

MUMPS AND MEASLES VIRUS IMMUNOGENS AND THEIR USE

CROSS-REFERENCE TO RELATED APPLICATION

This application claims the benefit of the earlier filing date of U.S. Provisional Application No. 62/946,902, filed December 11, 2019, which is incorporated herein by reference in its entirety.

FIELD

This disclosure relates to polypeptides, polynucleotides, compositions, and methods of their use, for elicitation and detection of an immune response to mumps virus (MuV) and measles virus (MeV).

BACKGROUND

MeV and MuV are highly contagious paramyxoviruses that can be transmitted by respiratory droplets from or direct contact with an infected person. The resulting diseases can lead to serious complications or death among children. The existing vaccines for MeV and MuV are live attenuated virus, which are administered in two subcutaneous doses at 1 year of age and as early as one month later. Two doses of a combination measles, mumps and rubella vaccine are 97% effective against measles and 88% effective against mumps. A single dose of a combination measles, mumps and rubella vaccine is 93% effective against measles and 78% effective against mumps.

Despite the effectiveness of the current licensed vaccines against MeV and MuV, incidences of both have increased in recent years. Contributing factors include reduced vaccination rates due to vaccine hesitancy and circulation of divergent strains against which the licensed MMR vaccine offers limited protection.

In the case of MuV, recent studies have shown that immunity wanes significantly after the second MMR vaccination which normally occurs in childhood. Additionally, there has been a drifting of genotypes in the current circulating MuV strains away from the Jeryl-Lynn strain in the standard Mumps vaccine. In response to recent recurring MuV disease outbreaks in the U.S. and Europe, the Advisory Committee on Immunization Practices is advising a third MMR vaccination to boost protection. However, existing immunity neutralizes a third MMR vaccination limiting its effectiveness.

SUMMARY

Disclosed herein are recombinant MuV F ectodomain trimers and recombinant MeV F ectodomain trimers comprising protomers comprising one or more modifications (such as amino acid substitutions) for stabilization in a prefusion conformation. Further provided are embodiments of the recombinant MuV F ectodomain trimers and recombinant MeV F ectodomain trimers linked to MuV HN ectodomain or a MeV H ectodomain. Chimeric proteins are provided, that include combinations of MuV F ectodomain trimer and MeV H ectodomain, or MeV F ectodomain trimer and MuV HN ectodomain. Embodiments of such proteins

are demonstrated to produce a superior immune response in animal models, and can be used, for example, to elicit or boost an immune response to MeV and/or MuV in a subject.

In some embodiments, the immunogen comprises a recombinant MuV F ectodomain trimer stabilized in a prefusion conformation by one or more amino acid substitutions in protomers of the trimer, the amino acid substitutions comprising cysteine substitutions that form a non-natural disulfide bond to stabilize the MuV F ectodomain trimer in the prefusion conformation. In some embodiments, the recombinant MuV F ectodomain trimer is stabilized in the prefusion conformation by a non-natural disulfide bond between the cysteine substitutions in protomers of the trimer at MuV F positions 206 and 223. In some embodiments, the protomers of the trimer further comprise a mutation to remove a F1/F2 furin cleavage site of the MuV F ectodomain. In some embodiments, the protomers of the recombinant MuV F ectodomain trimer are fused C-terminally to a trimerization domain, such as a GCN4 trimerization domain. In additional embodiments, the protomers of the recombinant MuV F ectodomain trimer are linked to a heterologous protein, such as a MuV HN ectodomain or a MeV H ectodomain.

In some embodiments, the immunogen comprises a recombinant MeV F ectodomain trimer stabilized in a prefusion conformation by one or more amino acid substitutions in protomers of the trimer, the amino acid substitutions comprising cysteine substitutions that form a non-natural disulfide bond to stabilize the MeV F ectodomain trimer in the prefusion conformation. In some embodiments, the recombinant MeV F ectodomain trimer is stabilized in the prefusion conformation by a non-natural disulfide bond between the cysteine substitutions in protomers of the trimer at MeV F positions 165 and 171. In some embodiments, the protomers of the trimer further comprise a mutation to remove a F1/F2 furin cleavage site of the MeV F ectodomain. In some embodiments, the protomers of the recombinant MeV F ectodomain trimer are fused C-terminally to a trimerization domain, such as a GCN4 trimerization domain. In additional embodiments, the protomers of the recombinant MeV F ectodomain trimer are linked to a heterologous protein, such as a MuV HN ectodomain or a MeV H ectodomain.

In some embodiments, an immunogen is provided that comprises a trimer of fusion proteins, each fusion protein comprising, in an N- to C-terminal direction: a trimerization domain and one or more MuV HN ectodomains or MeV H ectodomain.

In some embodiments, the immunogen comprises a dimer of a MeV H ectodomain head.

Nucleic acid molecules encoding the disclosed proteins are also provided, as are vectors including the nucleic acid molecules, and methods of their production.

Immunogenic compositions including a disclosed immunogen that are suitable for administration to a subject are also provided, and may also be contained in a unit dosage form. The immunogen may also contain a carrier to facilitate presentation to the immune system.

Methods of inducing an immune response in a subject are disclosed, as are methods of inhibiting or preventing a MuV or MeV infection in a subject, by administering to the subject an effective amount of a disclosed immunogen, nucleic acid molecule, or vector.

The foregoing and other features and advantages of this disclosure will become more apparent from the following detailed description of several embodiments which proceeds with reference to the accompanying figures.

5

BRIEF DESCRIPTION OF THE FIGURES

FIGs. 1A-1E. Structure-based design of a disulfide stabilized prefusion mumps F trimer. (FIG. 1A) Structure-based design of prefusion mumps F glycoprotein trimer by systematic screening of disulfides and GCN4 attachment positions, showing both yield and percentage of prefusion relative to postfusion F conformation as determined by negative-stain EM. (FIG. 1B) Homogenous prefusion F trimers were observed at high yield with combination of V206C/A223C with 476-GCN4, upper panel and postfusion is shown in the lower panel. (FIG. 1C) S200 gel filtration analysis shows monodispersal of prefusion F (PreF), glycosylated and deglycosylated, HN and prefusion F-HN. (FIG. 1D) Crystal structure to 2.16 Å resolution of prefusion mumps F trimer (SEQ ID NO: 11) with protomers shown, where residues that undergo >5 Å conformational change to transition to the postfusion conformation shown in black. GCN4 trimerization (TD) motif is shown in dotted line, connected to F residue 476. Zoom insets highlight mutated residues to stabilize the trimeric prefusion F structure. (FIG. 1E) A single protomer of the prefusion mumps trimer showing the D1-3 subregions and the locations of the six N-linked glycans per protomer.

FIGs. 2A-2H. Structural comparison of mumps and PIV5 prefusion Fs and analysis of mumps F and HN genotypic variation. (FIG. 2A) Structural superimposition of mumps prefusion F trimer and the related paramyxovirus PIV5 prefusion F trimer showing similar overall topology (RMSD = 1.68 Å), yet share 49% sequence identity. (FIG. 2B) The apical loops of the mumps preF associate in a 'closed cap' assembly, whereas in PIV5, these loops are splayed apart (top panel) and the residues that stabilize the mumps preF apex are T178, T179 and N181 (lower panel). (FIG. 2C) The mumps F glycoprotein is glycosylated with 6 glycans per protomer totaling 18 per trimer with 6 glycans in the conformationally mobile region (black) and 12 glycans in the less conformationally variable region, providing substantial glycan shielding to the trimer. (FIG. 2D) Phylogenetic analysis of fusion glycoproteins from mumps genotypes A-J. (FIG. 2E) Phylogenetic analysis of hemagglutinin-neuraminidase glycoproteins from mumps genotypes A-J. (FIG. 2F and 2G) Sequence alignment of Jeryl Lynn vaccine strain (genotype A, SEQ ID NO: 98) with fusion glycoproteins from mumps genotypes C (SEQ ID NO: 102), D (SEQ ID NO: 103), F (SEQ ID NO: 104), G (SEQ ID NO: 105), and H (SEQ ID NO: 106). (FIG. 2H) Structural mapping of mumps genotype variations on prefusion F (left panels) and HN (right panels), showing A-J genotype variation (top panels) and genotype G versus A (Jeryl Lynn (JL)) (lower panels), showing residue differences and glycan differences.

FIGs. 3A and 3B. Design and immunization scheme for mumps F and preF-HN immunogen variants. (FIG. 3A) Immunization in CB6F1/J mice was performed 3 times at 10 µg/dose with Poly(I:C) adjuvant at times 0, 3 and 10 weeks with blood sampling at 2, 5, 12 and 16 weeks. (FIG. 3B) Negative-stain characterization of mumps F and preF-HN proteins confirming the conformational and molecular identity of

the immunogens and mumps neutralization titers for postfusion F (left), prefusion F (center), and prefusion F-HN (right) immunogens elicited after 1, 2 or 3 immunizations in mice (shown in arrows), and PRN titers for genotype G virus, Jeryl Lynn genotype A virus, and genotype H virus.

FIGs. 4A-4E. Design of prefusion mumps F-HN immunogen, analysis of sera for specificity and durability of neutralization titers for three diverse mumps genotype viruses. (FIG. 4A) Negative-stain characterization of mumps preF-HN fusion protein, showing positions of preF head, GCN4 trimerization domain and three HN headgroups. (FIG. 4B) Binding titers for immunized mice using the mumps prefusion F probe and (FIG. 4C) HN probe using Octet Bioinferometry. (FIG. 4D) Durability analysis of mice immunized with three doses of 10µg prefusion F protein with Poly(I:C) with monthly serum sampling and PRNT analyses for six months against genotype G virus, Jeryl Lynn virus, and genotype H virus. (Fig. 4E) Durability analysis of mice immunized with three doses of 10µg prefusion F-HN protein with Poly(I:C) with monthly serum sampling and PRNT analyses for six months against genotype G virus, Jeryl Lynn virus and genotype H virus.

FIGs. 5A-5C. Structure-Based Design of prefusion Measles F. (FIG. 5A) Structure-based design of prefusion-stabilized measles F glycoprotein mutations that stabilize the prefusion conformation. (FIG. 5B) Negative stain EM analysis of measles F variants including the indicated non-native disulfide bond and a C-terminal GCN4 trimerization domain, and (FIG. 5C) Immunogenicity of prefusion (MeV F R165C/M171C-486-GCN4, SEQ ID NO: 38) and postfusion (native MeV F sequence with -486-GCN4) measles F glycoproteins.

FIGs. 6A-6C. Design of mumps pre-F-measles H chimeric immunogen. (FIG. 6A) Negative-stain characterization of measles H dimer (upper left), measles H trimer (upper middle), mumps HN trimer (upper right), and mumps pre-F with measles H (MuV F 206C/223C-476 + GCN4/Fd + MeV-H, SEQ ID NO: 28) (lower). (FIG. 6B) Measles plaque reduction neutralization titer assays (PRNT). (FIG. 6C) Mumps (genotype G) PRNT assays.

SEQUENCE LISTING

The nucleic and amino acid sequences listed in the accompanying sequence listing are shown using standard letter abbreviations for nucleotide bases, and three letter code for amino acids, as defined in 37 C.F.R. 1.822. Only one strand of each nucleic acid sequence is shown, but the complementary strand is understood as included by any reference to the displayed strand. The Sequence Listing is submitted as an ASCII text file in the form of the file named "Sequence.txt" (~456 kb), which was created on December 11, 2020, which is incorporated by reference herein.

STRUCTURAL COORDINATES

The atomic coordinates of the crystal structure of MuV F ectodomain trimer stabilized in a prefusion conformation are recited in Table 1 of U.S. Provisional Application No. 62/946,902, filed December 11,

2019, which is incorporated by reference herein in its entirety, and which is submitted therein as an ASCII text file in the form of the file named "Table_1.txt" (~555 KB), which was created on December 9, 2019.

DETAILED DESCRIPTION

5 I. Summary of Terms

Unless otherwise noted, technical terms are used according to conventional usage. Definitions of common terms in molecular biology may be found in Benjamin Lewin, *Genes X*, published by Jones & Bartlett Publishers, 2009; and Meyers *et al.* (eds.), *The Encyclopedia of Cell Biology and Molecular Medicine*, published by Wiley-VCH in 16 volumes, 2008; and other similar references. As used herein, the singular forms "a," "an," and "the," refer to both the singular as well as plural, unless the context indicates otherwise. For example, the term "an antigen" includes single or plural antigens and can be considered equivalent to the phrase "at least one antigen." As used herein, the term "comprises" means "includes." It is further to be understood that any and all base sizes or amino acid sizes, and all molecular weight or molecular mass values, given for nucleic acids or polypeptides are approximate, and are provided for descriptive purposes, unless otherwise indicated. Although many methods and materials similar or equivalent to those described herein can be used, particular suitable methods and materials are described below. In case of conflict, the present specification, including explanations of terms, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting. To facilitate review of the various embodiments, the following explanations of terms are provided:

20 **Adjuvant:** A vehicle used to enhance antigenicity. In some embodiments, an adjuvant includes a suspension of minerals (alum, aluminum hydroxide, or phosphate) on which antigen is adsorbed; or water-in-oil emulsion, for example, in which antigen solution is emulsified in mineral oil (Freund incomplete adjuvant), sometimes with the inclusion of killed mycobacteria (Freund's complete adjuvant) to further enhance antigenicity (inhibits degradation of antigen and/or causes influx of macrophages). In some
25 embodiments, the adjuvant used in a disclosed immunogenic composition is a combination of lecithin and carbomer homopolymer (such as the ADJUPLEX™ adjuvant available from Advanced BioAdjuvants, LLC, see also Wegmann, Clin Vaccine Immunol, 22(9): 1004-1012, 2015). Additional adjuvants for use in the disclosed immunogenic compositions include the QS21 purified plant extract, Matrix M, AS01, MF59, and ALFQ adjuvants. Immunostimulatory oligonucleotides (such as those including a CpG motif) can also be
30 used as adjuvants. Adjuvants include biological molecules (a "biological adjuvant"), such as costimulatory molecules. Exemplary adjuvants include IL-2, RANTES, GM-CSF, TNF- α , IFN- γ , G-CSF, LFA-3, CD72, B7-1, B7-2, OX-40L, 4-1BBL, immune stimulating complex (ISCOM) matrix, and toll-like receptor (TLR) agonists, such as TLR-9 agonists, Poly I:C, or PolyICLC. (See, *e.g.*, Singh (ed.) Vaccine Adjuvants and Delivery Systems. Wiley-Interscience, 2007).

35 **Administration:** The introduction of a composition into a subject by a chosen route. Administration can be local or systemic. For example, if the chosen route is intranasal, the composition (such as a composition including a disclosed recombinant MuV F ectodomain trimer or recombinant MeV F

ectodomain trimer) is administered by introducing the composition into the nasal passages of the subject. Exemplary routes of administration include, but are not limited to, oral, injection (such as subcutaneous, intramuscular, intradermal, intraperitoneal, and intravenous), sublingual, rectal, transdermal (for example, topical), intranasal, vaginal, and inhalation routes.

5 **Amino acid substitution:** The replacement of an amino acid in a polypeptide with one or more different amino acids. In the context of a protein sequence, an amino acid substitution is also referred to as a mutation.

Antibody: An immunoglobulin, antigen-binding fragment, or derivative thereof, that specifically binds and recognizes an analyte (antigen) such as MuV or MeV F protein, an antigenic fragment thereof, or a dimer or multimer of the antigen. The term “antibody” is used herein in the broadest sense and encompasses various antibody structures, including but not limited to monoclonal antibodies, polyclonal antibodies, multispecific antibodies (*e.g.*, bispecific antibodies), and antibody fragments, so long as they exhibit the desired antigen-binding activity. Non-limiting examples of antibodies include, for example, intact immunoglobulins and variants and fragments thereof that retain binding affinity for the antigen. Examples of antibody fragments include but are not limited to Fv, Fab, Fab', Fab'-SH, F(ab')₂; diabodies; linear antibodies; single-chain antibody molecules (*e.g.* scFv); and multispecific antibodies formed from antibody fragments. Antibody fragments include antigen binding fragments either produced by the modification of whole antibodies or those synthesized *de novo* using recombinant DNA methodologies (see, *e.g.*, Kontermann and Dubel (Ed), *Antibody Engineering*, Vols. 1-2, 2nd Ed., Springer Press, 2010).

20 **Carrier:** An immunogenic molecule to which an antigen can be linked. When linked to a carrier, the antigen may become more immunogenic. Carriers are chosen to increase the immunogenicity of the antigen and/or to elicit antibodies against the carrier which are diagnostically, analytically, and/or therapeutically beneficial. Useful carriers include polymeric carriers, which can be natural (for example, proteins from bacteria or viruses), semi-synthetic or synthetic materials containing one or more functional groups to which a reactant moiety can be attached.

25 **Conservative variants:** “Conservative” amino acid substitutions are those substitutions that do not substantially affect or decrease a function of a protein, such as the ability of the protein to induce an immune response when administered to a subject. The term conservative variation also includes the use of a substituted amino acid in place of an unsubstituted parent amino acid. Furthermore, individual substitutions, deletions or additions which alter, add or delete a single amino acid or a small percentage of amino acids (for instance less than 5%, in some embodiments less than 1%) in an encoded sequence are conservative variations where the alterations result in the substitution of an amino acid with a chemically similar amino acid.

30 The following six groups are examples of amino acids that are considered to be conservative substitutions for one another:

- 1) Alanine (A), Serine (S), Threonine (T);
- 2) Aspartic acid (D), Glutamic acid (E);

- 3) Asparagine (N), Glutamine (Q);
- 4) Arginine (R), Lysine (K);
- 5) Isoleucine (I), Leucine (L), Methionine (M), Valine (V); and
- 6) Phenylalanine (F), Tyrosine (Y), Tryptophan (W).

5 Non-conservative substitutions are those that reduce an activity or function of the recombinant MuV or MeV F ectodomain trimer, such as the ability to induce an immune response when administered to a subject. For instance, if an amino acid residue is essential for a function of the protein, even an otherwise conservative substitution may disrupt that activity. Thus, a conservative substitution does not alter the basic function of a protein of interest.

10 **Control:** A reference standard. In some embodiments, the control is a negative control sample obtained from a healthy patient. In other embodiments, the control is a positive control sample obtained from a patient diagnosed with MuV or MeV infection. In still other embodiments, the control is a historical control or standard reference value or range of values (such as a previously tested control sample, such as a group of MuV or MeV patients with known prognosis or outcome, or group of samples that represent

15 baseline or normal values).

A difference between a test sample and a control can be an increase or conversely a decrease. The difference can be a qualitative difference or a quantitative difference, for example a statistically significant difference. In some examples, a difference is an increase or decrease, relative to a control, of at least about 5%, such as at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about

20 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 100%, at least about 150%, at least about 200%, at least about 250%, at least about 300%, at least about 350%, at least about 400%, at least about 500%, or greater than 500%.

Degenerate variant: In the context of the present disclosure, a “degenerate variant” refers to a polynucleotide encoding a polypeptide that includes a sequence that is degenerate as a result of the genetic

25 code. There are 20 natural amino acids, most of which are specified by more than one codon. Therefore, all degenerate nucleotide sequences encoding a peptide are included as long as the amino acid sequence of the peptide encoded by the nucleotide sequence is unchanged.

Effective amount: An amount of agent, such as an immunogen, that is sufficient to elicit a desired response, such as an immune response in a subject. It is understood that to obtain a protective immune

30 response against an antigen of interest can require multiple administrations of a disclosed immunogen, and/or administration of a disclosed immunogen as the “prime” in a prime boost protocol wherein the boost immunogen can be different from the prime immunogen. Accordingly, an effective amount of a disclosed immunogen can be the amount of the immunogen sufficient to elicit a priming immune response in a subject that can be subsequently boosted with the same or a different immunogen to elicit a protective immune

35 response.

In one example, a desired response is to inhibit or reduce or prevent MuV infection. The MuV infection does not need to be completely eliminated or reduced or prevented for the method to be effective.

For example, administration of an effective amount of the agent can decrease the MuV infection (for example, as measured by infection of cells, or by number or percentage of subjects infected by MuV) by a desired amount, for example by at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, or even at least 100% (elimination or prevention of detectable MuV infection), as

5 compared to a suitable control.

In one example, a desired response is to inhibit or reduce or prevent MeV infection. The MeV infection does not need to be completely eliminated or reduced or prevented for the method to be effective. For example, administration of an effective amount of the agent can decrease the MeV infection (for example, as measured by infection of cells, or by number or percentage of subjects infected by MeV) by a
10 desired amount, for example by at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, or even at least 100% (elimination or prevention of detectable MeV infection), as compared to a suitable control.

In one example, a desired response is to inhibit or reduce or prevent both MuV and MeV infection. The MuV and MeV infections do not need to be completely eliminated or reduced or prevented for the
15 method to be effective. For example, administration of an effective amount of the agent can decrease the MuV and MeV infections (for example, as measured by infection of cells, or by number or percentage of subjects infected by MuV and/or MeV) by a desired amount, for example by at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, or even at least 100% (elimination or prevention of detectable MuV and/or MeV infection), as compared to a suitable control.

20 **Expression:** Transcription or translation of a nucleic acid sequence. For example, a gene is expressed when its DNA is transcribed into an RNA or RNA fragment, which in some examples is processed to become mRNA. A gene may also be expressed when its mRNA is translated into an amino acid sequence, such as a protein or a protein fragment. In a particular example, a heterologous gene is expressed when it is transcribed into an RNA. In another example, a heterologous gene is expressed when
25 its RNA is translated into an amino acid sequence. The term “expression” is used herein to denote either transcription or translation. Regulation of expression can include controls on transcription, translation, RNA transport and processing, degradation of intermediary molecules such as mRNA, or through activation, inactivation, compartmentalization or degradation of specific protein molecules after they are produced.

Expression Control Sequences: Nucleic acid sequences that regulate the expression of a
30 heterologous nucleic acid sequence to which it is operatively linked. Expression control sequences are operatively linked to a nucleic acid sequence when the expression control sequences control and regulate the transcription and, as appropriate, translation of the nucleic acid sequence. Thus expression control sequences can include appropriate promoters, enhancers, transcription terminators, a start codon (ATG) in front of a protein-encoding gene, splicing signal for introns, maintenance of the correct reading frame of that
35 gene to permit proper translation of mRNA, and stop codons. The term “control sequences” is intended to include, at a minimum, components whose presence can influence expression, and can also include

additional components whose presence is advantageous, for example, leader sequences and fusion partner sequences. Expression control sequences can include a promoter.

A promoter is a minimal sequence sufficient to direct transcription. Also included are those promoter elements which are sufficient to render promoter-dependent gene expression controllable for cell-type specific, tissue-specific, or inducible by external signals or agents; such elements may be located in the 5' or 3' regions of the gene. Both constitutive and inducible promoters are included (see for example, Bitter *et al.*, *Methods in Enzymology* 153:516-544, 1987). For example, when cloning in bacterial systems, inducible promoters such as pL of bacteriophage lambda, plac, ptrp, ptac (ptrp-lac hybrid promoter) and the like may be used. In one embodiment, when cloning in mammalian cell systems, promoters derived from the genome of mammalian cells (such as metallothionein promoter) or from mammalian viruses (such as the retrovirus long terminal repeat; the adenovirus late promoter; the vaccinia virus 7.5K promoter) can be used. Promoters produced by recombinant DNA or synthetic techniques may also be used to provide for transcription of the nucleic acid sequences.

Expression vector: A vector comprising a recombinant polynucleotide comprising expression control sequences operatively linked to a nucleotide sequence to be expressed. An expression vector comprises sufficient cis- acting elements for expression; other elements for expression can be supplied by the host cell or in an in vitro expression system. Expression vectors include all those known in the art, such as cosmids, plasmids (*e.g.*, naked or contained in liposomes) and viruses (*e.g.*, lentiviruses, retroviruses, adenoviruses, and adeno-associated viruses) that incorporate the recombinant polynucleotide.

GCN4 trimerization domain: A trimerization domain from the GCN4 protein that comprises a leucine zipper amino acid sequence that naturally forms a trimeric structure. Embodiments of the GCN4 trimerization domain is described, for example, Harbury *et al.* (1993 *Science* 262:1401-1407). In some examples, a GCN4 trimerization domain can be included in the amino acid sequence of a disclosed recombinant protein so that the recombinant protein will trimerize. A non-limiting example of a GCN4 trimerization domain sequence for use with the disclosed embodiments is provided as IEDKIEEILSKIYHIENEIARIKKLIGEAP (SEQ ID NO: 33).

Heterologous: Originating from a different genetic source.

Host cells: Cells in which a vector can be propagated and its nucleic acid expressed. The cell may be prokaryotic or eukaryotic. The term also includes any progeny of the subject host cell. It is understood that all progeny may not be identical to the parental cell since there may be mutations that occur during replication. However, such progeny are included when the term "host cell" is used.

Immune response: A response of a cell of the immune system, such as a B cell, T cell, or monocyte, to a stimulus. In one embodiment, the response is specific for a particular antigen (an "antigen-specific response"). In one embodiment, an immune response is a T cell response, such as a CD4+ response or a CD8+ response. In another embodiment, the response is a B cell response, and results in the production of specific antibodies.

Immunogen: A compound, composition, or substance (for example, a recombinant MuV or MeV F ectodomain trimer) that can elicit an immune response in an animal, including compositions that are injected or absorbed into an animal. Administration of an immunogen to a subject can lead to protective immunity against a pathogen of interest.

- 5 **Immunogenic composition:** A composition comprising a disclosed immunogen that induces a measurable CTL response against MuV or MeV, or induces a measurable B cell response (such as production of antibodies) against the MuV or MeV, when administered to a subject. It further refers to isolated nucleic acid molecules and vectors encoding a protomer of a disclosed recombinant MuV or MeV F ectodomain trimer that can be used to express the protomer (and thus be used to elicit an immune response
- 10 against recombinant MuV or MeV F ectodomain trimer). For *in vivo* use, the immunogenic composition will typically include the recombinant MuV or MeV F ectodomain trimer or a nucleic acid molecule encoding a protomer of the recombinant MuV or MeV F ectodomain trimer in a pharmaceutically acceptable carrier and may also include other agents, such as an adjuvant.

- Inhibiting or treating a disease:** Inhibiting the full development of a disease or condition, for
- 15 example, in a subject who is at risk for a disease such as MuV infection or MeV infection. “Treatment” refers to a therapeutic intervention that ameliorates a sign or symptom of a disease or pathological condition after it has begun to develop. The term “ameliorating,” with reference to a disease or pathological condition, refers to any observable beneficial effect of the treatment. Inhibiting a disease can include preventing or reducing the risk of the disease, such as preventing or reducing the risk of viral infection. The beneficial
- 20 effect can be evidenced, for example, by a delayed onset of clinical symptoms of the disease in a susceptible subject, a reduction in severity of some or all clinical symptoms of the disease, a slower progression of the disease, a reduction in the viral load, an improvement in the overall health or well-being of the subject, or by other parameters that are specific to the particular disease. A “prophylactic” treatment is a treatment administered to a subject who does not exhibit signs of a disease or exhibits only early signs for the purpose
- 25 of decreasing the risk of developing pathology.

- Isolated:** An “isolated” biological component has been substantially separated or purified away from other biological components, such as other biological components in which the component naturally occurs, such as other chromosomal and extrachromosomal DNA, RNA, and proteins. Proteins, peptides, nucleic acids, and viruses that have been “isolated” include those purified by standard purification methods.
- 30 Isolated does not require absolute purity, and can include protein, peptide, nucleic acid, or virus molecules that are at least 50% isolated, such as at least 75%, 80%, 90%, 95%, 98%, 99%, or even 99.9% isolated.

- Linker and Linked:** A bi-functional molecule that can be used to link two molecules into one contiguous molecule. Non-limiting examples of peptide linkers include glycine-serine peptide linkers. Unless context indicates otherwise, reference to “linking” a first polypeptide and a second polypeptide, or to
- 35 two polypeptides “linked” together, or to a first polypeptide having a “linkage” to a second polypeptide, refers to covalent linkage by peptide bond (for example via a peptide linker) such that the first and second polypeptides form a contiguous polypeptide chain. If a peptide linker is involved, the covalent linkage of

the first and second polypeptides can be to the N- and C-termini of the peptide linker. Typically, such linkage is accomplished using molecular biology techniques to genetically manipulate DNA encoding the first polypeptide linked to the second polypeptide by the peptide linker.

Native protein, sequence, or disulfide bond: A polypeptide, sequence or disulfide bond that has not been modified, for example, by selective mutation. For example, selective mutation to focus the antigenicity of the antigen to a target epitope, or to introduce a disulfide bond into a protein that does not occur in the native protein. Native protein or native sequence are also referred to as wild-type protein or wild-type sequence. A non-native disulfide bond is a disulfide bond that is not present in a native protein, for example, a disulfide bond that forms in a protein due to introduction of one or more cysteine residues into the protein by genetic engineering.

Measles: An infectious disease caused by measles virus. Symptoms usually develop 10–12 days after exposure to an infected person and last 7–10 days. Initial symptoms typically include fever, cough, runny nose, and inflamed eyes. Small white spots known as Koplik's spots may form inside the mouth two or three days after the start of symptoms. A red, flat rash which usually starts on the face and then spreads to the rest of the body typically begins three to five days after the start of symptoms. Common complications include diarrhea, middle ear infection, and pneumonia. These occur in part due to measles-induced immunosuppression. Less commonly seizures, blindness, or inflammation of the brain may occur.

Measles virus: A non-segmented, negative-stranded RNA virus of the family *Paramyxoviridae*, genus *Morbillivirus* that causes measles disease. Measles virus genomic RNA contains 6 linked transcription units that encode open reading frames for eight proteins: the nucleoprotein (N), phosphoprotein (P), C protein, V protein, matrix (M) protein, fusion (F) protein, hemagglutinin (H) protein, and the large (L) protein. There are at least 7 known genotypes of MeV, designated as genotypes A, B, C, D, F, G, and H, that are currently circulating globally.

MeV fusion (F) protein: An envelope glycoprotein of MeV that facilitates fusion of viral and cellular membranes. In nature, the F protein from MeV is initially synthesized as a single polypeptide precursor approximately 550 amino acids in length, designated F₀. F₀ includes an N-terminal signal peptide that directs localization to the endoplasmic reticulum, where the signal peptide is proteolytically cleaved. The remaining F₀ residues oligomerize to form a trimer and may be proteolytically processed by a cellular protease to generate two disulfide-linked fragments, F₁ and F₂. In MeV F the cleavage site is located approximately between residues 113/114. The smaller of these fragments, F₂, originates from the N-terminal portion of the F₀ precursor (approximately residues 24-113). The larger of these fragments, F₁, includes the C-terminal portion of the F₀ precursor (approximately residues 114-550) including an extracellular/luminal region (approximately residues 110-486), and a transmembrane and cytosolic regions (approximately residues 487-550). The extracellular portion of the MeV F protein is the MeV F ectodomain, which includes the F₂ protein and the F₁ ectodomain.

The MeV F protein exhibits remarkable sequence conservation within MeV strains. In view of this conservation, the person of ordinary skill in the art can easily compare amino acid positions of different

MeV F proteins. Unless context indicates otherwise, the numbering of MeV F amino acids is made with reference to SEQ ID NO: 36 (NCBI Reference Sequence P35973.1, which is incorporated by reference herein):

5 mglkvnvsaifmavllltlqtptgQIHWNLSKIGVVGIGSASYKVMTRSSHQSLVIKLPNITLLNNCTRVEIAEYRRL
RTVLEPIRDALNMTQNIQSVASSRRHKRFAGVVLGAAALGVATAAQITAGIALHQSMNLNSQAIQDNLRSLETTNQA
IEAIRQAGQEMILAVQGVQDYINNELIPSMNQLSCDLIGQKLGLKLLRYTEILSLFGPSLRDPISAEISIQALSALGG
DINKVLEKLGYSGGDLLGILESRIKARITHVDTESYLIVLSIAYPTLSEIKGVIVHRLEGVSYNIGSQEWYTTVPKYVA
TQGYLISNFDSECTFMPEGTVCSQNALYPMSPLLQECLRGSTKSCARTLVSGSFGNRFILSQGNLIANCASILCKCYTT
10 GTIINQDPDKILTYIAADHCPVVEVNGVTIQVGSRRYPDAVYLHRIDLGPPIILLERLDVGTNLGNIAKLEDAKELLE
DQILRSMKGLSSTCIVYILIAVCLGGLIGIPALICCCGRRCNKKGEQVGMSPGLKPDLTGTSKSYVRSL

Three MeV F protomers oligomerize in the mature F protein, which adopts a metastable **prefusion conformation** that is triggered to undergo a conformational change to a postfusion conformation upon contact with a target cell membrane. This conformational change exposes a hydrophobic sequence, known
15 as the fusion peptide, which is located at the N-terminus of the F₁ ectodomain, and which associates with the host cell membrane and promotes fusion of the membrane of the virus, or an infected cell, with the target cell membrane.

An MeV F ectodomain trimer “stabilized in a prefusion conformation” comprises one or more amino acid substitutions, deletions, or insertions compared to a corresponding native MeV F sequence that
20 provide for increased retention of the prefusion conformation compared to MeV F ectodomain trimers formed from a corresponding native MeV F sequence. The “stabilization” of the prefusion conformation can be, for example, energetic stabilization (for example, reducing the energy of the prefusion conformation relative to the postfusion open conformation) and/or kinetic stabilization (for example, reducing the rate of transition from the prefusion conformation to the postfusion conformation). Additionally, stabilization of
25 the MeV F ectodomain trimer in the prefusion conformation can include an increase in resistance to denaturation compared to a corresponding native MeV F sequence. Methods of determining if a MeV F ectodomain trimer is in the prefusion conformation are provided herein, and include (but are not limited to) negative stain electron microscopy and antibody binding assays using a prefusion conformation specific antibody. The term “pre-F” with reference to a MeV F protein describes a molecule that is a trimeric class I
30 fusion protein stabilized in the prefusion conformation by one or more amino acid substitutions.

MeV F prefusion specific antibody: An antibody that specifically binds to the MeV F protein in a prefusion conformation, but does not specifically bind to the MeV F protein in a postfusion conformation.

MeV hemagglutinin (H) protein: An MeV envelope glycoprotein that is a type II membrane protein and facilitates attachment of MeV to host cell membranes. The full-length H protein has an N-
35 terminal cytoplasmic tail and transmembrane domain (CT and TM, approximately amino acids 1-58), and an ectodomain (approximately amino acids 59-617) including stalk (approximately amino acids 59-179) and head (approximately amino acids 180-617) regions. An exemplary MeV H protein sequence is provided herein as SEQ ID NO: 49 (NCBI Reference Sequence AAA56644.1, which is incorporated by reference herein):

40 Mspqdrdrinafykdnphpkgsrvinrehlmidrpvyllavlfvmflsligllaiagirlhraaiytaeihkslstndlv
tnsiehqvkdvltplfkiiigdevglrtptqrftdlvkfisdskikflnppdreydfrdltwcinpperikldydqycadvaee

5 elmnaIvnstllletrttngFLAVSKGNCSGPTTIRGQFSNMSLSLLDLYLGRGYNVSSIVTMTSQGMYGGTYLVEKPNLS
 SKRSELSQLSMYRVFEVGVIRNPGLGAPVFHMTNYLEQPVSNDSLNCMVALGELKLAALCHGEDSITIPYQGSKGVSFQ
 LVKLGWKSPTDMQSWVPLSTDDPVIDRLYLSSHARGVIADNQAKWAVPTTRTDDKLRMETCFQQACKGKIQALCENPEWA
 PLKDNRIIPSYGVLSDLSLTVELKIKIASGFGLITHGSGMDLYKSNHNNVYWLTIPTPMKNLALGVINTLEWIPRFKVSF
 YLFTVPIKEAGEDCHAPTYPALAEVDGDVKLSSNLVILPGQDLQYVLATYDTSRVEHAVVYVYSPSRFSYFYFRLPIK
 GVPIELQVECFDWDQKLWCRHFCVLADSESGGHITHSGMVGMGVSCVTREDGTNRR

As used herein, MeV H residue positioning is made with reference to the sequence of the set forth as SEQ ID NO: 49.

10 **Mumps:** An infectious disease caused by mumps virus. Mumps is characterized by inflammation of the salivary glands, typically the parotid glands. Severe complications of mumps virus infection can occur, such as meningitis, encephalitis, pancreatitis, oophoritis (in females), orchitis (in males) and hearing loss.

Mumps virus (MuV): A non-segmented, negative-stranded RNA virus of the family *Paramyxoviridae*, subfamily *Paramyxovirinae*, genus *Rubulavirus* that causes mumps disease. Mumps virus genomic RNA contains 7 tandemly linked transcription units that encode open reading frames for the nucleoprotein (N), phosphoprotein (P), V protein, I protein, matrix (M) protein, fusion (F) protein, small hydrophobic (SH) protein, hemagglutinin-neuraminidase (HN) protein, and the large (L) protein. A schematic of the mumps virus genome is shown in FIG. 6. Due to RNA editing by insertion of guanine nucleotides, the P gene (also referred to as the “V/P/I gene”) results in three mRNA transcripts corresponding to the V, P and I proteins. Specifically, faithful transcription of the P gene produces the V protein, insertion of two guanine nucleotides produces an mRNA encoding the P protein, and insertion of four guanine residues results in an mRNA encoding the I protein. The SH gene is the most variable gene amongst different genotypes of MuV and is therefore generally used as the basis for genotyping. There are 12 known genotypes of MuV, designated as genotypes A, B, C, D, F, G, H, I, J, K, L and N, that are currently circulating globally. Most current MuV vaccines are based on genotype A (Jeryl Lynn), genotype B (Urabe-AM9) or undetermined genotype (Leningrad-Zagreb) viruses.

MuV fusion (F) protein: An envelope glycoprotein of MuV that facilitates fusion of viral and cellular membranes. In nature, the F protein from MuV is initially synthesized as a single polypeptide precursor approximately 538 amino acids in length, designated F₀. F₀ includes an N-terminal signal peptide that directs localization to the endoplasmic reticulum, where the signal peptide is proteolytically cleaved. The remaining F₀ residues oligomerize to form a trimer and may be proteolytically processed by a cellular protease to generate two disulfide-linked fragments, F₁ and F₂. In MuV F the cleavage site is located approximately between residues 103/104. The smaller of these fragments, F₂, originates from the N-terminal portion of the F₀ precursor (approximately residues 20-103). The larger of these fragments, F₁, includes the C-terminal portion of the F₀ precursor (approximately residues 104-538) including an extracellular/luminal region (approximately residues 110-483), and a transmembrane and cytosolic regions (approximately residues 484-538). The extracellular portion of the MuV F protein is the MuV F ectodomain, which includes the F₂ protein and the F₁ ectodomain.

The MuV F protein exhibits remarkable sequence conservation within MuV strains. In view of this conservation, the person of ordinary skill in the art can easily compare amino acid positions of different MuV F proteins. Unless context indicates otherwise, the numbering of MuV F amino acids is made with reference to SEQ ID NO: 1 (NCBI Reference Sequence P09458.1, which is incorporated by reference

5 herein):

10 mkafsvtclgfvfsssicVNINILQQIGYIKQQVRQLSYYSQSSSYIVVKLLPNIQPTDNSCEFKSVTQYNKTLNLL
LPIAENINNIA SPSPGSRHRKRFAGIAIGIAALGVATAAQVTA AVSLVQAQTNA RAIAAMKNSIQATNRAIFEVKEGTQQ
LAIAVQAIQDHINTIMNTQLNNMSCQILDNQLATYLGlyLTELTTVFQQLINPALSPISIQALRSLGSMTPAVVQATL
STISIAAEILSAGLMEGQIVSVLLDEMOMIVKINIPTIVTQSNALVIDFYSSISFINNQESIQLPDRILEIGNEQWSYP
AKNCKLTRHHIFCQYNEAERLSLESKLCLAGNISACVFSPIAGSYMRRFVALDGTIVANCRLTCLCKSPSYPIYQPDHH
AVTTIDLTTCQTLSDGLDFSIVLSNITYAENLTISLSQTINTQPIDISTELSKVNASLQNAVKYIKESNHQLQSVSVN
SKIGAIIVAALVLSILSIIISLLFCCWAYIATKEIRRINFKTNHINTISSVDDLIRY

15 Three MuV F protomers oligomerize in the mature F protein, which adopts a metastable **prefusion conformation** that is triggered to undergo a conformational change to a postfusion conformation upon contact with a target cell membrane. This conformational change exposes a hydrophobic sequence, known as the fusion peptide, which is located at the N-terminus of the F₁ ectodomain, and which associates with the host cell membrane and promotes fusion of the membrane of the virus, or an infected cell, with the target cell membrane.

20 An MuV F ectodomain trimer “stabilized in a prefusion conformation” comprises one or more amino acid substitutions, deletions, or insertions compared to a corresponding native MuV F sequence that provide for increased retention of the prefusion conformation compared to MuV F ectodomain trimers formed from a corresponding native MuV F sequence. The “stabilization” of the prefusion conformation can be, for example, energetic stabilization (for example, reducing the energy of the prefusion conformation relative to the postfusion open conformation) and/or kinetic stabilization (for example, reducing the rate of transition from the prefusion conformation to the postfusion conformation). Additionally, stabilization of the MuV F ectodomain trimer in the prefusion conformation can include an increase in resistance to denaturation compared to a corresponding native MuV F sequence. Methods of determining if a MuV F ectodomain trimer is in the prefusion conformation are provided herein, and include (but are not limited to)

25 negative stain electron microscopy and antibody binding assays using a prefusion conformation specific antibody. The term “pre-F” with reference to a MuV F protein describes a molecule that is a trimeric class I fusion protein stabilized in the prefusion conformation by one or more amino acid substitutions.

30 **MuV hemagglutinin-neuraminidase (HN) protein:** An MuV envelope glycoprotein that is a type II membrane protein and facilitates attachment of MuV to host cell membranes. The full-length MuV HN protein has an N-terminal cytoplasmic tail and transmembrane domain (CT and TM, approximately amino acids 1-53), and an ectodomain (approximately amino acids 54-582) including stalk (approximately amino acids 54-130) and head regions (approximately amino acids 131-582). An exemplary MuV HN protein sequence is provided herein as SEQ ID NO: 50 (NCBI Reference Sequence AQT03695.1, which is incorporated by reference herein):

40 mepskfftisdsatfapgpvsnaadkktfrtcfirilvlsqvavtlilvivltlgelvrmindgglsnqlssitdkiresat
miasavgvmnqvihgvtvslplqiegnqnqlatlatlaticasqkqvsnstNIPLVNDLRFINGINKFIEDYATHDFSIG

5 HPLNMPSFIPTATSPNGCTRIPSFSLGKTHWCYTHNVINANCKDHTSSNQYVSMGILVQTASGYPMFKTLKIQLSDGLN
 RKSCSIATVPDGCAMCYVSTQLETDDYAGSSPPTQKLTLLFYNDTVTERTISPSGLEGNWATLVPGVSGIYFENKLIF
 PAYGGVLEPNSTLGVKSAREFFRPVNPYNPCSGPQQDLQDALRSYFSPSYFSNRRIQSAFLVCAWNQILVTNCELVVPSSN
 QTMGAEGRVLLINNRLYYQRSTSWWPYELLYEISFTFTNSGPPSSVNMSWIPIYSFTRPGSGNCSGENVCPTACVSGVY
 LDPWPLTPYSHQSGINRNIFYFTGALLNSSTTRVNPTLYVSALNNLKVLAPYGTQGLFASYTTTTCFQDTGDASVYCVYIM
 ELASNIVGEFQILPVLTRLTIT

As used herein, MuV HN residue positioning is made with reference to the sequence of the set forth as SEQ ID NO: 50.

10 **MuV F prefusion specific antibody:** An antibody that specifically binds to the MuV F protein in a prefusion conformation, but does not specifically bind to the MuV F protein in a postfusion conformation.

Nucleic acid molecule: A polymeric form of nucleotides, which may include both sense and anti-sense strands of RNA, cDNA, genomic DNA, and synthetic forms and mixed polymers of the above. A nucleotide refers to a ribonucleotide, deoxynucleotide or a modified form of either type of nucleotide. The term “nucleic acid molecule” as used herein is synonymous with “nucleic acid” and “polynucleotide.” A nucleic acid molecule is usually at least 10 bases in length, unless otherwise specified. The term includes single- and double-stranded forms of DNA. A polynucleotide may include either or both naturally occurring and modified nucleotides linked together by naturally occurring and/or non-naturally occurring nucleotide linkages. “cDNA” refers to a DNA that is complementary or identical to an mRNA, in either single stranded or double stranded form. “Encoding” refers to the inherent property of specific sequences of nucleotides in a polynucleotide, such as a gene, a cDNA, or an mRNA, to serve as templates for synthesis of other polymers and macromolecules in biological processes having either a defined sequence of nucleotides (i.e., rRNA, tRNA and mRNA) or a defined sequence of amino acids and the biological properties resulting therefrom.

25 **Operably linked:** A first nucleic acid sequence is operably linked with a second nucleic acid sequence when the first nucleic acid sequence is placed in a functional relationship with the second nucleic acid sequence. For instance, a promoter is operably linked to a coding sequence if the promoter affects the transcription or expression of the coding sequence. Generally, operably linked nucleic acid sequences are contiguous and, where necessary to join two protein-coding regions, in the same reading frame.

30 **Pharmaceutically acceptable carriers:** The pharmaceutically acceptable carriers of use are conventional. *Remington's Pharmaceutical Sciences*, by E. W. Martin, Mack Publishing Co., Easton, PA, 19th Edition, 1995, describes compositions and formulations suitable for pharmaceutical delivery of the disclosed immunogens.

In general, the nature of the carrier will depend on the particular mode of administration being employed. For instance, parenteral formulations usually comprise injectable fluids that include pharmaceutically and physiologically acceptable fluids such as water, physiological saline, balanced salt solutions, aqueous dextrose, glycerol or the like as a vehicle. For solid compositions (e.g., powder, pill, tablet, or capsule forms), conventional non-toxic solid carriers can include, for example, pharmaceutical grades of mannitol, lactose, starch, or magnesium stearate. In addition to biologically neutral carriers, pharmaceutical compositions (such as immunogenic compositions) to be administered can contain minor

amounts of non-toxic auxiliary substances, such as wetting or emulsifying agents, preservatives, and pH buffering agents and the like, for example sodium acetate or sorbitan monolaurate. In particular embodiments, suitable for administration to a subject the carrier may be sterile, and/or suspended or otherwise contained in a unit dosage form containing one or more measured doses of the composition
5 suitable to induce the desired immune response. It may also be accompanied by medications for its use for treatment purposes. The unit dosage form may be, for example, in a sealed vial that contains sterile contents or a syringe for injection into a subject, or lyophilized for subsequent solubilization and administration or in a solid or controlled release dosage.

Polypeptide: Any chain of amino acids, regardless of length or post-translational modification (*e.g.*,
10 glycosylation or phosphorylation). “Polypeptide” applies to amino acid polymers including naturally occurring amino acid polymers and non-naturally occurring amino acid polymer as well as in which one or more amino acid residue is a non-natural amino acid, for example, an artificial chemical mimetic of a corresponding naturally occurring amino acid. A “residue” refers to an amino acid or amino acid mimetic incorporated in a polypeptide by an amide bond or amide bond mimetic. A polypeptide has an amino
15 terminal (N-terminal) end and a carboxy terminal (C-terminal) end. “Polypeptide” is used interchangeably with peptide or protein, and is used herein to refer to a polymer of amino acid residues.

Prime-boost vaccination: An immunotherapy including administration of a first immunogenic composition (the primer vaccine) followed by administration of a second immunogenic composition (the booster vaccine) to a subject to induce an immune response. The primer vaccine and/or the booster vaccine
20 include a vector (such as a viral vector, RNA, or DNA vector) expressing the antigen to which the immune response is directed. The booster vaccine is administered to the subject after the primer vaccine; a suitable time interval between administration of the primer vaccine and the booster vaccine, and examples of such timeframes are disclosed herein. In some embodiments, the primer vaccine, the booster vaccine, or both primer vaccine and the booster vaccine additionally include an adjuvant. In one non-limiting example, the
25 primer vaccine is a DNA-based vaccine (or other vaccine based on gene delivery), and the booster vaccine is a protein subunit or protein nanoparticle based vaccine.

