

Reagents and methods for preventing, treating or limiting henipavirus infection

5

Sequence Listing Statement

A computer readable form of the Sequence Listing is filed with this application by electronic submission and is incorporated into this application by reference in its entirety. The Sequence Listing is contained in the file created on August 19, 2024 having the file name
10 “23-1410-WO.xml” and is 81,889 bytes in size.

Background

Nipah virus (NiV) and Hendra virus (HeV) are bat-borne zoonotic pathogens of the *Henipavirus* (HNV) genus causing encephalitis and respiratory symptoms in humans,
15 with fatality rates between 50 and 100%. NiV was discovered in 1999 and over the past two decades NiV has spilled over into humans almost annually in Bangladesh (Bangladesh strain, NiV-B) and has also caused outbreaks in India (NiV-B) and the Philippines (Malaysia strain, NiV-M). The detection of cross-reactive HNV antibodies in humans and *Pteropus* bats in Africa underscored that 2 billion people worldwide live in regions threatened by HNV
20 spillovers. To date, no approved vaccines or therapeutics for use in humans exist against HNV infections.

HNV genomes are non-segmented single-stranded negative-sense RNA of 18.2 kb in length containing six genes encoding for the structural proteins N, P, M, F, G and L. HNV entry into host cells requires fusion of the viral membrane and the host plasma membrane
25 through the concerted action of the attachment (G) glycoprotein and the fusion (F) glycoprotein. The NiV and HeV entry receptors at the surface of host cells are the transmembrane protein tyrosine kinases ephrin-B2 or ephrin-B3. G and F undergo a cascade of conformational changes to promote membrane fusion upon receptor engagement.

The NiV G ectodomain contains an N-terminal stalk (residues 96 to 147), a neck domain (residues 148 to 165), a linker region (residues 166 to 177), and a C-terminal
30 head domain (residues 178 to 602). NiV G forms a 200-Å-long and 120-Å-wide intertwined homotetramer stabilized by interprotomer disulfide bonds formed in the neck and stalk. The overall NiV G architecture adopts a distinctive two-heads-up and two-heads-down conformation.

35

Summary

In a first aspect, the disclosure provides isolated polypeptides comprising:

- (a) an attachment glycoprotein G head domain (G HD) comprising an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID NO:1-2, optionally wherein the head domain comprises the amino acid sequence of SEQ ID NO:1, wherein at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or all 20 of the residues bounded by parentheses is the first listed residue; and
- (b) a multimerization domain capable of generating multimers comprising 2 to 60 copies of the isolated polypeptide.

In one embodiment, the multimerization domain generates 4 copies or 60 copies of the isolated polypeptide. In another embodiment, the multimerization domain comprises an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO:3-4, optionally wherein the multimerization domain comprises the amino acid sequence of SEQ ID NO:3, wherein at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 or all 18 of the residues bounded by parentheses is the first listed residue. In a further embodiment, the polypeptides comprise an amino acid linker between the head domain and the multimerization domain. In a still further embodiment, the head domain is carboxy-terminal to the multimerization domain.

In one embodiment, the polypeptide comprises an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence selected from the group consisting of SEQ ID NO:5-6, wherein n is 3-11, 4-11, 5-11, 6-11, 7-11, 8-11, 9-11, 10-11, 3-10, 4-10, 5-10, 6-10, 7-10, 8-10, 9-10, 3-9, 4-9, 5-9, 6-9, 7-9, 8-9, 3-8, 4-8, 5-8, 6-8, 7-8, 3, 4, 5, 6, 7, 8, 9, 10 or 11; optionally wherein n is 8-11, 9-11, 10-11, 8-10, 9-10, 8-9, 8, 9, 10, or 11. In another embodiment, the polypeptide comprises an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID NO:7-10 and 18.

The disclosure further provides multimers or scaffolds comprising 60 or more copies of a head domain (HD) comprising an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID NO:1-2. In one embodiment, the multimer comprises 60 or

more copies of the isolated polypeptide of any embodiment or combination of embodiments herein.

In various embodiments, the disclosure provides nucleic acid encoding the isolated polypeptide of any embodiment or combination of embodiments disclosed herein, a
5 recombinant expression vector comprising the nucleic acid operatively linked to a suitable control sequence, such as a promoter, and recombinant host cells comprising the polypeptide, the multimer, the scaffold, the nucleic acid, and/or the recombinant expression vector of any embodiment or combination of embodiments disclosed herein.

In one embodiment, the nucleic acid comprises mRNA. In another embodiment, the
10 mRNA comprises

- (a) a 5' cap;
- (b) a poly(A) tail of between 50 and 120 contiguous adenosine residues;
- (c) a 5' untranslated region comprising the nucleic acid sequence of SEQ ID NO:12 or 13;
- 15 (d) a 3' untranslated region comprising one or two copies of a beta globin mRNA 3'-UTR, optionally wherein the beta globin mRNA 3'-UTR comprises the nucleic acid sequence of SEQ ID NO:11; and/or
- (e) a signal sequence, optionally wherein the signal sequence is at the N-terminus of the encoded polypeptide, and optionally wherein the signal sequence comprises the amino
20 acid sequence of SEQ ID NO:14.

The disclosure also provides compositions comprising a plurality of mRNAs that encode 2, 3, or 4 polypeptides comprising an amino acid sequence at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence selected from the group consisting of SEQ ID NO:5-10 and 18.

25 The disclosure further provides pharmaceutical compositions comprising

(a) the polypeptide, the multimer, the scaffold, the nucleic acid, the recombinant expression vector, and/or the cell of any embodiment or combination of embodiments disclosed herein; and

(b) a pharmaceutically acceptable carrier.

30 In some embodiments, the pharmaceutical composition comprises:

(a) the mRNA of any embodiment or combination of embodiments disclosed herein; and

(b) a cationic lipid carrier, such as a liposome, or a cationic protein, such as protamine.

Also disclosed are methods for treating or limiting development of a henipavirus infection, or of generating an immune response, comprising administering to a subject infected with or at risk of a henipavirus an amount effective to treat or limit the infection, and/or to generate an immune response, of the polypeptide, the multimer, the scaffold, the nucleic acid, the recombinant expression vector, the cell, and/or the pharmaceutical composition of any embodiment or combination of embodiments disclosed herein.

The disclosure also provides methods for monitoring a henipavirus-induced disease in a subject and/or monitoring response of the subject to immunization by a henipavirus vaccine, comprising contacting the polypeptide, the multimer, the scaffold, the nucleic acid, the recombinant expression vector, the cell, and/or the pharmaceutical composition of any embodiment or combination of embodiments disclosed herein with a bodily fluid from the subject and detecting henipavirus-binding antibodies in the bodily fluid of the subject.

In another embodiment, the disclosure provides methods for detecting henipavirus binding antibodies, comprising

(a) contacting the polypeptide, the multimer, the scaffold, and/or the pharmaceutical composition of any embodiment or combination of embodiments disclosed herein with a composition comprising a candidate henipavirus binding antibody under conditions suitable for binding of henipavirus antibodies to the polypeptide, the multimer, the scaffold, and/or the pharmaceutical composition; and

(b) detecting henipavirus antibody complexes with the polypeptide, the multimer, the scaffold, and/or the pharmaceutical composition.

Description of the Figures

Figure 1. ELISA Positive control: Optical density (OD) values of anti-NiV G antibody at 7 serial volume dilutions starting with a concentration of 1 µg/mL

b. Optical density (OD) values of week 3 and 6 mouse serum samples at 1:50000 volume dilution.

Figure 3. Optical Density (OD) values of week 6 mouse serum samples at 1:50000 and 1:100000 volume dilutions.

Figure 4. Concentration-response curves in Vero-Fco cells. Vero-Fco cells were infected with recombinant virus rVSVΔG-NiV-G after neutralization with mouse sera. Relative fluorescence units (RFU) are shown on the y-axis. Each point on the graph represents mean values, and error bars indicate standard deviations of triplicate wells. (A)

Sera of mouse #1-3 at week 0 prior to immunization and serum of positive control (hamster that survived Nipah virus infection). (B) Sera of mouse #1-4 at week 3 after one dose. (C) Sera of mouse #5-8 at week 3 after one dose. (D) Sera of mouse #9-10 at week 3 after one dose and sera of mouse #1-2 at week 6 after prime/boost. (E) Sera of mouse #3-6 at week 6 after prime-boost. (F) Sera of mouse #7-10 at week 6 after prime-boost.

Figure 5. Representative images of the neutralization activity of anti-NiV G glycoprotein in Vero-Fco cells infected with recombinant virus rVSVΔG-NiV-G. Vero-Fco cells were treated with serial 5-fold dilutions of indicated mouse sera mixed with 100 TCID₅₀ of rVSVΔG-NiV-G. Fluorescence signal was measured after 72 hours. (A) Positive Control: serum of a hamster that survived Nipah virus infection. (B) Serum of mouse #1 at week 0 prior to immunization. (C) Serum of mouse #1 at week 3 after one dose. (D) Serum of mouse #1 at week 6 after prime/boost.

Detailed Description

All references cited are herein incorporated by reference in their entirety. As used herein, the singular forms “a”, “an” and “the” include plural referents unless the context clearly dictates otherwise.

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

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

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

The description of embodiments of the disclosure is not intended to be exhaustive or to limit the disclosure to the precise form disclosed. While the specific embodiments of, and

examples for, the disclosure are described herein for illustrative purposes, various equivalent modifications are possible within the scope of the disclosure, as those skilled in the relevant art will recognize.

As used throughout the present application, the term “henipavirus” is used to designate any highly pathogenic virus phylogenetically related to Nipah virus (NiV) or Hendra virus (HeV).

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

Any N-terminal amino acids in the polypeptides disclosed herein are optional, and may be present, or may be deleted.

