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(54) Title: INFLUENZA NUCLEIC ACID MOLECULES AND VACCINES MADE THEREFROM

(57) Abstract: Provided herein are nucleic acid sequences that encode novel consensus amino acid sequences of HA Influenza A of serotype H7N9 alone and in combination with HA hemagglutinin and/or influenza B hemagglutinin, as well as genetic constructs/vectors and vaccines expressing the sequences. Also provided herein are methods for generating an immune response against one or more influenza A serotypes and/or influenza B serotypes, or combinations thereof, using the vaccines that are provided.



INFLUENZA NUCLEIC ACID MOLECULES AND VACCINES MADE THEREFROM

FIELD OF THE INVENTION

The present invention relates to improved H7N9 influenza viral vaccines, improved
5 methods for inducing immune responses against influenza, improved methods for diagnosing
vaccinated vs. infected influenza mammalian hosts and for prophylactically and/or
therapeutically immunizing individuals against influenza.

BACKGROUND OF THE INVENTION

Influenza, commonly referred to as the flu, is an infectious disease caused by RNA
10 viruses of the family *Orthomyxoviridae*. Influenza or flu viruses infect birds and mammals.
Three of the five genera of *Orthomyxoviridae* are influenza viruses: Influenza A, Influenza B
and Influenza C. Of these, Influenza A is the most common.

Influenza is typically transmitted through the air in aerosols produced by coughs or
sneezes and by direct contact with body fluids containing the virus or contaminated surfaces.
15 Seasonal epidemics of influenza occur worldwide and result in hundreds of thousands of
deaths annually. In some years, pandemics occur and cause millions of deaths. In addition,
livestock, particularly poultry and swine, are also susceptible to annual epidemics and
occasional pandemics which cause large numbers of animal deaths and monetary losses. The
newly emerged Chinese avian influenza A (H7N9) virus is one subgroup among the larger
20 group of H7 viruses (10). As of July 4th, 2013, there have been a total of 133 laboratory-
confirmed cases and 43 people have died, indicating a very high mortality rate (32.3%

Structurally, influenza viruses are similar, having generally spherical or filamentous
virus particles of about 80–120 nm made up of similar molecular component. A central core
comprising viral proteins and viral RNA is covered by a viral envelope made up of two
25 different glycoproteins and a lipid coat derived from the cell that the viral particle is produced
in. Two additional different glycoproteins are anchored within the viral envelope and include
portions which project outward on the surface. The H7N9 virus exhibits several genetic
features of mammalian influenza viruses, including the specificity of their HA protein
binding to mammalian cellular receptors; a deletion in NA stalk associated with increased
30 virulence in mammals; and an important mutation in the PB2 protein that is essential for the
efficient replication of avian viruses in mammalian species. The efficient transmission of

H7N9 virus in ferrets suggested that human-to-human transmission of this virus might be possible under appropriate conditions. As a result, the emergence of the novel H7N9 has raised concerns about its pandemic potential, as well as that of related influenza viruses.

New synthetic DNA vaccines have emerged as an attractive approach against various infectious diseases and cancers. Conceptually, DNA vaccines have many useful attributes over traditional vaccination strategies, such as live-attenuated vaccines, protein/peptide-based vaccines. While DNA vaccines have been shown to be capable of eliciting balanced CD4+ and CD8+ T cell responses as well as humoral immune responses in small-animal models, their progress in the clinic historically has been hampered by difficulties in generating sufficiently potent T cell and humoral responses in humans. Until recently, the DNA platform has been used primarily in prime-boost strategies along with viral vectors and proteins, thus creating an inordinately long testing and development window for addressing emerging pandemics rapidly. In order to address the technical hurdles associated with weak vaccine-induced immunity, we have recently applied many synthetic DNA design strategies, including codon/RNA optimization, the addition of highly efficient immunoglobulin leader sequences, use of 'centralized' immunogens to broaden immunity and remove dependence on any individual viral sequence, new formulations combined with highly efficient DNA delivery methods such as in vivo electroporation (EP), to improve the induction of immune responses induced by DNA vaccines in small animals, macaques, and most importantly, in humans.

Accordingly, there remains a need for an immunogenic influenza consensus hemagglutinin protein, for nucleic acid constructs that encode such a protein and for compositions useful to induce immune responses in mammals that are broadly cross reactive against multiple strains of influenza. There remains a need for effective vaccines against influenza that are economical and effective across numerous influenza subtypes for treating individuals, including ability to cross protect against multiple strains of influenza.

SUMMARY OF THE INVENTION

The present invention is directed to an isolated nucleic acid comprising a nucleic acid sequence encoding an amino acid sequence selected from the group consisting of SEQ ID NO: 40, an amino acid sequence that is 95% identical over the entire length of the amino acid sequence of SEQ ID NO: 40, a fragment of SEQ ID NO: 40 comprising at least 30 amino acids, and an amino acid sequence that is 95% identical to a fragment of SEQ ID NO: 40 comprising at least 30 amino acids. The isolated nucleic acid can comprise a nucleic acid

sequence selected from the group consisting of SEQ ID NO: 39, a nucleic acid sequence that is 95% identical over the entire length of the nucleic acid sequence of SEQ ID NO: 39, a fragment of SEQ ID NO: 39 comprising at least 90 nucleotides, and a nucleic acid sequence that is 95% identical to a fragment of SEQ ID NO: 39 comprising at least 90 nucleotides.

- 5 The isolated nucleic acid can comprise a nucleic acid sequence selected from the group consisting of a nucleic acid sequence that is 95% identical over the entire length of the nucleic acid sequence of SEQ ID NO: 39 and a nucleic acid sequence that is 95% identical to a fragment of SEQ ID NO: 39 comprising at least 90 nucleotides. A recombinant vector can comprise the isolated nucleic acid. An expression vector can comprise the isolated nucleic acid operably linked to regulatory elements. The regulatory elements can be functional in a human cell. The expression vector can be a plasmid. The expression vector can be pGX0001.

- The present invention is directed to an isolated nucleic acid comprising a nucleic acid sequence encoding an amino acid sequence of SEQ ID NO: 40. The isolated nucleic acid can consist of a nucleic acid sequence encoding an amino acid sequence of SEQ ID NO: 40. The isolated nucleic acid can consist of a nucleic acid sequence of SEQ ID NO: 39. A recombinant vector can comprise the isolated nucleic acid. An expression vector can comprise the isolated nucleic acid operably linked to regulatory elements. The regulatory elements can be functional in a human cell. The expression vector can be a plasmid. The expression vector can be pGX0001.

- The present invention is directed to a composition comprising: a first nucleic acid sequence comprising one or more nucleotide sequences encoding an amino acid sequence selected from the group consisting of SEQ ID NO: 40, an amino acid sequence that is 95% identical over the entire length of the amino acid sequence of SEQ ID NO: 40, a fragment of SEQ ID NO: 40 comprising at least 30 amino acids, and an amino acid sequence that is 95% identical to a fragment of SEQ ID NO: 40 comprising at least 30 amino acids; and a second nucleic acid sequence that encodes a protein selected from the group consisting of one or more of: H1, H2, H3, H4, H5, H6, H7, H8, H9, H10, H11, H12, H13, H14, H15, H16, N1, N2, N3, N4, N5, N6, N7, N8 and N9. The first nucleic acid sequence can be selected from the group consisting of one or more of SEQ ID NO: 39, a nucleic acid sequence that is 95% identical over the entire length of the nucleic acid sequence of SEQ ID NO: 39, a fragment of SEQ ID NO: 39 comprising at least 90 nucleotides, and a nucleic acid sequence that is 95% identical to a fragment of SEQ ID NO: 39 comprising at least 90 nucleotides. The first nucleic acid sequence can be operably linked to regulatory elements that are functional in a

human cell. The composition can comprise a plasmid that comprises the first nucleic acid sequence operably linked to regulatory elements that are functional in a human cell. The second nucleic acid sequence can be selected from the group consisting of one or more of: SEQ ID NO: 1; a nucleic acid sequence that is 95% identical over the entire length of the
5 nucleic acid sequence of SEQ ID NO: 1; a fragment of SEQ ID NO: 1 comprising at least 90 nucleotides; a nucleic acid sequence that is 95% identical to a fragment of SEQ ID NO: 1 comprising at least 90 nucleotides; SEQ ID NO: 3; a nucleic acid sequence that is 95% identical over the entire length of the nucleic acid sequence of SEQ ID NO: 3; a fragment of
10 SEQ ID NO: 3 comprising at least 90 nucleotides; a nucleic acid sequence that is 95% identical to a fragment of SEQ ID NO: 3 comprising at least 90 nucleotides; SEQ ID NO: 6; a nucleic acid sequence that is 95% identical over the entire length of the nucleic acid sequence of SEQ ID NO: 6; a fragment of SEQ ID NO: 6 comprising at least 90 nucleotides; a nucleic acid sequence that is 95% identical to a fragment of SEQ ID NO: 6 comprising at least 90
15 nucleotides; SEQ ID NO: 8; a nucleic acid sequence that is 95% identical over the entire length of the nucleic acid sequence of SEQ ID NO: 8; a fragment of SEQ ID NO: 8 comprising at least 90 nucleotides; and a nucleic acid sequence that is 95% identical to a fragment of SEQ ID NO: 8 comprising at least 90 nucleotides. The composition can further comprise a peptide encoded by the nucleic acid sequence of SEQ ID NO: 39. The composition can further comprise a peptide comprising an amino acid sequence of SEQ ID
20 NO: 40.

The present invention is directed to a vaccine that can comprise said isolated nucleic acids, said recombinant vectors, said expression vector, or said compositions. The vaccine can further comprise a peptide encoded by the nucleic acid sequence of SEQ ID NO: 39. The vaccine can further comprise a peptide comprising an amino acid sequence of SEQ ID NO:
25 40.

The present invention is directed to a vaccine comprising a nucleic acid sequence encoding an amino acid sequence of SEQ ID NO: 40. The vaccine can further comprise a peptide encoded by the nucleic acid sequence of SEQ ID NO: 39. The vaccine can further comprise a peptide comprising an amino acid sequence of SEQ ID NO: 40.

30 The present invention is directed to a vaccine comprising a nucleic acid sequence of SEQ ID NO: 39. The vaccine can further comprise a peptide encoded by the nucleic acid sequence of SEQ ID NO: 39. The vaccine can further comprise a peptide comprising an amino acid sequence of SEQ ID NO: 40.

The present invention is directed to a vaccine against an influenza virus comprising a nucleic acid sequence encoding an amino acid sequence of SEQ ID NO: 40. The nucleic acid sequence can comprise a nucleic acid sequence of SEQ ID NO: 39. The influenza virus can be H7N9 influenza virus. The vaccine can further comprise a peptide encoded by the nucleic acid sequence of SEQ ID NO: 39. The vaccine can further comprise a peptide comprising an amino acid sequence of SEQ ID NO: 40.

The present invention is directed to a method of inducing an immune response comprising the step of: administering to an individual a nucleic acid molecule comprising said isolated nucleic acids. The method can further comprise administering to said individual a therapeutically effective amount of a peptide encoded by the nucleic acid sequence of SEQ ID NO: 39. The method can further comprise administering to said individual a therapeutically effective amount of a peptide comprising an amino acid sequence of SEQ ID NO: 40. The nucleic acid molecule can be a plasmid. The plasmid can be pGX0001. The nucleic acid molecule can be administered to said individual using electroporation

The present invention is directed to a method of inducing an immune response comprising the step of: administering to an individual said compositions. The method can further comprise administering to said individual a therapeutically effective amount of a peptide encoded by the nucleic acid sequence of SEQ ID NO: 39. The method can further comprise administering to said individual a therapeutically effective amount of a peptide comprising an amino acid sequence of SEQ ID NO: 40. The nucleic acid molecule can be a plasmid. The plasmid can be pGX0001. The nucleic acid molecule can be administered to said individual using electroporation

The present invention is directed to a method of protecting an individual against infection by an avian origin human influenza strain comprising the step of: administering to said individual a prophylactically effective amount of a nucleic acid molecule comprising said isolated nucleic acids; wherein the nucleic acid molecule is expressed in cells of said individual and an immune response against said protein is induced that is a protective immune response against avian origin human influenza. The method can further comprise administering to said individual a therapeutically effective amount of a peptide encoded by the nucleic acid sequence of SEQ ID NO: 39. The method can further comprise administering to said individual a therapeutically effective amount of a peptide comprising an amino acid sequence of SEQ ID NO: 40. The nucleic acid molecule can be a plasmid. The plasmid can be pGX0001. The nucleic acid molecule can be administered to said individual using electroporation

The present invention is directed to a method of protecting an individual against infection by an avian origin human influenza strain comprising the step of: administering to said individual a prophylactically effective amount of said compositions; wherein the first nucleic acid sequence is expressed in cells of said individual and an immune response against said first protein is induced that is a protective immune response against avian origin human influenza, the one or more second nucleic acid sequences are expressed in cells of said individual and immune responses against said one or more second proteins are induced. The method can further comprise administering to said individual a therapeutically effective amount of a peptide encoded by the nucleic acid sequence of SEQ ID NO: 39. The method can further comprise administering to said individual a therapeutically effective amount of a peptide comprising an amino acid sequence of SEQ ID NO: 40. The nucleic acid molecule can be a plasmid. The plasmid can be pGX0001. The nucleic acid molecule can be administered to said individual using electroporation

The present invention is directed to a method of treating an individual who has been infected by an avian origin human influenza strain comprising the step of: administering to said individual a therapeutically effective amount of said isolated nucleic acids. The method can further comprise administering to said individual a therapeutically effective amount of a peptide encoded by the nucleic acid sequence of SEQ ID NO: 39. The method can further comprise administering to said individual a therapeutically effective amount of a peptide comprising an amino acid sequence of SEQ ID NO: 40. The nucleic acid molecule can be a plasmid. The plasmid can be pGX0001. The nucleic acid molecule can be administered to said individual using electroporation

The present invention is directed to a method of treating an individual who has been infected by an avian origin human influenza H7N9 strain comprising the step of: administering to said individual a therapeutically effective amount of said compositions; wherein the first nucleic acid sequence is expressed in cells of said individual and an immune response against said first protein is induced that is a therapeutic immune response against avian origin human influenza, the one or more second nucleic acid sequences are expressed in cells of said individual and immune responses against said one or more second proteins are induced. The method can further comprise administering to said individual a therapeutically effective amount of a peptide encoded by the nucleic acid sequence of SEQ ID NO: 39. The method can further comprise administering to said individual a therapeutically effective amount of a peptide comprising an amino acid sequence of SEQ ID NO: 40. The nucleic acid

molecule can be a plasmid. The plasmid can be pGX0001. The nucleic acid molecule can be administered to said individual using electroporation.

In some aspects of the invention, methods are provided for treating an individual who has been infected by influenza. The methods comprise the step of: administering to said individual a therapeutically effective amount of such nucleic acid molecules and/or composition. In some embodiment, the immune response is broadly cross reactive against multiple strains of influenza.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 is a map of the 2999 basepair backbone vector plasmid pVAX1 (Invitrogen, Carlsbad CA). The CMV promoter is located at bases 137-724. The T7 promoter/priming site is at bases 664-683. Multiple cloning sites are at bases 696-811. Bovine GH polyadenylation signal is at bases 829-1053. The Kanamycin resistance gene is at bases 1226-2020. The pUC origin is at bases 2320-2993.

Based upon the sequence of pVAX1 available from Invitrogen, the following mutations were found in the sequence of pVAX1 that was used as the backbone for pGX2009:

	C>G	241	in CMV promoter
	C>T	1942	backbone, downstream of the bovine growth hormone polyadenylation signal (bGHpolyA)
20	A> -	2876	backbone, downstream of the Kanamycin gene
	C>T	3277	in pUC origin of replication (Ori) high copy number mutation (see Nucleic Acid Research 1985)
	G>C	3753	in very end of pUC Ori upstream of RNaseH site
25			Base pairs 2, 3 and 4 are changed from ACT to CTG in backbone, upstream of CMV promoter.

Figure 2 shows two maps of the plasmid pGX2009, which is also referred to as pH1HA09. The nucleic acid sequence of the plasmid pGX2009 (SEQ ID NO:5) includes the coding sequence for the consensus H1 protein construct (amino acid SEQ ID NO:4 encoded by SEQ ID NO:3) which includes the IgE leader(amino acid SEQ ID NO:17) linked to the N terminal of the consensus H1 amino acid sequence(amino acid SEQ ID NO:2 encoded by SEQ ID NO:1) which is linked at its C terminal to the HA Tag (SEQ ID NO:18). The

consensus H1 protein (amino acid SEQ ID NO:4 encoded by SEQ ID NO:3) is labeled SwiHum Con HA and H1HA09.

Figure 3 shows a maps of the plasmid pGX2006. The nucleic acid sequence of the plasmid pGX2006 (SEQ ID NO:8) includes the coding sequence for consensus H2 protein
5 (amino acid SEQ ID NO:7 encoded by SEQ ID NO:6) which is labeled H2HA.

Figure 4 shows data from hemagglutination inhibition assays performed with sera from immunized ferrets.

Figure 5 shows results of a challenge of immunized and unimmunized ferrets with a novel H1N1 strain.

10 Figure 6 displays an influenza H1 hemagglutinin (H1HA) genetic tree of the genes of strains over the last 20 years, showing the genetic relationship between the various H1HA influenza strains.

Figures 7A-G displays a number of graphs of inhibition titers generated from hemagglutination inhibition assays performed with sera from immunized mice (naïve, H1U
15 (SEQ ID NO: 35), HS09 (SEQ ID NO:1), ConBris (or H1Bris; SEQ ID NO:19), ConTT (or H1-TT; SEQ ID NO:21), Combo (all 4 H1U, HS09, ConBris, and ConTT)) against various seasonal viruses.

Figures 8A-C displays a number of graphs of inhibition titers generated from hemagglutination inhibition assays performed with sera from immunized mice (naïve, H1U
20 (SEQ ID NO: 35), HS09 (SEQ ID NO:1), ConBris (or H1Bris; SEQ ID NO:19), ConTT (or H1-TT; SEQ ID NO:21), Combo (all 4 H1U, HS09, ConBris, and ConTT)) against various pandemic viruses.

Figures 9A-B displays a number of graphs of inhibition titers generated from hemagglutination inhibition assays performed with sera from immunized guinea pigs (Combo
25 (all 4 H1U, HS09, ConBris, and ConTT)) against various seasonal viruses; 9A showing the data points; and 9B showing the mean and standard deviation.

Figures 10A-B displays a number of graphs of inhibition titers generated from hemagglutination inhibition assays performed with sera from immunized guinea pigs (Combo
30 (all 4 H1U, HS09, ConBris, and ConTT)) against various pandemic viruses; 10A showing the data points; and 10B showing the mean and standard deviation.

Figures 11A-C shows titer graphs generated from hemagglutination inhibition assays performed with sera from ferrets (immunized with combination of HS09 and H1U) against either 11A) A/Mexico/InDRE4108/2009 pandemic strain; and 11B)

A/Mexico/InDRE4487/2009 pandemic strain; and a graph showing percent of ferrets survival when challenged with 2009 H1 Mexico strain of influenza.

Figure 12 displays an influenza B hemagglutinin (BHA) genetic tree of the genes from strains of the last 20 years, showing the genetic relationship between the various BHA strains.

5 Figure 13A-F displays a number of graphs of inhibition titers generated from hemagglutination inhibition assays performed with sera from immunized mice (naïve, BHA-1 (SEQ ID NO: 13), BHA-2 (SEQ ID NO:25), Combo (both BHA-1 and BHA-2) against various viruses.

10 Figure 14A-C displays a number of graphs of inhibition titers generated from hemagglutination inhibition assays performed with sera from immunized mice (naïve, BHA-1 (SEQ ID NO: 13), BHA-2 (SEQ ID NO:25), Combo (both BHA-1 and BHA-2)) against various viruses.

15 Figure 15A-D displays a number of graphs of inhibition titers generated from hemagglutination inhibition assays performed with sera from immunized guinea pigs pre-immunization (“pre”), post-dose 1 (“PD1”), post-dose 2 (“PD2”), or post-dose 3 (“PD3”) (BHA-1 (SEQ ID NO: 13), BHA-2 (SEQ ID NO:25), Combo (both BHA-1 and BHA-2) against various viruses.

20 Figure 16A-C displays a number of graphs of inhibition titers generated from hemagglutination inhibition assays performed with sera from immunized guinea pigs pre-immunization (“pre”), post-dose 1 (“PD1”), post-dose 2 (“PD2”), or post-dose 3 (“PD3”) (BHA-1 (SEQ ID NO: 13), BHA-2 (SEQ ID NO:25), Combo (both BHA-1 and BHA-2) against various viruses.

25 Figure 17A-C displays a number of graphs of inhibition titers generated from hemagglutination inhibition assays performed with sera from immunized guinea pigs pre-immunization (“pre”), post-dose 1 (“PD1”), post-dose 2 (“PD2”), or post-dose 3 (“PD3”) (BHA-1 (SEQ ID NO: 13), BHA-2 (SEQ ID NO:25), Combo (both BHA-1 and BHA-2) against various viruses.

30 Note: for Figs 15A-D; 16A-C; and 17A-C “Combo” samples are guinea pigs that were immunized with combo separately (BHA-1 delivered separately from BHA-2); whereas with combo(mix) BHA-1 and BHA-2 were mixed and delivered simultaneously.

Figure 18 displays an influenza H3 hemagglutinin (H3HA) genetic tree of the genes from strains of the last 20 years, showing the genetic relationship between the various H3HA strains.

Figure 19A-E displays a number of graphs of inhibition titers generated from hemagglutination inhibition assays performed with sera from immunized mice (naïve, H3HA-1 (SEQ ID NO: 37), H3HA-2 (SEQ ID NO:23), Combo (both H3HA-1 and H3HA-2) against various viruses.

Figure 20A-F displays a number of graphs of inhibition titers generated from hemagglutination inhibition assays performed with sera from immunized mice (naïve, H3HA-1 (SEQ ID NO: 37), H3HA-2 (SEQ ID NO:23), Combo (both H3HA-1 and H3HA-2) against various viruses.

Figures 21A-21C show the H7N9 HA DNA vaccine design and its expression. (A) Phylogenetic tree based on neighbor-joining evaluation of H7HA alignment. Asterisks indicate location of consensus sequence. The strains used to generate the consensus HA are indicated. (B) The plasmid map of the H7HA plasmid. (C) Immunofluorescence assay of pH7HA. The transfected RD cells expressing H7HA protein showed typical red fluorescence. An anti-H7N9 HA mouse monoclonal antibody served as the source of primary antibody.

Figures 22A-22E show robust HA-specific IgG antibody titers and hemagglutination-inhibition titers in the sera of the immunized mice. (A) IgG antibodies against H7N9 A/Shanghai/1/2013 influenza HA protein. (B) IgG antibodies against H7N9 A/Anhui/1/2013 influenza HA protein. (C) IgG antibodies against H7N9 A/Hangzhou/1/2013 influenza HA protein. (D) HA-specific IgG endpoint titers. (E) Hemagglutination-inhibition titers against A/Anhui/1/2013. Each BALB/c mouse was immunized intramuscularly followed by electroporation with 25 µg of pH7HA DNA twice, three weeks apart. Mice (n=10) were bled before and two weeks after second immunization. IgG antibody titers and hemagglutination-inhibition titers were measured by endpoint enzyme-linked immunosorbent assay (ELISA) and hemagglutination-inhibition assay, respectively. Error bars represent 1 standard deviation from the mean.

Figures 23A-23B show HA-specific cellular immune response induced in the immunized mice. (A) Immunization schedule. (B) Total IFN-γ responses induced by pH7HA. Frequencies of HA-specific IFN-γ-secreting cells per million splenocytes after two DNA immunizations with pH7HA were determined by IFN-γ ELISpot assay. The splenocytes were isolated from each mouse (n=5), stimulated in vitro with four overlapping HA peptide pools for 24 h and IFN-γ secreting cells were determined by ELISpot assay. Naïve mice were included as a negative control. The values are the means ± standard error of the means.

Figures 24A-24C show cytokine frequencies and phenotypic profiles of specific CD4 T cells after pH7HA immunization. Cytokine recall responses to H7 HA were measured 4 weeks after last immunization by ICS and flow cytometry. CD4 T cells were identified by CD3 expression and further gated as CD44+. (A) The percentage of total CD44+CD4 T cells expressing IFN- γ in response to H7 HA stimulation. (B) Average percentage of HA-specific CD44+CD4 double-positive-producing cells. (C) Multiparameter flow cytometry was used to determine the percentages of multifunctional CD4 T cell cytokine profile of H7HA. The bar chart shows the percentage of specific CD44+CD4 T cells displayed as triple, double, or single positive CD4 T cells. Pie charts show the relative proportion of each cytokine subpopulation to H7 HA stimulation. Background staining from cells stimulated with medium alone has been subtracted. Data represent the mean $\pm$ SEM of five mice per group with ***P < 0.001, **P < 0.01, *P < 0.05 using Student's *t*-test.

Figures 25A-25D show cytokine frequencies and phenotypic profiles of specific CD8 T cells after pH7HA immunization. Cytokine recall responses to H7 HA were measured 4 weeks after last immunization by ICS and flow cytometry. CD8 T cells were identified by CD3 expression and further gated as CD44+. (A) The percentage of total CD44+CD8 T cells expressing IFN- γ in response to H7 HA stimulation. (B) Average percentage of HA-specific CD44+CD8 double-positive-producing cells. (C) Multiparameter flow cytometry was used to determine the percentages of multifunctional CD8 T cell cytokine profile of H7HA. The bar chart shows the percentage of specific CD44+CD8 T cells displayed as triple, double, or single positive CD8 T cells. Pie charts show the relative proportion of each cytokine subpopulation to H7 HA stimulation. (D) Antigen-specific cytolytic degranulation T cells were measured by degranulation marker expression, CD107a and IFN- γ . Background staining from cells stimulated with medium alone has been subtracted. Data represent the mean $\pm$ SEM of five mice per group with ***P < 0.001, **P < 0.01, *P < 0.05 using Student's *t*-test.

Figures 26A-26C show protection from H7N9 A/Anhui/12013 virus challenge in the immunized mice. (A) Experimental schedule of challenge study. The mice (n=10) were immunized with 25 μ g of pH7HA twice, three weeks apart. Four weeks after the second immunization, the mice were challenged intranasally with a lethal dose of A/Anhui/1/13 H7N9 virus and monitored daily for weight loss and mortality. (B) Weight loss of each individual surviving mice in both naïve and immunized groups. The data are plotted as

percentage of the weight on day 1. (C) Kaplan-Meier survival curve showing the percent survival following challenge. All surviving animals were monitored for a total of 28 days.

DETAILED DESCRIPTION

5 The present invention is directed to vaccines comprising consensus amino acid sequences of influenza H7N9 HA (referred to as “consensus H7N9 HA”; (SEQ ID NO: 40)). In some aspects, nucleic acid sequences are provided which encode proteins comprising the H7N9 HA consensus amino acid sequence of SEQ ID NO: 40.

10 Current vaccination platforms require significant development and production timelines to address pandemics. The synthetic H7N9 HA DNA vaccine is capable of eliciting robust cellular immune responses, broadly cross-reactive antibody responses and generating complete protection from lethal challenge with just a few week development and vaccination regime. The synthetic DNA vaccine platform eliminates many of the hurdles that limit the rapid development and deployment of a vaccine against an emerging pandemic. For example,
15 the lack of a requirement for any recombination or *in vivo* production, as with other technologies, quickens the production time line as well as eliminates a major potential source of errors in seed development, thus increasing safety by limiting potential mutations being induced during the immunogen development process.

20 The disclosed enhanced DNA approach provides as influenza vaccine platform that 1) can be rapidly and straightforwardly utilized to design an effective vaccine against multiple known and new strains, 2) includes a delivery mechanism that can quickly induce cellular and humoral immune responses against new antigens in humans, 3) is rapid from design to scale up and mass production, with a relevant clinical track record and 4) is very stable for distribution purposes. Using this approach, millions of doses can be produced at scale within
25 a few months of identification of the pathogen. In addition the stability of DNA and the lack of requirement of any viral amplification step ensure fidelity in the process.

30 While not being bound by scientific theory, a vaccine that can be used to elicit an immune response (humeral, cellular, or both) broadly against multiple H7N9 HA influenza subtypes can comprise one or more of the following: 1) a nucleic acid sequence that encodes a protein comprising the consensus H7N9 HA amino acid sequence; and 2) a protein comprising the consensus H7N9 HA amino acid sequence. Immunization methods can be performed and vaccines can be prepared which use and/or combine two or more of the following components: 1) a nucleic acid sequence that encodes a protein comprising the

consensus H7N9 HA amino acid sequence; and 2) a protein comprising the consensus H7N9 HA amino acid sequence.

The consensus H7N9 HA nucleic acid and/or amino acid sequence can be combined in a vaccine cocktail further comprising consensus amino acid sequences of influenza A H1 and H2 (referred to herein as “consensus H1S” or “HS09” (SEQ ID NO:2) and “consensus H2” or “H2HA” (SEQ ID NO:7), respectively), a synthetic hybrid consensus H1 influenza A hemagglutinin amino acid sequence (referred to herein as “consensus U2” or “H1U2” (SEQ ID NO:10)), a consensus amino acid sequence of influenza B hemagglutinin (referred to herein as “consensus BHA” or “BHA-1” (SEQ ID NO:13)), a consensus amino acid sequence H1Bris hemagglutinin “ConBris or “H1Bris” (SEQ ID NO:20), a consensus amino acid sequence H1TT hemagglutinin “ConTT” “H1TT” (SEQ ID NO:22), a consensus amino acid sequence H3 hemagglutinin or “H3HA-2” (SEQ ID NO:24), a consensus amino acid sequence influenza B hemagglutinin or “BHA-2” (SEQ ID NO:26), a consensus amino acid sequence H3 hemagglutinin or “H3HA-3” (SEQ ID NO:28), a consensus amino acid sequence H3 hemagglutinin or “H3HA-4” (SEQ ID NO:30), a consensus amino acid sequence influenza B hemagglutinin or “BHA-3” (SEQ ID NO:32), a consensus amino acid sequence influenza B hemagglutinin or “BHA-4” (SEQ ID NO:34), a synthetic hybrid consensus H1 influenza A hemagglutinin “consensus U” or “H1U” (SEQ ID NO:36), and a consensus amino acid sequence H3 hemagglutinin or “H3HA-1” (SEQ ID NO:38) are provided, which can provide protection of mammals against influenza. In addition, proteins are provided which comprise the consensus H1 amino acid sequence, the consensus H2 amino acid sequence, the consensus U2 amino acid sequence and/or the consensus BHA amino acid sequence. In some aspects, nucleic acid sequences are provided which encode proteins comprising the HS09 amino acid sequence (for example SEQ ID NO:1 or SEQ ID NO:3), the H2HA amino acid sequence (for example SEQ ID NO:6), the H1U2 amino acid sequence (for example SEQ ID NO:9 or SEQ ID NO:11), the BHA-1 amino acid sequence (for example SEQ ID NO:13) or (SEQ ID NO:15)), the H1Bris amino acid sequence (for example SEQ ID NO:19), the H1TT amino acid sequence (for example SEQ ID NO:21), the H3HA-2 amino acid sequence (for example SEQ ID NO:23), the BHA-2 amino acid sequence (for example SEQ ID NO:25), the H3HA-3 amino acid sequence (for example SEQ ID NO:27), the H3HA-4 amino acid sequence (for example SEQ ID NO:29), the BHA-3 amino acid sequence (for example SEQ ID NO:31), the BHA-4 amino acid sequence (for example SEQ ID NO:33), the H1U amino acid sequence (for example SEQ ID NO:35), and the H3HA-1 amino acid sequence (for example SEQ ID NO:37).

While not being bound by scientific theory, a combination H7N9 HA vaccine that can be used to elicit an immune response (humeral, cellular, or both) broadly against multiple influenza subtypes can comprise one or more of the following: 1) a nucleic acid sequence that encodes a protein comprising the consensus H7N9 HA amino acid sequence; 2) a protein comprising the consensus H7N9 HA amino acid sequence; 3) a nucleic acid sequence that encodes a protein comprising the consensus H1HA amino acid sequence; 4) a protein comprising the consensus H1HA amino acid sequence; 5) a nucleic acid sequence that encodes a protein comprising the consensus H2 amino acid sequence; 6) a protein comprising the consensus H2HA amino acid sequence; 7) a nucleic acid sequence that encodes a protein comprising the consensus H1U and/or H1U2 amino acid sequence; 8) a protein comprising the consensus H1U and/or H1U2 amino acid sequence; 9) a nucleic acid sequence that encodes a protein comprising the consensus BHA amino acid sequence; and 10) a protein comprising the consensus BHA amino acid sequence.

