles of various types did not yield assembled particles. We optimized the eFTop nanoparticle for secretion from mammalian cells by using forms of eFtop with both stabilizing mutations (eFTop-11.1) and with extra glycosylation sites, by

adding mutations to the lumazine synthase to stabilize the particle itself ("d41m3" mutations), and by using different DNA codon optimization schemes to improve expression levels. In our experience with secretion of these types of particles from mammalian cells, we have never obtained secreted particles unless they were glycosylated. Also, we have relied on the presence of glycans in the secreted particles to allow for the first purification step by lectin chromatography. Hence we believe that the glycosylation sites are likely at least partially occupied in these particles. With these advances, we were able to purify eFTop-nanoparticles containing from mammalian supernatant by lectin chromatography + size exclusion chromatography with a yield of ~13 mg/L for the eFtop11.1_g4b_d41m3_Nt_60mer construct. Binding of D25 Fab to the eFtop11.1_g4b_d41m3_Nt_60mer was validated by an SPR experiment in which the nanoparticles were captured on the sensor surface and D25 Fab was flowed as analyte.

Lumazine synthase fusions containing glycosylated forms of eFTop-11 were

eFtop11_g3_Ntf_60mer_m

eFtop11_g4a_Ntf_60mer_m

eFtop11_g4b_Ntf_60mer_m

The four Lumazine synthase fusions containing a form of eFTop11 with both stabilizing mutations (eFTop-11.1) and with additional glycans (g4a, g4b), and also containing stabilizing mutations added to the lumazine sythase itself ("d41m3" mutations), and also containing modified codon usage for improved expression were:

eFtop11.1_g4a_d41m3_Nt_60mer

eFtop11.1_g4a_d41m3_Ct_60mer

eFtop11.1_g4b_d41m3_Nt_60mer

eFtop11.1_g4b_d41m3_Ct_60mer

eFTop-10
AIASCEAVSKVLHLEGEVRKIKSALKSTNKAVVSLSNGVSVLTSKVLDLKNYIDKQLLPIVNK QSCSISNPETVKEFQQKNNRLLEITREFSVNAGVTTPVSTYMLTNSELLSLINDMPITNDQKKL MSNNVQIVRQQSYSVMSIIKEEVLAYVVCLPLPGHGGWYTSVITIELSNIKENKCNGTDAKVK LIKQELDKYKNAVTELQNLM (SEQ ID NO: 42)

eFTop-11
AIASGEAVCKVLHLEGEVRKIKSALKSTNKAVVSLSNGVSVLTSKVLDLKNYIDKQLLPIVNK QSCSISNPETVKEFQQKNNRLLEITREFSVNAGVTTPVSTYMLTNSELLSLINDMPITNDQKKL MSNNVQIVRQQSYSIMCIIKEEVLAYVVQLPLPGHGGWYTSVITIELSNIKENKCNGTDAKVK LIKQELDKYKNAVTELQNLM (SEQ ID NO: 43)

eFtop-11.1

AIASGEAVCKVLHLEGEVRKIKSALKSTNKAVVSLSNGVSVLTFKVLDLKNYIDKQLLPILNK
QSCSISNPETVKEFQQKNNRLLEITREFSVNAGVTTPVSTYMLTNSELLSLINDMPITNDQKKL
MSNNVQIVRQQSYSIMCIIKEEVLAYVVQLPLPGHGGWYTSVITIELSNIKENKCNGTDAKVK
LIKQELDKYKNAVTELQNLM (SEQ ID NO: 48)

eFtop-11.2
AIASGEAVCKVLHLEGEVRKIKSALKSTNKAVVSLSNGVSVLTSKVLDLKNYIDKQLLPIANK
QSCSISNPETLKEFQQKLNRFLEIAREFSVNAGVTTPVSTYMLTNSELLSLINDMPITNDQKKL
MSNNVQIVRQQSYSIMCIIKEEVLAYVVQLPLPGHGGWYTSVITIELSNIKENKCNGTDAKVK
LFKQEADKYKNAMTELQNLM (SEQ ID NO: 49)

eFtop11_g3
AIASGEAVCKVLHLEGEVRKIKSALKSTNKAVVSLSNGVSVLTSKVLDLKNYIDKQLLPIVNK
QSCSISNPNTTKEFQQKNNRLLEITREFSNNSGVTTPVSTYMLTNSELLSLINDMPITNDQKKL
MSNNVQIVRNQSYSIMCIIKEEVLAYVVQLPLPGHGGWYTSVITIELSNIKENKCNGTDAKVK
LIKQELDKYKNAVTELQNLM (SEQ ID NO: 50)

eFtop11_g4a
AIASGEAVCKVLHLEGEVRKIKSALKSTNKAVVSLSNGVSVLTSKVLDLKNYIDKQLLPIVNK
QSCSISNPNTTKEFQQKNNRLLEITREFSNNSGVTTPVSTYMLTNSELLSLINDMPITNDQKKL
MSNNVQIVRNQSYSIMCIIKEEVLAYVVQLPLPGHGGWYTSVITIELSNIKENKCNGTDAKVK
LIKQELDNYSNAVTELQNLM (SEQ ID NO: 51)

eFtop11_g4b
AIASGEAVCKVLHLEGEVRKIKSALKSTNKAVVSLSNGVSVLTSKVLDLKNYIDKQLLPIVNK
QSCSISNPNTTKEFQQKNNRLLNITREFSNNSGVTTPVSTYMLTNSELLSLINDMPITNDQKKL
MSNNVQIVRNQSYSIMCIIKEEVLAYVVQLPLPGHGGWYTSVITIELSNIKENKCNGTDAKVK
LIKQELDKYKNAVTELQNLM (SEQ ID NO: 52)

eFTop-11 3-mer
eFtop11_3mer-1gcm
AIASGEAVCKVLHLEGEVRKIKSALKSTNKAVVSLSNGVSVLTSKVLDLKNYIDKQLLPIVNK
QSCSISNPETVKEFQQKNNRLLEITREFSVNAGVTTPVSTYMLTNSELLSLINDMPITNDQKKL
MSNNVQIVRQQSYSIMCIIKEEVLAYVVQLPLPGHGGWYTSVITIELSNIKENKCNGTDAKVK
LIKQELDKYKNAVTELQNLMGGSGGSGGSGGG*RMKQIEDKIEEILSKIYHIENEIARIKKLIGER*
(SEQ ID NO: 53)

eFTop-11 4-mer
#uses 2b22
eFtop11_4mer
AIASGEAVCKVLHLEGEVRKIKSALKSTNKAVVSLSNGVSVLTSKVLDLKNYIDKQLLPIVNK
QSCSISNPETVKEFQQKNNRLLEITREFSVNAGVTTPVSTYMLTNSELLSLINDMPITNDQKKL
MSNNVQIVRQQSYSIMCIIKEEVLAYVVQLPLPGHGGWYTSVITIELSNIKENKCNGTDAKVK
LIKQELDKYKNAVTELQNLMGGSGGSGGSGGG*MKVKQLEDVVEELLSVNYHLENVVARLKKLV*
GER (SEQ ID NO: 54)

