

Engineered influenza neuraminidase antigens

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Cross Reference

This application claims priority to U.S. Provisional Patent Application Serial No. 62/986295 filed March 6, 2020, incorporated by reference herein in its entirety.

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Background

Influenza viruses claim countless lives and pose a significant public health and economic burden globally every year. The development of highly effective vaccines to ever-evolving influenza viruses has been a major public health priority. There are two major viral glycoproteins on the influenza virion, the hemagglutinin (HA) and the neuraminidase (NA), which facilitate viral entry and egress from host cells, respectively. NA is a homotetrameric, type II integral membrane protein, the N terminus of which is oriented towards the interior of the virus. The C-terminal globular head domain of NA contains six topologically identical beta sheets arranged in a propeller-like structure, comprises the discontinuous catalytic site, and is supported by a stalk domain. NA cleaves terminal sialic acid from glycans on the host cell surface, thereby releasing nascent viral particles. Additionally, it is believed that NA might play a role in viral entry. These characteristics make NA an attractive target as a vaccine immunogen, yet NA has historically been neglected in vaccine development.

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Summary

In one aspect, the disclosure provides non-naturally occurring mutant neuraminidase (NA) polypeptides that improve expression and/or modifies the open/closed tetrameric conformational state of the NA polypeptide. In one embodiment, wherein the polypeptide is

30 (a) at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the N1 NA polypeptide of SEQ ID NO:1, and wherein the non-naturally occurring polypeptide includes 1, 2, 3, 4, 5, 6, or all 7 of the following amino acid residues relative to SEQ ID NO:1 when aligned by protocol 1 or protocol 2:

161T/A, 105A, 165T/I, 166P, 196Q, 203Y, 444V; or

35 (b) at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the N1 NA polypeptide of SEQ ID NO:1, and wherein the non-naturally

occurring polypeptide includes 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, or all 17 of the following amino acid residues relative to SEQ ID NO:1 when aligned by protocol 1 or protocol 2:

- 161T/A/V/S/T, 100L, 408M, 419V, 99P, 103N, 105A, 131Q/M, 163I/L, 165T/S/V/A/I,**
 5 **166P, 177I, 196Q/T, 203Y, 205I, 442I, 444V.**

In another embodiment, the polypeptide is

- (a) at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%,
 or 99% identical to the N2 NA polypeptide of SEQ ID NO:2, and wherein the non-naturally
 occurring polypeptide includes 1, 2, 3, 4, 5, 6, 7, or all 8 of the following amino acid residues
 10 relative to SEQ ID NO:2 when aligned by protocol 1 or protocol 2:
 101C, 131M, 162P, 165S/T, 166V, 195Q, 202Y, 443S; or

- (b) at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%,
 or 99% identical to the N2 NA polypeptide of SEQ ID NO:2, and wherein the non-naturally
 occurring polypeptide includes 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or all 12 of the following amino
 15 acid residues relative to SEQ ID NO:2 when aligned by protocol 1 or protocol 2:
 101C/A, 103N, 105A, 106V, 131M, 162P, 163I, 165S/T/A/I, 166V, 195Q, 202Y, 443S.

In another embodiment, the polypeptide is

- (a) at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%,
 or 99% identical to the N3 NA polypeptide of SEQ ID NO:3, and wherein the non-naturally
 20 occurring polypeptide includes 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or all 11 of the following amino
 acid residues relative to SEQ ID NO:3 when aligned by protocol 1 or protocol 2:
 103N, 105S, 131Q/M, 157T, 165S/I, 166P, 196Q, 203Y, 205I, 443S, 445V; or

- (b) at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%,
 or 99% identical to the N3 NA polypeptide of SEQ ID NO:3, and wherein the non-naturally
 25 occurring polypeptide includes 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or all 13 of the following
 amino acid residues relative to SEQ ID NO:1 when aligned by protocol 1 or protocol 2:
 103N, 105S, 106V, 131Q/M, 157T, 163L, 165S/I/V/A, 166P/V, 196Q, 203Y, 205I, 443S,
 445V.

In a further embodiment, the polypeptide is

- (a) at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%,
 30 or 99% identical to the N4 NA polypeptide of SEQ ID NO:4, and wherein the non-naturally
 occurring polypeptide includes 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or all 16 of the
 following amino acid residues relative to SEQ ID NO:4 when aligned by protocol 1 or
 protocol 2:

160V/S/T/A, 409M, 102N, 104S/A, 105V, 112D, 130Q/M, 162L, 164S/T/A/I, 165P, 176I, 195Q, 202Y, 204I, 443I, 445V; or

- (b) at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the N4 NA polypeptide of SEQ ID NO:4, and wherein the non-naturally occurring polypeptide includes 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or all 16 of the following amino acid residues relative to SEQ ID NO:4 when aligned by protocol 1 or protocol 2:

160V/S/T/A, 409M, 102N, 104S/A, 105V, 112D, 130Q/M, 162L, 164S/T/A/I, 165P/V, 176I, 195Q, 202Y, 204I, 443I, 445V.

- 10 In one embodiment, the polypeptide is

- (a) at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the N5 NA polypeptide of SEQ ID NO:5, and wherein the non-naturally occurring polypeptide includes 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or all 13 of the following amino acid residues relative to SEQ ID NO:5 when aligned by protocol 1 or protocol 2:

- 15 **97L, 410M, 96P, 98A, 100N, 102S/A, 110D, 128Q/M, 160I, 162V/A/I, 163V/P, 193Q/T, 445I;** or

- (b) at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the N5 NA polypeptide of SEQ ID NO:5, and wherein the non-naturally occurring polypeptide includes 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or all 16 of the following amino acid residues relative to SEQ ID NO:5 when aligned by protocol 1 or protocol 2:

97L, 410M, 96P, 98A, 100N, 102S/A, 103V, 110D, 128Q/M, 154T, 160I, 162V/A/I/T, 163V/P, 174I, 193Q/T, 445I.

In a further embodiment, the polypeptide is

- 25 (a) at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the N6 NA polypeptide of SEQ ID NO:6, and wherein the non-naturally occurring polypeptide includes 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or all 11 of the following amino acid residues relative to SEQ ID NO:6 when aligned by protocol 1 or protocol 2:

99P, 103N, 113D, 131Q, 161I, 162P, 163I, 165S/T/V/A, 196Q, 203Y, 445S; or

- 30 (b) at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the N6 NA polypeptide of SEQ ID NO:6, and wherein the non-naturally occurring polypeptide includes 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or all 16 of the following amino acid residues relative to SEQ ID NO:6 when aligned by protocol 1 or protocol 2:

99P, 103N, 105S, 106V, 113D, 131Q, 157T, 161I, 162P, 163I/L, 165S/T/V/A/I, 166V/P, 196Q, 203Y, 445S.

In one embodiment, the polypeptide is

- (a) at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%,
5 or 99% identical to the N7 NA polypeptide of SEQ ID NO:7, and wherein the non-naturally occurring polypeptide includes 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or all 11 of the following amino acid residues relative to SEQ ID NO:7 when aligned by protocol 1 or protocol 2:

98P, 102N, 104S, 112D, 130Q, 156T, 162I, 164V/A/I, 165V, 202Y, 448V; or

- (b) at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%,
10 or 99% identical to the N7 NA polypeptide of SEQ ID NO:7, and wherein the non-naturally occurring polypeptide includes 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or all 13 of the following amino acid residues relative to SEQ ID NO:7 when aligned by protocol 1 or protocol 2:

98P, 102N, 104S, 112D, 130Q, 156T, 162I, 164V/A/I, 165V, 176I, 202Y, 446I, 448V.

In another embodiment, the polypeptide is

- (a) at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%,
15 or 99% identical to the N8 NA polypeptide of SEQ ID NO:8, and wherein the non-naturally occurring polypeptide includes 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or all 11 of the following amino acid residues relative to SEQ ID NO:8 when aligned by protocol 1 or protocol 2:

408M, 101N, 103A/S, 111D, 129Q, 161P/L, 163S/T/V/A/I, 164V, 194Q, 201Y, 442I; or

- (b) at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%,
20 or 99% identical to the N8 NA polypeptide of SEQ ID NO:8, and wherein the non-naturally occurring polypeptide includes 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or all 15 of the following amino acid residues relative to SEQ ID NO:8 when aligned by protocol 1 or protocol 2:

- 25 **98L**, **408M**, 101N, 103A/S, 104V, 111D, 129Q/M, 161P/L, 163S/T/V/A/I/S/T, 164V/P, 175I, 194Q, 201Y, 203I, 442I.

In one embodiment, the polypeptide is

- (a) at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%,
30 or 99% identical to the N9 NA polypeptide of SEQ ID NO:9, and wherein the non-naturally occurring polypeptide includes 1, 2, 3, 4, 5, 6, 7, 8, 9, or all 10 of the following amino acid residues relative to SEQ ID NO:9 when aligned by protocol 1 or protocol 2:

94P, 95L, 100S, 126Q/M, 160V/A/I, 161V, 191Q, 198Y, 439S, 441V; or

- (b) at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%,
or 99% identical to the N9 NA polypeptide of SEQ ID NO:9, and wherein the non-naturally

occurring polypeptide includes 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, or all 14 of the following amino acid residues relative to SEQ ID NO:9 when aligned by protocol 1 or protocol 2:

94P, 95L, 98N, 100S/A, 126Q/M, 152T, 158I, 160V/A/I/T, 161V, 191Q, 198Y, 200I, 439S, 441V.

- 5 In one embodiment, the polypeptide is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the N9 NA polypeptide of SEQ ID NO:9, and wherein the non-naturally occurring polypeptide includes a 160Q amino acid mutation relative to SEQ ID NO:9 when aligned by protocol 1, or may include a combination of 160Q/E and 172V amino acid residues relative to SEQ ID NO:9 when aligned by protocol
10 1 or protocol 2.

In a further embodiment, the disclosure provides compositions, comprising one or more of the polypeptides of any embodiment linked to a scaffold. In one embodiment, the scaffold comprises a protein scaffold. In a further embodiment, the polypeptide is covalently linked to a protein subunit of the protein scaffold to form a fusion protein.

- 15 In various further aspects, the disclosure provides nucleic acids encoding polypeptides or fusion proteins of the disclosure; expression vector comprising the nucleic acids of the disclosure operatively linked to a suitable control sequence; host cells comprising nucleic acids, expression vectors, and/or polypeptide or fusion proteins of the disclosure, and pharmaceutical compositions or vaccines, comprising

- 20 (a) one or more of the polypeptides, composition, nucleic acid, expression vector, and/or the host cell of the disclosure; and
(b) a pharmaceutically acceptable carrier.

- In another aspect, the disclosure provides methods for treating or limiting development of an influenza infection, comprising administering to a subject in need thereof
25 an amount effective to treat or limit development of the influenza infection of a polypeptide, fusion protein, composition, vaccine, nucleic acid, expression vector, host cell, pharmaceutical composition, and/or vaccine of any preceding claim.

Description of the Figures

- 30 **Figure 1(A-B).** Overview of Influenza Neuraminidase design and expression of soluble NA protein. (A) Schematic representation (H1N1 California 09pdm) of (1) wild type (WT) and two recombinant NA designs (2 and 3), that vary in amino acid length. The stalk domain of the WT was replaced by a 6xHis-tag, a hVSAP domain for protein tetramerization

(TD), a thrombin site and a GG linker. (B) Gel filtration chromatograms of His-purified H1N1 California 09pdm recorded at 280 nm wavelength.

Figure 2. Characterization of NA subtypes. Different subtype NAs differentially adapt to open and closed tetramers: A/California/07/2009 H1N1, A/New Caledonia/20/1999 H1N1, A/Michigan/45/2015 H1N1, A/WSN/1933 H1N1 and A/Sichuan/26221/2014 H5N6 are open. A/Netherlands/219/2003 H7N7, A/Jiangxi-Donghu/346-2/2013 H10N8 and A/Anhui/1/2013 H7N9 are mixed open/closed. A/Wisconsin/67/2005 H3N2, A/Swine/Missouri/2124514/2006 H2N3, A/Red knot/Delaware Bay/310/2016 H10N4 and A/Shorebird/Delaware Bay/309/2016 H10N5 are closed.

Figure 3. Design process of A/California/07/2009 H1N1 N1 neuraminidase, from loose to tight tetramers. Design 94 (SEQ ID NO:79) with ten different mutations resulted in 45% tight tetramer particles. Design 113 (SEQ ID NO:81) with 11 different mutations resulted in 80-90% tight tetramer particles. Design 155 (SEQ ID NO:13) with ten different mutations resulted in 90-100% tight tetramer particles.

Figure 4. Design process of A/Michigan/45/2015 H1N1 N1 neuraminidase, from loose to tight tetramers based on A/California/07/2009 H1N1 N1 design. Design 300 (SEQ ID NO:14) with ten mutations based on design 155 resulted in 75% tight tetramer particles. Design 174 (SEQ ID NO:18) with one additional mutation resulted in 100% tight tetramer particles. Three other distinct solutions for stabilizing mutations were found as well.

Figure 5. Design process of A/WSN/1933 H1N1 N1 neuraminidase, from loose to tight tetramers based on A/California/07/2009 H1N1 N1 design c155. Design 366 (SEQ ID NO:14) with 16 different mutations resulted in 80% tight tetramer particles. Ten of those mutation are the same mutations as in design c155 (SEQ ID NO:13).

Figure 6. Design process of A/Vietnam/1203/04 H5N1 N1 neuraminidase, from loose to tight tetramers based on A/California/07/2009 H1N1 N1 design c155. Design 348 resembles same mutations as design 155. Design 354 (SEQ ID NO:40) with 14 different mutations resulted in 100% tight tetramer particles. Ten of those mutation are the same mutations as in design c155.

Figure 7. Design process of A/Jiangxi-Donghu/346-2/2013 H10N8 N8 neuraminidase, from loose to tight tetramers based on A/California/07/2009 H1N1 N1 design. Design 285 (SEQ ID NO:36) with two space D mutations resulted in 100% tight tetramer particles.

Figure 8. Design process of A/Wisconsin/67/2005 H3N2 N2 neuraminidase, to destabilize the tight tetrameric particles based on A/California/07/2009 H1N1 N1 design.

Introducing two mutations in design 157 did not result in an open construct. Adding one additional mutation 165Q resulted in 0% tight tetramer particles with some unassembled subunits.

- Figure 9. BLASTp alignment of SEQ ID NO:1 with other N1 strain sequences that contain deletions or insertions.** BLASTp alignment allows for residue positions of reference strains to be matched with corresponding residue positions in strains from the same subtype, even when sequences contain arbitrary numbers of insertions and deletions relative to the reference strain.

10 Detailed Description

All references cited are herein incorporated by reference in their entirety. Within this application, unless otherwise stated, the techniques utilized may be found in any of several well-known references such as: *Molecular Cloning: A Laboratory Manual* (Sambrook, et al., 1989, Cold Spring Harbor Laboratory Press), *Gene Expression Technology* (Methods in Enzymology, Vol. 185, edited by D. Goeddel, 1991. Academic Press, San Diego, CA), "Guide to Protein Purification" in *Methods in Enzymology* (M.P. Deutscher, ed., (1990) Academic Press, Inc.); *PCR Protocols: A Guide to Methods and Applications* (Innis, et al. 1990. Academic Press, San Diego, CA), *Culture of Animal Cells: A Manual of Basic Technique, 2nd Ed.* (R.I. Freshney. 1987. Liss, Inc. New York, NY), *Gene Transfer and Expression Protocols*, pp. 109-128, ed. E.J. Murray, The Humana Press Inc., Clifton, N.J.), and the Ambion 1998 Catalog (Ambion, Austin, TX).

As used herein, the singular forms "a", "an" and "the" include plural referents unless the context clearly dictates otherwise.

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).

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.

5 The disclosure provides non-naturally occurring mutant neuraminidase (NA) polypeptides that improves expression and/or modifies the open/closed tetrameric conformational state of the NA polypeptide. As detailed in the examples that follow, the inventors have produced recombinant NA polypeptides in which head domains comprising stabilizing mutations are connected to tetramerization domains. We initially found that many
10 wild-type sequences of beta propeller head domains from certain NA subtypes adopted “open” conformations in which the head domains extended individually off of the stalk-like tetramerization domain, without forming the crystallographically observed “closed” tetramer. Constructs comprising the head domains from other NA subtypes formed closed tetramers naturally. Similar constructs from yet other subtypes formed mixtures of open and closed
15 tetramers. We identified specific mutations at multiple locations in NA sequences that dictate the open or closed conformational state of NA tetramers, and used these mutations to generate closed, stabilized tetramers from multiple NA subtypes. We also converted a naturally closed NA tetramer to a fully open conformation by substituting residues that we identified as pivotal for tetramer closure, confirming the importance of these residues for
20 determining the conformational state of NA. Monoclonal antibodies (mAbs) that bind across the interface of two neighboring protomers in the closed configuration bind better to closed tetramers than open tetramers. The NA polypeptides of the disclosure are improved vaccine antigens in either soluble form or when presented on scaffolds.

In a first aspect, the NA polypeptides are:

25 (a) at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the N1 NA polypeptide of SEQ ID NO:1, wherein the non-naturally occurring polypeptide includes 1, 2, 3, 4, 5, 6, or all 7 of the following amino acid residues relative to SEQ ID NO:1 when aligned by protocol 1 or protocol 2:

161T/A, 105A, 165T/I, 166P, 196Q, 203Y, 444V ; or

30 (b) at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the N1 NA polypeptide of SEQ ID NO:1, wherein the non-naturally occurring polypeptide includes 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, or all 17 of the following amino acid residues relative to SEQ ID NO:1 when aligned by protocol 1 or protocol 2:

161T/A/V/S/T, 100L, 408M, 419V, 99P, 103N, 105A, 131Q/M, 163I/L, 165T/S/V/A/I, 166P, 177I, 196Q/T, 203Y, 205I, 442I, 444V.

In this first aspect, the N1 NA reference sequence is based on N1 reference strain A/California/07/2009 from H1N1.

5 MNPNQKIITIGSVCMTIGMANLILQIGNIISIWISHSIQLGNQNQIETCNQSVITYENNTWVNQ
 TYVNISNTNFAAGQSVVS**VKLAGNSSLCPVSGWAIYSKDNSVRIGSKGDVVFVIREPFISCSPLE**
CRTFFLTQGALLNDKHSNGTIKDRSPYRTLMSCPIGEVPSFYNSRFESVAWSASACHDGINWLT
IGISGPDNGAVAVLKYNIGIITDTIKSWRNNILRTQSEACVNGSCFTVMTDGPNSNGQASYKIF
RIEKGKIVKSVEMNAPNYHYEECSYCPDSSEITCVCRDNWHGSRNPWVSFNQNLLEYQIGYICSG
 10 **IFGDNPRPNDKTGSCGPVSSNGANGVKGFSEFKYGNVWIGRTKSISSRNGFEMIWDPNGWTGTD**
NNFSIKQDIVGINEWSGYSGSFVQHPELTGLDCIRPCFWVELIRGRPKENTIWTSGSSISFCGV
NSDTVGWSWPDGAELPFTIDK (SEQ ID NO:1), wherein the bold region is the head
 region of the N1 HA protein

15 As used throughout this application (for all NA subtypes), "Protocol 1" and "Protocol 2" both permit alignment of polypeptide against the reference sequence (SEQ ID NO:1 in the above embodiment; SEQ ID NOs:2-9 in embodiments described below), taking insertions and deletions into account. Thus, the percent identity requirement is based on alignment with the reference sequence while discounting insertions or deletions relative to the reference
 20 polypeptide.

Protocol 1

1. To run a BLASTp alignment online, use the National Center for Biotechnology Information (NCBI) server (or see the article
 25 <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC146917/>).
 a. <https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE=Proteins>
2. Set up a BLAST alignment using the following settings:
 - Use the option for "Align two or more sequences"
 - Enter the subtype-specific reference strain sequence for the relevant NA
 30 subtype into the "Enter Query Sequence" section
 - Enter any sequence of the same NA subtype into the "Enter Subject Sequence" section
 - Algorithm: blastp (protein-protein BLAST)
 - Expect threshold: 0.1

- Word size: 6
- Max matches in a query range: 0
- Matrix: BLOSUM62
- Gap costs:
 - 5 -Existence: 11
 - Extension: 1
- Filter low complexity regions?: No
- Mask:
 - For lookup table only?: No
 - 10 -Lower case letters?: No
- 3. Run the analysis by clicking the "BLAST" button
- 4. Click on the "Alignments" tab to show the alignment between the two sequences
- 5. For each sequence position of interest, identify the number according to the "Query" sequence. Then identify the corresponding residue position in the "Sbjct" sequence that has been aligned with the position of the "Query" sequence.

Protocol 2

1. To run a protein BLASTp alignment on a personal computer or server, install BLAST for command line execution or identify a computer or server that already has it installed.
 - a. Directions for installation can be found in the user manual: "BLAST® Command Line Applications User Manual", National Center for Biotechnology Information (US). Bethesda, MD. 2008. <https://www.ncbi.nlm.nih.gov/books/NBK279690/>
2. Generate a file in FASTA format that contains the desired subtype-specific reference strain for the relevant NA subtype. In the command below, this file will be named "query.fasta".
3. Generate a second file in FASTA format that contains an NA sequence of interest from the same subtype. In the command below, this file will be named "sbjct.fasta".
4. Execute the following command using a program such as Terminal, iTerm2, Windows Console, Linux console or other similar terminal emulators. This will generate results in a file named "results.txt".

```
blastp -query query.fasta -subject sbjct.fasta -matrix BLOSUM62 -evalue
0.1 -word_size 6 -gapopen 11 -gapextend 1 -out results.txt
```

5. Open results.txt and view the section showing alignment of the two sequences.

For each sequence position of interest, identify the number according to the "Query" sequence. Then identify the corresponding residue position in the "Sbjct" sequence that has been aligned with the position of the "Query" sequence.

In another embodiment of any aspect, embodiment or combination of embodiments described herein (for all NA subtypes), the percent identity is based on an alignment of the polypeptide to the reference sequence using any protocol, and insertions and deletions relative to the reference polypeptide are not considered in determining percent identity. In a further embodiment or combination of embodiments described herein, the percent identity is based on an alignment of the polypeptide to the reference sequence using any protocol, and insertions and deletions relative to the reference polypeptide are considered in determining percent identity.

Throughout the application (for all NA subtypes), mutations that primarily improve expression of the engineered NA polypeptides are denoted in bold font.

Throughout the application (for all NA subtypes), mutations that primarily destabilize the engineered NA tetramers are denoted in italicized font.

In one embodiment of this first aspect, the polypeptides are

(a) at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the N1 NA polypeptide of SEQ ID NO:1, and wherein the non-naturally occurring polypeptide includes 1, 2, 3, 4, 5, 6, or all 7 of the following amino acid residues relative to SEQ ID NO:1 when aligned by protocol 1 or protocol 2:

- (i) **161T/A**, 105A, 157K, 165T/I, 166P, 196Q, 203Y, 444V; or
- (ii) 105A, 165T/I, 166P, 196Q, 203Y, 444V.

In another embodiment of the first aspect, the polypeptides are

(b) at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the N1 NA polypeptide of SEQ ID NO:1, and wherein the non-naturally occurring polypeptide includes 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, or all 17 of the following amino acid residues relative to SEQ ID NO:1 when aligned by protocol 1 or protocol 2:

- (i) **161T/A/V/S/T, 100L, 408M, 419V**, 99P, 103N, 105A, 131Q/M, 163I/L, 165T/S/V/A/I, 166P, 177I, 196Q/T, 203Y, 205I, 442I, 444V; or
- (ii) 99P, 103N, 105A, 131Q/M, 163I/L, 165T/S/V/A/I, 166P, 177I, 196Q/T, 203Y, 205I, 442I, 444V.

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In a further embodiment of this first aspect, the polypeptides include one or more of the following sets of amino acid residues relative to SEQ ID NO:1 when aligned by protocol 1 or protocol 2:

(i)

- 10 **161V/172A;**
100L/408M;
100L/408M/419V
 103N/105A;
 114I/166P;
- 15 101C/164C;
 101C/164C/174V;
 101C/122V/164C/174V/444V;
 122V/444V;
 131Q/442I;
- 20 101A/163L/444A;
 131M/442I;
 101A/131M/163L/442I/444A;
 100L/101A/131M/163L/442I/444A
 131M/163L/442I/444A;
- 25 100L/131M/163L/442I/444A;
 99P/100L/161V/165S/172A/177I/196T/205I/408M/419V;
 99P/100L/161V/165S/172A/177I/196T/408M/419V;
 99P/100L/131Q/161V/165S/172A/177I/196T/205I/408M/419V;
 99P/100L/131Q/161V/165S/172A/177I/196T/408M/419V;
- 30 99P/100L/101A/131M/161V/163L/165S/172A/177I/196T/205I/408M/419V/442I/444A;
 99P/100L/101A/131M/161V/163L/165S/172A/177I/196T/408M/419V/442I/444A;
 99P/161V/165S/172A/177I/196T/205I;
 99P/161V/165S/172A/177I/196T;
 99P/131Q/161V/165S/172A/177I/196T/205I;

- 99P/131Q/161V/165S/172A/177I/196T;
 99P/101A/131M/161V/163L/165S/172A/177I/196T/205I/442I/444A;
 99P/101A/131M/161V/163L/165S/172A/177I/196T/442I/444A;
 99P/196T;
- 5 99P/196T/205I;
 99P/177I/196T; and/or
 99P/177I/196T/205I; or
 (ii)
 103N/105A;
- 10 114I/166P;
 101C/164C;
 101C/164C/174V;
 101C/122V/164C/174V/444V;
 122V/444V;
- 15 131Q/442I;
 101A/1163L/444A;
 131M/442I;
 101A/131M/163L/442I/444A;
 100L/101A/131M/163L/442I/444A;
- 20 131M/163L/442I/444A;
 100L/131M/163L/442I/444A;
 99P/100L/161V/165S/172A/177I/196T/205I/408M/419V;
 99P/100L/131Q/161V/165S/172A/177I/196T/205I/408M/419V;
 99P/100L/131Q/161V/165S/172A/177I/196T/419V;
- 25 99P/100L/101A/131M/161V/163L/165S/172A/177I/196T/205I/408M/419V/442I/444A;
 99P/100L/101A/131M/161V/163L/165S/172A/177I/196T/408M/419V/442I/444A;
 99P/161V/165S/172A/177I/196T/205I;
 99P/161V/165S/172A/177I/196T;
 99P/131Q/161V/165S/172A/177I/196T/205I;
- 30 99P/131Q/161V/165S/172A/177I/196T;
 99P/101A/131M/161V/163L/165S/172A/177I/196T/205I/442I/444A;
 99P/101A/131M/161V/163L/165S/172A/177I/196T/442I/444A;
 99P/196T;
 99P/196T/205I;

99P/177I/196T; and/or

99P/177I/196T/205I.

5 In various embodiments, of this first aspect, the polypeptides includes 3, 4, 5, 6, 7, 8, 9 or more of the listed amino acid residues relative to SEQ ID NO:1 when aligned by protocol 1 or protocol 2.

In a further embodiment, the polypeptides further comprises 1, 2, 3, 4, 5, or all 6 of the following residues relative to SEQ ID NO:1 when aligned by protocol 1 or protocol 2: 105S, 106V, 163I, 166V, 210G, 442S.

10 In a still further embodiment of the first aspect, the polypeptides include one or more of the following sets of amino acid residues relative to SEQ ID NO:1 when aligned by protocol 1 or protocol 2:

99P/100L/161V/165S/172A/177I/196T/205I/408M/419V;

99P/100L/161V/165S/172A/177I/196T/408M/419V;

15 99P/100L/131Q/161V/165S/172A/177I/196T/205I/408M/419V;

99P/100L/131Q/161V/165S/172A/177I/196T/408M/419V;

99P/100L/101A/131M/161V/163L/165S/172A/177I/196T/205I/408M/419V/442I/444A;

99P/100L/101A/131M/161V/163L/165S/172A/177I/196T/408M/419V/442I/444A;

99P/161V/165S/172A/177I/196T/205I;

20 99P/161V/165S/172A/177I/196T;

99P/131Q/161V/165S/172A/177I/196T/205I;

99P/131Q/161V/165S/172A/177I/196T/;

99P/101A/131M/161V/163L/165S/172A/177I/196T/205I/442I/444A; and/or

99P/101A/131M/161V/163L/165S/172A/177I/196T/442I/444A.

25 In a still further embodiment, the polypeptides comprises an amino acid sequence at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid selected from the group consisting of N1 mutants listed in Table 1 (SEQ ID NO:10-32 and 39-95), when aligned by protocol 1 or protocol 2, wherein the polypeptide includes all of the residues listed in Table 1 for an individual N1 mutant listed in Table 1.

30

In a second aspect, the NA polypeptides are:

(a) at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the N2 NA polypeptide of SEQ ID NO:2, and wherein the non-naturally

occurring polypeptide includes 1, 2, 3, 4, 5, 6, 7, or all 8 of the following amino acid residues relative to SEQ ID NO:2 when aligned by protocol 1 or protocol 2:

101C, 131M, 162P, 165S/T, 166V, 195Q, 202Y, 443S; or

- (b) at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%,
5 or 99% identical to the N2 NA polypeptide of SEQ ID NO:2, and wherein the non-naturally occurring polypeptide includes 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or all 12 of the following amino acid residues relative to SEQ ID NO:2 when aligned by protocol 1 or protocol 2:

101C/A, 103N, 105A, 106V, 131M, 162P, 163I, 165S/T/A/I, 166V, 195Q, 202Y, 443S.

- In this second aspect, the N2 NA reference sequence is based on N2 reference strain
10 A/Wisconsin/67/2005 from H3N2.

15 MNPNQKIITIGSVSLTISTICFFMQIAILLITVTLHFRQYEFNSPPNNQVMLCEPTI IERNITEIVYL
TNTTIEKEICPKLA EYRNWSKPOCNITGFAPFSKDNSIRLSAGGDIWVTREPYVSCDEPDCKYQFALGQ
GTLLNNVHSNDTVHDRTPYRTLMLNELGVFPHLGTKQVCIWSSSSSCHDGKAWLHVCVTGDDKNATAS
FIYNGRLVDSIVSWSKEILRTQSEECVCINGTCTVVMTDGSASGKADTKILFIEEGKIVHTSTLSGSA
QHVEECSCYPRLGVRCVCRDNWKGSNRPVDINIKDYSIVSSYVCSGLVGDTPRKNDSSSSSHCLDP
NNEEGGHGVKGWAFDDGNDVVMGRTISEKLRSYETFKVIEGWSNPNSKLQINRQVIVDRGNRSGYSG
IFSVEGKSCINRCFYVELIRGRKEETEVLWTSNSIVVFCGTSPTYGTGSGWPDGADINLMPI (SEQ
ID NO:2), wherein the highlighted residues are the head region

20

In one embodiment of this second aspect, the polypeptides are:

- (a) at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%,
or 99% identical to the N2 NA polypeptide of SEQ ID NO:2, and wherein the non-naturally occurring polypeptide includes 1, 2, 3, 4, 5, 6, 7, or all 8 of the following amino acid residues
25 relative to SEQ ID NO:2 when aligned by protocol 1 or protocol 2:

101C, 131M, 162P, 165S/T, 166V, 195Q, 202Y, 443S.

In another embodiment, the polypeptides are:

- (b) at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%,
30 or 99% identical to the N2 NA polypeptide of SEQ ID NO:2, and wherein the non-naturally occurring polypeptide includes 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or all 12 of the following amino acid residues relative to SEQ ID NO:2 when aligned by protocol 1 or protocol 2:

101C/A, 103N, 105A, 106V, 131M, 162P, 163I, 165S/T/A/I, 166V, 195Q, 202Y, 443S.

In another embodiment of this second aspect, the polypeptides include one or more of the following sets of amino acid residues relative to SEQ ID NO:2 when aligned by protocol 1 or protocol 2:

- 103N/105A;
- 5 101C/164C;
- 101C/164C/173V;
- 101A/131M;
- 100L/101A/131M; and/or
- 100L/131M/163L.

- 10 In various embodiments of this second aspect, the polypeptides include 3, 4, 5, 6, 7, 8, 9, or more of the listed amino acid residues relative to SEQ ID NO:2 when aligned by protocol 1 or protocol 2. In another embodiment, the polypeptides of this second aspect comprises the amino acid sequence at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid selected from the group consisting
- 15 of N2 mutants listed in Table 1 (SEQ ID NO:33-34), when aligned by protocol 1 or protocol 2, wherein the polypeptide includes all of the residues listed in Table 1 for an individual N2 mutant listed in Table 1. In a still further embodiment of this second aspect, the polypeptides are at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the N2 NA polypeptide of SEQ ID NO:2, and wherein the non-naturally
- 20 occurring polypeptide includes a 165Q/E amino acid residues relative to SEQ ID NO:2 when aligned by protocol 1 or protocol 2, or includes 2 or 3 of 165Q/E, 176V, 195S amino acid residues relative to SEQ ID NO:2 when aligned by protocol 1 or protocol 2.

In a third aspect, the NA polypeptides are:

- 25 (a) at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the N3 NA polypeptide of SEQ ID NO:3, and wherein the non-naturally occurring polypeptide includes 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or all 11 of the following amino acid residues relative to SEQ ID NO:3 when aligned by protocol 1 or protocol 2:
- 103N, 105S, 131Q/M, 157T, 165S/I, 166P, 196Q, 203Y, 205I, 443S, 445V; or
- 30 (b) at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the N3 NA polypeptide of SEQ ID NO:3, and wherein the non-naturally occurring polypeptide includes 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or all 13 of the following amino acid residues relative to SEQ ID NO:1 when aligned by protocol 1 or protocol 2:

103N, 105S, 106V, 131Q/M, 157T, 163L, 165S/I/V/A, 166P/V, 196Q, 203Y, 205I, 443S, 445V.

