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] Provided herein are flu hemagglutinin (HA) polypeptides that induce a cross-protective immune response against the conserved HA stem domain (sometimes referred to herein as the "stalk" domain) of influenza viruses. The subject matter of the present invention is as defined in the attached set of claims. In one example, the disclosure concerns the design and construct of chimeric influenza virus hemagglutinin polypeptides having a stable HA stalk that displays a globular HA head heterologous to the stalk (i.e. chimeric influenza virus hemagglutinin polypeptides described herein). The HA immunogens designed for vaccination share the HA stalk region but are highly divergent in their globular heads. Such constructs are engineered into vaccine formulations such as live influenza viruses, killed influenza viruses, virus/viral-like particles ("VLPs"), subunit vaccines, split vaccines, etc., that elicit highly potent and broadly neutralizing antibodies against the conserved HA stalk. Such "universal" vaccines can be used to induce and/or boost cross-protective immune responses across influenza virus subtypes.

[0010] By way of background, neutralizing antibodies against influenza viruses target the HA glycoprotein and prevent either the binding or the fusion step involved in viral entry. Two basic subsets of neutralizing antibodies are elicited by exposure to influenza viruses: those directed to the strain-specific globular head (a domain that is non-conserved across the various strains and subtypes of influenza virus), and those directed to the highly conserved stem of the HA glycoprotein. The non-conserved HA globular head carries the immunodominant epitopes. Without being bound by theory, the strain-specific anti-globular head antibodies are thought to be more potent than anti-stem antibodies, thus explaining the largely strain-specific immunity conferred by infection with current vaccines.

[0011] The invention is based, in part, on the inventors' rational design strategies for influenza virus vaccines that elicit highly potent and broadly neutralizing antibodies against

addition of a glycosylation site to the globular head domain of an influenza virus HA of one subtype, wherein the glycosylation site is naturally found in the globular head domain of an influenza virus HA of another subtype. Another example of a non-naturally occurring glycosylation site is the addition of a glycosylation site to the globular head domain of an influenza virus HA from one strain, wherein the glycosylation site is naturally found in the globular head of an HA from another strain of influenza virus. Yet another example of a non-naturally occurring glycosylation site is the addition of a glycosylation site to the globular head of an HA from one strain, wherein the glycosylation site is not naturally found in the globular head of an HA from another subtype or strain of influenza virus. While not being bound by any particular theory of operation, it is believed that the additional glycosylation within the globular head domain will increase the immune response to the conserved stem domain, while reducing the immune response to the globular head domain.

[0018] It should be understood that use of the chimeric influenza hemagglutinin polypeptides described herein is advantageous because (i) said polypeptides are highly stable (by virtue of possessing an intact globular head domain) and (ii) the immune systems of the subjects to which said polypeptides are administered have not previously been exposed to the globular head domains of the chimeric influenza hemagglutinin, but have been exposed to the conserved epitopes of the stem domains of the chimeric influenza hemagglutinin.

[0019] In another example, provided herein is a flu HA polypeptide (e.g., influenza virus hemagglutinin stem domain polypeptides and non-chimeric influenza virus hemagglutinin polypeptides) that comprises a stem domain comprising one or more modified glycosylation sites that disrupt the binding of glycan to the stem domain.

[0020] In another example, provided herein is a flu HA polypeptide (e.g., influenza virus hemagglutinin stem domain polypeptides and non-chimeric influenza virus hemagglutinin polypeptides) that comprises a globular head domain comprising one or more non-naturally occurring glycosylation sites.

[0021] In yet another example, provided herein, is a flu HA polypeptide (e.g., influenza virus hemagglutinin stem domain polypeptides and non-chimeric influenza virus hemagglutinin polypeptides) that comprises (1) a stem domain comprising one or more modified glycosylation sites that disrupt the binding of glycan to the stem domain; and (2) a globular head domain comprising one or more non-naturally occurring glycosylation sites.

[0022] In a specific example, provided herein is a chimeric influenza virus hemagglutinin (HA) polypeptide comprising an HA stem domain and an HA globular head domain, wherein: (1) the HA globular head domain is heterologous to the HA stem domain;

and (2) the HA stem domain comprises one or more modified glycosylation site(s), wherein the modified glycosylation site(s) comprises a modification to a glycosylation site that disrupts/interferes with binding of a glycan to the glycosylation site in the stem domain. In specific examples, the modified glycosylation site comprises a modification of a naturally occurring glycosylation site having an amino acid sequence Asn-Xaa-Ser/Thr/Cys, and wherein Xaa is any amino acid. In certain examples, the HA globular head domain further comprises one or more non-naturally occurring glycosylation sites having an amino acid sequence Asn-Xaa-Ser/Thr/Cys, wherein Xaa is any amino acid.

[0023] In another example, provided herein is a non-chimeric influenza virus hemagglutinin (HA) polypeptide comprising an HA stem domain and an HA globular head domain, wherein: (1) the HA globular head domain is homologous to the HA stem domain, and (2) the HA stem domain comprises one or more modified glycosylation site(s), wherein the modified glycosylation site(s) comprises a modification to a glycosylation site that disrupts/interferes with binding of a glycan to the glycosylation site in the stem domain. In specific examples, the modified glycosylation site comprises a modification of a naturally occurring glycosylation site having an amino acid sequence Asn-Xaa-Ser/Thr/Cys, wherein the modification disrupts the ability of a glycan to attach to the modified glycosylation site, wherein Xaa is any amino acid.

[0024] In another example, provided herein is a an influenza virus hemagglutinin (HA) stem domain polypeptide comprising: 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 short stem segment; said HA1 domain in tertiary or quaternary association with an influenza hemagglutinin HA2 domain, wherein the influenza virus HA stem domain polypeptide domain further comprises one or more modified glycosylation site(s), wherein the modified glycosylation site(s) comprises a modification to a glycosylation site that disrupts/interferes with binding of a glycan to the glycosylation site in the stem domain. In specific examples, the modified glycosylation site comprises a modification of a naturally occurring glycosylation site having an amino acid sequence Asn-Xaa-Ser/Thr/Cys, where the modification disrupts the ability of a glycan to attach to the modified glycosylation site, and wherein Xaa is any amino acid.

[0025] In another example, provided herein is an influenza virus hemagglutinin (HA) stem domain polypeptide comprising: an influenza hemagglutinin HA1 domain that comprises an HA1 N-terminal long stem segment covalently linked to a linker of 1 to 50

heterologous residues that is in turn covalently linked to an HA1 C-terminal long stem segment; said HA1 domain in tertiary or quaternary association with an influenza hemagglutinin HA2 domain, wherein the influenza virus HA stem domain polypeptide domain further comprises one or more modified glycosylation site(s), wherein the modified glycosylation site(s) comprises a modification to a glycosylation site that disrupts/interferes with binding of a glycan to the glycosylation site in the stem domain. In specific examples, the modified glycosylation site comprises a modification of a naturally occurring glycosylation site having an amino acid sequence Asn-Xaa-Ser/Thr/Cys, where the modification disrupts the ability of a glycan to attach to the modified glycosylation site, and wherein Xaa is any amino acid.

