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(54) Title: NEUTRALIZING ANTIBODIES TO INFLUENZA HA AND THEIR USE AND IDENTIFICATION

(57) Abstract: Antibodies and antigen binding fragments that specifically bind to influenza HA protein and neutralize group 1 and group 2 influenza A viruses are disclosed. Nucleic acids encoding these antibodies, vectors and host cells are also provided. The use of these antibodies, antigen binding fragments, nucleic acids and vectors to prevent and/or treat an influenza A infection is disclosed. Additionally, methods of testing a vaccine for induction of an immune response that neutralizes group 1 and group 2 influenza A viruses are provided.

NEUTRALIZING ANTIBODIES TO INFLUENZA HA AND THEIR USE AND IDENTIFICATION

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims priority to U.S. Provisional Application No. 62/330,837, filed May 2, 2016, which is incorporated by reference in its entirety.

FIELD OF THE DISCLOSURE

This relates to monoclonal antibodies and antigen binding fragments that specifically bind to influenza A HA protein and their use, for example, in methods of treating a subject with an influenza A infection, as well as identification of such antibodies and/or an immune response that neutralizes group 1 and group 2 influenza A virus using genetic signatures.

BACKGROUND

Influenza A viruses can be categorized into two phylogenetic groups (group 1 and group 2), each containing diverse subtypes defined by their hemagglutinin ("HA") proteins. There are 16 HAs corresponding to 16 different influenza A subtypes (H1-H16) that are classified into group 1 (H1, H2, H5, H6, H8, H9, H11, H12, H13, and H16 subtypes), and group 2 (H3, H4, H7, H10, H14 and H15 subtypes). Currently, group 1 influenza A viruses from the H1 subtype (1918 and 2009 H1N1 pandemics), and the group 2 H3 subtype (1968 H3N2 pandemic), co-circulate and cause seasonal infections in over 10% of the human population each year. Other subtypes threaten to re-emerge, including the group 1 H2 subtype, endemic in humans from 1957-1968, the group 1 H5 subtype, which includes highly lethal avian strains, and the group 1 H6 and H9 and group 2 H7 and H10 subtypes, which have been associated with human infections and fatalities in recent years. Further, frequent zoonotic cross-overs that may cause pandemics of unpredictable frequency and severity highlight the need for a universal influenza vaccine that is capable of protecting against diverse subtypes of influenza A virus.

The neutralizing antibody response to Influenza A virus is typically specific for a given viral subtype. The H1 and H3 viruses continuously evolve generating new variants, a phenomenon called antigenic drift. As a consequence, antibodies produced in response to past viruses are poorly- or non-protective against new drifted viruses. As a consequence, a new vaccine has to be produced every year against H1 and H3 viruses that are predicted to emerge, a process that is very costly as well as not always efficient. The same applies to the production of a H5 influenza vaccine. Accordingly, there is a need for therapeutic antibodies that can neutralize a broad spectrum of influenza A viruses, as well as methods for the identification on influenza vaccines that can be used to produce an immune response protective against both group 1 and group 2 influenza viruses.

SUMMARY

Isolated monoclonal antibodies and antigen binding fragments that specifically bind to the influenza A HA protein and that neutralize group 1 and group 2 influenza A viruses are provided herein. In some embodiments, the antibody or antigen binding fragment comprises a heavy chain variable region (V_H) comprising a HCDR1, a HCDR2, and a HCDR3 of the V_H set forth as SEQ ID NO: 1 (54.f.01 V_H) and/or a light chain variable region (V_L) comprising a LCDR1, a LCDR2, and a LCDR3 of the V_L set forth as SEQ ID NO: 2 (54.f.01 V_L). In some embodiments, the antibody or antigen binding fragment comprises a V_H comprising a HCDR1, a HCDR2, and a

HCDR3 of the V_H set forth as SEQ ID NO: 3 (56.a.09 V_H) and/or a V_L comprising a LCDR1, a LCDR2, and a LCDR3 of the V_L set forth as SEQ ID NO: 4 (56.a.09 V_L). In some embodiments, the antibody or antigen binding fragment comprises a V_H comprising a HCDR1, a HCDR2, and a HCDR3 of the V_H set forth as SEQ ID NO: 5 (01.k.01 V_H) and/or a V_L comprising a LCDR1, a LCDR2, and a LCDR3 of the V_L set forth as SEQ ID NO: 6 (01.k.01 V_L). In some embodiments, the antibody or antigen binding fragment comprises a V_H comprising a HCDR1, a HCDR2, and a HCDR3 of the V_H set forth as SEQ ID NO: 7 (31.b.09 V_H) and/or a V_L comprising a LCDR1, a LCDR2, and a LCDR3 of the V_L set forth as SEQ ID NO: 8 (31.b.09 V_L). In some embodiments, the antibody or antigen binding fragment comprises a V_H comprising a HCDR1, a HCDR2, and a HCDR3 of the V_H set forth as SEQ ID NO: 9 (16.g.07 V_H) and/or a V_L comprising a LCDR1, a LCDR2, and a LCDR3 of the V_L set forth as SEQ ID NO: 10 (16.g.07 V_L). In some embodiments, the antibody or antigen binding fragment comprises a V_H comprising a HCDR1, a HCDR2, and a HCDR3 of the V_H set forth as SEQ ID NO: 11 (54.a.84 V_H) and/or a V_L comprising a LCDR1, a LCDR2, and a LCDR3 of the V_L set forth as SEQ ID NO: 12 (54.a.84 V_L). In some embodiments, the antibody or antigen binding fragment comprises a V_H comprising a HCDR1, a HCDR2, and a HCDR3 of the V_H set forth as SEQ ID NO: 13 (16.a.26 V_H) and/or a V_L comprising a LCDR1, a LCDR2, and a LCDR3 of the V_L set forth as SEQ ID NO: 14 (16.a.26 V_L). In some embodiments, the antibody or antigen binding fragment comprises a V_H comprising a HCDR1, a HCDR2, and a HCDR3 of the V_H set forth as SEQ ID NO: 15 (54.a.39 V_H) and/or a V_L comprising a LCDR1, a LCDR2, and a LCDR3 of the V_L set forth as SEQ ID NO: 16 (54.a.39 V_L). In some embodiments, the antibody or antigen binding fragment comprises a V_H comprising a HCDR1, a HCDR2, and a HCDR3 of the V_H set forth as SEQ ID NO: 17 (31.a.83 V_H) and/or a V_L comprising a LCDR1, a LCDR2, and a LCDR3 of the V_L set forth as SEQ ID NO: 18 (31.a.83 V_L). In some embodiments, the antibody or antigen binding fragment comprises a V_H comprising a HCDR1, a HCDR2, and a HCDR3 of the V_H set forth as SEQ ID NO: 71 (3150206_1A05 V_H) and/or a V_L comprising a LCDR1, a LCDR2, and a LCDR3 of the V_L set forth as SEQ ID NO: 72 (3150206_1A05 V_L). In some embodiments, the antibody or antigen binding fragment comprises a V_H comprising a HCDR1, a HCDR2, and a HCDR3 of the V_H set forth as SEQ ID NO: 73 (3155305_1A05 V_H) and/or a V_L comprising a LCDR1, a LCDR2, and a LCDR3 of the V_L set forth as SEQ ID NO: 74 (3155305_1A05 V_L). In some embodiments, the antibody or antigen binding fragment comprises a V_H comprising a HCDR1, a HCDR2, and a HCDR3 of the V_H set forth as SEQ ID NO: 75 (3155305_1B06 V_H) and/or a V_L comprising a LCDR1, a LCDR2, and a LCDR3 of the V_L set forth as SEQ ID NO: 76 (3155305_1B06 V_L). In some embodiments, the antibody or antigen binding fragment comprises a V_H comprising a HCDR1, a HCDR2, and a HCDR3 of the V_H set forth as SEQ ID NO: 363 (315-53-1F12 V_H) and/or a V_L comprising a LCDR1, a LCDR2, and a LCDR3 of the V_L set forth as SEQ ID NO: 364 (315-53-1F12 V_L). In some embodiments, the antibody or antigen binding fragment comprises a V_H comprising a HCDR1, a HCDR2, and a HCDR3 of the V_H set forth as SEQ ID NO: 365 (315-09-1B V_H) and/or a V_L comprising a LCDR1, a LCDR2, and a LCDR3 of the V_L set forth as SEQ ID NO: 366 (315-09-1B12 V_L). In some embodiments, the antibody or antigen binding fragment comprises a V_H comprising a HCDR1, a HCDR2, and a HCDR3 of the V_H set forth as SEQ ID NO: 367 (315-02-1E04 V_H) and/or a V_L comprising a LCDR1, a LCDR2, and a LCDR3 of the V_L set forth as SEQ ID NO: 368 (315-02-1E04 V_L).

In some embodiments, the glycosylation (for example, fucosylation) or sequence of a disclosed antibody or antigen binding fragment can be altered compared to that observed in nature. For example the glycosylation or sequence of a disclosed of the antibody or antigen binding fragment can be altered compared to that of native

antibodies to increase antibody half-life, antibody-dependent cell-mediated cytotoxic activity, and/or the neutralization profile of the antibody.

Also disclosed are compositions including the antibodies and antigen binding fragments, nucleic acids encoding the antibodies and antigen binding fragments, expression vectors comprising the nucleic acids, and isolated host cells that comprise the nucleic acids. In several embodiments, the nucleic acid molecule encoding a disclosed antibody or antigen binding fragment can be a cDNA molecule that encodes the antibody or antigen binding fragment. In additional embodiments, the nucleic acid molecule can be a bicistronic expression construct encoding the V_H and V_L of the antibody or antigen binding fragment.

The disclosed antibodies and antigen binding fragments potently neutralize influenza A infection in an accepted *in vitro* model of influenza A infection. Accordingly, a method is disclosed for treating or inhibiting an influenza A infection in a subject. The methods include administering a therapeutically effective amount of one or more of the disclosed antibodies, antigen binding fragments, nucleic acid molecules, vectors, or compositions, to the subject, such as a subject at risk of or having an influenza A infection.

The antibodies, antigen binding fragments, nucleic acid molecules, vectors, and compositions disclosed herein can be used for a variety of additional purposes, such as for detecting an influenza A infection or diagnosing influenza A infection in a subject, or detecting influenza A virus in a sample.

The foregoing and other features and advantages of this disclosure will become more apparent from the following detailed description of several embodiments which proceeds with reference to the accompanying figures.

BRIEF DESCRIPTION OF THE FIGURES

FIGs. 1A-1E are a set of graphs, diagrams, and tables showing that H5N1-vaccine recipients have cross-reactive B cells that utilize the same genetic elements and neutralize group 1 and 2 subtypes of influenza A virus. (FIG. 1A) Influenza A comprises two groups based on hemagglutinin (HA) sequence homology. Strains of the H1 and H3 subtypes currently co-circulate in the human population and are included in annual vaccine formulations, while both H5 and H7 subtypes have the potential to become human pandemic strains. Tree was generated using HA sequences (one per subtype) with program MEGA6 and visualized using Inkscape. Scale bar indicates distance for fractional nucleotide change. (FIG. 1B) Serum from 63 vaccinees, sampled two weeks after final H5N1 immunization was assessed for neutralization activity against the vaccine strain (A/Indonesia/5/2005) and heterologous group 2 HA strains (H3N2: A/Hong Kong/1-4-MA21-1/1968; H7N7: A/Netherlands/219/2003). Ten subjects with neutralization titers spanning low to high activity were selected for flow cytometric characterization. The limit of detection is indicated by the dotted line. (FIG. 1C) PBMC samples isolated from H5N1 vaccine recipients two weeks after final vaccination were co-stained with HA probes H5 (A/Indonesia/5/2005) and H3 (A/Perth/16/2009). Sizable populations of H5-H3 cross-reactive memory B cells were found in six of ten subjects (the six donors with significant B cells are indicated in (B) by filled shapes and the remaining four samples with open shapes) and single cell sorting and immunoglobulin sequencing was carried out. (FIG. 1D) Clonal diversity of H5-H3 cross-reactive B cells. The HV repertoire from each subject is shown as a pie chart; each slice represents a unique HV clone or clonally related family. The width of the slice is proportional to the frequency of clonal relatives, and HV-gene families are named for the largest HV groups and other selected families. The total number of HV sequences recovered per subject is indicated by the number in the center of the pie-chart. (FIG. 1E) Genetic and functional characteristics of selected antibodies recovered from H5-H3 cross-reactive B cells grouped by

number of genetic similarities. Antibodies which were structurally characterized are indicated by a ☼ symbol. All antibodies which expressed were assessed for binding to a set of H1 HAs followed by competition with CR9114. Antibodies were also assessed for pseudovirus entry inhibition against a panel of 14 Influenza A viruses and 1 Influenza B virus with HA subtype neutralization indicated.

FIGs. 2A-2G are a set of graphs, diagrams, and tables showing properties of a multidonor HV6-1+HD3-3 class of broadly neutralizing antibodies. (FIG. 2A) Immunoglobulin heavy chains utilizing germline genes HV6-1, HD3-3 and HJ4 or HJ5 were selected in three subjects. Nucleotides removed by exonuclease trimming are indicated with a line through the letters. The conserved HD3-3 encoded residues are highlighted in a box, as are the recurrent Ile100b$_{HC}$ which is derived by somatic hypermutation. HA amino acid contacts for the 56.a.09-HA co-crystal complex are indicated with open circles (○) denoting antibody main-chain-only contacts, open circles with rays (☼) denoting antibody side-chain-only contacts, and filled circles (●) denoting both main-chain and side-chain contacts. The sequences shown are HV6-1+HD3-3 (DNA, SEQ ID NO: 311; protein, SEQ ID NO: 312), 31.g.01 (DNA, SEQ ID NO: 313; protein, residues 98-115 of SEQ ID NO: 133), 54.f.01 (DNA, nucleotides 292-361 of SEQ ID NO: 83; protein, residues 98-115 of SEQ ID NO: 1), and 56.a.09 (DNA, nucleotides 292-361 of SEQ ID NO: 85; protein, residues 98-115 of SEQ ID NO: 3). (FIG. 2B) Neutralization breadth-potency curve for HV6-1+HD 3-3 antibodies, with breadth shown as percentage of pseudoviruses neutralized at each IC_{50} cutoff shown on the X-axis. The virus panel was made up of 15 strains and included common influenza A subtypes known to infect humans (H1, H2, H3, H5, H7, H9). (FIG. 2C) The co-crystal structure of Fab 56.a.09 in complex with an H3 HA monomer (A/Hong Kong/1-4-MA21-1/1968). The Fab heavy and light chains are shown and depicted in surface representation, while the H3 HA is depicted in ribbon and shown as a trimer (see FIGs. 14A-14D for HA crystal packing). Inset: The interacting CDR loops of the 56.a.09 Fab are shown in ribbon and sticks, with the antibody footprint outlined. (FIG. 2D) A five amino acid motif within the CDR H3, combining hydrophobic residues, inserts into the highly conserved Trp21 pocket of H3. (FIG. 2E) The HV6-1encoded CDR H2 provides an optimal interface with the HA fusion peptide and HV6-1 is the only HV gene which encodes a nine residue CDR H2. (FIG. 2F) Light chain interactions contribute to the antibody binding area but are seemingly not specific for KV3-20. (FIG. 2G) Analysis of the HA-antibody complex indicates that other CDR H3 residues may be compatible with HA binding. For example, 100$_{HC}$ could be a F, Y, or W residue (¥), 101$_{HC}$ could be a G, A, or S residue (x), and residues 102-104$_{HC}$ could be other residues as indicated. Analysis of the HD motif from residue 100$_{HC}$ to 100d$_{HC}$ indicated a set of HD genes that would encode compatible residues for a shorter three-residue motif but only HD3-3 encodes the five amino-acid motif. Given the genetic specificities in (FIGs. 2D-2F) 90% of humans carry the allelic potential for elicitation of HV6-1+HD3-3 class antibodies.

FIGs. 3A-3G are a set of graphs, diagrams, and tables showing properties of a multidonor HV1-18+HD3-9 class of broadly neutralizing antibodies. (FIG. 3A) Immunoglobulin recombination utilizing germline genes HV1-18, HD3-9 and HJ4 were selected in two subjects. Nucleotides removed by exonuclease trimming are indicated with a line through the letters. HA contacts for the 31.b.09 complex are indicated with open circles (○) denoting antibody main-chain-only contacts, open circles with rays (☼) denoting antibody side-chain-only contacts, and filled circles (●) denoting both main-chain and side-chain contacts. The sequences shown are HV1-18+HD3-9 (DNA, SEQ ID NO: 314; protein, SEQ ID NO: 315), 01.k.01 (DNA, nucleotides 286-336 of SEQ ID NO: 87; protein, residues 96-112 of SEQ ID NO: 5), and 31.b.09 (DNA, nucleotides 286-336 of SEQ ID NO: 89; protein, residues 96-112 of SEQ ID NO: 7). (FIG. 3B) Neutralization breadth-potency curve: HV1-18+HD 3-9 antibodies

on a panel of influenza A viruses that include all subtypes known to infect humans. (FIG. 3C) The co-crystal structure of Fab 31.b.09 in complex with an H1 trimer (A/California/04/2009). The Fab heavy and light chains shown and depicted in surface representation, while the H1 HA is depicted in ribbon. Inset: The interacting CDR loops of the 31.b.09 Fab are shown in ribbon and sticks, with the antibody footprint outlined. (FIG. 3D) A conserved motif within the CDR H3 inserts into the highly conserved Trp21 pocket of HA while also interacting with the HA fusion peptide. (FIG. 3E) The HV1-18 encoded CDR H2 also interacts with the opposing side of the HA fusion peptide. (FIG. 3F) Light chain interactions from CDR L3 and CDR L1 also contribute to the antibody binding surface area. (FIG. 3G) Given the genetic specificities in (FIGs. 3D-3F) 100% of humans carry the allelic potential for elicitation of HV1-18+HD3-9 class antibodies.

FIGs. 4A-4J are a set of graphs, diagrams, and tables showing properties of a multidonor HV1-18 class of broadly neutralizing antibodies with a shared Q-x-x-V motif. (FIG. 4A) Recombination of immunoglobulin germline genes HV1-18, HD2-2 or HD2-15 and HJ5 or HJ2 leads to highly similar CDR H3 sequences in multiple clonal families from two subjects (16 and 54). A common motif of a Gln98-x-x-Val100a (Q-x-x-V) was observed in all lineages. The sequences shown are HV1-18 Q-x-x-V (DNA, SEQ ID NO: 316; protein, SEQ ID NO: 317), 16.a.26 (DNA, nucleotides 286-336 of SEQ ID NO: 95; protein, residues 96-112 of SEQ ID NO: 13), 54.a.39 (DNA, nucleotides 286-336 of SEQ ID NO: 97; protein, residues 96-112 of SEQ ID NO: 15), HV1-18+HD2-15 (DNA, SEQ ID NO: 318; protein, SEQ ID NO: 319), 16.g.07 (DNA, nucleotides 286-336 of SEQ ID NO: 91; protein, residues 96-112 of SEQ ID NO: 9), and 54.a.84 (DNA, nucleotides 286-336 of SEQ ID NO: 93; protein, residues 96-112 of SEQ ID NO: 11). (FIG. 4B) Neutralization breadth-potency curve for HV1-18 (Q-x-x-V) class antibodies on a panel of influenza A viruses that include all subtypes known to infect humans. (FIG. 4C) Co-crystal structure of Fab 16.a.26 (HV1-18, HD2-2, HJ5) in complex with H3-HK68. The HA protomers are shown in ribbon representation. The heavy and light chain are depicted in surface representation. (FIG. 4D) The 16.a.26 CDR H3 is depicted with junction-encoded and mutated residues as in panel (A) and germline-encoded residues with the antibody footprint on the HA outlined in black. The conserved D-gene encoded Val100a inserts into a highly conserved pocket adjacent to the HA2 helix A. The group 2-conserved N-linked glycan at residue $Asn38_{HA2}$ (NAG 1038) is shown in white sticks. (FIG. 4E) The conserved, junction-encoded $Gln98_{HC}$ occupies a pocket formed by $Gly33_{HC}$ and $Ser52_{HC}$. The antibody heavy chain is depicted in surface with the CDR H3 loop shown in ribbon representation. For clarity only the N-terminal portion of the HA2 helix A (residues 37 through 42) and $Trp21_{HA2}$ are shown. 16.a.26 $Gln98_{HC}$ occupies a turn in the CDR H3, which interacts with the conserved residue $Gln42_{HA2}$. (FIG. 4F) Co-crystal structure of Fab 16.g.07 (HV1-18, HD2-15, and HJ2) in complex with H3-HK68 depicted as in panel (C). (FIG. 4G) The 16.g.07 CDR H3 is depicted as in panel (D) with the antibody footprint on the HA outlined in black. Again, the D-gene encoded $Val100a_{HC}$ inserts into a pocket beside helix A. (FIG. 4H) The 16.g.07 CDR H3 is depicted as in panel (E) with $Gln98_{HC}$ contacting $Gln42_{HA2}$. (FIG. 4I) Analysis of structural requirements of the CDR H3 sequence motif indicate that a D gene-encoded Val100a is critical for cross-group neutralization but this residue can be encoded by all HD genes. (FIG. 4J) Analysis of structural requirements of HV gene-encoded sequence indicates that while only $Tyr53_{HC}$ contacts HA, the pocket formed by $Gly33_{HC}$ and $Ser/Thr52_{HC}$ is uniquely encoded by the HV1-18 germline gene. It is estimated that 99% of the human population has the potential for elicitation of similar HV1-18 (Q-x-x-V) class antibodies.

FIGs. 5A-5H are a set of graphs, diagrams, and tables showing that a conserved site of group 1 and 2 influenza vulnerability is targeted by multidonor and lineage-unique antibodies. (FIG. 5A) Neutralization breadth-

potency curve for an HV3-23-derived antibodies, including one which neutralizes all influenza A subtypes. (FIG. 5B) Recombination of immunoglobulin germline genes HV3-23, HD3-9 and HJ6 can result in antibodies with broad neutralizing activity against diverse strains of influenza A. The recombination junctions for two HV3-23 derived antibodies, 31.a.83 and 56.h.01, are shown aligned to their germline genes. HA contacts for the 31.a.83 complex are indicated with open circles (○) denoting antibody main-chain-only contacts, open circles with rays (☼) denoting antibody side-chain-only contacts, and filled circles (●) denoting both main-chain and side-chain contacts. The CDR H3s of 31.a.83 and 56.h.01 are not similar in sequence or length despite utilizing identical HV, HD, and HJ genes. The sequences shown are HV3-23 (DNA, SEQ ID NO: 320; protein, SEQ ID NO: 321), 31.a.83 (DNA, nucleotides 286-336 of SEQ ID NO: 99; protein, residues 96-112 of SEQ ID NO: 17), HV3-23 (DNA, SEQ ID NO: 320; protein, SEQ ID NO: 321), and 56.h.01 (DNA, SEQ ID NO: 322; protein, SEQ ID NO: 323). (FIG. 5C) Co-crystal structure of Fab 31.a.83 in complex with HA (H3-HK68). The HA protomers are shown as ribbon representation with the antibody shown in surface representation. (FIG. 5D) The 31.a.83 CDR H3 is depicted with junction-encoded, mutated residues, and germline encoded residues, with the antibody footprint on HA outlined in black. (FIGs. 5E and 5F) Antibodies capable of neutralizing viruses from group 1 and 2 influenza A recognize overlapping epitopes within the HA stem. The HV-gene, degree of somatic hypermutation (% nucleotide changes) and length of CDR H3 are shown for each antibody. (FIG. 5G) Epitopes from all group 1 and 2-neutralizing HA-stem directed antibodies. One HA protomer is depicted in ribbon representation with larger ribbon diameter. Antibodies that neutralize both group 1 and 2 influenza A viruses bind primarily to an epitope which includes the HA2 helix A and fusion peptide, but avoids the group 2-conserved *N*-linked glycan at Asn 38_{HA2}. (FIG. 5H) Heavy and light chain orientation of both multidonor and unique human antibodies capable of neutralizing group 1 and 2 influenza A.

FIGs. 6A-6E are a set of graphs, diagrams, and tables showing properties of a sequence-signatures of multidonor antibody lineages. (FIG. 6A) Multidonor class antibodies capable of neutralizing group 1 and group 2 influenza A viruses: sequence signature, sequence-identified class sequences (number and frequency). Four singleton transcripts from 454-derived NGS data are italicized. (FIG. 6B) HV-HD germline-gene origin plots (one box per subject) of 645 sequences from 6 vaccinees of the 310 trial with observed multidonor class antibodies highlighted. Sequence frequencies are displayed according to their HV (horizontal) and HD (vertical) gene origin, with the size of the plotted circle corresponding to the number of antibody sequences as shown in key at left. Multidonor antibodies in different subjects are connected by lines. (FIG. 6C) Binding and competition MSD-ECLIA between HA and synthesized class antibodies identified from DeKoskey *et al.* (*Nature medicine* 21, 86-91, 2015) and Jiang *et al.* (*Science translational medicine* 5, 171ra119, 2013) databases competed with stem antibody F10. (FIG. 6D) Neutralization of influenza strains assessed using pseudovirus entry inhibition assays with class antibodies as in (C). The Median IC_{50} for each antibody is indicated by a horizontal line and the value (μg/ml) is shown at the base of the graph. (FIG. 6E) Clustering of antibodies based on neutralization fingerprint. Antibodies from the three multidonor classes were clustered together, suggesting that antibodies from each class have similar neutralization profiles.

FIGs. 7A-7G are a set of graphs and diagrams illustrating the vaccine induction of antibodies capable of neutralizing group 1 and 2 influenza A. (FIG. 7A) Frequencies of H5-H3 cross-reactive memory B cells pre- and post-H5N1 vaccination. The four subjects for which samples were no longer available for pre-immunization sorting are indicated with open symbol shapes, with the subject name and fold increase shown. (FIG. 7B) The percent

frequency of multidonor class sequences is shown for each donor. (FIG. 7C) The fold-increase in cross-reactive B cells is shown relative to the percentage of heavy chain sequences with three (out of a possible four) of the same heavy chain genetic elements with at least one sequence found in any of the other five donors (correlation method: Pearson); total number of sequences for each donor are shown in FIG. 1D. (FIG. 7D) The fold-increase in cross-reactive B cells for each donor is shown relative to the fold-increase in sera neutralization titer calculated based on the geometric mean over all tested influenza A strains (see FIGs. 1A-1E, FIGs. 8A and 8B), or a single H1N1/A/Singapore/8/1986 strain. (FIG. 7E) Left: percentage of multidonor class transcripts for each dataset; Right: number of multidonor class lineages divided by total transcript number (%) for each dataset. (FIG. 7F) Transcript frequency versus dataset and goal. Data points shown in the star sign are upper-bound estimates. (FIG. 7G) Multidonor antibodies are displayed in ribbon representation with class-conserved contact residues shown in stick representation. The antibody epitopes are shown (HV6-1+HD3-3), (HV1-18+HD3-9), and (HV1-18 with Q-x-x-V) with a black outline. Glycans are shown in surface representation.

FIGs. 8A and 8B are a set of tables showing VRC 310 subject sera neutralization of multiple influenza strains before and after H5 immunization, related to FIGs. 1A-1E. Significant increases in neutralization titer following H5N1 immunization across all subjects (black dots) are indicated by a *P* value above the pre-vaccination and 2 weeks post-boost neutralization titer values. (*P* values were determined using a Wilcoxon Ranked sum-test, horizontal line marks the mean of the samples, n=63).

FIGs. 9A-9C are a set of graphs showing results of flow cytometry cell sorting (FACS) gating strategy and analysis of PBMC samples from ten subjects, related to FIGs. 1A-1E. (FIG. 9A) Memory B cells were sorted by CD14-, CD3-, CD19+, IgD-, IgM-, CD27+, IgG+ reactivity prior to sorting based on HA reactivity. After gating for single lymphocytes and the exclusion of CD14+ monocytes, CD3+ T cells and dead cells, CD27+ IgG+ IgD- IgM+ CD19+ B cells were stained with recombinant HA probes to resolve H5(A/Indonesia/05/2005) / H1(A/New Caledonia/20/1999) or H5/ H3 (A/Perth/16/2009) cross-reactive B cell populations. (FIG. 9B) Pre-vaccination samples was available for six donors to allow group 1-2 B cell sorting. (FIG. 9C) Group 1 cross-reactive (H1+H5+) and group 1-2 cross-reactive (H3+H5+) FACS plots for ten subjects are shown.

FIGs. 10A-10E are a set of graphs and images showing results from influenza H1 HA binding competition, negative stain electron microscopy, and autoreactivity analyses, related to FIGs. 1A-6E. (FIGs. 10A and 10B) Antibodies isolated from vaccinated subjects were tested for binding to H1 HA (either from A/California/04/2009 (H1N1) or A/New Caledonia/20/1999 (H1N1)) starting at 10 μg/ml with three-fold dilutions. HA proteins were pre-incubated with CR9114 prior to antibody incubation to assess whether antibody binding was affected by a HA-stem antibody in a binding-competition assay. (FIG. 10C) Analysis of antibody Fab-HA complexes by negative stain electron microscopy. Aligned particle averages are shown with each image 28 nm x 28 nm in size. HA control and known HA-stem binding antibodies in complex (CR8020 and FI6v3) are shown for reference. Fab concentration was varied to allow one Fab to one HA complex formation for visualization of the side-view of the complexes. The HA head and stem domains are indicated in the top left panel and stem-binding HAs are indicated in all other images. (FIG. 10D) Hep-2 cell staining at 50 mg/ml antibody concentration is shown for representative antibodies from each convergent class and also for a set of HA stem-reactive antibodies and control antibodies. (FIG. 10E) Anti-cardiolipin ELISA (GPL units) results are shown for antibodies at a concentration of 33 μg/ml.

FIG. 11 is a graph illustrating the results of neutralization of influenza strains assessed using pseudovirus entry inhibition assay, related to FIGs. 1A-6E. Antibodies isolated from VRC310 donors were tested for

neutralization using a pseudovirus inhibition entry assay against fifteen influenza A strains and one influenza B strain. The antibody class is indicated at the top of the graph. The Median IC_{50} for each antibody is indicated by a horizontal line and the value (μg/ml) is shown at the base of the graph.

FIGs. 12A-12D are a set of images and diagrams illustrating the crystal structure of the antigen-binding fragment (Fab) for antibody 56.a.09, alone and in complex with HA0, related to FIGs. 2A-3G. (FIG. 12A) Negative-stain electron microscopy of monomeric and head-to-stem dimeric complex of 56.a.09 and the HA0 of A/Hong Kong/1-4-MA21-1/1968 (H3N2). (FIG. 12B) Crystal packing of the 56.a.09 and HA0 A/Hong Kong/1-4-MA21-1/1968 (H3N2) complex closely resembles the head-to-stem dimer observed by negative-stain electron microscopy. A complex and its nearby crystallographic symmetry mate are shown. (FIG. 12C) Crystal structure of 56.a.09. The antibody structure is depicted in ribbon conformation. The CDR H3 residues that are part of the conserved recombination sequence (Met-Ile-Phe-Gly-Ile) are shown in stick representation. The Phe residue points out away from the antibody while the conserved Met residue stacks against the antibody light chain. (FIG. 12D) Crystal packing of the 31.b.09 Fab and HA0 A/California/04/2009 (H1N1).

FIGs. 13A-13D are a set of sequence alignments and a graph illustrating HA group specific antigenic sites located on the HA stem, related to FIGs. 6A-6E. The HA molecule has many glycans (both conserved and variable) which can affect antibody binding. The antibody footprint of CR6261 and CR8020 are shown with the glycans which knock out binding of these group 1 and group 2 specific antibodies. Within the site of antigen recognition a structural pocket at Trp21HA2 is highly conserved in group 1 HAs, but is considerably smaller in group 2 HAs. This structural change between group 1 and group 2 HA stem has been shown to affect both neutralization and binding breadth of stem-directed antibodies ref18. In general, all of the group 1 and 2-neutralizing antibodies target a site of stem vulnerability which is similar to that recognized by antibodies capable of neutralizing only group 1 influenza A; however, the group 1 and 2 neutralizers critically avoid the N-linked glycan attached to $Asn38_{HA2}$ present on group 2 HAs, while maintaining interactions with both fusion peptide and the Trp 21 pocket.

FIGs. 14A-14D are a set of tables illustrating the convergence of the identified classes of antibodies, and junctional analysis for consensus sequences derived from NGS and correlation with cross-reactive memory B cells, related to FIGs. 6A-6E. (FIG. 14A) Consensus sequences derived from influenza-vaccinated donor NGS data (SRP015957) containing sequence signatures. (FIG. 14B) Consensus sequences derived from two of three normal donor NGS data sets (SRP026397 and SRP047462) containing sequence signatures. Each sequence label contains a unique index number along with the sequence signature class name and SRP number the sequence derives from. Nucleotides removed by exonuclease trimming are crossed out. All N, P additions occurring at the junctions are from JOINSOLVER (joinsolver.niaid.nih.gov). For the sequence belonging to the HV6-1+HD3-3 class, the HD3-3*01 germline D sequence was used instead of the HD3/OR15 germline D sequence even though the latter sequence provides a better match (by one nucleotide). (FIG. 14C) The percent frequency of convergent class antibodies in each donor is highly correlated with the frequency of H3-H5 cross-reactive memory B cells. Correlation method: Pearson with a two-tailed test (n=6).

FIGs. 15A-15D are a set of tables illustrating neutralization of representative antibodies (names based on subject.lineage.clone) from commonly elicited lineages and identified for NGS datasets using sequence signatures against a diverse panel of group 1 and group 2 influenza A viruses, related to FIGs. 1A-6E. The inferred HV gene followed by antibody name, and the tested viruses are indicated on the top row. (FIG. 15A) IC_{50} neutralization titers assessed using pseudovirus entry inhibition assay. (FIG. 15B) IC_{80} neutralization titers assessed using

pseudovirus entry inhibition assay. (FIG. 15C) IC_{50} neutralization titers assessed using micro-neutralization assay. (FIG. 15D) IC_{80} neutralization titers assessed using micro-neutralization assay

FIG. 16 is a table showing crystallographic data collection and refinement statistics.

FIGs. 17A-17D are a set of tables showing the functional complementation of heavy and light chains for multidonor class antibodies, related to FIGs. 2A-4J and 6A-6E. (FIG. 17A) Functional assessment of paired heavy and light chains. Light chains specified in columns were reconstituted with heavy chains specified in rows, expressed, purified, and tested for binding to HA as assessed by ECLIA-MSD. (FIG. 17B) Heavy chain amino acid identities for HV1-18 (Q-x-x-V) class antibodies. Identities greater than 0.78 are high-lighted in red. CDR H3 length for each antibody is listed in parentheses. Antibody pairs with unequal CDR H3 length is denoted as "-". (FIG. 17C) Functional assessment of NGS-derived HV1-18 (Q-x-x-V) class heavy chains. Light chains specified in columns were reconstituted with heavy chains specified in rows, expressed, purified, and tested for binding to HA as assessed by ECLIA-MSD. (FIG. 17D) Amino acid identities of NGS-derived HV1-18 (Q-x-x-V) class heavy chains. Identities greater than 0.78 are high-lighted in red. CDR H3 length for each antibody is listed in parentheses. Antibody pairs with unequal CDR H3 length is denoted as "-".

FIG. 18 is a set of graphs illustrating binding of the 3150206_1A05, 3155305P_1A05, and 3155305P _1B06 antibodies to HA proteins from different subtypes of influenza A virus.

FIG. 19 is a set of tables showing results from influenza A pseudovirus neutralization assays illustrating the neutralization profile of the 3150206_1A05, 3155305P_1A05, and 3155305P _1B06 antibodies.

FIGs. 20A-20G show sequence alignments of antibodies that bind to influenza HA and neutralizing group 1 and group 2 influenza HA viruses.

FIG. 21 is a set of graphs showing that the 315-53-1F12, 315-09-1B12, and 315-02-1E04 antibodies protect against infection with group 1 and group 2 influenza viruses. The antibodies were tested for protection of mice infected with A/California/07/2009 (H1N1) or A/Shanghai/02/2013 (H7N9). The mice were passively administered 5 mg/kg of the indicated antibody (10 mice/group) 24 hours before infection with A/Anhui/01/2013 (H7N9) or A/California/07/2009 (H1N1). As a control, one group received VRC01 IgG, an HIV-specific antibody. The left graph shows the Kaplan-Meier survival curve. There was greater survival in all groups that received an HA-stem binding antibody ($p \leq 0.001$, Fisher's exact test) compared to the control group. The middle graph shows the percent weight loss over time with each line representing one mouse. On the right the percent weight loss is shown for animals in each group on the last day when all animals infected with that strain had a recorded weight. The weight loss is statistically lower ($p < 0.0001$, Mann-Whitney t-test) in all groups that received an HA stem-binding antibody compared to the control group.

SEQUENCE LISTING

The nucleic and amino acid sequences listed in the accompanying sequence listing are shown using standard letter abbreviations for nucleotide bases, and three letter code for amino acids, as defined in 37 C.F.R. 1.822. Only one strand of each nucleic acid sequence is shown, but the complementary strand is understood as included by any reference to the displayed strand. The Sequence Listing is submitted as an ASCII text file in the form of the file named "Sequence.txt" (~275 kb), which was created on May 1, 2017, which is incorporated by reference herein. In the accompanying sequence listing:

SEQ ID NO: 1 is the amino acid sequence of the 54.f.01 V_H.
QVQLQQSGPRLVKPSQTLSLTCVISGDSVSSHSAWNWIRQSPSRGLEWLGRTYYRSKWYSDYAPSVKSRTTINADTSKNEI
SLYLNSVTPEDTAVYYCVRGSFMIFGIVMAFDQWGQGTLVTVSS

SEQ ID NO: 2 is the amino acid sequence of the 54.f.01 V_L.
EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQSPRLLIYGTSTRATGIPDRFSGSGSGTEFTLTITRLEP
EDFAVYYCQQFDGSHFTFGPGTKVDIK

SEQ ID NO: 3 is the amino acid sequence of the 56.a.09 V_H.
QVQLQQSGPGLVKPSQTLSLTCVISGDTVSSNRAAWNWIRQSPSRGLEWLGRTYYRSKWYTDYAVSVKSRITITPDTSKNQ
FSLQMKSVTPEDTAVYYCARGSAMIFGIVIILESWGQGTLVTVSS

SEQ ID NO: 4 is the amino acid sequence of the 56.a.09 V_L.
LRDRRVDTVSLSLSPGERATLSCRASQSVASSYLAWYQQKPGQAPRLLIYGASSRATGVPDRFSGSGSGTDFILTISRLEP
EDFAVYYCQQYDGSQYTFGQGTKLEIK

SEQ ID NO: 5 is the amino acid sequence of the 01.k.01 V_H.
HVHLVQSGAEVQESGASVKVSCKASGYSFSSHGISWVRQAPGQGLEWMGWISAYNGHTNYLQKFQGRVTLTTDTSTDTAYM
ELRSLRSDDTAVYYCARDRGNILTGCQFDYWGQGTRVTVSS

SEQ ID NO: 6 is the amino acid sequence of the 01.k.01 V_L.
DVVMTQSPLSLPVTLGQPASISCRSSQGLVFIDGSTYLNWFQQRPGQSPRRLIYEISKRDSGVPDRFSGSGSGTDFTLKIS
RVEAEDVGVYYCMQGTHWPITFGQGTRLEIK

SEQ ID NO: 7 is the amino acid sequence of the 31.b.09 V_H.
QVQLVQSGAEVKKPGASVKVSCKASGYSFSSYGISWVRQAPGQGLEWMGWISAYNGNTNYAQKLQGRVTMTTDTSTSTAYM
ELRSLRSDDTAVFYCARDRPHILTGFDFDYWGQGTLVTVSS

SEQ ID NO: 8 is the amino acid sequence of the 31.b.09 V_L.
DVVMTQSPVSLPVTLGQPASISCRSSQGLVYIDGNTYLNWFQQRPGQSPRRLIYNVFTRDSGVPDRFSGSGSGTDFTLKIT
TVEAEDVGVYYCMQGTHWPYTFGQGTKLEIK

SEQ ID NO: 9 is the amino acid sequence of the 16.g.07 V_H.
QVQLAQSENELKKPGASVKVSCKTSGYTFTRFGMSWVRQAPGQGLEWMGWISGYTGDTKYARSFQGRLTLTTDTSTGTAYM
ELRSLRSDDTAIYYCVRNRVQMEVSPATQSTWYMDLWGRGTLVSVSS

SEQ ID NO: 10 is the amino acid sequence of the 16.g.07 V_L.
DIQMTQTPFSLSASIGDRVTITCRASQDITRWLAWYQQKPGKAPELLIYAASTLQSGVPSRFRGRGSGTDFSLTISDLQAE
DFATYYCQQGSSFPYTFGQGTRLEIR

SEQ ID NO: 11 is the amino acid sequence of the 54.a.84 V_H.
QVQLVQSGPEVKKPGASVRVSCTASGYTFSRYGISWVRQAPGQGPEWMAWISAYTGDTHYARKFQGRVTVSTEASTATVYM
ELRSLRSDDTAVYYCARDRIQGVVALPKEQLNWFDPWGQGTLVTVTS

SEQ ID NO: 12 is the amino acid sequence of the 54.a.84 V_L.
EIVLTQSPAALSLSPGERATLSCRASHSISQFLAWYQQKPGQAPRLLIYGISNRATDVPARFRGSGSGTDFTLTISDLEPE
DFAVYYCQQGSNWPRTFGQGTKVEAK

SEQ ID NO: 13 is the amino acid sequence of the 16.a.26 V_H.
QVQLVQSGPEVKKPGASVKVSCKASGYSFSRYGISWVRQAPGQGLEWLGWISGYTGNTNYAQKFQGRVTMTTDTSTSTASM
ELRSLRSDDTAVYYCARDKKQGEVVLPAASFRWFAPWGQGTLVTVSS

SEQ ID NO: 14 is the amino acid sequence of the 16.a.26 V_L.
DIQMTQSPVSLSASVGDRVTITCRASQSIGKFLNWYQQKPGRAPKLLIYYASNLETGGPSRFSGRGSETEFSLTISSLQPE
DFATYYCQQSNNVPHTFGQGTKLEIK

SEQ ID NO: 15 is the amino acid sequence of the 54.a.39 V_H.
QVHLVQSGAEVKKPGASVKVSCKVSGYTFTSYGISWVRQAPGQGLEWMAWISAYTGNTNFAQKFKDRVTVSTDTSTTTAYM
ELRGLRYDDTAVYYCARDRIQGAVALPDKQVNWFDPWGQGTLVTVSS

SEQ ID NO: 16 is the amino acid sequence of the 54.a.39 V_L.
EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAPRLLIYGASNRATGIPARFRGSGSGTDFTLTINSLEPE
DFAVYYCQQHSNWPRTFGQGTKVETK

SEQ ID NO: 17 is the amino acid sequence of the 31.a.83 V_H.
EVQLLESGGGLVQPGESLRVSCAASGFTFRDSALSWVRQAPGKGLEWVSAISGNGGATYYADTVKGRFTISRDNSQTTLYL
QMNSLRADDTATYYCAKDESPPIYNLMPGYYSTYYYMDVWGKGTTVTVSS

SEQ ID NO: 18 is the amino acid sequence of the 31.a.83 V_L.
EIVMTPGPVTLSVSPGETATLSCRASQSVRSNLAWYQQKPGQAPRLLIYGASTRATDIPARFSGSGSGTEFTLSISSLQSD
DFAVYYCQQYNHWLRTFGQGTKLEIK

SEQ ID NOs: 19-70 are antibody CDR sequences.

SEQ ID NO: 71 is the amino acid sequence of the 3150206_1A05 V_H.
QVQLVQSGAEVKEPGASVKVSCQTSGYSLTGNYIHWIRQAPGQGPEWMGWINPKSGGTNFAEKFQGRVTLTSDTSVNTAYM
ELSRLGSADTAIYYCARDSGMRYFDWLSGYFDFWGQGTLITVSS

SEQ ID NO: 72 is the amino acid sequence of the 3150206_1A05 V_L.
EIVLTQSPGTLSLSPGERATLSCRTSQVLTTNYLAWYQQKPGQAPRLLIYGASTRATGIPDRFSGSGSGRDFTLTISRLEP
EDFAVFYCQVYDDLRVIFGGGTKVEIK

SEQ ID NO: 73 is the amino acid sequence of the 3155305_1A05 V_H.
QVQLQQSGPGLVKPSQTLSLTCLISGDSVSSNSAAWNWIRQSPSRGLEWLGRTYYRSKWYNDYADSVKSRITINPDTSKNQ
FSLQLNSVTPEDTAVYYCARAGIMIFGVIVGGLDVWGQGTTVTVSS

SEQ ID NO: 74 is the amino acid sequence of the 3155305_1A05 V_L.
DIQMTQSPSSLSASVGDRVTISCRASQSISSYLHWYQQKPGKAPELLIYGASNLHSGVPSRSSGSVSGTDFTLTISSLQPE
DSATYYCQQSSTKPGYTFGRGTKLEIK

SEQ ID NO: 75 is the amino acid sequence of the 3155305_1B06 V_H.
QVQLQQSGPGLVKPSQTLSLTCAISGDSVSSNSGAWDWIRQSPSRGLEWLGRTYYRSKWYNNYADFVKSRITINPDTSKNQ
FSLQLSSVTPEDTAVYFCARAGVTVFGVVVGAMDVWGQGTTVTVSS

SEQ ID NO: 76 is the amino acid sequence of the 3155305_1B06 V_L.
EIVLTQSPGTLSLSPGETAIISCRTSQYGSLAWYQQRPGQAPRLVIYSGSTRAAGIPDRFSGSRWGPDYNLTISNLESGDF
GVYYCQQYEFFGQGTKVQVDIK

SEQ ID NO: 77 is an exemplary nucleic acid sequence encoding the 3150206_1A05 V_H.
caggtgcagctggtgcagtctggggctgaggtgaaggagcctggggcctcagtgaaggtctcctgtcagacttctggatat
agcctcaccggcaactatatacactggatacgacaggcccctggacaagggcctgagtggatggggtggatcaaccctaaa
agtggtggcacaaactttgcagagaaatttcaaggcagagtcaccttgaccagcgacacgtccgtcaacacggcctacatg
gagttgagcagactgggatccgccgacacggccatctattactgtgcgagagactcgggtatgcgatattttgactggcta
agcggatactttgacttctggggccagggaaccctgatcaccgtctcctcagc

SEQ ID NO: 78 is an exemplary nucleic acid sequence encoding the 3150206_1A05 V_L.
gaaattgtgttgacgcagtctccaggcaccctgtctttgtctccaggggaaagagccaccctctcctgcaggaccagtcaa
gttcttacaaccaactacttagcctggtaccaacagaaacctggccaggctcccaggctcctcatctatggtgcatccacc
agggccactggcatcccagacaggttcagtggcagtgggtctgggagagacttcactctcaccatcagcagactggagcct
gaagattttgcagtgttttactgtcaggtgtatgatgatttacgtgtcattttcggcggagggaccaaggtggagatcaaa

SEQ ID NO: 79 is an exemplary nucleic acid sequence encoding the 3155305_1A05 V_H.
caggtacagctgcagcagtcaggtccaggactggtgaagccctcgcagaccctctcactcacctgtctcatctccggtgac
agtgtctctagcaacagcgctgcttggaattggatcaggcagtccccatcgagaggccttgagtggctgggaaggacatac
tacaggtccaagtggtataatgattatgcagattctgtgaaaagtcgaataaccatcaacccagacacatccaagaaccag
ttctccctgcagctgaactctgtgactcccgaggacacggctgtctattactgtgcaagagcgggcattatgatttttgga
gtgattgttggcggtttggacgtctggggtcaggggacaacagtaaccgtctccagt

SEQ ID NO: 80 is an exemplary nucleic acid sequence encoding the 3155305_1A05 V_L.

gacatccagatgacccagtctccatcctccctgtctgcatctgtaggagacagagtcaccatctcttgccgggcaagtcag
agcattagcagctatttacattggtatcaacagaaaccagggaaagcccctgaactcctgatctatggtgcctccaatttg
cacagtggagtcccatcaaggtccagtggcagtgtatctgggacagatttcactctcaccatcagcagtctgcaacctgag
gattctgcaacttactactgtcaacagagttccactaagcctgggtacacttttggccggggaccaagctggagatcaaa

SEQ ID NO: 81 is an exemplary nucleic acid sequence encoding the 3155305_1B06 V_H.

caggtacagctgcagcagtcaggtccaggactggtgaagccctcgcagaccctctcactcacctgtgccatctccggggac
agtgtctctagcaacagtggtgcttgggactggatcaggcagtccccatcgagaggccttgagtggctgggaaggacatac
tacaggtccaagtggtataataattatgcagattttgtgaaaagtcgaataaccatcaatccggacacatccaagaaccag
ttttccctgcaactgagctctgtgactcccgaggacacggctgtgtatttctgtgcaagagcgggagttacggtctttgga
gtggttgttggcgctatggacgtctggggtcaggggacaacagtaaccgtctccagt

SEQ ID NO: 82 is an exemplary nucleic acid sequence encoding the 3155305_1B06 V_L.

gaaattgtgttgacacagtctccaggcaccctgtctttgtctccaggggaaacagccatcatctcttgtcggaccagtcag
tatggttccttagcctggtatcaacagaggcccggccaggcccccaggctcgtcatctattcgggctctactcgggccgct
ggcatcccagacaggttcagcggcagtcggtgggggccagactacaatctcaccatcagcaacctggagtcgggagatttt
ggtgtttattattgccagcagtatgaatttttggccaggggaccaaggtccaggtcgacattaaa

SEQ ID NO: 83 is an exemplary nucleic acid sequence encoding the 54.f.01 V_H.

caggtgcagctgcagcagtcaggtccaagactggtgaagccctcgcagaccctctcactcacctgtgttatttccggggac
agtgtctctagtcacagtgcttggaactggatcaggcagtccccatcgagaggccttgagtggctgggaaggacatactac
aggtccaagtggtatagtgattatgcaccatctgtgaaaagtcgaacaaccatcaacgcagacacatccaagaacgagatc
tccctgtatttgaattctgtgactcccgaggacacggctgtctattactgtgtaagagggtcttttatgattttggaata
gtcatggcttttgaccagtggggccagggaaccctggtcaccgtc

SEQ ID NO: 84 is an exemplary nucleic acid sequence encoding the 54.f.01 V_L.

gaaattgtgttgacgcagtctccaggcaccctgtctttgtccccaggggaaagagccacctctcgtgcagggccagtcag
agtgtgagtagcagctacttagcctggtaccagcagaagcctggccagtctcccagactcctcatctatggtacttccacc
agggccactggcatccccgacaggttcagtggcagtgggtctgggacagagttcactctcaccatcaccagactggagcct
gaagattttgcagtgtattactgtcagcagtttgatggctcacacttcactttcggccctgggaccaaagtggatatcaaa

SEQ ID NO: 85 is an exemplary nucleic acid sequence encoding the 56.a.09 V_H.

caggtgcagttgcagcagtcaggtccaggactggtgaagccctcgcagaccctctcactcacctgtgtcatctccgggga
cactgtctctagcaaccgtgctgcttggaactggatcagacagtccccatcgagaggccttgagtggctgggaaggacat
actacaggtccaagtggtatactgattatgccgtatctgtgaaaagtcgaataaccatcaccccagacacatccaagaac
cagttctccctgcagatgaagtctgtgactcccgaggacacggctgtgtattattgtgcaagaggctcggctatgatttt
tggaatagttattatacttgagtcctggggccagggaaccctggtcaccgtc

SEQ ID NO: 86 is an exemplary nucleic acid sequence encoding the 56.a.09 V_L.

gaaattgtgttgacgcagtctccaggcaccctgtctttgtctccaggagaaagagccacctctcctgcagggccagtca
gagtgttgccagcagctacttagcctggtaccagcagaaacctggccaggctcccaggctcctcatctatggtgcatcca
gcagggccactggcgtcccagacaggttcagtggcagtgggtctgggacagacttcattctcaccatcagcagactggag
cctgaagattttgcagtgtattactgtcagcagtatgatggctcacagtacacttttggccaggggaccaagctggagat
caaa

SEQ ID NO: 87 is an exemplary nucleic acid sequence encoding the 01.k.01 V_H.

cacgttcacctggtgcagtctggagctgaggtgcaggagtctggggcctcagtgaaggtctcctgcaaggcttctggcta
cagctttagcagtcatggtatcagttgggtgcgacaggcccctggtcaagggcttgagtggatggggtggatcagcgctt
acaatggccacacaaattatctacagaaattccagggcagagtcaccttgaccacagacacatccacggacacagcctac
atggagttgaggagcctcagatctgacgacacggccgtctattactgtgcgagagaccggggcaatattttgactggttg
tcaatttgactactggggccagggaacccgggtcaccgtctcctccg

SEQ ID NO: 88 is an exemplary nucleic acid sequence encoding the 01.k.01 V_L.