In this third aspect, the N3 NA reference sequence is based on N3 reference strain A/Swine/Missouri/2124514/2006 from H2N3.

5

MNENQRIITIGVVNTTLLSTIALLLIGVGNLVFNTVIHEKIGDHQIVTYPIITTPAVPNCSDTIITYNNT
VINNITTTTITTEERPFKSPLPLCPFRGFFPFHKDNAILRGENKDVIVTREPYVSCDNDNCWSFALAQ
GALLGTHKHSNGTIKDRTPYRSLIRFPIGTAPVLGNYKEICIAWSSSSCFDGKEWMHVCMTGNDNDASA
QIIYGGRMTDSIKSWRKDILRTQESQCIDGTCVVAVTGPAANSADYRVYWIREGKIICKYENVPKT
10 KIQHLEECSCYVDIDVYICIRDNWKGSNRPWMRINNETILETGYVCSKFHSDTPRPADPSTMSCDSPS
NVNGGPGVKGFGEKAGDDVWLGRTVSTSGRSGFEIICKVTEGWINSFNHVKSIQTIVSNNDWSGYSGS
FIVKAKDCFPQCFYVELIRGRPNKNDVSWTSNSIVTFCGLDNEPGSGNWPFGSNIGFMFK (Head
region indicated by highlighting) (SEQ ID NO:3)

15

In one embodiment of this third aspect, the polypeptides are:

(a) at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the N3 NA polypeptide of SEQ ID NO:3, and wherein the non-naturally occurring polypeptide includes 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or all 11 of the following amino acid residues relative to SEQ ID NO:3 when aligned by protocol 1 or protocol 2:

20

103N, 105S, 131Q/M, 157T, 165S/I, 166P, 196Q, 203Y, 205I, 443S, 445V.

In another embodiment, the polypeptides are

(b) at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the N3 NA polypeptide of SEQ ID NO:3, and wherein the non-naturally occurring polypeptide includes 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or all 13 of the following amino acid residues relative to SEQ ID NO:3 when aligned by protocol 1 or protocol 2:

25

103N, 105S, 106V, 131Q/M, 157T, 163L, 165S/I/V/A, 166P/V, 196Q, 203Y, 205I, 443S, 445V.

In another embodiment of this third aspect, the polypeptide includes one or more of the following sets of amino acid residues relative to SEQ ID NO:3 when aligned by protocol 1 or protocol 2:

30

101C/164C;

101C/164C/174V;

101C/164C/174V/445V;

35

101A/445V;

- 101A/131M/445A;
 131M/445A;
 114I/166P;
 101A/163L/445V;
 5 101A/131M/163L/445A; and/or
 131M/163L/445A.

In various further embodiments of this third aspect, the polypeptides includes 3, 4, 5, 6, 7, 8, 9 or more of the listed amino acid residues relative to SEQ ID NO:3 when aligned by protocol 1 or protocol 2. In another embodiment, the polypeptide is at least 75%, 80%, 85%,
 10 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the N3 NA polypeptide of SEQ ID NO:3, and wherein the non-naturally occurring polypeptide includes one or both of the following amino acid residues relative to SEQ ID NO:3 when aligned by protocol 1 or protocol 2: 196S, 165Q/E.

15 In a fourth aspect, the NA polypeptides are:

(a) at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the N4 NA polypeptide of SEQ ID NO:4, and wherein the non-naturally occurring polypeptide includes 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or all 16 of the following amino acid residues relative to SEQ ID NO:4 when aligned by protocol 1 or

20 protocol 2:

160V/S/T/A, 409M, 102N, 104S/A, 105V, 112D, 130Q/M, 162L, 164S/T/A/I, 165P, 176I, 195Q, 202Y, 204I, 443I, 445V; or

(b) at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the N4 NA polypeptide of SEQ ID NO:4, and wherein the non-naturally occurring polypeptide includes 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or all 16 of the following amino acid residues relative to SEQ ID NO:4 when aligned by protocol 1 or
 25 protocol 2:

160V/S/T/A, 409M, 102N, 104S/A, 105V, 112D, 130Q/M, 162L, 164S/T/A/I, 165P/V, 176I, 195Q, 202Y, 204I, 443I, 445V

30 In this fourth aspect, the N4 NA reference sequence is based on N4 reference strain A/ruddy turnstone/Delaware Bay/141/2016 from H10N4.

MNPNQKIITIGSVSIILTTVGLLLQITSLCSIWFSHYNQVAQTHEQPCSNNTTNYNETFVNVTNVQN
 NYTTIAEPSAPDV**VHYSSGRDLCPIRGWAPLSKDNIGIRIGSRGEVVFIREPFISCSISECRTFFLTQG**

5 ALLNDKHSNGTVKDRSPFRTLMSCPIGVAPSPNSRFESVAWSATACSDGPGWLTTLGITGPDSTAVAV
LKYNGIITDTLKS WKGNIMRTQSEECVCQDEFCTTLVTDGPSDAQAFYKILKIRKGKIVSMKD VDATG
FHFEECSCYPSGTEIECVCRDNWRGSNRPWIRFNSDL DYQIGYVCSGIFGDNPRPVDGTGSCNGP VNN
GKGRYGVKGFSFRYGDGVWIGRTKSLESRS GFEMVWDANGWVSTDKDSNGVQDIIDNDNWSGYS GSFS
IRGETTGKNCTVPCFWVEMIRGQPKETIWTSGSSIAFCGVNSD TTGWSWPDGALLPFDIDK
 (Head sequence highlighted) (SEQ ID NO:4)

In one embodiment of this further aspect, the polypeptides are

- (a) at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the N4 NA polypeptide of SEQ ID NO:4, and wherein the non-naturally occurring polypeptide includes 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or all 16 of the following amino acid residues relative to SEQ ID NO:4 when aligned by protocol 1 or protocol 2:

- (i) **160V/S/T/A, 409M**, 102N, 104S/A, 105V, 112D, 130Q/M, 162L, 164S/T/A/I, 165P, 176I, 195Q, 202Y, 204I, 443I, 445V; or
- (ii) 102N, 104S/A, 105V, 112D, 130Q/M, 162L, 164S/T/A/I, 165P, 176I, 195Q, 202Y, 204I, 443I, 445V.

In another embodiment, the polypeptides are

- (b) at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the N4 NA polypeptide of SEQ ID NO:4, and wherein the non-naturally occurring polypeptide includes 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or all 16 of the following amino acid residues relative to SEQ ID NO:4 when aligned by protocol 1 or protocol 2:

- (i) **160V/S/T/A, 409M**, 102N, 104S/A, 105V, 112D, 130Q/M, 162L, 164S/T/A/I, 165P/V, 176I, 195Q, 202Y, 204I, 443I, 445V; or
- (ii) 102N, 104S/A, 105V, 112D, 130Q/M, 162L, 164S/T/A/I, 165P/V, 176I, 195Q, 202Y, 204I, 443I, 445V.

In another embodiment of this fourth aspect, the polypeptides include one or more of the following sets of amino acid residues relative to SEQ ID NO:4 when aligned by protocol 1 or protocol 2:

- (i)
- 160V/S/T/A;**
- 160V/171A;**

- 102N/104A;
 100C/163C;
 100C/163C/173V;
 100C/163C/173V/445V;
 5 130Q/443I;
 100A/162L/445A;
 130M/443I;
 100A/130M/162L/443I/445A;
 130M/162L/443I/445A;
 10 176I/204I;
 100C/121V/163C/173V/445V; and/or
 121V/445V; or
 (ii)
 102N/104A;
 15 100C/163C;
 100C/163C/173V;
 100C/163C/173V/445V;
 130Q/443I;
 100A/162L/445V;
 20 130M/443I;
 100A/130M/162L/443I/445A;
 130M/162L/443I/445A;
 176I/204I;;
 100C/121V/163C/173V/445V; and/or
 25 121V/445V.

- In various further embodiments of this fourth aspect, the polypeptides include 3, 4, 5, 6, 7, 8, 9 or more of the listed amino acid residues relative to SEQ ID NO:4 when aligned by protocol 1 or protocol 2. In a further embodiment, the polypeptides are at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the N4 NA
 30 polypeptide of SEQ ID NO:4, and wherein the non-naturally occurring polypeptide includes 1, or both of the following amino acid residues relative to SEQ ID NO:4 when aligned by protocol 1 or protocol 2: 164Q/E, 195S.

In a fifth aspect, the NA polypeptides are:

(a) at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the N5 NA polypeptide of SEQ ID NO:5, and wherein the non-naturally occurring polypeptide includes 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or all 13 of the following amino acid residues relative to SEQ ID NO:5 when aligned by protocol 1 or protocol 2:

5 **97L, 410M**, 96P, 98A, 100N, 102S/A, 110D, 128Q/M, 160I, 162V/A/I, 163V/P, 193Q/T, 445I; or

(b) at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the N5 NA polypeptide of SEQ ID NO:5, and wherein the non-naturally occurring polypeptide includes 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or all 16 of the following amino acid residues relative to SEQ ID NO:5 when aligned by protocol 1 or protocol 2:

10 **97L, 410M**, 96P, 98A, 100N, 102S/A, 103V, 110D, 128Q/M, 154T, 160I, 162V/A/I/T, 163V/P, 174I, 193Q/T, 445I.

In this fifth aspect, the N5 NA reference sequence is based on N5 reference strain

15 A/gull/Delaware Bay/218/2016 from H10N5.

MNENQKIITIGSVSLALVFNILLHIASIVIGIISVTKEISVSSTONTTEVYNETVRLETITIPINNT
VYIERESHQEPEFLNNTPELCNVSGFAIVSKDNGIRIGSRGHVVFVIREPFVACGPTECRFTFFLTQAL
LNDKHSNNTVKDRSPYRALMSVPLGSSFNAYQAKFESVAWSATACHDGKRWLAVGISGADDDAYAVIH
YGGMPTDVVRSWRKQILRTQESSCVCMKGNCYVWMTDGPANSQASYKIFKSHKGMVTNEREVSFQGGH
20 IEECSCYPNLGKVEVCVRDNWNGMNRPLVLTFFDEDLNYEVGYLCAGIPTDTPRVQDNSFIGSCTNAVGG
SGTNNGYVGKGFGRQNSVWAGRTVSISSRSGFEILLVEDGWVKTSKNVVKKVEVLNNKNWSGYSGAF
TIPITMTSKQCLVPCFWLEMIRGKPEERTSIWTSSSSTVFCGVSSSEVPGWSWDDGAILPFDIDKM

(Head sequence is highlighted) (SEQ ID NO:5)

In one embodiment of this fifth aspect, the polypeptides are

25 (a) at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the N5 NA polypeptide of SEQ ID NO:5, and wherein the non-naturally occurring polypeptide includes 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or all 13 of the following amino acid residues relative to SEQ ID NO:5 when aligned by protocol 1 or protocol 2:

(i) **97L, 410M**, 96P, 98A, 100N, 102S/A, 110D, 128Q/M, 160I, 162V/A/I, 163V/P, 193Q/T, 445I; or

30 (ii) 96P, 98A, 100N, 102S/A, 110D, 128Q/M, 160I, 162V/A/I, 163V/P, 193Q/T, 445I.

In another embodiment of this fifth aspect, the polypeptides are

- (b) at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the N5 NA polypeptide of SEQ ID NO:5, and wherein the non-naturally occurring polypeptide includes 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or all 16 of the following amino acid residues relative to SEQ ID NO:5 when aligned by protocol 1 or
- 5 protocol 2:
- (i) **97L, 410M**, 96P, 98A, 100N, 102S/A, 103V, 110D, 128Q/M, 154T, 160I, 162V/A/I/T, 163V/P, 174I, 193Q/T, 445I; or
- (ii) 96P, 98A, 100N, 102S/A, 103V, 110D, 128Q/M, 154T, 160I, 162V/A/I/T, 163V/P, 174I, 193Q/T, 445I.
- 10 In other embodiments of this fifth aspect, the polypeptides include one or more of the following sets of amino acid residues relative to SEQ ID NO:5 when aligned by protocol 1 or protocol 2:
- (i)
- 97L/410M;**
- 15 100N/102A;
- 98C/161C;
- 128Q/445I;
- 128M/445I;
- 98A/128M/445I/447A;
- 20 97L/98A/128M/445I/447A;
- 128M/445I/447A;
- 97L/128M/445I/447A;
- 96P/193T;
- 111I/163P;
- 25 96P/193T/202I;
- 96P/174I/193T; or
- 96P/174I/193T/202I; or
- (ii)
- 100N/102A;
- 30 98C/161C;
- 128Q/445I;
- 128M/445I;
- 98A/128M/445I/447A;
- 97L/98A/128M/445I/447A;

- 128M/445I/447A;
 97L/128M/445I/447A;
 96P/193T;
 111I/163P;
 5 96P/193T/202I;
 96P/174I/193T; and/or
 96P/174I/193T/202I.

In further embodiments of this fifth aspect, the polypeptides includes 3, 4, 5, 6, 7, 8, 9 or more of the listed amino acid residues relative to SEQ ID NO:5 when aligned by protocol 1 or protocol 2. In a further embodiment, the polypeptides are at least 75%, 80%, 85%, 90%,
 10 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the N5 NA polypeptide of SEQ ID NO:5, and wherein the non-naturally occurring polypeptide includes 1, or both of the following amino acid residues relative to SEQ ID NO:5 when aligned by protocol 1 or protocol 2: 162 Q/E, 200S.

15

In a sixth aspect, the NA polypeptides are:

- (a) at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the N6 NA polypeptide of SEQ ID NO:6, and wherein the non-naturally occurring polypeptide includes 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or all 11 of the following amino
 20 acid residues relative to SEQ ID NO:6 when aligned by protocol 1 or protocol 2:
 99P, 103N, 113D, 131Q, 161I, 162P, 163I, 165S/T/V/A, 196Q, 203Y, 445S; or
 (b) at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the N6 NA polypeptide of SEQ ID NO:6, and wherein the non-naturally occurring polypeptide includes 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or all 16 of the
 25 following amino acid residues relative to SEQ ID NO:6 when aligned by protocol 1 or protocol 2:
 99P, 103N, 105S, 106V, 113D, 131Q, 157T, 161I, 162P, 163I/L, 165S/T/V/A/L, 166V/P, 196Q, 203Y, 445S.

In this sixth aspect, the N6 NA reference sequence is based on N6 reference strain
 30 A/chicken/Sichuan/NCJPL1/2014 from H5N6.

MNFNQKITCISATGMTLSVVSMLIGIANLGLNIGLHYKVS DSTNINIPNMNETSPTTTTIINNHPQNNF
 TNITNIIIVTKTEEGHLLNLTKPLCEVNSWHILSKDNAIRIGEDAHIIIVTREPYLSCDPQGCRMFALSQ
GTTLRGKHANGTIHDRSPFRALVSWEMGQAPSPYNTRVEICIGWSSTSCHDGISRMSICISGPNNNASA
VVWYGGRPVTEIPSWAGNILRTQSECECVCHGGICPVVMTDGPANNRAETKIIYFKEGKIKKIEELKGD

AQHIEECSCYGASEMIKICRDNWKGANRPVITIDPEMMTHTSKYLCSKILTDTSRPNDPTNGKCEAP
ITGGSPDPGVKGFAFLDGENSWLGRTISKDSRSGYEMLKVPNAETDTQSGAISHQIIVNNQNWSGYSG
AFIDYWANKECFNPCFYVELIRGRPKESSVLWTSNSIVALCGSKERLGSWSWHDGAEIIYFK (Head
sequence highlighted) (SEQ ID NO:6)

5

In one embodiment of this sixth aspect, the polypeptides are:

- (a) at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the N6 NA polypeptide of SEQ ID NO:6, and wherein the non-naturally occurring polypeptide includes 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or all 11 of the following amino acid residues relative to SEQ ID NO:6 when aligned by protocol 1 or protocol 2:
- 99P, 103N, 113D, 131Q, 161I, 162P, 163I, 165S/T/V/A, 196Q, 203Y, 445S.

10

In another embodiment of this sixth aspect, the polypeptides are:

- (b) at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the N6 NA polypeptide of SEQ ID NO:6, and wherein the non-naturally occurring polypeptide includes 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or all 15 of the following amino acid residues relative to SEQ ID NO:6 when aligned by protocol 1 or protocol 2:

15

99P, 103N, 105S, 106V, 113D, 131Q, 157T, 161I, 162P, 163I/L, 165S/T/V/A/I, 166V/P, 196Q, 203Y, 445S.

20

In various further embodiments of this sixth aspect, the polypeptide include one or more of the following sets of amino acid residues relative to SEQ ID NO:6 when aligned by protocol 1 or protocol 2:

25

101C/164C;
101C/164C/174V;
101C/164C/174V/447V;
122V/447V;
101A/163L;
99P/196T; and/or
99P/196T/205I.

30

In further embodiments, the polypeptides include 3, 4, 5, 6, 7, 8, 9 or more of the listed amino acid residues relative to SEQ ID NO:6 when aligned by protocol 1 or protocol 2. In another embodiment, the polypeptides are at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the N6 NA polypeptide of SEQ ID NO:6, wherein the non-naturally occurring polypeptide includes a 165E amino acid mutation

relative to SEQ ID NO:6 when aligned by protocol 1, and optionally also includes a 177V amino acid mutation relative to SEQ ID NO:6 when aligned by protocol 1 or protocol 2.

In a seventh aspect, the NA polypeptides are:

- 5 (a) at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the N7 NA polypeptide of SEQ ID NO:7, and wherein the non-naturally occurring polypeptide includes 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or all 11 of the following amino acid residues relative to SEQ ID NO:7 when aligned by protocol 1 or protocol 2: 98P, 102N, 104S, 112D, 130Q, 156T, 162I, 164V/A/I, 165V, 202Y, 448V; or
- 10 (b) at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the N7 NA polypeptide of SEQ ID NO:7, and wherein the non-naturally occurring polypeptide includes 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or all 13 of the following amino acid residues relative to SEQ ID NO:7 when aligned by protocol 1 or protocol 2: 98P, 102N, 104S, 112D, 130Q, 156T, 162I, 164V/A/I, 165V, 176I, 202Y, 446I, 448V.
- 15 In this seventh aspect, the N7 NA reference sequence is based on N7 reference strain A/Netherlands/219/2003 from H7N7.

MNFNQKLFALSGVAIALSVLNLLIGISNVGLNVSLHLKEKGPKQEEENLTCTTINQNNTTVVENTYVNN
 TTIITKGTDLKTPSYLLLNKSLCNVEGWVVIKADNAVRFGESQIIIVTREPYVSCDPTGCKMYALHOG
 20 TTIRNKHSNGTIHDRTAFRGLISTPLGTPPTVSNSDFMCGWSSTTCHDGIARMTICIQGNNDNATAT
VYYNRRLTTTIKTWARNILRTQSEECVCHNGTCAVMTDGSASSQAYTKVMYFHKGLVVKEEELRGS
RHIEECSCYGHNQKVTVCVRDNWQGANRPIEIDMSTLEHTSRYVCTGILTDTSRPGDKSSGDCSNPI
TGSPGVPVGKFGFLNGDNTWLGRTISPRSRSGFEMLKIPNAGTDPNSRIAERQEIVDNNNWSGYSGS
FIDYWNDSNSECYNPCFYVELIRGRPEEAKYVWWASNSLIALCGSPFPVSGSGSPDGAQIQYFS
 25 (Head sequence highlighted) (SEQ ID NO:7)

In one embodiment of this seventh aspect, the polypeptides are

- (a) at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the N7 NA polypeptide of SEQ ID NO:7, and wherein the non-naturally occurring polypeptide includes 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or all 11 of the following amino acid residues relative to SEQ ID NO:7 when aligned by protocol 1 or protocol 2: 98P, 102N, 104S, 112D, 130Q, 156T, 162I, 164V/A/I, 165V, 202Y, 448V.

In another embodiment, the polypeptides are

- (b) at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the N7 NA polypeptide of SEQ ID NO:7, and wherein the non-naturally

occurring polypeptide includes 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or all 13 of the following amino acid residues relative to SEQ ID NO:7 when aligned by protocol 1 or protocol 2:
98P, 102N, 104S, 112D, 130Q, 156T, 162I, 164V/A/I, 165V, 176I, 202Y, 446I, 448V.

- In various further embodiments of this seventh aspect, the polypeptide includes one or
5 more of the following sets of amino acid residues relative to SEQ ID NO:7 when aligned by protocol 1 or protocol 2:

100C/163C;

100C/163C/173V;

100C/163C/173V/448V;

- 10 99L/446I/448A;

98P/195T;

130Q/446I;

446I/448A;

98P/195T/204I;

- 15 98P/176I/195T; and/or

98P/176I/195T/204I.

- In further embodiments, the polypeptides include 3, 4, 5, 6, 7, 8, 9, or more of the listed amino acid residues relative to SEQ ID NO:7 when aligned by protocol 1 or protocol 2. In another embodiment, the polypeptides are at least 75%, 80%, 85%, 90%, 91%, 92%, 93%,
20 94%, 95%, 96%, 97%, 98%, or 99% identical to the N7 NA polypeptide of SEQ ID NO:7, and wherein the non-naturally occurring polypeptide includes one or both of the following amino acid mutation relative to SEQ ID NO:7 when aligned by protocol 1 or protocol 2:
164Q/E, 195S.

- 25 In an eighth aspect, the NA polypeptides are:

- (a) at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the N8 NA polypeptide of SEQ ID NO:8, and wherein the non-naturally occurring polypeptide includes 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or all 11 of the following amino acid residues relative to SEQ ID NO:8 when aligned by protocol 1 or protocol 2:

- 30 **408M**, 101N, 103A/S, 111D, 129Q, 161P/L, 163S/T/V/A/I, 164V, 194Q, 201Y, 442I; or

- (b) at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the N8 NA polypeptide of SEQ ID NO:8, and wherein the non-naturally occurring polypeptide includes 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or all 15 of the

following amino acid residues relative to SEQ ID NO:8 when aligned by protocol 1 or protocol 2:

98L, 408M, 101N, 103A/S, 104V, 111D, 129Q/M, 161P/L, 163S/T/V/A/I/S/T, 164V/P, 175I, 194Q, 201Y, 203I, 442I

- 5 In this eighth aspect, the N8 NA reference sequence is based on N8 reference strain: A/Jiangxi/IPB13b/2013 from H10N8.

10 MNFNQKIITIGSVSLGLVILNILLHIVSITVTVLVLPNGNNGNESNETVIREYNETVRVEKVTQWHNT
 NVIEYTERPENDHFMNTEALCDAKGFAPFSKDNIGIRIGSRGHVFVIREPFVSCSPTECRTEFFLTQGS
LLNDKHSNGTVKDRSPYRTLMSVEIGQSPNVYQARFEAVAWSATACHDGKKWMTIGVTGPDAAVAVV
HYGGIPTDVINSWAGDILRTQESSCTCIQGEFCFWMTDGPANRQAQYRAFKAKQGKIVGQAEISFNNG
HIEECSCYPNEGKVECVCKDNWTGTNRPLVISPDLSYRVGYLCAGLPSTPRGEDSQFTGSCSTSPMG
NQGYGVKGFGRQGNVVMGRTISRTSRSGFEILKVRNGWVQNSKEQIKRQVVVDNLNWSGYSGSFTL
PAELTKRNCILVPCFWVEMIRGNPEEKTIWTSSSSIVMCGVDHEIADWSWHDGAILPFDIDKM (Head
 15 sequence highlighted) (SEQ ID NO:8)

In one embodiment of this eighth aspect, the polypeptides are:

- (a) at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the N8 NA polypeptide of SEQ ID NO:8, and wherein the non-naturally occurring polypeptide includes 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or all 11 of the following amino acid residues relative to SEQ ID NO:8 when aligned by protocol 1 or protocol 2:

- (i) **408M**, 101N, 103A/S, 111D, 129Q, 160P, 161L, 163S/T/V/A/I, 164V, 194Q, 201Y, 442I; or
 (ii) 101N, 103A/S, 111D, 129Q, 160P, 161L, 163S/T/V/A/I, 164V, 194Q, 201Y,
 25 442I.

In another embodiment, the polypeptides are

- (b) at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the N8 NA polypeptide of SEQ ID NO:8, and wherein the non-naturally occurring polypeptide includes 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or all 15 of the following amino acid residues relative to SEQ ID NO:8 when aligned by protocol 1 or protocol 2:

- (i) **98L, 408M**, 101N, 103A/S, 104V, 111D, 129Q/M, 160P, 161L, 163S/T/V/A/I/S/T, 164V/P, 175I, 194Q, 201Y, 203I, 442I; or
 (ii) 101N, 103A/S, 104V, 111D, 129Q/M, 160P, 161L, 163S/T/V/A/I/S/T,
 35 164V/P, 175I, 194Q, 201Y, 203I, 442I

In various further embodiments, the polypeptide include one or more of the following sets of amino acid residues relative to SEQ ID NO:8 when aligned by protocol 1 or protocol 2:

(i)

- 5 **98L/408M;**
160P/163S;
101N/103A;
99C/162C;
99C/162C/172V;
10 129Q/442I;
99A/161L;
99A/161L/442I;
161L/442I;
129M/442I;
15 99A/129M/161L/442I/444A;
98L/99A/129M/161L/442I/444A;
129M/161L/442I/444A;
98L/129M/161L/442I/444A; and/or
175I/203I; or

(ii)

- 20 160P/163S;
101N/103A;
99C/162C;
99C/162C/172V;
25 129Q/442I;
99A/161L;
99A/161L/442I;
161L/442I;
129M/442I;
30 99A/129M/161L/442I/444A;
98L/99A/129M/161L/442I/444A;
129M/161L/442I/444A;
98L/129M/161L/442I/444A; and/or
175I/203I.

In various embodiments, the polypeptide includes 3, 4, 5, 6, 7, 8, 9, or more of the listed amino acid residues relative to SEQ ID NO:8 when aligned by protocol 1 or protocol 2. In a further embodiment, the polypeptides comprises the amino acid sequence at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid selected from the group consisting of N8 mutants listed in Table 1 (SEQ ID NO:35-38), when aligned by protocol 1, wherein the polypeptide includes all of the residues listed in Table 1 for an individual N8 mutant listed in Table 1.

In various further embodiments, the polypeptides are at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the N8 NA polypeptide of SEQ ID NO:8, wherein the non-naturally occurring polypeptide includes a 163E amino acid mutation relative to SEQ ID NO:8 when aligned by protocol 1, and further may optionally include a 194S mutation relative to SEQ ID NO:8 when aligned by protocol 1 or protocol 2.

In a ninth aspect, the NA polypeptides are:

(a) at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the N9 NA polypeptide of SEQ ID NO:9, and wherein the non-naturally occurring polypeptide includes 1, 2, 3, 4, 5, 6, 7, 8, 9, or all 10 of the following amino acid residues relative to SEQ ID NO:9 when aligned by protocol 1 or protocol 2: 94P, 95L, 100S, 126Q/M, 160V/A/I, 161V, 191Q, 198Y, 439S, 441V; or

(b) at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the N9 NA polypeptide of SEQ ID NO:9, and wherein the non-naturally occurring polypeptide includes 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, or all 14 of the following amino acid residues relative to SEQ ID NO:9 when aligned by protocol 1 or protocol 2: 94P, 95L, 98N, 100S/A, 126Q/M, 152T, 158I, 160V/A/I/T, 161V, 191Q, 198Y, 200I, 439S, 441V.

In this ninth aspect, the N9 NA reference sequence is based on N9 reference strain: A/Anhui/1-YK_RG39/2013 from H7N9.

30 MNPNQKILCTSATAIIGAIIVLIGIANLGLNIGLHLKPGCNCSHSQPETTNTSQTIIINYYNETNITN
IQMEERTSENFNNLTGGLCTINSWHIYGKDNAVRIGESSDVLVTREPYVSCDPDECRFYALSQGTTR
GKHSNGTIHDRSQYRALISWPLSSPPTVYNSRVEICIGWSSTCHDGKSRMSICISGPNNNASAVVWYN
RRPVAEINTWARNILRTQSECEVCHNGVCPVVFTDGSATGPADTRIYYFKEGKILKWESLTGTAKHIE
ECSCYGERGTICTCRDNWQGSNRPVIQIDPVAMTHTSQYICSPVLTDPNPNPNIGKCNDDPYPGNN
NNGVKGFSYLDGANTWLGRTISTASRSGYEMLKVPNALTDERSKPIQGQTIVLNADWSGYSGSFMDYW

AEGDCYRACFYVELIRGRPKEDKVWWTNSIVSMCSSTEFLGQWNWPDGAKIEYFL (Read sequence is highlighted) (SEQ ID NO:9)

In one embodiment of this ninth aspect, the polypeptides are:

- (a) at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the N9 NA polypeptide of SEQ ID NO:9, and wherein the non-naturally occurring polypeptide includes 1, 2, 3, 4, 5, 6, 7, 8, 9, or all 10 of the following amino acid residues relative to SEQ ID NO:9 when aligned by protocol 1 or protocol 2:
94P, 95L, 100S, 126Q/M, 160V/A/I, 161V, 191Q, 198Y, 439S, 441V.

In another embodiment, the polypeptides are:

- (b) at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the N9 NA polypeptide of SEQ ID NO:9, and wherein the non-naturally occurring polypeptide includes 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, or all 14 of the following amino acid residues relative to SEQ ID NO:9 when aligned by protocol 1 or protocol 2:
94P, 95L, 98N, 100S/A, 126Q/M, 152T, 158I, 160V/A/I/T, 161V, 191Q, 198Y, 200I, 439S, 441V.

In various embodiments, the polypeptides include one or more of the following sets of amino acid residues relative to SEQ ID NO:9 when aligned by protocol 1 or protocol 2:

- 96C/159C;
96C/159C/441V;
96A/441A;
96A/126M/439I/441A;
95L/96A/126M/439I/441A;
126M/439I/441A;
95L/126M/439I/441A;
94P/191T;
98N/100A; and/or
94P/191T/200I.

In various embodiments, the polypeptides include 3, 4, 5, 6, 7, 8, 9 or more of the listed amino acid residues relative to SEQ ID NO:9 when aligned by protocol 1 or protocol 2.

- In a further embodiment, the polypeptides are is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the N9 NA polypeptide of SEQ ID NO:9, and wherein the non-naturally occurring polypeptide includes a 160Q amino acid mutation relative to SEQ ID NO:9 when aligned by protocol 1, or may include a combination

of 160Q/E and 172V amino acid residues relative to SEQ ID NO:9 when aligned by protocol 1 or protocol 2.

In another embodiment, the disclosure provides composition, comprising one or more of the non-naturally occurring polypeptides of any embodiment or combination of
5 embodiments disclosed herein linked to a scaffold. Linkage to scaffolds permits a plurality (2, 3, 4, 5, 6, 7, 8, 9, 10, or more) of the polypeptides to be displayed, which may enhance the immune response stimulated upon administration to a subject in need thereof, as described in the methods that follow. The compositions may comprise any scaffold suitable for an intended use. The one or more non-naturally occurring polypeptides may be linked
10 covalently or non-covalently to such a scaffold. In one embodiment, the scaffold comprises a protein scaffold; in this embodiment, the one or more non-naturally occurring polypeptides may be covalently linked to the protein scaffold, including but not limited to by being expressed as a fusion protein with a protein component of the scaffold.

In another aspect the disclosure provides nucleic acids encoding the polypeptide or
15 fusion protein of any embodiment or combination of embodiments of the disclosure. The nucleic acid sequence may comprise single stranded or double stranded RNA (such as an mRNA) or DNA in genomic or cDNA form, or DNA-RNA hybrids, each of which may include chemically or biochemically modified, non-natural, or derivatized nucleotide bases. Such nucleic acid sequences may comprise additional sequences useful for promoting
20 expression and/or purification of the encoded polypeptide, 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 a further aspect, the disclosure provides expression vectors comprising the nucleic acid of any aspect of the disclosure operatively linked to a suitable control sequence.
25 "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
30 sequences capable of effecting the expression of the nucleic acid molecules. The control sequences need not be contiguous with the nucleic acid sequences, so long as they function to direct the expression thereof. Thus, for example, intervening untranslated yet transcribed sequences can be present between a promoter sequence and the nucleic acid sequences and the promoter sequence can still be considered "operably linked" to the coding sequence.

Other such control sequences include, but are not limited to, polyadenylation signals, termination signals, and ribosome binding sites. Such expression vectors can be of any type, including but 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, or any other suitable expression vector.

In another aspect, the disclosure provides host cells that comprise the nucleic acids, expression vectors (i.e.: episomal or chromosomally integrated), non-naturally occurring polypeptides, fusion protein, or compositions disclosed herein, wherein the host cells can be either prokaryotic or eukaryotic. The cells can be transiently or stably engineered to incorporate the nucleic acids or 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.

In another aspect, the present disclosure provides pharmaceutical compositions, comprising one or more polypeptides, fusion proteins, compositions, nucleic acids, expression vectors, and/or host cells of the disclosure and a pharmaceutically acceptable carrier. The pharmaceutical compositions of the disclosure can be used, for example, in the methods of the disclosure described below. The pharmaceutical composition may comprise in addition to the polypeptide of the disclosure (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 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 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,

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
5 pharmaceutical composition may also include a tonicity adjusting agent, e.g., a compound 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
10 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.

The polypeptides, fusion proteins, compositions, nucleic acids, expression vectors,
15 and/or host cells may be the sole active agent in the pharmaceutical composition, or the composition or vaccine may further comprise one or more other active agents suitable for an intended use.