[0026] In another example, provided herein is an influenza virus hemagglutinin (HA) stem domain polypeptide comprising: 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 an influenza hemagglutinin HA2 domain, wherein the influenza virus HA stem domain polypeptide domain further comprises one or more modified glycosylation site(s), wherein the modified glycosylation site(s) comprises a modification to a glycosylation site that disrupts/interferes with binding of a glycan to the glycosylation site in the stem domain. In specific examples, the modified glycosylation site comprises a modification of a naturally occurring glycosylation site having an amino acid sequence Asn-Xaa-Ser/Thr/Cys, where the modification disrupts the ability of a glycan to attach to the modified glycosylation site, and wherein Xaa is any amino acid.

[0027] In another example, provided herein is an influenza virus hemagglutinin (HA) stem domain polypeptide comprising: an influenza hemagglutinin HA1 domain that comprises, linked in the following order: an HA1 N-terminal stem segment, a first linker of 1 to 50 heterologous residues, an HA1 intermediate stem segment, a second linker of 1 to 50 heterologous residues and an HA1 C-terminal stem segment; said HA1 domain in tertiary or quaternary association with an influenza hemagglutinin HA2 domain, wherein the influenza virus hemagglutinin (HA) stem domain polypeptide domain further comprises one or more modified glycosylation site(s), wherein the modified glycosylation site(s) comprises a modification to a glycosylation site that disrupts/interferes with binding of a glycan to the glycosylation site in the stem domain. In specific embodiments, the modified glycosylation site comprises a modification of a naturally occurring glycosylation site having an amino acid

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. Those of skill in the art will readily be able recognize the amino acid residues that correspond to the HA1 N-terminal stem segment of other influenza HA polypeptides, e.g., the amino acid residues that correspond to the HA1 N-terminal stem segment of HA1 from an H1 hemagglutinin (see, e.g., Figure 1).

[0042] "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 $HA1_{C\text{-}term}$ of an HA1 domain. $HA1_{C\text{-}term}$ 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 277-329 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. Those of skill in the art will readily be able recognize the amino acid residues that correspond to the HA1 C-terminal stem segment of other influenza HA polypeptides, e.g., the amino acid residues that correspond to the HA1 C-terminal stem segment of HA1 from an H1 hemagglutinin (see, e.g., Figure 1).

[0043] "HA1 C-terminal short stem segment" refers to a polypeptide segment that corresponds to the carboxyl-terminal portion of the stem domain of an influenza hemagglutinin HA1 polypeptide. In certain embodiments, an HA1 C-terminal short stem segment consists of amino acid residues corresponding approximately to amino acids B_q through $HA1_{C\text{-}term}$ of an HA1 domain. Residue B_q is identified in influenza A hemagglutinin polypeptides in FIG. 1. Exemplary HA1 C-terminal short stem segments are described herein. In certain embodiments, an HA1 C-terminal short stem segment consists of amino acid residues corresponding approximately to amino acids 305-329 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.

[0044] "HA1 N-terminal long 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 long stem segment consists of amino acid residues corresponding approximately to amino acids $HA1_{N\text{-}term}$ through C_p of an HA1 domain. C_p is a cysteine residue in the HA1 N-terminal long stem segment that is or is capable of being linked to an alanine residue in an HA1 C-terminal long stem segment. Residue C_p is identified in influenza A hemagglutinin polypeptides in FIG. 1. Exemplary HA1 N-terminal long stem segments are described herein. In certain embodiments, an HA1 N-terminal long stem segment consists of amino acid residues corresponding approximately to amino acids 1-97 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.

[0045] "HA1 C-terminal long stem segment" refers to a polypeptide segment that corresponds to the carboxyl-terminal portion of the stem domain of an influenza hemagglutinin HA1 polypeptide. In certain embodiments, an HA1 C-terminal long stem segment consists of amino acid residues corresponding approximately to amino acids C_q through $HA1_{C\text{-}term}$ of an HA1 domain. C_q is an alanine residue in the HA1 C-terminal long stem segment that is or is capable of being linked to a cysteine residue in an HA1 N-terminal long stem segment. Residue C_q is identified in influenza A hemagglutinin polypeptides in FIG. 1. Exemplary HA1 C-terminal long stem segments are described herein. In certain embodiments, an HA1 C-terminal long stem segment consists of amino acid residues corresponding approximately to amino acids 253-329 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.

[0046] "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.

B) Prime-boost regime with sequential administration of chimeric HAs consisting of a completely conserved stalk domain and a varying globular head domain.

[0084] **Fig. 10** describes generation of a chimeric HA with the stalk of an H1 HA and the globular head of an H3 HA. A chimeric HA consisting of the stalk domain of A/PR8/34 HA and the globular head domain of HK/68 (chimeric H3) as well as wild type HAs (PR8-HA and HK68 HA) were expressed in 293T cells. The upper Western blot was probed with a PR8-specific antibody whereas the blot on the lower side was probed with an antibody specific for H3.

[0085] **Fig. 11** depicts a sequence comparison of the hemagglutinin protein sequences of A/Hong Kong/1/1968 (H3), A/Perth/16/2009 (H3), A/PR/8/34 (H1), A/Cal/4/09 (H1), A/Viet Nam/1203/04 (H5), and A/mallard/Alberta/24/01 (H7). The Cys52 and Cys277 amino acid residues are specified (based on H3 numbering). The black shade indicates conserved amino acids. The black wavy line represents the globular head region of HAs. The starting points of HA1 and HA2 are indicated.

[0086] **Fig. 12** depicts a schematic of chimeric hemagglutinins. (A) Construction diagram of the chimeric PR8-cH1 HA. The chimeric HA was constructed by swapping the globular head domain located between Cys52 and Cys277 of A/PR/8/34(H1) HA with that of the A/California/4/09(H1) HA. The resulting chimeric HA has the stalk region of A/PR8/34 (H1) HA with a globular head domain of the A/California/4/09 (H1) HA designated as PR8-cH1. (B) Schematic of the folded structures of the different wild type and chimeric HAs, such as wild the type PR8 HA, the chimeric PR8-cH1 HA, the chimeric PR8-cH5 HA, the wild type Perth HA, and the chimeric Perth-cH7 HA (from left to right). The full-length HA structures were downloaded from the Protein Database (PDB): PR8 HA (PDB ID 1RU7) and Perth HA (represented by HK68 HA, PDB ID 1MQN). Final images were generated with PyMol (Delano Scientific).

