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).

[0061] "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 277-346 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. 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).

[0062] "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.

[0063] 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.

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.

[0097] **Fig. 9** 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).

[0098] **Fig. 10** 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).

[0099] **Fig. 11** depicts 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 (A), Cal/09 infection (B), DNA and Cal/09 infection (C), or Cal/09 split vaccine (D) were added to transfected cells and visualized by fluorescence microscopy following incubation with an Alexa Fluor 594-conjugated anti-mouse IgG.

[00100] **Fig. 12** 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 5×10^4 PFU of PR8 virus, instilled intranasally, and the weight of the animals was monitored for 14 days.

[00101] **Fig. 13** demonstrates reactivity of stalk specific antibodies to cH6 protein as determined by ELISA.

(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).

[00114] **Fig. 26** depicts 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. 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. (B) The mAb also inhibits entry of cH1/1 (black boxes) and cH5/1 (black triangles) 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.

[00115] **Fig. 27** demonstrates that sequential vaccination with cHA constructs elicits HA stalk-specific antibodies and provides protection from lethal challenge. Mice were primed with 20 μg of cH9/1 protein, administered with adjuvant intranasally and intraperitoneally. Three weeks later, mice were boosted with 20 μg cH6/1 protein, administered with adjuvant intranasally and intraperitoneally. As controls, mice were primed and boosted in a similar fashion using BSA, or given inactivated FM1 virus intramuscularly. Animals were bled and challenged three weeks after the last vaccination with 5LD50 of A/Fort Monmouth/1/1947 (FM1) virus. (A) ELISA plates were coated with cH5/1 N1 virus in order to assess the degree of stalk reactivity elicited through vaccination (BSA + BSA is the bottom line of the graph). (B) Mice were weighed daily for 14 days to assess protection from challenge. (C) Kaplan Meier curve depicting survival rate post challenge. cH9/1 + cH6/1 vaccinated mice had a statistically higher survival rate compared to BSA controls ($p = .0003$)

[00116] **Fig. 28** demonstrates that vaccination with cH6/1 elicits stalk-specific immunity that mediates protection from cH9/1 N1 viral challenge. Animals were inoculated with YAM-HA

tag or foldon domain or both. Useful cleavage sites include a thrombin cleavage site, for example one with the sequence LVPRGSP (SEQ ID NO:19). 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) (SEQ ID NO:20)).

[00172] In certain embodiments, the chimeric influenza hemagglutinin polypeptides described herein are soluble polypeptides.

[00173] 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.

[00174] 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}. 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}. For example, an HA1 N-terminal stem segment ending at A_{p-10} would end at Lys42 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}. 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}. 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.

[00175] In certain embodiments, the HA1 C-terminal stem segment of the chimeric influenza virus hemagglutinin polypeptides described herein does not start exactly 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}. 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}. For example, an HA1 C-terminal stem segment ending at A_{q-10} would start at Isoleucine262 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}. 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+3}, A_{q+3} to A_{q+5}, A_{q+5} to A_{q+8}, A_{q+8} to A_{q+10}. 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 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.

[00176] In one example, an HA1 N-terminal stem segment of a chimeric influenza virus hemagglutinin polypeptide described herein may end at any one of hemagglutinin amino acid positions 45-48 (using H3 numbering) and an HA1 C-terminal stem segment of the chimeric

human serum samples and to measure any neutralizing activity mediated by those stalk-specific antibodies.

[00497] 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. 17A). 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. 17B, 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).

[00498] 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. 14C). 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. 14B).

6.4.2.2 Stalk specific antibodies bind and neutralize cHA

[00499] In order to confirm that cH6/1 and cH9/1 proteins could be used as tools to detect stem antibodies, the use of these cHAs was first validated with an antibody known to react with the HA stalk. Indeed, mouse mAb C179, an antibody reactive with the stalk of H1 HA (Okuno *et al.*, 1993, *J Virol* 67, 2552), bound to baculovirus expressed cH6/1 and cH9/1 protein by ELISA in a dose dependent manner (Figs. 18A and B).

WHAT IS CLAIMED IS:

1. A chimeric influenza virus hemagglutinin (HA) polypeptide comprising the stem domain of the HA from influenza virus A/California/4/2009 (H1N1) and the globular head domain of the HA from an influenza virus of the H5 subtype.

2. The chimeric influenza virus hemagglutinin (HA) polypeptide of claim 1, wherein the H5 subtype is influenza virus A/Vietnam/1203/2004 (H5), A/Indonesia/5/2005 (H5), A/Anhui/1/2005 (H5), A/bar headed goose/Quinghai/1A/2005 (H5), A/turkey/Turkey/1/2005 (H5), or A/whooperswan/Mongolia/244/2005 (H5).

3. A chimeric influenza virus hemagglutinin (HA) polypeptide comprising the stem domain of the HA from influenza virus A/Victoria/361/2011 (H3N2), A/harbor seal/Massachusetts/1/2011 (H3N8), or A/Indiana/10/2011 (H3N2) and the globular head domain of the HA from an influenza virus of the H5 subtype.

4. The chimeric influenza virus hemagglutinin (HA) polypeptide of claim 3, wherein the H5 subtype is influenza virus HA of A/Vietnam/1203/2004 (H5), A/Indonesia/5/2005 (H5), A/Anhui/1/2005 (H5), A/bar headed goose/Quinghai/1A/2005 (H5), A/turkey/Turkey/1/2005 (H5), or A/whooperswan/Mongolia/244/2005 (H5).