eFTop-11 8-mer
#uses 2b22
eFtop11_8mer
AIASGEAVCKVLHLEGEVRKIKSALKSTNKAVVSLSNGVSVLTSKVLDLKNYIDKQLLPIVNK
QSCSISNPETVKEFQQKNNRLLEITREFSVNAGVTTPVSTYMLTNSELLSLINDMPITNDQKKL
MSNNVQIVRQQSYSIMCIIKEEVLAYVVQLPLPGHGGWYTSVITIELSNIKENKCNGTDAKVK
LIKQELDKYKNAVTELQNLMGGSGGSGGSGGG*MKVKQLEDVVEELLSVNYHLENVVARLKKLV*
*GER*GGSGGSGGSGGGAIASGEAVCKVLHLEGEVRKIKSALKSTNKAVVSLSNGVSVLTSKVL
DLKNYIDKQLLPIVNKQSCSISNPETVKEFQQKNNRLLEITREFSVNAGVTTPVSTYMLTNSEL

LSLINDMPITNDQKKLMSNNVQIVRQQSYSIMCIIKEEVLAYVVQLPLPGHGGWYTSVITIELS
NIKENKCNGTDAKVKLIKQELDKYKNAVTELQNLM (SEQ ID NO: 55)

eFtop11_g3_Ntf_60mer_m
MQIYEGKLTAEGLRFGIVASRFNHALVDRLVEGAIDAIVRHGGREEDITLVRVPGSWEIPVAA
GELARKEDIDAVIAIGVLIRGATPHFDYIASEVSKGLADLSLELRKPITFGVITADTLEQAIERA
GTKHGNKGWEAALSAIEMANLFKSLRGGSGGSGGSGGSGGSGGSGGGAIASGEAVCKVLHL
EGEVRKIKSALKSTNKAVVSLSNGVSVLTSKVLDLKNYIDKQLLPIVNKQSCSISNPNTTKEF
QQKNNRLLEITREFSNNSGVTTPVSTYMLTNSELLSLINDMPITNDQKKLMSNNVQIVRNQSY
SIMCIIKEEVLAYVVQLPLPGHGGWYTSVITIELSNIKENKCNGTDAKVKLIKQELDKYKNAV
TELQNLM** (SEQ ID NO: 56)

eFtop11_g4a_Ntf_60mer_m
MQIYEGKLTAEGLRFGIVASRFNHALVDRLVEGAIDAIVRHGGREEDITLVRVPGSWEIPVAA
GELARKEDIDAVIAIGVLIRGATPHFDYIASEVSKGLADLSLELRKPITFGVITADTLEQAIERA
GTKHGNKGWEAALSAIEMANLFKSLRGGSGGSGGSGGSGGSGGSGGGAIASGEAVCKVLHL
EGEVRKIKSALKSTNKAVVSLSNGVSVLTSKVLDLKNYIDKQLLPIVNKQSCSISNPNTTKEF
QQKNNRLLEITREFSNNSGVTTPVSTYMLTNSELLSLINDMPITNDQKKLMSNNVQIVRNQSY
SIMCIIKEEVLAYVVQLPLPGHGGWYTSVITIELSNIKENKCNGTDAKVKLIKQELDNYSNAV
TELQNLM** (SEQ ID NO: 57)

eFtop11_g4b_Ntf_60mer_m
MQIYEGKLTAEGLRFGIVASRFNHALVDRLVEGAIDAIVRHGGREEDITLVRVPGSWEIPVAA
GELARKEDIDAVIAIGVLIRGATPHFDYIASEVSKGLADLSLELRKPITFGVITADTLEQAIERA
GTKHGNKGWEAALSAIEMANLFKSLRGGSGGSGGSGGSGGSGGSGGGAIASGEAVCKVLHL
EGEVRKIKSALKSTNKAVVSLSNGVSVLTSKVLDLKNYIDKQLLPIVNKQSCSISNPNTTKEF
QQKNNRLLNITREFSNNSGVTTPVSTYMLTNSELLSLINDMPITNDQKKLMSNNVQIVRNQSY
SIMCIIKEEVLAYVVQLPLPGHGGWYTSVITIELSNIKENKCNGTDAKVKLIKQELDKYKNAV
TELQNLM** (SEQ ID NO: 58)

Connect C-term of d41m3 to N-term of eFTop-11.1_g4a
eFTop11.1_g4a_d41m3_Ct_60mer
MQIYEGKLTAEGLRFGIVASRANHALVDRLVEGAIDAIVRHGGREEDITLVRVCGSWEIPVA
AGELARKEDIDAVIAIGVLCRGATPSFDYIASEVSKGLADLSLELRKPITFGVITADTLEQAIEA
AGTCHGNKGWEAALCAIEMANLFKSLRGGSGGSGGSGGSGGGAIASGEAVCKVLHLEGEVR
KIKSALKSTNKAVVSLSNGVSVLTFKVLDLKNYIDKQLLPILNKQSCSISNPNTTKEFQQKNN
RLLEITREFSNNSGVTTPVSTYMLTNSELLSLINDMPITNDQKKLMSNNVQIVRNQSYSIMCII
KEEVLAYVVQLPLPGHGGWYTSVITIELSNIKENKCNGTDAKVKLIKQELDNYSNAVTELQN
LM** (SEQ ID NO: 39)

ATGCAGATCTACGAAGGAAAACTGACCGCTGAGGGACTGAGGTTCGGAATTGTCGCAAG
CCGCGCGAATCACGCACTGGTGGATAGGCTGGTGGAAGGCGCTATCGACGCAATTGTCC
GGCACGGCGGGAGAGAGGAAGACATCACACTGGTGAGAGTCTGCGGCAGCTGGGAGAT
TCCCGTGGCAGCTGGAGAACTGGCTCGAAAGGAGGACATCGATGCCGTGATCGCTATTG
GGGTCCTGTGCCGAGGAGCAACTCCCAGCTTCGACTACATCGCCTCAGAAGTGAGCAAG
GGGCTGGCTGATCTGTCCCTGGAGCTGAGGAAACCTATCACTTTTGGCGTGATTACTGCC
GACACCCTGGAACAGGCAATCGAGGCGGCCGGCACCTGCCATGGAAACAAAGGCTGGG
AAGCAGCCCTGTGCGCTATTGAGATGGCAAATCTGTTCAAATCTCTGCGA*GGAGGCTCCG*
*GAGGATCTGGAGGGAGTGGAGGCTCAGGAGGAGGC*GCCATCGCTAGCGGAGAGGCCGTGT
GCAAGGTCCTGCACCTGGAGGGCGAAGTGAGGAAGATCAAGTCTGCACTGAAGAGTACC
AACAAAGCCGTGGTCAGCCTGTCCAATGGCGTGTCCGTCCTGACATTCAAGGTGCTGGAC
CTGAAAAACTATATCGATAAGCAGCTGCTGCCAATTCTGAACAAGCAGTCTTGTAGTATC
TCAAATCCCAATACTACAAAAGAGTTCCAGCAGAAGAACAATCGGCTGCTGGAGATCAC
CAGAGAGTTCAGCAACAACTCTGGAGTCACCACCCCCGTGAGCACCTACATGCTGACCA
ATTCAGAGCTGCTGAGCCTGATCAACGACATGCCCATTACCAATGATCAGAAGAAACTG

