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(54) **Titre : VACCINS CONTRE LA GRIPPE**
(54) **Title: INFLUENZA VACCINES**

(57) **Abrégé/Abstract:**

Polypeptides, nucleic acid molecules, vectors, cells, fusion proteins, pharmaceutical compositions, combined preparations, and their use as vaccines against influenza are described. The polypeptides comprise a haemagglutinin subtype 5 (H5) globular head domain, and optionally a haemagglutinin stem domain, wherein the polypeptide comprises an amino acid sequence in which an amino acid residue at a position corresponding to residue position 144 or 145 of a wild-type H5 globular head domain has been deleted, or wherein the polypeptide comprises an amino acid sequence with an E amino acid residue at a position corresponding to residue position 238 of a wild-type H5 globular head domain.



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(54) Title: INFLUENZA VACCINES

(57) Abstract: Polypeptides, nucleic acid molecules, vectors, cells, fusion proteins, pharmaceutical compositions, combined preparations, and their use as vaccines against influenza are described. The polypeptides comprise a haemagglutinin subtype 5 (H5) globular head domain, and optionally a haemagglutinin stem domain, wherein the polypeptide comprises an amino acid sequence in which an amino acid residue at a position corresponding to residue position 144 or 145 of a wild-type H5 globular head domain has been deleted, or wherein the polypeptide comprises an amino acid sequence with an E amino acid residue at a position corresponding to residue position 238 of a wild-type H5 globular head domain.



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Influenza Vaccines

This invention relates to nucleic acid molecules, polypeptides, vectors, cells, fusion proteins, pharmaceutical compositions, combined preparations, and their use as vaccines against influenza.

5 Influenza is a highly contagious respiratory illness caused by the influenza virus infecting the epithelial cells within the upper respiratory tract. The infection is characterised by a sudden onset of high fever, headache, muscle ache and fatigue, sore throat, cough and rhinitis. For the majority of cases, influenza rarely lasts for over a week and is usually restricted to the upper respiratory tract. However, in medically vulnerable people, such as people over 65
10 years old and people with certain chronic medical conditions, influenza can cause complications and even result in death. There are around 9 million-45 million human infections. WHO estimates that seasonal influenza may result in 290 000-650 000 deaths each year due to respiratory diseases alone. Thus, the development of an effective flu vaccine is critical to the health of millions of people around the world.

15 The fundamental principal of a vaccine is to prepare the immune system for an encounter with a pathogen. A vaccine triggers the immune system to produce antibodies and T-cell responses, which helps to combat infection. Historically, once a pathogen was isolated and grown, it was either mass produced and killed or attenuated, and used as a vaccine. Later recombinant genes from isolated pathogens were used to generate recombinant proteins
20 that were mixed with adjuvants to stimulate immune responses. More recently the pathogen genes were cloned into vector systems (attenuated bacteria or viral delivery systems) to express and deliver the antigen *in vivo*. All of these strategies are dependent on pathogens isolated from past outbreaks to prevent future ones. For pathogens which do not change significantly, or slowly, this conventional technology is effective. However, some pathogens,
25 are prone to accelerated mutation rate and previously generated antibodies do not always recognise evolved strains of the same pathogen. New emerging and re-emerging pathogens often hide or disguise their vulnerable antigens from the immune system to escape the immune response.

Influenza is one of the best characterised re-emerging pathogens, and re-emerges each
30 season infecting up to 100 million people worldwide. Influenza is a member of the *Orthomyxoviridae* family and has a single-stranded negative sense RNA genome. RNA viruses generally have very high mutation rates compared to DNA viruses, because viral RNA polymerases lack the proofreading ability of DNA polymerases. This contributes towards antigenic drift, a continuous process of the accumulation of mutations in the genome
35 of an infectious agent resulting in minor changes in antigens presented to the immune system

of the host organism. Changes to antigenic regions of the proteins on the influenza virion result in its evasion of the host immune system and potentially increased pathogenicity and infectiousness. This is one reason why it is difficult to make effective vaccines to prevent influenza. Influenza can undergo antigenic shift, a process wherein there is a dramatic change in the antigens presented on the influenza virus. Gene segments from different subtypes of influenza can reassort and package into a new virion particle containing the genetic information from both of the subtypes. This can result in a virus that has antigenic characteristics not before seen in a human setting, to which we are naïve immunologically. The new quasispecies of the virus can cause a pandemic if no neutralising, or inhibitory antibodies to the new influenza virus are present in the human population.

There are multiple types of influenza viruses, the most common in humans being influenza A, influenza B, and influenza C. Influenza A viruses infect a wide variety of birds and mammals, including humans, horses, marine mammals, pigs, ferrets, and chickens. In their natural reservoirs in aquatic birds and bats, influenza A viruses show minimal evolution and cause unapparent disease; but once they transfer to a different species, influenza A viruses can evolve rapidly as they adapt to the new host, possibly causing pandemics or epidemics of acute respiratory disease in domestic poultry, lower animals and humans. In animals, most influenza A viruses cause mild localized infections of the respiratory and intestinal tract. However, highly pathogenic influenza A strains, such as some within the H5N1 subtype, can cause systemic infections in poultry with spill-over human cases, which can have high mortality rates. Influenza B and C are restricted to infecting humans, with no known animal reservoirs. Influenza B causes epidemic seasonal infections, with similar pathogenicity as influenza A. Influenza C viruses are usually associated with very mild or asymptomatic infections in humans.

At just over 100 years since the devastating 1918 influenza pandemic, there is still no optimal preventative or treatment against influenza A and B. Although they share some degree of similarity with antigen presentation on their surface, the highly heterologous nature of these antigens presents significant challenges in developing vaccines and treatments. During the 2019-2020 seasonal flu epidemic, quadrivalent vaccines were widely distributed. These gave protection against two influenza A viruses and two influenza B viruses. However, to prevent a potential outbreak of influenza in which the virus has rapidly evolved and hence unrecognisable by the host immune system, it is crucial that an influenza vaccine protects against many if not all potential influenza strains.

Influenza A has an outer envelope that is studded with three integral membrane proteins: hemagglutinin (HA); neuraminidase (NA); and matrix ion channel (M2), which overlay a

matrix protein (M1). The organisation of influenza B is similar, with HA and NA scattered across the lipid envelope, but with NB and BM2 transmembrane ion channels instead of M2.

Influenza A viruses are subtyped based on their combination of surface glycoproteins (GPs) namely HA and NA. Influenza B viruses, having much less antigenic variation than influenza A, are not. HA and NA are membrane bound envelope GPs, responsible for virus attachment, penetration of the viral particles into the cell, and release of the viral particle from the cell. They are the sources of the major immunodominant epitopes for virus neutralisation and protective immunity. Hence, both HA and NA proteins are considered the most important components for prophylactic influenza vaccines. During HA-mediated entry, binding of the GP to sialic acid-containing receptors on the host cell membrane initiates endocytosis of the virion into the cell. The low pH within the endosome induces a conformational change in HA to expose a hydrophobic region, termed the fusion peptide. The newly exposed fusion peptide then inserts into the endosomal membrane, thereby bringing the viral and endosomal membranes in close contact to allow membrane fusion and entry of the virus into the cytoplasm. This release into the cytoplasm allows viral proteins and RNA molecules to enter the nucleus for viral transcription and subsequent replication. Transcribed, positive sense mRNAs are exported from the nucleus to be translated into viral proteins, and replicated negative sense RNA is exported from the nucleus to re-assemble with the newly synthesised viral proteins to form a progeny virus particle. The virus buds from the apical cell membrane, taking with it host membrane to form a virion capable of infecting another cell.

HA exists as a homo-trimer on the virus surface, forming a cylinder-shaped molecule which projects externally from the virion and forms a type I transmembrane glycoprotein. Each monomer of the HA molecule consists of a single HA0 polypeptide chain with HA1 and HA2 regions linked by two disulphide bridges. Each HA0 polypeptide forms a globular head domain and a stem domain. The globular head domain comprises the most dominant epitopes, while the stem domain has less dominant, but important epitopes for broader antibody recognition. The amino acid sequence of these epitopes determines the binding affinity and specificity towards antibodies. The globular head domain consists of a part of HA1, including a receptor binding domain and an esterase domain, whereas the stem domain consists of parts of HA1 and HA2. Amino acid residues of HA1 that form the globular head domain fold into a motif of eight stranded antiparallel β -sheets which sits in a shallow pocket at the distal tip acting as the receptor binding site which is surrounded by antigenic sites. The remaining parts of the HA1 domain run down to the stem domain mainly comprising β -sheets. HA2 forms the majority of the stem domain and is folded into a helical coiled-coil structure forming the stem backbone. HA2 also contains the hydrophobic region required for

membrane fusion, and a long helical chain anchored to the surface membrane and a short cytosolic tail.

There are 18 different HA subtypes and 11 different NA subtypes within influenza A. Theoretically, there are potentially 198 different influenza A subtype combinations, some of which may be virulent in humans and other animals. As a result, there is significant concern that viruses from these subtypes could reassort with human transmissible viruses and initiate the next pandemic. In recent years, avian viruses of the H5, H7, H9, and H10 subtypes have caused zoonotic infections with H5 and H7 viruses often causing severe disease. The highly pathogenic Asian influenza (HPAI) outbreak of H5N1 of 1997 resulted in the killing of the entire domestic poultry population within Hong Kong. This panzootic also resulted in 860 confirmed infections and 454 fatalities in humans, demonstrating the ability of the avian-derived virus to transmit to humans and result in a high mortality rate. This HPAI of the H5N1 subtype frequently re-emerges and is of particular concern because of its 60% mortality rate, and because it continues to evolve and diversify. The last influenza pandemic, in 2009, was caused by a novel H1N1 influenza A virus, generated by circulating human influenza reassorting with human, porcine, and avian influenza. The virus was very different from H1N1 viruses that were circulating at the time of the pandemic. As a result, very few young people had any existing immunity to the virus, and around a third of people over the age of 60 had antibodies against the virus from past exposure of similar H1N1 viruses. The CDC (Centre for Disease Control and Prevention) estimate that the total number of deaths worldwide caused by the 2009 outbreak is ranged between 150,000 to 575,400. Influenza A is constantly evolving in multiple species and to prepare for this, virus characterization and translation into effective vaccines must be done in a timely manner.

Although they have less antigenic variation than influenza A viruses, influenza B viruses have recently emerged into two antigenically distinct lineages (B/Victoria/2/1987-like and B/Yamagata/16/1988-like), illustrating the fluidity with which influenza B can evolve, and how it is also now imperative to include viruses of both type A and B in seasonal flu vaccinations.

There is a need to provide improved influenza vaccines that protect against far more influenza strains than current vaccines. In particular, there is a need to provide vaccines against influenza A and B viruses that protect against several influenza A and B variants. In particular, there is a need to provide improved vaccines that elicit more broadly neutralising immune responses to influenza A H5 viruses. There is also a need to provide neutralising antibody protection against the H1N1 subtype of influenza A.

In particular, new vaccine strategies are needed to 1) successfully combat vaccine escape, and, 2) prevent the emergence and spread of new influenza pathogens in the human population. Envisioned herein is the use of large databases of different influenza virus sequences from not only humans, but also animals which are the source of new influenza virus re-assortments which give rise to new human pathogens.

Influenza A H5 viruses

While most viral genes have been replaced through reassortment yielding many different genotypes, the specific H5 gene has remained present in all influenza A isolates identified since its discovery in 1996. Thus, H5 provides a constant to which the evolving strains of influenza A may be effectively compared. A clade nomenclature system for H5 HA was developed to compare the evolutionary pattern of this gene. Circulating H5N1 viruses are grouped into numerous virus clades based on the characterisation and sequence homology of the HA gene. Clades will have a single common ancestor from which particular genetic changes have arisen. As the viruses within these clades continue to evolve, sub-lineages periodically emerge. Vaccines against influenza A H5 exist, however either these vaccines are unable to induce a neutralising immune response against the important H5 clades, or the affinity of the antigen to its neutralising antibody is sub-optimal. The computationally optimised broadly reactive antigen (COBRA) Tier 2 vaccine design (Nunez *et al*, *Vaccines*, 2020, **38**(4):830-839) is developed by consensus sequence alignment techniques using full-length sequences from H5N1 clade 2 infections isolated from both humans and birds. However, this design did not produce haemagglutinin inhibition (HAI) antibodies or protection against newer reassorted viruses across all H5N1 clades and sub-clades that were tested against the vaccine. The risk of human infection with avian influenza A(H5Nx), particularly from clade 2.3.4.4, is on the rise due to increasing human and avian contact and poor biosafety practices.

There is also a need, therefore, to provide improved vaccines that elicit more broadly neutralising immune responses to influenza A H5 viruses. In particular, there is a need to provide vaccines that elicit antibody responses that effectively neutralise influenza A clade 2.3.4.4.

The Applicant has identified amino acid sequences and their encoding nucleic acid molecules that induce a broadly neutralising immune response against important H5 clades of influenza A, including clade 2.3.4.4. The Applicant has further identified amino acid sequences and their encoding nucleic acid molecules responsible for stabilising the stem region of the H5 molecule both in the pre-fusion and post-fusion state.

H5 embodiments of the invention are described below.

According to the invention there is provided an isolated polypeptide comprising a haemagglutinin subtype 5 (H5) globular head domain, and optionally a haemagglutinin stem domain, with the following amino acid residues at positions 156, 157, 171, 172, and 205 of the head domain:

- 156: R;
- 157: P or S, preferably P;
- 171: D or N;
- 10 • 172: T or A, preferably T; and
- 205: K or R, preferably K

The applicant has found that such polypeptides elicit broadly neutralising antibody responses to a diverse panel of H5 influenza viruses, including viruses of several different clades.

- 15 Optionally a polypeptide of the invention comprises an amino acid sequence that has at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% amino acid identity along its entire length with the amino acid sequence of SEQ ID NO:7, 8, 10, 11, 1, or 3.

- 20 Optionally a polypeptide of the invention comprises the following amino acid residues at positions 156, 157, 171, 172, and 205 of the head domain:

- 156: R;
- 157: P;
- 171: D;
- 25 • 172: T; and
- 205: K

- 30 Optionally a polypeptide of the invention comprises an amino acid sequence of SEQ ID NO:7 or 8, or an amino acid sequence that has at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% amino acid identity along its entire length with the amino acid sequence of SEQ ID NO:7 or 8 and which has the following

amino acid residues at positions corresponding to positions 156, 157, 171, 172, and 205 of SEQ ID NO:7 or 8:

- 5
- 156: R;
 - 157: P;
 - 171: D;
 - 172: T; and
 - 205: K

Optionally a polypeptide of the invention comprises an amino acid sequence of SEQ ID NO:7 (FLU_T3_HA_1) (see Example 4 below).

- 10 Such polypeptides are particularly advantageous as they elicit broadly neutralising antibody responses to a diverse panel of H5 influenza viruses, including H5 influenza viruses of clades 2.3.4 and 7.1 arising from the Goose Guangdong (A/Goose/Guangdong/1/1996, GS/GD) lineage, which are currently in circulation in birds and humans.

- 15 Optionally a polypeptide of the invention comprises the following amino acid residues at positions 156, 157, 171, 172, and 205 of the head domain:

- 20
- 156: R;
 - 157: P;
 - 171: N;
 - 172: T; and
 - 205: K

- 25 Optionally a polypeptide of the invention comprises an amino acid sequence of SEQ ID NO:10 or 11, or an amino acid sequence that has at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% amino acid identity along its entire length with the amino acid sequence of SEQ ID NO:10 or 11 and which has the following amino acid residues at positions corresponding to positions 156, 157, 171, 172, and 205 of SEQ ID NO:10 or 11:

- 30
- 156: R;
 - 157: P;
 - 171: N;
 - 172: T; and
 - 205: K

Optionally a polypeptide of the invention comprises an amino acid sequence of SEQ ID NO:10 (FLU_T3_HA_2) (see Example 5 below).

Such polypeptides are particularly advantageous as they elicit broadly neutralising antibody responses to a diverse panel of H5 influenza viruses, including H5 influenza viruses of GS/GD clades 2.3.4 and 7.1, which are currently in circulation in birds.

Optionally a polypeptide of the invention comprises the following amino acid residues at positions 156, 157, 171, 172, and 205 of the head domain:

- 156: R;
- 157: S;
- 10 • 171: N;
- 172: A; and
- 205: R

Optionally a polypeptide of the invention comprises an amino acid sequence of SEQ ID NO:1 or 3, or an amino acid sequence that has at least 70%, 71%, 72%, 73%, 74%, 75%, 15 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% amino acid identity along its entire length with the amino acid sequence of SEQ ID NO:1 or 3 and which has the following amino acid residues at positions corresponding to positions 156, 157, 171, 172, and 205 of SEQ ID NO:1 or 3:

- 20 • 156: R;
- 157: S;
- 171: N;
- 172: A; and
- 205: R

25 Optionally a polypeptide of the invention comprises an amino acid sequence of SEQ ID NO:1 (FLU_T2_HA_1) (see Example 1 below).

Such polypeptides are particularly advantageous as they elicit broadly neutralising antibody responses to a diverse panel of H5 influenza viruses, including viruses of several different GS/GD clades.

30 Table 1 below summarises differences in amino acid sequence at positions A-E of the influenza haemagglutinin H5 for different embodiments of the invention, and differences at those positions compared with prior art COBRA sequences.

Table 1

Region	A		B		C	D	E	Example/ Figure
H5 Residue	156	157	171	172	205	416	434	
FLU_T2_HA_1	R	S	N	A	R	F	F	Ex1/Fig.2
FLU_T3_HA_1	R	P	D	T	K	F	F	Ex4/Fig.2
FLU_T3_HA_2	R	P	N	T	K	F	F	Ex5/Fig.2
COBRA (Human/Avian)	K	S	S	A	R	E	E	Fig.3
COBRA (Human)	S	P	N	T	R	E	E	Fig.3

The Applicant has also designed additional amino acid sequences and their encoding nucleic acid molecules that induce a broadly neutralising immune response against important H5 clades of influenza A. These polypeptides are referred to herein as

- 5 FLU_T3_HA_3, FLU_T3_HA_4, and FLU_T3_HA_5. Such polypeptides are particularly advantageous as they elicit broadly neutralising antibody responses to a diverse panel of H5 influenza viruses, as demonstrated by the results described Example 24, and Figure 23.

Figure 22 shows the amino acid sequences of FLU_T3_HA_3 (SEQ ID NO:27), FLU_T3_HA_4 (SEQ ID NO:35), and FLU_T3_HA_5 (SEQ ID NO:43) in alignment with the
 10 amino acid sequences of FLU_T2_HA_1 (also referred to as FLU_T2_HA_9), FLU_T3_HA_1, and FLU_T3_HA_2, and with the HA amino acid sequence of influenza A H5N1 strains A/whooper swan/Mongolia/244/2005 (H5_WSN) (SEQ ID NO:64), and A/gyrfalcon/Washington/41088-6/2014 (H5_GYR) (SEQ ID NO:65).

Figure 21 summarises differences in amino acid sequence at positions A-E of the influenza
 15 haemagglutinin H5 for: FLU_T2_HA_1 (also known as FLU_T2_HA_9), FLU_T3_HA_1, FLU_T3_HA_2, FLU_T3_HA_3, FLU_T3_HA_4, FLU_T3_HA_5).

According to the invention there is also provided an isolated polypeptide comprising a haemagglutinin subtype 5 (H5) globular head domain, and optionally a haemagglutinin stem domain, wherein the polypeptide comprises an amino acid sequence in which an
 20 amino acid residue at a position corresponding to residue position 144 or 145 of a wild-type H5 globular head domain has been deleted.

Optionally the polypeptide comprises an amino acid sequence in which an amino acid residue at a position corresponding to residue position 144 of the wild-type H5 globular head domain has been deleted.

Optionally the polypeptide comprises an amino acid sequence in which an amino acid residue at a position corresponding to residue position 145 of the wild-type H5 globular head domain has been deleted.

Optionally a polypeptide of the invention in which an amino acid residue at a position corresponding to residue position 144 or 145 of a wild-type H5 globular head domain has been deleted comprises an amino acid sequence that has at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% amino acid identity along its entire length with the amino acid sequence of SEQ ID NO:3.

- 10 Optionally an isolated polypeptide of the invention which comprises an H5 globular head domain retains at least some HA activity of a wild-type H5 globular head domain (for example, of an H5 WSN isolate - SEQ ID NO:64).

HA activity can be determined, for example, by blood agglutination assays, or by binding assays with sialic acid (SA). Suitable blood agglutination assays are referred to in Ustinov *et al.* (Biochemistry (Moscow), 2017, Vol. 82, No. 11, pp. 1234-1248: *The Power and Limitations of Influenza Virus Hemagglutinin Assays*). A suitable binding assay is described by Takemoto *et al.* (VIROLOGY 217, 452 – 458 (1996): *A Surface Plasmon Resonance Assay for the Binding of Influenza Virus*).

Influenza virions can agglutinate erythrocytes with the formation of a viscous gel. The agglutination occurs through the binding of virion-embedded HA to sialylated surface proteins of several erythrocytes at once. The number of agglutinated erythrocytes is proportional to the HA content and can be used for estimating the functional activity of the protein itself. The classical procedure uses 0.5-1.0% suspension of erythrocytes mixed and incubated with the virus suspension, with negative control containing erythrocytes only, and positive control containing erythrocytes and virions (Salk, J. E. (1944) A simplified procedure for titrating hemagglutinating capacity of influenza virus and the corresponding antibody, J. Immunol., 49, 87-98).

A hemagglutination test can be performed not only for influenza virions, but for isolated HA molecules as well if these molecules are in the form of trimers to provide the formation of a multiple-contact network. Thus, the HA ectodomain that exists in solely monomeric form does not agglutinate erythrocytes, while oligomerization-prone HA1 (a.a. 1-330) does (Khurana, *et al.*, (2010) *Properly folded bacterially expressed H1N1 hemagglutinin globular head and ectodomain vaccines protect ferrets against H1N1 pandemic influenza virus*, PLoS One, 5, e11548.). Removal of the HA1 N-terminal fragment (a.a. 1-8) that contains

the oligomerization signal Ile–Cys–Ile results in complete loss of the HA1 activity, while removal of the C-terminal portion (a.a. 321-330), on the contrary, stabilizes the trimer and facilitates hemagglutination. The larger HA1 fragment (a.a. 1-104) is also capable of oligomerization but does not agglutinate erythrocytes because of the absence of the SA binding site.

Optionally an isolated polypeptide of the invention which comprises an H5 globular head domain retains at least 25%, at least 50%, or at least 75% of HA activity of a wild-type H5 globular head domain (for example, of an H5 WSN isolate - SEQ ID NO:64).

Optionally an isolated polypeptide of the invention in which an amino acid residue at a position corresponding to residue position 144 or 145 of a wild-type H5 globular head domain has been deleted comprises an amino acid sequence with the following amino acid residues at positions corresponding to residues 156, 157, 171, 172, and 205 of the wild-type H5 globular head domain:

- 156: R;
- 157: S;
- 171: N;
- 172: A; and
- 205: R

Optionally an isolated polypeptide of the invention in which an amino acid residue at a position corresponding to residue position 144 or 145 of a wild-type H5 globular head domain has been deleted comprises an amino acid sequence of SEQ ID NO:27 or 29, or an amino acid sequence that has at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% amino acid identity along its entire length with the amino acid sequence of SEQ ID NO:27 or 29.

Optionally an isolated polypeptide of the invention in which an amino acid residue at a position corresponding to residue position 144 or 145 of a wild-type H5 globular head domain has been deleted comprises an amino acid sequence of SEQ ID NO:29.

Optionally an isolated polypeptide of the invention in which an amino acid residue at a position corresponding to residue position 144 or 145 of a wild-type H5 globular head domain has been deleted comprises an amino acid sequence of SEQ ID NO:27.

Optionally an isolated polypeptide of the invention in which an amino acid residue at a position corresponding to residue position 144 or 145 of a wild-type H5 globular head

domain has been deleted comprises a haemagglutinin stem domain, and wherein the polypeptide comprises an amino acid sequence with the following amino acid residues at positions corresponding to residue positions 416 and 434 of a wild-type H5 sequence:

- 416: F; and
- 5 • 434: F

Optionally the polypeptide comprises an amino acid sequence that has at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% amino acid identity along its entire length with the sequence of SEQ ID NO:27.

- 10 According to the invention there is also provided an isolated polypeptide comprising a haemagglutinin subtype 5 (H5) globular head domain, and optionally a haemagglutinin stem domain, wherein the polypeptide comprises an amino acid sequence with the following amino acid residues at positions corresponding to residue positions 148 and 149 of a wild-type H5 globular head domain:

- 15 • 148: V;
- 149: P.

Optionally a polypeptide of the invention which comprises an amino acid sequence with V at a position corresponding to residue position 148, and P at a position 149 corresponding to residue position 149, comprises an amino acid sequence with the following amino acid residue at a position corresponding to residue position 238 of a wild-type H5 globular head domain:

- 20 • 238: E.

- 25 Optionally a polypeptide of the invention which comprises an amino acid sequence with V at a position corresponding to residue position 148, and P at a position 149 corresponding to residue position 149, comprises an amino acid sequence that has at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% amino acid identity along its entire length with the amino acid sequence of SEQ ID NO:3.

- 30 Optionally an isolated polypeptide of the invention which comprises an H5 globular head domain retains at least some HA activity of a wild-type H5 globular head domain (for example, of an H5 WSN isolate - SEQ ID NO:64).

Optionally an isolated polypeptide of the invention which comprises an H5 globular head domain retains at least 25%, at least 50%, or at least 75% of HA activity of a wild-type H5 globular head domain (for example, of an H5 WSN isolate - SEQ ID NO:64).

5 Optionally a polypeptide of the invention which comprises an amino acid sequence with V at a position corresponding to residue position 148, and P at a position 149 corresponding to residue position 149, has reduced affinity for its receptor compared with the wild-type H5 globular head domain.

10 Optionally a polypeptide of the invention which comprises an amino acid sequence with V at a position corresponding to residue position 148, and P at a position 149 corresponding to residue position 149, comprises an amino acid sequence with the following amino acid residues at positions corresponding to residues 156, 157, 171, 172, and 205 of the wild-type H5 globular head domain:

- 156: R;
- 157: S;
- 15 • 171: N;
- 172: A; and
- 205: R.

20 Optionally a polypeptide of the invention which comprises an amino acid sequence with V at a position corresponding to residue position 148, and P at a position 149 corresponding to residue position 149, comprises an amino acid sequence of SEQ ID NO:35 or 37, or an amino acid sequence that has at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% amino acid identity along its entire length with the amino acid sequence of SEQ ID NO:35 or 37.

25 Optionally an isolated polypeptide of the invention comprises an amino acid sequence of SEQ ID NO:37.

Optionally an isolated polypeptide of the invention comprises an amino acid sequence of SEQ ID NO:35.

30 Optionally a polypeptide of the invention which comprises an amino acid sequence with V at a position corresponding to residue position 148, and P at a position 149 corresponding to residue position 149, comprises a haemagglutinin stem domain, and wherein the polypeptide comprises an amino acid sequence with the following amino acid residues at positions corresponding to residue positions 416 and 434 of a wild-type H5 sequence:

- 416: F; and
- 434: F

Optionally the polypeptide comprises an amino acid sequence that has at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%,
 5 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% amino acid identity along its entire length with the sequence of SEQ ID NO:35.

There is also provided according to the invention an isolated polypeptide comprising a haemagglutinin subtype 5 (H5) globular head domain, and optionally a haemagglutinin stem domain, wherein the polypeptide comprises an amino acid sequence with the
 10 following amino acid residue at a position corresponding to residue position 238 of a wild-type H5 globular head domain:

- 238: E

Optionally an isolated polypeptide of the invention which comprises an amino acid sequence with an E residue at a position corresponding to residue position 238 of a wild-
 15 type H5 globular head domain comprises an amino acid sequence with the following amino acid residues at positions corresponding to residue positions 148 and 149 of a wild-type H5 globular head domain:

- 148: S;
- 149: S.

20 Optionally an isolated polypeptide of the invention which comprises an amino acid sequence with an E residue at a position corresponding to residue position 238 of a wild-type H5 globular head domain comprises an amino acid sequence that has at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% amino
 25 acid identity along its entire length with the amino acid sequence of SEQ ID NO:3.

Optionally an isolated polypeptide of the invention which comprises an H5 globular head domain retains at least some HA activity of a wild-type H5 globular head domain (for example, of an H5 WSN isolate - SEQ ID NO:64).

30 Optionally an isolated polypeptide of the invention which comprises an H5 globular head domain retains at least 25%, at least 50%, or at least 75% of HA activity of a wild-type H5 globular head domain (for example, of an H5 WSN isolate - SEQ ID NO:64).

Optionally an isolated polypeptide of the invention which comprises an amino acid sequence with an E residue at a position corresponding to residue position 238 of a wild-type H5 globular head domain has reduced affinity for its receptor compared with the wild-type H5 globular head domain.

- 5 Optionally an isolated polypeptide of the invention which comprises an amino acid sequence with an E residue at a position corresponding to residue position 238 of a wild-type H5 globular head domain comprises an amino acid sequence with the following amino acid residues at positions corresponding to residues 156, 157, 171, 172, and 205 of the wild-type H5 globular head domain:
- 10
- 156: R;
 - 157: S;
 - 171: N;
 - 172: A; and
 - 205: R.
- 15 Optionally an isolated polypeptide of the invention which comprises an amino acid sequence with an E residue at a position corresponding to residue position 238 of a wild-type H5 globular head domain comprises an amino acid sequence of SEQ ID NO:43 or 45, or an amino acid sequence that has at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%,
- 20 93%, 94%, 95%, 96%, 97%, 98%, or 99% amino acid identity along its entire length with the amino acid sequence of SEQ ID NO:43 or 45.

Optionally an isolated polypeptide of the invention comprises an amino acid sequence of SEQ ID NO:45.

- 25 Optionally an isolated polypeptide of the invention comprises an amino acid sequence of SEQ ID NO:43.

Optionally an isolated polypeptide of the invention comprises an amino acid sequence with the following amino acid residues at positions corresponding to residue positions 279 and 298 of the wild-type H5 globular head domain:

- 30
- 279 A; and
 - 298 M

Optionally the polypeptide comprises an amino acid sequence that has at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%,

87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% amino acid identity along its entire length with the sequence of any of SEQ ID NO: 27, 35, or 43.

A polypeptide of the invention may comprise any suitable haemagglutinin stem domain, including a stem domain of any suitable influenza haemagglutinin subtype, including a non-
5 H5 subtype. Optionally the stem domain is an H5 stem domain.

Optionally a polypeptide of the invention comprises the following amino acid residues at positions 416 and 434 of the stem domain:

- 416: F; and
- 434: F

10 Optionally a polypeptide of the invention is up to 10,000, 9,000, 8,000, 7,000, 6,000, 5,000, 4,000, 3000, 2000, 1500, 1000, 900, 800, 700, 600, 590, 580, 570, 560, 550, 540, 530, 520, 510, 500, 490, 480, 470, 460, 450, 440, 430, 420, 410, 400, 390, 380, 370, 360, 350, 340, 330, 320, 310, 300, 290, 280, or 270 amino acid residues in length.

The Applicant has also appreciated that a polypeptide that includes a fragment of the H5
15 globular head domain with amino acid residues from positions A-C can also elicit an antibody response against H5 influenza viruses. For example, such a polypeptide may be used on its own, or grafted onto other HA subtype heads, or other proteins (for example with a similar folding motif) to generate a suitable antibody response.

Accordingly, there is also provided according to the invention an isolated polypeptide which
20 comprises the following amino acid sequence:

R(P/S)SFFRNVVWLIKKN(D/N)(T/A)YPTIKRSYNNTNQEDLLVLWGIHHPNDAAEQT(K/R)
(SEQ ID NO:13), or an amino acid sequence that has at least 70%, 71%, 72%, 73%, 74%,
75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%,
90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% amino acid identity along its
25 entire length with the sequence of SEQ ID NO:13 and which has the following amino acid
residues at positions corresponding to positions 1, 2, 16, 17, and 50 of SEQ ID NO:13:

- 1: R;
- 2: P or S, preferably P;
- 16: D or N;
- 30 • 17: T or A, preferably T; and
- 50: K or R, preferably K.

Optionally a polypeptide of the invention which comprises an amino acid sequence of SEQ ID NO:13, or an amino acid sequence that has at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% amino acid identity along its entire length with the sequence of SEQ ID NO:13, comprises the following amino acid residues at positions 1, 2, 16, 17, and 50 of the amino acid sequence, or at positions corresponding to positions 1, 2, 16, 17, and 50 of SEQ ID NO:13:

- 1: R;
- 2: P;
- 10 • 16: D;
- 17: T; and
- 50: K

Optionally a polypeptide of the invention which comprises an amino acid sequence of SEQ ID NO:13, or an amino acid sequence that has at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% amino acid identity along its entire length with the sequence of SEQ ID NO:13, comprises the following amino acid residues at positions 1, 2, 16, 17, and 50 of the amino acid sequence, or at positions corresponding to positions 1, 2, 16, 17, and 50 of SEQ ID NO:13:

- 20 • 1: R;
- 2: P;
- 16: N;
- 17: T; and
- 50: K

Optionally a polypeptide of the invention which comprises an amino acid sequence of SEQ ID NO:13, or an amino acid sequence that has at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% amino acid identity along its entire length with the sequence of SEQ ID NO:13, comprises the following amino acid residues at positions 1, 2, 16, 17, and 50 of the amino acid sequence, or at positions corresponding to positions 1, 2, 16, 17, and 50 of SEQ ID NO:13:

- 1: R;
- 2: S;
- 16: N;

- 17: A; and
- 50: R

Optionally a polypeptide of the invention which comprises an amino acid sequence of SEQ ID NO:13, or an amino acid sequence that has at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% amino acid identity along its entire length with the sequence of SEQ ID NO:13, is up to 570, 560, 550, 500, 450, 400, 350, 300, 250, 200, 150, 100, 90, 80, 70, 60, or 50 amino acid residues in length.

According to the invention there is also provided an isolated polypeptide which comprises an amino acid sequence of any of SEQ ID NOs:5, 9, or 12, or an amino acid sequence that has at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% amino acid identity along its entire length with the sequence of any of SEQ ID NOs:5, 9, or 12 and which has the following amino acid residues at positions corresponding to positions 148 and 166 of SEQ ID NO:5, 9, or 12:

- 148: F; and
- 166: F

The applicant has found that such polypeptides, when forming a stem region of a haemagglutinin molecule, stabilise the stem region in both the pre- and post-fusion state.

Such polypeptides may, for example, be provided with an H5 haemagglutinin head domain or a non-H5 head domain.

Optionally a polypeptide of the invention which comprises an amino acid sequence of any of SEQ ID NOs:5, 9, or 12, or an amino acid sequence that has at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% amino acid identity along its entire length with the sequence of any of SEQ ID NO:5, 9, or 12, is up to 10,000, 9,000, 8,000, 7,000, 6,000, 5,000, 4,000, 3000, 2000, 1500, 1000, 900, 800, 700, 600, 590, 580, 570, 560, 550, 540, 530, 520, 510, 500, 490, 480, 470, 460, 450, 440, 430, 420, 410, 400, 390, 380, 370, 360, 350, 340, 330, 320, 310, or 300 amino acid residues in length.

A polypeptide of the invention may include one or more conservative amino acid substitutions. Conservative amino acid substitutions are those substitutions that, when made, least interfere with the properties of the original protein, that is, the structure and especially the function of the protein is conserved and not significantly changed by such substitutions.

5 Examples of conservative substitutions are shown below:

	<u>Original Residue</u>	<u>Conservative Substitutions</u>
	Ala	Ser
	Arg	Lys
	Asn	Gln, His
10	Asp	Glu
	Cys	Ser
	Gln	Asn
	Glu	Asp
	His	Asn; Gln
15	Ile	Leu, Val
	Leu	Ile; Val
	Lys	Arg; Gln;
	Met	Leu; Ile
	Phe	Met; Leu; Tyr
20	Ser	Thr
	Thr	Ser
	Trp	Tyr
	Tyr	Trp; Phe
	Val	Ile; Leu

25 Conservative substitutions generally maintain (a) the structure of the polypeptide backbone in the area of the substitution, for example, as a sheet or helical conformation, (b) the charge or hydrophobicity of the molecule at the target site, or (c) the bulk of the side chain.

The substitutions which in general are expected to produce the greatest changes in protein properties will be non-conservative, for instance changes in which (a) a hydrophilic residue, for example, serine or threonine, is substituted for (or by) a hydrophobic residue, for example, leucine, isoleucine, phenylalanine, valine or alanine; (b) a cysteine or proline is substituted for (or by) any other residue; (c) a residue having an electropositive side chain, for example, lysine, arginine, or histidine, is substituted for (or by) an electronegative residue, for example, glutamate or aspartate; or (d) a residue having a bulky side chain, for example, phenylalanine, is substituted for (or by) one not having a side chain, for example, glycine.

30

35

There is also provided according to the invention an isolated nucleic acid molecule encoding a polypeptide of the invention, or the complement thereof.

There is also provided according to the invention an isolated nucleic acid molecule comprising a nucleotide sequence that is at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical over its entire length to a nucleic acid molecule of the invention encoding polypeptide of the invention, or the complement thereof.

Optionally nucleic acid molecule of the invention comprises a nucleotide sequence of SEQ ID NO:2, 4, or 6, or the complement thereof.

There is also provided according to the invention an isolated nucleic acid molecule comprising a nucleotide sequence that is at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical over its entire length with SEQ ID NO:2, 4, or 6, or the complement thereof.

There is also provided according to the invention an isolated nucleic acid molecule, which comprises a nucleotide sequence of SEQ ID NO:28, 30, 32, or 34, or which comprises nucleotide sequence of SEQ ID NOs:32 and 34, or a nucleotide sequence that is at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical with SEQ ID NO: 28, 30, 32, 34, or with SEQ ID NO:32 and 34, over its entire length, or the complement thereof.

Optionally an isolated nucleic acid molecule of the invention comprises a nucleotide sequence of SEQ ID NO:28, 30, 32, or 34, or nucleotide sequence of SEQ ID NOs:32 and 34, or the complement thereof.

Optionally an isolated nucleic acid molecule of the invention comprises a nucleotide sequence of SEQ ID NO:28, or the complement thereof.

Optionally an isolated nucleic acid molecule of the invention comprises a nucleotide sequence of SEQ ID NO:36, 38, 40, or 42, or which comprises nucleotide sequence of SEQ ID NOs:40 and 42, or a nucleotide sequence that is at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical with SEQ ID

NO: 36, 38, 40, or 42, or with SEQ ID NO:40 and 42, over its entire length, or the complement thereof.

Optionally an isolated nucleic acid molecule of the invention comprises a nucleotide sequence of SEQ ID NO:36, 38, 40, or 42, or nucleotide sequence of SEQ ID NOs:40 and
5 42, or the complement thereof.

Optionally an isolated nucleic acid molecule of the invention comprises a nucleotide sequence of SEQ ID NO:36, or the complement thereof.

Optionally an isolated nucleic acid molecule of the invention comprises a nucleotide sequence of SEQ ID NO:44, 46, 48, or 50, or nucleotide sequence of SEQ ID NOs 48 and
10 50, or a nucleotide sequence that is at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical with SEQ ID NO: 44, 46, 48, or 50, or with SEQ ID NO: 48 and 50, over its entire length, or the complement thereof.

Optionally an isolated nucleic acid molecule of the invention comprises a nucleotide
15 sequence of SEQ ID NO:44, 46, 48, or 50, or nucleotide sequence of SEQ ID NOs 48 and 50, or the complement thereof.

Optionally an isolated nucleic acid molecule of the invention comprises a nucleotide sequence of SEQ ID NO:44, or the complement thereof.

Optionally an isolated nucleic acid molecule of the invention comprises a nucleotide
20 sequence of SEQ ID NO:52, 54, 55, 56, or which comprises nucleotide sequence of SEQ ID NOs:52 and 54, or a nucleotide sequence that is at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical with SEQ ID NO: 52, 54, 55, 56, or with SEQ ID NO:52 and 54, over its entire length, or the complement thereof.

Optionally an isolated nucleic acid molecule of the invention comprises a nucleotide
25 sequence of SEQ ID NO:52, 54, 55, 56, or nucleotide sequence of SEQ ID NOs:52 and 54, or the complement thereof.

Optionally an isolated nucleic acid molecule of the invention comprises a nucleotide sequence of SEQ ID NO:55, or the complement thereof.

Optionally an isolated nucleic acid molecule of the invention comprises a nucleotide
30 sequence of SEQ ID NO:58, 60, 61, 62, or nucleotide sequence of SEQ ID NOs:58 and 60,

or a nucleotide sequence that is at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical with SEQ ID NO: 58, 60, 61, 62, or with SEQ ID NO:58 and 60, over its entire length, or the complement thereof.

- 5 Optionally an isolated nucleic acid molecule of the invention comprises a nucleotide sequence of SEQ ID NO:58, 60, 61, 62, or which comprises nucleotide sequence of SEQ ID NOs:58 and 60, or the complement thereof.

Optionally an isolated nucleic acid molecule of the invention comprises a nucleotide sequence of SEQ ID NO:61, or the complement thereof.

- 10 Optionally an isolated nucleic acid molecule of the invention comprises a messenger RNA (mRNA) molecule.

The term “broadly neutralising immune response” is used herein in respect of influenza A to include an immune response elicited in a subject that is sufficient to inhibit (i.e. reduce), neutralise or prevent infection, and/or progress of infection, of at least 3 antigenically distinct
 15 clades of influenza A. Optionally a broadly neutralising immune response is sufficient to inhibit, neutralise or prevent infection, and/or progress of infection, of different H5 clades of influenza A. Optionally, advantageously the different clades include clades 2.3.4 and/or 7.1. Optionally, the different clades include clade 2.3.4.4.

Additional H5 embodiments of the invention:

- 20 The Applicant has also designed additional amino acid sequences and their encoding nucleic acid molecules that induce a broadly neutralising immune response against important H5 clades of influenza A. The polypeptides comprising such amino acid sequence are referred to herein as FLU_T4_HA_1, FLU_T4_HA_2, and FLU_T4_HA_3
 25 polypeptides. Such polypeptides are particularly advantageous as they elicit broadly neutralising antibody responses to a diverse panel of H5 clade 2.3.4.4 influenza viruses influenza viruses, as discussed below.

Clade 2.3.4.4

- The Applicant has designed additional amino acid sequences and their encoding nucleic acid sequences that induce a broadly neutralising immune response against strains of clade
 30 2.3.4.4 of influenza A. These polypeptides are referred to herein as FLU_T4_HA_1, FLU_T4_HA_2, and FLU_T4_HA_3. Such polypeptides are particularly advantageous as they elicit broadly neutralising antibody responses to a diverse panel of clade 2.3.4.4

influenza viruses, as demonstrated by the results described Figures 29 to 34 and Example 34.

Figure 25 summarises novel differences in amino acid sequence for new H5 designs FLU_T4_HA_1, FLU_T4_HA_2, and FLU_T4_HA_3.

- 5 Figure 28 shows the amino acid sequences of FLU_T4_HA_1 (SEQ ID NO:71), FLU_T4_HA_2 (SEQ ID NO:80), and FLU_T4_HA_3 (SEQ ID NO:89) in alignment with the amino acid sequences of previously designed tier 3 (T3) H5 sequences, and with the H5 amino acid sequence of influenza A H5 strains. The residue positions on the alignment correspond to the residue positions of A/Sichuan/26221/2014.
- 10 Figures 29-34 show neutralisation assays in mice immunised with Tier 4 (T4) vaccine candidates, previously designed sequences, or WT strains vs challenge strain. Each of FLU_T4_HA_1, FLU_T4_HA_2, and FLU_T4_HA_3 elicits a comparable neutralising response to H5 strains which are homologous to the challenge strain, and a higher response to heterologous strains.
- 15 Table 2 below summarises amino acid residue differences between the H5 A/Sichuan/2014 isolate and tier 4 (T4) H5 designs of the invention.

Table 2

H5 residue position of A/Sichuan/2014	H5 residue of A/Sichuan/2014 (SEQ ID NO:100)	FLU_T4_HA_1 (SEQ ID NO:71)	FLU_T4_HA_2 (SEQ ID NO:80)	FLU_T4_HA_3 (SEQ ID NO:89)
107	Y	Y	<u>F</u>	<u>F</u>
142	E	E	E	<u>N</u>
200	A	A	A	<u>T</u>
231	T	T	T	<u>N</u>
238	Q	Q	<u>E</u>	Q

FLU_T4_HA_1 polypeptides and encoding nucleic acid molecules.

- 20 According to the invention there is provided an isolated polypeptide comprising an amino acid sequence of SEQ ID NO:71 (FLU_T4_HA_1: HA0 amino acid sequence).

According to the invention there is provided an isolated nucleic acid molecule comprising a nucleotide sequence that encodes an amino acid sequence of SEQ ID NO:71 (FLU_T4_HA_1: HA0 amino acid sequence), or the complement thereof.

- 25 Optionally the nucleotide sequence that encodes an amino acid sequence of SEQ ID NO:71 (FLU_T4_HA_1: HA0 amino acid sequence) is at least 70%, 71%, 72%, 73%, 74%, 75%,

76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical over its entire length with the nucleotide sequence of SEQ ID NO:72 (FLU_T4_HA_1: HA0 nucleic acid sequence), or the complement thereof.

- 5 According to the invention there is provided an isolated nucleic acid molecule comprising a nucleotide sequence of SEQ ID NO:72 (FLU_T4_HA_1: HA0 nucleic acid sequence), or the complement thereof.

According to the invention there is also provided an isolated polypeptide comprising an amino acid sequence of SEQ ID NO:73 (FLU_T4_HA_1: head region amino acid sequence).

- 10 According to the invention there is provided an isolated nucleic acid molecule comprising a nucleotide sequence that encodes an amino acid sequence of SEQ ID NO:73 (FLU_T4_HA_1: head region amino acid sequence), or the complement thereof.

- Optionally the nucleotide sequence that encodes an amino acid sequence of SEQ ID NO:73 (FLU_T4_HA_1: head region amino acid sequence) is at least 70%, 71%, 72%, 73%, 74%,
15 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical over its entire length with the nucleotide sequence SEQ ID NO:74 (FLU_T4_HA_1: head region nucleic acid sequence), or the complement thereof.

- According to the invention there is provided an isolated nucleic acid molecule comprising a
20 nucleotide sequence of SEQ ID NO:74 (FLU_T4_HA_1: head region nucleic acid sequence), or the complement thereof.

According to the invention there is provided an isolated polypeptide comprising an amino acid sequence of SEQ ID NO:75 (FLU_T4_HA_1: first stem region amino acid sequence).

- According to the invention there is provided an isolated nucleic acid molecule comprising a
25 nucleotide sequence that encodes an amino acid sequence of SEQ ID NO:75 (FLU_T4_HA_1: first stem region amino acid sequence), or the complement thereof.

- Optionally the nucleotide sequence that encodes an amino acid sequence of SEQ ID NO:75 (FLU_T4_HA_1: first stem region amino acid sequence) is at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%,
30 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical over its entire length

with the nucleotide sequence SEQ ID NO:76 (FLU_T4_HA_1: first stem region nucleic acid sequence), or the complement thereof.

According to the invention there is provided an isolated nucleic acid molecule comprising a nucleotide sequence of SEQ ID NO:76 (FLU_T4_HA_1: first stem region nucleic acid sequence), or the complement thereof.

According to the invention there is provided an isolated polypeptide comprising an amino acid sequence of SEQ ID NO:77 (FLU_T4_HA_1: second stem region amino acid sequence).

According to the invention there is provided an isolated nucleic acid molecule comprising a nucleotide sequence that encodes an amino acid sequence of SEQ ID NO:77 (FLU_T4_HA_1: second stem region amino acid sequence), or the complement thereof.

Optionally the nucleotide sequence that encodes an amino acid sequence of SEQ ID NO:77 (FLU_T4_HA_1: second stem region amino acid sequence) is at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical over its entire length with the nucleotide sequence SEQ ID NO:78 (FLU_T4_HA_1: second stem region nucleic acid sequence), or the complement thereof.

According to the invention there is provided an isolated nucleic acid molecule comprising a nucleotide sequence of SEQ ID NO:78 (FLU_T4_HA_1: second stem region nucleic acid sequence), or the complement thereof.

According to the invention there is provided an isolated nucleic acid molecule comprising a nucleotide sequence of SEQ ID NO:79 (pEVAC-FLU_T4_HA_1), or the complement thereof.

FLU_T4_HA_2 polypeptides and encoding nucleic acid molecules

According to the invention there is provided an isolated polypeptide comprising an amino acid sequence of SEQ ID NO:80 (FLU_T4_HA_2: HA0 amino acid sequence).

According to the invention there is provided an isolated nucleic acid molecule comprising a nucleotide sequence that encodes an amino acid sequence of SEQ ID NO:80 (FLU_T4_HA_2: HA0 amino acid sequence), or the complement thereof.

Optionally the nucleotide sequence that encodes an amino acid sequence of SEQ ID NO:80 (FLU_T4_HA_2: HA0 amino acid sequence) is at least 70%, 71%, 72%, 73%, 74%, 75%,

76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical over its entire length with the nucleotide sequence of SEQ ID NO:81 (FLU_T4_HA_2: HA0 nucleic acid sequence), or the complement thereof.

- 5 According to the invention there is provided an isolated nucleic acid molecule comprising a nucleotide sequence of SEQ ID NO:81 (FLU_T4_HA_2: HA0 nucleic acid sequence), or the complement thereof.

- According to the invention there is also provided an isolated polypeptide which comprises an amino acid sequence of SEQ ID NO:80 (FLU_T4_HA_2: HA0 amino acid sequence), or an amino acid sequence that has at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% amino acid identity along its entire length with the sequence of SEQ ID NO:80, and which comprises an amino acid residue F at a position corresponding to amino acid residue 107 of SEQ ID NO:100 (A/Sichuan/2014 H5), or an amino acid residue E at a position corresponding to amino acid residue 238 of SEQ ID NO:100 (A/Sichuan/2014 H5).

Optionally the polypeptide further comprises:

- an amino acid residue E at a position corresponding to amino acid residue 142 of SEQ ID NO:100 (A/Sichuan/2014 H5);
- 20 an amino acid residue A at a position corresponding to amino acid residue 172 of SEQ ID NO:100 (A/Sichuan/2014 H5);
- an amino acid residue A at a position corresponding to amino acid residue 200 of SEQ ID NO:100 (A/Sichuan/2014 H5);
- an amino acid residue T at a position corresponding to amino acid residue 231 of SEQ ID NO:100 (A/Sichuan/2014 H5);
- 25 an amino acid residue R at a position corresponding to amino acid residue 344 of SEQ ID NO:100 (A/Sichuan/2014 H5); or
- an amino acid residue K at a position corresponding to amino acid residue 345 of SEQ ID NO:100 (A/Sichuan/2014 H5).

According to the invention there is provided an isolated nucleic acid molecule comprising a nucleotide sequence that encodes an amino acid sequence of SEQ ID NO:80 (FLU_T4_HA_2: HA0 amino acid sequence), or an amino acid sequence that has at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% amino acid identity along its entire length with the sequence of SEQ ID NO:80, and which comprises an amino acid residue F at a position corresponding to amino acid residue 107 of SEQ ID NO:100 (A/Sichuan/2014 H5), or an amino acid residue E at a position corresponding to amino acid residue 238 of SEQ ID NO:100 (A/Sichuan/2014 H5), or the complement thereof.

- 10 According to the invention there is also provided an isolated polypeptide which comprises an amino acid sequence of SEQ ID NO:80 (FLU_T4_HA_2: HA0 amino acid sequence), or an amino acid sequence that has at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% amino acid identity along its entire length with the sequence of SEQ ID NO:80, and which comprises an amino acid residue F at a position corresponding to amino acid residue 107 of SEQ ID NO:100 (A/Sichuan/2014 H5), and an amino acid residue E at a position corresponding to amino acid residue 238 of SEQ ID NO:100 (A/Sichuan/2014 H5).

Optionally the polypeptide further comprises:

- 20 an amino acid residue E at a position corresponding to amino acid residue 142 of SEQ ID NO:100 (A/Sichuan/2014 H5);
- an amino acid residue A at a position corresponding to amino acid residue 172 of SEQ ID NO:100 (A/Sichuan/2014 H5);
- an amino acid residue A at a position corresponding to amino acid residue 200 of SEQ ID NO:100 (A/Sichuan/2014 H5);
- 25 an amino acid residue T at a position corresponding to amino acid residue 231 of SEQ ID NO:100 (A/Sichuan/2014 H5);
- an amino acid residue R at a position corresponding to amino acid residue 344 of SEQ ID NO:100 (A/Sichuan/2014 H5); or
- 30 an amino acid residue K at a position corresponding to amino acid residue 345 of SEQ ID NO:100 (A/Sichuan/2014 H5).

According to the invention there is provided an isolated nucleic acid molecule comprising a nucleotide sequence that encodes an amino acid sequence of SEQ ID NO:80 (FLU_T4_HA_2: HA0 amino acid sequence), or an amino acid sequence that has at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% amino acid identity along its entire length with the sequence of SEQ ID NO:80, and which comprises an amino acid residue F at a position corresponding to amino acid residue 107 of SEQ ID NO:100 (A/Sichuan/2014 H5), and an amino acid residue E at a position corresponding to amino acid residue 238 of SEQ ID NO:100 (A/Sichuan/2014 H5), or the complement thereof.

- 10 According to the invention there is also provided an isolated polypeptide comprising an amino acid sequence of SEQ ID NO:82 (FLU_T4_HA_2: head region amino acid sequence).

According to the invention there is provided an isolated nucleic acid molecule comprising a nucleotide sequence that encodes an amino acid sequence of SEQ ID NO:82 (FLU_T4_HA_2: head region amino acid sequence), or the complement thereof.

- 15 Optionally the nucleotide sequence that encodes an amino acid sequence of SEQ ID NO:82 (FLU_T4_HA_2: head region amino acid sequence) is at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical over its entire length with the nucleotide sequence SEQ ID NO:83 (FLU_T4_HA_2: head region nucleic acid sequence),
20 or the complement thereof.

According to the invention there is provided an isolated nucleic acid molecule comprising a nucleotide sequence of SEQ ID NO:83 (FLU_T4_HA_2: head region nucleic acid sequence), or the complement thereof.

- 25 According to the invention there is also provided an isolated polypeptide which comprises an amino acid sequence of SEQ ID NO:82 (FLU_T4_HA_2: head region amino acid sequence), or an amino acid sequence that has at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% amino acid identity along its entire length with the sequence of SEQ ID NO:82, and which comprises an amino acid residue F at a position
30 corresponding to amino acid residue 107 of SEQ ID NO:100 (A/Sichuan/2014 H5), or an amino acid residue E at a position corresponding to amino acid residue 238 of SEQ ID NO:100 (A/Sichuan/2014 H5).

Optionally the polypeptide further comprises:

an amino acid residue E at a position corresponding to amino acid residue 142 of SEQ ID NO:100 (A/Sichuan/2014 H5);

an amino acid residue A at a position corresponding to amino acid residue 172 of SEQ ID NO:100 (A/Sichuan/2014 H5);

- 5 an amino acid residue A at a position corresponding to amino acid residue 200 of SEQ ID NO:100 (A/Sichuan/2014 H5); or

an amino acid residue T at a position corresponding to amino acid residue 231 of SEQ ID NO:100 (A/Sichuan/2014 H5).

- 10 According to the invention there is provided an isolated nucleic acid molecule comprising a nucleotide sequence that encodes an amino acid sequence of SEQ ID NO:82 (FLU_T4_HA_2: head region amino acid sequence), or an amino acid sequence that has at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% amino acid identity along its entire length with the sequence of SEQ ID NO:82, and which
- 15 comprises an amino acid residue F at a position corresponding to amino acid residue 107 of SEQ ID NO:100 (A/Sichuan/2014 H5), or an amino acid residue E at a position corresponding to amino acid residue 238 of SEQ ID NO:100 (A/Sichuan/2014 H5), or the complement thereof.

- 20 According to the invention there is also provided an isolated polypeptide which comprises an amino acid sequence of SEQ ID NO:82 (FLU_T4_HA_2: head region amino acid sequence), or an amino acid sequence that has at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% amino acid identity along its entire length with the sequence of SEQ ID NO:82, and which comprises an amino acid residue F at a position
- 25 corresponding to amino acid residue 107 of SEQ ID NO:100 (A/Sichuan/2014 H5), and an amino acid residue E at a position corresponding to amino acid residue 238 of SEQ ID NO:100 (A/Sichuan/2014 H5).

Optionally the polypeptide further comprises:

- 30 an amino acid residue E at a position corresponding to amino acid residue 142 of SEQ ID NO:100 (A/Sichuan/2014 H5);

an amino acid residue A at a position corresponding to amino acid residue 172 of SEQ ID NO:100 (A/Sichuan/2014 H5);

an amino acid residue A at a position corresponding to amino acid residue 200 of SEQ ID NO:100 (A/Sichuan/2014 H5); or

- 5 an amino acid residue T at a position corresponding to amino acid residue 231 of SEQ ID NO:100 (A/Sichuan/2014 H5).

According to the invention there is provided an isolated nucleic acid molecule comprising a nucleotide sequence that encodes an amino acid sequence of SEQ ID NO:82 (FLU_T4_HA_2: head region amino acid sequence), or an amino acid sequence that has at
 10 least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% amino acid identity along its entire length with the sequence of SEQ ID NO:82, and which comprises an amino acid residue F at a position corresponding to amino acid residue 107 of SEQ ID NO:100 (A/Sichuan/2014 H5), and an amino acid residue E at a position
 15 corresponding to amino acid residue 238 of SEQ ID NO:100 (A/Sichuan/2014 H5), or the complement thereof.

According to the invention there is provided an isolated polypeptide comprising an amino acid sequence of SEQ ID NO:84 (FLU_T4_HA_2: first stem region amino acid sequence).

According to the invention there is provided an isolated nucleic acid molecule comprising a
 20 nucleotide sequence that encodes an amino acid sequence of SEQ ID NO:84 (FLU_T4_HA_2: first stem region amino acid sequence), or the complement thereof.

Optionally the nucleotide sequence that encodes an amino acid sequence of SEQ ID NO:84 (FLU_T4_HA_2: first stem region amino acid sequence) is at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%,
 25 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical over its entire length with the nucleotide sequence SEQ ID NO:85 (FLU_T4_HA_2: first stem region nucleic acid sequence), or the complement thereof.

According to the invention there is provided an isolated nucleic acid molecule comprising a nucleotide sequence of SEQ ID NO:85 (FLU_T4_HA_2: first stem region nucleic acid
 30 sequence), or the complement thereof.

According to the invention there is provided an isolated polypeptide comprising an amino acid sequence of SEQ ID NO:86 (FLU_T4_HA_2: second stem region amino acid sequence).

5 According to the invention there is provided an isolated nucleic acid molecule comprising a nucleotide sequence that encodes an amino acid sequence of SEQ ID NO:86 (FLU_T4_HA_2: second stem region amino acid sequence), or the complement thereof.

Optionally the nucleotide sequence that encodes an amino acid sequence of SEQ ID NO:86 (FLU_T4_HA_2: second stem region amino acid sequence) is at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%,
10 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical over its entire length with the nucleotide sequence SEQ ID NO:87 (FLU_T4_HA_2: second stem region nucleic acid sequence), or the complement thereof.

According to the invention there is provided an isolated nucleic acid molecule comprising a nucleotide sequence of SEQ ID NO:87 (FLU_T4_HA_2: second stem region nucleic acid
15 sequence), or the complement thereof.

According to the invention there is provided an isolated nucleic acid molecule comprising a nucleotide sequence of SEQ ID NO:88 (pEVAC-FLU_T4_HA_2), or the complement thereof.

There is also provided according to the invention an isolated nucleic acid molecule comprising a nucleotide sequence that encodes an amino acid sequence of any of the
20 FLU_T4_HA_2 polypeptides of the invention above, or the complement thereof.

FLU_T4_HA_3 polypeptides and encoding nucleic molecules

According to the invention there is provided an isolated polypeptide comprising an amino acid sequence of SEQ ID NO:89 (FLU_T4_HA_3: HA0 amino acid sequence).

25 According to the invention there is provided an isolated nucleic acid molecule comprising a nucleotide sequence that encodes an amino acid sequence of SEQ ID NO:89 (FLU_T4_HA_3: HA0 amino acid sequence), or the complement thereof.

Optionally the nucleotide sequence that encodes an amino acid sequence of SEQ ID NO:89 (FLU_T4_HA_3: HA0 amino acid sequence) is at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%,
30 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical over its entire length with the

nucleotide sequence of SEQ ID NO:90 (FLU_T4_HA_3: HA0 nucleic acid sequence), or the complement thereof.

According to the invention there is provided an isolated nucleic acid molecule comprising a nucleotide sequence of SEQ ID NO:90 (FLU_T4_HA_3: HA0 nucleic acid sequence), or the complement thereof.

According to the invention there is also provided an isolated polypeptide which comprises an amino acid sequence of SEQ ID NO:89, or an amino acid sequence that has at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% amino acid identity along its entire length with the sequence of SEQ ID NO:89, and which comprises an amino acid residue F at a position corresponding to amino acid residue 107 of SEQ ID NO:100 (A/Sichuan/2014 H5), an amino acid residue N at a position corresponding to amino acid residue 142 of SEQ ID NO:100 (A/Sichuan/2014 H5), an amino acid residue T at a position corresponding to amino acid residue 200 of SEQ ID NO:100 (A/Sichuan/2014 H5), or an amino acid residue N at a position corresponding to amino acid residue 231 of SEQ ID NO:100 (A/Sichuan/2014 H5).

Optionally the polypeptide further comprises:

an amino acid residue T at a position corresponding to amino acid residue 172 of SEQ ID NO:100 (A/Sichuan/2014 H5);

an amino acid residue Q at a position corresponding to amino acid residue 238 of SEQ ID NO:100 (A/Sichuan/2014 H5);

an amino acid residue R at a position corresponding to amino acid residue 344 of SEQ ID NO:100 (A/Sichuan/2014 H5); or

an amino acid residue K at a position corresponding to amino acid residue 345 of SEQ ID NO:100 (A/Sichuan/2014 H5).

According to the invention there is provided an isolated nucleic acid molecule comprising a nucleotide sequence that encodes an amino acid sequence of SEQ ID NO:89 (FLU_T4_HA_3: HA0 amino acid sequence), or an amino acid sequence that has at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% amino acid identity along its entire length with the sequence of SEQ ID NO:89, and which comprises

an amino acid residue F at a position corresponding to amino acid residue 107 of SEQ ID NO:100 (A/Sichuan/2014 H5), an amino acid residue N at a position corresponding to amino acid residue 142 of SEQ ID NO:100 (A/Sichuan/2014 H5), an amino acid residue T at a position corresponding to amino acid residue 200 of SEQ ID NO:100 (A/Sichuan/2014 H5),
 5 or an amino acid residue N at a position corresponding to amino acid residue 231 of SEQ ID NO:100 (A/Sichuan/2014 H5), or the complement thereof.

According to the invention there is also provided an isolated polypeptide which comprises an amino acid sequence of SEQ ID NO:89, or an amino acid sequence that has at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%,
 10 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% amino acid identity along its entire length with the sequence of SEQ ID NO:89, and which comprises an amino acid residue F at a position corresponding to amino acid residue 107 of SEQ ID NO:100 (A/Sichuan/2014 H5), an amino acid residue N at a position corresponding to amino acid residue 142 of SEQ ID NO:100 (A/Sichuan/2014 H5), an amino acid residue T at a
 15 position corresponding to amino acid residue 200 of SEQ ID NO:100 (A/Sichuan/2014 H5), and an amino acid residue N at a position corresponding to amino acid residue 231 of SEQ ID NO:100 (A/Sichuan/2014 H5).

Optionally the polypeptide further comprises:

an amino acid residue T at a position corresponding to amino acid residue 172 of SEQ ID
 20 NO:100 (A/Sichuan/2014 H5);

an amino acid residue Q at a position corresponding to amino acid residue 238 of SEQ ID NO:100 (A/Sichuan/2014 H5);

an amino acid residue R at a position corresponding to amino acid residue 344 of SEQ ID NO:100 (A/Sichuan/2014 H5); or

25 an amino acid residue K at a position corresponding to amino acid residue 345 of SEQ ID NO:100 (A/Sichuan/2014 H5).

According to the invention there is provided an isolated nucleic acid molecule comprising a nucleotide sequence that encodes an amino acid sequence of SEQ ID NO:89 (FLU_T4_HA_3: HA0 amino acid sequence), or an amino acid sequence that has at least
 30 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% amino acid identity along its entire length with the sequence of SEQ ID NO:89, and which comprises

an amino acid residue F at a position corresponding to amino acid residue 107 of SEQ ID NO:100 (A/Sichuan/2014 H5), an amino acid residue N at a position corresponding to amino acid residue 142 of SEQ ID NO:100 (A/Sichuan/2014 H5), an amino acid residue T at a position corresponding to amino acid residue 200 of SEQ ID NO:100 (A/Sichuan/2014 H5),
 5 and an amino acid residue N at a position corresponding to amino acid residue 231 of SEQ ID NO:100 (A/Sichuan/2014 H5), or the complement thereof.

According to the invention there is also provided an isolated polypeptide comprising an amino acid sequence of SEQ ID NO:91 (FLU_T4_HA_3: head region amino acid sequence).

10 According to the invention there is provided an isolated nucleic acid molecule comprising a nucleotide sequence that encodes an amino acid sequence of SEQ ID NO:91 (FLU_T4_HA_3: head region amino acid sequence), or the complement thereof.

Optionally the nucleotide sequence that encodes an amino acid sequence of SEQ ID NO:91 (FLU_T4_HA_3: head region amino acid sequence) is at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%,
 15 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical over its entire length with the nucleotide sequence SEQ ID NO:92 (FLU_T4_HA_3: head region nucleic acid sequence), or the complement thereof.

According to the invention there is provided an isolated nucleic acid molecule comprising a nucleotide sequence of SEQ ID NO:92 (FLU_T4_HA_3: head region nucleic acid sequence),
 20 or the complement thereof.

According to the invention there is also provided an isolated polypeptide which comprises an amino acid sequence of SEQ ID NO:91 (FLU_T4_HA_3: head region amino acid sequence), or an amino acid sequence that has at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%,
 25 94%, 95%, 96%, 97%, 98%, or 99% amino acid identity along its entire length with the sequence of SEQ ID NO:91, and which comprises an amino acid residue F at a position corresponding to amino acid residue 107 of SEQ ID NO:100 (A/Sichuan/2014 H5), an amino acid residue N at a position corresponding to amino acid residue 142 of SEQ ID NO:100 (A/Sichuan/2014 H5), an amino acid residue T at a position corresponding to amino acid
 30 residue 200 of SEQ ID NO:100 (A/Sichuan/2014 H5), or an amino acid residue N at a position corresponding to amino acid residue 231 of SEQ ID NO:100 (A/Sichuan/2014 H5).

Optionally the polypeptide further comprises:

an amino acid residue T at a position corresponding to amino acid residue 172 of SEQ ID NO:100 (A/Sichuan/2014 H5); or

an amino acid residue Q at a position corresponding to amino acid residue 238 of SEQ ID NO:100 (A/Sichuan/2014 H5).

- 5 According to the invention there is provided an isolated nucleic acid molecule comprising a nucleotide sequence that encodes an amino acid sequence of SEQ ID NO:91 (FLU_T4_HA_3: head region amino acid sequence), or an amino acid sequence that has at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% amino acid identity along its entire length with the sequence of SEQ ID NO:91, and which comprises an amino acid residue F at a position corresponding to amino acid residue 107 of SEQ ID NO:100 (A/Sichuan/2014 H5), an amino acid residue N at a position corresponding to amino acid residue 142 of SEQ ID NO:100 (A/Sichuan/2014 H5), an amino acid residue T at a position corresponding to amino acid residue 200 of SEQ ID NO:100 (A/Sichuan/2014 H5), or an amino acid residue N at a position corresponding to amino acid residue 231 of SEQ ID NO:100 (A/Sichuan/2014 H5), or the complement thereof.

According to the invention there is also provided an isolated polypeptide which comprises an amino acid sequence of SEQ ID NO:91 (FLU_T4_HA_3: head region amino acid sequence), or an amino acid sequence that has at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% amino acid identity along its entire length with the sequence of SEQ ID NO:91, and which comprises an amino acid residue F at a position corresponding to amino acid residue 107 of SEQ ID NO:100 (A/Sichuan/2014 H5), an amino acid residue N at a position corresponding to amino acid residue 142 of SEQ ID NO:100 (A/Sichuan/2014 H5), an amino acid residue T at a position corresponding to amino acid residue 200 of SEQ ID NO:100 (A/Sichuan/2014 H5), and an amino acid residue N at a position corresponding to amino acid residue 231 of SEQ ID NO:100 (A/Sichuan/2014 H5).

Optionally the polypeptide further comprises:

an amino acid residue T at a position corresponding to amino acid residue 172 of SEQ ID NO:100 (A/Sichuan/2014 H5); or

an amino acid residue Q at a position corresponding to amino acid residue 238 of SEQ ID NO:100 (A/Sichuan/2014 H5).

According to the invention there is provided an isolated nucleic acid molecule comprising a nucleotide sequence that encodes an amino acid sequence of SEQ ID NO:91 (FLU_T4_HA_3: head region amino acid sequence), or an amino acid sequence that has at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%,
 5 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% amino acid identity along its entire length with the sequence of SEQ ID NO:91, and which comprises an amino acid residue F at a position corresponding to amino acid residue 107 of SEQ ID NO:100 (A/Sichuan/2014 H5), an amino acid residue N at a position corresponding to amino acid residue 142 of SEQ ID NO:100 (A/Sichuan/2014 H5), an amino acid residue T
 10 at a position corresponding to amino acid residue 200 of SEQ ID NO:100 (A/Sichuan/2014 H5), and an amino acid residue N at a position corresponding to amino acid residue 231 of SEQ ID NO:100 (A/Sichuan/2014 H5), or the complement thereof.

According to the invention there is provided an isolated polypeptide comprising an amino acid sequence of SEQ ID NO:93 (FLU_T4_HA_3: first stem region amino acid sequence).

15 According to the invention there is provided an isolated nucleic acid molecule comprising a nucleotide sequence that encodes an amino acid sequence of SEQ ID NO:93 (FLU_T4_HA_3: first stem region amino acid sequence), or the complement thereof.

Optionally the nucleotide sequence that encodes an amino acid sequence of SEQ ID NO:93 (FLU_T4_HA_3: first stem region amino acid sequence) is at least 70%, 71%, 72%, 73%,
 20 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical over its entire length with the nucleotide sequence SEQ ID NO:94 (FLU_T4_HA_3: first stem region nucleic acid sequence), or the complement thereof.

According to the invention there is provided an isolated nucleic acid molecule comprising a
 25 nucleotide sequence of SEQ ID NO:94 (FLU_T4_HA_3: first stem region nucleic acid sequence), or the complement thereof.

According to the invention there is provided an isolated polypeptide comprising an amino acid sequence of SEQ ID NO:95 (FLU_T4_HA_3: second stem region amino acid sequence).

30 According to the invention there is provided an isolated nucleic acid molecule comprising a nucleotide sequence that encodes an amino acid sequence of SEQ ID NO:95 (FLU_T4_HA_3: second stem region amino acid sequence), or the complement thereof.

Optionally the nucleotide sequence that encodes an amino acid sequence of SEQ ID NO:95 (FLU_T4_HA_3: second stem region amino acid sequence) is at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical over its entire length
 5 with the nucleotide sequence SEQ ID NO:96 (FLU_T4_HA_3: second stem region nucleic acid sequence), or the complement thereof.

According to the invention there is provided an isolated nucleic acid molecule comprising a nucleotide sequence of SEQ ID NO:96 (FLU_T4_HA_3: second stem region nucleic acid sequence), or the complement thereof.

10 According to the invention there is provided an isolated nucleic acid molecule comprising a nucleotide sequence of SEQ ID NO:97 (pEVAC-FLU_T4_HA_3), or the complement thereof.

There is also provided according to the invention an isolated nucleic acid molecule comprising a nucleotide sequence that encodes an amino acid sequence of any of the FLU_T4_HA_3 polypeptides of the invention above, or the complement thereof.

15 M2

The extracellular domain of M2 has been identified as being almost invariant across all influenza A strains. This presents as a potential solution to the problem of creating a universal influenza A vaccine that elicits broad-spectrum protection against all influenza A infections.

The Applicant has identified amino acid sequences and their encoding nucleic acid
 20 molecules that induce a broadly neutralising immune response against M2 of influenza A.

M2 embodiments of the invention are described below.

According to the invention there is provided an isolated polypeptide which comprises an amino acid sequence of SEQ ID NO:14, or an amino acid sequence that has at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%,
 25 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% amino acid identity along its entire length with the sequence of SEQ ID NO:14.

There is also provided according to the invention an isolated nucleic acid molecule, comprising a nucleotide sequence of SEQ ID NO:15, or a nucleotide sequence that is at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%,
 30 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical with SEQ ID NO:15, over its entire length, or the complement thereof.

Neuraminidase

The Applicant has also identified amino acid sequences and their encoding nucleic acid molecules that include epitopes of neuraminidase that are conserved by several different influenza subtypes.

5 Neuraminidase embodiments of the invention are described below.

According to the invention there is provided an isolated polypeptide which comprises an amino acid sequence of SEQ ID NO:16, or an amino acid sequence that has at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% amino
10 acid identity along its entire length with the sequence of SEQ ID NO:16.

According to the invention there is provided an isolated polypeptide which comprises an amino acid sequence of SEQ ID NO:18, or an amino acid sequence that has at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% amino
15 acid identity along its entire length with the sequence of SEQ ID NO:18.

There is also provided according to the invention an isolated nucleic acid molecule, comprising a nucleotide sequence of SEQ ID NO:17, or a nucleotide sequence that is at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or
20 99% identical with SEQ ID NO:17, over its entire length, or the complement thereof.

There is also provided according to the invention an isolated nucleic acid molecule, comprising a nucleotide sequence of SEQ ID NO:19, or a nucleotide sequence that is at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or
25 99% identical with SEQ ID NO:19, over its entire length, or the complement thereof.

According to the invention there is provided an isolated polypeptide comprising an amino acid sequence of SEQ ID NO:98 (FLU_T3_NA_3 amino acid sequence).

According to the invention there is provided an isolated polypeptide which comprises an amino acid sequence of SEQ ID NO:98 (FLU_T3_NA_3 amino acid sequence), or an
30 amino acid sequence that has at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%,

94%, 95%, 96%, 97%, 98%, or 99% amino acid identity along its entire length with the sequence of SEQ ID NO:98.

According to the invention there is provided an isolated nucleic acid molecule comprising a nucleotide sequence that encodes an amino acid sequence of SEQ ID NO: 98

5 (FLU_T3_NA_3 amino acid sequence), or the complement thereof.

Optionally the nucleotide sequence that encodes an amino acid sequence of SEQ ID NO: 98 (FLU_T3_NA_3 amino acid sequence) is at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical over its entire length with the nucleotide sequence of SEQ ID NO:99 (FLU_T3_NA_3 nucleic acid sequence), or the complement thereof.

10

According to the invention there is provided an isolated nucleic acid molecule comprising a nucleotide sequence of SEQ ID NO:99 (FLU_T3_NA_3 nucleic acid sequence), or the complement thereof.

15 Influenza A H1

The Applicant has also designed amino acid sequences and their encoding nucleic acid molecules that can be used in vaccines to induce broad H1 immunity and protection against divergent strains of influenza A. The designed amino acid sequences are referred to as FLU_T2_HA_3_I3 and FLU_T2_HA_4 below.

20 H1 embodiments of the invention are described below.

FLU_T2_HA_3_I3:

FLU_T2_HA_3_I3 embodiments of the invention are described below.

There is also provided according to the invention an isolated polypeptide, which comprises an amino acid sequence of SEQ ID NO:22 (FLU_T2_HA_3_I3).

25 There is also provided according to the invention an isolated polynucleotide, which comprises a nucleotide sequence encoding an amino acid sequence of SEQ ID NO:22 (FLU_T2_HA_3_I3), or the complement thereof.

The nucleotide sequence may comprise a sequence of SEQ ID NO:23, or the complement thereof.

There is also provided according to the invention an isolated polypeptide, which comprises an amino acid sequence of SEQ ID NO:22 (FLU_T2_HA_3_I3), or an amino acid sequence that has at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% amino acid identity along its entire length with the sequence of SEQ ID NO:22.

There is also provided according to the invention an isolated polynucleotide, which comprises a nucleotide sequence of SEQ ID NO:23, or a nucleotide sequence that is at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical with SEQ ID NO:23, over its entire length, or the complement thereof.

FLU_T2_HA_4:

FLU_T2_HA_4 embodiments of the invention are described below.

There is also provided according to the invention an isolated polypeptide, which comprises an amino acid sequence of SEQ ID NO:68 (FLU_T2_HA_4).

There is also provided according to the invention an isolated polynucleotide, which comprises a nucleotide sequence encoding an amino acid sequence of SEQ ID NO:69 (FLU_T2_HA_4), or the complement thereof.

The nucleotide sequence may comprise a sequence of SEQ ID NO:69, or the complement thereof.

There is also provided according to the invention an isolated polypeptide, which comprises an amino acid sequence of SEQ ID NO:68 (FLU_T2_HA_4), or an amino acid sequence that has at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% amino acid identity along its entire length with the sequence of SEQ ID NO:68.

There is also provided according to the invention an isolated polynucleotide, which comprises a nucleotide sequence of SEQ ID NO:69, or a nucleotide sequence that is at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical with SEQ ID NO:69, over its entire length, or the complement thereof.

H5 and H1 embodiments of the invention are referred to collectively as HA embodiments below.

Combination Vaccines

To prevent vaccine escape more effectively, vaccines with a combination of 2 or more (preferably 3 or more) evolutionarily constrained, computationally designed viral antigen targets are provided, each designed to independently give the maximum breadth of vaccine protection. Vaccines of the invention may comprise ancestral antigen based designs of HA, NA and M2, either alone or in combination. Furthermore, combinations of modified HA and NA antigen structures that are not predominantly found to circulate widely as natural combinations in humans are provided (e.g. a group 1 HA combined with a group 2 NA not found to circulate and to co-evolve together, such as H1N1 or H3N2).

Polypeptides or nucleic acid molecules of the invention may be combined in any suitable combination (for example, HA and/or M2 and/or neuraminidase embodiments of the invention, H5 and/or M2 and/or neuraminidase embodiments of the invention, H1 and/or M2 and/or neuraminidase embodiments of the invention, or FLU_T2_HA_3_I3 and/or Flu_T2_NA_3 and/or Flu_T2_M2_1 embodiments of the invention, or FLU_T2_HA_4 and/or Flu_T2_NA_3 and/or Flu_T2_M2_1 embodiments of the invention) to provide an influenza vaccine that protects against far more influenza strains than current vaccines. In some embodiments such combination vaccines protect against several influenza A and B variants (especially those embodiments that include M2 embodiments, as M2 is better conserved between influenza A and B).

Optionally, one embodiment of each different category of embodiment is used in combination. For example, an HA embodiment (H5 or H1), and/or an M2 embodiment and/or a neuraminidase embodiment.

Optionally a trivalent vaccine combines H5, M2, and neuraminidase embodiments of the invention.

Optionally a trivalent vaccine of the invention combines an H5 embodiment, an M2 embodiment, and a neuraminidase embodiment of the invention.

Optionally a trivalent vaccine combines H1, M2, and neuraminidase embodiments of the invention.

Optionally a trivalent vaccine of the invention combines an H1 embodiment, an M2 embodiment, and a neuraminidase embodiment of the invention.

Optionally a nucleic acid vector of the invention comprises:

- 5 i) a nucleic acid molecule encoding a polypeptide which comprises an amino acid sequence of SEQ ID NO:7 or 8, or a nucleic acid molecule encoding a polypeptide which comprises an amino acid sequence of SEQ ID NO:10 or 11, or a nucleic acid molecule encoding a polypeptide which comprises an amino acid sequence of SEQ ID NO:1 or 3, or a nucleic acid molecule encoding a polypeptide which comprises an amino acid sequence of SEQ ID NO:27 or 29, or a nucleic acid molecule encoding a polypeptide which comprises an amino acid sequence of SEQ ID NO:35 or 37, or a nucleic acid molecule encoding a polypeptide which comprises an amino acid sequence of SEQ ID NO:43 or 45 (examples of H5 embodiments); and/or
 - 10 ii) a nucleic acid molecule encoding a polypeptide which comprises an amino acid sequence of SEQ ID NO:14 (examples of M2 embodiments); and/or
 - 15 iii) a nucleic acid molecule encoding a polypeptide which comprises an amino acid sequence of SEQ ID NO:16, or a nucleic acid molecule encoding a polypeptide which comprises an amino acid sequence of SEQ ID NO:18 (examples of neuraminidase embodiments).
- 20 Optionally a nucleic acid vector of the invention comprises:
- i) a nucleic acid molecule encoding a polypeptide which comprises an amino acid sequence of SEQ ID NO:22, or a nucleic acid molecule encoding a polypeptide which comprises an amino acid sequence of SEQ ID NO:68 (examples of H1 embodiments); and/or
 - 25 ii) a nucleic acid molecule encoding a polypeptide which comprises an amino acid sequence of SEQ ID NO:14 (examples of M2 embodiments); and/or
 - iii) a nucleic acid molecule encoding a polypeptide which comprises an amino acid sequence of SEQ ID NO:16, or a nucleic acid molecule encoding a polypeptide which comprises an amino acid sequence of SEQ ID NO:18 (examples of
 - 30 neuraminidase embodiments).

Optionally a nucleic acid vector of the invention comprises:

i) a nucleic acid molecule which comprises nucleotide sequence encoding FLU_T2_HA_3_I3 amino acid sequence (SEQ ID NO:22), or the complement thereof; and/or

5 ii) a nucleic acid molecule which comprises nucleotide sequence encoding FLU_T2_NA_3 amino acid sequence (SEQ ID NO:16), or the complement thereof; and/or

iii) a nucleic acid molecule which comprises nucleotide sequence encoding FLU_T2_M2_1 amino acid sequence (SEQ ID NO:14), or the complement thereof.

Optionally a nucleic acid vector of the invention comprises:

10 i) a nucleic acid molecule which comprises a nucleotide sequence encoding an amino acid sequence of SEQ ID NO:22 (FLU_T2_HA_3_I3 amino acid sequence), or an amino acid sequence that has at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% amino acid identity along its entire length with the sequence of SEQ ID NO:22, or the complement thereof; and/or

15 ii) a nucleic acid molecule which comprises a nucleotide sequence encoding an amino acid sequence of SEQ ID NO:16 (FLU_T2_NA_3 amino acid sequence), or an amino acid sequence that has at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% amino acid identity along its entire length with the sequence of SEQ ID NO:16, or the complement thereof; and/or

20 iii) a nucleic acid molecule which comprises a nucleotide sequence encoding an amino acid sequence of SEQ ID NO:14 (FLU_T2_M2_1 amino acid sequence), or an amino acid sequence that has at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% amino acid identity along its entire length with the sequence of SEQ ID NO:14, or the complement thereof.

25 30 Optionally the nucleic acid molecule of (i) comprises a nucleotide sequence encoding an amino acid sequence of SEQ ID NO:22 (FLU_T2_HA_3_I3 amino acid sequence), or the complement thereof.

Optionally the nucleic acid molecule of (ii) comprises a nucleotide sequence encoding an amino acid sequence of SEQ ID NO:16 (FLU_T2_NA_3 amino acid sequence), or the complement thereof.

5 Optionally the nucleic acid molecule of (iii) comprises a nucleotide sequence encoding an amino acid sequence of SEQ ID NO:14 (FLU_T2_M2_1 amino acid sequence), or the complement thereof.

Optionally the nucleotide sequence encoding FLU_T2_HA_3_I3 amino acid sequence (SEQ ID NO:22), or the complement thereof, comprises the nucleotide sequence of SEQ ID NO:23, or the complement thereof.

10 Optionally the nucleotide sequence encoding FLU_T2_NA_3 amino acid sequence (SEQ ID NO:16), or the complement thereof, comprises the nucleotide sequence of SEQ ID NO:17, or the complement thereof.

15 Optionally the nucleotide sequence encoding FLU_T2_M2_1 amino acid sequence (SEQ ID NO:14), or the complement thereof, comprises the nucleotide sequence of SEQ ID NO:15, or the complement thereof.

Optionally a vector of the invention further comprises a promoter operably linked to each nucleic acid molecule.

Optionally a vector of the invention is a pEVAC-based vector.

20 The immune response may be humoral and/or a cellular immune response. A cellular immune response is a response of a cell of the immune system, such as a B-cell, T-cell, macrophage or polymorphonucleocyte, to a stimulus such as an antigen or vaccine. An immune response can include any cell of the body involved in a host defence response, including for example, an epithelial cell that secretes an interferon or a cytokine. An immune response includes, but is not limited to, an innate immune response or inflammation.

25 Optionally a polypeptide of the invention induces a protective immune response. A protective immune response refers to an immune response that protects a subject from infection or disease (i.e. prevents infection or prevents the development of disease associated with infection). Methods of measuring immune responses are well known in the art and include, for example, measuring proliferation and/or activity of lymphocytes (such as B or T cells),
30 secretion of cytokines or chemokines, inflammation, or antibody production.

Optionally a polypeptide of the invention is able to induce the production of antibodies and/or a T-cell response in a human or non-human animal to which the polypeptide has been administered (either as a polypeptide or, for example, expressed from an administered nucleic acid expression vector).

- 5 The similarity between amino acid or nucleic acid sequences is expressed in terms of the similarity between the sequences, otherwise referred to as sequence identity. Sequence identity is frequently measured in terms of percentage identity (or similarity or homology); the higher the percentage, the more similar the two sequences are. Homologs or variants of a given gene or protein will possess a relatively high degree of sequence identity when
- 10 aligned using standard methods. Methods of alignment of sequences for comparison are well known in the art. Various programs and alignment algorithms are described in: Smith and Waterman, *Adv. Appl. Math.* 2:482, 1981; Needleman and Wunsch, *J. Mol. Biol.* 48:443, 1970; Pearson and Lipman, *Proc. Natl. Acad. Sci. U.S.A.* 85:2444, 1988; Higgins and Sharp, *Gene* 73:237-244, 1988; Higgins and Sharp, *CABIOS* 5:151-153, 1989; Corpet
- 15 et al., *Nucleic Acids' Research* 16:10881-10890, 1988; and Pearson and Lipman, *Proc. Natl. Acad. Sci. U.S.A.* 85:2444, 1988. Altschul et al., *Nature Genet.* 6:119-129, 1994. The NCBI Basic Local Alignment Search Tool (BLAST) (Altschul et al., *J. Mol. Biol.* 215:403-410, 1990) is available from several sources, including the National Center for Biotechnology Information (NCBI, Bethesda, MD) and on the Internet, for use in connection
- 20 with the sequence analysis programs blastp, blastn, blastx, tblastn and tblastx.