5. A chimeric influenza virus hemagglutinin (HA) polypeptide comprising the stem domain of the HA from influenza virus A/Victoria/361/2011 (H3N2), A/harbor seal/Massachusetts/1/2011 (H3N8), or A/Indiana/10/2011 (H3N2) and the globular head domain of the HA from an influenza virus of the H7 subtype.

6. The chimeric influenza virus hemagglutinin (HA) polypeptide of claim 5, wherein the H7 subtype is influenza virus A/Netherlands/219/03 (H7), A/Canada/504/04 (H7), A/Canada/444/04 (H7), A/chicken/Jalisco/CPA1/2012 (H7), A/mallard/Alberta/24/2001 (H7), A/rhea/NC/39482/93 (H7), or A/mallard/Netherlands/12/2000 (H7).

7. A chimeric influenza virus hemagglutinin (HA) polypeptide comprising the stem domain of the HA from influenza virus B/Malaysia/2506/2004, B/Florida/4/2006, B/Wisconsin/1/2010, or B/Brisbane/60/2008 and the globular head domain of the HA from an influenza virus of the H5 subtype.

8. The chimeric influenza virus hemagglutinin (HA) polypeptide of claim 7, wherein the H5 subtype is influenza virus A/Vietnam/1203/2004 (H5), A/Indonesia/5/2005 (H5), A/Anhui/1/2005 (H5), A/bar headed goose/Quinghai/1A/2005 (H5), A/turkey/Turkey/1/2005 (H5), or A/whooperswan/Mongolia/244/2005 (H5).

9. A chimeric influenza virus hemagglutinin (HA) polypeptide comprising the stem domain of the HA from influenza virus B/Malaysia/2506/2004, B/Florida/4/2006, B/Wisconsin/1/2010, or B/Brisbane/60/2008 and the globular head domain of the HA from an influenza virus of the H7 subtype.

10. The chimeric influenza virus hemagglutinin (HA) polypeptide of claim 9, wherein the H7 subtype is influenza virus A/Netherlands/219/03 (H7), A/Canada/504/04 (H7), A/Canada/444/04 (H7), A/chicken/Jalisco/CPA1/2012 (H7), A/mallard/Alberta/24/2001 (H7), A/rhea/NC/39482/93 (H7), or A/mallard/Netherlands/12/2000 (H7).

11. A chimeric influenza virus hemagglutinin (HA) polypeptide comprising the stem domain of the HA from influenza virus B/Malaysia/2506/2004, B/Florida/4/2006, B/Wisconsin/1/2010, or B/Brisbane/60/2008 and the globular head domain of the HA from a different strain of influenza B virus.

12. The chimeric influenza virus hemagglutinin (HA) polypeptide of claim 11, wherein the different strain of influenza B virus is B/Lee/1940 or B/seal/Netherlands/1/99.

13. A nucleic acid encoding the chimeric influenza virus hemagglutinin (HA) polypeptide of any one of claims 1 to 12.

14. A cell expressing the nucleic acid of claim 13.

15. A virus comprising a genome engineered to express the nucleic acid of claim 13.

16. A virus comprising the polypeptide of any one of claims 1 to 12.

17. The virus of claim 15, wherein the virus is an influenza virus, VSV, NDV, adenovirus, or baculovirus.

18. The virus of claim 16, wherein the virus is an influenza virus, VSV, NDV, adenovirus, or baculovirus.

19. The virus of claim 17, which is inactivated or split.

20. The virus of claim 18, which is inactivated or split.

21. A virus-like particle comprising the polypeptide of any one of claims 1 to 12.

22. An immunogenic composition comprising the polypeptide of any one of claims 1 to 12.

23. An immunogenic composition comprising the virus of claim 15.

24. An immunogenic composition comprising the virus of claim 16.

25. An immunogenic composition comprising the virus of claim 17.

26. An immunogenic composition comprising the virus of claim 18.

27. An immunogenic composition comprising the virus-like particle of claim 21.

28. An immunogenic composition comprising:

(i) a first chimeric influenza virus hemagglutinin (HA) polypeptide, wherein the polypeptide comprises the stem domain of the HA of influenza virus A/California/4/2009 (H1N1) and the globular head domain of the HA of influenza virus /Vietnam/1203/2004 (H5),

A/Indonesia/5/2005 (H5), A/Anhui/1/2005 (H5), A/bar headed goose/Quinghai/1A/2005 (H5), A/turkey/Turkey/1/2005 (H5), or A/whooperswan/Mongolia/244/2005 (H5); and

(ii) a second chimeric influenza virus hemagglutinin (HA) polypeptide, wherein the polypeptide comprises (a) the stem domain of the HA of influenza virus A/Victoria/361/2011 (H3N2), A/harbor seal/Massachusetts/1/2011 (H3N8), or A/Indiana/10/2011 (H3N2); and (b) the globular head domain of the HA of influenza virus A/Vietnam/1203/2004 (H5), A/Indonesia/5/2005 (H5), A/Anhui/1/2005 (H5), A/bar headed goose/Quinghai/1A/2005 (H5), A/turkey/Turkey/1/2005 (H5), or A/whooperswan/Mongolia/244/2005 (H5).