ATGAGCAACAATGTGCAGATCGTCCGGAATCAGTCTTACTCCATTATGTGCATCATCAAG
GAGGAAGTGCTGGCTTATGTGGTCCAGCTGCCACTGCCTGGGCATGGCGGATGGTACAC
ATCCGTGATCACTATTGAGCTGTCTAACATCAAGGAAAACAAATGTAACGGAACAGACG
CTAAGGTCAAACTGATTAAGCAGGAGCTGGATAACTATAGCAACGCAGTGACTGAACTG
CAGAATCTGATG**TGATAA** (SEQ ID NO: 44)

Connect C-term of eFTop-11.1_g4a to N-term of d41m3
eFTop11.1_g4a_d41m3_Nt_60mer
AIASGEAVCKVLHLEGEVRKIKSALKSTNKAVVSLSNGVSVLTFKVLDLKNYIDKQLLPILNK
QSCSISNPNTTKEFQQKNNRLLEITREFSNNSGVTTPVSTYMLTNSELLSLINDMPITNDQKKL
MSNNVQIVRNQSYSIMCIIKEEVLAYVVQLPLPGHGGWYTSVITIELSNIKENKCNGTDAKVK
LIKQELDNYSNAVTELQNLMGGSGGSGGSGGSGGGMQIYEGKLTAEGLRFGIVASRANHAL
VDRLVEGAIDAIVRHGGREEDITLVRVCGSWEIPVAAGELARKEDIDAVIAIGVLCRGATPSF
DYIASEVSKGLADLSLELRKPITFGVITADTLEQAIEAAGTCHGNKGWEAALCAIEMANLFKS
LR** (SEQ ID NO: 40)

GCCATCGCTAGCGGAGAGGCCGTGTGCAAGGTCCTGCACCTGGAGGGCGAAGTGAGGAA
GATCAAGTCTGCACTGAAGAGTACCAACAAAGCCGTGGTCAGCCTGTCCAATGGCGTGT
CCGTCCTGACATTCAAGGTGCTGGACCTGAAAAACTATATCGATAAGCAGCTGCTGCCAA
TTCTGAACAAGCAGTCTTGTAGTATCTCAAATCCCAATACTACAAAAGAGTTCCAGCAGA
AGAACAATCGGCTGCTGGAGATCACCAGAGAGTTCAGCAACAACTCTGGAGTCACCACC
CCCGTGAGCACCTACATGCTGACCAATTCAGAGCTGCTGAGCCTGATCAACGACATGCCC
ATTACCAATGATCAGAAGAAACTGATGAGCAACAATGTGCAGATCGTCCGGAATCAGTC
TTACTCCATTATGTGCATCATCAAGGAGGAAGTGCTGGCTTATGTGGTCCAGCTGCCACT
GCCTGGGCATGGCGGATGGTACACATCCGTGATCACTATTGAGCTGTCTAACATCAAGGA
AAACAAATGTAACGGAACAGACGCTAAGGTCAAACTGATTAAGCAGGAGCTGGATAACT
ATAGCAACGCAGTGACTGAACTGCAGAATCTGATG*GGAGGCTCCGGAGGATCTGGAGGGA*
*GTGGAGGCTCAGGAGGAGGC*ATGCAGATCTACGAAGGAAAACTGACCGCTGAGGGACTG
AGGTTCGGAATTGTCGCAAGCCGCGCGAATCACGCACTGGTGGATAGGCTGGTGGAAGG
CGCTATCGACGCAATTGTCCGGCACGGCGGGAGAGAGGAAGACATCACACTGGTGAGAG
TCTGCGGCAGCTGGGAGATTCCCGTGGCAGCTGGAGAACTGGCTCGAAAGGAGGACATC
GATGCCGTGATCGCTATTGGGGTCCTGTGCCGAGGAGCAACTCCCAGCTTCGACTACATC
GCCTCAGAAGTGAGCAAGGGGCTGGCTGATCTGTCCCTGGAGCTGAGGAAACCTATCAC
TTTTGGCGTGATTACTGCCGACACCCTGGAACAGGCAATCGAGGCGGCCGGCACCTGCC
ATGGAAACAAAGGCTGGGAAGCAGCCCTGTGCGCTATTGAGATGGCAAATCTGTTCAAA
TCTCTGCGA**TGATAA** (SEQ ID NO: 45)

Connect C-term of d41m3 to N-term of eFtop11.1_g4b
eFtop11.1_g4b_d41m3_Ct_60mer
MQIYEGKLTAEGLRFGIVASRANHALVDRLVEGAIDAIVRHGGREEDITLVRVCGSWEIPVA
AGELARKEDIDAVIAIGVLCRGATPSFDYIASEVSKGLADLSLELRKPITFGVITADTLEQAIEA
AGTCHGNKGWEAALCAIEMANLFKSLRGGSGGSGGSGGSGGGAIASGEAVCKVLHLEGEVR
KIKSALKSTNKAVVSLSNGVSVLTFKVLDLKNYIDKQLLPILNKQSCSISNPNTTKEFQQKNN
RLLNITREFSNNSGVTTPVSTYMLTNSELLSLINDMPITNDQKKLMSNNVQIVRNQSYSIMCII
KEEVLAYVVQLPLPGHGGWYTSVITIELSNIKENKCNGTDAKVKLIKQELDKYKNAVTELQN
LM** (SEQ ID NO: 41)

ATGCAGATCTACGAAGGAAAACTGACCGCTGAGGGACTGAGGTTCGGAATTGTCGCAAG
CCGCGCGAATCACGCACTGGTGGATAGGCTGGTGGAAGGCGCTATCGACGCAATTGTCC
GGCACGGCGGGAGAGAGGAAGACATCACACTGGTGAGAGTCTGCGGCAGCTGGGAGAT
TCCCGTGGCAGCTGGAGAACTGGCTCGAAAGGAGGACATCGATGCCGTGATCGCTATTG
GGGTCCTGTGCCGAGGAGCAACTCCCAGCTTCGACTACATCGCCTCAGAAGTGAGCAAG
GGGCTGGCTGATCTGTCCCTGGAGCTGAGGAAACCTATCACTTTTGGCGTGATTACTGCC
GACACCCTGGAACAGGCAATCGAGGCGGCCGGCACCTGCCATGGAAACAAAGGCTGGG
AAGCAGCCCTGTGCGCTATTGAGATGGCAAATCTGTTCAAATCTCTGCGA*GGAGGCTCCG*