Sequence identity between nucleic acid sequences, or between amino acid sequences, can be determined by comparing an alignment of the sequences. When an equivalent position in the compared sequences is occupied by the same nucleotide, or amino acid, then the molecules are identical at that position. Scoring an alignment as a percentage of

25 identity is a function of the number of identical nucleotides or amino acids at positions shared by the compared sequences. When comparing sequences, optimal alignments may require gaps to be introduced into one or more of the sequences to take into consideration possible insertions and deletions in the sequences. Sequence comparison methods may employ gap penalties so that, for the same number of identical molecules in sequences

30 being compared, a sequence alignment with as few gaps as possible, reflecting higher relatedness between the two compared sequences, will achieve a higher score than one with many gaps. Calculation of maximum percent identity involves the production of an optimal alignment, taking into consideration gap penalties.

Suitable computer programs for carrying out sequence comparisons are widely available in

35 the commercial and public sector. Examples include MatGat (Campanella et al., 2003,

BMC Bioinformatics 4: 29; program available from <http://bitincka.com/ledion/matgat>), Gap (Needleman & Wunsch, 1970, J. Mol. Biol. 48: 443-453), FASTA (Altschul et al., 1990, J. Mol. Biol. 215: 403-410; program available from <http://www.ebi.ac.uk/fasta>), Clustal W 2.0 and X 2.0 (Larkin et al., 2007, Bioinformatics 23: 2947-2948; program available from <http://www.ebi.ac.uk/tools/clustalw2>) and EMBOSS Pairwise Alignment Algorithms (Needleman & Wunsch, 1970, supra; Kruskal, 1983, In: Time warps, string edits and macromolecules: the theory and practice of sequence comparison, Sankoff & Kruskal (eds), pp 1-44, Addison Wesley; programs available from <http://www.ebi.ac.uk/tools/emboss/align>). All programs may be run using default parameters.

For example, sequence comparisons may be undertaken using the “needle” method of the EMBOSS Pairwise Alignment Algorithms, which determines an optimum alignment (including gaps) of two sequences when considered over their entire length and provides a percentage identity score. Default parameters for amino acid sequence comparisons (“Protein Molecule” option) may be Gap Extend penalty: 0.5, Gap Open penalty: 10.0, Matrix: Blosum 62.

The sequence comparison may be performed over the full length of the reference sequence.

Sequences described herein include reference to an amino acid sequence comprising amino acid residues “at positions corresponding to positions” of another amino acid sequence. Such corresponding positions may be identified, for example, from an alignment of the sequences using a sequence alignment method described herein, or another sequence alignment method known to the person of ordinary skill in the art.

Vectors

There is also provided according to the invention a vector comprising a nucleic acid molecule of the invention.

Optionally a vector of the invention comprises a nucleic acid molecule encoding a polypeptide which comprises an amino acid sequence of SEQ ID NO:27 or 29.

Optionally a vector of the invention comprises a nucleic acid molecule encoding a polypeptide which comprises an amino acid sequence of SEQ ID NO:35 or 37.

Optionally a vector of the invention comprises a nucleic acid molecule encoding a polypeptide which comprises an amino acid sequence of SEQ ID NO:43 or 45.

Optionally a vector of the invention comprises a nucleic acid molecule encoding a polypeptide which comprises an amino acid sequence of SEQ ID NO:7 or 8.

Optionally a vector of the invention comprises a nucleic acid molecule encoding a polypeptide which comprises an amino acid sequence of SEQ ID NO:10 or 11.

- 5 Optionally a vector of the invention further comprises a promoter operably linked to the nucleic acid.

Optionally the promoter is for expression of a polypeptide encoded by the nucleic acid in mammalian cells.

- 10 Optionally the promoter is for expression of a polypeptide encoded by the nucleic acid in yeast or insect cells.

Optionally the vector is a vaccine vector.

Optionally the vector is a viral vaccine vector, a bacterial vaccine vector, an RNA vaccine vector, an mRNA vaccine vector, or a DNA vaccine vector.

Optionally the vector is a DNA vector.

- 15 Optionally the vector is a mRNA vector.

A polynucleotide of the invention may comprise a DNA or an RNA molecule. For embodiments in which the polynucleotide comprises an RNA molecule, it will be appreciated that the nucleic acid sequence of the polynucleotide will be the same as that recited in the respective SEQ ID, or the complement thereof, but with each 'T' nucleotide replaced by 'U'.

- 20 As discussed in more detail below, a polynucleotide of the invention may include one or more modified nucleosides.

A polynucleotide of the invention may include one or more nucleotide analogs known to those of skill in the art.

- 25 A nucleic acid molecule of the invention may comprise a DNA or an RNA molecule. For embodiments in which the nucleic acid molecule comprises an RNA molecule, it will be appreciated that the molecule may comprise an RNA sequence that is at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical with, or identical with, any of SEQ ID NOs: 2, 4, or 6, in which each 'T' nucleotide is replaced by
30 'U', or the complement thereof.

For example, it will be appreciated that where an RNA vaccine vector comprising a nucleic acid of the invention is provided, the nucleic acid sequence of the nucleic acid of the invention will be an RNA sequence, so may comprise for example an RNA nucleic acid sequence that is at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical with, or identical with, any of SEQ ID NOs: 2, 4, or 6 in which each 'T' nucleotide is replaced by 'U', or the complement thereof.

Viral vaccine vectors use viruses to deliver nucleic acid (for example, DNA or RNA) into human or non-human animal cells. The nucleic acid contained in the virus encodes one or more antigens that, once expressed in the infected human or non-human animal cells, elicit an immune response. Both humoral and cell-mediated immune responses can be induced by viral vaccine vectors. Viral vaccine vectors combine many of the positive qualities of nucleic acid vaccines with those of live attenuated vaccines. Like nucleic acid vaccines, viral vaccine vectors carry nucleic acid into a host cell for production of antigenic proteins that can be tailored to stimulate a range of immune responses, including antibody, T helper cell (CD4⁺ T cell), and cytotoxic T lymphocyte (CTL, CD8⁺ T cell) mediated immunity. Viral vaccine vectors, unlike nucleic acid vaccines, also have the potential to actively invade host cells and replicate, much like a live attenuated vaccine, further activating the immune system like an adjuvant. A viral vaccine vector therefore generally comprises a live attenuated virus that is genetically engineered to carry nucleic acid (for example, DNA or RNA) encoding protein antigens from an unrelated organism. Although viral vaccine vectors are generally able to produce stronger immune responses than nucleic acid vaccines, for some diseases viral vectors are used in combination with other vaccine technologies in a strategy called heterologous prime-boost. In this system, one vaccine is given as a priming step, followed by vaccination using an alternative vaccine as a booster. The heterologous prime-boost strategy aims to provide a stronger overall immune response. Viral vaccine vectors may be used as both prime and boost vaccines as part of this strategy. Viral vaccine vectors are reviewed by Ura *et al.*, 2014 (*Vaccines* **2014**, 2, 624-641) and Choi and Chang, 2013 (*Clinical and Experimental Vaccine Research* 2013;2:97-105).

Optionally the viral vaccine vector is based on a viral delivery vector, such as a Poxvirus (for example, Modified Vaccinia Ankara (MVA), NYVAC, AVIPOX), herpesvirus (e.g. HSV, CMV, Adenovirus of any host species), Morbillivirus (e.g. measles), Alphavirus (e.g. SFV, Sendai), Flavivirus (e.g. Yellow Fever), or Rhabdovirus (e.g. VSV)-based viral delivery vector, a bacterial delivery vector (for example, Salmonella, *E.coli*), an RNA expression vector, or a DNA expression vector.

Adenoviruses are by far the most utilised and advanced viral vectors developed for SARS2 vaccines. They are non-enveloped double-stranded DNA (dsDNA) viruses with a packaging capacity of up to 7.5 kb of foreign genes. Recombinant Adenovirus vectors are widely used because of their high transduction efficiency, high level of transgene expression, and broad
 5 range of viral tropism. These vaccines are highly cell specific, highly efficient in gene transduction, and efficient at inducing an immune response. Adenovirus vaccines are effective at triggering and priming T cells, leading to long term and high level of antigenic protein expression and therefore long lasting protection.

Where viral vectors are used in accordance with the invention, it may be advantageous that
 10 each HA and/or M2 and/or neuraminidase embodiment of the invention, H5 and/or M2 and/or neuraminidase embodiment of the invention, H1 and/or M2 and/or neuraminidase embodiment of the invention, or FLU_T2_HA_3_I3 and/or Flu_T2_NA_3 and/or Flu_T2_M2_1 embodiment of the invention, or FLU_T2_HA_4 and/or Flu_T2_NA_3 and/or Flu_T2_M2_1 embodiment of the invention, is encoded as part of the same viral vaccine
 15 vector. For example, it may be easier (and less costly) to make a single vector encoding each of the H5, M2, and neuraminidase embodiments, than several different vectors, each encoding a different H5, M2, or neuraminidase embodiment.

Optionally the nucleic acid expression vector is a nucleic acid expression vector, and a viral pseudotype vector.

20 Optionally the nucleic acid expression vector is a vaccine vector.

Optionally the nucleic acid expression vector comprises, from a 5' to 3' direction: a promoter; a splice donor site (SD); a splice acceptor site (SA); and a terminator signal, wherein the multiple cloning site is located between the splice acceptor site and the terminator signal.

25 Optionally the promoter comprises a CMV immediate early 1 enhancer/promoter (CMV-IE-E/P) and/or the terminator signal comprises a terminator signal of a bovine growth hormone gene (Tbgh) that lacks a KpnI restriction endonuclease site.

Optionally the nucleic acid expression vector further comprises an origin of replication, and nucleic acid encoding resistance to an antibiotic. Optionally the origin of replication
 30 comprises a pUC-plasmid origin of replication and/or the nucleic acid encodes resistance to kanamycin.

Optionally the vector is a pEVAC-based expression vector.

Optionally the nucleic acid expression vector comprises a nucleic acid sequence of SEQ ID NO:21 (pEVAC). The pEVAC vector has proven to be a highly versatile expression vector for generating viral pseudotypes as well as direct DNA vaccination of animals and humans. The pEVAC expression vector is described in more detail in Example 11 below. Figure 8

5 shows a plasmid map for pEVAC.

The terms “polynucleotide” and “nucleic acid” are used interchangeably herein.

Optionally the, or each vaccine vector is an RNA vaccine vector.

Optionally the, or each vaccine vector is an mRNA vaccine vector.

A polynucleotide of the invention may comprise a DNA molecule.

- 10 The or each polynucleotide of a pharmaceutical composition, a combined preparation, or a vector, of the invention may comprise a DNA molecule.

A vector of the invention may be a DNA vector.

The or each vector of a pharmaceutical composition or a combined preparation of the invention may be a DNA vector.

- 15 A polynucleotide of the invention, or a polynucleotide of a pharmaceutical composition, a combined preparation, or a vector, of the invention, may be provided as part of a DNA vaccine.

There is also provided according to the invention a DNA vaccine which comprises a polynucleotide of the invention, a vector of the invention, or a pharmaceutical composition or

20 a combined preparation of the invention which comprises one or more polynucleotides, wherein the or each polynucleotide is a DNA molecule.

A polynucleotide of the invention may comprise an RNA molecule.

The or each polynucleotide of a pharmaceutical composition, a combined preparation, or a vector, of the invention may comprise an RNA molecule.

- 25 A vector of the invention may be an RNA vector.

The or each vector of a pharmaceutical composition or a combined preparation of the invention may be an RNA vector.

A polynucleotide of the invention, or a polynucleotide of a pharmaceutical composition, a combined preparation, or a vector, of the invention, may be provided as part of an RNA vaccine.

5 There is also provided according to the invention an RNA vaccine which comprises a polynucleotide of the invention, a vector of the invention, or a pharmaceutical composition or a combined preparation of the invention which comprises one or more polynucleotides, wherein the or each polynucleotide is an RNA molecule.

A polynucleotide of the invention may comprise an mRNA molecule.

10 The or each polynucleotide of a pharmaceutical composition, a combined preparation, or a vector, of the invention may comprise an mRNA molecule.

A vector of the invention may be an mRNA vector.

The or each vector of a pharmaceutical composition or a combined preparation of the invention may be an mRNA vector.

Messenger RNA (mRNA) vaccines

15 A polynucleotide of the invention, or a polynucleotide of a pharmaceutical composition, a combined preparation, or a vector, of the invention, may be provided as part of an mRNA vaccine.

20 There is also provided according to the invention an mRNA vaccine which comprises a polynucleotide of the invention, a vector of the invention, or a pharmaceutical composition or a combined preparation of the invention which comprises one or more polynucleotides, wherein the or each polynucleotide comprises an mRNA molecule.

Messenger RNA (mRNA) vaccines are a new form of vaccine (recently reviewed in Pardi *et al.*, *Nature Reviews Drug Discovery* Volume **17**, pages 261–279(2018); Wang *et al.*, *Molecular Cancer* (2021) **20**:33: mRNA vaccine: a potential therapeutic strategy). The first mRNA vaccines to be approved for use were BNT162b2 (BioNTech's vaccine manufactured by Pfizer) and mRNA-1273 (manufactured by Moderna) during the COVID-19 pandemic. mRNA vaccines have a unique feature of temporarily promoting the expression of antigen (typically days). The expression of the exogenous antigen is controlled by the lifetime of encoding mRNA, which is regulated by cellular degradation pathways. While this transient nature of protein expression requires repeated administration for the treatment of genetic diseases and cancers, it is extremely beneficial for vaccines, where prime or prime-boost

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vaccination is sufficient to develop highly specific adaptive immunity without any exposure to the contagion.

mRNA based vaccines trigger an immune response after the synthetic mRNA which encodes viral antigens transfects human cells. The cytosolic mRNA molecules are then translated by the host's own cellular machinery into specific viral antigens. These antigens may then be presented on the cell surface where they can be recognised by immune cells, triggering an immune response.

The structural elements of a vaccine vector mRNA molecule are similar to those of natural mRNA, comprising a 5' cap, 5' untranslated region (UTR), coding region (for example, comprising an open reading frame encoding a polypeptide of the invention), 3' UTR, and a poly(A) tail. The 5' UTR (also known as a leader sequence, transcript leader, or leader RNA) is the region of an mRNA that is directly upstream from the initiation codon. This region is important for the regulation of translation of a transcript. In many organisms, the 5' UTR forms complex secondary structure to regulate translation. The 5' UTR begins at the transcription start site and ends one nucleotide (nt) before the initiation sequence (usually AUG) of the coding region. In eukaryotes, the length of the 5' UTR tends to be anywhere from 100 to several thousand nucleotides long. The differing sizes are likely due to the complexity of the eukaryotic regulation which the 5' UTR holds as well as the larger pre-initiation complex that must form to begin translation. The eukaryotic 5' UTR contains the Kozak consensus sequence (ACCAAUG (initiation codon underlined) (SEQ ID NO:36), which contains the initiation codon AUG. The constructs described herein contain an elongated Kozak sequence: GCCACCAAUG (initiation codon underlined) (SEQ ID NO:37).

Two major types of RNA are currently studied as vaccines: non-replicating mRNA and virally derived, self-amplifying RNA. While both types of vaccines share a common structure in mRNA constructs, self-amplifying RNA vaccines contain additional sequences in the coding region for RNA replication, including RNA-dependent RNA polymerases.

BNT162b2 vaccine construct comprises a lipid nanoparticle (LNP) encapsulated mRNA molecule encoding trimerised full-length SARS2 S protein with a PP mutation (at residue positions 986-987). The mRNA is encapsulated in 80 nm ionizable cationic lipid nanoparticles. mRNA-1273 vaccine construct is also based on an LNP vector, but the synthetic mRNA encapsulated within the lipid construct encodes the full-length SARS2 S protein.

US Patent No. 10,702,600 B1 (ModernaTX) describes betacoronavirus mRNA vaccines, including suitable LNPs for use in such vaccines.

A nucleic acid vaccine (for example, a mRNA) of the invention may be formulated in a lipid nanoparticle.

mRNA vaccines have several advantages in comparison with conventional vaccines containing inactivated (or live attenuated) disease-causing organisms. Firstly, mRNA-based vaccines can be rapidly developed due to design flexibility and the ability of the constructs to mimic antigen structure and expression as seen in the course of a natural infection. mRNA vaccines can be developed within days or months based on sequencing information from a target virus, while conventional vaccines often take years and require a deep understanding of the target virus to make the vaccine effective and safe. Secondly, these novel vaccines can be rapidly produced. Due to high yields from *in vitro* transcription reactions, mRNA production can be rapid, inexpensive and scalable (due to chemical synthesis rather than biological growth of cells or bacteria). Thirdly, vaccine risks are low. mRNA does not contain infectious viral elements or cell debris that pose risks for infection and insertional mutagenesis (as the mRNA is generated synthetically). Anti-vector immunity is also avoided as mRNA is the minimally immunogenic genetic vector, allowing repeated administration of the vaccine. The challenge for effective application of mRNA vaccines lies in cytosolic delivery. mRNA isolates are rapidly degraded by extracellular RNases and cannot penetrate cell membranes to be transcribed in the cytosol. However, efficient *in vivo* delivery can be achieved by formulating mRNA into carrier molecules, allowing rapid uptake and expression in the cytoplasm. To date, numerous delivery methods have been developed including lipid-, polymer-, or peptide-based delivery, virus-like replicon particle, cationic nanoemulsion, naked mRNAs, and dendritic cell-based delivery (each reviewed in Wang *et al.*, *supra*). Decationic lipid nanoparticle (LNP) delivery is the most appealing and commonly used mRNA vaccine delivery tool.

Exogenous mRNA may be highly immunostimulatory. Single-stranded RNA (ssRNA) molecules are considered a pathogen associated molecular pattern (PAMP), and are recognised by various Toll-like receptors (TLR) which elicit a pro-inflammatory reaction. Although a strong cellular and humoral immune response is desirable in response to vaccination, the innate immune reaction elicited by exogenous mRNA may cause undesirable side-effects in the subject. The U-rich sequence of mRNA is a key element to activate TLR (Wang *et al.*, *supra*). Additionally, enzymatically synthesised mRNA preparations contain double stranded RNA (dsRNA) contaminants as aberrant products of the *in vitro* transcription (IVT) process. dsRNA is a potent PAMP, and elicits downstream reactions resulting in the inhibition of translation and the degradation of cellular mRNA and ribosomal RNA (Pardi *et al.*, *supra*). Thus, the mRNA may suppress antigen expression and thus reduce vaccine efficacy.

Studies over the past decade have shown that the immunostimulatory effect of mRNA can be shaped by the purification of IVT mRNA, the introduction of modified nucleosides, complexing the mRNA with various carrier molecules (Pardi *et al.*, *supra*), adding poly(A) tails or optimising mRNA with GC-rich sequence (Wang *et al.*, *supra*). Chemical modification of uridine is a common approach to minimise the immunogenicity of foreign mRNA. Incorporation of pseudouridine (ψ) and N1- methylpseudouridine (m1 ψ) to IVT mRNA prevents TLR activation and other innate immune sensors, thus reducing pro-inflammatory signalling in response to the exogenous mRNA. Such nucleoside modification also suppresses recognition of dsRNA species (Pardi *et al.*, *supra*) and can reduce innate immune sensing of exogenous mRNA translation (Hou *et al.* *Nature Reviews Materials*, 2021, <https://doi.org/10.1038/s41578-021-00358-0>).

Other nucleoside chemical modifications include, but are not limited to, 5-methylcytidine (m5C), 5-methyluridine (m5U), N1-methyladenosine (m1A), N6- methyladenosine (m6A), 2-thiouridine (s2U), and 5-methoxyuridine (5moU) (Wang *et al.*, *supra*).

The IVT mRNA molecules used in the mRNA-1273 and BNT162b2 COVID-19 vaccines were prepared by replacing uridine with m1 ψ , and their sequences were optimized to encode a stabilized pre- fusion spike protein with two pivotal proline substitutions (Hou *et al.*, *supra*). However, CureVac's mRNA vaccine candidate, CVnCoV, uses unmodified nucleosides and relies on a combination of mRNA sequence alterations to allow immune evasion without affecting the expressed protein. Firstly, CVnCoV has a higher GC content (63%) than rival vaccines (BNT162b2 has 56%) and the original SARS-CoV-2 virus itself (37%). Secondly, the vaccine comprises C-rich motifs which bind to poly(C)-binding protein, enhancing both the stability and expression of the mRNA. A further modification of CVnCoV is that it contains a histone stem-loop sequence as well as a poly(A) tail, to enhance the longevity and translation of the mRNA (Hubert, B., 2021. The CureVac Vaccine, and a brief tour through some of the wonders of nature. URL <https://berthub.eu/articles/posts/curevac-vaccine-and-wonders-of-biology/>.(accessed 15.09.21). However, the vaccine had disappointing results from phase III clinical trials, which experts assert are down to the decision not to incorporate chemically modified nucleosides into the mRNA sequence. Nonetheless, CureVac and Acuitas Therapeutics delivered erythropoietin (EPO)-encoding mRNA, which has rich GC codons, to pigs with lipid nanoparticles (LNPs). Their results indicated EPO-related responses were elicited without immunogenicity (Wang *et al.*, *supra*), suggesting that there is still scope for unmodified mRNA nucleoside-based vaccines.

A polynucleotide of the invention may comprise an mRNA molecule.

The or each polynucleotide of a pharmaceutical composition, a combined preparation, or a vector, of the invention may comprise an mRNA molecule.

A vector of the invention may be an mRNA vector.

5 The or each vector of a pharmaceutical composition or a combined preparation of the invention may be an mRNA vector.

A polynucleotide of the invention, or a polynucleotide of a pharmaceutical composition, a combined preparation, or a vector, of the invention, may be provided as part of an mRNA vaccine.

10 There is also provided according to the invention an mRNA vaccine which comprises a polynucleotide of the invention, a vector of the invention, or a pharmaceutical composition or a combined preparation of the invention which comprises one or more polynucleotides, wherein the or each polynucleotide comprises an mRNA molecule.

15 RNA or mRNA of a polynucleotide of the invention, or of a polynucleotide of a pharmaceutical composition, a combined preparation, a vector, or a vaccine, of the invention may be produced by *in vitro* transcription (IVT).

A polynucleotide of the invention, or a polynucleotide of a pharmaceutical composition, a combined preparation, a vector, or a vaccine, of the invention may comprise one or more modified nucleosides.

20 The one or more modified nucleosides may be present in DNA or RNA of a polynucleotide of the invention, or of a polynucleotide of a pharmaceutical composition, a combined preparation, a vector, or a vaccine, of the invention.

Optionally, at least one chemical modification is selected from pseudouridine, N1-methylpseudouridine, N1-ethylpseudouridine, 2-thiouridine, 4'-thiouridine, 5-methylcytosine, 5-methyluridine, 2-thio-1-methyl-1-deaza-pseudouridine, 2-thio-1-methyl-pseudouridine, 2-thio-5-aza-uridine, 2-thio-dihydropseudouridine, 2-thio-dihydrouridine, 2-thio-pseudouridine, 4-methoxy-2-thio-pseudouridine, 4-methoxy-pseudouridine, 4-thio-1-methyl-pseudouridine, 4-thio-pseudouridine, 5-aza-uridine, dihydropseudouridine, 5-methoxyuridine and 2'-O-methyl uridine. In some embodiments, the chemical modification is in the 5-position of the uracil. In some embodiments, the chemical modification is a N1-methylpseudouridine. In some embodiments, the chemical modification is a N1-ethylpseudouridine.

25

30

For example, an RNA or an mRNA of a polynucleotide of the invention, or of a polynucleotide of a pharmaceutical composition, a combined preparation, a vector, or a vaccine, of the invention may comprise one or more of the following modified nucleosides:

pseudouridine (ψ);

5 N1- methylpseudouridine (m1 ψ)

5-methylcytidine (m5C)

5-methyluridine (m5U)

N1-methyladenosine (m1A)

N6- methyladenosine (m6A)

10 2-thiouridine (s2U)

5- methoxyuridine (5moU)

In some embodiments, 100% of the uracil in the open reading frame have a chemical modification. In some embodiments, a chemical modification is in the 5-position of the uracil. In some embodiments, a chemical modification is a N1-methyl pseudouridine. In some
15 embodiments, 100% of the uracil in the open reading frame have a N1-methyl pseudouridine in the 5-position of the uracil.

The polynucleotide may contain from about 1% to about 100% modified nucleotides (or nucleosides) (either in relation to overall nucleotide content, or in relation to one or more types of nucleotide, i.e., any one or more of A, G, U or C) or any intervening percentage (e.g.,
20 from 1% to 20%, from 1% to 25%, from 1% to 50%, from 1% to 60%, from 1% to 70%, from 1% to 80%, from 1% to 90%, from 1% to 95%, from 10% to 20%, from 10% to 25%, from 10% to 50%, from 10% to 60%, from 10% to 70%, from 10% to 80%, from 10% to 90%, from 10% to 95%, from 10% to 100%, from 20% to 25%, from 20% to 50%, from 20% to 60%, from 20% to 70%, from 20% to 80%, from 20% to 90%, from 20% to 95%, from 20% to 100%,
25 from 50% to 60%, from 50% to 70%, from 50% to 80%, from 50% to 90%, from 50% to 95%, from 50% to 100%, from 70% to 80%, from 70% to 90%, from 70% to 95%, from 70% to 100%, from 80% to 90%, from 80% to 95%, from 80% to 100%, from 90% to 95%, from 90% to 100%, and from 95% to 100%). Any remaining percentage is accounted for by the presence of unmodified A, G, U, or C.

Optionally a polynucleotide of the invention, or of a polynucleotide of a pharmaceutical composition, a combined preparation, a vector, or a vaccine, of the invention, comprises an RNA molecule in which the nucleic acid sequence of the polynucleotide is the same as that recited in the respective SEQ ID, or the complement thereof, but with each 'U' replaced by m1 ψ .

Optionally a polynucleotide of the invention, or of a polynucleotide of a pharmaceutical composition, a combined preparation, a vector, or a vaccine, of the invention, comprises an mRNA molecule in which the nucleic acid sequence of the polynucleotide is the same as that recited in the respective SEQ ID, or the complement thereof, but with each 'U' replaced by m1 ψ .

Optionally a polynucleotide of the invention, or of a polynucleotide of a pharmaceutical composition, a combined preparation, a vector, or a vaccine, of the invention, comprises an RNA molecule in which the nucleic acid sequence of the polynucleotide is the same as that recited in the respective SEQ ID, or the complement thereof, but with at least 50% of the 'U's replaced by m1 ψ . The remaining 'U's may all be unmodified, or may comprise unmodified and one or more other modified nucleosides.

Optionally a polynucleotide of the invention, or of a polynucleotide of a pharmaceutical composition, a combined preparation, a vector, or a vaccine, of the invention, comprises an mRNA molecule in which the nucleic acid sequence of the polynucleotide is the same as that recited in the respective SEQ ID, or the complement thereof, but with at least 50% of the 'U's replaced by m1 ψ . The remaining 'U's may all be unmodified, or may comprise unmodified and one or more other modified nucleosides.

Optionally a polynucleotide of the invention, or of a polynucleotide of a pharmaceutical composition, a combined preparation, a vector, or a vaccine, of the invention, comprises an RNA molecule in which the nucleic acid sequence of the polynucleotide is the same as that recited in the respective SEQ ID, or the complement thereof, but with at least 90% of the 'U's replaced by m1 ψ . The remaining 'U's may all be unmodified, or may comprise unmodified and one or more other modified nucleosides.

Optionally a polynucleotide of the invention, or of a polynucleotide of a pharmaceutical composition, a combined preparation, a vector, or a vaccine, of the invention, comprises an mRNA molecule in which the nucleic acid sequence of the polynucleotide is the same as that recited in the respective SEQ ID, or the complement thereof, but with at least 90% of the 'U's replaced by m1 ψ . The remaining 'U's may all be unmodified, or may comprise unmodified and one or more other modified nucleosides.

mRNA vaccines of the invention may be co-administered with an immunological adjuvant, for example MF59 (Novartis), TriMix, RNActive (CureVac AG), RNAdjuvant (again reviewed in Wang *et al.*, *supra*).

Thus, in preferred embodiments, each vector of a pharmaceutical composition, or combined
5 preparation, of the invention is an mRNA vaccine vector.

There is also provided according to the invention an isolated cell comprising or transfected with a vector of the invention.

There is also provided according to the invention a fusion protein comprising a polypeptide of the invention.

10 There is also provided according to the invention a pseudotyped virus comprising a polypeptide of the invention.

Pharmaceutical Compositions

According to the invention there is also provided a pharmaceutical composition comprising a polypeptide of the invention, and a pharmaceutically acceptable carrier, excipient, or
15 diluent.

A pharmaceutical composition of the invention may include polypeptides of the invention in any suitable combination (for example, HA and/or M2 and/or neuraminidase embodiments of the invention, H5 and/or M2 and/or neuraminidase embodiments of the invention, H1 and/or M2 and/or neuraminidase embodiments of the invention, or FLU_T2_HA_3_I3 and/or
20 Flu_T2_NA_3 and/or Flu_T2_M2_1 embodiments of the invention, or FLU_T2_HA_4 and/or Flu_T2_NA_3 and/or Flu_T2_M2_1 embodiments of the invention).

Optionally, one embodiment of each different category of embodiment is used in combination. For example, an HA embodiment (H5 or H1), and/or an M2 embodiment and/or a neuraminidase embodiment. Optionally a pharmaceutical composition of the
25 invention comprises:

- i) a polypeptide which comprises an amino acid sequence of SEQ ID NO:7 or 8, or a polypeptide which comprises an amino acid sequence of SEQ ID NO:10 or 11, or a polypeptide which comprises an amino acid sequence of SEQ ID NO:1 or 3 (examples of H5 embodiments); and/or
- 30 ii) a polypeptide which comprises an amino acid sequence of SEQ ID NO:14 (examples of M2 embodiments); and/or

- iii) a polypeptide which comprises an amino acid sequence of SEQ ID NO:16, a polypeptide which comprises an amino acid sequence of SEQ ID NO:18 (examples of neuraminidase embodiments).

Optionally a pharmaceutical composition of the invention comprises:

- 5 i) a polypeptide which comprises an amino acid sequence of SEQ ID NO:7 or 8, or a polypeptide which comprises an amino acid sequence of SEQ ID NO:10 or 11, or a polypeptide which comprises an amino acid sequence of SEQ ID NO:1 or 3, or a polypeptide which comprises an amino acid sequence of SEQ ID NO:27 or 29, or a polypeptide which comprises an amino acid sequence of SEQ ID NO:35 or 37, or a polypeptide which comprises an amino acid sequence of SEQ ID NO:43 or 45 (examples of H5 embodiments); and/or
- 10 ii) a polypeptide which comprises an amino acid sequence of SEQ ID NO:14 (examples of M2 embodiments); and/or
- 15 iii) a polypeptide which comprises an amino acid sequence of SEQ ID NO:16, a polypeptide which comprises an amino acid sequence of SEQ ID NO:18 (examples of neuraminidase embodiments).

Optionally a pharmaceutical composition of the invention comprises:

- 20 i) a polypeptide which comprises an amino acid sequence of SEQ ID NO:22, or a polypeptide which comprises an amino acid sequence of SEQ ID NO:68 (examples of H1 embodiments); and/or
- ii) a polypeptide which comprises an amino acid sequence of SEQ ID NO:14 (examples of M2 embodiments); and/or
- 25 iii) a polypeptide which comprises an amino acid sequence of SEQ ID NO:16, or a polypeptide which comprises an amino acid sequence of SEQ ID NO:18 (examples of neuraminidase embodiments).

Optionally a pharmaceutical composition of the invention comprises:

- i) a polypeptide which comprises an amino acid sequence of SEQ ID NO:22 (FLU_T2_HA_3_I3 amino acid sequence); and/or
- 30 ii) a polypeptide which comprises an amino acid sequence of SEQ ID NO:14 (FLU_T2_M2_1 amino acid sequence); and/or
- iii) a polypeptide which comprises an amino acid sequence of SEQ ID NO:16 (FLU_T2_NA_3 amino acid sequence).

Optionally a pharmaceutical composition of the invention comprises:

- i) a polypeptide which comprises an amino acid sequence of SEQ ID NO:22 (FLU_T2_HA_3_I3 amino acid sequence), or an amino acid sequence that has at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% amino acid identity along its entire length with the sequence of SEQ ID NO:22; and/or
- ii) a polypeptide which comprises an amino acid sequence of SEQ ID NO:14 (FLU_T2_M2_1 amino acid sequence), or an amino acid sequence that has at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% amino acid identity along its entire length with the sequence of SEQ ID NO:14; and/or
- iii) a polypeptide which comprises an amino acid sequence of SEQ ID NO:16 (FLU_T2_NA_3 amino acid sequence), or an amino acid sequence that has at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% amino acid identity along its entire length with the sequence of SEQ ID NO:16.

Optionally the polypeptide of (i) comprises an amino acid sequence of SEQ ID NO:22 (FLU_T2_HA_3_I3 amino acid sequence).

Optionally the polypeptide of (ii) comprises an amino acid sequence of SEQ ID NO:14 (FLU_T2_M2_1 amino acid sequence).

Optionally the polypeptide of (iii) comprises an amino acid sequence of SEQ ID NO:16 (FLU_T2_NA_3 amino acid sequence).

According to the invention there is also provided a pharmaceutical composition comprising a nucleic acid of the invention, and a pharmaceutically acceptable carrier, excipient, or diluent.

A pharmaceutical composition of the invention may include nucleic acid molecules of the invention in any suitable combination (for example, HA and/or M2 and/or neuraminidase embodiments of the invention, H5 and/or M2 and/or neuraminidase embodiments of the invention, H1 and/or M2 and/or neuraminidase embodiments of the invention, or FLU_T2_HA_3_I3 and/or Flu_T2_NA_3 and/or Flu_T2_M2_1 embodiments of the invention, or FLU_T2_HA_4 and/or Flu_T2_NA_3 and/or Flu_T2_M2_1 embodiments of the invention).

Optionally, one embodiment of each different category of embodiment is used in combination. For example, an HA embodiment (H5 or H1), and/or an M2 embodiment and/or a neuraminidase embodiment.

Optionally a pharmaceutical composition of the invention comprises:

- 5 i) a nucleic acid molecule encoding a polypeptide which comprises an amino acid sequence of SEQ ID NO:7 or 8, or a nucleic acid molecule encoding a polypeptide which comprises an amino acid sequence of SEQ ID NO:10 or 11, or a nucleic acid molecule encoding a polypeptide which comprises an amino acid sequence of SEQ ID NO:1 or 3 (examples of H5 embodiments); and/or
- 10 ii) a nucleic acid molecule encoding a polypeptide which comprises an amino acid sequence of SEQ ID NO:14 (examples of M2 embodiments); and/or
- iii) a nucleic acid molecule encoding a polypeptide which comprises an amino acid sequence of SEQ ID NO:16, or a nucleic acid molecule encoding a polypeptide which comprises an amino acid sequence of SEQ ID NO:18 (examples of
- 15 neuraminidase embodiments).

Optionally a pharmaceutical composition of the invention comprises:

- i) a nucleic acid molecule encoding a polypeptide which comprises an amino acid sequence of SEQ ID NO:7 or 8, or a nucleic acid molecule encoding a polypeptide which comprises an amino acid sequence of SEQ ID NO:10 or 11,
- 20 or a nucleic acid molecule encoding a polypeptide which comprises an amino acid sequence of SEQ ID NO:1 or 3, or a nucleic acid molecule encoding a polypeptide which comprises an amino acid sequence of SEQ ID NO:27 or 29,
- ii) a nucleic acid molecule encoding a polypeptide which comprises an amino acid sequence of SEQ ID NO:35 or 37, or a nucleic acid molecule encoding a polypeptide which comprises an amino acid sequence of SEQ ID NO:43 or 45 (examples of H5 embodiments); and/or
- 25 or a nucleic acid molecule encoding a polypeptide which comprises an amino acid sequence of SEQ ID NO:14 (examples of M2 embodiments); and/or
- iii) a nucleic acid molecule encoding a polypeptide which comprises an amino acid sequence of SEQ ID NO:16, or a nucleic acid molecule encoding a polypeptide which comprises an amino acid sequence of SEQ ID NO:18 (examples of
- 30 neuraminidase embodiments).

Optionally a pharmaceutical composition of the invention comprises:

- i) a nucleic acid molecule encoding a polypeptide which comprises an amino acid sequence of SEQ ID NO:22, or a nucleic acid molecule encoding a polypeptide which comprises an amino acid sequence of SEQ ID NO:68 (examples of H1 embodiments); and/or
- 5 ii) a nucleic acid molecule encoding a polypeptide which comprises an amino acid sequence of SEQ ID NO:14 (examples of M2 embodiments); and/or
- iii) a nucleic acid molecule encoding a polypeptide which comprises an amino acid sequence of SEQ ID NO:16, or a nucleic acid molecule encoding a polypeptide which comprises an amino acid sequence of SEQ ID NO:18 (examples of
- 10 neuraminidase embodiments).

Optionally a pharmaceutical composition of the invention comprises:

- i) an isolated polynucleotide which comprises nucleotide sequence encoding FLU_T2_HA_3_I3 amino acid sequence (SEQ ID NO:22), or the complement thereof; and/or
- 15 ii) an isolated polynucleotide which comprises nucleotide sequence encoding FLU_T2_NA_3 amino acid sequence (SEQ ID NO:16), or the complement thereof; and/or
- iii) an isolated polynucleotide which comprises nucleotide sequence encoding FLU_T2_M2_1 amino acid sequence (SEQ ID NO:14), or the complement thereof.

20 According to the invention there is also provided a pharmaceutical composition which comprises:

- i) an isolated polynucleotide which comprises nucleotide sequence encoding FLU_T2_HA_3_I3 amino acid sequence (SEQ ID NO:22), or the complement thereof; and
- ii) an isolated polynucleotide which comprises nucleotide sequence encoding FLU_T2_NA_3 amino acid sequence (SEQ ID NO:16), or the complement thereof.
- 25 According to the invention there is also provided a pharmaceutical composition which comprises:
- i) an isolated polynucleotide which comprises nucleotide sequence encoding FLU_T2_HA_3_I3 amino acid sequence (SEQ ID NO:22), or the complement thereof; and
- ii) an isolated polynucleotide which comprises nucleotide sequence encoding
- 30 FLU_T2_M2_1 amino acid sequence (SEQ ID NO:14), or the complement thereof.

According to the invention there is also provided a pharmaceutical composition which comprises:

- i) an isolated polynucleotide which comprises nucleotide sequence encoding FLU_T2_NA_3 amino acid sequence (SEQ ID NO:16), or the complement thereof; and
- 5 ii) an isolated polynucleotide which comprises nucleotide sequence encoding FLU_T2_M2_1 amino acid sequence (SEQ ID NO:14), or the complement thereof.

According to the invention there is also provided a pharmaceutical composition which comprises:

- i) an isolated polynucleotide which comprises nucleotide sequence encoding
- 10 FLU_T2_HA_3_I3 amino acid sequence (SEQ ID NO:22), or the complement thereof;
- ii) an isolated polynucleotide which comprises nucleotide sequence encoding FLU_T2_NA_3 amino acid sequence (SEQ ID NO:16), or the complement thereof; and
- iii) an isolated polynucleotide which comprises nucleotide sequence encoding FLU_T2_M2_1 amino acid sequence (SEQ ID NO:14), or the complement thereof.

15 Optionally a pharmaceutical composition of the invention comprises:

- i) an isolated polynucleotide which comprises a nucleotide sequence encoding an amino acid sequence of SEQ ID NO:22 (FLU_T2_HA_3_I3 amino acid sequence), or an amino acid sequence that has at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 20 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% amino acid identity along its entire length with the sequence of SEQ ID NO:22, or the complement thereof; and/or
- ii) an isolated polynucleotide which comprises a nucleotide sequence encoding an amino acid sequence of SEQ ID NO:16 (FLU_T2_NA_3 amino acid sequence), 25 or an amino acid sequence that has at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% amino acid identity along its entire length with the sequence of SEQ ID NO:16, or the complement thereof; and/or
- 30 iii) an isolated polynucleotide which comprises a nucleotide sequence encoding an amino acid sequence of SEQ ID NO:14 (FLU_T2_M2_1 amino acid sequence), or an amino acid sequence that has at least 70%, 71%, 72%, 73%, 74%, 75%,

76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% amino acid identity along its entire length with the sequence of SEQ ID NO:14, or the complement thereof.

- 5 Optionally the polynucleotide of (i) comprises a nucleotide sequence encoding an amino acid sequence of SEQ ID NO:22 (FLU_T2_HA_3_I3 amino acid sequence), or the complement thereof.

- 10 Optionally the polynucleotide of (ii) comprises a nucleotide sequence encoding an amino acid sequence of SEQ ID NO:16 (FLU_T2_NA_3 amino acid sequence), or the complement thereof.

Optionally the polynucleotide of (iii) comprises a nucleotide sequence encoding an amino acid sequence of SEQ ID NO:14 (FLU_T2_M2_1 amino acid sequence), or the complement thereof.

- 15 Optionally the nucleotide sequence encoding FLU_T2_HA_3_I3 amino acid sequence (SEQ ID NO:22), or the complement thereof, comprises the nucleotide sequence of SEQ ID NO:23, or the complement thereof.

Optionally the nucleotide sequence encoding FLU_T2_NA_3 amino acid sequence (SEQ ID NO:16), or the complement thereof, comprises the nucleotide sequence of SEQ ID NO:17, or the complement thereof.

- 20 Optionally the nucleotide sequence encoding FLU_T2_M2_1 amino acid sequence (SEQ ID NO:14), or the complement thereof, comprises the nucleotide sequence of SEQ ID NO:15, or the complement thereof.

Optionally a pharmaceutical composition of the invention comprises:

- 25 i) an isolated polynucleotide which comprises nucleotide sequence encoding FLU_T2_HA_4 amino acid sequence (SEQ ID NO:68), or the complement thereof; and
- ii) an isolated polynucleotide which comprises nucleotide sequence encoding FLU_T2_NA_3 amino acid sequence (SEQ ID NO:16), or the complement thereof.

Optionally a pharmaceutical composition of the invention comprises:

- 30 i) an isolated polynucleotide which comprises nucleotide sequence encoding FLU_T2_HA_4 amino acid sequence (SEQ ID NO:68), or the complement thereof; and

ii) an isolated polynucleotide which comprises nucleotide sequence encoding FLU_T2_M2_1 amino acid sequence (SEQ ID NO:14), or the complement thereof.

Optionally a pharmaceutical composition of the invention comprises:

i) an isolated polynucleotide which comprises nucleotide sequence encoding
5 FLU_T2_NA_3 amino acid sequence (SEQ ID NO:16), or the complement thereof; and

ii) an isolated polynucleotide which comprises nucleotide sequence encoding FLU_T2_M2_1 amino acid sequence (SEQ ID NO:14), or the complement thereof.

Optionally a pharmaceutical composition of the invention comprises:

i) an isolated polynucleotide which comprises nucleotide sequence encoding
10 FLU_T2_HA_4 amino acid sequence (SEQ ID NO:68), or the complement thereof;

ii) an isolated polynucleotide which comprises nucleotide sequence encoding FLU_T2_NA_3 amino acid sequence (SEQ ID NO:16), or the complement thereof; and

iii) an isolated polynucleotide which comprises nucleotide sequence encoding FLU_T2_M2_1 amino acid sequence (SEQ ID NO:14), or the complement thereof.

15 Optionally the nucleotide sequence encoding FLU_T2_HA_4 amino acid sequence (SEQ ID NO:68), or the complement thereof, comprises the nucleotide sequence of SEQ ID NO:69, or the complement thereof.

Optionally the nucleotide sequence encoding FLU_T2_NA_3 amino acid sequence (SEQ ID NO:16), or the complement thereof, comprises the nucleotide sequence of SEQ ID
20 NO:17, or the complement thereof.

Optionally the nucleotide sequence encoding FLU_T2_M2_1 amino acid sequence (SEQ ID NO:14), or the complement thereof, comprises the nucleotide sequence of SEQ ID NO:15, or the complement thereof.

Optionally each polynucleotide comprises a DNA molecule.

25 Optionally each polynucleotide comprises a messenger RNA (mRNA) molecule.

According to the invention there is also provided a pharmaceutical composition comprising a vector of the invention, and a pharmaceutically acceptable carrier, excipient, or diluent.

Optionally a pharmaceutical composition of the invention further comprises an adjuvant for enhancing an immune response in a subject to the polypeptide, or to a polypeptide encoded by the nucleic acid, of the composition.

Each different nucleic acid molecule of a pharmaceutical composition of the invention may
5 be provided as part of a separate vector.

According to the invention there is also provided a pharmaceutical composition comprising a vector of the invention, and a pharmaceutically acceptable carrier, excipient, or diluent.

Optionally a pharmaceutical composition of the invention further comprises an adjuvant for enhancing an immune response in a subject to the polypeptides, or to polypeptides encoded
10 by the nucleic acids, of the composition.

According to the invention there is also provided a pharmaceutical composition comprising a vector of the invention, and a pharmaceutically acceptable carrier, excipient, or diluent.

Combined Preparations

The term "combined preparation" as used herein refers to a "kit of parts" in the sense that
15 the combination components (i) and (ii), or (i), (ii) and (iii), as defined herein, can be dosed independently or by use of different fixed combinations with distinguished amounts of the combination components (i) and (ii), or (i), (ii) and (iii). The components can be administered simultaneously or one after the other. If the components are administered one after the other, preferably the time interval between administration is chosen such that the therapeutic effect
20 of the combined use of the components is greater than the effect which would be obtained by use of only any one of the combination components (i) and (ii), or (i), (ii) and (iii).

The components of the combined preparation may be present in one combined unit dosage form, or as a first unit dosage form of component (i) and a separate, second unit dosage form of component (ii), or as a first unit dosage form of component (i), a separate, second unit
25 dosage form of component (ii), and a separate, third unit dosage form of component (iii). The ratio of the total amounts of the combination component (i) to the combination component (ii), or of the combination component (i) to the combination component (ii) and to the combination component (iii) to be administered in the combined preparation can be varied, for example in order to cope with the needs of a patient sub-population to be treated, or the
30 needs of the single patient, which can be due, for example, to the particular disease, age, sex, or body weight of the patient.

Preferably, there is at least one beneficial effect, for example an enhancing of the effect of the component (i), or an enhancing of the effect of the component (ii), or a mutual enhancing of the effect of the combination components (i) and (ii), or an enhancing of the effect of the component (i), or an enhancing of the effect of the component (ii), or an enhancing of the effect of the component (iii), or a mutual enhancing of the effect of the combination components (i), (ii), and (iii), for example a more than additive effect, additional advantageous effects, fewer side effects, less toxicity, or a combined therapeutic effect compared with an effective dosage of one or both of the combination components (i) and (ii), or (i), (ii), and (iii), and very preferably a synergism of the combination components (i) and (ii), or (i), (ii), and (iii).

A combined preparation of the invention may be provided as a pharmaceutical combined preparation for administration to a mammal, preferably a human. The component (i) may optionally be provided together with a pharmaceutically acceptable carrier, excipient, or diluent, and/or the component (ii) may optionally be provided together with a pharmaceutically acceptable carrier, excipient, or diluent, or the component (i) may optionally be provided together with a pharmaceutically acceptable carrier, excipient, or diluent, and/or the component (ii) may optionally be provided together with a pharmaceutically acceptable carrier, excipient, or diluent and/or the component (iii) may optionally be provided together with a pharmaceutically acceptable carrier, excipient, or diluent.

A combined preparation of the invention may include polypeptides of the invention in any suitable combination (for example, HA and/or M2 and/or neuraminidase embodiments of the invention, H5 and/or M2 and/or neuraminidase embodiments of the invention, H1 and/or M2 and/or neuraminidase embodiments of the invention, or FLU_T2_HA_3_I3 and/or Flu_T2_NA_3 and/or Flu_T2_M2_1 embodiments of the invention, or FLU_T2_HA_4 and/or Flu_T2_NA_3 and/or Flu_T2_M2_1 embodiments of the invention).

Optionally, one embodiment of each different category of embodiment is used in combination. For example, an HA embodiment (H5 or H1), and/or an M2 embodiment and/or a neuraminidase embodiment.

According to the invention there is provided a combined preparation, which comprises:

i) a polypeptide which comprises an amino acid sequence of SEQ ID NO:7 or 8, or a polypeptide which comprises an amino acid sequence of SEQ ID NO:10 or 11, or a polypeptide which comprises an amino acid sequence of SEQ ID NO:1 or 3 (examples of H5 embodiments); and/or

ii) a polypeptide which comprises an amino acid sequence of SEQ ID NO:14 (examples of M2 embodiments); and/or

iii) a polypeptide which comprises an amino acid sequence of SEQ ID NO:16, a polypeptide which comprises an amino acid sequence of SEQ ID NO:18 (examples of
5 neuraminidase embodiments).

According to the invention there is provided a combined preparation, which comprises:

i) a polypeptide which comprises an amino acid sequence of SEQ ID NO:7 or 8, or a polypeptide which comprises an amino acid sequence of SEQ ID NO:10 or 11, or a polypeptide which comprises an amino acid sequence of SEQ ID NO:1 or 3, or a polypeptide
10 which comprises an amino acid sequence of SEQ ID NO:27 or 29, or a polypeptide which comprises an amino acid sequence of SEQ ID NO:35 or 37, or a polypeptide which comprises an amino acid sequence of SEQ ID NO:43 or 45 (examples of H5 embodiments); and/or

ii) a polypeptide which comprises an amino acid sequence of SEQ ID NO:14
15 (examples of M2 embodiments); and/or

iii) a polypeptide which comprises an amino acid sequence of SEQ ID NO:16, a polypeptide which comprises an amino acid sequence of SEQ ID NO:18 (examples of neuraminidase embodiments).

According to the invention there is also provided a combined preparation, which comprises:

20 i) a polypeptide which comprises an amino acid sequence of SEQ ID NO:22, or a polypeptide which comprises an amino acid sequence of SEQ ID NO:68 (examples of H1 embodiments); and/or

ii) a polypeptide which comprises an amino acid sequence of SEQ ID NO:14 (examples of M2 embodiments); and/or

25 iii) a polypeptide which comprises an amino acid sequence of SEQ ID NO:16, or a polypeptide which comprises an amino acid sequence of SEQ ID NO:18 (examples of neuraminidase embodiments).

Optionally a combined preparation of the invention comprises:

i) a polypeptide which comprises an amino acid sequence of SEQ ID NO:22
30 (FLU_T2_HA_3_I3 amino acid sequence); and/or

ii) a polypeptide which comprises an amino acid sequence of SEQ ID NO:14 (FLU_T2_M2_1 amino acid sequence); and/or

iii) a polypeptide which comprises an amino acid sequence of SEQ ID NO:16 (FLU_T2_NA_3 amino acid sequence).

5 Optionally a combined preparation of the invention comprises:

i) a polypeptide which comprises an amino acid sequence of SEQ ID NO:22 (FLU_T2_HA_3_I3 amino acid sequence), or an amino acid sequence that has at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% amino acid identity along its entire length with the sequence of SEQ ID NO:22; and/or

10

ii) a polypeptide which comprises an amino acid sequence of SEQ ID NO:14 (FLU_T2_M2_1 amino acid sequence), or an amino acid sequence that has at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% amino acid identity along its entire length with the sequence of SEQ ID NO:14; and/or

15

iii) a polypeptide which comprises an amino acid sequence of SEQ ID NO:16 (FLU_T2_NA_3 amino acid sequence), or an amino acid sequence that has at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% amino acid identity along its entire length with the sequence of SEQ ID NO:16.

20

Optionally the polypeptide of (i) comprises an amino acid sequence of SEQ ID NO:22 (FLU_T2_HA_3_I3 amino acid sequence);

Optionally the polypeptide of (ii) comprises an amino acid sequence of SEQ ID NO:14 (FLU_T2_M2_1 amino acid sequence).

25 Optionally the polypeptide of (iii) comprises an amino acid sequence of SEQ ID NO:16 (FLU_T2_NA_3 amino acid sequence).

A combined preparation of the invention may include nucleic acid molecules of the invention in any suitable combination (for example, HA and/or M2 and/or neuraminidase embodiments of the invention, H5 and/or M2 and/or neuraminidase embodiments of the invention, H1 and/or M2 and/or neuraminidase embodiments of the invention, or FLU_T2_HA_3_I3 and/or

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Flu_T2_NA_3 and/or Flu_T2_M2_1 embodiments of the invention, or FLU_T2_HA_4 and/or Flu_T2_NA_3 and/or Flu_T2_M2_1 embodiments of the invention).

Optionally, one embodiment of each different category of embodiment is used in combination. For example, an HA embodiment (H5 or H1), and/or an M2 embodiment and/or
5 a neuraminidase embodiment.

Optionally a combined preparation of the invention comprises:

- i) a nucleic acid molecule encoding a polypeptide which comprises an amino acid sequence of SEQ ID NO:7 or 8, or a nucleic acid molecule encoding a polypeptide which comprises an amino acid sequence of SEQ ID NO:10 or 11, or a nucleic acid molecule
10 encoding a polypeptide which comprises an amino acid sequence of SEQ ID NO:1 or 3 (examples of H5 embodiments); and/or
- ii) a nucleic acid molecule encoding a polypeptide which comprises an amino acid sequence of SEQ ID NO:14 (examples of M2 embodiments); and/or
- iii) a nucleic acid molecule encoding a polypeptide which comprises an amino acid
15 sequence of SEQ ID NO:16, or a nucleic acid molecule encoding a polypeptide which comprises an amino acid sequence of SEQ ID NO:18 (examples of neuraminidase embodiments).

Optionally a combined preparation of the invention comprises:

- i) a nucleic acid molecule encoding a polypeptide which comprises an amino acid
20 sequence of SEQ ID NO:7 or 8, or a nucleic acid molecule encoding a polypeptide which comprises an amino acid sequence of SEQ ID NO:10 or 11, or a nucleic acid molecule encoding a polypeptide which comprises an amino acid sequence of SEQ ID NO:1 or 3, or a nucleic acid molecule encoding a polypeptide which comprises an amino acid sequence of SEQ ID NO:27 or 29, or a nucleic acid molecule encoding a polypeptide which
25 comprises an amino acid sequence of SEQ ID NO:35 or 37, or a nucleic acid molecule encoding a polypeptide which comprises an amino acid sequence of SEQ ID NO:43 or 45 (examples of H5 embodiments); and/or
- ii) a nucleic acid molecule encoding a polypeptide which comprises an amino acid sequence of SEQ ID NO:14 (examples of M2 embodiments); and/or
- iii) a nucleic acid molecule encoding a polypeptide which comprises an amino acid
30 sequence of SEQ ID NO:16, or a nucleic acid molecule encoding a polypeptide which

comprises an amino acid sequence of SEQ ID NO:18 (examples of neuraminidase embodiments).

Optionally a combined preparation of the invention comprises:

- 5 i) a nucleic acid molecule encoding a polypeptide which comprises an amino acid sequence of SEQ ID NO:22, or a nucleic acid molecule encoding a polypeptide which comprises an amino acid sequence of SEQ ID NO:68 (examples of H1 embodiments); and/or
- ii) a nucleic acid molecule encoding a polypeptide which comprises an amino acid sequence of SEQ ID NO:14 (examples of M2 embodiments); and/or
- 10 iii) a nucleic acid molecule encoding a polypeptide which comprises an amino acid sequence of SEQ ID NO:16, or a nucleic acid molecule encoding a polypeptide which comprises an amino acid sequence of SEQ ID NO:18 (examples of neuraminidase embodiments).

According to the invention there is also provided a combined preparation which comprises:

- 15 i) an isolated polynucleotide which comprises nucleotide sequence encoding FLU_T2_HA_3_I3 amino acid sequence (SEQ ID NO:22), or the complement thereof; and/or
- ii) an isolated polynucleotide which comprises nucleotide sequence encoding FLU_T2_NA_3 amino acid sequence (SEQ ID NO:16), or the complement thereof; and/or
- 20 iii) an isolated polynucleotide which comprises nucleotide sequence encoding FLU_T2_M2_1 amino acid sequence (SEQ ID NO:14), or the complement thereof.

According to the invention there is also provided a combined preparation which comprises:

- 25 i) an isolated polynucleotide which comprises a nucleotide sequence encoding an amino acid sequence of SEQ ID NO:22 (FLU_T2_HA_3_I3 amino acid sequence), or an amino acid sequence that has at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% amino acid identity along its entire length with the sequence of SEQ ID NO:22, or the complement thereof; and/or
- 30 ii) an isolated polynucleotide which comprises a nucleotide sequence encoding an amino acid sequence of SEQ ID NO:16 (FLU_T2_NA_3 amino acid sequence), or an amino acid sequence that has at least 70%, 71%, 72%, 73%, 74%, 75%,

76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% amino acid identity along its entire length with the sequence of SEQ ID NO:16, or the complement thereof; and/or

- 5 iii) an isolated polynucleotide which comprises a nucleotide sequence encoding an amino acid sequence of SEQ ID NO:14 (FLU_T2_M2_1 amino acid sequence), or an amino acid sequence that has at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% amino acid identity
- 10 along its entire length with the sequence of SEQ ID NO:14, or the complement thereof.

Optionally the polynucleotide of (i) comprises a nucleotide sequence encoding an amino acid sequence of SEQ ID NO:22 (FLU_T2_HA_3_I3 amino acid sequence), or the complement thereof.

- 15 Optionally the polynucleotide of (ii) comprises a nucleotide sequence encoding an amino acid sequence of SEQ ID NO:16 (FLU_T2_NA_3 amino acid sequence), or the complement thereof.

- Optionally the polynucleotide of (iii) comprises a nucleotide sequence encoding an amino acid sequence of SEQ ID NO:14 (FLU_T2_M2_1 amino acid sequence), or the
- 20 complement thereof.

According to the invention there is also provided a combined preparation which comprises:

- i) an isolated polynucleotide which comprises nucleotide sequence encoding FLU_T2_HA_3_I3 amino acid sequence (SEQ ID NO:22), or the complement thereof; and
- ii) an isolated polynucleotide which comprises nucleotide sequence encoding
- 25 FLU_T2_NA_3 amino acid sequence (SEQ ID NO:16), or the complement thereof.

According to the invention there is also provided a combined preparation which comprises:

- i) an isolated polynucleotide which comprises nucleotide sequence encoding FLU_T2_HA_3_I3 amino acid sequence (SEQ ID NO:22), or the complement thereof; and
- ii) an isolated polynucleotide which comprises nucleotide sequence encoding
- 30 FLU_T2_M2_1 amino acid sequence (SEQ ID NO:14), or the complement thereof.

According to the invention there is also provided a combined preparation which comprises:

- i) an isolated polynucleotide which comprises nucleotide sequence encoding
FLU_T2_NA_3 amino acid sequence (SEQ ID NO:16), or the complement thereof; and
- ii) an isolated polynucleotide which comprises nucleotide sequence encoding
5 FLU_T2_M2_1 amino acid sequence (SEQ ID NO:14), or the complement thereof.

According to the invention there is also provided a combined preparation which comprises:

- i) an isolated polynucleotide which comprises nucleotide sequence encoding
FLU_T2_HA_3_I3 amino acid sequence (SEQ ID NO:22), or the complement thereof;
- ii) an isolated polynucleotide which comprises nucleotide sequence encoding
10 FLU_T2_NA_3 amino acid sequence (SEQ ID NO:16), or the complement thereof; and
- iii) an isolated polynucleotide which comprises nucleotide sequence encoding
FLU_T2_M2_1 amino acid sequence (SEQ ID NO:14), or the complement thereof.

- Optionally the nucleotide sequence encoding FLU_T2_HA_3_I3 amino acid sequence
(SEQ ID NO:22), or the complement thereof, comprises the nucleotide sequence of SEQ
15 ID NO:23, or the complement thereof.

Optionally the nucleotide sequence encoding FLU_T2_NA_3 amino acid sequence (SEQ
ID NO:16), or the complement thereof, comprises the nucleotide sequence of SEQ ID
NO:17, or the complement thereof.

- Optionally the nucleotide sequence encoding FLU_T2_M2_1 amino acid sequence (SEQ
20 ID NO:14), or the complement thereof, comprises the nucleotide sequence of SEQ ID
NO:15, or the complement thereof.

According to the invention there is also provided a combined preparation which comprises:

- i) an isolated polynucleotide which comprises nucleotide sequence encoding
FLU_T2_HA_4 amino acid sequence (SEQ ID NO:68), or the complement thereof; and
- 25 ii) an isolated polynucleotide which comprises nucleotide sequence encoding
FLU_T2_NA_3 amino acid sequence (SEQ ID NO:16), or the complement thereof.

According to the invention there is also provided a combined preparation which comprises:

- i) an isolated polynucleotide which comprises nucleotide sequence encoding FLU_T2_HA_4 amino acid sequence (SEQ ID NO:68), or the complement thereof; and
- ii) an isolated polynucleotide which comprises nucleotide sequence encoding FLU_T2_M2_1 amino acid sequence (SEQ ID NO:14), or the complement thereof.

5 According to the invention there is also provided a combined preparation which comprises:

- i) an isolated polynucleotide which comprises nucleotide sequence encoding FLU_T2_NA_3 amino acid sequence (SEQ ID NO:16), or the complement thereof; and
- ii) an isolated polynucleotide which comprises nucleotide sequence encoding FLU_T2_M2_1 amino acid sequence (SEQ ID NO:14), or the complement thereof.

10 According to the invention there is also provided a combined preparation which comprises:

- i) an isolated polynucleotide which comprises nucleotide sequence encoding FLU_T2_HA_4 amino acid sequence (SEQ ID NO:68), or the complement thereof;
- ii) an isolated polynucleotide which comprises nucleotide sequence encoding FLU_T2_NA_3 amino acid sequence (SEQ ID NO:16), or the complement thereof; and

15 iii) an isolated polynucleotide which comprises nucleotide sequence encoding FLU_T2_M2_1 amino acid sequence (SEQ ID NO:14), or the complement thereof.

Optionally the nucleotide sequence encoding FLU_T2_HA_4 amino acid sequence (SEQ ID NO:68), or the complement thereof, comprises the nucleotide sequence of SEQ ID NO:69, or the complement thereof.

20 Optionally the nucleotide sequence encoding FLU_T2_NA_3 amino acid sequence (SEQ ID NO:16), or the complement thereof, comprises the nucleotide sequence of SEQ ID NO:17, or the complement thereof.

Optionally the nucleotide sequence encoding FLU_T2_M2_1 amino acid sequence (SEQ ID NO:14), or the complement thereof, comprises the nucleotide sequence of SEQ ID

25 NO:15, or the complement thereof.

Optionally each polynucleotide comprises a DNA molecule.

Optionally each polynucleotide comprises a messenger RNA (mRNA) molecule.

Each different nucleic acid molecule of a combined preparation of the invention may be provided as part of a separate vector.

- Optionally a combined preparation of the invention further comprises an adjuvant for enhancing an immune response in a subject to the polypeptides, or to the polypeptides encoded by the nucleic acids, of the combined preparation.

Strings

- Embodiments of the invention in which different polypeptides of the invention are encoded as part of the same polynucleotide (or nucleic acid), or are provided in the same polypeptide (i.e. as “strings” of different subunits, for example, HA and/or M2 and/or neuraminidase
- 10 embodiments of the invention, H5 and/or M2 and/or neuraminidase embodiments of the invention, H1 and/or M2 and/or neuraminidase embodiments of the invention, or FLU_T2_HA_3_I3 and/or Flu_T2_NA_3 and/or Flu_T2_M2_1 embodiments of the invention, or FLU_T2_HA_4 and/or Flu_T2_NA_3 and/or Flu_T2_M2_1 embodiments of the invention), are particularly advantageous since use of such a “string” as part of a vaccine requires testing
- 15 only of the single product containing the “string” for safety and efficacy, rather than testing each different subunit individually. This dramatically reduces the time and cost of developing the vaccine compared with individual subunits. In some embodiments, a combination of different strings (polynucleotide and/ or polypeptide), or a combination of one or more strings and one or more single subunits (polypeptide or encoded subunit) may be used.
- 20 Optionally, one embodiment of each different category of embodiment is used in combination. For example, an HA embodiment (H5 or H1), and/or an M2 embodiment and/or a neuraminidase embodiment.

- Strategies for multigene co-expression include introduction of multiple vectors, use of multiple promoters in a single vector, fusion proteins, proteolytic cleavage sites between
- 25 genes, internal ribosome entry sites (IRES), and “self-cleaving” 2A peptides. Multicistronic vectors based on IRES nucleotide sequence and self-cleaving 2A peptides are reviewed in Shaimardanova *et al.* (*Pharmaceutics* **2019**, *11*, 580; doi:10.3390/pharmaceutics11110580).

- In one embodiment of the invention, known as panH1N1 (described below in Example 15 below), a polypeptide comprising a string of the following subunits joined by self-cleaving 2A
- 30 peptides is provided:

FLU_T2_HA_3_I3 (amino acid SEQ ID NO:22), FLU_T2_NA_3 (amino acid SEQ ID NO:16), and FLU_T2_M2_1 (amino acid SEQ ID NO:14).

The amino acid sequence of panH1N1 is provided as SEQ ID NO:63.

According to the invention there is also provided an isolated polypeptide which comprises an amino acid sequence of SEQ ID NO:63.

According to the invention there is provided an isolated polypeptide which comprises an amino acid sequence that has at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% amino acid identity along its entire length with the amino acid sequence of SEQ ID NO:63.

Vaccines

Vaccines of the invention may be provided, for example, as nucleic acid vaccines, either as separate polynucleotides, each encoding a different subunit (HA and/or M2 and/or neuraminidase embodiment of the invention, for example a H5 and/or M2 and/or neuraminidase embodiment of the invention, a H1 and/or M2 and/or neuraminidase embodiment of the invention, or a FLU_T2_HA_3_I3 and/or Flu_T2_NA_3 and/or Flu_T2_M2_1 embodiment of the invention, or a FLU_T2_HA_4 and/or Flu_T2_NA_3 and/or Flu_T2_M2_1 embodiment of the invention) (for administration together or separately) or pieced together in a string as a single polynucleotide encoding all of the subunits. The separate polynucleotides may be administered as a mixture together (for example, as a pharmaceutical composition comprising the separate polynucleotides), or co-administered or administered sequentially in any order (in which case, the separate polynucleotides may be provided as a combined preparation for co-administration or sequential administration). Nucleic acid vaccines may be provided as DNA, RNA, or mRNA vaccines. Production and application of multicistronic constructs (for example, where the subunits are provided in a string as a single polynucleotide) is reviewed by Shaimardanova *et al.* (*Pharmaceutics* **2019**, *11*, 580; doi:10.3390/pharmaceutics11110580).

Vaccine constructs of the invention may also be provided, for example, either as separate polypeptides, each comprising a different subunit (for example, HA, M2, or neuraminidase embodiments of the invention, H5, M2, or neuraminidase embodiments of the invention, H1, M2, or neuraminidase embodiments of the invention, or FLU_T2_HA_3_I3, or Flu_T2_NA_3, or Flu_T2_M2_1 embodiments of the invention, or FLU_T2_HA_4, or Flu_T2_NA_3, or Flu_T2_M2_1 embodiments of the invention) or pieced together in a string as a single polypeptide comprising all of the subunits (for example, HA and M2 and neuraminidase embodiments of the invention, H5 and M2 and neuraminidase embodiments of the invention, H1 and M2 and neuraminidase embodiments of the invention, or FLU_T2_HA_3_I3 and Flu_T2_NA_3 and Flu_T2_M2_1 embodiments of the invention, or FLU_T2_HA_4 and

Flu_T2_NA_3 and Flu_T2_M2_1 embodiments of the invention). The separate polypeptides may be administered as a mixture together (for example, as a pharmaceutical composition comprising the separate polypeptides), or co-administered or administered sequentially in any order (in which case, the separate polypeptides may be provided as a combined preparation for co-administration or sequential administration).

Optionally, one embodiment of each different category of embodiment is used in combination. For example, an HA embodiment (H5 or H1), and/or an M2 embodiment and/or a neuraminidase embodiment.

Strategies for multigene co-expression include introduction of multiple vectors, use of multiple promoters in a single vector, fusion proteins, proteolytic cleavage sites between genes, internal ribosome entry sites (IRES), and “self-cleaving” 2A peptides. Multicistronic vectors based on IRES nucleotide sequence and self-cleaving 2A peptides are reviewed in Shaimardanova *et al.* (*Pharmaceutics* **2019**, 11, 580; doi:10.3390/pharmaceutics11110580).

In one embodiment of the invention (described below in Example 15), a nucleic acid molecule with a nucleotide sequence of SEQ ID NO:25 encoding a string of the following subunits joined by self-cleaving 2A peptides (known as panH1N1) is provided:

FLU_T2_HA_3_I3 (amino acid SEQ ID NO:22), FLU_T2_NA_3 (amino acid SEQ ID NO:16), and FLU_T2_M2_1 (amino acid SEQ ID NO:14).

There is also provided according to the invention an isolated polynucleotide comprising nucleotide sequence encoding FLU_T2_HA_3_I3 (amino acid SEQ ID NO:22), nucleotide sequence encoding FLU_T2_NA_3 (amino acid SEQ ID NO:16), and nucleotide sequence encoding FLU_T2_M2_1 (amino acid SEQ ID NO:14).

There is also provided according to the invention an isolated polynucleotide comprising nucleotide sequence of SEQ ID NO:23, nucleotide sequence of SEQ ID NO:17, and nucleotide sequence of SEQ ID NO:15.

According to the invention there is provided an isolated nucleic acid molecule, which comprises a nucleotide sequence encoding a polypeptide which comprises an amino acid sequence of SEQ ID NO:63, or the complement thereof.

There is also provided according to the invention an isolated nucleic acid molecule which comprises a nucleotide sequence of SEQ ID NO:25, or the complement thereof.

According to the invention there is also provided an isolated nucleic acid molecule, which comprises a nucleotide sequence encoding a polypeptide which comprises an amino acid

sequence that has at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% amino acid identity along its entire length with the amino acid sequence of SEQ ID NO:63, or the complement thereof.

- 5 There is also provided according to the invention an isolated nucleic acid molecule which comprises a nucleotide sequence of SEQ ID NO:25, or a nucleotide sequence which has at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity along its entire length with the nucleotide sequence of SEQ ID NO:25, which
10 encodes a polypeptide which comprises an amino acid sequence of SEQ ID NO:63, or the complement thereof.

- There is also provided according to the invention an isolated nucleic acid molecule which comprises a nucleotide sequence encoding a polypeptide which comprises an amino acid sequence of SEQ ID NO:63, or an amino acid sequence that has at least 70%, 71%, 72%,
15 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% amino acid identity along its entire length with the amino acid sequence of SEQ ID NO:63, wherein the nucleic acid molecule comprises a nucleotide sequence of SEQ ID NO:25, or a nucleotide sequence which has at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%,
20 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity along its entire length with the nucleotide sequence of SEQ ID NO:25, or the complement thereof.

- There is also provided according to the invention an isolated nucleic acid molecule which comprises a nucleotide sequence of SEQ ID NO:25, or a nucleotide sequence which has at
25 least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity along its entire length with the nucleotide sequence of SEQ ID NO:25, or the complement thereof.

- Optionally, an isolated nucleic acid molecule of the invention comprises a DNA molecule,
30 an RNA molecule, or an mRNA molecule.

Where mRNA vaccines are used in accordance with the invention, it is preferred that each designed subunit of a string of the invention is encoded as part of a separate mRNA vaccine vector.

Methods of treatment and uses

There is also provided according to the invention a method of inducing an immune response to an influenza virus in a subject, which comprises administering to the subject an effective amount of a polypeptide of the invention, a nucleic acid of the invention, a vector of the invention, a pharmaceutical composition, or a combined preparation, of the invention.

- 5 There is also provided according to the invention a method of immunising a subject against an influenza virus, which comprises administering to the subject an effective amount of a polypeptide of the invention, a nucleic acid of the invention, a vector of the invention, a pharmaceutical composition, or a combined preparation, of the invention.

10 An effective amount is an amount to produce an antigen-specific immune response in a subject.

There is further provided according to the invention a polypeptide of the invention, a nucleic acid of the invention, a vector of the invention, a pharmaceutical composition of the invention, or a combined preparation, for use as a medicament.

15 There is further provided according to the invention a polypeptide of the invention, a nucleic acid of the invention, a vector of the invention, a pharmaceutical composition of the invention, or a combined preparation, for use in the prevention, treatment, or amelioration of an influenza viral infection.

20 There is also provided according to the invention use of a polypeptide of the invention, a nucleic acid of the invention, a vector of the invention, or a pharmaceutical composition of the invention, or a combined preparation, in the manufacture of a medicament for the prevention, treatment, or amelioration of an influenza viral infection.

Administration

Any suitable route of administration may be used. Methods of administration include, but are not limited to, intradermal, intramuscular, intraperitoneal, parenteral, intravenous, 25 subcutaneous, vaginal, rectal, intranasal, inhalation or oral. Parenteral administration, such as subcutaneous, intravenous or intramuscular administration, is generally achieved by injection. Injectables can be prepared in conventional forms, either as liquid solutions or suspensions, solid forms suitable for solution or suspension in liquid prior to injection, or as emulsions. Injection solutions and suspensions can be prepared from sterile powders, 30 granules, and tablets of the kind previously described. Administration can be systemic or local. Routes for systemic administration in general include, for example, transdermal, oral, parenteral routes, including subcutaneous, intravenous, intramuscular, intraarterial, intradermal and intraperitoneal injections and/or intranasal administration routes. Routes for

local administration in general include, for example, topical administration routes but also intradermal, transdermal, subcutaneous, or intramuscular injections or intralesional, intracranial, intrapulmonal, intracardial, and sublingual injections.

5 Compositions may be administered in any suitable manner, such as with pharmaceutically acceptable carriers. Pharmaceutically acceptable carriers are determined in part by the particular composition being administered, as well as by the particular method used to administer the composition. Preparations for parenteral administration include sterile aqueous or nonaqueous solutions, suspensions, and emulsions. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and
10 injectable organic esters such as ethyl oleate. Aqueous carriers include water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. Parenteral vehicles include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's, or fixed oils. Intravenous vehicles include fluid and nutrient replenishers, electrolyte replenishers (such as those based on Ringer's dextrose),
15 and the like. Preservatives and other additives may also be present such as, for example, antimicrobials, anti-oxidants, chelating agents, and inert gases and the like.

Some of the compositions may potentially be administered as a pharmaceutically acceptable acid- or base-addition salt, formed by reaction with inorganic acids such as hydrochloric acid, hydrobromic acid, perchloric acid, nitric acid, thiocyanic acid, sulfuric
20 acid, and phosphoric acid, and organic acids such as formic acid, acetic acid, propionic acid, glycolic acid, lactic acid, pyruvic acid, oxalic acid, malonic acid, succinic acid, maleic acid, and fumaric acid, or by reaction with an inorganic base such as sodium hydroxide, ammonium hydroxide, potassium hydroxide, and organic bases such as mono-, di-, trialkyl and aryl amines and substituted ethanolamines.

25 Administration can be accomplished by single or multiple doses. The dose administered to a subject in the context of the present disclosure should be sufficient to induce a beneficial therapeutic response in a subject over time, or to inhibit or prevent infection. The dose required will vary from subject to subject depending on the species, age, weight and general condition of the subject, the severity of the infection being treated, the particular
30 composition being used and its mode of administration. An appropriate dose can be determined by one of ordinary skill in the art using only routine experimentation.

The present disclosure includes methods comprising administering an RNA vaccine, an mRNA vaccine, or a DNA vaccine to a subject in need thereof. The exact amount required will vary from subject to subject, depending on the species, age, and general condition of the

subject, the severity of the disease, the particular composition, its mode of administration, its mode of activity, and the like.

The RNA or DNA is typically formulated in dosage unit form for ease of administration and uniformity of dosage. It will be understood, however, that the total daily usage of the RNA may be decided by the attending physician within the scope of sound medical judgment. The specific therapeutically effective, prophylactically effective, or appropriate imaging dose level for any particular patient will depend upon a variety of factors including the disorder being treated and the severity of the disorder; the activity of the specific compound employed; the specific composition employed; the age, body weight, general health, sex and diet of the patient; the time of administration, route of administration, and rate of excretion of the specific compound employed; the duration of the treatment; drugs used in combination or coincidental with the specific compound employed; and like factors well known in the medical arts.

The effective amount of the RNA or DNA, as provided herein, may be as low as 20 pg, administered for example as a single dose or as two 10 pg doses. In some embodiments, the effective amount is a total dose of 20 µg-300 µg or 25 µg-300 µg. For example, the effective amount may be a total dose of 20 µg, 25 µg, 30 µg, 35 µg, 40 µg, 45 µg, 50 µg, 55 µg, 60 µg, 65 µg, 70 µg, 75 µg, 80 µg, 85 µg, 90 µg, 95 µg, 100 µg, 110 µg, 120 µg, 130 µg, 140 µg, 150 µg, 160 µg, 170 µg, 180 µg, 190 µg, 200 µg, 250 µg, or 300 µg. In some embodiments, the effective amount is a total dose of 20 µg. In some embodiments, the effective amount is a total dose of 25 pg. In some embodiments, the effective amount is a total dose of 50 µg. In some embodiments, the effective amount is a total dose of 75 µg. In some embodiments, the effective amount is a total dose of 100 µg. In some embodiments, the effective amount is a total dose of 150 µg. In some embodiments, the effective amount is a total dose of 200 µg. In some embodiments, the effective amount is a total dose of 250 pg. In some embodiments, the effective amount is a total dose of 300 µg.

The RNA or DNA described herein can be formulated into a dosage form described herein, such as an intranasal, intratracheal, or injectable (e.g., intravenous, intraocular, intravitreal, intramuscular, intradermal, intracardiac, intraperitoneal, and subcutaneous).

Optionally, an RNA (e.g., mRNA) or DNA vaccine is formulated in an effective amount to produce an antigen specific immune response in a subject.

In some embodiments, the effective amount is a total dose of 25 µg to 1000 µg, or 50 µg to 1000 µg. In some embodiments, the effective amount is a total dose of 100 µg. In some embodiments, the effective amount is a dose of 25 µg administered to the subject a total of

two times. In some embodiments, the effective amount is a dose of 100 µg administered to the subject a total of two times. In some embodiments, the effective amount is a dose of 400 µg administered to the subject a total of two times. In some embodiments, the effective amount is a dose of 500 µg administered to the subject a total of two times.

- 5 Optionally a dosage of between 10 µg/kg and 400 µg/kg of the nucleic acid vaccine is administered to the subject. In some embodiments the dosage of the RNA or DNA polynucleotide (or nucleic acid) is 1-5 µg, 5-10 µg, 10-15 µg, 15-20 µg, 10-25 µg, 20-25 µg, 20-50 µg, 30-50 µg, 40-50 µg, 40-60 µg, 60-80 µg, 60-100 µg, 50-100 µg, 80-120 µg, 40-120 µg, 40-150 µg, 50-150 µg, 50-200 µg, 80-200 µg, 100-200 µg, 120-250 µg, 150-250 µg, 180-
10 280 µg, 200-300 µg, 50-300 µg, 80-300 µg, 100-300 µg, 40-300 µg, 50-350 µg, 100-350 µg, 200-350 µg, 300-350 µg, 320-400 µg, 40-380 µg, 40-100 µg, 100-400 µg, 200-400 µg, or 300-400 µg per dose. In some embodiments, the nucleic acid vaccine is administered to the subject by intradermal or intramuscular injection. In some embodiments, the nucleic acid vaccine is administered to the subject on day zero. In some embodiments, a second dose of
15 the nucleic acid vaccine is administered to the subject on day twenty one.

Pharmaceutically acceptable carriers

- Pharmaceutically acceptable carriers include, but are not limited to, saline, buffered saline, dextrose, water, glycerol, ethanol, and combinations thereof. The carrier and composition can be sterile, and the formulation suits the mode of administration. The composition can
20 also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. The composition can be a liquid solution, suspension, emulsion, tablet, pill, capsule, sustained release formulation, or powder. The composition can be formulated as a suppository, with traditional binders and carriers such as triglycerides. Oral formulations can include standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium
25 stearate, sodium saccharine, cellulose, and magnesium carbonate. Any of the common pharmaceutical carriers, such as sterile saline solution or sesame oil, can be used. The medium can also contain conventional pharmaceutical adjunct materials such as, for example, pharmaceutically acceptable salts to adjust the osmotic pressure, buffers, preservatives and the like. Other media that can be used with the compositions and
30 methods provided herein are normal saline and sesame oil.

In some embodiments, the compositions comprise a pharmaceutically acceptable carrier and/or an adjuvant. For example, the adjuvant can be alum, Freund's complete adjuvant, a biological adjuvant or immunostimulatory oligonucleotides (such as CpG oligonucleotides).

The pharmaceutically acceptable carriers (vehicles) useful in this disclosure are conventional. Remington's Pharmaceutical Sciences, by E. W. Martin, Mack Publishing Co., Easton, PA, 15th Edition (1975), describes compositions and formulations suitable for pharmaceutical delivery of one or more therapeutic compositions, such as one or more
 5 influenza vaccines, and additional pharmaceutical agents.

In general, the nature of the carrier will depend on the particular mode of administration being employed. For instance, parenteral formulations usually comprise injectable fluids that include pharmaceutically and physiologically acceptable fluids such as water, physiological saline, balanced salt solutions, aqueous dextrose, glycerol or the like as a
 10 vehicle. For solid compositions (for example, powder, pill, tablet, or capsule forms), conventional non-toxic solid carriers can include, for example, pharmaceutical grades of mannitol, lactose, starch, or magnesium stearate. In addition to biologically-neutral carriers, pharmaceutical compositions to be administered can contain minor amounts of non-toxic auxiliary substances, such as wetting or emulsifying agents, preservatives, and pH
 15 buffering agents and the like, for example sodium acetate or sorbitan monolaurate.

Optionally a composition of the invention is administered intramuscularly.

Optionally the composition is administered intramuscularly, intradermally, subcutaneously by needle or by gene gun, or electroporation.

Aspects of the invention are defined in the following numbered paragraphs:

20

1. An isolated polypeptide comprising a haemagglutinin subtype 5 (H5) globular head domain, and optionally a haemagglutinin stem domain, with the following amino acid residues at positions 156, 157, 171, 172, and 205 of the head domain:

25

- 156: R;
- 157: P or S, preferably P;
- 171: D or N;
- 172: T or A, preferably T; and
- 205: K or R, preferably K

30

2. An isolated polypeptide according to paragraph 1, with the following amino acid residues at positions 156, 157, 171, 172, and 205 of the head domain:

- 156: R;
- 157: P;
- 171: D;

- 172: T; and
- 205: K

3. An isolated polypeptide according to paragraph 1 or 2, which comprises an amino acid sequence of SEQ ID NO:7 or 8, or an amino acid sequence that has at least 70%,

- 5 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% amino acid identity along its entire length with the amino acid sequence of SEQ ID NO:7 or 8 and which has the following amino acid residues at positions corresponding to positions 156, 157, 171, 172, and 205 of SEQ ID NO:7 or 8:

- 10
- 156: R;
 - 157: P;
 - 171: D;
 - 172: T; and
 - 205: K

- 15 4. An isolated polypeptide according to paragraph 1, with the following amino acid residues at positions 156, 157, 171, 172, and 205 of the head domain:

- 20
- 156: R;
 - 157: P;
 - 171: N;
 - 172: T; and
 - 205: K

5. An isolated polypeptide according to paragraph 1 or 4, which comprises an amino acid sequence of SEQ ID NO:10 or 11, or an amino acid sequence that has at least 70%,

- 25 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% amino acid identity along its entire length with the amino acid sequence of SEQ ID NO:10 or 11 and which has the following amino acid residues at positions corresponding to positions 156, 157, 171, 172, and 205 of SEQ ID NO:10 or 11:

- 30
- 156: R;
 - 157: P;
 - 171: N;
 - 172: T; and
 - 205: K

6. An isolated polypeptide according to paragraph 1, with the following amino acid residues at positions 156, 157, 171, 172, and 205 of the head domain:

- 156: R;
- 157: S;
- 171: N;
- 172: A; and
- 205: R

7. An isolated polypeptide according to paragraph 1 or 6, which comprises an amino acid sequence of SEQ ID NO:1 or 3, or an amino acid sequence that has at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% amino acid identity along its entire length with the amino acid sequence of SEQ ID NO:1 or 3 and which has the following amino acid residues at positions corresponding to positions 156, 157, 171, 172, and 205 of SEQ ID NO:1 or 3:

- 156: R;
- 157: S;
- 171: N;
- 172: A; and
- 205: R

8. An isolated polypeptide according to any preceding paragraph, with the following amino acid residues at positions 416 and 434 of the stem domain:

- 416: F; and
- 434: F

9. An isolated polypeptide which comprises the following amino acid sequence:

R(P/S)SFFRNVWLIKKN(D/N)(T/A)YPTIKRSYNNTNQEDLLVLWGIHHPNDAAEQT(K/R) (SEQ ID NO:13), or an amino acid sequence that has at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% amino acid identity along its entire length with the sequence of SEQ ID NO:13 and which has the following amino acid residues at positions corresponding to positions 1, 2, 16, 17, and 50 of SEQ ID NO:13:

- 1: R;
- 2: P or S, preferably P;

- 16: D or N;
- 17: T or A, preferably T; and
- 50: K or R, preferably K.

10. An isolated polypeptide according to paragraph 9, with the following amino acid
5 residues at positions 1, 2, 16, 17, and 50 of the amino acid sequence, or at positions corresponding to positions 1, 2, 16, 17, and 50 of SEQ ID NO:13:

- 1: R;
- 2: P;
- 16: D;
- 10 • 17: T; and
- 50: K

11. An isolated polypeptide according to paragraph 9, with the following amino acid
residues at positions 1, 2, 16, 17, and 50 of the amino acid sequence, or at positions
corresponding to positions 1, 2, 16, 17, and 50 of SEQ ID NO:13:

- 15 • 1: R;
- 2: P;
- 16: N;
- 17: T; and
- 50: K

20 12. An isolated polypeptide according to paragraph 9, with the following amino acid
residues at positions 1, 2, 16, 17, and 50 of the amino acid sequence, or at positions
corresponding to positions 1, 2, 16, 17, and 50 of SEQ ID NO:13:

- 1: R;
- 2: S;
- 25 • 16: N;
- 17: A; and
- 50: R

13. An isolated polypeptide which comprises an amino acid sequence of any of SEQ ID
NOs:5, 9, or 12, or an amino acid sequence that has at least 70%, 71%, 72%, 73%, 74%,
30 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%,
90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% amino acid identity along its
entire length with the sequence of any of SEQ ID NO:5, 9, or 12 and which has the

following amino acid residues at positions corresponding to positions 148 and 166 of SEQ ID NO:5, 9, or 12:

- 148: F; and
- 166: F

- 5 14. An isolated polypeptide which comprises an amino acid sequence of SEQ ID NO:14, or an amino acid sequence that has at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% amino acid identity along its entire length with the sequence of SEQ ID NO:14.
- 10 15. An isolated polypeptide which comprises an amino acid sequence of SEQ ID NO:16, or an amino acid sequence that has at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% amino acid identity along its entire length with the sequence of SEQ ID NO:16.
- 15 16. An isolated polypeptide which comprises an amino acid sequence of SEQ ID NO:18, or an amino acid sequence that has at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% amino acid identity along its entire length with the sequence of SEQ ID NO:18.
- 20 17. An isolated nucleic acid molecule encoding a polypeptide according to any of paragraphs 1 to 16, or an isolated nucleic acid molecule comprising a nucleotide sequence that is at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical with the nucleic acid molecule over its entire length, or the
- 25 complement thereof.
18. An isolated nucleic acid molecule according to paragraph 17, comprising a nucleotide sequence of SEQ ID NO:2, 4, or 6, or a nucleotide sequence that is at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%
- 30 identical with SEQ ID NO:2, 4, or 6, over its entire length, or the complement thereof.
19. An isolated nucleic acid molecule according to paragraph 17, comprising a nucleotide sequence of SEQ ID NO:15, or a nucleotide sequence that is at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%,

87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical with SEQ ID NO:15, over its entire length, or the complement thereof.