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

An abbreviated amino acid residue preceded or followed by a number indicates the position of the amino acid in a sequence of residues.

Nipah virus glycoprotein G head domain (NiV G HD) and Hendra virus glycoprotein G head domain (HeV G HD).

SEQ ID NO:17 is the reference sequence of NiV G HD (residues 178-602 of the Malaysia strain NiV G protein, Genbank AF212302):

VSNLVGLPNNICLQKTSNQILKPKLISYTLPVVGQSGTCITDPLLAMDEGYFAYSHLERIGS
CSRGVSKQRIIGVGEVLDRGDEVPSLFMTNVWTPPNPNTVYHCSAVYNNEFYVLCVAVSTVG
DPILNSTYWGSGLMMTRLAVKPKSNGGGYNQHQLALRSIEKGRYDKVMPYGPSGIKQGDPLY
FPAVGFLVRTEFKYNDSPITKQYKPKENRCLSMGIRPN SHYILRSGLLKYNLSDGENPK
VVFIEISDQRLSIGSPSKIYDSLGPVFYQASFSWDTMIKFGDVLTVNPLVVNWRNNTVISR
PGQSQCPRFNTCPEICWEGVYNDAFLIDRINWISAGVFLDSNQTAE NPVFTVFKDNEILYRA
QLASEDTNAQKTITNCFLKKNKIWCISLVEIYDTGDNVIRPKLFAVKIPEQCT (SEQ ID
NO:17) (NiV G HD Malaysia strain residues 178-602, Genbank AF212302)

The following is a list of NiV G HD mutations observed in Bangladesh, India and Malaysia (numbering relative to the numbering of the NiV G ectodomain sequence):

R236K,
P274S,
T299V,
I304V,
L335F,
S339N,
R344K,
I384V,
T385A,
K386E,
G421E,
P424S,
V426I,
V427I,
I444V,
L470Q,
N481D,
T498K,
I502V, and
I545V.

The above mutations are included in SEQ ID NO:1 of Nipah virus glycoprotein G head domain (NiV G HD) variants. Residue 1 in SEQ ID NO:1 corresponds to residue number 178 in the glycoprotein G. The same mutations using numbering starting with residue 1 in SEQ ID NO:1 is as follows:

R59K,
P97S,
T122V,
I127V,
L158F,
S162N,
R167K,

I207V,
T208A,
K209E,
G244E,
P247S,
V249I,
V250I,
I267V,
L293Q,
N304D,
T321K,
I325V, and
I368V.

VSNLVGLPNNICLQKTSNQILKPKLISYTLFVVGQSGTCITDPLLAMDEGYFAYSHLE (R/K)
) IGSCSRGVSKQRIIGVGEVLDRGDEVPSLFMTNVWTP (P/S) NPNTVYHCSAVYNNEFYV
LCAVS (T/V) VGDP (I/V) LNSTYWSGSLMMTRLAVKPKSNGGGYNQHQ (L/F) ALR (S/N)
IEKG (R/K) YDKVMPYGPSGIKQGDITLYFPAVGFLVRTEFKYND SNCP (I/V) (T/A) (K/E)
) CQYSKPENCRLSMGIRPNSHYILRSGLLKYNLS (G/E) EN (P/S) K (V/I) (V/I) FIEI
SDQRLSIGSPSK (I/V) YDSLGPVIFYQASFSWDTMIKFGDV (L/Q) TVNPLVNVNR (N/D)
NTVISRPGQSQCPRFN (T/K) CPE (I/V) CWEGVYNDAFLIDRINWISAGVFLDSNQTAENP
VFTVFKDNE (I/V) LYRAQLASEDTNAQKTITNCFLLNKIWCISLVEIYDTGDNVIRPKLF
AVKIQEQT

(SEQ ID NO:1) (NiV G HD variants)

SEQ ID NO:2 is the reference sequence of HeV G HD:

VSDLVGLPNQICLQKTTSTILKPRILISYTLPI NTREGVCITDPLLAVDNGFFAYSHLEKIGS
CTRGIAKQRIIGVGEVLDRGDKVPSMFMTNVWTPPNPSTIIHCSSTYHEDFYITLCAVSHVG
5 DPILNSTSWTESLSLIRLAVRPKSDSGDYNQKYIAITKVERGKYDKVMPYGPSGIKQGDITLY
FPAVGFLPRTEFQYND SNCP I I HCKYSKAENCR LSMGVNSKSHYILRSGLLKYNLSLGGDI I
LQFIEIADNRLTIGSPSKIYNSLGQPVIFYQASYWDTMIKLGVDVTDPLRVQWRNNSVISR
PGQSQCPRFNVCPVCWEGTYNDAFLIDRLNWVSAGVYLNSNQTAENPVFAVFKDNEILYQV
PLAEDDTNAQKTITDCFLLENVIWCISLVEIYDTGDSVIRPKLFAVKIPACSES (SEQ ID
10 NO:2) (HeV G HD)

In one aspect, the disclosure provides isolated polypeptide comprising:

- (a) a head domain (HD) comprising an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID NO:1-2; and
- (b) a multimerization domain capable of generating multimers comprising 2 to 60 copies of the isolated polypeptide.

The polypeptides of the disclosure comprise a multimerization domain. For example, the polypeptides can be engineered via genetic fusion to create 4-mer or 60-mer multimers. These constructs may be expressed, for example, in Chinese hamster ovary (CHO) cells and purified using standard nickel and size exclusion methods. By size exclusion chromatography with multi-angle light scattering (SEC-MALS), each construct is shown to have the correct molecular weight according to its intended multimeric state. The antigenic profiles of the constructs are tested and the results show binding to neutralizing antibodies.

The polypeptide is capable of multimerization and thus presenting multiple copies of the head domain to enhance the immune response generated when the polypeptide is administered to a subject. Any multimerization can be used that is capable of generating multimers as deemed suitable for an intended use.

In one embodiment the multimerization domain may comprise motifs from the leucine zipper domain of the general control protein GCN4 of the yeast *Saccharomyces cerevisiae*: RMKQIEDKIEEILSKIYHIENEIARIKKLIGER (SEQ ID NO:21), which is a coiled trimerization motif, or MKVKQLEDVVEELLSVNYHLENVVARLKKLVGER (SEQ ID NO:22), which is a tetramerization motif having 4 helices curling around each other in helical manner.

These multimerization domains can be used to generate multimers comprising 3, 4, 6 or 8 copies of the isolated polypeptide comprising a first domain of the disclosure.

A fusion with multimerization domain of SEQ ID NO:21 may comprise fusing one copy of a polypeptide comprising a first domain of the disclosure to the N-terminus of SEQ ID NO:21 with any suitable linker (such as those described below), including but not limited to the linker GGSGGSGGSGGSGGSGGG (SEQ ID NO:23) resulting in a 3-mer. A fusion with multimerization domain of SEQ ID NO:22 may comprise fusing one copy of a polypeptide comprising a first domain of the disclosure to the N-terminus of SEQ ID NO:22 with any suitable linker (such as those described below), including but not limited to the

linker GGSGGSGGSGGSGGSGGG (SEQ ID NO:23) resulting in a 4-mer. A fusion with multimerization domain may comprise fusing one copy of a polypeptide comprising a first domain of the disclosure to the N- terminus of SEQ ID NO:22 with any suitable linker (such as those described below), including but not limited to the linker

- 5 GGSGGSGGSGGSGGSGGG (SEQ ID NO:23) and a second copy of a polypeptide of the disclosure to the C- terminus of SEQ ID NO:22 with any suitable linker (such as those described below), including but not limited to the linker GGSGGSGGSGGSGGSGGG (SEQ ID NO:23), resulting in an 8-mer.

- In another embodiment, the multimerization domain comprises an amino acid
 10 sequence at least at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 3 or 4.
 MQIY (E/C) GK (L/C) (T/G) AEGLRFGIVASR (F/A) NHALVDRLVEGAIDAIV (R/C) (H /F/M) GGREEDITLV (R/C) V (P/C) GSWEIP (V/C) AAGELARKEDIDAVIAIGVL (I/C) RGA (T/C) (P/G) (H/S) FDYIASEVSKGLADLS (L/C) ELRKPIITFGVITA (D/C) TLEQA
 15 IE (R/A) AGT (K/C) HGKNGWEAAL (S/C) AIEMANLFKSLR (SEQ ID NO:3) (Lumazine synthase (LS) variants)

- MQIYEGKLTAEGLRFGIVASRANHALVDRLVEGAIDAIVRHGGREEDITLVRVCGSWEIPVA
 AAGELARKEDIDAVIAIGVLCRGATPSFDYIASEVSKGLADLSLELRKPITFGVITADTLEQA
 20 IEAAGTCHGKNGWEAALCAIEMANLFKSLR (SEQ ID NO:4) (Lumazine synthase (LS))

In this embodiment, the multimerization platform comprises lumazine synthase. The multimerization domains of SEQ ID NO: 3 and 4 can be used to generate multimers comprising 60 copies of the isolated polypeptides of the disclosure.

- 25 In one embodiment where the multimerization domain comprises SEQ ID NO:3, at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 or all 18 of the residues bounded by parentheses is the first listed residue.

- In another embodiment, the polypeptides of the disclosure may further comprise an amino acid linker between the head domain and the multimerization domain. Any amino
 30 acid linker may be used as suitable for an intended purpose. In one embodiment, the linker is a Gly-Ser rich linker (i.e.: 50%, 60%, 70%, 80%, 90%, 95%, or 100% made up of Gly or Ser residues). The combination of flexible and hydrophilic residues in these linkers limits the formation of secondary structures and reduces the likelihood that the linkers will interfere with the folding and function of the protein domains. In one specific embodiment, the linker

comprises or consists of (GGG)_nGGG, wherein n is 3-11, 4-11, 5-11, 6-11, 7-11, 8-11, 9-11, 10-11, 3-10, 4-10, 5-10, 6-10, 7-10, 8-10, 9-10, 3-9, 4-9, 5-9, 6-9, 7-9, 8-9, 3-8, 4-8, 5-8, 6-8, 7-8, 3, 4, 5, 6, 7, 8, 9, 10 or 11. In some embodiments, n is 8-11, 9-11, 10-11, 8-10, 9-10, 8-9, 8, 9, 10, or 11.