*GAGGATCTGGAGGGAGTGGAGGCTCAGGAGGAG*GCGCAATCGCATCCGGAGAGGCCGTGT
GCAAGGTGCTGCACCTGGAGGGCGAGGTGAGGAAGATCAAGAGCGCCCTGAAGTCCACC
AACAAGGCCGTGGTGAGCCTGTCCAATGGCGTGTCTGTGCTGACATTCAAGGTGCTGGAC
CTGAAGAACTATATCGATAAGCAGCTGCTGCCAATCCTGAATAAGCAGTCTTGTAGCATC
TCCAACCCCAATACCACAAAGGAGTTCCAGCAGAAGAACAATCGGCTGCTGAACATCAC
CAGAGAGTTTTCCAACAATTCTGGCGTGACCACCCCCGTGAGCACCTACATGCTGACAAA
TTCCGAGCTGCTGTCTCTGATCAACGACATGCCCATCACAAATGATCAGAAGAAGCTGAT
GAGCAACAATGTGCAGATCGTGCGGAACCAGTCTTACAGCATCATGTGCATCATCAAGG
AGGAGGTGCTGGCCTATGTGGTGCAGCTGCCACTGCCTGGCCACGGCGGCTGGTACACC
AGCGTGATCACAATCGAGCTGTCCAATATCAAGGAGAACAAGTGTAATGGCACCGACGC
CAAGGTGAAGCTGATCAAGCAGGAGCTGGATAAGTATAAGAACGCCGTGACAGAGCTGC
AGAATCTGATG**TGATAA** (SEQ ID NO: 46)

Connect C-term of eFtop11.1_g4b to N-term of d41m3
eFtop11.1_g4b_d41m3_Nt_60mer
AIASGEAVCKVLHLEGEVRKIKSALKSTNKAVVSLSNGVSVLTFKVLDLKNYIDKQLLPILNK
QSCSISNPNTTKEFQQKNNRLLNITREFSNNSGVTTPVSTYMLTNSELLSLINDMPITNDQKKL
MSNNVQIVRNQSYSIMCIIKEEVLAYVVQLPLPGHGGWYTSVITIELSNIKENKCNGTDAKVK
LIKQELDKYKNAVTELQNLMGGSGGSGGSGGSGGGMQIYEGKLTAEGLRFGIVASRANHAL
VDRLVEGAIDAIVRHGGREEDITLVRVCGSWEIPVAAGELARKEDIDAVIAIGVLCRGATPSF
DYIASEVSKGLADLSLELRKPITFGVITADTLEQAIEAAGTCHGNKGWEAALCAIEMANLFKS
LR**

GCAATCGCATCCGGAGAGGCCGTGTGCAAGGTGCTGCACCTGGAGGGCGAGGTGAGGAA
GATCAAGAGCGCCCTGAAGTCCACCAACAAGGCCGTGGTGAGCCTGTCCAATGGCGTGT
CTGTGCTGACATTCAAGGTGCTGGACCTGAAGAACTATATCGATAAGCAGCTGCTGCCAA
TCCTGAATAAGCAGTCTTGTAGCATCTCCAACCCCAATACCACAAAGGAGTTCCAGCAGA
AGAACAATCGGCTGCTGAACATCACCAGAGAGTTTTCCAACAATTCTGGCGTGACCACCC
CCGTGAGCACCTACATGCTGACAAATTCCGAGCTGCTGTCTCTGATCAACGACATGCCCA
TCACAAATGATCAGAAGAAGCTGATGAGCAACAATGTGCAGATCGTGCGGAACCAGTCT
TACAGCATCATGTGCATCATCAAGGAGGAGGTGCTGGCCTATGTGGTGCAGCTGCCACTG
CCTGGCCACGGCGGCTGGTACACCAGCGTGATCACAATCGAGCTGTCCAATATCAAGGA
GAACAAGTGTAATGGCACCGACGCCAAGGTGAAGCTGATCAAGCAGGAGCTGGATAAGT
ATAAGAACGCCGTGACAGAGCTGCAGAATCTGATG*GGAGGCTCCGGAGGATCTGGAGGGA*
*GTGGAGGCTCAGGAGGAGGC*ATGCAGATCTACGAAGGAAAACTGACCGCTGAGGGACTG
AGGTTCGGAATTGTCGCAAGCCGCGCGAATCACGCACTGGTGGATAGGCTGGTGGAAGG
CGCTATCGACGCAATTGTCCGGCACGGCGGGAGAGAGGAAGACATCACACTGGTGAGAG
TCTGCGGCAGCTGGGAGATTCCCGTGGCAGCTGGAGAACTGGCTCGAAAGGAGGACATC
GATGCCGTGATCGCTATTGGGGTCCTGTGCCGAGGAGCAACTCCCAGCTTCGACTACATC
GCCTCAGAAGTGAGCAAGGGGCTGGCTGATCTGTCCCTGGAGCTGAGGAAACCTATCAC
TTTTGGCGTGATTACTGCCGACACCCTGGAACAGGCAATCGAGGCGGCCGGCACCTGCC
ATGGAAACAAAGGCTGGGAAGCAGCCCTGTGCGCTATTGAGATGGCAAATCTGTTCAAA
TCTCTGCGA**TGATAA** (SEQ ID NO: 47)

We claim

1. A polypeptide comprising a first domain, wherein the first domain comprises the amino acid sequence of SEQ ID NO:1.

2. The polypeptide of claim 1, wherein the first domain comprises the amino acid sequence of SEQ ID NOS: 2-8.

3. The polypeptide of claim 1 or 2, wherein the first domain is present in two or more copies.

4. The polypeptide of any one of claims 1-3, further comprising a multimerization domain.

5. The polypeptide of claim 4, wherein the multimerization domain comprises the amino acid sequence selected from the group consisting of SEQ ID NOS:9-12.

6. The polypeptide of claim 4 or 5 wherein the multimerization domain comprises the amino acid sequence selected from the group consisting of SEQ ID NOS:13-28.

7. A polypeptide comprising:

(a) a multimerization domain comprising the amino acid sequence selected from the group consisting of SEQ ID NO:10, 14, and 15-28; and

(b) one or more copies of a respiratory syncytial virus (RSV) antigen.

8. The polypeptide of claim 7, wherein the multimerization domain comprises the amino acid sequence selected from the group consisting of SEQ ID NO: 10 and 15-28.