20. An isolated nucleic acid molecule according to paragraph 17, comprising a nucleotide sequence of SEQ ID NO:17, or a nucleotide sequence that is at least 70%, 71%,
5 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical with SEQ ID NO:17, over its entire length, or the complement thereof.

21. An isolated nucleic acid molecule according to paragraph 17, comprising a nucleotide sequence of SEQ ID NO:19, or a nucleotide sequence that is at least 70%, 71%,
10 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical with SEQ ID NO:19, over its entire length, or the complement thereof.

22. A vector comprising a nucleic acid molecule of any of paragraphs 17 to 21.

23. A vector according to paragraph 22, comprising a nucleic acid molecule encoding a
15 polypeptide of any of paragraphs 1 to 12.

24. A vector according to paragraph 22 or 23, comprising a nucleic acid molecule encoding a polypeptide of paragraph 14.

25. A vector according to any of paragraphs 22 to 24, comprising a nucleic acid molecule encoding a polypeptide of paragraph 15 or 16.

20 26. A vector according to any of paragraphs 22 to 25, comprising a nucleic acid molecule encoding a polypeptide which comprises an amino acid sequence of SEQ ID NO:7 or 8.

27. A vector according to any of paragraphs 22 to 26, comprising a nucleic acid molecule encoding a polypeptide which comprises an amino acid sequence of SEQ ID
25 NO:10 or 11.

28. A vector according to any of paragraphs 22 to 27, comprising a nucleic acid molecule encoding a polypeptide which comprises an amino acid sequence of SEQ ID NO:1 or 3.

29. A vector according to any of paragraphs 22 to 28, comprising a nucleic acid
30 molecule encoding a polypeptide which comprises an amino acid sequence of SEQ ID NO:14.

30. A vector according to any of paragraphs 22 to 29, comprising a nucleic acid molecule encoding a polypeptide which comprises an amino acid sequence of SEQ ID NO:16.
31. A vector according to any of paragraphs 22 to 30, comprising a nucleic acid molecule encoding a polypeptide which comprises an amino acid sequence of SEQ ID NO:18.
32. A vector according to any of paragraphs 22 to 31, which further comprises a promoter operably linked to the, or each nucleic acid molecule.
33. A vector according to paragraph 32, wherein the, or each promoter is for expression of a polypeptide encoded by the nucleic acid in mammalian cells.
34. A vector according to paragraph 33, wherein the, or each promoter is for expression of a polypeptide encoded by the nucleic acid in yeast or insect cells.
35. A vector according to any of paragraphs 22 to 34, which is a vaccine vector.
36. A vector according to paragraph 35, which is a viral vaccine vector, a bacterial vaccine vector, an RNA vaccine vector, or a DNA vaccine vector.
37. An isolated cell comprising a vector of any of paragraphs 22 to 36.
38. A fusion protein comprising a polypeptide according to any of paragraphs 1 to 16.
39. A pharmaceutical composition comprising a polypeptide according to any of paragraphs 1 to 16, and a pharmaceutically acceptable carrier, excipient, or diluent.
40. A pharmaceutical composition according to paragraph 39, comprising a polypeptide of any of paragraphs 1 to 12.
41. A pharmaceutical composition according to paragraph 39 or 40, comprising a polypeptide of paragraph 14.
42. A pharmaceutical composition according to any of paragraphs 39 to 41, comprising a polypeptide of paragraph 15 or 16.
43. A pharmaceutical composition according to any of paragraphs 39 to 42, comprising a polypeptide which comprises an amino acid sequence of SEQ ID NO:7 or 8.
44. A pharmaceutical composition according to any of paragraphs 39 to 43, comprising a polypeptide which comprises an amino acid sequence of SEQ ID NO:10 or 11.

45. A pharmaceutical composition according to any of paragraphs 39 to 44, comprising a polypeptide which comprises an amino acid sequence of SEQ ID NO:1 or 3.
46. A pharmaceutical composition according to any of paragraphs 39 to 45, comprising a polypeptide which comprises an amino acid sequence of SEQ ID NO:14.
- 5 47. A pharmaceutical composition according to any of paragraphs 39 to 46, comprising a polypeptide which comprises an amino acid sequence of SEQ ID NO:16.
48. A pharmaceutical composition according to any of paragraphs 39 to 47, comprising a polypeptide which comprises an amino acid sequence of SEQ ID NO:18.
49. A pharmaceutical composition comprising a nucleic acid according to any of
10 paragraphs 17 to 21, and a pharmaceutically acceptable carrier, excipient, or diluent.
50. A pharmaceutical composition according to paragraph 49, comprising a nucleic acid molecule encoding a polypeptide of any of paragraphs 1 to 12.
51. A pharmaceutical composition according to paragraph 49 or 50, comprising a nucleic acid molecule encoding a polypeptide of paragraph 14.
- 15 52. A pharmaceutical composition according to any of paragraphs 49 to 51, comprising a nucleic acid molecule encoding a polypeptide of paragraph 15 or 16.
53. A pharmaceutical composition according to any of paragraphs 49 to 52, comprising a nucleic acid molecule encoding a polypeptide which comprises an amino acid sequence of SEQ ID NO:7 or 8.
- 20 54. A pharmaceutical composition according to any of paragraphs 49 to 53, comprising a nucleic acid molecule encoding a polypeptide which comprises an amino acid sequence of SEQ ID NO:10 or 11.
55. A pharmaceutical composition according to any of paragraphs 49 to 54, comprising a nucleic acid molecule encoding a polypeptide which comprises an amino acid sequence
25 of SEQ ID NO:1 or 3.
56. A pharmaceutical composition according to any of paragraphs 49 to 55, comprising a nucleic acid molecule encoding a polypeptide which comprises an amino acid sequence of SEQ ID NO:14.

57. A pharmaceutical composition according to any of paragraphs 49 to 56, comprising a nucleic acid molecule encoding a polypeptide which comprises an amino acid sequence of SEQ ID NO:16.

58. A pharmaceutical composition according to any of paragraphs 49 to 57, comprising
5 a nucleic acid molecule encoding a polypeptide which comprises an amino acid sequence of SEQ ID NO:18.

59. A pharmaceutical composition comprising a vector according to any of paragraphs 22 to 36, and a pharmaceutically acceptable carrier, excipient, or diluent.

60. A pharmaceutical composition according to any of paragraphs 39 to 59, which
10 further comprises an adjuvant for enhancing an immune response in a subject to the polypeptide, or to a polypeptide encoded by the nucleic acid, of the composition.

61. A method of inducing an immune response to an influenza virus in a subject, which comprises administering to the subject an effective amount of a polypeptide according to any of paragraphs 1 to 16, a nucleic acid according to any of paragraphs 17 to 21, a vector
15 according to any of paragraphs 22 to 36, or a pharmaceutical composition according to any of paragraphs 39 to 60.

62. A method of immunising a subject against an influenza virus, which comprises administering to the subject an effective amount of a polypeptide according to any of paragraphs 1 to 16, a nucleic acid according to any of paragraphs 17 to 21, a vector
20 according to any of paragraphs 22 to 36, or a pharmaceutical composition according to any of paragraphs 39 to 60.

63. A polypeptide according to any of paragraphs 1 to 16, a nucleic acid according to any of paragraphs 17 to 21, a vector according to any of paragraphs 22 to 36, or a pharmaceutical composition according to any of paragraphs 39 to 60, for use as a
25 medicament.

64. A polypeptide according to any of paragraphs 1 to 16, a nucleic acid according to any of paragraphs 17 to 21, a vector according to any of paragraphs 22 to 36, or a pharmaceutical composition according to any of paragraphs 39 to 60, for use in the prevention, treatment, or amelioration of an influenza viral infection.

30 65. Use of a polypeptide according to any of paragraphs 1 to 16, a nucleic acid according to any of paragraphs 17 to 21, a vector according to any of paragraphs 22 to 36, or a pharmaceutical composition according to any of paragraphs 39 to 60, in the

manufacture of a medicament for the prevention, treatment, or amelioration of an influenza viral infection.

66. An isolated polypeptide, which comprises an amino acid sequence of SEQ ID NO:22 (FLU_T2_HA_3_I3).

5 67. An isolated polypeptide, which comprises an amino acid sequence of SEQ ID NO:16 (FLU_T2_NA_3).

68. An isolated polypeptide, which comprises an amino acid sequence of SEQ ID NO:14 (FLU_T2_M2_1).

10 69. An isolated polynucleotide, which comprises a nucleotide sequence encoding an amino acid sequence of SEQ ID NO:22 (FLU_T2_HA_3_I3), or the complement thereof.

70. A polynucleotide according to paragraph 69, wherein the nucleotide sequence comprises a sequence of SEQ ID NO:23, or the complement thereof.

71. An isolated polynucleotide, which comprises a nucleotide sequence encoding an amino acid sequence of SEQ ID NO:16 (FLU_T2_NA_3), or the complement thereof.

15 72. A polynucleotide according to paragraph 71, wherein the nucleotide sequence comprises a sequence of SEQ ID NO:17, or the complement thereof.

73. An isolated polynucleotide, which comprises a nucleotide sequence encoding an amino acid sequence of SEQ ID NO:14 (FLU_T2_M2_1), or the complement thereof.

20 74. A polynucleotide according to paragraph 73, wherein the nucleotide sequence comprises a sequence of SEQ ID NO:15, or the complement thereof.

75. An isolated polynucleotide which comprises nucleotide sequence encoding FLU_T2_HA_3_I3 amino acid sequence (SEQ ID NO:22), and FLU_T2_NA_3 amino acid sequence (SEQ ID NO:16), or the complement thereof.

25 76. An isolated polynucleotide which comprises nucleotide sequence encoding FLU_T2_HA_3_I3 amino acid sequence (SEQ ID NO:22), and FLU_T2_M2_1 amino acid sequence (SEQ ID NO:14), or the complement thereof.

77. An isolated polynucleotide which comprises nucleotide sequence encoding FLU_T2_NA_3 amino acid sequence (SEQ ID NO:16), and FLU_T2_M2_1 amino acid sequence (SEQ ID NO:14), or the complement thereof.

78. An isolated polynucleotide which comprises nucleotide sequence encoding FLU_T2_HA_3_I3 amino acid sequence (SEQ ID NO:22), FLU_T2_NA_3 amino acid sequence (SEQ ID NO:16), and FLU_T2_M2_1 amino acid sequence (SEQ ID NO:14), or the complement thereof.

5 79. A polynucleotide according to any of paragraphs 69 to 78, which comprises a DNA molecule.

80. A polynucleotide according to any of paragraphs 69 to 78, which comprises a messenger RNA (mRNA) molecule.

81. A pharmaceutical composition which comprises:

- 10 i) an isolated polynucleotide which comprises nucleotide sequence encoding FLU_T2_HA_3_I3 amino acid sequence (SEQ ID NO:22), or the complement thereof; and
- ii) an isolated polynucleotide which comprises nucleotide sequence encoding FLU_T2_NA_3 amino acid sequence (SEQ ID NO:16), or the complement thereof.

82. A pharmaceutical composition which comprises:

- 15 i) an isolated polynucleotide which comprises nucleotide sequence encoding FLU_T2_HA_3_I3 amino acid sequence (SEQ ID NO:22), or the complement thereof; and
- ii) an isolated polynucleotide which comprises nucleotide sequence encoding FLU_T2_M2_1 amino acid sequence (SEQ ID NO:14), or the complement thereof.

83. A pharmaceutical composition which comprises:

- 20 i) an isolated polynucleotide which comprises nucleotide sequence encoding FLU_T2_NA_3 amino acid sequence (SEQ ID NO:16), or the complement thereof; and
- ii) an isolated polynucleotide which comprises nucleotide sequence encoding FLU_T2_M2_1 amino acid sequence (SEQ ID NO:14), or the complement thereof.

84. A pharmaceutical composition which comprises:

- 25 i) an isolated polynucleotide which comprises nucleotide sequence encoding FLU_T2_HA_3_I3 amino acid sequence (SEQ ID NO:22), or the complement thereof;

ii) an isolated polynucleotide which comprises nucleotide sequence encoding FLU_T2_NA_3 amino acid sequence (SEQ ID NO:16), or the complement thereof; and

iii) an isolated polynucleotide which comprises nucleotide sequence encoding FLU_T2_M2_1 amino acid sequence (SEQ ID NO:14), or the complement thereof.

5 85. A pharmaceutical composition according to any of paragraphs 81, 82, or 84, wherein the nucleotide sequence encoding FLU_T2_HA_3_I3 amino acid sequence (SEQ ID NO:22), or the complement thereof, comprises the nucleotide sequence of SEQ ID NO:23, or the complement thereof.

86. A pharmaceutical composition according to any of paragraphs 81, 83, or 84,
10 wherein the nucleotide sequence encoding FLU_T2_NA_3 amino acid sequence (SEQ ID NO:16), or the complement thereof, comprises the nucleotide sequence of SEQ ID NO:17, or the complement thereof.

87. A pharmaceutical composition according to any of paragraphs 82, 83, or 84,
15 wherein the nucleotide sequence encoding FLU_T2_M2_1 amino acid sequence (SEQ ID NO:14), or the complement thereof, comprises the nucleotide sequence of SEQ ID NO:15, or the complement thereof.

88. A pharmaceutical composition according to any of paragraphs 81 to 87, wherein each polynucleotide comprises a DNA molecule.

89. A pharmaceutical composition according to any of paragraphs 81 to 87, wherein
20 each polynucleotide comprises a messenger RNA (mRNA) molecule.

90. A combined preparation which comprises:

i) an isolated polynucleotide which comprises nucleotide sequence encoding FLU_T2_HA_3_I3 amino acid sequence (SEQ ID NO:22), or the complement thereof; and

ii) an isolated polynucleotide which comprises nucleotide sequence encoding
25 FLU_T2_NA_3 amino acid sequence (SEQ ID NO:16), or the complement thereof.

91. A combined preparation which comprises:

i) an isolated polynucleotide which comprises nucleotide sequence encoding FLU_T2_HA_3_I3 amino acid sequence (SEQ ID NO:22), or the complement thereof; and

ii) an isolated polynucleotide which comprises nucleotide sequence encoding FLU_T2_M2_1 amino acid sequence (SEQ ID NO:14), or the complement thereof.

92. A combined preparation which comprises:

- i) an isolated polynucleotide which comprises nucleotide sequence encoding FLU_T2_NA_3 amino acid sequence (SEQ ID NO:16), or the complement thereof; and
- ii) an isolated polynucleotide which comprises nucleotide sequence encoding FLU_T2_M2_1 amino acid sequence (SEQ ID NO:14), or the complement thereof.

93. A combined preparation which comprises:

- i) an isolated polynucleotide which comprises nucleotide sequence encoding FLU_T2_HA_3_I3 amino acid sequence (SEQ ID NO:22), or the complement thereof;
- ii) an isolated polynucleotide which comprises nucleotide sequence encoding FLU_T2_NA_3 amino acid sequence (SEQ ID NO:16), or the complement thereof; and
- iii) an isolated polynucleotide which comprises nucleotide sequence encoding FLU_T2_M2_1 amino acid sequence (SEQ ID NO:14), or the complement thereof.

94. A combined preparation according to any of paragraphs 90, 91, or 93, wherein the nucleotide sequence encoding FLU_T2_HA_3_I3 amino acid sequence (SEQ ID NO:22), or the complement thereof, comprises the nucleotide sequence of SEQ ID NO:23, or the complement thereof.

95. A combined preparation according to any of paragraphs 90, 92, or 93, wherein the nucleotide sequence encoding FLU_T2_NA_3 amino acid sequence (SEQ ID NO:16), or the complement thereof, comprises the nucleotide sequence of SEQ ID NO:17, or the complement thereof.

96. A combined preparation according to any of paragraphs 91, 92, or 93, wherein the nucleotide sequence encoding FLU_T2_M2_1 amino acid sequence (SEQ ID NO:14), or the complement thereof, comprises the nucleotide sequence of SEQ ID NO:15, or the complement thereof.

97. A combined preparation according to any of paragraphs 90 to 96, wherein each polynucleotide comprises a DNA molecule.

98. A combined preparation according to any of paragraphs 90 to 96, wherein each polynucleotide comprises a messenger RNA (mRNA) molecule.

99. A vector comprising a polynucleotide of any of paragraphs 69 to 80.

100. A vector according to paragraph 99, which further comprises a promoter operably
5 linked to the nucleotide sequence.

101. A vector according to paragraph 99, which further comprises, for each nucleotide sequence of the vector encoding a separate polypeptide, a separate promoter operably linked to that nucleotide sequence.

102. A vector according to paragraph 99, which is a DNA vector.

10 103. A vector according to paragraph 99, which is a messenger (mRNA) vector.

104. A pharmaceutical composition which comprises:

i) a vector comprising a polynucleotide which comprises nucleotide sequence encoding FLU_T2_HA_3_I3 amino acid sequence (SEQ ID NO:22), or the complement thereof; and

15 ii) a vector comprising a polynucleotide which comprises nucleotide sequence encoding FLU_T2_NA_3 amino acid sequence (SEQ ID NO:16), or the complement thereof.

105. A pharmaceutical composition which comprises:

i) a vector comprising a polynucleotide which comprises nucleotide sequence encoding FLU_T2_HA_3_I3 amino acid sequence (SEQ ID NO:22), or the complement thereof; and

20 ii) a vector comprising a polynucleotide which comprises nucleotide sequence encoding FLU_T2_M2_1 amino acid sequence (SEQ ID NO:14), or the complement thereof.

106. A pharmaceutical composition which comprises:

i) a vector comprising a polynucleotide which comprises nucleotide sequence encoding FLU_T2_NA_3 amino acid sequence (SEQ ID NO:16), or the complement thereof; and

25 ii) a vector comprising a polynucleotide which comprises nucleotide sequence encoding FLU_T2_M2_1 amino acid sequence (SEQ ID NO:14), or the complement thereof.

107. A pharmaceutical composition which comprises:

i) a vector comprising a polynucleotide which comprises nucleotide sequence encoding FLU_T2_HA_3_I3 amino acid sequence (SEQ ID NO:22), or the complement thereof;

ii) a vector comprising a polynucleotide which comprises nucleotide sequence encoding FLU_T2_NA_3 amino acid sequence (SEQ ID NO:16), or the complement thereof; and

5 iii) a vector comprising a polynucleotide which comprises nucleotide sequence encoding FLU_T2_M2_1 amino acid sequence (SEQ ID NO:14), or the complement thereof.

108. A pharmaceutical composition according to any of paragraphs 104, 105, or 107, wherein the nucleotide sequence encoding FLU_T2_HA_3_I3 amino acid sequence (SEQ ID NO:22), or the complement thereof, comprises the nucleotide sequence of SEQ ID
10 NO:23, or the complement thereof.

109. A pharmaceutical composition according to any of paragraphs 104, 106, or 107, wherein the nucleotide sequence encoding FLU_T2_NA_3 amino acid sequence (SEQ ID NO:16), or the complement thereof, comprises the nucleotide sequence of SEQ ID NO:17, or the complement thereof.

15 110. A pharmaceutical composition according to any of paragraphs 105, 106, or 107, wherein the nucleotide sequence encoding FLU_T2_M2_1 amino acid sequence (SEQ ID NO:14), or the complement thereof, comprises the nucleotide sequence of SEQ ID NO:15, or the complement thereof.

20 111. A pharmaceutical composition according to any of paragraphs 104 to 110, wherein each vector comprises a promoter operably linked to the encoding nucleotide sequence.

112. A pharmaceutical composition according to any of paragraphs 104 to 110, wherein each vector is a DNA vector.

113. A pharmaceutical composition according to any of paragraphs 104 to 110, wherein each vector is a messenger (mRNA) vector.

25 114. A combined preparation which comprises:

i) a vector comprising a polynucleotide which comprises nucleotide sequence encoding FLU_T2_HA_3_I3 amino acid sequence (SEQ ID NO:22), or the complement thereof; and

ii) a vector comprising a polynucleotide which comprises nucleotide sequence encoding FLU_T2_NA_3 amino acid sequence (SEQ ID NO:16), or the complement thereof.

115. A combined preparation which comprises:

i) a vector comprising a polynucleotide which comprises nucleotide sequence encoding FLU_T2_HA_3_I3 amino acid sequence (SEQ ID NO:22), or the complement thereof; and

5 ii) a vector comprising a polynucleotide which comprises nucleotide sequence encoding FLU_T2_M2_1 amino acid sequence (SEQ ID NO:14), or the complement thereof.

116. A combined preparation which comprises:

i) a vector comprising a polynucleotide which comprises nucleotide sequence encoding FLU_T2_NA_3 amino acid sequence (SEQ ID NO:16), or the complement thereof; and

10 ii) a vector comprising a polynucleotide which comprises nucleotide sequence encoding FLU_T2_M2_1 amino acid sequence (SEQ ID NO:14), or the complement thereof.

117. A combined preparation which comprises:

i) a vector comprising a polynucleotide which comprises nucleotide sequence encoding FLU_T2_HA_3_I3 amino acid sequence (SEQ ID NO:22), or the complement thereof;

15 ii) a vector comprising a polynucleotide which comprises nucleotide sequence encoding FLU_T2_NA_3 amino acid sequence (SEQ ID NO:16), or the complement thereof; and

iii) a vector comprising a polynucleotide which comprises nucleotide sequence encoding FLU_T2_M2_1 amino acid sequence (SEQ ID NO:14), or the complement thereof.

118. A combined preparation according to any of paragraphs 114, 115, or 117, wherein the nucleotide sequence encoding FLU_T2_HA_3_I3 amino acid sequence (SEQ ID
20 NO:22), or the complement thereof, comprises the nucleotide sequence of SEQ ID NO:23, or the complement thereof.

119. A combined preparation according to any of paragraphs 114, 116, or 117, wherein the nucleotide sequence encoding FLU_T2_NA_3 amino acid sequence (SEQ ID NO:16), or the complement thereof, comprises the nucleotide sequence of SEQ ID NO:17, or the
25 complement thereof.

120. A combined preparation according to any of paragraphs 115, 116, or 117, wherein the nucleotide sequence encoding FLU_T2_M2_1 amino acid sequence (SEQ ID NO:14), or the complement thereof, comprises the nucleotide sequence of SEQ ID NO:15, or the complement thereof.

121. A combined preparation according to any of paragraphs 114 to 120, wherein each vector comprises a promoter operably linked to the encoding nucleotide sequence.

122. A combined preparation according to any of paragraphs 114 to 120, wherein each vector is a DNA vector.

5 123. A combined preparation according to any of paragraphs 114 to 120, wherein each vector is a messenger (mRNA) vector.

124. A vector according to paragraph 100 or 101, a pharmaceutical composition according to paragraph 111, or a combined preparation according to paragraph 121, wherein the, or each promoter is for expression of a polypeptide encoded by the polynucleotide in
10 mammalian cells.

125. A vector according to paragraph 100 or 101, a pharmaceutical composition according to paragraph 111, or a combined preparation according to paragraph 121, wherein the, or each promoter is for expression of a polypeptide encoded by the polynucleotide in yeast or insect cells.

15 126. A vector according to any of paragraphs 99-103, a pharmaceutical composition according to any of paragraphs 104-113, or a combined preparation according to any of paragraphs 114-123, wherein the, or each vector is a vaccine vector.

127. A vector, pharmaceutical composition, or combined preparation, according to paragraph 126, wherein the, or each vaccine vector is a viral vaccine vector, a bacterial
20 vaccine vector, an RNA vaccine vector, an mRNA vaccine vector, or a DNA vaccine vector.

128. A vector according to any of paragraphs 99-102, 124, 126, or 127, a pharmaceutical composition according to any of paragraphs 104, 105, 107-112, 124, 126, or 127, or a combined preparation according to any of paragraphs 114, 115, 117-122, 124, 126, or 127, wherein the vector comprising a polynucleotide which comprises nucleotide sequence
25 encoding FLU_T2_HA_3_I3 amino acid sequence (SEQ ID NO:22) comprises the nucleotide sequence of SEQ ID NO:24, or the complement thereof,

129. An isolated cell comprising a vector of any of paragraphs 99-103, 124, 126, or 127.

130. An isolated polypeptide which comprises FLU_T2_HA_3_I3 amino acid sequence (SEQ ID NO:22), FLU_T2_NA_3 amino acid sequence (SEQ ID NO:16), and
30 FLU_T2_M2_1 amino acid sequence (SEQ ID NO: 14).

131. An isolated polypeptide which comprises FLU_T2_HA_3_I3 amino acid sequence (SEQ ID NO:22), and FLU_T2_NA_3 amino acid sequence (SEQ ID NO:16).

132. An isolated polypeptide which comprises FLU_T2_HA_3_I3 amino acid sequence (SEQ ID NO:22), and FLU_T2_M2_1 amino acid sequence (SEQ ID NO: 14).

5 133. An isolated polypeptide which comprises FLU_T2_NA_3 amino acid sequence (SEQ ID NO:16), and FLU_T2_M2_1 amino acid sequence (SEQ ID NO: 14).

134. A pharmaceutical composition which comprises:

i) a polypeptide which comprises FLU_T2_HA_3_I3 amino acid sequence (SEQ ID NO:22);
and

10 ii) a polypeptide which comprises FLU_T2_NA_3 amino acid sequence (SEQ ID NO:16).

135. A pharmaceutical composition which comprises:

i) a polypeptide which comprises FLU_T2_HA_3_I3 amino acid sequence (SEQ ID NO:22);
and

ii) a polypeptide which comprises FLU_T2_M2_1 amino acid sequence (SEQ ID NO:14).

15 136. A pharmaceutical composition which comprises:

i) a polypeptide which comprises FLU_T2_NA_3 amino acid sequence (SEQ ID NO:16);
and

ii) a polypeptide which comprises FLU_T2_M2_1 amino acid sequence (SEQ ID NO:14).

137. A pharmaceutical composition which comprises:

20 i) a polypeptide which comprises FLU_T2_HA_3_I3 amino acid sequence (SEQ ID NO:22);

ii) a polypeptide which comprises FLU_T2_NA_3 amino acid sequence (SEQ ID NO:16);
and

iii) a polypeptide which comprises FLU_T2_M2_1 amino acid sequence (SEQ ID NO:14).

138. A combined preparation which comprises:

i) a polypeptide which comprises FLU_T2_HA_3_I3 amino acid sequence (SEQ ID NO:22);
and

ii) a polypeptide which comprises FLU_T2_NA_3 amino acid sequence (SEQ ID NO:16).

139. A combined preparation which comprises:

5 i) a polypeptide which comprises FLU_T2_HA_3_I3 amino acid sequence (SEQ ID NO:22);
and

ii) a polypeptide which comprises FLU_T2_M2_1 amino acid sequence (SEQ ID NO:14).

140. A combined preparation which comprises:

10 i) a polypeptide which comprises FLU_T2_NA_3 amino acid sequence (SEQ ID NO:16);
and

ii) a polypeptide which comprises FLU_T2_M2_1 amino acid sequence (SEQ ID NO:14).

141. A combined preparation which comprises:

i) a polypeptide which comprises FLU_T2_HA_3_I3 amino acid sequence (SEQ ID NO:22);

15 ii) a polypeptide which comprises FLU_T2_NA_3 amino acid sequence (SEQ ID NO:16);
and

iii) a polypeptide which comprises FLU_T2_M2_1 amino acid sequence (SEQ ID NO:14).

142. A pharmaceutical composition, which comprises an isolated polynucleotide according to any of paragraphs 69-80, and a pharmaceutically acceptable carrier, excipient, or diluent.

20 143. A pharmaceutical composition, which comprises a vector according to any of paragraphs 99-103, and a pharmaceutically acceptable carrier, excipient, or diluent.

144. A pharmaceutical composition, which comprises an isolated polypeptide according to any of paragraphs 66-68, or 130-133, and a pharmaceutically acceptable carrier, excipient, or diluent.

25 145. A pharmaceutical composition according to any of paragraphs 81-89, 104-113, 124-128, 134-137, or 142-144, which further comprises an adjuvant for enhancing an immune

response in a subject to a polypeptide, or to a polypeptide encoded by a nucleotide, of the composition.

146. A polynucleotide according to any of paragraphs 182-188, which comprises one or more modified nucleosides.

5 147. A vector according to any of paragraphs 99-103, or 124-128, wherein the polynucleotide of the vector comprises one or more modified nucleosides.

148. A pharmaceutical composition according to any of paragraphs 81-89, 104-113, 124-128, 134-137, or 142-145, wherein the or each polynucleotide of the composition comprises one or more modified nucleosides.

10 149. A combined preparation according to any of paragraphs 90-98, 114-128, or 138-141, wherein each nucleic acid of the combined preparation comprises one or more modified nucleosides.

150. A polynucleotide according to paragraph 146, a vector according to paragraph 147, a pharmaceutical composition according to paragraph 148, or a combined preparation
15 according to paragraph 149, wherein the or each polynucleotide comprises a messenger RNA (mRNA).

151. A polynucleotide according to paragraph 146 or 150, a vector according to paragraph 147 or 150, a pharmaceutical composition according to paragraph 148 or 150, or a combined preparation according to paragraph 149 or 150, wherein the one or more
20 modified nucleosides comprise a 1-methylpseudouridine modification.

152. A polynucleotide according to paragraph 146 or 150 or 151, a vector according to paragraph 147 or 150 or 151, a pharmaceutical composition according to paragraph 148 or 150 or 151, or a combined preparation according to paragraph 149 or 150 or 151, wherein the one or more modified nucleosides comprise a 1-methylpseudouridine modification.

25 153. A polynucleotide according to any of paragraphs 146 or 150-152, a vector according to any of paragraphs 147, or 150-152, a pharmaceutical composition according to any of paragraphs 148, or 150-152, or a combined preparation according to any of paragraphs 149-152, wherein at least 80% of the uridines in the open reading frame have been modified.

30 154. A fusion protein comprising a polypeptide according to any of paragraphs 66-68, or 130-133.

155. A pseudotyped virus particle comprising a polypeptide according to any of paragraphs 66-68, or 130-133.

156. A method of inducing an immune response to an influenza virus in a subject, which comprises administering to the subject an effective amount of a polypeptide according to any
5 of paragraphs 66-68, or 130-133, a polynucleotide according to any of paragraphs 69-80, 146, or 150-153, a vector according to any of paragraphs 99-103, 124-128, 147, or 150-153, a pharmaceutical composition according to any of paragraphs 81-89, 104-113, 124-128, 134-137, 142-145, 148, or 150-153, or a combined preparation according to any of paragraphs 90-98, 114-128, 138-141, or 149-153.

10 157. A method of immunising a subject against an influenza virus, which comprises administering to the subject an effective amount of a polypeptide according to any of paragraphs 66-68, or 130-133, a polynucleotide according to any of paragraphs 69-80, 146, or 150-153, a vector according to any of paragraphs 99-103, 124-128, 147, or 150-153, a pharmaceutical composition according to any of paragraphs 81-89, 104-113, 124-128, 134-
15 137, 142-145, 148, or 150-153, or a combined preparation according to any of paragraphs 90-98, 114-128, 138-141, or 149-153.

158. A polypeptide according to any of paragraphs 66-68, or 130-133, a polynucleotide according to any of paragraphs 69-80, 146, or 150-153, a vector according to any of paragraphs 99-103, 124-128, 147, or 150-153, a pharmaceutical composition according to
20 any of paragraphs 81-89, 104-113, 124-128, 134-137, 142-145, 148, or 150-153, or a combined preparation according to any of paragraphs 90-98, 114-128, 138-141, or 149-153, for use as a medicament.

159. A polypeptide according to any of paragraphs 66-68, or 130-133, a polynucleotide according to any of paragraphs 69-80, 146, or 150-153, a vector according to any of
25 paragraphs 99-103, 124-128, 147, or 150-153, a pharmaceutical composition according to any of paragraphs 81-89, 104-113, 124-128, 134-137, 142-145, 148, or 150-153, or a combined preparation according to any of paragraphs 90-98, 114-128, 138-141, or 149-153, for use in the prevention, treatment, or amelioration of an influenza viral infection.

160. Use of a polypeptide according to any of paragraphs 66-68, or 130-133, a
30 polynucleotide according to any of paragraphs 69-80, 146, or 150-153, a vector according to any of paragraphs 99-103, 124-128, 147, or 150-153, a pharmaceutical composition according to any of paragraphs 81-89, 104-113, 124-128, 134-137, 142-145, 148, or 150-153, or a combined preparation according to any of paragraphs 90-98, 114-128, 138-141, or

149-153, in the manufacture of a medicament for the prevention, treatment, or amelioration of an influenza viral infection.

161. A combined preparation, which comprises:

i) a polypeptide of any of paragraphs 1 to 12;

5 ii) a polypeptide of paragraph 14; and

iii) a polypeptide of paragraph 15 or 16.

162. A combined preparation, which comprises:

i) a polypeptide of any of paragraphs 1 to 12; and

ii) a polypeptide of paragraph 14.

10 163. A combined preparation, which comprises:

i) a polypeptide of any of paragraphs 1 to 12; and

ii) a polypeptide of paragraph 15 or 16.

164. A combined preparation, which comprises:

i) a polypeptide of paragraph 14; and

15 ii) a polypeptide of paragraph 15 or 16.

165. A combined preparation, which comprises:

i) a polynucleotide encoding a polypeptide of any of paragraphs 1 to 12;

ii) a polynucleotide encoding a polypeptide of paragraph 14; and

iii) a polynucleotide encoding a polypeptide of paragraph 15 or 16.

20 166. A combined preparation, which comprises:

i) a polynucleotide encoding a polypeptide of any of paragraphs 1 to 12; and

ii) a polynucleotide encoding a polypeptide of paragraph 14.

167. A combined preparation, which comprises:

- i) a polynucleotide encoding a polypeptide of any of paragraphs 1 to 12; and
- ii) a polynucleotide encoding a polypeptide of paragraph 15 or 16.

168. A combined preparation, which comprises:

- i) a polynucleotide encoding a polypeptide of paragraph 14; and
- 5 ii) a polynucleotide encoding a polypeptide of paragraph 15 or 16.

169. A combined preparation according to any of paragraphs 161, 162, or 163, which comprises a polypeptide which comprises an amino acid sequence of SEQ ID NO:7 or 8.

170. A combined preparation according to any of paragraphs 161, 162, or 163, comprising a polypeptide which comprises an amino acid sequence of SEQ ID NO:10 or
10 11.

171. A combined preparation according to any of paragraphs 161, 162, or 163, comprising a polypeptide which comprises an amino acid sequence of SEQ ID NO:1 or 3.

172. A combined preparation according to any of paragraphs 161, 162, or 164, comprising a polypeptide which comprises an amino acid sequence of SEQ ID NO:14.

15 173. A combined preparation according to any of paragraphs 161, 163, or 164, comprising a polypeptide which comprises an amino acid sequence of SEQ ID NO:16.

174. A combined preparation according to any of paragraphs 161, 163, or 164, comprising a polypeptide which comprises an amino acid sequence of SEQ ID NO:18.

175. A combined preparation according to any of paragraphs 165, 166, or 167, which
20 comprises a polynucleotide encoding a polypeptide which comprises an amino acid sequence of SEQ ID NO:7 or 8.

176. A combined preparation according to any of paragraphs 165, 166, or 167, comprising a polynucleotide encoding a polypeptide which comprises an amino acid sequence of SEQ ID NO:10 or 11.

25 177. A combined preparation according to any of paragraphs 165, 166, or 167, comprising a polynucleotide encoding a polypeptide which comprises an amino acid sequence of SEQ ID NO:1 or 3.

178. A combined preparation according to any of paragraphs 165, 166, or 168, comprising a polynucleotide encoding a polypeptide which comprises an amino acid sequence of SEQ ID NO:14.

5 179. A combined preparation according to any of paragraphs 165, 167, or 168, comprising a polynucleotide encoding a polypeptide which comprises an amino acid sequence of SEQ ID NO:16.

180. A combined preparation according to any of paragraphs 165, 167, or 168, comprising a polynucleotide encoding a polypeptide which comprises an amino acid sequence of SEQ ID NO:18.

10 181. A combined preparation according to any of paragraphs 165-168, or 175-180, wherein each polynucleotide comprises a DNA molecule.

182. A combined preparation according to any of paragraphs 165-168, or 175-180, wherein each polynucleotide comprises a messenger RNA (mRNA) molecule.

15 183. A combined preparation according to any of paragraphs 165-168, or 175-180, wherein each polynucleotide is provided by a vector.

184. A combined preparation according to paragraph 183, wherein each vector comprises a promoter operably linked to the encoding nucleotide sequence.

185. A combined preparation according to paragraph 183 or 184, wherein each vector is a DNA vector.

20 186. A combined preparation according to paragraph 183 or 184, wherein each vector is a messenger (mRNA) vector.

187. A combined preparation according to paragraph 183, wherein each promoter is for expression of a polypeptide encoded by the polynucleotide in mammalian cells.

25 188. A combined preparation according to paragraph 183, wherein each promoter is for expression of a polypeptide encoded by the polynucleotide in yeast or insect cells.

189. A combined preparation according to any of paragraphs 183-188, wherein each vector is a vaccine vector.

190. A combined preparation according to paragraph 189, wherein each vaccine vector is a viral vaccine vector, a bacterial vaccine vector, an RNA vaccine vector, an mRNA vaccine vector, or a DNA vaccine vector.

5 191. A combined preparation according to any of paragraphs 165-168, 175-190, wherein each nucleic acid of the combined preparation comprises one or more modified nucleosides.

192. A combined preparation according to paragraph 191, wherein each polynucleotide comprises a messenger RNA (mRNA).

10 193. A combined preparation according to paragraph 191 or 192, wherein the one or more modified nucleosides comprise a 1-methylpseudouridine modification.

194. A combined preparation according to paragraph 191 or 192 or 193, wherein the one or more modified nucleosides comprise a 1-methylpseudouridine modification.

195. A combined preparation according to any of paragraphs 191-194, wherein at least 80% of the uridines in the open reading frame have been modified.

15

Embodiments of the invention are now described, by way of example only, with reference to the accompanying drawings, in which:

Figure 1 shows the results of a neutralisation assay illustrating the strength of neutralising antibody responses to various pseudotyped viruses with H5 from different clades and sub-clades;

Figure 2 shows an amino acid sequence comparison of different embodiments of polypeptides of the invention;

Figure 3 shows an amino acid sequence comparison of different embodiments of polypeptides of the invention and prior art COBRA sequences;

Figure 4 shows the results of a flow cytometry-based immunofluorescence assay to test the ability of mouse sera, obtained following immunisation of mice with an embodiment of the invention, to target M2 molecules from various influenza A isolates;

Figure 5 shows the results of a Pseudotype-based Enzyme-Linked Lectin Assay (pELLA) using FLU_T2_NA_3;

Figure 6 shows the results of a pELLA using FLU_T2_NA_4;

Figure 7 shows the results of a pELLA with N9 mAbs;

Figure 8 shows a plasmid map for pEVAC vector;

Figure 9 shows $\log_{10}IC_{50}$ plot for pEVAC_Flu_T2_HA_3_I-3 and other controls;

Figure 10 shows inhibition of enzymatic activity of A/Brisbane/02/2018 neuraminidase by sera from mouse vaccinated by (A) PBS, (B) Primary strain - A/Brisbane/02/2018, (C) N1_Final_1, (D) N1_Final_2 (Flu_T2_NA_3);

Figures 11a and 11b show a vaccination protocol for panH1N1 in pigs;

Figure 12 shows nasal shedding of viral RNA in pigs post infection in four different vaccination groups, monitored daily by RRT-qPCR. Figure 12b illustrates virus titration measurements from bronchoalveolar lavage (BAL) fluid, turbinates, and trachea samples from pigs in each group;

Figure 13a shows the results of a HAI assay across four vaccination groups vs SW/EN/09 at different time points. Figure 13b shows the results of an NP competition ELISA (Idvet);

Figure 14 shows serum neutralising titers at different days post vaccination/infection vs SW/EN/09;

Figure 15a shows the results of a T-cell peptide stimulation assay; splenocytes were stimulated with the peptides spanning A/swine/England/1353/2009 strain and a/Victoria/2454/ HA. Figure 15b shows a HAI assay. The top panel shows distribution of the hemagglutinin inhibition titre 0 days, 28 days, 42 days and 63 days post vaccination and 8 days post infection. The titres were checked against A/swine/England/1353/2009 strain and a/Victoria/2454/2019 strain. The lower panel illustrates the mean values for each group;

Figure 16 shows a 3D model of DIOS panH1N1 designed vaccine, comprising HA, NA, and M2 polypeptides;

Figure 17a shows the results of a serum neutralisation assay in mice vs H1 pseudovirus panel using FLU_T2_HA_3_I3. Figure 17b shows the results of a HAI assay vs a panel of H1 wildtype viruses in mice;

Figure 18 shows viral RNA shedding in pigs vaccinated with panH1N1 and controls at a number of time points post infection with A/swine/EN/1353/09 10 weeks post-prime;

Figures 19a and 19b show the results of a serum neutralisation assay in pigs using panH1N1 vs H1 clades at various time points. Figure 19c shows the results of a neutralisation assay v a panel of H1 pseudoviruses using panH1N1 in pigs;

Figures 20a and 20b show an ELLA (Enzyme-Linked Lectin Assay) to assess the inhibition activity of the NA component of panH1N1 against A/swine/England/1353/2009 (Figure 20a) and A/England/195/2009 (Figure 20b) at a series of time points post-vaccination/infection. Figure 20c shows an ELLA against a panel of NA expressing pseudoviruses at 42 days post vaccination;

Figure 21 summarises differences in amino acid sequence of the influenza haemagglutinin H5 for different embodiments of the invention, including differences at positions A-E of H5 for the embodiments;

Figure 22 shows a multiple sequence alignment comparing the amino acid sequence of embodiments of the invention with two influenza isolates. In the figure, differences in amino acid residues are shown underlined, with amino acid differences across designed sequences FLU_T2_HA_1 and FLU_T3_HA_1/2/3/4/5 shown highlighted; and

Figure 23 shows serum neutralisation data for T3 H5 vaccine designs against a panel of 9 antigenically different H5Nx.

Figure 24 shows an updated Figure 17a, wherein two additional seasonal H1 wildtype strains are used as challenges against the designed panH1N1 vaccine.

Figure 25 summarises novel amino acid residue changes in FLU_T4_HA_1, FLU_T4_HA_2, and FLU_T4_HA_3 designed sequences. These novel amino acid residue changes are shown in bold and underline.

Figure 26 shows important amino acid residue positions of influenza H5. The residues shown in bold and underline format are novel amino acid residues in the H5 Tier 4 designs.

Figure 27 summarises amino acid residues of H5 FLU_T4_HA_1, FLU_T4_HA_2, and FLU_T4_HA_3, at further important residue positions of H5.

Figure 28 shows a multiple sequence alignment of H5 amino acid sequence for FLU_T4_HA_1, FLU_T4_HA_2, and FLU_T4_HA_3, known wild-type influenza H5 strains, and previously designed H5 sequences. The amino acid residue positions in the figure correspond to the amino acid residue positions of A/Sichuan/2014.

Figure 29A-I shows the neutralising activity of the candidate H5 vaccine antigens, previous designed sequences, and WT sequences, against a panel of clade 2.3.4.4 H5 viruses.

Figures 30A-I show individual neutralisation curves for mice immunised with designed sequences or WT sequences, vs A/gyrfalcon/Washington/41088-6/2014) clade 2.3.4.4c. challenge strain.

Figures 31A-I show individual neutralisation curves for mice immunised with designed sequences or WT sequences, vs A/Sichuan/26221/2014 clade 2.3.4.4a challenge strain.

Figures 32A-I show individual neutralisation curves for mice immunised with designed sequences or WT sequences, vs A/Anhui/2021-00011/2020 clade 2.3.4.4h challenge strain.

Figures 33A-I show individual neutralisation curves for mice immunised with designed sequences or WT sequences, vs A/mute swan/England/053054/2021 clade 2.3.4.4b. challenge strain.

Figures 34A-I show individual neutralisation curves for mice immunised with designed sequences or WT sequences, vs A/Hangzhou/01/2021 clade 2.3.4.4b. challenge strain.

Table of SEQ ID NOs:**SEQ ID NO: Description**

	1	FLU_T2_HA_1: HA0 amino acid sequence
	2	FLU_T2_HA_1: HA0 nucleic acid sequence
5	3	FLU_T2_HA_1: head region amino acid sequence
	4	FLU_T2_HA_1: head region nucleic acid sequence
	5	FLU_T2_HA_1: stem region amino acid sequence
	6	FLU_T2_HA_1: stem region nucleic acid sequence
	7	FLU_T3_HA_1: HA0 amino acid sequence
10	8	FLU_T3_HA_1: head region amino acid sequence
	9	FLU_T3_HA_1: stem region amino acid sequence
	10	FLU_T3_HA_2: HA0 amino acid sequence
	11	FLU_T3_HA_2: head region amino acid sequence
	12	FLU_T3_HA_2: stem region amino acid sequence
15	13	Fragment of H5 globular head domain
	14	FLU_T2_M2_1: amino acid sequence
	15	FLU_T2_M2_1: nucleic acid sequence
	16	FLU_T2_NA_3 (N1_FINAL_2): amino acid sequence
	17	FLU_T2_NA_3 (N1_FINAL_2): nucleic acid sequence
20	18	FLU_T2_NA_4 (N1_FINAL_3): amino acid sequence
	19	FLU_T2_NA_4 (N1_FINAL_3): nucleic acid sequence
	20	pEVAC multiple cloning site sequence
	21	Entire pEVAC sequence
	22	FLU_T2_HA_3_I3: amino acid sequence
25	23	FLU_T2_HA_3_I3: nucleic acid sequence
	24	pEVAC- FLU_T2_HA_3_I3: nucleic acid sequence
	25	panH1N1: nucleic acid sequence
	26	pEVAC_panH1N1: nucleic acid sequence
	27	FLU_T3_HA_3: HA0 amino acid sequence
30	28	FLU_T3_HA_3: HA0 nucleic acid sequence
	29	FLU_T3_HA_3: head region amino acid sequence
	30	FLU_T3_HA_3: head region nucleic acid sequence
	31	FLU_T3_HA_3: first stem region amino acid sequence
	32	FLU_T3_HA_3: first stem region nucleic acid sequence
35	33	FLU_T3_HA_3: second stem region amino acid sequence
	34	FLU_T3_HA_3: second stem region nucleic acid sequence
	35	FLU_T3_HA_4: HA0 amino acid sequence
	36	FLU_T3_HA_4: HA0 nucleic acid sequence
	37	FLU_T3_HA_4: head region amino acid sequence
40	38	FLU_T3_HA_4: head region nucleic acid sequence
	39	FLU_T3_HA_4: first stem region amino acid sequence
	40	FLU_T3_HA_4: first stem region nucleic acid sequence
	41	FLU_T3_HA_4: second stem region amino acid sequence
	42	FLU_T3_HA_4: second stem region nucleic acid sequence
45	43	FLU_T3_HA_5: HA0 amino acid sequence
	44	FLU_T3_HA_5: HA0 nucleic acid sequence
	45	FLU_T3_HA_5: head region amino acid sequence
	46	FLU_T3_HA_5: head region nucleic acid sequence
	47	FLU_T3_HA_5: first stem region amino acid sequence
50	48	FLU_T3_HA_5: first stem region nucleic acid sequence
	49	FLU_T3_HA_5: second stem region amino acid sequence

	50	FLU_T3_HA_5: second stem region nucleic acid sequence
	51	FLU_T3_HA_1: first stem region amino acid sequence
	52	FLU_T3_HA_1: first stem region nucleic acid sequence
	53	FLU_T3_HA_1: second stem region amino acid sequence
5	54	FLU_T3_HA_1: second stem region nucleic acid sequence
	55	FLU_T3_HA_1: HA0 nucleic acid sequence
	56	FLU_T3_HA_1: head region nucleic acid sequence
	57	FLU_T3_HA_2: first stem region amino acid sequence
	58	FLU_T3_HA_2: first stem region nucleic acid sequence
10	59	FLU_T3_HA_2: second stem region amino acid sequence
	60	FLU_T3_HA_2: second stem region nucleic acid sequence
	61	FLU_T3_HA_2: HA0 nucleic acid sequence
	62	FLU_T3_HA_2: head region nucleic acid sequence
	63	panH1N1: amino acid sequence
15	64	A/whooper swan/Mongolia/244/2005 H5 (H5_WSN)
	65	A/gyrfalcon/Washington/41088-6/2014 (H5_GYR)
	66	First 2A self-cleaving peptide sequence: GSGEGRGSLTCDVEENPGP
	67	Second 2A self-cleaving peptide sequence: GSGATNFSLLKQAGDVEENPGP
20	68	FLU_T2_HA_4 – amino acid sequence
	69	FLU_T2_HA_4 – nucleic acid sequence
	70	pEVAC-FLU_T2_HA_4 nucleic acid sequence
	71	FLU_T4_HA_1: HA0 amino acid sequence
	72	FLU_T4_HA_1: HA0 nucleic acid sequence
25	73	FLU_T4_HA_1: head region amino acid sequence
	74	FLU_T4_HA_1: head region nucleic acid sequence
	75	FLU_T4_HA_1: first stem region amino acid sequence
	76	FLU_T4_HA_1: first stem region nucleic acid sequence
	77	FLU_T4_HA_1: second stem region amino acid sequence
30	78	FLU_T4_HA_1: second stem region nucleic acid sequence
	79	pEVAC-FLU_T4_HA_1 – nucleic acid sequence
	80	FLU_T4_HA_2: HA0 amino acid sequence
	81	FLU_T4_HA_2: HA0 nucleic acid sequence
	82	FLU_T4_HA_2: head region amino acid sequence
35	83	FLU_T4_HA_2: head region nucleic acid sequence
	84	FLU_T4_HA_2: first stem region amino acid sequence
	85	FLU_T4_HA_2: first stem region nucleic acid sequence
	86	FLU_T4_HA_2: second stem region amino acid sequence
	87	FLU_T4_HA_2: second stem region nucleic acid sequence
40	88	pEVAC-FLU_T4_HA_2 – nucleic acid sequence
	89	FLU_T4_HA_3: HA0 amino acid sequence
	90	FLU_T4_HA_3: HA0 nucleic acid sequence
	91	FLU_T4_HA_3: head region amino acid sequence
	92	FLU_T4_HA_3: head region nucleic acid sequence
45	93	FLU_T4_HA_3: first stem region amino acid sequence
	94	FLU_T4_HA_3: first stem region nucleic acid sequence
	95	FLU_T4_HA_3: second stem region amino acid sequence
	96	FLU_T4_HA_3: second stem region nucleic acid sequence
	97	pEVAC-FLU_T4_HA_3 – nucleic acid sequence
50	98	FLU_T3_NA_3 amino acid sequence
	99	FLU_T3_NA_3 nucleic acid sequence
	100	A/Sichuan/2014 H5 amino acid sequence

Example 1 – FLU_T2_HA_1

This example provides amino acid sequences of the influenza haemagglutinin H5 head and stem regions for an embodiment of the invention known as FLU_T2_HA_1. In SEQ ID NO:1 below, the amino acid residues of the stem region are shown underlined. The amino acid residues of the head region are the remaining residues.

FLU_T2_HA_1 – HA0 amino acid sequence (SEQ ID NO:1):

MEKIVLLLAIVSLVKSDQICIGYHANNSTEQVDTIMEKNVTVTTHAQDILEKTHNGKLCDLDGV
 KPLILRDCSVAGWLLGNPMCEFINVPEWSYIVEKANPANDLCYPGNFNDYEELKHLLSRI
 NHFEKIQIIPKSSWSDHEASSGVSSACPYQGRSSFFRNVVWLIKNNAYPTIKRSYNNTNQ
 10 EDLLVLWGIHHPNDAAEQTRL YQNPTTYISVGTSTLNQRLVPKIATRSKVNGQSGRMEFF
 WTILKPNDAINFESNGNFIAPEYAYKIVKKGDSAIMKSELEYGNCNTKCQTPMGAINSSMPF
 HNIHPLTIGECPKYVKSNRLVLATGLRNSPQRERRRKKRGLFGAIAGFIEGGWQGMVDGW
YGYHHSNEQGSGYAADKESTQKAIDGVTNKVNSIIDKMNTQFEAVGREFNNLERRIENLN
KKMEDGFLDVWTYNAELLVLMENERTLDFHDSNVKNLYDKVRLQLRDNAKELGNGCFEF
 15 YHKCDNECMESVRNGTYDYPQYSEEARLKREEISGVKLESIGTYQILSIYSTVASSLALAIM
VAGLSLWMCSNGSLQCRICI

FLU_T2_HA_1 – HA0 nucleic acid sequence (SEQ ID NO:2):

atggaaaagattgtgctgctgctggccatcggtgccctggccaagagcgatcaaactgcatcggtaccacgccaacaacag
caccgaacaggtggacaccattatggaaaagaacgtgaccgtgacacacgcccaggacatcctggaaaagaccacaac
 20 ggcaagctgtgcgacctggatggcgtgaagcctctgacctgagagattgctctgtggccggctggctgctgggcaatcctatg
gcgacgagttcatcaacgtgcccagtggtcctatatctgtggaaaaggccaatcctgccaacgacctgtgctaccccgga
cttcaacgactacgaggaactgaaacatctgctgagccggatcaaccacttcgagaagatccagatcatccccaagtcctctt
ggagcgatcacgaggccttagcggagtgtctagcgctgtccttaccaggcagaagcagcttctccggaacgtgctgtgg
ctgatcaagaagaacaacgcttaccaccatcaagcggagctacaacaacaccaatcaaggagacctgtggtgctgtgg
 25 ggcatccaccatcctaataatgatgccgccgagcagacccggctgtaccagaatcctacaacctacatcagcgtgggcaccagc
acactgaaccagagactggtgcctaagatcgccaccagatccaaagtgaacggccagagcggccgatggaattcttctgg
accatcctgaagcctaacgacgccatcaactcgagagcaacggcaactttatgcccctgagtacgcctacaagatcgatga
agaaggcgacagcgccatcatgaagtccgagctggaatacggcaactgcaacaccaagtgtcagacccctatgggcgcc
atcaatagcagcatgccctccacaacattcaccctctgaccatcggcgagtgccccaaatacgtgaagtccaacagactggt
 30 cctggccaccggcctgagaaattctccacagagagagcggcgagaaagaagagaggcctgtttggagccattgccggctt
tatcgaaggcggctggcaaggcatggttgacggatggtacggctatcaccacagcaatgagcaaggctctggctacgccgc
cgacaaagagagcacacagaaagccatcgacggcgtgaccaacaaagtgaatagcatcatcgacaagatgaacaccca
gttcgaggccgtgggcagagagttcaacaacctggaaagacggatcgagaacctgaacaagaagatggaggacggcttc
ctggacgtgtggacctataatgccgagctgctggtcctgatggaaaacgagagaacctggacttcacgacagcaacgtga

agaacctgtacgacaaagtgcggctccagctgcgggacaatgccaaagaactcggcaacggctgcttcgagttctaccaca
agtgcgacaacgagtgcatggaaagcgtgcggaacggcacctacgactaccctcagtactctgaggaagcccggtgaag
agagaagagatcagcggagtggaagctggaatccatcgccacataccagatcctgagcatctacagaccgtggcctcttctc
ggcctggctattatggtggtggcctgagcctgtggatgtgctctaattggcagcctccagtgccggatctgcatc

5 *FLU_T2_HA_1 – head region amino acid sequence (SEQ ID NO:3):*

THNGKLCDLDGVKPLILRDCSVAGWLLGNPMCDEFINVPWSYIVEKANPANDLCYPGNF
 NDYEELKHLLSRINHFEKIQIIPKSSWSDHEASSGVSSACPYQGRSSFFRNVVWLIKNNA
 YPTIKRSYNNTNQEDLLVLWGIHHPNDAAEQTRLYQNPTTYISVGTSTLNQRLVPKIATRSK
 VNGQSGRMEFFWTILKPNDAINFESNGNFIAPEYAYKIVKKGDSAIMKSELEYGNCNTKCCQ

10 TPMGAINSSMPFHNIHPLTIGECF

The amino acid residues at positions 156, 157, 171, 172, and 205 are shown underlined in the above sequence (and are R, S, N, A, and R, respectively).

FLU_T2_HA_1 – head region nucleic acid sequence (SEQ ID NO:4):

15 acccacaacggcaagctgtgcgacctggatggcgtgaagcctctgatcctgagagattgctctgtggccggctggctgctggg
 caatcctatgtgcgacgagttcatcaacgtgcccagtggtctatatctgtgaaaaggccaatcctgccaacgacctgtgcta
 ccccggaacttcaacgactacgaggaactgaaacatctgctgagccggatcaaccacttcgagaagatccagatcatcccc
 aagtcctcttgagcgatcacgaggcctctagcggagtgcttagcgctgtcctaccaaggcagaagcagcttctccggaac
 gtcgtgtggctgatcaagaagaacaacgcttaccaccatcaagcggagctacaacaacaccaatcaagaggacctgctg
 gtgctgtggggcatccaccatcctaattgatgccgccgagcagaccggctgtaccagaatcctacaacctacatcagcgtgg
 20 gcaccagcacactgaaccagagactggtgcctaagatgccaccagatccaaagtgaacggccagagcggccggatgga
 attctctggaccatcctgaagcctaacgacgccatcaacttcgagagcaacggcaactttatcgcccctgagtacgcctacaa
 gatcgtgaagaagggcgacagcgccatcatgaagtcgagctggaatacggcaactgcaacaccaagtgtagaccctta
 tgggcgccatcaatagcagcatgccctccacaacattcacctctgaccatcggcgagtgcccc

FLU_T2_HA_1 – stem region amino acid sequence (SEQ ID NO:5):

25 MEKIVLLLAIVSLVKSDQICIGYHANNSTEQVDTIMEKNVTVTTHAQDILEKKYVKSRLVLAT
 GLRNSPQRERRRRKKRGLFGAIAFGIEGGWQGMVDGWYGYHHSNEQSGSYAADKESTQ
 KAIDGVTNKVNSIIDKMNTQFEAVGRENNLERRIENLNKKMEDGFLDVWTYNAELLVLME
 NERTLDFHDSNVKNLYDKVRLQLRDNAKELGNGCFEFYHKCDNECMESVRNGTYDYPQY
 SEEARLKREEISGVKLESIGTYQILSIYSTVASSLALAIMVAGLSLWMCSNGSLQCRICI

30 The amino acid residues at positions 416 and 434 (or at positions 148 and 166 if counting from the beginning of the stem region) are shown underlined in the above sequence (and are F and F, respectively).

FLU_T2_HA_1 – stem region nucleic acid sequence (SEQ ID NO:6):

atggaaaagattgtgctgctgctggccatcggtgccctggtcaagagcgatcaaactgcatcggtaccacgccaacaacag
 caccgaacaggtggacaccattatggaaaagaacgtgaccgtgacacacgcccaggacatcctggaaaagaaatacgtg
 aagtccaacagactggctcctggccaccggcctgagaaattctccacagagagagcggcgcagaaagaagagaggcctgtt
 5 tggagccattgccggctttatcgaaggcggctggcaaggcatggtgacggatggtacggctatcaccacagcaatgagcaa
 ggctctggctacgcccgcgacaaagagagcacacagaaagccatcgacggcgtgaccaacaaagtgaatagcatcatcg
 acaagatgaacacccagttcgaggccgtgggcagagagttcaacaacctggaaagacggatcgagaacctgaacaagaa
 gatggaggacggcttctggacgtgtggacctataatgccgagctgctggctctgatggaaaacgagagaacctggacttcc
 acgacagcaacgtgaagaacctgtacgacaaagtgcggctccagctgcgggacaatgccaaagaactcggcaacggctg
 10 cttcgagttctaccacaagtgcgacaacgagtgcatggaaagcgtgcggaacggcacctacgactaccctcagtactctgag
 gaagcccggtgaagagagaagagatcagcggagtgaagctggaatccatcggcacataccagatcctgagcatctacag
 caccgtggcctcttctctggccctggctattatggtggctggcctgagcctgtggatgtgctctaattggcagcctccagtgcggat
 ctgcatc

Example 2

15 FLU_T2_HA_1 was tested for its ability to elicit a broadly neutralising antibody response to pseudotyped viruses with H5 from different clades and sub-clades.

Immunisation of mice with DNA vaccine:

Female BALB/c mice, 8-10 weeks old, were immunised 4 times (week 0, week 2, week 4, week 6) and bled 6-7 times (week 0, week 2, week 4, week 6, week 8, week 10, week 12) with:

- 50µg FLU_T2_HA_1 DNA in pEVAC vector (see 'H5N1 Anc.' in Figure 1);
- 50µg A/whooper swan/Mongolia/244/2005 (H5) DNA in pEVAC vector (see 'WSN' in Figure 1), which is a primary isolate strain sequenced in 2005 from a whooper swan (i.e. an H5 control); or
- 25 • 50µl PBS.

DNA was injected subcutaneously into the rear flank of the mice. The DNA and the PBS are endotoxin free.

Ability of mouse sera to neutralise pseudotyped viruses with H5 from different clades and sub-clades:

30 Mouse sera collected following the immunisations was tested against the following pseudotyped viruses (with H5 from different clades and sub-clades):

- A/gyrfalcon/Washington/41088-6/2014 (H5, clade 2.3.4.4);
- A/turkey/Turkey/1/2005 (H5, clade 2.2.1);

- A/whooper Swan/Mongolia/244/2005 (H5, clade 2.2) – homologous to the H5 control;
- A/Indonesia/5/2005 (H5, clade 2.1.3.2);
- A/Vietnam/1194/2004 (H5, clade 1);
- A/goose/Guiyang/337/2006 (H5, clade 4);
- 5 • A/chicken/Vietnam/NCVD-016/2008 (H5, clade 7.1)

Figure 1 shows the results of a neutralisation assay illustrating the strength of neutralising antibody responses to the various pseudotyped viruses. The results illustrate the ability of each vaccine to elicit broadly neutralising antibody responses to a diverse panel of pseudotyped viruses with H5 from different clades and sub-clades.

- 10 The results show that administering mice the FLU_T2_HA_1 DNA vaccine gave a significantly greater cross-clade immune response than immunisation with the A/whooper swan/Mongolia/244/2005 H5 control vaccine, and the naïve mouse serum.

Example 3 – design of FLU_T3_HA_1 and FLU_T3_HA_2

- 15 This example describes the design of amino acid sequences of two further embodiments of the invention, FLU_T3_HA_1 and FLU_T3_HA_2.

- As described in Example 2 above, mouse sera obtained following immunisation with FLU_T2_HA_1 DNA vaccine neutralised many clades of H5 but was less effective against clades 2.3.4 and 7.1. These two clades are currently in circulation in birds, and are among the most dominant co-circulating H5N1 viruses in poultry in Asia, with sporadic cases of
20 infection occurring regularly in humans and other mammals.

Epitope regions in the H5 head region important for neutralisation of clade 2.3.4 and clade 7.1 were identified using available protein structural data. The amino acid sequences of these epitopes were compared with FLU_T2_HA_1 to identify amino acid positions that may have abrogated the neutralisation of these two clades by the mouse sera.

- 25 Amino acid positions within FLU_T2_HA_1 were identified that, when changed to particular amino acid residues, can elicit an antibody response that is able to neutralise clades 2.3.4 and 7.1 without abrogating the neutralisation of other clades. These positions are at amino acid residues 157, 171, 172, and 205 of the H5 protein (see positions A, B and C in Figure 2). The influence of these mutations on the stability of the HA protein, as well as its interaction
30 with known antibodies against clade 2.3.4 and clade 7, were checked by energetics calculations. The mutations that stabilised the protein and its interaction with such antibodies, while minimally altering the neutralisation of other clades, were selected for. The resulting new HA sequences are termed FLU_T3_HA_1 and FLU_T3_HA_2.

Figure 2 shows an amino acid sequence comparison of FLU_T2_HA_1 with FLU_T3_HA_1 and FLU_T3_HA_2. FLU_T3_HA_1 is described in more detail in Example 4, and FLU_T3_HA_2 is described in more detail in Example 5, below.

Example 4 - FLU_T3_HA_1

- 5 This example provides amino acid sequences of the influenza haemagglutinin H5 head and stem regions for an embodiment of the invention known as FLU_T3_HA_1. In SEQ ID NO:7 below, the amino acid residues of the stem region are shown underlined. The amino acid residues of the head region are the remaining residues.

FLU_T3_HA_1 – HA0 amino acid sequence (SEQ ID NO:7):

10 MEKIVLLLAIVSLVKSDQICIGYHANNSTEQVDTIMEKNVTVTHAQDILEKTHNGKLCDLDGV
KPLILRDCSVAGWLLGNPMCDEFINPEWSYIVEKANPANDLCYPGNFNDYEELKHLLSRI
NHFEKIQIIPKSSWSDHEASSGVSSACPYQGRPSFFRNVVWLIKNDTYPTIKRSYNNTNQ
EDLLVLWGIHHPNDAAEQTKLYQNPTTYISVGTSTLNQRLVPKIATRSKVNGQSGRMEFF
WTILKPNDAINFESNGNFIAPEYAYKIVKKGDSAIMKSELEYGNCNTKCQTPMGAINSSMPF
 15 HNIHPLTIGECPKYVKSNRLVLATGLRNSPQRRERRRKKRGLFGAAGFIEGGWQGMVDGW
YGYHHSNEQGSGYAADKESTQKAIDGVTNKVNSIIDKMNTQFEAVGREFNNLERRIENLN
KKMEDGFLDVWTYNAELLVLMENERTLDFHDSNVKNLYDKVRLQLRDNAKELGNGCFEF
YHKCDNECMESVRNGTYDYPQYSEEARLKREEISGVKLESIGTYQILSIYSTVASSLALAIM
VAGLSLWMCSNGSLQCRICI

20 *FLU_T3_HA_1 – head region amino acid sequence (SEQ ID NO:8):*

THNGKLCDLDGVKPLILRDCSVAGWLLGNPMCDEFINPEWSYIVEKANPANDLCYPGNF
 NDYEELKHLLSRINHFEKIQIIPKSSWSDHEASSGVSSACPYQGRPSFFRNVVWLIKNDT
 YPTIKRSYNNTNQEDLLVLWGIHHPNDAAEQTKLYQNPTTYISVGTSTLNQRLVPKIATR
 SKVNGQSGRMEFFWTILKPNDAINFESNGNFIAPEYAYKIVKKGDSAIMKSELEYGNCNTKCQ
 25 TPMGAINSSMPFHNIHPLTIGEC

The amino acid residues at positions 156, 157, 171, 172, and 205 are shown underlined in the above sequence (and are R, P, D, T, and K, respectively).

FLU_T3_HA_1 – stem region amino acid sequence (SEQ ID NO:9):

MEKIVLLLAIVSLVKSDQICIGYHANNSTEQVDTIMEKNVTVTHAQDILEKKYVKSNRLVLAT
 30 GLRNSPQRRERRRKKRGLFGAAGFIEGGWQGMVDGWYGYHHSNEQGSGYAADKESTQ
KAIDGVTNKVNSIIDKMNTQFEAVGRENNLERRIENLNKKMEDGFLDVWTYNAELLVLM

NERTLDFHDSNVKNLYDKVRLQLRDNAKELGNGCFEFYHKCDNECMESVRNGTYDYPQY
SEEARKREEISGVKLESIGTYQILSIYSTVASSLALAIMVAGLSLWMCSNGSLQCRICI

The amino acid residues at positions 416 and 434 are shown underlined in the above sequence (and are F and F, respectively).

5 Example 5 - Influenza H5 T3_HA_2

This example provides amino acid sequences of the influenza H5 head and stem regions for an embodiment of the invention known as FLU_T3_HA_2. In SEQ ID NO:4 below, the amino acid residues of the stem region are shown underlined. The amino acid residues of the head region are the remaining residues.

10 FLU_T3_HA_2 – HA0 amino acid sequence (SEQ ID NO:10):

MEKIVLLLAIVSLVKSDQICIGYHANNSTEQVDTIMEKNVTVTHAQDILEKTHNGKLCDLDGV
KPLILRDCSVAGWLLGNPMCDEFINVPESYIVEKANPANDLCYPGNFNDYEELKHLLSRI
NHFEKIQIIPKSSWSDHEASSGVSSACPYQGRPSFFRNVVWLIKNNPTYPTIKRSYNNTNQ
EDLLVLWGIHHPNDAAEQTKLYQNPTTYSVGTSTLNQRLVPKIATRISKVNGQSGRMEFF
15 WTILKPNDAINFESNGNFIAPYAYKIVKKGDSAIMKSELEYGNCNTKCQTPMGAINSSMPF
HNIHPLTIGECPKYVKSRLVLTGLRNSPQRRRRKKRGLFGAIGFIEGGWQGMVDGW
YGYHHSNEQGSGYAADKESTQKAIDGVTNKVNSIIDKMNTQFEAVGREFNNLERRIENLN
KKMEDGFLDVWTYNAELLVLMENERTLDFHDSNVKNLYDKVRLQLRDNAKELGNGCFEF
YHKCDNECMESVRNGTYDYPQYSEEARKREEISGVKLESIGTYQILSIYSTVASSLALAIM
20 VAGLSLWMCSNGSLQCRICI

FLU_T3_HA_2 – head region amino acid sequence (SEQ ID NO:11):

THNGKLCDLGDGVKPLILRDCSVAGWLLGNPMCDEFINVPESYIVEKANPANDLCYPGNF
NDYEELKHLLSRINHFEEKIQIIPKSSWSDHEASSGVSSACPYQGRPSFFRNVVWLIKNNT
YPTIKRSYNNTNQEDLLVLWGIHHPNDAAEQTKLYQNPTTYSVGTSTLNQRLVPKIATRISK
25 VNGQSGRMEFFWTILKPNDAINFESNGNFIAPYAYKIVKKGDSAIMKSELEYGNCNTKCQ
TPMGAINSSMPFHNIHPLTIGEC

The amino acid residues at positions 156, 157, 171, 172, and 205 are shown underlined in the above sequence (and are R, P, N, T, and K, respectively).

FLU_T3_HA_2 – stem region amino acid sequence (SEQ ID NO:12):

MEKIVLLLAIVSLVKSDQICIGYHANNSTEQVDTIMEKNVTVTTHAQDILEKKYVKSNRLVLAT
 GLRNSPQRERRRRKKRGLFGAIAGFIEGGWQGMVDGWYGYHHSNEQGSGYAADKESTQ
 KAIDGVTNKVNSIIDKMNTQFEAVGRENNLERRIENLNKKMEDGFLDVWTYNAELLVLME
 NERTLDFHDSNVKNLYDKVRLQLRDNAKELGNGCFEFYHKCDNECMESVRNGTYDYPQY
 5 SEEARLKREEISGVKLESIGTYQILSIYSTVASSLALAIMVAGLSLWMCSNGSLQCRICI

The amino acid residues at positions 416 and 434 are shown underlined in the above sequence (and are F and F, respectively).

Example 6 – comparison of FLU_T3_HA_1 and FLU_T3_HA_2 with prior art COBRA H5 Tier 2 design

- 10 Figure 3 shows an amino acid comparison of FLU_T3_HA_1 and FLU_T3_HA_2 with a prior art COBRA H5 Tier 2 design. There are amino acid differences at three positions (A, B, and C) in the head region which have been introduced in FLU_T3_HA_1 and FLU_T3_HA_2 to increase the affinity of the antigen towards antibodies of important clades. The amino acid differences are at residue numbers 156, 157, 171, 172, and 205 of
 15 the head region. There are additional amino acid differences at two positions (C and D) in the stem region which have been introduced in FLU_T3_HA_1 and FLU_T3_HA_2 to stabilise the stem region in both the pre- and post-fusion state. The amino acid differences are at residue numbers 416 and 434 of the stem region.

Example 7 - FLU_T2_M2_1

- 20 This example provides the amino acid and nucleic acid sequences of the influenza M2 region for an embodiment of the invention known as FLU_T2_M2_1.

FLU_T2_M2_1 – amino acid sequence (SEQ ID NO:14):

MSLLTEVETPTRNGWECRCSDSSDPLVIAASIIGILHLILWILDRLFFKCIYRRLKYGLKRGF
 STEGVPESMREEYRQKQQSAVDVDDGHFVNIELE

- 25 *FLU_T2_M2_1 – nucleic acid sequence (SEQ ID NO:15):*

ATGTCTCTGCTGACCGAGGTGGAAACCCCTACCAGAAATGGCTGGGAGTGCAGATGC
 AGCGACAGCAGCGATCCTCTGGTTATCGCCGCCAGCATCATCGGCATCCTGCACCTG
 ATCCTGTGGATCCTGGACCGGCTGTTCTTCAAGTGCATCTACCGGCGGCTGAAGTAC
 GGCCTGAAGAGAGGCCCTTCTACAGAGGGCGTGCCCGAGAGCATGCGGGAAGAGTA
 30 CAGACAGAAACAGCAGAGCGCCGTGGACGTGGACGATGGCCACTTCGTGAACATCGA
 GCTGGAA

Example 8 – Immune response elicited by FLU_T2_M2_1

This example describes a flow cytometry-based immunofluorescence assay to test the ability of mouse sera, obtained following immunisation of mice with FLU_T2_M2_1 DNA vaccine, to target M2 molecules from influenza A isolates of different subtypes.

5 Immunisation of mice with DNA vaccine:

4 groups of 6 Balb/c mice, 8-10 weeks old, were immunised 4 times (week 0, week 2, week 4, week 6) and bled 6 times (week 0, week 2, week 4, week 6, week 8, week 10) with:

- 50µg FLU_T2_M2_1 DNA in pEVAC vector (see 'M2 ancestor.' in Figure 5);
- 50µg FLU_T1_M2_1 DNA in pEVAC vector (M2 from H1N1pdm, see 'M2 H1N1' in Figure 5);
- 10 • 50µg FLU_T1_M2_2 DNA in pEVAC vector (M2 from H3N2, see 'M2 H3N2' in Figure 5); or
- 50µl PBS.

DNA was injected subcutaneously into the rear flank of the mice. The DNA and the PBS are
15 endotoxin free.

Ability of mouse sera to target M2 from influenza isolates of different subtypes:

HEK293T cells were transfected with pEVAC vector expressing M2 DNA from the following isolates:

- A/Brisbane/2/2018 (H1N1);
- 20 • A/Kansas/14/2017 (H3N2);
- A/England/195/2009(H1N1);
- A/Anhui/1/2013(H7N9); and
- A/Japan/WRAIR1059P/2008(H3N2)

Serum was pooled for each group (six mice per group), serially diluted and incubated with
25 cells for 30 minutes at room temperature. Mouse IgG isotype antibody was used as negative control staining. After incubation, cells were washed twice in PBS, and then incubated with Goat anti-mouse AF647 secondary antibody for 30 minutes at room temperature, in the dark. Before FACS analysis, cells were washed with PBS another two times. Analysis was performed using Attune NxT FACS (Thermo Fisher).

30 Figure 4 shows the results of a flow cytometry-based immunofluorescence assay illustrating the ability of the mouse serum antibodies to target M2s from the different influenza isolates. The results illustrate the ability of each vaccine to target M2 from influenza isolates of different subtypes.

The results show that administering mice the FLU_T2_M2_1 DNA vaccine (M2 ancestor) elicited a significantly greater immune response against M2 across different influenza subtypes than immunisation with M2 from H1N1 or H3N2 isolates, and the naïve mouse serum.

Example 9 - FLU_T2_NA_3 and FLU_T2_NA_4

- 5 This example provides the amino acid and nucleic acid sequences of the influenza neuraminidase region for embodiments of the invention known as FLU_T2_NA_3 and FLU_T2_NA_4.

FLU_T2_NA_3 (N1_FINAL_2) – *amino acid sequence (SEQ ID NO:16)*:

MNPNQKIITIGSICMVVGIIISLILQIGNIISIWVSHSIQTGNQNPETCNQSIITYENNTWWNQ
 10 YVNISNTNFVAEQAVASVALAGNSSLCPISGWAIYSKDNGIRIGSKGDVVFIREPFISCSHLE
 CRTFFLTQGALLNDKHSNGTVKDRSPYRTLMSCPVGEAPSPYNSRFESVAWSASACHDG
 ISWLTIGISGPDNGAVAVLKYNGIITDTIKSWRNNILRTQESECACINGSCFTIMTDGPSNGQ
 ASYKIFKIEKGKVVKSVELNAPNYHYEECSCYPDAGEVMCVCRDNWHGSNRPWVSFNQN
 LEYQIGYICSGVFGDNPRPNDGTGSCGPVSSNGAYGVKGFSEFKYGKGVWIGRTKSTSSR
 15 SGFEMIWDPNGWTETDSSFSVKQDIVAITDWSGYSGSFVQHPELTGLDCMRPCFWVELI
 RGRPKENTIWTSGSSISFCGVNSDTVGVSWPDGAELPFTIDK

FLU_T2_NA_3 (N1_FINAL_2) – *nucleic acid sequence (SEQ ID NO:17)*:

atgaatccaaatcagaaaataataaccattgggtcaatctgtatggtagttggaataatcagcctaattacaaattggaaca
 taatctcaatatgggttagccattcaattcagactggaaatcaaaaccaacctgaaacatgcaaccaaagcatcattacttatga
 20 aaacaacacttgggtgaatcaaacatatgttaacatcagcaataccaattttgttgctgaacaggctgtagcttcagtggcattag
 cgggcaattcctctctctgccccattagtggtgggtatatacagcaaggacaatggcataaggattggtccaaggagatgt
 attgtcataagagagccattcattcatgctccacttggaaatgcaggacctttttctgactcaaggagccttgtgaatgacaaa
 cattccaatggaaccgttaaagacagaagcccctacagaaccttaatgagctgtcctgttggtgaggctccctctccatacaatt
 caaggttgagtcggttgcttgctgagcaagtgtgcatgatggcattagctggtgacaattggaattccgggcccagacaat
 25 ggggcagtggtgtattgaaatacaatggcataataacagacactatcaaaagttggagaaacaacatattgaggacacaa
 gagtctgaatgtgcctgcataaatggttctgttactataatgaccgatggaccaagtaatgggcaggcctcatacaagattttc
 aagatagagaaggggaaggtagtcaaatcagtcgagttgaatggccctaattaccactacgaggaatgttctgttatcctgat
 gctggcgaagtaatgtgtgtgagggataattggcatggttcgaatcgaccatgggtgtcttcaatcaaaatctggagtatca
 aataggatacatatgcagtgggggtttcgagagacaatccacgccccaatgatggaacaggcagctgtggtccagtgtcttcaat
 30 ggagcatatggagtaaagggttttcatttaagtacggcaagggtgttgatagggagaactaagagcactagttccaggagt
 ggatttgagatgatttgggatcccaatggatggacagagacagatagtagtttctcagtgaagcaagatattgtagcaataactg
 attgtcaggatatagcgggagttttgtccaacatccagaattaacagggtgactgcatgaggccttgcttctgggtgaacta
 atcagaggacggcctaaggagaacacaatctggactagtgaggagcagcatttccttctgtggtgaaatagcgacactgtggg
 ttggtcttggccagacggtgctgagttgccattcaccattgacaag

FLU_T2_NA_4 (N1_FINAL_3) – *amino acid sequence (SEQ ID NO:18)*:

MNPNQKIITIGSICMVVGIIISLILQIGNIISIWVSHSIQTGNQNHHPETCNQSIITYENNTWWNQTYVNISNTNVVAGQDATSVILAGNSSLCPISGWAIYSKDNIGIRIGSKGDFVIREPFISCSHLE CRTFFLTQGALLNDKHSNGTVKDRSPYRTLMSCPVGEAPSPYNSRFESVAWSASACHDG
 5 MGWLTIGISGPDNGAVAVLKYNGIITDIKSWRNNILRTQESECACVNGSCFTIMTDGPSN GQASYKIFKIEKGKVIKSIELNAPNYHYEECSCTPDGKVMCVCRDNWHGSNRPWWSFDQ NLDYQIGYICSGVFGDNPRPNDGTGSCGPVSSNGANGVKGFSFRYGNGVWIGRTKSTSS RSGFEMIWDPNGWTTETDSSFSVKQDIVAITDWSGYSGSFVQHPELTGLDCMRPCFWWEL IRGQPKENTIWTSGSSISFCGVNSDTVGWSWPDGAELPFTIDK

10 FLU_T2_NA_4 (N1_FINAL_3) – *nucleic acid sequence (SEQ ID NO:19)*:

atgaatccaaatcaaaaaataataaccattgggtcaatctgtatggtagttggaataattagcctaattgcaaataagggaatat
 aatctcaatatgggttagccattcaattcaaactggaaatcaaaaccatcctgaaacatgcaaccaaagcatcattacctatga
 aaataacacctgggtgaatcaacatatgttaacattagcaataactaacgttgctgacaggatgcaacttcagtcatattag
 ccggcaattcctctctttgccccatcagtgggtgggtatatacagcaaagacaatggcataagaattggtccaaaggagacg
 15 ttttgcataagagagccatttattcatgctcacttggaatgcaggacctttttctgactcaaggcgcttgctgaatgacaagc
 attcaaatgggaccgtcaaggacagaagcccctatagaaccttaatgagctgccctgttggaagctccgtctccgtacaattc
 aagggtcgaatcggttgcttggtcagcaagtgcattgatggcatgggtggctaacaatcggaatttccggtccagataat
 ggagcagtggtgtattaaaatacaatgggtataataacagacaccatcaaaagttggaggaacaacataattgagaacgcaa
 gagtctgaatgtgcctgtgtaaatggtcatgtttactataatgaccgatggccaagtaatgggcaggcctctgacaaaatttc
 20 aagatagagaaggggaaggtattaaatcaattgagttgaatgcacctaattaccactacgaggaatgttctgttaccctgata
 caggtaaagtgatgtgtgtgcagagacaattggcatggttcgaatcgaccatgggtgtcttcgatcaaaatctggattatcaa
 ataggatacatctgcagtggtgggtttcggtgacaatccgctcccaatgatggaacaggcagctgtggtccagtgcttctaag
 agcaaatggagtaaagggattttcatttaggtatggaatggtgttgataggaagaactaaaagtaccagttccagaagcgg
 gtttgagatgatttgggatcctaattggtgacagagactgatagtagtttctgtgaaacaagatattgtagcaataactgattg
 25 gtcaggggtacagcgggagtttcgttcaacatcctgagctaacagggctggactgcatgaggcctgtcttctgggtgaattaatca
 ggggacaacctaaagagaacacaatctggactagtgggagcagcatttctttgtggcgtaaatagtatactgtaggttggtc
 ttggccagacgggtgctgagttgccattcaccattgacaag

Example 10 – Antibody inhibition of neuraminidase activity of FLU_T2_NA_3 and FLU_T2_NA_4

30 This example describes screening of neuraminidase polypeptides according to embodiments of the invention (FLU_T2_NA_3 and FLU_T2_NA_4) against a panel of monoclonal antibodies that recognise different neuraminidase epitopes.

Neuraminidase vaccines elicit binding antibodies or antibodies that inhibit the activity of the neuraminidase enzyme. This has been shown to correlate with reduction of severity of

disease, but not necessarily protection from infection. They also reduce transmission from infected vaccinated people, as the viruses require the NA activity to exit from infected cells.

Pseudotype based Enzyme-Linked Lectin Assay (pELLA)

5 Lentiviral pseudotypes are produced bearing the neuraminidase of selected influenza virus strains (e.g. the N9 from A/Shanghai/02/2013 (H7N9) or of a polypeptide according to an embodiment of the invention (e.g. T2_NA_3).

10 These pseudotypes bearing NA are used to digest the carbohydrate fetuin from pre-coated ELISA plates in a dilution series. The resulting product from the digested fetuin contains terminal galactose residues that can be recognised by the peanut lectin (conjugated to horseradish peroxidase).

The more the NA digests the fetuin, the more galactose is exposed, so more peanut lectin (HRPO) attaches to the galactose. An ELISA-based readout proportional to the enzymatic activity of the NA is obtained (Couzens *et al.*, *J Virol Methods*. 2014 Dec 15;210:7-14.)

15 The NA-pseudotypes are first titrated, then an inhibition assay is performed with antibodies or serum to 'knock down' the activity of the enzyme with antibodies. As this is a functional assay, it will only detect antibodies interfering with the enzymatic activity of the NA.

Figure 5:

Panel of monoclonal antibodies tested against FLU_T2_NA_3 (N1_FINAL_2):

- 20
- Strong inhibition of NA activity by: 2D4, Z2B3, 3H4, 1H8, 2D9, 3H10, 4E9, 4G2, 1H5, 2G6, A67C
 - Weak inhibition by: 3C2
 - No inhibition by: AF9C, 4C4, 2B5, 1C7, 3A2

FLU_T2_NA_3 (= N1_FINAL_2 = na2 = na2p1 in Figure 5)

Figure 6:

25 Panel of monoclonal antibodies tested against FLU_T2_NA_4 (N1_FINAL_3):

- Strong inhibition of NA activity by: Z2B3, 2D4, 1H8, 3H4, 2D9, 3H10, 4E9, 1H5, 2G6, 4G2, A67C
- Weak inhibition by: 4C4, 3C2
- No inhibition by: AF9C, 2B5, 1C7, 3A2

30 FLU_T2_NA_4 (= N1_FINAL_3 = p1na3 = na3 in Figure 6)

Figure 7:

Panel of monoclonal antibodies tested against FLU_T2_NA_18 (N9_FINAL_1),
FLU_T2_NA_19 (N9_FINAL_2), FLU_T2_NA_20 (N9_FINAL_3):

- Strong inhibition of NA activity by: 1E8, 7F8, 5H11, 7A4, 7F12, 2F6, Z2B3, 1E8
- 5 • Weak inhibition by: I2H3
- No inhibition by: N/A

For the wild type N9 (A/Shanghai/02/2013):

- Strong inhibition by: 1E8, 5H11, 7A4, 2F6, 7F12, Z2B3
- No inhibition by: 7F8 and I2H3

- 10 It was concluded from the results described above, and shown in Figures 5-7, that neuraminidase polypeptides according to embodiments of the invention (FLU_T2_NA_3 and FLU_T2_NA_4) contain epitopes conserved between N1 from seasonal H1N1, pandemic H1N1 and N1 from avian H5N1, as well as conserved epitope (Z2B3 mAb) between N1 and N9.

