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PCT

- FIG. 115

- (57) Abstract:** Provided herein are influenza hemagglutinin stem domain polypeptides, compositions comprising the same, vaccines comprising the same and methods of their use.

INFLUENZA VIRUS VACCINES AND USES THEREOF

[0001] This application claims priority benefit of U.S. Provisional Application No. 61/164,896, filed March 30, 2009 and U.S. Provisional Application No. 61/299,084, filed January 28, 2010, each of which is incorporated by reference in its entirety herein.

[0002] This invention was made, in part, with United States Government support under award number RC1 AI086061 from the National Institutes of Health (NIH) National Institute of Allergy and Infectious Diseases, award number U54 AI057158 from the NIH, award number HHSN266200700010C from the United States Department of Health and Human Services, and award number U01 AI070469 from the NIH. The United States Government may have certain rights in this invention.

1. INTRODUCTION

[0003] Provided herein are influenza hemagglutinin stem domain polypeptides, compositions comprising the same, vaccines comprising the same and methods of their use.

2. BACKGROUND

[0004] Influenza viruses are enveloped RNA viruses that belong to the family of Orthomyxoviridae (Palese and Shaw (2007) Orthomyxoviridae: The Viruses and Their Replication, 5th ed. Fields' Virology, edited by B.N. Fields, D.M. Knipe and P.M. Howley. Wolters Kluwer Health/Lippincott Williams & Wilkins, Philadelphia, USA, p1647-1689). The natural host of influenza viruses are avians, but influenza viruses (including those of avian origin) also can infect and cause illness in humans and other animal hosts (canines, pigs, horses, sea mammals, and mustelids). For example, the H5N1 avian influenza virus circulating in Asia has been found in pigs in China and Indonesia and has also expanded its host range to include cats, leopards, and tigers, which generally have not been considered susceptible to influenza A (CIDRAP - Avian Influenza: Agricultural and Wildlife Considerations). The occurrence of influenza virus infections in animals could potentially give rise to human pandemic influenza strains.

[0005] Influenza A and B viruses are major human pathogens, causing a respiratory disease that ranges in severity from sub-clinical infection to primary viral pneumonia which can result in death. The clinical effects of infection vary with the virulence of the

influenza strain and the exposure, history, age, and immune status of the host. The cumulative morbidity and mortality caused by seasonal influenza is substantial due to the relatively high attack rate. In a normal season, influenza can cause between 3-5 million cases of severe illness and up to 500,000 deaths worldwide (World Health Organization (2003) Influenza: Overview; <http://www.who.int/mediacentre/factsheets/fs211/en/>; March 2003). In the United States, influenza viruses infect an estimated 10-15% of the population (Glezen and Couch RB (1978) Interpandemic influenza in the Houston area, 1974-76. *N Engl J Med* 298: 587-592; Fox *et al.* (1982) Influenza virus infections in Seattle families, 1975-1979. II. Pattern of infection in invaded households and relation of age and prior antibody to occurrence of infection and related illness. *Am J Epidemiol* 116: 228-242) and are associated with approximately 30,000 deaths each year (Thompson WW *et al.* (2003) Mortality Associated with Influenza and Respiratory Syncytial Virus in the United States. *JAMA* 289: 179-186; Belshe (2007) Translational research on vaccines: influenza as an example. *Clin Pharmacol Ther* 82: 745-749).

[0006] In addition to annual epidemics, influenza viruses are the cause of infrequent pandemics. For example, influenza A viruses can cause pandemics such as those that occurred in 1918, 1957, 1968, and 2009. Due to the lack of pre-formed immunity against the major viral antigen, hemagglutinin (HA), pandemic influenza can affect greater than 50% of the population in a single year and often causes more severe disease than epidemic influenza. A stark example is the pandemic of 1918, in which an estimated 50-100 million people were killed (Johnson and Mueller (2002) Updating the Accounts: Global Mortality of the 1918-1920 "Spanish" Influenza Pandemic *Bulletin of the History of Medicine* 76: 105-115). Since the emergence of the highly pathogenic avian H5N1 influenza virus in the late 1990s (Claas *et al.* (1998) Human influenza A H5N1 virus related to a highly pathogenic avian influenza virus. *Lancet* 351: 472-7), there have been concerns that it may be the next pandemic virus.

[0007] An effective way to protect against influenza virus infection is through vaccination; however, current vaccination approaches rely on achieving a good match between circulating strains and the isolates included in the vaccine. Such a match is often difficult to attain due to a combination of factors. First, influenza viruses are constantly undergoing change: every 3-5 years the predominant strain of influenza A virus is replaced by a variant that has undergone sufficient antigenic drift to evade existing antibody responses. Isolates to be included in vaccine preparations must

therefore be selected each year based on the intensive surveillance efforts of the World Health Organization (WHO) collaborating centers. Second, to allow sufficient time for vaccine manufacture and distribution, strains must be selected approximately six months prior to the initiation of the influenza season. Often, the predictions of the vaccine strain selection committee are inaccurate, resulting in a substantial drop in the efficacy of vaccination.

[0008] The possibility of a novel subtype of influenza A virus entering the human population also presents a significant challenge to current vaccination strategies. Since it is impossible to predict what subtype and strain of influenza virus will cause the next pandemic, current, strain-specific approaches cannot be used to prepare a pandemic influenza vaccine.

3. SUMMARY

[0009] In one aspect, provided herein are influenza hemagglutinin stem domain polypeptides. In certain embodiments, the influenza hemagglutinin stem domain polypeptides lack globular head domains as described herein.

[0010] While not intending to be bound by any particular theory of operation, it is believed that the globular head domain of an influenza hemagglutinin comprises one or more highly immunogenic regions. These highly immunogenic regions might generate a host immune response. However, the highly immunogenic regions might also vary from strain to strain of influenza virus. Embodiments presented herein are based on, in part, the discovery that residues in influenza hemagglutinin stem domains are relatively conserved and immunogenic, and that antibodies binding to this region may be neutralizing. An influenza hemagglutinin stem domain polypeptide, lacking all or substantially all of an influenza hemagglutinin globular head domain, may be used to generate an immune response to one or more conserved epitopes of the stem domain polypeptide. Removal of the highly immunogenic regions of the globular head domain might expose one or more epitopes of the stem domain polypeptide to a host immune system. In addition, in certain embodiments, elimination of the glycosylation of the influenza hemagglutinin stem domain through alteration of glycosylation sites present therein may render the conserved regions of the stem domain more accessible to the host immune response.

[0011] If the one or more epitopes of the stem domain polypeptide are less immunogenic than the highly immunogenic regions of a globular head domain, the absence of a globular head domain in the stem domain polypeptide might allow an immune response against the one or more epitopes of the stem domain polypeptide to develop. Advantageously, since the amino acid sequences of influenza hemagglutinin stem domain polypeptides might be conserved or highly conserved across viral subtypes, an immune response against an influenza hemagglutinin stem domain polypeptide provided herein might cross react with one or more viral subtypes other than the subtype corresponding to the stem domain polypeptide. Accordingly, the influenza hemagglutinin stem domain polypeptides provided herein may be useful for immunogenic compositions (*e.g.* vaccines) capable of generating immune responses against a plurality of influenza virus strains.

[0012] Without being bound by any theory, influenza hemagglutinin stem domain polypeptides described herein are based, in part, on the inventors' discovery of polypeptides that lack the globular head domain of influenza hemagglutinin and maintain the stability of the pre-fusion conformation of influenza hemagglutinin. In one aspect, without being bound by theory, the inventors have discovered that the maintenance of cysteine residues identified as A_P and A_Q in influenza hemagglutinin polypeptides in FIG. 1 contributes the stability of the stalk region of influenza hemagglutinin. In another aspect, without being bound by theory, the inventors have discovered that influenza hemagglutinin stem domain polypeptides that maintain the pre-fusion conformation of influenza hemagglutinin polypeptides are more effective at inducing a protective effect in subjects. In certain aspects, the stability of the pre-fusion conformation can be conferred by introducing amino acid substitutions at certain residues, such as HA1 H17Y (H3 numbering).

[0012a] Although other aspects are described herein for completeness, the present invention particularly relates to vaccines utilising a polypeptide having a linker between an HA1 N-terminal stem segment and an HA1 C-terminal stem segment.

[0012b] Thus in one aspect the present invention provides a vaccine comprising a polypeptide, wherein said polypeptide comprises:

- (a) an influenza hemagglutinin HA1 domain that comprises an HA1 N-terminal stem

segment covalently linked to a linker of 1 to 50 heterologous residues that is in turn covalently linked to an HA1 C-terminal stem segment; said HA1 domain in tertiary or quaternary association with

(b) an influenza hemagglutinin HA2 domain, and

wherein the vaccine induces antibodies which are cross-reactive among HA subtypes.

[0012c] In another aspect the present invention provides a vaccine comprising a virus comprising a genome engineered to express a nucleic acid encoding a polypeptide, wherein said polypeptide comprises:

(a) an influenza hemagglutinin HA1 domain that comprises an HA1 N-terminal stem segment covalently linked to a linker of 1 to 50 heterologous residues that is in turn covalently linked to an HA1 C-terminal stem segment; said HA1 domain in tertiary or quaternary association with

(b) an influenza hemagglutinin HA2 domain, and

wherein the vaccine induces antibodies which are cross-reactive among HA subtypes.

[0012d] In a still further aspect the present invention provides a vaccine comprising a virus comprising a polypeptide, wherein said polypeptide comprises:

(a) an influenza hemagglutinin HA1 domain that comprises an HA1 N-terminal stem segment covalently linked to a linker of 1 to 50 heterologous residues that is in turn covalently linked to an HA1 C-terminal stem segment; said HA1 domain in tertiary or quaternary association with

(b) an influenza hemagglutinin HA2 domain, and

wherein the vaccine induces antibodies which are cross-reactive among HA subtypes.

[0012e] In another aspect provided herein are methods of inducing an immune response to an influenza virus hemagglutinin (HA), preventing an influenza virus disease and/or healing an influenza virus infection or disease, comprising administering an effective amount of the vaccine of the invention. In a related aspect provided is the use of a vaccine according to the invention in the manufacture of a medicament for the treatment or prevention of an influenza virus or influenza virus disease.

3.1 TERMINOLOGY

[0013] The terms “about” or “approximate,” when used in reference to an amino acid position refer to the particular amino acid position in a sequence or any amino acid that is within five, four, three, two or one residues of that amino acid position, either in an N-terminal direction or a C-terminal direction.

[0013a] Throughout the description of the invention, and in the claims that follow, except where the context requires otherwise due to express language or necessary implication, the word “comprise” or variations such as “comprises” or “comprising” is used in an inclusive sense, i.e. to specify the presence of the stated feature but not to preclude the presence or addition of further features in various embodiments of the invention.

[0014] As used herein, the term “about” or “approximately” when used in conjunction with a number refers to any number within 1, 5 or 10% of the referenced number.

[0015] The term “amino acid sequence identity” refers to the degree of identity or similarity between a pair of aligned amino acid sequences, usually expressed as a percentage. Percent identity is the percentage of amino acid residues in a candidate sequence that are identical (*i.e.*, the amino acid residues at a given position in the alignment are the same residue) or similar (*i.e.*, the amino acid substitution at a given position in the alignment is a conservative substitution, as discussed below), to the corresponding amino acid residue in the peptide after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence homology. Sequence homology, including percentages of sequence identity and similarity, are determined using sequence alignment techniques well-known in the art, preferably computer algorithms designed for this purpose, using the default parameters of said computer algorithms or the software packages containing them. Non-limiting examples of computer algorithms and software packages incorporating such algorithms include the following. The BLAST family of programs exemplify a particular, non-limiting example of a mathematical algorithm utilized for the comparison of two sequences (*e.g.*, Karlin & Altschul, 1990, *Proc. Natl. Acad. Sci. USA* 87:2264-2268 (modified as in Karlin & Altschul, 1993, *Proc. Natl. Acad. Sci. USA* 90:5873-5877), Altschul *et al.*, 1990, *J. Mol. Biol.* 215:403-410, (describing NBLAST and XBLAST), Altschul *et al.*, 1997, *Nucleic Acids Res.* 25:3389-3402 (describing Gapped BLAST, and PSI-Blast). Another particular example is the algorithm of Myers and Miller (1988 *CABIOS* 4:11-17) which is incorporated into the ALIGN program (version 2.0) and is available as part of the GCG sequence alignment software package. Also particular is the FASTA program (Pearson W.R. and Lipman D.J., *Proc. Nat. Acad. Sci. USA*, 85:2444-2448, 1988), available as part of the Wisconsin Sequence Analysis Package. Additional examples include BESTFIT, which uses the “local homology” algorithm of Smith and Waterman (*Advances in Applied Mathematics*, 2:482-489, 1981) to find best single region of similarity between two sequences, and which is preferable where the two sequences being compared are dissimilar in length; and GAP, which aligns two sequences by finding a “maximum similarity” according to the algorithm of Needleman and Wunsch (*J. Mol. Biol.* 48:443-354, 1970), and is preferable where the two sequences are approximately the same length and an alignment is expected over the entire length.

[0016] “Conservative substitution” refers to replacement of an amino acid of one class is with another amino acid of the same class. In particular embodiments, a conservative substitution does not alter the structure or function, or both, of a polypeptide. Classes of amino acids for the purposes of conservative substitution include hydrophobic (Met, Ala, Val, Leu, Ile), neutral hydrophilic (Cys, Ser, Thr), acidic (Asp, Glu), basic (Asn, Gln, His, Lys, Arg), conformation disrupters (Gly, Pro) and aromatic (Trp, Tyr, Phe).

[0017] As used herein, the terms “disease” and “disorder” are used interchangeably to refer to a condition in a subject. In some embodiments, the condition is a viral infection. In specific embodiments, a term “disease” refers to the pathological state resulting from the presence of the virus in a cell or a subject, or by the invasion of a cell or subject by the virus. In certain embodiments, the condition is a disease in a subject, the severity of which is decreased by inducing an immune response in the subject through the administration of an immunogenic composition.

[0018] As used herein, the term “effective amount” in the context of administering a therapy to a subject refers to the amount of a therapy which has a prophylactic and/or therapeutic effect(s). In certain embodiments, an “effective amount” in the context of administration of a therapy to a subject refers to the amount of a therapy which is sufficient to achieve one, two, three, four, or more of the following effects: (i) reduce or ameliorate the severity of an influenza virus infection, disease or symptom associated therewith; (ii) reduce the duration of an influenza virus infection, disease or symptom associated therewith; (iii) prevent the progression of an influenza virus infection, disease or symptom associated therewith; (iv) cause regression of an influenza virus infection, disease or symptom associated therewith; (v) prevent the development or onset of an influenza virus infection, disease or symptom associated therewith; (vi) prevent the recurrence of an influenza virus infection, disease or symptom associated therewith; (vii) reduce or prevent the spread of an influenza virus from one cell to another cell, one tissue to another tissue, or one organ to another organ; (ix) prevent or reduce the spread of an influenza virus from one subject to another subject; (x) reduce organ failure associated with an influenza virus infection; (xi) reduce hospitalization of a subject; (xii) reduce hospitalization length; (xiii) increase the survival of a subject with an influenza virus infection or disease associated therewith; (xiv) eliminate an influenza virus infection or disease associated therewith; (xv) inhibit or reduce influenza virus replication; (xvi) inhibit or reduce the entry of an influenza virus into a host cell(s);

(xviii) inhibit or reduce replication of the influenza virus genome; (xix) inhibit or reduce synthesis of influenza virus proteins; (xx) inhibit or reduce assembly of influenza virus particles; (xxi) inhibit or reduce release of influenza virus particles from a host cell(s); (xxii) reduce influenza virus titer; and/or (xxiii) enhance or improve the prophylactic or therapeutic effect(s) of another therapy.

[0019] In certain embodiments, the effective amount does not result in complete protection from an influenza virus disease, but results in a lower titer or reduced number of influenza viruses compared to an untreated subject. In certain embodiments, the effective amount results in a 0.5 fold, 1 fold, 2 fold, 4 fold, 6 fold, 8 fold, 10 fold, 15 fold, 20 fold, 25 fold, 50 fold, 75 fold, 100 fold, 125 fold, 150 fold, 175 fold, 200 fold, 300 fold, 400 fold, 500 fold, 750 fold, or 1,000 fold or greater reduction in titer of influenza virus relative to an untreated subject. In some embodiments, the effective amount results in a reduction in titer of influenza virus relative to an untreated subject of approximately 1 log or more, approximately 2 logs or more, approximately 3 logs or more, approximately 4 logs or more, approximately 5 logs or more, approximately 6 logs or more, approximately 7 logs or more, approximately 8 logs or more, approximately 9 logs or more, approximately 10 logs or more, 1 to 3 logs, 1 to 5 logs, 1 to 8 logs, 1 to 9 logs, 2 to 10 logs, 2 to 5 logs, 2 to 7 logs, 2 logs to 8 logs, 2 to 9 logs, 2 to 10 logs 3 to 5 logs, 3 to 7 logs, 3 to 8 logs, 3 to 9 logs, 4 to 6 logs, 4 to 8 logs, 4 to 9 logs, 5 to 6 logs, 5 to 7 logs, 5 to 8 logs, 5 to 9 logs, 6 to 7 logs, 6 to 8 logs, 6 to 9 logs, 7 to 8 logs, 7 to 9 logs, or 8 to 9 logs. Benefits of a reduction in the titer, number or total burden of influenza virus include, but are not limited to, less severe symptoms of the infection, fewer symptoms of the infection and a reduction in the length of the disease associated with the infection.

[0020] “Hemagglutinin” and “HA” refer to any hemagglutinin known to those of skill in the art. In certain embodiments, the hemagglutinin is influenza hemagglutinin, such as an influenza A hemagglutinin, an influenza B hemagglutinin or an influenza C hemagglutinin. A typical hemagglutinin comprises domains known to those of skill in the art including a signal peptide (optional herein), a stem domain, a globular head domain, a luminal domain (optional herein), a transmembrane domain (optional herein) and a cytoplasmic domain (optional herein). In certain embodiments, a hemagglutinin consists of a single polypeptide chain, such as HA0. In certain embodiments, a hemagglutinin consists of more than one polypeptide chain in quaternary association, *e.g.* HA1 and HA2. Those of skill in the art will recognize that an immature HA0 might

be cleaved to release a signal peptide (approximately 20 amino acids) yielding a mature hemagglutinin HA0. A hemagglutinin HA0 might be cleaved at another site to yield HA1 polypeptide (approximately 320 amino acids, including the globular head domain and a portion of the stem domain) and HA2 polypeptide (approximately 220 amino acids, including the remainder of the stem domain, a luminal domain, a transmembrane domain and a cytoplasmic domain). In certain embodiments, a hemagglutinin comprises a signal peptide, a transmembrane domain and a cytoplasmic domain. In certain embodiments, a hemagglutinin lacks a signal peptide, *i.e.* the hemagglutinin is a mature hemagglutinin. In certain embodiments, a hemagglutinin lacks a transmembrane domain or cytoplasmic domain, or both. As used herein, the terms “hemagglutinin” and “HA” encompass hemagglutinin polypeptides that are modified by post-translational processing such as signal peptide cleavage, disulfide bond formation, glycosylation (*e.g.*, *N*-linked glycosylation), protease cleavage and lipid modification (*e.g.* S-palmitoylation).

[0021] “HA1 N-terminal stem segment” refers to a polypeptide segment that corresponds to the amino-terminal portion of the stem domain of an influenza hemagglutinin HA1 polypeptide. In certain embodiments, an HA1 N-terminal stem segment consists of amino acid residues corresponding approximately to amino acids A_{N-term} through A_p of an HA1 domain. A_{N-term} is the N-terminal amino acid of HA1 as recognized by those of skill in the art. A_p is the cysteine residue in the HA1 N-terminal stem segment that forms or is capable of forming a disulfide bond with a cysteine residue in an HA1 C-terminal stem segment. Residue A_p is identified in influenza A hemagglutinin polypeptides in FIG. 1. Exemplary HA1 N-terminal stem segments are described herein. In certain embodiments, an HA1 N-terminal stem segment consists of amino acid residues corresponding approximately to amino acids 1-52 of HA1 from an H3 hemagglutinin. Note that, in this numbering system, 1 refers to the N-terminal amino acid of the mature HA0 protein, from which the signal peptide has been removed.

[0022] “HA1 C-terminal stem segment” refers to a polypeptide segment that corresponds to the carboxy-terminal portion of the stem domain of an influenza hemagglutinin HA1 polypeptide. In certain embodiments, an HA1 C-terminal stem segment consists of amino acid residues corresponding approximately to amino acids A_q through A_{C-term} of an HA1 domain. A_q is the cysteine residue in the HA1 C-terminal stem segment that forms or is capable of forming a disulfide bond with a cysteine residue in an HA1 N-terminal stem segment. A_{C-term} is the C-terminal amino acid of the

HA1 domain as recognized by those of skill in the art. Residue A_q is identified in influenza A hemagglutinin polypeptides in FIG. 1. Exemplary HA1 C-terminal stem segments are described herein. In certain embodiments, an HA1 C-terminal stem segment consists of amino acid residues corresponding approximately to amino acids 277-346 of HA1 from an H3 hemagglutinin. Note that, in this numbering system, 1 refers to the N-terminal amino acid of the mature HA0 protein, from which the signal peptide has been removed.

[0023] “HA2” refers to a polypeptide domain that corresponds to the HA2 domain of an influenza hemagglutinin polypeptide known to those of skill in the art. In certain embodiments, an HA2 consists of a stem domain, a luminal domain, a transmembrane domain and a cytoplasmic domain (*see, e.g., Scheiffle et al., 2007, EMBO J.* 16(18):5501-5508, the contents of which are incorporated by reference in their entirety). In certain embodiments, an HA2 consists of a stem domain, a luminal domain and a transmembrane domain. In certain embodiments, an HA2 consists of a stem domain and a luminal domain; in such embodiments, the HA2 might be soluble. In certain embodiments, an HA2 consists of a stem domain; in such embodiments, the HA2 might be soluble.

[0024] As used herein, the term “heterologous” in the context of a polypeptide, nucleic acid or virus refers to a polypeptide, nucleic acid or virus, respectively, that is not normally found in nature or not normally associated in nature with a polypeptide, nucleic acid or virus of interest. For example, a “heterologous polypeptide” may refer to a polypeptide derived from a different virus, *e.g.,* a different influenza strain or subtype, or an unrelated virus or different species.

[0025] As used herein, the term “in combination,” in the context of the administration of two or more therapies to a subject, refers to the use of more than one therapy (*e.g.,* more than one prophylactic agent and/or therapeutic agent). The use of the term “in combination” does not restrict the order in which therapies are administered to a subject. For example, a first therapy (*e.g.,* a first prophylactic or therapeutic agent) can be administered prior to (*e.g.,* 5 minutes, 15 minutes, 30 minutes, 45 minutes, 1 hour, 2 hours, 4 hours, 6 hours, 12 hours, 16 hours, 24 hours, 48 hours, 72 hours, 96 hours, 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 8 weeks, or 12 weeks before), concomitantly with, or subsequent to (*e.g.,* 5 minutes, 15 minutes, 30 minutes, 45 minutes, 1 hour, 2 hours, 4 hours, 6 hours, 12 hours, 16 hours, 24 hours, 48 hours, 72

hours, 96 hours, 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 8 weeks, or 12 weeks after) the administration of a second therapy to a subject.

[0026] As used herein, the term “infection” means the invasion by, multiplication and/or presence of a virus in a cell or a subject. In one embodiment, an infection is an “active” infection, *i.e.*, one in which the virus is replicating in a cell or a subject. Such an infection is characterized by the spread of the virus to other cells, tissues, and/or organs, from the cells, tissues, and/or organs initially infected by the virus. An infection may also be a latent infection, *i.e.*, one in which the virus is not replicating. In certain embodiments, an infection refers to the pathological state resulting from the presence of the virus in a cell or a subject, or by the invasion of a cell or subject by the virus.

[0027] As used herein, the term “influenza virus disease” refers to the pathological state resulting from the presence of an influenza (*e.g.*, influenza A or B virus) virus in a cell or subject or the invasion of a cell or subject by an influenza virus. In specific embodiments, the term refers to a respiratory illness caused by an influenza virus.

[0028] As used herein, the phrases “IFN deficient system” or “IFN-deficient substrate” refer to systems, *e.g.*, cells, cell lines and animals, such as pigs, mice, chickens, turkeys, rabbits, rats, etc., which do not produce IFN or produce low levels of IFN (*i.e.*, a reduction in IFN expression of 5-10%, 10-20%, 20-30%, 30-40%, 40-50%, 50-60%, 60-70%, 70-80%, 80-90% or more when compared to IFN-competent systems under the same conditions), do not respond or respond less efficiently to IFN, and/or are deficient in the activity of one or more antiviral genes induced by IFN.

[0029] As used herein, the numeric term “log” refers to $\log_{10}$.

[0030] As used herein, the phrase “multiplicity of infection” or “MOI” is the average number of infectious virus particles per infected cell. The MOI is determined by dividing the number of infectious virus particles added (ml added x PFU/ml) by the number of cells added (ml added x cells/ml).

[0031] As used herein, the term “nucleic acid” is intended to include DNA molecules (*e.g.*, cDNA or genomic DNA) and RNA molecules (*e.g.*, mRNA) and analogs of the DNA or RNA generated using nucleotide analogs. The nucleic acid can be single-stranded or double-stranded.

[0032] “Polypeptide” refers to a polymer of amino acids linked by amide bonds as is known to those of skill in the art. As used herein, the term can refer to a single polypeptide chain linked by covalent amide bonds. The term can also refer to multiple polypeptide chains associated by non-covalent interactions such as ionic contacts,

hydrogen bonds, Van der Waals contacts and hydrophobic contacts. Those of skill in the art will recognize that the term includes polypeptides that have been modified, for example by post-translational processing such as signal peptide cleavage, disulfide bond formation, glycosylation (*e.g.*, *N*-linked glycosylation), protease cleavage and lipid modification (*e.g.* S-palmitoylation).

[0033] As used herein, the terms “prevent,” “preventing” and “prevention” in the context of the administration of a therapy(ies) to a subject to prevent an influenza virus disease refer to one or more of the following effects resulting from the administration of a therapy or a combination of therapies: (i) the inhibition of the development or onset of an influenza virus disease or a symptom thereof; (ii) the inhibition of the recurrence of an influenza virus disease or a symptom associated therewith; and (iii) the reduction or inhibition in influenza virus infection and/or replication.

[0034] As used herein, the terms “purified” and “isolated” when used in the context of a polypeptide (including antibody) that is obtained from a natural source, *e.g.*, cells, refers to a polypeptide which is substantially free of contaminating materials from the natural source, *e.g.*, soil particles, minerals, chemicals from the environment, and/or cellular materials from the natural source, such as but not limited to cell debris, cell wall materials, membranes, organelles, the bulk of the nucleic acids, carbohydrates, proteins, and/or lipids present in cells. Thus, a polypeptide that is isolated includes preparations of a polypeptide having less than about 30%, 20%, 10%, 5%, 2%, or 1% (by dry weight) of cellular materials and/or contaminating materials. As used herein, the terms “purified” and “isolated” when used in the context of a polypeptide (including antibody) that is chemically synthesized refers to a polypeptide which is substantially free of chemical precursors or other chemicals which are involved in the syntheses of the polypeptide. In a specific embodiment, an influenza hemagglutinin stem domain polypeptide is chemically synthesized. In another specific embodiment, an influenza hemagglutinin stem domain polypeptide is isolated.

[0035] As used herein, the terms “replication,” “viral replication” and “virus replication” in the context of a virus refer to one or more, or all, of the stages of a viral life cycle which result in the propagation of virus. The steps of a viral life cycle include, but are not limited to, virus attachment to the host cell surface, penetration or entry of the host cell (*e.g.*, through receptor mediated endocytosis or membrane fusion), uncoating (the process whereby the viral capsid is removed and degraded by viral enzymes or host enzymes thus releasing the viral genomic nucleic acid), genome

replication, synthesis of viral messenger RNA (mRNA), viral protein synthesis, and assembly of viral ribonucleoprotein complexes for genome replication, assembly of virus particles, post-translational modification of the viral proteins, and release from the host cell by lysis or budding and acquisition of a phospholipid envelope which contains embedded viral glycoproteins. In some embodiments, the terms “replication,” “viral replication” and “virus replication” refer to the replication of the viral genome. In other embodiments, the terms “replication,” “viral replication” and “virus replication” refer to the synthesis of viral proteins.

[0036] “Stem domain polypeptide” refers to a derivative, *e.g.* an engineered derivative, of a hemagglutinin polypeptide that comprises one or more polypeptide chains that make up a stem domain of hemagglutinin. A stem domain polypeptide might be a single polypeptide chain, two polypeptide chains or more polypeptide chains. Typically, a stem domain polypeptide is a single polypeptide chain (*i.e.* corresponding to the stem domain of a hemagglutinin HA0 polypeptide) or two polypeptide chains (*i.e.* corresponding to the stem domain of a hemagglutinin HA1 polypeptide in association with a hemagglutinin HA2 polypeptide). In certain embodiments, a stem domain polypeptide is derived from an influenza hemagglutinin. Engineered stem domain polypeptides can comprise one or more linkers as described below.

[0037] As used herein, the terms “subject” or “patient” are used interchangeably to refer to an animal (*e.g.*, birds, reptiles, and mammals). In a specific embodiment, a subject is a bird. In another embodiment, a subject is a mammal including a non-primate (*e.g.*, a camel, donkey, zebra, cow, pig, horse, goat, sheep, cat, dog, rat, and mouse) and a primate (*e.g.*, a monkey, chimpanzee, and a human). In certain embodiments, a subject is a non-human animal. In some embodiments, a subject is a farm animal or pet. In another embodiment, a subject is a human. In another embodiment, a subject is a human infant. In another embodiment, a subject is a human child. In another embodiment, a subject is a human adult. In another embodiment, a subject is an elderly human. In another embodiment, a subject is a premature human infant.

[0038] As used herein, the term “premature human infant” refers to a human infant born at less than 37 weeks of gestational age.

[0039] As used herein, the term “human infant” refers to a newborn to 1 year old human.

[0040] As used herein, the term “human child” refers to a human that is 1 year to 18 years old.

[0041] As used herein, the term “human adult” refers to a human that is 18 years or older.

[0042] As used herein, the term “elderly human” refers to a human 65 years or older.

[0043] The terms “tertiary structure” and “quaternary structure” have the meanings understood by those of skill in the art. Tertiary structure refers to the three-dimensional structure of a single polypeptide chain. Quaternary structure refers to the three dimensional structure of a polypeptide having multiple polypeptide chains.

[0044] As used herein, the terms “therapies” and “therapy” can refer to any protocol(s), method(s), compound(s), composition(s), formulation(s), and/or agent(s) that can be used in the prevention or treatment of a viral infection or a disease or symptom associated therewith. In certain embodiments, the terms “therapies” and “therapy” refer to biological therapy, supportive therapy, and/or other therapies useful in treatment or prevention of a viral infection or a disease or symptom associated therewith known to one of skill in the art. In some embodiments, the term “therapy” refers to a nucleic acid encoding an influenza virus hemagglutinin stem domain polypeptide, an influenza virus hemagglutinin stem domain polypeptide, or a vector or composition comprising said nucleic acid encoding an influenza virus hemagglutinin stem domain polypeptide or an influenza hemagglutinin stem domain polypeptide. In some embodiments, the term “therapy” refers to an antibody that specifically binds to an influenza virus hemagglutinin polypeptide or an influenza virus hemagglutinin stem domain polypeptide.

[0045] As used herein, the terms “treat,” “treatment,” and “treating” refer in the context of administration of a therapy(ies) to a subject to treating an influenza virus disease to obtain a beneficial or therapeutic effect of a therapy or a combination of therapies. In specific embodiments, such terms refer to one, two, three, four, five or more of the following effects resulting from the administration of a therapy or a combination of therapies: (i) the reduction or amelioration of the severity of an influenza virus infection or a disease or a symptom associated therewith; (ii) the reduction in the duration of an influenza virus infection or a disease or a symptom associated therewith; (iii) the regression of an influenza virus infection or a disease or a symptom associated therewith; (iv) the reduction of the titer of an influenza virus; (v) the reduction in organ failure associated with an influenza virus infection or a disease associated therewith; (vi) the reduction in hospitalization of a subject; (vii) the reduction in hospitalization length; (viii) the increase in the survival of a subject; (ix) the elimination of an influenza virus

infection or a disease or symptom associated therewith; (x) the inhibition of the progression of an influenza virus infection or a disease or a symptom associated therewith; (xi) the prevention of the spread of an influenza virus from a cell, tissue, organ or subject to another cell, tissue, organ or subject; (xii) the inhibition or reduction in the entry of an influenza virus into a host cell(s); (xiii) the inhibition or reduction in the replication of an influenza virus genome; (xiv) the inhibition or reduction in the synthesis of influenza virus proteins; (xv) the inhibition or reduction in the release of influenza virus particles from a host cell(s); and/or (xvi) the enhancement or improvement the therapeutic effect of another therapy.

[0046] As used herein, in some embodiments, the phrase “wild-type” in the context of a virus refers to the types of a virus that are prevalent, circulating naturally and producing typical outbreaks of disease. In other embodiments, the term “wild-type” in the context of a virus refers to a parental virus.

4. BRIEF DESCRIPTION OF THE DRAWINGS

[0047] **Fig. 1** presents a sequence alignment by CLUSTALW of representative sequences of 16 subtypes of influenza virus A hemagglutinin (SEQ ID NOS:1-16, respectively).

[0048] **Fig. 2** presents a sequence alignment by CLUSTALW of a representative sequence of influenza virus B hemagglutinin (SEQ ID NO:17) aligned with influenza A HK68-H3N2 (SEQ ID NO:3) and PR8-H1N1 (SEQ ID NO:1) hemagglutinins.

[0049] **Fig. 3** provides exemplary nucleotide constructs encoding wild type HA and influenza HA stem domain polypeptides.

[0050] **Fig. 4** provides the putative structure of an influenza HA stem domain polypeptide.

[0051] **Figs. 5A and 5B** provide protein expression of exemplary influenza HA stem domain polypeptides.

[0052] **Fig. 6** provides an exemplary construct for expressing an influenza HA stem domain polypeptide with nucleotide (SEQ ID NO:169) and amino acid (SEQ ID NO:170) sequences. The glycine linker is underlined.

[0053] **Fig. 7** provides an exemplary construct for expressing an influenza HA stem domain polypeptide with nucleotide (SEQ ID NO:171) and amino acid (SEQ ID NO:172) sequences. The glycine linker is underlined.

[0054] **Fig. 8** provides an exemplary construct for expressing an influenza HA stem domain polypeptide with nucleotide (SEQ ID NO:173) and amino acid (SEQ ID NO:174) sequences. The proline-glycine linker is underlined.

[0055] **Fig. 9** provides an exemplary construct for expressing an influenza HA stem domain polypeptides with nucleotide (SEQ ID NO:175) and amino acid (SEQ ID NO:176) sequences. The glycine linker, thrombin cleavage site, foldon domain and HIS tag are underlined.

[0056] **Figs. 10A-10B.** Schematic of headless HA constructs. (A) Schematic of the linear structure of the full length influenza virus HA protein (top) and a generalized headless HA protein (bottom). Linker peptides tested in the context of the PR8 and HK68 HA sequences are shown. Inserted amino acids are shown in bold face font, while amino acids present in the native HA sequence are in regular font. (B) Schematic of the folded structures of the full length and headless HAs of PR8 virus (left panel) and HK68 virus (right panel). In both cases headless HAs carrying the 4G linker bridge are depicted. The HA1 subunit is colored dark grey and the HA2 subunit is light grey. The location of 4G linker sequences is indicated with an arrow in each panel. The full length HA structures were downloaded from the Protein Database (PDB): PR8 HA, PDB ID 1rvx and HK68 HA, PDB ID 1mgn. Schematics of headless HAs were generated using the full length HA coordinates as a starting point and 4G loops were manually docked into the headless HA carbon to close the discontinuous alpha carbon amino acid chain. Final images were generated by PyMol (Delano Scientific).

[0057] **Figs. 11A -11B.** Expression of headless HA constructs in transiently transfected cells. Headless HA constructs were expressed in 293T cells by plasmid transfection in the absence of exogenous trypsin. At 24 hours post-transfection, whole cell lysates were prepared and subjected to SDS-PAGE followed by Western blotting. HA proteins were detected using the polyclonal 3951 antiserum (for PR8) or the monoclonal 12D1 (for HK68). Molecular weight markers in kDa are shown to the left of each blot and transfected constructs are identified above the appropriate lane. "Mock" indicates untransfected cells; "Full" indicates the full length HA protein; for the headless HA constructs, the amino acid sequence bridging the N and C terminal strands of HA1 is shown. Letters in bold font indicate inserted amino acids, while letters in regular font represent residues present in the wild-type HA. In the region of the cys52 to cys277 disulfide bond, the wild-type sequences are as follows. PR8: K50L51C52...C277N278T279K280. HK68: K50I51C52...C277I278S279E280. PR8

based constructs are shown in panel (A) and HK68 based constructs are shown in panel (B). In (B) the molecular weight of the full length HK68 HA0 protein is indicated by an arrowhead.

[0058] Figs. 12A-12B. Detection of headless HA proteins on the surface of transfected cells. Full length and headless HA constructs were expressed in 293T cells by plasmid transfection. At 24 hours post-transfection, cells were trypsinized and HA proteins on the cell surface were stained using the polyclonal 3951 antiserum (for PR8) or the monoclonal 12D1 (for HK68) prior to analysis by flow cytometry. (A) Mock transfected cells stained with 3951 immune sera are compared to cells transfected with pDZ PR8 HA or cells transfected with pCAGGS PR8 2G, 4G or PG headless HA constructs. (B) Mock transfected cells stained with mAb 12D1 are compared to cells transfected with pCAGGS HK68 HA or cells transfected with pCAGGS HK68 2G, 4G or PG headless HA constructs.

[0059] Figs. 13A-13B. Incorporation of headless HA proteins into virus-like particles. The HA content of VLPs generated by co-transfection of HA constructs with pGagEGFP was assessed by Western blotting. (A) PR8 based VLPs were probed with the polyclonal 3951 antiserum. (B) HK68 based proteins were detected with the monoclonal 12D1. Bands are identified to the right of each blot. Note that VLPs were produced in the presence of exogenous trypsin resulting in the cleavage of HA0 to produce HA1 (not visualized here) and HA2. Ramps above the lanes indicate a 1/3 dilution of the sample: for each VLP, the left lane shows VLPs harvested from the equivalent of three 10 cm dishes of 293T cells, while the right lane shows VLPs harvested from one 10 cm dish.

[0060] Fig. 14. Vaccination of mice with headless HA constructs provides protection from death. The average body weight loss in each group of vaccinated mice following challenge with PR8 virus is shown. Error bars represent standard deviation. * indicates the death of a mouse.

[0061] Figs. 15A-15F. Anti-sera from mice vaccinated with the PR8 4G headless HA shows broad cross-reactivity by ELISA. The vaccine groups from which sera are derived are identified at the top of each column and the ELISA substrate used is indicated to the right of each row. Sera from vaccinated mice are shown in black with filled symbols. Each mouse is represented by a unique symbol which is the same in each panel. A rabbit anti-serum raised against whole PR8 virus is shown in grey with open triangles and a serum sample taken from a naïve mouse is shown in grey with open

squares. Reactivity of mouse sera to (A) whole PR8 virus, (B) purified recombinant A/New Caledonia/20/1999 HA protein, (C) purified recombinant A/California/04/2009 HA, (D) purified recombinant A/Singapore/1/1957 HA, (E) purified recombinant A/Viet Nam/1203/2004 HA, and (F) purified recombinant A/Hong Kong/1/1968 HA are shown.

[0062] Fig. 16A-16B. present schematic diagrams of representative headless molecules. (A) Headless HA construct based on the A/Hong Kong/68 hemagglutinin protein with the linker bridge positioned between amino acids 52 and 277 of the HA1 domain. (B) Headless HA construct based on the A/PR/8/34 hemagglutinin protein, with the linker bridge positioned between amino acids 46 and 276 of the HA1 domain.

[0063] Fig. 17A-17B. present schematic diagrams of the primary protein sequences of representative headless molecules. (A) Headless HA construct based on the A/Hong Kong/68 hemagglutinin protein with the linker bridge positioned between amino acids 52 and 277 of the HA1 domain. (B) Headless HA construct based on the A/PR/8/34 hemagglutinin protein, with the linker bridge positioned between amino acids 46 and 276 of the HA1 domain.

5. DETAILED DESCRIPTION

5.1 POLYPEPTIDES

[0064] Provided herein are influenza hemagglutinin stem domain polypeptides. While not intending to be bound by any particular theory of operation, it is believed that the influenza hemagglutinin stem domain polypeptides are useful for presenting one or more relatively conserved antigenic regions to a host immune system in order to generate an immune response that is capable of cross-reacting with a plurality of influenza strains. Since the one or more antigenic regions are well conserved across influenza hemagglutinin subtypes, such an immune response might cross-react with several subtypes of full-length influenza hemagglutinin polypeptides.

[0065] It is believed that full-length influenza hemagglutinin presents several highly antigenic segments in its globular head domain. These highly antigenic segments might be more accessible to a host immune system or more immunogenic in structure, or both. It is believed that a host immune system responds preferentially to these highly immunogenic segments compared to one or more epitopes in the stem domain of an influenza hemagglutinin. Further, since a globular head domain of an influenza hemagglutinin might be variable across subtypes and viral strains, an immune response

against one globular head domain subtype might be limited to the specific highly antigenic segments of that globular head domain. Strains with different globular head domains might not cross react with the same immune response. As such, the effectiveness of vaccines presenting hemagglutinin polypeptides might be limited to the specific strains presented in the vaccine. Hence, a given conventional influenza vaccine is likely only effective against the influenza strains predicted to be virulent during a given flu season.

[0066] Advantageously, influenza hemagglutinin stem domain polypeptides provided herein might be useful to generate an immune response against multiple influenza strains. The influenza hemagglutinin stem domain polypeptides generally do not comprise the highly antigenic, variable globular head domains of conventional influenza vaccine polypeptides. Thus, they should not generate immune responses limited to the variable segments of the globular head domains. Instead, they present one or more epitopes in the relatively conserved stem domain of influenza hemagglutinin. As such, they might be used to generate a host immune response against multiple influenza strains that carry the relatively conserved epitopes. Accordingly, the influenza hemagglutinin stem domain polypeptides find use as antigens in the compositions, vaccines and methods described in detail below. The influenza hemagglutinin stem domain polypeptides might be useful for generating a host immune response against any one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, fifteen or sixteen known influenza A hemagglutinin subtypes or a later identified influenza A hemagglutinin subtype. The influenza hemagglutinin stem domain polypeptides might also be useful for generating a host immune response against any influenza B hemagglutinin subtype now known or later identified.

[0067] Generally, the influenza hemagglutinin stem domain polypeptides provided herein are polypeptides that comprise or consist essentially of the stem domain of an influenza hemagglutinin polypeptide. The stem domain of an influenza hemagglutinin polypeptide is the stem domain that is generally recognized by those of skill in the art.

[0068] As is known to those of skill in the art, a full-length influenza hemagglutinin typically comprises an HA1 domain and an HA2 domain. The stem domain is formed by two segments of the HA1 domain and most or all of the HA2 domain. The two segments of the HA1 domain are separated, in primary sequence, by a globular head domain.

[0069] In certain embodiments, influenza hemagglutinin stem domain polypeptides comprise little or no globular head domain of an influenza hemagglutinin polypeptide. In certain embodiments, an influenza hemagglutinin stem domain polypeptides is an influenza hemagglutinin that has had its globular head domain deleted by any technique deemed suitable by one of skill in the art.

[0070] In certain embodiments, influenza hemagglutinin stem domain polypeptides described herein maintain the cysteine residues identified in influenza hemagglutinin polypeptides as A_p and A_q in FIG. 1. In certain embodiments, influenza hemagglutinin stem domain polypeptides described herein have greater stability at a pH lower than the hemagglutinin of a wild-type influenza virus (e.g., a pH less than 5.2, less than 5.1, less than 5.0, or less than 4.9, such as 4.8, 4.7, 4.6, 4.5, 4.4, 4.3, 4.2, 4.1, 4.0, 3.9, 3.8, etc.). In particular embodiments, influenza hemagglutinin stem domain polypeptides described herein undergo conformational changes from the pre-fusion to the fusion conformation at a pH lower than the hemagglutinin of wild-type influenza viruses. In some embodiments, influenza hemagglutinin stem domain polypeptides described herein comprise one or more amino acid substitutions, such as HA1 H17Y (H3 numbering) that increases the stability of the polypeptides at a low pH (e.g., a pH of between 4.9 to 5.2, 4.5 to 3.5, 3.5 to 2.5, 2.5 to 1.5, 1.5 to 0.5). The stability of influenza hemagglutinin stem domain polypeptides can be assessed using techniques known in the art, such as sensitivity of the hemagglutinin molecules to trypsin digestion, as described in, e.g., Thoennes et al., 2008, *Virology* 370: 403-414.

[0071] The influenza hemagglutinin stem domain polypeptides can be prepared according to any technique deemed suitable to one of skill, including techniques described below. In certain embodiments, the stem domain polypeptides are isolated.

[0072] The typical primary structure of an influenza hemagglutinin stem domain polypeptide provided herein comprises, in the following order, an HA1 N-terminal stem segment, a linker, an HA1 C-terminal stem segment and an HA2. The primary sequence might be formed by a single polypeptide, or it might be formed by multiple polypeptides. Typically, a single polypeptide is expressed by any technique deemed suitable by one of skill in the art. In single polypeptide embodiments, the HA1 segments and the HA2 are in tertiary association. As is known to those of skill in the art, a single HA polypeptide might be cleaved, for example by a protease, under appropriate expression conditions to yield two polypeptides in quaternary association. The cleavage

is typically between the HA1 C-terminal stem segment and the HA2. In certain embodiments, provided herein are multiple polypeptide, for example two polypeptide, influenza hemagglutinin stem domains. In multiple polypeptide embodiments, the HA1 segments and HA2 are in quaternary association.

[0073] In certain embodiments, an influenza hemagglutinin stem domain polypeptide provided herein is monomeric. In certain embodiments, an influenza hemagglutinin stem domain polypeptide provided herein is multimeric. In certain embodiments, an influenza hemagglutinin stem domain polypeptide provided herein is trimeric. Those of skill in the art will recognize that native influenza hemagglutinin polypeptides are capable of trimerization *in vivo* and that certain influenza hemagglutinin stem domain polypeptides provided herein are capable of trimerization. In particular embodiments described below, influenza hemagglutinin stem domain polypeptides provided herein comprise trimerization domains to facilitate trimerization.

[0074] In certain embodiments, an influenza hemagglutinin stem domain polypeptide comprises a signal peptide. Typically, the signal peptide is cleaved during or after polypeptide expression and translation to yield a mature influenza hemagglutinin stem domain polypeptide. The signal peptide might be advantageous for expression of the influenza hemagglutinin stem domain polypeptides. In certain embodiments, also provided herein are mature influenza hemagglutinin stem domain polypeptides that lack a signal peptide.

[0075] Influenza hemagglutinin HA2 typically comprises a stem domain, transmembrane domain and a cytoplasmic domain. In certain embodiments, provided herein are influenza hemagglutinin stem domain polypeptides that comprise an HA2 stem domain, an HA2 luminal domain, an HA2 transmembrane domain and an HA2 cytoplasmic domain. Such influenza hemagglutinin stem domain polypeptides might be expressed as membrane-bound antigens. In certain embodiments, provided herein are influenza hemagglutinin stem domain polypeptides that comprise an HA2 stem domain, an HA2 luminal domain, and an HA2 transmembrane domain but lack some or all of the typical cytoplasmic domain. Such influenza hemagglutinin stem domain polypeptides might be expressed as membrane-bound antigens. In certain embodiments, provided herein are influenza hemagglutinin stem domain polypeptides that comprise an HA2 stem domain and an HA2 luminal domain but lack both an HA2 transmembrane domain and an HA2 cytoplasmic domain. Such influenza hemagglutinin stem domain polypeptides might advantageously be expressed as soluble polypeptides. In certain

embodiments, provided herein are influenza hemagglutinin stem domain polypeptides that comprise an HA2 stem domain but lack an HA2 luminal domain, an HA2 transmembrane domain and an HA2 cytoplasmic domain. Such influenza hemagglutinin stem domain polypeptides might advantageously be expressed as soluble polypeptides. In certain embodiments, the influenza hemagglutinin stem domain polypeptides comprise an HA2 stem domain having at least 70%, 75%, 80%, 85%, 90%, 95%, 96% or 98% amino acid sequence identity to an influenza HA2 stem domain known to those of skill in the art. Exemplary known HA2 stem domains from known influenza A and influenza B hemagglutinins are provided in the tables below.

[0076] Also provided herein are influenza hemagglutinin stem domain polypeptides comprising deleted forms of HA2 stem domains wherein up to 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 amino acid residues are deleted from either or both termini of the HA2 stem domain. Further provided herein are influenza hemagglutinin stem domain polypeptides comprising altered forms of HA2 stem domains wherein up to 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 amino acid residues are conservatively substituted with other amino acids. Further provided are influenza hemagglutinin stem domain polypeptides comprising deleted and altered HA2 stem domains.

[0077] The HA1 N-terminal stem segment might be any HA1 N-terminal stem segment recognized by one of skill in the art based on the definition provided herein. Typically, an HA1 N-terminal stem segment corresponds to a polypeptide consisting of the N-terminal amino acid of a mature HA1 (*i.e.* an HA1 lacking a signal peptide) through the cysteine residue located in sequence at approximately the 52nd residue of the HA1. This cysteine residue, termed A_p herein, is generally capable of forming a disulfide bridge with a cysteine residue in the C-terminal stem segment of HA1. Sequences of 16 representative influenza A hemagglutinins are presented in FIG. 1, and residue A_p is identified in each.

[0078] In certain embodiments, the HA1 N-terminal stem segment does not end exactly at A_p (*e.g.*, Cys₅₂ of an HA1 subunit from an H3 hemagglutinin), but at a residue in sequence and structure vicinity to A_p. For example, in certain embodiments, the HA1 N-terminal stem segment ends at A_{p-1}, A_{p-2}, A_{p-3}, or A_{p-4}. In other embodiments, the HA1 N-terminal stem segment ends at A_{p+1}, A_{p+2}, A_{p+3}, A_{p+4} or A_{p+5}. The end of an HA1 N-terminal stem segment should be selected in conjunction with the end of the HA1 C-terminal stem segment and the linker so that the resulting linked HA1 stem

domain is capable of forming a three-dimensional structure similar, as described below, to an influenza hemagglutinin stem domain.

[0079] In certain embodiments, the influenza hemagglutinin stem domain polypeptides comprise an HA1 N-terminal stem segment having at least 70%, 75%, 80%, 85%, 90%, 95%, 96% or 98% amino acid sequence identity to an influenza HA1 N-terminal stem segment known to those of skill in the art. Exemplary known HA1 N-terminal stem segments are provided in the tables below.

[0080] Also provided herein are influenza hemagglutinin stem domain polypeptides comprising deleted forms of HA1 N-terminal stem segments wherein up to 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 amino acid residues are deleted from either or both termini of the HA1 N-terminal stem segment. In certain embodiments, provided herein are influenza hemagglutinin stem domain polypeptides that comprise expanded forms of HA1 N-terminal stem segments wherein 1, 2 or 3 residues are added to the C-terminus of the HA1 N-terminal stem segments; these added residues might be derived from the amino acid sequence of a globular head domain adjacent to an HA1 N-terminal stem segment. Further provided herein are influenza hemagglutinin stem domain polypeptides comprising altered forms of HA1 N-terminal stem segments wherein up to 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 amino acid residues are conservatively substituted with other amino acids. Further provided are influenza hemagglutinin stem domain polypeptides comprising deleted and altered HA1 N-terminal stem segments.

[0081] The HA1 C-terminal stem segment might be any HA1 C-terminal stem segment recognized by one of skill in the art based on the definition provided herein. Typically, an HA1 C-terminal stem segment corresponds to a polypeptide consisting of the cysteine residue located in sequence at approximately the 277th residue of an HA1 (using H3 numbering) through the C-terminal amino acid of the HA1. This cysteine residue, termed A_q herein, is generally capable of forming a disulfide bridge with cysteine residue A_p in the N-terminal stem segment of HA1. Sequences of 16 representative influenza A hemagglutinins are presented in FIG. 1, and residue A_q is identified in each.

[0082] In certain embodiments, the HA1 C-terminal stem segment does not start at A_q (e.g., Cys₂₇₇ of an HA1 subunit from an H3 hemagglutinin), but at a residue in sequence and structure vicinity to A_q. For example, in certain embodiments, the HA1 C-terminal stem segment starts at A_{q-1}, A_{q-2}, A_{q-3}, or A_{q-4}. In other embodiments, the HA1 C-terminal stem segment starts at A_{q+1}, A_{q+2}, A_{q+3}, A_{q+4} or A_{q+5}. The end of an HA1 N-

terminal stem segment should be selected in conjunction with the start of the HA1 C-terminal stem segment and the linker so that the resulting HA1 stem domain is capable of forming a three-dimensional structure similar, as described below, to an influenza hemagglutinin.

[0083] In certain embodiments, the influenza hemagglutinin stem domain polypeptides comprise an HA1 C-terminal stem segment having at least 70%, 75%, 80%, 85%, 90%, 95%, 96% or 98% amino acid sequence identity to an influenza HA1 C-terminal stem segment known to those of skill in the art. Exemplary known HA1 C-terminal stem segments are provided in the tables below.

[0084] In certain embodiments, the end of the N-terminal stem segment is A_{p-1} , and the start of the C-terminal stem segment is A_{q-1} . In certain embodiments, the end of the N-terminal stem segment is A_{p-2} , and the start of the C-terminal stem segment is A_{q-2} . In certain embodiments, the end of the N-terminal stem segment is A_{p-3} , and the start of the C-terminal stem segment is A_{q-3} . In certain embodiments, the end of the N-terminal stem segment is A_{p-4} , and the start of the C-terminal stem segment is A_{q-4} . In certain embodiments, the end of the N-terminal stem segment is A_{p-5} , and the start of the C-terminal stem segment is A_{q-5} .

[0085] In certain embodiments, the end of the N-terminal stem segment is A_{p+1} , and the start of the C-terminal stem segment is A_{q+1} . In certain embodiments, the end of the N-terminal stem segment is A_{p+2} , and the start of the C-terminal stem segment is A_{q+2} . In certain embodiments, the end of the N-terminal stem segment is A_{p+3} , and the start of the C-terminal stem segment is A_{q+3} . In certain embodiments, the end of the N-terminal stem segment is A_{p+4} , and the start of the C-terminal stem segment is A_{q+4} . In certain embodiments, the end of the N-terminal stem segment is A_{p+5} , and the start of the C-terminal stem segment is A_{q+5} .

[0086] In certain embodiments, the end of the N-terminal stem segment is A_{p-1} , and the start of the C-terminal stem segment is A_{q+1} . In certain embodiments, the end of the N-terminal stem segment is A_{p-2} , and the start of the C-terminal stem segment is A_{q+2} . In certain embodiments, the end of the N-terminal stem segment is A_{p-3} , and the start of the C-terminal stem segment is A_{q+3} . In certain embodiments, the end of the N-terminal stem segment is A_{p-4} , and the start of the C-terminal stem segment is A_{q+4} . In certain embodiments, the end of the N-terminal stem segment is A_{p-5} , and the start of the C-terminal stem segment is A_{q+5} .

[0087] In certain embodiments, the end of the N-terminal stem segment is A_p (i.e., the end of the N-terminal stem segment is Cysteine), and the start of the C-terminal stem segment is A_q (i.e., the start of the C-terminal stem segment is Cysteine). In certain embodiments, the end of the N-terminal stem segment is A_{p+1} , and the start of the C-terminal stem segment is A_{q-1} . In certain embodiments, the end of the N-terminal stem segment is A_{p+2} , and the start of the C-terminal stem segment is A_{q-2} . In certain embodiments, the end of the N-terminal stem segment is A_{p+3} , and the start of the C-terminal stem segment is A_{q-3} . In certain embodiments, the end of the N-terminal stem segment is A_{p+4} , and the start of the C-terminal stem segment is A_{q-4} . In certain embodiments, the end of the N-terminal stem segment is A_{p+5} , and the start of the C-terminal stem segment is A_{q-5} .

[0088] Also provided herein are influenza hemagglutinin stem domain polypeptides comprising deleted forms of HA1 C-terminal stem segments wherein up to 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 amino acid residues are deleted from either or both termini of the HA1 C-terminal stem segment. In certain embodiments, provided herein are influenza hemagglutinin stem domain polypeptides that comprise expanded forms of HA1 C-terminal stem segments wherein 1, 2 or 3 residues are added to the N-terminus of the HA1 C-terminal stem segments; these added residues might be derived from the amino acid sequence of a globular head domain adjacent to an HA1 C-terminal stem segment. In particular embodiments, if one residue is added to the C-terminal stem segment, then one residue is added to the N-terminal stem segment; if two residues are added to the C-terminal stem segment, then two residues are added to the N-terminal stem segment; if three residues are added to the C-terminal stem segment, then three residues are added to the N-terminal stem segment. Further provided herein are influenza hemagglutinin stem domain polypeptides comprising altered forms of HA1 C-terminal stem segments wherein up to 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 amino acid residues are conservatively substituted with other amino acids. Further provided are influenza hemagglutinin stem domain polypeptides comprising deleted and altered HA1 C-terminal stem segments.

[0089] The influenza hemagglutinin stem domain polypeptides might be based on (*i.e.* might have sequence identity, as described above) any influenza hemagglutinin known to those of skill or later discovered. In certain embodiments, influenza hemagglutinin stem domain polypeptides are based on an influenza A hemagglutinin. In certain embodiments, the influenza hemagglutinin stem domain polypeptides are based on an influenza A hemagglutinin selected from the group consisting of H1, H2, H3, H4,

H5, H6, H7, H8, H9, H10, H11, H12, H13, H14, H15 and H16. In certain embodiments, influenza hemagglutinin stem domain polypeptides are based on an influenza B hemagglutinin, as described in detail below.

[0090] The HA1 N-terminal stem segments might be based on (*i.e.* might have sequence identity, as described above) any HA1 N-terminal stem segments known to those of skill or later discovered. In certain embodiments, the HA1 N-terminal stem segments are based on influenza A HA1 N-terminal stem segments. In certain embodiments, the HA1 N-terminal stem segments are based on an influenza A hemagglutinin selected from the group consisting of H1, H2, H3, H4, H5, H6, H7, H8, H9, H10, H11, H12, H13, H14, H15 and H16. In certain embodiments, the HA1 N-terminal stem segment is selected from SEQ ID NOS:34-49. In certain embodiments, the HA1 N-terminal stem segment is selected from SEQ ID NOS:34-49, each having one amino acid deleted from its C-terminus. In certain embodiments, the HA1 N-terminal stem segment is selected from SEQ ID NOS:34-49, each having two amino acids deleted from its C-terminus. In certain embodiments, the HA1 N-terminal stem segment is selected from SEQ ID NOS:34-49, each having three amino acids deleted from its C-terminus. In certain embodiments, the HA1 N-terminal stem segment is selected from SEQ ID NOS:34-49, each having four amino acids deleted from its C-terminus. In certain embodiments, the HA1 N-terminal stem segment is selected from SEQ ID NOS:34-49, each having five amino acids deleted from its C-terminus. In certain embodiments, the HA1 N-terminal stem segment is selected from SEQ ID NOS:177-224.

[0091] The HA1 C-terminal stem segments might be based on (*i.e.* might have sequence identity, as described above) any HA1 C-terminal stem segments known to those of skill or later discovered. In certain embodiments, the HA1 C-terminal stem segments are based on influenza A HA1 C-terminal stem segments. In certain embodiments, the HA1 C-terminal stem segments are based on an influenza A hemagglutinin selected from the group consisting of H1, H2, H3, H4, H5, H6, H7, H8, H9, H10, H11, H12, H13, H14, H15 and H16. In certain embodiments, the HA1 C-terminal stem segment is selected from SEQ ID NOS:50-65. In certain embodiments, the HA1 C-terminal stem segment is selected from SEQ ID NOS: 50-65, each having one amino acid deleted from its N-terminus. In certain embodiments, the HA1 C-terminal stem segment is selected from SEQ ID NOS: 50-65, each having two amino acids deleted from its N-terminus. In certain embodiments, the HA1 C-terminal stem

segment is selected from SEQ ID NOS: 50-65, each having three amino acids deleted from its N-terminus. In certain embodiments, the HA1 C-terminal stem segment is selected from SEQ ID NOS: 50-65, each having four amino acids deleted from its N-terminus. In certain embodiments, the HA1 C-terminal stem segment is selected from SEQ ID NOS: 50-65, each having five amino acids deleted from its N-terminus. In certain embodiments, the HA1 C-terminal stem segment is selected from SEQ ID NOS:226-273.

[0092] The HA2 stem domains might be based on (*i.e.* might have sequence identity, as described above) any HA2 stem domains known to those of skill or later discovered. In certain embodiments, the HA2 stem domains are based on influenza A HA2 stem domains. In certain embodiments, the HA2 stem domains are based on an influenza A hemagglutinin selected from the group consisting of H1, H2, H3, H4, H5, H6, H7, H8, H9, H10, H11, H12, H13, H14, H15 and H16. In certain embodiments, the HA2 stem domain is selected from SEQ ID NOS:66-97.

[0093] In embodiments comprising a signal peptide, the signal peptide might be based on any influenza virus signal peptide known to those of skill in the art. In certain embodiments, the signal peptides are based on influenza A signal peptides. In certain embodiments, the signal peptides are based on an influenza A hemagglutinin selected from the group consisting of H1, H2, H3, H4, H5, H6, H7, H8, H9, H10, H11, H12, H13, H14, H15 and H16. In certain embodiments, the signal peptide might be any signal peptide deemed useful to one of skill in the art. In certain embodiments, the signal peptide is selected from SEQ ID NOS:18-33.

[0094] In embodiments comprising a luminal domain, the luminal domain might be based on any influenza luminal domain known to those of skill in the art. In certain embodiments, the luminal domains are based on influenza A luminal domains. In certain embodiments, the HA2 luminal domains are based on an influenza A hemagglutinin selected from the group consisting of H1, H2, H3, H4, H5, H6, H7, H8, H9, H10, H11, H12, H13, H14, H15 and H16. In certain embodiments, the luminal domain might be any luminal domain deemed useful to one of skill in the art. In certain embodiments, the luminal domain is selected from SEQ ID NOS:98-113.

[0095] In embodiments comprising a transmembrane domain, the transmembrane domain might be based on any influenza transmembrane domain known to those of skill in the art. In certain embodiments, the transmembrane domains are based on influenza A transmembrane domains. In certain embodiments, the HA2 transmembrane domains

are based on an influenza A hemagglutinin selected from the group consisting of H1, H2, H3, H4, H5, H6, H7, H8, H9, H10, H11, H12, H13, H14, H15 and H16. In certain embodiments, the transmembrane domain might be any transmembrane domain deemed useful to one of skill in the art. In certain embodiments, the transmembrane domain is selected from SEQ ID NOS:114-129.

[0096] In embodiments comprising a cytoplasmic domain, the cytoplasmic domain might be based on any influenza cytoplasmic domain known to those of skill in the art. In certain embodiments, the cytoplasmic domains are based on influenza A cytoplasmic domains. In certain embodiments, the HA2 cytoplasmic domains are based on an influenza A hemagglutinin selected from the group consisting of H1, H2, H3, H4, H5, H6, H7, H8, H9, H10, H11, H12, H13, H14, H15 and H16. In certain embodiments, the cytoplasmic domain might be any cytoplasmic domain deemed useful to one of skill in the art. In certain embodiments, the cytoplasmic domain is selected from SEQ ID NOS:130-145.

[0097] In certain embodiments, one or more of the glycosylation sites in the hemagglutinin stem domain are altered or deleted such that glycosylation at these sites will not occur during processing and maturation of the polypeptide. Those of skill in the art will recognize that influenza HA typically comprises one or more glycosylation sequences (*e.g.* Asn-Xaa-Ser/Thr/Cys, wherein Xaa is any amino acid other than Pro). In certain embodiments, one or more amino acid residues in a glycosylation sequence is conservatively substituted with an amino acid residue that disrupts the glycosylation sequence. In certain embodiments, one or more amino acid residues in a glycosylation sequence is substituted with any amino acid residue that disrupts the glycosylation sequence. In certain embodiments, one or more asparagine residues in a glycosylation sequence is substituted with alanine. In a particular embodiment, the asparagine at position 38 of an H3 hemagglutinin is changed to an alanine.

[0098] Table 1, below, identifies signal peptides, HA1 N-terminal stem segments, HA1 C-terminal stem segments and HA2 domains of influenza A hemagglutinin polypeptides. These signal peptides, stem segments and domains are useful in the polypeptides and methods described herein.

TABLE 1. Exemplary Influenza A Hemagglutinin Sequences

HA Subtype (Genbank No.)	Signal peptide	HA1 N-terminal Stem Segment	HA1 C-terminal Stem Segment	HA2 Domain

HA Subtype (Genbank No.)	Signal peptide	HA1 N-terminal Stem Segment	HA1 C-terminal Stem Segment	HA2 Domain
H1 PR8-H1N1 (EF467821.1)	MKAN LLVLL CALAA ADA [SEQ ID NO.:18]	DTICIGYHANN STDTVDTVLE KNVTVTHSVN LLED SHNGKL C [SEQ ID NO.:34]	CNTKCQTPLG AINSSLPYQNI HPVTIGECPKY VRS AKLRMVT GLRNNPSIQSR [SEQ ID NO.:50]	GLFGAIAGFIEGGW TGMIDGWYGYHHQ NEQSGSYAADQKST QNAINGITNKVNTVI EKMNIQFTAVGKEF NKLEKR MENLNKK VDDGFLDIWTYNAE LLV LLENERTLDFH DSNVKNLYE KVKSQ LKNNAKEIGNGCFE FYHKCDNECMESVR NGTYDYPKYSEESK LNREKVDGVKLES MGIYQILAIYSTVAS SLVLLVSLGAISFW MCSNGSLQCRICI [SEQ ID NO.:66]
H2 (L11136)	MAIY LILLFT AVRG [SEQ ID NO.:19]	DQICIGYHSNN STEKVD TILER NVTVTHAQNI LEKTHNGKLC [SEQ ID NO.:35]	CETKCQTPLG AINTTLPFHNV HPLTIGECPKY VKSERLVLAT GLRNV PQIESR [SEQ ID NO.:51]	GLFGAIAGFIEGGW QGMIDGWYGYHHS NDQSGSYAADKEST QKAIDGITNRVNSVI EKMNTQFEAVGKEF SNLEKRLENLNKKM EDGFLDVWTYNAE LLVLMENERTLDFH DSNVKNLYDRVRM QLRDNAKELGNGCF EFYHKCDDECMNS VKNGTYDYPKYEEE SKLNRNEIKGVKLS NMGVYQILAIYATV AGSLSLAIMIAGISL WMCSNGSLQCRICI [SEQ ID NO.:67]

HA Subtype (Genbank No.)	Signal peptide	HA1 N-terminal Stem Segment	HA1 C-terminal Stem Segment	HA2 Domain
H3 HK68-H3N2 (EF409245) PDB: 1HGJ	MKTII ALSYIF CLALG [SEQ ID NO.:20]	QDLPGNDNST ATLCLGHHAV PNGTLVKTITD DQIEVTNATEL VQSSSTGKIC [SEQ ID NO.:36]	CISECITPNGSI PNDKPFQNVN KITYGACPKY VKQNTLKLAT GMRNVPEKQT R [SEQ ID NO.52]	GLFGAIAGFIENGW EGMIDGWYGFRHQ NSEGTGQAADLKST QAAIDQINGKLN RVI EKTNEKFHQIEKFS EVEGRIQDLEKYVE DTKIDLWSYNAELL VALENQHTIDLTDS EMNKLFETRRLR ENAEDMGNGCFKIY HKCDNACIESIRNGT YDHDVYRDEALNN RFQIKGVELKSGYK DWILWISFAISCFL CVVLLGFIMWACQR GNIRCNICI [SEQ ID NO.:68]
H4 (D90302)	MLSIVI LFLIA ENSS [SEQ ID NO.:21]	QNYTGNPVIC MGHHAVANG TMVKTLADDQ VEVVTAQELV ESQNLPELC [SEQ ID NO.:37]	CVSKCHTDKG SLSTTKPFQNI SRIAVGDCPRY VKQGSLLKAT GMRNIPEKAS R [SEQ ID NO.:53]	GLFGAIAGFIENGW QGLIDGWYGFRHQ NAEGTGTAADLKST QAAIDQINGKLNRLI EKTNDKYHQIEKEF EQVEGRIQDLENYV EDTKIDLWSYNAEL LVALENQHTIDVTD SEMNKLFERVRRL RENAEDKGNGCFEI FHKCDNNCIESIRNG TYDHDYRDEAINN RFQIQGVKLTQGYK DIILWISFSISCFLV ALLAFILWACQNG NIRCQICI [SEQ ID NO.:69]

HA Subtype (Genbank No.)	Signal peptide	HA1 N-terminal Stem Segment	HA1 C-terminal Stem Segment	HA2 Domain
H5 (X07826)	MERIV LLLA VSLVK S [SEQ ID NO.:22]	DQICIGYHAN KSTKQVDTIM EKNVTVTTHAQ DILERTHNGKL C [SEQ ID NO.:38]	CDTKCQTPVG EINSSMPFHNI HPHTIGECPKY VKSDRLVLAT GLRNPQRKK R [SEQ ID NO.:54]	GLFGAIAGFIEGGW QGMVDGWYGYHH SNEQSGYAADKES TQKAIDGITNKNVSI IDKMNTRFEAVGKE FNNLERRVENLNKK MEDGFLDVWTYNV ELLVLMENERTLDF HDSNVNNLYDKVR LQLKDNARELGNGC FEFYHKCDNECMES VRNGTYDYPQYSEE ARLNREEISGVKLES MGVYQILSIYSTVAS SLALAIMIAGLSFW MCSNGSLQCRICI [SEQ ID NO.:70]
H6 (D90303)	MIAIIV VAILA TAGRS [SEQ ID NO.:23]	DKICIGYHAN NSTTQIDTILE KNVTVTTHSVE LLENQKEERF C [SEQ ID NO.:39]	CDATCQTVAG VLRTNKTQFN VSPLWIGECPK YVKSESLRLA TGLRNPQIET R [SEQ ID NO.:55]	GLFGAIAGFIEGGW TGMIDGWYGYHHE NSQSGYAADREST QKAVDGITNKNVSI DKMNTQFEAVDHE FSNLERRIDNLNKR MEDGFLDVWTYNA ELLVLENERTLDL HDANVKNLYERVK SQLRDNAMILGNGC FEFWHKCDDECMES VKNGTYDYPKYQD ESKLNREQIESVKLE SLGVYQILAIYSTVS SSLVLVGLIIVGLW MCSNGSMQCRICI [SEQ ID NO.:71]

HA Subtype (Genbank No.)	Signal peptide	HA1 N-terminal Stem Segment	HA1 C-terminal Stem Segment	HA2 Domain
H7 (M24457)	MNTQI LVFAL VAVIP TNA [SEQ ID NO.:24]	DKICLGHHAV SNGTKVNTLT ERGVEVVNAT ETVERTNIPKI C [SEQ ID NO.:40]	CEGECYHSGG TITSRLPFQIN SRAVGKCPRY VKQESLLLAT GMKNVPEPSK KRKKR [SEQ ID NO.:56]	GLFGAIAGFIENGW EGLVDGWYGFRHQ NAQGEGTAADYKS TQSAIDQITGKLNRL IEKTNQQFELIDNEF TEVEKQIGNLINWT KDSITEVWSYNAELI VAMENQHTIDLADS EMNRLYERVRKQL RENAEEDGTGCFEIF HKCDDDCMASIRNN TYDH SKYREEAMQ NRIQIDPVKLSSGYK DVILWFSFGASCFL LAIAMGLVFICVKN GNMRCTICI [SEQ ID NO.:72]
H8 (D90304)	MEKFI AIATL ASTNA Y [SEQ ID NO.:25]	DRICIGYQSN STDTVNTLIEQ NVPVTQTMEL VETEKHPAYC [SEQ ID NO.:41]	CNTKCQTYAG AINSSKPFQNA SRHYMGECPK YVKKASRLA VGLRNTPSVEP R [SEQ ID NO.:57]	GLFGAIAGFIEGGWS GMIDGWYGFHHSN SEGTGMAADQKST QEAIDKITNKVNNIV DKMNREFEVVNHEF SEVEKRINMINDKID DQIEDLWAYNAELL VLENQKTLDEHDS NVKNLFDEVKRRLS ANAIDAGNGCFDIL HKCDNECMETIKNG TYDHKEYEEEEAKLE RSKINGVKLEENTT YKILSIYSTVAASLC LAILIAGGLILGMQN GSCRCMFCI [SEQ ID NO.:73]

HA Subtype (Genbank No.)	Signal peptide	HA1 N-terminal Stem Segment	HA1 C-terminal Stem Segment	HA2 Domain
H9 (D90305)	METK AIIAAL LMVTA ANA [SEQ ID NO.:26]	DKICIGYQSTN STETVDTLTES NVPVTHTKEL LHTEHNGMLC [SEQ ID NO.:42]	CVVQCQTEKG GLNTTLPFHNI SKYAFGNCPK YVGVKSLKLP VGLRNVPAVS SR [SEQ ID NO.:58]	GLFGAIAGFIEGGWP GLVAGWYGFQHSN DQGVGMAADKGST QKAIDKITSKVNII DKMKNQYEVIDHEF NELEARLNMINKI DDQIQDIWAYNAEL LVLLENQKTLDEHD ANVNNLYNKVKRA LGSNAVEDGNGCFE LYHKCDDQCMETIR NGTYDRQKYQEESR LERQKIEGVKLESEG TYKILTIYSTVASSL VLAMGFAAFLFWA MSNGSCRCNICI [SEQ ID NO.:74]
H10 (M21647)	MYKV VVIIAL LGAVK G [SEQ ID NO.:27]	LDRICLGHHA VANGTIVKTL TNEQEEVTNA TETVESTNLN KLC [SEQ ID NO.:43]	CESKCFWRGG SINTKLFPQNL SPRTVGQCPK YVNQRSLLLA TGMRNVPEVV QGR [SEQ ID NO.:59]	GLFGAIAGFIENGW EGMVDGWYGFRHQ NAQGTGQAADYKS TQAAIDQITGKLNRL IEKTNTEFESIESEFS ETEHQIGNVINWTK DSITDIWTYNAELLV AMENQHTIDMADSE MLNLYERVRKQLR QNAEEDGKGCFEII HTCDDSCMESIRNN TYDHSQYREEALLN RLNINPVKLSSGYK DIILWFSFGESCFVL LAVVMGLVFFCLKN GNMRCTICI [SEQ ID NO.:75]

HA Subtype (Genbank No.)	Signal peptide	HA1 N-terminal Stem Segment	HA1 C-terminal Stem Segment	HA2 Domain
H11 (D90306)	MEKTL LFAAIF LCVKA [SEQ ID NO.:28]	DEICIGYLSNN STDKVDTHEN NVTVTSSVEL VETEHTGSFC [SEQ ID NO.:44]	CSTKCQTEIGG INTNKSFHNV HRNTIGDCPK YVNVKSLKLA TGPRNVPALAS R [SEQ ID NO.:60]	GLFGAIAGFIEGGWP GLINGWYGFQHRDE EGTGIAADKESTQK AIDQITSKVNNIVDR MNTNFESVQHEFSEI EERINQLSKHVDDSD VVDIWSYNAQLLVL LENEKTLDLHDSNV RNLHEKVRRMLKD NAKDEGNGCFTFYH KCDNKCIEVRNGT YDHKEFEEESKINR QEIEGVKLDSSGNV YKILSIYSCIASSLVL AALIMGFMFWACS NGSCRCTICI [SEQ ID NO.:76]
H12 (D90307)	MEKFII LSTVL AASFA Y [SEQ ID NO.:29]	DKICIGYQTN STETVNTLSEQ NVPVTQVEEL VHRGIDPILC [SEQ ID NO.:45]	CVTECQLNEG VMNTSKPFQN TSKHYYGKCPK YIPSGSLKLAI GLRNVQVQD R [SEQ ID NO.:61]	GLFGAIAGFIEGGWP GLVAGWYGFQHQ AEGTGIAADRSTQ RAIDNMQNKLNNVI DKMNKQFEVVNHE FSEVESRINMINSKI DDQITDIWAYNAEL LVLENQKTLDEHD ANVRNLHDRVRRV LRENAIDTGDGCFEI LHKCDNNCMDTIRN GTYNHKEYEEESKI ERQKVNGVKLEENS TYKILSIYSSVASSL VLLMIIGGFIFGCQ NGNVRCTFCI [SEQ ID NO.:77]

HA Subtype (Genbank No.)	Signal peptide	HA1 N-terminal Stem Segment	HA1 C-terminal Stem Segment	HA2 Domain
H13 (D90308)	MALN VIATL TLISVC VHA [SEQ ID NO.:30]	DRICVGYLSTN SSERVDTLLEN GVPVTSSIDLIE TNHTGTYC [SEQ ID NO.:46]	CNTKCQTSVG GINTNRTFQNI DKNALGDCPK YIKSGQLKLAT GLRNVPAISNR [SEQ ID NO.:62]	GLFGAIAGFIEGGWP GLINGWYGFQHQNE QGTGIAADKESTQK AIDQITTKINNIIDKM NGNYDSIRGEFNQV EKRLNMLADRIDDA VTDIWSYNAKLLVL LENDKTLDMDHAN VKNLHEQVRRELKD NAIDEGNGCFELLH KCNDSCMETIRNGT YDHTEYAEESKLKR QEIDGIKLKSEDNVY KALSIYSCIASSVVL VGLILSFIMWACSSG NCRFNVC [SEQ ID NO.:78]
H14 (M35997)	MIALIL VALAL SHTAY S [SEQ ID NO.:31]	QITNGTTGNPII CLGHHA VENG TSVKTLTDNH VEVVS AKELV ETNHTDELC [SEQ ID NO.:47]	CTSPCLTDKGS IQSDKPFQNV RIAIGNCPKYV KQGSMLLATG MRNIPGKQAK [SEQ ID NO.:63]	GLFGAIAGFIENGW QGLIDGWYGFRHQ NAEGTGTAADLKST QAAIDQINGKLNRLI EKTNEKYHQIEKEF EQVEGRIQDLEKYV EDTKIDLWSYNAEL LVALENQHTIDVTD SEMKNLFEVRRQL RENAEDQGNGCFEI FHQCDNNCIESIRNG TYDHNIRDEAINN RIKINPVTLTMGYK DIILWISFSMSCFVF VALILGFVLWACQN GNIRCQICI [SEQ ID NO.:79]

HA Subtype (Genbank No.)	Signal peptide	HA1 N-terminal Stem Segment	HA1 C-terminal Stem Segment	HA2 Domain
H15 (L43917)	MNTQI IVILVL GLSMV KS [SEQ ID NO.:32]	DKICLGHHAV ANGTKVNTLT ERGVEVVNAT ETVEITGIDKV C [SEQ ID NO.:48]	CEGECFYSGG TINSPLPFQNI SRAVGKCPRY VKQSSLPLAL GMKNVPEKIR TR [SEQ ID NO.:64]	GLFGAIAGFIENGW EGLIDGWYGFRHQN AQGGGTAADYKST QAAIDQITGKLNRLI EKTNKQFELIDNEFT EVEQQIGNVINWTR DSLTEIWSYNAELL VAMENQHTIDLADS EMNKLYERVRRQL RENAEEDGTGCFEIF HRCDDQCMESIRNN TYNHTEYRQEALQN RIMINPVKLSSGYKD VILWFSFGASCVML LAIAMGLIFMCVKN GNLRCTICI [SEQ ID NO.:80]
H16 (EU293865)	MMIK VLYFLI IVLGR YSKA [SEQ ID NO.:33]	DKICIGYLSNN SSDTVDLTLEN GVPVTSSVDL VETNHTGTYC [SEQ ID NO.:49]	CNTKCQTSLG GINTNKTFQNI ERNALGDGCPK YIKSGQLKLAT GLRNVPSIGER [SEQ ID NO.:65]	GLFGAIAGFIEGGWP GLINGWYGFQHQNE QGTGIAADKASTQK AINEITTKINNIIEKM NGNYDSIRGEFNQV EKRLNMLADRVDVA VTDIWSYNAKLLVL LENDRTLHLHDANV RNLHDQVKRALKS NAIDEGDGCNFLLH KCNDSCMETIRNGT YNHEDYREESQLKR QEIEGIKLKTEDNVY KVLSIYSCIASSIVLV GLILAFIMWACSNG SCRFNVC [SEQ ID NO.:81]

[0099] Table 1A, below, identifies useful HA1 N-terminal stem segments and HA1 C-terminal stem segments for the polypeptides and methods described herein.

TABLE 1A. Exemplary Influenza A Hemagglutinin Sequences

HA Subtype (Genbank No.)	HA1 N-terminal Stem Segment	HA1 C-terminal Stem Segment
H1 PR8-H1N1 (EF467821.1) No Cys	DTICIGYHANNSTDVDT VLEKNVTVTTHSVNLLD SHNGKL [SEQ ID NO.:177]	NTKCQTPLGAINSSLPYQNIHPVTIGEC PKYVRS AKLRMTGLRNNPSIQSR [SEQ ID NO.:226]
H1 PR8-H1N1 (EF467821.1) No Cys $\Delta 1$	DTICIGYHANNSTDVDT VLEKNVTVTTHSVNLLD SHNGKL [SEQ ID NO.:178]	TKCQTPLGAINSSLPYQNIHPVTIGEC KYVRS AKLRMTGLRNNPSIQSR [SEQ ID NO.:227]
H1 PR8-H1N1 (EF467821.1) No Cys $\Delta 3$	DTICIGYHANNSTDVDT VLEKNVTVTTHSVNLLD SHNGK [SEQ ID NO.:179]	KCQTPLGAINSSLPYQNIHPVTIGEC KYVRS AKLRMTGLRNNPSIQSR [SEQ ID NO.:228]
H1 PR8-H1N1 (EF467821.1) PR8-CON-A	DTICIGYHANNSTDVDT VLEKNVTVTTHSVNLLD SHNGKLCRLKC [SEQ ID NO.:309]	CKCQTPLGAINSSLPYQNIHPVTIGEC KYVRS AKLRMTGLRNNPSIQSRG [SEQ ID NO.:310]
H2 (L11136) No Cys	DQICIGYHSNNSTEKVD ILERNVTVTTHAQNILEKT HNGKL [SEQ ID NO.:180]	ETKCQTPLGAINTTLPFHNHPLTIGEC CPKYVKSERLVLATGLRNV PQIESR [SEQ ID NO.:229]
H2 (L11136) No Cys $\Delta 1$	DQICIGYHSNNSTEKVD ILERNVTVTTHAQNILEKT HNGKL [SEQ ID NO.:181]	TKCQTPLGAINTTLPFHNHPLTIGEC KYVKSERLVLATGLRNV PQIESR [SEQ ID NO.:230]
H2 (L11136) No Cys $\Delta 3$	DQICIGYHSNNSTEKVD ILERNVTVTTHAQNILEKT HNGK [SEQ ID NO.:182]	KCQTPLGAINTTLPFHNHPLTIGEC KYVKSERLVLATGLRNV PQIESR [SEQ ID NO.:231]
H3 HK68-H3N2 (EF409245) PDB: 1HGJ No Cys	QDLPGNDNSTATLCLGH HAVPNGTLVKTITDDQIE VTNATELVQSSSTGKI [SEQ ID NO.:183]	ISECITPNGSIPNDKPFQNVNKITYGAC PKYVKQNTLKLATGMRNVPEKQTR [SEQ ID NO.:232]
H3 HK68-H3N2 (EF409245) PDB: 1HGJ No Cys $\Delta 1$	QDLPGNDNSTATLCLGH HAVPNGTLVKTITDDQIE VTNATELVQSSSTGKI [SEQ ID NO.:184]	SECITPNGSIPNDKPFQNVNKITYGAC KYVKQNTLKLATGMRNVPEKQTR [SEQ ID NO.:233]

HA Subtype (Genbank No.)	HA1 N-terminal Stem Segment	HA1 C-terminal Stem Segment
H3 HK68-H3N2 (EF409245) PDB: 1HGJ No Cys $\Delta 3$	QDLPGNDNSTATLCLGH HAVPNGTLVKITITDDQIE VTNATELVQSSSTGK [SEQ ID NO.:185]	ECITPNGSIPNDKPFQNVNKITYGACP KYVKQNTLKLATGMRNVPEKQTR [SEQ ID NO.:234]
H3 HK68-H3N2 PDB: 1HGJ (EF409245) HK68-CON-A	STATLCLGHHAVPNGTL VKITITDDQIEVTNATELV QSSSTGKIC [SEQ ID NO.:308]	CISECITPNGSIPNDKPFQNVNKITYGA CPKYVKQNTLKLATGMRNVPEKQTR [SEQ ID NO.:52]
H4 (D90302) No Cys	QNYTGPNVICMGHHAV ANGTMVKTLLADDQVEV VTAQELVESQNLPEL [SEQ ID NO.:186]	VSKCHTDKGSLSSTTKPFQNISRIAVGD CPRYVKQGSLLKLATGMRNPEKASR [SEQ ID NO.:235]
H4 (D90302) No Cys $\Delta 1$	QNYTGPNVICMGHHAV ANGTMVKTLLADDQVEV VTAQELVESQNLPEL [SEQ ID NO.:187]	SKCHTDKGSLSSTTKPFQNISRIAVGDC PRYVKQGSLLKLATGMRNPEKASR [SEQ ID NO.:236]
H4 (D90302) No Cys $\Delta 3$	QNYTGPNVICMGHHAV ANGTMVKTLLADDQVEV VTAQELVESQNLPE [SEQ ID NO.:188]	KCHTDKGSLSSTTKPFQNISRIAVGDCP RYVKQGSLLKLATGMRNPEKASR [SEQ ID NO.:237]
H5 (X07826) No Cys	DQICIGYHANKSTKQVD TIMEKNVTVTTHAQDILE RTHNGKL [SEQ ID NO.:189]	DTKCQTPVGEINSSMPFHNIHPHTIGE CPKYVKSDRLVLATGLRNVPQRKKR [SEQ ID NO.:238]
H5 (X07826) No Cys $\Delta 1$	DQICIGYHANKSTKQVD TIMEKNVTVTTHAQDILE RTHNGKL [SEQ ID NO.:190]	TKCQTPVGEINSSMPFHNIHPHTIGEC PKYVKSDRLVLATGLRNVPQRKKR [SEQ ID NO.:239]
H5 (X07826) No Cys $\Delta 3$	DQICIGYHANKSTKQVD TIMEKNVTVTTHAQDILE RTHNGK [SEQ ID NO.:191]	KCQTPVGEINSSMPFHNIHPHTIGEC PKYVKSDRLVLATGLRNVPQRKKR [SEQ ID NO.:240]
H6 (D90303) No Cys	DKICIGYHANNSTTQIDT ILEKNVTVTTHSVELLENQ KEERF [SEQ ID NO.:192]	DATCQTVAGVLRNKTTFQNVSPWLIG ECPKYVKSESLRLATGLRNVPQIETR [SEQ ID NO.:241]
H6 (D90303) No Cys $\Delta 1$	DKICIGYHANNSTTQIDT ILEKNVTVTTHSVELLENQ KEERF [SEQ ID NO.:193]	ATCQTVAGVLRNKTTFQNVSPWLIGE CPKYVKSESLRLATGLRNVPQIETR [SEQ ID NO.:242]

HA Subtype (Genbank No.)	HA1 N-terminal Stem Segment	HA1 C-terminal Stem Segment
H6 (D90303) No Cys $\Delta 3$	DKICIGYHANNSTTQIDT ILEKNVTVTHSVELLENQ KEER [SEQ ID NO.:194]	TCQTVAGVLRNTNKTQNVSPWIGEC PKYVKSESLRLATGLRNVPQIETR [SEQ ID NO.:243]
H7 (M24457) No Cys	DKICLGHHAVSNGTKVN TLTERGVEVVNATETVE RTNIPKI [SEQ ID NO.:195]	EGECYHSGGTITSRLPFQINNSRAVGK CPRYVKQESLLLATGMKNVPEPSKKR KKR [SEQ ID NO.:244]
H7 (M24457) No Cys $\Delta 1$	DKICLGHHAVSNGTKVN TLTERGVEVVNATETVE RTNIPKI [SEQ ID NO.:196]	GECYHSGGTITSRLPFQINNSRAVGKC PRYVKQESLLLATGMKNVPEPSKKRK KR [SEQ ID NO.:245]
H7 (M24457) No Cys $\Delta 3$	DKICLGHHAVSNGTKVN TLTERGVEVVNATETVE RTNIPK [SEQ ID NO.:197]	ECYHSGGTITSRLPFQINNSRAVGKCP RYVKQESLLLATGMKNVPEPSKKRKR R [SEQ ID NO.:246]
H8 (D90304) No Cys	DRICIGYQSNNSTDVNT LIEQNVPTQTMELVET EKHPAY [SEQ ID NO.:198]	NTKCQTYAGAINSSKPFQNASRHYMG ECPKYVKKASRLAVGLRNTPSVEPR [SEQ ID NO.:247]
H8 (D90304) No Cys $\Delta 1$	DRICIGYQSNNSTDVNT LIEQNVPTQTMELVET EKHPAY [SEQ ID NO.:199]	TKCQTYAGAINSSKPFQNASRHYMGEC CPKYVKKASRLAVGLRNTPSVEPR [SEQ ID NO.:248]
H8 (D90304) No Cys $\Delta 3$	DRICIGYQSNNSTDVNT LIEQNVPTQTMELVET EKHPA [SEQ ID NO.:200]	KCQTYAGAINSSKPFQNASRHYMGEC PKYVKKASRLAVGLRNTPSVEPR [SEQ ID NO.:249]
H9 (D90305) No Cys	DKICIGYQSTNSTETVDT LTESNVPVTHTKELLHTE HNGML [SEQ ID NO.:201]	VVQCQTEKGGLNTTLPFHNISKYAFG NCPKYVGKSLKLPVGLRNVPVAVSSR [SEQ ID NO.:250]
H9 (D90305) No Cys $\Delta 1$	DKICIGYQSTNSTETVDT LTESNVPVTHTKELLHTE HNGML [SEQ ID NO.:202]	VQCQTEKGGLNTTLPFHNISKYAFGN CPKYVGKSLKLPVGLRNVPVAVSSR [SEQ ID NO.:251]
H9 (D90305) No Cys $\Delta 3$	DKICIGYQSTNSTETVDT LTESNVPVTHTKELLHTE HNGM [SEQ ID NO.:203]	QCQTEKGGLNTTLPFHNISKYAFGNCP KYVGKSLKLPVGLRNVPVAVSSR [SEQ ID NO.:252]

HA Subtype (Genbank No.)	HA1 N-terminal Stem Segment	HA1 C-terminal Stem Segment
H10 (M21647) No Cys	LDRICLGHHAVANGTIV KTLTNEQEEVTNATETV ESTNLNKL [SEQ ID NO.:204]	ESKCFWRGGSINTKLPPQNLSVRTVGQ CPKYVNQRSLLLATGMRNVPEVVQGR [SEQ ID NO.:253]
H10 (M21647) No Cys Δ1	LDRICLGHHAVANGTIV KTLTNEQEEVTNATETV ESTNLNKL [SEQ ID NO.:205]	SKCFWRGGSINTKLPPQNLSVRTVGQC PKYVNQRSLLLATGMRNVPEVVQGR [SEQ ID NO.:254]
H10 (M21647) No Cys Δ3	LDRICLGHHAVANGTIV KTLTNEQEEVTNATETV ESTNLNK [SEQ ID NO.:206]	KCFWRGGSINTKLPPQNLSVRTVGQCP KYVNQRSLLLATGMRNVPEVVQGR [SEQ ID NO.:255]
H11 (D90306) No Cys	DEICIGYLSNNSTDVKVDT IIENNVTVTSSVELVETE HTGSF [SEQ ID NO.:207]	STKCQTEIGGINTNKS FHNVHRNTIGD CPKYVNVKSLK LATGPRNVPAIASR [SEQ ID NO.:256]
H11 (D90306) No Cys Δ1	DEICIGYLSNNSTDVKVDT IIENNVTVTSSVELVETE HTGSF [SEQ ID NO.:208]	TKCQTEIGGINTNKS FHNVHRNTIGDC PKYVNVKSLK LATGPRNVPAIASR [SEQ ID NO.:257]
H11 (D90306) No Cys Δ3	DEICIGYLSNNSTDVKVDT IIENNVTVTSSVELVETE HTGS [SEQ ID NO.:209]	KCQTEIGGINTNKS FHNVHRNTIGDCP KYVNVKSLK LATGPRNVPAIASR [SEQ ID NO.:258]
H12 (D90307) No Cys	DKICIGYQTNNSTETVNT LSEQNPVTQVEELVHR GIDPIL [SEQ ID NO.:210]	VTECQLNEGVMNTSKPFQNTSKHYIG KCPKYIPSGSLK LAIGLRNV PQVQDR [SEQ ID NO.:259]
H12 (D90307) No Cys Δ1	DKICIGYQTNNSTETVNT LSEQNPVTQVEELVHR GIDPIL [SEQ ID NO.:211]	TECQLNEGVMNTSKPFQNTSKHYIGK CPKYIPSGSLK LAIGLRNV PQVQDR [SEQ ID NO.:260]
H12 (D90307) No Cys Δ3	DKICIGYQTNNSTETVNT LSEQNPVTQVEELVHR GIDPI [SEQ ID NO.:212]	ECQLNEGVMNTSKPFQNTSKHYIGKC PKYIPSGSLK LAIGLRNV PQVQDR [SEQ ID NO.:261]
H13 (D90308) No Cys	DRICVGYLSTNSSERVDT LLENGVPVTSSIDLITN HTGTY [SEQ ID NO.:213]	NTKCQTSVGGINTNRTFQNI DKNALG DCPKYIKSGQLK LATGLRNV PAISNR [SEQ ID NO.:262]

HA Subtype (Genbank No.)	HA1 N-terminal Stem Segment	HA1 C-terminal Stem Segment
H13 (D90308) No Cys $\Delta 1$	DRICVGYLSTNSSERVDT LLENGVPVTSSIDLIETN HTGTY [SEQ ID NO.:214]	TKCQTSVGGINTNRTFQNIIDKNALGD CPKYIKSGQLKLATGLRNVPAISNR [SEQ ID NO.:263]
H13 (D90308) No Cys $\Delta 3$	DRICVGYLSTNSSERVDT LLENGVPVTSSIDLIETN HTGT [SEQ ID NO.:215]	KCQTSVGGINTNRTFQNIIDKNALGDC PKYIKSGQLKLATGLRNVPAISNR [SEQ ID NO.:264]
H14 (M35997) No Cys	QITNGTTGNPIICLGHHA VENGTSVKTLTDNHVEV VSAKELVETNHTDEL [SEQ ID NO.:216]	TSPCLTDKGSIQSDKPFQNVSRIGNC PKYVKQGSMLLATGMRNIPGKQAK [SEQ ID NO.:265]
H14 (M35997) No Cys $\Delta 1$	QITNGTTGNPIICLGHHA VENGTSVKTLTDNHVEV VSAKELVETNHTDEL [SEQ ID NO.:217]	SPCLTDKGSIQSDKPFQNVSRIGNCP KYVKQGSMLLATGMRNIPGKQAK [SEQ ID NO.:266]
H14 (M35997) No Cys $\Delta 3$	QITNGTTGNPIICLGHHA VENGTSVKTLTDNHVEV VSAKELVETNHTDE [SEQ ID NO.:218]	PCLTDKGSIQSDKPFQNVSRIGNCPK YVKQGSMLLATGMRNIPGKQAK [SEQ ID NO.:267]
H15 (L43917) No Cys	DKICLGHHAVANGTKV NTLTERGVEVVNATETV EITGIDKV [SEQ ID NO.:219]	EGECFYSGGTINSPLPFQNIIDSRVKG CPRYVKQSSLPLALGMKNVPEKIRTR [SEQ ID NO.:268]
H15 (L43917) No Cys $\Delta 1$	DKICLGHHAVANGTKV NTLTERGVEVVNATETV EITGIDKV [SEQ ID NO.:220]	GECFYSGGTINSPLPFQNIIDSRVKGK PRYVKQSSLPLALGMKNVPEKIRTR [SEQ ID NO.:269]
H15 (L43917) No Cys $\Delta 3$	DKICLGHHAVANGTKV NTLTERGVEVVNATETV EITGIDK [SEQ ID NO.:221]	ECFYSGGTINSPLPFQNIIDSRVKGCP RYVKQSSLPLALGMKNVPEKIRTR [SEQ ID NO.:270]
H16 (EU293865) No Cys	DKICIGYLSNNSSDTVDT LTENGVPVTSSVDLVET NHTGTY [SEQ ID NO.:222]	NTKCQTSLGGINTNKTFFQNIERNALGD CPKYIKSGQLKLATGLRNVPSIGER [SEQ ID NO.:271]
H16 (EU293865) No Cys $\Delta 1$	DKICIGYLSNNSSDTVDT LTENGVPVTSSVDLVET NHTGTY [SEQ ID NO.:223]	TKCQTSLGGINTNKTFFQNIERNALGDC PKYIKSGQLKLATGLRNVPSIGER [SEQ ID NO.:272]

HA Subtype (Genbank No.)	HA1 N-terminal Stem Segment	HA1 C-terminal Stem Segment
H16 (EU293865) No Cys $\Delta 3$	DKICIGYLSNNSDVTDT LTENGVPVTSSVDLVET NHTGT [SEQ ID NO.:224]	KCQTSLGGINTNKTQNIERNALGDCP KYIKSGQLKLATGLRNVPSIGER [SEQ ID NO.:273]

[00100] Table 2, below, identifies putative stem domains, luminal domains, transmembrane domains and cytoplasmic domains of HA2 polypeptides.