The polypeptides, fusion proteins, compositions, pharmaceutical compositions, nucleic acids, expression vectors, and/or host cells of the disclosure may be used for any
20 suitable purpose, including but not limited to treat or limit development of influenza infections. For example, the polypeptides, fusion proteins, compositions, pharmaceutical compositions, nucleic acids, expression vectors, and/or host cells may be used to elicit an immune response to influenza virus. One type of immune response is a B-cell response, which results in the production of antibodies against the antigen that elicited the immune
25 response. While all antibodies are capable of binding to the antigen which elicited the immune response that resulted in antibody production, preferred antibodies are those that provide broad heterosubtypic protection against influenza virus. Thus, the methods may elicit antibodies that bind to an influenza NA protein from a virus selected from the group consisting of influenza A viruses, influenza B viruses, and influenza C viruses. These
30 methods may elicit antibodies that bind to an influenza NA protein from an influenza virus selected from the group consisting of H1, H2, H3, H4, H5, H6, H7, H8, H9, H10, H11, H12, H13, H14, H15, H16, H17, and H18 influenza A virus, and influenza B virus. The methods may elicit antibodies that bind to an influenza NA protein from a strain of influenza virus selected from the group consisting of influenza A/California/07/2009 (H1N1),

A/Michigan/45/2015 (H1N1), A/New Caledonia/20/1999 (H1N1), A/WSN/1933 (H1N1), A/Brevig Mission/1/1918 (H1N1), A/Vietnam/1203/2004 (H5N1), A/Wisconsin/67/2005 (H3N2), A/Swine/Missouri/2124514/2006 (H2N3), A/Red knot/Delaware Bay/310/2016 (H10N4), A/Shorebird/Delaware Bay/309/2016 (H10N5), A/chicken/Sichuan/NCJPL1/2014
 5 (H5N6), A/Netherlands/219/2003 (H7N7), A/Jiangxi/IPB13b/2013 (H10N8), A/Anhui/1-YK_RG39/2013 (H7N9), B/Phuket/3073/2013, B/Colorado/06/2017 and antigenic variants thereof.

Protective antibodies elicited by methods of this disclosure can protect against viral infections by affecting any step in the life cycle of the virus. For example, protective
 10 antibodies may prevent an influenza virus from attaching to a cell, entering a cell, releasing viral ribonucleoproteins into the cytoplasm, forming new viral particles in the infected cell, and/or budding new viral particles from the infected host cell membrane. Antibodies elicited by the methods of this disclosure preferably prevent influenza virus from attaching to or entering the host cell, prevent fusion of viral membranes with endosomal membranes, or
 15 prevent release of newly formed virus from the infected host cell.

One aspect of this disclosure is a vaccine composition (vaccine) comprising any polypeptide, fusion protein, or composition disclosed herein, to protect subjects against infection by influenza virus. Vaccine of this disclosure can also contain other components such as adjuvants, buffers and the like. Exemplary adjuvants include aluminum phosphate,
 20 benzyalkonium chloride, ubenimex, and QS21; genetic adjuvants such as the IL-2 gene or fragments thereof, the granulocyte macrophage colony-stimulating factor (GM-CSF) gene or fragments thereof, the IL-18 gene or fragments thereof, the chemokine (C-C motif) ligand 21 (CCL21) gene or fragments thereof, the IL-6 gene or fragments thereof, CpG, LPS, TLR agonists, and other immune stimulatory genes; protein adjuvants such IL-2 or fragments
 25 thereof, the granulocyte macrophage colony-stimulating factor (GM-CSF) or fragments thereof, IL-18 or fragments thereof, the chemokine (C-C motif) ligand 21 (CCL21) or fragments thereof, IL-6 or fragments thereof, CpG, LPS, TLR agonists and other immune stimulatory cytokines or fragments thereof; lipid adjuvants such as cationic liposomes, N3 (cationic lipid), monophosphoryl lipid A (MPL1); other adjuvants including cholera toxin,
 30 enterotoxin, Fms-like tyrosine kinase-3 ligand (Flt-3L), bupivacaine, marcaine, and levamisole.

The vaccines of this disclosure may include immunogenic portions of more than one Type, Group, subtype, or strain of influenza virus. Such vaccine may comprise nanoparticles, each of which comprises immunogenic portions from NA proteins from more than one Type,

Group, subtype, or strain of influenza virus. Such a vaccine is referred to as a multivalent vaccine. A multivalent vaccine can comprise immunogenic portions from as many influenza NA proteins as necessary to elicit production of an immune response sufficient to protect against a desired breadth of virus Types, Groups, subtypes, or strains. In one embodiment, the vaccine comprises immunogenic portions of NA proteins from at least two different influenza strains (i.e., a bivalent vaccine), or from at least three different influenza strains (i.e., a trivalent vaccine), or from at least four different influenza strains (i.e., a quadrivalent vaccine), or from at least five different influenza strains (i.e., a pentavalent vaccine). In one embodiment, the vaccine comprises immunogenic portions of NA proteins from at least six different influenza strains (hexavalent).

This disclosure provides methods of vaccinating a subject against influenza virus, the method comprising administering a polypeptides, compositions, pharmaceutical compositions, nucleic acids, expression vectors, and/or host cells to the subject such that an immune response against influenza virus is produced in the subject.

The subject may be any suitable subject, including but not limited to humans and other primates, including non-human primates such as chimpanzees and other apes and monkey species; farm animals such as cattle, sheep, pigs, seals, goats and horses; domestic mammals such as dogs and cats; laboratory animals including rodents such as mice, rats and guinea pigs; birds, including domestic, wild and game birds such as chickens, turkeys and other gallinaceous birds, ducks, geese, and the like.

In the vaccination methods of this disclosure, the subject being vaccinated may have been exposed to influenza virus. As used herein, the term "exposed" indicate the subject has come in contact with a person or animal that is known to be infected with an influenza virus. Vaccines of this disclosure may be administered by any suitable technique, by means including, but not limited to, traditional syringes, needleless injection devices, or microprojectile bombardment gene guns. Suitable routes of administration include, but are not limited to, parenteral delivery, such as intramuscular, intradermal, subcutaneous, or intramedullary injections, as well as intrathecal, direct intraventricular, intravenous, intraperitoneal, intranasal, or intraocular injection.

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.

Examples

We provide sequences of recombinant NA proteins in which head domains comprising stabilizing mutations are connected to tetramerization domains. We initially found that many wild-type sequences of beta propeller head domains from certain NA subtypes adopted “open” conformations in which the head domains extended individually off of the stalk-like tetramerization domain, without forming the crystallographically observed “closed” tetramer. Constructs comprising the head domains from other NA subtypes formed closed tetramers naturally. Similar constructs from yet other subtypes formed mixtures of open and closed tetramers. We identified specific mutations at multiple locations in NA sequences that dictate the open or closed conformational state of NA tetramers, and used these mutations to generate closed, stabilized tetramers from multiple NA subtypes. We also converted a naturally closed NA tetramer to a fully open conformation by substituting residues that we identified as pivotal for tetramer closure, confirming the importance of these residues for determining the conformational state of NA. Monoclonal antibodies (mAbs) that bind across the interface of two neighboring protomers in the closed configuration bind better to closed tetramers than open tetramers. The mutations we provide may be useful for stabilizing other NA proteins that naturally form open tetramers when produced recombinantly.

Together, the disclosure provides mutations at defined locations in NA proteins that close the open structures of various NA tetramers. These stabilized NA structures can be used as vaccine antigens in either soluble form or when presented on scaffolds.

Materials and Methods

Protein design and expression

NA constructs were expressed by transient transfection in Expi293F cells (ThermoFisher Scientific) at a density of 2.5×10^6 cells/ml using ExpiFectamine™ 293 Transfection Kit (ThermoFisher Scientific). The supernatants were harvested 5 days post transfection and centrifuged at 4000 rpm to remove cell debris. The culture supernatants were sterile filtered prior to purification by immobilized metal affinity chromatography (IMAC). Clarified supernatant was incubated for 2 h at room temperature with Ni Sepharose™ High Performance histidine-tagged protein purification resin (GE Healthcare) and separated

through affinity chromatography. Bound protein was eluted with 300 mM imidazole, 50 mM Tris-HCl and 0.5 M NaCl. Eluted protein was further purified by size exclusion chromatography into phosphate-buffered saline (PBS) using a Superdex™ 200 Increase 10/300 column (GE Life Sciences).

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NA antigenic characterization

Several mAbs are known that can be used to assess the antigenicity or conformational state of NA. We used multiple mAbs for this purpose, including CD6, a mAb that binds across the interface of two protomers in the closed, crystallographically observed C4-symmetric configuration (Wan et al., *Nat. Comms.* 6:6114). We found that CD6 bound better to recombinant NA proteins that formed closed tetramers than NA proteins that formed open tetramers.

A fortéBio Octet™ HTX instrument was used to measure binding of NA proteins to antibodies that target several antigenic sites. All assays were performed in PBS supplemented with 1% bovine serum albumin (BSA) to minimize nonspecific interactions. The final volume for all solutions was 50 µl/well. Assays were performed at 30 °C in solid black 384-well plates. NA was loaded for 300-600 s on HIS1K tips, which were then dipped to capture mAbs for 600 s. mAbs were then allowed to dissociate for 300-600 s in PBS + 1% BSA. Data analysis was carried out using Octet software, version 11. High capture levels of protein (same as reference proteins or higher) were part of the selection process for EM analysis. Binding of mAb to protein was the second step of the selection process for EM analysis.

NA activity assays

Neuraminidase activity was measured with the NA-Fluor Influenza Neuraminidase Assay Kit according to the manufacturer's protocol. Briefly, 50-100 µg/ml of protein was used as a start concentration and 2-fold dilutions were prepared in duplicate in a black 96-well, flat bottom plate for each protein sample. The wells in column 12 were left empty for controls. NA-Fluor Substrate was prepared according to the protocol and added to each well. Plates were incubated for 1 h at 37 °C and reactions were stopped with NA-Fluor Stop Solution. Plates were read using an excitation wavelength range of 350 nm to 365 nm and an emission wavelength range of 440 nm to 460 nm. Background control wells were subtracted for each protein serial dilution. Finally, protein dilutions were plotted versus relative fluorescence unit (RFU) values.

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EM sample preparation

We used negative stain electron microscopy and particle averaging to assess whether the head domain of recombinant NA proteins adopted the open or closed structure. Figure 2 shows representative examples of two-dimensional class averages of recombinant NA proteins that form open tetramers (N1_{Ca09}, N1_{NC99}, N1_{MI15}, N1_{WSN33} and N6_{Sichuan14}), mixtures of open and closed tetramers (N7_{NL03}, N8_{BD13}, and N9_{Anhui13}), and closed tetramers (N2_{W05}, N3_{Swine/Missouri}, N4_{Red knot/Delaware16}, and N5_{Shorebird/Delaware16}).

Proteins were diluted to a concentration of about 0.02 mg/ml with buffer containing 10 mM HEPES, pH 7.0, and 150 mM NaCl and adsorbed to a glow-discharged carbon-coated copper grid. The grid was washed with a drop of the same buffer three times and stained with 0.75% uranyl formate. Images were recorded at a nominal magnification of 100,000 (pixel size: 0.22 nm) using SerialEM software on an FEI Tecnai T20 electron microscope equipped with an FEI Eagle CCD camera and operated at 200 kV. Particles were selected from the micrographs automatically using in-house software (Yaroslav Tsybovsky, unpublished) and extracted into 128x128-pixel boxes. Reference-free 2D classification was performed using Relion 1.4.

Results

Referring to Figure 1, three different recombinant NA designs were expressed (Figure 1A), purified by IMAC, and examined by size exclusion chromatography (SEC). In the first recombinant NA configuration, an N-terminal 6xHis tag was appended to residues 35-469 of A/California/07/2009 (H1N1) NA (SEQ ID NO: 1), comprising the native stalk and head domains. A second recombinant NA configuration was tested in which the cytosolic, transmembrane, and stalk domains of the wild-type NA (residues 1-77 in SEQ ID NO: 1) were replaced by a 6xHis-tag, an hVSAP domain to drive protein tetramerization (Xu et al., J. Virol. 82:10493-10501), a thrombin cleavage site, and a two-residue Gly-Gly linker. A third recombinant NA configuration was tested in which the cytosolic, transmembrane, and stalk domains of the wild-type NA (residues 1-82 in SEQ ID NO: 1) was replaced by a 6xHis-tag, an hVSAP domain, a thrombin cleavage site, and a two-residue Gly-Gly linker. Recombinant NAs in which the head domain started at position 83 showed homogeneous SEC profiles (Figure 1B) with a major peak corresponding to the estimated molecular weight of NA tetramers, with minimal aggregation. Subsequent constructs were designed with head domains starting at position 83.

Referring to Figure 2, purified recombinant NA proteins from a number of subtypes were characterized structurally by negative stain EM. Representative NAs from the N2, N3, N4, N5 subtypes formed closed tetrameric structures in which the head domain resembled the C4-symmetric structure classically observed by X-ray crystallography. Representative NAs from the N1 and N6 subtypes formed open tetramers in which the head domains did not form a single, compact structure. Representative NAs from the N7, N8, and N9 subtypes formed mixtures of open and closed tetramers.

Referring to Figure 3, in one non-limiting example, we designed a series of recombinant NA mutants based on the wild-type A/California/07/2009 (H1N1) NA sequence that resulted in a protein that formed a closed tetramer. Introducing ten mutations into A/California/07/2009 (H1N1) NA (construct name 94_N1-Cal09_danf2 in Table 1; SEQ ID NO:79) resulted in 45% closed tetramers. Additional mutations and a reverse mutation result in a protein (construct name 155_N1-Cal09-cl30_T453V in Table 1; SEQ ID NO:13) that forms 90-100% closed recombinant NA tetramers. The 99P, 177L, 196T and 205I mutations provide improved hydrophobic packing at the inter-protomeric interface. The 165S mutation helps stabilize the closed conformation of a loop that participates in the inter-protomeric interface. The 161V and 172A mutations remove a cysteine and optimize packing, which improves expression. The 100L, 408M and 419V mutations improve expression by removing polar residues in a region of the protein that is partially hidden from solvent.

Referring to Figure 4, in another non-limiting example, stabilizing mutations introduced into the sequence of A/Michigan/45/2015 (H1N1) NA, which forms an open tetramer when the wild-type sequence for the head domain is used, result in a protein (construct name 174_N1-Mi15_cl155_T131Q in Table 1; SEQ ID NO:18) that forms 100% closed tetramers. The 131Q mutation optimizes packing and helps stabilize the closed conformation of a loop that participates in the inter-protomeric interface.

Referring to Figure 5, in another non-limiting example, stabilizing mutations introduced into the sequence of A/WSN/1933 (H1N1) NA, which forms an open tetramer when the wild-type sequence for the head domain is used, result in a protein (construct name 366_N1-WSN33_cl155_G105S_I106V_A157T_V163I_A166V_R210G in Table 1; SEQ ID NO:43) that forms 80% closed tetramers. The 157T and 166V mutations provide improved hydrophobic packing at the inter-protomeric interface. The 210G mutation removes electrostatic repulsion at the inter-protomeric interface. The 105S, 106V and 163I mutations help optimize packing to stabilize the closed conformation of a loop that participates in the inter-protomeric interface.

Referring to Figure 6, in another non-limiting example, stabilizing mutations introduced into the sequence of A/Vietnam/1203/04 (H5N1) NA, which forms an open tetramer when 10 stabilizing mutations originally identified in A/California/07/2009 (H1N1) NA are used, result in a protein bearing 14 mutations (construct name 354_N1-
 5 VN04_c155_I106V_T131Q_V163I_A166V in Table 1; SEQ ID NO:40) that forms 100% closed tetramers. The 106V, 131Q and 163I mutations help optimize packing to stabilize the closed conformation of a loop that participates in the inter-protomeric interface. The 166V mutation provides improved hydrophobic packing at the inter-protomeric interface.

Referring to Figure 7, two mutations introduced into the recombinant A/Jiangxi-Donghu/346-2/2013 (H10N8) N8 NA (construct name 285_N8-Jiangxi-Donghu2013_E162P-Q165S in Table 1; SEQ ID NO:36) resulted in the formation of 100% closed tetramers. The 162P and 165S mutation help stabilize the closed conformation of a loop that participates in the inter-protomeric interface.

Referring to Figure 8, we introduced substitutions at positions that are critical for
 15 stabilizing the closed tetrameric structure of NA into a naturally closed recombinant NA to open up the closed, compact structure. Three mutations at positions that had been identified to stabilize open tetramers resulted in the formation of exclusively open tetramers of the NA from A/Wisconsin/67/2005 (H3N2) (construct name 255_N2-Wis05_V165Q_I176V_T195S in Table 1; SEQ ID NO:34). The 176V and 195S mutations decrease the amount of inter-
 20 protomeric hydrophobic packing that is natively present. The 165Q mutation allows for increased flexibility of a loop that participates in the inter-protomeric interface.

Referring to Figure 9, a Basic Local Alignment Search Tool protein (BLASTp) alignment between reference sequences and any arbitrary sequence of the same subtype allows for identification of positions for the described mutations in any NA sequence.
 25 Sequence positions in arbitrary NA sequences are identified based on alignment to corresponding positions in reference sequences. Many sequence alignment tools are known to those of skill in the art and can be used to align arbitrary NA sequences to the provided reference sequences. Here we provide protocols for aligning sequences using the online BLASTp tool provided by the National Institute of Health (protocol 1) as well as a standalone
 30 executable version of the BLAST software that can be run locally on any computer (protocol 2).

Overall, we identified mutations that result in recombinant NA proteins that adopt closed, C4-symmetric, or open, non-symmetric conformations. Mutations that fill cavities in NA are helpful for tetramer closure. In some non-limiting examples, engineered disulfide

bonds are helpful for tetramer closure. Certain amino acid positions, including but not limited to position 165, appear most relevant for dictating the open or closed conformational state of NA tetramers. In addition, other mutations substantially improve overall protein expression levels.

5

Table 1

Name	Sequence	Mutations included	Mutations highlighted in uppercase	% Closure
94_N1-Cal09_d anfav2	VKLAGNSSLCPVSGWAPLSKDN SVRIGSKGEVFVIREPFISCS? LECRTFFLTQGALLNDKHSNGT IKDRSPYRTLMSCPIGSVSPSPS NSRFESVAWSASACHDGINWLT IGITGPDNGAVAILKYNGIITD TIKSWRNNILRTQESECACVNG SCFTVMTDGPNGQASYKIFRI EKGKIVKSVEMNAPNYHYEES CYPDSSEITCVCARDNWHGNSNR? WVSFNQNLLEYQIGYICSGIFGD NPRPNDKTGSCGPVSSNGANGV KGFSFKYNGVWIGRTKSISSR NGFEMIWDPNGWGTGTDNNFSIK QDIVGINEWSGYSGSFVMHPEL TGLDCIVPCFWVELIRGRPKEN TIWTSGSSISFCGVNSDTTGWS WPDGAELPFTIDK (SEQ ID NO:79)	N1 numbering: 99P/196T/205I, 113E/170S, 165S, 100L/408M/419V, 453T	vklaGnssLcPvsgwaPlskdn svrigskGEVfvirepfiscsp lecrtffltqgallndkhsngt ikdrspyrtlmscpigsvspSPS nsrfesvawsasachdginwlt igiTgpdngavaIlkyngiitd tikswrnnilrtqesecacvng scftvmtdgpsngqasykifri ekgkivksvemnapnyhyeecs cypdsseitcvcrdnwhgsnrp wvsfnqnleyqigyicsgifgd nprpndktgscgpvssngangv kgfsfkyngvwigrtksissr ngfemiwdpnngwtgtddnnfsik qdivginewsgysgsfvmhpel tgldciVpcfwvelirgrpken tiwtsgssisfcgvnsdtTgws wpdgaelpftidk (SEQ ID NO:79)	45%
112_N1-Cal09_d anfav2 no-space-B	VKLAGNSSLCPVSGWAPYSKDN SVRIGSKGEVFVIREPFISCS? LECRTFFLTQGALLNDKHSNGT IKDRSPYRTLMSCPIGSVSPSPS NSRFESVAWSASACHDGINWLT IGITGPDNGAVAILKYNGIITD TIKSWRNNILRTQESECACVNG SCFTVMTDGPNGQASYKIFRI EKGKIVKSVEMNAPNYHYEES CYPDSSEITCVCARDNWHGNSNR? WVSFNQNLLEYQIGYICSGIFGD NPRPNDKTGSCGPVSSNGANGV KGFSFKYNGVWIGRTKSISSR NGFEMIWDPNGWGTGTDNNFSIK QDIVGINEWSGYSGSFVQHPEL TGLDCIRPCFWVELIRGRPKEN TIWTSGSSISFCGVNSDTTGWS WPDGAELPFTIDK (SEQ ID NO:80)	N1 numbering: 99P/196T/205I, 113E/170S, 165S, 453T	vklaGnssLcPvsgwaPySkdn svrigskGEVfvirepfiscsp lecrtffltqgallndkhsngt ikdrspyrtlmscpigsvspSPS nsrfesvawsasachdginwlt igiTgpdngavaIlkyngiitd tikswrnnilrtqesecacvng scftvmtdgpsngqasykifri ekgkivksvemnapnyhyeecs cypdsseitcvcrdnwhgsnrp wvsfnqnleyqigyicsgifgd nprpndktgscgpvssngangv kgfsfkyngvwigrtksissr ngfemiwdpnngwtgtddnnfsik qdivginewsgysgsfvqhpel tgldcirpcfwvelirgrpken tiwtsgssisfcgvnsdtTgws wpdgaelpftidk (SEQ ID NO:80)	40%
113_N1-Cal09_D F2TI	VKLAGNSSLCPVSGWAPLSKDN SVRIGSKGEVFVIREPFISCS? LECRTFFLTQGALLNDKHSNGT IKDRSPYRTLMSCPIGSVSPPT NSRFESIAWSASACHDGINWLT IGITGPDNGAVAILKYNGIITD TIKSWRNNILRTQESECACVNG SCFTVMTDGPNGQASYKIFRI EKGKIVKSVEMNAPNYHYEES	N1 numbering: 99P/177I/196T/205I, 113E/170T, 165S, 100L/408M/419V, 453T	vklaGnssLcPvsgwaPlskdn svrigskGEVfvirepfiscsp lecrtffltqgallndkhsngt ikdrspyrtlmscpigsvspPT nsrfesiawsasachdginwlt igiTgpdngavaIlkyngiitd tikswrnnilrtqesecacvng scftvmtdgpsngqasykifri ekgkivksvemnapnyhyeecs	80-90%

	CYPDSSEITCVCARDNWHGSRNP WVSFNQNLLEYQIGYICSGIFGD NRPNDKTGSCGPVSSNGANGV KGFSFKYGNVWIGRTKSISSR NGFEMIWDPNWGTGTDNNFSIK QDIVGINEWSGYSGSFMHPEL TGLDCIVPCFWVELIRGRPKEN TIWTSGSSISFCGVNSDTTGWS WPDGAELPFTIDK (SEQ ID NO:81)		cypdsseitcvcardnwhgsrnp wvsfnqnleyqigyicsgifgd nprpndktgscgpvssngangv kgfsfkyngvwigrtksissr ngfemiwdpnwgtgtdnnfsik qdivginewsgysgsfvmhpel tgldciVpcfwvelirgrpken tiwtsgssisfcgvnsdtTgws wpdgaelpftidk (SEQ ID NO:81)	
114_N1- Cal09_D F2TI_T1 70S	VKLGNSSSLCPVSGWAPLSKDN SVRIGSKGEVFIREFPISCS? LECRTEFFLTQGALLNDKHSNGT IKDRSPYRTLMSCPIGSVSPSP NSRFESIAWSASACHDGINWLT IGITGPDNGAVAILKYNGIITD TIKSWRNNILRTQESACACVNG SCFTVMTDGPNGQASYKIFRI EKGKIVKSVEMNAPNYHYECS CYPDSSEITCVCARDNWHGSRNP WVSFNQNLLEYQIGYICSGIFGD NRPNDKTGSCGPVSSNGANGV KGFSFKYGNVWIGRTKSISSR NGFEMIWDPNWGTGTDNNFSIK QDIVGINEWSGYSGSFMHPEL TGLDCIVPCFWVELIRGRPKEN TIWTSGSSISFCGVNSDTTGWS WPDGAELPFTIDK (SEQ ID NO:82)	N1 numbering: 99P/177I/19 6T/205I, 113E/170S, 165S, 100L/408M/4 19V, 453T	vklagnssslcpvsgwaPlskdn svrigskgEvfvirepfiscsp lecrteffltqgallndkhsngt ikdrspyrtlmscpigsvpsps nsrfesilawsasachdginwlt igiTgpdngavaIlkyngiitd tikswrnnilrtqesecacvng scftvmtdgpsngqasykifri ekgkivksvemnapnyhyeecs cypdsseitcvcardnwhgsrnp wvsfnqnleyqigyicsgifgd nprpndktgscgpvssngangv kgfsfkyngvwigrtksissr ngfemiwdpnwgtgtdnnfsik qdivginewsgysgsfvmhpel tgldciVpcfwvelirgrpken tiwtsgssisfcgvnsdtTgws wpdgaelpftidk (SEQ ID NO:82)	100%
127_N1- Cal09_D F2TI_Cy sKO_no- space-B	VKLGNSSSLCPVSGWAPYSKDN SVRIGSKGEVFIREFPISCS? LECRTEFFLTQGALLNDKHSNGT IKDRSPYRTLMSVPIGSPSPPT NARFESIAWSASACHDGINWLT IGITGPDNGAVAILKYNGIITD TIKSWRNNILRTQESACACVNG SCFTVMTDGPNGQASYKIFRI EKGKIVKSVEMNAPNYHYECS CYPDSSEITCVCARDNWHGSRNP WVSFNQNLLEYQIGYICSGIFGD NRPNDKTGSCGPVSSNGANGV KGFSFKYGNVWIGRTKSISSR NGFEMIWDPNWGTGTDNNFSIK QDIVGINEWSGYSGSFVQHPEL TGLDCIRPCFWVELIRGRPKEN TIWTSGSSISFCGVNSDTTGWS WPDGAELPFTIDK (SEQ ID NO:83)	N1 numbering: 99P/177I/19 6T/205I, 113E/170T, 165S, 453T, 161V/172A	vklagnssslcpvsgwaPyskdn svrigskgEvfvirepfiscsp lecrteffltqgallndkhsngt ikdrspyrtlmsVpigsvpspt narfesilawsasachdginwlt igiTgpdngavaIlkyngiitd tikswrnnilrtqesecacvng scftvmtdgpsngqasykifri ekgkivksvemnapnyhyeecs cypdsseitcvcardnwhgsrnp wvsfnqnleyqigyicsgifgd nprpndktgscgpvssngangv kgfsfkyngvwigrtksissr ngfemiwdpnwgtgtdnnfsik qdivginewsgysgsfvqhpel tgldcirpcfwvelirgrpken tiwtsgssisfcgvnsdtTgws wpdgaelpftidk (SEQ ID NO:83)	30-40%
128_N1- Cal09_D F2TI_Cy sKO_T45 3V	VKLGNSSSLCPVSGWAPLSKDN SVRIGSKGEVFIREFPISCS? LECRTEFFLTQGALLNDKHSNGT IKDRSPYRTLMSVPIGSPSPPT NARFESIAWSASACHDGINWLT IGITGPDNGAVAILKYNGIITD TIKSWRNNILRTQESACACVNG SCFTVMTDGPNGQASYKIFRI EKGKIVKSVEMNAPNYHYECS CYPDSSEITCVCARDNWHGSRNP WVSFNQNLLEYQIGYICSGIFGD NRPNDKTGSCGPVSSNGANGV KGFSFKYGNVWIGRTKSISSR	N1 numbering: 99P/177I/19 6T/205I, 113E/170T, 165S, 100L/408M/4 19V, 161V/172A	vklagnssslcpvsgwaPlskdn svrigskgEvfvirepfiscsp lecrteffltqgallndkhsngt ikdrspyrtlmsVpigsvpspt narfesilawsasachdginwlt igiTgpdngavaIlkyngiitd tikswrnnilrtqesecacvng scftvmtdgpsngqasykifri ekgkivksvemnapnyhyeecs cypdsseitcvcardnwhgsrnp wvsfnqnleyqigyicsgifgd nprpndktgscgpvssngangv kgfsfkyngvwigrtksissr	90-100%

	NGFEMIWDPNNGWTGTDNNFSIK QDIVGINEWSGYSGSFVMHPPEL TGLDCIVPCFWVELIRGRPKEN TIWTSGSSISFCGVNSDTVGWS WPDGAELPFTIDK (SEQ ID NO:84)		ngfemiwdpnngwtgtdnnfsik qdivginewsgysgsfvmhpel tgldciVpcfwwelirgrpken tiwtsgsssisfcgvnsdtvgws wpdgaelpftidk (SEQ ID NO:84)	
130_N1- Cal09_D F2TI_Cy sKO_E11 3D_T170 Y	VKLAGNSSLCPVSGWAPLSKDN SVRIGSKGDFVIREPFISCS? LECRTEFFLTQGALLNDKHSNGT IKDRSPYRTLMSVPISGVSPPY NARFESIAWSASACHDGINWLT IGITGPDNGAVAILKYNGIITD TIKSWRNNILRTQESACVNG SCFTVMTDGPNGQASYKIFRI EKGKIVKSVEMNAPNYHYECS CYPDSSEITCVCRDNWHGSRP WVSFNQNLLEYQIGYICSGIFGD NPRPNDKTGSCGPVSSNGANGV KGFSFKYGNVWIGRTKSISSR NGFEMIWDPNNGWTGTDNNFSIK QDIVGINEWSGYSGSFVMHPPEL TGLDCIVPCFWVELIRGRPKEN TIWTSGSSISFCGVNSDTTGWWS WPDGAELPFTIDK (SEQ ID NO:85)	N1 numbering: 99P/177I/19 6T/205I, 165S, 100L/408M/4 19V, 453T, 161V/172A	vklaGnsslcPvsgwaPlskdn svrigskgdfvirepfiscsp lecrteffltqgallndkhsngt ikdrspyrtlmsVpigsVpspy nArfesIawsasachdginwlt igiTgpdngavaIlkyngiitd tikswrnnilrtqesecacvng scftvmtdgpsngqasykifri ekgkivksvemnapnyhyeecs cypdsseitcvcrdnwhgsnrp wvsfnqnleyqigiyicsgifgd nprpndktgscgpvssngangv kgfsfkygngvwigrtksissr ngfemiwdpnngwtgtdnnfsik qdivginewsgysgsfvmhpel tgldciVpcfwwelirgrpken tiwtsgsssisfcgvnsdtTgws wpdgaelpftidk (SEQ ID NO:85)	90-100%
131_N1- Cal09_D F2TI_Cy sKO_no- space- B_E113D _T170Y	VKLAGNSSLCPVSGWAPYSKDN SVRIGSKGDFVIREPFISCS? LECRTEFFLTQGALLNDKHSNGT IKDRSPYRTLMSVPISGVSPPY NARFESIAWSASACHDGINWLT IGITGPDNGAVAILKYNGIITD TIKSWRNNILRTQESACVNG SCFTVMTDGPNGQASYKIFRI EKGKIVKSVEMNAPNYHYECS CYPDSSEITCVCRDNWHGSRP WVSFNQNLLEYQIGYICSGIFGD NPRPNDKTGSCGPVSSNGANGV KGFSFKYGNVWIGRTKSISSR NGFEMIWDPNNGWTGTDNNFSIK QDIVGINEWSGYSGSFVQHPPEL TGLDCIRPCFWVELIRGRPKEN TIWTSGSSISFCGVNSDTTGWWS WPDGAELPFTIDK (SEQ ID NO:86)	N1 numbering: 99P/177I/19 6T/205I, 165S, 453T, 161V/172A	vklaGnsslcPvsgwaPySkdn svrigskgdfvirepfiscsp lecrteffltqgallndkhsngt ikdrspyrtlmsVpigsVpspy nArfesIawsasachdginwlt igiTgpdngavaIlkyngiitd tikswrnnilrtqesecacvng scftvmtdgpsngqasykifri ekgkivksvemnapnyhyeecs cypdsseitcvcrdnwhgsnrp wvsfnqnleyqigiyicsgifgd nprpndktgscgpvssngangv kgfsfkygngvwigrtksissr ngfemiwdpnngwtgtdnnfsik qdivginewsgysgsfVqhPel tgldciRpcfwwelirgrpken tiwtsgsssisfcgvnsdtTgws wpdgaelpftidk (SEQ ID NO:86)	90-100%
134_N1- Cal09_D F2TI_Cy sKO_S16 5E_T453 V_E113D _T170Y	VKLAGNSSLCPVSGWAPLSKDN SVRIGSKGDFVIREPFISCS? LECRTEFFLTQGALLNDKHSNGT IKDRSPYRTLMSVPIGEVPSPPY NARFESIAWSASACHDGINWLT IGITGPDNGAVAILKYNGIITD TIKSWRNNILRTQESACVNG SCFTVMTDGPNGQASYKIFRI EKGKIVKSVEMNAPNYHYECS CYPDSSEITCVCRDNWHGSRP WVSFNQNLLEYQIGYICSGIFGD NPRPNDKTGSCGPVSSNGANGV KGFSFKYGNVWIGRTKSISSR NGFEMIWDPNNGWTGTDNNFSIK QDIVGINEWSGYSGSFVMHPPEL TGLDCIVPCFWVELIRGRPKEN TIWTSGSSISFCGVNSDTVGWS	N1 numbering: 99P/177I/19 6T/205I, 100L/408M/4 19V, 161V/172A	vklaGnsslcPvsgwaPlskdn svrigskgdfvirepfiscsp lecrteffltqgallndkhsngt ikdrspyrtlmsVpigeVpspy nArfesIawsasachdginwlt igiTgpdngavaIlkyngiitd tikswrnnilrtqesecacvng scftvmtdgpsngqasykifri ekgkivksvemnapnyhyeecs cypdsseitcvcrdnwhgsnrp wvsfnqnleyqigiyicsgifgd nprpndktgscgpvssngangv kgfsfkygngvwigrtksissr ngfemiwdpnngwtgtdnnfsik qdivginewsgysgsfvmhpel tgldciVpcfwwelirgrpken tiwtsgsssisfcgvnsdtvgws	50%