15 **Monoclonal antibody panel:**

mAbs from Hongquan Wan, FDA:

	mAb_1E8	N9	Wan <i>et al.</i> , <i>Journal of Virology</i> , 2013, Vol. 87(16):9290–9300;
	mAb_7F8	N9	Wan <i>et al.</i> , <i>Journal of Virology</i> , 2018, Vol. 92(4):1-17;
	mAb_11B2	N9	Wan <i>et al.</i> , <i>Nat Commun.</i> , 2015, Feb 10;6:6114;
20	mAb_5H11	N9	
	mAb_7A4	N9	
	mAb_7F12	N9	
	mAb_2F6	N9	
	mAb_3A2	N1	
25	mAb_4G2	N1	
	mAb_1H5	N1	
	mAb_2G6	N1	
	mAb_2D9	N1	
	mAb_3H10	N1	
30	mAb_4E9	N1	
	mAb_1C7	N1	
	mAb_3C2	N1	
	mAb_2B5	N1	
	mAb_3H4	N1	
35	mAb_1H8	N1	
	mAb_2D4	N1	
	mAb_4C4	N1	

mAbs from Alain Townsend, Oxford:

- 40 mAb_AF9C N1 from seasonal and pandemic H1N1 Rijal *et al.*, *Journal of Virology*,
February 2020 Volume 94 Issue 4, 1-17;

mAb_Z2B3 N1 and N9 Rijal *et al.*, *Journal of Virology*, February 2020 Volume 94 Issue 4, 1-17

FACS binding assay:

The NA is expressed on the cell surface of HEK293T/17 cells and serum/mAbs are allowed to bind to it. Binding is detected with a secondary antibody directed to the mouse or human serum antibodies. The cells are passed through a Fluorescent activated cell sampler (FACS cytometer) and the amount of binding present in a sample is measured. This binding is irrespective of whether the antibodies interfere with the enzymatic activity. These may be antibodies that act through ADCC mechanisms through immune cells.

Example 11 - pEVAC Expression Vector

Figure 8 shows a map of the pEVAC expression vector. The sequence of the multiple cloning site of the vector is given below, followed by its entire nucleotide sequence.

Sequence of pEVAC Multiple Cloning Site (MCS) (SEQ ID NO:20):

```

pEVAC 1301 ACAGACTGTT CCTTCCATG GGTCTTTTCT GCAGTCACCG TCGGTACCGT
                                PstI      KpnI SalI
pEVAC 1351 CGACACGTGT GATCATCTAG AGGATCCGCG GCCGCAGATC T
                                BclI XbaI BamHI NotI BglII

```

Entire Sequence of pEVAC (SEQ ID NO:21):

CMV-IE-E/P:	248 - 989	CMV immediate early 1 enhancer / promoter
KanR:	3445 - 4098	Kanamycin resistance
SD:	990 - 1220	Splice donor
SA:	1221 - 1343	Splice acceptor
Tbgh:	1392 - 1942	Terminator signal from bovine growth hormone
pUC-ori:	2096 - 2769	pUC-plasmid origin of replication

```

30 1 TCGCGCGTTT CCGTGATGAC GGTGAAAACC TCTGACACAT GCAGCTCCCG
   51 GAGACGGTCA CAGCTTGTCT GTAAGCGGAT GCCGGGAGCA GACAAGCCCCG
  101 TCAGGGCGCG TCAGCGGGTG TTGGCGGGTG TCGGGGCTGG CTAACTATG
  151 CGGCATCAGA GCAGATTGTA CTGAGAGTGC ACCATATGCG GTGTGAAATA
  201 CCGCACAGAT GCGTAAGGAG AAAATACCGC ATCAGATTGG CTATTGGCCA
  251 TTGCATACGT TGTATCCATA TCATAATATG TACATTTATA TTGGCTCATG
  301 TCCAACATTA CCGCCATGTT GACATTGATT ATTGACTAGT TATTAATAGT
  351 AATCAATTAC GGGGTCATTA GTTCATAGCC CATATATGGA GTTCCGCGTT
  401 ACATAACTTA CCGTAAATGG CCCGCCGTCG TGACCGCCCA ACGACCCCGG
  451 CCCATTGACG TCAATAATGA CGTATGTTCC CATAGTAACG CCAATAGGGA
  501 CTTTCCATTG ACGTCAATGG GTGGAGTATT TACGGTAAAC TGCCCACTTG
  551 GCAGTACATC AAGTGTATCA TATGCCAAGT ACGCCCCCTA TTGACGTCAA
  601 TGACGGTAAA TGGCCCGCCT GGCATTATGC CCAGTACATG ACCTTATGGG
  651 ACTTTCCTAC TTGGCAGTAC ATCTACGTAT TAGTCATCGC TATTACCATG
  701 GTGATGCGGT TTTGGCAGTA CATCAATGGG CGTGGATAGC GGTTTGACTC
  751 ACGGGGATTT CCAAGTCTCC ACCCCATTGA CGTCAATGGG AGTTTGTTTT
  801 GGCACCAAAA TCAACGGGAC TTTCCAAAAT GTCGTAACAA CTCCGCCCCA
  851 TTGACGCAAA TGGGCGGTAG GCGTGTACGG TGGGAGGTCT ATATAAGCAG
  901 AGCTCGTTTA GTGAACCGTC AGATCGCCTG GAGACGCCAT CCACGCTGTT
  951 TTGACCTCCA TAGAAGACAC CGGGACCGAT CCAGCCTCCA TCGGCTCGCA
  1001 TCTCTCCTTC ACGCGCCCGC CGCCCTACCT GAGGCCGCCA TCCACGCCGG
  1051 TTGAGTCGCG TTCTGCCGCC TCCCGCCTGT GGTGCCCTCCT GAACTGCGTC
  1101 CGCCGTCTAG GTAAGTTTAA AGCTCAGGTC GAGACCGGGC CTTTGTCCGG
  1151 CGCTCCCTTG GAGCCTACCT AGACTCAGCC GGCTCTCCAC GCTTTGCCTG

```

	1201	ACCCTGCTTG	CTCAACTCTA	GTTAACGGTG	GAGGGCAGTG	TAGTCTGAGC
	1251	AGTACTCGTT	GCTGCCGCGC	GCGCCACCAG	ACATAATAGC	TGACAGACTA
	1301	ACAGACTGTT	CCTTTCCATG	GGTCTTTTCT	GCAGTCACCG	TCGGTACCGT
	1351	CGACACGTGT	GATCATCTAG	AGGATCCGCG	GCCGCAGATC	TGCTGTGCCT
5	1401	TCTAGTTGCC	AGCCATCTGT	TGTTTGCCCC	TCCCCCGTGC	CTTCCTTGAC
	1451	CCTGGAAGGT	GCCACTCCCA	CTGTCCTTTC	CTAATAAAAT	GAGGAAATTG
	1501	CATCGCATTG	TCTGAGTAGG	TGTCATTCTA	TTCTGGGGGG	TGGGGTGGGG
	1551	CAGGACAGCA	AGGGGGAGGA	TTGGGAAGAC	AATAGCAGGC	ATGCTGGGGA
10	1601	TGCGGTGGGC	TCTATGGCTA	CCCAGGTGCT	GAAGAATTGA	CCCGGTTCCCT
	1651	CCTGGGCCAG	AAAGAAGCAG	GCACATCCCC	TTCTCTGTGA	CACACCCTGT
	1701	CCACGCCCTT	GGTTCTTAGT	TCCAGCCCCA	CTCATAGGAC	ACTCATAGCT
	1751	CAGGAGGGCT	CCGCCCTCAA	TCCCACCCGC	TAAAGTACTT	GGAGCGGTCT
	1801	CTCCCTCCCT	CATCAGCCCA	CCAAACCAAA	CCTAGCCTCC	AAGAGTGGGA
	1851	AGAAATTAAA	GCAAGATAGG	CTATTAAAGT	CAGAGGGAGA	GAAAATGCCT
15	1901	CCAACATGTG	AGGAAGTAAT	GAGAGAAATC	ATAGAATTTT	AAGGCCATGA
	1951	TTTAAGGCCA	TCATGGCCTT	AATCTTCCGC	TTCCCTCGCTC	ACTGACTCGC
	2001	TGCGCTCGGT	CGTTCGGCTG	CGGCGAGCGG	TATCAGCTCA	CTCAAAGGCG
	2051	GTAATACGGT	TATCCACAGA	ATCAGGGGAT	AACGCAGGAA	AGAACATGTG
20	2101	AGCAAAAAGG	CAGCAAAAAG	CCAGGAACCG	TAAAAAGGCC	GCGTTGCTGG
	2151	CGTTTTTCCA	TAGGCTCCGC	CCCCCTGACG	AGCATCACAA	AAATCGACGC
	2201	TCAAGTCAGA	GGTGGCGAAA	CCCGACAGGA	CTATAAAGAT	ACCAGGCGTT
	2251	TCCCCCTGGA	AGCTCCCTCG	TGCGCTCTCC	TGTTCCGACC	CTGCCGCTTA
	2301	CCGGATACCT	GTCCGCCTTT	CTCCCTTCGG	GAAGCGTGGC	GCTTTCTCAT
	2351	AGCTCACGCT	GTAGGTATCT	CAGTTCGGTG	TAGGTCGTTT	GCTCCAAGCT
25	2401	GGGCTGTGTG	CACGAACCCC	CCGTTTCAGCC	CGACCGCTGC	GCCTTATCCG
	2451	GTAACATATC	TCTTGAGTCC	AACCCGTTAA	GACACGACTT	ATCGCCACTG
	2501	GCAGCAGCCA	CTGGTAACAG	GATTAGCAGA	GCGAGGTATG	TAGGCGGTGC
	2551	TACAGAGTTC	TTGAAGTGGT	GGCCTAACTA	CGGCTACACT	AGAAGAACAG
	2601	TATTTGGTAT	CTGCGCTCTG	CTGAAGCCAG	TTACCTTCGG	AAAAAGAGTT
30	2651	GGTAGCTCTT	GATCCGGCAA	ACAAACCACC	GCTGGTAGCG	GTGGTTTTTTT
	2701	TGTTTGCAAG	CAGCAGATTA	CGCGCAGAAA	AAAAGGATCT	CAAGAAGATC
	2751	CTTTGATCTT	TTCTACGGGG	TCTGACGCTC	AGTGGAACGA	AAACTCACGT
	2801	TAAGGGATTT	TGGTCATGAG	ATTATCAAAA	AGGATCTTCA	CCTAGATCCT
	2851	TTTAAATTAA	AAATGAAGTT	TTAAATCAAT	CTAAAGTATA	TATGAGTAAA
35	2901	CTTGGTCTGA	CAGTTACCAA	TGCTTAATCA	GTGAGGCACC	TATCTCAGCG
	2951	ATCTGTCTAT	TTCGTTTCATC	CATAGTTGCC	TGACTCGGGG	GGGGGGGGCG
	3001	CTGAGGTCTG	CCTCGTGAAG	AAGGTGTTGC	TGACTCATAC	CAGGCCTGAA
	3051	TCGCCCCATC	ATCCAGCCAG	AAAGTGAGGG	AGCCACGGTT	GATGAGAGCT
40	3101	TTGTTGTAGG	TGGACCAGTT	GGTGATTTTG	AACTTTTGCT	TTGCCACGGA
	3151	ACGGTCTGCG	TTGTCGGGAA	GATGCGTGAT	CTGATCCTTC	AACTCAGCAA
	3201	AAGTTCGATT	TATTCAACAA	AGCCGCCGTC	CCGTCAAGTC	AGCGTAATGC
	3251	TCTGCCAGTG	TTACAACCAA	TTAACCAATT	CTGATTAGAA	AAACTCATCG
	3301	ATGCATCAAT	GAAACTGCAA	TTTATTTCATA	TCAGGATTAT	CAATACCATA
	3351	TTTTTTGAAAA	AGCCGTTTCT	GTAATGAAGG	AGAAAACTCA	CCGAGGCAGT
45	3401	TCCATAGGAT	GGCAAGATCC	TGGTATCGGT	CTGCGATTCC	GACTCGTCCA
	3451	ACATCAATAC	AACCTATTAA	TTTCCCCCTC	TCAAAAATAA	GGTTATCAAG
	3501	TGAGAAATCA	CCATGAGTGA	CGACTGAATC	CGGTGAGAAT	GGCAAAAGCT
	3551	TATGCATTTT	TTTCCAGACT	TGTTCAACAG	GCCAGCCATT	ACGCTCGTCA
	3601	TCAAAATCAC	TCGCATCAAC	CAAACCGTTA	TTCATTTCGT	ATTGCGCCTG
50	3651	AGCGAGACGA	AATACGCGAT	CGCTGTTAAA	AGGACAATTA	CAAAACAGGAA
	3701	TCGAATGCAA	CCGGCGCAGG	AACACTGCCA	GCGCATCAAC	AATATTTTCA
	3751	CCTGAATCAG	GATATTCTTC	TAATACCTGG	AATGCTGTTT	TCCCGGGGAT
	3801	CGCAGTGGTG	AGTAACCATG	CATCATCAGG	AGTACGGATA	AAATGCTTGA
	3851	TGGTCGGAAG	AGGCATAAAT	TCCGTCAGCC	AGTTTAGTCT	GACCATCTCA
55	3901	TCTGTAAACAT	CATTGGCAAC	GCTACCTTTG	CCATGTTTCA	GAAACAACCTC
	3951	TGGCGCATCG	GGCTTCCCAT	ACAATCGATA	GATTGTTCGA	CCTGATTGCC
	4001	CGACATTATC	GCGAGCCCAT	TTATACCCAT	ATAAATCAGC	ATCCATGTTG
	4051	GAATTTAATC	GCGGCCTCGA	GCAAGACGTT	TCCCGTTGAA	TATGGCTCAT
	4101	AACACCCCTT	GTATTACTGT	TTATGTAAGC	AGACAGTTTT	ATTGTTTCATG
60	4151	ATGATATATT	TTTATCTTGT	GCAATGTAAC	ATCAGAGATT	TTGAGACACA
	4201	ACGTGGCTTT	CCCCCCCCCT	CCATTATTGA	AGCATTTATC	AGGGTTATTG
	4251	TCTCATGAGC	GGATACATAT	TTGAATGTAT	TTAGAAAAAT	AAACAAATAG

4301 GGGTTCCGCG CACATTTCCC CGAAAAGTGC CACCTGACGT CTAAGAAACC
 4351 ATTATTATCA TGACATTAAC CTATAAAAAT AGGCGTATCA CGAGGCCCTT
 4401 TCGTC

5 Example 12 - FLU_T2_HA_3_I3

This example provides the amino acid and nucleic acid sequences of the influenza H1 region for an embodiment of the invention known as FLU_T2_HA_3_I3.

FLU_T2_HA_3_I3 – *amino acid sequence (SEQ ID NO:22)*:

10 MKAILVLLYTFATANADTLICIGYHANNSTDVDTVLEKNVTVTHSVNLLEDKHNGKLCCKLRGVAPLHLGKCN
 AGWILGNPECESLSTASSWSYIVETSSSDNGTCYPGDFINYEELREQLSSVSSFERFEIFPKTSSWPNHDSNKG
 VTAACPHAGAKSFYKNLIWLKKGNSYPKLSKSYINDKGKEVLVLWGIHHPSTTADQQSLYQNADAYVFGTSR
 YSKKFKPEIAIRPKVRDQEGRMNYYWTLVEPGDKITFEATGNLVPRYAFAMERNAGSGIIISDTPVHDCNTTC
 QTPEGAINTSLPFQNIHPITIGKCPKYVKSTKLRLATGLRNVPSIQSRGLFGAIAGFIEGGWTGMVDGWYGYHH
 QNEQGSgyAADLKSTQNAIDKITNKVNSVIEKMNTQFTAVGKEFNHLEKRIENLNKKVDDGFLDIWTYNAELLV
 15 LLENERTLDYHDSNVKNLYEKVRNQLKNNKEIGNGCFEFYHKCDNTCMESVKNNGTYDPKYSEEAKLNREKID
 GVKLESTRIYQILAIYSTVASSLVLVSLGAISFWMCSNGSLQCRICI

FLU_T2_HA_3_I3 – *nucleic acid sequence (SEQ ID NO:23)*

ATGAAGGCTATTCTGGTGGTGCTGCTGTACACCTTCGCCACCGCCAATGCCGATACACTG
 TGTATTGGCTACCACGCCAACACAGCACCGACACCGTGGATACCGTGCTGGAAAAGAAC
 20 GTGACCGTGACACACAGCGTGAACCTGCTGGAAGATAAGCACAAACGGCAAGCTGTGCAAG
 CTGAGAGGCGTTGCACCTCTGCACCTGGGCAAGTGTAAATATCGCCGGCTGGATCCTGGGC
 AACCTGAGTGTGAAAGCCTGAGCACAGCCAGCAGCTGGTCCTACATCGTGGAAACCAGC
 AGCAGCGACAACGGCACATGCTACCCCGGCGACTTCATCAACTACGAGGAACTGAGAGAG
 CAGCTGAGCAGCGTCAGCAGCTTCGAGAGATTTCGAGATTTTCCCCAAGACCTCCAGCTGG
 25 CCCAACCACGATTCTAACAAGGGCGTGACAGCCGCCTGTCCTCATGCCGGCGCTAAGAGC
 TTCTACAAGAACCTGATCTGGCTGGTCAAGAAGGGCAACAGCTACCCAAGCTGAGCAAG
 AGCTACATCAACGACAAGGGCAAAGAGGTGCTGGTCCTCTGGGGCATCCACCATCCTTCT
 ACAACAGCCGACCAGCAGAGCCTGTACCAGAAATGCCGATGCCTACGTGTTCTGTTGGGCACC
 AGCAGATACAGCAAGAAGTTCAAGCCCGAGATCGCCATCAGACCCAAAGTGCGGGATCAA
 30 GAGGGCAGAATGAACTACTACTGGACCCTGGTGGAAACCCGGCGACAAGATCACATTTGAG
 GCCACAGGCAACCTGGTGGTCCCTAGATACGCCTTCGCCATGGAAAGAAATGCCGGCAGC
 GGCATCATCATCAGCGACACACCTGTGCACGACTGCAACACCACCTGTCAGACACCTGAG
 GCGCCATCAATACCAGCCTGCCTTTCCAGAACATTACCCCATCACCATCGGCAAGTGC
 CCCAAATACGTGAAGTCCACAAAGCTGAGACTGGCCACCGGCCTGAGAAATGTGCCTAGC
 35 ATCCAGAGCAGAGGCCTGTTTGGAGCCATTGCCGGCTTTATCGAAGGCGGCTGGACAGGC
 ATGGTTGACGGATGGTACGGCTACCACCATCAGAATGAGCAAGGCAGCGGATACGCCGCC
 GATCTGAAGTCTACACAGAACGCCATCGATAAGATCACCAACAAAGTGAACAGCGTGATC
 GAGAAGATGAACACCCAGTTCACCGCCGTGGGAAAAGAGTTCAACCACCTGGAAAAGCGC
 ATCGAGAACCTGAACAAGAAGGTGGACGACGGCTTCCTGGACATCTGGACCTATAATGCC

GAGCTGCTCGTGCTGCTCGAGAACGAGAGAACCCTGGACTACCACGACAGCAACGTGAAG
AACCTGTACGAGAAAAGTGCAGAACAGCTGAAGAACAACGCCAAAGAGATCGGCAACGGC
TGCTTCGAGTTCTACCACAAGTGCAGACAATACCTGCATGGAAAGCGTGAAGAATGGCACC
TACGACTACCCTAAGTACAGCGAGGAAGCCAAGCTGAACCGCGAGAAGATTGACGGCGTG
5 AAGCTGGAAAGCACCCGGATCTATCAGATCCTGGCCATCTACAGCACAGTGGCCTCTAGC
CTGGTGCTGGTGGTGTCTCTGGGAGCCATCAGCTTTTGGATGTGCAGCAATGGCAGCCTC
CAGTGCCCGGATCTGCATC

Example 13 – pEVAC-FLU_T2_HA-3-I-3

This example provides the nucleic acid sequence of pEVAC-FLU_T2_HA-3-I-3.

10 pEVAC-FLU_T2_HA-3-I-3 – nucleic acid sequence (SEQ ID NO:24):

LOCUS	17ADKK4C_I-3_pVRC8400EVAC_Ar	6083 bp	DNA
circular			
FEATURES	Location/Qualifiers		
15	promoter	complement(5925..5953) /label="AmpR_promoter"	
	promoter	868..987 /label="CMV2_promoter"	
	CDS	complement(4963..5778) /label="Kana (R) "	
20	rep_origin	complement(3818..4437) /label="pBR322_origin"	
	primer	complement(29..51) /label="pGEX_3_primer"	
	primer	855..875 /label="CMV_fwd_primer"	
25	primer	899..918 /label="pCEP_fwd_primer"	
	primer	901..925 /label="LNCX_primer"	
30	polyA_site	3071..3295 /label="BGH\pA"	
	promoter	394..904 /label="CMV_Promoter"	
	CDS	1343..3063 /label="I-3"	
35			

TCGCGCGTTTCGGTGATGACGGTGAAAACCTCTGACACATGCAGCTCCCGGAGACGGTCACAGCTTG
TCTGTAAGCGGATGCCGGGAGCAGACAAGCCCGTCAGGGCGCGTCAGCGGGTGTTGGCGGGTGTCGG
40 GGCTGGCTTAACTATGCGGCATCAGAGCAGATTGTACTGAGAGTGCACCATATGCGGTGTGAAATAC
CGCACAGATGCGTAAGGAGAAAATACCGCATCAGATTGGCTATTGGCCATTGCATACGTTGTATCCA
TATCATAATATGTACATTTATATTGGCTCATGTCCAACATTACCGCCATGTTGACATTGATTATTGA
CTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATAGCCCATATATGGAGTCCGCGTTAC
ATAACTTACGGTAAATGGCCCGCCTGGCTGACCGCCCAACGACCCCCGCCCATTTGACGTCAATAATG
ACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGAGTATTTACGGT
45 AAACCTGCCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGA
CGGTAAATGGCCCGCCTGGCATTATGCCCAGTACATGACCTTATGGGACTTTTCCTACTTGGCAGTAC
ATCTACGTATTAGTCATCGCTATTACCATGGTGATGCGGTTTTTGGCAGTACATCAATGGGCGTGGAT
AGCGGTTTGAATCACGGGGATTTCCAAGTCTCCACCCCATTTGACGTCAATGGGAGTTTGTTTTGGCA
CCAAAATCAACGGGACTTTCCAAAATGTCTGTAACAACTCCGCCCCATTGACGCAATGGGCGGTAGG

CGTGTACGGTGGGAGGTCTATATAAGCAGAGCTCGTTTTAGTGAACCGTCAGATCGCCTGGAGACGCC
ATCCACGCTGTTTTGACCTCCATAGAAGACACCGGGACCGATCCAGCCTCCATCGGCTCGCATCTCT
CCTTCACGCGCCCGCCGCCCTACCTGAGGCCGCCATCCACGCCGGTTGAGTCGCGTTCTGCCGCCCTC
CCGCCTGTGGTGCCTCCTGAACTGCGTCCGCCGTCTAGGTAAGTTTAAAGCTCAGGTCGAGACCGGG
5 CTTTGTCCGGCGCTCCCTTGGAGCCTACCTAGACTCAGCCGGCTCTCCACGCTTTGCCTGACCCTG
CTTGCTCAACTCTAGTTAACGGTGGAGGGCAGTGTAGTCTGAGCAGTACTCGTTGCTGCCGCGCGCG
CCACCAGACATAATAGCTGACAGACTAACAGACTGTTCCCTTTCCATGGGTCTTTTCTGCAGTCACCG
TCGGTACCGCCACCATGAAGGCTATTCTGGTGGTGTCTGTACACCTTCGCCACCGCCAATGCCGA
TACACTGTGTATTGGCTACCACGCCAACACAGCACCAGACACCGTGGATACCGTGTGGAAGAAGAAC
10 GTGACCGTGACACACAGCGTGAACCTGCTGGAAGATAAGCACAAACGGCAAGCTGTGCAAGCTGAGAG
GCGTTGCACCTCTGCACCTGGGCAAGTGTAAATATCGCCGGCTGGATCCTGGGCAACCTGAGTGTGA
AAGCCTGAGCACAGCCAGCAGCTGGTCTACATCGTGGAAACCAGCAGCAGCGACAACGGCACATGC
TACCCCGGCGACTTCATCAACTACGAGGAACTGAGAGAGCAGCTGAGCAGCGTCAGCAGCTTCGAGA
GATTCGAGATTTTCCCCAAGACCTCCAGCTGGCCCAACCACGATTCTAACAAGGGCGTGACAGCCGC
15 CTGTCTCATGCCGGCGCTAAGAGCTTCTACAAGAACCTGATCTGGCTGGTCAAGAAGGGCAACAGC
TACCCCAAGCTGAGCAAGAGCTACATCAACGACAAGGGCAAAGAGGTGCTGGTCTCTGGGGCATCC
ACCATCCTTCTACAACAGCCGACCAGCAGAGCCTGTACCAGAATGCCGATGCCTACGTGTTCTGTGGG
CACCAGCAGATACAGCAAGAAGTTCAAGCCCGAGATCGCCATCAGACCCAAAGTGCGGGATCAAGAG
GGCAGAATGAACTACTACTGGACCCTGGTGGAAACCCGGCGACAAGATCACATTTGAGGCCACAGGCA
20 ACCTGGTGGTCCCTAGATACGCCTTCGCCATGGAAAGAAATGCCGGCAGCGGCATCATCATCAGCGA
CACACCTGTGCACGACTGCAACACCACCTGTCAGACACCTGAGGGCGCCATCAATACCAGCCTGCCT
TTCCAGAACATTCACCCCATCACCATCGGCAAGTGCCCCAAATACGTGAAGTCCACAAAGCTGAGAC
TGGCCACCGGCCTGAGAAATGTGCCTAGCATCCAGAGCAGAGGCCTGTTTGGAGCCATTGCCGGCTT
TATCGAAGGCGGTGGACAGGCATGGTTGACGGATGGTACGGCTACCACCATCAGAATGAGCAAGGC
25 AGCGGATACGCCGCCGATCTGAAGTCTACACAGAACGCCATCGATAAGATCACCAACAAAGTGAACA
GCGTGATCGAGAAGATGAACACCCAGTTACCGCCGTGGGAAAAGAGTTCAACCACCTGGAAAAGCG
CATCGAGAACCTGAACAAGAAGGTGGACGACGGCTTCCTGGACATCTGGACCTATAATGCCGAGCTG
CTCGTGCTGCTCGAGAACGAGAGAACCCTGGACTACCACGACAGCAACGTGAAGAACCTGTACGAGA
AAGTGCGGAACCAGCTGAAGAACAACGCCAAAGAGATCGGCAACGGCTGCTTCGAGTTCTACCACAA
30 GTGCGACAATACCTGCATGGAAAGCGTGAAGAATGGCACCTACGACTACCCTAAGTACAGCGAGGAA
GCCAAGCTGAACCGCGAGAAGATTGACGGCGTGAAGCTGGAAGCACCCGGATCTATCAGATCCTGG
CCATCTACAGCACAGTGGCCTCTAGCCTGGTGTCTGGTGGTGTCTCTGGGAGCCATCAGCTTTTGGAT
GTGCAGCAATGGCAGCCTCCAGTGCCGGATCTGCATCTGAGCGGCCGAGATCTGCTGTGCCTTCTA
GTTGCCAGCCATCTGTTGTTTGGCCCTCCCCCGTGCCTTCCTTGACCCTGGAAGGTGCCACTCCCAC
35 TGTCCTTTTCTAATAAAATGAGGAAATTGCATCGCATTGTCTGAGTAGGTGTCAATTCTATTCTGGGG
GGTGGGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGAAGACAATAGCAGGCATGCTGGGGATGCGG
TGGGCTCTATGGCTACCCAGGTGCTGAAGAATTGACCCGGTTCTCTCTGGGCCAGAAAGAAGCAGGC
ACATCCCCTTCTCTGTGACACACCCTGTCCACGCCCTGGTTCTTAGTTCCAGCCCCACTCATAGGA
CACTCATAGCTCAGGAGGGCTCCGCCTTCAATCCCACCCGCTAAAGTACTTGGAGCGGTCTCTCCCT
40 CCCTCATCAGCCCACCAAACCAACCTAGCCTCCAAGAGTGGGAAGAAATTAAGCAAGATAGGCTA
TTAAGTGAGAGGGAGAGAAAATGCCTCCAACATGTGAGGAAGTAATGAGAGAAATCATAGAATTTT
AAGGCCATGATTTAAGGCCATCATGGCCTTAATCTTCCGCTTCCTCGCTCACTGACTCGCTGCGCTC
GGTCGTTCCGCTGCGGCGAGCGGTATCAGCTCACTCAAAGGCGGTAATACGGTTATCCACAGAATCA
GGGGATAACGCAGGAAGAACAATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCG
45 CGTTGCTGGCGTTTTTCCATAGGCTCCGCCCTTGACGAGCATCACAAAATCGACGCTCAAGTCA
GAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGC
TCTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCTTTCTCCCTTCGGGAAGCGTGCGC
TTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTGCTTCGCTCCAAGCTGGGCTGTGT
GCACGAACCCCCCGTTACGCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCG
50 GTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAG
GCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGAACAGTATTTGGTAT
CTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACC
ACCGCTGGTAGCGGTGGTTTTTTTTGTTTGCAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAG
AAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGGAACGAAAACCTCACGTTAAGGGATTTT
55 GGTGATGAGATTATCAAAAAGGATCTTCACCTAGATCCTTTTAAATTAATAAAGTATTAATCA
ATCTAAAGTATATATGAGTAACTTGGTCTGACAGTTACCAATGCTTAATCAGTGAGGCACCTATCT
CAGCGATCTGTCTATTTCTTCATCCATAGTTGCCTGACTCGGGGGGGGGGGCGCTGAGGTCTGCC

TCGTGAAGAAGGTGTTGCTGACTCATACCAGGCCTGAATCGCCCCATCATCCAGCCAGAAAGTGAGG
 GAGCCACGGTTGATGAGAGCTTTGTTGTAGGTGGACCAGTTGGTGATTTTGAACTTTGCCTTTGCCA
 CGGAACGGTCTGCGTTGTCTGGGAAGATGCGTGATCTGATCCTTCAACTCAGCAAAAGTTCGATTTAT
 TCAACAAAGCCGCCGTCCCGTCAAGTCAGCGTAATGCTCTGCCAGTGTTACAACCAATTAACCAATT
 5 CTGATTAGAAAACTCATCGAGCATCAAATGAACTGCAATTTATTATCATATCAGGATTATCAATACC
 ATATTTTTTGAAAAAGCCGTTTCTGTAATGAAGGAGAAAACTCACCGAGGCAGTTCCATAGGATGGCA
 AGATCCTGGTATCGGTCTGCGATTCCGACTCGTCCAACATCAATACAACCTATTAATTTCCCTCGT
 CAAAAATAAGGTTATCAAGTGAGAAATCACCATGAGTGACGACTGAATCCGGTGAGAATGGCAAAAG
 CTTATGCATTTCTTTCCAGACTTGTTCAACAGGCCAGCCATTACGCTCGTCATCAAAATCACTCGCA
 10 TCAACCAAACCGTTATTTCATTCGTGATTGCGCCTGAGCGAGACGAAATACGCGATCGCTGTTAAAAG
 GACAATTACAAACAGGAATCGAATGCAACCGGCGCAGGAACACTGCCAGCGCATCAACAATATTTTC
 ACCTGAATCAGGATATTCTTCTAATACCTGGAATGCTGTTTTCCCGGGGATCGCAGTGGTGAGTAAC
 CATGCATCATCAGGAGTACGGATAAAATGCTTGATGGTTCGGAAGAGGCATAAATTCCGTGAGCCAGT
 TTAGTCTGACCATCTCATCTGTAACATCATTGGCAACGCTACCTTTGCCATGTTTCAGAAACAACCTC
 15 TGGCGCATCGGGCTTCCCATAACAATCGATAGATTGTGCGACCTGATTGCCCGACATTATCGCGAGCC
 CATTTATACCCATATAAATCAGCATCCATGTTGGAATTTAATCGCGGCCCTCGAGCAAGACGTTTCCC
 GTTGAATATGGCTCATAACACCCCTTGTATTACTGTTTATGTAAGCAGACAGTTTTATTGTTTCATGA
 TGATATATTTTTATCTTGTGCAATGTAACATCAGAGATTTGAGACACAACGTGGCTTCCCCCCCC
 CCCATTATTGAAGCATTTATCAGGGTTATTGTCTCATGAGCGGATACATATTTGAATGTATTTAGA
 20 AAAATAAACAAATAGGGGTTCCGCGCACATTTCCCCGAAAAGTGCCACCTGACGTCTAAGAAACCAT
 TATTATCATGACATTAACCTATAAAAATAGGCGTATCACGAGGCCCTTTCGTC

//

25 **Example 14 – broad coverage H1N1 vaccine candidate**

This example provides a broad coverage H1N1 string-based vaccine construct (panH1N1 vaccine candidate). panH1N1 comprises an isolated polynucleotide comprising nucleotide sequence encoding FLU_T2_HA_3_I3 (SEQ ID NO:23), FLU_T2_NA_3 (SEQ ID NO:17), and FLU_T2_M2_1 (SEQ ID NO:15) designed subunits, covalently linked.

30 Figure 9 shows log₁₀IC₅₀ plot for pEVAC_Flu_T2_HA_3_I-3 and other controls.

Elicitation of neutralising antibodies by our vaccine candidate – Flu_T2_HA_3_I-3 against A/Brisbane/02/2018, A/California/07/2009, A/swine/Guangxi/2013, and A/swine/Henan/SN10/2018 was confirmed using pMN assay. Various controls used are: primary strains viz. A/Brisbane/02/2018, A/Michigan/45/2015, cobra design: H1N1 cobra, our
 35 seasonal H1N1 vaccine candidate: Flu_T2_HA_2 and monoclonal antibodies – mAb 4F8 and mAb FI6.

Figure 10 shows inhibition of enzymatic activity of A/Brisbane/02/2018 neuraminidase by sera from mouse vaccinated by (A) PBS, (B) Primary strain - A/Brisbane/02/2018, (C) N1_Final_1, (D) N1_Final_2 (Flu_T2_NA_3).

40 This data shows superiority in neutralisation breadth to some isolates, or equivalence in breadth to others, compared to the Cobra candidate.

Example 15 – panH1N1 vaccine candidate

This example provides the nucleic acid sequence of the broad coverage H1N1 vaccine candidate of the invention known as panH1N1. panH1N1 comprises an isolated polynucleotide comprising nucleotide sequence encoding FLU_T2_HA_3_I3 (SEQ ID NO:23), FLU_T2_NA_3 (SEQ ID NO:17), and FLU_T2_M2_1 (SEQ ID NO:15) designed subunits, covalently linked. The amino acid sequence of panH1N1 (SEQ ID NO:63) is also provided.

10 panH1N1 – nucleic acid sequence (SEQ ID NO:25)

ATGAAGGCTATTCTGGTGGTGCTGCTGTACACCTTCGCCACCGCCAATGCCGATACACTGTGTATTGGCTACCA
CGCCAACAACAGCACCGACACCGTGGATACCGTGCTGGAAAAGAACGTGACCGTGACACACAGCGTGAACCTGC
TGGAAGATAAGCACAAACGGCAAGCTGTGCAAGCTGAGAGGCGTTGCACCTCTGCACCTGGGCAAGTGTAAATATC
GCCGGCTGGATCCTGGGCAACCCTGAGTGTGAAAGCCTGAGCACAGCCAGCAGCTGGTCCTACATCGTGGAAAC
15 CAGCAGCAGCGACAACGGCACATGCTACCCCGGCGACTTCATCAACTACGAGGAACCTGAGAGAGCAGCTGAGCA
GCGTCAGCAGCTTCGAGAGATTGAGATTTTCCCCAAGACCTCCAGCTGGCCCAACCACGATTCCTAACAAGGGC
GTGACAGCCGCTGTCTCATGCCGGCGCTAAGAGCTTCTACAAGAACCTGATCTGGCTGGTCAAGAAGGGCAA
CAGCTACCCCAAGCTGAGCAAGAGCTACATCAACGACAAGGGCAAAGAGGTGCTGGTCCCTCTGGGGCATCCACC
ATCCTTCTACAACAGCCGACCAGCAGAGCCTGTACCAGAATGCCGATGCCTACGTGTTCTGGTGGCACCAGCAGA
20 TACAGCAAGAAGTTCAAGCCCGAGATCGCCATCAGACCCAAAGTGCGGGATCAAGAGGGCAGAATGAACACTA
CTGGACCTTGGTGGAAACCCGGCGACAAGATCACATTTGAGGGCCACAGGCAACCTGGTGGTCCCTAGATACGCCT
TCGCCATGGAAAGAAATGCCGGCAGCGGCATCATCATCAGCGACACACCTGTGCACGACTGCAACACCACCTGT
CAGACACCTGAGGGCGCCATCAATACCAGCCTGCCTTTCCAGAACATTCACCCCATCACCATCGGCAAGTGCCC
CAAATACGTGAAGTCCACAAAGCTGAGACTGGCCACCGGCCTGAGAAATGTGCCCTAGCATCCAGAGCAGAGGCC
25 TGTTTGGAGCCATTGCCGGCTTTATCGAAGGCGGCTGGACAGGCATGGTTGACGGATGGTACGGCTACCACCAT
CAGAATGAGCAAGGCAGCGGATACGCCGCCGATCTGAAGTCTACACAGAACGCCATCGATAAGATCACCACAA
AGTGAACAGCGTGATCGAGAAGATGAACACCCAGTTACCCGCCGTGGGAAAAGAGTTCAACCACCTGGAAAAGC
GCATCGAGAACCTGAACAAGAAGGTGGACGACGGCTTCCTGGACATCTGGACCTATAATGCCGAGCTGCTCGTG
CTGCTCGAGAACGAGAGAACCCTGGACTACCACGACAGCAACGTGAAGAACCCTGTACGAGAAAGTGCGGAACCA
30 GCTGAAGAACAACGCCAAAGAGATCGGCAACGGCTGCTTCGAGTTCTACCACAAGTGCGACAATACCTGCATGG
AAAGCGTGAAGAATGGCACCTACGACTACCCTAAGTACAGCGAGGAAGCCAAGCTGAACCGCGAGAAGATTGAC
GGCGTGAAGCTGGAAAACCCCGGATCTATCAGATCCTGGCCATCTACAGCACAGTGGCCTCTAGCCTGGTGCT
GGTGGTGTCTCTGGGAGCCATCAGCTTTTGGATGTGCAGCAATGGCAGCCTCCAGTGCCGGATCTGCATCGGAA
GCGGAGAAGGCAGAGGCAGCCTGCTGACATGCGGAGATGTGGAAGAGAATCCCGGACCTATGAATCCCAACCAG
35 AAGATCATCACCATCGGCAGCATCTGCATGGTCTGGGCATCATCAGCCTGATCCTCCAGATCGGCAACATCAT
CTCCATCTGGGTGTCCACAGCATCCAGACCGGCAATCAGAACCAGCCTGAGACATGCAACCAGTCCATCATCA
CCTACGAGAACAACACCTGGGTCAACCAGACCTACGTGAACATCAGCAACACCAACTTCGTGGCCGAACAGGCC
GTGGCTTCTGTTGCCCTGGCCGGAAATAGCTCTCTGTGCCCTATTAGCGGCTGGGCCATCTACAGCAAGGACAA
CGGCATCCGGATCGGCTCTAAGGGCGACGTGTTTCGTGATCAGAGAGCCCTTCATCAGCTGCTCCACCTGGAAT
40 GCCGGACATTCTTTCTGACCCAAGGCGCCCTGCTGAACGACAAGCACAGCAATGGCACCGTGAAGGACAGAAGC

CCCTACAGAACCCTGATGAGCTGCCCTGTGGGAGAAGCCCCATCTCCTTACAACAGCAGATTTCGAGTCCGTGGC
 TTGGAGCGCCTCTGCCTGTACGATGGAATCAGCTGGCTGACAATCGGCATCAGCGGCCCTGATAATGGCGCTG
 TGGCCGTGCTGAAGTACAACGGAATCATCACCGACACCATCAAGAGCTGGCGGAACAACATCCTGCGGACCCAA
 GAGTCCGAGTGCGCCTGTATCAATGGCAGCTGCTTCACCATCATGACAGACGGCCCTAGCAATGGCCAGGCCAG
 5 CTACAAGATTTTCAAGATCGAGAAGGGCAAAGTGGTCAAGAGCGTGGAACGAACGCCCCCTAACTACCACTACG
 AGGAATGCAGCTGCTACCCCGATGCCGGCGAAGTGATGTGCGTGTGCAGAGACAATTGGCACGGCAGCAACAGA
 CCTTGGGTGTCCTTCAACCAGAACCCTGGAATATCAGATCGGCTATATCTGCTCCGGCGTGTTCGGCGACAACCC
 CAGACCTAATGATGGCACAGGCAGCTGTGGCCCCGTGTCTAATGGCGCCTATGGCGTGAAGGGCTTCAGCT
 TTAAGTACGGCAAAGGCGTGTGGATCGGCCGGACCAAGAGCACCTCTAGCAGATCCGGCTTCGAGATGATCTGG
 10 GACCCCAACGGCTGGACCGAGACAGATAGCAGCTTCAGCGTGAAGCAGGACATCGTGGCCATCACCGATTGGAG
 CGGCTACAGCGGAAGCTTCGTGCAGCACCTGAACTGACAGGCCTGGACTGCATGAGGCCCTGCTTTTGGGTGCG
 AGCTGATCCGGGGCAGACCCAAAGAGAACACCATCTGGACAAGCGGCAGCAGCATCAGCTTTTGCGGCGTGAAC
 AGCGATACCGTCCGGCTGGTCTTGGCCTGATGGTGCCGAGCTGCCTTTCACCATCGACAAAGGATCCGGCGCCAC
 CAACTTTAGTCTGCTGAAACAGGCCGGCGACGTGGAAGAGAACCAGGTCTATGTCTCTGCTGACCGAGGTGG
 15 AAACCCCTACCAGAAATGGCTGGGAGTGCAGATGCAGCGACAGCAGCGATCCTCTGGTTATCGCCGCCAGCATC
 ATCGGCATCCTGCACCTGATCCTGTGGATCCTGGACCGGCTGTTCTTCAAGTGCATCTACCGCGGGCTGAAGTA
 CGGCCTGAAGAGAGGCCCTTCTACAGAGGGCGTGCCCGAGAGCATGCGGGAAGAGTACAGACAGAAACAGCAGA
 GCGCCGTGGACGTGGACGATGGCCACTTCGTGAACATCGAGCTGGAATGA

20 pEVAC_panH1N1 – *nucleic acid sequence (SEQ ID NO:26)*

TCGCGCGTTTTCGGTGATGACGGTGAAAACCTCTGACACATGCAGCTCCCGGAGACGGTCACAGCTTGTCTGTAA
 GCGGATGCCGGGAGCAGACAAGCCCGTCAGGGCGCGTCAGCGGGTGTGGCGGGTGTGGGGCTGGCTTAACTA
 TGCGGCATCAGAGCAGATTGTACTGAGAGTGCACCATATGCGGTGTGAAATACCGCACAGATGCGTAAGGAGAA
 AATACCGCATCAGATTGGCTATTGGCCATTGCATACGTTGTATCCATATCATAATATGTACATTTATATTGGCT
 25 CATGTCCAACATTACCGCCATGTTGACATTGATTATTGACTAGTTATTAATAGTAATCAATTACGGGGTCATTA
 GTTCATAGCCCATATATGGAGTTCCGCGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGACCGCCCAACGA
 CCCCCGCCCATTTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCATTGACGTCAAT
 GGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTATT
 GACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGCCAGTACATGACCTTATGGGACTTTCCTACTTGGCA
 30 GTACATCTACGTATTAGTCATCGTATTACCATGGTGATGCGGTTTTTGGCAGTACATCAATGGGCGTGGATAGC
 GGTTTGACTCACGGGGATTTCCAAGTCTCCACCCCATTTGACGTCAATGGGAGTTTGTTTTGGCACCAAAATCAA
 CGGGACTTTCCAATAATGTCGTAACAACCTCCGCCCATTTGACGCAAATGGGCGGTAGGCGTGTACGGTGGGAGGT
 CTATATAAGCAGAGCTCGTTTAGTGAACCGTCAGATCGCCTGGAGACGCCATCCACGCTGTTTTGACCTCCATA
 GAAGACACCGGGACCGATCCAGCCTCCATCGGCTCGCATCTCTCCTTCACGCGCCCGCCGCCCTACCTGAGGCC
 35 GCCATCCACGCGGTTGAGTCGCGTCTGCGCCCTCCCGCCTGTGGTGCCTCCTGAACTGCGTCCGCCGTCTAG
 GTAAGTTTTAAAGCTCAGGTCGAGACCGGGCCTTTGTCCGGCGCTCCCTTGGAGCCTACCTAGACTCAGCCGGCT
 CTCCACGCTTTGCTGACCTGCTTGTCTCAACTCTAGTTAACGGTGGAGGGCAGTGTAGTCTGAGCAGTACTCG
 TTGCTGCCGCGCGGCCACCAGACATAATAGCTGACAGACTAACAGACTGTTTCCTTTCCATGGGTCTTTTCTGC
 AGTCACCGTCCGTACCGCCACCATGAAGGCTATTCTGGTGGTGTGCTGTACACCTTCGCCACCGCCAATGCCG
 40 ATACACTGTGTATTGGCTACCACGCCAACACAGCACCGGACACCGTGGATACCGTGTGGAAAAGAACGTGACC
 GTGACACACAGCGTGAACCTGCTGGAAGATAAGCACACGGCAAGCTGTGCAAGCTGAGAGGCGTTGCACCTCT

GCACCTGGGCAAGTGTAATATCGCCGGCTGGATCCTGGGCAACCCTGAGTGTGAAAGCCTGAGCACAGCCAGCA
 GCTGGTCTTACATCGTGGAACCAGCAGCAGCGACAACGGCACATGCTACCCGGCGACTTCATCAACTACGAG
 GAACTGAGAGAGCAGCTGAGCAGCGTCAGCAGCTTCGAGAGATTTCGAGATTTTCCCCAAGACCTCCAGCTGGCC
 CAACCACGATTCTAACAAGGGCGTGACAGCCGCTGTCTCATGCCGGCGCTAAGAGCTTCTACAAGAACCTGA
 5 TCTGGCTGGTCAAGAAGGGCAACAGCTACCCCAAGCTGAGCAAGAGCTACATCAACGACAAGGGCAAAGAGGTG
 CTGGTCTCTGGGGCATCCACCATCCTTCTACAACAGCCGACCAGCAGAGCCTGTACCAGAATGCCGATGCCTA
 CGTGTTCTGTGGGCACCAGCAGATACAGCAAGAAGTTCAGCCCCGAGATCGCCATCAGACCCAAAGTGCGGGATC
 AAGAGGGCAGAATGAACTACTACTGGACCCTGGTGGAAACCCGGCGACAAGATCACATTTGAGGCCACAGGCAAC
 CTGGTGGTCCCTAGATACGCCTTCGCCATGGAAAAGAAATGCCGGCAGCGGCATCATCATCAGCGACACACCTGT
 10 GCACGACTGCAACACCACCTGTCAGACACCTGAGGGCGCCATCAATACCAGCCTGCCTTTCCAGAACATTACCC
 CCATCACCATCGGCAAGTGCCCCAAATACGTGAAGTCCACAAAGCTGAGACTGGCCACCGGCCCTGAGAAATGTG
 CCTAGCATCCAGAGCAGAGGCCCTGTTTGGAGCCATTGCCGGCTTTATCGAAGGCGGCTGGACAGGCATGGTTGA
 CGGATGGTACGGCTACCACCATCAGAATGAGCAAGGCAGCGGATACGCCGCCGATCTGAAGTCTACACAGAACG
 CCATCGATAAGATCACCAACAAAGTGAACAGCGTGATCGAGAAGATGAACACCCAGTTCACCGCCGTGGGAAAA
 15 GAGTTCAACCACCTGGAAAAGCGCATCGAGAACCTGAACAAGAAGGTGGACGACGGCTTCTGGACATCTGGAC
 CTATAATGCCGAGCTGCTCGTGCTGCTCGAGAACGAGAGAACCCTGGACTACCACGACAGCAACGTGAAGAACC
 TGTACGAGAAAGTGCGGAACCAGCTGAAGAACAACGCCAAAGAGATCGGCAACGGCTGCTTCGAGTTCTACCAC
 AAGTGCGACAATACCTGCATGGAAAGCGTGAAGAATGGCACCTACGACTACCCTAAGTACAGCGAGGAAGCCAA
 GCTGAACCGCGAGAAAGATTGACGGCGTGAAGCTGGAAAGCACCCGGATCTATCAGATCCTGGCCATCTACAGCA
 20 CAGTGGCCTCTAGCCTGGTGTGGTGGTGTCTCTGGGAGCCATCAGCTTTTGGATGTGCAGCAATGGCAGCCTC
 CAGTGCCGGATCTGCATCGGAAGCGGAGAAGGCAGAGGCAGCCTGCTGACATGCGGAGATGTGGAAGAGAATCC
 CGGACCTATGAATCCCAACCAGAAGATCATCACCATCGGCAGCATCTGCATGGTCTGTGGGCATCATCAGCCTGA
 TCCTCCAGATCGGCAACATCATCTCCATCTGGGTGTCCCACAGCATCCAGACCGGCAATCAGAACCAGCCTGAG
 ACATGCAACCAGTCCATCATCACCTACGAGAACAACACCTGGGTCAACCAGACCTACGTGAACATCAGCAACAC
 25 CAACTTCGTGGCCGAACAGGCCGTGGCTTCTGTTGCCCTGGCCGGAATAGCTCTCTGTGCCCTATTAGCGGCT
 GGGCCATCTACAGCAAGGACAACGGCATCCGGATCGGCTCTAAGGGCGACGTGTTCTGTGATCAGAGAGCCCTTC
 ATCAGCTGCTCCACCTGGAATGCCGGACATTCTTTCTGACCCAAGGCGCCCTGCTGAACGACAAGCACAGCAA
 TGGCACCGTGAAGGACAGAAGCCCCCTACAGAACCCTGATGAGCTGCCCTGTGGGAGAAGCCCCATCTCCTTACA
 ACAGCAGATTTCGAGTCCGTGGCTTGGAGCGCCTCTGCCTGTCACGATGGAATCAGCTGGCTGACAATCGGCATC
 30 AGCGGCCCTGATAATGGCGCTGTGGCCGTGCTGAAGTACAACGGAATCATCACCAGACCATCAAGAGCTGGCG
 GAACAACATCCTGCGGACCCAAGAGTCCGAGTGCGCCTGTATCAATGGCAGCTGCTTCACCATCATGACAGACG
 GCCCTAGCAATGGCCAGGCCAGCTACAAGATTTTCAAGATCGAGAAGGGCAAAGTGGTCAAGAGCGTGGAAGTGA
 AACGCCCCTAACCTACACTACGAGGAATGCAGCTGCTACCCCGATGCCGGCGAAGTGATGTGCGTGTGCAGAGA
 CAATTGGCACGGCAGCAACAGACCTTGGGTGTCTTCAACCAGAACCCTGGAATATCAGATCGGCTATATCTGCT
 35 CCGGCGTGTTTCGGCGACAACCCAGACCTAATGATGGCACAGGCAGCTGTGGCCCCGTGTCATCTAATGGCGCC
 TATGGCGTGAAGGGCTTCAGCTTTAAGTACGGCAAAGGCGTGTGGATCGGCCGACCAAGAGCACCTCTAGCAG
 ATCCGGCTTCGAGATGATCTGGGACCCCAACGGCTGGACCGAGACAGATAGCAGCTTCAGCGTGAAGCAGGACA
 TCGTGGCCATCACCGATTGGAGCGGCTACAGCGGAAGCTTCGTGCAGCACCCCTGAACTGACAGGCCTGGACTGC
 ATGAGGCCCTGCTTTTGGGTGAGCTGATCCGGGGCAGACCCAAAGAGAACACCATCTGGACAAGCGGCAGCAG
 40 CATCAGCTTTTTCGGCGTGAACAGCGATACCGTTCGGCTGGTCTTGGCCTGATGGTGGCGAGCTGCCTTTACCA
 TCGACAAAGGATCCGGCGCCACCAACTTTAGTCTGCTGAAACAGGCCGGCGACGTGGAAGAGAACCAGGTCTCT
 ATGTCTCTGCTGACCGAGGTGGAAACCCCTACCAGAAATGGCTGGGAGTGCAGATGCAGCGACAGCAGCGATCC

TCTGGTTATCGCCGCCAGCATCATCGGCATCCTGCACCTGATCCTGTGGATCCTGGACCGGCTGTTCTTCAAGT
 GCATCTACCGGCGGCTGAAGTACGGCCTGAAGAGAGGCCCTTCTACAGAGGGCGTGCCCGAGAGCATGCGGGAA
 GAGTACAGACAGAAACAGCAGAGCGCCGTGGACGTGGACGATGGCCACTTCGTGAACATCGAGCTGGAATGAGC
 GGCCGAGATCTGCTGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTGGCCCTCCCCCGTGCCCTTCCTTGACCCCT
 5 GGAAGGTGCCACTCCCCTGTCTTTTCTTAATAAAAATGAGGAAATTGCATCGCATTGTCTGAGTAGGTGTCATT
 CTATTCTGGGGGGTGGGGTGGGGCAGGACAGCAAGGGGAGGATTGGGAAGACAATAGCAGGCATGCTGGGGAT
 GCGGTGGGCTCTATGGCTACCCAGGTGCTGAAGAATTGACCCGGTTCCCTCCTGGGCCAGAAAGAAGCAGGCACA
 TCCCCCTTCTCTGTGACACACCCTGTCCACGCCCCCTGGTTCTTAGTTCCAGCCCCACTCATAGGACACTCATAGC
 TCAGGAGGGCTCCGCTTCAATCCACCCGCTAAAGTACTTGGAGCGGTCTCTCCCTCCCTCATCAGCCCACCA
 10 AACCAAACCTAGCCTCCAAGAGTGGGAAGAAATTAAAGCAAGATAGGCTATTAAGTGCAGAGGGAGAGAAAATG
 CCTCCAACATGTGAGGAAGTAATGAGAGAAATCATAGAATTTTAAGGCCATGATTTAAGGCCATCATGGCCTTA
 ATCTTCCGCTTCCTCGCTCACTGACTCGCTGCGCTCGGTCTGCTCGGCTGCGGCGAGCGGTATCAGCTCACTCAA
 AGGCGGTAAATACGGTTATCCACAGAATCAGGGGATAACGCAGGAAAGAACATGTGAGCAAAAGGCCAGCAAAAG
 GCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCTGACGAGCATCACAAAAA
 15 TCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATAACCAGGCGTTTTCCCCCTGGAAGCTCCC
 TCGTGCGCTCTCCTGTTCCGACCTGCCGCTTACCGGATACCTGTCCGCCTTTCTCCCTTCGGGAAGCGTGGCG
 CTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCTGCTCCAAGCTGGGCTGTGTGCACGA
 ACCCCCCGTTACGCCCCGACCGCTGCGCCTTATCCGTAACCTATCGTCTTGAGTCCAACCCGTAAGACACGACT
 TATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTG
 20 AAGTGGTGGCCTAACTACGGCTACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTT
 CGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTTGTTTGCAAGC
 AGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGG
 AACGAAAACCTCACGTTAAGGGATTTTGGTCATGAGATTATCAAAAAGGATCTTCACCTAGATCCTTTTAAATTA
 AAAATGAAGTTTTTAAATCAATCTAAAGTATATATGAGTAAACTTGGTCTGACAGTTACCAATGCTTAATCAGTG
 25 AGGCACCTATCTCAGCGATCTGTCTATTTTCGTTTCATCCATAGTTGCCTGACTCGGGGGGGGGGGCGCTGAGGT
 CTGCCTCGTGAAGAAGGTGTTGCTGACTCATACCAGGCCTGAATCGCCCCATCATCCAGCCAGAAAGTGAGGGA
 GCCACGGTTGATGAGAGCTTTGTTGTAGGTGGACCAGTTGGTGATTTTGAACTTTTGCTTTGCCACGGAACGGT
 CTGCGTTGTGCGGAAGATGCGTGATCTGATCCTTCAACTCAGCAAAAGTTTCGATTTATTCAACAAAGCCGCCGT
 CCCGTCAAGTCAGCGTAATGCTCTGCCAGTGTTACAACCAATTAACCAATTCTGATTAGAAAAACTCATCGAGC
 30 ATCAAAATGAACTGCAATTTATTTCATATCAGGATTATCAATACCATATTTTTGAAAAAGCCGTTTCTGTAATGA
 AGGAGAAAACTCACCGAGGCAGTTCCATAGGATGGCAAGATCCTGGTATCGGTCTGCGATTCCGACTCGTCCAA
 CATCAATACAACCTATTAATTTCCCCCTCGTCAAAAATAAGGTTATCAAGTGAGAAATCACCATGAGTGACGACT
 GAATCCGGTGAGAATGGCAAAAGCTTATGCATTTCTTTCCAGACTTGTTCAACAGGCCAGCCATTACGCTCGTC
 ATCAAAATCACTCGCATCAACCAAACCGTTATTCAATTCGTGATTGCGCCTGAGCGAGACGAAATACGCGATCGC
 35 TGTTAAAGGACAATTACAAACAGGAATCGAATGCAACCGGCGCAGGAACACTGCCAGCGCATCAACAATATTT
 TCACCTGAATCAGGATATTCTTCTAATACCTGGAATGCTGTTTTCCCGGGGATCGCAGTGGTGAGTAACCATGC
 ATCATCAGGAGTACGGATAAAATGCTTGATGGTCCGGAAGAGGCATAAATTCGGTCAGCCAGTTTAGTCTGACCA
 TCTCATCTGTAACATCATTTGGCAACGCTACCTTTGCCATGTTTTAGAAACAACCTCTGGCGCATCGGGCTTCCCA
 TACAATCGATAGATTGTCGCACCTGATTGCCCCGACATTATCGCGAGCCCATTATACCCATATAAATCAGCATC
 40 CATGTTGGAATTTAATCGCGCCTCGAGCAAGACGTTTCCCGTTGAATATGGCTCATAACACCCCTTGTATTAC
 TGTTTATGTAAGCAGACAGTTTTATTGTTTCATGATGATATATTTTTATCTTGTGCAATGTAACATCAGAGATTT
 TGAGACACAACGTGGCTTTCCCCCCCCCCCCATTATTGAAGCATTTATCAGGGTTATTGTCTCATGAGCGGATA

CATATTTGAATGTATTTAGAAAAATAAACAAATAGGGGTTCCGCGCACATTTCCCCGAAAAGTGCCACCTGACG
TCTAAGAAACCATTATTATCATGACATTAACTATAAAAAATAGGCGTATCACGAGGCCCTTTCGTC

panH1N1 - *amino acid sequence* (SEQ ID NO:63)

5 MKAILVLLYTFATANADTLICIGYHANNSTDVTDVLEKNVTVTHSVNLLLEDKHNGKLCKLRGVAPLHLGKCNI
AGWILGNPECESLSTASSWSYIVETSSSDNGTCYPGDFINYEELREQLSSVSSFERFEIFPKTSSWPNHDSNKG
VTAACPHAGAKSFYKNLIWLKKGNSYPKLSKSYINDKGKEVLVLWGIHHPSTTADQQSLYQNADAYFVVGTSR
YSKKFKPEIAIRPKVRDQEGRMNYYWTLVEPGDKITFEATGNLVVPRYAFAMERNAGSGIIISDTPVHDCNTTC
QTPEGAINTSLPFQNIHPITIGKCPKYVKSTKLRLATGLRNVPISIQRGLFGAIAGFIEGGWTGMVDGWYGYHH
10 QNEQGSYGAADLKSTQNAIDKITNKVNSVIEKMNTQFTAVGKEFNHLEKRIENLNKKVDDGFLDIWTYNAELLV
LLENERTLDYHDSNVKNLYEKVRNQLKNNAKEIGNGCFEFYHKCDNTCMESVKNGTYDYPKYSEEAKLNREKID
GVKLESTRIYQILAIYSTVASSLVLVVSLGAISFWMCSNGSLQCRICI **GSGEGRGSLLTCGDVEENPGP** MNPNQ
KIITIGSICMVVGIIISLILQIGNIISIWVSHSIQTGNQNQPETCNQSIITYENNTWVNQTYVNIISNTNFVAEQA
VASVALAGNSSLCPISGWAIYSKDNIGIRIGSKGDVVFVIREPFISCSHLECRTFFLTQGALLNDKHSNGTVKDRS
15 PYRTLMSCPVGEAPSPYNSRFESVAWSASACHDGISWLTIGISGPDNGAVAVLKYNGIITDTIKSWRNNILRTQ
ESECACINGSCFTIMTDGPSNGQASYKIFKIEKGKVVKSVELNAPNYHYEECSYCPDAGEVMCVCRDNWHGNSR
PWVSFNQNLLEYQIGYICSGVFGDNPRPNDGTGSCGPVSSNGAYGVKGFsfkygkgvwigrtkstssrsgfemiw
DPNGWTETDSSFSVKQDIVAITDWSGYSGSFVQHPELTGLDCMRPCFWVELIRGRPKENTIWTSGSSISFCGVN
SDTVGWSWPDGAELPFTIDK **GSGATNFSLLKQAGDVEENPGP** MSLLTEVETPTRNGWECRCSDSSDPLVIAASI
20 IGILHLILWILDRLFFKCIYRRLKYGLKRG PSTEGVPESMREEYRQKQSAVDVDDGHFVNIELE

The panH1N1 amino acid sequence (SEQ ID NO:63) shown above includes a first 2A self-
cleaving peptide sequence (GSGEGRGSLLTCGDVEENPGP; SEQ ID NO:66), shown
highlighted in bold, between the amino acid sequences of the FLU_T2_HA_3_I3 and
25 FLU_T2_NA_3 subunits, and a second 2A self-cleaving peptide sequence
(GSGATNFSLLKQAGDVEENPGP; SEQ ID NO:67), shown highlighted in bold, between the
amino acid sequences of the FLU_T2_NA_3 and FLU_T2_M2_1 subunits.

Strategies for multigene co-expression include introduction of multiple vectors, use of
multiple promoters in a single vector, fusion proteins, proteolytic cleavage sites between
30 genes, internal ribosome entry sites (IRES), and “self-cleaving” 2A peptides. Multicistronic
vectors based on IRES nucleotide sequence and self-cleaving 2A peptides are reviewed in
Shaimardanova *et al.* (*Pharmaceutics* **2019**, *11*, 580; doi:10.3390/pharmaceutics11110580).

2A self-cleaving peptides are 18–22 amino-acid-long viral oligopeptides that mediate
“cleavage” of polypeptides during translation in eukaryotic cells (Liu *et al.*, *Scientific Reports*
35 **7**, Article number: 2193 (2017)). The designation “2A” refers to a specific region of the viral
genome and different viral 2As have generally been named after the virus they were derived
from. The first discovered 2A was F2A (foot-and-mouth disease virus), after which E2A
(equine rhinitis A virus), P2A (porcine teschovirus-1 2A), and T2A (thossea asigna virus 2A)

were also identified. The mechanism of 2A-mediated “self-cleavage” is ribosome skipping the formation of a glycyl-prolyl peptide bond at the C-terminus of the 2A. A highly conserved sequence GDVEXNPGP is shared by different 2As at the C-terminus, and is essential for the creation of steric hindrance and ribosome skipping. There are three possibilities for a 2A-mediated skipping event: (1) Successful skipping and recommencement of translation results in two “cleaved” proteins: the protein upstream of the 2A is attached to the complete 2A peptide except for the C-terminal proline, and the protein downstream of the 2A is attached to one proline at the N-terminus; (2) Successful skipping but ribosome fall-off and discontinued translation results in only the protein upstream of 2A; (3) Unsuccessful skipping and continued translation resulting in a fusion protein. Overall, 2A peptides lead to relatively high levels of downstream protein expression compared to other strategies for multi-gene co-expression, and they are small in size thus bearing a lower risk of interfering with the function of co-expressed genes.

Example 16 - Immunogenicity and efficacy of a broadly reactive H1N1 Influenza vaccine in pigs

Background:

The continued antigenic change (drift) of influenza A virus strains over time in the human population necessitates twice-yearly updates to the human seasonal vaccine composition. Development of a broadly cross-reactive ‘universal’ vaccine that does not require such frequent updates would be a considerable advantage. The aim of this study was to assess a novel broadly cross-reactive vaccine technology in the pig model of influenza.

Methods:

The test vaccine in this study was a structure-based computational synthetic multi-gene antigen of human-origin H1N1 influenza A virus, panH1N1 (also referred to as DIOSynVax-H1N1). It was administered needle-free to 5 pigs as DNA intradermally (ID) using the PharmaJet® Tropis® system. Two control whole, inactivated virus (WIV) vaccines of the same pandemic lineage, A/swine/England/1353/2009 (WIV₁₃₅₃) and A/Victoria/2454/2019 (WIV_{vic}) in oil-in-water adjuvant were administered intramuscularly to 5 pigs each at the same 4-week interval. Six weeks following the second immunisation all groups were challenged with the swine-origin pH1N1 strain A/swine/England/1353/2009 (1.7×10^6 TCID₅₀ per pig intranasally). Pigs were monitored daily post-inoculation (dpi) until end of experiment on 8 or 9dpi. The immunisation and bleed protocol of pigs tested is illustrated in Figure 11a and b.

Results:

Nasal shedding of viral RNA was monitored daily by RRT-qPCR (Figure 12). All challenged animals shed viral RNA, reaching a peak between 2 to 5dpi by RRT-qPCR (Figure 12a). Area under the curve analysis showed significant reduction in the nasal shedding of viral RNA in the animal groups vaccinated with the broadly reactive panH1N1 (P=0.0012) or WIV₁₃₅₃ (P=0.0003) vaccines when compared to the naïve control or WIV_{vic} vaccinated groups (Figure 12b). All pigs resolved the infection by 8-9dpi, as shown in the lower graph of Figure 12b which illustrates virus titration measurements from bronchoalveolar lavage (BAL) fluid, turbinates, and trachea samples from pigs of each group.

Influenza virus-specific serum antibody levels were monitored longitudinally by Haemagglutinin inhibition Assay (HAI; Figure 13a), and NP ELISA (Figure 13b). Both assays revealed significant antibody levels in the WIV-vaccinated groups, even after a single vaccination. The panH1N1 immunised pigs mounted an equivalent HA antibody response to the WIV vaccines after the boost vaccination (i.e. after D28). Antibodies were found to be neutralising, as shown in the serum neutralisation assay in Figure 14. Further evidence that the panH1N1 vaccine provides similar protection to WIV₁₃₅₃ is shown in the ELISopt and HAI assays of Figure 15. In particular, Figure 15a shows a T-cell peptide stimulation assay, wherein splenocytes were stimulated with the peptides spanning A/Swine/england/1353/2009 HA and A/Victoria/2545/2019 HA. Higher the values on the y-axis indicate a higher T-cell response. Each point corresponds to one pig. panH1N1 vaccinated group has better T-cell response than the controls post infection. Figure 15b shows a HAI assay. The top panel shows distribution of the hemagglutinin inhibition titre 0 days, 28 days, 42 days and 63 days post vaccination and 8 days post infection. The titres were checked against A/swine/England/1353/2009 strain and a/Victoria/2454/2019 strain. The lower panel illustrates the mean values for each group.

Conclusion:

Importantly this study demonstrated proof-of-concept that pigs immunised with the broadly neutralising panH1N1 vaccine were protected as well as a whole virion, inactivated adjuvanted vaccine homologous to the challenge strain (WIV₁₃₅₃). In contrast, a WIV vaccine made from a human-origin strain from the same pH1N1 1A.3.3.2 lineage (WIV_{vic}), failed to show any protection in the presence of significant antibody levels.

Example 17 – Optimised vaccine generates neutralising immune responses and protects against human and swine H1N1 influenza in mice and pigs

Background

Influenza A's (IAV) zoonotic transmission and constant evolution in multiple species especially birds and pigs heighten the potential emergence of novel strains at the human-animal interface. The cornerstone of influenza prevention and control is still strain-specific vaccination, however pitfalls associated with this have decreased vaccine effectiveness. To cover seasonal, zoonotic, and pandemic threats, we present an elegant Digitally designed, Immune Optimised, Synthetic (DIOS) vaccine to induce broad H1, N1 and M2 subtype-specific immunity and protection against divergent strains in mouse and pig models.

Methods

For mouse immunogenicity studies, individual immunogen, FLU_T2_HA_3_I3, was injected subcutaneously 4 times in 2-week intervals and terminal bleeds taken 10 weeks post-first immunization. For the pig challenge, a prime-boost regimen (4 week interval) was employed and the panH1N1 vaccine candidate administered intradermally via PharmaJet® Tropis. Controls delivered intramuscularly included whole inactivated virus (WIV) representing swine and human influenza. Pigs were challenged with A/swine/EN/1353/09 10 weeks post-prime. Efficacy was measured as reduced viral shedding. Serum neutralizing titers were monitored using pseudotype neutralization (pMN), enzyme-linked lectin assay (ELLA) and hemagglutination inhibition (HAI).

Results

Figure 16 shows surface representations of hemagglutinin (HA), neuraminidase (NA) and M2 from A/swine/EN/1353/09, A/Victoria/2454/2019 H1N1 strains and our DIOS vaccine candidate, panH1N1. Coloured residues show defined antigenic sites with non-conserved residues between swine/EN/09 and panH1N1 highlighted in red, and between swine/EN/09 and Victoria/19 in magenta.

As shown in Figure 17a and Figure 24, we observed excellent immune responses in all mice (n=6) vaccinated with FLU_T2_HA_3_I3 (referred to as DIOS(HA) in the Figure 17, and H1N1dpm in Figure 24) against all H1N1 strains tested that are comparable or superior to the control A/Michigan/45/15(H1) (*p<0.05). Values are calculated as the fold dilution of sera that resulted in 50% neutralization of virus via pMN. Figure 17b shows administration of FLU_T2_HA_3_I3 in mice elicits effective antibody binding responses to six H1 wild-type influenza viruses tested.

In pigs, reduced viral shedding in nasal swabs (expressed as mean log Relative equivalent units (REU) of viral RNA) was observed post-challenge in the panH1N1 (n=5) and WIV₁₃₅₃ (homologous to the challenge strain) (n=5) groups (Figure 18). Serum neutralising antibodies

against HA and NA of A/swine/England/1353/2009 and other relevant H1N1 viruses were also detected in groups given panH1N1 and WIV₁₃₅₃. Figures 19a and Figure 19b show serum neutralising titers vs VI/2570/19 and EN/195/09 as monitored at specific times, Figure 19c shows serum neutralisation with panH1N1 vaccination against a panel of H1 expressing pseudoviruses at 42 days post vaccination.

Figures 20a and 20b show an ELLA (Enzyme-Linked Lectin Assay) to assess the inhibition activity of the NA component of panH1N1 against A/swine/England/1353/2009 (Figure 20a) and A/England/195/2009 (Figure 20b) at a series of time points post-vaccination/infection. Figure 20c shows an ELLA against a panel of NA expressing pseudoviruses at 42 days post vaccination.

PanH1N1 is referred to as DIOS in Figures 16 and 18, 19, and 20.

Conclusion

We have shown the immunogenicity and efficacy of the DIOS (panH1N1 and individual FLU_T2_HA3_I3) vaccine against relevant IAV H1N1 strains *in vitro* and *in vivo* in mice and pigs. This approach may target different aspects of influenza leading to broadened protection within the same subtype. This can support pandemic preparedness whilst protecting against circulating human influenza. This platform can be translated into other subtypes with the goal of producing a universal influenza vaccine.

Example 18 - FLU_T3_HA_3

This example provides amino acid sequences of the influenza haemagglutinin H5 head and stem regions for an embodiment of the invention known as FLU_T3_HA_3. In SEQ ID NO:27 below, the amino acid residues of the stem region are shown underlined. The amino acid residues of the head region are the remaining residues. Similarly, in SEQ ID NO:28 below, the nucleic acid residues of the stem region are shown underlined. The nucleic acid residues of the head region are the remaining residues.

FLU_T3_HA_3 – HA0 amino acid sequence (SEQ ID NO:27):

MEKIVLLLLAIVSLVKSDQICIGYHANNSTEQVDTIMEKNVTVTTHAQDILEKTHNGKLCDL
DGVKPLILRDCSVAGWLLGNPMCDEFINVPESYIVEKANPANDLCYPGNFNDYEELKHL
LSRINHFEKIQIIPKSSWSDHEAS/GVSSACPYQGRSSFFRNVVWLIKNNAYPTIKRSY
NNTNQEDLLVLWGIHHPNDAAEQTRLYQNPTTYISVGTSTLNQRLVPKIATRISKVNGQSG
RMEFFWTILKPNDAINFESNGNFIAPYAYKIVKKGDSAIMKSELEYGNCNTKCQTPMGA
INSSMPFHNHPLTIGECPKYVKSRLVLAATGLRNSPQRERRRKKRGLFGAIAGFIEGGW
QGMVDGWYGYHHSNEQGSYAADKESTQKAIDGVTNKVNSIIDKMNTQFEAVGREFNNLE
RRIENLNKKMEDGFLDVWTYNAELLVLMENERTLDFHDSNVKNLYDKVRLQLRDNAKELG

NGCFEFYHKCDNECMESVRNGTYDYPQYSEEARLKREEISGVKLESIGTYQILSIYSTVA
SSLALAIMVAGLSLWMCSNGSLQCRICI

FLU_T3_HA_3 – HA0 nucleic acid sequence (SEQ ID NO:28)

5 ATGGAAAAGATTGTGCTGCTGCTGGCCATCGTGTCCCTGGTCAAGAGCGATCAAATCTGC
ATCGGCTACCAACGCCAACACAGCACCGAACAGGTGGACACCATTATGGAAAAGAACGTG
ACCGTGACACACGCCCAGGACATCCTGGAAAAGACCCACAACGGCAAGCTGTGCGACCTG
GATGGCGTGAAGCCTCTGATCCTGAGAGATTGCTCTGTGGCCGGCTGGCTGCTGGGCAAT
CCTATGTGCGACGAGTTCATCAACGTGCCCCGAGTGGTCCTATATCGTGGAAAAGGCCAAT
10 CCTGCCAACGACCTGTGCTACCCCGGCAACTTCAACGACTACGAGGAAGTGAACATCTG
CTGAGCCGGATCAACCACTTCGAGAAGATCCAGATCATCCCCAAGTCCTCTTGAGCGAT
CACGAGGCCTCTGGAGTGTCTAGCGCCTGTCCTTACCAAGGCAGAAGCAGCTTCTTCCGG
AACGTCTGTGGCTGATCAAGAAGAACAACGCTTACCCACCATCAAGCGGAGCTACAAC
AACACCAATCAAGAGGACCTGCTGGTGCTGTGGGGCATCCACCATCCTAATGATGCCGCC
15 GAGCAGACCCGGCTGTACCAGAATCCTACAACCTACATCAGCGTGGGCACCAGCACACTG
AACCAGAGACTGGTGCCTAAGATCGCCACCAGATCCAAAGTGAACGGCCAGAGCGGCCGG
ATGGAATTCTTCTGGACCATCCTGAAGCCTAACGACGCCATCAACTTCGAGAGCAACGGC
AACTTTATCGCCCCTGAGTACGCCTACAAGATCGTGAAGAAGGGCGACAGCGCCATCATG
AAGTCCGAGCTGGAATACGGCAACTGCAACACCAAGTGTGAGACCCCTATGGGCGCCATC
20 AATAGCAGCATGCCCTTCCACAACATTCACCCTCTGACCATCGGCGAGTGCCCCAAATAC
GTGAAGTCCAACAGACTGGTCCTGGCCACCGGCCTGAGAAATTCTCCACAGAGAGAGCGG
CGCAGAAAGAAGAGAGGCCTGTTTGGAGCCATTGCCGGCTTTATCGAAGGCGGCTGGCAA
GGCATGGTTGACGGATGGTACGGCTATCACCACAGCAATGAGCAAGGCTCTGGCTACGCC
GCCGACAAAGAGAGCACACAGAAAGCCATCGACGGCGTGACCAACAAAGTGAATAGCATC
25 ATCGACAAGATGAACACCCAGTTCGAGGCCGTGGGCAGAGAGTTCAACAACCTGGAAAGA
CGGATCGAGAACCTGAACAAGAAGATGGAGGACGGCTTCCTGGACGTGTGGACCTATAAT
GCCGAGCTGCTGGTCCTGATGGAAAACGAGAGAACCCTGGACTTCCACGACAGCAACGTG
AAGAACCTGTACGACAAAGTGC GGCTCCAGCTGCGGGACAATGCCAAAGAACTCGGCAAC
GGCTGCTTCGAGTTCTACCACAAGTGCGACAACGAGTG CATGGAAAGCGTGCGGAACGGC
30 ACCTACGACTACCCTCAGTACTCTGAGGAAGCCCGGCTGAAGAGAGAAGAGATCAGCGGA
GTGAAGCTGGAATCCATCGGCACATACCAGATCCTGAGCATCTACAGCACCGTGGCCTCT
TCTCTGGCCCTGGCTATTATGGTGGCTGGCCTGAGCCTGTGGATGTGCTCTAATGGCAGC
CTCCAGTGCCGGATCTGCATCTGA

FLU_T3_HA_3 – head region amino acid sequence (SEQ ID NO:29)

35 THNGKLCDDLGVKPLILRDCSVAGWLLGNPMCDEFINVPESYIVEKANPANDLCYPGNFNNDYEELK
HLLSRINHFEKIQIIPKSSWSDEAS/GVSSACPYQGRSSFFRNVVWLIKNNAYPTIKRSYNNTNQ

EDLLVLWGIHHPNDAAEQTELYQNPTTYISVGTSTLNQRLVPKIATRSKVNQSGRMEFFWTILKPN
DAINFESNGNFIAPEYAYKIVKKGDSAIMKSELEYGNCNTKCQTPMGAINSSMPFHNIHPLTIGECF

- 5 The amino acid residues at positions 156, 157, 171, 172, and 205 are shown underlined and highlighted in grey in the above sequence (and are R, S, N, A, and R, respectively). The deleted amino acid residue at residue 144 or 145 is shown as “/”, and highlighted in greyscale.

FLU_T3_HA_3 – head region nucleic acid sequence (SEQ ID NO:30)

10 ACCCACAAACGGCAAGCTGTGCGACCTGGATGGCGTGAAGCCTCTGATCCTGAGAGATTGCTCTGTGG
CCGGCTGGCTGCTGGGCAATCCTATGTGCGACGAGTTCATCAACGTGCCCGAGTGGTCCTATATCGT
GGAAAAGGCCAATCCTGCCAACGACCTGTGCTACCCCGGCAACTTCAACGACTACGAGGAACTGAAA
CATCTGCTGAGCCGGATCAACCACTTCGAGAAGATCCAGATCATCCCCAAGTCCTCTTGAGCGATC
ACGAGGCCTCTGGAGTGTCTAGCGCCTGTCTTACCAAGGCAGAAGCAGCTTCTTCCGGAACGTCGT
GTGGCTGATCAAGAAGAACAACGCTTACCCACCATCAAGCGGAGCTACAACAACACCAATCAAGAG
15 GACCTGCTGGTGTGTGGGGCATCCACCATCCTAATGATGCCGCCGAGCAGACCCGGCTGTACCAGA
ATCCTACAACCTACATCAGCGTGGGCACCAGCACACTGAACCAGAGACTGGTGCCTAAGATCGCCAC
CAGATCCAAAGTGAACGGCCAGAGCGGCCGGATGGAATTCTTCTGGACCATCCTGAAGCCTAACGAC
GCCATCAACTTCGAGAGCAACGGCAACTTTATCGCCCCCTGAGTACGCCTACAAGATCGTGAAGAAGG
GCGACAGCGCCATCATGAAGTCCGAGCTGGAATACGGCAACTGCAACACCAAGTGTGAGACCCCTAT
20 GGGCGCCATCAATAGCAGCATGCCCTTCCACAACATTCACCCTCTGACCATCGGCGAGTGCCCC

FLU_T3_HA_3 – first stem region amino acid sequence (SEQ ID NO:31)

MEKIVLLLAIVSLVKSDQICIGYHANNSTEQVDTIMEKNVTVTTHAQDILEK

FLU_T3_HA_3 – first stem region nucleic acid sequence (SEQ ID NO:32)

25 ATGGAAAAGATTGTGCTGCTGCTGGCCATCGTGTCCCTGGTCAAGAGCGATCAAATCTGCATCGGCT
ACCACGCCAACCAACAGCACCGAACAGGTGGACACCATTATGGAAAAGAACGTGACCGTGACACACGC
CCAGGACATCCTGGAAAAG

FLU_T3_HA_3 – second stem region amino acid sequence (SEQ ID NO:33)

30 KYVKSRLVLATGLRNSPQRERRRKKRGLFGAIAGFIEGGWQGMVDGWYGYHHSNEQSGYAADKES
TQKAIDGVTNKVNSIIDKMNTQFEAVGRENNLERRIENLNKKMEDGELDVWTYNAELLVLMENERT
LDFHDSNVKNLYDKVRLQLRDNAKELGNGCFEFYHKCDNECMESVRNGTYDYPQYSEEARLKREEIS
GVKLESIGTYQILSIYSTVASSLALAIMVAGLSLWMCSNGSLQCRICI

The amino acid residues at positions 416 and 434 (or at positions 148 and 166 if counting from the beginning of the stem region) are shown underlined in the above sequence (and are F and F, respectively).

5 FLU_T3_HA_3 – second stem region nucleic acid sequence (SEQ ID NO:34)

AAATACGTGAAGTCCAACAGACTGGTCCTGGCCACCGGCCTGAGAAATTCTCCACAGAGAGAGCGGC
GCAGAAAGAAGAGAGGCCTGTTTGGAGCCATTGCCGGCTTTATCGAAGGCGGCTGGCAAGGCATGGT
TGACGGATGGTACGGCTATCACCACAGCAATGAGCAAGGCTCTGGCTACGCCGCCGACAAAGAGAGC
10 ACACAGAAAGCCATCGACGGCGTGACCAACAAAGTGAATAGCATCATCGACAAGATGAACACCCAGT
TCGAGGCCGTGGGCAGAGAGTTCAACAACCTGGAAAGACGGATCGAGAACCTGAACAAGAAGATGGA
GGACGGCTTCTGGACGTGTGGACCTATAATGCCGAGCTGCTGGTCCTGATGGAAAACGAGAGAACC
CTGGACTTCCACGACAGCAACGTGAAGAACCTGTACGACAAAGTGCGGCTCCAGCTGCGGGACAATG
CCAAAGAACTCGGCAACGGCTGCTTCGAGTTCTACCACAAGTGCGACAACGAGTGCATGGAAAGCGT
15 GCGGAACGGCACCTACGACTACCCTCAGTACTCTGAGGAAGCCCGGCTGAAGAGAGAAGAGATCAGC
GGAGTGAAGCTGGAATCCATCGGCACATACCAGATCCTGAGCATCTACAGCACCGTGGCCTCTCTCT
GGCCCTGGCTATTATGGTGGCTGGCCTGAGCCTGTGGATGTGCTCTAATGGCAGCCTCCAGTGCCGG
ATCTGCATCTGA

20 **Example 19 - FLU_T3_HA_4**

This example provides amino acid sequences of the influenza haemagglutinin H5 head and stem regions for an embodiment of the invention known as FLU_T3_HA_4. In SEQ ID NO:35 below, the amino acid residues of the stem region are shown underlined. The amino acid residues of the head region are the remaining residues. Similarly, in SEQ ID NO:36 below,
25 the nucleic acid residues of the stem region are shown underlined. The nucleic acid residues of the head region are the remaining residues.