[0087] **Fig. 13** depicts the surface expression and functional analysis of chimeric HA constructs. (A) Surface expression of chimeric HA constructs was evaluated in transiently transfected cells. At 24 h post-transfection, 293T cells were trypsinized and cell surface expression of chimeric HA proteins were analyzed by flow cytometry. In the upper panels, mock-transfected cells (left shaded region) are compared to cells transfected with PR8 HA (right) or cells transfected with PR8-cH1 (right) or PR8-cH5 (right). In the bottom panels, mock-transfected cells (left shaded region) are compared to cells transfected with Perth and Perth-cH7 constructs (right). (B) Luciferase-encoding pseudo-particles expressing chimeric HAs were used to infect MDCK cells. The relative light units (RLU) generated in the

luciferase assay indicate that pseudo-particles expressing chimeric HAs were able to enter the cells.

[0088] **Fig. 14** describes the generation of recombinant viruses bearing chimeric hemagglutinins. (A) Western blot analysis of the recombinant viruses. Extracts from MDCK cells mock infected or infected with the indicated viruses (16 hpi) at an MOI of 2 were prepared and probed with antibodies: anti-A/PR8/HA(H1) (PY102), anti-A/Cal/09/HA(H1) (29C1), anti-A/VN/HA(H5) (M08), anti-H3/HA (12D1), anti-H7 (NR-3125), anti-A/NP (HT103) and anti-GAPDH as an internal loading control. (B) Immunofluorescence analysis of the MDCK cells infected with recombinant viruses using antibodies: anti-A/NP (HT103), anti-A/H1 HA (6F12), anti-A/PR8/HA (PY102), anti-A/Cal/09/HA (29C1), anti-A/VN/HA (M08), anti-H3/HA (12D1), and anti-A/H7 virus (NR-3152).

[0089] **Fig. 15** describes the growth kinetics and plaque phenotypes of recombinant viruses. (A) 10-day old embryonated chicken eggs were infected with wild-type or recombinant virus with 100 pfu per egg and viral growth monitored for 72 hours post infection. (B) The plaque phenotype of recombinant viruses was assessed by plaque assay. MDCK cells were infected with either a wild-type or recombinant virus and at 48 hours post infection immuno-stained to reveal plaque phenotype using the antibody against A/NP (HT103).

[0090] **Fig. 16A-Fig. 16F** depict an immunofluorescence analysis of cells transfected with chimeric H6 hemagglutinin. 293T cells were transfected with 1 μg of pCAGGS plasmid expressing chimeric H6 hemagglutinin. Sera from animals that received DNA (Fig. 16A), Cal/09 infection (Fig. 16B), DNA and Cal/09 infection (Fig. 16C), or Cal/09 split vaccine (Fig. 16D) were added to transfected cells and visualized by fluorescence microscopy following incubation with an Alexa Fluor 594-conjugated anti-mouse IgG. As controls, the cross-reactive H1 stem antibody C179 (Fig. 16E) or the antibody PY102 (Fig. 16F) directed against the globular head of PR8 were added to transfected cells and visualized by fluorescence microscopy followed by incubation with an Alexa Fluor 594-conjugated anti-mouse IgG.

[0091] **Fig. 17** demonstrates that DNA prime and chimeric virus boost confer protection to animals challenged with lethal influenza virus challenge. Animals were either treated with DNA alone, chimeric H9 virus alone, DNA prime and chimeric H9 virus boost, or with inactivated PR8 virus. Mice were then challenged with 5 x 10^4 PFU of PR8 virus, instilled intranasally, and the weight of the animals was monitored for 14 days.

[0092] **Fig. 18** demonstrates reactivity of stalk specific antibodies to cH6 protein as determined by ELISA.

[0093] **Fig. 19** depicts the structure of A/PR/8/34 H1 hemagglutinin trimer having a stem domain and globular head domain. The hemagglutinin trimer is depicted without glycans

A/PR8/HA (PY102), anti-A/Cal/09/HA (29E3), anti-A/VN/HA (mAb #8), anti-H3/HA (12D1), and anti-A/H7 virus (NR-3152).

[00111] **Fig. 37** depicts the growth kinetics and plaque phenotypes of wild type and recombinant viruses. (A) 10-day old embryonated chicken eggs were infected with 100 pfu per egg of wild-type or recombinant virus and viral growth was monitored for 72 hours post infection. Data points represent the average and standard deviation of experimental replicates. (B) The plaque phenotypes of recombinant viruses were assessed by plaque assay. MDCK cells were infected with either a wild-type or recombinant virus. Cells were fixed 48 hours post infection and immunostained to reveal plaque phenotypes using the antibody against A/NP (HT103).

[00112] **Figs. 38A-38B** depict that stalk-specific monoclonal antibody neutralizes cHA-expressing viruses and pseudotype particles. The ability of a mAb (KB2) to neutralize cHA-expressing viruses or pseudotype particles was assessed by plaque reduction assay or pseudotype particle inhibition assay. MDCK cells were infected or transduce with cHA-expressing viruses or pseudotype particles in the presence of the indicated amount (ug/mL) of the mAb or without antibody. Plaque formation or luciferase activity was used as a readout to determine the degree of inhibition by the mAb. (Fig. 38A) The mAb neutralizes cH1/1 (black boxes) and cH5/1 (black triangles) virus replication in a dose dependent manner, with 100% inhibition at concentrations above 100 ug/mL. Data points represent the average and standard deviation of experimental replicates. (Fig. 38B) The mAb also inhibits entry of cH1/1 (black boxes) pseudotype particles in a dose dependent manner, with complete inhibition above 4ug/mL. Data points represent the average and standard deviation of experimental replicates. The pseudotype inhibition assays were processed independently.

[00113] **Fig. 39** demonstrates that NJ/76 vaccine recipients had elevated anti-HA stalk antibodies prior to Cal/09 vaccination. Serial dilutions of serum from NJ/76 vaccinees (n = 20) or age-matched control subjects (n = 15) were tested for their reactivity to A) cH6/1 HA or B) NC/99 HA by ELISA and IgG endpoint titers were calculated. Due to limited quantities of available serum, pre-Cal/09 vaccination IgG endpoint titers were also determined for pooled NJ/76 vaccinees (N=5) and control subjects (n=7) against C) cH6/1 and D) NC/99. Each pool consisted of all individuals from each group for whom both pre- and post-Cal/09 vaccination samples were available. Unpaired Student T-tests were performed and two-tailed p-values <0.05 were considered statistically significant. N.D. = not detected. N.S = not significant. *statistically significant.

[00140] It should be understood that use of the chimeric influenza hemagglutinin polypeptides described herein is advantageous because (i) said polypeptides are highly stable (by virtue of possessing an intact globular head domain) and (ii) the immune systems of the subjects to which said polypeptides are administered have not previously been exposed to the globular head domains of the chimeric influenza hemagglutinin, but have been exposed to the conserved epitopes of the stem domains of the chimeric influenza hemagglutinin.