TABLE 2 Exemplary Influenza A Hemagglutinin Sequences

HA2 domain Subtype (Genbank No.)	Stem domain	Luminal domain	Transmembrane domain	Cytoplasmic domain
H1 PR8-H1N1 (EF467821.1)	GLFGAIAGFIEGGWT GMIDGWYGYHHQNE QSGGYAADQKSTQN AINGITNKVNTVIEK MNIQFTAVGKEFNKL EKR MENLNKKVDDG FLDIWTYNAELLVLL ENERTLDFHDSNVKN LYEKVKSQKNNAK EIGNGCFEFYHKCDN ECMESVRNGTYDYP KYSEESKLNREKVDG VKLES [SEQ ID NO.:82]	MGIYQ [SEQ ID NO.:98]	ILAIYSTVASSL VLLVSLGAISF WMCS [SEQ ID NO.:114]	NGSLQCRI CI [SEQ ID NO.:130]
H2 (L11136)	GLFGAIAGFIEGGWQ GMIDGWYGYHHSND QSGGYAADKESTQK AIDGITNRVNSVIEK MNTQFEAVGKEFSNL EKRL ENLNKKMEDG FLDVWTYNAELLVL MENERTLDFHDSNV KNLYDRVRMQLRDN AKELGNGCFEFYHKC DDECMNSVKNGTYD YPKYEEESKLNREI KGVKLSN [SEQ ID NO.:83]	MGVYQ [SEQ ID NO.:99]	ILAIYATVAGSL SLAIMIAGISLW MCS [SEQ ID NO.:115]	NGSLQCRI CI [SEQ ID NO.:131]
H3 HK68-H3N2	GLFGAIAGFIENGWE GMIDGWYGFRHQNS	SGYKD	WILWISFAISCF LLCVVLLGFIM	RGNIRCNI CI

HA2 domain Subtype (Genbank No.)	Stem domain	Luminal domain	Transmembrane domain	Cytoplasmic domain
(EF409245) PDB: 1HGJ	EGTGQAADLKSTQA AIDQINGKLN RVIEKT NEKFHQIEKEFSEVE GRIQDLEKYVEDTKI DLWSYNAELLVALE NQHTIDLT DSEMKNL FEKTRRQLRENAED MGNGCFKIYHKCDN ACIESIRNGTYDHDV YRDEALNNRFQIKGV ELK [SEQ ID NO.:84]	[SEQ ID NO.:100]	WACQ [SEQ ID NO.:116]	[SEQ ID NO.:132]
H4 (D90302)	GLFGAIAGFIENGWQ GLIDGWYGRHQN A EGTGTAADLKSTQA AIDQINGKLNRLIEKT NDKYHQIEKEFEQVE GRIQDLENYVEDTKI DLWSYNAELLVALE NQHTIDVT DSEMKNL FERVRRQLRENAEDK GNGCFEIFHKCDNNC IESIRNGTYDHDIIYRD EAINNRFQIQGVKLT [SEQ ID NO.:85]	QGYKD [SEQ ID NO.:101]	IILWISFSISCFL L VALLAFILWA CQ [SEQ ID NO.:117]	NGNIRCQI CI [SEQ ID NO.:133]
H5 (X07826)	GLFGAIAGFIEGGWQ GMVDGWYGYHHSN EQGSGYAADKESTQ KAIDGITNKVNSIIDK MNTRFEAVGKEFNN LERRVENLNKKMED GFLDVWTYNVELLV LMENERTLDFHDSNV NNLYDKVRLQLKDN ARELGNGCFEFYHKC DNECMESVRNGTYD YPQYSEEARLNREEIS GVKLES [SEQ ID NO.:86]	MGVYQ [SEQ ID NO.:102]	ILSIYSTVASSL ALAIMIAGLSF WMCS [SEQ ID NO.:118]	NGSLQCRI CI [SEQ ID NO.:134]
H6 (D90303)	GLFGAIAGFIEGGWT GMIDGWYGYHHENS QGSGYAADRESTQK AVDGITNKVNSIIDK MNTQFEAVDHEFSNL ERRIDNLNKRMEDGF LDVWTYNAELLVLL	LG VYQ [SEQ ID NO.:103]	ILAIYSTVSSSL VLVGLIIAVGL WMCS [SEQ ID NO.:119]	NGSMQCR ICI [SEQ ID NO.:135]

HA2 domain Subtype (Genbank No.)	Stem domain	Luminal domain	Transmembrane domain	Cytoplasmic domain
	ENERTLDLHDANVK NLYERVKSQLRDNA MILGNGCFEFWHKC DDECMEVKNGTYD YPKYQDESKLNRQEI ESVKLES [SEQ ID NO.:87]			
H7 (M24457)	GLFGAIAGFIENGWE GLVDGWYGFRHQNA QGEGTAADYKSTQS AIDQITGKLNRLIEKT NQQFELIDNEFTEVE KQIGNLINWTKDSITE VWSYNAELIVAMEN QHTIDLADSEMNRLY ERVRKQLRENAEED GTGCFEIFHKCDDDC MASIRNNTYDHSKYR EEAMQNRIQIDPVKL S [SEQ ID NO.:88]	SGYKD [SEQ ID NO.:104]	VILWFSFGASCF LLLAIAMGLVFI CVK [SEQ ID NO.:120]	NGNMRCIT ICI [SEQ ID NO.:136]
H8 (D90304)	GLFGAIAGFIEGGWS GMIDGWYGFHHSNS EGTGMAADQKSTQE AIDKITNKVNNIVDK MNFEEVVNHEFSEV EKRLNMINDKIDDQIE DLWAYNAELLVLE NQKTLDEHDSNVKN LFDEVKRRLSANAID AGNGCFDILHKCDNE CMETIKNGTYDHKE YEEEAKLERSKINGV KLEE [SEQ ID NO.:89]	NTTYK [SEQ ID NO.:105]	ILSIYSTVAASL CLAILIAGGLIL GMQ [SEQ ID NO.:121]	NGSCRCM FCI [SEQ ID NO.:137]
H9 (D90305)	GLFGAIAGFIEGGWP GLVAGWYGFQHSND QGVGMAADKGSTQK AIDKITSKVNNIIDKM NKQYEVIDHEFNELE ARLNMINKIDDQIQ DIWAYNAELLVLEN QKTLDEHDANVNNL YNKVKRALGSNAVE DGNGCFELYHKCDD QCMETIRNGTYDRQ	EGTYK [SEQ ID NO.:106]	ILTIYSTVASSL VLAMGFAAFLF WAMS [SEQ ID NO.:122]	NGSCRCNI CI [SEQ ID NO.:138]

HA2 domain Subtype (Genbank No.)	Stem domain	Luminal domain	Transmembrane domain	Cytoplasmic domain
	KYQEESRLERQKIEG VKLES [SEQ ID NO.:90]			
H10 (M21647)	GLFGAIAGFIENGWE GMVDGWYGFQRHQN AQGTGQAADYKSTQ AAIDQITGKLNRLIEK TNTEFESIESEFSETEH QIGNVINWTKDSITDI WTYNAELLVAMENQ HTIDMADSEMLNLYE RVRKQLRQNAEEDG KGCFEIYHTCDDSCM ESIRNNTYDHSQYRE EALLNRLNINPVKLS [SEQ ID NO.:91]	SGYKD [SEQ ID NO.:107]	IILWFSFGESCF VLLAVVMGLV FFCLK [SEQ ID NO.:123]	NGNMRC TICI [SEQ ID NO.:139]
H11 (D90306)	GLFGAIAGFIEGGWP GLINGWYGFQHRDE EGTGIAADKESTQKA IDQITSKVNIVDRM NTNFESVQHEFSEIEE RINQLSKHVDDSVVD IWSYNAQLLVLENE KTLDLHDSNVRNLHE KVRMLKDNAKDEG NGCFTFYHKCDNKCI ERVRNGTYDHKEFEE ESKINRQEIEGVKLDS S [SEQ ID NO.:92]	GNVYK [SEQ ID NO.:108]	ILSIYSCIASSLV LAALIMGFMFW ACS [SEQ ID NO.:124]	NGSCRCTI CI [SEQ ID NO.:140]
H12 (D90307)	GLFGAIAGFIEGGWP GLVAGWYGFQHQNA EGTGIAADRSTQRA IDNMQNKLNNVIDK MNKQFEVNVNHEFSE VESRINMINSKIDDQI TDIWAYNAELLVLE NQKTLDEHDANVRN LHDRVRRVLRENAID TGDGCFEILHKCDNN CMDTIRNGTYNHKE YEEESKIERQKVNGV KLEE [SEQ ID NO.:93]	NSTYK [SEQ ID NO.:109]	ILSIYSSVASSLV LLLMIIGGFIFG CQN [SEQ ID NO.:125]	GNVRCTF CI [SEQ ID NO.:141]
H13 (D90308)	GLFGAIAGFIEGGWP GLINGWYGFQHQNE	DNVYK [SEQ ID	ALSIYSCIASSV VLVGLILSFIM	GNCRFNV CI

HA2 domain Subtype (Genbank No.)	Stem domain	Luminal domain	Transmembrane domain	Cytoplasmic domain
	QGTGIAADKESTQKA IDQITTKINNIIDKMN GNYDSIRGEFNQVEK RINMLADRIDDAVTD IWSYNAKLLVLEND KTLDMDHDANVKNLH EQVRRELKDNAIDEG NGCFELLHKCNDSC METIRNGTYDHTEYA EESKLKRQEIDGIKLS E [SEQ ID NO.:94]	NO.:110]	WACSS [SEQ ID NO.:126]	[SEQ ID NO.:142]
H14 (M35997)	GLFGAIAGFIENGWQ GLIDGWYGF RHQNA EGTGTAADLKSTQA AIDQINGKLNRLIEKT NEKYHQIEKEFEQVE GRIQDLEKYVEDTKI DLWSYNAELLVALE NQHTIDVTDSEMKNL FERVRRQLRENAEDQ GNGCFEIFHQCDNNC IESIRNGTYDHNIYRD EAINNRIKINPVTLT [SEQ ID NO.:95]	MGYKD [SEQ ID NO.:111]	IILWISFSMSCF VFVALILGFVL WACQ [SEQ ID NO.:127]	NGNIRCQI CI [SEQ ID NO.:143]
H15 (L43917)	GLFGAIAGFIENGWE GLIDGWYGF RHQNA QGQGTAAADYKSTQA AIDQITGKLNRLIEKT NKQFELIDNEFTEVE QQIGNVINWTRDSL EIWSYNAELLVAME NQHTIDLADSEMKNL YERVRRQLRENAEED GTGCFEIFHRCDDQC MESIRNNTYNHTEYR QEALQNRIMINPVKLS S [SEQ ID NO.:96]	SGYKD [SEQ ID NO.:112]	VILWFSFGASC VMLLAIAMGLI FMCVKN [SEQ ID NO.:128]	GNLRCTIC I [SEQ ID NO.:144]
H16 (EU293865)	GLFGAIAGFIEGGWP GLINGWYGFQHNE QGTGIAADKASTQKA INEITTKINNIIEKMNG NYDSIRGEFNQVEKR INMLADRVDDAVTDI WSYNAKLLVLEND	DNVYK [SEQ ID NO.:113]	VLSIYSCIASSIV LVGLILAFIMW ACS [SEQ ID NO.:129]	NGSCRFN VCI [SEQ ID NO.:145]

HA2 domain Subtype (Genbank No.)	Stem domain	Luminal domain	Transmembrane domain	Cytoplasmic domain
	RTLDLHDANVRNLH DQVKRALKSNAIDEG DGCFNLLHKCNDS METIRNGTYNHEDYR EESQLKRQEIEGIK LKTE [SEQ ID NO.:97]			

[00101] In certain embodiments, the influenza hemagglutinin stem domain polypeptides comprise one or more immunogenic epitopes in the tertiary or quaternary structure of an influenza hemagglutinin polypeptide.

[00102] In certain embodiments, the HA1 N-terminal stem segment comprises the amino acid sequence $A_{17}-A_{18}-(Xaa)_n-A_{38}$ (SEQ ID NO:146), wherein

A_{17} is Y or H;

A_{18} is H, L, or Q;

$(Xaa)_n$ represents a sequence of 18-20 amino acid residues; and

A_{38} is H, S, Q, T or N.

[00103] In certain embodiments, the HA1 C-terminal stem segment comprises the amino acid sequence $A_{291}-A_{292}$ (SEQ ID NO:147), wherein

A_{291} is T, S, N, D, P or K; and

A_{292} is L, M, K or R.

[00104] In certain embodiments, the HA2 domain comprises the amino acid sequence $A_{18}-A_{19}-A_{20}-A_{21}$ (SEQ ID NO:148), wherein

A_{18} is V or I;

A_{19} is D, N or A;

A_{20} is G, and

A_{21} is W.

[00105] In certain embodiments, the HA2 domain comprises the amino acid sequence $A_{38}-A_{39}-A_{40}-A_{41}-A_{42}-A_{43}-A_{44}-A_{45}-A_{46}-A_{47}-A_{48}-A_{49}-A_{50}-A_{51}-A_{52}-A_{53}-A_{54}-A_{55}-A_{56}$ (SEQ ID NO:149), wherein

A_{38} is K, Q, R, L or Y;

A_{39} is any amino acid residue;

A_{40} is any amino acid residue;

A₄₁ is T;
A₄₂ is Q;
A₄₃ is any amino acid residue;
A₄₄ is A;
A₄₅ is I;
A₄₆ is D;
A₄₇ is any amino acid residue;
A₄₈ is I, V or M;
A₄₉ is T, Q or N;
A₅₀ is any amino acid residue;
A₅₁ is K;
A₅₂ is V or L;
A₅₃ is N;
A₅₄ is any amino acid residue;
A₅₅ is V, I or L; and
A₅₆ is V or I.

[00106] In certain embodiments, the influenza stem domain polypeptides comprise two amino acid sequences selected from SEQ ID NOS:146-149. In certain embodiments, the influenza stem domain polypeptides comprise three amino acid sequences selected from SEQ ID NOS:146-149. In certain embodiments, the influenza stem domain polypeptides comprise four amino acid sequences selected from SEQ ID NOS:146-149.

[00107] In certain embodiments, the HA1 N-terminal stem segments are based on an influenza B hemagglutinin. In certain embodiments, the HA1 N-terminal stem segment is selected from SEQ ID NOS:154-157, presented in Table 3 below.

[00108] In certain embodiments, the HA1 C-terminal stem segments are based on an influenza B hemagglutinin. In certain embodiments, the HA1 C-terminal stem segment is selected from SEQ ID NOS:158-159, presented in Table 3 below.

[00109] In certain embodiments, the HA2 stem domains are based on an influenza B hemagglutinin. Exemplary residues for the end of an N-terminal stem segment and the end of a C-terminal stem segment of an influenza B hemagglutinin are indicated in FIG. 2. In certain embodiments, the HA2 stem domain is according to SEQ ID NO:160, presented in Tables 3 and 4 below.

[00110] In particular embodiments, the boundaries of the influenza B virus HA1 N-terminal stem segment and influenza B virus HA1 C-terminal segment are defined with respect to three pairs of amino acid residues: Arg₅₀ and Ser₂₇₇; Ala₆₆ and Trp₂₇₁; and Lys₈₀ and Ser₂₇₇. The residue numbers are based on the numbering of the B-HA from influenza virus B as described in Protein Data Bank accession No. 3BT6. The amino acid sequence corresponding to the X-ray crystal structure of the B-HA protein in Protein Data Bank accession No. 3BT6 is aligned with representative H1 and H3 amino acid sequence and shown in FIG. 2. Positions of the three pairs of residues are also highlighted in FIG. 2.

[00111] In certain embodiments, an influenza B virus HA1 N-terminal stem segment starts at residue 1 (based on numbering of an influenza B virus HA1 subunit as in PDB file 3BT6) and ends at Arg₅₀. In certain embodiments, an influenza B virus HA1 N-terminal stem segment starts at residue 1 and ends at Ala₆₆. In some embodiments, an influenza B virus HA1 N-terminal stem segment starts at residue 1 and ends at Lys₈₀. In some embodiments, an influenza B virus N-terminal stem segment starts at residue 1 and ends at Arg₈₀.

[00112] In some embodiments, an influenza B virus HA1 N-terminal stem segment has an amino acid sequence according to any one of SEQ ID NOS:154-157, as illustrated in TABLE 3. In some embodiments, an influenza B virus HA1 N-terminal stem segment has an amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96% or 98% identical to any one of the amino acid sequences of any one of SEQ ID NOS:154-157.

[00113] In some embodiments, an influenza B virus HA1 N-terminal stem segment has an amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96% or 98% identical to the amino acid sequence SEQ ID NO:154, which corresponds to residues 1-50 of the influenza B virus HA1.

[00114] In some embodiments, an influenza B virus HA1 N-terminal stem segment has an amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96% or 98% identical to the amino acid sequence SEQ ID NO:155, which corresponds to residues 1-66 of the influenza B virus HA1.

[00115] In some embodiments, an influenza B virus HA1 N-terminal stem segment has an amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96% or 98% identical to the amino acid sequence SEQ ID NO:156, which corresponds to residues 1-80 of the influenza B virus HA1.

[00116] In some embodiments, an influenza B virus HA1 N-terminal stem segment has an amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96% or 98% identical to the amino acid sequence SEQ ID NO:157, which corresponds to residues 1-80 of the influenza B virus HA1.

[00117] In some embodiments, an influenza B virus HA1 C-terminal stem segment has an amino acid sequence that starts at Ser₂₇₇ residue or Trp₂₇₁, or corresponding residues in other influenza B virus HA subtypes.

[00118] In some embodiments, an influenza B virus HA1 C-terminal stem segment has an amino acid sequence according to any one of SEQ ID NOS:158-159, as illustrated in TABLE 3. In some embodiments, an influenza B virus HA1 C-terminal stem segment has an amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96% or 98% identical to SEQ ID NO:158, which correspond to residues 277-344 of influenza B virus HA1. In some embodiments, an influenza B virus HA1 C-terminal stem segment has an amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 96% or 98% identical to SEQ ID NO.:159, which correspond to residues 271-344 of influenza B virus HA1.

[00119] In some embodiments, an influenza B virus HA1 C-terminal stem segment starts at residue-276, residue-275, residue-274, residue-273, or residue-272. In other embodiments, an influenza B virus HA1 C-terminal stem segment starts at residue-278, residue-279, residue-280, residue-281, or residue-282.

[00120] In certain embodiments, the influenza B virus HA2 domain is in tertiary or quaternary association with the influenza B virus HA1 domain through the influenza B virus HA1 N-terminal segment, the influenza B virus HA1 C-terminal segment, or both.

[00121] In some embodiments, the influenza B virus HA1 C-terminal segment and the influenza B virus HA2 subunit are covalently linked. For example, at its C-terminus (*e.g.*, at the ending residue of the second sequence), the influenza B virus HA1 C-terminal segment is covalently linked to the influenza B virus HA2 domain in such embodiments. In some embodiments, the influenza B virus HA1 C-terminal segment and influenza B virus HA2 domain form a continuous polypeptide chain.

[00122] In some embodiments, the influenza B virus HA2 domain has the amino acid sequence of SEQ ID NO:160 or 161, as illustrated in TABLE 3 or 4. In some embodiments, the amino acid sequence of the HA2 domain is at least 70%, 75%, 80%, 85%, 90%, 95%, 96% or 98% identical to any one of SEQ ID NOS:160-161.

[00123] In certain embodiments, the influenza B stem domain polypeptides comprise a signal peptide. The signal peptide can be any signal peptide deemed suitable to those of skill in the art, including any signal peptide described herein. In certain embodiments, the signal peptide is at least 70%, 75%, 80%, 85%, 90%, 95%, 96% or 98% identical to any of SEQ ID NOS:150-153. In certain embodiments, the signal peptide is according to any of SEQ ID NOS:150-153.

[00124] In certain embodiments, the influenza B stem domain polypeptides comprise a luminal domain. The luminal domain can be any luminal domain deemed suitable to those of skill in the art, including any luminal domain described herein. In certain embodiments, the luminal is at least 60% or 80%, identical to SEQ ID NO:162. In certain embodiments, the luminal domain is according to SEQ ID NO:162.

[00125] In certain embodiments, the influenza B stem domain polypeptides comprise a transmembrane domain. The transmembrane domain can be any transmembrane domain deemed suitable to those of skill in the art, including any transmembrane domain described herein. In certain embodiments, the transmembrane domain is at least 70%, 75%, 80%, 85%, 90%, 95%, 96% or 98% identical to SEQ ID NO:163. In certain embodiments, the transmembrane domain is according to SEQ ID NO:163.

[00126] In certain embodiments, the influenza B stem domain polypeptides comprise a cytoplasmic domain. The cytoplasmic domain can be any cytoplasmic domain deemed suitable to those of skill in the art, including any cytoplasmic domain described herein. In certain embodiments, the cytoplasmic domain is at least 70%, 75%, 80%, 85%, 90%, 95%, 96% or 98% identical to SEQ ID NO:164. In certain embodiments, the cytoplasmic domain is according to SEQ ID NO:164.

TABLE 3: Exemplary Influenza B Hemagglutinin Sequences

HA construct variants	Signal peptide	HA1 N-terminal Stem Segment	HA1 C-terminal Stem Segment	HA2 Domain
Arg50-Ser277	MKAIIVILMV VTSNA [SEQ ID NO.:150]	DRICTGITSSNS PHVVKATATQG EVNVTGVIPLT TTPTKSHFANL KGTETR [SEQ ID NO.:154]	SKVIKGSPLI GEADCLHEKY GGLNKSPPYY TGEHAKAIGN CPIWVKTPLKL ANGTKYRPPA KLLKER [SEQ ID NO.:158]	GFFGAIAGFLEGG WEGMIAGWHGY TSHGAHGVAVAA DLKSTQEAINKIT KNLNSLSELEVKN LQRLSGAMDELH NEILELDEKVDDL RADTISSQIELAVL LSNEGIINSEDEHL

HA construct variants	Signal peptide	HA1 N-terminal Stem Segment	HA1 C-terminal Stem Segment	HA2 Domain
				LALERKLKKMLG PSAVEIGNGCFET KHKCNQTCLDRI AAGTFDAGEFSLP TFDSL NITAASLN DDGLDNHTILLYY STAASSLAVTLM I AIFVVY MVSRDN VSCSICL [SEQ ID NO.:160]
Ala66- Trp271	MKAIIIVILMV VTSNA [SEQ ID NO.:151]	DRICTGITSSNS PHVVK TATQG EVNVTGVIPLT TTPTKSHFANL KGTETRGKLC PKCLNCTDLD VA [SEQ ID NO.:155]	WCASGRSKVI KGSLPLIGEAD CLHEKYGGLN KSKPYTGEH AKAIGNCPIW VKTPLKLANG TKYRPPAKLL KER [SEQ ID NO.:159]	GFFGAIAGFLEGG WEGMIAGWHGY TSHGAHGVAVAA DLKSTQEAINKIT KNLNSLSELEVKN LQRLSGAMDELH NEILELDEKVDDL RADTISSQIELAVL LSNEGIINSEDEHL LALERKLKKMLG PSAVEIGNGCFET KHKCNQTCLDRI AAGTFDAGEFSLP TFDSL NITAASLN DDGLDNHTILLYY STAASSLAVTLM I AIFVVY MVSRDN VSCSICL [SEQ ID NO.:160]
Lys80- Ser277	MKAIIIVILMV VTSNA [SEQ ID NO.:152]	DRICTGITSSNS PHVVK TATQG EVNVTGVIPLT TTPTKSHFANL KGTETRGKLC PKCLNCTDLD VALGRP KCTG KIPSAK [SEQ ID NO.:156]	SKVIKGS LPLI GEADCLHEKY GGLN KSKPYY TGEHAKAIGN CPIWVK TPLKL ANGTKYRPPA KLLKER [SEQ ID NO.:158]	GFFGAIAGFLEGG WEGMIAGWHGY TSHGAHGVAVAA DLKSTQEAINKIT KNLNSLSELEVKN LQRLSGAMDELH NEILELDEKVDDL RADTISSQIELAVL LSNEGIINSEDEHL LALERKLKKMLG PSAVEIGNGCFET KHKCNQTCLDRI AAGTFDAGEFSLP TFDSL NITAASLN DDGLDNHTILLYY STAASSLAVTLM I AIFVVY MVSRDN VSCSICL

HA construct variants	Signal peptide	HA1 N-terminal Stem Segment	HA1 C-terminal Stem Segment	HA2 Domain
				[SEQ ID NO.:160]
Arg80-Ser277	MKAIIIVILMV VTSNA [SEQ ID NO.:153]	DRICTGITSSNS PHVVKTATQG EVNVTGVIPLT TTPTKSHFANL KGTETRGKLC PKCLNCTDLD VALGRPCKTG KIPSAR [SEQ ID NO.:157]	SKVIKGSPLI GEADCLHEKY GGLNKSPPY TGEHAKAIGN CPIWVKTPKL ANGTKYRPPA KLLKER [SEQ ID NO.:158]	GFFGAIAGFLEGG WEGMIAGWHGY TSHGAHGVAVAA DLKSTQEAINKIT KNLNSLSELEVKN LQRLSGAMDELH NEILELDEKVDDL RADTISSQIELAVL LSNEGIINSEDEHL LALERKLKMLG PSAVEIGNGCFET KHKCNQTCLDRI AAGTFDAGEFSLP TFDSL NITAASLN DDGLDNHTILLY STAASSLAVTLMI AIFVVMVSRDN VSCSICL [SEQ ID NO.:160]

[00127] Table 4 provides the putative stem domain, luminal domain, transmembrane domain and cytoplasmic domain of HA from influenza B.

TABLE 4: Exemplary Influenza B Hemagglutinin Sequences

HA2 domain Subtype (Genbank No.)	Stem domain	Luminal domain	Transmembrane domain	Cytoplasmic domain
HA2 (AY096185)	GFFGAIAGFLEG GWEGMIAGWH GYTSHGAHGV AVAADLKSTQE AINKITKNLNSL SELEVKNLQRL SGAMDELHNEI LELDEKVDDL ADTISSQIELAV LLSNEGIINSE EHLALERKLK KMLGPSAVEIG NGCFETKHKCN QTCLDRIAAGT FDAGEFSLPTFD	DGLDN [SEQ ID NO.:162]	HTILLYYSTAAS SLAVTLMIAIFV VYMV [SEQ ID NO.:163]	SRDNVSCSIC L [SEQ ID NO.:164]

HA2 domain Subtype (Genbank No.)	Stem domain	Luminal domain	Transmembrane domain	Cytoplasmic domain
	SLNITAASLND [SEQ ID NO.:161]			

[00128] As illustrated in FIGS. 1 and 2, HA1 N-terminal stem segments share sequence identity between influenza A and influenza B and additionally across influenza A subtypes. Similarly, HA1 C-terminal stem segments also share sequence identity between influenza A and influenza B and additionally across influenza A subtypes. Further, HA2 domains also share sequence identity between influenza A and influenza B and additionally across influenza A subtypes.

[00129] In some embodiments, the influenza hemagglutinin stem domain polypeptide is a hybrid polypeptide that comprises or consists essentially of segments and/or domains from a plurality of influenza strains or subtypes. For example, an influenza hemagglutinin stem domain polypeptide might comprise HA1 N-terminal and HA1 C-terminal stem segments from different influenza A virus HA subtypes. In some embodiments, the HA1 N-terminal stem segment is from influenza A virus while the HA1 C-terminal stem segment is from influenza B virus. Similarly, HA2 may also be from influenza A virus while the HA1 N-terminal and/or C-terminal stem segment is from influenza B virus.

[00130] It will be understood that any combination of the sequence elements listed in Tables 1-4 or the variants thereof may be used to form the hemagglutinin HA stem domain polypeptides of the present invention.

[00131] In an influenza stem domain polypeptide provided herein, a linker covalently connects the HA1 N-terminal stem segment to the HA1 C-terminal stem segment. In certain embodiments, the linker is a direct bond. In certain embodiments, the linker is a peptide that comprises one amino acid residue, two or fewer amino acid residues, three or fewer amino acid residues, four or fewer amino acid residues, five or fewer amino acid residues, ten or fewer amino acid residues, 15 or fewer amino acid residues, 20 or fewer amino acid residues, 30 or fewer amino acid residues, 40 or fewer amino acid residues, or 50 or fewer amino acid residues. In certain embodiments, the linker peptide comprises 50 or more amino acid residues. In certain embodiments the linker substantially lacks a globular head domain. In other words, the linker comprises no

more than 10, 9, 8, 7, 6, 5 or 4 contiguous, sequential amino acid residues from the amino acid sequence of an influenza globular head domain. In certain embodiments, the linker is other than Lys-Leu-Asn-Gly-Ser-Gly-Ile-Met-Lys-Thr-Glu-Gly-Thr-Leu-Glu-Asn (SEQ ID NO:311). In certain embodiments, the linker is other than Asn-Asn-Ile-Asp-Thr or Lys-Leu-Asn-Gly-Ser-Gly-Ile-Met-Lys-Thr-Glu-Gly-Thr-Leu-Glu-Asn (SEQ ID NO:312). In certain embodiments, the linker is other than Asn-Asn-Ile-Asp-Thr (SEQ ID NO:315).

[00132] In certain embodiments, the linker is covalently connected, at one end, to the C-terminus of the HA1 N-terminal stem segment. The linker peptide is also covalently connected, at the other end, to the N-terminus of the HA1 C-terminal stem segment. In certain embodiments, one of the covalent links is an amide bond. In certain embodiments, both covalent links are amide bonds.

[00133] The linker might be any linker deemed suitable by one of skill in the art. In certain embodiments, the linker is selected based on the HA1 N-terminal stem segment and the HA1 C-terminal stem segment. In these embodiments, the linker might be selected with molecular modeling programs such as InsightII and Quanta, both from Accelrys. In certain embodiments, the linker is a structural motif that allows structural alignment of the HA1 N-terminal stem segment and the HA1 C-terminal stem segment that is consistent with the structure of a hemagglutinin stem domain as recognized by those of skill in the art. In certain embodiments, the linker is selected from a library of candidate linkers. In certain embodiments, the library includes three dimensional polypeptide structures in a publicly available database such as the Protein Data Bank (PDB) or the Macromolecular Structure Database at the European Molecular Biology Laboratory (EMBL) or European Bioinformatics Institute (EBI). In certain embodiments, the library includes proprietary three-dimensional polypeptide structures associated with commercial programs such as InsightII and Quanta, both from Accelrys. Additionally, any databases or collections of protein structures or structural elements can be used to select the linker. Exemplary database or collections of protein structural elements include but are not limited to the Structural Classification of Proteins (SCOP, maintained by and available through Cambridge University); the database of protein families (Pfam, maintained by and available through the Wellcome Trust Sanger Institute); the Universal Protein Resource (UniProt, maintained by and available through the UniProt Consortium); the Integrated resource for protein families (InterPro; maintained by and available through EMBL-EBI); the Class Architecture Topology

Homologous superfamily (CATH, maintained by and available through Institute of Structural and Molecular Biology at the University College London); and the families of structurally similar proteins (FSSP, maintained by and available through EBI). Any algorithm deemed suitable by one of skill in the art may be used to select the linker, including but not limited by those used by SCOP, CATH and FSSP. Useful examples include but are not limited to Pymol (Delano Scientific LLC), InsightII and Quanta (both from Accelrys), MIDAS (University of California, San Francisco), SwissPDB viewer (Swiss Institute of Bioinformatics), TOPOFIT (Northeastern University), CBSU LOOPP (Cornell University), and SuperPose (University of Alberta, Edmonton).

[00134] In certain embodiments, the linker is a direct bond. In certain embodiments, the linker is selected from the group consisting of Gly, Gly-Gly, Gly-Gly-Gly, Gly-Gly-Gly-Gly and Gly-Gly-Gly-Gly-Gly. In certain embodiments, the linker is selected from the group consisting of Gly-Pro and Pro-Gly. In certain embodiments, the linker is a 281 turn loop, *e.g.* having the sequence ITPNGSIPNDKPFQNVNKITYGA (SEQ ID NO:165).

[00135] In certain embodiments the linker comprises a glycosylation sequence. In certain embodiments, the linker comprises an amino acid sequence according to Asn-Xaa-Ser/Thr where Xaa is any amino acid other than proline and Ser/Thr is serine or threonine. In certain embodiments, the linker comprises the amino acid sequence Asn-Ala-Ser. In certain embodiments the linker is a glycosylation sequence. In certain embodiments, the linker is an amino acid sequence according to Asn-Xaa-Ser/Thr where Xaa is any amino acid other than proline and Ser/Thr is serine or threonine. In certain embodiments, the linker is the amino acid sequence Asn-Ala-Ser.

[00136] In certain embodiments, influenza hemagglutinin stem domain polypeptides are capable of forming a three dimensional structure that is similar to the three dimensional structure of the stem domain of a native influenza hemagglutinin.

Structural similarity might be evaluated based on any technique deemed suitable by those of skill in the art. For instance, reaction, *e.g.* under non-denaturing conditions, of an influenza hemagglutinin stem domain polypeptide with a neutralizing antibody or antiserum that recognizes a native influenza hemagglutinin might indicate structural similarity. Useful neutralizing antibodies or antisera are described in, *e.g.*, Sui, *et al.*, 2009, *Nat. Struct. Mol. Biol.* 16(3):265-273, Ekiert *et al.*, February 26, 2009, *Science* [DOI: 10.1126/science.1171491], Wang *et al.* (2010) "Broadly Protective Monoclonal Antibodies against H3 Influenza Viruses following Sequential Immunization with

Different Hemagglutinins,” PLOS Pathogens 6(2):1-9, and Kashyap *et al.*, 2008, *Proc. Natl. Acad. Sci. USA* 105(16):5986-5991, the contents of which are hereby incorporated by reference in their entireties. In certain embodiments, the antibody or antiserum is an antibody or antiserum that reacts with a non-contiguous epitope (*i.e.*, not contiguous in primary sequence) that is formed by the tertiary or quaternary structure of a hemagglutinin.

[00137] In certain embodiments, structural similarity might be assessed by spectroscopic techniques such as circular dichroism, Raman spectroscopy, NMR, 3D NMR and X-ray crystallography. Known influenza hemagglutinin structures determined by X-ray crystallography are described in structural coordinates in Protein Data Bank files including but not limited to 1HGJ (an HA H3N2 strain) and 1RUZ (an HA H1N1 strain).

[00138] In certain embodiments, structural similarity is evaluated by RMS deviation between corresponding superimposed portions of two structures. In order to create a meaningful superimposition, in certain embodiments the coordinates of at least 20 corresponding atoms, 25 corresponding atoms, 30 corresponding atoms, 40 corresponding atoms, 50 corresponding atoms, 60 corresponding atoms, 70 corresponding atoms, 80 corresponding atoms, 90 corresponding atoms, 100 corresponding atoms, 120 corresponding atoms, 150 corresponding atoms, 200 corresponding atoms, or 250 corresponding atoms are used to calculate an RMS deviation.

[00139] In certain embodiments, the coordinates of all corresponding atoms in amino acid backbones are used to calculate an RMS deviation. In certain embodiments, the coordinates of all corresponding alpha carbon-atoms in the amino acid backbones are used to calculate an RMS deviation. In certain embodiments, the coordinates of all corresponding identical residues, including side chains, are used to calculate an RMS deviation.

[00140] In certain embodiments, coordinates of all or a portion of the corresponding atoms in a HA1 N-terminal segment are used to calculate an RMS deviation. In certain embodiments, coordinates of all or a portion of the corresponding atoms in a HA1 C-terminal segment are used to calculate an RMS deviation. In certain embodiments, coordinates of all or a portion of the corresponding atoms in both a HA1 N-terminal segment and a C-terminal segment are used to calculate an RMS deviation. In certain

embodiments, coordinates of all or a portion of corresponding atoms in HA2 domains are used to calculate an RMS deviation.

[00141] In certain embodiments, the RMS deviation between the structures of a influenza hemagglutinin stem domain polypeptide and corresponding portions of a known influenza A virus hemagglutinin stem domain (*e.g.*, from 1HGJ or 1RUZ) is 5 Å or less, 4 Å or less, 3 Å or less, 2.5 Å or less, 2 Å or less, 1.5 Å or less, 1 Å or less, 0.75 Å or less, 0.5 Å or less, 0.3 Å or less, 0.2 Å or less, or 0.1 Å or less. Commercially available or open source software might be used to perform the structural superimpositions and/or RMS deviation calculations. Useful examples include but are not limited to Pymol (Delano Scientific LLC), InsightII and Quanta (both from Accelrys), MIDAS (University of California, San Francisco), SwissPDB viewer (Swiss Institute of Bioinformatics), TOPOFIT (Northeastern University), CBSU LOOPP (Cornell University), and SuperPose (University of Alberta, Edmonton).

[00142] In certain embodiments, any influenza hemagglutinin stem domain polypeptide provided herein can further comprise one or more polypeptide domains deemed suitable to those of skill in the art. Useful polypeptide domains include domains that facilitate purification, folding and cleavage of portions of a polypeptide. For example, a His tag (His-His-His-His-His-His, SEQ ID NO:166), FLAG epitope or other purification tag can facilitate purification of a polypeptide provided herein. A foldon, or trimerization, domain from bacteriophage T4 fibrin can facilitate trimerization of polypeptides provided herein. The foldon domain can have any foldon sequence known to those of skill in the art (*see, e.g.*, Papanikolopoulou *et al.*, 2004, *J. Biol. Chem.* 279(10):8991-8998, the contents of which are hereby incorporated by reference in their entirety. Examples include GSGYIPEAPRDGQAYVRKDGEWVLLSTFL (SEQ ID NO:167). A foldon domain can be useful to facilitate trimerization of soluble polypeptides provided herein. Cleavage sites can be used to facilitate cleavage of a portion of a polypeptide, for example cleavage of a purification tag or foldon domain or both. Useful cleavage sites include a thrombin cleavage site, for example one with the sequence LVPRGSP (SEQ ID NO:168).

[00143] In certain embodiments, provided are influenza hemagglutinin stem domain polypeptides comprising an elastase cleavage site. Those of skill in the art will recognize that the trypsin cleavage site at the linkage between HA1 and HA2 can be mutated to an elastase cleavage site by substituting valine for the arginine or lysine at the HA1-HA2 cleavage site in a hemagglutinin sequence (*see, e.g.*, Stech *et al.*, 2005,

Nature Med. 11(6):683-689). Accordingly, provided herein are influenza hemagglutinin stem domain polypeptides having a valine substitution at the C-terminus of the C-terminal stem segment (i.e., the C-terminus of the HA1 domain). In particular embodiments, provided herein are influenza hemagglutinin stem domain polypeptides comprising any of SEQ ID NOS:50-65 or 158-159 wherein the C-terminal amino acid residue, *e.g.* arginine or lysine, of SEQ ID NOS:50-65 or 158-159 is substituted with a valine residue.

[00144] In certain embodiments, provided herein are influenza hemagglutinin stem domain polypeptides that are predicted to be resistant to protease cleavage at the junction between HA1 and HA2. Those of skill in the art should recognize that the Arg-Gly sequence spanning HA1 and HA2 is a recognition site for trypsin and is typically cleaved for hemagglutinin activation. Since the stem domain polypeptides described herein need not be activated, provided herein are influenza hemagglutinin stem domain polypeptides that are predicted to be resistant to protease cleavage. In certain embodiments, provided is any influenza hemagglutinin stem domain polypeptide described herein wherein the protease site spanning HA1 and HA2 is mutated to a sequence that is resistant to protease cleavage. In certain embodiments, provided is any influenza hemagglutinin stem domain polypeptide described herein wherein the C-terminal residue of the HA1 C-terminal stem segment is any residue other than Lys or Arg. In certain embodiments, provided is any influenza hemagglutinin stem domain polypeptide described herein wherein the N-terminal residue of the HA2 domain is proline. In certain embodiments, provided is any influenza hemagglutinin stem domain polypeptide described herein wherein the C-terminal residue of the HA1 C-terminal stem segment is Ala and the N-terminal residue of the HA2 domain is also Ala.

[00145] In certain embodiments, provided herein are influenza hemagglutinin stem domain polypeptides consisting of an HA1 N-terminal stem segment covalently linked to a linker, in turn covalently linked to an HA1 C-terminal stem segment in binding association with an HA2 stem domain. In certain embodiments, provided herein are influenza hemagglutinin stem domain polypeptides consisting of an HA1 N-terminal stem segment covalently linked to a linker, in turn covalently linked to an HA1 C-terminal stem segment, in turn covalently linked to an HA2 stem domain. In certain embodiments, provided herein are influenza hemagglutinin stem domain polypeptides consisting of a signal peptide covalently linked to an HA1 N-terminal stem segment

covalently linked to a linker, in turn covalently linked to an HA1 C-terminal stem segment, in turn covalently linked to an HA2 stem domain.

[00146] In certain embodiments, provided herein are influenza hemagglutinin stem domain polypeptides consisting of an HA1 N-terminal stem segment covalently linked to a linker, in turn covalently linked to an HA1 C-terminal stem segment in binding association with an HA2 stem domain that is covalently linked to an HA2 luminal domain. In certain embodiments, provided herein are influenza hemagglutinin stem domain polypeptides consisting of an HA1 N-terminal stem segment covalently linked to a linker, in turn covalently linked to an HA1 C-terminal stem segment, in turn covalently linked to an HA2 stem domain that is covalently linked to an HA2 luminal domain. In certain embodiments, provided herein are influenza hemagglutinin stem domain polypeptides consisting of a signal peptide covalently linked to an HA1 N-terminal stem segment covalently linked to a linker, in turn covalently linked to an HA1 C-terminal stem segment, in turn covalently linked to an HA2 stem domain that is covalently linked to an HA2 luminal domain.

[00147] In certain embodiments, provided herein are influenza hemagglutinin stem domain polypeptides consisting of an HA1 N-terminal stem segment covalently linked to a linker, in turn covalently linked to an HA1 C-terminal stem segment in binding association with an HA2 stem domain that is covalently linked to, in sequence, a thrombin cleavage site, a foldon domain and a His tag. In certain embodiments, provided herein are influenza hemagglutinin stem domain polypeptides consisting of an HA1 N-terminal stem segment covalently linked to a linker, in turn covalently linked to an HA1 C-terminal stem segment, in turn covalently linked to an HA2 stem domain that is covalently linked to, in sequence, a thrombin cleavage site, a foldon domain and a His tag. In certain embodiments, provided herein are influenza hemagglutinin stem domain polypeptides consisting of a signal peptide covalently linked to an HA1 N-terminal stem segment covalently linked to a linker, in turn covalently linked to an HA1 C-terminal stem segment, in turn covalently linked to an HA2 stem domain that is covalently linked to, in sequence, a thrombin cleavage site, a foldon domain and a His tag.

[00148] In certain embodiments, provided herein are influenza hemagglutinin stem domain polypeptides consisting of an HA1 N-terminal stem segment covalently linked to a linker, in turn covalently linked to an HA1 C-terminal stem segment in binding association with an HA2 stem domain that is covalently linked to an HA2 luminal domain that is covalently linked to, in sequence, a thrombin cleavage site, a foldon

domain and a His tag. In certain embodiments, provided herein are influenza hemagglutinin stem domain polypeptides consisting of an HA1 N-terminal stem segment covalently linked to a linker, in turn covalently linked to an HA1 C-terminal stem segment, in turn covalently linked to an HA2 stem domain that is covalently linked to an HA2 luminal domain that is covalently linked to, in sequence, a thrombin cleavage site, a foldon domain and a His tag. In certain embodiments, provided herein are influenza hemagglutinin stem domain polypeptides consisting of a signal peptide covalently linked to an HA1 N-terminal stem segment covalently linked to a linker, in turn covalently linked to an HA1 C-terminal stem segment, in turn covalently linked to an HA2 stem domain that is covalently linked to an HA2 luminal domain that is covalently linked to, in sequence, a thrombin cleavage site, a foldon domain and a His tag.

[00149] In certain embodiments, provided herein are influenza hemagglutinin stem domain polypeptides consisting of an HA1 N-terminal stem segment covalently linked to a linker, in turn covalently linked to an HA1 C-terminal stem segment in binding association with an HA2 stem domain that is covalently linked to an HA2 luminal domain that is in turn covalently linked to an HA2 transmembrane domain. In certain embodiments, provided herein are influenza hemagglutinin stem domain polypeptides consisting of an HA1 N-terminal stem segment covalently linked to a linker, in turn covalently linked to an HA1 C-terminal stem segment, in turn covalently linked to an HA2 stem domain that is covalently linked to an HA2 luminal domain that is in turn covalently linked to an HA2 transmembrane domain. In certain embodiments, provided herein are influenza hemagglutinin stem domain polypeptides consisting of a signal peptide covalently linked to an HA1 N-terminal stem segment covalently linked to a linker, in turn covalently linked to an HA1 C-terminal stem segment, in turn covalently linked to an HA2 stem domain that is covalently linked to an HA2 luminal domain that is in turn covalently linked to an HA2 transmembrane domain.

[00150] In certain embodiments, provided herein are influenza hemagglutinin stem domain polypeptides consisting of an HA1 N-terminal stem segment covalently linked to a linker, in turn covalently linked to an HA1 C-terminal stem segment in binding association with an HA2 stem domain that is covalently linked to an HA2 luminal domain that is in turn covalently linked to an HA2 transmembrane domain that is in turn covalently linked to an HA2 cytoplasmic domain. In certain embodiments, provided herein are influenza hemagglutinin stem domain polypeptides consisting of an HA1 N-

terminal stem segment covalently linked to a linker, in turn covalently linked to an HA1 C-terminal stem segment, in turn covalently linked to an HA2 stem domain that is covalently linked to an HA2 luminal domain that is in turn covalently linked to an HA2 transmembrane domain that is in turn covalently linked to an HA2 cytoplasmic domain. In certain embodiments, provided herein are influenza hemagglutinin stem domain polypeptides consisting of a signal peptide covalently linked to an HA1 N-terminal stem segment covalently linked to a linker, in turn covalently linked to an HA1 C-terminal stem segment, in turn covalently linked to an HA2 stem domain that is covalently linked to an HA2 luminal domain that is in turn covalently linked to an HA2 transmembrane domain that is in turn covalently linked to an HA2 cytoplasmic domain.

[00151] In certain embodiments, provided herein is an influenza hemagglutinin stem domain polypeptide having a sequence selected from the group consisting of:

(SEQ ID NO:34)-LL-(SEQ ID NO:50)-(SEQ ID NO:66),
 (SEQ ID NO:35)-LL-(SEQ ID NO:51)-(SEQ ID NO:67),
 (SEQ ID NO:36)-LL-(SEQ ID NO:52)-(SEQ ID NO:68),
 (SEQ ID NO:37)-LL-(SEQ ID NO:53)-(SEQ ID NO:69),
 (SEQ ID NO:38)-LL-(SEQ ID NO:54)-(SEQ ID NO:70),
 (SEQ ID NO:39)-LL-(SEQ ID NO:55)-(SEQ ID NO:71),
 (SEQ ID NO:40)-LL-(SEQ ID NO:56)-(SEQ ID NO:72),
 (SEQ ID NO:41)-LL-(SEQ ID NO:57)-(SEQ ID NO:73),
 (SEQ ID NO:42)-LL-(SEQ ID NO:58)-(SEQ ID NO:74),
 (SEQ ID NO:43)-LL-(SEQ ID NO:59)-(SEQ ID NO:75),
 (SEQ ID NO:44)-LL-(SEQ ID NO:60)-(SEQ ID NO:76),
 (SEQ ID NO:45)-LL-(SEQ ID NO:61)-(SEQ ID NO:77),
 (SEQ ID NO:46)-LL-(SEQ ID NO:62)-(SEQ ID NO:78),
 (SEQ ID NO:47)-LL-(SEQ ID NO:63)-(SEQ ID NO:79),
 (SEQ ID NO:48)-LL-(SEQ ID NO:64)-(SEQ ID NO:80), and
 (SEQ ID NO:49)-LL-(SEQ ID NO:65)-(SEQ ID NO:81),

wherein each sequence above is linked to the adjacent sequence as described herein and wherein LL is a linker as described herein. In particular, the HA1 C-terminal segments can be covalently or non-covalently linked to the HA2 domains. In certain embodiments, LL is selected from the group consisting of a direct bond, Gly, Gly-Gly, Gly-Gly-Gly, Gly-Gly-Gly-Gly, (Gly)_n (wherein *n* is any number of Glycine residues so long as there is flexibility in the peptide linker; in certain embodiments, *n* is 2, 3, 4, 5, 6,

or 7 Glycine residues), Gly-Pro, ITPNGSIPNDKPFQNVNKITYGA (SEQ ID NO:165) and Asn-Ala-Ser.

[00152] In certain embodiments, provided herein is an influenza hemagglutinin stem domain polypeptide having a sequence selected from the group consisting of:

(SEQ ID NO:34)-LL-(SEQ ID NO:50)-(SEQ ID NO:82),
 (SEQ ID NO:35)-LL-(SEQ ID NO:51)-(SEQ ID NO:83),
 (SEQ ID NO:36)-LL-(SEQ ID NO:52)-(SEQ ID NO:84),
 (SEQ ID NO:37)-LL-(SEQ ID NO:53)-(SEQ ID NO:85),
 (SEQ ID NO:38)-LL-(SEQ ID NO:54)-(SEQ ID NO:86),
 (SEQ ID NO:39)-LL-(SEQ ID NO:55)-(SEQ ID NO:87),
 (SEQ ID NO:40)-LL-(SEQ ID NO:56)-(SEQ ID NO:88),
 (SEQ ID NO:41)-LL-(SEQ ID NO:57)-(SEQ ID NO:89),
 (SEQ ID NO:42)-LL-(SEQ ID NO:58)-(SEQ ID NO:90),
 (SEQ ID NO:43)-LL-(SEQ ID NO:59)-(SEQ ID NO:91),
 (SEQ ID NO:44)-LL-(SEQ ID NO:60)-(SEQ ID NO:92),
 (SEQ ID NO:45)-LL-(SEQ ID NO:61)-(SEQ ID NO:93),
 (SEQ ID NO:46)-LL-(SEQ ID NO:62)-(SEQ ID NO:94),
 (SEQ ID NO:47)-LL-(SEQ ID NO:63)-(SEQ ID NO:95),
 (SEQ ID NO:48)-LL-(SEQ ID NO:64)-(SEQ ID NO:96), and
 (SEQ ID NO:49)-LL-(SEQ ID NO:65)-(SEQ ID NO:97),

wherein each sequence above is linked to the adjacent sequence as described herein and wherein LL is a linker as described herein. In particular, the HA1 C-terminal segments can be covalently or non-covalently linked to the HA2 domains. In certain embodiments, LL is selected from the group consisting of a direct bond, Gly, Gly-Gly, Gly-Gly-Gly, Gly-Gly-Gly-Gly, (Gly)_n, Gly-Pro, ITPNGSIPNDKPFQNVNKITYGA (SEQ ID NO:165) and Asn-Ala-Ser.

[00153] In certain embodiments, provided herein is an influenza hemagglutinin stem domain polypeptide having a sequence selected from the group consisting of:

(SEQ ID NO:34)-LL-(SEQ ID NO:50)-(SEQ ID NO:82)-(SEQ ID NO:98),
 (SEQ ID NO:35)-LL-(SEQ ID NO:51)-(SEQ ID NO:83)-(SEQ ID NO:99),
 (SEQ ID NO:36)-LL-(SEQ ID NO:52)-(SEQ ID NO:84)-(SEQ ID NO:100),
 (SEQ ID NO:37)-LL-(SEQ ID NO:53)-(SEQ ID NO:85)-(SEQ ID NO:101),
 (SEQ ID NO:38)-LL-(SEQ ID NO:54)-(SEQ ID NO:86)-(SEQ ID NO:102),
 (SEQ ID NO:39)-LL-(SEQ ID NO:55)-(SEQ ID NO:87)-(SEQ ID NO:103),

(SEQ ID NO:40)-LL-(SEQ ID NO:56)-(SEQ ID NO:88)-(SEQ ID NO:104),
 (SEQ ID NO:41)-LL-(SEQ ID NO:57)-(SEQ ID NO:89)-(SEQ ID NO:105),
 (SEQ ID NO:42)-LL-(SEQ ID NO:58)-(SEQ ID NO:90)-(SEQ ID NO:106),
 (SEQ ID NO:43)-LL-(SEQ ID NO:59)-(SEQ ID NO:91)-(SEQ ID NO:107),
 (SEQ ID NO:44)-LL-(SEQ ID NO:60)-(SEQ ID NO:92)-(SEQ ID NO:108),
 (SEQ ID NO:45)-LL-(SEQ ID NO:61)-(SEQ ID NO:93)-(SEQ ID NO:109),
 (SEQ ID NO:46)-LL-(SEQ ID NO:62)-(SEQ ID NO:94)-(SEQ ID NO:110),
 (SEQ ID NO:47)-LL-(SEQ ID NO:63)-(SEQ ID NO:95)-(SEQ ID NO:111),
 (SEQ ID NO:48)-LL-(SEQ ID NO:64)-(SEQ ID NO:96)-(SEQ ID NO:112), and
 (SEQ ID NO:49)-LL-(SEQ ID NO:65)-(SEQ ID NO:97)-(SEQ ID NO:113),

wherein each sequence above is linked to the adjacent sequence as described herein and wherein LL is a linker as described herein. In particular, the HA1 C-terminal segments can be covalently or non-covalently linked to the HA2 domains. In certain embodiments, LL is selected from the group consisting of a direct bond, Gly, Gly-Gly, Gly-Gly-Gly, Gly-Gly-Gly-Gly, (Gly)_n, Gly-Pro, ITPNGSIPNDKPFQNVNKITYGA (SEQ ID NO:165) and Asn-Ala-Ser.

[00154] In certain embodiments, provided herein is an influenza hemagglutinin stem domain polypeptide having a sequence selected from the group consisting of:

(SEQ ID NO:34)-LL-(SEQ ID NO:50)-(SEQ ID NO:82)-(SEQ ID NO:168)-(SEQ ID NO:167)-(SEQ ID NO:166),
 (SEQ ID NO:35)-LL-(SEQ ID NO:51)-(SEQ ID NO:83)-(SEQ ID NO:168)-(SEQ ID NO:167)-(SEQ ID NO:166),
 (SEQ ID NO:36)-LL-(SEQ ID NO:52)-(SEQ ID NO:84)-(SEQ ID NO:168)-(SEQ ID NO:167)-(SEQ ID NO:166),
 (SEQ ID NO:37)-LL-(SEQ ID NO:53)-(SEQ ID NO:85)-(SEQ ID NO:168)-(SEQ ID NO:167)-(SEQ ID NO:166),
 (SEQ ID NO:38)-LL-(SEQ ID NO:54)-(SEQ ID NO:86)-(SEQ ID NO:168)-(SEQ ID NO:167)-(SEQ ID NO:166),
 (SEQ ID NO:39)-LL-(SEQ ID NO:55)-(SEQ ID NO:87)-(SEQ ID NO:168)-(SEQ ID NO:167)-(SEQ ID NO:166),
 (SEQ ID NO:40)-LL-(SEQ ID NO:56)-(SEQ ID NO:88)-(SEQ ID NO:168)-(SEQ ID NO:167)-(SEQ ID NO:166),
 (SEQ ID NO:41)-LL-(SEQ ID NO:57)-(SEQ ID NO:89)-(SEQ ID NO:168)-(SEQ ID NO:167)-(SEQ ID NO:166),

(SEQ ID NO:42)-LL-(SEQ ID NO:58)-(SEQ ID NO:90)-(SEQ ID NO:168)-(SEQ ID NO:167)-(SEQ ID NO:166),

(SEQ ID NO:43)-LL-(SEQ ID NO:59)-(SEQ ID NO:91)-(SEQ ID NO:168)-(SEQ ID NO:167)-(SEQ ID NO:166),

(SEQ ID NO:44)-LL-(SEQ ID NO:60)-(SEQ ID NO:92)-(SEQ ID NO:168)-(SEQ ID NO:167)-(SEQ ID NO:166),

(SEQ ID NO:45)-LL-(SEQ ID NO:61)-(SEQ ID NO:93)-(SEQ ID NO:168)-(SEQ ID NO:167)-(SEQ ID NO:166),

(SEQ ID NO:46)-LL-(SEQ ID NO:62)-(SEQ ID NO:94)-(SEQ ID NO:168)-(SEQ ID NO:167)-(SEQ ID NO:166),

(SEQ ID NO:47)-LL-(SEQ ID NO:63)-(SEQ ID NO:95)-(SEQ ID NO:168)-(SEQ ID NO:167)-(SEQ ID NO:166),

(SEQ ID NO:48)-LL-(SEQ ID NO:64)-(SEQ ID NO:96)-(SEQ ID NO:168)-(SEQ ID NO:167)-(SEQ ID NO:166), and

(SEQ ID NO:49)-LL-(SEQ ID NO:65)-(SEQ ID NO:97)-(SEQ ID NO:168)-(SEQ ID NO:167)-(SEQ ID NO:166),

wherein each sequence above is linked to the adjacent sequence as described herein and wherein LL is a linker as described herein. In particular, the HA1 C-terminal segments can be covalently or non-covalently linked to the HA2 domains. In certain embodiments, LL is selected from the group consisting of a direct bond, Gly, Gly-Gly, Gly-Gly-Gly, Gly-Gly-Gly-Gly, (Gly)_n, Gly-Pro, ITPNGSIPNDKPFQNVNKITYGA (SEQ ID NO:165) and Asn-Ala-Ser.

[00155] In certain embodiments, provided herein is an influenza hemagglutinin stem domain polypeptide having a sequence selected from the group consisting of:

(SEQ ID NO:34)-LL-(SEQ ID NO:50)-(SEQ ID NO:82)-(SEQ ID NO:98)-(SEQ ID NO:168)-(SEQ ID NO:167)-(SEQ ID NO:166),

(SEQ ID NO:35)-LL-(SEQ ID NO:51)-(SEQ ID NO:83)-(SEQ ID NO:99)-(SEQ ID NO:168)-(SEQ ID NO:167)-(SEQ ID NO:166),

(SEQ ID NO:36)-LL-(SEQ ID NO:52)-(SEQ ID NO:84)-(SEQ ID NO:100)-(SEQ ID NO:168)-(SEQ ID NO:167)-(SEQ ID NO:166),

(SEQ ID NO:37)-LL-(SEQ ID NO:53)-(SEQ ID NO:85)-(SEQ ID NO:101)-(SEQ ID NO:168)-(SEQ ID NO:167)-(SEQ ID NO:166),

(SEQ ID NO:38)-LL-(SEQ ID NO:54)-(SEQ ID NO:86)-(SEQ ID NO:102)-(SEQ ID NO:168)-(SEQ ID NO:167)-(SEQ ID NO:166),

(SEQ ID NO:39)-LL-(SEQ ID NO:55)-(SEQ ID NO:87)-(SEQ ID NO:103)-(SEQ ID NO:168)-(SEQ ID NO:167)-(SEQ ID NO:166),
 (SEQ ID NO:40)-LL-(SEQ ID NO:56)-(SEQ ID NO:88)-(SEQ ID NO:104)-(SEQ ID NO:168)-(SEQ ID NO:167)-(SEQ ID NO:166),
 (SEQ ID NO:41)-LL-(SEQ ID NO:57)-(SEQ ID NO:89)-(SEQ ID NO:105)-(SEQ ID NO:168)-(SEQ ID NO:167)-(SEQ ID NO:166),
 (SEQ ID NO:42)-LL-(SEQ ID NO:58)-(SEQ ID NO:90)-(SEQ ID NO:106)-(SEQ ID NO:168)-(SEQ ID NO:167)-(SEQ ID NO:166),
 (SEQ ID NO:43)-LL-(SEQ ID NO:59)-(SEQ ID NO:91)-(SEQ ID NO:107)-(SEQ ID NO:168)-(SEQ ID NO:167)-(SEQ ID NO:166),
 (SEQ ID NO:44)-LL-(SEQ ID NO:60)-(SEQ ID NO:92)-(SEQ ID NO:108)-(SEQ ID NO:168)-(SEQ ID NO:167)-(SEQ ID NO:166),
 (SEQ ID NO:45)-LL-(SEQ ID NO:61)-(SEQ ID NO:93)-(SEQ ID NO:109)-(SEQ ID NO:168)-(SEQ ID NO:167)-(SEQ ID NO:166),
 (SEQ ID NO:46)-LL-(SEQ ID NO:62)-(SEQ ID NO:94)-(SEQ ID NO:110)-(SEQ ID NO:168)-(SEQ ID NO:167)-(SEQ ID NO:166),
 (SEQ ID NO:47)-LL-(SEQ ID NO:63)-(SEQ ID NO:95)-(SEQ ID NO:111)-(SEQ ID NO:168)-(SEQ ID NO:167)-(SEQ ID NO:166),
 (SEQ ID NO:48)-LL-(SEQ ID NO:64)-(SEQ ID NO:96)-(SEQ ID NO:112)-(SEQ ID NO:168)-(SEQ ID NO:167)-(SEQ ID NO:166), and
 (SEQ ID NO:49)-LL-(SEQ ID NO:65)-(SEQ ID NO:97)-(SEQ ID NO:113)-(SEQ ID NO:168)-(SEQ ID NO:167)-(SEQ ID NO:166),
 wherein each sequence above is linked to the adjacent sequence as described herein and wherein LL is a linker as described herein. In particular, the HA1 C-terminal segments can be covalently or non-covalently linked to the HA2 domains. In certain embodiments, LL is selected from the group consisting of a direct bond, Gly, Gly-Gly, Gly-Gly-Gly, Gly-Gly-Gly-Gly, Gly-Pro, ITPNGSIPNDKPFQNVNKITYGA (SEQ ID NO:165) and Asn-Ala-Ser.

[00156] In certain embodiments, provided herein is an influenza hemagglutinin stem domain polypeptide having a sequence selected from the group consisting of:

(SEQ ID NO:177)-LL-(SEQ ID NO:226)-(SEQ ID NO:66),
 (SEQ ID NO:178)-LL-(SEQ ID NO:227)-(SEQ ID NO:66),
 (SEQ ID NO:179)-LL-(SEQ ID NO:228)-(SEQ ID NO:66),
 (SEQ ID NO:180)-LL-(SEQ ID NO:229)-(SEQ ID NO:67),

(SEQ ID NO:181)-LL-(SEQ ID NO:230)-(SEQ ID NO:67),
(SEQ ID NO:182)-LL-(SEQ ID NO:231)-(SEQ ID NO:67),
(SEQ ID NO:183)-LL-(SEQ ID NO:232)-(SEQ ID NO:68),
(SEQ ID NO:184)-LL-(SEQ ID NO:233)-(SEQ ID NO:68),
(SEQ ID NO:185)-LL-(SEQ ID NO:234)-(SEQ ID NO:68),
(SEQ ID NO:186)-LL-(SEQ ID NO:235)-(SEQ ID NO:69),
(SEQ ID NO:187)-LL-(SEQ ID NO:236)-(SEQ ID NO:69),
(SEQ ID NO:188)-LL-(SEQ ID NO:237)-(SEQ ID NO:69),
(SEQ ID NO:189)-LL-(SEQ ID NO:238)-(SEQ ID NO:70),
(SEQ ID NO:190)-LL-(SEQ ID NO:239)-(SEQ ID NO:70),
(SEQ ID NO:191)-LL-(SEQ ID NO:240)-(SEQ ID NO:70),
(SEQ ID NO:192)-LL-(SEQ ID NO:241)-(SEQ ID NO:71),
(SEQ ID NO:193)-LL-(SEQ ID NO:242)-(SEQ ID NO:71),
(SEQ ID NO:194)-LL-(SEQ ID NO:243)-(SEQ ID NO:71),
(SEQ ID NO:195)-LL-(SEQ ID NO:244)-(SEQ ID NO:72),
(SEQ ID NO:196)-LL-(SEQ ID NO:245)-(SEQ ID NO:72),
(SEQ ID NO:197)-LL-(SEQ ID NO:246)-(SEQ ID NO:72),
(SEQ ID NO:198)-LL-(SEQ ID NO:247)-(SEQ ID NO:73),
(SEQ ID NO:199)-LL-(SEQ ID NO:248)-(SEQ ID NO:73),
(SEQ ID NO:200)-LL-(SEQ ID NO:249)-(SEQ ID NO:73),
(SEQ ID NO:201)-LL-(SEQ ID NO:250)-(SEQ ID NO:74),
(SEQ ID NO:202)-LL-(SEQ ID NO:251)-(SEQ ID NO:74),
(SEQ ID NO:203)-LL-(SEQ ID NO:252)-(SEQ ID NO:74),
(SEQ ID NO:204)-LL-(SEQ ID NO:253)-(SEQ ID NO:75),
(SEQ ID NO:205)-LL-(SEQ ID NO:254)-(SEQ ID NO:75),
(SEQ ID NO:206)-LL-(SEQ ID NO:255)-(SEQ ID NO:75),
(SEQ ID NO:207)-LL-(SEQ ID NO:256)-(SEQ ID NO:76),
(SEQ ID NO:208)-LL-(SEQ ID NO:257)-(SEQ ID NO:76),
(SEQ ID NO:209)-LL-(SEQ ID NO:258)-(SEQ ID NO:76),
(SEQ ID NO:210)-LL-(SEQ ID NO:259)-(SEQ ID NO:77),
(SEQ ID NO:211)-LL-(SEQ ID NO:260)-(SEQ ID NO:77),
(SEQ ID NO:212)-LL-(SEQ ID NO:261)-(SEQ ID NO:77),
(SEQ ID NO:213)-LL-(SEQ ID NO:262)-(SEQ ID NO:78),
(SEQ ID NO:214)-LL-(SEQ ID NO:263)-(SEQ ID NO:78),

(SEQ ID NO:215)-LL-(SEQ ID NO:264)-(SEQ ID NO:78),
 (SEQ ID NO:216)-LL-(SEQ ID NO:265)-(SEQ ID NO:79),
 (SEQ ID NO:217)-LL-(SEQ ID NO:266)-(SEQ ID NO:79),
 (SEQ ID NO:218)-LL-(SEQ ID NO:267)-(SEQ ID NO:79),
 (SEQ ID NO:219)-LL-(SEQ ID NO:268)-(SEQ ID NO:80),
 (SEQ ID NO:220)-LL-(SEQ ID NO:269)-(SEQ ID NO:80),
 (SEQ ID NO:221)-LL-(SEQ ID NO:270)-(SEQ ID NO:80),
 (SEQ ID NO:222)-LL-(SEQ ID NO:271)-(SEQ ID NO:81),
 (SEQ ID NO:223)-LL-(SEQ ID NO:272)-(SEQ ID NO:81),
 (SEQ ID NO:224)-LL-(SEQ ID NO:273)-(SEQ ID NO:81),
 (SEQ ID NO:309)-LL-(SEQ ID NO:310)-(SEQ ID NO:66), and
 (SEQ ID NO:308)-LL-(SEQ ID NO:52)-(SEQ ID NO:68),

(wherein each sequence above is linked to the adjacent sequence as described herein and wherein LL is a linker as described herein. In particular, the HA1 C-terminal segments can be covalently or non-covalently linked to the HA2 domains. In certain embodiments, LL is selected from the group consisting of a direct bond, Gly, Gly-Gly, Gly-Gly-Gly, Gly-Gly-Gly-Gly, (Gly)_n, Gly-Pro, ITPNGSIPNDKPFQNVNKITYGA (SEQ ID NO:165) and Asn-Ala-Ser.

[00157] In certain embodiments, provided herein is an influenza hemagglutinin stem domain polypeptide having a sequence selected from the group consisting of:

(SEQ ID NO:154)-LL-(SEQ ID NO:158)-(SEQ ID NO:160),
 (SEQ ID NO:155)-LL-(SEQ ID NO:159)-(SEQ ID NO:160),
 (SEQ ID NO:156)-LL-(SEQ ID NO:158)-(SEQ ID NO:160), and
 (SEQ ID NO:157)-LL-(SEQ ID NO:159)-(SEQ ID NO:160),

wherein each sequence above is linked to the adjacent sequence as described herein and wherein LL is a linker as described herein. In particular, the HA1 C-terminal segments can be covalently or non-covalently linked to the HA2 domains. In certain embodiments, LL is selected from the group consisting of a direct bond, Gly, Gly-Gly, Gly-Gly-Gly, Gly-Gly-Gly-Gly, (Gly)_n, Gly-Pro, ITPNGSIPNDKPFQNVNKITYGA (SEQ ID NO:165) and Asn-Ala-Ser.

[00158] In certain embodiments, provided herein is an influenza hemagglutinin stem domain polypeptide having a sequence selected from the group consisting of:

(SEQ ID NO:154)-LL-(SEQ ID NO:158)-(SEQ ID NO:161),
 (SEQ ID NO:155)-LL-(SEQ ID NO:159)-(SEQ ID NO:161),

(SEQ ID NO:156)-LL-(SEQ ID NO:158)-(SEQ ID NO:161), and

(SEQ ID NO:157)-LL-(SEQ ID NO:159)-(SEQ ID NO:161),

wherein each sequence above is linked to the adjacent sequence as described herein and wherein LL is a linker as described herein. In particular, the HA1 C-terminal segments can be covalently or non-covalently linked to the HA2 domains. In certain embodiments, LL is selected from the group consisting of a direct bond, Gly, Gly-Gly, Gly-Gly-Gly, Gly-Gly-Gly-Gly, (Gly)_n, Gly-Pro, ITPNGSIPNDKPFQNVNKITYGA (SEQ ID NO:165) and Asn-Ala-Ser.

[00159] In certain embodiments, provided herein is an influenza hemagglutinin stem domain polypeptide having a sequence selected from the group consisting of:

(SEQ ID NO:154)-LL-(SEQ ID NO:158)-(SEQ ID NO:161)-(SEQ ID NO:162),

(SEQ ID NO:155)-LL-(SEQ ID NO:159)-(SEQ ID NO:161)-(SEQ ID NO:162),

(SEQ ID NO:156)-LL-(SEQ ID NO:158)-(SEQ ID NO:161)-(SEQ ID NO:162), and

(SEQ ID NO:157)-LL-(SEQ ID NO:159)-(SEQ ID NO:161)-(SEQ ID NO:162),

wherein each sequence above is linked to the adjacent sequence as described herein and wherein LL is a linker as described herein. In particular, the HA1 C-terminal segments can be covalently or non-covalently linked to the HA2 domains. In certain embodiments, LL is selected from the group consisting of a direct bond, Gly, Gly-Gly, Gly-Gly-Gly, Gly-Gly-Gly-Gly, (Gly)_n, Gly-Pro, ITPNGSIPNDKPFQNVNKITYGA (SEQ ID NO:165) and Asn-Ala-Ser.

[00160] In certain embodiments, provided herein is an influenza hemagglutinin stem domain polypeptide having a sequence selected from the group consisting of:

(SEQ ID NO:154)-LL-(SEQ ID NO:158)-(SEQ ID NO:161)-(SEQ ID NO:168)-(SEQ ID NO:167)-SEQ ID NO:166),

(SEQ ID NO:155)-LL-(SEQ ID NO:159)-(SEQ ID NO:161)-(SEQ ID NO:168)-(SEQ ID NO:167)-SEQ ID NO:166),

(SEQ ID NO:156)-LL-(SEQ ID NO:158)-(SEQ ID NO:161)-(SEQ ID NO:168)-(SEQ ID NO:167)-SEQ ID NO:166), and

(SEQ ID NO:157)-LL-(SEQ ID NO:159)-(SEQ ID NO:161)-(SEQ ID NO:168)-(SEQ ID NO:167)-SEQ ID NO:166),

wherein each sequence above is linked to the adjacent sequence as described herein and wherein LL is a linker as described herein. In particular, the HA1 C-terminal segments can be covalently or non-covalently linked to the HA2 domains. In certain embodiments, LL is selected from the group consisting of a direct bond, Gly, Gly-Gly,

Gly-Gly-Gly, Gly-Gly-Gly-Gly, (Gly)_n, Gly-Pro, ITPNGSIPNDKPFQNVNKITYGA (SEQ ID NO:165) and Asn-Ala-Ser.

[00161] In certain embodiments, provided herein is an influenza hemagglutinin stem domain polypeptide having a sequence selected from the group consisting of:

(SEQ ID NO:154)-LL-(SEQ ID NO:158)-(SEQ ID NO:161)-(SEQ ID NO:162)-(SEQ ID NO:168)-(SEQ ID NO:167)-SEQ ID NO:166),

(SEQ ID NO:155)-LL-(SEQ ID NO:159)-(SEQ ID NO:161)-(SEQ ID NO:162)-(SEQ ID NO:168)-(SEQ ID NO:167)-SEQ ID NO:166),

(SEQ ID NO:156)-LL-(SEQ ID NO:158)-(SEQ ID NO:161)-(SEQ ID NO:162)-(SEQ ID NO:168)-(SEQ ID NO:167)-SEQ ID NO:166), and

(SEQ ID NO:157)-LL-(SEQ ID NO:159)-(SEQ ID NO:161)-(SEQ ID NO:162)-(SEQ ID NO:168)-(SEQ ID NO:167)-SEQ ID NO:166),

wherein each sequence above is linked to the adjacent sequence as described herein and wherein LL is a linker as described herein. In particular, the HA1 C-terminal segments can be covalently or non-covalently linked to the HA2 domains. In certain embodiments, LL is selected from the group consisting of a direct bond, Gly, Gly-Gly, Gly-Gly-Gly, Gly-Gly-Gly-Gly, (Gly)_n, Gly-Pro, ITPNGSIPNDKPFQNVNKITYGA (SEQ ID NO:165) and Asn-Ala-Ser.

[00162] In certain embodiments, the influenza hemagglutinin polypeptides described herein do not comprise polypeptides having the amino acid sequence of either Thr-Gly-Leu-Arg-Asn (SEQ ID NO:313) or Gly-Ile-Thr-Asn-Lys-Val-Asn-Ser-Val-Ile-Glu-Lys (SEQ ID NO:314). In certain embodiments, the influenza hemagglutinin polypeptides described herein do not comprise polypeptides having the amino acid sequence of Thr-Gly-Leu-Arg-Asn (SEQ ID NO:313) and Gly-Ile-Thr-Asn-Lys-Val-Asn-Ser-Val-Ile-Glu-Lys (SEQ ID NO:314). In certain embodiments, the influenza hemagglutinin polypeptides described herein do not comprise polypeptides having the amino acid sequence of either Thr-Gly-Met-Arg-Asn (SEQ ID NO:316) or Gln-Ile-Asn-Gly-Lys-Leu-Asn-Arg-Leu-Ile-Glu-Lys (SEQ ID NO:317). In certain embodiments, the influenza hemagglutinin polypeptides described herein do not comprise polypeptides having the amino acid sequence of Thr-Gly-Met-Arg-Asn (SEQ ID NO:316) and Gln-Ile-Asn-Gly-Lys-Leu-Asn-Arg-Leu-Ile-Glu-Lys (SEQ ID NO:317). In certain embodiments, the influenza hemagglutinin polypeptides described herein do not comprise polypeptides having the amino acid sequence of either Thr-Gly-Met-Arg-Asn (SEQ ID NO:316) or Gln-Ile-Asn-Gly-Lys-Leu-Asn-Arg-Val-Ile-Glu-Lys (SEQ ID

NO:318). In certain embodiments, the influenza hemagglutinin polypeptides described herein do not comprise polypeptides having the amino acid sequence of Thr-Gly-Met-Arg-Asn (SEQ ID NO:316) and Gln-Ile-Asn-Gly-Lys-Leu-Asn-Arg-Val-Ile-Glu-Lys (SEQ ID NO:318).

[00163] In certain embodiments, the influenza hemagglutinin polypeptides described herein are not recognized or bound by the antibody C179 (produced by hybridoma FERM BP-4517; clones sold by Takara Bio, Inc. (Otsu, Shiga, Japan)) or by the antibody AI3C (FERM BP-4516).

5.2 NUCLEIC ACIDS ENCODING INFLUENZA HEMAGGLUTININ STEM DOMAIN POLYPEPTIDES

[00164] Provided herein are nucleic acids that encode an influenza hemagglutinin stem domain polypeptide. In a specific embodiment, provided herein is a nucleic acid that encodes an influenza virus hemagglutinin stem domain polypeptide. Due to the degeneracy of the genetic code, any nucleic acid that encodes an influenza hemagglutinin stem domain polypeptide described herein is encompassed herein. In certain embodiments, nucleic acids corresponding to naturally occurring influenza virus nucleic acids encoding an HA1 N-terminal stem segment, an HA1 C-terminal stem segment, HA2 domain, luminal domain, transmembrane domain, and/or cytoplasmic domain are used to produce an influenza hemagglutinin stem domain polypeptide.

[00165] Also provided herein are nucleic acids capable of hybridizing to a nucleic acid encoding an influenza hemagglutinin stem domain polypeptide. In certain embodiments, provided herein are nucleic acids capable of hybridizing to a fragment of a nucleic acid encoding an influenza hemagglutinin stem domain polypeptide. In other embodiments, provided herein are nucleic acids capable of hybridizing to the full length of a nucleic acid encoding an influenza hemagglutinin stem domain polypeptide. General parameters for hybridization conditions for nucleic acids are described in Sambrook *et al.*, Molecular Cloning - A Laboratory Manual (2nd Ed.), Vols. 1-3, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York (1989), and in Ausubel *et al.*, Current Protocols in Molecular Biology, vol. 2, Current Protocols Publishing, New York (1994). Hybridization may be performed under high stringency conditions, medium stringency conditions, or low stringency conditions. Those of skill in the art will understand that low, medium and high stringency conditions are contingent upon multiple factors all of which interact and are also dependent upon the nucleic acids in

question. For example, high stringency conditions may include temperatures within 5°C melting temperature of the nucleic acid(s), a low salt concentration (e.g., less than 250 mM), and a high co-solvent concentration (e.g., 1-20% of co-solvent, e.g., DMSO). Low stringency conditions, on the other hand, may include temperatures greater than 10°C below the melting temperature of the nucleic acid(s), a high salt concentration (e.g., greater than 1000 mM) and the absence of co-solvents.

[00166] In some embodiments, a nucleic acid encoding an influenza virus hemagglutinin stem domain polypeptide is isolated. In certain embodiments, an “isolated” nucleic acid refers to a nucleic acid molecule which is separated from other nucleic acid molecules which are present in the natural source of the nucleic acid. In other words, the isolated nucleic acid can comprise heterologous nucleic acids that are not associated with it in nature. In other embodiments, an “isolated” nucleic acid, such as a cDNA molecule, can be substantially free of other cellular material, or culture medium when produced by recombinant techniques, or substantially free of chemical precursors or other chemicals when chemically synthesized. The term “substantially free of cellular material” includes preparations of nucleic acid in which the nucleic acid is separated from cellular components of the cells from which it is isolated or recombinantly produced. Thus, nucleic acid that is substantially free of cellular material includes preparations of nucleic acid having less than about 30%, 20%, 10%, or 5% (by dry weight) of other nucleic acids. The term “substantially free of culture medium” includes preparations of nucleic acid in which the culture medium represents less than about 50%, 20%, 10%, or 5% of the volume of the preparation. The term “substantially free of chemical precursors or other chemicals” includes preparations in which the nucleic acid is separated from chemical precursors or other chemicals which are involved in the synthesis of the nucleic acid. In specific embodiments, such preparations of the nucleic acid have less than about 50%, 30%, 20%, 10%, 5% (by dry weight) of chemical precursors or compounds other than the nucleic acid of interest.

[00167] In addition, provided herein are nucleic acids encoding the individual components of an influenza hemagglutinin stem domain polypeptide. In specific embodiments, nucleic acids encoding an HA1 N-terminal stem segment, an HA1 C-terminal stem segment and/or HA2 domain are provided. Nucleic acids encoding components of an influenza hemagglutinin stem domain polypeptide may be assembled using standard molecular biology techniques known to the one of skill in the art.

5.3 EXPRESSION OF INFLUENZA HEMAGGLUTININ STEM DOMAIN POLYPEPTIDES

[00168] Provided herein are vectors, including expression vectors, containing a nucleic acid encoding an influenza hemagglutinin stem domain polypeptide. In a specific embodiment, the vector is an expression vector that is capable of directing the expression of a nucleic acid encoding an influenza hemagglutinin stem domain polypeptide. Non-limiting examples of expression vectors include, but are not limited to, plasmids and viral vectors, such as replication defective retroviruses, adenoviruses, adeno-associated viruses and baculoviruses.

[00169] In some embodiments, provided herein are expression vectors encoding components of an influenza hemagglutinin stem domain polypeptide (*e.g.*, HA1 N-terminal stem segment, an HA1 C-terminal stem segment and/or an HA2). Such vectors may be used to express the components in one or more host cells and the components may be isolated and conjugated together with a linker using techniques known to one of skill in the art.

[00170] An expression vector comprises a nucleic acid encoding an influenza hemagglutinin stem domain polypeptide in a form suitable for expression of the nucleic acid in a host cell. In a specific embodiment, an expression vector includes one or more regulatory sequences, selected on the basis of the host cells to be used for expression, which is operably linked to the nucleic acid to be expressed. Within an expression vector, "operably linked" is intended to mean that a nucleic acid of interest is linked to the regulatory sequence(s) in a manner which allows for expression of the nucleic acid (*e.g.*, in an *in vitro* transcription/translation system or in a host cell when the vector is introduced into the host cell). Regulatory sequences include promoters, enhancers and other expression control elements (*e.g.*, polyadenylation signals). Regulatory sequences include those which direct constitutive expression of a nucleic acid in many types of host cells, those which direct expression of the nucleic acid only in certain host cells (*e.g.*, tissue-specific regulatory sequences), and those which direct the expression of the nucleic acid upon stimulation with a particular agent (*e.g.*, inducible regulatory sequences). It will be appreciated by those skilled in the art that the design of the expression vector can depend on such factors as the choice of the host cell to be transformed, the level of expression of protein desired, etc. The term "host cell" is intended to include a particular subject cell transformed or transfected with a nucleic acid and the progeny or potential progeny of such a cell. Progeny of such a cell may not

be identical to the parent cell transformed or transfected with the nucleic acid due to mutations or environmental influences that may occur in succeeding generations or integration of the nucleic acid into the host cell genome.

[00171] Expression vectors can be designed for expression of an influenza hemagglutinin stem domain polypeptide using prokaryotic (*e.g.*, *E. coli*) or eukaryotic cells (*e.g.*, insect cells (using baculovirus expression vectors, see, *e.g.*, Treanor et al., 2007, JAMA, 297(14):1577-1582 incorporated by reference herein in its entirety), yeast cells, plant cells, algae or mammalian cells). Examples of mammalian host cells include, but are not limited to, Crucell Per.C6 cells, Vero cells, CHO cells, VERY cells, BHK cells, HeLa cells, COS cells, MDCK cells, 293 cells, 3T3 cells or WI38 cells. In certain embodiments, the host cells are myeloma cells, *e.g.*, NS0 cells, 45.6 TG1.7 cells, AF-2 clone 9B5 cells, AF-2 clone 9B5 cells, J558L cells, MOPC 315 cells, MPC-11 cells, NCI-H929 cells, NP cells, NS0/1 cells, P3 NS1 Ag4 cells, P3/NS1/1-Ag4-1 cells, P3U1 cells, P3X63Ag8 cells, P3X63Ag8.653 cells, P3X63Ag8U.1 cells, RPMI 8226 cells, Sp20-Ag14 cells, U266B1 cells, X63AG8.653 cells, Y3.Ag.1.2.3 cells, and YO cells. Non-limiting examples of insect cells include *Sf9*, *Sf21*, *Trichoplusia ni*, *Spodoptera frugiperda* and *Bombyx mori*. In a particular embodiment, a mammalian cell culture system (*e.g.* Chinese hamster ovary or baby hamster kidney cells) is used for expression of an influenza hemagglutinin stem domain polypeptide. In another embodiment, a plant cell culture system is used for expression of an influenza hemagglutinin stem domain polypeptide. See, *e.g.*, U.S. Patent Nos. 7,504,560; 6,770,799; 6,551,820; 6,136,320; 6,034,298; 5,914,935; 5,612,487; and 5,484,719, and U.S. patent application publication Nos. 2009/0208477, 2009/0082548, 2009/0053762, 2008/0038232, 2007/0275014 and 2006/0204487 for plant cells and methods for the production of proteins utilizing plant cell culture systems.

[00172] An expression vector can be introduced into host cells via conventional transformation or transfection techniques. Such techniques include, but are not limited to, calcium phosphate or calcium chloride co-precipitation, DEAE-dextran-mediated transfection, lipofection, and electroporation. Suitable methods for transforming or transfecting host cells can be found in Sambrook *et al.*, 1989, Molecular Cloning - A Laboratory Manual, 2nd Edition, Cold Spring Harbor Press, New York, and other laboratory manuals. In certain embodiments, a host cell is transiently transfected with an expression vector containing a nucleic acid encoding an influenza hemagglutinin stem domain polypeptide. In other embodiments, a host cell is stably transfected with an

expression vector containing a nucleic acid encoding an influenza hemagglutinin stem domain polypeptide.

[00173] For stable transfection of mammalian cells, it is known that, depending upon the expression vector and transfection technique used, only a small fraction of cells may integrate the foreign DNA into their genome. In order to identify and select these integrants, a nucleic acid that encodes a selectable marker (*e.g.*, for resistance to antibiotics) is generally introduced into the host cells along with the nucleic acid of interest. Examples of selectable markers include those which confer resistance to drugs, such as G418, hygromycin and methotrexate. Cells stably transfected with the introduced nucleic acid can be identified by drug selection (*e.g.*, cells that have incorporated the selectable marker gene will survive, while the other cells die).