- 5 The multimerization domain may be N-terminal or C-terminal to the head domain. In one specific embodiment, the head domain is carboxy-terminal to the multimerization domain.

In other embodiments, the polypeptide comprises the amino acid sequence selected from the group consisting of SEQ ID NO: 5-6, wherein n is 3-11, 4-11, 5-11, 6-11, 7-11, 8-11, 9-11, 10-11, 3-10, 4-10, 5-10, 6-10, 7-10, 8-10, 9-10, 3-9, 4-9, 5-9, 6-9, 7-9, 8-9, 3-8, 4-8, 5-8, 6-8, 7-8, 3, 4, 5, 6, 7, 8, 9, 10 or 11. In some embodiments, n is 8-11, 9-11, 10-11, 8-10, 9-10, 8-9, 8, 9, 10, or 11.

MQIY (E/C) GK (L/C) (T/G) AEGLRFGIVASR (F/A) NHALVDRLVEGAIDAIV (R/C) (H /F/M) GGREEDITLV (R/C) V (P/C) GSWEIP (V/C) AAGELARKEDIDAVIAIGVL (I/C)
 15 RGA (T/C) (P/G) (H/S) FDYIASEVSKGLADLS (L/C) ELRKPITFGVITA (D/C) TLEQA IE (R/A) AGT (K/C) HGKKGWEAAL (S/C) AIEMANLFKSLR (GGG)_nGGGVSNLVGLPNNIC LQKTSNQILKPKLISYTLPPVVGQSGTCITDPLLAMDEGYFAYSHLE (R/K) IGSCSRGVSKQ RIIGVGEVLDRGDEVPSLFMTNVWTP (P/S) NPNTVYHCSAVYNNEFYVLCVAVS (T/V) VG DP (I/V) LNSTYWSGSLMMTRLAVKPKSNGGGYNQHQ (L/F) ALR (S/N) IEKG (R/K) YDK
 20 VMPYGPSPGIKQGDITLYFPAVGFLVRTEFKYNDSNCP (I/V) (T/A) (K/E) CQYSKPENCR L SMGIRPNSHYILRSGLLKYNLSD (G/E) EN (P/S) K (V/I) (V/I) FIEISDQRLSIGSPSK (I/V) YDSLQGPVFYQASFSWDTMIKFGDV (L/Q) TVNPLVVNR (N/D) NTVISRPGQSQC PRFN (T/K) CPE (I/V) CWEGVYNDAFLIDRINWISAGVFLDSNQTAENPVFTVFKDNE (I /V) LYRAQLASEDTNAQKTITNCFLLKNKIWCISLVEIYDTGDNVIRPKLFAVKIPEQCT
 25 (SEQ ID NO:5) (LS variants-linker-NiV G HD variants)

MQIY (E/C) GK (L/C) (T/G) AEGLRFGIVASR (F/A) NHALVDRLVEGAIDAIV (R/C) (H /F/M) GGREEDITLV (R/C) V (P/C) GSWEIP (V/C) AAGELARKEDIDAVIAIGVL (I/C)
 30 RGA (T/C) (P/G) (H/S) FDYIASEVSKGLADLS (L/C) ELRKPITFGVITA (D/C) TLEQA IE (R/A) AGT (K/C) HGKKGWEAAL (S/C) AIEMANLFKSLR (GGG)_nGGGVSDLVGLPNQIC LQKTTSTILKPRLLISYTLPI NTREGVCITDPLLAVDNGFFAYSHLEKIGSCTRGIAKQRIIG VGEVLDRGDKVPSMFMTNVWTPPNPSTIIHCSSTYHEDFYITLCAVSHVGDPI LNSTSWTES LSLIRLAVRPKSDSGDYNQKYIAITKVERGKYDKVMPYGPSPGIKQGDITLYFPAVGFLPRTEF QYNDSNCP I I HCKYKAENCR L SMGVNSKSHYILRSGLLKYNLSLGGDIILQFIEIADNRLT

IGSPSKIYNSLGQPVFYQASYSWDTMIKLGVDVTDPLRVQWRNNSVISRPGQSQCPRFNV
 PEVCWEGTYNDAFLIDRLNWVSAGVYLNNSQTAENPVFAVFKDNEILYQVPLAEDDTNAQKT
 ITDCFLLENVWCISLVEIYDTGDSVIRPKLFAVKIPAQCSES (SEQ ID NO:6) (LS
 variants-linker-HeV G HD)

5

In specific embodiments, the polypeptide comprises the amino acid sequence of any
 one of SEQ ID NO:7-10 and 18.

MQIYEGKLTAEGLRFGIVASRANHALVDRLVEGAIDAIVRHGGREEDITLVRVCGSWEIPVA
 AGELARKEDIDAVIAIGVLCRGATPSFDYIASEVSKGLADLSLELRKPITFGVITADTLEQA
 10 IEAAGTCHGNKGWEAALCAIEMANLFKSLRGGSGGSGGSGGSGGSGGSGGSGGSGGSGGVS
 GLPNNICLQKTSNQILKPKLISYTLFVVGQSGTCITDPLLAMDEGYFAYSHLE (R/K) IGSC
 SRGVSKQRIIGVGEVLDRGDEVPSLFMTNVWTP (P/S) NPNTVYHCSAVYNNEFYVLCVAVS
 (T/V) VGDP (I/V) LNSTYWSGSLMMTRLAVKPKSNGGGYNQHQ (L/F) ALR (S/N) IEKG (R/K)
 YDKVMPYGPSGIKQGDITLYFPAVGFLVRTEFKYNDNSNCP (I/V) (T/A) (K/E) CQYS
 15 KPENCRLSMGIRPNSHYILRSGLLKYNLS (G/E) EN (P/S) K (V/I) (V/I) FIEISDQRL
 SIGSPSK (I/V) YDSLQGPVFIYQASFSWDTMIKFGDV (L/Q) TVNPLVVNWR (N/D) NTVIS
 RFGQSQCPRFN (T/K) CPE (I/V) CWEGVYNDAFLIDRINWISAGVFLDSNQTAENPVFTVF
 KDNE (I/V) LYRAQLASEDTNAQKTITNCFLLKNKIWCISLVEIYDTGDNVIRPKLFAVKIP
 EQCT (SEQ ID NO:7) (LS-linker-NiV G HD variants)

20

MQIYEGKLTAEGLRFGIVASRANHALVDRLVEGAIDAIVRHGGREEDITLVRVCGSWEIPVA
 AGELARKEDIDAVIAIGVLCRGATPSFDYIASEVSKGLADLSLELRKPITFGVITADTLEQA
 IEAAGTCHGNKGWEAALCAIEMANLFKSLRGGSGGSGGSGGSGGSGGSGGSGGSGGSGGVS
 GLPNQICLQKTTSTILKPKRLISYTLPI NTREGVCITDPLLAVDNGFFAYSHLEKIGSCTRGI
 25 AKQRIIGVGEVLDRGDKVPSMFMTNVWTPPNPSTIIHCSSTYHEDFYITLCAVSHVGDPI
 LNSTWTESLSLIRLAVRPKSDSGDYNQKYIAITKVERGKYDKVMPYGPSGIKQGDITLYFPAVG
 FLPRTEFQYNDNSNCP IIHCKYKAENCRLSMGVNSKSHYILRSGLLKYNLSLGGDIILQFIE
 IADNRLTIGSPSKIYNSLGQPVFYQASYSWDTMIKLGVDVTDPLRVQWRNNSVISRPGQSQC
 CPRFNVCEVCWEGTYNDAFLIDRLNWVSAGVYLNNSQTAENPVFAVFKDNEILYQVPLAED
 30 DTNAQKTITDCFLLENVWCISLVEIYDTGDSVIRPKLFAVKIPAQCSES (SEQ ID NO:8)
 (LS-linker-HeV G HD)

MKVKQLEDVVEELLSVNYHLENVVARLKKLVGERGGSGGSGGSGGSGGSGGSGGSGGSGGVSNLVGL
 PNNICLQKTSNQILKPKLISYTLPPVVGQSGTCITDPLLAMDEGYFAYSHLE (R/K) IGSCSR
 GVSKQRIIGVGEVLDRGDEVPSLFMTNVWTP (P/S) NPNTVYHCSAVYNNEFYVLCVAVS (T
 /V) VGDP (I/V) LNSTYWSGSLMMTRLAVKPKSNGGGYNQHQ (L/F) ALR (S/N) IEKG (R/
 5 K) YDKVMPYGPSGIKQGD TLYFPAVGFLVRTEFKYND SNCP (I/V) (T/A) (K/E) CQYSKP
 ENCRLSMGIRPN SHYILRSGLLKYNLSD (G/E) EN (P/S) K (V/I) (V/I) FIEISDQRLSI
 GSPSK (I/V) YDSLQGPVIFYQASFSWDTMIKFGDV (L/Q) TVNPLVVNWR (N/D) NTVISRP
 GQSQCPRFN (T/K) CPE (I/V) CWEGVYNDAFLIDRINWISAGVFLDSNQTAENPVFTVFKD
 NE (I/V) LYRAQLASEDTNAQKTITNCFLLKNKIWCISLVEIYDTGDNVIRPKLFAVKIPEQ
 10 CT (SEQ ID NO:9) (tetra motif-linker-NiV G HD variants)