[00141] The chimeric influenza virus hemagglutinin (HA) polypeptide is illustrated by the working Examples (e.g., Section 6.2) which demonstrate the construction of a chimeric influenza HA polypeptide comprising an HA stem and displaying a heterologous HA head, and the production of a stable chimeric HA protein from this polypeptide that cross-reacts with antibodies to both the stem domain and the head domain.

[00142] In one aspect, provided herein are chimeric influenza virus hemagglutinin polypeptides comprising an influenza virus hemagglutinin head domain polypeptide and an influenza virus hemagglutinin stem domain polypeptide, wherein said influenza virus hemagglutinin head domain polypeptide is heterologous to said influenza virus hemagglutinin stem domain polypeptide (e.g., the influenza virus hemagglutinin head domain polypeptide and the influenza virus hemagglutinin stem domain polypeptide are derived from different influenza virus hemagglutinin subtypes). The chimeric influenza virus hemagglutinin polypeptides provided herein may be generated by combining an influenza virus hemagglutinin head domain polypeptide (see Section 5.2) with an influenza virus hemagglutinin stem domain polypeptide (Section 5.3). That is, using the principles of the invention, the influenza virus hemagglutinin head domain polypeptides described herein (see Section 5.1.1, infra) and the influenza virus hemagglutinin stem domain polypeptides described herein (Section 5.1.2, infra) can be mixed and matched so as to generate a chimeric influenza virus hemagglutinin polypeptide.

[00143] In another aspect, provided herein are flu hemagglutinin (HA) polypeptides (*e.g.*, chimeric influenza virus hemagglutinin (HA) polypeptides, influenza virus hemagglutinin (HA) stem domain polypeptides) comprising one or more modified glycosylation sites and/or one or more non-naturally occurring glycosylation sites. As shown in Fig. 19B, glycosylation of wild type hemagglutinin occurs in both the globular head and stem domains. It is believed that glycosylation within these domains can mask antigenic regions, thereby allowing an influenza virus to evade a host immune system response. For example, seasonal influenza virus strains (e.g., H1N1 and H3N2) have been known to acquire additional glycosylation sites overtime in immunodominant antigenic regions of the globular head

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). In certain embodiments, the cleavage site is a cleavage site recognized by Tobacco Etch Virus (TEV) protease (e.g., amino acid sequence Glu-Asn-Leu-Tyr-Phe-Gln-(Gly/Ser)).

[00199] In certain embodiments, the chimeric influenza hemagglutinin hemagglutinin polypeptides are soluble polypeptides, such as those described in Examples 6 and 9, infra.

[00200] In certain embodiments, the influenza hemagglutinin stem domain polypeptides of the chimeric influenza virus hemagglutinin polypeptides described herein maintain the cysteine residues identified in influenza hemagglutinin polypeptides as A_p and A_q in FIG. 1, i.e., the cysteine residues identified in influenza hemagglutinin polypeptides as A_p and A_q in FIG. 1 are maintained in the chimeric influenza virus hemagglutinin polypeptides described herein. Thus, in certain embodiments, in the primary sequence of a chimeric influenza virus hemagglutinin polypeptide described herein: (i) the N-terminal segment of an influenza hemagglutinin stem domain polypeptide ends at the cysteine residue identified as A_p in FIG. 1, (ii) the C-terminal segment of an influenza hemagglutinin stem domain polypeptide begins at the cysteine residue identified as A_q in FIG. 1; and (iii) the influenza hemagglutinin head domain polypeptide (which is heterologous to the influenza hemagglutinin stem domain polypeptide) is between the N-terminal and C-terminal segments of the influenza hemagglutinin stem domain polypeptide. Influenza hemagglutinin stem domain polypeptides are described in detail in Section 5.1.2, infra.

[00201] In certain embodiments, the HA1 N-terminal stem segment of the chimeric influenza virus hemagglutinin polypeptides described herein does not end exactly at A_p (*e.g.*, Cys_{52} of an HA1 subunit from an H3 hemagglutinin), but at a residue in sequence and structural vicinity to A_p. For example, in certain embodiments, the HA1 N-terminal stem segment of the chimeric influenza virus hemagglutinin polypeptides described herein ends at A_{p-1}, A_{p-2}, A_{p-3}, A_{p-4}, A_{p-5}, A_{p-6}, A_{p-7}, A_{p-8}, A_{p-9}, A_{p-10}, A_{p-11}, A_{p-12}, A_{p-13}, A_{p-14}, A_{p-15}, A_{p-16}, A_{p-17}, A_{p-18}, A_{p-19}, A_{p-20}, A_{p-21}, A_{p-22}, A_{p-23}, A_{p-23}, A_{p-24}, A_{p-25}, A_{p-26}, A_{p-27}, A_{p-28}, A_{p-29}, A_{p-30}. In certain embodiments, the HA1 N-terminal stem segment of the chimeric influenza virus hemagglutinin polypeptides described herein ends in the range of A_{p-1} to A_{p-3}, A_{p-3} to A_{p-5}, A_{p-5} to A_{p-8}, A_{p-8} to A_{p-10}, A_{p-10} to A_{p-15}, A_{p-15} to A_{p-20}, A_{p-20} to A_{p-30}, A_{p-30} to A_{p-40}. For example, an HA1 N-terminal stem segment ending at A_{p-10} would end at Leu42 of an H3 hemagglutinin. In certain embodiments, the HA1 N-terminal stem segment of the chimeric

influenza virus hemagglutinin polypeptides described herein ends at A_{p+1}, A_{p+2}, A_{p+3}, A_{p+4}, A_{p+5}, A_{p+6}, A_{p+7}, A_{p+8}, A_{p+9}, A_{p+10}, A_{p+11}, A_{p+12}, A_{p+13}, A_{p+14}, A_{p+15}, A_{p+16}, A_{p+17}, A_{p+18}, A_{p+19}, A_{p+20}, A_{p+21}, A_{p+22}, A_{p+23}, A_{p+24}, A_{p+25}, A_{p+26}, A_{p+27}, A_{p+28}, A_{p+29}, A_{p+30}, A_{p+31}, A_{p+32}, A_{p+33}, A_{p+34}, A_{p+35}, A_{p+36}, A_{p+37}, A_{p+38}, A_{p+39}, A_{p+40}. In certain embodiments, the HA1 N-terminal stem segment of the chimeric influenza virus hemagglutinin polypeptides described herein ends in the range of A_{p+1} to A_{p+5}, A_{p+5} to A_{p+10}, A_{p+10} to A_{p+15}, A_{p+15} to A_{p+20}, A_{p+20} to A_{p+25}, A_{p+25} to A_{p+30}, A_{p+30} to A_{p+35}, A_{p+35} to A_{p+40}, or A_{p+40} to A_{p+50}. For example, an HA1 N-terminal stem segment ending at A_{p+38} would end at Arg90 of an H3 hemagglutinin. 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 influenza hemagglutinin head domain polypeptide so that the resulting chimeric influenza virus hemagglutinin polypeptide is capable of forming a three-dimensional structure similar to a wild-type influenza hemagglutinin. In such embodiments, an influenza hemagglutinin head domain polypeptide (which is heterologous to the influenza hemagglutinin stem domain polypeptide) is located, in primary sequence, between the N-terminal and C-terminal segments of the influenza hemagglutinin stem domain polypeptide.