[00174] As an alternative to recombinant expression of an influenza hemagglutinin stem domain polypeptide using a host cell, an expression vector containing a nucleic acid encoding an influenza hemagglutinin stem domain polypeptide can be transcribed and translated *in vitro* using, *e.g.*, T7 promoter regulatory sequences and T7 polymerase. In a specific embodiment, a coupled transcription/translation system, such as Promega TNT®, or a cell lysate or cell extract comprising the components necessary for transcription and translation may be used to produce an influenza hemagglutinin stem domain polypeptide.

[00175] Once an influenza hemagglutinin stem domain polypeptide has been produced, it may be isolated or purified by any method known in the art for isolation or purification of a protein, for example, by chromatography (*e.g.*, ion exchange, affinity, particularly by affinity for the specific antigen, by Protein A, and sizing column chromatography), centrifugation, differential solubility, or by any other standard technique for the isolation or purification of proteins. In certain embodiments, an influenza hemagglutinin stem domain polypeptide may be conjugated to heterologous proteins, *e.g.*, a major histocompatibility complex (MHC) with or without heat shock proteins (*e.g.*, Hsp10, Hsp20, Hsp30, Hsp40, Hsp60, Hsp70, Hsp90, or Hsp100). In certain embodiments, an influenza hemagglutinin stem domain polypeptide may be conjugated to immunomodulatory molecules, such as proteins which would target the influenza hemagglutinin stem domain polypeptide to immune cells such as B cells (*e.g.*, C3d) or T cells. In certain embodiments, an influenza hemagglutinin stem domain polypeptide may be conjugated to proteins which stimulate the innate immune system such as interferon type I, alpha, beta, or gamma interferon, colony stimulating factors

such as granulocyte-macrophage colony-stimulating factor (GM-CSF), interleukin (IL)-1, IL-2, IL-4, IL-5, IL-6, IL-7, IL-12, IL-15, IL-18, IL-21, IL-23, tumor necrosis factor (TNF)- β , TNF α , B7.1, B7.2, 4-1BB, CD40 ligand (CD40L), and drug-inducible CD40 (iCD40).

[00176] Accordingly, provided herein are methods for producing an influenza hemagglutinin stem domain polypeptide. In one embodiment, the method comprises culturing a host cell containing a nucleic acid encoding the polypeptide in a suitable medium such that the polypeptide is produced. In some embodiments, the method further comprises isolating the polypeptide from the medium or the host cell.

5.4 INFLUENZA VIRUS VECTORS

[00177] In one aspect, provided herein are influenza viruses containing an influenza hemagglutinin stem domain polypeptide. In a specific embodiment, the influenza hemagglutinin stem domain polypeptide is incorporated into the virions of the influenza virus. The influenza viruses may be conjugated to moieties that target the viruses to particular cell types, such as immune cells. In some embodiments, the virions of the influenza virus have incorporated into them or express a heterologous polypeptide in addition to an influenza hemagglutinin stem domain polypeptide. The heterologous polypeptide may be a polypeptide that has immunopotentiating activity, or that targets the influenza virus to a particular cell type, such as an antibody that binds to an antigen on a specific cell type or a ligand that binds a specific receptor on a specific cell type.

[00178] Influenza viruses containing an influenza hemagglutinin stem domain polypeptide may be produced by supplying in *trans* the influenza hemagglutinin stem domain polypeptide during production of virions using techniques known to one skilled in the art, such as reverse genetics and helper-free plasmid rescue. Alternatively, the replication of a parental influenza virus comprising a genome engineered to express an influenza hemagglutinin stem domain polypeptide in cells susceptible to infection with the virus wherein hemagglutinin function is provided in *trans* will produce progeny influenza viruses containing the influenza hemagglutinin stem domain polypeptide.

[00179] In another aspect, provided herein are influenza viruses comprising a genome engineered to express an influenza hemagglutinin stem domain polypeptide. In a specific embodiment, the genome of a parental influenza virus is engineered to encode an influenza hemagglutinin stem domain polypeptide, which is expressed by progeny

influenza virus. In another specific embodiment, the genome of a parental influenza virus is engineered to encode an influenza hemagglutinin stem domain polypeptide, which is expressed and incorporated into the virions of progeny influenza virus. Thus, the progeny influenza virus resulting from the replication of the parental influenza virus contain an influenza hemagglutinin stem domain polypeptide. The virions of the parental influenza virus may have incorporated into them an influenza virus hemagglutinin polypeptide that is from the same or a different type, subtype or strain of influenza virus. Alternatively, the virions of the parental influenza virus may have incorporated into them a moiety that is capable of functionally replacing one or more of the activities of influenza virus hemagglutinin polypeptide (*e.g.*, the receptor binding and/or fusogenic activities of influenza virus hemagglutinin). In certain embodiments, one or more of the activities of the influenza virus hemagglutinin polypeptide is provided by a fusion protein comprising (i) an ectodomain of a polypeptide heterologous to influenza virus fused to (ii) a transmembrane domain, or a transmembrane domain and a cytoplasmic domain of an influenza virus hemagglutinin polypeptide. In a specific embodiment, the virions of the parental influenza virus may have incorporated into them a fusion protein comprising (i) an ectodomain of a receptor binding/fusogenic polypeptide of an infectious agent other than influenza virus fused to (ii) a transmembrane domain, or a transmembrane domain and a cytoplasmic domain of an influenza virus hemagglutinin. For a description of fusion proteins that provide one or more activities of an influenza virus hemagglutinin polypeptide and methods for the production of influenza viruses engineered to express such fusion proteins, see, *e.g.*, International patent application Publication No. WO 2007/064802, published June 7, 2007, which is incorporated herein by reference in its entirety.

[00180] In some embodiments, the virions of the parental influenza virus have incorporated into them a heterologous polypeptide. In certain embodiments, the genome of a parental influenza virus is engineered to encode a heterologous polypeptide and an influenza virus hemagglutinin stem domain polypeptide, which are expressed by progeny influenza virus. In specific embodiments, the influenza hemagglutinin stem domain polypeptide, the heterologous polypeptide or both are incorporated into virions of the progeny influenza virus.

[00181] The heterologous polypeptide may be a polypeptide that targets the influenza virus to a particular cell type, such as an antibody that recognizes an antigen on a specific cell type or a ligand that binds a specific receptor on a specific cell type. In

some embodiments, the targeting polypeptide replaces the target cell recognition function of the virus. In a specific embodiment, the heterologous polypeptide targets the influenza virus to the same cell types that influenza virus infects in nature. In other specific embodiments, the heterologous polypeptide targets the progeny influenza virus to immune cells, such as B cells, T cells, macrophages or dendritic cells. In some embodiments, the heterologous polypeptide recognizes and binds to cell-specific markers of antigen presenting cells, such as dendritic cells (*e.g.*, such as CD44). In one embodiment, the heterologous polypeptide is DC-SIGN which targets the virus to dendritic cells. In another embodiment, the heterologous polypeptide is an antibody (*e.g.*, a single-chain antibody) that targets the virus to an immune cell, which may be fused with a transmembrane domain from another polypeptide so that it is incorporated into the influenza virus virion. In some embodiments, the antibody is a CD20 antibody, a CD34 antibody, or an antibody against DEC-205. Techniques for engineering viruses to express polypeptides with targeting functions are known in the art. See, *e.g.*, Yang *et al.*, 2006, PNAS 103: 11479-11484 and United States patent application Publication No. 20080019998, published January 24, 2008, and No. 20070020238, published January 25, 2007, the contents of each of which are incorporated herein in their entirety.

[00182] In another embodiment, the heterologous polypeptide is a viral attachment protein. Non-limiting examples of viruses whose attachment protein(s) can be used in this aspect are viruses selected from the group of: Lassa fever virus, Hepatitis B virus, Rabies virus, Newcastle disease virus (NDV), a retrovirus such as human immunodeficiency virus, tick-borne encephalitis virus, vaccinia virus, herpesvirus, poliovirus, alphaviruses such as Semliki Forest virus, Ross River virus, and Aura virus (which comprise surface glycoproteins such as E1, E2, and E3), Borna disease virus, Hantaan virus, foamyvirus, and SARS-CoV virus.

[00183] In one embodiment, a flavivirus surface glycoprotein may be used, such as Dengue virus (DV) E protein. In some embodiments, a Sindbis virus glycoprotein from the alphavirus family is used (K. S. Wang, R. J. Kuhn, E. G. Strauss, S. Ou, J. H. Strauss, J. Virol. 66, 4992 (1992)). In certain embodiments, the heterologous polypeptide is derived from an NDV HN or F protein; a human immunodeficiency virus (HIV) gp160 (or a product thereof, such as gp41 or gp120); a hepatitis B virus surface antigen (HBsAg); a glycoprotein of herpesvirus (*e.g.*, gD, gE); or VP1 of poliovirus.

[00184] In another embodiment, the heterologous polypeptide is derived from any non-viral targeting system known in the art. In certain embodiments, a protein of a

nonviral pathogen such as an intracellular bacteria or protozoa is used. In some embodiments, the bacterial polypeptide is provided by, *e.g.*, Chlamydia, Rickettsia, Coxiella, Listeria, Brucella, or Legionella. In some embodiments, protozoan polypeptide is provided by, *e.g.*, Plasmodia species, *Leishmania spp.*, *Toxoplasma gondii*, or *Trypanosoma cruzi*. Other exemplary targeting systems are described in Waehler *et al.*, 2007, "Engineering targeted viral vectors for gene therapy," Nature Reviews Genetics 8: 573-587, which is incorporated herein in its entirety.

[00185] In certain embodiments, the heterologous polypeptide expressed by an influenza virus has immunopotentiating (immune stimulating) activity. Non-limiting examples of immunopotentiating polypeptides include, but are not limited to, stimulation molecules, cytokines, chemokines, antibodies and other agents such as Flt-3 ligands. Specific examples of polypeptides with immunopotentiating activity include: interferon type 1, alpha, beta, or gamma interferon, colony stimulating factors such as granulocyte-macrophage colony-stimulating factor (GM-CSF), interleukin (IL)-1, IL-2, IL-4, IL-5, IL-6, IL-7, IL-12, IL-15, IL-18, IL-21, IL-23, tumor necrosis factor (TNF)- β , TNF α , B7.1, B7.2, 4-1BB, CD40 ligand (CD40L), and drug-inducible CD40 (iCD40) (see, *e.g.*, Hanks, B. A., *et al.* 2005. Nat Med 11:130-137, which is incorporated herein by reference in its entirety.)

[00186] Since the genome of influenza A and B viruses consist of eight (8) single-stranded, negative sense segments (influenza C viruses consist of seven (7) single-stranded, negative sense segments), the genome of a parental influenza virus may be engineered to express an influenza hemagglutinin stem domain polypeptide (and any other polypeptide, such as a heterologous polypeptide) using a recombinant segment and techniques known to one skilled in the art, such a reverse genetics and helper-free plasmid rescue. In one embodiment, the recombinant segment comprises a nucleic acid encoding the influenza hemagglutinin stem domain polypeptide as well as the 3' and 5' incorporation signals which are required for proper replication, transcription and packaging of the vRNAs (Fujii *et al.*, 2003, Proc. Natl. Acad. Sci. USA 100:2002-2007; Zheng, *et al.*, 1996, Virology 217:242-251, both of which are incorporated by reference herein in their entireties). In a specific embodiment, the recombinant segment uses the 3' and 5' noncoding and/or nontranslated sequences of segments of influenza viruses that are from a different or the same type, subtype or strain as the parental influenza virus. In some embodiments, the recombinant segment comprises the 3' noncoding region of an influenza virus hemagglutinin polypeptide, the untranslated regions of an

influenza virus hemagglutinin polypeptide, and the 5' non-coding region of an influenza virus hemagglutinin polypeptide. In specific embodiments, the recombinant segment comprises the 3' and 5' noncoding and/or nontranslated sequences of the HA segment of an influenza virus that is the same type, subtype or strain as the influenza virus type, subtype or strain as the HA1 N-terminal stem segment, the HA1 C-terminal stem segment and/or the HA2 of an influenza hemagglutinin stem domain polypeptide. In certain embodiments, the recombinant segment encoding the influenza hemagglutinin stem domain polypeptide may replace the HA segment of a parental influenza virus. In some embodiments, the recombinant segment encoding the influenza hemagglutinin stem domain polypeptide may replace the NS1 gene of the parental influenza virus. In some embodiments, the recombinant segment encoding the influenza hemagglutinin stem domain polypeptide may replace the NA gene of the parental influenza virus. Exemplary influenza virus strains that can be used to express the influenza hemagglutinin stem domain polypeptides include Ann Arbor/1/50, A/Puerto Rico/8/34, A/South Dakota/6/2007, A/Uruguay/716/2007, and B/Brisbane/60/2008.

[00187] In some embodiments, the genome of a parental influenza virus may be engineered to express an influenza hemagglutinin stem domain polypeptide using a recombinant segment that is bicistronic. Bicistronic techniques allow the engineering of coding sequences of multiple proteins into a single mRNA through the use of internal ribosome entry site (IRES) sequences. IRES sequences direct the internal recruitment of ribosomes to the RNA molecule and allow downstream translation in a cap independent manner. Briefly, a coding region of one protein is inserted into the open reading frame (ORF) of a second protein. The insertion is flanked by an IRES and any untranslated signal sequences necessary for proper expression and/or function. The insertion must not disrupt the ORF, polyadenylation or transcriptional promoters of the second protein (see, *e.g.*, García-Sastre *et al.*, 1994, J. Virol. 68:6254-6261 and García-Sastre *et al.*, 1994 Dev. Biol. Stand. 82:237-246, each of which is hereby incorporated by reference in its entirety). See also, *e.g.*, U.S. Patent No. 6,887,699, U.S. Patent No. 6,001,634, U.S. Patent No. 5,854,037 and U.S. Patent No. 5,820,871, each of which is incorporated herein by reference in its entirety. Any IRES known in the art or described herein may be used in accordance with the invention (*e.g.*, the IRES of BiP gene, nucleotides 372 to 592 of GenBank database entry HUMGRP78; or the IRES of encephalomyocarditis virus (EMCV), nucleotides 1430-2115 of GenBank database entry CQ867238.). Thus, in certain embodiments, a parental influenza virus is engineered to contain a bicistronic

RNA segment that expresses the influenza hemagglutinin stem domain polypeptide and another polypeptide, such as gene expressed by the parental influenza virus. In some embodiments, the parental influenza virus gene is the HA gene. In some embodiments, the parental influenza virus gene is the NA gene. In some embodiments, the parental influenza virus gene is the NS1 gene.

[00188] Techniques known to one skilled in the art may be used to produce an influenza virus containing an influenza hemagglutinin stem domain polypeptide and an influenza virus comprising a genome engineered to express an influenza hemagglutinin stem domain polypeptide. For example, reverse genetics techniques may be used to generate such an influenza virus. Briefly, reverse genetics techniques generally involve the preparation of synthetic recombinant viral RNAs that contain the non-coding regions of the negative-strand, viral RNA which are essential for the recognition by viral polymerases and for packaging signals necessary to generate a mature virion. The recombinant RNAs are synthesized from a recombinant DNA template and reconstituted *in vitro* with purified viral polymerase complex to form recombinant ribonucleoproteins (RNPs) which can be used to transfect cells. A more efficient transfection is achieved if the viral polymerase proteins are present during transcription of the synthetic RNAs either *in vitro* or *in vivo*. The synthetic recombinant RNPs can be rescued into infectious virus particles. The foregoing techniques are described in U.S. Patent No. 5,166,057 issued November 24, 1992; in U.S. Patent No. 5,854,037 issued December 29, 1998; in European Patent Publication EP 0702085A1, published February 20, 1996; in U.S. Patent Application Serial No. 09/152,845; in International Patent Publications PCT WO 97/12032 published April 3, 1997; WO 96/34625 published November 7, 1996; in European Patent Publication EP A780475; WO 99/02657 published January 21, 1999; WO 98/53078 published November 26, 1998; WO 98/02530 published January 22, 1998; WO 99/15672 published April 1, 1999; WO 98/13501 published April 2, 1998; WO 97/06270 published February 20, 1997; and EPO 780 475A1 published June 25, 1997, each of which is incorporated by reference herein in its entirety.

[00189] Alternatively, helper-free plasmid technology may be used to produce an influenza virus containing an influenza hemagglutinin stem domain polypeptide and an influenza virus comprising a genome engineered to express an influenza hemagglutinin stem domain polypeptide. Briefly, full length cDNAs of viral segments are amplified using PCR with primers that include unique restriction sites, which allow the insertion of the PCR product into the plasmid vector (Flandorfer *et al.*, 2003, J. Virol. 77:9116-9123;

Nakaya *et al.*, 2001, J. Virol. 75:11868-11873; both of which are incorporated herein by reference in their entireties). The plasmid vector is designed so that an exact negative (vRNA sense) transcript is expressed. For example, the plasmid vector may be designed to position the PCR product between a truncated human RNA polymerase I promoter and a hepatitis delta virus ribozyme sequence such that an exact negative (vRNA sense) transcript is produced from the polymerase I promoter. Separate plasmid vectors comprising each viral segment as well as expression vectors comprising necessary viral proteins may be transfected into cells leading to production of recombinant viral particles. In another example, plasmid vectors from which both the viral genomic RNA and mRNA encoding the necessary viral proteins are expressed may be used. For a detailed description of helper-free plasmid technology see, *e.g.*, International Publication No. WO 01/04333; U.S. Patent Nos. 6,951,754, 7,384,774, 6,649,372, and 7,312,064; Fodor *et al.*, 1999, J. Virol. 73:9679-9682; Quinlivan *et al.*, 2005, J. Virol. 79:8431-8439; Hoffmann *et al.*, 2000, Proc. Natl. Acad. Sci. USA 97:6108-6113; and Neumann *et al.*, 1999, Proc. Natl. Acad. Sci. USA 96:9345-9350, which are incorporated herein by reference in their entireties.

[00190] The influenza viruses described herein may be propagated in any substrate that allows the virus to grow to titers that permit their use in accordance with the methods described herein. In one embodiment, the substrate allows the viruses to grow to titers comparable to those determined for the corresponding wild-type viruses. In certain embodiments, the substrate is one which is biologically relevant to the influenza virus or to the virus from which the HA function is derived. In a specific embodiment, an attenuated influenza virus by virtue of, *e.g.*, a mutation in the NS1 gene, may be propagated in an IFN-deficient substrate. For example, a suitable IFN-deficient substrate may be one that is defective in its ability to produce or respond to interferon, or is one which An IFN-deficient substrate may be used for the growth of any number of viruses which may require interferon-deficient growth environment. See, for example, U.S. Patent Nos. 6,573,079, issued June 3, 2003, 6,852,522, issued February 8, 2005, and 7,494,808, issued February 24, 2009, the entire contents of each of which is incorporated herein by reference in its entirety.

[00191] The influenza viruses described herein may be isolated and purified by any method known to those of skill in the art. In one embodiment, the virus is removed from cell culture and separated from cellular components, typically by well known clarification procedures, *e.g.*, such as gradient centrifugation and column

chromatography, and may be further purified as desired using procedures well known to those skilled in the art, *e.g.*, plaque assays.

[00192] In certain embodiments, the influenza viruses, or influenza virus polypeptides, genes or genome segments for use as described herein are obtained or derived from an influenza A virus. In certain embodiments, the influenza viruses, or influenza virus polypeptides, genes or genome segments for use as described herein are obtained or derived from a single influenza A virus subtype or strain. In other embodiments, the influenza viruses, or influenza virus polypeptides, genes or genome segments for use as described herein are obtained or derived from two or more influenza A virus subtypes or strains.

[00193] In some embodiments, the influenza viruses, or influenza virus polypeptides, genes or genome segments for use as described herein are obtained or derived from an influenza B virus. In certain embodiments, the influenza viruses, or influenza virus polypeptides, genes or genome segments for use as described herein are obtained or derived from a single influenza B virus subtype or strain. In other embodiments, the influenza viruses, or influenza virus polypeptides, genes or genome segments for use as described herein are obtained or derived from two or more influenza B virus subtypes or strains. In other embodiments, the influenza viruses, or influenza virus polypeptides, genes or genome segments for use as described herein are obtained or derived from a combination of influenza A and influenza B virus subtypes or strains.

[00194] In some embodiments, the influenza viruses, or influenza virus polypeptides, genes or genome segments for use as described herein are obtained or derived from an influenza C virus. In certain embodiments, the influenza viruses, or influenza virus polypeptides, genes or genome segments for use as described herein are obtained or derived from a single influenza C virus subtype or strain. In other embodiments, the influenza viruses, or influenza virus polypeptides, genes or genome segments for use as described herein are obtained or derived from two or more influenza C virus subtypes or strains. In other embodiments, the influenza viruses, or influenza virus polypeptides, genes or genome segments for use as described herein are obtained or derived from a combination of influenza C virus and influenza A virus and/or influenza B virus subtypes or strains.

[00195] Non-limiting examples of influenza A viruses include subtype H10N4, subtype H10N5, subtype H10N7, subtype H10N8, subtype H10N9, subtype H11N1, subtype H11N13, subtype H11N2, subtype H11N4, subtype H11N6, subtype H11N8,

subtype H11N9, subtype H12N1, subtype H12N4, subtype H12N5, subtype H12N8, subtype H13N2, subtype H13N3, subtype H13N6, subtype H13N7, subtype H14N5, subtype H14N6, subtype H15N8, subtype H15N9, subtype H16N3, subtype H1N1, subtype H1N2, subtype H1N3, subtype H1N6, subtype H1N9, subtype H2N1, subtype H2N2, subtype H2N3, subtype H2N5, subtype H2N7, subtype H2N8, subtype H2N9, subtype H3N1, subtype H3N2, subtype H3N3, subtype H3N4, subtype H3N5, subtype H3N6, subtype H3N8, subtype H3N9, subtype H4N1, subtype H4N2, subtype H4N3, subtype H4N4, subtype H4N5, subtype H4N6, subtype H4N8, subtype H4N9, subtype H5N1, subtype H5N2, subtype H5N3, subtype H5N4, subtype H5N6, subtype H5N7, subtype H5N8, subtype H5N9, subtype H6N1, subtype H6N2, subtype H6N3, subtype H6N4, subtype H6N5, subtype H6N6, subtype H6N7, subtype H6N8, subtype H6N9, subtype H7N1, subtype H7N2, subtype H7N3, subtype H7N4, subtype H7N5, subtype H7N7, subtype H7N8, subtype H7N9, subtype H8N4, subtype H8N5, subtype H9N1, subtype H9N2, subtype H9N3, subtype H9N5, subtype H9N6, subtype H9N7, subtype H9N8, and subtype H9N9.

[00196] Specific examples of strains of influenza A virus include, but are not limited to: A/sw/Iowa/15/30 (H1N1); A/WSN/33 (H1N1); A/eq/Prague/1/56 (H7N7); A/PR/8/34; A/mallard/Potsdam/178-4/83 (H2N2); A/herring gull/DE/712/88 (H16N3); A/sw/Hong Kong/168/1993 (H1N1); A/mallard/Alberta/211/98 (H1N1); A/shorebird/Delaware/168/06 (H16N3); A/sw/Netherlands/25/80 (H1N1); A/sw/Germany/2/81 (H1N1); A/sw/Hannover/1/81 (H1N1); A/sw/Potsdam/1/81 (H1N1); A/sw/Potsdam/15/81 (H1N1); A/sw/Potsdam/268/81 (H1N1); A/sw/Finistere/2899/82 (H1N1); A/sw/Potsdam/35/82 (H3N2); A/sw/Cote d'Armor/3633/84 (H3N2); A/sw/Gent/1/84 (H3N2); A/sw/Netherlands/12/85 (H1N1); A/sw/Karrenzien/2/87 (H3N2); A/sw/Schwerin/103/89 (H1N1); A/turkey/Germany/3/91 (H1N1); A/sw/Germany/8533/91 (H1N1); A/sw/Belgium/220/92 (H3N2); A/sw/Gent/V230/92 (H1N1); A/sw/Leipzig/145/92 (H3N2); A/sw/Re220/92hp (H3N2); A/sw/Bakum/909/93 (H3N2); A/sw/Schleswig-Holstein/1/93 (H1N1); A/sw/Scotland/419440/94 (H1N2); A/sw/Bakum/5/95 (H1N1); A/sw/Best/5C/96 (H1N1); A/sw/England/17394/96 (H1N2); A/sw/Jena/5/96 (H3N2); A/sw/Oedenrode/7C/96 (H3N2); A/sw/Lohne/1/97 (H3N2); A/sw/Cote d'Armor/790/97 (H1N2); A/sw/Bakum/1362/98 (H3N2); A/sw/Italy/1521/98 (H1N2); A/sw/Italy/1553-2/98 (H3N2); A/sw/Italy/1566/98 (H1N1); A/sw/Italy/1589/98 (H1N1); A/sw/Bakum/8602/99 (H3N2); A/sw/Cotes d'Armor/604/99 (H1N2); A/sw/Cote

d'Armor/1482/99 (H1N1); A/sw/Gent/7625/99 (H1N2); A/Hong Kong/1774/99 (H3N2);
 A/sw/Hong Kong/5190/99 (H3N2); A/sw/Hong Kong/5200/99 (H3N2); A/sw/Hong
 Kong/5212/99 (H3N2); A/sw/Ille et Villaine/1455/99 (H1N1); A/sw/Italy/1654-1/99
 (H1N2); A/sw/Italy/2034/99 (H1N1); A/sw/Italy/2064/99 (H1N2);
 A/sw/Berlin/1578/00 (H3N2); A/sw/Bakum/1832/00 (H1N2); A/sw/Bakum/1833/00
 (H1N2); A/sw/Cote d'Armor/800/00 (H1N2); A/sw/Hong Kong/7982/00 (H3N2);
 A/sw/Italy/1081/00 (H1N2); A/sw/Belzig/2/01 (H1N1); A/sw/Belzig/54/01 (H3N2);
 A/sw/Hong Kong/9296/01 (H3N2); A/sw/Hong Kong/9745/01 (H3N2);
 A/sw/Spain/33601/01 (H3N2); A/sw/Hong Kong/1144/02 (H3N2); A/sw/Hong
 Kong/1197/02 (H3N2); A/sw/Spain/39139/02 (H3N2); A/sw/Spain/42386/02 (H3N2);
 A/Switzerland/8808/2002 (H1N1); A/sw/Bakum/1769/03 (H3N2);
 A/sw/Bissendorf/IDT1864/03 (H3N2); A/sw/Ehren/IDT2570/03 (H1N2);
 A/sw/Gescher/IDT2702/03 (H1N2); A/sw/Haselünne/2617/03hp (H1N1);
 A/sw/Löningen/IDT2530/03 (H1N2); A/sw/IVD/IDT2674/03 (H1N2);
 A/sw/Nordkirchen/IDT1993/03 (H3N2); A/sw/Nordwalde/IDT2197/03 (H1N2);
 A/sw/Norden/IDT2308/03 (H1N2); A/sw/Spain/50047/03 (H1N1);
 A/sw/Spain/51915/03 (H1N1); A/sw/Vechta/2623/03 (H1N1);
 A/sw/Visbek/IDT2869/03 (H1N2); A/sw/Waltersdorf/IDT2527/03 (H1N2);
 A/sw/Damme/IDT2890/04 (H3N2); A/sw/Geldern/IDT2888/04 (H1N1);
 A/sw/Granstedt/IDT3475/04 (H1N2); A/sw/Greven/IDT2889/04 (H1N1);
 A/sw/Gudensberg/IDT2930/04 (H1N2); A/sw/Gudensberg/IDT2931/04 (H1N2);
 A/sw/Lohne/IDT3357/04 (H3N2); A/sw/Nortrup/IDT3685/04 (H1N2);
 A/sw/Seesen/IDT3055/04 (H3N2); A/sw/Spain/53207/04 (H1N1); A/sw/Spain/54008/04
 (H3N2); A/sw/Stolzenau/IDT3296/04 (H1N2); A/sw/Wedel/IDT2965/04 (H1N1);
 A/sw/Bad Griesbach/IDT4191/05 (H3N2); A/sw/Cloppenburg/IDT4777/05 (H1N2);
 A/sw/Dötlingen/IDT3780/05 (H1N2); A/sw/Dötlingen/IDT4735/05 (H1N2);
 A/sw/Eggldham/IDT5250/05 (H3N2); A/sw/Harkenblek/IDT4097/05 (H3N2);
 A/sw/Hertzen/IDT4317/05 (H3N2); A/sw/Krogel/IDT4192/05 (H1N1);
 A/sw/Laer/IDT3893/05 (H1N1); A/sw/Laer/IDT4126/05 (H3N2);
 A/sw/Merzen/IDT4114/05 (H3N2); A/sw/Muesleringen-S./IDT4263/05 (H3N2);
 A/sw/Osterhofen/IDT4004/05 (H3N2); A/sw/Sprengel/IDT3805/05 (H1N2);
 A/sw/Stadtlohn/IDT3853/05 (H1N2); A/sw/Voglarn/IDT4096/05 (H1N1);
 A/sw/Wohlerst/IDT4093/05 (H1N1); A/sw/Bad Griesbach/IDT5604/06 (H1N1);

A/sw/Herzlake/IDT5335/06 (H3N2); A/sw/Herzlake/IDT5336/06 (H3N2);

A/sw/Herzlake/IDT5337/06 (H3N2); and A/wild boar/Germany/R169/2006 (H3N2).

[00197] Other specific examples of strains of influenza A virus include, but are not limited to: A/Toronto/3141/2009 (H1N1); A/Regensburg/D6/2009 (H1N1); A/Bayern/62/2009 (H1N1); A/Bayern/62/2009 (H1N1); A/Bradenburg/19/2009 (H1N1); A/Bradenburg/20/2009 (H1N1); A/Distrito Federal/2611/2009 (H1N1); A/Mato Grosso/2329/2009 (H1N1); A/Sao Paulo/1454/2009 (H1N1); A/Sao Paulo/2233/2009 (H1N1); A/Stockholm/37/2009 (H1N1); A/Stockholm/41/2009 (H1N1); A/Stockholm/45/2009 (H1N1); A/swine/Alberta/OTH-33-1/2009 (H1N1); A/swine/Alberta/OTH-33-14/2009 (H1N1); A/swine/Alberta/OTH-33-2/2009 (H1N1); A/swine/Alberta/OTH-33-21/2009 (H1N1); A/swine/Alberta/OTH-33-22/2009 (H1N1); A/swine/Alberta/OTH-33-23/2009 (H1N1); A/swine/Alberta/OTH-33-24/2009 (H1N1); A/swine/Alberta/OTH-33-25/2009 (H1N1); A/swine/Alberta/OTH-33-3/2009 (H1N1); A/swine/Alberta/OTH-33-7/2009 (H1N1); A/Beijing/502/2009 (H1N1); A/Firenze/10/2009 (H1N1); A/Hong Kong/2369/2009 (H1N1); A/Italy/85/2009 (H1N1); A/Santo Domingo/572N/2009 (H1N1); A/Catalonia/385/2009 (H1N1); A/Catalonia/386/2009 (H1N1); A/Catalonia/387/2009 (H1N1); A/Catalonia/390/2009 (H1N1); A/Catalonia/394/2009 (H1N1); A/Catalonia/397/2009 (H1N1); A/Catalonia/398/2009 (H1N1); A/Catalonia/399/2009 (H1N1); A/Sao Paulo/2303/2009 (H1N1); A/Akita/1/2009 (H1N1); A/Castro/JXP/2009 (H1N1); A/Fukushima/1/2009 (H1N1); A/Israel/276/2009 (H1N1); A/Israel/277/2009 (H1N1); A/Israel/70/2009 (H1N1); A/Iwate/1/2009 (H1N1); A/Iwate/2/2009 (H1N1); A/Kagoshima/1/2009 (H1N1); A/Osaka/180/2009 (H1N1); A/Puerto Montt/Bio87/2009 (H1 N1); A/Sao Paulo/2303/2009 (H1N1); A/Sapporo/1/2009 (H1N1); A/Stockholm/30/2009 (H1N1); A/Stockholm/31/2009 (H1N1); A/Stockholm/32/2009 (H1N1); A/Stockholm/33/2009 (H1N1); A/Stockholm/34/2009 (H1N1); A/Stockholm/35/2009 (H1N1); A/Stockholm/36/2009 (H1N1); A/Stockholm/38/2009 (H1N1); A/Stockholm/39/2009 (H1N1); A/Stockholm/40/2009 (H1N1); A/Stockholm/42/2009 (H1N1); A/Stockholm/43/2009 (H1N1); A/Stockholm/44/2009 (H1N1); A/Utsunomiya/2/2009 (H1N1); A/WRAIR/0573N/2009 (H1N1); and A/Zhejiang/DTID-ZJU01/2009 (H1N1).

[00198] Non-limiting examples of influenza B viruses include strain Aichi/5/88, strain Akita/27/2001, strain Akita/5/2001, strain Alaska/16/2000, strain Alaska/1777/2005, strain Argentina/69/2001, strain Arizona/146/2005, strain Arizona/148/2005, strain Bangkok/163/90, strain Bangkok/34/99, strain

Bangkok/460/03, strain Bangkok/54/99, strain Barcelona/215/03, strain Beijing/15/84, strain Beijing/184/93, strain Beijing/243/97, strain Beijing/43/75, strain Beijing/5/76, strain Beijing/76/98, strain Belgium/WV106/2002, strain Belgium/WV107/2002, strain Belgium/WV109/2002, strain Belgium/WV114/2002, strain Belgium/WV122/2002, strain Bonn/43, strain Brazil/952/2001, strain Bucharest/795/03, strain Buenos Aires/161/00), strain Buenos Aires/9/95, strain Buenos Aires/SW16/97, strain Buenos Aires/VL518/99, strain Canada/464/2001, strain Canada/464/2002, strain Chaco/366/00, strain Chaco/R113/00, strain Cheju/303/03, strain Chiba/447/98, strain Chongqing/3/2000, strain clinical isolate SA1 Thailand/2002, strain clinical isolate SA10 Thailand/2002, strain clinical isolate SA100 Philippines/2002, strain clinical isolate SA101 Philippines/2002, strain clinical isolate SA110 Philippines/2002), strain clinical isolate SA112 Philippines/2002, strain clinical isolate SA113 Philippines/2002, strain clinical isolate SA114 Philippines/2002, strain clinical isolate SA2 Thailand/2002, strain clinical isolate SA20 Thailand/2002, strain clinical isolate SA38 Philippines/2002, strain clinical isolate SA39 Thailand/2002, strain clinical isolate SA99 Philippines/2002, strain CNIC/27/2001, strain Colorado/2597/2004, strain Cordoba/VA418/99, strain Czechoslovakia/16/89, strain Czechoslovakia/69/90, strain Daegu/10/97, strain Daegu/45/97, strain Daegu/47/97, strain Daegu/9/97, strain B/Du/4/78, strain B/Durban/39/98, strain Durban/43/98, strain Durban/44/98, strain B/Durban/52/98, strain Durban/55/98, strain Durban/56/98, strain England/1716/2005, strain England/2054/2005), strain England/23/04, strain Finland/154/2002, strain Finland/159/2002, strain Finland/160/2002, strain Finland/161/2002, strain Finland/162/03, strain Finland/162/2002, strain Finland/162/91, strain Finland/164/2003, strain Finland/172/91, strain Finland/173/2003, strain Finland/176/2003, strain Finland/184/91, strain Finland/188/2003, strain Finland/190/2003, strain Finland/220/2003, strain Finland/WV5/2002, strain Fujian/36/82, strain Geneva/5079/03, strain Genoa/11/02, strain Genoa/2/02, strain Genoa/21/02, strain Genova/54/02, strain Genova/55/02, strain Guangdong/05/94, strain Guangdong/08/93, strain Guangdong/5/94, strain Guangdong/55/89, strain Guangdong/8/93, strain Guangzhou/7/97, strain Guangzhou/86/92, strain Guangzhou/87/92, strain Gyeonggi/592/2005, strain Hannover/2/90, strain Harbin/07/94, strain Hawaii/10/2001, strain Hawaii/1990/2004, strain Hawaii/38/2001, strain Hawaii/9/2001, strain Hebei/19/94, strain Hebei/3/94), strain Henan/22/97, strain Hiroshima/23/2001, strain Hong Kong/110/99, strain Hong Kong/1115/2002, strain Hong Kong/112/2001, strain

Hong Kong/123/2001, strain Hong Kong/1351/2002, strain Hong Kong/1434/2002, strain Hong Kong/147/99, strain Hong Kong/156/99, strain Hong Kong/157/99, strain Hong Kong/22/2001, strain Hong Kong/22/89, strain Hong Kong/336/2001, strain Hong Kong/666/2001, strain Hong Kong/9/89, strain Houston/1/91, strain Houston/1/96, strain Houston/2/96, strain Hunan/4/72, strain Ibaraki/2/85, strain ncheon/297/2005, strain India/3/89, strain India/77276/2001, strain Israel/95/03, strain Israel/WV187/2002, strain Japan/1224/2005, strain Jiangsu/10/03, strain Johannesburg/1/99, strain Johannesburg/96/01, strain Kadoma/1076/99, strain Kadoma/122/99, strain Kagoshima/15/94, strain Kansas/22992/99, strain Khazkov/224/91, strain Kobe/1/2002, strain, strain Kouchi/193/99, strain Lazio/1/02, strain Lee/40, strain Leningrad/129/91, strain Lissabon/2/90), strain Los Angeles/1/02, strain Lusaka/270/99, strain Lyon/1271/96, strain Malaysia/83077/2001, strain Maputo/1/99, strain Mar del Plata/595/99, strain Maryland/1/01, strain Memphis/1/01, strain Memphis/12/97-MA, strain Michigan/22572/99, strain Mie/1/93, strain Milano/1/01, strain Minsk/318/90, strain Moscow/3/03, strain Nagoya/20/99, strain Nanchang/1/00, strain Nashville/107/93, strain Nashville/45/91, strain Nebraska/2/01, strain Netherland/801/90, strain Netherlands/429/98, strain New York/1/2002, strain NIB/48/90, strain Ningxia/45/83, strain Norway/1/84, strain Oman/16299/2001, strain Osaka/1059/97, strain Osaka/983/97-V2, strain Oslo/1329/2002, strain Oslo/1846/2002, strain Panama/45/90, strain Paris/329/90, strain Parma/23/02, strain Perth/211/2001, strain Peru/1364/2004, strain Philippines/5072/2001, strain Pusan/270/99, strain Quebec/173/98, strain Quebec/465/98, strain Quebec/7/01, strain Roma/1/03, strain Saga/S172/99, strain Seoul/13/95, strain Seoul/37/91, strain Shangdong/7/97, strain Shanghai/361/2002), strain Shiga/T30/98, strain Sichuan/379/99, strain Singapore/222/79, strain Spain/WV27/2002, strain Stockholm/10/90, strain Switzerland/5441/90, strain Taiwan/0409/00, strain Taiwan/0722/02, strain Taiwan/97271/2001, strain Tehran/80/02, strain Tokyo/6/98, strain Trieste/28/02, strain Ulan Ude/4/02, strain United Kingdom/34304/99, strain USSR/100/83, strain Victoria/103/89, strain Vienna/1/99, strain Wuhan/356/2000, strain WV194/2002, strain Xuanwu/23/82, strain Yamagata/1311/2003, strain Yamagata/K500/2001, strain Alaska/12/96, strain GA/86, strain NAGASAKI/1/87, strain Tokyo/942/96, and strain Rochester/02/2001.

[00199] Non-limiting examples of influenza C viruses include strain Aichi/1/81, strain Ann Arbor/1/50, strain Aomori/74, strain California/78, strain England/83, strain

Greece/79, strain Hiroshima/246/2000, strain Hiroshima/252/2000, strain Hyogo/1/83, strain Johannesburg/66, strain Kanagawa/1/76, strain Kyoto/1/79, strain Mississippi/80, strain Miyagi/1/97, strain Miyagi/5/2000, strain Miyagi/9/96, strain Nara/2/85, strain NewJersey/76, strain pig/Beijing/115/81, strain Saitama/3/2000), strain Shizuoka/79, strain Yamagata/2/98, strain Yamagata/6/2000, strain Yamagata/9/96, strain BERLIN/1/85, strain ENGLAND/892/8, strain GREAT LAKES/1167/54, strain JJ/50, strain PIG/BEIJING/10/81, strain PIG/BEIJING/439/82), strain TAYLOR/1233/47, and strain C/YAMAGATA/10/81.

[00200] In certain embodiments, the influenza viruses provided herein have an attenuated phenotype. In specific embodiments, the attenuated influenza virus is based on influenza A virus. In other embodiments, the attenuated influenza virus is based on influenza B virus. In yet other embodiments, the attenuated influenza virus is based on influenza C virus. In other embodiments, the attenuated influenza virus may comprise genes or genome segments from one or more strains or subtypes of influenza A, influenza B, and/or influenza C virus. In some embodiments, the attenuated backbone virus comprises genes from an influenza A virus and an influenza B virus.

[00201] In specific embodiments, attenuation of influenza virus is desired such that the virus remains, at least partially, infectious and can replicate *in vivo*, but only generate low titers resulting in subclinical levels of infection that are non-pathogenic. Such attenuated viruses are especially suited for embodiments described herein wherein the virus or an immunogenic composition thereof is administered to a subject to induce an immune response. Attenuation of the influenza virus can be accomplished according to any method known in the art, such as, *e.g.*, selecting viral mutants generated by chemical mutagenesis, mutation of the genome by genetic engineering, selecting reassortant viruses that contain segments with attenuated function, or selecting for conditional virus mutants (*e.g.*, cold-adapted viruses). Alternatively, naturally occurring attenuated influenza viruses may be used as influenza virus backbones for the influenza virus vectors.

[00202] In one embodiment, an influenza virus may be attenuated, at least in part, by virtue of substituting the HA gene of the parental influenza virus with an influenza hemagglutinin stem domain polypeptide described herein. In some embodiments, an influenza virus may be attenuated, at least in part, by engineering the influenza virus to express a mutated NS1 gene that impairs the ability of the virus to antagonize the cellular interferon (IFN) response. Examples of the types of mutations that can be

introduced into the influenza virus NS1 gene include deletions, substitutions, insertions and combinations thereof. One or more mutations can be introduced anywhere throughout the NS1 gene (*e.g.*, the N-terminus, the C-terminus or somewhere in between) and/or the regulatory element of the NS1 gene. In one embodiment, an attenuated influenza virus comprises a genome having a mutation in an influenza virus NS1 gene resulting in a deletion consisting of 5, preferably 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 75, 80, 85, 90, 95, 99, 100, 105, 110, 115, 120, 125, 126, 130, 135, 140, 145, 150, 155, 160, 165, 170 or 175 amino acid residues from the C-terminus of NS1, or a deletion of between 5-170, 25-170, 50-170, 100-170, 100-160, or 105-160 amino acid residues from the C-terminus. In another embodiment, an attenuated influenza virus comprises a genome having a mutation in an influenza virus NS1 gene such that it encodes an NS1 protein of amino acid residues 1-130, amino acid residues 1-126, amino acid residues 1-120, amino acid residues 1-115, amino acid residues 1-110, amino acid residues 1-100, amino acid residues 1-99, amino acid residues 1-95, amino acid residues 1-85, amino acid residues 1-83, amino acid residues 1-80, amino acid residues 1-75, amino acid residues 1-73, amino acid residues 1-70, amino acid residues 1-65, or amino acid residues 1-60, wherein the N-terminus amino acid is number 1. For examples of NS1 mutations and influenza viruses comprising a mutated NS1, see, *e.g.*, U.S. Patent Nos. 6,468,544 and 6,669,943; and Li *et al.*, 1999, J. Infect. Dis. 179:1132-1138, each of which is incorporated by reference herein in its entirety.

5.5 NON-INFLUENZA VIRUS VECTORS

[00203] In one aspect, provided herein are non-influenza viruses containing an influenza hemagglutinin stem domain polypeptide. In a specific embodiment, the influenza hemagglutinin stem domain polypeptide is incorporated into the virions of the non-influenza virus. The non-influenza viruses may be conjugated to moieties that target the viruses to particular cell types, such as immune cells. In some embodiments, the virions of the non-influenza virus have incorporated into them or express a heterologous polypeptide in addition to an influenza hemagglutinin stem domain polypeptide. The heterologous polypeptide may be a polypeptide that has immunopotentiating activity, or that targets the non-influenza virus to a particular cell type, such as an antibody that recognizes an antigen on a specific cell type or a ligand

that binds a specific receptor on a specific cell type. See Section 5.4 *supra* for examples of such heterologous polypeptides.

[00204] Non-influenza viruses containing an influenza hemagglutinin stem domain polypeptide may be produced by supplying in *trans* the influenza hemagglutinin stem domain polypeptide during production of virions using techniques known to one skilled in the art. Alternatively, the replication of a parental non-influenza virus comprising a genome engineered to express an influenza hemagglutinin stem domain polypeptide in cells susceptible to infection with the virus wherein hemagglutinin function is provided in *trans* will produce progeny viruses containing the influenza hemagglutinin stem domain polypeptide.

[00205] Any virus type, subtype or strain including, but not limited to, naturally occurring strains, variants or mutants, mutagenized viruses, reassortants and/or genetically modified viruses may be used as a non-influenza virus vector. In a specific embodiment, the parental non-influenza virus is not a naturally occurring virus. In another specific embodiment, the parental non-influenza virus is a genetically engineered virus. In certain embodiments, an enveloped virus is preferred for the expression of a membrane bound influenza hemagglutinin stem domain polypeptide described herein.

[00206] In an exemplary embodiment, the non-influenza virus vector is a Newcastle disease virus (NDV). In another embodiment, the non-influenza virus vector is a vaccinia virus. In other exemplary, non-limiting, embodiments, the non-influenza virus vector is adenovirus, adeno-associated virus (AAV), hepatitis B virus, retrovirus (such as, *e.g.*, a gammaretrovirus such as Mouse Stem Cell Virus (MSCV) genome or Murine Leukemia Virus (MLV), *e.g.*, Moloney murine leukemia virus, oncoretrovirus, or lentivirus), an alphavirus (*e.g.*, Venezuelan equine encephalitis virus), a rhabdovirus, such as vesicular stomatitis virus or papillomaviruses, poxvirus (such as, *e.g.*, vaccinia virus, a MVA-T7 vector, or fowlpox), metapneumovirus, measles virus, herpesvirus, such as herpes simplex virus, or foamyvirus. *See, e.g.*, Lawrie and Tumin, 1993, Cur. Opin. Genet. Develop. 3, 102-109 (retroviral vectors); Bett *et al.*, 1993, J. Virol. 67, 5911 (adenoviral vectors); Zhou *et al.*, 1994, J. Exp. Med. 179, 1867 (adeno-associated virus vectors); Dubensky *et al.*, 1996, J. Virol. 70, 508-519 (viral vectors from the pox family including vaccinia virus and the avian pox viruses and viral vectors from the alpha virus genus such as those derived from Sindbis and Semliki Forest Viruses); U.S. Pat. No. 5,643,576 (Venezuelan equine encephalitis virus); WO 96/34625 (VSV); Ohe *et*

al., 1995, Human Gene Therapy 6, 325-333; Woo *et al.*, WO 94/12629; Xiao & Brandsma, 1996, Nucleic Acids. Res. 24, 2630-2622 (papillomaviruses); and Bukreyev and Collins, 2008, Curr Opin Mol Ther. 10:46-55 (NDV), each of which is incorporated by reference herein in its entirety.

[00207] In a specific embodiment, the non-influenza virus vector is NDV. Any NDV type, subtype or strain may serve as the backbone that is engineered to express an influenza hemagglutinin stem domain polypeptide, including, but not limited to, naturally-occurring strains, variants or mutants, mutagenized viruses, reassortants and/or genetically engineered viruses. In a specific embodiment, the NDV that serves as the backbone for genetic engineering is a naturally-occurring strain. In certain embodiments, the NDV that serves as the backbone for genetic engineering is a lytic strain. In other embodiments, the NDV that serves as the backbone for genetic engineering is a non-lytic strain. In certain embodiments, the NDV that serves as the backbone for genetic engineering is lentogenic strain. In some embodiments, the NDV that serves as the backbone for genetic engineering is a mesogenic strain. In other embodiments, the NDV that serves as the backbone for genetic engineering is a velogenic strain. Specific examples of NDV strains include, but are not limited to, the 73-T strain, Ulster strain, MTH-68 strain, Italien strain, Hickman strain, PV701 strain, Hitchner B1 strain, La Sota strain, YG97 strain, MET95 strain, and F48E9 strain. In a specific embodiment, the NDV that serves as the backbone for genetic engineering is the Hitchner B1 strain. In another specific embodiment, the NDV that serves as the backbone for genetic engineering is the La Sota strain.

[00208] In one embodiment, the NDV used as the backbone for a non-influenza virus vector is engineered to express a modified F protein in which the cleavage site of the F protein is replaced with one containing one or two extra arginine residues, allowing the mutant cleavage site to be activated by ubiquitously expressed proteases of the furin family. Specific examples of NDVs that express such a modified F protein include, but are not limited to, rNDV/F2aa and rNDV/F3aa. For a description of mutations introduced into a NDV F protein to produce a modified F protein with a mutated cleavage site, see, e.g., Park *et al.* (2006) "Engineered viral vaccine constructs with dual specificity: Avian influenza and Newcastle disease." PNAS USA 103: 8203-2808, which is incorporated herein by reference in its entirety.

[00209] In one embodiment, the non-influenza virus vector is a poxvirus. A poxvirus vector may be based on any member of the poxviridae, in particular, a vaccinia virus or

an avipox virus (*e.g.*, such as canarypox, fowlpox, etc.) that provides suitable sequences for vaccine vectors. In a specific embodiment, the poxviral vector is a vaccinia virus vector. Suitable vaccinia viruses include, but are not limited to, the Copenhagen (VC-2) strain (Goebel, *et al.*, Virol 179: 247-266, 1990; Johnson, *et al.*, Virol. 196: 381-401, 1993), modified Copenhagen strain (NYVAC) (U.S. Pat. No. 6,265,189), the WYETH strain and the modified Ankara (MVA) strain (Antoine, *et al.*, Virol. 244: 365-396, 1998). Other suitable poxviruses include fowlpox strains such as ALVAC and TROVAC vectors that provide desirable properties and are highly attenuated (*see, e.g.*, U.S. Pat. No. 6,265,189; Tartaglia *et al.*, In AIDS Research Reviews, Koff, *et al.*, eds., Vol. 3, Marcel Dekker, N.Y., 1993; and Tartaglia *et al.*, 1990, Reviews in Immunology 10: 13-30, 1990).

[00210] Methods of engineering non-influenza viruses to express an influenza hemagglutinin stem domain polypeptide are well known in the art, as are methods for attenuating, propagating, and isolating and purifying such viruses. For such techniques with respect to NDV vectors, *see, e.g.*, International Publication No. WO 01/04333; U.S. Patent Nos. 7,442,379, 6,146,642, 6,649,372, 6,544,785 and 7,384,774; Swayne *et al.* (2003). Avian Dis. 47:1047-1050; and Swayne *et al.* (2001). J. Virol. 11868-11873, each of which is incorporated by reference in its entirety. For such techniques with respect to poxviruses, *see, e.g.*, Piccini, *et al.*, Methods of Enzymology 153: 545-563, 1987; International Publication No. WO 96/11279; U.S. Pat. No. 4,769,330; U.S. Pat. No. 4,722,848; U.S. Pat. No. 4,769,330; U.S. Pat. No. 4,603,112; U.S. Pat. No. 5,110,587; U.S. Pat. No. 5,174,993; EP 83 286; EP 206 920; Mayr *et al.*, Infection 3: 6-14, 1975; and Sutter and Moss, Proc. Natl. Acad. Sci. USA 89: 10847-10851, 1992. In certain embodiments, the non-influenza virus is attenuated.

[00211] Exemplary considerations for the selection of a non-influenza virus vector, particularly for use in compositions for administration to a subject, are safety, low toxicity, stability, cell type specificity, and immunogenicity, particularly, antigenicity of the influenza hemagglutinin stem domain polypeptide expressed by the non-influenza virus vector.

5.6 VIRAL-LIKE PARTICLES AND VIROSOMES

[00212] Influenza hemagglutinin stem domain polypeptides can be incorporated into viral-like particle (VLP) vectors. VLPs generally comprise a viral polypeptide(s)

typically derived from a structural protein(s) of a virus. In some embodiments, the VLPs are not capable of replicating. In certain embodiments, the VLPs may lack the complete genome of a virus or comprise a portion of the genome of a virus. In some embodiments, the VLPs are not capable of infecting a cell. In some embodiments, the VLPs express on their surface one or more of viral (*e.g.*, virus surface glycoprotein) or non-viral (*e.g.*, antibody or protein) targeting moieties known to one skilled in the art or described herein. In some embodiments, the VLPs comprise an influenza hemagglutinin stem domain polypeptide and a viral structural protein, such as HIV gag. In a specific embodiment, the VLPs comprise an influenza hemagglutinin stem domain polypeptide and an HIV gag polypeptide, such as described in Example 2 in Section 6.2 below.

[00213] Methods for producing and characterizing recombinantly produced VLPs have been described based on several viruses, including influenza virus (Bright *et al.* (2007) Vaccine. 25:3871), human papilloma virus type 1 (Hagnese *et al.* (1991) J. Virol. 67:315), human papilloma virus type 16 (Kirnbauer *et al.* Proc. Natl. Acad. Sci. (1992)89:12180), HIV-1 (Haffer *et al.*, (1990) J. Virol. 64:2653), and hepatitis A (Winokur (1991) 65:5029), each of which is incorporated herein in its entirety. Methods for expressing VLPs that contain NDV proteins are provided by Pantua *et al.* (2006) J. Virol. 80:11062-11073, and in United States patent application Publication No. 20090068221, published March 12, 2009, each of which is incorporated in its entirety herein.

[00214] In a specific embodiment, an influenza hemagglutinin stem domain polypeptide may be incorporated into a virosome. A virosome containing an influenza hemagglutinin stem domain polypeptide may be produced using techniques known to those skilled in the art. For example, a virosome may be produced by disrupting a purified virus, extracting the genome, and reassembling particles with the viral proteins (*e.g.*, an influenza hemagglutinin stem domain polypeptide) and lipids to form lipid particles containing viral proteins.

5.7 BACTERIAL VECTORS

[00215] In a specific embodiment, bacteria may be engineered to express an influenza hemagglutinin stem domain polypeptide described herein. Suitable bacteria for expression of an influenza virus hemagglutinin stem domain include, but are not limited to, *Listeria*, *Salmonella*, *Shigella sp.*, *Mycobacterium tuberculosis*, *E. coli*, *Neisseria*

meningitides, *Brucella abortus*, *Brucella melitensis*, *Borrelia burgdorferi*, and *Francisella tularensis*. In a specific embodiment, the bacteria engineered to express an influenza hemagglutinin stem domain polypeptide are attenuated. Techniques for the production of bacteria engineered to express a heterologous polypeptide are known in the art and can be applied to the expression of an influenza hemagglutinin stem domain polypeptide. See, e.g., United States Patent Application Publication No. 20080248066, published October 9, 2008, and United States Patent Application Publication No. 20070207171, published September 6, 2007, each of which are incorporated by reference herein in their entirety.

5.8 PLANT AND ALGAE VECTORS

[00216] In certain embodiments, plants (e.g., plants of the genus *Nicotiana*) may be engineered to express an influenza hemagglutinin stem domain polypeptide described herein. In specific embodiments, plants are engineered to express an influenza hemagglutinin stem domain polypeptide described herein via an agroinfiltration procedure using methods known in the art. For example, nucleic acids encoding a gene of interest, e.g., a gene encoding influenza hemagglutinin stem domain polypeptide described herein, are introduced into a strain of *Agrobacterium*. Subsequently the strain is grown in a liquid culture and the resulting bacteria are washed and suspended into a buffer solution. The plants are then exposed (e.g., via injection or submersion) to the *Agrobacterium* that comprises the nucleic acids encoding an influenza hemagglutinin stem domain polypeptide described herein such that the *Agrobacterium* transforms the gene of interest to a portion of the plant cells. The influenza hemagglutinin stem domain polypeptide is then transiently expressed by the plant and can be isolated using methods known in the art and described herein. (For specific examples see Shoji et al., 2008, Vaccine, 26(23):2930-2934; and D'Aoust et al., 2008, J. Plant Biotechnology, 6(9):930-940). In a specific embodiment, the plant is a tobacco plant (i.e., *Nicotiana tabacum*). In another specific embodiment, the plant is a relative of the tobacco plant (e.g., *Nicotiana benthamiana*).

[00217] In other embodiments, algae (e.g., *Chlamydomonas reinhardtii*) may be engineered to express an influenza hemagglutinin stem domain polypeptide described herein (see, e.g., Rasala et al., 2010, Plant Biotechnology Journal (Published online March 7, 2010)).

5.9 GENERATION OF ANTIBODIES AGAINST INFLUENZA HEMAGGLUTININ STEM DOMAIN POLYPEPTIDE

[00218] The influenza hemagglutinin stem domain polypeptides, nucleic acids encoding such polypeptides, or vectors comprising such nucleic acids or polypeptides described herein may be used to elicit neutralizing antibodies against influenza, for example, against the stalk region of influenza virus hemagglutinin polypeptide. In a specific embodiment, the influenza hemagglutinin stem domain polypeptides, nucleic acids encoding such polypeptides, or vectors comprising such nucleic acids or polypeptides described herein may be administered to a non-human subject (*e.g.*, a mouse, rabbit, rat, guinea pig, etc.) to induce an immune response that includes the production of antibodies which may be isolated using techniques known to one of skill in the art (*e.g.*, immunoaffinity chromatography, centrifugation, precipitation, etc.).

[00219] Alternatively, influenza hemagglutinin stem domain polypeptides described herein may be used to screen for antibodies from antibody libraries. For example, an isolated influenza hemagglutinin stem domain polypeptide may be immobilized to a solid support (*e.g.*, a silica gel, a resin, a derivatized plastic film, a glass bead, cotton, a plastic bead, a polystyrene bead, an alumina gel, or a polysaccharide, a magnetic bead), and screened for binding to antibodies. As an alternative, the antibodies may be immobilized to a solid support and screened for binding to the isolated influenza hemagglutinin stem domain polypeptide. Any screening assay, such as a panning assay, ELISA, surface plasmon resonance, or other antibody screening assay known in the art may be used to screen for antibodies that bind to the influenza hemagglutinin stem domain. The antibody library screened may be a commercially available antibody library, an *in vitro* generated library, or a library obtained by identifying and cloning or isolating antibodies from an individual infected with influenza. In particular embodiments, the antibody library is generated from a survivor of an influenza virus outbreak. Antibody libraries may be generated in accordance with methods known in the art. In a particular embodiment, the antibody library is generated by cloning the antibodies and using them in phage display libraries or a phagemid display library.

[00220] Antibodies identified in the methods described herein may be tested for neutralizing activity and lack of autoreactivity using the biological assays known in the art or described herein. In one embodiment, an antibody isolated from a non-human animal or an antibody library neutralizes a hemagglutinin polypeptide from more than one influenza subtype. In some embodiments, an antibody elicited or identified using an

influenza hemagglutinin stem domain polypeptide, a nucleic acid encoding such a polypeptide, or a vector encoding such a nucleic acid or polypeptide neutralizes an influenza H3 virus. In some embodiments, an antibody elicited or identified using an influenza hemagglutinin stem domain polypeptide, a nucleic acid encoding such a polypeptide, or a vector comprising such a nucleic acid or polypeptide neutralizes 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or 16 or more subtypes or strains of influenza virus. In one embodiment, the neutralizing antibody neutralizes one or more influenza A viruses and one or more influenza B viruses. In particular embodiments, the neutralizing antibody is not, or does not bind the same epitope as CR6261, CR6325, CR6329, CR6307, CR6323, 2A, D7, D8, F10, G17, H40, A66, D80, E88, E90, H98, C179 (produced by hybridoma FERM BP-4517; clones sold by Takara Bio, Inc. (Otsu, Shiga, Japan)), AI3C (produced by hybridoma FERM BP-4516) or any other antibody described in Ekiert DC *et al.* (2009) Antibody Recognition of a Highly Conserved Influenza Virus Epitope. *Science* (published in *Science Express* February 26, 2009); Kashyap *et al.* (2008) Combinatorial antibody libraries from survivors of the Turkish H5N1 avian influenza outbreak reveal virus neutralization strategies. *Proc Natl Acad Sci U S A* 105: 5986-5991; Sui *et al.* (2009) Structural and functional bases for broad-spectrum neutralization of avian and human influenza A viruses. *Nat Struct Mol Biol* 16: 265-273; U.S. Patent Nos. 5,589,174, 5,631,350, 6,337,070, and 6,720,409; International Application No. PCT/US2007/068983 published as International Publication No. WO 2007/134237; International Application No. PCT/US2008/075998 published as International Publication No. WO 2009/036157; International Application No. PCT/EP2007/059356 published as International Publication No. WO 2008/028946; and International Application No. PCT/US2008/085876 published as International Publication No. WO 2009/079259. In other embodiments, the neutralizing antibody is not an antibody described in Wang *et al.* (2010) "Broadly Protective Monoclonal Antibodies against H3 Influenza Viruses following Sequential Immunization with Different Hemagglutinins," *PLOS Pathogens* 6(2):1-9. In particular embodiments, the neutralizing antibody does not use the Ig VH1-69 segment. In some embodiments, the interaction of the neutralizing antibody with the antigen is not mediated exclusively by the heavy chain.

[00221] Antibodies identified or elicited using an influenza hemagglutinin stem domain polypeptide, a nucleic acid encoding such a polypeptide, or a vector comprising such a nucleic acid or polypeptide include immunoglobulin molecules and

immunologically active portions of immunoglobulin molecules, *i.e.*, molecules that contain an antigen binding site that specifically binds to a hemagglutinin polypeptide. The immunoglobulin molecules may be of any type (*e.g.*, IgG, IgE, IgM, IgD, IgA and IgY), class (*e.g.*, IgG₁, IgG₂, IgG₃, IgG₄, IgA₁ and IgA₂) or subclass of immunoglobulin molecule. Antibodies include, but are not limited to, monoclonal antibodies, multispecific antibodies, human antibodies, humanized antibodies, chimeric antibodies, single-chain Fvs (scFv), single chain antibodies, Fab fragments, F(ab') fragments, disulfide-linked Fvs (sdFv), and anti-idiotypic (anti-Id) antibodies (including, *e.g.*, anti-Id antibodies to antibodies elicited or identified using a method described herein), and epitope-binding fragments of any of the above.

[00222] Antibodies elicited or identified using an influenza hemagglutinin stem domain polypeptide, nucleic acids encoding such a polypeptide or a vector comprising such a nucleic acid or polypeptide may be used in diagnostic immunoassays, passive immunotherapy, and generation of antiidiotypic antibodies. The antibodies before being used in passive immunotherapy may be modified, *e.g.*, the antibodies may be chimerized or humanized. *See, e.g.*, U.S. Patent Nos. 4,444,887 and 4,716,111; and International Publication Nos. WO 98/46645, WO 98/50433, WO 98/24893, WO 98/16654, WO 96/34096, WO 96/33735, and WO 91/10741, each of which is incorporated herein by reference in its entirety, for reviews on the generation of chimeric and humanized antibodies. In addition, the ability of the antibodies to neutralize hemagglutinin polypeptides and the specificity of the antibodies for the polypeptides may be tested prior to using the antibodies in passive immunotherapy. *See* Section 5.11 *infra* for a discussion regarding use of neutralizing antibodies for the prevention or treatment of disease caused by influenza virus infection.

[00223] Antibodies elicited or identified using an influenza hemagglutinin stem domain polypeptide, a nucleic acid encoding such a polypeptide, or a vector comprising such a nucleic acid or polypeptide may be used to monitor the efficacy of a therapy and/or disease progression. Any immunoassay system known in the art may be used for this purpose including, but not limited to, competitive and noncompetitive assay systems using techniques such as radioimmunoassays, ELISA (enzyme linked immunosorbent assays), "sandwich" immunoassays, precipitin reactions, gel diffusion precipitin reactions, immunodiffusion assays, agglutination assays, complement fixation assays, immunoradiometric assays, fluorescent immunoassays, protein A immunoassays and immunoelectrophoresis assays, to name but a few.

[00224] Antibodies elicited or identified using an influenza hemagglutinin stem domain polypeptide, a nucleic acid encoding such a polypeptide, or a vector comprising such a nucleic acid or polypeptide may be used in the production of antiidiotypic antibody. The antiidiotypic antibody can then in turn be used for immunization, in order to produce a subpopulation of antibodies that bind a particular antigen of influenza, *e.g.*, a neutralizing epitope of a hemagglutinin polypeptide (Jerne, 1974, Ann. Immunol. (Paris) 125c:373; Jerne *et al.*, 1982, EMBO J. 1:234, incorporated herein by reference in its entirety).

5.10 STIMULATION OF CELLS WITH INFLUENZA HEMAGGLUTININ STEM DOMAIN PEPTIDE

[00225] In another aspect, provided herein are methods for stimulating cells *ex vivo* with an influenza hemagglutinin stem domain polypeptide described herein. Such cells, *e.g.*, dendritic cells, may be used *in vitro* to generate antibodies against the influenza hemagglutinin stem domain polypeptide or may themselves be administered to a subject by, *e.g.*, an adoptive transfer technique known in the art. *See, e.g.*, United States patent application Publication No. 20080019998, published January 24, 2008, which is incorporated herein by reference in its entirety, for a description of adoptive transfer techniques. In certain embodiments, when cells that have been stimulated *ex vivo* with an influenza hemagglutinin stem domain polypeptide described herein are administered to a subject, the cells are not mammalian cells (*e.g.*, CB-1 cells).

[00226] In one non-limiting example, a vector, *e.g.*, an influenza virus vector, engineered to express an influenza hemagglutinin stem domain polypeptide described herein can be used to generate dendritic cells (DCs) that express the influenza hemagglutinin stem domain polypeptide and display immunostimulatory properties directed against an influenza virus hemagglutinin polypeptide. Such DCs may be used to expand memory T cells and are potent stimulators of T cells, including influenza hemagglutinin stem domain polypeptide-specific cytotoxic T lymphocyte clones. *See Strobel et al.*, 2000, Human Gene Therapy 11:2207-2218, which is incorporated herein by reference in its entirety.

[00227] An influenza hemagglutinin stem domain polypeptide described herein may be delivered to a target cell in any way that allows the polypeptide to contact the target cell, *e.g.*, a DC, and deliver the polypeptide to the target cell. In certain embodiments, the influenza hemagglutinin stem domain polypeptide is delivered to a subject, as

described herein. In some such embodiments, cells contacted with the polypeptide may be isolated and propagated.

[00228] In certain embodiments, an influenza hemagglutinin stem domain polypeptide is delivered to a target cell *in vitro*. Techniques known to one of skill in the art may be used to deliver the polypeptide to target cells. For example, target cells may be contacted with the polypeptide in a tissue culture plate, tube or other container. The polypeptide may be suspended in media and added to the wells of a culture plate, tube or other container. The media containing the polypeptide may be added prior to plating of the cells or after the cells have been plated. The target cells are preferably incubated with the polypeptide for a sufficient amount of time to allow the polypeptide to contact the cells. In certain embodiments, the cells are incubated with the polypeptide for about 1 hour or more, about 5 hours or more, about 10 hours or more, about 12 hours or more, about 16 hours or more, about 24, hours or more, about 48 hours or more, about 1 hour to about 12 hours, about 3 hours to about 6 hours, about 6 hours to about 12 hours, about 12 hours to about 24 hours, or about 24 hours to about 48 hours. In certain embodiments, wherein the influenza hemagglutinin stem domain polypeptide is in a virus, the contacting of the target cells comprises infecting the cells with the virus.

[00229] The target cells may be from any species, including, e.g., humans, mice, rats, rabbits and guinea pigs. In some embodiments, target cells are DCs obtained from a healthy subject or a subject in need of treatment. In certain embodiments, target cells are DCs obtained from a subject in whom it is desired to stimulate an immune response to the polypeptide. Methods of obtaining cells from a subject are well known in the art.

5.11 COMPOSITIONS

[00230] The nucleic acids, vectors, polypeptides, bacteria, antibodies, or cells described herein (sometimes referred to herein as “active compounds”) may be incorporated into compositions. In a specific embodiment, the compositions are pharmaceutical compositions, such as immunogenic compositions (*e.g.*, vaccine formulations). The pharmaceutical compositions provided herein can be in any form that allows for the composition to be administered to a subject. In a specific embodiment, the pharmaceutical compositions are suitable for veterinary and/or human administration. The compositions may be used in methods of preventing or treating an influenza virus disease.

[00231] In one embodiment, a pharmaceutical composition comprises an influenza hemagglutinin stem domain polypeptide, in an admixture with a pharmaceutically acceptable carrier. In another embodiment, a pharmaceutical composition comprises a nucleic acid encoding an influenza hemagglutinin stem domain polypeptide described herein, in an admixture with a pharmaceutically acceptable carrier. In another embodiment, a pharmaceutical composition comprises an expression vector comprising a nucleic acid encoding an influenza hemagglutinin stem domain polypeptide, in an admixture with a pharmaceutically acceptable carrier. In another embodiment, a pharmaceutical composition comprises an influenza virus or non-influenza virus containing an influenza hemagglutinin stem domain polypeptide, in an admixture with a pharmaceutically acceptable carrier. In another embodiment, a pharmaceutical composition comprises an influenza virus or non-influenza virus having a genome engineered to express an influenza hemagglutinin stem domain polypeptide, in admixture with a pharmaceutically acceptable carrier. In another embodiment, a pharmaceutical composition comprises a viral-like particle or virosome containing an influenza hemagglutinin stem domain polypeptide, in an admixture with a pharmaceutically acceptable carrier. In another embodiment, a pharmaceutical composition comprises a bacteria expressing or engineered to express an influenza hemagglutinin stem domain polypeptide, in an admixture with a pharmaceutically acceptable carrier. In another embodiment, a pharmaceutical composition comprises cells stimulated with an influenza hemagglutinin stem domain polypeptide, in an admixture with a pharmaceutically acceptable carrier.

[00232] In some embodiments, a pharmaceutical composition may comprise one or more other therapies in addition to an active compound.

[00233] As used herein, the term "pharmaceutically acceptable" means approved by a regulatory agency of the Federal or a state government or listed in the U.S.

Pharmacopeia or other generally recognized pharmacopeiae for use in animals, and more particularly in humans. The term "carrier" refers to a diluent, adjuvant, excipient, or vehicle with which the pharmaceutical composition is administered. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Suitable excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. Examples of suitable pharmaceutical carriers are described in

"Remington's Pharmaceutical Sciences" by E.W. Martin. The formulation should suit the mode of administration.

[00234] In a specific embodiment, pharmaceutical compositions are formulated to be suitable for the intended route of administration to a subject. For example, the pharmaceutical composition may be formulated to be suitable for parenteral, oral, intradermal, transdermal, colorectal, intraperitoneal, and rectal administration. In a specific embodiment, the pharmaceutical composition may be formulated for intravenous, oral, intraperitoneal, intranasal, intratracheal, subcutaneous, intramuscular, topical, intradermal, transdermal or pulmonary administration.

[00235] In certain embodiments, biodegradable polymers, such as ethylene vinyl acetate, polyanhydrides, polyethylene glycol (PEGylation), polymethyl methacrylate polymers, polylactides, poly(lactide-co-glycolides), polyglycolic acid, collagen, polyorthoesters, and polylactic acid, may be used as carriers. In some embodiments, the active compounds are prepared with carriers that increase the protection of the compound against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Methods for preparation of such formulations will be apparent to those skilled in the art. Liposomes or micelles can also be used as pharmaceutically acceptable carriers. These can be prepared according to methods known to those skilled in the art, for example, as described in U.S. Pat. No. 4,522,811. In certain embodiments, the pharmaceutical compositions comprise one or more adjuvants.

[00236] In specific embodiments, immunogenic compositions described herein are monovalent formulations. In other embodiments, immunogenic compositions described herein are multivalent formulations. In one example, a multivalent formulation comprises one or more vectors expressing an influenza hemagglutinin stem domain polypeptide derived from an influenza A virus hemagglutinin polypeptide and one or more vectors expressing an influenza hemagglutinin stem domain polypeptide derived from an influenza B virus hemagglutinin polypeptide. In another example, a multivalent formulation comprises a vector expressing an influenza hemagglutinin stem domain polypeptide derived from an influenza A virus H3 antigen and a vector expressing an influenza hemagglutinin stem domain polypeptide derived from an influenza A virus H1 antigen. In another example, a multivalent formulation comprises a vector expressing an influenza hemagglutinin stem domain polypeptide derived from an influenza A virus H3 antigen, a vector expressing an influenza hemagglutinin stem domain polypeptide

derived from an influenza A virus H1 antigen, and a vector expressing an influenza hemagglutinin stem domain polypeptide derived from an influenza B virus HA antigen. In certain embodiments, a multivalent formulation may comprise one or more different influenza hemagglutinin stem domain polypeptides expressed using a single vector.

[00237] In certain embodiments, the pharmaceutical compositions described herein additionally comprise a preservative, e.g., the mercury derivative thimerosal. In a specific embodiment, the pharmaceutical compositions described herein comprises 0.001% to 0.01% thimerosal. In other embodiments, the pharmaceutical compositions described herein do not comprise a preservative. In a specific embodiment, thimerosal is used during the manufacture of a pharmaceutical composition described herein and the thimerosal is removed via purification steps following production of the pharmaceutical composition, i.e., the pharmaceutical composition contains trace amounts of thimerosal (<0.3 µg of mercury per dose after purification; such pharmaceutical compositions are considered thimerosal-free products).

[00238] In certain embodiments, the pharmaceutical compositions described herein additionally comprise egg protein (e.g., ovalbumin or other egg proteins). The amount of egg protein in the pharmaceutical compositions described herein may range from about 0.0005 to about 1.2. µg of egg protein to 1 ml of pharmaceutical composition. In other embodiments, the pharmaceutical compositions described herein do not comprise egg protein.

[00239] In certain embodiments, the pharmaceutical compositions described herein additionally comprise one or more antimicrobial agents (e.g., antibiotics) including, but not limited to gentamicin, neomycin, polymyxin (e.g., polymyxin B), and kanamycin, streptomycin. In other embodiments, the pharmaceutical compositions described herein do not comprise any antibiotics.

[00240] In certain embodiments, the pharmaceutical compositions described herein additionally comprise one or more components used to inactivate a virus, e.g., formalin or formaldehyde or a detergent such as sodium deoxycholate, octoxynol 9 (Triton X-100), and octoxynol 10. In other embodiments, the pharmaceutical compositions described herein do not comprise any components used to inactivate a virus.

[00241] In certain embodiments, the pharmaceutical compositions described herein additionally comprise gelatin. In other embodiments, the pharmaceutical compositions described herein do not comprise gelatin.

[00242] In certain embodiments, the pharmaceutical compositions described herein additionally comprise one or more buffers, e.g., phosphate buffer and sucrose phosphate glutamate buffer. In other embodiments, the pharmaceutical compositions described herein do not comprise buffers.

[00243] In certain embodiments, the pharmaceutical compositions described herein additionally comprise one or more salts, e.g., sodium chloride, calcium chloride, sodium phosphate, monosodium glutamate, and aluminum salts (e.g., aluminum hydroxide, aluminum phosphate, alum (potassium aluminum sulfate), or a mixture of such aluminum salts). In other embodiments, the pharmaceutical compositions described herein do not comprise salts.

[00244] In specific embodiments, the the pharmaceutical compositions described herein are low-additive influenza virus vaccines, i.e., the pharmaceutical compositions do not comprise one or more additives commonly found in influenza virus vaccines. Low-additive influenza vaccines have been described (see, e.g., International Application No. PCT/IB2008/002238 published as International Publication No. WO 09/001217 which is herein incorporated by reference in its entirety).

[00245] The pharmaceutical compositions described herein can be included in a container, pack, or dispenser together with instructions for administration.

[00246] The pharmaceutical compositions described herein can be stored before use, e.g., the pharmaceutical compositions can be stored frozen (e.g., at about -20°C or at about -70°C); stored in refrigerated conditions (e.g., at about 4°C); or stored at room temperature (see International Application No. PCT/IB2007/001149 published as International Publication No. WO 07/110776, which is herein incorporated by reference in its entirety, for methods of storing compositions comprising influenza vaccines without refrigeration).

[00247] In certain embodiments, when the active compound in a pharmaceutical composition described herein is a cell engineered to express an influenza hemagglutinin stem domain polypeptide, the cells in the pharmaceutical composition are not mammalian cells (e.g., CB-1 cells).

5.11.1 Subunit Vaccines

[00248] In a specific embodiment, provided herein are subunit vaccines comprising an influenza hemagglutinin stem domain polypeptide described herein. In some embodiments, a subunit vaccine comprises an influenza hemagglutinin stem domain

polypeptide and one or more surface glycoproteins (*e.g.*, influenza virus neuraminidase), other targeting moieties or adjuvants. In specific embodiments, a subunit vaccine comprises a single influenza hemagglutinin stem domain polypeptide. In other embodiments, a subunit vaccine comprises two, three, four or more influenza hemagglutinin stem domain polypeptides. In specific embodiments, the influenza hemagglutinin stem domain polypeptide(s) used in a subunit vaccine is not membrane-bound, *i.e.*, it is soluble.

[00249] In certain embodiments, provided herein are subunit vaccines comprising about 10 µg to about 60 µg of one or more influenza hemagglutinin stem domain polypeptides described herein, about 0.001% to 0.01% thimerosal, about 0.1 µg to about 1.0 µg chicken egg protein, about 1.0 µg to about 5.0 µg polymyxin, about 1.0 µg to about 5.0 µg neomycin, about 0.1 µg to about 0.5 µg betapropiolactone, and about .001 to about .05 % w/v of nonylphenol ethoxylate per dose.

[00250] In a specific embodiment, a subunit vaccine provided herein comprises or consists of a 0.5 ml dose that comprises 45 µg of influenza hemagglutinin stem domain polypeptide(s) provided herein, ≤ 1.0 µg of mercury (from thimerosal), ≤ 1.0 µg chicken egg protein (*i.e.*, ovalbumin), ≤ 3.75 µg polymyxin, and ≤ 2.5 µg neomycin. In some embodiments, a subunit vaccine provided herein additionally comprises or consists of not more than 0.5 µg betapropiolactone, and not more than 0.015 % w/v of nonylphenol ethoxylate per dose. In some embodiments, the 0.5 ml dose subunit vaccine is packaged in a pre-filled syringe.

[00251] In a specific embodiment, a subunit vaccine provided herein consists of a 5.0 ml multidose vial (0.5 ml per dose) that comprises 45 µg of influenza hemagglutinin stem domain polypeptide(s) provided herein, 25.0 µg of mercury (from thimerosal), ≤ 1.0 µg chicken egg protein (*i.e.*, ovalbumin), ≤ 3.75 µg polymyxin, and ≤ 2.5 µg neomycin. In some embodiments, a subunit vaccine provided herein additionally comprises or consists of not more than 0.5 µg betapropiolactone, and not more than 0.015 % w/v of nonylphenol ethoxylate per dose.

[00252] In a specific embodiment, the subunit vaccine is prepared using influenza virus that was propagated in embryonated chicken eggs (*i.e.*, the components of the subunit vaccine (*e.g.*, a hemagglutinin stem domain polypeptide) are isolated from virus that was propagated in embryonated chicken eggs). In another specific embodiment, the subunit vaccine is prepared using influenza virus that was not propagated in embryonated chicken eggs (*i.e.*, the components of the subunit vaccine (*e.g.*, a

hemagglutinin stem domain polypeptide) are isolated from virus that was not propagated in embryonated chicken eggs). In another specific embodiment, the subunit vaccine is prepared using influenza virus that was propagated in mammalian cells, e.g., immortalized human cells (see, e.g., International Application No. PCT/EP2006/067566 published as International Publication No. WO 07/045674 which is herein incorporated by reference in its entirety) or canine kidney cells such as MDCK cells (see, e.g., International Application No. PCT/IB2007/003536 published as International Publication No. WO 08/032219 which is herein incorporated by reference in its entirety) (i.e., the components of the subunit vaccine (e.g., a hemagglutinin stem domain polypeptide) are isolated from virus that was propagated in mammalian cells). In another specific embodiment, the hemagglutinin stem domain polypeptide(s) in a subunit vaccine are prepared using an expression vector, e.g., a viral vector, plant vector or a bacterial vector (i.e., the hemagglutinin stem domain polypeptide(s) in the subunit vaccine are obtained/isolated from an expression vector).

5.11.2 Live Virus Vaccines

[00253] In one embodiment, provided herein are immunogenic compositions (e.g., vaccines) comprising live virus containing an influenza hemagglutinin stem domain polypeptide. In another embodiment, provided herein are immunogenic compositions (e.g., vaccines) comprising live virus that is engineered to encode an influenza hemagglutinin stem domain polypeptide, which is expressed by progeny virus produced in the subjects administered the compositions. In specific embodiments, the influenza hemagglutinin stem domain polypeptide is membrane-bound. In other specific embodiments, the influenza virus hemagglutinin stem domain polypeptide is not membrane-bound, i.e., soluble. In particular embodiments, the live virus is an influenza virus, such as described in Section 5.4, *supra*. In other embodiments, the live virus is a non-influenza virus, such as described in Section 5.5, *supra*. In some embodiments, the live virus is attenuated. In some embodiments, an immunogenic composition comprises two, three, four or more live viruses containing or engineered to express two, three, four or more different influenza hemagglutinin stem domain polypeptides.

[00254] In certain embodiments, provided herein are immunogenic compositions (e.g., vaccines) comprising about 10^5 to about 10^{10} fluorescent focus units (FFU) of live attenuated influenza virus containing one or more influenza hemagglutinin stem domain polypeptides described herein, about 0.1 to about 0.5 mg monosodium glutamate, about

1.0 to about 5.0 mg hydrolyzed procine gelatin, about 1.0 to about 5.0 mg arginine, about 10 to about 15 mg sucrose, about 1.0 to about 5.0 mg dibasic potassium phosphate, about 0.5 to about 2.0 mg monobasic potassium phosphate, and about 0.001 to about 0.05 µg/ml gentamicin sulfate per dose. In some embodiments, the immunogenic compositions (*e.g.*, vaccines) are packaged as pre-filled sprayers containing single 0.2 ml doses.

[00255] In a specific embodiment, provided herein are immunogenic compositions (*e.g.*, vaccines) comprising $10^{6.5}$ to $10^{7.5}$ FFU of live attenuated influenza virus containing one or more influenza hemagglutinin stem domain polypeptides described herein, 0.188 mg monosodium glutamate, 2.0 mg hydrolyzed procine gelatin, 2.42 mg arginine, 13.68 mg sucrose, 2.26 mg dibasic potassium phosphate, 0.96 mg monobasic potassium phosphate, and < 0.015 µg/ml gentamicin sulfate per dose. In some embodiments, the immunogenic compositions (*e.g.*, vaccines) are packaged as pre-filled sprayers containing single 0.2 ml doses.

[00256] In a specific embodiment, the live virus that contains an influenza hemagglutinin stem domain polypeptide is propagated in embryonated chicken eggs before its use in an immunogenic composition described herein. In another specific embodiment, the live virus that contains an influenza hemagglutinin stem domain polypeptide is not propagated in embryonated chicken eggs before its use in an immunogenic composition described herein. In another specific embodiment, the live virus that contains an influenza hemagglutinin stem domain polypeptide is propagated in mammalian cells, *e.g.*, immortalized human cells (see, *e.g.*, International Application No. PCT/EP2006/067566 published as International Publication No. WO 07/045674 which is herein incorporated by reference in its entirety) or canine kidney cells such as MDCK cells (see, *e.g.*, International Application No. PCT/IB2007/003536 published as International Publication No. WO 08/032219 which is herein incorporated by reference in its entirety) before its use in an immunogenic composition described herein.

[00257] An immunogenic composition comprising a live virus for administration to a subject may be preferred because multiplication of the virus in the subject may lead to a prolonged stimulus of similar kind and magnitude to that occurring in natural infections, and therefore, confer substantial, long lasting immunity.

5.11.3 Inactivated Virus Vaccines

[00258] In one embodiment, provided herein are immunogenic compositions (*e.g.*, vaccines) comprising an inactivated virus containing an influenza hemagglutinin stem domain polypeptide. In specific embodiments, the influenza hemagglutinin stem domain polypeptide is membrane-bound. In particular embodiments, the inactivated virus is an influenza virus, such as described in Section 5.4, *supra*. In other embodiments, the inactivated virus is a non-influenza virus, such as described in Section 5.5, *supra*. In some embodiments, an immunogenic composition comprises two, three, four or more inactivated viruses containing two, three, four or more different influenza hemagglutinin stem domain polypeptides. In certain embodiments, the inactivated virus immunogenic compositions comprise one or more adjuvants.

[00259] Techniques known to one of skill in the art may be used to inactivate viruses containing an influenza hemagglutinin stem domain polypeptide. Common methods use formalin, heat, or detergent for inactivation. *See, e.g.*, U.S. Patent No. 6,635,246, which is herein incorporated by reference in its entirety. Other methods include those described in U.S. Patent Nos. 5,891,705; 5,106,619 and 4,693,981, which are incorporated herein by reference in their entireties.

[00260] In certain embodiments, provided herein are immunogenic compositions (*e.g.*, vaccines) comprising inactivated influenza virus such that each dose of the immunogenic composition comprises about 15 to about 60 μ g of influenza hemagglutinin stem domain polypeptide described herein, about 1.0 to about 5.0 mg sodium chloride, about 20 to about 100 μ g monobasic sodium phosphate, about 100 to about 500 μ g dibasic sodium phosphate, about 5 to about 30 μ g monobasic potassium phosphate, about 5 to about 30 μ g potassium chloride, and about .5 to about 3.0 μ g calcium chloride. In some embodiments, the immunogenic compositions (*e.g.*, vaccines) are packaged as single 0.25 ml or single 0.5 ml doses. In other embodiments, the immunogenic compositions (*e.g.*, vaccines) are packaged as multi-dose formulations.

[00261] In certain embodiments, provided herein are immunogenic compositions (*e.g.*, vaccines) comprising inactivated influenza virus such that each dose of the immunogenic composition comprises about 15 to about 60 μ g of influenza hemagglutinin stem domain polypeptide described herein, about 0.001% to 0.01% thimerosal, about 1.0 to about 5.0 mg sodium chloride, about 20 to about 100 μ g monobasic sodium phosphate, about 100 to about 500 μ g dibasic sodium phosphate, about 5 to about 30 μ g monobasic potassium phosphate, about 5 to about 30 μ g potassium chloride, and about 0.5 to about 3.0 μ g calcium chloride per dose. In some

embodiments, the immunogenic compositions (*e.g.*, vaccines) are packaged as single 0.25 ml or single 0.5 ml doses. In other embodiments, the immunogenic compositions (*e.g.*, vaccines) are packaged as multi-dose formulations.

[00262] In a specific embodiment, immunogenic compositions (*e.g.*, vaccines) provided herein are packaged as single 0.25 ml doses and comprise 22.5 µg of influenza hemagglutinin stem domain polypeptide described herein, 2.05 mg sodium chloride, 40 µg monobasic sodium phosphate, 150 µg dibasic sodium phosphate, 10 µg monobasic potassium phosphate, 10 µg potassium chloride, and 0.75 µg calcium chloride per dose.

[00263] In a specific embodiment, immunogenic compositions (*e.g.*, vaccines) provided herein are packaged as single 0.5 ml doses and comprise 45 µg of influenza hemagglutinin stem domain polypeptide described herein, 4.1 mg sodium chloride, 80 µg monobasic sodium phosphate, 300 µg dibasic sodium phosphate, 20 µg monobasic potassium phosphate, 20 µg potassium chloride, and 1.5 µg calcium chloride per dose.

[00264] In a specific embodiment, immunogenic compositions (*e.g.*, vaccines) are packaged as multi-dose formulations comprising or consisting of 5.0 ml of vaccine (0.5 ml per dose) and comprise 24.5 µg of mercury (from thimerosal), 45 µg of influenza hemagglutinin stem domain polypeptide described herein, 4.1 mg sodium chloride, 80 µg monobasic sodium phosphate, 300 µg dibasic sodium phosphate, 20 µg monobasic potassium phosphate, 20 µg potassium chloride, and 1.5 µg calcium chloride per dose.

[00265] In a specific embodiment, the inactivated virus that contains an influenza hemagglutinin stem domain polypeptide was propagated in embryonated chicken eggs before its inactivation and subsequent use in an immunogenic composition described herein. In another specific embodiment, the inactivated virus that contains an influenza hemagglutinin stem domain polypeptide was not propagated in embryonated chicken eggs before its inactivation and subsequent use in an immunogenic composition described herein. In another specific embodiment, the inactivated virus that contains an influenza hemagglutinin stem domain polypeptide was propagated in mammalian cells, *e.g.*, immortalized human cells (see, *e.g.*, International Application No.

PCT/EP2006/067566 published as International Publication No. WO 07/045674 which is herein incorporated by reference in its entirety) or canine kidney cells such as MDCK cells (see, *e.g.*, International Application No. PCT/IB2007/003536 published as International Publication No. WO 08/032219 which is herein incorporated by reference in its entirety) before its inactivation and subsequent use in an immunogenic composition described herein.

5.11.4 Split Virus Vaccines

[00266] In one embodiment, an immunogenic composition comprising an influenza hemagglutinin stem domain polypeptide is a split virus vaccine. In some embodiments, split virus vaccine contains two, three, four or more different influenza hemagglutinin stem domain polypeptides. In certain embodiments, the influenza hemagglutinin stem domain polypeptide is/was membrane-bound. In certain embodiments, the split virus vaccines comprise one or more adjuvants.

[00267] Techniques for producing split virus vaccines are known to those skilled in the art. By way of non-limiting example, an influenza virus split vaccine may be prepared using inactivated particles disrupted with detergents. One example of a split virus vaccine that can be adapted for use in accordance with the methods described herein is the Fluzone®, Influenza Virus Vaccine (Zonal Purified, Subvirion) for intramuscular use, which is formulated as a sterile suspension prepared from influenza viruses propagated in embryonated chicken eggs. The virus-containing fluids are harvested and inactivated with formaldehyde. Influenza virus is concentrated and purified in a linear sucrose density gradient solution using a continuous flow centrifuge. The virus is then chemically disrupted using a nonionic surfactant, octoxinol-9, (Triton® X-100 - A registered trademark of Union Carbide, Co.) producing a “split virus.” The split virus is then further purified by chemical means and suspended in sodium phosphate-buffered isotonic sodium chloride solution.

[00268] In certain embodiments, provided herein are split virus vaccines comprising about 10 µg to about 60 µg of one or more influenza hemagglutinin stem domain polypeptides described herein, about 0.01 to about 1.0 mg octoxynol-10 (TRITON X-100®), about 0.5 to 0.5 mg α-tocopheryl hydrogen succinate, about 0.1 to 1.0 mg polysorbate 80 (Tween 80), about 0.001 to about 0.003 µg hydrocortisone, about 0.05 to about 0.3 µg gentamicin sulfate, about 0.5 to about 2.0 µg chicken egg protein (ovalbumin), about 25 to 75 µg formaldehyde, and about 25 to 75 µg sodium deoxycholate.

[00269] In a specific embodiment, a split virus vaccine provided herein comprises or consists of a 0.5 ml dose that comprises 45 µg of influenza hemagglutinin stem domain polypeptide(s) provided herein, ≤ 0.085 mg octoxynol-10 (TRITON X-100®), ≤ 0.1 mg α-tocopheryl hydrogen succinate, ≤ .415 mg polysorbate 80 (Tween 80), ≤ 0.0016 µg hydrocortisone, ≤ 0.15 µg gentamicin sulfate, ≤ 1.0 chicken egg protein (ovalbumin), ≤

50 µg formaldehyde, and ≤ 50 µg sodium deoxycholate. In some embodiments, the 0.5 ml dose subunit vaccine is packaged in a pre-filled syringe.