29. An immunogenic composition comprising:

(i) a first chimeric influenza virus hemagglutinin (HA) polypeptide, wherein the polypeptide comprises the stem domain of the HA of influenza virus A/California/4/2009 (H1N1) and the globular head domain of the HA of influenza virus A/Vietnam/1203/2004 (H5), A/Indonesia/5/2005 (H5), A/Anhui/1/2005 (H5), A/bar headed goose/Quinghai/1A/2005 (H5), A/turkey/Turkey/1/2005 (H5), or A/whooperswan/Mongolia/244/2005 (H5); and

(ii) a second chimeric influenza virus hemagglutinin (HA) polypeptide, wherein the polypeptide comprises (a) the stem domain of the HA of influenza virus A/Victoria/361/2011 (H3N2), A/harbor seal/Massachusetts/1/2011 (H3N8), or A/Indiana/10/2011 (H3N2); and (b) the globular head domain of the HA of influenza virus B/Malaysia/2506/2004 and the globular head domain of the HA of influenza virus A/Netherlands/219/03 (H7), A/Canada/504/04 (H7), A/Canada/444/04 (H7), A/chicken/Jalisco/CPA1/2012 (H7), A/mallard/Alberta/24/2001 (H7), A/rhea/NC/39482/93 (H7), or A/mallard/Netherlands/12/2000 (H7).

30. The immunogenic composition of claim 28 or 29, further comprising a third chimeric influenza virus hemagglutinin (HA) polypeptide, wherein the polypeptide comprises (a) the stem domain of the HA of influenza virus B/Malaysia/2506/2004, B/Florida/4/2006, B/Wisconsin/1/2010; or B/Brisbane/60/2008; and (b) the globular head domain of the HA of influenza virus A/Vietnam/1203/2004 (H5), A/Indonesia/5/2005 (H5), A/Anhui/1/2005 (H5), A/bar headed goose/Quinghai/1A/2005 (H5), A/turkey/Turkey/1/2005 (H5), A/whooperswan/Mongolia/244/2005 (H5), B/Lee/1940, or B/Seal/Netherlands/1/99.

31. An immunogenic composition comprising:

(i) a first virus comprising a genome engineered to express a first nucleic acid that encodes a chimeric influenza virus hemagglutinin (HA) polypeptide, wherein the polypeptide comprises the stem domain of the HA of influenza virus A/California/4/2009 (H1N1) and the globular head domain of the HA of influenza virus A/Vietnam/1203/2004 (H5), A/Indonesia/5/2005 (H5), A/Anhui/1/2005 (H5), A/bar headed goose/Quinghai/1A/2005 (H5), A/turkey/Turkey/1/2005 (H5), or A/whooperswan/Mongolia/244/2005 (H5); and

(ii) a second virus comprising a genome engineered to express a second nucleic acid that encodes a chimeric influenza virus hemagglutinin (HA) polypeptide, wherein the polypeptide comprises (a) the stem domain of the HA of influenza virus A/Victoria/361/2011 (H3N2), A/harbor seal/Massachusetts/1/2011 (H3N8), or A/Indiana/10/2011 (H3N2); and (b) the globular head domain of the HA of influenza virus A/Vietnam/1203/2004 (H5), A/Indonesia/5/2005 (H5), A/Anhui/1/2005 (H5), A/bar headed goose/Quinghai/1A/2005 (H5), A/turkey/Turkey/1/2005 (H5), or A/whooperswan/Mongolia/244/2005 (H5).

32. An immunogenic composition comprising:

(i) a first virus comprising a genome engineered to express a first nucleic acid that encodes a chimeric influenza virus hemagglutinin (HA) polypeptide, wherein the polypeptide comprises the stem domain of the HA of influenza virus A/California/4/2009 (H1N1) and the globular head domain of the HA of influenza virus A/Vietnam/1203/2004 (H5), A/Indonesia/5/2005 (H5), A/Anhui/1/2005 (H5), A/bar headed goose/Quinghai/1A/2005 (H5), A/turkey/Turkey/1/2005 (H5), or A/whooperswan/Mongolia/244/2005 (H5); and

(ii) a second virus comprising a genome engineered to express a second nucleic acid that encodes a chimeric influenza virus hemagglutinin (HA) polypeptide, wherein the polypeptide comprises (a) the stem domain of the HA of influenza virus A/Victoria/361/2011 (H3N2), A/harbor seal/Massachusetts/1/2011 (H3N8), or A/Indiana/10/2011 (H3N2); and (b) the globular head domain of the HA of influenza virus B/Malaysia/2506/2004 and the globular head domain of the HA of A/Netherlands/219/03 (H7), A/Canada/504/04 (H7), A/Canada/444/04 (H7), A/chicken/Jalisco/CPA1/2012 (H7), A/mallard/Alberta/24/2001 (H7), A/rhea/NC/39482/93 (H7), or A/mallard/Netherlands/12/2000 (H7).

33. The immunogenic composition of claim 31 or 32, further comprising a third virus comprising a genome engineered to express a third nucleic acid that encodes a chimeric influenza virus hemagglutinin (HA) polypeptide, wherein the polypeptide comprises (a) the stem domain of the HA of influenza virus B/Malaysia/2506/2004, B/Florida/4/2006, B/Wisconsin/1/2010; or B/Brisbane/60/2008; and (b) the globular head domain of the HA of influenza virus A/Vietnam/1203/2004 (H5), A/Indonesia/5/2005 (H5), A/Anhui/1/2005 (H5), A/bar headed goose/Quinghai/1A/2005 (H5), A/turkey/Turkey/1/2005 (H5), A/whooperswan/Mongolia/244/2005 (H5), B/Lee/1940, or B/Seal/Netherlands/1/99.