[00202] In certain embodiments, the HA1 C-terminal stem segment of the chimeric influenza virus hemagglutinin polypeptides described herein does not start at A_q (*e.g.*, Cys_{277} of an HA1 subunit from an H3 hemagglutinin), but at a residue in sequence and structural vicinity to A_q. For example, in certain embodiments, the HA1 C-terminal stem segment of the chimeric influenza virus hemagglutinin polypeptides described herein starts at about A_{q-1}, A_{q-2}, A_{q-3}, A_{q-4}, A_{q-5}, A_{q-6}, A_{q-7}, A_{q-8}, A_{q-9}, A_{q-10}, A_{q-11}, A_{q-12}, A_{q-13}, A_{q-14}, A_{q-15}, A_{q-20}, A_{q-25}, A_{q-30}, A_{q-35}, A_{q-40}, A_{q-45}, A_{q-50}, A_{q-55}, A_{q-60}, A_{q-65}, A_{q-70}, A_{q-75}, or A_{q-80}. In certain embodiments, the HA1 C-terminal stem segment of the chimeric influenza virus hemagglutinin polypeptides described herein starts in the range of A_{q-1} to A_{q-5}, A_{q-5} to A_{q-10}, A_{q-10} to A_{q-15}, A_{q-15} to A_{q-20}, A_{q-20} to A_{q-25}, A_{q-25} to A_{q-30}, A_{q-30} to A_{q-35}, A_{q-35} to A_{q-40}, A_{q-40} to A_{q-45}, A_{q-45} to A_{q-50}, A_{q-50} to A_{q-55}, A_{q-55} to A_{q-60}, A_{q-60} to A_{q-65}, A_{q-65} to A_{q-70}, A_{q-75} to A_{q-80}. For example, an HA1 C-terminal stem segment ending at A_{q-77} would start at Gly200 of an H3 hemagglutinin; and an HA1 C-terminal stem segment ending at A_{q-10} would start at Isoleucine267 of an H3 hemagglutinin. In certain embodiments, the HA1 C-terminal stem segment of the chimeric influenza virus hemagglutinin polypeptides described herein starts at A_{q+1}, A_{q+2}, A_{q+3}, A_{q+4}, A_{q+5}, A_{q+6}, A_{q+7}, A_{q+8}, A_{q+9}, A_{q+10}, A_{q+11}, A_{q+12}, A_{q+13}, A_{q+14}, A_{q+15}, A_{q+16}, A_{q+17}, A_{q+18}, A_{q+19}, A_{q+20}, A_{q+21}, A_{q+22}, A_{q+23}, A_{q+24}, A_{q+25}, A_{q+26}, A_{q+27}, A_{q+28}, A_{q+29}, A_{q+30}. In certain embodiments, the HA1 C-terminal stem segment of the chimeric influenza

85, 80, 75, 70, 65, 60, 55, 50, 45, 40, 35, 30, 25, 20, 15, 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 long stem domain polypeptides comprising deleted and altered HA1 N-terminal long stem segments.

[00382] The HA1 C-terminal long stem segment can be any HA1 C-terminal long stem segment recognized by one of skill in the art based on the definition provided herein. Typically, an HA1 C-terminal long stem segment corresponds to a polypeptide consisting of the alanine residue located in sequence at approximately the 253rd residue of an HA1 (using H3 numbering) through the C-terminal amino acid of the HA1. This alanine residue, termed C_q herein, is generally capable of being linked to a cysteine residue C_p in the N-terminal long stem segment of HA1. Sequences of 16 representative influenza A hemagglutinins are presented in FIG. 1, and residue C_q is identified in each.

[00383] In certain embodiments, the HA1 C-terminal long stem segment does not start at C_q (*e.g.*, Ala_{253} of an HA1 subunit from an H3 hemagglutinin), but at a residue in sequence and structural vicinity to C_q. For example, in certain embodiments, the HA1 C-terminal long stem segment starts at C_{q-1}, C_{q-2}, C_{q-3}, or C_{q-4}. In other embodiments, the HA1 C-terminal long stem segment starts at C_{q+1}, C_{q+2}, C_{q+3}, C_{q+4} or C_{q+5}. The end of an HA1 N-terminal long stem segment should be selected in conjunction with the start of the HA1 C-terminal long 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.

[00384] In certain embodiments, the influenza hemagglutinin long stem domain polypeptides comprise an HA1 C-terminal long stem segment having at least 70%, 75%, 80%, 85%, 90%, 95%, 96% or 98% amino acid sequence identity to an influenza HA1 C-terminal long stem segment known to those of skill in the art. Exemplary known HA1 C-terminal long stem segments are provided in the tables below.

[00385] In certain embodiments, the end of the N-terminal long stem segment is C_{p-1}, and the start of the C-terminal long stem segment is C_{q-1}. In certain embodiments, the end of the N-terminal long stem segment is A_{p-2}, and the start of the C-terminal long stem segment is C_{q-2}. In certain embodiments, the end of the N-terminal long stem segment is C_{p-3}, and the start of the C-terminal long stem segment is C_{q-3}. In certain embodiments, the end of the N-terminal long stem segment is C_{p-4}, and the start of the C-terminal long stem segment is C_{q-4}. In certain embodiments, the end of the N-terminal long stem segment is C_{p-5}, and the start of the C-terminal long stem segment is C_{q-5}.

likely be negative for hemagglutination inhibition (HI) activity against H6 or H9 viruses (due to lack of prior exposure to these virus subtypes), yet would be reactive with the cH6/1 or cH9/1 constructs due to prior exposure to the H1 hemagglutinin stalk. These tools, recombinantly expressed cHA proteins and viruses expressing the cHAs, could be used to assess relative amounts of stalk antibodies in human serum samples and to measure any neutralizing activity mediated by those stalk-specific antibodies.