[00270] In a specific embodiment, the split virus vaccine is prepared using influenza virus that was propagated in embryonated chicken eggs. In another specific embodiment, the split virus vaccine is prepared using influenza virus that was not propagated in embryonated chicken eggs. In another specific embodiment, the split virus vaccine is prepared using influenza virus that was propagated in mammalian cells, e.g., immortalized human cells (see, e.g., PCT/EP2006/067566 published as WO 07/045674 which is herein incorporated by reference in its entirety) or canine kidney cells such as MDCK cells (see, e.g., PCT/IB2007/003536 published as WO 08/032219 which is herein incorporated by reference in its entirety).

5.11.5 Adjuvants

[00271] In certain embodiments, the compositions described herein comprise, or are administered in combination with, an adjuvant. The adjuvant for administration in combination with a composition described herein may be administered before, concomitantly with, or after administration of said composition. In some embodiments, the term “adjuvant” refers to a compound that when administered in conjunction with or as part of a composition described herein augments, enhances and/or boosts the immune response to an influenza hemagglutinin stem domain polypeptide, but when the compound is administered alone does not generate an immune response to the polypeptide. In some embodiments, the adjuvant generates an immune response to the polypeptide and does not produce an allergy or other adverse reaction. Adjuvants can enhance an immune response by several mechanisms including, e.g., lymphocyte recruitment, stimulation of B and/or T cells, and stimulation of macrophages.

[00272] In certain embodiments, an adjuvant augments the intrinsic response to the influenza hemagglutinin stem domain polypeptide without causing conformational changes in the polypeptide that affect the qualitative form of the response. Specific examples of adjuvants include, but are not limited to, aluminum salts (alum) (such as aluminum hydroxide, aluminum phosphate, and aluminum sulfate), 3 De-O-acylated monophosphoryl lipid A (MPL) (see GB 2220211), MF59 (Novartis), AS03 (GlaxoSmithKline), AS04 (GlaxoSmithKline), polysorbate 80 (Tween 80; ICL Americas, Inc.), imidazopyridine compounds (see International Application No. PCT/US2007/064857, published as International Publication No. WO2007/109812),

imidazoquinoxaline compounds (see International Application No. PCT/US2007/064858, published as International Publication No. WO2007/109813) and saponins, such as QS21 (see Kensil *et al.*, in Vaccine Design: The Subunit and Adjuvant Approach (eds. Powell & Newman, Plenum Press, NY, 1995); U.S. Pat. No. 5,057,540). In some embodiments, the adjuvant is Freund's adjuvant (complete or incomplete). Other adjuvants are oil in water emulsions (such as squalene or peanut oil), optionally in combination with immune stimulants, such as monophosphoryl lipid A (see Stoute *et al.*, N. Engl. J. Med. 336, 86-91 (1997)). Another adjuvant is CpG (Bioworld Today, Nov. 15, 1998). Such adjuvants can be used with or without other specific immunostimulating agents such as MPL or 3-DMP, QS21, polymeric or monomeric amino acids such as polyglutamic acid or polylysine, or other immunopotentiating agents described in Section 5.4, *supra*. It should be understood that different formulations of influenza hemagglutinin stem domain polypeptide may comprise different adjuvants or may comprise the same adjuvant.

5.12 PROPHYLACTIC AND THERAPEUTIC USES

[00273] In one aspect, provided herein are methods for inducing an immune response in a subject utilizing an active compound, i.e., an influenza hemagglutinin stem domain polypeptide described herein, a nucleic acid encoding such a polypeptide, a vector (*e.g.*, a viral vector, or a bacteria) containing or expressing such a polypeptide, or cells stimulated with such a polypeptide. In a specific embodiment, a method for inducing an immune response to an influenza virus hemagglutinin polypeptide in a subject comprises administering to a subject in need thereof an effective amount of an influenza virus hemagglutinin stem domain polypeptide or an immunogenic composition thereof. In another embodiment, a method for inducing an immune response to an influenza virus hemagglutinin polypeptide in a subject comprises administering to a subject in need thereof an effective amount of a nucleic acid encoding an influenza hemagglutinin stem domain polypeptide or an immunogenic composition thereof. In another embodiment, a method for inducing an immune response to an influenza virus hemagglutinin polypeptide in a subject comprises administering to a subject in need thereof an effective amount of a viral vector containing or expressing an influenza hemagglutinin stem domain polypeptide or an immunogenic composition thereof. In yet another embodiment, a method for inducing an immune response to an influenza virus

hemagglutinin polypeptide in a subject comprises administering to a subject in need thereof an effective amount of cells stimulated with an influenza hemagglutinin stem domain polypeptide or a pharmaceutical composition thereof. In certain embodiments, an influenza hemagglutinin stem domain polypeptide used in the method is a purified influenza hemagglutinin stem domain polypeptide derived from a mammalian cell, a plant cell, or an insect cell.

[00274] In a specific embodiment, a method for inducing an immune response to an influenza virus hemagglutinin polypeptide in a subject comprises administering to a subject in need thereof a subunit vaccine described herein. In another embodiment, a method for inducing an immune response to an influenza virus hemagglutinin polypeptide in a subject comprises administering to a subject in need thereof a live virus vaccine described herein. In particular embodiments, the live virus vaccine comprises an attenuated virus. In another embodiment, a method for inducing an immune response to an influenza virus hemagglutinin polypeptide in a subject comprises administering to a subject in need thereof an inactivated virus vaccine described herein. In another embodiment, a method for inducing an immune response to an influenza virus hemagglutinin polypeptide in a subject comprises administering to a subject in need thereof a split virus vaccine described herein. In another embodiment, a method for inducing an immune response to an influenza virus hemagglutinin polypeptide in a subject comprises administering to a subject in need thereof a viral-like particle vaccine described herein. In another embodiment, a method for inducing an immune response to an influenza hemagglutinin polypeptide comprises administering to a subject in need thereof a virosome described herein. In another embodiment, a method for inducing an immune response to an influenza hemagglutinin polypeptide comprises administering to a subject in need thereof a bacteria expressing or engineered to express an influenza hemagglutinin stem domain polypeptide or a composition thereof. In certain embodiments, an influenza hemagglutinin stem domain polypeptide used in the method is a purified influenza hemagglutinin stem domain polypeptide derived from a mammalian cell, a plant cell, or an insect cell.

[00275] In some embodiments, the immune response induced by an active compound or a composition described herein is effective to prevent and/or treat an influenza virus infection caused by any subtype or strain of influenza virus. In certain embodiments, the immune response induced by an active compound or a composition described herein is effective to prevent and/or treat an influenza virus infection caused by a subtype of

influenza virus that belongs to one HA group (e.g., Group 1, which comprises H1, H2, H5, H6, H8, H9, H11, H12, H13, and H16) and not the other HA group (e.g., Group 2, which comprises H3, H4, H7, H10, H14, and H15). For example, the immune response induced may be effective to prevent and/or treat an influenza virus infection caused by an influenza virus that belongs to the HA group consisting of H11, H13, H16, H9, H8, H12, H6, H1, H5 and H2. Alternatively, the immune response induced may be effective to prevent and/or treat an influenza virus infection caused by an influenza virus that belongs to the HA group consisting of H3, H4, H14, H10, H15 and H7. In some embodiments, the immune response induced by an active compound or a composition described herein is effective to prevent and/or treat an influenza virus infection caused by one, two, three, four or five subtypes of influenza virus. In certain embodiments, the immune response induced by an active compound or a composition described herein is effective to prevent and/or treat an influenza virus infection caused by six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen or fifteen subtypes of influenza virus. In some embodiments, the immune response induced by an active compound or a composition described herein is effective to prevent and/or treat an influenza virus infection caused by one or more variants within the same subtype of influenza virus.

[00276] In some embodiments, the immune response induced by an active compound or a composition described herein is effective to prevent and/or treat an influenza virus infection caused by both H1N1 and H2N2 subtypes. In other embodiments, the immune response induced by an active compound or a composition described herein is not effective to prevent and/or treat an influenza virus infection caused by both H1N1 and H2N2 subtypes. In some embodiments, the immune response induced by an active compound or a composition described herein is effective to prevent and/or treat an influenza virus infection caused by H1N1, H2N2, and H3N2 subtypes. In some embodiments, the immune response induced by an active compound or a composition described herein is effective to prevent and/or treat an influenza virus infection caused by H3N2 subtypes. In other embodiments, the immune response induced by an active compound or a composition described herein is not effective to prevent and/or treat an influenza virus infection caused by H3N2 subtypes.

[00277] In some embodiments, the immune response induced by an active compound or a composition described herein is effective to prevent and/or treat an influenza virus disease caused by any subtype or strain of influenza virus. In certain embodiments, the immune response induced by an active compound or a composition described herein is

effective to prevent and/or treat an influenza virus disease caused by a subtype of influenza virus that belongs to one HA group and not the other HA group. For example, the immune response induced may be effective to prevent and/or treat an influenza virus disease caused by an influenza virus that belongs to the HA group consisting of H11, H13, H16, H9, H8, H12, H6, H1, H5 and H2. Alternatively, the immune response induced may be effective to prevent and/or treat an influenza virus disease caused by an influenza virus that belongs to the HA group consisting of H3, H4, H14, H10, H15 and H7. In some embodiments, the immune response induced by an active compound or a composition described herein is effective to prevent and/or treat an influenza virus disease caused by any of one, two, three, four or five subtypes of influenza virus. In certain embodiments, the immune response induced by an active compound or a composition described herein is effective to prevent and/or treat an influenza virus disease caused by any of six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen or fifteen subtypes of influenza virus. In some embodiments, the immune response induced by an active compound or a composition described herein is effective to prevent and/or treat an influenza virus disease caused by one or more variants within the same subtype of influenza virus.

[00278] In some embodiments, the immune response induced by an active compound or a composition described herein is effective to reduce symptoms resulting from an influenza virus disease/infection. Symptoms of influenza virus disease/infection include, but are not limited to, body aches (especially joints and throat), fever, nausea, headaches, irritated eyes, fatigue, sore throat, reddened eyes or skin, and abdominal pain.

[00279] In some embodiments, the immune response induced by an active compound or a composition described herein is effective to reduce the hospitalization of a subject suffering from an influenza virus disease/infection. In some embodiments, the immune response induced by an active compound or a composition described herein is effective to reduce the duration of hospitalization of a subject suffering from an influenza virus disease/infection.

[00280] In another aspect, provided herein are methods for preventing and/or treating an influenza virus infection in a subject utilizing an active compound (e.g., an influenza hemagglutinin stem domain polypeptide described herein, a nucleic acid encoding such a polypeptide, a vector containing or expressing such a polypeptide, or cells stimulated with such a polypeptide) or a composition described herein. In one embodiment, a

method for preventing or treating an influenza virus infection in a subject comprises administering to a subject in need thereof an influenza hemagglutinin stem domain polypeptide, a nucleic acid encoding such a polypeptide, a vector containing or expressing such a polypeptide, or a composition of any one of the foregoing. In a specific embodiment, a method for preventing or treating an influenza virus infection in a subject comprises administering to a subject in need thereof a subunit vaccine, a live virus vaccine, an inactivated virus vaccine, a split virus vaccine or a viral-like particle vaccine.

[00281] In another aspect, provided herein are methods for preventing and/or treating an influenza virus disease in a subject utilizing an influenza hemagglutinin stem domain polypeptide described herein, a nucleic acid encoding such a polypeptide, a vector containing or expressing such a polypeptide, or cells stimulated with such a polypeptide. In a specific embodiment, a method for preventing or treating an influenza virus disease in a subject comprises administering to a subject in need thereof an effective amount of an influenza hemagglutinin stem domain polypeptide or an immunogenic composition thereof. In another embodiment, a method for preventing or treating an influenza virus disease in a subject comprises administering to a subject in need thereof an effective amount of a nucleic acid encoding an influenza hemagglutinin stem domain polypeptide or an immunogenic composition thereof. In another embodiment, a method for preventing or treating an influenza virus disease in a subject comprises administering to a subject in need thereof an effective amount of a viral vector containing or expressing an influenza hemagglutinin stem domain polypeptide or an immunogenic composition thereof. In yet another embodiment, a method for preventing or treating an influenza virus disease in a subject comprises administering to a subject in need thereof an effective amount of cells stimulated with an influenza hemagglutinin stem domain polypeptide or a pharmaceutical composition thereof.

[00282] In a specific embodiment, a method for preventing or treating an influenza virus disease in a subject comprises administering to a subject in need thereof a subunit vaccine described herein. In another embodiment, a method for preventing or treating an influenza virus disease in a subject comprises administering to a subject in need thereof a live virus vaccine described herein. In particular embodiments, the live virus vaccine comprises an attenuated virus. In another embodiment, a method for preventing or treating an influenza virus disease in a subject comprises administering to a subject in need thereof an inactivated virus vaccine described herein. In another embodiment, a

method for preventing or treating an influenza virus disease in a subject comprises administering to a subject in need thereof a split virus vaccine described herein. In another embodiment, a method for preventing or treating an influenza virus disease comprises administering to a subject in need thereof a viral-like particle vaccine described herein. In another embodiment, a method for preventing or treating an influenza virus disease in a subject, comprising administering to a subject in need thereof a virosome described herein. In another embodiment, a method for preventing or treating an influenza virus disease in a subject comprising administering to a subject in need thereof a bacteria expressing or engineered to express an influenza hemagglutinin stem domain polypeptide or a composition thereof.

[00283] In another aspect, provided herein are methods of preventing and/or treating an influenza virus disease in a subject by administering neutralizing antibodies described herein. In a specific embodiment, a method for preventing or treating an influenza virus disease in a subject comprises administering to a subject in need thereof an effective amount of a neutralizing antibody described herein, or a pharmaceutical composition thereof. In particular embodiments, the neutralizing antibody is a monoclonal antibody. In certain embodiments, the neutralizing antibody is not CR6261, CR6325, CR6329, CR6307, CR6323, 2A, D7, D8, F10, G17, H40, A66, D80, E88, E90, H98, C179 (FERM BP-4517), AI3C (FERM BP-4516) or any other antibody described in Ekiert *DC et al.* (2009) Antibody Recognition of a Highly Conserved Influenza Virus Epitope. *Science* (published in *Science Express* February 26, 2009); Kashyap *et al.* (2008) Combinatorial antibody libraries from survivors of the Turkish H5N1 avian influenza outbreak reveal virus neutralization strategies. *Proc Natl Acad Sci U S A* 105: 5986-5991; Sui *et al.* (2009) Structural and functional bases for broad-spectrum neutralization of avian and human influenza A viruses. *Nat Struct Mol Biol* 16: 265-273; U.S. Patent Nos. 5,589,174, 5,631,350, 6,337,070, and 6,720,409; International Application No. PCT/US2007/068983 published as International Publication No. WO 2007/134237; International Application No. PCT/US2008/075998 published as International Publication No. WO 2009/036157; International Application No. PCT/EP2007/059356 published as International Publication No. WO 2008/028946; and International Application No. PCT/US2008/085876 published as International Publication No. WO 2009/079259. In other embodiments, the neutralizing antibody is not an antibody described in Wang *et al.* (2010) "Broadly Protective Monoclonal Antibodies against H3

Influenza Viruses following Sequential Immunization with Different Hemagglutinins,” PLOS Pathogens 6(2):1-9.

[00284] In certain embodiments, the methods for preventing or treating an influenza virus disease or infection in a subject (e.g., a human or non-human animal) provided herein result in a reduction in the replication of the influenza virus in the subject as measured by *in vivo* and *in vitro* assays known to those of skill in the art and described herein. In some embodiments, the replication of the influenza virus is reduced by approximately 1 log or more, approximately 2 logs or more, approximately 3 logs or more, approximately 4 logs or more, approximately 5 logs or more, approximately 6 logs or more, approximately 7 logs or more, approximately 8 logs or more, approximately 9 logs or more, approximately 10 logs or more, 1 to 3 logs, 1 to 5 logs, 1 to 8 logs, 1 to 9 logs, 2 to 10 logs, 2 to 5 logs, 2 to 7 logs, 2 logs to 8 logs, 2 to 9 logs, 2 to 10 logs 3 to 5 logs, 3 to 7 logs, 3 to 8 logs, 3 to 9 logs, 4 to 6 logs, 4 to 8 logs, 4 to 9 logs, 5 to 6 logs, 5 to 7 logs, 5 to 8 logs, 5 to 9 logs, 6 to 7 logs, 6 to 8 logs, 6 to 9 logs, 7 to 8 logs, 7 to 9 logs, or 8 to 9 logs.

5.12.1 Combination therapies

[00285] In various embodiments, an influenza hemagglutinin stem domain polypeptide described herein, a nucleic acid encoding such a polypeptide, a vector (e.g., a viral vector or a bacteria) containing or expressing such a polypeptide, cells stimulated with such a polypeptide, or a neutralizing antibody may be administered to a subject in combination with one or more other therapies (e.g., antiviral, antibacterial, or immunomodulatory therapies). In some embodiments, a pharmaceutical composition (e.g., an immunogenic composition) described herein may be administered to a subject in combination with one or more therapies. The one or more other therapies may be beneficial in the treatment or prevention of an influenza virus disease or may ameliorate a symptom or condition associated with an influenza virus disease. In some embodiments, the one or more other therapies are pain relievers, anti-fever medications, or therapies that alleviate or assist with breathing. In certain embodiments, the therapies are administered less than 5 minutes apart, less than 30 minutes apart, 1 hour apart, at about 1 hour apart, at about 1 to about 2 hours apart, at about 2 hours to about 3 hours apart, at about 3 hours to about 4 hours apart, at about 4 hours to about 5 hours apart, at about 5 hours to about 6 hours apart, at about 6 hours to about 7 hours apart, at about 7 hours to about 8 hours apart, at about 8 hours to about 9 hours apart, at about 9 hours to

about 10 hours apart, at about 10 hours to about 11 hours apart, at about 11 hours to about 12 hours apart, at about 12 hours to 18 hours apart, 18 hours to 24 hours apart, 24 hours to 36 hours apart, 36 hours to 48 hours apart, 48 hours to 52 hours apart, 52 hours to 60 hours apart, 60 hours to 72 hours apart, 72 hours to 84 hours apart, 84 hours to 96 hours apart, or 96 hours to 120 hours part. In specific embodiments, two or more therapies are administered within the same patent visit.

[00286] Any anti-viral agents well-known to one of skill in the art may used in combination with an active compound or pharmaceutical composition described herein. Non-limiting examples of anti-viral agents include proteins, polypeptides, peptides, fusion proteins antibodies, nucleic acid molecules, organic molecules, inorganic molecules, and small molecules that inhibit and/or reduce the attachment of a virus to its receptor, the internalization of a virus into a cell, the replication of a virus, or release of virus from a cell. In particular, anti-viral agents include, but are not limited to, nucleoside analogs (*e.g.*, zidovudine, acyclovir, gancyclovir, vidarabine, idoxuridine, trifluridine, and ribavirin), foscarnet, amantadine, peramivir, rimantadine, saquinavir, indinavir, ritonavir, alpha-interferons and other interferons, AZT, zanamivir (Relenza®), and oseltamivir (Tamiflu®). Other anti-viral agents include influenza virus vaccines, *e.g.*, Fluarix® (GlaxoSmithKline), FluMist® (MedImmune Vaccines), Fluvirin® (Chiron Corporation), Flulaval® (GlaxoSmithKline), Afluria® (CSL Biotherapies Inc.), Agriflu® (Novartis) or Fluzone® (Aventis Pasteur).

[00287] In specific embodiments, the anti-viral agent is an immunomodulatory agent that is specific for a viral antigen. In particular embodiments, the viral antigen is an influenza virus polypeptide other than a hemagglutinin polypeptide. In other embodiments, the viral antigen is an influenza virus hemagglutinin polypeptide.

[00288] Any anti-bacterial agents known to one of skill in the art may used in combination with an active compound or pharmaceutical composition described herein. Non-limiting examples of anti-bacterial agents include Amikacin, Amoxicillin, Amoxicillin-clavulanic acid, Amphotericin-B, Ampicillin, Ampicillin-sulbactam, Apramycin, Azithromycin, Aztreonam, Bacitracin, Benzylpenicillin, Caspofungin, Cefaclor, Cefadroxil, Cefalexin, Cefalothin, Cefazolin, Cefdinir, Cefepime, Cefixime, Cefmenoxime, Cefoperazone, Cefoperazone-sulbactam, Cefotaxime, Cefoxitin, Cefpirome, Cefpodoxime, Cefpodoxime-clavulanic acid, Cefpodoxime-sulbactam, Cefprozil, Cefquinome, Ceftazidime, Ceftibutin, Ceftiofur, Cefetobiprole, Ceftriaxon, Cefuroxime, Chloramphenicol, Florfenicol, Ciprofloxacin, Clarithromycin,

Clinafloxacin, Clindamycin, Cloxacillin, Colistin, Cotrimoxazol (Trimthoprim/sulphamethoxazole), Dalbavancin, Dalfopristin/Quinopristin, Daptomycin, Dibekacin, Dicloxacillin, Doripenem, Doxycycline, Enrofloxacin, Ertapenem, Erythromycin, Flucloxacillin, Fluconazol, Flucytosin, Fosfomycin, Fusidic acid, Garenoxacin, Gatifloxacin, Gemifloxacin, Gentamicin, Imipenem, Itraconazole, Kanamycin, Ketoconazole, Levofloxacin, Lincomycin, Linezolid, Loracarbef, Mecillnam (amdinocillin), Meropenem, Metronidazole, Meziocillin, Mezlocillin-sulbactam, Minocycline, Moxifloxacin, Mupirocin, Nalidixic acid, Neomycin, Netilmicin, Nitrofurantoin, Norfloxacin, Ofloxacin, Oxacillin, Pefloxacin, Penicillin V, Piperacillin, Piperacillin-sulbactam, Piperacillin-tazobactam, Rifampicin, Roxythromycin, Sparfloxacin, Spectinomycin, Spiramycin, Streptomycin, Sulbactam, Sulfamethoxazole, Teicoplanin, Telavancin, Telithromycin, Temocillin, Tetracyclin, Ticarcillin, Ticarcillin-clavulanic acid, Tigecycline, Tobramycin, Trimethoprim, Trovafloxacin, Tylosin, Vancomycin, Virginiamycin, and Voriconazole.

[00289] In some embodiments, a combination therapy comprises active immunization with an influenza hemagglutinin stem domain polypeptide, or one or more vectors described in Sections 5.2–5.7 and passive immunization with one or more neutralizing antibodies described in Section 5.9. In some embodiments, a combination therapy comprises immunization with one or more vectors described in Sections 5.2–5.7 and administration of cells (*e.g.*, by adoptive transfer) described in Section 5.9.

[00290] In some embodiments, a combination therapy comprises administration of two or more different vectors described in Sections 5.2–5.7. In one example, one or more vectors expressing an influenza hemagglutinin stem domain polypeptide derived from an influenza A virus hemagglutinin polypeptide and one or more vectors expressing an influenza hemagglutinin stem domain polypeptide derived from an influenza B virus hemagglutinin polypeptide are administered in combination. In some embodiments, a combination therapy comprises administration of a vector expressing an influenza hemagglutinin stem domain polypeptide derived from an influenza A virus H3 antigen and a vector expressing an influenza hemagglutinin stem domain polypeptide derived from an influenza A virus H1 antigen. In some embodiments, the combination therapy comprises administration of a vector expressing an influenza hemagglutinin stem domain polypeptide derived from an influenza A virus H3 antigen, a vector expressing an influenza hemagglutinin stem domain polypeptide derived from an

influenza A virus H1 antigen, and a vector expressing an influenza hemagglutinin stem domain polypeptide derived from an influenza B virus hemagglutinin polypeptide.

[00291] In some embodiments, a combination therapy comprises active immunization with an active compound that induces an immune response to one, two, three, or more HA subtypes in one HA group (e.g., Group 1) in combination with an active compound that induces an immune response to one, two, three, or more HA subtypes in the other HA group (e.g., Group 2).

[00292] In some embodiments, a combination therapy comprises active immunization with two or more influenza hemagglutinin stem domain polypeptides described in Section 5.1.

[00293] In certain embodiments, a combination therapy comprises active immunization with one, two, or more influenza hemagglutinin stem domain polypeptides derived from an influenza A virus and one or more influenza hemagglutinin stem domain polypeptides derived from an influenza B virus.

5.12.2 Patient Populations

[00294] In certain embodiments, an active compound or composition described herein may be administered to a naïve subject, *i.e.*, a subject that does not have a disease caused by influenza virus infection or has not been and is not currently infected with an influenza virus infection. In one embodiment, an active compound or composition described herein is administered to a naïve subject that is at risk of acquiring an influenza virus infection. In one embodiment, an active compound or composition described herein is administered to a subject that does not have a disease caused by the specific influenza virus, or has not been and is not infected with the specific influenza virus to which the influenza hemagglutinin stem domain polypeptide induces an immune response. An active compound or composition described herein may also be administered to a subject that is and/or has been infected with the influenza virus or another type, subtype or strain of the influenza virus to which the influenza hemagglutinin stem domain polypeptide induces an immune response.

[00295] In certain embodiments, an active compound or composition described herein is administered to a patient who has been diagnosed with an influenza virus infection. In some embodiments, an active compound or composition described herein is administered to a patient infected with an influenza virus before symptoms manifest or symptoms become severe (e.g., before the patient requires hospitalization). In some

embodiments, an active compound or composition described herein is administered to a patient that is infected with or has been diagnosed with a different type of influenza virus than that of the influenza virus from which the HA stem domain polypeptide of the active compound or composition was derived.

[00296] In certain embodiments, an active compound or composition described herein is administered to a patient that may be or is infected with an influenza virus that belongs to the same HA group as that of the influenza hemagglutinin stem domain polypeptide. In certain embodiments, an active compound or composition described herein is administered to a patient that may be or is infected with an influenza virus of the same subtype as that of the influenza hemagglutinin stem domain polypeptide.

[00297] In some embodiments, a subject to be administered an active compound or composition described herein is an animal. In certain embodiments, the animal is a bird. In certain embodiments, the animal is a canine. In certain embodiments, the animal is a feline. In certain embodiments, the animal is a horse. In certain embodiments, the animal is a cow. In certain embodiments, the animal is a mammal, *e.g.*, a horse, swine, mouse, or primate, preferably a human.

[00298] In certain embodiments, a subject to be administered an active compound or composition described herein is a human adult. In certain embodiments, a subject to be administered an active compound or composition described herein is a human adult more than 50 years old. In certain embodiments, a subject to be administered an active compound or composition described herein is an elderly human subject.

[00299] In certain embodiments, a subject to be administered an active compound or composition described herein is a human child. In certain embodiments, a subject to be administered an active compound or composition described herein is a human infant. In certain embodiments, a subject to whom an active compound or composition described herein is administered is not an infant of less than 6 months old. In a specific embodiment, a subject to be administered an active compound or composition described herein is 2 years old or younger.

[00300] In specific embodiments, a subject to be administered an active compound or composition described herein is any infant or child more than 6 months of age and any adult over 50 years of age. In other embodiments, the subject is an individual who is pregnant. In another embodiment, the subject is an individual who may or will be pregnant during the influenza season (*e.g.*, November to April). In specific

embodiments, a subject to be administered an active compound or composition described herein is a woman who has given birth 1, 2, 3, 4, 5, 6, 7, or 8 weeks earlier.

[00301] In some embodiments, the human subject to be administered an active compound or composition described herein is any individual at increased risk of influenza virus infection or disease resulting from influenza virus infection (*e.g.*, an immunocompromised or immunodeficient individual). In some embodiments, the human subject to be administered an active compound or composition described herein is any individual in close contact with an individual with increased risk of influenza virus infection or disease resulting from influenza virus infection (*e.g.*, immunocompromised or immunosuppressed individuals).

[00302] In some embodiments, the human subject to be administered an active compound or composition described herein is an individual affected by any condition that increases susceptibility to influenza virus infection or complications or disease resulting from influenza virus infection. In other embodiments, an active compound or composition described herein is administered to a subject in which an influenza virus infection has the potential to increase complications of another condition that the individual is affected by, or for which they are at risk. In particular embodiments, such conditions that increase susceptibility to influenza virus complications or for which influenza virus increases complications associated with the condition are, *e.g.*, conditions that affect the lung, such as cystic fibrosis, emphysema, asthma, or bacterial infections (*e.g.*, infections caused by *Haemophilus influenzae*, *Streptococcus pneumoniae*, *Legionella pneumophila*, and *Chlamydia trachomatis*); cardiovascular disease (*e.g.*, congenital heart disease, congestive heart failure, and coronary artery disease); endocrine disorders (*e.g.*, diabetes), neurological and neuron-developmental conditions (*e.g.*, disorders of the brain, the spinal cord, the peripheral nerve, and muscle (such as cerebral palsy, epilepsy (seizure disorders), stroke, intellectual disability (*e.g.*, mental retardation), muscular dystrophy, and spinal cord injury)).

[00303] In some embodiments, the human subject to be administered an active compound or composition described herein is an individual that resides in a group home, such as a nursing home. In some embodiments, the human subject to be administered an active compound or composition described herein works in, or spends a significant amount of time in, a group home, *e.g.*, a nursing home. In some embodiments, the human subject to be administered an active compound or composition described herein is a health care worker (*e.g.*, a doctor or nurse). In some embodiments, the human

subject to be administered an active compound or composition described herein is a smoker. In a specific embodiment, the human subject to be administered an active compound or composition described herein is immunocompromised or immunosuppressed.

[00304] In addition, subjects at increased risk of developing complications from influenza who may be administered an active compound or composition described herein include: any individual who can transmit influenza viruses to those at high risk for complications, such as, *e.g.*, members of households with high-risk individuals, including households that will include infants younger than 6 months, individuals coming into contact with infants less than 6 months of age, or individuals who will come into contact with individuals who live in nursing homes or other long-term care facilities; individuals with long-term disorders of the lungs, heart, or circulation; individuals with metabolic diseases (*e.g.*, diabetes); individuals with kidney disorders; individuals with blood disorders (including anemia or sickle cell disease); individuals with weakened immune systems (including immunosuppression caused by medications, malignancies such as cancer, organ transplant, or HIV infection); children who receive long-term aspirin therapy (and therefore have a higher chance of developing Reye syndrome if infected with influenza).

[00305] In other embodiments, subjects for administration of an active compound or composition described herein include healthy individuals six months of age or older, who: plan to travel to foreign countries and areas where flu outbreaks may be occurring, such, *e.g.*, as the tropics and the Southern Hemisphere from April through September; travel as a part of large organized tourist groups that may include persons from areas of the world where influenza viruses are circulating; attend school or college and reside in dormitories, or reside in institutional settings; or wish to reduce their risk of becoming ill with influenza.

[00306] In some embodiments, a subject for whom administration of an active compound or composition described herein is contraindicated include any individual for whom influenza vaccination is contraindicated, such as: infants younger than six months of age; and individuals who have had an anaphylactic reaction (allergic reactions that cause difficulty breathing, which is often followed by shock) to eggs, egg products, or other components used in the production of the immunogenic formulation. In certain embodiments, when administration of an active compound or composition described herein is contraindicated due to one or more components used in the production of the

immunogenic formulation (e.g., due to the presence of egg or egg products), the active compound or composition may be produced in a manner that does not include the component that causes the administration of an active compound or composition to be contraindicated (e.g., the active compound or composition may be produced without the use of eggs or egg products).

[00307] In some embodiments, it may be advisable not to administer a live virus vaccine to one or more of the following patient populations: elderly humans; infants younger than 6 months old; pregnant individuals; infants under the age of 1 years old; children under the age of 2 years old; children under the age of 3 years old; children under the age of 4 years old; children under the age of 5 years old; adults under the age of 20 years old; adults under the age of 25 years old; adults under the age of 30 years old; adults under the age of 35 years old; adults under the age of 40 years old; adults under the age of 45 years old; adults under the age of 50 years old; elderly humans over the age of 70 years old; elderly humans over the age of 75 years old; elderly humans over the age of 80 years old; elderly humans over the age of 85 years old; elderly humans over the age of 90 years old; elderly humans over the age of 95 years old; children and adolescents (2-17 years of age) receiving aspirin or aspirin-containing medications, because of the complications associated with aspirin and wild-type influenza virus infections in this age group; individuals with a history of asthma or other reactive airway diseases; individuals with chronic underlying medical conditions that may predispose them to severe influenza infections; individuals with a history of Guillain-Barre syndrome; individuals with acute serious illness with fever; or individuals who are moderately or severely ill. For such individuals, administration of inactivated virus vaccines, split virus vaccines, subunit vaccines, virosomes, viral-like particles or the non-viral vectors described herein may be preferred. In certain embodiments, subjects preferably administered a live virus vaccine may include healthy children and adolescents, ages 2-17 years, and healthy adults, ages 18-49.

[00308] In certain embodiments, an immunogenic formulation comprising a live virus vector is not given concurrently with other live-virus vaccines.

5.13 MODES OF ADMINISTRATION

5.13.1 Routes of Delivery

[00309] An active compound or composition described herein may be delivered to a subject by a variety of routes. These include, but are not limited to, intranasal, intratracheal, oral, intradermal, intramuscular, intraperitoneal, transdermal, intravenous, conjunctival and subcutaneous routes. In some embodiments, a composition is formulated for topical administration, for example, for application to the skin. In specific embodiments, the route of administration is nasal, *e.g.*, as part of a nasal spray. In certain embodiments, a composition is formulated for intramuscular administration. In some embodiments, a composition is formulated for subcutaneous administration. In certain embodiments, a composition is not formulated for administration by injection. In specific embodiments for live virus vaccines, the vaccine is formulated for administration by a route other than injection.

[00310] In cases where the antigen is a viral vector, a virus-like particle vector, or a bacterial vector, for example, it may be preferable to introduce an immunogenic composition via the natural route of infection of the backbone virus or bacteria from which the vector was derived. Alternatively, it may be preferable to introduce an influenza hemagglutinin stem domain polypeptide via the natural route of infection of the influenza virus from which polypeptide is derived. The ability of an antigen, particularly a viral vector, to induce a vigorous secretory and cellular immune response can be used advantageously. For example, infection of the respiratory tract by a viral vector may induce a strong secretory immune response, for example in the urogenital system, with concomitant protection against an influenza virus. In addition, in a preferred embodiment it may be desirable to introduce the pharmaceutical compositions into the lungs by any suitable route. Pulmonary administration can also be employed, *e.g.*, by use of an inhaler or nebulizer, and formulation with an aerosolizing agent for use as a spray.

[00311] In a specific embodiment, a subunit vaccine is administered intramuscularly. In another embodiment, a live influenza virus or live NDV vaccine is administered intranasally. In another embodiment, an inactivated influenza virus vaccine, or a split influenza virus vaccine is administered intramuscularly. In another embodiment, an inactivated NDV virus vaccine or a split NDV virus vaccine is administered intramuscularly. In another embodiment, a viral-like particle or composition thereof is administered intramuscularly.

[00312] In some embodiments, cells stimulated with an influenza hemagglutinin stem domain polypeptide *in vitro* may be introduced (or re-introduced) into a subject using

techniques known to one of skill in the art. In some embodiments, the cells can be introduced into the dermis, under the dermis, or into the peripheral blood stream. In some embodiments, the cells introduced into a subject are preferably cells derived from that subject, to avoid an adverse immune response. In other embodiments, cells also can be used that are derived from a donor host having a similar immune background. Other cells also can be used, including those designed to avoid an adverse immunogenic response.

5.13.2 Dosage and Frequency of Administration

[00313] The amount of an active compound or composition which will be effective in the treatment and/or prevention of an influenza virus infection or an influenza virus disease will depend on the nature of the disease, and can be determined by standard clinical techniques.

[00314] The precise dose to be employed in the formulation will also depend on the route of administration, and the seriousness of the infection or disease caused by it, and should be decided according to the judgment of the practitioner and each subject's circumstances. For example, effective doses may also vary depending upon means of administration, target site, physiological state of the patient (including age, body weight, health), whether the patient is human or an animal, other medications administered, and whether treatment is prophylactic or therapeutic. Usually, the patient is a human but nonhuman mammals including transgenic mammals can also be treated. Treatment dosages are optimally titrated to optimize safety and efficacy.

[00315] In certain embodiments, an *in vitro* assay is employed to help identify optimal dosage ranges. Effective doses may be extrapolated from dose response curves derived from *in vitro* or animal model test systems.

[00316] Exemplary doses for nucleic acids encoding influenza hemagglutinin stem domain polypeptides range from about 10 ng to 1 g, 100 ng to 100 mg, 1 µg to 10 mg, or 30-300 µg nucleic acid, *e.g.*, DNA, per patient.

[00317] In certain embodiments, exemplary doses for influenza hemagglutinin stem domain polypeptides (*e.g.*, as provided in split virus vaccines and subunit vaccines) range from about 5 µg to 100 mg, 15 µg to 50 mg, 15 µg to 25 mg, 15 µg to 10 mg, 15 µg to 5 mg, 15 µg to 1 mg, 15 µg to 100 µg, 15 µg to 75 µg, 5 µg to 50 µg, 10 µg to 50 µg, 15 µg to 45 µg, 20 µg to 40 µg, or 25 to 35 µg per kilogram of the patient. In other embodiments, exemplary doses for influenza hemagglutinin stem domain

polypeptides range from about 1 µg to 50 mg, 5 µg to 50 mg, 1 µg to 100 mg, 5 µg to 100 mg, 15 µg to 50 mg, 15 µg to 25 mg, 15 µg to 10 mg, 15 µg to 5 mg, 15 µg to 1 mg, 15 µg to 100 µg, 15 µg to 75 µg, 5 µg to 50 µg, 10 µg to 50 µg, 15 µg to 45 µg, 20 µg to 40 µg, or 25 to 35 µg of influenza hemagglutinin stem domain polypeptides per dose.

[00318] Doses for infectious viral vectors may vary from 10-100, or more, virions per dose. In some embodiments, suitable dosages of a virus vector are 10^2 , 5×10^2 , 10^3 , 5×10^3 , 10^4 , 5×10^4 , 10^5 , 5×10^5 , 10^6 , 5×10^6 , 10^7 , 5×10^7 , 10^8 , 5×10^8 , 1×10^9 , 5×10^9 , 1×10^{10} , 5×10^{10} , 1×10^{11} , 5×10^{11} or 10^{12} pfu, and can be administered to a subject once, twice, three or more times with intervals as often as needed.

[00319] In certain embodiments, exemplary doses for VLPs range from about 0.01 µg to about 100 mg, about 0.1 µg to about 100 mg, about 5 µg to about 100 mg, about 15 µg to about 50 mg, about 15 µg to about 25 mg, about 15 µg to about 10 mg, about 15 µg to about 5 mg, about 15 µg to about 1 mg, about 15 µg to about 100 µg, about 15 µg to about 75 µg, about 5 µg to about 50 µg, about 10 µg to about 50 µg, about 15 µg to about 45 µg, about 20 µg to about 40 µg, or about 25 to about 35 µg per kilogram of the patient. In other embodiments, exemplary doses for influenza hemagglutinin stem domain polypeptides range from about 1 µg to about 50 mg, about 5 µg to about 50 mg, about 1 µg to about 100 mg, about 5 µg to about 100 mg, about 15 µg to about 50 mg, about 15 µg to about 25 mg, about 15 µg to about 10 mg, about 15 µg to about 5 mg, about 15 µg to about 1 mg, about 15 µg to about 100 µg, about 15 µg to about 75 µg, about 5 µg to about 50 µg, about 10 µg to about 50 µg, about 15 µg to about 45 µg, about 20 µg to about 40 µg, or about 25 to about 35 µg of influenza hemagglutinin stem domain polypeptides per dose, and can be administered to a subject once, twice, three or more times with intervals as often as needed.

[00320] In one embodiment, an inactivated vaccine is formulated such that it contains about 5 µg to about 50 µg, about 10 µg to about 50 µg, about 15 µg to about 100 µg, about 15 µg to about 75 µg, about 15 µg to about 50 µg, about 15 µg to about 30 µg, about 20 µg to about 50 µg, about 25 µg to about 40 µg, about 25 µg to about 35 µg of an influenza hemagglutinin stem domain polypeptide. Such a vaccine may contain a combination of one or more different influenza hemagglutinin stem domain polypeptides, for example, one or more influenza hemagglutinin stem domain polypeptides from an influenza A virus and one or more influenza hemagglutinin stem domain polypeptides from an influenza B virus. In some embodiments, influenza hemagglutinin stem domain polypeptides derived from, *e.g.*, A/H1N1, A/H3N2, and B

hemagglutinin polypeptides are included in a trivalent inactivated vaccine (TIV), formulated such that a 0.5-mL dose contains 15 µg each of influenza hemagglutinin stem domain polypeptide. In one embodiment, a live attenuated influenza vaccine (LAIV) is formulated such that a 0.2-mL dose contains $10^{6.5-7.5}$ fluorescent focal units of live attenuated influenza viruses from three strains expressing at least one influenza hemagglutinin stem domain polypeptide.

[00321] In certain embodiments, an active compound or composition is administered to a subject once as a single dose. In certain embodiments, an active compound or composition is administered to a subject as a single dose followed by a second dose 3 to 6 weeks later. In accordance with these embodiments, booster inoculations may be administered to the subject at 6 to 12 month intervals following the second inoculation. In certain embodiments, the booster inoculations may utilize a different active compound or composition. In some embodiments, the administration of the same active compound or composition may be repeated and the administrations may be separated by at least 1 day, 2 days, 3 days, 5 days, 10 days, 15 days, 30 days, 45 days, 2 months, 75 days, 3 months, or at least 6 months. In certain embodiments, an active compound or composition is administered to a subject as a single dose once per year.

[00322] In specific embodiments for administration to children, two doses of an active compound or composition, given at least one month apart, are administered to a child. In specific embodiments for administration to adults, a single dose is given. In another embodiment, two doses of an active compound or composition, given at least one month apart, are administered to an adult. In another embodiment, a young child (six months to nine years old) may be administered an active compound or composition for the first time in two doses given one month apart. In a particular embodiment, a child who received only one dose in their first year of vaccination should receive two doses in the following year. In some embodiments, two doses administered 4 weeks apart are preferred for children 2 -8 years of age who are administered an influenza vaccine, *e.g.*, an immunogenic formulation described herein, for the first time. In certain embodiments, for children 6-35 months of age, a half dose (0.25 ml) may be preferred, in contrast to 0.5 ml which may be preferred for subjects over three years of age.

[00323] In particular embodiments, an active compound or composition is administered to a subject in the fall or winter, *i.e.*, prior to or during the influenza season in each hemisphere. In one embodiment, children are administered their first dose early

in the season, *e.g.*, late September or early October, so that the second dose can be given prior to the peak of the influenza season.

[00324] For passive immunization with an antibody, the dosage ranges from about 0.0001 to 100 mg/kg, and more usually 0.01 to 5 mg/kg, of the patient body weight. For example, dosages can be 1 mg/kg body weight or 10 mg/kg body weight or within the range of 1-10 mg/kg or in other words, 70 mg or 700 mg or within the range of 70-700 mg, respectively, for a 70 kg patient. An exemplary treatment regime entails administration once per every two weeks or once a month or once every 3 to 6 months for a period of one year or over several years, or over several year-intervals. In some methods, two or more monoclonal antibodies with different binding specificities are administered simultaneously, in which case the dosage of each antibody administered falls within the ranges indicated. Antibody is usually administered on multiple occasions. Intervals between single dosages can be weekly, monthly or yearly. Intervals can also be irregular as indicated by measuring blood levels of antibody to the influenza hemagglutinin stem domain polypeptide in the patient.

5.14 BIOLOGICAL ASSAYS

5.14.1 Assays for Testing Activity of Influenza Hemagglutinin Stem Domain Polypeptide

[00325] Assays for testing the expression of a influenza hemagglutinin stem domain polypeptide in a vector disclosed herein may be conducted using any assay known in the art. For example, an assay for incorporation into a viral vector comprises growing the virus as described in this section or Sections 5.4 or 5.5, purifying the viral particles by centrifugation through a sucrose cushion, and subsequent analysis for influenza hemagglutinin stem domain polypeptide expression by an immunoassay, such as Western blotting, using methods well known in the art.

[00326] In one embodiment, an influenza hemagglutinin stem domain polypeptide disclosed herein is assayed for proper folding and functionality by testing its ability to bind specifically to a neutralizing antibody directed to an influenza virus hemagglutinin polypeptide, such as the stalk region of the polypeptide, using any assay for antibody-antigen interaction known in the art. Neutralizing antibodies for use in such assays include, for example, the neutralizing antibodies described in Ekiert *et al.*, 2009, *Science Express*, 26 February 2009; Kashyap *et al.*, 2008, *Proc Natl Acad Sci U S A* 105: 5986-5991; Sui *et al.* 2009, *Nature Structural and Molecular Biology*, 16:265-273; Wang *et*

al., 2010, *PLOS Pathogens* 6(2):1-9; U.S. Patent Nos. 5,589,174, 5,631,350, 6,337,070, and 6,720,409; International Application No. PCT/US2007/068983 published as International Publication No. WO 2007/134237; International Application No. PCT/US2008/075998 published as International Publication No. WO 2009/036157; International Application No. PCT/EP2007/059356 published as International Publication No. WO 2008/028946; and International Application No. PCT/US2008/085876 published as International Publication No. WO 2009/079259.. These antibodies include CR6261, CR6325, CR6329, CR6307, CR6323, 2A, D7, D8, F10, G17, H40, A66, D80, E88, E90, H98, C179 (FERM BP-4517), AI3C (FERM BP-4516), among others.

[00327] In another embodiment, an influenza hemagglutinin stem domain polypeptide disclosed herein is assayed for proper folding by determination of the structure or conformation of the influenza hemagglutinin stem domain polypeptide using any method known in the art such as, *e.g.*, NMR, X-ray crystallographic methods, or secondary structure prediction methods, *e.g.*, circular dichroism.

5.14.2 Assays for Testing Activity of Antibodies Generated using Influenza Hemagglutinin Stem Domain Polypeptide

[00328] Antibodies described herein may be characterized in a variety of ways known to one of skill in the art (*e.g.* ELISA, Surface Plasmon resonance display (BIAcore), Western blot, immunofluorescence, immunostaining and/or microneutralization assays). In some embodiments, antibodies are assayed for the ability to specifically bind to an influenza virus hemagglutinin polypeptide, or a vector comprising said polypeptide. Such an assay may be performed in solution (*e.g.*, Houghten, 1992, *Bio/Techniques* 13:412 421), on beads (Lam, 1991, *Nature* 354:82 84), on chips (Fodor, 1993, *Nature* 364:555 556), on bacteria (U.S. Patent No. 5,223,409), on spores (U.S. Patent Nos. 5,571,698; 5,403,484; and 5,223,409), on plasmids (Cull *et al.*, 1992, *Proc. Natl. Acad. Sci. USA* 89:1865 1869) or on phage (Scott and Smith, 1990, *Science* 249:386 390; Cwirla *et al.*, 1990, *Proc. Natl. Acad. Sci. USA* 87:6378 6382; and Felici, 1991, *J. Mol. Biol.* 222:301 310) (each of these references is incorporated herein in its entirety by reference).

[00329] Specific binding of an antibody to the influenza virus hemagglutinin polypeptide and cross-reactivity with other antigens can be assessed by any method known in the art. Immunoassays which can be used to analyze specific binding and cross-reactivity include, but are not limited to, competitive and non-competitive assay

systems using techniques such as western blots, radioimmunoassays, ELISA (enzyme linked immunosorbent assay), “sandwich” immunoassays, immunoprecipitation assays, precipitin reactions, gel diffusion precipitin reactions, immunodiffusion assays, agglutination assays, complement-fixation assays, immunoradiometric assays, fluorescent immunoassays, protein A immunoassays, to name but a few. Such assays are routine and well known in the art (see, e.g., Ausubel *et al.*, eds., 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York, which is incorporated by reference herein in its entirety).

[00330] The binding affinity of an antibody to an influenza virus hemagglutinin polypeptide and the off-rate of an antibody-antigen interaction can be determined by competitive binding assays. One example of a competitive binding assay is a radioimmunoassay comprising the incubation of labeled antigen (e.g., ^3H or ^{125}I) with the antibody of interest in the presence of increasing amounts of unlabeled antigen, and the detection of the antibody bound to the labeled antigen. The affinity of the antibody for an influenza virus hemagglutinin polypeptide and the binding off-rates can be determined from the data by Scatchard plot analysis. Competition with a second antibody can also be determined using radioimmunoassays. In this case, an influenza virus hemagglutinin polypeptide is incubated with the test antibody conjugated to a labeled compound (e.g., ^3H or ^{125}I) in the presence of increasing amounts of an unlabeled second antibody.

[00331] In certain embodiments, antibody binding affinity and rate constants are measured using the KinExA 3000 System (Sapidyne Instruments, Boise, ID). In some embodiments, surface plasmon resonance (e.g., BIAcore kinetic) analysis is used to determine the binding on and off rates of the antibodies to an influenza virus hemagglutinin polypeptide. BIAcore kinetic analysis comprises analyzing the binding and dissociation of influenza virus hemagglutinin polypeptide from chips with immobilized antibodies to an influenza virus hemagglutinin polypeptide on their surface. A typical BIAcore kinetic study involves the injection of 250 μL of an antibody reagent (mAb, Fab) at varying concentration in HBS buffer containing 0.005% Tween-20 over a sensor chip surface, onto which has been immobilized the influenza virus hemagglutinin polypeptide. The flow rate is maintained constant at 75 $\mu\text{L}/\text{min}$. Dissociation data is collected for 15 min or longer as necessary. Following each injection/dissociation cycle, the bound antibody is removed from the influenza virus hemagglutinin polypeptide surface using brief, 1 min pulses of dilute acid, typically 10-100 mM HCl, though other

regenerants are employed as the circumstances warrant. More specifically, for measurement of the rates of association, k_{on} , and dissociation, k_{off} , the polypeptide is directly immobilized onto the sensor chip surface through the use of standard amine coupling chemistries, namely the EDC/NHS method (EDC= N-diethylaminopropyl)-carbodiimide). Briefly, a 5-100 nM solution of the polypeptide in 10 mM NaOAc, pH 4 or pH 5 is prepared and passed over the EDC/NHS-activated surface until approximately 30-50 RU's worth of polypeptide are immobilized. Following this, the unreacted active esters are "capped" off with an injection of 1M Et-NH₂. A blank surface, containing no polypeptide, is prepared under identical immobilization conditions for reference purposes. Once an appropriate surface has been prepared, a suitable dilution series of each one of the antibody reagents is prepared in HBS/Tween-20, and passed over both the polypeptide and reference cell surfaces, which are connected in series. The range of antibody concentrations that are prepared varies, depending on what the equilibrium binding constant, K_D , is estimated to be. As described above, the bound antibody is removed after each injection/dissociation cycle using an appropriate regenerant.

[00332] The neutralizing activity of an antibody can be determined utilizing any assay known to one skilled in the art. Antibodies described herein can be assayed for their ability to inhibit the binding of an influenza virus, or any other composition comprising influenza virus hemagglutinin polypeptide (*e.g.*, a VLP, liposome, or detergent extract), to its host cell receptor (*i.e.*, sialic acid) using techniques known to those of skill in the art. For example, cells expressing influenza virus receptors can be contacted with a composition comprising influenza virus hemagglutinin polypeptide in the presence or absence of the antibody and the ability of the antibody to inhibit the antigen's binding can be measured by, for example, flow cytometry or a scintillation assay. The composition comprising an influenza virus hemagglutinin polypeptide or the antibody can be labeled with a detectable compound such as a radioactive label (*e.g.*, ³²P, ³⁵S, and ¹²⁵I) or a fluorescent label (*e.g.*, fluorescein isothiocyanate, rhodamine, phycoerythrin, phycocyanin, allophycocyanin, o-phthaldehyde and fluorescamine) to enable detection of an interaction between the composition comprising an influenza virus hemagglutinin polypeptide and a cell receptor. Alternatively, the ability of antibodies to inhibit an influenza virus hemagglutinin polypeptide from binding to its receptor can be determined in cell-free assays. For example, a composition comprising an influenza virus hemagglutinin polypeptide can be contacted with an antibody and the ability of the antibody to inhibit the composition comprising an influenza virus hemagglutinin

polypeptide from binding to a cell receptor can be determined. In a specific embodiment, the antibody is immobilized on a solid support and the composition comprising an influenza virus hemagglutinin polypeptide is labeled with a detectable compound. Alternatively, a composition comprising an influenza virus hemagglutinin polypeptide is immobilized on a solid support and the antibody is labeled with a detectable compound. In certain embodiments, the ability of an antibody to inhibit an influenza virus hemagglutinin polypeptide from binding to a cell receptor is determined by assessing the percentage of binding inhibition of the antibody relative to a control (e.g., an antibody known to inhibit the influenza virus hemagglutinin polypeptide from binding to the cell receptor).

[00333] In other embodiments, an antibody suitable for use in the methods described herein does not inhibit influenza virus receptor binding, yet is still found to be neutralizing in an assay described herein. In some embodiments, an antibody suitable for use in accordance with the methods described herein reduces or inhibits virus-host membrane fusion in an assay known in the art or described herein.

[00334] In one embodiment, virus-host membrane fusion is assayed in an *in vitro* assay using an influenza virus containing a reporter and a host cell capable of being infected with the virus. An antibody inhibits fusion if reporter activity is inhibited or reduced compared to a negative control (e.g., reporter activity in the presence of a control antibody or in the absence of antibody).

[00335] In one embodiment, virus-host membrane fusion is detected using a model system of cell fusion. In an exemplary cell fusion assay, cells (e.g., HeLa cells) are transfected with a plasmid encoding an influenza hemagglutinin polypeptide and contacted and exposed to a buffer that allows the hemagglutinin polypeptide fusion function (e.g., pH 5.0 buffer) in the presence of an antibody. An antibody is neutralizing if it reduces or inhibits syncytia formation compared to a negative control (e.g., syncytia formation in the presence of a control antibody or in the absence of antibody).

[00336] In other embodiments, virus-host membrane fusion is assayed using an *in vitro* liposome-based assay. In an exemplary assay, the host cell receptor is reconstituted into liposomes containing one half of a reporter. Influenza hemagglutinin polypeptide is reconstituted into another set of liposomes containing another half of a reporter. When the two liposome populations are mixed together, fusion is detected by reconstitution of the reporter, for example, an enzymatic reaction that can be detected colorimetrically. The antibody inhibits fusion if reporter activity is reduced or inhibited

compared to reporter activity in an assay conducted in the absence of antibody or in the presence of a control antibody. In certain embodiments, the ability of an antibody to inhibit fusion is determined by assessing the percentage of fusion in the presence of the antibody relative to the percentage of fusion in the presence a control.

5.14.3 Assays for Testing Activity of Stimulated Cells

[00337] Cells stimulated in accordance with the methods described herein may be analyzed, for example, for integration, transcription and/or expression of the polynucleotide or gene(s) of interest, the number of copies of the gene integrated, and the location of the integration. Such analysis may be carried out at any time and may be carried out by any methods known in the art. In other embodiments, successful stimulation of the target cell with an influenza hemagglutinin stem domain polypeptide described herein is determined by detecting production of neutralizing antibodies against the influenza hemagglutinin stem domain polypeptide using methods known in the art or described herein.

[00338] In certain embodiments, subjects in which the stimulated cells, e.g., DCs, are administered can be analyzed for location of the cells, expression of a vector-delivered polynucleotide or gene encoding the influenza hemagglutinin stem domain polypeptide, stimulation of an immune response (e.g., production of neutralizing antibodies against the influenza hemagglutinin stem domain polypeptide), and/or monitored for symptoms associated with influenza virus infection or a disease associated therewith by any methods known in the art or described herein.

[00339] Reporter assays can be used to determine the specificity of the targeting of the influenza hemagglutinin stem domain polypeptide. For example, a mixed population of bone marrow cells can be obtained from a subject and cultured in vitro. The influenza hemagglutinin stem domain polypeptide can be administered to the mixed population of bone marrow cells, and expression of a reporter gene associated with the influenza hemagglutinin stem domain polypeptide can be assayed in the cultured cells. In some embodiments, at least about 50%, more preferably at least about 60%, 70%, 80% or 90%, still more preferably at least about 95% of stimulated cells in the mixed cell population are dendritic cells.

5.14.4 Antiviral Activity Assays

[00340] Antibodies described herein or compositions thereof can be assessed *in vitro* for antiviral activity. In one embodiment, the antibodies or compositions thereof are tested *in vitro* for their effect on growth of an influenza virus. Growth of influenza virus can be assessed by any method known in the art or described herein (e.g. in cell culture). In a specific embodiment, cells are infected at a MOI of 0.0005 and 0.001, 0.001 and 0.01, 0.01 and 0.1, 0.1 and 1, or 1 and 10, or a MOI of 0.0005, 0.001, 0.005, 0.01, 0.05, 0.1, 0.5, 1, 5 or 10 and incubated with serum free media supplemented. Viral titers are determined in the supernatant by hemagglutinin plaques or any other viral assay described herein. Cells in which viral titers can be assessed include, but are not limited to, EFK-2 cells, Vero cells, MDCK cells, primary human umbilical vein endothelial cells (HUVEC), H292 human epithelial cell line and HeLa cells. *In vitro* assays include those that measure altered viral replication (as determined, e.g., by plaque formation) or the production of viral proteins (as determined, e.g., by Western blot analysis) or viral RNAs (as determined, e.g., by RT-PCR or northern blot analysis) in cultured cells *in vitro* using methods which are well known in the art or described herein.

[00341] In one non-limiting example, a monolayer of the target mammalian cell line is infected with different amounts (e.g., multiplicity of 3 plaque forming units (pfu) or 5 pfu) of virus (e.g., influenza) and subsequently cultured in the presence or absence of various dilutions of antibodies (e.g., 0.1 µg/ml, 1 µg/ml, 5 µg/ml, or 10 µg/ml). Infected cultures are harvested 48 hours or 72 hours post infection and titered by standard plaque assays known in the art on the appropriate target cell line (e.g., Vero cells).

[00342] In a non-limiting example of a hemagglutination assay, cells are contacted with an antibody and are concurrently or subsequently infected with the virus (e.g., at an MOI of 1) and the virus is incubated under conditions to permit virus replication (e.g., 20-24 hours). The antibodies are preferably present throughout the course of infection. Viral replication and release of viral particles is then determined by hemagglutination assays using 0.5% chicken red blood cells. See, e.g., Kashyap *et al.*, PNAS USA 105: 5986-5991. In some embodiments, a compound is considered an inhibitor of viral replication if it reduces viral replication by at least 2 wells of HA, which equals approximately a 75% reduction in viral titer. In specific embodiments, an inhibitor reduces viral titer in this assay by 50% or more, by 55% or more, by 60% or more, by 65% or more, by 70% or more, by 75% or more, by 80% or more, by 85% or more, by 90% or more, or by 95% or more. In other specific embodiments an inhibitor results in a reduction of approximately 1 log or more, approximately 2 logs or more, approximately

3 logs or more, approximately 4 logs or more, approximately 5 logs or more, approximately 6 logs or more, approximately 7 logs or more, approximately 8 logs or more, approximately 9 logs or more, approximately 10 logs or more, 1 to 3 logs, 1 to 5 logs, 1 to 8 logs, 1 to 9 logs, 2 to 10 logs, 2 to 5 logs, 2 to 7 logs, 2 logs to 8 logs, 2 to 9 logs, 2 to 10 logs 3 to 5 logs, 3 to 7 logs, 3 to 8 logs, 3 to 9 logs, 4 to 6 logs, 4 to 8 logs, 4 to 9 logs, 5 to 6 logs, 5 to 7 logs, 5 to 8 logs, 5 to 9 logs, 6 to 7 logs, 6 to 8 logs, 6 to 9 logs, 7 to 8 logs, 7 to 9 logs, or 8 to 9 logs in influenza virus titer in the subject. The log-reduction in Influenza virus titer may be as compared to a negative control, as compared to another treatment, or as compared to the titer in the patient prior to antibody administration.

5.14.5 Cytotoxicity Assays

[00343] Many assays well-known in the art can be used to assess viability of cells (infected or uninfected) or cell lines following exposure to an active compound or a composition thereof and, thus, determine the cytotoxicity of the compound or composition. For example, cell proliferation can be assayed by measuring Bromodeoxyuridine (BrdU) incorporation (*See, e.g.,* Hoshino *et al.*, 1986, *Int. J. Cancer* 38, 369; Campana *et al.*, 1988, *J. Immunol. Meth.* 107:79), (3H) thymidine incorporation (*See, e.g.,* Chen, J., 1996, *Oncogene* 13:1395-403; Jeoung, J., 1995, *J. Biol. Chem.* 270:18367-73), by direct cell count, or by detecting changes in transcription, translation or activity of known genes such as proto-oncogenes (*e.g.,* fos, myc) or cell cycle markers (Rb, cdc2, cyclin A, D1, D2, D3, E, etc). The levels of such protein and mRNA and activity can be determined by any method well known in the art. For example, protein can be quantitated by known immunodiagnostic methods such as ELISA, Western blotting or immunoprecipitation using antibodies, including commercially available antibodies. mRNA can be quantitated using methods that are well known and routine in the art, for example, using northern analysis, RNase protection, or polymerase chain reaction in connection with reverse transcription. Cell viability can be assessed by using trypan-blue staining or other cell death or viability markers known in the art. In a specific embodiment, the level of cellular ATP is measured to determine cell viability.

[00344] In specific embodiments, cell viability is measured in three-day and seven-day periods using an assay standard in the art, such as the CellTiter-Glo Assay Kit (Promega) which measures levels of intracellular ATP. A reduction in cellular ATP is

indicative of a cytotoxic effect. In another specific embodiment, cell viability can be measured in the neutral red uptake assay. In other embodiments, visual observation for morphological changes may include enlargement, granularity, cells with ragged edges, a filmy appearance, rounding, detachment from the surface of the well, or other changes. These changes are given a designation of T (100% toxic), PVH (partially toxic–very heavy–80%), PH (partially toxic–heavy–60%), P (partially toxic–40%), Ps (partially toxic–slight–20%), or 0 (no toxicity–0%), conforming to the degree of cytotoxicity seen. A 50% cell inhibitory (cytotoxic) concentration (IC_{50}) is determined by regression analysis of these data.

[00345] In a specific embodiment, the cells used in the cytotoxicity assay are animal cells, including primary cells and cell lines. In some embodiments, the cells are human cells. In certain embodiments, cytotoxicity is assessed in one or more of the following cell lines: U937, a human monocyte cell line; primary peripheral blood mononuclear cells (PBMC); Huh7, a human hepatoblastoma cell line; 293T, a human embryonic kidney cell line; and THP-1, monocytic cells. In certain embodiments, cytotoxicity is assessed in one or more of the following cell lines: MDCK, MEF, Huh 7.5, Detroit, or human tracheobronchial epithelial (HTBE) cells.

[00346] Active compounds or compositions thereof can be tested for *in vivo* toxicity in animal models. For example, animal models, described herein and/or others known in the art, used to test the activities of active compounds can also be used to determine the *in vivo* toxicity of these compounds. For example, animals are administered a range of concentrations of active compounds. Subsequently, the animals are monitored over time for lethality, weight loss or failure to gain weight, and/or levels of serum markers that may be indicative of tissue damage (*e.g.*, creatine phosphokinase level as an indicator of general tissue damage, level of glutamic oxalic acid transaminase or pyruvic acid transaminase as indicators for possible liver damage). These *in vivo* assays may also be adapted to test the toxicity of various administration mode and/or regimen in addition to dosages.

[00347] The toxicity and/or efficacy of an active compound can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, *e.g.*, for determining the LD_{50} (the dose lethal to 50% of the population) and the ED_{50} (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD_{50}/ED_{50} . An active compound that exhibits large therapeutic indices is preferred.

While an active compound that exhibits toxic side effects may be used, care should be taken to design a delivery system that targets such agents to the site of affected tissue in order to minimize potential damage to uninfected cells and, thereby, reduce side effects.

[00348] The data obtained from the cell culture assays and animal studies can be used in formulating a range of dosage of an active compound for use in humans. The dosage of such agents lies preferably within a range of circulating concentrations that include the ED₅₀ with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. For any active compound used in a method described herein, the effective dose can be estimated initially from cell culture assays. A dose may be formulated in animal models to achieve a circulating plasma concentration range that includes the IC₅₀ (*i.e.*, the concentration of the test compound that achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Levels in plasma may be measured, for example, by high-performance liquid chromatography. Additional information concerning dosage determination is provided herein.

[00349] Further, any assays known to those skilled in the art can be used to evaluate the prophylactic and/or therapeutic utility of the active compounds and compositions described herein, for example, by measuring viral infection or a condition or symptoms associated therewith.

5.14.6 In vivo Antiviral Activity

[00350] Active compounds and compositions thereof are preferably assayed *in vivo* for the desired therapeutic or prophylactic activity prior to use in humans. For example, *in vivo* assays can be used to determine whether it is preferable to administer an active compound or composition thereof and/or another therapy. For example, to assess the use of an active compound or composition thereof to prevent an influenza virus disease, the composition can be administered before the animal is infected with influenza virus. Alternatively, or in addition, an active compound or composition thereof can be administered to the animal at the same time that the animal is infected with influenza virus. To assess the use of an active compound or composition thereof to treat an influenza virus infection or disease associated therewith, the compound or composition may be administered after infecting the animal with influenza virus. In a specific

embodiment, an active compound or composition thereof is administered to the animal more than one time.

[00351] Active compounds and compositions thereof can be tested for antiviral activity in animal model systems including, but are not limited to, rats, mice, chicken, cows, monkeys, pigs, goats, sheep, dogs, rabbits, guinea pigs, etc. In a specific embodiment, active compounds and compositions thereof are tested in a mouse model system. Such model systems are widely used and well-known to the skilled artisan. In a specific embodiment, active compounds and compositions thereof are tested in a mouse model system. Non-limiting examples of animal models for influenza virus are provided in this section.

[00352] In general, animals are infected with influenza virus and concurrently or subsequently treated with an active compound or composition thereof, or placebo. Alternatively, animals are treated with an active compound or composition thereof or placebo and subsequently infected with influenza virus. Samples obtained from these animals (*e.g.*, serum, urine, sputum, semen, saliva, plasma, or tissue sample) can be tested for viral replication via well known methods in the art, *e.g.*, those that measure altered viral titers (as determined, *e.g.*, by plaque formation), the production of viral proteins (as determined, *e.g.*, by Western blot, ELISA, or flow cytometry analysis) or the production of viral nucleic acids (as determined, *e.g.*, by RT-PCR or northern blot analysis). For quantitation of virus in tissue samples, tissue samples are homogenized in phosphate-buffered saline (PBS), and dilutions of clarified homogenates are adsorbed for 1 hour at 37°C onto monolayers of cells (*e.g.*, Vero, CEF or MDCK cells). In other assays, histopathologic evaluations are performed after infection, preferably evaluations of the organ(s) the virus is known to target for infection. Virus immunohistochemistry can be performed using a viral-specific monoclonal antibody.

[00353] The effect of an active compound or composition thereof on the virulence of a virus can also be determined using *in vivo* assays in which the titer of the virus in an infected subject administered an active compound or composition thereof, the length of survival of an infected subject administered an active compound or composition thereof, the immune response in an infected subject administered an active compound or composition thereof, the number, duration and/or severity of the symptoms in an infected subject administered an active compound or composition thereof, and/or the time period before onset of one or more symptoms in an infected subject administered an active compound or composition thereof, is assessed. Techniques known to one of

skill in the art can be used to measure such effects. In certain embodiments, an active compound or composition thereof results in a 0.5 fold, 1 fold, 2 fold, 4 fold, 6 fold, 8 fold, 10 fold, 15 fold, 20 fold, 25 fold, 50 fold, 75 fold, 100 fold, 125 fold, 150 fold, 175 fold, 200 fold, 300 fold, 400 fold, 500 fold, 750 fold, or 1,000 fold or greater reduction in titer of influenza virus relative to an untreated subject. In some embodiments, an active compound or composition thereof results in a reduction in titer of influenza virus relative to an untreated subject of approximately 1 log or more, approximately 2 logs or more, approximately 3 logs or more, approximately 4 logs or more, approximately 5 logs or more, approximately 6 logs or more, approximately 7 logs or more, approximately 8 logs or more, approximately 9 logs or more, approximately 10 logs or more, 1 to 3 logs, 1 to 5 logs, 1 to 8 logs, 1 to 9 logs, 2 to 10 logs, 2 to 5 logs, 2 to 7 logs, 2 logs to 8 logs, 2 to 9 logs, 2 to 10 logs 3 to 5 logs, 3 to 7 logs, 3 to 8 logs, 3 to 9 logs, 4 to 6 logs, 4 to 8 logs, 4 to 9 logs, 5 to 6 logs, 5 to 7 logs, 5 to 8 logs, 5 to 9 logs, 6 to 7 logs, 6 to 8 logs, 6 to 9 logs, 7 to 8 logs, 7 to 9 logs, or 8 to 9 logs.

[00354] Influenza virus animal models, such as ferret, mouse, guinea pig, squirrel monkey, macaque, and chicken, developed for use to test antiviral agents against influenza virus have been described. *See, e.g.,* Sidwell *et al.*, Antiviral Res., 2000, 48:1-16; Lowen A.C. *et al.* PNAS., 2006, 103: 9988-92; and McCauley *et al.*, Antiviral Res., 1995, 27:179-186 and Rimmelzmann *et al.*, Avian Diseases, 2003, 47:931-933. For mouse models of influenza, non-limiting examples of parameters that can be used to assay antiviral activity of active compounds administered to the influenza-infected mice include pneumonia-associated death, serum α 1-acid glycoprotein increase, animal weight, lung virus assayed by hemagglutinin, lung virus assayed by plaque assays, and histopathological change in the lung. Statistical analysis is carried out to calculate significance (*e.g.*, a P value of 0.05 or less).

[00355] In other assays, histopathologic evaluations are performed after infection of an animal model subject. Nasal turbinates and trachea may be examined for epithelial changes and subepithelial inflammation. The lungs may be examined for bronchiolar epithelial changes and peribronchiolar inflammation in large, medium, and small or terminal bronchioles. The alveoli are also evaluated for inflammatory changes. The medium bronchioles are graded on a scale of 0 to 3+ as follows: 0 (normal: lined by medium to tall columnar epithelial cells with ciliated apical borders and basal pseudostratified nuclei; minimal inflammation); 1+ (epithelial layer columnar and even in outline with only slightly increased proliferation; cilia still visible on many cells); 2+

(prominent changes in the epithelial layer ranging from attenuation to marked proliferation; cells disorganized and layer outline irregular at the luminal border); 3+ (epithelial layer markedly disrupted and disorganized with necrotic cells visible in the lumen; some bronchioles attenuated and others in marked reactive proliferation).

[00356] The trachea is graded on a scale of 0 to 2.5+ as follows: 0 (normal: Lined by medium to tall columnar epithelial cells with ciliated apical border, nuclei basal and pseudostratified. Cytoplasm evident between apical border and nucleus. Occasional small focus with squamous cells); 1+ (focal squamous metaplasia of the epithelial layer); 2+ (diffuse squamous metaplasia of much of the epithelial layer, cilia may be evident focally); 2.5+ (diffuse squamous metaplasia with very few cilia evident).

[00357] Virus immunohistochemistry is performed using a viral-specific monoclonal antibody (e.g. NP-, N- or HN-specific monoclonal antibodies). Staining is graded 0 to 3+ as follows: 0 (no infected cells); 0.5+ (few infected cells); 1+ (few infected cells, as widely separated individual cells); 1.5+ (few infected cells, as widely separated singles and in small clusters); 2+ (moderate numbers of infected cells, usually affecting clusters of adjacent cells in portions of the epithelial layer lining bronchioles, or in small sublobular foci in alveoli); 3+ (numerous infected cells, affecting most of the epithelial layer in bronchioles, or widespread in large sublobular foci in alveoli).

[00358] In one example, the ability to induce lung lesions and cause infection in an animal model of virus infection is compared using wild-type virus and mock virus. Lung lesions can be assessed as a percentage of lung lobes that are healthy by visual inspection. Animals are euthanized 5 days p.i. by intravenous administration of pentobarbital, and their lungs are removed in toto. The percentage of the surface of each pulmonary lobe that is affected by macroscopic lesions is estimated visually. The percentages are averaged to obtain a mean value for the 7 pulmonary lobes of each animal. In other assays, nasal swabs can be tested to determine virus burden or titer. Nasal swabs can be taken during necropsy to determine viral burden post-infection.

[00359] In one embodiment, virus is quantified in tissue samples. For example, tissue samples are homogenized in phosphate-buffered saline (PBS), and dilutions of clarified homogenates adsorbed for 1 h at 37°C onto monolayers of cells (e.g., MDCK cells). Infected monolayers are then overlaid with a solution of minimal essential medium containing 0.1% bovine serum albumin (BSA), 0.01% DEAE-dextran, 0.1% NaHCO₃, and 1% agar. Plates are incubated 2 to 3 days until plaques could be visualized. Tissue culture infectious dose (TCID) assays to titrate virus from PR8-infected samples are

carried out as follows. Confluent monolayers of cells (e.g., MDCK cells) in 96-well plates are incubated with log dilutions of clarified tissue homogenates in media. Two to three days after inoculation, 0.05-ml aliquots from each well are assessed for viral growth by hemagglutination assay (HA assay).

5.14.6.1.1 Assays in Humans

[00360] In one embodiment, an active compound or composition thereof that modulates replication an influenza virus are assessed in infected human subjects. In accordance with this embodiment, an active compound or composition thereof is administered to the human subject, and the effect of the active compound or composition on viral replication is determined by, *e.g.*, analyzing the level of the virus or viral nucleic acids in a biological sample (*e.g.*, serum or plasma). An active compound or composition thereof that alters virus replication can be identified by comparing the level of virus replication in a subject or group of subjects treated with a control to that in a subject or group of subjects treated with an active compound or composition thereof. Alternatively, alterations in viral replication can be identified by comparing the level of the virus replication in a subject or group of subjects before and after the administration of an active compound or composition thereof. Techniques known to those of skill in the art can be used to obtain the biological sample and analyze the mRNA or protein expression.

[00361] In another embodiment, the effect of an active compound or composition thereof on the severity of one or more symptoms associated with an influenza virus infection/disease are assessed in an infected subject. In accordance with this embodiment, an active compound or composition thereof or a control is administered to a human subject suffering from influenza virus infection and the effect of the active compound or composition on one or more symptoms of the virus infection is determined. An active compound or composition thereof that reduces one or more symptoms can be identified by comparing the subjects treated with a control to the subjects treated with the active compound or composition. In another embodiment, an active compound or composition thereof is administered to a healthy human subject and monitored for efficacy as a vaccine (*e.g.*, the subject is monitored for the onset of symptoms of influenza virus infection; the ability of influenza virus to infect the subject; and/or a reduction in/absence of one or more symptoms associated with influenza virus infection). Techniques known to physicians familiar with infectious diseases can be

used to determine whether an active compound or composition thereof reduces one or more symptoms associated with the influenza virus disease.

5.15 KITS

[00362] Provided herein is a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions described herein, such as one or more active compounds provided herein. Optionally associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration.

[00363] The kits encompassed herein can be used in the above methods. In one embodiment, a kit comprises an active compound described herein, preferably one or more influenza hemagglutinin stem domain polypeptides, in one or more containers. In certain embodiments, a kit comprises a vaccine described herein, e.g., a split virus vaccine, a subunit vaccine, an inactivated influenza virus vaccine, or a live influenza virus vaccine.

6. EXAMPLES

6.1 EXAMPLE 1: INFLUENZA HEMAGGLUTININ STEM DOMAIN POLYPEPTIDES

[00364] This example describes the generation of constructs that express influenza hemagglutinin stem domain polypeptides. The influenza hemagglutinin stem domain polypeptides lack the globular head domain of influenza virus hemagglutinin and maintain the structural integrity of the stalk region of the influenza virus hemagglutinin. Since the stalk region of influenza virus hemagglutinin is relatively conserved among influenza viruses, the influenza hemagglutinin stem domain polypeptides should induce neutralizing antibodies against the stalk region of hemagglutinin that are cross-reactive with influenza virus hemagglutinin from different influenza virus subtypes and strains.

[00365] FIG. 3 depicts two schematic nucleotide constructs for expressing an influenza HA stem domain polypeptide from influenza A HK68-H3N2. FIG. 3 also depicts a schematic of a construct (WT HA) for expressing full length influenza HA. The first construct ("Membrane Bound HA") provides a nucleotide sequence encoding

the N-terminal and C-terminal segments, linker peptides, and an HA2 domain. The first construct also encodes a transmembrane (TM) domain and a cytoplasmic (CT) domain. The first construct also encodes a signal peptide (SP).

[00366] Additionally, a second construct ("Soluble HA") provides a nucleotide sequence not encoding the SP, TM or CT in order to generate soluble form of the influenza HA stem domain polypeptide. The second construct includes, after the sequence encoding the HA2 domain, a nucleotide sequence encoding a thrombin cleavage site, a trimerization domain, and a His-tag.

[00367] FIG. 4 illustrates the location of the linker peptide in a putative three dimensional structure of an influenza HA stem domain polypeptide.

[00368] Constructs:

[00369] Construct #1: The nucleotide sequence encoding amino acids 53 to 276 of the influenza HA1 domain was deleted from the full-length influenza virus A/Puerto Rico/8/34 (PR8; H1N1) hemagglutinin and replaced by a linker sequence encoding two glycine residues (GG). Fig. 6 provides the PR8 HA construct (PR8 HAΔGHD (2G)) with a GG linker with both nucleotide (SEQ ID NO:169) and amino acid (SEQ ID NO:170) sequences. A similar construct was made using the full-length influenza virus A/Hong Kong/1/68 (HK68; H3N2) hemagglutinin polypeptide (HK68 HAΔGHD (2G)) and the construct was inserted in the pCAGGS expression vector. The PR8 HAΔGHD (2G) and HK68 HAΔGHD (2G) constructs were each inserted into a pPol1 vector for use in the rescue of recombinant influenza virus.

[00370] Construct #2: The nucleotide sequence encoding amino acids 53 to 276 of the influenza HA1 domain was deleted from the full-length PR8 hemagglutinin and replaced by a linker sequence encoding four glycine residues (GGGG). FIG. 7 provides the PR8 HA construct (PR8 HAΔGHD (4G)) with a GGGG linker with both nucleotide (SEQ ID NO:171) and amino acid (SEQ ID NO:172) sequences. A similar construct was made using the full-length influenza virus HK68, H3N2 hemagglutinin polypeptide (HK68 HAΔGHD (4G)). The PR8 HAΔGHD (4G) and HK68 HAΔGHD (4G) constructs were each inserted in the pCAGGS expression vector. The PR8 HAΔGHD (4G) and HK68 HAΔGHD (4G) constructs were each inserted into a pPol1 vector for use in the rescue of recombinant influenza virus.

[00371] Construct #3: The nucleotide sequence encoding amino acids 53 to 276 of the influenza HA1 domain was deleted from the full-length PR8 hemagglutinin and replaced by a linker sequence encoding a proline residue immediately followed by a

glycine residue (PG). Fig. 8 provides the PR8 HA construct (PR8 HA Δ GHD (PG)) with a PG linker with both nucleotide (SEQ ID NO:173) and amino acid (SEQ ID NO:174) sequences. A similar construct was made using the full-length influenza virus HK68, H3N2 hemagglutinin polypeptide (HK68 HA Δ GHD (PG)). The PR8 HA Δ GHD (PG) and HK68 HA Δ GHD (PG) constructs were each inserted in the pCAGGS expression vector. The PR8 HA Δ GHD (PG) and HK68 HA Δ GHD (PG) constructs were each inserted into a pPol1 vector for use in the rescue of recombinant influenza virus.

[00372] Construct #4: The nucleotide sequence encoding amino acids 53 to 276 of the influenza HA1 domain is deleted from the full-length PR8 and replaced by a linker sequence encoding four glycine residues (GGGG). FIG. 9 provides the PR8 HA construct with a GGGG linker with both nucleotide (SEQ ID NO:175) and amino acid (SEQ ID NO:176) sequences. The PR8 HA construct in Fig. 9 also encodes, after the influenza HA2 domain, a thrombin cleavage site, a foldon domain for trimerization and a HIS₆ tag. A similar construct may be made using the influenza virus HK68, H3N2 hemagglutinin polypeptide.

[00373] Expression of Constructs:

[00374] The pCAGGS expression vectors containing either the HK68 HA Δ GHD (2G) construct, PR8 HA Δ GHD (4G) construct, HK68 HA Δ GHD (4G) construct, PR8 HA Δ GHD (PG) construct, or HK68 HA Δ GHD (PG) construct were transiently transfected into 293T cells in the absence of exogenous trypsin. Influenza HA stem domain polypeptides HA Δ GHD were shown to be expressed in human 293T cell cultures. At 24 hours post-transfection, cells were lysed and lysates were subjected to SDS-PAGE followed by Western blotting. Either a rabbit polyclonal antiserum raised against a HK68 influenza A virus HA2 preparation or a mouse monoclonal raised against multiple H3 HA proteins was used as a primary antibody, as indicated at the bottom of each blot shown in FIGS. 5A and 5B.

[00375] As shown in FIG. 5A, polyclonal antibodies against a HK68 influenza A virus HA2 preparation recognized full length HA0 expressed by the control construct (lane 2) and truncated HA0 (HA0 Δ GHD) expressed by the PR8 HA Δ GHD (4G) construct (lane 3) and PR8 HA Δ GHD (PG) construct (lane 4).

[00376] As shown in FIG. 5B, monoclonal antibodies against multiple H3 HA proteins also recognized full length HA0 expressed by the control construct (lane 2) and truncated HA0 (HA0 Δ GHD) expressed by the HK68 HA Δ GHD (2G) construct (lane 3), HK68 HA Δ GHD (4G) construct (lane 4) and HK68 HA Δ GHD (PG) construct (lane 5).

6.2 **EXAMPLE 2: INFLUENZA VIRUS VACCINE BASED ON CONSERVED HEMAGGLUTININ STALK DOMAIN**

[00377] This example describes the effectiveness of an influenza hemagglutinin stem domain polypeptide (sometimes referred to herein as a “headless HA”) vaccine in inducing an immune response that provides full protection against death and partial protection against disease following lethal viral challenge.

6.2.1 **Materials and Methods**

[00378] **Plasmids**

[00379] pGag-EGFP was generously provided by Carol Carter, Stonybrook University (Hermida-Matsumoto, L., and M. D. Resh. 2000. Localization of human immunodeficiency virus type 1 Gag and Env at the plasma membrane by confocal imaging. *J Virol* 74:8670-9). The pCAGGS expression plasmid was kindly provided by J. Miyazaki, Osaka University (Miyazaki, J., S. Takaki, K. Araki, F. Tashiro, A. Tominaga, K. Takatsu, and K. Yamamura. 1989. Expression vector system based on the chicken beta-actin promoter directs efficient production of interleukin-5. *Gene* 79:269-77). The plasmids pDZ PR8 HA and pDZ PR8 NA were constructed previously as described in reference (Quinlivan, M., D. Zamarin, A. Garcia-Sastre, A. Cullinane, T. Chambers, and P. Palese. 2005. Attenuation of equine influenza viruses through truncations of the NS1 protein. *J Virol* 79:8431-9). For the construction of pCAGGS HK68 HA and pCAGGS HK68 NA, viral genes were reverse transcribed (Transcriptor RT, Roche) from purified virion RNA, amplified (PFU turbo, Stratagene) and cloned into the vector pPOL1 (Fodor, E., L. Devenish, O. G. Engelhardt, P. Palese, G. G. Brownlee, and A. Garcia-Sastre. 1999. Rescue of influenza A virus from recombinant DNA. *J Virol* 73:9679-82) following the recombinational protocol described by Wang et al. (Wang, S., Q. Liu, J. Pu, Y. Li, L. Keleta, Y. W. Hu, J. Liu, and E. G. Brown. 2008. Simplified recombinational approach for influenza A virus reverse genetics. *J Virol Methods* 151:74-8). Protein coding regions were then amplified with primers carrying the appropriate restriction enzyme sites and subcloned into the multiple cloning site of pCAGGS between the Not1 and Nhe1 sites. Headless HA constructs were generated by either excise or fusion PCR methods. Excise PCR was performed on the pPOL1 PR8 HA or pPOL1 HK68 HA plasmids. The resulting PCR products were circularized by ligation and the open reading frame of the headless HA was then subcloned into pCAGGS at the Not1 and Nhe1 sites. Fusion PCR was performed on pDZ PR8 HA or

pCAGGS HK68 plasmid templates and products were inserted into pCAGGS at the NotI and NruI sites. Primer sequences used are as follows Table 5 below.

Table 5: Summary of Sequences of Primers Used in the Construction of Headless HAs Listed in Figure 10

Construct	PCR method ^a	Upstream primer ^b	Downstream primer ^c
PR8 2G	Excise	ACATAGTTTTCCTGTTGGCTGCTTCGAGC [SEQ ID NO: 274]	GGAGGCTGTAACACGAAAGTGTCAAAACACCCCTGGGAGCTATA AACA [SEQ ID NO: 291]
PR8 4G	Excise	ACATAGTTTTCCTGTTGGCTGCTTCGAGC [SEQ ID NO: 275]	GGAGGCGGAGGTTGTAAACACGAAAGTGTCAAAACACCCCTGGGA GCTATAAACA [SEQ ID NO: 292]
PR8 PG	Excise	ACATAGTTTTCCTGTTGGCTGCTTCGAGC [SEQ ID NO: 276]	CCAGGCTGTAACACGAAAGTGTCAAAACACCCCTGGGAGCTATA AACA [SEQ ID NO: 293]
PR8 No Cys 1G	Fusion	TGACACTTCGTGTACCTAGTTTTCGTTGTGGCTG [SEQ ID NO: 277]	GGTAACACGAAAGTGTCAAAACAC [SEQ ID NO: 294]
PR8 No Cys 2G	Fusion	ACTTCGTGTTCCGCCCTAGTTTTCGTTGTGGCTG [SEQ ID NO: 278]	GGCGGAAACACGAAAGTGTCAAAACAC [SEQ ID NO: 295]
PR8 No Cys 3G	Fusion	CGTGTACCTCCGCCCTAGTTTTCGTTGTGGCTG [SEQ ID NO: 279]	GGCGGAGGTAACACGAAAGTGTCAAAACAC [SEQ ID NO: 296]
PR8 No Cys	Fusion	TGTTTGACACTTCGTGTTAGTTTTCGTTGTGGCTGTC [SEQ ID NO: 280]	GCCACAACGGGAAAACTAAACAGGAAAGTGTCAAAACACCCC [SEQ ID NO: 297]
PR8 No Cys Δ1	Fusion	GGTGTGACACTTCGTGTTAGTTTTCGTTGTGGCTGTC [SEQ ID NO: 281]	GCCACAACGGGAAAACTAAACAGGAAAGTGTCAAAACACCCCTG [SEQ ID NO: 298]
PR8 No Cys Δ3	Fusion	AGGGGTGTTGACACTTTTTCGTTGTGGCTGTCTTC [SEQ ID NO: 282]	ACAGCCACAACGGGAAAAAAGTGTCAAAACACCCCTGGGA [SEQ ID NO: 299]

PR8 No Cys NAS	Fusion	ACTTCGTGTTGGAGGCGTTTAGTTTCCGTTGTGGCTG [SEQ ID NO: 283]	AACGCCTCAACACGAAAGTGTCAAAACAC [SEQ ID NO: 300]
HK68 2G	Excise	GCUCUCAACGGGGAAAAUAUGCGGA [SEQ ID NO: 284]	GGCTGTATTTCTGAATGCATCACTCC [SEQ ID NO: 301]
HK68 4G	Excise	GCUCUCAACGGGGAAAAUAUGCGGAGGC [SEQ ID NO: 285]	GGAGGTGTATTTCTGAATGCATCACTCC [SEQ ID NO: 302]
HK68 PG	Excise	GCUCUCAACGGGGAAAAUAUGCCCA [SEQ ID NO: 286]	GGCTGTATTTCTGAATGCATCACTCC [SEQ ID NO: 303]
HK68 No Cys	Fusion	GAGTGATGCATTCAGAAAATTATTTCCCGTTGAGGAGC [SEQ ID NO: 287]	CCTCAACGGGGAAAAATAATTTCTGAATGCATCACTCCA [SEQ ID NO: 304]
HK68 No Cys $\Delta 1$	Fusion	TGGAGTGATGCATTCAGATATTTTCCCGTTGAGGAGC [SEQ ID NO: 288]	CCTCAACGGGGAAAAATATCTGAATGCATCACTCCAAAT [SEQ ID NO: 305]
HK68 No Cys $\Delta 3$	Fusion	ATTGGAGTGATGCATTTTCCCGTTGAGGAGCTC [SEQ ID NO: 289]	CTCCTCAACGGGGAAAAAGAAATGCATCACTCCAAATGG [SEQ ID NO: 306]
HK68 No Cys NAS	Fusion	TTCAGAAATGGAGGCGTTTATTTCCCGTTGAGGAG [SEQ ID NO: 290]	AACGCCTCCATTTCTGAATGCATCACTCC [SEQ ID NO: 307]

^a In the Excise PCR method, primers flanking the sequence targeted for deletion are used to amplify the remainder of the gene and plasmid vector. Thus, the downstream primer is the forward primer in the PCR reaction and the upstream primer is the reverse primer. A linear fragment is produced which lacks the intervening sequence. A short foreign sequence can be introduced into the deletion site by adding it to the 5' end of one of the primers. Once generated, the PCR product is purified and self-ligated in order to produce a circular plasmid carrying the modified gene of interest.

In the Fusion PCR method two fragments of the desired product are PCR amplified independently. One fragment corresponds to the sequence upstream of the introduced mutation and the second corresponds to the sequence downstream of the mutation. The upstream fragment is amplified using the upstream primer in combination with a 5' outside primer, while the downstream fragment is produced using the downstream primer together with a 3' outside primer. The nucleotide sequence to be introduced at the site of mutation is added to both fragments through inclusion in both the upstream and downstream primers. The two pieces are then fused in a subsequent PCR reaction using only the 5' and 3' outside primers. The PR8 outside primers used for all PR8 fusion PCRs were PR8 5': cgagctcatgaaggcaaacctactgg and PR8 3': cgcagatcttcagatgcattctgcact. The HK68 outside primers used for all HK68 fusion PCRs were HK68 5'not1: ggaagcggcgcgcgatgaagaccatcattgttgag and HK68 3'nrul: gggcgctgcgatcaaatgcaatgttgacacataa.

^b These primers anneal immediately upstream of the mutation site in both Excise and Fusion PCR methods.

^c These primers anneal immediately downstream of the mutation site in both Excise and Fusion PCR method

[00380] Antibodies

[00381] Monoclonal antibody (mAb) 12D1 was generated by sequential intramuscular immunization of Balb/C mice with plasmid DNAs encoding the HK68 HA, the A/Alabama/1/1981 (H3N2) HA, and the A/Beijing/46/1992 (H3N2) HA, followed by a boost with whole A/Wyoming/03/2003 (H3N2) virus. See U.S. provisional application Serial Nos. 61/181,263 and 61/224,302, filed May 26, 2009 and July 9, 2009, which are incorporated herein by reference in their entirety, for a description of the 12D1 mAb. This mAb binds multiple H3 HA proteins and maps to the HA2 subunit. Rabbit polyclonal serum 3951 was raised against PR8 virus from which the HA1 subunit had been removed by treatment with acid and DTT (Graves, P. N., J. L. Schulman, J. F. Young, and P. Palese. 1983. Preparation of influenza virus subviral particles lacking the HA1 subunit of hemagglutinin: unmasking of cross-reactive HA2 determinants. *Virology* 126:106-16).

[00382] Cells and Viruses

[00383] 293T cells were obtained from the ATCC and were maintained in Dulbecco's modified Eagles medium (DMEM; Gibco) supplemented with 10% fetal bovine serum (FBS; Clontech).