Protein nanoparticle: A self-assembling, multi-subunit, protein-based polyhedron shaped structure. The subunits are each composed of proteins or polypeptides (for example a glycosylated polypeptide), and, optionally of single or multiple features of the following: nucleic acids, prosthetic groups,
30 organic and inorganic compounds. Non-limiting examples of protein nanoparticles include ferritin nanoparticles (see, *e.g.*, Zhang, *Y. Int. J. Mol. Sci.*, 12:5406-5421, 2011, incorporated by reference herein), encapsulin nanoparticles (see, *e.g.*, Sutter *et al.*, *Nature Struct. and Mol. Biol.*, 15:939-947, 2008, incorporated by reference herein), Sulfur Oxygenase Reductase (SOR) nanoparticles (see, *e.g.*, Urich *et al.*, *Science*, 311:996-1000, 2006, incorporated by reference herein), lumazine synthase nanoparticles (see, *e.g.*,
35 Zhang *et al.*, *J. Mol. Biol.*, 306: 1099-1114, 2001) or pyruvate dehydrogenase nanoparticles (see, *e.g.*, Izard *et al.*, *PNAS* 96: 1240-1245, 1999, incorporated by reference herein). Ferritin, encapsulin, SOR, lumazine synthase, and pyruvate dehydrogenase are monomeric proteins that self-assemble into a globular protein

complexes that in some cases consists of 24, 60, 24, 60, and 60 protein subunits, respectively. In some examples, ferritin, encapsulin, SOR, lumazine synthase, or pyruvate dehydrogenase monomers are linked to a recombinant MuV or MeV F ectodomain and self-assemble into a protein nanoparticle presenting the recombinant MuV or MeV F ectodomain trimer on its surface, which can be administered to a subject to stimulate an immune response to the antigen.

Recombinant: A recombinant nucleic acid molecule is one that has a sequence that is not naturally occurring, for example, includes one or more nucleic acid substitutions, deletions or insertions, and/or has a sequence that is made by an artificial combination of two otherwise separated segments of sequence. This artificial combination can be accomplished by chemical synthesis or, more commonly, by the artificial manipulation of isolated segments of nucleic acids, for example, by genetic engineering techniques.

A recombinant virus is one that includes a genome that includes a recombinant nucleic acid molecule.

A recombinant protein is one that has a sequence that is not naturally occurring or has a sequence that is made by an artificial combination of two otherwise separated segments of sequence. In several embodiments, a recombinant protein is encoded by a heterologous (for example, recombinant) nucleic acid that has been introduced into a host cell, such as a bacterial or eukaryotic cell, or into the genome of a recombinant virus.

Sequence identity: The similarity between amino acid sequences is expressed in terms of the similarity between the sequences, otherwise referred to as sequence identity. Sequence identity is frequently measured in terms of percentage identity; the higher the percentage, the more similar the two sequences are. Homologs, orthologs, or variants of a polypeptide will possess a relatively high degree of sequence identity when aligned using standard methods.

Methods of alignment of sequences for comparison are well known in the art. Various programs and alignment algorithms are described in: Smith & Waterman, *Adv. Appl. Math.* 2:482, 1981; Needleman & Wunsch, *J. Mol. Biol.* 48:443, 1970; Pearson & Lipman, *Proc. Natl. Acad. Sci. USA* 85:2444, 1988; Higgins & Sharp, *Gene*, 73:237-44, 1988; Higgins & Sharp, *CABIOS* 5:151-3, 1989; Corpet *et al.*, *Nuc. Acids Res.* 16:10881-90, 1988; Huang *et al.* *Computer Appls. In the Biosciences* 8, 155-65, 1992; and Pearson *et al.*, *Meth. Mol. Bio.* 24:307-31, 1994. Altschul *et al.*, *J. Mol. Biol.* 215:403-10, 1990, presents a detailed consideration of sequence alignment methods and homology calculations.

Variants of a polypeptide are typically characterized by possession of at least about 75%, for example, at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity counted over the full length alignment with the amino acid sequence of interest. Proteins with even greater similarity to the reference sequences will show increasing percentage identities when assessed by this method, such as at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99% sequence identity. When less than the entire sequence is being compared for sequence identity, homologs and variants will typically possess at least 80% sequence identity over short windows of 10-20 amino acids, and may possess sequence identities of at least 85% or at least 90% or 95% depending on their similarity to

the reference sequence. Methods for determining sequence identity over such short windows are available at the NCBI website on the internet.

As used herein, reference to “at least 90% identity” (or similar language) refers to “at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or even 100% identity” to a specified reference sequence.

Signal Peptide: A short amino acid sequence (*e.g.*, approximately 18-25 amino acids in length) that directs newly synthesized secretory or membrane proteins to and through membranes (for example, the endoplasmic reticulum membrane). Signal peptides are typically located at the N-terminus of a polypeptide and are removed by signal peptidases after the polypeptide has crossed the membrane. Signal peptide sequences typically contain three common structural features: an N-terminal polar basic region (n-region), a hydrophobic core, and a hydrophilic c-region). An exemplary signal peptide sequence is set forth as MKAFSVTCLSFVAVFSSSIC (residues 1-19 of SEQ ID NO: 2).

Specifically bind: When referring to the formation of an antibody:antigen protein complex, or a protein:protein complex, refers to a binding reaction which determines the presence of a target protein, peptide, or polysaccharide (for example, a glycoprotein), in the presence of a heterogeneous population of proteins and other biologics. Thus, under designated conditions, a particular antibody or protein binds preferentially to a particular target protein, peptide or polysaccharide (such as an antigen present on the surface of a pathogen, for example, an antigenic site at the membrane distal apex of the prefusion conformation of the MuV F or MeV F ectodomain timer) and does not bind in a significant amount to other proteins or polysaccharides present in the sample or subject or to alternative conformations of the same protein (for example, the postfusion conformation of the MuV or MeV F proteins). Specific binding can be determined by methods known in the art. A first protein or antibody specifically binds to a target protein when the interaction has a K_D of less than 10^{-6} Molar, such as less than 10^{-7} Molar, less than 10^{-8} Molar, less than 10^{-9} , or even less than 10^{-10} Molar.

Soluble protein: A protein capable of dissolving in aqueous liquid at room temperature and remaining dissolved. The solubility of a protein may change depending on the concentration of the protein in the water-based liquid, the buffering condition of the liquid, the concentration of other solutes in the liquid, for example salt and protein concentrations, and the heat of the liquid. In several embodiments, a soluble protein is one that dissolves to a concentration of at least 0.5 mg/ml in phosphate buffered saline (pH 7.4) at room temperature and remains dissolved for at least 48 hours.

Subject: Living multi-cellular vertebrate organisms, a category that includes human and non-human mammals. In an example, a subject is a human. In a particular example, the subject is a newborn infant. In an additional example, a subject is selected that is in need of inhibiting of a MuV or MeV infection. For example, the subject is either uninfected and at risk of MuV or MeV infection or is infected in need of treatment.

T4 fibrin trimerization domain: Also referred to as a “foldon” domain, the T4 fibrin trimerization domain comprises an amino acid sequence that naturally forms a trimeric structure. In some

examples, a T4 fibrin trimerization domain can be included in the amino acid sequence of a disclosed recombinant protein so that the antigen will form a trimer. In one example, a T4 fibrin trimerization domain comprises the amino acid sequence set forth as GYIPEAPRDGQAYVRKDGEWVLLSTFL (SEQ ID NO: 34). Several embodiments include a T4 fibrin trimerization domain that can be cleaved from a purified protein, for example by incorporation of a thrombin cleave site adjacent to the T4 fibrin trimerization domain that can be used for cleavage purposes.

Transmembrane domain: An amino acid sequence that inserts into a lipid bilayer, such as the lipid bilayer of a cell or virus or virus-like particle. A transmembrane domain can be used to anchor an antigen to a membrane. In some examples a transmembrane domain is a MuV F transmembrane domain. In other examples a transmembrane domain is a MeV F transmembrane domain.

Under conditions sufficient for: A phrase that is used to describe any environment that permits a desired activity.

Vaccine: A preparation of immunogenic material capable of stimulating an immune response, administered for the prevention, amelioration, or treatment of infectious or other types of disease. The immunogenic material may include attenuated or killed microorganisms (such as bacteria or viruses), or antigenic proteins, peptides, or DNA derived from them. A vaccine may include a disclosed immunogen (such as a recombinant MuV or MeV F ectodomain trimer or nucleic acid molecule encoding same), a virus, a cell or one or more cellular constituents. Vaccines may elicit both prophylactic (preventative or protective) and therapeutic responses. Methods of administration vary according to the vaccine, but may include inoculation, ingestion, inhalation or other forms of administration. Vaccines may be administered with an adjuvant to boost the immune response. In one specific, non-limiting example, a vaccine prevents and/or reduces the severity of the symptoms associated with MuV infection and/or decreases the viral load compared to a control. In another specific, non-limiting example, a vaccine prevents and/or reduces the severity of the symptoms associated with MeV infection and/or decreases the viral load compared to a control.

Vector: An entity containing a DNA or RNA molecule bearing a promoter(s) that is operationally linked to the coding sequence of an antigen(s) of interest and can express the coding sequence. Non-limiting examples include a naked or packaged (lipid and/or protein) DNA, a naked or packaged RNA, a subcomponent of a virus or bacterium or other microorganism that may be replication-incompetent, or a virus or bacterium or other microorganism that may be replication-competent. A vector is sometimes referred to as a construct. Recombinant DNA vectors are vectors having recombinant DNA. A vector can include nucleic acid sequences that permit it to replicate in a host cell, such as an origin of replication. A vector can also include one or more selectable marker genes and other genetic elements known in the art. Viral vectors are recombinant nucleic acid vectors having at least some nucleic acid sequences derived from one or more viruses.

Virus-like particle (VLP): A non-replicating, viral shell, derived from any of several viruses. VLPs are generally composed of one or more viral proteins, such as, but not limited to, those proteins

referred to as capsid, coat, shell, surface and/or envelope proteins, or particle-forming polypeptides derived from these proteins. VLPs can form spontaneously upon recombinant expression of the protein in an appropriate expression system. Methods for producing particular VLPs are known in the art. The presence of VLPs following recombinant expression of viral proteins can be detected using conventional techniques known in the art, such as by electron microscopy, biophysical characterization, and the like. Further, VLPs can be isolated by known techniques, *e.g.*, density gradient centrifugation and identified by characteristic density banding. See, for example, Baker *et al.* (1991) *Biophys. J.* 60:1445-1456; and Hagensee *et al.* (1994) *J. Virol.* 68:4503-4505; Vincente, *J Invertebr Pathol.*, 2011; Schneider-Ohrum and Ross, *Curr. Top. Microbiol. Immunol.*, 354: 53073, 2012).

II. Immunogens

A. Recombinant MuV F Ectodomain Trimers

Recombinant MuV F ectodomain trimers are disclosed herein that are modified from a native form (*e.g.*, by introduction of one or more amino acid substitutions) to be stabilized in a prefusion conformation.

As described in the Examples, embodiments of the disclosed MuV F ectodomain trimers have been selected through multiple rounds of structure based design for optimized solubility, stability, expression, and immunogenicity. The recombinant MuV F ectodomain trimers are useful to induce an immune response in a vertebrate animal (such humans) to MuV. Exemplary embodiments are shown to produce a superior immune response in an animal model compared to corresponding MuV F ectodomain trimers that are not stabilized in the prefusion conformation.

In some embodiments, the immunogen comprises a recombinant MuV F ectodomain trimer comprising protomers comprising one or more amino acid substitutions or deletions that stabilize the MuV F ectodomain trimer in the prefusion conformation.

In some embodiments, the immunogen comprises a recombinant MuV F ectodomain trimer stabilized in a prefusion conformation by one or more amino acid substitutions in protomers of the trimer, the amino acid substitutions comprising cysteine substitutions that form a non-natural disulfide bond to stabilize the MuV F ectodomain trimer in the prefusion conformation. A non-natural disulfide bond is one that does not occur in a native MuV F protein, and is introduced by protein engineering (*e.g.*, by including one or more substituted cysteine residues that form the non-natural disulfide bond). For example, in some embodiments, any of the disclosed recombinant MuV F proteins can be stabilized in a prefusion conformation by any one of 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 non-natural disulfide bonds.

The cysteine residues that form the disulfide bond can be introduced into a native MuV F sequence by one or more amino acid substitutions. For example, in some embodiments, a single amino acid substitution introduces a cysteine that forms a disulfide bond with a cysteine residue present in the native MuV F sequence. Alternately, two cysteine residues can be introduced into a native MuV F sequence to form the disulfide bond. The location of the cysteine (or cysteines) of the non-natural disulfide bond can be

determined by the person of ordinary skill in the art using the disclosed structure of the MuV F ectodomain trimer in a prefusion conformation.

The amino acid positions of the cysteines are typically within a sufficiently close distance for formation of a disulfide bond in the prefusion conformation of the MuV F protein trimer. Methods of using three-dimensional structure data (for example, as provided in Table 1) to determine if two residues are within a sufficiently close distance to one another for disulfide bond formation are known (see, e.g., Peterson *et al.*, *Protein engineering*, 12:535-548, 1999 and Dombkowski, *Bioinformatics*, 19:1852-1853, 3002 (disclosing DISULFIDE BY DESIGN™), each of which is incorporated by reference herein). Residues can be selected manually, based on the three dimensional structure of the MuV F trimer in a prefusion conformation provided herein, or a software, such as DISULFIDE BY DESIGN™, can be used. Without being bound by theory, ideal distances for formation of a disulfide bond are generally considered to be about ~5.6 Å for Cα-Cα distance, ~2.02 Å for Sγ-Sγ distance, and 3.5-4.25 Å for Cβ-Cβ distance (using the optimal rotamer). The person of ordinary skill in the art will appreciate that variations from these distances are included when selecting residues in a three dimensional structure that can be substituted for cysteines for introduction of a disulfide bond. For example, in some embodiments the selected residues have a Cα-Cα distance of less than 7.0 Å and/or a Cβ-Cβ distance of less than 4.7 Å. In some embodiments the selected residues have a Cα-Cα distance of from 2.0-8.0 Å and/or a Cβ-Cβ distance of from 2.0-6.0 Å.

In some embodiments, the protomers of the recombinant MuV F ectodomain trimer comprise cysteine substitutions at MuV F positions 86 and 215 (such as N86C and A215C substitutions) that form a non-natural intra-protomer disulfide bond for stabilization in the prefusion conformation.

In some embodiments, the protomers of the recombinant MuV F ectodomain trimer comprise cysteine substitutions at MuV F positions 155 and 161 (such as K155C and L161C substitutions) that form a non-natural intra-protomer disulfide bond for stabilization in the prefusion conformation.

In some embodiments, the protomers of the recombinant MuV F ectodomain trimer comprise cysteine substitutions at MuV F positions 165 and 231 (such as V165C and M231C substitutions) that form a non-natural intra-protomer disulfide bond for stabilization in the prefusion conformation.

In some embodiments, the protomers of the recombinant MuV F ectodomain trimer comprise cysteine substitutions at MuV F positions 206 and 223 (such as V206C and A223C substitutions) that form a non-natural intra-protomer disulfide bond for stabilization in the prefusion conformation.

In some embodiments, the protomers of the recombinant MuV F ectodomain trimer comprise cysteine substitutions at MuV F positions 209 and 214 (such as P209C and P214C substitutions) that form a non-natural intra-protomer disulfide bond for stabilization in the prefusion conformation.

In some embodiments, the protomers of the recombinant MuV F ectodomain trimer comprise cysteine substitutions at MuV F positions 221 and 255 (such as I221C and M255C substitutions) that form a non-natural intra-protomer disulfide bond for stabilization in the prefusion conformation.

Any of the above recombinant MuV F proteins can further comprise modification to eliminate the protease cleavage site between the F1 and F2 polypeptides to generate a “single chain” recombinant F

protein. For example, any of the above recombinant MuV proteins can comprise deletion of MuV F positions 101-103 with positions 100 and 104 fused by a peptide linker. This modification removes the F2/F1 furin cleavage site and also removed the first residue of the fusion peptide (which is hydrophobic). Any suitable peptide linker may be used that fuses the F2 and F1 ectodomain and allows folding of the F
 5 ectodomain into the prefusion conformation. In some embodiments, the peptide linker is a glycine, serine, or glycine-serine peptide linker. In some embodiments, the peptide linker is a Gly-Gly-Gly linker.

In a non-limiting example, a recombinant MuV F ectodomain trimer is provided that includes protomers with V206C and A223C substitutions to form a non-natural disulfide bond and a deletion of MuV F positions 101-103 with positions 100 and 104 fused by a Gly-Gly-Gly peptide linker.

10 In several embodiments, the protomers of the recombinant MuV F ectodomain can comprise one or more additional amino acid substitutions, for example, to increase stabilization of the prefusion conformation, or for other purposes, such as to increase solubility or to reduce and unwanted immune response.

The above-listed non-native disulfide bonds stabilize the membrane-distal portion of the MuV F
 15 ectodomain in its prefusion conformation. Any of these mutations can be combined with modifications to the membrane proximal portion (such as the stem) of the MuV F ectodomain, for example, to increase trimerization of the ectodomain.

In several embodiments, the N-terminal position of the recombinant F₂ polypeptide in the protomer can be one of MuV F positions 20-30 (such as position 20), and the C-terminal position of the F₁ ectodomain
 20 can be from the stem region of the ectodomain, such as one of MuV F positions 469-483 (such as position 476).

In a non-limiting example, a recombinant MuV F ectodomain trimer is provided that includes protomers including MuV positions 20-476 with V206C and A223C substitutions to form a non-natural disulfide bond and a deletion of MuV F positions 101-103 with positions 100 and 104 fused by a Gly-Gly-
 25 Gly peptide linker.

Non-limiting examples of protomers of a MuV F ectodomain trimer including amino acid substitutions for stabilization in the prefusion conformation are provided herein. In some embodiments, the protomers of the MuV F ectodomain trimer comprise an amino acid sequence at least 90% identical to residues 20-483 of any one of SEQ ID NOs: 3-8, residues 20-476 of any one of SEQ ID NOs: 11-16, 26, or
 30 51, or residues 20-469 of any one of SEQ ID NOs: 19-24; wherein the protomers comprise the one or more amino acid substitutions that stabilize the MuV F ectodomain trimer in the prefusion conformation. In some embodiments, the protomers of the MuV F ectodomain trimer comprise residues 20-483 of any one of SEQ ID NOs: 3-8, residues 20-476 of any one of SEQ ID NOs: 11-16, 26, or 51, or residues 20-469 of any one of SEQ ID NOs: 19-4.

35 In several embodiments, the recombinant MuV F ectodomain trimer is a soluble protein complex, for example, for use as a recombinant subunit vaccine. In several such embodiments, the protomers of the recombinant MuV F ectodomain trimer can each comprise a C-terminal linkage to a trimerization domain,

such as a GCN4 trimerization domain or a T4 fibrin trimerization domain, or both. The trimerization domain promotes trimerization and stabilization of the membrane proximal aspect of the recombinant MuV F ectodomain trimer. For example, a C-terminal residue of the protomers of the recombinant MuV F ectodomain trimer (such as a residue of the stem region of the trimer) can be directly linked to the trimerization domain, or indirectly linked to the trimerization domain via a peptide linker. Exemplary linkers include glycine and glycine-serine linkers. Non-limiting examples of exogenous multimerization domains that promote stable trimers of soluble recombinant proteins include: the GCN4 leucine zipper, a T4 fibrin trimerization domain, the trimerization motif from the lung surfactant protein (Hoppe *et al.* 1994 *FEBS Lett* 344:191-195) or collagen (McAlinden *et al.* 2003 *J Biol Chem* 278:42200-42207), any of which can be linked to the C-terminus of the protomers of a recombinant MuV F ectodomain to promote trimerization, as long as the recombinant MuV F ectodomain trimer retains the prefusion conformation. In some examples, the protomers of the recombinant MuV F ectodomain trimer can be linked to a MuV trimerization domain, for example, each protomer in the trimer can include a C-terminal linkage to the GCN4 trimerization domain, such as a linkage to any one of MuV F positions 469-483, such as MuV F position 469, MuV F position 476, or MuV F position 483. In specific examples, the GCN4 trimerization domain comprises or consists of the amino acid sequence IEDKIEEILSKIYHIENEIARIKKLIGEAP (SEQ ID NO: 33). In specific examples, the T4 fibrin trimerization domain comprises or consists of the amino acid sequence GYIPEAPRDGQAYVRKDGWVLLSTFL (SEQ ID NO: 34). In specific examples, the GCN4 trimerization domain fused to the fibrin trimerization domain comprises or consists of the amino acid sequence IEDKIEEILSKIYHIENEIARIKKLIGEAPSGYIPEAPRDGQAYVRKDGWVLLSTFL (SEQ ID NO: 35).

In a non-limiting example, a recombinant MuV F ectodomain trimer is provided that includes protomers including MuV positions 20-476 with V206C and A223C substitutions to form a non-natural disulfide bond, a deletion of MuV F positions 101-103 with positions 100 and 104 fused by a Gly-Gly peptide linker, and a GCN4 trimerization domain linked to the C-terminus of the protomers in the ectodomain.

Non-limiting examples of protomers of a MuV F ectodomain trimer including amino acid substitutions for stabilization in the prefusion conformation as well as a C-terminal linkage to a trimerization domain are provided herein. In some embodiments, the protomers of the MuV F ectodomain trimer comprise an amino acid sequence at least 90% identical to residues 20-513 of any one of SEQ ID NOs: 3-8, residues 20-506 of any one of SEQ ID NOs: 11-16, 26, or 51, or residues 20-499 of any one of SEQ ID NOs: 19-24; and wherein the protomers comprise the one or more amino acid substitutions that stabilize the MuV F ectodomain trimer in the prefusion conformation. In some embodiments, the protomers of the MuV F ectodomain trimer comprise residues 20-513 of any one of SEQ ID NOs: 3-8, residues 20-506 of any one of SEQ ID NOs: 11-16, 26, or 51, or residues 20-499 of any one of SEQ ID NOs: 19-24.

In some embodiments, the recombinant MuV F ectodomain trimer can be a membrane anchored protein complex, for example, for use in an attenuated virus or virus like particle vaccine. Membrane

anchoring can be accomplished, for example, by C-terminal linkage of the protomers of the recombinant MuV F ectodomain trimer to a transmembrane domain and optionally a cytoplasmic tail, such as a MuV F transmembrane domain and cytoplasmic tail. In some embodiments, one or more peptide linkers (such as a gly-ser linker, for example, a 10 amino acid glycine-serine peptide linker can be used to link the protomers of the recombinant MuV F ectodomain trimer to the transmembrane domain. A non-limiting example of a transmembrane domain for use with the disclosed embodiments includes a MuV F transmembrane domain, such as GAIIVAALVLSILSIIISLLFCCW (SEQ ID NO: 44). A non-limiting example of a transmembrane domain and cytoplasmic tail for use with the disclosed embodiments includes a MuV F transmembrane domain and cytoplasmic tail, such as

5 GAIIVAALVLSILSIIISLLFCCWAYIATKEIRRINFKTNHINTISSSVDDLIRY (SEQ ID NO: 99).

Native MuV F proteins from different MuV strains, as well as nucleic acid sequences encoding such proteins and methods, are known and can be altered using the description provided herein to generate a recombinant MuV F ectodomain trimer.

The recombinant MuV F ectodomain trimer can be derivatized or linked to another molecule (such as another peptide or protein). In general, the recombinant MuV F ectodomain is derivatized such that the binding to neutralizing antibodies to a trimer of the recombinant MuV F protein is not affected adversely by the derivatization or labeling. For example, the recombinant MuV F ectodomain can be functionally linked (by chemical coupling, genetic fusion, non-covalent association or otherwise) to one or more other molecular entities, such as a carrier protein, an antibody, a heterologous protein, or a detection tag.

20 In some embodiments, the recombinant MuV F ectodomain trimers are fused to one or more MuV HN ectodomains, such as the ectodomain head of the MuV HN sequence set forth as:

MuV HN ectodomain head (genotype G, Arkansas16, GenBank ARM65482.1 (SEQ ID NO: 30)

25 NIPLVNDLRFINGINKFIIEDYATHDFSIGHPLNMPFSIPTATSPNGCTRIPSFSLGKTHWCYTHNVINANCKDHTSSNQ
YVSMGILVQTASGYPMFKTLKIQYLSGDLNRKSCSIATVPDGCAMCYVSTQLETDDYAGSSPPTQKLTLFFYNDTVTER
TISPSGLEGNWATLVPGVSGGIYFENKLIFPAYGGVLPNSTLGVKSAREFFRPVNPYNPCSGFPQQDLQRALRSYFPSYF
SNRRIQSAFLVCAWNQILVTNCELVVPSSNQTMGAEGRVLLINNRLLYQIRSTSWWPYELLYEISFTFTNSGPSSVNMS
WIFIYSFTRPGSGNCSENVCPACVSGVYLDPWPLTPYSHQSGINRNIFYFTGALLNSSTTRVNPPLYVSALNNLKVLP
30 YGTQGLFASYTTTTCFQDTGDASVYCVYIMELASNIVGEFQILPVLTRLTIT

In some embodiments, the recombinant MuV F ectodomain trimers are fused to one or more MuV HN ectodomains, such as the ectodomain stalk and head of any one of MuV HN positions 54-130 to MuV HN position 582 (such as MuV HN positions 54-63 to MuV HN position 582, for example, positions 54-582, 61-582, 63-582, or 55-582). In some embodiments, the recombinant MuV F ectodomain trimers are fused to one or more MuV HN ectodomains, such as the ectodomain stalk and head of the sequence set forth as residues 22-550 of SEQ ID NO: 90, residues 22-543 of SEQ ID NO: 91, residues 22-541 of SEQ ID NO: 92, or residues 22-549 of SEQ ID NO: 93.

For example, the protomers of the recombinant MuV F ectodomain trimer are each fused to a MuV HN ectodomain. The fusion can be direct or via a peptide linker. In some embodiments, the MuV HN ectodomain can be fused, directly or indirectly via a peptide linker, to the C-terminus of the protomers of the

MuV F ectodomain trimer. In some such embodiments, the MuV HN ectodomain can be fused, directly or indirectly via a peptide linker, to the C-terminus of a trimerization domain (such as a GCN4 or T4 fibrin trimerization domain) fused to the C-terminus of the protomers of the MuV F ectodomain trimer. In some such embodiments, the protomers of the MuV F ectodomain trimer linked to the trimerization domain and

5 the MuV HN ectodomain comprise an amino acid sequence set forth as residues 20-966 of SEQ ID NO: 27, or an amino acid sequence at least 90% identical to residues 20-966 of SEQ ID NO: 27.

In some embodiments, the recombinant MuV F ectodomain trimers are fused to one or more MeV H ectodomains, such as the ectodomain head of the H sequence set forth as:

MeV H ectodomain head (SEQ ID NO: 31)

10 FLAVSKGNCSGPTTIRGQFSNMSLSLLDLYLGRGYNVSSIVTMTSQGMYGGTYLVEKPNLSSKRSELSQLSMYRVFEVGV
IRNPGLGAPVFHMTNYLEQPVSNDSLNCMVAGELKLAALCHGEDSITIPYQGSQKGVSFQVLKLGWVKSPADMQSWVPL
STDDPVIDRLYLSSHARGVIADNQAKWAVPTTRTDDKLRMETCFQQACKGKIQTLCENPEWAPLKDNRIPSYGVLSDLSL
TVELKIKIASGFGLITHGSGMDLYKSNHNNVWLTIPPMKNLALGVINTLEWIPRFKVSPLYFTVPIKEAGEDCHAPT
15 LPAEVDGDVKLSSNLVILPGQDLQYVLATYDTSRVEHAVVYVYSPGRSFSYFYFRLPIKGVPIELQVECFCTWDQKLWC
RHFVCLADSESGGHI THSGMVGMGVSCVTREDGTNR

MeV H ectodomain head (SEQ ID NO: 32)

ADVAEELMNALVNSTLLEARATNQFLAVSKGNCSGPTTIRGQFSNMSLSLLDLYLSRGYNVSSIVTMTSQGMYGGTYLV
GKPNLSSKGSELSQLSMHRVFEVGVIRNPGLGAPVFHMTNYFEQPVSNDFSNCMVAGELKFAALCHREDSITIPYQGSQ
20 KGVSFQVLKLGWVKSPDMRSWVPLSTDDPVIDRLYLSSHARGVIADNQAKWAVPTTRTDDKLRMETCFQQACKGKNQALC
ENPEWAPLKDNRIPSYGVLSDLSLVELKIKIASGFGLITHGSGMDLYKTNHNNVWLTIPPMKNLALGVINTLEWIP
RFKVSPLYFTVPIKEAGEDCHAPTYPALPAEVDGDVKLSSNLVILPGQDLQYVLATYDTSRVEHAVVYVYSPGRSFSYFYF
FRLPIKGVPIELQVECFCTWDKKLWCRHFVCLADSESGGHI THSGMVGMGVSCVTREDGTNR

25 In some embodiments, the recombinant MuV F ectodomain trimers are fused to one or more MeV H ectodomains, such as the ectodomain stalk and head of any one of MeV H positions 59-179 to MeV H position 617 (such as any one of MeV H positions 59-67 to MeV H position 617, for example positions 59-617, 62-617, 60-617, or 67-617). In some embodiments, the recombinant MuV F ectodomain trimers are fused to one or more MeV H ectodomains, such as the ectodomain stalk and head of the sequence set forth

30 as residues 22-580 of SEQ ID NO: 86, residues 22-577 of SEQ ID NO: 87, residues 22-579 of SEQ ID NO: 88, or residues 22-572 of SEQ ID NO: 89.

For example, the protomers of the recombinant MuV F ectodomain trimer stabilized in the prefusion conformation are each fused to a MeV H ectodomain. The fusion can be direct or via a peptide linker. In some embodiments, the MeV H ectodomain can be fused, directly or indirectly via a peptide linker, to the C-

35 terminus of the protomers of the prefusion MuV F ectodomain trimer. In some such embodiments, the MeV H ectodomain can be fused, directly or indirectly via a peptide linker, to the C-terminus of a trimerization domain (such as a GCN4 or T4 fibrin trimerization domain) fused to the C-terminus of the protomers of the MuV F ectodomain trimer. In some such embodiments, the protomers of the MuV F ectodomain trimer linked to the trimerization domain and the MeV H ectodomain comprise an amino acid sequence set forth as

40 residues 21-981 of SEQ ID NO: 28 or residues 20-1006 of SEQ ID NO: 29, or an amino acid sequence at least 90% identical to residues 21-981 of SEQ ID NO: 28 or residues 20-1006 of SEQ ID NO: 29.

Non-limiting examples of sequences containing a MuV F ectodomain with amino acid substitutions for stabilization in a prefusion conformation are provided as follows:

- MuV F V206C/A223C-GGG-483-GCN4 (SEQ ID NO: 3)
mkafsvtclsfavfsssicvninilqqigyikqqvrqlsyysqssssyivvklpnigptddscfksvstqynktnsl
lpiaeninniaspagsrrrhGGGagaaigialgvataaavslvqaqtnaraiaamknsiqatnraavfevkegtq
laiavqaiqdhintimntqlnmqscqildnqlatslglylteltt**C**fqpqltnpalpsisic**C**lrslsgsmtpavvqatl
5 stsisaaeilsaglmegqiisvllldemqmivkiniptivtqsnalvidfysissfinnqesiqlpdrileigneqwsyp
aknckltrrhifcqyneaeerlslesklclagnisacvfspiagsymrrfvaldgtivancrsltclckspspyiqpdhh
avttidltacqtlslldgldfsivslsnityaenltislsqtlntqpidistelskvnaslqnnavkyikesnhqlqsvsvn
ski IEDKIEEILSKIYHIENEIARIKKLIGEP
- 10 MuV F N86C/A215C-GGG-483-GCN4 (SEQ ID NO: 4)
mkafsvtclsfavfsssicvninilqqigyikqqvrqlsyysqssssyivvklpnigptddscfksvstqynktnsl
lpiaeninniaspagsrrrhGGGagaaigialgvataaavslvqaqtnaraiaamknsiqatnraavfevkegtq
laiavqaiqdhintimntqlnmqscqildnqlatslglylteltt**C**fqpqltnp**C**lpsisigalrslsgsmtpavvqatl
15 stsisaaeilsaglmegqiisvllldemqmivkiniptivtqsnalvidfysissfinnqesiqlpdrileigneqwsyp
aknckltrrhifcqyneaeerlslesklclagnisacvfspiagsymrrfvaldgtivancrsltclckspspyiqpdhh
avttidltacqtlslldgldfsivslsnityaenltislsqtlntqpidistelskvnaslqnnavkyikesnhqlqsvsvn
ski IEDKIEEILSKIYHIENEIARIKKLIGEP
- MuV F P209C/P214C-GGG-483-GCN4 (SEQ ID NO: 5)
20 mkafsvtclsfavfsssicvninilqqigyikqqvrqlsyysqssssyivvklpnigptddscfksvstqynktnsl
lpiaeninniaspagsrrrhGGGagaaigialgvataaavslvqaqtnaraiaamknsiqatnraavfevkegtq
laiavqaiqdhintimntqlnmqscqildnqlatslglylteltt**C**qltn**C**alspisigalrslsgsmtpavvqatl
stsisaaeilsaglmegqiisvllldemqmivkiniptivtqsnalvidfysissfinnqesiqlpdrileigneqwsyp
25 aknckltrrhifcqyneaeerlslesklclagnisacvfspiagsymrrfvaldgtivancrsltclckspspyiqpdhh
avttidltacqtlslldgldfsivslsnityaenltislsqtlntqpidistelskvnaslqnnavkyikesnhqlqsvsvn
ski IEDKIEEILSKIYHIENEIARIKKLIGEP
- MuV F K155C/L161C-GGG-483-GCN4 (SEQ ID NO: 6)
30 mkafsvtclsfavfsssicvninilqqigyikqqvrqlsyysqssssyivvklpnigptddscfksvstqynktnsl
lpiaeninniaspagsrrrhGGGagaaigialgvataaavslvqaqtnaraiaamknsiqatnraavfev**C**egttq
Caiavqaiqdhintimntqlnmqscqildnqlatslglylteltt**C**qltn**C**alspisigalrslsgsmtpavvqatl
stsisaaeilsaglmegqiisvllldemqmivkiniptivtqsnalvidfysissfinnqesiqlpdrileigneqwsyp
aknckltrrhifcqyneaeerlslesklclagnisacvfspiagsymrrfvaldgtivancrsltclckspspyiqpdhh
35 avttidltacqtlslldgldfsivslsnityaenltislsqtlntqpidistelskvnaslqnnavkyikesnhqlqsvsvn
ski IEDKIEEILSKIYHIENEIARIKKLIGEP
- MuV F V165C/M231C-GGG-483-GCN4 (SEQ ID NO: 7)
mkafsvtclsfavfsssicvninilqqigyikqqvrqlsyysqssssyivvklpnigptddscfksvstqynktnsl
lpiaeninniaspagsrrrhGGGagaaigialgvataaavslvqaqtnaraiaamknsiqatnraavfevkegtq
40 laia**C**aiavqaiqdhintimntqlnmqscqildnqlatslglylteltt**C**fqpqltnpalpsisigalrslsg**C**tpavvqatl
stsisaaeilsaglmegqiisvllldemqmivkiniptivtqsnalvidfysissfinnqesiqlpdrileigneqwsyp
aknckltrrhifcqyneaeerlslesklclagnisacvfspiagsymrrfvaldgtivancrsltclckspspyiqpdhh
avttidltacqtlslldgldfsivslsnityaenltislsqtlntqpidistelskvnaslqnnavkyikesnhqlqsvsvn
45 ski IEDKIEEILSKIYHIENEIARIKKLIGEP
- MuV F I221C/M255C-GGG-483-GCN4 (SEQ ID NO: 8)
mkafsvtclsfavfsssicvninilqqigyikqqvrqlsyysqssssyivvklpnigptddscfksvstqynktnsl
lpiaeninniaspagsrrrhGGGagaaigialgvataaavslvqaqtnaraiaamknsiqatnraavfevkegtq
laiavqaiqdhintimntqlnmqscqildnqlatslglylteltt**C**fqpqltnpalpsis**C**qalrslsgsmtpavvqatl
50 stsisaaeilsag**C**egqiisvllldemqmivkiniptivtqsnalvidfysissfinnqesiqlpdrileigneqwsyp
aknckltrrhifcqyneaeerlslesklclagnisacvfspiagsymrrfvaldgtivancrsltclckspspyiqpdhh
avttidltacqtlslldgldfsivslsnityaenltislsqtlntqpidistelskvnaslqnnavkyikesnhqlqsvsvn
ski IEDKIEEILSKIYHIENEIARIKKLIGEP
- 55 MuV F V206C/A223C-GGG-476-GCN4 (SEQ ID NO: 11)
mkafsvtclsfavfsssicvninilqqigyikqqvrqlsyysqssssyivvklpnigptddscfksvstqynktnsl
lpiaeninniaspagsrrrhGGGagaaigialgvataaavslvqaqtnaraiaamknsiqatnraavfevkegtq
laiavqaiqdhintimntqlnmqscqildnqlatslglylteltt**C**fqpqltnpalpsisic**C**lrslsgsmtpavvqatl
60 stsisaaeilsaglmegqiisvllldemqmivkiniptivtqsnalvidfysissfinnqesiqlpdrileigneqwsyp
aknckltrrhifcqyneaeerlslesklclagnisacvfspiagsymrrfvaldgtivancrsltclckspspyiqpdhh
avttidltacqtlslldgldfsivslsnityaenltislsqtlntqpidistelskvnaslqnnavkyikesnhqlq
IEDKIEEILSKIYHIENEIARIKKLIGEP

MuV F N86C/A215C-GGG-476-GCN4 (SEQ ID NO: 12)
 mkafsvtclsfavfsssicvnnilqqigiyikqvrqlsyysqssssyivvklpnigptdddscefksvtqynktnsl
 lpiaeCinniasp spgsrrhGGGAgiaigiaalgvataaqvtaavslvgaqtnaraiaamknsiqatnnavfevkegtq
 laiaavqaiqdhintimntqlnnmscqildnqlatslglylteltttvfqpqltnpCalspisiqalrslsgmtpavvqat
 stsisaaeilsaglmegqiisvlldemqmivkiniptivtqsnalvidfysissfinngesiqlpdrileigneqwsyp
 aknckltrhhifcqyneaerlslesklclagnisacvfspiagsymrrfvaldgtivancrsltcclckspysyipyqpdh
 avttidltacqtlslsdgldfsivslsnityaenltislsqtintqpidistelskvnaslqnavkyikesnhqlqs
 IEDKIEEILSKIYHIENEIARIKKLIGEAP

MuV F P209C/P214C-GGG-476-GCN4 (SEQ ID NO: 13)
 mkafsvtclsfavfsssicvnnilqqigiyikqvrqlsyysqssssyivvklpnigptdddscefksvtqynktnsl
 lpiaeninniasp spgsrrhGGGAgiaigiaalgvataaqvtaavslvgaqtnaraiaamknsiqatnnavfevkegtq
 laiaavqaiqdhintimntqlnnmscqildnqlatslglylteltttvfqpqltnpCqqltnCalspisiqalrslsgmtpavvqat
 stsisaaeilsaglmegqiisvlldemqmivkiniptivtqsnalvidfysissfinngesiqlpdrileigneqwsyp
 aknckltrhhifcqyneaerlslesklclagnisacvfspiagsymrrfvaldgtivancrsltcclckspysyipyqpdh
 avttidltacqtlslsdgldfsivslsnityaenltislsqtintqpidistelskvnaslqnavkyikesnhqlqs
 IEDKIEEILSKIYHIENEIARIKKLIGEAP

MuV F K155C/L161C-GGG-476-GCN4 (SEQ ID NO: 14)
 mkafsvtclsfavfsssicvnnilqqigiyikqvrqlsyysqssssyivvklpnigptdddscefksvtqynktnsl
 lpiaeninniasp spgsrrhGGGAgiaigiaalgvataaqvtaavslvgaqtnaraiaamknsiqatnnavfevCegtq
 C₁aiaavqaiqdhintimntqlnnmscqildnqlatslglylteltttvfqpqltnpalspisiqalrslsgmtpavvqat
 stsisaaeilsaglmegqiisvlldemqmivkiniptivtqsnalvidfysissfinngesiqlpdrileigneqwsyp
 aknckltrhhifcqyneaerlslesklclagnisacvfspiagsymrrfvaldgtivancrsltcclckspysyipyqpdh
 avttidltacqtlslsdgldfsivslsnityaenltislsqtintqpidistelskvnaslqnavkyikesnhqlqs
 IEDKIEEILSKIYHIENEIARIKKLIGEAP

MuV F V165C/M231C-GGG-476-GCN4 (SEQ ID NO: 15)
 mkafsvtclsfavfsssicvnnilqqigiyikqvrqlsyysqssssyivvklpnigptdddscefksvtqynktnsl
 lpiaeninniasp spgsrrhGGGAgiaigiaalgvataaqvtaavslvgaqtnaraiaamknsiqatnnavfevkegtq
 laiaaCqaiqdhintimntqlnnmscqildnqlatslglylteltttvfqpqltnpalspisiqalrslsgmtpavvqat
 stsisaaeilsaglmegqiisvlldemqmivkiniptivtqsnalvidfysissfinngesiqlpdrileigneqwsyp
 aknckltrhhifcqyneaerlslesklclagnisacvfspiagsymrrfvaldgtivancrsltcclckspysyipyqpdh
 avttidltacqtlslsdgldfsivslsnityaenltislsqtintqpidistelskvnaslqnavkyikesnhqlqs
 IEDKIEEILSKIYHIENEIARIKKLIGEAP

MuV F I221C/M255C-GGG-476-GCN4 (SEQ ID NO: 16)
 mkafsvtclsfavfsssicvnnilqqigiyikqvrqlsyysqssssyivvklpnigptdddscefksvtqynktnsl
 lpiaeninniasp spgsrrhGGGAgiaigiaalgvataaqvtaavslvgaqtnaraiaamknsiqatnnavfevkegtq
 laiaavqaiqdhintimntqlnnmscqildnqlatslglylteltttvfqpqltnpalspisCqalrslsgmtpavvqat
 stsisaaeilsaglmegqiisvlldemqmivkiniptivtqsnalvidfysissfinngesiqlpdrileigneqwsyp
 aknckltrhhifcqyneaerlslesklclagnisacvfspiagsymrrfvaldgtivancrsltcclckspysyipyqpdh
 avttidltacqtlslsdgldfsivslsnityaenltislsqtintqpidistelskvnaslqnavkyikesnhqlqs
 IEDKIEEILSKIYHIENEIARIKKLIGEAP

MuV F V206C/A223C-GGG-469-GCN4 (SEQ ID NO: 19)
 mkafsvtclsfavfsssicvnnilqqigiyikqvrqlsyysqssssyivvklpnigptdddscefksvtqynktnsl
 lpiaeninniasp spgsrrhGGGAgiaigiaalgvataaqvtaavslvgaqtnaraiaamknsiqatnnavfevkegtq
 laiaavqaiqdhintimntqlnnmscqildnqlatslglylteltttCfpqqltnpalspisiqCrlslsgmtpavvqat
 stsisaaeilsaglmegqiisvlldemqmivkiniptivtqsnalvidfysissfinngesiqlpdrileigneqwsyp
 aknckltrhhifcqyneaerlslesklclagnisacvfspiagsymrrfvaldgtivancrsltcclckspysyipyqpdh
 avttidltacqtlslsdgldfsivslsnityaenltislsqtintqpidistelskvnaslqnavkyike
 IEDKIEEILSKIYHIENEIARIKKLIGEAP

MuV F N86C/A215C-GGG-469-GCN4 (SEQ ID NO: 20)
 mkafsvtclsfavfsssicvnnilqqigiyikqvrqlsyysqssssyivvklpnigptdddscefksvtqynktnsl
 lpiaeCinniasp spgsrrhGGGAgiaigiaalgvataaqvtaavslvgaqtnaraiaamknsiqatnnavfevkegtq
 laiaavqaiqdhintimntqlnnmscqildnqlatslglylteltttvfqpqltnpCalspisiqalrslsgmtpavvqat
 stsisaaeilsaglmegqiisvlldemqmivkiniptivtqsnalvidfysissfinngesiqlpdrileigneqwsyp
 aknckltrhhifcqyneaerlslesklclagnisacvfspiagsymrrfvaldgtivancrsltcclckspysyipyqpdh
 avttidltacqtlslsdgldfsivslsnityaenltislsqtintqpidistelskvnaslqnavkyike
 IEDKIEEILSKIYHIENEIARIKKLIGEAP

- MuV F P209C/P214C-GGG-469-GCN4 (SEQ ID NO: 21)
 mkafsvtclsfavfsssicvninilqqigiyikqqvrqlsyysqssssyivvklpnigptddscfksvstqynktnsl
 lpiaeninniaspspgsrrrhGGGagiaigiaalgvataaavtaavslvqaqtnaraiaamknsiqatnra
 laiaavqaiqdhintimntqlnnmscildnqlatslglylteitvtfqCqqltnCalspisiqalrsl
 stsisaaeilsaglmegqiisvllldemqmivkiniptivtqsnalvidfysissfinnqesi
 5 aknckltrrhifcgyneaeerlslesklclagnisacvfspiagsymrrfvaldgtivancrsltc
 avttidltacqtlslldgldfsivslsnityaenltislsqtintqpidistelskvnaslqnavkyike
 IEDKIEEILSKIYHIENEIARIKKLIGEAP
- MuV F K155C/L161C-GGG-469-GCN4 (SEQ ID NO: 22)
 mkafsvtclsfavfsssicvninilqqigiyikqqvrqlsyysqssssyivvklpnigptddscfksvstqynktnsl
 lpiaeninniaspspgsrrrhGGGagiaigiaalgvataaavtaavslvqaqtnaraiaamknsiqatnra
 laiaavqaiqdhintimntqlnnmscildnqlatslglylteitvtfqCqqltnCalspisiqalrsl
 stsisaaeilsaglmegqiisvllldemqmivkiniptivtqsnalvidfysissfinnqesi
 10 C
 15 aknckltrrhifcgyneaeerlslesklclagnisacvfspiagsymrrfvaldgtivancrsltc
 avttidltacqtlslldgldfsivslsnityaenltislsqtintqpidistelskvnaslqnavkyike
 IEDKIEEILSKIYHIENEIARIKKLIGEAP
- MuV F V165C/M231C-GGG-469-GCN4 (SEQ ID NO: 23)
 mkafsvtclsfavfsssicvninilqqigiyikqqvrqlsyysqssssyivvklpnigptddscfksvstqynktnsl
 lpiaeninniaspspgsrrrhGGGagiaigiaalgvataaavtaavslvqaqtnaraiaamknsiqatnra
 laiaCqaiqdhintimntqlnnmscildnqlatslglylteitvtfqCqqltnCalspisiqalrsl
 stsisaaeilsaglmegqiisvllldemqmivkiniptivtqsnalvidfysissfinnqesi
 20 C
 25 aknckltrrhifcgyneaeerlslesklclagnisacvfspiagsymrrfvaldgtivancrsltc
 avttidltacqtlslldgldfsivslsnityaenltislsqtintqpidistelskvnaslqnavkyike
 IEDKIEEILSKIYHIENEIARIKKLIGEAP
- MuV F I221C/M255C-GGG-469-GCN4 (SEQ ID NO: 24)
 mkafsvtclsfavfsssicvninilqqigiyikqqvrqlsyysqssssyivvklpnigptddscfksvstqynktnsl
 lpiaeninniaspspgsrrrhGGGagiaigiaalgvataaavtaavslvqaqtnaraiaamknsiqatnra
 laiaavqaiqdhintimntqlnnmscildnqlatslglylteitvtfqCqqltnCalspisiqalrsl
 stsisaaeilsaglmegqiisvllldemqmivkiniptivtqsnalvidfysissfinnqesi
 30 C
 35 aknckltrrhifcgyneaeerlslesklclagnisacvfspiagsymrrfvaldgtivancrsltc
 avttidltacqtlslldgldfsivslsnityaenltislsqtintqpidistelskvnaslqnavkyike
 IEDKIEEILSKIYHIENEIARIKKLIGEAP
- MuV-JL F 206C/A223C-GGG-476-GCN4 (SEQ ID NO: 26)
 mkafsvtclsfavfsssicvninilqqigiyikqqvrqlsyysqssssyVvklpnigptdNscfksvstqynktnsl
 lpiaeninniaspspgsrrrhGGGagiaigiaalgvataaavtaavslvqaqtnaraiaamknsiqatnra
 laiaavqaiqdhintimntqlnnmscildnqlatslglylteitvtfqCqqltnCalspisiqalrsl
 stsisaaeilsaglmegqiisvllldemqmivkiniptivtqsnalvidfysissfinnqesi
 40 C
 45 aknckltrrhifcgyneaeerlsleTklclagnisacvfspiagsymrrfvaldgtivancrsltc
 avttidltacqtlslldgldfsivslsnityaenltislsqtintqpidistelskvnaslqnavkyikesnhqlqs
 IEDKIEEILSKIYHIENEIARIKKLIGEAP
- MuV-IL17 F 206C/A223C-GGG-476-GCN4 (SEQ ID NO: 51)
 mkvslvtclglfavfsssicvninilqqigiyikqqvrqlsyysqssssyivvklpnigptdNscfksvstqynktnsl
 lpiaeninniaspspgsrrrhGGGagiaigiaalgvataaavtaavslvqaqtnaraiaamknsiqatnra
 laiaavqaiqdhintimntqlnnmscildnqlatslglylteLTTCfqpqlinpalspisiqCLRSllgsmt
 stsisaaeilsaglmegqiisvllldemqmivkiniptivtqsnalvidfysissfinnqesi
 50 C
 aknckltrrhifcgyneaeerlslesklclagnisacvfspiagsymrrfvaldgtivancrsltc
 avttidltacqtlslldgldfsivslsnityaenltislsqtintqpidistelskvnaslqnavkyikesnhqlqs
 IEDKIEEILSKIYHIENEIARIKKLIGEAP

- 55 The above sequences include an N-terminal signal peptide, a MuV F ectodomain, and a GCN4
 trimerization domain. It will be appreciated that an alternative trimerization domain can be used, such as a
 T4 Fibrin trimerization domain. Additionally, many of the above sequence include a GGG linker to
 remove the native furin cleavage site separating the F1 and F2 subunits. Alternate glycine linkers can also

be used, such as GSG, GGS, or SGG. Additionally, in any of the sequences, the native furin cleavage site can be included instead of the GGG linker. It will be appreciated that the N-terminal signal peptide is removed during cellular processing and is not present in the purified protein. Additionally, the MuV F ectodomain of any of the above sequences can be included in a full-length MuV F protein to provide a

5 membrane anchored version of the prefusion MuV F protein, for example for mRNA immunization.