FLU_T3_HA_4 – HA0 amino acid sequence (SEQ ID NO:35):

MEKIVLLLAIVSLVKSDQICIGYHANNSTEQVDTIMEKNVTVTTHAQDILEKTHNGKLCDLGDKPLI
30 LRDCSVAGWLLGNPMCDEFINPEWSYIVEKANPANDLCYPGNFNDYEELKHLISRINHFEKIQTIP
KSSWSDHEASSGVVPACPYQGRSSFFRNVVWLIKNNAYPTIKRSYNNNTNQEDLLVLWGIHHPNDAA
EQTRLYQNPTTYISVGTSTLNQRLVPKIAATRSKVNNGESGRMEFFWTILKPNDAINFESNGNFIAPY
AYKIVKKGDSAIMKSELEYGNCNTKCQTPMGAINSSMPFHNIHPLTIGECPKYVKS NRLVLATGLRN
SPQRRERRRKRGLFGAIAGFIEGGWQGMVDGWYGYHHSNEQGSYAADKESTQKAIDGVTNKVNSII
35 DKMNTQFEAVGREFNNLERRIENLNKKMEDGFLDVWVTYNAELLVLMENERTLDFHDSNVKNLYDKVR
LQLRDNAKELGNGCFEFYHKCDNECMESVRNGTYDYPQYSEEARLKREEISGVKLESIGTYQILSIY
STVASSLALAIMVAGLSLWMCSNGSLQCRICI

FLU_T3_HA_4 – HA0 nucleic acid sequence (SEQ ID NO:36)

ATGGAAAAGATTGTGCTGCTGCTGGCCATCGTGTCCCTGGTCAAGAGCGATCAAATCTGCATCGGCT
 ACCACGCCAACACAGCACCGAACAGGTGGACACCATTATGGAAAAGAACGTGACCGTGACACACGC
 5 CCAGGACATCCTGGAAAAGACCCACAACGGCAAGCTGTGCGACCTGGATGGCGTGAAGCCTCTGATC
 CTGAGAGATTGCTCTGTGGCCGGCTGGCTGCTGGGCAATCCTATGTGCGACGAGTTCATCAACGTGC
 CCGAGTGGTCTTATATCGTGGAAAAGGCCAATCCTGCCAACGACCTGTGCTACCCCGGCAACTTCAA
 CGACTACGAGGAACTGAAACATCTGCTGAGCCGGATCAACCACTTCGAGAAGATCCAGATCATCCCC
 AAGTCCTCTTGGAGCGATCACGAGGCCTCTAGCGGAGTGGTGCCGGCCTGTCCTTACCAAGGCAGAA
 10 GCAGCTTCTTCCGGAACGTCTGTGGCTGATCAAGAAGAACAACGCTTACCCACCATCAAGCGGAG
 CTACAACAACACCAATCAAGAGGACCTGCTGGTGCTGTGGGGCATCCACCATCCTAATGATGCCGCC
 GAGCAGACCCGGCTGTACCAGAATCCTACAACCTACATCAGCGTGGGCACCAGCACACTGAACCAGA
 GACTGGTGCCCTAAGATCGCCACCAGATCCAAAGTGAACGGCGAAAGCGGCCGGATGGAATTCTTCTG
 GACCATCCTGAAGCCTAACGACGCCATCAACTTCGAGAGCAACGGCAACTTTATCGCCCCTGAGTAC
 15 GCCTACAAGATCGTGAAGAAGGGCGACAGCGCCATCATGAAGTCCGAGCTGGAATACGGCAACTGCA
 ACACCAAGTGTGAGACCCCTATGGGCGCCATCAATAGCAGCATGCCCTTCCACAACATTACCCCTCT
 GACCATCGGCGAGTGCCCCAAATACGTGAAGTCCAACAGACTGGTCCTGGCCACCGGCCTGAGAAAT
 TCTCCACAGAGAGAGCGGCGCAGAAAGAAGAGAGGCCTGTTTGGAGCCATTGCCGGCTTTATCGAAG
 GCGGCTGGCAAGGCATGGTTGACGGATGGTACGGCTATCACCACAGCAATGAGCAAGGCTCTGGCTA
 20 CGCCGCCGACAAAGAGAGCACACAGAAAGCCATCGACGGCGTGACCAACAAAGTGAATAGCATCATC
 GACAAGATGAACACCCAGTTCGAGGCGCTGGGCAGAGAGTTCAACAACCTGGAAAGACGGATCGAGA
 ACCTGAACAAGAAGATGGAGGACGGCTTCCTGGACGTGTGGACCTATAATGCCGAGCTGCTGGTCCT
 GATGGAAAACGAGAGAACCCTGGACTTCCACGACAGCAACGTGAAGAACCTGTACGACAAAGTGCGG
 CTCCAGCTGCGGGACAATGCCAAAGAACTCGGCAACGGCTGCTTCGAGTTCTACCACAAGTGCGACA
 25 ACGAGTGCATGGAAAGCGTGCGGAACGGCACCTACGACTACCCTCAGTACTCTGAGGAAGCCCGGCT
 GAAGAGAGAAGAGATCAGCGGAGTGAAGCTGGAATCCATCGGCACATACCAGATCCTGAGCATCTAC
 AGCACCGTGGCCTCTTCTCTGGCCCTGGCTATTATGGTGGCTGGCCTGAGCCTGTGGATGTGCTCTA
 ATGGCAGCCTCCAGTGCCGGATCTGCATCTGA

30 FLU_T3_HA_4 – head region amino acid sequence (SEQ ID NO:37)

THNGKLCDLDGVKPLILRDCSVAGWLLGNPMCDEFINVPESYIVEKANPANDLCYPGNFNDYEELK
 HLLSRINHFEKIQIIPKSSWSDHEASSGVVPACPYQGRSSFFRNVVWLIKNNAYPTIKRSYNNTNQ
 EDLLVLWGIHHPNDAAEQTFLYQNPTTYISVGTSTLNQRLVPKIAATRSKVNGETSGRMEFFWTILKPN
 DAINFESNGNFIAPYAYKIVKKGDSAIMKSELEYGNCNTKCQTPMGAINSSMPFHNHPLTIGECF

35

The amino acid residues at positions 156, 157, 171, 172, and 205 are highlighted in the above sequence (and are R, S, N, A, and R, respectively). The amino acid residues at positions 148, 149, and 238 are also highlighted, and are V, P, and E, respectively.

FLU_T3_HA_4 – head region nucleic acid sequence (SEQ ID NO:38)

ACCCACAAACGGCAAGCTGTGCGACCTGGATGGCGTGAAGCCTCTGATCCTGAGAGATTGCTCTGTGG
 CCGGCTGGCTGCTGGGCAATCCTATGTGCGACGAGTTCATCAACGTGCCCGAGTGGTCTATATCGT
 GGAAAAGGCCAATCCTGCCAACGACCTGTGCTACCCCGGCAACTTCAACGACTACGAGGAACTGAAA
 5 CATCTGCTGAGCCGGATCAACCACTTCGAGAAGATCCAGATCATCCCCAAGTCTCTTGGAGCGATC
 ACGAGGCCTCTAGCGGAGTGGTGCCGGCCTGTCCTTACCAAGGCAGAAGCAGCTTCTTCCGGAACGT
 CGTGTGGCTGATCAAGAAGAACAACGCTTACCCCAACATCAAGCGGAGCTACAACAACACCAATCAA
 GAGGACCTGCTGGTGTGTGGGCGATCCACCATCCTAATGATGCCGCCGAGCAGACCCGGCTGTACC
 AGAATCCTACAACCTACATCAGCGTGGGCACCAGCACACTGAACCAGAGACTGGTGCCTAAGATCGC
 10 CACCAGATCCAAAGTGAACGGCGAAAGCGGCCGGATGGAATTCTTCTGGACCATCCTGAAGCCTAAC
 GACGCCATCAACTTCGAGAGCAACGGCAACTTTATCGCCCCTGAGTACGCCTACAAGATCGTGAAGA
 AGGGCGACAGCGCCATCATGAAGTCCGAGCTGGAATACGGCAACTGCAACACCAAGTGTGAGACCCC
 TATGGGCGCCATCAATAGCAGCATGCCCTTCCACAACATTCACCCTCTGACCATCGGCGAGTGCCCC

FLU_T3_HA_4 – first stem region amino acid sequence (SEQ ID NO:39)

15 MEKIVLLLAIVSLVKSDQICIGYHANNSTEQVDTIMEKNVTVTTHAQDILEK

FLU_T3_HA_4 – first stem region nucleic acid sequence (SEQ ID NO:40)

ATGGAAAAGATTGTGCTGCTGCTGGCCATCGTGTCCCTGGTCAAGAGCGATCAAATCTGCATCGGCT
 ACCACGCCAACACAGCACCGAACAGGTGGACACCATTATGGAAAAGAACGTGACCGTGACACACGC
 CCAGGACATCCTGGAAAAG

20 FLU_T3_HA_4 – second stem region amino acid sequence (SEQ ID NO:41)

KYVKSRLVLATGLRNSPQRRERRRKRGLFGAIAGFIEGGWQGMVDGWYGYHHSNEQSGYAADKES
 TQKAIDGVTNKNVNSIIDKMNTQFEAVGRENNLERRIENLNKKMEDGFLDVWVTYNAELLVLMENERT
 LDFHDSNVKNLYDKVRLQLRDNAKELGNGCFEFYHKCDNECMESVRNGTYDYPQYSEEARLKREEIS
 GVKLESIGTYQILSIYSTVASSLALAIMVAGLSLWMCSNGSLQCRICI

25

The amino acid residues at positions 416 and 434 (or at positions 148 and 166 if counting from the beginning of the stem region) are shown underlined in the above sequence (and are F and F, respectively).

30 FLU_T3_HA_4 – second stem region nucleic acid sequence (SEQ ID NO:42)

AAATACGTGAAGTCCAACAGACTGGTCCTGGCCACCGGCCTGAGAAATTCTCCACAGAGAGAGCGGC
 GCAGAAAGAAGAGAGGCCTGTTTGGAGCCATTGCCGGCTTTATCGAAGGCGGCTGGCAAGGCATGGT
 TGACGGATGGTACGGCTATCACCACAGCAATGAGCAAGGCTCTGGCTACGCCGCCGACAAAGAGAGC
 35 ACACAGAAAGCCATCGACGGCGTGACCAACAAAGTGAATAGCATCATCGACAAGATGAACACCCAGT
 TCGAGGCCGTGGGCAGAGAGTTCAACAACCTGGAAAGACGGATCGAGAACCTGAACAAGAAGATGGA

GGACGGCTTCCTGGACGTGTGGACCTATAATGCCGAGCTGCTGGTCCTGATGGAAAACGAGAGAACC
 CTGGACTTCCACGACAGCAACGTGAAGAACCTGTACGACAAAGTGC GGCTCCAGCTGCGGGACAATG
 CCAAAGAACTCGGCAACGGCTGCTTCGAGTTCTACCACAAGTGC GACACGAGTGCATGGAAAGCGT
 GCGGAACGGCACCTACGACTACCCTCAGTACTCTGAGGAAGCCCGGCTGAAGAGAGAAGAGATCAGC
 5 GGAGTGAAGCTGGAATCCATCGGCACATAACCAGATCCTGAGCATCTACAGCACCGTGGCCTCTTCTC
 TGGCCCTGGCTATTATGGTGGCTGGCCTGAGCCTGTGGATGTGCTCTAATGGCAGCCTCCAGTGCCG
 GATCTGCATCTGA

Example 20 - FLU_T3_HA_5

10 This example provides amino acid sequences of the influenza haemagglutinin H5 head and
 stem regions for an embodiment of the invention known as FLU_T3_HA_5. In SEQ ID NO:43
 below, the amino acid residues of the stem region are shown underlined. The amino acid
 residues of the head region are the remaining residues. Similarly, in SEQ ID NO:44 below,
 the nucleic acid residues of the stem region are shown underlined. The nucleic acid residues
 15 of the head region are the remaining residues.

FLU_T3_HA_5 – HA0 amino acid sequence (SEQ ID NO:43):

MEKIVLLLAIVSLVKSDQICIGYHANNSTEQVDTIMEKNVTVTHAQDILEKTHNGKLCDLDGVKPLI
LRDCSVAGWLLGNPMCDEFINVEPSYIVEKANPANDLCYPGNFNDYEELKHLLSRINHFEKIQIIP
 20 KSSWSDHEASSGVSSACPYQGRSSFFRNVWLIKKNNAYPTIKRSYNNTNQEDLLVLWGIHHPNDAA
EQTRLYQNPTTYISVGTSTLNQRLVPKIATRSKVNGESGRMEFFWTILKPNDAINFESNGNFIAP
AYKIVKKGDSAIMKSELEYGNCNTKCQTPMGAINSSMPFHNIHPLTIGECPKYVKSNRLVLATGLRN
SPQRRERRRKKRGLFGAIAGFIEGGWQGMVDGWYGYHHSNEQGSGYAADKESTQKAIDGVTNKVNSII
DKMNTQFEAVGREFNNLERRIENLNKKMEDGFLDVWTYNAELLVLMENERTLDFHDSNVKNLYDKVR
 25 LQLRDNAKELGNGCFEFYHKCDNECMESVRNGTYDYPQYSEEARLKREEISGVKLESIGTYQILSIY
STVASSLALAIMVAGLSLWMCSNGLQCRICI

FLU_T3_HA_5 – HA0 nucleic acid sequence (SEQ ID NO:44)

ATGGAAAAGATTGTGCTGCTGCTGGCCATCGTGTCCCTGGTCAAGAGCGATCAAATCTGCATCGGCT
 30 ACCACGCCAACACAGCACCGAACAGGTGGACACCATTATGGAAAAGAACGTGACCGTGACACACGC
CCAGGACATCCTGGAAAAGACCCACAACGGCAAGCTGTGCGACCTGGATGGCGTGAAGCCTCTGATC
CTGAGAGATTGCTCTGTGGCCGGCTGGCTGCTGGGCAATCCTATGTGCGACGAGTTCATCAACGTGC
CCGAGTGGTCTATATCGTGGAAAAGGCCAATCCTGCCAACGACCTGTGCTACCCCGGCAACTTCAA
CGACTACGAGGAACTGAAACATCTGCTGAGCCGGATCAACCACTTCGAGAAGATCCAGATCATCCCC
 35 AAGTCCTCTTGGAGCGATCACGAGGCCTCTAGCGAGTGTCTAGCGCCTGTCCTTACCAAGGCAGAA
GCAGCTTCTTCCGGAACGTCTGTGGCTGATCAAGAAGAACAACGCTTACCCACCATCAAGCGGAG
CTACAACAACACCAATCAAGAGGACCTGCTGGTGTGCTGTGGGGCATCCACCATCCTAATGATGCCGCC

GAGCAGACCCGGCTGTACCAGAATCCTACAACCTACATCAGCGTGGGCACCAGCACACTGAACCAGA
 GACTGGTGCCTAAGATCGCCACCAGATCCAAAGTGAACGGCGAAAGCGGCCGGATGGAATTCTTCTG
 GACCATCCTGAAGCCTAACGACGCCATCAACTTCGAGAGCAACGGCAACTTTATCGCCCCTGAGTAC
 GCCTACAAGATCGTGAAGAAGGGCGACAGCGCCATCATGAAGTCCGAGCTGGAATACGGCAACTGCA
 5 ACACCAAGTGTGAGACCCCTATGGGCGCCATCAATAGCAGCATGCCCTTCCACAACATTCACCCTCT
 GACCATCGGCGAGTGCCCCAAATACGTGAAGTCCAACAGACTGGTCCTGGCCACCGGCCTGAGAAAT
 TCTCCACAGAGAGAGCGGCGCAGAAAGAAGAGAGGCCTGTTTGGAGCCATTGCCGGCTTTATCGAAG
 GCGGCTGGCAAGGCATGGTTGACGGATGGTACGGCTATCACCACAGCAATGAGCAAGGCTCTGGCTA
 CGCCGCCGACAAAGAGAGCACACAGAAAGCCATCGACGGCGTGACCAACAAAGTGAATAGCATCATC
 10 GACAAGATGAACACCCAGTTCGAGGCCGTGGGCAGAGAGTTCAACAACCTGGAAGACGGATCGAGA
 ACCTGAACAAGAAGATGGAGGACGGCTTCCTGGACGTGTGGACCTATAATGCCGAGCTGCTGGTCCT
 GATGGAAAACGAGAGAACCCTGGACTTCCACGACAGCAACGTGAAGAACCTGTACGACAAAGTGGCG
 CTCCAGCTGCGGGACAATGCCAAAGAACTCGGCAACGGCTGCTTCGAGTTCTACCACAAGTGGCACA
 ACGAGTGCATGGAAAGCGTGCGGAACGGCACCTACGACTACCCTCAGTACTCTGAGGAAGCCCGGCT
 15 GAAGAGAGAAGAGATCAGCGGAGTGAAGCTGGAATCCATCGGCACATACCAGATCCTGAGCATCTAC
 AGCACCCTGGCCTCTTCTCTGGCCCTGGCTATTATGGTGGCTGGCCTGAGCCTGTGGATGTGCTCTA
 ATGGCAGCCTCCAGTGCCGGATCTGCATCTGA

FLU_T3_HA_5 – head region amino acid sequence (SEQ ID NO:45)

20 THNGKLCDLGKPLILRDCSVAGWLLGNPMCDEFINVPESYIVEKANPANDLCYPGNFNDYEELK
 HLLSRINHFEDIQIIIPKSSWSDHEASSGVSSACPYQGRSSFFRNVVWLIKNNAYPTIKRSYNNNTNQ
 EDLLVLWGIHHPNDAAEQTELYQNPTTYISVGTSTLNQRLVPKIATRSKVNGESGRMEFFWTILKPN
 DAINFESNGNFIAPYAYKIVKKGDSAIMKSELEYGNCNTKCQTPMGAINSSMPFHNIHPLTIGECF

25 The amino acid residues at positions 156, 157, 171, 172, and 205 are highlighted in
 the above sequence (and are R, S, N, A, and R, respectively). The amino acid residues at
 positions 148, 149, and 238 are also highlighted, and are S, S, and E, respectively.

FLU_T3_HA_5 – head region nucleic acid sequence (SEQ ID NO:46)

ACCCACAAACGGCAAGCTGTGCGACCTGGATGGCGTGAAGCCTCTGATCCTGAGAGATTGCTCTGTGG
 30 CCGGCTGGCTGCTGGGCAATCCTATGTGCGACGAGTTCATCAACGTGCCCGAGTGGTCCTATATCGT
 GGAAAAGGCCAATCCTGCCAACGACCTGTGCTACCCCGGCAACTTCAACGACTACGAGGAACTGAAA
 CATCTGCTGAGCCGGATCAACCACTTCGAGAAGATCCAGATCATCCCCAAGTCCTCTTGAGCGATC
 ACGAGGCCTCTAGCGGAGTGTCTAGCGCCTGTCTTACCAAGGCAGAAGCAGCTTCTTCCGGAACGT
 CGTGTGGCTGATCAAGAAGAACAACGCTTACCCACCATCAAGCGGAGCTACAACAACACCAATCAA
 35 GAGGACCTGCTGGTGTGTGGGCATCCACCATCCTAATGATGCCGCCGAGCAGACCCGGCTGTACC
 AGAATCCTACAACCTACATCAGCGTGGGCACCAGCACACTGAACCAGAGACTGGTGCCTAAGATCGC
 CACCAGATCCAAAGTGAACGGCGAAAGCGGCCGGATGGAATTCTTCTGGACCATCCTGAAGCCTAAC

GACGCCATCAACTTCGAGAGCAACGGCAACTTTATCGCCCCTGAGTACGCCTACAAGATCGTGAAGA
 AGGGCGACAGCGCCATCATGAAGTCCGAGCTGGAATACGGCAACTGCAACACCAAGTGTGAGACCCC
 TATGGGCGCCATCAATAGCAGCATGCCCTTCCACAACATTACCCCTCTGACCATCGGCGAGTGCCCC

5 FLU_T3_HA_5 – first stem region amino acid sequence (SEQ ID NO:47)

MEKIVLLLAIVSLVKSDQICIGYHANNSTEQVDTIMEKNVTVTTHAQDILEK

FLU_T3_HA_5 – first stem region nucleic acid sequence (SEQ ID NO:48)

ATGGAAAAGATTGTGCTGCTGCTGGCCATCGTGTCCCTGGTCAAGAGCGATCAAATCTGCATCGGCT
 ACCACGCCAACACAGCACCGAACAGGTGGACACCATTATGGAAAAGAACGTGACCGTGACACACGC
 10 CCAGGACATCCTGAAAAG

FLU_T3_HA_5 – second stem region amino acid sequence (SEQ ID NO:49)

KYVKSRLVLATGLRNSPQRRERRRKKRGLFGAIAGFIEGGWQGMVDGWYGYHHSNEQSGSYAADKES
 TQKAIDGVTNKVNSIIDKMNTQFEAVGREENNLERRIENLNKKMEDGEELLDVWVTYNAELLVLMENERT
 LDFHDSNVKNLYDKVRLQLRDNAKELGNGCFEFYHKCDNECMESVRNGTYDYPQYSEEARLKREEIS
 15 GVKLESIGTYQILSIYSTVASSLALAIMVAGLSLWMCSNGSLQCRICI

The amino acid residues at positions 416 and 434 (or at positions 148 and 166 if counting from the beginning of the stem region) are shown underlined in the above sequence (and are F and F, respectively).

20

FLU_T3_HA_5 – second stem region nucleic acid sequence (SEQ ID NO:50)

AAATACGTGAAGTCCAACAGACTGGTCCTGGCCACCGGCCTGAGAAATTCTCCACAGAGAGAGCGGC
 GCAGAAAGAAGAGAGGCCTGTTTGGAGCCATTGCCGGCTTTATCGAAGGCGGCTGGCAAGGCATGGT
 25 TGACGGATGGTACGGCTATCACCACAGCAATGAGCAAGGCTCTGGCTACGCCGCCGACAAAGAGAGC
 ACACAGAAAGCCATCGACGGCGTGACCAACAAAGTGAATAGCATCATCGACAAGATGAACACCCAGT
 TCGAGGCCGTGGGCAGAGAGTTCAACAACCTGGAAAGACGGATCGAGAACCTGAACAAGAAGATGGA
 GGACGGCTTCCTGGACGTGTGGACCTATAATGCCGAGCTGCTGGTCCTGATGAAAACGAGAGAACC
 CTGGACTTCCACGACAGCAACGTGAAGAACCTGTACGACAAAGTGCGGCTCCAGCTGCGGGACAATG
 30 CCAAAGAACTCGGCAACGGCTGCTTCGAGTTCTACCACAAGTGCGACAACGAGTGCATGGAAAGCGT
 GCGGAACGGCACCTACGACTACCCTCAGTACTCTGAGGAAGCCCGGCTGAAGAGAGAAGAGATCAGC
 GGAGTGAAGCTGGAATCCATCGGCACATACCAGATCCTGAGCATCTACAGCACCGTGCCCTCTTCTC
 TGGCCCTGGCTATTATGGTGGCTGGCCTGAGCCTGTGGATGTGCTCTAATGGCAGCCTCCAGTGCCG
 GATCTGCATCTGA

35

Example 21 - FLU_T3_HA_1

This example provides amino acid and nucleic acid sequences of the influenza haemagglutinin H5 stem regions for an embodiment of the invention known as FLU_T3_HA_1. Example 4 above provides the amino acid and nucleic acid sequences for the composite stem region of FLU_T3_HA_1, however the stem regions are separated by a head region. This example also provides the nucleic acid sequence of the H5 head and stem regions, with the stem regions underlined.

FLU_T3_HA_1 – first stem region amino acid sequence (SEQ ID NO:51)

MEKIVLLLAIVSLVKSDQICIGYHANNSTEQVDTIMEKNVTVTTHAQDILEK

10 FLU_T3_HA_1 – first stem region nucleic acid sequence (SEQ ID NO:52)

ATGGAAAAGATCGTGCTGCTGCTGGCCATCGTGTCCCTGGTCAAGAGCGACCAAATCTGCATCGGCT
ACCACGCCAACACAGCACCGAACAGGTGGACACCATTATGGAAAAGAACGTCACCGTGACACACGC
CCAGGACATCCTGGAAAAG

15 FLU_T3_HA_1 – second stem region amino acid sequence (SEQ ID NO:53)

KYVKS NRLVLATGLRNSPQRRERRRKRGLFGAIAGFIEGGWQGMVDGWYGYHHSNEQSGYAADKES
TQKAIDGVTNKVNSIIDKMNTQFEAVGREFNNLERRIENLNKKMEDGFLDVWVTYNAELLVLMENERT
LDFHDSNVKNLYDKVRLQLRDNAKELGNGCFEFYHKCDNECMESVRNGTYDYPQYSEEARLKREEIS
GVKLESIGTYQILSIYSTVASSLALAIMVAGLSLWMCSNGLQCRICI

20

FLU_T3_HA_1 – second stem region nucleic acid sequence (SEQ ID NO:54)

AAATACGTGAAGTCCAACAGACTGGTCCTGGCCACCGGCCTGAGAACTCTCCCCAGCGCGAGCGGA
GAAGAAAGAAGAGAGGCCTGTTTGGCGCCATTGCCGGCTTTATCGAAGGCGGCTGGCAAGGCATGGT
GGACGGATGGTACGGCTATCACCACAGCAACGAGCAAGGCTCTGGATACGCCGCCGACAAAGAGAGC
25 ACCCAGAAAGCCATTGACGGCGTGACCAACAAAGTCAACAGCATCATCGACAAGATGAACACCCAGT
TCGAGGCCGTGGGCAGAGAGTTCAACAACCTGGAACGGCGGATCGAGAACCTGAACAAGAAGATGGA
GGACGGCTTCCTGGACGTGTGGACCTACAATGCCGAGCTGCTGGTCCTGATGGAAAACGAGAGAACC
CTGGACTTCCACGACAGCAACGTGAAGAACCTGTACGACAAAGTGCGGCTCCAGCTGCGGGACAACG
CCAAAGAACTCGGCAACGGCTGCTTCGAGTTCTACCACAAGTGCGACAACGAGTGCATGGAAAGCGT
30 GCGGAACGGCACCTACGACTACCCTCAGTACAGCGAGGAAGCCCGGCTGAAGAGGGAAGAGATCAGC
GGAGTGAAGCTGGAATCCATCGGCACATACCAGATCCTGAGCATCTACAGCACCGTGCCCTCTTCTC
TGGCCCTGGCCATTATGGTGGCTGGCCTGTCTCTGTGGATGTGCAGCAATGGCAGCCTCCAGTGCCG
GATCTGCATCTGA

35 FLU_T3_HA_1 – HA0 nucleic acid sequence (SEQ ID NO:55)

ATGGAAAAGATCGTGCTGCTGCTGGCCATCGTGTCCCTGGTCAAGAGCGACCAAATCTGCATCGGCT
ACCACGCCAACACAGCACCGAACAGGTGGACACCATTATGGAAAAGAACGTCACCGTGACACACGC
CCAGGACATCCTGGAAAAGACCCACAACGGCAAGCTGTGCGACCTGGATGGCGTGAAGCCTCTGATC

CTGAGAGATTGCTCTGTGGCCGGATGGCTGCTGGGCAATCCCATGTGCGACGAGTTCATCAACGTGC
 CCGAGTGGTCTTATATCGTGGAAGAGGCAATCCTGCCAACGACCTGTGCTACCCCGGCAACTTCAA
 CGACTACGAGGAACTGAAGCACCTCCTGAGCCGGATCAACCACTTCGAGAAGATCCAGATCATCCCC
 AAGTCCTCTTGGAGCGACCACGAAGCCTCTAGCGGAGTGTCTAGCGCCTGTCCTTACCAAGGCAGAC
 5 CCAGCTTCTTCCGGAACGTCTGTGGCTGATCAAGAAGAAGACACATACCCACCATCAAGCGGAG
 CTACAACAACACCAATCAAGAGGACCTGCTGGTGCTGTGGGGCATCCACCATCCTAATGATGCCGCC
 GAGCAGACCAAGCTGTATCAGAACCCACCACCTACATCAGCGTGGGCACCAGCACACTGAACCAGA
 GACTGGTGCCCTAAGATCGCCACCAGATCCAAAGTGAACGGCCAGAGCGGCAGAATGGAATTCTTCTG
 GACCATCCTGAAGCCTAACGACGCCATCAACTTCGAGAGCAACGGCAACTTTATCGCCCCTGAGTAC
 10 GCCTACAAGATCGTGAAGAAGGGCGACAGCGCCATCATGAAGTCCGAGCTGGAATACGGCAACTGCA
 ACACCAAGTGTGAGACCCCTATGGGCGCCATCAATAGCAGCATGCCCTTCCACAACATTACCCCTCT
 GACCATCGGCGAGTGCCCCAAATACGTGAAGTCCAACAGACTGGTCCTGGCCACCGGCCTGAGAAAC
TCTCCCCAGCGGAGCGGAGAAGAAAGAAGAGAGGCCTGTTTGGCGCCATTGCCGGCTTTATCGAAG
GCGGCTGGCAAGGCATGGTGGACGGATGGTACGGCTATCACCACAGCAACGAGCAAGGCTCTGGATA
 15 CGCCGCCGACAAAGAGAGCACCCAGAAAGCCATTGACGGCGTGACCAACAAAGTCAACAGCATCATC
GACAAGATGAACACCCAGTTCGAGGCGGTGGGCAGAGAGTTCAACAACCTGGAACGGCGGATCGAGA
ACCTGAACAAGAAGATGGAGGACGGCTTCCTGGACGTGTGGACCTACAATGCCGAGCTGCTGGTCCT
GATGGAAAACGAGAGAACCCTGGACTTCACGACAGCAACGTGAAGAACCTGTACGACAAAGTGCGG
CTCCAGCTGCGGGACAACGCCAAAGAACTCGGCAACGGCTGCTTCGAGTTCTACCACAAGTGCGACA
 20 ACGAGTGCATGGAAAGCGTGCGGAACGGCACCTACGACTACCCTCAGTACAGCGAGGAAGCCCGGCT
GAAGAGGGGAAGAGATCAGCGGAGTGAAGCTGGAATCCATCGGCACATACCAGATCCTGAGCATCTAC
AGCACCGTGGCCTCTTCTCTGGCCCTGGCCATTATGGTGGCTGGCCTGTCTCTGTGGATGTGCAGCA
ATGGCAGCCTCCAGTGCCGGATCTGCATCTGA

25 FLU_T3_HA_1 – head region nucleic acid sequence (SEQ ID NO:56)

ACCCACAACGGCAAGCTGTGCGACCTGGATGGCGTGAAGCCTCTGATCCTGAGAGATTGCTCTGTGG
 CCGGATGGCTGCTGGGCAATCCCATGTGCGACGAGTTCATCAACGTGCCCGAGTGGTCTTATATCGT
 GGAAAAGGCCAATCCTGCCAACGACCTGTGCTACCCCGGCAACTTCAACGACTACGAGGAACTGAAG
 CACCTCCTGAGCCGGATCAACCACTTCGAGAAGATCCAGATCATCCCCAAGTCCTCTTGGAGCGAC
 30 CACGAAGCCTCTAGCGGAGTGTCTAGCGCCTGTCCTTACCAAGGCAGACCCAGCTTCTTCCGGAACG
 TCGTGTGGCTGATCAAGAAGAAGACACATACCCACCATCAAGCGGAGCTACAACAACACCAATCA
 AGAGGACCTGCTGGTGCTGTGGGGCATCCACCATCCTAATGATGCCGCCGAGCAGACCAAGCTGTAT
 CAGAACCCACCACCTACATCAGCGTGGGCACCAGCACACTGAACCAGAGACTGGTGCCCTAAGATCG
 CCACCAGATCCAAAGTGAACGGCCAGAGCGGCAGAATGGAATTCTTCTGGACCATCCTGAAGCCTAA
 35 CGACGCCATCAACTTCGAGAGCAACGGCAACTTTATCGCCCCTGAGTACGCCTACAAGATCGTGAAG
 AAGGGCGACAGCGCCATCATGAAGTCCGAGCTGGAATACGGCAACTGCAACACCAAGTGTGAGACCC
 CTATGGGCGCCATCAATAGCAGCATGCCCTTCCACAACATTACCCCTCTGACCATCGGCGAGTGCCC
 C

Example 22 - FLU_T3_HA_2

This example provides amino acid and nucleic acid sequences of the influenza haemagglutinin H5 stem regions for an embodiment of the invention known as
 5 FLU_T3_HA_2. Example 5 above provides the amino acid and nucleic acid sequences for the composite stem region of FLU_T3_HA_2, however the stem regions are separated by a head region. This example also provides the nucleic acid sequence of the H5 head and stem regions, with the stem regions underlined.

FLU_T3_HA_2 – first stem region amino acid sequence (SEQ ID NO:57)

10 MEKIVLLLAIVSLVKSDQICIGYHANNSTEQVDTIMEKNVTVTTHAQDILEK

FLU_T3_HA_2 – first stem region nucleic acid sequence (SEQ ID NO:58)

ATGGAAAAGATCGTGCTGCTGCTGGCCATCGTGTCCCTGGTCAAGAGCGACCAAATCTGCATCGGCT
 ACCACGCCAACACAGCACCGAACAGGTGGACACCATTATGGAAAAGAACGTCACCGTGACACACGC
 15 CCAGGACATCCTGGAAAAG

FLU_T3_HA_2 – second stem region amino acid sequence (SEQ ID NO:59)

KYVKSNRLVLATGLRNSPQRRERRRKKRGLFGAIAGFIEGGWQGMVDGWYGYHHSNEQSGSYAADKES
 20 TQKAIDGVTNKNVNSIIDKMNTQFEAVGREFNNLERRIENLNKKMEDGFLDVWVTYNAELLVLMENERT
 LDFHDSNVKNLYDKVRLQLRDNAKELGNGCFEFYHKCDNECMESVRNGTYDYPQYSEEARLKREEIS
 GVKLESIGTYQILSIYSTVASSLALAIMVAGLSLWMCSNGSLQCRICI

FLU_T3_HA_2 – second stem region nucleic acid sequence (SEQ ID NO:60)

25 AAATACGTGAAGTCCAACAGACTGGTCCTGGCCACCGGCCTGAGAACTCTCCCCAGCGCGAGCGGA
 GAAGAAAGAAGAGAGGCCTGTTTGGCGCCATTGCCGGCTTTATCGAAGCGGGCTGGCAAGGCATGGT
 GGACGGATGGTACGGCTATCACCACAGCAACGAGCAAGGCTCTGGATACGCCGCCGACAAAGAGAGC
 ACCCAGAAAGCCATTGACGGCGTGACCAACAAAGTCAACAGCATCATCGACAAGATGAACACCCAGT
 TCGAGGCCGTGGGCAGAGAGTTCAACAACCTGGAACGGCGGATCGAGAACCTGAACAAGAAGATGGA
 30 GGACGGCTTCTTGGACGTGTGGACCTACAATGCCGAGCTGCTGGTCCTGATGGAAAACGAGAGAACC
 CTGGACTTCCACGACAGCAACGTGAAGAACCTGTACGACAAAGTGCGGCTCCAGCTGCGGGACAACG
 CCAAAGAACTCGGCAACGGCTGCTTCGAGTTCTACCACAAGTGCGACAACGAGTGATGGAAAGCGT
 GCGGAACGGCACCTACGACTACCCTCAGTACAGCGAGGAAGCCCGGCTGAAGAGGGAAGAGATCAGC
 GGAGTGAAGCTGGAATCCATCGGCACATACCAGATCCTGAGCATCTACAGCACCGTGCCCTCTTCTC
 35 TGGCCCTGGCCATTATGGTGGCTGGCCTGTCTCTGTGGATGTGCAGCAATGGCAGCCTCCAGTGCCG
 GATCTGCATCTGA

FLU_T3_HA_2 – HA0 nucleic acid sequence (SEQ ID NO:61)

ATGGAAGATCGTGCTGCTGCTGGCCATCGTGTCCCTGGTCAAGAGCGACCAAATCTGCATCGGCT
ACCACGCCAACACAGCACCGAACAGGTGGACACCATTATGGAAAAGAACGTACCCGTGACACACGC
CCAGGACATCCTGGAAAAGACCCACAACGGCAAGCTGTGCGACCTGGATGGCGTGAAGCCTCTGATC
 CTGAGAGATTGCTCTGTGGCCGGATGGCTGCTGGGCAATCCCATGTGCGACGAGTTCATCAACGTGC
 5 CCGAGTGGTCTATATCGTGGAAAAGGCCAATCCTGCCAACGACCTGTGCTACCCCGGCAACTTCAA
 CGACTACGAGGAACTGAAGCACCTCCTGAGCCGGATCAACCACTTCGAGAAGATCCAGATCATCCCC
 AAGTCCTCTTGGAGCGACCACGAAGCCTCTAGCGGAGTGTCTAGCGCCTGTCCTTACCAAGGCAGAC
 CCAGCTTCTTCCGGAACGTCTGTGTGGCTGATCAAGAAGAACAACACATACCCACCATCAAGCGGAG
 CTACAACAACACCAATCAAGAGGACCTGCTGGTGTGTGGGGCATCCACCATCCTAATGATGCCGCC
 10 GAGCAGACCAAGCTGTATCAGAACCCACACCTACATCAGCGTGGGCACCAGCACACTGAACCAGA
 GACTGGTGCCTAAGATCGCCACCAGATCCAAAGTGAACGGCCAGAGCGGCAGAATGGAATTCTTCTG
 GACCATCCTGAAGCCTAACGACGCCATCAACTTCGAGAGCAACGGCAACTTTATCGCCCCTGAGTAC
 GCCTACAAGATCGTGAAGAAGGGCGACAGCGCCATCATGAAGTCCGAGCTGGAATACGGCAACTGCA
 ACACCAAGTGTGAGACCCCTATGGGCGCCATCAATAGCAGCATGCCCTTCCACAACATTCACCCTCT
 15 GACCATCGGCGAGTGCCCCAAATACGTGAAGTCCAACAGACTGGTCCTGGCCACCGGCCTGAGAAAC
TCTCCCCAGCGCGAGCGGAGAAGAAAGAGAGGCCTGTTTGGCGCCATTGCCGGCTTTATCGAAG
GCGGCTGGCAAGGCATGGTGGACGGATGGTACGGCTATCACCACAGCAACGAGCAAGGCTCTGGATA
CGCCGCCGACAAAGAGAGCACCCAGAAAGCCATTGACGGCGTGACCAACAAAGTCAACAGCATCATC
GACAAGATGAACACCCAGTTCGAGGCCGTGGGCAGAGAGTTCAACAACCTGGAACGGCGGATCGAGA
 20 ACCTGAACAAGAAGATGGAGGACGGCTTCCTGGACGTGTGGACCTACAATGCCGAGCTGCTGGTCCT
GATGGAAAACGAGAGAACCCTGGACTTCCACGACAGCAACGTGAAGAACCTGTACGACAAAGTGCGG
CTCCAGCTGCGGGACAACGCCAAAGAACTCGGCAACGGCTGCTTCGAGTTCTACCACAAGTGCGACA
ACGAGTGCATGGAAAGCGTGCGGAACGGCACCTACGACTACCCTCAGTACAGCGAGGAAGCCCGGCT
GAAGAGGGAAGAGATCAGCGGAGTGAAGCTGGAATCCATCGGCACATACCAGATCCTGAGCATCTAC
 25 AGCACCGTGGCCTCTTCTCTGGCCCTGGCCATTATGGTGGCTGGCCTGTCTCTGTGGATGTGCAGCA
ATGGCAGCCTCCAGTGCCGGATCTGCATCTGA

FLU_T3_HA_2 – head region nucleic acid sequence (SEQ ID NO:62)

30 ACCACAACGGCAAGCTGTGCGACCTGGATGGCGTGAAGCCTCTGATCCTGAGAGATTGCTCTGTGG
 CCGGATGGCTGCTGGGCAATCCCATGTGCGACGAGTTCATCAACGTGCCCGAGTGGTCTATATCGT
 GGAAAAGGCCAATCCTGCCAACGACCTGTGCTACCCCGGCAACTTCAACGACTACGAGGAACTGAAG
 CACCTCCTGAGCCGGATCAACCACTTCGAGAAGATCCAGATCATCCCCAAGTCCTCTTGGAGCGACC
 ACGAAGCCTCTAGCGGAGTGTCTAGCGCCTGTCCTTACCAAGGCAGACCCAGCTTCTTCCGGAACGT
 35 CGTGTGGCTGATCAAGAAGAACAACACATACCCACCATCAAGCGGAGCTACAACAACACCAATCAA
 GAGGACCTGCTGGTGTGTGGGGCATCCACCATCCTAATGATGCCGCCGAGCAGACCAAGCTGTATC
 AGAACCCACACCTACATCAGCGTGGGCACCAGCACACTGAACCAGAGACTGGTGCCCTAAGATCGC
 CACCAGATCCAAAGTGAACGGCCAGAGCGGCAGAATGGAATTCTTCTGGACCATCCTGAAGCCTAAC
 GACGCCATCAACTTCGAGAGCAACGGCAACTTTATCGCCCCTGAGTACGCCTACAAGATCGTGAAGA

AGGGCGACAGCGCCATCATGAAGTCCGAGCTGGAATACGGCAACTGCAACACCAAGTGTGAGACCCC
TATGGGCGCCATCAATAGCAGCATGCCCTTCCACAACATTACCCCTCTGACCATCGGCGAGTGCCCC

Example 23 – Residue differences in amino acid sequence of influenza Tier 3 H5 vaccine candidates FLU_T3_HA_1 to FLU_T3_HA_5, and influenza Tier 2 H5 design FLU_T2_HA_1

Figure 21 summarises differences in amino acid sequence of the influenza haemagglutinin H5 for different embodiments of the invention, including differences at positions A-E of H5 for the embodiments. Positions A, B, and C of H5 are at epitope regions in the head region, and the mutations shown in the figure at these positions increase the affinity of H5 towards binding antibodies. Positions D and E are in the H5 stem region, and the mutations at these positions increase the stability of the stem region both in the pre-fusion and post-fusion state. The amino acid residue mutations at positions 148, 149, and 238 of FLU_T3_HA_4, and at position 238 of FLU_T3_HA_5, are at receptor binding sites. These residue mutations reduce the affinity of HA to its receptor (sialic acid) on the surface of target cells, thus increasing the bioavailability of HA for antigen presentation.

Figure 22 shows a multiple sequence alignment of HA amino acid sequence for FLU_T2_HA_1, FLU_T3_HA_1 to FLU_T3_HA_5, and two influenza isolates H5_WSN (SEQ ID NO:64) and H5_GYR (SEQ ID NO:65). In the figure, differences in amino acid residues are shown underlined, with amino acid differences across designed sequences FLU_T2_HA_1 and FLU_T3_HA_1/2/3/4/5 shown highlighted. The amino acid residues at positions A, B, and C of the head region, and D and E of the stem region, are shown in boxes. These amino acid residues are at residue positions 156, 157, 171, 172, and 205 of the head region, and at residue positions 416 and 434 of the stem region.

>A/WSN/Mongolia/244/2005 (SEQ ID NO:64)

Amino acid sequence

MEKIVLLLAIVSLVKSDQICIGYHANNSTEQVDTIMEKNVTVTTHAQDILEKTHNGKLCDL
DGVKPLILRDCSVAGWLLGNPMCDEFLNVPEWSYIVEKINPANDLCYPGNFNDYEELKHL
LSRINHFEKIQIIPKSSWSDHEASSGVSSACPYQGRSSFFRNVVWLIKKNAYPTIKRSY
NNTNQEDLLVLWGIHHPNDAAEQTRLYQNPTTYISVGTSTLNQRLVPKIATRISKVNGQSG
RMEFFWTILKPNDAINFESNGNFIAPENAYKIVKKG DSTIMKSELEYGNCNTKCQTPIGA
INSSMPFHNHPLTIGEC PKYVKS NRLVLATGLRNSPQGE-RRRRKRGLFGAIAGFIEGG
WQGMVDGWYGYHHSNEQGS GYAADKESTQKAIDGVTNKVNSIIDKMNTQFEAVGREFNNL
ERRIENLNKKMEDGFLDVWTYNAELLVLMENERTLDFHDSNVKNLYDKVRLQLRDN AKEL
GNGCFEFYHRCNECMESVRNGTYDYPQYSEEARLKREEISGVKLESIGTYQILSIYSTV
ASSLALAIMVAGLSLWMC SNGSLQCRICI

>A/gyrfalcon/Washington/41088-6/2014_H5N8(FLU_T1_HA_9; SEQ ID NO:65))

Amino acid sequence

MEKIVLLLVAVISLVKSDQICIGYHANNSTKQVDTIMEKNVTVTTHAQDILEKTHNGKLCDL
 5 NGVKPLILKDCSVAGWLLGNPMCDEFIRVPEWSYIVERANPANDLCYPGTLNDYEELKHL
 LSRINHFEKTLIIPRSSWPNHETSLGVSAACPYQGASSFFRNVVWLIKNDAYPTIKISY
 NNTNREDLLILWGIHHSNNAEQTNLYKNPDTYVSVGTSTLNQRLVPKIATRSQVNGQSG
 RMDFFWTILKPNDAIHFESNGNFIAPYAYKIVKKG DSTIMKSEMEYGH CNTKCQTPIGA
 INSSMPFHNHPLTIGECPKYVKS NKLVLATGLRNSPLRERRRKRGLFGAIAGFIEGGWQ
 10 GMVDGWYGYHHSNEQSGSYAADKESTQKAIDGVTNKVNSIIDKMNTQFEAVGREFNNLER
 RIENLNKKMEDGFLDVWTYNAELLVLMENERTLDFHDSNVKNLYDKVRLQLRDN AKELGN
 GCFEFYHKCDNECMESVRNGTYDYPKYSEEAILKREEISGVKLESIGTYQILSIYSTVAS
 SLALAIIVAGLSLWMCSNGSLQCRICI

Example 24 – Iteratively designed H5Nx antigens generate broad immune response in mice

Background

Yearly outbreak of avian influenza (H5Nx) has a huge socio-economic impact worldwide. In addition, there is a constant threat of spill overs to naïve human population leading to a pandemic. Constant antigenic drift in the surface glycoprotein of influenza – hemagglutinin, and recombination with different neuraminidase subtypes add a complex dimension to design of universal H5 influenza vaccine antigens that can provide broad protection to H5Nx. Here we discuss our H5Nx antigen designs that has been iteratively optimised to increase the coverage of H5Nx.

Methods

Available H5Nx sequences from NCBI virus database were downloaded, cleaned, and trimmed to generate a non-redundant dataset of H5 sequences. Phylogenetic relationship between these sequences were estimated and a phylogenetically optimised sequence was designed as our first vaccine candidate FLU_T2_HA_1 (referred to as DIOS-T2_HA_9 in Figure 23). Immunogenicity of the vaccine design was confirmed in Balb/c mice. Mice sera were tested for neutralisation using pseudotype neutralisation assays against multiple H5 viruses. Based on these results, FLU_T2_HA_1 was further optimised to generate a panel of next tier vaccine designs FLU_T3_HA_1/2/3/4/5 (referred to as DIOS-T3_HA_1/2/3/4/5 in Figure 23) using epitope optimisation to achieve broad neutralisation.

Results

Our vaccine candidate FLU_T2_HA_1 generates better immune response in comparison to the wild-type control (A/whooper swan/Mongolia/244/2005) against different H5Nx, except a

few H5Nx, where both our design and the WT do not show robust neutralisation. We further improved FLU_T2_HA_1 to broaden the immune response to H5Nx clades missed earlier. The improved designs (FLU_T3_HA_1/2/3/4/5) showed a better neutralisation profile against a panel of 9 antigenically different H5Nx (Figure 23). American non-goose Guangdong (Am-
 5 nonGsGd) is a low pathogenic lineage of H5Nx viruses.

Conclusion

A promising panel of platform independent vaccine candidates that can provide a broader protection against multiple antigenically different H5Nx. The vaccine candidate can be a useful tool in keeping in check the recurrent yearly avian influenza outbreak and
 10 preparedness of future spill over into human population.

Example 25 - FLU_T2_HA_4

This example provides the amino acid and nucleic acid sequences of the influenza H1 region for an embodiment of the invention known as FLU_T2_HA_4.

FLU_T2_HA_4 – *amino acid sequence (SEQ ID NO:68):*

15 MKVKLLVLLCTFTATYADTICIGYHANNSTDTVDTVLEKNVTVTHSVNLLLED SHNGKLCLLKGIAPL
 QLGNC SVAGWILGNPECELLISKESWSYIVETPNPENGTCYPGYFADYEELREQLSSVSSFERFEIF
 PKESSWPNHTVTSGVSASCSHNGKSSFYRNLLWLTGKNGLYPNLSKSYANNKEKEVLVLWGVHHPN
 IGDQRALYHTENAYVSVVSSHYSRRFTPEIAKRPKVRDQEGRINYWTLLPEGDTIIFEANGNLIAP
 RYAFALSRGFGSGIINSNAPMDECDAKCQTPQGAINSSLPFQNVHPVTIGECPKYVRS AKLRMVTGL
 20 RNIPSIQSRGLFGAIAGFIEGGWTGMVDGWYGYHHQNEQSGYAADQKSTQNAINGITNKVNSVIEK
 MNTQFTAVGKEFNKLERRMENLNKKVDDGFIDIWTYNAELLVLL ENERTLDFHDSNVKNLYEKVKSQ
 LKNNAKEIGNGCFEFYHKCNDECMESVKNGTYDYPKYSEESKLNREKIDGVKLESMGVYQILAIYST
 VASSLVLLVSLGAISFWMCSNGSLQCRICI

FLU_T2_HA_4 – *nucleic acid sequence (SEQ ID NO:69)*

25 ATGAAGGTCAAAC TGGTGCTGCTGTGCACCTTACCGCCACATACGCCGATACCATCTGTATCG
 GCTACCACGCCAACACAGCACCGACACCGTGGATACCGTGTGGAAAAGAACGTGACCGTGACACA
 CAGCGTGAACCTGCTGGAAGATAGCCACAACGGCAAGCTGTGCCTGCTGAAGGGAATTGCCCTCTC
 CAGCTGGGAAATTGCTCTGTGGCTGGCTGGATCCTGGGCAATCCTGAGTGCGAGCTGCTGATCTCCA
 AAGAGAGCTGGTCCTACATCGTCGAGACACCCAATCCAGAGAACGGCACATGCTACCCCGGCTACTT
 30 CGCCGACTATGAGGA ACTGAGAGAGCAGCTGAGCAGCGTCAGCAGCTTCGAGAGATTCGAGATTTTC
 CCCAAAGAGTCCAGCTGGCCCAACCACACAGTGACAAGCGGAGTGTCTGCCAGCTGTTCCCACAATG
 GCAAGAGCAGCTTCTACAGAAACCTGCTGTGGCTGACCGGCAAGAACGGACTGTACCCCAACCTGAG
 CAAGAGCTACGCTAACAACAAAGAGAAAGAGGTCCTGGTCCTCTGGGGCGTGACCATCCTCCAAAT
 ATCGGAGATCAGAGAGCCCTGTACCACACCGAGAATGCCTACGTGTCCGTGGTGTCCAGCCACTACA

GCAGAAGATTACCCCTGAGATCGCCAAGCGGCCCAAAGTGCGAGATCAAGAGGGCAGAATCAACTA
 CTACTGGACACTGCTGGAACCCGGCGACACCATCATCTTCGAGGCCAACGGAAACCTGATCGCCCCCT
 AGATACGCCTTCGCTCTGAGCAGAGGCTTTGGCAGCGGCATCATCAACAGCAACGCCCTTATGGATG
 AGTGCGACGCCAAGTGTCAAACACCCCAGGGCGCTATCAACAGCTCCCTGCCTTTTCAGAACGTGCA
 5 CCCTGTGACCATCGGCGAGTGTCTTAAATATGTGCGGAGCGCCAAGCTGAGAATGGTCACCGGCCTG
 AGAAACATCCCCAGCATCCAGTCTAGAGGCCTGTTTGGCGCCATTGCCGGCTTTATCGAAGGCGGAT
 GGACAGGCATGGTGGACGGATGGTACGGCTATCACCACCAGAATGAGCAAGGCAGCGGCTACGCCGC
 CGATCAGAAATCTACCCAGAACGCCATCAACGGGATCACCAACAAAGTGAACAGCGTGATCGAGAAG
 ATGAACACCCAGTTTACCGCCGTGGGCAAAGAGTTCAACAAGCTGGAAAGACGGATGGAAAACCTGA
 10 ACAAGAAGGTGGACGACGGCTTCATCGACATCTGGACCTACAACGCTGAGCTGCTGGTCTCTGGA
 AAACGAGAGAACCCTGGACTTCCACGACAGCAACGTGAAGAACCTGTACGAGAAAGTGAAGTCCCAG
 CTGAAGAACAACGCCAAAGAGATCGGCAACGGCTGCTTCGAGTTCTACCACAAGTGCAACGACGAGT
 GCATGGAAAGCGTGAAGAATGGCACCTACGACTACCCCAAGTACAGCGAGGAAAGCAAGCTGAACCG
 CGAGAAGATCGACGGCGTGAAGCTGGAATCTATGGGCGTGTACCAGATCCTGGCCATCTACAGCACA
 15 GTGGCTTCTAGCCTGGTGCTCCTGGTGTCTCTGGGAGCCATCAGCTTTTGGATGTGCAGCAATGGCA
 GCCTCCAGTGCCGGATCTGCATC

Example 26 – pEVAC-FLU_T2_HA_4

This example provides the nucleic acid sequence of pEVAC-FLU_T2_HA_4.

20 pEVAC-FLU_T2_HA_4 – *nucleic acid sequence (SEQ ID NO:70)*:

LOCUS	17ADKK4C_I-3_pVRC8400EVAC_Ar	6083 bp	DNA
circular			
FEATURES	Location/Qualifiers		
25	promoter	complement(5925..5953) /label="AmpR_promoter"	
	promoter	868..987 /label="CMV2_promoter"	
	CDS	complement(4963..5778) /label="Kana(R)"	
30	rep_origin	complement(3818..4437) /label="pBR322_origin"	
	primer	complement(29..51) /label="pGEX_3_primer"	
	primer	855..875 /label="CMV_fwd_primer"	
35	primer	899..918 /label="pCEP_fwd_primer"	
	primer	901..925 /label="LNCX_primer"	
40	polyA_site	3071..3295 /label="BGH\pA"	
	promoter	394..904 /label="CMV_Promoter"	
	CDS	1343..3063	

/label="I-3"

ORIGIN							
5	1	TCGCGCGTTT	CGGTGATGAC	GGTGAAAACC	TCTGACACAT	GCAGCTCCCG	GAGACGGTCA
	61	CAGCTTGTCT	GTAAGCGGAT	GCCGGGAGCA	GACAAGCCCG	TCAGGGCGCG	TCAGCGGGTG
	121	TTGGCGGGTG	TCGGGGCTGG	CTTAACATATG	CGGCATCAGA	GCAGATTGTA	CTGAGAGTGC
	181	ACCATATGCG	GTGTGAAATA	CCGCACAGAT	GCGTAAGGAG	AAAATACCGC	ATCAGATTGG
10	241	CTATTGGCCA	TTGCATACGT	TGTATCCATA	TCATAATATG	TACATTTATA	TTGGCTCATG
	301	TCCAACATTA	CCGCCATGTT	GACATTGATT	ATTGACTAGT	TATTAATAGT	AATCAATTAC
	361	GGGGTCATTA	GTTTCATAGCC	CATATATGGA	GTTCCGCGTT	ACATAACTTA	CGGTAAATGG
	421	CCCGCCTGGC	TGACCGCCCA	ACGACCCCGG	CCCATTGACG	TCAATAATGA	CGTATGTTCC
15	481	CATAGTAACG	CCAATAGGGA	CTTTCCATTG	ACGTCAATGG	GTGGAGTATT	TACGGTAAAC
	541	TGCCCACCTG	GCAGTACATC	AAGTGTATCA	TATGCCAAGT	ACGCCCCCTA	TTGACGTCAA
	601	TGACGGTAAA	TGGCCCGCCT	GGCATTATGC	CCAGTACATG	ACCTTATGGG	ACTTTCCTAC
	661	TTGGCAGTAC	ATCTACGTAT	TAGTCATCGC	TATTACCATG	GTGATGCGGT	TTTGGCAGTA
20	721	CATCAATGGG	CGTGGATAGC	GGTTTGACTC	ACGGGGATT	CCAAGTCTCC	ACCCCATGTA
	781	CGTCAATGGG	AGTTTGTTTT	GGCACCAAAA	TCAACGGGAC	TTTCCAAAAT	GTCGTAACAA
	841	CTCCGCCCCA	TTGACGCAAA	TGGGCGGTAG	GCGTGTACGG	TGGGAGGTCT	ATATAAGCAG
	901	AGCTCGTTTA	GTGAACCGTC	AGATCGCCTG	GAGACGCCAT	CCACGCTGTT	TTGACCTCCA
25	961	TAGAACGAC	CGGGACCGAT	CCAGCCTCCA	TCGGCTCGCA	TCTCTCCTTC	ACGCGCCCGC
	1021	CGCCCTACCT	GAGGCCGCCA	TCCACGCCGG	TTGAGTCGCG	TTCTGCCGCC	TCCCGCCTGT
	1081	GGTGCCTCCT	GAAGTGCCTC	CGCCGTCTAG	GTAAGTTTAA	AGCTCAGGTC	GAGACCGGGC
	1141	CTTTGTCCGG	CGCTCCCTTG	GAGCCTACCT	AGACTCAGCC	GGCTCTCCAC	GCTTTGCCTG
30	1201	ACCCTGCTTG	CTCAACTCTA	GTTAACGGTG	GAGGGCAGTG	TAGTCTGAGC	AGTACTCGTT
	1261	GCTGCCGCGC	GCGCCACCAG	ACATAATAGC	TGACAGACTA	ACAGACTGTT	CCTTTCCATG
	1321	GGTCTTTTCT	GCAGTCACCG	TCGGTACCAG	CACCATGAAG	GTCAAACCTG	TGGTGTGCT
	1381	GTGCACCTTC	ACCGCCACAT	ACGCCGATAC	CATCTGTATC	GGCTACCACG	CCAACAACAG
35	1441	CACCGACACC	GTGGATACCG	TGCTGGAAAA	GAACGTGACC	GTGACACACA	GCGTGAACCT
	1501	GCTGGAAGAT	AGCCACAACG	GCAAGCTGTG	CCTGCTGAAG	GGAATTGCCC	CTCTCCAGCT
	1561	GGGAAATTGC	TCTGTGGCTG	GCTGGATCCT	GGGCAATCCT	GAGTGCAGAC	TGCTGATCTC
	1621	CAAAGAGAGC	TGGTCTCTAC	TCGTGAGAC	ACCCAATCCA	GAGAACGGCA	CATGCTACCC
40	1681	CGGCTACTTC	CGGACTATG	AGGAAGTGTG	AGAGCAGCTG	AGCAGCGTCA	GCAGCTTCGA
	1741	GAGATTCTGAG	ATTTTCCCA	AAGAGTCCAG	CTGGCCCAAC	CACACAGTGA	CAAGCGGAGT
	1801	GTCTGCCAGC	TGTTCCCA	ATGGCAAGAG	CAGCTTCTAC	AGAAACCTGC	TGTGGCTGAC
	1861	CGGCAAGAAC	GGACTGTACC	CCAACCTGAG	CAAGAGCTAC	GCTAACAACA	AAGAGAAAGA
45	1921	GGTCTGTGTC	CTCTGGGGCG	TGCACCATCC	TCCAAATATC	GGAGATCAGA	GAGCCCTGTA
	1981	CCACACCGAG	AATGCCTACG	TGTCCGTGGT	GTCCAGCCAC	TACAGCAGAA	GATTACCCCC
	2041	TGAGATCGCC	AAGCGGCCCA	AAGTGCAGAG	TCAAGAGGGC	AGAATCAACT	ACTACTGGAC
	2101	ACTGCTGGAA	CCCGGCGACA	CCATCATCTT	CGAGGCCAAC	GGAAACCTGA	TCGCCCCTAG
50	2161	ATACGCTTTC	GCTCTGAGCA	GAGGCTTTGG	CAGCGGCATC	ATCAACAGCA	ACGCCCTAT
	2221	GGATGAGTGC	GACGCCAAGT	GTCAAACACC	CCAGGGCGCT	ATCAACAGCT	CCCTGCCTTT
	2281	TCAGAACGTG	CACCTGTGA	CCATCGGCCG	GTGTCTTAAA	TATGTGCGGA	GCGCCAAGCT
	2341	GAGAATGGTC	ACCGGCCTGA	GAAACATCCC	CAGCATCCAG	TCTAGAGGCC	TGTTTGGCGC
55	2401	CATTGCCGGC	TTTATCGAAG	GCGGATGGAC	AGGCATGGTG	GACGGATGGT	ACGGCTATCA
	2461	CCACAGAAAT	GAGCAAGGCA	GCGGCTACGC	CGCCGATCAG	AAATCTACCC	AGAAGGCCAT
	2521	CAACGGGATC	ACCAACAAAG	TGAACAGCGT	GATCGAGAAG	ATGAACACCC	AGTTACCGCG
	2581	CGTGGGCAAA	GAGTTCAACA	AGCTGGAAAG	ACGGATGGAA	AACCTGAACA	AGAAGGTGGA
60	2641	CGACGGCTTC	ATCGACATCT	GGACCTACAA	CGCTGAGCTG	CTGGTCTCTC	TGGAAAACGA
	2701	GAGAACCCTG	GACTTCCACG	ACAGCAACGT	GAAGAACCTG	TACGAGAAAG	TGAAGTCCCA
	2761	GCTGAAGAAC	AACGCCAAAG	AGATCGGCCA	CGGCTGCTTC	GAGTTCTACC	ACAAGTGCAA
	2821	CGACGAGTGC	ATGGAAAGCG	TGAAGAATGG	CACCTACGAC	TACCCCAAGT	ACAGCGAGGA
65	2881	AAGCAAGCTG	AACCGCGAGA	AGATCGACGG	CGTGAAGCTG	GAATCTATGG	GCGTGTACCA
	2941	GATCCTGGCC	ATCTACAGCA	CAGTGGCTTC	TAGCCTGGTG	CTCCTGGTGT	CTCTGGGAGC
	3001	CATCAGCTTT	TGGATGTGCA	GCAATGGCAG	CCTCCAGTGC	CGGATCTGCA	TCTGAGCGGC
	3061	CGCAGATCTG	CTGTGCCTTC	TAGTTGCCAG	CCATCTGTTG	TTTGCCCCCT	CCCCGTGCCT
70	3121	TCCTTGACCC	TGGAAGGTGC	CACTCCCCTC	GTCTTTTCCT	AATAAAATGA	GGAAATTGCA
	3181	TCGCATTGTC	TGAGTAGGTG	TCATTCTATT	CTGGGGGGTG	GGGTGGGGCA	GGACAGCAAG
	3241	GGGGAGGATT	GGGAAGACAA	TAGCAGGCAT	GCTGGGGATG	CGGTGGGCTC	TATGGCTACC
	3301	CAGGTGCTGA	AGAATTGACC	CGGTTCTCTC	TGGGCCAGAA	AGAAGCAGGC	ACATCCCTTT
75	3361	CTCTGTGACA	CACCTGTGCC	ACGCCCCCTG	TTCTTAGTTC	CAGCCCCACT	CATAGGACAC
	3421	TCATAGCTCA	GGAGGGCTCC	GCCTTCAATC	CCACCCGCTA	AAGTACTTGG	AGCGGTCTCT
	3481	CCCTCCCTCA	TCAGCCCACC	AAACCAAACC	TAGCCTCCAA	GAGTGGGAAG	AAATTAAAGC
	3541	AAGATAGGCT	ATTAAGTGCA	GAGGGAGAGA	AAATGCCTCC	AACATGTGAG	GAAGTAATGA
80	3601	GAGAAATCAT	AGAATTTTAA	GGCCATGATT	TAAGGCCATC	ATGGCCTTAA	TCTTCCGCTT
	3661	CCTCGCTCAC	TGACTCGCTG	CGCTCGGTCG	TTGGGCTGCG	GCGAGCGGTA	TCAGCTCACT
	3721	CAAAGCGGGT	AATACGGTTA	TCCACAGAAT	CAGGGGATAA	CGCAGGAAAG	AACATGTGAG

3781 CAAAAGGCCA GCAAAGGCC AGGAACCGTA AAAAGGCCGC GTTGCTGGCG TTTTCCATA
 3841 GGCTCCGCCC CCCTGACGAG CATCACAAAA ATCGACGCTC AAGTCAGAGG TGGCGAAACC
 3901 CGACAGGACT ATAAAGATAC CAGGCGTTTC CCCCTGGAAG CTCCCTCCTG CGTCTCCTG
 3961 TTCCGACCCCT GCCGCTTACC GGATACCTGT CCGCCTTTCT CCCTTCGGGA AGCGTGGCGC
 5 4021 TTTCTCATAG CTCACGCTGT AGGTATCTCA GTTCGGTGTA GGTCGTTCGC TCCAAGCTGG
 4081 GCTGTGTGCA CGAACCCCC GTTCAGCCCG ACCGCTGCGC CTTATCCGGT AACTATCGTC
 4141 TTGAGTCCAA CCCGGTAAGA CACGACTTAT CGCCACTGGC AGCAGCCACT GGTAACAGGA
 4201 TTAGCAGAGC GAGGTATGTA GGCGGTGCTA CAGAGTTCTT GAAGTGGTGG CCTAACTACG
 4261 GCTACACTAG AAGAACAGTA TTTGGTATCT GCGCTCTGCT GAAGCCAGTT ACCTTCGGAA
 10 4321 AAAGAGTTGG TAGCTCTTGA TCCGGCAAAAC AAACCACCGC TGGTAGCGGT GGTTTTTTTG
 4381 TTTGCAAGCA GCAGATTACG CGCAGAAAAA AAGGATCTCA AGAAGATCCT TTGATCTTTT
 4441 CTACGGGGTC TGACGCTCAG TGGAACGAAA ACTCACGTTA AGGGATTTTG GTCATGAGAT
 4501 TATCAAAAAG GATCTTCACC TAGATCCTTT TAAATTAAAA ATGAAGTTT AAATCAATCT
 4561 AAAGTATATA TGAGTAACT TGGTCTGACA GTTACCAATG CTTAATCAGT GAGGCACCTA
 15 4621 TCTCAGCGAT CTGTCTATTT CGTTCATCCA TAGTTGCCTG ACTCGGGGGG GGGGGGCGCT
 4681 GAGGTCTGCC TCGTGAAGAA GGTGTTGCTG ACTCATACCA GGCCTGAATC GCGCCATCAT
 4741 CCAGCCAGAA AGTGAGGGAG CCACGTTTGA TGAGAGCTTT GTTGTAGGTG GACCAAGTTG
 4801 TGATTTTGAA CTTTTGCTTT GCCACGGAAC GGTCTGCGTT GTCGGGAAGA TCGGTGATCT
 4861 GATCCTTCAA CTCAGCAAAA GTTCGATTTA TTCAACAAAG CCGCCGTCCC GTCAAGTCAG
 20 4921 CGTAATGCTC TGCCAGTGTT ACAACCAATT AACCAATTCT GATTAGAAAA ACTCATCGAG
 4981 CATCAAATGA AACTGCAATT TATTCATATC AGGATTATCA ATACCATATT TTTGAAAAAG
 5041 CCGTTTCTGT AATGAAGGAG AAAACTCACC GAGGCAGTTC CATAGGATGG CAAGATCCTG
 5101 GTATCGGTCT GCGATTCCGA CTCGTCCAAC ATCAATACAA CCTATTAATT TCCCTCGTC
 5161 AAAAATAAGG TTATCAAGTG AGAAATCACC ATGAGTGACG ACTGAATCCG GTGAGAATGG
 25 5221 CAAAAGCTTA TGCATTTCTT TCCAGACTTG TTCAACAGGC CAGCCATTAC GCTCGTCATC
 5281 AAAATCACTC GCATCAACCA AACCGTTATT CATTCTGTAT TGCGCCTGAG CGAGACGAAA
 5341 TACGCGATCG CTGTTAAAAG GACAATTACA AACAGGAATC GAATGCAACC GCGCGAGGAA
 5401 CACTGCCAGC GCATCAACAA TATTTTCACC TGAATCAGGA TATTCTTCTA ATACCTGGAA
 5461 TGCTGTTTTT CCGGGGATCG CAGTGGTGAG TAACCATGCA TCATCAGGAG TACGGATAAA
 30 5521 ATGCTTGATG GTCGGAAGAG GCATAAATTC CGTCAGCCAG TTTAGTCTGA CCATCTCATC
 5581 TGTAACATCA TTGGCAACGC TACCTTTGCC ATGTTTCAGA AACAACTCTG GCGCATCGGG
 5641 CTTCCCATAC AATCGATAGA TTGTCGCACC TGATTGCCCC ACATTATCGC GAGCCCATT
 5701 ATACCCATAT AAATCAGCAT CCATGTTGGA ATTTAATCGC GGCCTCGAGC AAGACGTTTC
 5761 CCGTTGAATA TGGCTCATAA CACCCCTTGT ATTACTGTTT ATGTAAGCAG ACAGTTTAT
 35 5821 TGTTTCATGAT GATATATTTT TATCTTGTGC AATGTAACAT CAGAGATTTT GAGACACAAC
 5881 GTGGCTTTCC CCCCCCCCC ATTATTGAAG CATTATCAG GGTATTGTC TCATGAGCGG
 5941 ATACATATTT GAATGTATTT AGAAAAATAA ACAAATAGGG GTTCCGCGCA CATTCCCCG
 6001 AAAAGTGCCA CCTGACGTCT AAGAAACCAT TATTATCATG ACATTAACCT ATAAAAATAG
 6061 GCGTATCACG AGGCCCTTTC GTC
 40 //

Example 27 - FLU_T4_HA_1

This example provides amino acid and nucleic acid sequences of the influenza haemagglutinin H5 head and stem regions for an embodiment of the invention known as
 45 FLU_T4_HA_1. In SEQ ID NO:71 below, the amino acid residues of the stem region are shown underlined. The amino acid residues of the head region are the remaining residues. Similarly, in SEQ ID NO:72 below, the nucleic acid residues of the stem region are shown underlined. The nucleic acid residues of the head region are the remaining residues.

FLU_T4_HA_1 – HA0 amino acid sequence (SEQ ID NO:71)

50 MEKIVLLLAIIVSLVKSDQICIGYHANNSTEQVDTIMEKNVTVTHAQDILEKTHNGKLCDLNGVKPLILKDCS
 VAGWLLGNPMCEDEFIRVPEWSYIVERANPANDLCYPGNLNDYEELKHLLSRINHFEKILII PKSSWPNHETS
 LGVSAACPYQGTPSFVRNVWLIKKNDAIPTIKISYNNTNREDLLILWGIHHSNNAEQTNLYKNPTTYISV
 GTSTLNQRLVPKIATRSQVNGQRGRMDFFWTILKPNDIAHFESNGNFIAPEYAYKIVKKGDSTIMKSEVEYG
 HCN TKCQTPIGAINSSMPFHNIHPLTIGECPKYVKSNKLVLATGLRNSPLREKRRRKKRGLFGAIAGFIEGG
 55 WQGMVDGWYGYHHSNEQSGYAADKESTQKAIDGVTNKVNSIIDKMNTQFEAVGREFNNLERRIENLNKKME

DGFLDVWTYNAELLVLMENERTLDHFDSNVKNLYDKVRLQLRDNAKELGNGCFEFYHKCDNECMESVRNGTY
DYPQYSEEARKREEISGVKLESIGTYQILSIYSTVASSLALAIMVAGLSLWMC SNGLQCRICI

FLU_T4_HA_1 – HA0 nucleic acid sequence (SEQ ID NO:72)

5 GTACCGCCACCATGGAAAAGATCGTGCTGCTGCTGGCCATCGTGTCCTGGTCAAGAGCGACCAAATCTGCATC
GGCTACCACGCCAACACAGCACCGAACAGGTGGACACCATTATGGAAAAGAACGTCACCGTGACACACGCCCA
GGACATCTTGAAAAGACCCACAACGGCAAGCTGTGCGACCTGAACGGCGTGAAGCCTCTGATCCTGAAGGATT
GCTCTGTGGCCGGATGGCTGCTGGGCAATCCCATGTGCGACGAGTTCATCAGAGTGCCCGAGTGGTCCTACATC
GTGGAAGAGCCAATCCTGCCAACGACCTGTGCTACCCCGGCAACCTGAACGACTACGAGGAACCTGAAGCACCT
10 CCTGAGCCGGATCAACCACTTCGAGAAGATCCTGATCATCCCCAAGAGCAGCTGGCCCAACCACGAGACATCTC
TGGGAGTGTCTGCCGCATGTCCATACCAGGGCACCCCTAGCTTTTTCGGAACGTCGTGTGGCTGATCAAGAAG
AACGACGCTTACCCACCATCAAGATCAGCTACAACAACACCAACCGCGAGGACCTGCTGATCCTGTGGGAAT
CCACCACAGCAACAATGCCGCCGAGCAGACCAACCTGTACAAGAACCCACACCTACATCAGCGTGGGCACCA
GCACACTGAACCAGAGACTGGTGCCTAAGATCGCCACACGGTCCCAAGTGAATGGCCAGAGGGGCAGAATGGAC
15 TTCTTCTGGACCATCCTGAAGCCTAACGACGCCATCCACTTTGAGAGCAACGGCAACTTTATCGCCCCCTGAGTA
CGCCTACAAGATCGTGAAGAAGGGCGACAGCACCATCATGAAGTCCGAGGTGGAATACGGCCACTGCAACACCA
AGTGTGACAGCCCTATCGGCGCCATCAACTCCAGCATGCCCTTCCACAACATTACCCCTCTGACCATCGGCGAG
TGCCCCAAATACGTGAAGTCCAACAAGCTGGTGTGGCTACCGGCTGAGAAACAGCCCTCTGAGAGAGAAGCG
CAGACGGAAGAAGAGAGGCTGTTTGGCGCCATTGCCGGCTTTATCGAAGGCGGCTGGCAAGGCATGGTGGACG
20 GATGGTACGGCTACCATCACAGCAACGAGCAAGGCTCTGGATACGCCGCCGACAAAGAGAGCACCCAGAAAGCC
ATTGACGGCGTGACCAACAAAGTGAACAGCATCATCGACAAGATGAACACCCAGTTCGAGGCGGTGGGCAGAGA
GTTCAACAACCTGGAACGGCGGATCGAGAATCTGAACAAGAAGATGGAGGACGGCTTCCTGGACGTGTGGACCT
ACAAATGCCGAGCTGCTGGTCCTGATGGAAAACGAGAGAACCCTGGACTTCCACGACTCCAACGTGAAGAACCTG
TACGACAAAGTGCGGCTCCAGCTGCGGGACAACGCCAAAGAACTCGGCAACGGCTGCTTCGAGTTCTACCACAA
25 GTGCGACAACGAGTGTCATGGAAAGCGTGCGGAACGGCACCTACGACTACCCCTCAGTACAGCGAGGAAGCCCGGC
TGAAGAGAGAAGAGATCAGCGGAGTGAAGCTGGAATCCATCGGCACATACCAGATCCTGTCCATCTACAGCACC
GTGGCCTCTTCTCTGGCCCTGGCCATTATGGTGGCTGGCCTGTCTCTGTGGATGTGCAGCAATGGCAGCCTCCA
GTGCCGGATCTGCATCTGAGCGGCC

FLU_T4_HA_1 – Head region amino acid sequence (SEQ ID NO:73)

30 THNGKLCDLNGVKPLILKDCSVAGWLLGNPMCEDEFIRVPEWSYIVERANPANDLCYPGNLNDYEELKHLISRIN
HFEKILIIIPKSSWPNHETSLGVSAACPYQGTSPSFFRNVVWLIKNDAYPTIKISYNNTNREDLLILWGIHHSNN
AAEQTNLYKNPTTYISVGTSTLNQRLVPKIIATRSQVNGQGRMDFFWTILKPNDAIHFESNGNFIAPYAYKIV
KKGDSTIMKSEVEYGH CNTKCTPIGAINSSMPFHNHPLTIGCEP

FLU_T4_HA_1 – Head region nucleic acid sequence (SEQ ID NO:74)

35 TCCTGGAAAAGACCCACAACGGCAAGCTGTGCGACCTGAACGGCGTGAAGCCTCTGATCCTGAAGGATTGCTCT
GTGGCCGGATGGCTGCTGGGCAATCCCATGTGCGACGAGTTCATCAGAGTGCCCGAGTGGTCCTACATCGTGGA
AAGAGCCAATCCTGCCAACGACCTGTGCTACCCCGGCAACCTGAACGACTACGAGGAACCTGAAGCACCTCCTGA
GCCGGATCAACCACTTCGAGAAGATCCTGATCATCCCCAAGAGCAGCTGGCCCAACCACGAGACATCTCTGGGA

GTGTCTGCCGCATGTCCATACCAGGGCACCCCTAGCTTTTTCCGGAACGTCGTGTGGCTGATCAAGAAGAACGA
 CGCTTACCCACCATCAAGATCAGCTACAACAACACCAACCGCGAGGACCTGCTGATCCTGTGGGGAATCCACC
 ACAGCAACAATGCCGCCGAGCAGACCAACCTGTACAAGAACCCACCACCTACATCAGCGTGGGCACCAGCACA
 CTGAACCAGAGACTGGTGCCTAAGATCGCCACACGGTCCCAAGTGAATGGCCAGAGGGGCAGAATGGACTTCTT
 5 CTGGACCATCCTGAAGCCTAACGACGCCATCCACTTTGAGAGCAACGGCAACTTTATCGCCCCCTGAGTACGCCT
 ACAAGATCGTGAAGAAGGGCGACAGCACCATCATGAAGTCCGAGGTGGAATACGGCCACTGCAACACCAAGTGT
 CAGACCCCTATCGGCGCCATCAACTCCAGCATGCCCTTCCACAACATTACCCTCTGACCATCGGCGAGTGCCC
 CAAATACGTG

FLU_T4_HA_1 – *First stem region amino acid sequence (SEQ ID NO:75)*

10 MEKIVLLLLAIIVSLVKSDQICIGYHANNSTEQVDTIMEKNVTVTHAQDILEK

FLU_T4_HA_1 – *First stem region nucleic acid sequence (SEQ ID NO:76)*

GTACCGCCACCATGGAAAAGATCGTGCTGCTGCTGGCCATCGTGTCCCTGGTCAAGAGCGACCAAATCTGCATC
 GGCTACCACGCCAACACAGCACCGAACAGGTGGACACCATTATGGAAAAGAACGTCACCGTGACACACGCCCA
 GGACA

15 FLU_T4_HA_1 – *Second stem region amino acid sequence (SEQ ID NO:77)*

KYVKSNNKLVLATGLRNSPLREKRRRKRGLFGAIAGFIEGGWQGMVDGWYGYHHSNEQSGYAADKESTQKAID
 GVTNKVNSIIDKMNTQFEAVGREFNLERRIENLNKKMEDGFLDVWITYNAELLLVMENERTLDFHDSNVKNLYD
 KVRQLQLRDNAKELGNGCFEFYHKDNECMESVRNGTYDYPQYSEEARLKREEISGVKLESIGTYQILSIYSTVA
 SSLALAIMVAGLSLWMCSNGLQCRICI

20 FLU_T4_HA_1 – *Second stem region nucleic acid sequence (SEQ ID NO:78)*

AAGTCCAACAAGCTGGTGCTGGCTACCGGCCTGAGAAACAGCCCTCTGAGAGAGAAGCGCAGACGGAAGAAGAG
 AGGCCTGTTTGGCGCCATTGCCGGCTTTATCGAAGGCGGCTGGCAAGGCATGGTGGACGGATGGTACGGCTACC
 ATCACAGCAACGAGCAAGGCTCTGGATACGCCGCCGACAAAGAGAGCACCCAGAAAGCCATTGACGGCGTGACC
 AACAAAGTGAACAGCATCATCGACAAGATGAACACCCAGTTCGAGGCCGTGGGCAGAGAGTTCAACAACCTGGA
 25 ACGGCGGATCGAGAATCTGAACAAGAAGATGGAGGACGGCTTCCTGGACGTGTGGACCTACAATGCCGAGCTGC
 TGGTCTCTGATGGAAAACGAGAGAACCCTGGACTTCCACGACTCCAACGTGAAGAACCTGTACGACAAAGTGC GG
 CTCCAGCTGCGGGACAACGCCAAAGAACTCGGCAACGGCTGCTTCGAGTTCTACCACAAGTGCGACAACGAGTG
 CATGGAAAGCGTGCGGAACGGCACCTACGACTACCCCTCAGTACAGCGAGGAAGCCCGGCTGAAGAGAGAAGAGA
 TCAGCGGAGTGAAGCTGGAATCCATCGGCACATACCAGATCCTGTCCATCTACAGCACCGTGGCCTCTTCTCTG
 30 GCCCTGGCCATTATGGTGGCTGGCCTGTCTCTGTGGATGTGCAGCAATGGCAGCCTCCAGTGCCGGATCTGCAT
 CTGAGCGGCC

Example 28 – pEVAC-FLU_T4_HA_1

This example provides the nucleic acid sequence of pEVAC-FLU_T4_HA_1.

pEVAC-FLU_T4_HA_1 – *nucleic acid sequence (SEQ ID NO:79):*

WO 2023/057766

PCT/GB2022/052534

LOCUS pVRC8400EVAC_Ar_pEVAC-Flu_T4_HA_1 6092 bp DNA
linear SYN 08-SEP-2022

FEATURES Location/Qualifiers

CDS 1344..3070

5 /label="Flu_T4_HA_1"
/note="CI:V1=KpnI,V2=NotI,I1=KpnI,I2=NotI"

ORIGIN

1 TCGCGCGTTT CGGTGATGAC GGTGAAAACC TCTGACACAT GCAGCTCCCG GAGACGGTCA
61 CAGCTTGTCT GTAAGCGGAT GCCGGGAGCA GACAAGCCCG TCAGGGCGCG TCAGCGGGTG
10 121 TTGGCGGGTG TCGGGGCTGG CTTAACTATG CGGCATCAGA GCAGATTGTA CTGAGAGTGC
181 ACCATATGCG GTGTGAAATA CCGCACAGAT GCGTAAGGAG AAAATACCGC ATCAGATTGG
241 CTATTGGCCA TTGCATACGT TGTATCCATA TCATAATATG TACATTTATA TTGGCTCATG
301 TCCAACATTA CCGCCATGTT GACATTGATT ATTGACTAGT TATTAATAGT AATCAATTAC
361 GGGGTCATTA GTTCATAGCC CATATATGGA GTTCCGCGTT ACATAACTTA CGGTAAATGG
15 421 CCCGCCTGGC TGACCGCCCA ACGACCCCG CCCATTGACG TCAATAATGA CGTATGTTCC
481 CATAGTAACG CCAATAGGGA CTTTCCATTG ACGTCAATGG GTGGAGTATT TACGGTAAAC
541 TGCCCACTTG GCAGTACATC AAGTGTATCA TATGCCAAGT ACGCCCCCTA TTGACGTCAA
601 TGACGGTAAA TGGCCCGCCT GGCATTATGC CCAGTACATG ACCTTATGGG ACTTTCCTAC
661 TTGGCAGTAC ATCTACGTAT TAGTCATCGC TATTACCATG GTGATGCGGT TTTGGCAGTA
20 721 CATCAATGGG CGTGGATAGC GGTTTGACTC ACGGGGATTT CCAAGTCTCC ACCCCATTGA
781 CGTCAATGGG AGTTTGTTTT GGCACCAAAA TCAACGGGAC TTTCCAAAAT GTCGTAACAA
841 CTCCGCCCCA TTGACGCAA TGGGCGGTAG GCGTGTACGG TGGGAGGTCT ATATAAGCAG
901 AGCTCGTTTA GTGAACCGTC AGATCGCCTG GAGACGCCAT CCACGCTGTT TTGACCTCCA
961 TAGAAGACAC CGGGACCGAT CCAGCCTCCA TCGGCTCGCA TCTCTCCTTC ACGCGCCCGC
25 1021 CGCCCTACCT GAGGCCGCCA TCCACGCCGG TTGAGTCGCG TTCTGCCGCC TCCCGCCTGT
1081 GGTGCCTCCT GAACTGCGTC CGCCGTCTAG GTAAGTTTAA AGCTCAGGTC GAGACCGGGC
1141 CTTTGTCCGG CGCTCCCTTG GAGCCTACCT AGACTCAGCC GGCTCTCCAC GCTTTGCCTG
1201 ACCCTGCTTG CTCAACTCTA GTTAACGGTG GAGGGCAGTG TAGTCTGAGC AGTACTCGTT
1261 GCTGCCGCGC GCGCCACCAG ACATAATAGC TGACAGACTA ACAGACTGTT CCTTTCCATG
30 1321 GGTCTTTTCT GCAGTCACCG TCGGTACCGC CACCATGGAA AAGATCGTGC TGCTGCTGGC
1381 CATCGTGTCC CTGGTCAAGA GCGACCAAAT CTGCATCGGC TACCACGCCA ACAACAGCAC
1441 CGAACAGGTG GACACCATTA TGGAAAAGAA CGTCACCGTG ACACACGCCC AGGACATCCT
1501 GGAAAAGACC CACAACGGCA AGCTGTGCGA CCTGAACGGC GTGAAGCCTC TGATCCTGAA
1561 GGATTGCTCT GTGGCCGGAT GGCTGCTGGG CAATCCCATG TGCGACGAGT TCATCAGAGT
35 1621 GCCCGAGTGG TCCTACATCG TGGAAAGAGC CAATCCTGCC AACGACCTGT GCTACCCCGG
1681 CAACCTGAAC GACTACGAGG AACTGAAGCA CCTCCTGAGC CGGATCAACC ACTTCGAGAA
1741 GATCCTGATC ATCCCAAGA GCAGCTGGCC CAACCACGAG ACATCTCTGG GAGTGTCTGC
1801 CGCATGTCCA TACCAGGGCA CCCCTAGCTT TTTCCGGAAC GTCGTGTGGC TGATCAAGAA
1861 GAACGACGCT TACCCACCA TCAAGATCAG CTACAACAAC ACCAACC GCG AGGACCTGCT

1921 GATCCTGTGG GGAATCCACC ACAGCAACAA TGCCGCCGAG CAGACCAACC TGTACAAGAA
 1981 CCCCACCACC TACATCAGCG TGGGCACCAG CACACTGAAC CAGAGACTGG TGCCTAAGAT
 2041 CGCCACACGG TCCCAAGTGA ATGGCCAGAG GGGCAGAATG GACTTCTTCT GGACCATCCT
 2101 GAAGCCTAAC GACGCCATCC ACTTTGAGAG CAACGGCAAC TTTATCGCCC CTGAGTACGC
 5 2161 CTACAAGATC GTGAAGAAGG GCGACAGCAC CATCATGAAG TCCGAGGTGG AATACGGCCA
 2221 CTGCAACACC AAGTGTGAGA CCCCTATCGG CGCCATCAAC TCCAGCATGC CCTTCCACAA
 2281 CATTACCCCT CTGACCATCG GCGAGTGCCC CAAATACGTG AAGTCCAACA AGCTGGTGCT
 2341 GGCTACCGGC CTGAGAAACA GCCCTCTGAG AGAGAAGCGC AGACGGAAGA AGAGAGGCCT
 2401 GTTTGGCGCC ATTGCCGGCT TTATCGAAGG CGGCTGGCAA GGCATGGTGG ACGGATGGTA
 10 2461 CGGCTACCAT CACAGCAACG AGCAAGGCTC TGGATACGCC GCCGACAAAG AGAGCACCCA
 2521 GAAAGCCATT GACGGCGTGA CCAACAAAGT GAACAGCATC ATCGACAAGA TGAACACCCA
 2581 GTTCGAGGCC GTGGGCAGAG AGTTCAACAA CCTGGAACGG CGGATCGAGA ATCTGAACAA
 2641 GAAGATGGAG GACGGCTTCC TGGACGTGTG GACCTACAAT GCCGAGCTGC TGGTCTGAT
 2701 GGAAAACGAG AGAACCCTGG ACTTCCACGA CTCCAACGTG AAGAACCCTGT ACGACAAAGT
 15 2761 GCGGCTCCAG CTGCGGGACA ACGCCAAAGA ACTCGGCAAC GGCTGCTTCG AGTTCTACCA
 2821 CAAGTGCGAC AACGAGTGCA TGGAAAGCGT GCGGAACGGC ACCTACGACT ACCCTCAGTA
 2881 CAGCGAGGAA GCGCGGCTGA AGAGAGAAGA GATCAGCGGA GTGAAGCTGG AATCCATCGG
 2941 CACATAACCAG ATCCTGTCCA TCTACAGCAC CGTGGCCTCT TCTCTGCCCC TGGCCATTAT
 3001 GGTGGCTGGC CTGTCTCTGT GGATGTGCAG CAATGGCAGC CTCCAGTGCC GGATCTGCAT
 20 3061 CTGAGCGGCC GCAGATCTGC TGTGCCTTCT AGTTGCCAGC CATCTGTTGT TTGCCCCCTCC
 3121 CCCGTGCCTT CCTTGACCCT GGAAGGTGCC ACTCCCCTG TCCTTTCCCTA ATAAAATGAG
 3181 GAAATTGCAT CGCATTGTCT GAGTAGGTGT CATTCTATTC TGGGGGGTGG GGTGGGGCAG
 3241 GACAGCAAGG GGGAGGATTG GGAAGACAAT AGCAGGCATG CTGGGGATGC GGTGGGCTCT
 3301 ATGGCTACCC AGGTGCTGAA GAATTGACCC GGTTCCCTCCT GGGCCAGAAA GAAGCAGGCA
 25 3361 CATCCCCTTC TCTGTGACAC ACCCTGTCCA CGCCCCTGGT TCTTAGTTCC AGCCCCACTC
 3421 ATAGGACACT CATAGCTCAG GAGGGCTCCG CCTTCAATCC CACCCGCTAA AGTACTTGGA
 3481 GCGGTCTCTC CCTCCCTCAT CAGCCCACCA AACCAAACCT AGCCTCCAAG AGTGGGAAGA
 3541 AATTAAAGCA AGATAGGCTA TTAAGTGAG AGGGAGAGAA AATGCCTCCA ACATGTGAGG
 3601 AAGTAATGAG AGAAATCATA GAATTTTAAG GCCATGATTT AAGGCCATCA TGGCCTTAAT
 30 3661 CTTCCGCTTC CTCGCTCACT GACTCGCTGC GCTCGGTCGT TCGGCTGCGG CGAGCGGTAT
 3721 CAGCTCACTC AAAGGCGGTA ATACGGTTAT CCACAGAATC AGGGGATAAC GCAGGAAAGA
 3781 ACATGTGAGC AAAAGGCCAG CAAAAGGCCA GGAACCGTAA AAAGGCCGCG TTGCTGGCGT
 3841 TTTTCCATAG GCTCCGCCCC CCTGACGAGC ATCACAAAAA TCGACGCTCA AGTCAGAGGT
 3901 GGCGAAACCC GACAGGACTA TAAAGATACC AGGCGTTTCC CCCTGGAAGC TCCCTCGTGC
 35 3961 GCTCTCCTGT TCCGACCCTG CCGCTTACCG GATACCTGTC CGCCTTTCTC CCTTCGGGAA
 4021 GCGTGGCGCT TTCTCATAGC TCACGCTGTA GGTATCTCAG TTCGGTGTAG GTCGTTTCGT
 4081 CCAAGCTGGG CTGTGTGCAC GAACCCCCCG TTCAGCCCGA CCGCTGCGCC TTATCCGGTA
 4141 ACTATCGTCT TGAGTCCAAC CCGGTAAGAC ACGACTTATC GCCACTGGCA GCAGCCACTG
 4201 GTAACAGGAT TAGCAGAGCG AGGTATGTAG GCGGTGCTAC AGAGTTCTTG AAGTGGTGGC

4261 CTAAC TACGG CTACACTAGA AGAACAGTAT TTGGTATCTG CGCTCTGCTG AAGCCAGTTA
 4321 CCTTCGGAAA AAGAGTTGGT AGCTCTTGAT CCGGCAAACA AACCACCGCT GGTAGCGGTG
 4381 GTTTTTTTGT TTGCAAGCAG CAGATTACGC GCAGAAAAAA AGGATCTCAA GAAGATCCTT
 4441 TGATCTTTTC TACGGGGTCT GACGCTCAGT GGAACGAAAA CTCACGTTAA GGGATTTTGG
 5 4501 TCATGAGATT ATCAAAAAGG ATCTTCACCT AGATCCTTTT AAATTAATAA TGAAGTTTAA
 4561 AATCAATCTA AAGTATATAT GAGTAAACTT GGTCTGACAG TTACCAATGC TTAATCAGTG
 4621 AGGCACCTAT CTCAGCGATC TGTCTATTTT GTTCATCCAT AGTTGCCTGA CTCGGGGGGG
 4681 GGGGGCGCTG AGGTCTGCCT CGTGAAGAAG GTGTTGCTGA CTCATACCAG GCCTGAATCG
 4741 CCCCATCATC CAGCCAGAAA GTGAGGGAGC CACGGTTGAT GAGAGCTTTG TTGTAGGTGG
 10 4801 ACCAGTTGGT GATTTTGAAC TTTTGCTTTG CCACGGAACG GTCTGCGTTG TCGGGAAGAT
 4861 GCGTGATCTG ATCCTTCAAC TCAGCAAAAAG TTCGATTTAT TCAACAAAAG CGCCGTCCCG
 4921 TCAAGTCAGC GTAATGCTCT GCCAGTGTTA CAACCAATTA ACCAATTCTG ATTAGAAAAA
 4981 CTCATCGAGC ATCAAATGAA ACTGCAATTT ATTCATATCA GGATTATCAA TACCATATTT
 5041 TTGAAAAAGC CGTTTCTGTA ATGAAGGAGA AAAC TACCG AGGCAGTTCC ATAGGATGGC
 15 5101 AAGATCCTGG TATCGGTCTG CGATTCCGAC TCGTCCAACA TCAATACAAC CTATTAATTT
 5161 CCCCTCGTCA AAAATAAGGT TATCAAGTGA GAAATCACCA TGAGTGACGA CTGAATCCGG
 5221 TGAGAATGGC AAAAGCTTAT GCATTTCTTT CCAGACTTGT TCAACAGGCC AGCCATTACG
 5281 CTCGT CATCA AAATCACTCG CATCAACCAA ACCGTTATTC ATTCGTGATT GCGCCTGAGC
 5341 GAGACGAAAT ACGCGATCGC TGTTAAAAGG ACAATTACAA ACAGGAATCG AATGCAACCG
 20 5401 GCGCAGGAAC ACTGCCAGCG CATCAACAAT ATTTTCACCT GAATCAGGAT ATTCTTCTAA
 5461 TACCTGGAAT GCTGTTTTCC CGGGGATCGC AGTGGTGAGT AACCATGCAT CATCAGGAGT
 5521 ACGGATAAAA TGCTTGATGG TCGGAAGAGG CATAAATTCC GTCAGCCAGT TTAGTCTGAC
 5581 CATCTCATCT GTAACATCAT TGGCAACGCT ACCTTTGCCA TGTTTCAGAA ACAACTCTGG
 5641 CGCATCGGGC TTCCCATAACA ATCGATAGAT TGTCGCACCT GATTGCCCCA CATTATCGCG
 25 5701 AGCCCATTTA TACCCATATA AATCAGCATC CATGTTGGAA TTTAATCGCG GCCTCGAGCA
 5761 AGACGTTTCC CGTTGAATAT GGCTCATAAC ACCCCTTGTA TTAAGTTTAA TGTAAGCAGA
 5821 CAGTTTTATT GTTCATGATG ATATATTTTT ATCTTGTGCA ATGTAACATC AGAGATTTTG
 5881 AGACACAACG TGGCTTTCCC CCCCCCCCCA TTATTGAAGC ATTTATCAGG GTTATTGTCT
 5941 CATGAGCGGA TACATATTTG AATGTATTTA GAAAAATAAA CAAATAGGGG TTCCGCGCAC
 30 6001 ATTTCCCCGA AAAGTGCCAC CTGACGTCTA AGAAACCATT ATTATCATGA CATTAAACCTA
 6061 TAAAAATAGG CGTATCACGA GGCCCTTTTCG TC

//

Example 29 - FLU_T4_HA_2

This example provides amino acid and nucleic acid sequences of the influenza
 35 haemagglutinin H5 head and stem regions for an embodiment of the invention known as
 FLU_T4_HA_2. In SEQ ID NO:80 below, the amino acid residues of the stem region are
 shown underlined. The amino acid residues of the head region are the remaining residues.