MKVKQLEDVVEELLSVNYHLENVVARLKKLVGERGGSGGSGGSGGSGGSGGSGGSGGSGGVSNDLVGL
 PNQICLQKTTSTILKPRILISYTLPI NTREGVCITDPLLAVDNGFFAYSHLEKIGSCTRGIAK
 QRIIGVGEVLDRGDKVP SMFMTNVWTPPNPSTIIHCSSTYHEDFYITLCAVSHVGPILNST
 15 SWTESLSLIRLAVRPKSDSGDYNQKYIAITKVERGKYDKVMPYGPSGIKQGD TLYFPAVGFL
 PRTEFQYND SNCP I IHCYSKAENCRLSMGVNSKSHYILRSGLLKYNLSLGGDIILQFIEIA
 DNRLTIGSPSKIYNSLGQPVIFYQASYSWDTMIKLGVDVTDPLRVQWRNNSVISRPGQSQC
 RFNVCPEVCWEGTYNDAFLIDRLNWVSAGVYLN SNQTAENPVFAVFKDNEILYQVPLAEDDT
 NAQKTITDCFLENNVIWCISLVEIYDTGDSVIRPKLFAVKI PAQCSES (SEQ ID NO:10)
 20 (tetra motif-linker-HeV G HD)

MQIYEGKLTAEGLRFGIVASRANHVLVDRLVEGAIDAIVRHGGREEDITLVRVCGSWEIPVA
 AGELARKEDIDAVIAIGVLCRGATPSFDYIASEVSKGLADLSLELRKPITFGVITADTLEQA
 IEAAGTCHGNKGWEAALCAIEMANLFKSLRGGSGGSGGSGGSGGSGGSGGSGGSGGSGGVSNLV
 25 GLPNNICLQKTSNQILKPKLISYTLPPVVGQSGTCITDPLLAMDEGYFAYSHLERIGSCSRGV
 SKQRIIGVGEVLDRGDEVPSLFMTNVWTPPNPNTVYHCSAVYNNEFYVLCVAVSTVGPILN
 STYWSGSLMMTRLAVKPKSNGGGYNQHQ LALRSIEKGRYDKVMPYGPSGIKQGD TLYFPAVG
 FLVRTEFKYND SNCPITKQYSKPENCRLSMGIRPN SHYILRSGLLKYNLSDGENPKVVFIE
 ISDQRLSIGSPSKIYDSLQGPVIFYQASFSWDTMIKFGDVLTVNPLVVNWRNNTVISRPGQSQ
 30 CPRFNTCPEICWEGVYNDAFLIDRINWISAGVFLDSNQTAENPVFTVFKDNEILYRAQLASE
 DTNAQKTITNCFLLKNKIWCISLVEIYDTGDNVIRPKLFAVKIPEQCT (SEQ ID NO:18)
 (LS-linker-NiV G HD Malaysia strain residues 178-602)

In specific embodiments the Nipah virus head domain comprises the amino acid sequence of SEQ ID NO:5, 7 and 9, wherein at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, or all 18 of the residues bounded by parentheses is the first listed residue.

In another aspect, the disclosure provides multimers, comprising two or more copies of the isolated polypeptide of any embodiment or combination of embodiments disclosed herein. The multimers may be formed in any suitable manner, including but not limited to by inclusion of multimerization domains in the primary amino acid sequence, or by linking the polypeptides to a scaffold. In one embodiment, the multimer comprises between 2 and 60 copies of the isolated polypeptide. In various embodiments, the multimer may comprise 2, 3, 4, 6, 8, 60, or more copies of the polypeptide. In one embodiment, the disclosure provides scaffolds comprising two or more isolated polypeptides of any embodiment or combination of embodiments disclosed herein on a surface of the scaffold. Any suitable scaffolds may be used, whether polypeptide scaffolds, virus-like particles, beads, or other scaffold materials. The polypeptides may be linked to the scaffolds in any suitable matter. In one embodiment, the two or more isolated polypeptides are all identical polypeptides. In another embodiment, the two or more isolated polypeptides include different polypeptides, permitting delivery of a multivalent composition to a subject in need thereof.

In another aspect, the disclosure provides isolated nucleic acids encoding the isolated polypeptide of any embodiment or combination of embodiments disclosed herein. The isolated nucleic acid sequence may comprise RNA or DNA. Such isolated nucleic acid sequences may comprise additional sequences useful for promoting expression and/or purification of the encoded protein, including but not limited to polyA sequences, modified Kozak sequences, and sequences encoding epitope tags, export signals, and secretory signals, nuclear localization signals, and plasma membrane localization signals. It will be apparent to those of skill in the art, based on the teachings herein, what nucleic acid sequences will encode the polypeptides of the disclosure.

In another aspect, the present disclosure provides expression vectors comprising the nucleic acid of any aspect of the disclosure operatively linked to a suitable control sequence. "Expression vector" includes vectors that operatively link a nucleic acid coding region or gene to any control sequences capable of effecting expression of the gene product. "Control sequences" operably linked to the nucleic acid sequences of the disclosure are nucleic acid sequences capable of effecting the expression of the nucleic acid molecules. The control sequences need not be contiguous with the nucleic acid sequences, so long as they function to direct the expression thereof. Thus, for example, intervening untranslated yet transcribed

sequences can be present between a promoter sequence and the nucleic acid sequences and the promoter sequence can still be considered "operably linked" to the coding sequence. Other such control sequences include, but are not limited to, polyadenylation signals, termination signals, and ribosome binding sites. Such expression vectors include but are not limited to, plasmid and viral-based expression vectors. The control sequence used to drive expression of the disclosed nucleic acid sequences in a mammalian system may be constitutive (driven by any of a variety of promoters, including but not limited to, CMV, SV40, RSV, actin, EF) or inducible (driven by any of a number of inducible promoters including, but not limited to, tetracycline, ecdysone, steroid-responsive). The expression vector must be replicable in the host organisms either as an episome or by integration into host chromosomal DNA. In various embodiments, the expression vector may comprise a plasmid, viral-based vector (including but not limited to a retroviral vector or oncolytic virus), or any other suitable expression vector. In some embodiments, the expression vector can be administered in the methods of the disclosure to express the polypeptides *in vivo* for therapeutic benefit.

In a further aspect, the present disclosure provides host cells that comprise the polypeptides, nucleic acids, expression vectors and/or nucleic acids disclosed herein, wherein the host cells can be either prokaryotic or eukaryotic. The cells can be transiently or stably engineered to incorporate the expression vector of the disclosure, using techniques including but not limited to bacterial transformations, calcium phosphate co-precipitation, electroporation, or liposome mediated-, DEAE dextran mediated-, polycationic mediated-, or viral mediated transfection. (See, for example, *Molecular Cloning: A Laboratory Manual* (Sambrook, et al., 1989, Cold Spring Harbor Laboratory Press); *Culture of Animal Cells: A Manual of Basic Technique, 2nd Ed.* (R.I. Freshney, 1987, Liss, Inc. New York, NY)). A method of producing a polypeptide according to the disclosure is an additional part of the disclosure. The method comprises the steps of (a) culturing a host according to this aspect of the disclosure under conditions conducive to the expression of the polypeptide, and (b) optionally, recovering the expressed polypeptide. The expressed polypeptide can be recovered from the cell free extract, but preferably they are recovered from the culture medium.

In one embodiment of the nucleic acids of the disclosure, the nucleic acid comprises mRNA. Messenger RNA (mRNA) offers a relatively safe and efficient alternative to the polypeptide therapeutics and vaccines of the disclosure. After mRNA *in vivo* injection and uptake by professional antigen-presenting cells (APCs) in various tissues, the head domain is

expressed in APCs and displayed for the immune response.

Various modifications of mRNA may be used in order to counter the degradation of a head domain mRNA therapeutic or vaccine disclosed herein.

In one embodiment, the mRNA comprises a 5' cap. The 5' cap is a specially
5 altered nucleotide on the 5' end of mRNA. This process, known as mRNA capping, is highly regulated and vital in the creation of stable and mature messenger RNA able to undergo translation during protein synthesis. In eukaryotes, the 5' cap consists of a guanine nucleotide connected to mRNA via an unusual 5' to 5' triphosphate linkage. This guanosine is methylated directly after capping *in vivo* by a methyl transferase. In multicellular eukaryotes,
10 further modifications exist, including the methylation of the first 2 ribose sugars of the 5' end of the mRNA. The 5' cap is chemically similar to the 3' end of an RNA molecule and this provides significant resistance to 5'exonucleases. Eukaryotic RNA undergoes a series of modifications in order to be exported from the nucleus and successfully translated into function proteins, many of which are dependent on mRNA capping, the first mRNA
15 modification to take place. Various versions of 5' caps can be added during or after the transcription reaction using various capping enzymes such as a vaccinia virus capping enzyme or by incorporating a synthetic cap or anti-reverse cap analogues.

In another embodiment, the mRNA further comprises a poly(A) tail of between 50 and 120 contiguous adenosine residues. Polyadenylation helps protect the mRNA 3' end
20 against degradation by exonucleases, the export of mature mRNA to the cytoplasmic environment, and also for mRNA translation.