34. An immunogenic composition comprising:

(i) a first virus comprising a first chimeric influenza virus hemagglutinin (HA) polypeptide, wherein the polypeptide comprises the stem domain of the HA of influenza virus A/California/4/2009 (H1N1) and the globular head domain of the HA of influenza virus A/Vietnam/1203/2004 (H5), A/Indonesia/5/2005 (H5), A/Anhui/1/2005 (H5), A/bar headed goose/Quinghai/1A/2005 (H5), A/turkey/Turkey/1/2005 (H5), or A/whooperswan/Mongolia/244/2005 (H5); and

(ii) a second virus comprising a second chimeric influenza virus hemagglutinin (HA) polypeptide, wherein the polypeptide comprises (a) the stem domain of the HA of influenza virus A/Victoria/361/2011 (H3N2), A/harbor seal/Massachusetts/1/2011 (H3N8), or A/Indiana/10/2011 (H3N2); and (b) the globular head domain of the HA of influenza virus A/Vietnam/1203/2004 (H5), A/Indonesia/5/2005 (H5), A/Anhui/1/2005 (H5), A/bar headed goose/Quinghai/1A/2005 (H5), A/turkey/Turkey/1/2005 (H5), or A/whooperswan/Mongolia/244/2005 (H5).

35. An immunogenic composition comprising:

(i) a first virus comprising a first chimeric influenza virus hemagglutinin (HA) polypeptide, wherein the polypeptide comprises the stem domain of the HA of influenza virus A/California/4/2009 (H1N1) and the globular head domain of the HA of influenza virus A/Vietnam/1203/2004 (H5), A/Indonesia/5/2005 (H5), A/Anhui/1/2005 (H5), A/bar headed goose/Quinghai/1A/2005 (H5), A/turkey/Turkey/1/2005 (H5), or A/whooperswan/Mongolia/244/2005 (H5); and

(ii) a second virus comprising a second chimeric influenza virus hemagglutinin (HA) polypeptide, wherein the polypeptide comprises (a) the stem domain of the HA of influenza virus A/Victoria/361/2011 (H3N2), A/harbor seal/Massachusetts/1/2011 (H3N8), or A/Indiana/10/2011 (H3N2); and (b) the globular head domain of the HA of influenza virus A/Netherlands/219/03 (H7), A/Canada/504/04 (H7), A/Canada/444/04 (H7), A/chicken/Jalisco/CPA1/2012 (H7), A/mallard/Alberta/24/2001 (H7), A/rhea/NC/39482/93 (H7), or A/mallard/Netherlands/12/2000 (H7); and

36. The immunogenic composition of claim 34 or 35, further comprising a third virus comprising a third chimeric influenza virus hemagglutinin (HA) polypeptide, wherein the polypeptide comprises (a) the stem domain of the HA of influenza virus B/Malaysia/2506/2004, B/Florida/4/2006, B/Wisconsin/1/2010; or B/Brisbane/60/2008; and (b) the globular head domain of the HA of influenza virus A/Vietnam/1203/2004 (H5), A/Indonesia/5/2005 (H5), A/Anhui/1/2005 (H5), A/bar headed goose/Quinghai/1A/2005 (H5), A/turkey/Turkey/1/2005 (H5), A/whooperswan/Mongolia/244/2005 (H5), B/Lee/1940, or B/Seal/Netherlands/1/99.

37. The immunogenic composition of any one of claims 31-36, wherein each of the viruses in the immunogenic composition is an influenza virus, VSV, NDV, or adenovirus.

38. The immunogenic composition of any one of claims 31-36, wherein each of the viruses in the immunogenic composition is inactivated or split.

39. The immunogenic composition of claim 37, wherein each of the influenza viruses is inactivated or split.

40. A method of immunizing a subject against influenza virus comprising administering to the subject an effective amount of the immunogenic composition of any one of claims 22-39.

41. A method of immunizing a subject against influenza virus comprising administering to the subject a first dose of an effective amount of an immunogenic composition and administering to the subject a second dose of an effective amount of the immunogenic

composition 30 days to 6 months after the subject has received the first dose, wherein the immunogenic composition is the composition of any one of claims 22-39.

42. The method of claim 41, further comprising administering to the subject a third dose of an effective amount of the immunogenic composition 30 days to 6 months after the subject has received the second dose.

43. A method of immunizing a subject against influenza virus comprising administering to the subject a first dose of an effective amount of a first immunogenic composition and administering to the subject a second dose of an effective amount of a second immunogenic composition 30 days to 6 months after the subject has received the first dose, wherein the immunogenic compositions are the compositions of any one of claims 22-39, and wherein the globular head domain of the chimeric influenza virus hemagglutinin (HA) polypeptide present in the first immunogenic composition is different than the globular head domain of the chimeric influenza virus hemagglutinin (HA) polypeptide present in the second immunogenic composition.

44. A method of preventing an influenza virus disease comprising administering to a subject an effective amount of the immunogenic composition of any one of claims 22-39.

45. A method of treating an influenza virus infection or an influenza virus disease comprising administering to a subject an effective amount of the immunogenic composition of any one of claims 22-39.

46. The method of any one of claims 40-45, wherein the subject is a human.

47. The method of claim 41 or 43, wherein the subject is a 1-5 year old human subject.

48. The method of claim 41 or 43, wherein the subject is an elderly human subject.

49. The method of any one of claims 40-48, wherein the immunogenic composition is administered intramuscularly or intranasally to the subject.