	WPDGAELPFTIDK (SEQ ID NO:10)		wpdgaelpftidk (SEQ ID NO:10)	
140_N1-Mi15_DF2TI	VKLAGNSSLCPVSGWAPLSKDN SVRIGSKGEVFIREFPISCS? LECRFFLTQGALLNDKHSNGT IKDRSPYRTLMSCPIGSVSPST NSRFESIAWSASACHDGINWLT IGITGPDGSAVAILKYNGIITD TIKSWRNNILRTQESACACVNG SCFTIMTDGPGSDGQASYKIFRI EKGKIIKSVEMKAPNYHYECS CYPDSSEITCVCARDNWHGSRNP WVSFNQNLLEYQMGYICSGVFGD NPRPNDKTGSCGPVSSNGANGV KGFSFKYGNVWIGRTKSISR KGFEMIWDPNGTGTDNKFSIK QDIVGINEWSGYSGSFVMHPEL TGLDCIVPCFWVELIRGRPEEN TIWTSGSSISFCGVNSDTTGWS WPDGAELPFTIDK (SEQ ID NO:11)	N1 numbering: 99P/177I/19 6T/205I, 113E/170T, 165S, 100L/408M/4 19V, 453T	vkla g n s s l c p v s g w a P l s k d n s v r i g s k g E v f i r e p f i s c s p l e c r t f f l t q g a l l n d k h s n g t i k d r s p y r t l m s c p i g s v s p s t n s r f e s i a w s a s a c h d g i n w l t i g i T g p d s g a v a i l k y n g i i t d t i k s w r n n i l r t q e s e c a c v n g s c f t i m t d g p s d g q a s y k i f r i e k g k i i k s v e m k a p n y h y e e c s c y p d s s e i t c v c r d n w h g s n r p w v s f n q n l e y q m g y i c s g v f g d n p r p n d k t g s c g p v s s n g a n g v k g f s f k y g n v w i g r t k s i s s r k g f e m i w d p n g w t g t d n k f s i k q d i v g i n e w s g y s g s f v m h p e l t g l d c i v p c f w v e l i r g r p e e n t i w t s g s s i s f c g v n s d t t g w s w p d g a e l p f t i d k (S E Q I D N O : 1 1)	10-20%
141_N1-Mi15_DF2TI_no-space-B	VKLAGNSSLCPVSGWAPYSKDN SVRIGSKGEVFIREFPISCS? LECRFFLTQGALLNDKHSNGT IKDRSPYRTLMSCPIGSVSPST NSRFESIAWSASACHDGINWLT IGITGPDGSAVAILKYNGIITD TIKSWRNNILRTQESACACVNG SCFTIMTDGPGSDGQASYKIFRI EKGKIIKSVEMKAPNYHYECS CYPDSSEITCVCARDNWHGSRNP WVSFNQNLLEYQMGYICSGVFGD NPRPNDKTGSCGPVSSNGANGV KGFSFKYGNVWIGRTKSISR KGFEMIWDPNGTGTDNKFSIK QDIVGINEWSGYSGSFVQHPEL TGLDCIRPCFWVELIRGRPEEN TIWTSGSSISFCGVNSDTTGWS WPDGAELPFTIDK (SEQ ID NO:12)	N1 numbering: 99P/177I/19 6T/205I, 113E/170T, 165S, 453T	vkla g n s s l c p v s g w a P y s k d n s v r i g s k g E v f i r e p f i s c s p l e c r t f f l t q g a l l n d k h s n g t i k d r s p y r t l m s c p i g s v s p s t n s r f e s i a w s a s a c h d g i n w l t i g i T g p d s g a v a i l k y n g i i t d t i k s w r n n i l r t q e s e c a c v n g s c f t i m t d g p s d g q a s y k i f r i e k g k i i k s v e m k a p n y h y e e c s c y p d s s e i t c v c r d n w h g s n r p w v s f n q n l e y q m g y i c s g v f g d n p r p n d k t g s c g p v s s n g a n g v k g f s f k y g n v w i g r t k s i s s r k g f e m i w d p n g w t g t d n k f s i k q d i v g i n e w s g y s g s f v q h p e l t g l d c i r p c f w v e l i r g r p e e n t i w t s g s s i s f c g v n s d t t g w s w p d g a e l p f t i d k (S E Q I D N O : 1 2)	40%
155_N1-Cal09-cl30-T453V	VKLAGNSSLCPVSGWAPLSKDN SVRIGSKGDVFIREFPISCS? LECRFFLTQGALLNDKHSNGT IKDRSPYRTLMSVPISGVSPPY NARFESIAWSASACHDGINWLT IGITGPDNGAVAILKYNGIITD TIKSWRNNILRTQESACACVNG SCFTVMTDGPSPNGQASYKIFRI EKGKIVKSVEMNAPNYHYECS CYPDSSEITCVCARDNWHGSRNP WVSFNQNLLEYQIGYICSGIFGD NPRPNDKTGSCGPVSSNGANGV KGFSFKYGNVWIGRTKSISR NGFEMIWDPNGTGTDNFNSIK QDIVGINEWSGYSGSFVMHPEL TGLDCIVPCFWVELIRGRPKEN TIWTSGSSISFCGVNSDTVGWS WPDGAELPFTIDK (SEQ ID NO:13)	N1 numbering: 99P/177I/19 6T/205I, 165S, 100L/408M/4 19V, 161V/172A	vkla g n s s l c p v s g w a P l s k d n s v r i g s k g d v f i r e p f i s c s p l e c r t f f l t q g a l l n d k h s n g t i k d r s p y r t l m s v p i g s v s p p y n a r f e s i a w s a s a c h d g i n w l t i g i T g p d n g a v a i l k y n g i i t d t i k s w r n n i l r t q e s e c a c v n g s c f t v m t d g p s n g q a s y k i f r i e k g k i v k s v e m n a p n y h y e e c s c y p d s s e i t c v c r d n w h g s n r p w v s f n q n l e y q i g y i c s g i f g d n p r p n d k t g s c g p v s s n g a n g v k g f s f k y g n v w i g r t k s i s s r n g f e m i w d p n g w t g t d n n f s i k q d i v g i n e w s g y s g s f v m h p e l t g l d c i v p c f w v e l i r g r p k e n t i w t s g s s i s f c g v n s d t v g w s w p d g a e l p f t i d k (S E Q I D N O : 1 3)	90-100%
300_Mi15-cl55	VKLAGNSSLCPVSGWAPLSKDN SVRIGSKGDVFIREFPISCS?	N1 numbering:	vkla g n s s l c p v s g w a P l s k d n s v r i g s k g d v f i r e p f i s c s p	70%

	LECRTEFFLTQGALLNDKHSNGT IKDRSPYRTLMSVPIGSVPSPY NARFESIAWSASACHDGINWLT IGITGPDGSAVAILKYNGIITD TIKSWRNNILRTQESACVNG SCFTIMTDGPSDQASYKIFRI EKGKIKSVEMKAPNYHYECS CYPDSSEITCVCARDNWHGSRNP WVSFNQNLQYQMGYICSGVFGD NPRPNDKTGSCGPVSSNGANGV KGFSFKYNGVWIGRTKSISSR KGFEMIWDPNGWGTGDNKFSIK QDIVGINEWSGYSGSFVMHPEL TGLDCIVPCFWVELIRGRPEEN TIWTSGSSISFCGVNSDTVGWS WPDGAELPFTIDK (SEQ ID NO:14)	99P/177I/19 6T/205I, 165S, 100L/408M/4 19V, 161V/172A	lecrteffltqgallndkhsngt ikdrspyrtlmsVpigSvpspy nArfesIawsasachdginwlt igiTgpdsgavaIlkyngiitd tikswrnnilrtqesecacvng scftimtdgpsdgqasykifri ekgkiiksvemkapnyhyeecs cypdsseitcvcrdnwhgsnrp wvsfnqnleyqmgycsgvfgd nprpndktgscgpvssngangv kgfsfkyngvwigrtksissr kgfemiwdpnngwtgtdnkfsik qdivginewsgysgsfvmhpel tgldciVpcfwwelirgrpeen tiwtsgssisfcgvnsdtvgws wpdgaelpftidk (SEQ ID NO:14)	
164_N1- Mi15_c1 55_D103 N_S105A	VKLGNSSSLCPVSGWAPLSKNN AVRIGSKGDVFIREFPISCS? LECRTEFFLTQGALLNDKHSNGT IKDRSPYRTLMSVPIGSVPSPY NARFESIAWSASACHDGINWLT IGITGPDGSAVAILKYNGIITD TIKSWRNNILRTQESACVNG SCFTIMTDGPSDQASYKIFRI EKGKIKSVEMKAPNYHYECS CYPDSSEITCVCARDNWHGSRNP WVSFNQNLQYQMGYICSGVFGD NPRPNDKTGSCGPVSSNGANGV KGFSFKYNGVWIGRTKSISSR KGFEMIWDPNGWGTGDNKFSIK QDIVGINEWSGYSGSFVMHPEL TGLDCIVPCFWVELIRGRPEEN TIWTSGSSISFCGVNSDTVGWS WPDGAELPFTIDK (SEQ ID NO:15)	N1 numbering: 99P/177I/19 6T/205I, 165S, 100L/408M/4 19V, 161V/172A, 103N/105A	vklnssslcpvsgwaPLskNn AvrigskgdvfiRefpiscsp lecrteffltqgallndkhsngt ikdrspyrtlmsVpigSvpspy nArfesIawsasachdginwlt igiTgpdsgavaIlkyngiitd tikswrnnilrtqesecacvng scftimtdgpsdgqasykifri ekgkiiksvemkapnyhyeecs cypdsseitcvcrdnwhgsnrp wvsfnqnleyqmgycsgvfgd nprpndktgscgpvssngangv kgfsfkyngvwigrtksissr kgfemiwdpnngwtgtdnkfsik qdivginewsgysgsfvmhpel tgldciVpcfwwelirgrpeen tiwtsgssisfcgvnsdtvgws wpdgaelpftidk (SEQ ID NO:15)	80-90%
165_N1- Mi15_c1 55_V114 I_V166P	VKLGNSSSLCPVSGWAPLSKDN SVRIGSKGDI FVIREFPISCS? LECRTEFFLTQGALLNDKHSNGT IKDRSPYRTLMSVPIGSPPSPY NARFESIAWSASACHDGINWLT IGITGPDGSAVAILKYNGIITD TIKSWRNNILRTQESACVNG SCFTIMTDGPSDQASYKIFRI EKGKIKSVEMKAPNYHYECS CYPDSSEITCVCARDNWHGSRNP WVSFNQNLQYQMGYICSGVFGD NPRPNDKTGSCGPVSSNGANGV KGFSFKYNGVWIGRTKSISSR KGFEMIWDPNGWGTGDNKFSIK QDIVGINEWSGYSGSFVMHPEL TGLDCIVPCFWVELIRGRPEEN TIWTSGSSISFCGVNSDTVGWS WPDGAELPFTIDK (SEQ ID NO:16)	N1 numbering: 99P/177I/19 6T/205I, 165S, 100L/408M/4 19V, 161V/172A, 114I/166P	vklnssslcpvsgwaPLskdn svrigskgdiFvirefpiscsp lecrteffltqgallndkhsngt ikdrspyrtlmsVpigSPpspy nArfesIawsasachdginwlt igiTgpdsgavaIlkyngiitd tikswrnnilrtqesecacvng scftimtdgpsdgqasykifri ekgkiiksvemkapnyhyeecs cypdsseitcvcrdnwhgsnrp wvsfnqnleyqmgycsgvfgd nprpndktgscgpvssngangv kgfsfkyngvwigrtksissr kgfemiwdpnngwtgtdnkfsik qdivginewsgysgsfvmhpel tgldciVpcfwwelirgrpeen tiwtsgssisfcgvnsdtvgws wpdgaelpftidk (SEQ ID NO:16)	100%
167_N1- Mi15_c1 55_S101 C_I122V _G164C_	VKLGNSSSLCPVSGWAPLCKDN SVRIGSKGDVFIREFVSCSP LECRTEFFLTQGALLNDKHSNGT IKDRSPYRTLMSVPICSVPSPY NARVESIAWSASACHDGINWLT IGITGPDGSAVAILKYNGIITD	N1 numbering: 99P/177I/19 6T/205I, 165S, 100L/408M/4	vklnssslcpvsgwaPLCkdn svrigskgdvfiRefpVscsp lecrteffltqgallndkhsngt ikdrspyrtlmsVpiCSvpspy nArVesIawsasachdginwlt igiTgpdsgavaIlkyngiitd	100%

F174V_S 444V	TIKSWRNNILRTQSEECACVNG SCFTIMTDGPGSDGQASYKIFRI EKGKIIKSVMKAPNYHYECS CYPDSSEITCVCARDNWHGSRNP WVSFNQNLLEYQMGYICSGVFGD NPRPNDKTGSCGPVSSNGANGV KGFsfkygngvwigrtksissr KGFEMIWDPNGWGTGTDNKFSIK QDIVGINEWSGYSGSFMHPPEL TGLDCIVPCFWVELIRGRPEEN TIWTSGSSISFCGVNSDTVGWS WPDGAELPFTIDK (SEQ ID NO:17)	19V, 161V/172A, 101C/122V/1 64C/174V/44 4V	tikswrnnilrtqesecacvng scftimtdgpgsdgqasykifri ekgkiiksvenkapnyhyeecs cypdsseitcvcrdnwhgsnrp wvsfnqnleyqmgycsgvfgd nprpndktgscgpvssngangv kgfsfkygngvwigrtksissr kgfemiwdpnngwtgtndkfsik qdivginewsgysgsfvmhpel tgldciVpcfvelirgrpeen tiwtsgssivfcgvnsdtvgws wpdgaelpftidk (SEQ ID NO:17)	
174_N1- Mi15_c1 55_T131 Q	VKLGNSSSLCPVSGWAPLSKDN SVRIGSKGDVFIREFPISCS? LECRQFFLTQGALLNDKHSNGT IKDRSPYRTLMSVPIGSPSPY NARFESIAWSASACHDGINWLT IGITGPDGSAVAILKYNGIITD TIKSWRNNILRTQSEECACVNG SCFTIMTDGPGSDGQASYKIFRI EKGKIIKSVMKAPNYHYECS CYPDSSEITCVCARDNWHGSRNP WVSFNQNLLEYQMGYICSGVFGD NPRPNDKTGSCGPVSSNGANGV KGFsfkygngvwigrtksissr KGFEMIWDPNGWGTGTDNKFSIK QDIVGINEWSGYSGSFMHPPEL TGLDCIVPCFWVELIRGRPEEN TIWTSGSSISFCGVNSDTVGWS WPDGAELPFTIDK (SEQ ID NO:18)	N1 numbering: 99P/177I/19 6T/205I, 165S, 100L/408M/4 19V, 161V/172A, 131Q	vklnagnssslcpvsgwaPlskdn svrigskgdvfiorefiscsp lecrQffltqgallndkhsngt ikdrspyrtlmsVpigSvpspy nArfesIawsasachdginwlt igiTgpdsgavaIlkyngiitd tikswrnnilrtqesecacvng scftimtdgpgsdgqasykifri ekgkiiksvenkapnyhyeecs cypdsseitcvcrdnwhgsnrp wvsfnqnleyqmgycsgvfgd nprpndktgscgpvssngangv kgfsfkygngvwigrtksissr kgfemiwdpnngwtgtndkfsik qdivginewsgysgsfvmhpel tgldciVpcfvelirgrpeen tiwtsgssisfcgvnsdtvgws wpdgaelpftidk (SEQ ID NO:18)	100%
175_N1- Mi15_c1 55_D103 N_S105A _T131Q	VKLGNSSSLCPVSGWAPLSKNN AVRIGSKGDVFIREFPISCS? LECRQFFLTQGALLNDKHSNGT IKDRSPYRTLMSVPIGSPSPY NARFESIAWSASACHDGINWLT IGITGPDGSAVAILKYNGIITD TIKSWRNNILRTQSEECACVNG SCFTIMTDGPGSDGQASYKIFRI EKGKIIKSVMKAPNYHYECS CYPDSSEITCVCARDNWHGSRNP WVSFNQNLLEYQMGYICSGVFGD NPRPNDKTGSCGPVSSNGANGV KGFsfkygngvwigrtksissr KGFEMIWDPNGWGTGTDNKFSIK QDIVGINEWSGYSGSFMHPPEL TGLDCIVPCFWVELIRGRPEEN TIWTSGSSISFCGVNSDTVGWS WPDGAELPFTIDK (SEQ ID NO:19)	N1 numbering: 99P/177I/19 6T/205I, 165S, 100L/408M/4 19V, 161V/172A, 103N/105A, 131Q	vklnagnssslcpvsgwaPlskNn Avrigskgdvfiorefiscsp lecrQffltqgallndkhsngt ikdrspyrtlmsVpigSvpspy nArfesIawsasachdginwlt igiTgpdsgavaIlkyngiitd tikswrnnilrtqesecacvng scftimtdgpgsdgqasykifri ekgkiiksvenkapnyhyeecs cypdsseitcvcrdnwhgsnrp wvsfnqnleyqmgycsgvfgd nprpndktgscgpvssngangv kgfsfkygngvwigrtksissr kgfemiwdpnngwtgtndkfsik qdivginewsgysgsfvmhpel tgldciVpcfvelirgrpeen tiwtsgssisfcgvnsdtvgws wpdgaelpftidk (SEQ ID NO:19)	90-100%
176_N1- Mi15_c1 55_V114 I_V166P _T131Q	VKLGNSSSLCPVSGWAPLSKDN SVRIGSKGDI FVIREPFISCS? LECRQFFLTQGALLNDKHSNGT IKDRSPYRTLMSVPIGSPSPY NARFESIAWSASACHDGINWLT IGITGPDGSAVAILKYNGIITD TIKSWRNNILRTQSEECACVNG SCFTIMTDGPGSDGQASYKIFRI EKGKIIKSVMKAPNYHYECS CYPDSSEITCVCARDNWHGSRNP	N1 numbering: 99P/177I/19 6T/205I, 165S, 100L/408M/4 19V, 161V/172A, 131Q, 114I/166P	vklnagnssslcpvsgwaPlskdn svrigskgdIfvirepfiscsp lecrQffltqgallndkhsngt ikdrspyrtlmsVpigSPpspy nArfesIawsasachdginwlt igiTgpdsgavaIlkyngiitd tikswrnnilrtqesecacvng scftimtdgpgsdgqasykifri ekgkiiksvenkapnyhyeecs cypdsseitcvcrdnwhgsnrp	100%

	WVSFNQNLEYQMGYICSGVFGD NPRPNDKTGSCGPVSSNGANGV KGFSFKYGNVWIGRTKSISSR KGFEMIWDPNGWGTGTDNKFISIK QDIVGINEWSGYSGSFMHPHEL TGLDCIVPCFWVELIRGRPEEN TIWTSGSSISFCGVNSDTVGWS WPDGAELPFTIDK (SEQ ID NO:20)		wvsfnqnleyqmgycsgvfgd nprpndktgscgpvssngangv kgfsfkyngvwigrtksissr kgfemiwdpngwtgtdnkfsik qdivginewsgysgsfvmhpel tgldciVpcfwvelirgrpeen tiwtsgssisfcgvnsdtvgws wpdgaelpftidk (SEQ ID NO:20)	
178_N1- Mi15_c1 55_T131 Q_S442I	VKLAGNSSLCPVSGWAPLSKDN SVRIGSKGDVFIREFFISCS? LECRQFFLTQGALLNDKHSNGT IKDRSPYRTLMSVPIGSVPSPY NARFESIAWSASACHDGINWLT IGITGPDGSAVAILKYNGIITD TIKSWRNNILRTQESACVNG SCFTIMTDGPSDQASYKIFRI EKGKIIKSVMKAPNYHYECS CYPDSSEITCVCARDNWHGSRNP WVSFNQNLEYQMGYICSGVFGD NPRPNDKTGSCGPVSSNGANGV KGFSFKYGNVWIGRTKSISSR KGFEMIWDPNGWGTGTDNKFISIK QDIVGINEWSGYSGSFMHPHEL TGLDCIVPCFWVELIRGRPEEN TIWTSGSIISFCGVNSDTVGWS WPDGAELPFTIDK (SEQ ID NO:21)	N1 numbering: 99P/177I/19 6T/205I, 165S, 100L/408M/4 19V, 161V/172A, 131Q/442I	vklaGnsslcPvsgwaPlskdn svrigskgdvfiRefFiscsp lecrQffltqgallndkhsngt ikdrspyrtlmsVpigSvpspy nArfesIawsasachdginwlt igiTgpdsgavaIlkyngiitd tikswrnnilrtqesecacvng scftimtdgpsdgqasykifri ekgkiiksvemkapnyhyeecs cypdsseitcvcrdnwhgsnrp wvsfnqnleyqmgycsgvfgd nprpndktgscgpvssngangv kgfsfkyngvwigrtksissr kgfemiwdpngwtgtdnkfsik qdivginewsgysgsfvmhpel tgldciVpcfwvelirgrpeen tiwtsgsIisfcgvnsdtvgws wpdgaelpftidk (SEQ ID NO:21)	90-100%
181_N1- Mi15_c1 55_T131 M_S442I	VKLAGNSSLCPVSGWAPLSKDN SVRIGSKGDVFIREFFISCS? LECRMFFLTQGALLNDKHSNGT IKDRSPYRTLMSVPIGSVPSPY NARFESIAWSASACHDGINWLT IGITGPDGSAVAILKYNGIITD TIKSWRNNILRTQESACVNG SCFTIMTDGPSDQASYKIFRI EKGKIIKSVMKAPNYHYECS CYPDSSEITCVCARDNWHGSRNP WVSFNQNLEYQMGYICSGVFGD NPRPNDKTGSCGPVSSNGANGV KGFSFKYGNVWIGRTKSISSR KGFEMIWDPNGWGTGTDNKFISIK QDIVGINEWSGYSGSFMHPHEL TGLDCIVPCFWVELIRGRPEEN TIWTSGSIISFCGVNSDTVGWS WPDGAELPFTIDK (SEQ ID NO:22)	N1 numbering: 99P/177I/19 6T/205I, 165S, 100L/408M/4 19V, 161V/172A, 131M/442I	vklaGnsslcPvsgwaPlskdn svrigskgdvfiRefFiscsp lecrMffltqgallndkhsngt ikdrspyrtlmsVpigSvpspy nArfesIawsasachdginwlt igiTgpdsgavaIlkyngiitd tikswrnnilrtqesecacvng scftimtdgpsdgqasykifri ekgkiiksvemkapnyhyeecs cypdsseitcvcrdnwhgsnrp wvsfnqnleyqmgycsgvfgd nprpndktgscgpvssngangv kgfsfkyngvwigrtksissr kgfemiwdpngwtgtdnkfsik qdivginewsgysgsfvmhpel tgldciVpcfwvelirgrpeen tiwtsgsIisfcgvnsdtvgws wpdgaelpftidk (SEQ ID NO:22)	90-100%
182_N1- Mi15_c1 55_D103 N_S105A _T131M- S442I	VKLAGNSSLCPVSGWAPLSKNN AVRIGSKGDVFIREFFISCS? LECRMFFLTQGALLNDKHSNGT IKDRSPYRTLMSVPIGSVPSPY NARFESIAWSASACHDGINWLT IGITGPDGSAVAILKYNGIITD TIKSWRNNILRTQESACVNG SCFTIMTDGPSDQASYKIFRI EKGKIIKSVMKAPNYHYECS CYPDSSEITCVCARDNWHGSRNP WVSFNQNLEYQMGYICSGVFGD NPRPNDKTGSCGPVSSNGANGV KGFSFKYGNVWIGRTKSISSR KGFEMIWDPNGWGTGTDNKFISIK	N1 numbering: 99P/177I/19 6T/205I, 165S, 100L/408M/4 19V, 161V/172A, 103N/105A, 131M/442I	vklaGnsslcPvsgwaPlskNn AvrigskgdvfiRefFiscsp lecrMffltqgallndkhsngt ikdrspyrtlmsVpigSvpspy nArfesIawsasachdginwlt igiTgpdsgavaIlkyngiitd tikswrnnilrtqesecacvng scftimtdgpsdgqasykifri ekgkiiksvemkapnyhyeecs cypdsseitcvcrdnwhgsnrp wvsfnqnleyqmgycsgvfgd nprpndktgscgpvssngangv kgfsfkyngvwigrtksissr kgfemiwdpngwtgtdnkfsik	90-100%

	QDIVGINEWSGYSGSFMHPEL TGLDCIVPCFWVELIRGRPEEN TIWTSGSIIISFCGVNSDTVGWS WPDGAELPFTIDK (SEQ ID NO:23)		qdivginewsgygsfvmhpel tgldciVpcfwwelirgrpeen tiwtsgsIisfcgvnsdtvgws wpdgaelpftidk (SEQ ID NO:23)	
183 N1- Mi15_c1 55_S101 A_T131M I163L S442I_S 444A	VKLAGNSSLCPVSGWAPLAKDN SVRIGSKGDFVIREPFISCS? LECRMFFLTQGALLNDKHSNGT IKDRSPYRTLMSVPLGSPSPY NARFESIAWSASACHDGINWLT IGITGPDGSGAVAILKYNGIITD TIKSWRNNILRTQSEECACVNG SCFTIMTDGPGSDGQASYKIFRI EKGKIIKSVMKAPNYHYEES CYPDSSEITCVCARDNWHGSRNP WVSFNQNLQMGYICSGVFGD NPRPNDKTGSCGPVSSNGANGV KGFSFKYGNVWIGRTKSISR KGFEMIWDPNGTGTDNKFSIK QDIVGINEWSGYSGSFMHPEL TGLDCIVPCFWVELIRGRPEEN TIWTSGSIIISFCGVNSDTVGWS WPDGAELPFTIDK (SEQ ID NO:24)	N1 numbering: 99P/177I/19 6T/205I, 165S, 100L/408M/4 19V, 161V/172A, 101A/131M/1 63L/442I/44 4A	vkla g n s s l c p v s g w a P L A k d n s v r i g s k g d f v i r e p f i s c s ? l e c r m f f l t q g a l l n d k h s n g t i k d r s p y r t l m s v p l g s p s p y n a r f e s i a w s a s a c h d g i n w l t i g i t g p d s g a v a i l k y n g i i t d t i k s w r n n i l r t q e s e c a c v n g s c f t i m t d g p s d g q a s y k i f r i e k g k i i k s v m k a p n y h y e e c s c y p d s s e i t c v c r d n w h g s n r p w v s f n q n l e y q m g y i c s g v f g d n p r p n d k t g s c g p v s s n g a n g v k g f s f k y g n v w i g r t k s i s s r k g f e m i w d p n g w t g t d n k f s i k q d i v g i n e w s g y s g s f m h p e l t g l d c i v p c f w v e l i r g r p e e n t i w t s g s I i s f c g v n s d t v g w s w p d g a e l p f t i d k (S E Q I D N O : 2 4)	95-100%
185 N1- Mi15_DF 2TI_V11 4I_V166 P	VKLAGNSSLCPVSGWAPLSKDN SVRIGSKGEIVIREPFISCS? LECRFFLTQGALLNDKHSNGT IKDRSPYRTLMSCPIGSPSPPT NSRFESIAWSASACHDGINWLT IGITGPDGSGAVAILKYNGIITD TIKSWRNNILRTQSEECACVNG SCFTIMTDGPGSDGQASYKIFRI EKGKIIKSVMKAPNYHYEES CYPDSSEITCVCARDNWHGSRNP WVSFNQNLQMGYICSGVFGD NPRPNDKTGSCGPVSSNGANGV KGFSFKYGNVWIGRTKSISR KGFEMIWDPNGTGTDNKFSIK QDIVGINEWSGYSGSFMHPEL TGLDCIVPCFWVELIRGRPEEN TIWTSGSIIISFCGVNSDTTGWWS WPDGAELPFTIDK (SEQ ID NO:25)	N1 numbering: 99P/177I/19 6T/205I, 113E/170T, 165S, 100L/408M/4 19V, 453T, 114I/166P	vkla g n s s l c p v s g w a P L s k d n s v r i g s k g E i v i r e p f i s c s p l e c r t f f l t q g a l l n d k h s n g t i k d r s p y r t l m s c p i g s p s p t n s r f e s i a w s a s a c h d g i n w l t i g i t g p d s g a v a i l k y n g i i t d t i k s w r n n i l r t q e s e c a c v n g s c f t i m t d g p s d g q a s y k i f r i e k g k i i k s v m k a p n y h y e e c s c y p d s s e i t c v c r d n w h g s n r p w v s f n q n l e y q m g y i c s g v f g d n p r p n d k t g s c g p v s s n g a n g v k g f s f k y g n v w i g r t k s i s s r k g f e m i w d p n g w t g t d n k f s i k q d i v g i n e w s g y s g s f m h p e l t g l d c i v p c f w v e l i r g r p e e n t i w t s g s s i s f c g v n s d t T g w s w p d g a e l p f t i d k (S E Q I D N O : 2 5)	40%
194 N1- Mi15_DF 2TI_T13 1Q	VKLAGNSSLCPVSGWAPLSKDN SVRIGSKGEVIREPFISCS? LECRQFFLTQGALLNDKHSNGT IKDRSPYRTLMSCPIGSPSPPT NSRFESIAWSASACHDGINWLT IGITGPDGSGAVAILKYNGIITD TIKSWRNNILRTQSEECACVNG SCFTIMTDGPGSDGQASYKIFRI EKGKIIKSVMKAPNYHYEES CYPDSSEITCVCARDNWHGSRNP WVSFNQNLQMGYICSGVFGD NPRPNDKTGSCGPVSSNGANGV KGFSFKYGNVWIGRTKSISR KGFEMIWDPNGTGTDNKFSIK QDIVGINEWSGYSGSFMHPEL TGLDCIVPCFWVELIRGRPEEN TIWTSGSIIISFCGVNSDTTGWWS	N1 numbering: 99P/177I/19 6T/205I, 113E/170T, 165S, 100L/408M/4 19V, 453T, T131Q	vkla g n s s l c p v s g w a P L s k d n s v r i g s k g E v i r e p f i s c s p l e c r Q f f l t q g a l l n d k h s n g t i k d r s p y r t l m s c p i g s p s p t n s r f e s i a w s a s a c h d g i n w l t i g i t g p d s g a v a i l k y n g i i t d t i k s w r n n i l r t q e s e c a c v n g s c f t i m t d g p s d g q a s y k i f r i e k g k i i k s v m k a p n y h y e e c s c y p d s s e i t c v c r d n w h g s n r p w v s f n q n l e y q m g y i c s g v f g d n p r p n d k t g s c g p v s s n g a n g v k g f s f k y g n v w i g r t k s i s s r k g f e m i w d p n g w t g t d n k f s i k q d i v g i n e w s g y s g s f m h p e l t g l d c i v p c f w v e l i r g r p e e n t i w t s g s s i s f c g v n s d t T g w s	70-80%