In another embodiment, the mRNA comprises a 5' untranslated region (including the start codon) comprising the sequence GGGAGACUGCCACCAUG (SEQ ID NO:12) or GGGAGACUGCCAAGAUG (SEQ ID NO:13). The 5'-untranslated region (5'-UTR) of mRNA
25 of this embodiment contains structural elements, which are recognized by cell-specific RNA-binding proteins, thereby affecting the translation of the molecule. To create recombinant RNA transcripts with short synthetic 5'-UTRs, the corresponding DNA sequences may be cloned into a plasmid vector upstream of the head domain gene. Table 1 lists the positions of different bases in the mRNA relative to the start codon. T7 promoter
30 (TAATACGACTCACTATA (SEQ ID NO: 15)) may be combined with the Kozak element consensus sequence upstream of the start codon (ATG). Transcription from T7 promoter begins with the first G after the TATA element. The following six bases after the TATA element (GGGAGA) help provide high yields and homogenous 5'mRNA ends during *in vitro*

transcription. This template-sequence results in an RNA, which has the sequence GGGAGACUGCCA (C/A) (C/G) AUG (SEQ ID NO: 16) as its 5'-UTR.

Table 1 shows two minimal UTRs with best results as 5'-UTRs.

5 Table 1. Sequences of Synthetic 5'-Untranslated Region

Minimal UTR	Promoter	Transcription start site	Extra nucleotides	Kozak sequence	Start codon
UTR1	T7	GGGAGA	CT	GCCACC	ATG
UTR2	T7	GGGAGA	CT	GCCAAG	ATG

10 In a further embodiment, the mRNA comprises a 3' untranslated region comprising one or two copies of a beta globin mRNA 3'-UTR. Any beta globin mRNA 3'-UTR may be used as deemed suitable for an intended purpose. In one embodiment, the beta globin mRNA 3'-UTR comprises the amino acid sequence:

GCUCGCUUUCUUGCUGUCCAAUUUCUAUUAAAGGUUCCUUUGUUCCCUAAGUCCAACUACUA
AACUGGGGGGAUUAUUAUGAAGGGCCUUGAGCAUCUGGAUUCUGCCUAAUAAAAACAUUUAUU
UUCAUUGC (SEQ ID NO:11)

- 15 In another aspect, the disclosure provides pharmaceutical compositions comprising
- (a) the polypeptide, the multimer, the scaffold, the nucleic acid, the recombinant expression vector, and/or the cell of a preceding claim; and
 - (b) a pharmaceutically acceptable carrier.

20 The pharmaceutical compositions of the disclosure may be used, for example, in the methods of the disclosure. In one embodiment, the composition comprises the pharmaceutically acceptable carrier and:

- 25 (a) the isolated polypeptide of SEQ ID NO:5; or
- (b) the isolated polypeptide of SEQ ID NO:6; or
 - (c) the isolated polypeptide of SEQ ID NO:7; or
 - (d) the isolated polypeptide of SEQ ID NO:8; or
 - (e) the isolated polypeptide of SEQ ID NO:9; or
 - (f) the isolated polypeptide of SEQ ID NO:10.

In another embodiment, compositions comprise

- (a) the mRNA of any embodiment or combination of embodiments herein; and
- (b) a cationic lipid carrier, such as a liposome, or a cationic protein, such as protamine.

Any cationic lipid carrier or cationic protein may be used as deemed appropriate for an intended use, including but not limited to liposomes and protamine.

In one such embodiment, the mRNA encodes:

- (a) the isolated polypeptide of SEQ ID NO:5; or
- (b) the isolated polypeptide of SEQ ID NO:6; or
- (c) the isolated polypeptide of SEQ ID NO:7; or
- 10 (d) the isolated polypeptide of SEQ ID NO:8; or
- (e) the isolated polypeptide of SEQ ID NO:9; or
- (f) the isolated polypeptide of SEQ ID NO:10.

The pharmaceutical composition may further comprise (a) a lyoprotectant; (b) a surfactant; (c) a bulking agent; (d) a tonicity adjusting agent; (e) a stabilizer; (f) a preservative and/or (g) a buffer.

In some embodiments, the buffer in the pharmaceutical composition is a Tris buffer, a histidine buffer, a phosphate buffer, a citrate buffer or an acetate buffer. The pharmaceutical composition may also include a lyoprotectant, e.g. sucrose, sorbitol or trehalose. In certain
20 embodiments, the pharmaceutical composition includes a preservative e.g. benzalkonium chloride, benzethonium, chlorohexidine, phenol, m-cresol, benzyl alcohol, methylparaben, propylparaben, chlorobutanol, o-cresol, p-cresol, chlorocresol, phenylmercuric nitrate, thimerosal, benzoic acid, and various mixtures thereof. In other embodiments, the pharmaceutical composition includes a bulking agent, like glycine. In yet other embodiments,
25 the pharmaceutical composition includes a surfactant e.g., polysorbate-20, polysorbate-40, polysorbate- 60, polysorbate-65, polysorbate-80 polysorbate-85, poloxamer-188, sorbitan monolaurate, sorbitan monopalmitate, sorbitan monostearate, sorbitan monooleate, sorbitan trilaurate, sorbitan tristearate, sorbitan trioleate, or a combination thereof. The pharmaceutical composition may also include a tonicity-adjusting agent, e.g., a compound
30 that renders the formulation substantially isotonic or isoosmotic with human blood. Exemplary tonicity adjusting agents include sucrose, sorbitol, glycine, methionine, mannitol, dextrose, inositol, sodium chloride, arginine and arginine hydrochloride. In other embodiments, the pharmaceutical composition additionally includes a stabilizer, e.g., a molecule which, when combined with a protein of interest substantially prevents or reduces

chemical and/or physical instability of the protein of interest in lyophilized or liquid form. Exemplary stabilizers include sucrose, sorbitol, glycine, inositol, sodium chloride, methionine, arginine, and arginine hydrochloride.

5 The polypeptide, the multimer, the scaffold, the nucleic acid, the recombinant expression vector, and/or the cell of any embodiment or combination of embodiments herein may be the sole active agent in the pharmaceutical composition, or the composition may further comprise one or more other active agents suitable for an intended use.

In another aspect, the disclosure provides methods for treating a henipavirus infection, comprising administering to a subject infected with a henipavirus an amount effective to treat
10 the infection of the polypeptide, the multimer, the scaffold, the nucleic acid, the recombinant expression vector, the cell, and/or the pharmaceutical composition of any claim herein.

As used herein, "treat" or "treating" means accomplishing one or more of the following in an individual that already has a henipavirus infection: (a) reducing the severity of the infection; (b) limiting or preventing development of symptoms characteristic of the infection being treated; (c) inhibiting worsening of symptoms characteristic of the infection;
15 and (d) limiting or preventing recurrence of symptoms in patients that were previously symptomatic for the infection.

In another aspect, the disclosure provides methods for limiting development of a henipavirus infection, comprising administering to a subject at risk of henipavirus infection
20 an amount effective to limit development of a henipavirus infection of the polypeptide, the multimer, the scaffold, the nucleic acid, the recombinant expression vector, the cell, and/or the pharmaceutical composition of any claim herein.

As used herein, "limiting" or "limiting development of" means accomplishing one or more of the following in an individual that does not have a henipavirus infection: (a)
25 preventing infection; (b) reducing the severity a subsequent infection; and (c) limiting or preventing development of symptoms after a subsequent infection.

In a further aspect, the disclosure provides methods for generating an immune response in a subject, comprising administering to the subject an amount effective to generate an immune response of the polypeptide, the multimer, the scaffold, the nucleic acid, the
30 recombinant expression vector, the cell, and/or the pharmaceutical composition of any claim herein.

In this aspect, generating an immune response can be used to prevent infection, treat an existing infection or limit development of a subsequent infection.

In all of the above aspects, an “amount effective” refers to an amount of the therapeutic that is effective for treating and/or limiting the infection. The polypeptide, the multimer, the scaffold, the nucleic acid, the recombinant expression vector, the cell, and/or the pharmaceutical composition may be administered by any suitable route. In one embodiment of all of these aspects, the polypeptide, the multimer, the scaffold, the nucleic acid, the recombinant expression vector, the cell, and/or the pharmaceutical composition may be administered by subcutaneous, intradermal or intramuscular injection. In one embodiment of the above aspects, the nucleic acid is administered with a needle-free injection system.

The subject in any of the methods disclosed herein may be any subject infected with or at risk of a henipavirus infection, including but not limited to a human subject.

In another aspect, the disclosure provides methods for monitoring a henipavirus-induced disease in a subject and/or monitoring response of the subject to immunization by a henipavirus vaccine, comprising contacting the polypeptide, the multimer, the scaffold, the nucleic acid, the recombinant expression vector, the cell, and/or the pharmaceutical composition of any claim herein with a bodily fluid from the subject and detecting henipavirus-binding antibodies in the bodily fluid of the subject. In this embodiment, a change in henipavirus-binding antibodies in the bodily fluid of the subject can be monitored over time after the therapeutic or prophylactic methods disclosed herein, or any other therapeutic or prophylactic methods to treat or limit development of a henipavirus-induced disease.

In one embodiment, the bodily fluid comprises serum or whole blood.

In a further aspect, the disclosure provides methods for detecting henipavirus binding antibodies, comprising

(a) contacting the polypeptide, the multimer, the scaffold, and/or the pharmaceutical composition of any claim herein with a composition comprising a candidate henipavirus binding antibody under conditions suitable for binding of henipavirus antibodies to the polypeptide, the multimer, the scaffold, and/or the pharmaceutical composition; and

(b) detecting henipavirus antibody complexes with the polypeptide, the multimer, the scaffold, and/or the pharmaceutical composition. In this embodiment, the reagents disclosed herein can be used in testing a subject for henipavirus infection.

In one embodiment, the method further comprises isolating the henipavirus antibodies that can be used, for example, as therapeutic antibodies to treat a subject having a henipavirus infection.

In a further embodiment, the disclosure provides methods for producing henipavirus antibodies, comprising

- (a) administering to a subject an amount effective to generate an antibody response of the polypeptide, the multimer, the scaffold, the nucleic acid, the recombinant expression vector, the cell, and/or the pharmaceutical composition of any claim herein; and
- (b) isolating antibodies produced by the subject. In this aspect, antibodies may be isolated and used, for example, as therapeutic antibodies to treat a subject having a henipavirus infection.