50. The chimeric influenza virus hemagglutinin (HA) polypeptide of any one of claims 1 to 12, wherein the polypeptide is soluble.

51. The chimeric influenza virus hemagglutinin (HA) polypeptide of claim 50, wherein the polypeptide comprises a trimerization domain.

52. The immunogenic composition of any one of claims 22-39 for use in a method of immunizing a subject against influenza virus.

53. The immunogenic composition of any one of claims 22-39 for use in a method of immunizing a subject against influenza virus, wherein said method comprises administering to the subject a first dose of an effective amount of an immunogenic composition and administering to the subject a second dose of an effective amount of the immunogenic composition 30 days to 6 months after the subject has received the first dose.

54. The immunogenic composition of claim 53, wherein said method further comprises administering to the subject a third dose of an effective amount of the immunogenic composition 30 days to 6 months after the subject has received the second dose.

55. The immunogenic composition of any one of claims 22-39 for use in a method of immunizing a subject against influenza virus, wherein said method comprises administering to the subject a first dose of an effective amount of a first immunogenic composition and administering to the subject a second dose of an effective amount of a second immunogenic composition 30 days to 6 months after the subject has received the first dose, and wherein the globular head domain of the chimeric influenza virus hemagglutinin (HA) polypeptide present in the first immunogenic composition is different than the globular head domain of the chimeric influenza virus hemagglutinin (HA) polypeptide present in the second immunogenic composition.

56. The immunogenic composition of any one of claims 22-39 for use in a method of preventing an influenza virus disease in a subject.

57. The immunogenic composition of any one of claims 22-39 for use in a method of treating an influenza virus infection or an influenza virus disease in a subject.

58. The immunogenic composition of any one of claims 52-57, wherein the subject is a human.

59. Use of the immunogenic composition of any one of claims 22-39 for the manufacture of a medicament for use in a method of immunizing a subject against influenza virus.

60. Use of the immunogenic composition of any one of claims 22-39 for the manufacture of a medicament for use in a method of immunizing a subject against influenza virus, wherein said method comprises administering to the subject a first dose of an effective amount of an immunogenic composition and administering to the subject a second dose of an effective amount of the immunogenic composition 30 days to 6 months after the subject has received the first dose.

61. The use of claim 60, wherein said method further comprises administering to the subject a third dose of an effective amount of the immunogenic composition 30 days to 6 months after the subject has received the second dose.

62. Use of the immunogenic composition of any one of claims 22-39 for the manufacture of a medicament for use in a method of immunizing a subject against influenza virus, wherein said method comprises administering to the subject a first dose of an effective amount of a first immunogenic composition and administering to the subject a second dose of an effective amount of a second immunogenic composition 30 days to 6 months after the subject has received the first dose, and wherein the globular head domain of the chimeric influenza virus hemagglutinin (HA) polypeptide present in the first immunogenic composition is different than

the globular head domain of the chimeric influenza virus hemagglutinin (HA) polypeptide present in the second immunogenic composition.

63. Use of the immunogenic composition of any one of claims 22-39 for the manufacture of a medicament for use in a method of preventing an influenza virus disease in a subject.

64. Use of the immunogenic composition of any one of claims 22-39 for the manufacture of a medicament for use in a method of treating an influenza virus infection or an influenza virus disease in a subject.

65. Use of the immunogenic composition of any one of claims 59-64, wherein the subject is human.

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).

[0061] "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).

[0062] "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.

[0063] 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.

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.

[0097] **Fig. 9** 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).

[0098] **Fig. 10** 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).

[0099] **Fig. 11** depicts 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. 11A), Cal/09 infection (Fig. 11B), DNA and Cal/09 infection (Fig. 11C), or Cal/09 split vaccine (Fig. 11D) 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. 11E) or the antibody PY102 (Fig. 11F) 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.

[00100] **Fig. 12** 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 5×10^4 PFU of PR8 virus, instilled intranasally, and the weight of the animals was monitored for 14 days.

[00101] **Fig. 13** demonstrates reactivity of stalk specific antibodies to cH6 protein as determined by ELISA.

(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).

[00114] **Fig. 26** depicts 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. (A) 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. (B) 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.

[00115] **Fig. 27** demonstrates that sequential vaccination with cHA constructs elicits HA stalk-specific antibodies and provides protection from lethal challenge. Mice were primed with 20 µg of cH9/1 protein, administered with adjuvant intranasally and intraperitoneally. Three weeks later, mice were boosted with 20 µg cH6/1 protein, administered with adjuvant intranasally and intraperitoneally. As controls, mice were primed and boosted in a similar fashion using BSA, or given inactivated FM1 virus intramuscularly. Animals were bled and challenged three weeks after the last vaccination with 5LD50 of A/Fort Monmouth/1/1947 (FM1) virus. (A) ELISA plates were coated with cH5/1 N1 virus in order to assess the degree of stalk reactivity elicited through vaccination (BSA + BSA is the bottom line of the graph). (B) Mice were weighed daily for 14 days to assess protection from challenge. (C) Kaplan Meier curve depicting survival rate post challenge. cH9/1 + cH6/1 vaccinated mice had a statistically higher survival rate compared to BSA controls ($p = .0003$)

[00116] **Fig. 28** demonstrates that vaccination with cH6/1 elicits stalk-specific immunity that mediates protection from cH9/1 N1 viral challenge. Animals were inoculated with YAM-HA

tag or foldon domain or both. Useful cleavage sites include a thrombin cleavage site, for example one with the sequence LVPRGSP (SEQ ID NO:19). 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) (SEQ ID NO:20)).