	WPDGAELPFTIDK (SEQ ID NO:26)		wpdgaelpftidk (SEQ ID NO:26)	
195 N1-Mi15_DF 2TI_D10 3N_S105 A_T131Q	VKLAGNSSLCPVSGWAPLSKNN AVRIGSKGEVFIREFPISCS? LECRQFFLTQGALLNDKHSNGT IKDRSPYRTLMSCPIGSVSPST NSRFESIAWSASACHDGINWLT IGITGPDGSAVAILKYNGIITD TIKSWRNNILRTQESACACVNG SCFTIMTDGPGSDGQASYKIFRI EKGKIIKSVEMKAPNYHYECS CYPDSSEITCVCARDNWHGSRNP WVSFNQNLLEYQMGYICSGVFGD NPRPNDKTGSCGPVSSNGANGV KGFSFKYGNVWIGRTKSISR KGFEMIWDPNGTGTDNKFSIK QDIVGINEWSGYSGSFMHPEL TGLDCIVPCFWVELIRGRPEEN TIWTSGSSISFCGVNSDTTGWS WPDGAELPFTIDK (SEQ ID NO:27)	N1 numbering: 99P/177I/19 6T/205I, 113E/170T, 165S, 100L/408M/4 19V, 453T, 103N/105A T131Q	vkla g n s s l c p v s g w a P L s k N n A v r i g s k g E v f i r e p f i s c s p l e c r Q f f l t q g a l l n d k h s n g t i k d r s p y r t l m s c p i g s v s p s t n s r f e s i a w s a s a c h d g i n w l t i g i T g p d s g a v a i l k y n g i i t d t i k s w r n n i l r t q e s e c a c v n g s c f t i m t d g p s d g q a s y k i f r i e k g k i i k s v e m k a p n y h y e e c s c y p d s s e i t c v c r d n w h g s n r p w v s f n q n l e y q m g y i c s g v f g d n p r p n d k t g s c g p v s s n g a n g v k g f s f k y g n v w i g r t k s i s s r k g f e m i w d p n g w t g t d n k f s i k q d i v g i n e w s g y s g s f v m h p e l t g l d c i v p c f w v e l i r g r p e e n t i w t s g s s i s f c g v n s d t t g w s w p d g a e l p f t i d k (S E Q I D N O : 2 7)	50-60%
196 N1-Mi15_DF 2TI_V11 4I_V166 P_T131Q	VKLAGNSSLCPVSGWAPLSKDN SVRIGSKGEIFVIREPFISCS? LECRQFFLTQGALLNDKHSNGT IKDRSPYRTLMSCPIGSPSPST NSRFESIAWSASACHDGINWLT IGITGPDGSAVAILKYNGIITD TIKSWRNNILRTQESACACVNG SCFTIMTDGPGSDGQASYKIFRI EKGKIIKSVEMKAPNYHYECS CYPDSSEITCVCARDNWHGSRNP WVSFNQNLLEYQMGYICSGVFGD NPRPNDKTGSCGPVSSNGANGV KGFSFKYGNVWIGRTKSISR KGFEMIWDPNGTGTDNKFSIK QDIVGINEWSGYSGSFMHPEL TGLDCIVPCFWVELIRGRPEEN TIWTSGSSISFCGVNSDTTGWS WPDGAELPFTIDK (SEQ ID NO:28)	N1 numbering: 99P/177I/19 6T/205I, 113E/170T, 165S, 100L/408M/4 19V, 453T, 114I/166P, T131Q	vkla g n s s l c p v s g w a P L s k d n s v r i g s k g E i f v i r e p f i s c s p l e c r Q f f l t q g a l l n d k h s n g t i k d r s p y r t l m s c p i g s p s p s t n s r f e s i a w s a s a c h d g i n w l t i g i T g p d s g a v a i l k y n g i i t d t i k s w r n n i l r t q e s e c a c v n g s c f t i m t d g p s d g q a s y k i f r i e k g k i i k s v e m k a p n y h y e e c s c y p d s s e i t c v c r d n w h g s n r p w v s f n q n l e y q m g y i c s g v f g d n p r p n d k t g s c g p v s s n g a n g v k g f s f k y g n v w i g r t k s i s s r k g f e m i w d p n g w t g t d n k f s i k q d i v g i n e w s g y s g s f v m h p e l t g l d c i v p c f w v e l i r g r p e e n t i w t s g s s i s f c g v n s d t t g w s w p d g a e l p f t i d k (S E Q I D N O : 2 8)	40%
198 N1-Mi15_DF 2TI_T13 1Q_S442 I	VKLAGNSSLCPVSGWAPLSKDN SVRIGSKGEVFIREFPISCS? LECRQFFLTQGALLNDKHSNGT IKDRSPYRTLMSCPIGSVSPST NSRFESIAWSASACHDGINWLT IGITGPDGSAVAILKYNGIITD TIKSWRNNILRTQESACACVNG SCFTIMTDGPGSDGQASYKIFRI EKGKIIKSVEMKAPNYHYECS CYPDSSEITCVCARDNWHGSRNP WVSFNQNLLEYQMGYICSGVFGD NPRPNDKTGSCGPVSSNGANGV KGFSFKYGNVWIGRTKSISR KGFEMIWDPNGTGTDNKFSIK QDIVGINEWSGYSGSFMHPEL TGLDCIVPCFWVELIRGRPEEN TIWTSGSSISFCGVNSDTTGWS WPDGAELPFTIDK (SEQ ID NO:29)	N1 numbering: 99P/177I/19 6T/205I, 113E/170T, 165S, 100L/408M/4 19V, 453T, T131Q/442I	vkla g n s s l c p v s g w a P L s k d n s v r i g s k g E v f i r e p f i s c s p l e c r Q f f l t q g a l l n d k h s n g t i k d r s p y r t l m s c p i g s v s p s t n s r f e s i a w s a s a c h d g i n w l t i g i T g p d s g a v a i l k y n g i i t d t i k s w r n n i l r t q e s e c a c v n g s c f t i m t d g p s d g q a s y k i f r i e k g k i i k s v e m k a p n y h y e e c s c y p d s s e i t c v c r d n w h g s n r p w v s f n q n l e y q m g y i c s g v f g d n p r p n d k t g s c g p v s s n g a n g v k g f s f k y g n v w i g r t k s i s s r k g f e m i w d p n g w t g t d n k f s i k q d i v g i n e w s g y s g s f v m h p e l t g l d c i v p c f w v e l i r g r p e e n t i w t s g s i s f c g v n s d t t g w s w p d g a e l p f t i d k (S E Q I D N O : 2 9)	20-30%
201 N1-Mi15_DF	VKLAGNSSLCPVSGWAPLSKDN SVRIGSKGEVFIREFPISCS?	N1 numbering:	vkla g n s s l c p v s g w a P L s k d n s v r i g s k g E v f i r e p f i s c s p	50%

2TI T13 1M_S442 I	LECRMFFLTQGALLNDKHSNGT IKDRSPYRTLMSCPIGSVSPST NSRFESIAWSASACHDGINWLT IGITGPDGSAVAILKYNGIITD TIKSWRNNILRTQESACVNG SCFTIMTDGPSDQASYKIFRI EKGKI IKSVMKAPNYHYECS CYPDSSEITCVCARDNWHGSRNP WVSFNQNL EYQMGYICSGVFGD NPRPNDKTGSCGPVSSNGANGV KGFsfkygngvwigrtksissr KGFEMIWDPNGWGTGDNKFSIK QDIVGINEWSGYSGSFMHPEL TGLDCIVPCFWVELIRGRPEEN TIWTSGSIISFCGVNSDTTGWS WPDGAELPFTIDK (SEQ ID NO:30)	99P/177I/19 6T/205I, 113E/170T, 165S, 100L/408M/4 19V, 453T, T131M/442I	lecrMffltqgallndkhsngt ikdrspyrtlmscpigsvspst nsrfesiawsasachdginwlt igitgpdsgavaIlkyngiitd tikswrnnilrtqesecacvng scftimtdgpsdgqasykifri ekgkiiksvekapnyhyeecs cypdsseitcvcrdnwhgsnrp wvsfnqnleyqmgycsgvfgd nprpndktgscgpvssngangv kgfsfkygngvwigrtksissr kgfemiwdpnngwtgtdnkfsik qdivginewsgysgsfvmhpel tgldciVpcfwwelirgrpeen tiwtsgsIisfcgvnsdtTgws wpdgaelpftidk (SEQ ID NO:30)	
202 N1- Mi15_DF 2TI D10 3N_S105 A_T131M _S442I	VKLAGNSSLCPVSGWAPLskNN AVRIGSKGEVfVIREPFISCS? LECRMFFLTQGALLNDKHSNGT IKDRSPYRTLMSCPIGSVSPST NSRFESIAWSASACHDGINWLT IGITGPDGSAVAILKYNGIITD TIKSWRNNILRTQESACVNG SCFTIMTDGPSDQASYKIFRI EKGKI IKSVMKAPNYHYECS CYPDSSEITCVCARDNWHGSRNP WVSFNQNL EYQMGYICSGVFGD NPRPNDKTGSCGPVSSNGANGV KGFsfkygngvwigrtksissr KGFEMIWDPNGWGTGDNKFSIK QDIVGINEWSGYSGSFMHPEL TGLDCIVPCFWVELIRGRPEEN TIWTSGSIISFCGVNSDTTGWS WPDGAELPFTIDK (SEQ ID NO:31)	N1 numbering: 99P/177I/19 6T/205I, 113E/170T, 165S, 100L/408M/4 19V, 453T, 103N/105A, T131M/442I	vklaGnsslcpvsgwaPlskNn AvrigskgEvfvirepfiscsp lecrMffltqgallndkhsngt ikdrspyrtlmscpigsvspst nsrfesiawsasachdginwlt igitgpdsgavaIlkyngiitd tikswrnnilrtqesecacvng scftimtdgpsdgqasykifri ekgkiiksvekapnyhyeecs cypdsseitcvcrdnwhgsnrp wvsfnqnleyqmgycsgvfgd nprpndktgscgpvssngangv kgfsfkygngvwigrtksissr kgfemiwdpnngwtgtdnkfsik qdivginewsgysgsfvmhpel tgldciVpcfwwelirgrpeen tiwtsgsIisfcgvnsdtTgws wpdgaelpftidk (SEQ ID NO:31)	20%
203 N1- Mi15_DF 2TI_S10 1A_T131 M_I163L _S442I - S444A	VKLAGNSSLCPVSGWAPLAKDN SVRIGSKGEVfVIREPFISCS? LECRMFFLTQGALLNDKHSNGT IKDRSPYRTLMSCPVSPST NSRFESIAWSASACHDGINWLT IGITGPDGSAVAILKYNGIITD TIKSWRNNILRTQESACVNG SCFTIMTDGPSDQASYKIFRI EKGKI IKSVMKAPNYHYECS CYPDSSEITCVCARDNWHGSRNP WVSFNQNL EYQMGYICSGVFGD NPRPNDKTGSCGPVSSNGANGV KGFsfkygngvwigrtksissr KGFEMIWDPNGWGTGDNKFSIK QDIVGINEWSGYSGSFMHPEL TGLDCIVPCFWVELIRGRPEEN TIWTSGSIISFCGVNSDTTGWS WPDGAELPFTIDK (SEQ ID NO:32)	N1 numbering: 99P/177I/19 6T/205I, 113E/170T, 165S, 100L/408M/4 19V, 453T, 101A/163L/T 131M/442I/4 44A	vklaGnsslcpvsgwaPlakdn svrigskgEvfvirepfiscsp lecrMffltqgallndkhsngt ikdrspyrtlmscpVgsvspst nsrfesiawsasachdginwlt igitgpdsgavaIlkyngiitd tikswrnnilrtqesecacvng scftimtdgpsdgqasykifri ekgkiiksvekapnyhyeecs cypdsseitcvcrdnwhgsnrp wvsfnqnleyqmgycsgvfgd nprpndktgscgpvssngangv kgfsfkygngvwigrtksissr kgfemiwdpnngwtgtdnkfsik qdivginewsgysgsfvmhpel tgldciVpcfwwelirgrpeen tiwtsgsIiAfcgvnsdtTgws wpdgaelpftidk (SEQ ID NO:32)	90%
249 N2- Wis05_V 165Q	EYRNWSKPQCNIITGFAPFSKDN SIRLSAGGDIWVTREPVS CD? DKCYQFALGQGTTLNNVHSNDT VHDRTPYRTLMLNELGQPFHLG TKQVCIAWSSSSCHDGAWLHV CVTGDDKNATASFIYNGRLVDS	N2 numbering: 165Q (mutations promote)	eyrnwskpqcnitgfapfskdn sirlsaggdiwvtrepvscdp dkcyqfalgggttlnnvhsndt vhdrtpyrllmnelgqpflhg tkqvciawsssschdgkawlhv cvtgddknatasfiyngrlvds	50% closed (50% open)

	IVSWSKEILRTQSEECVCINGT CTVVMTDGSASGKADTKILFIE EGKIVHTSTLGSQAQHVEEESC YPRYLGVRVCVRDNWKGSNRPI VDINIKDYSIVSSYVCSGLVGD TPRKNDSSSSSHCLDPNNEEGG HGVKGWAFDDGNDVVMGRTISE KLRSGETFKVIEGWSNPNSKL QINRQVIVDRGNRSGYSGIFSV EGKSCINRCFYVELIRGRKEET EVLWTSNSIVVFCGTSPTYGTG SWPDGADINLMP1 (SEQ ID NO:33)	open state of NA)	ivswskeilrtqesecvcingt ctvvmtdgsasgkadtakilfie egkivhtstlsgsaqhveesc yprylgvrcvcrdnwkgssnrpi vdinikdysivssyvcsglvgd tprkndssssshcldpnneegg hgvkgwafddgndvwmgrtise klrsgyetfkviiegwsnpnsl qinrqvivdrgnrsgysgifs egkscinrcfyvelirgrkeet evlwtssnivvfcgtsptygtg swpdgadinlmpi (SEQ ID NO:33)	
255_N2- Wis05_V 165Q_I1 76V_T19 5S	EYRNWSKPQCNTGFAPFSKDN SIRLSAGGDIWVTREPVS CD? DKCYQFALGGQTLLNNVHSDT VHDRTPYRTLMLNELGQPFHLG TKQVCVAVSSSCHDGAWLHV CVSGDDKNATASFIYNGRLVDS IVSWSKEILRTQSEECVCINGT CTVVMTDGSASGKADTKILFIE EGKIVHTSTLGSQAQHVEEESC YPRYLGVRVCVRDNWKGSNRPI VDINIKDYSIVSSYVCSGLVGD TPRKNDSSSSSHCLDPNNEEGG HGVKGWAFDDGNDVVMGRTISE KLRSGETFKVIEGWSNPNSKL QINRQVIVDRGNRSGYSGIFSV EGKSCINRCFYVELIRGRKEET EVLWTSNSIVVFCGTSPTYGTG SWPDGADINLMP1 (SEQ ID NO:34)	N2 numbering: 165Q, 176I, 195S (mutations promote open state of NA)	eyrnwskpqcntgfapfskdn sirlsaggdiwvtrepvscdp dkcyqfalggqgtllnnvhsndt vhdrtpyrllmnelgqpfhl tkqvcvawssschdgawlhv cvsgddknatasfiyngrlvds ivswskeilrtqesecvcingt ctvvmtdgsasgkadtakilfie egkivhtstlsgsaqhveesc yprylgvrcvcrdnwkgssnrpi vdinikdysivssyvcsglvgd tprkndssssshcldpnneegg hgvkgwafddgndvwmgrtise klrsgyetfkviiegwsnpnsl qinrqvivdrgnrsgysgifs egkscinrcfyvelirgrkeet evlwtssnivvfcgtsptygtg swpdgadinlmpi (SEQ ID NO:34)	0% closed (100% open)
282_N8- Jiangxi - Donghu2 013_Q16 3S	HFMNTEALCDAGFAPFSKDN GIRIGSRGHVFVIREPFVSCS? TECRTFFLTQGSLLNDKHSNGT VKDRSPYRTLMSVEIGSSPNVY QARFEAVAWSATACHDGKKWMT IGVTGPDAAVAVVHYGGIPTD VINSWAGDILRTQESSCTCIQG ECFWVMTDGPANRQAQYRAFA KQGKIVGQAEISFNGGHIIECS CYPNEGKVECVCKDNWTGTRP VLVISPDLSYRVGYLCAGLPSD TPRGDSQFTGSGTSPMGNQGY GVKGFGFRQGNVVMGRTISRT SRSGFEILKVRNGWVQNSKEQI KRQVVVDNLNWSGYSGSFTLPA ELTKRNCVPCFWVEMIRGNPE EKTIWTSSSSIVMCGVDHEIAD WSWHDGAILPFDIDKM (SEQ ID NO:35)	N8 numbering: 163S	hfmntealcdagfapfskdn girigsrghvfvirepfvscsp tecrtflltqgsllndkhsngt vkdrspyrtlmsveigsspnvy qarfeavawsatachdgkkwmt igvtgpdakavavvhyggiptd vinswagdiltqessctciqg ecfwvmtdgpandrqaqyrafka kqgkivgqaeisfngghiecs cypnegkvecvckdnwtgtrp vlvispdlsyrvgyllcaglpd tprgdsqftgsgtspmgnqgy gvkgfgfrqgndvwmgrtisrt srsgfeilkvrngwvqnskeqi krqvvdnlwnsgysgsftlpa eltkrncvpcfwvemirgnpe ektiwtssssivmcgvdheiad swwdhgailpfdidkm (SEQ ID NO:35)	60%
285_N8- Jiangxi - Donghu2 013_E16 0P- Q163S	HFMNTEALCDAGFAPFSKDN GIRIGSRGHVFVIREPFVSCS? TECRTFFLTQGSLLNDKHSNGT VKDRSPYRTLMSVPIGSSPNVY QARFEAVAWSATACHDGKKWMT IGVTGPDAAVAVVHYGGIPTD VINSWAGDILRTQESSCTCIQG ECFWVMTDGPANRQAQYRAFA KQGKIVGQAEISFNGGHIIECS CYPNEGKVECVCKDNWTGTRP	N8 numbering: 160P, 163S	hfmntealcdagfapfskdn girigsrghvfvirepfvscsp tecrtflltqgsllndkhsngt vkdrspyrtlmsvpi gsspnvy qarfeavawsatachdgkkwmt igvtgpdakavavvhyggiptd vinswagdiltqessctciqg ecfwvmtdgpandrqaqyrafka kqgkivgqaeisfngghiecs cypnegkvecvckdnwtgtrp	100%

	VLVISPDLSYRVGYLCAGLPST TPRGEDSQFTGSCSPMGNGGY GVKGFGRQGNVWVGRTISRT SRSGFEILKVRNGWVQNSKEQI KRQVVVDNLNWSGYSGSFTLPA ELTKRNCVPCFWVEMIRGNPE EKTIWTSSSSIVMCGVDHEIAD WSWHDGAILPFDIDKM (SEQ ID NO:36)		vlvispdlsyrvgylcaglpst tprgedsqftgsctspmngnggy gvkgfgrqgndvwmgrtisrt srsgeilkvrgwvqnskeqi krqvvvdnlwnsgysgsftlpa eltkrncvpcfwvemirgnpe ektiwtsssivmcgvdheiad wswhdgailpfdidkm (SEQ ID NO:36)	
288_N8- Jiangxi - Donghu2 013_E16 0P- Q163S- V175I	HFMNTEALCDAKGFAPFSKDN GIRIGSRGHVFVIREPFVSCSP TECRTFFLTQGSLLNDKHSNGT VKDRSPYRTLMSVPIGSSPNVY QARFEALAWSATACHDGKKWMT IGVTGPDAAVAVVHYGGIPTD VINWAGDILRTQESSCTCIQG ECFWMTDGPANRQAQYRAFA KQGGKIVGQAEISFNGGHEECS CYPNEGKVECVCKDNWTGTRNP VLVISPDLSYRVGYLCAGLPST TPRGEDSQFTGSCSPMGNGGY GVKGFGRQGNVWVGRTISRT SRSGFEILKVRNGWVQNSKEQI KRQVVVDNLNWSGYSGSFTLPA ELTKRNCVPCFWVEMIRGNPE EKTIWTSSSSIVMCGVDHEIAD WSWHDGAILPFDIDKM (SEQ ID NO:37)	N8 numbering: 160P, 163S, 175I	hfmntealcda kgfapfskdn girigsrghvf virepfvscsp tecrtf fltqgsllndkhsngt vkdrspyrtlmsvpigsspnvy qarfealawsatachdgkkwmt igvtgpdakavavhyggiptd vinwagdilrtqessctciqg ecfwmt dgp anrqayrafka kqgkivgqaeisfngghieecs cypnegkvecvckdnwtgtrnp vlvispdlsyrvgylcaglpst tprgedsqftgsctspmngnggy gvkgfgrqgndvwmgrtisrt srsgeilkvrgwvqnskeqi krqvvvdnlwnsgysgsftlpa eltkrncvpcfwvemirgnpe ektiwtsssivmcgvdheiad wswhdgailpfdidkm (SEQ ID NO:37)	
289_N8- Jiangxi - Donghu2 013_E16 2P- Q165S- V176I- V204I	HFMNTEALCDAKGFAPFSKDN GIRIGSRGHVFVIREPFVSCSP TECRTFFLTQGSLLNDKHSNGT VKDRSPYRTLMSVPIGSSPNVY QARFEALAWSATACHDGKKWMT IGVTGPDAAVAIVHYGGIPTD VINWAGDILRTQESSCTCIQG ECFWMTDGPANRQAQYRAFA KQGGKIVGQAEISFNGGHEECS CYPNEGKVECVCKDNWTGTRNP VLVISPDLSYRVGYLCAGLPST TPRGEDSQFTGSCSPMGNGGY GVKGFGRQGNVWVGRTISRT SRSGFEILKVRNGWVQNSKEQI KRQVVVDNLNWSGYSGSFTLPA ELTKRNCVPCFWVEMIRGNPE EKTIWTSSSSIVMCGVDHEIAD WSWHDGAILPFDIDKM (SEQ ID NO:38)	N8 numbering: 160P, 163S, 175I, 203I	hfmntealcda kgfapfskdn girigsrghvf virepfvscsp tecrtf fltqgsllndkhsngt vkdrspyrtlmsvpigsspnvy qarfealawsatachdgkkwmt igvtgpdakavai vhyggiptd vinwagdilrtqessctciqg ecfwmt dgp anrqayrafka kqgkivgqaeisfngghieecs cypnegkvecvckdnwtgtrnp vlvispdlsyrvgylcaglpst tprgedsqftgsctspmngnggy gvkgfgrqgndvwmgrtisrt srsgeilkvrgwvqnskeqi krqvvvdnlwnsgysgsftlpa eltkrncvpcfwvemirgnpe ektiwtsssivmcgvdheiad wswhdgailpfdidkm (SEQ ID NO:38)	70%
315_N1- BrevMis s1918_c 155_s10 1A_G105 S_T131M _V163L_ A166V_S 442I_S4 44A	VILTGNSSLCPISGWAPLAKDN SIRIGSKGDVFVIREPFISCSH LECRMFFLTQGALLNDKHSNGT VKDRSPYRTLMSVPLGSPSPY NARFESIAWSASACHDGMGWL IGITGPDNGAVAILKYNGIITD TIKSWRNNILRTQESACVNG SCFTIMTDGSPNGQASYKILKI EKGKVTKSIELNAPNYHYECS CYPDTGKVMCVRDNWHGSRNP WVSFDQNLDYQIGYICSGVFGD NRPNDGTGSCGPVSSNGANGI KGFSFRYDNGVWIGRTKSTSSR SGFEMIWDPNGWTTETDSSFSVR	N1 numbering: 99P/177I/19 6T/205I, 165S, 100L/408M/4 19V, 161V/172A, 101A/131M/1 63L/442I/44 4A, 105S	viltgnsslcpi sgwapl akdn sirigskgdvf virepfiscsh lecrmffltqgallndkhsngt vkdrspyrtlmsvplgsvspy narfeslawsasachdgmgwlt igitgpdngavailkyngiitd tikswrnnilrtqesecavng scftimtdgpsngqasykilki ekgkvtsielnapnyhyeecs cypdtgkvmcvrndwhgsnrp wvsfdqnl dyqigyicsgvfgd nprpndgtgscgpvssngangi kgfsfrydngvwigrtkstssr sgfemiwdpn gwtetdssfsvr	20-30%

	QDIVAITDWSGYSGSFVMHPEL TGLDCMVPCFWVELIRGQPKEN TIWTSGSIIAFCGVNSDTVGWS WPDGAELPFSIDK (SEQ ID NO:39)		qdivaitdwsygsgsfvmhpel tgldcmVpcfwvelirgqpkEN tiwtsgsIiAfcgvnsdtvgws wpdgaelpfsidk (SEQ ID NO:39)	
354 N1- VN04_c1 55_I106 V_T131Q _V163I- A166V	VKLAGNSSLCPINGWAPLSKDN SVRIGSKGDVFIREFPISCSH LECRQFFLTQGALLNDKHSNGT VKDRSPHRTLMSVPIGSPSPY NARFESIAWSASACHDGTSWLT IGITGPDNGAVAILKYNGIITD TIKSWRNNILRTQSEECACVNG SCFTVMTDGPNGQASYKIFKM EKGKVVKSVELDAPNYHYEES CYPNAGEITCVCARDNWHGSRP WVSFNQNLLEYQIGYICSGVFGD NPRPNDGTGSCGPVSSNGAYGV KGFSFKYNGVWIGRTKSTNSR SGFEMIWDPNGTETDSSFSVK QDIVAITDWSGYSGSFVMHPEL TGLDCIVPCFWVELIRGRPKES TIWTSGSSISFCGVNSDTVGWS WPDGAELPFTIDK (SEQ ID NO:40)	N1 numbering: 99P/177I/19 6T/205I, 165S, 100L/408M/4 19V, 161V/172A, 131Q, 106V, 163I, 166V	vklaGnsslcpingwaPlskdn sVrigskgdvfiRefpiscsh lecrQffltqgallndkhsngt vkdrspHrtlmsVpIgSPspY nArfesIawsasachdgtswlt igiTgpdngavaIlkyngiitd tikswrnnilrtqesecacvng scftvmtDgPngqasykifkm ekgkvksvelDapnyhyeecs cypnAgeitcvcrdnwhgsnrp wvsfnqnleyqigyicsgvfgd nprpndgtgscgpvssngaygv kgfsfkyngvwigrtkstnsr sgfemiwdpngtetdssfsvk qdivaitdwsygsgsfvmhpel tgldciVpcfwvelirgrpkEs tiwtsgssisfcgvnsdtvgws wpdgaelpftidk (SEQ ID NO:40)	100%
356 N1- VN04_c1 55_I106 V_V114I _T131Q _V163I_V 166P	VKLAGNSSLCPINGWAPLSKDN SVRIGSKGDI FVIREPFISCSH LECRQFFLTQGALLNDKHSNGT VKDRSPHRTLMSVPIGSPSPY NARFESIAWSASACHDGTSWLT IGITGPDNGAVAILKYNGIITD TIKSWRNNILRTQSEECACVNG SCFTVMTDGPNGQASYKIFKM EKGKVVKSVELDAPNYHYEES CYPNAGEITCVCARDNWHGSRP WVSFNQNLLEYQIGYICSGVFGD NPRPNDGTGSCGPVSSNGAYGV KGFSFKYNGVWIGRTKSTNSR SGFEMIWDPNGTETDSSFSVK QDIVAITDWSGYSGSFVMHPEL TGLDCIVPCFWVELIRGRPKES TIWTSGSSISFCGVNSDTVGWS WPDGAELPFTIDK (SEQ ID NO:41)	N1 numbering: 99P/177I/19 6T/205I, 165S, 100L/408M/4 19V, 161V/172A, 131Q, 106V, 163I, 114I/166P	vklaGnsslcpingwaPlskdn sVrigskgdIfvirepfiscsh lecrQffltqgallndkhsngt vkdrspHrtlmsVpIgSPspY nArfesIawsasachdgtswlt igiTgpdngavaIlkyngiitd tikswrnnilrtqesecacvng scftvmtDgPngqasykifkm ekgkvksvelDapnyhyeecs cypnAgeitcvcrdnwhgsnrp wvsfnqnleyqigyicsgvfgd nprpndgtgscgpvssngaygv kgfsfkyngvwigrtkstnsr sgfemiwdpngtetdssfsvk qdivaitdwsygsgsfvmhpel tgldciVpcfwvelirgrpkEs tiwtsgssisfcgvnsdtvgws wpdgaelpftidk (SEQ ID NO:41)	80%
357 N1- VN04_c1 55_I106 V_V114I _V163I_V 166P_sm allCal0 9pack	VKLAGNSSLCPINGWAPLSKDN SVRIGSKGDI FVIREPFISCSH LECRQFFLTQGALLNDKHSNGT VKDRSPHRTLMSVPIGSPSPY NARFESIAWSASACHDGTSWLT IGITGPDNGAVAILKYNGIITD TIKSWRNNILRTQSEECACVNG SCFTVMTDGPNGQASYKIFKI EKGKIVKSVEMDAPNYHYEES CYPNAGEITCVCARDNWHGSRP WVSFNQNLLEYQIGYICSGVFGD NPRPNDGTGSCGPVSSNGAYGV KGFSFKYNGVWIGRTKSTNSR SGFEMIWDPNGTETDSSFSVK QDIVAITDWSGYSGSFVMHPEL TGLDCIVPCFWVELIRGRPKES TIWTSGSSISFCGVNSDTVGWS	N1 numbering: 99P/177I/19 6T/205I, 165S, 100L/408M/4 19V, 161V/172A, 131Q, 106V, 163I, 114I/166P, 126P, 210G, 257I, 262I, 268M	vklaGnsslcpingwaPlskdn sVrigskgdIfvirepfiscsP lecrQffltqgallndkhsngt vkdrspHrtlmsVpIgSPspY nArfesIawsasachdgtswlt igiTgpdngavaIlkyngiitd tikswrnnilrtqesecacvng scftvmtDgPngqasykifkI ekgkIvksveMDapnyhyeecs cypnAgeitcvcrdnwhgsnrp wvsfnqnleyqigyicsgvfgd nprpndgtgscgpvssngaygv kgfsfkyngvwigrtkstnsr sgfemiwdpngtetdssfsvk qdivaitdwsygsgsfvmhpel tgldciVpcfwvelirgrpkEs tiwtsgssisfcgvnsdtvgws	70%

	WPDGAELPFTIDK (SEQ ID NO:42)		wpdgaelpftidk (SEQ ID NO:42)	
366_N1-WSN33_c155_G105S_I106V_A157T_V163I_A166V_R210G	VILTGNSSLCPIRGWAPLSKDN SVRIGSKGDVFIREFPISCSH LECRFFLTQGALLNDKHSRGT FKDRSPYRTLMSVPIGSPSPY NARFESIAWSASACHDGMGWL IGITGPDGDAVAILKYNGIITE TIKSWRKNILRTQSECTCVNG SCFTIMTDGPGDGLASYKIFKI EKGKVTKSIELNAPNSHYECS CYPDTGKVMCVCARDNWHGSRP WVSFDQNL DYKIGYICSGVFGD NPRPKDGTGSCGPVSADGANGV KGFSYKYGNGVWIGRTKSDSSR HGFEMIWDPNGTETDSRFSMR QDVVAITNRSYGSGSFMHPEL TGLDCMVPCFWVELIRGLPEED AIWTSGSIISFCGVNSDTVDWS WPDGAELPFTIDK (SEQ ID NO:43)	N1 numbering: 99P/177I/19 6T/205I, 165S, 100L/408M/4 19V, 161V/172A, 105S, 106V, 157T, 163I, 166V, 210G	viltgnssslcpirgwaplskdn SVrigskgdvfvirepfiscsh lecrffltqgallndkhsrgt fkdrspyrTlmsVpIgSVpspy nArfesIawsasachdgmgwlt igiTgpdgdgavaIlkynGiite tikswrkniltqseectcvng scftimtdgpgsdglasykifki ekgkvtskielnapnshyeecs cypdtgkvmcvcrdnwhgsnrp wvsfdqnl dykigyicsgvfgd nprpkdgtgscgpvsadgangv kgfsy:xyngvwigrtksdssr hgfemiwdpngwtetdsrfsmr qdvvaitnrsgysgsfvmhpel tgldcmVpcfwwelirglpeed aiwtsgsiisfcgvnsdtvdws wpdgaelpftidk (SEQ ID NO:43)	80%
367_N1-WSN33_c155_G105S_I106V_A157T_V163I_A166V_R210G	VILTGNSSLCPIRGWAPLSKDN SVRIGSKGDVFIREFPISCSH LECRQFFLTQGALLNDKHSRGT FKDRSPYRTLMSVPIGSPSPY NARFESIAWSASACHDGMGWL IGITGPDGDAVAILKYNGIITE TIKSWRKNILRTQSECTCVNG SCFTIMTDGPGDGLASYKIFKI EKGKVTKSIELNAPNSHYECS CYPDTGKVMCVCARDNWHGSRP WVSFDQNL DYKIGYICSGVFGD NPRPKDGTGSCGPVSADGANGV KGFSYKYGNGVWIGRTKSDSSR HGFEMIWDPNGTETDSRFSMR QDVVAITNRSYGSGSFMHPEL TGLDCMVPCFWVELIRGLPEED AIWTSGSIISFCGVNSDTVDWS WPDGAELPFTIDK (SEQ ID NO:44)	N1 numbering: 99P/177I/19 6T/205I, 165S, 100L/408M/4 19V, 161V/172A, 131Q, 105S, 106V, 157T, 163I, 166V, 210G	viltgnssslcpirgwaplskdn SVrigskgdvfvirepfiscsh lecrQffltqgallndkhsrgt fkdrspyrTlmsVpIgSVpspy nArfesIawsasachdgmgwlt igiTgpdgdgavaIlkynGiite tikswrkniltqseectcvng scftimtdgpgsdglasykifki ekgkvtskielnapnshyeecs cypdtgkvmcvcrdnwhgsnrp wvsfdqnl dykigyicsgvfgd nprpkdgtgscgpvsadgangv kgfsy:xyngvwigrtksdssr hgfemiwdpngwtetdsrfsmr qdvvaitnrsgysgsfvmhpel tgldcmVpcfwwelirglpeed aiwtsgsiisfcgvnsdtvdws wpdgaelpftidk (SEQ ID NO:44)	
369_N1-WSN33_c155_G105S_I106V_A157T_V163I_A166V_R210G_I442S	VILTGNSSLCPIRGWAPLSKDN SVRIGSKGDVFIREFPISCSH LECRQFFLTQGALLNDKHSRGT FKDRSPYRTLMSVPIGSPSPY NARFESIAWSASACHDGMGWL IGITGPDGDAVAILKYNGIITE TIKSWRKNILRTQSECTCVNG SCFTIMTDGPGDGLASYKIFKI EKGKVTKSIELNAPNSHYECS CYPDTGKVMCVCARDNWHGSRP WVSFDQNL DYKIGYICSGVFGD NPRPKDGTGSCGPVSADGANGV KGFSYKYGNGVWIGRTKSDSSR HGFEMIWDPNGTETDSRFSMR QDVVAITNRSYGSGSFMHPEL TGLDCMVPCFWVELIRGLPEED AIWTSGSIISFCGVNSDTVDWS WPDGAELPFTIDK (SEQ ID NO:45)	N1 numbering: 99P/177I/19 6T/205I, 165S, 100L/408M/4 19V, 161V/172A, 131Q, 105S, 106V, 157T, 163I, 166V, 210G, 442S	viltgnssslcpirgwaplskdn SVrigskgdvfvirepfiscsh lecrQffltqgallndkhsrgt fkdrspyrTlmsVpIgSVpspy nArfesIawsasachdgmgwlt igiTgpdgdgavaIlkynGiite tikswrkniltqseectcvng scftimtdgpgsdglasykifki ekgkvtskielnapnshyeecs cypdtgkvmcvcrdnwhgsnrp wvsfdqnl dykigyicsgvfgd nprpkdgtgscgpvsadgangv kgfsy:xyngvwigrtksdssr hgfemiwdpngwtetdsrfsmr qdvvaitnrsgysgsfvmhpel tgldcmVpcfwwelirglpeed aiwtsgsSisfcgvnsdtvdws wpdgaelpftidk (SEQ ID NO:45)	70%
371_N1-WSN33_c	VILTGNSSLCPIRGWAPLSKDN SVRIGSKGDI FVIREPFISCSH	N1 numbering:	viltgnssslcpirgwaplskdn SVrigskgdIfvirepfiscsh	80%