[00769] In order to generate cH6/1 and cH9/1 protein for analytical assays, plasmids coding for the chimeric HAs were generated and expressed as soluble proteins in a baculovirus expression system. Coomassie staining of 2μg of total protein suggests a high degree of purity in these preparations (Fig. 29A). The slight delayed migration of cH9/1 is thought to be the result of an increased number of occupied glycosylation sites on the cH9/1 head. The cHA proteins were further characterized by western blot analysis using antibodies reactive with various parts of the HA. As shown in Fig. 29B, only cH6/1, cH9/1 and full length HA from PR8 reacted with a rabbit polyclonal antiserum specific for the PR8 stalk. Monoclonal antibodies against the head domains of H6, H9, and PR8 confirmed that the chimeric constructs were expressing exotic heads atop a PR8 stalk. H3 protein was used as a negative control, and was only detected when using the pan-H3 antibody 12D1 (Wang *et al.*, 2010, *PLoS Pathog* 6, e1000796).

[00770] Recombinant viruses expressing the chimeric molecules were also rescued for the purpose of detecting neutralizing stalk antibodies. Because all human influenza viruses over the last century have encoded NA of the N1 or N2 subtype, it was reasoned that the rescue of the cH9/1N3 reassortant virus would allow for the assessment of the neutralizing capability of stalk-specific antibodies, while not measuring any (N1 or N2) neuraminidase antibody activity. The cH9/1N3 virus (expressing the H1 stalk with H9 globular head HA with an N3 subtype neuraminidase) was rescued using reverse genetics and grown to high titers in embryonated chicken eggs. The plaque assay phenotype of this virus was similar to that of PR8 wild type virus (Fig. 29C). To confirm the presence of the H9 head after virus passage, cells were infected with cH9/1N3 virus. Infected cells were then probed with mouse mAb G1-26, an antibody specific for H9 subtype hemagglutinin proteins. A pan-H1 stalk specific antibody, 6F12, was used to detect both wild type PR8 and cH9/1N3 virus infected cells (Fig. 26B).

Stalk specific antibodies bind and neutralize cHA

constructs. This "double peak" expression pattern was observed only in transfection conditions, and was reproducible. It was not detected upon infection with either cH7/3 or cH5/3-expressing recombinant viruses (Fig. 33). Therefore, these data indicate that the cHAs can be transported through the Golgi complex to the cell surface.

[00800] Next, the entry characteristics of the different cHAs through transduction of MDCK cells were examined using retroviral pseudotype particles that contained a luciferase reporter construct and expressed the cHA and wild-type B/Yamagata/16/88 virus NA on the particle surface. The entry efficiency mediated by the cHA proteins was detected by the luciferase read-out. Comparable levels of pseudotype particle-mediated luciferase expression were observed for cH5/1, cH7/3 and cH5/3 chimeric HAs and the corresponding wild type proteins (Fig. 34). Particles encoding the cH1/1 HA expressed lower luciferase levels compared to the other HA constructs, which could be due to either the lower expression of the cH1/1 in the producer cell line and hence fewer HA trimers per particle or the less efficient entry properties of the cH1/1 HA. It is also possible that when normalizing the pseudotype particles to 4 hemagglutinin units, the actual amount of pseudotype particles may vary due to differences in binding to red blood cells.

Generation of recombinant influenza viruses bearing chimeric hemagglutinins

[00801] Because it had determined that our cHA constructs were efficiently expressed and transported to the cell surface, a study was performed to assess whether a recombinant influenza virus that encodes a cHA could be rescued. Viruses containing the different cHAs were successfully generated using previously published protocols (*see, e.g.*, Fodor *et al.*, 1999, *J Virol* 73:9679-9682; and Hai *et al.*, 2008, *J Virol* 82:10580-10590). The resulting viruses were plaque purified, amplified in 10 day old embryonated eggs and the chimeric segments were analyzed by RT-PCR and sequenced. In all cases, the virus was found to have the expected chimeric HA segment and no other HA segment (data not shown).

[00802] The presence of the cHAs in rescued viruses was further confirmed by Western blot and indirect immunofluorescence of infected cells (Figure 35 and 36). MDCK cells were infected with rWT PR8, wild-type Perth/09, cH1/1, cH5/1, cH7/3 and cH5/3 viruses (Figure 35 and 36). cH1/1 and cH5/1 chimeric HA proteins were detected in the corresponding samples using antibodies reactive against the head domains of Cal/09 (H1) HA (29E3) (Medina *et al.*, 2010, *Nature Communications* 1:28) or VN/04 (H5) HA (mAb #8) (Steel *et al.*, 2009, *J Virol* 83:1742-53) respectively (Figure 35). Comparable expression levels among the cH7/3, cH5/3 and wild type Perth HA using 12D1, a pan-H3 anti-stalk mAb (*see*

WHAT IS CLAIMED IS:

1. A chimeric influenza virus hemagglutinin (HA) polypeptide comprising an HA stem domain and an HA globular head domain, wherein the HA globular head domain is from a different influenza virus subtype or strain than the influenza virus subtype or strain of the HA stem domain, wherein

(a) the HA stem domain comprises (i) an HA1 N-terminal stem segment, wherein the HA1 N-terminal stem segment consists of amino acid residues $HA1_{N\text{-}term}$ through A_{p-1}, A_{p-2}, A_{p-3}, A_{p-4}, A_{p-5}, A_{p-6}, A_{p-7}, A_{p-8}, A_{p-9}, Ap_{-10}, A_p, A_{p+1}, Ap_{+2}, A_{p+3}, A_{p+4}, A_{p+5}, A_{p+6}, A_{p+7}, A_{p+8}, A_{p+9}, or A_{p+10}; (ii) an HA1 C-terminal stem segment, wherein the HA1 C-terminal stem segment consists of amino acid residues A_{q-1}, A_{q-2}, A_{q-3}, A_{q-4}, A_{q-5}, A_{q-6}, A_{q-7}, A_{q-8}, A_{q-9}, A_{q-10}, A_q, A_{q+1}, A_{q+2}, A_{q+3}, A_{q+4}, A_{q+5}, A_{q+6}, A_{q+7}, A_{q+8}, A_{q+9}, or A_{q+10} through $HA1_{C\text{-}term}$; and (iii) an HA2 stem domain; and

(b) the HA globular head domain comprises the amino acid residues between (i) A_{p-1}, A_{p-2}, A_{p-3}, A_{p-4}, A_{p-5}, A_{p-6}, A_{p-7}, A_{p-8}, A_{p-9}, Ap_{-10}, A_p, A_{p+1}, Ap_{+2}, A_{p+3}, A_{p+4}, A_{p+5}, A_{p+6}, A_{p+7}, A_{p+8}, A_{p+9}, or A_{p+10} and (ii) A_{q-1}, A_{q-2}, A_{q-3}, A_{q-4}, A_{q-5}, A_{q-6}, A_{q-7}, A_{q-8}, A_{q-9}, A_{q-10}, A_q, A_{q+1}, A_{q+2}, A_{q+3}, A_{q+4}, A_{q+5}, A_{q+6}, A_{q+7}, A_{q+8}, A_{q+9}, or A_{q+10} of an HA1 domain; wherein $HA1_{N\text{-}term}$ is the N-terminal amino acid of a mature HA0 protein lacking a signal peptide; wherein HA1C-term is the C-terminal amino acid of an HA1 domain; and wherein A_p is the Cys that corresponds to amino acid position 52 of an HA1 domain using H3 numbering; wherein Aq is the Cys that corresponds to amino acid position 277 of an HA1 domain using H3 numbering.