Non-limiting examples of sequences containing a MuV F ectodomain with amino acid substitutions for stabilization in a prefusion conformation linked to a MuV HN ectodomain or a MeV H ectodomain are provided as follows:

- 10 MuV F 206C/223C-GGG-476 + GCN4 + MuV HN_G (SEQ ID NO: 27)
 Mkafsvtclsfavfsssicvnninilqqigiyikqqvrqlsyysqssssyivvklpnigptddscfksvvtqynktnsl
 lpiaeninniaspssgrrhGGGagaaigaaalgvataaavslvqaqtnaraiaamknsiqatnraavfevkegtqq
 laiaavqaiqdhintimntqlnnmscgildnqlatslglyltelttCfqpqltnpalpsisicClrsllgsmtpavvqatl
 stsisaaeilsaglmegqiisvllldemqmivkiniptivtqsnalvidfysissfinnqesiqlpdrileigneqwsyp
 15 aknckltrhhifcgyneaelrslslesklclagnisacvfspiagsymrrfvaldgtivancrsltcclckspspyiqpdhh
 avttidltacqtlslldgldfsivslsnityaenltislsqtintqpidistelskvnaslgnavkyikesnhqlqs
 IEDKIEEILSKIYHIENEIARIKKLIGEAP GSGGGGGG
 Niplvndlrifinginkfiiedyathdfisighplnmpsfipatspngctripsfslgkthwcythnvinanckdhtssnq
 yvsmgilvqtasgypmfktlkiqylsdglnrkscsiatvpdgcamycyvstqletddyagssptqkltllfyndvtvter
 20 tispsglegnwatlvpgvgsgiyfenklifpayggvlpnstlgvksareffrpvnypnpscgppqddlqralrsyfpsyf
 snrriqsafvlvcawnqilvtncelvvpsnqtmgaegrvllinnrlllyqqrstswwpelyeistftnsgpssvnms
 wipiysftrpgsgnscgenvcptacvsgvldpwppltpyshqsginrnfyftgallnssttrvntplyvsalnlnklvap
 ygtqglfasyttttcfqdtgdasvycvymelasnivgefqlipvltrltit
- 25 MuV F 206C/223C-GGG-476 + GCN4/Fd + MeV_H (SEQ ID NO: 28)
 Mkafsvtclsfavfsssicvnninilqqigiyikqqvrqlsyysqssssyivvklpnigptddscfksvvtqynktnsl
 lpiaeninniaspssgrrhGGGagaaigaaalgvataaavslvqaqtnaraiaamknsiqatnraavfevkegtqq
 laiaavqaiqdhintimntqlnnmscgildnqlatslglyltelttCfqpqltnpalpsisicClrsllgsmtpavvqatl
 stsisaaeilsaglmegqiisvllldemqmivkiniptivtqsnalvidfysissfinnqesiqlpdrileigneqwsyp
 30 aknckltrhhifcgyneaelrslslesklclagnisacvfspiagsymrrfvaldgtivancrsltcclckspspyiqpdhh
 avttidltacqtlslldgldfsivslsnityaenltislsqtintqpidistelskvnaslgnavkyikesnhqlqs
 IEDKIEEILSKIYHIENEIARIKKLIGEAP GS GYIPEAPRDGQAYVRKDGEWVLLSTFL GSGGGGGG
 Flavskgncsgpttirgqfnsmslslldlylgrgynvssivtmtsqgmyggtlylveknlsksrslsqslmsrvfevgv
 irnpglgapvfhmtnyleqpvsndlsncmvalgelklaalchgedsitipyqsgkgvsvqlvklgvkwsadmqswwpl
 35 stddpvidrlylsshrgviadnqakwvpttrtdklrmetcfqackgkiqtlcenpewaplkdnrripsygvlsvdsl
 tvelkikiasgfgplithgsgmdlykshnnvywltippmknlalgvintlewi prfkvspylftvpikaeagedchapt
 ylpaevdgdvklssnlvilpgqdlqyvlatydt srvehavvyvyspgrsfysyfpfrlpikgvpieqlqvecftwdqklw
 rhfcvldasesgghithsgmvgmvsctvtredgtnrr
- 40 MuV F 206C/223C-GGG-476 + GCN4/Fd + MeV_H (SEQ ID NO: 29)
 Mkafsvtclsfavfsssicvnninilqqigiyikqqvrqlsyysqssssyivvklpnigptddscfksvvtqynktnsl
 lpiaeninniaspssgrrhGGGagaaigaaalgvataaavslvqaqtnaraiaamknsiqatnraavfevkegtqq
 laiaavqaiqdhintimntqlnnmscgildnqlatslglyltelttCfqpqltnpalpsisicClrsllgsmtpavvqatl
 stsisaaeilsaglmegqiisvllldemqmivkiniptivtqsnalvidfysissfinnqesiqlpdrileigneqwsyp
 45 aknckltrhhifcgyneaelrslslesklclagnisacvfspiagsymrrfvaldgtivancrsltcclckspspyiqpdhh
 avttidltacqtlslldgldfsivslsnityaenltislsqtintqpidistelskvnaslgnavkyikesnhqlqs
 IEDKIEEILSKIYHIENEIARIKKLIGEAP GS GYIPEAPRDGQAYVRKDGEWVLLSTFL GSGGGGGG
 Advaaeelmnalvnstllearatnqflavskgncsgpttirgqfnsmslslldlylrgynvssivtmtsqgmyggtlyl
 gkpnlskgselsqlsmhrvfevgvirnpglgapvfhmtnyfeqpvsndfsncmvalgelkfaalchreditsitipyqsg
 50 kgvsvqlvklgvksptdmrswvplstddpvidrlylsshrgviadnqakwvpttrtdklrmetcfqackgkgnqalc
 enpewaplkdnrripsygvlsvnslstvelkikiasgfgplithgsgmdlykshnnvywltippmknlalgvintlewi
 rfkvspnlftvpikaeagedchaptylpaevdgdvklssnlvilpgqdlqyvlatydt srvehavvyvyspsrfsyfp
 frlpikgvpieqlqvecftwdkklwcrhfcvldasesgghithsgmvgmvsctvtredgtnrr

The above sequences include an N-terminal signal peptide and a MuV F ectodomain in combination with various other elements, including a GCN4 trimerization domain, a T4-fibrin trimerization domain, peptide cleavage sites (e.g., thrombin), a HIS tag, a Strep tag, as well as various linker residues between segments. Purified forms of these proteins typically lack the N-terminal signal peptide and C-terminal residues removed by peptide cleavage.

B. Recombinant MeV F Ectodomain Trimers

Recombinant MeV F ectodomain trimers are disclosed herein that are modified from a native form (e.g., by introduction of one or more amino acid substitutions) to be stabilized in a prefusion conformation. As described in the Examples, embodiments of the disclosed MeV F ectodomain trimers have been selected through multiple rounds of structure based design for optimized solubility, stability, expression, and immunogenicity. The recombinant MeV F ectodomain trimers are useful to induce an immune response in a vertebrate animal (such humans) to MeV. Exemplary embodiments are shown to produce a superior immune response in an animal model compared to corresponding MeV F ectodomain trimers that are not stabilized in the prefusion conformation.

In some embodiments, the immunogen comprises a recombinant MeV F ectodomain trimer comprising protomers comprising one or more amino acid substitutions or deletions that stabilize the MeV F ectodomain trimer in the prefusion conformation.

In some embodiments, the protomers of the recombinant MeV F ectodomain trimer comprise cysteine substitutions at MeV F positions 48 and 284 (such as R48C and A284C substitutions) that form a non-natural intra-protomer disulfide bond for stabilization in the prefusion conformation.

In some embodiments, the protomers of the recombinant MeV F ectodomain trimer comprise cysteine substitutions at MeV F positions 90 and 225 (such as A90C and I225C substitutions) that form a non-natural intra-protomer disulfide bond for stabilization in the prefusion conformation.

In some embodiments, the protomers of the recombinant MeV F ectodomain trimer comprise cysteine substitutions at MeV F positions 141 and 270 (such as M141C and T270C substitutions) that form a non-natural intra-protomer disulfide bond for stabilization in the prefusion conformation.

In some embodiments, the protomers of the recombinant MeV F ectodomain trimer comprise cysteine substitutions at MeV F positions 165 and 171 (such as R165C and M171C substitutions) that form a non-natural intra-protomer disulfide bond for stabilization in the prefusion conformation.

In some embodiments, the protomers of the recombinant MeV F ectodomain trimer comprise cysteine substitutions at MeV F positions 173 and 245 (such as L173C and V245C substitutions) that form a non-natural intra-protomer disulfide bond for stabilization in the prefusion conformation.

In some embodiments, the protomers of the recombinant MeV F ectodomain trimer comprise cysteine substitutions at MeV F positions 175 and 241 (such as V175C and D241C substitutions) that form a non-natural intra-protomer disulfide bond for stabilization in the prefusion conformation.

In some embodiments, the protomers of the recombinant MeV F ectodomain trimer comprise cysteine substitutions at MeV F positions 212 and 236 (such as E212C and Y236C substitutions) that form a non-natural intra-protomer disulfide bond for stabilization in the prefusion conformation.

5 In some embodiments, the protomers of the recombinant MeV F ectodomain trimer comprise cysteine substitutions at MeV F positions 216 and 233 (such as L216C and A233C substitutions) that form a non-natural intra-protomer disulfide bond for stabilization in the prefusion conformation.

In some embodiments, the protomers of the recombinant MeV F ectodomain trimer comprise cysteine substitutions at MeV F positions 219 and 224 (such as P219C and P224C substitutions) that form a non-natural intra-protomer disulfide bond for stabilization in the prefusion conformation.

10 In some embodiments, the protomers of the recombinant MeV F ectodomain trimer comprise cysteine substitutions at MeV F positions 99 and 117 (such as R99C and V117C substitutions) that form a non-natural intra-protomer disulfide bond for stabilization in the prefusion conformation.

In some embodiments, the protomers of the recombinant MeV F ectodomain trimer comprise cysteine substitutions at MeV F positions 100 and 117 (such as P100C and V117C substitutions) that form a non-natural intra-protomer disulfide bond for stabilization in the prefusion conformation.

15 In some embodiments, the protomers of the recombinant MeV F ectodomain trimer comprise cysteine substitutions at MeV F positions 101 and 117 (such as V101C and V117C substitutions) that form a non-natural intra-protomer disulfide bond for stabilization in the prefusion conformation.

In some embodiments, the protomers of the recombinant MeV F ectodomain trimer comprise cysteine substitutions at MeV F positions 102 and 117 (such as Q102C and V117C substitutions) that form a non-natural intra-protomer disulfide bond for stabilization in the prefusion conformation.

In some embodiments, the protomers of the recombinant MeV F ectodomain trimer comprise cysteine substitutions at MeV F positions 103 and 117 (such as S103C and V117C substitutions) that form a non-natural intra-protomer disulfide bond for stabilization in the prefusion conformation.

25 In some embodiments, the protomers of the recombinant MeV F ectodomain trimer comprise cysteine substitutions at MeV F positions 165 and 171 (such as R165C and M171C substitutions) and positions 141 and 270 (such as M141C and T270C substitutions) that form a non-natural intra-protomer disulfide bond for stabilization in the prefusion conformation.

In some embodiments, the protomers of the recombinant MeV F ectodomain trimer comprise cysteine substitutions at MeV F positions 165 and 171 (such as R165C and M171C substitutions) and positions 212 and 236 (such as E212C and Y236C substitutions) that form a non-natural intra-protomer disulfide bond for stabilization in the prefusion conformation.

30 In some embodiments, the protomers of the recombinant MeV F ectodomain trimer comprise cysteine substitutions at MeV F positions 165 and 171 (such as R165C and M171C substitutions) and positions 48 and 284 (such as R48C and A284C substitutions) that form a non-natural intra-protomer disulfide bond for stabilization in the prefusion conformation.

In some embodiments, the protomers of the recombinant MeV F ectodomain trimer comprise a phenylalanine at MeV F position 175 (such as a V175F substitution) for stabilization in the prefusion conformation. The phenylalanine substitution can be combined with any of the disclosed cysteine substitutions or the proline substitution at MeV F position 194 to stabilize the recombinant MeV F ectodomain trimer in the prefusion conformation.

In some embodiments, the protomers of the recombinant MeV F ectodomain trimer comprise a proline substitution at MeV F position 194 (such as a S194P substitution) for stabilization in the prefusion conformation. The proline substitution can be combined with any of the disclosed cysteine substitutions to stabilize the recombinant MeV F ectodomain trimer in the prefusion conformation.

Any of the above recombinant MeV F proteins can further comprise modification to eliminate the protease cleavage site between the F1 and F2 polypeptides to generate a “single chain” recombinant F protein. For example, any of the above recombinant MeV F proteins can comprise deletion of MeV F positions 111-113 with positions 110 and 114 fused by a peptide linker. This modification removes the F2/F1 furin cleavage site and also removed the first residue of the fusion peptide (which is hydrophobic).

Any suitable peptide linker may be used that fuses the F2 and F1 ectodomain and allows folding of the F ectodomain into the prefusion conformation. In some embodiments, the peptide linker is a glycine, serine, or glycine-serine peptide linker. In some embodiments, the peptide linker is a Gly-Gly-Gly linker.

In a non-limiting example, a recombinant MeV F ectodomain trimer is provided that includes protomers with R165C and M171C substitutions to form a non-natural disulfide bond and a deletion of MeV F positions 111-113 with positions 110 and 114 fused by a Gly-Gly-Gly peptide linker.

In several embodiments, the protomers of the recombinant MeV F ectodomain can comprise one or more additional amino acid substitutions, for example, to increase stabilization of the prefusion conformation, or for other purposes, such as to increase solubility or to reduce and unwanted immune response.

The above-listed non-native disulfide bonds stabilize the membrane-distal portion of the MeV F ectodomain in its prefusion conformation. Any of these mutations can be combined with modifications to the membrane proximal portion (such as the stem) of the MeV F ectodomain, for example, to increase trimerization of the ectodomain.

In several embodiments, the N-terminal position of the recombinant F₂ polypeptide in the protomer can be one of MeV F positions 24-34 (such as position 24), and the C-terminal position of the F₁ ectodomain can be from the stem region of the ectodomain, such as one of MeV F positions 472-486 (such as position 486).

In a non-limiting example, a recombinant MeV F ectodomain trimer is provided that includes protomers including MeV positions 24-486 with R165C and M171C substitutions to form a non-natural disulfide bond and a deletion of MeV F positions 111-113 with positions 110 and 114 fused by a Gly-Gly-Gly peptide linker.

Non-limiting examples of protomers of a MeV F ectodomain trimer including amino acid substitutions for stabilization in the prefusion conformation are provided herein. In some embodiments, the protomers of the MeV F ectodomain trimer comprise an amino acid sequence at least 90% identical to residues 21-483 of any one of SEQ ID NOs: 37-43 or 53-55; wherein the protomers comprise the one or
 5 more amino acid substitutions that stabilize the MeV F ectodomain trimer in the prefusion conformation. In some embodiments, the protomers of the MeV F ectodomain trimer comprise residues 21-483 of any one of SEQ ID NOs: 37-43 or 53-55.

In several embodiments, the recombinant MeV F ectodomain trimer is a soluble protein complex, for example, for use as a recombinant subunit vaccine. In several such embodiments, the protomers of the
 10 recombinant MeV F ectodomain trimer can each comprise a C-terminal linkage to a trimerization domain, such as a GCN4 trimerization domain or a T4 fibrin trimerization domain, or both. The trimerization domain promotes trimerization and stabilization of the membrane proximal aspect of the recombinant MeV F ectodomain trimer. For example, a C-terminal residue of the protomers of the recombinant MeV F ectodomain trimer (such as a residue of the stem region of the trimer) can be directly linked to the
 15 trimerization domain, or indirectly linked to the trimerization domain via a peptide linker. Exemplary linkers include glycine and glycine-serine linkers. Non-limiting examples of exogenous multimerization domains that promote stable trimers of soluble recombinant proteins include: the GCN4 leucine zipper, a T4 fibrin trimerization domain, the trimerization motif from the lung surfactant protein (Hoppe *et al.* 1994 *FEBS Lett* 344:191-195) or collagen (McAlinden *et al.* 2003 *J Biol Chem* 278:42200-42207), any of which
 20 can be linked to the C-terminus of the protomers of a recombinant MeV F ectodomain to promote trimerization, as long as the recombinant MeV F ectodomain trimer retains the prefusion conformation. In some examples, the protomers of the recombinant MeV F ectodomain trimer can be linked to a MeV trimerization domain, for example, each protomer in the trimer can include a C-terminal linkage to the GCN4 trimerization domain, such as a linkage to any one of MeV F positions 472-486, such as MeV F
 25 position 486. In specific examples, the GCN4 trimerization domain comprises or consists of the amino acid sequence IEDKIEEILSKIYHIENEIARIKKLIGEAP (SEQ ID NO: 33). In specific examples, the T4 fibrin trimerization domain comprises or consists of the amino acid sequence
 GYIPEAPRDGQAYVRKDGWVLLSTFL (SEQ ID NO: 34). In specific examples, the GCN4 trimerization domain fused to the fibrin trimerization domain comprises or consists of the amino acid sequence
 30 IEDKIEEILSKIYHIENEIARIKKLIGEAPGSYIPEAPRDGQAYVRKDGWVLLSTFL (SEQ ID NO: 35).

In a non-limiting example, a recombinant MeV F ectodomain trimer is provided that includes protomers including MeV positions 24-486 with R165C and M171C substitutions to form a non-natural disulfide bond and a deletion of MeV F positions 111-113 with positions 110 and 114 fused by a Gly-Gly-Gly peptide linker, and a GCN4 trimerization domain linked to the C-terminus of the protomers in the
 35 ectodomain.

Non-limiting examples of protomers of a MeV F ectodomain trimer including amino acid substitutions for stabilization in the prefusion conformation as well as a C-terminal linkage to a trimerization

domain are provided herein. In some embodiments, the protomers of the MeV F ectodomain trimer comprise an amino acid sequence at least 90% identical to residues 21-513 of any one of SEQ ID NOs: 37-43 or 53-55; wherein the protomers comprise the one or more amino acid substitutions that stabilize the MeV F ectodomain trimer in the prefusion conformation. In some embodiments, the protomers of the MeV F ectodomain trimer comprise residues 21-513 of any one of SEQ ID NOs: 37-43 or 53-55.

In some embodiments, the recombinant MeV F ectodomain trimer can be a membrane anchored protein complex, for example, for use in an attenuated virus or virus like particle vaccine. Membrane anchoring can be accomplished, for example, by C-terminal linkage of the protomers of the recombinant MeV F ectodomain trimer to a transmembrane domain and optionally a cytoplasmic tail, such as a MeV F transmembrane domain and cytoplasmic tail. In some embodiments, one or more peptide linkers (such as a gly-ser linker, for example, a 10 amino acid glycine-serine peptide linker can be used to link the protomers of the recombinant MeV F ectodomain trimer to the transmembrane domain. A non-limiting example of a transmembrane domain for use with the disclosed embodiments includes a MeV F transmembrane domain, such as CIVYILIAVCLGGLIGI (SEQ ID NO: 100). A non-limiting example of a transmembrane domain for use with the disclosed embodiments includes a MeV F transmembrane domain, such as CIVYILIAVCLGGLIGIPALICCCGRGRCNKKGEQVGMSRPGPKPDLTGTSKSYVRSL (SEQ ID NO: 101).

Native MeV F proteins from different MeV strains, as well as nucleic acid sequences encoding such proteins and methods, are known and can be altered using the description provided herein to generate a recombinant MeV F ectodomain trimer.

The recombinant MeV F ectodomain trimer can be derivatized or linked to another molecule (such as another peptide or protein). In general, the recombinant MeV F ectodomain is derivatized such that the binding to cross-neutralizing antibodies to a trimer of the recombinant MeV F protein is not affected adversely by the derivatization or labeling. For example, the recombinant MeV F ectodomain can be functionally linked (by chemical coupling, genetic fusion, non-covalent association or otherwise) to one or more other molecular entities, such as a carrier protein, an antibody, a heterologous protein, or a detection tag.

In some embodiments, the recombinant MeV F ectodomain trimers are fused to one or more MuV HN ectodomains, such as the ectodomain of the MuV HN sequence set forth as:

MuV HN ectodomain head (SEQ ID NO: 30)
 NIPLVNDLRFINGINKFIIEDYATHDFSIGHPLNMPSEFIPTATSPNGCTRIPSFSLGKTHWCYTHNVINANCKDHTSSNQ
 YVSMGILVQTASGYPMFKTLKIQLSDGLNRKSCSIATVPDGCAMCYVSTQLEDDYAGSSPPTQKLTLLFYNDTVTER
 TISPSGLEGNWATLVPGVSGGIYFENKLIFPAYGGVLPNSTLGVKSAREFFRPVNPYNPCSGPQQDLQRLRSYFPSYF
 SNRRIQSAFLVCAWNQILVTNCELVPSSNQTMGAEGRVLLINNRLYYQRSTSWWPYELLYEISFTFTNSGPSSVNMS
 WIPIYSFTRPGSGNCSENVCPACVSGVYLDPWPLTPYSHQSGINRNFYFTGALLNSSTTRVNPTLYVSALNNLKVLP
 YGTQGLFASYTTTTTCFQDTGDASVYCVYIMELASNIVGEFQILFVLTRLTIT

For example, the protomers of the recombinant MeV F ectodomain trimer are each fused to a MuV HN ectodomain. The fusion can be direct or via a peptide linker. In some embodiments, the MuV HN ectodomain can be fused, directly or indirectly via a peptide linker, to the C-terminus of the protomers of the

MeV F ectodomain trimer. In some such embodiments, the MuV HN ectodomain can be fused, directly or indirectly via a peptide linker, to the C-terminus of a trimerization domain (such as a GCN4 or T4 fibrin trimerization domain) fused to the C-terminus of the protomers of the MeV F ectodomain trimer. In some such embodiments, the protomers of the MeV F ectodomain trimer linked to the trimerization domain and the MuV HN ectodomain comprise an amino acid sequence set forth as residues 20-966 of SEQ ID NO: 27, or an amino acid sequence at least 90% identical to residues 20-966 of SEQ ID NO: 27.

In some embodiments, the recombinant MeV F ectodomain trimers are fused to one or more MeV H ectodomains, such as the ectodomain of the H sequence set forth as:

MeV H ectodomain (SEQ ID NO: 31)

FLAVSKGNCSGPTTIRGQFSNMSLSLLDLYLGRGVNVSIVTMTSQGMYGGTYLVEKPNLSSKRSELSQLSMYRVFEVGV
IRNPGLGAPVVFHMTNYLEQPVSNDSLNCMVVALGELKLAALCHGEDSITIPYQGSQKGVVSFQVLKLGWVKSPADMQSWVPL
STDDPVIDRLYLSSHARGVIADNQAKWAVPTTRTDDKLRMETCFQQACKGKIQTLCENPEWAPLKDNRIPSYGVLSDLSL
TVELKIKIASGFGLITHGSGMDLYKSNHNNVYWLTIIPPMKNLALGVINTLEWIPRFKVSPYLFVPIKEAGEDCHAPTY
LPAEVDGDVKLSSNLVILPGQDLQYVLATYDTSRVEHAVVYVYSPGRSFSYFYFRLPIKGVPIELQVECFCTWDQKLWC
RHFVCLADSESGGHI THSGMVGMGVSCTVTREDGTNR

MeV H ectodomain (SEQ ID NO: 32)

ADVAAEELMNALVNSTLLEARATNQFLAVSKGNCSGPTTIRGQFSNMSLSLLDLYLSRGYNVSIVTMTSQGMYGGTYLV
GKPNLSSKGSSELSQLSMHRVFEVGVIRNPGLGAPVVFHMTNYFEQPVSNDFSNCMVVALGELKFAALCHREDSITIPYQGSQ
KGVVSFQVLKLGWVKSPDMRSWVPLSTDDPVIDRLYLSSHARGVIADNQAKWAVPTTRTDDKLRMETCFQQACKGKNQALC
ENPEWAPLKDNRIPSYGVLSDLSLVELKIKIASGFGLITHGSGMDLYKTNHNNVYWLTIIPPMKNLALGVINTLEWIP
RFKVSPNLFTVPIKEAGEDCHAPTYLPAEVDGDVKLSSNLVILPGQDLQYVLATYDTSRVEHAVVYVYSPGRSFSYFYF
FRLPIKGVPIELQVECFCTWDKKLWCRHFVCLADSESGGHI THSGMVGMGVSCTVTREDGTNR

For example, the protomers of the recombinant MeV F ectodomain trimer are each fused to a MeV H ectodomain. The fusion can be direct or via a peptide linker. In some embodiments, the MeV H ectodomain can be fused, directly or indirectly via a peptide linker, to the C-terminus of the protomers of the MeV F ectodomain trimer. In some such embodiments, the MeV H ectodomain can be fused, directly or indirectly via a peptide linker, to the C-terminus of a trimerization domain (such as a GCN4 or T4 fibrin trimerization domain) fused to the C-terminus of the protomers of the MeV F ectodomain trimer. In some such embodiments, the protomers of the MeV F ectodomain trimer linked to the trimerization domain and the MeV H ectodomain comprise an amino acid sequence set forth as residues 21-959 of SEQ ID NO: 56 or 21-973 of SEQ ID NO 57 or an amino acid sequence at least 90% identical thereto. .

Non-limiting examples of sequences containing a MeV F ectodomain with amino acid substitutions for stabilization in a prefusion conformation are provided as follows:

MeV F R48C/A284C-GGG-486-GCN4 (SEQ ID NO: 53)

mymqglascvtltlvtllvnsqihwgnlskigvvgigsasykvmtCsshqslviklmpnitllnnctrveiaeyrrllrtv
lepardalnavtqnirpvgvassrrrhGGGagvvlagaalgvataaqitagialhqsmnsqaidnlaslettnqiaiea
irgagqgemilavqgvqdyinnelipsmnqlscdligqklglkllryyteilsfpgpslrdpisaeisigalsyalggdin
kvleklgysggdlgilesrgikarithvdtesyfivlsiCpytlseikgvivhrlegvsnignsqewyttvpkyvatqg
ylisnfdesctfmpegtvcnalympspilqecrlgstkscartlvsgsfgnrfilsqgnliancasilckcyttgti
inqdpdkiltyiaadhcpvvevngvtiqvgsrrypdavylhridlgppislerldvgtnlgnaiakledakellessdqi
lsa IEDKIEEILSKIYHIENEIARIKKLIGEAP

MeV F A90C/I225C-GGG-486-GCN4 (SEQ ID NO: 37)

mymqglascvtltlvtllvnsqihwgnlskigvvgigsasykvmttrsshqslviklmpnitllnnctrveiaeyrrllrtv
lepardClnavtqnirpvgvassrrrhGGGagvvlagaalgvataaqitagialhqsmnsqaidnlaslettnqiaiea
irgagqgemilavqgvqdyinnelipsmnqlscdligqklglkllryyteilsfpgpslrdpCsaeisigalsyalggdin

- kvleklysgggdlgilesgikarithvdtesyfivlsiaayptlseikgvivhrlegvsynigsqewyttvpkyvatgg
ylisnfdesstfmpegtvcsgnalypmspllqecrlrgstkscartlvsgsfgnrfilsqgnlianacasilckcyttgti
inqdpdkiltyiaadhcpvvevngvtiqvgsrrypdavylhridlgppislerldvgtnlgnaiakledakellessdqi
lsa IEDKIEEILSKIYHIENEIARIKKLIGEP
- 5 MeV F M141C/T270C-GGG-486-GCN4 (SEQ ID NO: 54)
mysmqlascvtltlvllvnsqihwgnlskigvvgigsasykvmttrsshqslviklmpnitllnnctrveiaeyrrllrtv
lepardalnavtqnirpvqsvassrrhGGGagvvlagaaalgvataaqitagialhqsc^lnsqaidnlaslettnqaeia
irqagqemilavqgvqdyinnelipsmnqlscdligqklglkllryyteils^lfgpslrdpisaaisiqalsyalggdⁿ
10 kvleklysgggdlgilesgikarithvdtesyfivlsiaayptlseikgvivhrlegvsynigsqewyttvpkyvatgg
ylisnfdesstfmpegtvcsgnalypmspllqecrlrgstkscartlvsgsfgnrfilsqgnlianacasilckcyttgti
inqdpdkiltyiaadhcpvvevngvtiqvgsrrypdavylhridlgppislerldvgtnlgnaiakledakellessdqi
lsa IEDKIEEILSKIYHIENEIARIKKLIGEP
- 15 MeV F R165C/M171C-GGG-486-GCN4 (SEQ ID NO: 38)
mysmqlascvtltlvllvnsqihwgnlskigvvgigsasykvmttrsshqslviklmpnitllnnctrveiaeyrrllrtv
lepardalnavtqnirpvqsvassrrhGGGagvvlagaaalgvataaqitagialhqsm^lnsqaidnlaslettnqaeia
i^cqagq^ceilavqgvqdyinnelipsmnqlscdligqklglkllryyteils^lfgpslrdpisaaisiqalsyalggdⁿ
10 kvleklysgggdlgilesgikarithvdtesyfivlsiaayptlseikgvivhrlegvsynigsqewyttvpkyvatgg
ylisnfdesstfmpegtvcsgnalypmspllqecrlrgstkscartlvsgsfgnrfilsqgnlianacasilckcyttgti
inqdpdkiltyiaadhcpvvevngvtiqvgsrrypdavylhridlgppislerldvgtnlgnaiakledakellessdqi
lsa IEDKIEEILSKIYHIENEIARIKKLIGEP
- 20 MeV F L173C/V245C-GGG-486-GCN4 (SEQ ID NO: 39)
mysmqlascvtltlvllvnsqihwgnlskigvvgigsasykvmttrsshqslviklmpnitllnnctrveiaeyrrllrtv
lepardalnavtqnirpvqsvassrrhGGGagvvlagaaalgvataaqitagialhqsm^lnsqaidnlaslettnqaeia
irqagqemi^cavqgvqdyinnelipsmnqlscdligqklglkllryyteils^lfgpslrdpisaaisiqalsyalggdⁿ
20 kvleklysgggdlgilesgikarithvdtesyfivlsiaayptlseikgvivhrlegvsynigsqewyttvpkyvatgg
ylisnfdesstfmpegtvcsgnalypmspllqecrlrgstkscartlvsgsfgnrfilsqgnlianacasilckcyttgti
inqdpdkiltyiaadhcpvvevngvtiqvgsrrypdavylhridlgppislerldvgtnlgnaiakledakellessdqi
lsa IEDKIEEILSKIYHIENEIARIKKLIGEP
- 25 MeV F L173C/V245C-GGG-486-GCN4 (SEQ ID NO: 39)
mysmqlascvtltlvllvnsqihwgnlskigvvgigsasykvmttrsshqslviklmpnitllnnctrveiaeyrrllrtv
lepardalnavtqnirpvqsvassrrhGGGagvvlagaaalgvataaqitagialhqsm^lnsqaidnlaslettnqaeia
irqagqemi^cavqgvqdyinnelipsmnqlscdligqklglkllryyteils^lfgpslrdpisaaisiqalsyalggdⁿ
30 kvleklysgggdlgilesgikarithvdtesyfivlsiaayptlseikgvivhrlegvsynigsqewyttvpkyvatgg
ylisnfdesstfmpegtvcsgnalypmspllqecrlrgstkscartlvsgsfgnrfilsqgnlianacasilckcyttgti
inqdpdkiltyiaadhcpvvevngvtiqvgsrrypdavylhridlgppislerldvgtnlgnaiakledakellessdqi
lsa IEDKIEEILSKIYHIENEIARIKKLIGEP
- 35 MeV F V175C/D241C-GGG-486-GCN4 (SEQ ID NO: 40)
mysmqlascvtltlvllvnsqihwgnlskigvvgigsasykvmttrsshqslviklmpnitllnnctrveiaeyrrllrtv
lepardalnavtqnirpvqsvassrrhGGGagvvlagaaalgvataaqitagialhqsm^lnsqaidnlaslettnqaeia
irqagqemila^cqgvqdyinnelipsmnqlscdligqklglkllryyteils^lfgpslrdpisaaisiqalsyalgg^c
35 kvleklysgggdlgilesgikarithvdtesyfivlsiaayptlseikgvivhrlegvsynigsqewyttvpkyvatgg
ylisnfdesstfmpegtvcsgnalypmspllqecrlrgstkscartlvsgsfgnrfilsqgnlianacasilckcyttgti
inqdpdkiltyiaadhcpvvevngvtiqvgsrrypdavylhridlgppislerldvgtnlgnaiakledakellessdqi
40 lsa IEDKIEEILSKIYHIENEIARIKKLIGEP
- 45 MeV F E212C/Y236C-GGG-486-GCN4 (SEQ ID NO: 55)
mysmqlascvtltlvllvnsqihwgnlskigvvgigsasykvmttrsshqslviklmpnitllnnctrveiaeyrrllrtv
lepardalnavtqnirpvqsvassrrhGGGagvvlagaaalgvataaqitagialhqsm^lnsqaidnlaslettnqaeia
irqagqemilavqgvqdyinnelipsmnqlscdligqklglkllryyt^cilslfgpslrdpisaaisiqals^calggdⁿ
45 kvleklysgggdlgilesgikarithvdtesyfivlsiaayptlseikgvivhrlegvsynigsqewyttvpkyvatgg
ylisnfdesstfmpegtvcsgnalypmspllqecrlrgstkscartlvsgsfgnrfilsqgnlianacasilckcyttgti
inqdpdkiltyiaadhcpvvevngvtiqvgsrrypdavylhridlgppislerldvgtnlgnaiakledakellessdqi
50 lsa IEDKIEEILSKIYHIENEIARIKKLIGEP
- 55 MeV F L216C/A233C-GGG-486-GCN4 (SEQ ID NO: 41)
mysmqlascvtltlvllvnsqihwgnlskigvvgigsasykvmttrsshqslviklmpnitllnnctrveiaeyrrllrtv
lepardalnavtqnirpvqsvassrrhGGGagvvlagaaalgvataaqitagialhqsm^lnsqaidnlaslettnqaeia
irqagqemilavqgvqdyinnelipsmnqlscdligqklglkllryyteils^cfgpslrdpisaaisiq^clsyalggdⁿ
55 kvleklysgggdlgilesgikarithvdtesyfivlsiaayptlseikgvivhrlegvsynigsqewyttvpkyvatgg
ylisnfdesstfmpegtvcsgnalypmspllqecrlrgstkscartlvsgsfgnrfilsqgnlianacasilckcyttgti
inqdpdkiltyiaadhcpvvevngvtiqvgsrrypdavylhridlgppislerldvgtnlgnaiakledakellessdqi
lsa IEDKIEEILSKIYHIENEIARIKKLIGEP
- 60 MeV F P219C/P224C-GGG-486-GCN4 (SEQ ID NO: 42)
mysmqlascvtltlvllvnsqihwgnlskigvvgigsasykvmttrsshqslviklmpnitllnnctrveiaeyrrllrtv
lepardalnavtqnirpvqsvassrrhGGGagvvlagaaalgvataaqitagialhqsm^lnsqaidnlaslettnqaeia

irqagqemilavqgvqdyinnelipsmnqlscdligqklglkllryyteilsflgCslrdCisaeisigalsyalggdin
 kvleklgysggdlhgilesrgikarithvdtesyfivlsiaayptlseikgvivhrlegvsynigsqewyttvpkyvatqg
 ylisnfdesstfmpegtvcsqnalypmspllqecclrgstkscartlvsgsfgnrfilsqgnliancasilckcyttgti
 inqdpdkiltyiaadhcpvvevngvtiqvgsrrypdavylhridlgppislerldvgtnlgnaiakledakellessdqi
 5 lsa IEDKIEEILSKIYHIENEIARIKKLIGEAP

MeV F S194P-GGG-486-GCN4 (SEQ ID NO: 43)
 mysmqlascvtltlavlvsqihwgnlskigvvgigsasykvmttrsshqslviklmpnitllnnctrveiaeyrrllrtv
 lepirdalnavtqnirpvcvassrrhGGGagvClagaaalgvataaqitagialhqsmlnsqaidnlaslettnqaiea
 10 irqagqemilavqgvqdyinnelipsmnqlscdligqklglkllryyteilsflgpslrdpisaeisigalsyalggdin
 kvleklgysggdlhgilesrgikarithvdtesyfivlsiaayptlseikgvivhrlegvsynigsqewyttvpkyvatqg
 ylisnfdesstfmpegtvcsqnalypmspllqecclrgstkscartlvsgsfgnrfilsqgnliancasilckcyttgti
 inqdpdkiltyiaadhcpvvevngvtiqvgsrrypdavylhridlgppislerldvgtnlgnaiakledakellessdqi
 lsa IEDKIEEILSKIYHIENEIARIKKLIGEAP

15 MeV F S103C/V117C-GGG-486-GCN4 (SEQ ID NO: 62)
 mysmqlascvtltlavlvsqihwgnlskigvvgigsasykvmttrsshqslviklmpnitllnnctrveiaeyrrllrtv
 lepirdalnavtqnirpvcvassrrhGGGagvClagaaalgvataaqitagialhqsmlnsqaidnlaslettnqaiea
 irqagqemilavqgvqdyinnelipsmnqlscdligqklglkllryyteilsflgpslrdpisaeisigalsyalggdin
 20 kvleklgysggdlhgilesrgikarithvdtesyfivlsiaayptlseikgvivhrlegvsynigsqewyttvpkyvatqg
 ylisnfdesstfmpegtvcsqnalypmspllqecclrgstkscartlvsgsfgnrfilsqgnliancasilckcyttgti
 inqdpdkiltyiaadhcpvvevngvtiqvgsrrypdavylhridlgppislerldvgtnlgnaiakledakellessdqi
 lsa IEDKIEEILSKIYHIENEIARIKKLIGEAP

25 MeV F V175F-GGG-486-GCN4 (SEQ ID NO: 63)
 mysmqlascvtltlavlvsqihwgnlskigvvgigsasykvmttrsshqslviklmpnitllnnctrveiaeyrrllrtv
 lepirdalnavtqnirpvcvassrrhGGGagvClagaaalgvataaqitagialhqsmlnsqaidnlaslettnqaiea
 irqagqemilavqgvqdyinnelipsmnqlscdligqklglkllryyteilsflgpslrdpisaeisigalsyalggdin
 30 kvleklgysggdlhgilesrgikarithvdtesyfivlsiaayptlseikgvivhrlegvsynigsqewyttvpkyvatqg
 ylisnfdesstfmpegtvcsqnalypmspllqecclrgstkscartlvsgsfgnrfilsqgnliancasilckcyttgti
 inqdpdkiltyiaadhcpvvevngvtiqvgsrrypdavylhridlgppislerldvgtnlgnaiakledakellessdqi
 lsa IEDKIEEILSKIYHIENEIARIKKLIGEAP

MeV F S103C/V117C, V175F, S194P-GGG-486-GCN4 (SEQ ID NO: 64)
 35 mysmqlascvtltlavlvsqihwgnlskigvvgigsasykvmttrsshqslviklmpnitllnnctrveiaeyrrllrtv
 lepirdalnavtqnirpvcvassrrhGGGagvClagaaalgvataaqitagialhqsmlnsqaidnlaslettnqaiea
 irqagqemilavqgvqdyinnelipsmnqlscdligqklglkllryyteilsflgpslrdpisaeisigalsyalggdin
 kvleklgysggdlhgilesrgikarithvdtesyfivlsiaayptlseikgvivhrlegvsynigsqewyttvpkyvatqg
 40 ylisnfdesstfmpegtvcsqnalypmspllqecclrgstkscartlvsgsfgnrfilsqgnliancasilckcyttgti
 inqdpdkiltyiaadhcpvvevngvtiqvgsrrypdavylhridlgppislerldvgtnlgnaiakledakellessdqi
 lsa IEDKIEEILSKIYHIENEIARIKKLIGEAP

MeV F S103C/V117C, R165C/M171C, S194P-GGG-486-GCN4 (SEQ ID NO: 65)
 45 mysmqlascvtltlavlvsqihwgnlskigvvgigsasykvmttrsshqslviklmpnitllnnctrveiaeyrrllrtv
 lepirdalnavtqnirpvcvassrrhGGGagvClagaaalgvataaqitagialhqsmlnsqaidnlaslettnqaiea
 irqagqemilavqgvqdyinnelipsmnqlscdligqklglkllryyteilsflgpslrdpisaeisigalsyalggdin
 kvleklgysggdlhgilesrgikarithvdtesyfivlsiaayptlseikgvivhrlegvsynigsqewyttvpkyvatqg
 ylisnfdesstfmpegtvcsqnalypmspllqecclrgstkscartlvsgsfgnrfilsqgnliancasilckcyttgti
 50 inqdpdkiltyiaadhcpvvevngvtiqvgsrrypdavylhridlgppislerldvgtnlgnaiakledakellessdqi
 lsa IEDKIEEILSKIYHIENEIARIKKLIGEAP

MeV F R99C/V117C-GGG-486-GCN4 (SEQ ID NO: 66)
 55 mysmqlascvtltlavlvsqihwgnlskigvvgigsasykvmttrsshqslviklmpnitllnnctrveiaeyrrllrtv
 lepirdalnavtqnirpvcvassrrhGGGagvClagaaalgvataaqitagialhqsmlnsqaidnlaslettnqaiea
 irqagqemilavqgvqdyinnelipsmnqlscdligqklglkllryyteilsflgpslrdpisaeisigalsyalggdin
 kvleklgysggdlhgilesrgikarithvdtesyfivlsiaayptlseikgvivhrlegvsynigsqewyttvpkyvatqg
 ylisnfdesstfmpegtvcsqnalypmspllqecclrgstkscartlvsgsfgnrfilsqgnliancasilckcyttgti
 inqdpdkiltyiaadhcpvvevngvtiqvgsrrypdavylhridlgppislerldvgtnlgnaiakledakellessdqi
 60 lsa IEDKIEEILSKIYHIENEIARIKKLIGEAP

MeV F R99C/V117C, V175F, S194P-GGG-486-GCN4 (SEQ ID NO: 67)