Immunization methods can be performed and vaccines can be prepared which use and/or combine two or more of the following components: 1) a nucleic acid sequence that encodes a protein comprising the consensus H7N9 HA amino acid sequence; 2) a protein comprising the consensus H7N9 HA amino acid sequence; 3) a nucleic acid sequence that encodes a protein comprising the consensus H1 amino acid sequence; 4) a protein comprising the consensus H1 amino acid sequence; 5) a nucleic acid sequence that encodes a protein comprising the consensus H2 amino acid sequence, 6) a protein comprising the consensus H2 amino acid sequence; 7) a nucleic acid sequence that encodes a protein comprising the consensus U2 amino acid sequence, 8) a protein comprising the consensus U2 amino acid sequence, 9) a nucleic acid sequence that encodes a protein comprising the consensus BHA amino acid sequence, and 10) a protein comprising the consensus BHA amino acid sequence.

For more broad based treatments against influenza, immunization methods can be performed and vaccines can be prepared which use and/or combine one or more other influenza proteins such as influenza H7N9, influenza A H1-H16, influenza A N1-N9, influenza B hemagglutinin, influenza B neuraminidase and/or genes encoding these proteins together with one or more of the following components: 1) a nucleic acid sequence that encodes a protein comprising the consensus H7N9 amino acid sequence; 2) a protein comprising the consensus H7N9 amino acid sequence; 3) a nucleic acid sequence that encodes a protein comprising the consensus H1 amino acid sequence; 4) a protein comprising the consensus H1 amino acid sequence; 5) a nucleic acid sequence that encodes a protein comprising the consensus H2 amino acid sequence, 6) a protein comprising the consensus H2 amino acid sequence; 7) a

nucleic acid sequence that encodes a protein comprising the consensus U2 amino acid sequence, 8) a protein comprising the consensus U2 amino acid sequence, 9) a nucleic acid sequence that encodes a protein comprising the consensus BHA amino acid sequence, and 10) a protein comprising the consensus BHA amino acid sequence.

5

1. Definitions.

The terminology used herein is for the purpose of describing particular embodiments only and is not intended to be limiting. As used in the specification and the appended claims, the singular forms “a,” “an” and “the” include plural referents unless the context clearly dictates otherwise.

10

For recitation of numeric ranges herein, each intervening number there between with the same degree of precision is explicitly contemplated. For example, for the range of 6-9, the numbers 7 and 8 are contemplated in addition to 6 and 9, and for the range 6.0-7.0, the numbers 6.0, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, and 7.0 are explicitly contemplated.

15

a. Adjuvant

“Adjuvant” as used herein means any molecule added to the DNA plasmid vaccines described herein to enhance the immunogenicity of the antigens encoded by the DNA plasmids and the encoding nucleic acid sequences described hereinafter.

b. Antibody

20

“Antibody” as used herein means an antibody of classes IgG, IgM, IgA, IgD or IgE, or fragments, fragments or derivatives thereof, including Fab, F(ab')₂, Fd, and single chain antibodies, diabodies, bispecific antibodies, bifunctional antibodies and derivatives thereof. The antibody can be an antibody isolated from the serum sample of mammal, a polyclonal antibody, affinity purified antibody, or mixtures thereof which exhibits sufficient binding specificity to a desired epitope or a sequence derived therefrom.

25

c. Coding Sequence

“Coding sequence” or “encoding nucleic acid” as used herein means the nucleic acids (RNA or DNA molecule) that comprise a nucleotide sequence which encodes a protein. The coding sequence can further include initiation and termination signals operably linked to regulatory elements including a promoter and polyadenylation signal capable of directing expression in the cells of an individual or mammal to which the nucleic acid is administered.

30

d. Complement

“Complement” or “complementary” as used herein means a nucleic acid can mean Watson-Crick (*e.g.*, A-T/U and C-G) or Hoogsteen base pairing between nucleotides or nucleotide analogs of nucleic acid molecules.

5 e. Consensus or Consensus Sequence

“Consensus” or “consensus sequence” as used herein means a polypeptide sequence based on analysis of an alignment of multiple subtypes of a particular influenza antigen.

Nucleic acid sequences that encode a consensus polypeptide sequence can be prepared.

Vaccines comprising proteins that comprise consensus sequences and/or nucleic acid

10 molecules that encode such proteins can be used to induce broad immunity against multiple subtypes or serotypes of a particular influenza antigen. Consensus influenza antigens can include influenza H7N9 HA consensus amino acid sequences, influenza A consensus hemagglutinin amino acid sequences, including for example consensus H1, consensus H2, or influenza B consensus hemagglutinin amino acid sequences.

15 f. Constant Current

“Constant current” as used herein means a current that is received or experienced by a tissue, or cells defining said tissue, over the duration of an electrical pulse delivered to same tissue. The electrical pulse is delivered from the electroporation devices described herein.

This current remains at a constant amperage in said tissue over the life of an electrical pulse

20 because the electroporation device provided herein has a feedback element, preferably having instantaneous feedback. The feedback element can measure the resistance of the tissue (or cells) throughout the duration of the pulse and cause the electroporation device to alter its electrical energy output (*e.g.*, increase voltage) so current in same tissue remains constant throughout the electrical pulse (on the order of microseconds), and from pulse to pulse. In
25 some embodiments, the feedback element comprises a controller.

g. Current Feedback or Feedback

“Current feedback” or “feedback” can be used interchangeably and means the active response of the provided electroporation devices, which comprises measuring the current in tissue between electrodes and altering the energy output delivered by the EP device

30 accordingly in order to maintain the current at a constant level. This constant level is preset by a user prior to initiation of a pulse sequence or electrical treatment. The feedback can be accomplished by the electroporation component, *e.g.*, controller, of the electroporation device, as the electrical circuit therein is able to continuously monitor the current in tissue between electrodes and compare that monitored current (or current within tissue) to a preset

current and continuously make energy-output adjustments to maintain the monitored current at preset levels. The feedback loop can be instantaneous as it is an analog closed-loop feedback.

h. Decentralized Current

5 “Decentralized current” as used herein means the pattern of electrical currents delivered from the various needle electrode arrays of the electroporation devices described herein, wherein the patterns minimize, or preferably eliminate, the occurrence of electroporation related heat stress on any area of tissue being electroporated.

i. Electroporation

10 “Electroporation,” “electro-permeabilization,” or “electro-kinetic enhancement” (“EP”) as used interchangeably herein means the use of a transmembrane electric field pulse to induce microscopic pathways (pores) in a bio-membrane; their presence allows biomolecules such as plasmids, oligonucleotides, siRNA, drugs, ions, and water to pass from one side of the cellular membrane to the other.

j. Feedback Mechanism

15 “Feedback mechanism” as used herein means a process performed by either software or hardware (or firmware), which process receives and compares the impedance of the desired tissue (before, during, and/or after the delivery of pulse of energy) with a present value, preferably current, and adjusts the pulse of energy delivered to achieve the preset
20 value. A feedback mechanism can be performed by an analog closed loop circuit.

k. Fragment

“Fragment” as used herein with respect to nucleic acid sequences means a nucleic acid sequence or a portion thereof, that encodes a polypeptide capable of eliciting an immune response in a mammal that cross reacts with a full length wild type strain influenza antigen,
25 including, e.g., an influenza H7N9 HA antigen, an influenza A H1 hemagglutinin, an influenza A H2 hemagglutinin or an influenza B hemagglutinin. The fragments can be DNA fragments selected from at least one of the various nucleotide sequences that encode the consensus amino acid sequences and constructs comprising such sequences, including SEQ ID NOS: 1, 6, 9, 13, 15, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37 and 39; and nucleotide
30 sequences encoding SEQ ID NOs: 2, 7, 10, 14, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38 and 40. DNA fragments can comprise coding sequences for the immunoglobulin leader such as IgE or IgG sequences. The DNA fragments can be 30 or more nucleotides in length, 45 or more, 60 or more, 75 or more, 90 or more, 120 or more, 150 or more, 180 or more, 210 or more, 240 or more, 270 or more, 300 or more, 360 or more, 420 or more, 480 or more, 540 or more,

600 or more, 660 or more, 720 or more, 780 or more, 840 or more, 900 or more, 960 or more, 1020 or more, 1080 or more, 1140 or more, 1200 or more, 1260 or more, 1320 or more, 1380 or more, 1440 or more, 1500 or more, 1560 or more, 1620 or more, 1680 or more, 1740 or more, 1800 or more, 1860 or more, 1820 or more, 1880 or more, 1940 or more, 2000 or more, 2600 or more, 2700 or more, 2800 or more, 2900 or more, 2910 or more, 2920 or more, 2930 or more, 2931 or more, 2932 or more, 2933 or more, 2934 or more, 2935 or more, 2936 or more, 2937 or more, or 2938 or more in length. DNA fragments can be fewer than 10 nucleotides, fewer than 20, fewer than 30, fewer than 40, fewer than 50, fewer than 60, fewer than 75, fewer than 90, fewer than 120, fewer than 150, fewer than 180, fewer than 210, fewer than 240, fewer than 270, fewer than 300, fewer than 360, fewer than 420, fewer than 480, fewer than 540, fewer than 600, fewer than 660, fewer than 720, fewer than 780, fewer than 840, fewer than 900, fewer than 960, fewer than 1020, fewer than 1080, fewer than 1140, fewer than 1200, fewer than 1260, fewer than 1320, fewer than 1380, fewer than 1440, fewer than 1500, fewer than 1560, fewer than 1620, fewer than 1680, or fewer than 1740 nucleotides, fewer than 1800, fewer than 1860, fewer than 1820, fewer than 1880, fewer than 1940, fewer than 2000, fewer than 2600, fewer than 2700, fewer than 2800, fewer than 2900, fewer than 2910, fewer than 2920, fewer than 2930, fewer than 2931, fewer than 2932, fewer than 2933, fewer than 2934, fewer than 2935, fewer than 2936, fewer than 2937, or fewer than 2938.

“Fragment” with respect to polypeptide sequences means a polypeptide capable of eliciting an immune response in a mammal that cross reacts with a full length wild type strain influenza antigen, including, e.g., an influenza A H1 hemagglutinin, an influenza A H2 hemagglutinin or an influenza B hemagglutinin. The fragment can be polypeptide fragment selected from at least one of the various polypeptide sequences of the present invention, including SEQ ID NOs: 2, 7, 10, 14, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38 and 40.

Polypeptide fragments can be analyzed to contact at least one antigenic epitope as provided by a publicly available database such as the Los Alamos National Laboratory’s HA Sequence Database. Polypeptides HA fragments can further comprise amino acid sequences for the immunoglobulin leader such as IgE or IgG. The polypeptide fragments can be 30 or more amino acids in length, 45 or more, 60 or more, 75 or more, 90 or more, 120 or more, 150 or more, 180 or more, 210 or more, 240 or more, 270 or more, 300 or more, 360 or more, 420 or more, 480 or more, 540 or more, 600 or more, 660 or more, or 710 amino acids or more in length. Polypeptide fragments can be fewer than 10 amino acids, fewer than 20, fewer than 30, fewer than 40, fewer than 50, fewer than 60, fewer than 75, fewer than 90, fewer than

120, fewer than 150, fewer than 180, fewer than 210, fewer than 240, fewer than 270, fewer than 300, fewer than 360, fewer than 420, fewer than 480, fewer than 540, fewer than 600, fewer than 660, fewer than 700, fewer than 701, fewer than 702, fewer than 703, fewer than 704, fewer than 705, fewer than 706, fewer than 707, fewer than 708, fewer than 709, or
5 fewer than 710 amino acids in length.

l. Genetic construct

As used herein, the term "genetic construct" refers to the DNA or RNA molecules that comprise a nucleotide sequence which encodes a protein. The coding sequence includes initiation and termination signals operably linked to regulatory elements including a promoter
10 and polyadenylation signal capable of directing expression in the cells of the individual to whom the nucleic acid molecule is administered. As used herein, the term "expressible form" refers to gene constructs that contain the necessary regulatory elements operable linked to a coding sequence that encodes a protein such that when present in the cell of the individual, the coding sequence will be expressed.

m. Identical

"Identical" or "identity" as used herein in the context of two or more nucleic acids or polypeptide sequences, means that the sequences have a specified percentage of residues that are the same over a specified region. The percentage can be calculated by optimally aligning the two sequences, comparing the two sequences over the specified region, determining the
20 number of positions at which the identical residue occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the specified region, and multiplying the result by 100 to yield the percentage of sequence identity. In cases where the two sequences are of different lengths or the alignment produces one or more staggered ends and the specified region of comparison
25 includes only a single sequence, the residues of single sequence are included in the denominator but not the numerator of the calculation. When comparing DNA and RNA, thymine (T) and uracil (U) can be considered equivalent. Identity can be performed manually or by using a computer sequence algorithm such as BLAST or BLAST 2.0.

n. Impedance

30 "Impedance" can be used when discussing the feedback mechanism and can be converted to a current value according to Ohm's law, thus enabling comparisons with the preset current.

o. Immune Response

“Immune response” as used herein means the activation of a host’s immune system, e.g., that of a mammal, in response to the introduction of antigen such as an influenza hemagglutinin consensus antigen. The immune response can be in the form of a cellular or humoral response, or both.

p. Nucleic Acid

“Nucleic acid” or “oligonucleotide” or “polynucleotide” as used herein means at least two nucleotides covalently linked together. The depiction of a single strand also defines the sequence of the complementary strand. Thus, a nucleic acid also encompasses the complementary strand of a depicted single strand. Many variants of a nucleic acid can be used for the same purpose as a given nucleic acid. Thus, a nucleic acid also encompasses substantially identical nucleic acids and complements thereof. A single strand provides a probe that can hybridize to a target sequence under stringent hybridization conditions. Thus, a nucleic acid also encompasses a probe that hybridizes under stringent hybridization conditions.

Nucleic acids can be single stranded or double stranded, or can contain portions of both double stranded and single stranded sequence. The nucleic acid can be DNA, both genomic and cDNA, RNA, or a hybrid, where the nucleic acid can contain combinations of deoxyribo- and ribo-nucleotides, and combinations of bases including uracil, adenine, thymine, cytosine, guanine, inosine, xanthine hypoxanthine, isocytosine and isoguanine. Nucleic acids can be obtained by chemical synthesis methods or by recombinant methods.

q. Operably Linked

“Operably linked” as used herein means that expression of a gene is under the control of a promoter with which it is spatially connected. A promoter can be positioned 5' (upstream) or 3' (downstream) of a gene under its control. The distance between the promoter and a gene can be approximately the same as the distance between that promoter and the gene it controls in the gene from which the promoter is derived. As is known in the art, variation in this distance can be accommodated without loss of promoter function.

r. Promoter

“Promoter” as used herein means a synthetic or naturally-derived molecule which is capable of conferring, activating or enhancing expression of a nucleic acid in a cell. A promoter can comprise one or more specific transcriptional regulatory sequences to further enhance expression and/or to alter the spatial expression and/or temporal expression of same. A promoter can also comprise distal enhancer or repressor elements, which can be located as

much as several thousand base pairs from the start site of transcription. A promoter can be derived from sources including viral, bacterial, fungal, plants, insects, and animals. A promoter can regulate the expression of a gene component constitutively, or differentially with respect to cell, the tissue or organ in which expression occurs or, with respect to the developmental stage at which expression occurs, or in response to external stimuli such as physiological stresses, pathogens, metal ions, or inducing agents. Representative examples of promoters include the bacteriophage T7 promoter, bacteriophage T3 promoter, SP6 promoter, lac operator-promoter, tac promoter, SV40 late promoter, SV40 early promoter, RSV-LTR promoter, CMV IE promoter, SV40 early promoter or SV40 late promoter and the CMV IE promoter.

s. Stringent Hybridization Conditions

“Stringent hybridization conditions” as used herein means conditions under which a first nucleic acid sequence (e.g., probe) will hybridize to a second nucleic acid sequence (e.g., target), such as in a complex mixture of nucleic acids. Stringent conditions are sequence-dependent and will be different in different circumstances. Stringent conditions can be selected to be about 5-10°C lower than the thermal melting point (T_m) for the specific sequence at a defined ionic strength pH. The T_m can be the temperature (under defined ionic strength, pH, and nucleic concentration) at which 50% of the probes complementary to the target hybridize to the target sequence at equilibrium (as the target sequences are present in excess, at T_m , 50% of the probes are occupied at equilibrium). Stringent conditions can be those in which the salt concentration is less than about 1.0 M sodium ion, such as about 0.01-1.0 M sodium ion concentration (or other salts) at pH 7.0 to 8.3 and the temperature is at least about 30°C for short probes (e.g., about 10-50 nucleotides) and at least about 60°C for long probes (e.g., greater than about 50 nucleotides). Stringent conditions can also be achieved with the addition of destabilizing agents such as formamide. For selective or specific hybridization, a positive signal can be at least 2 to 10 times background hybridization. Exemplary stringent hybridization conditions include the following: 50% formamide, 5x SSC, and 1% SDS, incubating at 42°C, or, 5x SSC, 1% SDS, incubating at 65°C, with wash in 0.2x SSC, and 0.1% SDS at 65°C.

t. Substantially Complementary

“Substantially complementary” as used herein means that a first sequence is at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98% or 99% identical to the complement of a second sequence over a region of 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22,

23, 24, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 180, 270, 360, 450, 540, 630, 720, 810, 900, 990, 1080, 1170, 1260, 1350, 1440, 1530, 1620, 1710, 1800, 1890, 1980, 2070 or more nucleotides or amino acids, or that the two sequences hybridize under stringent hybridization conditions.

5 **u. Substantially Identical**

“Substantially identical” as used herein means that a first and second sequence are at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98% or 99% identical over a region of 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 180, 270, 360, 450, 540, 630, 720, 810, 900, 990, 1080, 1170, 1260, 1350, 1440, 1530, 1620, 1710, 1800, 1890, 1980, 2070 or more nucleotides or amino acids, or over the entire length of the nucleic acid sequence or amino acid sequence, if the first sequence is substantially complementary to the complement of the second sequence.

v. Subtype or Serotype

“Subtype” or “serotype”: as used herein, interchangeably, and in reference to influenza virus, means genetic variants of an influenza virus such that one subtype is recognized by an immune system apart from a different subtype.

w. Variant

“Variant” used herein with respect to a nucleic acid means (i) a portion or fragment of a referenced nucleotide sequence; (ii) the complement of a referenced nucleotide sequence or portion thereof; (iii) a nucleic acid that is substantially identical to a referenced nucleic acid or the complement thereof; or (iv) a nucleic acid that hybridizes under stringent conditions to the referenced nucleic acid, complement thereof, or a sequences substantially identical thereto.

“Variant” with respect to a peptide or polypeptide that differs in amino acid sequence by the insertion, deletion, or conservative substitution of amino acids, but retain at least one biological activity. Variant can also mean a protein with an amino acid sequence that is substantially identical to a referenced protein with an amino acid sequence that retains at least one biological activity. A conservative substitution of an amino acid, i.e., replacing an amino acid with a different amino acid of similar properties (e.g., hydrophilicity, degree and distribution of charged regions) is recognized in the art as typically involving a minor change. These minor changes can be identified, in part, by considering the hydropathic index of amino acids, as understood in the art. Kyte et al., J. Mol. Biol. 157:105-132 (1982). The hydropathic index of an amino acid is based on a consideration of its hydrophobicity and charge. It is known in the art that amino acids of similar hydropathic indexes can be

substituted and still retain protein function. In one aspect, amino acids having hydrophobic indexes of ± 2 are substituted. The hydrophilicity of amino acids can also be used to reveal substitutions that would result in proteins retaining biological function. A consideration of the hydrophilicity of amino acids in the context of a peptide permits calculation of the greatest local average hydrophilicity of that peptide, a useful measure that has been reported to correlate well with antigenicity and immunogenicity. U.S. Patent No. 4,554,101, incorporated fully herein by reference. Substitution of amino acids having similar hydrophilicity values can result in peptides retaining biological activity, for example immunogenicity, as is understood in the art. Substitutions can be performed with amino acids having hydrophilicity values within ± 2 of each other. Both the hydrophobicity index and the hydrophilicity value of amino acids are influenced by the particular side chain of that amino acid. Consistent with that observation, amino acid substitutions that are compatible with biological function are understood to depend on the relative similarity of the amino acids, and particularly the side chains of those amino acids, as revealed by the hydrophobicity, hydrophilicity, charge, size, and other properties.

x. Vector

"Vector" as used herein means a nucleic acid sequence containing an origin of replication. A vector can be a vector, bacteriophage, bacterial artificial chromosome or yeast artificial chromosome. A vector can be a DNA or RNA vector. A vector can be a self-replicating extrachromosomal vector, and preferably, is a DNA plasmid.

2. H7N9 HA Influenza Antigen

Provided herein are H7N9 antigens capable of eliciting an immune response in a mammal against one or more H7N9 influenza strains. The H7N9 antigen can be an hemagglutinin antigen (HA). The antigen can be capable of eliciting an immune response in a mammal against one or more H7N9 influenza strains, including against one or more pandemic strains, such as the Chinese avian influenza A (H7N9). The antigen can comprise epitopes that make them particularly effective as immunogens against which, anti-H7N9 influenza immune responses can be induced.

The H7N9 antigen can comprise the full length translation product HA of H7N9, a variant thereof, a fragment thereof or a combination thereof. The influenza hemagglutinin H7N9 antigen can be a consensus sequence derived from multiple strains of influenza A serotype H7N9. The H7N9 antigen can contain at least one antigenic epitope that can be effective against particular influenza immunogens against which an immune response can be induced. The H7N9 antigen can provide an entire repertoire of immunogenic sites and

epitopes present in an intact influenza H7N9 virus. The H7N9 antigen can be a consensus hemagglutinin antigen sequence that can be derived from hemagglutinin antigen sequences from a plurality of influenza A virus strains of one serotype H7N9. The antigen can be a hybrid consensus H7N9 hemagglutinin antigen sequence that can be derived from combining
 5 two different consensus H7N9 hemagglutinin antigen sequences or portions thereof. Each of two different consensus H7N9 hemagglutinin antigen sequences can be derived from a different set of a plurality of influenza A H7N9 virus strains of one serotype. The antigen can be a consensus H7N9 hemagglutinin antigen sequence that can be derived from H7N9 hemagglutinin antigen sequences from a plurality of influenza A H7N9 virus strains.

10 The consensus H7N9 hemagglutinin antigen may be encoded by an isolated nucleic acid. The isolated nucleic acid can be a nucleic acid sequence encoding an amino acid sequence selected from the group consisting of SEQ ID NO: 40, an amino acid sequence that is 95% identical over the entire length of the amino acid sequence of SEQ ID NO: 40, a fragment of SEQ ID NO: 40 comprising at least 30 amino acids, and an amino acid sequence
 15 that is 95% identical to a fragment of SEQ ID NO: 40 comprising at least 30 amino acids. The isolated nucleic acid can be a nucleic acid sequence encoding an amino acid sequence of SEQ ID NO: 40. The isolated nucleic acid can be a nucleic acid sequence of SEQ ID NO: 39.

The consensus H7N9 hemagglutinin antigen can be a protein comprising SEQ ID NO:
 20 40 (the consensus HA amino acid sequence of Chinese avian influenza A (H7N9)) wherein amino acids 1-560 correspond to the HA consensus amino acid sequence.

The consensus H7N9 hemagglutinin antigen can further comprise one or more additional amino acid sequence elements. The consensus H7N9 hemagglutinin antigen can further comprise on its N-terminal an IgE or IgG leader amino acid sequence. The IgE leader
 25 amino acid sequence can be SEQ ID NO: 17. The consensus H7N9 hemagglutinin antigen can further comprise an immunogenic tag which is a unique immunogenic epitope that can be detected by readily available antibodies. An example of such an immunogenic tag is the 9 amino acid influenza HA Tag which can be linked on the consensus H7N9 hemagglutinin antigen terminus. The HA Tag amino acid sequence can be SEQ ID NO:18. In some
 30 embodiments, consensus H7N9 hemagglutinin antigen can further comprise on its N-terminal an IgE or IgG leader amino acid sequence and on its C terminal an HA tag.

The consensus H7N9 hemagglutinin antigen can be a consensus H7N9 hemagglutinin HA protein that consists of consensus influenza amino acid sequences or fragments and variants thereof. The consensus H7N9 hemagglutinin antigen can be a consensus

hemagglutinin protein that comprises non-influenza protein sequences and influenza protein sequences or fragments and variants thereof.

Examples of a consensus H7N9 hemagglutinin antigen HA protein include those that can consist of the consensus H7N9 hemagglutinin antigen amino acid sequence (SEQ ID NO:40) or those that further comprise additional elements such as an IgE leader sequence, or an HA Tag or both an IgE leader sequence and an HA Tag. An example of the consensus H7N9 hemagglutinin antigen HA protein can include both an IgE leader sequence and an HA Tag, which comprises the consensus H7N9 hemagglutinin antigen HA amino acid coding sequence (SEQ ID NO:2) linked to the IgE leader amino acid sequence (SEQ ID NO: 17) at its N terminal and linked to the HA Tag (SEQ ID NO:18) at its C terminal.

The consensus H7N9 hemagglutinin protein can be encoded by a consensus H7N9 hemagglutinin nucleic acid, a variant thereof or a fragment thereof. Unlike the consensus H7N9 hemagglutinin protein which can be a consensus sequence derived from a plurality of different H7N9 hemagglutinin sequences from different strains and variants, the consensus H7N9 hemagglutinin nucleic acid refers to a nucleic acid sequence that encodes a consensus protein sequence and the coding sequences used can differ from those used to encode the particular amino acid sequences in the plurality of different H7N9 hemagglutinin sequences from which the consensus H7N9 hemagglutinin protein sequence is derived. The consensus nucleic acid sequence can be codon optimized and/or RNA optimized. The consensus H7N9 hemagglutinin nucleic acid sequence can comprise a Kozak's sequence in the 5' untranslated region. The consensus H7N9 hemagglutinin nucleic acid sequence can comprise nucleic acid sequences that encode a leader sequence. The coding sequence of an N terminal leader sequence is 5' of the H7N9 hemagglutinin coding sequence. The N-terminal leader can facilitate secretion. The N-terminal leader can be an IgE leader or an IgG leader. The consensus H7N9 hemagglutinin nucleic acid sequence can comprise nucleic acid sequences that encode an immunogenic tag. The immunogenic tag can be on the C terminus of the protein and the sequence encoding it is 3' of the HA coding sequence. The immunogenic tag provides a unique epitope for which there are readily available antibodies so that such antibodies can be used in assays to detect and confirm expression of the protein. The immunogenic tag can be an H Tag at the C-terminus of the protein.

Consensus H7N9 hemagglutinin nucleic acid can have a polynucleotide sequence that encodes a protein that comprises the amino acid sequence of SEQ ID NO: 40. The consensus H7N9 hemagglutinin nucleic acid can comprise the nucleic sequence of SEQ ID NO: 39. The consensus H7N9 hemagglutinin nucleic acid can further comprise a polynucleotide

sequence encoding the IgE leader amino acid sequence, or a polynucleotide sequence encoding an HA Tag amino acid sequence, or both. SEQ ID NO: 17 is an IgE leader polypeptide sequence. SEQ ID NO: 18 is an HA Tag polypeptide sequence. Examples of H7N9 hemagglutinin consensus nucleic acids that further comprise polynucleotide sequences encoding an IgE leader sequence and an HA Tag include nucleic acid molecules that encode proteins that comprise amino acid sequence of SEQ ID NO: 40.

The synthetic H7N9 HA nucleic acid is capable of eliciting robust cellular immune responses, broadly cross-reactive antibody responses and generating complete protection from lethal challenge with just a few week development and vaccination regime due to the consensus sequence across many different strains of H7N9. For example, the disclosed enhanced DNA approach provides as influenza vaccine platform that 1) can be rapidly and straightforwardly utilized to design an effective vaccine against multiple known and new strains, 2) includes a delivery mechanism that can quickly induce cellular and humoral immune responses against new antigens in humans, 3) is rapid from design to scale up and mass production, with a relevant clinical track record and 4) is very stable for distribution purposes. Using this approach, millions of doses can be produced at scale within a few months of identification of the pathogen. In addition the stability of DNA and the lack of requirement of any viral amplification step ensure fidelity in the process.

3. Combinational Influenza Antigens

Provided herein are antigens capable of eliciting an immune response in a mammal against one or more influenza serotypes in combination with the H7N9 antigen as discussed above. The antigen that can be combined with the H7N9 antigen can be capable of eliciting an immune response in a mammal against one or more influenza serotypes, including against one or more pandemic strains, such as 2009 H1N1 swine or H7N9 avian originated influenza. The antigen can be capable of eliciting an immune response in a mammal against one or more influenza serotype, including against one or more strains of swine or avian derived human influenza. The antigen can comprise epitopes that make them particularly effective as immunogens against which anti-influenza immune responses can be induced.

The antigen can comprise the full length translation product HA0, subunit HA1, subunit HA2, a variant thereof, a fragment thereof or a combination thereof. The influenza hemagglutinin antigen can be a consensus sequence derived from multiple strains of influenza A serotype H1, a consensus sequence derived from multiple strains of influenza A serotype H2, a hybrid sequence containing portions of two different consensus sequences

derived from different sets of multiple strains of influenza A serotype H1 or a consensus
 sequence derived from multiple strains of influenza B. The influenza hemagglutinin antigen
 can be from influenza B. The antigen can contain at least one antigenic epitope that can be
 effective against particular influenza immunogens against which an immune response can be
 5 induced. The antigen can provide an entire repertoire of immunogenic sites and epitopes
 present in an intact influenza virus. The antigen can be a consensus hemagglutinin antigen
 sequence that can be derived from hemagglutinin antigen sequences from a plurality of
 influenza A virus strains of one serotype such as a plurality of influenza A virus strains of
 serotype H1 or of serotype H2. The antigen can be a hybrid consensus hemagglutinin antigen
 10 sequence that can be derived from combining two different consensus hemagglutinin antigen
 sequences or portions thereof. Each of two different consensus hemagglutinin antigen
 sequences can be derived from a different set of a plurality of influenza A virus strains of one
 serotype such as a plurality of influenza A virus strains of serotype H1. The antigen can be a
 consensus hemagglutinin antigen sequence that can be derived from hemagglutinin antigen
 15 sequences from a plurality of influenza B virus strains.

The consensus hemagglutinin antigen can be a protein comprising SEQ ID NO: 2 (the
 consensus H1 amino acid sequence) wherein amino acids 1-343 correspond to the HA1
 subunit of the precursor HA0 consensus H1 amino acid sequence and amino acids 344-566
 correspond to the HA2 subunit of the HA0 consensus H1 amino acid sequence. The
 20 consensus hemagglutinin antigen can also be a consensus hemagglutinin protein derived from
 hemagglutinin sequences from H1HA strains, such as a protein comprising SEQ ID NO: 20
 (H1Bris) or SEQ ID NO:22 (H1TT). The consensus hemagglutinin antigen can be a protein
 comprising SEQ ID NO: 7 (H2HA). The consensus hemagglutinin antigen can be a
 consensus hemagglutinin protein derived from hemagglutinin sequences from H3HA strains,
 25 such as a protein comprising SEQ ID NO:24 (H3HA-2), SEQ ID NO:28 (H3HA-3), SEQ ID
 NO:30 (H3HA-4), or SEQ ID NO: 38 (H3HA-1). The consensus hemagglutinin antigen can
 be a synthetic hybrid consensus H1 sequences comprising portions of two different consensus
 H1 sequences which are each derived from a different set of sequences from the other. An
 example of a consensus HA antigen that is a synthetic hybrid consensus H1 protein is a
 30 protein comprising SEQ ID NO: 10 (H1U2) or SEQ ID NO:36 (H1U). The consensus
 hemagglutinin antigen can be a consensus hemagglutinin protein derived from hemagglutinin
 sequences from influenza B strains, such as a protein comprising SEQ ID NO: 14 (BHA-1),
 SEQ ID NO: 26 (BHA-2), SEQ ID NO: 32 (BHA-3), or SEQ ID NO: 34 (BHA-4).