Gatgttgtgatgactcagtctccactctccctgcccgtcacccttggacagccggcctccatctcctgcaggtctagtca
aggcctcgttttcattgatggaagcacctacttgaactggtttcagcagaggccaggccagtctcccaggcgcctaattt
atgagatttctaagcgggactctggggtcccagacagattcagcggcagtgggtcaggcactgatttcacactgaagatc
agcagggtggaggctgaggatgttggggtttattactgcatgcaaggtacacactggccgatcaccttcggccaagggac
acgactggagattaaac

SEQ ID NO: 89 is an exemplary nucleic acid sequence encoding the 31.b.09 V_H.
caggtgcagctggtgcagtctggagctgaggtgaagaagcctggggcctcagtgaaggtctcctgcaaggcttctggata
tagttttagcagttacggtattagctgggtgcgacaggcccctggacaagggcttgagtggatgggatggatcagcgctt
acaacggtaacacaaactatgcgcagaaactccagggcagagtcaccatgaccacggacacatccacgagcacagcctac
atggagctgaggagcctgagatctgacgacacggccgtgttttactgtgcgagagatcggccccatattttgacgggttt
tgactttgactactggggccagggaaccctggtcaccgtc

SEQ ID NO: 90 is an exemplary nucleic acid sequence encoding the 31.b.09 V_L.
gatgttgtgatgactcagtctccagtctccctgcccgtcacccttggacagccggcctccatctcctgcaggtctagtca
aggcctcgtatatattgatggaaacacctacttgaattggtttcagcagaggccaggccaatctccaaggcgcctaattt
ataacgtttttacccgggactctggggtcccagacagattcagcggcagtgggtcaggcactgatttcacactgaagatc
accacggtggaggctgaggatgttggggtttattattgcatgcaaggtacacactggccgtacacttttggccaggggac
caagctggagatcaaa

SEQ ID NO: 91 is an exemplary nucleic acid sequence encoding the 16.g.07 V_H.
caggtgcagctggcgcagtctgaaaatgaactgaagaagcctggggcctcagtgaaggtctcctgcaagacttctggtta
cacctttaccaggtttggtatgagttgggtgcgacaggcccctggacaagggcttgagtggatgggatggatcagcggtt
acacaggtgacacaaagtatgcacggagtttccagggcagactcaccttgaccacggacacgtccacgggcacagcctac
atggaattgaggagccttagatctgacgacacggccatatattactgtgtgagaaacagagttcagatggaggtgtcacc
tgctacccaatcgacctggtacatggatctctggggccgtggcaccctggtctctgtc

SEQ ID NO: 92 is an exemplary nucleic acid sequence encoding the 16.g.07 V_L.
gacatccagatgacccagactccattctccctgtctgcttctataggagacagagtcaccattacttgtcgggcgagtca
ggatattactaggtggttagcctggtatcagcagaaaccagggaaagcccctgaactcctgatctatgctgcatccactt
tgcaaagtggggtcccctcaagattccgcggccgtggatctgggacagatttcagtctcaccatcagcgacctgcaggct
gaagattttgcaacttattattgtcagcagggtagcagtttcccgtacacctttggccaggggaccaggctggagattag
a

SEQ ID NO: 93 is an exemplary nucleic acid sequence encoding the 54.a.84 V_H.
caggttcagctggtgcagtctggacctgaggtgaagaagcctggggcctcagtgagggtctcctgcacggcttcaggtta
cacctttagcagatatggtatcagctgggtgcgacaggcccctggacaggggcctgagtggatggcttggatcagtgctt
acactggtgacacacattatgcccggaagttccagggcagagtcaccgtctccacagaggcttccacggccaccgtctac
atggagttgcggagtctgagatctgacgacacggccgtctattattgtgcgagagatcgaatccagggtgtagttgcgct
acctaaggaacaactcaattggttcgacccctggggccaggggaccctggtcaccgtc

SEQ ID NO: 94 is an exemplary nucleic acid sequence encoding the 54.a.84 V_L.
gaaattgtgttgacacagtctccagctgccctgtctttgtctccaggggagagagccaccctctcgtgcagggccagtca
cagtattagccagttcttagcctggtaccaacagaaacctggccaggctccccggctcctcatctatggtatatctaaca
gggccactgacgtcccagccaggttccgtggcagtgggtctgggacagacttcactctcaccatcagcgacctcgagcct
gaagattttgcagtttattactgtcagcagggtagcaactggcctcggacgttcggccaagggaccaaagtggaagccaa
a

SEQ ID NO: 95 is an exemplary nucleic acid sequence encoding the 16.a.26 V_H.
caggttcagttggtacagtctggacctgaggtgaagaagcctggggcctcagtgaaggtctcctgcaaggcttctggtta
cagtttttccagatatggtatcagctgggtgcgacaggcccctggacaagggcttgagtggctggggtggatcagcggtt
acactggcaacacaaactatgcacagaagttccagggcagagtcaccatgaccacagacacatccacgagcacagcctcc
atggagctgaggagtctgagatctgacgacacggccgtgtattactgtgcgagagacaagaagcaaggggaagtagtgct
accagctgctagtttccgttggttcgccccctggggccagggaaccctggtcaccgtc

SEQ ID NO: 96 is an exemplary nucleic acid sequence encoding the 16.a.26 V_L.
gacatccagatgacccagtctccagtctccctgtctgcttctgttggagacagagtcaccattacttgccgggcaagtca
gagcattggcaaatttttaaattggtatcagcagaaaccagggagagcccctaaactcctaatctattatgcatccaatt
tagagactgggggcccatcaaggttcagtggccgtggatctgagacagaattcagtctcaccatcagcagtctgcaacct
gaagattttgcaacttactactgtcaacagagtaacaatgtccctcacacttttggccaggggaccaagctggagatcaa
a

SEQ ID NO: 97 is an exemplary nucleic acid sequence encoding the 54.a.39 V_H.
caggttcacttggtgcagtctggagctgaggtgaagaagcctggggcctcagtgaaggtctcctgcaaggtttctggtta
cacgtttaccagttatggtatcagttgggtgcgacaggcccctggacaagggcttgagtggatggcatggatcagcgctt
acactggtaacacaaattttgcacagaagttcaaggacagagtcaccgtgtccacagacacatccacgaccacagcctac
atggagctgcggggcctgagatatgacgacacggccgtgtattactgtgcgagagatcggatccagggtgctgtcgcact
acctgataaacaggtgaactggttcgacccctggggccagggaaccctggtcaccgtctcctcg

SEQ ID NO: 98 is an exemplary nucleic acid sequence encoding the 54.a.39 V_L.

gaaattgtgttgacacagtctccagccaccctgtctttgtctccaggggaaagagccaccctctcctgcagggccagtca
gagtgttagcagctacttagcctggtaccaacagaaacctggccaggctccccggctcctcatctatggtgcatccaaca
gggccactggcatcccagccaggttccgtggcagtgggtctgggacagacttcactctcaccatcaacagcctagagcct
gaagattttgcagtttattactgtcagcagcatagcaactggcctcgaacgttcggccaagggaccaaggtggaaaccaa
a

SEQ ID NO: 99 is an exemplary nucleic acid sequence encoding the 31.a.83 V_H.

gaggtgcagctgctggagtctgggggaggcttggtgcagcctggggagtccctgagagtctcctgtgcagcctccggatt
caccttta gagactctgccctgagctgggtccgccaggctccagggaaggggctggagtgggtctcagctattagtggta
atggtggtgccacatactacgcggacaccgtgaagggccgattcaccatctccagagacaattcccagaccacactgtat
ctgcaaatgaacagcctgagagccgacgacacggccacatattactgtgcgaaagatgaaagtcccccgatttacaatct
tatgcctggttattactccacatattactacatggacgtctggggcaaagggaccacggtcaccgtctcctca

SEQ ID NO: 100 is an exemplary nucleic acid sequence encoding the 31.a.83 V_L.

gaaatagtgatgacgcagtctccagtcaccctgtctgtgtctccaggggaaacagccaccctctcctgcagggccagtca
gagtgttcgcagtaacttagcctggtaccagcagaaacctggccaggctccgaggctcctcatctatggtgcatccacca
gggccactgatatcccagccaggttcagtggcagtgggtctgggacagagttcactctctccatcagcagcctgcagtct
gacgattttgcagtttattactgtcagcagtataatcattggctccgaacttttggccaggggaccaagctggagatcaa
ac

SEQ ID NOs: 101-115 are antibody CDR sequences.

SEQ ID NOs: 116-121 and 128 are consensus CDR sequences of HV6-1/HD3-36 class consensus.

SEQ ID NOs: 122-127 and 129 are consensus CDR sequences of HV1-18/HD3-9 class antibodies.

SEQ ID NOs: 130 and 131 are consensus sequences of HV1-18 (Q-x-x-V) class antibodies.

SEQ ID NOs: 132-362 are anti-influenza HA antibody sequences.

SEQ ID NO: 363 is the amino acid sequence of the 315-53-1F12 V_H.

QVQLQQSGPGLVKPSQTLSLTCVISGDTVSSNTATWNWIRQSPSRGLEWLGRTYYRSKWYNDYGDSVKSRISISPDTSNNH
FSLHLKSVTPEDAAVYFCARAGIRIFGLIVGGLDVWGQGTTVTVSS

SEQ ID NO: 364 is the amino acid sequence of the 315-53-1F12 V_L.

DIQMTQSPSSLSASVGDRVTITCRTSQSVSSYLHWYQQKPGKAPELLIYATSNLHSGVPSRFSGSGSGTDFSLTISNLQPD
DFATYFCQQSSTNPGYTFGRGTKLEIK

SEQ ID NO: 365 is the amino acid sequence of the 315-09-1B12 V_H.

QVQLVQSAPEVKRPGASVRLSCKASGYTFNTYGIVWVRQAPGQGLEWMGWISAYTGNTNYAQKVQGRVTMTTDITTSTAYL
ELRGLRSDDTAVYYCARGLLQGAVILDSYHYALDFWGQGTMVTVSS

SEQ ID NO: 366 is the amino acid sequence of the 315-09-1B12 V_L.

EIVLTQSPGTLSLSPGERAALSCRASQSVTNRFIAWYQHKPGQSPRLLIYGASSRATGIPDRFSGRGSGTDFTLTISRLEP
EDFAVYYCQQYDTSPRWTFGRGTKLEIK

SEQ ID NO: 367 is the amino acid sequence of the 315-02-1E04 V_H.

QVHLVQSGPEVKKPGASVKVSCKVSGYTFTNYGVSWMRQAPGQGLEWIGWISAYNGHANSAQNFQDRVTMTTDKSTNTAYL
DLRGLTSDDTAVYYCARDRSNVLTGYLLDHWGQGTLVTVSS

SEQ ID NO: 368 is the amino acid sequence of the 315-02-1E04 V_L.

DVVMTQSPVSLPVTLGQSASISCRSSQGLVHIDGNIYLSWFHQRPGQSPRRLIYKVSNRDSGVPDRFTGSGSGTEFTLEIS
RVEAEDVGVYYCMQGTHRRLTFGEGTKVEIK

SEQ ID NO: 369 is an exemplary codon-optimized (not naturally occurring) nucleic acid sequence encoding the 315-53-1F12 V_H.

caggtgcagctgcagcagagcggaccaggcctggtgaagccttcccagaccctgtctctgacatgcgtgatctccggcga
caccgtgagctccaacaccgccacatggaattggatcaggcagtccccatctaggggcctggagtggctgggacggacat

actatagatctaagtggtacaacgactatggcgattctgtgaagagccggatcagcatctccccagacaccagcaacaat
cacttctccctgcacctgaagtctgtgacacccgaggatgcagccgtgtacttctgtgcaagggcaggaatccgcatctt
tggcctgatcgtgggaggcctggacgtgtggggacagggcaccacagtgaccgtgtctagc

SEQ ID NO: 370 is an exemplary codon-optimized (not naturally occurring) nucleic acid sequence encoding the 315-53-1F12 V_L.

gacatccagatgacacagagccctagctccctgagcgcctccgtgggcgatcgggtgaccatcacatgcagaacctctcag
agcgtgtctagctacctgcactggtatcagcagaagcccggcaaggcccctgagctgctgatctacgccacatccaacctg
cactctggagtgccaagcaggttctccggatctggaagcggaaccgactttccctgacaatctctaacctgcagccagac
gatttcgccacctacttttgtcagcagtcctctaccaatcccggctatacattcggccggggcaccaagctggagatcaag

SEQ ID NO: 371 is an exemplary codon-optimized (not naturally occurring) nucleic acid sequence encoding the 315-09-1B12 V_H.

caggtgcagctggtgcagtctgcacctgaggtaaagaggcctggggcctcagtgaggctctcctgcaaggcgtctggtta
caccttaacacctatggtattgtctgggtgcgccaggcccctggacaagggcttgagtggatggggtggatcagcgctt
acactggaaacacaaactatgcacagaaagtccagggcagagtcaccatgaccacagacattaccacgagcacagcctat
ctggaactgaggggtctcagatctgacgacacggccgtgtattattgtgcgagagggctccttcagggagctgttatcct
cgactcctaccactacgctttggacttctggggccaagggacaatggtcaccgtctcttca

SEQ ID NO: 372 is an exemplary codon-optimized (not naturally occurring) nucleic acid sequence encoding the 315-09-1B12 V_L.

gaaattgtgttgacgcagtctccaggcaccctgtctttgtctccaggggaaagagccgccctctcctgcagggccagtcag
agtgttaccaacaggttcatagcctggtaccaacataaacctggccagtctcccaggctcctcatctatggtgcatccagc
agggccactggcatcccagacaggttcagtggcagggggtctgggacagacttcactctcaccatcagcagactggagcct
gaagattttgcagtgtattactgtcagcagtatgatacctcacctcggtggacttttggccgggggaccaagctggagatc
aaa

SEQ ID NO: 373 is an exemplary codon-optimized (not naturally occurring) nucleic acid sequence encoding the 315-02-1E04 V_H.

caggttcacctggtgcagtctggacctgaggtgaagaagcctggggcctcagtgaaggtctcttgcaaggtttctggttac
acctttaccaattacggcgtcagttggatgcggcaggccccgggacaggggcttgagtggatcggatggatcagtgcatac
aatgggcatgcaaactctgcacaaaatttccaggacagagtcaccatgaccacagacaaatccacgaacacagcctatttg
gacctgaggggcctgacatctgacgacacggccgtctattactgtgcgagagatcggtccaacgtcttgactggttatttg
cttgaccattggggccaaggaaccctggtcaccgtctcctca

SEQ ID NO: 374 is an exemplary codon-optimized (not naturally occurring) nucleic acid sequence encoding the 315-02-1E04 V_L.

gatgttgtgatgactcagtctccagtctccctgcccgtcacccttggacagtcggcctccatctcctgcaggtctagtcaa
ggcctcgtacacattgatggaaacatctacttgagttggtttcaccagaggccaggccaatctccaaggcgcctaatttat
aaggtttctaaccgggactctggggtcccagacagattcaccggcagtgggtcaggcactgaattcacactggagatcagc
agagtggaggctgaagatgttggggtttactactgcatgcaaggcacacaccggcggctcactttcggcgaagggaccaag
gtggagatcaaa

DETAILED DESCRIPTION

I. Summary of Terms

Unless otherwise noted, technical terms are used according to conventional usage. Definitions of common terms in molecular biology may be found in Benjamin Lewin, *Genes X*, published by Jones & Bartlett Publishers, 2009; and Meyers *et al.* (eds.), *The Encyclopedia of Cell Biology and Molecular Medicine*, published by Wiley-VCH in 16 volumes, 2008; and other similar references.

As used herein, the singular forms "a," "an," and "the," refer to both the singular as well as plural, unless the context clearly indicates otherwise. For example, the term "an antigen" includes single or plural antigens and

can be considered equivalent to the phrase "at least one antigen." As used herein, the term "comprises" means "includes." It is further to be understood that any and all base sizes or amino acid sizes, and all molecular weight or molecular mass values, given for nucleic acids or polypeptides are approximate, and are provided for descriptive purposes, unless otherwise indicated. Although many methods and materials similar or equivalent to those described herein can be used, particular suitable methods and materials are described herein. In case of conflict, the present specification, including explanations of terms, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting. To facilitate review of the various embodiments, the following explanations of terms are provided:

Administration: The introduction of a composition into a subject by a chosen route. Administration can be local or systemic. For example, if the chosen route is intravenous, the composition is administered by introducing the composition into a vein of the subject. Exemplary routes of administration include, but are not limited to, oral, injection (such as subcutaneous, intramuscular, intradermal, intraperitoneal, and intravenous), sublingual, rectal, transdermal (for example, topical), intranasal, vaginal, and inhalation routes.

Amplification: A technique that increases the number of copies of a nucleic acid molecule (such as an RNA or DNA). An example of amplification is the polymerase chain reaction, in which a biological sample is contacted with a pair of oligonucleotide primers, under conditions that allow for the hybridization of the primers to a nucleic acid template in the sample. The primers are extended under suitable conditions, dissociated from the template, and then re-annealed, extended, and dissociated to amplify the number of copies of the nucleic acid. The product of amplification can be characterized by electrophoresis, restriction endonuclease cleavage patterns, oligonucleotide hybridization or ligation, and/or nucleic acid sequencing using standard techniques. Other examples of amplification include strand displacement amplification, as disclosed in U.S. Patent No. 5,744,311; transcription-free isothermal amplification, as disclosed in U.S. Patent No. 6,033,881; repair chain reaction amplification, as disclosed in WO 90/01069; ligase chain reaction amplification, as disclosed in EP-A-320 308; gap filling ligase chain reaction amplification, as disclosed in U.S. Patent No. 5,427,930; and NASBA™ RNA transcription-free amplification, as disclosed in U.S. Patent No. 6,025,134.

Antibody: An immunoglobulin, antigen-binding fragment, or derivative thereof, that specifically binds and recognizes an analyte (antigen) such as influenza HA. The term "antibody" is used herein in the broadest sense and encompasses various antibody structures, including but not limited to monoclonal antibodies, polyclonal antibodies, multispecific antibodies (*e.g.*, bispecific antibodies), and antibody fragments, so long as they exhibit the desired antigen-binding activity.

Non-limiting examples of antibodies include, for example, intact immunoglobulins and variants and fragments thereof known in the art that retain binding affinity for the antigen. Examples of antibody fragments include but are not limited to Fv, Fab, Fab', Fab'-SH, $F(ab')_2$; diabodies; linear antibodies; single-chain antibody molecules (*e.g.*, scFv); and multispecific antibodies formed from antibody fragments. Antibody fragments include antigen binding fragments either produced by the modification of whole antibodies or those synthesized *de novo* using recombinant DNA methodologies (see, *e.g.*, Kontermann and Dubel (Ed), Antibody Engineering, Vols. 1-2, 2nd Ed., Springer Press, 2010).

A single-chain antibody (scFv) is a genetically engineered molecule containing the V_H and V_L domains of one or more antibody(ies) linked by a suitable polypeptide linker as a genetically fused single chain molecule (see, for example, Bird *et al.*, *Science*, 242:423-426, 1988; Huston *et al.*, *Proc. Natl. Acad. Sci.*, 85:5879-5883, 1988;

Ahmad *et al.*, *Clin. Dev. Immunol.*, 2012, doi:10.1155/2012/980250; Marbry, *IDrugs*, 13:543-549, 2010). The intramolecular orientation of the V_H-domain and the V_L-domain in a scFv, is typically not decisive for scFvs. Thus, scFvs with both possible arrangements (V_H-domain-linker domain-V_L-domain; V_L-domain-linker domain-V_H-domain) may be used.

In a dsFv the V_H and V_L have been mutated to introduce a disulfide bond to stabilize the association of the chains. Diabodies also are included, which are bivalent, bispecific antibodies in which V_H and V_L domains are expressed on a single polypeptide chain, but using a linker that is too short to allow for pairing between the two domains on the same chain, thereby forcing the domains to pair with complementary domains of another chain and creating two antigen binding sites (see, for example, Holliger *et al.*, *Proc. Natl. Acad. Sci.,* 90:6444-6448, 1993; Poljak *et al.*, *Structure,* 2:1121-1123, 1994).

Antibodies also include genetically engineered forms such as chimeric antibodies (such as humanized murine antibodies) and heteroconjugate antibodies (such as bispecific antibodies). See also, *Pierce Catalog and Handbook*, 1994-1995 (Pierce Chemical Co., Rockford, IL); Kuby, J., *Immunology*, 3rd Ed., W.H. Freeman & Co., New York, 1997.

An "antibody that binds to the same epitope" as a reference antibody refers to an antibody that blocks binding of the reference antibody to its antigen in a competition assay by 50% or more, and conversely, the reference antibody blocks binding of the antibody to its antigen in a competition assay by 50% or more. Antibody competition assays are known, and an exemplary competition assay is provided herein.

An antibody may have one or more binding sites. If there is more than one binding site, the binding sites may be identical to one another or may be different. For instance, a naturally-occurring immunoglobulin has two identical binding sites, a single-chain antibody or Fab fragment has one binding site, while a bispecific or bifunctional antibody has two different binding sites.

Typically, a naturally occurring immunoglobulin has heavy (H) chains and light (L) chains interconnected by disulfide bonds. Immunoglobulin genes include the kappa, lambda, alpha, gamma, delta, epsilon and mu constant region genes, as well as the myriad immunoglobulin variable domain genes. There are two types of light chain, lambda (λ) and kappa (κ). There are five main heavy chain classes (or isotypes) which determine the functional activity of an antibody molecule: IgM, IgD, IgG, IgA and IgE.

Each heavy and light chain contains a constant region (or constant domain) and a variable region (or variable domain; see, *e.g.,* Kindt *et al.* Kuby Immunology, 6th ed., W.H. Freeman and Co., page 91 (2007).) In several embodiments, the V_H and V_L combine to specifically bind the antigen. In additional embodiments, only the V_H is required. For example, naturally occurring camelid antibodies consisting of a heavy chain only are functional and stable in the absence of light chain (see, *e.g.*, Hamers-Casterman *et al.*, *Nature*, 363:446-448, 1993; Sheriff *et al.*, *Nat. Struct. Biol.*, 3:733-736, 1996). Any of the disclosed antibodies can include a heterologous constant domain. For example the antibody can include constant domain that is different from a native constant domain, such as a constant domain including one or more modifications (such as the "LS" mutations) to increase half-life.

References to "V_H" or "VH" refer to the variable region of an antibody heavy chain, including that of an antigen binding fragment, such as Fv, scFv, dsFv or Fab. References to "V_L" or "VL" refer to the variable domain of an antibody light chain, including that of an Fv, scFv, dsFv or Fab.

The V_H and V_L contain a "framework" region interrupted by three hypervariable regions, also called "complementarity-determining regions" or "CDRs" (see, *e.g.*, Kabat *et al.*, *Sequences of Proteins of Immunological*

Interest, U.S. Department of Health and Human Services, 1991). The sequences of the framework regions of different light or heavy chains are relatively conserved within a species. The framework region of an antibody, that is the combined framework regions of the constituent light and heavy chains, serves to position and align the CDRs in three-dimensional space.

The CDRs are primarily responsible for binding to an epitope of an antigen. The amino acid sequence boundaries of a given CDR can be readily determined using any of a number of well-known schemes, including those described by Kabat *et al.* ("Sequences of Proteins of Immunological Interest," 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD, 1991; "Kabat" numbering scheme), Al-Lazikani *et al.*, (JMB 273,927-948, 1997; "Chothia" numbering scheme), and Lefranc *et al.* ("IMGT unique numbering for immunoglobulin and T cell receptor variable domains and Ig superfamily V-like domains," Dev. Comp. Immunol., 27:55-77, 2003; "IMGT" numbering scheme). The CDRs of each chain are typically referred to as CDR1, CDR2, and CDR3 (from the N-terminus to C-terminus), and are also typically identified by the chain in which the particular CDR is located. Thus, a V_H CDR3 is the CDR3 from the V_H of the antibody in which it is found, whereas a V_L CDR1 is the CDR1 from the V_L of the antibody in which it is found. Light chain CDRs are sometimes referred to as LCDR1, LCDR2, and LCDR3. Heavy chain CDRs are sometimes referred to as HCDR1, HCDR2, and HCDR3.

A "monoclonal antibody" is an antibody obtained from a population of substantially homogeneous antibodies, that is, the individual antibodies comprising the population are identical and/or bind the same epitope, except for possible variant antibodies, for example, containing naturally occurring mutations or arising during production of a monoclonal antibody preparation, such variants generally being present in minor amounts. In contrast to polyclonal antibody preparations, which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody of a monoclonal antibody preparation is directed against a single determinant on an antigen. Thus, the modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies may be made by a variety of techniques, including but not limited to the hybridoma method, recombinant DNA methods, phage-display methods, and methods utilizing transgenic animals containing all or part of the human immunoglobulin loci, such methods and other exemplary methods for making monoclonal antibodies being described herein. In some examples monoclonal antibodies are isolated from a subject. Monoclonal antibodies can have conservative amino acid substitutions which have substantially no effect on antigen binding or other immunoglobulin functions. (See, for example, Harlow & Lane, *Antibodies, A Laboratory Manual*, 2nd ed. Cold Spring Harbor Publications, New York (2013).)

A "humanized" antibody or antigen binding fragment includes a human framework region and one or more CDRs from a non-human (such as a mouse, rat, or synthetic) antibody or antigen binding fragment. The non-human antibody or antigen binding fragment providing the CDRs is termed a "donor," and the human antibody or antigen binding fragment providing the framework is termed an "acceptor." In one embodiment, all the CDRs are from the donor immunoglobulin in a humanized immunoglobulin. Constant regions need not be present, but if they are, they can be substantially identical to human immunoglobulin constant regions, such as at least about 85-90%, such as about 95% or more identical. Hence, all parts of a humanized antibody or antigen binding fragment, except possibly the CDRs, are substantially identical to corresponding parts of natural human antibody sequences.

A "chimeric antibody" is an antibody which includes sequences derived from two different antibodies, which typically are of different species. In some examples, a chimeric antibody includes one or more CDRs and/or framework regions from one human antibody and CDRs and/or framework regions from another human antibody.

A "fully human antibody" or "human antibody" is an antibody which includes sequences from (or derived from) the human genome, and does not include sequence from another species. In some embodiments, a human antibody includes CDRs, framework regions, and (if present) an Fc region from (or derived from) the human genome. Human antibodies can be identified and isolated using technologies for creating antibodies based on sequences derived from the human genome, for example by phage display or using transgenic animals (see, *e.g.*, Barbas *et al. Phage display: A Laboratory Manuel.* 1st Ed. New York: Cold Spring Harbor Laboratory Press, 2004. Print.; Lonberg, Nat. Biotech., 23: 1117-1125, 2005; Lonenberg, Curr. Opin. Immunol., 20:450-459, 2008)

Antibody or antigen binding fragment that neutralizes influenza A: An antibody or antigen binding fragment that specifically binds to the HA protein of influenza A in such a way as to inhibit a biological function associated with the HA protein (such as binding to its target receptor) that reduces and/or destroys the infectivity of the influenza A. In several embodiments, an antibody or antigen binding fragment that neutralizes influenza A reduces the infectious titer of influenza A.

An antibody can neutralize the activity of an infectious agent, such as influenza A virus at various points during the lifecycle of the virus. For example, an antibody may interfere with viral attachment to a target cell by interfering with the interaction of the virus and one or more cell surface receptors. Alternately, an antibody may interfere with one or more post-attachment interactions of the virus with its receptors, for example, by interfering with viral internalization by receptor-mediated endocytosis. In one embodiment, the antibody or binding fragment thereof neutralizes the activity of influenza A by interfering with the fusion process, for example, by interfering with fusion of the viral and endosomal membranes. In another embodiment, the antibody or binding fragment thereof interferes with protease mediated cleavage of HA0, thus interfering with viral maturation and the formation of the HA2 viral fusion peptide. For example, in one embodiment, the antibody or binding fragment thereof interferes with protease mediated HA0 cleavage, necessary for activation of the influenza A virus.

In some embodiments, an antibody or antigen binding fragment that specifically binds to influenza HA protein can neutralize at least one subtype (such as one, two, or three subtypes) of influenza A from each of group 1 (H1, H2, H5, H6, H8, H9, H11, H12, H13, and H16 subtypes) and group 2 (H3, H4, H7, H10, H14 and H15 subtypes) influenza A viruses. For example, in several embodiments, the antibody or antigen binding fragment can neutralize H1 and H3 subtypes of influenza A viruses.

Antibodyome: The entire repertoire of expressed antibody heavy and light chain sequence in an individual. The individual can be an individual infected with a pathogen, for example, influenza A.

Antibody self-reactivity or autoreactivity: A property of an antibody, whereby the antibody reacts with self-epitopes, that is epitopes of proteins and/or lipids that are produced by the subject. An antibody that does not have self-reactivity does not substantially bind to epitopes or lipids present on the membrane of a cell from a subject. Methods of determining if an antibody reacts with self epitopes are known to the person of ordinary skill in the art. In one example, antibody self reactivity is evaluated using HEp-2 cell staining, a cardiolipin binding assay, or an anti-nuclear antigen (ANA) assay. The anti-ANA assay can include an anti-ANA LUMINEX® assay or an ANA cell-staining assay, for example. In several embodiments, a disclosed antibody is not self-reactive (or autoreactive), or is minimally self-reactive, for example as measured using HEp-2 cell staining, cardiolipin binding,

an anti-ANA LUMINEX® assay, or an ANA cell-staining assay. In another non-limiting example, a disclosed antibody noes not have self reactivity above background levels, for example, as measured using an anti-ANA LUMINEX® assay or an ANA cell-staining assay.

B Cell and Memory B cell: B cells are a subset of lymphocytes, that is, white blood cells (leukocytes). Mature B cells differentiate into plasma cells, which produces antibodies, and memory B cells. A "B cell progenitor" is a cell that can develop into a mature B cell. B cell progenitors include stem cells, early pro-B cells, late pro-B cells, large pre-B cells, small pre-B cells, and immature B cells and transitional B cells. Generally, early pro-B cells (that express, for example, CD43 or B220) undergo immunoglobulin heavy chain rearrangement to become late pro B and pre B cells, and further undergo immunoglobulin light chain rearrangement to become an immature B cells. In humans, immature B cells (for example, immature peripheral transitional B cells) include $CD38^{hi}$, IgD^{+}, $CD10^{+}$, $CD24^{hi}$, $CD44^{lo}$, $CD23^{lo}$ and $CD1^{lo}$ cells. Thus, immature B cells include B220 (CD45R) expressing cells wherein the light and the heavy chain immunoglobulin genes are rearranged. In one embodiment, immature B cells express CD45R, class II, IgM, CD19 and CD40. Immature B cells can develop into mature B cells, which can produce immunoglobulins (*e.g.*, IgA, IgG or IgM). Mature B cells have acquired surface IgM and IgD, are capable of responding to antigen, and express characteristic markers such as CD21 and CD23 ($CD23^{hi}CD21^{hi}$ cells). Plasma cells are terminally differentiated B cells that are the predominant antibody-secreting cells.

After a B cell progenitor (*e.g.*, a pre-committed small lymphocyte) is stimulated by an antigen, it differentiates into a blast cell, which differentiates into an immature plasma cell that can differentiate into either a mature plasma cell or a memory B cell. A "mature plasma cell" secretes immunoglobulins in response to a specific antigen.

B cells can be activated by agents such as lippopolysaccharide (LPS) or IL-4 and antibodies to IgM. Common biological sources of B cells and B cell progenitors include bone marrow, peripheral blood, spleen and lymph nodes.

A "memory B cell" is a B cell that undergoes isotype switching and somatic hypermutation that are generally found during a secondary immune response (a subsequent antigen exposure following a primary exposure) but can also be detected during a primary antigen response. Generation of memory B cells generally requires helper T cells. The development of memory B cells takes place in germinal centers (GC) of lymphoid follicles where antigen-driven lymphocytes undergo somatic hypermutation and affinity selection, presumably under the influence of helper T cells. Typically, memory B cells express high affinity antigen specific immunoglobulin (B cell receptor) on their cell surface. In one embodiment, memory B cells include cells that express CD19. In some embodiments, B cells are selected that express CD19, but do not express IgA, IgD or IgM ($CD19^{+}IgA^{-}IgD^{-}IgM^{-}$ cells).

B cell repertoire: The B cells in a sample or in a subject specific for antigen of interest.

Biological sample: A sample obtained from a subject. Biological samples include all clinical samples useful for detection of disease or infection (for example, influenza HA infection) in subjects, including, but not limited to, cells, tissues, and bodily fluids, such as blood, derivatives and fractions of blood (such as serum), cerebrospinal fluid; as well as biopsied or surgically removed tissue, for example tissues that are unfixed, frozen, or fixed in formalin or paraffin. In a particular example, a biological sample is obtained from a subject having or suspected of having an influenza HA infection.

Bispecific antibody: A recombinant molecule composed of two different antigen binding domains that consequently binds to two different antigenic epitopes. Bispecific antibodies include chemically or genetically linked molecules of two antigen-binding domains. The antigen binding domains can be linked using a linker. The antigen binding domains can be monoclonal antibodies, antigen-binding fragments (*e.g.*, Fab, scFv), or combinations thereof. A bispecific antibody can include one or more constant domains, but does not necessarily include a constant domain.

Conditions sufficient to form an immune complex: Conditions which allow an antibody or antigen binding fragment to bind to its cognate epitope to a detectably greater degree than, and/or to the substantial exclusion of, binding to substantially all other epitopes. Conditions sufficient to form an immune complex are dependent upon the format of the binding reaction and typically are those utilized in immunoassay protocols or those conditions encountered *in vivo*. See Harlow & Lane, *Antibodies, A Laboratory Manual*, 2nd ed. Cold Spring Harbor Publications, New York (2013), for a description of immunoassay formats and conditions. The conditions employed in the methods are "physiological conditions" which include reference to conditions (*e.g.*, temperature, osmolarity, pH) that are typical inside a living mammal or a mammalian cell. While it is recognized that some organs are subject to extreme conditions, the intra-organismal and intracellular environment normally lies around pH 7 (*e.g.*, from pH 6.0 to pH 8.0, more typically pH 6.5 to 7.5), contains water as the predominant solvent, and exists at a temperature above 0°C and below 50°C. Osmolarity is within the range that is supportive of cell viability and proliferation.

The formation of an immune complex can be detected through conventional methods known to the skilled artisan, for instance immunohistochemistry, immunoprecipitation, flow cytometry, immunofluorescence microscopy, ELISA, immunoblotting (for example, Western blot), magnetic resonance imaging, CT scans, X-ray and affinity chromatography. Immunological binding properties of selected antibodies may be quantified using methods well known in the art.

Conjugate: A complex of two molecules linked together, for example, linked together by a covalent bond. In one embodiment, an antibody is linked to an effector molecule; for example, an antibody that specifically binds to influenza HA covalently linked to an effector molecule. The linkage can be by chemical or recombinant means. In one embodiment, the linkage is chemical, wherein a reaction between the antibody moiety and the effector molecule has produced a covalent bond formed between the two molecules to form one molecule. A peptide linker (short peptide sequence) can optionally be included between the antibody and the effector molecule. Because conjugates can be prepared from two molecules with separate functionalities, such as an antibody and an effector molecule, they are also sometimes referred to as "chimeric molecules."

Conservative variants: "Conservative" amino acid substitutions are those substitutions that do not substantially affect or decrease a function of a protein, such as the ability of the protein to interact with a target protein. For example, an influenza A-specific antibody can include up to 1, 2, 3, 4, 5, 6, 7, 8, 9, or up to 10 conservative substitutions compared to a reference antibody sequence and retain specific binding activity for influenza HA, and/or influenza A neutralization activity. The term conservative variation also includes the use of a substituted amino acid in place of an unsubstituted parent amino acid.

Furthermore, one of ordinary skill will recognize that individual substitutions, deletions or additions which alter, add or delete a single amino acid or a small percentage of amino acids (for instance less than 5%, in some

embodiments less than 1%) in an encoded sequence are conservative variations where the alterations result in the substitution of an amino acid with a chemically similar amino acid.

Conservative amino acid substitution tables providing functionally similar amino acids are well known to one of ordinary skill in the art. The following six groups are examples of amino acids that are considered to be conservative substitutions for one another:

1) Alanine (A), Serine (S), Threonine (T);

2) Aspartic acid (D), Glutamic acid (E);

3) Asparagine (N), Glutamine (Q);

4) Arginine (R), Lysine (K);

5) Isoleucine (I), Leucine (L), Methionine (M), Valine (V); and

6) Phenylalanine (F), Tyrosine (Y), Tryptophan (W).

Non-conservative substitutions are those that reduce an activity or function of the influenza HA-specific antibody, such as the ability to specifically bind to influenza HA. For instance, if an amino acid residue is essential for a function of the protein, even an otherwise conservative substitution may disrupt that activity. Thus, a conservative substitution does not alter the basic function of a protein of interest.

Contacting: Placement in direct physical association; includes both in solid and liquid form, which can take place either *in vivo* or *in vitro*. Contacting includes contact between one molecule and another molecule, for example the amino acid on the surface of one polypeptide, such as an antigen, that contacts another polypeptide, such as an antibody. Contacting can also include contacting a cell for example by placing an antibody in direct physical association with a cell.

Control: A reference standard. In some embodiments, the control is a negative control, such as sample obtained from a healthy patient not infected with influenza A. In other embodiments, the control is a positive control, such as a tissue sample obtained from a patient diagnosed with influenza A infection. In still other embodiments, the control is a historical control or standard reference value or range of values (such as a previously tested control sample, such as a group of influenza A patients with known prognosis or outcome, or group of samples that represent baseline or normal values).

A difference between a test sample and a control can be an increase or conversely a decrease. The difference can be a qualitative difference or a quantitative difference, for example a statistically significant difference. In some examples, a difference is an increase or decrease, relative to a control, of at least about 5%, such as at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 100%, at least about 150%, at least about 200%, at least about 250%, at least about 300%, at least about 350%, at least about 400%, or at least about 500%.

Degenerate variant: In the context of the present disclosure, a "degenerate variant" refers to a polynucleotide encoding a protein (for example, an antibody that specifically binds influenza HA or a variable region thereof) that includes a sequence that is degenerate as a result of the genetic code. There are twenty natural amino acids, most of which are specified by more than one codon. Therefore, all degenerate nucleotide sequences are included as long as the amino acid sequence of the antibody that binds influenza HA encoded by the nucleotide sequence is unchanged.

Detectable marker: A detectable molecule (also known as a label) that is conjugated directly or indirectly to a second molecule, such as an antibody, to facilitate detection of the second molecule. For example, the detectable marker can be capable of detection by ELISA, spectrophotometry, flow cytometry, microscopy or diagnostic imaging techniques (such as CT scans, MRIs, ultrasound, fiberoptic examination, and laparoscopic examination). Specific, non-limiting examples of detectable markers include fluorophores, chemiluminescent agents, enzymatic linkages, radioactive isotopes and heavy metals or compounds (for example super paramagnetic iron oxide nanocrystals for detection by MRI). In one example, a "labeled antibody" refers to incorporation of another molecule in the antibody. For example, the label is a detectable marker, such as the incorporation of a radiolabeled amino acid or attachment to a polypeptide of biotinyl moieties that can be detected by marked avidin (for example, streptavidin containing a fluorescent marker or enzymatic activity that can be detected by optical or colorimetric methods). Various methods of labeling polypeptides and glycoproteins are known in the art and may be used. Examples of labels for polypeptides include, but are not limited to, the following: radioisotopes or radionuclides (such as ^{35}S or ^{131}I), fluorescent labels (such as fluorescein isothiocyanate (FITC), rhodamine, lanthanide phosphors), enzymatic labels (such as horseradish peroxidase, beta-galactosidase, luciferase, alkaline phosphatase), chemiluminescent markers, biotinyl groups, predetermined polypeptide epitopes recognized by a secondary reporter (such as a leucine zipper pair sequences, binding sites for secondary antibodies, metal binding domains, epitope tags), or magnetic agents, such as gadolinium chelates. In some embodiments, labels are attached by spacer arms of various lengths to reduce potential steric hindrance. Methods for using detectable markers and guidance in the choice of detectable markers appropriate for various purposes are discussed for example in Sambrook *et al.* (Molecular Cloning: A Laboratory Manual, 4th ed, Cold Spring Harbor, New York, 2012) and Ausubel *et al.* (In Current Protocols in Molecular Biology, John Wiley & Sons, New York, through supplement 104, 2013).

Detecting: To identify the existence, presence, or fact of something. General methods of detecting are known to the skilled artisan and may be supplemented with the protocols and reagents disclosed herein. For example, included herein are methods of detecting a cell that expresses influenza HA in a subject.

DNA sequencing: The process of determining the nucleotide order of a given DNA molecule. In exemplary embodiments, sequencing can be performed using automated Sanger sequencing (AB13730xl genome analyzer), pyrosequencing on a solid support (454 sequencing, Roche), sequencing-by-synthesis with reversible terminations (ILLUMINA® Genome Analyzer), sequencing-by-ligation (ABI SOLiD®) or sequencing-by-synthesis with virtual terminators (HELISCOPE®). .

In some embodiments, DNA sequencing is performed using a chain termination method developed by Frederick Sanger, and thus termed "Sanger based sequencing" or "SBS." This technique uses sequence-specific termination of a DNA synthesis reaction using modified nucleotide substrates. Extension is initiated at a specific site on the template DNA by using a short oligonucleotide primer complementary to the template at that region. The oligonucleotide primer is extended using DNA polymerase in the presence of the four deoxynucleotide bases (DNA building blocks), along with a low concentration of a chain terminating nucleotide (most commonly a di-deoxynucleotide). Limited incorporation of the chain terminating nucleotide by the DNA polymerase results in a series of related DNA fragments that are terminated only at positions where that particular nucleotide is present. The fragments are then size-separated by electrophoresis a polyacrylamide gel, or in a narrow glass tube (capillary)

filled with a viscous polymer. An alternative to using a labeled primer is to use labeled terminators instead; this method is commonly called "dye terminator sequencing."

"Pyrosequencing" is an array based method, which has been commercialized by 454 Life Sciences (Branford, CT). In some embodiments of the array-based methods, single-stranded DNA is annealed to beads and amplified via EmPCR®. These DNA-bound beads are then placed into wells on a fiber-optic chip along with enzymes that produce light in the presence of ATP. When free nucleotides are washed over this chip, light is produced as the PCR amplification occurs and ATP is generated when nucleotides join with their complementary base pairs. Addition of one (or more) nucleotide(s) results in a reaction that generates a light signal that is recorded, such as by the charge coupled device (CCD) camera, within the instrument. The signal strength is proportional to the number of nucleotides, for example, homopolymer stretches, incorporated in a single nucleotide flow.

Effector molecule: A molecule intended to have or produce a desired effect; for example, a desired effect on a cell to which the effector molecule is targeted. Effector molecules can include, for example, polypeptides and small molecules. In one non-limiting example, the effector molecule is a toxin. The skilled artisan will understand that some effector molecules may have or produce more than one desired effect.

Epitope: An antigenic determinant. These are particular chemical groups or peptide sequences on a molecule that are antigenic, *i.e.* that elicit a specific immune response. An antibody specifically binds a particular antigenic epitope on a polypeptide. In some examples a disclosed antibody specifically binds to an epitope on influenza HA.

Expression: Transcription or translation of a nucleic acid sequence. For example, an encoding nucleic acid sequence (such as a gene) can be expressed when its DNA is transcribed into an RNA or RNA fragment, which in some examples is processed to become mRNA. An encoding nucleic acid sequence (such as a gene) may also be expressed when its mRNA is translated into an amino acid sequence, such as a protein or a protein fragment. In a particular example, a heterologous gene is expressed when it is transcribed into an RNA. In another example, a heterologous gene is expressed when its RNA is translated into an amino acid sequence. Regulation of expression can include controls on transcription, translation, RNA transport and processing, degradation of intermediary molecules such as mRNA, or through activation, inactivation, compartmentalization or degradation of specific protein molecules after they are produced.

Expression Control Sequences: Nucleic acid sequences that regulate the expression of a heterologous nucleic acid sequence to which it is operatively linked. Expression control sequences are operatively linked to a nucleic acid sequence when the expression control sequences control and regulate the transcription and, as appropriate, translation of the nucleic acid sequence. Thus expression control sequences can include appropriate promoters, enhancers, transcription terminators, a start codon (ATG) in front of a protein-encoding gene, splicing signal for introns, maintenance of the correct reading frame of that gene to permit proper translation of mRNA, and stop codons. The term "control sequences" is intended to include, at a minimum, components whose presence can influence expression, and can also include additional components whose presence is advantageous, for example, leader sequences and fusion partner sequences. Expression control sequences can include a promoter.

A promoter is a minimal sequence sufficient to direct transcription. Also included are those promoter elements which are sufficient to render promoter-dependent gene expression controllable for cell-type specific, tissue-specific, or inducible by external signals or agents; such elements may be located in the 5' or 3' regions of the gene. Both constitutive and inducible promoters are included (see for example, Bitter *et al.*, *Methods in*

Enzymology 153:516-544, 1987). For example, when cloning in bacterial systems, inducible promoters such as pL of bacteriophage lambda, plac, ptrp, ptac (ptrp-lac hybrid promoter) and the like may be used. In one embodiment, when cloning in mammalian cell systems, promoters derived from the genome of mammalian cells (such as metallothionein promoter) or from mammalian viruses (such as the retrovirus long terminal repeat; the adenovirus late promoter; the vaccinia virus 7.5K promoter) can be used. Promoters produced by recombinant DNA or synthetic techniques may also be used to provide for transcription of the nucleic acid sequences.

A polynucleotide can be inserted into an expression vector that contains a promoter sequence which facilitates the efficient transcription of the inserted genetic sequence of the host. The expression vector typically contains an origin of replication, a promoter, as well as specific nucleic acid sequences that allow phenotypic selection of the transformed cells.