- 5
15
120
125
130
135
140
145
150
155
160
- mysmqlascvtltlvtllvnsqihwgnlskigvvgigsasykvmttrsshqslviklmpnitllnnctrveiaeyrrllrtv
lepardalnavtqniCpvqsvassrrrhGGGagvC¹lagaalgvataaqitagialhqsmnlsqaidnlraslettnqaiea
irqagqemilaFggvqdyinnelipsmnqLPcdliqgklglkllryyteilsfpgpslrdpisaaisiqalsyalggdin
kvleklgysggdllgilesrgikarithvdtesyfivlsiaayptlseikgvivhrlegvsynigsqewyttvpkyvatqg
ylisnfdesstfmpegtvcsqnalypmspllqecrlrgstkscartlvsgsfgnrfilsqgnliancasilckcyttgti
inqdpdkiltyiaadhcpvvevngvtiqvgsrrypdavylhridlppislerldvgtnlgnaiakledakellessdqi
lsa IEDKIEEILSKIYHIENEIARIKKLIGEAP
- MeV F R99C/V117C, R165C/M171C, S194P-GGG-486-GCN4 (SEQ ID NO: 68)
- mysmqlascvtltlvtllvnsqihwgnlskigvvgigsasykvmttrsshqslviklmpnitllnnctrveiaeyrrllrtv
lepardalnavtqniCpvqsvassrrrhGGGagvC¹lagaalgvataaqitagialhqsmnlsqaidnlraslettnqaiea
iCqagqeCilaFggvqdyinnelipsmnqLPcdliqgklglkllryyteilsfpgpslrdpisaaisiqalsyalggdin
kvleklgysggdllgilesrgikarithvdtesyfivlsiaayptlseikgvivhrlegvsynigsqewyttvpkyvatqg
ylisnfdesstfmpegtvcsqnalypmspllqecrlrgstkscartlvsgsfgnrfilsqgnliancasilckcyttgti
inqdpdkiltyiaadhcpvvevngvtiqvgsrrypdavylhridlppislerldvgtnlgnaiakledakellessdqi
lsa IEDKIEEILSKIYHIENEIARIKKLIGEAP
- MeV F R99C/V117C-GGG-493-GCN4 (SEQ ID NO: 69)
- mysmqlascvtltlvtllvnsqihwgnlskigvvgigsasykvmttrsshqslviklmpnitllnnctrveiaeyrrllrtv
lepardalnavtqniCpvqsvassrrrhGGGagvC¹lagaalgvataaqitagialhqsmnlsqaidnlraslettnqaiea
irqagqemilaFggvqdyinnelipsmnqLPcdliqgklglkllryyteilsfpgpslrdpisaaisiqalsyalggdin
kvleklgysggdllgilesrgikarithvdtesyfivlsiaayptlseikgvivhrlegvsynigsqewyttvpkyvatqg
ylisnfdesstfmpegtvcsqnalypmspllqecrlrgstkscartlvsgsfgnrfilsqgnliancasilckcyttgti
inqdpdkiltyiaadhcpvvevngvtiqvgsrrypdavylhridlppislerldvgtnlgnaiakledakellessdqi
l rsmkgllsa IEDKIEEILSKIYHIENEIARIKKLIGEAP
- MeV F R99C/V117C, V175F, S194P-GGG-493-GCN4 (SEQ ID NO: 70)
- mysmqlascvtltlvtllvnsqihwgnlskigvvgigsasykvmttrsshqslviklmpnitllnnctrveiaeyrrllrtv
lepardalnavtqniCpvqsvassrrrhGGGagvC¹lagaalgvataaqitagialhqsmnlsqaidnlraslettnqaiea
iCqagqeCilaFggvqdyinnelipsmnqLPcdliqgklglkllryyteilsfpgpslrdpisaaisiqalsyalggdin
kvleklgysggdllgilesrgikarithvdtesyfivlsiaayptlseikgvivhrlegvsynigsqewyttvpkyvatqg
ylisnfdesstfmpegtvcsqnalypmspllqecrlrgstkscartlvsgsfgnrfilsqgnliancasilckcyttgti
inqdpdkiltyiaadhcpvvevngvtiqvgsrrypdavylhridlppislerldvgtnlgnaiakledakellessdqi
l rsmkgllsa IEDKIEEILSKIYHIENEIARIKKLIGEAP
- MeV F R99C/V117C, R165C/M171C, S194P-GGG-493-GCN4 (SEQ ID NO: 71)
- mysmqlascvtltlvtllvnsqihwgnlskigvvgigsasykvmttrsshqslviklmpnitllnnctrveiaeyrrllrtv
lepardalnavtqniCpvqsvassrrrhGGGagvC¹lagaalgvataaqitagialhqsmnlsqaidnlraslettnqaiea
iCqagqeCilaFggvqdyinnelipsmnqLPcdliqgklglkllryyteilsfpgpslrdpisaaisiqalsyalggdin
kvleklgysggdllgilesrgikarithvdtesyfivlsiaayptlseikgvivhrlegvsynigsqewyttvpkyvatqg
ylisnfdesstfmpegtvcsqnalypmspllqecrlrgstkscartlvsgsfgnrfilsqgnliancasilckcyttgti
inqdpdkiltyiaadhcpvvevngvtiqvgsrrypdavylhridlppislerldvgtnlgnaiakledakellessdqi
l rsmkgllsa IEDKIEEILSKIYHIENEIARIKKLIGEAP
- MeV F P100C/V117C, R165C/M171C, S194P-GGG-486-GCN4 (SEQ ID NO: 72)
- mysmqlascvtltlvtllvnsqihwgnlskigvvgigsasykvmttrsshqslviklmpnitllnnctrveiaeyrrllrtv
lepardalnavtqnirCqsvassrrrhGGGagvC¹lagaalgvataaqitagialhqsmnlsqaidnlraslettnqaiea
iCqagqeCilaFggvqdyinnelipsmnqLPcdliqgklglkllryyteilsfpgpslrdpisaaisiqalsyalggdin
kvleklgysggdllgilesrgikarithvdtesyfivlsiaayptlseikgvivhrlegvsynigsqewyttvpkyvatqg
ylisnfdesstfmpegtvcsqnalypmspllqecrlrgstkscartlvsgsfgnrfilsqgnliancasilckcyttgti
inqdpdkiltyiaadhcpvvevngvtiqvgsrrypdavylhridlppislerldvgtnlgnaiakledakellessdqi
lsa IEDKIEEILSKIYHIENEIARIKKLIGEAP
- MeV F V101C/V117C, R165C/M171C, S194P-GGG-486-GCN4 (SEQ ID NO: 73)
- mysmqlascvtltlvtllvnsqihwgnlskigvvgigsasykvmttrsshqslviklmpnitllnnctrveiaeyrrllrtv
lepardalnavtqnirCqsvassrrrhGGGagvC¹lagaalgvataaqitagialhqsmnlsqaidnlraslettnqaiea
iCqagqeCilaFggvqdyinnelipsmnqLPcdliqgklglkllryyteilsfpgpslrdpisaaisiqalsyalggdin
kvleklgysggdllgilesrgikarithvdtesyfivlsiaayptlseikgvivhrlegvsynigsqewyttvpkyvatqg
ylisnfdesstfmpegtvcsqnalypmspllqecrlrgstkscartlvsgsfgnrfilsqgnliancasilckcyttgti
inqdpdkiltyiaadhcpvvevngvtiqvgsrrypdavylhridlppislerldvgtnlgnaiakledakellessdqi
lsa IEDKIEEILSKIYHIENEIARIKKLIGEAP

- MeV F Q102C/V117C, R165C/M171C, S194P-GGG-486-GCN4 (SEQ ID NO: 74)
mymqglascvtltlvllvnsqihwgnlskigvvgigsasykvmtrsshqslviklmpnitllnnctrveiaeyrrllrtv
lepardalnavtqnirpvcsvassrrhGGGagvC^lagaaalgvataaqitagialhqsmlnsqaidnlraslettnqaeia
iC^qagaggeCilaF^qggvqdyinnelipsmnqlP^cdligqklglkllryyteilsf^gpslrdpisaaisiqalsyalggdin
5 kvleklysggdllgilesrgikarithvdtesyfivlsiaayptlseikgvivhrlegvsynigsqewyttvpkyvatgg
ylisnfdesstfmpegtvcsqnalypmspllqecrlrgstkscartlvsgsfgnrfilsqgnliancasilckcyttgti
inqdpdkiltyiaadhcpvvevngvtiqvgsrrypdavylhrldlppislerldvgtnlgnaiakledakeillessdqi
lsa IEDKIEEILSKIYHIENEIARIKKLIGEAP
- 10 MeV F R165C/M171C, M141C/T270C-GGG-486-GCN4 (SEQ ID NO: 75)
mymqglascvtltlvllvnsqihwgnlskigvvgigsasykvmtrsshqslviklmpnitllnnctrveiaeyrrllrtv
lepardalnavtqnirpvcsvassrrhGGGagvvlagaalgvataaqitagialhqsC^lnsqaidnlraslettnqaeia
iC^qagaggeCilavqgvqdyinnelipsmnqlscdligqklglkllryyteilsf^gpslrdpisaaisiqalsyalggdin
kvleklysggdllgilesrgikariC^hvdtesyfivlsiaayptlseikgvivhrlegvsynigsqewyttvpkyvatgg
15 ylisnfdesstfmpegtvcsqnalypmspllqecrlrgstkscartlvsgsfgnrfilsqgnliancasilckcyttgti
inqdpdkiltyiaadhcpvvevngvtiqvgsrrypdavylhrldlppislerldvgtnlgnaiakledakeillessdqi
lsa IEDKIEEILSKIYHIENEIARIKKLIGEAP
- MeV F R165C/M171C, E212C/Y236C-GGG-486-GCN4 (SEQ ID NO: 76)
20 mymqglascvtltlvllvnsqihwgnlskigvvgigsasykvmtrsshqslviklmpnitllnnctrveiaeyrrllrtv
lepardalnavtqnirpvcsvassrrhGGGagvvlagaalgvataaqitagialhqsmlnsqaidnlraslettnqaeia
iC^qagaggeCilavqgvqdyinnelipsmnqlscdligqklglkllryytC^lilsf^gpslrdpisaaisiqalsC^qalggdin
kvleklysggdllgilesrgikarithvdtesyfivlsiaayptlseikgvivhrlegvsynigsqewyttvpkyvatgg
ylisnfdesstfmpegtvcsqnalypmspllqecrlrgstkscartlvsgsfgnrfilsqgnliancasilckcyttgti
25 inqdpdkiltyiaadhcpvvevngvtiqvgsrrypdavylhrldlppislerldvgtnlgnaiakledakeillessdqi
lsa IEDKIEEILSKIYHIENEIARIKKLIGEAP
- MeV F R165C/M171C, R48C/A284C -GGG-486-GCN4 (SEQ ID NO: 77)
30 mymqglascvtltlvllvnsqihwgnlskigvvgigsasykvmT^csshqslviklmpnitllnnctrveiaeyrrllrtv
lepardalnavtqnirpvcsvassrrhGGGagvvlagaalgvataaqitagialhqsmlnsqaidnlraslettnqaeia
iC^qagaggeCilavqgvqdyinnelipsmnqlscdligqklglkllryyteilsf^gpslrdpisaaisiqalsyalggdin
kvleklysggdllgilesrgikarithvdtesyfivlsiC^yptlseikgvivhrlegvsynigsqewyttvpkyvatgg
ylisnfdesstfmpegtvcsqnalypmspllqecrlrgstkscartlvsgsfgnrfilsqgnliancasilckcyttgti
35 inqdpdkiltyiaadhcpvvevngvtiqvgsrrypdavylhrldlppislerldvgtnlgnaiakledakeillessdqi
lsa IEDKIEEILSKIYHIENEIARIKKLIGEAP
- MeV F R99C/V117C, native furin-486-GCN4 (SEQ ID NO: 78)
mymqglascvtltlvllvnsqihwgnlskigvvgigsasykvmtrsshqslviklmpnitllnnctrveiaeyrrllrtv
lepardalnavtqnirC^pvcsvassrrhkrfagvC^lagaaalgvataaqitagialhqsmlnsqaidnlraslettnqaeia
40 irqagqgemilavqgvqdyinnelipsmnqlscdligqklglkllryyteilsf^gpslrdpisaaisiqalsyalggdin
kvleklysggdllgilesrgikarithvdtesyfivlsiaayptlseikgvivhrlegvsynigsqewyttvpkyvatgg
ylisnfdesstfmpegtvcsqnalypmspllqecrlrgstkscartlvsgsfgnrfilsqgnliancasilckcyttgti
inqdpdkiltyiaadhcpvvevngvtiqvgsrrypdavylhrldlppislerldvgtnlgnaiakledakeillessdqi
45 lsa IEDKIEEILSKIYHIENEIARIKKLIGEAP
- MeV F R99C/V117C, V175F, S194P, native furin-486-GCN4 (SEQ ID NO: 79)
mymqglascvtltlvllvnsqihwgnlskigvvgigsasykvmtrsshqslviklmpnitllnnctrveiaeyrrllrtv
lepardalnavtqnirC^pvcsvassrrhkrfagvC^lagaaalgvataaqitagialhqsmlnsqaidnlraslettnqaeia
50 irqagqgemilaF^qggvqdyinnelipsmnqlP^cdligqklglkllryyteilsf^gpslrdpisaaisiqalsyalggdin
kvleklysggdllgilesrgikarithvdtesyfivlsiaayptlseikgvivhrlegvsynigsqewyttvpkyvatgg
ylisnfdesstfmpegtvcsqnalypmspllqecrlrgstkscartlvsgsfgnrfilsqgnliancasilckcyttgti
inqdpdkiltyiaadhcpvvevngvtiqvgsrrypdavylhrldlppislerldvgtnlgnaiakledakeillessdqi
lsa IEDKIEEILSKIYHIENEIARIKKLIGEAP
- 55 MeV F R99C/V117C, R165C/M171C, S194P, native furin-486-GCN4 (SEQ ID NO: 80)
mymqglascvtltlvllvnsqihwgnlskigvvgigsasykvmtrsshqslviklmpnitllnnctrveiaeyrrllrtv
lepardalnavtqnirC^pvcsvassrrhkrfagvC^lagaaalgvataaqitagialhqsmlnsqaidnlraslettnqaeia
iC^qagaggeCilaF^qggvqdyinnelipsmnqlP^cdligqklglkllryyteilsf^gpslrdpisaaisiqalsyalggdin
kvleklysggdllgilesrgikarithvdtesyfivlsiaayptlseikgvivhrlegvsynigsqewyttvpkyvatgg
60 ylisnfdesstfmpegtvcsqnalypmspllqecrlrgstkscartlvsgsfgnrfilsqgnliancasilckcyttgti
inqdpdkiltyiaadhcpvvevngvtiqvgsrrypdavylhrldlppislerldvgtnlgnaiakledakeillessdqi
lsa IEDKIEEILSKIYHIENEIARIKKLIGEAP

- The above sequences include an N-terminal signal peptide, a MeV F ectodomain, and a GCN4 trimerization domain. It will be appreciated that an alternative trimerization domain can be used, such as a T4 Fibrin trimerization domain. Additionally, many of the above sequence include a GGG linker to
- 5 remove the native furin cleavage site separating the F1 and F2 subunits. Alternate glycine linkers can also be used, such as GSG, GGS, or SGG. Additionally, in any of the sequences, the native furin cleavage site can be included instead of the GGG linker. It will be appreciated that the N-terminal signal peptide is removed during cellular processing and is not present in the purified protein. Additionally, the MeV F ectodomain of any of the above sequences can be included in a full-length MeV F protein to provide a
- 10 membrane anchored version of the prefusion MeV F protein, for example for mRNA immunization.

Non-limiting examples of sequences containing a MeV F ectodomain with amino acid substitutions for stabilization in a prefusion conformation linked to a MuV HN ectodomain or a MeV H ectodomain are provided as follows:

- 15 MeV F R165C/M171C-GGG-486-GCN4 + MeV_H (SEQ ID NO: 56)
 mysmqlascvtltlvtllvnsqihwgnlskigvvgigsasykvmtsrsshqslviklmpnitllnnctrveiaeyrrllrtv
 lepardalnnavtqnirpvqsvassrrrhGGGagvvlagaalgvataaqitagialhqsmnlsqaidnlraslettnqaiea
 iCqagqgeCilavqgvqdyinnelipsmnqlscdligqklglkllryyteilsfsgpslrdpisaaisiqalsyalggdin
 kvleklgysggdllgilesrgikarithvdtesyfivlsiaayptlseikgvivhrlegvsynigsqewyttvpkyvatqg
 20 ylisnfdesstfmpegtvcsqnalypmspllqecrlrgstkscartlvsgsfgnrfilsqgnliancasilckcyttgti
 inqdpdkiltyiaadhcpvvevngvtiqvgsrrypdavylhridlgppislerldvgtnlgnaiakledakellessdqi
 lsa IEDKIEEILSKIYHIENEIARIKKLIGEAP GSGGGGGG
 flavskgncsgpttirgqfsnmsslldlylgrgynvssivtmtsqgmyggtylvekpnlsksrseqlsmyrvfevgv
 irnpglgapvfhmtnyleqpvsndlnscmvalgelklaalchgedsitipyqsgkgvvsfqlvklgvwkspadmqswwpl
 25 stddpvidrlylsshrgviadnqakwvpttrtdddklrmetcfqgackgkiqtlcenpewaplkdnrripsygvlsvdsl
 tvelkikiasgfgplithsgmdlyksnhnnvywltippmknalalgvintlewi prfkvspylftvpik eagedchapt
 lpaevgdvklssnlvilpgqdlqyvatydt srhevavvyvyspgsrfsyfprlpikgvpielqvceftwdqklwc
 rhfcvldsesgghithsgmvgmgsctvtvredgtnr
- 30 MeV F R165C/M171C-GGG-486-GCN4 + MuV_HN (SEQ ID NO: 57)
 mysmqlascvtltlvtllvnsqihwgnlskigvvgigsasykvmtsrsshqslviklmpnitllnnctrveiaeyrrllrtv
 lepardalnnavtqnirpvqsvassrrrhGGGagvvlagaalgvataaqitagialhqsmnlsqaidnlraslettnqaiea
 iCqagqgeCilavqgvqdyinnelipsmnqlscdligqklglkllryyteilsfsgpslrdpisaaisiqalsyalggdin
 kvleklgysggdllgilesrgikarithvdtesyfivlsiaayptlseikgvivhrlegvsynigsqewyttvpkyvatqg
 35 ylisnfdesstfmpegtvcsqnalypmspllqecrlrgstkscartlvsgsfgnrfilsqgnliancasilckcyttgti
 inqdpdkiltyiaadhcpvvevngvtiqvgsrrypdavylhridlgppislerldvgtnlgnaiakledakellessdqi
 lsa IEDKIEEILSKIYHIENEIARIKKLIGEAPG sggggg
 niplvndlr finginkfiiedyathdfsighplnmpsfiptat spngctripsfslgkthwcythnvinanckdhtssnq
 yvsmgilvqtasgypmfktlkiqylsdglnrkscsiatvpdgcamycyvstqletddyagssptqkltllyfndvtvtr
 40 tispsglegnwatlvpgvgsgiyfenklifpayggvlpnstlgvksareffrpvnpyncpsgpgqddqralrsyfpsyf
 snriqsafllvcawnqilvtncelvpssngtmmgaegrvllinnrlllyqrstswwpelyellieisftftnsgpssvnms
 wipiysftrpgsgncsgenvcptacvsgvyldpwltpyshqsginrnfyftgallnssttrvnptlyvsalnlnklvap
 ygtqglfasytthttcfqdtgdasvvcyimelasnivgefqiilpvltrltit
- 45 MeV F R165C/M171C-GGG-486-GCN4/Fd + MeV_H (SEQ ID NO: 81)
 mysmqlascvtltlvtllvnsqihwgnlskigvvgigsasykvmtsrsshqslviklmpnitllnnctrveiaeyrrllrtv
 lepardalnnavtqnirpvqsvassrrrhGGGagvvlagaalgvataaqitagialhqsmnlsqaidnlraslettnqaiea
 iCqagqgeCilavqgvqdyinnelipsmnqlscdligqklglkllryyteilsfsgpslrdpisaaisiqalsyalggdin
 kvleklgysggdllgilesrgikarithvdtesyfivlsiaayptlseikgvivhrlegvsynigsqewyttvpkyvatqg
 50 ylisnfdesstfmpegtvcsqnalypmspllqecrlrgstkscartlvsgsfgnrfilsqgnliancasilckcyttgti
 inqdpdkiltyiaadhcpvvevngvtiqvgsrrypdavylhridlgppislerldvgtnlgnaiakledakellessdqi
 lsa IEDKIEEILSKIYHIENEIARIKKLIGEAP GS GYIPEAPRDGQAYVRKDG EWVLLSTFL GSGGGGGG
 flavskgncsgpttirgqfsnmsslldlylgrgynvssivtmtsqgmyggtylvekpnlsksrseqlsmyrvfevgvirnpg
 lgapvfhmtnyleqpvsndlnscmvalgelklaalchgedsitipyqsgkgvvsfqlvklgvwkspadmqswwplstddp

vidrlylssshrgviadngakwvpttrtdddklrmetcfqqackgkiqtlcenpewaplkdnrripsygvlsvdlsitvelk
 ikiasgfgplithgsgmdlyksnhnnvyltippmknalgvintlewi prfkvspylftvpik eagedchaptylpaev
 dgdvklssnlvilpgqdlqyvltydtsrvehavvyvyspgrsfyfprlpikgvpielqvecftwdqklwcrhfcv
 ladesegghithsgmvgmgvscvtvredgt nrr

5

The above sequences include an N-terminal signal peptide, a MeV F ectodomain, a trimerization domain (GCN4 and/or T4Fibritin), and a MeV H ectodomain head or a MuV HN ectodomain head region. The MeV F ectodomain sequence includes a GGG linker to remove the native furin cleavage site separating the F1 and F2 subunits. Alternate glycine linkers can also be used, such as GSG, GGS, or SGG.

10 Additionally, in any of the sequences, the native furin cleavage site can be included instead of the GGG linker. It will be appreciated that the N-terminal signal peptide is removed during cellular processing and is not present in the purified protein.

C. *MuV HN and MuV H Multimers*

15 In some embodiments, an immunogen is provided that comprises a multimer of the MuV HN ectodomain and/or the MeV H ectodomain. The H or HN ectodomain can include the ectodomain head or the ectodomain stalk and head.

In some embodiments, the immunogen comprises a trimer of fusion proteins, each fusion protein comprising one or more MuV HN ectodomains or MeV H ectodomains and a trimerization domain (such as a GCN4 trimerization domain, a T4 fibritin trimerization domain, or a GCN4 trimerization domain fused to a T4 fibritin trimerization domain).

20 In some embodiments, the fusion protein comprises, in an N- to C-terminal direction, a trimerization domain (such as a GCN4 trimerization domain, a T4 fibritin trimerization domain, or a GCN4 trimerization domain fused to a T4 fibritin trimerization domain) and one or more (such as one, two, or three) MuV HN
 25 ectodomains or Mev H ectodomains. The trimerization domains interact to form the trimer. In some embodiments, a fragment of the ectodomain is included, for example the head region of the MuV HN ectodomain or the head region of the MeV H ectodomain can be fused to the trimerization domain, optionally by a peptide linker. In some embodiment, the fusion proteins in the trimer comprise or consist of an amino acid sequence set forth as residues 24-510 of SEQ ID NO: 58 or residues 24-496 of SEQ ID NO:
 30 59, or a sequence at least 90% identical to any one of residues 25-510 of SEQ ID NO: 58 or residues 25-496 of SEQ ID NO: 59.

In some embodiments, the fusion protein comprises, in an N- to C-terminal direction, one or more (such as one, two, or three) MuV HN ectodomains or Mev H ectodomains, a trimerization domain (such as a GCN4 trimerization domain, a T4 fibritin trimerization domain, or a GCN4 trimerization domain fused to a
 35 T4 fibritin trimerization domain), and one or more (such as one, two, or three) MuV HN ectodomains or Mev H ectodomains. The trimerization domains interact to form the trimer. In some embodiments, a fragment of the ectodomain is included, for example the head region of the MuV HN ectodomain or the head region of the MeV H ectodomain can be fused to the trimerization domain, optionally by a peptide linker. In some embodiment, the fusion proteins in the trimer comprise or consist of an amino acid sequence set forth
 40 as residues 22-950 of SEQ ID NO: 82, residues 25-985 of SEQ ID NO: 83, residues 22-948 of SEQ ID NO:

84, or residues 22-981 of SEQ ID NO: 85, or a sequence at least 90% identical to any one of residues 22-950 of SEQ ID NO: 82, residues 25-985 of SEQ ID NO: 83, residues 22-948 of SEQ ID NO: 84, or residues 22-981 of SEQ ID NO: 85.

In some embodiments, the multimer is a dimer of MeV H ectodomain head regions. The MeV H ectodomain head region can be expressed in mammalian cells and spontaneously forms a dimer in physiological solution. This dimer can then be purified and used as an immunogen. In some embodiments, the subunits of the dimer comprise or consist of an amino acid sequence set forth as residues 22-459 of SEQ ID NO: 60 or a sequence at least 90% identical to residues 22-459 of SEQ ID NO: 60.

In some embodiments, the multimer is a dimer of MeV H ectodomain stalk and head regions. The MeV H ectodomain stalk and head region can be expressed in mammalian cells and spontaneously form a dimer in physiological solution. This dimer can then be purified and used as an immunogen. In some embodiments, the subunits of the dimer comprise or consist of an amino acid sequence of any one of MeV H positions 59-197 to MeV H position 617 (such as any one of MeV H positions 59-67 to MeV H position 617, for example positions 59-617, 62-617, 60-617, or 67-617). In some embodiments, the subunits of the dimer comprise or consist of an amino acid sequence set forth as residues 22-580 of SEQ ID NO: 86, residues 22-577 of SEQ ID NO: 87, residues 22-579 of SEQ ID NO: 88, or residues 22-572 of SEQ ID NO: 89, or a sequence at least 90% identical to residues 22-580 of SEQ ID NO: 86, residues 22-577 of SEQ ID NO: 87, residues 22-579 of SEQ ID NO: 88, or residues 22-572 of SEQ ID NO: 89.

In some embodiments, the multimer is a dimer of MuV HN ectodomain head regions. In some embodiments, the subunits of the dimer comprise or consist of an amino acid sequence set forth as SEQ ID NO: 30 or a sequence at least 90% identical to SEQ ID NO: 30.

In some embodiments, the multimer is a dimer of MuV H ectodomain stalk and head regions. In some embodiments, the subunits of the dimer comprise or consist of an amino acid sequence of any one of MuV H positions 54-130 to MuV HN position 582 (such as any one of MuV H positions 54-63 to MuV HN position 582, for example positions 54-582, 61-582, 63-582, or 55-582). In some embodiments, the subunits of the dimer comprise or consist of an amino acid sequence set forth as residues 22-550 of SEQ ID NO: 90, residues 22-543 of SEQ ID NO: 91, residues 22-541 of SEQ ID NO: 92, or residues 22-549 of SEQ ID NO: 93, or a sequence at least 90% identical to residues 22-550 of SEQ ID NO: 90, residues 22-543 of SEQ ID NO: 91, residues 22-541 of SEQ ID NO: 92, or residues 22-549 of SEQ ID NO: 93.

D. Additional Description

The protomers in the recombinant MuV or MeV F ectodomain trimer can comprise modifications of the native MuV F or MeV F sequence in addition to those noted above, such as amino acid substitutions, deletions or insertions, glycosylation and/or covalent linkage to unrelated proteins (*e.g.*, a protein tag), as long as the recombinant MuV or MeV F ectodomain trimer remains stabilized in the prefusion conformation and retains immunogenicity. Further, in embodiments including a heterologous MuV HN ectodomain or a MeV H ectodomain, or a multimer of a MuV HN ectodomain or a MeV H ectodomain, the HN or H

ectodomain can include modifications of the native HN or H sequence, such as amino acid substitutions, deletions or insertions, glycosylation and/or covalent linkage to unrelated proteins (*e.g.*, a protein tag), as long as the HN or H ectodomain retains immunogenicity. These variations in sequence can be naturally occurring variations or they can be engineered through the use of genetic engineering technique known to those skilled in the art. Examples of such techniques are found in see, *e.g.*, Sambrook *et al.* (Molecular Cloning: A Laboratory Manual, 4th ed., Cold Spring Harbor, New York, 2012) and Ausubel *et al.* (In Current Protocols in Molecular Biology, John Wiley & Sons, New York, through supplement 104, 2013, both of which are incorporated herein by reference in their entirety.

In some embodiments, the protomers in the recombinant MuV F ectodomain trimer or comprise one or more amino acid substitutions compared to a corresponding native MuV F sequence. For example, in some embodiments, the F₂ polypeptide, F₁ ectodomain, or both, can include up to 20 (such as up to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, or 19) amino acid substitutions (such as conservative amino acid substitutions) compared to a native MuV F sequence.

In some embodiments, the protomers in the recombinant MeV F ectodomain trimer or comprise one or more amino acid substitutions compared to a corresponding native MeV F sequence. For example, in some embodiments, the F₂ polypeptide, F₁ ectodomain, or both, can include up to 20 (such as up to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, or 19) amino acid substitutions (such as conservative amino acid substitutions) compared to a native MeV F sequence.

In some embodiments, the MuV HN ectodomain comprises one or more amino acid substitutions compared to a corresponding native MuV HN ectodomain sequence. For example, in some embodiments, the MuV HN ectodomain includes up to 20 (such as up to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, or 19) amino acid substitutions (such as conservative amino acid substitutions) compared to a native MuV HN ectodomain sequence.

In some embodiments, the MeV H ectodomain comprises one or more amino acid substitutions compared to a corresponding native MeV H ectodomain sequence. For example, in some embodiments, the MeV H ectodomain includes up to 20 (such as up to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, or 19) amino acid substitutions (such as conservative amino acid substitutions) compared to a native MeV H ectodomain sequence.

The simplest modifications involve the substitution of one or more amino acids for amino acids having similar biochemical properties, such as conservative amino acid substitutions. Such substitutions are likely to have minimal impact on the activity of the resultant protein.

In some embodiments, protomers in the recombinant MeV F ectodomain trimer or the MuV F ectodomain trimer can be joined at either end to other unrelated sequences (for example non-MuV F or MeV F protein sequences, non-viral envelope, or non-viral protein sequences)

In several embodiments, the recombinant MuV F ectodomain trimer or MeV F ectodomain trimer, or fusions of these protomers with heterologous proteins such as MuV HN ectodomain or MeV H ectodomain is soluble in aqueous solution. In some embodiments, the recombinant MuV F ectodomain

trimer MeV F ectodomain trimer, or corresponding fusions with heterologous proteins such as MuV HN ectodomain or MeV H ectodomain dissolves to a concentration of at least 0.5 mg/ml (such as at least 1.0 mg/ml, 1.5 mg/ml, 2.0 mg/ml, 3.0 mg/ml, 4.0 mg/ml or at least 5.0 mg/ml) in aqueous solution (such as phosphate buffered saline (pH 7.4) or 350 mM NaCl (pH 7.0)) at room temperature (*e.g.*, 20-22 degrees Celsius) and remain dissolved for at least 12 hours (such as at least 24 hours, at least 48 hours, at least one week, at least two weeks, at least one month, or more time). In one embodiment, the phosphate buffered saline includes NaCl (137 mM), KCl (2.7 mM), Na₂HPO₄ (10 mM), KH₂PO₄ (1.8 mM) at pH 7.4. In some embodiments, the phosphate buffered saline further includes CaCl₂ (1 mM) and MgCl₂ (0.5 mM). The person of skill in the art is familiar with methods of determining if a protein remains in solution over time.

For example, the concentration of the protein dissolved in an aqueous solution can be tested over time using standard methods.

In some embodiments, the immunogen is provided as a homogenous population of soluble trimers that are substantially in the prefusion conformation with limited to no MuV F ectodomain trimer and/or MeV F ectodomain trimer in a postfusion conformation. The conformation of the MeV F ectodomain trimer or the MuV F ectodomain trimer can be detected, for example, by negative stain electron microscopy and/or specific binding by appropriate pre- or post-fusion specific antibody. In some embodiments, at least about 95% of the recombinant MuV F ectodomain trimer or MeV F ectodomain trimer (such as at least about 95%, 96%, 97%, 98%, 99% or 99.9% of the MuV or MeV F proteins) in the homogeneous population are stabilized in the prefusion conformation.

In some embodiments, the recombinant MuV F ectodomain trimer or MeV F ectodomain trimer retains specific binding for a prefusion specific antibody following incubation at 50°C for one hour in phosphate buffered saline. In some embodiments, the recombinant MuV F ectodomain trimer or MeV F ectodomain trimer retains specific binding for a prefusion specific antibody following incubation at 4°C for six months in phosphate buffered saline.

In certain embodiments, an immunogen provided herein may be further modified to contain additional nonproteinaceous moieties that are known in the art and readily available. The moieties suitable for derivatization of the immunogen include but are not limited to water soluble polymers. Non-limiting examples of water soluble polymers include, but are not limited to, polyethylene glycol (PEG), copolymers of ethylene glycol/propylene glycol, carboxymethylcellulose, dextran, polyvinyl alcohol, polyvinyl pyrrolidone, poly-1,3-dioxolane, poly-1,3,6-trioxane, ethylene/maleic anhydride copolymer, polyaminoacids (either homopolymers or random copolymers), and dextran or poly(n-vinyl pyrrolidone)polyethylene glycol, propylene glycol homopolymers, polypropylene oxide/ethylene oxide co-polymers, polyoxyethylated polyols (*e.g.*, glycerol), polyvinyl alcohol, and mixtures thereof. Polyethylene glycol propionaldehyde may have advantages in manufacturing due to its stability in water. The polymer may be of any molecular weight, and may be branched or unbranched. The number of polymers attached to the antibody may vary, and if more than one polymer are attached, they can be the same or different molecules. In general, the number and/or type of polymers used for derivatization can be determined based on considerations

including, but not limited to, the particular properties or functions of the immunogen to be improved or altered, whether the immunogen derivative will be used in a therapy under defined conditions, etc.

Some of the sequences including recombinant MuV F ectodomain trimer, or MeV F ectodomain trimer, or MuV HN ectodomain or MeV H ectodomain provided herein include the sequence of protease cleavage sites (such as thrombin sites), protein tags (such as a His tag, a Strep Tag II, a Avi tag, etc.), and signal peptides; such sequences can be removed from an isolated immunogen including a recombinant MuV F ectodomain trimer, or MeV F ectodomain trimer, or MuV HN ectodomain or MeV H ectodomain for therapeutic use.

10 E. Protein Nanoparticles

In some embodiments, a protein nanoparticle is provided that includes one or more of the disclosed recombinant MuV F ectodomain trimers or recombinant MeV F ectodomain trimers, or MuV HN, or MeV H multimers, or chimera thereof.

In some embodiments, the protein nanoparticle comprises the MeV F ectodomain trimer or MuV F ectodomain trimer displayed on a two-component self-assembling nanoparticle platform as described in Marcandalli et al. "Induction of potent neutralizing antibody responses by a designed protein nanoparticle vaccine for respiratory syncytial virus," Cell, 176(6):1420-1431, 2019, which is incorporated by reference herein.

In additional non-limiting example of nanoparticles include ferritin nanoparticles, encapsulin nanoparticles, Sulfur Oxygenase Reductase (SOR) nanoparticles, and lumazine synthase nanoparticles, which are comprised of an assembly of monomeric subunits including ferritin proteins, encapsulin proteins, SOR proteins, and lumazine synthase, respectively. To construct such protein nanoparticles a protomer of the recombinant MuV F ectodomain trimer or recombinant MeV F ectodomain trimer, or a subunit of a MuV HN or MeV H multimer, is linked to a subunit of the protein nanoparticle (such as a ferritin protein, an encapsulin protein, a SOR protein, or a lumazine synthase protein) and expressed in cells under appropriate conditions. The fusion protein self-assembles into a nanoparticle any can be purified.

In some embodiments, a protomer of a disclosed recombinant MuV F ectodomain trimer or recombinant MeV F ectodomain trimer, or a subunit of a MuV HN or MeV H multimer, can be linked to a ferritin subunit to construct a ferritin nanoparticle. Ferritin nanoparticles and their use for immunization purposes (e.g., for immunization against influenza antigens) have been disclosed in the art (see, e.g., Kanekiyo et al., Nature, 499:102-106, 2013, incorporated by reference herein in its entirety). The globular form of the ferritin nanoparticle is made up of monomeric subunits, which are polypeptides having a molecule weight of approximately 17-20 kDa. Following production, these monomeric subunit proteins self-assemble into the globular ferritin protein. Thus, the globular form of ferritin comprises 24 monomeric, subunit proteins, and has a capsid-like structure having 432 symmetry. Methods of constructing ferritin nanoparticles are further described herein (see, e.g., Zhang, Int. J. Mol. Sci., 12:5406-5421, 2011, which is

incorporated herein by reference in its entirety). An example of the amino acid sequence of one such monomeric ferritin subunit is represented by:

DI IKLLNEQVNKEMQSSNLYMSMSSWCYTHSLDGAGLFLFDHAAEEYEHAKKLIIFLNENNVPVQLTSISAP
 5 EH KFEGLTQIFQKAYEHEQHISESINNIVDHAISKDHATFNFLQWYVAEQHEEEVLFDKILDKIELIGNEN
 HGLYLADQYVKGIAKSRKS (SEQ ID NO: 45)

In specific examples, the ferritin polypeptide is *E. coli* ferritin, *Helicobacter pylori* ferritin, human light chain ferritin, bullfrog ferritin or a hybrid thereof, such as *E. coli*-human hybrid ferritin, *E. coli*-bullfrog hybrid ferritin, or human-bullfrog hybrid ferritin. Exemplary amino acid sequences of ferritin polypeptides and nucleic acid sequences encoding ferritin polypeptides for use to make a ferritin nanoparticle including a recombinant MuV or MeV F ectodomain trimer can be found in GENBANK®, for example at accession numbers ZP_03085328, ZP_06990637, EJB64322.1, AAA35832, NP_000137 AAA49532, AAA49525, AAA49524 and AAA49523, which are specifically incorporated by reference herein in their entirety as available April 10, 2015. In some embodiments, a protomer of a recombinant MuV or MeV F ectodomain trimer can be linked to a ferritin subunit including an amino acid sequence at least 80% (such as at least 85%, at least 90%, at least 95%, or at least 97%) identical to amino acid sequence set forth as SEQ ID NO: 45.

In some embodiments, a protomer of a disclosed recombinant MuV F ectodomain trimer or recombinant MuV or MeV F ectodomain trimer, or a subunit of a MuV HN or MeV H multimer, can be linked to a lumazine synthase subunit to construct a lumazine synthase nanoparticle. The globular form of lumazine synthase nanoparticle is made up of monomeric subunits; an example of the sequence of one such lumazine synthase subunit is provides as the amino acid sequence set forth as:

MQIYEGKLTAEGLRFGIVASRFNHALVDRLVEGAIDCIVRHGGREEDITLVRVPGSWEIPVAAGELARKEDI
 25 DAVIAIGVLIRGATPHFDYIASEVSKGLADLSLELRKPITFGVITADTLEQAIERAGTKHGNKGWEAALSAL
 EMANLFKSLR (SEQ ID NO: 46).

In some embodiments, a protomer of a disclosed recombinant MuV F ectodomain trimer or recombinant MeV F ectodomain trimer, or a subunit of a MuV HN or MeV H multimer, can be linked to a lumazine synthase subunit including an amino acid sequence at least 80% (such as at least 85%, at least 90%, at least 95%, or at least 97%) identical to amino acid sequence set forth as SEQ ID NO: 46.

In some embodiments, a protomer of a disclosed recombinant MuV F ectodomain trimer or recombinant MeV F ectodomain trimer, or a subunit of a MuV HN or MeV H multimer, can be linked to an encapsulin nanoparticle subunit to construct an encapsulin nanoparticle. The globular form of the encapsulin nanoparticle is made up of monomeric subunits; an example of the sequence of one such encapsulin subunit is provides as the amino acid sequence set forth as

MEFLKRSEFAPLTEKQWQEIDNRAREIFKTQLYGRKFVDVEGPGWEYAAHPLGEVEVLSDENEVVKWGLRKS
 35 LPLIELRATFTLDLWELDNLERGKPNVDLSSLEETVRKVAEFEDEVIFRGCEKSGVKGLLSFEERKIECGST
 PKDLLEAIVRALSIFS KDGIEGPYTLVINTDRWINFLKEEAGHYPLEKRVEECLRGKIIITPRIEDALVVS
 ERGGDFKLILGQDLSIGYEDREKDAVRLFITETFTFQVNPALILLKF (SEQ ID NO: 47).

In some embodiments, a protomer of a disclosed recombinant MuV F ectodomain trimer or recombinant MeV F ectodomain trimer, or a subunit of a MuV HN or MeV H multimer, can be linked to an

encapsulin subunit including an amino acid sequence at least 80% (such as at least 85%, at least 90%, at least 95%, or at least 97%) identical to amino acid sequence set forth as SEQ ID NO: 47.

Encapsulin proteins are a conserved family of bacterial proteins also known as linocin-like proteins that form large protein assemblies that function as a minimal compartment to package enzymes. The encapsulin assembly is made up of monomeric subunits, which are polypeptides having a molecule weight of approximately 30 kDa. Following production, the monomeric subunits self-assemble into the globular encapsulin assembly including 60, or in some cases, 180 monomeric subunits. Methods of constructing encapsulin nanoparticles are further described (see, for example, Sutter *et al.*, Nature Struct. and Mol. Biol., 15:939-947, 2008, which is incorporated by reference herein in its entirety). In specific examples, the encapsulin polypeptide is bacterial encapsulin, such as *Thermotoga maritime* or *Pyrococcus furiosus* or *Rhodococcus erythropolis* or *Myxococcus xanthus* encapsulin.

In some embodiments, a protomer of a disclosed recombinant MuV F ectodomain trimer or recombinant MeV F ectodomain trimer, or a subunit of a MuV HN or MeV H multimer, can be linked to a Sulfur Oxygenase Reductase (SOR) subunit to construct a recombinant SOR nanoparticle. In some embodiments, the SOR subunit can include the amino acid sequence set forth as

MEFLKRSFAPLTEKQWQEI DN RAREIFKTQLYGRKFVDVEGPYGWEYAAHPLGEVEVLSDENEVVKWGLRKS
LPLIELRATFTLDLWELDNLERGKPNVDLSSLEETVRKVAEFEDEVIFRGCEKSGVKGLLSFEERKIECGST
PKDLLEAIVRALSIFS KDGIEGPYTLVINTDRWINFLKEEAGHYPLEKRVEECLRGGKIITTPRIEDALVVS
ERGGDFKLILGQDLSIGYEDREKDAVRLFITETFTFQVVNPEALILLKF (SEQ ID NO: 48).

In some embodiments, a protomer of a disclosed recombinant MuV F ectodomain trimer or recombinant MeV F ectodomain trimer, or a subunit of a MuV HN or MeV H multimer, can be linked to a SOR subunit including an amino acid sequence at least 80% (such as at least 85%, at least 90%, at least 95%, or at least 97%) identical to amino acid sequence set forth as SEQ ID NO: 48.

SOR proteins are microbial proteins (for example from the thermoacidophilic archaeon *Acidianus ambivalens* that form 24 subunit protein assemblies. Methods of constructing SOR nanoparticles are described in Ulrich *et al.*, Science, 311:996-1000, 2006, which is incorporated by reference herein in its entirety. An example of an amino acid sequence of a SOR protein for use to make SOR nanoparticles is set forth in Ulrich *et al.*, Science, 311:996-1000, 2006, which is incorporated by reference herein in its entirety.

For production purposes, the recombinant MuV F ectodomain or recombinant MeV F ectodomain, or the subunit of the MuV HN or MeV H multimer, linked to the nanoparticle subunit can include an N-terminal signal peptide that is cleaved during cellular processing. For example, the recombinant MuV F ectodomain protomer, or the recombinant MeV F ectodomain protomer, linked to the protein nanoparticle subunit can include a signal peptide at its N-terminus including, for example, a native MuV or MeV F signal peptide.

The protein nanoparticles can be expressed in appropriate cells (e.g., HEK 293 Freestyle cells) and fusion proteins are secreted from the cells self-assembled into nanoparticles. The nanoparticles can be purified using known techniques, for example by a few different chromatography procedures, e.g. Mono Q (anion exchange) followed by size exclusion (SUPEROSE® 6) chromatography.

The fusion proteins need not comprise the full-length sequence of a monomeric subunit polypeptide of a ferritin, encapsulin, SOR, or lumazine synthase protein. Portions, or regions, of the monomeric subunit polypeptide can be utilized so long as the portion comprises amino acid sequences that direct self-assembly of monomeric subunits into the globular form of the protein.

5

II. Polynucleotides and Expression

Also provided are polynucleotides encoding any of the disclosed immunogens. For example, a polynucleotide encoding a protomer of a MuV F ectodomain trimer stabilized in the prefusion conformation, a protomer of a MeV F ectodomain trimer stabilized in the prefusion conformation, a chimera of one of these protomers linked to a MuV HN or MeV H ectodomain, or a subunit of a self-assembling protein nanoparticle containing a recombinant MuV or MeV F ectodomain. These polynucleotides include DNA, cDNA and RNA sequences, including vectors including the DNA, cDNA and RNA sequences, such as a DNA or RNA vector used for immunization. The genetic code can be used to construct a variety of functionally equivalent nucleic acids, such as nucleic acids which differ in sequence but which encode the same protein sequence, or encode a conjugate or fusion protein including the nucleic acid sequence.