The consensus hemagglutinin antigen can further comprise one or more additional amino acid sequence elements. The consensus hemagglutinin antigen can further comprise on its N-terminal an IgE or IgG leader amino acid sequence. The IgE leader amino acid sequence can be SEQ ID NO: 17. The consensus hemagglutinin antigen can further comprise
5 an immunogenic tag which is a unique immunogenic epitope that can be detected by readily available antibodies. An example of such an immunogenic tag is the 9 amino acid influenza HA Tag which can be linked on the consensus hemagglutinin C terminus. The HA Tag amino acid sequence can be SEQ ID NO:18. In some embodiments, consensus hemagglutinin antigen can further comprise on its N-terminal an IgE or IgG leader amino
10 acid sequence and on its C terminal an HA tag.

The consensus hemagglutinin antigen can be a consensus hemagglutinin protein that consists of consensus influenza amino acid sequences or fragments and variants thereof. The consensus hemagglutinin antigen can be a consensus hemagglutinin protein that comprises non-influenza protein sequences and influenza protein sequences or fragments and variants
15 thereof.

Examples of a consensus H1 protein include those that can consist of the consensus H1 amino acid sequence (SEQ ID NO:2) or those that further comprise additional elements such as an IgE leader sequence, or an HA Tag or both an IgE leader sequence and an HA Tag. An example of the consensus H1 protein that includes both an IgE leader sequence and
20 an HA Tag is SEQ ID NO: 4, which comprises the consensus H1 amino acid coding sequence (SEQ ID NO:2) linked to the IgE leader amino acid sequence (SEQ ID NO: 17) at its N terminal and linked to the HA Tag (SEQ ID NO:18) at its C terminal.

Examples of consensus H2 proteins include those that can consist of the consensus H2 amino acid sequence (SEQ ID NO:7) or those that further comprise an IgE leader sequence,
25 or an HA Tag, or both an IgE leader sequence and an HA Tag.

Examples of hybrid consensus H1 proteins include those that can consist of the consensus U2 amino acid sequence (SEQ ID NO:10) or those that further comprise an IgE leader sequence, or an HA Tag, or both an IgE leader sequence and an HA Tag. An example of the consensus U2 protein is SEQ ID NO:12, which comprises the consensus U2 amino
30 acid sequence (SEQ ID NO:10) linked to the IgE leader amino acid sequence (SEQ ID NO: 17) at its N terminal and linked to the HA Tag (SEQ ID NO:18) at its C terminal.

Examples of hybrid consensus influenza B hemagglutinin proteins include those that can consist of the consensus BHA amino acid sequence (SEQ ID NO:14) or it can comprise an IgE leader sequence, or a an HA Tag, or both an IgE leader sequence and an HA Tag. An

example of the consensus BHA protein is SEQ ID NO:16 which comprises the consensus BHA amino acid sequence (SEQ ID NO:14) linked to the IgE leader amino acid sequence (SEQ ID NO: 17) at its N terminal and linked to the HA Tag (SEQ ID NO:18) at its C terminal.

5 The consensus hemagglutinin protein can be encoded by a consensus hemagglutinin nucleic acid, a variant thereof or a fragment thereof. Unlike the consensus hemagglutinin protein which can be a consensus sequence derived from a plurality of different hemagglutinin sequences from different strains and variants, the consensus hemagglutinin nucleic acid refers to a nucleic acid sequence that encodes a consensus protein sequence and
10 the coding sequences used can differ from those used to encode the particular amino acid sequences in the plurality of different hemagglutinin sequences from which the consensus hemagglutinin protein sequence is derived. The consensus nucleic acid sequence can be codon optimized and/or RNA optimized. The consensus hemagglutinin nucleic acid sequence can comprise a Kozak's sequence in the 5' untranslated region. The consensus
15 hemagglutinin nucleic acid sequence can comprise nucleic acid sequences that encode a leader sequence. The coding sequence of an N terminal leader sequence is 5' of the hemagglutinin coding sequence. The N-terminal leader can facilitate secretion. The N-terminal leader can be an IgE leader or an IgG leader. The consensus hemagglutinin nucleic acid sequence can comprise nucleic acid sequences that encode an immunogenic tag. The
20 immunogenic tag can be on the C terminus of the protein and the sequence encoding it is 3' of the HA coding sequence. The immunogenic tag provides a unique epitope for which there are readily available antibodies so that such antibodies can be used in assays to detect and confirm expression of the protein. The immunogenic tag can be an H Tag at the C-terminus of the protein.

25 Consensus hemagglutinin nucleic acid can have a polynucleotide sequence that encodes a protein that comprises the amino acid sequence of SEQ ID NO: 2, SEQ ID NO:7, SEQ ID NO:10, SEQ ID NO:14, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, or SEQ ID NO:38. A consensus hemagglutinin nucleic acid that encodes SEQ ID NO: 2, SEQ ID NO:7, SEQ ID
30 NO:10, SEQ ID NO:14, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, or SEQ ID NO:38 can be SEQ ID NO:1, SEQ ID NO:6, SEQ ID NO:9, SEQ ID NO:13, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, or SEQ ID NO:37 respectively. The consensus hemagglutinin nucleic acid

can further comprise a polynucleotide sequence encoding the IgE leader amino acid sequence, or a polynucleotide sequence encoding an HA Tag amino acid sequence, or both. SEQ ID NO: 17 is an IgE leader polypeptide sequence. SEQ ID NO: 18 is an HA Tag polypeptide sequence. Examples of hemagglutinin consensus nucleic acids that further
 5 comprise polynucleotide sequences encoding an IgE leader sequence and an HA Tag include nucleic acid molecules that encode proteins that comprise the amino acid sequence of SEQ ID NO:4, SEQ ID NO:12 or SEQ ID NO:16. A consensus hemagglutinin nucleic acid that encodes SEQ ID NO:4, SEQ ID NO:12 or SEQ ID NO:16 can be SEQ ID NO:3, SEQ ID NO:11 or SEQ ID NO:15, respectively.

10

4. Genetic Constructs and Plasmids

Provided herein are genetic constructs that can comprise a nucleic acid sequence that encodes the hemagglutinin H7N9 antigen and hemagglutinin from other influenza serotypes as described above. The genetic construct can be present in the cell as a functioning
 15 extrachromosomal molecule comprising the nucleic acid encoding the hemagglutinin antigen. The genetic construct comprising the nucleic acid encoding the hemagglutinin antigen can be linear minichromosome including centromere, telomers or plasmids or cosmid.

The genetic construct can also be part of a genome of a recombinant viral vector, including recombinant adenovirus, recombinant adenovirus associated virus and recombinant
 20 vaccinia. The genetic construct can be part of the genetic material in attenuated live microorganisms or recombinant microbial vectors which live in cells.

The genetic constructs can comprise regulatory elements for gene expression of the hemagglutinin nucleic acid. The regulatory elements can be a promoter, an enhancer an initiation codon, a stop codon, or a polyadenylation signal.

25 Compositions can comprise nucleic acid sequences of one or more of: SEQ ID NO:1, SEQ ID NO:6, SEQ ID NO:9, SEQ ID NO:13, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, or SEQ ID NO: 39. Compositions can comprise a first nucleic acid sequence which encodes the hemagglutinin consensus antigen selected from the group consisting of
 30 one or more of: influenza A consensus hemagglutinin H7N9, influenza A consensus hemagglutinin H1 antigen, influenza A consensus hemagglutinin H2 antigen, influenza A consensus hemagglutinin H1U and/or H1U2 antigen, and influenza B consensus hemagglutinin protein BHA, which can include SEQ ID NO:1, SEQ ID NO:6, SEQ ID NO:9, SEQ ID NO:13, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID

NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO: 39 or nucleic acid sequences that encode one or more of: SEQ ID NO:2, SEQ ID NO:7, SEQ ID NO:10, SEQ ID NO:14, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, or SEQ ID NO: 40; and can further comprise one or more additional nucleic acid sequence(s) that encodes one or more protein(s) selected from the group consisting of: influenza A hemagglutinin proteins H1, H2, H3, H4, H5, H6, H7, H8, H9, H10, H11, H12, H13, H14, H15, H16, H7N9, influenza A neuraminidase N1, N2, N3, N4, N5, N6, N7, N8, N9, influenza B hemagglutinin (BHA) and influenza B neuraminidase (BNA), including one or more of the consensus sequences provided herein. The first and additional nucleic acid sequences can be present on the same nucleic acid molecule or different nucleic acid molecules. The first and additional nucleic acid sequences can be under the control of regulatory elements that function in a human cell. The additional coding sequence can encode one or more H1, H2, H3, H4, H5, H6, H7, H8, H9, H10, H11, H12, H13, H14, H15, H16, H7N9, N1, N2, N3, N4, N5, N6, N7, N8, N9, BHA and BNA from one or more strains of influenza, or be a consensus derived from a plurality of strains having the serotype, or be a hybrid which includes sequences from two or more consensus sequences.

The nucleic acid sequences can make up a genetic construct that can be a vector. The vector can be capable of expressing a consensus hemagglutinin antigen in the cell of a mammal in a quantity effective to elicit an immune response in the mammal. The vector can be recombinant. The vector can comprise heterologous nucleic acid encoding the consensus hemagglutinin antigen. The vector can be a plasmid. The vector can be useful for transfecting cells with nucleic acid encoding a consensus hemagglutinin antigen, which the transformed host cell is cultured and maintained under conditions wherein expression of the consensus hemagglutinin antigen takes place.

The vector can comprise heterologous nucleic acid encoding a consensus hemagglutinin antigen and can further comprise an initiation codon, which can be upstream of the consensus hemagglutinin coding sequence, and a stop codon, which can be downstream of the consensus hemagglutinin coding sequence. The initiation and termination codon can be in frame with the consensus hemagglutinin coding sequence. The vector can also comprise a promoter that is operably linked to the consensus hemagglutinin coding sequence. The promoter operably linked to the consensus hemagglutinin coding sequence can be a promoter from simian virus 40 (SV40), a mouse mammary tumor virus (MMTV) promoter, a human immunodeficiency virus (HIV) promoter such as the bovine

immunodeficiency virus (BIV) long terminal repeat (LTR) promoter, a Moloney virus promoter, an avian leukosis virus (ALV) promoter, a cytomegalovirus (CMV) promoter such as the CMV immediate early promoter, Epstein Barr virus (EBV) promoter, or a Rous sarcoma virus (RSV) promoter. The promoter can also be a promoter from a human gene
5 such as human actin, human myosin, human hemoglobin, human muscle creatine, or human metallothionein. The promoter can also be a tissue specific promoter, such as a muscle or skin specific promoter, natural or synthetic. Examples of such promoters are described in US patent application publication no.US20040175727, the contents of which are incorporated herein in its entirety.

10 The vector can also comprise a polyadenylation signal, which can be downstream of the HA coding sequence. The polyadenylation signal can be a SV40 polyadenylation signal, LTR polyadenylation signal, bovine growth hormone (bGH) polyadenylation signal, human growth hormone (hGH) polyadenylation signal, or human β -globin polyadenylation signal. The SV40 polyadenylation signal can be a polyadenylation signal from a pCEP4 vector
15 (Invitrogen, San Diego, CA).

The vector can also comprise an enhancer upstream of the consensus hemagglutinin coding. The enhancer can be necessary for DNA expression. The enhancer can be human actin, human myosin, human hemoglobin, human muscle creatine or a viral enhancer such as one from CMV, HA, RSV or EBV. Polynucleotide function enhances are described in U.S.
20 Patent Nos. 5,593,972, 5,962,428, and WO94/016737, the contents of each are fully incorporated by reference.

The vector can also comprise a mammalian origin of replication in order to maintain the vector extrachromosomally and produce multiple copies of the vector in a cell. The vector can be pVAX1 (Figure 1), pCEP4 or pREP4 from Invitrogen (San Diego, CA), which
25 can comprise the Epstein Barr virus origin of replication and nuclear antigen EBNA-1 coding region, which can produce high copy episomal replication without integration. The vector can be pVAX1 with changes such as those described in the paragraph referring to Figure 1 in the Brief Description of the Figures section above. The backbone of the vector can be pAV0242. The vector can be a replication defective adenovirus type 5 (Ad5) vector.

30 The vector can also comprise a regulatory sequence, which can be well suited for gene expression in a mammalian or human cell into which the vector is administered. The consensus hemagglutinin coding sequence can comprise a codon, which can allow more efficient transcription of the coding sequence in the host cell.

The vector can be pSE420 (Invitrogen, San Diego, Calif.), which can be used for protein production in *Escherichia coli* (*E. coli*). The vector can also be pYES2 (Invitrogen, San Diego, Calif.), which can be used for protein production in *Saccharomyces cerevisiae* strains of yeast. The vector can also be of the MAXBAC™ complete baculovirus expression system (Invitrogen, San Diego, Calif.), which can be used for protein production in insect cells. The vector can also be pcDNA I or pcDNA3 (Invitrogen, San Diego, Calif.), which can be used for protein production in mammalian cells such as Chinese hamster ovary (CHO) cells. The vector can be expression vectors or systems to produce protein by routine techniques and readily available starting materials including Sambrook et al., Molecular Cloning: A Laboratory Manual, Second Ed., Cold Spring Harbor (1989), which is incorporated fully by reference.

The vector can be pGX2009 or pGX2006, which can be used for expressing the consensus hemagglutinin antigen. The vector pGX2009 (4739 bp, Figure 2; SEQ ID NO: 5) is a modified pVAX1 plasmid with a nucleic acid sequence that encodes a consensus H1 protein (amino acid SEQ ID NO:4 encoded by SEQ ID NO:3) that comprises an IgE leader sequence (amino acid SEQ ID NO:12 encoded by SEQ ID NO:11) linked to a consensus H1 amino acid sequence (amino acid SEQ ID NO:2 encoded by SEQ ID NO:1). The vector pGX2006 (4628 bp; Figure 3, SEQ ID NO:8) is a pVAX1 plasmid with a nucleic acid sequence that encodes a consensus H2 protein (amino acid SEQ ID NO:7 encoded by SEQ ID NO:6). Alternatively, in a similar DNA plasmid backbone pVAX1 as pGX2006 (or pVAX (Invitrogen), a nucleic acid insert can replace the H2HA sequence with any one of the following: SEQ ID NO:1, SEQ ID NO:6, SEQ ID NO:9, SEQ ID NO:13, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, or nucleic acid sequences that encode one or more of: SEQ ID NO:2, SEQ ID NO:7, SEQ ID NO:10, SEQ ID NO:14, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, or SEQ ID NO:38; or any fragments and variants described herein.

The genetic constructs and components disclosed herein which include consensus hemagglutinin coding sequences can be used to express other influenza proteins such as influenza A H1, H2, H3, H4, H5, H6, H7, H8, H9, H10, H11, H12, H13, H14, H15, H16, H7N9, N1, N2, N3, N4, N5, N6, N7, N8, N9, influenza B hemagglutinin or neuraminidase protein whereby coding sequences for influenza A proteins H1, H2, H3, H4, H5, H6, H7, H8, H9, H10, H11, H12, H13, H14, H15, H16, H7N9, N1, N2, N3, N4, N5, N6, N7, N8, N9,

influenza B hemagglutinin or neuraminidase protein are included in place of consensus hemagglutinin coding sequences.

5. Pharmaceutical compositions

5 Provided herein are pharmaceutical compositions according to the present invention which comprise about 1 nanogram to about 10 mg of DNA. In some embodiments, pharmaceutical compositions according to the present invention comprise from between: 1) at least 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95 or 100 nanograms, or at least 1, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 105, 110, 10 115, 120, 125, 130, 135, 140, 145, 150, 155, 160, 165, 170, 175, 180, 185, 190, 195, 200, 205, 210, 215, 220, 225, 230, 235, 240, 245, 250, 255, 260, 265, 270, 275, 280, 285, 290, 295, 300, 305, 310, 315, 320, 325, 330, 335, 340, 345, 350, 355, 360, 365, 370, 375, 380, 385, 390, 395, 400, 405, 410, 415, 420, 425, 430, 435, 440, 445, 450, 455, 460, 465, 470, 475, 480, 485, 490, 495, 500, 605, 610, 615, 620, 625, 630, 635, 640, 645, 650, 655, 660, 15 665, 670, 675, 680, 685, 690, 695, 700, 705, 710, 715, 720, 725, 730, 735, 740, 745, 750, 755, 760, 765, 770, 775, 780, 785, 790, 795, 800, 805, 810, 815, 820, 825, 830, 835, 840, 845, 850, 855, 860, 865, 870, 875, 880, 885, 890, 895, 900, 905, 910, 915, 920, 925, 930, 935, 940, 945, 950, 955, 960, 965, 970, 975, 980, 985, 990, 995 or 1000 micrograms, or at least 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5 or 10 mg or more; and 2) up 20 to and including 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95 or 100 nanograms, or up to and including 1, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 105, 110, 115, 120, 125, 130, 135, 140, 145, 150, 155, 160, 165, 170, 175, 180, 185, 190, 195, 200, 205, 210, 215, 220, 225, 230, 235, 240, 245, 250, 255, 260, 265, 270, 275, 280, 285, 290, 295, 300, 305, 310, 315, 320, 325, 330, 335, 340, 345, 350, 355, 25 360, 365, 370, 375, 380, 385, 390, 395, 400, 405, 410, 415, 420, 425, 430, 435, 440, 445, 450, 455, 460, 465, 470, 475, 480, 485, 490, 495, 500, 605, 610, 615, 620, 625, 630, 635, 640, 645, 650, 655, 660, 665, 670, 675, 680, 685, 690, 695, 700, 705, 710, 715, 720, 725, 730, 735, 740, 745, 750, 755, 760, 765, 770, 775, 780, 785, 790, 795, 800, 805, 810, 815, 820, 825, 830, 835, 840, 845, 850, 855, 860, 865, 870, 875, 880, 885, 890, 895, 900, 905, 30 910, 915, 920, 925, 930, 935, 940, 945, 950, 955, 960, 965, 970, 975, 980, 985, 990, 995, or 1000 micrograms, or up to and including 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5 or 10 mg. In some embodiments, pharmaceutical compositions according to the present invention comprise about 5 nanogram to about 10 mg of DNA. In some embodiments, pharmaceutical compositions according to the present invention comprise

about 25 nanogram to about 5 mg of DNA. In some embodiments, the pharmaceutical compositions contain about 50 nanograms to about 1 mg of DNA. In some embodiments, the pharmaceutical compositions contain about 0.1 to about 500 micrograms of DNA. In some embodiments, the pharmaceutical compositions contain about 1 to about 350 micrograms of DNA. In some embodiments, the pharmaceutical compositions contain about 5 to about 250 micrograms of DNA. In some embodiments, the pharmaceutical compositions contain about 10 to about 200 micrograms of DNA. In some embodiments, the pharmaceutical compositions contain about 15 to about 150 micrograms of DNA. In some embodiments, the pharmaceutical compositions contain about 20 to about 100 micrograms of DNA. In some embodiments, the pharmaceutical compositions contain about 25 to about 75 micrograms of DNA. In some embodiments, the pharmaceutical compositions contain about 30 to about 50 micrograms of DNA. In some embodiments, the pharmaceutical compositions contain about 35 to about 40 micrograms of DNA. In some embodiments, the pharmaceutical compositions contain about 100 to about 200 microgram DNA. In some embodiments, the pharmaceutical compositions comprise about 10 microgram to about 100 micrograms of DNA. In some embodiments, the pharmaceutical compositions comprise about 20 micrograms to about 80 micrograms of DNA. In some embodiments, the pharmaceutical compositions comprise about 25 micrograms to about 60 micrograms of DNA. In some embodiments, the pharmaceutical compositions comprise about 30 nanograms to about 50 micrograms of DNA. In some embodiments, the pharmaceutical compositions comprise about 35 nanograms to about 45 micrograms of DNA. In some preferred embodiments, the pharmaceutical compositions contain about 0.1 to about 500 micrograms of DNA. In some preferred embodiments, the pharmaceutical compositions contain about 1 to about 350 micrograms of DNA. In some preferred embodiments, the pharmaceutical compositions contain about 25 to about 250 micrograms of DNA. In some preferred embodiments, the pharmaceutical compositions contain about 100 to about 200 microgram DNA.

The pharmaceutical compositions according to the present invention are formulated according to the mode of administration to be used. In cases where pharmaceutical compositions are injectable pharmaceutical compositions, they are sterile, pyrogen free and particulate free. An isotonic formulation is preferably used. Generally, additives for isotonicity can include sodium chloride, dextrose, mannitol, sorbitol and lactose. In some cases, isotonic solutions such as phosphate buffered saline are preferred. Stabilizers include gelatin and albumin. In some embodiments, a vasoconstriction agent is added to the formulation.

a. Vaccines

Preferably the pharmaceutical composition is a vaccine, and more preferably a DNA vaccine.

Provided herein is a vaccine capable of generating in a mammal an immune response against one or more influenza serotypes. The vaccine can comprise the genetic construct as discussed above. The vaccine can comprise a plurality of the vectors each directed to one or more Influenza A serotypes such as H7N9 or H1-H16 Influenza B hemagglutinin or combinations thereof. The vaccine can comprise one or more nucleic acid sequences that encode one or more consensus hemagglutinin antigens. When the vaccine comprises more than one consensus hemagglutinin nucleic acid sequences, all such sequences can be present on a single nucleic acid molecule or each such sequences can be present on a different nucleic acid molecule. Alternatively, vaccines that comprise more than one consensus hemagglutinin nucleic acid sequences can comprise nucleic acid molecules with a single consensus hemagglutinin nucleic acid sequences and nucleic acid molecules with more than one consensus hemagglutinin nucleic acid sequences. In addition, vaccines comprising one or more consensus hemagglutinin nucleic acid sequences can further comprise coding sequences for one or more proteins selected from the group consisting of H7N9, H1, H2, H3, H4, H5, H6, H7, H8, H9, H10, H11, H12, H13, H14, H15, H16, N1, N2, N3, N4, N5, N6, N7, N8, N9 and influenza B neuraminidase.

In some embodiments, vaccines can comprise proteins. Some vaccines can comprise one or more consensus hemagglutinin antigens such as H7N9, H1, H2, U2 and BHA. The vaccines can comprise one or more other proteins selected from the group consisting of H7N9, H1, H2, H3, H4, H5, H6, H7, H8, H9, H10, H11, H12, H13, H14, H15, H16, N1, N2, N3, N4, N5, N6, N7, N8, N9 and influenza B neuraminidase. The vaccines can comprise one or more consensus hemagglutinin antigens in combination with one or more other proteins selected from the group consisting of H7N9, H1, H2, H3, H4, H5, H6, H7, H8, H9, H10, H11, H12, H13, H14, H15, H16, N1, N2, N3, N4, N5, N6, N7, N8, N9, influenza B hemagglutinin and neuraminidase.

The vaccine can be a DNA vaccine. The DNA vaccine can comprise a plurality of the same or different plasmids comprising one or more of consensus hemagglutinin nucleic acid sequences. The DNA vaccine can comprise one or more nucleic acid sequences that encode one or more consensus hemagglutinin antigens. When the DNA vaccine comprises more than one consensus hemagglutinin nucleic acid sequences, all such sequences can be present on a single plasmid, or each such sequences can be present on a different plasmids, or some

plasmids can comprise a single consensus hemagglutinin nucleic acid sequences while other plasmids have more than one consensus hemagglutinin nucleic acid sequences. In addition, DNA vaccines can further comprise one or more consensus coding sequences for one or more proteins selected from the group consisting of influenza A H7N9, H1, H2, H3, H4, H5, H6, H7, H8, H9, H10, H11, H12, H13, H14, H15, H16, N1, N2, N3, N4, N5, N6, N7, N8, N9, influenza B hemagglutinin and neuraminidase. Such additional coding sequences can be on the same or different plasmids from each other and from the plasmids comprising one or more of consensus hemagglutinin nucleic acid sequences.

In some embodiments, vaccines can comprise nucleic acid sequences that encode influenza antigens in combination with influenza antigens. In some embodiments, the nucleic acid sequences encode one or more consensus hemagglutinin antigens such as H7N9, H1 (including H1U and H1U2), H2, H3, and BHA. In some embodiments, the nucleic acid sequences encode one or more one or more other proteins selected from the group consisting of, influenza A H7N9, H1, H2, H3, H4, H5, H6, H7, H8, H9, H10, H11, H12, H13, H14, H15, H16, N1, N2, N3, N4, N5, N6, N7, N8, N9, influenza B hemagglutinin and neuraminidase. In some embodiments, the vaccines comprise one or more consensus hemagglutinin antigens such as H7N9, H1 (including H1U and H1U2), H2, H3, and BHA. In some embodiments, the vaccines comprise one or more one or more other proteins selected from the group consisting of influenza A H7N9, H1, H2, H3, H4, H5, H6, H7, H8, H9, H10, H11, H12, H13, H14, H15, H16, N1, N2, N3, N4, N5, N6, N7, N8, N9, influenza B hemagglutinin and neuraminidase.

In some embodiments, vaccines comprise a combination of four or more consensus hemagglutinin nucleic acid sequences including those encoding one or more of H7N9, H1 (including H1U and H1U2), H2, and BHA. In some embodiments, vaccines comprise a combination of four or more hemagglutinin nucleic acid sequences including those encoding consensus H7N9, H1U and/or H1U2, consensus BHA and an H3 hemagglutinin. In some embodiments, vaccines comprise a combination of four or more hemagglutinin nucleic acid sequences including those encoding consensus H7N9, BHA, an H1 hemagglutinin and an H3 hemagglutinin. In some embodiments, vaccines comprise one or more nucleic acid sequences that encode one or more influenza antigens disclosed in U.S. Serial No. 12/375,518, which is incorporated herein by reference and/or U.S. Serial No. 12/269,824, which is incorporated herein by reference. In some embodiments, vaccines comprise a nucleic acid sequence that encodes an H7N9 hemagglutinin (SEQ ID NO: 39). In some embodiments, vaccines comprise a nucleic acid sequence that encodes an H1 hemagglutinin

from U.S. Serial No. 12/375,518 (SEQ ID NO:36 therein) and/or U.S. Serial No. 12/269,824 (SEQ ID NO:9 therein). In some embodiments, vaccines comprise a nucleic acid sequence that encodes an H3 hemagglutinin from U.S. Serial No. 12/269,824 (SEQ ID NO:11 therein).

In some embodiments, vaccines comprise a combination of four or more consensus
 5 hemagglutinin proteins including one or more of H7N9, H1, H2, U2 and BHA. In some
 embodiments, vaccines comprise a combination of four or more hemagglutinin proteins
 including consensus H7N9, U2, consensus BHA and an H3 hemagglutinin. In some
 embodiments, vaccines comprise a combination of four or more hemagglutinin proteins
 including consensus H7N9, BHA, an H1 hemagglutinin and an H3 hemagglutinin. In some
 10 embodiments, vaccines comprise one or more antigens from U.S. Serial No. 12/375,518
 and/or U.S. Serial No. 12/269,824. In some embodiments, vaccines comprise H7N9
 hemagglutinin (SEQ ID NO: 40). In some embodiments, vaccines comprise an H1
 hemagglutinin disclosed in U.S. Serial No. 12/375,518 (SEQ ID NO:37 therein) and/or U.S.
 Serial No. 12/269,824 (SEQ ID NO:10 therein). In some embodiments, vaccines comprise an
 15 H3 hemagglutinin disclosed in U.S. Serial No. 12/269,824 (SEQ ID NO:12 therein).

In some embodiments, vaccines comprise a combination of 1) the consensus
 hemagglutinin H7N9 protein (SEQ ID NO: 40) and/or a nucleic acid sequences encoding the
 consensus hemagglutinin H7N9 protein (SEQ ID NO: 39), 2) the consensus hemagglutinin
 U2 protein and/or a nucleic acid sequences encoding the consensus hemagglutinin U2
 20 protein, 3) the consensus hemagglutinin BHA protein and/or a nucleic acid sequences
 encoding the consensus hemagglutinin BHA protein, and 4) a hemagglutinin H3 protein
 disclosed in U.S. Serial No. 12/269,824 (SEQ ID NO:12 therein) and/or a nucleic acid
 sequences encoding hemagglutinin H3 protein disclosed in U.S. Serial No. 12/269,824 (SEQ
 ID NO:11 therein).

In some embodiments, vaccines comprise a combination of 1) the consensus
 hemagglutinin H7N9 protein (SEQ ID NO: 40) and/or a nucleic acid sequences encoding the
 consensus hemagglutinin H7N9 protein (SEQ ID NO: 39), 2) the consensus hemagglutinin
 BHA protein and/or a nucleic acid sequences encoding the consensus hemagglutinin BHA
 protein, 3) a hemagglutinin H1 protein disclosed in U.S. Serial No. 12/269,824 (SEQ ID
 30 NO:10 therein) or U.S. Serial No. 12/375,518 (SEQ ID NO:37 therein) and/or a nucleic acid
 sequences encoding hemagglutinin H1 protein disclosed in U.S. Serial No. 12/269,824 (SEQ
 ID NO:9 therein) or U.S. Serial No. 12/375,518 (SEQ ID NO:36 therein), and 4) a
 hemagglutinin H3 protein disclosed in U.S. Serial No. 12/269,824 (SEQ ID NO:12 therein)

and/or a nucleic acid sequences encoding hemagglutinin H3 protein disclosed in U.S. Serial No. 12/269,824 (SEQ ID NO:11 therein).

Preferably, combinations of antigens provided herein can be formulated to a vaccine that causes seroconversion in vaccinated mammals that provide cross-reactivity against a broad range of seasonal strains of influenza and also pandemic strains of influenza. The seroconversion and broad cross-reactivity can be determined by measuring inhibiting titers against different hemagglutinin strains of influenza. Preferred combinations include at least one antigen from the following groups: 1) consensus H7N9 hemagglutinin; 2) consensus H1 hemagglutinin; 3) consensus H2 hemagglutinin; 4) consensus H3 hemagglutinin; and 5) influenza B hemagglutinin; and more preferred combinations include at least one antigen from the following groups: 1) consensus H7N9 hemagglutinin; 2) consensus H1 hemagglutinin; 3) consensus H3 hemagglutinin; and 4) influenza B hemagglutinin.