Expression vector: A vector comprising a recombinant polynucleotide comprising expression control sequences operatively linked to a nucleotide sequence to be expressed. An expression vector comprises sufficient cis- acting elements for expression; other elements for expression can be supplied by the host cell or in an in vitro expression system. Expression vectors include all those known in the art, such as cosmids, plasmids (*e.g.*, naked or contained in liposomes) and viruses (*e.g.*, lentiviruses, retroviruses, adenoviruses, and adeno-associated viruses) that incorporate the recombinant polynucleotide.

Fc polypeptide: The polypeptide including the constant region of an antibody excluding the first constant region immunoglobulin domain. Fc region generally refers to the last two constant region immunoglobulin domains of IgA, IgD, and IgG, and the last three constant region immunoglobulin domains of IgE and IgM. An Fc region may also include part or all of the flexible hinge N-terminal to these domains. For IgA and IgM, an Fc region may or may not include the tailpiece, and may or may not be bound by the J chain. For IgG, the Fc region includes immunoglobulin domains Cgamma2 and Cgamma3 (Cγ2 and Cγ3) and the lower part of the hinge between Cgamma1 (Cγ1) and Cγ2. Although the boundaries of the Fc region may vary, the human IgG heavy chain Fc region is usually defined to include residues C226 or P230 to its carboxyl-terminus, wherein the numbering is according to the EU index as in Kabat. For IgA, the Fc region includes immunoglobulin domains Calpha2 and Calpha3 (Cα2 and Cα3) and the lower part of the hinge between Calpha1 (Cα1) and Cα2.

Germline Origin: Nucleic acid sequences encoding different domains of immunoglobulins in their unrearranged state rather than the rearranged sequences (such as rearranged nucleic acid sequences for production of immunoglobulins or T cell receptor molecules), and the amino acids encoded therein. Three separate loci encode, respectively, the Ig κ light chain, the Ig λ light chain, and all the Ig heavy chain germline sequences. Each germline Ig locus is made up of at least three different types of gene segments, the variable (V), constant (C), and joining segments (J), that are separated from one another in the genome by large stretches of DNA that are never transcribed. The germline organization of Ig loci exists in all cell types of the body. However, germline genes cannot be transcribed into mRNA that gives rise to functional antibodies. These are created only in developing B and T lymphocytes by rearrangement of DNA that make the V, C, and J segments contiguous; the human V, D and J segments recombine during B cell development. Germline sequences of immunoglobulins do not include sequences wherein V-D-J recombination has occurred, and do not contain somatic hypermutations. The V regions from light and heavy chain germline sequences have been cloned (see, for example, Tomlinson *et al.* (*J. Mol. Biol.*, 227:776-798, 1992, and Cox *et al., Eur. J. Immunol.,* 24:827-836, 1994, which are incorporated herein by reference).

Standard methods of determining the germline origin of a particular antibody sequence (DNA or amino acid sequence) are available, including, for example, using IgBlast (Ye *et al.*, *Nucleic acids research,* 41, W34-40, 2013) to assign germline origin.

HV6-1/HD3-3 heavy chain variable region: An antibody heavy chain variable region having a germline origin based on recombination and somatic mutation of the HV6-1 and HD3-3 antibody germline genes. The 56.a.09 antibody disclosed herein has a HV6-1/HD3-3 heavy chain variable region.

HV1-18/HD3-9 heavy chain variable region: An antibody heavy chain variable region having a germline origin based on recombination and somatic mutation of the HV1-18 and HD3-9 antibody germline genes. The 31.b.09 antibody disclosed herein has a HV1-18/HD3-9 heavy chain variable region.

HV1-18 heavy chain variable region: An antibody heavy chain variable region having a germline origin based on recombination and somatic mutation of HV1-18 antibody germline gene. The 16.g.07 antibody disclosed herein has a HV1-18 heavy chain variable region.

Influenza Hemagglutinin (HA) protein: An influenza virus surface glycoprotein that is a homotrimeric integral membrane glycoprotein. HA mediates binding of the virus particle to a host cell and subsequent entry of the virus into the host cell. The nucleotide and amino acid sequences of numerous influenza HA proteins are known in the art and are publically available, such as through the NCBI Influenza Virus Resource database (Bao *et al.*, *J Virol* 82:596-601, 2008). HA (along with NA) is one of the two major influenza virus antigenic determinants. The three identical monomers that constitute HA are constructed into a central α helix coil; three spherical heads contain the sialic acid binding sites. In nature, HA monomers are synthesized as precursors that are then glycosylated and cleaved into two smaller polypeptides: the HA1 and HA2 subunits. Each HA monomer consists of a long, helical chain anchored in the membrane by HA2 and topped by a large HA1 globular head which contains the sialic acid receptor binding sites. The HA2 protein chain facilitates membrane fusion; the C-terminal end of the protein is embedded in the viral membrane. The stalk of HA is comprised of portions of HA1 and HA2.

Influenza virus: A segmented negative-strand RNA virus that belongs to the *Orthomyxoviridae* family. There are three types of influenza viruses, A, B and C. Influenza A viruses infect a wide variety of birds and mammals, including humans, horses, marine mammals, pigs, ferrets, and chickens. In animals, most influenza A viruses cause mild localized infections of the respiratory and intestinal tract. However, highly pathogenic influenza A strains, such as H5N1, cause systemic infections in poultry in which mortality may reach 100%. H1N1 influenza was the most common cause of human influenza in 2009, and this strain was referred to as "swine flu," 2009 H1N1 pandemic (pdm) flu, or A(H1N1)pdm09 influenza virus. H1N1 influenza A viruses were also responsible for the Spanish flu pandemic in 1918, the Fort Dix outbreak in 1976, and the Russian flu epidemic in 1977-1978.

Influenza A viruses are categorized into subtypes based on the type of two proteins, hemagglutinin (HA) and neuraminidase (NA) that are on the surface of the viral envelope. Different influenza viruses encode for different HA and NA proteins. There are 16 different HA subtypes and 11 different NA subtypes, H1 through H18 and N1 through N11 respectively.

Influenza A viruses that have caused disease in humans include the following subtypes: H1N1, H2N2, and H3N2. Other subtypes of viruses that have infected humans but are not transmitted from person to person are: H5N1 (bird flu), H6N1, H7N7, H1N2, H9N2, H7N2, H7N3, H10N7 and H10N8. There are different lineages of HA and NA within each subtype that are distinguished by amino acid sequence. For example, H1N1 viruses that circulated before 2009 and swine flu H1N1 viruses that have circulated amongst humans since 2009, represent

different HA and NA lineages. Within each lineage, virus variants of different "clades" circulate in the population each winter. The H1N1 related viruses currently circulating have HA and NA antigens that are antigenically similar to the A(H1N1)pdm09 virus and are called A(H1N1)pdm09-like viruses.

Antibodies that bind HA can block virus attachment to receptors or virus entry. Antibodies to NA, particularly those that inhibit its enzyme activity, reduce virus replication because newly formed virus particles cannot be released from the infected cell. Antibodies to HA and NA are associated with resistance to influenza disease.

IgA: A polypeptide belonging to the class of antibodies that are substantially encoded by a recognized immunoglobulin alpha gene. In humans, this class or isotype comprises IgA_1 and IgA_2. IgA antibodies can exist as monomers, polymers (referred to as pIgA) of predominantly dimeric form, and secretory IgA. The constant chain of wild-type IgA contains an 18-amino-acid extension at its C-terminus called the tail piece (tp). Polymeric IgA is secreted by plasma cells with a 15-kDa peptide called the J chain linking two monomers of IgA through the conserved cysteine residue in the tail piece.

IgG: A polypeptide belonging to the class or isotype of antibodies that are substantially encoded by a recognized immunoglobulin gamma gene. In humans, this class comprises IgG_1, IgG_2, IgG_3, and IgG_4. In mice, this class comprises IgG_1, IgG_{2a}, IgG_{2b}, IgG_3.

Immune complex: The binding of antibody or antigen binding fragment (such as a scFv) to a soluble antigen forms an immune complex. The formation of an immune complex can be detected through conventional methods known to the skilled artisan, for instance immunohistochemistry, immunoprecipitation, flow cytometry, immunofluorescence microscopy, ELISA, immunoblotting (for example, Western blot), magnetic resonance imaging, CT scans, X-ray and affinity chromatography. Immunological binding properties of selected antibodies may be quantified using methods well known in the art.

Isolated: A biological component (such as a nucleic acid, peptide, protein or protein complex, for example an antibody) that has been substantially separated, produced apart from, or purified away from other biological components in the cell of the organism in which the component naturally occurs, that is, other chromosomal and extra-chromosomal DNA and RNA, and proteins. Thus, isolated nucleic acids, peptides and proteins include nucleic acids and proteins purified by standard purification methods. The term also embraces nucleic acids, peptides and proteins prepared by recombinant expression in a host cell, as well as, chemically synthesized nucleic acids. A isolated nucleic acid, peptide or protein, for example an antibody, can be at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% pure.

Kabat position: A position of a residue in an amino acid sequence that follows the numbering convention delineated by Kabat *et al.* (*Sequences of Proteins of Immunological Interest*, 5th Edition, Department of Health and Human Services, Public Health Service, National Institutes of Health, Bethesda, NIH Publication No. 91-3242, 1991).

Linker: A bi-functional molecule that can be used to link two molecules into one contiguous molecule, for example, to link an effector molecule to an antibody. In some embodiments, the provided conjugates include a linker between the effector molecule or detectable marker and an antibody. In some cases, a linker is a peptide within an antigen binding fragment (such as an Fv fragment) which serves to indirectly bond the V_H and V_L. Non-limiting examples of peptide linkers include glycine-serine linkers.

The terms "conjugating," "joining," "bonding," or "linking" can refer to making two molecules into one contiguous molecule; for example, linking two polypeptides into one contiguous polypeptide, or covalently attaching an effector molecule or detectable marker radionuclide or other molecule to a polypeptide, such as an scFv. In the specific context, the terms include reference to joining a ligand, such as an antibody moiety, to an effector molecule. The linkage can be either by chemical or recombinant means. "Chemical means" refers to a reaction between the antibody moiety and the effector molecule such that there is a covalent bond formed between the two molecules to form one molecule.

Nucleic acid: A polymer composed of nucleotide units (ribonucleotides, deoxyribonucleotides, related naturally occurring structural variants, and synthetic non-naturally occurring analogs thereof) linked via phosphodiester bonds, related naturally occurring structural variants, and synthetic non-naturally occurring analogs thereof. Thus, the term includes nucleotide polymers in which the nucleotides and the linkages between them include non-naturally occurring synthetic analogs, such as, for example and without limitation, phosphorothioates, phosphoramidates, methyl phosphonates, chiral-methyl phosphonates, 2-O-methyl ribonucleotides, peptide-nucleic acids (PNAs), and the like. Such polynucleotides can be synthesized, for example, using an automated DNA synthesizer. The term "oligonucleotide" typically refers to short polynucleotides, generally no greater than about 50 nucleotides. It will be understood that when a nucleotide sequence is represented by a DNA sequence (i.e., A, T, G, C), this also includes an RNA sequence (i.e., A, U, G, C) in which "U" replaces "T. "

Conventional notation is used herein to describe nucleotide sequences: the left-hand end of a single-stranded nucleotide sequence is the 5'-end; the left-hand direction of a double-stranded nucleotide sequence is referred to as the 5'-direction. The direction of 5' to 3' addition of nucleotides to nascent RNA transcripts is referred to as the transcription direction. The DNA strand having the same sequence as an mRNA is referred to as the "coding strand;" sequences on the DNA strand having the same sequence as an mRNA transcribed from that DNA and which are located 5' to the 5'-end of the RNA transcript are referred to as "upstream sequences;" sequences on the DNA strand having the same sequence as the RNA and which are 3' to the 3' end of the coding RNA transcript are referred to as "downstream sequences."

"cDNA" refers to a DNA that is complementary or identical to an mRNA, in either single stranded or double stranded form.

"Encoding" refers to the inherent property of specific sequences of nucleotides in a polynucleotide, such as a gene, a cDNA, or an mRNA, to serve as templates for synthesis of other polymers and macromolecules in biological processes having either a defined sequence of nucleotides (i.e., rRNA, tRNA and mRNA) or a defined sequence of amino acids and the biological properties resulting therefrom. Thus, a gene encodes a protein if transcription and translation of mRNA produced by that gene produces the protein in a cell or other biological system. Both the coding strand, the nucleotide sequence of which is identical to the mRNA sequence and is usually provided in sequence listings, and non-coding strand, used as the template for transcription, of a gene or cDNA can be referred to as encoding the protein or other product of that gene or cDNA. Unless otherwise specified, a "nucleotide sequence encoding an amino acid sequence" includes all nucleotide sequences that are degenerate versions of each other and that encode the same amino acid sequence. Nucleotide sequences that encode proteins and RNA may include introns.

The nucleotides can be ribonucleotides, deoxyribonucleotides, or modified forms of either nucleotide. The term includes single- and double- stranded forms of DNA.

Operably linked: A first nucleic acid sequence is operably linked with a second nucleic acid sequence when the first nucleic acid sequence is placed in a functional relationship with the second nucleic acid sequence. For instance, a promoter, such as the CMV promoter, is operably linked to a coding sequence if the promoter affects the transcription or expression of the coding sequence. Generally, operably linked DNA sequences are contiguous and, where necessary to join two protein-coding regions, in the same reading frame.

Pharmaceutically acceptable carriers: The pharmaceutically acceptable carriers of use are conventional. *Remington's Pharmaceutical Science, 22th ed.*, Pharmaceutical Press, London, UK (2012), describes compositions and formulations suitable for pharmaceutical delivery of the disclosed agents.

In general, the nature of the carrier will depend on the particular mode of administration being employed. For instance, parenteral formulations usually include 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 vehicle. For solid compositions (*e.g.*, 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, added preservatives (such as non-natural preservatives), and pH buffering agents and the like, for example sodium acetate or sorbitan monolaurate. In particular examples, the pharmaceutically acceptable carrier is sterile and suitable for parenteral administration to a subject for example, by injection. In some embodiments, the active agent and pharmaceutically acceptable carrier are provided in a unit dosage form such as a pill or in a selected quantity in a vial. Unit dosage forms can include one dosage or multiple dosages (for example, in a vial from which metered dosages of the agents can selectively be dispensed).

Polypeptide: A polymer in which the monomers are amino acid residues that are joined together through amide bonds. When the amino acids are alpha-amino acids, either the L-optical isomer or the D-optical isomer can be used, the L-isomers being preferred. The terms "polypeptide" or "protein" as used herein are intended to encompass any amino acid sequence and include modified sequences such as glycoproteins. A polypeptide includes both naturally occurring proteins, as well as those that are recombinantly or synthetically produced. A polypeptide has an amino terminal (N-terminal) end and a carboxy-terminal end. In some embodiments, the polypeptide is a disclosed antibody or a fragment thereof.

Polypeptide modifications: polypeptides can be modified by a variety of chemical techniques to produce derivatives having essentially the same activity and conformation as the unmodified peptides, and optionally having other desirable properties. For example, carboxylic acid groups of the protein, whether carboxyl-terminal or side chain, may be provided in the form of a salt of a pharmaceutically-acceptable cation or esterified to form a C_1-C_{16} ester, or converted to an amide of formula NR_1R_2 wherein R_1 and R_2 are each independently H or C_1-C_{16} alkyl, or combined to form a heterocyclic ring, such as a 5- or 6- membered ring. Amino groups of the peptide, whether amino-terminal or side chain, may be in the form of a pharmaceutically-acceptable acid addition salt, such as the HCl, HBr, acetic, benzoic, toluene sulfonic, maleic, tartaric and other organic salts, or may be modified to C_1-C_{16} alkyl or dialkyl amino or further converted to an amide.

Hydroxyl groups of the peptide side chains can be converted to C_1-C_{16} alkoxy or to a C_1-C_{16} ester using well-recognized techniques. Phenyl and phenolic rings of the peptide side chains can be substituted with one or more halogen atoms, such as F, Cl, Br or I, or with C_1-C_{16} alkyl, C_1-C_{16} alkoxy, carboxylic acids and esters thereof,

or amides of such carboxylic acids. Methylene groups of the peptide side chains can be extended to homologous C_2-C_4 alkylenes. Thiols can be protected with any one of a number of well-recognized protecting groups, such as acetamide groups.

Recombinant: A recombinant nucleic acid is one that has a sequence that is not naturally occurring or has a sequence that is made by an artificial combination of two otherwise separated segments of sequence. This artificial combination can be accomplished by chemical synthesis or, more commonly, by the artificial manipulation of isolated segments of nucleic acids, for example, by genetic engineering techniques. A recombinant protein is one that has a sequence that is not naturally occurring or has a sequence that is made by an artificial combination of two otherwise separated segments of sequence. In several embodiments, a recombinant protein is encoded by a heterologous (for example, recombinant) nucleic acid that has been introduced into a host cell, such as a bacterial or eukaryotic cell. The nucleic acid can be introduced, for example, on an expression vector having signals capable of expressing the protein encoded by the introduced nucleic acid or the nucleic acid can be integrated into the host cell chromosome.

Sequence identity: The similarity between amino 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 polypeptide will possess a relatively high degree of sequence identity when 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, 1988; Higgins and Sharp, *CABIOS* 5:151, 1989; Corpet *et al.*, *Nucleic Acids Research* 16:10881, 1988; and Pearson and Lipman, *Proc. Natl. Acad. Sci. U.S.A.* 85:2444, 1988. Altschul *et al.*, *Nature Genet.* 6:119, 1994, presents a detailed consideration of sequence alignment methods and homology calculations.

The NCBI Basic Local Alignment Search Tool (BLAST) (Altschul *et al.*, *J. Mol. Biol.* 215:403, 1990) is available from several sources, including the National Center for Biotechnology Information (NCBI, Bethesda, MD) and on the internet, for use in connection with the sequence analysis programs blastp, blastn, blastx, tblastn and tblastx. A description of how to determine sequence identity using this program is available on the NCBI website on the internet.

Homologs and variants of a V_L or a V_H of an antibody that specifically binds a polypeptide are typically characterized by possession of at least about 75%, for example at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity counted over the full length alignment with the amino acid sequence of interest. Proteins with even greater similarity to the reference sequences will show increasing percentage identities when assessed by this method, such as at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99% sequence identity. When less than the entire sequence is being compared for sequence identity, homologs and variants will typically possess at least 80% sequence identity over short windows of 10-20 amino acids, and may possess sequence identities of at least 85% or at least 90% or 95% depending on their similarity to the reference sequence. Methods for determining sequence identity over such short windows are available at the NCBI website on the internet. One of skill in the art will appreciate that these sequence identity

ranges are provided for guidance only; it is entirely possible that strongly significant homologs could be obtained that fall outside of the ranges provided.

Terms used to describe sequence relationships between two or more nucleotide sequences or amino acid sequences include "reference sequence," "selected from," "comparison window," "identical," "percentage of sequence identity," "substantially identical," "complementary," and "substantially complementary."

For sequence comparison of nucleic acid sequences, typically one sequence acts as a reference sequence, to which test sequences are compared. When using a sequence comparison algorithm, test and reference sequences are entered into a computer, subsequence coordinates are designated, if necessary, and sequence algorithm program parameters are designated. Default program parameters are used. Methods of alignment of sequences for comparison are well known in the art. Optimal alignment of sequences for comparison can be conducted, *e.g.*, by the local homology algorithm of Smith & Waterman, *Adv. Appl. Math.* 2:482, 1981, by the homology alignment algorithm of Needleman & Wunsch, *J. Mol. Biol.* 48:443, 1970, by the search for similarity method of Pearson & Lipman, *Proc. Nat'l. Acad. Sci. USA* 85:2444, 1988, by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, WI), or by manual alignment and visual inspection (see, *e.g.*, Sambrook *et al.* (Molecular Cloning: A Laboratory Manual, 4th ed, Cold Spring Harbor, New York, 2012) and Ausubel *et al.* (In Current Protocols in Molecular Biology, John Wiley & Sons, New York, through supplement 104, 2013). One example of a useful algorithm is PILEUP. PILEUP uses a simplification of the progressive alignment method of Feng & Doolittle, *J. Mol. Evol.* 35:351-360, 1987. The method used is similar to the method described by Higgins & Sharp, *CABIOS* 5:151-153, 1989. Using PILEUP, a reference sequence is compared to other test sequences to determine the percent sequence identity relationship using the following parameters: default gap weight (3.00), default gap length weight (0.10), and weighted end gaps. PILEUP can be obtained from the GCG sequence analysis software package, *e.g.*, version 7.0 (Devereaux *et al., Nuc. Acids Res.* 12:387-395, 1984.

Another example of algorithms that are suitable for determining percent sequence identity and sequence similarity are the BLAST and the BLAST 2.0 algorithm, which are described in Altschul *et al., J. Mol. Biol.* 215:403-410, 1990 and Altschul *et al., Nucleic Acids Res.* 25:3389-3402, 1977. Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information (ncbi.nlm.nih.gov). The BLASTN program (for nucleotide sequences) uses as defaults a word length (W) of 11, alignments (B) of 50, expectation (E) of 10, M=5, N=-4, and a comparison of both strands. The BLASTP program (for amino acid sequences) uses as defaults a word length (W) of 3, and expectation (E) of 10, and the BLOSUM62 scoring matrix (see Henikoff & Henikoff, *Proc. Natl. Acad. Sci. USA* 89:10915, 1989). An oligonucleotide is a linear polynucleotide sequence of up to about 100 nucleotide bases in length.

Specifically bind: When referring to an antibody or antigen binding fragment, refers to a binding reaction which determines the presence of a target protein, peptide, or polysaccharide in the presence of a heterogeneous population of proteins and other biologics. Thus, under designated conditions, an antibody binds preferentially to a particular target protein, peptide or polysaccharide (such as an antigen present on the surface of a pathogen, for example influenza HA) and does not bind in a significant amount to other proteins or polysaccharides present in the sample or subject. Specific binding can be determined by methods known in the art. With reference to an antibody-antigen complex, specific binding of the antigen and antibody has a K_d of less than about 10^{-7} Molar, such as less than about 10^{-8} Molar, 10^{-9}, or even less than about 10^{-10} Molar.

K_d refers to the dissociation constant for a given interaction, such as a polypeptide ligand interaction or an antibody antigen interaction. For example, for the bimolecular interaction of an antibody or antigen binding fragment and an antigen it is the concentration of the individual components of the bimolecular interaction divided by the concentration of the complex.

The antibodies disclosed herein specifically bind to a defined target (or multiple targets, in the case of a bispecific antibody). Thus, an antibody that specifically binds to an epitope on influenza HA is an antibody that binds substantially to influenza HA, including cells or tissue expressing influenza HA, substrate to which the influenza HA is attached, or influenza HA in a biological specimen. It is, of course, recognized that a certain degree of non-specific interaction may occur between an antibody or conjugate including an antibody (such as an antibody that specifically binds influenza HA or conjugate including such antibody) and a non-target (such as a cell that does not express influenza HA). Typically, specific binding results in a much stronger association between the antibody and protein or cells bearing the antigen than between the antibody and protein or cells lacking the antigen. Specific binding typically results in greater than 2-fold, such as greater than 5-fold, greater than 10-fold, or greater than 100-fold increase in amount of bound antibody (per unit time) to a protein including the epitope or cell or tissue expressing the target epitope as compared to a protein or cell or tissue lacking this epitope. Specific binding to a protein under such conditions requires an antibody that is selected for its specificity for a particular protein. A variety of immunoassay formats are appropriate for selecting antibodies or other ligands specifically immunoreactive with a particular protein. For example, solid-phase ELISA immunoassays are routinely used to select monoclonal antibodies specifically immunoreactive with a protein. See Harlow & Lane, *Antibodies, A Laboratory Manual*, 2nd ed., Cold Spring Harbor Publications, New York (2013), for a description of immunoassay formats and conditions that can be used to determine specific immunoreactivity.

Subject: Living multi-cellular vertebrate organisms, a category that includes human and non-human mammals. In an example, a subject is a human. In a particular example, the subject is a newborn infant. In an additional example, a subject is selected that is in need of inhibiting of an influenza A infection. For example, the subject is either uninfected and at risk of influenza A infection or is infected in need of treatment.

Therapeutically effective amount: The amount of agent, such as a disclosed influenza HA specific antibody or antigen binding fragment that is sufficient to prevent, treat (including prophylaxis), reduce and/or ameliorate the symptoms and/or underlying causes of a disorder or disease, for example to prevent, inhibit, and/or treat influenza A infection. For instance, this can be the amount necessary to inhibit or prevent influenza A replication or to measurably alter outward symptoms of the influenza A infection. In general, this amount will be sufficient to measurably inhibit influenza A replication or infectivity. Ideally, a therapeutically effective amount provides a therapeutic effect without causing a substantial cytotoxic effect in the subject.

In some embodiments, administration of a therapeutically effective amount of a disclosed antibody or antigen binding fragment that binds to influenza HA can reduce or inhibit an influenza A infection (for example, as measured by infection of cells, or by number or percentage of subjects infected by influenza A, or by an increase in the survival time of infected subjects) by a desired amount, for example by at least 10%, at least 20%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, or even at least 100% (elimination or prevention of detectable influenza A infection), as compared to a suitable control.

Several preparations disclosed herein are administered in therapeutically effective amounts. A therapeutically effective amount of an antibody or antigen binding fragment that specifically binds influenza HA

that is administered to a subject will vary depending upon a number of factors associated with that subject, for example the overall health and/or weight of the subject. A therapeutically effective amount can be determined by varying the dosage and measuring the resulting therapeutic response, such as, for example, a reduction in viral titer. Therapeutically effective amounts also can be determined through various *in vitro*, *in vivo* or *in situ* immunoassays.

A therapeutically effective amount encompasses a fractional dose that contributes in combination with previous or subsequent administrations to attaining a therapeutic response. For example, a therapeutically effective amount of an agent can be administered in a single dose, or in several doses, for example daily, during a course of treatment lasting several days or weeks. However, the therapeutically effective amount can depend on the subject being treated, the severity and type of the condition being treated, and the manner of administration. A unit dosage form of the agent can be packaged in a therapeutic amount, or in multiples of the therapeutic amount, for example, in a vial (*e.g.*, with a pierceable lid) or syringe having sterile components.

Transformed: A transformed cell is a cell into which a nucleic acid molecule has been introduced by molecular biology techniques. As used herein, the term transformation encompasses all techniques by which a nucleic acid molecule might be introduced into such a cell, including transfection with viral vectors, transformation with plasmid vectors, and introduction of DNA by electroporation, lipofection, and particle gun acceleration.

Treating or preventing a disease: Inhibiting the full development of a disease or condition, for example, in a subject who is at risk of or has an influenza HA infection. "Treatment" refers to a therapeutic intervention that ameliorates a sign or symptom of a disease or pathological condition after it has begun to develop. The term "ameliorating," with reference to a disease or pathological condition, refers to any observable beneficial effect of the treatment. The beneficial effect can be evidenced, for example, by a delayed onset of clinical symptoms of the disease in a susceptible subject, a reduction in severity of some or all clinical symptoms of the disease, a slower progression of the disease, a reduction in the viral load, an improvement in the overall health or well-being of the subject, or by other parameters well known in the art that are specific to the particular disease. A "prophylactic" treatment is a treatment administered to a subject who does not exhibit signs of a disease for the purpose of reducing the risk of developing pathology.

The term "prevents" does not necessarily mean that an agent completely eliminates the disease or condition, so long as at least one characteristic of the disease or condition is eliminated. Thus, an antibody that inhibits or prevents an infection, can, but does not necessarily completely, eliminate such an infection, so long as the infection is measurably diminished, for example, by at least about 50%, such as by at least about 70%, or about 80%, or even by about 90% the infection in the absence of the agent, or in comparison to a reference agent.

Vector: Recombinant DNA vectors are vectors having recombinant DNA. A vector can include nucleic acid sequences that permit it to replicate in a host cell, such as an origin of replication. A vector can also include one or more selectable marker genes and other genetic elements known in the art. Viral vectors are recombinant nucleic acid vectors having at least some nucleic acid sequences derived from one or more viruses. In some embodiments, a viral vector is provided that comprises one or more nucleic acid molecules encoding a disclosed antibody or antigen binding fragment that specifically binds to influenza HA and neutralizes influenza A. In some embodiments, the viral vector can be an adeno-associated virus (AAV) vector. A replication deficient viral vector is a vector that requires complementation of one or more regions of the viral genome required for replication due to a deficiency in at least one replication-essential gene function. For example, such that the viral vector does not

replicate in typical host cells, especially those in a human patient that could be infected by the viral vector in the course of a therapeutic method.

Vaccine: An immunogenic composition that, when administered to a subject, induces an immune response that inhibits a disorder or disease, including prevention of the disease of disorder (such as a viral infection), or reduces the risk of the disease or disorder (such as the risk of contracting the viral infection). In one specific, non-limiting embodiment, a vaccine induces an immune response that inhibits infection by group 1 and group 2 influenza A viruses.

II. Description of Several Embodiments

A. Neutralizing Monoclonal Antibodies to Influenza AH and Antigen Binding Fragments Thereof

Isolated monoclonal antibodies and antigen binding fragments that specifically bind an epitope on influenza A HA protein are provided. The antibodies and antigen binding fragments can be fully human. The antibodies and antigen binding fragments can neutralize group 1 and group 2 influenza A viruses. Also disclosed herein are compositions including the antibodies and antigen binding fragments and a pharmaceutically acceptable carrier. Nucleic acids encoding the antibodies or antigen binding fragments, expression vectors (such as adeno-associated virus (AAV) viral vectors) including these nucleic acids are also provided. The antibodies, antigen binding fragments, nucleic acid molecules, host cells, and compositions can be used for research, diagnostic and therapeutic purposes. For example, the monoclonal antibodies and antigen binding fragments can be used to diagnose or treat a subject with an influenza A infection, or can be administered prophylactically to prevent influenza A infection in a subject.

In some embodiments, the antibodies and antigen binding fragments include a variable heavy (V_H) and a variable light (V_L) chain and specifically bind to influenza A HA protein and neutralize group 1 and group 2 influenza A viruses. In several embodiments, the antibodies and antigen binding fragments include a heavy chain comprising a heavy chain complementarity determining region (HCDR)1, a HCDR2 and an HCDR3, and a light chain comprising a light chain complementarity determining region (LCDR) 1, a LCDR2, and a LCDR3 and specifically bind to influenza A HA protein and optionally also neutralize group 1 and group 2 influenza A viruses. In several embodiments, the antibody or antigen binding fragment includes heavy and light chain variable regions including the HCDR1, HCDR2, and HCDR3, and LCDR1, LCDR2, and LCDR3, respectively, of one of the 54.f.01, 56.a.09, 01.k.01, 31.b.09, 16.g.07, 54.a.84, 16.a.26, 54.a.39, 31.a.83, 3150206_1A05, 3155305P_1A05, 3155305P _1B06, 315-09-1B12, 315-02-1E04, or 315-02-1E04 antibodies, and specifically binds to influenza HA protein and optionally also neutralizes group 1 and group 2 influenza A viruses.

The discussion of monoclonal antibodies below refers to isolated monoclonal antibodies that include heavy and/or light chain variable domains (or antigen binding fragments thereof) including a CDR1, CDR2, and/or CDR3 with reference to the IMGT numbering scheme (unless the context indicates otherwise). The person of ordinary skill in the art will understand that various CDR numbering schemes (such as the Kabat, Chothia or IMGT numbering schemes) can be used to determine CDR positions. The amino acid sequence and the CDR positions of the heavy and light chain of the 54.f.01, 56.a.09, 01.k.01, 31.b.09, 16.g.07, 54.a.84, 16.a.26, 54.a.39, 31.a.83, 3150206_1A05, 3155305P_1A05, 3155305P _1B06, 315-09-1B12, 315-02-1E04, and 315-02-1E04 monoclonal antibodies according to the IMGT numbering schemes are shown in Table 1 (IMGT).

Table 1. IMGT CDR sequences of HA specific antibodies

54.f.01 V_H			
V_H	SEQ ID NO: 1 residues	A.A. Sequence	CDR SEQ ID NO
HCDR1	26-34	GDSVSSHSA	19
HCDR2	52-58	TYYRSKWYS	20
HCDR3	97-110	VRGSFMIFGIVMAFDQ	21
54.f.01 V_L			
V_L	SEQ ID NO: 2 residues	A.A. Sequence	CDR SEQ ID NO
LCDR1	27-33	QSVSSSY	22
LCDR2	51-53	GTS	23
LCDR3	90-98	QQFDGSHFT	24
56.a.09 V_H			
V_H	SEQ ID NO: 3 residues	A.A. Sequence	CDR SEQ ID NO
HCDR1	26-35	GDTVSSNRAA	25
HCDR2	53-61	TYYRSKWYT	26
HCDR3	100-115	ARGSAMIFGIVIILES	27
56.a.09 V_L			
V_L	SEQ ID NO: 4 residues	A.A. Sequence	CDR SEQ ID NO
LCDR1	27-33	QSVASSY	28
LCDR2	51-53	GAS	29
LCDR3	90-98	QQYDGSQYT	30
01.k.01 V_H			
V_H	SEQ ID NO: 5 residues	A.A. Sequence	CDR SEQ ID NO
HCDR1	26-33	GYSFSSHG	31
HCDR2	51-58	ISAYNGHT	32
HCDR3	97-111	ARDRGNILTGCQFDY	33
01.k.01 V_L			
V_L	SEQ ID NO: 6 residues	A.A. Sequence	CDR SEQ ID NO
LCDR1	27-37	QGLVFIDGSTY	34
LCDR2	55-57	EIS	35
LCDR3	94-102	MQGTHWPIT	36
31.b.09 V_H			
V_H	SEQ ID NO: 7 Residues	A.A. Sequence	CDR SEQ ID NO
HCDR1	26-33	GYSFSSYG	37
HCDR2	51-58	ISAYNGNT	38
HCDR3	97-111	ARDRPHILTGFDFDY	39
31.b.09 V_L			
V_L	SEQ ID NO: 8 Residues	A.A. Sequence	CDR SEQ ID NO
LCDR1	27-37	QGLVYIDGNTY	40
LCDR2	55-57	NVF	41
LCDR3	94-102	MQGTHWPYT	42
16.g.07 V_H			
V_H	SEQ ID NO: 9 Residues	A.A. Sequence	CDR SEQ ID NO
HCDR1	26-33	GYTFTRFG	43
HCDR2	51-58	ISGYTGDT	44
HCDR3	98-117	VRNRVQMEVSPATQSTWYMDL	45

16.g.07 V_L			
V_L	SEQ ID NO: 10 Residues	A.A. Sequence	CDR SEQ ID NO
LCDR1	27-32	QDITRW	46
LCDR2	50-52	AAS	47
LCDR3	89-97	QQGSSFPYT	48
54.a.84 V_H			
V_H	SEQ ID NO: 11 Residues	A.A. Sequence	CDR SEQ ID NO
HCDR1	26-33	GYTFSRYG	49
HCDR2	51-58	ISAYTGDT	50
HCDR3	97-117	ARDRIQGVVALPKEQLNWFDP	51
54.a.84 V_L			
V_L	SEQ ID NO: 12 Residues	A.A. Sequence	CDR SEQ ID NO
LCDR1	27-32	HSISQF	52
LCDR2	50-52	GIS	53
LCDR3	89-97	QQGSNWPRT	54
16.a.26 V_H			
V_H	SEQ ID NO: 13 Residues	A.A. Sequence	CDR SEQ ID NO
HCDR1	26-33	GYSFSRYG	55
HCDR2	51-58	ISGYTGNT	56
HCDR3	97-117	ARDKKQGEVVLPAASFRWFAP	57
16.a.26 V_L			
V_L	SEQ ID NO: 14 Residues	A.A. Sequence	CDR SEQ ID NO
LCDR1	27-32	QSIGKF	58
LCDR2	50-52	YAS	59
LCDR3	89-97	QQSNNVPHT	60
54.a.39 V_H			
V_H	SEQ ID NO: 15 Residues	A.A. Sequence	CDR SEQ ID NO
HCDR1	26-33	GYTFTSYG	61
HCDR2	51-58	ISAYTGNT	62
HCDR3	97-117	ARDRIQGAVALPDKQVNWFDP	63
54.a.39 V_L			
V_L	SEQ ID NO: 16 Residues	A.A. Sequence	CDR SEQ ID NO
LCDR1	27-32	QSVSSY	64
LCDR2	50-52	GAS	29
LCDR3	89-97	QQHSNWPRT	65
31.a.83 V_H			
V_H	SEQ ID NO: 17 Residues	A.A. Sequence	CDR SEQ ID NO
HCDR1	26-33	GFTFRDSA	66
HCDR2	51-58	ISGNGGAT	67
HCDR3	97-120	AKDESPPIYNLMPGYYSTYYYMDV	68
31.a.83 V_L			
V_L	SEQ ID NO: 18 Residues	A.A. Sequence	CDR SEQ ID NO
LCDR1	27-32	QSVRSN	69
LCDR2	50-52	GAS	29
LCDR3	89-97	QQYNHWLRT	70
3150206_1A05 V_H			
V_H	SEQ ID NO: 71 Residues	A.A. Sequence	CDR SEQ ID NO
HCDR1	26-33	GYSLTGNY	101

HCDR2	51-58	INPKSGGT	102
HCDR3	97-114	ARDSGMRYFDWLSGYFDF	103
3150206_1A05 V_L			
V_L	SEQ ID NO: 72 Residues	A.A. Sequence	CDR SEQ ID NO
LCDR1	27-33	QVLTTNY	104
LCDR2	51-53	GAS	29
LCDR3	90-98	QVYDDLRVI	105
3155305_1A05 V_H			
V_H	SEQ ID NO: 73 Residues	A.A. Sequence	CDR SEQ ID NO
HCDR1	26-35	GDSVSSNSAA	106
HCDR2	53-61	TYYRSKWYN	107
HCDR3	100-116	ARAGIMIFGVIVGGLDV	108
3155305_1A05 V_L			
V_L	SEQ ID NO: 74 Residues	A.A. Sequence	CDR SEQ ID NO
LCDR1	27-32	QSISSY	109
LCDR2	50-52	GAS	29
LCDR3	89-98	QQSSTKPGYT	110
3155305_1B06 V_H			
V_H	SEQ ID NO: 75 Residues	A.A. Sequence	CDR SEQ ID NO
HCDR1	26-35	GDSVSSNSGA	111
HCDR2	53-61	TYYRSKWYN	107
HCDR3	100-116	ARAGVTVFGVVVGAMDV	112
3155305P_1B06 V_L			
V_L	SEQ ID NO: 76 Residues	A.A. Sequence	CDR SEQ ID NO
LCDR1	27-30	QYGS	113
LCDR2	48-50	SGS	114
LCDR3	87-91	QQYEF	115
315-53-1F12 V_H			
V_H	SEQ ID NO: 363 Residues	A.A. Sequence	CDR SEQ ID NO
HCDR1	26-35	GDTVSSNTAT	375
HCDR2	53-58	TYYRSKWYN	107
HCDR3	100-116	ARAGIRIFGLIVGGLDV	376
315-53-1F12 V_L			
V_L	SEQ ID NO: 364 Residues	A.A. Sequence	CDR SEQ ID NO
LCDR1	27-32	QSVSSY	64
LCDR2	50-52	ATS	377
LCDR3	89-98	QQSSTNPGYT	378
315-09-1B12 V_H			
V_H	SEQ ID NO: 365 Residues	A.A. Sequence	CDR SEQ ID NO
HCDR1	26-33	GYTFNTYG	379
HCDR2	51-61	ISAYTGNT	62
HCDR3	97-116	ARGLLQGAVILDSYHYALDF	380
315-09-1B12 V_L			
V_L	SEQ ID NO: 366 Residues	A.A. Sequence	CDR SEQ ID NO
LCDR1	27-33	QSVTNRF	381
LCDR2	51-53	GAS	29
LCDR3	90-99	QQYDTSPRWT	382

315-02-1E04 V_H			
V_H	SEQ ID NO: 367 Residues	A.A. Sequence	CDR SEQ ID NO
HCDR1	26-33	GYTFTNYG	383
HCDR2	51-61	ISAYNGHA	384
HCDR3	97-111	ARDRSNVLTGYLLDH	385
315-02-1E04 V_L			
V_L	SEQ ID NO: 368 Residues	A.A. Sequence	CDR SEQ ID NO
LCDR1	27-37	QGLVHIDGNIY	386
LCDR2	55-57	KVS	387
LCDR3	94-102	MQGTHRRLT	388

54.f.01

In some embodiments, the antibody or antigen binding fragment can be based on or derived from the 54.f.01 antibody, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. For example, the antibody or antigen binding fragment can comprise a V_H and a V_L comprising the HCDR1, the HCDR2, and the HCDR3, and the LCDR1, the LCDR2, and the LCDR3, respectively (for example, according to IMGT or kabat), of the 54.f.01 antibody, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses.

In some embodiments, the antibody or antigen binding fragment can comprise a V_H comprising the HCDR1, the HCDR2, and the HCDR3 of the 54.f.01 V_H as set forth in Table 1, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. In some embodiments, the antibody or antigen binding fragment can comprise a V_L comprising the LCDR1, the LCDR2, and the LCDR3 of the 54.f.01 V_L as set forth in Table 1, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. In some embodiments, the antibody or antigen binding fragment can comprise a V_H and a V_L comprising the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2, and the LCDR3 of the 54.f.01 V_H and V_L as set forth in Table 1, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses.

In some embodiments, the antibody or antigen binding fragment includes at least one CDR (such as an HCDR3) with a sequence that has at least 95% (such as at least 96%, at least 97%, at least 98%, at least 99%, or even 100%) sequence identity to any one of the heavy or light chain CDRs of the 54.f.01 V_H or V_L as shown in Table 1, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. In some embodiments, the antibody or antigen binding fragment includes a V_H comprising a HCDR1, a HCDR2, and a HCDR3 comprising amino acid sequences at least 90% (such as at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical to amino acids 26-34, 52-58, and 97-110, respectively, of SEQ ID NO: 1, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. In some embodiments, the antibody or antigen binding fragment includes a V_L comprising a LCDR1, a LCDR2, and a LCDR3 comprising amino acid sequences at least 90% (such as at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical to amino acids amino acids 27-33, 51-53, and 90-98, respectively, of SEQ ID NO: 2, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. In additional embodiments, the antibody or antigen binding fragment includes a V_H comprising a HCDR1, a HCDR2, and a HCDR3 comprising amino acid sequences at least 90% (such as at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical to amino acids 26-34, 52-58, and 97-110, respectively, of SEQ ID NO: 1, and a V_L comprising a LCDR1,

a LCDR2, and a LCDR3 comprising amino acid sequences at least 90% (such as at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical to amino acids amino acids 27-33, 51-53, and 90-98, respectively, of SEQ ID NO: 2, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses.

In some embodiments, the antibody or antigen binding fragment includes a V_H comprising an amino acid sequence at least 90% (such as at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical to the amino acid sequence set forth as SEQ ID NO: 1, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. In more embodiments, the antibody or antigen binding fragment includes a V_L comprising an amino acid sequence at least 90% (such as at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical to the amino acid sequence set forth as SEQ ID NO: 2, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. In additional embodiments, the antibody or antigen binding fragment includes a V_H and a V_L independently comprising amino acid sequences at least 90% (such as at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical to the amino acid sequences set forth as SEQ ID NOs: 1 and 2, respectively, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses.

In additional embodiments, the antibody or antigen binding fragment includes a V_H comprising the amino acid sequence set forth as one of SEQ ID NO: 1, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. In more embodiments, the antibody or antigen binding fragment includes a V_L comprising the amino acid sequence set forth as SEQ ID NO: 2, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. In some embodiments, the antibody or antigen binding fragment includes a V_H and a V_L comprising the amino acid sequences set forth as SEQ ID NOs: 1 and 2, respectively, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses.

56.a.09

In some embodiments, the antibody or antigen binding fragment can be based on or derived from the 56.a.09 antibody, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. For example, the antibody or antigen binding fragment can comprise a V_H and a V_L comprising the HCDR1, the HCDR2, and the HCDR3, and the LCDR1, the LCDR2, and the LCDR3, respectively (for example, according to IMGT or kabat), of the 56.a.09 antibody, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses.

In some embodiments, the antibody or antigen binding fragment can comprise a V_H comprising the HCDR1, the HCDR2, and the HCDR3 of the 56.a.09 V_H as set forth in Table 1, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. In some embodiments, the antibody or antigen binding fragment can comprise a V_L comprising the LCDR1, the LCDR2, and the LCDR3 of the 56.a.09 V_L as set forth in Table 1, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. In some embodiments, the antibody or antigen binding fragment can comprise a V_H and a V_L comprising the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2, and the LCDR3 of the 56.a.09 V_H and V_L as set forth in Table 1, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses.

In some embodiments, the antibody or antigen binding fragment includes at least one CDR (such as an HCDR3) with a sequence that has at least 95% (such as at least 96%, at least 97%, at least 98%, at least 99%, or

even 100%) sequence identity to any one of the heavy or light chain CDRs of the 56.a.09 V_H or V_L as shown in Table 1, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. In some embodiments, the antibody or antigen binding fragment includes a V_H comprising a HCDR1, a HCDR2, and a HCDR3 comprising amino acid sequences at least 90% (such as at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical to amino acids 26-35, 53-61, and 100-115, respectively, of SEQ ID NO: 3, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. In some embodiments, the antibody or antigen binding fragment includes a V_L comprising a LCDR1, a LCDR2, and a LCDR3 comprising amino acid sequences at least 90% (such as at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical to amino acids amino acids 27-33, 51-53, and 90-98, respectively, of SEQ ID NO: 4, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. In additional embodiments, the antibody or antigen binding fragment includes a V_H comprising a HCDR1, a HCDR2, and a HCDR3 comprising amino acid sequences at least 90% (such as at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical to amino 26-35, 53-61, and 100-115, respectively, of SEQ ID NO: 3, and a V_L comprising a LCDR1, a LCDR2, and a LCDR3 comprising amino acid sequences at least 90% (such as at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical to amino acids amino acids 27-33, 51-53, and 90-98, respectively, of SEQ ID NO: 4, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses.

In some embodiments, the antibody or antigen binding fragment includes a V_H comprising an amino acid sequence at least 90% (such as at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical to the amino acid sequence set forth as SEQ ID NO: 3, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. In more embodiments, the antibody or antigen binding fragment includes a V_L comprising an amino acid sequence at least 90% (such as at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical to the amino acid sequence set forth as SEQ ID NO: 4, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. In additional embodiments, the antibody or antigen binding fragment includes a V_H and a V_L independently comprising amino acid sequences at least 90% (such as at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical to the amino acid sequences set forth as SEQ ID NOs: 3 and 4, respectively, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses.

In additional embodiments, the antibody or antigen binding fragment includes a V_H comprising the amino acid sequence set forth as one of SEQ ID NO: 3, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. In more embodiments, the antibody or antigen binding fragment includes a V_L comprising the amino acid sequence set forth as SEQ ID NO: 4, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. In some embodiments, the antibody or antigen binding fragment includes a V_H and a V_L comprising the amino acid sequences set forth as SEQ ID NOs: 3 and 4, respectively, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses.

01.k.01

In some embodiments, the antibody or antigen binding fragment can be based on or derived from the 01.k.01 antibody, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. For example, the antibody or antigen binding fragment can comprise a V_H and a V_L comprising the HCDR1, the HCDR2, and the HCDR3, and the LCDR1, the LCDR2, and the LCDR3, respectively (for example,

according to IMGT or kabat), of the 01.k.01 antibody, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses.

In some embodiments, the antibody or antigen binding fragment can comprise a V_H comprising the HCDR1, the HCDR2, and the HCDR3 of the 01.k.01 V_H as set forth in Table 1, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. In some embodiments, the antibody or antigen binding fragment can comprise a V_L comprising the LCDR1, the LCDR2, and the LCDR3 of the 01.k.01 V_L as set forth in Table 1, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. In some embodiments, the antibody or antigen binding fragment can comprise a V_H and a V_L comprising the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2, and the LCDR3 of the 01.k.01 V_H and V_L as set forth in Table 1, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses.

In some embodiments, the antibody or antigen binding fragment includes at least one CDR (such as an HCDR3) with a sequence that has at least 95% (such as at least 96%, at least 97%, at least 98%, at least 99%, or even 100%) sequence identity to any one of the heavy or light chain CDRs of the 01.k.01 V_H or V_L as shown in Table 1, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. In some embodiments, the antibody or antigen binding fragment includes a V_H comprising a HCDR1, a HCDR2, and a HCDR3 comprising amino acid sequences at least 90% (such as at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical to amino acids 26-35, 51-58, and 97-111, respectively, of SEQ ID NO: 5, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. In some embodiments, the antibody or antigen binding fragment includes a V_L comprising a LCDR1, a LCDR2, and a LCDR3 comprising amino acid sequences at least 90% (such as at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical to amino acids amino acids 27-37, 55-57, and 94-102, respectively, of SEQ ID NO: 6, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. In additional embodiments, the antibody or antigen binding fragment includes a V_H comprising a HCDR1, a HCDR2, and a HCDR3 comprising amino acid sequences at least 90% (such as at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical to amino 26-35, 51-58, and 97-111, respectively, of SEQ ID NO: 5, and a V_L comprising a LCDR1, a LCDR2, and a LCDR3 comprising amino acid sequences at least 90% (such as at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical to amino acids amino acids 27-37, 55-57, and 94-102, respectively, of SEQ ID NO: 6, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses.