[00172] In certain embodiments, the chimeric influenza hemagglutinin polypeptides described herein are soluble polypeptides.

[00173] 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.

[00174] 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}. 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}. 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}. 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 $A_{p+10.}$ 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.

[00175] In certain embodiments, the HA1 C-terminal stem segment of the chimeric influenza virus hemagglutinin polypeptides described herein does not start exactly 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}. 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}. For example, 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}. 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+3}, A_{q+3} to A_{q+5}, A_{q+5} to A_{q+8}, A_{q+8} to A_{q+10}. 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 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.

[00176] In one example, an HA1 N-terminal stem segment of a chimeric influenza virus hemagglutinin polypeptide described herein may end at any one of hemagglutinin amino acid positions 45-48 (using H3 numbering) and an HA1 C-terminal stem segment of the chimeric

human serum samples and to measure any neutralizing activity mediated by those stalk-specific antibodies.

[00497] 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. 17A). 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. 17B, 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).

[00498] 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. 17C). 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. 14B).

6.4.2.2 Stalk specific antibodies bind and neutralize cHA

[00499] In order to confirm that cH6/1 and cH9/1 proteins could be used as tools to detect stem antibodies, the use of these cHAs was first validated with an antibody known to react with the HA stalk. Indeed, mouse mAb C179, an antibody reactive with the stalk of H1 HA (Okuno *et al*., 1993, *J Virol* 67, 2552), bound to baculovirus expressed cH6/1 and cH9/1 protein by ELISA in a dose dependent manner (Figs. 18A and B).

CLAIMS

1. A chimeric influenza virus hemagglutinin (HA) polypeptide comprising the stem domain of the HA from influenza virus A/California/4/2009 (H1N1) and the globular head domain of the HA from an influenza virus of the H5 subtype, wherein the H5 subtype is influenza virus A/Vietnam/1203/2004 (H5), A/Indonesia/5/2005 (H5), A/Anhui/1/2005 (H5), A/bar headed goose/Quinghai/1A/2005 (H5), A/turkey/Turkey/1/2005 (H5), or A/whooperswan/Mongolia/244/2005 (H5).

2. The chimeric influenza virus HA polypeptide of claim 1, wherein the stem domain of the HA maintains the cysteine residues A_p and A_q, wherein A_p is the Cys that corresponds to amino acid position 52 of an HA1 domain using H3 numbering, and wherein A_q is the Cys that corresponds to amino acid position 277 of an HA1 domain using H3 numbering.

3. The chimeric influenza virus HA polypeptide of claim 1 or 2, 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; and (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

(b) the HA globular head domain comprises the amino acid residues between A_p and A_q 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 $HA1_{C\text{-}term}$ 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; and 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 HA polypeptide of claim 1 or 2, 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 $A_{p\text{-}1}$, $A_{p\text{-}2}$, $A_{p\text{-}3}$, $A_{p\text{-}4}$, $A_{p\text{-}5}$, $A_{p\text{-}6}$, $A_{p\text{-}7}$, $A_{p\text{-}8}$, $A_{p\text{-}9}$, $A_{p\text{-}10}$, A_p, 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}, or A_{p+10}; and (ii) an HA1 C-terminal stem segment, wherein the HA1 C-terminal stem segment consists of amino acid residues $A_{q\text{-}1}$, $A_{q\text{-}2}$, $A_{q\text{-}3}$, $A_{q\text{-}4}$, $A_{q\text{-}5}$, $A_{q\text{-}6}$, $A_{q\text{-}7}$,

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

(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}, A_{p-10}, A_p, 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}, 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 $HA1_{C\text{-}term}$ 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; and wherein A_q is the Cys that corresponds to amino acid position 277 of an HA1 domain using H3 numbering.

5. The chimeric influenza virus HA polypeptide of any one of claims 1 to 4, wherein the polypeptide is soluble.

6. The chimeric influenza virus HA polypeptide of claim 5, wherein the polypeptide comprises a trimerization domain.

7. The chimeric influenza virus hemagglutinin (HA) polypeptide of claim 5, wherein the polypeptide does not comprise a transmembrane domain or an endodomain of an influenza virus HA.

8. The chimeric influenza virus hemagglutinin (HA) polypeptide of claim 7, wherein the polypeptide further comprises (i) a thrombin cleavage site and a T4 trimerization domain; or (ii) a HIS tag.

9. The chimeric influenza virus HA polypeptide of any one of claims 1 to 8, which is expressed and isolated from a cell.

10. The chimeric influenza virus HA polypeptide of claim 9, wherein the cell is a mammalian cell.

11. A nucleic acid encoding the chimeric influenza virus hemagglutinin (HA) polypeptide of any one of claims 1 to 8.

12. A cell expressing the nucleic acid of claim 11.

13. The cell of claim 12, wherein the cell is a mammalian cell or an insect cell.

14. A cell line expressing the nucleic acid of claim 11.

15. The cell line of claim 14, wherein the cell line is a mammalian or insect cell line.

16. The cell line of claim 15, which is a Crucell Per.C6 cell line, Vero cell line, CHO cell line, VERY cell line, BHK cell line, HeLa cell line, COS cell line, MDCK cell line, 293 cell line, 3T3 cell line, WI38 cell line, NS0 cell line, 45.6 TG1.7 cell line, AF-2 clone 9B5 cell line, AF-2 clone 9B5 cell line, J558L cell line, MOPC 315 cell line, MPC-11 cell line, NCI-H929 cell line, NP cell line, NS0/1 cell line, P3 NS1 Ag4 cell line, P3/NS1/1-Ag4-1 cell line, P3U1 cell line, P3X63Ag8 cell line, P3X63Ag8.653 cell line, P3X63Ag8U.1 cell line, RPMI 8226 cell line, Sp20-Ag14 cell line, U266B1 cell line, X63AG8.653 cell line, Y3.Ag.1.2.3 cell line, YO cell line, Sf9 cell line, Sf21 cell line, Ao/Tn 38 cell line, High Five cell line, Trichoplusia ni cell line, Spodoptera frugiperda cell line, or Bombyx mori cell line.