[00384] Influenza A/Puerto Rico/8/1934 (H1N1) virus was obtained by reverse genetics as previously described (Steel, J., S. V. Burmakina, C. Thomas, E. Spackman, A. Garcia-Sastre, D. E. Swayne, and P. Palese. 2008. A combination in-ovo vaccine for avian influenza virus and Newcastle disease virus. *Vaccine* 26:522-31) using plasmids encoding the eight genes defined by accession numbers AF389115 to AF189122 (A/Puerto Rico/8/34/Mount Sinai) in the NCBI database. The virus was amplified in 10-11 day old embryonated chickens eggs and titrated by plaque assay.

[00385] Western blotting

[00386] To assess expression levels of HA-based proteins, 293T cells were transfected with 2 µg of the appropriate plasmid using Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions. At 24 h post-transfection, cells were lysed in 2x protein loading buffer (125 mM Tris-HCl [pH 6.8], 4% sodium dodecyl sulfate, 20% glycerol, 10% β-mercaptoethanol, and 0.1% bromophenol blue). Lysates were heated at 100°C for 5 minutes and then separated on a 10% sodium dodecyl sulfate-polyacrylamide gel and transferred to a nitrocellulose membrane (Whatman, Inc.). To detect HA-based proteins in VLP preparations, pelleted VLPs were suspended in phosphate buffered saline (PBS), lysed through 1:1 mixing with 2x protein loading

buffer, boiled for 5 minutes, separated on a 10% sodium dodecyl sulfate-polyacrylamide gel and transferred to a nitrocellulose membrane. Membranes were then probed with mAb 12D1 or 3951 antiserum at a 1:2000 dilutions.

[00387] Flow Cytometric Analysis

[00388] To assess levels of HA-based proteins at the cell surface, 293T cells were transfected with 1 µg of the appropriate plasmid using Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions. At 24 hours post-transfection, cells were trypsinized and resuspended in PBS containing 2% FBS prior to staining with 3951 antiserum at a 1/250 dilution or mAb 12D1 at a 1/200 dilution. Stained cells were enumerated on a Beckman Coulter Cytomics FC 500 flow cytometer and results were analyzed using Flow Jo software.

[00389] Generation of Virus Like Particles

[00390] For the production of virus like particles, 6×10^6 293T cells were seeded into a 10 cm dish in 8 ml of DMEM with 10% FBS. While still in suspension, cells were transfected with Lipofectamine 2000 (Invitrogen) combined with the desired plasmid DNAs at a 4:1 ratio and as per the manufacturer's instructions. The amounts of plasmid DNA used were as follows: for Gag-only VLPs, 7.5 µg pGagEGFP was transfected; for gag + PR8 4G VLPs, 4.5 µg of pGagEGFP plus 4.5 µg of pCAGGS PR8 4G was used; for Gag + HK68 4G VLPs, 4.5 µg of pGagEGFP plus 4.5 µg of pCAGGS HK68 4G was used; and for Gag + PR8 HA + PR8 NA VLPs, 3 µg of pGagEGFP was combined with 3 µg each of pDZ PR8 HA and pDZ PR8 NA. At 6 hours post-transfection, medium was changed to fresh DMEM containing 10% FBS or to Opti-MEM (Gibco) supplemented with 3% bovine serum albumin (Sigma) and 10 µg/ml TPCK-treated trypsin (Sigma). VLPs were harvested at 28 hours post-transfection by layering clarified cell culture supernatant over a cushion of 30% sucrose in NTE buffer (1M sodium chloride, 0.1 M Tris, 0.01 M EDTA, pH 7.4) and centrifuging for 2 h at 4°C and 25,000 RPM in an SW28 rotor (Beckman). Pellets were resuspended in PBS and HA protein content was assessed by Western blotting in parallel with serial dilutions of a known amount of gradient purified PR8 or HK68 virus (prepared as described in Palese, P., and J. L. Schulman. 1976. Differences in RNA patterns of influenza A viruses. J Virol 17:876-84). VLPs shown in Figure 13 were produced in the presence of exogenous trypsin, while those used to boost mice were produced without the addition of trypsin.

[00391] Mouse Vaccine-Challenge Experiment

[00392] Female, 6 to 8-week-old C57BL/6 mice (Charles River Laboratories) were initially vaccinated by intramuscular administration of plasmid DNA followed immediately by the application of electrical stimulation (TriGrid Delivery System, Ichor Medical Systems (Luxembourg, A., C. F. Evans, and D. Hannaman. 2007. Electroporation-based DNA immunisation: translation to the clinic. *Expert Opin Biol Ther* 7:1647-64; Luxembourg, A., D. Hannaman, E. Nolan, B. Ellefsen, G. Nakamura, L. Chau, O. Tellez, S. Little, and R. Bernard. 2008. Potentiation of an anthrax DNA vaccine with electroporation. *Vaccine* 26:5216-22)). The spacing of the TriGrid electrode array is 2.5 mm, and the electrical field is applied at an amplitude of 250 V/cm of electrode spacing for six pulses totaling 40 msec duration applied over a 400 msec interval. Each DNA vaccination comprised 37.5 µg of pGagEGFP alone or in combination with 75 µg of pDZ PR8 HA, pCAGGS PR8 4G or pCAGGS HK68 4G. Three weeks later a DNA boost was performed following the same procedure. Five weeks after the second dose of DNA was administered, the mice were boosted a second time with VLPs. For the HA-containing VLPs, HA content was normalized such that each mouse received approximately 150 ng HA protein. Prior to intraperitoneal administration, VLP suspensions were combined in a 1:1 ratio with complete Freund's adjuvant (Pierce) and emulsified by multiple passes through two linked syringes. Mice were challenged three weeks following the VLP boost via intranasal inoculation of 2 MLD50 (50% mouse lethal dose) of PR8 virus in a total volume of 50 µl PBS. Body weight was monitored daily and mice losing greater than 25% of their initial weight were sacrificed and scored as dead.

[00393] Serological Assays

[00394] Sera were collected from mice immediately prior to challenge and at 21 days post-challenge. To remove non-specific inhibitors of hemagglutination, trypsin-heat-periodate treatment was performed as described previously (Lowen, A. C., J. Steel, S. Mubareka, E. Carnero, A. Garcia-Sastre, and P. Palese. 2009. Blocking inter-host transmission of influenza virus by vaccination in the guinea pig model. *J Virol*. 83: 2803-18). For hemagglutination inhibition assays, sera from each vaccination group were pooled; for ELISA, sera collected from individual mice were evaluated separately. HI assays were carried out as described previously (Lowen, A. C., J. Steel, S. Mubareka, E. Carnero, A. Garcia-Sastre, and P. Palese. 2009. Blocking inter-host transmission of influenza virus by vaccination in the guinea pig model. *J Virol*. 83: 2803-18). For ELISA, 96-well plates (Co-Star) were coated with 0.25 µg per well of PR8 virus or with

0.1 µg per well of purified recombinant HA protein in PBS. PR8 virus was prepared from allantoic fluid by concentration through a 30% sucrose cushion as previously described (Lowen, A. C., J. Steel, S. Mubareka, E. Carnero, A. Garcia-Sastre, and P. Palese. 2009. Blocking inter-host transmission of influenza virus by vaccination in the guinea pig model. *J Virol.* 83: 2803-18). The recombinant HA proteins of the A/California/04/2009, A/Viet Nam/1203/2004, and A/Singapore/1/1957 viruses were obtained from BEI Resources; the A/Hong Kong/1/1968 HA was the generous gift of Ian Wilson; and the A/New Caledonia/20/99 HA was purchased from Feldan-Bio. Five-fold serial dilutions of anti-sera were incubated on the plates and, after extensive washing, bound antibody was detected with an alkaline phosphatase linked anti-mouse IgG antibody (Caltag) and PNPP substrate (Sigma). In each assay a rabbit immune serum raised against whole PR8 virus was included as a positive control, and serum obtained from a naïve C57BL/6 mouse was included as a negative control.

6.2.2 Results

[00395] Design and Construction of Headless HA Constructs

[00396] The goal was to generate an immunogen consisting of the complete HA2 polypeptide plus the regions of HA1 contributing to the stalk region, but lacking the globular head domain of HA1. With this aim in mind, the existence of a conserved disulfide bond linking cysteines 52 and 277 (H3 numbering) of HA1 was noted. The loop flanked by these two cysteines comprises the bulk of the globular head domain, while the N-terminal 51 and the C-terminal 52 amino acids of HA1 extend downward from the cysteine bridge and contribute to the stalk region. Due to the proximity of cysteines 52 and 277 in the three-dimensional structure of HA (Stevens, J., A. L. Corper, C. F. Basler, J. K. Taubenberger, P. Palese, and I. A. Wilson. 2004. Structure of the uncleaved human H1 hemagglutinin from the extinct 1918 influenza virus. *Science* 303:1866-70 and Figure 10), it was predicted that replacement of the intervening loop with a short linker peptide would not disrupt the folding of the remainder of the molecule. Based on this principle, a panel of headless HA constructs was designed (Figure 10).

[00397] First, sequences encoding linker peptides of two glycines (2G), four glycines (4G) or a proline and a glycine (PG) were inserted into the open reading frames of the A/Puerto Rico/8/1934 (H1N1) (PR8) and the A/Hong Kong/1968 (H3N2) (HK68) hemagglutinins in place of the respective nucleotide sequences encoding amino acids 53

to 276. These three linker peptides were selected to have a range of flexibilities, with 4G predicted to be the most flexible and PG the most rigid. To test whether insertion of a linker in the absence of a disulfide bond at this position would yield a more stable product, three additional constructs in the context of the PR8 HA were designed: sequences encoding one, two or three glycines were inserted in place of the sequences encoding amino acids 52 to 277 (that is, both the cysteines and the connecting loop were replaced). Based on the hypothesis that glycosylation may improve trafficking through the Golgi, the insertion of a glycosylation site (NAS) in place of amino acids 52 to 277 in both the PR8 and the HK68 backgrounds was also tested. Finally, a series of three constructs were made in each of the PR8 and the HK68 HAs in which existing wild-type amino acids were directly linked: amino acid 51 to 278, 51 to 279, or 50 to 280. Constructs were made in the context of an H1 (representative of group 1) and an H3 (representative of group 2) HA since the activity of neutralizing antibodies targeting the stalk region appears to be limited to HA subtypes within the same major phylogenetic group (Ekiert, D. C., G. Bhabha, M. A. Elsliger, R. H. Friesen, M. Jongeneelen, M. Throsby, J. Goudsmit, and I. A. Wilson. 2009. Antibody recognition of a highly conserved influenza virus epitope. *Science* 324:246-51; Kashyap, A. K., J. Steel, A. F. Oner, M. A. Dillon, R. E. Swale, K. M. Wall, K. J. Perry, A. Faynboym, M. Ilhan, M. Horowitz, L. Horowitz, P. Palese, R. R. Bhatt, and R. A. Lerner. 2008. Combinatorial antibody libraries from survivors of the Turkish H5N1 avian influenza outbreak reveal virus neutralization strategies. *Proc Natl Acad Sci U S A* 105:5986-91; Okuno, Y., Y. Isegawa, F. Sasao, and S. Ueda. 1993. A common neutralizing epitope conserved between the hemagglutinins of influenza A virus H1 and H2 strains. *J Virol* 67:2552-8; and Sui, J., W. C. Hwang, S. Perez, G. Wei, D. Aird, L. M. Chen, E. Santelli, B. Stec, G. Cadwell, M. Ali, H. Wan, A. Murakami, A. Yammanuru, T. Han, N. J. Cox, L. A. Bankston, R. O. Donis, R. C. Liddington, and W. A. Marasco. 2009. Structural and functional bases for broad-spectrum neutralization of avian and human influenza A viruses. *Nat Struct Mol Biol* 16:265-73).

[00398] These constructs are summarized in the Table 6 below.

Table 6: Summary of Constructs

<u>Name</u>	<u>HA1 N-terminal Stem Segment</u>	<u>Linker</u>	<u>HA1 C-terminal Stem Segment</u>	<u>HA2 Domain</u>
PR8-2G	SEQ ID NO:34	Gly-Gly	SEQ ID NO:50	SEQ ID NO:66
PR8-4G	SEQ ID NO:34	Gly-Gly-Gly-Gly	SEQ ID NO:50	SEQ ID NO:66
PR8-PG	SEQ ID NO:34	Pro-Gly	SEQ ID NO:50	SEQ ID NO:66
PR8-No Cys-1G	SEQ ID NO:177	Gly	SEQ ID NO:226	SEQ ID NO:66
PR8-No Cys 2G	SEQ ID NO:177	Gly-Gly	SEQ ID NO:226	SEQ ID NO:66
PR8-No Cys 3G	SEQ ID NO:177	Gly-Gly-Gly	SEQ ID NO:226	SEQ ID NO:66
PR8-No Cys	SEQ ID NO:177	direct bond	SEQ ID NO:226	SEQ ID NO:66
PR8-No Cys Δ 1	SEQ ID NO:178	direct bond	SEQ ID NO:227	SEQ ID NO:66
PR8-No Cys Δ 3	SEQ ID NO:179	direct bond	SEQ ID NO:228	SEQ ID NO:66
PR8-No Cys NAS	SEQ ID NO:177	Asn-Ala-Ser	SEQ ID NO:226	SEQ ID NO:66
PR8-CON-A	SEQ ID NO:309	Gly-Gly-Gly-Gly	SEQ ID NO:310	SEQ ID NO:66
HK68-2G	SEQ ID NO:36	Gly-Gly	SEQ ID NO:52	SEQ ID NO:68
HK68-4G	SEQ ID NO:36	Gly-Gly-Gly-Gly	SEQ ID NO:52	SEQ ID NO:68
HK68-PG	SEQ ID NO:36	Pro-Gly	SEQ ID NO:52	SEQ ID NO:68
HK68-No Cys	SEQ ID NO:183	direct bond	SEQ ID NO:232	SEQ ID NO:68
HK68-No Cys Δ 1	SEQ ID NO:184	direct bond	SEQ ID NO:233	SEQ ID NO:68
HK68-No Cys Δ 3	SEQ ID NO:185	direct bond	SEQ ID NO:234	SEQ ID NO:68
HK68-No Cys NAS	SEQ ID NO:183	Asn-Ala-Ser	SEQ ID NO:232	SEQ ID NO:68
HK68-CON-A	SEQ ID NO:308	Gly-Gly-Gly-Gly	SEQ ID NO:52	SEQ ID NO:68

[00399] Expression of Headless HA Constructs in Transfected Cell Cultures

[00400] As a preliminary test of protein integrity and stability, levels of the headless HA constructs expressed in transiently transfected cells were assessed by Western blotting. As shown in Figure 11A, headless HA constructs based on the PR8 HA protein were expressed to levels comparable to the corresponding full length protein at 24 hours post-transfection. Within the panel of constructs tested, those which retained cys 52 and 277 and carried the linker peptides 2G, 4G or PG exhibited the highest steady state levels. In the context of the HK68 HA (Figure 11B) similar results were seen: all HK68 headless HAs tested were detected using an antibody specific to the stalk domain, and those carrying the 2G, 4G or PG linker between cys 52 and cys 277 were the most abundant. For both HK68 and PR8, the least abundant constructs were those with the direct linkage between amino acids 50 and 280 and those with the inserted glycosylation site (Figure 11).

[00401] To test whether the headless HA constructs were also being transported to the cell surface, FACS analysis of transiently transfected 293T cells was performed following surface staining with HA2-specific antibodies. Only the 2G, 4G and PG constructs, which showed high levels by Western blotting, were tested in this assay. As shown in Figure 12, the three PR8 and the three HK68 based constructs were detected, indicating that transport through the Golgi to the cell surface was not disrupted by the removal of the globular head domain. No marked differences among the three linker bridges were noted in either the Western blotting or FACS based assays. The constructs carrying the 4G linker bridge were selected for further characterization.

[00402] Incorporation of Headless HA into Viral Like Particles

[00403] As a further test of the functionality of the headless HA molecules, their ability to bud from the cell surface to produce virus like particles (VLP) was assessed. While transient expression of the headless HA constructs alone in 293T cells was not found to result in VLP production, co-transfection with an HIV Gag-based construct did lead to the production of headless HA-containing particles. Specifically, when either the PR8 or HK68 4G headless HA constructs was co-expressed in 293T cells with a Gag-EGFP (enhanced green fluorescent protein) fusion protein, particles capable of sedimenting through a 30% sucrose cushion and containing headless HA proteins were released into the cell culture medium (Figure 13). Similar results were obtained in the presence (as in Figure 13) or absence of exogenous trypsin. Unlike the full-length HA protein, and as expected based on the lack of a globular head domain, the release of headless HA containing particles was not found to be dependent on the presence of neuraminidase activity.

[00404] Vaccination with the PR8 Headless HA Provides Protection Against Homologous Challenge in Mice

[00405] The potential of vaccination with a headless HA construct to induce a protective immune response was evaluated in the mouse model. A three-dose vaccine regimen was followed in which mice received plasmid DNA on days 0 and 21 and VLP preparations delivered with Freund's adjuvant on day 56. Each DNA vaccine comprised pGagEGFP alone or in combination with a protein expression vector encoding the full length PR8 HA, the PR8 4G headless HA or the HK68 4G headless HA and was administered intramuscularly with electroporation. For a final boost, VLP preparations with an HA content of 150 ng (or an equivalent amount of Gag-only VLP) were combined with Freund's complete adjuvant and administered intraperitoneally to each

mouse. On day 77, mice were challenged intranasally with PR8 virus and then monitored daily for morbidity and mortality for 10 days. In the Gag-only vaccinated group, three out of four mice lost >25% of their initial body weight and were therefore scored as dead and the fourth animal was seen to lose 15% body weight. By contrast, all mice vaccinated with the PR8 4G headless HA survived and experienced a maximum, on average, of only 6% weight loss (Figure 14).

[00406] Vaccination of Mice with the PR8 Headless

HA Elicits Cross-Reactive Anti-Sera

[00407] The reactivity of serum collected from vaccinated mice against influenza virus HA proteins was assessed by hemagglutination inhibition (HAI) assay and ELISA. As expected based on the absence of a globular head domain in the vaccine constructs, the pooled sera from mice immunized with Gag alone, the HK68 4G headless HA or the PR8 4G headless HA did not show HAI activity against PR8 virus prior to challenge. In contrast, pre-challenge sera obtained from mice that received the full length PR8 HA vaccine, as well as all post-challenge sera, were strongly reactive against PR8 virus in the HAI assay (Table 7).

[00408] Table 7. Lack of Hemagglutination Inhibition Activity in Immune Sera of Headless HA Vaccinated Mice.

Vaccine	Fold-Increase Over Gag-Only Pre-Challenge Serum	
	Pre-Challenge	Post-Challenge
Gag-only	-	8
HK68 4G headless HA plus Gag	1	8
PR8 4G headless HA plus Gag	1	8
PR8 full length HA plus Gag	≥128	≥128

[00409] By ELISA, pre-challenge sera were tested against a panel of HA substrates in order to evaluate the breadth of reactivity (Figure 15). Against concentrated PR8 virion (Figure 15A), Gag-only and HK68 4G anti-sera showed only a low level of background activity at the lowest dilution (1:50), while sera from the PR8 full length HA vaccinated animals gave a positive signal at a 1:6250 dilution. Antisera against the PR8 4G headless HA were less potent than those against the full length HA, but reacted positively at a 1:50 dilution. When tested against recombinant HA proteins derived from a recent seasonal H1N1 (A/New Caledonia/20/1999; Figure 12B) and a 2009

pandemic H1N1 (A/California/04/2009; Figure 15C) influenza virus, Gag-only and HK68 4G anti-sera were negative, while sera from mice that received the full length PR8 HA were either negative or showed a low level of reactivity. On these heterologous H1 substrates, the PR8 4G anti-sera showed the highest level of reactivity, with the sera from two of the five mice in particular demonstrating high titers. Similar results were seen with recombinant HA proteins of the H2 and H5 subtypes: against the A/Singapore/1/1957 (H2N2) and A/Viet Nam/1203/2004 (H5N1) HAs, sera derived from PR8 4G vaccinated mice showed moderate to high activity, while sera from the remaining groups (including the full length HA) were largely negative (Figures 15D and 15E). Finally, against the H3 subtype HA of A/Hong Kong/1/1968 (H3N2) only the HK68 4G anti-sera produced a positive signal (Figure 15F). Thus, overall, sera obtained from mice vaccinated with the headless PR8 HA showed greater activity against heterologous strains than did sera from full length PR8 HA vaccinated animals. While serum titers of PR8 4G vaccinated mice appeared to be higher against the heterologous HA proteins than against the homologous PR8 virus, a direct comparison should not be made due to differing substrates used (purified HA versus whole virus). Within the PR8 4G group, the sera from two mice in particular consistently showed relatively high titers by ELISA. These serological findings correlated with the protection data in that these same two mice were fully protected from disease while their three remaining counterparts each exhibited some weight loss after challenge.

6.2.3 Conclusion

[00410] This example describes influenza hemagglutinin (HA) stem domain polypeptides (“headless HA constructs”) which lack the highly immunogenic globular head of the HA protein and are thereby designed to present the conserved HA stalk region to immune cells. These headless HA constructs can be stably expressed in mammalian cells and targeted to the cell surface in a similar manner to full length HA polypeptides. Immunization of mice with a PR8-based HA stem domain polypeptide in plasmid DNA and VLP formats provided full protection against death and partial protection from disease following a lethal homologous challenge.

[00411] Serological analysis revealed that the PR8 4G influenza headless HA construct, but not the full-length PR8 HA vaccine, induced antibodies which are cross-reactive among group 1 HA subtypes. This finding suggests that the globular head domain of an intact HA molecule inhibits recognition of the stem region by immune

cells, either through steric shielding or due to the immune dominance of the membrane distal portion of the protein. These data furthermore suggest that vaccination with an headless HA construct can lead to protection against divergent influenza strains.

6.3 EXAMPLE 3: CHALLENGE WITH HETEROLOGOUS VIRUSES

[00412] The data described in Example 2 above shows that mice vaccinated with a PR8 headless HA construct are protected against challenge with PR8 virus (that is, protected against homologous challenge). These data indicate that an influenza virus hemagglutinin stem domain polypeptide (sometimes referred to herein as a “headless HA”) is sufficiently immunogenic to act as a vaccine but do not provide information on the breadth of protection achieved. To test whether an influenza virus hemagglutinin stem domain polypeptide can elicit an immune response which will protect against challenge with a range of heterologous viruses, mice will be vaccinated through intraperitoneal injection of 5 µg of a purified influenza virus hemagglutinin stem domain polypeptide or, as a control, 5 µg of full length HA in the context of whole inactivated influenza virus preparations. In both cases, the vaccine will be combined with MF-59 adjuvant prior to administration. At three weeks post-vaccination, groups of 8 mice will be challenged by intranasal inoculation with 10 MLD₅₀ (50% mouse lethal dose) of the virus strains identified in Table 8. Mice will be monitored daily up to 14 days post-challenge for changes in body weight and death. The influenza virus hemagglutinin stem domain polypeptide vaccines are expected to provide superior protection from death and disease following heterologous virus challenges compared to the conventional whole inactivated virus vaccines.

[00413] **Table 8. Summary of Challenge Experiments**

Vaccine	Challenge Virus	Mouse Model ^a
PR8 4G headless HA	A/Puerto Rico/8/1934 (H1N1)	C57BL/6
	A/Netherlands/602/2009 (novel H1N1)	DBA-2
	A/Viet Nam/1203/2004 (H5N1) ^b	C57BL/6
PR8 virus	A/Puerto Rico/8/1934 (H1N1)	C57BL/6
	A/Netherlands/602/2009 (novel H1N1)	DBA-2
	A/Viet Nam/1203/2004 (H5N1)	C57BL/6
HK68 4G headless HA	X31 (H3N2)	DBA-2
	A/Rhea/North Carolina 39482/1993 (H7N1)	DBA-2

X31 ^c virus	X31 (H3N2)	DBA-2
	A/Rhea/North Carolina 39482/1993 (H7N1)	DBA-2

^a The strain of inbred mouse to be used is based on the lethality of the challenge viruses. Virus strains that are less pathogenic to mice must be used in the more susceptible DBA-2 model.

^b Rather than the wild-type virus, a reassortant virus carrying the HA and NA genes of A/Viet Nam/1203/04 and the remaining six genes from PR8 virus will be used. In addition, the multibasic cleavage site in the HA segment of this virus is mutated to a low pathogenic form. These changes do not affect the antigenicity of the HA protein.

^c X31 is a mouse adapted virus carrying the HA and NA genes of A/Hong Kong/1/1968 (H3N2) virus and the remaining six genes from PR8.

[00414] All publications, patents and patent applications cited in this specification are herein incorporated by reference as if each individual publication or patent application were specifically and individually indicated to be incorporated by reference. However, reference to such publications, patents and patent applications should not be taken as an admission that said publications, patents and patent applications form part of the prior art.

[00415] Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be readily apparent to those of ordinary skill in the art in light of the teachings of this invention that certain changes and modifications may be made thereto without departing from the spirit or scope of the appended claims.

The claims defining the invention are as follows:

1. A vaccine comprising a polypeptide, wherein said polypeptide comprises:
 - (a) an influenza hemagglutinin HA1 domain that comprises an HA1 N-terminal stem segment covalently linked to a linker of 1 to 50 heterologous residues that is in turn covalently linked to an HA1 C-terminal stem segment; said HA1 domain in tertiary or quaternary association with
 - (b) an influenza hemagglutinin HA2 domain, andwherein the vaccine induces antibodies which are cross-reactive among HA subtypes.
2. A vaccine comprising a virus comprising a genome engineered to express a nucleic acid encoding a polypeptide, wherein said polypeptide comprises:
 - (a) an influenza hemagglutinin HA1 domain that comprises an HA1 N-terminal stem segment covalently linked to a linker of 1 to 50 heterologous residues that is in turn covalently linked to an HA1 C-terminal stem segment; said HA1 domain in tertiary or quaternary association with
 - (b) an influenza hemagglutinin HA2 domain, andwherein the vaccine induces antibodies which are cross-reactive among HA subtypes.
3. A vaccine comprising a virus comprising a polypeptide, wherein said polypeptide comprises:
 - (a) an influenza hemagglutinin HA1 domain that comprises an HA1 N-terminal stem segment covalently linked to a linker of 1 to 50 heterologous residues that is in turn covalently linked to an HA1 C-terminal stem segment; said HA1 domain in tertiary or quaternary association with

(b) an influenza hemagglutinin HA2 domain, and

wherein the vaccine induces antibodies which are cross-reactive among HA subtypes.

4. The vaccine of claim 2 or 3, wherein the virus is an influenza virus, a Newcastle disease virus (NDV), a vaccinia virus, an adenovirus, an adeno-associated virus (AAV), or a retrovirus.
5. The vaccine of claim 3, wherein said virus is inactivated or split.
6. The vaccine of any one of claims 1 to 5, wherein the HA1 domains contact the HA2 domain.
7. The vaccine of any one of claims 1 to 6, wherein the HA1 C-terminal stem segment is covalently linked to the HA2 domain.
8. The vaccine of any one of claims 1 to 7, wherein the polypeptide in said vaccine has a tertiary or quaternary structure having 0-5 Å RMS deviation from the tertiary or quaternary structure of the corresponding polypeptide of 1RUZ.
9. The vaccine of any one of claims 1 to 8, wherein the polypeptide in said vaccine selectively binds neutralizing antiserum capable of binding an influenza hemagglutinin .
10. The vaccine of any one of claims 1 to 9, wherein the polypeptide in said vaccine lacks an influenza globular head domain.
11. The vaccine of any one of claims 1 to 10, wherein (i) the amino acid sequences of the HA1 segments are at least 70%, 75%, 80%, 85%, 90%, 95%, 96% or 98% identical to the amino acid sequences of the corresponding segments of an HA1 from an H1, H2, H3, H4, H5, H6, H7, H8, H9, H10, H11, H12, H13, H14, H15 or H16 influenza A; or (ii) the amino acid sequence of the HA2 domain is at least 70%, 75%, 80%, 85%, 90%, 95%, 96% or 98% identical to the amino acid sequence of an HA2 from an H1, H2, H3, H4, H5, H6, H7, H8, H9, H10, H11, H12, H13, H14, H15 or H16 influenza A.

12. The vaccine of any one of claims 1-11, wherein the polypeptide is soluble.
13. The vaccine of claim 12, wherein the polypeptide comprises a thrombin cleavage site and a foldon domain, and (b) lacks an influenza virus HA signal peptide, transmembrane domain, and cytoplasmic tail.
14. The vaccine of any one of claims 1 to 13, wherein the HA1 N-terminal stem segment comprises a cysteine residue covalently linked to a cysteine residue of the HA1 C-terminal stem segment via a disulfide bridge.
15. The vaccine of any one of claims 1 to 14, wherein:
 - (a) the HA1 N-terminal stem segment comprises the amino acid sequence A₁₇-A₁₈-(Xaa)_n-A₃₈ (SEQ ID NO:146), wherein
 - A₁₇ is Y or H;
 - A₁₈ is H, L, or Q;
 - (Xaa)_n represents a sequence of 18-20 amino acid residues; and
 - A₃₈ is H, S, Q, T or N;
 - (b) the HA1 C-terminal stem segment comprises the amino acid sequence A₂₉₁-A₂₉₂, wherein
 - A₂₉₁ is T, S, N, D, P or K; and
 - A₂₉₂ is L, M, K or R;
 - (c) the HA2 domain comprises the amino acid sequence A₁₈-A₁₉-A₂₀-A₂₁, wherein
 - A₁₈ is V or I;
 - A₁₉ is D, N or A;
 - A₂₀ is G, and
 - A₂₁ is W; or
 - (d) the HA2 domain comprises the amino acid sequence A₃₈-A₃₉-A₄₀-A₄₁-A₄₂-A₄₃-A₄₄-A₄₅-A₄₆-A₄₇-A₄₈-A₄₉-A₅₀-A₅₁-A₅₂-A₅₃-A₅₄-A₅₅-A₅₆ (SEQ ID NO:149), wherein
 - A₃₈ is K, Q, R, L or Y;

A₃₉ is any amino acid residue;
A₄₀ is any amino acid residue;
A₄₁ is T;
A₄₂ is Q;
A₄₃ is any amino acid residue;
A₄₄ is A;
A₄₅ is I;
A₄₆ is D;
A₄₇ is any amino acid residue;
A₄₈ is I, V or M;
A₄₉ is T, Q or N;
A₅₀ is any amino acid residue;
A₅₁ is K;
A₅₂ is V or L;
A₅₃ is N;
A₅₄ is any amino acid residue;
A₅₅ is V, I or L; and
A₅₆ is V or I.

16. The vaccine of any one of claims 1 to 15, wherein said linker is of 1 to 40, 1 to 30 residues, 1 to 20 residues, 1 to 10 residues, 1 to 5 residues, 1 to 4 residues, 1 to 3 residues, 1 to 2 residues or 1 residue.
17. The vaccine of any one of claims 1 to 16, wherein said linker is GG, PG, GGG, GGGG, GGGGG, ITPNGSIPNDKPFQNVNKITYGA, or NAS.
18. The vaccine of any one of claims 1 to 17, wherein

(a) the amino acid sequence of the HA1 N-terminal stem segment of the polypeptide in said vaccine consists of amino acid residues A_{N-term} through A_p, wherein A_{N-term} is the N-terminal amino acid of a mature HA0 protein lacking a signal peptide, and wherein A_p is Cys that corresponds to amino acid position 52 of an HA1 domain using H3 numbering, and

(b) the amino acid sequence of the HA1 C-terminal stem segment of the polypeptide in said vaccine consists of amino acid residues A_q through A_{C-term} of the HA1 domain, wherein A_q is Cys that corresponds to amino acid position 277 of an HA1 domain using H3 numbering, and wherein A_{C-term} is the C-terminal amino acid of the HA1 domain.

19. The vaccine of claim 18, wherein A_p forms a disulfide bond with A_q .
20. The vaccine of claim 18 or 19, wherein up to 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid residues are deleted from either or both termini of the HA1 N-terminal stem segment and/or wherein up to 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid residues are deleted from either or both termini of the HA1 C-terminal stem segment.
21. The vaccine of claim 18 or 19, wherein 1, 2, or 3 residues are added to a C-terminus of the HA1 N-terminal stem segment and/or wherein 1, 2, or 3 residues are added to an N-terminus of the HA1 C-terminal stem segment.
22. The vaccine of any one of claims 1 to 21, wherein the HA1 N-terminal stem segment, HA1 C-terminal stem segment, and HA2 domain are obtained or derived from a single influenza virus subtype or strain.
23. The vaccine of any one of claims 1 to 22 further comprising an adjuvant.
24. A method of inducing an immune response to an influenza virus hemagglutinin (HA) in a subject comprising administering to the subject an effective amount of the vaccine of any one of claims 1 to 23.
25. The method of claim 24, wherein the immune response is effective to prevent and/or treat an influenza virus disease or an influenza virus infection caused by a subtype of an influenza virus that belongs to one HA group and not by a subtype of an influenza virus that belongs to the other group.
26. A method of preventing an influenza virus disease comprising administering to a subject an effective amount of the vaccine of any one of claims 1 to 23.

27. The method of claim 26, wherein the influenza virus disease that is prevented is an influenza virus disease caused by a subtype of an influenza virus that belongs to one HA group and not by a subtype of an influenza virus that belongs to the other group.
28. A method of treating an influenza virus infection or an influenza virus disease comprising administering to a subject an effective amount of the vaccine of any one of claims 1 to 23.
29. The method of claim 28, wherein the influenza virus infection or influenza virus disease that is treated is an influenza virus infection or an influenza virus disease caused by a subtype of an influenza virus that belongs to one HA group and not by a subtype of an influenza virus that belongs to the other group.
30. The method of any one of claims 25, 27, or 29, wherein said one HA group consists of:
 - (i) H1, H2, H5, H6, H8, H9, H11, H12, H13, and H16; or
 - (ii) H3, H4, H7, H10, H14, and H15.
31. The method of any one of claims 24 to 30, wherein the vaccine is administered intramuscularly or intranasally to the subject.
32. The method of any one of claims 24 to 31, wherein the subject is a human.
33. Use of the vaccine according to any one of claims 1 to 23, in the manufacture of a medicament for the treatment or prevention of an influenza virus or influenza virus disease.

▼(Mature residue 1)

H1 MKANLLVLLCALA-----AADADTICIGYHANNSTDTVDTVLEKNVTVTHSVNLLLE
H2 --MAIYLLILFT-----AVRGDQICIGYHSNNSTEKVDITILERNVTVTHAQNILE
H3 MKTIIALSYIFCLALGQDLPGNDNSTATLCLGHHA VPNGTLVKITITDDQIEVTNATELVQ
H4 MLSIVILFLLIAENS----SQNYTGNPVICMGHHAVANGTMVKTLADDQVEVVTAQELVE
H5 -MERIVLLLAIVS-----LVKSDQICIGYHANKSTKQVDTIMEKNVTVTHAQDILE
H6 -MIAIIVVAILAT-----AGRSDDKICIGYHANNSTTQIDTILEKNVTVTHSVELLE
H7 MNTQILVLFALVAVIPTN-----ADKICLGHHA VSNGTKVNTLTERGVEVVNATETVE
H8 --MEKFIAIAT-LAS-----TNAYDRICIGYQSNNSTDTVNTLIEQNVPTQTMEELVE
H9 -METKAI IAALLMVT-----AANADKICIGYQSTNSTETVDTLTESNVPVTHTKELLH
H10 MYKVVVIIALLGAVKG-----LDRICLGHHA VANGTIVKTLTNEQEVTNATETVE
H11 -MEKTLFAAIFL-----CVKADEICIGYLSNNSTDKVDTIIENNVTVTSSVELVE
H12 --MEKFIIILSTVLAA-----SFAYDKICIGYQTNNSTETVNTLSEQNVPTQVEELVH
H13 MALNV IATLTLIS-V-----CVHADRICVGYLSTNSSERVDTLLENGVPVTSSIDLIE
H14 MIALIIVLALALSH TAYSQITNGTGNPIICLGHHA VENGTSVKTLTDNHVEVVSAKELVE
H15 MNTQIIVILVGLSMVK-----SDKICLGHHA VANGTKVNTLTERGVEVVNATETVE
H16 MMIKVLYFLIIVLGR-----YSKADKICIGYLSNNSSDTVDTLTENGVPVTSSVDLVE

▲(Mature residue 1)

▼(Residue Ap)

H1 DSHNGKLCRLKGIAPLQLGKCNIA GWLLGNPECDPLLPVRSWSYIVETPNSENGICYPGD
H2 KTHNGKLCCKLNGI PPLELGDCSIAGWLLGNPECDRLLTVPESWYIMEKENPRNGLCYPGS
H3 SSSTGKICNN- PHRI LDGIDCTLIDALLGDPHCDVFQN-ETWDLFVERSKAFS-NCYPYD
H4 SQNLPELCPS-PLRLVDGQTCDIINGALGSPGCDHLNG-AEWDVFIERPNAVD-TCYPFD
H5 RTHNGKLCSLNGVKPLILRDCSVAGWLLGNPMCEFLNLPWLYIVEKDNPI NSLCYPGD
H6 NQKEERFCKILKKAPLDLKGCTIEGWILGNPQCDLLLGDSWSYIVERPTAQNGICYPGV
H7 RTNIPKICSK-GKRITDLGQCGLLGTITGPPQCDQFLE-FSADLIERREGND-VCYPGK
H8 TEKHPAYCNTDLGAPLELRDCKIEAVIYGNPKCDIHLKDQGSYIVERPSAPEGMCYPGS
H9 TEHNGMLCATDLGHPLILDTCTIEGLIYGNPSCDILLGGKEWSYIVERSSAVNGMCYPGN
H10 STNLNKLCKM-GRSYKDLGNCHPVGMLIGTPVCDPHLT-GTWDTLIERENAI A-HCYPGA
H11 TEHTGSFCSINGKQPI SLGDCSFAGWILGNPMCEDELIGKTSWSYIVEKPNPTNGICYPGT
H12 RGIDPILCGTELGSPLVLDDCSLEGLILGNPKCDLYLNGREWSYIVERPKEMEGVCYPGS
H13 TNHTGTYSCLNGVSPVHLGDCSFEGWIVGNPACTSNFGIREWSYLIEDPAAPHGLCYPGE
H14 TNHTDELCP-PLKLVDGQDCHLINGALGSPGCDRLQD-TTWDVFIERPTAVD-TCYPFD
H15 ITGIDKVCTK-GKKAVDLGSCGILGTIIGPPQCDLHLE-FKADLIERRNSSD-ICYPGR
H16 TNHTGTYSCLNGISPIHLGDCSFEGWIVGNPSCATNINIREWSYLIEDPNAPNKFCYPGE

▲(Residue Ap)

H1 FIDYEELREQLSSVSSFERFEIFPKESSWP NHNTNGV-TAAC SHE-GKSSFYRNLLWLTE
H2 FNDYEELKHLSSVTHFEKVKILPK-DRWTQHTTTGG-SRACAVS-GNPSFFRN MVWLTK
H3 VPDYASLRSLVAS-S-GTLEFITEGFTW-TGVTQNGGSNACKRGPG-NGFFSRLNWLTK
H4 VPEYQSLRSILAN-N-GKFEFIAEEFQW-NTVKQNGKSGACKRANV-DDFFNRLNWLVK
H5 FNDYEELKYLSSSTNHFEKIRIIPR-SSWSNH DASSGVSSACPYI-GRSSFLRN VVWLK
H6 LNEVEELKALIGSGERVERFEMFPK-STWTGVDTS SGVTRACPYN-SGSSFYRNLLWIIK
H7 FVNEEALRQILRG-S-GGIDKETMGFTY-SGIRTNGTTSACRRSG--SSFYAEME WLLS
H8 VENLEELRFLVFSSAASYKRIRLFDY-SRWNVTRS--GTSKACNASTGGQSFYRSINWLTK
H9 VENLEELRSLFSSAKSYKRIQIFPD-KTWNVTYS--GTSRACSN----SFYRSMRWLTH
H10 TINEEALRQKIME-S-GGISKMSTGFTYGSITSAGTTKACMRNGG-DSFYAELKWLVLS
H11 LESEEEELRLKFSGVLEFNKFEVFTS-NGWGAVNSGVGVTAACKFG-GSNSFFRN MVWLTH
H12 IENQEELRSLFSSIKKYERVKMFD- TKWNVTYT--GTSKACNNTSNQGSFYRSMRWLTL
H13 LNNNGELRHLFSGIRSF SRTELIPP-TSWG EVLD--GTTACRDNTGTNSFYRNLVWFIK
H14 VPDYQSLRSILAS-S-GSLEFIAEQFTW-NGVKVDGSSSACL RGGR-NSFFSRLNWLTK
H15 FTNEEALRQIIRE-S-GGIDKESMGFRY-SGIRTDGATSACKRTV--SSFYSEM KWLLS
H16 LDNNGELRHLFSGVNSFSRTELINP-SKWGNVLD--GVTASCLDR-GASSFYRNLVWIVK

H1 -K-EGSYPKLKNSYVNKKGKEVLVLWGIHHPNSKEQQONLYQENAYVSVVTSNYNRRFT
H2 -K-GSNIPIAKGSYNNTSGEQMLIIWGVHHPNDETEQRTLYQNVGTYSIGTSTLNKRSI
H3 S--GSTYPVLNVTMPNNDNFDKLIWGVHHPSTNQEQTSLYVQESGRVTVSTRSQSII
H4 SD-GNAYPLQNLTKINNGDYARLYIWGVHHPSTSTEQTNLYKNNPGRVTVSTKTSQTSVV
H5 -K-NNTYPTIKRSYNNTNQEDLLI LWGIHHPNDAAEQTKLYQNPTTYVSVGTSTLNQRSI
H6 TK-SAAYSVMIKGAYNNTGNQPILYFWGVHHPDPTNEQNTLYGSGDRYVRMGTESMNFAS
H7 NTDNASFPQMTKSYKNTRRRESALIVWGIHHSGSTTEQTKLYGSGNKLI TVGSSKYHQSFV
H8 KE-PD TYDFNEGAYVN NEDGDIIFLWGIHHPDPTKEQTTLYKNANTLSSVTTNTINRSFQ

FIG. 1A

H9 K--SNSYPFQNAHYTNNERENILFMWGIHHPPTDTEQTDLYKNADTTTSVTTEDINRTFK
H10 KTKGQNFPPQTTNTYRNTDTAEHLIIWGIHHPSTQEKNDLYGTQSLISVESSTYQNNFV
H11 -Q-SGTYPVIKRTFNNTKGRDVLIVWGIHHPATLTEHQDLYKDDSSYVAVGSETYNRRFT
H12 K--SGQFPVQTDDEYKNTRDSDIVFTWAIHHPPTSDEQVKLYKNPDTLSSVTTVEINRSFK
H13 -K-NTRYPVISKTYNNTTGRDVLVLWGIHHPVSVDETKTLYVNSDPYTLVSTKSWSEKYK
H14 AT-NGNYGPIINVTKENTGSYVRLYLWGVHHPSSDNEQTDLYKVATGRVTVSTRSDQISIV
H15 SMNNQVFPQLNQTYRNTRKEPALIVWGVHSSSLDEQNKLYGTGNKLI TVGSSKYQSSFS
H16 -K-DEKYPVIKGDYNNNTTGRDVLVLWGIHHPDTETTATNLYVNKNPYTLVSTKEWSKRYE

H1 PEIAERPVKVRDQAGRMNYYWTLTKPGDTII FEANGNLIAPMYAFALSRGFG-----S
H2 PVIATRPKVNGQGGRMEFSWTIILDIWDTINFESTGNLIAPEYGFRI SKRGS-----S
H3 PNIGSRPWVRGQSSRSISYWTIVKPGDVLVINSNGNLIAPRGYFKMRTG-----KS
H4 PDIGSRPLVRGQSGRVSFYWTIVEPGDLIVFNTIGNLIAAPRGHYKLNNQK-----KS
H5 PEIATRPKVNGQSGRMEFFWTILKPNDAINFESNGNFIAPRYAYKIVKKG-----S
H6 PEIAARPAVNGQGRIDYYSILKPGETLNVESNGNLIAPWYAFRFVSTSNK-----G
H7 PSPGTRPQINGQSGRIDFHWLILDPNDTIVTFNFNGAFIAPNRASFLR-----GKS
H8 PNIGRPLVRGQQGRMDYYWGILKRGETLKIRTNGLIAPYGHVLTGESHG-----R
H9 PVIATRPKVNGQSGRIDYYSVILKPGQTLRIRSNGLIAPWYGHVLTGESHG-----R
H10 PVVGARPVNGQSGRIDFHWTLVQPGDNITFSDNGGLIAPSRVSKLT-----GRD
H11 PEINTRPRVNGQAGRMTFYWKIVKPGESITFESNGAFLAPRYAFEIVSVGN-----G
H12 PNIGRPLVRGQQGRMDYYWAVLKPQGTVKIQTNGLIAPYGHVLTGESHG-----R
H13 LETGVRPGYNGQSRWMIYWSLIHPGEMITFESNGGFLAPRYGYIIIEYK-----G
H14 PNIGSRPRVRNQSGRISYWTLVNPGDSII FNSIGNLIAPRGHYKISKST-----KS
H15 PSPGARPKVNGQAGRIDFHWMLLDPGDVTFTFNGAFIAPDRATFLRSNAPSGIEYNGKS
H16 LEIGTRIG-DGQRSWMLKYWHLMHPGERIMFESNGGLIAPRYGYIIIEKYGT-----G

▼(Residue Ag)

H1 GIITSNASMHE-CNTKCQTPLGAINSSLPYQNIHPVTIGECPKYVRS AKLRMVTGLRNNP
H2 GIMKTEGTLEN-CETKCQTPLGAINSSLPYQNIHPVTIGECPKYVRS AKLRMVTGLRNNP
H3 SIMSSDAPIDT-CISECITPNGSIPNDKPFQNVNKITYGACPKYVKQNTLKLATGMRNVP
H4 TILNTAIPIGS-CVSKCHTDKGSSTTKPFQNISRIAVGDCPRYVKQGS LKLATGMRNIP
H5 AIMKSGLAYGN-CDTKCQTPVGEINSSMPFHNHPHTIGECPKYVKS DRLVLATGLRNV
H6 AVFKSNLPIEN-CDATCQTVAGVLRNTKTFQNVSPWIGECPKYVKSESLRLATGLRNV
H7 MGIQSDVQVDANCEGECYHSGGTITSRLPFQNI NSRAVGKCPRYVKQES LLLATGMKNVP
H8 IIQNEDIPIGN-CNTKCQTYAGAINS SKPFQNASRHYMGECPKYVVKAS LRLAVGLRNT
H9 ILKT-DLNNGN-CVVQCQTEKGG LNTTLPFHNISKYAFGNCPKYVGVKSLKLPVGLRNV
H10 LGIQSEALIDNSCESKCFWRGGSINTKLPFQNLSPRTVGGCPKYVNRSL LLLATGMKNVP
H11 KLFRSELNIES-CSTKCQTEIGGINTNKS FHNVRNTIGDCPKYVNVKSLKLATGPRNVP
H12 ILKN-NLPMGQ-CVTECQLNEGVMNTSKPFQNTSKHYIGKCPKYIPSGSLKLALGLRNV
H13 RIFQSRIRMSR-CNTKCQTSVGGINTNR TFQNI DNKALGDCPKYIKSGQLKLATGLRNV
H14 TVLKS DKKRIGS-CTSPCLTDKGS IQSDKPFQNVSR IAGNCPKYVKQGS LMLATGMKNIP
H15 LGIQSDAQIDESCEGECFYSGGTINSPLPFQNI DSRVAGKCPRYVKQSS LPLALGMKNVP
H16 RIFQSGVRMAR-CNTKCQTS LGGINTNKT FQNIERNALGDCPKYIKSGQLKLATGLRNV

▲(Residue Ag)

▼(HA2 domain starts)

H1 ----SIQSRGLFGAIAAGFIEGGWTGMIDGWYGYHHQNEQSGYAADQKSTQNAINGITNK
H2 ----QIESRGLFGAIAAGFIEGGWQGMIDGWYGYHHSNDQSGYAADKESTQKAIDGITNR
H3 ----EKQTRGLFGAIAAGFIENGWEGMIDGWYGFHRHQNSEGTGQAADLKSTQAAIDQINGK
H4 ----EKASRGLFGAIAAGFIENGWQGLIDGWYGFHRHQNAEGTGTAADLKSTQAAIDQINGK
H5 ----QRKKRGLFGAIAAGFIEGGWQGMVDGWYGYHHSNEQSGYAADKESTQKAIDGITNK
H6 ----QIETRGLFGAIAAGFIEGGWTGMIDGWYGYHHNSQSGYAADRESTQKAIDGITNK
H7 EPSKKRKKRGLFGAIAAGFIENGWGLVDGWYGFHRHQNAQEGTAAADYKSTQSAIDQITGK
H8 ----SVEPRGLFGAIAAGFIEGGWSGMIDGWYGFHHSNSEGTGMAADQKSTQEAIDKITNK
H9 ----AVSSRGLFGAIAAGFIEGGWPLVAGWYGFQHSNDQGVGMAADKSTQKAIDKITSK
H10 ---EVVQGRGLFGAIAAGFIENGWEGMVDGWYGFHRHQNAQGTGQAADYKSTQAAIDQITGK
H11 ----AIASRGLFGAIAAGFIEGGWPLINGWYGFQHRDEEGTGIAADKESTQKAIDQITSK
H12 ----QVQDRGLFGAIAAGFIEGGWPLVAGWYGFQHQNAEGTGIAADRDSTQRAIDNMQNK
H13 ----AISNRGLFGAIAAGFIEGGWPLINGWYGFQHQNEQGTGIAADKESTQKAIDQITTK
H14 ----GKQAKGLFGAIAAGFIENGWQGLIDGWYGFHRHQNAEGTGTAADLKSTQAAIDQINGK
H15 ----EKIRTRGLFGAIAAGFIENGWGLVDGWYGFHRHQNAQGGTAAADYKSTQAAIDQITGK
H16 ----SIGERGLFGAIAAGFIEGGWPLINGWYGFQHQNEQGTGIAADKASTQKAINEITTK

▲(HA2 domain starts)

FIG. 1B

H1 VNTVIEKMNIQFTAVGKEFNKLEKRMENLNKKVDDGFLDIWYNAELLVLENERTLDFH
H2 VNSVIEKMNTQFEAVGKEFSNLEKRLLENLNKKMEDGFLDVWYNAELLVLMENERTLDFH
H3 LNRVIEKTNEKFHQIEKEFESEVEGRIQDLEKYVEDTKIDLWSYNAELLVALENQHTIDL
H4 LNRLIEKTNNDKYHQIEKEFEQVEGRIQDLENYVEDTKIDLWSYNAELLVALENQHTIDVT
H5 VNSIIDKMNTQFEAVGKEFNLERVENLNKKMEDGFLDVWYTNVELLVLMENERTLDFH
H6 VNSIIDKMNTQFEAVDHEFSNLERRIDNLNKRMEDGFLDVWYNAELLVLENERTLDFH
H7 LNRLIEKTNQQFELIDNEFTEVEKQIGNLINWTKDSITEVWSYNAELIVAMENQHTIDLA
H8 VNNIVDKMNRFEFVNHEFSEVEKIRINMINDKIDDQIEDLWAYNAELLVLENQKTLDH
H9 VNNIIDKMNKQYEVIDHEFNELEARNMINNKIDDQIQDIWAYNAELLVLENQKTLDH
H10 LNRLIEKTNTEFESIESEFSETEHQIGNVINWTKDSITDIWYNAELLVAMENQHTIDMA
H11 VNNIVDRMNTNFESVQHEFSEIEERINQLSKHVDDSVVDIWSYNAQLLVLENQKTLDH
H12 LNNVIDKMNKQFEVNVHEFSEVESRINMINSKIDDQITDIWAYNAELLVLENQKTLDH
H13 INNIIDKMNGNYDSIRGEFNQVEKIRINMLADRIDDVTDIWSYNAKLLVLENDKTLDMH
H14 LNRLIEKTNKYHQIEKEFEQVEGRIQDLEKYVEDTKIDLWSYNAELLVALENQHTIDVT
H15 LNRLIEKTNKQFELIDNEFTEVEQQIGNVINWTRDSLTEIWSYNAELLVAMENQHTIDLA
H16 INNIIEKMNGNYDSIRGEFNQVEKIRINMLADRVDVTDIWSYNAKLLVLENDRTLDH

H1 DSNVKNLYEKVKSQKLNNAKEIENGCGFEFYHKCDNECMESVRNGTYDYPKYSEESKLNRE
H2 DSNVKNLYDRVRMQLRDNNAKELGNGCGFEFYHKCDNECMNSVKNGTYDYPKYSEESKLNRN
H3 DSEMKNLFETRRLRENAEDMGNGCFKIYHKCDNACIESIRNGTYDHDVYRDEALNNRF
H4 DSEMKNLFERVRRLRENAEDKGNGCFEIFHKCDNNCIESIRNGTYDHDVYRDEALNNRF
H5 DSNVNNLYDKVRLQLKDNARELGNGCGFEFYHKCDNECMESVRNGTYDYPQYSEEARLNRE
H6 DANVKNLYERVKSQKLRDNAMILGNGCGFEFWHKCDNECMESVKNGTYDYPKYQDESKLNRO
H7 DSEMKNLYERVRLRENAEEDGTGCGFEIFHKCDNDCMASIRNNTYDHSKYREEAMQNR
H8 DSNVKNLFDEVKRRLSANAIIDAGNGCFDILHKCDNECMETIKNGTYDHKEEYEEAKLERS
H9 DANVNNLYNKVKRALGSNAVEDGNGCGFELYHKCDNDCMETIRNGTYDRQKYQEEARLRO
H10 DSEMLNLYERVRLRQNAEEDGKCGFEIYHTCDDSCMESIRNNTYDHSQYREEALNRL
H11 DSNVKNLHEKVRRLKDNNAKDEGNGCFEYHKCDNCKIERVRNGTYDHKEFEESKLNRO
H12 DANVRNLHDRVRLRENAIDTGDGCGFEILHKCDNCCMDTIRNGTYNHKEEYEEESKIERO
H13 DANVKNLHEGVRRLKDNNAIDEGNGCFELHCKNDCMETIRNGTYDHTYAEESKLRRO
H14 DSEMKNLFERVRRLRENAEDQNGCGFEIFHQCDNNCIESIRNGTYDHNIRDEALNNRI
H15 DSEMKNLYERVRLRQNAEEDGTGCGFEIFHRCDNDCMESIRNNTYHNHTYRQEALQNR
H16 DANVRNLHDQVKRALKSNAIDEGDGCNLLHCKNDCMETIRNGTYNHEDYREESQKLRRO

H1 KVDGVKLESMSG-IYQILAIYSTVASSLVLLVSLGAISFWMCSNGSLQCRICI
H2 EIKGVKLSNMG-VYQILAIYATVAGSLSLAIMIAGISLWMCNSNGSLQCRICI
H3 QIKGVKLSG--KDWILWISFAISCFLLCVLLGFIMWACQNGNIRCNICI
H4 QIQGVKLTQGY--KDIILWISFISCFLLVALLAFILWACQNGNIRCNICI
H5 EISGVKLESMSG-VYQILAIYSTVASSLALAIMIAGLSFWMCSNGSLQCRICI
H6 EIESGVKLESIG-VYQILAIYSTVSSSLVVLGLIIAVGLWMCNSNGSMQCRICI
H7 QIDPVKLSSGY--KDVILWFSFGASCFLLLAIAMGLVFCVKNGNMRCTICI
H8 KINGVKLEENT-TYKILSIYSTVAASLCLAILIAGGLIILGMQNGSCRCMFCI
H9 KIEGVKLESEG-TYKILTIYSTVASSLVLAMGFAAFLFWAMSGNRCRCNICI
H10 NINPVKLSSGY--KDIILWFSFGESCFVLLAVVMGLVFFCLKNGNMRCTICI
H11 EIEGVKLDSSGNVYKILSIYSCIASSLVLAALIMGMFWACSGNRCRCNICI
H12 KVNGVKLEENS-TYKILSIYSSVASSLVLLMIIGGFI FGQNGNVRCTFCI
H13 EIDGIKLKSEDNVYKALSIIYSCIASSVVLVGLILSFIMWACSSGNCRFNVCI
H14 KINPVTLTMGY--KDIILWISFMSCFVVALILGFVLWACQNGNIRCNICI
H15 MINPVKLSSGY--KDVILWFSFGASCVMLLAIAMGLIFMCVKNGNLRCTICI
H16 EIEGIKLKTEDNVYKVLIIYSCIASSIVLVGLILAFIMWACSGNRCRFNVCI

FIG. 1C

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H1  MK--ANLLVLLCALAAADAD-----TICIGYHANNSTDTVDTVLEKNVTVTHSVNLL
H3  MKTIIALSIFYFCLALGQDLPGNDNSTATLCLGHHAVPNGTLVKTITDDQIEVTNATELVQ
HB  MK---AIIVILMVVTSNADR-----ICTGITSSNSPHVVKATATQGEVNVTVGVIPLTT

      ▼(Cys52, HA1)
H1  DSHNGKLCRLKG-----IAPLQLGKCNIAGWLLGNPECDPLLVRWSYIVETPNSENG-
H3  SSSTGKICNNP-----HRILDGIDCTLIDALLGDPHCD-VFQNETWDLFVERSKAFS--
HB  TPTKSHFANLKGTETRGKLCPKCLNCTDLDVALGRPKCTGKIP SARVSILHEVRPVTSGC
      ▲(Arg50, B-HA1)  ▲(Ala66, B-HA1) ▲(Arg80, B-HA1)

H1  ICYPGDFIDYEELREQLSSVSSF--ERFEIFPKESSWPNHNTNGVTAACS-HEGKSSFYR
H3  NCYPYDVPDYASLRSLVASSG----T-LEFITEGFTWTGVTQNGGSNACK-RGPGNGFFS
HB  FPIMHRTKIRQLPNLLRGYEHIRLSTHNVINAENAPGGPYKIGTSGSCPNI TNGNGFFA

H1  NLLWLTEKE-----GSYPKLKNSYVNKKGKEVLVLWGIHHPNSKEQQONLYQENAYVS
H3  RLNLWTKSG-----STYPVLNVTMPNNDNFDKLYIWGVVHHPSTNQEQTSLYVQESGRVT
HB  TMAWAVPKNDKNKTATNPLTIEVPYICTEGEDQITVWGFHSDSETQMAKLYGDSKPQKFT

H1  VVTSNYYNRFTPEIAERP KVRD-----QAGRMNYYWTLLKPGDTIIFEANGNLIAPMYAF
H3  VSTRRSQQSIIPNIGSRPWVRG-----QSSRSIYWTIVKPGDVLVINSNGNLIAPRGYF
HB  SSANGVTTHYVSQIGGFNPQTEDGGLPQSGRIVVDYMVQKSGKTGTITYQRGILLPQKVW
      ▲(Trp271)

H1  ALSRFGSGGIITSNASMHECNTKQCOTPLGAINSSLPYQNIHPVTIGECPKYVRSAKLRMV
H3  KMRTGKSS-IMSSDAPI DTCISECITPNGSIPNDKPFQNVNKITYGACPKYVKQNTLKL
HB  CASGRSKV-IKGSPLIGEADCLHEKYGGLNKS KPYTGEHAKAIGNCPIVWKTP-LKLA
      ▲(Ser277, B-HA1)

      ▼(HA2 domain starts)
H1  TGLRNNP---SIQSRGLFGAIAAGFIEGGWTGMIDGWYGYHHQNEQSGYAADQKSTQNAI
H3  TGMRNVP---EKQTRGLFGAIAAGFIENGWEGMIDGWYGFRHQNSEGTGQAADLKSTQAAI
HB  NGTKYRPPAKLLKERGFFGAIAGFLEGGWEGMIAGWHGYTSHGAHGVAVAADLKSTQEAI
      ▲(B-HA2 domain starts)

H1  NGITNKVNTVIEKMNIQFTAVGKEFNKLEKRMENLNKKVDDGFLDIWTYNAELLVLLNE
H3  DQINGKLN RVIEKTNEKFHQIEKEFSEVEGRIQDLEKYVEDTKIDLWSYNAELLVALENQ
HB  NKITKNLNSLSELEVKNLQRLSGAMDELHNEILELDEKVDDL RADTISSQIELAVLLSNE

H1  RTLDFHDSNVKNLYEKVKSQ LKNNAKEIGNGCFEFYHKCDNECMESVRNGTYDYPKYSEE
H3  HTIDLTDSEMKNLF EKTRRQLRENAEDMGNGCFKIYHKCDNACIESIRNGTYDHDVYRDE
HB  GIINSEDEHLLALERKLKMLGPSAVEIGNGCFETKHKCNQTC LDRIAAGTFDAGEFSLP

H1  SKLNR-EKVDGVKLES MGIYQILAIYSTVASSLVLLVSLGAISFWMCSNGSLQCRICI
H3  ALNNR-FQIKGVELKSGYKDWILWISFAISCFLLCVVLLGFI-MWACQQRGNIRCNICI
HB  TFDSLNIT AASLNDDGLDNHTILLYYSTAASSLAVTLMIAIFVVYMVSRDNVSCSICL

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FIG. 2

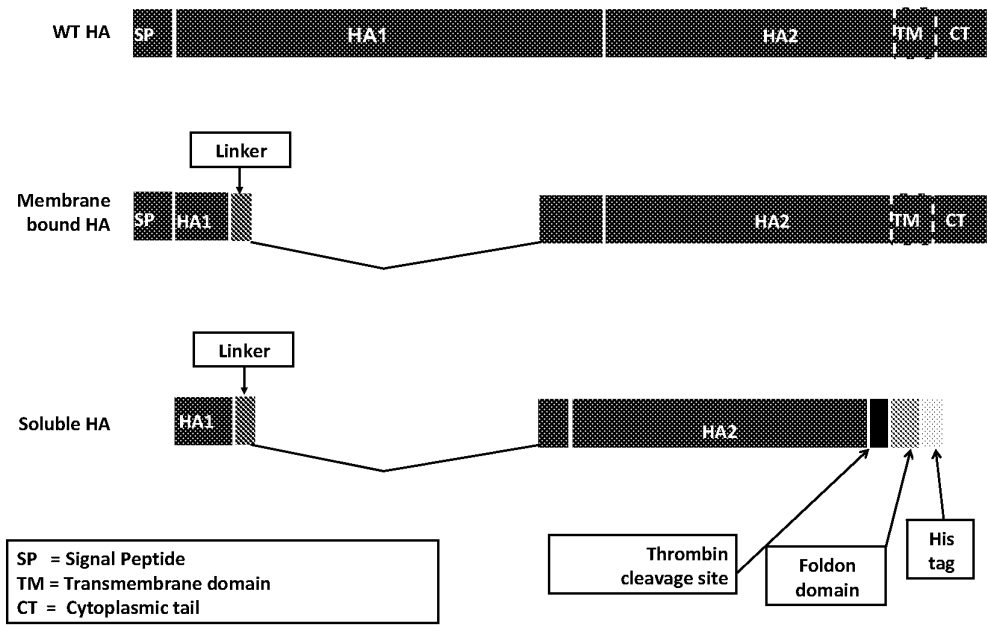


FIG. 3

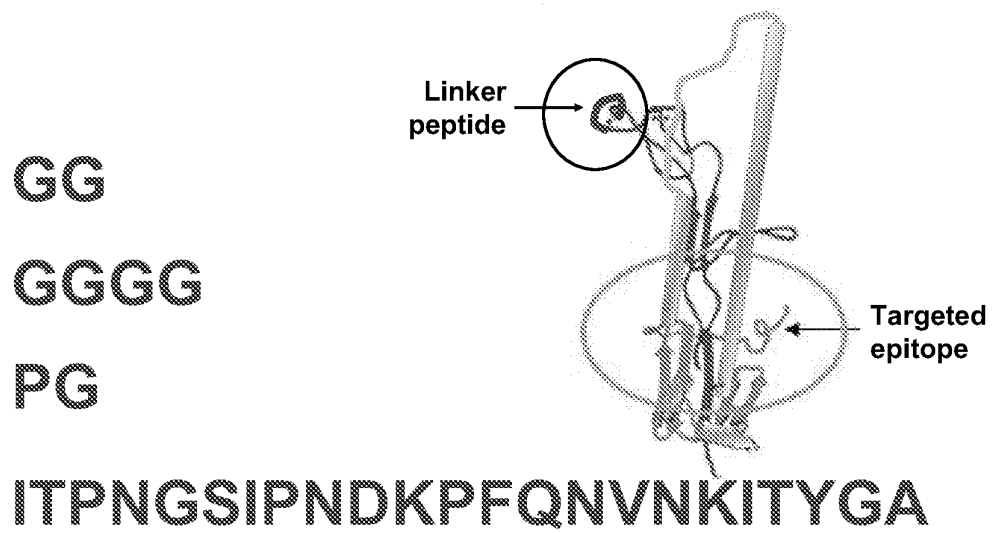


FIG. 4

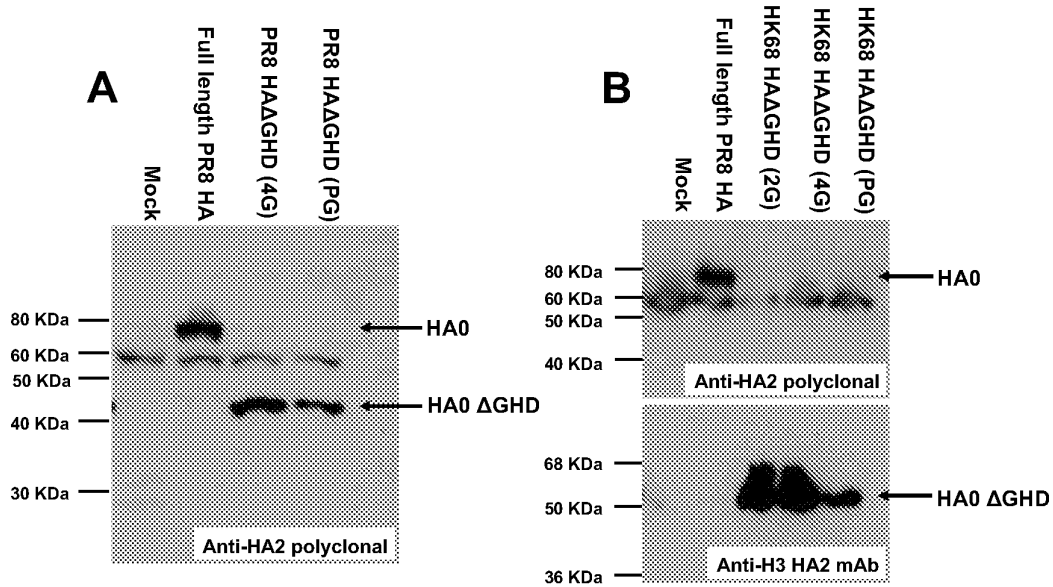


FIG. 5

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AGCAAAAGCA GGGGAAAATA AAAACAACCA AAATGAAGGC AAACCTACTG
                                M K A N L L