In some embodiments the vaccines can have a combination as such:

a) one or more of a first influenza nucleic acid sequence H7N9 hemagglutinin capable of generating broadly cross reactive immune response in mammals against multiple strains of influenza virus, which is/are selected from the following:

a nucleic acid sequence selected from the group consisting of: SEQ ID NO: 39, a nucleic acid sequence that is 95% identical over the entire length of the nucleic acid sequence of SEQ ID NO: 39; a fragment of SEQ ID NO: 39; a nucleic acid sequence that is 95% identical to a fragment of SEQ ID NO: 39; nucleic acid sequences encoding SEQ ID NO:40, a nucleic acid sequence that is 95% identical over the entire length of the nucleic acid sequence encoding SEQ ID NO: 40; a fragment of nucleic acid sequences encoding SEQ ID NO: 40; and a nucleic acid sequence that is 95% identical to a fragment of nucleic acid sequences encoding SEQ ID NO: 40.

b) one or more of a first influenza nucleic acid sequence H1 hemagglutinin capable of generating broadly cross reactive immune response in mammals against multiple strains of influenza virus, which is/are selected from the following:

a nucleic acid sequence selected from the group consisting of: SEQ ID NO:1, a nucleic acid sequence that is 95% identical over the entire length of the nucleic acid sequence of SEQ ID NO:1; a fragment of SEQ ID NO:1; a nucleic acid sequence that is 95% identical to a

fragment of SEQ ID NO:1; SEQ ID NO:9, a nucleic acid sequence that is 95% identical over the entire length of the nucleic acid sequence of SEQ ID NO:9; a fragment of SEQ ID NO:9; a nucleic acid sequence that is 95% identical to a fragment of SEQ ID NO:9; SEQ ID NO:19; a nucleic acid sequence that is 95% identical over the entire length of the nucleic acid sequence of SEQ ID NO:19; a fragment of SEQ ID NO:19; a nucleic acid sequence that is 95% identical to a fragment of SEQ ID NO:19; SEQ ID NO:21; a nucleic acid sequence that is 95% identical over the entire length of the nucleic acid sequence of SEQ ID NO:21; a fragment of SEQ ID NO:21; a nucleic acid sequence that is 95% identical to a fragment of SEQ ID NO:21; SEQ ID NO:35; a nucleic acid sequence that is 95% identical over the entire length of the nucleic acid sequence of SEQ ID NO:35; a fragment of SEQ ID NO:35; a nucleic acid sequence that is 95% identical to a fragment of SEQ ID NO:35; nucleic acid sequences encoding SEQ ID NO:2, a nucleic acid sequence that is 95% identical over the entire length of the nucleic acid sequence encoding SEQ ID NO:2; a fragment of nucleic acid sequences encoding SEQ ID NO:2; a nucleic acid sequence that is 95% identical over the entire length of the nucleic acid sequence of a fragment of nucleic acid sequences encoding SEQ ID NO:2; nucleic acid sequences encoding SEQ ID NO:10, a nucleic acid sequence that is 95% identical over the entire length of the nucleic acid sequence encoding SEQ ID NO:10; a fragment of nucleic acid sequences encoding SEQ ID NO:10; a nucleic acid sequence that is 95% identical to a fragment of nucleic acid sequences encoding SEQ ID NO:10; nucleic acid sequences encoding SEQ ID NO:20; a nucleic acid sequence that is 95% identical over the entire length of the nucleic acid sequence encoding SEQ ID NO:20; a fragment of nucleic acid sequences encoding SEQ ID NO:20; a nucleic acid sequence that is 95% identical to a fragment of nucleic acid sequences encoding SEQ ID NO:20; nucleic acid sequences encoding SEQ ID NO:22; a nucleic acid sequence that is 95% identical over the entire length of the nucleic acid sequence encoding SEQ ID NO:22; a fragment of nucleic acid sequences encoding SEQ ID NO:22; a nucleic acid sequence that is 95% identical to a fragment of nucleic acid sequences encoding SEQ ID NO:22; nucleic acid sequences encoding SEQ ID NO:36; a nucleic acid sequence that is 95% identical over the entire length of the nucleic acid sequence encoding SEQ ID NO:36; a fragment of nucleic acid sequences encoding SEQ ID NO:36; and a nucleic acid sequence that is 95% identical to a fragment of nucleic acid sequences encoding SEQ ID NO:36.

c) one or more of a first influenza nucleic acid sequence H3 hemagglutinin capable of generating broadly cross reactive immune response in mammals against multiple strains of influenza virus, which is/are selected from the following:

- 5 a nucleic acid sequence selected from the group consisting of: SEQ ID NO:23, a nucleic acid sequence that is 95% identical over the entire length of the nucleic acid sequence of SEQ ID NO:23; a fragment of SEQ ID NO:23; a nucleic acid sequence that is 95% identical to a fragment of SEQ ID NO:23; SEQ ID NO:27, a nucleic acid sequence that is 95% identical over the entire length of the nucleic acid sequence of SEQ ID NO:27; a fragment of SEQ ID
10 NO:27; a nucleic acid sequence that is 95% identical to a fragment of SEQ ID NO:27; SEQ ID NO:29; a nucleic acid sequence that is 95% identical over the entire length of the nucleic acid sequence of SEQ ID NO:29; a fragment of SEQ ID NO:29; a nucleic acid sequence that is 95% identical to a fragment of SEQ ID NO:29; SEQ ID NO:37; a nucleic acid sequence that is 95% identical over the entire length of the nucleic acid sequence of SEQ ID NO:37; a fragment of SEQ ID NO:37; a nucleic acid sequence that is 95% identical to a fragment of
15 SEQ ID NO:37; nucleic acid sequences encoding SEQ ID NO:24, a nucleic acid sequence that is 95% identical over the entire length of the nucleic acid sequence encoding SEQ ID NO:24; a fragment of nucleic acid sequences encoding SEQ ID NO:24; a nucleic acid sequence that is 95% identical to a fragment of nucleic acid sequences encoding SEQ ID
20 NO:24; nucleic acid sequences encoding SEQ ID NO:28; a nucleic acid sequence that is 95% identical over the entire length of the nucleic acid sequence encoding SEQ ID NO: 28; a fragment of nucleic acid sequences encoding SEQ ID NO: 28; a nucleic acid sequence that is 95% identical to a fragment of nucleic acid sequences encoding SEQ ID NO: 28; nucleic acid sequences encoding SEQ ID NO:30; a nucleic acid sequence that is 95% identical over the
25 entire length of the nucleic acid sequence encoding SEQ ID NO: 30; a fragment of nucleic acid sequences encoding SEQ ID NO: 30; a nucleic acid sequence that is 95% identical to a fragment of nucleic acid sequences encoding SEQ ID NO: 30; nucleic acid sequences encoding SEQ ID NO:38; a nucleic acid sequence that is 95% identical over the entire length of the nucleic acid sequence encoding SEQ ID NO: 38; a fragment of nucleic acid sequences
30 encoding SEQ ID NO: 38; and a nucleic acid sequence that is 95% identical to a fragment of nucleic acid sequences encoding SEQ ID NO: 38.

d) one or more of a first influenza nucleic acid sequence influenza B hemagglutinin capable of generating broadly cross reactive immune response in mammals against multiple strains of influenza virus, which is/are selected from the following:

- 5 a nucleic acid sequence selected from the group consisting of: SEQ ID NO:13, a nucleic acid sequence that is 95% identical over the entire length of the nucleic acid sequence of SEQ ID NO: 13; a fragment of SEQ ID NO: 13; a nucleic acid sequence that is 95% identical to a fragment of SEQ ID NO: 13; SEQ ID NO:25, a nucleic acid sequence that is 95% identical over the entire length of the nucleic acid sequence of SEQ ID NO: 25; a fragment of SEQ ID
- 10 NO: 25; a nucleic acid sequence that is 95% identical to a fragment of SEQ ID NO: 25; SEQ ID NO:31; a nucleic acid sequence that is 95% identical over the entire length of the nucleic acid sequence of SEQ ID NO: 31; a fragment of SEQ ID NO: 31; a nucleic acid sequence that is 95% identical to a fragment of SEQ ID NO: 31; SEQ ID NO:33; a nucleic acid sequence that is 95% identical over the entire length of the nucleic acid sequence of SEQ ID NO: 33; a
- 15 fragment of SEQ ID NO: 33; a nucleic acid sequence that is 95% identical to a fragment of SEQ ID NO: 33; nucleic acid sequences encoding SEQ ID NO:14, a nucleic acid sequence that is 95% identical over the entire length of the nucleic acid sequence encoding SEQ ID NO: 14; a fragment of nucleic acid sequences encoding SEQ ID NO: 14; a nucleic acid sequence that is 95% identical to a fragment of nucleic acid sequences encoding SEQ ID NO:
- 20 14; nucleic acid sequences encoding SEQ ID NO:26; a nucleic acid sequence that is 95% identical over the entire length of the nucleic acid sequence encoding SEQ ID NO: 26; a fragment of nucleic acid sequences encoding SEQ ID NO: 26; a nucleic acid sequence that is 95% identical to a fragment of nucleic acid sequences encoding SEQ ID NO: 26; nucleic acid sequences encoding SEQ ID NO:32; a nucleic acid sequence that is 95% identical over the
- 25 entire length of the nucleic acid sequence encoding SEQ ID NO: 32; a fragment of nucleic acid sequences encoding SEQ ID NO: 32; a nucleic acid sequence that is 95% identical to a fragment of nucleic acid sequences encoding SEQ ID NO: 32; nucleic acid sequences encoding SEQ ID NO:34; a nucleic acid sequence that is 95% identical over the entire length of the nucleic acid sequence encoding SEQ ID NO: 34; a fragment of nucleic acid sequences
- 30 encoding SEQ ID NO: 34; and a nucleic acid sequence that is 95% identical to a fragment of nucleic acid sequences encoding SEQ ID NO: 34.

In some embodiments, in addition to a), b), c), and d), above, the combination can also include one or more of an influenza nucleic acid sequence H2 hemagglutinin capable of generating broadly cross reactive immune response in mammals against multiple strains of

influenza virus, which is/are selected from the following: SEQ ID NO:6, a nucleic acid sequence that is 95% identical over the entire length of the nucleic acid sequence of SEQ ID NO: 6; a fragment of SEQ ID NO: 6; a nucleic acid sequence that is 95% identical to a fragment of SEQ ID NO: 6; nucleic acid sequences encoding SEQ ID NO:7; a nucleic acid sequence that is 95% identical over the entire length of the nucleic acid sequence encoding SEQ ID NO: 7; a fragment of nucleic acid sequences encoding SEQ ID NO: 7; and a nucleic acid sequence that is 95% identical to a fragment of nucleic acid sequences encoding SEQ ID NO: 7.

DNA vaccines are disclosed in US Patent Nos. 5,593,972, 5,739,118, 5,817,637, 5,830,876, 5,962,428, 5,981,505, 5,580,859, 5,703,055, and 5,676,594, which are incorporated herein fully by reference. The DNA vaccine can further comprise elements or reagents that inhibit it from integrating into the chromosome. The vaccine can be an RNA of the hemagglutinin antigen. The RNA vaccine can be introduced into the cell.

The vaccine can be a recombinant vaccine comprising the genetic construct or antigen described above. The vaccine can also comprise one or more consensus hemagglutinin antigen in the form of one or more protein subunits, one or more killed influenza particles comprising one or more consensus hemagglutinin antigens, or one or more attenuated influenza particles comprising one or more consensus hemagglutinin antigens. The attenuated vaccine can be attenuated live vaccines, killed vaccines and vaccines that use recombinant vectors to deliver foreign genes that encode one or more consensus hemagglutinin antigens, and well as subunit and glycoprotein vaccines. Examples of attenuated live vaccines, those using recombinant vectors to deliver foreign antigens, subunit vaccines and glycoprotein vaccines are described in U.S. Patent Nos.: 4,510,245; 4,797,368; 4,722,848; 4,790,987; 4,920,209; 5,017,487; 5,077,044; 5,110,587; 5,112,749; 5,174,993; 5,223,424; 5,225,336; 5,240,703; 5,242,829; 5,294,441; 5,294,548; 5,310,668; 5,387,744; 5,389,368; 5,424,065; 5,451,499; 5,453,364; 5,462,734; 5,470,734; 5,474,935; 5,482,713; 5,591,439; 5,643,579; 5,650,309; 5,698,202; 5,955,088; 6,034,298; 6,042,836; 6,156,319 and 6,589,529, which are each incorporated herein by reference.

The vaccine can comprise vectors and/or proteins directed to Influenza A serotypes from particular regions in the world, for example, Asia. The vaccine can also be directed against Influenza A serotypes of swine or avian origin that now infect humans. The vaccine can comprise vectors and/or proteins directed to Influenza B from particular regions in the world. The vaccine can also be directed against Influenza B that infect humans. The vaccine

can comprise one or more vectors and/or one or more proteins directed to one or more strains of Influenza A and/or B.

The vaccine provided can be used to induce immune responses including therapeutic or prophylactic immune responses. Antibodies and/or killer T cells can be generated which
5 are directed to the consensus hemagglutinin antigen, and also broadly across multiple subtypes of influenza viruses. Such antibodies and cells can be isolated.

The vaccine can further comprise a pharmaceutically acceptable excipient. The pharmaceutically acceptable excipient can be functional molecules as vehicles, adjuvants, carriers, or diluents. The pharmaceutically acceptable excipient can be a transfection
10 facilitating agent, which can include surface active agents, such as immune-stimulating complexes (ISCOMS), Freund's incomplete adjuvant, LPS analog including monophosphoryl lipid A, muramyl peptides, quinone analogs, vesicles such as squalene and squalene, hyaluronic acid, lipids, liposomes, calcium ions, viral proteins, polyanions, polycations, or nanoparticles, or other known transfection facilitating agents.

The transfection facilitating agent is a polyanion, polycation, including poly-L-glutamate (LGS), or lipid. The transfection facilitating agent is poly-L-glutamate, and more preferably, the poly-L-glutamate is present in the vaccine at a concentration less than 6 mg/ml. The transfection facilitating agent can also include surface active agents such as immune-stimulating complexes (ISCOMS), Freund's incomplete adjuvant, LPS analog
20 including monophosphoryl lipid A, muramyl peptides, quinone analogs and vesicles such as squalene and squalene, and hyaluronic acid can also be used administered in conjunction with the genetic construct. In some embodiments, the DNA vector vaccines can also include a transfection facilitating agent such as lipids, liposomes, including lecithin liposomes or other liposomes known in the art, as a DNA-liposome mixture (see for example W09324640),
25 calcium ions, viral proteins, polyanions, polycations, or nanoparticles, or other known transfection facilitating agents. Preferably, the transfection facilitating agent is a polyanion, polycation, including poly-L-glutamate (LGS), or lipid. Concentration of the transfection agent in the vaccine is less than 4 mg/ml, less than 2 mg/ml, less than 1 mg/ml, less than 0.750 mg/ml, less than 0.500 mg/ml, less than 0.250 mg/ml, less than 0.100 mg/ml, less than
30 0.050 mg/ml, or less than 0.010 mg/ml.

The pharmaceutically acceptable excipient can be an adjuvant. The adjuvant can be other genes that are expressed in alternative plasmid or are delivered as proteins in combination with the plasmid above in the vaccine. The adjuvant can be selected from the group consisting of: α -interferon (IFN- α), β -interferon (IFN- β), γ -interferon, platelet derived

growth factor (PDGF), TNF α , TNF β , GM-CSF, epidermal growth factor (EGF), cutaneous T cell-attracting chemokine (CTACK), epithelial thymus-expressed chemokine (TECK), mucosae-associated epithelial chemokine (MEC), IL-12, IL-15, MHC, CD80, CD86 including IL-15 having the signal sequence deleted and optionally including the signal peptide from IgE. The adjuvant can be IL-12, IL-15, IL-28, CTACK, TECK, platelet derived growth factor (PDGF), TNF α , TNF β , GM-CSF, epidermal growth factor (EGF), IL-1, IL-2, IL-4, IL-5, IL-6, IL-10, IL-12, IL-18, or a combination thereof.

Other genes which can be useful adjuvants include those encoding: MCP-1, MIP-1 α , MIP-1 β , IL-8, RANTES, L-selectin, P-selectin, E-selectin, CD34, GlyCAM-1, MadCAM-1, LFA-1, VLA-1, Mac-1, p150.95, PECAM, ICAM-1, ICAM-2, ICAM-3, CD2, LFA-3, M-CSF, G-CSF, IL-4, mutant forms of IL-18, CD40, CD40L, vascular growth factor, fibroblast growth factor, IL-7, nerve growth factor, vascular endothelial growth factor, Fas, TNF receptor, Flt, Apo-1, p55, WSL-1, DR3, TRAMP, Apo-3, AIR, LARD, NGRF, DR4, DR5, KILLER, TRAIL-R2, TRICK2, DR6, Caspase ICE, Fos, c-jun, Sp-1, Ap-1, Ap-2, p38, p65Rel, MyD88, IRAK, TRAF6, I κ B, Inactive NIK, SAP K, SAP-1, JNK, interferon response genes, NF κ B, Bax, TRAIL, TRAILrec, TRAILrecDRC5, TRAIL-R3, TRAIL-R4, RANK, RANK LIGAND, O $\times$ 40, O $\times$ 40 LIGAND, NKG2D, MICA, MICB, NKG2A, NKG2B, NKG2C, NKG2E, NKG2F, TAP1, TAP2 and functional fragments thereof.

The vaccine can further comprise a genetic vaccine facilitator agent as described in U.S. Serial No. 021,579 filed April 1, 1994, which is fully incorporated by reference.

6. Methods of Delivery

Provided herein is a method for delivering the pharmaceutical formulations, preferably vaccines, for providing genetic constructs and proteins of the hemagglutinin antigen which comprise epitopes that make them particular effective immunogens against which an immune response to influenza viral infections can be induced. The method of delivering the vaccine, or vaccination, can be provided to induce a therapeutic and/or prophylactic immune response. The vaccination process can generate in the mammal an immune response against a plurality of influenza subtypes, including a H1N1 serotype, such as the 2009 swine originated H1N1 or avian H7N9, or other seasonal and/or pandemic varieties. The vaccine can be delivered to an individual to modulate the activity of the mammal's immune system and enhance the immune response. The delivery of the vaccine can be the transfection of the HA antigen as a nucleic acid molecule that is expressed in the cell and delivered to the surface of the cell upon which the immune system recognized and induces a cellular, humoral, or cellular and humoral response. The delivery of the vaccine

can be used to induce or elicit an immune response in mammals against a plurality of influenza viruses by administering to the mammals the vaccine as discussed herein.

Upon delivery of the vaccine to the mammal, and thereupon the vector into the cells of the mammal, the transfected cells will express and secrete the corresponding influenza protein, including at least one of the consensus antigens, and preferably H7N9, H1, H2, U2, and BHA. These secreted proteins, or synthetic antigens, will be recognized as foreign by the immune system, which will mount an immune response that can include: antibodies made against the antigens, and T-cell response specifically against the antigen. In some examples, a mammal vaccinated with the vaccines discussed herein will have a primed immune system and when challenged with an influenza viral strain, the primed immune system will allow for rapid clearing of subsequent influenza viruses, whether through the humoral, cellular, or both. The vaccine can be delivered to an individual to modulate the activity of the individual's immune system thereby enhancing the immune response.

The vaccine can be delivered in the form of a DNA vaccine and methods of delivering a DNA vaccines are described in U.S. Patent Nos. 4,945,050 and 5,036,006, which are both incorporated fully by reference.

The vaccine can be administered to a mammal to elicit an immune response in a mammal. The mammal can be human, non-human primate, cow, pig, sheep, goat, antelope, bison, water buffalo, bovids, deer, hedgehogs, elephants, llama, alpaca, mice, rats, or chicken, and preferably human, cow, pig, or chicken.

a. Combination with other Antigens and Adjuvants

The pharmaceutical compositions, preferably vaccines, described herein can be administered in combination with one or more other influenza proteins or genes encoding influenza A H7N9, H1, H2, H3, H4, H5, H6, H7, H8, H9, H10, H11, H12, H13, H14, H15, H16, N1, N2, N3, N4, N5, N6, N7, N8, N9, influenza B hemagglutinin and neuraminidase. The vaccine can be administered in combination with proteins or genes encoding adjuvants, which can include: α -interferon (IFN- α), β -interferon (IFN- β), γ -interferon, IL-12, IL-15, IL-21, IL-23, IL-28, IL-33, CTACK, TECK, platelet derived growth factor (PDGF), TNF α , TNF β , GM-CSF, epidermal growth factor (EGF), IL-1, IL-2, IL-4, IL-5, IL-6, IL-10, IL-18, MCP-1, MIP-1a, MIP-1p, IL-8, RANTES, L-selectin, P-selectin, E-selectin, CD34, GlyCAM-1, MadCAM-1, LFA-1, VLA-1, Mac-1, p150.95, PECAM, ICAM-1, ICAM-2, ICAM-3, CD2, LFA-3, M-CSF, G-CSF, IL-4, mutant forms of IL-18, CD40, CD40L, vascular growth factor, fibroblast growth factor, IL-7, nerve growth factor, vascular endothelial growth factor, Fas,

TNF receptor, Flt, Apo-1, p55, WSL-1, DR3, TRAMP, Apo-3, AIR, LARD, NGRF, DR4, DR5, KILLER, TRAIL-R2, TRICK2, DR6, Caspase ICE, Fos, c-jun, Sp-1, Ap-1, Ap-2, p38, p65Rel, MyD88, IRAK, TRAF6, Ikb, Inactive NIK, SAP K, SAP-1, JNK, interferon response genes, NFkB, Bax, TRAIL, TRAILrec, TRAILrecDRC5, TRAIL-R3, TRAIL-R4, 5 RANK, RANK LIGAND, Ox40, Ox40 LIGAND, NKG2D, MICA, MICB, NKG2A, NKG2B, NKG2C, NKG2E, NKG2F, TAP1, or TAP2, or functional fragments thereof.

b. Routes of Administration

The vaccine can be administered by different routes including orally, parenterally, sublingually, transdermally, rectally, transmucosally, topically, via inhalation, via buccal 10 administration, intrapleurally, intravenous, intraarterial, intraperitoneal, subcutaneous, intramuscular, intranasal intrathecal, and intraarticular or combinations thereof. For veterinary use, the composition can be administered as a suitably acceptable formulation in accordance with normal veterinary practice. The veterinarian can readily determine the dosing regimen and route of administration that is most appropriate for a particular animal. 15 The vaccine can be administered by traditional syringes, needleless injection devices, "microprojectile bombardment gone guns", or other physical methods such as electroporation ("EP"), "hydrodynamic method", or ultrasound.

The vector of the vaccine can be delivered to the mammal by several well known technologies including DNA injection (also referred to as DNA vaccination) with and without 20 in vivo electroporation, liposome mediated, nanoparticle facilitated, recombinant vectors such as recombinant adenovirus, recombinant adenovirus associated virus and recombinant vaccinia. The HA antigen can be delivered via DNA injection and along with in vivo electroporation.

c. Electroporation

25 Administration of the vaccine via electroporation of the plasmids of the vaccine can be accomplished using electroporation devices that can be configured to deliver to a desired tissue of a mammal a pulse of energy effective to cause reversible pores to form in cell membranes, and preferable the pulse of energy is a constant current similar to a preset current input by a user. The electroporation device can comprise an electroporation component and 30 an electrode assembly or handle assembly. The electroporation component can include and incorporate one or more of the various elements of the electroporation devices, including: controller, current waveform generator, impedance tester, waveform logger, input element, status reporting element, communication port, memory component, power source, and power switch. The electroporation can be accomplished using an in vivo electroporation device, for

example CELLECTRA® EP system (VGX Pharmaceuticals, Blue Bell, PA) or Elgen electroporator (Genetronics, San Diego, CA) to facilitate transfection of cells by the plasmid.

The electroporation component can function as one element of the electroporation devices, and the other elements are separate elements (or components) in communication with the electroporation component. The electroporation component can function as more than one element of the electroporation devices, which can be in communication with still other elements of the electroporation devices separate from the electroporation component. The elements of the electroporation devices existing as parts of one electromechanical or mechanical device can not limited as the elements can function as one device or as separate elements in communication with one another. The electroporation component can be capable of delivering the pulse of energy that produces the constant current in the desired tissue, and includes a feedback mechanism. The electrode assembly can include an electrode array having a plurality of electrodes in a spatial arrangement, wherein the electrode assembly receives the pulse of energy from the electroporation component and delivers same to the desired tissue through the electrodes. At least one of the plurality of electrodes is neutral during delivery of the pulse of energy and measures impedance in the desired tissue and communicates the impedance to the electroporation component. The feedback mechanism can receive the measured impedance and can adjust the pulse of energy delivered by the electroporation component to maintain the constant current.

A plurality of electrodes can deliver the pulse of energy in a decentralized pattern. The plurality of electrodes can deliver the pulse of energy in the decentralized pattern through the control of the electrodes under a programmed sequence, and the programmed sequence is input by a user to the electroporation component. The programmed sequence can comprise a plurality of pulses delivered in sequence, wherein each pulse of the plurality of pulses is delivered by at least two active electrodes with one neutral electrode that measures impedance, and wherein a subsequent pulse of the plurality of pulses is delivered by a different one of at least two active electrodes with one neutral electrode that measures impedance.

The feedback mechanism can be performed by either hardware or software. The feedback mechanism can be performed by an analog closed-loop circuit. The feedback occurs every 50 μ s, 20 μ s, 10 μ s or 1 μ s, but is preferably a real-time feedback or instantaneous (i.e., substantially instantaneous as determined by available techniques for determining response time). The neutral electrode can measure the impedance in the desired tissue and communicates the impedance to the feedback mechanism, and the feedback

mechanism responds to the impedance and adjusts the pulse of energy to maintain the constant current at a value similar to the preset current. The feedback mechanism can maintain the constant current continuously and instantaneously during the delivery of the pulse of energy.

5 Examples of electroporation devices and electroporation methods that can facilitate delivery of the DNA vaccines of the present invention, include those described in U.S. Patent No. 7,245,963 by Draghia-Akli, et al., U.S. Patent Pub. 2005/0052630 submitted by Smith, et al., the contents of which are hereby incorporated by reference in their entirety. Other electroporation devices and electroporation methods that can be used for facilitating delivery
10 of the DNA vaccines include those provided in co-pending and co-owned U.S. Patent Application, Serial No. 11/874072, filed October 17, 2007, which claims the benefit under 35 USC 119(e) to U.S. Provisional Applications Ser. Nos. 60/852,149, filed October 17, 2006, and 60/978,982, filed October 10, 2007, all of which are hereby incorporated in their entirety.

U.S. Patent No. 7,245,963 by Draghia-Akli, et al. describes modular electrode
15 systems and their use for facilitating the introduction of a biomolecule into cells of a selected tissue in a body or plant. The modular electrode systems can comprise a plurality of needle electrodes; a hypodermic needle; an electrical connector that provides a conductive link from a programmable constant-current pulse controller to the plurality of needle electrodes; and a power source. An operator can grasp the plurality of needle electrodes that are mounted on a
20 support structure and firmly insert them into the selected tissue in a body or plant. The biomolecules are then delivered via the hypodermic needle into the selected tissue. The programmable constant-current pulse controller is activated and constant-current electrical pulse is applied to the plurality of needle electrodes. The applied constant-current electrical pulse facilitates the introduction of the biomolecule into the cell between the plurality of
25 electrodes. The entire content of U.S. Patent No. 7,245,963 is hereby incorporated by reference.

U.S. Patent Pub. 2005/0052630 submitted by Smith, et al. describes an electroporation device which can be used to effectively facilitate the introduction of a biomolecule into cells of a selected tissue in a body or plant. The electroporation device comprises an electro-kinetic
30 device ("EKD device") whose operation is specified by software or firmware. The EKD device produces a series of programmable constant-current pulse patterns between electrodes in an array based on user control and input of the pulse parameters, and allows the storage and acquisition of current waveform data. The electroporation device also comprises a replaceable electrode disk having an array of needle electrodes, a central injection channel for

an injection needle, and a removable guide disk. The entire content of U.S. Patent Pub. 2005/0052630 is hereby incorporated by reference.

The electrode arrays and methods described in U.S. Patent No. 7,245,963 and U.S. Patent Pub. 2005/0052630 can be adapted for deep penetration into not only tissues such as muscle, but also other tissues or organs. Because of the configuration of the electrode array, the injection needle (to deliver the biomolecule of choice) is also inserted completely into the target organ, and the injection is administered perpendicular to the target issue, in the area that is pre-delineated by the electrodes. The electrodes described in U.S. Patent No. 7,245,963 and U.S. Patent Pub. 2005/005263 are preferably 20 mm long and 21 gauge.

Additionally, contemplated in some embodiments that incorporate electroporation devices and uses thereof, there are electroporation devices that are those described in the following patents: US Patent 5,273,525 issued December 28, 1993, US Patents 6,110,161 issued August 29, 2000, 6,261,281 issued July 17, 2001, and 6,958,060 issued October 25, 2005, and US patent 6,939,862 issued September 6, 2005. Furthermore, patents covering subject matter provided in US patent 6,697,669 issued February 24, 2004, which concerns delivery of DNA using any of a variety of devices, and US patent 7,328,064 issued February 5, 2008, drawn to method of injecting DNA are contemplated herein. The above-patents are incorporated by reference in their entirety.

d. Method of Preparing Vaccine

Provided herein is methods for preparing the DNA plasmids that comprise the DNA vaccines discussed herein. The DNA plasmids, after the final subcloning step into the mammalian expression plasmid, can be used to inoculate a cell culture in a large scale fermentation tank, using known methods in the art.

The DNA plasmids for use with the EP devices of the present invention can be formulated or manufactured using a combination of known devices and techniques, but preferably they are manufactured using an optimized plasmid manufacturing technique that is described in a licensed, co-pending U.S. provisional application U.S. Serial No. 60/939,792, which was filed on Can 23, 2007. In some examples, the DNA plasmids used in these studies can be formulated at concentrations greater than or equal to 10 mg/mL. The manufacturing techniques also include or incorporate various devices and protocols that are commonly known to those of ordinary skill in the art, in addition to those described in U.S. Serial No. 60/939792, including those described in a licensed patent, US Patent No. 7,238,522, which issued on July 3, 2007. The above-referenced application and patent, US Serial No.

60/939,792 and US Patent No. 7,238,522, respectively, are hereby incorporated in their entirety.

EXAMPLES

5 The present invention is further illustrated in the following Examples. It should be understood that these Examples, while indicating preferred embodiments of the invention, are given by way of illustration only. From the above discussion and these Examples, one skilled in the art can ascertain the essential characteristics of this invention, and without departing from the spirit and scope thereof, can make various changes and modifications of the
10 invention to adapt it to various usages and conditions. Thus, various modifications of the invention in addition to those shown and described herein will be apparent to those skilled in the art from the foregoing description. Such modifications are also intended to fall within the scope of the appended claims.

Example 1

15 **pGX2009 (pH1HA09) – Plasmid Encoding 2009 H1N1 Influenza (Swine Flu) Hemagglutinin Antigen**

 The backbone of pGX2009 (H1HA09) is the modified expression vector pVAX1 (Invitrogen, Carlsbad, CA) under the control of the cytomegalovirus immediate-early (CMV) promoter. The original pVAX1 was purchased from Invitrogen (Catalog number V260-20)
20 and maintained at -20°C. As noted above, sequence analysis revealed differences between the sequence of pVAX1 used as the backbone of pGX2009 and the pVAX1 sequence available from Invitrogen. The differences are set forth above.

 Plasmid pGX2009, also referred to as pH1HA09, comprises a nucleic acid sequence that encodes a consensus 2009 H1N1 influenza (swine flu) hemagglutinin molecule. The 79
25 primary sequences used to generate the consensus sequence were selected from The Influenza Sequence Database.

 The accession numbers for nucleotide sequences encoding the amino acid sequence for the various influenza A hemagglutinin H1 proteins as well as the amino acid sequences encoded by the nucleotide sequences are in the GenBank database corresponding to the
30 following accession numbers. The accession numbers not in parentheses disclose nucleotide sequences and additional list amino acid sequences encoded by them. The accession numbers in parentheses are for entries of the corresponding amino acid sequence in GenBank's protein database.

The accession numbers are as follows: GQ323579.1 (ACS72657.1), GQ323564.1 (ACS72654.1), GQ323551.1 (ACS72652.1), GQ323530.1 (ACS72651.1), GQ323520.1 (ACS72650.1), GQ323495.1 (ACS72648.1), GQ323489.1 (ACS72647.1), GQ323486.1 (ACS72646.1), GQ323483.1 (ACS72645.1), GQ323455.1 (ACS72641.1), GQ323451.1 (ACS72640.1), GQ323443.1 (ACS72638.1), GQ293077.1 (ACS68822.1), GQ288372.1 (ACS54301.1), GQ287625.1 (ACS54262.1), GQ287627.1 (ACS54263.1), GQ287623.1 (ACS54261.1), GQ287621.1 (ACS54260.1), GQ286175.1 (ACS54258.1), GQ283488.1 (ACS50088.1), GQ280797.1 (ACS45035.1), GQ280624.1 (ACS45017.1), GQ280121.1 (ACS45189.1), GQ261277.1 (ACS34968.1), GQ253498.1 (ACS27787.1), GQ323470.1 (ACS72643.1), GQ253492.1 (ACS27780.1), FJ981613.1 (ACQ55359.1), FJ971076.1 (ACP52565.1), FJ969540.1 (ACP44189.1), FJ969511.1 (ACP44150.1), FJ969509.1 (ACP44147.1), GQ255900.1 (ACS27774.1), GQ255901.1 (ACS27775.1), FJ966974.1 (ACP41953.1), GQ261275.1 (ACS34967.1), FJ966960.1 (ACP41935.1), FJ966952.1 (ACP41926.1), FJ966082.1 (ACP41105.1), GQ255897.1 (ACS27770.1), CY041645.1 (ACS27249.1), CY041637.1 (ACS27239.1), CY041629 (ACS27229.1), GQ323446.1 (ACS72639.1), CY041597.1 (ACS27189.1), CY041581.1 (ACS14726.1), CY040653.1 (ACS14666.1), CY041573.1 (ACS14716.1), CY041565.1 (ACS14706.1), CY041541.1 (ACS14676.1), GQ258462.1 (ACS34667.1), CY041557.1 (ACS14696.1), CY041549.1 (ACS14686.1), GQ283484.1 (ACS50084.1), GQ283493.1 (ACS50095.1), GQ303340.1 (ACS71656.1), GQ287619.1 (ACS54259.1), GQ267839.1 (ACS36632.1), GQ268003.1 (ACS36645.1), CY041621.1 (ACS27219.1), CY041613.1 (ACS27209.1), CY041605.1 (ACS27199.1), FJ966959.1 (ACP41934.1), FJ966982.1 (ACP41963.1), CY039527.2 (ACQ45338.1), FJ981612.1 (ACQ55358.1), FJ981615.1 (ACQ55361.1), FJ982430.1 (ACQ59195.1), FJ998208.1 (ACQ73386.1), GQ259909.1 (ACS34705.1), GQ261272.1 (ACS34966.1), GQ287621.1 (ACS54260.1), GQ290059.1 (ACS66821.1), GQ323464.1 (ACS72642.1), GQ323473.1 (ACS72644.1), GQ323509.1 (ACS72649.1), GQ323560.1 (ACS72653.1), GQ323574.1 (ACS72655.1), and GQ323576.1 (ACS72656.1). The amino

acid sequences were downloaded from the NCBI Sequence Database, and an alignment and consensus sequence generated using Clustal X. A highly efficient leader sequence, the IgE leader, was fused in frame upstream of the start codon to facilitate the expression. In order to have a higher level of expression, the codon usage of this fusion gene was adapted to the codon bias of *Homo sapiens* genes. In addition, RNA optimization was also performed: regions of very high (>80%) or very low (<30%) GC content and the cis-acting sequence motifs such as internal TATA boxes, chi-sites and ribosomal entry sites were avoided. The

entire sequence was synthetically produced at Geneart (Regensburg, Germany). The synthetic engineered H1HA09 gene was 1818 bp in length (SEQ ID NO:1) and was cloned into pVAX1 at BamHI and XhoI sites by Geneart (Figure 2).