2. The chimeric influenza virus hemagglutinin (HA) polypeptide of claim 1, wherein

(a) the HA stem domain comprises (i) an HA1 N-terminal stem segment, wherein the HA1 N-terminal stem segment consists of amino acid residues $HA1_{N\text{-}term}$ through A_{p-1}, A_{p-2}, A_{p-3}, A_{p-4}, A_{p-5}, A_{p-6}, A_{p-7}, A_{p-8}, A_{p-9}, Ap_{-10}, A_p, A_{p+1}, Ap_{+2}, A_{p+3}, A_{p+4}, A_{p+5}, A_{p+6}, A_{p+7}, A_{p+8}, A_{p+9}, or A_{p+10}; (ii) an HA1 C-terminal stem segment, wherein the HA1 C-terminal stem segment consists of amino acid residues A_{q-1}, A_{q-2}, A_{q-3}, A_{q-4}, A_q, A_{q+1}, A_{q+2}, or A_{q+3} through $HA1_{C\text{-}term}$; and (iii) an HA2 stem domain; and

(b) the HA globular head domain comprises the amino acid residues between (i) Ap-1, Ap-2, Ap-3, Ap-4, Ap-5, Ap-6, Ap-7, Ap-8, Ap-9, Ap-10, Ap, Ap+1, Ap+2, Ap+3, Ap+4, Ap+5, Ap+6, Ap+7, Ap+8, Ap+9, or Ap+10 and (ii) Aq-1, Aq-2, Aq-3, Aq-4, Aq, Aq+1, Aq+2, or Aq+3 of an HA1 domain; and wherein $HA1_{N\text{-}term}$ is the N-terminal amino acid of a mature HA0 protein

lacking a signal peptide; wherein $HA1_{C\text{-}term}$ is the C-terminal amino acid of an HA1 domain; and wherein Ap is the Cys that corresponds to amino acid position 52 of an HA1 domain using H3 numbering; wherein Aq is the Cys that corresponds to amino acid position 277 of an HA1 domain using H3 numbering.

3. A chimeric influenza virus hemagglutinin (HA) polypeptide comprising an HA stem domain and an HA globular head domain, wherein the HA globular head domain is from a different influenza virus strain or subtype than the influenza virus strain or subtype of the HA stem domain, wherein

(a) the HA stem domain comprises (i) an HA1 N-terminal stem segment, wherein the HA1 N-terminal stem segment consists of amino acid residues $HA1_{N\text{-}term}$ through A_p; (ii) an HA1 C-terminal stem segment, wherein the HA1 C-terminal stem segment consists of amino acid residues A_q through $HA1_{C\text{-}term}$; and (iii) an HA2 stem domain; and

(b) the HA globular head domain comprises the amino acid residues between A_p and A_q of an HA1 domain; and wherein $HA1_{N\text{-}term}$ is the N-terminal amino acid of a mature HA0 protein lacking a signal peptide; wherein $HA1_{C\text{-}term}$ is the C-terminal amino acid of an HA1 domain; and wherein Ap is the Cys that corresponds to amino acid position 52 of an HA1 domain using H3 numbering; wherein A_q is the Cys that corresponds to amino acid position 277 of an HA1 domain using H3 numbering.

4. The chimeric influenza virus hemagglutinin (HA) polypeptide of any one of claims 1 to 3, wherein the HA globular head domain is from a different influenza virus strain than the influenza virus strain of the HA stem domain.

5. The chimeric influenza virus HA polypeptide of any one of claims 1 to 3, wherein the HA stem domain is from a different subtype than the influenza subtype of the HA globular head domain.

6. The chimeric influenza virus HA polypeptide of any one of claims 1 to 5, wherein the HA stem domain is the HA stem domain of an influenza virus of subtype H1.

7. The chimeric influenza virus HA polypeptide of claim 6, wherein the H1 subtype is influenza virus A/California/04/09.

8. The chimeric influenza virus HA polypeptide of any one of claims 1 to 5, wherein the HA stem domain is the HA stem domain of an influenza virus of subtype H3.

9. The chimeric influenza virus HA polypeptide of claim 8, wherein the H3 subtype is influenza virus A/Perth/16/2009.

10. The chimeric influenza virus HA polypeptide of any one of claims 1 to 9, wherein the HA globular head domain is the HA globular head domain of an influenza virus of subtype H4, H6, H7, H8, H9, H10, H11, H12, H13, H14, H15, H16, or H17.

11. The chimeric influenza virus HA polypeptide of any one of claims 1 to 9, wherein the HA globular head domain is the HA globular head domain of an influenza virus of subtype H5.

12. The chimeric influenza virus HA polypeptide of claim 6 or 7, wherein the HA globular head domain is the HA globular head domain of an influenza virus of subtype H2, H6, H8, H9, H11, H12, H13, or H16.

13. The chimeric influenza virus HA polypeptide of claim 6 or 7, wherein the HA globular head domain is the HA globular head domain of an influenza virus of subtype H8, H11, or H12.

14. The chimeric influenza virus HA polypeptide of claim 8 or 9, wherein the HA globular head domain is the HA globular head domain of an influenza virus of subtype H4, H7, H10, H14, or H15.

15. The chimeric influenza virus HA polypeptide of claim 9, wherein the HA globular head domain is the HA globular head domain of influenza virus A/Vietnam/1203/04 (H5).

16. The chimeric influenza virus HA polypeptide of claim 9, wherein the HA globular head domain is the HA globular head domain of influenza virus A/Alberta/24/01 (H7).

17. The chimeric influenza virus HA polypeptide of any one of claims 1 to 16, wherein the chimeric influenza virus HA polypeptide further comprises an HA2 luminal domain, and HA2 transmembrane domain and an HA2 cytoplasmic domain.

18. The chimeric influenza virus HA polypeptide of claim 17, wherein the HA2 luminal domain, the HA2 transmembrane domain and the HA2 cytoplasmic domain are from the same influenza virus as the HA stem domain.

19. The chimeric influenza virus HA polypeptide of any of claims 1 to 16, which comprises an HA2 domain.

20. The chimeric influenza virus HA polypeptide of any of claims 1 to 16, which is soluble.

21. The chimeric influenza virus HA polypeptide of any of claims 1 to 16 or 20, which lacks an HA2 luminal domain, an HA2 transmembrane domain and an HA2 cytoplasmic domain.