15

An exemplary nucleic acid sequence encoding full-length MeV F protein is provided as SEQ ID

NO: 94:

atgggtctcaagggtgaacgtctctgccatattcatggcagtagtctgtaactctccaaacacccaccgggtcaaatccattg
gggcaatctctctaagataggggtggttaggaataggaagtgcagctacaaagttatgactcggtccagccatcaatcat
tagtcataaaattaatgcccaatataactctcctcaataactgcacgagggtagagattgcagaatacaggagactactg
agaacagttttggaaccaattagagatgcacttaatgcaatgaccagaatataagaccggttcagagtgtagcttcaag
taggagacacaaagatttgccgggagtagtcctggcagggtgcggccctaggcgttgccacagctgctcagataacagccg
gcattgcacttcaccagtcctatgctgaactctcaagccatcgacaatctgagagcgagcctggaaactactaatcaggca
attgaggcaatcagacaagcagggcaggagatgatattggctgttcagggtgtccaagactacatcaataatgagctgat
accgtctatgaaccaactatcttgtgatttaactcgccagaagctcgggctcaaattgctcagatactatacagaaatcc
tgtcattatattggccccagcttacgggaccccatatctgcggagatatctatccaggctttgagctatgcgcttggagga
gacatcaataaggtgttagaaaagctcggatcacgtggaggtgatttactgggcatcttagagagcagaggaataaaggc
ccggataactcacgtcgacacagagtcctacttaattgtcctcagtagcctatccgacgctgtccgagattaaggggg
tgattgtccaccggctagaggggtctcgtacaacataggctctcaagagtggtataccactgtgcccaagtatgttgca
acccaaggggtaccttatctcgaattttgatgagtcctgtgactttcatgccagaggggactgtgtgcagccaaaatgc
cttgtaccgatgagtcctctgctccaagaatgcctcgggggtccaccaagtcctgtgctcgtacactcgtatccgggt
cttttggaaccgggttcattttatcacaagggaacctaatagccaattgtgcatcaatcctttgcaagtggttacacaaca
ggaacgatcattaatcaagaccctgacaagatcctaacatacattgctgccgatcactgcccggtagtcgagggtgaacgg
cgtgaccatccaagtcgggagcaggaggtatccagacgctgtgtacttgacagaattgacctcggctcctccatattat
tggagaggttggacgtagggacaaaatctggggaatgcaattgctaagttggaggatgccaaaggaattgttggagtcacg
gaccagatattgaggagtagaaagggttatcgagcacttgcatagctacatcctgattgcagtggtcttggagggtt
gatagggatccccgctttaatatgttgctgcagggggcgtttgtaacaaaagggaacaagttggtatgtcagaccag
gcctaaagcctgatcttacgggaacatcaaaatcctatgtaagggtcgctctga

40

An exemplary nucleic acid sequence encoding full-length MuV F protein is provided as SEQ ID

NO: 95:

atgaaggccttttcagttacttgcttgggctttgcagtcctttcgtcttctatatgtgtgaatatcaacatcttgcagca
aattggatatatcaagcaacaagtcaggcaactaagctattactcacaaagttctagctcctacatagtggtcaagcttt
taccgaatatccaaccactgataacagctgtgaatttaagagtgttaactcaatacaataagaccttgagtaacttgctt
cttcccatgtcagaaaaacataaacaatattgcatcgccctcacctgggtcaagacgtcataaaagggttggctggcattgc
cattggcattgtgcgctcgggtgttgcgaccgcagcacaggtaactgcgctgtctcattagttcaagcacagacaaaatg
cacgtgcaatagcggcgatgaaaaattcaatacaggcaactaatcgggcaatcttcgaagtgaagggaaggcaccacaag
ttagctatagcgggtacaagcaatacaagaccacatcaatactattatgaacaccaattgaacaatatgtcttgcagat
ccttgataaaccagcttgcaacctacctaggattatacctaacagaatttaacaacagtggttcagccacaatttaataatc

45

cggcattgtcaccgattagtagatatacaagccttgaggctcttctgcttgggaagtatgacgcctgcagtggttcaagcaacatta
 tctacgtcaatttctgctgctgaaataactaagtgcgggtctaatggagggtcagattggttctgctgctagatgagat
 gcagatgatagtcgaagataaataattccaaccattgtcacacaatcaaatgcattggtgattgacttctactcaatttcca
 5 gctttatataataatcaagaatccataattcaattgccagacaggatcctagagatcggaatgaacaatggagctatcca
 gctaaaaattgttaagttgacaagacaccacatattctgccaatacaatgaggcagagaggctgagcctagaatcaaaact
 atgccttgccaggtaatataagtgccctgtgtgttctcaccatagcaggaggattatagaggcgattttagcactggatg
 gaacaattggttcaaaactgtcgaagtctaactgtctatgcaagagtcctattctctatataaccaactgaccatcat
 10 gcagtcacgaccattgatctaaccacatgtcaaacattgtccctagacggattggacttcagcattgtctcttaagcaa
 catcacttacgctgagaaccttaccatttctattgtctcaaacatcaatactcaaccattgacatatcaactgaactga
 gtaaaagttaatgcatccctccaaaatgccgttaagtacataaaaggagagcaaccatcaactccaatctgtgagtgtaaat
 tccaaaattggagctataattgtagcagccttagttttagcatcctgtcaattatcatttgcgtattgttttgcgtgctg
 ggcttacattgcaactaaagaatcagaagaatcaacttcaaaccaaatcatatcaacacaatatcgagtagtgctgatg
 atctcatcaggtactaa

15 An exemplary nucleic acid sequence encoding full-length MeV H protein is provided as SEQ ID

NO: 96:

atgtcaccacaacgagaccggataaatgccttctacaaagataacccccatcccaagggaagtaggatagtcattaacag
 agaacatcttatgattgatagacctatgttttgcctggtgttctgttctgtcatgtttctgagcttgatcggttgctag
 ccattgcaggcattagacttcatcgggcagccatctacaccgcagagatccataaaagcctcagcaccatctagatgta
 20 actaactcaatcgagcatcaggtcaaggacgtgctgacaccactcttcaaaatcatcggtgatgaagtgggctgaggac
 acctcagagattcactgacctagtgaattctctgtacaaagattaaattccttaatccggataggagtagcagcttca
 gagatctcacttgggtgatcaaccgcagagagaatcaaatggattatgatcaatactgtgcagatgtggctgctgaa
 gagctcatgaatgattggtgaactcaactctactggagaccagaacaaccaatcagttcctagctgtctcaaaaggaaa
 ctgctcagggcccactacaatcagaggtcaattctcaaacatgtcgcgtgtccctgttagacttgtatttaggtcgaggtt
 25 acaatgtgtcatctatagtcactatgacatcccagggaatgtatgggggaacttacctagtggaaaagcctaactctgagc
 agcaaaagggtcagagttgtcacaactgagcatgtaccgagtggttgaagtagggtttatcagaatccgggttgggggc
 tccgggtgttccatagacaaactatcttgagcaaccagtcagtaatgatctcagcaactgtatggtggcttgggggagc
 tcaaaactcgcagcccttctgcacggggaagattctatcacaattccctatcagggatcagggaagggtgtcagcttccag
 ctgctcaagctagggtgtctggaaatccccaacgcacatgcaatcctgggtcccttatcaacggatgatccagtgataga
 30 caggcttacctctcatctcacagaggtgttatcgctgacaatcaagcaaaatgggctgtcccgacaacacgaacagatg
 acaagttggaatggagacatgcttccaacaggcgtgtaagggttaaaatccaagcactctgcgagaatcccgagtgaggca
 ccattgaaggataacaggattccttcatacggggtctgtctgttgatctgagctgacagttgagcttaaaatcaaaat
 tgcttcgggattcggggccattgatcacacacgggttcagggatggacctatacaaatccaaccacaacaatgtgtattggc
 tgactatcccgcaatgaagaacctagccttaggtgtaatacaacattggagtgataccgagattcaagggtagctccc
 35 tactcttcaactgtcccaattaaaggaagcaggcgaagactgcatgcccaacatacctaactgcggaggtggatggtga
 tgtcaaaactcagttccaatctggtgattctacctggtcaagatctccaatattgttttggcaacctacgatacttccaggg
 ttgaacatgctgtgggtttattacgtttacagcccaagcgcctatcttcttactttatccttttaggttgctataaaag
 ggggtcccatcgaattacaagtgggaatgcttcacatgggacaaaactctggtgcccgtcacttctgtgtgcttgcgga
 40 ctcagaatctggtggacatatcactcactctgggatggtgggcagtgaggagtcagctgcacagtcacccggaagatggaa
 ccaatcgcagatag

An exemplary nucleic acid sequence encoding full-length MuV HN protein is provided as SEQ ID

NO: 97:

atggagccctcgaaattcttcacaatatcggaacagtccacaccttgcacctgggcctgtcagcaatcgcgctgacaagaa
 45 gacattccgaacctgcttccgaatactggtactatctgtacaagctgtcacccctcatattggttattgtcactttagggtg
 agcttgaaggatgatcaatgatcaaggcttgagcaatcagttgtcttcaattacagacaagataagagagtcagctact
 atgattgcatctgctgtgggagtaaatgaatcaagttattcatggagtaaacggtatccttaccctacaaattgagggaaa
 ccaaaatcaattgttagccacacttgccacaatctgcgcagccaaaaacaagctcctcaaaactgctctacaaacatccct
 50 tagtcaatgacctcaggtttataatgggatcaataaattattattgaagattacgcaactcatgatttctctatcggc
 catccactcaatatgccagctttatcccaactgcaacttcaccaatggttgcaacaagaattccatccttttctttagg
 taagacacactggtgctacacacataatgtaattaatgccaactgcaaggaccatacttctgcttaaccaatattgtgtcca
 tggggattctcgttcagaccgcgtcagggtatcctatgttcaaaccttaaaaatccaatatctcagtgatggcctgaat
 cggaaaagctgctcaattgcaacagtcctgatgggtgcgcgatgtactgttatgtctctactcaacttgaaccgcagca
 55 ctatgcgggggtccagtcacccaccccaaaaacttacctgttattctataatgacacgctcacagaaaggacaatatctc
 catctggtcttgaagggaattgggctactttggtgccaggagtggggagtgggatataatttggagaataagttgatcttc
 cctgcatatgggggtgcttggccaatagtcacactcggggttaaatcagcaagagaatttttccgctgtttaatccata
 taatccatggttcaggaccacaacaagatttagatcagcgtgctttgaggtcacttcccaagttacttcttaactcgaa
 gaatacagagtgcatcttctgtctgtgcctggaatcagatccttagttacaaaattgtgagctagttgtcccctcaagcaat
 60 cagacaatgatgggtgcagaaggagaggtttattgatcaataatcgactattatattatcagagaagtagcagctggtg
 gccgatgaactcctctacagatatcattcacatttcaaaactctggtccatcatctgtaaatatgtcctggatacctaa

5 tatattcattcactcgtcctgggtcaggcaattgcagtggtgaaaatgtgtgcccgactgcttgtgtgtcaggggtttat
 ctgatccctggccattactccatagccaccaatcaggtattacagaaatttctatttcacaggtgcactattaaa
 ttcaagtacaactagagtaaaccctaccctttatgtctctgcccttaataatcttaaagtactagccccatatggtagctc
 aaggactgtttgcctcgtacaccacaaccacctgctttcaagataccggtgatgctagtgtgtattgtgtttatattatg
 gagctagcatcaaatattgttgagaattccaaattctacctgtgctaactagattgactatcacttga

These exemplary nucleic acid sequences (or a corresponding RNA sequence) can be modified to encode any of the immunogens provided herein.

In several embodiments, the nucleic acid molecule encodes a precursor of a protomer of the MuV or MeV F ectodomain trimer or a promoter of the MeV F ectodomain trimer or a chimera of such a protomer with a MuV HN or MeV H ectodomain, or a subunit of a MuV HN or MeV H multimer, that, when expressed in an appropriate cell, is processed into a protomer of the F ectodomain trimer, or subunit of a MuV HN or MeV H multimer, that can self-assemble into the corresponding trimer or multimer. For example, the nucleic acid molecule can encode a protomer of the MuV or MeV F ectodomain trimer or a promoter of the MeV F ectodomain trimer including a N-terminal signal sequence for entry into the cellular secretory system that is proteolytically cleaved in the during processing of the recombinant F ectodomain in the cell.

In some embodiments, the nucleic acid molecule encodes a F₀ polypeptide that, when expressed in an appropriate cell, is processed into a protomer of the MuV or MeV F ectodomain trimer or a promoter of the MeV F ectodomain trimer including an F₂ polypeptide linked to a F₁ ectodomain, wherein the recombinant F₂-F₁ ectodomain protomer includes any of the prefusion-stabilizing modifications described herein, and optionally can be linked to a trimerization domain, such as a GCN4 trimerization domain and/or a T4 fibrin trimerization domain.

In some embodiments, the nucleic acid molecule encodes a full-length F₀ polypeptide that, when expressed in an appropriate cell, is processed into a protomer of the MuV or MeV F ectodomain trimer or a promoter of the MuV or MeV F ectodomain trimer including an F₂ polypeptide linked to a F₁ polypeptide including the F₁ transmembrane and cytosolic tail, wherein the recombinant F₂-F₁ ectodomain protomer includes any of the prefusion-stabilizing modifications described herein.

Exemplary nucleic acids can be prepared by cloning techniques. Examples of appropriate cloning and sequencing techniques, and instructions sufficient to direct persons of skill through many cloning exercises are known (see, *e.g.*, Sambrook *et al.* (Molecular Cloning: A Laboratory Manual, 4th ed, Cold Spring Harbor, New York, 2012) and Ausubel *et al.* (In Current Protocols in Molecular Biology, John Wiley & Sons, New York, through supplement 104, 2013).

Nucleic acids can also be prepared by amplification methods. Amplification methods include polymerase chain reaction (PCR), the ligase chain reaction (LCR), the transcription-based amplification system (TAS), the self-sustained sequence replication system (3SR). A wide variety of cloning methods, host cells, and *in vitro* amplification methodologies are well known to persons of skill.

The polynucleotides encoding a protomer of the MuV or MeV F ectodomain trimer, or a subunit of a MuV HN or MeV H multimer, can include a recombinant DNA which is incorporated into a vector (such

as an expression vector) into an autonomously replicating plasmid or virus or into the genomic DNA of a prokaryote or eukaryote, or which exists as a separate molecule (such as a cDNA) independent of other sequences. The nucleotides can be ribonucleotides, deoxyribonucleotides, or modified forms of either nucleotide. The term includes single and double forms of DNA.

- 5 Polynucleotide sequences encoding a protomer of the MuV or MeV F ectodomain trimer or a promoter of the MeV F ectodomain trimer or a chimera of such a protomer with a MuV HN or MeV H ectodomain, or a subunit of a MuV HN or MeV H multimer, can be operatively linked to expression control sequences. An expression control sequence operatively linked to a coding sequence is ligated such that expression of the coding sequence is achieved under conditions compatible with the expression control
- 10 sequences. The expression control sequences include, but are not limited to, appropriate promoters, enhancers, transcription terminators, a start codon (*i.e.*, ATG) in front of a protein-encoding gene, splicing signal for introns, maintenance of the correct reading frame of that gene to permit proper translation of mRNA, and stop codons.

- DNA sequences encoding the protomer of the MuV or MeV F ectodomain trimer or a promoter of the MeV F ectodomain trimer or a chimera of such a protomer with a MuV HN or MeV H ectodomain, or a subunit of a MuV HN or MeV H multimer, can be expressed *in vitro* by DNA transfer into a suitable host cell. The cell may be prokaryotic or eukaryotic. The term also includes any progeny of the subject host cell. It is understood that all progeny may not be identical to the parental cell since there may be mutations that occur during replication. Methods of stable transfer, meaning that the foreign DNA is continuously
- 15 maintained in the host, are known in the art.

- Hosts can include microbial, yeast, insect and mammalian organisms. Methods of expressing DNA sequences having eukaryotic or viral sequences in prokaryotes are well known in the art. Non-limiting examples of suitable host cells include bacteria, archaea, insect, fungi (for example, yeast), plant, and animal cells (for example, mammalian cells, such as human). Exemplary cells of use include *Escherichia coli*,
- 25 *Bacillus subtilis*, *Saccharomyces cerevisiae*, *Salmonella typhimurium*, SF9 cells, C129 cells, 293 cells, *Neurospora*, and immortalized mammalian myeloid and lymphoid cell lines. Techniques for the propagation of mammalian cells in culture are well-known (see, *e.g.*, Helgason and Miller (Eds.), 2012, Basic Cell Culture Protocols (Methods in Molecular Biology), 4th Ed., Humana Press). Examples of commonly used mammalian host cell lines are VERO and HeLa cells, CHO cells, and WI38, BHK, and
- 30 COS cell lines, although cell lines may be used, such as cells designed to provide higher expression, desirable glycosylation patterns, or other features. In some embodiments, the host cells include HEK293 cells or derivatives thereof, such as GnTI^{-/-} cells (ATCC® No. CRL-3022), or HEK-293F cells.

- Transformation of a host cell with recombinant DNA can be carried out by conventional techniques. In some embodiments where the host is prokaryotic, such as, but not limited to, *E. coli*, competent cells
- 35 which are capable of DNA uptake can be prepared from cells harvested after exponential growth phase and subsequently treated by the CaCl₂ method. Alternatively, MgCl₂ or RbCl can be used. Transformation can also be performed after forming a protoplast of the host cell if desired, or by electroporation.

When the host is a eukaryote, such methods of transfection of DNA as calcium phosphate coprecipitates, conventional mechanical procedures such as microinjection, electroporation, insertion of a plasmid encased in liposomes, or viral vectors can be used. Eukaryotic cells can also be co-transformed with polynucleotide sequences encoding a disclosed antigen, and a second foreign DNA molecule encoding a selectable phenotype, such as the herpes simplex thymidine kinase gene. Another method is to use a eukaryotic viral vector, such as simian virus 40 (SV40) or bovine papilloma virus, to transiently infect or transform eukaryotic cells and express the protein (see for example, *Viral Expression Vectors*, Springer press, Muzyczka ed., 2011). Appropriate expression systems such as plasmids and vectors of use in producing proteins in cells including higher eukaryotic cells such as the COS, CHO, HeLa and myeloma cell lines.

In one non-limiting example, a disclosed immunogen is expressed using the pVRC8400 vector (described in Barouch *et al.*, *J. Virol.*, 79, 8828-8834, 2005, which is incorporated by reference herein).

Modifications can be made to a nucleic acid encoding a disclosed immunogen without diminishing its biological activity. Some modifications can be made to facilitate the cloning, expression, or incorporation of the targeting molecule into a fusion protein. Exemplary modifications include termination codons, a methionine added at the amino terminus to provide an initiation site, additional amino acids placed on either terminus to create conveniently located restriction sites, or additional amino acids (such as poly His) to aid in purification steps.

In some embodiments, the nucleic acid encoding the protomer of the MuV or MeV F ectodomain trimer or the promoter of the MeV F ectodomain trimer or a chimera of such a protomer with a MuV HN or MeV H ectodomain, or a subunit of a MuV HN or MeV H multimer, can be expressed in cells under conditions where the protomers self-assemble into trimers which are secreted from the cells into the cell media, for example as described for RSV F proteins (see, *e.g.*, PCT Pub. WO2014160463, McLellan *et al.*, *Science*, 340:1113-1117, 2013; McLellan *et al.*, *Science*, 342:592-598, 2013, each of which is incorporated by reference herein in its entirety). In such embodiments, the protomer contains a leader sequence (signal peptide) that causes the protein to enter the secretory system, and the signal peptide is cleaved and the protomers form a trimer, before being secreted in the cell media. The medium can be centrifuged and recombinant MuV or MeV F ectodomain trimer or recombinant MuV or MeV F ectodomain trimer or a chimera thereof with a MuV HN or MeV H ectodomain purified from the supernatant.

III. Viral Vectors

A nucleic acid molecule encoding a disclosed immunogen can be included in a viral vector, for example, for expression of the immunogen in a host cell, or for immunization of a subject as disclosed herein. In some embodiments, the viral vectors are administered to a subject as part of a prime-boost vaccination. Typically such viral vectors include a nucleic acid molecule encoding an immunogen that contains a transmembrane domain. In several embodiments, the viral vectors are included in a vaccine, such as a primer vaccine or a booster vaccine for use in a prime-boost vaccination.

In some examples, the viral vector can be replication-competent. For example, the viral vector can have a mutation (*e.g.*, insertion of nucleic acid encoding the protomer) in the viral genome that attenuates, but does not completely block viral replication in host cells.

In several embodiments, the viral vector can be delivered via the respiratory tract. For example, a hPIV vector, such as bovine parainfluenza virus (BPIV) vector (*e.g.*, a BPIV1, BPIV2, or BPIV3 vector) or human hPIV vector (*e.g.*, a hPIV3 vector), a metapneumovirus (MPV) vector, a Sendia virus vector, a New Castle Disease Virus (NCDV) vector, a mumps virus vector, a measles virus vector, or another paramyxovirus or pneumovirus vector is used to express a disclosed antigen.

Additional viral vectors are also available for expression of the disclosed antigens, including polyoma, *i.e.*, SV40 (Madzak *et al.*, 1992, *J. Gen. Virol.*, 73:1533-1536), adenovirus (Berkner, 1992, *Curr. Top. Microbiol. Immunol.*, 158:39-6; Berliner *et al.*, 1988, *Bio Techniques*, 6:616-629; Gorziglia *et al.*, 1992, *J. Virol.*, 66:4407-4412; Quantin *et al.*, 1992, *Proc. Natl. Acad. Sci. USA*, 89:2581-2584; Rosenfeld *et al.*, 1992, *Cell*, 68:143-155; Wilkinson *et al.*, 1992, *Nucl. Acids Res.*, 20:2233-2239; Stratford-Perricaudet *et al.*, 1990, *Hum. Gene Ther.*, 1:241-256), vaccinia virus (Mackett *et al.*, 1992, *Biotechnology*, 24:495-499), adeno-associated virus (Muzyczka, 1992, *Curr. Top. Microbiol. Immunol.*, 158:91-123; On *et al.*, 1990, *Gene*, 89:279-282), herpes viruses including HSV and EBV and CMV (Margolskee, 1992, *Curr. Top. Microbiol. Immunol.*, 158:67-90; Johnson *et al.*, 1992, *J. Virol.*, 66:2952-2965; Fink *et al.*, 1992, *Hum. Gene Ther.* 3:11-19; Breakfield *et al.*, 1987, *Mol. Neurobiol.*, 1:337-371; Fresse *et al.*, 1990, *Biochem. Pharmacol.*, 40:2189-2199), Sindbis viruses (H. Herweijer *et al.*, 1995, *Human Gene Therapy* 6:1161-1167; U.S. Pat. Nos. 5,091,309 and 5,2217,879), alphaviruses (S. Schlesinger, 1993, *Trends Biotechnol.* 11:18-22; I. Frolov *et al.*, 1996, *Proc. Natl. Acad. Sci. USA* 93:11371-11377) and retroviruses of avian (Brandyopadhyay *et al.*, 1984, *Mol. Cell Biol.*, 4:749-754; Petropoulos *et al.*, 1992, *J. Virol.*, 66:3391-3397), murine (Miller, 1992, *Curr. Top. Microbiol. Immunol.*, 158:1-24; Miller *et al.*, 1985, *Mol. Cell Biol.*, 5:431-437; Sorge *et al.*, 1984, *Mol. Cell Biol.*, 4:1730-1737; Mann *et al.*, 1985, *J. Virol.*, 54:401-407), and human origin (Page *et al.*, 1990, *J. Virol.*, 64:5370-5276; Buchsachler *et al.*, 1992, *J. Virol.*, 66:2731-2739). Baculovirus (*Autographa californica* multinuclear polyhedrosis virus; AcMNPV) vectors are also known in the art, and may be obtained from commercial sources (such as PharMingen, San Diego, Calif.; Protein Sciences Corp., Meriden, Conn.; Stratagene, La Jolla, Calif.).

IV. Virus-Like Particles

In some embodiments, a virus-like particle (VLP) is provided that includes a disclosed immunogen. Typically such VLPs include an immunogen containing a transmembrane domain, for example, a recombinant MuV F ectodomain trimer with protomers containing a MuV F transmembrane domain and cytosolic tail, or a recombinant MeV F ectodomain trimer with protomers containing a MeV F transmembrane domain and cytosolic tail. VLPs lack the viral components that are required for virus replication and thus represent a highly attenuated, replication-incompetent form of a virus. However, the VLP can display a polypeptide (*e.g.*, a recombinant MuV or MeV F ectodomain trimer) that is analogous to

that expressed on infectious virus particles and can eliciting an immune response to MuV or MeV when administered to a subject. Exemplary virus like particles and methods of their production, as well as viral proteins from several viruses that are known to form VLPs, including human papillomavirus, HIV (Kang *et al.*, Biol. Chem. 380: 353-64 (1999)), Semliki-Forest virus (Notka *et al.*, Biol. Chem. 380: 341-52 (1999)),

5 human polyomavirus (Goldmann *et al.*, J. Virol. 73: 4465-9 (1999)), rotavirus (Jiang *et al.*, Vaccine 17: 1005-13 (1999)), parvovirus (Casal, Biotechnology and Applied Biochemistry, Vol 29, Part 2, pp 141-150 (1999)), canine parvovirus (Hurtado *et al.*, J. Virol. 70: 5422-9 (1996)), hepatitis E virus (Li *et al.*, J. Virol. 71: 7207-13 (1997)), and Newcastle disease virus. The formation of such VLPs can be detected by any suitable technique. Examples of suitable techniques for detection of VLPs in a medium include, *e.g.*,

10 electron microscopy techniques, dynamic light scattering (DLS), selective chromatographic separation (*e.g.*, ion exchange, hydrophobic interaction, and/or size exclusion chromatographic separation of the VLPs) and density gradient centrifugation.

V. Immunogenic Compositions

15 Immunogenic compositions comprising a disclosed immunogen (*e.g.*, recombinant MuV F ectodomain trimer, a recombinant MeV F ectodomain trimer, or corresponding fusions with MuV HN ectodomain or MeV H ectodomain, or a MuV HN or MeV H multimer) and a pharmaceutically acceptable carrier are also provided. Such compositions can be administered to subjects by a variety of administration modes, for example, intramuscular, subcutaneous, intravenous, intra-arterial, intra-articular, intraperitoneal,

20 or parenteral routes. In several embodiments, a pharmaceutical composition including one or more of the disclosed immunogens are immunogenic compositions. Actual methods for preparing administrable compositions are described in more detail in such publications as *Remingtons Pharmaceutical Sciences*, 19th Ed., Mack Publishing Company, Easton, Pennsylvania, 1995.

Thus, an immunogen described herein can be formulated with pharmaceutically acceptable carriers

25 to help retain biological activity while also promoting increased stability during storage within an acceptable temperature range. Potential carriers include, but are not limited to, physiologically balanced culture medium, phosphate buffer saline solution, water, emulsions (*e.g.*, oil/water or water/oil emulsions), various types of wetting agents, cryoprotective additives or stabilizers such as proteins, peptides or hydrolysates (*e.g.*, albumin, gelatin), sugars (*e.g.*, sucrose, lactose, sorbitol), amino acids (*e.g.*, sodium glutamate), or

30 other protective agents. The resulting aqueous solutions may be packaged for use as is or lyophilized. Lyophilized preparations are combined with a sterile solution prior to administration for either single or multiple dosing.

Formulated compositions, especially liquid formulations, may contain a bacteriostat to prevent or minimize degradation during storage, including but not limited to effective concentrations (usually $\leq 1\%$

35 w/v) of benzyl alcohol, phenol, m-cresol, chlorobutanol, methylparaben, and/or propylparaben. A bacteriostat may be contraindicated for some patients; therefore, a lyophilized formulation may be reconstituted in a solution either containing or not containing such a component.

The immunogenic compositions of the disclosure can contain as pharmaceutically acceptable vehicles substances as required to approximate physiological conditions, such as pH adjusting and buffering agents, tonicity adjusting agents, wetting agents and the like, for example, sodium acetate, sodium lactate, sodium chloride, potassium chloride, calcium chloride, sorbitan monolaurate, and triethanolamine oleate.

5 The immunogenic composition may optionally include an adjuvant to enhance an immune response of the host. Adjuvants, such as aluminum hydroxide (e.g., ALHYDROGEL®, available from Brenntag Biosector, Copenhagen, Denmark and Amphogel®, Wyeth Laboratories, Madison, NJ), Freund's adjuvant, MPL™ (3-O-deacylated monophosphoryl lipid A; Corixa, Hamilton, IN) and IL-12 (Genetics Institute, Cambridge, MA), TLR agonists (such as TLR-9 agonists, for example cytidine-phospho-guanosine
10 oligodeoxynucleotide (CpG-ODN)1018), among many other suitable adjuvants well known in the art, can be included in the compositions. Suitable adjuvants are, for example, toll-like receptor agonists, alum, AlPO₄, alhydrogel, Lipid-A and derivatives or variants thereof, oil-emulsions, saponins, neutral liposomes, liposomes containing the vaccine and cytokines, non-ionic block copolymers, and chemokines. Non-ionic block polymers containing polyoxyethylene (POE) and polyxylpropylene (POP), such as POE-POP-POE
15 block copolymers, MPL™ (3-O-deacylated monophosphoryl lipid A; Corixa, Hamilton, IN) and IL-12 (Genetics Institute, Cambridge, MA), may be used as an adjuvant (Newman et al., 1998, *Critical Reviews in Therapeutic Drug Carrier Systems* 15:89-142). These adjuvants have the advantage in that they help to stimulate the immune system in a non-specific way, thus enhancing the immune response to a pharmaceutical product.

20 In some instances, the adjuvant formulation is a mineral salt, such as a calcium or aluminum (alum) salt, for example calcium phosphate, aluminum phosphate or aluminum hydroxide. In some embodiments, the disclosed immunogen comprises one or more phosphoserine modifications and is used with a Alum adjuvant. In some embodiments, the adjuvant includes an oil and water emulsion, e.g., an oil-in-water emulsion (such as MF59 (Novartis) or AS03 (GlaxoSmithKline)). One example of an oil-in-water emulsion
25 comprises a metabolisable oil, such as squalene, a tocol such as a tocopherol, e.g., alpha-tocopherol, and a surfactant, such as sorbitan trioleate (Span 85) or polyoxyethylene sorbitan monooleate (Tween 80), in an aqueous carrier.

In some instances, it may be desirable to combine a disclosed immunogen with other pharmaceutical products (e.g., vaccines) which induce protective responses to other agents. For example, a composition
30 including a recombinant MuV F ectodomain trimer, a recombinant MeV F ectodomain trimer, or corresponding fusions with MuV HN ectodomain or MeV H ectodomain as described herein can be administered simultaneously (typically separately) or sequentially with other vaccines recommended by the Advisory Committee on Immunization Practices (ACIP; [cdc.gov/vaccines/acip/index.html](https://www.cdc.gov/vaccines/acip/index.html)) for the targeted age group (e.g., infants from approximately one to six months of age). As such, a disclosed immunogen
35 described herein may be administered simultaneously or sequentially with vaccines against, for example, hepatitis B (HepB), diphtheria, tetanus and pertussis (DTaP), pneumococcal bacteria (PCV), *Haemophilus influenzae* type b (Hib), polio, influenza and rotavirus.

In some embodiments, the composition can be provided as a sterile composition. The immunogenic composition typically contains an effective amount of a disclosed immunogen and can be prepared by conventional techniques. Typically, the amount of immunogen in each dose of the immunogenic composition is selected as an amount which induces an immune response without significant, adverse side effects. In some embodiments, the composition can be provided in unit dosage form for use to induce an immune response in a subject, for example, to inhibit MuV and/or MeV infection in the subject. A unit dosage form contains a suitable single preselected dosage for administration to a subject, or suitable marked or measured multiples of two or more preselected unit dosages, and/or a metering mechanism for administering the unit dose or multiples thereof.

VI. Methods of Inducing an Immune Response

The disclosed immunogens (*e.g.*, recombinant MuV F ectodomain trimer, a recombinant MeV F ectodomain trimer, or corresponding fusions with MuV HN ectodomain or MeV H ectodomain, MuV HN or MeV H multimer, a nucleic acid molecule (such as an RNA molecule) encoding a disclosed immunogen, or a protein nanoparticle or virus like particle comprising the immunogen) can be administered to a subject to induce an immune response to MuV and/or MeV in the subject. In a particular example, the subject is a human. The immune response can be a protective immune response, for example a response that inhibits subsequent infection with MuV and/or MeV. Elicitation of the immune response can also be used to treat or inhibit MuV and/or MeV infection and illnesses associated therewith.

A subject can be selected for treatment that has, or is at risk for developing MuV or MeV infection, for example because of exposure or the possibility of exposure to MuV or MeV. Following administration of a disclosed immunogen, the subject can be monitored for the MuV and/or MeV infection or symptoms associated therewith, or both.

Typical subjects intended for treatment with the therapeutics and methods of the present disclosure include humans. In some embodiments, the subject is a human subject that is seronegative for MuV and/or MeV specific antibodies. In some embodiments, the subject is a human subject that is seropositive for MuV and/or MeV specific antibodies, and the immunogen is administered to boost the immune response to MeV and/or MuV in the subject. To identify subjects for treatment according to the methods of the disclosure, accepted screening methods are employed to determine risk factors associated with a targeted or suspected disease or condition, or to determine the status of an existing disease or condition in a subject. These screening methods include, for example, conventional work-ups to determine environmental, familial, occupational, and other such risk factors that may be associated with the targeted or suspected disease or condition, as well as diagnostic methods, such as various ELISA and other immunoassay methods to detect and/or characterize MuV and/or MeV infection. These and other routine methods allow the clinician to select patients in need of therapy using the methods and immunogenic compositions of the disclosure. In accordance with these methods and principles, a composition can be administered according to the teachings

herein, or other conventional methods, as an independent prophylaxis or treatment program, or as a follow-up, adjunct or coordinate treatment regimen to other treatments.

The administration of a disclosed immunogen can be for prophylactic or therapeutic purpose. When provided prophylactically, the immunogen can be provided in advance of any symptom, for example in advance of infection. The prophylactic administration serves to prevent or ameliorate any subsequent infection. In some embodiments, the methods can involve selecting a subject at risk for contracting MuV and/or MeV infection, and administering a therapeutically effective amount of a disclosed immunogen to the subject. The immunogen can be provided prior to the anticipated exposure to MuV and/or MeV so as to attenuate the anticipated severity, duration or extent of an infection and/or associated disease symptoms, after exposure or suspected exposure to the virus, or after the actual initiation of an infection. Populations that benefit from prophylactic use of the disclosed immunogen (for example, as a boost immunization) include children entering school (e.g., 5 years old) and adolescents entering high school or college or military (e.g., 15-18 years old). Transplant recipients may also need to be revaccinated or immunocompromised children like HIV+ would benefit from a protein vaccine as opposed to a live-attenuated virus that may not be safe including pregnant women.

When provided therapeutically, the disclosed immunogens are provided at or after the onset of a symptom of MuV and/or MeV infection, or after diagnosis of MuV and/or MeV infection. Treatment of MuV by inhibiting MuV replication or infection can include delaying and/or reducing signs or symptoms of MuV infection in a subject. Treatment of MeV by inhibiting MeV replication or infection can include delaying and/or reducing signs or symptoms of MeV infection in a subject. In some examples, treatment using the methods disclosed herein prolongs the time of survival of the subject.

In some embodiments, administration of a disclosed immunogen to a subject can elicit the production of an immune response that is protective against or reduces symptoms of disease when the subject is subsequently infected or re-infected with a wild-type MuV and/or MeV. While the naturally circulating virus may still be capable of causing infection there can be a reduced possibility of serious or life-threatening symptoms as a result of the vaccination and a possible boosting of resistance by subsequent infection by wild-type virus. Following vaccination, there are detectable levels of host engendered serum and secretory antibodies which are capable of neutralizing homologous (of the same subgroup) wild-type virus *in vitro* and *in vivo*. In many instances the host antibodies will also neutralize wild-type virus of a different, non-vaccine subgroup.

The immunogens described herein, and immunogenic compositions thereof, are provided to a subject in an amount effective to induce or enhance an immune response against MuV and/or MeV in the subject, preferably a human. The actual dosage of disclosed immunogen will vary according to factors such as the disease indication and particular status of the subject (for example, the subject's age, size, fitness, extent of symptoms, susceptibility factors, and the like), time and route of administration, other drugs or treatments being administered concurrently, as well as the specific pharmacology of the composition for

eliciting the desired activity or biological response in the subject. Dosage regimens can be adjusted to provide an optimum prophylactic or therapeutic response.

An immunogenic composition including one or more of the disclosed immunogens can be used in coordinate (or prime-boost) vaccination protocols or combinatorial formulations. In certain embodiments, novel combinatorial immunogenic compositions and coordinate immunization protocols employ separate immunogens or formulations, each directed toward eliciting an anti-viral immune response, such as an immune response to MuV F protein and/or MeV F protein. Separate immunogenic compositions that elicit the anti-viral immune response can be combined in a polyvalent immunogenic composition administered to a subject in a single immunization step, or they can be administered separately (in monovalent immunogenic compositions) in a coordinate (or prime-boost) immunization protocol.

There can be several boosts, and each boost can be a different disclosed immunogen. In some examples that the boost may be the same immunogen as another boost, or the prime. The prime and boost can be administered as a single dose or multiple doses, for example two doses, three doses, four doses, five doses, six doses or more can be administered to a subject over days, weeks or months. Multiple boosts can also be given, such one to five (*e.g.*, 1, 2, 3, 4 or 5 boosts), or more. Different dosages can be used in a series of sequential immunizations. For example a relatively large dose in a primary immunization and then a boost with relatively smaller doses.

In some embodiments, the boost can be administered about two, about three to eight, or about four, weeks following the prime, or about several months after the prime. In some embodiments, the boost can be administered about 5, about 6, about 7, about 8, about 10, about 12, about 18, about 24, months after the prime, or more or less time after the prime. Periodic additional boosts can also be used at appropriate time points to enhance the subject's "immune memory." The adequacy of the vaccination parameters chosen, *e.g.*, formulation, dose, regimen and the like, can be determined by taking aliquots of serum from the subject and assaying antibody titers during the course of the immunization program. In addition, the clinical condition of the subject can be monitored for the desired effect, *e.g.*, inhibition of MuV and/or MeV infection or improvement in disease state (*e.g.*, reduction in viral load). If such monitoring indicates that vaccination is sub-optimal, the subject can be boosted with an additional dose of immunogenic composition, and the vaccination parameters can be modified in a fashion expected to potentiate the immune response.

In some embodiments, the prime-boost method can include DNA-primer and protein-boost vaccination protocol to a subject. The method can include two or more administrations of the nucleic acid molecule or the protein.

For protein therapeutics, typically, each human dose will comprise 1-1000 µg of protein, such as from about 1 µg to about 100 µg, for example, from about 1 µg to about 50 µg, such as about 1 µg, about 2 µg, about 5 µg, about 10 µg, about 15 µg, about 20 µg, about 25 µg, about 30 µg, about 40 µg, or about 50 µg.

The amount utilized in an immunogenic composition is selected based on the subject population (*e.g.*, infant or elderly). An optimal amount for a particular composition can be ascertained by standard

studies involving observation of antibody titers and other responses in subjects. It is understood that an effective amount of a disclosed immunogen, such as a recombinant MuV or MeV F ectodomain trimer or recombinant MeV F ectodomain trimer or a chimera thereof with a MuV HN or MeV H ectodomain, viral vector, or nucleic acid molecule in an immunogenic composition, can include an amount that is ineffective at eliciting an immune response by administration of a single dose, but that is effective upon administration of multiple dosages, for example in a prime-boost administration protocol.

Upon administration of a disclosed immunogen the immune system of the subject typically responds to the immunogenic composition by producing antibodies specific for viral protein. Such a response signifies that an immunologically effective dose was delivered to the subject.

For each particular subject, specific dosage regimens can be evaluated and adjusted over time according to the individual need and professional judgment of the person administering or supervising the administration of the immunogenic composition. The dosage and number of doses will depend on the setting, for example, in an adult or anyone primed by prior MuV and/or MeV infection or immunization, a single dose may be a sufficient booster. In naïve subjects, in some examples, at least two doses would be given, for example, at least three doses. In some embodiments, an annual boost is given, for example, along with an annual influenza vaccination.

In some embodiments, the antibody response of a subject will be determined in the context of evaluating effective dosages/immunization protocols. In most instances it will be sufficient to assess the antibody titer in serum or plasma obtained from the subject. Decisions as to whether to administer booster inoculations and/or to change the amount of the therapeutic agent administered to the individual can be at least partially based on the antibody titer level. The antibody titer level can be based on, for example, an immunobinding assay which measures the concentration of antibodies in the serum which bind to an antigen including, for example, a MuV F protein and/or a MeV F protein.

Determination of effective dosages is typically based on animal model studies followed up by human clinical trials and is guided by administration protocols that significantly reduce the occurrence or severity of targeted disease symptoms or conditions in the subject, or that induce a desired response in the subject (such as a neutralizing immune response). Suitable models in this regard include, for example, murine, rat, porcine, feline, ferret, non-human primate, and other accepted animal model subjects known in the art. Alternatively, effective dosages can be determined using *in vitro* models (for example, immunologic and histopathologic assays). Using such models, only ordinary calculations and adjustments are required to determine an appropriate concentration and dose to administer an effective amount of the composition (for example, amounts that are effective to elicit a desired immune response or alleviate one or more symptoms of a targeted disease). In alternative embodiments, an effective amount or effective dose of the composition may simply inhibit or enhance one or more selected biological activities correlated with a disease or condition, as set forth herein, for either therapeutic or diagnostic purposes.

Administration of an immunogenic composition that elicits an immune response to reduce or prevent an infection, can, but does not necessarily completely, eliminate such an infection, so long as the

infection is measurably diminished. For example, administration of an effective amount of the agent can decrease the MuV or MeV infection (for example, as measured by infection of cells, or by number or percentage of subjects infected by MuV or MeV by a desired amount, for example by at least 10%, at least 20%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, or even
5 at least 100% (elimination or prevention of detectable MuV or MeV infection, as compared to a suitable control, also referred to as sterilizing immunity).

In some embodiments, administration of an effective amount of one or more of the disclosed immunogens to a subject induces a neutralizing immune response in the subject. To assess neutralization activity, following immunization of a subject, serum can be collected from the subject at appropriate time
10 points, frozen, and stored for neutralization testing. Methods to assay for neutralization activity include, but are not limited to, plaque reduction neutralization titer (PRNT) assays, microneutralization assays, flow cytometry-based assays, single-cycle infection assays. In some embodiments, the serum neutralization activity can be assayed using a panel of MuV and/or MeV pseudoviruses.

One approach to administration of nucleic acids is direct immunization with plasmid DNA, such as
15 with a mammalian expression plasmid. Immunization by nucleic acid constructs is well known in the art and taught, for example, in U.S. Patent No. 5,643,578 (which describes methods of immunizing vertebrates by introducing DNA encoding a desired antigen to elicit a cell-mediated or a humoral response), and U.S. Patent No. 5,593,972 and U.S. Patent No. 5,817,637 (which describe operably linking a nucleic acid sequence encoding an antigen to regulatory sequences enabling expression). U.S. Patent No. 5,880,103
20 describes several methods of delivery of nucleic acids encoding immunogenic peptides or other antigens to an organism. The methods include liposomal delivery of the nucleic acids (or of the synthetic peptides themselves), and immune-stimulating constructs, or ISCOMSTM, negatively charged cage-like structures of 30-40 nm in size formed spontaneously on mixing cholesterol and Quil ATM (saponin). Protective immunity has been generated in a variety of experimental models of infection, including toxoplasmosis and Epstein-
25 Barr virus-induced tumors, using ISCOMSTM as the delivery vehicle for antigens (Mowat and Donachie, *Immunol. Today* 12:383, 1991). Doses of antigen as low as 1 µg encapsulated in ISCOMSTM have been found to produce Class I mediated CTL responses (Takahashi *et al.*, *Nature* 344:873, 1990).

In some embodiments, a plasmid DNA vaccine is used to express a disclosed immunogen in a subject. For example, a nucleic acid molecule encoding a disclosed immunogen can be administered to a
30 subject to elicit an immune response to the F protein of MuV or MeV. In some embodiments, the nucleic acid molecule can be included on a plasmid vector for DNA immunization, such as the pVRC8400 vector (described in Barouch *et al.*, *J. Virol.* 79, 8828-8834, 2005, which is incorporated by reference herein).

In another approach to using nucleic acids for immunization, a disclosed immunogen can be expressed by attenuated viral hosts (such as an attenuated MuV or MeV vector) or vectors or bacterial
35 vectors. Recombinant vaccinia virus, adeno-associated virus (AAV), herpesvirus, retrovirus, cytomeglovirus, paramyxovirus, pneumovirus, or other viral vectors can be used to express the peptide or protein, thereby eliciting a CTL response. For example, vaccinia vectors and methods useful in

immunization protocols are described in U.S. Patent No. 4,722,848. BCG (Bacillus Calmette Guerin) provides another vector for expression of the peptides (see Stover, *Nature* 351:456-460, 1991).

In another example, a disclosed immunogen can be administered to a subject using RNA immunization, such as with a lipid-encapsulated mRNA immunization platform (see, e.g., Roth et al., “A Modified mRNA Vaccine Targeting Immunodominant NS Eitopes Protects Against Dengue Virus Infection in HLA Class I Transgenic Mice,” *Frot Immunol.*, June 21, 2019, Vol. 10, Article 1424; Jagger et al., *J Infect Dis*, “Protective Efficacy of Nucleic Acid Vaccines Against Transmission of Zika Virus During Pregnancy in Mice,” *jiz338*, Jul 1, 2019; Feldman et al., “mRNA vaccines against H10N8 and H7N9 influenza viruses of pandemic potential are immunogenic and well tolerated in healthy adults in phase 1 randomized clinical trials,” *Vaccine*, 37(25), 3326-3334, 2019; and Hassett et al., “Optimization of Lipid Nanoparticles for Intramuscular Administration of mRNA Vaccines,” *Mol Ther Nucleic Acids*, 15: 1-11, 2019.

In one embodiment, a nucleic acid encoding a protomer of a disclosed MuV F or MeV F ectodomain trimer is introduced directly into cells. For example, the nucleic acid can be loaded onto gold microspheres by standard methods and introduced into the skin by a device such as Bio-Rad’s HELIOS™ Gene Gun. The nucleic acids can be “naked,” consisting of plasmids under control of a strong promoter. Typically, the DNA is injected into muscle, although it can also be injected directly into other sites. Dosages for injection are usually around 0.5 µg/kg to about 50 mg/kg, and typically are about 0.005 mg/kg to about 5 mg/kg (see, e.g., U.S. Patent No. 5,589,466).

In another embodiment, an mRNA-based immunization protocol can be used to deliver a nucleic acid encoding a disclosed immunogen directly into cells. In some embodiments, nucleic acid-based vaccines based on mRNA may provide a potent alternative to the previously mentioned approaches. mRNA vaccines preclude safety concerns about DNA integration into the host genome and can be directly translated in the host cell cytoplasm. Moreover, the simple cell-free, *in vitro* synthesis of RNA avoids the manufacturing complications associated with viral vectors. Two exemplary forms of RNA-based vaccination that can be used to deliver a nucleic acid encoding a disclosed immunogen include conventional non-amplifying mRNA immunization (see, e.g., Petsch et al., “Protective efficacy of *in vitro* synthesized, specific mRNA vaccines against influenza A virus infection,” *Nature biotechnology*, 30(12):1210–6, 2012) and self-amplifying mRNA immunization (see, e.g., Geall et al., “Nonviral delivery of self-amplifying RNA vaccines,” *PNAS*, 109(36): 14604-14609, 2012; Magini et al., “Self-Amplifying mRNA Vaccines Expressing Multiple Conserved Influenza Antigens Confer Protection against Homologous and Heterosubtypic Viral Challenge,” *PLoS One*, 11(8):e0161193, 2016; and Brito et al., “Self-amplifying mRNA vaccines,” *Adv Genet.*, 89:179-233, 2015).

In some embodiments, a lipid nanoparticle including mRNA encoding a disclosed immunogen is used in the method of eliciting an immune response, for example, as described in WO2017070626, US2019/0192646, and for the mRNA-1273 vaccine described in Jackson *et al.* “An mRNA vaccine against SARS-CoV2 – preliminary report,” *N. Engl. J. Med.*, 383(20):1920-1931, 2020, each of which is

incorporated by reference herein. As described in WO2017070626, the mRNA encoding the immunogen can be formulated in lipid nanoparticles with 50 mol% ionizable lipid, 10 mol% DSPC, 38.5 mol% cholesterol, and 1.5 mol% (PEG2000 DMG). Further, the mRNA encoding the immunogen can be a modified mRNA with 1-methylpseudouridine in place of uridine and a 7mG(5')ppp(5')N1mpNp cap (enzymatic), as well as a 5'UTR, a 3'UTR, and a polyA tail.

EXAMPLES

The following examples are provided to illustrate particular features of certain embodiments, but the scope of the claims should not be limited to those features exemplified.

EXAMPLE 1

MuV F Proteins Stabilized in a Prefusion Conformation and Fusions Thereof with MuV HN ectodomain

This example illustrates embodiments of a MuV F ectodomain trimer stabilized in a prefusion conformation by one or more amino acid substitutions. Further provided are MuV F ectodomain trimers linked to a MuV HN ectodomain. The prefusion-stabilized MuV F ectodomain trimers and corresponding fusions with MuV HN ectodomain are useful, for example, for inducing a neutralizing immune response to MuV in a subject.

Before the introduction of mumps-containing vaccines in the late 1960s, mumps caused widespread morbidity characterized by fever, parotitis and less commonly, orchiditis, meningitis, encephalitis and deafness. The combination measles, mumps and rubella vaccine (MMR) dramatically reduced the incidence of mumps throughout the world. Two doses of MMR vaccine are approximately 88% effective for the prevention of mumps disease however since 2006, there has been a resurgence in the number of cases of mumps disease in the world among highly-vaccinated populations, with >30,000 affected individuals in the U.S. Contributing factors may include waning immunity, poorly effective antibody responses, and antigenic differences between the Jeryl Lynn strain used in the MMR vaccine and circulating wildtype strains. The predominant mumps genotype characterizing outbreaks in the USA and Europe in recent years has been genotype G.

As described herein, structure-based design was used to engineer a MuV F glycoprotein stabilized in the prefusion conformation. A crystal structure of mumps fusion glycoprotein at 2.16 Å resolution reveals the basis for prefusion conformational stabilization. Potent cross-mumps genotype plaque reduction neutralizing titers (PRNT) were elicited in mice from the mumps prefusion-stabilized F glycoprotein or a chimeric fusion glycoprotein of prefusion-stabilized mumps F trimer linked to genotype G mumps hemagglutinin neuraminidase (HN). The prefusion F-HN mumps chimera could elicit the highest PRNT to genotypes A, G and H mumps viruses, greater than 100-fold the reported human protective titer. Additionally, monoclonal antibodies to mumps prefusion F and HN were isolated from immunized mice, which were capable of neutralizing genotype G mumps virus with a spectrum of potencies. Structural and

binding analyses of these prefusion F-specific antibodies revealed binding to four discrete neutralizing antigenic sites. The engineered immunogens are vaccine candidates for mumps either as novel or as booster vaccines.

5 RESULTS

Disulfide bond and membrane-proximal coiled coil stabilization robustly stabilize and allow production of a soluble prefusion mumps F trimer.

When produced in cells, the MuV F ectodomain linked to a C-terminal GCN4 trimerization domain forms trimers that spontaneously transition to the prefusion conformation. Unstabilized recombinant MuV F-GCN4 is so unstable, 100% of molecules have transitioned to the postfusion conformation at the point of evaluation (EM). Also protein expression is substantially reduced without stabilization. Accordingly, structure-based vaccine design was used to identify mutations for the stabilization of the MuV F ectodomain in a prefusion conformation and also to eliminate the F1/F2 cleavage site to produce a “single chain” MuV F protein with increased expression.

The crystal structures of the simian prefusion parainfluenza virus 5 (PIV5) F glycoprotein (PDB IDs 4GIP, 4WSG) (Welch, B. D. *et al. Proc Natl Acad Sci U S A* 109, 16672-16677, 2012) were used to construct a homology model for the prefusion mumps F protein, consisting of three intertwined monomers forming a quaternary assembly of DI, DII, DIII and HRB domains.