Similarly, in SEQ ID NO:81 below, the nucleic acid residues of the stem region are shown underlined. The nucleic acid residues of the head region are the remaining residues.

FLU_T4_HA_2 – HA0 amino acid sequence (SEQ ID NO:80)

5 MEKIVLLLLAIVSLVKSDQICIGYHANNSTEQVDTIMEKNVTVTHAQDILEKTHNGKLCDLNGVKPLILKDCS
VAGWLLGNPMCEDEFIRVPEWSYIVERANPANDLCFPGNLNDYEELKHLLSRINHFEEKILIIIPKSSWPNHETS
LGVSAACPYQGTSPFFRNVVWLIKKNDAIPTIKISYNNTNREDLLILWGIHHSNNAAEQTNLYKNPTTYISV
GTSTLNQRLVPKIATRSQVNGERGRMDFFWTILKPNDAIHFESNGNFIAPEYAYKIVKKGDSTIMKSEVEYG
HCNTKCQTPIGAINSSMPFHNHPLTIGECPKYVKSSNKLVLATGLRNSPLREKRRRKKRGLFGAAGFIEGG
WQGMVDGWYGYHHSNEQSGSYAADKESTQKAIDGVTNKVNSIIDKMNTQFEAVGREFNNLERRIENLNKKME
10 DGFLDVWTYNAELLVLMENERTLDFHDSNVKNLYDKVRLQLRDNAKELGNCGFEFYHKCDNECMESVRNGTY
DYPQYSEEARKREEISGVKLESIGTYQILSIYSTVASSLALAIMVAGLSLWMCSNGSLQCRICI

FLU_T4_HA_2 – HA0 nucleic acid sequence (SEQ ID NO:81)

GTACCGCCACCATGGAAAAGATCGTGCTGCTGCTGGCCATCGTGTCCTGGTCAAGAGCGACCAAATCTGCATC
GGCTACCACGCCAACACAGCACCGAACAGGTGGACACCATTTATGGAAAAGAACGTCACCGTGACACAGCCCCA
15 GGACATCTCTGGAAAAGACCCACAACGGCAAGCTGTGCGACCTGAACGGCGTGAAGCCTCTGATCCTGAAGGATT
GCTCTGTGGCCGGATGGCTGCTGGGCAATCCCATGTGCGACGAGTTCATCAGAGTGCCCGAGTGGTCCTACATC
GTGGAAAGAGCCAATCCTGCCAACGACCTGTGCTTCCCCGGCAACCTGAACGACTACGAGGAACCTGAAGCACCT
CCTGAGCCGGATCAACCACTTCGAGAAGATCCTGATCATCCCCAAGAGCAGCTGGCCCAACCACGAGACATCTC
TGGGAGTGTCTGCCGCATGTCCATACCAGGGCACCCCTAGCTTTTTTCCGGAACGTCGTGTGGCTGATCAAGAAG
20 AACGACGCTTACCCCAACCATCAAGATCAGCTACAACAACACCAACCGCGAGGACCTGCTGATCCTGTGGGAAT
CCACCACAGCAACAATGCCGCCGAGCAGACCAACCTGTACAAGAACCCACACCTACATCAGCGTGGGCACCA
GCACACTGAACCAGAGACTGGTGCCCTAAGATCGCCACACGGTCCCAAGTGAATGGCGAGAGGGGAGCAATGGAC
TTCTTCTGGACCATCCTGAAGCCTAACGACGCCATCCACTTTGAGAGCAACGGCAACTTTATCGCCCCCTGAGTA
CGCCTACAAGATCGTGAAGAAGGGGACAGCACCATCATGAAGTCCGAGGTGGAATACGGCCACTGCAACACCA
25 AGTGTGACACCCCTATCGGCGCCATCAACTCCAGCATGCCCTTCCACAACATTCACCCCTCTGACCATCGGCGAG
TGCCCCAAATACGTGAAGTCCAACAAGCTGGTGCTGGCTACCGGCCTGAGAAACAGCCCTCTGAGAGAGAAGCG
CAGACGGAAGAAGAGAGAGGCTGTTTGGCGCCATTGCCGGCTTTATCGAAGGCGGCTGGCAAGGCATGGTGGACG
GATGGTACGGCTACCATCACAGCAACGAGCAAGGCTCTGGATACGCCGCCGACAAAGAGAGCACCCAGAAAGCC
ATTGACGGCGTGACCAACAAAGTGAAACAGCATCATCGACAAGATGAACACCCAGTTTCGAGGCCGTGGGCAGAGA
30 GTTCAACAACCTGGAACGGCGGATCGAGAACTCTGAACAAGAAGATGGAGGACGGCTTCCTGGACGTGTGGACCT
ACAATGCCGAGCTGCTGGTCCTGATGGAAAACGAGAGAACCCTGGACTTCCACGACTCCAACGTGAAGAACCTG
TACGACAAAGTGCGGCTCCAGCTGCGGGACAACGCCAAAGAACTCGGCAACGGCTGCTTCGAGTTCTTACCACAA
GTGCGACAACGAGTGCATGGAAAGCGTGCGGAACGGCACCTACGACTACCCCTCAGTACAGCGAGGAAGCCCGGC
TGAAGAGAGAAAGAGATCAGCGGAGTGAAGCTGGAATCCATCGGCACATACCAGATCCTGTCCATCTACAGCACC
35 GTGGCCTCTTCTCTGGCCCTGGCCATTATGGTGGCTGGCCTGTCTCTGTGGATGTGCAGCAATGGCAGCCTCCA
GTGCCGGATCTGCATCTGAGCGGCC

FLU_T4_HA_2 – Head region amino acid sequence (SEQ ID NO:82)

THNGKLCDLNGVKPLILKDCSVAGWLLGNPMCDEFIRVPEWSYIVERANPANDLCFPGNLNDYEELKHLLSRIN
HFEKILIIIPKSSWPNHETSLGVSAACPYPQGTPSFRRNVVWLIKKNDAIPTIKISYNNNTNREDLLILWGIHHSNN
AAEQTNLYKNPTTYISVGTSTLNQRLVPKIATRSQVNGERGRMDFFWTILKPNDAIHFESNGNFIAPEYAYKIV
KKGDSTIMKSEVEYGH CNTKCTPIGAINSSMPFHNHPLTIGECF

5 FLU_T4_HA_2 – *Head region nucleic acid sequence (SEQ ID NO:83)*

TCCTGGAAAAGACCCACAACGGCAAGCTGTGCGACCTGAACGGCGTGAAGCCTCTGATCCTGAAGGATTGCTCT
GTGGCCGGATGGCTGCTGGGCAATCCCATGTGCGACGAGTTCATCAGAGTGCCCGAGTGGTCTACATCGTGGA
AAGAGCCAATCCTGCCAACGACCTGTGCTTCCCCGGCAACCTGAACGACTACGAGGAACCTGAAGCACCTCCTGA
GCCGGATCAACCACTTCGAGAAGATCCTGATCATCCCCAAGAGCAGCTGGCCCAACCACGAGACATCTCTGGGA
10 GTGTCTGCCGCATGTCCATACCAGGGCACCCTAGCTTTTCCGGAACGTCGTGTGGCTGATCAAGAAGAACGA
CGCTTACCCCACCATCAAGATCAGCTACAACAACACCAACCGCGAGGACCTGCTGATCCTGTGGGGAATCCACC
ACAGCAACAATGCCGCCGAGCAGACCAACCTGTACAAGAACCCACACCTACATCAGCGTGGGCACCAGCACA
CTGAACCAGAGACTGGTGCCTAAGATCGCCACACGGTCCCAAGTGAATGGCGAGAGGGGCAGAATGGACTTCTT
CTGGACCATCCTGAAGCCTAACGACGCCATCCACTTTGAGAGCAACGGCAACTTTATCGCCCCCTGAGTACGCCT
15 ACAAGATCGTGAAGAAGGGCGACAGCACCATCATGAAGTCCGAGGTGGAATACGGCCACTGCAACACCAAGTGT
CAGACCCCTATCGGCGCCATCAACTCCAGCATGCCCTTCCACAACATTCACCCTCTGACCATCGGCGAGTGCCC
CAAATACGTG

FLU_T4_HA_2 – *First stem region amino acid sequence (SEQ ID NO:84)*

MEKIVLLLLAIIVSLVKSQDQICIGYHANNSTEQVDTIMEKNVTVTTHAQDILEK

20 FLU_T4_HA_2 – *First stem region nucleic acid sequence (SEQ ID NO:85)*

GTACCGCCACCATGGAAAAGATCGTGCTGCTGCTGGCCATCGTGTCCCTGGTCAAGAGCGACCAAATCTGCATC
GGCTACCACGCCAACAAACAGCACCGAACAGGTGGACACCATTATGGAAAAGAACGTCACCGTGACACACGCCCA
GGACA

FLU_T4_HA_2 – *Second stem region amino acid sequence (SEQ ID NO:86)*

25 KYVKSNNKLVLATGLRNSPLREKRRRKRGLFGAIAGFIEGGWQGMVDGWYGYHHSNEQSGSYAADKESTQKAID
GVTNKVNSIIDKMNTQFEAVGREFNNLERRIENLNKKMEDGFLDVWITYNAELLVLMENERTLDFHDSNVKNLYD
KVRLQLRDNAKELGNGCFEFYHKCDNECMESVRNGTYDYPQYSEEARLKREEISGVKLESIGTYQILSIYSTVA
SSLALAIMVAGLSLWMCSNGSLQCRICI

FLU_T4_HA_2 – *Second stem region nucleic acid sequence (SEQ ID NO:87)*

30 AAGTCCAACAAGCTGGTGCTGGCTACCGGCCTGAGAAACAGCCCTCTGAGAGAGAAGCGCAGACGGAAGAAGAG
AGGCCTGTTTGGCGCCATTGCCGGCTTTATCGAAGGCGGCTGGCAAGGCATGGTGGACGGATGGTACGGCTACC
ATCACAGCAACGAGCAAGGCTCTGGATACGCCGCCGACAAAGAGAGCACCAGAAAGCCATTGACGGCGTGACC
AACAAAGTGAACAGCATCATCGACAAGATGAACACCCAGTTCGAGGCCGTGGGCAGAGAGTTCAACAACCTGGA
ACGGCGGATCGAGAATCTGAACAAGAAGATGGAGGACGGCTTCCTGGACGTGTGGACCTACAATGCCGAGCTGC
35 TGGTCTCTGATGGAAAACGAGAGAACCTGGACTTCCACGACTCCAACGTGAAGAACCTGTACGACAAAGTGCGG
CTCCAGCTGCGGGACAACGCCAAAGAACTCGGCAACGGCTGCTTCGAGTTCTACCACAAGTGCGACAACGAGTG

CATGGAAAGCGTGCGGAACGGCACCTACGACTACCCTCAGTACAGCGAGGAAGCCCGGCTGAAGAGAGAAGAGA
 TCAGCGGAGTGAAGCTGGAATCCATCGGCACATACCAGATCCTGTCCATCTACAGCACCGTGGCCTCTTCTCTG
 GCCCTGGCCATTATGGTGGCTGGCCTGTCTCTGTGGATGTGCAGCAATGGCAGCCTCCAGTGCCGGATCTGCAT
 CTGAGCGGCC

5 Example 30 – pEVAC-FLU_T4_HA_2

This example provides the nucleic acid sequence of pEVAC-FLU_T4_HA_2.

pEVAC-FLU_T4_HA_2 – *nucleic acid sequence (SEQ ID NO:88)*:

LOCUS pVRC8400EVAC_Ar_pEVAC-Flu_T4_HA_2 6092 bp DNA
 linear SYN 08-SEP-2022

10 FEATURES Location/Qualifiers
 CDS 1344..3070
 /label="Flu_T4_HA_2"
 /note="CI:V1=KpnI,V2=NotI,I1=KpnI,I2=NotI"

ORIGIN

15 1 TCGCGCGTTT CGGTGATGAC GGTGAAAACC TCTGACACAT GCAGCTCCCG GAGACGGTCA
 61 CAGCTTGTCT GTAAGCGGAT GCCGGGAGCA GACAAGCCCG TCAGGGCGCG TCAGCGGGTG
 121 TTGGCGGGTG TCGGGGCTGG CTTAACTATG CGGCATCAGA GCAGATTGTA CTGAGAGTGC
 181 ACCATATGCG GTGTGAAATA CCGCACAGAT GCGTAAGGAG AAAATACCGC ATCAGATTGG
 241 CTATTGGCCA TTGCATACGT TGTATCCATA TCATAATATG TACATTTATA TTGGCTCATG
 20 301 TCCAACATTA CCGCCATGTT GACATTGATT ATTGACTAGT TATTAATAGT AATCAATTAC
 361 GGGGTCATTA GTTCATAGCC CATATATGGA GTTCCGCGTT ACATAACTTA CGGTAAATGG
 421 CCCGCCTGGC TGACCGCCCA ACGACCCCG CCCATTGACG TCAATAATGA CGTATGTTCC
 481 CATAGTAACG CCAATAGGGA CTTTCCATTG ACGTCAATGG GTGGAGTATT TACGGTAAAC
 541 TGCCCACTTG GCAGTACATC AAGTGTATCA TATGCCAAGT ACGCCCCCTA TTGACGTCAA
 25 601 TGACGGTAAA TGGCCCGCCT GGCATTATGC CCAGTACATG ACCTTATGGG ACTTTCCTAC
 661 TTGGCAGTAC ATCTACGTAT TAGTCATCGC TATTACCATG GTGATGCGGT TTTGGCAGTA
 721 CATCAATGGG CGTGGATAGC GGTTTGACTC ACGGGGATTT CCAAGTCTCC ACCCCATTGA
 781 CGTCAATGGG AGTTTGTTTT GGCACCAAAA TCAACGGGAC TTTCCAAAAT GTCGTAACAA
 841 CTCCGCCCCA TTGACGCAAA TGGGCGGTAG GCGTGTACGG TGGGAGGTCT ATATAAGCAG
 30 901 AGCTCGTTTA GTGAACGTC AGATCGCCTG GAGACGCCAT CCACGCTGTT TTGACCTCCA
 961 TAGAAGACAC CGGGACCGAT CCAGCCTCCA TCGGCTCGCA TCTCTCCTTC ACGCGCCCGC
 1021 CGCCCTACCT GAGGCCGCCA TCCACGCCGG TTGAGTCGCG TTCTGCCGCC TCCCGCCTGT
 1081 GGTGCCTCCT GAACTGCGTC CGCCGTCTAG GTAAGTTTAA AGCTCAGGTC GAGACCGGGC
 1141 CTTTGTCCGG CGCTCCCTTG GAGCCTACCT AGACTCAGCC GGCTCTCCAC GCTTTGCCTG
 35 1201 ACCCTGCTTG CTCAACTCTA GTTAACGGTG GAGGGCAGTG TAGTCTGAGC AGTACTCGTT

1261 GCTGCCGCGC GCGCCACCAG ACATAATAGC TGACAGACTA ACAGACTGTT CCTTTCCATG
 1321 GGTCTTTTCT GCAGTCACCG TCGGTACCGC CACCATGGAA AAGATCGTGC TGCTGCTGGC
 1381 CATCGTGTCC CTGGTCAAGA GCGACCAAAT CTGCATCGGC TACCACGCCA ACAACAGCAC
 1441 CGAACAGGTG GACACCATTA TGGAAAAGAA CGTCACCGTG ACACACGCCC AGGACATCCT
 5 1501 GGAAAAGACC CACAACGGCA AGCTGTGCGA CCTGAACGGC GTGAAGCCTC TGATCCTGAA
 1561 GGATTGCTCT GTGGCCGGAT GGCTGCTGGG CAATCCCATG TGCGACGAGT TCATCAGAGT
 1621 GCCCGAGTGG TCCTACATCG TGGAAAGAGC CAATCCTGCC AACGACCTGT GCTTCCCCGG
 1681 CAACCTGAAC GACTACGAGG AACTGAAGCA CCTCCTGAGC CGGATCAACC ACTTCGAGAA
 1741 GATCCTGATC ATCCCCAAGA GCAGCTGGCC CAACCACGAG ACATCTCTGG GAGTGTCTGC
 10 1801 CGCATGTCCA TACCAGGGCA CCCCTAGCTT TTTCCGGAAC GTCGTGTGGC TGATCAAGAA
 1861 GAACGACGCT TACCCACCA TCAAGATCAG CTACAACAAC ACCAACCGCG AGGACCTGCT
 1921 GATCCTGTGG GGAATCCACC ACAGCAACAA TGCCGCCGAG CAGACCAACC TGTACAAGAA
 1981 CCCCACCACC TACATCAGCG TGGGCACCAG CACACTGAAC CAGAGACTGG TGCCTAAGAT
 2041 CGCCACACGG TCCCAAGTGA ATGGCGAGAG GGGCAGAATG GACTTCTTCT GGACCATCCT
 15 2101 GAAGCCTAAC GACGCCATCC ACTTTGAGAG CAACGGCAAC TTTATCGCCC CTGAGTACGC
 2161 CTACAAGATC GTGAAGAAGG GCGACAGCAC CATCATGAAG TCCGAGGTGG AATACGGCCA
 2221 CTGCAACACC AAGTGTGAGA CCCCTATCGG CGCCATCAAC TCCAGCATGC CCTTCCACAA
 2281 CATTCACCTT CTGACCATCG GCGAGTGCCC CAAATACGTG AAGTCCAACA AGCTGGTGCT
 2341 GGCTACCGGC CTGAGAAACA GCCCTCTGAG AGAGAAGCGC AGACGGAAGA AGAGAGGCCT
 20 2401 GTTTGGCGCC ATTGCCGGCT TTATCGAAGG CGGCTGGCAA GGCATGGTGG ACGGATGGTA
 2461 CGGCTACCAT CACAGCAACG AGCAAGGCTC TGGATACGCC GCCGACAAAG AGAGCACCCA
 2521 GAAAGCCATT GACGGCGTGA CCAACAAAGT GAACAGCATC ATCGACAAGA TGAACACCCA
 2581 GTTCGAGGCC GTGGGCAGAG AGTTCAACAA CCTGGAACGG CGGATCGAGA ATCTGAACAA
 2641 GAAGATGGAG GACGGCTTCC TGGACGTGTG GACCTACAAT GCCGAGCTGC TGGTCCTGAT
 25 2701 GGAAAACGAG AGAACCCTGG ACTTCCACGA CTCCAACGTG AAGAACCCTGT ACGACAAAGT
 2761 GCGGCTCCAG CTGCGGGACA ACGCCAAAGA ACTCGGCAAC GGCTGCTTCG AGTTCTACCA
 2821 CAAGTGCGAC AACGAGTGCA TGGAAAGCGT GCGGAACGGC ACCTACGACT ACCCTCAGTA
 2881 CAGCGAGGAA GCCCGGCTGA AGAGAGAAGA GATCAGCGGA GTGAAGCTGG AATCCATCGG
 2941 CACATACCAG ATCCTGTCCA TCTACAGCAC CGTGGCCTCT TCTCTGGCCC TGGCCATTAT
 30 3001 GGTGGCTGGC CTGTCTCTGT GGATGTGCAG CAATGGCAGC CTCCAGTGCC GGATCTGCAT
 3061 CTGAGCGGCC GCAGATCTGC TGTGCCTTCT AGTTGCCAGC CATCTGTTGT TTGCCCTCC
 3121 CCCGTGCCTT CCTTGACCCT GGAAGGTGCC ACTCCCACTG TCCTTTCCTA ATAAAATGAG
 3181 GAAATTGCAT CGCATTGTCT GAGTAGGTGT CATTCTATTC TGGGGGTGG GGTGGGGCAG
 3241 GACAGCAAGG GGGAGGATTG GGAAGACAAT AGCAGGCATG CTGGGGATGC GGTGGGCTCT
 35 3301 ATGGCTACCC AGGTGCTGAA GAATTGACCC GGTTCCTCCT GGGCCAGAAA GAAGCAGGCA
 3361 CATCCCTTC TCTGTGACAC ACCCTGTCCA CGCCCCTGGT TCTTAGTTCC AGCCCCACTC
 3421 ATAGGACACT CATAGCTCAG GAGGGCTCCG CCTTCAATCC CACCCGCTAA AGTACTTGGA

3481 GCGGTCTCTC CCTCCCTCAT CAGCCCACCA AACCAAACCT AGCCTCCAAG AGTGGGAAGA
 3541 AATTAAAGCA AGATAGGCTA TTAAGTGCAG AGGGAGAGAA AATGCCTCCA ACATGTGAGG
 3601 AAGTAATGAG AGAAATCATA GAATTTTAAAG GCCATGATTT AAGGCCATCA TGGCCTTAAT
 3661 CTTCCGCTTC CTCGCTCACT GACTCGCTGC GCTCGGTCGT TCGGCTGCGG CGAGCGGTAT
 5 3721 CAGCTCACTC AAAGGCGGTA ATACGGTTAT CCACAGAATC AGGGGATAAC GCAGGAAAGA
 3781 ACATGTGAGC AAAAGGCCAG CAAAAGGCCA GGAACCGTAA AAAGGCCGCG TTGCTGGCGT
 3841 TTTTCCATAG GCTCCGCCCC CCTGACGAGC ATCACAAAAA TCGACGCTCA AGTCAGAGGT
 3901 GGC GAAACCC GACAGGACTA TAAAGATACC AGGCGTTTCC CCCTGGAAGC TCCCTCGTGC
 3961 GCTCTCCTGT TCCGACCCTG CCGCTTACCG GATACCTGTC CGCCTTTCTC CCTTCGGGAA
 10 4021 GCGTGGCGCT TTCTCATAGC TCACGCTGTA GGTATCTCAG TTCGGTGTAG GTCGTTGCGT
 4081 CCAAGCTGGG CTGTGTGCAC GAACCCCCCG TTCAGCCCGA CCGCTGCGCC TTATCCGGTA
 4141 ACTATCGTCT TGAGTCCAAC CCGGTAAGAC ACGACTTATC GCCACTGGCA GCAGCCACTG
 4201 GTAACAGGAT TAGCAGAGCG AGGTATGTAG GCGGTGCTAC AGAGTTCTTG AAGTGGTGGC
 4261 CTA ACTACG CTACACTAGA AGAACAGTAT TTGGTATCTG CGCTCTGCTG AAGCCAGTTA
 15 4321 CCTTCGGA AAGAGTTGGT AGCTCTTGAT CCGGCAAACA AACCACCGCT GGTAGCGGTG
 4381 GTTTTTTTGT TTGCAAGCAG CAGATTACGC GCAGAAAAAA AGGATCTCAA GAAGATCCTT
 4441 TGATCTTTTC TACGGGGTCT GACGCTCAGT GGAACGAAAA CTCACGTTAA GGGATTTTGG
 4501 TCATGAGATT ATCAAAAAGG ATCTTCACCT AGATCCTTTT AAATTAAAAA TGAAGTTTAA
 4561 AATCAATCTA AAGTATATAT GAGTAAACTT GGTCTGACAG TTACCAATGC TTAATCAGTG
 20 4621 AGGCACCTAT CTCAGCGATC TGTCTATTTT GTTCATCCAT AGTTGCCTGA CTCGGGGGGG
 4681 GGGGGCGCTG AGGTCTGCCT CGTGAAGAAG GTGTTGCTGA CTCATACCAG GCCTGAATCG
 4741 CCCCATCATC CAGCCAGAAA GTGAGGGAGC CACGGTTGAT GAGAGCTTTG TTGTAGGTGG
 4801 ACCAGTTGGT GATTTTGAAC TTTTGCTTTG CCACGGAACG GTCTGCGTTG TCGGGAAGAT
 4861 GCGTGATCTG ATCCTTCAAC TCAGCAAAAG TTCGATTTAT TCAACAAAGC CGCCGTCCCG
 25 4921 TCAAGTCAGC GTAATGCTCT GCCAGTGTTA CAACCAATTA ACCAATTCTG ATTAGAAAAA
 4981 CTCATCGAGC ATCAAATGAA ACTGCAATTT ATTCATATCA GGATTATCAA TACCATATTT
 5041 TTGAAAAAGC CGTTTCTGTA ATGAAGGAGA AAACCTACCG AGGCAGTTCC ATAGGATGGC
 5101 AAGATCCTGG TATCGGTCTG CGATTCCGAC TCGTCCAACA TCAATACAAC CTATTAATTT
 5161 CCCCTCGTCA AAAATAAGGT TATCAAGTGA GAAATCACCA TGAGTGACGA CTGAATCCGG
 30 5221 TGAGAATGGC AAAAGCTTAT GCATTTCTTT CCAGACTTGT TCAACAGGCC AGCCATTACG
 5281 CTCGTATCA AAATCACTCG CATCAACCAA ACCGTTATTC ATTCGTGATT GCGCCTGAGC
 5341 GAGACGAAAT ACGCGATCGC TGTTAAAAGG ACAATTACAA ACAGGAATCG AATGCAACCG
 5401 GCGCAGGAAC ACTGCCAGCG CATCAACAAT ATTTTCACCT GAATCAGGAT ATTCTTCTAA
 5461 TACCTGGAAT GCTGTTTTCC CGGGGATCGC AGTGGTGAGT AACCATGCAT CATCAGGAGT
 35 5521 ACGGATAAAA TGCTTGATGG TCGGAAGAGG CATAAATTCC GTCAGCCAGT TTAGTCTGAC
 5581 CATCTCATCT GTAACATCAT TGGCAACGCT ACCTTTGCCA TGTTTCAGAA ACAACTCTGG
 5641 CGCATCGGGC TTCCCATACA ATCGATAGAT TGTCGCACCT GATTGCCCCG CATTATCGCG

5701 AGCCCATTTA TACCCATATA AATCAGCATC CATGTTGGAA TTTAATCGCG GCCTCGAGCA
 5761 AGACGTTTCC CGTTGAATAT GGCTCATAAC ACCCCTTGTA TTAAGTTTA TGTAAGCAGA
 5821 CAGTTTTATT GTTCATGATG ATATATTTTT ATCTTGTGCA ATGTAACATC AGAGATTTTG
 5881 AGACACAACG TGGCTTTCCC CCCCCCCCCA TTATTGAAGC ATTTATCAGG GTTATTGTCT
 5941 CATGAGCGGA TACATATTG AATGTATTTA GAAAAATAAA CAAATAGGGG TTCCGCGCAC
 6001 ATTTCCCCGA AAAGTGCCAC CTGACGTCTA AGAAACCATT ATTATCATGA CATTAAACCTA
 6061 TAAAAATAGG CGTATCACGA GGCCCTTTCG TC

Example 31 - FLU_T4_HA_3

This example provides amino acid and nucleic acid sequences of the influenza haemagglutinin H5 head and stem regions for an embodiment of the invention known as FLU_T4_HA_3. In SEQ ID NO:89 below, the amino acid residues of the stem region are shown underlined. The amino acid residues of the head region are the remaining residues. Similarly, in SEQ ID NO:90 below, the nucleic acid residues of the stem region are shown underlined. The nucleic acid residues of the head region are the remaining residues.

FLU_T4_HA_3 – HA0 amino acid sequence (SEQ ID NO:89)

MEKIVLLLAIVSLVKSDQICIGYHANNSTEQVDTIMEKNVTVTHAQDILEKTHNGKLCDLNGVKPLILKDCS
 VAGWLLGNPMCDFIRVPEWSYIVERANPANDLCFPGNLNDYEELKHLISRINHFEEKILII PKSSWPNHNTS
 LGVSAACPYQGTSPFRNVVWLIKNDTYPTIKISYNNNTNREDLLILWGIHHSNNTAEQTNLYKNPTTYISV
 GTSTLNQRLVPKIANRSQVNGQRGRMDFFWTILKPNDAIHFESNGNFIAPEYAYKIVKKGDSTIMKSEVEYG
 HCNTKCQTPIGAINSSMPFHNHPLTIGECPKYVKSSNKLVLATGLRNSPLREKRRRKKRGLFGAIAAGFIEGG
WQGMVDGWYGYHHSNEQSGSYAADKESTQKAIDGVTNKVNSIIDKMNTQFEAVGREFNNLERRIENLNKKME
DGFLDVWTYNAELLVLMENERTLDFHDSNVKNLYDKVRLQLRDNAKELGNGCFEFYHKCDNECMESVRNGTY
DYPQYSEEARKREEISGVKLESIGTYQILSIYSTVASSLALAIMVAGLSLWMCSNGSLQCRICI

FLU_T4_HA_3 – HA0 nucleic acid sequence (SEQ ID NO:90)

GTACCGCCACCATGGAAAAGATCGTGCTGCTGCTGGCCATCGTGTCCCTGGTCAAGAGCGACCAAAT
CTGCATCGGCTACCACGCCAACAACAGCACCGAACAGGTGGACACCATTTATGGAAAAGAACGTCACC
GTGACACACGCCCAGGACATCCTGGAAAAGACCCACAACGGCAAGCTGTGCGACCTGAACGGCGTGA
 AGCCTCTGATCCTGAAGGATTGCTCTGTGGCCGATGGCTGCTGGGCAATCCCATGTGCGACGAGTT
 CATCAGAGTGCCCGAGTGGTCCTACATCGTGGAAGAGCCAATCCTGCCAACGACCTGTGCTTCCCC
 GGCAACCTGAACGACTACGAGGAAGTGAAGCACCTCCTGAGCCGGATCAACCACTTCGAGAAGATCC
 TGATCATCCCCAAGAGCAGCTGGCCCAACCACAATACCAGCCTGGGAGTGTCTGCCGCATGTCCATA
 TCAGGGCACCCCTAGCTTTTTCCGGAACGTCGTGTGGCTGATCAAGAAGAACGACACATACCCACC
 ATCAAGATCAGCTACAACAACACCAACCGGAGGACCTGCTGATCCTGTGGGGAATCCACCACAGCA
 ACAATACCGCCGAGCAGACCAACCTGTACAAGAACCCACCACCTACATCAGCGTGGGCACCAGCAC

ACTGAACCAGAGACTGGTGCCTAAGATCGCCAACCGCAGCCAAGTGAATGGCCAGAGGGGCAGAATG
 GACTTCTTCTGGACCATCCTGAAGCCTAACGACGCCATCCACTTTGAGAGCAACGGCAACTTTATCG
 CCCCTGAGTACGCCTACAAGATCGTGAAGAAGGGCGACAGCACCATCATGAAGTCCGAGGTGGAATA
 CGGCCACTGCAACACCAAGTGTGAGACCCCTATCGGCGCCATCAACTCCAGCATGCCCTTCCACAAC
 5 ATTCACCCTCTGACCATCGGCGAGTGCCCCAAATACGTGAAGTCCAACAAGCTGGTGTGGCTACCG
GCCTGAGAAACAGCCCTCTGAGAGAGAAGCGCAGACGGAAGAAGAGAGGCCTGTTTGGCGCCATTGC
CGGCTTTATCGAAGGCGGCTGGCAAGGCA TGGTGGACGGATGGTACGGCTACCATCACAGCAACGAG
CAAGGCTCTGGCTACGCCGCCGACAAAGAGAGCACACAGAAAGCCATCGACGGCGTGACCAACAAAG
TGAACAGCATCATCGACAAGATGAACACCCAGTTTCGAGGCCGTGGGCAGAGAGTTCAACAACCTGGA
 10 ACGGCGGATCGAGAATCTGAACAAGAAGATGGAGGACGGCTTCCTGGACGTGTGGACCTACAATGCC
GAGCTGCTGGTCTGATGGAAAACGAGAGAACCCTGGACTTCCACGACTCCAACGTGAAGAACCTGT
ACGACAAAGTGCGGCTCCAGCTGCGGGACAACGCCAAAGAACTCGGCAACGGCTGCTTCGAGTTCTA
CCACAAGTGCAGACAACGAGTGCATGGAAAGCGTGCAGAACGGCACCTACGACTACCCCTCAGTACAGC
GAGGAAGCCCGGCTGAAGAGAGAAGAGATCAGCGGAGTGAAGCTGGAATCCATCGGCACATACCAGA
 15 TCCTGTCCATCTACAGCACCGTGGCCTCTTCTCTGGCCCTGGCCATTATGGTGGCTGGCCTGTCTCT
GTGGATGTGCAGCAATGGCAGCCTCCAGTGCCGGATCTGCATCTGAGCGGCC

FLU_T4_HA_3 – Head region amino acid sequence (SEQ ID NO:91)

THNGKLCDLNGVKPLILKDCSVAGWLLGNPMCEDEFIRVPEWSYIVERANPANDLCFPGNLNDYEELKHLLSRIN
 HFEKILIIIPKSSWPNHNTSLGVSAACPYQGTSPSFFRNWVWLIKNDTYPTIKISYNNNTNREDLLILWGIHHSNN
 20 TAEQTNLYKNPTTYISVGTSTLNQRLVPKIANRSQVNGQRGRMDFFWTILKPNDAIHFESNGNFIAPYAYKIV
 KKG DSTIMKSEVEYGH CNTKCQTPIGAINSSMPFHNIHPLTIGCEP

FLU_T4_HA_3 – Head region nucleic acid sequence (SEQ ID NO:92)

TCCTGGAAAAGACCCACAACGGCAAGCTGTGCGACCTGAACGGCGTGAAGCCTCTGATCCTGAAGGA
 TTGCTCTGTGGCCGGATGGCTGCTGGGCAATCCCATGTGCGACGAGTTCATCAGAGTGCCCGAGTGG
 25 TCCTACATCGTGGAAGAGCCAATCCTGCCAACGACCTGTGCTTCCCCGGCAACCTGAACGACTACG
 AGGAACTGAAGCACCTCCTGAGCCGGATCAACCACTTCGAGAAGATCCTGATCATCCCCAAGAGCAG
 CTGGCCCAACCAACAATACCAGCCTGGGAGTGTCTGCCGCATGTCCATATCAGGGCACCCCTAGCTTT
 TTCCGGAACGTCTGTGTGGCTGATCAAGAAGAACGACACATACCCCAACCATCAAGATCAGCTACAACA
 ACACCAACCGCGAGGACCTGCTGATCCTGTGGGGAATCCACCACAGCAACAATACCGCCGAGCAGAC
 30 CAACCTGTACAAGAACCCCAACCTACATCAGCGTGGGCACCAGCACACTGAACCAGAGACTGGTG
 CCTAAGATCGCCAACCGCAGCCAAGTGAATGGCCAGAGGGGCAGAATGGACTTCTTCTGGACCATCC
 TGAAGCCTAACGACGCCATCCACTTTGAGAGCAACGGCAACTTTATCGCCCCTGAGTACGCCTACAA
 GATCGTGAAGAAGGGCGACAGCACCATCATGAAGTCCGAGGTGGAATACGGCCACTGCAACACCAAG
 TGTGAGACCCCTATCGGCGCCATCAACTCCAGCATGCCCTTCCACAACATTACCCCTCTGACCATCG
 35 GCGAGTGCCCCAAATACGTG

FLU_T4_HA_3 – First stem region amino acid sequence (SEQ ID NO:93)

MEKIVLLLLAIVSLVKSDQICIGYHANNSTEQVDTIMEKNVTVTTHAQDILEK

FLU_T4_HA_3 – First stem region nucleic acid sequence (SEQ ID NO:94)

GTACCGCCACCATGGAAAAGATCGTGCTGCTGCTGGCCATCGTGTCCCTGGTCAAGAGCGACCAAAT
CTGCATCGGCTACCACGCCAACACAGCACCGAACAGGTGGACACCATATTATGGAAAAGAACGTCACC
5 GTGACACACGCCCAGGACA

FLU_T4_HA_3 – Second stem region amino acid sequence (SEQ ID NO:95)

KYVKSNNKLVLATGLRNSPLREKRRRRKKRGLFGAIAAGFIEGGWQGMVDGWYGYHHSNEQSGYAADKESTQKAID
GVTNKVNSIIDKMNTQFEAVGREFNNLERRIENLNKKMEDGFLDVWTYNAELLVLMENERTLDFHDSNVKNLYD
KVRLQLRDNAKELGNGCFEFYHKCDNECMESVRNGTYDYPQYSEEARLKREEISGVKLESIGTYQILSIYSTVA
10 SSLALAIMVAGLSLWMCSNGSLQCRICI

FLU_T4_HA_3 – Second stem region nucleic acid sequence (SEQ ID NO:96)

AAGTCCAACAAGCTGGTGCTGGCTACCGGCCTGAGAAACAGCCCTCTGAGAGAGAAGCGCAGACGGA
AGAAGAGAGGCCTGTTTGGCGCCATTGCCGGCTTTATCGAAGGCGGCTGGCAAGGCATGGTGGACGG
15 ATGGTACGGCTACCATCACAGCAACGAGCAAGGCTCTGGCTACGCCGCCGACAAAGAGAGCACACAG
AAAGCCATCGACGGCGTGACCAACAAAGTGAACAGCATCATCGACAAGATGAACACCCAGTTCGAGG
CCGTGGGCAGAGAGTTCAACAACCTGGAACGGCGGATCGAGAATCTGAACAAGAAGATGGAGGACGG
CTTCTGGACGTGTGGACCTACAATGCCGAGCTGCTGGTCCTGATGGAAAACGAGAGAACCCTGGAC
TTCCACGACTCCAACGTGAAGAACCTGTACGACAAAGTGCGGCTCCAGCTGCGGGACAACGCCAAAG
20 AACTCGGCAACGGCTGCTTCGAGTTCTACCACAAGTGCGACAACGAGTGCATGGAAAGCGTGCGGAA
CGGCACCTACGACTACCCTCAGTACAGCGAGGAAGCCCGGCTGAAGAGAGAAGAGATCAGCGGAGTG
AAGCTGGAATCCATCGGCACATACCAGATCCTGTCCATCTACAGCACCGTGGCCTCTTCTCTGGCCC
TGGCCATTATGGTGGCTGGCCTGTCTCTGTGGATGTGCAGCAATGGCAGCCTCCAGTGCCGGATCTG
CATCTGAGCGGCC

25 Example 32 – pEVAC-FLU_T4_HA_3

This example provides the nucleic acid sequence of pEVAC-FLU_T4_HA_3.

pEVAC-FLU_T4_HA_3 – nucleic acid sequence (SEQ ID NO:97):

LOCUS pVRC8400EVAC_Ar_pEVAC-Flu_T4_HA_3 6092 bp DNA
linear SYN 08-SEP-2022

30 FEATURES Location/Qualifiers
CDS 1344..3070
/label="Flu_T4_HA_3"
/note="CI:V1=KpnI,V2=NotI,I1=KpnI,I2=NotI"

ORIGIN

1 TCGCGCGTTT CGGTGATGAC GGTGAAAACC TCTGACACAT GCAGCTCCCG GAGACGGTCA
 61 CAGCTTGTCT GTAAGCGGAT GCCGGGAGCA GACAAGCCCG TCAGGGCGCG TCAGCGGGTG
 121 TTGGCGGGTG TCGGGGCTGG CTTAACTATG CGGCATCAGA GCAGATTGTA CTGAGAGTGC
 5 181 ACCATATGCG GTGTGAAATA CCGCACAGAT GCGTAAGGAG AAAATACCGC ATCAGATTGG
 241 CTATTGGCCA TTGCATACGT TGTATCCATA TCATAATATG TACATTTATA TTGGCTCATG
 301 TCCAACATTA CCGCCATGTT GACATTGATT ATTGACTAGT TATTAATAGT AATCAATTAC
 361 GGGGTCATTA GTTCATAGCC CATATATGGA GTTCCGCGTT ACATAACTTA CGGTAAATGG
 421 CCCGCCTGGC TGACCGCCCA ACGACCCCG CCCATTGACG TCAATAATGA CGTATGTTCC
 10 481 CATAGTAACG CCAATAGGGA CTTTCCATTG ACGTCAATGG GTGGAGTATT TACGGTAAAC
 541 TGCCCACTTG GCAGTACATC AAGTGTATCA TATGCCAAGT ACGCCCCCTA TTGACGTCAA
 601 TGACGGTAAA TGGCCCGCCT GGCATTATGC CCAGTACATG ACCTTATGGG ACTTTCCTAC
 661 TTGGCAGTAC ATCTACGTAT TAGTCATCGC TATTACCATG GTGATGCGGT TTTGGCAGTA
 721 CATCAATGGG CGTGGATAGC GGTTTGACTC ACGGGGATTT CCAAGTCTCC ACCCCATTGA
 15 781 CGTCAATGGG AGTTTGTTTT GGCACCAAAA TCAACGGGAC TTTCCAAAAT GTCGTAACAA
 841 CTCCGCCCCA TTGACGCAAA TGGGCGGTAG GCGTGTACGG TGGGAGGTCT ATATAAGCAG
 901 AGCTCGTTTA GTGAACCGTC AGATCGCCTG GAGACGCCAT CCACGCTGTT TTGACCTCCA
 961 TAGAAGACAC CGGGACCGAT CCAGCCTCCA TCGGCTCGCA TCTCTCCTTC ACGCGCCCGC
 1021 CGCCCTACCT GAGGCCGCCA TCCACGCCGG TTGAGTCGCG TTCTGCCGCC TCCCGCCTGT
 20 1081 GGTGCCTCCT GAACTGCGTC CGCCGTCTAG GTAAGTTTAA AGCTCAGGTC GAGACCGGGC
 1141 CTTTGTCCGG CGCTCCCTTG GAGCCTACCT AGACTCAGCC GGCTCTCCAC GCTTTGCCTG
 1201 ACCCTGCTTG CTCAACTCTA GTTAACGGTG GAGGGCAGTG TAGTCTGAGC AGTACTCGTT
 1261 GCTGCCGCGC GCGCCACCAG ACATAATAGC TGACAGACTA ACAGACTGTT CCTTTCCATG
 1321 GGTCTTTTCT GCAGTCACCG TCGGTACCGC CACCATGGAA AAGATCGTGC TGCTGCTGGC
 25 1381 CATCGTGTCC CTGGTCAAGA GCGACCAAAT CTGCATCGGC TACCACGCCA ACAACAGCAC
 1441 CGAACAGGTG GACACCATTA TGGAAAAGAA CGTCACCGTG ACACACGCCC AGGACATCCT
 1501 GGAAAAGACC CACAACGGCA AGCTGTGCGA CCTGAACGGC GTGAAGCCTC TGATCCTGAA
 1561 GGATTGCTCT GTGGCCGGAT GGCTGCTGGG CAATCCCATG TGCGACGAGT TCATCAGAGT
 1621 GCCCGAGTGG TCCTACATCG TGGAAAGAGC CAATCCTGCC AACGACCTGT GCTTCCCCGG
 30 1681 CAACCTGAAC GACTACGAGG AACTGAAGCA CCTCCTGAGC CGGATCAACC ACTTCGAGAA
 1741 GATCCTGATC ATCCCCAAGA GCAGCTGGCC CAACCACAAT ACCAGCCTGG GAGTGTCTGC
 1801 CGCATGTCCA TATCAGGGCA CCCCTAGCTT TTTCCGGAAC GTCGTGTGGC TGATCAAGAA
 1861 GAACGACACA TACCCACCA TCAAGATCAG CTACAACAAC ACCAACCGCG AGGACCTGCT
 1921 GATCCTGTGG GGAATCCACC ACAGCAACAA TACCGCCGAG CAGACCAACC TGTACAAGAA
 35 1981 CCCCACCACC TACATCAGCG TGGGCACCAG CACACTGAAC CAGAGACTGG TGCCTAAGAT
 2041 CGCCAACCGC AGCCAAGTGA ATGGCCAGAG GGGCAGAATG GACTTCTTCT GGACCATCCT
 2101 GAAGCCTAAC GACGCCATCC ACTTTGAGAG CAACGGCAAC TTTATCGCCC CTGAGTACGC

2161 CTACAAGATC GTGAAGAAGG GCGACAGCAC CATCATGAAG TCCGAGGTGG AATACGGCCA
 2221 CTGCAACACC AAGTGTGAGA CCCCTATCGG CGCCATCAAC TCCAGCATGC CCTTCCACAA
 2281 CATTACCCCT CTGACCATCG GCGAGTGGCC CAAATACGTG AAGTCCAACA AGCTGGTGCT
 2341 GGCTACCGGC CTGAGAAACA GCCCTCTGAG AGAGAAGCGC AGACGGAAGA AGAGAGGCCT
 5 2401 GTTTGGCGCC ATTGCCGGCT TTATCGAAGG CGGCTGGCAA GGCATGGTGG ACGGATGGTA
 2461 CGGCTACCAT CACAGCAACG AGCAAGGCTC TGGCTACGCC GCCGACAAAG AGAGCACACA
 2521 GAAAGCCATC GACGGCGTGA CCAACAAAGT GAACAGCATC ATCGACAAGA TGAACACCCA
 2581 GTTCGAGGCC GTGGGCAGAG AGTTCAACAA CCTGGAACGG CGGATCGAGA ATCTGAACAA
 2641 GAAGATGGAG GACGGCTTCC TGGACGTGTG GACCTACAAT GCCGAGCTGC TGGTCCTGAT
 10 2701 GGAAAACGAG AGAACCCTGG ACTTCCACGA CTCCAACGTG AAGAACCTGT ACGACAAAGT
 2761 GCGGCTCCAG CTGCGGGACA ACGCCAAAGA ACTCGGCAAC GGCTGCTTCG AGTTCTACCA
 2821 CAAGTGCAC AACGAGTGCA TGGAAGCGT GCGGAACGGC ACCTACGACT ACCCTCAGTA
 2881 CAGCGAGGAA GCCCGGCTGA AGAGAGAAGA GATCAGCGGA GTGAAGCTGG AATCCATCGG
 2941 CACATACCAG ATCCTGTCCA TCTACAGCAC CGTGGCCTCT TCTCTGGCCC TGGCCATTAT
 15 3001 GGTGGCTGGC CTGTCTCTGT GGATGTGCAG CAATGGCAGC CTCCAGTGCC GGATCTGCAT
 3061 CTGAGCGGCC GCAGATCTGC TGTGCCTTCT AGTTGCCAGC CATCTGTTGT TTGCCCCCTCC
 3121 CCCGTGCCTT CCTTGACCCT GGAAGGTGCC ACTCCCACTG TCCTTTCCTA ATAAAATGAG
 3181 GAAATTGCAT CGCATTGTCT GAGTAGGTGT CATTCTATTC TGGGGGGTGG GGTGGGGCAG
 3241 GACAGCAAGG GGGAGGATTG GGAAGACAAT AGCAGGCATG CTGGGGATGC GGTGGGCTCT
 20 3301 ATGGCTACCC AGGTGCTGAA GAATTGACCC GGTTCCCTCCT GGGCCAGAAA GAAGCAGGCA
 3361 CATCCCTTC TCTGTGACAC ACCCTGTCCA CGCCCCTGGT TCTTAGTTCC AGCCCCACTC
 3421 ATAGGACACT CATAGCTCAG GAGGGCTCCG CCTTCAATCC CACCCGCTAA AGTACTTGGA
 3481 GCGGTCTCTC CCTCCCTCAT CAGCCCACCA AACC AACCT AGCCTCCAAG AGTGGGAAGA
 3541 AATTAAAGCA AGATAGGCTA TTAAGTGCAG AGGGAGAGAA AATGCCTCCA ACATGTGAGG
 25 3601 AAGTAATGAG AGAAATCATA GAATTTTAAG GCCATGATTT AAGGCCATCA TGGCCTTAAT
 3661 CTTCCGCTTC CTCGCTCACT GACTCGCTGC GCTCGGTCGT TCGGCTGCGG CGAGCGGTAT
 3721 CAGCTCACTC AAAGGCGGTA ATACGGTTAT CCACAGAATC AGGGGATAAC GCAGGAAAGA
 3781 ACATGTGAGC AAAAGGCCAG CAAAAGGCCA GGAACCGTAA AAAGGCCGCG TTGCTGGCGT
 3841 TTTTCCATAG GCTCCGCCCC CCTGACGAGC ATCACAAAAA TCGACGCTCA AGTCAGAGGT
 30 3901 GGCGAACCC GACAGGACTA TAAAGATACC AGGCGTTTCC CCCTGGAAGC TCCCTCGTGC
 3961 GCTCTCCTGT TCCGACCCTG CCGCTTACCG GATACCTGTC CGCCTTTCTC CCTTCGGGAA
 4021 GCGTGGCGCT TTCTCATAGC TCACGCTGTA GGTATCTCAG TTCGGTGTAG GTCGTTGCT
 4081 CCAAGCTGGG CTGTGTGCAC GAACCCCCCG TTCAGCCCGA CCGCTGCGCC TTATCCGGTA
 4141 ACTATCGTCT TGAGTCCAAC CCGGTAAGAC ACGACTTATC GCCACTGGCA GCAGCCACTG
 35 4201 GTAACAGGAT TAGCAGAGCG AGGTATGTAG GCGGTGCTAC AGAGTTCTTG AAGTGGTGGC
 4261 CTAACCTACG CTACACTAGA AGAACAGTAT TTGGTATCTG CGCTCTGCTG AAGCCAGTTA
 4321 CCTTCGGAAA AAGAGTTGGT AGCTCTTGAT CCGGCAAACA AACCACCGCT GGTAGCGGTG

4381 GTTTTTTTGT TTGCAAGCAG CAGATTACGC GCAGAAAAAA AGGATCTCAA GAAGATCCTT
 4441 TGATCTTTTC TACGGGGTCT GACGCTCAGT GGAACGAAAA CTCACGTTAA GGGATTTTGG
 4501 TCATGAGATT ATCAAAAAGG ATCTTCACCT AGATCCTTTT AAATTAATAA TGAAGTTTAA
 4561 AATCAATCTA AAGTATATAT GAGTAAACTT GGTCTGACAG TTACCAATGC TTAATCAGTG
 5 4621 AGGCACCTAT CTCAGCGATC TGTCTATTTT GTTCATCCAT AGTTGCCTGA CTCGGGGGGG
 4681 GGGGGCGCTG AGGTCTGCCT CGTGAAGAAG GTGTTGCTGA CTCATACCAG GCCTGAATCG
 4741 CCCCATCATC CAGCCAGAAA GTGAGGGAGC CACGGTTGAT GAGAGCTTTG TTGTAGGTGG
 4801 ACCAGTTGGT GATTTTGAAC TTTTGCTTTG CCACGGAACG GTCTGCGTTG TCGGGAAGAT
 4861 GCGTGATCTG ATCCTTCAAC TCAGCAAAAG TTCGATTAT TCAACAAAGC CGCCGTCCCG
 10 4921 TCAAGTCAGC GTAATGCTCT GCCAGTGTTA CAACCAATTA ACCAATCTG ATTAGAAAAA
 4981 CTCATCGAGC ATCAAATGAA ACTGCAATTT ATTCATATCA GGATTATCAA TACCATATTT
 5041 TTGAAAAAGC CGTTTCTGTA ATGAAGGAGA AAACCTACCG AGGCAGTTCC ATAGGATGGC
 5101 AAGATCCTGG TATCGGTCTG CGATTCCGAC TCGTCCAACA TCAATACAAC CTATTAATTT
 5161 CCCCTCGTCA AAAATAAGGT TATCAAGTGA GAAATCACCA TGAGTGACGA CTGAATCCGG
 15 5221 TGAGAATGGC AAAAGCTTAT GCATTTCTTT CCAGACTTGT TCAACAGGCC AGCCATTACG
 5281 CTCGTCATCA AAATCACTCG CATCAACCAA ACCGTTATTC ATTCGTGATT GCGCCTGAGC
 5341 GAGACGAAAT ACGCGATCGC TGTTAAAAGG ACAATTACAA ACAGGAATCG AATGCAACCG
 5401 GCGCAGGAAC ACTGCCAGCG CATCAACAAT ATTTTCACCT GAATCAGGAT ATTCTTCTAA
 5461 TACCTGGAAT GCTGTTTTCC CGGGGATCGC AGTGGTGAGT AACCATGCAT CATCAGGAGT
 20 5521 ACGGATAAAA TGCTTGATGG TCGGAAGAGG CATAAATTCC GTCAGCCAGT TTAGTCTGAC
 5581 CATCTCATCT GTAACATCAT TGGCAACGCT ACCTTTGCCA TGTTTCAGAA ACAACTCTGG
 5641 CGCATCGGGC TTCCCATAACA ATCGATAGAT TGTCGCACCT GATTGCCCCG CATTATCGCG
 5701 AGCCCATTTA TACCCATATA AATCAGCATC CATGTTGGAA TTTAATCGCG GCCTCGAGCA
 5761 AGACGTTTCC CGTTGAATAT GGCTCATAAC ACCCCTTGTA TTAAGTTTGA TGTAAGCAGA
 25 5821 CAGTTTTATT GTTCATGATG ATATATTTTT ATCTTGTCGA ATGTAACATC AGAGATTTTG
 5881 AGACACAACG TGGCTTTCCC CCCCCCCCCA TTATTGAAGC ATTTATCAGG GTTATTGTCT
 5941 CATGAGCGGA TACATATTTG AATGTATTTA GAAAAATAAA CAAATAGGGG TTCCGCGCAC
 6001 ATTTCCCCGA AAAGTGCCAC CTGACGTCTA AGAAACCATT ATTATCATGA CATTACCTA
 6061 TAAAAATAGG CGTATCACGA GGCCCTTTTCG TC

Example 33 – Residue differences in amino acid sequence of influenza Tier 4 H5 vaccine candidates FLU_T4_HA_1, FLU_T4_HA_2, and FLU_T4_HA_3, compared influenza Tier 2 and Tier 3 H5 designs, and wild-type H5 strains

Figure 25 summarises novel amino acid residue changes in influenza haemagglutinin H5 for embodiments of the invention relating to FLU_T4_HA_1, FLU_T4_HA_2, and FLU_T4_HA_3 designed sequences. These novel amino acid residue changes are shown in bold and underline for each of FLU_T4_HA_1, FLU_T4_HA_2, and FLU_T4_HA_3. In particular,

amino acid residues at positions 107, 142, 200, and 231 of A/Sichuan/2014 H5 have been changed in some or all of the designed sequences according to the invention. These residue alterations are not present at the corresponding residue positions in the known wild type H5 sequences or previous H5 designed sequences shown in Figure 25. Residue positions 107, 142, 200, and 231 are at epitope regions in the H5 head region, and the amino acid changes at these positions in the new T4 H5 designs alter the affinity of H5 towards binding antibodies.

Figure 26 shows important amino acid residue positions of H5, in particular, residue positions 107, 142, 172, 200, 231, 238, 344, and 345, corresponding to amino acid residue positions of A/Sichuan/2014. Positions 142, 172, 200, and 231 of H5 are at epitope regions in the head region, and positions 344-345 are at an epitope region in the stem region. Position 107 is at a receptor binding site. Amino acid residue changes at these positions alter the affinity of H5 towards binding antibodies. Position 238 is at a receptor binding site in the head region. Mutation at this residue reduces the affinity of HA to its receptor (sialic acid) on the surface of target cells, thus increasing the bioavailability of HA for antigen presentation. The residues shown in bold and underline format are novel amino acid residues at the positions disclosed above, which are not present in the H5 wild type sequences shown or in previous designed H5 sequences.

Figure 27 summarises amino acid residues of H5 FLU_T4_HA_1, FLU_T4_HA_2, and FLU_T4_HA_3, at important residue positions of H5. Positions A, B, and C of H5 are at epitope regions in the head region, and residue changes at these positions alter the affinity of H5 towards binding antibodies. Positions D and E are in the H5 stem region, and mutations at these positions alter the stability of the stem region both in the pre-fusion and post-fusion state. The amino acid residues at positions 148, 149, and 238 are at receptor binding sites. Amino acid changes at these residue positions alter the affinity of HA to its receptor (sialic acid) on the surface of target cells, thus increasing or decreasing the bioavailability of HA for antigen presentation.

Figure 28 shows a multiple sequence alignment of H5 amino acid sequence for FLU_T4_HA_1, FLU_T4_HA_2, and FLU_T4_HA_3, known wild-type influenza H5 strains, and previously designed H5 sequences. The amino acid residue positions in the figure correspond to the amino acid residue positions of A/Sichuan/2014 (SEQ ID NO:100):

> EPI533583_A/Sichuan/26221/2014_H5N6 (SEQ ID NO:100)

MEKIVLLLAIVSLVKGDQICIGYHANNSTEQVDTIMEKNVTVTTHAQDILEKTHNGKLCDLNG
VKPLILKDCSVAGWLLGNPMCDEFIRVPEWSYIVERANPANDLCYPGNLNDYEELKHLLSR
INHFEEKILIIPKSSWTNHETSLGVSAACPYQGTSPFFRNVWLIKKNDAYPTIKISYNNTNQE
DLLILWGVHHSNNAAEQTNLYKNPTTYISVGTSTLNQRLVPKIATRSQVNGQRGRMDFFW

TILKPNDIAHFESNGNFIAP EYAYKIVKKG DSTIMKSEMEYGH CNTKCQTP IGA INSSMPFH
 NIHPLTIG ECPKYVKS NKLVLATGLR NSPLREKRRKRGLFGA IAGFIEGGWQGMVDGWYG
 YHHSNEQGS GYAADKESTQKAIDGV TNKVN SIIDKMNTQFEAVGREFN NLERRIENLNKK
 MEDGFLDVW TYNAELLVLMENERTLDFHDSNVKNLYDKVRLQLR DNAKELGNGCFEFYH
 5 KCDNKCME SVRNGTYDYPQYSEEARKREEISGVKLESIGTYQILSIYSTVASSLALAIIVAG
 LSLWMCSNGSLQCRICI

Example 34

Designed candidate H5 vaccine antigens induce broad neutralising responses against
 10 panel of influenza H5 clade 2.3.4.4.

Mice (n=6) immunised with our DIOS H5 DNA vaccines twice at 30 day interval:

- H5_Anc_4 [T4_HA_1]
- H5_Anc_4_mut1 [T4_HA_2]
- H5_Anc_4_mut2 [T4_HA_3]

15 Figure 29a is a summary of the neutralising activity of the candidate H5 vaccine antigens against a panel of clade 2.3.4.4. H5 viruses. The figure shows that the neutralising response elicited by immunisation by any one of the three designed sequences vs five clade 2.3.4.4 H5Nx strains, is comparable to the controls wherein the subjects were immunised with antigens from the same clade as the challenge strain (ns>0.05). These immune responses
 20 are broadly-neutralising and cover the 2.3.4.4 sub-clades. It is important to note that our designs generate good neutralising responses against one of the recent human H5 strains (A/Hangzhou/01/2021). The “ns” label denotes that the non-significant difference between our DIOS candidate and either the matched strain or the matched H5 clade is $P < 0.05$ (Kruskal Wallis).

25 Figures 29B shows neutralisation assay data for the vaccine designs vs controls. Figure 29B shows that the DIOS candidates elicit equivalent responses to homologous strain viz. A/Sichuan/2014 but higher responses than the heterologous strain A/gyr/WSA and A/Anhui/2020 (left). The DIOS candidates also elicit equivalent responses to homologous strain viz. A/gyr/WSA but higher responses than the heterologous strain A/Anhui/2020 and
 30 A/Sichuan/2014 (right). Our best candidates from previous tier, FLU_T2_HA_9 (H5_ANC_1), and FLU_T3_HA_2 (H5_ANC_3), show poor response.

Figure 29C shows that the DIOS candidates elicit equivalent responses to homologous strain viz. A/Anhui/ 2020 but higher responses than the heterologous strain A/gyr/WSA and

A/Sichuan/2014 (left). The figure also shows that the DIOS candidates (H5_ANC_4 and H5_ANC_4_mut1) elicit better responses to clade 2.3.4.4b (A/mute swan/England/053054/2021) challenge in comparison to H5 controls viz. A/gyr/WSA, A/Anhui/2020 and A/Sichuan/2014, and the DIOS candidate H5_ANC_4_mut2 elicits a comparative neutralisation response to the challenge. Our best candidates from previous tier, FLU_T2_HA_9 (H5_ANC_1), and FLU_T3_HA_2 (H5_ANC_3), show poor response to clade 2.3.4.4b.

Figure 29D is a repeat neutralisation assay of the assay performed in Figure 29c right panel, and shows that the DIOS candidates (H5_ANC_4 and H5_ANC_4_mut1) again elicit better responses to clade 2.3.4.4b (A/mute swan/England/053054/2021) challenge in comparison to H5 controls viz. A/gyr/WSA, A/Anhui/2020 and A/Sichuan/2014, and the DIOS candidate H5_ANC_4_mut2 elicits a comparative neutralisation response to the challenge.

Figures 30A-I show individual neutralisation curves for mice immunised with a control PBS vaccine (A), DIOS candidate vaccine designs H5_ANC_4 (B), H5_ANC_4_mut1 (C), H5_ANC_4_mut2 (D), previous H5 vaccine designs H5_ANC_1 (T2_HA_9)(E), H5_ANC_3 (T3_HA_2)(F), or homologous (H) or heterologous (G or I) WT strains vs A/gyrfalcon/Washington/41088-6/2014) clade 2.3.4.4c. challenge strain.

Figures 31A-I similarly show individual neutralisation curves for mice immunised with either control PBS vaccine (A), new vaccine designs (B, C, D), previous DIOS vaccine candidates (E, F), or homologous (G) or heterologous WT strain (H and I) vs A/Sichuan/26221/2014 clade 2.3.4.4a challenge strain.

Figures 32A-I similarly show individual neutralisation curves for mice immunised with either control PBS vaccine (A), new vaccine designs (B, C, D), previous DIOS vaccine candidates (E, F), or homologous (I) or heterologous strain (G, H) vs A/Anhui/2021-00011/2020 clade 2.3.4.4h challenge strain.

Figures 33A-I show individual neutralisation curves for mice immunised with a control PBS vaccine (A), DIOS candidate vaccine designs H5_ANC_4 (B), H5_ANC_4_mut1 (C), H5_ANC_4_mut2 (D), previous H5 vaccine designs H5_ANC_1 (T2_HA_9)(E), H5_ANC_3 (T3_HA_2)(F), or heterologous (G, H or I) strains vs A/mute swan/England/053054/2021 clade 2.3.4.4b. challenge strain.

Figures 34A-I show individual neutralisation curves for mice immunised with a control PBS vaccine (A), DIOS candidate vaccine designs H5_ANC_4 (B), H5_ANC_4_mut1 (C), H5_ANC_4_mut2 (D), previous H5 vaccine designs H5_ANC_1 (T2_HA_9)(E), H5_ANC_3

(T3_HA_2)(F), or heterologous (G, H or I) strains vs A/Hangzhou/01/2021 clade 2.3.4.4b. challenge strain.

Example 35 – FLU_T3_NA_3

- 5 This example provides the amino acid and nucleic acid sequences of the influenza neuraminidase region for the embodiment of the invention known as FLU_T3_NA_3.

FLU_T3_NA_3 amino acid sequence (SEQ ID NO:98)

MNPNQKIITIGSICMVVGIISLILQIGNIISIWVSHSIQTGNQNH PETCNQSIITYENNTWVNQTYVNI SNTNF
VAEQDVT SVVL AGNSSLCPI SGWAIYSKDN GIRIGSKGDV FVIREPFISCSHLECR TFFLTQ GALLNDKHSNGT
10 VKDRSPYRTL MSCPVGEAPSPYNSRFESVAWSASACHD GMSWLTIGISGPD SGAVAVLKYNGIITDTIKSWRNN
ILRTQESE CACINGSCFTIMTDGPSD GQASYKIFKIEKGKVKSVELNAPNYHYEECS CYPDAGKVMCVCRDNW
HGSNRPWVSFDQ NLEYQIGYICSGVFGDNPRPNDGTGSCGPVSSNGANGVKGFSFRYNGVWIGRTKSISSRKG
FEMIWDPN GWTETDSSFSVKQDIVGINEWSGYSGSFVQHPELTGLDCMRPCFWVELIRGRPEENTIWTSGSSIS
FCGVNSD TVGWSWPDGAELPFTIDK

15 FLU_T3_NA_3 nucleic acid sequence (SEQ ID NO:98)

ATGAACCCAAATCAGAAGATTATCACTATTGGTTCTATCTGTATGGTGGTAGGCATCATTTCACTTA
TCCTCCAGATTGGAACATTATATCCATTTGGGTGTACACAGTATTCAGACTGGGAACCAGAACCA
TCCTGAGACTTGTAATCAATCCATCATTACATACGAAAACAACACCTGGGTCAATCAGACCTATGTG
AACATAAGCAATACAACTTTGTGGCCGAGCAGGACGTGACATCCGTGGTCCCTTG CAGGAACTCCA
20 GCCTGTGTCCCATTAGCGGTTGGGCAATTTACTCAAAGGATAACGGCATCAGGATTGGTTCCAAGGG
TGACGTGTTCTGTAATCAGGGAGCCATTTATTTCTCTGCTCACACCTCGAATGCAGAACCTTCTTCTCTG
ACTCAGGGGGCACTCCTGAATGATAAGCATTCGAATGGAACAGTGAAAGACCGCTCCCCCTATAGGA
CATTGATGTCTCTGTCTGTTGGTGAGGCCCATCTCCTTATAATAGTAGGTTTGAGAGTGTGGCCTG
GTCCGCAAGTGCTTGTACGATGGGATGTCTGGCTGACCATTGGTATTTCTGGTCCAGACTCTGGA
25 GCCGTGGCTGTTCTGAAATATAACGGAATAATCACTGACACAATCAAAAGTTGGCGAAATAATATCC
TGAGGACCCAGGAGAGCGAGTGTGCTTGCATAAATGGAAGTTGTTTCACTATTATGACCGATGGGCC
ATCCGATGGGCAGGCTTCATATAAAATCTTCAAATCGAAAAGGGTAAGGTTGTGAAGTCCGTCGAA
CTGAATGCTCCTAATTACCATTTACGAAGAATGCTCCTGCTACCCCGACGCTGGCAAAGTGATGTGCG
TATGTGAGATAACTGGCACGGGAGTAATAGACCTTGGGTGTCCTTCGACCAAACTTGGAATACCA
30 AATAGGCTACATTTGTTTCAGGGGTGTTCTGGCGACAATCCTCGGCCAAACGATGGGACAGGTTCTCTGT
GGGCCAGTTTCTTCAAACGGAGCCAATGGGGTCAAAGGCTTCAGTTTCAGATACGGCAACGGGGTGT
GGATTGGCCGAACCAAGAGCATTTCCAGCCGAAAGGGATTGAGATGATTTGGGACCTAACGGGTG
GACCGAGACGGACAGTTCTTTTCAGTGAAACAAGATATTGTGGGCATCAACGAATGGAGCGGATAT
AGCGGGTCTTCTGTCAGCACCCAGAACTCACAGGACTGGATTGTATGCGGCCCTGTTTCTGGGTAG
35 AACTCATTAGAGGCAGACCCGAAGAGAACACAATCTGGACATCAGGCAGTTCCATTTCTTCTGCGG
GGTGAATAGCGATACAGTGGGATGGTCTTGGCCTGATGGTGCCGAATTGCCATTCACAATAGATAAG

Claims

1. An isolated polypeptide comprising a haemagglutinin subtype 5 (H5) globular head domain, and optionally a haemagglutinin stem domain, wherein the polypeptide comprises an amino acid sequence in which an amino acid residue at a position corresponding to
5 residue position 144 or 145 of a wild-type H5 globular head domain has been deleted.
2. An isolated polypeptide according to claim 1, wherein the polypeptide comprises an amino acid sequence in which an amino acid residue at a position corresponding to residue position 144 of the wild-type H5 globular head domain has been deleted.
3. An isolated polypeptide according to claim 1, wherein the polypeptide comprises an
10 amino acid sequence in which an amino acid residue at a position corresponding to residue position 145 of the wild-type H5 globular head domain has been deleted.
4. An isolated polypeptide according to any of claims 1 to 3, wherein the polypeptide comprises an amino acid sequence that has at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%,
15 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% amino acid identity along its entire length with the amino acid sequence of SEQ ID NO:3.
5. An isolated polypeptide according to any preceding claim, which retains at least some HA activity of a wild-type H5 globular head domain.
6. An isolated polypeptide according to any preceding claim, wherein the polypeptide
20 comprises an amino acid sequence with the following amino acid residues at positions corresponding to residues 156, 157, 171, 172, and 205 of the wild-type H5 globular head domain:
 - 156: R;
 - 157: S;
 - 25 • 171: N;
 - 172: A; and
 - 205: R
7. An isolated polypeptide according to any preceding claim, which comprises an amino acid sequence of SEQ ID NO:27 or 29, or an amino acid sequence that has at least
30 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%

amino acid identity along its entire length with the amino acid sequence of SEQ ID NO:27 or 29.

8. An isolated polypeptide according to any preceding claim, which comprises an amino acid sequence of SEQ ID NO:29.

5 9. An isolated polypeptide according to any preceding claim, which comprises an amino acid sequence of SEQ ID NO:27.

10. An isolated polypeptide according to any preceding claim, wherein the polypeptide comprises a haemagglutinin stem domain, and wherein the polypeptide comprises an amino acid sequence with the following amino acid residues at positions corresponding to
10 residue positions 416 and 434 of a wild-type H5 sequence:

- 416: F; and
- 434: F

11. An isolated polypeptide according to claim 10, wherein the polypeptide comprises an amino acid sequence that has at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%,
15 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% amino acid identity along its entire length with the sequence of SEQ ID NO:27.

12. An isolated polypeptide comprising a haemagglutinin subtype 5 (H5) globular head domain, and optionally a haemagglutinin stem domain, wherein the polypeptide comprises
20 an amino acid sequence with the following amino acid residues at positions corresponding to residue positions 148 and 149 of a wild-type H5 globular head domain:

- 148: V;
- 149: P.

13. An isolated polypeptide according to claim 12, wherein the polypeptide comprises
25 an amino acid sequence with the following amino acid residue at a position corresponding to residue position 238 of a wild-type H5 globular head domain:

- 238: E.

14. An isolated polypeptide according to claim 12 or 13, wherein the polypeptide comprises an amino acid sequence that has at least 70%, 71%, 72%, 73%, 74%, 75%,
30 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%,

91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% amino acid identity along its entire length with the amino acid sequence of SEQ ID NO:3.

15. An isolated polypeptide according to any of claims 12 to 14, which retains at least some HA activity of a wild-type H5 globular head domain.

5 16. An isolated polypeptide according to any of claims 12 to 15, which has reduced affinity for its receptor compared with the wild-type H5 globular head domain.

17. An isolated polypeptide according to any of claims 12 to 16, wherein the polypeptide comprises an amino acid sequence with the following amino acid residues at positions corresponding to residues 156, 157, 171, 172, and 205 of the wild-type H5 globular head domain:

- 156: R;
- 157: S;
- 171: N;
- 172: A; and
- 15 • 205: R.

18. An isolated polypeptide according to any of claims 12 to 17, which comprises an amino acid sequence of SEQ ID NO:35 or 37, or an amino acid sequence that has at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% amino acid identity along its entire length with the amino acid sequence of SEQ ID NO:35 or 37.

19. An isolated polypeptide according to any of claims 12 to 18, which comprises an amino acid sequence of SEQ ID NO:37.

20. An isolated polypeptide according to any of claims 12 to 19, which comprises an amino acid sequence of SEQ ID NO:35.

21. An isolated polypeptide according to any of claims 12 to 20, wherein the polypeptide comprises a haemagglutinin stem domain, and wherein the polypeptide comprises an amino acid sequence with the following amino acid residues at positions corresponding to residue positions 416 and 434 of a wild-type H5 sequence:

- 30 • 416: F; and
- 434: F

22. An isolated polypeptide according to claim 21, wherein the polypeptide comprises an amino acid sequence that has at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% amino acid identity along its entire length with the sequence of SEQ ID NO:35.

23. An isolated polypeptide comprising a haemagglutinin subtype 5 (H5) globular head domain, and optionally a haemagglutinin stem domain, wherein the polypeptide comprises an amino acid sequence with the following amino acid residue at a position corresponding to residue position 238 of a wild-type H5 globular head domain:

- 238: E

24. An isolated polypeptide according to claim 23, wherein the polypeptide comprises an amino acid sequence with the following amino acid residues at positions corresponding to residue positions 148 and 149 of a wild-type H5 globular head domain:

- 148: S;
- 149: S.

25. An isolated polypeptide according to claim 23 or 24, wherein the polypeptide comprises an amino acid sequence that has at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% amino acid identity along its entire length with the amino acid sequence of SEQ ID NO:3.

26. An isolated polypeptide according to any of claims 23 to 25, which retains at least some HA activity of a wild-type H5 globular head domain.

27. An isolated polypeptide according to any of claims 23 to 26, which has reduced affinity for its receptor compared with the wild-type H5 globular head domain.

28. An isolated polypeptide according to any of claims 23 to 27, wherein the polypeptide comprises an amino acid sequence with the following amino acid residues at positions corresponding to residues 156, 157, 171, 172, and 205 of the wild-type H5 globular head domain:

- 156: R;
- 157: S;
- 171: N;

- 172: A; and
- 205: R.

29. An isolated polypeptide according to any of claims 23 to 28, which comprises an amino acid sequence of SEQ ID NO:43 or 45, or an amino acid sequence that has at least
 5 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% amino acid identity along its entire length with the amino acid sequence of SEQ ID NO:43 or 45.

30. An isolated polypeptide according to any of claims 23 to 29, which comprises an
 10 amino acid sequence of SEQ ID NO:45.

31. An isolated polypeptide according to any of claims 23 to 30, which comprises an amino acid sequence of SEQ ID NO:43.

32. An isolated polypeptide according to any preceding claim, wherein the polypeptide comprises an amino acid sequence with the following amino acid residues at positions
 15 corresponding to residue positions 279 and 298 of the wild-type H5 globular head domain:

- 279 A; and
- 298 M

33. An isolated polypeptide according to any preceding claim, wherein the polypeptide comprises a haemagglutinin stem domain, and wherein the polypeptide comprises an
 20 amino acid sequence with the following amino acid residues at positions corresponding to residue positions 416 and 434 of a wild-type H5 sequence:

- 416: F; and
- 434: F

34. An isolated polypeptide according to claim 33, wherein the polypeptide comprises
 25 an amino acid sequence that has at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% amino acid identity along its entire length with the sequence of any of SEQ ID NO: 27, 35, or 43.

35. An isolated nucleic acid molecule which comprises a nucleotide sequence encoding
 30 a polypeptide according to any of claims 1 to 34, or the complement thereof.

36. An isolated nucleic acid molecule according to claim 35, which comprises a nucleotide sequence of SEQ ID NO:28, 30, 32, or 34, or which comprises nucleotide sequence of SEQ ID NOs:32 and 34, or a nucleotide sequence that is at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical with SEQ ID NO: 28, 30, 32, 34, or with SEQ ID NO:32 and 34, over its entire length, or the complement thereof.
37. An isolated nucleic acid molecule according to claim 35, which comprises a nucleotide sequence of SEQ ID NO:28, 30, 32, or 34, or which comprises nucleotide sequence of SEQ ID NOs:32 and 34, or the complement thereof.
38. An isolated nucleic acid molecule according to claim 35, which comprises a nucleotide sequence of SEQ ID NO:28, or the complement thereof.
39. An isolated nucleic acid molecule according to claim 35, which comprises a nucleotide sequence of SEQ ID NO:36, 38, 40, or 42, or which comprises nucleotide sequence of SEQ ID NOs:40 and 42, or a nucleotide sequence that is at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical with SEQ ID NO: 36, 38, 40, or 42, or with SEQ ID NO:40 and 42, over its entire length, or the complement thereof.
40. An isolated nucleic acid molecule according to claim 35, which comprises a nucleotide sequence of SEQ ID NO:36, 38, 40, or 42, or which comprises nucleotide sequence of SEQ ID NOs:40 and 42, or the complement thereof.
41. An isolated nucleic acid molecule according to claim 35, which comprises a nucleotide sequence of SEQ ID NO:36, or the complement thereof.
42. An isolated nucleic acid molecule according to claim 35, which comprises a nucleotide sequence of SEQ ID NO:44, 46, 48, or 50, or which comprises nucleotide sequence of SEQ ID NOs 48 and 50, or a nucleotide sequence that is at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical with SEQ ID NO: 44, 46, 48, or 50, or with SEQ ID NO: 48 and 50, over its entire length, or the complement thereof.

43. An isolated nucleic acid molecule according to claim 35, which comprises a nucleotide sequence of SEQ ID NO:44, 46, 48, or 50, or which comprises nucleotide sequence of SEQ ID NOs 48 and 50, or the complement thereof.

44. An isolated nucleic acid molecule according to claim 35, which comprises a nucleotide sequence of SEQ ID NO:44, or the complement thereof.

45. An isolated nucleic acid molecule which comprises a nucleotide sequence of SEQ ID NO:52, 54, 55, 56, or which comprises nucleotide sequence of SEQ ID NOs:52 and 54, or a nucleotide sequence that is at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical with SEQ ID NO: 52, 54, 55, 56, or with SEQ ID NO:52 and 54, over its entire length, or the complement thereof.

46. An isolated nucleic acid molecule which comprises a nucleotide sequence of SEQ ID NO:52, 54, 55, 56, or which comprises nucleotide sequence of SEQ ID NOs:52 and 54, or the complement thereof.

47. An isolated nucleic acid molecule which comprises a nucleotide sequence of SEQ ID NO:55, or the complement thereof.

48. An isolated nucleic acid molecule which comprises a nucleotide sequence of SEQ ID NO:58, 60, 61, 62, or which comprises nucleotide sequence of SEQ ID NOs:58 and 60, or a nucleotide sequence that is at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical with SEQ ID NO: 58, 60, 61, 62, or with SEQ ID NO:58 and 60, over its entire length, or the complement thereof.

49. An isolated nucleic acid molecule which comprises a nucleotide sequence of SEQ ID NO:58, 60, 61, 62, or which comprises nucleotide sequence of SEQ ID NOs:58 and 60, or the complement thereof.

50. An isolated nucleic acid molecule which comprises a nucleotide sequence of SEQ ID NO:61, or the complement thereof.

51. An isolated nucleic acid molecule according to any of claims 35 to 50, which comprises a messenger RNA (mRNA) molecule.

52. A vector comprising a nucleic acid molecule of any of claims 35 to 51.

53. A vector according to claim 52, comprising a nucleic acid molecule encoding a polypeptide of any of claims 1 to 34.
54. A vector according to claim 52 or 53, comprising a nucleic acid molecule encoding a polypeptide which comprises an amino acid sequence of SEQ ID NO:27 or 29.
- 5 55. A vector according to any of claims 52 to 54, comprising a nucleic acid molecule encoding a polypeptide which comprises an amino acid sequence of SEQ ID NO:35 or 37.
56. A vector according to any of claims 52 to 55, comprising a nucleic acid molecule encoding a polypeptide which comprises an amino acid sequence of SEQ ID NO:43 or 45.
57. A vector according to any of claims 52 to 56, comprising a nucleic acid molecule
10 encoding a polypeptide which comprises an amino acid sequence of SEQ ID NO:7 or 8.
58. A vector according to any of claims 52 to 57, comprising a nucleic acid molecule encoding a polypeptide which comprises an amino acid sequence of SEQ ID NO:10 or 11.
59. A vector according to any of claims 52 to 57, which further comprises a promoter operably linked to the, or each nucleic acid molecule.
- 15 60. A vector according to claim 59, wherein the, or each promoter is for expression of a polypeptide encoded by the nucleic acid in mammalian cells.
61. A vector according to claim 59, wherein the, or each promoter is for expression of a polypeptide encoded by the nucleic acid in yeast or insect cells.
62. A vector according to any of claims 52 to 61, which is a vaccine vector.
- 20 63. A vector according to claim 62, which is a viral vaccine vector, a bacterial vaccine vector, an RNA vaccine vector, a messenger RNA (mRNA) vector, or a DNA vaccine vector.
64. An isolated cell comprising a vector of any of claims 52 to 63.
65. A fusion protein comprising a polypeptide according to any of claims 1 to 34.
- 25 66. A pharmaceutical composition comprising a polypeptide according to any of claims 1 to 34, and a pharmaceutically acceptable carrier, excipient, or diluent.
67. A pharmaceutical composition according to claim 66, comprising a polypeptide which comprises an amino acid sequence of SEQ ID NO:27 or 29.