51 GTCCTGTTAA GTGCACTTGC AGCTGCAGAT GCAGACACAA TATGTATAGG
   V L L S A L A A A D A D T I C I G

101 CTACCATGCG AACAAATTCAA CCGACACTGT TGACACAGTA CTCGAGAAGA
    Y H A N N S T D T V D T V L E K N

151 ATGTGACAGT GACACACTCT GTTAACCTGC TCGAAGACAG CCACAACGGA
    V T V T H S V N L L E D S H N G

201 AAACATATGTG GAGGCTGTAA CACGAAGTGT CAAACACCCC TGGGAGCTAT
    K L C G G C N T K C Q T P L G A I

251 AAACAGCAGT CTCCCTTACC AGAATATACA CCCAGTCACA ATAGGAGAGT
    N S S L P Y Q N I H P V T I G E C

301 GCCCAAAATA CGTCAGGAGT GCCAAATTGA GGATGGTTAC AGGACTAAGG
    P K Y V R S A K L R M V T G L R

351 AACACTCCGT CCATTCAATC CAGAGGTCTA TTTGGAGCCA TTGCCGGTTT
    N T P S I Q S R G L F G A I A G F

401 TATTGAAGGG GGATGGACTG GAATGATAGA TGGATGGTAT GGTTATCATC
    I E G G W T G M I D G W Y G Y H H

451 ATCAGAATGA ACAGGGATCA GGCTATGCAG CGGATCAAAA AAGCACACAA
    Q N E Q G S G Y A A D Q K S T Q

501 AATGCCATTA ACGGGATTAC AAACAAGGTG AACACTGTTA TCGAGAAAAT
    N A I N G I T N K V N T V I E K M

551 GAACATTCAA TTCACAGCTG TGGGTAAAGA ATTCAACAAA TTAGAAAAAA
    N I Q F T A V G K E F N K L E K R

601 GGATGGAAAA TTTAAATAAA AAAGTTGATG ATGGATTTCT GGACATTTGG
    M E N L N K K V D D G F L D I W

651 ACATATAATG CAGAATTGTT AGTTCTACTG GAAAATGAAA GGACTCTGGA
    T Y N A E L L V L L E N E R T L D

701 TTTCCATGAC TCAAATGTGA AGAATCTGTA TGAGAAAGTA AAAAGCCAAT
    F H D S N V K N L Y E K V K S Q L

751 TAAAGAATAA TGCCAAAGAA ATCGGAAATG GATGTTTTGA GTTCTACCAC
    K N N A K E I G N G C F E F Y H

```

FIG. 6A

801 AAGTGTGACA ATGAATGCAT GGAAAGTGTA AGAAATGGGA CTTATGATTA
K C D N E C M E S V R N G T Y D Y

851 TCCCAAATAT TCAGAAGAGT CAAAGTTGAA CAGGGAAAAG GTAGATGGAG
P K Y S E E S K L N R E K V D G V

901 TGAAATTGGA ATCAATGGGG ATCTATCAGA TTCTGGCGAT CTACTCAACT
K L E S M G I Y Q I L A I Y S T

951 GTCGCCAGTT CACTGGTGCT TTTGGTCTCC CTGGGGGCAA TCAGTTTCTG
V A S S L V L L V S L G A I S F W

1001 GATGTGTTCT AATGGATCTT TGCAGTGCAG AATATGCATC TGAGATTAGA
M C S N G S L Q C R I C I *

1051 ATTTCAGAAA TATGAGGAAA AACACCCTTG TTTCTACT

FIG. 6B

1 AGCAAAAGCA GGGGAAAATA AAAACAACCA AAATGAAGGC AAACCTACTG
M K A N L L

51 GTCCTGTTAA GTGCACTTGC AGCTGCAGAT GCAGACACAA TATGTATAGG
V L L S A L A A A D A D T I C I G

101 CTACCATGCG AACAATTCAA CCGACACTGT TGACACAGTA CTCGAGAAGA
Y H A N N S T D T V D T V L E K N

151 ATGTGACAGT GACACACTCT GTTAACCTGC TCGAAGACAG CCACAACGGA
V T V T H S V N L L E D S H N G

201 AAACATATGTG GAGGCGGAGG TTGTAACACG AAGTGTCAAA CACCCCTGGG
K L C G G G G C N T K C Q T P L G

251 AGCTATAAAC AGCAGTCTCC CTTACCAGAA TATACACCCA GTCACAATAG
A I N S S L P Y Q N I H P V T I G

301 GAGAGTGCCC AAAATACGTC AGGAGTGCCA AATTGAGGAT GGTTACAGGA
E C P K Y V R S A K L R M V T G

351 CTAAGGAACA CTCCGTCCAT TCAATCCAGA GGTCTATTTG GAGCCATTGC
L R N T P S I Q S R G L F G A I A

401 CGGTTTTATT GAAGGGGGAT GGACTGGAAT GATAGATGGA TGGTATGTTT
G F I E G G W T G M I D G W Y G Y

451 ATCATCATCA GAATGAACAG GGATCAGGCT ATGCAGCGGA TCAAAAAAGC
H H Q N E Q G S G Y A A D Q K S

501 ACACAAAATG CCATTAACGG GATTACAAAC AAGGTGAACA CTGTTATCGA
T Q N A I N G I T N K V N T V I E

551 GAAAATGAAC ATTCAATTCA CAGCTGTGGG TAAAGAATTC AACAAATTAG
K M N I Q F T A V G K E F N K L E

601 AAAAAAGGAT GGAAAATTTA AATAAAAAAG TTGATGATGG ATTTCTGGAC
K R M E N L N K K V D D G F L D

651 ATTTGGACAT ATAATGCAGA ATTGTTAGTT CTAATGGAAA ATGAAAGGAC
I W T Y N A E L L V L L E N E R T

701 TCTGGATTTT CATGACTCAA ATGTGAAGAA TCTGTATGAG AAAGTAAAAA
L D F H D S N V K N L Y E K V K S

751 GCCAATTAAA GAATAATGCC AAAGAAATCG GAAATGGATG TTTTGAGTTC
Q L K N N A K E I G N G C F E F

FIG. 7A

801 TACCACAAGT GTGACAATGA ATGCATGGAA AGTGTAAGAA ATGGGACTTA
Y H K C D N E C M E S V R N G T Y

851 TGATTATCCC AAATATTCAG AAGAGTCAAA GTTGAACAGG GAAAAGGTAG
D Y P K Y S E E S K L N R E K V D

901 ATGGAGTGAA ATTGGAATCA ATGGGGATCT ATCAGATTCT GGCGATCTAC
G V K L E S M G I Y Q I L A I Y

951 TCAACTGTCG CCAGTTCACT GGTGCTTTTG GTCTCCCTGG GGGCAATCAG
S T V A S S L V L L V S L G A I S

1001 TTTCTGGATG TGTTCATATG GATCTTTGCA GTGCAGAATA TGCATCTGAG
F W M C S N G S L Q C R I C I *

1051 ATTAGAATTT CAGAAATATG AGGAAAAACA CCCTTGTTTC TACT

FIG. 7B

1 AGCAAAAGCA GGGGAAAATA AAAACAACCA AAATGAAGGC AAACCTACTG
M K A N L L

51 GTCCTGTTAA GTGCACTTGC AGCTGCAGAT GCAGACACAA TATGTATAGG
V L L S A L A A A D A D T I C I G

101 CTACCATGCG AACAAATTCAA CCGACACTGT TGACACAGTA CTCGAGAAGA
Y H A N N S T D T V D T V L E K N

151 ATGTGACAGT GACACACTCT GTTAACCTGC TCGAAGACAG CCACAACGGA
V T V T H S V N L L E D S H N G

201 AAACATATGTC CAGGCTGTAA CACGAAGTGT CAAACACCCC TGGGAGCTAT
K L C P G C N T K C Q T P L G A I

251 AAACAGCAGT CTCCCTTACC AGAATATACA CCCAGTCACA ATAGGAGAGT
N S S L P Y Q N I H P V T I G E C

301 GCCCAAAATA CGTCAGGAGT GCCAAATTGA GGATGGTTAC AGGACTAAGG
P K Y V R S A K L R M V T G L R

351 AACACTCCGT CCATTCAATC CAGAGGTCTA TTTGGAGCCA TTGCCGGTTT
N T P S I Q S R G L F G A I A G F

401 TATTGAAGGG GGATGGACTG GAATGATAGA TGGATGGTAT GGTTATCATC
I E G G W T G M I D G W Y G Y H H

451 ATCAGAATGA ACAGGGATCA GGCTATGCAG CGGATCAAAA AAGCACACAA
Q N E Q G S G Y A A D Q K S T Q

501 AATGCCATTA ACGGGATTAC AAACAAGGTG AACACTGTTA TCGAGAAAAT
N A I N G I T N K V N T V I E K M

551 GAACATTCAA TTCACAGCTG TGGGTAAAGA ATTCAACAAA TTAGAAAAAA
N I Q F T A V G K E F N K L E K R

601 GGATGGAAAA TTTAAATAAA AAAGTTGATG ATGGATTTCT GGACATTTGG
M E N L N K K V D D G F L D I W

651 ACATATAATG CAGAATTGTT AGTTCTACTG GAAAATGAAA GGACTCTGGA
T Y N A E L L V L L E N E R T L D

701 TTTCCATGAC TCAAATGTGA AGAATCTGTA TGAGAAAGTA AAAAGCCAAT
F H D S N V K N L Y E K V K S Q L

751 TAAAGAATAA TGCCAAAGAA ATCGGAAATG GATGTTTTGA GTTCTACCAC
K N N A K E I G N G C F E F Y H

FIG. 8A

801 AAGTGTGACA ATGAATGCAT GGAAAGTGTA AGAAATGGGA CTTATGATTA
K C D N E C M E S V R N G T Y D Y

851 TCCCAAATAT TCAGAAGAGT CAAAGTTGAA CAGGGAAAAG GTAGATGGAG
P K Y S E E S K L N R E K V D G V

901 TGAAATTGGA ATCAATGGGG ATCTATCAGA TTCTGGCGAT CTACTCAACT
K L E S M G I Y Q I L A I Y S T

951 GTCGCCAGTT CACTGGTGCT TTTGGTCTCC CTGGGGGCAA TCAGTTTCTG
V A S S L V L L V S L G A I S F W

1001 GATGTGTTCT AATGGATCTT TGCAGTGCAG AATATGCATC TGAGATTAGA
M C S N G S L Q C R I C I *

1051 ATTTCAGAAA TATGAGGAAA AACACCCTTG TTTCTACT

FIG. 8B

1 ATGGACACAA TATGTATAGG CTACCATGCG AACAAATTCAA CCGACACTGT
 M D T I C I G Y H A N N S T D T V
 51 TGACACAGTA CTCGAGAAGA ATGTGACAGT GACACACTCT GTTAACCTGC
 D T V L E K N V T V T H S V N L L
 101 TCGAAGACAG CCACAACGGA AAACCTATGTG GAGGCGGAGG TTGTAACACG
 E D S H N G K L C G G G G C N T
 151 AAGTGTCAAA CACCCCTGGG AGCTATAAAC AGCAGTCTCC CTTACCAGAA
 K C Q T P L G A I N S S L P Y Q N
 201 TATACACCCA GTCACAATAG GAGAGTGCCC AAAATACGTC AGGAGTGCCA
 I H P V T I G E C P K Y V R S A K
 251 AATTGAGGAT GGTTACAGGA CTAAGGAACA CTCCGTCCAT TCAATCCAGA
 L R M V T G L R N T P S I Q S R
 301 GGTCTATTTG GAGCCATTGC CGGTTTTATT GAAGGGGGAT GGACTGGAAT
 G L F G A I A G F I E G G W T G M
 351 GATAGATGGA TGGTATGGTT ATCATCATCA GAATGAACAG GGATCAGGCT
 I D G W Y G Y H H Q N E Q G S G Y
 401 ATGCAGCGGA TCAAAAAGC ACACAAAATG CCATTAACGG GATTACAAAC
 A A D Q K S T Q N A I N G I T N
 451 AAGGTGAACA CTGTTATCGA GAAAATGAAC ATTCAATTCA CAGCTGTGGG
 K V N T V I E K M N I Q F T A V G
 501 TAAAGAATTC AACAAATTAG AAAAAAGGAT GGAAAATTTA AATAAAAAAG
 K E F N K L E K R M E N L N K K V
 551 TTGATGATGG ATTTCTGGAC ATTTGGACAT ATAATGCAGA ATTGTTAGTT
 D D G F L D I W T Y N A E L L V
 601 CTA CTG GAAA ATGAAAGGAC TCTGGATTTT CATGACTCAA ATGTGAAGAA
 L L E N E R T L D F H D S N V K N
 651 TCTGTATGAG AAAGTAAAAA GCCAATTAAA GAATAATGCC AAAGAAATCG
 L Y E K V K S Q L K N N A K E I G
 701 GAAATGGATG TTTTGAGTTC TACCACAAGT GTGACAATGA ATGCATGGAA
 N G C F E F Y H K C D N E C M E
 751 AGTGTAAGAA ATGGGACTTA TGATTATCCC AAATATTCAG AAGAGTCAAA
 S V R N G T Y D Y P K Y S E E S K

FIG. 9A

801 GTTGAACAGG GAAAAGGTAG ATGGAGTGC G TTCTCTGGTT CCGCGTGGTT
L N R E K V D G V R S L V P R G S

851 CTCCGGGTTC TGGTTACATC CCGGAAGCTC CGCGTGACGG TCAGGCTTAC
P G S G Y I P E A P R D G Q A Y

901 GTTCGTAAAG ACGGTGAATG GGTTCTGCTG TCTACCTTCC TGCACCACCA
V R K D G E W V L L S T F L H H H

951 CCACCACCAC TGA
H H H *

FIG. 9B

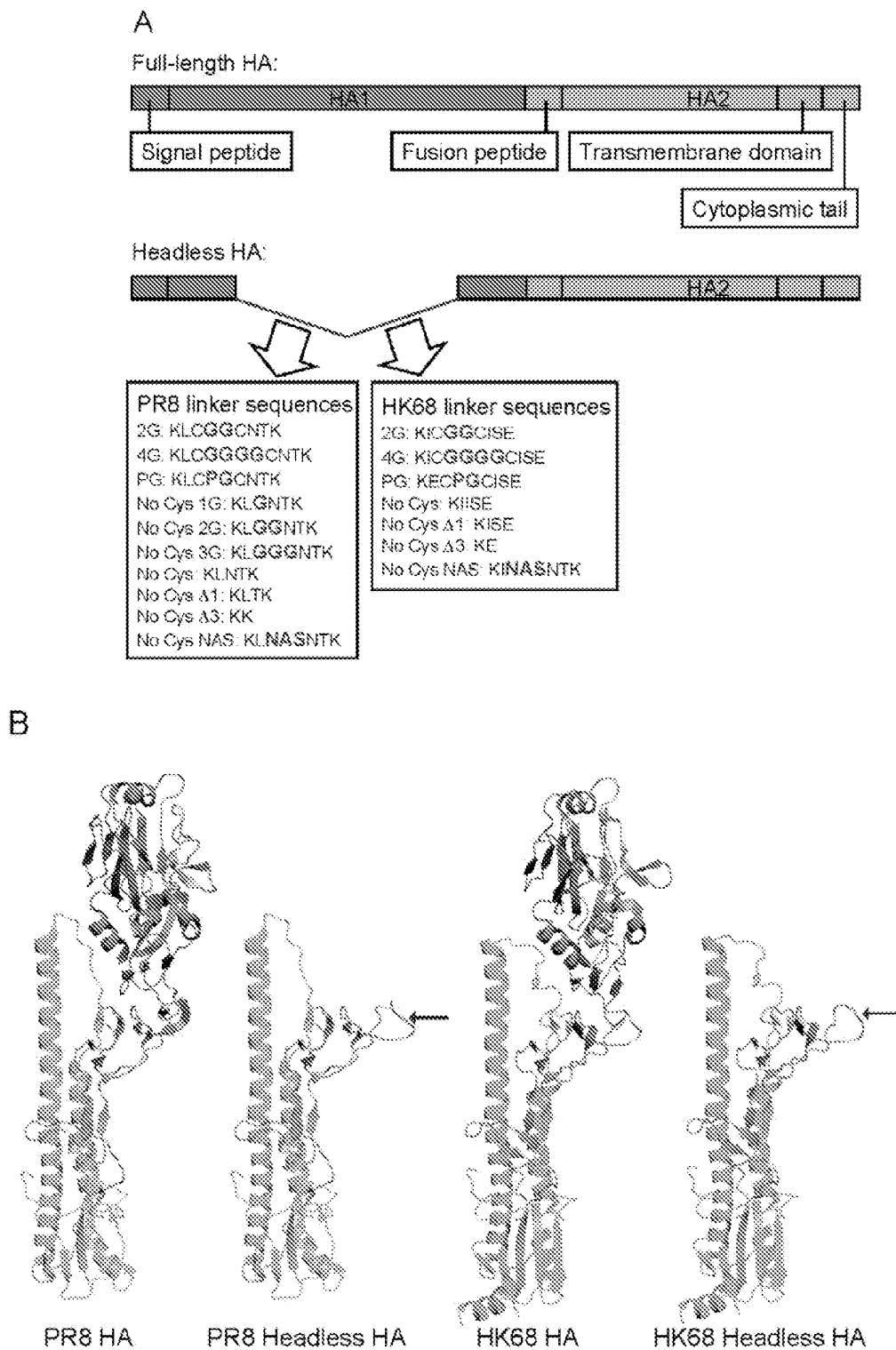


FIG. 10A – 10B

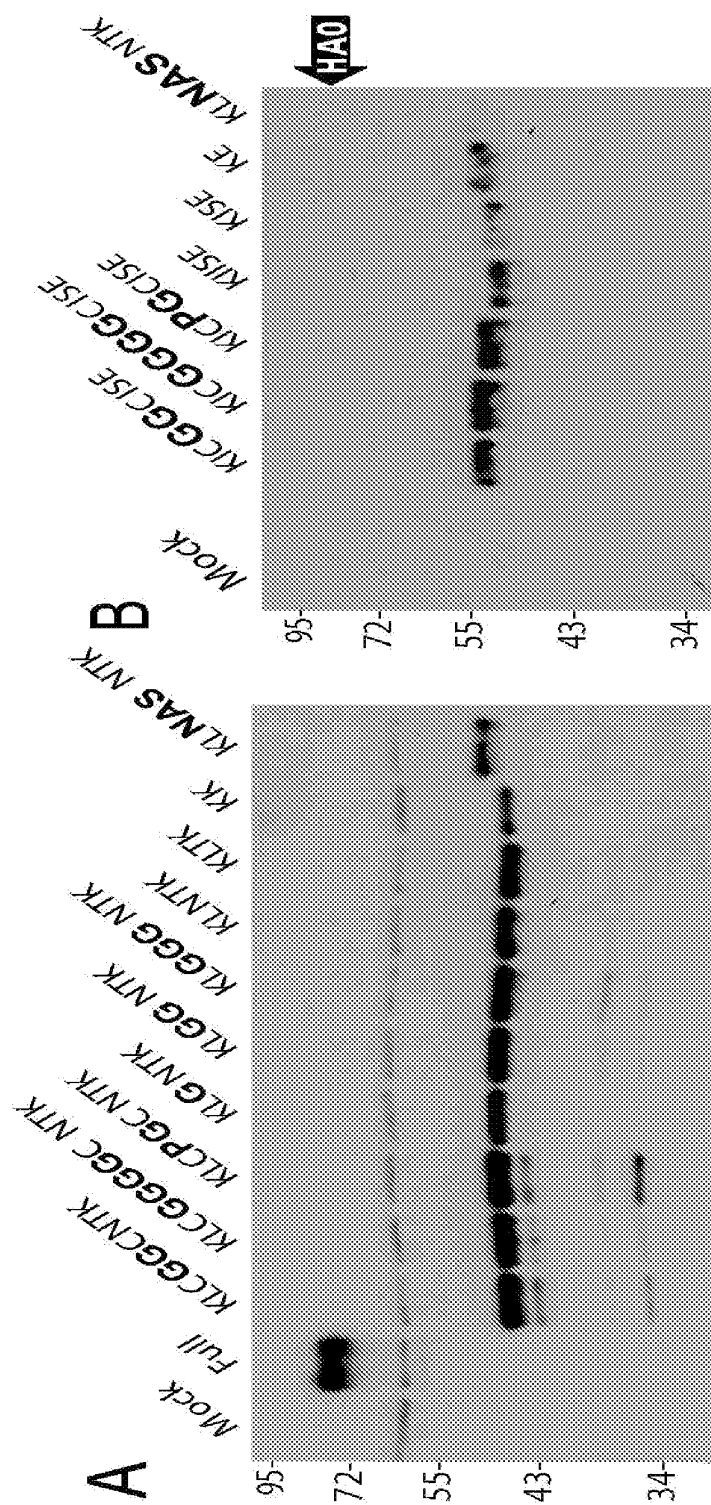


FIG. 11A – 11B

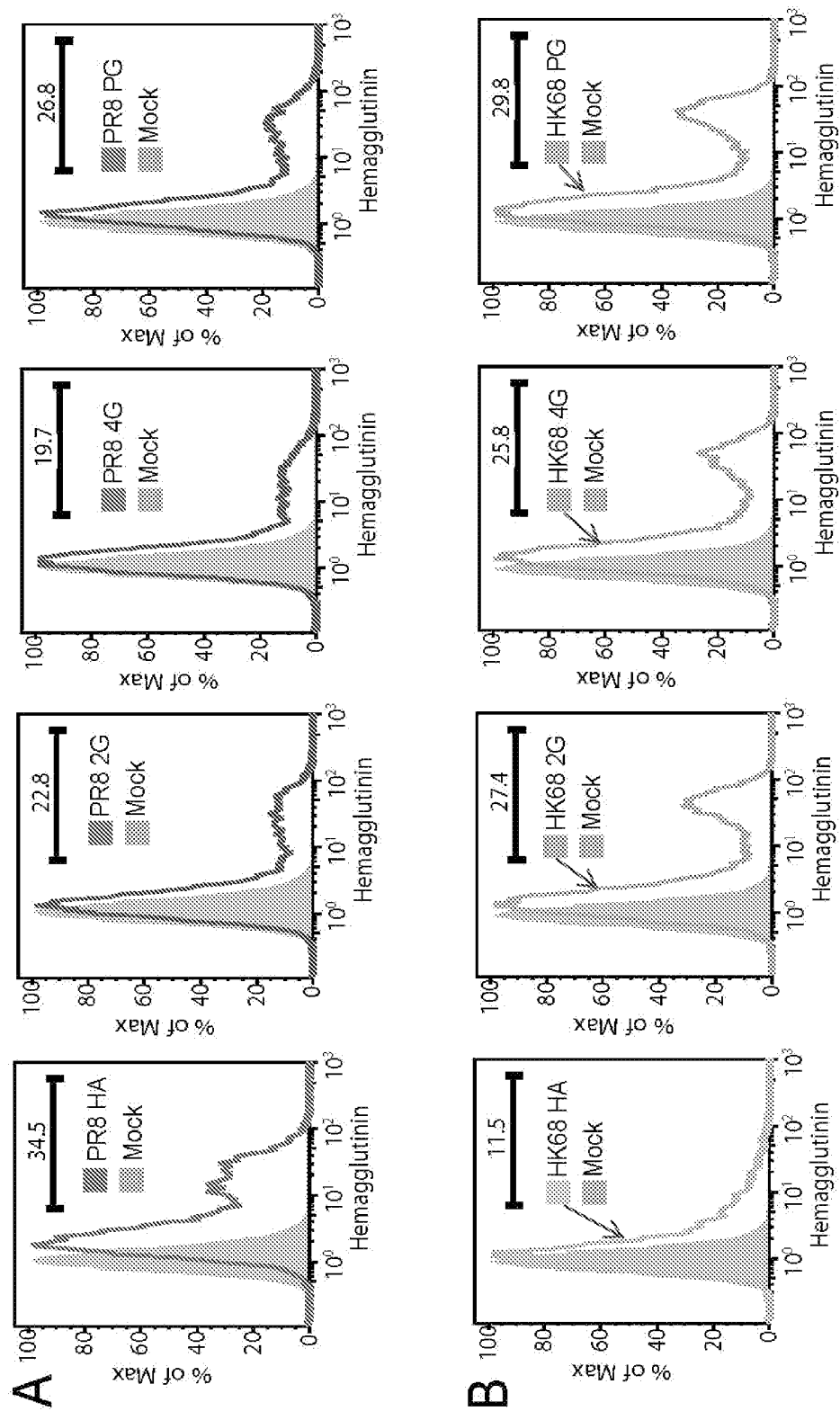


FIG. 12A – 12B

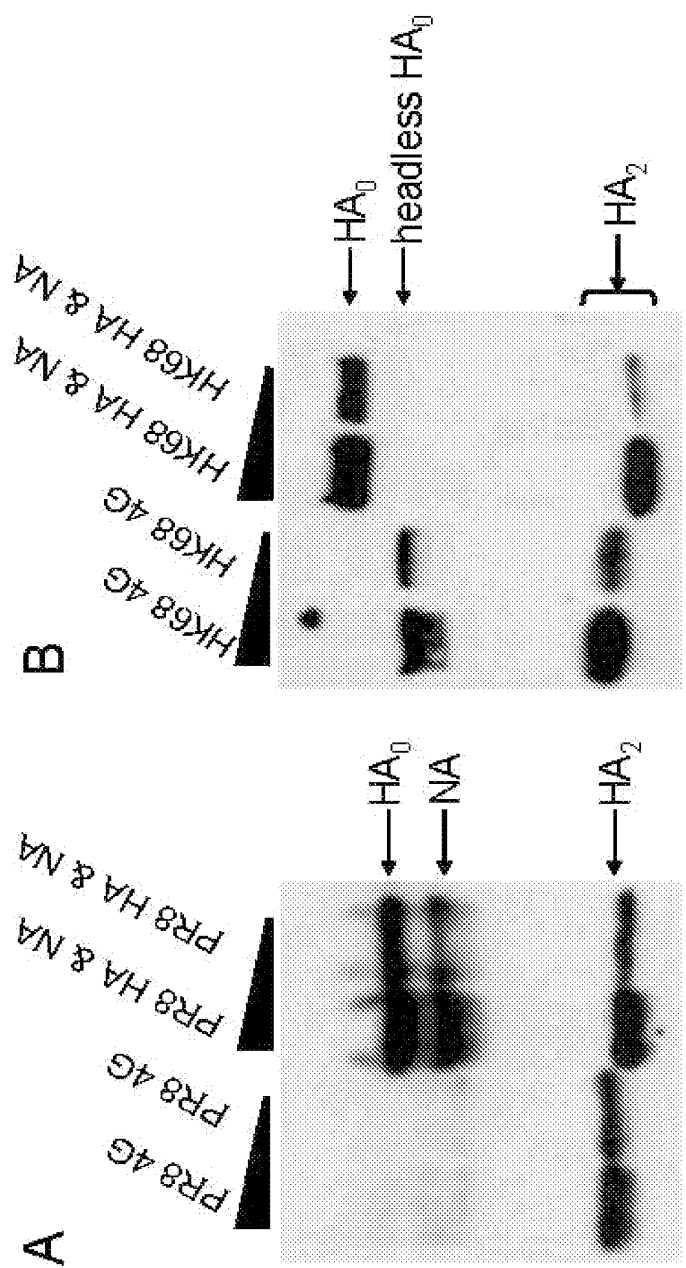


FIG. 13A - 13B

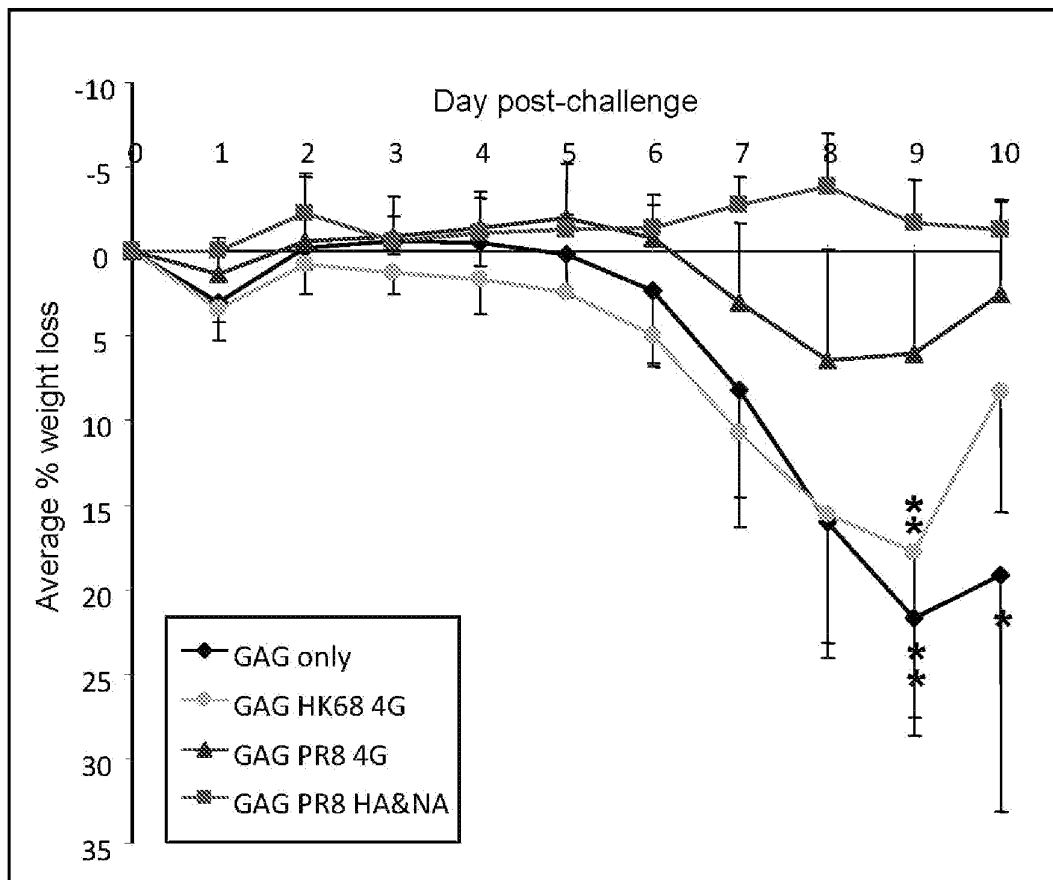


FIG. 14

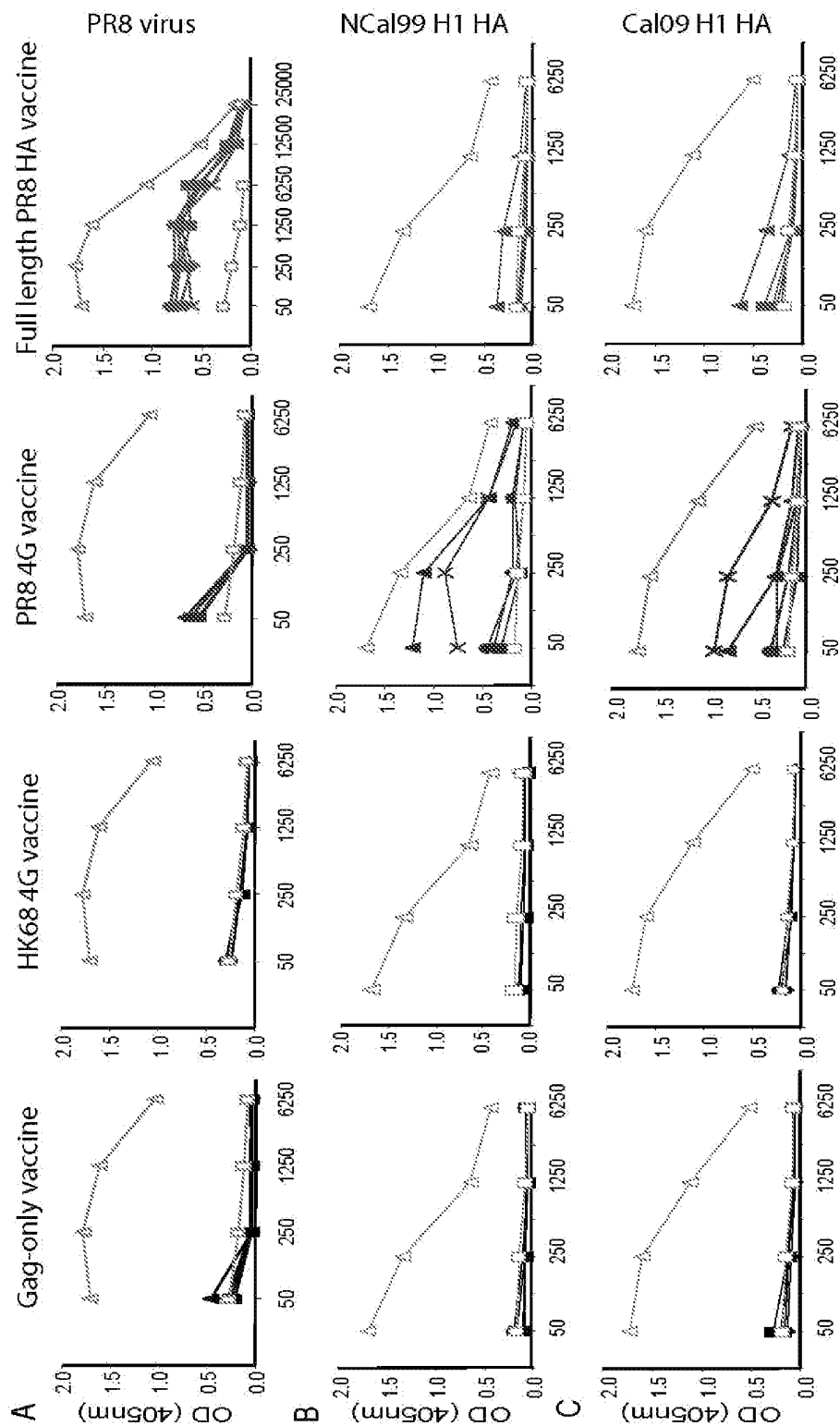


FIG. 15A - 15C

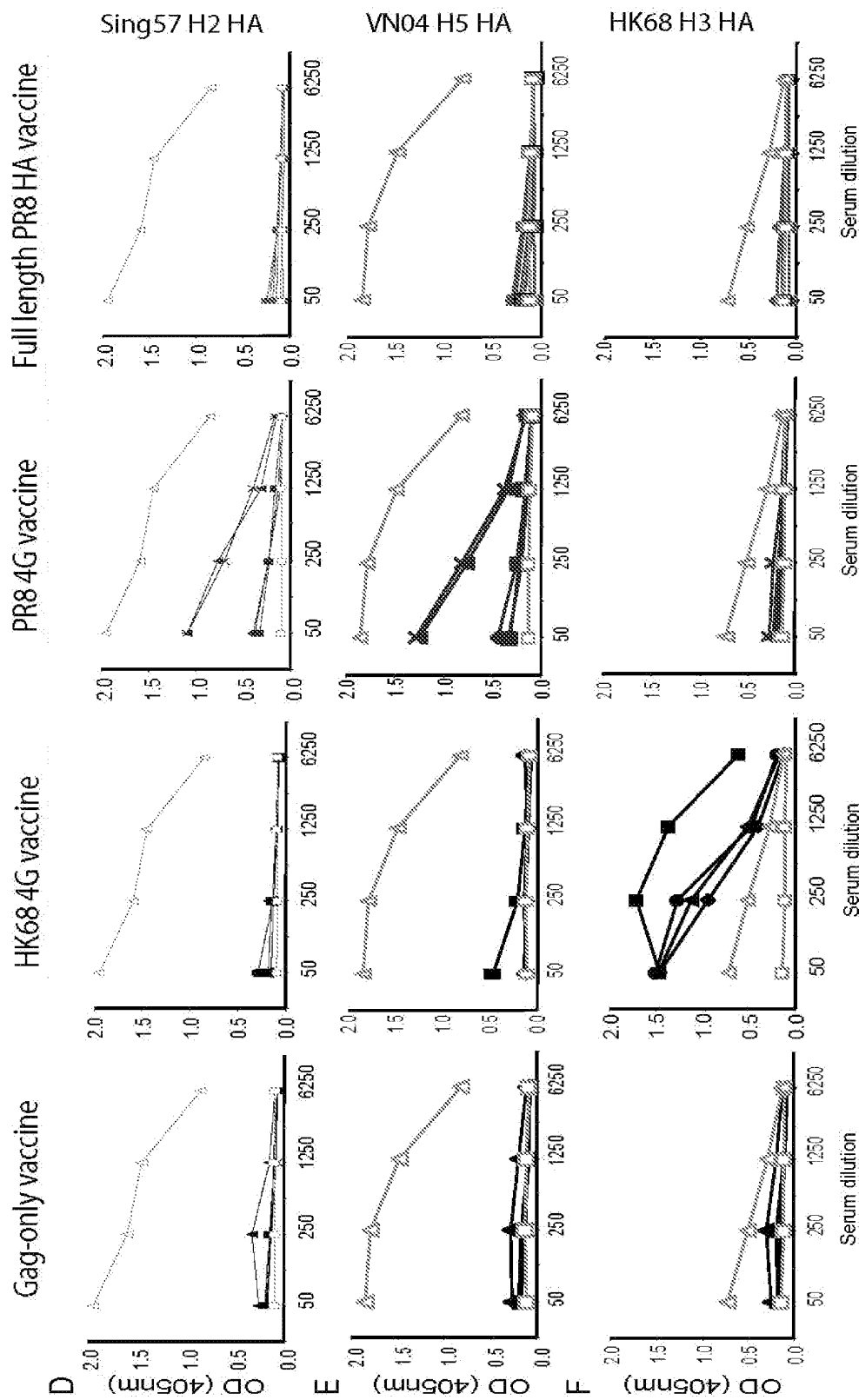


FIG. 15D – 15F

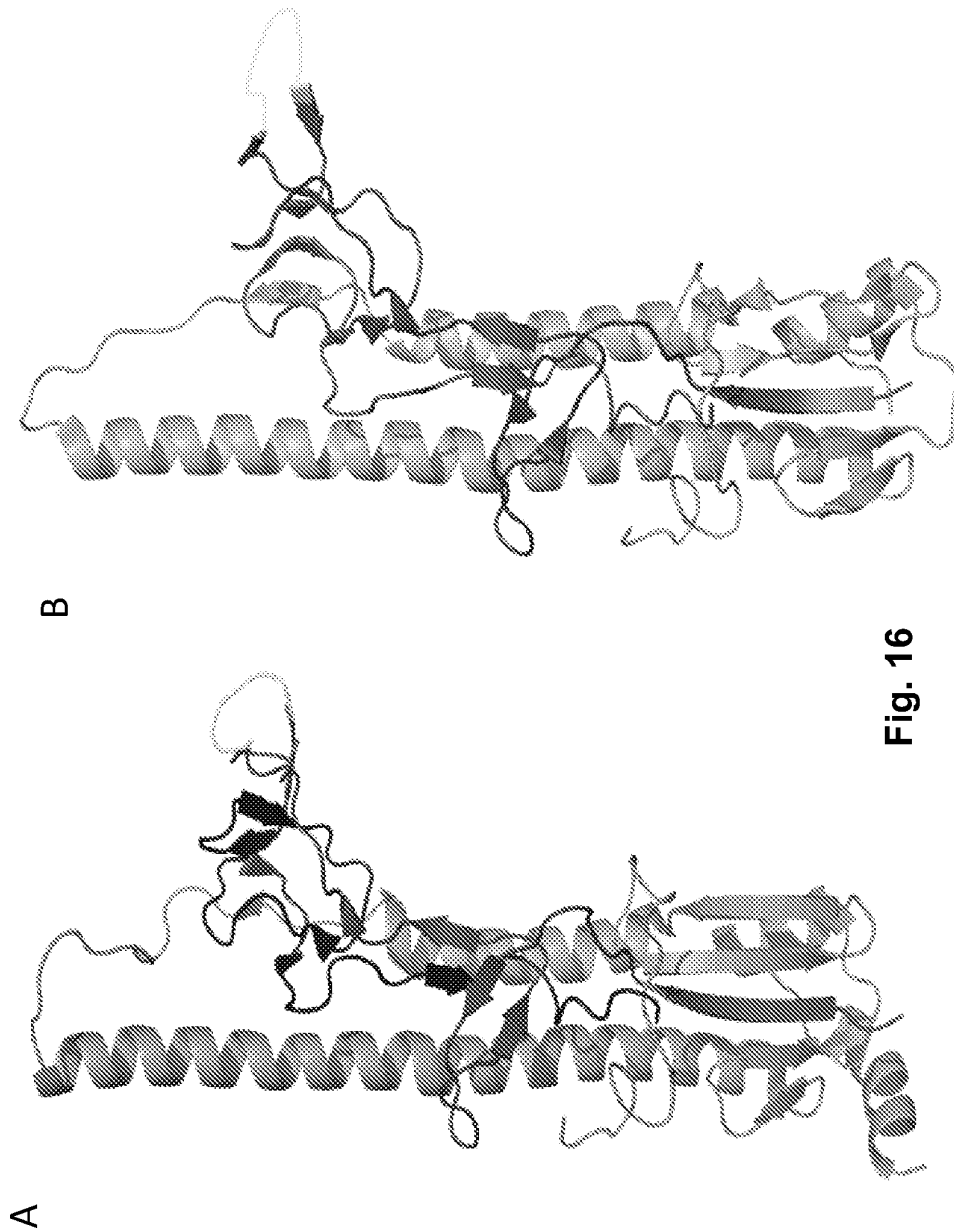


Fig. 16

Schematic of HA construct: primary protein sequence

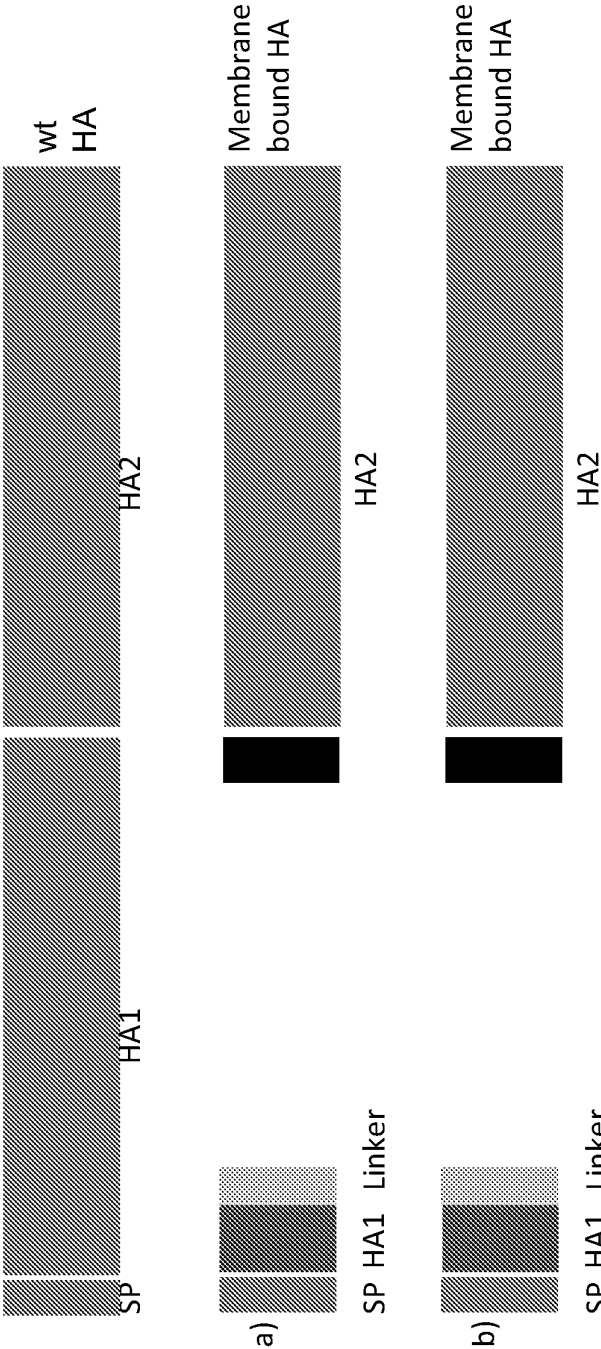


Fig. 17

Sequence Listing - 2010234849

SEQUENCE LISTING

<110> Mount Sinai School of Medicine of New York University
Lowen, Anice C.
Steel, John F.
Garcia-Sastre, Adolfo
Palese, Peter

<120> INFLUENZA VIRUS VACCINES AND USES THEREOF

<130> 6923-168-228

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<150> 61/164,896

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<212> PRT

<213> influenza A virus

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<223> influenza virus A hemagglutinin subtype H1

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Val Asp Thr Val Leu Glu Lys Asn Val Thr Val Thr His Ser Val Asn
 35      40      45
Leu Leu Glu Asp Ser His Asn Gly Lys Leu Cys Arg Leu Lys Gly Ile
 50      55      60
Ala Pro Leu Gln Leu Gly Lys Cys Asn Ile Ala Gly Trp Leu Leu Gly
 65      70      75      80
Asn Pro Glu Cys Asp Pro Leu Leu Pro Val Arg Ser Trp Ser Tyr Ile
 85      90      95
Val Glu Thr Pro Asn Ser Glu Asn Gly Ile Cys Tyr Pro Gly Asp Phe
100      105      110
Ile Asp Tyr Glu Glu Leu Arg Glu Gln Leu Ser Ser Val Ser Ser Phe
115      120      125
Glu Arg Phe Glu Ile Phe Pro Lys Glu Ser Ser Trp Pro Asn His Asn
130      135      140
Thr Asn Gly Val Thr Ala Ala Cys Ser His Glu Gly Lys Ser Ser Phe
145      150      155      160
Tyr Arg Asn Leu Leu Trp Leu Thr Glu Lys Glu Gly Ser Tyr Pro Lys
165      170      175
Leu Lys Asn Ser Tyr Val Asn Lys Lys Gly Lys Glu Val Leu Val Leu
180      185      190
Trp Gly Ile His His Pro Pro Asn Ser Lys Glu Gln Gln Asn Leu Tyr
195      200      205
Gln Asn Glu Asn Ala Tyr Val Ser Val Val Thr Ser Asn Tyr Asn Arg
210      215      220
Arg Phe Thr Pro Glu Ile Ala Glu Arg Pro Lys Val Arg Asp Gln Ala
225      230      235      240
Gly Arg Met Asn Tyr Trp Thr Leu Leu Lys Pro Gly Asp Thr Ile
245      250      255
Ile Phe Glu Ala Asn Gly Asn Leu Ile Ala Pro Met Tyr Ala Phe Ala
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Sequence Listing - 2010234849

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Ser 290 Leu Pro Tyr Gln Asn 295 Ile His Pro Val Thr 300 Ile Gly Glu Cys Pro
305 Lys Tyr Val Arg Ser 310 Ala Lys Leu Arg Met 315 Val Thr Gly Leu Arg Asn
Asn Pro Ser 325 Ile Gln Ser Arg Gly 330 Phe Gly Ala Ile Ala Gly Phe
Ile Glu Gly 340 Gly Trp Thr Gly Met 345 Ile Asp Gly Trp Tyr Gly Tyr His
His Gln 355 Asn Glu Gln Gly Ser 360 Gly Tyr Ala Ala Asp 365 Gln Lys Ser Thr
Gln 370 Asn Ala Ile Asn Gly 375 Ile Thr Asn Lys Val Asn Thr Val Ile Glu
385 Lys Met Asn Ile Gln 390 Phe Thr Ala Val Gly 395 Lys Glu Phe Asn Lys Leu
Glu Lys Arg Met 405 Glu Asn Leu Asn Lys 410 Lys Val Asp Asp Gly 415 Phe Leu
Asp Ile Trp Thr Tyr Asn Ala Glu 425 Leu Leu Val Leu Leu Glu Asn Glu
435 Arg Thr Leu Asp Phe His Asp 440 Ser Asn Val Lys Asn 445 Leu Tyr Glu Lys
Val 450 Lys Ser Gln Leu Lys 455 Asn Asn Ala Lys Glu 460 Ile Gly Asn Gly Cys
465 Phe Glu Phe Tyr His 470 Lys Cys Asp Asn Glu 475 Cys Met Glu Ser Val Arg
Asn Gly Thr Tyr Asp Tyr Pro Lys Tyr 485 Ser Glu Glu Ser Lys 495 Leu Asn
Arg Glu Lys 500 Val Asp Gly Val Lys 505 Leu Glu Ser Met Gly 510 Ile Tyr Gln
Ile Leu Ala Ile Tyr Ser Thr 515 Val Ala Ser Ser Leu 520 Val Leu Leu Val
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545 Cys Arg Ile Cys Ile 550 555 560
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Thr Ile Leu Glu Arg Asn Val Thr Val Thr His Ala Gln Asn Ile Leu
35 40 45
Glu Lys Thr His Asn Gly Lys Leu Cys Lys Leu Asn Gly Ile Pro Pro
50 55 60
Leu Glu Leu Gly Asp Cys Ser Ile Ala Gly Trp Leu Leu Gly Asn Pro
65 70 75 80
Glu Cys Asp Arg Leu Leu Thr Val Pro Glu Trp Ser Tyr Ile Met Glu
85 90 95
Lys Glu Asn Pro Arg Asn Gly Leu Cys Tyr Pro Gly Ser Phe Asn Asp
100 105 110
Tyr Glu Glu Leu Lys His Leu Leu Ser Ser Val Thr His Phe Glu Lys
115 120 125
Val Lys Ile Leu Pro Lys Asp Arg Trp Thr Gln His Thr Thr Thr Gly
130 135 140

```

Sequence Listing - 2010234849

Gly	Ser	Arg	Ala	Cys	Ala	Val	Ser	Gly	Asn	Pro	Ser	Phe	Phe	Arg	Asn
145					150					155					160
Met	Val	Trp	Leu	Thr	Lys	Lys	Gly	Ser	Asn	Tyr	Pro	Ile	Ala	Lys	Gly
				165					170					175	
Ser	Tyr	Asn	Asn	Thr	Ser	Gly	Glu	Gln	Met	Leu	Ile	Ile	Trp	Gly	Val
			180					185					190		
His	His	Pro	Asn	Asp	Glu	Thr	Glu	Gln	Arg	Thr	Leu	Tyr	Gln	Asn	Val
		195					200					205			
Gly	Thr	Tyr	Val	Ser	Ile	Gly	Thr	Ser	Thr	Leu	Asn	Lys	Arg	Ser	Ile
	210					215					220				
Pro	Val	Ile	Ala	Thr	Arg	Pro	Lys	Val	Asn	Gly	Gln	Gly	Gly	Arg	Met
225					230					235					240
Glu	Phe	Ser	Trp	Thr	Ile	Leu	Asp	Ile	Trp	Asp	Thr	Ile	Asn	Phe	Glu
				245					250					255	
Ser	Thr	Gly	Asn	Leu	Ile	Ala	Pro	Glu	Tyr	Gly	Phe	Arg	Ile	Ser	Lys
			260					265					270		
Arg	Gly	Ser	Ser	Gly	Ile	Met	Lys	Thr	Glu	Gly	Thr	Leu	Glu	Asn	Cys
		275					280					285			
Glu	Thr	Lys	Cys	Gln	Thr	Pro	Leu	Gly	Ala	Ile	Asn	Thr	Thr	Leu	Pro
	290					295					300				
Phe	His	Asn	Val	His	Pro	Leu	Thr	Ile	Gly	Glu	Cys	Pro	Lys	Tyr	Val
305					310					315					320
Lys	Ser	Glu	Arg	Leu	Val	Leu	Ala	Thr	Gly	Leu	Arg	Asn	Val	Pro	Gln
				325					330					335	
Ile	Glu	Ser	Arg	Gly	Leu	Phe	Gly	Ala	Ile	Ala	Gly	Phe	Ile	Glu	Gly
			340					345					350		
Gly	Trp	Gln	Gly	Met	Ile	Asp	Gly	Trp	Tyr	Gly	Tyr	His	His	Ser	Asn
		355					360					365			
Asp	Gln	Gly	Ser	Gly	Tyr	Ala	Ala	Asp	Lys	Glu	Ser	Thr	Gln	Lys	Ala
	370					375					380				
Ile	Asp	Gly	Ile	Thr	Asn	Arg	Val	Asn	Ser	Val	Ile	Glu	Lys	Met	Asn
385					390					395					400
Thr	Gln	Phe	Glu	Ala	Val	Gly	Lys	Glu	Phe	Ser	Asn	Leu	Glu	Lys	Arg
				405					410					415	
Leu	Glu	Asn	Leu	Asn	Lys	Lys	Met	Glu	Asp	Gly	Phe	Leu	Asp	Val	Trp
		420						425					430		
Thr	Tyr	Asn	Ala	Glu	Leu	Leu	Val	Leu	Met	Glu	Asn	Glu	Arg	Thr	Leu
		435					440					445			
Asp	Phe	His	Asp	Ser	Asn	Val	Lys	Asn	Leu	Tyr	Asp	Arg	Val	Arg	Met
	450					455					460				
Gln	Leu	Arg	Asp	Asn	Ala	Lys	Glu	Leu	Gly	Asn	Gly	Cys	Phe	Glu	Phe
465					470					475					480
Tyr	His	Lys	Cys	Asp	Asp	Glu	Cys	Met	Asn	Ser	Val	Lys	Asn	Gly	Thr
				485					490					495	
Tyr	Asp	Tyr	Pro	Lys	Tyr	Glu	Glu	Glu	Ser	Lys	Leu	Asn	Arg	Asn	Glu
			500					505					510		
Ile	Lys	Gly	Val	Lys	Leu	Ser	Asn	Met	Gly	Val	Tyr	Gln	Ile	Leu	Ala
		515					520					525			
Ile	Tyr	Ala	Thr	Val	Ala	Gly	Ser	Leu	Ser	Leu	Ala	Ile	Met	Ile	Ala
	530					535					540				
Gly	Ile	Ser	Leu	Trp	Met	Cys	Ser	Asn	Gly	Ser	Leu	Gln	Cys	Arg	Ile
545					550					555					560
Cys	Ile														

<210> 3

<211> 566

<212> PRT

<213> influenza A virus

<220>

<223> influenza virus A hemagglutinin subtype H3

<400> 3

Met	Lys	Thr	Ile	Ile	Ala	Leu	Ser	Tyr	Ile	Phe	Cys	Leu	Ala	Leu	Gly
1				5					10					15	
Gln	Asp	Leu	Pro	Gly	Asn	Asp	Asn	Ser	Thr	Ala	Thr	Leu	Cys	Leu	Gly

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			20				25			30					
His	His	Ala	Val	Pro	Asn	Gly	Thr	Leu	Val	Lys	Thr	Ile	Thr	Asp	Asp
		35					40					45			
Gln	Ile	Glu	Val	Thr	Asn	Ala	Thr	Glu	Leu	Val	Gln	Ser	Ser	Ser	Thr
	50					55					60				
Gly	Lys	Ile	Cys	Asn	Asn	Pro	His	Arg	Ile	Leu	Asp	Gly	Ile	Asp	Cys
65				70				75						80	
Thr	Leu	Ile	Asp	Ala	Leu	Leu	Gly	Asp	Pro	His	Cys	Asp	Val	Phe	Gln
			85					90					95		
Asn	Glu	Thr	Trp	Asp	Leu	Phe	Val	Glu	Arg	Ser	Lys	Ala	Phe	Ser	Asn
			100					105					110		
Cys	Tyr	Pro	Tyr	Asp	Val	Pro	Asp	Tyr	Ala	Ser	Leu	Arg	Ser	Leu	Val
		115					120					125			
Ala	Ser	Ser	Gly	Thr	Leu	Glu	Phe	Ile	Thr	Glu	Gly	Phe	Thr	Trp	Thr
	130					135					140				
Gly	Val	Thr	Gln	Asn	Gly	Gly	Ser	Asn	Ala	Cys	Lys	Arg	Gly	Pro	Gly
145				150						155				160	
Asn	Gly	Phe	Phe	Ser	Arg	Leu	Asn	Trp	Leu	Thr	Lys	Ser	Gly	Ser	Thr
			165					170					175		
Tyr	Pro	Val	Leu	Asn	Val	Thr	Met	Pro	Asn	Asn	Asp	Asn	Phe	Asp	Lys
			180					185					190		
Leu	Tyr	Ile	Trp	Gly	Val	His	His	Pro	Ser	Thr	Asn	Gln	Glu	Gln	Thr
		195				200						205			
Ser	Leu	Tyr	Val	Gln	Glu	Ser	Gly	Arg	Val	Thr	Val	Ser	Thr	Arg	Arg
	210					215					220				
Ser	Gln	Gln	Ser	Ile	Ile	Pro	Asn	Ile	Gly	Ser	Arg	Pro	Trp	Val	Arg
225				230						235				240	
Gly	Gln	Ser	Ser	Arg	Ile	Ser	Ile	Tyr	Trp	Thr	Ile	Val	Lys	Pro	Gly
			245					250					255		
Asp	Val	Leu	Val	Ile	Asn	Ser	Asn	Gly	Asn	Leu	Ile	Ala	Pro	Arg	Gly
			260					265				270			
Tyr	Phe	Lys	Met	Arg	Thr	Gly	Lys	Ser	Ser	Ile	Met	Ser	Ser	Asp	Ala
		275					280					285			
Pro	Ile	Asp	Thr	Cys	Ile	Ser	Glu	Cys	Ile	Thr	Pro	Asn	Gly	Ser	Ile
	290					295					300				
Pro	Asn	Asp	Lys	Pro	Phe	Gln	Asn	Val	Asn	Lys	Ile	Thr	Tyr	Gly	Ala
305				310						315				320	
Cys	Pro	Lys	Tyr	Val	Lys	Gln	Asn	Thr	Leu	Lys	Leu	Ala	Thr	Gly	Met
			325						330					335	
Arg	Asn	Val	Pro	Glu	Lys	Gln	Thr	Arg	Gly	Leu	Phe	Gly	Ala	Ile	Ala
			340					345				350			
Gly	Phe	Ile	Glu	Asn	Gly	Trp	Glu	Gly	Met	Ile	Asp	Gly	Trp	Tyr	Gly
		355					360				365				
Phe	Arg	His	Gln	Asn	Ser	Glu	Gly	Thr	Gly	Gln	Ala	Ala	Asp	Leu	Lys
	370					375					380				
Ser	Thr	Gln	Ala	Ala	Ile	Asp	Gln	Ile	Asn	Gly	Lys	Leu	Asn	Arg	Val
385					390					395				400	
Ile	Glu	Lys	Thr	Asn	Glu	Lys	Phe	His	Gln	Ile	Glu	Lys	Glu	Phe	Ser
			405					410					415		
Glu	Val	Glu	Gly	Arg	Ile	Gln	Asp	Leu	Glu	Lys	Tyr	Val	Glu	Asp	Thr
			420					425				430			
Lys	Ile	Asp	Leu	Trp	Ser	Tyr	Asn	Ala	Glu	Leu	Leu	Val	Ala	Leu	Glu
		435					440					445			
Asn	Gln	His	Thr	Ile	Asp	Leu	Thr	Asp	Ser	Glu	Met	Asn	Lys	Leu	Phe
	450					455					460				
Glu	Lys	Thr	Arg	Arg	Gln	Leu	Arg	Glu	Asn	Ala	Glu	Asp	Met	Gly	Asn
465				470						475				480	
Gly	Cys	Phe	Lys	Ile	Tyr	His	Lys	Cys	Asp	Asn	Ala	Cys	Ile	Glu	Ser
			485					490					495		
Ile	Arg	Asn	Gly	Thr	Tyr	Asp	His	Asp	Val	Tyr	Arg	Asp	Glu	Ala	Leu
		500						505				510			
Asn	Asn	Arg	Phe	Gln	Ile	Lys	Gly	Val	Glu	Leu	Lys	Ser	Gly	Tyr	Lys
		515					520					525			
Asp	Trp	Ile	Leu	Trp	Ile	Ser	Phe	Ala	Ile	Ser	Cys	Phe	Leu	Leu	Cys
	530					535					540				
Val	Val	Leu	Leu	Gly	Phe	Ile	Met	Trp	Ala	Cys	Gln	Arg	Gly	Asn	Ile
545				550						555				560	
Arg	Cys	Asn	Ile	Cys	Ile										

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565

<210> 4
 <211> 564
 <212> PRT
 <213> influenza A virus

<220>
 <223> influenza virus A hemagglutinin subtype H4

<400> 4
 Met Leu Ser Ile Val Ile Leu Phe Leu Leu Ile Ala Glu Asn Ser Ser
 1 5 10 15
 Gln Asn Tyr Thr Gly Asn Pro Val Ile Cys Met Gly His His Ala Val
 20 25 30
 Ala Asn Gly Thr Met Val Lys Thr Leu Ala Asp Asp Gln Val Glu Val
 35 40 45
 Val Thr Ala Gln Glu Leu Val Glu Ser Gln Asn Leu Pro Glu Leu Cys
 50 55 60
 Pro Ser Pro Leu Arg Leu Val Asp Gly Gln Thr Cys Asp Ile Ile Asn
 65 70 75 80
 Gly Ala Leu Gly Ser Pro Gly Cys Asp His Leu Asn Gly Ala Glu Trp
 85 90 95
 Asp Val Phe Ile Glu Arg Pro Asn Ala Val Asp Thr Cys Tyr Pro Phe
 100 105 110
 Asp Val Pro Glu Tyr Gln Ser Leu Arg Ser Ile Leu Ala Asn Asn Gly
 115 120 125
 Lys Phe Glu Phe Ile Ala Glu Glu Phe Gln Trp Asn Thr Val Lys Gln
 130 135 140
 Asn Gly Lys Ser Gly Ala Cys Lys Arg Ala Asn Val Asp Asp Phe Phe
 145 150 155 160
 Asn Arg Leu Asn Trp Leu Val Lys Ser Asp Gly Asn Ala Tyr Pro Leu
 165 170 175
 Gln Asn Leu Thr Lys Ile Asn Asn Gly Asp Tyr Ala Arg Leu Tyr Ile
 180 185 190
 Trp Gly Val His His Pro Ser Thr Ser Thr Glu Gln Thr Asn Leu Tyr
 195 200 205
 Lys Asn Asn Pro Gly Arg Val Thr Val Ser Thr Lys Thr Ser Gln Thr
 210 215 220
 Ser Val Val Pro Asp Ile Gly Ser Arg Pro Leu Val Arg Gly Gln Ser
 225 230 235 240
 Gly Arg Val Ser Phe Tyr Trp Thr Ile Val Glu Pro Gly Asp Leu Ile
 245 250 255
 Val Phe Asn Thr Ile Gly Asn Leu Ile Ala Pro Arg Gly His Tyr Lys
 260 265 270
 Leu Asn Asn Gln Lys Lys Ser Thr Ile Leu Asn Thr Ala Ile Pro Ile
 275 280 285
 Gly Ser Cys Val Ser Lys Cys His Thr Asp Lys Gly Ser Leu Ser Thr
 290 295 300
 Thr Lys Pro Phe Gln Asn Ile Ser Arg Ile Ala Val Gly Asp Cys Pro
 305 310 315 320
 Arg Tyr Val Lys Gln Gly Ser Leu Lys Leu Ala Thr Gly Met Arg Asn
 325 330 335
 Ile Pro Glu Lys Ala Ser Arg Gly Leu Phe Gly Ala Ile Ala Gly Phe
 340 345 350
 Ile Glu Asn Gly Trp Gln Gly Leu Ile Asp Gly Trp Tyr Gly Phe Arg
 355 360 365
 His Gln Asn Ala Glu Gly Thr Gly Thr Ala Ala Asp Leu Lys Ser Thr
 370 375 380
 Gln Ala Ala Ile Asp Gln Ile Asn Gly Lys Leu Asn Arg Leu Ile Glu
 385 390 395 400
 Lys Thr Asn Asp Lys Tyr His Gln Ile Glu Lys Glu Phe Glu Gln Val
 405 410 415
 Glu Gly Arg Ile Gln Asp Leu Glu Asn Tyr Val Glu Asp Thr Lys Ile
 420 425 430
 Asp Leu Trp Ser Tyr Asn Ala Glu Leu Val Ala Leu Glu Asn Gln
 435 440 445

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His Thr Ile Asp Val Thr Asp Ser Glu Met Asn Lys Leu Phe Glu Arg
 450 455 460
Val Arg Arg Gln Leu Arg Glu Asn Ala Glu Asp Lys Gly Asn Gly Cys
465 470 475 480
Phe Glu Ile Phe His Lys Cys Asp Asn Asn Cys Ile Glu Ser Ile Arg
 485 490 495
Asn Gly Thr Tyr Asp His Asp Ile Tyr Arg Asp Glu Ala Ile Asn Asn
 500 505 510
Arg Phe Gln Ile Gln Gly Val Lys Leu Thr Gln Gly Tyr Lys Asp Ile
 515 520 525
Ile Leu Trp Ile Ser Phe Ser Ile Ser Cys Phe Leu Leu Val Ala Leu
 530 535 540
Leu Leu Ala Phe Ile Leu Trp Ala Cys Gln Asn Gly Asn Ile Arg Cys
545 550 555 560
Gln Ile Cys Ile

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<210> 5

<211> 564

<212> PRT

<213> influenza A virus

<220>

<223> influenza virus A hemagglutinin subtype H5

<400> 5

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

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          325          330          335
Pro  Gln  Arg  Lys  Arg  Gly  Leu  Phe  Gly  Ala  Ile  Ala  Gly  Phe  Ile
          340          345          350
Glu  Gly  Gly  Trp  Gln  Gly  Met  Val  Asp  Gly  Trp  Tyr  Gly  Tyr  His  His
          355          360          365
Ser  Asn  Glu  Gln  Gly  Ser  Gly  Tyr  Ala  Ala  Asp  Lys  Glu  Ser  Thr  Gln
          370          375          380
Lys  Ala  Ile  Asp  Gly  Ile  Thr  Asn  Lys  Val  Asn  Ser  Ile  Ile  Asp  Lys
385          390          395          400
Met  Asn  Thr  Arg  Phe  Glu  Ala  Val  Gly  Lys  Glu  Phe  Asn  Asn  Leu  Glu
          405          410          415
Arg  Arg  Val  Glu  Asn  Leu  Asn  Lys  Lys  Met  Glu  Asp  Gly  Phe  Leu  Asp
          420          425          430
Val  Trp  Thr  Tyr  Asn  Val  Glu  Leu  Leu  Val  Leu  Met  Glu  Asn  Glu  Arg
          435          440          445
Thr  Leu  Asp  Phe  His  Asp  Ser  Asn  Val  Asn  Asn  Leu  Tyr  Asp  Lys  Val
          450          455          460
Arg  Leu  Gln  Leu  Lys  Asp  Asn  Ala  Arg  Glu  Leu  Gly  Asn  Gly  Cys  Phe
465          470          475          480
Glu  Phe  Tyr  His  Lys  Cys  Asp  Asn  Glu  Cys  Met  Glu  Ser  Val  Arg  Asn
          485          490          495
Gly  Thr  Tyr  Asp  Tyr  Pro  Gln  Tyr  Ser  Glu  Glu  Ala  Arg  Leu  Asn  Arg
          500          505          510
Glu  Glu  Ile  Ser  Gly  Val  Lys  Leu  Glu  Ser  Met  Gly  Val  Tyr  Gln  Ile
          515          520          525
Leu  Ser  Ile  Tyr  Ser  Thr  Val  Ala  Ser  Ser  Leu  Ala  Leu  Ala  Ile  Met
          530          535          540
Ile  Ala  Gly  Leu  Ser  Phe  Trp  Met  Cys  Ser  Asn  Gly  Ser  Leu  Gln  Cys
545          550          555          560
Arg  Ile  Cys  Ile

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<210> 6

<211> 566

<212> PRT

<213> influenza A virus

<220>

<223> influenza virus A hemagglutinin subtype H6

<400> 6

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Met  Ile  Ala  Ile  Ile  Val  Val  Ala  Ile  Leu  Ala  Thr  Ala  Gly  Arg  Ser
1          5          10          15
Asp  Lys  Ile  Cys  Ile  Gly  Tyr  His  Ala  Asn  Asn  Ser  Thr  Thr  Gln  Ile
          20          25          30
Asp  Thr  Ile  Leu  Glu  Lys  Asn  Val  Thr  Val  Thr  His  Ser  Val  Glu  Leu
          35          40          45
Leu  Glu  Asn  Gln  Lys  Glu  Glu  Arg  Phe  Cys  Lys  Ile  Leu  Lys  Lys  Ala
          50          55          60
Pro  Leu  Asp  Leu  Lys  Gly  Cys  Thr  Ile  Glu  Gly  Trp  Ile  Leu  Gly  Asn
65          70          75          80
Pro  Gln  Cys  Asp  Leu  Leu  Leu  Gly  Asp  Gln  Ser  Trp  Ser  Tyr  Ile  Val
          85          90          95
Glu  Arg  Pro  Thr  Ala  Gln  Asn  Gly  Ile  Cys  Tyr  Pro  Gly  Val  Leu  Asn
          100          105          110
Glu  Val  Glu  Glu  Leu  Lys  Ala  Leu  Ile  Gly  Ser  Gly  Glu  Arg  Val  Glu
          115          120          125
Arg  Phe  Glu  Met  Phe  Pro  Lys  Ser  Thr  Trp  Thr  Gly  Val  Asp  Thr  Ser
          130          135          140
Ser  Gly  Val  Thr  Arg  Ala  Cys  Pro  Tyr  Asn  Ser  Gly  Ser  Ser  Phe  Tyr
145          150          155          160
Arg  Asn  Leu  Leu  Trp  Ile  Ile  Lys  Thr  Lys  Ser  Ala  Ala  Tyr  Ser  Val
          165          170          175
Ile  Lys  Gly  Ala  Tyr  Asn  Asn  Thr  Gly  Asn  Gln  Pro  Ile  Leu  Tyr  Phe
          180          185          190
Trp  Gly  Val  His  His  Pro  Pro  Asp  Thr  Asn  Glu  Gln  Asn  Thr  Leu  Tyr
          195          200          205

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

<210> 7

<211> 563

<212> PRT

<213> influenza A virus

<220>

<223> influenza virus A hemagglutinin subtype H7

<400> 7

Met	Asn	Thr	Gln	Ile	Leu	Val	Phe	Ala	Leu	Val	Ala	Val	Ile	Pro	Thr
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Asn	Ala	Asp	Lys	Ile	Cys	Leu	Gly	His	Ala	Val	Ser	Asn	Gly	Thr	
			20					25				30			
Lys	Val	Asn	Thr	Leu	Thr	Glu	Arg	Gly	Val	Glu	Val	Val	Asn	Ala	Thr
		35					40					45			
Glu	Thr	Val	Glu	Arg	Thr	Asn	Ile	Pro	Lys	Ile	Cys	Ser	Lys	Gly	Lys
	50					55					60				
Arg	Thr	Thr	Asp	Leu	Gly	Gln	Cys	Gly	Leu	Leu	Gly	Thr	Ile	Thr	Gly
65					70					75					80
Pro	Pro	Gln	Cys	Asp	Gln	Phe	Leu	Glu	Phe	Ser	Ala	Asp	Leu	Ile	Ile

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<210> 8
<211> 565
<212> PRT
<213> influenza A virus
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<220>

<223> influenza virus A hemagglutinin subtype H8

<400> 8

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Met Glu Lys Phe Ile Ala Ile Ala Thr Leu Ala Ser Thr Asn Ala Tyr
 1      5      10      15
Asp Arg Ile Cys Ile Gly Tyr Gln Ser Asn Asn Ser Thr Asp Thr Val
 20      25      30
Asn Thr Leu Ile Glu Gln Asn Val Pro Val Thr Gln Thr Met Glu Leu
 35      40      45
Val Glu Thr Glu Lys His Pro Ala Tyr Cys Asn Thr Asp Leu Gly Ala
 50      55      60
Pro Leu Glu Leu Arg Asp Cys Lys Ile Glu Ala Val Ile Tyr Gly Asn
 65      70      75      80
Pro Lys Cys Asp Ile His Leu Lys Asp Gln Gly Trp Ser Tyr Ile Val
 85      90      95
Glu Arg Pro Ser Ala Pro Glu Gly Met Cys Tyr Pro Gly Ser Val Glu
100      105
Asn Leu Glu Glu Leu Arg Phe Val Phe Ser Ser Ala Ala Ser Tyr Lys
115      120      125
Arg Ile Arg Leu Phe Asp Tyr Ser Arg Trp Asn Val Thr Arg Ser Gly
130      135      140
Thr Ser Lys Ala Cys Asn Ala Ser Thr Gly Gly Gln Ser Phe Tyr Arg
145      150      155
Ser Ile Asn Trp Leu Thr Lys Lys Glu Pro Asp Thr Tyr Asp Phe Asn
165      170      175
Glu Gly Ala Tyr Val Asn Asn Glu Asp Gly Asp Ile Ile Phe Leu Trp
180      185      190
Gly Ile His His Pro Pro Asp Thr Lys Glu Gln Thr Thr Leu Tyr Lys
195      200      205
Asn Ala Asn Thr Leu Ser Ser Val Thr Thr Asn Thr Ile Asn Arg Ser
210      215      220
Phe Gln Pro Asn Ile Gly Pro Arg Pro Leu Val Arg Gly Gln Gln Gly
225      230      235      240
Arg Met Asp Tyr Tyr Trp Gly Ile Leu Lys Arg Gly Glu Thr Leu Lys
245      250      255
Ile Arg Thr Asn Gly Asn Leu Ile Ala Pro Glu Phe Gly Tyr Leu Leu
260      265      270
Lys Gly Glu Ser Tyr Gly Arg Ile Ile Gln Asn Glu Asp Ile Pro Ile
275      280      285
Gly Asn Cys Asn Thr Lys Cys Gln Thr Tyr Ala Gly Ala Ile Asn Ser
290      295      300
Ser Lys Pro Phe Gln Asn Ala Ser Arg His Tyr Met Gly Glu Cys Pro
305      310      315      320
Lys Tyr Val Lys Lys Ala Ser Leu Arg Leu Ala Val Gly Leu Arg Asn
325      330      335
Thr Pro Ser Val Glu Pro Arg Gly Leu Phe Gly Ala Ile Ala Gly Phe
340      345      350
Ile Glu Gly Gly Trp Ser Gly Met Ile Asp Gly Trp Tyr Gly Phe His
355      360      365
His Ser Asn Ser Glu Gly Thr Gly Met Ala Ala Asp Gln Lys Ser Thr
370      375      380
Gln Glu Ala Ile Asp Lys Ile Thr Asn Lys Val Asn Asn Ile Val Asp
385      390      395      400
Lys Met Asn Arg Glu Phe Glu Val Val Asn His Glu Phe Ser Glu Val
405      410      415
Glu Lys Arg Ile Asn Met Ile Asn Asp Lys Ile Asp Asp Gln Ile Glu
420      425      430
Asp Leu Trp Ala Tyr Asn Ala Glu Leu Leu Val Leu Leu Glu Asn Gln
435      440      445
Lys Thr Leu Asp Glu His Asp Ser Asn Val Lys Asn Leu Phe Asp Glu
450      455      460
Val Lys Arg Arg Leu Ser Ala Asn Ala Ile Asp Ala Gly Asn Gly Cys
465      470      475      480
Phe Asp Ile Leu His Lys Cys Asp Asn Glu Cys Met Glu Thr Ile Lys
485      490      495
Asn Gly Thr Tyr Asp His Lys Glu Tyr Glu Glu Glu Ala Lys Leu Glu
500      505      510

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Arg	Ser	Lys	Ile	Asn	Gly	Val	Lys	Leu	Glu	Glu	Asn	Thr	Thr	Tyr	Lys
		515					520					525			
Ile	Leu	Ser	Ile	Tyr	Ser	Thr	Val	Ala	Ala	Ser	Leu	Cys	Leu	Ala	Ile
	530					535					540				
Leu	Ile	Ala	Gly	Gly	Leu	Ile	Leu	Gly	Met	Gln	Asn	Gly	Ser	Cys	Arg
545					550					555					560
Cys	Met	Phe	Cys	Ile											
				565											

<210> 9

<211> 560

<212> PRT

<213> influenza A virus

<220>

<223> influenza virus A hemagglutinin subtype H9

<400> 9

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

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385	Tyr	Glu	Val	Ile	Asp	390	His	Glu	Phe	Asn	Glu	395	Leu	Glu	Ala	Arg	Leu	400	Asn
					405						410						415		
Met	Ile	Asn	Asn	Lys	Ile	Asp	Asp	Gln	Ile	Gln	Asp	Ile	Trp	Ala	Tyr				
			420					425						430					
Asn	Ala	Glu	Leu	Leu	Val	Leu	Leu	Glu	Asn	Gln	Lys	Thr	Leu	Asp	Glu				
		435					440					445							
His	Asp	Ala	Asn	Val	Asn	Asn	Leu	Tyr	Asn	Lys	Val	Lys	Arg	Ala	Leu				
	450				455						460								
Gly	Ser	Asn	Ala	Val	Glu	Asp	Gly	Asn	Gly	Cys	Phe	Glu	Leu	Tyr	His				
465					470				475						480				
Lys	Cys	Asp	Asp	Gln	Cys	Met	Glu	Thr	Ile	Arg	Asn	Gly	Thr	Tyr	Asp				
				485					490						495				
Arg	Gln	Lys	Tyr	Gln	Glu	Glu	Ser	Arg	Leu	Glu	Arg	Gln	Lys	Ile	Glu				
			500					505					510						
Gly	Val	Lys	Leu	Glu	Ser	Glu	Gly	Thr	Tyr	Lys	Ile	Leu	Thr	Ile	Tyr				
		515					520					525							
Ser	Thr	Val	Ala	Ser	Ser	Leu	Val	Leu	Ala	Met	Gly	Phe	Ala	Ala	Phe				
	530					535					540								
Leu	Phe	Trp	Ala	Met	Ser	Asn	Gly	Ser	Cys	Arg	Cys	Asn	Ile	Cys	Ile				
545					550					555					560				

<210> 10

<211> 561

<212> PRT

<213> influenza A virus

<220>

<223> influenza virus A hemagglutinin subtype H10

<400> 10

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

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Ser	Lys	Cys	Phe	Trp	Arg	Gly	Gly	Ser	Ile	Asn	Thr	Lys	Leu	Pro	Phe
290	295					295				300					
Gln	Asn	Leu	Ser	Pro	Arg	Thr	Val	Gly	Gln	Cys	Pro	Lys	Tyr	Val	Asn
305					310					315					320
Gln	Arg	Ser	Leu	Leu	Leu	Ala	Thr	Gly	Met	Arg	Asn	Val	Pro	Glu	Val
				325					330					335	
Val	Gln	Gly	Arg	Gly	Leu	Phe	Gly	Ala	Ile	Ala	Gly	Phe	Ile	Glu	Asn
			340					345					350		
Gly	Trp	Glu	Gly	Met	Val	Asp	Gly	Trp	Tyr	Gly	Phe	Arg	His	Gln	Asn
		355					360					365			
Ala	Gln	Gly	Thr	Gly	Gln	Ala	Ala	Asp	Tyr	Lys	Ser	Thr	Gln	Ala	Ala
		370				375					380				
Ile	Asp	Gln	Ile	Thr	Gly	Lys	Leu	Asn	Arg	Leu	Ile	Glu	Lys	Thr	Asn
385					390					395					400
Thr	Glu	Phe	Glu	Ser	Ile	Glu	Ser	Glu	Phe	Ser	Glu	Thr	Glu	His	Gln
				405					410					415	
Ile	Gly	Asn	Val	Ile	Asn	Trp	Thr	Lys	Asp	Ser	Ile	Thr	Asp	Ile	Trp
			420					425					430		
Thr	Tyr	Asn	Ala	Glu	Leu	Leu	Val	Ala	Met	Glu	Asn	Gln	His	Thr	Ile
		435					440					445			
Asp	Met	Ala	Asp	Ser	Glu	Met	Leu	Asn	Leu	Tyr	Glu	Arg	Val	Arg	Lys
	450					455					460				
Gln	Leu	Arg	Gln	Asn	Ala	Glu	Glu	Asp	Gly	Lys	Gly	Cys	Phe	Glu	Ile
465					470					475					480
Tyr	His	Thr	Cys	Asp	Asp	Ser	Cys	Met	Glu	Ser	Ile	Arg	Asn	Asn	Thr
				485					490					495	
Tyr	Asp	His	Ser	Gln	Tyr	Arg	Glu	Glu	Ala	Leu	Leu	Asn	Arg	Leu	Asn
			500					505					510		
Ile	Asn	Pro	Val	Lys	Leu	Ser	Ser	Gly	Tyr	Lys	Asp	Ile	Ile	Leu	Trp
		515					520					525			
Phe	Ser	Phe	Gly	Glu	Ser	Cys	Phe	Val	Leu	Leu	Ala	Val	Val	Met	Gly
	530					535					540				
Leu	Val	Phe	Phe	Cys	Leu	Lys	Asn	Gly	Asn	Met	Arg	Cys	Thr	Ile	Cys
545					550					555					560
Ile															

<210> 11
 <211> 565
 <212> PRT
 <213> influenza A virus

<220>
 <223> influenza virus A hemagglutinin subtype H11

<400> 11	Met	Glu	Lys	Thr	Leu	Leu	Phe	Ala	Ala	Ile	Phe	Leu	Cys	Val	Lys	Ala
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Asp	Glu	Ile	Cys	Ile	Gly	Tyr	Leu	Ser	Asn	Asn	Ser	Thr	Asp	Lys	Val	
			20					25					30			
Asp	Thr	Ile	Ile	Glu	Asn	Asn	Val	Thr	Val	Thr	Ser	Ser	Val	Glu	Leu	
		35					40					45				
Val	Glu	Thr	Glu	His	Thr	Gly	Ser	Phe	Cys	Ser	Ile	Asn	Gly	Lys	Gln	
	50					55					60					
Pro	Ile	Ser	Leu	Gly	Asp	Cys	Ser	Phe	Ala	Gly	Trp	Ile	Leu	Gly	Asn	
65				70						75					80	
Pro	Met	Cys	Asp	Glu	Leu	Ile	Gly	Lys	Thr	Ser	Trp	Ser	Tyr	Ile	Val	
			85						90					95		
Glu	Lys	Pro	Asn	Pro	Thr	Asn	Gly	Ile	Cys	Tyr	Pro	Gly	Thr	Leu	Glu	
			100					105					110			
Ser	Glu	Glu	Glu	Leu	Arg	Leu	Lys	Phe	Ser	Gly	Val	Leu	Glu	Phe	Asn	
		115					120					125				
Lys	Phe	Glu	Val	Phe	Thr	Ser	Asn	Gly	Trp	Gly	Ala	Val	Asn	Ser	Gly	
	130					135					140					
Val	Gly	Val	Thr	Ala	Ala	Cys	Lys	Phe	Gly	Gly	Ser	Asn	Ser	Phe	Phe	
145					150					155					160	
Arg	Asn	Met	Val	Trp	Leu	Ile	His	Gln	Ser	Gly	Thr	Tyr	Pro	Val	Ile	

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165
Lys Arg Thr Phe Asn Asn Thr Lys Gly 170 Asp Val Leu Ile 175
180
Gly Ile His His Pro Ala Thr Leu Thr 185 Glu His Gln Asp Leu Tyr Lys
195
Lys Asp Ser Ser Tyr Val Ala Val Gly Ser Glu Thr Tyr Asn Arg Arg
210
Phe Thr Pro Glu Ile Asn Thr Arg Pro Arg Val Asn Gly Gln Ala Gly
225
Arg Met Thr Phe Tyr Trp Lys Ile Val Lys Pro Gly Glu Ser Ile Thr
245
Phe Glu Ser Asn Gly Ala Phe Leu Ala Pro Arg Tyr Ala Phe Glu Ile
260
Val Ser Val Gly Asn Gly Lys Leu Phe Arg Ser Glu Leu Asn Ile Glu
275
Ser Cys Ser Thr Lys Cys Gln Thr Glu Ile Gly Gly Ile Asn Thr Asn
290
Lys Ser Phe His Asn Val His Arg Asn Thr Ile Gly Asp Cys Pro Lys
305
Tyr Val Asn Val Lys Ser Leu Lys Leu Ala Thr Gly Pro Arg Asn Val
325
Pro Ala Ile Ala Ser Arg Gly Leu Phe Gly Ala Ile Ala Gly Phe Ile
340
Glu Gly Gly Trp Pro Gly Leu Ile Asn Gly Trp Tyr Gly Phe Gln His
355
Arg Asp Glu Glu Gly Thr Gly Ile Ala Ala Asp Lys Glu Ser Thr Gln
370
Lys Ala Ile Asp Gln Ile Thr Ser Lys Val Asn Asn Ile Val Asp Arg
385
Met Asn Thr Asn Phe Glu Ser Val Gln His Glu Phe Ser Glu Ile Glu
405
Glu Arg Ile Asn Gln Leu Ser Lys His Val Asp Asp Ser Val Val Asp
420
Ile Trp Ser Tyr Asn Ala Gln Leu Leu Val Leu Leu Glu Asn Glu Lys
435
Thr Leu Asp Leu His Asp Ser Asn Val Arg Asn Leu His Glu Lys Val
450
Arg Arg Met Leu Lys Asp Asn Ala Lys Asp Glu Gly Asn Gly Cys Phe
465
Thr Phe Tyr His Lys Cys Asp Asn Lys Cys Ile Glu Arg Val Arg Asn
485
Gly Thr Tyr Asp His Lys Glu Phe Glu Glu Ser Lys Ile Asn Arg
500
Gln Glu Ile Glu Gly Val Lys Leu Asp Ser Ser Gly Asn Val Tyr Lys
515
Ile Leu Ser Ile Tyr Ser Cys Ile Ala Ser Ser Leu Val Leu Ala Ala
530
Leu Ile Met Gly Phe Met Phe Trp Ala Cys Ser Asn Gly Ser Cys Arg
545
Cys Thr Ile Cys Ile 550
565

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<210> 12

<211> 564

<212> PRT

<213> influenza A virus

<220>

<223> influenza virus A hemagglutinin subtype H12

<400> 12

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Met Glu Lys Phe Ile Ile Leu Ser Thr Val Leu Ala Ala Ser Phe Ala
1
Tyr Asp Lys Ile Cys Ile Gly Tyr Gln Thr Asn Asn Ser Thr Glu Thr
20
Val Asn Thr Leu Ser Glu Gln Asn Val Pro Val Thr Gln Val Glu Glu
35
40
45

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Leu	Val	His	Arg	Gly	Ile	Asp	Pro	Ile	Leu	Cys	Gly	Thr	Glu	Leu	Gly
50						55				60					
Ser	Pro	Leu	Val	Leu	Asp	Asp	Cys	Ser	Leu	Glu	Gly	Leu	Ile	Leu	Gly
65					70					75					80
Asn	Pro	Lys	Cys	Asp	Leu	Tyr	Leu	Asn	Gly	Arg	Glu	Trp	Ser	Tyr	Ile
				85					90					95	
Val	Glu	Arg	Pro	Lys	Glu	Met	Glu	Gly	Val	Cys	Tyr	Pro	Gly	Ser	Ile
			100					105					110		
Glu	Asn	Gln	Glu	Glu	Leu	Arg	Ser	Leu	Phe	Ser	Ser	Ile	Lys	Lys	Tyr
		115					120					125			
Glu	Arg	Val	Lys	Met	Phe	Asp	Phe	Thr	Lys	Trp	Asn	Val	Thr	Tyr	Thr
	130					135					140				
Gly	Thr	Ser	Lys	Ala	Cys	Asn	Asn	Thr	Ser	Asn	Gln	Gly	Ser	Phe	Tyr
145					150					155					160
Arg	Ser	Met	Arg	Trp	Leu	Thr	Leu	Lys	Ser	Gly	Gln	Phe	Pro	Val	Gln
				165					170					175	
Thr	Asp	Glu	Tyr	Lys	Asn	Thr	Arg	Asp	Ser	Asp	Ile	Val	Phe	Thr	Trp
			180					185					190		
Ala	Ile	His	His	Pro	Pro	Thr	Ser	Asp	Glu	Gln	Val	Lys	Leu	Tyr	Lys
		195					200					205			
Asn	Pro	Asp	Thr	Leu	Ser	Ser	Val	Thr	Thr	Val	Glu	Ile	Asn	Arg	Ser
	210					215					220				
Phe	Lys	Pro	Asn	Ile	Gly	Pro	Arg	Pro	Leu	Val	Arg	Gly	Gln	Gln	Gly
225					230					235					240
Arg	Met	Asp	Tyr	Tyr	Trp	Ala	Val	Leu	Lys	Pro	Gly	Gln	Thr	Val	Lys
				245					250					255	
Ile	Gln	Thr	Asn	Gly	Asn	Leu	Ile	Ala	Pro	Glu	Tyr	Gly	His	Leu	Ile
			260					265					270		
Thr	Gly	Lys	Ser	His	Gly	Arg	Ile	Leu	Lys	Asn	Asn	Leu	Pro	Met	Gly
		275					280					285			
Gln	Cys	Val	Thr	Glu	Cys	Gln	Leu	Asn	Glu	Gly	Val	Met	Asn	Thr	Ser
	290					295					300				
Lys	Pro	Phe	Gln	Asn	Thr	Ser	Lys	His	Tyr	Ile	Gly	Lys	Cys	Pro	Lys
305					310					315					320
Tyr	Ile	Pro	Ser	Gly	Ser	Leu	Lys	Leu	Ala	Ile	Gly	Leu	Arg	Asn	Val
				325					330					335	
Pro	Gln	Val	Gln	Asp	Arg	Gly	Leu	Phe	Gly	Ala	Ile	Ala	Gly	Phe	Ile
			340					345					350		
Glu	Gly	Gly	Trp	Pro	Gly	Leu	Val	Ala	Gly	Trp	Tyr	Gly	Phe	Gln	His
		355					360					365			
Gln	Asn	Ala	Glu	Gly	Thr	Gly	Ile	Ala	Ala	Asp	Arg	Asp	Ser	Thr	Gln
	370					375					380				
Arg	Ala	Ile	Asp	Asn	Met	Gln	Asn	Lys	Leu	Asn	Asn	Val	Ile	Asp	Lys
385					390					395					400
Met	Asn	Lys	Gln	Phe	Glu	Val	Val	Asn	His	Glu	Phe	Ser	Glu	Val	Glu
				405					410					415	
Ser	Arg	Ile	Asn	Met	Ile	Asn	Ser	Lys	Ile	Asp	Asp	Gln	Ile	Thr	Asp
			420					425					430		
Ile	Trp	Ala	Tyr	Asn	Ala	Glu	Leu	Leu	Val	Leu	Leu	Glu	Asn	Gln	Lys
		435					440					445			
Thr	Leu	Asp	Glu	His	Asp	Ala	Asn	Val	Arg	Asn	Leu	His	Asp	Arg	Val
	450					455					460				
Arg	Arg	Val	Leu	Arg	Glu	Asn	Ala	Ile	Asp	Thr	Gly	Asp	Gly	Cys	Phe
465					470					475					480
Glu	Ile	Leu	His	Lys	Cys	Asp	Asn	Asn	Cys	Met	Asp	Thr	Ile	Arg	Asn
				485					490					495	
Gly	Thr	Tyr	Asn	His	Lys	Glu	Tyr	Glu	Glu	Glu	Ser	Lys	Ile	Glu	Arg
			500					505					510		
Gln	Lys	Val	Asn	Gly	Val	Lys	Leu	Glu	Glu	Asn	Ser	Thr	Tyr	Lys	Ile
		515					520					525			
Leu	Ser	Ile	Tyr	Ser	Ser	Val	Ala	Ser	Ser	Leu	Val	Leu	Leu	Leu	Met
	530					535					540				
Ile	Ile	Gly	Gly	Phe	Ile	Phe	Gly	Cys	Gln	Asn	Gly	Asn	Val	Arg	Cys
545					550					555					560
Thr	Phe	Cys	Ile												

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<210> 13
 <211> 566
 <212> PRT
 <213> influenza A virus

<220>
 <223> influenza virus A hemagglutinin subtype H13

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 His Ala Asp Arg Ile Cys Val Gly Tyr Leu Ser Thr Asn Ser Ser Glu
 20 25 30
 Arg Val Asp Thr Leu Leu Glu Asn Gly Val Pro Val Thr Ser Ser Ile
 35 40 45
 Asp Leu Ile Glu Thr Asn His Thr Gly Thr Tyr Cys Ser Leu Asn Gly
 50 55 60
 Val Ser Pro Val His Leu Gly Asp Cys Ser Phe Glu Gly Trp Ile Val
 65 70 75 80
 Gly Asn Pro Ala Cys Thr Ser Asn Phe Gly Ile Arg Glu Trp Ser Tyr
 85 90 95
 Leu Ile Glu Asp Pro Ala Ala Pro His Gly Leu Cys Tyr Pro Gly Glu
 100 105 110
 Leu Asn Asn Asn Gly Glu Leu Arg His Leu Phe Ser Gly Ile Arg Ser
 115 120 125
 Phe Ser Arg Thr Glu Leu Ile Pro Pro Thr Ser Trp Gly Glu Val Leu
 130 135 140
 Asp Gly Thr Thr Ser Ala Cys Arg Asp Asn Thr Gly Thr Asn Ser Phe
 145 150 155 160
 Tyr Arg Asn Leu Val Trp Phe Ile Lys Lys Asn Thr Arg Tyr Pro Val
 165 170 175
 Ile Ser Lys Thr Tyr Asn Asn Thr Thr Gly Arg Asp Val Leu Val Leu
 180 185 190
 Trp Gly Ile His His Pro Val Ser Val Asp Glu Thr Lys Thr Leu Tyr
 195 200 205
 Val Asn Ser Asp Pro Tyr Thr Leu Val Ser Thr Lys Ser Trp Ser Glu
 210 215 220
 Lys Tyr Lys Leu Glu Thr Gly Val Arg Pro Gly Tyr Asn Gly Gln Arg
 225 230 235 240
 Ser Trp Met Lys Ile Tyr Trp Ser Leu Ile His Pro Gly Glu Met Ile
 245 250 255
 Thr Phe Glu Ser Asn Gly Gly Phe Leu Ala Pro Arg Tyr Gly Tyr Ile
 260 265 270
 Ile Glu Glu Tyr Gly Lys Gly Arg Ile Phe Gln Ser Arg Ile Arg Met
 275 280 285
 Ser Arg Cys Asn Thr Lys Cys Gln Thr Ser Val Gly Gly Ile Asn Thr
 290 295 300
 Asn Arg Thr Phe Gln Asn Ile Asp Lys Asn Ala Leu Gly Asp Cys Pro
 305 310 315 320
 Lys Tyr Ile Lys Ser Gly Gln Leu Lys Leu Ala Thr Gly Leu Arg Asn
 325 330 335
 Val Pro Ala Ile Ser Asn Arg Gly Leu Phe Gly Ala Ile Ala Gly Phe
 340 345 350
 Ile Glu Gly Gly Trp Pro Gly Leu Ile Asn Gly Trp Tyr Gly Phe Gln
 355 360 365
 His Gln Asn Glu Gln Gly Thr Gly Ile Ala Ala Asp Lys Glu Ser Thr
 370 375 380
 Gln Lys Ala Ile Asp Gln Ile Thr Thr Lys Ile Asn Asn Ile Ile Asp
 385 390 395 400
 Lys Met Asn Gly Asn Tyr Asp Ser Ile Arg Gly Glu Phe Asn Gln Val
 405 410 415
 Glu Lys Arg Ile Asn Met Leu Ala Asp Arg Ile Asp Asp Ala Val Thr
 420 425 430
 Asp Ile Trp Ser Tyr Asn Ala Lys Leu Leu Val Leu Leu Glu Asn Asp
 435 440 445
 Lys Thr Leu Asp Met His Asp Ala Asn Val Lys Asn Leu His Glu Gln
 450 455 460
 Val Arg Arg Glu Leu Lys Asp Asn Ala Ile Asp Glu Gly Asn Gly Cys

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465           470           475           480
Phe Glu Leu Leu His Lys Cys Asn Asp Ser Cys Met Glu Thr Ile Arg
           485           490           495
Asn Gly Thr Tyr Asp His Thr Glu Tyr Ala Glu Glu Ser Lys Leu Lys
           500           505           510
Arg Gln Glu Ile Asp Gly Ile Lys Leu Lys Ser Glu Asp Asn Val Tyr
           515           520           525
Lys Ala Leu Ser Ile Tyr Ser Cys Ile Ala Ser Ser Val Val Leu Val
           530           535           540
Gly Leu Ile Leu Ser Phe Ile Met Trp Ala Cys Ser Ser Gly Asn Cys
545           550           555           560
Arg Phe Asn Val Cys Ile
           565

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<210> 14

<211> 568

<212> PRT

<213> influenza A virus

<220>

<223> influenza virus A hemagglutinin subtype H14

<400> 14

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

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Ile	Ala	Gly	Phe	Ile	Glu	Asn	Gly	Trp	Gln	Gly	Leu	Ile	Asp	Gly	Trp
		355					360				365				
Tyr	Gly	Phe	Arg	His	Gln	Asn	Ala	Glu	Gly	Thr	Gly	Thr	Ala	Ala	Asp
	370					375					380				
Leu	Lys	Ser	Thr	Gln	Ala	Ala	Ile	Asp	Gln	Ile	Asn	Gly	Lys	Leu	Asn
385					390					395					400
Arg	Leu	Ile	Glu	Lys	Thr	Asn	Glu	Lys	Tyr	His	Gln	Ile	Glu	Lys	Glu
				405					410					415	
Phe	Glu	Gln	Val	Glu	Gly	Arg	Ile	Gln	Asp	Leu	Glu	Lys	Tyr	Val	Glu
			420					425					430		
Asp	Thr	Lys	Ile	Asp	Leu	Trp	Ser	Tyr	Asn	Ala	Glu	Leu	Leu	Val	Ala
		435					440					445			
Leu	Glu	Asn	Gln	His	Thr	Ile	Asp	Val	Thr	Asp	Ser	Glu	Met	Asn	Lys
	450					455					460				
Leu	Phe	Glu	Arg	Val	Arg	Arg	Gln	Leu	Arg	Glu	Asn	Ala	Glu	Asp	Gln
465					470					475					480
Gly	Asn	Gly	Cys	Phe	Glu	Ile	Phe	His	Gln	Cys	Asp	Asn	Asn	Cys	Ile
				485					490					495	
Glu	Ser	Ile	Arg	Asn	Gly	Thr	Tyr	Asp	His	Asn	Ile	Tyr	Arg	Asp	Glu
			500					505					510		
Ala	Ile	Asn	Asn	Arg	Ile	Lys	Ile	Asn	Pro	Val	Thr	Leu	Thr	Met	Gly
		515					520					525			
Tyr	Lys	Asp	Ile	Ile	Leu	Trp	Ile	Ser	Phe	Ser	Met	Ser	Cys	Phe	Val
	530					535					540				
Phe	Val	Ala	Leu	Ile	Leu	Gly	Phe	Val	Leu	Trp	Ala	Cys	Gln	Asn	Gly
545					550					555					560
Asn	Ile	Arg	Cys	Gln	Ile	Cys	Ile								
				565											

<210> 15
 <211> 570
 <212> PRT
 <213> influenza A virus

<220>
 <223> influenza virus A hemagglutinin subtype H15

<400> 15

Met	Asn	Thr	Gln	Ile	Ile	Val	Ile	Leu	Val	Leu	Gly	Leu	Ser	Met	Val
1				5				10					15		
Lys	Ser	Asp	Lys	Ile	Cys	Leu	Gly	His	His	Ala	Val	Ala	Asn	Gly	Thr
			20					25					30		
Lys	Val	Asn	Thr	Leu	Thr	Glu	Arg	Gly	Val	Glu	Val	Val	Asn	Ala	Thr
		35					40					45			
Glu	Thr	Val	Glu	Ile	Thr	Gly	Ile	Asp	Lys	Val	Cys	Thr	Lys	Gly	Lys
	50					55					60				
Lys	Ala	Val	Asp	Leu	Gly	Ser	Cys	Gly	Ile	Leu	Gly	Thr	Ile	Ile	Gly
65				70						75				80	
Pro	Pro	Gln	Cys	Asp	Leu	His	Leu	Glu	Phe	Lys	Ala	Asp	Leu	Ile	Ile
				85					90					95	
Glu	Arg	Arg	Asn	Ser	Ser	Asp	Ile	Cys	Tyr	Pro	Gly	Arg	Phe	Thr	Asn
			100					105					110		
Glu	Glu	Ala	Leu	Arg	Gln	Ile	Ile	Arg	Glu	Ser	Gly	Gly	Ile	Asp	Lys
		115				120						125			
Glu	Ser	Met	Gly	Phe	Arg	Tyr	Ser	Gly	Ile	Arg	Thr	Asp	Gly	Ala	Thr
	130					135					140				
Ser	Ala	Cys	Lys	Arg	Thr	Val	Ser	Ser	Phe	Tyr	Ser	Glu	Met	Lys	Trp
145					150					155					160
Leu	Ser	Ser	Ser	Met	Asn	Asn	Gln	Val	Phe	Pro	Gln	Leu	Asn	Gln	Thr
				165					170					175	
Tyr	Arg	Asn	Thr	Arg	Lys	Glu	Pro	Ala	Leu	Ile	Val	Trp	Gly	Val	His
			180					185					190		
His	Ser	Ser	Ser	Leu	Asp	Glu	Gln	Asn	Lys	Leu	Tyr	Gly	Thr	Gly	Asn
		195					200					205			
Lys	Leu	Ile	Thr	Val	Gly	Ser	Ser	Lys	Tyr	Gln	Gln	Ser	Phe	Ser	Pro
	210					215					220				
Ser	Pro	Gly	Ala	Arg	Pro	Lys	Val	Asn	Gly	Gln	Ala	Gly	Arg	Ile	Asp

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

<210> 16

<211> 565

<212> PRT

<213> influenza A virus

<220>

<223> influenza virus A hemagglutinin subtype H16

<400> 16

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

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Glu	Leu	Asp	Asn	Asn	Gly	Glu	Leu	Arg	His	Leu	Phe	Ser	Gly	Val	Asn
		115				120						125			
Ser	Phe	Ser	Arg	Thr	Glu	Leu	Asn	Pro	Ser	Lys	Trp	Gly	Asn	Val	
	130					135				140					
Leu	Asp	Gly	Val	Thr	Ala	Ser	Cys	Leu	Asp	Arg	Gly	Ala	Ser	Ser	Phe
145					150					155					160
Tyr	Arg	Asn	Leu	Val	Trp	Ile	Val	Lys	Lys	Asp	Glu	Lys	Tyr	Pro	Val
				165					170					175	
Ile	Lys	Gly	Asp	Tyr	Asn	Asn	Thr	Thr	Gly	Arg	Asp	Val	Leu	Val	Leu
			180					185					190		
Trp	Gly	Ile	His	His	Pro	Asp	Thr	Glu	Thr	Thr	Ala	Thr	Asn	Leu	Tyr
		195					200					205			
Val	Asn	Lys	Asn	Pro	Tyr	Thr	Leu	Val	Ser	Thr	Lys	Glu	Trp	Ser	Lys
	210					215					220				
Arg	Tyr	Glu	Leu	Glu	Ile	Gly	Thr	Arg	Ile	Gly	Asp	Gly	Gln	Arg	Ser
225					230					235					240
Trp	Met	Lys	Leu	Tyr	Trp	His	Leu	Met	His	Pro	Gly	Glu	Arg	Ile	Met
				245					250					255	
Phe	Glu	Ser	Asn	Gly	Gly	Leu	Ile	Ala	Pro	Arg	Tyr	Gly	Tyr	Ile	Ile
			260					265					270		
Glu	Lys	Tyr	Gly	Thr	Gly	Arg	Ile	Phe	Gln	Ser	Gly	Val	Arg	Met	Ala
		275					280					285			
Arg	Cys	Asn	Thr	Lys	Cys	Gln	Thr	Ser	Leu	Gly	Gly	Ile	Asn	Thr	Asn
	290					295					300				
Lys	Thr	Phe	Gln	Asn	Ile	Glu	Arg	Asn	Ala	Leu	Gly	Asp	Cys	Pro	Lys
305					310					315					320
Tyr	Ile	Lys	Ser	Gly	Gln	Leu	Lys	Leu	Ala	Thr	Gly	Leu	Arg	Asn	Val
				325					330					335	
Pro	Ser	Ile	Gly	Glu	Arg	Gly	Leu	Phe	Gly	Ala	Ile	Ala	Gly	Phe	Ile
			340					345					350		
Glu	Gly	Gly	Trp	Pro	Gly	Leu	Ile	Asn	Gly	Trp	Tyr	Gly	Phe	Gln	His
		355					360					365			
Gln	Asn	Glu	Gln	Gly	Thr	Gly	Ile	Ala	Ala	Asp	Lys	Ala	Ser	Thr	Gln
	370					375					380				
Lys	Ala	Ile	Asn	Glu	Ile	Thr	Thr	Lys	Ile	Asn	Asn	Ile	Ile	Glu	Lys
385					390					395					400
Met	Asn	Gly	Asn	Tyr	Asp	Ser	Ile	Arg	Gly	Glu	Phe	Asn	Gln	Val	Glu
				405					410					415	
Lys	Arg	Ile	Asn	Met	Leu	Ala	Asp	Arg	Val	Asp	Asp	Ala	Val	Thr	Asp
			420					425					430		
Ile	Trp	Ser	Tyr	Asn	Ala	Lys	Leu	Leu	Val	Leu	Leu	Glu	Asn	Asp	Arg
		435					440					445			
Thr	Leu	Asp	Leu	His	Asp	Ala	Asn	Val	Arg	Asn	Leu	His	Asp	Gln	Val
	450					455					460				
Lys	Arg	Ala	Leu	Lys	Ser	Asn	Ala	Ile	Asp	Glu	Gly	Asp	Gly	Cys	Phe
465					470					475					480
Asn	Leu	Leu	His	Lys	Cys	Asn	Asp	Ser	Cys	Met	Glu	Thr	Ile	Arg	Asn
				485					490					495	
Gly	Thr	Tyr	Asn	His	Glu	Asp	Tyr	Arg	Glu	Glu	Ser	Gln	Leu	Lys	Arg
			500					505					510		
Gln	Glu	Ile	Glu	Gly	Ile	Lys	Leu	Lys	Thr	Glu	Asp	Asn	Val	Tyr	Lys
		515					520					525			
Val	Leu	Ser	Ile	Tyr	Ser	Cys	Ile	Ala	Ser	Ser	Ile	Val	Leu	Val	Gly
	530					535					540				
Leu	Ile	Leu	Ala	Phe	Ile	Met	Trp	Ala	Cys	Ser	Asn	Gly	Ser	Cys	Arg
545					550					555					560
Phe	Asn	Val	Cys	Ile											
				565											

<210> 17

<211> 585

<212> PRT

<213> influenza B virus

<220>

<223> influenza virus B hemagglutinin

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<400> 17

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Met Lys Ala Ile Ile Val Ile Leu Met Val Val Thr Ser Asn Ala Asp
1      5      10      15
Arg Ile Cys Thr Gly Ile Thr Ser Ser Asn Ser Pro His Val Val Lys
      20      25      30
Thr Ala Thr Gln Gly Glu Val Asn Val Thr Gly Val Ile Pro Leu Thr
      35      40      45
Thr Thr Pro Thr Lys Ser His Phe Ala Asn Leu Lys Gly Thr Glu Thr
      50      55      60
Arg Gly Lys Leu Cys Pro Lys Cys Leu Asn Cys Thr Asp Leu Asp Val
65      70      75      80
Ala Leu Gly Arg Pro Lys Cys Thr Gly Lys Ile Pro Ser Ala Arg Val
      85      90      95
Ser Ile Leu His Glu Val Arg Pro Val Thr Ser Gly Cys Phe Pro Ile
      100      105      110
Met His Asp Arg Thr Lys Ile Arg Gln Leu Pro Asn Leu Leu Arg Gly
      115      120      125
Tyr Glu His Ile Arg Leu Ser Thr His Asn Val Ile Asn Ala Glu Asn
      130      135      140
Ala Pro Gly Gly Pro Tyr Lys Ile Gly Thr Ser Gly Ser Cys Pro Asn
145      150      155      160
Ile Thr Asn Gly Asn Gly Phe Phe Ala Thr Met Ala Trp Ala Val Pro
      165      170      175
Lys Asn Asp Lys Asn Lys Thr Ala Thr Asn Pro Leu Thr Ile Glu Val
      180      185      190
Pro Tyr Ile Cys Thr Glu Gly Glu Asp Gln Ile Thr Val Trp Gly Phe
      195      200      205
His Ser Asp Ser Glu Thr Gln Met Ala Lys Leu Tyr Gly Asp Ser Lys
      210      215      220
Pro Gln Lys Phe Thr Ser Ser Ala Asn Gly Val Thr Thr His Tyr Val
225      230      235      240
Ser Gln Ile Gly Gly Phe Pro Asn Gln Thr Glu Asp Gly Gly Leu Pro
      245      250      255
Gln Ser Gly Arg Ile Val Val Asp Tyr Met Val Gln Lys Ser Gly Lys
      260      265      270
Thr Gly Thr Ile Thr Tyr Gln Arg Gly Ile Leu Leu Pro Gln Lys Val
      275      280      285
Trp Cys Ala Ser Gly Arg Ser Lys Val Ile Lys Gly Ser Leu Pro Leu
290      295      300
Ile Gly Glu Ala Asp Cys Leu His Glu Lys Tyr Gly Gly Leu Asn Lys
305      310      315      320
Ser Lys Pro Tyr Tyr Thr Gly Glu His Ala Lys Ala Ile Gly Asn Cys
      325      330      335
Pro Ile Trp Val Lys Thr Pro Leu Lys Leu Ala Asn Gly Thr Lys Tyr
      340      345      350
Arg Pro Pro Ala Lys Leu Leu Lys Glu Arg Gly Phe Phe Gly Ala Ile
      355      360      365
Ala Gly Phe Leu Glu Gly Gly Trp Glu Gly Met Ile Ala Gly Trp His
      370      375      380
Gly Tyr Thr Ser His Gly Ala His Gly Val Ala Val Ala Ala Asp Leu
385      390      395      400
Lys Ser Thr Gln Glu Ala Ile Asn Lys Ile Thr Lys Asn Leu Asn Ser
      405      410      415
Leu Ser Glu Leu Glu Val Lys Asn Leu Gln Arg Leu Ser Gly Ala Met
      420      425      430
Asp Glu Leu His Asn Glu Ile Leu Glu Leu Asp Glu Lys Val Asp Asp
      435      440      445
Leu Arg Ala Asp Thr Ile Ser Ser Gln Ile Glu Leu Ala Val Leu Leu
      450      455      460
Ser Asn Glu Gly Ile Ile Asn Ser Glu Asp Glu His Leu Leu Ala Leu
465      470      475      480
Glu Arg Lys Leu Lys Lys Met Leu Gly Pro Ser Ala Val Glu Ile Gly
      485      490      495
Asn Gly Cys Phe Glu Thr Lys His Lys Cys Asn Gln Thr Cys Leu Asp
      500      505      510
Arg Ile Ala Ala Gly Thr Phe Asp Ala Gly Glu Phe Ser Leu Pro Thr
      515      520      525
Phe Asp Ser Leu Asn Ile Thr Ala Ala Ser Leu Asn Asp Asp Gly Leu

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530	Asp	Asn	His	Thr	Ile	Leu	Leu	Tyr	Tyr	Ser	Thr	Ala	Ala	Ser	Ser	Leu
545	Ala	Val	Thr	Leu	Met	Ile	Ala	Ile	Phe	Val	Val	Tyr	Met	Val	Ser	Arg
	Asp	Asn	Val	Ser	Cys	Ser	Ile	Cys	Leu							
				580					585							

<210> 18
 <211> 17
 <212> PRT
 <213> influenza A virus

<220>
 <223> signal peptide of HA subtype PR8-H1N1

<400> 18	Met	Lys	Ala	Asn	Leu	Leu	Val	Leu	Leu	Cys	Ala	Leu	Ala	Ala	Ala	Asp
	1				5					10					15	
	Ala															

<210> 19
 <211> 15
 <212> PRT
 <213> influenza A virus

<220>
 <223> signal peptide of HA subtype H2N2

<400> 19	Met	Ala	Ile	Ile	Tyr	Leu	Ile	Leu	Leu	Phe	Thr	Ala	Val	Arg	Gly
	1				5					10					15

<210> 20
 <211> 16
 <212> PRT
 <213> influenza A virus

<220>
 <223> signal peptide of HA subtype HK68-H3N2

<400> 20	Met	Lys	Thr	Ile	Ile	Ala	Leu	Ser	Tyr	Ile	Phe	Cys	Leu	Ala	Leu	Gly
	1				5					10					15	

<210> 21
 <211> 16
 <212> PRT
 <213> influenza A virus

<220>
 <223> signal peptide of HA subtype H4

<400> 21	Met	Leu	Ser	Ile	Val	Ile	Leu	Phe	Leu	Leu	Ile	Ala	Glu	Asn	Ser	Ser
	1				5					10					15	

<210> 22
 <211> 16
 <212> PRT
 <213> influenza A virus

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<220>

<223> signal peptide of HA subtype H5

<400> 22

Met Glu Arg Ile Val Leu Leu Leu Ala Ile Val Ser Leu Val Lys Ser
1 5 10 15

<210> 23

<211> 16

<212> PRT

<213> influenza A virus

<220>

<223> signal peptide of HA subtype H6

<400> 23

Met Ile Ala Ile Ile Val Val Ala Ile Leu Ala Thr Ala Gly Arg Ser
1 5 10 15

<210> 24

<211> 18

<212> PRT

<213> influenza A virus

<220>

<223> signal peptide of HA subtype H7

<400> 24

Met Asn Thr Gln Ile Leu Val Phe Ala Leu Val Ala Val Ile Pro Thr
1 5 10 15
Asn Ala

<210> 25

<211> 16

<212> PRT

<213> influenza A virus

<220>

<223> signal peptide of HA subtype H8

<400> 25

Met Glu Lys Phe Ile Ala Ile Ala Thr Leu Ala Ser Thr Asn Ala Tyr
1 5 10 15

<210> 26

<211> 18

<212> PRT

<213> influenza A virus

<220>

<223> signal peptide of HA subtype H9

<400> 26

Met Glu Thr Lys Ala Ile Ile Ala Ala Leu Leu Met Val Thr Ala Ala
1 5 10 15
Asn Ala

<210> 27

<211> 16

<212> PRT

<213> influenza A virus

Sequence Listing - 2010234849

<220>

<223> signal peptide of HA subtype H10

<400> 27

Met Tyr Lys Val Val Val Ile Ile Ala Leu Leu Gly Ala Val Lys Gly
1 5 10 15

<210> 28

<211> 16

<212> PRT

<213> influenza A virus

<220>

<223> signal peptide of HA subtype H11

<400> 28

Met Glu Lys Thr Leu Leu Phe Ala Ala Ile Phe Leu Cys Val Lys Ala
1 5 10 15

<210> 29

<211> 17

<212> PRT

<213> influenza A virus

<220>

<223> signal peptide of HA subtype H12

<400> 29

Met Glu Lys Phe Ile Ile Leu Ser Thr Val Leu Ala Ala Ser Phe Ala
1 5 10 15
Tyr

<210> 30

<211> 18

<212> PRT

<213> influenza A virus

<220>

<223> signal peptide of HA subtype H13

<400> 30

Met Ala Leu Asn Val Ile Ala Thr Leu Thr Leu Ile Ser Val Cys Val
1 5 10 15
His Ala

<210> 31

<211> 17

<212> PRT

<213> influenza A virus

<220>

<223> signal peptide of HA subtype H14

<400> 31

Met Ile Ala Leu Ile Leu Val Ala Leu Ala Leu Ser His Thr Ala Tyr
1 5 10 15
Ser

<210> 32

<211> 18

Sequence Listing - 2010234849

<212> PRT
 <213> influenza A virus
 <220>
 <223> signal peptide of HA subtype H15
 <400> 32
 Met Asn Thr Gln Ile Ile Val Ile Leu Val Leu Gly Leu Ser Met Val
 1 5 10 15
 Lys Ser

<210> 33
 <211> 19
 <212> PRT
 <213> influenza A virus
 <220>
 <223> signal peptide of HA subtype H16
 <400> 33
 Met Met Ile Lys Val Leu Tyr Phe Leu Ile Ile Val Leu Gly Arg Tyr
 1 5 10 15
 Ser Lys Ala