9. The polypeptide of any one of claims 4-8, further comprising an amino acid linker between the first domain and the multimerization domain, or between the multimerization domain and the RSV antigen.

10. The polypeptide of any one of claims 1-9, comprising the amino acid sequence selected from the group consisting of SEQ ID NOS: 29-41.

11. The polypeptide of any one of claims 1-10, comprising the amino acid sequence selected from the group consisting of SEQ ID NOS: 29-31.

12. A multimer, comprising two or more copies of the polypeptide of any one of claims 4-11.

13. The multimer of claim 12, comprising eight or more copies of the polypeptide of any one of claims 4-11

14. A nucleic acid encoding the polypeptide of any one of claims 1-11.

15. The nucleic acid of claim 14, wherein the nucleic acid comprises the sequence selected from the group consisting of SEQ ID NOS: 44-47.

16. A recombinant expression vector comprising the nucleic acid of claim 14 or 15 operatively linked to a suitable control sequence.

17. A recombinant host cell comprising the recombinant expression vector of claim 16.

18. A pharmaceutical composition comprising

(a) the polypeptide of any one of claims 1-11, the multimer of claims 12-13, the nucleic acid of claim 14 or 15, the recombinant expression vector of claim 16, or the recombinant host cell of claim 17; and

(b) a pharmaceutically acceptable carrier.

19. A method for treating a respiratory syncytial virus (RSV) infection, comprising administering to a subject infected with RSV an amount effective to treat the infection of the polypeptide of any one of claims 1-11, the multimer of claims 12-13, the nucleic acid of claim 14 or 15, the recombinant expression vector of claim 16, the recombinant host cell of claim 17, or the pharmaceutical composition of claim 18.

20. A method for limiting development of an RSV infection, comprising administering to a subject at risk of RSV infection an amount effective to limit development of an RSV infection of the polypeptide of any one of claims 1-11, the multimer of claims 12-13, the

nucleic acid of claim 14 or 15, the recombinant expression vector of claim 16, the recombinant host cell of claim 17, or the pharmaceutical composition of claim 18.

21. A method for generating an immune response in a subject, comprising administering to the subject an amount effective to generate an immune response of the polypeptide of any one of claims 1-11, the multimer of claims 12-13, the nucleic acid of claim 14 or 15, the recombinant expression vector of claim 16, the recombinant host cell of claim 17, or the pharmaceutical composition of claim 18.

22. A method for monitoring an RSV-induced disease in a subject and/or monitoring response of the subject to immunization by an RSV vaccine, comprising contacting the polypeptide of any one of claims 1-11, the multimer of claims 12-13, the nucleic acid of claim 14 or 15, the recombinant expression vector of claim 16, the recombinant host cell of claim 17, or the pharmaceutical composition of claim 18 with a bodily fluid from the subject and detecting RSV-binding antibodies in the bodily fluid of the subject.

23. The method of claim 22, wherein the bodily fluid comprises serum or whole blood.

24. A method for detecting RSV binding antibodies, comprising

(a) contacting the polypeptide of any one of claims 1-11, the multimer of claims 12-13, the nucleic acid of claim 14 or 15, the recombinant expression vector of claim 16, the recombinant host cell of claim 17, or the pharmaceutical composition of claim 18 with a composition comprising a candidate RSV binding antibody under conditions suitable for binding of RSV antibodies to the polypeptide, multimer, or composition; and

(b) detecting RSV antibody complexes with the polypeptide, multimer, or composition.

25. The method of claim 24, further comprising isolating the RSV antibodies.

26. A method for producing RSV antibodies, comprising

(a) administering to a subject an amount effective to generate an antibody response of the polypeptide of any one of claims 1-11, the multimer of claims 12-13, the nucleic acid of claim 14 or 15, the recombinant expression vector of claim 16, the recombinant host cell of claim 17, or the pharmaceutical composition of claim 18; and

(b) isolating antibodies produced by the subject.

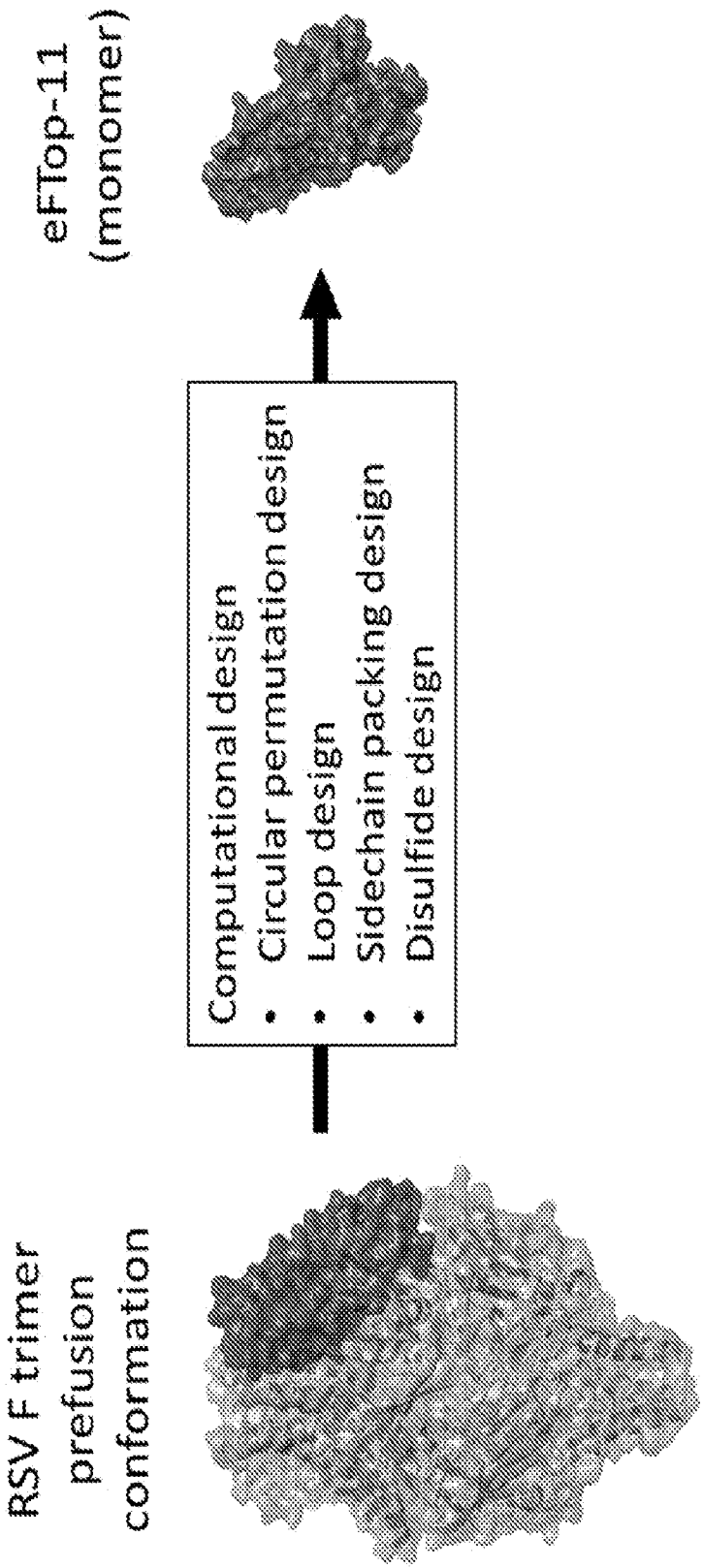
RSV F trimer prefusion conformation
Computational design
• Circular permutation design
• Loop design
• Sidechain packing design
• Disulfide design
eFTop-11 (monomer)