22. The chimeric influenza virus HA polypeptide of claim 20, wherein the polypeptide comprises a foldon or trimerization domain.

23. The chimeric influenza virus HA polypeptide of any one of claims 1 to 22, wherein the HA2 stem domain is resistant to protease cleavage at the junction between HA1 and HA2.

24. The chimeric influenza virus HA polypeptide of any one of claims 1 to 23, which has fusogenic activity of an influenza virus HA.

25. The chimeric influenza virus HA polypeptide of any one of claims 1 to 24, which is expressed in and isolated from a mammalian cell.

26. A nucleic acid sequence encoding the polypeptide of any of claims 1 to 24.

27. An expression vector containing the nucleic acid sequence of claim 25.

28. The expression vector of claim 27, which is a replication-defective retrovirus, an adenovirus, an adeno-associated virus or a baculovirus.

29. An isolated cell expressing the nucleic acid sequence of claim 26.

30. A virus comprising a genome engineered to express the nucleic acid sequence of claim 26.

31. A virus comprising the polypeptide of any one of claims 1 to 24.

32. The virus of claim 30 or 31, wherein the virus is an influenza virus.

33. The virus of claim 32, wherein the influenza virus is an influenza A virus.

34. The virus of claim 30 or 31, which is a Newcastle disease virus, a vaccinia virus, adenovirus, adeno-associated virus, hepatitis B virus, a retrovirus, an alphavirus, a rhabdovirus, a poxvirus, a metapneumovirus virus, a measles virus, a herpes virus or foamyvirus.

35. The virus of any one of claims 30 to 34, which is inactivated or split.

36. The virus of any one of claims 30 to 34, which is live attenuated.

37. A virus-like particle comprising the polypeptide of any one of claims 1 to 24.

38. An immunogenic composition comprising the polypeptide of any one of claims 1 to 25.

39. An immunogenic composition comprising the virus of claim 30 to 36.

40. An immunogenic composition comprising the virus-like particle of claim 37.

41. An immunogenic composition comprising the nucleic acid of claim 26.

42. An immunogenic composition comprising the expression vector of claim 27 or 28.

43. The immunogenic composition of claim 39, which further comprises an adjuvant.

44. The immunogenic composition of claim 43, wherein the adjuvant is AS03, an aluminum salt, 3 De-O-acylated monophosphoryl lipid A (MPL), MF59, AS04, polysorbate 80, an imidazopyridine compound, an imidazoquinoxaline compound, a saponin, Freund's adjuvant, an oil in water emulsion, or CpG.

45. The immunogenic composition of claim 38, 40, 41 or 42, which further comprises an adjuvant.

46. The immunogenic composition of claim 45, wherein the adjuvant is AS03, an aluminum salt, 3 De-O-acylated monophosphoryl lipid A (MPL), MF59, AS04, polysorbate 80, an imidazopyridine compound, an imidazoquinoxaline compound, a saponin, Freund's adjuvant, an oil in water emulsion, or CpG.

47. The immunogenic composition of claim 39, 43 or 44, which is formulated for intramuscular or intranasal administration.

48. The immunogenic composition of any one of claims 38, 40 to 42, 46 or 47, which is formulated for intramuscular or intranasal administration.

49. Use of the immunogenic composition of any one of claims 38 to 48 in the manufacture of a medicament for immunizing a subject against influenza virus infection or influenza virus disease.

50. Use of the immunogenic composition of any one of claims 38 to 48 in the manufacture of a medicament for inducing an immune response to an influenza virus HA polypeptide in a subject.

51. Use of the immunogenic composition of any one of claims 38 to 48 in the manufacture of a medicament for preventing influenza disease in a subject.

52. Use of the immunogenic composition of any one of claims 38 to 48 in the manufacture of a medicament for treating influenza virus infection or influenza virus disease in a subject.

53. Use of the immunogenic composition of any one of claims 38 to 48 in the manufacture of a medicament for immunizing a subject against an influenza virus infection or an influenza virus disease, wherein the immunogenic composition is to be administered to the subject in combination with a second immunogenic composition comprising a second chimeric influenza virus HA polypeptide, wherein the second chimeric influenza virus HA polypeptide comprises a second HA globular domain and a second HA stem domain, wherein the second HA globular head domain is from a different influenza virus strain or subtype than the influenza virus strain or subtype of the second HA stem domain, and wherein the second chimeric influenza virus HA polypeptide comprises the same HA stem domain as the chimeric influenza virus HA polypeptide.

54. The use of claim 53, wherein the second HA globular head domain is from a different influenza virus strain than the influenza virus strain of the second HA stem domain.

55. The use of claim 53, wherein the second HA globular head domain is from a different influenza virus subtype than the influenza virus subtype of the second HA stem domain.

56. The use of any one of claims 53 to 55, wherein the HA globular head domain of the chimeric influenza virus HA polypeptide of the immunogenic composition is different than

the second HA globular head domain of the second chimeric influenza virus HA polypeptide of the second immunogenic composition.

57. The use of claim 49, wherein the immunogenic composition is to be administered to the subject in combination with a second virus, wherein the second virus is an influenza virus, wherein the chimeric influenza virus HA polypeptide comprises the same HA stem domain as the HA polypeptide of the second virus.

58. The use of claim 57, wherein the HA globular head domain of the HA polypeptide of the second virus and the chimeric influenza virus HA polypeptide are heterologous.

59. The immunogenic composition of any one of claims 38 to 48 for use in therapy.

60. The use of any one of claims 49 to 58 or the immunogenic composition for use of claim 59, wherein the subject is human.

61. The use of any one of claims 49 to 58 or the immunogenic composition for use of claim 59, wherein the subject is a human infant.

62. The use of any one of claims 49 to 58 or the immunogenic composition for use of claim 59, wherein the subject is a human adult.

63. The use of any one of claims 49 to 58 or the immunogenic composition for use of claim 59, wherein the subject is a human child.

64. The use of any one of claims 49 to 58 or the immunogenic composition for use of claim 59, wherein the subject is an elderly human.

65. An *in vitro* method for producing the virus of any one of claims 30 to 34 or 36, comprising (a) propagating the virus of any one of claims 30 to 34 or 36 in an embryonated egg or cell or cell line; and (b) isolating the virus from the embryonated egg or cell or cell line.

66. A method for producing the chimeric influenza virus HA polypeptide of any one of claims 1 to 24, comprising: (a) culturing the cell of claim 29; and (b) isolating the polypeptide from the medium or isolated cell.

67. The method of claim 65 or 66, wherein the cell is an insect cell.

68. The method of claim 65 or 66, wherein the cell is a mammalian cell.