Multiple stabilization strategies were employed to “lock” the MuV F ectodomain in the prefusion conformation, including introduction of disulfide bonds and proline substitutions. The selection of residues to mutate to cysteines in MuV F were based on homology design from the PIV5 prefusion F structure (PDB 4WSG), based on residues that would be predicted to undergo conformational change in transitioning from prefusion to postfusion conformation. The residue pairs C β atoms were identified to be within 5 angstrom and orientated such that the formation of a disulfide bond might be possible. In total, approximately 60 different mutants were designed, expressed, purified, assessed for expression level, and assessed for prefusion conformation by negative stain EM.

The mutations were introduced into a MuV F ectodomain (based on C-terminal truncations at MuV F position 469, 476, or 483, and linked to a C-terminal GCN4 trimerization domain, and the resulting mutants were screened as noted above. The ectodomain also included a mutation to remove the F1/F2 furin cleavage site. The prefusion stabilizing mutations assessed included: cysteine substitutions at one or more of MuV F positions 86 and 215, 155 and 161, 163 and 235, 165 and 231, 206 and 223, 209 and 214, 221 and 255 that form a non-natural disulfide bond; and a proline substitution at MuV F position 184. Relevant sequences are shown below. In FIG. 1A, a summary of the initial successful prefusion-stabilizing mutations are reported.

The expression and purification of the single chain and prefusion-stabilized MuV F proteins showed a substantial increase in expression level compared to the unmodified MuV F.

As illustrated in FIGs. 1A and B, negative stain EM can be used to distinguish MuV F ectodomain trimers that are in the prefusion conformation from those that are in the postfusion conformation.

By creating a matrix of disulfide substitutions and C-terminal coiled-coil-GCN4 attachment positions, the level of protein expression and the proportion of protein adopting the prefusion or postfusion trimer conformation was evaluated using negative-stain EM (FIG. 1A). It was observed that while five combinations of disulfide bonds and GCN4 attachment positions gave rise to 100% prefusion trimers, one such combination gave rise to high-yield protein expression (V206C-A223C with 476-GCN4) at about 4.8mg/L from Expi293 cells. The negative stain EM 2D average of this design (FIG. 1B, top inset) contrasts with the postfusion mumps F glycoprotein trimer (FIG. 1B, lower inset) and was consistent with prior observations for parainfluenza virus F protein conformations.

Crystal structure of prefusion mumps F glycoprotein trimer to 2.16 Å resolution reveals a stabilizing disulfide design and the location of polymorphic residues. Size-exclusion chromatography of the prefusion-stabilized mumps F protein trimer (MuV F V206C-A223C-GGG-476-GCN4, SEQ ID NO: 11) revealed a homogeneous peak (FIG. 1C), which when expressed in the presence of kifunensine, could be deglycosylated and crystallized. The 3-dimensional coordinates for the structure are provided herewith in Table 1. The x-ray structure to 2.16 Å resolution (FIGs. 1D and 1E) revealed a similar overall architecture to prefusion PIV5 and other paramyxovirus prefusion F trimers (Stewart-Jones *et al.*, *Proc Natl Acad Sci U S A* 115, 12265-12270, 2018; Welch, B. D. *et al.* *Proc Natl Acad Sci U S A* 109, 16672-16677, 2012; Xu K. *et al.* *PLoS Pathog* 11(12):e1005322, 2015), however adopts a ‘closed cap’ structure comprising of loops N177-S184 at the trimer apex of the mumps prefusion F trimer, formed by interactions between side chains T178, Q179 and N181 (FIGs. 2A and 2B). The remaining N-acetyl glucosamine moieties from the six glycans at positions N73, N182, N352, N427, N433 and N457 were visible in the electron density and a glycosylated model built (FIG. 2C). The V206C-A223C disulfide was clearly defined by the electron density with a Cα-Cα atomic distance of 4.8 Å whereas in the homologous postfusion PIV3 F trimer structure [PDB ID 1ZTM], Cα-Cα atoms are positioned 5.8 Å apart. The DI-DIII domains enclosed a large aqueous cavity measuring ~20,000 Å³ in volume and protomers bury a 6,500 Å² interface, similar to that observed in PIV5, PIV3 and nipah prefusion F structures (Stewart-Jones *et al.*, *Proc Natl Acad Sci U S A* 115, 12265-12270, 2018; Welch, B. D. *et al.* *Proc Natl Acad Sci U S A* 109, 16672-16677, 2012; Xu K. *et al.* *PLoS Pathog* 11(12):e1005322, 2015). The residues thought to undergo conformational change between prefusion and postfusion conformations (based on PIV5 pre-F structure [PDB ID 4WSG] and PIV3 post-F [PDB ID 1ZTM]) are shown in black in Figure 1E and correspond to MuV F residues 92-253.

While the sequence identities of mumps F and HN are relatively high between genotypes (FIGs. 2A-2C), polymorphic variation to the structures of prefusion mumps F and HN was mapped (FIG. 2D). Mapping the variation to prefusion F for all genotypes and specifically between genotype G and Jeryl Lynn (genotype A), much of the protein surface exposed for antibody recognition was conserved, while numerous variable amino acids were located within the aqueous cavity in the core of the prefusion trimer (FIG. 2D, left). In contrast, the mumps HN dimer structures revealed that most polymorphic amino acids are exposed

to the solvent (FIG. 2D, right), including a glycan variation at position N464 between Jeryl Lynn and genotype G HN. The predominance of solvent-exposed polymorphic residues on HN versus prefusion F suggests that genotype G resistance to Jeryl Lynn vaccine-elicited humoral immunity could be accounted for more from HN than from prefusion F.

- 5 **Cross-strain effectiveness of stabilizing mutations.** To show that the prefusion stabilizing mutations were effective for F from across MuV strains, these mutations were tested in F from several different MuV strains. MuV F V206C-A223C-GGG-476-GCN4 (SEQ ID NO: 11) is based on a genotype C MuV F. Introduction of these prefusion-stabilizing mutations into a genotype A (Jeryl Lynn) MuV F protein (MuV-JL F 206C-A223C-GGG -476-GCN4 (SEQ ID NO: 26) and genotype G MuV F protein (MuV-IL17
- 10 F 206C-A223C-GGG -476-GCN4 (SEQ ID NO: 51) also provided prefusion stabilization. Further, introduction of these prefusion-stabilizing mutations into F protein from the following MuV stains also resulted in prefusion stabilization as measured by negative stain EM and/or prefusion specific antibody binding: Canada (URABE), Albany (genotype A), Hoshino (genotype B), India (genotype C), Netherlands (genotype D), China (genotype F), NethL11 (genotype G), NY14 (genotype G), IA14 (genotype G), MA16
- 15 (genotype G), LA17 (genotype G), IL17 (genotype G), Virginia (genotype H), Taiwan (genotype J), Taiwan (genotype K), Netherlands (genotype L), MG15 (genotype A)

- F-HN chimera.** To increase the immunogenic footprint of the MuV F ectodomain trimer, a MuV HN ectodomain was genetically fused to the C-terminus of the trimerization domain of each protomer of the trimer. The format is illustrated in FIG. 4A. Corresponding sequences are shown below. Negative stain
- 20 EM shows that the F ectodomain maintains the prefusion conformation, with the three HN ectodomains (one linked to each F protomer) arrayed C-terminal to the trimerization domain. This design yielded approximately 0.3mg/L from Expi293 cells and was monodispersed on size exclusion chromatography and displayed expected assembly from negative-stain EM (FIG. 1C and FIG. 4A).

- Prefusion-stabilized mumps F and prefusion-stabilized F-HN chimeric trimers elicit high-titer**
- 25 **neutralizing antibodies in mice.** The ability of prefusion-stabilized mumps F to elicit neutralizing antibodies compared to other mumps immunogens was assessed. Groups of 10 CB6F1/J mice were immunized with 10 µg doses of mumps glycoproteins combined with 10 µg polyinosinic-polycytidylic acid (poly-I:C) adjuvant at weeks 0, 3 and 10 and the ability of sera to prevent mumps virus infection of HEp-2 cells was measured (Fig. 3A).

- 30 The immunogens assessed were a MuV F ectodomain trimer in a post-fusion conformation (native ectodomain with -476-GCN4), a MuV F ectodomain trimer in a pre-fusion conformation (MuV F V206C-A223C-GGG-476-GCN4, SEQ ID NO: 11), MuV HN ectodomain monomer, and a MuV F ectodomain trimer in a pre-fusion conformation with the protomers of the trimer fused to MuV HN ectodomain (MuV F 206C-223C-GGG-476 + GCN4 + MuV HN_G (SEQ ID NO: 27).

- 35 Where mice were immunized with prefusion mumps F-containing immunogens (preF or preF-HN), specific responses to preF were detected in sera, with lower levels of binding to postfusion-immunized mice (FIG. 4B). Recombinant HN monomer bound only HN-immunized mouse sera, however monomeric HN

immunized mice showed little binding whereas sera from preF-HN immunized mice showed substantial levels of HN binding suggesting multivalency of HN was driving a robust humoral responses (FIG. 4C).

To analyze the elicitation of neutralizing antibody titers from three immunizations with either postfusion F, prefusion F or prefusion F-HN, the PRNT 2 weeks following each immunization was analyzed (FIG. 3B). Incremental increases in neutralizing titers were observed after each immunization, however the third immunization showed a more marginal increase than after the second immunization. Neutralizing antibodies were observed with postfusion immunization, however the prefusion F immunogen showed a 3.5 and 2.5-fold higher neutralization than postfusion F after the second and third immunization respectively (geometric mean infective dose (ID₆₀ values) of 322 and 789 respectively to genotype G mumps virus). The preF-HN chimeric variant elicited neutralizing titers after the third immunization 12-fold higher than postfusion F and 5-fold higher than prefusion F, with an ID₆₀ value of 3930 to genotype G virus.

Next, the PRNT to Jeryl Lynn and genotype H viruses was evaluated to characterize cross-neutralizing antibodies elicited from the recombinant immunogens. For postfusion and prefusion F, a greater level of neutralization was observed for Jeryl Lynn virus than to genotype G virus, whereas for the prefusion F-HN chimera equivalent PRNTs were observed. At week 16, sera from both preF and preF-HN groups showed robust PRNT to genotype H virus. This indicates these recombinant immunogens can elicit antibodies that can cross-neutralize numerous mumps genotypes. the durability of PRNT to these three mumps viruses was monitored for an additional 6 months and it was found that while there is an a reduction in titers, ID₆₀ plateaus form after 3 months and the preF-HN had geometric mean PRNTs for genotype G, Jeryl Lynn and genotype H viruses of approximately 640, 800 and 1700 respectively (FIG. 4D). The ID₆₀ PRNT plateaus formed with the preF immunogen against genotype G, Jeryl Lynn and genotype H viruses were approximately 100, 660 and 3153 (FIG. 4E). Overall, prefusion stabilized F mumps linked to HN yielded neutralization titers that were higher than F alone and represents a design strategy to combine both viral surface antigens in a single immunogen stoichiometrically.

DISCUSSION

Human immunity to mumps following MMR vaccination is characterized by PRNT typically around 220 for Jeryl Lynn and 40 for genotype G (Rasheed *et al.*, *Proc Natl Acad Sci U S A* 116(38):19071-19076, 2019). The results provided herein for the disclosed immunogens appear to provide increased effectiveness as measured by PNRT, assuming that the mouse model data correlated with response in a human.

Due to mumps outbreaks among two-dose vaccine recipients, improvements are needed to the current mumps vaccine to reduce disease incidence and the burden on public health resources. A third dose of mumps-containing vaccine has been recommended by the Advisory Committee on Immunization Practices (ACIP) to individuals at risk of contracting mumps in an outbreak setting. A third dose of live attenuated MMR has resulted in a temporary elevation of neutralization titers, lasting about 12 months (Fiebelkorn A. P. *et al.* *Open Forum Infect Dis* 1(3):ofu094, 2014). Additionally, there was a 78% reduced risk of contracting mumps infection after a third dose of MMR compared to individuals who had received

two doses of MMR (Cardemil C. *et al*, Effectiveness of a Third Dose of MMR Vaccine for Mumps Outbreak Control. *N Engl J Med.* **377** (10): 947–956, 2017). The recombinant protein vaccine candidates described in this example provide an alternative vaccine modality than administration of MMR in a mumps outbreak setting and provide increased durability and effectiveness.

- 5 The prefusion F-HN chimera, comprising the two key neutralization targets on mumps virions is capable of eliciting potent cross-genotype neutralizing responses, including to the dominant mumps genotype G causing outbreaks and two other genotypes, A and H represents a universal vaccine candidate for global mumps strains.

10 **Sequences:**

MuV F A163C/V235C-GGG-483-GCN4 (SEQ ID NO: 2)
 mkafsvtclsfavfsssicvnnilqqigyikqvrqlsyysqssssyivvklpnigptddscefksvtqynktnl
 lpiaeninniaspspgsrrhGGGagiaigiaalgvataaavtaavslvqaqtnaraiaamknsiqatnra
 15 vfevkegtqqlaCavqaiqdhintimntqlnnmscgildnqlatslglyltelttvfqqltnpalpsisigalrsl
 lgsmtmpaCvqatlstsisaaeilsaglmegqiisvllldemqmivkiniptivtqsnalvidfysissfinnq
 esiqlpdrileigneqwsypaknckltrhhifcqyneaeerlslesklclagnisacvfspiagsymrrfval
 dgtivancrsltlclckspspyiqpdhhavttidltacqtlslldgldfsivslsnityaenltislsqtint
 qpidistelskvnaslqnnavkyikesnhqlqsvsvnski IEDKIEEILSKIYHIENEIARIKKLIGEAP

MuV F V206C/A223C-GGG-483-GCN4 (SEQ ID NO: 3)
 mkafsvtclsfavfsssicvnnilqqigyikqvrqlsyysqssssyivvklpnigptddscefksvtqynktnl
 lpiaeninniaspspgsrrhGGGagiaigiaalgvataaavtaavslvqaqtnaraiaamknsiqatnra
 20 vfevkegtqqlaiavqaiqdhintimntqlnnmscgildnqlatslglyltelttCfqpqltnpalpsisig
 Crlslrgsmtmpavvqatlstsisaaeilsaglmegqiisvllldemqmivkiniptivtqsnalvidfysissfinnq
 25 esiqlpdrileigneqwsypaknckltrhhifcqyneaeerlslesklclagnisacvfspiagsymrrfval
 dgtivancrsltlclckspspyiqpdhhavttidltacqtlslldgldfsivslsnityaenltislsqtint
 qpidistelskvnaslqnnavkyikesnhqlqsvsvnski IEDKIEEILSKIYHIENEIARIKKLIGEAP

MuV F N86C/A215C-GGG-483-GCN4 (SEQ ID NO: 4)
 mkafsvtclsfavfsssicvnnilqqigyikqvrqlsyysqssssyivvklpnigptddscefksvtqynktnl
 lpiaeninniaspspgsrrhGGGagiaigiaalgvataaavtaavslvqaqtnaraiaamknsiqatnra
 30 vfevkegtqqlaiavqaiqdhintimntqlnnmscgildnqlatslglyltelttvfqqltnpCclpsisig
 alrslrgsmtmpavvqatlstsisaaeilsaglmegqiisvllldemqmivkiniptivtqsnalvidfysissfinnq
 35 esiqlpdrileigneqwsypaknckltrhhifcqyneaeerlslesklclagnisacvfspiagsymrrfval
 dgtivancrsltlclckspspyiqpdhhavttidltacqtlslldgldfsivslsnityaenltislsqtint
 qpidistelskvnaslqnnavkyikesnhqlqsvsvnski IEDKIEEILSKIYHIENEIARIKKLIGEAP

MuV F P209C/P214C-GGG-483-GCN4 (SEQ ID NO: 5)
 mkafsvtclsfavfsssicvnnilqqigyikqvrqlsyysqssssyivvklpnigptddscefksvtqynktnl
 lpiaeninniaspspgsrrhGGGagiaigiaalgvataaavtaavslvqaqtnaraiaamknsiqatnra
 40 vfevkegtqqlaiavqaiqdhintimntqlnnmscgildnqlatslglyltelttvfqCqltnCalspisig
 alrslrgsmtmpavvqatlstsisaaeilsaglmegqiisvllldemqmivkiniptivtqsnalvidfysissfinnq
 45 esiqlpdrileigneqwsypaknckltrhhifcqyneaeerlslesklclagnisacvfspiagsymrrfval
 dgtivancrsltlclckspspyiqpdhhavttidltacqtlslldgldfsivslsnityaenltislsqtint
 qpidistelskvnaslqnnavkyikesnhqlqsvsvnski IEDKIEEILSKIYHIENEIARIKKLIGEAP

MuV F K155C/L161C-GGG-483-GCN4 (SEQ ID NO: 6)
 mkafsvtclsfavfsssicvnnilqqigyikqvrqlsyysqssssyivvklpnigptddscefksvtqynktnl
 lpiaeninniaspspgsrrhGGGagiaigiaalgvataaavtaavslvqaqtnaraiaamknsiqatnra
 50 vfevCegtqqCaiaavqaiqdhintimntqlnnmscgildnqlatslglyltelttvfqqltnpalpsisig
 alrslrgsmtmpavvqatlstsisaaeilsaglmegqiisvllldemqmivkiniptivtqsnalvidfysissfinnq
 esiqlpdrileigneqwsypaknckltrhhifcqyneaeerlslesklclagnisacvfspiagsymrrfval
 dgtivancrsltlclckspspyiqpdhhavttidltacqtlslldgldfsivslsnityaenltislsqtint
 55 qpidistelskvnaslqnnavkyikesnhqlqsvsvnski IEDKIEEILSKIYHIENEIARIKKLIGEAP

- MuV F V165C/M231C-GGG-483-GCN4 (SEQ ID NO: 7)
 mkafsvtclsfavfsssicvnninilqqigiyikqqvrqlsyysqssssyivvklpnigptddscfksvstqynktnlsl
 lpiaeninniaspogrrhGGGagaaigialgvataaavtaavslvqaqtnaraiaamknsiqatnraavfevkegtq
 laiaa**C**qaiqgdhintimntqlnmscildnqlatslglyltelttvfpqqltnpalpsisqalrslslgs**C**tpavvqatl
 stsisaaeilsaglmegqiisvllldemqmivkiniptivtqsnalvidfysissfinnqesiqlpdrileigneqwsyp
 aknckltrhhifcgyneaeerlslesklclagnisacvfspiagsymrrfvaldgtivancrsltclckspspyiqpdhh
 avttidltacqtlslldgldfsivslsnityaenltislsqtintqpidistelskvnaslqnnavkyikesnhqlqsvsvn
 ski IEDKIEEILSKIYHIENEIARIKKLIGEP
- 10 MuV F I221C/M255C-GGG-483-GCN4 (SEQ ID NO: 8)
 mkafsvtclsfavfsssicvnninilqqigiyikqqvrqlsyysqssssyivvklpnigptddscfksvstqynktnlsl
 lpiaeninniaspogrrhGGGagaaigialgvataaavtaavslvqaqtnaraiaamknsiqatnraavfevkegtq
 laiaavqaiqgdhintimntqlnmscildnqlatslglyltelttvfpqqltnpalpsis**C**qalrslslgsmtpavvqatl
 stsisaaeilsaglb**C**egqiisvllldemqmivkiniptivtqsnalvidfysissfinnqesiqlpdrileigneqwsyp
 aknckltrhhifcgyneaeerlslesklclagnisacvfspiagsymrrfvaldgtivancrsltclckspspyiqpdhh
 avttidltacqtlslldgldfsivslsnityaenltislsqtintqpidistelskvnaslqnnavkyikesnhqlqsvsvn
 ski IEDKIEEILSKIYHIENEIARIKKLIGEP
- 15 MuV F S184P/GGG-483-GCN4 (SEQ ID NO: 9)
 mkafsvtclsfavfsssicvnninilqqigiyikqqvrqlsyysqssssyivvklpnigptddscfksvstqynktnlsl
 lpiaeninniaspogrrhGGGagaaigialgvataaavtaavslvqaqtnaraiaamknsiqatnraavfevkegtq
 laiaavqaiqgdhintimntqlnms**P**cqildnqlatslglyltelttvfpqqltnpalpsisqalrslslgsmtpavvqatl
 stsisaaeilsaglmegqiisvllldemqmivkiniptivtqsnalvidfysissfinnqesiqlpdrileigneqwsyp
 aknckltrhhifcgyneaeerlslesklclagnisacvfspiagsymrrfvaldgtivancrsltclckspspyiqpdhh
 avttidltacqtlslldgldfsivslsnityaenltislsqtintqpidistelskvnaslqnnavkyikesnhqlqsvsvn
 ski IEDKIEEILSKIYHIENEIARIKKLIGEP
- 20 MuV F A163C/V235C-GGG-476-GCN4 (SEQ ID NO: 10)
 mkafsvtclsfavfsssicvnninilqqigiyikqqvrqlsyysqssssyivvklpnigptddscfksvstqynktnlsl
 lpiaeninniaspogrrhGGGagaaigialgvataaavtaavslvqaqtnaraiaamknsiqatnraavfevkegtq
 la**C**avqaiqgdhintimntqlnmscildnqlatslglyltelttvfpqqltnpalpsisqalrslslgsmtpa**C**vqatl
 stsisaaeilsaglmegqiisvllldemqmivkiniptivtqsnalvidfysissfinnqesiqlpdrileigneqwsyp
 aknckltrhhifcgyneaeerlslesklclagnisacvfspiagsymrrfvaldgtivancrsltclckspspyiqpdhh
 avttidltacqtlslldgldfsivslsnityaenltislsqtintqpidistelskvnaslqnnavkyikesnhqlq
 ski IEDKIEEILSKIYHIENEIARIKKLIGEP
- 25 MuV F V206C/A223C-GGG-476-GCN4 (SEQ ID NO: 11)
 mkafsvtclsfavfsssicvnninilqqigiyikqqvrqlsyysqssssyivvklpnigptddscfksvstqynktnlsl
 lpiaeninniaspogrrhGGGagaaigialgvataaavtaavslvqaqtnaraiaamknsiqatnraavfevkegtq
 laiaavqaiqgdhintimntqlnmscildnqlatslglylteltt**C**fqpqltnpalpsisq**C**lrlslgsmtpavvqatl
 stsisaaeilsaglmegqiisvllldemqmivkiniptivtqsnalvidfysissfinnqesiqlpdrileigneqwsyp
 aknckltrhhifcgyneaeerlslesklclagnisacvfspiagsymrrfvaldgtivancrsltclckspspyiqpdhh
 avttidltacqtlslldgldfsivslsnityaenltislsqtintqpidistelskvnaslqnnavkyikesnhqlq
 ski IEDKIEEILSKIYHIENEIARIKKLIGEP
- 30 MuV F N86C/A215C-GGG-476-GCN4 (SEQ ID NO: 12)
 mkafsvtclsfavfsssicvnninilqqigiyikqqvrqlsyysqssssyivvklpnigptddscfksvstqynktnlsl
 lpiaeb**C**inniaspogrrhGGGagaaigialgvataaavtaavslvqaqtnaraiaamknsiqatnraavfevkegtq
 laiaavqaiqgdhintimntqlnmscildnqlatslglyltelttvfpqqltnp**C**lspisqalrslslgsmtpavvqatl
 stsisaaeilsaglmegqiisvllldemqmivkiniptivtqsnalvidfysissfinnqesiqlpdrileigneqwsyp
 aknckltrhhifcgyneaeerlslesklclagnisacvfspiagsymrrfvaldgtivancrsltclckspspyiqpdhh
 avttidltacqtlslldgldfsivslsnityaenltislsqtintqpidistelskvnaslqnnavkyikesnhqlq
 ski IEDKIEEILSKIYHIENEIARIKKLIGEP
- 35 MuV F P209C/P214C-GGG-476-GCN4 (SEQ ID NO: 13)
 mkafsvtclsfavfsssicvnninilqqigiyikqqvrqlsyysqssssyivvklpnigptddscfksvstqynktnlsl
 lpiaeninniaspogrrhGGGagaaigialgvataaavtaavslvqaqtnaraiaamknsiqatnraavfevkegtq
 laiaavqaiqgdhintimntqlnmscildnqlatslglyltelttv**C**qqltn**C**alspisqalrslslgsmtpavvqatl
 stsisaaeilsaglmegqiisvllldemqmivkiniptivtqsnalvidfysissfinnqesiqlpdrileigneqwsyp
 aknckltrhhifcgyneaeerlslesklclagnisacvfspiagsymrrfvaldgtivancrsltclckspspyiqpdhh
 avttidltacqtlslldgldfsivslsnityaenltislsqtintqpidistelskvnaslqnnavkyikesnhqlq
 ski IEDKIEEILSKIYHIENEIARIKKLIGEP
- 40
- 45
- 50
- 55
- 60

- MuV F K155C/L161C-GGG-476-GCN4 (SEQ ID NO: 14)
 mkafsvtclsfavfsssicvnninilqqigiyikqqvrqlsyysqssssyivvklpnigptddscfksvqtqynktnlsl
 lpiaeninniaspspgsrrrhGGGagaaigialgvataaavtaavslvqaqtnaraiaamknsiqatnraavfevkegtq
 5 **C**aiaavqaiqdhintimntqlnmscildnqlatslglyltelttvfqpqltnpalpsisqalrslslgsmtpavvqatl
 stsisaaeilsaglmegqiisvllldemqmivkiniptivtqsnalvidfysissfinnqesiqlpdrileigneqwsyp
 aknckltrrhifcgyneaeerlslesklclagnisacvfspiagsymrrfvaldgtivancrsltclckspspyiqpdhh
 avttidltacqtlslldgldfsivslsnityaenltislsqtintqpidistelskvnaslqnnavkyikesnhqlqs
 IEDKIEEILSKIYHIENEIARIKKLIGEAP
- MuV F V165C/M231C-GGG-476-GCN4 (SEQ ID NO: 15)
 mkafsvtclsfavfsssicvnninilqqigiyikqqvrqlsyysqssssyivvklpnigptddscfksvqtqynktnlsl
 lpiaeninniaspspgsrrrhGGGagaaigialgvataaavtaavslvqaqtnaraiaamknsiqatnraavfevkegtq
 10 **C**aiaavqaiqdhintimntqlnmscildnqlatslglyltelttvfqpqltnpalpsisqalrslslgsmtpavvqatl
 stsisaaeilsaglmegqiisvllldemqmivkiniptivtqsnalvidfysissfinnqesiqlpdrileigneqwsyp
 15 aknckltrrhifcgyneaeerlslesklclagnisacvfspiagsymrrfvaldgtivancrsltclckspspyiqpdhh
 avttidltacqtlslldgldfsivslsnityaenltislsqtintqpidistelskvnaslqnnavkyikesnhqlqs
 IEDKIEEILSKIYHIENEIARIKKLIGEAP
- MuV F I221C/M255C-GGG-476-GCN4 (SEQ ID NO: 16)
 mkafsvtclsfavfsssicvnninilqqigiyikqqvrqlsyysqssssyivvklpnigptddscfksvqtqynktnlsl
 lpiaeninniaspspgsrrrhGGGagaaigialgvataaavtaavslvqaqtnaraiaamknsiqatnraavfevkegtq
 20 **C**aiaavqaiqdhintimntqlnmscildnqlatslglyltelttvfqpqltnpalpsisqalrslslgsmtpavvqatl
 stsisaaeilsaglmegqiisvllldemqmivkiniptivtqsnalvidfysissfinnqesiqlpdrileigneqwsyp
 25 aknckltrrhifcgyneaeerlslesklclagnisacvfspiagsymrrfvaldgtivancrsltclckspspyiqpdhh
 avttidltacqtlslldgldfsivslsnityaenltislsqtintqpidistelskvnaslqnnavkyikesnhqlqs
 IEDKIEEILSKIYHIENEIARIKKLIGEAP
- MuV F S184P-GGG-476-GCN4 (SEQ ID NO: 17)
 mkafsvtclsfavfsssicvnninilqqigiyikqqvrqlsyysqssssyivvklpnigptddscfksvqtqynktnlsl
 lpiaeninniaspspgsrrrhGGGagaaigialgvataaavtaavslvqaqtnaraiaamknsiqatnraavfevkegtq
 30 **C**aiaavqaiqdhintimntqlnmscildnqlatslglyltelttvfqpqltnpalpsisqalrslslgsmtpavvqatl
 stsisaaeilsaglmegqiisvllldemqmivkiniptivtqsnalvidfysissfinnqesiqlpdrileigneqwsyp
 35 aknckltrrhifcgyneaeerlslesklclagnisacvfspiagsymrrfvaldgtivancrsltclckspspyiqpdhh
 avttidltacqtlslldgldfsivslsnityaenltislsqtintqpidistelskvnaslqnnavkyikesnhqlqs
 IEDKIEEILSKIYHIENEIARIKKLIGEAP
- MuV F A163C/V235C-GGG-469-GCN4 (SEQ ID NO: 18)
 mkafsvtclsfavfsssicvnninilqqigiyikqqvrqlsyysqssssyivvklpnigptddscfksvqtqynktnlsl
 lpiaeninniaspspgsrrrhGGGagaaigialgvataaavtaavslvqaqtnaraiaamknsiqatnraavfevkegtq
 40 **C**aiaavqaiqdhintimntqlnmscildnqlatslglyltelttvfqpqltnpalpsisqalrslslgsmtpavvqatl
 stsisaaeilsaglmegqiisvllldemqmivkiniptivtqsnalvidfysissfinnqesiqlpdrileigneqwsyp
 45 aknckltrrhifcgyneaeerlslesklclagnisacvfspiagsymrrfvaldgtivancrsltclckspspyiqpdhh
 avttidltacqtlslldgldfsivslsnityaenltislsqtintqpidistelskvnaslqnnavkyikesnhqlqs
 IEDKIEEILSKIYHIENEIARIKKLIGEAP
- MuV F V206C/A223C-GGG-469-GCN4 (SEQ ID NO: 19)
 mkafsvtclsfavfsssicvnninilqqigiyikqqvrqlsyysqssssyivvklpnigptddscfksvqtqynktnlsl
 lpiaeninniaspspgsrrrhGGGagaaigialgvataaavtaavslvqaqtnaraiaamknsiqatnraavfevkegtq
 50 **C**aiaavqaiqdhintimntqlnmscildnqlatslglyltelttvfqpqltnpalpsisqalrslslgsmtpavvqatl
 stsisaaeilsaglmegqiisvllldemqmivkiniptivtqsnalvidfysissfinnqesiqlpdrileigneqwsyp
 55 aknckltrrhifcgyneaeerlslesklclagnisacvfspiagsymrrfvaldgtivancrsltclckspspyiqpdhh
 avttidltacqtlslldgldfsivslsnityaenltislsqtintqpidistelskvnaslqnnavkyikesnhqlqs
 IEDKIEEILSKIYHIENEIARIKKLIGEAP
- MuV F N86C/A215C-GGG-469-GCN4 (SEQ ID NO: 20)
 mkafsvtclsfavfsssicvnninilqqigiyikqqvrqlsyysqssssyivvklpnigptddscfksvqtqynktnlsl
 lpiaeninniaspspgsrrrhGGGagaaigialgvataaavtaavslvqaqtnaraiaamknsiqatnraavfevkegtq
 60 **C**aiaavqaiqdhintimntqlnmscildnqlatslglyltelttvfqpqltnpalpsisqalrslslgsmtpavvqatl
 stsisaaeilsaglmegqiisvllldemqmivkiniptivtqsnalvidfysissfinnqesiqlpdrileigneqwsyp
 aknckltrrhifcgyneaeerlslesklclagnisacvfspiagsymrrfvaldgtivancrsltclckspspyiqpdhh
 avttidltacqtlslldgldfsivslsnityaenltislsqtintqpidistelskvnaslqnnavkyikesnhqlqs
 IEDKIEEILSKIYHIENEIARIKKLIGEAP

- MuV F P209C/P214C-GGG-469-GCN4 (SEQ ID NO: 21)
mkafsvtclsfavfsssicvnninilqqigiyikqqvrqlsyysqssssyivvklpnigptddscfksvqtynktnsl
lpiaeninniaspssgrrrhGGGagaaigialgvataaavslvqaqtnaraiaamknsiqatnraavfevkegtq
laiavqaiqdhintimntqlnmscildnqlatslglylteitvtfqCqltnCalspisiqalrslsgsmtpavvqatl
5 stsisaaeilsaglmegqiisvllldemqmivkiniptivtqsnalvidfysissfinnqesiqlpdrileigneqwsyp
aknckltrhhifcgyneaeerlslesklclagnisacvfspiagsymrrfvaldgtivancrsltclckspspyiyqpddh
avttidltacqtlslldgldfsivslsnityaenltislsqtintqpidistelskvnaslqnnavkyike
IEDKIEEILSKIYHIENEIARIKKLIGEAP
- 10 MuV F K155C/L161C-GGG-469-GCN4 (SEQ ID NO: 22)
mkafsvtclsfavfsssicvnninilqqigiyikqqvrqlsyysqssssyivvklpnigptddscfksvqtynktnsl
lpiaeninniaspssgrrrhGGGagaaigialgvataaavslvqaqtnaraiaamknsiqatnraavfevCegtq
Caiavqaiqdhintimntqlnmscildnqlatslglylteitvtfqpqltnpalspisiqalrslsgsmtpavvqatl
stsisaaeilsaglmegqiisvllldemqmivkiniptivtqsnalvidfysissfinnqesiqlpdrileigneqwsyp
15 aknckltrhhifcgyneaeerlslesklclagnisacvfspiagsymrrfvaldgtivancrsltclckspspyiyqpddh
avttidltacqtlslldgldfsivslsnityaenltislsqtintqpidistelskvnaslqnnavkyike
IEDKIEEILSKIYHIENEIARIKKLIGEAP
- MuV F V165C/M231C-GGG-469-GCN4 (SEQ ID NO: 23)
20 mkafsvtclsfavfsssicvnninilqqigiyikqqvrqlsyysqssssyivvklpnigptddscfksvqtynktnsl
lpiaeninniaspssgrrrhGGGagaaigialgvataaavslvqaqtnaraiaamknsiqatnraavfevkegtq
laiavqaiqdhintimntqlnmscildnqlatslglylteitvtfqpqltnpalspisiqalrslsgsmtpavvqatl
stsisaaeilsaglmegqiisvllldemqmivkiniptivtqsnalvidfysissfinnqesiqlpdrileigneqwsyp
aknckltrhhifcgyneaeerlslesklclagnisacvfspiagsymrrfvaldgtivancrsltclckspspyiyqpddh
25 avttidltacqtlslldgldfsivslsnityaenltislsqtintqpidistelskvnaslqnnavkyike
IEDKIEEILSKIYHIENEIARIKKLIGEAP
- MuV F I221C/M255C-GGG-469-GCN4 (SEQ ID NO: 24)
mkafsvtclsfavfsssicvnninilqqigiyikqqvrqlsyysqssssyivvklpnigptddscfksvqtynktnsl
lpiaeninniaspssgrrrhGGGagaaigialgvataaavslvqaqtnaraiaamknsiqatnraavfevkegtq
laiavqaiqdhintimntqlnmscildnqlatslglylteitvtfqpqltnpalspisiqalrslsgsmtpavvqatl
stsisaaeilsaglcCegqiisvllldemqmivkiniptivtqsnalvidfysissfinnqesiqlpdrileigneqwsyp
aknckltrhhifcgyneaeerlslesklclagnisacvfspiagsymrrfvaldgtivancrsltclckspspyiyqpddh
30 avttidltacqtlslldgldfsivslsnityaenltislsqtintqpidistelskvnaslqnnavkyike
IEDKIEEILSKIYHIENEIARIKKLIGEAP
- 35 MuV F S184P-GGG-469-GCN4 (SEQ ID NO: 25)
mkafsvtclsfavfsssicvnninilqqigiyikqqvrqlsyysqssssyivvklpnigptddscfksvqtynktnsl
lpiaeninniaspssgrrrhGGGagaaigialgvataaavslvqaqtnaraiaamknsiqatnraavfevkegtq
laiavqaiqdhintimntqlnmscildnqlatslglylteitvtfqpqltnpalspisiqalrslsgsmtpavvqatl
stsisaaeilsaglmegqiisvllldemqmivkiniptivtqsnalvidfysissfinnqesiqlpdrileigneqwsyp
aknckltrhhifcgyneaeerlslesklclagnisacvfspiagsymrrfvaldgtivancrsltclckspspyiyqpddh
35 avttidltacqtlslldgldfsivslsnityaenltislsqtintqpidistelskvnaslqnnavkyike
IEDKIEEILSKIYHIENEIARIKKLIGEAP
- MuV F S184P-GGG-469-GCN4 (SEQ ID NO: 25)
mkafsvtclsfavfsssicvnninilqqigiyikqqvrqlsyysqssssyivvklpnigptddscfksvqtynktnsl
lpiaeninniaspssgrrrhGGGagaaigialgvataaavslvqaqtnaraiaamknsiqatnraavfevkegtq
laiavqaiqdhintimntqlnmscildnqlatslglylteitvtfqpqltnpalspisiqalrslsgsmtpavvqatl
stsisaaeilsaglmegqiisvllldemqmivkiniptivtqsnalvidfysissfinnqesiqlpdrileigneqwsyp
aknckltrhhifcgyneaeerlslesklclagnisacvfspiagsymrrfvaldgtivancrsltclckspspyiyqpddh
40 avttidltacqtlslldgldfsivslsnityaenltislsqtintqpidistelskvnaslqnnavkyike
IEDKIEEILSKIYHIENEIARIKKLIGEAP
- 45 MuV-JL F 206C/A223C-GGG-476-GCN4 (SEQ ID NO: 26)
mkafsvtclsfavfsssicvnninilqqigiyikqqvrqlsyysqssssyVvklpnigptdNscefsvqtynktnsl
lpiaeninniaspssgrrrhGGGagaaigialgvataaavslvqaqtnaraiaamknsiqatnraavfevkegtq
laiavqaiqdhintimntqlnmscildnqlatslglylteitvtfqpqltnpalspisiqalrslsgsmtpavvqatl
stsisaaeilsaglmegqiisvllldemqmivkiniptivtqsnalvidfysissfinnqesiqlpdrileigneqwsyp
50 aknckltrhhifcgyneaeerlsleTKlclagnisacvfspiagsymrrfvaldgtivancrsltclckspspyiyqpddh
avttidltScqtlslldgldfsivslsnityaenltislsqtintqpidistelskvnaslqnnavkyikesnhqlqs
IEDKIEEILSKIYHIENEIARIKKLIGEAP
- 55 MuV-IL17 F 206C/A223C-GGG-476-GCN4 (SEQ ID NO: 51)
mkvslvtclgfvfsssicvnninilqqigiyikqqvrqlsyysqssssyivvklpnigptdNscefsvqtynktnsl
lpiaeninniaspssgrrrhGGGagaaigialgvataaavslvqaqtnaraiaamknsiqatnraavfevkegtq
laiavqaiqdhintimntqlnmscildnqlatslglylteLTCfqpqltnpalspisiqCLRslsgsmtpavvqatl
stsisaaeilsaglmegqiisvllldemqmivkiniptivtqsnalvidfysissfinnqesiqlpdrileigneqwsyp
60 aknckltrhhifcgyneaeerlslesklclagnisacvfspiagsymrrfvaldgtivancrsltclckspspyiyqpddh
avttidltacqtlslldgldfsivslsnityaenltislsqtintqpidistelskvnaslqnnavkyikesnhqlqs
IEDKIEEILSKIYHIENEIARIKKLIGEAP

- Postfusion MuV F ectodomain (SEQ ID NO: 52)
 mkafsvtclsfavfsssicvninilqqigiyikqqvrqlsyysqsssyivvklpniqptddscefksvtqynktnll
 lpiaeninniaspspgsrrhGGGagialgiaalgvataaavslvqaqtnaraiaamknsiqatnraavfevCegtqg
 Caiavqaiqdhintimntqlnmqscqildnqlatslglyltelttvfqpqltnpalpsisqalrslgsmtpavvqatl
 5 stsisaaeilsaglmegqiisvllldemqmivkiniptivtqsnalvidfysissfinnqesiqlpdrileigneqwsyp
 aknckltrhhifcqyneaeerlslesklclagnisacvfspiagsymrrfvaldgtivancrsltclckspspyiqpdhh
 avttidltacqtlslldgldfsivslsnityaenltislsqtintqpidistelskvnaslqnnavkyikesnhqlqsvsn
 skigaiIEDKIEEILSKIYHIENEIARIKKLIGEAP
- 10 MuV F 206C/223C-GGG-476 + GCN4 + MuV HN_G (SEQ ID NO: 27)
 mkafsvtclsfavfsssicvninilqqigiyikqqvrqlsyysqsssyivvklpniqptddscefksvtqynktnll
 lpiaeninniaspspgsrrhGGGagialgiaalgvataaavslvqaqtnaraiaamknsiqatnraavfevkegtqg
 laiavqaiqdhintimntqlnmqscqildnqlatslglyltelttcfqpqltnpalpsisqclrslgsmtpavvqatl
 stsisaaeilsaglmegqiisvllldemqmivkiniptivtqsnalvidfysissfinnqesiqlpdrileigneqwsyp
 15 aknckltrhhifcqyneaeerlslesklclagnisacvfspiagsymrrfvaldgtivancrsltclckspspyiqpdhh
 avttidltacqtlslldgldfsivslsnityaenltislsqtintqpidistelskvnaslqnnavkyikesnhqlqs
 IEDKIEEILSKIYHIENEIARIKKLIGEAP GSGGGGGG
 niplvndlrfringinkfiiedyathdfsighplnmpsfiptatspngctripsfslgkthwcythvinanckdhtssnq
 yvsmgilvqtasgypmftlkiqylsdglnrkscsiatvpdgcamycyvstqletddyagssptqkltllfyndtvtter
 20 tispsglegnwatlvpgvgsgiyfenklifpayggvlpnstlgvksareffrpvpnpynpcsgpqqdldqralrsyfyf
 snriqsafllvcawnqilvtncelvpssngtmmgaegrvllinnrllyyqrstswwpelyellieisftftnsgpssvnms
 wipiystrpgsgncsgenvcptacvsgvyldpwppltpyshqsginrnfytgallnssttrvnptlyvsalnlnkvlap
 ygtqglfasyttttcfqdtgdasvycvyimelasnivgefqiilpvltrtit
- 25 The above sequences include an N-terminal signal peptide, a MuV F ectodomain, a GCN4
 trimerization domain, optionally a MuV HN ectodomain, a thrombin cleavage site, a HIS tag and a Strep tag,
 as well as various linker residues between segments.

Example 2

30 MeV F Proteins Stabilized in a Prefusion Conformation and Fusions Thereof with MeV H Ectodomain or MuV HN ectodomain

The example illustrates embodiments of a MeV F ectodomain trimer stabilized in a prefusion
 conformation by one or more amino acid substitutions. Further provided are MeV F ectodomain trimers
 linked to a MeV H ectodomain. The prefusion-stabilized MeV F ectodomain trimers and corresponding
 35 fusions with MeV H ectodomain are useful, for example, for inducing a neutralizing immune response to
 MeV in a subject.

When produced in cells, the MeV F ectodomain linked to a C-terminal GCN4 trimerization domain
 forms trimers that spontaneously transition to the prefusion conformation. Unstabilized recombinant MeV
 F-GCN4 is so unstable, 100% of molecules have transitioned to the postfusion conformation at the point of
 40 evaluation (EM). Also protein expression is substantially reduced without stabilization.

Accordingly, structure-based vaccine design was used to identify mutations for the stabilization of
 the MeV F ectodomain in a prefusion conformation (based on prefusion PIV5 F structure PDB ID 4WSG
 and MeV F structure PDB ID 5YXW), and also to eliminate the F1/F2 cleavage site to produce a “single
 chain” MeV F protein with increased expression. Multiple stabilization strategies were employed to “lock”
 45 the MeV F ectodomain in the prefusion conformation, including introduction of disulfide bonds and proline
 substitutions. In total, approximately 40 different mutants were designed, expressed, purified, assessed for
 expression level, and assessed for prefusion conformation by negative stain EM.

The mutations were introduced into a MeV F ectodomain (based on C-terminal truncations at MeV F position 486, and linked to a C-terminal GCN4 trimerization domain, and the resulting mutants were screened as noted above. The ectodomain also included a mutation to remove the F1/F2 furin cleavage site. The prefusion stabilizing mutations assessed included: cysteine substitutions at one or more of MeV F positions 48 and 284, 90 and 225, 141 and 270, 165 and 171, 173 and 245, 175 and 241, 212 and 236, 216 and 233, and 219 and 224 that form a non-natural disulfide bond; and a proline substitution at MeV F position 194. Relevant sequences are shown below.

- The expression and purification of the single chain and prefusion-stabilized MeV F proteins showed a substantial increase in expression level compared to the unmodified MeV F.
- As illustrated in FIG. 5, negative EM can be used to distinguish MeV F ectodomain trimers that are in the prefusion conformation from those that are in the postfusion conformation. Also, MeV F R165C-M171C-486-GCN4 (SEQ ID NO: 38) showed an excellent combination of prefusion stabilization and protein expression, and purified as a monodispersed protein by S200 gel filtration. This construct and others were analyzed by electron microscopy to further confirm the prefusion conformation.
- Immunization assays were conducted with a MeV F ectodomain trimer in a post-fusion conformation and a MeV F ectodomain trimer in a pre-fusion conformation (MeV F R165C-M171C-486-GCN4 (SEQ ID NO: 38)). The immunization protocol was according to that shown in FIG. 3A. Groups of 10 CB6F1/J mice were immunized with 10 µg/dose of protein in Poly IC adjuvant at weeks 0 and 3, and the neutralization titer of week 5 sera from the immunized mice were assessed. The immune sera were assessed in a MeV neutralization assay (FIG. 5C), which showed that immune sera from animals immunized with the MeV F R165C-M171C-486-GCN4 (SEQ ID NO: 38) neutralized MeV more than 200-fold higher than sera from animals immunized with the postfusion MeV F, and above a protective threshold.