68. A pharmaceutical composition according to claim 66, comprising a polypeptide which comprises an amino acid sequence of SEQ ID NO:27.
69. A pharmaceutical composition according to any of claims 66 to 68, comprising a polypeptide which comprises an amino acid sequence of SEQ ID NO:35 or 37.
- 5 70. A pharmaceutical composition according to any of claims 66 to 69, comprising a polypeptide which comprises an amino acid sequence of SEQ ID NO:35.
71. A pharmaceutical composition according to any of claims 66 to 70, comprising a polypeptide which comprises an amino acid sequence of SEQ ID NO:43 or 45.
72. A pharmaceutical composition according to any of claims 66 to 71, comprising a
10 polypeptide which comprises an amino acid sequence of SEQ ID NO:43.
73. A pharmaceutical composition comprising a nucleic acid according to any of claims 35 to 51, and a pharmaceutically acceptable carrier, excipient, or diluent.
74. A pharmaceutical composition according to claim 73, comprising a nucleic acid molecule encoding a polypeptide of any of claims 1 to 34.
- 15 75. A pharmaceutical composition according to claim 73 or 74, comprising a nucleic acid molecule encoding a polypeptide which comprises an amino acid sequence of SEQ ID NO: 27 or 29.
76. A pharmaceutical composition according to claim 75, which comprises a nucleic acid molecule comprising a nucleotide sequence of SEQ ID NO:28 or 30.
- 20 77. A pharmaceutical composition according to any of claims 73 to 76, comprising a nucleic acid molecule encoding a polypeptide which comprises an amino acid sequence of SEQ ID NO: 27.
78. A pharmaceutical composition according to claim 77, which comprises a nucleic acid molecule comprising a nucleotide sequence of SEQ ID NO:28.
- 25 79. A pharmaceutical composition according to any of claims 73 to 78, comprising a nucleic acid molecule encoding a polypeptide which comprises an amino acid sequence of SEQ ID NO: 35 or 37.
80. A pharmaceutical composition according to any of claims 73 to 79, comprising a nucleic acid molecule comprising a nucleotide sequence of SEQ ID NO: 36 or 38.

81. A pharmaceutical composition according to any of claims 73 to 80, comprising a nucleic acid molecule encoding a polypeptide which comprises an amino acid sequence of SEQ ID NO: 35.
82. A pharmaceutical composition according to any of claims 73 to 81, comprising a
5 nucleic acid molecule comprising a nucleotide sequence of SEQ ID NO: 36.
83. A pharmaceutical composition according to any of claims 73 to 82, comprising a nucleic acid molecule encoding a polypeptide which comprises an amino acid sequence of SEQ ID NO: 43 or 45.
84. A pharmaceutical composition according to any of claims 73 to 83, comprising a
10 nucleic acid molecule comprising a nucleotide sequence of SEQ ID NO: 44 or 46.
85. A pharmaceutical composition according to any of claims 73 to 84, comprising a nucleic acid molecule encoding a polypeptide which comprises an amino acid sequence of SEQ ID NO: 43.
86. A pharmaceutical composition according to any of claims 73 to 85, comprising a
15 nucleic acid molecule comprising a nucleotide sequence of SEQ ID NO: 44.
87. A pharmaceutical composition comprising a vector according to any of claims 52 to 63, and a pharmaceutically acceptable carrier, excipient, or diluent.
88. A pharmaceutical composition according to any of claims 73 to 86, wherein the nucleic acid comprises a messenger RNA (mRNA) molecule.
89. A pharmaceutical composition according to claim 87 or 88, wherein the vector is a
20 messenger (mRNA) vector.
90. A pharmaceutical composition according to any of claims 66 to 89, which further comprises an adjuvant for enhancing an immune response in a subject to the polypeptide, or to a polypeptide encoded by the nucleic acid, of the composition.
91. A method of inducing an immune response to an influenza virus in a subject, which
25 comprises administering to the subject an effective amount of a polypeptide according to any of claims 1 to 34, a nucleic acid according to any of claims 35 to 51, a vector according to any of claims 52 to 63, or a pharmaceutical composition according to any of claims 66 to 90.

92. A method of immunising a subject against an influenza virus, which comprises administering to the subject an effective amount of a polypeptide according to any of claims 1 to 34, a nucleic acid according to any of claims 35 to 51, a vector according to any of claims 52 to 63, or a pharmaceutical composition according to any of claims 66 to 90.

5 93. A polypeptide according to any of claims 1 to 34, a nucleic acid according to any of claims 35 to 51, a vector according to any of claims 52 to 63, or a pharmaceutical composition according to any of claims 66 to 90, for use as a medicament.

94. A polypeptide according to any of claims 1 to 34, a nucleic acid according to any of claims 35 to 51, a vector according to any of claims 52 to 63, or a pharmaceutical
10 composition according to any of claims 66 to 90, for use in the prevention, treatment, or amelioration of an influenza viral infection.

95. Use of a polypeptide according to any of claims 1 to 34, a nucleic acid according to any of claims 35 to 51, a vector according to any of claims 52 to 63, or a pharmaceutical composition according to any of claims 66 to 90, in the manufacture of a medicament for
15 the prevention, treatment, or amelioration of an influenza viral infection.

96. An isolated polypeptide which comprises an amino acid sequence of SEQ ID NO:63, or an amino acid sequence that has at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% amino acid identity along its entire
20 length with the amino acid sequence of SEQ ID NO:63.

97. An isolated polypeptide which comprises an amino acid sequence of SEQ ID NO:63.

98. An isolated nucleic acid molecule, which comprises a nucleotide sequence encoding a polypeptide according to claim 96 or 97, or the complement thereof.

25 99. An isolated nucleic acid molecule according to claim 98, which comprises a nucleotide sequence of SEQ ID NO:25, or a nucleotide sequence which has at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity along its entire length with the nucleotide sequence of SEQ ID NO:25, or the complement
30 thereof.

100. An isolated nucleic acid molecule which comprises a nucleotide sequence of SEQ ID NO:25, or a nucleotide sequence which has at least 70%, 71%, 72%, 73%, 74%, 75%,

76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity along its entire length with the nucleotide sequence of SEQ ID NO:25, or the complement thereof.

101. An isolated nucleic acid molecule which comprises a nucleotide sequence of SEQ
5 ID NO:25, or the complement thereof.

102. An isolated nucleic acid molecule according to any of claims 98 to 101, which comprises a DNA molecule.

103. An isolated nucleic acid molecule according to any of claims 98 to 101, which comprises a messenger RNA (mRNA) molecule.

10 104. A vector comprising a nucleic acid molecule according to any of claims 98 to 103.

105. A vector according to claim 104, which comprises a nucleotide sequence of SEQ ID NO:26.

106. A vector according to claim 104 or 105, comprising a nucleic acid molecule encoding a polypeptide which comprises an amino acid sequence of SEQ ID NO:63.

15 107. A vector according to any of claims 104 to 106, which further comprises a promoter operably linked to the nucleic acid molecule.

108. A vector according to claim 107, wherein the promoter is for expression of a polypeptide encoded by the nucleic acid in mammalian cells.

20 109. A vector according to claim 108, wherein the promoter is for expression of a polypeptide encoded by the nucleic acid in yeast or insect cells.

110. A vector according to any of claims 104 to 109, which is a vaccine vector.

111. A vector according to claim 110, which is a viral vaccine vector, a bacterial vaccine vector, an RNA vaccine vector, a messenger RNA (mRNA) vector, or a DNA vaccine vector.

25 112. An isolated cell comprising a vector of any of claims 104 to 111.

113. A fusion protein comprising a polypeptide according to claim 96 or 97.

114. A pharmaceutical composition which comprises an isolated polypeptide according to claim 96 or 97, and a pharmaceutically acceptable carrier, excipient, or diluent.

115. A pharmaceutical composition which comprises an isolated nucleic acid molecule according to any of claims 98 to 103, and a pharmaceutically acceptable carrier, excipient, or diluent.

116. A pharmaceutical composition according to claim 115, comprising a nucleic acid molecule encoding a polypeptide comprising an amino acid sequence of SEQ ID NO:63, or the complement thereof.

117. A pharmaceutical composition comprising a vector according to any of claims 104 to 111, and a pharmaceutically acceptable carrier, excipient, or diluent.

118. A pharmaceutical composition according to claim 117, comprising a vector comprising a nucleotide sequence of SEQ ID NO:26.

119. A pharmaceutical composition according to claim 115 or 116, wherein the nucleic acid comprises a messenger RNA (mRNA) molecule.

120. A pharmaceutical composition according to claim 117 or 118, wherein the vector is a messenger (mRNA) vector.

121. A pharmaceutical composition according to any of claims 114 to 120, which further comprises an adjuvant for enhancing an immune response in a subject to the polypeptide, or to a polypeptide encoded by the nucleic acid, of the composition.

122. A method of inducing an immune response to an influenza virus in a subject, which comprises administering to the subject an effective amount of a polypeptide according to claim 96 or 97, a nucleic acid molecule according to any of claims 98 to 103, a vector according to any of claims 104 to 111, or a pharmaceutical composition according to any of claims 114 to 121.

123. A method of immunising a subject against an influenza virus, which comprises administering to the subject an effective amount of a polypeptide according to claim 96 or 97, a nucleic acid molecule according to any of claims 98 to 103, a vector according to any of claims 104 to 111, or a pharmaceutical composition according to any of claims 114 to 121.

124. A polypeptide according to claim 96 or 97, a nucleic acid molecule according to any of claims 98 to 103, a vector according to any of claims 104 to 111, or a pharmaceutical composition according to any of claims 114 to 121, for use as a medicament.

125. A polypeptide according to claim 96 or 97, a nucleic acid molecule according to any of claims 98 to 103, a vector according to any of claims 104 to 111, or a pharmaceutical composition according to any of claims 114 to 121, for use in the prevention, treatment, or amelioration of an influenza viral infection.

- 5 126. Use of a polypeptide according to claim 96 or 97, a nucleic acid molecule according to any of claims 98 to 103, a vector according to any of claims 104 to 111, or a pharmaceutical composition according to any of claims 114 to 121, in the manufacture of a medicament for the prevention, treatment, or amelioration of an influenza viral infection.

Assays using mouse sera

- FLU002 (H5)
- H5N1 Anc. and VSN

Figure 1

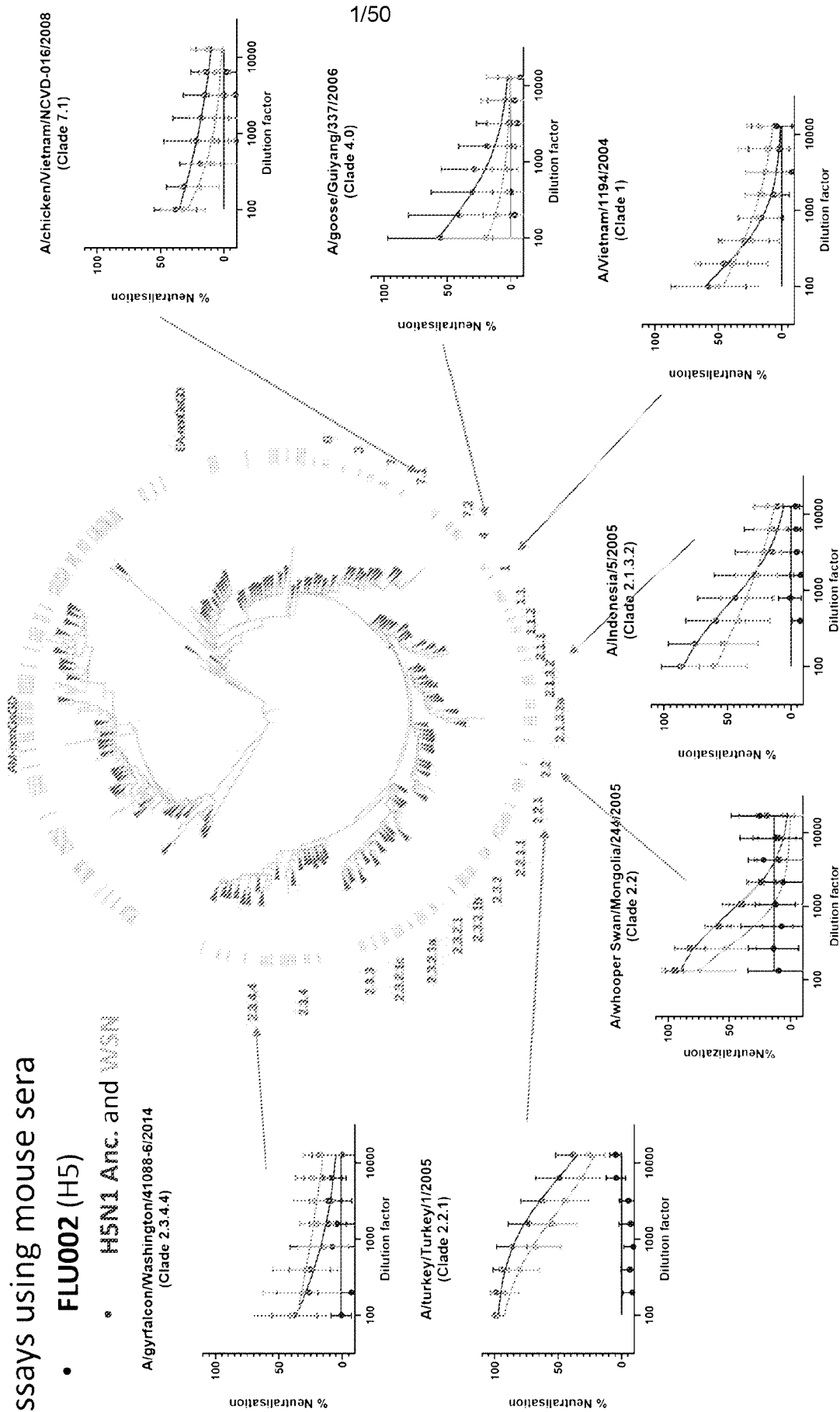


Figure 2

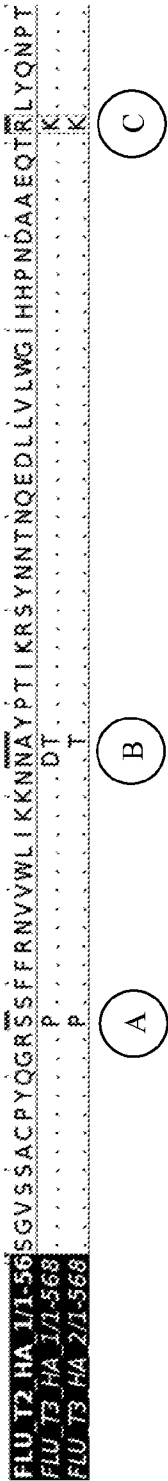
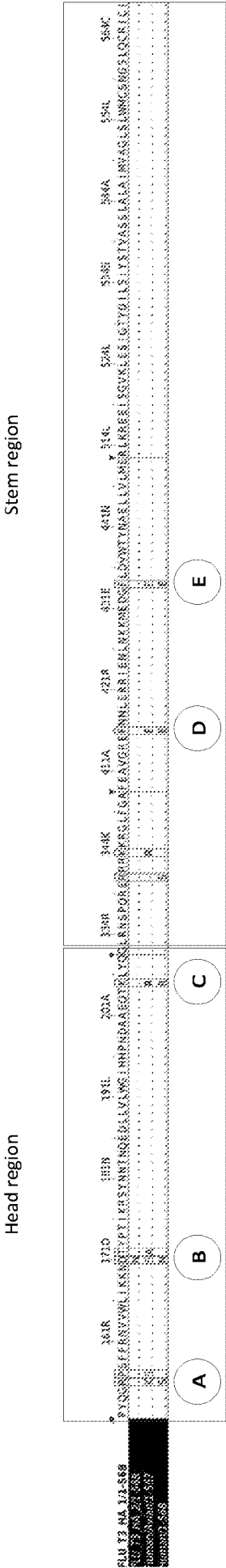


Figure 3

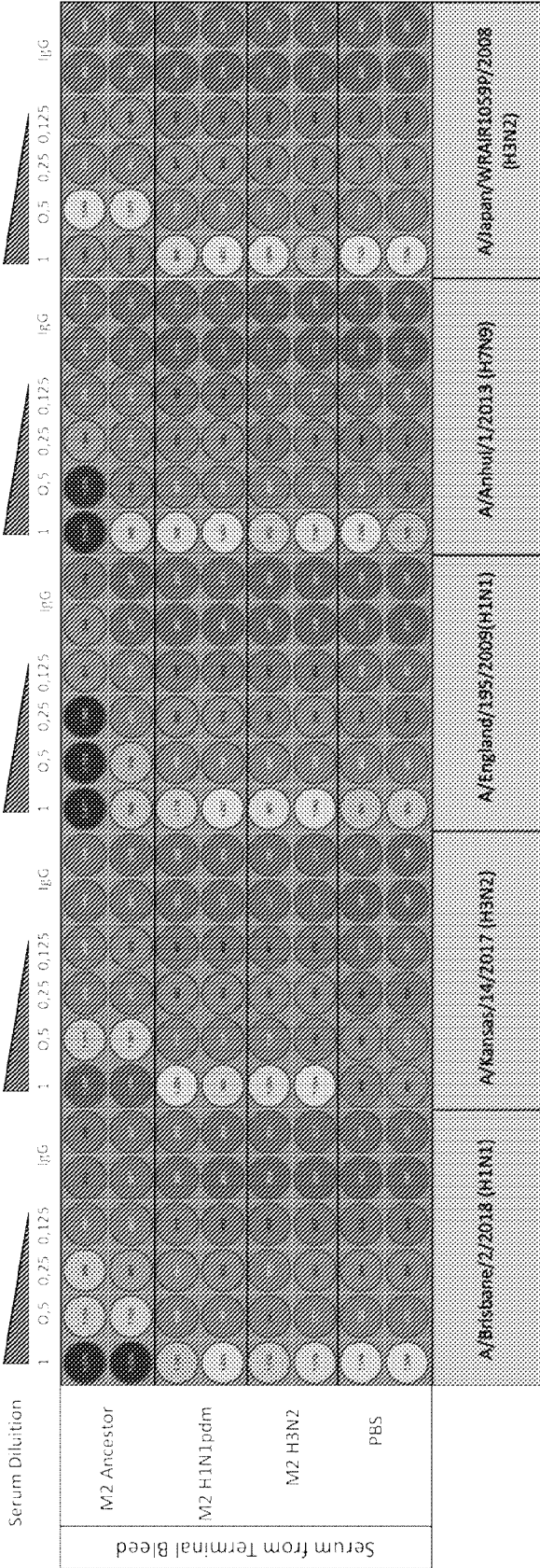


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Flu_T3 – Our design
Human/avian and Human Cobra 2 design

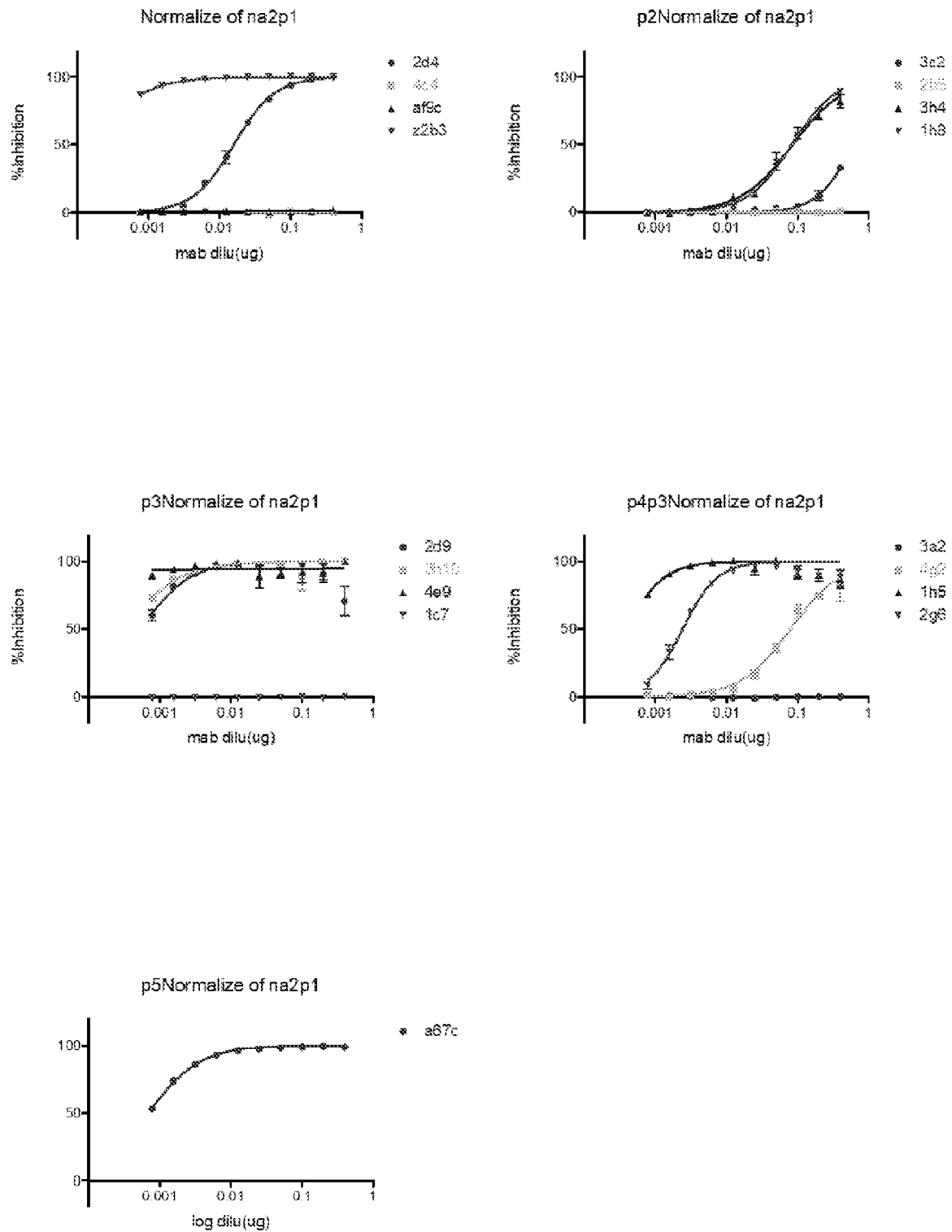
- A B C Mutations in the epitope to increase the affinity towards the antibody
- D E Increases the stability of the stem region both in the pre-fusion and post-fusion state

Figure 4



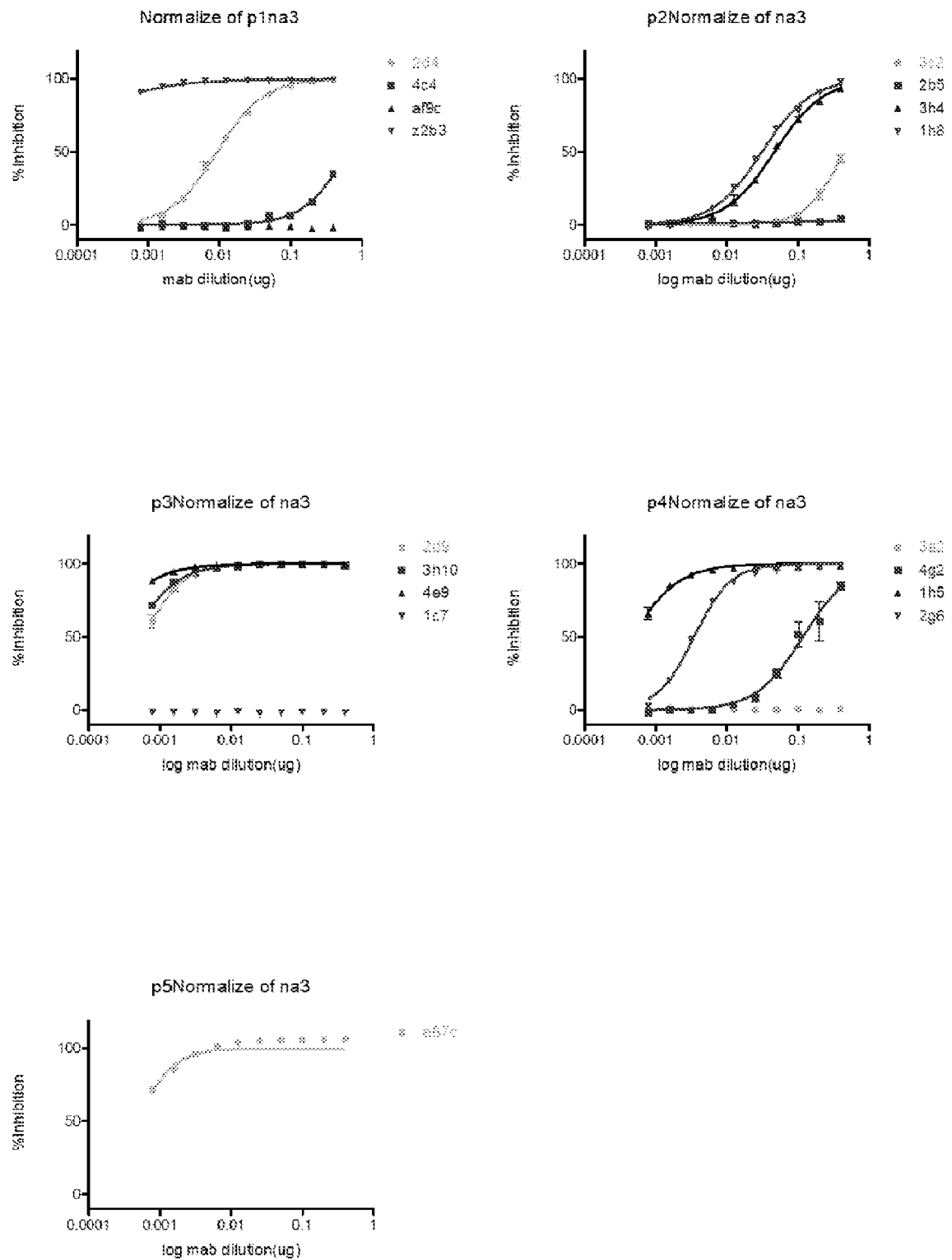
5/50

Figure 5



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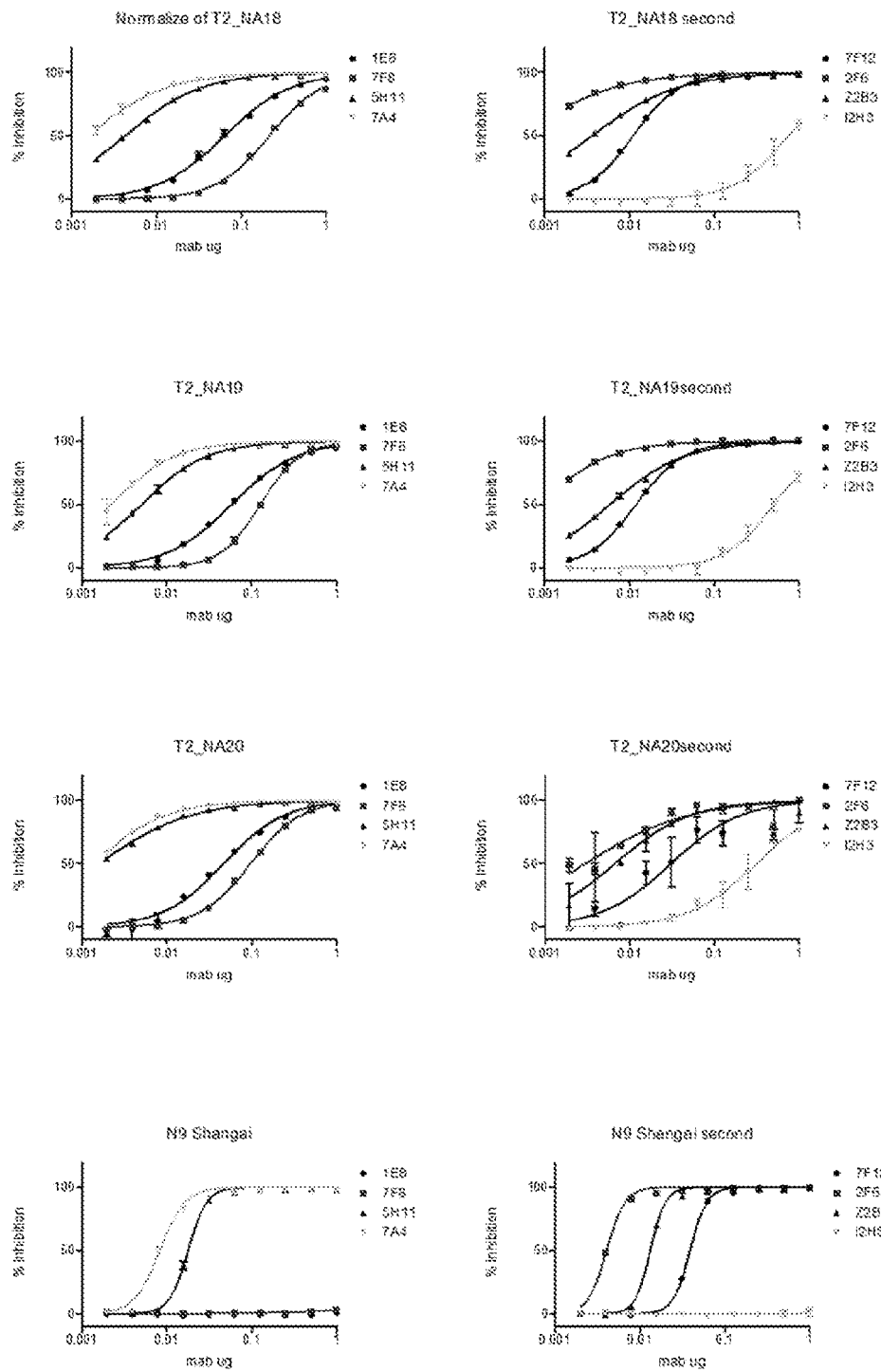
Figure 6



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Figure 7

pELLA Inhibition N9 mAbs



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Figure 8

pEVAC

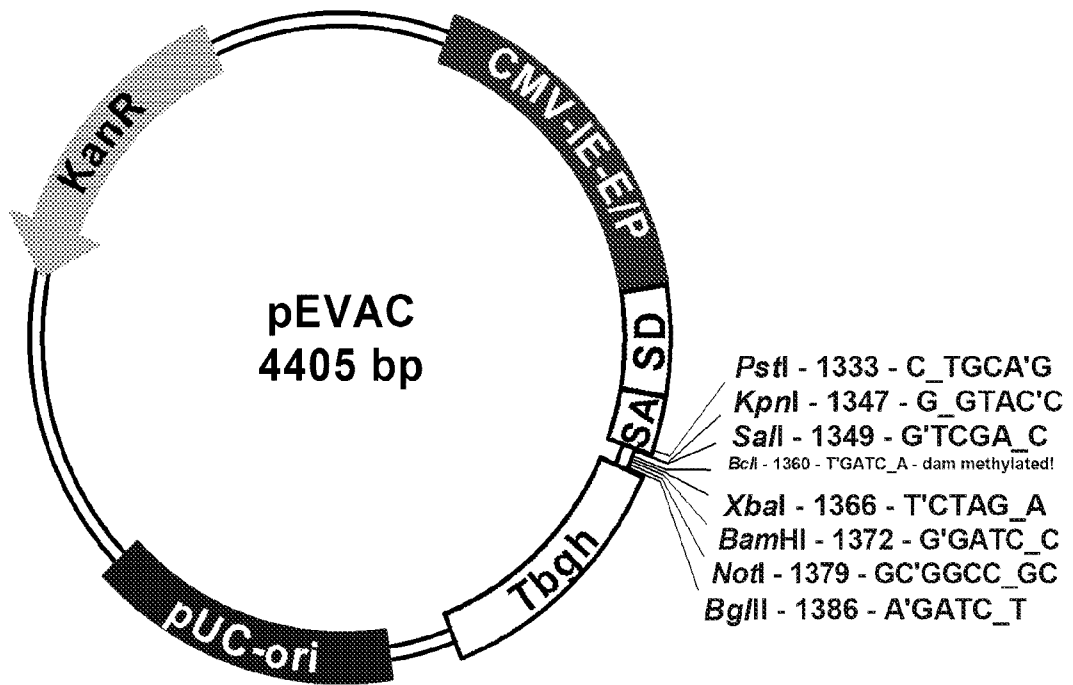


Figure 9
log_eIC₅₀ plot for pEVAC_Flu_T2_HA_3_I-3 and other controls

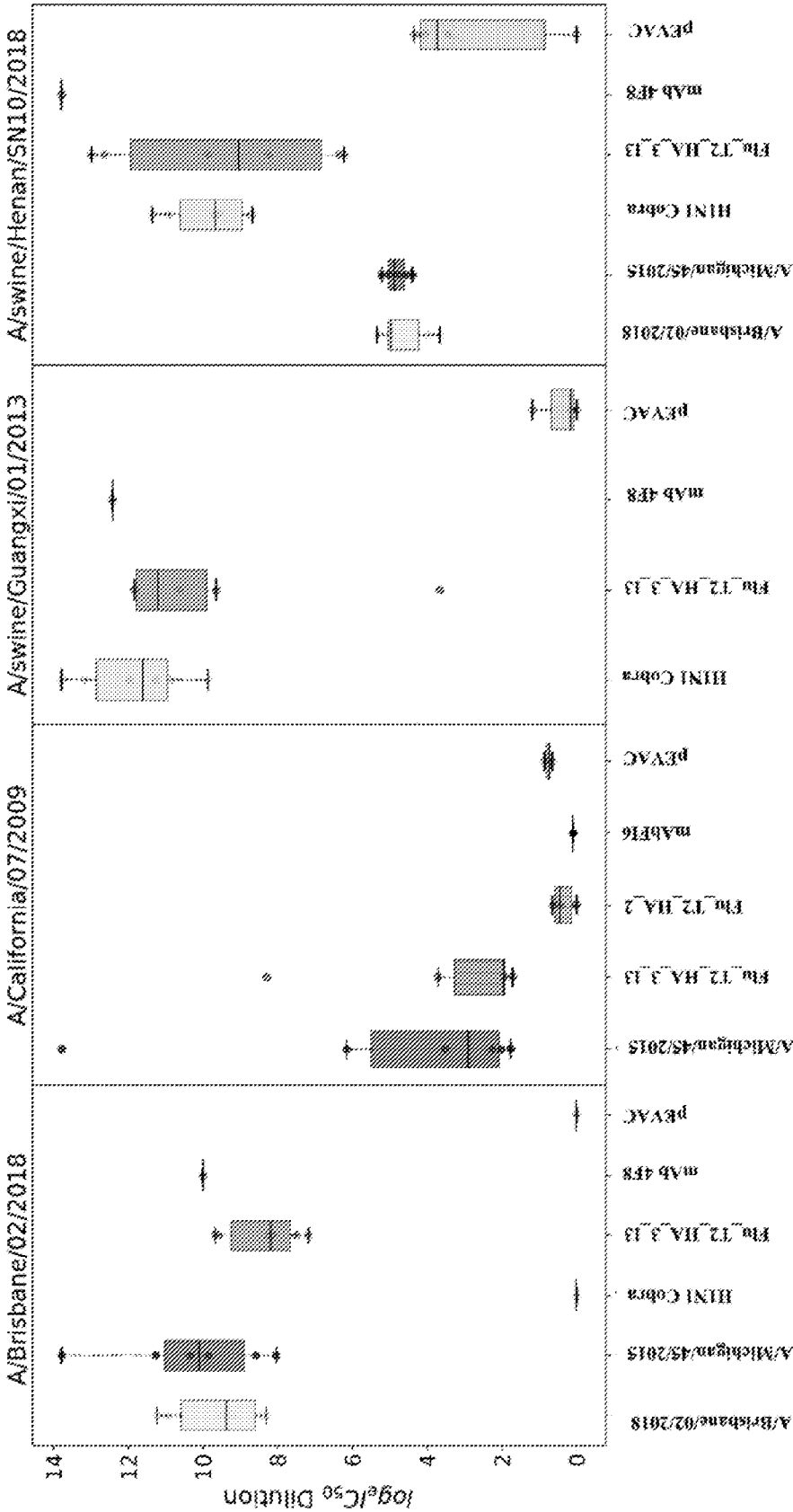
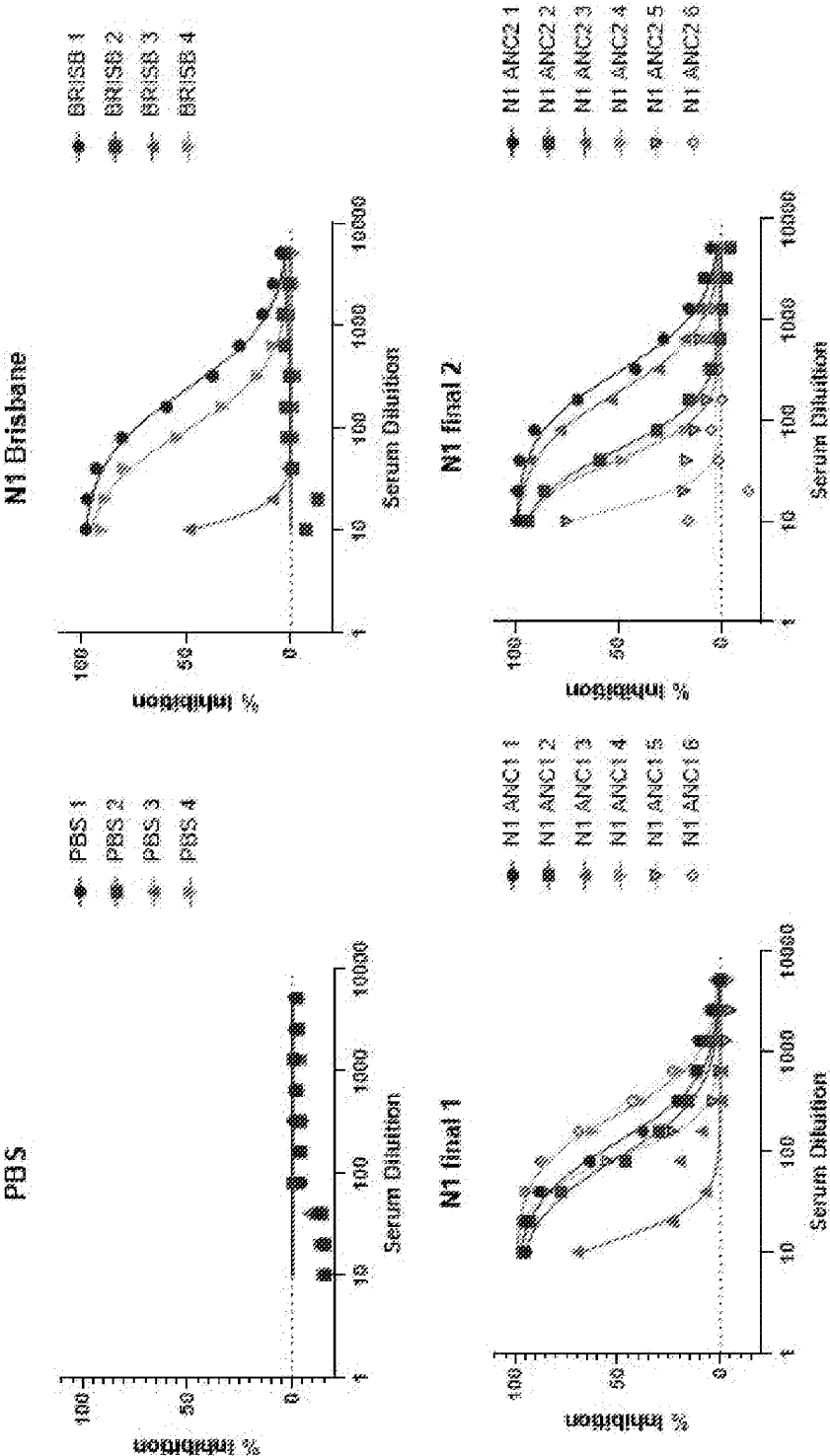


Figure 10



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Figure 11

a)

In ACDP2 accommodation (B357)

Group 1 n=5 Test vaccine group; DNA prime (ID), DNA boost

Group 2 n=5 control vaccine 1, WIV₁₃₅₃ in adjuvant (IM)Group 3 n=5 control vaccine 2, WIV_{Vic} in adjuvant (IM)

Group 4 n=5 naïve control

Total: 20 pigs

Test vaccine : synthetic multi-gene antigen containing gene sequences encoding M1, M2 and M2 antigens of human H1N1 influenza A virus of the pandemic 2009 lineage. Prime and boost vaccination administered as naked DNA intradermally (ID) by a Pharmajet delivery device

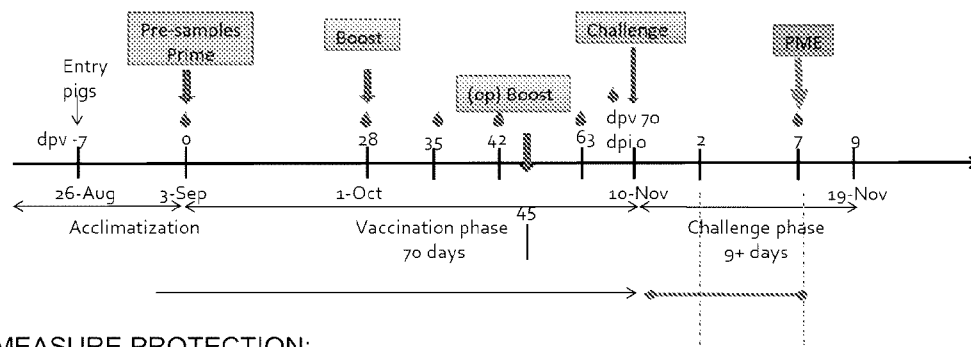
Control vaccine 1 : Whole Inactivated Virus **A/swine/England/1353/2009** pH1N1 in adjuvant

Adjuvant : TS6, a commercially available adjuvant licensed for adjuvanted pig vaccines or Addavax an approved, licenced adjuvant for many mammalian species

Control vaccine 2 : Whole Inactivated Virus **A/Victoria/2454/2019** pH1N1 in adjuvant

Challenge strain : **A/swine/England/1353/2009** (swine pdm09 H1N1) virus

b)

*** Non-Lethal Challenge with **A/swine/England/1353/2009** (swine pdm09 H1N1)

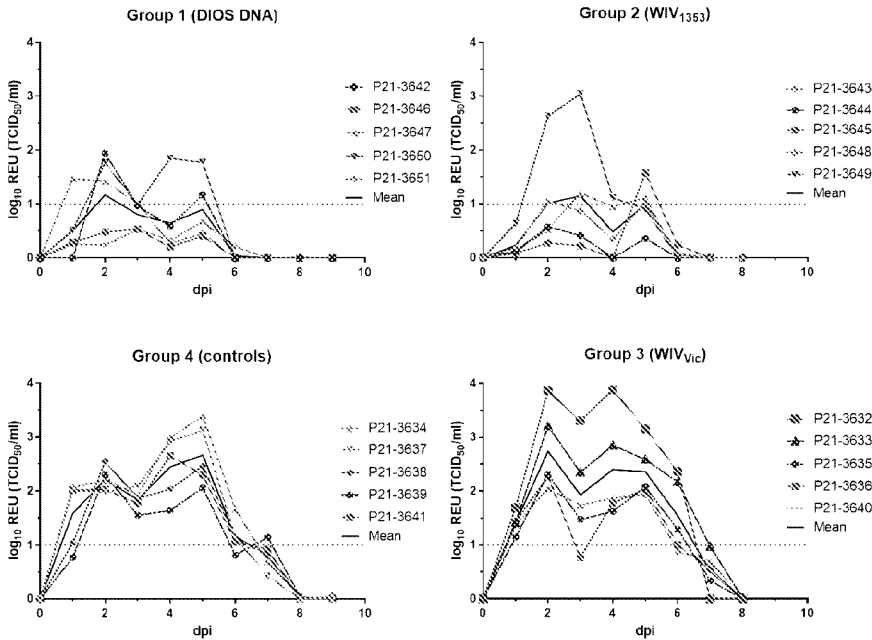
HOW TO MEASURE PROTECTION:

1. Reduction in Viral Shedding via qRT-PCR
2. HI Titers: Seroprotective Titer ≥ 40
3. Virus Neutralization: pMN titers

Figure 12

a)

Nasal Shedding per group



Nasal Shedding

b)

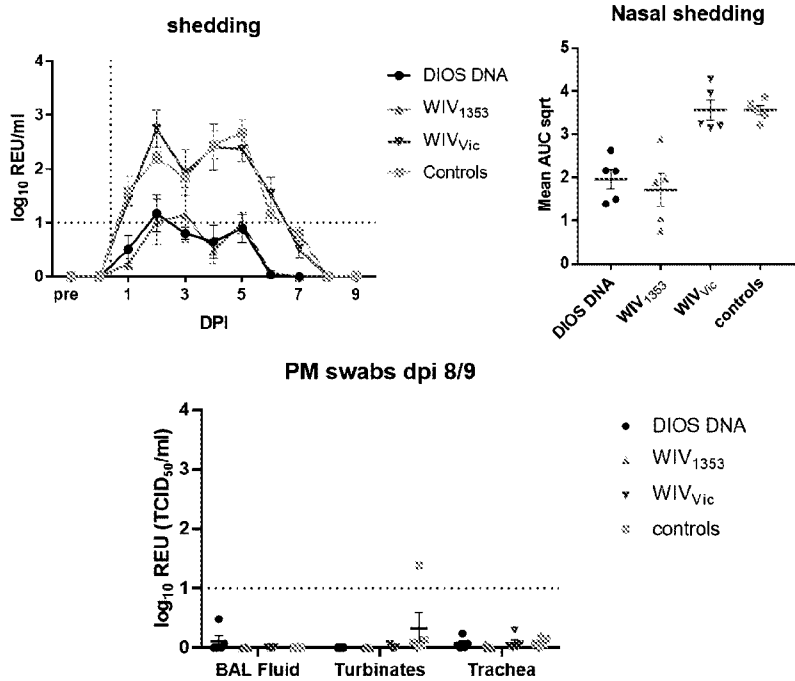
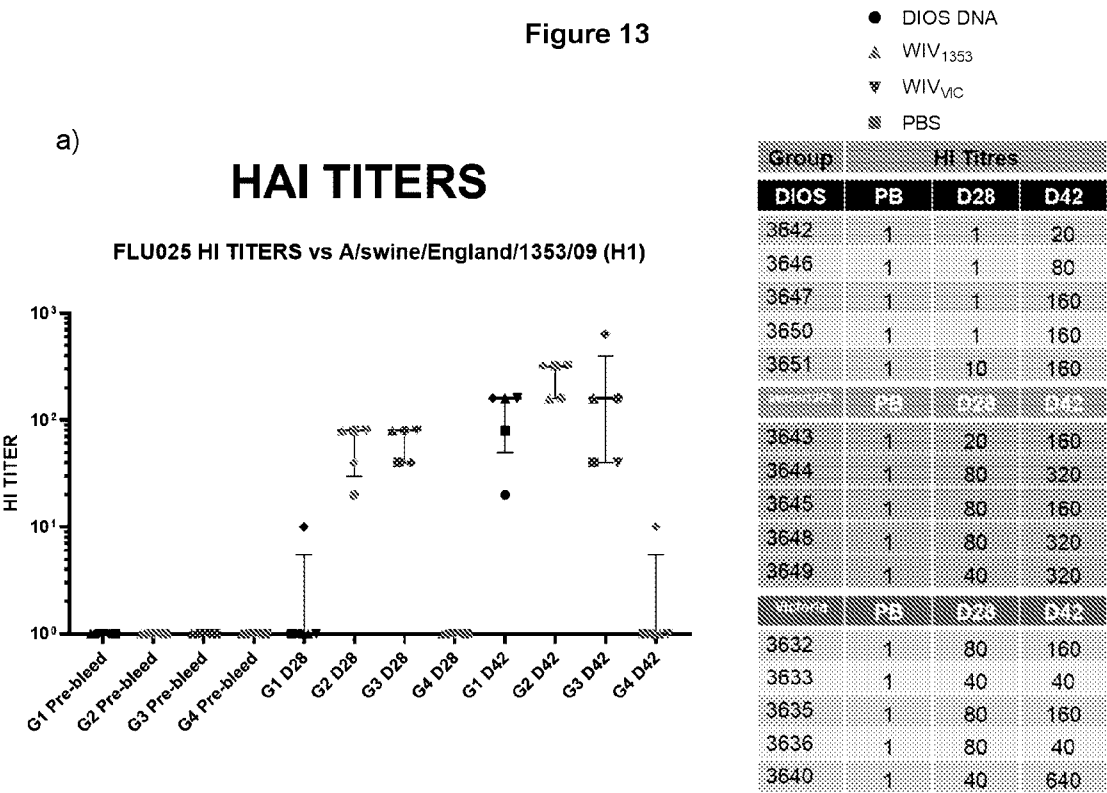
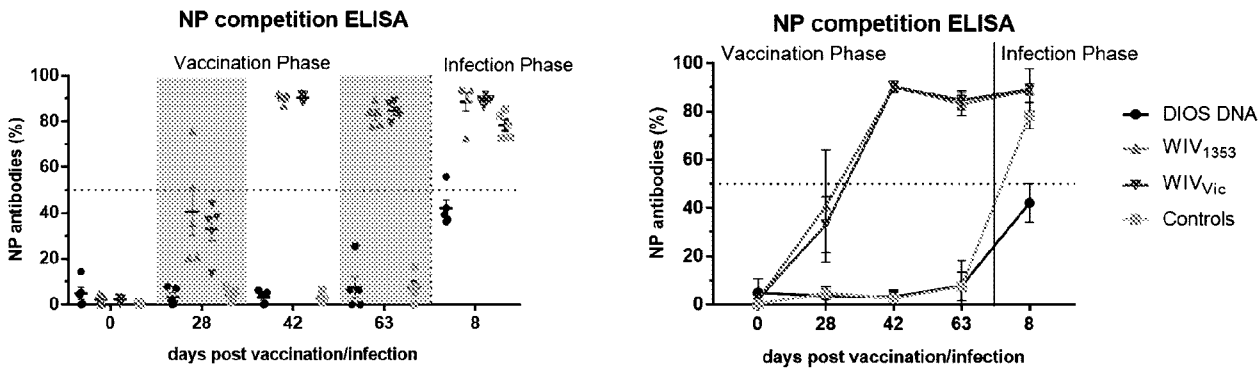


Figure 13



b)

NP competition ELISA (Idvet)



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Figure 14

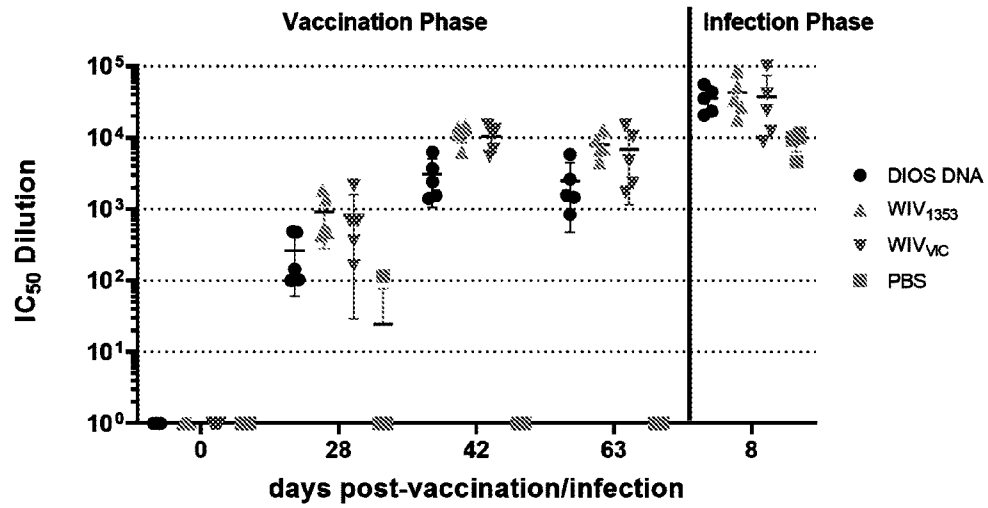
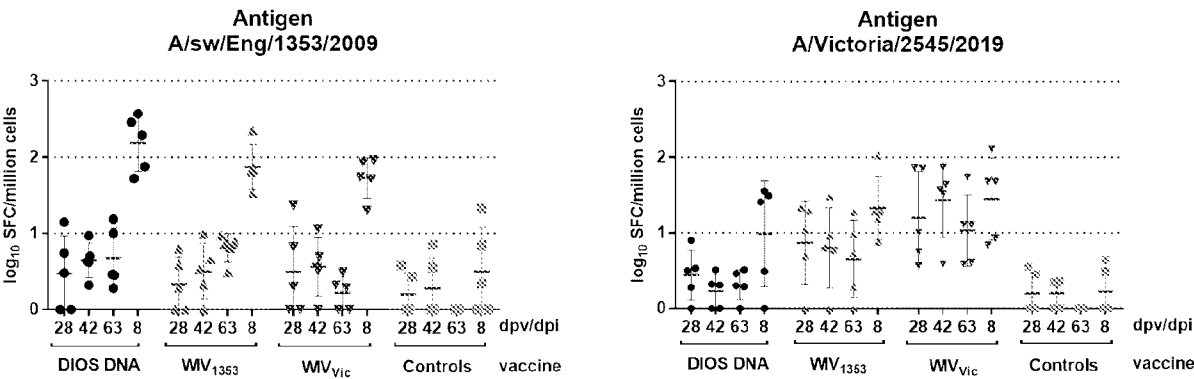
Serum neutralizing titers vs A/swine/England/1353/09 (H1)

Figure 15

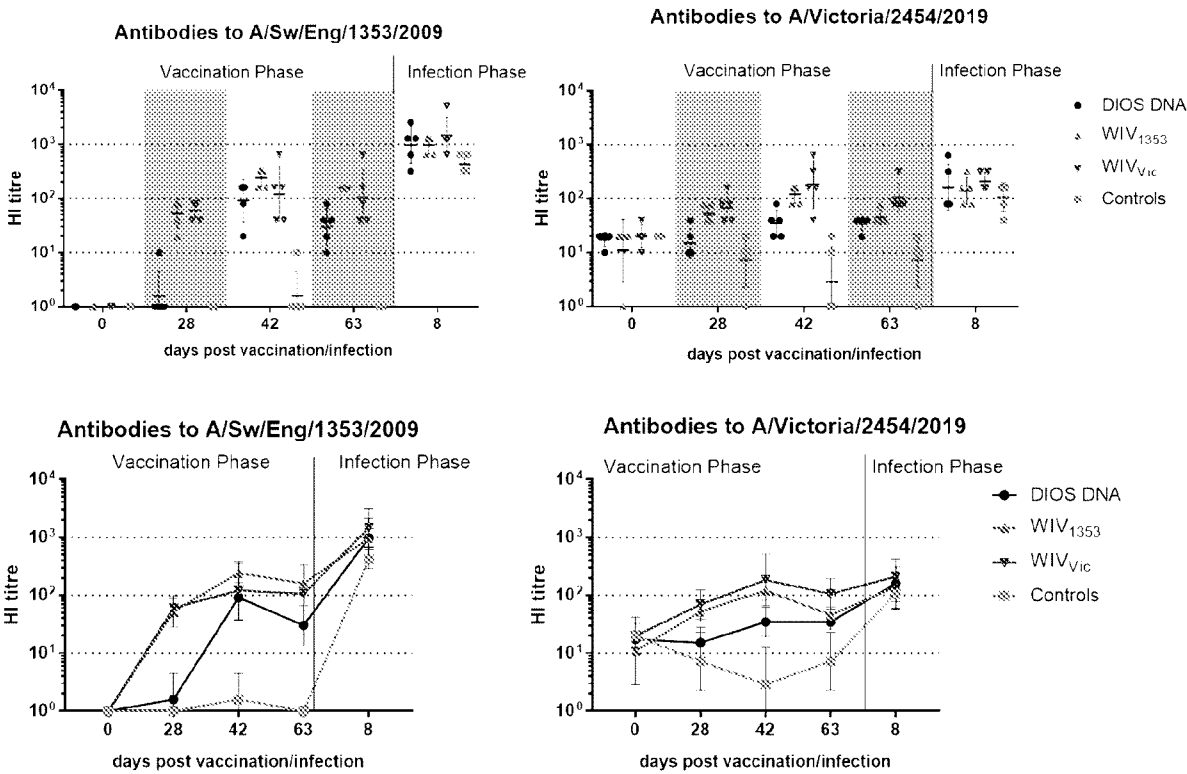
ELISpot

a)



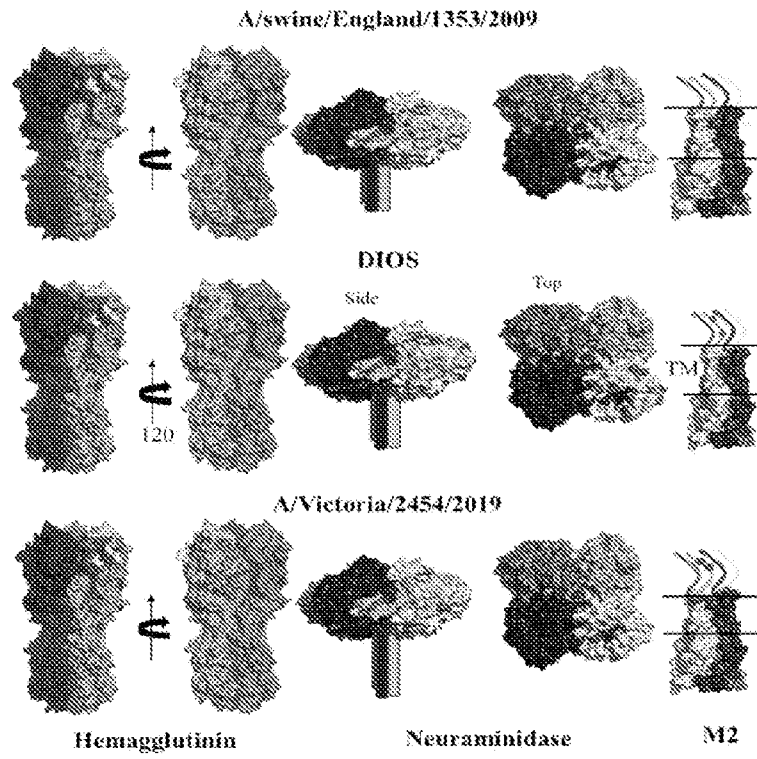
b)

HAIt



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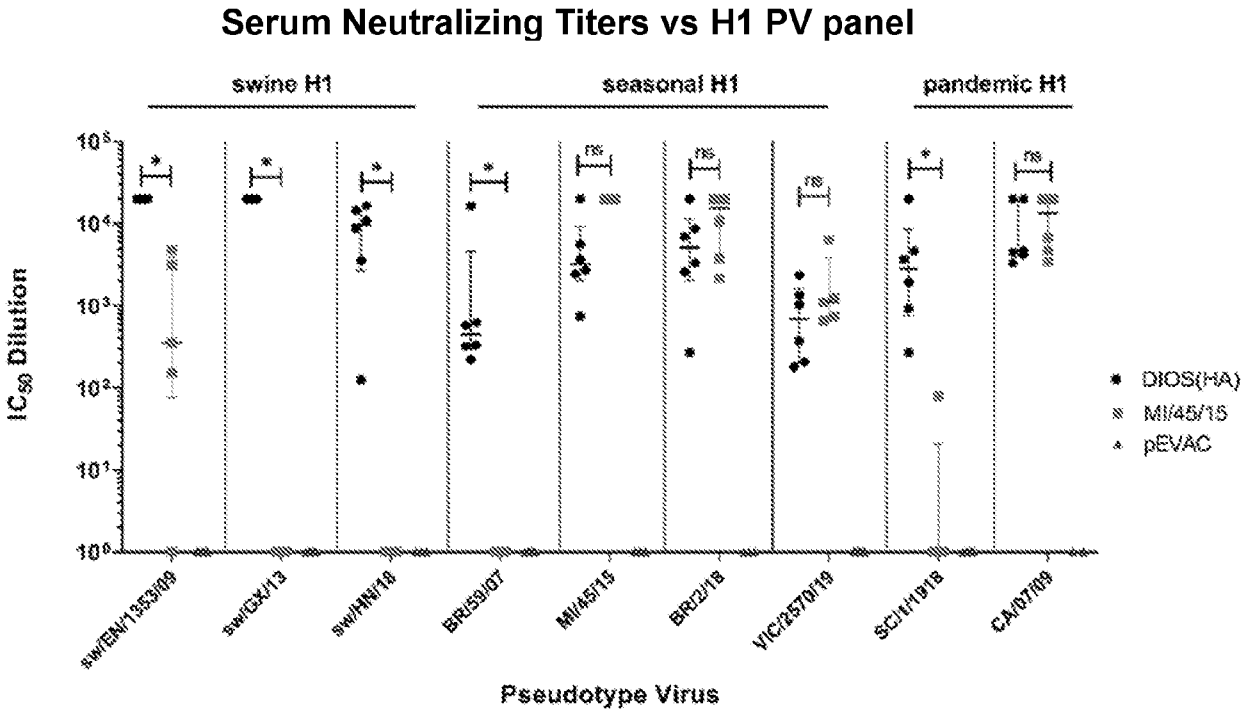
Figure 16



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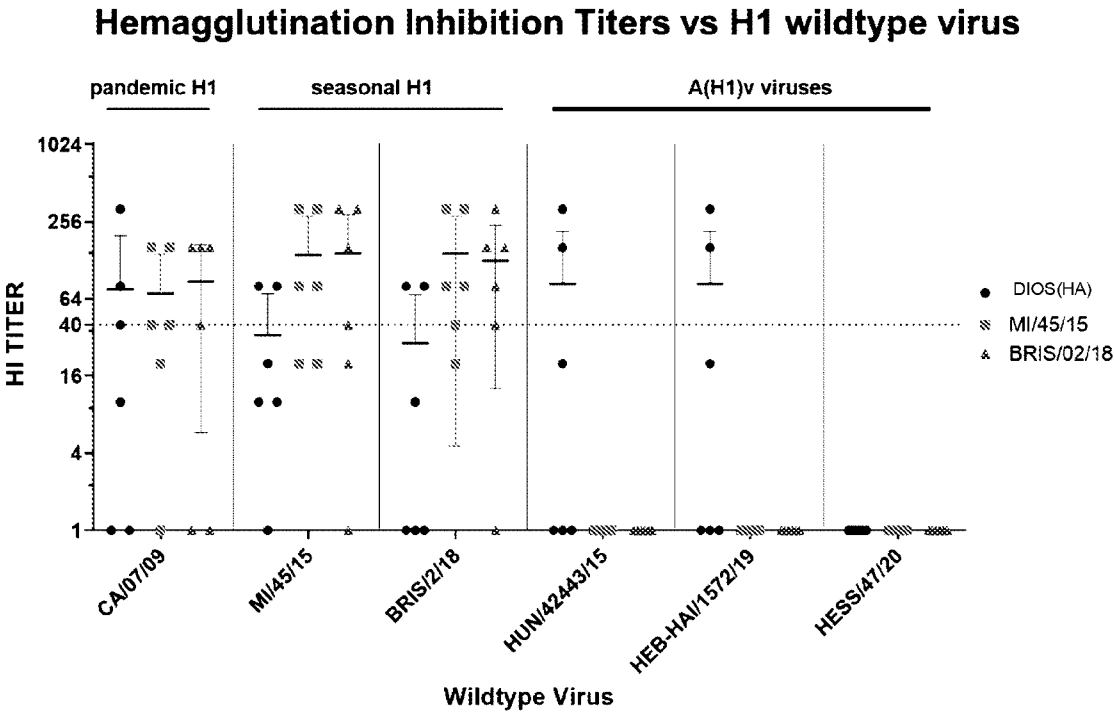
Figure 17

a)



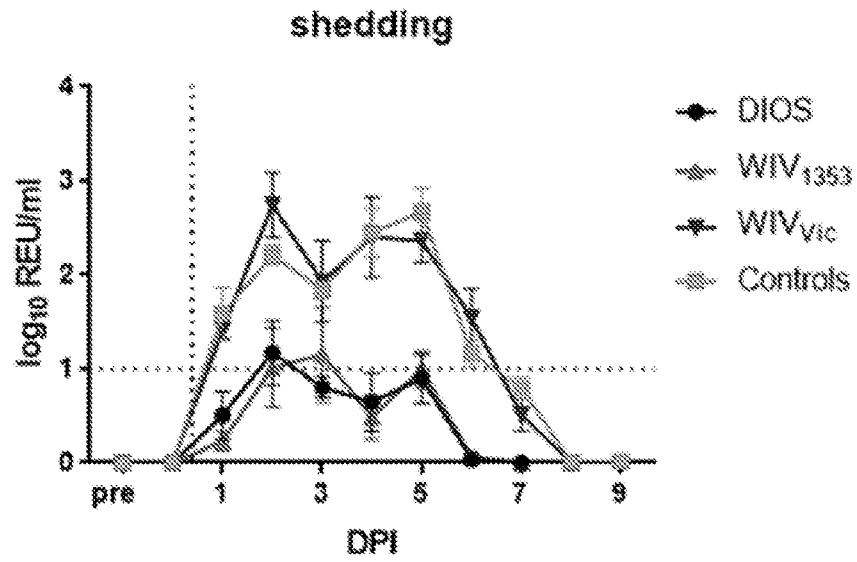
NOTE: * is $p < 0.05$.

b)



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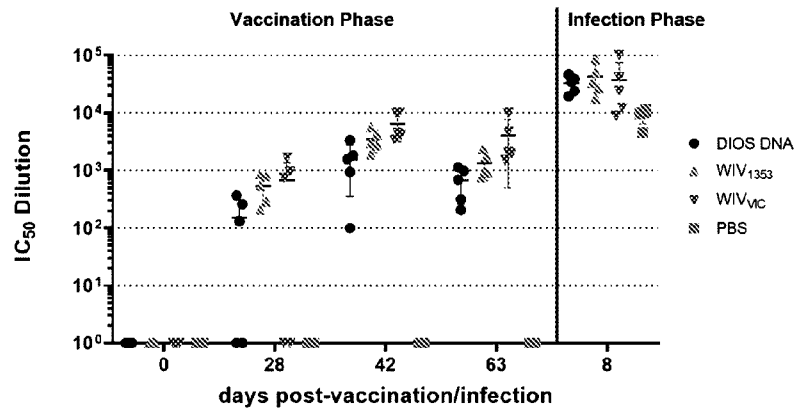
Figure 18



19/50
Figure 19

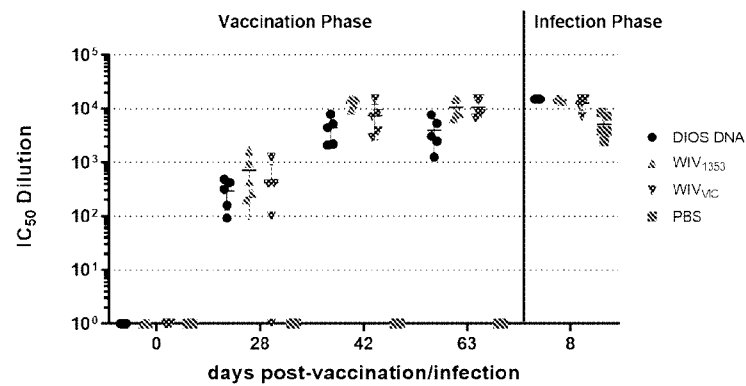
a)

Serum neutralizing titers vs A/Victoria/2570/19 (H1)



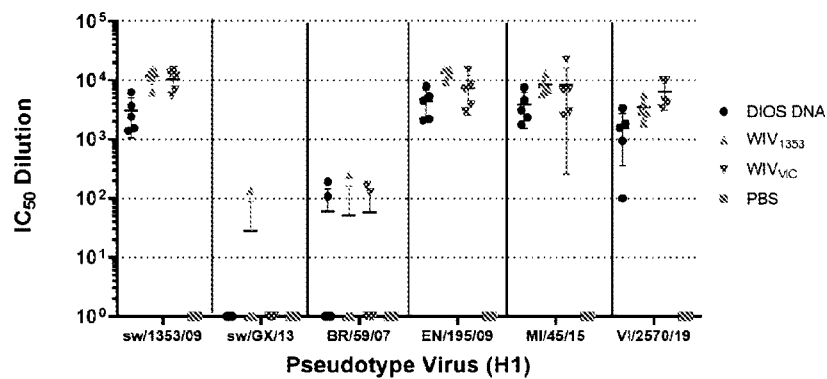
b)

Serum neutralizing titers vs A/England/195/2009 (H1)



c)

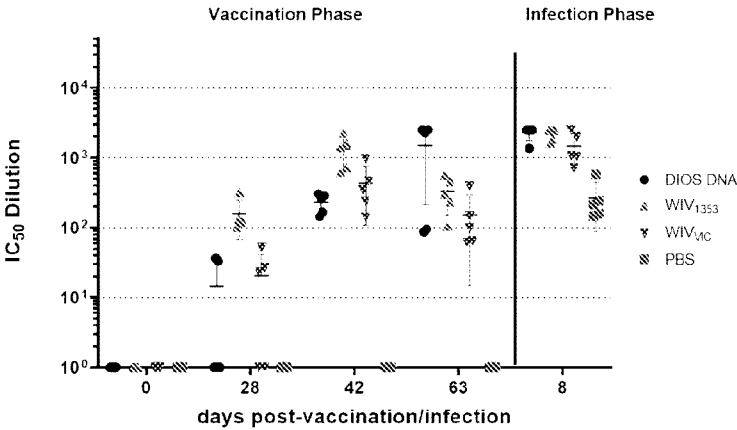
Serum neutralizing titers (FLU025 day 42) vs H1 PV panel



20/50
Figure 20

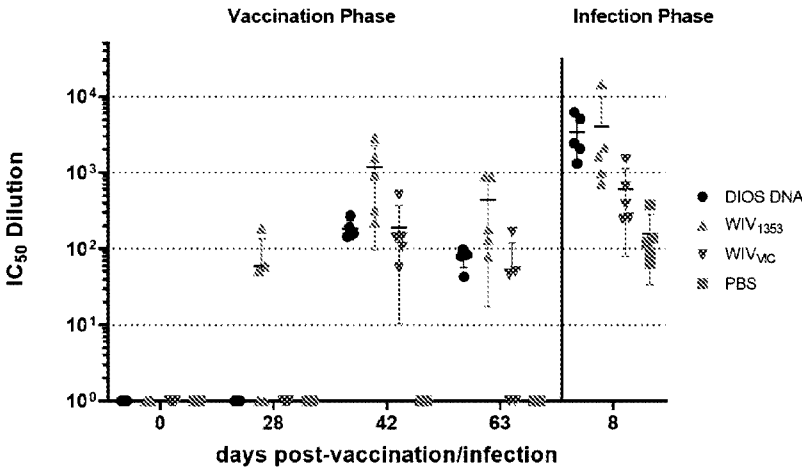
a)

Neuraminidase Inhibition Titers (Pig model)
vs A/swine/England/1353/2009 (N1)



Neuraminidase Inhibition Titers (Pig model)
vs A/England/195/2009 (N1)

b)



c)

Serum neutralizing titers (FLU025 day 42) vs N1 PV panel

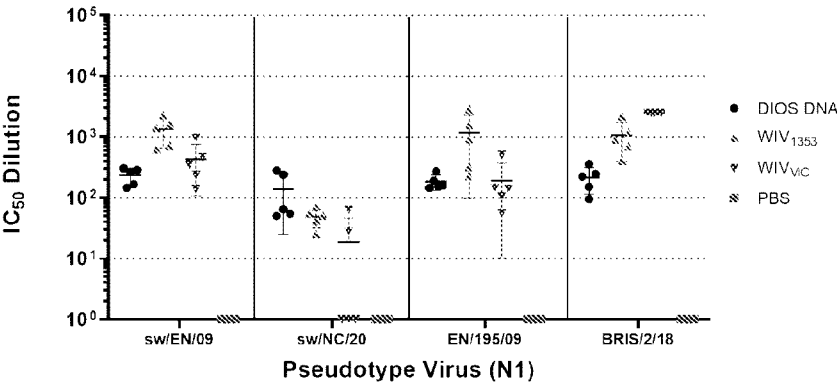


Figure 21

Region			A		B		C		D	E	Example/Figure
H5 Residue	148	149	156	157	171	172	205	238	416	434	
FLU_T2_HA_1	S	S	R	S	N	A	R	Q	F	F	Ex1/Fig.2
FLU_T3_HA_1	S	S	R	P	D	T	K	Q	F	F	Ex4/Fig.2
FLU_T3_HA_2	S	S	R	P	N	T	K	Q	F	F	Ex5/Fig.2
FLU_T3_HA_3	S	S	R	S	N	A	R	Q	F	F	Ex18
FLU_T3_HA_4	V	P	R	S	N	A	R	E	F	F	Ex19
FLU_T3_HA_5	S	S	R	S	N	A	R	E	F	F	Ex20

Figure 22

MEKIVLLLA	VSLVKSQIC	IGYHANNSTE	QVDTIMEKNV	TVTHAQDILE	KTHNGKLCDL	60	FLU_T2_HA_1
MEKIVLLLA	VSLVKSQIC	IGYHANNSTE	QVDTIMEKNV	TVTHAQDILE	KTHNGKLCDL	60	FLU_T3_HA_1
MEKIVLLLA	VSLVKSQIC	IGYHANNSTE	QVDTIMEKNV	TVTHAQDILE	KTHNGKLCDL	60	FLU_T3_HA_2
MEKIVLLLA	VSLVKSQIC	IGYHANNSTE	QVDTIMEKNV	TVTHAQDILE	KTHNGKLCDL	60	FLU_T3_HA_3
MEKIVLLLA	VSLVKSQIC	IGYHANNSTE	QVDTIMEKNV	TVTHAQDILE	KTHNGKLCDL	60	FLU_T3_HA_4
MEKIVLLLA	VSLVKSQIC	IGYHANNSTE	QVDTIMEKNV	TVTHAQDILE	KTHNGKLCDL	60	FLU_T3_HA_5
MEKIVLLLA	VSLVKSQIC	IGYHANNSTE	QVDTIMEKNV	TVTHAQDILE	KTHNGKLCDL	60	H5_WSN
MEKIVLLLA	VSLVKSQIC	IGYHANNSTE	QVDTIMEKNV	TVTHAQDILE	KTHNGKLCDL	60	H5_GYR
DGVKPLLRD	CSVAGWLLGN	PMCDEFINVP	EWSYIVEKAN	PANDLCYPGN	FNDYEELKHL	120	FLU_T2_HA_1
DGVKPLLRD	CSVAGWLLGN	PMCDEFINVP	EWSYIVEKAN	PANDLCYPGN	FNDYEELKHL	120	FLU_T3_HA_1
DGVKPLLRD	CSVAGWLLGN	PMCDEFINVP	EWSYIVEKAN	PANDLCYPGN	FNDYEELKHL	120	FLU_T3_HA_2
DGVKPLLRD	CSVAGWLLGN	PMCDEFINVP	EWSYIVEKAN	PANDLCYPGN	FNDYEELKHL	120	FLU_T3_HA_3
DGVKPLLRD	CSVAGWLLGN	PMCDEFINVP	EWSYIVEKAN	PANDLCYPGN	FNDYEELKHL	120	FLU_T3_HA_4
DGVKPLLRD	CSVAGWLLGN	PMCDEFINVP	EWSYIVEKAN	PANDLCYPGN	FNDYEELKHL	120	FLU_T3_HA_5
DGVKPLLRD	CSVAGWLLGN	PMCDEFINVP	EWSYIVEKAN	PANDLCYPGN	FNDYEELKHL	120	H5_WSN
DGVKPLLRD	CSVAGWLLGN	PMCDEFINVP	EWSYIVEKAN	PANDLCYPGN	FNDYEELKHL	120	H5_GYR
LSRINHFEKI	QIIPKSSWSD	HEASSGVSSA	CPYQGRSFF	RNVVWLIKKN	NAYPTIKRSY	180	FLU_T2_HA_1
LSRINHFEKI	QIIPKSSWSD	HEASSGVSSA	CPYQGRSFF	RNVVWLIKKN	NAYPTIKRSY	180	FLU_T3_HA_1
LSRINHFEKI	QIIPKSSWSD	HEASSGVSSA	CPYQGRSFF	RNVVWLIKKN	NAYPTIKRSY	180	FLU_T3_HA_2
LSRINHFEKI	QIIPKSSWSD	HEASSGVSSA	CPYQGRSFF	RNVVWLIKKN	NAYPTIKRSY	179	FLU_T3_HA_3
LSRINHFEKI	QIIPKSSWSD	HEASSGVSSA	CPYQGRSFF	RNVVWLIKKN	NAYPTIKRSY	180	FLU_T3_HA_4
LSRINHFEKI	QIIPKSSWSD	HEASSGVSSA	CPYQGRSFF	RNVVWLIKKN	NAYPTIKRSY	180	FLU_T3_HA_5
LSRINHFEKI	QIIPKSSWSD	HEASSGVSSA	CPYQGRSFF	RNVVWLIKKD	NAYPTIKRSY	180	H5_WSN
LSRINHFEKI	QIIPKSSWSD	HEASSGVSSA	CPYQGRSFF	RNVVWLIKKD	NAYPTIKRSY	180	H5_GYR
NNTNQEDLLV	LWGIHHPNDA	AEQTRLYQNP	TTYISVGTST	LNQRLVPKIA	TRSKVNGQSG	240	FLU_T2_HA_1
NNTNQEDLLV	LWGIHHPNDA	AEQTRLYQNP	TTYISVGTST	LNQRLVPKIA	TRSKVNGQSG	240	FLU_T3_HA_1
NNTNQEDLLV	LWGIHHPNDA	AEQTRLYQNP	TTYISVGTST	LNQRLVPKIA	TRSKVNGQSG	240	FLU_T3_HA_2
NNTNQEDLLV	LWGIHHPNDA	AEQTRLYQNP	TTYISVGTST	LNQRLVPKIA	TRSKVNGQSG	239	FLU_T3_HA_3
NNTNQEDLLV	LWGIHHPNDA	AEQTRLYQNP	TTYISVGTST	LNQRLVPKIA	TRSKVNGQSG	240	FLU_T3_HA_4
NNTNQEDLLV	LWGIHHPNDA	AEQTRLYQNP	TTYISVGTST	LNQRLVPKIA	TRSKVNGQSG	240	FLU_T3_HA_5
NNTNQEDLLV	LWGIHHPNDA	AEQTRLYQNP	TTYISVGTST	LNQRLVPKIA	TRSKVNGQSG	240	H5_WSN
NNTNQEDLLV	LWGIHHPNDA	AEQTRLYQNP	TTYISVGTST	LNQRLVPKIA	TRSKVNGQSG	240	H5_GYR

Figure 22 continued

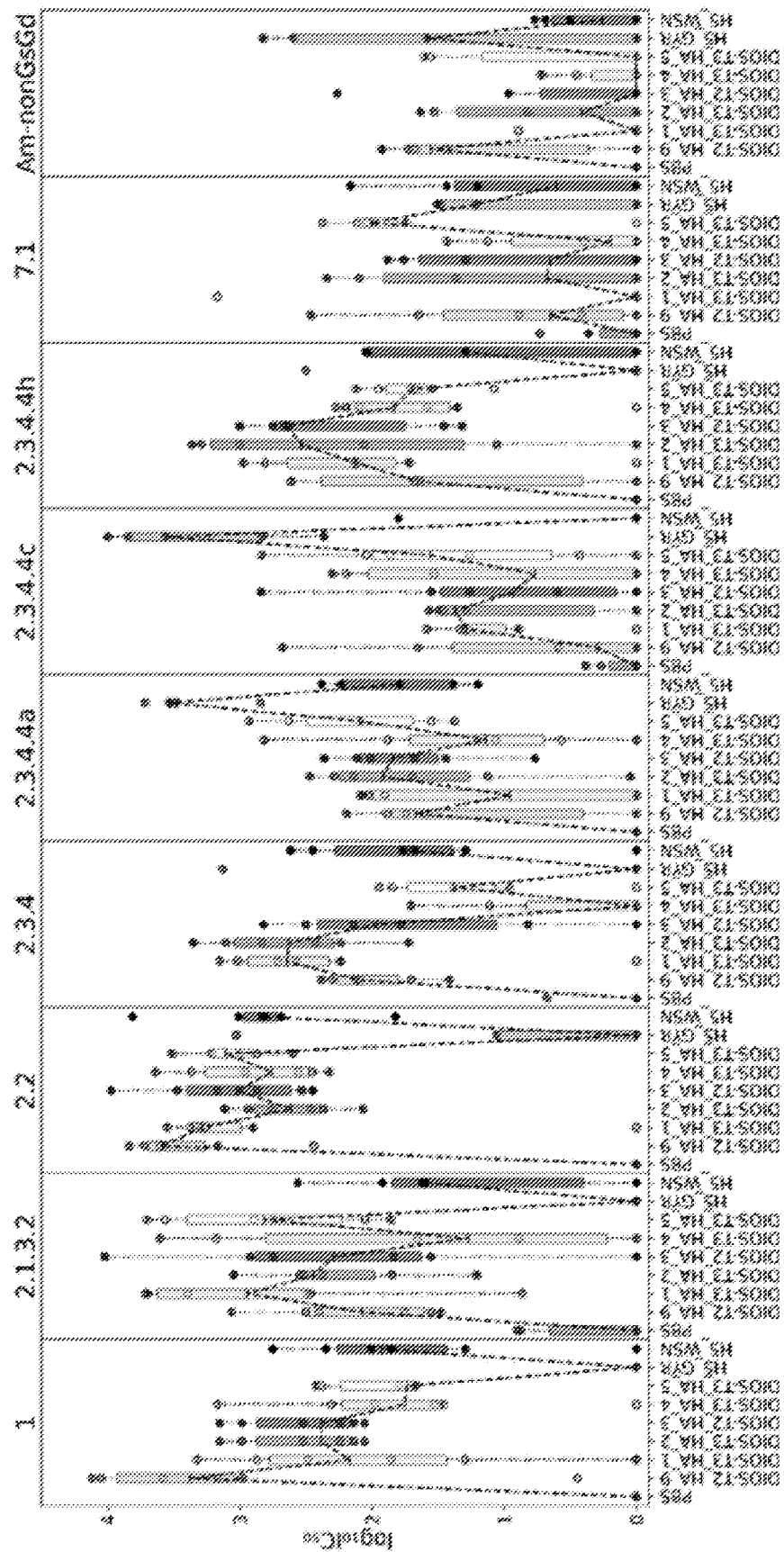
RMEFFWTILK	PNDAINFESN	GNFIAPEYAY	KIVKKGDSAI	MKSELEYGNC	NTKCQTPMGA	300	FLU_T2_HA_1
RMEFFWTILK	PNDAINFESN	GNFIAPEYAY	KIVKKGDSAI	MKSELEYGNC	NTKCQTPMGA	300	FLU_T3_HA_1
RMEFFWTILK	PNDAINFESN	GNFIAPEYAY	KIVKKGDSAI	MKSELEYGNC	NTKCQTPMGA	300	FLU_T3_HA_2
RMEFFWTILK	PNDAINFESN	GNFIAPEYAY	KIVKKGDSAI	MKSELEYGNC	NTKCQTPMGA	299	FLU_T3_HA_3
RMEFFWTILK	PNDAINFESN	GNFIAPEYAY	KIVKKGDSAI	MKSELEYGNC	NTKCQTPMGA	300	FLU_T3_HA_4
RMEFFWTILK	PNDAINFESN	GNFIAPEYAY	KIVKKGDSAI	MKSELEYGNC	NTKCQTPMGA	300	FLU_T3_HA_5
RMEFFWTILK	PNDAINFESN	GNFIAPEYAY	KIVKKGDSAI	MKSELEYGNC	NTKCQTPMGA	300	H5_WSN
RMEFFWTILK	PNDAINFESN	GNFIAPEYAY	KIVKKGDSAI	MKSELEYGNC	NTKCQTPMGA	300	H5_GYR
INSSMPFHNI	HPLTIGECPC	YVKSRLVLA	TGLRNSPQRE	RRRKRGLFG	AIAGFTIEGGW	360	FLU_T2_HA_1
INSSMPFHNI	HPLTIGECPC	YVKSRLVLA	TGLRNSPQRE	RRRKRGLFG	AIAGFTIEGGW	360	FLU_T3_HA_1
INSSMPFHNI	HPLTIGECPC	YVKSRLVLA	TGLRNSPQRE	RRRKRGLFG	AIAGFTIEGGW	360	FLU_T3_HA_2
INSSMPFHNI	HPLTIGECPC	YVKSRLVLA	TGLRNSPQRE	RRRKRGLFG	AIAGFTIEGGW	359	FLU_T3_HA_3
INSSMPFHNI	HPLTIGECPC	YVKSRLVLA	TGLRNSPQRE	RRRKRGLFG	AIAGFTIEGGW	360	FLU_T3_HA_4
INSSMPFHNI	HPLTIGECPC	YVKSRLVLA	TGLRNSPQRE	RRRKRGLFG	AIAGFTIEGGW	360	FLU_T3_HA_5
INSSMPFHNI	HPLTIGECPC	YVKSRLVLA	TGLRNSPQRE	RRRKRGLFG	AIAGFTIEGGW	360	H5_WSN
INSSMPFHNI	HPLTIGECPC	YVKSRLVLA	TGLRNSPQRE	RRRKRGLFG	AIAGFTIEGGW	359	H5_GYR
QGMVDGWYGY	HHSNEQGSY	AADKESTQKA	IDGVTNKVNS	IIDKMNTQFE	AVGRENNLE	420	FLU_T2_HA_1
QGMVDGWYGY	HHSNEQGSY	AADKESTQKA	IDGVTNKVNS	IIDKMNTQFE	AVGRENNLE	420	FLU_T3_HA_1
QGMVDGWYGY	HHSNEQGSY	AADKESTQKA	IDGVTNKVNS	IIDKMNTQFE	AVGRENNLE	420	FLU_T3_HA_2
QGMVDGWYGY	HHSNEQGSY	AADKESTQKA	IDGVTNKVNS	IIDKMNTQFE	AVGRENNLE	419	FLU_T3_HA_3
QGMVDGWYGY	HHSNEQGSY	AADKESTQKA	IDGVTNKVNS	IIDKMNTQFE	AVGRENNLE	420	FLU_T3_HA_4
QGMVDGWYGY	HHSNEQGSY	AADKESTQKA	IDGVTNKVNS	IIDKMNTQFE	AVGRENNLE	420	FLU_T3_HA_5
QGMVDGWYGY	HHSNEQGSY	AADKESTQKA	IDGVTNKVNS	IIDKMNTQFE	AVGRENNLE	420	H5_WSN
QGMVDGWYGY	HHSNEQGSY	AADKESTQKA	IDGVTNKVNS	IIDKMNTQFE	AVGRENNLE	419	H5_GYR
RRIENLNKKM	EDGFLDVWTY	NAELLVLNEN	ERTLDFHDSN	VKNLYDKVRL	QLRDNAKELG	480	FLU_T2_HA_1
RRIENLNKKM	EDGFLDVWTY	NAELLVLNEN	ERTLDFHDSN	VKNLYDKVRL	QLRDNAKELG	480	FLU_T3_HA_1
RRIENLNKKM	EDGFLDVWTY	NAELLVLNEN	ERTLDFHDSN	VKNLYDKVRL	QLRDNAKELG	480	FLU_T3_HA_2
RRIENLNKKM	EDGFLDVWTY	NAELLVLNEN	ERTLDFHDSN	VKNLYDKVRL	QLRDNAKELG	479	FLU_T3_HA_3
RRIENLNKKM	EDGFLDVWTY	NAELLVLNEN	ERTLDFHDSN	VKNLYDKVRL	QLRDNAKELG	480	FLU_T3_HA_4
RRIENLNKKM	EDGFLDVWTY	NAELLVLNEN	ERTLDFHDSN	VKNLYDKVRL	QLRDNAKELG	480	FLU_T3_HA_5
RRIENLNKKM	EDGFLDVWTY	NAELLVLNEN	ERTLDFHDSN	VKNLYDKVRL	QLRDNAKELG	480	H5_WSN
RRIENLNKKM	EDGFLDVWTY	NAELLVLNEN	ERTLDFHDSN	VKNLYDKVRL	QLRDNAKELG	479	H5_GYR