Fig. 1

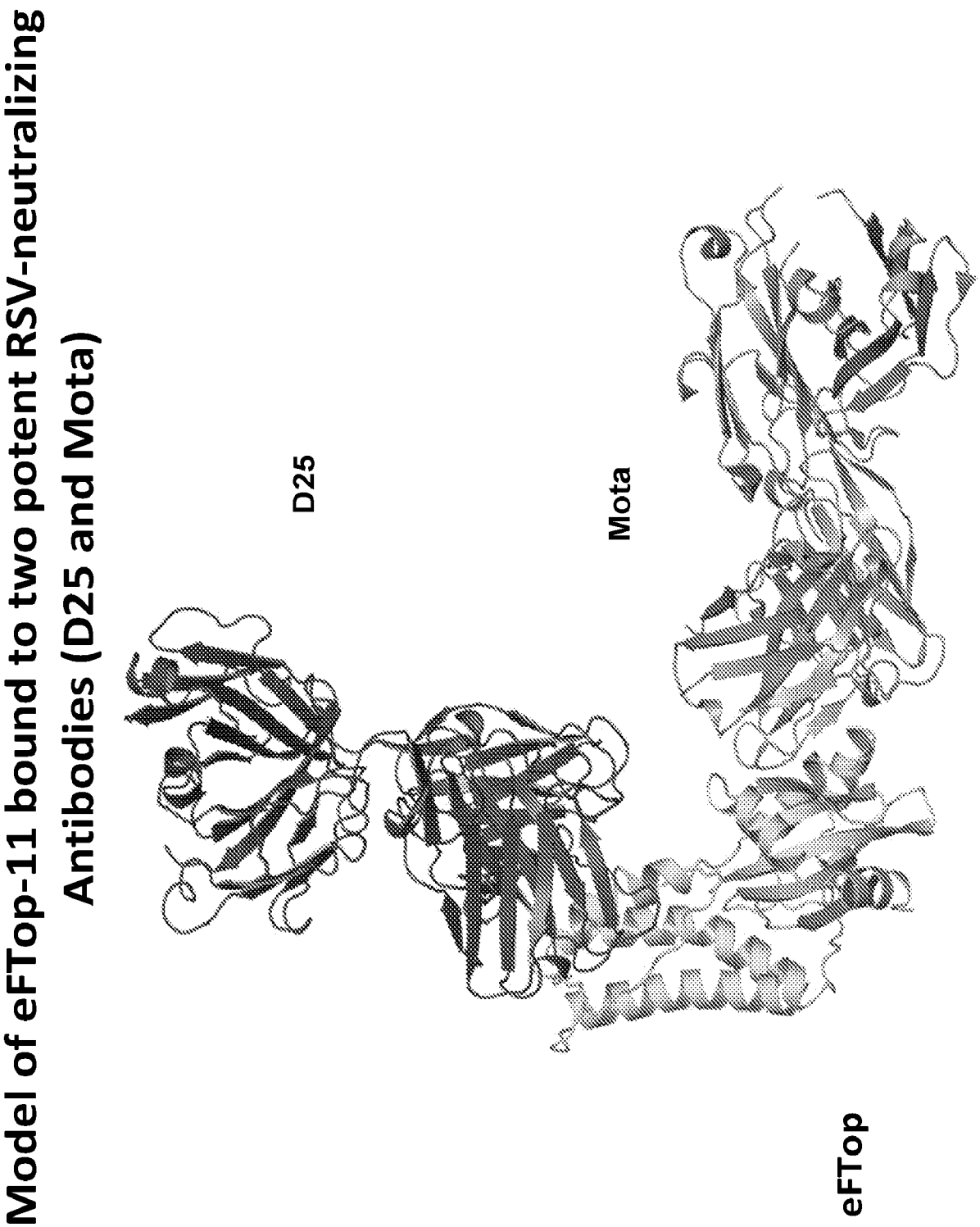
Model of eFTop-11 bound to two potent RSV-neutralizing Antibodies (D25 and Mota)
D25
Mota
eFTop