155_G10 5S_I106 V_V114I T131Q A157T_V 163I_A1 66P_R21 0G_I442 S	LECRQFFLTQGALLNDKHSRGT FKDRSPYRTLMSVPIGSPSPY NARFESIAWSASACHDGMGWL IGITGPDGAVAILKYNIGIITE TIKSWRKNILRTQSEECTCVNG SCFTIMTDGPGSDGLASYKIFKI EKGKVTKSIELNAPNSHYECS CYPDTGKVMCVRDNWHGSRNP WVSFDQNLDYKIGYICSGVFGD NPRPKDGTGSCGPVSADGANGV KGFSYKYGNGVWIGRTKSDSSR HGFEMIWDPNGWETEDSRFSMR QDVVAITNRSGYSGSFVMHPEL TGLDCMVPCFWVELIRGLPEED AIWTSGSSISFCGVNSDTVDS WPDGAELPFTIDK (SEQ ID NO:46)	99P/177I/19 6T/205I, 165S, 100L/408M/4 19V, 161V/172A, 114I/166P, 131Q, 105S, 106V, 157T, 163I, 210G, 442S	lecrQffltqgallndkhsrgt fkdrspyrTlmsVpIgSPspy nArfesIawsasachdgmgt igiTgpdgavaIlkynGiite tikswrkniltqseectcvng scftimtdgpsdglasykifki ekgkvksielnapnshyeecs cypdtgkvmcvcrdnwhgsnrp wvsfdqnlidykigyicsgvfgd nprpkdgtgscgpvsadgangv kgfsyxygngvwigrtksdssr hgfemiwdpngwetedsrfsmr qdvvaitnrsgysgsfvMhpel tgldcmVpcfwwelirglpeed aiwtsgsSisfcgvnsdtvds wpdgaelpftidk (SEQ ID NO:46)	
399_N1- Cal09- c155_I2 05V	VKLAGNSSLCPVSGWAPLSKDN SVRIGSKGDVFIREFPISCS? LECRFFLTQGALLNDKHSNGT IKDRSPYRTLMSVPIGSPSPY NARFESIAWSASACHDGINWLT IGITGPDNGAVAVLKYNIGIITD TIKSWRNNILRTQSEECACVNG SCFTVMTDGPNGQASYKIFRI EKGKIVKSVEMNAPNYHYECS CYPDSSEITCVRDNWHGSRNP WVSFNQNLLEYQIGYICSGIFGD NPRPNDKTGSCGPVSSNGANGV KGFSFKYGNGVWIGRTKSISR NGFEMIWDPNGWGTGTDNNFSIK QDIVGINEWSGYSGSFVMHPEL TGLDCIVPCFWVELIRGRPKEN TIWTSGSSISFCGVNSDTVGWS WPDGAELPFTIDK (SEQ ID NO:47)	N1 numbering: 99P/177I/19 6T, 165S, 100L/408M/4 19V, 161V/172A	vklaGnsslcPvsgwaPlskdn svrigskgdvfiRefpiscsp lecrffltqgallndkhsngt ikdrspyrTlmsVpIgSPspy nArfesIawsasachdginwlt igiTgpdngavavlkyngiitd tikswrnniltqseecacvng scftvmtdgpsngqasykifri ekgkivksvemnapnyhyeecs cypdsseitcvrdnwhgsnrp wvsfnqnleyqigyicsgifgd nprpndktgscgpvssngangv kgfsfkygngvwigrtksissr ngfemiwdpngwgtgtdnnfsik qdivginewsgysgsfvMhpel tgldciVpcfwwelirgrpken tiwtsgssisfcgvnsdtvgws wpdgaelpftidk (SEQ ID NO:47)	
400_N1- Cal09- c155_I1 77V_I20 5V	VKLAGNSSLCPVSGWAPLSKDN SVRIGSKGDVFIREFPISCS? LECRFFLTQGALLNDKHSNGT IKDRSPYRTLMSVPIGSPSPY NARFESVAWSASACHDGINWLT IGITGPDNGAVAVLKYNIGIITD TIKSWRNNILRTQSEECACVNG SCFTVMTDGPNGQASYKIFRI EKGKIVKSVEMNAPNYHYECS CYPDSSEITCVRDNWHGSRNP WVSFNQNLLEYQIGYICSGIFGD NPRPNDKTGSCGPVSSNGANGV KGFSFKYGNGVWIGRTKSISR NGFEMIWDPNGWGTGTDNNFSIK QDIVGINEWSGYSGSFVMHPEL TGLDCIVPCFWVELIRGRPKEN TIWTSGSSISFCGVNSDTVGWS WPDGAELPFTIDK (SEQ ID NO:48)	N1 numbering: 99P/196T, 165S, 100L/408M/4 19V, 161V/172A	vklaGnsslcPvsgwaPlskdn svrigskgdvfiRefpiscsp lecrffltqgallndkhsngt ikdrspyrTlmsVpIgSPspy nArfesvawsasachdginwlt igiTgpdngavavlkyngiitd tikswrnniltqseecacvng scftvmtdgpsngqasykifri ekgkivksvemnapnyhyeecs cypdsseitcvrdnwhgsnrp wvsfnqnleyqigyicsgifgd nprpndktgscgpvssngangv kgfsfkygngvwigrtksissr ngfemiwdpngwgtgtdnnfsik qdivginewsgysgsfvMhpel tgldciVpcfwwelirgrpken tiwtsgssisfcgvnsdtvgws wpdgaelpftidk (SEQ ID NO:48)	
401_N1- Cal09- c155_P9 9I_I177 V_T195S I205V	VKLAGNSSLCPVSGWAILSKDN SVRIGSKGDVFIREFPISCS? LECRFFLTQGALLNDKHSNGT IKDRSPYRTLMSVPIGSPSPY NARFESVAWSASACHDGINWLT IGISGPDNGAVAVLKYNIGIITD	N1 numbering: 165S, 100L/408M/4 19V, 161V/172A	vklaGnsslcPvsgwaiLskdn svrigskgdvfiRefpiscsp lecrffltqgallndkhsngt ikdrspyrTlmsVpIgSPspy nArfesvawsasachdginwlt igisgpdngavavlkyngiitd	

	TIKSWRNNILRTQSEECACVNG SCFTVMTDGPNGQASYKIFRI EKGKIVKSVEMNAPNYHYECS CYPDSSEITCVCARDNWHGSRNP WVSFNQNLLEYQIGYICSGIFGD NPRPNDKTGSCGPVSSNGANGV KGFSFKYGNVWIGRTKSISSR NGFEMIWDPNWGTGTDNNFSIK QDIVGINEWSGYSGSFVMHPPEL TGLDCIVPCFWVELIRGRPKEN TIWTSGSSISFCGVNSDTVGWS WPDGAELPFTIDK (SEQ ID NO:49)		tikswrnnilrtqesecacvng scftvmtdgpsngqasykifri ekgkivksvemnapnyhyeecs cypdsseitcvcrdnwhgsnrp wvsfnqnleyqigyicsgifgd nprpndktgscgpvssngangv kgfsfkyngvwigrtksissr ngfemiwdpngwtgtdnnfsik qdivginewsgysgsfvmhpel tgldciVpcfvelirgrpken tiwtsgssisfcgvnsdtvgws wpdgaelpftidk (SEQ ID NO:49)	
402_N1- Cal09- c155_P9 9I_I177 V_T195S _I205V_ no- spaceB	VKLAGNSSLCPVSGWAIYSKDN SVRIGSKGDVFVIREPFISCS? LECRFFLTQGALLNDKHSNGT IKDRSPYRTLMSVPISGSPSPY NARFESVAWSASACHDGINWLT IGISGPDNGAVAVLKYNGIITD TIKSWRNNILRTQSEECACVNG SCFTVMTDGPNGQASYKIFRI EKGKIVKSVEMNAPNYHYECS CYPDSSEITCVCARDNWHGSRNP WVSFNQNLLEYQIGYICSGIFGD NPRPNDKTGSCGPVSSNGANGV KGFSFKYGNVWIGRTKSISSR NGFEMIWDPNWGTGTDNNFSIK QDIVGINEWSGYSGSFVQHPPEL TGLDCIRPCFWVELIRGRPKEN TIWTSGSSISFCGVNSDTVGWS WPDGAELPFTIDK (SEQ ID NO:50)	N1 numbering: 165S, 161V/172A	vklaGSSlcpvsgwaiyskdn svrigskgdvfvirepfiscsp lecrffltqgallndkhsngt ikdrspyrtlmsVpigSvpspy nArfesvawsasachdginwlt igisgpdngavavlkyngiitd tikswrnnilrtqesecacvng scftvmtdgpsngqasykifri ekgkivksvemnapnyhyeecs cypdsseitcvcrdnwhgsnrp wvsfnqnleyqigyicsgifgd nprpndktgscgpvssngangv kgfsfkyngvwigrtksissr ngfemiwdpngwtgtdnnfsik qdivginewsgysgsfVqhpel tgldcirpcfwvelirgrpken tiwtsgssisfcgvnsdtvgws wpdgaelpftidk (SEQ ID NO:50)	
403_N1- Cal09- c155_P9 9I_I177 V_T195S _I205V_ no- spaceB V114I_V 166P	VKLAGNSSLCPVSGWAIYSKDN SVRIGSKGDI FVIREPFISCS? LECRFFLTQGALLNDKHSNGT IKDRSPYRTLMSVPISGSPSPY NARFESVAWSASACHDGINWLT IGISGPDNGAVAVLKYNGIITD TIKSWRNNILRTQSEECACVNG SCFTVMTDGPNGQASYKIFRI EKGKIVKSVEMNAPNYHYECS CYPDSSEITCVCARDNWHGSRNP WVSFNQNLLEYQIGYICSGIFGD NPRPNDKTGSCGPVSSNGANGV KGFSFKYGNVWIGRTKSISSR NGFEMIWDPNWGTGTDNNFSIK QDIVGINEWSGYSGSFVQHPPEL TGLDCIRPCFWVELIRGRPKEN TIWTSGSSISFCGVNSDTVGWS WPDGAELPFTIDK (SEQ ID NO:51)	N1 numbering: 165S, 161V/172A, 114/166P	vklaGSSlcpvsgwaiyskdn svrigskgdIfvirepfiscsp lecrffltqgallndkhsngt ikdrspyrtlmsVpigSvpspy nArfesvawsasachdginwlt igisgpdngavavlkyngiitd tikswrnnilrtqesecacvng scftvmtdgpsngqasykifri ekgkivksvemnapnyhyeecs cypdsseitcvcrdnwhgsnrp wvsfnqnleyqigyicsgifgd nprpndktgscgpvssngangv kgfsfkyngvwigrtksissr ngfemiwdpngwtgtdnnfsik qdivginewsgysgsfVqhpel tgldcirpcfwvelirgrpken tiwtsgssisfcgvnsdtvgws wpdgaelpftidk (SEQ ID NO:51)	
404_N1- Cal09- c155_P9 9I_I177 V_T195S _I205V_ no- spaceB T131Q	VKLAGNSSLCPVSGWAIYSKDN SVRIGSKGDVFVIREPFISCS? LECRQFFLTQGALLNDKHSNGT IKDRSPYRTLMSVPISGSPSPY NARFESVAWSASACHDGINWLT IGISGPDNGAVAVLKYNGIITD TIKSWRNNILRTQSEECACVNG SCFTVMTDGPNGQASYKIFRI EKGKIVKSVEMNAPNYHYECS CYPDSSEITCVCARDNWHGSRNP	N1 numbering: 165S, 161V/172A, 131Q	vklaGSSlcpvsgwaiyskdn svrigskgdvfvirepfiscsp lecrQffltqgallndkhsngt ikdrspyrtlmsVpigSvpspy nArfesvawsasachdginwlt igisgpdngavavlkyngiitd tikswrnnilrtqesecacvng scftvmtdgpsngqasykifri ekgkivksvemnapnyhyeecs cypdsseitcvcrdnwhgsnrp	

	WVSFNQNLEYQIGYICSGIFGD NPRPNDKTGSCGPVSSNGANGV KGFSFKYGNVWIGRTKSISSR NGFEMIWDPNGWGTGTDNNFSIK QDIVGINEWSGYSGSFVQHPHEL TGLDCIRPCFWVELIRGRPKEN TIWTSGSSISFCGVNSDTVGWS WPDGAELPFTIDK (SEQ ID NO:52)		wvsfnqnleyqigiyicsgifgd nprpndktgscgpvssngangv kgfsfkyngvwigrtksissr ngfemiwdpngwtgtdnnfsik qdivginewsgysgsfvsqhpel tgldcirpcfwvelirgrpken tiwtsgssisfcgvnsdtvgws wpdgaelpftidk (SEQ ID NO:52)	
405_N1- Cal09- c155_P9 9I_I177 V_T195S _I205V_ no- spaceB_ V114I_T 131Q_V1 66P	VKLAGNSSLCPVSGWAIYSKDN SVRIGSKGDI FVIREPFISCS? LECRQFFLTQGALLNDKHSNGT IKDRSPYRTLMSVPIGSPSPY NARFESVAWSASACHDGINWLT IGISGPDNGAVAVLKYNGIITD TIKSWRNNILRTQESACVNG SCFTVMTDGPNGQASYKIFRI EKGKIVKSVEMNAPNYHYECS CYPDSSEITCVCARDNWHGSRNP WVSFNQNLEYQIGYICSGIFGD NPRPNDKTGSCGPVSSNGANGV KGFSFKYGNVWIGRTKSISSR NGFEMIWDPNGWGTGTDNNFSIK QDIVGINEWSGYSGSFVQHPHEL TGLDCIRPCFWVELIRGRPKEN TIWTSGSSISFCGVNSDTVGWS WPDGAELPFTIDK (SEQ ID NO:53)	N1 numbering: 165S, 161V/172A, 114I/166P, 131Q	vkla g n s s l c p v s g w a i y s k d n s v r i g s k g d i f v i r e p f i s c s p l e c r q f f l t q g a l l n d k h s n g t i k d r s p y r t l m s v p i g s p s p y n a r f e s v a w s a s a c h d g i n w l t i g i s g p d n g a v a v l k y n g i i t d t i k s w r n n i l r t q e s e c a c v n g s c f t v m t d g p s n g q a s y k i f r i e k g k i v k s v e m n a p n y h y e e c s c y p d s s e i t c v c r d n w h g s n r p w v s f n q n l e y q i g y i c s g i f g d n p r p n d k t g s c g p v s s n g a n g v k g f s f k y g n v w i g r t k s i s s r n g f e m i w d p n g w t g t d n n f s i k q d i v g i n e w s g y s g s f v q h p e l t g l d c i r p c f w v e l i r g r p k e n t i w t s g s s i s f c g v n s d t v g w s w p d g a e l p f t i d k (S E Q I D N O : 5 3)	
406_N1- Cal09- c155_P9 9I_I177 V_T195S _I205V_ no- spaceB_ Y100L_S 101A_T1 31M_I16 3L_S442 I_S444A	VKLAGNSSLCPVSGWAILAKDN SVRIGSKGDV FVIREPFISCS? LECRMFFLTQGALLNDKHSNGT IKDRSPYRTLMSVPLGSPSPY NARFESVAWSASACHDGINWLT IGISGPDNGAVAVLKYNGIITD TIKSWRNNILRTQESACVNG SCFTVMTDGPNGQASYKIFRI EKGKIVKSVEMNAPNYHYECS CYPDSSEITCVCARDNWHGSRNP WVSFNQNLEYQIGYICSGIFGD NPRPNDKTGSCGPVSSNGANGV KGFSFKYGNVWIGRTKSISSR NGFEMIWDPNGWGTGTDNNFSIK QDIVGINEWSGYSGSFVQHPHEL TGLDCIRPCFWVELIRGRPKEN TIWTSGSIIAFCGVNSDTVGWS WPDGAELPFTIDK (SEQ ID NO:54)	N1 numbering: 165S, 161V/172A, 100L/101A/1 31M/163L/44 2I/444A	vkla g n s s l c p v s g w a i l a k d n s v r i g s k g d v f v i r e p f i s c s p l e c r m f f l t q g a l l n d k h s n g t i k d r s p y r t l m s v p l g s p s p y n a r f e s v a w s a s a c h d g i n w l t i g i s g p d n g a v a v l k y n g i i t d t i k s w r n n i l r t q e s e c a c v n g s c f t v m t d g p s n g q a s y k i f r i e k g k i v k s v e m n a p n y h y e e c s c y p d s s e i t c v c r d n w h g s n r p w v s f n q n l e y q i g y i c s g i f g d n p r p n d k t g s c g p v s s n g a n g v k g f s f k y g n v w i g r t k s i s s r n g f e m i w d p n g w t g t d n n f s i k q d i v g i n e w s g y s g s f v q h p e l t g l d c i r p c f w v e l i r g r p k e n t i w t s g s i i a f c g v n s d t v g w s w p d g a e l p f t i d k (S E Q I D N O : 5 4)	
407_N1- Cal09- c155_P9 9I_I177 V_T195S _I205V_ no- spaceB_ Y100L_S 101A_V1 14I_T13 1M_I163 L_V166P	VKLAGNSSLCPVSGWAILAKDN SVRIGSKGDI FVIREPFISCS? LECRMFFLTQGALLNDKHSNGT IKDRSPYRTLMSVPLGSPSPY NARFESVAWSASACHDGINWLT IGISGPDNGAVAVLKYNGIITD TIKSWRNNILRTQESACVNG SCFTVMTDGPNGQASYKIFRI EKGKIVKSVEMNAPNYHYECS CYPDSSEITCVCARDNWHGSRNP WVSFNQNLEYQIGYICSGIFGD NPRPNDKTGSCGPVSSNGANGV KGFSFKYGNVWIGRTKSISSR NGFEMIWDPNGWGTGTDNNFSIK	N1 numbering: 165S, 161V/172A, 114I/166P, 100L/101A/1 31M/163L/44 2I/444A	vkla g n s s l c p v s g w a i l a k d n s v r i g s k g d i f v i r e p f i s c s p l e c r m f f l t q g a l l n d k h s n g t i k d r s p y r t l m s v p l g s p s p y n a r f e s v a w s a s a c h d g i n w l t i g i s g p d n g a v a v l k y n g i i t d t i k s w r n n i l r t q e s e c a c v n g s c f t v m t d g p s n g q a s y k i f r i e k g k i v k s v e m n a p n y h y e e c s c y p d s s e i t c v c r d n w h g s n r p w v s f n q n l e y q i g y i c s g i f g d n p r p n d k t g s c g p v s s n g a n g v k g f s f k y g n v w i g r t k s i s s r n g f e m i w d p n g w t g t d n n f s i k	

S442I S444A	QDIVGINEWSGYSGSFVQHPEL TGLDCIRPCFWVELIRGRPKEN TIWTSGSIIAFCGVNSDTVGWS WPDGAELPFTIDK (SEQ ID NO:55)		qdivginewsgysgsfVqhpeL tgldcirpcfwvelirgrpken tiwtsgsIIAfcgvnsdtvgws wpdgaelpftidk (SEQ ID NO:55)	
408_N1- Cal09- c155_V1 14I_V16 6P	VKLAGNSSLCPVSGWAPLSKDN SVRIGSKGDI FVIREPFISCS? LECRFFLTQGALLNDKHSNGT IKDRSPYRTLMSVPIGSPSPY NARFESIAWSASACHDGINWLT IGITGPDNGAVAILKYNGIITD TIKSWRNNILRTQESECACVNG SCFTVMTDGPNGQASYKIFRI EKGKIVKSVEMNAPNYHYECS CYPDSSEITCVCARDNWHGSRNP WVSFNQNL EYQIGYICSGIFGD NPRPNDKTGSCGPVSSNGANGV KGFSFKYGNVWIGRTKSISR NGFEMIWDPNGTGT DNNFSIK QDIVGINEWSGYSGSFVMHPEL TGLDCIVPCFWVELIRGRPKEN TIWTSGSSISFCGVNSDTVGWS WPDGAELPFTIDK (SEQ ID NO:56)	N1 numbering: 99P/177I/19 6T/205I, 165S, 100L/408M/4 19V, 161V/172A, 114I/166P	vklaGnssLcpvsgwaPlskdn svrigskgdIfvirepfiscsp lecrffltqgallndkhsngt ikdrspyrtlmsVpigSPpspy nArfesIawsasachdginwlt igiTgpdngavaIlkyngiitd tikswrnnilrtqesecacvng scftvmtdgpsngqasykifri ekgkivksvemnapnyhyeecs cypdsseitcvcrdnwhgsnrp wvsfnqnleyqigiyicsgifgd nprpndktgscgpvssngangv kgfsfkYgnvgwigrtksissr ngfemiwdpngwtgt dnnfsik qdivginewsgysgsfVmhpeL tgldciVpcfwvelirgrpken tiwtsgssisfcgvnsdtvgws wpdgaelpftidk (SEQ ID NO:56)	
409_N1- Cal09- c155_T1 31Q	VKLAGNSSLCPVSGWAPLSKDN SVRIGSKGDV FVIREPFISCS? LECRQFFLTQGALLNDKHSNGT IKDRSPYRTLMSVPIGSVPSY NARFESIAWSASACHDGINWLT IGITGPDNGAVAILKYNGIITD TIKSWRNNILRTQESECACVNG SCFTVMTDGPNGQASYKIFRI EKGKIVKSVEMNAPNYHYECS CYPDSSEITCVCARDNWHGSRNP WVSFNQNL EYQIGYICSGIFGD NPRPNDKTGSCGPVSSNGANGV KGFSFKYGNVWIGRTKSISR NGFEMIWDPNGTGT DNNFSIK QDIVGINEWSGYSGSFVMHPEL TGLDCIVPCFWVELIRGRPKEN TIWTSGSSISFCGVNSDTVGWS WPDGAELPFTIDK (SEQ ID NO:57)	N1 numbering: 99P/177I/19 6T/205I, 165S, 100L/408M/4 19V, 161V/172A, 131Q	vklaGnssLcpvsgwaPlskdn svrigskgdvfvirepfiscsp lecrQffltqgallndkhsngt ikdrspyrtlmsVpigSVpspy nArfesIawsasachdginwlt igiTgpdngavaIlkyngiitd tikswrnnilrtqesecacvng scftvmtdgpsngqasykifri ekgkivksvemnapnyhyeecs cypdsseitcvcrdnwhgsnrp wvsfnqnleyqigiyicsgifgd nprpndktgscgpvssngangv kgfsfkYgnvgwigrtksissr ngfemiwdpngwtgt dnnfsik qdivginewsgysgsfVmhpeL tgldciVpcfwvelirgrpken tiwtsgssisfcgvnsdtvgws wpdgaelpftidk (SEQ ID NO:57)	
410_N1- Cal09- c155_V1 14I_T13 1Q_V166 P	VKLAGNSSLCPVSGWAPLSKDN SVRIGSKGDI FVIREPFISCS? LECRQFFLTQGALLNDKHSNGT IKDRSPYRTLMSVPIGSPSPY NARFESIAWSASACHDGINWLT IGITGPDNGAVAILKYNGIITD TIKSWRNNILRTQESECACVNG SCFTVMTDGPNGQASYKIFRI EKGKIVKSVEMNAPNYHYECS CYPDSSEITCVCARDNWHGSRNP WVSFNQNL EYQIGYICSGIFGD NPRPNDKTGSCGPVSSNGANGV KGFSFKYGNVWIGRTKSISR NGFEMIWDPNGTGT DNNFSIK QDIVGINEWSGYSGSFVMHPEL TGLDCIVPCFWVELIRGRPKEN TIWTSGSSISFCGVNSDTVGWS	N1 numbering: 99P/177I/19 6T/205I, 165S, 100L/408M/4 19V, 161V/172A, 114I/166P, 131Q	vklaGnssLcpvsgwaPlskdn svrigskgdIfvirepfiscsp lecrQffltqgallndkhsngt ikdrspyrtlmsVpigSPpspy nArfesIawsasachdginwlt igiTgpdngavaIlkyngiitd tikswrnnilrtqesecacvng scftvmtdgpsngqasykifri ekgkivksvemnapnyhyeecs cypdsseitcvcrdnwhgsnrp wvsfnqnleyqigiyicsgifgd nprpndktgscgpvssngangv kgfsfkYgnvgwigrtksissr ngfemiwdpngwtgt dnnfsik qdivginewsgysgsfVmhpeL tgldciVpcfwvelirgrpken tiwtsgssisfcgvnsdtvgws	

	WPDGAELPFTIDK (SEQ ID NO:58)		wpdgaelpftidk (SEQ ID NO:58)	
411_N1-Cal09-c155_S101A_T131M_I163L_S442I_S444A	VKLAGNSSLCPVSGWAPLAKDN SVRIGSKGDVFVIREPFISCS? LECRMFFLTQGALLNDKHSNGT IKDRSPYRTLMSVPLGSPSPY NARFESIAWSASACHDGINWLT IGITGPDNGAVAILKYNGIITD TIKSWRNNILRTQESECACVNG SCFTVMTDGPNGQASYKIFRI EKGKIVKSVEMNAPNYHYECS CYPDSSEITCVCARDNWHGSRNP WVSFNQNLLEYQIGYICSGIFGD NPRPNDKTGSCGPVSSNGANGV KGFSFYKNGVWIGRTKSISR NGFEMIWDPNWGTGTDDNFSIK QDIVGINEWSGYSGSFVMHPEL TGLDCIVPCFWVELIRGRPKEN TIWTSGSIIAFCGVNSDTVGWS WPDGAELPFTIDK (SEQ ID NO:59)	N1 numbering: 99P/177I/19 6T/205I, 165S, 100L/408M/4 19V, 161V/172A, 101A/131M/1 63L/442I/44 4A	vkla g n s s l c p v s g w a P L A k d n s v r i g s k g d v f v i r e p f i s c s p l e c r m f f l t q g a l l n d k h s n g t i k d r s p y r t l m s v p l g s p s p y n a r f e s i a w s a s a c h d g i n w l t i g i t g p d n g a v a i l k y n g i i t d t i k s w r n n i l r t q e s e c a c v n g s c f t v m t d g p s n g q a s y k i f r i e k g k i v k s v e m n a p n y h y e e c s c y p d s s e i t c v c r d n w h g s n r p w v s f n q n l e y q i g y i c s g i f g d n p r p n d k t g s c g p v s s n g a n g v k g f s f y k n g v w i g r t k s i s s r n g f e m i w d p n g w t g t d d n n f s i k q d i v g i n e w s g y s g s f v m h p e l t g l d c i v p c f w v e l i r g r p k e n t i w t s g s i i a f c g v n s d t v g w s w p d g a e l p f t i d k (S E Q I D N O : 5 9)	
412_N1-Cal09-c155_S101A_V114I_T131M_I163L_V166P_S442I_S444A	VKLAGNSSLCPVSGWAPLAKDN SVRIGSKGDIFVIREPFISCS? LECRMFFLTQGALLNDKHSNGT IKDRSPYRTLMSVPLGSPSPY NARFESIAWSASACHDGINWLT IGITGPDNGAVAILKYNGIITD TIKSWRNNILRTQESECACVNG SCFTVMTDGPNGQASYKIFRI EKGKIVKSVEMNAPNYHYECS CYPDSSEITCVCARDNWHGSRNP WVSFNQNLLEYQIGYICSGIFGD NPRPNDKTGSCGPVSSNGANGV KGFSFYKNGVWIGRTKSISR NGFEMIWDPNWGTGTDDNFSIK QDIVGINEWSGYSGSFVMHPEL TGLDCIVPCFWVELIRGRPKEN TIWTSGSIIAFCGVNSDTVGWS WPDGAELPFTIDK (SEQ ID NO:60)	N1 numbering: 99P/177I/19 6T/205I, 165S, 100L/408M/4 19V, 161V/172A, 114I/166P, 101A/131M/1 63L/442I/44 4A	vkla g n s s l c p v s g w a P L A k d n s v r i g s k g d i f v i r e p f i s c s p l e c r m f f l t q g a l l n d k h s n g t i k d r s p y r t l m s v p l g s p s p y n a r f e s i a w s a s a c h d g i n w l t i g i t g p d n g a v a i l k y n g i i t d t i k s w r n n i l r t q e s e c a c v n g s c f t v m t d g p s n g q a s y k i f r i e k g k i v k s v e m n a p n y h y e e c s c y p d s s e i t c v c r d n w h g s n r p w v s f n q n l e y q i g y i c s g i f g d n p r p n d k t g s c g p v s s n g a n g v k g f s f y k n g v w i g r t k s i s s r n g f e m i w d p n g w t g t d d n n f s i k q d i v g i n e w s g y s g s f v m h p e l t g l d c i v p c f w v e l i r g r p k e n t i w t s g s i i a f c g v n s d t v g w s w p d g a e l p f t i d k (S E Q I D N O : 6 0)	
N1-Cal09_E165S	VKLAGNSSLCPVSGWAIYSKDN SVRIGSKGDVFVIREPFISCS? LECRFFLTQGALLNDKHSNGT IKDRSPYRTLMSCPIGSVSPY NSRFESVAWSASACHDGINWLT IGISGPDNGAVAVLKYNIGIITD TIKSWRNNILRTQESECACVNG SCFTVMTDGPNGQASYKIFRI EKGKIVKSVEMNAPNYHYECS CYPDSSEITCVCARDNWHGSRNP WVSFNQNLLEYQIGYICSGIFGD NPRPNDKTGSCGPVSSNGANGV KGFSYKNGVWIGRTKSISR NGFEMIWDPNWGTGTDDNFSIK QDIVGINEWSGYSGSFVQHPPEL TGLDCIRPCFWVELIRGRPKEN TIWTSGSSISFCGVNSDTVGWS WPDGAELPFTIDK (SEQ ID NO:61)	N1 numbering: 165S	vkla g n s s l c p v s g w a i y s k d n s v r i g s k g d v f v i r e p f i s c s p l e c r t f f l t q g a l l n d k h s n g t i k d r s p y r t l m s c p i g s v s p y n s r f e s v a w s a s a c h d g i n w l t i g i s g p d n g a v a v l k y n i g i i t d t i k s w r n n i l r t q e s e c a c v n g s c f t v m t d g p s n g q a s y k i f r i e k g k i v k s v e m n a p n y h y e e c s c y p d s s e i t c v c r d n w h g s n r p w v s f n q n l e y q i g y i c s g i f g d n p r p n d k t g s c g p v s s n g a n g v k g f s y k n g v w i g r t k s i s s r n g f e m i w d p n g w t g t d d n n f s i k q d i v g i n e w s g y s g s f v q h p e l t g l d c i r p c f w v e l i r g r p k e n t i w t s g s s i s f c g v n s d t v g w s w p d g a e l p f t i d k (S E Q I D N O : 6 1)	