10 Example

Immunogenicity in mouse of Nipah mRNA vaccine encoding for amino acid sequence of SEQ ID NO:18.

The following experiments confirmed that the mRNA vaccine VX4004R2 encoding for the amino acid sequence of SEQ ID NO:18 elicits neutralizing antibodies in vaccinated mice.

Construct in pUC19 plasmid

A construct with the T7 RNA polymerase promoter, the 5' minimal untranslated region UTR2 of Table 1, the N-terminal human IL-2 signal sequence encoding for the amino acid sequence MYRMQLLSICIALSLALVTNS (SEQ ID NO:14), a nucleotide sequence encoding the amino acid sequence of SEQ ID NO:18, the 3'UTR region of SEQ ID NO:11, a poly(A) tail of 80 adenosine residues and the BsmBI restriction site was cloned into the pUC19 vector. The sequence of this construct is SEQ ID NO:19.

SEQ ID NO:19

25 Construct cloned into pUC19 vector

taatacgactcactatagggagactgccaagatgtacagaatgcagctgctgagctgcatcgccctga
gcttggcccttggtgaccaacagcatgcagatctacgagggcaagctgaccgccgagggcctgagattc
ggcatcgtggccagcagagccaaccacgccttggtggacagactggtggagggcgccatcgacgccat
30 cgtgagacacggcggcagagaggaggacatcaccttggtgagagtgtgcggcagctgggagatccccg
tggccgcggcgagctggccagaaaggaggacatcgacgccgtgatcgccatcgccgtgctgtgcaga
ggcgccacccccagcttcgactacatcgccagcgaggtgagcaagggcctggccgacctgagcctgga
gctgagaaagcccatcaccttcggcgtgatcacgcgcgacacctggagcaggccatcgaggccgcg
gcacctgccacggcaacaagggctgggaggccgcctgtgcgccatcgagatggccaacctgttcaag
35 agcctgagaggaggctccggaggatctggagggagtggaggctcaggcggcagcggaggggtccggcgg

aagcgggggctctggaggaggcgtgagcaacctgggtgggcctgcccacaacatctgcctgcagaaga
ccagcaaccagatcctgaagcccaagctgatcagctacacctgcccgtgggtggccagagcggcacc
tgcatacccgacccccctgctggccatggacgagggctacttcgcctacagccacctggagagaatcgg
cagctgcagcagaggcgtgagcaagcagagaatcatcggcgtgggcgaggtgctggacagaggcgacg
5 aggtgccagcctgttcatagaccaacgtgtggaccccccccaaccccaacaccgtgtaccactgcagc
gccgtgtacaacaacgagttctactacgtgctgtgcccgtgagcaacctgggcgaaccccatcctgaa
cagcacctactggagcggcagcctgatgatgaccagactggccgtgaagcccaagagcaacggcggcg
gctacaaccagcaccagctggccctgagaagcatcgagaaggcagatacgacaagggtgatgcctac
ggccccagcggcatcaagcagggcgacacctgtacttccccgcgtgggcttctgggtgagaaccga
10 gttcaagtacaacgacagcaactgccccatcaccaagtgccagtacagcaagcccgagaactgcagac
tgagcatgggcatcagacccaacagccactacatcctgagaagcggcctgctgaagtacaacctgagc
gacggcgagaacccccaaagggtgggtgttcatacgagatcagcgaccagagactgagcatcggcagccccag
caagatctacgacagcctgggcccagcccgtgttctaccaggccagcttcagctgggacaccatgatca
agttcggcgacgtgctgaccgtgaacccccctgggtgggtgaactggagaaacaacaccgtgatcagcaga
15 cccggccagagccagtgcaccagattcaacacctgccccgagatctgctgggagggcgtgtacaacga
cgcttctgatcgacagaatcaactggatcagcgccggcgtgttctggacagcaaccagaccgccg
agaaccccgtgttcacogtgttcaaggacaacgagatcctgtacagagcccagctggccagcagggac
accaacgcccagaagaccatcaccaactgcttctgctgaagaacaagatctggtgcatcagcctgggt
ggagatctacgacaccggcgacaacgtgatcagacccaagctgttcgcccgtgaagatccccgagcagt
20 gcacctgagctcgctttcttctgtgtccaatttctattaaaggttcctttgttccttaagtccaactac
taaactgggggatattatgaagggccttgagcatctggattctgcctaataaaaaacattttattttca
ttgcaaa
aaaaaaaaaaaaaaaaaaagagacg

25 mRNA transcription and capping

The supercoiled pUC19 DNA was upscaled, linearized with the enzyme BsmBI, and purified. In vitro transcription was performed with T7 polymerase in a 5mL reaction. The mRNA was capped with a cap 1 structure on the 5' end by vaccinia 2'-O-methyltransferase enzymatic capping. Capped mRNA was purified by reverse phase chromatography using
30 POROS™ resin (ThermoFisher) followed by tangential flow filtration (TFF). Final yield of purified mRNA was 5.51 g/l of IVT reaction. RNA integrity was analyzed by denaturing PAGE. mRNA sample and ladder were denatured at 70°C for 2 minutes and kept on ice, then 200 ng of mRNA mixed with diluent marker was loaded on gel. The sequence of this mRNA is SEQ ID NO:20.

35

SEQ ID NO:20

mRNA Sequence

GGGAGACUGCCAAGAUGUACAGAAUGCAGCUGCUGAGCUGCAUCGCCCUGAGCCUGGCCCCUG
GUGACCAACAGCAUGCAGAUUCUACGAGGGCAAGCUGACCGCCGAGGGCCUGAGAUUCGGCAU
CGUGGCCAGCAGAGCCAACCACGCCCUGGUGGACAGACUGGUGGAGGGCGCCAUCGACGCCA
5 UCGUGAGACACGGCGGCAGAGAGGAGGACAUCACCCUGGUGAGAGUGUGCGGCAGCUGGGAG
AUCCCCGUGGCCCGCGCGAGCUGGCCAGAAAGGAGGACAUCGACGCCGUGAUCGCCAUCGG
CGUGCUGUGCAGAGGCGCCACCCCAGCUUCGACUACAUCGCCAGCGAGGUGAGCAAGGGCC
UGGCCGACCUGAGCCUGGAGCUGAGAAAGCCCCAUCACCUUCGGCGUGAUCACCGCCGACACC
CUGGAGCAGGCCAUCGAGGCCGCGGCCACCUGCCACGGCAACAAGGGCUGGGAGGCCGCCCU
10 GUGCGCCAUCGAGAUGGCCAACCUGUUCAAGAGCCUGAGAGGAGGCUCGCGAGGAUCUGGAG
GGAGUGGAGGCUCAGGCGGCAGCGGAGGGUCCGGCGGAAGCGGGGCGUCUGGAGGAGGCGUG
AGCAACCUGGUGGGCCUGCCCAACAACAUCUGCCUGCAGAAGACCAGCAACCAGAUCCUGAA
GCCCAAGCUGAUCAGCUACACCCUGCCCUGGUGGGCCAGAGCGGCACCUGCAUCACCGACC
CCCUGCUGGCCAUGGACGAGGGCUACUUCGCCUACAGCCACCUGGAGAGAAUCGGCAGCUGC
15 AGCAGAGGCGUGAGCAAGCAGAGAAUCAUCGGCGUGGGCGAGGUGCUGGACAGAGGCGACGA
GGUGCCCAGCCUGUUCAUGACCAACGUGUGGACCCCCCCCCAACCCCAACACCGUGUACCACU
GCAGCGCCGUGUACAACAACGAGUUCUACUACGUGCUGUGCGCCGUGAGCACCGUGGGCGAC
CCCAUCCUGAACAGCACCUACUGGAGCGGCAGCCUGAUGAUGACCAGACUGGCCCGUGAAGCC
CAAGAGCAACGGCGGGCGGCUACAACCAGCACAGCUGGCCCCUGAGAAGCAUCGAGAAGGGCA
20 GAUACGACAAGGUGAUGCCCUACGGCCCCAGCGGCAUCAAGCAGGGCGACACCCUGUACUUC
CCCGCCGUGGGCUUCUGGUGAGAACCGAGUUCAAGUACAACGACAGCAACUGCCCCAUCAC
CAAGUGCCAGUACAGCAAGCCCGAGAACUGCAGACUGAGCAUGGGCAUCAGACCCAACAGCC
ACUACAUCUGAGAAGCGGCCUGCUGAAGUACAACCUGAGCGACGGCGAGAACCCCAAGGUG
GUGUUCAUCGAGAUACGCGACCAGAGACUGAGCAUCGGCAGCCCCAGCAAGAUCAACGACAG
25 CCUGGGCCAGCCCGUGUUCUACCAGGCCAGCUUCAGCUGGGACACCAUGAUCAAGUUCGGCG
ACGUGCUGACCGUGAACCCCCUGGUGGUGAACUGGAGAAACAACACCGUGAUCAGCAGACCC
GGCCAGAGCCAGUGCCCCAGAUUCAACAACUGCCCCGAGAUUCGUGGGAGGGCGUGUACAA
CGACGCCUUCUGAUCGACAGAAUCAACUGGAUCAGCGCCGGCGUGUUCUGGACAGCAACC
AGACCGCCGAGAACCCCGUGUUCACCGUGUUCAAGGACAACGAGAUCUGUACAGAGCCCAG
30 CUGGCCAGCGAGGACACCAACGCCCAGAAGACCAUCACCAACUGCUUCCUGCUGAAGAACAA
GAUCUGGUGCAUCAGCCUGGUGGAGAUCAACGACACCGGCGACAACGUGAUCAGACCCAAGC
UGUUCGCCGUGAAGAUCGCCGAGCAGUGCACCGAGCUCGCUUUCUUGCUGUCCAUUUUCUA
UUAAGGUUCCUUGUUCUUAAGUCCAACUACUAAACUGGGGGAUAUUAUGAAGGGCCUUG

AGCAUCUGGAUUCUGCCUAAUAAAAACAUUUAUUUUCAUUGCAAAAAAAAAAAAAAAAAAAAA
 AAA

Vaccine formulation

- 5 The mRNA was complexed with the polycationic protein protamine by addition of protamine to the mRNA at a mass ratio of 1:5. The vaccine was prepared on each injection day with final mRNA concentration of 420µg/mL. mRNA integrity prior to and after formulation was analyzed on 1% agarose gel with SYBR safe stain (ThermoFisher).