In some embodiments, the antibody or antigen binding fragment includes a V_H comprising an amino acid sequence at least 90% (such as at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical to the amino acid sequence set forth as SEQ ID NO: 5, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. In more embodiments, the antibody or antigen binding fragment includes a V_L comprising an amino acid sequence at least 90% (such as at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical to the amino acid sequence set forth as SEQ ID NO: 6, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. In additional embodiments, the antibody or antigen binding fragment includes a V_H and a V_L independently comprising amino acid sequences at least 90% (such as at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical to the amino acid sequences set forth as SEQ ID NOs: 5 and 6, respectively, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses.

In additional embodiments, the antibody or antigen binding fragment includes a V_H comprising the amino acid sequence set forth as one of SEQ ID NO: 5, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. In more embodiments, the antibody or antigen binding fragment includes a V_L comprising the amino acid sequence set forth as SEQ ID NO: 6, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. In some embodiments, the antibody or antigen binding fragment includes a V_H and a V_L comprising the amino acid sequences set forth as SEQ ID NOs: 5 and 6, respectively, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses.

31.b.09

In some embodiments, the antibody or antigen binding fragment can be based on or derived from the 31.b.09 antibody, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. For example, the antibody or antigen binding fragment can comprise a V_H and a V_L comprising the HCDR1, the HCDR2, and the HCDR3, and the LCDR1, the LCDR2, and the LCDR3, respectively (for example, according to IMGT or kabat), of the 31.b.09 antibody, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses.

In some embodiments, the antibody or antigen binding fragment can comprise a V_H comprising the HCDR1, the HCDR2, and the HCDR3 of the 31.b.09 V_H as set forth in Table 1, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. In some embodiments, the antibody or antigen binding fragment can comprise a V_L comprising the LCDR1, the LCDR2, and the LCDR3 of the 31.b.09 V_L as set forth in Table 1, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. In some embodiments, the antibody or antigen binding fragment can comprise a V_H and a V_L comprising the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2, and the LCDR3 of the 31.b.09 V_H and V_L as set forth in Table 1, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses.

In some embodiments, the antibody or antigen binding fragment includes at least one CDR (such as an HCDR3) with a sequence that has at least 95% (such as at least 96%, at least 97%, at least 98%, at least 99%, or even 100%) sequence identity to any one of the heavy or light chain CDRs of the 31.b.09 V_H or V_L as shown in Table 1, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. In some embodiments, the antibody or antigen binding fragment includes a V_H comprising a HCDR1, a HCDR2, and a HCDR3 comprising amino acid sequences at least 90% (such as at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical to amino acids 26-33, 51-58, and 97-111, respectively, of SEQ ID NO: 7, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. In some embodiments, the antibody or antigen binding fragment includes a V_L comprising a LCDR1, a LCDR2, and a LCDR3 comprising amino acid sequences at least 90% (such as at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical to amino acids amino acids 27-37, 55-57, and 94-102, respectively, of SEQ ID NO: 8, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. In additional embodiments, the antibody or antigen binding fragment includes a V_H comprising a HCDR1, a HCDR2, and a HCDR3 comprising amino acid sequences at least 90% (such as at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical to amino 26-33, 51-58, and 97-111, respectively, of SEQ ID NO: 7, and a V_L comprising a LCDR1, a LCDR2, and a LCDR3 comprising amino acid sequences at least 90% (such as at least 95%, at least 96%, at least

97%, at least 98%, or at least 99%) identical to amino acids amino acids 27-37, 55-57, and 94-102, respectively, of SEQ ID NO: 8, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses.

In some embodiments, the antibody or antigen binding fragment includes a V_H comprising an amino acid sequence at least 90% (such as at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical to the amino acid sequence set forth as SEQ ID NO: 7, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. In more embodiments, the antibody or antigen binding fragment includes a V_L comprising an amino acid sequence at least 90% (such as at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical to the amino acid sequence set forth as SEQ ID NO: 8, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. In additional embodiments, the antibody or antigen binding fragment includes a V_H and a V_L independently comprising amino acid sequences at least 90% (such as at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical to the amino acid sequences set forth as SEQ ID NOs: 7 and 8, respectively, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses.

In additional embodiments, the antibody or antigen binding fragment includes a V_H comprising the amino acid sequence set forth as one of SEQ ID NO: 7, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. In more embodiments, the antibody or antigen binding fragment includes a V_L comprising the amino acid sequence set forth as SEQ ID NO: 8, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. In some embodiments, the antibody or antigen binding fragment includes a V_H and a V_L comprising the amino acid sequences set forth as SEQ ID NOs: 7 and 8, respectively, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses.

16.g.07

In some embodiments, the antibody or antigen binding fragment can be based on or derived from the 16.g.07 antibody, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. For example, the antibody or antigen binding fragment can comprise a V_H and a V_L comprising the HCDR1, the HCDR2, and the HCDR3, and the LCDR1, the LCDR2, and the LCDR3, respectively (for example, according to IMGT or kabat), of the 16.g.07 antibody, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses.

In some embodiments, the antibody or antigen binding fragment can comprise a V_H comprising the HCDR1, the HCDR2, and the HCDR3 of the 16.g.07 V_H as set forth in Table 1, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. In some embodiments, the antibody or antigen binding fragment can comprise a V_L comprising the LCDR1, the LCDR2, and the LCDR3 of the 16.g.07 V_L as set forth in Table 1, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. In some embodiments, the antibody or antigen binding fragment can comprise a V_H and a V_L comprising the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2, and the LCDR3 of the 16.g.07 V_H and V_L as set forth in Table 1, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses.

In some embodiments, the antibody or antigen binding fragment includes at least one CDR (such as an HCDR3) with a sequence that has at least 95% (such as at least 96%, at least 97%, at least 98%, at least 99%, or even 100%) sequence identity to any one of the heavy or light chain CDRs of the 16.g.07 V_H or V_L as shown in

Table 1, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. In some embodiments, the antibody or antigen binding fragment includes a V_H comprising a HCDR1, a HCDR2, and a HCDR3 comprising amino acid sequences at least 90% (such as at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical to amino acids 26-33, 51-58, and 97-117, respectively, of SEQ ID NO: 9, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. In some embodiments, the antibody or antigen binding fragment includes a V_L comprising a LCDR1, a LCDR2, and a LCDR3 comprising amino acid sequences at least 90% (such as at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical to amino acids amino acids 27-32, 50-52, and 89-97, respectively, of SEQ ID NO: 10, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. In additional embodiments, the antibody or antigen binding fragment includes a V_H comprising a HCDR1, a HCDR2, and a HCDR3 comprising amino acid sequences at least 90% (such as at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical to amino 26-33, 51-58, and 97-117, respectively, of SEQ ID NO: 9, and a V_L comprising a LCDR1, a LCDR2, and a LCDR3 comprising amino acid sequences at least 90% (such as at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical to amino acids amino acids 27-32, 50-52, and 89-97, respectively, of SEQ ID NO: 10, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses.

In some embodiments, the antibody or antigen binding fragment includes a V_H comprising an amino acid sequence at least 90% (such as at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical to the amino acid sequence set forth as SEQ ID NO: 9, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. In more embodiments, the antibody or antigen binding fragment includes a V_L comprising an amino acid sequence at least 90% (such as at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical to the amino acid sequence set forth as SEQ ID NO: 10, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. In additional embodiments, the antibody or antigen binding fragment includes a V_H and a V_L independently comprising amino acid sequences at least 90% (such as at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical to the amino acid sequences set forth as SEQ ID NOs: 9 and 10, respectively, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses.

In additional embodiments, the antibody or antigen binding fragment includes a V_H comprising the amino acid sequence set forth as one of SEQ ID NO: 9, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. In more embodiments, the antibody or antigen binding fragment includes a V_L comprising the amino acid sequence set forth as SEQ ID NO: 10, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. In some embodiments, the antibody or antigen binding fragment includes a V_H and a V_L comprising the amino acid sequences set forth as SEQ ID NOs: 9 and 10, respectively, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses.

54.a.84

In some embodiments, the antibody or antigen binding fragment can be based on or derived from the 54.a.84 antibody, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. For example, the antibody or antigen binding fragment can comprise a V_H and a V_L comprising the HCDR1, the HCDR2, and the HCDR3, and the LCDR1, the LCDR2, and the LCDR3, respectively (for example,

according to IMGT or kabat), of the 54.a.84 antibody, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses.

In some embodiments, the antibody or antigen binding fragment can comprise a V_H comprising the HCDR1, the HCDR2, and the HCDR3 of the 54.a.84 V_H as set forth in Table 1, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. In some embodiments, the antibody or antigen binding fragment can comprise a V_L comprising the LCDR1, the LCDR2, and the LCDR3 of the 54.a.84 V_L as set forth in Table 1, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. In some embodiments, the antibody or antigen binding fragment can comprise a V_H and a V_L comprising the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2, and the LCDR3 of the 54.a.84 V_H and V_L as set forth in Table 1, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses.

In some embodiments, the antibody or antigen binding fragment includes at least one CDR (such as an HCDR3) with a sequence that has at least 95% (such as at least 96%, at least 97%, at least 98%, at least 99%, or even 100%) sequence identity to any one of the heavy or light chain CDRs of the 54.a.84 V_H or V_L as shown in Table 1, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. In some embodiments, the antibody or antigen binding fragment includes a V_H comprising a HCDR1, a HCDR2, and a HCDR3 comprising amino acid sequences at least 90% (such as at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical to amino acids 26-33, 51-58, and 97-117, respectively, of SEQ ID NO: 11, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. In some embodiments, the antibody or antigen binding fragment includes a V_L comprising a LCDR1, a LCDR2, and a LCDR3 comprising amino acid sequences at least 90% (such as at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical to amino acids amino acids 27-32, 50-52, and 89-97, respectively, of SEQ ID NO: 12, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. In additional embodiments, the antibody or antigen binding fragment includes a V_H comprising a HCDR1, a HCDR2, and a HCDR3 comprising amino acid sequences at least 90% (such as at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical to amino 26-33, 51-58, and 97-117, respectively, of SEQ ID NO: 11, and a V_L comprising a LCDR1, a LCDR2, and a LCDR3 comprising amino acid sequences at least 90% (such as at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical to amino acids amino acids 27-32, 50-52, and 89-97, respectively, of SEQ ID NO: 12, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses.

In some embodiments, the antibody or antigen binding fragment includes a V_H comprising an amino acid sequence at least 90% (such as at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical to the amino acid sequence set forth as SEQ ID NO: 11, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. In more embodiments, the antibody or antigen binding fragment includes a V_L comprising an amino acid sequence at least 90% (such as at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical to the amino acid sequence set forth as SEQ ID NO: 12, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. In additional embodiments, the antibody or antigen binding fragment includes a V_H and a V_L independently comprising amino acid sequences at least 90% (such as at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical to the amino acid

sequences set forth as SEQ ID NOs: 11 and 12, respectively, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses.

In additional embodiments, the antibody or antigen binding fragment includes a V_H comprising the amino acid sequence set forth as one of SEQ ID NO: 11, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. In more embodiments, the antibody or antigen binding fragment includes a V_L comprising the amino acid sequence set forth as SEQ ID NO: 12, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. In some embodiments, the antibody or antigen binding fragment includes a V_H and a V_L comprising the amino acid sequences set forth as SEQ ID NOs: 11 and 12, respectively, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses.

16.a.26

In some embodiments, the antibody or antigen binding fragment can be based on or derived from the 16.a.26 antibody, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. For example, the antibody or antigen binding fragment can comprise a V_H and a V_L comprising the HCDR1, the HCDR2, and the HCDR3, and the LCDR1, the LCDR2, and the LCDR3, respectively (for example, according to IMGT or kabat), of the 16.a.26 antibody, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses.

In some embodiments, the antibody or antigen binding fragment can comprise a V_H comprising the HCDR1, the HCDR2, and the HCDR3 of the 16.a.26 V_H as set forth in Table 1, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. In some embodiments, the antibody or antigen binding fragment can comprise a V_L comprising the LCDR1, the LCDR2, and the LCDR3 of the 16.a.26 V_L as set forth in Table 1, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. In some embodiments, the antibody or antigen binding fragment can comprise a V_H and a V_L comprising the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2, and the LCDR3 of the 16.a.26 V_H and V_L as set forth in Table 1, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses.

In some embodiments, the antibody or antigen binding fragment includes at least one CDR (such as an HCDR3) with a sequence that has at least 95% (such as at least 96%, at least 97%, at least 98%, at least 99%, or even 100%) sequence identity to any one of the heavy or light chain CDRs of the 16.a.26 V_H or V_L as shown in Table 1, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. In some embodiments, the antibody or antigen binding fragment includes a V_H comprising a HCDR1, a HCDR2, and a HCDR3 comprising amino acid sequences at least 90% (such as at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical to amino acids 26-33, 51-58, and 97-117, respectively, of SEQ ID NO: 13, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. In some embodiments, the antibody or antigen binding fragment includes a V_L comprising a LCDR1, a LCDR2, and a LCDR3 comprising amino acid sequences at least 90% (such as at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical to amino acids amino acids 27-32, 50-52, and 89-97, respectively, of SEQ ID NO: 14, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. In additional embodiments, the antibody or antigen binding fragment includes a V_H comprising a HCDR1, a HCDR2, and a HCDR3 comprising amino acid sequences at least 90% (such as at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%)

identical to amino 26-33, 51-58, and 97-117, respectively, of SEQ ID NO: 13, and a V_L comprising a LCDR1, a LCDR2, and a LCDR3 comprising amino acid sequences at least 90% (such as at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical to amino acids amino acids 27-32, 50-52, and 89-97, respectively, of SEQ ID NO: 14, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses.

In some embodiments, the antibody or antigen binding fragment includes a V_H comprising an amino acid sequence at least 90% (such as at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical to the amino acid sequence set forth as SEQ ID NO: 13, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. In more embodiments, the antibody or antigen binding fragment includes a V_L comprising an amino acid sequence at least 90% (such as at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical to the amino acid sequence set forth as SEQ ID NO: 14, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. In additional embodiments, the antibody or antigen binding fragment includes a V_H and a V_L independently comprising amino acid sequences at least 90% (such as at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical to the amino acid sequences set forth as SEQ ID NOs: 13 and 14, respectively, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses.

In additional embodiments, the antibody or antigen binding fragment includes a V_H comprising the amino acid sequence set forth as one of SEQ ID NO: 13, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. In more embodiments, the antibody or antigen binding fragment includes a V_L comprising the amino acid sequence set forth as SEQ ID NO: 14, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. In some embodiments, the antibody or antigen binding fragment includes a V_H and a V_L comprising the amino acid sequences set forth as SEQ ID NOs: 13 and 14, respectively, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses.

54.a.39

In some embodiments, the antibody or antigen binding fragment can be based on or derived from the 54.a.39 antibody, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. For example, the antibody or antigen binding fragment can comprise a V_H and a V_L comprising the HCDR1, the HCDR2, and the HCDR3, and the LCDR1, the LCDR2, and the LCDR3, respectively (for example, according to IMGT or kabat), of the 54.a.39 antibody, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses.

In some embodiments, the antibody or antigen binding fragment can comprise a V_H comprising the HCDR1, the HCDR2, and the HCDR3 of the 54.a.39 V_H as set forth in Table 1, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. In some embodiments, the antibody or antigen binding fragment can comprise a V_L comprising the LCDR1, the LCDR2, and the LCDR3 of the 54.a.39 V_L as set forth in Table 1, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. In some embodiments, the antibody or antigen binding fragment can comprise a V_H and a V_L comprising the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2, and the LCDR3 of the 54.a.39 V_H and V_L as set forth in Table 1, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses.

In some embodiments, the antibody or antigen binding fragment includes at least one CDR (such as an HCDR3) with a sequence that has at least 95% (such as at least 96%, at least 97%, at least 98%, at least 99%, or even 100%) sequence identity to any one of the heavy or light chain CDRs of the 54.a.39 V_H or V_L as shown in Table 1, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. In some embodiments, the antibody or antigen binding fragment includes a V_H comprising a HCDR1, a HCDR2, and a HCDR3 comprising amino acid sequences at least 90% (such as at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical to amino acids 26-33, 51-58, and 97-117, respectively, of SEQ ID NO: 15, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. In some embodiments, the antibody or antigen binding fragment includes a V_L comprising a LCDR1, a LCDR2, and a LCDR3 comprising amino acid sequences at least 90% (such as at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical to amino acids amino acids 27-32, 50-52, and 89-97, respectively, of SEQ ID NO: 16, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. In additional embodiments, the antibody or antigen binding fragment includes a V_H comprising a HCDR1, a HCDR2, and a HCDR3 comprising amino acid sequences at least 90% (such as at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical to amino 26-33, 51-58, and 97-117, respectively, of SEQ ID NO: 15, and a V_L comprising a LCDR1, a LCDR2, and a LCDR3 comprising amino acid sequences at least 90% (such as at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical to amino acids amino acids 27-32, 50-52, and 89-97, respectively, of SEQ ID NO: 16, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses.

In some embodiments, the antibody or antigen binding fragment includes a V_H comprising an amino acid sequence at least 90% (such as at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical to the amino acid sequence set forth as SEQ ID NO: 15, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. In more embodiments, the antibody or antigen binding fragment includes a V_L comprising an amino acid sequence at least 90% (such as at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical to the amino acid sequence set forth as SEQ ID NO: 16, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. In additional embodiments, the antibody or antigen binding fragment includes a V_H and a V_L independently comprising amino acid sequences at least 90% (such as at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical to the amino acid sequences set forth as SEQ ID NOs: 15 and 16, respectively, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses.

In additional embodiments, the antibody or antigen binding fragment includes a V_H comprising the amino acid sequence set forth as one of SEQ ID NO: 15, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. In more embodiments, the antibody or antigen binding fragment includes a V_L comprising the amino acid sequence set forth as SEQ ID NO: 16, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. In some embodiments, the antibody or antigen binding fragment includes a V_H and a V_L comprising the amino acid sequences set forth as SEQ ID NOs: 15 and 16, respectively, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses.

31.a.83

In some embodiments, the antibody or antigen binding fragment can be based on or derived from the 31.a.83 antibody, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. For example, the antibody or antigen binding fragment can comprise a V_H and a V_L comprising the HCDR1, the HCDR2, and the HCDR3, and the LCDR1, the LCDR2, and the LCDR3, respectively (for example, according to IMGT or kabat), of the 31.a.83 antibody, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses.

In some embodiments, the antibody or antigen binding fragment can comprise a V_H comprising the HCDR1, the HCDR2, and the HCDR3 of the 31.a.83 V_H as set forth in Table 1, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. In some embodiments, the antibody or antigen binding fragment can comprise a V_L comprising the LCDR1, the LCDR2, and the LCDR3 of the 31.a.83 V_L as set forth in Table 1, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. In some embodiments, the antibody or antigen binding fragment can comprise a V_H and a V_L comprising the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2, and the LCDR3 of the 31.a.83 V_H and V_L as set forth in Table 1, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses.

In some embodiments, the antibody or antigen binding fragment includes at least one CDR (such as an HCDR3) with a sequence that has at least 95% (such as at least 96%, at least 97%, at least 98%, at least 99%, or even 100%) sequence identity to any one of the heavy or light chain CDRs of the 31.a.83 V_H or V_L as shown in Table 1, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. In some embodiments, the antibody or antigen binding fragment includes a V_H comprising a HCDR1, a HCDR2, and a HCDR3 comprising amino acid sequences at least 90% (such as at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical to amino acids 26-33, 51-58, and 97-120, respectively, of SEQ ID NO: 17, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. In some embodiments, the antibody or antigen binding fragment includes a V_L comprising a LCDR1, a LCDR2, and a LCDR3 comprising amino acid sequences at least 90% (such as at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical to amino acids amino acids 27-32, 50-52, and 89-97, respectively, of SEQ ID NO: 18, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. In additional embodiments, the antibody or antigen binding fragment includes a V_H comprising a HCDR1, a HCDR2, and a HCDR3 comprising amino acid sequences at least 90% (such as at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical to amino 26-33, 51-58, and 97-120, respectively, of SEQ ID NO: 17, and a V_L comprising a LCDR1, a LCDR2, and a LCDR3 comprising amino acid sequences at least 90% (such as at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical to amino acids amino acids 27-32, 50-52, and 89-97, respectively, of SEQ ID NO: 18, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses.

In some embodiments, the antibody or antigen binding fragment includes a V_H comprising an amino acid sequence at least 90% (such as at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical to the amino acid sequence set forth as SEQ ID NO: 17, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. In more embodiments, the antibody or antigen binding fragment includes a V_L comprising an amino acid sequence at least 90% (such as at least 95%, at least 96%, at least 97%, at

least 98%, or at least 99%) identical to the amino acid sequence set forth as SEQ ID NO: 18, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. In additional embodiments, the antibody or antigen binding fragment includes a V_H and a V_L independently comprising amino acid sequences at least 90% (such as at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical to the amino acid sequences set forth as SEQ ID NOs: 17 and 18, respectively, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses.

In additional embodiments, the antibody or antigen binding fragment includes a V_H comprising the amino acid sequence set forth as one of SEQ ID NO: 17, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. In more embodiments, the antibody or antigen binding fragment includes a V_L comprising the amino acid sequence set forth as SEQ ID NO: 18, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. In some embodiments, the antibody or antigen binding fragment includes a V_H and a V_L comprising the amino acid sequences set forth as SEQ ID NOs: 17 and 18, respectively, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses.

3150206_1A05

In some embodiments, the antibody or antigen binding fragment can be based on or derived from the 3150206_1A05 antibody, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. For example, the antibody or antigen binding fragment can comprise a V_H and a V_L comprising the HCDR1, the HCDR2, and the HCDR3, and the LCDR1, the LCDR2, and the LCDR3, respectively (for example, according to IMGT or kabat), of the 3150206_1A05 antibody, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses.

In some embodiments, the antibody or antigen binding fragment can comprise a V_H comprising the HCDR1, the HCDR2, and the HCDR3 of the 3150206_1A05 V_H as set forth in Table 1, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. In some embodiments, the antibody or antigen binding fragment can comprise a V_L comprising the LCDR1, the LCDR2, and the LCDR3 of the 3150206_1A05 V_L as set forth in Table 1, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. In some embodiments, the antibody or antigen binding fragment can comprise a V_H and a V_L comprising the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2, and the LCDR3 of the 3150206_1A05 V_H and V_L as set forth in Table 1, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses.

In some embodiments, the antibody or antigen binding fragment includes at least one CDR (such as an HCDR3) with a sequence that has at least 95% (such as at least 96%, at least 97%, at least 98%, at least 99%, or even 100%) sequence identity to any one of the heavy or light chain CDRs of the 3150206_1A05 V_H or V_L as shown in Table 1, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. In some embodiments, the antibody or antigen binding fragment includes a V_H comprising a HCDR1, a HCDR2, and a HCDR3 comprising amino acid sequences at least 90% (such as at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical to amino acids 26-33, 51-58, and 97-114, respectively, of SEQ ID NO: 71, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. In some embodiments, the antibody or antigen binding fragment includes a V_L comprising a LCDR1, a LCDR2, and a LCDR3 comprising amino acid sequences at least 90% (such as at least 95%, at least 96%, at least 97%, at least

98%, or at least 99%) identical to amino acids amino acids 27-33, 51-53, and 90-98, respectively, of SEQ ID NO: 72, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. In additional embodiments, the antibody or antigen binding fragment includes a V_H comprising a HCDR1, a HCDR2, and a HCDR3 comprising amino acid sequences at least 90% (such as at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical to amino acids 26-33, 51-58, and 97-114, respectively, of SEQ ID NO: 71, and a V_L comprising a LCDR1, a LCDR2, and a LCDR3 comprising amino acid sequences at least 90% (such as at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical to amino acids amino acids 27-33, 51-53, and 90-98, respectively, of SEQ ID NO: 72, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses.

In some embodiments, the antibody or antigen binding fragment includes a V_H comprising an amino acid sequence at least 90% (such as at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical to the amino acid sequence set forth as SEQ ID NO: 71, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. In more embodiments, the antibody or antigen binding fragment includes a V_L comprising an amino acid sequence at least 90% (such as at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical to the amino acid sequence set forth as SEQ ID NO: 72, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. In additional embodiments, the antibody or antigen binding fragment includes a V_H and a V_L independently comprising amino acid sequences at least 90% (such as at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical to the amino acid sequences set forth as SEQ ID NOs: 71 and 72, respectively, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses.

In additional embodiments, the antibody or antigen binding fragment includes a V_H comprising the amino acid sequence set forth as one of SEQ ID NO: 71, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. In more embodiments, the antibody or antigen binding fragment includes a V_L comprising the amino acid sequence set forth as SEQ ID NO:72, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. In some embodiments, the antibody or antigen binding fragment includes a V_H and a V_L comprising the amino acid sequences set forth as SEQ ID NOs: 71 and 72, respectively, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses.

3155305P_1A05

In some embodiments, the antibody or antigen binding fragment can be based on or derived from the 3155305P_1A05 antibody, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. For example, the antibody or antigen binding fragment can comprise a V_H and a V_L comprising the HCDR1, the HCDR2, and the HCDR3, and the LCDR1, the LCDR2, and the LCDR3, respectively (for example, according to IMGT or kabat), of the 3155305P_1A05 antibody, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses.

In some embodiments, the antibody or antigen binding fragment can comprise a V_H comprising the HCDR1, the HCDR2, and the HCDR3 of the 3155305P_1A05 V_H as set forth in Table 1, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. In some embodiments, the antibody or antigen binding fragment can comprise a V_L comprising the LCDR1, the LCDR2, and the LCDR3 of the 3155305P_1A05 V_L as set forth in Table 1, and can specifically bind to influenza HA protein and neutralize group

1 and 2 influenza A viruses. In some embodiments, the antibody or antigen binding fragment can comprise a V_H and a V_L comprising the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2, and the LCDR3 of the 3155305P_1A05 V_H and V_L as set forth in Table 1, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses.

In some embodiments, the antibody or antigen binding fragment includes at least one CDR (such as an HCDR3) with a sequence that has at least 95% (such as at least 96%, at least 97%, at least 98%, at least 99%, or even 100%) sequence identity to any one of the heavy or light chain CDRs of the 3155305P_1A05 V_H or V_L as shown in Table 1, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. In some embodiments, the antibody or antigen binding fragment includes a V_H comprising a HCDR1, a HCDR2, and a HCDR3 comprising amino acid sequences at least 90% (such as at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical to amino acids 26-35, 53-61, and 100-116, respectively, of SEQ ID NO: 73, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. In some embodiments, the antibody or antigen binding fragment includes a V_L comprising a LCDR1, a LCDR2, and a LCDR3 comprising amino acid sequences at least 90% (such as at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical to amino acids amino acids 27-32, 50-52, and 89-98, respectively, of SEQ ID NO: 74, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. In additional embodiments, the antibody or antigen binding fragment includes a V_H comprising a HCDR1, a HCDR2, and a HCDR3 comprising amino acid sequences at least 90% (such as at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical to amino acids 26-35, 53-61, and 100-116, respectively, of SEQ ID NO: 73, and a V_L comprising a LCDR1, a LCDR2, and a LCDR3 comprising amino acid sequences at least 90% (such as at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical to amino acids amino acids 27-32, 50-52, and 89-98, respectively, of SEQ ID NO: 74, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses.

In some embodiments, the antibody or antigen binding fragment includes a V_H comprising an amino acid sequence at least 90% (such as at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical to the amino acid sequence set forth as SEQ ID NO: 73, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. In more embodiments, the antibody or antigen binding fragment includes a V_L comprising an amino acid sequence at least 90% (such as at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical to the amino acid sequence set forth as SEQ ID NO: 74, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. In additional embodiments, the antibody or antigen binding fragment includes a V_H and a V_L independently comprising amino acid sequences at least 90% (such as at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical to the amino acid sequences set forth as SEQ ID NOs: 73 and 74, respectively, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses.

In additional embodiments, the antibody or antigen binding fragment includes a V_H comprising the amino acid sequence set forth as one of SEQ ID NO: 73, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. In more embodiments, the antibody or antigen binding fragment includes a V_L comprising the amino acid sequence set forth as SEQ ID NO: 74, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. In some embodiments, the antibody or antigen binding fragment

includes a V_H and a V_L comprising the amino acid sequences set forth as SEQ ID NOs: 73 and 74, respectively, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses.

3155305P_1B06

In some embodiments, the antibody or antigen binding fragment can be based on or derived from the 3155305P_1B06 antibody, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. For example, the antibody or antigen binding fragment can comprise a V_H and a V_L comprising the HCDR1, the HCDR2, and the HCDR3, and the LCDR1, the LCDR2, and the LCDR3, respectively (for example, according to IMGT or kabat), of the 3155305P_1B06 antibody, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses.

In some embodiments, the antibody or antigen binding fragment can comprise a V_H comprising the HCDR1, the HCDR2, and the HCDR3 of the 3155305P_1B06 V_H as set forth in Table 1, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. In some embodiments, the antibody or antigen binding fragment can comprise a V_L comprising the LCDR1, the LCDR2, and the LCDR3 of the 3155305P_1B06 V_L as set forth in Table 1, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. In some embodiments, the antibody or antigen binding fragment can comprise a V_H and a V_L comprising the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2, and the LCDR3 of the 3155305P_1B06 V_H and V_L as set forth in Table 1, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses.

In some embodiments, the antibody or antigen binding fragment includes at least one CDR (such as an HCDR3) with a sequence that has at least 95% (such as at least 96%, at least 97%, at least 98%, at least 99%, or even 100%) sequence identity to any one of the heavy or light chain CDRs of the 3155305P_1B06 V_H or V_L as shown in Table 1, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. In some embodiments, the antibody or antigen binding fragment includes a V_H comprising a HCDR1, a HCDR2, and a HCDR3 comprising amino acid sequences at least 90% (such as at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical to amino acids 26-35, 53-61, and 100-116, respectively, of SEQ ID NO: 75, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. In some embodiments, the antibody or antigen binding fragment includes a V_L comprising a LCDR1, a LCDR2, and a LCDR3 comprising amino acid sequences at least 90% (such as at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical to amino acids amino acids 27-30, 48-50, and 87-91, respectively, of SEQ ID NO: 76, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. In additional embodiments, the antibody or antigen binding fragment includes a V_H comprising a HCDR1, a HCDR2, and a HCDR3 comprising amino acid sequences at least 90% (such as at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical to amino acids 26-35, 53-61, and 100-116, respectively, of SEQ ID NO: 75, and a V_L comprising a LCDR1, a LCDR2, and a LCDR3 comprising amino acid sequences at least 90% (such as at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical to amino acids amino acids 27-30, 48-50, and 87-91, respectively, of SEQ ID NO: 76, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses.

In some embodiments, the antibody or antigen binding fragment includes a V_H comprising an amino acid sequence at least 90% (such as at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical to

the amino acid sequence set forth as SEQ ID NO: 75, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. In more embodiments, the antibody or antigen binding fragment includes a V_L comprising an amino acid sequence at least 90% (such as at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical to the amino acid sequence set forth as SEQ ID NO: 76, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. In additional embodiments, the antibody or antigen binding fragment includes a V_H and a V_L independently comprising amino acid sequences at least 90% (such as at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical to the amino acid sequences set forth as SEQ ID NOs: 75 and 76, respectively, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses.

In additional embodiments, the antibody or antigen binding fragment includes a V_H comprising the amino acid sequence set forth as one of SEQ ID NO: 75, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. In more embodiments, the antibody or antigen binding fragment includes a V_L comprising the amino acid sequence set forth as SEQ ID NO: 76, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. In some embodiments, the antibody or antigen binding fragment includes a V_H and a V_L comprising the amino acid sequences set forth as SEQ ID NOs: 75 and 76, respectively, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses.

315-53-1F12

In some embodiments, the antibody or antigen binding fragment can be based on or derived from the 315-53-1F12 antibody, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. For example, the antibody or antigen binding fragment can comprise a V_H and a V_L comprising the HCDR1, the HCDR2, and the HCDR3, and the LCDR1, the LCDR2, and the LCDR3, respectively (for example, according to IMGT or kabat), of the 315-53-1F12 antibody, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses.

In some embodiments, the antibody or antigen binding fragment can comprise a V_H comprising the HCDR1, the HCDR2, and the HCDR3 of the 315-53-1F12 V_H as set forth in Table 1, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. In some embodiments, the antibody or antigen binding fragment can comprise a V_L comprising the LCDR1, the LCDR2, and the LCDR3 of the 315-53-1F12 V_L as set forth in Table 1, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. In some embodiments, the antibody or antigen binding fragment can comprise a V_H and a V_L comprising the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2, and the LCDR3 of the 315-53-1F12 V_H and V_L as set forth in Table 1, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses.

In some embodiments, the antibody or antigen binding fragment includes at least one CDR (such as an HCDR3) with a sequence that has at least 95% (such as at least 96%, at least 97%, at least 98%, at least 99%, or even 100%) sequence identity to any one of the heavy or light chain CDRs of the 315-53-1F12 V_H or V_L as shown in Table 1, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. In some embodiments, the antibody or antigen binding fragment includes a V_H comprising a HCDR1, a HCDR2, and a HCDR3 comprising amino acid sequences at least 90% (such as at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical to amino acids 26-35, 53-58, and 100-116, respectively, of SEQ ID NO: 363, and

can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. In some embodiments, the antibody or antigen binding fragment includes a V_L comprising a LCDR1, a LCDR2, and a LCDR3 comprising amino acid sequences at least 90% (such as at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical to amino acids amino acids 27-32, 50-52, and 89-98, respectively, of SEQ ID NO: 364, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. In additional embodiments, the antibody or antigen binding fragment includes a V_H comprising a HCDR1, a HCDR2, and a HCDR3 comprising amino acid sequences at least 90% (such as at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical to amino 26-35, 53-58, and 100-116, respectively, of SEQ ID NO: 363, and a V_L comprising a LCDR1, a LCDR2, and a LCDR3 comprising amino acid sequences at least 90% (such as at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical to amino acids amino acids 27-32, 50-52, and 89-98, respectively, of SEQ ID NO: 364, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses.

In some embodiments, the antibody or antigen binding fragment includes a V_H comprising an amino acid sequence at least 90% (such as at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical to the amino acid sequence set forth as SEQ ID NO: 363, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. In more embodiments, the antibody or antigen binding fragment includes a V_L comprising an amino acid sequence at least 90% (such as at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical to the amino acid sequence set forth as SEQ ID NO: 364, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. In additional embodiments, the antibody or antigen binding fragment includes a V_H and a V_L independently comprising amino acid sequences at least 90% (such as at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical to the amino acid sequences set forth as SEQ ID NOs: 363 and 364, respectively, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses.

In additional embodiments, the antibody or antigen binding fragment includes a V_H comprising the amino acid sequence set forth as one of SEQ ID NO: 363, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. In more embodiments, the antibody or antigen binding fragment includes a V_L comprising the amino acid sequence set forth as SEQ ID NO: 364, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. In some embodiments, the antibody or antigen binding fragment includes a V_H and a V_L comprising the amino acid sequences set forth as SEQ ID NOs: 363 and 364, respectively, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses.

315-09-1B12

In some embodiments, the antibody or antigen binding fragment can be based on or derived from the 315-09-1B12 antibody, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. For example, the antibody or antigen binding fragment can comprise a V_H and a V_L comprising the HCDR1, the HCDR2, and the HCDR3, and the LCDR1, the LCDR2, and the LCDR3, respectively (for example, according to IMGT or kabat), of the 315-09-1B12 antibody, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses.

In some embodiments, the antibody or antigen binding fragment can comprise a V_H comprising the HCDR1, the HCDR2, and the HCDR3 of the 315-09-1B12 V_H as set forth in Table 1, and can specifically bind to

influenza HA protein and neutralize group 1 and 2 influenza A viruses. In some embodiments, the antibody or antigen binding fragment can comprise a V_L comprising the LCDR1, the LCDR2, and the LCDR3 of the 315-09-1B12 V_L as set forth in Table 1, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. In some embodiments, the antibody or antigen binding fragment can comprise a V_H and a V_L comprising the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2, and the LCDR3 of the 315-09-1B12 V_H and V_L as set forth in Table 1, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses.

In some embodiments, the antibody or antigen binding fragment includes at least one CDR (such as an HCDR3) with a sequence that has at least 95% (such as at least 96%, at least 97%, at least 98%, at least 99%, or even 100%) sequence identity to any one of the heavy or light chain CDRs of the 315-09-1B12 V_H or V_L as shown in Table 1, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. In some embodiments, the antibody or antigen binding fragment includes a V_H comprising a HCDR1, a HCDR2, and a HCDR3 comprising amino acid sequences at least 90% (such as at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical to amino acids 26-33, 51-61, and 97-116, respectively, of SEQ ID NO: 365, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. In some embodiments, the antibody or antigen binding fragment includes a V_L comprising a LCDR1, a LCDR2, and a LCDR3 comprising amino acid sequences at least 90% (such as at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical to amino acids amino acids 27-33, 51-53, and 90-99, respectively, of SEQ ID NO: 366, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. In additional embodiments, the antibody or antigen binding fragment includes a V_H comprising a HCDR1, a HCDR2, and a HCDR3 comprising amino acid sequences at least 90% (such as at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical to amino 26-33, 51-61, and 97-116, respectively, of SEQ ID NO: 365, and a V_L comprising a LCDR1, a LCDR2, and a LCDR3 comprising amino acid sequences at least 90% (such as at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical to amino acids amino acids 27-33, 51-53, and 90-99, respectively, of SEQ ID NO: 366, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses.

In some embodiments, the antibody or antigen binding fragment includes a V_H comprising an amino acid sequence at least 90% (such as at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical to the amino acid sequence set forth as SEQ ID NO: 365, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. In more embodiments, the antibody or antigen binding fragment includes a V_L comprising an amino acid sequence at least 90% (such as at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical to the amino acid sequence set forth as SEQ ID NO: 366, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. In additional embodiments, the antibody or antigen binding fragment includes a V_H and a V_L independently comprising amino acid sequences at least 90% (such as at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical to the amino acid sequences set forth as SEQ ID NOs: 365 and 366, respectively, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses.

In additional embodiments, the antibody or antigen binding fragment includes a V_H comprising the amino acid sequence set forth as one of SEQ ID NO: 365, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. In more embodiments, the antibody or antigen binding fragment includes a V_L

comprising the amino acid sequence set forth as SEQ ID NO: 366, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. In some embodiments, the antibody or antigen binding fragment includes a V_H and a V_L comprising the amino acid sequences set forth as SEQ ID NOs: 365 and 366, respectively, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses.

315-02-1E04

In some embodiments, the antibody or antigen binding fragment can be based on or derived from the 315-02-1E04 antibody, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. For example, the antibody or antigen binding fragment can comprise a V_H and a V_L comprising the HCDR1, the HCDR2, and the HCDR3, and the LCDR1, the LCDR2, and the LCDR3, respectively (for example, according to IMGT or kabat), of the 315-02-1E04 antibody, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses.

In some embodiments, the antibody or antigen binding fragment can comprise a V_H comprising the HCDR1, the HCDR2, and the HCDR3 of the 315-02-1E04 V_H as set forth in Table 1, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. In some embodiments, the antibody or antigen binding fragment can comprise a V_L comprising the LCDR1, the LCDR2, and the LCDR3 of the 315-02-1E04 V_L as set forth in Table 1, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. In some embodiments, the antibody or antigen binding fragment can comprise a V_H and a V_L comprising the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2, and the LCDR3 of the 315-02-1E04 V_H and V_L as set forth in Table 1, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses.

In some embodiments, the antibody or antigen binding fragment includes at least one CDR (such as an HCDR3) with a sequence that has at least 95% (such as at least 96%, at least 97%, at least 98%, at least 99%, or even 100%) sequence identity to any one of the heavy or light chain CDRs of the 315-02-1E04 V_H or V_L as shown in Table 1, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. In some embodiments, the antibody or antigen binding fragment includes a V_H comprising a HCDR1, a HCDR2, and a HCDR3 comprising amino acid sequences at least 90% (such as at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical to amino acids 26-33, 51-61, and 97-111, respectively, of SEQ ID NO: 367, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. In some embodiments, the antibody or antigen binding fragment includes a V_L comprising a LCDR1, a LCDR2, and a LCDR3 comprising amino acid sequences at least 90% (such as at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical to amino acids amino acids 27-37, 55-57, and 94-102, respectively, of SEQ ID NO: 368, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. In additional embodiments, the antibody or antigen binding fragment includes a V_H comprising a HCDR1, a HCDR2, and a HCDR3 comprising amino acid sequences at least 90% (such as at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical to amino 26-33, 51-61, and 97-111, respectively, of SEQ ID NO: 367, and a V_L comprising a LCDR1, a LCDR2, and a LCDR3 comprising amino acid sequences at least 90% (such as at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical to amino acids amino acids 27-37, 55-57, and 94-102, respectively, of SEQ ID NO: 368, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses.

In some embodiments, the antibody or antigen binding fragment includes a V_H comprising an amino acid sequence at least 90% (such as at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical to the amino acid sequence set forth as SEQ ID NO: 367, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. In more embodiments, the antibody or antigen binding fragment includes a V_L comprising an amino acid sequence at least 90% (such as at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical to the amino acid sequence set forth as SEQ ID NO: 368, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. In additional embodiments, the antibody or antigen binding fragment includes a V_H and a V_L independently comprising amino acid sequences at least 90% (such as at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical to the amino acid sequences set forth as SEQ ID NOs: 367 and 368, respectively, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses.

In additional embodiments, the antibody or antigen binding fragment includes a V_H comprising the amino acid sequence set forth as one of SEQ ID NO: 367, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. In more embodiments, the antibody or antigen binding fragment includes a V_L comprising the amino acid sequence set forth as SEQ ID NO: 368, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. In some embodiments, the antibody or antigen binding fragment includes a V_H and a V_L comprising the amino acid sequences set forth as SEQ ID NOs: 367 and 368, respectively, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses.

HV6-1/HD3-36 class consensus

The sequence similarity and structural information disclosed herein for the HV6-1/HD3-36 class of antibodies allows elucidation of a consensus sequence of such antibodies that retains binding affinity for influenza HA and neutralizes group 1 and group 2 influenza A viruses. Accordingly, in several embodiments, an isolated antibody or antigen binding fragment is provided that comprises a heavy chain variable region comprising a HCDR1, a HCDR2, and a HCDR3, and a light chain variable region comprising a LCDR1, a LCDR2, and a LCDR3, wherein the heavy chain variable region comprises the following by kabat positioning: a Gln at position 1; a Val at position 2; a Gly at position 26; an Asp at position 27; a Asn, His or Tyr at position 32; a Ser at position 33; an Ala, Ser or Thr, or no residue at position 35; an Arg at position 50; a Tyr at position 52; an Arg at position 52b; a Ser at position 53; a Tyr at position 56; an Asp at position 58; an Arg at position 94; an Ala, Phe, Val, or Pro at position 97; a Met at position 98; an Ile at position 99; a Phe at position 100; a Gly at position 100a; an Ile or Val at position 100b; a Val, Leu, or Asp at position 100c; and a Met, Ile, or Val at position 100d; and the light chain variable region comprises the following by kabat positioning: a Glu at position 1; a Gln or Arg at position 27; a Ser at position 27a; an Ala, Ser, Gly, or Val at position 29; an Arg or Ser at position 30; a Val or Ser at position 31; a Tyr at position 32; a Phe, Tyr, or Lys at position 49; a Ser, Thr, Pro, or Asn at position 56; a Tyr, Phe, or His at positon 91; an Asp or Gly at position 92; a Gly, Ser, or Val at position 93; a Ser at position 94; and a Phe or Tyr at position 96. The antibody or antigen binding fragment specifically binds to influenza A HA protein and neutralizes group 1 and group 2 influenza A viruses.

In some such embodiments, the light chain variable region comprises the following by kabat positioning: a Glu at position 1; a Gln or Arg at position 27; a Ser at position 27a; an Ala, Ser, or Gly at position 29; a Ser at position 30; a Ser at position 31; a Tyr at position 32; a Phe or Tyr at position 49; a Ser, Thr, Pro, or Asn at position

56; a Tyr or Phe at positon 91; an Asp or Gly at position 92; a Gly or Ser at position 93; a Ser at position 94; and a Phe or Tyr at position 96.

In some embodiments, the HCDR1 comprises the amino acid sequence set forth as $GDX_1VSSX_2SAX_3$ (SEQ ID NO: 116); the HCDR2 comprises the amino acid sequence set forth as $TYYRSX_4WYX_5$ (SEQ ID NO: 117); the HCDR3 comprises the amino acid sequence set forth as $ARX_6SX_7MIFGX_8X_9X_{10}X_{11}X_{12}X_{13}X_{14}X_{15}$ (SEQ ID NO: 118); the LCDR1 comprises the amino acid sequence set forth as $X_{16}SVX_{17}X_{18}X_{19}Y$ (SEQ ID NO: 119); the LCDR2 comprises the amino acid sequence set forth as $GX_{20}S$ (SEQ ID NO: 120); the LCDR3 comprises the amino acid sequence set forth as $QX_{21}X_{22}X_{23}X_{24}SX_{25}X_{26}T$ (SEQ ID NO: 121). The variable positions are defined as follows: X_1 is Ser or Thr; X_2 is Asn, His or Tyr; X_3 is Ala, Ser, Thr, or no amino acid; X_4 is Lys or Arg; X_5 is Ser, Asn, Thr, Tyr, Gly, Ser, or Thr; X_6 is Gly, Ala, or Val; X_7 is Ala, Phe, Val, or Pro; X_8 is Ile or Val; X_9 is Val, Leu, or Asp; X_{10} is Met, Ile, or Val; X_{11} is Gly or no amino acid; X_{12} is Ala, Ile, Val, or Glu; X_{13} is Phe, Leu, or Met; X_{14} is Asp or Glu; X_{15} is Gln, Phe, Cys, Ser, Tyr, or Leu; X_{16} is Gln or Arg; X_{17} is Ala, Ser, Gly, or Val; X_{18} is Ser or Arg; X_{19} is Ser or Val; X_{20} is Ala, Val, or Thr; X_{21} is Gln or Arg; X_{22} is Tyr, Phe, or His; X_{23} is Asp or Gly; X_{24} is Gly, Ser, or Val; X_{25} is Gln, His, or Arg; and X_{26} is Tyr or Phe.

In some such embodiments, the heavy chain variable region or the antibody or antigen binding fragment comprises a germline origin of HV6-1, HD3-3, and HJ4 or HJ5 genes, and the light chain variable region of the antibody or antigen binding fragment comprises a germline origin of KV3-20 and KJ4 or KJ5 genes.

HV1-18/HD3-9 class consensus

The sequence similarity and structural information disclosed herein for the HV1-18/HD3-9 class of antibodies allows elucidation of a consensus sequence of such antibodies that retains binding affinity for influenza HA and neutralizes group 1 and group 2 influenza A viruses. Accordingly, in several embodiments, an isolated antibody or antigen binding fragment is provided that comprises a heavy chain variable region comprising a HCDR1, a HCDR2, and a HCDR3, and a light chain variable region comprising a LCDR1, a LCDR2, and a LCDR3, wherein the heavy chain variable region comprises the following by kabat positioning: a Trp at position 50; a Tyr at position 53; an Asn at position 54; an Ala or Gly at position 55; a Asn or His at position 56; an Asn or Gln at position 58; an Ala, Asp, His, Asn, Thr at position 98; an Ile at position 99; a Leu at position 100; and a Thr at position 100a; and the light chain variable region comprises the following by kabat positioning a Gln at position 27; a Gly at position 27a; a Leu at position 27b; a Leu or Val at position 27c; a Phe, His, or Tyr at position 27d; an Ile at position 27e; a Asp at position 28; a Gly at position 29; a Thr at position 31; an Asn, Glu, His, or Lys at position 50; a Val or Ile at position 51; a Ser or Phe at position 52; a Ser at position 65; a Gly at position 66; a Ser at position 67; a Gly at position 68; a Thr at position 92; a His or Tyr at position 93; a Trp at position 94. The antibody or antigen binding fragment specifically binds to influenza A HA protein and neutralizes group 1 and group 2 influenza A viruses.