5

Example 2

HS09 influenza (pGX2009) immunized Ferrets

Experiments were carried out using ferrets, a preferred model for influenza. The ferrets were immunized using plasmid pGX2009 (SEQ ID NO :5, including insert HS09 (SEQ ID NO :1)).

10

Animals: 4 groups x 5 animals/group, plus one control group with 4 animals = 24 ferrets total (male)

Duration: 18 weeks (including challenge)

Dose: .2mg plasmid

Protocol Summary: Ferrets were allocated randomly into DNA vaccine groups.

15

Animals were immunized at Study Day 0, Day 28, and Day 56. Animals were anesthetized with ketamine/midazolam cocktail, isoflurane or equivalent according to approved anesthesia protocols and vaccinated IM with influenza DNA vaccine combinations. Groups 1 and 2 were immediately electroporated using CELLECTRA® adaptive constant current electroporation (EP) device at 0.5 Amp, 52 millisecond pulses, 0.2 sec between pulses, 4 sec firing delay, 3 total pulses. Control animals were naïve controls (no plasmid, no EP). Ferrets were allowed to recover from anesthesia in their cages and were closely monitored for 24 hours to ensure full recovery.

20

Food and water was available ad libitum for the length of the study. On Day 84, animals were challenged by intranasal infection with 1 ml of MX10 (A/Mexico/InDRE4487/2009; 5×10^5 PFU/ml). Animals were monitored daily for clinical signs (weight, temperature, etc.), using an established and approved scoring sheet. On 1, 3, 6, 9 and 15 dpi nasal washes and rectal swabs were collected. Lungs were collected at day 15. Samples were stored in RNAlater for virus load by real-time PCR, medium for infectious virus (TCID₅₀) and formalin for histology when appropriated.

25

30

HAI Titers

The ferrets were bled and sera samples immediately stored on dry-ice before being shipped to BIOQUAL, Rockville, MD for processing. Sera was treated with receptor-destroying enzyme by diluting 1 part serum with 3 parts enzyme and were incubated

overnight at 37°C water bath. The enzyme was inactivated by 30-min incubation at 56°C, followed by the addition of 6 parts phosphate-buffered saline for a final dilution of 1/10. HAI assays were performed in V-bottomed 96-well microtiter plates, using 4 hemagglutination units of virus and 1 % red blood cells. Virus (H1N1/Mexico/InDRE4487/2009 strain) used for the HAI assays are obtained from the influenza branch of the CDC. Figure 4 shows a Hemagglutination Inhibition assay performed with sera from immunized ferrets (3 immunizations). A titer of >1:40 is considered "protective". A dotted line indicates the 1:40 mark. All animals were above the 1:40 mark after 3 immunizations.

Challenge Studies

Figure 5 shows results of a challenge of immunized and unimmunized ferrets with a novel H1N1 strain MX10 (A/Mexico/InDRE4487/2009). All immunized ferrets survived, while 75% of the naive ferrets died within the 15 day period.

Example 3

H1 hemagglutinin combination studies

Experiments were carried out using various animals (mouse, guinea pigs and ferrets). The animals were immunized using plasmid constructs with backbone pVAX1, with each construct having a different insert:

In Figures 7A-G, 8A-C, 9A-B, 10A-B, 11A-C: naive (pVAX1 only); H1U1 (pVAX1 with SEQ ID NO:35 insert); HS09 (SEQ ID NO:5); ConBris (or H1Bris) (pVAX1 with SEQ ID NO:19 insert); ConTT (or H1TT) (pVAX1 with SEQ ID NO:21 insert);

In Figures 13A-F, 14A-C, 15A-D, 16A-C, 17A-C: naive (pVAX1 only); BHA-1 (pVAX1 with SEQ ID NO:13 insert); BHA-2 (pVAX1 with SEQ ID NO:25 insert); BHA-3 (pVAX1 with SEQ ID NO:31 insert); BHA-4 (pVAX1 with SEQ ID NO:33 insert).

In Figures 19A-E, 20A-F: naive (pVAX1 only); H3HA-1 (pVAX1 with SEQ ID NO:37 insert); H3HA-2 (pVAX1 with SEQ ID NO:23 insert); H3HA-3 (pVAX1 with SEQ ID NO:27 insert); H3HA-4 (pVAX1 with SEQ ID NO:29 insert).

Dose: 0.2mg plasmid

Protocol Summary: animals were allocated randomly into DNA vaccine groups. Animals were immunized at Study Day 0, Day 28, and Day 56. Animals were anesthetized

with ketamine/midazolam cocktail, isoflurane or equivalent according to approved anesthesia protocols and vaccinated IM with influenza DNA vaccine combinations. Groups 1 and 2 were immediately electroporated using CELLECTRA adaptive constant current electroporation (EP) device (Inovio Pharmaceuticals, Blue Bell, PA) at 0.5 Amp, 52
5 millisecond pulses, 0.2 sec between pulses, 4 sec firing delay, 3 total pulses. Control animals were naïve controls (no plasmid, no EP). The animals were allowed to recover from anesthesia in their cages and were closely monitored for 24 hours to ensure full recovery.

HAI Assays

10 Animals were bled and sera samples immediately stored on dry-ice before being shipped to BIOQUAL, Rockville, MD for processing. Sera was treated with receptor-destroying enzyme by diluting 1 part serum with 3 parts enzyme and were incubated overnight at 37°C water bath. The enzyme was inactivated by 30-min incubation at 56°C, followed by the addition of 6 parts phosphate-buffered saline for a final dilution of 1/10. HAI assays were performed in
15 V-bottomed 96-well microtiter plates, using 4 hemagglutination units of virus and 1 % red blood cells. Virus (H1N1/Mexico/2009 strain) used for the HAI assays are obtained from the influenza branch of the CDC.

H1 studies:

The experiments showed that mice vaccinated with the combo (all four H1 antigens)
20 showed protective titers against 9 out of 10 strains (Fig 7A-7F, and Fig 8A-C). This shows robust cross-reactivity across strains. Similarly the experiments showed that guinea pigs with the combo showed protective titers against all 10 strains (see Fig 9A-B and 10A-B).

Flu B studies:

The experiments showed that mice vaccinated with the combo (both BHA-1 and
25 BHA-2 antigens) showed protective titers against 4 out of 9 strains (Fig 13A-13C, and Fig 14C); while 2 strains showed borderline 1:40 (Fig 13D and Fig 14A). The individual antigens showed protective titers against 4 of 9 strains (BHA-1) and 3 out of 9 strains (BHA-2). Similarly the experiments showed that guinea pigs with the combo showed protective titers against all 10 strains (see Fig 9A-B and 10A-B).

30 The experiments showed that guinea pigs vaccinated with the combo (both BHA-1 and BHA-2 antigens) showed protective titers against all 10 of 10 strains (looking at post-dose 3 “PD3”). Whereas, on the other hand, immunization with single antigens did not

provide such cross-protection, with BHA-1 providing only protective titers against 7 of 10 strains (Fig 15A-D and Fig. 16A-C).

H3 studies:

The experiments showed that mice vaccinated with the combo (both H3HA-1 and H3HA-2 antigens) showed protective titers against 4 of 11 strains (Fig 19A-D), and near protective titers against 2 strains (Fig 19E and Fig 20F). Whereas, on the other hand, the single antigens display cross-protection against 4 of 11 strains in the case of H3HA-1 (Fig 19A-D) but no HAI titer exhibited in any of the remaining strains. The combo H3 mix showed slightly broader cross-protection than one H3 antigen alone.

10

Challenge Studies

Food and water was available ad libitum for the length of the study. On Day 84, ferrets were challenged by intranasal infection with 1 ml of MX10 (A/Mexico/2009; 5×10^5 PFU/ml). Animals were monitored daily for clinical signs (weight, temperature, etc.), using an established and approved scoring sheet. On 1, 3, 6, 9 and 15 dpi nasal washes and rectal swabs were collected. Lungs were collected at day 15. Samples were stored in RNAlater for virus load by real-time PCR, medium for infectious virus (TCID₅₀) and formalin for histology when appropriated.

20 H1HA vaccinated – challenge

The ferrets vaccinated with HS09 and H1U showed immunoprotection as all five ferrets survive 14 days post-infection; whereas only one out of five naive survived. See Figs 11A-C.

Example 4

25 Influenza H7N9 Hemagglutinin DNA Vaccine Design

To design a H7N9 hemagglutinin DNA vaccine, the hemagglutinin (HA) sequences of the first four identified H7N9 human isolates were retrieved from The Global Initiative on Sharing All Influenza Data (GISAID). All HA sequences were aligned using MegAlign (DNASTAR, Madison, WI) and a consensus HA sequence (H7HA) was developed, codon/RNA optimized and subsequently synthesized by GenScript. The H7N9 HA

30

consensus sequence (SEQ ID NO: 40) was generated from the first four H7N9 HA sequences.

After generating the consensus HA sequence, codon and RNA optimizations were performed, as previously described (Yan et al., (2007) Mol Ther 15: 411). In just a few days, the synthetic H7HA gene (SEQ ID NO: 39), which is 1683 bp in length, was synthesized, sequence verified, and subcloned into the expression vector pGX0001 at *Bam*HI and *Not*I sites and named pH7HA (Fig. 21B). The expression vector pGX0001 is under the control of the cytomegalovirus immediate-early promoter.

Example 5

Phylogenetic Analysis of H7N9 HA protein sequences

Twenty-four primary HA protein sequences of human H7N9 virus isolates were retrieved from GISAID. The alignment applied in the phylogenetic study was performed using Clustal X (version 2.0) and a phylogenetic tree was constructed based on Neighbor-joining evaluation of the alignment. Phylogenetic analysis indicated that the sequence identity between any two H7 HA proteins of these human isolates could be as low as 95.9%, while the identities between the consensus HA and primary HA proteins were 97.5% and above. The consensus approach to antigen development for influenza was not dependent upon any one viral isolate that can not represent the totality of the pandemic in real time. The relevant placement of the consensus H7 HA sequence is indicated in Fig. 21A.

Example 6

Expression of pH7HA DNA vaccine

To determine the expression of the H7HA construct, an indirect immunofluorescent assay was carried out using an anti-H7N9 HA mouse monoclonal antibody. Briefly, human rhabdomyosarcoma (RD) cells were plated on two-well chamber slides (BD Biosciences), at a density to obtain 60-70% confluency the next day in complete DMEM medium with 10% FBS (GIBCO) and allowed to adhere overnight. The cells were transfected with pH7HA and the control plasmid pGX0001 (1 µg/well) using TurboFectinTM8.0 Transfection Reagent (OriGene) according to the manufacturer's instructions. Forty-eight hours later, the cells were washed gently three times with 1XPBS and fixed on slides using ice cold methanol for 10 min. The cells were incubated with anti-H7N9 HA mouse monoclonal antibody (Sino Biological Inc., Cat# 11082-MM04) at a 1:400 dilution for 2 hrs at room temperature. The slides were then incubated with the Alexa 555-conjugated anti-mouse secondary antibody

(Cell Signaling Technology) for 60 min in the dark, and analyzed by fluorescent microscopy (Leica DM4000B, Leica Microsystems Inc., USA) using the SPOT Advanced software program (SPOTTM Diagnostic Instruments, Inc.).

High membrane expression was observed by fluorescent microscopy in the pH7HA-transfected cells (Fig. 21C), supporting the idea that the expressed HA protein was a surface protein and the protein exhibited a relatively native conformation. As a control, expression was not detected in pGX0001 (null vector)-transfected cells.

Example 7

Induction of cross-reactive antibodies by H7HA DNA immunogen

Immunization of Mice: Upon confirming expression of the pH7HA construct, the ability of this immunogen to drive cross-reactive antibody responses was investigated. Female 8-week-old BALB/c mice were purchased from Jackson Laboratory. Their care was in accordance with the guidelines of the National Institutes of Health and the University of Pennsylvania Institutional Animal Care and Use Committee (IACUC). Mice were immunized twice, three weeks apart, with pH7HA and the serum samples collected at pre-vaccination and at two weeks post the second immunization were utilized for the ELISA assay. Mice were immunized with 25 µg of pH7HA by intramuscular injection (IM) into the quadriceps muscle followed by *in vivo* electroporation (EP) using the CELLECTRA[®] adaptive constant current electroporation device (Inovio Pharmaceuticals Inc.). Two 0.1 Amp constant current square-wave pulses were delivered through a triangular 3-electrode array consisting of 26-gauge solid stainless steel electrodes. Each pulse was 52 milliseconds in length with a 1 second delay between pulses. The mice received two immunizations, three weeks apart. Serum samples were collected pre-immunization and two weeks after the second vaccination time point for the ELISA and Hemagglutination Inhibition (HAI) assays. Four weeks after the second immunization, the mice were sacrificed for analysis of cellular immune responses.

H7N9 HA-specific Antibody Determination: The measurement of IgG antibodies specific for H7HA was performed by ELISA in both immunized and control mice. The plates were coated with 0.5 µg/ml of H7N9 A/Shanghai/1/2013 influenza HA protein, A/Anhui/1/2013 influenza HA1 protein and A/Hangzhou/1/2013 HA1 protein (Sino Biological Inc.), respectively, and standard ELISA was carried out (Yan et al., (2007) *Mol Ther* **15**: 411). Endpoint titers were determined, as previously described (Frey et al., (1998) *J Immunol Methods* **221**: 35). The mathematical formula used to calculate the endpoint titer

cutoffs is expressed as the standard deviation multiplied by a factor that was based on the number of negative controls (naïve sera) (n = 10) and the confidence level (95%). The endpoint titer is reported as the reciprocal of the last dilution that remained above the endpoint cutoff.

As shown in Figs. 22A, 22B and 22C, high-titer vaccine-induced antibody responses were detected against three different HA proteins in the immunized mice. The average HA-specific antibody endpoint titers against A/Shanghai/1/2013 HA, A/Anhui/1/2013 HA1 and A/Hangzhou/1/2013 HA1 were 222,074 (range 50,000-1,581,111), 368,019 (range 50,000-1,581,111) and 315,542 (range 50,000-1,581,111), respectively (Fig. 22D). These data indicated that vaccination with pH7HA elicited strong and broad antibody responses.

The surface HA protein of the influenza virus contains two structural elements (head and stalk) that differ in their potential utility as vaccine targets. Antibodies targeting areas of the HA globular head region (HA1) can drive HAI activity and viral neutralization. HAI titers against A/Anhui/1/2013 were tested. However, high-titer cross-reactive binding antibodies against A/Anhui/1/2013 HA1 and A/Hangzhou/1/2013 HA1 regions were confirmed in all vaccinated mice. High titer binding was also observed against A/Shanghai/2013. Therefore, vaccination with pH7HA DNA vaccine appears to induce broadly reactive antibodies against multiple H7N9 strains.

Example 8

Induction of protective antibodies by H7HA DNA immunogen

In order to test whether the cross-reactive antibodies can be protective, a standard Hemagglutination Inhibition assay (HAI) was used to test the ability of the pH7N9 plasmid to generate functional antibodies with HAI activity as HAI antibody is the major correlate of protection for flu vaccines. Sera samples were treated with receptor-destroying enzyme (RDE, 1:3 ratio) at 37°C overnight for 18-20 hrs followed by complement inactivation at 56°C for 45 min. Starting with a 1:10 dilution in PBS, twofold serial dilutions of RDE-treated serum were serially diluted down on 96-well V-bottom microtiter plates. Four hemagglutinating dose of A/Anhui/1/13 was added to each well and the serum-virus mixture were incubated at room temperature for 1.5 hrs. Following incubation, 50 µl horse red blood cells (1% cells + 0.5% Bovine Serum Albumin Fraction V in 0.85% saline solution) were added to each well and incubated for 1.25 hrs at room temperature. The HAI antibody titer was scored as the reciprocal of the highest dilution that did not exhibit agglutination of red blood cells. Each assay was performed in duplicate.

The A/Anhui/1/2013 H7N9 strain was quantified and used to determine a serum HAI antibody titer induced by the vaccination. As depicted in Fig. 22E, two weeks after two immunizations, pH7HA induced protective HAI titers ($\geq 1:40$) in every immunized animal (n=10) with a GMT titer of 1:130 in the pH7HA-immunized mice. The strong and long-
 5 lasting antibodies measured can allow for the development of HAI antibody dependent influenza DNA vaccines. Furthermore, a potential human dose could be in the range of just 500 μ g to 1 mg per immunization.

Example 9

Induction of potent antigen-specific effector T cell memory responses

10 Cell mediated immunity can be involved with virus clearance. The number of IFN- γ secreting cells correlates with the efficacy of live, attenuated influenza vaccine in children. The ability of pH7HA to induce antigen-specific cellular immune responses was explored.

IFN- γ ELISpot assay: Mouse IFN- γ ELISpot assay was performed, as described previously (Yan et al., (2007) *Mol Ther* **15**: 411). A set of peptides spanning the entire
 15 consensus H7HA protein, each containing 15 amino acid residues overlapping by 8 amino acids, were synthesized from GenScript (Piscataway, NJ). The entire set of peptides was pooled at a concentration of 2 μ g/ml/peptide into 4 pools as antigens for specific stimulation of the IFN- γ release. Concavalin A (Sigma-Aldrich, St. Louis, MO), at 5 μ g /ml, and complete culture medium were used as positive and negative control, respectively. The
 20 average number of spot forming cells (SFC) was adjusted to 1×10^6 splenocytes.

C57BL/6 mice were immunized twice with pH7HA, sacrificed four weeks post the second immunization (Fig. 23A), and the IFN- γ ELISpot assay was performed. As shown in Fig. 23B, the average response against four pools of H7HA overlapping peptides in the mice immunized with pH7HA was 504 ± 132 SFU/ 10^6 splenocytes, while minimal background
 25 spots were observed in naïve mice. Strong IFN- γ responses were induced by vaccination with pH7HA.

Intracellular Cytokine stain for Flow Cytometry: The phenotype and cytokine profile production of the memory T cells generated was characterized. Splenocytes were added to a 96-well plate (1×10^6 /well) and were stimulated with H7 peptide for 5-6 hrs at 37°C/5% CO₂
 30 in the presence of Protein Transport Inhibitor Cocktail (Brefeldin A and Monensin) (eBioscience) according to the manufacturers' instructions. The Cell Stimulation Cocktail (plus protein transport inhibitors) (phorbol 12-myristate 13-acetate (PMA), ionomycin,

brefeldin A and monensin) (eBioscience) was used as a positive control. R10 media was used as a negative control. In cultures being used to measure degranulation, anti-CD107a (FITC; clone 1D4B; Biolegend) was added at this time to enhance staining. The cells were then fixed and stained. Briefly, the cells were washed in FACS buffer (PBS containing 0.1% sodium azide and 1% FCS) before surface staining with fluorochrome-conjugated antibodies. Cells were washed with FACS buffer fixation and permeabilization using the BD Cytotfix/CytopermTM (BD, San Diego, CA, USA) according to the manufacturer's protocol followed by intracellular staining.

The following antibodies were used for surface staining: LIVE/DEAD Fixable Violet Dead Cell stain kit (Invitrogen), CD19 (V50; clone 1D3; BD Biosciences) CD4 (V500; clone RM4-5; BD Biosciences), CD8 (PE-TexasRed; clone 53-6.7; Abcam), CD44 (A700; clone IM7; Biolegend). For intracellular staining the following antibodies were used: IFN- γ (APC; clone XMGI.2; Biolegend), TNF- α (PE; clone MP6-XT22; eBioscience), IL-2 (PeCy7; clone JES6-SH4; eBioscience), CD3 (PerCP/Cy5.5; clone 145-2C11; Biolegend). All data were collected using a LSRII flow cytometer (BD Biosciences) and analyzed using FlowJo software (Tree Star, Ashland, OR) and SPICE v5.2. Boolean gating was performed using FlowJo software to examine the polyfunctionality of the T cells from vaccinated animals. For flow cytometry, cells were gated on singlets using FSC-H by FSC-A followed by gating on LIVE-DEAD (dump channel), CD3⁺ CD4⁺ CD8⁻ T and CD3⁺ CD8⁺ CD4⁻ T cells to examine the CD4⁺ and CD8⁺ T-cell populations. Standard and paired student's t-tests were applied to analyze statistical significance of all quantitative data produced in this study. A $p < 0.05$ was considered statistically significant.

The longevity and quality of CD4 and CD8 effector T cell memory induced by the H7HA DNA vaccine was studied. Four weeks after the last vaccination, mice were sacrificed and splenocytes were stimulated *in vitro* with H7HA pooled peptides and the production of IFN- γ , TNF- α , and IL-2 by CD4⁺ CD44⁺ and CD8⁺ CD44⁺ T cells was analyzed. The H7HA DNA vaccine elicited significant HA-specific CD4⁺ T cells producing all three cytokines (Fig. 24A), and a significant number of these cells were double positive (67%) and triple-positive (7%) (Figs. 24B-C). The triple positive and TNF α ⁺IL2⁺ double positive T cell phenotypes normally represent effector memory and central memory T cells, indicating the induction of a memory like CD4 T cell immune response by the HA vaccine. In terms of CD8 T cells, vaccination with pH7HA elicited substantially higher frequencies of HA-specific IFN γ ⁺CD8 T cells, with up to 3.7% of total splenic CD44⁺CD8⁺ T cells (Fig. 25A)

producing either IFN- γ ⁺ alone (0.76%), dual IFN γ ⁺/TNF α ⁺ (2.7%) or triple IFN γ ⁺/IL-2⁺/TNF α ⁺ (0.28%) (Figs. 25A-C). To further characterize the vaccine-induced T cells, the cytotoxic potential of the induced CD8⁺ T cells undergoing degranulation was analyzed. Cultures were stained with an antibody to CD107a, which is a marker for degranulation, and

5 CD8 T cells from the vaccinated mice showed a significant increase of antigen-specific (IFN γ ⁺CD107a⁺: 3.8%) degranulation compared to naïve mice.

These results are indicative of the vaccine's potent ability to induce cytolytic T cell responses with the potential ability to clear H7N9 influenza infected cells. In addition, the vaccine also induced a high frequency of TNF- α producing HA-specific CD4 (0.4%) and

10 CD8 T cells (3.2%), with a modest but significant increase in IL-2 responses (0.2% and 0.3%, respectively) compared to the naïve group (Figs. 24A and 25A). Interestingly, the proportional order of effector CD4 and CD8 T cell subpopulations in response to H7 HA stimulation was similar, with IFN γ ⁺/TNF α ⁺ being greater than IFN γ ⁺/IL2⁺/TNF α ⁺. The high frequencies of effector cells secreting IFN γ (CD8: 3.7%; CD4: 0.3%), IFN γ ⁺/TNF α ⁺ (CD8:

15 3.1%; CD4: 0.24%), and IFN γ ⁺/IL2⁺/TNF α ⁺ among the HA-specific CD4 and CD8 T cell populations are indicative of strong vaccine potency induced by pH7HA vaccination (Figs. 24 and 25). Overall, the frequency hierarchy of effector CD4 and CD8 memory T cells expressing one (1+), any combination of two (2+), or all three cytokine (3+) by flow cytometry were CD4⁺CD44⁺: 1+ (26%), 2+ (67%), 3+ (7%) and CD8+CD44+: 1+ (50%), 2+

20 (35%), 3+ (15%). Taken together, strong effector and memory T cells, as well as protective antibody responses induced by this vaccination approach were observed. The magnitude of the T cell responses are similar to those induced by viral infection rather than weak responses which are generated by traditional DNA immunization.

pH7HA was shown to induce both potent polyfunctional effector CD4 and CD8 T cell

25 memory responses. The percentage of antigen-specific CD8 cells secreting IFN γ +CD107a+ increased significantly, indicating potential ability of this vaccine to induce cytolytic T cell responses to clear influenza H7N9 infected cells. These data support the idea that the synthetic vaccine could induce both antigen-specific antibody and T cell responses, which would be important for protection during a pandemic.

Example 10

Vaccination with pH7HA elicits complete protection against H7N9 virus challenge

To determine if vaccination with pH7HA could elicit protective immunity to a lethal viral challenge, mice were immunized with pH7HA twice, three weeks apart, then challenged with a lethal dose of the H7N9 virus A/Anhui/1/2013 four weeks post the final immunization, and then monitored for weight loss for 14 days (Fig. 26A). Twenty female BALB/c mice were divided into two groups (n=10): the naïve and immunized group. The mice in the immunized group were immunized with 25 µg of pH7HA twice, three weeks apart. Four weeks after the second immunization, the mice were anesthetized with isoflurane and subsequently challenged by intranasal administration (bolus delivery into the nostrils using a standard micropipette) of 100 X LD₅₀ of A/Anhui/1/13 influenza virus in 50 µl Dulbecco's Modified Eagle Medium (DMEM) plus 2% Fetal Bovine Serum (FBS). After challenge, the animals were weighed daily for 14 days and monitored for clinical signs of influenza infection using an approved scoring sheet. All surviving animals were monitored for a total of 28 days. All procedures and the scoring method were approved by the Institutional Animal Care Committee at the National Microbiology Laboratory (NML) of the Public Health Agency of Canada (PHAC) according to the guidelines of the Canadian Council on Animal Care. All infectious work was performed in the 'Biosafety Level 4' (BSL4) facility.

According to the protocol established prior to the study, animals falling below the threshold of 70% of their initial body weight were humanely euthanized. As shown in Figs. 26B and 26C, in naïve group, one mouse lost 30% of its body weight by day 6 post-challenge, five mice exhibited 30% weight loss by day 7 post-challenge, and the rest of four mice lost 30% of their body weight by day 8. In contrast, all vaccinated mice (n=10) survived to day 28 post challenge with no observed pathogenic effects or weight loss at which time the study was ended. These data support that the synthetic DNA vaccine induced immune responses that protect against influenza H7N9 infection.

The rapid development of a synthetic H7N9 HA DNA vaccine efficiently delivered *in vivo* by EP capable of eliciting not only strong effector T cell memory responses, but also broadly reactive antibody responses. Immunization with the pH7HA DNA vaccine induced protective HAI titers in all immunized animals and resulted in 100% protection from mortality and morbidity due to influenza H7N9 infection. The H7N9 HA vaccine protected against newly emergent influenza subtypes. The development of a highly potent, synthetic, H7N9 HA DNA vaccine which in combination with an adaptive constant current

electroporation delivery platform was capable of eliciting robust cellular immune responses, broadly cross-reactive antibody responses and generating complete protection from lethal challenge with just a few week development and vaccination regime.

5 It is understood that the foregoing detailed description and accompanying examples are merely illustrative and are not to be taken as limitations upon the scope of the invention, which is defined solely by the appended claims and their equivalents.

Various changes and modifications to the disclosed embodiments will be apparent to those skilled in the art. Such changes and modifications, including without limitation those relating to the chemical structures, substituents, derivatives, intermediates, syntheses,
10 compositions, formulations, or methods of use of the invention, can be made without departing from the spirit and scope thereof.

CLAIMS

What is claimed is:

1. An isolated nucleic acid comprising a nucleic acid sequence encoding an amino acid sequence selected from the group consisting of SEQ ID NO: 40, an amino acid sequence
5 that is 95% identical over the entire length of the amino acid sequence of SEQ ID NO: 40, a fragment of SEQ ID NO: 40 comprising at least 30 amino acids, and an amino acid sequence that is 95% identical to a fragment of SEQ ID NO: 40 comprising at least 30 amino acids.

2. The isolated nucleic acid of claim 1, wherein the isolated nucleic acid comprises a nucleic acid sequence selected from the group consisting of SEQ ID NO: 39, a nucleic acid
10 sequence that is 95% identical over the entire length of the nucleic acid sequence of SEQ ID NO: 39, a fragment of SEQ ID NO: 39 comprising at least 90 nucleotides, and a nucleic acid sequence that is 95% identical to a fragment of SEQ ID NO: 39 comprising at least 90 nucleotides.

3. The isolated nucleic acid of claim 1, wherein the isolated nucleic acid comprises a nucleic acid sequence selected from the group consisting of a nucleic acid sequence that is
15 95% identical over the entire length of the nucleic acid sequence of SEQ ID NO: 39 and a nucleic acid sequence that is 95% identical to a fragment of SEQ ID NO: 39 comprising at least 90 nucleotides.

4. A recombinant vector comprising the isolated nucleic acid of claim 1.

20 5. An expression vector comprising the isolated nucleic acid of claim 1 operably linked to regulatory elements.

6. An expression vector comprising the isolated nucleic acid of claim 1 operably linked to regulatory elements that are functional in a human cell.

7. The expression vector of claim 6, wherein the expression vector is a plasmid.

25 8. The expression vector of claim 6, wherein the expression vector is pGX0001.

9. An isolated nucleic acid comprising a nucleic acid sequence encoding an amino acid sequence of SEQ ID NO: 40.

10. The isolated nucleic acid of claim 9, wherein the isolated nucleic acid consists of a nucleic acid sequence encoding an amino acid sequence of SEQ ID NO: 40

11. The isolated nucleic acid of claim 9, wherein the isolated nucleic acid consists of a nucleic acid sequence of SEQ ID NO: 39.

12. A recombinant vector comprising the isolated nucleic acid of claim 9.

13. An expression vector comprising the isolated nucleic acid of claim 9 operably
5 linked to regulatory elements.

14. An expression vector comprising the isolated nucleic acid of claim 9 operably linked to regulatory elements that are functional in a human cell.

15. The expression vector of claim 14, wherein the expression vector is a plasmid.

16. The expression vector of claim 15, wherein the expression vector is pGX0001.

10 17. A composition comprising:

- 15 (a) a first nucleic acid sequence comprising one or more nucleotide sequences encoding an amino acid sequence selected from the group consisting of SEQ ID NO: 40, an amino acid sequence that is 95% identical over the entire length of the amino acid sequence of SEQ ID NO: 40, a fragment of SEQ ID NO: 40 comprising at least 30 amino acids, and an amino acid sequence that is 95% identical to a fragment of SEQ ID NO: 40 comprising at least 30 amino acids; and
- 20 (b) a second nucleic acid sequence that encodes a protein selected from the group consisting of one or more of: H1, H2, H3, H4, H5, H6, H7, H8, H9, H10, H11, H12, H13, H14, H15, H16, N1, N2, N3, N4, N5, N6, N7, N8 and N9.

18. The composition of claim 17, wherein the first nucleic acid sequence is selected from the group consisting of one or more of SEQ ID NO: 39, a nucleic acid sequence that is 95% identical over the entire length of the nucleic acid sequence of SEQ ID NO: 39, a
25 fragment of SEQ ID NO: 39 comprising at least 90 nucleotides, and a nucleic acid sequence that is 95% identical to a fragment of SEQ ID NO: 39 comprising at least 90 nucleotides.

19. The composition of claim 17, wherein the first nucleic acid sequence is operably linked to regulatory elements that are functional in a human cell.

20. The composition of claim 19 comprising a plasmid that comprises the first nucleic
30 acid sequence operably linked to regulatory elements that are functional in a human cell.