Sequences:

- MeV F A90C/I225C-GGG-486-GCN4 (SEQ ID NO: 37)
 mysmqlascvtltlvllvnsqihwgnlskigvvgigsasykvmtsrsshqslviklmpnitllnnctrveiaeyrrllrtv
 lepir**C**lnavtqnirpvqsvassrrhGGGagvvlagaalgvataaqitagialhqsmnlsqaidnlaslettnqaiea
 irqagqgemilavqgvqdyinnelipsmnqlscdligqklglklrryyteilsflgpslrdp**C**saeisigalsyalggdin
 kvleklysggdllgilesrgikarithvdtesyfivlsiaayptlseikgvivhrlegvsnigsgewyttvpkyvatqg
 ylisnfdesstfmpegtvcsqnalypmspllqecrlrgstkscartlvsgsfgnrfilsqgnliancasilckcyttgti
 inqdpdkiltyiaadhcpvvevngvtiqvgsrrypdavylhrldlgppislerldvgtlnlgnaiakledakellessdqi
 lsa IEDKIEEILSKIYHIENEIARIKKLIGEAP
- MeV F R165C/M171C-GGG-486-GCN4 (SEQ ID NO: 38)
 mysmqlascvtltlvllvnsqihwgnlskigvvgigsasykvmtsrsshqslviklmpnitllnnctrveiaeyrrllrtv
 lepir**C**lnavtqnirpvqsvassrrhGGGagvvlagaalgvataaqitagialhqsmnlsqaidnlaslettnqaiea
 i**C**qagqge**C**ilavqgvqdyinnelipsmnqlscdligqklglklrryyteilsflgpslrdp**C**saeisigalsyalggdin
 kvleklysggdllgilesrgikarithvdtesyfivlsiaayptlseikgvivhrlegvsnigsgewyttvpkyvatqg
 ylisnfdesstfmpegtvcsqnalypmspllqecrlrgstkscartlvsgsfgnrfilsqgnliancasilckcyttgti
 inqdpdkiltyiaadhcpvvevngvtiqvgsrrypdavylhrldlgppislerldvgtlnlgnaiakledakellessdqi
 lsa IEDKIEEILSKIYHIENEIARIKKLIGEAP
- MeV F L173C/V245C-GGG-486-GCN4 (SEQ ID NO: 39)
 mysmqlascvtltlvllvnsqihwgnlskigvvgigsasykvmtsrsshqslviklmpnitllnnctrveiaeyrrllrtv
 lepir**C**lnavtqnirpvqsvassrrhGGGagvvlagaalgvataaqitagialhqsmnlsqaidnlaslettnqaiea
 irqagqgem**C**avqgvqdyinnelipsmnqlscdligqklglklrryyteilsflgpslrdp**C**saeisigalsyalggdin

- 5 kCleklygysggdlilgilesrgikarithvdtesyfivlsiaayptlseikgvivhrlegvsynigsqewyttvpkyvatqg
ylisnfdesstfmpegtvcsgnalypmspllqecrlrgstkscartlvsgsfgnrfilsqgnlianacasilckcyttgti
inqdpdkiltyiaadhcpvvevngvtiqvgsrrypdavylhridlgppislerldvgtnlgnaiakledakellessdqi
lsa IEDKIEEILSKIYHIENEIARIKKLIGEAP
- 10 MeV F V175C/D241C-GGG-486-GCN4 (SEQ ID NO: 40)
mysmqlascvtltlvllvnsqihwgnlskigvvgigsasykvmttrsshqslviklmpnitllnnctrveiaeyrrllrtv
lepardalnavtqnirpvqsvassrrhGGGagvvlagaalgvataaqitagialhqsmnlsqaidnlaslettnqiaiea
irqagqemilaCggvqdyinnelipsmnqlscdligqklglkllryyteilsfpgpslrldpisaaisiqalsyalggCin
kvleklygysggdlilgilesrgikarithvdtesyfivlsiaayptlseikgvivhrlegvsynigsqewyttvpkyvatqg
ylisnfdesstfmpegtvcsgnalypmspllqecrlrgstkscartlvsgsfgnrfilsqgnlianacasilckcyttgti
inqdpdkiltyiaadhcpvvevngvtiqvgsrrypdavylhridlgppislerldvgtnlgnaiakledakellessdqi
lsa IEDKIEEILSKIYHIENEIARIKKLIGEAP
- 15 MeV F L216C/A233C-GGG-486-GCN4 (SEQ ID NO: 41)
mysmqlascvtltlvllvnsqihwgnlskigvvgigsasykvmttrsshqslviklmpnitllnnctrveiaeyrrllrtv
lepardalnavtqnirpvqsvassrrhGGGagvvlagaalgvataaqitagialhqsmnlsqaidnlaslettnqiaiea
irqagqemilavqgvqdyinnelipsmnqlscdligqklglkllryyteilsCfpgpslrldpisaaisiqCalsyalggdin
kvleklygysggdlilgilesrgikarithvdtesyfivlsiaayptlseikgvivhrlegvsynigsqewyttvpkyvatqg
20 ylisnfdesstfmpegtvcsgnalypmspllqecrlrgstkscartlvsgsfgnrfilsqgnlianacasilckcyttgti
inqdpdkiltyiaadhcpvvevngvtiqvgsrrypdavylhridlgppislerldvgtnlgnaiakledakellessdqi
lsa IEDKIEEILSKIYHIENEIARIKKLIGEAP
- 25 MeV F P219C/P224C-GGG-486-GCN4 (SEQ ID NO: 42)
mysmqlascvtltlvllvnsqihwgnlskigvvgigsasykvmttrsshqslviklmpnitllnnctrveiaeyrrllrtv
lepardalnavtqnirpvqsvassrrhGGGagvvlagaalgvataaqitagialhqsmnlsqaidnlaslettnqiaiea
irqagqemilavqgvqdyinnelipsmnqlscdligqklglkllryyteilsfpgCslrdCisaaisiqalsyalggdin
kvleklygysggdlilgilesrgikarithvdtesyfivlsiaayptlseikgvivhrlegvsynigsqewyttvpkyvatqg
ylisnfdesstfmpegtvcsgnalypmspllqecrlrgstkscartlvsgsfgnrfilsqgnlianacasilckcyttgti
30 inqdpdkiltyiaadhcpvvevngvtiqvgsrrypdavylhridlgppislerldvgtnlgnaiakledakellessdqi
lsa IEDKIEEILSKIYHIENEIARIKKLIGEAP
- 35 MeV F S194P-GGG-486-GCN4 (SEQ ID NO: 43)
mysmqlascvtltlvllvnsqihwgnlskigvvgigsasykvmttrsshqslviklmpnitllnnctrveiaeyrrllrtv
lepardalnavtqnirpvqsvassrrhGGGagvvlagaalgvataaqitagialhqsmnlsqaidnlaslettnqiaiea
irqagqemilavqgvqdyinnelipsmnqlPcdligqklglkllryyteilsfpgpslrldpisaaisiqalsyalggdin
kvleklygysggdlilgilesrgikarithvdtesyfivlsiaayptlseikgvivhrlegvsynigsqewyttvpkyvatqg
ylisnfdesstfmpegtvcsgnalypmspllqecrlrgstkscartlvsgsfgnrfilsqgnlianacasilckcyttgti
40 inqdpdkiltyiaadhcpvvevngvtiqvgsrrypdavylhridlgppislerldvgtnlgnaiakledakellessdqi
lsa IEDKIEEILSKIYHIENEIARIKKLIGEAP
- 50 Postfusion MeV F (SEQ ID NO: 61)
MYSMQLASCVTTLTLVLLVNSQIHWGNLSKIGVVGIGSASYKVMTRSSHQSLVIKLMPNITLLNNCTRVEIAEYRRLRLRTV
LEPIRDALNAVTONIRPVQSVASSSGSGGSAQITAGIALHQSMNLSQAIDNLRASLETTNQAIEAIRQAGQEMILAVQGV
45 QDYINNELIPSMNQLSCDLIGQKLGLKLLRYYTEILSLFGPSLRDPISAEISIQALSALGGDINKVLEKLYSGGDLG
ILESRIKARITHVDTESYFIVLSIAYPTLSEIKGVIVHRLEGVSYNIGSQEWYTTVPKYVATQGYLISNFDESSCTFMP
EGTVCSQNALYPMSPLLQECLRGSTKSCARTLVSGSFGNRFILSQGNLIANACASILCKCYTTGTIINQDPDKILTYIAD
HCPVVEVNGVTIQVGSRRYPDAVYLHRLDLPISLERLDVGTNLGNATAKLEDAKELLESSDQILSAIGGYIPEAPRDG
QAYVRKDGEWVLLSTFL
- 55 MeV F S103C/V117C-GGG-486-GCN4 (SEQ ID NO: 62)
mysmqlascvtltlvllvnsqihwgnlskigvvgigsasykvmttrsshqslviklmpnitllnnctrveiaeyrrllrtv
lepardalnavtqnirpvqCvassrrhGGGagvCclagaalgvataaqitagialhqsmnlsqaidnlaslettnqiaiea
irqagqemilavqgvqdyinnelipsmnqlscdligqklglkllryyteilsfpgpslrldpisaaisiqalsyalggdin
kvleklygysggdlilgilesrgikarithvdtesyfivlsiaayptlseikgvivhrlegvsynigsqewyttvpkyvatqg
ylisnfdesstfmpegtvcsgnalypmspllqecrlrgstkscartlvsgsfgnrfilsqgnlianacasilckcyttgti
inqdpdkiltyiaadhcpvvevngvtiqvgsrrypdavylhridlgppislerldvgtnlgnaiakledakellessdqi
lsa IEDKIEEILSKIYHIENEIARIKKLIGEAP
- 60 MeV F V175F-GGG-486-GCN4 (SEQ ID NO: 63)
mysmqlascvtltlvllvnsqihwgnlskigvvgigsasykvmttrsshqslviklmpnitllnnctrveiaeyrrllrtv
lepardalnavtqnirpvqsvassrrhGGGagvvlagaalgvataaqitagialhqsmnlsqaidnlaslettnqiaiea

irqagqemilaFggvqdyinnelipsmnqlscdligqklglkllryyteilsflgpslrdpisaeisigalsyalggdin
 kvleklgysggdllgilesrgikarithvdtesyfivlsiaayptlseikgvivhrlegvsynigsqewyttvpkyvatqg
 ylisnfdessctfmpegtvcsqnalypmspllqecclrgstkscartlvsgsfgnrflsqgnliancasilckcyttgti
 inqdpdkiltyiaadhcpvvevngvtiqvgsrrypdavylhridlgppislerldvgtnlgnaiakledakellessdqi
 5 lsa IEDKIEEILSKIYHIENEIARIKKLIGEAP

MeV F S103C/V117C, V175F, S194P-GGG-486-GCN4 (SEQ ID NO: 64)
 mysmqlascvtltlvtllvnsqihwgnlskigvvgigsasykvmttrsshqslviklmpnitllnnctrveiaeyrrllrtv
 lepirdalnavtqnirpvgCvassrrhGGGagvClagaaalgvataaqitagialhqsmlnsqaidnlaslettnqiaiea
 10 irqagqemilaFggvqdyinnelipsmnqlPcdligqklglkllryyteilsflgpslrdpisaeisigalsyalggdin
 kvleklgysggdllgilesrgikarithvdtesyfivlsiaayptlseikgvivhrlegvsynigsqewyttvpkyvatqg
 ylisnfdessctfmpegtvcsqnalypmspllqecclrgstkscartlvsgsfgnrflsqgnliancasilckcyttgti
 inqdpdkiltyiaadhcpvvevngvtiqvgsrrypdavylhridlgppislerldvgtnlgnaiakledakellessdqi
 15 lsa IEDKIEEILSKIYHIENEIARIKKLIGEAP

MeV F S103C/V117C, R165C/M171C, S194P-GGG-486-GCN4 (SEQ ID NO: 65)
 mysmqlascvtltlvtllvnsqihwgnlskigvvgigsasykvmttrsshqslviklmpnitllnnctrveiaeyrrllrtv
 lepirdalnavtqnirpvgCvassrrhGGGagvClagaaalgvataaqitagialhqsmlnsqaidnlaslettnqiaiea
 iCqagqeCilaFggvqdyinnelipsmnqlPcdligqklglkllryyteilsflgpslrdpisaeisigalsyalggdin
 20 kvleklgysggdllgilesrgikarithvdtesyfivlsiaayptlseikgvivhrlegvsynigsqewyttvpkyvatqg
 ylisnfdessctfmpegtvcsqnalypmspllqecclrgstkscartlvsgsfgnrflsqgnliancasilckcyttgti
 inqdpdkiltyiaadhcpvvevngvtiqvgsrrypdavylhridlgppislerldvgtnlgnaiakledakellessdqi
 25 lsa IEDKIEEILSKIYHIENEIARIKKLIGEAP

MeV F R99C/V117C-GGG-486-GCN4 (SEQ ID NO: 66)
 mysmqlascvtltlvtllvnsqihwgnlskigvvgigsasykvmttrsshqslviklmpnitllnnctrveiaeyrrllrtv
 lepirdalnavtqnirpvgCvassrrhGGGagvClagaaalgvataaqitagialhqsmlnsqaidnlaslettnqiaiea
 irqagqemilavggvqdyinnelipsmnqlscdligqklglkllryyteilsflgpslrdpisaeisigalsyalggdin
 kvleklgysggdllgilesrgikarithvdtesyfivlsiaayptlseikgvivhrlegvsynigsqewyttvpkyvatqg
 30 ylisnfdessctfmpegtvcsqnalypmspllqecclrgstkscartlvsgsfgnrflsqgnliancasilckcyttgti
 inqdpdkiltyiaadhcpvvevngvtiqvgsrrypdavylhridlgppislerldvgtnlgnaiakledakellessdqi
 35 lsa IEDKIEEILSKIYHIENEIARIKKLIGEAP

MeV F R99C/V117C, V175F, S194P-GGG-486-GCN4 (SEQ ID NO: 67)
 mysmqlascvtltlvtllvnsqihwgnlskigvvgigsasykvmttrsshqslviklmpnitllnnctrveiaeyrrllrtv
 lepirdalnavtqnirpvgCvassrrhGGGagvClagaaalgvataaqitagialhqsmlnsqaidnlaslettnqiaiea
 irqagqemilaFggvqdyinnelipsmnqlPcdligqklglkllryyteilsflgpslrdpisaeisigalsyalggdin
 kvleklgysggdllgilesrgikarithvdtesyfivlsiaayptlseikgvivhrlegvsynigsqewyttvpkyvatqg
 ylisnfdessctfmpegtvcsqnalypmspllqecclrgstkscartlvsgsfgnrflsqgnliancasilckcyttgti
 40 inqdpdkiltyiaadhcpvvevngvtiqvgsrrypdavylhridlgppislerldvgtnlgnaiakledakellessdqi
 45 lsa IEDKIEEILSKIYHIENEIARIKKLIGEAP

MeV F R99C/V117C, R165C/M171C, S194P-GGG-486-GCN4 (SEQ ID NO: 68)
 mysmqlascvtltlvtllvnsqihwgnlskigvvgigsasykvmttrsshqslviklmpnitllnnctrveiaeyrrllrtv
 lepirdalnavtqnirpvgCvassrrhGGGagvClagaaalgvataaqitagialhqsmlnsqaidnlaslettnqiaiea
 iCqagqeCilaFggvqdyinnelipsmnqlPcdligqklglkllryyteilsflgpslrdpisaeisigalsyalggdin
 kvleklgysggdllgilesrgikarithvdtesyfivlsiaayptlseikgvivhrlegvsynigsqewyttvpkyvatqg
 ylisnfdessctfmpegtvcsqnalypmspllqecclrgstkscartlvsgsfgnrflsqgnliancasilckcyttgti
 50 inqdpdkiltyiaadhcpvvevngvtiqvgsrrypdavylhridlgppislerldvgtnlgnaiakledakellessdqi
 55 lsa IEDKIEEILSKIYHIENEIARIKKLIGEAP

MeV F R99C/V117C-GGG-493-GCN4 (SEQ ID NO: 69)
 mysmqlascvtltlvtllvnsqihwgnlskigvvgigsasykvmttrsshqslviklmpnitllnnctrveiaeyrrllrtv
 lepirdalnavtqnirpvgCvassrrhGGGagvClagaaalgvataaqitagialhqsmlnsqaidnlaslettnqiaiea
 irqagqemilavggvqdyinnelipsmnqlscdligqklglkllryyteilsflgpslrdpisaeisigalsyalggdin
 kvleklgysggdllgilesrgikarithvdtesyfivlsiaayptlseikgvivhrlegvsynigsqewyttvpkyvatqg
 ylisnfdessctfmpegtvcsqnalypmspllqecclrgstkscartlvsgsfgnrflsqgnliancasilckcyttgti
 inqdpdkiltyiaadhcpvvevngvtiqvgsrrypdavylhridlgppislerldvgtnlgnaiakledakellessdqi
 60 l rsmkgllsa IEDKIEEILSKIYHIENEIARIKKLIGEAP

MeV F R99C/V117C, V175F, S194P-GGG-493-GCN4 (SEQ ID NO: 70)

- 5
10
15
20
25
30
35
40
45
50
55
60
- mysmq¹las²cv³tl⁴tl⁵vl⁶lv⁷ns⁸qih⁹wgn¹⁰lsk¹¹ig¹²vv¹³gigs¹⁴asy¹⁵kv¹⁶mr¹⁷ssh¹⁸qsl¹⁹vik²⁰lmp²¹nit²²l²³nn²⁴ctr²⁵veia²⁶ey²⁷rr²⁸ll²⁹rtv³⁰
le³¹pir³²dal³³nav³⁴tq³⁵ni³⁶r³⁷pv³⁸qsv³⁹ass⁴⁰rrrh⁴¹GGG⁴²agv⁴³cl⁴⁴aga⁴⁵alg⁴⁶vata⁴⁷aa⁴⁸qita⁴⁹gial⁵⁰hqs⁵¹mlns⁵²qaid⁵³nl⁵⁴ras⁵⁵lett⁵⁶nq⁵⁷aiea⁵⁸
ir⁵⁹qag⁶⁰qem⁶¹ila⁶²F⁶³ggv⁶⁴qdy⁶⁵inn⁶⁶elips⁶⁷mnq⁶⁸l⁶⁹P⁷⁰cd⁷¹lig⁷²qkl⁷³gl⁷⁴kl⁷⁵lry⁷⁶yte⁷⁷il⁷⁸sl⁷⁹fg⁸⁰ps⁸¹lr⁸²dp⁸³isa⁸⁴e⁸⁵is⁸⁶iq⁸⁷als⁸⁸yal⁸⁹gg⁹⁰din⁹¹
kv⁹²lek⁹³lgys⁹⁴ggd⁹⁵ll⁹⁶giles⁹⁷rgi⁹⁸kar⁹⁹ith¹⁰⁰vd¹⁰¹tes¹⁰²y¹⁰³fi¹⁰⁴vl¹⁰⁵sia¹⁰⁶ypt¹⁰⁷lse¹⁰⁸ik¹⁰⁹gv¹¹⁰iv¹¹¹hr¹¹²leg¹¹³vsyn¹¹⁴igs¹¹⁵qew¹¹⁶yt¹¹⁷tp¹¹⁸ky¹¹⁹vat¹²⁰qg¹²¹
yl¹²²is¹²³nf¹²⁴des¹²⁵sct¹²⁶fm¹²⁷peg¹²⁸tv¹²⁹cs¹³⁰nal¹³¹yp¹³²ms¹³³pll¹³⁴qec¹³⁵lr¹³⁶gst¹³⁷ksc¹³⁸art¹³⁹lv¹⁴⁰sgs¹⁴¹fgn¹⁴²r¹⁴³fil¹⁴⁴sq¹⁴⁵gn¹⁴⁶li¹⁴⁷anc¹⁴⁸asil¹⁴⁹ck¹⁵⁰cy¹⁵¹tt¹⁵²gt¹⁵³i¹⁵⁴
in¹⁵⁵qdp¹⁵⁶kil¹⁵⁷tyia¹⁵⁸adh¹⁵⁹cp¹⁶⁰vve¹⁶¹nv¹⁶²gt¹⁶³iq¹⁶⁴vg¹⁶⁵srr¹⁶⁶yp¹⁶⁷dav¹⁶⁸yl¹⁶⁹hr¹⁷⁰idl¹⁷¹gpp¹⁷²is¹⁷³ler¹⁷⁴ld¹⁷⁵vg¹⁷⁶tn¹⁷⁷lgn¹⁷⁸ai¹⁷⁹ak¹⁸⁰led¹⁸¹ak¹⁸²el¹⁸³less¹⁸⁴dqi¹⁸⁵
l¹⁸⁶rsm¹⁸⁷kg¹⁸⁸lssa¹⁸⁹IEDKIEEILSKIYHIENEIARIKKLIGEAP
- MeV F R99C/V117C, R165C/M171C, S194P-GGG-493-GCN4 (SEQ ID NO: 71)
- mysmq¹las²cv³tl⁴tl⁵vl⁶lv⁷ns⁸qih⁹wgn¹⁰lsk¹¹ig¹²vv¹³gigs¹⁴asy¹⁵kv¹⁶mr¹⁷ssh¹⁸qsl¹⁹vik²⁰lmp²¹nit²²l²³nn²⁴ctr²⁵veia²⁶ey²⁷rr²⁸ll²⁹rtv³⁰
le³¹pir³²dal³³nav³⁴tq³⁵ni³⁶r³⁷pv³⁸qsv³⁹ass⁴⁰rrrh⁴¹GGG⁴²agv⁴³cl⁴⁴aga⁴⁵alg⁴⁶vata⁴⁷aa⁴⁸qita⁴⁹gial⁵⁰hqs⁵¹mlns⁵²qaid⁵³nl⁵⁴ras⁵⁵lett⁵⁶nq⁵⁷aiea⁵⁸
i⁵⁹C⁶⁰qag⁶¹qe⁶²Cila⁶³F⁶⁴ggv⁶⁵qdy⁶⁶inn⁶⁷elips⁶⁸mnq⁶⁹l⁷⁰P⁷¹cd⁷²lig⁷³qkl⁷⁴gl⁷⁵kl⁷⁶lry⁷⁷yte⁷⁸il⁷⁹sl⁸⁰fg⁸¹ps⁸²lr⁸³dp⁸⁴isa⁸⁵e⁸⁶is⁸⁷iq⁸⁸als⁸⁹yal⁹⁰gg⁹¹din⁹²
kv⁹³lek⁹⁴lgys⁹⁵ggd⁹⁶ll⁹⁷giles⁹⁸rgi⁹⁹kar¹⁰⁰ith¹⁰¹vd¹⁰²tes¹⁰³y¹⁰⁴fi¹⁰⁵vl¹⁰⁶sia¹⁰⁷ypt¹⁰⁸lse¹⁰⁹ik¹¹⁰gv¹¹¹iv¹¹²hr¹¹³leg¹¹⁴vsyn¹¹⁵igs¹¹⁶qew¹¹⁷yt¹¹⁸tp¹¹⁹ky¹²⁰vat¹²¹qg¹²²
yl¹²³is¹²⁴nf¹²⁵des¹²⁶sct¹²⁷fm¹²⁸peg¹²⁹tv¹³⁰cs¹³¹nal¹³²yp¹³³ms¹³⁴pll¹³⁵qec¹³⁶lr¹³⁷gst¹³⁸ksc¹³⁹art¹⁴⁰lv¹⁴¹sgs¹⁴²fgn¹⁴³r¹⁴⁴fil¹⁴⁵sq¹⁴⁶gn¹⁴⁷li¹⁴⁸anc¹⁴⁹asil¹⁵⁰ck¹⁵¹cy¹⁵²tt¹⁵³gt¹⁵⁴i¹⁵⁵
in¹⁵⁶qdp¹⁵⁷kil¹⁵⁸tyia¹⁵⁹adh¹⁶⁰cp¹⁶¹vve¹⁶²nv¹⁶³gt¹⁶⁴iq¹⁶⁵vg¹⁶⁶srr¹⁶⁷yp¹⁶⁸dav¹⁶⁹yl¹⁷⁰hr¹⁷¹idl¹⁷²gpp¹⁷³is¹⁷⁴ler¹⁷⁵ld¹⁷⁶vg¹⁷⁷tn¹⁷⁸lgn¹⁷⁹ai¹⁸⁰ak¹⁸¹led¹⁸²ak¹⁸³el¹⁸⁴less¹⁸⁵dqi¹⁸⁶
l¹⁸⁷rsm¹⁸⁸kg¹⁸⁹lssa¹⁹⁰IEDKIEEILSKIYHIENEIARIKKLIGEAP
- MeV F P100C/V117C, R165C/M171C, S194P-GGG-486-GCN4 (SEQ ID NO: 72)
- mysmq¹las²cv³tl⁴tl⁵vl⁶lv⁷ns⁸qih⁹wgn¹⁰lsk¹¹ig¹²vv¹³gigs¹⁴asy¹⁵kv¹⁶mr¹⁷ssh¹⁸qsl¹⁹vik²⁰lmp²¹nit²²l²³nn²⁴ctr²⁵veia²⁶ey²⁷rr²⁸ll²⁹rtv³⁰
le³¹pir³²dal³³nav³⁴tq³⁵ni³⁶r³⁷pv³⁸qsv³⁹ass⁴⁰rrrh⁴¹GGG⁴²agv⁴³cl⁴⁴aga⁴⁵alg⁴⁶vata⁴⁷aa⁴⁸qita⁴⁹gial⁵⁰hqs⁵¹mlns⁵²qaid⁵³nl⁵⁴ras⁵⁵lett⁵⁶nq⁵⁷aiea⁵⁸
i⁵⁹C⁶⁰qag⁶¹qe⁶²Cila⁶³F⁶⁴ggv⁶⁵qdy⁶⁶inn⁶⁷elips⁶⁸mnq⁶⁹l⁷⁰P⁷¹cd⁷²lig⁷³qkl⁷⁴gl⁷⁵kl⁷⁶lry⁷⁷yte⁷⁸il⁷⁹sl⁸⁰fg⁸¹ps⁸²lr⁸³dp⁸⁴isa⁸⁵e⁸⁶is⁸⁷iq⁸⁸als⁸⁹yal⁹⁰gg⁹¹din⁹²
kv⁹³lek⁹⁴lgys⁹⁵ggd⁹⁶ll⁹⁷giles⁹⁸rgi⁹⁹kar¹⁰⁰ith¹⁰¹vd¹⁰²tes¹⁰³y¹⁰⁴fi¹⁰⁵vl¹⁰⁶sia¹⁰⁷ypt¹⁰⁸lse¹⁰⁹ik¹¹⁰gv¹¹¹iv¹¹²hr¹¹³leg¹¹⁴vsyn¹¹⁵igs¹¹⁶qew¹¹⁷yt¹¹⁸tp¹¹⁹ky¹²⁰vat¹²¹qg¹²²
yl¹²³is¹²⁴nf¹²⁵des¹²⁶sct¹²⁷fm¹²⁸peg¹²⁹tv¹³⁰cs¹³¹nal¹³²yp¹³³ms¹³⁴pll¹³⁵qec¹³⁶lr¹³⁷gst¹³⁸ksc¹³⁹art¹⁴⁰lv¹⁴¹sgs¹⁴²fgn¹⁴³r¹⁴⁴fil¹⁴⁵sq¹⁴⁶gn¹⁴⁷li¹⁴⁸anc¹⁴⁹asil¹⁵⁰ck¹⁵¹cy¹⁵²tt¹⁵³gt¹⁵⁴i¹⁵⁵
in¹⁵⁶qdp¹⁵⁷kil¹⁵⁸tyia¹⁵⁹adh¹⁶⁰cp¹⁶¹vve¹⁶²nv¹⁶³gt¹⁶⁴iq¹⁶⁵vg¹⁶⁶srr¹⁶⁷yp¹⁶⁸dav¹⁶⁹yl¹⁷⁰hr¹⁷¹idl¹⁷²gpp¹⁷³is¹⁷⁴ler¹⁷⁵ld¹⁷⁶vg¹⁷⁷tn¹⁷⁸lgn¹⁷⁹ai¹⁸⁰ak¹⁸¹led¹⁸²ak¹⁸³el¹⁸⁴less¹⁸⁵dqi¹⁸⁶
lsa¹⁸⁷IEDKIEEILSKIYHIENEIARIKKLIGEAP
- MeV F V101C/V117C, R165C/M171C, S194P-GGG-486-GCN4 (SEQ ID NO: 73)
- mysmq¹las²cv³tl⁴tl⁵vl⁶lv⁷ns⁸qih⁹wgn¹⁰lsk¹¹ig¹²vv¹³gigs¹⁴asy¹⁵kv¹⁶mr¹⁷ssh¹⁸qsl¹⁹vik²⁰lmp²¹nit²²l²³nn²⁴ctr²⁵veia²⁶ey²⁷rr²⁸ll²⁹rtv³⁰
le³¹pir³²dal³³nav³⁴tq³⁵ni³⁶r³⁷pv³⁸qsv³⁹ass⁴⁰rrrh⁴¹GGG⁴²agv⁴³cl⁴⁴aga⁴⁵alg⁴⁶vata⁴⁷aa⁴⁸qita⁴⁹gial⁵⁰hqs⁵¹mlns⁵²qaid⁵³nl⁵⁴ras⁵⁵lett⁵⁶nq⁵⁷aiea⁵⁸
i⁵⁹C⁶⁰qag⁶¹qe⁶²Cila⁶³F⁶⁴ggv⁶⁵qdy⁶⁶inn⁶⁷elips⁶⁸mnq⁶⁹l⁷⁰P⁷¹cd⁷²lig⁷³qkl⁷⁴gl⁷⁵kl⁷⁶lry⁷⁷yte⁷⁸il⁷⁹sl⁸⁰fg⁸¹ps⁸²lr⁸³dp⁸⁴isa⁸⁵e⁸⁶is⁸⁷iq⁸⁸als⁸⁹yal⁹⁰gg⁹¹din⁹²
kv⁹³lek⁹⁴lgys⁹⁵ggd⁹⁶ll⁹⁷giles⁹⁸rgi⁹⁹kar¹⁰⁰ith¹⁰¹vd¹⁰²tes¹⁰³y¹⁰⁴fi¹⁰⁵vl¹⁰⁶sia¹⁰⁷ypt¹⁰⁸lse¹⁰⁹ik¹¹⁰gv¹¹¹iv¹¹²hr¹¹³leg¹¹⁴vsyn¹¹⁵igs¹¹⁶qew¹¹⁷yt¹¹⁸tp¹¹⁹ky¹²⁰vat¹²¹qg¹²²
yl¹²³is¹²⁴nf¹²⁵des¹²⁶sct¹²⁷fm¹²⁸peg¹²⁹tv¹³⁰cs¹³¹nal¹³²yp¹³³ms¹³⁴pll¹³⁵qec¹³⁶lr¹³⁷gst¹³⁸ksc¹³⁹art¹⁴⁰lv¹⁴¹sgs¹⁴²fgn¹⁴³r¹⁴⁴fil¹⁴⁵sq¹⁴⁶gn¹⁴⁷li¹⁴⁸anc¹⁴⁹asil¹⁵⁰ck¹⁵¹cy¹⁵²tt¹⁵³gt¹⁵⁴i¹⁵⁵
in¹⁵⁶qdp¹⁵⁷kil¹⁵⁸tyia¹⁵⁹adh¹⁶⁰cp¹⁶¹vve¹⁶²nv¹⁶³gt¹⁶⁴iq¹⁶⁵vg¹⁶⁶srr¹⁶⁷yp¹⁶⁸dav¹⁶⁹yl¹⁷⁰hr¹⁷¹idl¹⁷²gpp¹⁷³is¹⁷⁴ler¹⁷⁵ld¹⁷⁶vg¹⁷⁷tn¹⁷⁸lgn¹⁷⁹ai¹⁸⁰ak¹⁸¹led¹⁸²ak¹⁸³el¹⁸⁴less¹⁸⁵dqi¹⁸⁶
lsa¹⁸⁷IEDKIEEILSKIYHIENEIARIKKLIGEAP
- MeV F Q102C/V117C, R165C/M171C, S194P-GGG-486-GCN4 (SEQ ID NO: 74)
- mysmq¹las²cv³tl⁴tl⁵vl⁶lv⁷ns⁸qih⁹wgn¹⁰lsk¹¹ig¹²vv¹³gigs¹⁴asy¹⁵kv¹⁶mr¹⁷ssh¹⁸qsl¹⁹vik²⁰lmp²¹nit²²l²³nn²⁴ctr²⁵veia²⁶ey²⁷rr²⁸ll²⁹rtv³⁰
le³¹pir³²dal³³nav³⁴tq³⁵ni³⁶r³⁷pv³⁸qsv³⁹ass⁴⁰rrrh⁴¹GGG⁴²agv⁴³cl⁴⁴aga⁴⁵alg⁴⁶vata⁴⁷aa⁴⁸qita⁴⁹gial⁵⁰hqs⁵¹mlns⁵²qaid⁵³nl⁵⁴ras⁵⁵lett⁵⁶nq⁵⁷aiea⁵⁸
i⁵⁹C⁶⁰qag⁶¹qe⁶²Cila⁶³F⁶⁴ggv⁶⁵qdy⁶⁶inn⁶⁷elips⁶⁸mnq⁶⁹l⁷⁰P⁷¹cd⁷²lig⁷³qkl⁷⁴gl⁷⁵kl⁷⁶lry⁷⁷yte⁷⁸il⁷⁹sl⁸⁰fg⁸¹ps⁸²lr⁸³dp⁸⁴isa⁸⁵e⁸⁶is⁸⁷iq⁸⁸als⁸⁹yal⁹⁰gg⁹¹din⁹²
kv⁹³lek⁹⁴lgys⁹⁵ggd⁹⁶ll⁹⁷giles⁹⁸rgi⁹⁹kar¹⁰⁰ith¹⁰¹vd¹⁰²tes¹⁰³y¹⁰⁴fi¹⁰⁵vl¹⁰⁶sia¹⁰⁷ypt¹⁰⁸lse¹⁰⁹ik¹¹⁰gv¹¹¹iv¹¹²hr¹¹³leg¹¹⁴vsyn¹¹⁵igs¹¹⁶qew¹¹⁷yt¹¹⁸tp¹¹⁹ky¹²⁰vat¹²¹qg¹²²
yl¹²³is¹²⁴nf¹²⁵des¹²⁶sct¹²⁷fm¹²⁸peg¹²⁹tv¹³⁰cs¹³¹nal¹³²yp¹³³ms¹³⁴pll¹³⁵qec¹³⁶lr¹³⁷gst¹³⁸ksc¹³⁹art¹⁴⁰lv¹⁴¹sgs¹⁴²fgn¹⁴³r¹⁴⁴fil¹⁴⁵sq¹⁴⁶gn¹⁴⁷li¹⁴⁸anc¹⁴⁹asil¹⁵⁰ck¹⁵¹cy¹⁵²tt¹⁵³gt¹⁵⁴i¹⁵⁵
in¹⁵⁶qdp¹⁵⁷kil¹⁵⁸tyia¹⁵⁹adh¹⁶⁰cp¹⁶¹vve¹⁶²nv¹⁶³gt¹⁶⁴iq¹⁶⁵vg¹⁶⁶srr¹⁶⁷yp¹⁶⁸dav¹⁶⁹yl¹⁷⁰hr¹⁷¹idl¹⁷²gpp¹⁷³is¹⁷⁴ler¹⁷⁵ld¹⁷⁶vg¹⁷⁷tn¹⁷⁸lgn¹⁷⁹ai¹⁸⁰ak¹⁸¹led¹⁸²ak¹⁸³el¹⁸⁴less¹⁸⁵dqi¹⁸⁶
lsa¹⁸⁷IEDKIEEILSKIYHIENEIARIKKLIGEAP
- MeV F R165C/M171C, M141C/T270C-GGG-486-GCN4 (SEQ ID NO: 75)
- mysmq¹las²cv³tl⁴tl⁵vl⁶lv⁷ns⁸qih⁹wgn¹⁰lsk¹¹ig¹²vv¹³gigs¹⁴asy¹⁵kv¹⁶mr¹⁷ssh¹⁸qsl¹⁹vik²⁰lmp²¹nit²²l²³nn²⁴ctr²⁵veia²⁶ey²⁷rr²⁸ll²⁹rtv³⁰
le³¹pir³²dal³³nav³⁴tq³⁵ni³⁶r³⁷pv³⁸qsv³⁹ass⁴⁰rrrh⁴¹GGG⁴²agv⁴³cl⁴⁴aga⁴⁵alg⁴⁶vata⁴⁷aa⁴⁸qita⁴⁹gial⁵⁰hqs⁵¹mlns⁵²qaid⁵³nl⁵⁴ras⁵⁵lett⁵⁶nq⁵⁷aiea⁵⁸
i⁵⁹C⁶⁰qag⁶¹qe⁶²Cila⁶³v⁶⁴qg⁶⁵v⁶⁶qdy⁶⁷inn⁶⁸elips⁶⁹mnq⁷⁰l⁷¹sc⁷²d⁷³lig⁷⁴qkl⁷⁵gl⁷⁶kl⁷⁷lry⁷⁸yte⁷⁹il⁸⁰sl⁸¹fg⁸²ps⁸³lr⁸⁴dp⁸⁵isa⁸⁶e⁸⁷is⁸⁸iq⁸⁹als⁹⁰yal⁹¹gg⁹²din⁹³
kv⁹⁴lek⁹⁵lgys⁹⁶ggd⁹⁷ll⁹⁸giles⁹⁹rgi¹⁰⁰kar¹⁰¹i¹⁰²ch¹⁰³vd¹⁰⁴tes¹⁰⁵y¹⁰⁶fi¹⁰⁷vl¹⁰⁸sia¹⁰⁹ypt¹¹⁰lse¹¹¹ik¹¹²gv¹¹³iv¹¹⁴hr¹¹⁵leg¹¹⁶vsyn¹¹⁷igs¹¹⁸qew¹¹⁹yt¹²⁰tp¹²¹ky¹²²vat¹²³qg¹²⁴
yl¹²⁵is¹²⁶nf¹²⁷des¹²⁸sct¹²⁹fm¹³⁰peg¹³¹tv¹³²cs¹³³nal¹³⁴yp¹³⁵ms¹³⁶pll¹³⁷qec¹³⁸lr¹³⁹gst¹⁴⁰ksc¹⁴¹art¹⁴²lv¹⁴³sgs¹⁴⁴fgn¹⁴⁵r¹⁴⁶fil¹⁴⁷sq¹⁴⁸gn¹⁴⁹li¹⁵⁰anc¹⁵¹asil¹⁵²ck¹⁵³cy¹⁵⁴tt¹⁵⁵gt¹⁵⁶i¹⁵⁷
in¹⁵⁸qdp¹⁵⁹kil¹⁶⁰tyia¹⁶¹adh¹⁶²cp¹⁶³vve¹⁶⁴nv¹⁶⁵gt¹⁶⁶iq¹⁶⁷vg¹⁶⁸srr¹⁶⁹yp¹⁷⁰dav¹⁷¹yl¹⁷²hr¹⁷³idl¹⁷⁴gpp¹⁷⁵is¹⁷⁶ler¹⁷⁷ld¹⁷⁸vg¹⁷⁹tn¹⁸⁰lgn¹⁸¹ai¹⁸²ak¹⁸³led¹⁸⁴ak¹⁸⁵el¹⁸⁶less¹⁸⁷dqi¹⁸⁸
lsa¹⁸⁹IEDKIEEILSKIYHIENEIARIKKLIGEAP
- MeV F R165C/M171C, E212C/Y236C-GGG-486-GCN4 (SEQ ID NO: 76)
- mysmq¹las²cv³tl⁴tl⁵vl⁶lv⁷ns⁸qih⁹wgn¹⁰lsk¹¹ig¹²vv¹³gigs¹⁴asy¹⁵kv¹⁶mr¹⁷ssh¹⁸qsl¹⁹vik²⁰lmp²¹nit²²l²³nn²⁴ctr²⁵veia²⁶ey²⁷rr²⁸ll²⁹rtv³⁰
le³¹pir³²dal³³nav³⁴tq³⁵ni³⁶r³⁷pv³⁸qsv³⁹ass⁴⁰rrrh⁴¹GGG⁴²agv⁴³cl⁴⁴aga⁴⁵alg⁴⁶vata⁴⁷aa⁴⁸qita⁴⁹gial⁵⁰hqs⁵¹mlns⁵²qaid⁵³nl⁵⁴ras⁵⁵lett⁵⁶nq⁵⁷aiea⁵⁸
i⁵⁹C⁶⁰qag⁶¹qe⁶²Cila⁶³v⁶⁴qg⁶⁵v⁶⁶qdy⁶⁷inn⁶⁸elips⁶⁹mnq⁷⁰l⁷¹sc⁷²d⁷³lig⁷⁴qkl⁷⁵gl⁷⁶kl⁷⁷lry⁷⁸yte⁷⁹il⁸⁰sl⁸¹fg⁸²ps⁸³lr⁸⁴dp⁸⁵isa⁸⁶e⁸⁷is⁸⁸iq⁸⁹als⁹⁰yal⁹¹gg⁹²din⁹³
kv⁹⁴lek⁹⁵lgys⁹⁶ggd⁹

- MeV F R165C/M171C, R48C/A284C-GGG-486-GCN4 (SEQ ID NO: 77)
 mysmqlascvtltlvllvnsqihwgnlskigvvgigsasykvmtCsshqslviklmpnitllnnctrveiaeyrrllrtv
 lepirdalnavtqnirpvgvassrrrhGGGagvvlagaaalgvataaqitagialhqsmnlsqaidnlaslettnqaiea
 5 iCqagqgeCilavqgvqdyinnelipsmnqlscdligqklglkllryyteilsfpgpslrdpisaeisigalsyalggdin
 kvleklgysggdllgilesrgikarithvdtesyfivlsiCpytlseikgvivhrlegvsnynigsqewyttvpkyvatqg
 ylisnfdessctfmpegtvcsqnalypmspllqecclrgstkscartlvsgsfgnrfilsqgnlianacasilckcyttgti
 inqdpdkiltyiaadhcpvvevngvtiqvgsrrypdavylhridlgppislerldvgtnlgnaiakledakellessdqi
 10 lsa IEDKIEEILSKIYHIENEIARIKKLIGEAP
- MeV F R99C/V117C, native furin-486-GCN4 (SEQ ID NO: 78)
 mysmqlascvtltlvllvnsqihwgnlskigvvgigsasykvmttrsshqslviklmpnitllnnctrveiaeyrrllrtv
 lepirdalnavtqnirpvgvassrrrhkrfagvClagaaalgvataaqitagialhqsmnlsqaidnlaslettnqaiea
 15 irqagqgemilavqgvqdyinnelipsmnqlscdligqklglkllryyteilsfpgpslrdpisaeisigalsyalggdin
 kvleklgysggdllgilesrgikarithvdtesyfivlsiaayptlseikgvivhrlegvsnynigsqewyttvpkyvatqg
 ylisnfdessctfmpegtvcsqnalypmspllqecclrgstkscartlvsgsfgnrfilsqgnlianacasilckcyttgti
 inqdpdkiltyiaadhcpvvevngvtiqvgsrrypdavylhridlgppislerldvgtnlgnaiakledakellessdqi
 20 lsa IEDKIEEILSKIYHIENEIARIKKLIGEAP
- MeV F R99C/V117C, V175F, S194P, native furin-486-GCN4 (SEQ ID NO: 79)
 mysmqlascvtltlvllvnsqihwgnlskigvvgigsasykvmttrsshqslviklmpnitllnnctrveiaeyrrllrtv
 lepirdalnavtqnirpvgvassrrrhkrfagvClagaaalgvataaqitagialhqsmnlsqaidnlaslettnqaiea
 25 irqagqgemilaFggvqdyinnelipsmnqlPcdligqklglkllryyteilsfpgpslrdpisaeisigalsyalggdin
 kvleklgysggdllgilesrgikarithvdtesyfivlsiaayptlseikgvivhrlegvsnynigsqewyttvpkyvatqg
 ylisnfdessctfmpegtvcsqnalypmspllqecclrgstkscartlvsgsfgnrfilsqgnlianacasilckcyttgti
 inqdpdkiltyiaadhcpvvevngvtiqvgsrrypdavylhridlgppislerldvgtnlgnaiakledakellessdqi
 30 lsa IEDKIEEILSKIYHIENEIARIKKLIGEAP
- MeV F R99C/V117C, R165C/M171C, S194P, native furin-486-GCN4 (SEQ ID NO: 80)
 mysmqlascvtltlvllvnsqihwgnlskigvvgigsasykvmttrsshqslviklmpnitllnnctrveiaeyrrllrtv
 lepirdalnavtqnirpvgvassrrrhkrfagvClagaaalgvataaqitagialhqsmnlsqaidnlaslettnqaiea
 35 iCqagqgeCilaFggvqdyinnelipsmnqlPcdligqklglkllryyteilsfpgpslrdpisaeisigalsyalggdin
 kvleklgysggdllgilesrgikarithvdtesyfivlsiaayptlseikgvivhrlegvsnynigsqewyttvpkyvatqg
 ylisnfdessctfmpegtvcsqnalypmspllqecclrgstkscartlvsgsfgnrfilsqgnlianacasilckcyttgti
 inqdpdkiltyiaadhcpvvevngvtiqvgsrrypdavylhridlgppislerldvgtnlgnaiakledakellessdqi
 lsa IEDKIEEILSKIYHIENEIARIKKLIGEAP

The above sequences include an N-terminal signal peptide, a MeV F ectodomain, a GCN4 trimerization domain, as well as various linker residues between segments.

40

Additionally, chimeric constructs with the MeV F ectodomain with amino acid substitutions for stabilization in a prefusion conformation linked to a MuV HN ectodomain or a MeV H ectodomain were designed as follows:

- MeV F R165C/M171C-GGG-486-GCN4 + MeV_H (SEQ ID NO: 56)
 mysmqlascvtltlvllvnsqihwgnlskigvvgigsasykvmttrsshqslviklmpnitllnnctrveiaeyrrllrtv
 lepirdalnavtqnirpvgvassrrrhGGGagvvlagaaalgvataaqitagialhqsmnlsqaidnlaslettnqaiea
 45 iCqagqgeCilavqgvqdyinnelipsmnqlscdligqklglkllryyteilsfpgpslrdpisaeisigalsyalggdin
 kvleklgysggdllgilesrgikarithvdtesyfivlsiaayptlseikgvivhrlegvsnynigsqewyttvpkyvatqg
 50 ylisnfdessctfmpegtvcsqnalypmspllqecclrgstkscartlvsgsfgnrfilsqgnlianacasilckcyttgti
 inqdpdkiltyiaadhcpvvevngvtiqvgsrrypdavylhridlgppislerldvgtnlgnaiakledakellessdqi
 lsa IEDKIEEILSKIYHIENEIARIKKLIGEAP GSGGGGGG
 flavskgncsgpttirgqfsnmslsldlylgrgynvssivtmtsqgmyggtylvekpnlsskrseqlsmyrvfevgv
 irnpglgapvfhmtnyleqpvsnldsnmvalgelklaalchgedsitipyqsgkgvsgfqlvklgvwkspadmqswwpl
 55 stddpvidrlylsshrgviadnqakwvpttrtdklrmetcfqqackgkiqtlcenpewaplkdnrripsygvlsvdsl
 tvelkikiasgfgplithgsgmdlyksnhnnvywltippmknalgvintlewi prfkvspylftvpikagedchapt
 lpaevdgdvklssnlvilpgqdlqyvatydt srvehavvyvyspgsfsyfprlpikgvpielqvecftwdqklwc
 rhfcvladesesgghithsgmvgmgsctvtredgtnrr

MeV F R165C/M171C-GGG-486-GCN4 + MuV_HN (SEQ ID NO: 57)
 mysmqlascvtltlvtllvnsqihwgnlskigvvgigsasykvmttrsshqslviklmpnitllnnctrveiaeyrrllrtv
 lepiraldnavtqnirpvgvassrrrhGGGagvvlagaalgvataaqitagialhqsmnlsqaidnlaslettnqaeia
 5 iCgagqgeCilavqgvqdyinnelipsmnqlscdligqklglkllryyteilsfpgslrdpisaieisigalsyalggdin
 kvleklysgsgdillgilesrgikarithvdtesyfivlsiaayptlseikgvivhrlegvsvynigsqewyttvpkyvatqg
 ylisnfdessctfmpegtvcsqnalypmspllqecclrgstkscartlvsgsfgnrfilsqgnliancasilckcyttgti
 inqdpdkiltyiaadhcpvvevngvtiqvgsrrypdavylhrldlgppislerldvgtnlgnaiakledakellessdqi
 10 lsa IEDKIEEILSKIYHIENEIARIKKLIGEAPG sgggggg
 niplvndlrfininkfiiedyathdfsighplnmpsfiptatpncgtripsfslgkthwcythnvinanckdhtssnq
 yvsmgilvqtasgypmfktlkiqylsdglnrkscsiatvpdgcamycyvstqletddyagssptqkltlflfyndtvtter
 tispsglegnwatlvpgvgsgiyfenklifpayggvlpnstlgvksareffrpvpnpynpcsgpqqdldqralrsyfpsyf
 snrriqsafllvcawnqilvtncelvpssnqtmmaegrvllinnrlllyyqrstswwpellyeistftfnsgpssvnms
 wipiysftrpgsgncsgenvcptacvsgvylpwppltpyshqsginrnfytgallnssttrvnptlyvsalnlnklvlap
 15 ygtqglfasytthttcfqdtgdasvycvyimelasnivgefqlpvltrltit

MeV F R165C/M171C-GGG-486-GCN4/Fd + MeV_H (SEQ ID NO: 81)
 MYSMQLASCVTTLTLVLLVNSQIHWGNLSKIGVVGIGSASYKVMTRSSHQSLVIKLMFNITLLNNCTRVEIAEYRRLRTV
 LEPIRDALNAVTONIRPVQSVASSRRHGGGAGVVLAGAALGVATAAQITAGIALHQSMNLSQAIDNLRASLETNNQAIEA
 20 ICQAGQECILAVQGVQDYINNELIPSMNQLSCDLIGQKLGLKLLRYYTEILSLFGPSLRDPISAEISIQALSALGGDIN
 KVLEKLYSGGDLGILESRIKARITHVDTESYFIVLSIAAYPTLSEIKGVIVHRLEGVSVYNIGSQEWYTTVPKYVATQG
 YLISNFDSSCTFMPEGTVCSQNALYPMSPLLQECLRGSTKSCARTLVSGSFGNRFILSQGNLIANCASILCKCYTTGTI
 INQDPDKILTYIAADHCPVVEVNGVTIQVGSRRYPDAVYLHRIDLGPPISELERLDVGTNLGNIAIAKLEDKELLESSDQI
 LSA IEDKIEEILSKIYHIENEIARIKKLIGEAP GS GYIPEAPRDGQAYVRKDGWVLLSTFL GSGGGGGGg
 25 FLAVSKGNCSGPTTIRGQFSNMSLSLLDLYLGRGYNVSSIVTMTSQGMYGGTYLVEKPNLSSKRSELSQLSMYRVFEVGV
 IRNPGLGAPVVFHMTNYLEQPVSNDLSCMVVALGELKLAALCHGEDSITIPYQGSKGVSFQLVKLGWVKSADMQSWVPL
 STDDPVIDRLYLSSHGVIADNQAQWAVPTTRTDDKLRMETCFQACKGKIQTLCENPEWAPLKDNRIPSYGLVSDLSL
 TVELKIKIASGFGLITHGSGMDLYKSNHNNVYWLTIPTPMKNLALGVINTLEWIPRFKVSPLYFTVPIKEAGEDCHAPTY
 LPAEVDGDVKLSSNLVILPGQDLQYVLATYDTSRVEHAVVYVYSPGRSFSYFYPRLPIKGVPIELQVECTWDQKLWC
 30 RHFCVLADSESGGHIHSGMVGMGVSCVTREDGTNR

The above sequences include an N-terminal signal peptide, a MeV F ectodomain, a GCN4 trimerization domain, optionally a T4 Fibrin trimerization domain, a MeV H ectodomain, as well as various linker residues between segments.