In some embodiments, the HCDR1 comprises the amino acid sequence set forth as $GYX_1FX_2X_3X_4G$ (SEQ ID NO: 122); the HCDR2 comprises the amino acid sequence set forth as $X_5SX_6YNX_7X_8X_9$ (SEQ ID NO: 123); the HCDR3 comprises the amino acid sequence set forth as $X_{10}RDX_{11}X_{12}X_{13}ILTGX_{14}X_{15}X_{16}DX_{17}$ (SEQ ID NO: 124); the LCDR1 comprises the amino acid sequence set forth as $QGLX_{18}X_{19}IDGX_{20}X_{21}Y$ (SEQ ID NO: 125); the LCDR2 comprises the amino acid sequence set forth as $X_{22}X_{23}X_{24}$ (SEQ ID NO: 126); and/or the LCDR3 comprises the amino acid sequence set forth as $X_{25}QGTX_{26}WPX_{27}T$ (SEQ ID NO: 127). The variable positions are

defined as follows: X_1 is Arg, Thr, Asp, or Ser; X_2 is Ser, Thr, or Asn; X_3 is Ser, Thr, or Asn; X_4 is Tyr, Ser, Phe, or His; X_5 is Ile or Val; X_6 is Ala or Gly; X_7 is Gly or Ala; X_8 is His or Asn; X_9 is Thr or Ile; X_{10} is Ala or Thr; X_{11} is Gln or Arg; X_{12} is Arg, Tyr, Gly, Phe, Ser, or Pro; X_{13} is Asp, Ala, Asn, Thr, or His; X_{14} is Gly, Pro, Ser, Tyr, Asp, Phe, or Cys; X_{15} is Leu, Asn, Ala, Arg, Asp, His, or Gln; X_{16} is Phe, Gly, Thr, Asp, or Leu; X_{17} is Cys, Tyr, His, Asp, Phe, Ser, or Ile; X_{18} is Val or Leu; X_{19} is His, Tyr, or Phe; X_{20} is Asn or Ser; X_{21} is Thr or Ile; X_{22} is Asn, His, Lys, or Glu; X_{23} is Val, or Ile; X_{24} is Phe or Ser; X_{25} is Met or Leu; X_{26} is Tyr or His; and X_{27} is Tyr, Leu, Arg, or Ile.

In some such embodiments, the heavy chain variable region or the antibody or antigen binding fragment comprises a germline origin of HV1-18, HD3-9, and HJ4 genes, and the light chain variable region comprises a germline origin of KV2-30 and KJ2 or KJ5 genes.

Reference to FIG. 20

In some embodiments, the antibody or antigen binding fragment can be based on or derived from any one of the antibodies shown in FIGs. 20A-20G, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. For example, the antibody or antigen binding fragment can comprise a V_H and a V_L comprising the HCDR1, the HCDR2, and the HCDR3, and the LCDR1, the LCDR2, and the LCDR3, respectively (for example, according to IMGT or kabat), of any one of the antibodies shown in FIGs. 20A-20G, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses.

In some embodiments, the antibody or antigen binding fragment can comprise a V_H comprising the HCDR1, the HCDR2, and the HCDR3 of the VH of any one of the antibodies shown in FIGs. 20A-20G, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. In some embodiments, the antibody or antigen binding fragment can comprise a V_L comprising the LCDR1, the LCDR2, and the LCDR3 of the VL of any one of the antibodies shown in FIGs. 20A-20G, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. In some embodiments, the antibody or antigen binding fragment can comprise a V_H and a V_L comprising the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2, and the LCDR3 of the VH and VL any one of the antibodies shown in FIGs. 20A-20G, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses.

In some embodiments, the antibody or antigen binding fragment includes at least one CDR (such as an HCDR3) with a sequence that has at least 95% (such as at least 96%, at least 97%, at least 98%, at least 99%, or even 100%) sequence identity to any one of the heavy or light chain CDRs of any the VH and/or VL of any one of the antibodies shown in FIGs. 20A-20G, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. In some embodiments, the antibody or antigen binding fragment includes a V_H comprising a HCDR1, a HCDR2, and a HCDR3 comprising amino acid sequences at least 90% (such as at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical to the CDRs of the VH of any one of the antibodies shown in FIGs. 20A-20G. In some embodiments, the antibody or antigen binding fragment includes a V_L comprising a LCDR1, a LCDR2, and a LCDR3 comprising amino acid sequences at least 90% (such as at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical to the CDRs of the VL of any one of the antibodies shown in FIGs. 20A-20G.

In additional embodiments, the antibody or antigen binding fragment includes a V_H comprising a HCDR1, a HCDR2, and a HCDR3 comprising amino acid sequences at least 90% (such as at least 95%, at least 96%, at least

97%, at least 98%, or at least 99%) identical to the CDRs of the VH of any one of the antibodies shown in FIGs. 20A-20G, and a V_L comprising a LCDR1, a LCDR2, and a LCDR3 comprising amino acid sequences at least 90% (such as at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical to the CDRs of the VL of any one of the antibodies shown in FIGs. 20A-20G, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses.

In some embodiments, the antibody or antigen binding fragment includes a V_H comprising an amino acid sequence at least 90% (such as at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical to the VH of any one of the antibodies shown in FIGs. 20A-20G, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. In more embodiments, the antibody or antigen binding fragment includes a V_L comprising an amino acid sequence at least 90% (such as at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical to the VL of any one of the antibodies shown in FIGs. 20A-20G, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. In additional embodiments, the antibody or antigen binding fragment includes a V_H and a V_L independently comprising amino acid sequences at least 90% (such as at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) identical to the VH and VL, respectively, of any one of the antibodies shown in FIGs. 20A-20G.

In additional embodiments, the antibody or antigen binding fragment includes a V_H comprising the amino acid sequence set forth as the VH of any one of the antibodies shown in FIGs. 20A-20G, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. In more embodiments, the antibody or antigen binding fragment includes a V_L comprising the amino acid sequence set forth as the VL of any one the of the antibodies shown in FIGs. 20A-20G, and can specifically bind to influenza HA protein and neutralize group 1 and 2 influenza A viruses. In some embodiments, the antibody or antigen binding fragment includes a V_H and a V_L comprising the amino acid sequences set forth as the VH and VL of any one of the antibodies shown in FIGs. 20A-20G.

Additional embodiments

Clause 1. An isolated antibody, comprising:

a heavy chain variable region comprising a HCDR1, a HCDR2, and a HCDR3, and a light chain variable region comprising a LCDR1, a LCDR2, and a LCDR3, wherein:

the heavy chain variable region comprises the following by kabat positioning: a Gln at position 1; a Val at position 2; a Gly at position 26; an Asp at position 27; a Asn, His or Tyr at position 32; a Ser at position 33; an Ala, Ser or Thr, or no residue at position 35; an Arg at position 50; a Tyr at position 52; an Arg at position 52b; a Ser at position 53; a Tyr at position 56; an Asp at position 58; an Arg at position 94; an Ala, Phe, Val, or Pro at position 97; a Met at position 98; an Ile at position 99; a Phe at position 100; a Gly at position 100a; an Ile or Val at position 100b; a Val, Leu, or Asp at position 100c;and a Met, Ile, or Val at position 100d;

the light chain variable region comprises the following by kabat positioning: a Glu at position 1; a Gln or Arg at position 27; a Ser at position 27a; an Ala, Ser, Gly, or Val at position 29; an Arg or Ser at position 30; a Val or Ser at position 31; a Tyr at position 32; a Phe, Tyr, or Lys at position 49; a Ser, Thr, Pro, or Asn at position 56; a Tyr, Phe, or His at positon 91; an Asp or Gly at position 92; a Gly, Ser, or Val at position 93; a Ser at position 94; and a Phe or Tyr at position 96; and

the antibody specifically binds to influenza A HA protein and neutralizes group 1 and group 2 influenza A viruses.

Clause 2. The isolated antibody of clause 1, wherein the light chain variable region comprises the following by kabat positioning: a Glu at position 1; a Gln or Arg at position 27; a Ser at position 27a; an Ala, Ser, or Gly at position 29; a Ser at position 30; a Ser at position 31; a Tyr at position 32; a Phe or Tyr at position 49; a Ser, Thr, Pro, or Asn at position 56; a Tyr or Phe at positon 91; an Asp or Gly at position 92; a Gly or Ser at position 93; a Ser at position 94; and a Phe or Tyr at position 96.

Clause 3. The isolated antibody of clause 1 or clause 2, wherein

the HCDR1 comprises the amino acid sequence set forth as $GDX_1VSSX_2SAX_3$ (SEQ ID NO: 116);

the HCDR2 comprises the amino acid sequence set forth as $TYYRSX_4WYX_5$ (SEQ ID NO: 117);

the HCDR3 comprises the amino acid sequence set forth as $ARX_6SX_7MIFGX_8X_9X_{10}X_{11}X_{12}X_{13}X_{14}X_{15}$ (SEQ ID NO: 118);

the LCDR1 comprises the amino acid sequence set forth as $X_{16}SVX_{17}X_{18}X_{19}Y$ (SEQ ID NO: 119);

the LCDR2 comprises the amino acid sequence set forth as $GX_{20}S$ (SEQ ID NO: 120);

the LCDR3 comprises the amino acid sequence set forth as $QX_{21}X_{22}X_{23}X_{24}SX_{25}X_{26}T$ (SEQ ID NO: 121); and wherein

X_1 is Ser or Thr; X_2 is Asn, His or Tyr; X_3 is Ala, Ser, Thr, or no amino acid; X_4 is Lys or Arg; X_5 is Ser, Asn, Thr, Tyr, Gly, Ser, or Thr; X_6 is Gly, Ala, or Val; X_7 is Ala, Phe, Val, or Pro; X_8 is Ile or Val; X_9 is Val, Leu, or Asp; X_{10} is Met, Ile, or Val; X_{11} is Gly or no amino acid; X_{12} is Ala, Ile, Val, or Glu; X_{13} is Phe, Leu, or Met; X_{14} is Asp or Glu; X_{15} is Gln, Phe, Cys, Ser, Tyr, or Leu; X_{16} is Gln or Arg; X_{17} is Ala, Ser, Gly, or Val; X_{18} is Ser or Arg; X_{19} is Ser or Val; X_{20} is Ala, Val, or Thr; X_{21} is Gln or Arg; X_{22} is Tyr, Phe, or His;X_{23} is Asp or Gly; X_{24} is Gly, Ser, or Val; X_{25} is Gln, His, or Arg; and X_{26} is Tyr or Phe.

Clause 4. The isolated antibody of any of clauses 1-3, wherein the heavy chain variable region comprises a germline origin of HV6-1, HD3-3, and HJ4 or HJ5 genes, and the light chain variable region comprises a germline origin of KV3-20 and KJ4 or KJ5 genes.

Clause 5. An isolated antibody, comprising:

a heavy chain variable region comprising a HCDR1, a HCDR2, and a HCDR3, and a light chain variable region comprising a LCDR1, a LCDR2, and a LCDR3, wherein:

the heavy chain variable region comprises the following by kabat positioning: a Trp at position 50; a Tyr at position 53; an Asn at position 54; an Ala or Gly at position 55; a Asn or His at position 56; an Asn or Gln at position 58; an Ala, Asp, His, Asn, Thr at position 98; an Ile at position 99; a Leu at position 100; and a Thr at position 100a;

the light chain variable region comprises the following by kabat positioning a Gln at position 27; a Gly at position 27a; a Leu at position 27b; a Leu or Val at position 27c; a Phe, His, or Tyr at position 27d; an Ile at position 27e; a Asp at position 28; a Gly at position 29; a Thr at position 31; an Asn, Glu, His, or Lys at position

50; a Val or Ile at position 51; a Ser or Phe at position 52; a Ser at position 65; a Gly at position 66; a Ser at position 67; a Gly at position 68; a Thr at position 92; a His or Tyr at position 93; a Trp at position 94; and

wherein the antibody specifically binds to influenza A HA protein and neutralizes group 1 and group 2 influenza A viruses.

Clause 6. The isolated antibody of clause 5, wherein

the HCDR1 comprises the amino acid sequence set forth as $GYX_1FX_2X_3X_4G$ (SEQ ID NO: 122);

the HCDR2 comprises the amino acid sequence set forth as $X_5SX_6YNX_7X_8X_9$ (SEQ ID NO: 123);

the HCDR3 comprises the amino acid sequence set forth as $X_{10}RDX_{11}X_{12}X_{13}ILTGX_{14}X_{15}X_{16}DX_{17}$ (SEQ ID NO: 124);

the LCDR1 comprises the amino acid sequence set forth as $QGLX_{18}X_{19}IDGX_{20}X_{21}Y$ (SEQ ID NO: 125);

the LCDR2 comprises the amino acid sequence set forth as $X_{22}X_{23}X_{24}$ (SEQ ID NO: 126);

the LCDR3 comprises the amino acid sequence set forth as $X_{25}QGTX_{26}WPX_{27}T$ (SEQ ID NO: 127); and

wherein X_1 is Arg, Thr, Asp, or Ser; X_2 is Ser, Thr, or Asn; X_3 is Ser, Thr, or Asn; X_4 is Tyr, Ser, Phe, or His; X_5 is Ile or Val; X_6 is Ala or Gly; X_7 is Gly or Ala; X_8 is His or Asn; X_9 is Thr or Ile; X_{10} is Ala or Thr; X_{11} is Gln or Arg; X_{12} is Arg, Tyr, Gly, Phe, Ser, or Pro; X_{13} is Asp, Ala, Asn, Thr, or His; X_{14} is Gly, Pro, Ser, Tyr, Asp, Phe, or Cys; X_{15} is Leu, Asn, Ala, Arg, Asp, His, or Gln; X_{16} is Phe, Gly, Thr, Asp, or Leu; X_{17} is Cys, Tyr, His, Asp, Phe, Ser, or Ile; X_{18} is Val or Leu; X_{19} is His, Tyr, or Phe; X_{20} is Asn or Ser; X_{21} is Thr or Ile; X_{22} is Asn, His, Lys, or Glu; X_{23} is Val, or Ile; X_{24} is Phe or Ser; X_{25} is Met or Leu; X_{26} is Tyr or His; and X_{27} is Tyr, Leu, Arg, or Ile.

Clause 7. The isolated antibody of clause 5 or clause 6, wherein the heavy chain variable region comprises a germline origin of HV1-18, HD3-9, and HJ4 genes, and the light chain variable region comprises a germline origin of KV2-30 and KJ2 or KJ5 genes.

1. Additional Description of Antibodies and Antigen Binding Fragments

The antibody or antigen binding fragment can be a human antibody or fragment thereof. Chimeric antibodies are also provided. The antibody or antigen binding fragment can include any suitable framework region, such as (but not limited to) a human framework region. Human framework regions, and mutations that can be made in a human antibody framework regions, are known in the art (see, for example, in U.S. Patent No. 5,585,089, which is incorporated herein by reference). Alternatively, a heterologous framework region, such as, but not limited to a mouse or monkey framework region, can be included in the heavy or light chain of the antibodies. (See, for example, Jones *et al.*, *Nature* 321:522, 1986; Riechmann *et al.*, *Nature* 332:323, 1988; Verhoeyen *et al.*, *Science* 239:1534, 1988; Carter *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 89:4285, 1992; Sandhu, *Crit. Rev. Biotech.*12:437, 1992; and Singer *et al.*, *J. Immunol.*150:2844, 1993.)

The antibody can be of any isotype. The antibody can be, for example, an IgM or an IgG antibody, such as IgG_1, IgG_2, IgG_3, or IgG_4. The class of an antibody that specifically binds influenza HA can be switched with another. In one aspect, a nucleic acid molecule encoding V_L or V_H is isolated using methods well-known in the art, such that it does not include any nucleic acid sequences encoding the constant region of the light or heavy chain, respectively. A nucleic acid molecule encoding V_L or V_H is then operatively linked to a nucleic acid sequence

encoding a C_L or C_H from a different class of immunoglobulin molecule. This can be achieved using a vector or nucleic acid molecule that comprises a C_L or C_H chain, as known in the art. For example, an antibody that specifically binds influenza A HA protein, that was originally IgG may be class switched to an IgM. Class switching can be used to convert one IgG subclass to another, such as from IgG_1 to IgG_2, IgG_3, or IgG_4.

In some examples, the disclosed antibodies are oligomers of antibodies, such as dimers, trimers, tetramers, pentamers, hexamers, septamers, octomers and so on.

(a) Binding affinity

In several embodiments, the antibody or antigen binding fragment can specifically bind influenza HA with an affinity (*e.g.*, measured by K_d) of no more than 1.0×10^{-8} M, no more than 5.0×10^{-8} M, no more than 1.0×10^{-9} M, no more than 5.0×10^{-9} M, no more than 1.0×10^{-10} M, no more than 5.0×10^{-10} M, or no more than 1.0×10^{-11} M. K_d can be measured, for example, by a radiolabeled antigen binding assay (RIA) performed with the Fab version of an antibody of interest and its antigen using known methods. In one assay, solution binding affinity of Fabs for antigen is measured by equilibrating Fab with a minimal concentration of (^{125}I)-labeled antigen in the presence of a titration series of unlabeled antigen, then capturing bound antigen with an anti-Fab antibody-coated plate (see, *e.g.*, Chen *et al.*, *J. Mol. Biol.* 293:865-881 (1999)). To establish conditions for the assay, MICROTITER® multi-well plates (Thermo Scientific) are coated overnight with 5 µg/ml of a capturing anti-Fab antibody (Cappel Labs) in 50 mM sodium carbonate (pH 9.6), and subsequently blocked with 2% (w/v) bovine serum albumin in PBS for two to five hours at room temperature (approximately 23° C.). In a non-adsorbent plate (Nunc #269620), 100 µM or 26 pM [^{125}I]-antigen are mixed with serial dilutions of a Fab of interest (*e.g.*, consistent with assessment of the anti-VEGF antibody, Fab-12, in Presta *et al.*, *Cancer Res.* 57:4593-4599 (1997)). The Fab of interest is then incubated overnight; however, the incubation may continue for a longer period (*e.g.*, about 65 hours) to ensure that equilibrium is reached. Thereafter, the mixtures are transferred to the capture plate for incubation at room temperature (*e.g.*, for one hour). The solution is then removed and the plate washed eight times with 0.1% polysorbate 20 (TWEEN-20®) in PBS. When the plates have dried, 150 µl/well of scintillant (MICROSCINT-20™; Packard) is added, and the plates are counted on a TOPCOUNT™ gamma counter (Packard) for ten minutes. Concentrations of each Fab that give less than or equal to 20% of maximal binding are chosen for use in competitive binding assays.

In another assay, K_d can be measured using surface plasmon resonance assays using a BIACORE®-2000 or a BIACORE®-3000 (BIAcore, Inc., Piscataway, N.J.) at 25° C with immobilized antigen CM5 chips at ~10 response units (RU). Briefly, carboxymethylated dextran biosensor chips (CM5, BIACORE®, Inc.) are activated with N-ethyl-N′-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS) according to the supplier's instructions. Antigen is diluted with 10 mM sodium acetate, pH 4.8, to 5 µg/ml (~0.2 µM) before injection at a flow rate of 5 l/minute to achieve approximately 10 response units (RU) of coupled protein. Following the injection of antigen, 1 M ethanolamine is injected to block unreacted groups. For kinetics measurements, two-fold serial dilutions of Fab (0.78 nM to 500 nM) are injected in PBS with 0.05% polysorbate 20 (TWEEN-20™) surfactant (PBST) at 25° C at a flow rate of approximately 25 l/min. Association rates (k_{on}) and dissociation rates (k_{off}) are calculated using a simple one-to-one Langmuir binding model (BIACORE® Evaluation Software version 3.2) by simultaneously fitting the association and dissociation sensorgrams. The equilibrium dissociation constant (Kd) is calculated as the ratio k_{off}/k_{on}. See, *e.g.*, Chen *et al.*, *J. Mol. Biol.* 293:865-881 (1999).

If the on-rate exceeds 106 M^{-1} s^{-1} by the surface plasmon resonance assay above, then the on-rate can be determined by using a fluorescent quenching technique that measures the increase or decrease in fluorescence emission intensity (excitation=295 nm; emission=340 nm, 16 nm band-pass) at 25° C. of a 20 nM anti-antigen antibody (Fab form) in PBS, pH 7.2, in the presence of increasing concentrations of antigen as measured in a spectrometer, such as a stop-flow equipped spectrophometer (Aviv Instruments) or a 8000-series SLM-AMINCO™ spectrophotometer (ThermoSpectronic) with a stirred cuvette.

(b) Neutralization

In some embodiments, the antibody or antigen binding fragment can also be distinguished by neutralization breadth. In some embodiments, an antibody or antigen binding fragment that specifically binds to influenza HA protein can neutralize at least one (such as 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10) of H1, H2, H5, H6, H8, H9, H11, H12, H13, and H16 group influenza A subtypes and at least one (such as 1, 2, 3, 4, 5, or 6) of H3, H4, H7, H10, H14 and H15 subtypes group 2 influenza A subtypes. For example, in several embodiments, the antibody or antigen binding fragment can neutralize H1 and H3 subtypes of influenza A viruses. In several embodiments, the antibody or antigen binding fragment can neutralize the group 1 and group 2 influenza A viruses with an IC_{50} of less than 50 μg/ml. Exemplary pseudovirus neutralization assays and panels of influenza A pseudovirus are described in Example 1.

(c) Multispecific antibodies

In some embodiments, the antibody or antigen binding fragment is included on a multispecific antibody, such as a bi-specific antibody. Such multispecific antibodies can be produced by known methods, such as crosslinking two or more antibodies, antigen binding fragments (such as scFvs) of the same type or of different types. Exemplary methods of making multispecific antibodies include those described in PCT Pub. No. WO2013/163427, which is incorporated by reference herein in its entirety. Suitable crosslinkers include those that are heterobifunctional, having two distinctly reactive groups separated by an appropriate spacer (such as m-maleimidobenzoyl-N-hydroxysuccinimide ester) or homobifunctional (such as disuccinimidyl suberate). Such linkers are available from Pierce Chemical Company, Rockford, Ill.

Various types of multi-specific antibodies are known. Bispecific single chain antibodies can be encoded by a single nucleic acid molecule. Examples of bispecific single chain antibodies, as well as methods of constructing such antibodies are known in the art (see, *e.g.*, U.S. Pat. Nos. 8,076,459, 8,017,748, 8,007,796, 7,919,089, 7,820,166, 7,635,472, 7,575,923, 7,435,549, 7,332,168, 7,323,440, 7,235,641, 7,229,760, 7,112,324, 6,723,538, incorporated by reference herein). Additional examples of bispecific single chain antibodies can be found in PCT application No. WO 99/54440; Mack, *J. Immunol.*, 158:3965-3970, 1997; Mack, *PNAS*, 92:7021-7025, 1995; Kufer, *Cancer Immunol. Immunother.*, 45:193-197, 1997; Loffler, *Blood*, 95:2098-2103, 2000; and Bruhl, *J. Immunol.*, 166:2420-2426, 2001. Production of bispecific Fab-scFv ("bibody") molecules are described, for example, in Schoonjans *et al.* (J. Immunol. 165:7050-57, 2000) and Willems *et al.* (J Chromatogr B Analyt Technol Biomed Life Sci. 786:161-76, 2003). For bibodies, a scFv molecule can be fused to one of the VL-CL (L) or VH-CH1 chains, *e.g.*, to produce a bibody one scFv is fused to the C-term of a Fab chain.

(d) Fragments

Antigen binding fragments are encompassed by the present disclosure, such as Fab, $F(ab')_2$, and Fv which include a heavy chain and V_L and specifically bind influenza HA. These antibody fragments retain the ability to selectively bind with the antigen and are "antigen-binding" fragments. Non-limiting examples of such fragments include:

(1) Fab, the fragment which contains a monovalent antigen-binding fragment of an antibody molecule, can be produced by digestion of whole antibody with the enzyme papain to yield an intact light chain and a portion of one heavy chain;

(2) Fab', the fragment of an antibody molecule can be obtained by treating whole antibody with pepsin, followed by reduction, to yield an intact light chain and a portion of the heavy chain; two Fab' fragments are obtained per antibody molecule;

(3) $(Fab')_2$, the fragment of the antibody that can be obtained by treating whole antibody with the enzyme pepsin without subsequent reduction; $F(ab')_2$ is a dimer of two Fab' fragments held together by two disulfide bonds;

(4) Fv, a genetically engineered fragment containing the V_L and V_L expressed as two chains; and

(5) Single chain antibody (such as scFv), defined as a genetically engineered molecule containing the V_H and the V_L linked by a suitable polypeptide linker as a genetically fused single chain molecule (see, *e.g.*, Ahmad *et al.*, Clin. Dev. Immunol., 2012, doi:10.1155/2012/980250; Marbry, IDrugs, 13:543-549, 2010). The intramolecular orientation of the V_H-domain and the V_L-domain in a scFv, is not decisive for the provided antibodies (*e.g.*, for the provided multispecific antibodies). Thus, scFvs with both possible arrangements (V_H-domain-linker domain-V_L-domain; V_L-domain-linker domain-V_H-domain) may be used.

(6) A dimer of a single chain antibody ($scFV_2$), defined as a dimer of a scFV. This has also been termed a "miniantibody."

Methods of making these fragments are known in the art (see for example, Harlow and Lane, *Antibodies: A Laboratory Manual*, 2nd, Cold Spring Harbor Laboratory, New York, 2013).

In some embodiments, the antigen binding fragment can be an Fv antibody, which is typically about 25 kDa and contain a complete antigen-binding site with three CDRs per each heavy chain and each light chain. To produce F_V antibodies, the V_H and the V_L can be expressed from two individual nucleic acid constructs in a host cell. If the V_H and the V_L are expressed non-contiguously, the chains of the Fv antibody are typically held together by noncovalent interactions. However, these chains tend to dissociate upon dilution, so methods have been developed to crosslink the chains through glutaraldehyde, intermolecular disulfides, or a peptide linker. Thus, in one example, the Fv can be a disulfide stabilized Fv (dsFv), wherein the V_H and the V_L are chemically linked by disulfide bonds. In an additional example, the Fv fragments include V_H and V_L chains connected by a peptide linker. These single-chain antigen binding proteins (scFv) can be prepared by constructing a nucleic acid molecule encoding the V_H and V_L domains connected by an oligonucleotide. The nucleic acid molecule is inserted into an expression vector, which is subsequently introduced into a host cell such as a mammalian cell. The recombinant host cells synthesize a single polypeptide chain with a linker peptide bridging the two V domains. Methods for producing scFvs are known in the art (see Whitlow *et al.*, *Methods: a Companion to Methods in Enzymology*, Vol. 2, page 97, 1991; Bird *et al.*, *Science* 242:423, 1988; U.S. Patent No. 4,946,778; Pack *et al.*, *Bio/Technology*

11:1271, 1993; Ahmad *et al.*, *Clin. Dev. Immunol.*, 2012, doi:10.1155/2012/980250; Marbry, *IDrugs*, 13:543-549, 2010). Dimers of a single chain antibody ($scFV_2$), are also contemplated.

Antigen binding fragments can be prepared by proteolytic hydrolysis of the antibody or by expression in a host cell (such as an *E. coli* cell) of DNA encoding the fragment. Antigen binding fragments can also be obtained by pepsin or papain digestion of whole antibodies by conventional methods. For example, antigen binding fragments can be produced by enzymatic cleavage of antibodies with pepsin to provide a 5S fragment denoted $F(ab')_2$. This fragment can be further cleaved using a thiol reducing agent, and optionally a blocking group for the sulfhydryl groups resulting from cleavage of disulfide linkages, to produce 3.5S Fab' monovalent fragments. Alternatively, an enzymatic cleavage using pepsin produces two monovalent Fab' fragments and an Fc fragment directly (see U.S. Patent No. 4,036,945 and U.S. Patent No. 4,331,647, and references contained therein; Nisonhoff *et al.*, *Arch. Biochem. Biophys.* 89:230, 1960; Porter, *Biochem. J.* 73:119, 1959; Edelman *et al.*, *Methods in Enzymology*, Vol. 1, page 422, Academic Press, 1967; and Coligan *et al.* at sections 2.8.1-2.8.10 and 2.10.1-2.10.4).

Other methods of cleaving antibodies, such as separation of heavy chains to form monovalent light-heavy chain fragments, further cleavage of fragments, or other enzymatic, chemical, or genetic techniques may also be used, so long as the fragments bind to the antigen that is recognized by the intact antibody.

Antigen binding single V_H domains, called domain antibodies (dAb), have also been identified from a library of murine V_H genes amplified from genomic DNA of immunized mice (Ward *et al. Nature 341*:544-546, 1989). Human single immunoglobulin variable domain polypeptides capable of binding antigen with high affinity have also been described (see, for example, PCT Publication Nos. WO 2005/035572 and WO 2003/002609). The CDRs disclosed herein can also be included in a dAb.

In some embodiments, one or more of the heavy and/or light chain complementarity determining regions (CDRs) from a disclosed antibody (such as the 54.f.01, 56.a.09, 01.k.01, 31.b.09, 16.g.07, 54.a.84, 16.a.26, 54.a.39, 31.a.83, 3150206_1A05, 3155305P_1A05, 3155305P _1B06, 315-09-1B12, 315-02-1E04, or 315-02-1E04 antibody) is expressed on the surface of another protein, such as a scaffold protein. The expression of domains of antibodies on the surface of a scaffolding protein are known in the art (see *e.g.*, Liu *et al., J. Virology* 85(17): 8467-8476, 2011). Such expression creates a chimeric protein that retains the binding for influenza HA. In some specific embodiments, one or more of the heavy chain CDRs is grafted onto a scaffold protein, such as one or more of heavy chain CDR1, CDR2, and/or CDR3. One or more CDRs can also be included in a diabody or another type of single chain antibody molecule.

(f) Variants

In certain embodiments, amino acid sequence variants of the antibodies provided herein are contemplated. For example, it may be desirable to improve the binding affinity and/or other biological properties of the antibody. Amino acid sequence variants of an antibody may be prepared by introducing appropriate modifications into the nucleotide sequence encoding the antibody, or by peptide synthesis. Such modifications include, for example, deletions from, and/or insertions into and/or substitutions of residues within the amino acid sequences of the antibody. Any combination of deletion, insertion, and substitution can be made to arrive at the final construct, provided that the final construct possesses the desired characteristics, *e.g.*, antigen-binding.

In certain embodiments, antibody variants having one or more amino acid substitutions are provided. Sites of interest for substitutional mutagenesis include the CDRs and the framework regions. Amino acid substitutions may be introduced into an antibody of interest and the products screened for a desired activity, *e.g.*, retained/improved antigen binding, decreased immunogenicity, or improved ADCC or CDC.

The variants typically retain amino acid residues necessary for correct folding and stabilizing between the V_H and the V_L regions, and will retain the charge characteristics of the residues in order to preserve the low pI and low toxicity of the molecules. Amino acid substitutions can be made in the V_H and the V_L regions to increase yield.

In some embodiments, the heavy chain of the antibody includes up to 10 (such as up to 1, up to 2, up to 3, up to 4, up to 5, up to 6, up to 7, up to 8, or up to 9) amino acid substitutions (such as conservative amino acid substitutions) compared to the amino acid sequence set forth as one of SEQ ID NOs : 1, 3, 5, 7, 9, 11, 13, 15, 17, 71, 73, 75, 363, 365, or 367. In some embodiments, the light chain of the antibody includes up to 10 (such as up to 1, up to 2, up to 3, up to 4, up to 5, up to 6, up to 7, up to 8, or up to 9) amino acid substitutions (such as conservative amino acid substitutions) compared to the amino acid sequence set forth as one of SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, 16, 18, 72, 74, 76, 364, 366, or 368.

In some embodiments, the antibody or antigen binding fragment can include up to 10 (such as up to 1, up to 2, up to 3, up to 4, up to 5, up to 6, up to 7, up to 8, or up to 9) amino acid substitutions (such as conservative amino acid substitutions) in the framework regions of the heavy chain of the antibody, or the light chain of the antibody, or the heavy and light chains of the antibody, compared to a known framework region, or compared to a known framework region, or compared to the framework regions of the 54.f.01, 56.a.09, 01.k.01, 31.b.09, 16.g.07, 54.a.84, 16.a.26, 54.a.39, 31.a.83, 3150206_1A05, 3155305P_1A05, or 3155305P _1B06, 315-09-1B12, 315-02-1E04, or 315-02-1E04 antibody, and maintain the specific binding activity for HA.

In certain embodiments, substitutions, insertions, or deletions may occur within one or more CDRs so long as such alterations do not substantially reduce the ability of the antibody to bind antigen. For example, conservative alterations (*e.g.*, conservative substitutions as provided herein) that do not substantially reduce binding affinity may be made in CDRs. In certain embodiments of the variant V_H and V_L sequences provided above, each CDR either is unaltered, or contains no more than one, two or three amino acid substitutions.

To increase binding affinity of the antibody, the V_L and V_H segments can be randomly mutated, such as within HCDR3 region or the LCDR3 region, in a process analogous to the *in vivo* somatic mutation process responsible for affinity maturation of antibodies during a natural immune response. Thus *in vitro* affinity maturation can be accomplished by amplifying V_H and V_L regions using PCR primers complementary to the HCDR3 or LCDR3, respectively. In this process, the primers have been "spiked" with a random mixture of the four nucleotide bases at certain positions such that the resultant PCR products encode V_H and V_L segments into which random mutations have been introduced into the V_H and/or V_L CDR3 regions. These randomly mutated V_H and V_L segments can be tested to determine the binding affinity for influenza A HA protein. In particular examples, the V_H amino acid sequence is one of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, 17, 71, 73, 315-09-1B12, 315-02-1E04, and 315-02-1E04. In other examples, the V_L amino acid sequence is one of SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, 16, 18, 72, 74, 76, 364, 366, or 368. Methods of in vitro affinity maturation are known (see, *e.g.*, Chowdhury, *Methods Mol. Biol.* 207:179-196 (2008)), and Hoogenboom *et al.* in *Methods in Molecular Biology* 178:1-37 (O'Brien *et al.*, ed., Human Press, Totowa, N.J., (2001).)

In certain embodiments, an antibody or antigen binding fragment is altered to increase or decrease the extent to which the antibody or antigen binding fragment is glycosylated. Addition or deletion of glycosylation sites may be conveniently accomplished by altering the amino acid sequence such that one or more glycosylation sites is created or removed.

Where the antibody comprises an Fc region, the carbohydrate attached thereto may be altered. Native antibodies produced by mammalian cells typically comprise a branched, biantennary oligosaccharide that is generally attached by an N-linkage to Asn297 of the CH_2 domain of the Fc region. See, *e.g.*, Wright *et al.* *TIBTECH* 15:26-32 (1997). The oligosaccharide may include various carbohydrates, *e.g.*, mannose, N-acetyl glucosamine (GlcNAc), galactose, and sialic acid, as well as a fucose attached to a GlcNAc in the "stem" of the biantennary oligosaccharide structure. In some embodiments, modifications of the oligosaccharide in an antibody may be made in order to create antibody variants with certain improved properties.

In one embodiment, antibody variants are provided having a carbohydrate structure that lacks fucose attached (directly or indirectly) to an Fc region. For example, the amount of fucose in such antibody may be from 1% to 80%, from 1% to 65%, from 5% to 65% or from 20% to 40%. The amount of fucose is determined by calculating the average amount of fucose within the sugar chain at Asn297, relative to the sum of all glycostructures attached to Asn 297 (*e.g.*, complex, hybrid and high mannose structures) as measured by MALDI-TOF mass spectrometry, as described in WO 2008/077546, for example. Asn297 refers to the asparagine residue located at about position 297 in the Fc region; however, Asn297 may also be located about ±3 amino acids upstream or downstream of position 297, i.e., between positions 294 and 300, due to minor sequence variations in antibodies. Such fucosylation variants may have improved ADCC function. See, *e.g.*, US Patent Publication Nos. US 2003/0157108 (Presta, L.); US 2004/0093621 (Kyowa Hakko Kogyo Co., Ltd). Examples of publications related to "defucosylated" or "fucose-deficient" antibody variants include: US 2003/0157108; WO 2000/61739; WO 2001/29246; US 2003/0115614; US 2002/0164328; US 2004/0093621; US 2004/0132140; US 2004/0110704; US 2004/0110282; US 2004/0109865; WO 2003/085119; WO 2003/084570; WO 2005/035586; WO 2005/035778; WO2005/053742; WO2002/031140; Okazaki *et al. J. Mol. Biol.* 336:1239-1249 (2004); Yamane-Ohnuki *et al.* *Biotech. Bioeng.* 87: 614 (2004). Examples of cell lines capable of producing defucosylated antibodies include Lec 13 CHO cells deficient in protein fucosylation (Ripka *et al. Arch. Biochem. Biophys.* 249:533-545 (1986); US Pat Appl No US 2003/0157108 A1, Presta, L; and WO 2004/056312 A1, Adams *et al.*, especially at Example 11), and knockout cell lines, such as alpha-1,6-fucosyltransferase gene, FUT8, knockout CHO cells (see, *e.g.*, Yamane-Ohnuki *et al. Biotech. Bioeng.* 87: 614 (2004); Kanda, Y. *et al.*, *Biotechnol. Bioeng.*, 94(4):680-688 (2006); and WO2003/085107).

Antibodies variants are further provided with bisected oligosaccharides, *e.g.*, in which a biantennary oligosaccharide attached to the Fc region of the antibody is bisected by GlcNAc. Such antibody variants may have reduced fucosylation and/or improved ADCC function. Examples of such antibody variants are described, *e.g.*, in WO 2003/011878 (Jean-Mairet *et al.*); U.S. Pat. No. 6,602,684 (Umana *et al.*); and US 2005/0123546 (Umana *et al.*). Antibody variants with at least one galactose residue in the oligosaccharide attached to the Fc region are also provided. Such antibody variants may have improved CDC function. Such antibody variants are described, *e.g.*, in WO 1997/30087 (Patel *et al.*); WO 1998/58964 (Raju, S.); and WO 1999/22764 (Raju, S.).

In several embodiments, the constant region of the antibody includes one or more amino acid substitutions to optimize *in vivo* half-life of the antibody. The serum half-life of IgG Abs is regulated by the neonatal Fc

receptor (FcRn). Thus, in several embodiments, the antibody includes an amino acid substitution that increases binding to the FcRn. Several such substitutions are known to the person of ordinary skill in the art, such as substitutions at IgG constant regions T250Q and M428L (see, *e.g.*, Hinton *et al., J Immunol.,* 176:346-356, 2006); M428L and N434S (the "LS" mutation, see, *e.g.*, Zalevsky, *et al., Nature Biotechnology, 28:157-159,* 2010); N434A (see, *e.g.*, Petkova *et al., Int. Immunol.*, 18:1759-1769, 2006); T307A, E380A, and N434A (see, *e.g.*, Petkova *et al., Int. Immunol.*, 18:1759-1769, 2006); and M252Y, S254T, and T256E (see, *e.g.*, Dall'Acqua *et al., J. Biol. Chem.*, 281:23514-23524, 2006).The disclosed antibodies and antigen binding fragments can be linked to a Fc polypeptide including any of the substitutions listed above, for example, the Fc polypeptide can include the M428L and N434S substitutions.

In some embodiments, the constant region of the antibody includes one of more amino acid substitutions to optimize antibody-dependent cell-mediated cytotoxicity (ADCC). ADCC is mediated primarily through a set of closely related Fcγ receptors. In some embodiments, the antibody includes one or more amino acid substitutions that increase binding to FcγRIIIa. Several such substitutions are known to the person of ordinary skill in the art, such as substitutions at IgG constant regions S239D and I332E (see, *e.g.*, Lazar *et al., Proc. Natl., Acad. Sci. U.S.A.*, 103:4005-4010, 2006); and S239D, A330L, and I332E (see, *e.g.*, Lazar *et al., Proc. Natl., Acad. Sci. U.S.A.*, 103:4005-4010, 2006).

Combinations of the above substitutions are also included, to generate an IgG constant region with increased binding to FcRn and FcγRIIIa. The combinations increase antibody half-life and ADCC. For example, such combination include antibodies with the following amino acid substitution in the Fc region: (1) S239D/I332E and T250Q/M428L; (2) S239D/I332E and M428L/N434S; (3) S239D/I332E and N434A; (4) S239D/I332E and T307A/E380A/N434A; (5) S239D/I332E and M252Y / S254T/T256E; (6) S239D/A330L/I332E and 250Q/M428L; (7) S239D/A330L/I332E and M428L/N434S; (8) S239D/A330L/I332E and N434A; (9) S239D/A330L/I332E and T307A/E380A/N434A; or (10) S239D/A330L/I332E and M252Y/S254T/T256E. In some examples, the antibodies, or an antigen binding fragment thereof is modified such that it is directly cytotoxic to infected cells, or uses natural defenses such as complement, antibody dependent cellular cytotoxicity (ADCC), or phagocytosis by macrophages.

In certain embodiments, an antibody provided herein may be further modified to contain additional nonproteinaceous moieties that are known in the art and readily available. The moieties suitable for derivatization of the antibody include but are not limited to water soluble polymers. Non-limiting examples of water soluble polymers include, but are not limited to, polyethylene glycol (PEG), copolymers of ethylene glycol/propylene glycol, carboxymethylcellulose, dextran, polyvinyl alcohol, polyvinyl pyrrolidone, poly-1,3-dioxolane, poly-1,3,6-trioxane, ethylene/maleic anhydride copolymer, polyaminoacids (either homopolymers or random copolymers), and dextran or poly(n-vinyl pyrrolidone)polyethylene glycol, propropylene glycol homopolymers, prolypropylene oxide/ethylene oxide co-polymers, polyoxyethylated polyols (*e.g.*, glycerol), polyvinyl alcohol, and mixtures thereof. Polyethylene glycol propionaldehyde may have advantages in manufacturing due to its stability in water. The polymer may be of any molecular weight, and may be branched or unbranched. The number of polymers attached to the antibody may vary, and if more than one polymer are attached, they can be the same or different molecules. In general, the number and/or type of polymers used for derivatization can be determined based on considerations including, but not limited to, the particular properties or functions of the antibody to be improved, whether the antibody derivative will be used in a therapy under defined conditions, etc.

The antibody or antigen binding fragment can be derivatized or linked to another molecule (such as another peptide or protein). In general, the antibody or antigen binding fragment is derivatized such that the binding to influenza HA is not affected adversely by the derivatization or labeling. For example, the antibody or antigen binding fragment can be functionally linked (by chemical coupling, genetic fusion, noncovalent association or otherwise) to one or more other molecular entities, such as another antibody (for example, a bi-specific antibody or a diabody), a detectable marker, an effector molecule, or a protein or peptide that can mediate association of the antibody or antibody portion with another molecule (such as a streptavidin core region or a polyhistidine tag).

B. Conjugates

The antibodies and antigen binding fragments that specifically bind to an epitope on influenza A HA protein can be conjugated to an agent, such as an effector molecule or detectable marker, using any number of means known to those of skill in the art. Both covalent and noncovalent attachment means may be used. One of skill in the art will appreciate that various effector molecules and detectable markers can be used, including (but not limited to) toxins and radioactive agents such as ^{125}I, ^{32}P, ^{14}C, ^{3}H and ^{35}S and other labels, target moieties and ligands, etc. The choice of a particular effector molecule or detectable marker depends on the particular target molecule or cell, and the desired biological effect.

The choice of a particular effector molecule or detectable marker depends on the particular target molecule or cell, and the desired biological effect. Thus, for example, the effector molecule can be a cytotoxin that is used to bring about the death of a particular target cell (such as an influenza virus infected cell).

The procedure for attaching an effector molecule or detectable marker to an antibody or antigen binding fragment varies according to the chemical structure of the effector. Polypeptides typically contain a variety of functional groups; such as carboxylic acid (COOH), free amine (-NH_2) or sulfhydryl (-SH) groups, which are available for reaction with a suitable functional group on a polypeptide to result in the binding of the effector molecule or detectable marker. Alternatively, the antibody or antigen binding fragment is derivatized to expose or attach additional reactive functional groups. The derivatization may involve attachment of any of a number of known linker molecules such as those available from Pierce Chemical Company, Rockford, IL. The linker can be any molecule used to join the antibody or antigen binding fragment to the effector molecule or detectable marker. The linker is capable of forming covalent bonds to both the antibody or antigen binding fragment and to the effector molecule or detectable marker. Suitable linkers are well known to those of skill in the art and include, but are not limited to, straight or branched-chain carbon linkers, heterocyclic carbon linkers, or peptide linkers. Where the antibody or antigen binding fragment and the effector molecule or detectable marker are polypeptides, the linkers may be joined to the constituent amino acids through their side groups (such as through a disulfide linkage to cysteine) or to the alpha carbon amino and carboxyl groups of the terminal amino acids.

In view of the large number of methods that have been reported for attaching a variety of radiodiagnostic compounds, radiotherapeutic compounds, labels (such as enzymes or fluorescent molecules), toxins, and other agents to antibodies one skilled in the art will be able to determine a suitable method for attaching a given agent to an antibody or antigen binding fragment or other polypeptide. For example, the antibody or antigen binding fragment can be conjugated with effector molecules such as small molecular weight drugs such as Monomethyl Auristatin E (MMAE), Monomethyl Auristatin F (MMAF), maytansine, maytansine derivatives, including the derivative of maytansine known as DM1 (also known as mertansine), or other agents to make an antibody drug

conjugate (ADC). In several embodiments, conjugates of an antibody or antigen binding fragment and one or more small molecule toxins, such as a calicheamicin, maytansinoids, dolastatins, auristatins, a trichothecene, and CC1065, and the derivatives of these toxins that have toxin activity, are provided.

The antibody or antigen binding fragment can be conjugated with a detectable marker; for example, a detectable marker capable of detection by ELISA, spectrophotometry, flow cytometry, microscopy or diagnostic imaging techniques (such as computed tomography (CT), computed axial tomography (CAT) scans, magnetic resonance imaging (MRI), nuclear magnetic resonance imaging NMRI), magnetic resonance tomography (MTR), ultrasound, fiberoptic examination, and laparoscopic examination). Specific, non-limiting examples of detectable markers include fluorophores, chemiluminescent agents, enzymatic linkages, radioactive isotopes and heavy metals or compounds (for example super paramagnetic iron oxide nanocrystals for detection by MRI). For example, useful detectable markers include fluorescent compounds, including fluorescein, fluorescein isothiocyanate, rhodamine, 5-dimethylamine-1-napthalenesulfonyl chloride, phycoerythrin, lanthanide phosphors and the like. Bioluminescent markers are also of use, such as luciferase, Green fluorescent protein (GFP), Yellow fluorescent protein (YFP). An antibody or antigen binding fragment can also be conjugated with enzymes that are useful for detection, such as horseradish peroxidase, β- galactosidase, luciferase, alkaline phosphatase, glucose oxidase and the like. When an antibody or antigen binding fragment is conjugated with a detectable enzyme, it can be detected by adding additional reagents that the enzyme uses to produce a reaction product that can be discerned. For example, when the agent horseradish peroxidase is present the addition of hydrogen peroxide and diaminobenzidine leads to a colored reaction product, which is visually detectable. An antibody or antigen binding fragment may also be conjugated with biotin, and detected through indirect measurement of avidin or streptavidin binding. It should be noted that the avidin itself can be conjugated with an enzyme or a fluorescent label.

The antibody or antigen binding fragment can be conjugated with a paramagnetic agent, such as gadolinium. Paramagnetic agents such as superparamagnetic iron oxide are also of use as labels. Antibodies can also be conjugated with lanthanides (such as europium and dysprosium), and manganese. An antibody or antigen binding fragment may also be labeled with a predetermined polypeptide epitopes recognized by a secondary reporter (such as leucine zipper pair sequences, binding sites for secondary antibodies, metal binding domains, epitope tags).

The antibody or antigen binding fragment can also be conjugated with a radiolabeled amino acid. The radiolabel may be used for both diagnostic and therapeutic purposes. For instance, the radiolabel may be used to detect influenza virus HA expressing cells by x-ray, emission spectra, or other diagnostic techniques. Examples of labels for polypeptides include, but are not limited to, the following radioisotopes or radionucleotides: ^{3}H, ^{14}C, ^{15}N, ^{35}S, ^{90}Y, ^{99}Tc, ^{111}In, ^{125}I, ^{131}I.

Means of detecting such detectable markers are well known to those of skill in the art. Thus, for example, radiolabels may be detected using photographic film or scintillation counters, fluorescent markers may be detected using a photodetector to detect emitted illumination. Enzymatic labels are typically detected by providing the enzyme with a substrate and detecting the reaction product produced by the action of the enzyme on the substrate, and colorimetric labels are detected by simply visualizing the colored label.

The average number of effector molecule or detectable marker moieties per antibody or antigen binding fragment in a conjugate can range, for example, from 1 to 20 moieties per antibody or antigen binding fragment. In certain embodiments, the average number of effector molecule or detectable marker moieties per antibody or

antigen binding fragment in a conjugate range from about 1 to about 2, from about 1 to about 3, about 1 to about 8; from about 2 to about 6; from about 3 to about 5; or from about 3 to about 4. The loading (for example, effector molecule/antibody ratio) of an conjugate may be controlled in different ways, for example, by: (i) limiting the molar excess of effector molecule-linker intermediate or linker reagent relative to antibody, (ii) limiting the conjugation reaction time or temperature, (iii) partial or limiting reductive conditions for cysteine thiol modification, (iv) engineering by recombinant techniques the amino acid sequence of the antibody such that the number and position of cysteine residues is modified for control of the number or position of linker-effector molecule attachments.

D. Polynucleotides and Expression

Nucleic acids molecules (for example, cDNA molecules) encoding the amino acid sequences of antibodies, antigen binding fragments, CARs and conjugates that specifically bind influenza virus HA are provided. Nucleic acids encoding these molecules can readily be produced by one of skill in the art, using the amino acid sequences provided herein (such as the CDR sequences and V_H and V_L sequences), sequences available in the art (such as framework or constant region sequences), and the genetic code. In several embodiments, a nucleic acid molecules can encode the V_H, the V_L, or both the V_H and V_L (for example in a bicistronic expression vector) of a disclosed antibody or antigen binding fragment. In several embodiments, the nucleic acid molecules can be expressed in a host cell (such as a mammalian cell) to produce a disclosed antibody or antigen binding fragment.