21. The composition of claim 17, wherein the second nucleic acid sequence is selected from the group consisting of one or more of:

SEQ ID NO: 1;

a nucleic acid sequence that is 95% identical over the entire length of the nucleic acid sequence of SEQ ID NO: 1;

a fragment of SEQ ID NO: 1 comprising at least 90 nucleotides;

a nucleic acid sequence that is 95% identical to a fragment of SEQ ID NO: 1 comprising at least 90 nucleotides;

SEQ ID NO: 3;

a nucleic acid sequence that is 95% identical over the entire length of the nucleic acid sequence of SEQ ID NO: 3;

a fragment of SEQ ID NO: 3 comprising at least 90 nucleotides;

a nucleic acid sequence that is 95% identical to a fragment of SEQ ID NO: 3 comprising at least 90 nucleotides;

SEQ ID NO: 6;

a nucleic acid sequence that is 95% identical over the entire length of the nucleic acid sequence of SEQ ID NO: 6;

a fragment of SEQ ID NO: 6 comprising at least 90 nucleotides;

a nucleic acid sequence that is 95% identical to a fragment of SEQ ID NO: 6 comprising at least 90 nucleotides;

SEQ ID NO: 8;

a nucleic acid sequence that is 95% identical over the entire length of the nucleic acid sequence of SEQ ID NO: 8;

a fragment of SEQ ID NO: 8 comprising at least 90 nucleotides; and

a nucleic acid sequence that is 95% identical to a fragment of SEQ ID NO: 8 comprising at least 90 nucleotides.

22. The composition of claim 17, further comprising a peptide encoded by the nucleic acid sequence of SEQ ID NO: 39.

23. The composition of claim 17, further comprising a peptide comprising an amino acid sequence of SEQ ID NO: 40.

24. A vaccine comprising the isolated nucleic acid of any one of claim 1-3 or 9-11, the recombinant vector of any one of claims 4 or 12, the expression vector of any one of claims 5-8 or 13-16, or the composition of any one of claims 17-23.

25. The vaccine of claim 24 further comprising a peptide encoded by the nucleic acid sequence of SEQ ID NO: 39.

26. The vaccine of claim 24 further comprising a peptide comprising an amino acid sequence of SEQ ID NO: 40.

5 27. A vaccine comprising a nucleic acid sequence encoding an amino acid sequence of SEQ ID NO: 40.

28. A vaccine comprising a nucleic acid sequence of SEQ ID NO: 39.

29. A vaccine against an influenza virus comprising a nucleic acid sequence encoding an amino acid sequence of SEQ ID NO: 40.

10 30. The vaccine of claim 29, wherein the nucleic acid sequence comprises a nucleic acid sequence of SEQ ID NO: 39.

31. The vaccine of claim 29, where the influenza virus is H7N9 influenza virus.

32. The vaccine of any one of claims 27-31 further comprising a peptide encoded by the nucleic acid sequence of SEQ ID NO: 39.

15 33. The vaccine of any one of claims 27-31 further comprising a peptide comprising an amino acid sequence of SEQ ID NO: 40.

34. A method of inducing an immune response comprising the step of: administering to an individual a nucleic acid molecule comprising the isolated nucleic acid of claim 1.

20 35. A method of inducing an immune response comprising the step of: administering to an individual the composition of claim 17.

36. A method of protecting an individual against infection by an avian origin human influenza strain comprising the step of: administering to said individual a prophylactically effective amount of a nucleic acid molecule comprising the isolated nucleic acid of claim 1; wherein the nucleic acid molecule is expressed in cells of said individual and an immune
25 response against said protein is induced that is a protective immune response against avian origin human influenza.

37. A method of protecting an individual against infection by an avian origin human influenza strain comprising the step of: administering to said individual a prophylactically effective amount of the composition of claim 17; wherein the first nucleic acid sequence is
30 expressed in cells of said individual and an immune response against said first protein is

induced that is a protective immune response against avian origin human influenza, the one or more second nucleic acid sequences are expressed in cells of said individual and immune responses against said one or more second proteins are induced.

38. A method of treating an individual who has been infected by an avian origin human influenza strain comprising the step of: administering to said individual a therapeutically effective amount of the isolated nucleic acid of claim 1.

39. A method of treating an individual who has been infected by an avian origin human influenza H7N9 strain comprising the step of: administering to said individual a therapeutically effective amount of the composition of claim 17; wherein the first nucleic acid sequence is expressed in cells of said individual and an immune response against said first protein is induced that is a therapeutic immune response against avian origin human influenza, the one or more second nucleic acid sequences are expressed in cells of said individual and immune responses against said one or more second proteins are induced.

40. The method of any one of claims 34-39 further comprising administering to said individual a therapeutically effective amount of a peptide encoded by the nucleic acid sequence of SEQ ID NO: 39.

41. The method of any one of claims 34-39 further comprising administering to said individual a therapeutically effective amount of a peptide comprising an amino acid sequence of SEQ ID NO: 40.

42. The method of any one of claims 34-39 wherein said nucleic acid molecule is a plasmid.

43. The method of any one of claims 34-39 wherein said nucleic acid molecule is a plasmid that is pGX0001.

44. The method of any one of claims 34-39 wherein said nucleic acid molecule is administered to said individual using electroporation.

FIGURE 1

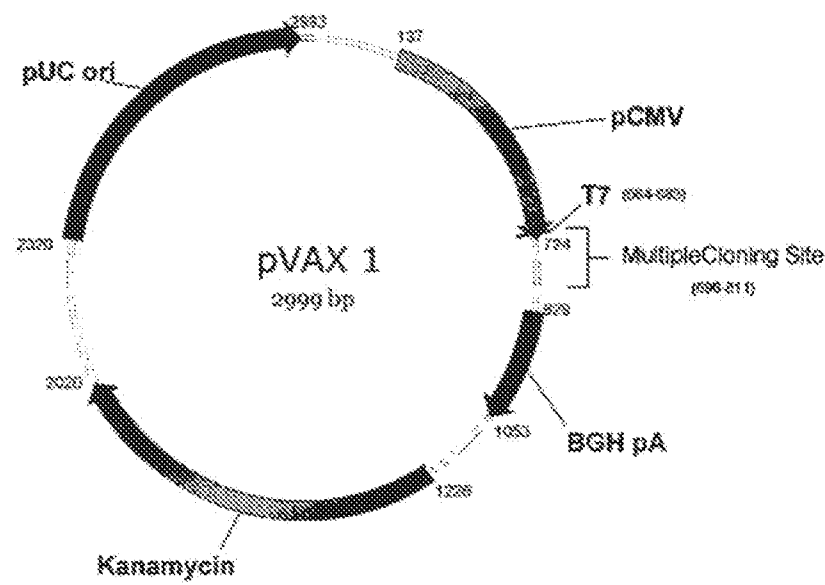


FIGURE 2

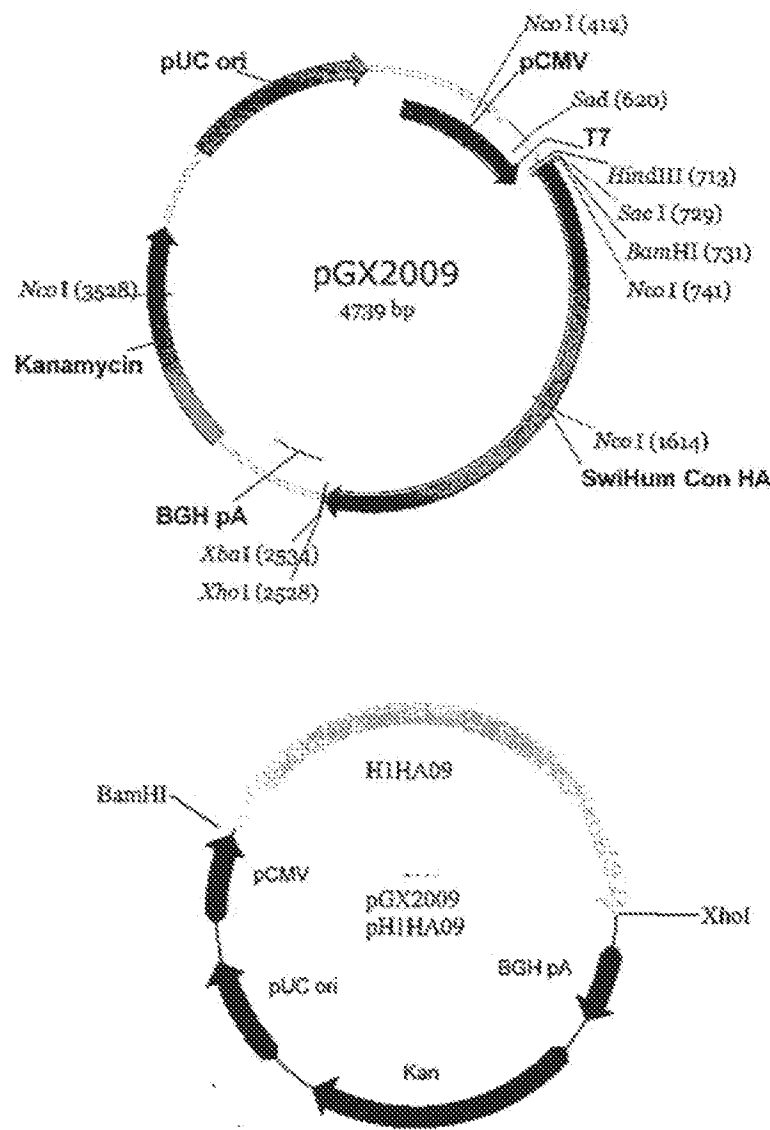


FIGURE 3

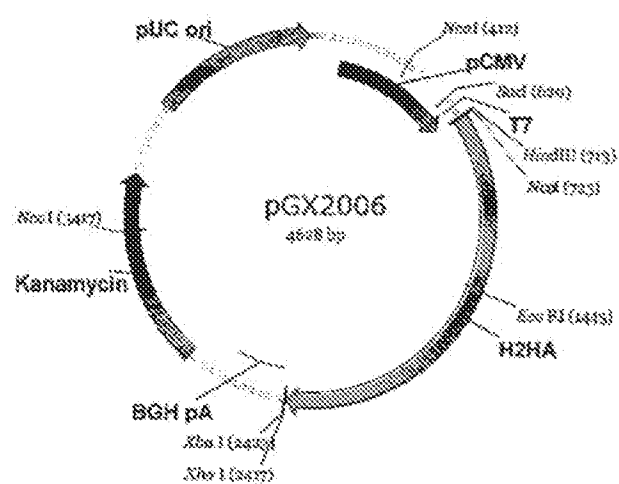


FIGURE 4

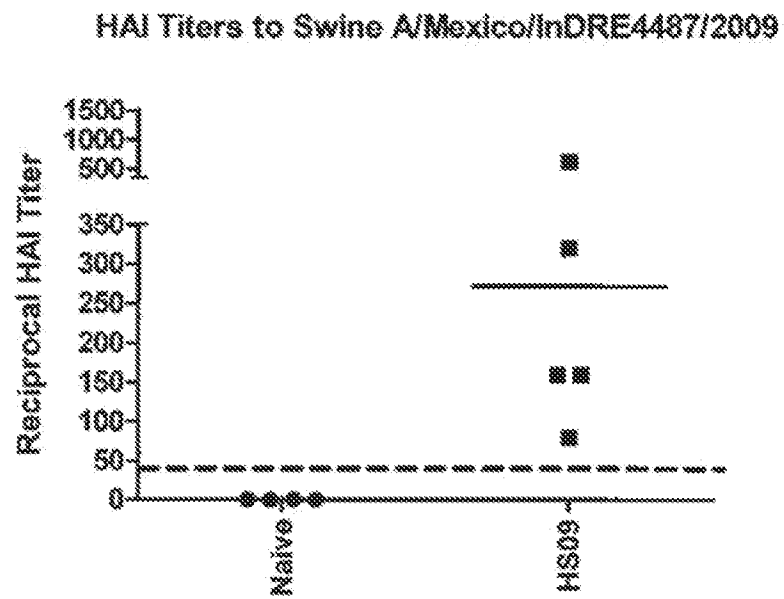
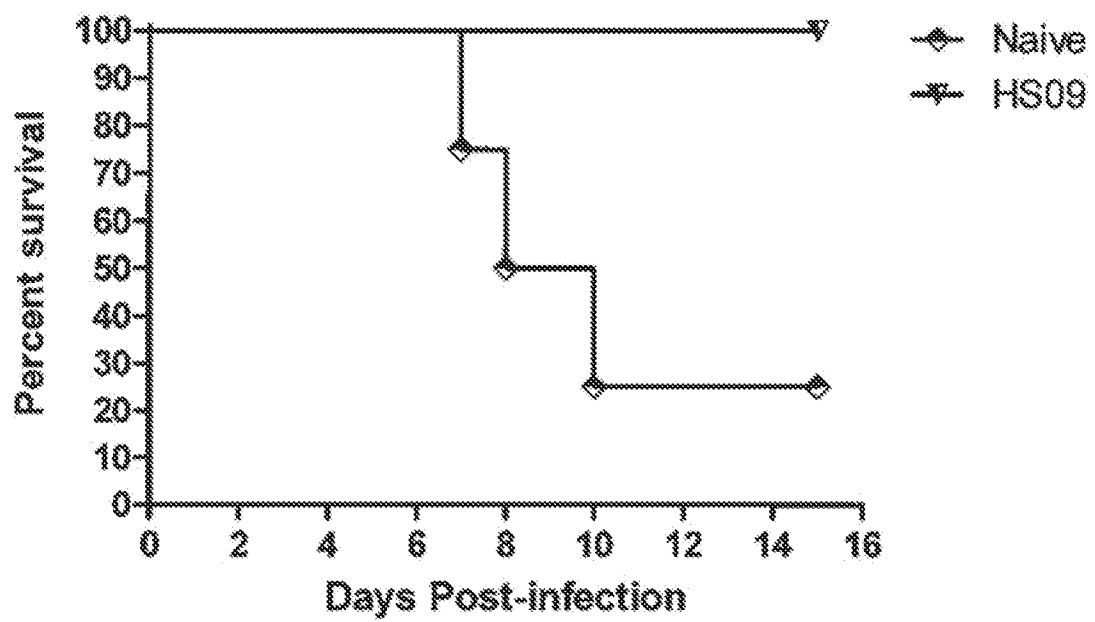
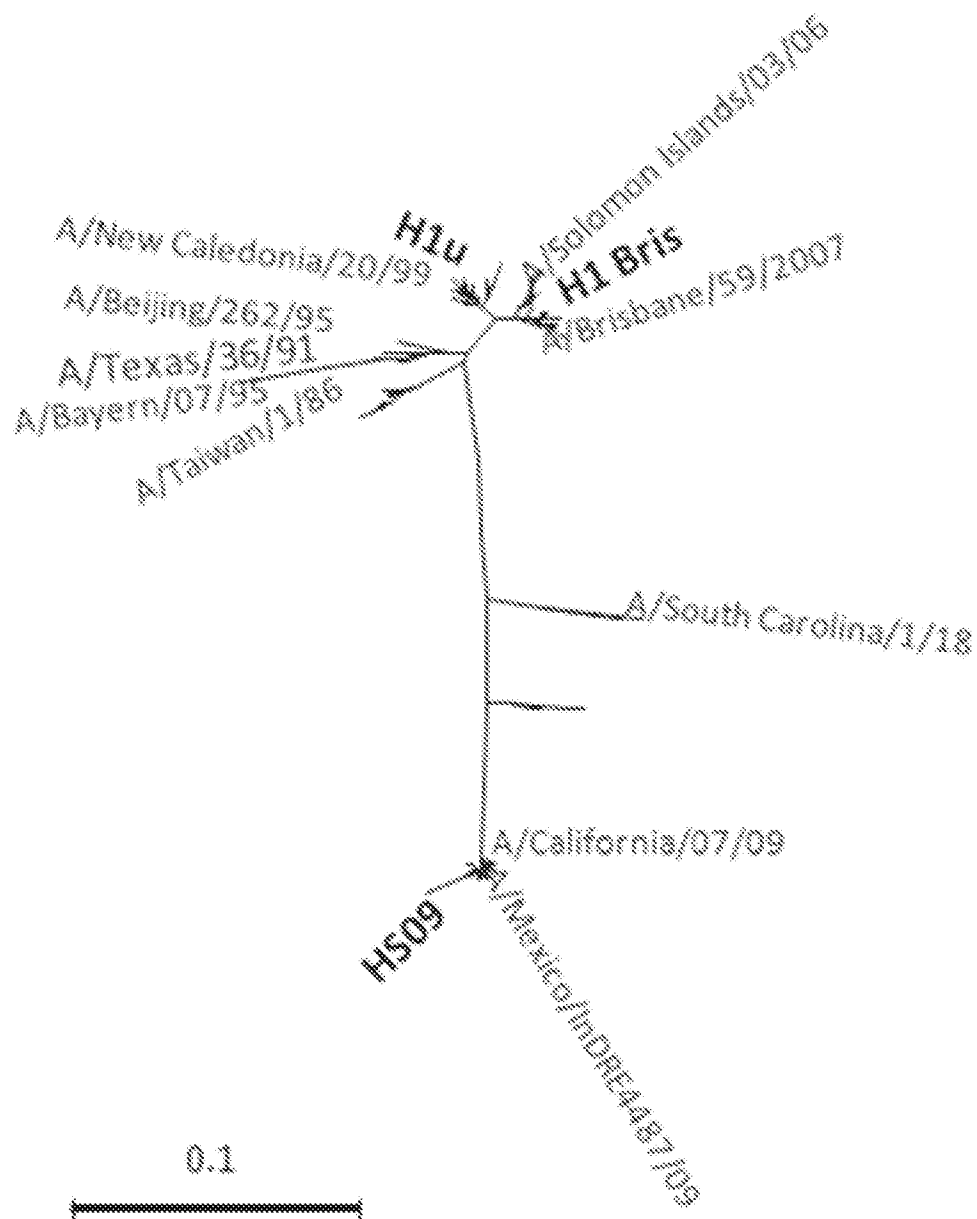


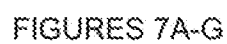
FIGURE 5

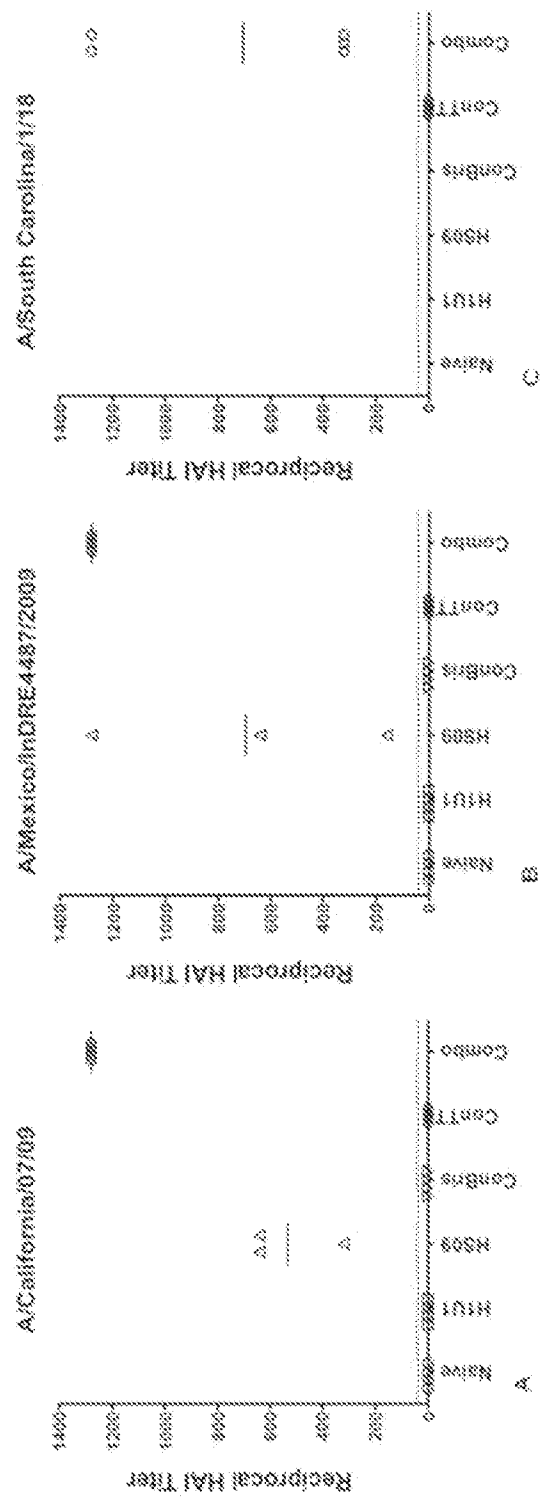




H1 tree of all viruses from last 20 years as well as South Carolina and where the constructs are located

FIGURE 6





Mouse HIs against pandemic viruses

FIGURES 8A-C

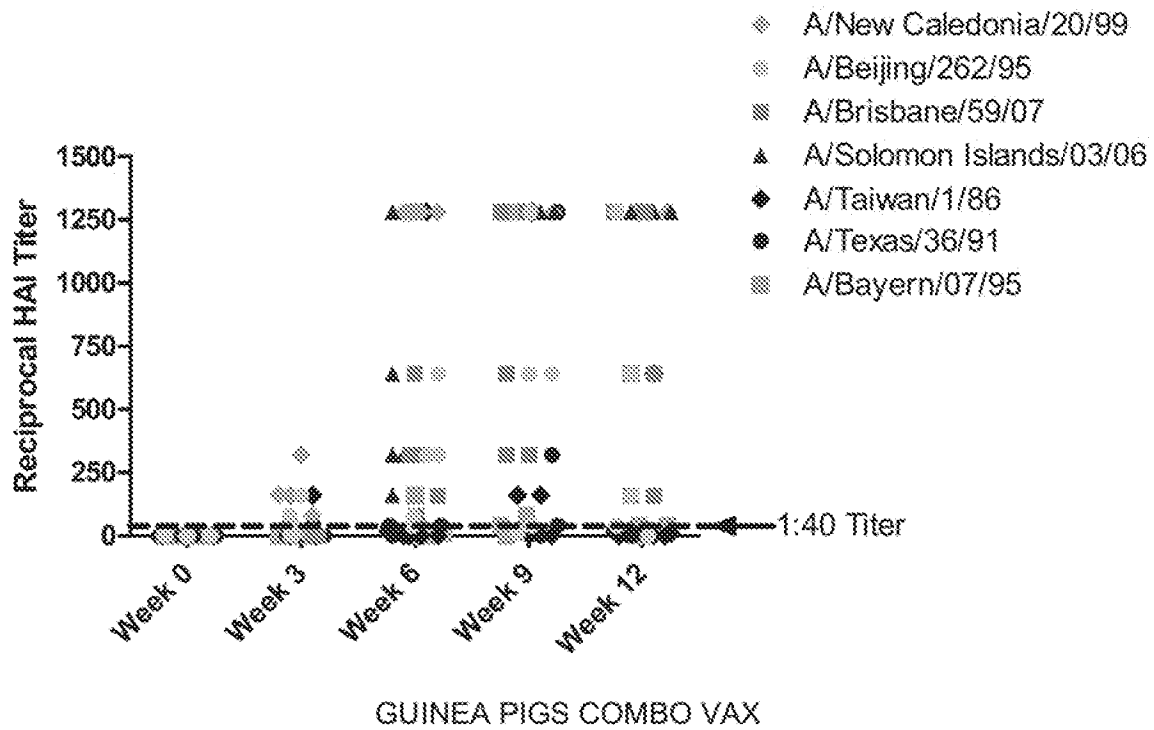


FIGURE 9A

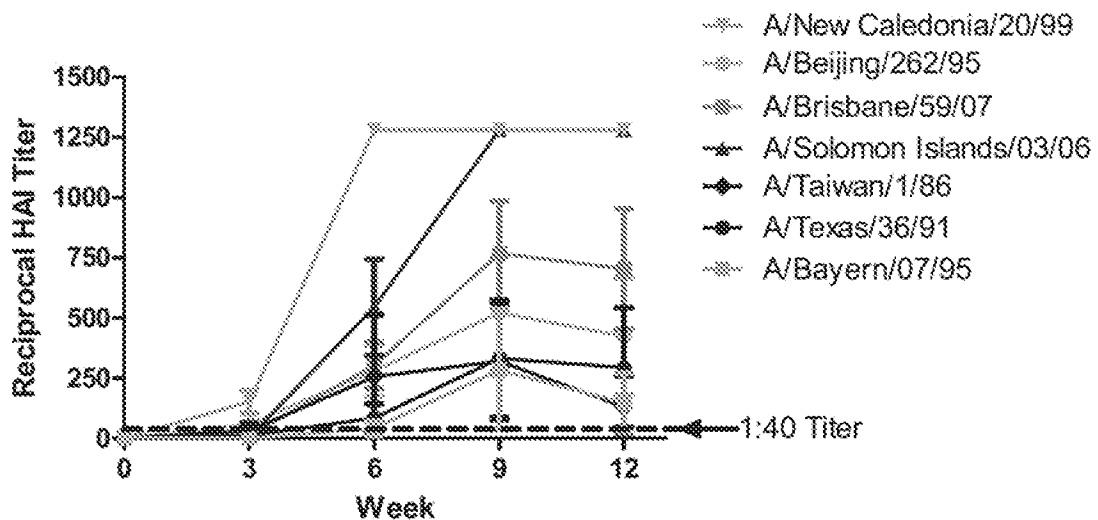
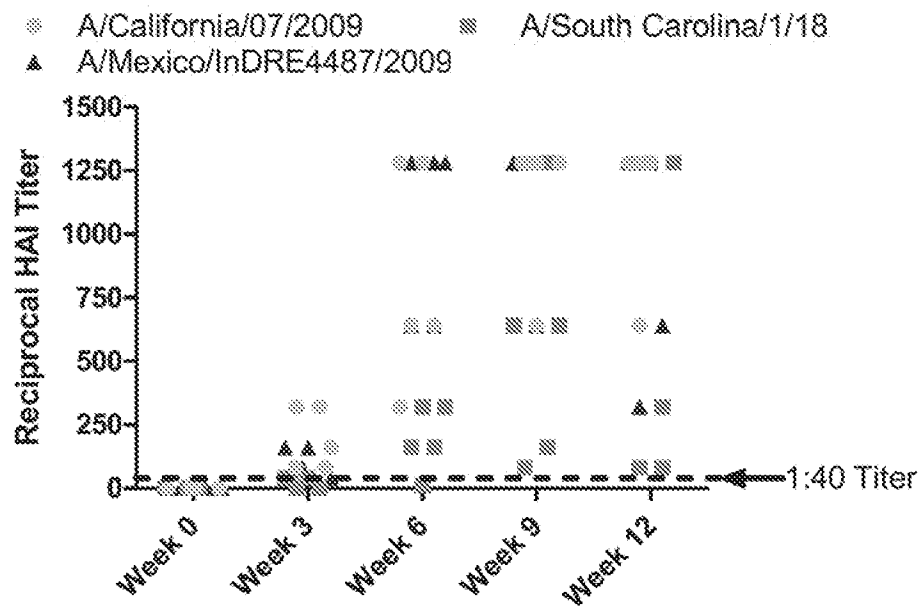
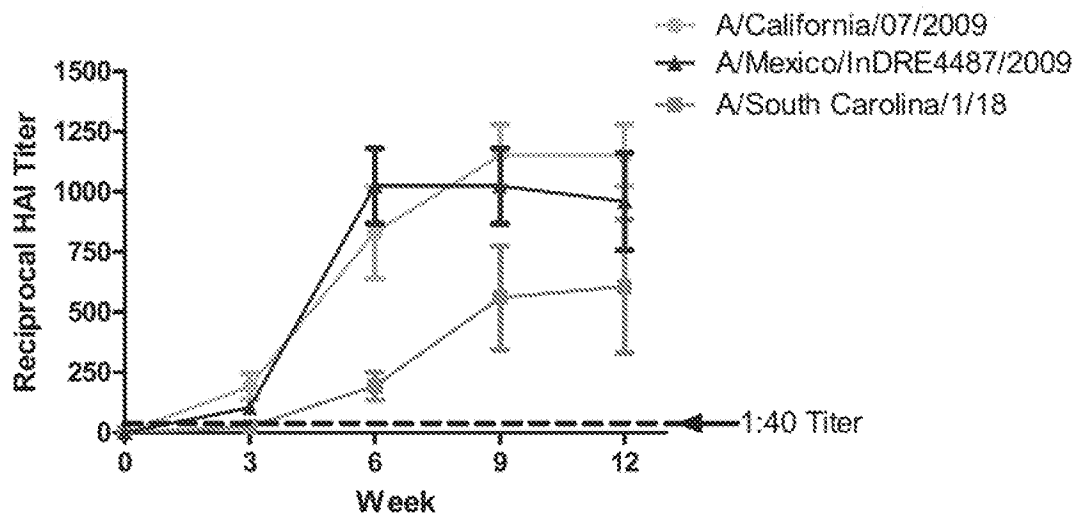