<210> 34
 <211> 42
 <212> PRT
 <213> influenza A virus
 <220>
 <223> N-terminal stem segment of HA subtype H1
 <400> 34
 Asp Thr Ile Cys Ile Gly Tyr His Ala Asn Asn Ser Thr Asp Thr Val
 1 5 10 15
 Asp Thr Val Leu Glu Lys Asn Val Thr Val Thr His Ser Val Asn Leu
 20 25 30
 Leu Glu Asp Ser His Asn Gly Lys Leu Cys
 35 40

<210> 35
 <211> 42
 <212> PRT
 <213> influenza A virus
 <220>
 <223> N-terminal stem segment of HA subtype H2
 <400> 35
 Asp Gln Ile Cys Ile Gly Tyr His Ser Asn Asn Ser Thr Glu Lys Val
 1 5 10 15
 Asp Thr Ile Leu Glu Arg Asn Val Thr Val Thr His Ala Gln Asn Ile
 20 25 30
 Leu Glu Lys Thr His Asn Gly Lys Leu Cys
 35 40

<210> 36
 <211> 52
 <212> PRT
 <213> influenza A virus
 <220>

Sequence Listing - 2010234849

<223> N-terminal stem segment of HA subtype H3

<400> 36

Gln	Asp	Leu	Pro	Gly	Asn	Asp	Asn	Ser	Thr	Ala	Thr	Leu	Cys	Leu	Gly
1				5				10					15		
His	His	Ala	Val	Pro	Asn	Gly	Thr	Leu	Val	Lys	Thr	Ile	Thr	Asp	Asp
		20						25				30			
Gln	Ile	Glu	Val	Thr	Asn	Ala	Thr	Glu	Leu	Val	Gln	Ser	Ser	Ser	Thr
		35					40					45			
Gly	Lys	Ile	Cys												
	50														

<210> 37

<211> 48

<212> PRT

<213> influenza A virus

<220>

<223> N-terminal stem segment of HA subtype H4

<400> 37

Gln	Asn	Tyr	Thr	Gly	Asn	Pro	Val	Ile	Cys	Met	Gly	His	His	Ala	Val
1				5				10						15	
Ala	Asn	Gly	Thr	Met	Val	Lys	Thr	Leu	Ala	Asp	Asp	Gln	Val	Glu	Val
		20						25				30			
Val	Thr	Ala	Gln	Glu	Leu	Val	Glu	Ser	Gln	Asn	Leu	Pro	Glu	Leu	Cys
		35					40					45			

<210> 38

<211> 42

<212> PRT

<213> influenza A virus

<220>

<223> N-terminal stem segment of HA subtype H5

<400> 38

Asp	Gln	Ile	Cys	Ile	Gly	Tyr	His	Ala	Asn	Lys	Ser	Thr	Lys	Gln	Val
1				5				10						15	
Asp	Thr	Ile	Met	Glu	Lys	Asn	Val	Thr	Val	Thr	His	Ala	Gln	Asp	Ile
		20						25				30			
Leu	Glu	Arg	Thr	His	Asn	Gly	Lys	Leu	Cys						
		35					40								

<210> 39

<211> 42

<212> PRT

<213> influenza A virus

<220>

<223> N-terminal stem segment of HA subtype H6

<400> 39

Asp	Lys	Ile	Cys	Ile	Gly	Tyr	His	Ala	Asn	Asn	Ser	Thr	Thr	Gln	Ile
1				5				10						15	
Asp	Thr	Ile	Leu	Glu	Lys	Asn	Val	Thr	Val	Thr	His	Ser	Val	Glu	Leu
		20						25				30			
Leu	Glu	Asn	Gln	Lys	Glu	Glu	Arg	Phe	Cys						
		35					40								

<210> 40

<211> 42

<212> PRT

<213> influenza A virus

Sequence Listing - 2010234849

<220>

<223> N-terminal stem segment of HA subtype H7

<400> 40

Asp	Lys	Ile	Cys	Leu	Gly	His	His	Ala	Val	Ser	Asn	Gly	Thr	Lys	Val
1				5					10					15	
Asn	Thr	Leu	Thr	Glu	Arg	Gly	Val	Glu	Val	Val	Asn	Ala	Thr	Glu	Thr
			20					25					30		
Val	Glu	Arg	Thr	Asn	Ile	Pro	Lys	Ile	Cys						
			35				40								

<210> 41

<211> 42

<212> PRT

<213> influenza A virus

<220>

<223> N-terminal stem segment of HA subtype H8

<400> 41

Asp	Arg	Ile	Cys	Ile	Gly	Tyr	Gln	Ser	Asn	Asn	Ser	Thr	Asp	Thr	Val
1				5					10					15	
Asn	Thr	Leu	Ile	Glu	Gln	Asn	Val	Pro	Val	Thr	Gln	Thr	Met	Glu	Leu
			20					25					30		
Val	Glu	Thr	Glu	Lys	His	Pro	Ala	Tyr	Cys						
			35				40								

<210> 42

<211> 42

<212> PRT

<213> influenza A virus

<220>

<223> N-terminal stem segment of HA subtype H9

<400> 42

Asp	Lys	Ile	Cys	Ile	Gly	Tyr	Gln	Ser	Thr	Asn	Ser	Thr	Glu	Thr	Val
1				5					10					15	
Asp	Thr	Leu	Thr	Glu	Ser	Asn	Val	Pro	Val	Thr	His	Thr	Lys	Glu	Leu
			20					25					30		
Leu	His	Thr	Glu	His	Asn	Gly	Met	Leu	Cys						
			35				40								

<210> 43

<211> 43

<212> PRT

<213> influenza A virus

<220>

<223> N-terminal stem segment of HA subtype H10

<400> 43

Leu	Asp	Arg	Ile	Cys	Leu	Gly	His	His	Ala	Val	Ala	Asn	Gly	Thr	Ile
1				5					10					15	
Val	Lys	Thr	Leu	Thr	Asn	Glu	Gln	Glu	Glu	Val	Thr	Asn	Ala	Thr	Glu
			20					25					30		
Thr	Val	Glu	Ser	Thr	Asn	Leu	Asn	Lys	Leu	Cys					
			35				40								

<210> 44

<211> 42

<212> PRT

<213> influenza A virus

Sequence Listing - 2010234849

<220>

<223> N-terminal stem segment of HA subtype H11

<400> 44

Asp	Glu	Ile	Cys	Ile	Gly	Tyr	Leu	Ser	Asn	Asn	Ser	Thr	Asp	Lys	Val
1				5					10					15	
Asp	Thr	Ile	Ile	Glu	Asn	Asn	Val	Thr	Val	Thr	Ser	Ser	Val	Glu	Leu
			20					25					30		
Val	Glu	Thr	Glu	His	Thr	Gly	Ser	Phe	Cys						
			35				40								

<210> 45

<211> 42

<212> PRT

<213> influenza A virus

<220>

<223> N-terminal stem segment of HA subtype H12

<400> 45

Asp	Lys	Ile	Cys	Ile	Gly	Tyr	Gln	Thr	Asn	Asn	Ser	Thr	Glu	Thr	Val
1				5					10					15	
Asn	Thr	Leu	Ser	Glu	Gln	Asn	Val	Pro	Val	Thr	Gln	Val	Glu	Glu	Leu
			20					25					30		
Val	His	Arg	Gly	Ile	Asp	Pro	Ile	Leu	Cys						
			35				40								

<210> 46

<211> 42

<212> PRT

<213> influenza A virus

<220>

<223> N-terminal stem segment of HA subtype H13

<400> 46

Asp	Arg	Ile	Cys	Val	Gly	Tyr	Leu	Ser	Thr	Asn	Ser	Ser	Glu	Arg	Val
1				5					10					15	
Asp	Thr	Leu	Leu	Glu	Asn	Gly	Val	Pro	Val	Thr	Ser	Ser	Ile	Asp	Leu
			20					25					30		
Ile	Glu	Thr	Asn	His	Thr	Gly	Thr	Tyr	Cys						
			35				40								

<210> 47

<211> 51

<212> PRT

<213> influenza A virus

<220>

<223> N-terminal stem segment of HA subtype H14

<400> 47

Gln	Ile	Thr	Asn	Gly	Thr	Thr	Gly	Asn	Pro	Ile	Ile	Cys	Leu	Gly	His
1				5					10					15	
His	Ala	Val	Glu	Asn	Gly	Thr	Ser	Val	Lys	Thr	Leu	Thr	Asp	Asn	His
			20					25					30		
Val	Glu	Val	Val	Ser	Ala	Lys	Glu	Leu	Val	Glu	Thr	Asn	His	Thr	Asp
			35				40					45			
Glu	Leu	Cys													
			50												

<210> 48

<211> 42

Sequence Listing - 2010234849

<212> PRT
<213> influenza A virus

<220>
<223> N-terminal stem segment of HA subtype H15

<400> 48
Asp Lys Ile Cys Leu Gly His His Ala Val Ala Asn Gly Thr Lys Val
1 5 10 15
Asn Thr Leu Thr Glu Arg Gly Val Glu Val Val Asn Ala Thr Glu Thr
20 25 30
Val Glu Ile Thr Gly Ile Asp Lys Val Cys
35 40

<210> 49
<211> 42
<212> PRT
<213> influenza A virus

<220>
<223> N-terminal stem segment of HA subtype H16

<400> 49
Asp Lys Ile Cys Ile Gly Tyr Leu Ser Asn Asn Ser Ser Asp Thr Val
1 5 10 15
Asp Thr Leu Thr Glu Asn Gly Val Pro Val Thr Ser Ser Val Asp Leu
20 25 30
Val Glu Thr Asn His Thr Gly Thr Tyr Cys
35 40

<210> 50
<211> 53
<212> PRT
<213> influenza A virus

<220>
<223> C-terminal stem segment of HA subtype H1

<400> 50
Cys Asn Thr Lys Cys Gln Thr Pro Leu Gly Ala Ile Asn Ser Ser Leu
1 5 10 15
Pro Tyr Gln Asn Ile His Pro Val Thr Ile Gly Glu Cys Pro Lys Tyr
20 25 30
Val Arg Ser Ala Lys Leu Arg Met Val Thr Gly Leu Arg Asn Asn Pro
35 40 45
Ser Ile Gln Ser Arg
50

<210> 51
<211> 53
<212> PRT
<213> influenza A virus

<220>
<223> C-terminal stem segment of HA subtype H2

<400> 51
Cys Glu Thr Lys Cys Gln Thr Pro Leu Gly Ala Ile Asn Thr Thr Leu
1 5 10 15
Pro Phe His Asn Val His Pro Leu Thr Ile Gly Glu Cys Pro Lys Tyr
20 25 30
Val Lys Ser Glu Arg Leu Val Leu Ala Thr Gly Leu Arg Asn Val Pro
35 40 45
Gln Ile Glu Ser Arg
50

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<210> 52
 <211> 53
 <212> PRT
 <213> influenza A virus

<220>
 <223> C-terminal stem segment of HA subtype H3

<400> 52
 Cys Ile Ser Glu Cys Ile Thr Pro Asn Gly Ser Ile Pro Asn Asp Lys
 1 5 10 15
 Pro Phe Gln Asn Val Asn Lys Ile Thr Tyr Gly Ala Cys Pro Lys Tyr
 20 25 30
 Val Lys Gln Asn Thr Leu Lys Leu Ala Thr Gly Met Arg Asn Val Pro
 35 40 45
 Glu Lys Gln Thr Arg
 50

<210> 53
 <211> 53
 <212> PRT
 <213> influenza A virus

<220>
 <223> C-terminal stem segment of HA subtype H4

<400> 53
 Cys Val Ser Lys Cys His Thr Asp Lys Gly Ser Leu Ser Thr Thr Lys
 1 5 10 15
 Pro Phe Gln Asn Ile Ser Arg Ile Ala Val Gly Asp Cys Pro Arg Tyr
 20 25 30
 Val Lys Gln Gly Ser Leu Lys Leu Ala Thr Gly Met Arg Asn Ile Pro
 35 40 45
 Glu Lys Ala Ser Arg
 50

<210> 54
 <211> 53
 <212> PRT
 <213> influenza A virus

<220>
 <223> C-terminal stem segment of HA subtype H5

<400> 54
 Cys Asp Thr Lys Cys Gln Thr Pro Val Gly Glu Ile Asn Ser Ser Met
 1 5 10 15
 Pro Phe His Asn Ile His Pro His Thr Ile Gly Glu Cys Pro Lys Tyr
 20 25 30
 Val Lys Ser Asp Arg Leu Val Leu Ala Thr Gly Leu Arg Asn Val Pro
 35 40 45
 Gln Arg Lys Lys Arg
 50

<210> 55
 <211> 53
 <212> PRT
 <213> influenza A virus

<220>
 <223> C-terminal stem segment of HA subtype H6

<400> 55

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Cys Asp Ala Thr Cys Gln Thr Val Ala Gly Val Leu Arg Thr Asn Lys
 1      5      10      15
Thr Phe Gln Asn Val Ser Pro Leu Trp Ile Gly Glu Cys Pro Lys Tyr
      20      25      30
Val Lys Ser Glu Ser Leu Arg Leu Ala Thr Gly Leu Arg Asn Val Pro
      35      40      45
Gln Ile Glu Thr Arg
      50

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<210> 56
 <211> 57
 <212> PRT
 <213> influenza A virus

<220>
 <223> C-terminal stem segment of HA subtype H7

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<400> 56
Cys Glu Gly Glu Cys Tyr His Ser Gly Gly Thr Ile Thr Ser Arg Leu
 1      5      10      15
Pro Phe Gln Asn Ile Asn Ser Arg Ala Val Gly Lys Cys Pro Arg Tyr
      20      25      30
Val Lys Gln Glu Ser Leu Leu Leu Ala Thr Gly Met Lys Asn Val Pro
      35      40      45
Glu Pro Ser Lys Lys Arg Lys Lys Arg
      50      55

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<210> 57
 <211> 53
 <212> PRT
 <213> influenza A virus

<220>
 <223> C-terminal stem segment of HA subtype H8

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<400> 57
Cys Asn Thr Lys Cys Gln Thr Tyr Ala Gly Ala Ile Asn Ser Ser Lys
 1      5      10      15
Pro Phe Gln Asn Ala Ser Arg His Tyr Met Gly Glu Cys Pro Lys Tyr
      20      25      30
Val Lys Lys Ala Ser Leu Arg Leu Ala Val Gly Leu Arg Asn Thr Pro
      35      40      45
Ser Val Glu Pro Arg
      50

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<210> 58
 <211> 53
 <212> PRT
 <213> influenza A virus

<220>
 <223> C-terminal stem segment of HA subtype H9

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<400> 58
Cys Val Val Gln Cys Gln Thr Glu Lys Gly Gly Leu Asn Thr Thr Leu
 1      5      10      15
Pro Phe His Asn Ile Ser Lys Tyr Ala Phe Gly Asn Cys Pro Lys Tyr
      20      25      30
Val Gly Val Lys Ser Leu Lys Leu Pro Val Gly Leu Arg Asn Val Pro
      35      40      45
Ala Val Ser Ser Arg
      50

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<210> 59

Sequence Listing - 2010234849

<211> 54
<212> PRT
<213> influenza A virus

<220>
<223> C-terminal stem segment of HA subtype H10

<400> 59
Cys Glu Ser Lys Cys Phe Trp Arg Gly Gly Ser Ile Asn Thr Lys Leu
1 5 10 15
Pro Phe Gln Asn Leu Ser Pro Arg Thr Val Gly Gln Cys Pro Lys Tyr
20 25 30
Val Asn Gln Arg Ser Leu Leu Ala Thr Gly Met Arg Asn Val Pro
35 40 45
Glu Val Val Gln Gly Arg
50

<210> 60
<211> 53
<212> PRT
<213> influenza A virus

<220>
<223> C-terminal stem segment of HA subtype H11

<400> 60
Cys Ser Thr Lys Cys Gln Thr Glu Ile Gly Gly Ile Asn Thr Asn Lys
1 5 10 15
Ser Phe His Asn Val His Arg Asn Thr Ile Gly Asp Cys Pro Lys Tyr
20 25 30
Val Asn Val Lys Ser Leu Lys Leu Ala Thr Gly Pro Arg Asn Val Pro
35 40 45
Ala Ile Ala Ser Arg
50

<210> 61
<211> 53
<212> PRT
<213> influenza A virus

<220>
<223> C-terminal stem segment of HA subtype H12

<400> 61
Cys Val Thr Glu Cys Gln Leu Asn Glu Gly Val Met Asn Thr Ser Lys
1 5 10 15
Pro Phe Gln Asn Thr Ser Lys His Tyr Ile Gly Lys Cys Pro Lys Tyr
20 25 30
Ile Pro Ser Gly Ser Leu Lys Leu Ala Ile Gly Leu Arg Asn Val Pro
35 40 45
Gln Val Gln Asp Arg
50

<210> 62
<211> 53
<212> PRT
<213> influenza A virus

<220>
<223> C-terminal stem segment of HA subtype H13

<400> 62
Cys Asn Thr Lys Cys Gln Thr Ser Val Gly Gly Ile Asn Thr Asn Arg
1 5 10 15
Thr Phe Gln Asn Ile Asp Lys Asn Ala Leu Gly Asp Cys Pro Lys Tyr
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Ile Lys Ser Gly Gln Leu Lys Leu Ala Thr Gly Leu Arg Asn Val Pro
 20 25 30
 35 40 45
 Ala Ile Ser Asn Arg
 50

<210> 63
 <211> 53
 <212> PRT
 <213> influenza A virus

<220>
 <223> C-terminal stem segment of HA subtype H14

<400> 63
 Cys Thr Ser Pro Cys Leu Thr Asp Lys Gly Ser Ile Gln Ser Asp Lys
 1 5 10 15
 Pro Phe Gln Asn Val Ser Arg Ile Ala Ile Gly Asn Cys Pro Lys Tyr
 20 25 30
 Val Lys Gln Gly Ser Leu Met Leu Ala Thr Gly Met Arg Asn Ile Pro
 35 40 45
 Gly Lys Gln Ala Lys
 50

<210> 64
 <211> 54
 <212> PRT
 <213> influenza A virus

<220>
 <223> C-terminal stem segment of HA subtype H15

<400> 64
 Cys Glu Gly Glu Cys Phe Tyr Ser Gly Gly Thr Ile Asn Ser Pro Leu
 1 5 10 15
 Pro Phe Gln Asn Ile Asp Ser Arg Ala Val Gly Lys Cys Pro Arg Tyr
 20 25 30
 Val Lys Gln Ser Ser Leu Pro Leu Ala Leu Gly Met Lys Asn Val Pro
 35 40 45
 Glu Lys Ile Arg Thr Arg
 50

<210> 65
 <211> 53
 <212> PRT
 <213> influenza A virus

<220>
 <223> C-terminal stem segment of HA subtype H16

<400> 65
 Cys Asn Thr Lys Cys Gln Thr Ser Leu Gly Gly Ile Asn Thr Asn Lys
 1 5 10 15
 Thr Phe Gln Asn Ile Glu Arg Asn Ala Leu Gly Asp Cys Pro Lys Tyr
 20 25 30
 Ile Lys Ser Gly Gln Leu Lys Leu Ala Thr Gly Leu Arg Asn Val Pro
 35 40 45
 Ser Ile Gly Glu Arg
 50

<210> 66
 <211> 222
 <212> PRT
 <213> influenza A virus

Sequence Listing - 2010234849

<220>

<223> HA2 domain of HA subtype H1

<400> 66

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Gly Leu Phe Gly Ala Ile Ala Gly Phe Ile Glu Gly Gly Trp Thr Gly
 1      5      10      15
Met Ile Asp Gly Trp Tyr Gly Tyr His His Gln Asn Glu Gln Gly Ser
 20      25      30
Gly Tyr Ala Ala Asp Gln Lys Ser Thr Gln Asn Ala Ile Asn Gly Ile
 35      40      45
Thr Asn Lys Val Asn Thr Val Ile Glu Lys Met Asn Ile Gln Phe Thr
 50      55      60
Ala Val Gly Lys Glu Phe Asn Lys Leu Glu Lys Arg Met Glu Asn Leu
 65      70      75      80
Asn Lys Lys Val Asp Asp Gly Phe Leu Asp Ile Trp Thr Tyr Asn Ala
 85      90      95
Glu Leu Leu Val Leu Leu Glu Asn Glu Arg Thr Leu Asp Phe His Asp
100      105      110
Ser Asn Val Lys Asn Leu Tyr Glu Lys Val Lys Ser Gln Leu Lys Asn
115      120      125
Asn Ala Lys Glu Ile Gly Asn Gly Cys Phe Glu Phe Tyr His Lys Cys
130      135      140
Asp Asn Glu Cys Met Glu Ser Val Arg Asn Gly Thr Tyr Asp Tyr Pro
145      150      155      160
Lys Tyr Ser Glu Glu Ser Lys Leu Asn Arg Glu Lys Val Asp Gly Val
165      170      175
Lys Leu Glu Ser Met Gly Ile Tyr Gln Ile Leu Ala Ile Tyr Ser Thr
180      185      190
Val Ala Ser Ser Leu Val Leu Leu Val Ser Leu Gly Ala Ile Ser Phe
195      200      205
Trp Met Cys Ser Asn Gly Ser Leu Gln Cys Arg Ile Cys Ile
210      215      220

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<210> 67

<211> 222

<212> PRT

<213> influenza A virus

<220>

<223> HA2 domain of HA subtype H2

<400> 67

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

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Val Ala Gly Ser Leu Ser Leu Ala Ile Met Ile Ala Gly Ile Ser Leu
 195 200 205
 Trp Met Cys Ser Asn Gly Ser Leu Gln Cys Arg Ile Cys Ile
 210 215 220

<210> 68
 <211> 221
 <212> PRT
 <213> influenza A virus

<220>
 <223> HA2 domain of HA subtype H3

<400> 68
 Gly Leu Phe Gly Ala Ile Ala Gly Phe Ile Glu Asn Gly Trp Glu Gly
 1 5 10 15
 Met Ile Asp Gly Trp Tyr Gly Phe Arg His Gln Asn Ser Glu Gly Thr
 20 25 30
 Gly Gln Ala Ala Asp Leu Lys Ser Thr Gln Ala Ala Ile Asp Gln Ile
 35 40 45
 Asn Gly Lys Leu Asn Arg Val Ile Glu Lys Thr Asn Glu Lys Phe His
 50 55 60
 Gln Ile Glu Lys Glu Phe Ser Glu Val Glu Gly Arg Ile Gln Asp Leu
 65 70 75 80
 Glu Lys Tyr Val Glu Asp Thr Lys Ile Asp Leu Trp Ser Tyr Asn Ala
 85 90 95
 Glu Leu Leu Val Ala Leu Glu Asn Gln His Thr Ile Asp Leu Thr Asp
 100 105 110
 Ser Glu Met Asn Lys Leu Phe Glu Lys Thr Arg Arg Gln Leu Arg Glu
 115 120 125
 Asn Ala Glu Asp Met Gly Asn Gly Cys Phe Lys Ile Tyr His Lys Cys
 130 135 140
 Asp Asn Ala Cys Ile Glu Ser Ile Arg Asn Gly Thr Tyr Asp His Asp
 145 150 155 160
 Val Tyr Arg Asp Glu Ala Leu Asn Asn Arg Phe Gln Ile Lys Gly Val
 165 170 175
 Glu Leu Lys Ser Gly Tyr Lys Asp Trp Ile Leu Trp Ile Ser Phe Ala
 180 185 190
 Ile Ser Cys Phe Leu Leu Cys Val Val Leu Leu Gly Phe Ile Met Trp
 195 200 205
 Ala Cys Gln Arg Gly Asn Ile Arg Cys Asn Ile Cys Ile
 210 215 220

<210> 69
 <211> 221
 <212> PRT
 <213> influenza A virus

<220>
 <223> HA2 domain of HA subtype H4

<400> 69
 Gly Leu Phe Gly Ala Ile Ala Gly Phe Ile Glu Asn Gly Trp Gln Gly
 1 5 10 15
 Leu Ile Asp Gly Trp Tyr Gly Phe Arg His Gln Asn Ala Glu Gly Thr
 20 25 30
 Gly Thr Ala Ala Asp Leu Lys Ser Thr Gln Ala Ala Ile Asp Gln Ile
 35 40 45
 Asn Gly Lys Leu Asn Arg Leu Ile Glu Lys Thr Asn Asp Lys Tyr His
 50 55 60
 Gln Ile Glu Lys Glu Phe Glu Gln Val Glu Gly Arg Ile Gln Asp Leu
 65 70 75 80
 Glu Asn Tyr Val Glu Asp Thr Lys Ile Asp Leu Trp Ser Tyr Asn Ala
 85 90 95
 Glu Leu Leu Val Ala Leu Glu Asn Gln His Thr Ile Asp Val Thr Asp
 100 105 110

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Ser Glu Met Asn Lys Leu Phe Glu Arg Val Arg Arg Gln Leu Arg Glu
    115 120 125
Asn Ala Glu Asp Lys Gly Asn Gly Cys Phe Glu Ile Phe His Lys Cys
    130 135 140
Asp Asn Asn Cys Ile Glu Ser Ile Arg Asn Gly Thr Tyr Asp His Asp
    145 150 155 160
Ile Tyr Arg Asp Glu Ala Ile Asn Asn Arg Phe Gln Ile Gln Gly Val
    165 170 175
Lys Leu Thr Gln Gly Tyr Lys Asp Ile Leu Trp Ile Ser Phe Ser
    180 185 190
Ile Ser Cys Phe Leu Leu Val Ala Leu Leu Leu Ala Phe Ile Leu Trp
    195 200 205
Ala Cys Gln Asn Gly Asn Ile Arg Cys Gln Ile Cys Ile
    210 215 220

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<210> 70
 <211> 222
 <212> PRT
 <213> influenza A virus

<220>
 <223> HA2 domain of HA subtype H5

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<400> 70
Gly Leu Phe Gly Ala Ile Ala Gly Phe Ile Glu Gly Gly Trp Gln Gly
 1      5      10      15
Met Val Asp Gly Trp Tyr Gly Tyr His His Ser Asn Glu Gln Gly Ser
    20      25      30
Gly Tyr Ala Ala Asp Lys Glu Ser Thr Gln Lys Ala Ile Asp Gly Ile
    35      40      45
Thr Asn Lys Val Asn Ser Ile Ile Asp Lys Met Asn Thr Arg Phe Glu
    50      55      60
Ala Val Gly Lys Glu Phe Asn Asn Leu Glu Arg Arg Val Glu Asn Leu
    65      70      75      80
Asn Lys Lys Met Glu Asp Gly Phe Leu Asp Val Trp Thr Tyr Asn Val
    85      90      95
Glu Leu Leu Val Leu Met Glu Asn Glu Arg Thr Leu Asp Phe His Asp
    100      105      110
Ser Asn Val Asn Asn Leu Tyr Asp Lys Val Arg Leu Gln Leu Lys Asp
    115      120      125
Asn Ala Arg Glu Leu Gly Asn Gly Cys Phe Glu Phe Tyr His Lys Cys
    130      135      140
Asp Asn Glu Cys Met Glu Ser Val Arg Asn Gly Thr Tyr Asp Tyr Pro
    145      150      155      160
Gln Tyr Ser Glu Glu Ala Arg Leu Asn Arg Glu Glu Ile Ser Gly Val
    165      170      175
Lys Leu Glu Ser Met Gly Val Tyr Gln Ile Leu Ser Ile Tyr Ser Thr
    180      185      190
Val Ala Ser Ser Leu Ala Leu Ala Ile Met Ile Ala Gly Leu Ser Phe
    195      200      205
Trp Met Cys Ser Asn Gly Ser Leu Gln Cys Arg Ile Cys Ile
    210      215      220

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<210> 71
 <211> 222
 <212> PRT
 <213> influenza A virus

<220>
 <223> HA2 domain of HA subtype H6

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<400> 71
Gly Leu Phe Gly Ala Ile Ala Gly Phe Ile Glu Gly Gly Trp Thr Gly
 1      5      10      15
Met Ile Asp Gly Trp Tyr Gly Tyr His His Glu Asn Ser Gln Gly Ser
    20      25      30

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Gly	Tyr	Ala	Ala	Asp	Arg	Glu	Ser	Thr	Gln	Lys	Ala	Val	Asp	Gly	Ile
		35					40					45			
Thr	Asn	Lys	Val	Asn	Ser	Ile	Ile	Asp	Lys	Met	Asn	Thr	Gln	Phe	Glu
	50					55					60				
Ala	Val	Asp	His	Glu	Phe	Ser	Asn	Leu	Glu	Arg	Arg	Ile	Asp	Asn	Leu
65					70					75					80
Asn	Lys	Arg	Met	Glu	Asp	Gly	Phe	Leu	Asp	Val	Trp	Thr	Tyr	Asn	Ala
				85					90					95	
Glu	Leu	Leu	Val	Leu	Leu	Glu	Asn	Glu	Arg	Thr	Leu	Asp	Leu	His	Asp
			100					105					110		
Ala	Asn	Val	Lys	Asn	Leu	Tyr	Glu	Arg	Val	Lys	Ser	Gln	Leu	Arg	Asp
		115					120					125			
Asn	Ala	Met	Ile	Leu	Gly	Asn	Gly	Cys	Phe	Glu	Phe	Trp	His	Lys	Cys
	130					135					140				
Asp	Asp	Glu	Cys	Met	Glu	Ser	Val	Lys	Asn	Gly	Thr	Tyr	Asp	Tyr	Pro
145					150					155					160
Lys	Tyr	Gln	Asp	Glu	Ser	Lys	Leu	Asn	Arg	Gln	Glu	Ile	Glu	Ser	Val
				165					170					175	
Lys	Leu	Glu	Ser	Leu	Gly	Val	Tyr	Gln	Ile	Leu	Ala	Ile	Tyr	Ser	Thr
			180					185					190		
Val	Ser	Ser	Ser	Leu	Val	Leu	Val	Gly	Leu	Ile	Ile	Ala	Val	Gly	Leu
		195					200					205			
Trp	Met	Cys	Ser	Asn	Gly	Ser	Met	Gln	Cys	Arg	Ile	Cys	Ile		
	210					215					220				

<210> 72

<211> 221

<212> PRT

<213> influenza A virus

<220>

<223> HA2 domain of HA subtype H7

<400> 72

Gly	Leu	Phe	Gly	Ala	Ile	Ala	Gly	Phe	Ile	Glu	Asn	Gly	Trp	Glu	Gly
1				5					10					15	
Leu	Val	Asp	Gly	Trp	Tyr	Gly	Phe	Arg	His	Gln	Asn	Ala	Gln	Gly	Glu
			20					25					30		
Gly	Thr	Ala	Ala	Asp	Tyr	Lys	Ser	Thr	Gln	Ser	Ala	Ile	Asp	Gln	Ile
		35					40					45			
Thr	Gly	Lys	Leu	Asn	Arg	Leu	Ile	Glu	Lys	Thr	Asn	Gln	Gln	Phe	Glu
	50					55					60				
Leu	Ile	Asp	Asn	Glu	Phe	Thr	Glu	Val	Glu	Lys	Gln	Ile	Gly	Asn	Leu
65					70					75					80
Ile	Asn	Trp	Thr	Lys	Asp	Ser	Ile	Thr	Glu	Val	Trp	Ser	Tyr	Asn	Ala
				85					90					95	
Glu	Leu	Ile	Val	Ala	Met	Glu	Asn	Gln	His	Thr	Ile	Asp	Leu	Ala	Asp
			100					105					110		
Ser	Glu	Met	Asn	Arg	Leu	Tyr	Glu	Arg	Val	Arg	Lys	Gln	Leu	Arg	Glu
		115					120					125			
Asn	Ala	Glu	Glu	Asp	Gly	Thr	Gly	Cys	Phe	Glu	Ile	Phe	His	Lys	Cys
	130					135					140				
Asp	Asp	Asp	Cys	Met	Ala	Ser	Ile	Arg	Asn	Asn	Thr	Tyr	Asp	His	Ser
145					150					155					160
Lys	Tyr	Arg	Glu	Glu	Ala	Met	Gln	Asn	Arg	Ile	Gln	Ile	Asp	Pro	Val
				165					170					175	
Lys	Leu	Ser	Ser	Gly	Tyr	Lys	Asp	Val	Ile	Leu	Trp	Phe	Ser	Phe	Gly
			180					185					190		
Ala	Ser	Cys	Phe	Leu	Leu	Leu	Ala	Ile	Ala	Met	Gly	Leu	Val	Phe	Ile
		195					200					205			
Cys	Val	Lys	Asn	Gly	Asn	Met	Arg	Cys	Thr	Ile	Cys	Ile			
	210					215					220				

<210> 73

<211> 222

<212> PRT

Sequence Listing - 2010234849

<213> influenza A virus

<220>

<223> HA2 domain of HA subtype H8

<400> 73

Gly	Leu	Phe	Gly	Ala	Ile	Ala	Gly	Phe	Ile	Glu	Gly	Gly	Trp	Ser	Gly	1	5	10	15
Met	Ile	Asp	Gly	Trp	Tyr	Gly	Phe	His	His	Ser	Asn	Ser	Glu	Gly	Thr	20	25	30	
Gly	Met	Ala	Ala	Asp	Gln	Lys	Ser	Thr	Gln	Glu	Ala	Ile	Asp	Lys	Ile	35	40	45	
Thr	Asn	Lys	Val	Asn	Asn	Ile	Val	Asp	Lys	Met	Asn	Arg	Glu	Phe	Glu	50	55	60	
Val	Val	Asn	His	Glu	Phe	Ser	Glu	Val	Glu	Lys	Arg	Ile	Asn	Met	Ile	65	70	75	
Asn	Asp	Lys	Ile	Asp	Asp	Gln	Ile	Glu	Asp	Leu	Trp	Ala	Tyr	Asn	Ala	85	90	95	
Glu	Leu	Leu	Val	Leu	Leu	Glu	Asn	Gln	Lys	Thr	Leu	Asp	Glu	His	Asp	100	105	110	
Ser	Asn	Val	Lys	Asn	Leu	Phe	Asp	Glu	Val	Lys	Arg	Arg	Leu	Ser	Ala	115	120	125	
Asn	Ala	Ile	Asp	Ala	Gly	Asn	Gly	Cys	Phe	Asp	Ile	Leu	His	Lys	Cys	130	135	140	
Asp	Asn	Glu	Cys	Met	Glu	Thr	Ile	Lys	Asn	Gly	Thr	Tyr	Asp	His	Lys	145	150	155	
Glu	Tyr	Glu	Glu	Glu	Ala	Lys	Leu	Glu	Arg	Ser	Lys	Ile	Asn	Gly	Val	165	170	175	
Lys	Leu	Glu	Glu	Asn	Thr	Thr	Tyr	Lys	Ile	Leu	Ser	Ile	Tyr	Ser	Thr	180	185	190	
Val	Ala	Ala	Ser	Leu	Cys	Leu	Ala	Ile	Leu	Ile	Ala	Gly	Gly	Leu	Ile	195	200	205	
Leu	Gly	Met	Gln	Asn	Gly	Ser	Cys	Arg	Cys	Met	Phe	Cys	Ile			210	215	220	

<210> 74

<211> 222

<212> PRT

<213> influenza A virus

<220>

<223> HA2 domain of HA subtype H9

<400> 74

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

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Val	Ala	Ser	180	Leu	Val	Leu	Ala	185	Gly	Phe	Ala	Ala	190	Phe	Leu	Phe
Trp	Ala	Met	195	Ser	Asn	Gly	Ser	200	Cys	Arg	Cys	Asn	Ile	205	Cys	Ile
	210						215						220			

<210> 75
 <211> 221
 <212> PRT
 <213> influenza A virus

<220>
 <223> HA2 domain of HA subtype H10

Gly	Leu	Phe	Gly	Ala	Ile	Ala	Gly	Phe	Ile	Glu	Asn	Gly	Trp	Glu	Gly
1			5					10					15		
Met	Val	Asp	Gly	Trp	Tyr	Gly	Phe	Arg	His	Gln	Asn	Ala	Gln	Gly	Thr
		20						25					30		
Gly	Gln	Ala	Ala	Asp	Tyr	Lys	Ser	Thr	Gln	Ala	Ala	Ile	Asp	Gln	Ile
	35						40					45			
Thr	Gly	Lys	Leu	Asn	Arg	Leu	Ile	Glu	Lys	Thr	Asn	Thr	Glu	Phe	Glu
	50					55					60				
Ser	Ile	Glu	Ser	Glu	Phe	Ser	Glu	Thr	Glu	His	Gln	Ile	Gly	Asn	Val
65				70						75				80	
Ile	Asn	Trp	Thr	Lys	Asp	Ser	Ile	Thr	Asp	Ile	Trp	Thr	Tyr	Asn	Ala
			85						90					95	
Glu	Leu	Leu	Val	Ala	Met	Glu	Asn	Gln	His	Thr	Ile	Asp	Met	Ala	Asp
			100					105					110		
Ser	Glu	Met	Leu	Asn	Leu	Tyr	Glu	Arg	Val	Arg	Lys	Gln	Leu	Arg	Gln
		115					120					125			
Asn	Ala	Glu	Glu	Asp	Gly	Lys	Gly	Cys	Phe	Glu	Ile	Tyr	His	Thr	Cys
	130					135					140				
Asp	Asp	Ser	Cys	Met	Glu	Ser	Ile	Arg	Asn	Asn	Thr	Tyr	Asp	His	Ser
145					150					155					160
Gln	Tyr	Arg	Glu	Glu	Ala	Leu	Leu	Asn	Arg	Leu	Asn	Ile	Asn	Pro	Val
			165						170					175	
Lys	Leu	Ser	Ser	Gly	Tyr	Lys	Asp	Ile	Ile	Leu	Trp	Phe	Ser	Phe	Gly
			180						185					190	
Glu	Ser	Cys	Phe	Val	Leu	Leu	Ala	Val	Val	Met	Gly	Leu	Val	Phe	Phe
		195					200					205			
Cys	Leu	Lys	Asn	Gly	Asn	Met	Arg	Cys	Thr	Ile	Cys	Ile			
	210					215					220				

<210> 76
 <211> 223
 <212> PRT
 <213> influenza A virus

<220>
 <223> HA2 domain of HA subtype H11

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

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Ser	Asn	Val	Arg	Asn	Leu	His	Glu	Lys	Val	Arg	Arg	Met	Leu	Lys	Asp
		115					120					125			
Asn	Ala	Lys	Asp	Glu	Gly	Asn	Gly	Cys	Phe	Thr	Phe	Tyr	His	Lys	Cys
	130					135					140				
Asp	Asn	Lys	Cys	Ile	Glu	Arg	Val	Arg	Asn	Gly	Thr	Tyr	Asp	His	Lys
145					150					155					160
Glu	Phe	Glu	Glu	Glu	Ser	Lys	Ile	Asn	Arg	Gln	Glu	Ile	Glu	Gly	Val
			165					170						175	
Lys	Leu	Asp	Ser	Ser	Gly	Asn	Val	Tyr	Lys	Ile	Leu	Ser	Ile	Tyr	Ser
			180					185					190		
Cys	Ile	Ala	Ser	Ser	Leu	Val	Leu	Ala	Ala	Leu	Ile	Met	Gly	Phe	Met
	195						200					205			
Phe	Trp	Ala	Cys	Ser	Asn	Gly	Ser	Cys	Arg	Cys	Thr	Ile	Cys	Ile	
210					215						220				

<210> 77

<211> 222

<212> PRT

<213> influenza A virus

<220>

<223> HA2 domain of HA subtype H12

<400> 77

Gly	Leu	Phe	Gly	Ala	Ile	Ala	Gly	Phe	Ile	Glu	Gly	Gly	Trp	Pro	Gly
1				5				10					15		
Leu	Val	Ala	Gly	Trp	Tyr	Gly	Phe	Gln	His	Gln	Asn	Ala	Glu	Gly	Thr
		20					25					30			
Gly	Ile	Ala	Ala	Asp	Arg	Asp	Ser	Thr	Gln	Arg	Ala	Ile	Asp	Asn	Met
		35				40						45			
Gln	Asn	Lys	Leu	Asn	Asn	Val	Ile	Asp	Lys	Met	Asn	Lys	Gln	Phe	Glu
	50				55					60					
Val	Val	Asn	His	Glu	Phe	Ser	Glu	Val	Glu	Ser	Arg	Ile	Asn	Met	Ile
65				70				75						80	
Asn	Ser	Lys	Ile	Asp	Gln	Ile	Thr	Asp	Ile	Trp	Ala	Tyr	Asn	Ala	
			85					90					95		
Glu	Leu	Leu	Val	Leu	Leu	Glu	Asn	Gln	Lys	Thr	Leu	Asp	Glu	His	Asp
			100					105					110		
Ala	Asn	Val	Arg	Asn	Leu	His	Asp	Arg	Val	Arg	Arg	Val	Leu	Arg	Glu
		115					120					125			
Asn	Ala	Ile	Asp	Thr	Gly	Asp	Gly	Cys	Phe	Glu	Ile	Leu	His	Lys	Cys
	130					135					140				
Asp	Asn	Asn	Cys	Met	Asp	Thr	Ile	Arg	Asn	Gly	Thr	Tyr	Asn	His	Lys
145					150					155					160
Glu	Tyr	Glu	Glu	Glu	Ser	Lys	Ile	Glu	Arg	Gln	Lys	Val	Asn	Gly	Val
			165					170						175	
Lys	Leu	Glu	Glu	Asn	Ser	Thr	Tyr	Lys	Ile	Leu	Ser	Ile	Tyr	Ser	Ser
			180					185					190		
Val	Ala	Ser	Ser	Leu	Val	Leu	Leu	Leu	Met	Ile	Ile	Gly	Gly	Phe	Ile
		195				200						205			
Phe	Gly	Cys	Gln	Asn	Gly	Asn	Val	Arg	Cys	Thr	Phe	Cys	Ile		
210					215						220				

<210> 78

<211> 223

<212> PRT

<213> influenza A virus

<220>

<223> HA2 domain of HA subtype H13

<400> 78

Gly	Leu	Phe	Gly	Ala	Ile	Ala	Gly	Phe	Ile	Glu	Gly	Gly	Trp	Pro	Gly
1				5				10					15		
Leu	Ile	Asn	Gly	Trp	Tyr	Gly	Phe	Gln	His	Gln	Asn	Glu	Gln	Gly	Thr

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			20					25					30		
Gly	Ile	Ala	Ala	Asp	Lys	Glu	Ser	Thr	Gln	Lys	Ala	Ile	Asp	Gln	Ile
		35					40					45			
Thr	Thr	Lys	Ile	Asn	Asn	Ile	Ile	Asp	Lys	Met	Asn	Gly	Asn	Tyr	Asp
	50					55					60				
Ser	Ile	Arg	Gly	Glu	Phe	Asn	Gln	Val	Glu	Lys	Arg	Ile	Asn	Met	Leu
65					70					75					80
Ala	Asp	Arg	Ile	Asp	Asp	Ala	Val	Thr	Asp	Ile	Trp	Ser	Tyr	Asn	Ala
				85					90					95	
Lys	Leu	Leu	Val	Leu	Leu	Glu	Asn	Asp	Lys	Thr	Leu	Asp	Met	His	Asp
			100					105					110		
Ala	Asn	Val	Lys	Asn	Leu	His	Glu	Gln	Val	Arg	Arg	Glu	Leu	Lys	Asp
		115					120					125			
Asn	Ala	Ile	Asp	Glu	Gly	Asn	Gly	Cys	Phe	Glu	Leu	Leu	His	Lys	Cys
	130					135					140				
Asn	Asp	Ser	Cys	Met	Glu	Thr	Ile	Arg	Asn	Gly	Thr	Tyr	Asp	His	Thr
					150					155					160
Glu	Tyr	Ala	Glu	Glu	Ser	Lys	Leu	Lys	Arg	Gln	Glu	Ile	Asp	Gly	Ile
				165					170					175	
Lys	Leu	Lys	Ser	Glu	Asp	Asn	Val	Tyr	Lys	Ala	Leu	Ser	Ile	Tyr	Ser
			180					185					190		
Cys	Ile	Ala	Ser	Ser	Val	Val	Leu	Val	Gly	Leu	Ile	Leu	Ser	Phe	Ile
		195					200					205			
Met	Trp	Ala	Cys	Ser	Ser	Gly	Asn	Cys	Arg	Phe	Asn	Val	Cys	Ile	
	210					215					220				

<210> 79

$\langle 211 \rangle$ 221

<212> PRT

<213> influenza A virus

 $\langle 220 \rangle$

<223> HA2 domain of HA subtype H14

<400> 79

Gly 1	Leu	Phe	Gly	Ala 5	Ile	Ala	Gly	Phe	Ile 10	Glu	Asn	Gly	Trp	Gln 15	Gly
Leu	Ile	Asp	Gly 20	Trp	Tyr	Gly	Phe	Arg 25	His	Gln	Asn	Ala	Glu 30	Gly	Thr
Gly	Thr	Ala 35	Ala	Asp	Leu	Lys	Ser 40	Thr	Gln	Ala	Ala	Ile 45	Asp	Gln	Ile
Asn	Gly 50	Lys	Leu	Asn	Arg	Leu 55	Ile	Glu	Lys	Thr	Asn 60	Glu	Lys	Tyr	His
Gln 65	Ile	Glu	Lys	Glu	Phe 70	Glu	Gln	Val	Glu	Gly 75	Arg	Ile	Gln	Asp	Leu 80
Glu	Lys	Tyr	Val 85	Glu	Asp	Thr	Lys	Ile	Asp 90	Leu	Trp	Ser	Tyr	Asn 95	Ala
Glu	Leu	Leu	Val 100	Ala	Leu	Glu	Asn	Gln 105	His	Thr	Ile	Asp 110	Val	Thr	Asp
Ser	Glu	Met 115	Asn	Lys	Leu	Phe	Glu 120	Arg	Val	Arg	Arg	Gln 125	Leu	Arg	Glu
Asn	Ala 130	Glu	Asp	Gln	Gly	Asn 135	Gly	Cys	Phe	Glu	Ile 140	Phe	His	Gln	Cys
Asp 145	Asn	Asn	Cys	Ile	Glu 150	Ser	Ile	Arg	Asn	Gly 155	Thr	Tyr	Asp	His	Asn 160
Ile	Tyr	Arg	Asp 165	Glu	Ala	Ile	Asn	Asn	Arg 170	Ile	Lys	Ile	Asn	Pro 175	Val
Thr	Leu	Thr	Met 180	Gly	Tyr	Lys	Asp	Ile 185	Ile	Leu	Trp	Ile	Ser 190	Phe	Ser
Met	Ser	Cys 195	Phe	Val	Phe	Val	Ala 200	Leu	Ile	Leu	Gly	Phe 205	Val	Leu	Trp
Ala	Cys 210	Gln	Asn	Gly	Asn	Ile 215	Arg	Cys	Gln	Ile	Cys 220	Ile			

$\langle 210 \rangle$ 80

$\langle 211 \rangle$ 221

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<212> PRT

<213> influenza A virus

<220>

<223> HA2 domain of HA subtype H15

<400> 80

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Gly Leu Phe Gly Ala Ile Ala Gly Phe Ile Glu Asn Gly Trp Glu Gly
 1      5      10      15
Leu Ile Asp Gly Trp Tyr Gly Phe Arg His Gln Asn Ala Gln Gly Gln
      20      25      30
Gly Thr Ala Ala Asp Tyr Lys Ser Thr Gln Ala Ala Ile Asp Gln Ile
      35      40      45
Thr Gly Lys Leu Asn Arg Leu Ile Glu Lys Thr Asn Lys Gln Phe Glu
      50      55      60
Leu Ile Asp Asn Glu Phe Thr Glu Val Glu Gln Gln Ile Gly Asn Val
      65      70      75      80
Ile Asn Trp Thr Arg Asp Ser Leu Thr Glu Ile Trp Ser Tyr Asn Ala
      85      90      95
Glu Leu Leu Val Ala Met Glu Asn Gln His Thr Ile Asp Leu Ala Asp
      100      105      110
Ser Glu Met Asn Lys Leu Tyr Glu Arg Val Arg Arg Gln Leu Arg Glu
      115      120      125
Asn Ala Glu Glu Asp Gly Thr Gly Cys Phe Glu Ile Phe His Arg Cys
      130      135      140
Asp Asp Gln Cys Met Glu Ser Ile Arg Asn Asn Thr Tyr Asn His Thr
      145      150      155      160
Glu Tyr Arg Gln Glu Ala Leu Gln Asn Arg Ile Met Ile Asn Pro Val
      165      170      175
Lys Leu Ser Ser Gly Tyr Lys Asp Val Ile Leu Trp Phe Ser Phe Gly
      180      185      190
Ala Ser Cys Val Met Leu Leu Ala Ile Ala Met Gly Leu Ile Phe Met
      195      200      205
Cys Val Lys Asn Gly Asn Leu Arg Cys Thr Ile Cys Ile
      210      215      220

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<210> 81

<211> 223

<212> PRT

<213> influenza A virus

<220>

<223> HA2 domain of HA subtype H16

<400> 81

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

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Lys Leu Lys Thr Glu Asp Asn Val Tyr Lys Val Leu Ser Ile Tyr Ser
 180 185 190
 Cys Ile Ala Ser Ser Ile Val Leu Val Gly Leu Ile Leu Ala Phe Ile
 195 200 205
 Met Trp Ala Cys Ser Asn Gly Ser Cys Arg Phe Asn Val Cys Ile
 210 215 220

<210> 82
 <211> 180
 <212> PRT
 <213> influenza A virus

<220>
 <223> Stem domain of HA2 domain subtype H1

<400> 82
 Gly Leu Phe Gly Ala Ile Ala Gly Phe Ile Glu Gly Gly Trp Thr Gly
 1 5 10 15
 Met Ile Asp Gly Trp Tyr Gly Tyr His His Gln Asn Glu Gln Gly Ser
 20 25 30
 Gly Tyr Ala Ala Asp Gln Lys Ser Thr Gln Asn Ala Ile Asn Gly Ile
 35 40 45
 Thr Asn Lys Val Asn Thr Val Ile Glu Lys Met Asn Ile Gln Phe Thr
 50 55 60
 Ala Val Gly Lys Glu Phe Asn Lys Leu Glu Lys Arg Met Glu Asn Leu
 65 70 75 80
 Asn Lys Lys Val Asp Asp Gly Phe Leu Asp Ile Trp Thr Tyr Asn Ala
 85 90 95
 Glu Leu Leu Val Leu Leu Glu Asn Glu Arg Thr Leu Asp Phe His Asp
 100 105 110
 Ser Asn Val Lys Asn Leu Tyr Glu Lys Val Lys Ser Gln Leu Lys Asn
 115 120 125
 Asn Ala Lys Glu Ile Gly Asn Gly Cys Phe Glu Phe Tyr His Lys Cys
 130 135 140
 Asp Asn Glu Cys Met Glu Ser Val Arg Asn Gly Thr Tyr Asp Tyr Pro
 145 150 155 160
 Lys Tyr Ser Glu Glu Ser Lys Leu Asn Arg Glu Lys Val Asp Gly Val
 165 170 175
 Lys Leu Glu Ser
 180

<210> 83
 <211> 180
 <212> PRT
 <213> influenza A virus

<220>
 <223> Stem domain of HA2 domain subtype H2

<400> 83
 Gly Leu Phe Gly Ala Ile Ala Gly Phe Ile Glu Gly Gly Trp Gln Gly
 1 5 10 15
 Met Ile Asp Gly Trp Tyr Gly Tyr His His Ser Asn Asp Gln Gly Ser
 20 25 30
 Gly Tyr Ala Ala Asp Lys Glu Ser Thr Gln Lys Ala Ile Asp Gly Ile
 35 40 45
 Thr Asn Arg Val Asn Ser Val Ile Glu Lys Met Asn Thr Gln Phe Glu
 50 55 60
 Ala Val Gly Lys Glu Phe Ser Asn Leu Glu Lys Arg Leu Glu Asn Leu
 65 70 75 80
 Asn Lys Lys Met Glu Asp Gly Phe Leu Asp Val Trp Thr Tyr Asn Ala
 85 90 95
 Glu Leu Leu Val Leu Met Glu Asn Glu Arg Thr Leu Asp Phe His Asp
 100 105 110
 Ser Asn Val Lys Asn Leu Tyr Asp Arg Val Arg Met Gln Leu Arg Asp
 115 120 125

Sequence Listing - 2010234849

Asn	Ala	Lys	Glu	Leu	Gly	Asn	Gly	Cys	Phe	Glu	Phe	Tyr	His	Lys	Cys
130						135				140					
Asp	Asp	Glu	Cys	Met	Asn	Ser	Val	Lys	Asn	Gly	Thr	Tyr	Asp	Tyr	Pro
145					150					155					160
Lys	Tyr	Glu	Glu	Glu	Ser	Lys	Leu	Asn	Arg	Asn	Glu	Ile	Lys	Gly	Val
				165					170					175	
Lys	Leu	Ser	Asn												
			180												

<210> 84
 <211> 179
 <212> PRT
 <213> influenza A virus

<220>
 <223> Stem domain of HA2 domain subtype H3

<400>	84														
Gly	Leu	Phe	Gly	Ala	Ile	Ala	Gly	Phe	Ile	Glu	Asn	Gly	Trp	Glu	Gly
1				5				10						15	
Met	Ile	Asp	Gly	Trp	Tyr	Gly	Phe	Arg	His	Gln	Asn	Ser	Glu	Gly	Thr
			20					25					30		
Gly	Gln	Ala	Ala	Asp	Leu	Lys	Ser	Thr	Gln	Ala	Ala	Ile	Asp	Gln	Ile
		35					40					45			
Asn	Gly	Lys	Leu	Asn	Arg	Val	Ile	Glu	Lys	Thr	Asn	Glu	Lys	Phe	His
50						55					60				
Gln	Ile	Glu	Lys	Glu	Phe	Ser	Glu	Val	Glu	Gly	Arg	Ile	Gln	Asp	Leu
65					70					75				80	
Glu	Lys	Tyr	Val	Glu	Asp	Thr	Lys	Ile	Asp	Leu	Trp	Ser	Tyr	Asn	Ala
			85						90					95	
Glu	Leu	Leu	Val	Ala	Leu	Glu	Asn	Gln	His	Thr	Ile	Asp	Leu	Thr	Asp
			100					105					110		
Ser	Glu	Met	Asn	Lys	Leu	Phe	Glu	Lys	Thr	Arg	Arg	Gln	Leu	Arg	Glu
		115					120					125			
Asn	Ala	Glu	Asp	Met	Gly	Asn	Gly	Cys	Phe	Lys	Ile	Tyr	His	Lys	Cys
130						135					140				
Asp	Asn	Ala	Cys	Ile	Glu	Ser	Ile	Arg	Asn	Gly	Thr	Tyr	Asp	His	Asp
145					150					155					160
Val	Tyr	Arg	Asp	Glu	Ala	Leu	Asn	Asn	Arg	Phe	Gln	Ile	Lys	Gly	Val
				165					170					175	
Glu	Leu	Lys													

<210> 85
 <211> 179
 <212> PRT
 <213> influenza A virus

<220>
 <223> Stem domain of HA2 domain subtype H4

<400>	85														
Gly	Leu	Phe	Gly	Ala	Ile	Ala	Gly	Phe	Ile	Glu	Asn	Gly	Trp	Gln	Gly
1				5				10						15	
Leu	Ile	Asp	Gly	Trp	Tyr	Gly	Phe	Arg	His	Gln	Asn	Ala	Glu	Gly	Thr
			20					25					30		
Gly	Thr	Ala	Ala	Asp	Leu	Lys	Ser	Thr	Gln	Ala	Ala	Ile	Asp	Gln	Ile
		35					40					45			
Asn	Gly	Lys	Leu	Asn	Arg	Leu	Ile	Glu	Lys	Thr	Asn	Asp	Lys	Tyr	His
50						55					60				
Gln	Ile	Glu	Lys	Glu	Phe	Glu	Gln	Val	Glu	Gly	Arg	Ile	Gln	Asp	Leu
65					70					75				80	
Glu	Asn	Tyr	Val	Glu	Asp	Thr	Lys	Ile	Asp	Leu	Trp	Ser	Tyr	Asn	Ala
			85						90					95	
Glu	Leu	Leu	Val	Ala	Leu	Glu	Asn	Gln	His	Thr	Ile	Asp	Val	Thr	Asp
			100					105					110		

Sequence Listing - 2010234849

Ser Glu Met Asn Lys Leu Phe Glu Arg Val Arg Arg Gln Leu Arg Glu
115 120 125
Asn Ala Glu Asp Lys Gly Asn Gly Cys Phe Glu Ile Phe His Lys Cys
130 135 140
Asp Asn Asn Cys Ile Glu Ser Ile Arg Asn Gly Thr Tyr Asp His Asp
145 150 155 160
Ile Tyr Arg Asp Glu Ala Ile Asn Asn Arg Phe Gln Ile Gln Gly Val
165 170 175
Lys Leu Thr

<210> 86
<211> 180
<212> PRT
<213> influenza A virus

<220>
<223> Stem domain of HA2 domain subtype H5

<400> 86
Gly Leu Phe Gly Ala Ile Ala Gly Phe Ile Glu Gly Gly Trp Gln Gly
1 5 10 15
Met Val Asp Gly Trp Tyr Gly Tyr His His Ser Asn Glu Gln Gly Ser
20 25 30
Gly Tyr Ala Ala Asp Lys Glu Ser Thr Gln Lys Ala Ile Asp Gly Ile
35 40 45
Thr Asn Lys Val Asn Ser Ile Ile Asp Lys Met Asn Thr Arg Phe Glu
50 55 60
Ala Val Gly Lys Glu Phe Asn Asn Leu Glu Arg Arg Val Glu Asn Leu
65 70 75 80
Asn Lys Lys Met Glu Asp Gly Phe Leu Asp Val Trp Thr Tyr Asn Val
85 90 95
Glu Leu Leu Val Leu Met Glu Asn Glu Arg Thr Leu Asp Phe His Asp
100 105 110
Ser Asn Val Asn Asn Leu Tyr Asp Lys Val Arg Leu Gln Leu Lys Asp
115 120 125
Asn Ala Arg Glu Leu Gly Asn Gly Cys Phe Glu Phe Tyr His Lys Cys
130 135 140
Asp Asn Glu Cys Met Glu Ser Val Arg Asn Gly Thr Tyr Asp Tyr Pro
145 150 155 160
Gln Tyr Ser Glu Glu Ala Arg Leu Asn Arg Glu Glu Ile Ser Gly Val
165 170 175
Lys Leu Glu Ser
180

<210> 87
<211> 180
<212> PRT
<213> influenza A virus

<220>
<223> Stem domain of HA2 domain subtype H6

<400> 87
Gly Leu Phe Gly Ala Ile Ala Gly Phe Ile Glu Gly Gly Trp Thr Gly
1 5 10 15
Met Ile Asp Gly Trp Tyr Gly Tyr His His Glu Asn Ser Gln Gly Ser
20 25 30
Gly Tyr Ala Ala Asp Arg Glu Ser Thr Gln Lys Ala Val Asp Gly Ile
35 40 45
Thr Asn Lys Val Asn Ser Ile Ile Asp Lys Met Asn Thr Gln Phe Glu
50 55 60
Ala Val Asp His Glu Phe Ser Asn Leu Glu Arg Arg Ile Asp Asn Leu
65 70 75 80
Asn Lys Arg Met Glu Asp Gly Phe Leu Asp Val Trp Thr Tyr Asn Ala
85 90 95

Sequence Listing - 2010234849

```

Glu Leu Leu Val Leu Leu Glu Asn Glu Arg Thr Leu Asp Leu His Asp
100 105 110
Ala Asn Val Lys Asn Leu Tyr Glu Arg Val Lys Ser Gln Leu Arg Asp
115 120 125
Asn Ala Met Ile Leu Gly Asn Gly Cys Phe Glu Phe Trp His Lys Cys
130 135 140
Asp Asp Glu Cys Met Glu Ser Val Lys Asn Gly Thr Tyr Asp Tyr Pro
145 150 155 160
Lys Tyr Gln Asp Glu Ser Lys Leu Asn Arg Gln Glu Ile Glu Ser Val
165 170 175
Lys Leu Glu Ser
180

```

<210> 88
 <211> 179
 <212> PRT
 <213> influenza A virus

<220>
 <223> Stem domain of HA2 domain subtype H7

```

<400> 88
Gly Leu Phe Gly Ala Ile Ala Gly Phe Ile Glu Asn Gly Trp Glu Gly
1 5 10 15
Leu Val Asp Gly Trp Tyr Gly Phe Arg His Gln Asn Ala Gln Gly Glu
20 25 30
Gly Thr Ala Ala Asp Tyr Lys Ser Thr Gln Ser Ala Ile Asp Gln Ile
35 40 45
Thr Gly Lys Leu Asn Arg Leu Ile Glu Lys Thr Asn Gln Gln Phe Glu
50 55 60
Leu Ile Asp Asn Glu Phe Thr Glu Val Glu Lys Gln Ile Gly Asn Leu
65 70 75 80
Ile Asn Trp Thr Lys Asp Ser Ile Thr Glu Val Trp Ser Tyr Asn Ala
85 90 95
Glu Leu Ile Val Ala Met Glu Asn Gln His Thr Ile Asp Leu Ala Asp
100 105 110
Ser Glu Met Asn Arg Leu Tyr Glu Arg Val Arg Lys Gln Leu Arg Glu
115 120 125
Asn Ala Glu Glu Asp Gly Thr Gly Cys Phe Glu Ile Phe His Lys Cys
130 135 140
Asp Asp Asp Cys Met Ala Ser Ile Arg Asn Asn Thr Tyr Asp His Ser
145 150 155 160
Lys Tyr Arg Glu Glu Ala Met Gln Asn Arg Ile Gln Ile Asp Pro Val
165 170 175
Lys Leu Ser

```

<210> 89
 <211> 180
 <212> PRT
 <213> influenza A virus

<220>
 <223> Stem domain of HA2 domain subtype H8

```

<400> 89
Gly Leu Phe Gly Ala Ile Ala Gly Phe Ile Glu Gly Gly Trp Ser Gly
1 5 10 15
Met Ile Asp Gly Trp Tyr Gly Phe His Ser Asn Ser Glu Gly Thr
20 25 30
Gly Met Ala Ala Asp Gln Lys Ser Thr Gln Glu Ala Ile Asp Lys Ile
35 40 45
Thr Asn Lys Val Asn Asn Ile Val Asp Lys Met Asn Arg Glu Phe Glu
50 55 60
Val Val Asn His Glu Phe Ser Glu Val Glu Lys Arg Ile Asn Met Ile
65 70 75 80

```

Sequence Listing - 2010234849

```

Asn Asp Lys Ile Asp Asp Gln Ile Glu Asp Leu Trp Ala Tyr Asn Ala
85 90
Glu Leu Leu Val Leu Leu Glu Asn Gln Lys Thr Leu Asp Glu His Asp
100 105 110
Ser Asn Val Lys Asn Leu Phe Asp Glu Val Lys Arg Arg Leu Ser Ala
115 120 125
Asn Ala Ile Asp Ala Gly Asn Gly Cys Phe Asp Ile Leu His Lys Cys
130 135 140
Asp Asn Glu Cys Met Glu Thr Ile Lys Asn Gly Thr Tyr Asp His Lys
145 150 155 160
Glu Tyr Glu Glu Glu Ala Lys Leu Glu Arg Ser Lys Ile Asn Gly Val
165 170 175
Lys Leu Glu Glu
180

```

<210> 90
 <211> 180
 <212> PRT
 <213> influenza A virus

<220>
 <223> Stem domain of HA2 domain subtype H9

```

<400> 90
Gly Leu Phe Gly Ala Ile Ala Gly Phe Ile Glu Gly Gly Trp Pro Gly
1 5 10 15
Leu Val Ala Gly Trp Tyr Gly Phe Gln His Ser Asn Asp Gln Gly Val
20 25 30
Gly Met Ala Ala Asp Lys Gly Ser Thr Gln Lys Ala Ile Asp Lys Ile
35 40 45
Thr Ser Lys Val Asn Asn Ile Ile Asp Lys Met Asn Lys Gln Tyr Glu
50 55 60
Val Ile Asp His Glu Phe Asn Glu Leu Glu Ala Arg Leu Asn Met Ile
65 70 75 80
Asn Asn Lys Ile Asp Asp Gln Ile Gln Asp Ile Trp Ala Tyr Asn Ala
85 90 95
Glu Leu Leu Val Leu Leu Glu Asn Gln Lys Thr Leu Asp Glu His Asp
100 105 110
Ala Asn Val Asn Asn Leu Tyr Asn Lys Val Lys Arg Ala Leu Gly Ser
115 120 125
Asn Ala Val Glu Asp Gly Asn Gly Cys Phe Glu Leu Tyr His Lys Cys
130 135 140
Asp Asp Gln Cys Met Glu Thr Ile Arg Asn Gly Thr Tyr Asp Arg Gln
145 150 155 160
Lys Tyr Gln Glu Glu Ser Arg Leu Glu Arg Gln Lys Ile Glu Gly Val
165 170 175
Lys Leu Glu Ser
180

```

<210> 91
 <211> 179
 <212> PRT
 <213> influenza A virus

<220>
 <223> Stem domain of HA2 domain subtype H10

```

<400> 91
Gly Leu Phe Gly Ala Ile Ala Gly Phe Ile Glu Asn Gly Trp Glu Gly
1 5 10 15
Met Val Asp Gly Trp Tyr Gly Phe Arg His Gln Asn Ala Gln Gly Thr
20 25 30
Gly Gln Ala Ala Asp Tyr Lys Ser Thr Gln Ala Ala Ile Asp Gln Ile
35 40 45
Thr Gly Lys Leu Asn Arg Leu Ile Glu Lys Thr Asn Thr Glu Phe Glu
50 55 60

```

Sequence Listing - 2010234849

```

Ser Ile Glu Ser Glu Phe Ser Glu Thr Glu His Gln Ile Gly Asn Val
65      70      75
Ile Asn Trp Thr Lys Asp Ser Ile Thr Asp Ile Trp Thr Tyr Asn Ala
85      90      95
Glu Leu Leu Val Ala Met Glu Asn Gln His Thr Ile Asp Met Ala Asp
100     105     110
Ser Glu Met Leu Asn Leu Tyr Glu Arg Val Arg Lys Gln Leu Arg Gln
115     120     125
Asn Ala Glu Glu Asp Gly Lys Gly Cys Phe Glu Ile Tyr His Thr Cys
130     135     140
Asp Asp Ser Cys Met Glu Ser Ile Arg Asn Asn Thr Tyr Asp His Ser
145     150     155     160
Gln Tyr Arg Glu Glu Ala Leu Leu Asn Arg Leu Asn Ile Asn Pro Val
165     170     175
Lys Leu Ser

```

<210> 92
 <211> 181
 <212> PRT
 <213> influenza A virus

<220>
 <223> Stem domain of HA2 domain subtype H11

```

<400> 92
Gly Leu Phe Gly Ala Ile Ala Gly Phe Ile Glu Gly Gly Trp Pro Gly
1      5      10      15
Leu Ile Asn Gly Trp Tyr Gly Phe Gln His Arg Asp Glu Gly Thr
20     25     30
Gly Ile Ala Ala Asp Lys Glu Ser Thr Gln Lys Ala Ile Asp Gln Ile
35     40     45
Thr Ser Lys Val Asn Asn Ile Val Asp Arg Met Asn Thr Asn Phe Glu
50     55     60
Ser Val Gln His Glu Phe Ser Glu Ile Glu Glu Arg Ile Asn Gln Leu
65     70     75     80
Ser Lys His Val Asp Asp Ser Val Val Asp Ile Trp Ser Tyr Asn Ala
85     90     95
Gln Leu Leu Val Leu Leu Glu Asn Glu Lys Thr Leu Asp Leu His Asp
100    105    110
Ser Asn Val Arg Asn Leu His Glu Lys Val Arg Arg Met Leu Lys Asp
115    120    125
Asn Ala Lys Asp Glu Gly Asn Gly Cys Phe Thr Phe Tyr His Lys Cys
130    135    140
Asp Asn Lys Cys Ile Glu Arg Val Arg Asn Gly Thr Tyr Asp His Lys
145    150    155    160
Glu Phe Glu Glu Glu Ser Lys Ile Asn Arg Gln Glu Ile Glu Gly Val
165    170    175
Lys Leu Asp Ser Ser
180

```

<210> 93
 <211> 180
 <212> PRT
 <213> influenza A virus

<220>
 <223> Stem domain of HA2 domain subtype H12

```

<400> 93
Gly Leu Phe Gly Ala Ile Ala Gly Phe Ile Glu Gly Gly Trp Pro Gly
1      5      10      15
Leu Val Ala Gly Trp Tyr Gly Phe Gln His Gln Asn Ala Glu Gly Thr
20     25     30
Gly Ile Ala Ala Asp Arg Asp Ser Thr Gln Arg Ala Ile Asp Asn Met
35     40     45

```

Sequence Listing - 2010234849

```

Gln Asn Lys Leu Asn Asn Val Ile Asp Lys Met Asn Lys Gln Phe Glu
 50 55 60
Val Val Asn His Glu Phe Ser Glu Val Glu Ser Arg Ile Asn Met Ile
65 70 75 80
Asn Ser Lys Ile Asp Asp Gln Ile Thr Asp Ile Trp Ala Tyr Asn Ala
 85 90 95
Glu Leu Leu Val Leu Leu Glu Asn Gln Lys Thr Leu Asp Glu His Asp
100 105 110
Ala Asn Val Arg Asn Leu His Asp Arg Val Arg Arg Val Leu Arg Glu
115 120 125
Asn Ala Ile Asp Thr Gly Asp Gly Cys Phe Glu Ile Leu His Lys Cys
130 135 140
Asp Asn Asn Cys Met Asp Thr Ile Arg Asn Gly Thr Tyr Asn His Lys
145 150 155 160
Glu Tyr Glu Glu Glu Ser Lys Ile Glu Arg Gln Lys Val Asn Gly Val
165 170 175
Lys Leu Glu Glu
180

```

<210> 94
 <211> 181
 <212> PRT
 <213> influenza A virus

<220>
 <223> Stem domain of HA2 domain subtype H13

```

<400> 94
Gly Leu Phe Gly Ala Ile Ala Gly Phe Ile Glu Gly Gly Trp Pro Gly
 1 5 10 15
Leu Ile Asn Gly Trp Tyr Gly Phe Gln His Gln Asn Glu Gln Gly Thr
20 25 30
Gly Ile Ala Ala Asp Lys Glu Ser Thr Gln Lys Ala Ile Asp Gln Ile
35 40 45
Thr Thr Lys Ile Asn Asn Ile Asp Lys Met Asn Gly Asn Tyr Asp
50 55 60
Ser Ile Arg Gly Glu Phe Asn Gln Val Glu Lys Arg Ile Asn Met Leu
65 70 75 80
Ala Asp Arg Ile Asp Asp Ala Val Thr Asp Ile Trp Ser Tyr Asn Ala
85 90 95
Lys Leu Leu Val Leu Leu Glu Asn Asp Lys Thr Leu Asp Met His Asp
100 105 110
Ala Asn Val Lys Asn Leu His Glu Gln Val Arg Arg Glu Leu Lys Asp
115 120 125
Asn Ala Ile Asp Glu Gly Asn Gly Cys Phe Glu Leu Leu His Lys Cys
130 135 140
Asn Asp Ser Cys Met Glu Thr Ile Arg Asn Gly Thr Tyr Asp His Thr
145 150 155 160
Glu Tyr Ala Glu Glu Ser Lys Leu Lys Arg Gln Glu Ile Asp Gly Ile
165 170 175
Lys Leu Lys Ser Glu
180

```

<210> 95
 <211> 179
 <212> PRT
 <213> influenza A virus

<220>
 <223> Stem domain of HA2 domain subtype H14

```

<400> 95
Gly Leu Phe Gly Ala Ile Ala Gly Phe Ile Glu Asn Gly Trp Gln Gly
 1 5 10 15
Leu Ile Asp Gly Trp Tyr Gly Phe Arg His Gln Asn Ala Glu Gly Thr
20 25 30

```


Sequence Listing - 2010234849

```

Gly Thr Ala Ala Asp Leu Lys Ser Thr Gln Ala Ala Ile Asp Gln Ile
   35         40         45
Asn Gly Lys Leu Asn Arg Leu Ile Glu Lys Thr Asn Glu Lys Tyr His
   50         55         60
Gln Ile Glu Lys Glu Phe Glu Gln Val Glu Gly Arg Ile Gln Asp Leu
   65         70         75
Glu Lys Tyr Val Glu Asp Thr Lys Ile Asp Leu Trp Ser Tyr Asn Ala
   85         90         95
Glu Leu Leu Val Ala Leu Glu Asn Gln His Thr Ile Asp Val Thr Asp
  100        105        110
Ser Glu Met Asn Lys Leu Phe Glu Arg Val Arg Arg Gln Leu Arg Glu
  115        120        125
Asn Ala Glu Asp Gln Gly Asn Gly Cys Phe Glu Ile Phe His Gln Cys
  130        135        140
Asp Asn Asn Cys Ile Glu Ser Ile Arg Asn Gly Thr Tyr Asp His Asn
  145        150        155        160
Ile Tyr Arg Asp Glu Ala Ile Asn Asn Arg Ile Lys Ile Asn Pro Val
  165        170        175
Thr Leu Thr

```

<210> 96
 <211> 179
 <212> PRT
 <213> influenza A virus

<220>
 <223> Stem domain of HA2 domain subtype H15

```

<400> 96
Gly Leu Phe Gly Ala Ile Ala Gly Phe Ile Glu Asn Gly Trp Glu Gly
  1         5         10        15
Leu Ile Asp Gly Trp Tyr Gly Phe Arg His Gln Asn Ala Gln Gly Gln
  20        25        30
Gly Thr Ala Ala Asp Tyr Lys Ser Thr Gln Ala Ala Ile Asp Gln Ile
  35        40        45
Thr Gly Lys Leu Asn Arg Leu Ile Glu Lys Thr Asn Lys Gln Phe Glu
  50        55        60
Leu Ile Asp Asn Glu Phe Thr Glu Val Glu Gln Gln Ile Gly Asn Val
  65        70        75        80
Ile Asn Trp Thr Arg Asp Ser Leu Thr Glu Ile Trp Ser Tyr Asn Ala
  85        90        95
Glu Leu Leu Val Ala Met Glu Asn Gln His Thr Ile Asp Leu Ala Asp
  100       105       110
Ser Glu Met Asn Lys Leu Tyr Glu Arg Val Arg Arg Gln Leu Arg Glu
  115       120       125
Asn Ala Glu Glu Asp Gly Thr Gly Cys Phe Glu Ile Phe His Arg Cys
  130       135       140
Asp Asp Gln Cys Met Glu Ser Ile Arg Asn Asn Thr Tyr Asn His Thr
  145       150       155       160
Glu Tyr Arg Gln Glu Ala Leu Gln Asn Arg Ile Met Ile Asn Pro Val
  165       170       175
Lys Leu Ser

```

<210> 97
 <211> 181
 <212> PRT
 <213> influenza A virus

<220>
 <223> Stem domain of HA2 domain subtype H16

```

<400> 97
Gly Leu Phe Gly Ala Ile Ala Gly Phe Ile Glu Gly Gly Trp Pro Gly
  1         5         10        15

```

Sequence Listing - 2010234849

```

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

```

<210> 98
 <211> 5
 <212> PRT
 <213> influenza A virus

<220>
 <223> Luminal domain of HA2 domain subtype H1

<400> 98
 Met Gly Ile Tyr Gln
 1 5

<210> 99
 <211> 5
 <212> PRT
 <213> influenza A virus

<220>
 <223> Luminal domain of HA2 domain subtype H2

<400> 99
 Met Gly Val Tyr Gln
 1 5

<210> 100
 <211> 5
 <212> PRT
 <213> influenza A virus

<220>
 <223> Luminal domain of HA2 domain subtype H3

<400> 100
 Ser Gly Tyr Lys Asp
 1 5

<210> 101
 <211> 5
 <212> PRT
 <213> influenza A virus

Sequence Listing - 2010234849

<220>
<223> Luminal domain of HA2 domain subtype H4

<400> 101
Gln Gly Tyr Lys Asp
1 5

<210> 102
<211> 5
<212> PRT
<213> influenza A virus

<220>
<223> Luminal domain of HA2 domain subtype H5

<400> 102
Met Gly Val Tyr Gln
1 5

<210> 103
<211> 5
<212> PRT
<213> influenza A virus

<220>
<223> Luminal domain of HA2 domain subtype H6

<400> 103
Leu Gly Val Tyr Gln
1 5

<210> 104
<211> 5
<212> PRT
<213> influenza A virus

<220>
<223> Luminal domain of HA2 domain subtype H7

<400> 104
Ser Gly Tyr Lys Asp
1 5

<210> 105
<211> 5
<212> PRT
<213> influenza A virus

<220>
<223> Luminal domain of HA2 domain subtype H8

<400> 105
Asn Thr Thr Tyr Lys
1 5

<210> 106
<211> 5
<212> PRT
<213> influenza A virus

<220>
<223> Luminal domain of HA2 domain subtype H9

Sequence Listing - 2010234849

<400> 106
Glu Gly Thr Tyr Lys
1 5

<210> 107
<211> 5
<212> PRT
<213> influenza A virus

<220>
<223> Luminal domain of HA2 domain subtype H10

<400> 107
Ser Gly Tyr Lys Asp
1 5

<210> 108
<211> 5
<212> PRT
<213> influenza A virus

<220>
<223> Luminal domain of HA2 domain subtype H11

<400> 108
Gly Asn Val Tyr Lys
1 5

<210> 109
<211> 5
<212> PRT
<213> influenza A virus

<220>
<223> Luminal domain of HA2 domain subtype H12

<400> 109
Asn Ser Thr Tyr Lys
1 5

<210> 110
<211> 5
<212> PRT
<213> influenza A virus

<220>
<223> Luminal domain of HA2 domain subtype H13

<400> 110
Asp Asn Val Tyr Lys
1 5

<210> 111
<211> 5
<212> PRT
<213> influenza A virus

<220>
<223> Luminal domain of HA2 domain subtype H14

<400> 111
Met Gly Tyr Lys Asp
1 5

Sequence Listing - 2010234849

<210> 112
 <211> 5
 <212> PRT
 <213> influenza A virus

<220>
 <223> Luminal domain of HA2 domain subtype H15

<400> 112
 Ser Gly Tyr Lys Asp
 1 5

<210> 113
 <211> 5
 <212> PRT
 <213> influenza A virus

<220>
 <223> Luminal domain of HA2 domain subtype H16

<400> 113
 Asp Asn Val Tyr Lys
 1 5

<210> 114
 <211> 27
 <212> PRT
 <213> influenza A virus

<220>
 <223> Transmembrane domain of HA2 domain subtype H1

<400> 114
 Ile Leu Ala Ile Tyr Ser Thr Val Ala Ser Ser Leu Val Leu Leu Val
 1 5 10 15
 Ser Leu Gly Ala Ile Ser Phe Trp Met Cys Ser
 20 25

<210> 115
 <211> 27
 <212> PRT
 <213> influenza A virus

<220>
 <223> Transmembrane domain of HA2 domain subtype H2

<400> 115
 Ile Leu Ala Ile Tyr Ala Thr Val Ala Gly Ser Leu Ser Leu Ala Ile
 1 5 10 15
 Met Ile Ala Gly Ile Ser Leu Trp Met Cys Ser
 20 25

<210> 116
 <211> 27
 <212> PRT
 <213> influenza A virus

<220>
 <223> Transmembrane domain of HA2 domain subtype H3

<400> 116
 Trp Ile Leu Trp Ile Ser Phe Ala Ile Ser Cys Phe Leu Leu Cys Val

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```

1           5           10           15
Val Leu Leu Gly Phe Ile Met Trp Ala Cys Gln
      20           25

```

<210> 117
 <211> 27
 <212> PRT
 <213> influenza A virus

<220>
 <223> Transmembrane domain of HA2 domain subtype H4

```

<400> 117
Ile Ile Leu Trp Ile Ser Phe Ser Ile Ser Cys Phe Leu Leu Val Ala
1           5           10           15
Leu Leu Leu Ala Phe Ile Leu Trp Ala Cys Gln
      20           25

```

<210> 118
 <211> 27
 <212> PRT
 <213> influenza A virus

<220>
 <223> Transmembrane domain of HA2 domain subtype H5

```

<400> 118
Ile Leu Ser Ile Tyr Ser Thr Val Ala Ser Ser Leu Ala Leu Ala Ile
1           5           10           15
Met Ile Ala Gly Leu Ser Phe Trp Met Cys Ser
      20           25

```

<210> 119
 <211> 27
 <212> PRT
 <213> influenza A virus

<220>
 <223> Transmembrane domain of HA2 domain subtype H6

```

<400> 119
Ile Leu Ala Ile Tyr Ser Thr Val Ser Ser Ser Leu Val Leu Val Gly
1           5           10           15
Leu Ile Ile Ala Val Gly Leu Trp Met Cys Ser
      20           25

```

<210> 120
 <211> 27
 <212> PRT
 <213> influenza A virus

<220>
 <223> Transmembrane domain of HA2 domain subtype H7

```

<400> 120
Val Ile Leu Trp Phe Ser Phe Gly Ala Ser Cys Phe Leu Leu Leu Ala
1           5           10           15
Ile Ala Met Gly Leu Val Phe Ile Cys Val Lys
      20           25

```

<210> 121
 <211> 27
 <212> PRT

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<213> influenza A virus

<220>

<223> Transmembrane domain of HA2 domain subtype H8

<400> 121

Ile	Leu	Ser	Ile	Tyr	Ser	Thr	Val	Ala	Ala	Ser	Leu	Cys	Leu	Ala	Ile
1				5				10						15	
Leu	Ile	Ala	Gly	Gly	Leu	Ile	Leu	Gly	Met	Gln					
			20					25							

<210> 122

<211> 27

<212> PRT

<213> influenza A virus

<220>

<223> Transmembrane domain of HA2 domain subtype H9

<400> 122

Ile	Leu	Thr	Ile	Tyr	Ser	Thr	Val	Ala	Ser	Ser	Leu	Val	Leu	Ala	Met
1				5				10						15	
Gly	Phe	Ala	Ala	Phe	Leu	Phe	Trp	Ala	Met	Ser					
			20					25							

<210> 123

<211> 27

<212> PRT

<213> influenza A virus

<220>

<223> Transmembrane domain of HA2 domain subtype H10

<400> 123

Ile	Ile	Leu	Trp	Phe	Ser	Phe	Gly	Glu	Ser	Cys	Phe	Val	Leu	Leu	Ala
1				5				10						15	
Val	Val	Met	Gly	Leu	Val	Phe	Phe	Cys	Leu	Lys					
			20					25							

<210> 124

<211> 27

<212> PRT

<213> influenza A virus

<220>

<223> Transmembrane domain of HA2 domain subtype H11

<400> 124

Ile	Leu	Ser	Ile	Tyr	Ser	Cys	Ile	Ala	Ser	Ser	Leu	Val	Leu	Ala	Ala
1				5				10						15	
Leu	Ile	Met	Gly	Phe	Met	Phe	Trp	Ala	Cys	Ser					
			20					25							

<210> 125

<211> 28

<212> PRT

<213> influenza A virus

<220>

<223> Transmembrane domain of HA2 domain subtype H12

<400> 125

Ile	Leu	Ser	Ile	Tyr	Ser	Ser	Val	Ala	Ser	Ser	Leu	Val	Leu	Leu	Leu
1				5				10						15	

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Met Ile Ile Gly Gly Phe Ile Phe Gly Cys Gln Asn
 20 25

<210> 126
 <211> 28
 <212> PRT
 <213> influenza A virus

<220>
 <223> Transmembrane domain of HA2 domain subtype H13

<400> 126
 Ala Leu Ser Ile Tyr Ser Cys Ile Ala Ser Ser Val Val Leu Val Gly
 1 5 10 15
 Leu Ile Leu Ser Phe Ile Met Trp Ala Cys Ser Ser
 20 25

<210> 127
 <211> 27
 <212> PRT
 <213> influenza A virus

<220>
 <223> Transmembrane domain of HA2 domain subtype H14

<400> 127
 Ile Ile Leu Trp Ile Ser Phe Ser Met Ser Cys Phe Val Phe Val Ala
 1 5 10 15
 Leu Ile Leu Gly Phe Val Leu Trp Ala Cys Gln
 20 25

<210> 128
 <211> 28
 <212> PRT
 <213> influenza A virus

<220>
 <223> Transmembrane domain of HA2 domain subtype H15

<400> 128
 Val Ile Leu Trp Phe Ser Phe Gly Ala Ser Cys Val Met Leu Leu Ala
 1 5 10 15
 Ile Ala Met Gly Leu Ile Phe Met Cys Val Lys Asn
 20 25

<210> 129
 <211> 27
 <212> PRT
 <213> influenza A virus

<220>
 <223> Transmembrane domain of HA2 domain subtype H16

<400> 129
 Val Leu Ser Ile Tyr Ser Cys Ile Ala Ser Ser Ile Val Leu Val Gly
 1 5 10 15
 Leu Ile Leu Ala Phe Ile Met Trp Ala Cys Ser
 20 25

<210> 130
 <211> 10
 <212> PRT
 <213> influenza A virus

Sequence Listing - 2010234849

<220>

<223> Cytoplasmic domain of HA2 domain subtype H1

<400> 130

Asn Gly Ser Leu Gln Cys Arg Ile Cys Ile
1 5 10

<210> 131

<211> 10

<212> PRT

<213> influenza A virus

<220>

<223> Cytoplasmic domain of HA2 domain subtype H2

<400> 131

Asn Gly Ser Leu Gln Cys Arg Ile Cys Ile
1 5 10

<210> 132

<211> 10

<212> PRT

<213> influenza A virus

<220>

<223> Cytoplasmic domain of HA2 domain subtype H3

<400> 132

Arg Gly Asn Ile Arg Cys Asn Ile Cys Ile
1 5 10

<210> 133

<211> 10

<212> PRT

<213> influenza A virus

<220>

<223> Cytoplasmic domain of HA2 domain subtype H4

<400> 133

Asn Gly Asn Ile Arg Cys Gln Ile Cys Ile
1 5 10

<210> 134

<211> 10

<212> PRT

<213> influenza A virus

<220>

<223> Cytoplasmic domain of HA2 domain subtype H5

<400> 134

Asn Gly Ser Leu Gln Cys Arg Ile Cys Ile
1 5 10

<210> 135

<211> 10

<212> PRT

<213> influenza A virus

<220>

<223> Cytoplasmic domain of HA2 domain subtype H6

Sequence Listing - 2010234849

<400> 135

Asn Gly Ser Met Gln Cys Arg Ile Cys Ile
1 5 10

<210> 136

<211> 10

<212> PRT

<213> influenza A virus

<220>

<223> Cytoplasmic domain of HA2 domain subtype H7

<400> 136

Asn Gly Asn Met Arg Cys Thr Ile Cys Ile
1 5 10

<210> 137

<211> 10

<212> PRT

<213> influenza A virus

<220>

<223> Cytoplasmic domain of HA2 domain subtype H8

<400> 137

Asn Gly Ser Cys Arg Cys Met Phe Cys Ile
1 5 10

<210> 138

<211> 10

<212> PRT

<213> influenza A virus

<220>

<223> Cytoplasmic domain of HA2 domain subtype H9

<400> 138

Asn Gly Ser Cys Arg Cys Asn Ile Cys Ile
1 5 10

<210> 139

<211> 10

<212> PRT

<213> influenza A virus

<220>

<223> Cytoplasmic domain of HA2 domain subtype H10

<400> 139

Asn Gly Asn Met Arg Cys Thr Ile Cys Ile
1 5 10

<210> 140

<211> 10

<212> PRT

<213> influenza A virus

<220>

<223> Cytoplasmic domain of HA2 domain subtype H11

<400> 140

Asn Gly Ser Cys Arg Cys Thr Ile Cys Ile
1 5 10

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<210> 141
 <211> 9
 <212> PRT
 <213> influenza A virus

<220>
 <223> Cytoplasmic domain of HA2 domain subtype H12

<400> 141
 Gly Asn Val Arg Cys Thr Phe Cys Ile
 1 5

<210> 142
 <211> 9
 <212> PRT
 <213> influenza A virus

<220>
 <223> Cytoplasmic domain of HA2 domain subtype H13

<400> 142
 Gly Asn Cys Arg Phe Asn Val Cys Ile
 1 5

<210> 143
 <211> 10
 <212> PRT
 <213> influenza A virus

<220>
 <223> Cytoplasmic domain of HA2 domain subtype H14

<400> 143
 Asn Gly Asn Ile Arg Cys Gln Ile Cys Ile
 1 5 10

<210> 144
 <211> 9
 <212> PRT
 <213> influenza A virus

<220>
 <223> Cytoplasmic domain of HA2 domain subtype H15

<400> 144
 Gly Asn Leu Arg Cys Thr Ile Cys Ile
 1 5

<210> 145
 <211> 10
 <212> PRT
 <213> influenza A virus

<220>
 <223> Cytoplasmic domain of HA2 domain subtype H16

<400> 145
 Asn Gly Ser Cys Arg Phe Asn Val Cys Ile
 1 5 10

<210> 146

Sequence Listing - 2010234849

```

<211> 23
<212> PRT
<213> Artificial Sequence

<220>
<223> HA1 N-terminal Stem Segment

<220>
<221> VARIANT
<222> 1
<223> Xaa = Tyr or His

<220>
<221> PEPTIDE
<222> 1
<223> Xaa is position 17 of HA1 N-terminal stem segment

<220>
<221> VARIANT
<222> 2
<223> Xaa = His, Leu, or Gln

<220>
<221> PEPTIDE
<222> 2
<223> Xaa is position 18 of HA1 N-terminal stem segment

<220>
<221> VARIANT
<222> (3)...(20)
<223> Xaa = any amino acid

<220>
<221> VARIANT
<222> (21)...(21)
<223> Xaa = any amino acid or absent

<220>
<221> VARIANT
<222> (22)...(22)
<223> Xaa = any amino acid or absent

<220>
<221> VARIANT
<222> (23)...(23)
<223> Xaa = His, Ser, Gln, Thr or Asn

<400> 146
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
 1           5           10          15
Xaa Xaa Xaa Xaa Xaa Xaa Xaa
      20

<210> 147
<211> 2
<212> PRT
<213> Artificial Sequence

<220>
<223> HA1 C-terminal stem segment

<220>
<221> VARIANT
<222> 1
<223> Xaa = Thr, Ser, Asn, Asp. Pro or Lys

```

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<220>
 <221> VARIANT
 <222> 1
 <223> position 291 or HA1 C-terminal stem segment

<220>
 <221> VARIANT
 <222> 2
 <223> Xaa = Leu, Met, Lys or Arg

<220>
 <221> VARIANT
 <222> 2
 <223> position 292 or HA1 C-terminal stem segment

<220>
 <221> VARIANT
 <222> 1, 2
 <223> Xaa = Any Amino Acid

<400> 147
 Xaa Xaa
 1

<210> 148
 <211> 4
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> HA2 domain

<220>
 <221> PEPTIDE
 <222> (1)...(4)
 <223> position 18 to 21 of HA2 domain

<220>
 <221> VARIANT
 <222> 1
 <223> Xaa = Val or Ile

<220>
 <221> VARIANT
 <222> 2
 <223> Xaa = Asp, Asn or Ala

<220>
 <221> VARIANT
 <222> 1, 2
 <223> Xaa = Any Amino Acid

<400> 148
 Xaa Xaa Gly Trp
 1

<210> 149
 <211> 19
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> HA2 domain

<220>
 <221> PEPTIDE

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```

<222> (1)...(19)
<223> position 38 to 56 of HA2 domain

<220>
<221> VARIANT
<222> 1
<223> Xaa = Lys, Gln, Arg, Leu or Tyr

<220>
<221> VARIANT
<222> 11
<223> Xaa = Ile, Val or Met

<220>
<221> VARIANT
<222> 12
<223> Xaa - Thr, Gln or Asn

<220>
<221> VARIANT
<222> 15
<223> Xaa = Val or Leu

<220>
<221> VARIANT
<222> (18)...(18)
<223> Xaa = Val, Ile or Leu

<220>
<221> VARIANT
<222> (19)...(19)
<223> Xaa = Val or Ile

<220>
<221> VARIANT
<222> 2, 3, 6, 10, 13, 17
<223> Xaa = any amino acid

<400> 149
Xaa Xaa Xaa Thr Gln Xaa Ala Ile Asp Xaa Xaa Xaa Xaa Lys Xaa Asn
 1             5             10             15
Xaa Xaa Xaa