N1- Cal09_E 165T	VKLAGNSSLCPVSGWAIYSKDN SVRIGSKGDFVIREPFISCS? LECRTFFLTQGALLNDKHSNGT IKDRSPYRTLMSCPIGTVPSPY NSRFESVAWSASACHDGINWLT IGISGPDNGAVAVLKYNGIITD TIKSWRNNILRTQESSECACVNG SCFTVMTDGPSSNGQASYKIFRI EKGKIVKSVEMNAPNYHYEECS CYPDSSEITCVCARDNWHGSRNP WVSFNQNLLEYQIGYICSGIFGD NPRPNDKTGSCGPVSSNGANGV KGFSYKYNGVWIGRTKSISSR NGFEMIWDPNGWGTGTDNNFSIK QDIVGINEWSGYSGSFVQHPHEL TGLDCIRPCFWVELIRGRPKEN TIWTSGSSISFCGVNSDTVGWS WPDGAELPFTIDK (SEQ ID NO:62)	N1 numbering: 165T	vklaGnssslcpvsgwaiyskdn svrigskgdfvirepfiscsp lecrtffltqgallndkhsngt ikdrspyrtlmscpigTvpSPY nsrfesvawsasachdginwlt igisgpdngavavlkyngiitd tikswrnnilrtqesecacvng scftvmtdgpssngqasykifri ekgkivksvemnapnyhyeecs cypdsseitcvcrdnwhgsnrp wvsfnqnleyqigyicsgifgd nprpndktgscgpvssngangv kgfsYxygngvwigrtksissr ngfemiwdpnngwtgtdnnfsik qdivginewsgysgsfvqhpel tgldcirpcfwvelirgrpken tiwtsgssisfcgvnsdtvgws wpdgaelpftidk (SEQ ID NO:62)	
N1- Cal09_E 165V	VKLAGNSSLCPVSGWAIYSKDN SVRIGSKGDFVIREPFISCS? LECRTFFLTQGALLNDKHSNGT IKDRSPYRTLMSCPIGVPSPY NSRFESVAWSASACHDGINWLT IGISGPDNGAVAVLKYNGIITD TIKSWRNNILRTQESSECACVNG SCFTVMTDGPSSNGQASYKIFRI EKGKIVKSVEMNAPNYHYEECS CYPDSSEITCVCARDNWHGSRNP WVSFNQNLLEYQIGYICSGIFGD NPRPNDKTGSCGPVSSNGANGV KGFSYKYNGVWIGRTKSISSR NGFEMIWDPNGWGTGTDNNFSIK QDIVGINEWSGYSGSFVQHPHEL TGLDCIRPCFWVELIRGRPKEN TIWTSGSSISFCGVNSDTVGWS WPDGAELPFTIDK (SEQ ID NO:63)	N1 numbering: 165V	vklaGnssslcpvsgwaiyskdn svrigskgdfvirepfiscsp lecrtffltqgallndkhsngt ikdrspyrtlmscpigVvpSPY nsrfesvawsasachdginwlt igisgpdngavavlkyngiitd tikswrnnilrtqesecacvng scftvmtdgpssngqasykifri ekgkivksvemnapnyhyeecs cypdsseitcvcrdnwhgsnrp wvsfnqnleyqigyicsgifgd nprpndktgscgpvssngangv kgfsYxygngvwigrtksissr ngfemiwdpnngwtgtdnnfsik qdivginewsgysgsfvqhpel tgldcirpcfwvelirgrpken tiwtsgssisfcgvnsdtvgws wpdgaelpftidk (SEQ ID NO:63)	
N1- Cal09_E 165A	VKLAGNSSLCPVSGWAIYSKDN SVRIGSKGDFVIREPFISCS? LECRTFFLTQGALLNDKHSNGT IKDRSPYRTLMSCPIGAVPSPY NSRFESVAWSASACHDGINWLT IGISGPDNGAVAVLKYNGIITD TIKSWRNNILRTQESSECACVNG SCFTVMTDGPSSNGQASYKIFRI EKGKIVKSVEMNAPNYHYEECS CYPDSSEITCVCARDNWHGSRNP WVSFNQNLLEYQIGYICSGIFGD NPRPNDKTGSCGPVSSNGANGV KGFSYKYNGVWIGRTKSISSR NGFEMIWDPNGWGTGTDNNFSIK QDIVGINEWSGYSGSFVQHPHEL TGLDCIRPCFWVELIRGRPKEN TIWTSGSSISFCGVNSDTVGWS WPDGAELPFTIDK (SEQ ID NO:64)	N1 numbering: 165A	vklaGnssslcpvsgwaiyskdn svrigskgdfvirepfiscsp lecrtffltqgallndkhsngt ikdrspyrtlmscpigAvpSPY nsrfesvawsasachdginwlt igisgpdngavavlkyngiitd tikswrnnilrtqesecacvng scftvmtdgpssngqasykifri ekgkivksvemnapnyhyeecs cypdsseitcvcrdnwhgsnrp wvsfnqnleyqigyicsgifgd nprpndktgscgpvssngangv kgfsYxygngvwigrtksissr ngfemiwdpnngwtgtdnnfsik qdivginewsgysgsfvqhpel tgldcirpcfwvelirgrpken tiwtsgssisfcgvnsdtvgws wpdgaelpftidk (SEQ ID NO:64)	
N1- Cal09_E 165I	VKLAGNSSLCPVSGWAIYSKDN SVRIGSKGDFVIREPFISCS? LECRTFFLTQGALLNDKHSNGT IKDRSPYRTLMSCPIGIVPSPY	N1 numbering: 165I	vklaGnssslcpvsgwaiyskdn svrigskgdfvirepfiscsp lecrtffltqgallndkhsngt ikdrspyrtlmscpigIvpSPY	

	NSRFESVAWSASACHDGINWLT IGISGPDNGAVAVLKYNGIITD TIKSWRNNILRTQESECACVNG SCFTVMTDGPSPNGQASYKIFRI EKGKIVKSVEMNAPNYHYEES CYPDSSEITCVCARDNWHGSRNP WVSFNQNLLEYQIGYICSGIFGD NPRPNDKTGSCGPVSSNGANGV KGFSYKYNGVWIGRTKSISSR NGFEMIWDPNGWGTGTDNNFSIK QDIVGINEWSGYSGSFVQHP TGLDCIRPCFWVELIRGRPKEN TIWTSGSSISFCGVNSDTVGWS WPDGAELPFTIDK (SEQ ID NO:65)		nsrfesvawsasachdginwlt igisgpdngavavlkynngiitd tikswrnnilrtqesecacvng scftvmtdgpsngqasykifri ekgkivksvemnapnyhyeecs cypdsseitcvcrdnwhgsnrp wvsfnqnleyqigyicsgifgd nprpndktgscgpvssngangv kgfsYkyngvwigrtksissr ngfemiwdpngwtgtdnnfsik qdivginewsgysgsfvqhpe tgldcirpcfwvelirgrpken tiwtsgssisfcgvnsdtvgws wpdgaelpftidk (SEQ ID NO:65)	
N1- Cal09- c155_s1 65T	VKLGNSSSLCPVSGWAPLSKDN SVRIGSKGDFVIREPFISCS? LECRTEFFLTQGALLNDKHSNGT IKDRSPYRTLMSVPiGTVPSPY NARFESIAWSASACHDGINWLT IGITGPDNGAVAILKYNGIITD TIKSWRNNILRTQESECACVNG SCFTVMTDGPSPNGQASYKIFRI EKGKIVKSVEMNAPNYHYEES CYPDSSEITCVCARDNWHGSRNP WVSFNQNLLEYQIGYICSGIFGD NPRPNDKTGSCGPVSSNGANGV KGFSFKYNGVWIGRTKSISSR NGFEMIWDPNGWGTGTDNNFSIK QDIVGINEWSGYSGSFVMHP TGLDCIVPCFWVELIRGRPKEN TIWTSGSSISFCGVNSDTVGWS WPDGAELPFTIDK (SEQ ID NO:66)	N1 numbering: 99P/177I/19 6T/205I, 165T, 100L/408M/4 19V, 161V/172A	vklnnssslcpvsgwaPlskdn svrigskgdfvirepfiscsp lecrteffltqgallndkhsngt ikdrspyrtlmsVpiGTvpspy nArfesIawsasachdginwlt igiTgpdngavaIlkynngiitd tikswrnnilrtqesecacvng scftvmtdgpsngqasykifri ekgkivksvemnapnyhyeecs cypdsseitcvcrdnwhgsnrp wvsfnqnleyqigyicsgifgd nprpndktgscgpvssngangv kgfsfkyngvwigrtksissr ngfemiwdpngwtgtdnnfsik qdivginewsgysgsfvMhp tgldciVpcfwvelirgrpken tiwtsgssisfcgvnsdtvgws wpdgaelpftidk (SEQ ID NO:66)	
N1- Cal09- c155_s1 65V	VKLGNSSSLCPVSGWAPLSKDN SVRIGSKGDFVIREPFISCS? LECRTEFFLTQGALLNDKHSNGT IKDRSPYRTLMSVPiGVVPSY NARFESIAWSASACHDGINWLT IGITGPDNGAVAILKYNGIITD TIKSWRNNILRTQESECACVNG SCFTVMTDGPSPNGQASYKIFRI EKGKIVKSVEMNAPNYHYEES CYPDSSEITCVCARDNWHGSRNP WVSFNQNLLEYQIGYICSGIFGD NPRPNDKTGSCGPVSSNGANGV KGFSFKYNGVWIGRTKSISSR NGFEMIWDPNGWGTGTDNNFSIK QDIVGINEWSGYSGSFVMHP TGLDCIVPCFWVELIRGRPKEN TIWTSGSSISFCGVNSDTVGWS WPDGAELPFTIDK (SEQ ID NO:67)	N1 numbering: 99P/177I/19 6T/205I, 165V, 100L/408M/4 19V, 161V/172A	vklnnssslcpvsgwaPlskdn svrigskgdfvirepfiscsp lecrteffltqgallndkhsngt ikdrspyrtlmsVpiGVvpspy nArfesIawsasachdginwlt igiTgpdngavaIlkynngiitd tikswrnnilrtqesecacvng scftvmtdgpsngqasykifri ekgkivksvemnapnyhyeecs cypdsseitcvcrdnwhgsnrp wvsfnqnleyqigyicsgifgd nprpndktgscgpvssngangv kgfsfkyngvwigrtksissr ngfemiwdpngwtgtdnnfsik qdivginewsgysgsfvMhp tgldciVpcfwvelirgrpken tiwtsgssisfcgvnsdtvgws wpdgaelpftidk (SEQ ID NO:67)	
N1- Cal09- c155_s1 65A	VKLGNSSSLCPVSGWAPLSKDN SVRIGSKGDFVIREPFISCS? LECRTEFFLTQGALLNDKHSNGT IKDRSPYRTLMSVPiGAVPSY NARFESIAWSASACHDGINWLT IGITGPDNGAVAILKYNGIITD TIKSWRNNILRTQESECACVNG SCFTVMTDGPSPNGQASYKIFRI	N1 numbering: 99P/177I/19 6T/205I, 165A, 100L/408M/4 19V, 161V/172A	vklnnssslcpvsgwaPlskdn svrigskgdfvirepfiscsp lecrteffltqgallndkhsngt ikdrspyrtlmsVpiGAvpspy nArfesIawsasachdginwlt igiTgpdngavaIlkynngiitd tikswrnnilrtqesecacvng scftvmtdgpsngqasykifri	

	EKGKIVKSVEMNAPNYHYECS CYPDSSEITCVCARDNWHGSRNP WVSFNQNLLEYQIGYICSGIFGD NPRPNDKTGSCGPVSSNGANGV KGFSFKYGNVWIGRTKSISSR NGFEMIWDPNWGTGTDNNFSIK QDIVGINEWSGYSGSFVMHPEL TGLDCIVPCFWVELIRGRPKEN TIWTSGSSISFCGVNSDTVGWS WPDGAELPFTIDK (SEQ ID NO:68)		ekgkivksvemnapnyhyeecs cypdsseitcvcardnwhgsrnp wvsfnqnleyqigyicsgifgd nprpndktgscgpvssngangv kgfsfkyngvwigrtksissr ngfemiwdpnwgtgtdnnfsik qdivginewsgysgsfvmhpel tgldciVpcfwwelirgrpken tiwtsgssisfcgvnsdtvgws wpdgaelpftidk (SEQ ID NO:68)	
N1- Cal09- c155_s1 65I	VKLAGNSSLCPVSGWAPLSKDN SVRIGSKGDVFIREFPISCS? LECRFFLTQGALLNDKHSNGT IKDRSPYRTLMSVPIGIVSPY NARFESIAWSASACHDGINWLT IGITGPDNGAVAILKYNGIITD TIKSWRNNILRTQESACVNG SCFTVMTDGPNSGQASYKIFRI EKGKIVKSVEMNAPNYHYECS CYPDSSEITCVCARDNWHGSRNP WVSFNQNLLEYQIGYICSGIFGD NPRPNDKTGSCGPVSSNGANGV KGFSFKYGNVWIGRTKSISSR NGFEMIWDPNWGTGTDNNFSIK QDIVGINEWSGYSGSFVMHPEL TGLDCIVPCFWVELIRGRPKEN TIWTSGSSISFCGVNSDTVGWS WPDGAELPFTIDK (SEQ ID NO:69)	N1 numbering: 99P/177I/19 6T/205I, 165I, 100L/408M/4 19V, 161V/172A	vklaGnssLcpvsgwApLskdn svrigskgdvfiRefpiscsp lecrffltqgallndkhsngt ikdrspyrtlmsVpiGivspy nArfesIawsasachdginwlt igiTgpdngavaIlkyngiitd tikswrnnilrtqesecacvng scftvmtdgpsngqasykifri ekgkivksvemnapnyhyeecs cypdsseitcvcardnwhgsrnp wvsfnqnleyqigyicsgifgd nprpndktgscgpvssngangv kgfsfkyngvwigrtksissr ngfemiwdpnwgtgtdnnfsik qdivginewsgysgsfvmhpel tgldciVpcfwwelirgrpken tiwtsgssisfcgvnsdtvgws wpdgaelpftidk (SEQ ID NO:69)	
N1- Cal09_C ysKO	VKLAGNSSLCPVSGWAIYSKDN SVRIGSKGDVFIREFPISCS? LECRFFLTQGALLNDKHSNGT IKDRSPYRTLMSVPIGEVSPY NARFESVAWSASACHDGINWLT IGISGPDNGAVAVLKYNIGIITD TIKSWRNNILRTQESACVNG SCFTVMTDGPNSGQASYKIFRI EKGKIVKSVEMNAPNYHYECS CYPDSSEITCVCARDNWHGSRNP WVSFNQNLLEYQIGYICSGIFGD NPRPNDKTGSCGPVSSNGANGV KGFSFKYGNVWIGRTKSISSR NGFEMIWDPNWGTGTDNNFSIK QDIVGINEWSGYSGSFVQHPEL TGLDCIRPCFWVELIRGRPKEN TIWTSGSSISFCGVNSDTVGWS WPDGAELPFTIDK (SEQ ID NO:70)	N1 numbering: 161V/172A	vklaGnssLcpvsgwaiyskdn svrigskgdvfiRefpiscsp lecrffltqgallndkhsngt ikdrspyrtlmsVpigeVspy nArfesvawsasachdginwlt igisgpdngavavlkynigitt tikswrnnilrtqesecacvng scftvmtdgpsngqasykifri ekgkivksvemnapnyhyeecs cypdsseitcvcardnwhgsrnp wvsfnqnleyqigyicsgifgd nprpndktgscgpvssngangv kgfsfkyngvwigrtksissr ngfemiwdpnwgtgtdnnfsik qdivginewsgysgsfvqhpeL tgldcirpcfwvelirgrpken tiwtsgssisfcgvnsdtvgws wpdgaelpftidk (SEQ ID NO:70)	
N1- Cal09- c155_s1 01C_I12 2V_G164 C_F174V _S444V	VKLAGNSSLCPVSGWAPLCKDN SVRIGSKGDVFIREFVSCSP LECRFFLTQGALLNDKHSNGT IKDRSPYRTLMSVPICSVPSPY NARVESIAWSASACHDGINWLT IGITGPDNGAVAILKYNGIITD TIKSWRNNILRTQESACVNG SCFTVMTDGPNSGQASYKIFRI EKGKIVKSVEMNAPNYHYECS CYPDSSEITCVCARDNWHGSRNP WVSFNQNLLEYQIGYICSGIFGD NPRPNDKTGSCGPVSSNGANGV	N1 numbering: 99P/177I/19 6T/205I, 165S, 100L/408M/4 19V, 161V/172A, 101C/122V/1 64C/174V/44 4V	vklaGnssLcpvsgwApLCKdn svrigskgdvfiRefVscsp lecrffltqgallndkhsngt ikdrspyrtlmsVpiCSVpspy nArVesIawsasachdginwlt igiTgpdngavaIlkyngiitd tikswrnnilrtqesecacvng scftvmtdgpsngqasykifri ekgkivksvemnapnyhyeecs cypdsseitcvcardnwhgsrnp wvsfnqnleyqigyicsgifgd nprpndktgscgpvssngangv	

	KGFSFKYGNVWIGRTKSISSR NGFEMIWDPNWGTGTDNNFSIK QDIVGINEWSGYSGSFMHPPEL TGLDCIVPCFWVELIRGRPKEN TIWTSGSSIVFCGVNSDTVGS WPDGAELPFTIDK (SEQ ID NO:71)		kgfsfkygngvwigrtksissr ngfemiwdpngwtgtdnnfsik qdivginewsgysgsfvmhpel tgldciVpcfwwelirgrpken tiwtsgssiVfcgvnsdtvgws wpdgaelpftidk (SEQ ID NO:71)	
N1- Cal09- c155_V1 14I_V16 6P_S101 C_I122V _G164C _F174V_S 444V	VKLGNSSSLCPVSGWAPLCKDN SVRIGSKGDI FVIREPFVSCSP LECRFFLTQGALLNDKHSNGT IKDRSPYRTLMSVPICSPSPY NARVESIAWSASACHDGINWLT IGITGPDNGAVAILKYNGIITD TIKSWRNNILRTQESACVNG SCFTVMTDGPNGQASYKIFRI EKGKIVKSVEMNAPNYHYECS CYPDSSEITCVCARDNWHGSRP WVSFNQNLLEYQIGYICSGIFGD NPRPNDKTGSCGPVSSNGANGV KGFSFKYGNVWIGRTKSISSR NGFEMIWDPNWGTGTDNNFSIK QDIVGINEWSGYSGSFMHPPEL TGLDCIVPCFWVELIRGRPKEN TIWTSGSSIVFCGVNSDTVGS WPDGAELPFTIDK (SEQ ID NO:72)	N1 numbering: 99P/177I/19 6T/205I, 165S, 100L/408M/4 19V, 161V/172A, 114I/166P, 101C/122V/1 64C/174V/44 4V	vklnagnssslcpvsgwaPLCKdn svrigskgdIfvirepfVscsp lecrffltqgallndkhsngt ikdrspyrtlmsVpiCSppspy nArVesIawsasachdginwlt igiTgpdngavaIlkyngiitd tikswrnnilrtqesecacvng scftvmtdgpnsngqasykifri ekgkivksvemnapnyhyeecs cypdsseitcvcrdnwhgsnrp wvsfnqnleyqigiyicsgifgd nprpndktgscgpvssngangv kgfsfkygngvwigrtksissr ngfemiwdpngwtgtdnnfsik qdivginewsgysgsfvmhpel tgldciVpcfwwelirgrpken tiwtsgssiVfcgvnsdtvgws wpdgaelpftidk (SEQ ID NO:72)	
N1- Cal09- c155_T1 31Q_S10 1C_I122 V_G164C _F174V - S444V	VKLGNSSSLCPVSGWAPLCKDN SVRIGSKGDI FVIREPFVSCSP LECRQFFLTQGALLNDKHSNGT IKDRSPYRTLMSVPICSPSPY NARVESIAWSASACHDGINWLT IGITGPDNGAVAILKYNGIITD TIKSWRNNILRTQESACVNG SCFTVMTDGPNGQASYKIFRI EKGKIVKSVEMNAPNYHYECS CYPDSSEITCVCARDNWHGSRP WVSFNQNLLEYQIGYICSGIFGD NPRPNDKTGSCGPVSSNGANGV KGFSFKYGNVWIGRTKSISSR NGFEMIWDPNWGTGTDNNFSIK QDIVGINEWSGYSGSFMHPPEL TGLDCIVPCFWVELIRGRPKEN TIWTSGSSIVFCGVNSDTVGS WPDGAELPFTIDK (SEQ ID NO:73)	N1 numbering: 99P/177I/19 6T/205I, 165S, 100L/408M/4 19V, 161V/172A, 131Q, 101C/122V/1 64C/174V/44 4V	vklnagnssslcpvsgwaPLCKdn svrigskgdIfvirepfVscsp lecrQffltqgallndkhsngt ikdrspyrtlmsVpiCSppspy nArVesIawsasachdginwlt igiTgpdngavaIlkyngiitd tikswrnnilrtqesecacvng scftvmtdgpnsngqasykifri ekgkivksvemnapnyhyeecs cypdsseitcvcrdnwhgsnrp wvsfnqnleyqigiyicsgifgd nprpndktgscgpvssngangv kgfsfkygngvwigrtksissr ngfemiwdpngwtgtdnnfsik qdivginewsgysgsfvmhpel tgldciVpcfwwelirgrpken tiwtsgssiVfcgvnsdtvgws wpdgaelpftidk (SEQ ID NO:73)	
N1- Cal09- c155_V1 14I_V16 6P_T131 Q_S101C _I122V _G164C_F 174V_S4 44V	VKLGNSSSLCPVSGWAPLCKDN SVRIGSKGDI FVIREPFVSCSP LECRQFFLTQGALLNDKHSNGT IKDRSPYRTLMSVPICSPSPY NARVESIAWSASACHDGINWLT IGITGPDNGAVAILKYNGIITD TIKSWRNNILRTQESACVNG SCFTVMTDGPNGQASYKIFRI EKGKIVKSVEMNAPNYHYECS CYPDSSEITCVCARDNWHGSRP WVSFNQNLLEYQIGYICSGIFGD NPRPNDKTGSCGPVSSNGANGV KGFSFKYGNVWIGRTKSISSR NGFEMIWDPNWGTGTDNNFSIK QDIVGINEWSGYSGSFMHPPEL TGLDCIVPCFWVELIRGRPKEN	N1 numbering: 99P/177I/19 6T/205I, 165S, 100L/408M/4 19V, 161V/172A, 114I/166P, 131Q, 101C/122V/1 64C/174V/44 4V	vklnagnssslcpvsgwaPLCKdn svrigskgdIfvirepfVscsp lecrQffltqgallndkhsngt ikdrspyrtlmsVpiCSppspy nArVesIawsasachdginwlt igiTgpdngavaIlkyngiitd tikswrnnilrtqesecacvng scftvmtdgpnsngqasykifri ekgkivksvemnapnyhyeecs cypdsseitcvcrdnwhgsnrp wvsfnqnleyqigiyicsgifgd nprpndktgscgpvssngangv kgfsfkygngvwigrtksissr ngfemiwdpngwtgtdnnfsik qdivginewsgysgsfvmhpel tgldciVpcfwwelirgrpken	

	TIWTSGSSIVFCGVNSDTVGWS WPDGAELPFTIDK (SEQ ID NO:74)		tiwtsgssivfcgvnsdtvgws wpdgaelpftidk (SEQ ID NO:74)	
N1- Cal09- c155_T1 31M_I16 3L_S442 I_S444A _S101C _G164C_F 174V	VKLAGNSSLCPVSGWAPLCKDN SVRIGSKGDFVIREPFISCS? LECRMFFLTQGALLNDKHSNGT IKDRSPYRTLMSVPLCSVPSPY NARVESIAWSASACHDGINWLT IGITGPDNGAVAILKYNGIITD TIKSWRNNILRTQESACVNG SCFTVMTDGPNSNGQASYKIFRI EKGKIVKSVMENAPNYHYECS CYPDSSEITCVCARDNWHGSRNP WVSFNQNLLEYQIGYICSGIFGD NPRPNDKTGSCGPVSSNGANGV KGFSFKYNGVWIGRTKSISR NGFEMIWDPNGTGTDDNFSIK QDIVGINEWSGYSGSFMHPPEL TGLDCIVPCFWVELIRGRPKEN TIWTSGSIIAFCGVNSDTVGWS WPDGAELPFTIDK (SEQ ID NO:75)	N1 numbering: 99P/177I/19 6T/205I, 165S, 100L/408M/4 19V, 161V/172A, 131M/163L/4 42I/444A, 101C/164C/1 74V	vklaGnssLcPvsgwaPLCkdn svrigskgdvfvirepfiscsp lecrMffltqgallndkhsngt ikdrspyrtlmsVpLCSVpspy nArVesIawsasachdginwlt igiTgpdngavaIlkyngiitd tikswrnnilrtqesecacvng scftvmtdgpsngqasykifri ekgkivksvemnapnyhyeecs cypdsseitcvcrdnwhgsnrp wvsfnqnleyqigiyicsgifgd nprpndktgscgpvssngangv kgfsfkyngvwigrtksissr ngfemiwdpngwtgtddnfsik qdivginewsgysgsfvmhpel tgldciVpcfvelirgrpken tiwtsgsIIAfcgvnsdtvgws wpdgaelpftidk (SEQ ID NO:75)	
N1- Cal09- c155_T1 31M_S44 2I_S101 C_I122V _G164C _F174V_S 444V	VKLAGNSSLCPVSGWAPLCKDN SVRIGSKGDFVIREPFISCS? LECRMFFLTQGALLNDKHSNGT IKDRSPYRTLMSVPLCSVPSPY NARVESIAWSASACHDGINWLT IGITGPDNGAVAILKYNGIITD TIKSWRNNILRTQESACVNG SCFTVMTDGPNSNGQASYKIFRI EKGKIVKSVMENAPNYHYECS CYPDSSEITCVCARDNWHGSRNP WVSFNQNLLEYQIGYICSGIFGD NPRPNDKTGSCGPVSSNGANGV KGFSFKYNGVWIGRTKSISR NGFEMIWDPNGTGTDDNFSIK QDIVGINEWSGYSGSFMHPPEL TGLDCIVPCFWVELIRGRPKEN TIWTSGSIIVFCGVNSDTVGWS WPDGAELPFTIDK (SEQ ID NO:76)	N1 numbering: 99P/177I/19 6T/205I, 165S, 100L/408M/4 19V, 161V/172A, 131M/442I, 101C/122V/1 64C/174V/44 4V	vklaGnssLcPvsgwaPLCkdn svrigskgdvfvirepfiscsp lecrMffltqgallndkhsngt ikdrspyrtlmsVpLCSVpspy nArVesIawsasachdginwlt igiTgpdngavaIlkyngiitd tikswrnnilrtqesecacvng scftvmtdgpsngqasykifri ekgkivksvemnapnyhyeecs cypdsseitcvcrdnwhgsnrp wvsfnqnleyqigiyicsgifgd nprpndktgscgpvssngangv kgfsfkyngvwigrtksissr ngfemiwdpngwtgtddnfsik qdivginewsgysgsfvmhpel tgldciVpcfvelirgrpken tiwtsgsIIvfcgvnsdtvgws wpdgaelpftidk (SEQ ID NO:76)	
N1- Cal09- c155_V1 14I_V16 6P_T131 M_I163L _S442I _S444A_S 101C_G1 64C_F17 4V	VKLAGNSSLCPVSGWAPLCKDN SVRIGSKGDFVIREPFISCS? LECRMFFLTQGALLNDKHSNGT IKDRSPYRTLMSVPLCSPPSPY NARVESIAWSASACHDGINWLT IGITGPDNGAVAILKYNGIITD TIKSWRNNILRTQESACVNG SCFTVMTDGPNSNGQASYKIFRI EKGKIVKSVMENAPNYHYECS CYPDSSEITCVCARDNWHGSRNP WVSFNQNLLEYQIGYICSGIFGD NPRPNDKTGSCGPVSSNGANGV KGFSFKYNGVWIGRTKSISR NGFEMIWDPNGTGTDDNFSIK QDIVGINEWSGYSGSFMHPPEL TGLDCIVPCFWVELIRGRPKEN TIWTSGSIIAFCGVNSDTVGWS WPDGAELPFTIDK (SEQ ID NO:77)	N1 numbering: 99P/177I/19 6T/205I, 165S, 100L/408M/4 19V, 161V/172A, 114I/166P, 131M/163L/4 42I/444A, 101C/164C/1 74V	vklaGnssLcPvsgwaPLCkdn svrigskgdIfvirepfiscsp lecrMffltqgallndkhsngt ikdrspyrtlmsVpLCSppspy nArVesIawsasachdginwlt igiTgpdngavaIlkyngiitd tikswrnnilrtqesecacvng scftvmtdgpsngqasykifri ekgkivksvemnapnyhyeecs cypdsseitcvcrdnwhgsnrp wvsfnqnleyqigiyicsgifgd nprpndktgscgpvssngangv kgfsfkyngvwigrtksissr ngfemiwdpngwtgtddnfsik qdivginewsgysgsfvmhpel tgldciVpcfvelirgrpken tiwtsgsIIAfcgvnsdtvgws wpdgaelpftidk (SEQ ID NO:77)	

N1- Cal09- c155_V1 14I_V16 6P_T131 M_S442I _S101C_ I122V_G 164C_F1 74V_S44 4V	VKLAGNSSLCPVSGWAPLCKDN SVRIGSKGDI FVIREPFVSCSP LECRMFFLTQGALLNDKHSNGT IKDRSPYRTLMSVPICSPSPSY NARVESIAWSASACHDGINWLT IGITGPDNGAVAILKYNGIITD TIKSWRNNILRTQESACVNG SCFTVMTDGPNGQASYKIFRI EKGKIVKSVEMNAPNYHYECS CYPDSSEITCVCARDNWHGSRNP WVSFNQNL EYQIGYICSGIFGD NPRPNDKTGSCGPVSSNGANGV KGFSFKYNGVWIGRTKSISR NGFEMIWDPNGTGTDNFNSIK QDIVGINEWSGYSGSFVMHP TGLDCIVPCFWVELIRGRPKEN TIWTSGSII VFCGVNSDTVGWS WPDGAELPFTIDK (SEQ ID NO:78)	N1 numbering: 99P/177I/19 6T/205I, 165S, 100L/408M/4 19V, 161V/172A, 114I/166P, 131M/442I, 101C/122V/1 64C/174V/44 4V	vklagNSSLCpvsgwaPLCkdn svrigskgdIfvirepfVscsp lecrMffltqgallndkhsngt ikdrspyrtlmsVpiCSPPSPY nArVesIawsasachdginwlt igiTgpdngavaIlkyngiitd tikswrnnilrtqesecacvng scftvmtdgpsngqasykifri ekgkivksvemnapnyhyeecs cypdsseitcvcrdnwhgsnrp wvsfnqnleyqigiyicsgifgd nprpndktgscgpvssngangv kgfsfkyngvwigrtksissr ngfemiwdpngwtgtDNFNSIK qdivginewsgysgsfvMhpel tgldciVpcfvelirgrpken tiwtsgsII Vfcgvnsdtvgws wpdgaelpftidk (SEQ ID NO:78)	
N1- Cal09- c130_T4 53V_I20 5V	VKLAGNSSLCPVSGWAPLSKDN SVRIGSKGDFVIREPFISCS? LECRFFLTQGALLNDKHSNGT IKDRSPYRTLMSVPIGSVPSPY NARFESIAWSASACHDGINWLT IGITGPDNGAVAVLKYNIGIITD TIKSWRNNILRTQESACVNG SCFTVMTDGPNGQASYKIFRI EKGKIVKSVEMNAPNYHYECS CYPDSSEITCVCARDNWHGSRNP WVSFNQNL EYQIGYICSGIFGD NPRPNDKTGSCGPVSSNGANGV KGFSFKYNGVWIGRTKSISR NGFEMIWDPNGTGTDNFNSIK QDIVGINEWSGYSGSFVMHP TGLDCIVPCFWVELIRGRPKEN TIWTSGSSISFCGVNSDTVGWS WPDGAELPFTIDK (SEQ ID NO:87)	N1 numbering: 99P/177I/19 6T, 165S, 100L/408M/4 19V, 161V/172A	vklagNSSLCpvsgwaPLskdn svrigskgdvfvirepfiscsp lecrffltqgallndkhsngt ikdrspyrtlmsVpigSvpspy nArfesIawsasachdginwlt igiTgpdngavavlkynigiitd tikswrnnilrtqesecacvng scftvmtdgpsngqasykifri ekgkivksvemnapnyhyeecs cypdsseitcvcrdnwhgsnrp wvsfnqnleyqigiyicsgifgd nprpndktgscgpvssngangv kgfsfkyngvwigrtksissr ngfemiwdpngwtgtDNFNSIK qdivginewsgysgsfvMhpel tgldciVpcfvelirgrpken tiwtsgssisfcgvnsdtvgws wpdgaelpftidk (SEQ ID NO:87)	
Mi15_c1 55_I205 V	VKLAGNSSLCPVSGWAPLSKDN SVRIGSKGDFVIREPFISCS? LECRFFLTQGALLNDKHSNGT IKDRSPYRTLMSVPIGSVPSPY NARFESIAWSASACHDGINWLT IGITGPDNGAVAVLKYNIGIITD TIKSWRNNILRTQESACVNG SCFTIMTDGPSDGQASYKIFRI EKGKI IKSVMKAPNYHYECS CYPDSSEITCVCARDNWHGSRNP WVSFNQNL EYQMGYICSGVFGD NPRPNDKTGSCGPVSSNGANGV KGFSFKYNGVWIGRTKSISR KGFEMIWDPNGTGTDNKFSIK QDIVGINEWSGYSGSFVMHP TGLDCIVPCFWVELIRGRPEEN TIWTSGSSISFCGVNSDTVGWS WPDGAELPFTIDK (SEQ ID NO:88)	N1 numbering: 99P/177I/19 6T, 165S, 100L/408M/4 19V, 161V/172A	vklagNSSLCpvsgwaPLskdn svrigskgdvfvirepfiscsp lecrffltqgallndkhsngt ikdrspyrtlmsVpigSvpspy nArfesIawsasachdginwlt igiTgpdsgavavlkynigiitd tikswrnnilrtqesecacvng scftimtdgpsdgqasykifri ekgkiiksvekmkpnhyhyeecs cypdsseitcvcrdnwhgsnrp wvsfnqnleyqmgycsgvfgd nprpndktgscgpvssngangv kgfsfkyngvwigrtksissr kgfemiwdpngwtgtDNKFSIK qdivginewsgysgsfvMhpel tgldciVpcfvelirgrpeen tiwtsgssisfcgvnsdtvgws wpdgaelpftidk (SEQ ID NO:88)	
N1- Mi15_c1 55_T131 Q_I205V	VKLAGNSSLCPVSGWAPLSKDN SVRIGSKGDFVIREPFISCS? LECRQFFLTQGALLNDKHSNGT IKDRSPYRTLMSVPIGSVPSPY	N1 numbering: 99P/177I/19 6T, 165S,	vklagNSSLCpvsgwaPLskdn svrigskgdvfvirepfiscsp lecrQffltqgallndkhsngt ikdrspyrtlmsVpigSvpspy	