One of skill in the art can readily use the genetic code to construct a variety of functionally equivalent nucleic acids, such as nucleic acids which differ in sequence but which encode the same antibody sequence, or encode a conjugate or fusion protein including the V_L and/or V_H nucleic acid sequence.

In a non-limiting example, an isolated nucleic acid molecule encodes the V_H of a disclosed antibody or antigen binding fragment and includes the nucleic acid sequence set forth as any one of SEQ ID NOs: 77, 79, 81, 83, 85, 87, 89, 91, 93, 95, 97, 99, 369, 371, or 373. In a non-limiting example, an isolated nucleic acid molecule encodes the V_L of a disclosed antibody or antigen binding fragment and includes the nucleic acid sequence set forth as any one of SEQ ID NOs: 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 370, 372, or 374. In a non-limiting example, an isolated nucleic acid molecule encodes the V_H and V_L of a disclosed antibody or antigen binding fragment and includes the nucleic acid sequences set forth as any one of SEQ ID NOs: 77 and 78, respectively, 79 and 80, respectively, 81 and 82, respectively, 83 and 84, respectively, 85 and 86, respectively, 87 and 88, respectively, 89 and 90, respectively, 91 and 92, respectively, 93 and 94, respectively, 95 and 96, respectively, 97 and 98, respectively, 99 and 100, respectively, 369 and 370, respectively, 371 and 372, respectively, or 373 and 374, respectively.

Nucleic acid sequences encoding the of antibodies, antigen binding fragments, and conjugates that specifically bind influenza virus HA can be prepared by any suitable method including, for example, cloning of appropriate sequences or by direct chemical synthesis by methods such as the phosphotriester method of Narang *et al., Meth. Enzymol.* 68:90-99, 1979; the phosphodiester method of Brown *et al., Meth. Enzymol.* 68:109-151, 1979; the diethylphosphoramidite method of Beaucage *et al., Tetra. Lett.* 22:1859-1862, 1981; the solid phase phosphoramidite triester method described by Beaucage & Caruthers, *Tetra. Letts.* 22(20):1859-1862, 1981, for example, using an automated synthesizer as described in, for example, Needham-VanDevanter *et al., Nucl. Acids Res.* 12:6159-6168, 1984; and, the solid support method of U.S. Patent No. 4,458,066. Chemical synthesis produces

a single stranded oligonucleotide. This can be converted into double stranded DNA by hybridization with a complementary sequence or by polymerization with a DNA polymerase using the single strand as a template.

Exemplary nucleic acids can be prepared by cloning techniques. Examples of appropriate cloning and sequencing techniques, and instructions sufficient to direct persons of skill through many cloning exercises are known (see, e.g, Sambrook *et al.* (Molecular Cloning: A Laboratory Manual, 4th ed, Cold Spring Harbor, New York, 2012) and Ausubel *et al.* (In Current Protocols in Molecular Biology, John Wiley & Sons, New York, through supplement 104, 2013). Product information from manufacturers of biological reagents and experimental equipment also provide useful information. Such manufacturers include the SIGMA Chemical Company (Saint Louis, MO), R&D Systems (Minneapolis, MN), Pharmacia Amersham (Piscataway, NJ), CLONTECH Laboratories, Inc. (Palo Alto, CA), Chem Genes Corp., Aldrich Chemical Company (Milwaukee, WI), Glen Research, Inc., GIBCO BRL Life Technologies, Inc. (Gaithersburg, MD), Fluka Chemica-Biochemika Analytika (Fluka Chemie AG, Buchs, Switzerland), Invitrogen (Carlsbad, CA), and Applied Biosystems (Foster City, CA), as well as many other commercial sources known to one of skill.

Nucleic acids can also be prepared by amplification methods. Amplification methods include polymerase chain reaction (PCR), the ligase chain reaction (LCR), the transcription-based amplification system (TAS), the self-sustained sequence replication system (3SR). A wide variety of cloning methods, host cells, and *in vitro* amplification methodologies are well known to persons of skill.

The nucleic acid molecules can be expressed in a recombinantly engineered cell such as bacteria, plant, yeast, insect and mammalian cells. The antibodies, antigen binding fragments, and conjugates can be expressed as individual V_H and/or V_L chain (linked to an effector molecule or detectable marker as needed), or can be expressed as a fusion protein. Methods of expressing and purifying antibodies and antigen binding fragments are known and further described herein (see, *e.g.*, Al-Rubeai (ed), *Antibody Expression and Production*, Springer Press, 2011). An immunoadhesin can also be expressed. Thus, in some examples, nucleic acids encoding a V_H and V_L, and immunoadhesin are provided. The nucleic acid sequences can optionally encode a leader sequence.

To create a scFv the V_H- and V_L-encoding DNA fragments can be operatively linked to another fragment encoding a flexible linker, *e.g.*, encoding the amino acid sequence $(Gly_4\text{-}Ser)_3$, such that the V_H and V_L sequences can be expressed as a contiguous single-chain protein, with the V_L and V_H domains joined by the flexible linker (see, *e.g.,* Bird *et al., Science* 242:423-426, 1988; Huston *et al., Proc. Natl. Acad. Sci. USA* 85:5879-5883, 1988; McCafferty *et al., Nature* 348:552-554, 1990; Kontermann and Dubel (Ed), Antibody Engineering, Vols. 1-2, 2nd Ed., Springer Press, 2010; Harlow and Lane, *Antibodies: A Laboratory Manual*, 2nd, Cold Spring Harbor Laboratory, New York, 2013,). Optionally, a cleavage site can be included in a linker, such as a furin cleavage site.

The nucleic acid encoding a V_H and/or the V_L optionally can encode an Fc domain (immunoadhesin). The Fc domain can be an IgA, IgM or IgG Fc domain. The Fc domain can be an optimized Fc domain, as described in U.S. Published Patent Application No. 20100/093979, incorporated herein by reference. In one example, the immunoadhesin is an IgG_1 Fc.

The single chain antibody may be monovalent, if only a single V_H and V_L are used, bivalent, if two V_H and V_L are used, or polyvalent, if more than two V_H and V_L are used. Bispecific or polyvalent antibodies may be generated that bind specifically to influenza virus HA and another antigen. The encoded V_H and V_L optionally can include a furin cleavage site between the V_H and V_L domains.

Those of skill in the art are knowledgeable in the numerous expression systems available for expression of proteins including *E. coli*, other bacterial hosts, yeast, and various higher eukaryotic cells such as the COS, CHO, HeLa and myeloma cell lines.

One or more DNA sequences encoding the antibodies, antigen binding fragments, or conjugates can be expressed *in vitro* by DNA transfer into a suitable host cell. The cell may be prokaryotic or eukaryotic. The term also includes any progeny of the subject host cell. It is understood that all progeny may not be identical to the parental cell since there may be mutations that occur during replication. Methods of stable transfer, meaning that the foreign DNA is continuously maintained in the host, are known in the art. Hybridomas expressing the antibodies of interest are also encompassed by this disclosure.

The expression of nucleic acids encoding the antibodies and antigen binding fragments described herein can be achieved by operably linking the DNA or cDNA to a promoter (which is either constitutive or inducible), followed by incorporation into an expression cassette. The promoter can be any promoter of interest, including a cytomegalovirus promoter. Optionally, an enhancer, such as a cytomegalovirus enhancer, is included in the construct. The cassettes can be suitable for replication and integration in either prokaryotes or eukaryotes. Typical expression cassettes contain specific sequences useful for regulation of the expression of the DNA encoding the protein. For example, the expression cassettes can include appropriate promoters, enhancers, transcription and translation terminators, initiation sequences, a start codon (*i.e.*, ATG) in front of a protein-encoding gene, splicing signal for introns, sequences for the maintenance of the correct reading frame of that gene to permit proper translation of mRNA, and stop codons. The vector can encode a selectable marker, such as a marker encoding drug resistance (for example, ampicillin or tetracycline resistance).

To obtain high level expression of a cloned gene, it is desirable to construct expression cassettes which contain, at the minimum, a strong promoter to direct transcription, a ribosome binding site for translational initiation (internal ribosomal binding sequences), and a transcription/translation terminator. For *E. coli*, this can include a promoter such as the T7, trp, lac, or lambda promoters, a ribosome binding site, and preferably a transcription termination signal. For eukaryotic cells, the control sequences can include a promoter and/or an enhancer derived from, for example, an immunoglobulin gene, HTLV, SV40 or cytomegalovirus, and a polyadenylation sequence, and can further include splice donor and/or acceptor sequences (for example, CMV and/or HTLV splice acceptor and donor sequences). The cassettes can be transferred into the chosen host cell by well-known methods such as transformation or electroporation for *E. coli* and calcium phosphate treatment, electroporation or lipofection for mammalian cells. Cells transformed by the cassettes can be selected by resistance to antibiotics conferred by genes contained in the cassettes, such as the amp, gpt, neo and hyg genes.

When the host is a eukaryote, such methods of transfection of DNA as calcium phosphate coprecipitates, conventional mechanical procedures such as microinjection, electroporation, insertion of a plasmid encased in liposomes, or virus vectors may be used. Eukaryotic cells can also be cotransformed with polynucleotide sequences encoding the antibody, labeled antibody, or antigen biding fragment, and a second foreign DNA molecule encoding a selectable phenotype, such as the herpes simplex thymidine kinase gene. Another method is to use a eukaryotic viral vector, such as simian virus 40 (SV40) or bovine papilloma virus, to transiently infect or transform eukaryotic cells and express the protein (see for example, Viral Expression Vectors, Springer press, Muzyczka ed., 2011). One of skill in the art can readily use an expression systems such as plasmids and vectors of

use in producing proteins in cells including higher eukaryotic cells such as the COS, CHO, HeLa and myeloma cell lines.

Also provided is a population of cells comprising at least one host cell described herein. The population of cells can be a substantially homogeneous population, in which the population comprises mainly host cells (*e.g.*, consisting essentially of) comprising the recombinant expression vector. The population also can be a clonal population of cells, in which all cells of the population are clones of a single host cell comprising a recombinant expression vector, such that all cells of the population comprise the recombinant expression vector. In one embodiment of the invention, the population of cells is a clonal population comprising host cells comprising a recombinant expression vector as described herein.

Modifications can be made to a nucleic acid encoding a polypeptide described herein without diminishing its biological activity. Some modifications can be made to facilitate the cloning, expression, or incorporation of the targeting molecule into a fusion protein. Such modifications are well known to those of skill in the art and include, for example, termination codons, a methionine added at the amino terminus to provide an initiation, site, additional amino acids placed on either terminus to create conveniently located restriction sites, or additional amino acids (such as poly His) to aid in purification steps. In addition to recombinant methods, the immunoconjugates, effector moieties, and antibodies of the present disclosure can also be constructed in whole or in part using standard peptide synthesis well known in the art.

Once expressed, the antibodies, antigen binding fragments, and conjugates can be purified according to standard procedures in the art, including ammonium sulfate precipitation, affinity columns, column chromatography, and the like (see, generally, Simpson ed., Basic methods in Protein Purification and Analysis: A laboratory Manual, Cold Harbor Press, 2008). The antibodies, antigen binding fragment, and conjugates need not be 100% pure. Once purified, partially or to homogeneity as desired, if to be used therapeutically, the polypeptides should be substantially free of endotoxin.

Methods for expression of the antibodies, antigen binding fragments, and conjugates, and/or refolding to an appropriate active form, from mammalian cells, and bacteria such as *E. coli* have been described and are well-known and are applicable to the antibodies disclosed herein. See, *e.g.*, Harlow and Lane, *Antibodies: A Laboratory Manual*, 2nd, Cold Spring Harbor Laboratory, New York, 2013, Simpson ed., Basic methods in Protein Purification and Analysis: A laboratory Manual, Cold Harbor Press, 2008, and Ward *et al.*, *Nature* 341:544, 1989.

In addition to recombinant methods, the antibodies, antigen binding fragments, and/or conjugates can also be constructed in whole or in part using standard peptide synthesis. Solid phase synthesis of the polypeptides can be accomplished by attaching the C-terminal amino acid of the sequence to an insoluble support followed by sequential addition of the remaining amino acids in the sequence. Techniques for solid phase synthesis are described by Barany & Merrifield, *The Peptides: Analysis, Synthesis, Biology. Vol. 2: Special Methods in Peptide Synthesis, Part A.* pp. 3-284; Merrifield *et al.*, *J. Am. Chem. Soc.* 85:2149-2156, 1963, and Stewart *et al., Solid Phase Peptide Synthesis, 2nd ed.*, Pierce Chem. Co., Rockford, Ill., 1984. Proteins of greater length may be synthesized by condensation of the amino and carboxyl termini of shorter fragments. Methods of forming peptide bonds by activation of a carboxyl terminal end (such as by the use of the coupling reagent N, N'-dicylohexylcarbodimide) are well known in the art.

E. Methods and Compositions

1. Therapeutic methods

Methods are disclosed herein for the prevention or treatment of an influenza A infection. Prevention can include inhibition of infection with influenza A. The methods include administering to a subject a therapeutically effective amount of a disclosed antibody, antigen binding fragment, conjugate, or a nucleic acid encoding such an antibody, antigen binding fragment, or conjugate, to a subject with or at risk of an influenza A infection. For example the methods can be used pre-exposure (for example, to prevent or inhibit influenza A infection) or in post-exposure prophylaxis.

Influenza A infection does not need to be completely eliminated for the method to be effective. For example, a method can decrease influenza A infection by a desired amount, for example by at least 10%, at least 20%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, or even at least 100% (elimination of detectable influenza A infection) as compared to influenza A infection in the absence of the treatment. In some embodiments, the subject can also be treated with a therapeutically effective amount of an additional agent, such as anti-viral agent.

In one embodiment, administration of a disclosed antibody, antigen binding fragment, conjugate, or nucleic acid molecule, results in a reduction in the establishment of influenza A infection and/or reducing subsequent influenza A disease progression in a subject. A reduction in the establishment of influenza A infection and/or a reduction in subsequent influenza A disease progression encompass any statistically significant reduction in influenza A activity.

In some examples, a subject is administered the DNA encoding the antibody or antigen binding fragments thereof, to provide *in vivo* antibody production, for example using the cellular machinery of the subject. Immunization by nucleic acid constructs is well known in the art and taught, for example, in U.S. Patent No. 5,643,578, and U.S. Patent No. 5,593,972 and U.S. Patent No. 5,817,637. U.S. Patent No. 5,880,103 describes several methods of delivery of nucleic acids encoding to an organism. One approach to administration of nucleic acids is direct administration with plasmid DNA, such as with a mammalian expression plasmid. The nucleotide sequence encoding the disclosed antibody, or antigen binding fragments thereof, can be placed under the control of a promoter to increase expression. The methods include liposomal delivery of the nucleic acids. Such methods can be applied to the production of an antibody, or antigen binding fragments thereof, by one of ordinary skill in the art. In some embodiments, a disclosed antibody or antigen binding fragment is expressed in a subject using the pVRC8400 vector (described in Barouch *et al.*, J. Virol, 79 ,8828-8834, 2005, which is incorporated by reference herein).

The nucleic acid molecules encoding the disclosed antibodies or antigen binding fragments can be included in a viral vector, for example for expression of the antibody or antigen binding fragment in a host cell, or a subject (such as a subject with or at risk of influenza A infection). A number of viral vectors have been constructed, that can be used to express the disclosed antibodies or antigen binding fragments, such as a retroviral vector, an adenoviral vector, or an adeno-associated virus (AAV) vector. In several examples, the viral vector can be replication-competent. For example, the viral vector can have a mutation in the viral genome that does not inhibit viral replication in host cells. The viral vector also can be conditionally replication-competent. In other examples, the viral vector is replication-deficient in host cells.

In several embodiments, a subject (such as a human subject with or at risk of influenza A infection) can be administered a therapeutically effective amount of an adeno-associated virus (AAV) viral vector that includes one or more nucleic acid molecules encoding a disclosed antibody or antigen binding fragment. The AAV viral vector is designed for expression of the nucleic acid molecules encoding a disclosed antibody or antigen binding fragment, and administration of the therapeutically effective amount of the AAV viral vector to the subject leads to expression of a therapeutically effective amount of the antibody or antigen binding fragment in the subject. Non-limiting examples of AAV viral vectors that can be used to express a disclosed antibody or antigen binding fragment in a subject include those provided in Johnson *et al* ("Vector-mediated gene transfer engenders long-lived neutralizing activity and protection against SIV infection in monkeys," *Nat. Med.*, 15(8):901-906, 2009) and Gardner *et al.* ("AAV-expressed eCD4-Ig provides durable protection from multiple SHIV challenges," *Nature*, 519(7541): 87-91, 2015), each of which is incorporated by reference herein in its entirety.

In one embodiment, a nucleic acid encoding a disclosed antibody, or antigen binding fragments thereof, is introduced directly into cells. For example, the nucleic acid can be loaded onto gold microspheres by standard methods and introduced into the skin by a device such as Bio-Rad's HELIOS™ Gene Gun. The nucleic acids can be "naked," consisting of plasmids under control of a strong promoter.

Typically, the DNA is injected into muscle, although it can also be injected directly into other sites. Dosages for injection are usually around 0.5 μg/kg to about 50 mg/kg, and typically are about 0.005 mg/kg to about 5 mg/kg (see, *e.g.*, U.S. Patent No. 5,589,466).

2. Dosages

A therapeutically effective amount of an influenza HA-specific antibody, antigen binding fragment, conjugate, or nucleic acid molecule encoding such molecules, will depend upon the severity of the disease and/or infection and the general state of the patient's health. A therapeutically effective amount is that which provides either subjective relief of a symptom(s) or an objectively identifiable improvement as noted by the clinician or other qualified observer. The influenza HA-specific antibody, antigen binding fragment, conjugate, or nucleic acid molecule encoding such molecules, can be administered in conjunction with another therapeutic agent, either simultaneously or sequentially.

Single or multiple administrations of a composition including a disclosed influenza HA-specific antibody, antigen binding fragment, conjugate, or nucleic acid molecule encoding such molecules, can be administered depending on the dosage and frequency as required and tolerated by the patient. Compositions including the influenza HA-specific antibody, antigen binding fragment, conjugate, or nucleic acid molecule encoding such molecules, should provide a sufficient quantity of at least one of the influenza HA-specific antibody, antigen binding fragment, conjugate, or nucleic acid molecule encoding such molecules to effectively treat the patient. The dosage can be administered once, but may be applied periodically until either a therapeutic result is achieved or until side effects warrant discontinuation of therapy. In one example, a dose of the antibody or antigen binding fragment is infused for thirty minutes every other day. In this example, about one to about ten doses can be administered, such as three or six doses can be administered every other day. In a further example, a continuous infusion is administered for about five to about ten days. The subject can be treated at regular intervals, such as monthly, until a desired therapeutic result is achieved. Generally, the dose is sufficient to treat or ameliorate symptoms or signs of disease without producing unacceptable toxicity to the patient.

Data obtained from cell culture assays and animal studies can be used to formulate a range of dosage for use in humans. The dosage normally lies within a range of circulating concentrations that include the ED_{50}, with little or minimal toxicity. The dosage can vary within this range depending upon the dosage form employed and the route of administration utilized. The therapeutically effective dose can be determined from cell culture assays and animal studies.

In certain embodiments, the influenza HA-specific antibody, antigen binding fragment, conjugate, or nucleic acid molecule encoding such molecules, or a composition including such molecules, is administered at a dose in the range of from about 5 or 10 nmol/kg to about 300 nmol/kg, or from about 20 nmol/kg to about 200 nmol/kg, or at a dose of about 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 110, 120, 125, 130, 140, 150, 160, 170, 175, 180, 190, 200, 210, 220, 230, 240, 250, 260, 270, 280, 290, 300, 350, 400, 450, 500, 750, 1000, 1250, 1500, 1750 or 2000 nmol/kg, or at a dose of about 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950 or 1000 μg/kg, or about 1, 1.25, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5 or 10 mg/kg, or other dose deemed appropriate by the treating physician. In some embodiments, the antibody or antigen binding fragment can be administered to a subject at a dose of from about 0.5 to about 40 mg/kg, such as about 1 to about 30, about 1 to about 20, about 1 to about 15, about 1 to about 10, about 1 to about 5, about 1 to about 3, about 0.5 to about 40 mg/kg, such as about 0.5 to about 30, about 0.5 to about 20, about 0.5 to about 15, about 0.5 to about 10, about 0.5 to about 5, about 0.5 to about 3, about 3 to about 7, about 8 to about 12, about 15 to about 25, about 18 to about 22, about 28 to about 32, about 10 to about 20, about 5 to about 15, or about 20 to about 40 mg/kg. The doses described herein can be administered according to the dosing frequency/frequency of administration described herein, including without limitation daily, 2 or 3 times per week, weekly, every 2 weeks, every 3 weeks, monthly, every other month, etc.

In some embodiments, a disclosed therapeutic agent may be administered intravenously, subcutaneously or by another mode daily or multiple times per week for a period of time, followed by a period of no treatment, then the cycle is repeated. In some embodiments, the initial period of treatment (*e.g.*, administration of the therapeutic agent daily or multiple times per week) is for 3 days, 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 7 weeks, 8 weeks, 9 weeks, 10 weeks, 11 weeks or 12 weeks. In a related embodiment, the period of no treatment lasts for 3 days, 1 week, 2 weeks, 3 weeks or 4 weeks. In certain embodiments, the dosing regimen of the therapeutic agent is daily for 3 days followed by 3 days off; or daily or multiple times per week for 1 week followed by 3 days or 1 week off; or daily or multiple times per week for 2 weeks followed by 1 or 2 weeks off; or daily or multiple times per week for 3 weeks followed by 1, 2 or 3 weeks off; or daily or multiple times per week for 4, 5, 6, 7, 8, 9, 10, 11 or 12 weeks followed by 1, 2, 3 or 4 weeks off.

3. Modes of Administration

The influenza HA-specific antibody, antigen binding fragment, conjugate, or nucleic acid molecule encoding such molecules, or a composition including such molecules, as well as additional agents, can be administered to subjects in various ways, including local and systemic administration, such as, *e.g.*, by injection subcutaneously, intravenously, intra-arterially, intraperitoneally, intramuscularly, intradermally, or intrathecally. In an embodiment, a therapeutic agent is administered by a single subcutaneous, intravenous, intra-arterial,

intraperitoneal, intramuscular, intradermal or intrathecal injection once a day. The therapeutic agent can also be administered by direct injection at or near the site of disease.

The influenza HA-specific antibody, antigen binding fragment, conjugate, or nucleic acid molecule encoding such molecules may also be administered orally in the form of microspheres, microcapsules, liposomes (uncharged or charged (*e.g.*, cationic)), polymeric microparticles (*e.g.*, polyamides, polylactide, polyglycolide, poly(lactide-glycolide)), microemulsions, and the like.

A further method of administration is by osmotic pump (*e.g.*, an Alzet pump) or mini-pump (*e.g.*, an Alzet mini-osmotic pump), which allows for controlled, continuous and/or slow-release delivery of the therapeutic agent or pharmaceutical composition over a pre-determined period. The osmotic pump or mini-pump can be implanted subcutaneously, or near a target site.

It will be apparent to one skilled in the art that the influenza HA-specific antibody, antigen binding fragment, conjugate, or nucleic acid molecule encoding such molecules, or a composition including such molecules can also be administered by other modes. Determination of the most effective mode of administration is within the skill of the skilled artisan. The influenza HA-specific antibody, antigen binding fragment, conjugate, or nucleic acid molecule encoding such molecules, or a composition including such molecules can be administered as pharmaceutical formulations suitable for, *e.g.*, oral (including buccal and sub-lingual), rectal, nasal, topical, pulmonary, vaginal or parenteral (including intramuscular, intraarterial, intrathecal, subcutaneous and intravenous) administration, or in a form suitable for administration by inhalation or insufflation. Depending on the intended mode of administration, the pharmaceutical formulations can be in the form of solid, semi-solid or liquid dosage forms, such as tablets, suppositories, pills, capsules, powders, liquids, suspensions, emulsions, creams, ointments, lotions, and the like. The formulations can be provided in unit dosage form suitable for single administration of a precise dosage. The formulations comprise an effective amount of a therapeutic agent, and one or more pharmaceutically acceptable excipients, carriers and/or diluents, and optionally one or more other biologically active agents.

4. Compositions

Compositions are provided that include one or more of the influenza HA-specific antibody, antigen binding fragment, conjugate, or nucleic acid molecule encoding such molecules, that are disclosed herein in a carrier. The compositions are useful, for example, for example, for the treatment or detection of an influenza A infection. The compositions can be prepared in unit dosage forms for administration to a subject. The amount and timing of administration are at the discretion of the treating physician to achieve the desired purposes. The influenza HA-specific antibody, antigen binding fragment, conjugate, or nucleic acid molecule encoding such molecules can be formulated for systemic or local administration. In one example, the influenza HA-specific antibody, antigen binding fragment, conjugate, or nucleic acid molecule encoding such molecules, is formulated for parenteral administration, such as intravenous administration.

In some embodiments, the compositions comprise an antibody, antigen binding fragment, or conjugate thereof, in at least 70% (such as at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% purity. In certain embodiments, the compositions contain less than 10% (such as less than 5%, less than 4%, less than 3%, less than 2%, less than 1%, less than 0.5%, or even less) of macromolecular contaminants, such as other mammalian (*e.g.*, human) proteins.

The compositions for administration can include a solution of the influenza HA-specific antibody, antigen binding fragment, conjugate, or nucleic acid molecule encoding such molecules, dissolved in a pharmaceutically acceptable carrier, such as an aqueous carrier. A variety of aqueous carriers can be used, for example, buffered saline and the like. These solutions are sterile and generally free of undesirable matter. These compositions may be sterilized by conventional, well known sterilization techniques. The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions such as pH adjusting and buffering agents, toxicity adjusting agents and the like, for example, sodium acetate, sodium chloride, potassium chloride, calcium chloride, sodium lactate and the like. The concentration of antibody in these formulations can vary widely, and will be selected primarily based on fluid volumes, viscosities, body weight and the like in accordance with the particular mode of administration selected and the subject's needs.

A typical composition for intravenous administration includes about 0.01 to about 30 mg/kg of antibody or antigen binding fragment or conjugate per subject per day (or the corresponding dose of a conjugate including the antibody or antigen binding fragment). Actual methods for preparing administrable compositions will be known or apparent to those skilled in the art and are described in more detail in such publications as *Remington's Pharmaceutical Science, 22th ed.*, Pharmaceutical Press, London, UK (2012). In some embodiments, the composition can be a liquid formulation including one or more antibodies, antigen binding fragments (such as an antibody or antigen binding fragment that specifically binds to influenza HA), in a concentration range from about 0.1 mg/ml to about 20 mg/ml, or from about 0.5 mg/ml to about 20 mg/ml, or from about 1 mg/ml to about 20 mg/ml, or from about 0.1 mg/ml to about 10 mg/ml, or from about 0.5 mg/ml to about 10 mg/ml, or from about 1 mg/ml to about 10 mg/ml.

Antibodies, or an antigen binding fragment thereof or a conjugate or a nucleic acid encoding such molecules, can be provided in lyophilized form and rehydrated with sterile water before administration, although they are also provided in sterile solutions of known concentration. The antibody solution, or an antigen binding fragment or a nucleic acid encoding such antibodies or antigen binding fragments, can then be added to an infusion bag containing 0.9% sodium chloride, USP, and typically administered at a dosage of from 0.5 to 15 mg/kg of body weight. Considerable experience is available in the art in the administration of antibody drugs, which have been marketed in the U.S. since the approval of RITUXAN® in 1997. Antibodies, antigen binding fragments, conjugates, or a nucleic acid encoding such molecules, can be administered by slow infusion, rather than in an intravenous push or bolus. In one example, a higher loading dose is administered, with subsequent, maintenance doses being administered at a lower level. For example, an initial loading dose of 4 mg/kg may be infused over a period of some 90 minutes, followed by weekly maintenance doses for 4-8 weeks of 2 mg/kg infused over a 30 minute period if the previous dose was well tolerated.

Controlled-release parenteral formulations can be made as implants, oily injections, or as particulate systems. For a broad overview of protein delivery systems see, Banga, A.J., *Therapeutic Peptides and Proteins: Formulation, Processing, and Delivery Systems*, Technomic Publishing Company, Inc., Lancaster, PA, (1995). Particulate systems include microspheres, microparticles, microcapsules, nanocapsules, nanospheres, and nanoparticles. Microcapsules contain the therapeutic protein, such as a cytotoxin or a drug, as a central core. In microspheres the therapeutic is dispersed throughout the particle. Particles, microspheres, and microcapsules smaller than about 1 μm are generally referred to as nanoparticles, nanospheres, and nanocapsules, respectively. Capillaries have a diameter of approximately 5 μm so that only nanoparticles are administered intravenously.

Microparticles are typically around 100 μm in diameter and are administered subcutaneously or intramuscularly. See, for example, Kreuter, J., *Colloidal Drug Delivery Systems*, J. Kreuter, ed., Marcel Dekker, Inc., New York, NY, pp. 219-342 (1994); and Tice & Tabibi, *Treatise on Controlled Drug Delivery*, A. Kydonieus, ed., Marcel Dekker, Inc. New York, NY, pp. 315-339, (1992).

Polymers can be used for ion-controlled release of the antibody compositions disclosed herein. Various degradable and nondegradable polymeric matrices for use in controlled drug delivery are known in the art (Langer, *Accounts Chem. Res.* 26:537-542, 1993). For example, the block copolymer, polaxamer 407, exists as a viscous yet mobile liquid at low temperatures but forms a semisolid gel at body temperature. It has been shown to be an effective vehicle for formulation and sustained delivery of recombinant interleukin-2 and urease (Johnston *et al.*, *Pharm. Res.* 9:425-434, 1992; and Pec *et al., J. Parent. Sci. Tech.* 44(2):58-65, 1990). Alternatively, hydroxyapatite has been used as a microcarrier for controlled release of proteins (Ijntema *et al.*, *Int. J. Pharm.*112:215-224, 1994). In yet another aspect, liposomes are used for controlled release as well as drug targeting of the lipid-capsulated drug (Betageri *et al., Liposome Drug Delivery Systems*, Technomic Publishing Co., Inc., Lancaster, PA (1993)). Numerous additional systems for controlled delivery of therapeutic proteins are known (see U.S. Patent No. 5,055,303; U.S. Patent No. 5,188,837; U.S. Patent No. 4,235,871; U.S. Patent No. 4,501,728; U.S. Patent No. 4,837,028; U.S. Patent No. 4,957,735; U.S. Patent No. 5,019,369; U.S. Patent No. 5,055,303; U.S. Patent No. 5,514,670; U.S. Patent No. 5,413,797; U.S. Patent No. 5,268,164; U.S. Patent No. 5,004,697; U.S. Patent No. 4,902,505; U.S. Patent No. 5,506,206; U.S. Patent No. 5,271,961; U.S. Patent No. 5,254,342 and U.S. Patent No. 5,534,496).

5. Methods of detection and diagnosis

Methods are also provided for the detection of the expression of influenza HA *in vitro* or *in vivo*. In one example, expression of influenza HA is detected in a biological sample, and can be used to detect influenza A infection. The sample can be any sample, including, but not limited to, tissue from biopsies, autopsies and pathology specimens. Biological samples also include sections of tissues, for example, frozen sections taken for histological purposes. Biological samples further include body fluids, such as blood, serum, plasma, sputum, spinal fluid or urine. The method of detection can include contacting a cell or sample, or administering to a subject, an antibody or antigen binding fragment that specifically binds to influenza HA, or conjugate there of (*e.g.*, a conjugate including a detectable marker) under conditions sufficient to form an immune complex, and detecting the immune complex (*e.g.*, by detecting a detectable marker conjugated to the antibody or antigen binding fragment.

In several embodiments, a method is provided for detecting influenza A infection in a subject. The disclosure provides a method for detecting influenza A in a biological sample, wherein the method includes contacting a biological sample from a subject with a disclosed antibody or antigen binding fragment under conditions sufficient for formation of an immune complex, and detecting the immune complex, to detect the influenza HA in the biological sample. In one example, detection of influenza HA in the sample confirms a diagnosis of influenza A infection in the subject.

In some embodiments, the disclosed antibodies or antigen binding fragments thereof are used to test vaccines. For example to test if a vaccine composition including an influenza HA protein or fragment thereof assumes a conformation including the epitope of a disclosed antibody. Thus provided herein is a method for testing a vaccine, wherein the method includes contacting a sample containing the vaccine, such as an influenza HA

immunogen, with a disclosed antibody or antigen binding fragment under conditions sufficient for formation of an immune complex, and detecting the immune complex, to detect the vaccine with an influenza HA immunogen including the epitope in the sample. In one example, the detection of the immune complex in the sample indicates that vaccine component, such as a influenza HA immunogen assumes a conformation capable of binding the antibody or antigen binding fragment.

In one embodiment, the antibody or antigen binding fragment is directly labeled with a detectable marker. In another embodiment, the antibody that binds influenza HA (the first antibody) is unlabeled and a second antibody or other molecule that can bind the antibody that binds the first antibody is utilized for detection. As is well known to one of skill in the art, a second antibody is chosen that is able to specifically bind the specific species and class of the first antibody. For example, if the first antibody is a human IgG, then the secondary antibody may be an anti-human-IgG. Other molecules that can bind to antibodies include, without limitation, Protein A and Protein G, both of which are available commercially.

Suitable labels for the antibody, antigen binding fragment or secondary antibody are described above, and include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, magnetic agents and radioactive materials. Non-limiting examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, beta-galactosidase, or acetylcholinesterase. Non-limiting examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin. Non-limiting examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin. A non-limiting exemplary luminescent material is luminol; a non-limiting exemplary a magnetic agent is gadolinium, and non-limiting exemplary radioactive labels include ^{125}I, ^{131}I, ^{35}S or ^{3}H.

F. Kits

Kits are also provided. For example, kits for treating a subject with an influenza A infection, or for detecting influenza A in a sample or in a subject. The kits will typically include a disclosed influenza HA-specific antibody, antigen binding fragment, conjugate, or nucleic acid molecule encoding such molecules, or compositions including such molecules. More than one of the disclosed influenza HA-specific antibody, antigen binding fragment, conjugate, or nucleic acid molecule encoding such molecules, or compositions including such molecules can be included in the kit.

In one embodiment, the kit is a diagnostic kit and includes an immunoassay. Although the details of the immunoassays may vary with the particular format employed, the method of detecting influenza HA in a biological sample generally includes the steps of contacting the biological sample with an antibody which specifically reacts, under conditions sufficient to form an immune complex, to influenza HA. The antibody is allowed to specifically bind under immunologically reactive conditions to form an immune complex, and the presence of the immune complex (bound antibody) is detected directly or indirectly.

The kit can include a container and a label or package insert on or associated with the container. Suitable containers include, for example, bottles, vials, syringes, etc. The containers may be formed from a variety of materials such as glass or plastic. The container typically holds a composition including one or more of the disclosed antibodies, antigen binding fragments, conjugates, nucleic acid molecules, or compositions. In several embodiments the container may have a sterile access port (for example the container may be an intravenous

solution bag or a vial having a stopper pierceable by a hypodermic injection needle). A label or package insert indicates that the composition is used for treating the particular condition.

The label or package insert typically will further include instructions for use of the antibodies, antigen binding fragments, conjugates, nucleic acid molecules, or compositions included in the kit. The package insert typically includes instructions customarily included in commercial packages of therapeutic products that contain information about the indications, usage, dosage, administration, contraindications and/or warnings concerning the use of such therapeutic products. The instructional materials may be written, in an electronic form (such as a computer diskette or compact disk) or may be visual (such as video files). The kits may also include additional components to facilitate the particular application for which the kit is designed. Thus, for example, the kit may additionally contain means of detecting a label (such as enzyme substrates for enzymatic labels, filter sets to detect fluorescent labels, appropriate secondary labels such as a secondary antibody, or the like). The kits may additionally include buffers and other reagents routinely used for the practice of a particular method. Such kits and appropriate contents are well known to those of skill in the art.

G. Genetic Signatures

Disclosed herein are the structural determinants and ontongeny of several classes of anti-HA antibodies that can neutralize group 1 and group 2 influenza A viruses. These discoveries allowed for elucidation of genetic signatures of antibody variable region genes that correlate with an immune response to influenza A that can neutralize group 1 and group 2 influenza A viruses. Identification of these genetic signatures in a subject immunized with an influenza A vaccine that includes the HA protein indicates that the subject has produced (or is producing) an immune response that neutralizes group 1 and group 2 influenza A viruses. Accordingly, the identified genetic signatures can be used to screen influenza A vaccines and/or vaccine compositions for those that induce an immune response that neutralizes group 1 and group 2 influenza A viruses, as well as to confirm that a subject immunized with an influenza A vaccine has produced an immune response that neutralizes group 1 and group 2 influenza A viruses.

Genetic signature of HV6-1 +HD3-3 class antibodies

As discussed in Example 1, three distinct memory B cell lineages were observed, from subjects 31, 54 and 56, to share heavy chain sequences derived from a recombination of HV6-1 with HD3-3, and HJ4 or HJ5, to yield highly similar amino acid sequences in the CDR H3 and also to share similar neutralization signatures. These three lineages shared the same CDR H3 length (16 amino acids) and a conserved ^{98}MIFGI motif, with a somatic hypermuation at Val100$_a$ILE and Met98 at the V-D junction. Structural analysis of 56.a.09, a prototypic antibody of this class, revealed that heavy chain binding involved the HD3-3-encoded CDR H3 (FIG. 2C) with Phe100$_{HC}$, Gly100a$_{HC}$ and Val100c$_{HC}$ contributing ~240 Å^2 of BSA and the SHM-altered Val100bIle$_{HC}$ inserting directly into the Trp21$_{HA2}$ pocket (contributing over 100 Å^2 of interactive surface). In addition, Met98$_{HC}$ of 56.a.09 interacted with a conserved aromatic residue present on all light chains, helping to orient the CDR H3. These observations suggested this stretch of CDR H3 residues to contribute to the binding of the 56.a.09 antibody to HA.

Thus, for the HV6-1+HD3-3 class, a five amino acid motif (^{98}MIFGI) was identified in addition to HV and HD gene requirements and a CDR H3 length of 16 amino acids as the signature for this class of antibodies. As illustrated in Example 1, this signature can be used to identify a subject with an immune response to an influenza A

vaccine that neutralizes group 1 and group 2 influenza A viruses, and also to identify antibodies that neutralize group 1 and group 2 influenza A viruses.

In some embodiments, a method of identifying a vaccine that induces an immune response in a subject that neutralizes group 1 and group 2 influenza A viruses is provided. The method includes obtaining test sequence reads of nucleic acid molecules encoding antibody heavy chain variable regions from memory B cells from a subject immunized with an influenza A vaccine comprising an HA protein, and screening the test sequence reads for the identified genetic signature of HV6-1+HD3-3 class antibodies that neutralize group 1 and group 2 influenza viruses. Thus, the method can include screening the test sequence reads for nucleic acid sequences encoding a HV6-1/HD3-3 heavy chain variable region comprising an HCDR3 according to IMGT that is 16 amino acids in length, comprises the amino acid sequence set forth as xxxxxxMIFGIxxxxx (SEQ ID NO: 128), wherein x is any amino acid, and is flanked by an N-terminal Cys and a C-terminal Trp. In some embodiments, the HCDR3 comprises the amino acid sequence set forth as $ARX_6SX_7MIFGX_8X_9X_{10}X_{11}X_{12}X_{13}X_{14}X_{15}$ (SEQ ID NO: 118), wherein X_6 is Gly, Ala, or Val; X_7 is Ala, Phe, Val, or Pro; X_8 is Ile; X_9 is Val, Leu, or Asp; X_{10} is Met, Ile, or Val; X_{11} is Gly or no amino acid; X_{12} is Ala, Ile, Val, or Glu; X_{13} is Phe, Leu, or Met; X_{14} is Asp or Glu; X_{15} is Gln, Phe, Cys, Ser, Tyr, or Leu. Identification of the genetic signature of HV6-1+HD3-3 class antibodies in the test sequence reads indicates that the vaccine can induce an immune response in a subject that neutralizes group 1 and group 2 influenza A viruses.

In some embodiments, the method can further comprise obtaining control sequence reads of nucleic acid molecules encoding antibody heavy chain variable regions from memory B cells from one or more subjects that have not been immunized with the influenza A vaccine comprising the HA protein, and screening the control sequence reads for nucleic acid sequences encoding the identified genetic signature of HV6-1+HD3-3 class antibodies that neutralize group 1 and group 2 influenza viruses. If a ratio of the presence of nucleic acid sequences encoding the identified genetic signature of HV6-1+HD3-3 class antibodies that neutralize group 1 and group 2 influenza viruses in the test sequence reads to the control sequence reads is greater than 2:1 (such as greater than 5:1 or greater than 10:1), then the vaccine has induced an immune response in a subject that neutralizes group 1 and group 2 influenza A viruses.

Genetic signature of HV1-18+HD3-9 class antibodies

As discussed in Example 1, two distinct memory B cell lineages from subjects 1 and 31 were observed to share immunoglobulin heavy chain sequence derived from recombination of HV1-18 with HD3-9 and HJ4, to have highly similar amino acid sequences in a CDR H3 of 15-amino acids in length, and to share similar neutralization signatures. Notably an $Arg96_{HC}$ residue was encoded by n-nucleotide addition in both cases and a ^{99}ILTG motif was conserved in both cases.

Thus, for the HV1-18+HD3-9 class of antibodies a seven amino acid signature (R-x-x-I-L-T-G, beginning with $R96_{HC}$) was identified in addition to HV and HD gene requirements and a CDR H3 length of 15 amino acids as the signature for this class of antibodies. As illustrated in Example 1, this signature can be used to identify a subject with an immune response to an influenza A vaccine that neutralizes group 1 and group 2 influenza A viruses, and also to identify antibodies that neutralize group 1 and group 2 influenza A viruses.

In some embodiments, a method of identifying a vaccine that induces an immune response in a subject that neutralizes group 1 and group 2 influenza A viruses is provided. The method includes obtaining test sequence

reads of nucleic acid molecules encoding antibody heavy chain variable regions from memory B cells from a subject immunized with an influenza A vaccine comprising an HA protein, and screening the test sequence reads for the identified genetic signature of HV1-18+HD3-9 class antibodies that neutralize group 1 and group 2 influenza viruses. The method can include screening the test sequence reads for nucleic acid sequences encoding a HV1-18+HD3-9 heavy chain variable region comprising an HCDR3 according to IMGT that is 15 amino acids in length, comprises the amino acid sequence set forth as xxxRxxILTGxxxxx (SEQ ID NO: 129), wherein x is any amino acid, and is flanked by an N-terminal Cys and a C-terminal Trp. In some embodiments, the HCDR3 comprises the amino acid sequence set forth as $X_{10}RDX_{11}X_{12}X_{13}ILTGX_{14}X_{15}X_{16}DX_{17}$ (SEQ ID NO: 124), wherein X_{10} is Ala or Thr; X_{11} is Gln or Arg; X_{12} is Arg, Tyr, Gly, Phe, Ser, or Pro; X_{13} is Asp, Ala, Asn, Thr, or His; X_{14} is Gly, Pro, Ser, Tyr, Asp, Phe, or Cys; X_{15} is Leu, Asn, Ala, Arg, Asp, His, or Gln; X_{16} is Phe, Gly, Thr, Asp, or Leu; X_{17} is Cys, Tyr, His, Asp, Phe, Ser, or Ile. Identification of the genetic signature of HV1-18+HD3-9 class antibodies in the test sequence reads indicates that the vaccine can induce an immune response in a subject that neutralizes group 1 and group 2 influenza A viruses.

In some embodiments, the method can further comprise obtaining control sequence reads of nucleic acid molecules encoding antibody heavy chain variable regions from memory B cells from one or more subjects that have not been immunized with the influenza A vaccine comprising the HA protein, and screening the control sequence reads for nucleic acid sequences encoding the identified genetic signature of the HV1-18+HD3-9 class of antibodies that neutralize group 1 and group 2 influenza viruses. If a ratio of the presence of nucleic acid sequences encoding the identified genetic signature of the HV1-18+HD3-9 class of antibodies that neutralize group 1 and group 2 influenza viruses in the test sequence reads to the control sequence reads is greater than 2:1 (such as greater than 5:1 or greater than 10:1), then the vaccine has induced an immune response in a subject that neutralizes group 1 and group 2 influenza A viruses.

Genetic signature of HV1-18 (Q-x-x-V) class antibodies

As illustrated in Example 1, multiple B cell lineages were observed in two subjects (16 and 54) to produce distinctive HV1-18-derived immunoglobulins sharing five genetic elements and having a CDR H3 of 21 amino acids derived from recombination with either HD2-2 or HD2-15 genes, and to share similar neutralization signatures. These sequences all contained a ^{98}Q-x-x-V (SEQ ID NO: 130) motif in CDR H3, and crystal structures of two prototypic antibodies, 16.a.26 and 16.g.09, displayed similar recognition modes. The location of $Gln98_{HC}$ within the framework pocket likely stabilized the perpendicular orientation of the CDR H3 relative to the antibody-framework regions and may allow for CDR H3-motifs derived from diverse D genes to bind to this conserved hydrophobic groove. The conserved $Val100a_{HC}$ inserted into a pocket present on both group 1 and group 2 HA. Further, $Tyr53_{HC}$, which is encoded in HV1-18 germline gene, contributed the largest BSA among all heavy chain residues, and $Thr54_{HC}$, a conserved SHM-change, was present in 309 of the 318 VRC 310 sequences. Additionally, antibodies from subject 01 with an HV1-18 germline gene and a Gln98-x-x-Val100a motif in a 17 amino acid CDR H3 were able to neutralize H3 and H5 strains of influenza.

Thus, for the HV1-18 (Q-x-x-V) class, a 2-residue CDR H2 signature ($Tyr53_{HC}$ and $Thr54_{HC}$) was combined with a four amino acid CDR H3 signature (Q-x-x-V (SEQ ID NO: 130), beginning with $Q98_{HC}$), in addition to the HV1-18 germline requirement and a CDR H3 length between 17 and 21 amino acids, as the signature for this class of antibodies.

In some embodiments, a method of identifying a vaccine that induces an immune response in a subject that neutralizes group 1 and group 2 influenza A viruses is provided. The method includes obtaining test sequence reads of nucleic acid molecules encoding antibody heavy chain variable regions from memory B cells from a subject immunized with an influenza A vaccine comprising an HA protein, and screening the test sequence reads for the identified genetic signature of HV1-18 (Q-x-x-V) class antibodies that neutralize group 1 and group 2 influenza viruses. The method can include screening the test sequence reads for nucleic acid sequences encoding a HV1-18 heavy chain variable region comprising a tyrosine at kabat position 53, a threonine at kabat position 54 and a HCDR3 according to IMGT that is 17-21 amino acids in length, comprises the amino acid sequence set forth as xxxxxQxxV$(x)_n$ (SEQ ID NO: 131), wherein x is any amino acid and n is 8-12, and is flanked by an N-terminal Cys and a C-terminal Trp. Identification of the genetic signature of HV1-18+HD3-9 class antibodies in the test sequence reads indicates that the vaccine can induce an immune response in a subject that neutralizes group 1 and group 2 influenza A viruses.

In some embodiments, the method can further comprise obtaining control sequence reads of nucleic acid molecules encoding antibody heavy chain variable regions from memory B cells from one or more subjects that have not been immunized with the influenza A vaccine comprising the HA protein, and screening the control sequence reads for nucleic acid sequences encoding the identified genetic signature of the HV1-18 (Q-x-x-V) class antibodies that neutralize group 1 and group 2 influenza viruses. If a ratio of the presence of nucleic acid sequences encoding the identified genetic signature of the HV1-18 (Q-x-x-V) class antibodies that neutralize group 1 and group 2 influenza viruses in the test sequence reads to the control sequence reads is greater than 2:1 (such as greater than 5:1 or greater than 10:1), then the vaccine has induced an immune response in a subject that neutralizes group 1 and group 2 influenza A viruses.

Additional description

Memory B cells can be isolated from biological samples such as blood or a fraction of the blood, according to methods known in the art. For example, memory B cells can be isolated from whole blood, serum, or PBMCs (peripheral blood mononuclear cells) by various cell separation methods known in the art, such as differential centrifugation, filtration, flow cytometry sorting, immuno-affinity techniques; or magnetic sorting. The memory B cells may be isolated from other subsets of B cells using cell surface markers by using positive or negative selection procedures. In several embodiments, the method can comprise collecting the memory B cells from the subject following immunization with the vaccine. In some embodiments, the B cells can be isolated from a subject from one week to six months (such as one week to four weeks) following immunization with the influenza vaccine. In some embodiments, the B cells can be isolated from a subject at least two weeks (such as such as 2 weeks to six months, or 2 weeks, 3 weeks, 4 weeks, or 5 weeks), following immunization with the influenza vaccine

In several embodiments, nucleic acid molecules are isolated from the memory B cells. For example, genomic DNA is isolated from the sample and the genomic sequences which encode the antibodies are analyzed or sequenced by various DNA sequencing methods known in the art (i.e., next generation sequencing platforms). In another embodiment, RNA (i.e., mRNA) can be isolated from the B cells and reverse-transcribed using art-recognized methods into cDNA. Isolated nucleic acid can be amplified as needed, for example, using the "Vh_all" family of primers (Doria-Rose *et al., Nature,* 509, 55-62, 2014) as described in (Zhu *et al., PNAS,* 110, E4088-

4097, 2013) or through the use of 5' RACE as described in Bonsignori *et al.*, (Maturation pathway from germline to broad HIV-1 neutralizer of a CD4-Mimic antibody, Cell, 2016).