FIGURE 9B



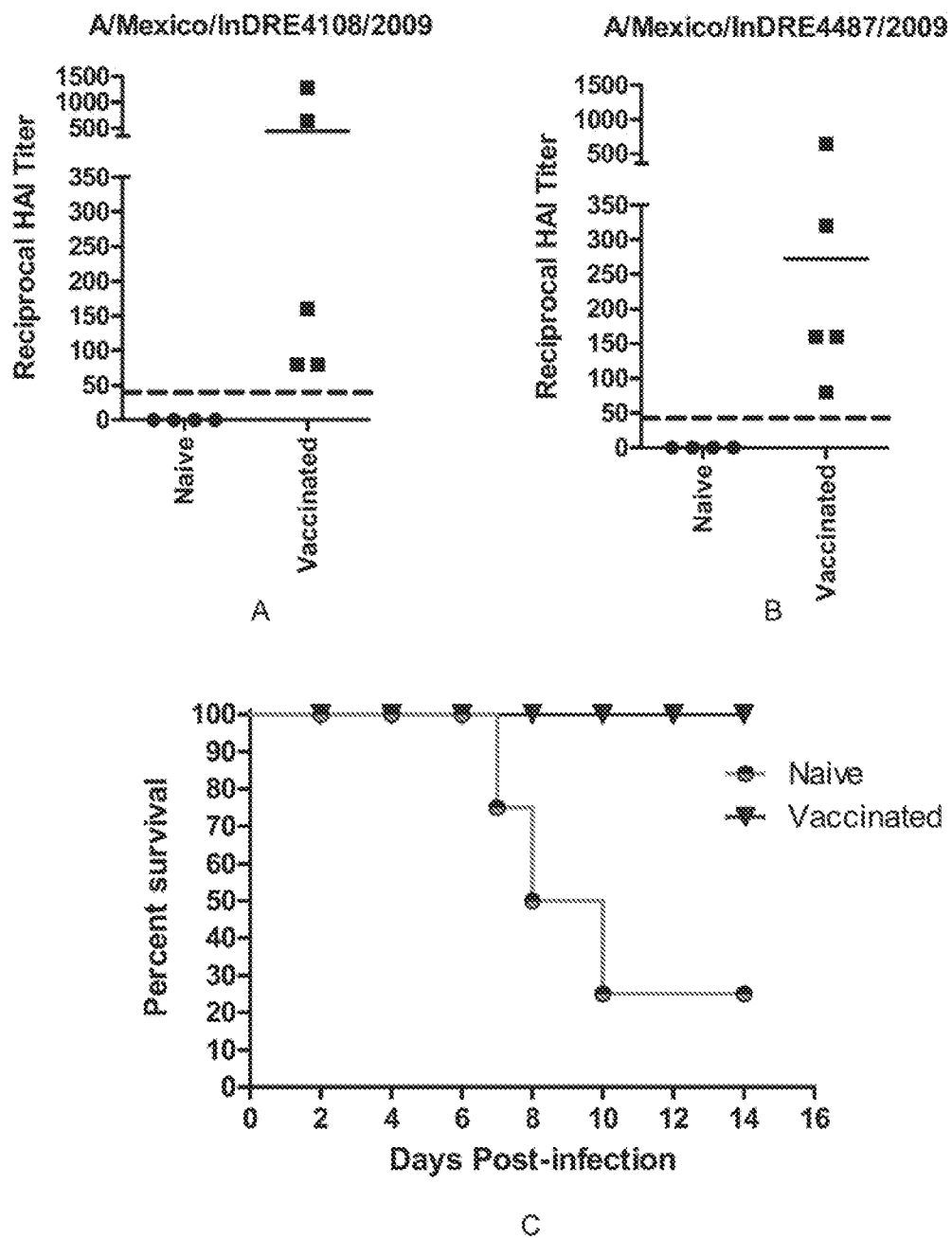
GUINEA PIGS COMBO VAX

FIGURE 10A



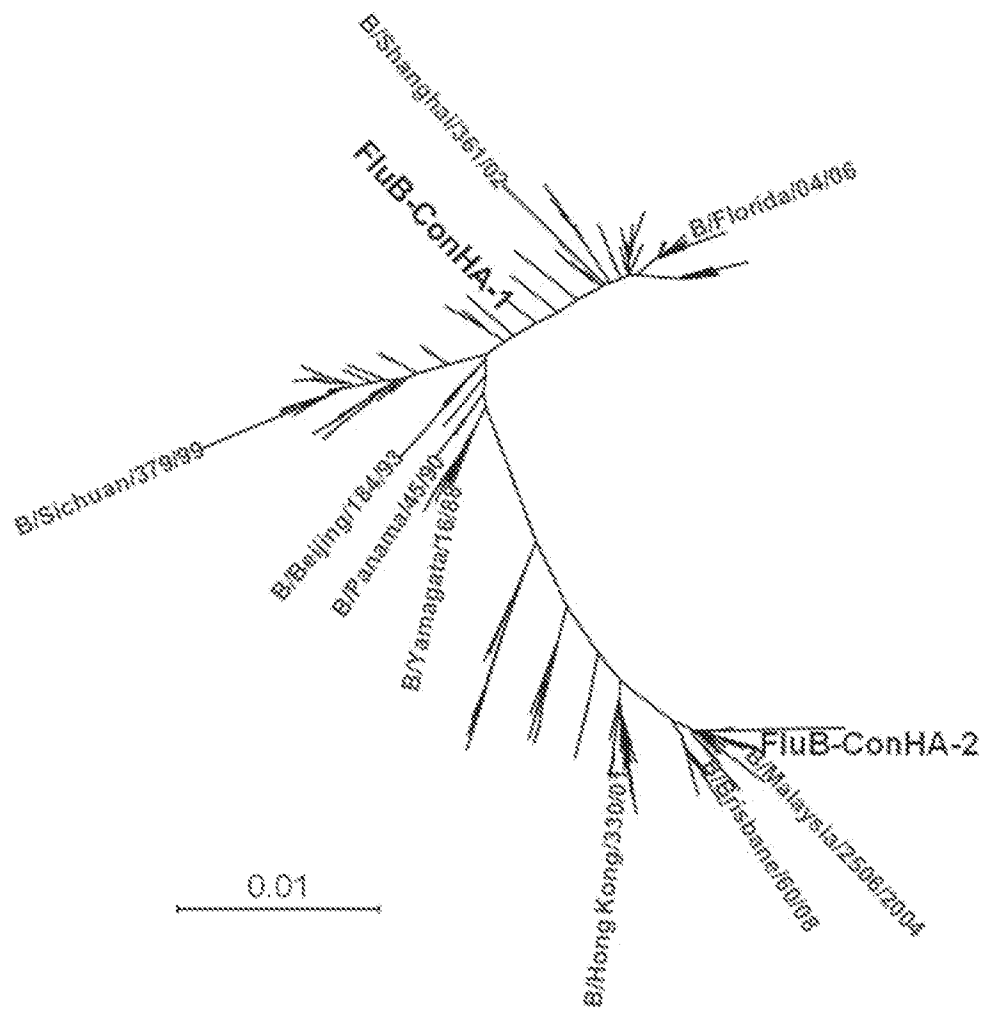
GUINEA PIGS COMBO VAX

FIGURE 10B



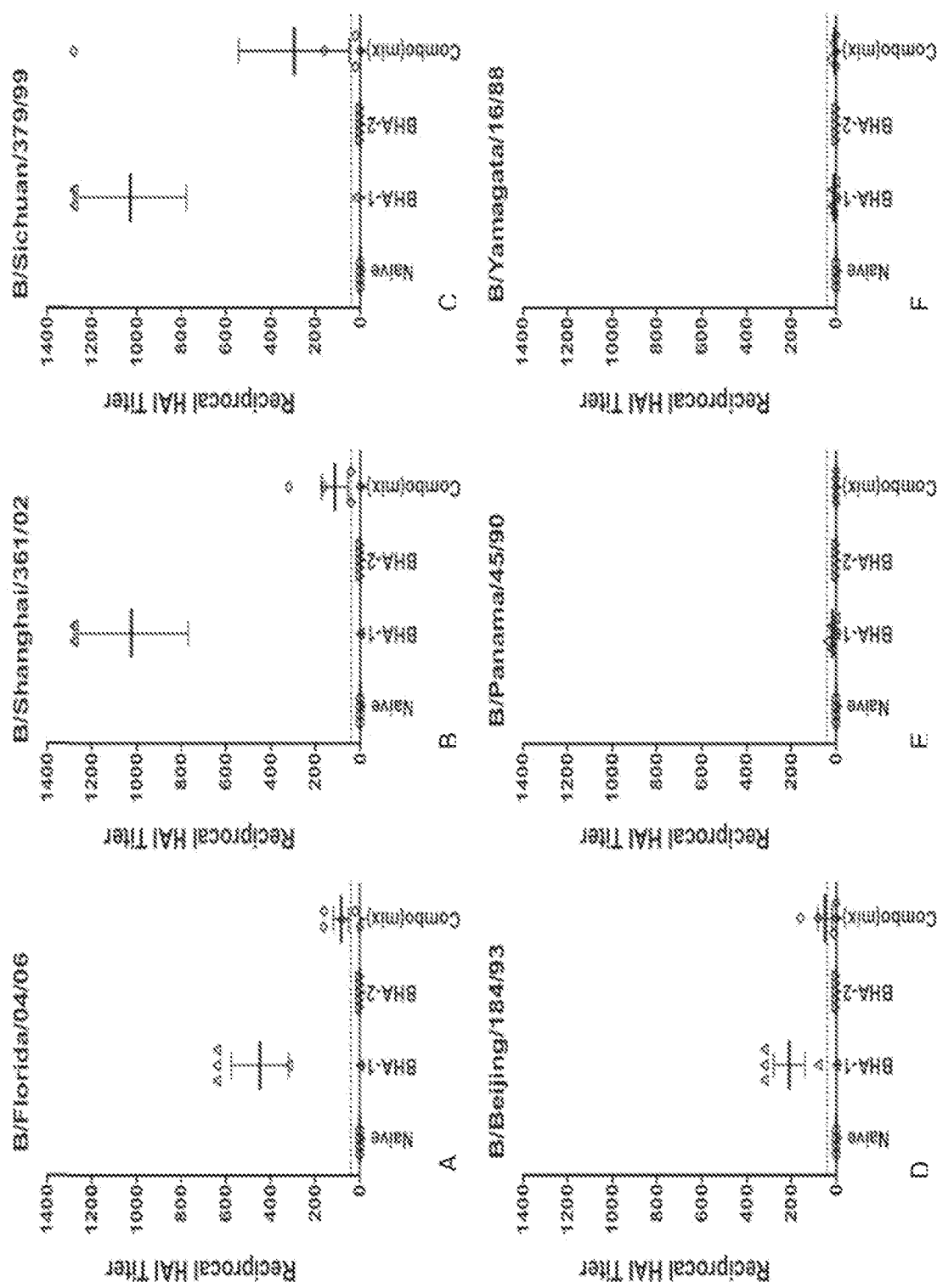
FERRETS VAX WITH HS09 & H1U, CHALLENGE STUDY

FIGURES 11A-C

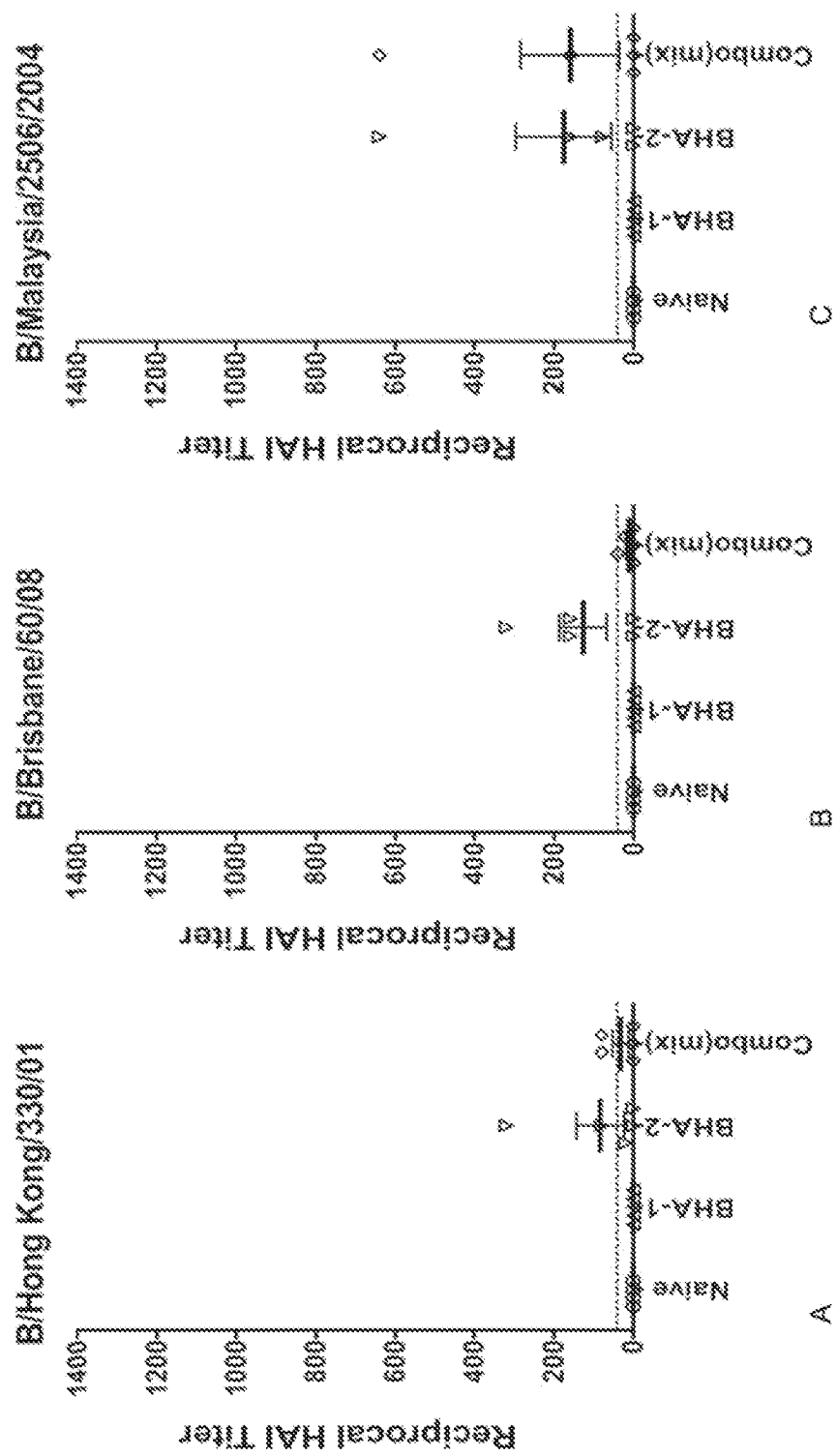


FluB tree of all viruses from last 20 years and where the constructs are located

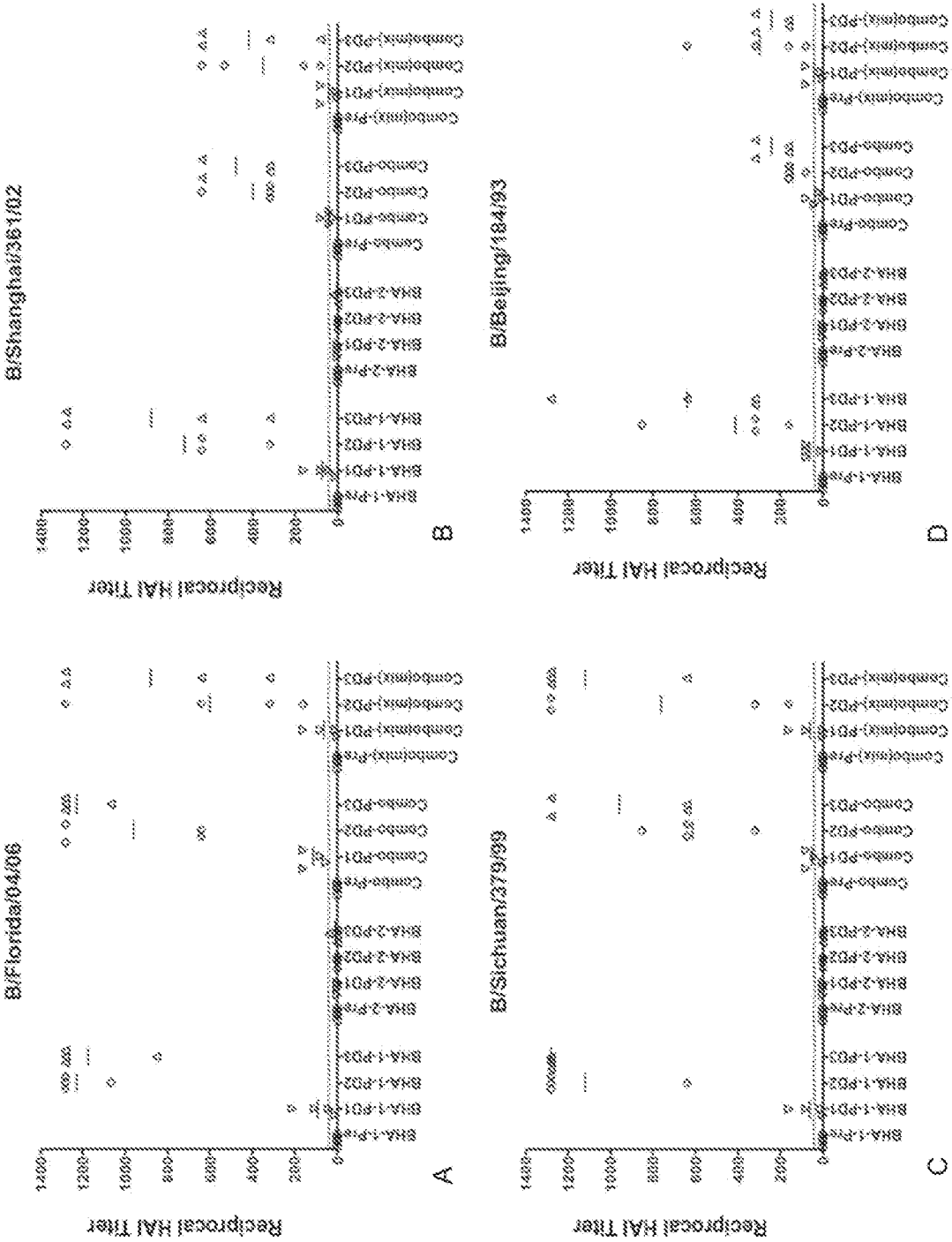
FIGURE 12



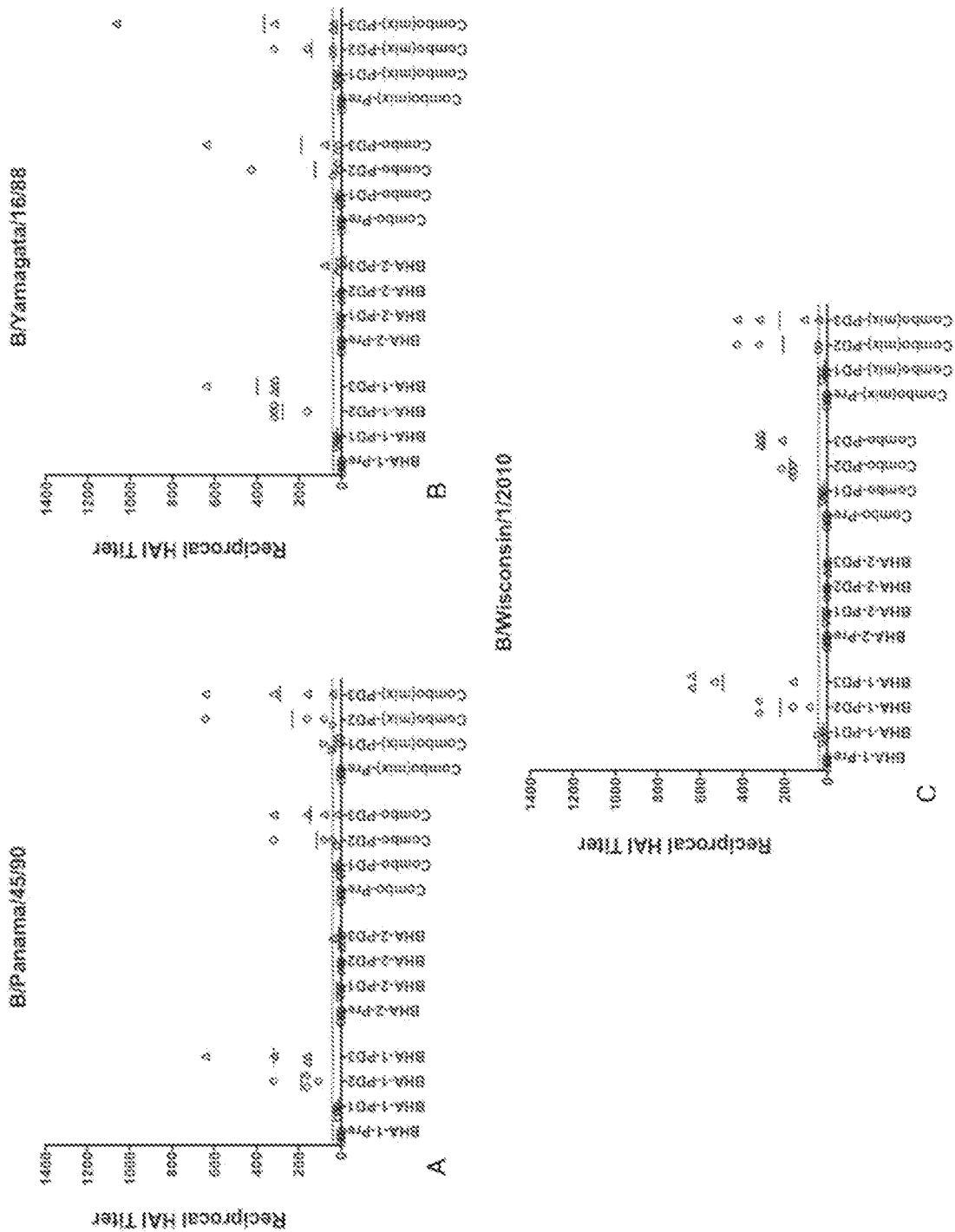
Mouse HIs
FIGURES 13A-F



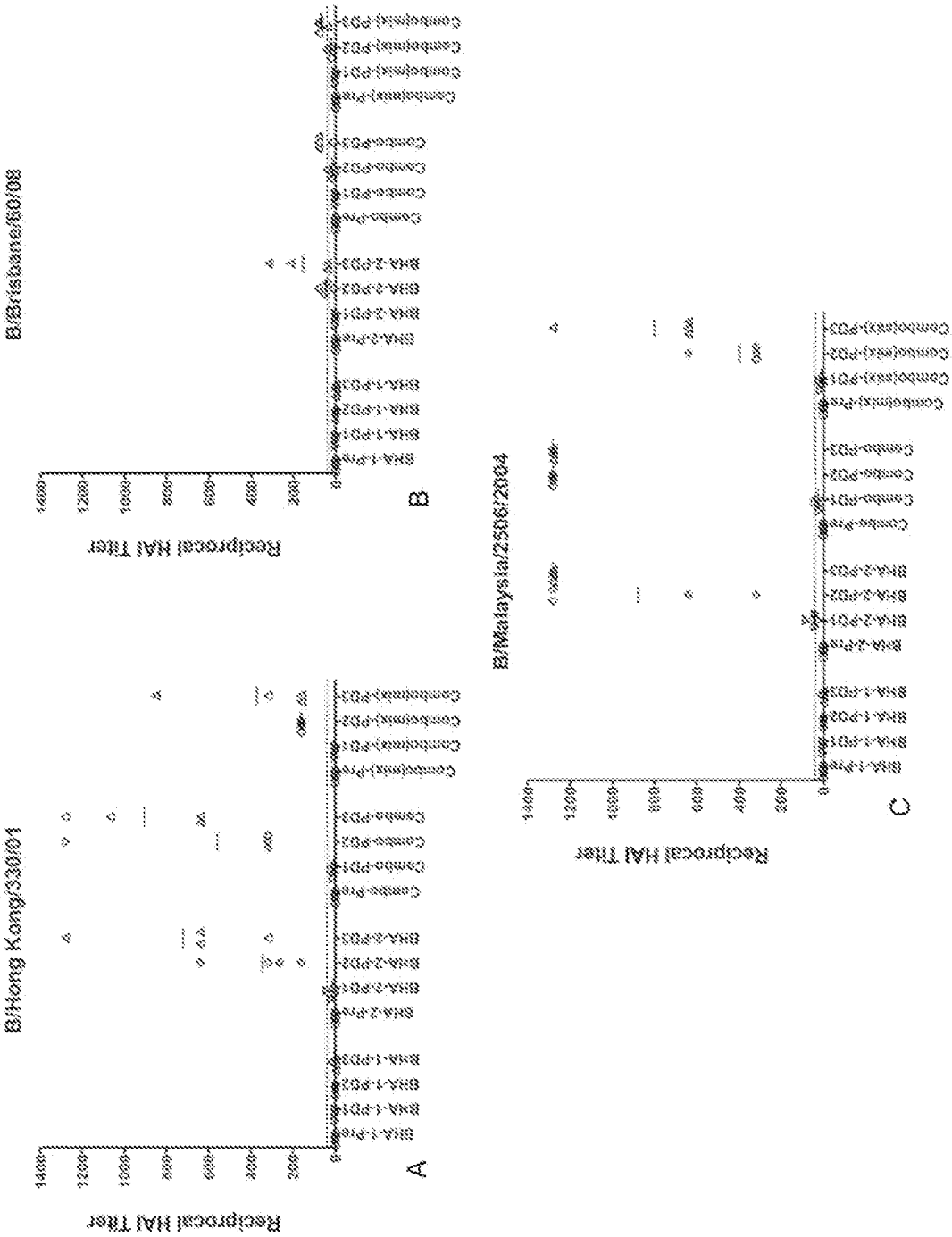
Mouse HAIs
FIGURES 14A-C



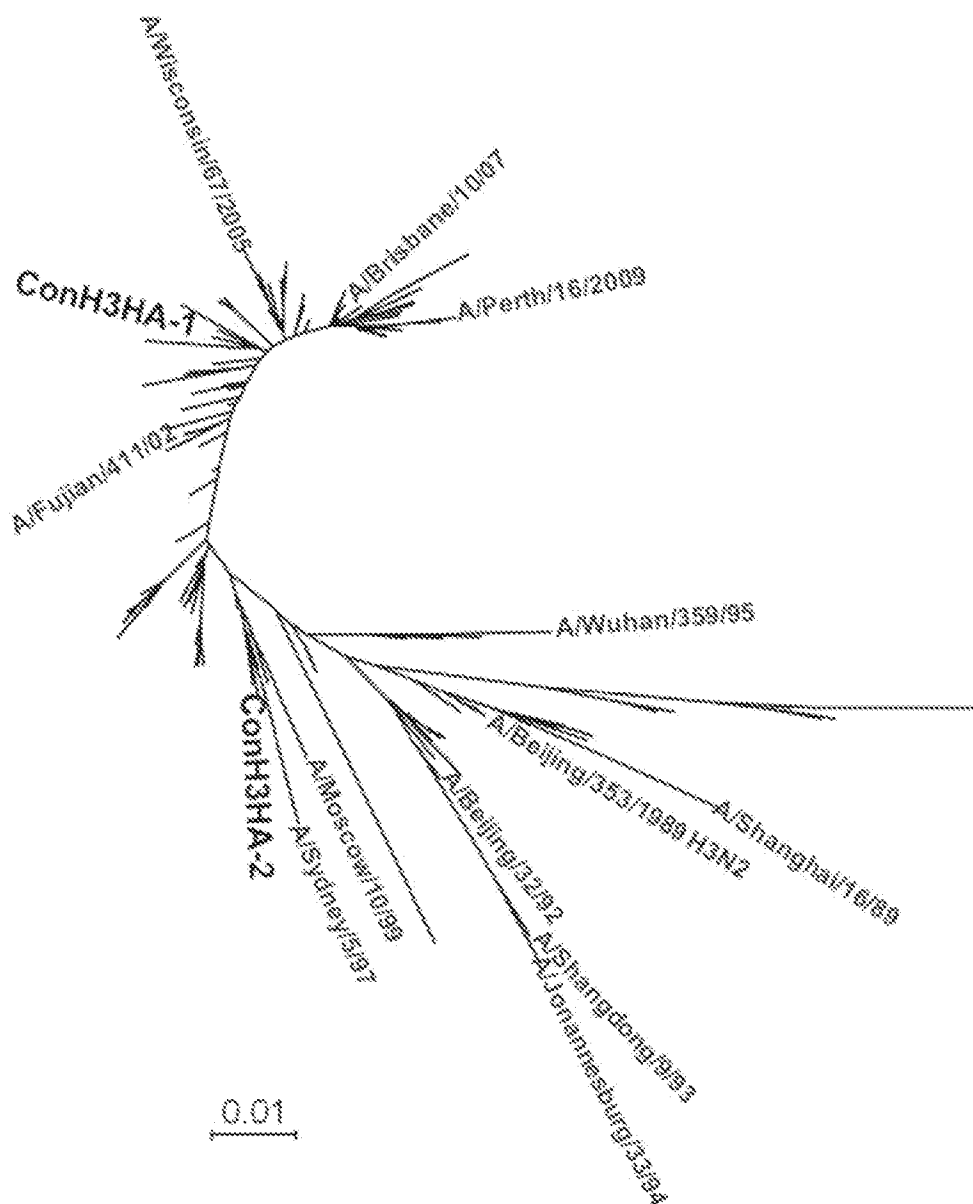
GUINEA PIG DATA
FIGURES 15A-D



GUINEA PIG DATA
FIGURES 16A-C

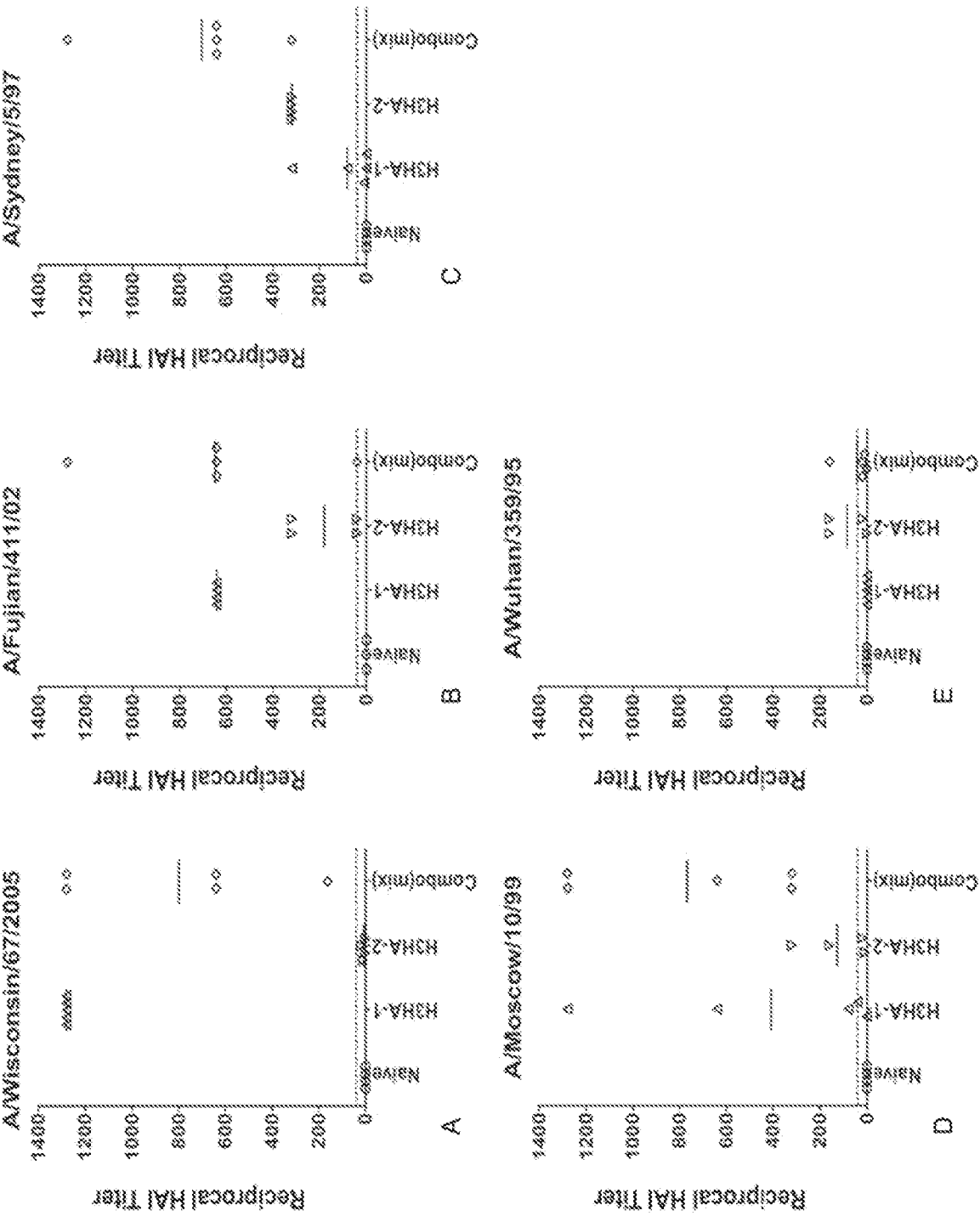


GUINEA PIG DATA
FIGURES 17A-C



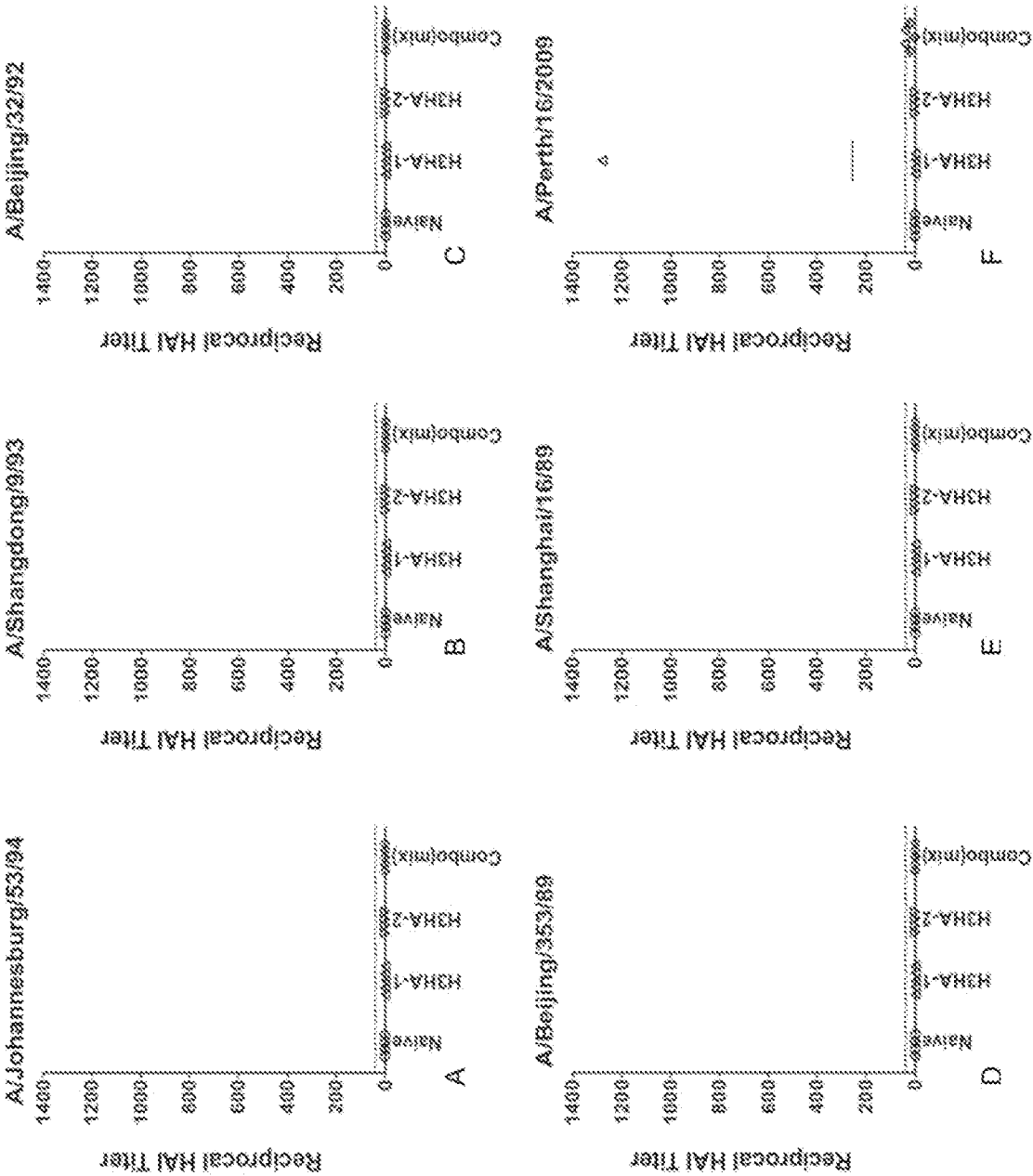
Flu H3 tree of all viruses from last 20 years and where the constructs are located