```

```

<210> 150
<211> 15
<212> PRT
<213> influenza B virus

```

```

<220>
<223> Signal peptide of HA construct variant

```

```

<400> 150
Met Lys Ala Ile Ile Val Ile Leu Met Val Val Thr Ser Asn Ala
 1             5             10             15

```

```

<210> 151
<211> 15
<212> PRT
<213> influenza B virus

```

```

<220>
<223> Signal peptide of HA construct variant

```

```

<400> 151
Met Lys Ala Ile Ile Val Ile Leu Met Val Val Thr Ser Asn Ala

```

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```

1              5              10              15

<210> 152
<211> 15
<212> PRT
<213> influenza B virus

<220>
<223> Signal peptide of HA construct variant

<400> 152
Met Lys Ala Ile Ile Val Ile Leu Met Val Val Thr Ser Asn Ala
 1              5              10              15

<210> 153
<211> 15
<212> PRT
<213> influenza B virus

<220>
<223> Signal peptide of HA construct variant

<400> 153
Met Lys Ala Ile Ile Val Ile Leu Met Val Val Thr Ser Asn Ala
 1              5              10              15

<210> 154
<211> 50
<212> PRT
<213> influenza B virus

<220>
<223> HA1 N-terminal stem segment of HA construct variant

<400> 154
Asp Arg Ile Cys Thr Gly Ile Thr Ser Ser Asn Ser Pro His Val Val
 1              5              10              15
Lys Thr Ala Thr Gln Gly Glu Val Asn Val Thr Gly Val Ile Pro Leu
              20              25              30
Thr Thr Thr Pro Thr Lys Ser His Phe Ala Asn Leu Lys Gly Thr Glu
              35              40              45
Thr Arg
 50

<210> 155
<211> 66
<212> PRT
<213> influenza B virus

<220>
<223> HA1 N-terminal stem segment of HA construct variant

<400> 155
Asp Arg Ile Cys Thr Gly Ile Thr Ser Ser Asn Ser Pro His Val Val
 1              5              10              15
Lys Thr Ala Thr Gln Gly Glu Val Asn Val Thr Gly Val Ile Pro Leu
              20              25              30
Thr Thr Thr Pro Thr Lys Ser His Phe Ala Asn Leu Lys Gly Thr Glu
              35              40              45
Thr Arg Gly Lys Leu Cys Pro Lys Cys Leu Asn Cys Thr Asp Leu Asp
              50              55              60
Val Ala
65

```

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<210> 156
 <211> 80
 <212> PRT
 <213> influenza B virus

<220>
 <223> HA1 N-terminal stem segment of HA construct variant

```
<400> 156
Asp Arg Ile Cys Thr Gly Ile Thr Ser Ser Asn Ser Pro His Val Val
 1          5          10          15
Lys Thr Ala Thr Gln Gly Glu Val Asn Val Thr Gly Val Ile Pro Leu
          20          25          30
Thr Thr Thr Pro Thr Lys Ser His Phe Ala Asn Leu Lys Gly Thr Glu
          35          40          45
Thr Arg Gly Lys Leu Cys Pro Lys Cys Leu Asn Cys Thr Asp Leu Asp
          50          55          60
Val Ala Leu Gly Arg Pro Lys Cys Thr Gly Lys Ile Pro Ser Ala Lys
65          70          75          80
```

<210> 157
 <211> 80
 <212> PRT
 <213> influenza B virus

<220>
 <223> HA1 N-terminal stem segment of HA construct variant

```
<400> 157
Asp Arg Ile Cys Thr Gly Ile Thr Ser Ser Asn Ser Pro His Val Val
 1          5          10          15
Lys Thr Ala Thr Gln Gly Glu Val Asn Val Thr Gly Val Ile Pro Leu
          20          25          30
Thr Thr Thr Pro Thr Lys Ser His Phe Ala Asn Leu Lys Gly Thr Glu
          35          40          45
Thr Arg Gly Lys Leu Cys Pro Lys Cys Leu Asn Cys Thr Asp Leu Asp
          50          55          60
Val Ala Leu Gly Arg Pro Lys Cys Thr Gly Lys Ile Pro Ser Ala Arg
65          70          75          80
```

<210> 158
 <211> 68
 <212> PRT
 <213> influenza B virus

<220>
 <223> HA1 C-terminal stem segment of HA construct variant

```
<400> 158
Ser Lys Val Ile Lys Gly Ser Leu Pro Leu Ile Gly Glu Ala Asp Cys
 1          5          10          15
Leu His Glu Lys Tyr Gly Gly Leu Asn Lys Ser Lys Pro Tyr Tyr Thr
          20          25          30
Gly Glu His Ala Lys Ala Ile Gly Asn Cys Pro Ile Trp Val Lys Thr
          35          40          45
Pro Leu Lys Leu Ala Asn Gly Thr Lys Tyr Arg Pro Pro Ala Lys Leu
          50          55          60
Leu Lys Glu Arg
65
```

<210> 159
 <211> 74
 <212> PRT
 <213> influenza B virus

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<220>

<223> HA1 C-terminal stem segment of HA construct variant

<400> 159

```

Trp Cys Ala Ser Gly Arg Ser Lys Val Ile Lys Gly Ser Leu Pro Leu
 1      5      10     15
Ile Gly Glu Ala Asp Cys Leu His Glu Lys Tyr Gly Gly Leu Asn Lys
 20     25     30
Ser Lys Pro Tyr Tyr Thr Gly Glu His Ala Lys Ala Ile Gly Asn Cys
 35     40     45
Pro Ile Trp Val Lys Thr Pro Leu Lys Leu Ala Asn Gly Thr Lys Tyr
 50     55     60
Arg Pro Pro Ala Lys Leu Leu Lys Glu Arg
65      70

```

<210> 160

<211> 223

<212> PRT

<213> influenza B virus

<220>

<223> HA2 domain of HA construct variant

<400> 160

```

Gly Phe Phe Gly Ala Ile Ala Gly Phe Leu Glu Gly Gly Trp Glu Gly
 1      5      10     15
Met Ile Ala Gly Trp His Gly Tyr Thr Ser His Gly Ala His Gly Val
 20     25     30
Ala Val Ala Ala Asp Leu Lys Ser Gln Glu Ala Ile Asn Lys Ile
 35     40     45
Thr Lys Asn Leu Asn Ser Leu Ser Glu Leu Glu Val Lys Asn Leu Gln
 50     55     60
Arg Leu Ser Gly Ala Met Asp Glu Leu His Asn Glu Ile Leu Glu Leu
65      70     75     80
Asp Glu Lys Val Asp Asp Leu Arg Ala Asp Thr Ile Ser Ser Gln Ile
 85     90     95
Glu Leu Ala Val Leu Leu Ser Asn Glu Gly Ile Ile Asn Ser Glu Asp
100    105    110
Glu His Leu Leu Ala Leu Glu Arg Lys Leu Lys Lys Met Leu Gly Pro
115    120    125
Ser Ala Val Glu Ile Gly Asn Gly Cys Phe Glu Thr Lys His Lys Cys
130    135    140
Asn Gln Thr Cys Leu Asp Arg Ile Ala Ala Gly Thr Phe Asp Ala Gly
145    150    155    160
Glu Phe Ser Leu Pro Thr Phe Asp Ser Leu Asn Ile Thr Ala Ala Ser
165    170    175
Leu Asn Asp Asp Gly Leu Asp Asn His Thr Ile Leu Leu Tyr Tyr Ser
180    185    190
Thr Ala Ala Ser Ser Leu Ala Val Thr Leu Met Ile Ala Ile Phe Val
195    200    205
Val Tyr Met Val Ser Arg Asp Asn Val Ser Cys Ser Ile Cys Leu
210    215    220

```

<210> 161

<211> 179

<212> PRT

<213> influenza B virus

<220>

<223> stem domain of HA2 domain subtype HA2

<400> 161

```

Gly Phe Phe Gly Ala Ile Ala Gly Phe Leu Glu Gly Gly Trp Glu Gly
 1      5      10     15
Met Ile Ala Gly Trp His Gly Tyr Thr Ser His Gly Ala His Gly Val

```

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```

      20      25      30
Ala Val Ala Asp Leu Lys Ser Thr Gln Glu Ala Ile Asn Lys Ile
      35      40      45
Thr Lys Asn Leu Asn Ser Leu Ser Glu Leu Glu Val Lys Asn Leu Gln
      50      55      60
Arg Leu Ser Gly Ala Met Asp Glu Leu His Asn Glu Ile Leu Glu Leu
      65      70      75
Asp Glu Lys Val Asp Asp Leu Arg Ala Asp Thr Ile Ser Ser Gln Ile
      80      85      90
Glu Leu Ala Val Leu Leu Ser Asn Glu Gly Ile Ile Asn Ser Glu Asp
      95     100     105
Glu His Leu Leu Ala Leu Glu Arg Lys Leu Lys Lys Met Leu Gly Pro
     110     115     120
Ser Ala Val Glu Ile Gly Asn Gly Cys Phe Glu Thr Lys His Lys Cys
     125     130     135
Asn Gln Thr Cys Leu Asp Arg Ile Ala Ala Gly Thr Phe Asp Ala Gly
     140     145     150
Glu Phe Ser Leu Pro Thr Phe Asp Ser Leu Asn Ile Thr Ala Ala Ser
     155     160     165
Leu Asn Asp

```

<210> 162
 <211> 5
 <212> PRT
 <213> influenza B virus

<220>
 <223> Luminal domain of HA2 domain subtype HA2

<400> 162
 Asp Gly Leu Asp Asn
 1 5

<210> 163
 <211> 28
 <212> PRT
 <213> influenza B virus

<220>
 <223> transmembrane domain of HA2 domain subtype HA2

```

<400> 163
His Thr Ile Leu Leu Tyr Tyr Ser Thr Ala Ala Ser Ser Leu Ala Val
  1      5      10      15
Thr Leu Met Ile Ala Ile Phe Val Val Tyr Met Val
      20      25

```

<210> 164
 <211> 11
 <212> PRT
 <213> influenza B virus

<220>
 <223> cytoplasmic domain of HA2 domain subtype HA2

<400> 164
 Ser Arg Asp Asn Val Ser Cys Ser Ile Cys Leu
 1 5 10

<210> 165
 <211> 23
 <212> PRT
 <213> Artificial Sequence

Sequence Listing - 2010234849

<220>

<223> linker sequence

<400> 165

Ile Thr Pro Asn Gly Ser Ile Pro Asn Asp Lys Pro Phe Gln Asn Val
 1 5 10 15
 Asn Lys Ile Thr Tyr Gly Ala
 20

<210> 166

<211> 6

<212> PRT

<213> Artificial Sequence

<220>

<223> His tag

<400> 166

His His His His His His
 1 5

<210> 167

<211> 29

<212> PRT

<213> Artificial Sequence

<220>

<223> foldon domain

<400> 167

Gly Ser Gly Tyr Ile Pro Glu Ala Pro Arg Asp Gly Gln Ala Tyr Val
 1 5 10 15
 Arg Lys Asp Gly Glu Trp Val Leu Leu Ser Thr Phe Leu
 20 25

<210> 168

<211> 7

<212> PRT

<213> Artificial Sequence

<220>

<223> thrombin cleavage site

<400> 168

Leu Val Pro Arg Gly Ser Pro
 1 5

<210> 169

<211> 1088

<212> DNA

<213> Artificial Sequence

<220>

<223> construct for expressing an influenza HA stem domain (fig.6)

<400> 169

agcaaaagca ggggaaaata aaaacaacca aaatgaaggc aaacctactg gtcctgttaa 60
 gtgcacttgc agctgcagat gcagacacaa tatgtatagg ctaccatgcg aacaattcaa 120
 ccgacactgt tgacacagta ctcgagaaga atgtgacagt gacacactct gttaacctgc 180
 tcgaagacag ccacaacgga aaactatgtg gaggctgtaa cacgaagtgt caaacacccc 240
 tgggagctat aaacagcagt ctcccttacc agaataataccc cccagtcaca ataggagagt 300
 gcccaaaaata cgtcaggagt gccaaattga ggatgggttac aggactaagg aacactccgt 360
 ccattcaatc cagaggtcta tttggagcca ttgccggttt tattgaaggg ggatggactg 420

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```

gaatgataga tggatggtat gggtatcatc atcagaatga acagggatca ggctatgcag 480
cggatcaaaa aagcacacaa aatgccatta acgggattac aaacaagggtg aacactgtta 540
tcgagaaaaa gaacattcaa ttcacagctg tgggtaaaaga attcaacaaa ttagaaaaaa 600
ggatggaaaaa tttaaataaa aaagtgtgat atggatttct ggacatttgg acatataatg 660
cagaattgtt agttctactg gaaaatgaaa ggactctgga tttccatgac tcaaattgtga 720
agaatctgta tgagaaagta aaaagccaat taaagaataa tgccaaagaa atcggaaatg 780
gatgttttga gttctaccac aagtgtgaca atgaatgcat ggaaagtgtg agaaatggga 840
cttatgatta tcccaaatat tcagaagagt caaagttgaa cagggaaaag gtagatggag 900
tgaaattgga atcaatgggg atctatcaga ttctggcgat ctactcaact gtcgccagtt 960
cactgggtgct tttgggtctcc ctgggggcaa tcagtttctg gatgtgttct aatggatctt 1020
tgcagtgcag aatatgcatc tgagattaga atttcagaaa tatgaggaaa aacacccttg 1080
tttctact

```

<210> 170

<211> 336

<212> PRT

<213> Artificial Sequence

<220>

<223> construct for expressing an influenza HA stem domain (fig.6)

<400> 170

```

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

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<210> 171

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<211> 1094

<212> DNA

<213> Artificial Sequence

<220>

<223> construct for expressing an influenza HA stem domain (fig.7)

<400> 171

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agcaaaagca ggggaaaata aaaacaacca aaatgaaggc aaacctactg gtcctgttaa 60
gtgcacttgc agctgcagat gcagacacaa tatgtatagg ctaccatgcg aacaattcaa 120
ccgacactgt tgacacagta ctcgagaaga atgtgacagt gacacactct gttaacctgc 180
tcgaagacag ccacaacgga aaactatgtg gaggcggagg ttgtaacacg aagtgtcaaa 240
cacccttggg agctataaac agcagtcctcc cttaccagaa tatacaccca gtcacaatag 300
gagagtgtcc aaaatacgtc aggagtgtcc aattgaggat gggtacagga ctaaggaaca 360
ctccgtccat tcaatccaga ggtctatttg gagccattgc cggttttatt gaagggggat 420
ggactggaat gatagatgga tggatgtggt atcatcatca gaatgaacag ggatcaggct 480
atgcagcggg tcaaaaaagc acacaaaatg ccattaacgg gattacaaac aaggtgaaca 540
ctgttatcga gaaaatgaac attcaattca cagctgtggg taaagaattc aacaaattag 600
aaaaaaggat ggaaaattta aataaaaaag ttgatgatgg atttctggac atttggacat 660
ataatgcaga attgttagtt ctactggaaa atgaaaggac tctggatttc catgactcaa 720
atgtgaagaa tctgtatgag aaagtataaa gccaatataa gaataatgcc aaagaaatcg 780
gaaatggatg ttttgagttc taccacaagt gtgacaatga atgcatggaa agtgaagaa 840
atgggactta tgattatccc aaatattcag aagagtcaaa gttgaacagg gaaaaggtag 900
atggagttaa attggaatca atggggatct atcagattct ggcgatctac tcaactgtcg 960
ccagttcact ggtgcttttg gtctccctgg gggcaatcag tttctggatg tgttctaatt 1020
gatctttgca gtgcagaata tgcattctgag attagaattt cagaaatatg aggaaaaaca 1080
cccttgtttc tact                                     1094

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<210> 172

<211> 338

<212> PRT

<213> Artificial Sequence

<220>

<223> construct for expressing an influenza HA stem domain (fig.7)

<400> 172

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Met Lys Ala Asn Leu Leu Val Leu Leu Ser Ala Leu Ala Ala Ala Asp
 1          5          10          15
Ala Asp Thr Ile Cys Ile Gly Tyr His Ala Asn Asn Ser Thr Asp Thr
 20          25          30
Val Asp Thr Val Leu Glu Lys Asn Val Thr Val Thr His Ser Val Asn
 35          40          45
Leu Leu Glu Asp Ser His Asn Gly Lys Leu Cys Gly Gly Gly Gly Cys
 50          55          60
Asn Thr Lys Cys Gln Thr Pro Leu Gly Ala Ile Asn Ser Ser Leu Pro
 65          70          75          80
Tyr Gln Asn Ile His Pro Val Thr Ile Gly Glu Cys Pro Lys Tyr Val
 85          90          95
Arg Ser Ala Lys Leu Arg Met Val Thr Gly Leu Arg Asn Thr Pro Ser
100          105          110          115
Ile Gln Ser Arg Gly Leu Phe Gly Ala Ile Ala Gly Phe Ile Glu Gly
120          125
Gly Trp Thr Gly Met Ile Asp Gly Trp Tyr Gly Tyr His His Gln Asn
130          135          140
Glu Gln Gly Ser Gly Tyr Ala Ala Asp Gln Lys Ser Thr Gln Asn Ala
145          150          155          160
Ile Asn Gly Ile Thr Asn Lys Val Asn Thr Val Ile Glu Lys Met Asn
165          170          175
Ile Gln Phe Thr Ala Val Gly Lys Glu Phe Asn Lys Leu Glu Lys Arg
180          185          190
Met Glu Asn Leu Asn Lys Lys Val Asp Asp Gly Phe Leu Asp Ile Trp
195          200          205
Thr Tyr Asn Ala Glu Leu Leu Val Leu Leu Glu Asn Glu Arg Thr Leu
210          215          220
Asp Phe His Asp Ser Asn Val Lys Asn Leu Tyr Glu Lys Val Lys Ser
225          230          235          240
Gln Leu Lys Asn Asn Ala Lys Glu Ile Gly Asn Gly Cys Phe Glu Phe

```

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Tyr His Lys Cys 245 Asp Asn Glu Cys Met 250 Glu Ser Val Arg Asn 255 Gly Thr
 Tyr Asp Tyr Pro 260 Lys Tyr Ser Glu Glu Ser Lys Leu Asn Arg 270 Glu Lys
 Val Asp Gly Val Lys Leu Glu Ser Met Gly Ile Tyr Gln Ile Leu Ala
 Ile Tyr Ser Thr Val Ala Ser Ser Leu Val Leu Val Ser Leu Gly
 305 Ala Ile Ser Phe Trp 310 Met Cys Ser Asn Gly Ser Leu Gln Cys Arg Ile
 Cys Ile 325 330 335

<210> 173
 <211> 1088
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> construct for expressing an influenza HA stem domain (fig.8)

<400> 173
 agcaaaagca ggggaaaata aaaacaacca aaatgaaggc aaacctactg gtcctgttaa 60
 gtgcacttgc agctgcagat gcagacacaa tatgtatagg ctaccatgcg aacaattcaa 120
 ccgacactgt tgacacagta ctcgagaaga atgtgacagt gacacactct gttaacctgc 180
 tcgaagacag ccacaacgga aaactatgtc caggctgtaa cacgaagtgt caaacacccc 240
 tgggagctat aaacagcagt ctccccttacc agaataataca cccagtcaca ataggagagt 300
 gcccaaaata cgtcaggagt gccaaattga ggatggttac aggactaagg aacactccgt 360
 ccattcaatc cagaggtcta tttggagcca ttgccggttt tattgaaggg ggatggactg 420
 gaatgataga tggatgggat gggtatcatc atcagaatga acagggatca ggctatgcag 480
 cggtatcaaaa aagcacacaa aatgccatta acgggattac aaacaagggtg aacactgtta 540
 tcgagaaaaat gaacattcaa ttcacagctg tgggtaaaga attcaacaaa ttagaaaaaa 600
 ggatggaaaaa tttaaataaa aaagttgatg atggatttct ggacatttgg acatataatg 660
 cagaattgtt agttctactg gaaaatgaaa ggactctgga tttccatgac tcaaattgtga 720
 agaattctgta tgagaaagta aaaagccaat taaagaataa tgccaaagaa atcggaaatg 780
 gatgttttga gttctaccac aagtgtgaca atgaatgcat ggaaagtgtg agaaatggga 840
 cttatgatta tcccaaatat tcagaagagt caaagttgaa cagggaaaag gtagatggag 900
 tgaaattgga atcaatgggg atctatcaga ttctggcgat ctactcaact gtcgccagtt 960
 cactggtgct tttggtctcc ctgggggcaa tcagtttctg gatgtgttct aatggatctt 1020
 tgcagtgcag aatatgcac tgcagattaga atttcagaaa tatgaggaaa aacacccttg 1080
 tttctact 1088

<210> 174
 <211> 336
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> construct for expressing an influenza HA stem domain (fig.8)

<400> 174
 Met Lys Ala Asn Leu Leu Val Leu Leu Ser Ala Leu Ala Ala Ala Asp
 1 5 10 15
 Ala Asp Thr Ile Cys Ile Gly Tyr His Ala Asn Asn Ser Thr Asp Thr
 20 25 30
 Val Asp Thr Val Leu Glu Lys Asn Val Thr Val Thr His Ser Val Asn
 35 40 45
 Leu Leu Glu Asp Ser His Asn Gly Lys Leu Cys Pro Gly Cys Asn Thr
 50 55 60
 Lys Cys Gln Thr Pro Leu Gly Ala Ile Asn Ser Ser Leu Pro Tyr Gln
 65 70 75 80
 Asn Ile His Pro Val Thr Ile Gly Glu Cys Pro Lys Tyr Val Arg Ser
 85 90 95
 Ala Lys Leu Arg Met Val Thr Gly Leu Arg Asn Thr Pro Ser Ile Gln
 100 105 110
 Ser Arg Gly Leu Phe Gly Ala Ile Ala Gly Phe Ile Glu Gly Gly Trp

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		115				120				125					
Thr	Gly	Met	Ile	Asp	Gly	Trp	Tyr	Gly	Tyr	His	His	Gln	Asn	Glu	Gln
	130					135					140				
Gly	Ser	Gly	Tyr	Ala	Ala	Asp	Gln	Lys	Ser	Thr	Gln	Asn	Ala	Ile	Asn
145					150					155					160
Gly	Ile	Thr	Asn	Lys	Val	Asn	Thr	Val	Ile	Glu	Lys	Met	Asn	Ile	Gln
				165					170					175	
Phe	Thr	Ala	Val	Gly	Lys	Glu	Phe	Asn	Lys	Leu	Glu	Lys	Arg	Met	Glu
			180					185					190		
Asn	Leu	Asn	Lys	Lys	Val	Asp	Asp	Gly	Phe	Leu	Asp	Ile	Trp	Thr	Tyr
		195				200					205				
Asn	Ala	Glu	Leu	Leu	Val	Leu	Leu	Glu	Asn	Glu	Arg	Thr	Leu	Asp	Phe
210						215					220				
His	Asp	Ser	Asn	Val	Lys	Asn	Leu	Tyr	Glu	Lys	Val	Lys	Ser	Gln	Leu
225					230					235					240
Lys	Asn	Asn	Ala	Lys	Glu	Ile	Gly	Asn	Gly	Cys	Phe	Glu	Phe	Tyr	His
				245					250					255	
Lys	Cys	Asp	Asn	Glu	Cys	Met	Glu	Ser	Val	Arg	Asn	Gly	Thr	Tyr	Asp
			260					265					270		
Tyr	Pro	Lys	Tyr	Ser	Glu	Glu	Ser	Lys	Leu	Asn	Arg	Glu	Lys	Val	Asp
		275					280					285			
Gly	Val	Lys	Leu	Glu	Ser	Met	Gly	Ile	Tyr	Gln	Ile	Leu	Ala	Ile	Tyr
	290					295					300				
Ser	Thr	Val	Ala	Ser	Ser	Leu	Val	Leu	Leu	Val	Ser	Leu	Gly	Ala	Ile
305					310					315					320
Ser	Phe	Trp	Met	Cys	Ser	Asn	Gly	Ser	Leu	Gln	Cys	Arg	Ile	Cys	Ile
			325						330					335	

<210> 175

<211> 963

<212> DNA

<213> Artificial Sequence

<220>

<223> construct for expressing an influenza HA stem domain (fig.9)

<400> 175

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atggacacaa tatgtatagg ctaccatgcg aacaattcaa cgcacactgt tgacacagta 60
ctcgagaaga atgtgacagt gacacactct gttaacctgc tcgaagacag ccacaacgga 120
aaactatgtg gaggcggagg ttgtaacacg aagtgtcaaa caccctggg agctataaac 180
agcagtcctc cttaccagaa tatacaccca gtcacaatag gagagtgcc aaaatacgtc 240
aggagtgcga aattgaggat ggttacagga ctaaggaaca ctccgtccat tcaatccaga 300
ggtctatttg gagccattgc cggttttatt gaagggggat ggactggaat gatagatgga 360
tggtatgggt atcatcatca gaatgaacag ggatcaggct atgcagcgg tcaaaaaagc 420
acacaaaatg ccattaacgg gattacaaac aaggtgaaca ctgttatcga gaaaatgaac 480
attcaattca cagctgtggg taaagaattc aacaaattag aaaaaaggat ggaaaattta 540
aataaaaaag ttgatgatgg atttctggac atttggacat ataatgcaga attgttagtt 600
ctactggaaa atgaaaggac tctggatttc catgactcaa atgtgaagaa tctgtatgag 660
aaagtataaaa gccaatataa gaataatgcc aaagaaatcg gaaatggatg ttttgagttc 720
taccacaagt gtgacaatga atgcatggaa agtgaagaa atgggactta tgattatccc 780
aaatattcag aagagtcaaa gttgaacagg gaaaaggtag atggagtgcg ttctctgggt 840
ccgcgtggtt ctccgggttc tggttacatc ccggaagctc cgcgtgacgg tcaggcttac 900
gttcgtaaag acggtgaatg ggttctgctg tctaccttc tgcaccacca ccaccaccac 960
tga

```

<210> 176

<211> 320

<212> PRT

<213> Artificial Sequence

<220>

<223> construct for expressing an influenza HA stem domain (fig.9)

<400> 176

Met	Asp	Thr	Ile	Cys	Ile	Gly	Tyr	His	Ala	Asn	Asn	Ser	Thr	Asp	Thr
1				5					10					15	
Val	Asp	Thr	Val	Leu	Glu	Lys	Asn	Val	Thr	Val	Thr	His	Ser	Val	Asn

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```

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

```

<210> 177

<211> 41

<212> PRT

<213> Artificial Sequence

<220>

<223> HA1 N-terminal stem segment of HA subtype H1

<400> 177

```

Asp Thr Ile Cys Ile Gly Tyr His Ala Asn Asn Ser Thr Asp Thr Val
 1      5      10      15
Asp Thr Val Leu Glu Lys Asn Val Thr Val Thr His Ser Val Asn Leu
      20      25      30
Leu Glu Asp Ser His Asn Gly Lys Leu
      35      40

```

<210> 178

<211> 41

<212> PRT

<213> Artificial Sequence

<220>

<223> HA1 N-terminal stem segment of HA subtype H1

<400> 178

```

Asp Thr Ile Cys Ile Gly Tyr His Ala Asn Asn Ser Thr Asp Thr Val
 1      5      10      15
Asp Thr Val Leu Glu Lys Asn Val Thr Val Thr His Ser Val Asn Leu

```


Sequence Listing - 2010234849

Leu Glu Asp Ser His Asn Gly Lys Leu
 20 25 30
 35 40

<210> 179
 <211> 40
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> HA1 N-terminal stem segment of HA subtype H1

<400> 179
 Asp Thr Ile Cys Ile Gly Tyr His Ala Asn Asn Ser Thr Asp Thr Val
 1 5 10 15
 Asp Thr Val Leu Glu Lys Asn Val Thr Val Thr His Ser Val Asn Leu
 20 25 30
 Leu Glu Asp Ser His Asn Gly Lys
 35 40

<210> 180
 <211> 41
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> HA1 N-terminal stem segment of HA subtype H2

<400> 180
 Asp Gln Ile Cys Ile Gly Tyr His Ser Asn Asn Ser Thr Glu Lys Val
 1 5 10 15
 Asp Thr Ile Leu Glu Arg Asn Val Thr Val Thr His Ala Gln Asn Ile
 20 25 30
 Leu Glu Lys Thr His Asn Gly Lys Leu
 35 40

<210> 181
 <211> 41
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> HA1 N-terminal stem segment of HA subtype H2

<400> 181
 Asp Gln Ile Cys Ile Gly Tyr His Ser Asn Asn Ser Thr Glu Lys Val
 1 5 10 15
 Asp Thr Ile Leu Glu Arg Asn Val Thr Val Thr His Ala Gln Asn Ile
 20 25 30
 Leu Glu Lys Thr His Asn Gly Lys Leu
 35 40

<210> 182
 <211> 40
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> HA1 N-terminal stem segment of HA subtype H2

<400> 182
 Asp Gln Ile Cys Ile Gly Tyr His Ser Asn Asn Ser Thr Glu Lys Val
 1 5 10 15
 Asp Thr Ile Leu Glu Arg Asn Val Thr Val Thr His Ala Gln Asn Ile
 Page 74

Sequence Listing - 2010234849

Leu Glu Lys Thr His Asn Gly Lys
 20 25 30
 35 40

<210> 183
 <211> 51
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> HA1 N-terminal stem segment of HA subtype H3

<400> 183
 Gln Asp Leu Pro Gly Asn Asp Asn Ser Thr Ala Thr Leu Cys Leu Gly
 1 5 10 15
 His His Ala Val Pro Asn Gly Thr Leu Val Lys Thr Ile Thr Asp Asp
 20 25 30
 Gln Ile Glu Val Thr Asn Ala Thr Glu Leu Val Gln Ser Ser Thr
 35 40 45
 Gly Lys Ile
 50

<210> 184
 <211> 51
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> HA1 N-terminal stem segment of HA subtype H3

<400> 184
 Gln Asp Leu Pro Gly Asn Asp Asn Ser Thr Ala Thr Leu Cys Leu Gly
 1 5 10 15
 His His Ala Val Pro Asn Gly Thr Leu Val Lys Thr Ile Thr Asp Asp
 20 25 30
 Gln Ile Glu Val Thr Asn Ala Thr Glu Leu Val Gln Ser Ser Thr
 35 40 45
 Gly Lys Ile
 50

<210> 185
 <211> 50
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> HA1 N-terminal stem segment of HA subtype H3

<400> 185
 Gln Asp Leu Pro Gly Asn Asp Asn Ser Thr Ala Thr Leu Cys Leu Gly
 1 5 10 15
 His His Ala Val Pro Asn Gly Thr Leu Val Lys Thr Ile Thr Asp Asp
 20 25 30
 Gln Ile Glu Val Thr Asn Ala Thr Glu Leu Val Gln Ser Ser Ser Thr
 35 40 45
 Gly Lys
 50

<210> 186
 <211> 47
 <212> PRT
 <213> Artificial Sequence

<220>

Sequence Listing - 2010234849

<223> HA1 N-terminal stem segment of HA subtype H4

<400> 186

Gln	Asn	Tyr	Thr	Gly	Asn	Pro	Val	Ile	Cys	Met	Gly	His	His	Ala	Val
1				5				10						15	
Ala	Asn	Gly	Thr	Met	Val	Lys	Thr	Leu	Ala	Asp	Asp	Gln	Val	Glu	Val
		20						25					30		
Val	Thr	Ala	Gln	Glu	Leu	Val	Glu	Ser	Gln	Asn	Leu	Pro	Glu	Leu	
		35					40					45			

<210> 187

<211> 47

<212> PRT

<213> Artificial Sequence

<220>

<223> HA1 N-terminal stem segment of HA subtype H4

<400> 187

Gln	Asn	Tyr	Thr	Gly	Asn	Pro	Val	Ile	Cys	Met	Gly	His	His	Ala	Val
1				5				10						15	
Ala	Asn	Gly	Thr	Met	Val	Lys	Thr	Leu	Ala	Asp	Asp	Gln	Val	Glu	Val
		20						25					30		
Val	Thr	Ala	Gln	Glu	Leu	Val	Glu	Ser	Gln	Asn	Leu	Pro	Glu	Leu	
		35					40					45			

<210> 188

<211> 46

<212> PRT

<213> Artificial Sequence

<220>

<223> HA1 N-terminal stem segment of HA subtype H4

<400> 188

Gln	Asn	Tyr	Thr	Gly	Asn	Pro	Val	Ile	Cys	Met	Gly	His	His	Ala	Val
1				5				10						15	
Ala	Asn	Gly	Thr	Met	Val	Lys	Thr	Leu	Ala	Asp	Asp	Gln	Val	Glu	Val
		20						25					30		
Val	Thr	Ala	Gln	Glu	Leu	Val	Glu	Ser	Gln	Asn	Leu	Pro	Glu		
		35					40					45			

<210> 189

<211> 41

<212> PRT

<213> Artificial Sequence

<220>

<223> HA1 N-terminal stem segment of HA subtype H5

<400> 189

Asp	Gln	Ile	Cys	Ile	Gly	Tyr	His	Ala	Asn	Lys	Ser	Thr	Lys	Gln	Val
1				5				10						15	
Asp	Thr	Ile	Met	Glu	Lys	Asn	Val	Thr	Val	Thr	His	Ala	Gln	Asp	Ile
		20						25					30		
Leu	Glu	Arg	Thr	His	Asn	Gly	Lys	Leu							
		35					40								

<210> 190

<211> 41

<212> PRT

<213> Artificial Sequence

<220>

Sequence Listing - 2010234849

<223> HA1 N-terminal stem segment of HA subtype H5

<400> 190

Asp	Gln	Ile	Cys	Ile	Gly	Tyr	His	Ala	Asn	Lys	Ser	Thr	Lys	Gln	Val
1				5					10					15	
Asp	Thr	Ile	Met	Glu	Lys	Asn	Val	Thr	Val	Thr	His	Ala	Gln	Asp	Ile
			20					25					30		
Leu	Glu	Arg	Thr	His	Asn	Gly	Lys	Leu							
		35					40								

<210> 191

<211> 40

<212> PRT

<213> Artificial Sequence

<220>

<223> HA1 N-terminal stem segment of HA subtype H5

<400> 191

Asp	Gln	Ile	Cys	Ile	Gly	Tyr	His	Ala	Asn	Lys	Ser	Thr	Lys	Gln	Val
1				5					10					15	
Asp	Thr	Ile	Met	Glu	Lys	Asn	Val	Thr	Val	Thr	His	Ala	Gln	Asp	Ile
			20					25					30		
Leu	Glu	Arg	Thr	His	Asn	Gly	Lys								
		35					40								

<210> 192

<211> 41

<212> PRT

<213> Artificial Sequence

<220>

<223> HA1 N-terminal stem segment of HA subtype H6

<400> 192

Asp	Lys	Ile	Cys	Ile	Gly	Tyr	His	Ala	Asn	Asn	Ser	Thr	Thr	Gln	Ile
1				5					10					15	
Asp	Thr	Ile	Leu	Glu	Lys	Asn	Val	Thr	Val	Thr	His	Ser	Val	Glu	Leu
			20					25					30		
Leu	Glu	Asn	Gln	Lys	Glu	Glu	Arg	Phe							
		35					40								

<210> 193

<211> 41

<212> PRT

<213> Artificial Sequence

<220>

<223> HA1 N-terminal stem segment of HA subtype H6

<400> 193

Asp	Lys	Ile	Cys	Ile	Gly	Tyr	His	Ala	Asn	Asn	Ser	Thr	Thr	Gln	Ile
1				5					10					15	
Asp	Thr	Ile	Leu	Glu	Lys	Asn	Val	Thr	Val	Thr	His	Ser	Val	Glu	Leu
			20					25					30		
Leu	Glu	Asn	Gln	Lys	Glu	Glu	Arg	Phe							
		35					40								

<210> 194

<211> 40

<212> PRT

<213> Artificial Sequence

<220>

Sequence Listing - 2010234849

<223> HA1 N-terminal stem segment of HA subtype H6

<400> 194

Asp	Lys	Ile	Cys	Ile	Gly	Tyr	His	Ala	Asn	Asn	Ser	Thr	Thr	Gln	Ile
1				5					10					15	
Asp	Thr	Ile	Leu	Glu	Lys	Asn	Val	Thr	Val	Thr	His	Ser	Val	Glu	Leu
			20					25					30		
Leu	Glu	Asn	Gln	Lys	Glu	Glu	Arg								
		35					40								

<210> 195

<211> 41

<212> PRT

<213> Artificial Sequence

<220>

<223> HA1 N-terminal stem segment of HA subtype H7

<400> 195

Asp	Lys	Ile	Cys	Leu	Gly	His	His	Ala	Val	Ser	Asn	Gly	Thr	Lys	Val
1				5					10					15	
Asn	Thr	Leu	Thr	Glu	Arg	Gly	Val	Glu	Val	Val	Asn	Ala	Thr	Glu	Thr
			20					25					30		
Val	Glu	Arg	Thr	Asn	Ile	Pro	Lys	Ile							
		35					40								

<210> 196

<211> 41

<212> PRT

<213> Artificial Sequence

<220>

<223> HA1 N-terminal stem segment of HA subtype H7

<400> 196

Asp	Lys	Ile	Cys	Leu	Gly	His	His	Ala	Val	Ser	Asn	Gly	Thr	Lys	Val
1				5					10					15	
Asn	Thr	Leu	Thr	Glu	Arg	Gly	Val	Glu	Val	Val	Asn	Ala	Thr	Glu	Thr
			20					25					30		
Val	Glu	Arg	Thr	Asn	Ile	Pro	Lys	Ile							
		35					40								

<210> 197

<211> 40

<212> PRT

<213> Artificial Sequence

<220>

<223> HA1 N-terminal stem segment of HA subtype of H7

<400> 197

Asp	Lys	Ile	Cys	Leu	Gly	His	His	Ala	Val	Ser	Asn	Gly	Thr	Lys	Val
1				5					10					15	
Asn	Thr	Leu	Thr	Glu	Arg	Gly	Val	Glu	Val	Val	Asn	Ala	Thr	Glu	Thr
			20					25					30		
Val	Glu	Arg	Thr	Asn	Ile	Pro	Lys								
		35					40								

<210> 198

<211> 41

<212> PRT

<213> Artificial Sequence

<220>

Sequence Listing - 2010234849

<223> HA1 N-terminal stem segment of HA subtype of H8

<400> 198

Asp	Arg	Ile	Cys	Ile	Gly	Tyr	Gln	Ser	Asn	Asn	Ser	Thr	Asp	Thr	Val
1				5					10				15		
Asn	Thr	Leu	Ile	Glu	Gln	Asn	Val	Pro	Val	Thr	Gln	Thr	Met	Glu	Leu
			20					25					30		
Val	Glu	Thr	Glu	Lys	His	Pro	Ala	Tyr							
		35					40								

<210> 199

<211> 41

<212> PRT

<213> Artificial Sequence

<220>

<223> HA1 N-terminal stem segment of HA subtype of H8

<400> 199

Asp	Arg	Ile	Cys	Ile	Gly	Tyr	Gln	Ser	Asn	Asn	Ser	Thr	Asp	Thr	Val
1				5					10				15		
Asn	Thr	Leu	Ile	Glu	Gln	Asn	Val	Pro	Val	Thr	Gln	Thr	Met	Glu	Leu
			20					25					30		
Val	Glu	Thr	Glu	Lys	His	Pro	Ala	Tyr							
		35					40								

<210> 200

<211> 40

<212> PRT

<213> Artificial Sequence

<220>

<223> HA1 N-terminal stem segment of HA subtype of H8

<400> 200

Asp	Arg	Ile	Cys	Ile	Gly	Tyr	Gln	Ser	Asn	Asn	Ser	Thr	Asp	Thr	Val
1				5					10				15		
Asn	Thr	Leu	Ile	Glu	Gln	Asn	Val	Pro	Val	Thr	Gln	Thr	Met	Glu	Leu
			20					25					30		
Val	Glu	Thr	Glu	Lys	His	Pro	Ala								
		35					40								

<210> 201

<211> 41

<212> PRT

<213> Artificial Sequence

<220>

<223> HA1 N-terminal stem segment of HA subtype of H9

<400> 201

Asp	Lys	Ile	Cys	Ile	Gly	Tyr	Gln	Ser	Thr	Asn	Ser	Thr	Glu	Thr	Val
1				5					10				15		
Asp	Thr	Leu	Thr	Glu	Ser	Asn	Val	Pro	Val	Thr	His	Thr	Lys	Glu	Leu
			20					25					30		
Leu	His	Thr	Glu	His	Asn	Gly	Met	Leu							
		35					40								

<210> 202

<211> 41

<212> PRT

<213> Artificial Sequence

<220>

Sequence Listing - 2010234849

<223> HA1 N-terminal stem segment of HA subtype of H9

<400> 202

Asp	Lys	Ile	Cys	Ile	Gly	Tyr	Gln	Ser	Thr	Asn	Ser	Thr	Glu	Thr	Val
1				5					10				15		
Asp	Thr	Leu	Thr	Glu	Ser	Asn	Val	Pro	Val	Thr	His	Thr	Lys	Glu	Leu
			20					25					30		
Leu	His	Thr	Glu	His	Asn	Gly	Met	Leu							
		35					40								

<210> 203

<211> 40

<212> PRT

<213> Artificial Sequence

<220>

<223> HA1 N-terminal stem segment of HA subtype of H9

<400> 203

Asp	Lys	Ile	Cys	Ile	Gly	Tyr	Gln	Ser	Thr	Asn	Ser	Thr	Glu	Thr	Val
1				5					10				15		
Asp	Thr	Leu	Thr	Glu	Ser	Asn	Val	Pro	Val	Thr	His	Thr	Lys	Glu	Leu
			20					25					30		
Leu	His	Thr	Glu	His	Asn	Gly	Met								
		35					40								

<210> 204

<211> 42

<212> PRT

<213> Artificial Sequence

<220>

<223> HA1 N-terminal stem segment of HA subtype of H10

<400> 204

Leu	Asp	Arg	Ile	Cys	Leu	Gly	His	His	Ala	Val	Ala	Asn	Gly	Thr	Ile
1				5					10				15		
Val	Lys	Thr	Leu	Thr	Asn	Glu	Gln	Glu	Glu	Val	Thr	Asn	Ala	Thr	Glu
			20					25					30		
Thr	Val	Glu	Ser	Thr	Asn	Leu	Asn	Lys	Leu						
		35					40								

<210> 205

<211> 42

<212> PRT

<213> Artificial Sequence

<220>

<223> HA1 N-terminal stem segment of HA subtype of H10

<400> 205

Leu	Asp	Arg	Ile	Cys	Leu	Gly	His	His	Ala	Val	Ala	Asn	Gly	Thr	Ile
1				5					10				15		
Val	Lys	Thr	Leu	Thr	Asn	Glu	Gln	Glu	Glu	Val	Thr	Asn	Ala	Thr	Glu
			20					25					30		
Thr	Val	Glu	Ser	Thr	Asn	Leu	Asn	Lys	Leu						
		35					40								

<210> 206

<211> 41

<212> PRT

<213> Artificial Sequence

<220>

Sequence Listing - 2010234849

<223> HA1 N-terminal stem segment of HA subtype of H10

<400> 206

```

Leu Asp Arg Ile Cys Leu Gly His His Ala Val Ala Asn Gly Thr Ile
 1           5           10          15
Val Lys Thr Leu Thr Asn Glu Gln Glu Val Thr Asn Ala Thr Glu
          20          25          30
Thr Val Glu Ser Thr Asn Leu Asn Lys
          35          40

```

<210> 207

<211> 41

<212> PRT

<213> Artificial Sequence

<220>

<223> HA1 N-terminal stem segment of HA subtype of H11

<400> 207

```

Asp Glu Ile Cys Ile Gly Tyr Leu Ser Asn Asn Ser Thr Asp Lys Val
 1           5           10          15
Asp Thr Ile Ile Glu Asn Asn Val Thr Val Thr Ser Ser Val Glu Leu
          20          25          30
Val Glu Thr Glu His Thr Gly Ser Phe
          35          40

```

<210> 208

<211> 41

<212> PRT

<213> Artificial Sequence

<220>

<223> HA1 N-terminal stem segment of HA subtype of H11

<400> 208

```

Asp Glu Ile Cys Ile Gly Tyr Leu Ser Asn Asn Ser Thr Asp Lys Val
 1           5           10          15
Asp Thr Ile Ile Glu Asn Asn Val Thr Val Thr Ser Ser Val Glu Leu
          20          25          30
Val Glu Thr Glu His Thr Gly Ser Phe
          35          40

```

<210> 209

<211> 40

<212> PRT

<213> Artificial Sequence

<220>

<223> HA1 N-terminal stem segment of HA subtype of H11

<400> 209

```

Asp Glu Ile Cys Ile Gly Tyr Leu Ser Asn Asn Ser Thr Asp Lys Val
 1           5           10          15
Asp Thr Ile Ile Glu Asn Asn Val Thr Val Thr Ser Ser Val Glu Leu
          20          25          30
Val Glu Thr Glu His Thr Gly Ser
          35          40

```

<210> 210

<211> 41

<212> PRT

<213> Artificial Sequence

<220>

Sequence Listing - 2010234849

<223> HA1 N-terminal stem segment of HA subtype of H12

<400> 210

```

Asp Lys Ile Cys Ile Gly Tyr Gln Thr Asn Asn Ser Thr Glu Thr Val
 1           5           10          15
Asn Thr Leu Ser Glu Gln Asn Val Pro Val Thr Gln Val Glu Glu Leu
 20          25          30
Val His Arg Gly Ile Asp Pro Ile Leu
 35          40

```

<210> 211

<211> 41

<212> PRT

<213> Artificial Sequence

<220>

<223> HA1 N-terminal stem segment of HA subtype of H12

<400> 211

```

Asp Lys Ile Cys Ile Gly Tyr Gln Thr Asn Asn Ser Thr Glu Thr Val
 1           5           10          15
Asn Thr Leu Ser Glu Gln Asn Val Pro Val Thr Gln Val Glu Glu Leu
 20          25          30
Val His Arg Gly Ile Asp Pro Ile Leu
 35          40

```

<210> 212

<211> 40

<212> PRT

<213> Artificial Sequence

<220>

<223> HA1 N-terminal stem segment of HA subtype of H12

<400> 212

```

Asp Lys Ile Cys Ile Gly Tyr Gln Thr Asn Asn Ser Thr Glu Thr Val
 1           5           10          15
Asn Thr Leu Ser Glu Gln Asn Val Pro Val Thr Gln Val Glu Glu Leu
 20          25          30
Val His Arg Gly Ile Asp Pro Ile
 35          40

```

<210> 213

<211> 41

<212> PRT

<213> Artificial Sequence

<220>

<223> HA1 N-terminal stem segment of HA subtype of H13

<400> 213

```

Asp Arg Ile Cys Val Gly Tyr Leu Ser Thr Asn Ser Ser Glu Arg Val
 1           5           10          15
Asp Thr Leu Leu Glu Asn Gly Val Pro Val Thr Ser Ser Ile Asp Leu
 20          25          30
Ile Glu Thr Asn His Thr Gly Thr Tyr
 35          40

```

<210> 214

<211> 41

<212> PRT

<213> Artificial Sequence

<220>

Sequence Listing - 2010234849

<223> HA1 N-terminal stem segment of HA subtype of H13

<400> 214

Asp	Arg	Ile	Cys	Val	Gly	Tyr	Leu	Ser	Thr	Asn	Ser	Ser	Glu	Arg	Val
1				5					10				15		
Asp	Thr	Leu	Leu	Glu	Asn	Gly	Val	Pro	Val	Thr	Ser	Ser	Ile	Asp	Leu
			20					25					30		
Ile	Glu	Thr	Asn	His	Thr	Gly	Thr	Tyr							
		35					40								

<210> 215

<211> 40

<212> PRT

<213> Artificial Sequence

<220>

<223> HA1 N-terminal stem segment of HA subtype of H13

<400> 215

Asp	Arg	Ile	Cys	Val	Gly	Tyr	Leu	Ser	Thr	Asn	Ser	Ser	Glu	Arg	Val
1				5					10				15		
Asp	Thr	Leu	Leu	Glu	Asn	Gly	Val	Pro	Val	Thr	Ser	Ser	Ile	Asp	Leu
			20					25					30		
Ile	Glu	Thr	Asn	His	Thr	Gly	Thr								
		35					40								

<210> 216

<211> 50

<212> PRT

<213> Artificial Sequence

<220>

<223> HA1 N-terminal stem segment of HA subtype of H14

<400> 216

Gln	Ile	Thr	Asn	Gly	Thr	Thr	Gly	Asn	Pro	Ile	Ile	Cys	Leu	Gly	His
1				5				10					15		
His	Ala	Val	Glu	Asn	Gly	Thr	Ser	Val	Lys	Thr	Leu	Thr	Asp	Asn	His
			20					25					30		
Val	Glu	Val	Val	Ser	Ala	Lys	Glu	Leu	Val	Glu	Thr	Asn	His	Thr	Asp
		35					40					45			
Glu	Leu														
	50														

<210> 217

<211> 50

<212> PRT

<213> Artificial Sequence

<220>

<223> HA1 N-terminal stem segment of HA subtype of H14

<400> 217

Gln	Ile	Thr	Asn	Gly	Thr	Thr	Gly	Asn	Pro	Ile	Ile	Cys	Leu	Gly	His
1				5				10					15		
His	Ala	Val	Glu	Asn	Gly	Thr	Ser	Val	Lys	Thr	Leu	Thr	Asp	Asn	His
			20					25					30		
Val	Glu	Val	Val	Ser	Ala	Lys	Glu	Leu	Val	Glu	Thr	Asn	His	Thr	Asp
		35					40					45			
Glu	Leu														
	50														

<210> 218

<211> 49

Sequence Listing - 2010234849

<212> PRT
<213> Artificial Sequence

<220>
<223> HA1 N-terminal stem segment of HA subtype of H14

<400> 218
Gln Ile Thr Asn Gly Thr Thr Gly Asn Pro Ile Ile Cys Leu Gly His
1 5 10 15
His Ala Val Glu Asn Gly Thr Ser Val Lys Thr Leu Thr Asp Asn His
20 25 30
Val Glu Val Val Ser Ala Lys Glu Leu Val Glu Thr Asn His Thr Asp
35 40 45
Glu

<210> 219
<211> 41
<212> PRT
<213> Artificial Sequence

<220>
<223> HA1 N-terminal stem segment of HA subtype of H15

<400> 219
Asp Lys Ile Cys Leu Gly His His Ala Val Ala Asn Gly Thr Lys Val
1 5 10 15
Asn Thr Leu Thr Glu Arg Gly Val Glu Val Val Asn Ala Thr Glu Thr
20 25 30
Val Glu Ile Thr Gly Ile Asp Lys Val
35 40

<210> 220
<211> 41
<212> PRT
<213> Artificial Sequence

<220>
<223> HA1 N-terminal stem segment of HA subtype of H15

<400> 220
Asp Lys Ile Cys Leu Gly His His Ala Val Ala Asn Gly Thr Lys Val
1 5 10 15
Asn Thr Leu Thr Glu Arg Gly Val Glu Val Val Asn Ala Thr Glu Thr
20 25 30
Val Glu Ile Thr Gly Ile Asp Lys Val
35 40

<210> 221
<211> 40
<212> PRT
<213> Artificial Sequence

<220>
<223> HA1 N-terminal stem segment of HA subtype of H15

<400> 221
Asp Lys Ile Cys Leu Gly His His Ala Val Ala Asn Gly Thr Lys Val
1 5 10 15
Asn Thr Leu Thr Glu Arg Gly Val Glu Val Val Asn Ala Thr Glu Thr
20 25 30
Val Glu Ile Thr Gly Ile Asp Lys
35 40

Sequence Listing - 2010234849

<210> 222
 <211> 41
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> HA1 N-terminal stem segment of HA subtype of H16

<400> 222
 Asp Lys Ile Cys Ile Gly Tyr Leu Ser Asn Asn Ser Ser Asp Thr Val
 1 5 10 15
 Asp Thr Leu Thr Glu Asn Gly Val Pro Val Thr Ser Ser Val Asp Leu
 20 25 30
 Val Glu Thr Asn His Thr Gly Thr Tyr
 35 40

<210> 223
 <211> 41
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> HA1 N-terminal stem segment of HA subtype of H16

<400> 223
 Asp Lys Ile Cys Ile Gly Tyr Leu Ser Asn Asn Ser Ser Asp Thr Val
 1 5 10 15
 Asp Thr Leu Thr Glu Asn Gly Val Pro Val Thr Ser Ser Val Asp Leu
 20 25 30
 Val Glu Thr Asn His Thr Gly Thr Tyr
 35 40

<210> 224
 <211> 40
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> HA1 N-terminal stem segment of HA subtype of H16

<400> 224
 Asp Lys Ile Cys Ile Gly Tyr Leu Ser Asn Asn Ser Ser Asp Thr Val
 1 5 10 15
 Asp Thr Leu Thr Glu Asn Gly Val Pro Val Thr Ser Ser Val Asp Leu
 20 25 30
 Val Glu Thr Asn His Thr Gly Thr
 35 40

<210> 225

<400> 225
 000

<210> 226
 <211> 52
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> HA1 C-terminal stem segment of HA subtype of H1

<400> 226
 Asn Thr Lys Cys Gln Thr Pro Leu Gly Ala Ile Asn Ser Ser Leu Pro
 1 5 10 15

Sequence Listing - 2010234849

Tyr Gln Asn Ile His Pro Val Thr Ile Gly Glu Cys Pro Lys Tyr Val
 20 25 30
 Arg Ser Ala Lys Leu Arg Met Val Thr Gly Leu Arg Asn Asn Pro Ser
 35 40 45
 Ile Gln Ser Arg
 50

<210> 227
 <211> 51
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> HA1 C-terminal stem segment of HA subtype of H1

<400> 227
 Thr Lys Cys Gln Thr Pro Leu Gly Ala Ile Asn Ser Ser Leu Pro Tyr
 1 5 10 15
 Gln Asn Ile His Pro Val Thr Ile Gly Glu Cys Pro Lys Tyr Val Arg
 20 25 30
 Ser Ala Lys Leu Arg Met Val Thr Gly Leu Arg Asn Asn Pro Ser Ile
 35 40 45
 Gln Ser Arg
 50

<210> 228
 <211> 50
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> HA1 C-terminal stem segment of HA subtype of H1

<400> 228
 Lys Cys Gln Thr Pro Leu Gly Ala Ile Asn Ser Ser Leu Pro Tyr Gln
 1 5 10 15
 Asn Ile His Pro Val Thr Ile Gly Glu Cys Pro Lys Tyr Val Arg Ser
 20 25 30
 Ala Lys Leu Arg Met Val Thr Gly Leu Arg Asn Asn Pro Ser Ile Gln
 35 40 45
 Ser Arg
 50

<210> 229
 <211> 52
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> HA1 C-terminal stem segment of HA subtype of H2

<400> 229
 Glu Thr Lys Cys Gln Thr Pro Leu Gly Ala Ile Asn Thr Thr Leu Pro
 1 5 10 15
 Phe His Asn Val His Pro Leu Thr Ile Gly Glu Cys Pro Lys Tyr Val
 20 25 30
 Lys Ser Glu Arg Leu Val Leu Ala Thr Gly Leu Arg Asn Val Pro Gln
 35 40 45
 Ile Glu Ser Arg
 50

<210> 230
 <211> 51
 <212> PRT

Sequence Listing - 2010234849

<213> Artificial Sequence

<220>

<223> HA1 C-terminal stem segment of HA subtype of H2

<400> 230

```

Thr Lys Cys Gln Thr Pro Leu Gly Ala Ile Asn Thr Thr Leu Pro Phe
 1           5           10           15
His Asn Val His Pro Leu Thr Ile Gly Glu Cys Pro Lys Tyr Val Lys
      20           25           30
Ser Glu Arg Leu Val Leu Ala Thr Gly Leu Arg Asn Val Pro Gln Ile
      35           40           45
Glu Ser Arg
      50

```

<210> 231

<211> 50

<212> PRT

<213> Artificial Sequence

<220>

<223> HA1 C-terminal stem segment of HA subtype of H2

<400> 231

```

Lys Cys Gln Thr Pro Leu Gly Ala Ile Asn Thr Thr Leu Pro Phe His
 1           5           10           15
Asn Val His Pro Leu Thr Ile Gly Glu Cys Pro Lys Tyr Val Lys Ser
      20           25           30
Glu Arg Leu Val Leu Ala Thr Gly Leu Arg Asn Val Pro Gln Ile Glu
      35           40           45
Ser Arg
      50

```

<210> 232

<211> 52

<212> PRT

<213> Artificial Sequence

<220>

<223> HA1 C-terminal stem segment of HA subtype of H3

<400> 232

```

Ile Ser Glu Cys Ile Thr Pro Asn Gly Ser Ile Pro Asn Asp Lys Pro
 1           5           10           15
Phe Gln Asn Val Asn Lys Ile Thr Tyr Gly Ala Cys Pro Lys Tyr Val
      20           25           30
Lys Gln Asn Thr Leu Lys Leu Ala Thr Gly Met Arg Asn Val Pro Glu
      35           40           45
Lys Gln Thr Arg
      50

```

<210> 233

<211> 51

<212> PRT

<213> Artificial Sequence

<220>

<223> HA1 C-terminal stem segment of HA subtype of H3

<400> 233

```

Ser Glu Cys Ile Thr Pro Asn Gly Ser Ile Pro Asn Asp Lys Pro Phe
 1           5           10           15
Gln Asn Val Asn Lys Ile Thr Tyr Gly Ala Cys Pro Lys Tyr Val Lys
      20           25           30
Gln Asn Thr Leu Lys Leu Ala Thr Gly Met Arg Asn Val Pro Glu Lys

```

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Gln Thr Arg
35
50

40

45

<210> 234
<211> 50
<212> PRT
<213> Artificial Sequence

<220>
<223> HA1 C-terminal stem segment of HA subtype of H3

<400> 234
Glu Cys Ile Thr Pro Asn Gly Ser Ile Pro Asn Asp Lys Pro Phe Gln
1 5 10 15
Asn Val Asn Lys Ile Thr Tyr Gly Ala Cys Pro Lys Tyr Val Lys Gln
20 25 30
Asn Thr Leu Lys Leu Ala Thr Gly Met Arg Asn Val Pro Glu Lys Gln
35 40 45
Thr Arg
50

<210> 235
<211> 52
<212> PRT
<213> Artificial Sequence

<220>
<223> HA1 C-terminal stem segment of HA subtype of H4

<400> 235
Val Ser Lys Cys His Thr Asp Lys Gly Ser Leu Ser Thr Thr Lys Pro
1 5 10 15
Phe Gln Asn Ile Ser Arg Ile Ala Val Gly Asp Cys Pro Arg Tyr Val
20 25 30
Lys Gln Gly Ser Leu Lys Leu Ala Thr Gly Met Arg Asn Ile Pro Glu
35 40 45
Lys Ala Ser Arg
50

<210> 236
<211> 51
<212> PRT
<213> Artificial Sequence

<220>
<223> HA1 C-terminal stem segment of HA subtype of H4

<400> 236
Ser Lys Cys His Thr Asp Lys Gly Ser Leu Ser Thr Thr Lys Pro Phe
1 5 10 15
Gln Asn Ile Ser Arg Ile Ala Val Gly Asp Cys Pro Arg Tyr Val Lys
20 25 30
Gln Gly Ser Leu Lys Leu Ala Thr Gly Met Arg Asn Ile Pro Glu Lys
35 40 45
Ala Ser Arg
50

<210> 237
<211> 50
<212> PRT
<213> Artificial Sequence

<220>

Sequence Listing - 2010234849

<223> HA1 C-terminal stem segment of HA subtype of H4

<400> 237

```
Lys Cys His Thr Asp Lys Gly Ser Leu Ser Thr Thr Lys Pro Phe Gln
 1           5           10           15
Asn Ile Ser Arg Ile Ala Val Gly Asp Cys Pro Arg Tyr Val Lys Gln
          20           25           30
Gly Ser Leu Lys Leu Ala Thr Gly Met Arg Asn Ile Pro Glu Lys Ala
      35           40           45
Ser Arg
    50
```

<210> 238

<211> 52

<212> PRT

<213> Artificial Sequence

<220>

<223> HA1 C-terminal stem segment of HA subtype of H5

<400> 238

```
Asp Thr Lys Cys Gln Thr Pro Val Gly Glu Ile Asn Ser Ser Met Pro
 1           5           10           15
Phe His Asn Ile His Pro His Thr Ile Gly Glu Cys Pro Lys Tyr Val
          20           25           30
Lys Ser Asp Arg Leu Val Leu Ala Thr Gly Leu Arg Asn Val Pro Gln
      35           40           45
Arg Lys Lys Arg
    50
```

<210> 239

<211> 51

<212> PRT

<213> Artificial Sequence

<220>

<223> HA1 C-terminal stem segment of HA subtype of H5

<400> 239

```
Thr Lys Cys Gln Thr Pro Val Gly Glu Ile Asn Ser Ser Met Pro Phe
 1           5           10           15
His Asn Ile His Pro His Thr Ile Gly Glu Cys Pro Lys Tyr Val Lys
          20           25           30
Ser Asp Arg Leu Val Leu Ala Thr Gly Leu Arg Asn Val Pro Gln Arg
      35           40           45
Lys Lys Arg
    50
```

<210> 240

<211> 50

<212> PRT

<213> Artificial Sequence

<220>

<223> HA1 C-terminal stem segment of HA subtype of H5

<400> 240

```
Lys Cys Gln Thr Pro Val Gly Glu Ile Asn Ser Ser Met Pro Phe His
 1           5           10           15
Asn Ile His Pro His Thr Ile Gly Glu Cys Pro Lys Tyr Val Lys Ser
          20           25           30
Asp Arg Leu Val Leu Ala Thr Gly Leu Arg Asn Val Pro Gln Arg Lys
      35           40           45
Lys Arg
    50
```


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<210> 241
 <211> 52
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> HA1 C-terminal stem segment of HA subtype of H6

<400> 241
 Asp Ala Thr Cys Gln Thr Val Ala Gly Val Leu Arg Thr Asn Lys Thr
 1 5 10 15
 Phe Gln Asn Val Ser Pro Leu Trp Ile Gly Glu Cys Pro Lys Tyr Val
 20 25 30
 Lys Ser Glu Ser Leu Arg Leu Ala Thr Gly Leu Arg Asn Val Pro Gln
 35 40 45
 Ile Glu Thr Arg
 50

<210> 242
 <211> 51
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> HA1 C-terminal stem segment of HA subtype of H6

<400> 242
 Ala Thr Cys Gln Thr Val Ala Gly Val Leu Arg Thr Asn Lys Thr Phe
 1 5 10 15
 Gln Asn Val Ser Pro Leu Trp Ile Gly Glu Cys Pro Lys Tyr Val Lys
 20 25 30
 Ser Glu Ser Leu Arg Leu Ala Thr Gly Leu Arg Asn Val Pro Gln Ile
 35 40 45
 Glu Thr Arg
 50

<210> 243
 <211> 50
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> HA1 C-terminal stem segment of HA subtype of H6

<400> 243
 Thr Cys Gln Thr Val Ala Gly Val Leu Arg Thr Asn Lys Thr Phe Gln
 1 5 10 15
 Asn Val Ser Pro Leu Trp Ile Gly Glu Cys Pro Lys Tyr Val Lys Ser
 20 25 30
 Glu Ser Leu Arg Leu Ala Thr Gly Leu Arg Asn Val Pro Gln Ile Glu
 35 40 45
 Thr Arg
 50

<210> 244
 <211> 56
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> HA1 C-terminal stem segment of HA subtype of H7

<400> 244

Sequence Listing - 2010234849

Glu Gly Glu Cys Tyr His Ser Gly Gly Thr Ile Thr Ser Arg Leu Pro
 1 5 10 15
 Phe Gln Asn Ile Asn Ser Arg Ala Val Gly Lys Cys Pro Arg Tyr Val
 20 25 30
 Lys Gln Glu Ser Leu Leu Leu Ala Thr Gly Met Lys Asn Val Pro Glu
 35 40 45
 Pro Ser Lys Lys Arg Lys Lys Arg
 50 55

<210> 245

<211> 55

<212> PRT

<213> Artificial Sequence

<220>

<223> HA1 C-terminal stem segment of HA subtype of H7

<400> 245

Gly Glu Cys Tyr His Ser Gly Gly Thr Ile Thr Ser Arg Leu Pro Phe
 1 5 10 15
 Gln Asn Ile Asn Ser Arg Ala Val Gly Lys Cys Pro Arg Tyr Val Lys
 20 25 30
 Gln Glu Ser Leu Leu Leu Ala Thr Gly Met Lys Asn Val Pro Glu Pro
 35 40 45
 Ser Lys Lys Arg Lys Lys Arg
 50 55

<210> 246

<211> 54

<212> PRT

<213> Artificial Sequence

<220>

<223> HA1 C-terminal stem segment of HA subtype of H7

<400> 246

Glu Cys Tyr His Ser Gly Gly Thr Ile Thr Ser Arg Leu Pro Phe Gln
 1 5 10 15
 Asn Ile Asn Ser Arg Ala Val Gly Lys Cys Pro Arg Tyr Val Lys Gln
 20 25 30
 Glu Ser Leu Leu Leu Ala Thr Gly Met Lys Asn Val Pro Glu Pro Ser
 35 40 45
 Lys Lys Arg Lys Lys Arg
 50

<210> 247

<211> 52

<212> PRT

<213> Artificial Sequence

<220>

<223> HA1 C-terminal stem segment of HA subtype of H8

<400> 247

Asn Thr Lys Cys Gln Thr Tyr Ala Gly Ala Ile Asn Ser Ser Lys Pro
 1 5 10 15
 Phe Gln Asn Ala Ser Arg His Tyr Met Gly Glu Cys Pro Lys Tyr Val
 20 25 30
 Lys Lys Ala Ser Leu Arg Leu Ala Val Gly Leu Arg Asn Thr Pro Ser
 35 40 45
 Val Glu Pro Arg
 50

<210> 248

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<211> 51
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> HA1 C-terminal stem segment of HA subtype of H8

<400> 248
 Thr Lys Cys Gln Thr Tyr Ala Gly Ala Ile Asn Ser Ser Lys Pro Phe
 1 5 10 15
 Gln Asn Ala Ser Arg His Tyr Met Gly Glu Cys Pro Lys Tyr Val Lys
 20 25 30
 Lys Ala Ser Leu Arg Leu Ala Val Gly Leu Arg Asn Thr Pro Ser Val
 35 40 45
 Glu Pro Arg
 50

<210> 249
 <211> 50
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> HA1 C-terminal stem segment of HA subtype of H8

<400> 249
 Lys Cys Gln Thr Tyr Ala Gly Ala Ile Asn Ser Ser Lys Pro Phe Gln
 1 5 10 15
 Asn Ala Ser Arg His Tyr Met Gly Glu Cys Pro Lys Tyr Val Lys Lys
 20 25 30
 Ala Ser Leu Arg Leu Ala Val Gly Leu Arg Asn Thr Pro Ser Val Glu
 35 40 45
 Pro Arg
 50

<210> 250
 <211> 52
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> HA1 C-terminal stem segment of HA subtype of H9

<400> 250
 Val Val Gln Cys Gln Thr Glu Lys Gly Gly Leu Asn Thr Thr Leu Pro
 1 5 10 15
 Phe His Asn Ile Ser Lys Tyr Ala Phe Gly Asn Cys Pro Lys Tyr Val
 20 25 30
 Gly Val Lys Ser Leu Lys Leu Pro Val Gly Leu Arg Asn Val Pro Ala
 35 40 45
 Val Ser Ser Arg
 50

<210> 251
 <211> 51
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> HA1 C-terminal stem segment of HA subtype of H9

<400> 251
 Val Gln Cys Gln Thr Glu Lys Gly Gly Leu Asn Thr Thr Leu Pro Phe
 1 5 10 15
 His Asn Ile Ser Lys Tyr Ala Phe Gly Asn Cys Pro Lys Tyr Val Gly

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```

                20          25          30
Val Lys Ser  Leu Lys Leu Pro Val Gly Leu Arg Asn Val Pro Ala Val
                35          40          45
Ser Ser Arg
                50

```

<210> 252
 <211> 50
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> HA1 C-terminal stem segment of HA subtype of H9

```

<400> 252
Gln Cys Gln Thr Glu Lys Gly Gly Leu Asn Thr Thr Leu Pro Phe His
 1          5          10          15
Asn Ile Ser Lys Tyr Ala Phe Gly Asn Cys Pro Lys Tyr Val Gly Val
                20          25          30
Lys Ser Leu Lys Leu Pro Val Gly Leu Arg Asn Val Pro Ala Val Ser
                35          40          45
Ser Arg
                50

```

<210> 253
 <211> 53
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> HA1 C-terminal stem segment of HA subtype of H10

```

<400> 253
Glu Ser Lys Cys Phe Trp Arg Gly Gly Ser Ile Asn Thr Lys Leu Pro
 1          5          10          15
Phe Gln Asn Leu Ser Pro Arg Thr Val Gly Gln Cys Pro Lys Tyr Val
                20          25          30
Asn Gln Arg Ser Leu Leu Leu Ala Thr Gly Met Arg Asn Val Pro Glu
                35          40          45
Val Val Gln Gly Arg
                50

```

<210> 254
 <211> 52
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> HA1 C-terminal stem segment of HA subtype of H10

```

<400> 254
Ser Lys Cys Phe Trp Arg Gly Gly Ser Ile Asn Thr Lys Leu Pro Phe
 1          5          10          15
Gln Asn Leu Ser Pro Arg Thr Val Gly Gln Cys Pro Lys Tyr Val Asn
                20          25          30
Gln Arg Ser Leu Leu Leu Ala Thr Gly Met Arg Asn Val Pro Glu Val
                35          40          45
Val Gln Gly Arg
                50

```

<210> 255
 <211> 51
 <212> PRT
 <213> Artificial Sequence

Sequence Listing - 2010234849

<220>

<223> HA1 C-terminal stem segment of HA subtype of H10

<400> 255

```

Lys Cys Phe Trp Arg Gly Gly Ser Ile Asn Thr Lys Leu Pro Phe Gln
 1           5           10           15
Asn Leu Ser Pro Arg Thr Val Gly Gln Cys Pro Lys Tyr Val Asn Gln
          20           25           30
Arg Ser Leu Leu Leu Ala Thr Gly Met Arg Asn Val Pro Glu Val Val
          35           40           45
Gln Gly Arg
      50

```

<210> 256

<211> 52

<212> PRT

<213> Artificial Sequence

<220>

<223> HA1 C-terminal stem segment of HA subtype of H11

<400> 256

```

Ser Thr Lys Cys Gln Thr Glu Ile Gly Gly Ile Asn Thr Asn Lys Ser
 1           5           10           15
Phe His Asn Val His Arg Asn Thr Ile Gly Asp Cys Pro Lys Tyr Val
          20           25           30
Asn Val Lys Ser Leu Lys Leu Ala Thr Gly Pro Arg Asn Val Pro Ala
          35           40           45
Ile Ala Ser Arg
      50

```

<210> 257

<211> 51

<212> PRT

<213> Artificial Sequence

<220>

<223> HA1 C-terminal stem segment of HA subtype of H11

<400> 257

```

Thr Lys Cys Gln Thr Glu Ile Gly Gly Ile Asn Thr Asn Lys Ser Phe
 1           5           10           15
His Asn Val His Arg Asn Thr Ile Gly Asp Cys Pro Lys Tyr Val Asn
          20           25           30
Val Lys Ser Leu Lys Leu Ala Thr Gly Pro Arg Asn Val Pro Ala Ile
          35           40           45
Ala Ser Arg
      50

```

<210> 258

<211> 50

<212> PRT

<213> Artificial Sequence

<220>

<223> HA1 C-terminal stem segment of HA subtype of H11

<400> 258

```

Lys Cys Gln Thr Glu Ile Gly Gly Ile Asn Thr Asn Lys Ser Phe His
 1           5           10           15
Asn Val His Arg Asn Thr Ile Gly Asp Cys Pro Lys Tyr Val Asn Val
          20           25           30
Lys Ser Leu Lys Leu Ala Thr Gly Pro Arg Asn Val Pro Ala Ile Ala
          35           40           45

```

Sequence Listing - 2010234849

Ser Arg
50

<210> 259
<211> 52
<212> PRT
<213> Artificial Sequence

<220>
<223> HA1 C-terminal stem segment of HA subtype of H12

<400> 259
Val Thr Glu Cys Gln Leu Asn Glu Gly Val Met Asn Thr Ser Lys Pro
1 5 10 15
Phe Gln Asn Thr Ser Lys His Tyr Ile Gly Lys Cys Pro Lys Tyr Ile
20 25 30
Pro Ser Gly Ser Leu Lys Leu Ala Ile Gly Leu Arg Asn Val Pro Gln
35 40 45
Val Gln Asp Arg
50

<210> 260
<211> 51
<212> PRT
<213> Artificial Sequence

<220>
<223> HA1 C-terminal stem segment of HA subtype of H12

<400> 260
Thr Glu Cys Gln Leu Asn Glu Gly Val Met Asn Thr Ser Lys Pro Phe
1 5 10 15
Gln Asn Thr Ser Lys His Tyr Ile Gly Lys Cys Pro Lys Tyr Ile Pro
20 25 30
Ser Gly Ser Leu Lys Leu Ala Ile Gly Leu Arg Asn Val Pro Gln Val
35 40 45
Gln Asp Arg
50

<210> 261
<211> 50
<212> PRT
<213> Artificial Sequence

<220>
<223> HA1 C-terminal stem segment of HA subtype of H12

<400> 261
Glu Cys Gln Leu Asn Glu Gly Val Met Asn Thr Ser Lys Pro Phe Gln
1 5 10 15
Asn Thr Ser Lys His Tyr Ile Gly Lys Cys Pro Lys Tyr Ile Pro Ser
20 25 30
Gly Ser Leu Lys Leu Ala Ile Gly Leu Arg Asn Val Pro Gln Val Gln
35 40 45
Asp Arg
50

<210> 262
<211> 52
<212> PRT
<213> Artificial Sequence

<220>
<223> HA1 C-terminal stem segment of HA subtype of H13

Sequence Listing - 2010234849

<400> 262

```

Asn Thr Lys Cys Gln Thr Ser Val Gly Gly Ile Asn Thr Asn Arg Thr
 1           5           10           15
Phe Gln Asn Ile Asp Lys Asn Ala Leu Gly Asp Cys Pro Lys Tyr Ile
           20           25           30
Lys Ser Gly Gln Leu Lys Leu Ala Thr Gly Leu Arg Asn Val Pro Ala
           35           40           45
Ile Ser Asn Arg
      50

```

<210> 263

<211> 51

<212> PRT

<213> Artificial Sequence

<220>

<223> HA1 C-terminal stem segment of HA subtype of H13

<400> 263

```

Thr Lys Cys Gln Thr Ser Val Gly Gly Ile Asn Thr Asn Arg Thr Phe
 1           5           10           15
Gln Asn Ile Asp Lys Asn Ala Leu Gly Asp Cys Pro Lys Tyr Ile Lys
           20           25           30
Ser Gly Gln Leu Lys Leu Ala Thr Gly Leu Arg Asn Val Pro Ala Ile
           35           40           45
Ser Asn Arg
      50

```

<210> 264

<211> 50

<212> PRT

<213> Artificial Sequence

<220>

<223> HA1 C-terminal stem segment of HA subtype of H13

<400> 264

```

Lys Cys Gln Thr Ser Val Gly Gly Ile Asn Thr Asn Arg Thr Phe Gln
 1           5           10           15
Asn Ile Asp Lys Asn Ala Leu Gly Asp Cys Pro Lys Tyr Ile Lys Ser
           20           25           30
Gly Gln Leu Lys Leu Ala Thr Gly Leu Arg Asn Val Pro Ala Ile Ser
           35           40           45
Asn Arg
      50

```

<210> 265

<211> 52

<212> PRT

<213> Artificial Sequence

<220>

<223> HA1 C-terminal stem segment of HA subtype of H14

<400> 265

```

Thr Ser Pro Cys Leu Thr Asp Lys Gly Ser Ile Gln Ser Asp Lys Pro
 1           5           10           15
Phe Gln Asn Val Ser Arg Ile Ala Ile Gly Asn Cys Pro Lys Tyr Val
           20           25           30
Lys Gln Gly Ser Leu Met Leu Ala Thr Gly Met Arg Asn Ile Pro Gly
           35           40           45
Lys Gln Ala Lys
      50

```

Sequence Listing - 2010234849

<210> 266
 <211> 51
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> HA1 C-terminal stem segment of HA subtype of H14

<400> 266
 Ser Pro Cys Leu Thr Asp Lys Gly Ser Ile Gln Ser Asp Lys Pro Phe
 1 5 10 15
 Gln Asn Val Ser Arg Ile Ala Ile Gly Asn Cys Pro Lys Tyr Val Lys
 20 25 30
 Gln Gly Ser Leu Met Leu Ala Thr Gly Met Arg Asn Ile Pro Gly Lys
 35 40 45
 Gln Ala Lys
 50

<210> 267
 <211> 50
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> HA1 C-terminal stem segment of HA subtype of H14

<400> 267
 Pro Cys Leu Thr Asp Lys Gly Ser Ile Gln Ser Asp Lys Pro Phe Gln
 1 5 10 15
 Asn Val Ser Arg Ile Ala Ile Gly Asn Cys Pro Lys Tyr Val Lys Gln
 20 25 30
 Gly Ser Leu Met Leu Ala Thr Gly Met Arg Asn Ile Pro Gly Lys Gln
 35 40 45
 Ala Lys
 50

<210> 268
 <211> 53
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> HA1 C-terminal stem segment of HA subtype of H15

<400> 268
 Glu Gly Glu Cys Phe Tyr Ser Gly Gly Thr Ile Asn Ser Pro Leu Pro
 1 5 10 15
 Phe Gln Asn Ile Asp Ser Arg Ala Val Gly Lys Cys Pro Arg Tyr Val
 20 25 30
 Lys Gln Ser Ser Leu Pro Leu Ala Leu Gly Met Lys Asn Val Pro Glu
 35 40 45
 Lys Ile Arg Thr Arg
 50

<210> 269
 <211> 52
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> HA1 C-terminal stem segment of HA subtype of H15

<400> 269
 Gly Glu Cys Phe Tyr Ser Gly Gly Thr Ile Asn Ser Pro Leu Pro Phe

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```

1           5           10           15
Gln Asn Ile Asp Ser Arg Ala Val Gly Lys Cys Pro Arg Tyr Val Lys
      20      25      30
Gln Ser Ser Leu Pro Leu Ala Leu Gly Met Lys Asn Val Pro Glu Lys
      35      40      45
Ile Arg Thr Arg
      50

```

<210> 270
 <211> 51
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> HA1 C-terminal stem segment of HA subtype of H15

```

<400> 270
Glu Cys Phe Tyr Ser Gly Gly Thr Ile Asn Ser Pro Leu Pro Phe Gln
1           5           10           15
Asn Ile Asp Ser Arg Ala Val Gly Lys Cys Pro Arg Tyr Val Lys Gln
      20      25      30
Ser Ser Leu Pro Leu Ala Leu Gly Met Lys Asn Val Pro Glu Lys Ile
      35      40      45
Arg Thr Arg
      50

```

<210> 271
 <211> 52
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> HA1 C-terminal stem segment of HA subtype of H16

```

<400> 271
Asn Thr Lys Cys Gln Thr Ser Leu Gly Gly Ile Asn Thr Asn Lys Thr
1           5           10           15
Phe Gln Asn Ile Glu Arg Asn Ala Leu Gly Asp Cys Pro Lys Tyr Ile
      20      25      30
Lys Ser Gly Gln Leu Lys Leu Ala Thr Gly Leu Arg Asn Val Pro Ser
      35      40      45
Ile Gly Glu Arg
      50

```

<210> 272
 <211> 51
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> HA1 C-terminal stem segment of HA subtype of H16

```

<400> 272
Thr Lys Cys Gln Thr Ser Leu Gly Gly Ile Asn Thr Asn Lys Thr Phe
1           5           10           15
Gln Asn Ile Glu Arg Asn Ala Leu Gly Asp Cys Pro Lys Tyr Ile Lys
      20      25      30
Ser Gly Gln Leu Lys Leu Ala Thr Gly Leu Arg Asn Val Pro Ser Ile
      35      40      45
Gly Glu Arg
      50

```

<210> 273
 <211> 50

Sequence Listing - 2010234849

<212> PRT
 <213> Artificial Sequence

<220>
 <223> HA1 C-terminal stem segment of HA subtype of H16

<400> 273
 Lys Cys Gln Thr Ser Leu Gly Gly Ile Asn Thr Asn Lys Thr Phe Gln
 1 5 10 15
 Asn Ile Glu Arg Asn Ala Leu Gly Asp Cys Pro Lys Tyr Ile Lys Ser
 20 25 30
 Gly Gln Leu Lys Leu Ala Thr Gly Leu Arg Asn Val Pro Ser Ile Gly
 35 40 45
 Glu Arg
 50

<210> 274
 <211> 31
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Upstream primer used in Excise PCR method for construct PR8 2G

<400> 274
 acatagtttt ccgttgtggc tgtcttcgag c 31

<210> 275
 <211> 46
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Upstream primer used in Excise PCR method for construct PR8 4G

<400> 275
 ggaggctgta acacgaagtg tcaaacaccc ctgggagcta taaaca 46

<210> 276
 <211> 31
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Upstream primer used in Excise PCR method for construct PR8 PG

<400> 276
 acatagtttt ccgttgtggc tgtcttcgag c 31

<210> 277
 <211> 52
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Upstream primer used in Fusion PCR method for construct PR8 No Cys 1G

<400> 277
 ggaggcggag gttgtaacac gaagtgtcaa acaccctgg gagctataaa ca 52

<210> 278
 <211> 31
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Upstream primer used in Fusion PCR method for construct PR8 No Cys 2G

Sequence Listing - 2010234849

```

<400> 278
acatagtttt ccgttggtggc tgtcttcgag c
31

<210> 279
<211> 46
<212> DNA
<213> Artificial Sequence

<220>
<223> Upstream primer used in Fusion PCR method for construct PR8 No Cys 3G

<400> 279
ccaggctgta acacgaagtg tcaaacaccc ctgggagcta taaaca
46

<210> 280
<211> 36
<212> DNA
<213> Artificial Sequence

<220>
<223> Upstream primer used in Fusion PCR method for construct PR8 No Cys

<400> 280
tgacacttcg tgttacctag ttttccgttg tggctg
36

<210> 281
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Upstream primer used in Fusion PCR method for construct PR8 No Cys
delta1

<400> 281
ggtaacacga agtgtcaaac ac
22

<210> 282
<211> 35
<212> DNA
<213> Artificial Sequence

<220>
<223> Upstream primer used in Fusion PCR method for construct PR8 No Cys
delta3

<400> 282
acttcgtgtt tccgcctagt tttccgttgt ggctg
35

<210> 283
<211> 25
<212> DNA
<213> Artificial Sequence

<220>
<223> Upstream primer used in Fusion PCR method for construct PR8 No Cys NAS

<400> 283
ggcggaaaca cgaagtgta aacac
25

<210> 284
<211> 34
<212> DNA
<213> Artificial Sequence

<220>
<223> Upstream primer used in Excise PCR method for construct HK68 2G

```

Sequence Listing - 2010234849

```

<400> 284
cgtgttacct cgcctagtt ttccgttggtg gctg
34

<210> 285
<211> 28
<212> DNA
<213> Artificial Sequence

<220>
<223> Upstream primer used in Excise PCR method for construct HK68 4G

<400> 285
ggcggaggta acacgaagtg tcaaacac
28

<210> 286
<211> 39
<212> DNA
<213> Artificial Sequence

<220>
<223> Upstream primer used in Excise PCR method for construct HK68 PG

<400> 286
tgtttgacac ttcgtgttta gttttccgtt gtggctgtc
39

<210> 287
<211> 38
<212> DNA
<213> Artificial Sequence

<220>
<223> Upstream primer used in Fusion PCR method for construct HK68 No Cys

<400> 287
gccacaacgg aaaactaaac acgaagtgtc aaacaccc
38

<210> 288
<211> 38
<212> DNA
<213> Artificial Sequence

<220>
<223> Upstream primer used in Fusion PCR method for construct HK68 No Cys
delta1

<400> 288
ggtgtttgac acttcgtag ttttccgttg tggctgtc
38

<210> 289
<211> 38
<212> DNA
<213> Artificial Sequence

<220>
<223> Upstream primer used in Fusion PCR method for construct HK68 No Cys
delta3

<400> 289
gccacaacgg aaaactaacg aagtgtcaaa caccctgt
38

<210> 290
<211> 38
<212> DNA
<213> Artificial Sequence

<220>
<223> Upstream primer used in Fusion PCR method for construct HK68 No Cys
NAS

```

Sequence Listing - 2010234849

```

<400> 290
aggggtgttt gacacttttt tccgttgtgg ctgtcttc           38

<210> 291
<211> 38
<212> DNA
<213> Artificial Sequence

<220>
<223> downstream primer used in Excise PCR method for construct PR8 2G

<400> 291
acagccacaa cggaaaaaag tgtcaaacac ccctggga           38

<210> 292
<211> 38
<212> DNA
<213> Artificial Sequence

<220>
<223> downstream primer used in Excise PCR method for construct PR8 4G

<400> 292
acttcgtgtt ggaggcgttt agttttccgt tgtggctg           38

<210> 293
<211> 28
<212> DNA
<213> Artificial Sequence

<220>
<223> downstream primer used in Excise PCR method for construct PR8 PG

<400> 293
aacgcctcca acacgaagtg tcaaacac           28

<210> 294
<211> 26
<212> DNA
<213> Artificial Sequence

<220>
<223> downstream primer used in Fusion PCR method for construct PR8 No Cys 1G

<400> 294
gcuccucaac ggggaaaaua ugcgga           26

<210> 295
<211> 26
<212> DNA
<213> Artificial Sequence

<220>
<223> downstream primer used in Fusion PCR method for construct PR8 No Cys 2G

<400> 295
ggctgtattt ctgaatgcat cactcc           26

<210> 296
<211> 29
<212> DNA
<213> Artificial Sequence

<220>
<223> downstream primer used in Fusion PCR method for construct PR8 No Cys 3G

<400> 296

```

Sequence Listing - 2010234849

gcuccucaac ggggaaaaua ugcggaggc 29

<210> 297
 <211> 29
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> downstream primer used in Fusion PCR method for construct PR8 No Cys

<400> 297
 ggaggttgta tttctgaatg catcactcc 29

<210> 298
 <211> 26
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> downstream primer used in Fusion PCR method for construct PR8 No Cys
 delta1

<400> 298
 gcuccucaac ggggaaaaua ugccca 26

<210> 299
 <211> 26
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> downstream primer used in Fusion PCR method for construct PR8 No Cys
 delta3

<400> 299
 ggctgtatatt ctgaatgcat cactcc 26

<210> 300
 <211> 39
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> downstream primer used in Fusion PCR method for construct PR8 No Cys
 NAS

<400> 300
 gagtgatgca ttcagaaatt attttccccg ttgaggagc 39

<210> 301
 <211> 38
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> downstream primer used in Excise PCR method for construct HK68 2G

<400> 301
 cctcaacggg gaaaataatt tctgaatgca tcaactcca 38

<210> 302
 <211> 38
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> downstream primer used in Excise PCR method for construct HK68 4G

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```

<400> 302
tgagatgatg cattcagata ttttccccgt tgaggagc          38

<210> 303
<211> 38
<212> DNA
<213> Artificial Sequence

<220>
<223> downstream primer used in Excise PCR method for construct HK68 PG

<400> 303
cctcaacggg gaaaatatct gaatgcatca ctccaaat          38

<210> 304
<211> 37
<212> DNA
<213> Artificial Sequence

<220>
<223> downstream primer used in Fusion PCR method for construct HK68 No Cys

<400> 304
atttgagtg atgcattctt tccccgttga ggagctc          37

<210> 305
<211> 36
<212> DNA
<213> Artificial Sequence

<220>
<223> downstream primer used in Fusion PCR method for construct HK68 No Cys
delta1

<400> 305
ctcctcaacg gggaaagaat gcatcactcc aaatgg          36

<210> 306
<211> 37
<212> DNA
<213> Artificial Sequence

<220>
<223> downstream primer used in Fusion PCR method for construct HK68 No Cys
delta3

<400> 306
ttcagaaatg gaggcgttta ttttccccgt tgaggag          37

<210> 307
<211> 29
<212> DNA
<213> Artificial Sequence

<220>
<223> downstream primer used in Fusion PCR method for construct HK68 No Cys
NAS

<400> 307
aacgcctcca tttctgaatg catcactcc          29

<210> 308
<211> 44
<212> PRT
<213> Artificial Sequence

<220>

```

Sequence Listing - 2010234849

<223> HA1 N-terminal Stem Segment of HK68-CON-A

<400> 308

```
Ser Thr Ala Thr Leu Cys Leu Gly His His Ala Val Pro Asn Gly Thr
 1           5           10          15
Leu Val Lys Thr Ile Thr Asp Asp Gln Ile Glu Val Thr Asn Ala Thr
          20          25          30
Glu Leu Val Gln Ser Ser Ser Thr Gly Lys Ile Cys
      35          40
```

<210> 309

<211> 46

<212> PRT

<213> Artificial Sequence

<220>

<223> HA1 N-terminal Stem Segment of PR8-CON-A

<400> 309

```
Asp Thr Ile Cys Ile Gly Tyr His Ala Asn Asn Ser Thr Asp Thr Val
 1           5           10          15
Asp Thr Val Leu Glu Lys Asn Val Thr Val Thr His Ser Val Asn Leu
          20          25          30
Leu Glu Asp Ser His Asn Gly Lys Leu Cys Arg Leu Lys Cys
      35          40          45
```

<210> 310

<211> 52

<212> PRT

<213> Artificial Sequence

<220>

<223> HA1 C-terminal Stem Segment of PR8-CON-A

<400> 310

```
Cys Lys Cys Gln Thr Pro Leu Gly Ala Ile Asn Ser Ser Leu Pro Tyr
 1           5           10          15
Gln Asn Ile His Pro Val Thr Ile Gly Glu Cys Pro Lys Tyr Val Arg
          20          25          30
Ser Ala Lys Leu Arg Met Val Thr Gly Leu Arg Asn Asn Pro Ser Ile
      35          40          45
Gln Ser Arg Gly
      50
```

<210> 311

<211> 16

<212> PRT

<213> influenza A virus

<220>

<223> influenza A polypeptide

<400> 311

```
Lys Leu Asn Gly Ser Gly Ile Met Lys Thr Glu Gly Thr Leu Glu Asn
 1           5           10          15
```

<210> 312

<211> 20

<212> PRT

<213> influenza A virus

<220>

<223> influenza A polypeptide

Sequence Listing - 2010234849

<220>

<221> VARIANT

<222> 5

<223> Xaa = Thr or Lys

<400> 312

Asn	Asn	Ile	Asp	Xaa	Leu	Asn	Gly	Ser	Gly	Ile	Met	Lys	Thr	Glu	Gly
1				5					10					15	
Thr	Leu	Glu	Asn												
			20												

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