The antibody genetic signature of the B cells or the sample can be determined by nucleic acid analysis techniques. Non-limiting examples of suitable nucleic acid analysis techniques include RT-PCR, quantitative PCR analysis, and next generation sequencing technologies, including Illumina sequencing platforms, Solexa sequencing platforms, 454 pyrosequencing, SOLiD, Ion Torrent (proton), PacBio SMRT, or Nanopore.

In some embodiments, nucleic acids from B cells may be sequenced to generate sequence reads. In some cases, a full or substantially full sequence is obtained and sometimes a partial sequence is obtained. Sequencing, mapping and related analytical methods are known in the art (*e.g.*, United States Patent Application Publication US2009/0029377, incorporated by reference). Certain aspects of such processes are described hereafter.

As used herein, "reads" (i.e., "a read", "a sequence read") are short nucleotide sequences produced by any sequencing process described herein or known in the art. Reads can be generated from one end of nucleic acid fragments ("single-end reads"), and sometimes are generated from both ends of nucleic acids (*e.g.*, paired-end reads, double-end reads).

Reads generally are representations of nucleotide sequences in a physical nucleic acid. For example, in a read containing an ATGC depiction of a sequence, "A" represents an adenine nucleotide, "T" represents a thymine nucleotide, "G" represents a guanine nucleotide and "C" represents a cytosine nucleotide, in a physical nucleic acid. In certain embodiments, "obtaining" sequence reads of a sample from a subject and/or "obtaining" sequence reads of a biological specimen from one or more reference persons can involve directly sequencing nucleic acid to obtain the sequence information. In some embodiments, "obtaining" can involve receiving sequence information obtained directly from a nucleic acid by another.

The number of sequence reads in a data base of sequence (*e.g.*, from B-cells from an individual) having a specified sequence or "signature" can be referred to as counts. In some embodiments, counts can be manipulated or transformed (*e.g.*, normalized, combined, added, filtered, selected, averaged, derived as a mean, the like, or a combination thereof). In some embodiments, counts can be transformed to produce normalized counts.

In some embodiments, one nucleic acid sample from one individual is sequenced. In certain embodiments, nucleic acid samples from two or more biological samples, where each biological sample is from one individual or two or more individuals, are pooled and the pool is sequenced. In the latter embodiments, a nucleic acid sample from each biological sample often is identified by one or more unique identification tags.

Any sequencing method suitable for conducting methods described herein can be utilized. In some embodiments, a high-throughput sequencing method is used. High-throughput sequencing methods generally involve clonally amplified DNA templates or single DNA molecules that are sequenced in a massively parallel fashion within a flow cell (*e.g.*, as described in Metzker M Nature Rev 11:31-46 (2010); Volkerding *et al.* Clin. Chem. 55:641-658 (2009)). Such sequencing methods also can provide digital quantitative information, where each sequence read is a countable "sequence tag" or "count" representing an individual clonal DNA template, a single DNA molecule, bin or chromosome. Next generation sequencing techniques capable of sequencing DNA in a massively parallel fashion are collectively referred to herein as "massively parallel sequencing" (MPS). High-throughput sequencing technologies include, for example, sequencing-by-synthesis with reversible dye terminators, sequencing by oligonucleotide probe ligation, pyrosequencing and real time sequencing. Non-limiting examples of MPS include Massively Parallel Signature Sequencing (MPSS), Polony sequencing, Pyrosequencing, Illumina

(Solexa) sequencing, SOLiD sequencing, Ion semiconductor sequencing, DNA nanoball sequencing, Helioscope single molecule sequencing, single molecule real time (SMRT) sequencing, nanopore sequencing, ION Torrent and RNA polymerase (RNAP) sequencing.

Systems utilized for high-throughput sequencing methods are commercially available and include, for example, the Roche 454 platform, the Applied Biosystems SOLID platform, the Helicos True Single Molecule DNA sequencing technology, the sequencing-by-hybridization platform from Affymetrix Inc., the single molecule, real-time (SMRT) technology of Pacific Biosciences, the sequencing-by-synthesis platforms from 454 Life Sciences, Illumina/Solexa and Helicos Biosciences, and the sequencing-by-ligation platform from Applied Biosystems. The ION TORRENT technology from Life technologies and nanopore sequencing also can be used in high-throughput sequencing approaches.

In some embodiments, first generation technology, such as, for example, Sanger sequencing including the automated Sanger sequencing, can be used in a method provided herein. Additional sequencing technologies that include the use of developing nucleic acid imaging technologies (*e.g.*, transmission electron microscopy (TEM) and atomic force microscopy (AFM)), also are contemplated herein. Examples of various sequencing technologies are described below.

Antibody variable region sequences obtained by sequencing the isolated B-cells can be filtered (*e.g.*, by minimum read length) and processed as needed before analysis for genetic signature. Germline genes are assigned to sequence reads using available methods, such as IgBlast (Ye *et al.*, *Nucleic acids research,* 41, W34-40, 2013). As discussed above, in several embodiments, sequence reads containing the heavy chain germline genes HV6-1 and HV1-18 are placed into one of three classes based on their heavy chain germline gene assignment, CDR H3 length (measured between the canonical cysteine and tryptophan) and a sequence signature in the CDR H3 junction. For the first class, all reads are required to contain the HV6-1 and HD3-3 germline gene assignments along with a CDR H3 length of 16. In addition, the following sequence signature in the CDR H3 junction (where 'x' denotes any amino acid) is required: xxxxxMIFGIxxxxxx (SEQ ID NO: 128) flanked by an N-terminal Cys and a C-terminal Trp. For the second class, all sequences had the HV1-18 germline gene assignment and a CDR H3 length of 15. The specific sequence signature associated with the second class was (where 'x' denotes any amino acid): xxxRxxILTGxxxxx (SEQ ID NO: 129), flanked by an N-terminal Cys and a C-terminal Trp. For the third and final class, all sequences had the HV1-18 germline gene assignment and a CDR H3 length of between 17-21 amino acids. The sequence signature associated with the third class was (where 'x' denotes any amino acid): xxxxxQxxV$(x)_n$ (SEQ ID NO: 131), where n=8-12, flanked by an N-terminal Cys and a C-terminal Trp. In addition to the CDR H3 sequence signature, residues 53 and 54 (kabat numbering) are required to be a tyrosine and threonine, receptively. Identifying the indicated antibody heavy chain signatures indicates that the B cells from which the nucleic acid molecules were obtained express antibodies that neutralize group 1 and group 2 influenza A viruses.

Also provided are methods for analyzing the occurrence or frequency of anti-influenza antibodies elicited in pre-vaccinated and post-vaccinated B-cell samples from subjects to determine the efficacy of a particular influenza vaccine for a particular subject. Specifically, B cell samples from the subject are obtained and prepared using methods known in the art to analyze secreted immunoglobulins before vaccination and after vaccination. The presence or absence, levels, or frequency of anti-influenza antibodies that fall within one of the disclosed classes of broadly neutralizing influenza antibodies can be determined by assaying for the genetic signature of such

antibodies. A higher level or frequency of antibodies having one or more structural determinant after vaccination indicates that the vaccine was successful in the elicitation of broadly neutralizing HA antibodies that can neutralize group 1 and group 2 influenza A viruses.

Also provided are methods for screening an immunogen or vaccine composition to induce broadly neutralizing influenza virus antibodies that bind to the influenza HA protein by presenting the immunogen or vaccine composition to a population of B cells under conditions for elicitation of antibodies from the B cells, and determining the presence or absence or level of antibodies secreted from the B cells that fall within one of the classes of broadly neutralizing anti-influenza antibodies as described herein. These methods may be particularly useful for identifying immunogens or vaccine compositions that can or cannot elicit such broadly neutralizing antibodies. In one embodiment, the B cells are in culture and antibody production is induced in culture. Methods for eliciting antibody production from a population of B cells in culture are well known. In some aspects, methods for eliciting antibody production may involve using antigen-presenting cells (i.e., dendritic cells) that present the immunogen or vaccine composition to be tested. In another embodiment, the population of B cells is in a subject, such as a human subject, or a recombinant mouse having human antibody variable region genes.

Furthermore, the identification of a subject that has been responsive to an influenza vaccine can also be determined by the presence or absence of a disclosed antibody genetic signature.

Next generation sequencing can also be used to determine the presence, absence, or level of any of the nucleic acid sequences described herein that contribute to the genetic signature. In some embodiments, it is useful to calculate a ratio or absolute frequency of immunoglobulins that have the broadly neutralizing antibody genetic signature for each population of B cells tested. The comparison of these ratios or absolute frequency, for example between naive and memory B cell populations, indicates whether the subject has had prior immunologic exposure and memory to influenza virus. Alternatively, the ratio or absolute frequency indicates the antigen responsiveness of a subject to a vaccine or influenza virus vaccine. These ratios can be derived from analysis of antibody sequence libraries generated by next generation sequencing.

Machines, Software and Interfaces

Certain processes and methods described herein often cannot be performed without a computer, processor, software, module or other apparatus. Methods described herein can be computer-implemented methods, and one or more portions of a method sometimes are performed by one or more processors. Embodiments pertaining to methods described in this document generally are applicable to the same or related processes implemented by instructions in systems, apparatus and computer program products described herein. In some embodiments, processes and methods described herein (*e.g.*, quantifying, counting and/or determining sequence reads, counts, elevations and/or profiles) are performed by automated methods. In some embodiments, an automated method is embodied in software, modules, processors, peripherals and/or an apparatus comprising the like, that determine sequence reads, counts, mapping, mapped sequence tags, elevations, profiles, normalizations, comparisons, range setting, categorization, adjustments, plotting, outcomes, transformations and identifications. As used herein, software refers to computer readable program instructions that, when executed by a processor, perform computer operations, as described herein.

Sequence reads and counts derived from a test subject (*e.g.*, a subject immunized with an influenza A vaccine) and/or from a reference subject can be further analyzed and processed to determine the presence or

absence of a antibody genetic signature variation. In some embodiments, data or data sets of sequence reads and counts can be characterized by one or more features or variables (*e.g.*, a particular heavy or light chain germline origin, a particular genetic signature, etc.). In certain embodiments, data or data sets of sequence reads and counts can be organized into a matrix having two or more dimensions based on one or more features or variables. Data organized into matrices can be organized using any suitable features or variables. A non-limiting example of data in a matrix includes data that is organized by immunization status and presence or absence of genetic signature for one of the classes of broadly neutralizing antibodies as described herein. In certain embodiments, data sets characterized by one or more features or variables sometimes are processed after counting.

Apparatuses, software and interfaces may be used to conduct methods described herein. Using apparatuses, software and interfaces, a user may enter, request, query or determine options for using particular information, programs or processes (*e.g.*, processing sequence reads for the presence or absence of a genetic signature of interest), which can involve implementing statistical analysis algorithms, statistical significance algorithms, statistical algorithms, iterative steps, validation algorithms, and graphical representations, for example. In some embodiments, a data set may be entered by a user as input information, a user may download one or more data sets by a suitable hardware media (*e.g.*, flash drive), and/or a user may send a data set from one system to another for subsequent processing and/or providing an outcome (*e.g.*, send sequence read data from a sequencer to a computer system for sequence read analysis; send analyzed sequence data to a computer system for processing and yielding an outcome and/or report).

A system typically comprises one or more apparatus. Each apparatus comprises one or more of memory, one or more processors, and instructions. Where a system includes two or more apparatus, some or all of the apparatus may be located at the same location, some or all of the apparatus may be located at different locations, all of the apparatus may be located at one location and/or all of the apparatus may be located at different locations. Where a system includes two or more apparatus, some or all of the apparatus may be located at the same location as a user, some or all of the apparatus may be located at a location different than a user, all of the apparatus may be located at the same location as the user, and/or all of the apparatus may be located at one or more locations different than the user.

A system sometimes comprises a computing apparatus and a sequencing apparatus, where the sequencing apparatus is configured to receive physical nucleic acid and generate sequence reads, and the computing apparatus is configured to process the reads from the sequencing apparatus. The computing apparatus sometimes is configured to determine the presence or absence of a genetic signature of a class of antibodies that neutralizes influenza A as described herein from the sequence reads.

A user may, for example, place a query to software which then may acquire a data set via internet access, and in certain embodiments, a programmable processor may be prompted to acquire a suitable data set based on given parameters. A programmable processor also may prompt a user to select one or more data set options selected by the processor based on given parameters. A programmable processor may prompt a user to select one or more data set options selected by the processor based on information found via the internet, other internal or external information, or the like. Options may be chosen for selecting one or more data feature selections, one or more statistical algorithms, one or more statistical analysis algorithms, one or more statistical significance algorithms, iterative steps, one or more validation algorithms, and one or more graphical representations of methods, apparatuses, or computer programs.

Systems addressed herein may comprise general components of computer systems, such as, for example, network servers, laptop systems, desktop systems, handheld systems, personal digital assistants, computing kiosks, and the like. A computer system may comprise one or more input means such as a keyboard, touch screen, mouse, voice recognition or other means to allow the user to enter data into the system. A system may further comprise one or more outputs, including, but not limited to, a display screen (*e.g.*, CRT or LCD), speaker, FAX machine, printer (*e.g.*, laser, ink jet, impact, black and white or color printer), or other output useful for providing visual, auditory and/or hardcopy output of information (*e.g.*, outcome and/or report).

In a system, input and output means may be connected to a central processing unit which may comprise among other components, a microprocessor for executing program instructions and memory for storing program code and data. In some embodiments, processes may be implemented as a single user system located in a single geographical site. In certain embodiments, processes may be implemented as a multi-user system. In the case of a multi-user implementation, multiple central processing units may be connected by means of a network. The network may be local, encompassing a single department in one portion of a building, an entire building, span multiple buildings, span a region, span an entire country or be worldwide. The network may be private, being owned and controlled by a provider, or it may be implemented as an internet based service where the user accesses a web page to enter and retrieve information. Accordingly, in certain embodiments, a system includes one or more machines, which may be local or remote with respect to a user. More than one machine in one location or multiple locations may be accessed by a user, and data may be mapped and/or processed in series and/or in parallel. Thus, a suitable configuration and control may be utilized for mapping and/or processing data using multiple machines, such as in local network, remote network and/or "cloud" computing platforms.

A system can include a communications interface in some embodiments. A communications interface allows for transfer of software and data between a computer system and one or more external devices. Non-limiting examples of communications interfaces include a modem, a network interface (such as an Ethernet card), a communications port, a PCMCIA slot and card, and the like. Software and data transferred via a communications interface generally are in the form of signals, which can be electronic, electromagnetic, optical and/or other signals capable of being received by a communications interface. Signals often are provided to a communications interface via a channel. A channel often carries signals and can be implemented using wire or cable, fiber optics, a phone line, a cellular phone link, an RF link and/or other communications channels. Thus, in an example, a communications interface may be used to receive signal information that can be detected by a signal detection module.

Data may be input by a suitable device and/or method, including, but not limited to, manual input devices or direct data entry devices (DDEs). Non-limiting examples of manual devices include keyboards, concept keyboards, touch sensitive screens, light pens, mouse, tracker balls, joysticks, graphic tablets, scanners, digital cameras, video digitizers and voice recognition devices. Non-limiting examples of DDEs include bar code readers, magnetic strip codes, smart cards, magnetic ink character recognition, optical character recognition, optical mark recognition, and turnaround documents.

In some embodiments, output from a sequencing apparatus may serve as data that can be input via an input device. In certain embodiments, analyzed sequence reads may serve as data that can be input via an input device. In certain embodiments, simulated data is generated by an in silico process and the simulated data serves as data that can be input via an input device. The term "in silico" refers to research and experiments performed using a

computer. In silico processes include, but are not limited to, analyzing sequence reads and processing analyzed sequence reads according to processes described herein.

A system may include software useful for performing a process described herein, and software can include one or more modules for performing such processes (*e.g.*, sequencing module, logic processing module, data display organization module). The term "software" refers to computer readable program instructions that, when executed by a computer, perform computer operations. Instructions executable by the one or more processors sometimes are provided as executable code, that when executed, can cause one or more processors to implement a method described herein. A module described herein can exist as software, and instructions (*e.g.*, processes, routines, subroutines) embodied in the software can be implemented or performed by a processor. For example, a module (*e.g.*, a software module) can be a part of a program that performs a particular process or task. The term "module" refers to a self-contained functional unit that can be used in a larger apparatus or software system. A module can comprise a set of instructions for carrying out a function of the module. A module can transform data and/or information. Data and/or information can be in a suitable form. For example, data and/or information can be digital or analogue. In some cases, data and/or information can be packets, bytes, characters, or bits. In some embodiments, data and/or information can be any gathered, assembled or usable data or information. Non-limiting examples of data and/or information include a suitable media, pictures, video, sound (*e.g.*, frequencies, audible or non-audible), numbers, constants, a value, objects, time, functions, instructions, maps, references, sequences, reads, analyzed reads, signals, displays, representations, or transformations thereof. A module can accept or receive data and/or information, transform the data and/or information into a second form, and provide or transfer the second form to an apparatus, peripheral, component or another module. A module can perform one or more of the following non-limiting functions: analyzing sequence reads for genetic signature, providing counts, providing a count profile, normalizing (*e.g.*, normalizing reads, normalizing counts, and the like), providing a normalized count profile, providing uncertainty values, categorizing, plotting, and/or determining an outcome, for example. A processor can, in some cases, carry out the instructions in a module. In some embodiments, one or more processors are required to carry out instructions in a module or group of modules. A module can provide data and/or information to another module, apparatus or source and can receive data and/or information from another module, apparatus or source.

A computer program product sometimes is embodied on a tangible computer-readable medium, and sometimes is tangibly embodied on a non-transitory computer-readable medium. A module sometimes is stored on a computer readable medium (*e.g.*, disk, drive) or in memory (*e.g.*, random access memory). A module and processor capable of implementing instructions from a module can be located in an apparatus or in different apparatus. A module and/or processor capable of implementing an instruction for a module can be located in the same location as a user (*e.g.*, local network) or in a different location from a user (*e.g.*, remote network, cloud system). In embodiments in which a method is carried out in conjunction with two or more modules, the modules can be located in the same apparatus, one or more modules can be located in different apparatus in the same physical location, and one or more modules may be located in different apparatus in different physical locations.

An apparatus, in some embodiments, comprises at least one processor for carrying out the instructions in a module. Counts of sequence reads analyzed for a genetic signature as described herein sometimes are accessed by a processor that executes instructions configured to carry out a method described herein. Counts that are accessed by a processor can be within memory of a system, and the counts can be accessed and placed into the memory of

the system after they are obtained. In some embodiments, an apparatus includes a processor (*e.g.*, one or more processors) which processor can perform and/or implement one or more instructions (*e.g.*, processes, routines and/or subroutines) from a module. In some embodiments, an apparatus includes multiple processors, such as processors coordinated and working in parallel. In some embodiments, an apparatus operates with one or more external processors (*e.g.*, an internal or external network, server, storage device and/or storage network (*e.g.*, a cloud)). In some embodiments, an apparatus comprises a module. Sometimes an apparatus comprises one or more modules. An apparatus comprising a module often can receive and transfer one or more of data and/or information to and from other modules. In some cases, an apparatus comprises peripherals and/or components. Sometimes an apparatus can comprise one or more peripherals or components that can transfer data and/or information to and from other modules, peripherals and/or components. Sometimes an apparatus interacts with a peripheral and/or component that provides data and/or information. Sometimes peripherals and components assist an apparatus in carrying out a function or interact directly with a module. Non-limiting examples of peripherals and/or components include a suitable computer peripheral, I/O or storage method or device including but not limited to scanners, printers, displays (*e.g.*, monitors, LED, LCT or CRTs), cameras, microphones, pads (*e.g.*, ipads, tablets), touch screens, smart phones, mobile phones, USB I/O devices, USB mass storage devices, keyboards, a computer mouse, digital pens, modems, hard drives, jump drives, flash drives, a processor, a server, CDs, DVDs, graphic cards, specialized I/O devices (*e.g.*, sequencers, photo cells, photo multiplier tubes, optical readers, sensors, etc.), one or more flow cells, fluid handling components, network interface controllers, ROM, RAM, wireless transfer methods and devices (Bluetooth, WiFi, and the like,), the world wide web (www), the internet, a computer and/or another module.

One or more of a sequencing module, logic processing module and data display organization module can be utilized in a method described herein. Sometimes a logic processing module, sequencing module or data display organization module, or an apparatus comprising one or more such modules, gather, assemble, receive, provide and/or transfer data and/or information to or from another module, apparatus, component, peripheral or operator of an apparatus. For example, sometimes an operator of an apparatus provides a constant, a threshold value, a formula or a predetermined value to a logic processing module, sequencing module or data display organization module. A logic processing module, sequencing module or data display organization module can receive data and/or information from another module, non-limiting examples of which include a logic processing module, sequencing module, data display organization module, sequencing module, counting module, normalization module, comparison module, range setting module, categorization module, adjustment module, plotting module, outcome module, data display organization module and/or logic processing module, the like or combination thereof. Data and/or information derived from or transformed by a logic processing module, sequencing module or data display organization module can be transferred from a logic processing module, sequencing module or data display organization module to a sequencing module, counting module, normalization module, comparison module, range setting module, categorization module, adjustment module, plotting module, outcome module, data display organization module, logic processing module or other suitable apparatus and/or module. A sequencing module can receive data and/or information form a logic processing module and/or sequencing module and transfer data and/or information to a logic processing module and/or a mapping module, for example. Sometimes a logic processing module orchestrates, controls, limits, organizes, orders, distributes, partitions, transforms and/or regulates data and/or information or the transfer of data and/or information to and from one or more other modules,

peripherals or devices. A data display organization module can receive data and/or information form a logic processing module and/or plotting module and transfer data and/or information to a logic processing module, plotting module, display, peripheral or device. An apparatus comprising a logic processing module, sequencing module or data display organization module can comprise at least one processor. In some embodiments, data and/or information are provided by an apparatus that includes a processor (*e.g.*, one or more processors) which processor can perform and/or implement one or more instructions (*e.g.*, processes, routines and/or subroutines) from the logic processing module, sequencing module and/or data display organization module. In some embodiments, a logic processing module, sequencing module or data display organization module operates with one or more external processors (*e.g.*, an internal or external network, server, storage device and/or storage network (*e.g.*, a cloud)).

Software often is provided on a program product containing program instructions recorded on a computer readable medium, including, but not limited to, magnetic media including floppy disks, hard disks, and magnetic tape; and optical media including CD-ROM discs, DVD discs, magneto-optical discs, flash drives, RAM, floppy discs, the like, and other such media on which the program instructions can be recorded. In online implementation, a server and web site maintained by an organization can be configured to provide software downloads to remote users, or remote users may access a remote system maintained by an organization to remotely access software. Software may obtain or receive input information. Software may include a module that specifically obtains or receives data (*e.g.*, a data receiving module that receives sequence read data and/or data for sequence reads analyzed for a genetic signature as described herein) and may include a module that specifically processes the data (*e.g.*, a processing module that processes received data (*e.g.*, filters, normalizes, provides an outcome and/or report). The terms "obtaining" and "receiving" input information refers to receiving data (*e.g.*, sequence reads, mapped reads) by computer communication means from a local, or remote site, human data entry, or any other method of receiving data. The input information may be generated in the same location at which it is received, or it may be generated in a different location and transmitted to the receiving location. In some embodiments, input information is modified before it is processed (*e.g.*, placed into a format amenable to processing (*e.g.*, tabulated)). In some embodiments, provided are computer program products, such as, for example, a computer program product comprising a computer usable medium having a computer readable program code embodied therein, the computer readable program code adapted to be executed to implement a method comprising: (a) obtaining sequence reads of sample nucleic acid from a test subject; (b) screening the sequence reads obtained in (a) for a genetic signature as described herein; (c) counting the sequence reads that match an identified genomic signature; (d) generating a sample normalized count profile by normalizing the counts obtained in (c); and (e) determining the if there is an increase in the normalized number of sequence reads containing the genetic signature compared to a control.

A system may include one or more processors in certain embodiments. A processor can be connected to a communication bus. A computer system may include a main memory, often random access memory (RAM), and can also include a secondary memory. Memory in some embodiments comprises a non-transitory computer-readable storage medium. Secondary memory can include, for example, a hard disk drive and/or a removable storage drive, representing a floppy disk drive, a magnetic tape drive, an optical disk drive, memory card and the like. A removable storage drive often reads from and/or writes to a removable storage unit. Non-limiting examples of removable storage units include a floppy disk, magnetic tape, optical disk, and the like, which can be read by and written to by, for example, a removable storage drive. A removable storage unit can include a computer-usable storage medium having stored therein computer software and/or data.

A processor may implement software in a system. In some embodiments, a processor may be programmed to automatically perform a task described herein that a user could perform. Accordingly, a processor, or algorithm conducted by such a processor, can require little to no supervision or input from a user (*e.g.*, software may be programmed to implement a function automatically). In some embodiments, the complexity of a process is so large that a single person or group of persons could not perform the process in a timeframe short enough for determining the presence or absence of a genetic variation.

In some embodiments, secondary memory may include other similar means for allowing computer programs or other instructions to be loaded into a computer system. For example, a system can include a removable storage unit and an interface device. Non-limiting examples of such systems include a program cartridge and cartridge interface (such as that found in video game devices), a removable memory chip (such as an EPROM, or PROM) and associated socket, and other removable storage units and interfaces that allow software and data to be transferred from the removable storage unit to a computer system.

In some embodiments, a method is provided, comprising, using a computer, receiving test sequence reads of nucleic acid molecules encoding antibody heavy chain variable regions from memory B cells from a subject immunized with an influenza A vaccine comprising an HA protein, and screening the test sequence reads for nucleic acid sequences encoding:

(A) a HV6-1/HD3-3 heavy chain variable region comprising an HCDR3 according to IMGT that is 16 amino acids in length, comprises the amino acid sequence set forth as xxxxxxMIFGIxxxxx (SEQ ID NO: 128), wherein x is any amino acid, and is flanked by an N-terminal Cys and a C-terminal Trp;

(B) a HV1-18/HD3-9 heavy chain variable region comprising an HCDR3 according to IMGT that is 15 amino acids in length, comprises the amino acid sequence set forth as xxxRxxILTGxxxxx (SEQ ID NO: 129), wherein x is any amino acid, and is flanked by an N-terminal Cys and a C-terminal Trp; and/or

(C) a HV1-18 heavy chain variable region comprising a tyrosine at kabat position 53, a threonine at kabat position 54 and a HCDR3 according to IMGT that is 17-21 amino acids in length, comprises the amino acid sequence set forth as xxxxxQxxV$(x)_n$ (SEQ ID NO: 131), wherein x is any amino acid and n is 8-12, and is flanked by an N-terminal Cys and a C-terminal Trp.

The number of counts of (A), (B), and/or (C) in the sequence reads can be reported to a user, or stored, for example, in system memory. In some embodiments, the method further comprises, using the computer, calculating a ratio of counts of the nucleic acid sequences encoding (A), (B), or (C) in the test sequence reads to counts of the nucleic acid sequences encoding (A), (B), or (C) in control sequence reads of nucleic acid molecules encoding antibody heavy chain variable regions from memory B cells from one or more subjects that have not been immunized with the influenza A vaccine comprising the HA protein. The ratio can be provided to a user, or stored, for example, in system memory. A non-transitory computer readable medium having a computer readable program code stored therein, the computer readable program code adapted to be executed to carry out such methods is also provided.

Additional embodiments

Clause 1. A method of identifying a vaccine that induces an immune response in a subject that neutralizes group 1 and group 2 influenza A viruses, comprising:

obtaining test sequence reads of nucleic acid molecules encoding antibody heavy chain variable regions from memory B cells from a subject immunized with an influenza A vaccine comprising an HA protein;

screening the test sequence reads for nucleic acid sequences encoding:

(A) a HV6-1/HD3-3 heavy chain variable region comprising an HCDR3 according to IMGT that is 16 amino acids in length, comprises the amino acid sequence set forth as xxxxxxMIFGIxxxxx (SEQ ID NO: 128), wherein x is any amino acid, and is flanked by an N-terminal Cys and a C-terminal Trp;

(B) a HV1-18/HD3-9 heavy chain variable region comprising an HCDR3 according to IMGT that is 15 amino acids in length, comprises the amino acid sequence set forth as xxxRxxILTGxxxxx (SEQ ID NO: 129), wherein x is any amino acid, and is flanked by an N-terminal Cys and a C-terminal Trp; and/or

(C) a HV1-18 heavy chain variable region comprising a tyrosine at kabat position 53, a threonine at kabat position 54, and a HCDR3 according to IMGT that is 17-21 amino acids in length, comprises the amino acid sequence set forth as xxxxxQxxV$(x)_n$ (SEQ ID NO: 131), wherein x is any amino acid and n is 8-12, and is flanked by an N-terminal Cys and a C-terminal Trp; and

wherein the presence of nucleic acid sequences encoding (A), (B), and/or (C) in the test sequence reads identifies the vaccine as a vaccine that can induce an immune response in a subject that neutralizes group 1 and group 2 influenza A viruses.

Clause 2. The method of clause 1, further comprising

obtaining control sequence reads of nucleic acid molecules encoding antibody heavy chain variable regions from memory B cells from one or more subjects that have not been immunized with the influenza A vaccine comprising the HA protein;

screening the control sequence reads for nucleic acid sequences encoding (A), (B), and/or (C);

determining a ratio of the presence of nucleic acid sequences encoding (A), (B), and/or (C) in the test sequence reads to the control sequence reads; and

identifying the vaccine as a vaccine that can induce an immune response in a subject that neutralizes group 1 and group 2 influenza A viruses if the ratio is greater than 2:1.

Clause 3. The method of clause 2, comprising identifying the vaccine as a vaccine that can induce an immune response in a subject that neutralizes group 1 and group 2 influenza A viruses if the ratio is greater than 10:1.

Clause 4. The method of any of clauses 1-3, comprising screening the test sequence reads for nucleic acid sequences encoding (A).

Clause 5. The method of any of clauses 1-4, comprising screening for nucleic acid sequences encoding (A) and wherein the HCDR3 comprises the amino acid sequence set forth as $ARX_6SX_7MIFGX_8X_9X_{10}X_{11}X_{12}X_{13}X_{14}X_{15}$ (SEQ ID NO: 119), wherein X_6 is Gly, Ala, or Val; X_7 is Ala, Phe, Val, or Pro; X_8 is Ile; X_9 is Val, Leu, or Asp; X_{10} is Met, Ile, or Val; X_{11} is Gly or no amino acid; X_{12} is Ala, Ile, Val, or Glu; X_{13} is Phe, Leu, or Met; X_{14} is Asp or Glu; X_{15} is Gln, Phe, Cys, Ser, Tyr, or Leu.

Clause 6. The method of any of clauses 1-3, comprising screening the sequence reads for nucleic acid sequences encoding (B).

Clause 7. The method of any of clauses 1-3 or 6, comprising screening for nucleic acid sequences encoding (B) and wherein the HCDR3 comprises the amino acid sequence set forth as $X_{10}RDX_{11}X_{12}X_{13}ILTGX_{14}X_{15}X_{16}DX_{17}$ (SEQ ID NO: 124), wherein X_{10} is Ala or Thr; X_{11} is Gln or Arg; X_{12} is Arg, Tyr, Gly, Phe, Ser, or Pro; X_{13} is Asp, Ala, Asn, Thr, or His; X_{14} is Gly, Pro, Ser, Tyr, Asp, Phe, or Cys; X_{15} is Leu, Asn, Ala, Arg, Asp, His, or Gln; X_{16} is Phe, Gly, Thr, Asp, or Leu; X_{17} is Cys, Tyr, His, Asp, Phe, Ser, or Ile.

Clause 8. The method of any of clauses 1-3, comprising screening the sequence reads for nucleic acid sequences encoding (C).

Clause 9. The method of any of clauses 1-8, further comprising sequencing the nucleic acid molecules encoding antibody heavy chain variable regions from memory B cells to obtain the test and/or control sequence reads.

Clause 10. The method of any of clauses 1-9, further comprising collecting the memory B cells from the subject following immunization with the vaccine.

Clause 11. The method of any of clauses 1-10, further comprising administering the vaccine to the subject to induce the immune response in the subject.

Clause 12. The method of any of clauses 1-11, wherein the memory B cells were obtained from the subject from one week to six months following immunization with the influenza A vaccine.

Clause 13. The method of clause 12, wherein the memory B cells were obtained from the subject from one week to four weeks following immunization with the influenza A vaccine.

Clause 14. The method of clause 12, wherein the memory B cells were obtained from the subject two weeks following immunization with the influenza A vaccine.

Clause 15. The method of any of clauses 1-14, wherein the subject is a human.

Clause 16. The method of any of clauses 1-15, further comprising selecting the vaccine for administration to a subject to prevent or inhibit influenza A infection.

III. EXAMPLES

The following examples are provided to illustrate particular features of certain embodiments, but the scope of the claims should not be limited to those features exemplified.

EXAMPLE 1

This example illustrates antibodies that specifically bind to influenza A HA protein and neutralize group 1 and group 2 influenza viruses, as well as gene signatures for identifying such antibodies.

From subjects enrolled in an H5N1 DNA/MIV-prime-boost influenza vaccine trial, hemagglutinin-cross reactive memory-B cells were sorted and three convergent antibody classes were identified, each capable of neutralizing group 1 and 2-influenza A viruses. Co-crystal structures with hemagglutinin revealed that each class utilized characteristic germline genes and convergent sequence motifs to recognize overlapping epitopes in the hemagglutinin stem. All six analyzed subjects had sequences from at least one multidonor class, and in half the subjects, multidonor-class sequences were recovered from >40% of cross reactive-B cells. By contrast, these multidonor-class sequences were rare in published antibody datasets. Vaccination with a divergent hemagglutinin can thus increase the frequency of B cells encoding broad influenza A-neutralizing antibodies; the sequence signature-quantified prevalence of these B cells can be used as a metric to guide universal influenza A-immunization strategies. Influenza A viruses can be categorized into two phylogenetic groups (group 1 and group 2), each containing diverse subtypes (FIG. 1A).

Currently, group 1 influenza viruses from the H1 subtype (1918 and 2009 H1N1 pandemics), and the group 2 H3 subtype (1968 H3N2 pandemic), co-circulate and cause seasonal infections in over 10% of the human population each year. Other subtypes have emerged or threaten to re-emerge including the group 1 H2 subtype, endemic in humans from 1957-1968, the group 1 H5 subtype, which includes highly lethal avian strains, and the group 1 H6 and H9 and group 2 H7 and H10 subtypes, which have been associated with human infections and fatalities in recent years. Frequent zoonotic cross-overs that may cause pandemics of unpredictable frequency and severity highlight the need for a universal influenza vaccine that is capable of protecting against diverse subtypes of influenza A virus.

Potential approaches to a universal influenza vaccine involve the elicitation of neutralizing antibodies that recognize the influenza hemagglutinin (HA) from multiple subtypes, thereby providing broad protection from divergent influenza viruses. One means to accomplish this involves ontogeny-based strategies, which seek to identify antibodies of reproducible classes and to induce similar antibodies by vaccination. Antibodies are considered to be of the same class when they recognize the same region, employ the same structural mode of recognition, and develop through similar recombination and maturation pathways (Kwong and Mascola, Immunity, 37(3): 412-425, 2012). Reproducible classes, which are observed in multiple individuals, represent immunological solutions to the challenge of broad influenza A neutralization that might be available to the general human population.

The influenza A-neutralizing stem-directed antibodies that utilize the HV1-69 germline gene are one such multidonor class (Sui *et al.*, *Nat. Struct. Mol. Biol.,* 16, 265-273, 2009; Throsby *et al, PloS one* 3, e3942, 2008;

Ekiert *et al., Science* 324, 246-251, 2009; Corti *et al., J Clin. Invest.,* 120, 1663-1673, 2010; Wrammert *et al., J Experimental Med.,* 208, 181-193, 2011). In terms of reproducibility, the HV1-69-derived antibodies have the additional advantage of utilizing heavy chain-only recognition, and prior studies have shown their vaccine-induced elicitation (Ellebedy *et al., PNAS,* 111, 13133-13138, 2014; Halliley *et al., J Infect. Dis.*, 2015; Khurana *et al., J Infect. Dis.,* 208, 413-417, 2013; Ledgerwood *et al., J Infect. Dis.,* 208, 418-422, 2013; Ledgerwood *et al., Lancet Infect Dis* 11, 916-924, 2011; Sui *et al., Nat. Struct. Mol. Biol.,* 16, 265-273, 2009; Wheatley *et al., J Immunol* 195, 602-610, 2015; Whittle *et al., J Virol.* 88, 4047-4057, 2014).

However, an issue with the HV1-69-derived antibodies is that they generally do not neutralize both group 1 and 2 strains of influenza A. Only a single HV1-69-derived antibody has been identified (CR9114) capable of neutralizing both group 1 and 2 strains of influenza A (Dreyfus *et al., Science* 337, 1343-1348, 2012). Other broadly neutralizing antibodies have been identified, such as FI6v3 and 39.29, both of which derived from the HV3-30 germline gene; while these two antibodies target overlapping epitopes in the HA stem, co-crystal structures with HA reveal different modes of recognition (Corti *et al., Science* 333, 850-856, 2011; Nakamura *et al., Cell Host Microbe* 14, 93-103, 2013), and they are thus not members of the same class. Indeed, a reproducible antibody class capable of neutralizing both group 1 and 2 influenza A virus has not been observed in multiple donors.

It was previously found that subjects enrolled in the phase I clinical trial, VRC 310 – who received an A/Indonesia/05/2005 monovalent inactivated virus (MIV) vaccine primed by an H5 DNA plasmid vaccine (Ledgerwood *et al., J Infect. Dis.,* 208, 418-422, 2013; Ledgerwood *et al., Lancet Infect Dis* 11, 916-924, 2011) (Table 2A) –showed transient expansion of H1- and H5-cross reactive memory B cells specific to the HA stem (Wheatley *et al., J Immunol* 195, 602-610, 2015; Whittle *et al., J Virol.* 88, 4047-4057, 2014). To determine whether these memory B cells might encode multidonor class antibodies capable of neutralizing group 1 and 2 influenza A virus, memory B cells that reacted with both H5 (group 1) and H3 (group 2) HAs were sorted. Immunoglobulin transcripts from post vaccination cross-reactive memory B cells were sequenced and the encoded antibodies were synthesized and characterized. Specifically, influenza neutralization breadth and potency was assessed, representative crystal structures in complex with HA were determined, sequence convergence based upon V(D)J-gene recombination and somatic hypermutation was analyzed, and sequence signatures for their ability to identify other group 1 and 2 neutralizing antibodies were assayed. The findings reveal common immunological pathways that achieve broadly reactive stem-directed antibodies, and support a method of quantifying convergent B cell lineages to guide universal influenza A vaccine strategies.

Results

Identification of memory B cells cross-reactive with group 1 and 2 influenza A HAs. Ten subjects from the VRC 310 H5N1 vaccine trial who displayed a range of vaccine-elicited serum H5N1 neutralization activity were studied, as well as varied but detectable responses against group 2 strains A/Hong Kong/1-4-MA21-1/1968 (H3N2) or A/Netherlands/219/2003 (H7N7) potentially indicative of antibody responses with inter-group specificity, were studied (FIGs. 1B and 8, Table 2B).

Recombinant group 1- (H5) and group 2- (H3) specific HA probes – modified to prevent sialic acid binding (HA□SA) (Wheatley *et al., J Immunol* 195, 602-610, 2015; Whittle *et al., J Virol.* 88, 4047-4057, 2014) – were used

to co-stain and sort PBMCs isolated two weeks post H5N1 MIV boost (FIGs. 1C and 9). Sequences of memory B cell immunoglobulin gene transcripts were recovered from six of the ten studied subjects (FIG. 1D).

The sequence repertoire of each subject was generally dominated by clonally related transcripts comprising a small number of clonal expansions. Transcripts derived from diverse HV genes including HV 1-18, 3-23, 3-64D, 4-30, 4-34 and 6-1 (FIGs. 1D and 1E). Notably, transcripts from HV1-69, which often dominate group 1-specific stem-reactive antibodies, comprised only 2.5% of this set of group 1 (H5+)-group 2 (H3+) double-positive memory B cells.

Group 1 and 2-neutralizing antibodies from different vaccinees are genetically similar. Immunoglobulin sequences recovered from H5+ and H3+ cross-reactive memory B cells showed surprising similarity among subjects. Notably, many immunoglobulin sequences from different donors contained identical genetic elements (FIG. 1E). To analyze commonalities of immunoglobulin transcripts between subjects, the following seven genetic elements were considered: inferred HV (Heavy Variable), HD (Heavy Diversity), and HJ (Heavy Joining) genes, and CDR H3 length for the heavy chain-gene transcript, and inferred LV (Light Variable) and LJ (Light Joining) genes, and CDR L3 length for the corresponding light chain transcripts (FIG. 1E). Frequentist analysis indicated that the presence of four or more of the same genetic elements in separate lineages to be statistically significant ($P \leq 0.001$) (FIG. 1E), and representative antibodies were therefore cloned and expressed from such lineages. Of note, the antibody nomenclature specifies donor, lineage and clone; *e.g.*, antibody 56.a.09 (FIG. 1E, third row) is named for subject (56), lineage within this subject (a) and clone within this lineage (09). Representative antibodies from HV families 3-30 and 4-34 that were previously identified as common among cross-reactive stem-directed antibodies (Corti *et al., Science* 333, 850-856, 2011; Wyrzucki *et al., J Virol.,* 88, 7083-7092, 2014; Hu *et al., Virology* 435, 320-328, 2013) were also expressed. All of the expressed antibodies bound HA except for one HV3-23- and three HV1-69-derived antibodies. All antibodies that bound HA could be competed for binding with the antigen-binding fragment (Fab) of the stem-directed antibody CR9114 (Dreyfus *et al., Science* 337, 1343-1348, 2012) or F10 (Sui *et al., Nat. Struct. Mol. Biol.,* 16, 265-273, 2009) and could be visualized by negative stain EM as binding to the HA stem (FIG. 10). Most of these antibodies neutralized viruses from both group 1 and 2 HA, including H1, H3, H5 and H7 subtypes, with select antibodies also demonstrating neutralization of viruses from subtypes H2, H9 and H10 (FIGs. 1E, 11, and 15). Notably, in pseudovirus assays, the breadth and potency for several of the newly identified antibodies was comparable to that of CR9114, but lower than FI6v3. The newly identified antibodies were also tested for neutralization of influenza A viruses (not pseudotypes); neutralization was observed with both group 1 and 2 influenza A strains. Notably, this neutralization paralleled the neutralization observed with CR9114 and FI6v3 (FIG. 15).

To understand the immunological basis of these highly similar humoral responses against influenza A, the recombination, somatic hypermutation, and structural constraints which drove selection of these antibodies was analyzed.

A multidonor class of broadly neutralizing antibodies with HV6-1+HD3-3 germline genes. Three distinct memory B cell lineages, from subjects 31, 54 and 56, shared heavy chain sequences derived from a recombination of HV6-1 with HD3-3, and HJ4 or HJ5, to yield highly similar amino acid sequences in the CDR H3 (FIG. 1E, top row; FIGs. 20A-20B). In each case, the heavy chain was paired with a light chain sequence resulting from KV3-20, KJ2 or KJ3 and a CDR L3 of 9 amino acids. Similar affinity maturation patterns were observed: a Val100bIleHC alteration of an HD-gene encoded section of the CDR H3 was completely conserved in all three lineages (FIG. 2A); (for clarity, each residue number is followed by a subscript denoting parent molecule: HC for

heavy chain; LC for light chain; HA1, HA2 or HA for either HA subunit or HA in general). Notably, these antibodies displayed neutralization breadth and potency that rivaled that of CR9114 and FI6v3 and exceeded that of the group-specific CR6261 or CR8020 or that of the head-directed antibody CH65 (Whittle *et al., PNAS,* 108, 14216-14221, 2011) (FIG. 2B).

To provide insight into the structural basis for the similarity between these HV6-1+HD3-3 antibodies, the crystal structure of the antigen-binding fragment (Fab) for antibody, 56.a.09, alone, and in complex with A/Hong Kong/1-4-MA21-1/1968 (H3N2) HA was determined at 3.3 Å resolution (FIG. 2C, FIG. 12, and FIG. 16, Table 3). Unexpectedly, the HA was not trimeric; the crystallographic asymmetric unit for the Fab-H3 complex comprised an HA head of one protomer interacting with the HA stem of an adjacent protomer in a "head-to-stem" dimeric arrangement (FIGs. 12B and 12C). Despite this non-trimeric arrangement, the Cα-RMSD between the 56.a.09-bound HA and the ligand-free HA was less than 1 Å; for clarity, the 56.a.09 bound complex is depicted as a typical HA trimer (FIG. 2C).

antibody 56.a.09 recognized a conserved region on the HA stem, with a footprint overlapping the prototypical group 1-neutralizing HV1-69 antibody, CR6261, as well as the group 2-neutralizing antibody, CR8020 (Throsby *et al, PloS one* 3, e3942, 2008; Ekiert *et al., Science* 324, 246-251, 2009; Ekiert *et al., Science* 333, 843-850, 2011). Most group 1 stem-neutralizing antibodies derived from HV1-69 are blocked by glycan Asn38HA1, which is conserved on group 2 viruses. Group 2-neutralizing antibodies such as CR8020 and CR8043 are blocked by glycan Asn21HA1 conserved on group 1 viruses. Notably, the HV6-1+HD3-3 epitope avoids these two glycans, and its neutralization is insensitive to the presence or absence of glycan. Antibody 56.a.09 bound primarily with its heavy chain (934 $Å^2$ buried surface area (BSA) versus 386 $Å^2$ BSA for the light chain). Heavy chain binding involved the HD3-3-encoded CDR H3 (FIG. 2C) with $Phe100_{HC}$ and $Gly100a_{HC}$ contributing ~240 $Å^2$ of BSA and the somatic hypermutation-altered $Val100bIle_{HC}$ inserting directly into the $Trp21_{HA2}$ pocket (contributing over 100 $Å^2$ of interactive surface). In addition, $Met98_{HC}$ interacted with a conserved aromatic residue present on all light chains, helping to orient the CDR H3 (FIG. 2D). Heavy-chain binding also involved the HV6-1 germline-encoded CDR H2, which uniquely encodes a 9 amino acid CDR H2, contributed 182 $Å^2$ of BSA, was unmutated in contact residues in all three subjects (FIG. 13), and interacted with the conserved fusion peptide (FIG. 2E). With respect to light chain, the largely unmutated KV3-20-derived V genes (4-6% SHM) observed in lineages from these three subjects interacted with the HA stem through both CDR L1 and CDR L3 (FIG. 2F, Table 3), with $Tyr33_{LC}$ contributed the largest BSA among all light chain residues (73.1 $Å^2$).

The antibodies derived from HV6-1+HD3-3 germline genes were found in three independent donors, shared genetic elements in both the heavy and light chain, displayed convergent affinity maturation, and appeared to share the same mode of recognition (additional structure-function analysis of the antibody interface and important SHM change are provided in FIG. 13). The functional complementation of the heavy and light chains derived from HV6-1+HD3-3 class antibodies was tested; swapping of heavy and light chains between three antibodies of the putative class resulted in six functional antibodies from nine possible pairings, with the three containing the heavy chain of antibody 31.g.01 failing to express (FIG. 17). Overall, these results indicate the HV6-1+HD3-3-derived antibodies to form a multidonor class. While structural constraints likely drove the heavy chain-selection and -affinity maturation of the HV6-1+HD3-3 recombinants, structural analysis indicated numerous light chains to be compatible with binding. Although the genetic requirements of this class are quite specific, approximately 99% of the human

population (Genomes Project *et al., Nature* 491, 56-65, 2012) possess alleles of the HV6-1 and HD3-3 genes compatible with the class elicitation and recognition described here (FIGs. 2D-2G).

A second multidonor class of broadly neutralizing antibodies with HV1-18+HD3-9 germline genes. Two distinct memory B cell lineages from subjects 1 and 31 shared immunoglobulin heavy chain sequence derived from recombination of HV1-18 with HD3-9 and HJ4 to yield highly similar amino acid sequences in a CDR H3 of 15-amino acids in length (FIGs. 1E and 13). Notably an $Arg96_{HC}$ residue was encoded by n-nucleotide addition in both cases (FIG. 3A). In each donor, the heavy chain was paired with a light chain derived from KV2-30. Encoded immunoglobulins were expressed and shown to neutralize primarily group 1 strains of influenza A, although a few group 2 strains were neutralized (FIG. 11). Overall neutralization from these HV1-18+HD3-9 antibodies appeared more similar to the group 1-specific antibody CR6261, than to the very broad FI6v3, CR9114 or HV6-1-derived antibodies, however, neutralization breadth was greater than 50% of influenza A subtypes that commonly infect humans (FIG. 3B).