	NARFESIAWSASACHDGINWLT IGITGPDGSAVAVLKYNGIITD TIKSWRNNILRTQESECACVNG SCFTIMTDGPGSDGQASYKIFRI EKGKIIKSVEMKAPNYHYEES CYPDSSEITCVCARDNWHGSRNP WVSFNQNLLEYQMGYICSGVFGD NPRPNDKTGSCGPVSSNGANGV KGFSFKYGNVWIGRTKSISSR KGFEMIWDPNGTGTDNKFSIK QDIVGINEWSGYSGSFMHP TGLDCIVPCFWVELIRGRPEEN TIWTSGSSISFCGVNSDTVGWS WPDGAELPFTIDK (SEQ ID NO:89)	100L/408M/4 19V, 161V/172A, 131Q	nArfesIawsasachdginwlt igiTgpdsgavavlkyngiitd tikswrnnilrtqesecacvng scftimtdgpgsdgqasykifri ekgkiiksvemkapnyhyeecs cypdsseitcvcrdnwhgsnrp wvsfnqnleyqmgycsgvfgd nprpndktgscgpvssngangv kgfsfkygngvwigrtksisr kgfemiwdpngwtgtdnkfsik qdivginewsgysgsfvmhpel tgldciVpcfwwelirgrpeen tiwtsgssisfcgvnsdtvgws wpdgaelpftidk (SEQ ID NO:89)	
N1- Mi15_c1 55_S101 A_T131M I163L S442I_S 444A_I2 05V	VKLGNSSSLCPVSGWAPLAKDN SVRIGSKGDVFIREFFISCS LECRMFFLTQGALLNDKHSNGT IKDRSPYRTLMSVPLGSPSPY NARFESIAWSASACHDGINWLT IGITGPDGSAVAVLKYNGIITD TIKSWRNNILRTQESECACVNG SCFTIMTDGPGSDGQASYKIFRI EKGKIIKSVEMKAPNYHYEES CYPDSSEITCVCARDNWHGSRNP WVSFNQNLLEYQMGYICSGVFGD NPRPNDKTGSCGPVSSNGANGV KGFSFKYGNVWIGRTKSISSR KGFEMIWDPNGTGTDNKFSIK QDIVGINEWSGYSGSFMHP TGLDCIVPCFWVELIRGRPEEN TIWTSGSSIIAFCGVNSDTVGWS WPDGAELPFTIDK (SEQ ID NO:90)	N1 numbering: 99P/177I/19 6T, 165S, 100L/408M/4 19V, 161V/172A, 101A/131M/1 63L/442I/44 4A	vklnssslcpvsgwaPLAkd svrigskgdvfiorefiscsp lecrMffltqgallndkhsngt ikdrspyrTlmsVpLgSvpspy nArfesIawsasachdginwlt igiTgpdsgavavlkyngiitd tikswrnnilrtqesecacvng scftimtdgpgsdgqasykifri ekgkiiksvemkapnyhyeecs cypdsseitcvcrdnwhgsnrp wvsfnqnleyqmgycsgvfgd nprpndktgscgpvssngangv kgfsfkygngvwigrtksisr kgfemiwdpngwtgtdnkfsik qdivginewsgysgsfvmhpel tgldciVpcfwwelirgrpeen tiwtsgsIIAfcgvnsdtvgws wpdgaelpftidk (SEQ ID NO:90)	
N1- VN04_c1 55_I106 V_T131Q V163I A166V_I 205V	VKLGNSSSLCPINGWAPLSKDN SVRIGSKGDVFIREFFISCSH LECRQFFLTQGALLNDKHSNGT VKDRSPHRTLMSVPIGSPSPY NARFESIAWSASACHDGTSWLT IGITGPDNGAVAVLKYNGIITD TIKSWRNNILRTQESECACVNG SCFTVMTDGPNGQASYKIFKM EKGKVKSVELDAPNYHYEES CYPNAGEITCVCARDNWHGSRNP WVSFNQNLLEYQIGYICSGVFGD NPRPNDGTGSCGPVSSNGAYGV KGFSFKYGNVWIGRTKSTNSR SGFEMIWDPNGTETDSSFSVK QDIVAITDWSGYSGSFMHP TGLDCIVPCFWVELIRGRPKES TIWTSGSSISFCGVNSDTVGWS WPDGAELPFTIDK (SEQ ID NO:91)	N1 numbering: 99P/177I/19 6T, 165S, 100L/408M/4 19V, 161V/172A, 131Q, 106V, 163I, 166V	vklnssslcpingwaPLskdn svrigskgdvfiorefiscsh lecrQffltqgallndkhsngt vkdrspyrTlmsVpIgSvpspy nArfesIawsasachdgtswlt igiTgpdngavavlkyngiitd tikswrnnilrtqesecacvng scftvmtdgpsngqasykifkm ekgkvksveldapnyhyeecs cypnageitcvcrdnwhgsnrp wvsfnqnleyqigyicsgvfgd nprpndgtgscgpvssngaygv kgfsfkygngvwigrtkstnsr sgfemiwdpngwtetdssfsvk qdivaitdwsygsfvmhpel tgldciVpcfwwelirgrpk tiwtsgssisfcgvnsdtvgws wpdgaelpftidk (SEQ ID NO:91)	
N1- WSN33_c 155_G10 5S_I106 V_A157T V163I A166V_R	VILTNSSSLCPIRGWAPLSKDN SVRIGSKGDVFIREFFISCSH LECRFFLTQGALLNDKHSRGT FKDRSPYRTLMSVPIGSPSPY NARFESIAWSASACHDGMGWL IGITGPDGSAVAVLKYNGIITE TIKSWRNNILRTQESECTCVNG SCFTIMTDGPGDGLASYKIFKI	N1 numbering: 99P/177I/19 6T, 165S, 100L/408M/4 19V, 161V/172A, 105S, 106V,	viltgnssslcpirgwaPLskdn SVrigskgdvfiorefiscsh lecrtffltqgallndkhsrgt fkdrspyrTlmsVpIgSvpspy nArfesIawsasachdgmgwlt igiTgpdgavavlkyngiite tikswrnnilrtqesectcvng scftimtdgpgdglasykifki	

210G_I2 05V	EKGKVTKSIELNAPNSHYECS CYPDTGKVMCVCRDNWHGNSRP WVSFDQNL DYKIGYICSGVFGD NPRPKDGTGSCGPVSADGANGV KGFSYKYGNVWIGRTKSDSSR HGFEMIWDPNGWETETDSRFSMR QDVVAITNRSGYSGSFMHPEL TGLDCMVPCFWVELIRGLPEED AIWTSGSIISFCGVNSDTVDS WPDGAELPFTIDK (SEQ ID NO:92)	157T, 163I, 166V, 210G	ekgkvtksielnapnshyeecs cypdtgkvmcvcrdnwhgsnrp wvsfdqnl dykigyicsgvfgd nprpkdgtgscgpvsadgangv kgfsykyngvwigrtksdssr hgfemiwdpngwtetdsrfsmr qdvvaitnrsgysgsfvmhpel tgldcmVpcfwwelirglpeed aiwtsgsiisfcgvnsdtvds wpdgaelpftidk (SEQ ID NO:92)	
N1- WSN33_c 155_G10 5S_I106 V_T131Q _A157T_ V163I_A 166V_R2 10G_I20 5V	VILTGNSSLCPIRGWAPlskdn SVRIGSKGDVfVIREPFISCSH LECRQFFLTQGALLNDKHSRGT FKDRSPYRTLMSVPiGsvpspy NARFESIAWSASACHDGMWLT IGITGPDGAVAVLKynGIITE TIKSWRNILRTQESectcVNG SCFTIMTDGpsdGLasyKIFKI EKGKVTKSIELNAPNSHYECS CYPDTGKVMCVCRDNWHGNSRP WVSFDQNL DYKIGYICSGVFGD NPRPKDGTGSCGPVSADGANGV KGFSYKYGNVWIGRTKSDSSR HGFEMIWDPNGWETETDSRFSMR QDVVAITNRSGYSGSFMHPEL TGLDCMVPCFWVELIRGLPEED AIWTSGSIISFCGVNSDTVDS WPDGAELPFTIDK (SEQ ID NO:93)	N1 numbering: 99P/177I/19 6T, 165S, 100L/408M/4 19V, 161V/172A, 131Q, 105S, 106V, 157T, 163I, 166V, 210G	viltgnssslcpirgwaPlskdn SVrigskgdvfvirepfiscsh lecrQffltqgallndkhsrgt fkdrspyrTlmsVpIgSVpspy nArfesIawsasachdgmwlt igiTgpdgavavlkynGiite tikswrknilrtqesectcVng scftimtdgpsdGLasykifki ekgkvtksielnapnshyeecs cypdtgkvmcvcrdnwhgsnrp wvsfdqnl dykigyicsgvfgd nprpkdgtgscgpvsadgangv kgfsykyngvwigrtksdssr hgfemiwdpngwtetdsrfsmr qdvvaitnrsgysgsfvmhpel tgldcmVpcfwwelirglpeed aiwtsgsiisfcgvnsdtvds wpdgaelpftidk (SEQ ID NO:93)	
N1- Ca109- c155_T1 31Q_I20 5V	VKLGNSSSLCPVSGWAPlskdn SVRIGSKGDVfVIREPFISCS? LECRQFFLTQGALLNDKHSNGT IKDRSPYRTLMSVPiGsvpspy NARFESIAWSASACHDGINWLT IGITGPDNGAVAVLKynGIITD TIKSWRNILRTQESecacVNG SCFTVMTDgpsNGQasyKIFRI EKGKIVKSVEMNAPNYHYECS CYPDSSEITCVCRDNWHGNSRP WVSFNQNL EYQIGYICSGIFGD NPRPNDKTGSCGPVSSNGANGV KGFSFKYGNVWIGRTKSISSR NGFEMIWDPNGWGTGTDNNFSIK QDIVGINEWSGYSGSFMHPEL TGLDCIVPCFWVELIRGRPKEN TIWTSGSSISFCGVNSDTVGS WPDGAELPFTIDK (SEQ ID NO:94)	N1 numbering: 99P/177I/19 6T, 165S, 100L/408M/4 19V, 161V/172A, 131Q	vklnssslcpvsgwaPlskdn svrigskgdvfvirepfiscsp lecrQffltqgallndkhsngt ikdrspyrTlmsVpIgSVpspy nArfesIawsasachdginwlt igiTgpdngavavlkynGIITD tikswrnnilrtqesecacVng scftvmtdgpsngQasykifri ekgkivksvemnapnyhyeecs cypdsseitcvcrdnwhgsnrp wvsfnqnleyqigyicsgifgd nprpnDKtgscgpvssngangv kgfsfkyngvwigrtksissr ngfemiwdpngwtgtdnnfsik qdivginewsgysgsfvmhpel tgldciVpcfwwelirgrpken tiwtsgssisfcgvnsdtvgws wpdgaelpftidk (SEQ ID NO:94)	
N1- Ca109- c155_S1 01A_T13 1M_I163 L_S442I _S444A_ I205V	VKLGNSSSLCPVSGWAPlakdn SVRIGSKGDVfVIREPFISCS? LECRMFFLTQGALLNDKHSNGT IKDRSPYRTLMSVPLGsvpspy NARFESIAWSASACHDGINWLT IGITGPDNGAVAVLKynGIITD TIKSWRNILRTQESecacVNG SCFTVMTDgpsNGQasyKIFRI EKGKIVKSVEMNAPNYHYECS CYPDSSEITCVCRDNWHGNSRP WVSFNQNL EYQIGYICSGIFGD NPRPNDKTGSCGPVSSNGANGV	N1 numbering: 99P/177I/19 6T, 165S, 100L/408M/4 19V, 161V/172A, 101A/131M/1 63L/442I/44 4A	vklnssslcpvsgwaPlakdn svrigskgdvfvirepfiscsp lecrMffltqgallndkhsngt ikdrspyrTlmsVpLgSVpspy nArfesIawsasachdginwlt igiTgpdngavavlkynGIITD tikswrnnilrtqesecacVng scftvmtdgpsngQasykifri ekgkivksvemnapnyhyeecs cypdsseitcvcrdnwhgsnrp wvsfnqnleyqigyicsgifgd nprpnDKtgscgpvssngangv	

KGFSFKYNGVWIGRTKSISSR NGFEMIWDPNGWTGTDNNFSIK QDIVGINEWSGYSGSFVMHPEL TGLDCIVPCFWVELIRGRPKEN TIWTSGSIIAFCGVNSDTVGWS WPDGAELPFTIDK (SEQ ID NO:95)	kgfsfkyngvwigrtksissr ngfemiwdpngwtgtdnnfsik qdivginewsgysgsfvMhpel tgldciVpcfvelirgrpken tiwtsgsIiAfcgvnsdtvgws wpdgaelpftidk (SEQ ID NO:95)
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- 100C/163C;
 100C/163C/173V;
 100C/163C/173V/445V;
 130Q/443I;
 5 100A/162L/445V;
 130M/443I;
 100A/130M/162L/443I/445A;
 130M/162L/443I/445A;
 176I/204I;
 10 100C/121V/163C/173V/445V; and/or
 121V/445V.

36. The polypeptide of any one of claim 32-34, wherein the polypeptide includes three or more of the listed amino acid residues relative to SEQ ID NO:4 when aligned by protocol 1
 15 or protocol 2.

37. The polypeptide of any one of claim 32-34, wherein the polypeptide includes five or more of the listed amino acid residues relative to SEQ ID NO:4 when aligned by protocol 1
 20 or protocol 2.

38. The polypeptide of any one of claim 32-34, wherein the polypeptide includes seven or more of the listed amino acid residues relative to SEQ ID NO:4 when aligned by protocol 1
 or protocol 2.

39. The polypeptide of any one of claim 32-34, wherein the polypeptide includes nine or more of the listed amino acid residues relative to SEQ ID NO:4 when aligned by protocol 1
 25 or protocol 2.

40. The non-naturally occurring polypeptide of claim 1, wherein the polypeptide is at
 30 least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the N4 NA polypeptide of SEQ ID NO:4, and wherein the non-naturally occurring polypeptide includes 1, or both of the following amino acid residues relative to SEQ ID NO:4 when aligned by protocol 1 or protocol 2: 164Q/E, 195S.

41. The non-naturally occurring polypeptide of claim 1, wherein the polypeptide is
- (a) at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the N5 NA polypeptide of SEQ ID NO:5, and wherein the non-naturally occurring polypeptide includes 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or all 13 of the following amino acid residues relative to SEQ ID NO:5 when aligned by protocol 1 or protocol 2:
- 97L, 410M**, 96P, 98A, 100N, 102S/A, 110D, 128Q/M, 160I, 162V/A/I, 163V/P, 193Q/T, 445I; or
- (b) at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the N5 NA polypeptide of SEQ ID NO:5, and wherein the non-naturally occurring polypeptide includes 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or all 16 of the following amino acid residues relative to SEQ ID NO:5 when aligned by protocol 1 or protocol 2:
- 97L, 410M**, 96P, 98A, 100N, 102S/A, 103V, 110D, 128Q/M, 154T, 160I, 162V/A/I/T, 163V/P, 174I, 193Q/T, 445I.
42. The polypeptide of claim 41, wherein the polypeptide is
- (a) at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the N5 NA polypeptide of SEQ ID NO:5, and wherein the non-naturally occurring polypeptide includes 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or all 13 of the following amino acid residues relative to SEQ ID NO:5 when aligned by protocol 1 or protocol 2:
- (i) **97L, 410M**, 96P, 98A, 100N, 102S/A, 110D, 128Q/M, 160I, 162V/A/I, 163V/P, 193Q/T, 445I; or
- (ii) 96P, 98A, 100N, 102S/A, 110D, 128Q/M, 160I, 162V/A/I, 163V/P, 193Q/T, 445I.
43. The polypeptide of claim 41, wherein the polypeptide is
- (b) at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the N5 NA polypeptide of SEQ ID NO:5, and wherein the non-naturally occurring polypeptide includes 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or all 16 of the following amino acid residues relative to SEQ ID NO:5 when aligned by protocol 1 or protocol 2:
- (i) **97L, 410M**, 96P, 98A, 100N, 102S/A, 103V, 110D, 128Q/M, 154T, 160I, 162V/A/I/T, 163V/P, 174I, 193Q/T, 445I; or

(ii) 96P, 98A, 100N, 102S/A, 103V, 110D, 128Q/M, 154T, 160I, 162V/A/I/T, 163V/P, 174I, 193Q/T, 445I.

44. The polypeptide of any one of claim 41-43, wherein the polypeptide includes one or
5 more of the following sets of amino acid residues relative to SEQ ID NO:5 when aligned by protocol 1 or protocol 2:

(i)

97L/410M;
100N/102A;
10 98C/161C;
128Q/445I;
128M/445I;
98A/128M/445I/447A;
97L/98A/128M/445I/447A;
15 128M/445I/447A;
97L/128M/445I/447A;
96P/193T;
111I/163P;
96P/193T/202I;
20 96P/174I/193T; and/or
96P/174I/193T/202I; or

(ii)

100N/102A;
98C/161C;
25 128Q/445I;
128M/445I;
98A/128M/445I/447A;
97L/98A/128M/445I/447A;
128M/445I/447A;
30 97L/128M/445I/447A;
96P/193T;
111I/163P;
96P/193T/202I;
96P/174I/193T; and/or

96P/174I/193T/202L

45. The polypeptide of any one of claim 41-43, wherein the polypeptide includes three or more of the listed amino acid residues relative to SEQ ID NO:5 when aligned by protocol 1 or protocol 2.
46. The polypeptide of any one of claim 41-43, wherein the polypeptide includes five or more of the listed amino acid residues relative to SEQ ID NO:5 when aligned by protocol 1 or protocol 2.
47. The polypeptide of any one of claim 41-43, wherein the polypeptide includes seven or more of the listed amino acid residues relative to SEQ ID NO:5 when aligned by protocol 1 or protocol 2.
48. The polypeptide of any one of claim 41-43, wherein the polypeptide includes nine or more of the listed amino acid residues relative to SEQ ID NO:5 when aligned by protocol 1 or protocol 2.
49. The non-naturally occurring polypeptide of claim 1, wherein the polypeptide is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the N5 NA polypeptide of SEQ ID NO:5, and wherein the non-naturally occurring polypeptide includes 1, or both of the following amino acid residues relative to SEQ ID NO:5 when aligned by protocol 1 or protocol 2: 162 Q/E, 200S.
50. The non-naturally occurring polypeptide of claim 1, wherein the polypeptide is
- (a) at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the N6 NA polypeptide of SEQ ID NO:6, and wherein the non-naturally occurring polypeptide includes 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or all 11 of the following amino acid residues relative to SEQ ID NO:6 when aligned by protocol 1 or protocol 2:
- 99P, 103N, 113D, 131Q, 161I, 162P, 163I, 165S/T/V/A, 196Q, 203Y, 445S; or
- (b) at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the N6 NA polypeptide of SEQ ID NO:6, and wherein the non-naturally occurring polypeptide includes 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or all 16 of the

following amino acid residues relative to SEQ ID NO:6 when aligned by protocol 1 or protocol 2:

99P, 103N, 105S, 106V, 113D, 131Q, 157T, 161I, 162P, 163I/L, 165S/T/V/A/I, 166V/P, 196Q, 203Y, 445S.

5

51. The polypeptide of claim 50, wherein the polypeptide is

(a) at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the N6 NA polypeptide of SEQ ID NO:6, and wherein the non-naturally occurring polypeptide includes 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or all 11 of the following amino

10 acid residues relative to SEQ ID NO:6 when aligned by protocol 1 or protocol 2:

99P, 103N, 113D, 131Q, 161I, 162P, 163I, 165S/T/V/A, 196Q, 203Y, 445S.

52. The polypeptide of claim 50, wherein the polypeptide is

(b) at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the N6 NA polypeptide of SEQ ID NO:6, and wherein the non-naturally occurring polypeptide includes 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or all 15 of the

15 following amino acid residues relative to SEQ ID NO:6 when aligned by protocol 1 or protocol 2:

99P, 103N, 105S, 106V, 113D, 131Q, 157T, 161I, 162P, 163I/L, 165S/T/V/A/I,

20 166V/P, 196Q, 203Y, 445S.

53. The polypeptide of any one of claim 50-52, wherein the polypeptide includes one or more of the following sets of amino acid residues relative to SEQ ID NO:6 when aligned by protocol 1 or protocol 2:

25 101C/164C;

101C/164C/174V;

101C/164C/174V/447V;

122V/447V;

101A/163L;

30 99P/196T; and/or

99P/196T/205I.

54. The polypeptide of any one of claim 50-52, wherein the polypeptide includes three or more of the listed amino acid residues relative to SEQ ID NO:6 when aligned by protocol 1 or protocol 2.
- 5 55. The polypeptide of any one of claim 50-52, wherein the polypeptide includes five or more of the listed amino acid residues relative to SEQ ID NO:6 when aligned by protocol 1 or protocol 2.
56. The polypeptide of any one of claim 50-52, wherein the polypeptide includes seven or
10 more of the listed amino acid residues relative to SEQ ID NO:6 when aligned by protocol 1 or protocol 2.
57. The polypeptide of any one of claim 50-52, wherein the polypeptide includes nine or more of the listed amino acid residues relative to SEQ ID NO:6 when aligned by protocol 1
15 or protocol 2.
58. The non-naturally occurring polypeptide of claim 1, wherein the polypeptide is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the N6 NA polypeptide of SEQ ID NO:6, and wherein the non-naturally occurring
20 polypeptide includes a 165E amino acid mutation relative to SEQ ID NO:6 when aligned by protocol 1, and optionally also includes a 177V amino acid mutation relative to SEQ ID NO:6 when aligned by protocol 1 or protocol 2.
59. The non-naturally occurring polypeptide of claim 1, wherein the polypeptide is
25 (a) at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the N7 NA polypeptide of SEQ ID NO:7, and wherein the non-naturally occurring polypeptide includes 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or all 11 of the following amino acid residues relative to SEQ ID NO:7 when aligned by protocol 1 or protocol 2:
98P, 102N, 104S, 112D, 130Q, 156T, 162I, 164V/A/I, 165V, 202Y, 448V; or
30 (b) at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the N7 NA polypeptide of SEQ ID NO:7, and wherein the non-naturally occurring polypeptide includes 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or all 13 of the following amino acid residues relative to SEQ ID NO:7 when aligned by protocol 1 or protocol 2:
98P, 102N, 104S, 112D, 130Q, 156T, 162I, 164V/A/I, 165V, 176I, 202Y, 446I, 448V.

60. The polypeptide of claim 59, wherein the polypeptide is
 (a) at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%,
 or 99% identical to the N7 NA polypeptide of SEQ ID NO:7, and wherein the non-naturally
 occurring polypeptide includes 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or all 11 of the following amino
 5 acid residues relative to SEQ ID NO:7 when aligned by protocol 1 or protocol 2:
 98P, 102N, 104S, 112D, 130Q, 156T, 162I, 164V/A/I, 165V, 202Y, 448V.

61. The polypeptide of claim 59, wherein the polypeptide is
 (b) at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%,
 10 or 99% identical to the N7 NA polypeptide of SEQ ID NO:7, and wherein the non-naturally
 occurring polypeptide includes 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or all 13 of the following
 amino acid residues relative to SEQ ID NO:7 when aligned by protocol 1 or protocol 2:
 98P, 102N, 104S, 112D, 130Q, 156T, 162I, 164V/A/I, 165V, 176I, 202Y, 446I, 448V.

15 62. The polypeptide of any one of claim 59-61, wherein the polypeptide includes one or
 more of the following sets of amino acid residues relative to SEQ ID NO:7 when aligned by
 protocol 1 or protocol 2:
 100C/163C;
 100C/163C/173V;
 20 100C/163C/173V/448V;
 99L/446I/448A;
 98P/195T;
 130Q/446I;
 446I/448A;
 25 98P/195T/204I;
 98P/176I/195T; and/or
 98P/176I/195T/204I.

30 63. The polypeptide of any one of claim 59-61, wherein the polypeptide includes three or
 more of the listed amino acid residues relative to SEQ ID NO:7 when aligned by protocol 1
 or protocol 2.

64. The polypeptide of any one of claim 59-61, wherein the polypeptide includes five or more of the listed amino acid residues relative to SEQ ID NO:7 when aligned by protocol 1 or protocol 2.
- 5 65. The polypeptide of any one of claim 59-61, wherein the polypeptide includes seven or more of the listed amino acid residues relative to SEQ ID NO:7 when aligned by protocol 1 or protocol 2.
66. The polypeptide of any one of claim 59-61, wherein the polypeptide includes nine or
10 more of the listed amino acid residues relative to SEQ ID NO:7 when aligned by protocol 1 or protocol 2.
67. The non-naturally occurring polypeptide of claim 1, wherein the polypeptide is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical
15 to the N7 NA polypeptide of SEQ ID NO:7, and wherein the non-naturally occurring polypeptide includes one or both of the following amino acid mutation relative to SEQ ID NO:7 when aligned by protocol 1 or protocol 2: 164Q/E, 195S.
68. The non-naturally occurring polypeptide of claim 1, wherein the polypeptide is
20 (a) at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the N8 NA polypeptide of SEQ ID NO:8, and wherein the non-naturally occurring polypeptide includes 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or all 11 of the following amino acid residues relative to SEQ ID NO:8 when aligned by protocol 1 or protocol 2:
408M, 101N, 103A/S, 111D, 129Q, 161P/L, 163S/T/V/A/I, 164V, 194Q, 201Y, 442I; or
25 (b) at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the N8 NA polypeptide of SEQ ID NO:8, and wherein the non-naturally occurring polypeptide includes 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or all 15 of the following amino acid residues relative to SEQ ID NO:8 when aligned by protocol 1 or protocol 2:
30 **98L**, **408M**, 101N, 103A/S, 104V, 111D, 129Q/M, 161P/L, 163S/T/V/A/I/S/T, 164V/P, 175I, 194Q, 201Y, 203I, 442I

69. The polypeptide of claim 68, wherein the polypeptide is

- (a) at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the N8 NA polypeptide of SEQ ID NO:8, and wherein the non-naturally occurring polypeptide includes 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or all 11 of the following amino acid residues relative to SEQ ID NO:8 when aligned by protocol 1 or protocol 2:

(i) **408M**, 101N, 103A/S, 111D, 129Q, 160P, 161L, 163S/T/V/A/I, 164V, 194Q, 201Y, 442I; or

(ii) 101N, 103A/S, 111D, 129Q, 160P, 161L, 163S/T/V/A/I, 164V, 194Q, 201Y, 442I.

70. The polypeptide of claim 68, wherein the polypeptide is

- (b) at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the N8 NA polypeptide of SEQ ID NO:8, and wherein the non-naturally occurring polypeptide includes 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or all 15 of the following amino acid residues relative to SEQ ID NO:8 when aligned by protocol 1 or protocol 2:

(i) **98L, 408M**, 101N, 103A/S, 104V, 111D, 129Q/M, 160P, 161L, 163S/T/V/A/I/S/T, 164V/P, 175I, 194Q, 201Y, 203I, 442I; or

(ii) 101N, 103A/S, 104V, 111D, 129Q/M, 160P, 161L, 163S/T/V/A/I/S/T,

164V/P, 175I, 194Q, 201Y, 203I, 442I

71. The polypeptide of any one of claim 68-70, wherein the polypeptide includes one or more of the following sets of amino acid residues relative to SEQ ID NO:8 when aligned by protocol 1 or protocol 2:

- (i)

98L/408M;

160P/163S;

101N/103A;

99C/162C;

- 99C/162C/172V;

129Q/442I;

99A/161L;

99A/161L/442I;

161L/442I;

- 129M/442I;
 99A/129M/161L/442I/444A;
 98L/99A/129M/161L/442I/444A;
 129M/161L/442I/444A;
 5 98L/129M/161L/442I/444A; and/or
 175I/203I; or
 (ii)
 160P/163S;
 101N/103A;
 10 99C/162C;
 99C/162C/172V;
 129Q/442I;
 99A/161L;
 99A/161L/442I;
 15 161L/442I;
 129M/442I
 99A/129M/161L/442I/444A;
 98L/99A/129M/161L/442I/444A;
 129M/161L/442I/444A;
 20 98L/129M/161L/442I/444A; and/or
 175I/203I.

72. The polypeptide of any one of claim 68-70, wherein the polypeptide includes three or more of the listed amino acid residues relative to SEQ ID NO:8 when aligned by protocol 1
 25 or protocol 2.

73. The polypeptide of any one of claim 68-70, wherein the polypeptide includes five or more of the listed amino acid residues relative to SEQ ID NO:8 when aligned by protocol 1
 30 or protocol 2.

74. The polypeptide of any one of claim 68-70, wherein the polypeptide includes seven or more of the listed amino acid residues relative to SEQ ID NO:8 when aligned by protocol 1
 or protocol 2.

75. The polypeptide of any one of claim 68-70, wherein the polypeptide includes nine or more of the listed amino acid residues relative to SEQ ID NO:8 when aligned by protocol 1 or protocol 2.

- 5 76. The non-naturally occurring polypeptide of any one of claims 80-85, wherein the polypeptide comprises the amino acid sequence at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid selected from the group consisting of N8 mutants listed in Table 1 (SEQ ID NO:35-38), when aligned by protocol 1, wherein the polypeptide includes all of the residues listed in Table 1 for an individual N8
10 mutant listed in Table 1.

77. The non-naturally occurring polypeptide of claim 1, wherein the polypeptide is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the N8 NA polypeptide of SEQ ID NO:8, and wherein the non-naturally occurring
15 polypeptide includes a 163E amino acid mutation relative to SEQ ID NO:8 when aligned by protocol 1, and further may optionally include a 194S mutation relative to SEQ ID NO:8 when aligned by protocol 1 or protocol 2.

78. The non-naturally occurring polypeptide of claim 1, wherein the polypeptide is
20 (a) at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the N9 NA polypeptide of SEQ ID NO:9, and wherein the non-naturally occurring polypeptide includes 1, 2, 3, 4, 5, 6, 7, 8, 9, or all 10 of the following amino acid residues relative to SEQ ID NO:9 when aligned by protocol 1 or protocol 2:
94P, 95L, 100S, 126Q/M, 160V/A/I, 161V, 191Q, 198Y, 439S, 441V; or
25 (b) at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the N9 NA polypeptide of SEQ ID NO:9, and wherein the non-naturally occurring polypeptide includes 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, or all 14 of the following amino acid residues relative to SEQ ID NO:9 when aligned by protocol 1 or protocol 2:
94P, 95L, 98N, 100S/A, 126Q/M, 152T, 158I, 160V/A/I/T, 161V, 191Q, 198Y, 200I, 439S,
30 441V.

79. The polypeptide of claim 78, wherein the polypeptide is
(a) at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the N9 NA polypeptide of SEQ ID NO:9, and wherein the non-naturally

occurring polypeptide includes 1, 2, 3, 4, 5, 6, 7, 8, 9, or all 10 of the following amino acid residues relative to SEQ ID NO:9 when aligned by protocol 1 or protocol 2:

94P, 95L, 100S, 126Q/M, 160V/A/I, 161V, 191Q, 198Y, 439S, 441V.

5 80. The polypeptide of claim 78, wherein the polypeptide is

(b) at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the N9 NA polypeptide of SEQ ID NO:9, and wherein the non-naturally occurring polypeptide includes 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, or all 14 of the following amino acid residues relative to SEQ ID NO:9 when aligned by protocol 1 or protocol 2:

10 94P, 95L, 98N, 100S/A, 126Q/M, 152T, 158I, 160V/A/I/T, 161V, 191Q, 198Y, 200I, 439S, 441V.

81. The polypeptide of any one of claim 78-80, wherein the polypeptide includes one or more of the following sets of amino acid residues relative to SEQ ID NO:9 when aligned by protocol 1 or protocol 2:

15 96C/159C;

96C/159C/441V;

96A/441A;

96A/126M/439I/441A;

20 95L/96A/126M/439I/441A;

126M/439I/441A;

95L/126M/439I/441A;

94P/191T;

98N/100A; and/or

25 94P/191T/200I.

82. The polypeptide of any one of claim 78-80, wherein the polypeptide includes three or more of the listed amino acid residues relative to SEQ ID NO:9 when aligned by protocol 1 or protocol 2.

30

83. The polypeptide of any one of claim 78-80, wherein the polypeptide includes five or more of the listed amino acid residues relative to SEQ ID NO:9 when aligned by protocol 1 or protocol 2.

84. The polypeptide of any one of claim 78-80, wherein the polypeptide includes seven or more of the listed amino acid residues relative to SEQ ID NO:9 when aligned by protocol 1 or protocol 2.
- 5 85. The polypeptide of any one of claim 78-80, wherein the polypeptide includes nine or more of the listed amino acid residues relative to SEQ ID NO:9 when aligned by protocol 1 or protocol 2.
86. The non-naturally occurring polypeptide of claim 1, wherein the polypeptide is at
10 least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the N9 NA polypeptide of SEQ ID NO:9, and wherein the non-naturally occurring polypeptide includes a 160Q amino acid mutation relative to SEQ ID NO:9 when aligned by protocol 1, or may include a combination of 160Q/E and 172V amino acid residues relative to SEQ ID NO:9 when aligned by protocol 1 or protocol 2.
- 15 87. A composition, comprising one or more of the polypeptides of any preceding claim linked to a scaffold.
88. The composition of claim 87, wherein the scaffold comprises a protein scaffold.
20
89. The composition of claim 88, wherein the polypeptide is covalently linked to a protein subunit of the protein scaffold to form a fusion protein.
90. A nucleic acid encoding the polypeptide of any preceding claim, or the fusion protein
25 of claim 89.
91. An expression vector comprising the nucleic acid of claim 90 operatively linked to a suitable control sequence.
- 30 92. A host cell comprising the nucleic acid of claim 90, the expression vector of claim 91, and/or the polypeptide of any preceding claim.
93. A pharmaceutical composition, comprising

(a) one or more of the polypeptides, fusion protein, composition, nucleic acid, expression vector, and/or the host cell of any preceding claim; and

(b) a pharmaceutically acceptable carrier.

5 94. A vaccine comprising

(a) one or more of the polypeptides, fusion protein, composition, nucleic acid, expression vector, and/or the host cell of any preceding claim; and

(b) a pharmaceutically acceptable carrier.

10 95. A method for treating or limiting development of an influenza infection, comprising administering to a subject in need thereof an amount effective to treat or limit development of the influenza infection of a polypeptide, fusion protein, composition, vaccine, nucleic acid, expression vector, host cell, pharmaceutical composition, and/or vaccine of any preceding claim.

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