Immunization

- 10 A group of N=10 CB6F1/J female mice 7 weeks old from The Jackson Laboratory (SN 100007) was dosed by intramuscular injection in the caudal thigh with a needle-free injection system (Tropis™ injector prototype modified for mouse injection, PharmaJet) under 1-5% isoflurane anesthesia at week 0 and 3. Dose was 42µg/100µL.

Blood collection

- 15 Blood was collected into clot activator tubes via retro-orbital capillary tube collection under 1-5% isoflurane anesthesia at 200 µL per collection in week 0 (prior to dose) and 3 (prior to second dose), and terminal cardiac puncture (500 µL) in week 6. All blood samples were allowed to clot at room temperature, centrifuged ambient (20°C) at 3000 RPM for 15 minutes, and serum supernatant was stored frozen at -80 °C.

Anti-NiV G antibody detection enzyme-linked immunosorbent assay (ELISA).

- 20 Serum samples of week 0, 3 and 6 were analyzed with anti-NiV G antibody detection ELISA. The sandwich ELISA detects any antibodies directed to the NiV glycoprotein G. The ELISA has four key components: a nickel chelate-coated 96-well plate (Pierce ThermoFisher, Cat #15442), a NiV G protein (Malaysia strain residues 71-602, Genbank AF212302)
 25 produced in HEK293 cells with polyhistidine tag at the N-terminus used for plate capture (Acro Biosystems, Cat #GLN-N52H3), a horseradish peroxidase (HRP) conjugated goat anti-mouse immunoglobulin G fragment crystallizable region (IgG Fc) secondary antibody (ThermoFisher, Cat #SA510276), and a mouse monoclonal anti-NiV G antibody (JB3) (The Native Antigen, Cat #MAB12308) used as positive control.

- 30 First, the nickel chelate-coated plate was pre-washed. The plate was then be coated with NiV G protein by adding 0.5µg of polyhistidine-tagged NiV G per well and incubating with shaking for 1 hour at room temperature (20-25°C). After washing steps, the positive control with a series of dilutions, the negative control (phosphate buffered saline, PBS) and the 30 serum samples were added in duplicate to the wells and incubated for 1 hour at room

temperature. After washing steps antibodies in mouse sera targeting NiV G remained bound to the plate. The HRP-conjugated secondary anti-mouse IgG Fc antibody was added to detect the mouse antibodies on the plate and incubated for 1 hour at room temperature. After washing steps 3,3',5,5'-tetramethylbenzidine (TMB) solution was added and the plate was
5 incubated in dark at 20°C for 15 min. The reaction was quenched by adding 0.16M sulfuric acid and the final solution read immediately at 450 nm in a microtiter plate reader. The absorbance of the sample was dependent on the concentration of anti-NiV G antibodies in the sample as indicated by the positive control curve.

Neutralization assays

10 NiV is a highly pathogenic virus with a high case fatality rate and its study requires biosafety level 4 (BSL4) containment. We used a novel neutralization assay developed for use in biosafety level 2 laboratories. The assay uses a recombinant vesicular stomatitis virus (VSV) expressing NiV glycoprotein G and the fluorescent protein ZsGreenTM (ZsG). The recombinant virus propagates as a replication-competent virus in a cell line (Vero-Fco)
15 constitutively expressing NiV fusion (F) protein. This system was used to evaluate the neutralization activity of serum from immunized mice as a surrogate for using pathogenic NiV. Sera from all 10 mice collected at week 0, 3 and 6 were tested with this neutralization assay to determine 50% effective inhibition concentration (IC₅₀) values.

Vero-Fco cells were created with a codon-optimized sequence encoding the F protein
20 of NiV strain Malaysia (Genbank AF212302) inserted into the cell genome using the Flp-InTM system (Invitrogen). Continuing expression of NiV F protein in Vero-Fco cells was verified by immunofluorescence assays (IFA) performed on non-infected Vero-Fco and Vero-E6 cells using anti-NiV F protein antibody (12B2) and representative IFA images (magnification 10x) of non-infected Vero-Fco and Vero-E6 cells collected using EVOS
25 microscope.

The recombinant rVSVΔG-NiV-G virus contains ZsGreenTM (ZsG) fluorescent protein fused to VSV nucleoprotein (N) via a porcine teschovirus-1 2A (P2A), VSV phosphoprotein (P), VSV matrix protein (M), NiV glycoprotein G, and VSV-polymerase (L). Plasmids encoding VSVΔG-NiV-G, VSV-L, VSV-N, VSV-P, and VSV-G were co-
30 transfected into Huh-7 cells to rescue rVSVΔG-NiV-G. The supernatant was blind-passaged onto Vero-Fco cells and the resulting virus stock titrated using TCID₅₀ determination method and sequenced by next-generation sequencing. Replication of recombinant virus in Vero-Fco cells was verified with representative images (magnification 10x, EVOS microscope) of Vero-Fco infected with rVSVΔG-NiV-G or mock infected.

For the neutralization assay, 10,000 cells/well were seeded onto 96-well plates one day prior to the experiment. Serial 5-fold dilutions of mouse serum samples were mixed with 100 TCID₅₀ of rVSVΔG-NiV-G. After the virus-sample mixture incubation for 1 h at 37°C, it was added to Vero-Fco monolayers in triplicate. Cells were incubated for 72 h at 37°C and the fluorescence signal was measured using a plate reader (BioTek). Data were analyzed using GraphPad Prism™ v9 (GraphPad Software). Fluorescence values of ZsG expression were normalized to fluorescence in non-infected cells. The resulting normalized values were used to fit a 4-parameter equation to semi-log plots of the concentration-response data. Dilution of the sera samples inhibiting 50% of ZsG™ expression (IC₅₀) was calculated by interpolating the values.

ELISA results

The sandwich ELISA detected any antibodies targeting the Nipah virus G glycoprotein (Malaysia strain). A positive control mouse anti-NiV G monoclonal antibody indicated a sensitivity of the assay below 1 ng/ml of antibody and optical density (OD) a linear function of antibody concentration in the 5-100 ng/ml range (Fig. 1). In all 10 mice immunized with VX4004R2 vaccine a high concentration of antibodies (>100 ng/ml) targeting NiV G was detected in week 3 and week 6 sera, with OD = 4.0 in all week 3 and week 6 mouse sera samples at volume dilution ratios of 1:10, 1:100, 1:1000 and 1:5000, and in 10/10 week 6 and 9/10 week 3 mouse sera at a volume dilution ratio of 1:10000. At a volume dilution ratio of 1:50000 OD results were significantly different between week 3 (mean 1.39, sd 0.46) and week 6 (mean 3.79, sd 0.33) mouse sera (Fig. 2), this difference was equivalent to a 15-fold serum dilution according to the positive control curve. At a volume dilution ratio of 1:100000 all week 6 sera were in the linear part of the positive control curve with OD ranging from 1.33 to 3.57 (mean 2.54, sd 0.72) (Fig. 3) and the extrapolated average concentration of anti-NiV G antibodies in mouse sera was 2 mg/ml.

Neutralization assay results

In all 10 mice immunized with VX4004R2 week 3 and week 6 sera neutralized rVSV-ΔG-NiV G in a dose-dependent manner (Fig. 4B-F, Fig. 5 and Tables 3-4). All 10/10 week 6 sera samples and 7/10 week 3 sera samples neutralized the pseudotyped virus in an efficient manner (Fig. 4B-F and Tables 3-4). 50% effective inhibition concentration (IC₅₀) values of mouse sera against rVSVΔG-NiV-G at week 6 ranged from 1120 to 5302 (mean 1984, sd 1310) (Table 3). IC₅₀ values of mouse sera against rVSVΔG-NiV-G at week 6 were on

average more than 4-fold IC₅₀ value of the serum of a Syrian hamster (*Mesocricetus auratus*) surviving Nipah virus infection used as a positive control, with a range from more than 2-fold to more than 11-fold. As expected, no neutralization activity, even at lower dilutions, was observed in mouse sera samples collected at week 0 prior to immunization (Fig. 4A, Fig. 5 and Table 2). For 7/10 mouse sera at week 3 50% effective inhibition concentration (IC₅₀) ranged from 117 to 546 (mean 249, sd 144) and concentration-response curves in Vero-Fco cells were comparable to the positive control serum curve (Fig. 4B-D and Table 3), indicating efficient neutralization activity 3 weeks after the first dose and prior to the second dose.

10

Serum #	IC ₅₀ (dilution factor)
Week 0-1	0
Week 0-2	0
Week 0-3	0
Positive Control	465

Table 2. 50% effective inhibition concentration (IC₅₀) values of each indicated mouse serum at week 0 and IC₅₀ value of positive control against rVSVΔG-NiV-G. The positive control is serum of a hamster that survived a Nipah virus challenge.

15

Serum #	IC ₅₀ (dilution factor)
Week 3-1	60
Week 3-2	18
Week 3-3	117
Week 3-4	52
Week 3-5	230
Week 3-6	139
Week 3-7	249
Week 3-8	546
Week 3-9	174
Week 3-10	287

Table 3. 50% effective inhibition concentration (IC₅₀) values of each indicated mouse serum at week 3 against rVSVΔG-NiV-G.