17. A virus comprising a genome engineered to express the nucleic acid of claim 11.

18. A virus comprising the polypeptide of any one of claims 1 to 8.

19. The virus of claim 17, wherein the virus is an influenza virus, vesicular stomatitis virus (VSV), Newcastle disease virus (NDV), adenovirus, or baculovirus.

20. The virus of claim 18, wherein the virus is an influenza virus, vesicular stomatitis virus (VSV), Newcastle disease virus (NDV), adenovirus, or baculovirus.

21. The virus of claim 19, which is inactivated, live attenuated, or split.

22. The virus of claim 20, which is inactivated, live attenuated, or split.

23. A virus-like particle comprising the polypeptide of any one of claims 1 to 8.

24. An embryonated egg comprising the virus of any one of claims 17 to 20.

25. The embryonated egg of claim 24, which is a chicken embryonated egg.

26. A method for producing the chimeric influenza virus HA polypeptide of any one of claims 1 to 8, comprising (a) culturing the cell or cell line of any one of claims 12 to 16; and (b) isolating the chimeric influenza virus HA polypeptide.

27. A method for producing the virus of any one of claims 17 to 20, comprising (a) propagating the virus of any one of claims 17 to 20 in an embryonated egg; and (b) isolating the virus from the embryonated egg.

28. An immunogenic composition comprising the polypeptide of any one of claims 1 to 8.

29. An immunogenic composition comprising the virus of any one of claims 17 to 22.

30. An immunogenic composition comprising the virus-like particle of claim 23.

31. The immunogenic composition of any one of claims 28-30 further comprising an adjuvant.

32. The immunogenic composition of claim 31, wherein the adjuvant is an aluminum salt, 3 De-O-acylated monophosphoryl lipid A, MF59, AS03, AS04, polysorbate 80, an imidazopyridine compound, an imidazoquinoxaline compound, Matrix-M, MVA, ISCOMATRIX, AddaVax, polyI:C, in vitro transcribed RNA hairpin from Sendai virus Cantell strain defective interfering RNA, a saponin, Freund's adjuvant, an oil in water emulsion, CpG, a physical adjuvant, or a Toll-like receptor stimulatory molecule.

33. The immunogenic composition of any one of claims 28-32 for use in a method of immunizing a subject against influenza virus.

34. The immunogenic composition of any one of claims 28-32 for use in a method of immunizing a subject against influenza virus, wherein said method comprises

administering to the subject a first dose of an effective amount of the immunogenic composition and administering to the subject a second dose of an effective amount of the immunogenic composition 30 days to 6 months after the subject has received the first dose.

35. The immunogenic composition of any one of claims 28-32 for use in a method of immunizing a subject against influenza virus, wherein said method comprises administering to the subject a first dose of an effective amount of a first immunogenic composition comprising a first chimeric influenza virus hemagglutinin (HA) polypeptide and administering to the subject a second dose of an effective amount of a second immunogenic composition comprising a second chimeric influenza virus hemagglutinin (HA) polypeptide 30 days to 6 months after the subject has received the first dose, and wherein the globular head domain of the first chimeric influenza virus hemagglutinin (HA) polypeptide present in the first immunogenic composition is different than the globular head domain of the second chimeric influenza virus hemagglutinin (HA) polypeptide present in the second immunogenic composition.

36. The immunogenic composition of any one of claims 28-32 for use in a method of preventing an influenza virus disease in a subject.

37. The immunogenic composition of any one of claims 28-32 for use in a method of preventing an influenza virus disease in a subject, wherein said method comprises administering to the subject a first dose of an effective amount of the immunogenic composition and administering to the subject a second dose of an effective amount of the immunogenic composition 30 days to 6 months after the subject has received the first dose.

38. The immunogenic composition of any one of claims 28-32 for use in a method of preventing an influenza virus disease in a subject, wherein said method comprises administering to the subject a first dose of an effective amount of a first immunogenic composition comprising a first chimeric influenza virus hemagglutinin (HA) polypeptide and administering to the subject a second dose of an effective amount of a second immunogenic composition comprising a second chimeric influenza virus hemagglutinin (HA) polypeptide 30 days to 6 months after the subject has received the first dose, and wherein the globular head domain of the first chimeric influenza virus hemagglutinin (HA) polypeptide present in the first immunogenic composition is different than the globular head domain of the second

chimeric influenza virus hemagglutinin (HA) polypeptide present in the second immunogenic composition.

39. The immunogenic composition of any one of claims 28-32 for use in a method of treating an influenza virus infection or an influenza virus disease in a subject.

40. The immunogenic composition of any one of claims 28-32 for use in a method of treating an influenza virus infection or an influenza virus disease in a subject, wherein said method comprises administering to the subject a first dose of an effective amount of the immunogenic composition and administering to the subject a second dose of an effective amount of the immunogenic composition 30 days to 6 months after the subject has received the first dose.