Figure 22 continued

NGCFEFYHKC	DNECMESVRN	GTYPDYPQYSE	EARLKREEIS	GVKLESIGTY	QILSIYSTVA	540	FLU_T2_HA_1
NGCFEFYHKC	DNECMESVRN	GTYPDYPQYSE	EARLKREEIS	GVKLESIGTY	QILSIYSTVA	540	FLU_T3_HA_1
NGCFEFYHKC	DNECMESVRN	GTYPDYPQYSE	EARLKREEIS	GVKLESIGTY	QILSIYSTVA	540	FLU_T3_HA_2
NGCFEFYHKC	DNECMESVRN	GTYPDYPQYSE	EARLKREEIS	GVKLESIGTY	QILSIYSTVA	539	FLU_T3_HA_3
NGCFEFYHKC	DNECMESVRN	GTYPDYPQYSE	EARLKREEIS	GVKLESIGTY	QILSIYSTVA	540	FLU_T3_HA_4
NGCFEFYHKC	DNECMESVRN	GTYPDYPQYSE	EARLKREEIS	GVKLESIGTY	QILSIYSTVA	540	FLU_T3_HA_5
NGCFEFYHRC	DNECMESVRN	GTYPDYPQYSE	EARLKREEIS	GVKLESIGTY	QILSIYSTVA	540	H5_WSN
NGCFEFYHKC	DNECMESVRN	GTYPDYPKYSE	EARLKREEIS	GVKLESIGTY	QILSIYSTVA	539	H5_GYR
SSLALAIMVA	GLSLWMCNSG	SLQCRICI	668	FLU_T2_HA_1			
SSLALAIMVA	GLSLWMCNSG	SLQCRICI	668	FLU_T3_HA_1			
SSLALAIMVA	GLSLWMCNSG	SLQCRICI	668	FLU_T3_HA_2			
SSLALAIMVA	GLSLWMCNSG	SLQCRICI	667	FLU_T3_HA_3			
SSLALAIMVA	GLSLWMCNSG	SLQCRICI	668	FLU_T3_HA_4			
SSLALAIMVA	GLSLWMCNSG	SLQCRICI	668	FLU_T3_HA_5			
SSLALAIMVA	GLSLWMCNSG	SLQCRICI	668	H5_WSN			
SSLALAIIVA	GLSLWMCNSG	SLQCRICI	667	H5_GYR			

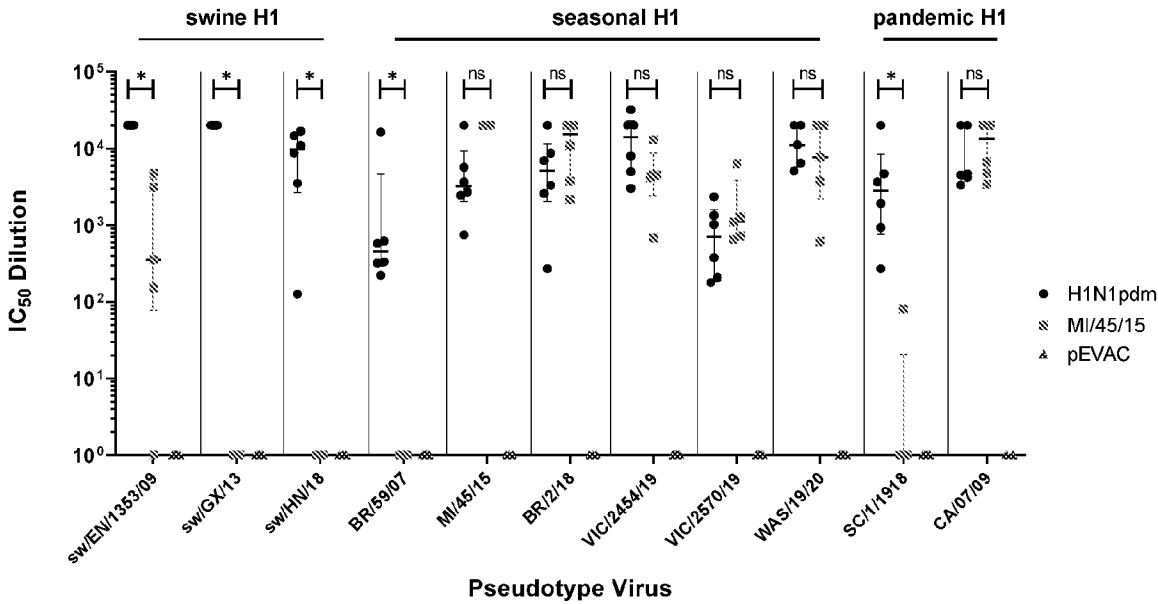
25/50

Figure 23



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Figure 24



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Figure 25

Region						Example/Figure
H5 residue of A/Sichuan/2014	107	142	200	231	238	
A/Sichuan/2014	Y	E	A	T	Q	
FLU_T4_HA_1	Y	E	A	T	Q	Example 27
FLU_T4_HA_2	<u>E</u>	E	A	T	<u>E</u>	Example 29
FLU_T4_HA_3	<u>E</u>	<u>N</u>	<u>I</u>	<u>N</u>	Q	Example 31
FLU_T2_HA_1	Y	E	A	T	Q	Ex1/Fig.2
FLU_T3_HA_1	Y	E	A	T	Q	Ex4/Fig.2
FLU_T3_HA_2	Y	E	A	T	Q	Ex5/Fig.2
FLU_T3_HA_3	Y	E	A	T	Q	Ex18
FLU_T3_HA_4	Y	E	A	T	E	Ex19
FLU_T3_HA_5	Y	E	A	T	E	Ex20
A/gyr/Washington/2014	Y	E	A	T	Q	
A/Anhui/2020	Y	T	V	T	Q	
A/Mute_swan/2021	Y	E	A	T	Q	
A/WSN/2005	Y	E	A	T	Q	
A/Hangzhou/2021	Y	E	A	T	Q	
H5N1_COBRA	Y	E	A	T	Q	
Hu/AV_COBRA2	Y	E	A	T	Q	

28/50

Figure 26

Region								
H5 residue of A/Sichuan/2014	107	142	172	200	231	238	344	345
A/Sichuan/2014	Y	E	A	A	T	Q	K	R
FLU_T4_HA_1	Y	E	A	A	T	Q	R	K
FLU_T4_HA_2	<u>E</u>	E	A	A	T	<u>E</u>	R	K
FLU_T4_HA_3	<u>E</u>	<u>N</u>	T	<u>I</u>	<u>N</u>	Q	R	K
FLU_T2_HA_1	Y	E	A	A	T	Q	K	K
FLU_T3_HA_1	Y	E	T	A	T	Q	K	K
FLU_T3_HA_2	Y	E	T	A	T	Q	K	K
FLU_T3_HA_3	Y	E	A	A	T	Q	K	K
FLU_T3_HA_4	Y	E	A	A	T	E	K	K
FLU_T3_HA_5	Y	E	A	A	T	E	K	K
A/gyr/Washington/2014	Y	E	A	A	T	Q	K	R
A/Anhui/2020	Y	T	A	V	T	Q	K	R
A/Mute_swan/2021	Y	E	A	A	T	Q	K	R
A/WSN/2005	Y	E	A	A	T	Q	R	R
A/Hangzhou/2021	Y	E	A	A	T	Q	K	R
H5N1_COBRA	Y	E	T	A	T	Q	K	K
Hu/AV_COBRA2	Y	E	A	A	T	Q	K	R

Figure 27

Region			A	B		C		D	E	Example/Figure
	148	149	156	157	171	172	205	238	416	434
H5 Residue of FLU_T2_HA_1	S	S	R	S	N	A	R	Q	F	F
FLU_T2_HA_1	S	S	R	P	D	T	K	Q	F	Ex1/Fig.2
FLU_T3_HA_1	S	S	R	P	N	T	K	Q	F	Ex4/Fig.2
FLU_T3_HA_2	S	S	R	S	N	A	R	Q	F	Ex5/Fig.2
FLU_T3_HA_3	S	S	R	S	N	A	R	Q	F	Ex18
FLU_T3_HA_4	V	P	R	S	N	A	R	E	F	Ex19
FLU_T3_HA_5	S	S	R	S	N	A	R	E	F	Ex20
FLU_T4_HA_1	S	A	T	P	D	A	N	Q	F	Ex 27
FLU_T4_HA_2	S	A	T	P	D	A	N	E	F	Ex 29
FLU_T4_HA_3	S	A	T	P	D	T	N	Q	F	Ex 31
A/Sichuan/2014	S	A	T	P	D	A	N	Q	F	F

30/50

Figure 28

A/Sichuan/26221/2014	MEKIVLLLAIVSLVKGDQICIGYHANNSTEQVDTIMEKN	39
<i>A/gyr/Washington/41088-6/2014</i>	V.I.....S.....K.....	39
<i>A/Anhui/2021-00011/2020</i>	SV.G.....S.....	39
<i>A/mute_swan/England/053054/2021</i>	..N.....N.....S.....	39
<i>A/WSN/Mongolia/244/2005</i>	S.....	39
<i>A/Hangzhou/01/2021</i>	..N..F.....S.....	39
<i>FLU_T2_HA_9</i>	S.....	39
<i>FLU_T3_HA_1</i>	S.....	39
<i>FLU_T3_HA_2</i>	S.....	39
<i>FLU_T3_HA_3</i>	S.....	39
<i>FLU_T3_HA_4</i>	S.....	39
<i>FLU_T3_HA_5</i>	S.....	39
<i>Flu_T4_HA_1</i>	S.....	39
<i>Flu_T4_HA_2</i>	S.....	39
<i>Flu_T4_HA_3</i>	S.....	39
<i>H5N1_COBRA</i>	S.....	39
<i>Hu/AV_COBRA2</i>	S.....	39
A/Sichuan/26221/2014	VTVTHAQDILEKTHNGKLCDLNGVKPLILKDCSVAGWLL	78
<i>A/gyr/Washington/41088-6/2014</i>	N.....	78
<i>A/Anhui/2021-00011/2020</i>	N.....	78
<i>A/mute_swan/England/053054/2021</i>	D.....R.....	78
<i>A/WSN/Mongolia/244/2005</i>	D.....R.....	78
<i>A/Hangzhou/01/2021</i>	D.....R.....	78
<i>FLU_T2_HA_9</i>	D.....R.....	78
<i>FLU_T3_HA_1</i>	D.....R.....	78
<i>FLU_T3_HA_2</i>	D.....R.....	78
<i>FLU_T3_HA_3</i>	D.....R.....	78
<i>FLU_T3_HA_4</i>	D.....R.....	78
<i>FLU_T3_HA_5</i>	D.....R.....	78
<i>Flu_T4_HA_1</i>	D.....R.....	78
<i>Flu_T4_HA_2</i>	D.....R.....	78
<i>Flu_T4_HA_3</i>	D.....R.....	78
<i>H5N1_COBRA</i>	D.....R.....	78
<i>Hu/AV_COBRA2</i>	D.....R.....	78
A/Sichuan/26221/2014	GNPMCDFEIRVPEWSYIVERANPANDLCYPGNLNDYEEL	117
<i>A/gyr/Washington/41088-6/2014</i>	T.....	117
<i>A/Anhui/2021-00011/2020</i>	N.....	117
<i>A/mute_swan/England/053054/2021</i>	S.....	117
<i>A/WSN/Mongolia/244/2005</i>	LN.....K.I.....F.....	117
<i>A/Hangzhou/01/2021</i>	S.....	117
<i>FLU_T2_HA_9</i>	N.....K.....F.....	117
<i>FLU_T3_HA_1</i>	N.....K.....F.....	117
<i>FLU_T3_HA_2</i>	N.....K.....F.....	117
<i>FLU_T3_HA_3</i>	N.....K.....F.....	117
<i>FLU_T3_HA_4</i>	N.....K.....F.....	117
<i>FLU_T3_HA_5</i>	N.....K.....F.....	117
<i>Flu_T4_HA_1</i>	N.....K.....F.....	117
<i>Flu_T4_HA_2</i>	T.....	117
<i>Flu_T4_HA_3</i>	T.....	117
<i>H5N1_COBRA</i>	N.....K.S.....F.....	117
<i>Hu/AV_COBRA2</i>	N.....K.....F.....	117

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Figure 28 continued

A/Sichuan/26221/2014	KHLLSRINHFELIIPKSSWTNHETSLGVSAACPYQGT	156
<i>A/gyr/Washington/41088-6/2014</i>	T.....R.....P.....	A 156
<i>A/Anhui/2021-00011/2020</i>	TQ.....E.....S.....T.....	V 155
<i>A/mute swan/England/053054/2021</i>	P.....	156
<i>A/WSN/Mongolia/244/2005</i>	Q.....SD.....A.....S.....S.....	R 156
<i>A/Hangzhou/01/2021</i>	P.....	A 156
<i>Flu_T2_HA_9</i>	Q.....SD.....A.....S.....S.....	R 156
<i>Flu_T3_HA_1</i>	Q.....SD.....A.....S.....S.....	R 156
<i>Flu_T3_HA_2</i>	Q.....SD.....A.....S.....S.....	R 156
<i>Flu_T3_HA_3</i>	Q.....SD.....A.....S.....S.....	R 155
<i>Flu_T3_HA_4</i>	Q.....SD.....A.....S.....VP.....	R 156
<i>Flu_T3_HA_5</i>	Q.....SD.....A.....S.....S.....	R 156
<i>Flu_T4_HA_1</i>	P.....	156
<i>Flu_T4_HA_2</i>	P.....	156
<i>Flu_T4_HA_3</i>	P.....N.....	156
<i>HSN1_COBRA</i>	Q.....S.....A.....S.....S.....	R 156
<i>Hu/AV_COBRA2</i>	Q.....SD.....A.....S.....S.....	K 156
A/Sichuan/26221/2014	PSFFRNVVWLIRKNDAYPTIKISYNNNTNOEDLLILWGVH	195
<i>A/gyr/Washington/41088-6/2014</i>	S.....R.....	I 195
<i>A/Anhui/2021-00011/2020</i>	A.....T.....K.....K.....	I 194
<i>A/mute swan/England/053054/2021</i>	R.....	I 195
<i>A/WSN/Mongolia/244/2005</i>	S.....DN.....R.....V.....	I 195
<i>A/Hangzhou/01/2021</i>	R.....	I 195
<i>Flu_T2_HA_9</i>	S.....N.....R.....V.....	I 195
<i>Flu_T3_HA_1</i>	T.....R.....V.....	I 195
<i>Flu_T3_HA_2</i>	NT.....R.....V.....	I 195
<i>Flu_T3_HA_3</i>	S.....N.....R.....V.....	I 194
<i>Flu_T3_HA_4</i>	S.....N.....R.....V.....	I 195
<i>Flu_T3_HA_5</i>	S.....N.....R.....V.....	I 195
<i>Flu_T4_HA_1</i>	R.....	I 195
<i>Flu_T4_HA_2</i>	R.....	I 195
<i>Flu_T4_HA_3</i>	T.....R.....	I 195
<i>HSN1_COBRA</i>	S.....ST.....R.....V.....	I 195
<i>Hu/AV_COBRA2</i>	S.....S.....R.....V.....	I 195
A/Sichuan/26221/2014	HSNNAAEQTNLYKNFTTYISVGTSTLNORLVPKIATRSG	234
<i>A/gyr/Washington/41088-6/2014</i>	D.....V.....	234
<i>A/Anhui/2021-00011/2020</i>	SVE.....D.....V.....	233
<i>A/mute swan/England/053054/2021</i>	E.....	234
<i>A/WSN/Mongolia/244/2005</i>	P.D.....R.....Q.....	K 234
<i>A/Hangzhou/01/2021</i>	E.....I.....S.....	234
<i>Flu_T2_HA_9</i>	P.D.....R.....Q.....	K 234
<i>Flu_T3_HA_1</i>	P.D.....K.....Q.....	K 234
<i>Flu_T3_HA_2</i>	P.D.....K.....Q.....	K 234
<i>Flu_T3_HA_3</i>	P.D.....R.....Q.....	K 233
<i>Flu_T3_HA_4</i>	P.D.....R.....Q.....	K 234
<i>Flu_T3_HA_5</i>	P.D.....R.....Q.....	K 234
<i>Flu_T4_HA_1</i>		234
<i>Flu_T4_HA_2</i>		234
<i>Flu_T4_HA_3</i>	T.....N.....	234
<i>HSN1_COBRA</i>	P.D.....K.....Q.....	K 234
<i>Hu/AV_COBRA2</i>	P.D.....R.....Q.....	K 234
A/Sichuan/26221/2014	VNGQRGRMOFFWTILKPNDALHFESNGNFIAPAYAYKIV	273
<i>A/gyr/Washington/41088-6/2014</i>	S.....	273
<i>A/Anhui/2021-00011/2020</i>	R.....	I 272
<i>A/mute swan/England/053054/2021</i>	D.....	273
<i>A/WSN/Mongolia/244/2005</i>	S.....E.....N.....N.....	273
<i>A/Hangzhou/01/2021</i>	D.....	273
<i>Flu_T2_HA_9</i>	S.....E.....N.....	273
<i>Flu_T3_HA_1</i>	S.....E.....N.....	273
<i>Flu_T3_HA_2</i>	S.....E.....N.....	273
<i>Flu_T3_HA_3</i>	S.....E.....N.....	272
<i>Flu_T3_HA_4</i>	ES.....E.....N.....	273
<i>Flu_T3_HA_5</i>	ES.....E.....N.....	273
<i>Flu_T4_HA_1</i>		273
<i>Flu_T4_HA_2</i>	E.....	273
<i>Flu_T4_HA_3</i>		273
<i>HSN1_COBRA</i>	S.....E.....N.....	273
<i>Hu/AV_COBRA2</i>	S.....E.....N.....	273

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Figure 28 continued

A/Sichuan/26221/2014	KKGDSTIMKSEMEYGHCHTKCQTPIGAINSSMPFHNHP	312
<i>A/gyr/Washington/41088-6/2014</i>		312
<i>A/Anhui/2021-00011/2020</i>	..T.....I...D..S.....	311
<i>A/mute_swan/England/053054/2021</i>	GV.....V.....	312
<i>A/WSN/Mongolia/244/2005</i>	L...N.....	312
<i>A/Hangzhou/01/2021</i>	GV.....V.....	312
<i>FLU_T2_HA_9</i>	A.....L...N.....M.....	312
<i>FLU_T3_HA_1</i>	A.....L...N.....M.....	312
<i>FLU_T3_HA_2</i>	A.....L...N.....M.....	312
<i>FLU_T3_HA_3</i>	A.....L...N.....M.....	311
<i>FLU_T3_HA_4</i>	A.....L...N.....M.....	312
<i>FLU_T3_HA_5</i>	A.....L...N.....M.....	312
<i>Flu_T4_HA_1</i>	V.....	312
<i>Flu_T4_HA_2</i>	V.....	312
<i>Flu_T4_HA_3</i>	V.....	312
<i>MSN1_COBRA</i>	L...N.....M.....	312
<i>Hu/AV_COBRA2</i>	A.....L...N.....M.....	312
A/Sichuan/26221/2014	LTIGECPKYVKSNEKLVLATGLRNSPLRE·KRR·KRGLEFG	349
<i>A/gyr/Washington/41088-6/2014</i>	R.....	349
<i>A/Anhui/2021-00011/2020</i>	R.....	349
<i>A/mute_swan/England/053054/2021</i>		349
<i>A/WSN/Mongolia/244/2005</i>	R.....QG·R·S.....	350
<i>A/Hangzhou/01/2021</i>		349
<i>FLU_T2_HA_9</i>	R.....Q·R·K.....	350
<i>FLU_T3_HA_1</i>	R.....Q·R·K.....	350
<i>FLU_T3_HA_2</i>	R.....Q·R·K.....	350
<i>FLU_T3_HA_3</i>	R.....Q·R·K.....	349
<i>FLU_T3_HA_4</i>	R.....Q·R·K.....	350
<i>FLU_T3_HA_5</i>	R.....Q·R·K.....	350
<i>Flu_T4_HA_1</i>	KR·K.....	351
<i>Flu_T4_HA_2</i>	KR·K.....	351
<i>Flu_T4_HA_3</i>	KR·K.....	351
<i>MSN1_COBRA</i>	R.....Q·R·K.....	350
<i>Hu/AV_COBRA2</i>	R.....Q·R·K.....	349
A/Sichuan/26221/2014	AIAQFIEGGWQGMVGGWYGYHHSNEQQSGYAAOKESTQK	388
<i>A/gyr/Washington/41088-6/2014</i>		388
<i>A/Anhui/2021-00011/2020</i>	R.....	387
<i>A/mute_swan/England/053054/2021</i>		388
<i>A/WSN/Mongolia/244/2005</i>		389
<i>A/Hangzhou/01/2021</i>		388
<i>FLU_T2_HA_9</i>		389
<i>FLU_T3_HA_1</i>		389
<i>FLU_T3_HA_2</i>		389
<i>FLU_T3_HA_3</i>		388
<i>FLU_T3_HA_4</i>		389
<i>FLU_T3_HA_5</i>		389
<i>Flu_T4_HA_1</i>		390
<i>Flu_T4_HA_2</i>		390
<i>Flu_T4_HA_3</i>		390
<i>MSN1_COBRA</i>		389
<i>Hu/AV_COBRA2</i>		388
A/Sichuan/26221/2014	AIDGVTNEVNSIIIDKMNTQFEAVGREFNNLERRIENLNK	427
<i>A/gyr/Washington/41088-6/2014</i>		427
<i>A/Anhui/2021-00011/2020</i>	S.....	426
<i>A/mute_swan/England/053054/2021</i>		427
<i>A/WSN/Mongolia/244/2005</i>		428
<i>A/Hangzhou/01/2021</i>		427
<i>FLU_T2_HA_9</i>		428
<i>FLU_T3_HA_1</i>		428
<i>FLU_T3_HA_2</i>		428
<i>FLU_T3_HA_3</i>		427
<i>FLU_T3_HA_4</i>		428
<i>FLU_T3_HA_5</i>		428
<i>Flu_T4_HA_1</i>		429
<i>Flu_T4_HA_2</i>		429
<i>Flu_T4_HA_3</i>		429
<i>MSN1_COBRA</i>		428
<i>Hu/AV_COBRA2</i>	E.....	427

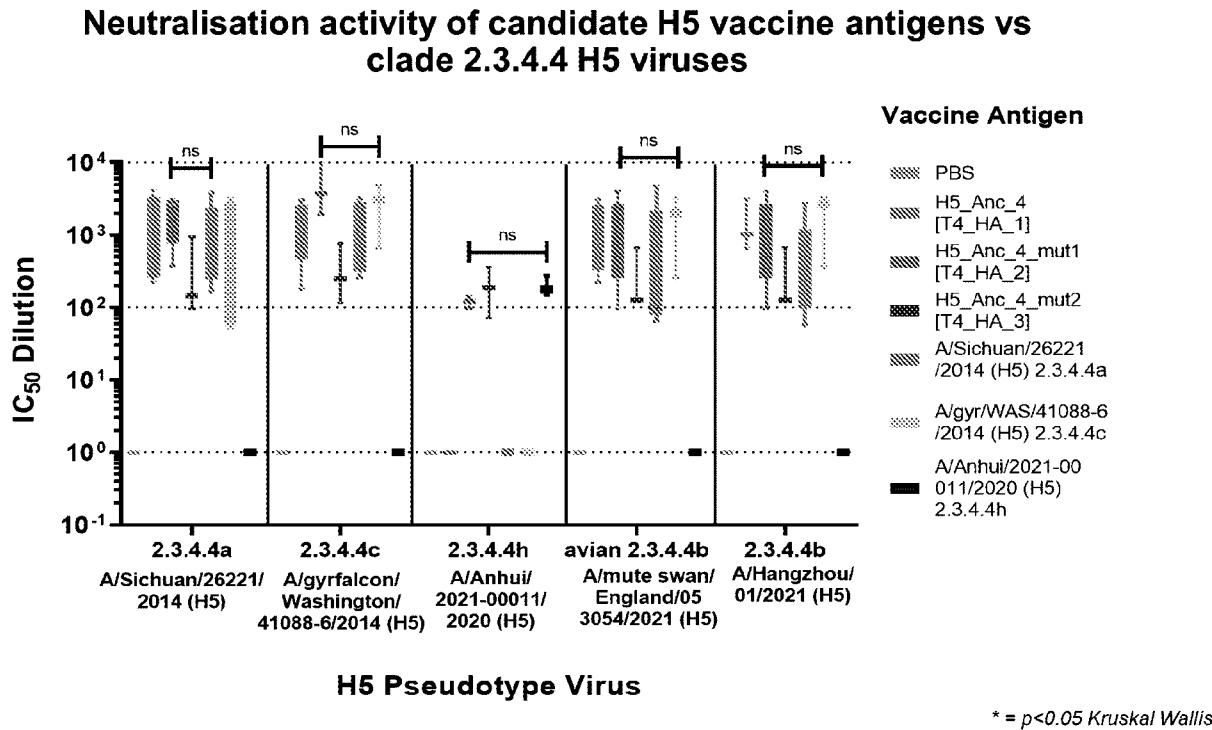
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Figure 28 continued

A/Sichuan/26221/2014	KMEDGFLDVWYTNAEILLVLMENEATLOPHDSNVKNLYDK	466
<i>A/gyr/Washington/41088-6/2014</i>		466
<i>A/Anhui/2021-00011/2020</i>		466
<i>A/mute_swan/England/053054/2021</i>		466
<i>A/WSN/Mongolia/244/2005</i>		467
<i>A/Hangzhou/01/2021</i>		466
<i>Flu_T2_HA_9</i>		467
<i>Flu_T3_HA_1</i>		467
<i>Flu_T3_HA_2</i>		467
<i>Flu_T3_HA_3</i>		466
<i>Flu_T3_HA_4</i>		467
<i>Flu_T3_HA_5</i>		467
<i>Flu_T4_HA_1</i>		468
<i>Flu_T4_HA_2</i>		468
<i>Flu_T4_HA_3</i>		468
<i>HSN1_COBRA</i>		467
<i>Hu/AV_COBRA2</i>	E.....	466
A/Sichuan/26221/2014	VRLQLRDNAKELONGCFEFYHKCONKCMESVRNGTYDYP	505
<i>A/gyr/Washington/41088-6/2014</i>	m.....	505
<i>A/Anhui/2021-00011/2020</i>	mm.....	504
<i>A/mute_swan/England/053054/2021</i>	mm.....	505
<i>A/WSN/Mongolia/244/2005</i>	B.....	506
<i>A/Hangzhou/01/2021</i>	mm.....	505
<i>Flu_T2_HA_9</i>	mm.....	506
<i>Flu_T3_HA_1</i>	mm.....	506
<i>Flu_T3_HA_2</i>	mm.....	506
<i>Flu_T3_HA_3</i>	mm.....	505
<i>Flu_T3_HA_4</i>	mm.....	506
<i>Flu_T3_HA_5</i>	mm.....	506
<i>Flu_T4_HA_1</i>	mm.....	507
<i>Flu_T4_HA_2</i>	mm.....	507
<i>Flu_T4_HA_3</i>	mm.....	507
<i>HSN1_COBRA</i>	mm.....K.....	506
<i>Hu/AV_COBRA2</i>	mm.....	505
A/Sichuan/26221/2014	QYSEEARLKREEISQVKLESIGTYOILSIYSTVASSLAL	544
<i>A/gyr/Washington/41088-6/2014</i>	K.....I.....	544
<i>A/Anhui/2021-00011/2020</i>	T.....F.....V.....	543
<i>A/mute_swan/England/053054/2021</i>	A.....	544
<i>A/WSN/Mongolia/244/2005</i>		545
<i>A/Hangzhou/01/2021</i>	A.....	544
<i>Flu_T2_HA_9</i>		545
<i>Flu_T3_HA_1</i>		545
<i>Flu_T3_HA_2</i>		545
<i>Flu_T3_HA_3</i>		544
<i>Flu_T3_HA_4</i>		545
<i>Flu_T3_HA_5</i>		545
<i>Flu_T4_HA_1</i>		546
<i>Flu_T4_HA_2</i>		546
<i>Flu_T4_HA_3</i>		546
<i>HSN1_COBRA</i>	N.....	545
<i>Hu/AV_COBRA2</i>		544
A/Sichuan/26221/2014	AIIVAGLSLWMCSNGSLQCRICI	567
<i>A/gyr/Washington/41088-6/2014</i>		567
<i>A/Anhui/2021-00011/2020</i>	M.....	566
<i>A/mute_swan/England/053054/2021</i>	MM.....	567
<i>A/WSN/Mongolia/244/2005</i>	M.....	568
<i>A/Hangzhou/01/2021</i>	MI.....	567
<i>Flu_T2_HA_9</i>	M.....	568
<i>Flu_T3_HA_1</i>	M.....	568
<i>Flu_T3_HA_2</i>	M.....	568
<i>Flu_T3_HA_3</i>	M.....	567
<i>Flu_T3_HA_4</i>	M.....	568
<i>Flu_T3_HA_5</i>	M.....	568
<i>Flu_T4_HA_1</i>	M.....	569
<i>Flu_T4_HA_2</i>	M.....	569
<i>Flu_T4_HA_3</i>	M.....	569
<i>HSN1_COBRA</i>	M.....	568
<i>Hu/AV_COBRA2</i>	M.....	567

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Figure 29

A)



TIER 4 DESIGNS vs clade 2.3.4.4x

B)

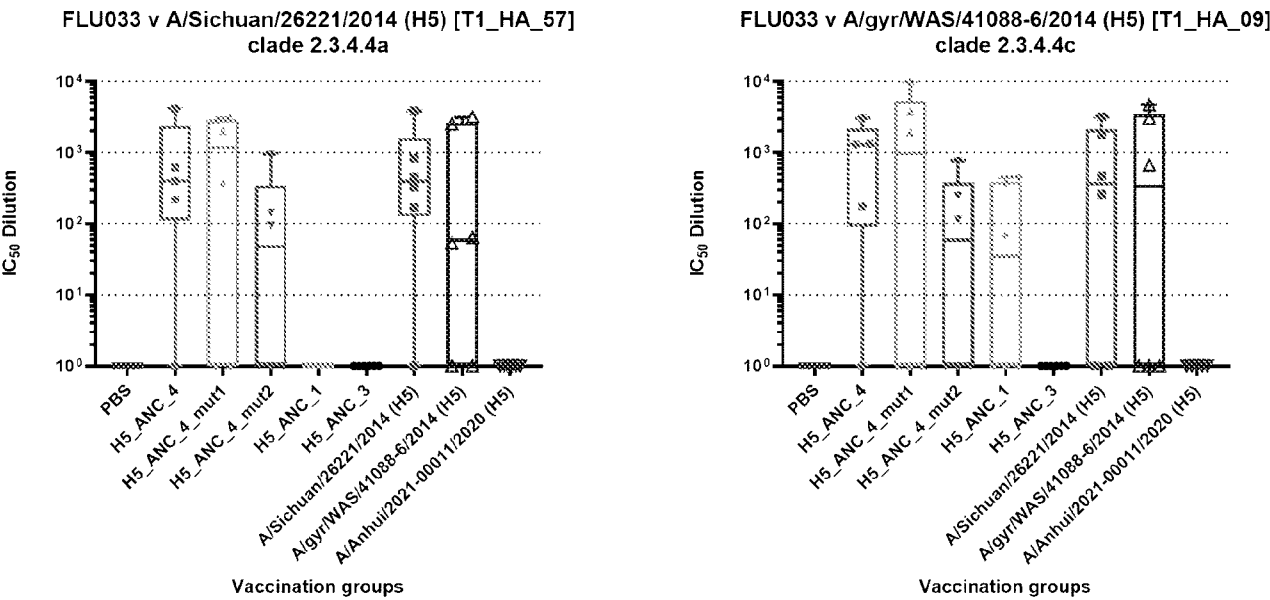
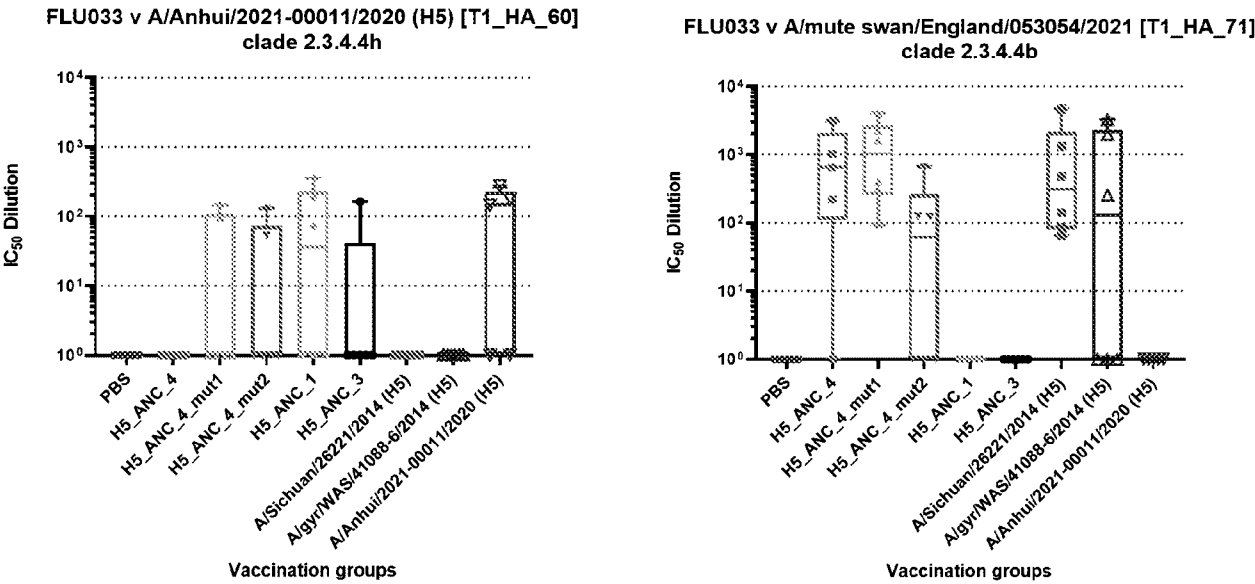


Figure 29 Continued

C)

TIER 4 DESIGNS vs clade 2.3.4.4x



TIER 4 DESIGNS vs clade 2.3.4.4x

D)

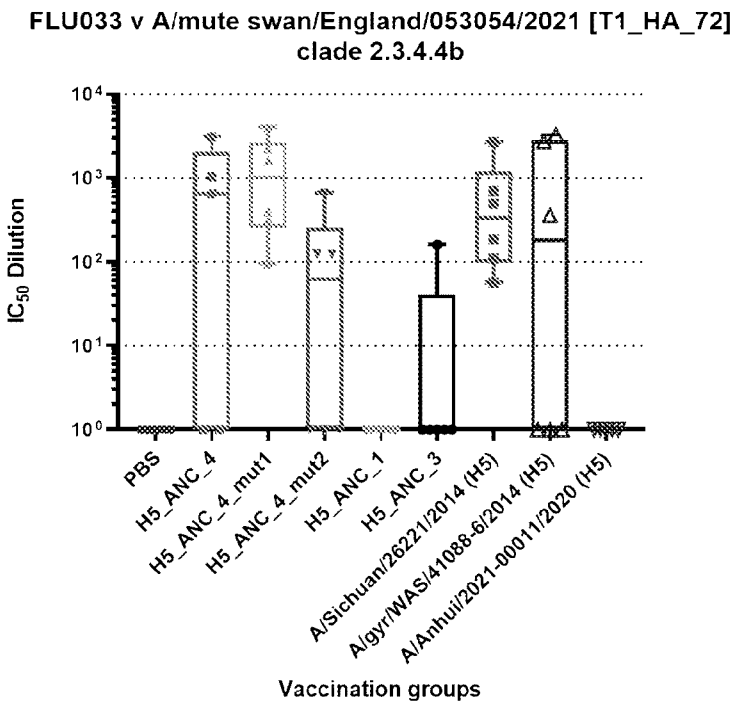


Figure 30

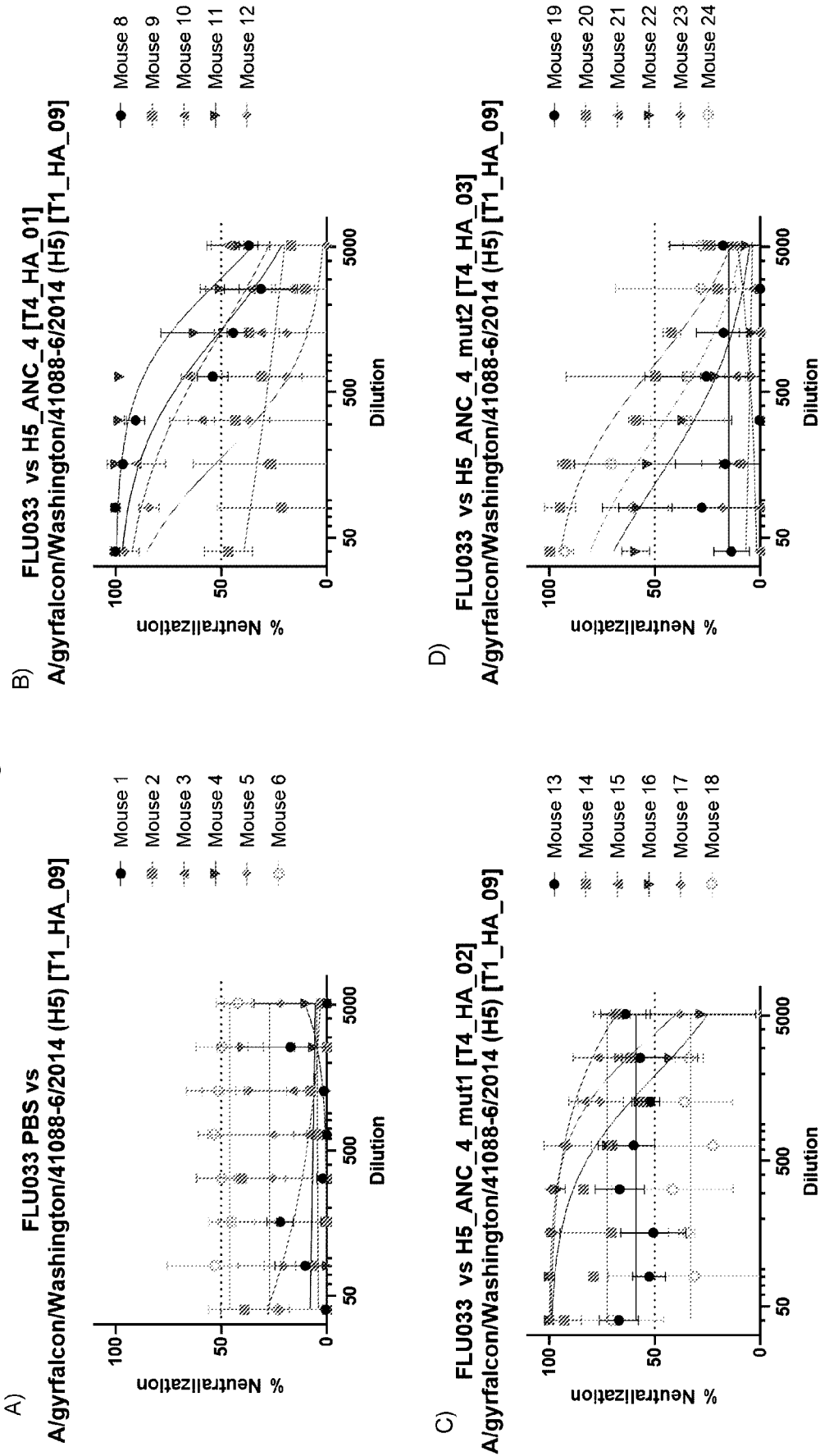
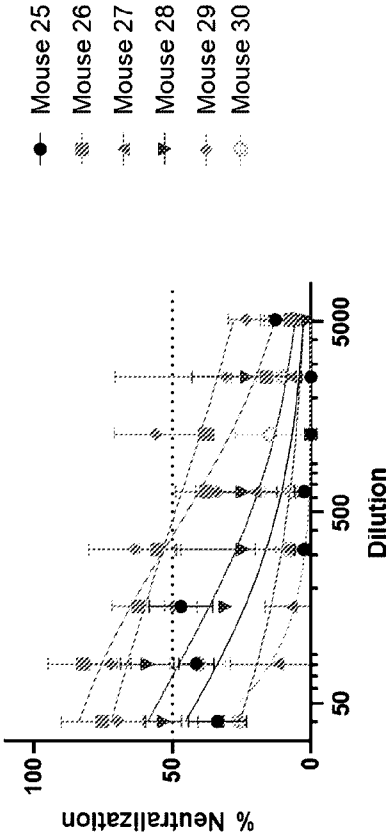
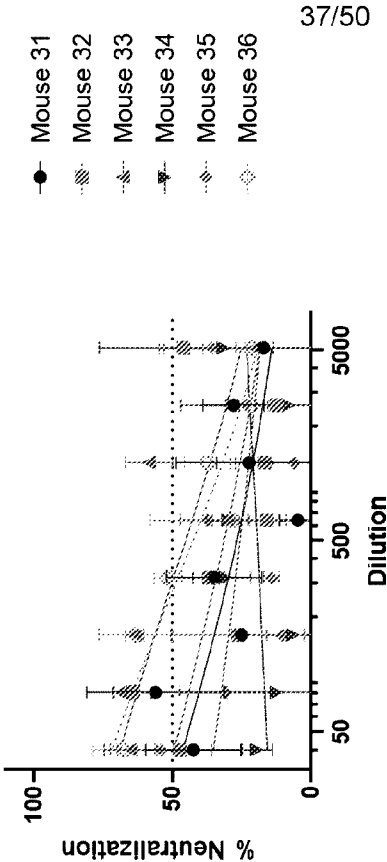


Figure 30 continued

E) FLU033 vs H5_ANC_1 [T2_HA_09]
A/gyrfalcon/Washington/41088-6/2014 (H5) [T1_HA_09]

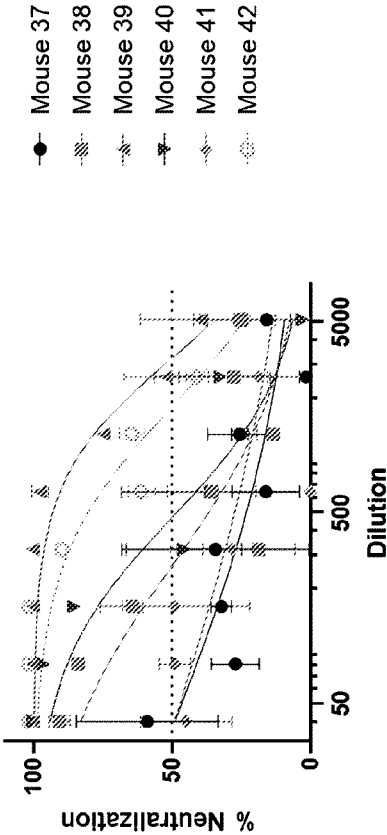


F) FLU033 H5_ANC_3 [T3_HA_02] vs
A/gyrfalcon/Washington/41088-6/2014 (H5) [T1_HA_09]

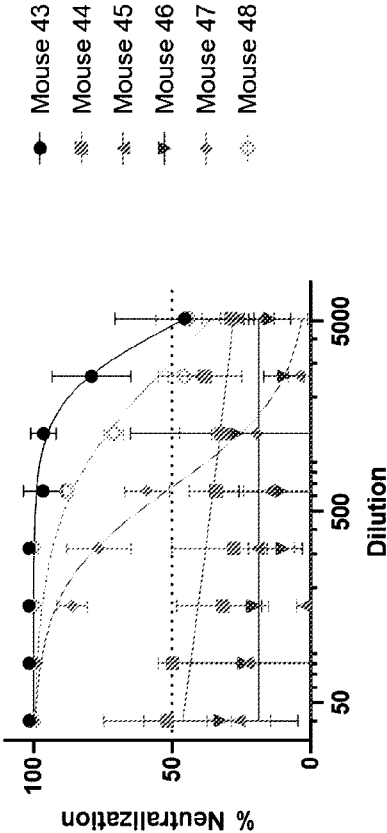


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G) FLU033 A/Sichuan/26221/2014 (H5) [T1_HA_57] vs
A/gyrfalcon/Washington/41088-6/2014 (H5) [T1_HA_09]



H) FLU033 A/gyrfalcon/Washington/41088-6/2014 (H9) [T1_HA_09]
vs A/gyrfalcon/Washington/41088-6/2014 (H5) [T1_HA_09]



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Figure 30 continued

l)

**FLU033 A/Anhui/2021-00011/2020 (H5) [T1_HA_60] vs
A/gyrfalcon/Washington/41088-6/2014 (H5) [T1_HA_09]**

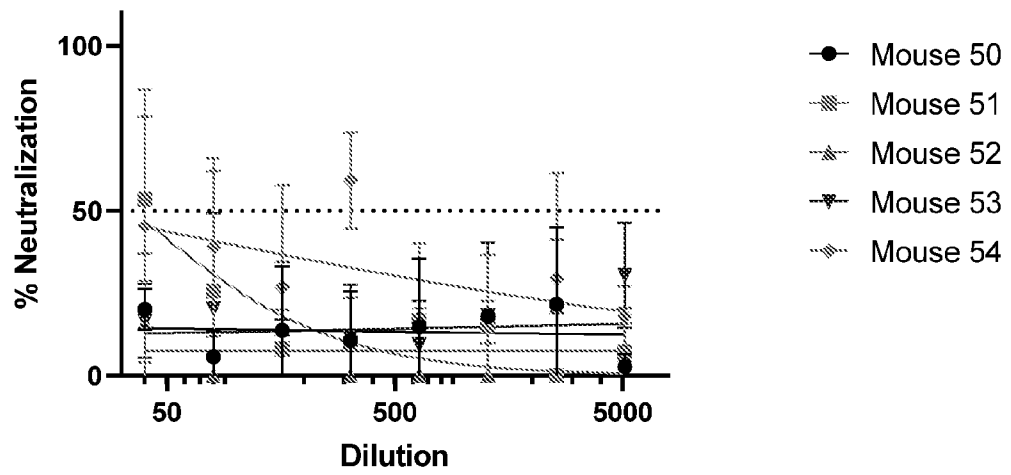
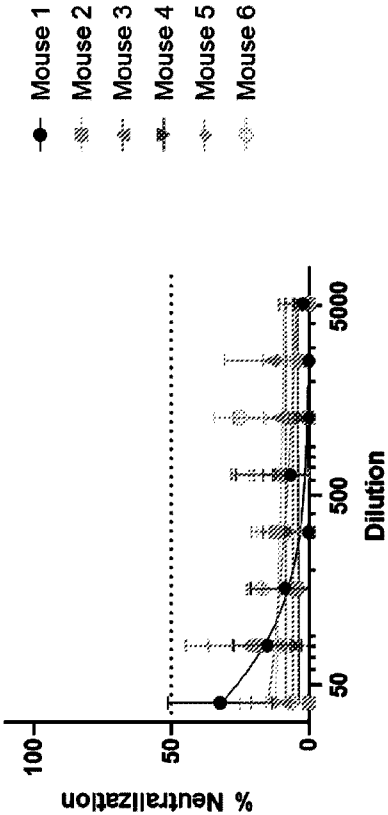


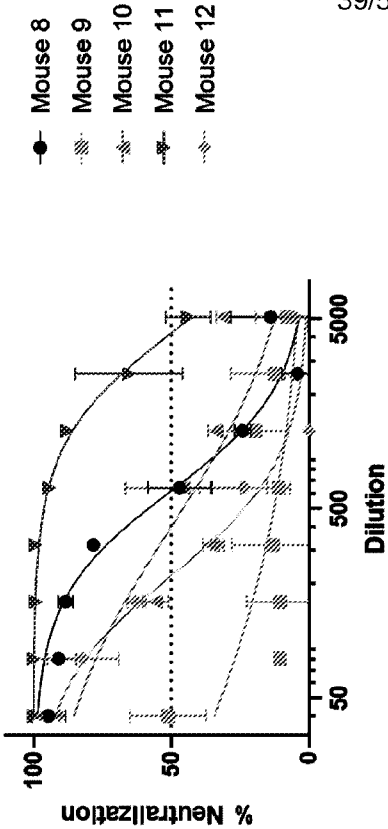
Figure 31

A) FLU033 PBS vs
A/Sichuan/26221/2014 (H5) [T1_HA_57]



B)

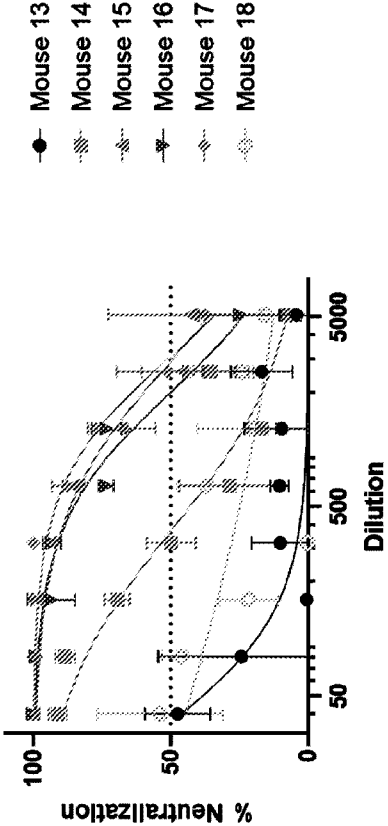
FLU033 vs H5_ANC_4 [T4_HA_01]
A/Sichuan/26221/2014 (H5) [T1_HA_57]



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D)

C) FLU033 vs H5_ANC_4_mut1 [T4_HA_02]
A/Sichuan/26221/2014 (H5) [T1_HA_57]



FLU033 vs H5_ANC_4_mut2 [T4_HA_03]
A/Sichuan/26221/2014 (H5) [T1_HA_57]

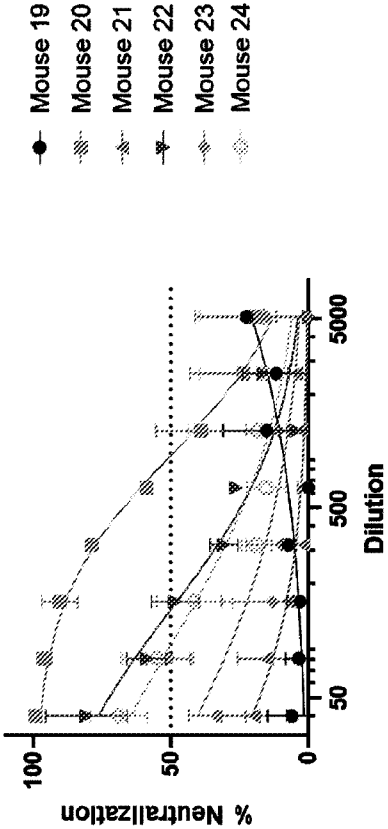
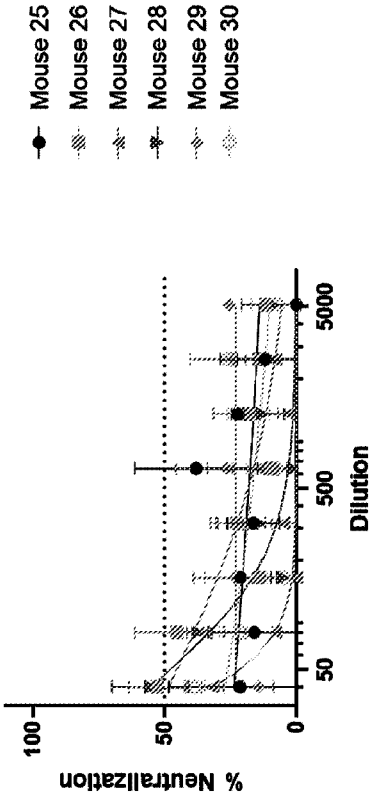


Figure 31 continued

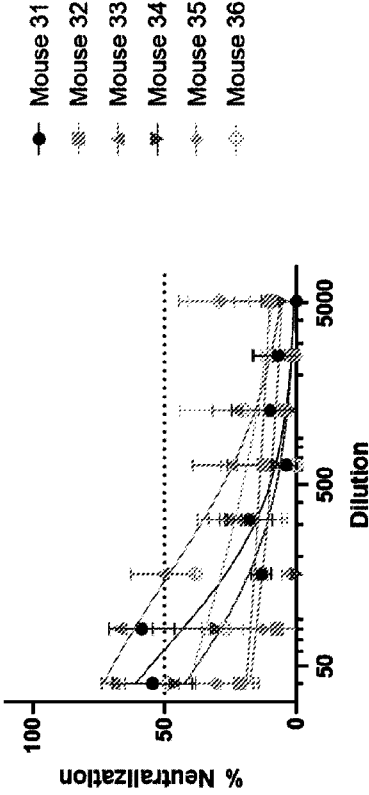
E)

FLU033 vs H5_ANC_1 [T2_HA_09]
A/Sichuan/26221/2014 (H5) [T1_HA_57]



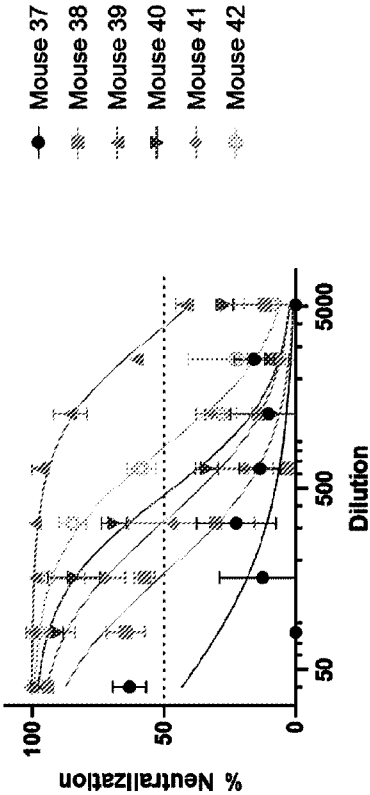
F)

FLU033 H5_ANC_3 [T3_HA_02] vs
A/Sichuan/26221/2014 (H5) [T1_HA_57]



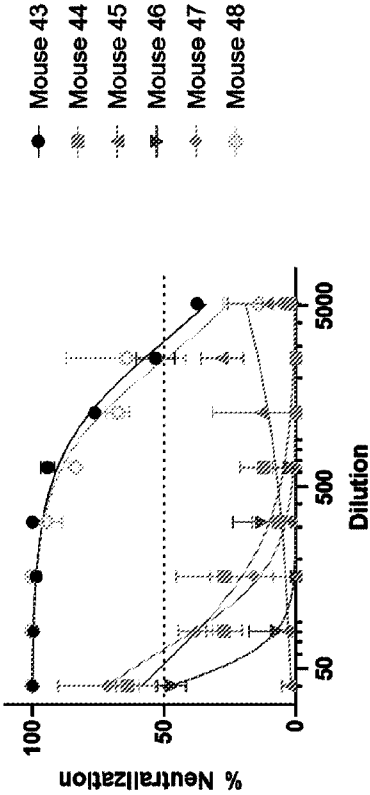
G)

FLU033 A/Sichuan/26221/2014 (H5) [T1_HA_57] vs
A/Sichuan/26221/2014 (H5) [T1_HA_57]



H)

FLU033 A/gyrfalcon/Washington/41088-6/2014 (H9) [T1_HA_09]
vs A/Sichuan/26221/2014 (H5) [T1_HA_57]



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Figure 31 continued

l)

FLU033 A/Anhui/2021-00011/2020 (H5) [T1_HA_60] vs
A/Sichuan/26221/2014 (H5) [T1_HA_57]

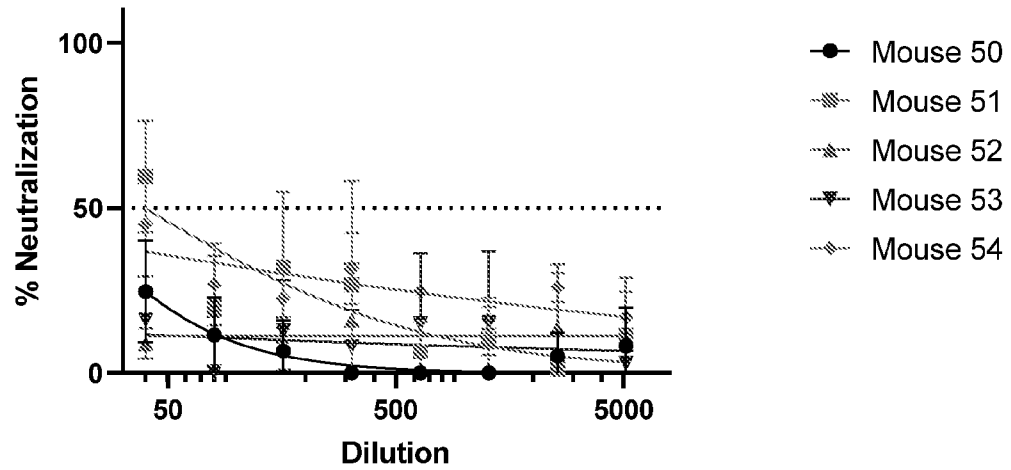


Figure 32

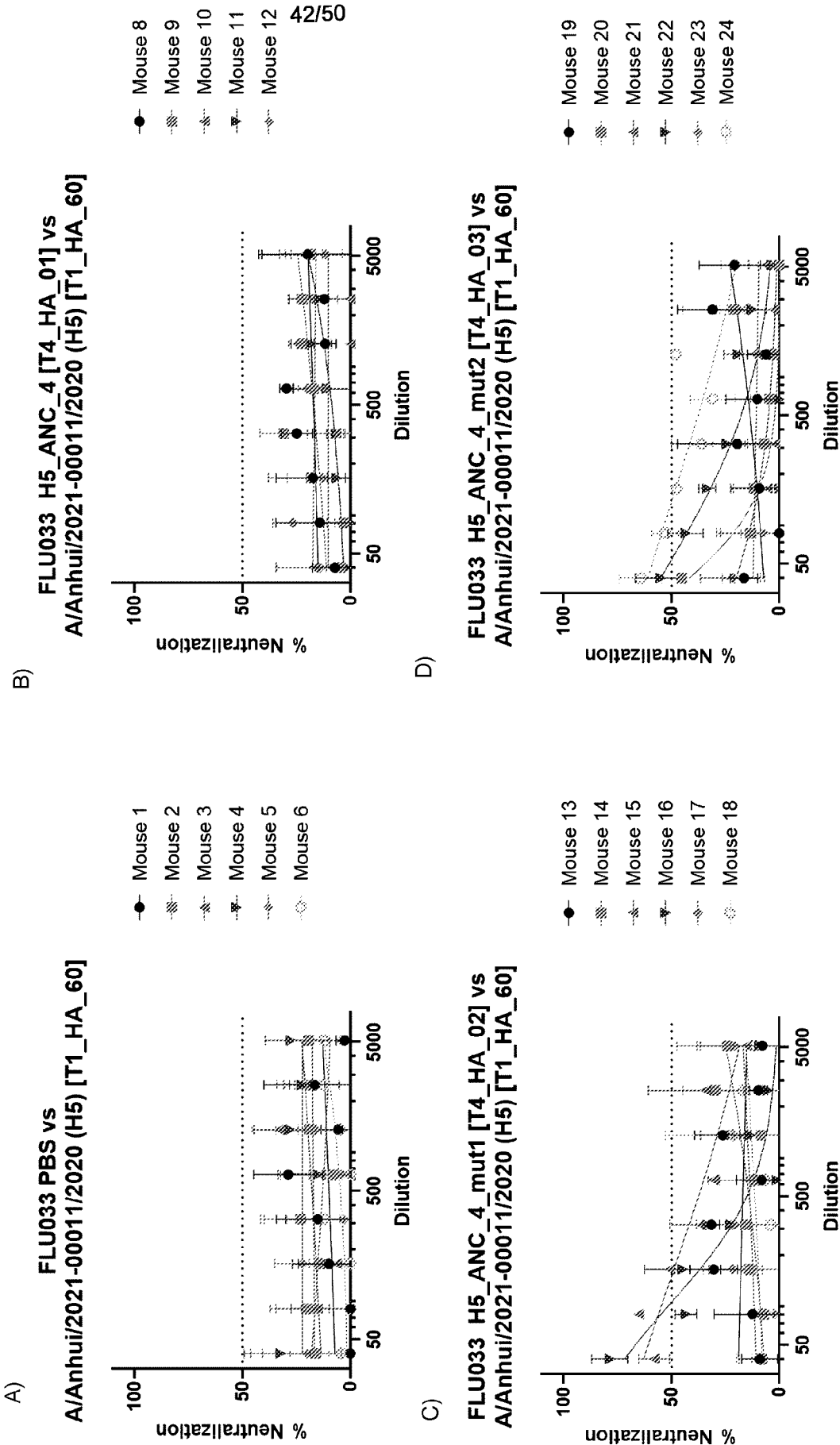
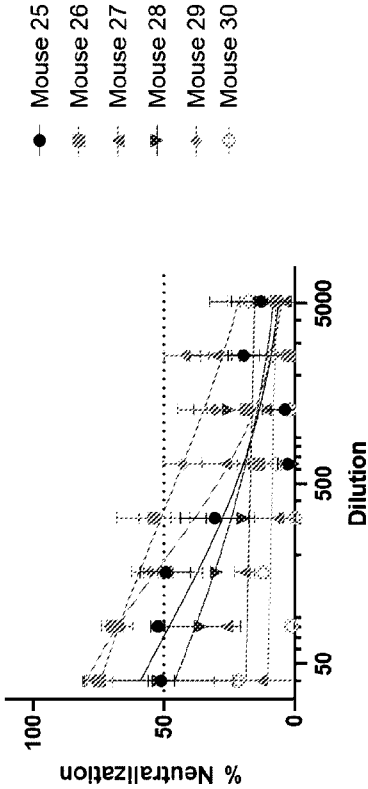
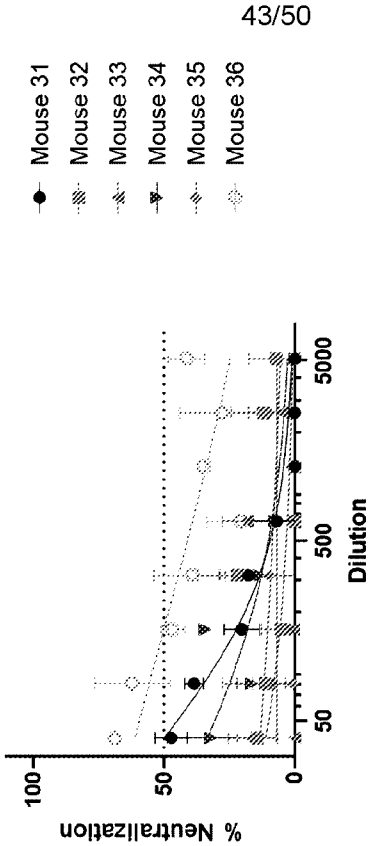


Figure 32 continued

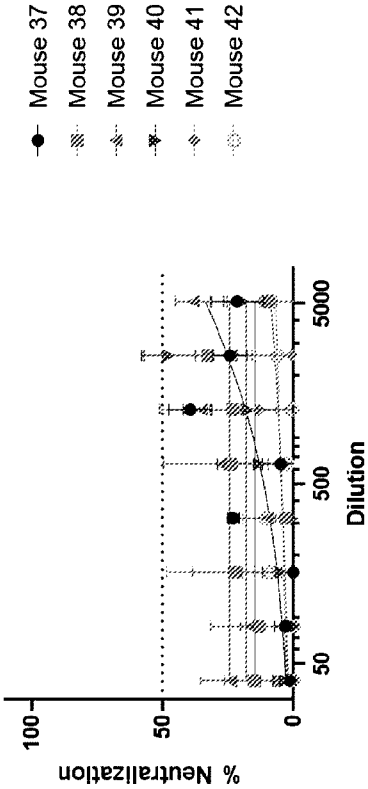
E) FLU033 H5_ANC_1 [T2_HA_09] vs
A/Anhui/2021-00011/2020 (H5) [T1_HA_60]



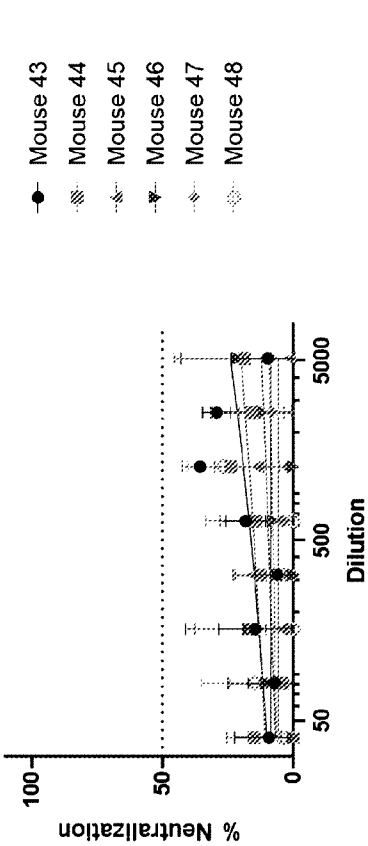
F) FLU033 H5_ANC_3 [T3_HA_02] vs
A/Anhui/2021-00011/2020 (H5) [T1_HA_60]



G) FLU033 A/Sichuan/26221/2014 (H5) [T1_HA_57] vs
A/Anhui/2021-00011/2020 (H5) [T1_HA_60]



H) FLU033 A/gyrfalcon/Washington/41088-6/2014 (H9) [T1_HA_09]
vs A/Anhui/2021-00011/2020 (H5) [T1_HA_60]



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Figure 32 continued

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FLU033 A/Anhui/2021-00011/2020 (H5) [T1_HA_60] vs
A/Anhui/2021-00011/2020 (H5) [T1_HA_60]

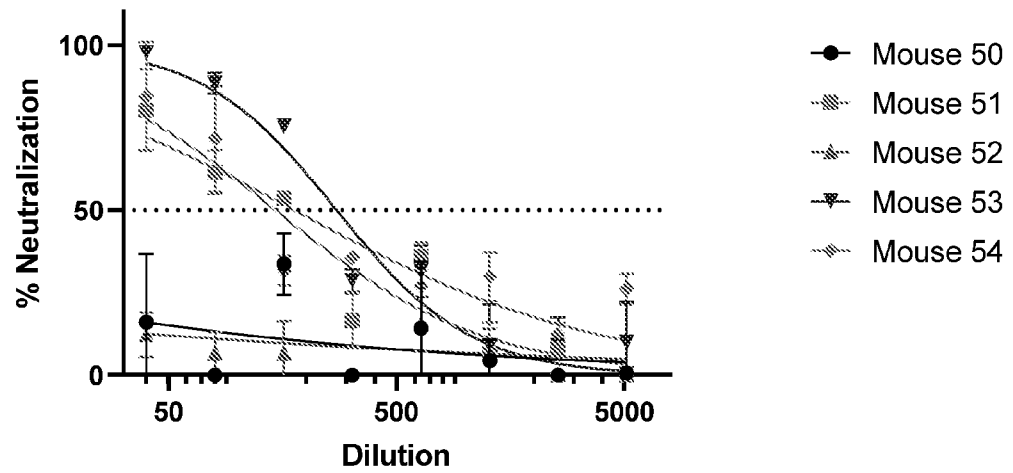
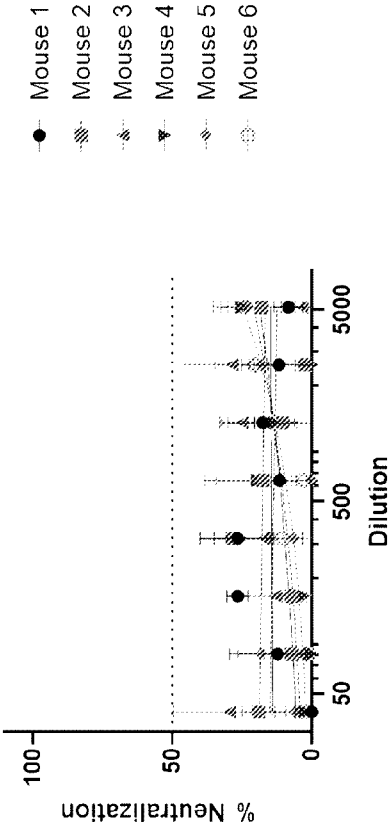
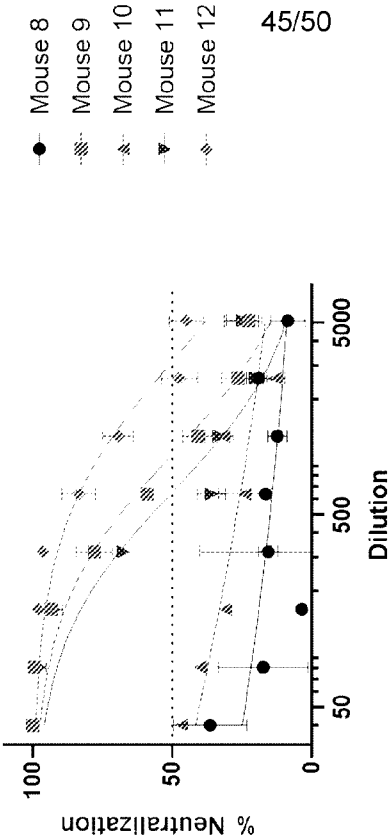


Figure 33

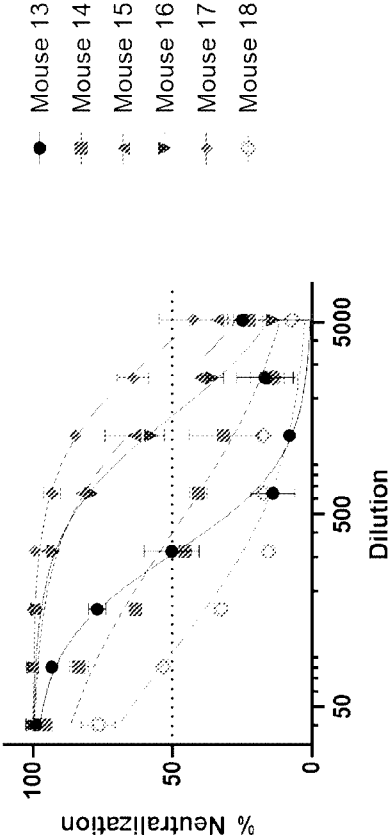
A) FLU033 PBS vs
A/mute swan/England/053054/2021 (H5) [T1_HA_71]



B) FLU033 H5_Anc_4 [T4_HA_01] vs
A/mute swan/England/053054/2021 (H5) [T1_HA_71]



C) FLU033 H5_Anc_4_mut1 [T4_HA_02] vs
A/mute swan/England/053054/2021 (H5) [T1_HA_71]



D) FLU033 H5_Anc_4_mut2 [T4_HA_03] vs
A/mute swan/England/053054/2021 (H5) [T1_HA_71]

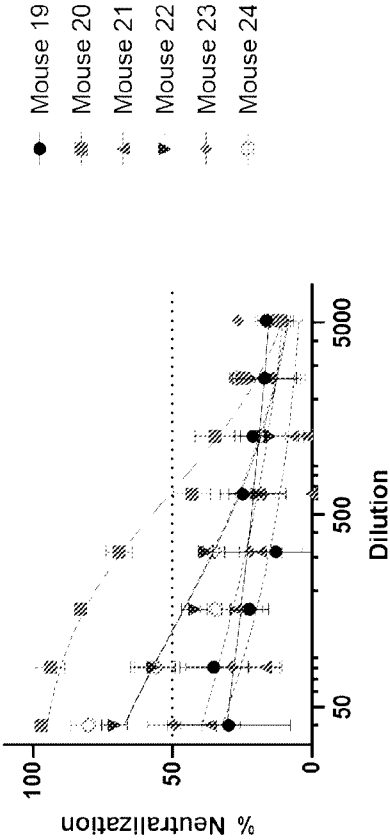
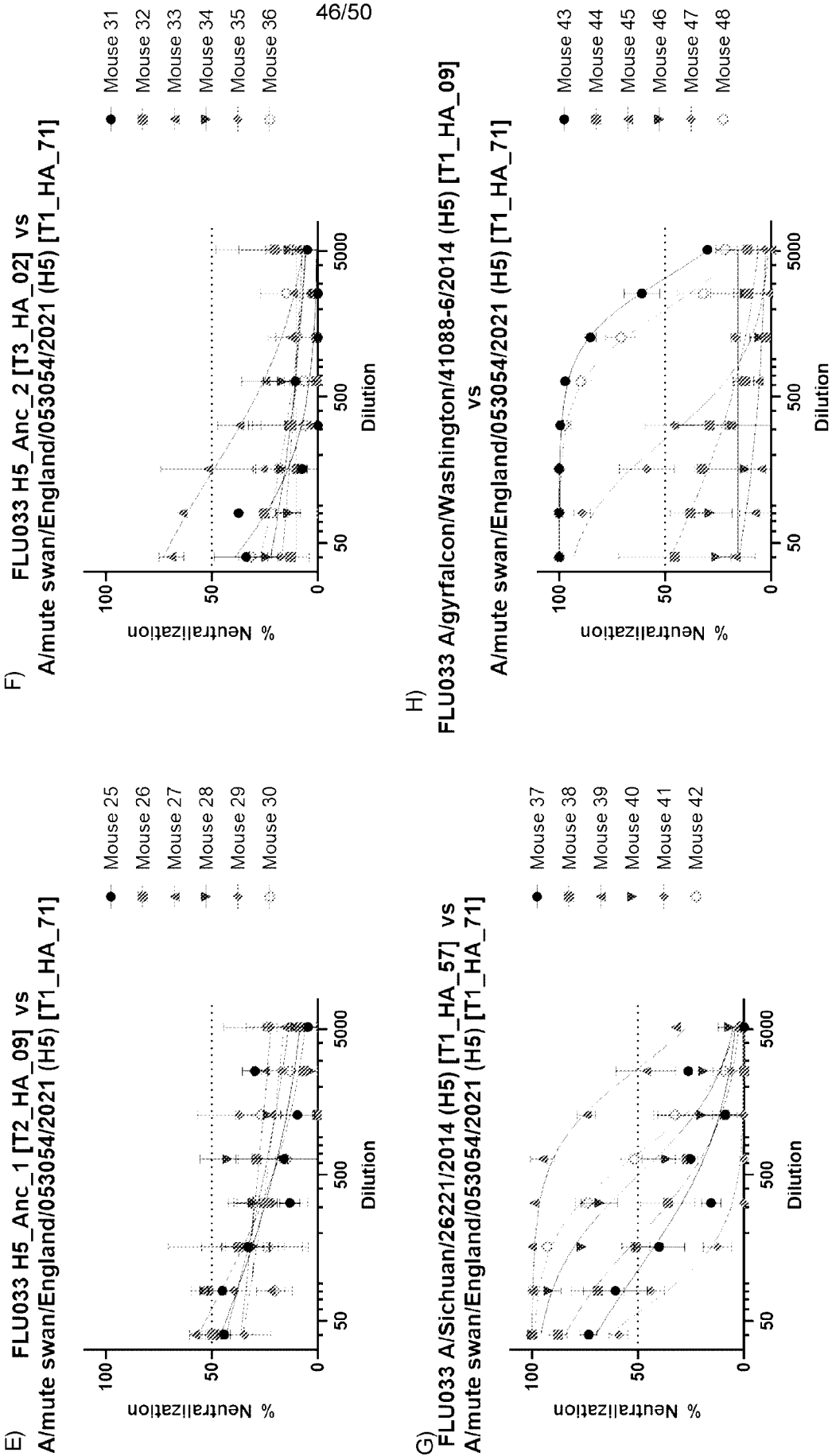


Figure 33 continued



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Figure 33 continued

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FLU033 A/Anhui/2021-00011/2020 (H5) [T1_HA_60] vs
A/mute swan/England/053054/2021 (H5) [T1_HA_71]

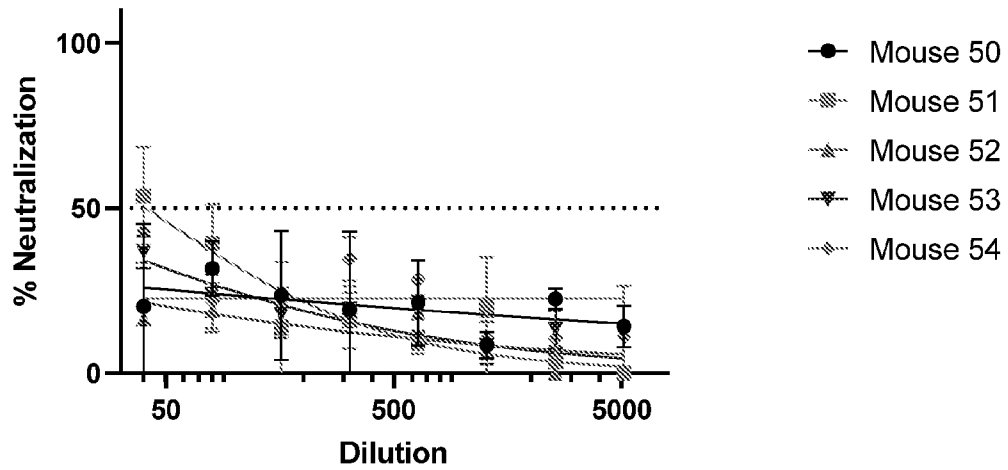


Figure 34

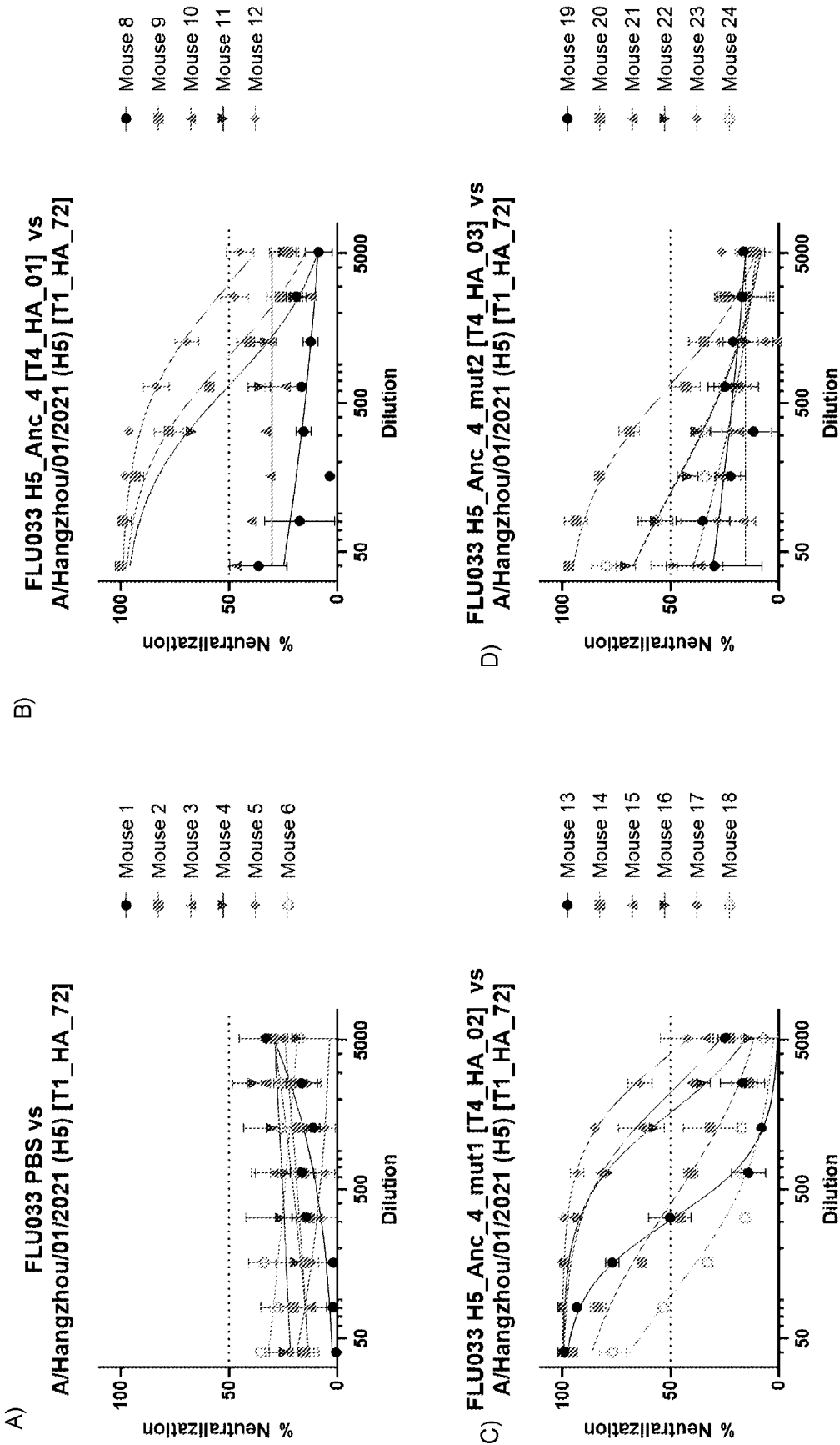
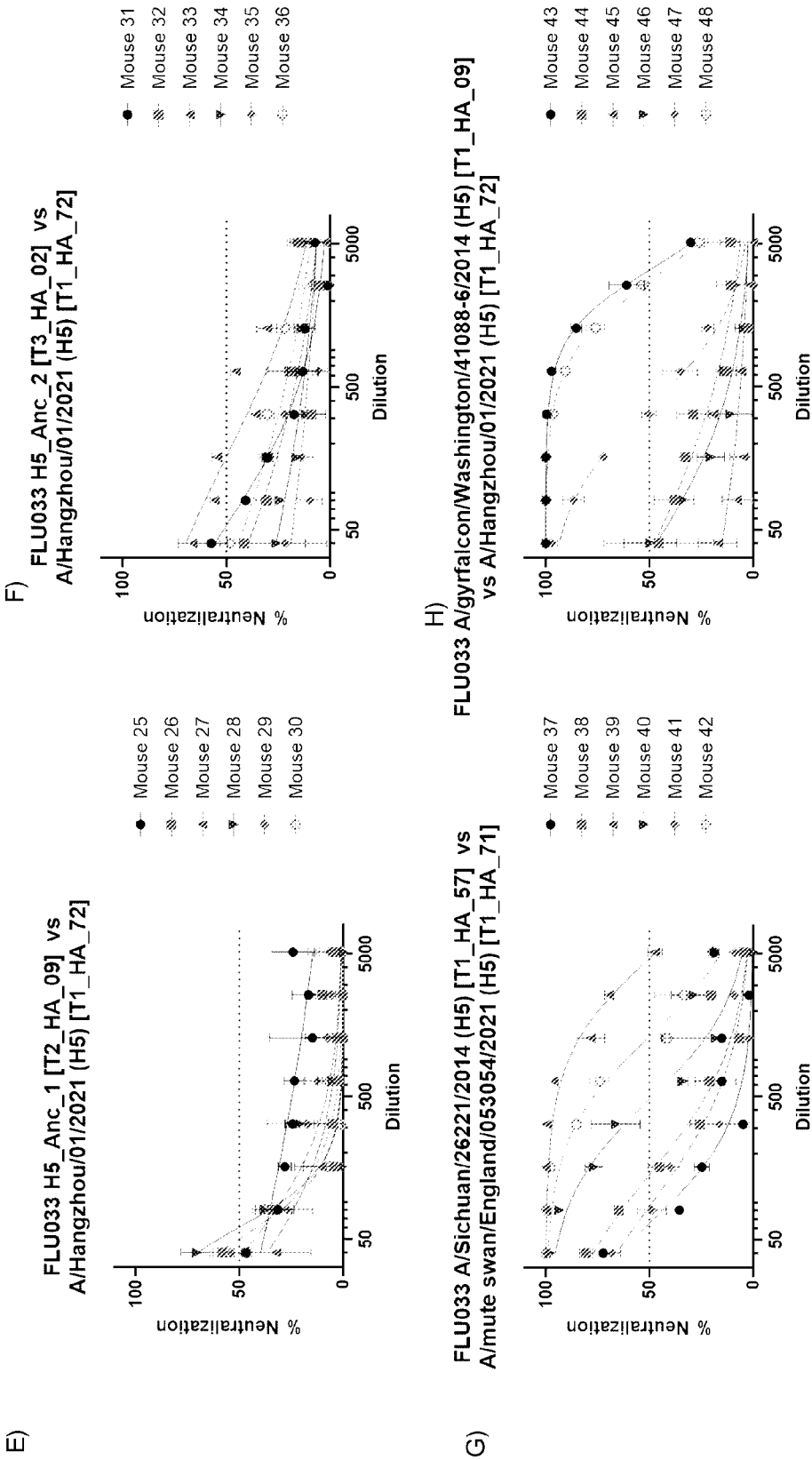


Figure 34 continued



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Figure 34 continued

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FLU033 A/Anhui/2021-00011/2020 (H5) [T1_HA_60] vs
A/Hangzhou/01/2021 (H5) [T1_HA_72]