Fig. 2

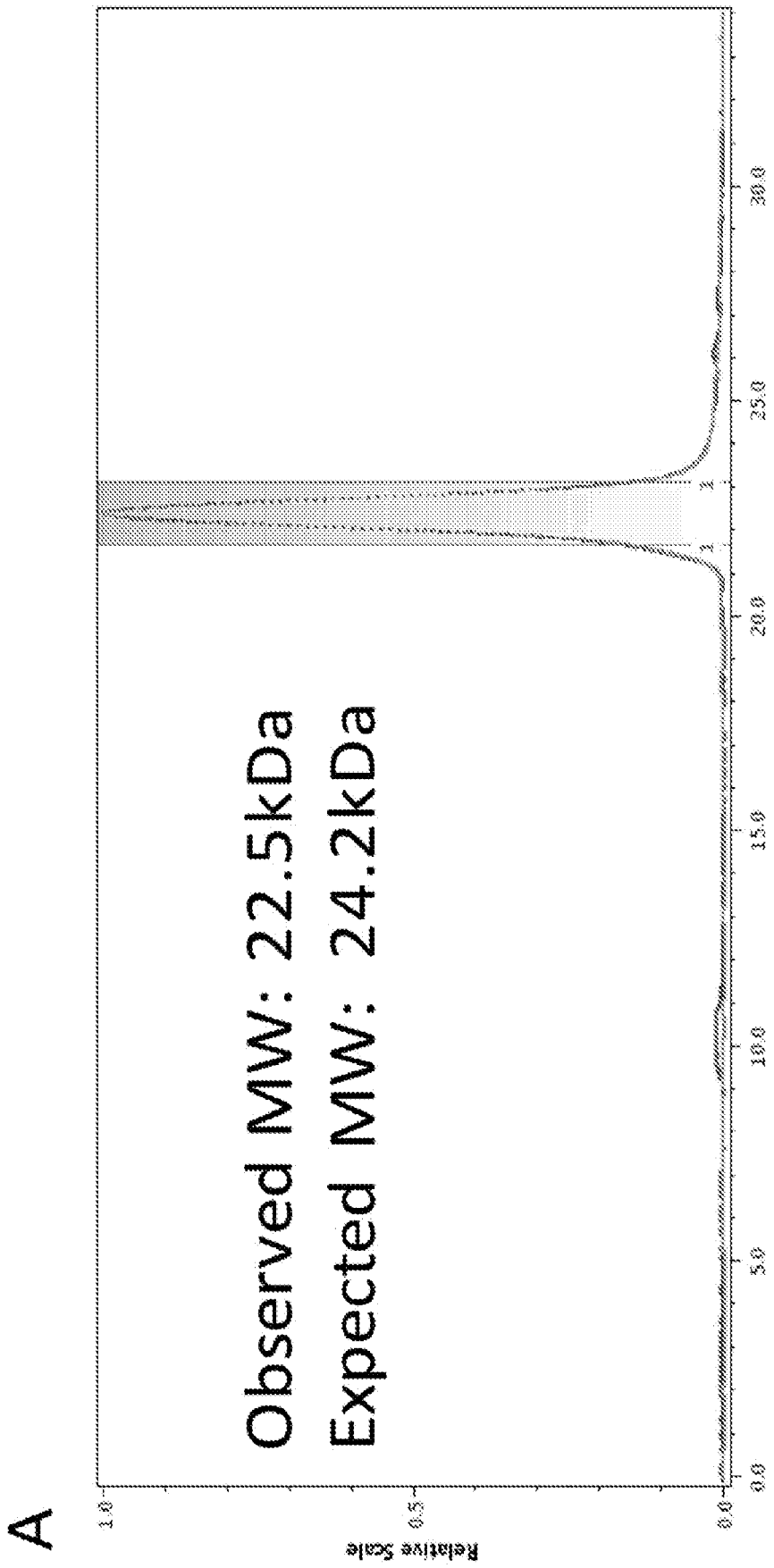
A
Biophysical characterization of eFtop
Observed MW: 22.5kDa
Expected MW: 24.2kDa
Relative Scale
time (min)
0.0
0.5
1.0
5.0
10.0
15.0
20.0
25.0
30.0

Fig. 3a

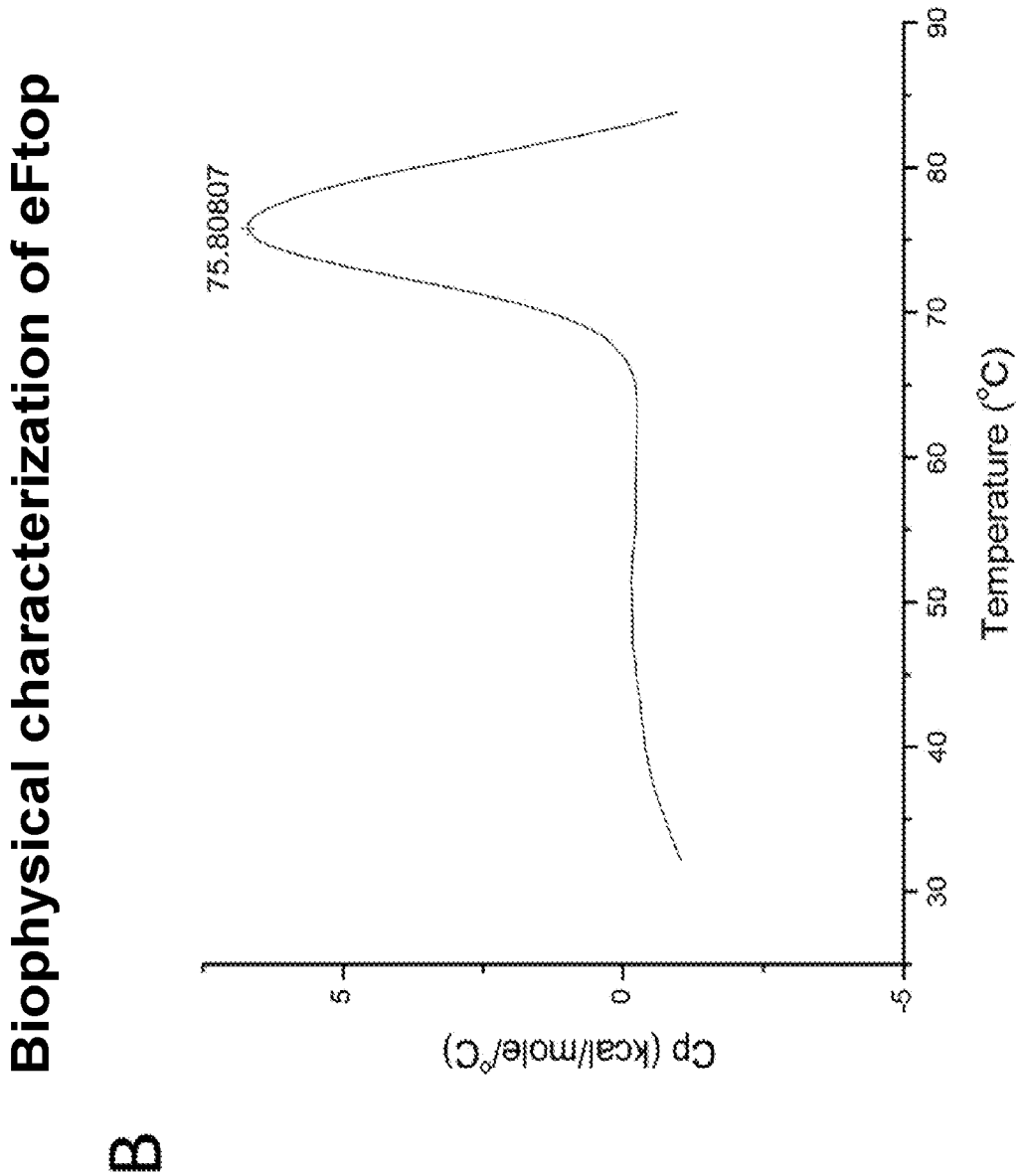
Biophysical characterization of eFtop
B
75.80807
Cp (kcal/mole/°C)
Temperature (°C)
5
0
-5
30
40
50
60
70
80
90