FIGURE 18



Mouse HAIs

FIGURES 19A-E



Mouse HAIs
FIGURES 20A-F

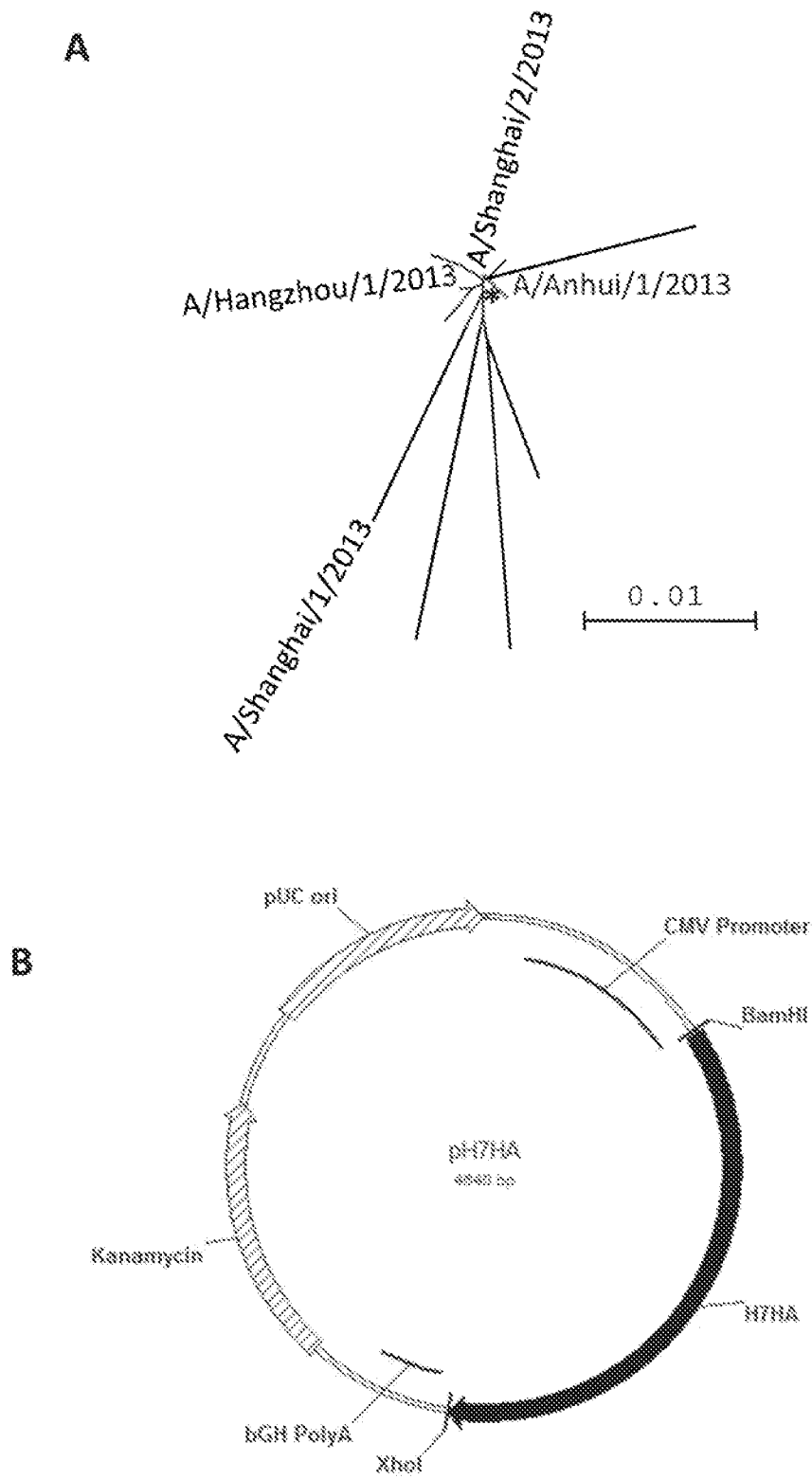


Figure 21

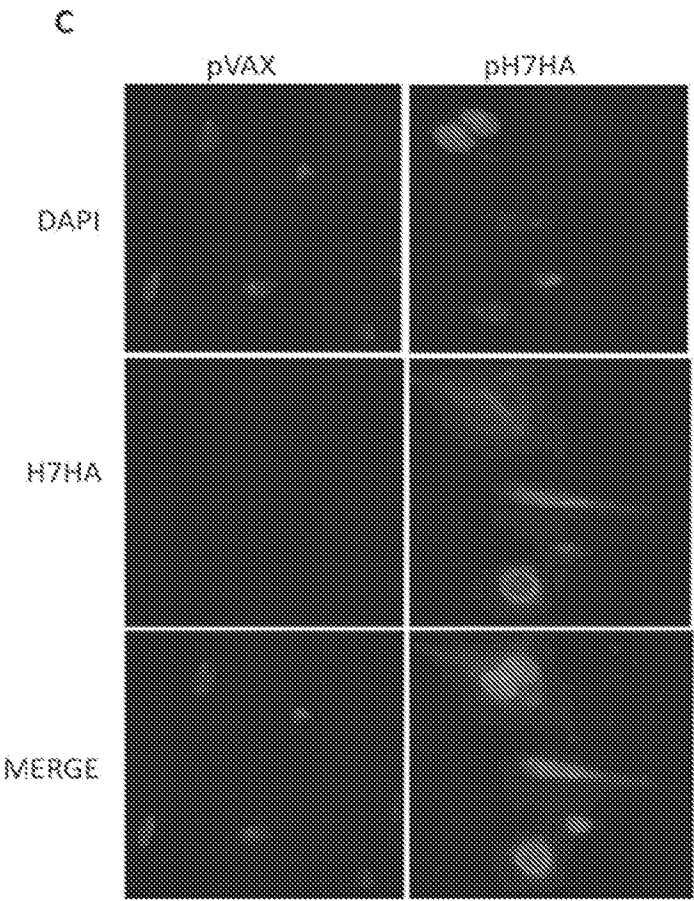


Figure 21 (continued)

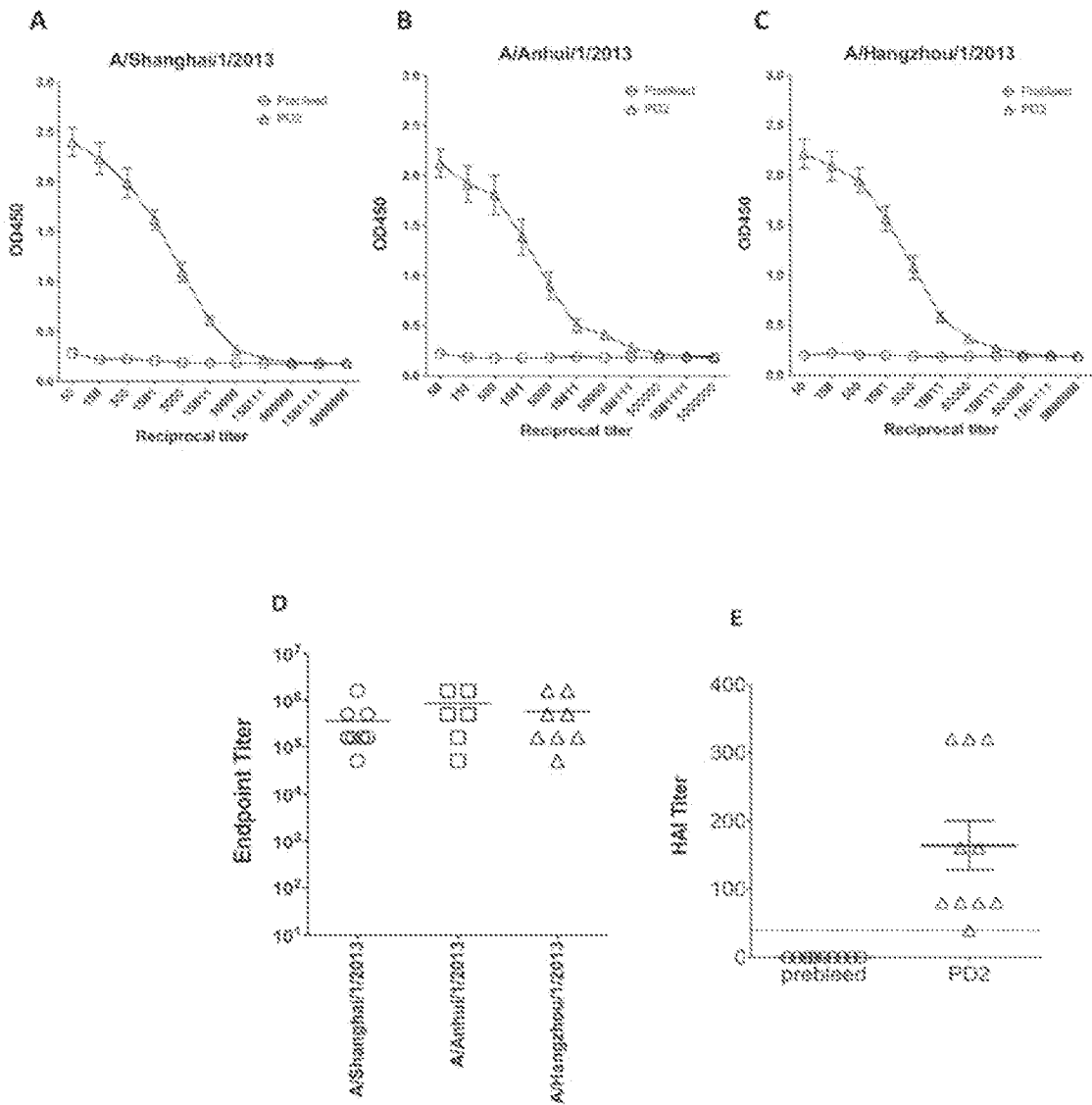


Figure 22

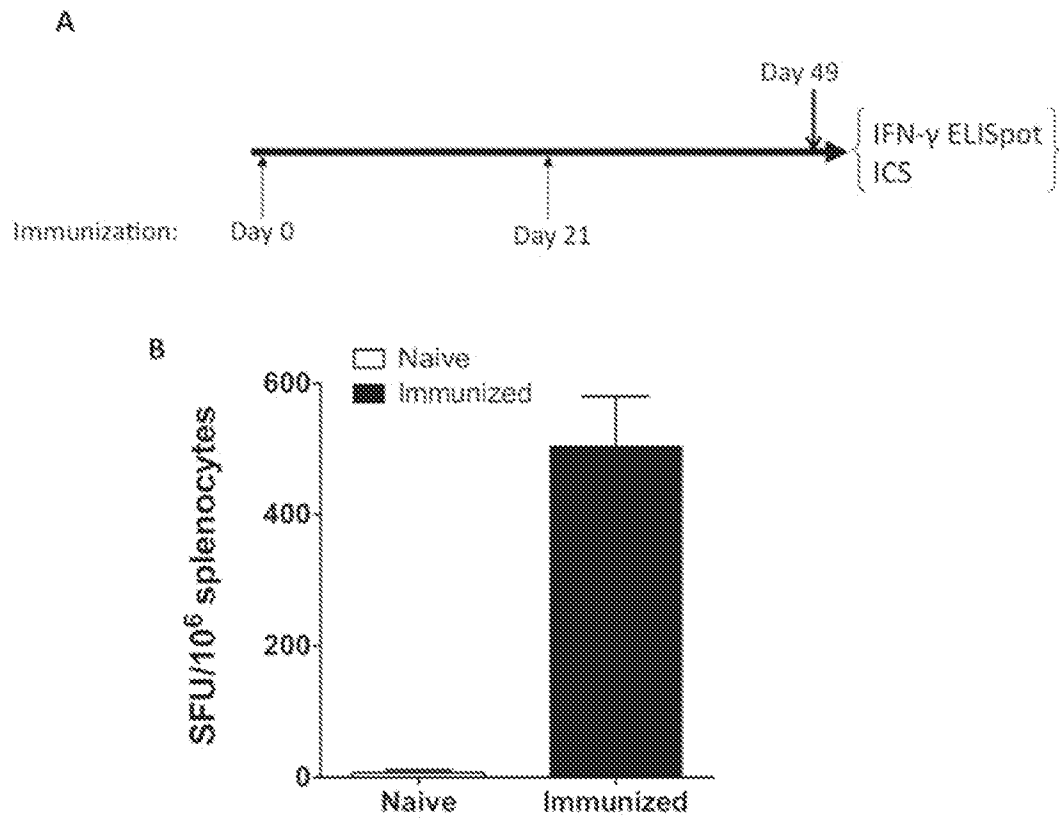


Figure 23

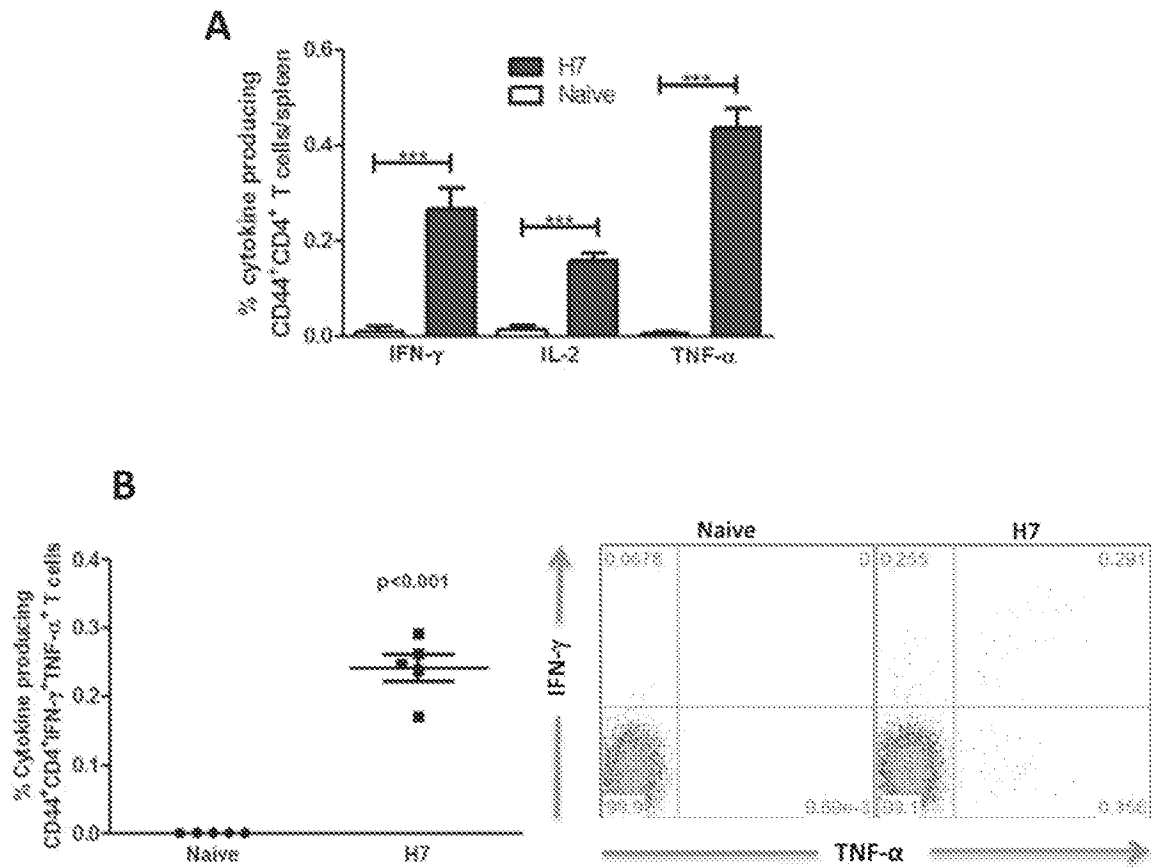


Figure 24

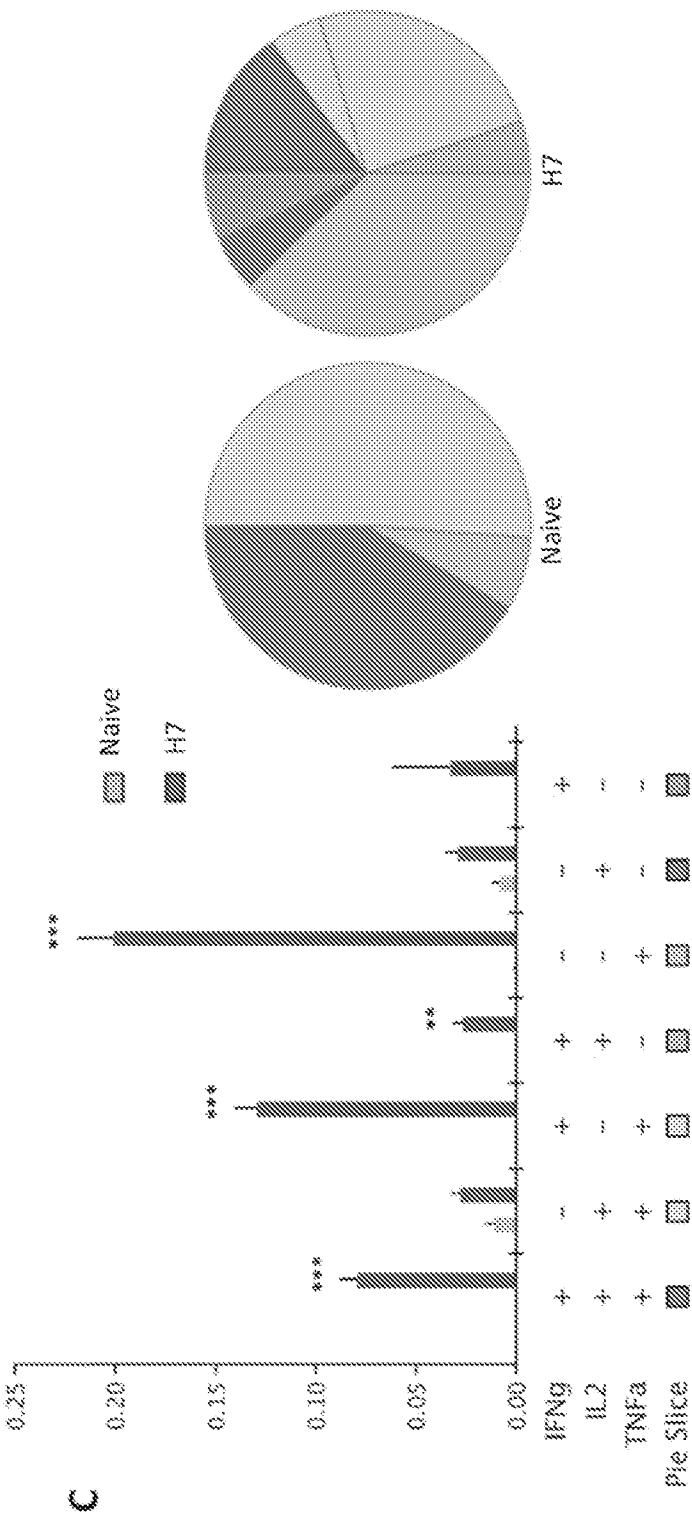


Figure 24 (continued)

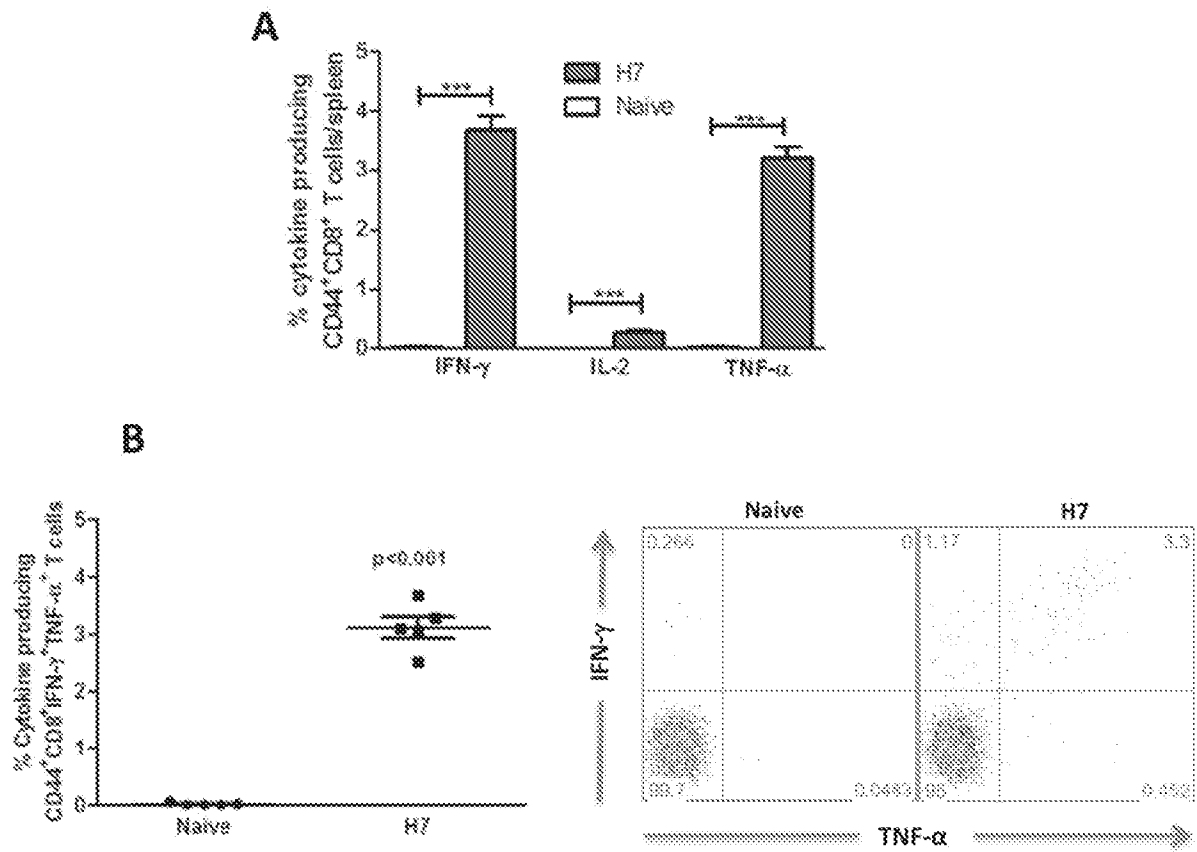


Figure 25

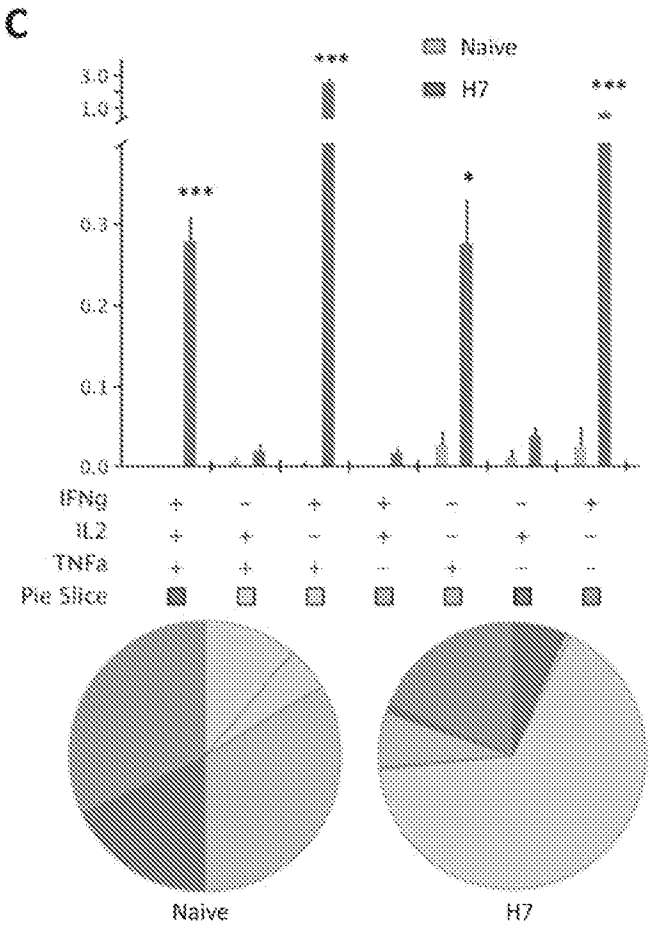


Figure 25 (continued)

D

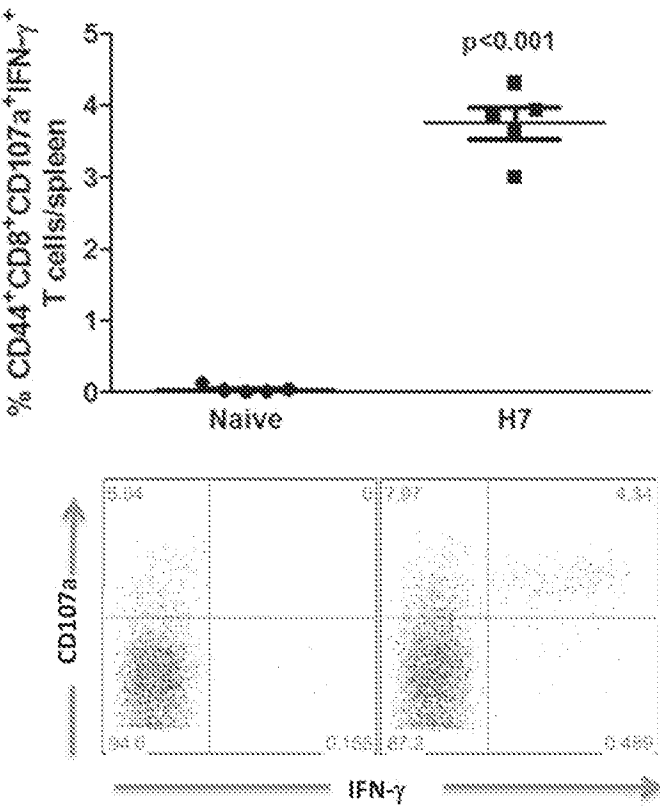


Figure 25 (continued)

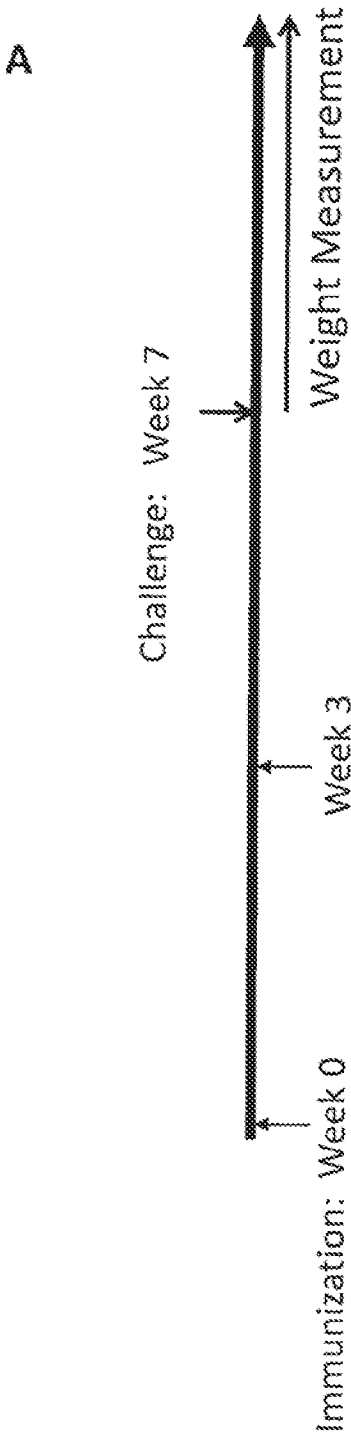


Figure 26

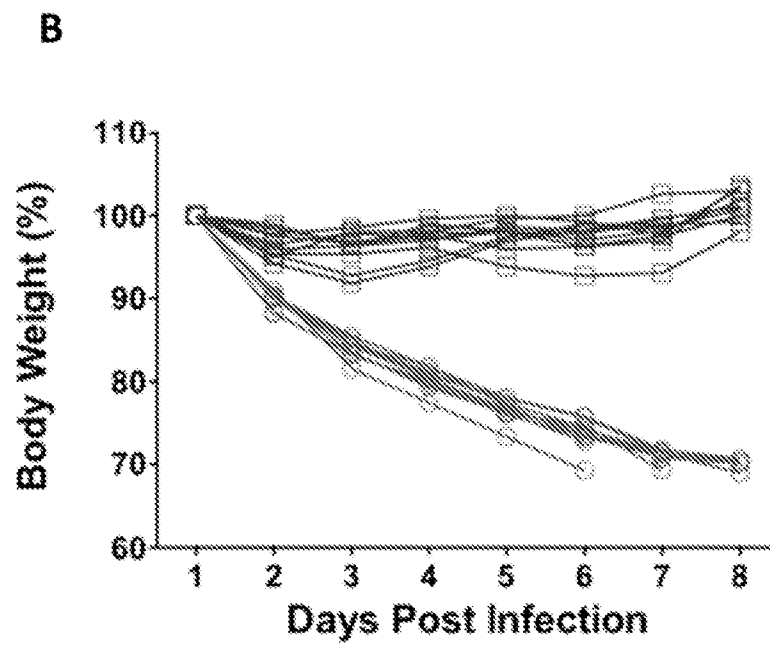


Figure 26 (continued)

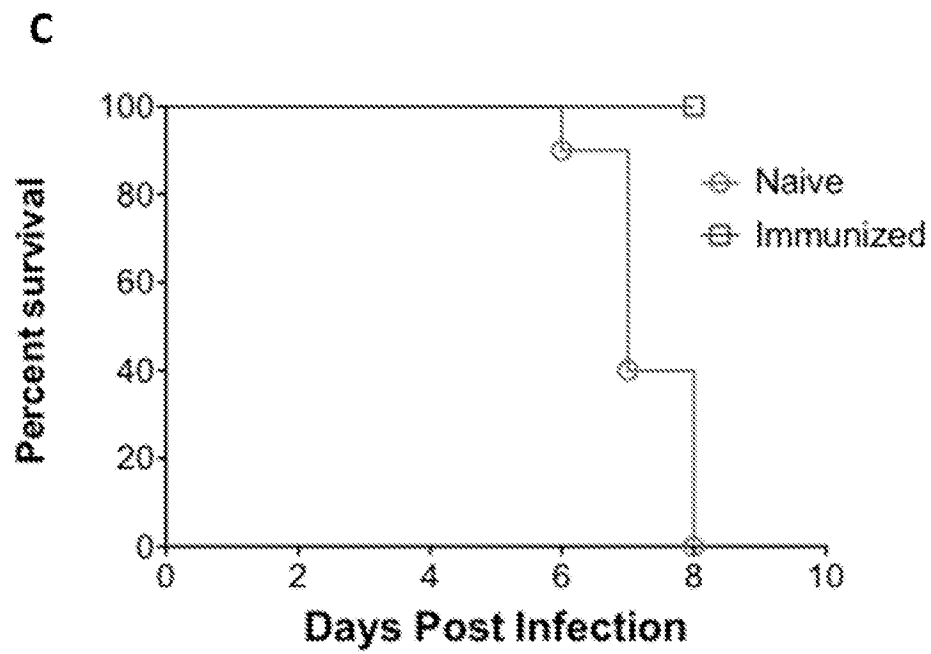


Figure 26 (continued)