41. The immunogenic composition of any one of claims 28-32 for use in a method of treating an influenza virus infection or an influenza virus disease in a subject, wherein said method comprises administering to the subject a first dose of an effective amount of a first immunogenic composition comprising a first chimeric influenza virus hemagglutinin (HA) polypeptide and administering to the subject a second dose of an effective amount of a second immunogenic composition comprising a second chimeric influenza virus hemagglutinin (HA) polypeptide 30 days to 6 months after the subject has received the first dose, and wherein the globular head domain of the first chimeric influenza virus hemagglutinin (HA) polypeptide present in the first immunogenic composition is different than the globular head domain of the second chimeric influenza virus hemagglutinin (HA) polypeptide present in the second immunogenic composition.

42. The immunogenic composition of claim 33 or 34, wherein said method further comprises administering to the subject a third dose of an effective amount of the immunogenic composition 30 days to 6 months after the subject has received the second dose.

43. The immunogenic composition of any one of claims 33-42, wherein the subject is a human.

44. The immunogenic composition of any one of claims 33-42, wherein the subject is a 1-5 year old human subject.

45. The immunogenic composition of any one of claims 33-42, wherein the subject is an elderly human subject.

46. Use of the immunogenic composition of any one of claims 28-32 in the manufacture of a medicament for immunizing a subject against influenza virus.

47. Use of the immunogenic composition of any one of claims 28-32 in the manufacture of a medicament for immunizing a subject against influenza virus, wherein a first dose of an effective amount of an immunogenic composition is followed by a second dose of an effective amount of the immunogenic composition 30 days to 6 months after the subject has received the first dose.

48. The use of claim 47, further comprising a third dose of an effective amount of the immunogenic composition 30 days to 6 months after the subject has received the second dose.

49. Use of the immunogenic composition of any one of claims 28-32 in the manufacture of a medicament for immunizing a subject against influenza virus, wherein a first dose of an effective amount of a first immunogenic composition comprising a first chimeric influenza virus HA polypeptide is followed by a second dose of an effective amount of a second immunogenic composition comprising a second chimeric influenza virus HA polypeptide 30 days to 6 months after the subject has received the first dose, and wherein the globular head domain of the first chimeric influenza virus hemagglutinin (HA) polypeptide present in the first immunogenic composition is different than the globular head domain of the second chimeric influenza virus hemagglutinin (HA) polypeptide present in the second immunogenic composition.

50. Use of the immunogenic composition of any one of claims 28-32 in the manufacture of a medicament for preventing an influenza virus disease in a subject.

51. Use of the immunogenic composition of any one of claims 28-32 in the manufacture of a medicament for preventing an influenza virus disease in a subject, wherein a first dose of an effective amount of an immunogenic composition is followed by a second dose of an effective amount of the immunogenic composition 30 days to 6 months after the subject has received the first dose.

52. The use of claim 51, further comprising a third dose of an effective amount of the immunogenic composition 30 days to 6 months after the subject has received the second dose.

53. Use of the immunogenic composition of any one of claims 28-32 in the manufacture of a medicament for preventing an influenza virus disease in a subject, wherein a first dose of an effective amount of a first immunogenic composition comprising a first chimeric influenza virus HA polypeptide is followed by a second dose of an effective amount of a second immunogenic composition comprising a second chimeric influenza virus HA polypeptide 30 days to 6 months after the subject has received the first dose, and wherein the globular head domain of the first chimeric influenza virus hemagglutinin (HA) polypeptide present in the first immunogenic composition is different than the globular head domain of the second chimeric influenza virus hemagglutinin (HA) polypeptide present in the second immunogenic composition.

54. Use of the immunogenic composition of any one of claims 28-32 in the manufacture of a medicament for treating an influenza virus infection or an influenza virus disease in a subject.

55. Use of the immunogenic composition of any one of claims 28-32 in the manufacture of a medicament for treating an influenza virus infection or an influenza virus disease in a subject, wherein a first dose of an effective amount of an immunogenic composition is followed by a second dose of an effective amount of the immunogenic composition 30 days to 6 months after the subject has received the first dose.

56. The use of claim 55, further comprising a third dose of an effective amount of the immunogenic composition 30 days to 6 months after the subject has received the second dose.

57. Use of the immunogenic composition of any one of claims 28-32 in the manufacture of a medicament for treating an influenza virus infection or an influenza virus disease in a subject, wherein a first dose of an effective amount of a first immunogenic composition comprising a first chimeric influenza virus HA polypeptide is followed by a second dose of an effective amount of a second immunogenic composition comprising a second chimeric influenza virus HA polypeptide 30 days to 6 months after the subject has received the first dose, and wherein the globular head domain of the first chimeric influenza virus hemagglutinin (HA) polypeptide present in the first immunogenic composition is different than the globular head domain of the second chimeric influenza virus hemagglutinin (HA) polypeptide present in the second immunogenic composition.

58. The use of the immunogenic composition of any one of claims 46-57, wherein the subject is human.

59. The use of any one of claims 46-57, wherein the subject is a 1-5 year old human subject.

60. The use of any one of claims 46-57, wherein the subject is an elderly human subject.

61. The immunogenic composition of any one of claims 33-42, wherein the subject is a human infant, wherein the human infant is a newborn to 1 year old human.

62. The use of any one of claims 46-57, wherein the subject is a human infant, wherein the human infant is a newborn to 1 year old human.