Fig. 3b

Biophysical characterization of eFtop

C

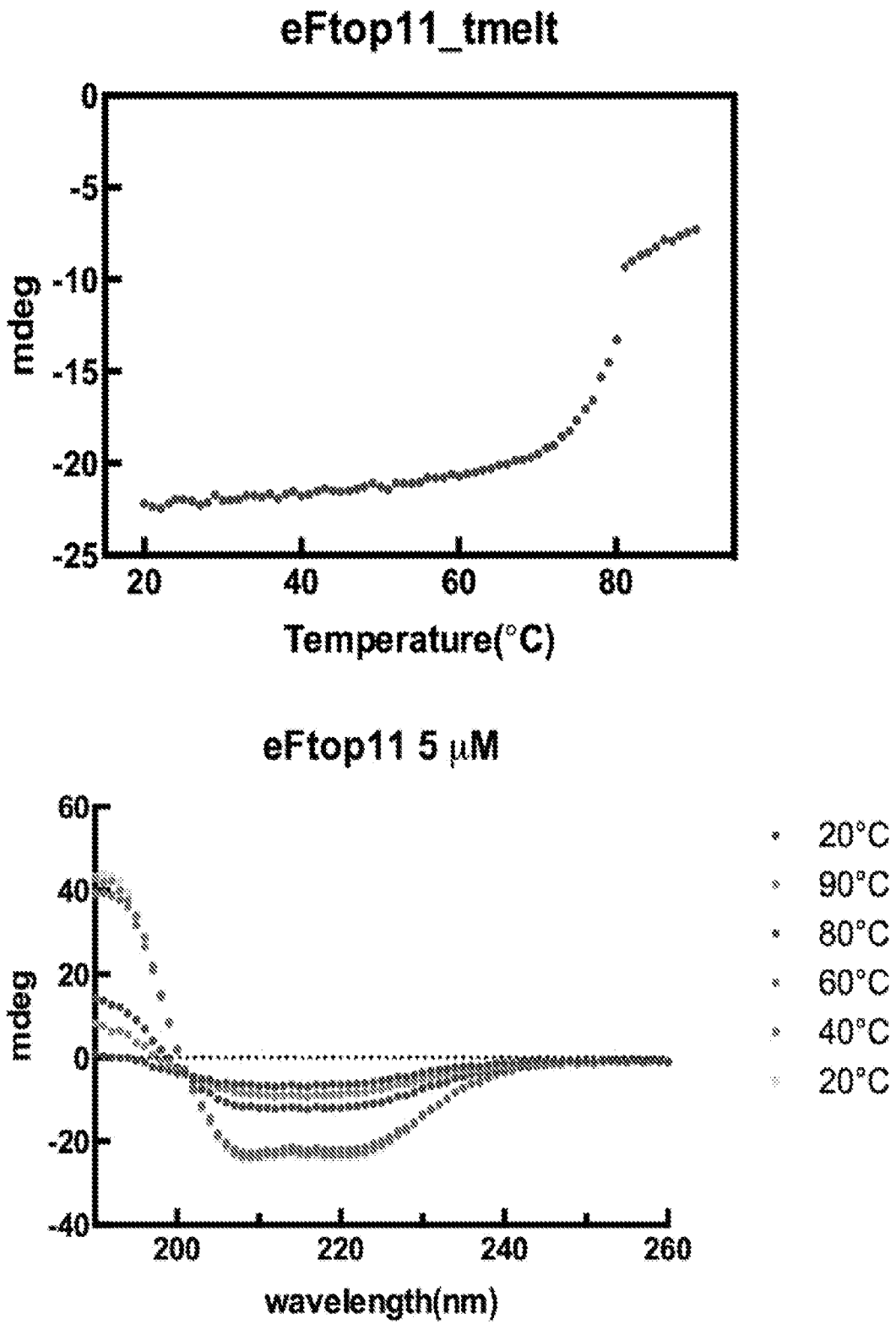

Fig. 3c

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US18/23463

A. CLASSIFICATION OF SUBJECT MATTER
IPC - A61K 39/245, 39/12; C07K 14/00 (2018.01)
CPC - A61K 39/245, 39/12; C07K 14/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
See Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
See Search History document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2016/0303224 A1 (THE UNITED STATES OF AMERICA, AS REPRESENTED BY THE SECRETARY, DEPARTMENT OF HEALTH AND HUMAN SERVICES) 20 October 2016; Paragraphs [0094], [0218]; Claim 53; SEQ ID NO:26	7, 8
Y	US 2016/0046675 A1 (THE USA, AS REPRESENTED BY THE SECRETARY, DEPARTMENT OF HEALTH & HUMAN SERVICES) 18 February 2016; Abstract; Paragraph [0002], SEQ ID NO: 798	7, 8
A	WO 2014/160463 A1 (THE UNITED STATES OF AMERICA, AS REPRESENTED BY THE SECRETARY, DEPARTMENT OF HEALTH & HUMAN SERVICES) 02 October 2014; Abstract; Claim 52; SEQ ID NO: 1188	1, 2, 3/1, 3/2
A	US 8,563,002 B2 (BAUDOUX, GJMFP, et al.) 22 October 2013; Abstract; Claims 1-4; SEQ ID NO:2	1, 2, 3/1, 3/2
A	US 8,580,270 B2 (MORRISON, TG) 12 November 2013; Abstract; Column 7, Lines 38-44; SEQ ID NO:1	1, 2, 3/1, 3/2
A	US 8,846,051 B2 (KEW, OM, et al.) 30 September 2014; Column 8, Lines 20-25; SEQ ID NO:32	1, 2, 3/1, 3/2

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
12 July 2018 (12.07.2018)	20 JUL 2018
Name and mailing address of the ISA/ Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer Shane Thomas PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (January 2015)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US18/23463

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 4-6, 9-26
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

-***-Please See Supplemental Page-***-

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

-***-Please See Supplemental Page-***-

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/US18/23463

-***-Continued from Box No. III: Observations Where Unity of Invention Is Lacking:

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Groups I+, Claims 1-3, 7, 8 and SEQ ID NO: 10, limited to wherein residue 5 is E, residue 8 is L, residue 9 is T, residue 40 is R, residue 41 is H, residue 52 is R, residue 54 is P, residue 61 is V, residue 86 is T, residue 87 is P and residue 105 is L (first exemplary multimerization domain sequence). Applicant is invited to elect additional multimerization domain sequence(s), with specified SEQ ID NO: for each, or with specified substitution(s) or residue(s) at specified site(s) of a SEQ ID NO., such that the sequence of each elected species is fully specified (i.e. no optional or variable residues or substituents), to be searched. Additional multimerization domain sequence(s) will be searched upon the payment of additional fees. It is believed that claims 1-3, 7 (in-part) and 8 (in-part) encompass this first named invention and thus these claims will be searched without fee to the extent that they encompass SEQ ID NO: 10, limited to wherein residue 5 is E, residue 8 is L, residue 9 is T, residue 40 is R, residue 41 is H, residue 52 is R, residue 54 is P, residue 61 is V, residue 86 is T, residue 87 is P and residue 105 is L (multimerization domain sequence). Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched/examined. An exemplary election would be a multimerization domain encompassing SEQ ID NO: 14 (multimerization domain sequence). (It should be noted that the sequences of Claims 1 and 2 will be searched as a part of the first embodiment of Groups I+).

No technical features are shared between the multimerization domain sequences of Groups I+ and, accordingly, these groups lack unity a priori.