35

Example 3

Multimeric MuV HN, Multimeric MeV H, and MuV pre-F-MeV H Chimera

This example describes an embodiment of a recombinant MuV pre-F ectodomain trimer linked to MeV H ectodomain to provide a chimeric immunogen that elicits a cross-neutralizing immune response to MeV and MuV. Additionally, multimeric MuV HN and multimeric MeV H are described.

40

To increase the immunogenic footprint of the MuV F ectodomain trimer, a MeV H ectodomain was genetically fused to the C-terminus of the trimerization domain of each protomer of the trimer. The assessed construct included a MuV F ectodomain containing V206C-A223C, a mutation to eliminate the F1/F2 furin cleavage site, a trimerization domain fused to position 476 of the ectodomain, and a MeV H ectodomain
 45 linked to the C-terminus of the trimerization domain. In this embodiment, the trimerization domain included both a GCN4 trimerization domain and a T4 fibrin trimerization domain in series; however, either of these domains can also be used on their own. The format is illustrated in Figure 6A. A corresponding sequence is shown below.

MuV F 206C/223C-476 + GCN4/Fd + MeV-H (SEQ ID NO: 28):
 Mkafsvtclsfavfsssicvninilqqigiyikqqvrqlsyysqsssyivvklpnigptddscfksvstqynktnlsl
 lpiaeninniaspspgsrrrhGGGagiaigiaalgvataaavslvgaqtnaraiaamknsiqatnrafvfvevkegtqg
 laiavqaiqdhintimntqlnmscqidnqlatslglyltelttCfqpqltnpalspisiqClrslslgsmtpavvqatl
 5 stsisaaeilsaglmegqiisvllldemqmivkiniptivtqsnalvidfysissfinnqesiqlpdrileigneqwsyp
 aknckltrhhifcgyneaeerlslesklclagnisacvfspiagsymrrfvaldgtivancrsltlclckspisyipyqpdhh
 avttidltacqtllsldgldfsivslsnityaenltislsqintqpidistelskvnaslqnavkyikesnhqlqs
 IEDKIEEILSKIYHIENEIARIKKLIGEAP GS GYIPEAPRDGQAYVRKDGWVLLSTFL GSGGGGGGg
 Flavskgncsgpttirgqfsnmslslldlylgrgynvssivtmtsqgmyggtylvekpnlsksrseqlsmyrvfevgv
 10 irnpglgapvfhmtnyleqpvsndlsncmvalgelklaalchgedsitipyqsgkgvsvfqlvklgvwkspadmqswvpl
 stddpvidrlylsshrvgviadnqakwvpttrtdklrmetcfqqackgkiqtlcenpewaplkdnrripsygvlsvdsl
 tvelkikiasgfgplithgsgmdlyksnhnnvyltippmknalalgvintlewi prfkvspyflftvpik eagedchapy
 lpaevdgdvklssnlvilpgqdlqyvlatydt srvehavvyyvyspgrsf syfypfrlpikgvpielqvecftwdqklwc
 rhfcvldadesegghithsgmvgmvsctvtredgtnrr

15

Negative stain EM of purified MuV F 206C-223C-476 + GCN4/Fd + MeV-H (SEQ ID NO: 28)

shows that the F ectodomain maintains the prefusion conformation, with the three H ectodomains (one
 linked to each F protomer) arrayed C-terminal to the trimerization domain (FIG. 6A). The negative stain
 EM shows that this construct assembles with a conformation similar to MuV F-MuV HN described in

20 Example 1.

Additional immunogens were constructed containing multimers of the MeV H ectodomain head
 region, or the MuV HN ectodomain head region.

Trimeric MuV HN ectodomain head region and trimeric MeV H ectodomain head region were
 constructed by linking the N-terminus of the head region to a T4 fibrin trimerization domain. The

25 sequences are provided as follows:

Fd-MuV HN (trimeric MuV HN, SEQ ID NO: 58)
 mysmqlascvtltlavlvsq GS GYIPEAPRDGQAYVRKDGWVLLSTFL GSGGGGGGg
 NIPLVNDLRIFINGINKFIIEDYATHDFSIGHPLNMPFSIPTATSPNGCTRIPSFSLGKTHWCYTHNVINANCKDHTSSNQ
 YVSMGILVQTASGYPMFKTLKIQYLSLSDGLNRKSCSIATVPDGCAMCYVSTQLETDDYAGSSPTQKLTLLFYNDVTTER
 30 TISPSGLEGNWATLVPGVSGSIYFENKLIFPAYGGVLPNSTLGVKSAREFFRPVNPYNPCSGPQQDLQDQALRSYFPSYF
 SNRRIQSAFLVCAWNQILVTNCELVVPSSNQTMGAEGRVLLINNRLLYQQRSTSWWPYELLYEISFTFTNSGPSSVNMS
 WIPIYSFTRPGSGNCSGENVCPTACVSGVYLDPWPLTPYSHQSGINRNFYFTGALLNSSTTRVNPTLYVSALNNLKVLP
 YGTQGLFASVTTTTCTCFQDTGDASVYCVYIMELASNIVGEFQILPVLTRLTIT

35 Fd-MeV H (trimeric MeV H, SEQ ID NO: 59)

mysmqlascvtltlavlvsq GS GYIPEAPRDGQAYVRKDGWVLLSTFL GSGGGGGGg
 FLAVSKGNCSGPTTIRGQFSNMSLSLLDLYLGRGYNVSSIVTMTSQGMYGGTYLVEKPNLSKRSLSQLSMYRVFEVGV
 IRNPGLGAPVFHMNTNLYEQPVSNDSNCMVALGELKLAALCHGEDSITIPYQSGKGVSFQLVKLGVWKS PADMQSWVPL
 40 STDDPVIDRLYLSSHRVGVIADNQAKWVPTTRTDKLRMETCFQQACKGKIQTLCENPEWAPLKDNRIPS YGVLSVDLSL
 TVELKIKIASGFGPLITHGSGMDLYKSNHNNVYLTIPPMMKNALALGVINTLEWIPRFKVSPLYFTVPIKEAGEDCHAPTY
 LPAEVDGDVKLSSNLVILPGQDLQYVLATYDTSRVEHAVVYVYSPGRSFSYFYPFRLPKGVPIELQVECFTWDQKLWC
 RHFCVLADSESGGHI THSGMVGMSCTVTREDGTNRR

Dimeric MeV H was constructed by expressing the MeV H ectodomain head region in mammalian
 45 cells and purifying the resulting protein complex; the MeV H head dimerizes in physiological solution. The
 sequence of the MeV H head region is provided as follows:

Dimeric MeV H head region (SEQ ID NO: 60)

50 mysmqlascvtltlavlvsq flavskgncsgpttirgqfsnmslslldlylgrgynvssivtmtsqgmyggtylvek
 pnlsksrseqlsmyrvfevgvirnpglgapvfhmtnyleqpvsndlsncmvalgelklaalchgedsitipyqsgkg

vsfqlvklgvwkspadmqswvplstddpvidrlylsshrgviadnqakwvpttrtdklrmetcfqqackgkiqtlcen
pewaplkdnrripsygvlsvdlsltvelkikiasgfgplithgsgmdlyksnhnnvywltippmknalgvintlewi
prfkvspylftvpikaeagedchaptylpaevdgdvklssnlvilpgqdlqyvladydtsrvehavvyvyspsr
sfyfprlpikgvpielqvecftwdqklwcrhfcvldasesgghithsgmvgmgsctvtredgtnrr

5

Dimeric MeV H including the stalk and head regions can be constructed by expressing the MeV H ectodomain stalk and head regions in mammalian cells and purifying the resulting protein complex; the MeV H stalk and head dimerizes in physiological solution. Exemplary sequences of MeV H stalk and head regions are provided as follows:

10

MeV H stalk and head (SEQ ID NO: 86)
mysmqglascvtltlvllvnsq RLHraaiytaeihkslstnldvtnsiehqvkdvltpfkiigdevglrtppqrftdlv
kfisdki flnpdreydfrdltwcinpperikldydqycadvaeeelmnalvnstlletrttnqFLAVskgncsgpttir
gqfsnmslsldlylgrgynvssivtmtsggmyggtylveknlskselsqlsmyrvfevgvirnpglgapvfhmtny
leqpvsndlsncmvalgelklaalchgedsitipyqsgkgvsvfqlvklgvwksptdmqswvplstddpvidrlylssh
rgviadnqakwvpttrtdklrmetcfqqackgkiqalcentpewaplkdnrripsygvlsvdlsltvelkikiasgfgpli
thgsgmdlyksnhnnvywltippmknalgvintlewi prfkvspylfnvpikaeagedchaptylpaevdgdvklssnlv
ilpgqdlqyvladydtsrvehavvyvyspsrfsyfprlpikgvpielqvecftwdqklwcrhfcvldasesgghith
hsgmegmgsctvtredgtnrr

20

MeV H stalk and head 2 (SEQ ID NO: 87)
mysmqglascvtltlvllvnsq raaiytaeihkslstnldvtnsiehqvkdvltpfkiigdevglrtppqrftdlvkfi
sdki flnpdreydfrdltwcinpperikldydqycadvaeeelmnalvnstlletrttnqFLAVskgncsgpttirgqf
snmslsldlylgrgynvssivtmtsggmyggtylveknlskselsqlsmyrvfevgvirnpglgapvfhmtnyleq
pvsndlsncmvalgelklaalchgedsitipyqsgkgvsvfqlvklgvwksptdmqswvplstddpvidrlylssh
rgviadnqakwvpttrtdklrmetcfqqackgkiqalcentpewaplkdnrripsygvlsvdlsltvelkikiasgfgpli
thgsgmdlyksnhnnvywltippmknalgvintlewi prfkvspylfnvpikaeagedchaptylpaevdgdvklssnlv
ilpgqdlqyvladydtsrvehavvyvyspsrfsyfprlpikgvpielqvecftwdqklwcrhfcvldasesgghith
hsgmegmgsctvtredgtnrr

30

MeV H stalk and head 3 (SEQ ID NO: 88)
mysmqglascvtltlvllvnsq LHraaiytaeihkslstnldvtnsiehqvkdvltpfkiigdevglrtppqrftdlvk
fisdki flnpdreydfrdltwcinpperikldydqycadvaeeelmnalvnstlletrttnqFLAVskgncsgpttirg
qfsnmslsldlylgrgynvssivtmtsggmyggtylveknlskselsqlsmyrvfevgvirnpglgapvfhmtnyl
eqpvsndlsncmvalgelklaalchgedsitipyqsgkgvsvfqlvklgvwksptdmqswvplstddpvidrlylssh
rgviadnqakwvpttrtdklrmetcfqqackgkiqalcentpewaplkdnrripsygvlsvdlsltvelkikiasgfgpli
thgsgmdlyksnhnnvywltippmknalgvintlewi prfkvspylfnvpikaeagedchaptylpaevdgdvklssnlv
ilpgqdlqyvladydtsrvehavvyvyspsrfsyfprlpikgvpielqvecftwdqklwcrhfcvldasesgghith
hsgmegmgsctvtredgtnrr

40

MeV H stalk and head 4 (SEQ ID NO: 89)
mysmqglascvtltlvllvnsq taeihkslstnldvtnsiehqvkdvltpfkiigdevglrtppqrftdlvkfisdki
flnpdreydfrdltwcinpperikldydqycadvaeeelmnalvnstlletrttnqFLAVskgncsgpttirgqfsnmsl
sldlylgrgynvssivtmtsggmyggtylveknlskselsqlsmyrvfevgvirnpglgapvfhmtnyleqpvsnd
lsncmvalgelklaalchgedsitipyqsgkgvsvfqlvklgvwksptdmqswvplstddpvidrlylsshrgviadnq
akwvpttrtdklrmetcfqqackgkiqalcentpewaplkdnrripsygvlsvdlsltvelkikiasgfgplithgsgmdl
yksnhnnvywltippmknalgvintlewi prfkvspylfnvpikaeagedchaptylpaevdgdvklssnlvilpgqdlq
yvladydtsrvehavvyvyspsrfsyfprlpikgvpielqvecftwdqklwcrhfcvldasesgghithsgmegmgs
ctvtredgtnrr

50

MuV HN including the stalk and head regions can be constructed by expressing the MuV HN ectodomain stalk and head regions in mammalian cells and purifying the resulting protein. Exemplary sequences of MuV HN stalk and head regions are provided as follows:

55

- MuV HN stalk and head (SEQ ID NO: 90)
mysmqlascvttltlvllvnsq ELVRmindqglsnqlssitdkiresatmiasavgvnmqvihgvtvslplqiegnqnq
 5 llatlatiactsqkqvsnstNIPlvndlrifinginkfiiedyathdfsigbplnmfpiptatpangctripsfslgkth
 wcythnvinanckdhtssnqyvsimgilvqtasgypmfktlkiqylsdglnrkscsiatvpdgcamycyvstqletddyag
 sspptqkltllfyndtvttertispqlegnwatlvpgvgsgiyfenklifpayggvlpnstlgvksareffrpvnpync
 sgpgqlddqralsyfsyfsnrriqsafllvcawnqilvtncelvpssnqtmgaegrllinnrlllyqrstswpye
 llyeisftftnsgpssvnmwipiysftrpgsgncsgenvcptacvsgvldpwltpyshqsginrnfytgallnsst
 trvnptlyvsalnnlkvlapygtqglfasyttttcfqdtgdasvycvymelasnivgefqlpvltrltit
- MuV HN stalk and head 2 (SEQ ID NO: 91)
mysmqlascvttltlvllvnsq dqglsnqlssitdkiresatmiasavgvnmqvihgvtvslplqiegnqnqllatlat
 10 ictsqkqvsnstNIPlvndlrifinginkfiiedyathdfsigbplnmfpiptatpangctripsfslgkthwcythn
 inanckdhtssnqyvsimgilvqtasgypmfktlkiqylsdglnrkscsiatvpdgcamycyvstqletddyagssptqk
 ltllyfyndtvttertispqlegnwatlvpgvgsgiyfenklifpayggvlpnstlgvksareffrpvnpyncsgpgqld
 15 dqralsyfsyfsnrriqsafllvcawnqilvtncelvpssnqtmgaegrllinnrlllyqrstswpyellieisf
 tftnsgpssvnmwipiysftrpgsgncsgenvcptacvsgvldpwltpyshqsginrnfytgallnssttrvnptl
 yvsalnnlkvlapygtqglfasyttttcfqdtgdasvycvymelasnivgefqlpvltrltit
- MuV HN stalk and head 3 (SEQ ID NO: 92)
mysmqlascvttltlvllvnsq glsnqlssitdkiresatmiasavgvnmqvihgvtvslplqiegnqnqllatlat
 20 tlatlatiactsqkqvsnstNIPlvndlrifinginkfiiedyathdfsigbplnmfpiptatpangctripsfslgkth
 wcythnvinanckdhtssnqyvsimgilvqtasgypmfktlkiqylsdglnrkscsiatvpdgcamycyvstqletddyag
 sspptqkltllfyndtvttertispqlegnwatlvpgvgsgiyfenklifpayggvlpnstlgvksareffrpvnpync
 sgpgqlddqralsyfsyfsnrriqsafllvcawnqilvtncelvpssnqtmgaegrllinnrlllyqrstswpyellieisf
 25 tftnsgpssvnmwipiysftrpgsgncsgenvcptacvsgvldpwltpyshqsginrnfytgallnssttrvnptl
 yvsalnnlkvlapygtqglfasyttttcfqdtgdasvycvymelasnivgefqlpvltrltit
- MuV HN stalk and head 4 (SEQ ID NO: 93)
mysmqlascvttltlvllvnsq LVRmindqglsnqlssitdkiresatmiasavgvnmqvihgvtvslplqiegnqnq
 30 llatlatiactsqkqvsnstNIPlvndlrifinginkfiiedyathdfsigbplnmfpiptatpangctripsfslgkth
 wcythnvinanckdhtssnqyvsimgilvqtasgypmfktlkiqylsdglnrkscsiatvpdgcamycyvstqletddyag
 sspptqkltllfyndtvttertispqlegnwatlvpgvgsgiyfenklifpayggvlpnstlgvksareffrpvnpync
 sgpgqlddqralsyfsyfsnrriqsafllvcawnqilvtncelvpssnqtmgaegrllinnrlllyqrstswpyel
 35 llyeisftftnsgpssvnmwipiysftrpgsgncsgenvcptacvsgvldpwltpyshqsginrnfytgallnsst
 trvnptlyvsalnnlkvlapygtqglfasyttttcfqdtgdasvycvymelasnivgefqlpvltrltit

Additionally, chimeric versions of the trimeric MuV HN ectodomain head region and trimeric MeV
 H ectodomain head region were constructed by linking these molecules to the N- and C- termini of a T4
 fibrin trimerization domain and/or GCN4 trimerization domain. The sequences are provided as follows:

- 40 MuV HN-Fd-MeV H (SEQ ID NO: 82)
mysmqlascvttltlvllvnsq NIPlvndlrifinginkfiiedyathdfsigbplnmfpiptatpangctripsfslg
 kthwcythnvinanckdhtssnqyvsimgilvqtasgypmfktlkiqylsdglnrkscsiatvpdgcamycyvstqletdd
 yagssptqkltllfyndtvttertispqlegnwatlvpgvgsgiyfenklifpayggvlpnstlgvksareffrpvnpy
 45 npcsgpgqlddqralsyfsyfsnrriqsafllvcawnqilvtncelvpssnqtmgaegrllinnrlllyqrstsw
 pyellieisftftnsgpssvnmwipiysftrpgsgncsgenvcptacvsgvldpwltpyshqsginrnfytgalln
 ssttrvnptlyvsalnnlkvlapygtqglfasyttttcfqdtgdasvycvymelasnivgefqlpvltrltitgg
 GSGYIPEAPRDGQAYVRKDGEWVLLSTFLSGGGGGGg
 flavskgncsgpttirgqfsmnslldlylgrgynvssivtmtsqgmyggtylvekpnlsksrslsqsmyrvfevgv
 50 irnpglgapvfhmtnyleqpvsnclsnmvalgelklaalchgedsitipyqsgkgvsvqlvklgwkspdtmqswvpl
 stddpvidrlylsshrgviadnqakwvpttrtdklrmetcfqqackgkialcenpewaplkdnripsygvlsvdsl
 tvelkikiasgfgplithgsgmdlyksnhnnvywltippmknalgvintlewiiprfkvspylfnvpkeagedchapy
 lpaevdgdvklssnlvilpgqdlqyvladytsrvehavvyvyspsrsfsyfyfprlpikgvpielqvecftwdqklwc
 rhfcvladesegghithsgmegmgsctvtredgtnrr
- 55 MuV HN-GCN4/Fd-MeV H (SEQ ID NO: 83)
mysmqlascvttltlvllvnsq NIPlvndlrifinginkfiiedyathdfsigbplnmfpiptatpangctripsfslg
 kthwcythnvinanckdhtssnqyvsimgilvqtasgypmfktlkiqylsdglnrkscsiatvpdgcamycyvstqletdd
 yagssptqkltllfyndtvttertispqlegnwatlvpgvgsgiyfenklifpayggvlpnstlgvksareffrpvnpy

npcsgpqqdldqralrsyfspyfsnrriqsaflvcawnqilvtncelvpssnqtmgaegrvllinnrlllyqrstsw
 pyellyeisftftnsgpssvnmwipiysftrpgsgncsgenvcptacvsgvyldpwppltpyshqsginrnfyftgalln
 ssttrvnptlyvsalnnklvlapygtqglfasyttttcfqdtgdasvycvyimelasnivgefqlpvltrltit gg
 KLMKQIEDKIEEILSKIYHIENEIARIKKLIGEAP GSGYIPEAPRDGQAYVRKDGEWVLLSTFL GSGGGGGg
 5 flavskgncsgpttirgqfsnmslsldlylgrgynvssivtmtsqgmyggtylvekpnlsksrseqlsmyrvfevgv
 irnpglgapvfhmtnyleqpvsndlsncmvalgelklaalchgedsitipyqgsgkgvsvfqlvklgvksptdmqswvpl
 stddpvidrlylsshrvgiadnqakwavpttrtdcklrmctcfqgackgkigalcenpewaplkdnripsygvlsvdsl
 tvelkikiasgfgplithgsgmdlyksnhnnvywltippmknalgvintlewiwprfkvsvpylfnvpikagedchapt
 10 lpaevdgdvklssnlvilpgqdlqyvatydtservehavvyvyspsrsfsyfprlpikgvpielqvecftwdqklwc
 rhfcvladesesgghithsgmegmgvscvtvredgtmr

MeV H-Fd-MuV HN (SEQ ID NO: 84)
 mysmqlascvtltlrvllvnsq FLAVskgncsgpttirgqfsnmslsldlylgrgynvssivtmtsqgmyggtylvek
 pnlsksrseqlsmyrvfevgvirnpglgapvfhmtnyleqpvsndlsncmvalgelklaalchgedsitipyqgsgkg
 15 vsvfqlvklgvksptdmqswvplstddpvidrlylsshrvgiadnqakwavpttrtdcklrmctcfqgackgkigalcen
 pewaplkdnripsygvlsvdslstvelkikiasgfgplithgsgmdlyksnhnnvywltippmknalgvintlewiwprf
 kvsvpylfnvpikagedchaptlpaevdgdvklssnlvilpgqdlqyvatydtservehavvyvyspsrsfsyfpr
 lpikgvpielqvecftwdqklwcrhfcvladesesgghithsgmegmgvscvtvredgtmr
 GSGYIPEAPRDGQAYVRKDGEWVLLSTFL GSGGGGGg
 20 NIPlvndlrifinginkfiiedyathdfsigplnmpsfiptatpngctripsfslgkthwcythnvinanckdhtssnq
 yvsmgilvqtasgypmfktlkiqylsdglnrkscsiatvpdgcamycyvtqletddyagssptqkltllfyndtvtter
 tispsglegnwatlvpgvgsgiyfenklifpayggvlpnstlgvksareffrpvpnpynpcsgpqqdldqralrsyfspyf
 snrriqsaflvcawnqilvtncelvpssnqtmgaegrvllinnrlllyqrstswpyellyeisftftnsgpssvnmw
 25 wipiysftrpgsgncsgenvcptacvsgvyldpwppltpyshqsginrnfyftgallnssttrvnptlyvsalnnklvlap
 ygtqglfasyttttcfqdtgdasvycvyimelasnivgefqlpvltrltit

MeV H-GCN4/Fd-MuV HN (SEQ ID NO: 85)
 mysmqlascvtltlrvllvnsq FLAVskgncsgpttirgqfsnmslsldlylgrgynvssivtmtsqgmyggtylvek
 pnlsksrseqlsmyrvfevgvirnpglgapvfhmtnyleqpvsndlsncmvalgelklaalchgedsitipyqgsgkg
 30 vsvfqlvklgvksptdmqswvplstddpvidrlylsshrvgiadnqakwavpttrtdcklrmctcfqgackgkigalcen
 pewaplkdnripsygvlsvdslstvelkikiasgfgplithgsgmdlyksnhnnvywltippmknalgvintlewiwprf
 kvsvpylfnvpikagedchaptlpaevdgdvklssnlvilpgqdlqyvatydtservehavvyvyspsrsfsyfpr
 lpikgvpielqvecftwdqklwcrhfcvladesesgghithsgmegmgvscvtvredgtmr
 MKQIEDKIEEILSKIYHIENEIARIKKLIGEAP GSGYIPEAPRDGQAYVRKDGEWVLLSTFL GSGGGGGg
 35 NIPlvndlrifinginkfiiedyathdfsigplnmpsfiptatpngctripsfslgkthwcythnvinanckdhtssnq
 yvsmgilvqtasgypmfktlkiqylsdglnrkscsiatvpdgcamycyvtqletddyagssptqkltllfyndtvtter
 tispsglegnwatlvpgvgsgiyfenklifpayggvlpnstlgvksareffrpvpnpynpcsgpqqdldqralrsyfspyf
 snrriqsaflvcawnqilvtncelvpssnqtmgaegrvllinnrlllyqrstswpyellyeisftftnsgpssvnmw
 40 wipiysftrpgsgncsgenvcptacvsgvyldpwppltpyshqsginrnfyftgallnssttrvnptlyvsalnnklvlap
 ygtqglfasyttttcfqdtgdasvycvyimelasnivgefqlpvltrltit

The MeV H ectodomain head dimer (SEQ ID NO: 60), MeV H ectodomain head trimer (SEQ ID NO: 59), and MuV HN ectodomain head trimer (SEQ ID NO: 58) were designed, expressed, purified and characterized by negative stain EM (see FIG. 6A).

45 Mice were immunized with the purified constructs and sera assessed for MeV and MuV neutralization by PRNT. The immunogens assessed were the MeV H dimer or trimer (SEQ ID NO: 59 or 60), prefusion MuV F ectodomain trimer (MuV F V206C-A223C-GGG-476-GCN4, SEQ ID NO: 11), MuV pre-F-MeV H chimera (MuV F 206C-223C-476 + GCN4/Fd + MeV_H_3INB-tHS (SEQ ID NO: 28), and MuV HN trimer (SEQ ID NO: 58).

50 For MeV neutralization (FIG. 6B), the MeV H dimer elicited PRNT ID₆₀ averaging 66,000, the MeV H trimer 30,000, and the trimerized H on the C-terminus of prefusion MuV F 35,000. These results were quite surprising given the much lower neutralization titers observed from immunization with prefusion MeV F trimer (PRNT of 873; see FIG. 5C). Human PRNT titers following 2 x MMR vaccination average

approximately 650. The very high MeV PRNT titer for the MeV H designs was not expected since the prefusion F ectodomain trimer was initially believed to provide a better immune response (similar to other paramyxoviruses, such as RSV). Unexpectedly, the multimeric MeV H immunogens showed very good immunogenicity, being up to 75-fold more potent than prefusion-stabilized MeV F ectodomain trimer and
5 approximately 100-fold higher than human responses following MMR vaccination.

For MuV neutralization (FIG. 6C), each of the assessed immunogens elicited an immune response above the protective threshold, and the soluble trimerized MuV HN elicited a very potent immune response, which is quite surprising given the minimal response elicited by soluble MuV HN monomer (FIG. 4C). Surprisingly, there was a 13.4-fold increase in neutralization potency between the MuV HN ectodomain
10 monomer and the trimeric MuV HN ectodomain.

Protein production, analysis and immunization were performed as noted above.

It will be apparent that the precise details of the methods or compositions described may be varied or modified without departing from the spirit of the described embodiments. We claim all such modifications and variations that fall within the scope and spirit of the claims below.

It is claimed:

1. An immunogen, comprising:
a recombinant Mumps virus (MuV) F ectodomain trimer stabilized in a prefusion conformation by one or more amino acid substitutions in protomers of the trimer, the amino acid substitutions comprising cysteine substitutions that form a non-natural disulfide bond to stabilize the MuV F ectodomain trimer in the prefusion conformation.
2. The immunogen of claim 1, wherein the cysteine substitutions are located at one or more of MuV F positions 86 and 215, positions 155 and 161, positions 165 and 231, positions 206 and 223, positions 209 and 214, and positions 221 and 255.
3. The immunogen of claim 1 or claim 2, wherein the recombinant MuV F ectodomain trimer is stabilized in the prefusion conformation by a non-natural disulfide bond between the cysteine substitutions in protomers of the trimer at MuV F positions 206 and 223.
4. The immunogen of any one of the prior claims, wherein the cysteine substitutions at:
MuV F positions 86 and 215 are N86C and A215C substitutions;
MuV F positions 155 and 161 are K155C and L161C substitutions;
MuV F positions 165 and 231 are V165C and M231C substitutions;
MuV F positions 206 and 223 are V206C and A223C substitutions;
MuV F positions 209 and 214 are P209C and P214C substitutions; and
MuV F positions 221 and 255 are I221C and M255C substitutions.
5. The immunogen of any one of the prior claims, wherein the protomers of the recombinant MuV F ectodomain trimer comprise a F₂ protein comprising or consisting of MuV F positions 20-100 and a F₁ ectodomain comprising or consisting of MuV F positions 104-469, 104-476, or 104-483.
6. The immunogen of any one of the prior claims, wherein the protomers of the trimer further comprise a mutation to remove a F1/F2 furin cleavage site of the MuV F ectodomain and optionally an a first residue of a fusion peptide of a F1 ectodomain.
7. The immunogen of claim 6, wherein the mutation to remove the F1/F2 furin cleavage site and the first residue of the fusion peptide comprise a deletion of MuV F positions 101-103 with positions 100 and 104 fused by a peptide linker.
8. The immunogen of claim 7, wherein the peptide linker is a Gly-Gly-Gly linker.
9. The immunogen of any one of the prior claims, wherein the positioning of the amino acid substitutions is according to a reference MuV F proteins sequence set forth as SEQ ID NO: 1.

10. The immunogen of any one of the prior claims, wherein the protomers of the MuV F ectodomain trimer comprise an amino acid sequence at least 90% identical to
residues 20-483 of any one of SEQ ID NOs: 3-8,
residues 20-476 of any one of SEQ ID NOs: 11-16, 26, or 51, or
residues 20-469 of any one of SEQ ID NOs: 19-24; and
wherein the protomers comprise the one or more amino acid substitutions that stabilize the MuV F ectodomain trimer in the prefusion conformation.

11. The immunogen of claim 10, wherein the protomers of the MuV F ectodomain trimer comprise or consist of the amino acid sequence set forth as
residues 20-483 of any one of SEQ ID NOs: 3-8,
residues 20-476 of any one of SEQ ID NOs: 11-16, 26, or 51, or
residues 20-469 of any one of SEQ ID NOs: 19-24.

12. The immunogen of any one of the prior claims, wherein the protomers of the recombinant MuV F ectodomain trimer are fused C-terminally to a trimerization domain.

13. The immunogen of claim 12, wherein the trimerization domain comprises a GCN4 trimerization domain, a T4 fibrin trimerization domain, or both.

14. The immunogen of claim 13, wherein
the GCN4 trimerization domain comprises an amino acid sequence set forth as
IEDKIEEILSKIYHIENEIARIKKLIGGEAP (SEQ ID NO: 33).
the T4 fibrin trimerization domain comprises an amino acid sequence set forth as
GYIPEAPRDGQAYVRKDGWVLLSTFL (SEQ ID NO: 34).
the trimerization domain comprising both the GCN4 and T4 fibrin trimerization domains
comprises an amino acid sequence set forth as
IEDKIEEILSKIYHIENEIARIKKLIGGEAPGSGYIPEAPRDGQAYVRKDGWVLLSTFL (SEQ ID NO: 35).

15. The immunogen of any one of claims 12-14, wherein the protomers of the MuV F ectodomain trimer fused to the trimerization domain comprise an amino acid sequence at least 90% identical to
residues 20-513 of any one of SEQ ID NOs: 3-8,
residues 20-506 of any one of SEQ ID NOs: 11-16, 26, or 51, or
residues 20-499 of any one of SEQ ID NOs: 19-24; and
wherein the protomers comprise the one or more amino acid substitutions that stabilize the MuV F ectodomain trimer in the prefusion conformation.

16. The immunogen of claim 15, wherein the protomers of the MuV F ectodomain trimer fused to the trimerization domain comprise or consist of the amino acid sequence set forth as
residues 20-513 of any one of SEQ ID NOs: 3-8,
residues 20-506 of any one of SEQ ID NOs: 11-16, 26, or 51, or
residues 20-499 of any one of SEQ ID NOs: 19-24.
17. The immunogen of any one of the prior claims, wherein the protomers of the recombinant MuV F ectodomain trimer are linked to a heterologous protein.
18. The immunogen of claim 17, wherein the heterologous protein is an ectodomain head or ectodomain stalk and head of a MeV H protein or a MuV HN protein.
19. The immunogen of claim 18, wherein the ectodomain head or ectodomain stalk and head of the MeV H protein or the MuV HN protein is fused C-terminally to a trimerization domain that is fused C-terminally to the protomer of the recombinant MuV F ectodomain trimer.
20. The immunogen of claim 19, wherein the protomers of the MuV F ectodomain trimer linked to the trimerization domain and the ectodomain of the MeV H protein or the ectodomain of a MuV HN protein comprise an amino acid sequence set forth as residues 20-966 of SEQ ID NO: 27, residues 21-981 of SEQ ID NO: 28, or residues 20-1006 of SEQ ID NO: 29.
21. An immunogen, comprising:
a recombinant Measles virus (MeV) F ectodomain trimer stabilized in a prefusion conformation by one or more amino acid substitutions in protomers of the trimer, the amino acid substitutions comprising one or more of the following:
cysteine substitutions at one or more of MeV F positions 48 and 284, positions 90 and 225, positions 141 and 270, positions 165 and 171, positions 173 and 245, positions 175 and 241, positions 212 and 236, positions 216 and 233, positions 219 and 224, positions 99 and 117, positions 100 and 117, positions 101 and 117, positions 102 and 117, and positions 103 and 117 that form a non-natural disulfide bond;
a phenylalanine substitution at MeV F position 175; and
a proline substitution at MeV F position 194.
22. The immunogen of claim 21, wherein the recombinant MeV F ectodomain trimer is stabilized in the prefusion conformation by a non-natural disulfide bond between the cysteine substitutions in protomers of the trimer at MeV F positions 165 and 171.

23. The immunogen of claim 21 or claim 22, wherein:

the cysteine substitutions at:

MeV F positions 48 and 284 are R48C and A284C substitutions;

MeV F positions 90 and 225 are A90C and I225C substitutions;

MeV F positions 141 and 270 are M141C and T270C substitutions;

MeV F positions 165 and 171 are R165C and M171C substitutions;

MeV F positions 173 and 245 are L173C and V245C substitutions;

MeV F positions 175 and 241 are V175C and D241C substitutions;

MeV F positions 212 and 236 are E212C and Y236C substitutions;

MeV F positions 216 and 233 are L216C and A233C substitutions;

MeV F positions 219 and 224 are P219C and P224C substitutions;

MeV F positions 99 and 117 are R99C-V117C substitutions;

MeV F positions 100 and 117 are P100C-V117C substitutions;

MeV F positions 101 and 117 are V101C-V117C substitutions;

MeV F positions 102 and 117 are Q102C-V117C substitutions;

MeV F positions 103 and 117 are S103C-V117C substitutions;

the phenylalanine substitution at MeV F position 175 is a V175F substitution; and

the proline substitution at MeV F position 194 is a S194P substitution.

24. The immunogen of any one of claims 21-23, wherein the protomers of the recombinant MeV F ectodomain trimer comprise a F₂ protein comprising or consisting of MeV F positions 24-110 and a F₁ ectodomain comprising or consisting of MeV F positions 114-486.

25. The immunogen of any one of claims 21-24, wherein the protomers of the trimer further comprise a mutation to remove a F1/F2 furin cleavage site of the MeV F ectodomain and optionally a first residue of a fusion peptide of a F1 ectodomain.

26. The immunogen of claim 25, wherein the mutation to remove the F1/F2 furin cleavage site and the first residue of the fusion peptide comprise a deletion of MeV F positions 111-113 with positions 110 and 114 fused by a peptide linker.

27. The immunogen of claim 26, wherein the peptide linker is a Gly-Gly-Gly linker.

28. The immunogen of any one of claims 21-27, wherein the positioning of the amino acid substitutions is according to a reference MeV F proteins sequence set forth as SEQ ID NO: 36.

29. The immunogen of any one of claims 21-28, wherein the protomers of the MeV F ectodomain trimer comprise an amino acid sequence at least 90% identical to residues 21-483 of any one of SEQ ID NOs: 37-43, 53-55, 62-68, or 72-80 or residues 21-490 of any one of SEQ ID NOs: 70-72; and wherein the protomers comprise the one or more amino acid substitutions that stabilize the MeV F ectodomain trimer in the prefusion conformation.

30. The immunogen of claim 29, wherein the protomers of the MeV F ectodomain trimer comprise or consist of the amino acid sequence set forth as 21-483 of any one of SEQ ID NOs: 37-43, 53-55, 62-68, or 72-80 or residues 21-490 of any one of SEQ ID NOs: 70-72.

31. The immunogen of any one claims 21-30, wherein the protomers of the recombinant MeV F ectodomain trimer are fused C-terminally to a trimerization domain.

32. The immunogen of claim 31, wherein the trimerization domain comprises a GCN4 trimerization domain, a T4 fibrin trimerization domain, or both.

33. The immunogen of claim 32, wherein
the GCN4 trimerization domain comprises an amino acid sequence set forth as
IEDKIEEILSKIYHIENEIARIKKLIGEAP (SEQ ID NO: 33).
the T4 fibrin trimerization domain comprises an amino acid sequence set forth as
GYIPEAPRDGQAYVRKDGWVLLSTFL (SEQ ID NO: 34).
the trimerization domain comprising both the GCN4 and T4 fibrin trimerization domains
comprises an amino acid sequence set forth as
IEDKIEEILSKIYHIENEIARIKKLIGEAPSGYIPEAPRDGQAYVRKDGWVLLSTFL (SEQ ID NO: 34).

34. The immunogen of any one of claims 31-33, wherein the protomers of the MeV F ectodomain trimer fused to the trimerization domain comprise an amino acid sequence at least 90% identical to residues 21-513 of any one of SEQ ID NOs: 37-43, 53-55, 62-68, or 72-80 or residues 21-520 of any one of SEQ ID NOs: 70-72; and wherein the protomers comprise the one or more amino acid substitutions that stabilize the MeV F ectodomain trimer in the prefusion conformation.

35. The immunogen of claim 34, wherein the protomers of the MeV F ectodomain trimer fused to the trimerization domain comprise or consist of the amino acid sequence set forth as residues 21-513 of any one of SEQ ID NOs: 37-43, 53-55, 62-68, or 72-80 or residues 21-520 of any one of SEQ ID NOs: 70-72.

36. The immunogen of any one of claims 21-35, wherein the protomers of the recombinant MeV F ectodomain trimer are linked to a heterologous protein.

37. The immunogen of claim 36, wherein the heterologous protein is an ectodomain head or ectodomain stalk and head of a MeV H protein or a MuV HN protein.

38. The immunogen of claim 37, wherein the ectodomain head or ectodomain stalk and head of the MeV H protein or the MuV HN protein is fused C-terminally to a trimerization domain that is fused C-terminally to the protomer of the recombinant MuV F ectodomain trimer.

39. The immunogen of claim 38, wherein the protomers of the MeV F ectodomain trimer linked to the trimerization domain and the ectodomain of the MeV H protein or the MuV HN protein comprise an amino acid sequence set forth as residues 21-959 of SEQ ID NO: 56 or 21-973 of SEQ ID NO 57 or residues 21-988 of SEQ ID NO: 81.

40. The immunogen of any one of the prior claims, wherein the protomers of the recombinant MeV F ectodomain trimer or the recombinant MuV F ectodomain trimer further comprise one or more additional amino acid substitutions.

41. An isolated immunogen, comprising:

a trimer of fusion proteins, each fusion protein comprising or consisting of, in an N- to C-terminal direction, a trimerization domain, an optional peptide linker, and one or more copies of a MuV HN ectodomain head or a MeV H ectodomain head; or

a trimer of fusion proteins, each fusion protein comprising or consisting of, in an N- to C-terminal direction, one or more copies of a MuV HN ectodomain head or a MeV H ectodomain head, an optional peptide linker, a trimerization domain, an optional peptide linker, and one or more copies of a MuV HN ectodomain head or a MeV H ectodomain head; or

a trimer of fusion proteins, each fusion protein comprising or consisting of, in an N- to C-terminal direction, one or more copies of a MuV HN ectodomain head or stalk and head or a MeV H ectodomain head or stalk and head, an optional peptide linker, a trimerization domain, an optional peptide linker, and one or more copies of a MuV HN ectodomain head or stalk and head or a MeV H ectodomain head or stalk and head.

42. The immunogen of claim 41, wherein

the MuV HN ectodomain head comprises or consists of an amino acid sequence set forth as residues 59-510 of SEQ ID NO: 58, or a sequence at least 90% identical to residues 59-510 of SEQ ID NO: 58 ;

the MuV HN ectodomain stalk and head comprises residues 22-550 of SEQ ID NO: 90, residues 22-543 of SEQ ID NO: 91, residues 22-541 of SEQ ID NO: 92, or residues 22-549 of SEQ ID NO: 93, or a sequence at least 90% identical to residues 22-550 of SEQ ID NO: 90, residues 22-543 of SEQ ID NO: 91, residues 22-541 of SEQ ID NO: 92, or residues 22-549 of SEQ ID NO: 93;

the MeV H ectodomain head comprises or consists of an amino acid sequence set forth as residues 59-496 of SEQ ID NO: 59, or a sequence at least 90% identical to residues 59-496 of SEQ ID NO: 59; or

the MeV H ectodomain stalk and head comprises residues 22-580 of SEQ ID NO: 86, residues 22-577 of SEQ ID NO: 87, residues 22-579 of SEQ ID NO: 88, or residues 22-572 of SEQ ID NO: 89, or a sequence at least 90% identical to residues 22-580 of SEQ ID NO: 86, residues 22-577 of SEQ ID NO: 87, residues 22-579 of SEQ ID NO: 88, or residues 22-572 of SEQ ID NO: 89.

43. The immunogen of claim 41 or claim 42, wherein

the GCN4 trimerization domain comprises an amino acid sequence set forth as

IEDKIEEILSKIYHIENEIARIKKLIGEAP (SEQ ID NO: 33).

the T4 fibrin trimerization domain comprises an amino acid sequence set forth as

GYIPEAPRDGQAYVRKDGWVLLSTFL SEQ ID NO: 34).

the trimerization domain comprising both the GCN4 and T4 fibrin trimerization domains comprises an amino acid sequence set forth as

IEDKIEEILSKIYHIENEIARIKKLIGEAPSGYIPEAPRDGQAYVRKDGWVLLSTFL (SEQ ID NO: 35).

44. The immunogen of any one of claims 41-43, wherein the fusion proteins in the trimer comprise or consist of residues 24-510 of SEQ ID NO: 58, residues 24-496 of SEQ ID NO: 59, residues 22-950 of SEQ ID NO: 82, residues 25-985 of SEQ ID NO: 83, residues 22-948 of SEQ ID NO: 84, or residues 22-981 of SEQ ID NO: 85, or a sequence at least 90% identical to any one of residues 24-510 of SEQ ID NO: 58, residues 24-496 of SEQ ID NO: 59, residues 22-950 of SEQ ID NO: 82, residues 25-985 of SEQ ID NO: 83, residues 22-948 of SEQ ID NO: 84, or residues 22-981 of SEQ ID NO: 85.

45. An isolated immunogen, comprising:

a dimer of a MeV H ectodomain head;

a dimer of a MeV H ectodomain stalk and head;.

a dimer of a MuV HN ectodomain head; or

a dimer of a MuV HN ectodomain stalk and head.

46. The immunogen of claim 45, wherein

the MeV H ectodomain head comprises or consists of the amino acid sequence set forth as residues 22-459 of SEQ ID NO: 60, or a sequence at least 90% identical to residues 22-459 of SEQ ID NO: 60;

the MeV H ectodomain stalk and head comprises residues 22-580 of SEQ ID NO: 86, residues 22-577 of SEQ ID NO: 87, residues 22-579 of SEQ ID NO: 88, or residues 22-572 of SEQ ID NO: 89, or a sequence at least 90% identical to residues 22-580 of SEQ ID NO: 86, residues 22-577 of SEQ ID NO: 87, residues 22-579 of SEQ ID NO: 88, or residues 22-572 of SEQ ID NO: 89.

the MuV H ectodomain head comprises or consists of the amino acid sequence set forth as SEQ ID NO: 30, or a sequence at least 90% identical to SEQ ID NO: 30; or

the MuV HN ectodomain stalk and head comprises or consists of residues 22-550 of SEQ ID NO: 90, residues 22-543 of SEQ ID NO: 91, residues 22-541 of SEQ ID NO: 92, or residues 22-549 of SEQ ID NO: 93, or a sequence at least 90% identical to residues 22-550 of SEQ ID NO: 90, residues 22-543 of SEQ ID NO: 91, residues 22-541 of SEQ ID NO: 92, or residues 22-549 of SEQ ID NO: 93.

47. The immunogen of any one of the prior claims, conjugated to a heterologous carrier.
48. The immunogen of any one of the prior claims, wherein the immunogen is soluble.
49. The immunogen of any one of prior claims, wherein the protomers of the recombinant MeV F ectodomain trimer, the protomers of the recombinant MuV F ectodomain trimer, the trimer of fusion proteins, or the dimer of the MeV H ectodomain head are fused to a transmembrane domain by a peptide linker, or directly fused to the transmembrane domain.
50. The immunogen of claim 49, wherein the protomers of the recombinant MeV F ectodomain trimer or recombinant MuV F ectodomain trimer comprise a full-length F₁ protein.
51. A virus-like particle comprising the immunogen of any one or the prior claims.
52. A self-assembling protein nanoparticle comprising the immunogen of any one or the prior claims.
53. A nucleic acid molecule encoding the immunogen of any one of the prior claims.
54. The nucleic acid molecule of claim 53, operably linked to a promoter.
55. A vector comprising the nucleic acid molecule of claim 54.
56. The vector of claim 55, wherein the vector is an RNA vector.

57. A method of producing an immunogen, comprising:
expressing the nucleic acid molecule of any one of claims 55-56 in a host cell; and
purifying the immunogen.
58. The immunogen produced by the method of claim 57.
59. An immunogenic composition, comprising the immunogen, the nucleic acid molecule, the vector, or the virus like particle, of any one of claims 1-56 and 58, and a pharmaceutically acceptable carrier.
60. A method of eliciting an immune response to MuV F, MeV F, MuV NH, and/or MeV H in a subject, comprising administering to the subject an effective amount of the immunogenic composition of claim 59 to elicit the immune response.
61. The method of claim 60, wherein the immune response inhibits MuV and/or MeV infection in the subject.
62. The method of claim 60 or 61, wherein the subject is an adult who was previously immunized with a live attenuated vaccine to MeV and/or MuV and the administration boosts the immune response to the MuV and/or MeV.