20

Serum #	IC ₅₀ (dilution factor)
Week 6-1	5302

Week 6-2	1424
Week 6-3	1471
Week 6-4	1418
Week 6-5	1399
Week 6-6	1394
Week 6-7	1120
Week 6-8	1555
Week 6-9	1485
Week 6-10	3276

Table 4. 50% effective inhibition concentration (IC_{50}) values of each indicated mouse serum at week 6 against rVSV Δ G-NiV-G.

I claim

1. An isolated polypeptide comprising:
 - (a) an attachment glycoprotein G head domain (G HD) comprising an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID NO:1-2, optionally wherein the head domain comprises the amino acid sequence of SEQ ID NO:1, wherein at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or all 20 of the residues bounded by parentheses is the first listed residue; and
 - (b) a multimerization domain capable of generating multimers comprising 2 to 60 copies of the isolated polypeptide.
2. The isolated polypeptide of claim 1, wherein the multimerization domain generates 4 copies or 60 copies of the isolated polypeptide.
3. The isolated polypeptide of claim 1 or 2, wherein the multimerization domain comprises an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO:3-4, optionally wherein the multimerization domain comprises the amino acid sequence of SEQ ID NO:3, wherein at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 or all 18 of the residues bounded by parentheses is the first listed residue.
4. The isolated polypeptide of any one of claims 1-3, further comprising an amino acid linker between the head domain and the multimerization domain.
5. The isolated polypeptide of claim 4, wherein the linker comprises or consists of (GGS)_nGGG, wherein n is 3-11, 4-11, 5-11, 6-11, 7-11, 8-11, 9-11, 10-11, 3-10, 4-10, 5-10, 6-10, 7-10, 8-10, 9-10, 3-9, 4-9, 5-9, 6-9, 7-9, 8-9, 3-8, 4-8, 5-8, 6-8, 7-8, 3, 4, 5, 6, 7, 8, 9, 10 or 11; optionally wherein n is 8-11, 9-11, 10-11, 8-10, 9-10, 8-9, 8, 9, 10, or 11.
6. The isolated polypeptide of any one of claims 1-5, wherein the head domain is carboxy-terminal to the multimerization domain.
7. The isolated polypeptide of any one of claims 1-6 comprising an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or

100% identical to the amino acid sequence selected from the group consisting of SEQ ID NO:5-6, wherein n is 3-11, 4-11, 5-11, 6-11, 7-11, 8-11, 9-11, 10-11, 3-10, 4-10, 5-10, 6-10, 7-10, 8-10, 9-10, 3-9, 4-9, 5-9, 6-9, 7-9, 8-9, 3-8, 4-8, 5-8, 6-8, 7-8, 3, 4, 5, 6, 7, 8, 9, 10 or 11; optionally wherein n is 8-11, 9-11, 10-11, 8-10, 9-10, 8-9, 8, 9, 10, or 11.

5

8. The isolated polypeptide of any one of claims 1-7 comprising an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID NO:7-10 and 18.

10 9. A multimer comprising 60 or more copies of a head domain (HD) comprising an amino acid sequence at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of any one of SEQ ID NO:1-2.

15 10. The multimer of claim 9, comprising 60 or more copies of the isolated polypeptide of any one of claims 1-8.

11. A scaffold, comprising 60 or more isolated polypeptides of any one of claims 1-8 on a surface of the scaffold.

20

12. The scaffold of claim 11, wherein the isolated polypeptides are all identical polypeptides, or wherein the isolated polypeptides include different polypeptides.

13. A nucleic acid encoding the isolated polypeptide of any one of claims 1-8.

25

14. A recombinant expression vector comprising the nucleic acid of claim 13 operatively linked to a suitable control sequence.

15. A recombinant host cell comprising the polypeptide, the multimer, the scaffold, the
30 nucleic acid, and/or the recombinant expression vector of any claim herein.

16. The nucleic acid of claim 13 wherein the nucleic acid comprises mRNA.

17. The nucleic acid of claim 16, wherein the mRNA comprises:

- (a) a 5' cap;
- (b) a poly(A) tail of between 50 and 120 contiguous adenosine residues;
- (c) a 5' untranslated region comprising the nucleic acid sequence of SEQ ID NO:12 or 13;
- 5 (d) a 3' untranslated region comprising one or two copies of a beta globin mRNA 3'-UTR, optionally wherein the beta globin mRNA 3'-UTR comprises the nucleic acid sequence of SEQ ID NO:11; and/or
- (e) a signal sequence, optionally wherein the signal sequence is at the N-terminus of the encoded polypeptide, and optionally wherein the signal sequence comprises the amino
- 10 acid sequence of SEQ ID NO:14.
18. The nucleic acid of claim 16, wherein the mRNA comprises:
- (a) a 5' cap;
- (b) a poly(A) tail of between 50 and 120 contiguous adenosine residues;
- 15 (c) a 5' untranslated region comprising the nucleic acid sequence of SEQ ID NO:12 or 13;
- (d) a 3' untranslated region comprising one or two copies of a beta globin mRNA 3'-UTR, wherein the beta globin mRNA 3'-UTR comprises the nucleic acid sequence of SEQ ID NO:11; and
- 20 (e) a signal sequence at the N-terminus of the encoded polypeptide, comprising the amino acid sequence of SEQ ID NO:14.
19. A composition comprising a plurality of mRNA that encode 2, 3, or 4 polypeptides comprising an amino acid sequence at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%,
- 25 98%, 99%, or 100% identical to the amino acid sequence selected from the group consisting of SEQ ID NO:5-10 and 18.
20. A pharmaceutical composition comprising
- (a) the polypeptide, the multimer, the scaffold, the nucleic acid, the recombinant
- 30 expression vector, and/or the cell of an preceding claim; and
- (b) a pharmaceutically acceptable carrier.
21. The pharmaceutical composition of claim 20, comprising the pharmaceutically acceptable carrier and:

- (a) the isolated polypeptide of SEQ ID NO:5; or
 (b) the isolated polypeptide of SEQ ID NO:6; or
 (c) the isolated polypeptide of SEQ ID NO:7; or
 (d) the isolated polypeptide of SEQ ID NO:8; or
 5 (e) the isolated polypeptide of SEQ ID NO:9; or
 (f) the isolated polypeptide of SEQ ID NO:10; or
 (g) the isolated polypeptide of SEQ ID NO:18;
 or multimers or scaffolds thereof.
- 10 22. The pharmaceutical composition of claim 20, comprising:
 (a) the mRNA of any one of claims 16-18, or the composition of claim 19; and
 (b) a cationic lipid carrier, such as a liposome, or a cationic protein, such as
 protamine.
- 15 23. The pharmaceutical composition of claim 22, wherein the mRNA encodes:
 (a) the isolated polypeptide of SEQ ID NO:5;
 (b) the isolated polypeptide of SEQ ID NO:6;
 (c) the isolated polypeptide of SEQ ID NO:7;
 (d) the isolated polypeptide of SEQ ID NO:8;
 20 (e) the isolated polypeptide of SEQ ID NO:9;
 (f) the isolated polypeptide of SEQ ID NO:10; and/or
 (g) the isolated polypeptide of SEQ ID NO:18.
24. A method for treating a henipavirus infection, comprising administering to a subject
 25 infected with a henipavirus an amount effective to treat the infection of the polypeptide, the
 multimer, the scaffold, the nucleic acid, the recombinant expression vector, the cell, and/or
 the pharmaceutical composition of any claim herein.
25. A method for limiting development of a henipavirus infection, comprising
 30 administering to a subject at risk of henipavirus infection an amount effective to limit
 development of a henipavirus infection of the polypeptide, the multimer, the scaffold, the
 nucleic acid, the recombinant expression vector, the cell, and/or the pharmaceutical
 composition of any claim herein.

26. A method for generating an immune response in a subject, comprising administering to the subject an amount effective to generate an immune response of the polypeptide, the multimer, the scaffold, the nucleic acid, the recombinant expression vector, the cell, and/or the pharmaceutical composition of any claim herein.

5

27. The method of any one of claims 25-26, wherein the method comprises administering to the subject an amount effective of the pharmaceutical composition of claim 22 or 23 by subcutaneous, intradermal or intramuscular injection, optionally administering with a needle-free injection system.

10

28. A method for monitoring a henipavirus-induced disease in a subject and/or monitoring response of the subject to immunization by a henipavirus vaccine, comprising contacting the polypeptide, the multimer, the scaffold, the nucleic acid, the recombinant expression vector, the cell, and/or the pharmaceutical composition of any claim herein with a
15 bodily fluid from the subject and detecting henipavirus-binding antibodies in the bodily fluid of the subject.

29. The method of claim 28, wherein the bodily fluid comprises serum or whole blood.

20

30. A method for detecting henipavirus binding antibodies, comprising
(a) contacting the polypeptide, the multimer, the scaffold, and/or the pharmaceutical composition of any claim herein with a composition comprising a candidate henipavirus binding antibody under conditions suitable for binding of henipavirus antibodies to the polypeptide, the multimer, the scaffold, and/or the pharmaceutical composition; and

25

(b) detecting henipavirus antibody complexes with the polypeptide, the multimer, the scaffold, and/or the pharmaceutical composition.

31. The method of claim 30, further comprising isolating the henipavirus antibodies.

30

32. A method for producing henipavirus antibodies, comprising
(a) administering to a subject an amount effective to generate an antibody response of the polypeptide, the multimer, the scaffold, the nucleic acid, the recombinant expression vector, the cell, and/or the pharmaceutical composition of any claim herein; and
(b) isolating antibodies produced by the subject.