The crystal structure of Fab 31.b.09 in complex with A/California/04/2009 H1 was determined (FIGs. 3C and 16, Table 3). Similar to the 56.a.09-H3 complex structure, the crystallized hemagglutinin in the Fab 31.b.09 complex was not a trimer, but a molecular dimer (FIG. 16). Despite this unexpected non-trimeric arrangement, the Cα-RMSD between the 31.b.09-bound HA and the ligand-free HA in the stem region was 0.6 Å; for clarity the 31.b.09 bound complex is shown in a more typical trimeric arrangement (FIG. 3C). The HV1-18+HD3-9-derived antibody 31.b.09 bound an epitope that overlapped the HV6-1+HD3-3 class epitope, but with antibody rotated ~105° degrees (mostly involving a rotation perpendicular to the trimer axis) (FIG. 3C). Antibody 31.b.09 bound using both heavy and light chains (343 $Å^2$ BSA for heavy chain and 540 $Å^2$ BSA for the light chain). Heavy chain interactions were generated through CDR H2 and H3 loops. In CDR H2 (127 $Å^2$ BSA), the HV1-18 germline encoded $Tyr53_{HC}$ recognized the fusion peptide of HA2 and $Asn56_{HC}$ recognized helix A of HA1, while the CDR H3 (216 $Å^2$ BSA) was positioned over the fusion peptide-helix A interface with $Ile99_{HC}$ and $Leu100_{HC}$ inserting into the hydrophobic groove between these two conserved elements (FIGs. 3C-3E). Light chain interactions were generated through CDR L1 and CDR L3, which recognized helix A (FIG. 3F). Functional complementation was tested; swapping of heavy and light chains between two antibodies of the putative class resulted in four functional antibodies from the four possible pairings (FIG. 17). Overall the results indicate the HV1-18+HD3-9-derived antibodies to form a multidonor class. The light chain had a 16 amino acid CDR L1, which could be encoded by twelve other LV genes. The major contact residue $Ile27e_{LC}$ was generated by somatic hypermutation and could be found in multiple light chains. The multiple alternative D gene alleles that could be used to generate the CDR H3 for this multidonor class, in combination with a large number of possible light chains, led to a calculated distribution of potential HV1-18 combinations in the human population of close to 100% (Genomes Project *et al., Nature* 491, 56-65, 2012) (FIG. 3G).

A third multidonor class of broadly neutralizing antibodies with HV1-18 germline gene and CDR H3 Q-x-x-V motif. Multiple B cell lineages in two subjects (16 and 54) produced distinctive HV1-18-derived immunoglobulins sharing five genetic elements and having a CDR H3 of 21 amino acids derived from recombination with either HD2-2 or HD2-15 genes (FIGs. 4A and 13, FIG. 20). This set of immunoglobulins all shared an SHM-derived $Thr54_{HC}$,a $Gln98_{HC}$ encoded by n-nucleotide addition, and a germline HD-encoded aliphatic residue three residues later at $position100a_{HC}$ (Q-x-x-V motif). Neutralization breadth and potency for this set of immunoglobulins were similar to that of antibody CR9114, neutralizing all of the common human-infecting

subtypes of influenza A except H2, and substantially exceeded the breadth of group 1-specific or group 2-specific stem antibodies or the head-directed antibody CH65 (FIG. 4B). To understand the basis of their recognition, representative antibodies were crystallized with HA. Co-crystal structures of representative HV1-18+HD2-2 (16.a.26) and HV1-18+HD2-15 (16.g.07) Fabs in complex with the A/Hong Kong/1-4-MA21-1/1968 (H3N2) HA revealed highly similar epitopes (FIG. 4C-4H and 16, Table 3). Antibody recognition occurred primarily through the heavy chain to a conserved region of the HA stem. In the case of 16.a.26, the heavy chain contributed 514 $Å^2$ of BSA while the light chain contributed 283 $Å^2$ of BSA, and in the case of 16.g.07, the heavy chain contributed 646 $Å^2$ of BSA while the light chain contributed 329 $Å^2$ of BSA.

Heavy chain interactions were generated primarily through the CDR H2 (~150 $Å^2$ BSA for 16.a.26 and ~200 $Å^2$ BSA for 16.g.07) and the CDR H3 (~300 $Å^2$ BSA for 16.a.26 and ~450 $Å^2$ BSA for 16.g.07) with the HV1-18 germline-encoded $Tyr53_{HC}$ and the SHM-derived $Thr54_{HC}$ recognizing the N-terminal region of HA1 and the hydrophobic groove between helix A and the fusion peptide of HA2 (FIGs. 4D and 4G, Table 3). The CDR H3s of these antibodies also bound a conserved hydrophobic groove along helix A adjacent to $Trp21_{HA2}$. The conserved $Val100a_{HC}$ inserted into a pocket present on both group 1 and group 2 HAs, just above $Trp21_{HA2}$ and proximal to $Ile\ 48_{HA2}$. Despite differences between the residues encoded by HD2-2 (16.a.26) and HD2-15 (16.g.07), the CDR H3s from both antibodies were highly similar. Both were oriented perpendicular to the Fab axis and interacted with HA similarly. The conserved $Gln98_{HC}$ interacted with $Gln42_{HA2}$ by utilizing a germline-encoded pocket unique to HV1-18-encoded antibodies, which was formed by antibody framework residues $Gly33_{HC}$ and $Ser\ 52_{HC}$ (FIGs. 4E and 4H). The location of $Gln98_{HC}$ within the framework pocket likely stabilized the perpendicular orientation of the CDR H3 relative to the antibody-framework regions, and may allow for CDR H3-motifs derived from diverse D genes to bind to this conserved hydrophobic groove. In this regard, it is noted that sequences from subject 01 with an HV1-18 germline gene and a Gln98-x-x-Val100a motif in a 17 amino acid CDR H3 were able to neutralize H3 and H5 strains of influenza. Although light chains of antibodies in this class contribute about one-third of the total buried surface area, analysis of antibodies of this class revealed light chain sequences to derive from diverse LV genes with only KV3-11 appearing twice. Also, the recently reported HV1-18-derived group 1 and group 2-neutralizing antibody CT149 uses a $Gln98_{HC}$-x-x-$Val100a_{HC}$ motif with a 19 amino acid CDR H3 to bind the HA stem (Wu *et al., Nat Commun* 6, 7708, 2015) to recognize HA in a manner highly similar to both 16.a.26 and 16.g.07. The functional complementation for antibodies 16.a.26, 16.g.07, 54.a.39, 54.a.84, and CT149 from donors 16, 54, and SH-K1 (the source of antibody CT149) as tested. Swapping of heavy and light chains between these five antibodies resulted in 10 functional antibodies from the 25 possible pairings (FIG. 17). Functionality correlated strongly with HV gene identity and CDR H3 length (FIG. 17), suggesting a requirement for specific heavy-light chain interactions. Despite these requirements, the HV1-18 antibodies with ^{54}Thr and CDR H3 ^{98}Q-x-x-V motif appeared to form a multidonor class. Thus the recombination of HV1-18 with many alternative D gene segments gives rise to a Q-x-x-V motif in the CDR H3, which may comprise a common solution for neutralization of group1 and group 2 influenza A viruses. The many alternative HD gene alleles that can be used to generate CDR H3s for this multidonor class in combination with no obvious light chain bias leads to a calculated distribution of potential HV1-18 combinations in the human population of close to 100% (Genomes Project *et al., Nature* 491, 56-65, 2012) (FIGs. 4I and 4J).

Multidonor and unique lineages recognize similar epitopes. The most highly expanded lineage of antibodies from any of the six subjects that was sequenced was lineage "a" from subject 31, from which 104 clones were sequenced (FIG. 1E). The 31.a.83 antibody from this lineage derived from HV3-23, HD3-9 and HJ6 germline

genes and displayed the highest neutralization breadth of all the antibodies that were sequenced and expressed, neutralizing influenza A viruses from all six of the common subtypes that infect humans (FIG. 5A). Structural characterization of antibody 31.a.83 in complex with HA-HK68 (FIG. 16) revealed binding to the conserved HA stem, primarily through CDR H3 residues, many of which were somatically hypermutated (FIGs. 5B-5D, Table 3). The mode of 31.a.83 recognition was similar to other stem directed antibodies that recognize an epitope adjacent to helix A and involving the Trp21 pocket. Antibody 31.a.83 utilized hydrophobic residues to contact HA and oriented its CDR H3 parallel to helix A in a manner that enabled it to avoid the conserved N-glycan at $Asn38_{HA2}$ found in group 2 HAs.

A second lineage, lineage "h" from subject 56, also derived from the same HV3-23, HD3-9 and HJ6 germline genes as antibody 31.a.83 (FIGs. 5B and 13). However, critical CDR H3 contact residues, $Ile100_{HC}$-x-x-Leu-Met of antibody 31.a.83 were not conserved in this second 56.h lineage, suggesting lineage 56.h to have a different mode of recognition. Moreover, the representative antibody that was expressed and analyzed from this second lineage, antibody 56.h.01, neutralized H1, H2 and H9 subtypes, but not H3, H5, or H7 subtypes (FIG. 1E, 11, and 15).

These results provide an example of influenza A-targeting antibodies that derive from the same heavy chain-VDJ germline genes, but do not use the same mode of recognition nor share convergent development. Thus antibody lineages may be multidonor (common or public), meaning that they are observed in different individuals and share the same genetic elements and mode of recognition or unique (uncommon or private), meaning that they have only been observed a single time. When epitopes recognized by HA stem-directed group 1 and group 2 neutralizing antibodies were compared, both newly identified in this study and previously described in the literature (FIGs. 5E-5H); no substantial epitope difference was observed between multidonor and the unique antibodies capable of neutralizing group 1 and 2-influenza A viruses (FIG. 5G). A segregation in antibody approach to HA used by multidonor or unique antibodies was not observed (FIG. 5H). However, antibodies from multi-donor lineages did have lower somatic hypermutation (averaging 8% for multidonor versus 11% for the unique). The lower somatic hypermutation suggested multidonor antibodies to undergo more parsimonious routes of development. As the number of donors with sequenced cross-reactive memory B cells increases, some of the antibodies described here as "unique" would be expected to be observed in other donors. It is noted that unique lineages accounted for the majority of cross-reactive B cells in 4 of the 6 analyzed VRC 310 donors (1, 31, 36 and 56); in light of the positive functional characteristics of several of unique lineages (*e.g.*, 31.a.83) it seems likely that these antibodies would contribute to a protective response. It remains to be seen whether the unique lineages will continue to constitute roughly half of the response, or if different vaccination schemes will alter the ratio of multidonor and unique lineages.

Specificity of sequence signatures for multidonor antibody classes. Once multidonor classes were identified, sequence signatures, specific to each multidonor antibody class, were be used to identify group 1 and 2 neutralizing antibodies on the basis of sequence alone. The multidonor antibodies identified here were analyzed for class specific-sequence signatures (FIG. 6A). Heavy chain-only signatures were focused on, as such signatures would be easier to identify in next-generation sequencing (NGS) data. For the HV6-1+HD3-3 class, a five amino acid signature (M-I-F-G-I, beginning with $M98_{HC}$) in addition to HV and HD gene requirements and a CDR H3 length of 16 amino acids was identified; this signature matched 21 sequences in subjects 31, 54 and 56 (FIG. 6B). For the HV1-18+HD3-9 class, a seven amino acid signature (R-x-x-I-L-T-G, beginning with $R96_{HC}$) in addition to the HV and HD gene requirements and a CDR H3 length of 15 amino acids was identified, which matched 16 sequences

in subjects 1 and 31. And for the HV1-18 (Q-x-x-V motif), a two amino-acid VH-gene signature ($Tyr53_{HC}$ and $Thr54_{HC}$) was combined with a four amino acid CDR H3 signature (Q-x-x-V, beginning with $Q98_{HC}$), in addition to a HV1-18 germline requirement and a CDR H3 length between 17 and 21 amino acids; this signature matched 309 sequences in subjects 1, 16, 36 and 54.

To assess the specificity of these sequence signatures, published human antibody datasets were searched, both those with paired heavy-light sequences (DeKosky *et al., Nature medicine* 21, 86-91, 2015), as well as those with heavy-chain only sequences (Jiang *et al., Science translational medicine* 5, 171ra119, 2013) (FIG. 6A). Searches with the HV6-1+HD3-3 signature did not yield sequence matches in paired heavy-light sequences, but in the published heavy chain-only datasets, 13 sequence matches were found, which appeared to derive from a single lineage (FIG. 13). Consensus as well as the sequence closest to consensus were synthesized and reconstituted with light chains of HV6-1+HD3-3 class antibodies from subjects 54 and 56. One of these reconstituted antibodies did not express, but the other three did and bound H1 HA in a manner that could be competed with antibody F10 (FIG. 6C). Two of these antibodies were tested and both neutralized group 1 and 2 influenza A strains (FIG. 6D, FIG. 20). The neutralization signatures (Georgiev *et al., Science* 340, 751-756, 2013) of the synthesized antibodies clustered in a dendrogram with the known HV6-1+HD3-3 class antibodies (FIG. 6E).

With the HV1-18+HD3-9 signature, 17 sequence matches were found in published paired heavy-light chain sequences, which appeared to derive from a single lineage. Consensus sequences were synthesized and published heavy and light chains as well as the published heavy chain and light chain of this class from subject 31 were reconstituted. Both of these reconstituted antibodies bound H1 hemagglutinin in a manner that could be competed with antibody F10 (FIG. 6C), neutralized group 1 influenza A strains (FIGs. 6D and 15), and clustered in neutralization dendrograms with the known HV1-18+HD3-9 class antibodies (FIG. 6E). The neutralization breadth of these antibodies was lower than those isolated from VRC 310 subjects, likely due to the use of germline sequences to complete the CDR L1 and CDR L2 regions of this antibody (the somatic mutation of $27E_{LC}$ to Ile is required for optimal recognition (FIG. 3F)).

With the HV1-18 (Q-x-x-V) signature, searches with this signature did not yield sequence matches in paired heavy-light sequences, but in the published heavy chain-only datasets, 242 sequence matches were found, which appeared to derive from 3 lineages (FIGs. 6A and 14). Four sequences were synthesized (consensus or closest NGS read from each lineage) and reconstituted with the five light chains used previously in the swapping experiments. None of these reconstituted antibodies bound a set of HAs (FIG. 6C) or neutralized any of the 15 viruses in the neutralization panel. Analysis of the tested heavy chains indicated that their CDR H3 length matched that of CT149 in three of four cases, but was below the 78% identity threshold that correlated with function in heavy-light complementation of this class (FIG. 17).

Altogether the results indicate sequence signatures with sufficient specificity to identify other functional class members by sequence alone could be obtained for two multidonor classes, HV6-1+HD3-3 and HV1-18+HD3-9. The sequence signature for the third class, HV1-18 (Q-x-x-V), was complicated by incompatibility of some heavy-light pairs from this class; nonetheless, the sequence searches for this third class place an upper limit on the prevalence of this multidonor antibody class in the searched databases.

Vaccine induction of multidonor broadly neutralizing antibodies. In the VRC 310 trial, a significant expansion (P=0.0284) of H5+H3+ memory B cells following H5 DNA prime-MIV boost was observed, ranging from an increase of 1.2- to 10.6-fold (FIG. 7A). Notably subjects with the largest increases and the highest frequencies of

multidonor antibodies had the largest percentages of antibodies belonging to the three multidonor classes identified here (FIG. 7B). The initial observation of a high number of transcripts with multiple genetic commonalities may be explained by the preferential expansion of multidonor class transcripts; indeed, the fold-increase in cross-reactive B cells by donor correlated with the percentage of sequences with multiple genetic commonalities (FIG. 7C). Importantly, the frequency of cross-reactive memory B cells post-VRC 310 vaccination correlated with the sequence signature-identified prevalence of multidonor class antibodies (P=0.0045) (FIG. 13C). Moreover, while significant correlation between fold increase in sera titer versus an increase in cross-reactive memory B cells was not observed, a significant correlation relevant to the titers for H1N1 virus A/Singapore/8/1986 was observed. The H1N1 virus A/Singapore/8/1986 virus was previously found to be especially sensitive to neutralization by stem-directed antibodies (FIG. 7D).

To quantify the frequency of multidonor class antibodies in unvaccinated subjects, NGS-determined memory B cell transcripts from healthy normal donors were examined. In 1,739,891 heavy chain-only transcripts, no HV6-1+HD3-3 class sequences, 64 HV1-18+HD3-9 class sequences (from 2 lineages), and 2 HV1-18 (Q-x-x-V) class sequences (from 1 lineage) were observed. By contrast, from 515,594 sorted memory B cells from VRC 310-vaccinated subjects, 21 HV6-1+HD3-3 class sequences (3 lineages), 16 HV1-18+HD3-9 class sequences (2 lineages), and 309 HV1-18 (Q-x-x-V) class sequences (from 14 lineages) were observed (FIG. 6A). To compare frequencies from VRC 310-vaccinated versus unvaccinated subjects, the total number of sorted memory B cells was equated with the total number of transcripts (assuming all of the class sequences from the VRC 310 subjects were present in the 645 sequenced cross-reactive memory B cells). A substantially higher transcript frequency (and to a lesser degree, a higher lineage frequency) was observed for multidonor class sequences upon H5N1 vaccination in the VRC 310 trial (FIG. 7E).

Published antibody sequences from subjects immunized with the 2009 or 2010 seasonal influenza vaccine (Jiang *et al., Science translational medicine* 5, 171ra119, 2013) were also examined.. In 759,337 heavy chain-only transcripts prior to vaccination, no class sequences were observed (FIG. 6A). By contrast, in 3,045,513 heavy chain-only transcripts after seasonal vaccination 13 HV6-1+HD3-3 class sequences (from a single lineage), 123 HV1-18+HD3-9 class sequences (from 3 lineages), and 242 HV1-18 (Q-x-x-V) class sequences (from 3 lineages) were observed (FIGs. 6A, 7E, and 13). Overall, transcripts matching the HV1-18+HD3-9 signature were ~10x more prevalent prior to vaccination than the other multidonor transcripts; however, this class appeared not to expand upon either seasonal or VRC 310 vaccination. By contrast, transcripts matching HV6-1+HD3-3 and HV1-18 (Q-x-x-V) signatures appeared to be present at low frequencies prior to vaccination and to increase upon seasonal vaccination - and up to a 1000-fold upon VRC 310 vaccination (FIG. 7F).

Discussion

While the human immune system can generate broadly neutralizing antibodies against influenza, such antibodies appeared to be relatively rare, and their induction by vaccination had not been reported. In this study, three multidonor classes of antibodies capable of neutralizing group 1 and group 2 influenza A viruses were identified in VRC 310-vaccinated subjects. One multidonor class utilized HV6-1+HD3-3 germline genes (FIG. 2); a second class utilized HV1-18+HD3-9 germline genes and a 15-amino acid CDR H3 (FIG. 3); and a third class utilized HV1-18 germline gene and a $Gln98_{HC}$-x-x-$Val100a_{HC}$ motif (FIG. 4). For each class, sequence signatures were delineated (FIG. 6). Despite the lack of clear serological indicators of vaccine-induced improvement in cross

neutralization with VRC 310-vaccinated subjects (FIG. 9), vaccine-induced expansion of cross-reactive memory B cells was observed (FIG. 7A) as was a clear increase in the frequency of transcripts for two of the multidonor antibody classes (FIG. 7F). These findings demonstrate the vaccine induction of broadly neutralizing influenza A antibodies.

Stereotypic antibody signatures have been reported for some bacterial polysaccharide antigens (Adderson *et al., J Clinical Iinvest.,* 91, 2734-2743, 1993), CD4-induced, V1V2-directed and VRC01-classes of HIV-1-neutralizing antibodies, and both stem and head-directed influenza neutralizing antibodies. Thus, despite the potential repertoire of human immunoglobulins being large and somatic hypermutation further increasing diversity to the point where highly similar modes of antigen recognition might be expected very infrequently, the findings presented herein demonstrate that multidonor classes can be induced by vaccination in humans.

The coexistence of multiple vaccine-induced pathways to generate influenza group 1 and group 2 neutralizing antibodies is encouraging for efforts aimed at achieving analogous responses in genetically diverse human populations. The stem-directed antibodies induced here potently neutralize in pseudotype assays (FIG. 11), but less potently in live influenza A-virus assays (FIG. 15), and assessment of *in vivo* concentrations required for protective efficacy may require passive infusion trials in humans. The NGS-based assessment of transcript frequency indicated vaccination to boost the frequency of transcripts from two multidonor classes substantially towards the target goal, and increases after seasonal vaccination were even observed (FIG. 7F). In this regard, sequence signature-based quantification provides a suitably sensitive technology to detect increases in the transcript frequency of group 1 and 2 neutralization antibodies, to measure their expression in appropriate memory B cell subsets and long-lived plasma cells, and to assess their durability. Appropriate SHM is an additional aspect, and the recognition of germline-reverted versions of each of the three multidonor classes as well as the effect of mutational analysis of critical contacts was analyzed (FIGs. 13-14). Further studies aimed at increasing the prevalence of group 1 and 2-neutralizing influenza antibody lineages using the sequence signatures identified here may provide a means to achieving the protective efficacy required of a universal influenza A vaccine. In this regards, it is helpful to know that 100-1000-fold increases in the transcript frequencies for two multidonor classes of influenza A-neutralizing antibodies could be achieved through immunization with a divergent influenza (FIG. 6A and 7F), likely by enhancing the immune focus to the HA-stem region (FIG. 7G).

Methods

VRC 310 study design. The VRC 310 study (ClinicalTrials.gov identifier NCT01086657) (Ledgerwood *et al., J Infect. Dis.,* 208, 418-422, 2013; Ledgerwood *et al., Lancet Infect Dis* 11, 916-924, 2011) was conducted at the National Institutes of Health, Bethesda, MD by the Vaccine Research Center, NIAID, NIH, DHHS. This Phase I study examined the safety and immunogenicity of H5N1 prime-boost vaccination. One group received inactivated H5N1 vaccine (A/Indonesia/05/2005) for both prime and boost, whilst five other groups were primed with a DNA vaccine expressing H5 (A/Indonesia/05/2005) followed by boosting with inactivated H5N1 vaccine at increasing intervals ranging from 1 to 6 months.

Production of pseudotyped lentiviral vectors and measurement of antibody neutralizing activity. Influenza pseudotyped lentiviral vectors expressing a luciferase reporter gene were produced as described (Yang *et al., Science* 317, 825-828, 2007). Briefly, 293T cells were co-transfected by using the following plasmids: 17.5 μg of pCMV-R8.2, 17.5 μg of pHR'CMV-Luc, 1 μg CMV/R H1 South Carolina/1/1918, H1 Puerto Rico/8/1934, H1

New Caledonia/20/1999, H1 California/04/2009-Mut, H2 Canada/720/2005, H3 Hong Kong/1/1968, H3 Perth/16/2009, H5 Indonesia/05/2005, H7 Netherlands219/2003, H7 Anhui/1/2013) or H9 Hong Kong 1074/1999, or INF-BBrisbane/60/2008; and 0.125ug of the corresponding NA (18 million cells in a 15cm dish). For the production of H1N1, H2N2, H3N2, H7N7, H7N9 and H9N2 pseudovirus, a human type II transmembrane serine protease TMPRSS2 gene was included in transfection for the proteolytic activation of HA (Bottcher *et al., J. Virol.,* 80, 9896-9898, 2006). Cells were transfected overnight and replenished with fresh medium. Forty-eight hours later, supernatants were harvested, filtered through a 0.45-μm syringe filter, aliquoted, and frozen at -80°C before use.

The neutralization assays were carried out as follows: monoclonal Abs at various dilutions were mixed with pseudoviruses for 45 min and then added to 293A cells in 96-well dishes (10,000 cells per well). Additional fresh medium was added 2h later. Three days after infection, cells were lysed in 20 μl of cell culture lysis buffer (Promega, Madison, WI). Luciferase assay reagent (50 μl; Promega) was added to the cell lysate prior to measuring luciferase activity. The protocol for human sera neutralization assays is identical to that for monoclonal antibodies, with one exception. Human sera is initially pretreated with receptor destroying enzyme II (Denka Seiken, Japan) to eliminate serum nonspecific inhibitors in accordance with the manufacturer's protocol prior to beginning the neutralization assays.

Expression of HA probes. HA constructs consisting of the extracellular domain of HA modified to ablate sialic acid binding and C-terminally fused to a T4 fibritin trimerization motif, biotinylatable AviTag sequence and a hexahistidine affinity tag were synthesised (Genescript) and cloned into a CMV expression plasmid as previously described (Whittle *et al., J Virol.* 88, 4047-4057, 2014). Plasmid DNA was prepared by Maxiprep or Megaprep (Qiagen). Expi293 cells (Life Technologies) were diluted to 1.2×10^6 cells/ml and transfected with 500 μg/liter of HA expression plasmids using Expifectamine transfection reagent. At day 5, the media was clarified by centrifugation at 2,000 × g and filtered, concentrated, diafiltered against 4 volumes of phosphate-buffered saline (PBS) with 20 mM imidazole (pH 8), and loaded on Ni Sepharose Fast Flow resin (GE Healthcare) by gravity flow. The resin was washed with 6 column volumes of PBS with 60 mM imidazole and the protein was eluted in 5 column volumes of PBS with 500 mM imidazole. The eluted protein was stored at 4°C overnight, concentrated with a centrifugal concentrator, and loaded on a Superdex 200 16/60 column and fractions corresponding to trimeric HA were pooled and concentrated. Eight hundred microliters of protein in 10 mM Tris (pH 8.0) was biotinylated using biotin protein ligase (Avidity) by the addition of 100 μl of Biomix-A, 100 μl of Biomix-B, and 2.5 μl of biotin ligase BirA and incubated at 37°C for 1 h. The resulting biotinylated protein was exchanged into PBS with a centrifugal concentrator to remove excess biotin. Biotinylation was confirmed by capture ELISA with streptavidin-coated using anti-HA antibodies for detection.

Sequencing and immunoglobulin expression from HA-specific B cells. Cryopreserved PBMC samples from VRC 310 participants isolated 2wks post-immunization were stained and sorted on a FACS Aria II using fluorescently labelled recombinant H1 (A/New Caledonia/20/1999), H5 (A/Indonesia/05/2005) or H3 (A/Perth/16/2009) probes as previously described (Whittle *et al., J Virol.* 88, 4047-4057, 2014). The sequencing and cloning of BCRs from single sorted B cells was performed as previously described (Whittle *et al., J Virol.* 88, 4047-4057, 2014; Tiller *et al., J Immunol. Meth.* 329, 112-124, 2008). Heavy and light chain expression plasmids were transfected into Expi293F cells using ExpiFectamine (Invitrogen) and monoclonal antibodies were purified from culture supernatants using sepharose Protein-A or G (Pierce) as per the manufacturer's instructions.

Flow cytometry and cell sorting. Labelling of HA probes was achieved by the sequential addition of phycoerythrin (PE) or allophycocyanin (APC) labelled streptavidin (Life Technologies). Cryopreserved PBMC samples from VRC 310 participants isolated at baseline or 2wks post-final immunization were stained with the following labelled monoclonal antibodies: CD3-QD655, CD14-QD800, and CD27-QD605 (Molecular Probes/Life Technologies); CD19-ECD (Beckman Coulter); IgD-Cy7PE, IgM-Cy5.5-PerCP, and IgG-BV421 (BD Pharmingen) and ~0.05μg each HA probe per sample. Cell viability was assessed using Aqua amine-reactive dye (Life Technologies). Samples were collected using a FACS Aria II instrument (BD Immunocytometry Systems) configured to detect 18 fluorochromes and analyzed using FlowJo software version 9.5.2 (TreeStar). Single memory B cells binding to both H5 and H3 probes were sorted and sequencing of immunoglobulin genes by multiplex PCR performed as previously described (Whittle *et al., J Virol.* 88, 4047-4057, 2014; Tiller *et al., J Immunol. Meth.* 329, 112-124, 2008). PCR products were sequenced by Beckman Genomics, analyzed using IGMT/V-QUEST (Giudicelli, Brochet & Lefranc, *Cold Spring Harbor protocols,* 2011, 695-715, 2011; Brochet, Lefranc & Giudicelli, *Nucleic Acids Res.,* 36, W503-508, 2008), and grouped into clonal families based upon common genetic elements and motifs within the complementarity-determining region H3 (CDR-H3).

Cloning of antibodies. Immunoglobulin heavy chain or light chain sequences were constructed by gene synthesis and cloned into human IgG1, lambda or kappa expression plasmids as previously described (Whittle *et al., J Virol.* 88, 4047-4057, 2014; Tiller *et al., J Immunol. Meth.* 329, 112-124, 2008). Heavy and light chain expression plasmid DNA was produced using maxiprep kits (Qiagen) and transfected into Expi293F cells using ExpiFectamine (Life Technologies). Monoclonal antibodies were purified from culture supernatants using sepharose Protein-A or G (Pierce) as per the manufacturer's instructions.

HA antibody binding competition assay. Meso Scale Discovery (MSD) 384 well Streptavidin coated SECTOR® Imager 2400 Reader Plates (Standard) (Cat# L25SA-1) were blocked with 35 μL of 5% (W/V) MSD Blocker A (Cat # R93BA-4) for 30 to 60 min and then washed three times with 0.05%Tween PBS (wash buffer). The plates were coated with biotinylated HA protein (H1N1 NC99 or CA09) at 2 μg/mL concentration for 1 hour and then washed with the wash buffer. 1% MSD Blocker A was used as assay diluent in the assay. CR9114 or F10 Fab at 4 μg/mL concentration was used to block the stem region of the HA. VRC01 ScFv at 4 μg/mL was used as a negative control for the inhibition step. After an hour of incubation with the Fab or ScFv, the plates were washed and then the test antibodies at 10 μg/mL and 3-fold serial dilutions were added to the plates. The plates were again washed with the wash buffer after an hour of incubation with the antibodies and SULFO-TAG conjugated Anti-Hu/NHP IgG secondary detection antibody (MSD Cat# D20JL-6) was used for detection at 1 μg/mL. After an hour of incubation, the unbound secondary detection antibody was washed off the plates and the plates were read using 1X MSD Read Buffer (MSD Cat# R92TC-2) using a MSD sector imager 2400.

Negative-stain electron microscopy. Fabs of antibodies were produced and purified as described in the X-ray crystallography methods. In addition to H3 HK68, constructs encoding the soluble extracellular domain of hemagglutinins (HA) of A/Perth/16/2009 (H3N2) (H3 PE09) and A/Albany/14/1957 (H1N1) (H1 Alb57) with an added C-terminal Thrombin cleavage site followed by a T4 fibritin (foldon) trimerization motif and a His_6 purification tag or a tandem His8-Strep-tag® III purification tag were also produced and purified as described in the X-ray crystallography methods. To achieve desired orientations for imaging, Fab and HA proteins were mixed at varied ratios and often a ratio of approximately 1 Fab per trimer provided optimal orientations.

Samples of each complex were diluted to ~0.03 mg/ml, adsorbed to a freshly glow-discharged carbon-film grid for 15s, and stained with 0.7% uranyl formate. Images were collected at 0° and 45° tilt semi-automatically using SerialEM (Mastronarde, *J Struct. Biol.,* 152, 36-51, 2005) on a FEI Tecnai T20 microscope operating at 200 kV and equipped with a 2k x 2k Eagle CCD camera. The pixel size was 0.22 nm/px. Particles were picked manually or using the swarm mode in e2boxer from the EMAN2 software package (Tang *et al., J Struct. Biol.,* 157, 38-46, 2007). Reference-free 2D class averages were calculated with EMAN2 and SPIDER (Shaikh *et al., Nature protocols,* 3, 1941-1974, 2008).

X-ray crystallography. Constructs encoding the hemagglutinin (HA) of A/Hong Kong/1-4-MA21-1/1968 (H3N2) (H3 HK68) or the HA of A/California/04/2009 (H1N1) (H1 CA09) spanning residues 1 to 521 with a C-terminal Thrombin cleavage site followed by a T4 fibritin (foldon) trimerization motif and a His6 purification tag or a tandem His8-Strep-tag® III purification tag was transiently transfected in HEK293S GnTI-/- cells. Cultures were incubated at 33°C for seven day, after which supernatants were harvested. Protein was purified from supernatant by NiNTA affinity chromatography (GE Healthcare 17-5318-02) and dialyzed into 1x PBS, pH 7.4. The C-terminal Foldon was proteolytically cleaved using Thrombin (EMD Millipore Cat No. 69671) at room temperature overnight. In some cases, purified HA was simultaneously deglycosylated using endoglycosidase H. The resulting mixture was purified by size exclusion chromatography (Superdex 200; GE Healthcare) in PBS, pH 7.4. All HA proteins used for crystallization were in the uncleaved, HA0 precursor form and were not proteolytically cleaved into the HA1 and HA2 chains.

Monoclonal antibodies used in crystallization studies were expressed in HEK Expi293™ using transient co-transfection of constructs encoding the IgG heavy and light chains, respectively. Cultures were fed with fresh 293FreeStyle media (Life Technologies) 4 hr post-transfection and with HyClone SFM4HEK293 enriched medium (HyClone) containing valproic acid (4mM final concentration) 24 hr after transfection. Cultures were incubated at 33°C for six days, after which supernatants were harvested. IgG protein was purified from supernatant using Protein A affinity chromatography and dialyzed against PBS, pH 7.4. The fragments antigen binding regions (Fab) were proteolytically cleaved from the fragment crystallizable regions (Fc) using endoproteinase Lys-C digestion at 37°C for 4 hours. The resulting Fc molecules and any remaining IgG was removed from the mixture using Protein A chromatography and the Fab molecules were purified by size exclusion chromatography (Superdex 75; GE Healthcare) in PBS, pH 7.4. For crystals of Fabs, purified Fab was concentrated to 7-10 mg/ml and used for crystallization screening. For crystals of Fabs in complex with HA, excess Fab was mixed with purified, glycosylated or deglycosylated HA. After 30 min at room temperature or overnight at 4°C, the complexes were purified by size exclusion chromatography and concentrated to 7-10 mg/ml.

Crystallization screening was carried out using a TTP Labtech mosquito® Crystal robot, using the sitting well vapor diffusion method at 20° C by mixing 0.1 µl of protein complex with 0.1 µl of reservoir solution. Once initial crystal conditions were observed, further crystallization trials to improve crystal size and shape were carried out by hand using a 1:1 ratio of protein and reservoir solution. Optimized crystals were briefly soaked in mother liquor supplemented with a cryoprotectant and frozen in liquid nitrogen prior to collection of x-ray diffraction data. Crystals of the 56.a.09 Fab were obtained using the hanging drop vapor diffusion method with an 8.1 mg/ml protein solution and a reservoir solution of 0.1M sodium acetate, pH 5.5, 25% (w/v) PEG-400 and 11% (w/v) PEG-8000. Mother liquor containing 50% (w/v) PEG-400 was used as a cryoprotectant. Crystals of the 56.a.09 Fab in complex with glycosylated H3 HK68 were obtained using the hanging drop vapor diffusion method with a 7.9

mg/ml protein solution and a reservoir solution of 0.28M ammonium citrate, pH 8.5, and 12.5% (w/v) PEG-8000. Mother liquor supplemented with 27% glycerol was used as a cryoprotectant. Crystals of the 31.d.09 Fab in complex with glycosylated H1 CA09 were obtained using the hanging drop vapor diffusion method with a 7.2 mg/ml protein solution and a reservoir solution of 0.1M imidazole, pH 6.5, 0.1M MgCl2, 1.75M NaCl, and 15% PEG-3350. Mother liquor supplemented with 22% (v/v) glycerol was used as a cryoprotectant. Crystals of 16.a.26 Fab in complex with deglycosylated H3 HK68 were obtained using the sitting well vapor diffusion method with a 8.6 mg/ml protein solution and a reservoir solution of 0.01M L-cysteine, 0.09M sodium cacodylate, pH 6.5, 0.18M magnesium acetate, and 7.9% (w/v) PEG-8000. Mother liquor supplemented with 15% (2R,3R)-butanediol was used as a cryoprotectant. Crystals of 16.g.07 Fab in complex with deglycosylated H3 HK68 were obtained using the sitting well vapor diffusion method with a 8.6 mg/ml protein solution and a reservoir solution of 0.010M Tris(2-carboxyethyl)phosphine HCl (TCEP), 0.09 M 2-(N-morpholino)ethanesulfonic acid (MES), pH 6.0, 12% (w/v) PEG-6000. Mother liquor supplemented with 22% ethylene glycol was used as a cryoprotectant. Crystals of 31.a.83 Fab in complex with deglycosylated H3 HK68 were obtained using the sitting well vapor diffusion method with a 6.9 mg/ml protein solution and a reservoir solution of 0.1M Tris, pH 8.5, 5.0% (w/v) PEG-8000, 20% (v/v) PEG-300, and 10% (v/v) glycerol. Mother liquor supplemented with an additional 15% (v/v) PEG-300 was used as a cryoprotectant.

Data for all crystals were collected at a wavelength of 1.00 Å at SER-CAT beamlines BM-22 and ID-22 (Advanced Photon Source, Argonne National Laboratory). All diffraction data were processed with the HKL2000 suite (Otwinowski & Minor, *Method Enzymol,* 276, 307-326, 1997) and truncated at CC1/2 of 0.5 in the highest resolution shell (Karplus & Diederichs, *Science,* 336, 1030-1033, 2012). Diffraction of crystals of 31.a.83 Fab in complex with deglycosylated H3 HK68 and 31.b.09 in complex with glycosylated H1 pCA09 was anisotropic and resolution limits were respectively set at 4.00 Å, 3.71 Å, 3.71 Å and 3.38 Å, 3.38 Å, 3.40 Å, in the a*, b*, and c* axes. Diffraction data was then rescaled using the UCLA Diffraction Anisotropy Server (Strong *et al., PNAS,* 103, 8060-8065, 2006).

Resolutions of the structures are reported as the highest resolution shell with at least 50% completeness and an I/σI of two or higher. Structures were solved by molecular replacement using PHASER 45, and iterative model building and refinement were performed in COOT (Emsley *et al.*, *Acta crystallog. D, Biol. Crystallog.,* 66, 486-501, 2010) and Phenix (Adams *et al. Acta crystallog. D, Biol. Crystallog.,* 66, 213-221, 2010) or BUSTER (Bricogne *et al., Global Phasing Ltd., Cambridge, United Kingdom,* 2011), respectively. Prior to refinement, a cross validation (Rfree) test set consisting of 5% of the reflections was selected and used to assess the model accuracy throughout the refinement process. For the 56.a.09 Fab crystals, PDB ID 1BVK was used as a search model for the Fab variable domain (Fv), and PDB ID 4LSS was used as a search model for the Fab constant domain (CH1-CL). For the crystals of 56.a.09 Fab in complex with glycosylated H3 HK68, PDB ID 4FNK was used as a search model for the HA with separate searches for the head (HA1 residues 55-274) and stem (HA1 residues 9-54, 275-326 and all of HA2) domains, and the refined 56.a.09 Fab structure was used as a search model for the Fab. For the crystals of 31.b.09 Fab in complex with glycosylated H1 pCA09, PDB ID 1WT5 was used as a search model for the Fv domain, and an aligned ensemble of PDB IDs 3AL4, 3LZG, 3UBE, 3UBJ, 3UBN, 3UBQ, 3ZTN, 4JTV, 4JTX, 4JUO was used as a search model for the HA with separate searches for the head and stem as described above. PDB ID 4LSS was used as a search model for the CH1-CL domain. For the crystals of 16.a.26 Fab in complex with deglycosylated H3 HK68, PDB ID 4FNK was used as a search model for the HA, PDB ID

1XIW was used as a search model for the Fab Fv domain, and PDB ID 4LSS was used as a search model for the Fab CH1-CL domain. For the crystals of 16.g.07 Fab in complex with deglycosylated H3 HK68, the Fv domain and H3 trimer from the crystal structure of 16.a.26 Fab in complex with HA, and the CH1-CL domain of PDB ID 4LSS were used as search models. For the crystals of 31.a.83 Fab in complex with deglycosylated H3 HK68, PDB ID 4FNK was used as a search model for the HA with separate searches for the head and stem domains as described above, PDB ID 1FGV was used as a search model for the Fab Fv domain, and PDB ID 4LSS was used as a search model for the Fab CH1-CL domain. The Ramachandran plot as determined by MOLPROBITY78 showed 91-97% of all residues in favored regions and 97-99% of all residues in allowed regions.

Population analysis for compatible germline genes for each multi-donor antibody classes. The percentage of human population that have the potential to produce antibodies from each of the three multi-donor antibody classes (defined in Figure 2, 3, and 4) was estimated based on the population genetics data from 1000 Genomes Project (Genomes Project *et al., Nature* 491, 56-65, 2012). For the HV6-1-HD3-3 class, about 91% of alleles make a full length HV6-1 and 9% of the alleles include a stop codon. Since humans have two alleles, ~99% $(1-(0.09)^2)$ of the population should have a functional HV6-1 allele. For the HD3-3 virtually the entire human population has this gene. This implies that about 99% of the human population should have alleles compatible with the HV6-1-HD3-3 class. For the HV1-18 Q-X-X-V class, only less than 0.1% of the alleles contain premature stop codons in the HV1-18 gene, suggesting that more than 99.9% of human population have the potential to produce this class of antibodies. Similar analysis showed that more than 99.9% of human population has the potential to produce HV1-18 HD3-9 class antibodies.

Delineation of sequence signatures

HV6-1 +HD3-3 class

Three distinct memory B cell lineages were observed, from subjects 31, 54 and 56, to share heavy chain sequences derived from a recombination of HV6-1 with HD3-3, and HJ4 or HJ5, to yield highly similar amino acid sequences in the CDR H3 (FIG. 1E, top row; FIG. 20) and also to share similar neutralization signatures (FIG. 6E). These three lineages shared the same CDR H3 length (16 amino acids) and a conserved ^{98}MIFGI motif, with a somatic hypermuation at Val100$_a$ILE and Met98 at the V-D junction. Structural analysis of 56.a.09, a prototypic antibody of this class, revealed that heavy chain binding involved the HD3-3-encoded CDR H3 (FIG. 2C) with Phe100$_{HC}$, Gly100a$_{HC}$ and Val100c$_{HC}$ contributing ~240 Å^2 of BSA and the SHM-altered Val100bIle$_{HC}$ inserting directly into the Trp21$_{HA2}$ pocket (contributing over 100 Å^2 of interactive surface). In addition, Met98$_{HC}$ interacted with a conserved aromatic residue present on all light chains, helping to orient the CDR H3 (FIG. 2D). These observations suggested this stretch of CDR H3 residues to be critical to the binding of the antibody to HA.

Thus, for the HV6-1+HD3-3 class, a five amino acid motif (^{98}MIFGI) was chosen in addition to HV and HD gene requirements and a CDR H3 length of 16 amino acids as the signature for this class of antibodies.

HV1-18+HD3-9 class

Two distinct memory B cell lineages from subjects 1 and 31 were observed to share immunoglobulin heavy chain sequence derived from recombination of HV1-18 with HD3-9 and HJ4, to have highly similar amino acid sequences in a CDR H3 of 15-amino acids in length (FIG. 1E, 13), and to share similar neutralization signatures (FIG. 6E). Notably an Arg96$_{HC}$ residue was encoded by n-nucleotide addition in both cases (FIG. 3A)

and a ^{99}ILTG motif was conserved in both cases. Thus, for the HV1-18+HD3-9 class a seven amino acid signature (R-x-x-I-L-T-G, beginning with $R96_{HC}$) was chose in addition to the HV gene requirement and a CDR H3 length of 15 amino acids as the signature for this class of antibodies.

HV1-18 (Q-x-x-V)

Multiple B cell lineages were observed in two subjects (16 and 54) to produce distinctive HV1-18-derived immunoglobulins sharing five genetic elements and having a CDR H3 of 21 amino acids derived from recombination with either HD2-2 or HD2-15 genes (FIGs. 4A and 13, FIG. 20) and to share similar neutralization signatures (FIG. 6E). These sequences all contained a ^{98}Q-x-x-V motif in CDR H3, and crystal structures of two prototypic antibodies, 16.a.26 and 16.g.09, displayed similar recognition modes (FIG. 4D and 4G). The location of $Gln98_{HC}$ within the framework pocket likely stabilized the perpendicular orientation of the CDR H3 relative to the antibody-framework regions and may allow for CDR H3-motifs derived from diverse D genes to bind to this conserved hydrophobic groove. The conserved $Val100a_{HC}$ inserted into a pocket present on both group 1 and group 2 HA. In addition, the recently reported HV1-18-derived group 1 and group 2-neutralizing antibody CT149 uses ^{98}Q-x-x-V motif with a 19 amino acid CDR H3 to bind the HA stem (Wu *et al., Nat Commun* 6, 7708, 2015) and to recognize HA in a mode similar to both 16.a.26 and 16.g.07. Furthermore, it is noted that antibodies from subject 01 with an HV1-18 germline gene and a Gln98-x-x-Val100a motif in a 17 amino acid CDR H3 were able to neutralize H3 and H5 strains of influenza. Thus, a four amino acid CDR H3 signature (Q-x-x-V, beginning with $Q98_{HC}$) was specified, in addition to an HV1-18 germline requirement and a CDR H3 length between 17 and 21 amino acids, as the initial signature of this class of antibodies. Almost half of the sequences (318) of cross-reactive B cells from the VRC 310 trial were observed to have this initial signature. Transcripts corresponding to this signature were also present in other data sets (FIG. 6A). $Tyr53_{HC}$, which is encoded in HV1-18 germline gene, was selected as an additional component of the signature for this class as it contributed the largest BSA among all heavy chain residues (and three post-TIV Jiang *et al.* (*Science translational medicine* 5, 171ra119, 2013) sequences had a Cys at this position). Thr54HC, a conserved SHM-change present in 309 of the 318 VRC 310 sequences, but in less than half of the transcripts in other data sets that otherwise satisfied the initial signature, was also selected as an additional component of the signature for this class. Thus, for the HV1-18 (Q-x-x-V) class, a 2-residue CDR H2 signature ($Tyr53_{HC}$ and $Thr54_{HC}$) was combined with a four amino acid CDR H3 signature (Q-x-x-V, beginning with $Q98_{HC}$), in addition to the HV1-18 germline requirement and a CDR H3 length between 17 and 21 amino acids, as the refined signature for this class of antibodies.

Using bioinformatics and sequence signatures to sieve NGS data. Three next-generation sequencing (NGS) data sets from normal donors were obtained using either a 454 pyrosequencing or Illumina paired-end sequencing. Amplicons for use with the 454 pyrosequencing platform used either the "Vh_all" family of primers (Doria-Rose *et al., Nature,* 509, 55-62, 2014) as described in (Zhu *et al., PNAS,* 110, E4088-4097, 2013) or through the use of 5' RACE as described in (Bonsignori *et al.*, Maturation pathway from germline to broad HIV-1 neutralizer of a CD4-Mimic antibody, Cell, 2016)). Amplicons for use with the Illumina platform were generated using 5' RACE in a manner similar to that used for 454 pyrosequencing as described in (Bonsignori *et al.*, Maturation pathway from germline to broad HIV-1 neutralizer of a CD4-Mimic antibody, Cell, 2016). Paired-end reads from the Illumina platform were merged using the program FLASH (Magoc, FLASH: fast length adjustment of short reads to improve genome assemblies. Bioinformatics *27*, 2957-2963, 2011); to ensure consistency after

merging, all non-duplicate reads were filtered out. All reads were filtered using a minimum read length of 350 nucleotides.

NGS data was also analyzed from influenza-vaccinated donors (Jiang *et al., Science translational medicine* 5, 171ra119, 2013). As in the case for normal donors, all reads with less than 350 nucleotides and with Phred scores of less than 20 occurring over 80% of the read were discarded. The program Flexbar (Dodt *et al.*, *Biology* 1, 895-905, 2012) was used to demultiplex the NGS data using barcode information available from the Sequence Read Archive (SRA) (see SRX190717 for details). The demulitplexed NGS data was then separated into pre and post influenza-vaccinated data sets.

Germline genes were assigned to all filtered reads using IgBlast (Ye *et al.*, *Nucleic acids research,* 41, W34-40, 2013). Reads containing the heavy chain germline genes HV6-1 and HV1-18 were placed into one of three classes based on their heavy chain germline gene assignment, CDR H3 length (measured between the canonical cysteine and tryptophan) and a sequence signature in the CDR H3 junction. For the first class, all reads were required to contain the HV6-1 and HD3-3 germline gene assignments along with a CDR H3 length of 16. In addition, the following sequence signature appeared in the CDR H3 junction (where 'x' denotes any amino acid): xxxxxMIFGIxxxxxx (SEQ ID NO: 128 flanked by an N-terminal Cys and a C-terminal Trp. For the second class, all sequences had the HV1-18 germline gene assignment and a CDR H3 length of 15. The specific sequence signature associated with the second class was (where 'x' denotes any amino acid): xxxRxxILTGxxxxx (SEQ ID NO: 129) flanked by an N-terminal Cys and a C-terminal Trp. For the third and final class, all sequences had the HV1-18 germline gene assignment and a CDR H3 length of between 17-21 amino acids. The sequence signature associated with the third class was (where 'x' denotes any amino acid): $xxxxxQxxV(x)_n$ (SEQ ID NO: 131) flanked by an N-terminal Cys and a C-terminal Trp, where n=8-12. In addition to the CDR H3 sequence signature for the third class, residue 54 in the CDR H2 region was required to be a threonine.

After assigning all reads (sequences) to their respective classes, any sequences containing stop codons in the VDJ region were discarded. The final pool of sequences in each of the three classes was then clustered based on their nucleotide sequence in the CDR H3 junction region with the program CD-HIT (Fu *et al.*, *Bioinformatics* 28, 3150-3152, 2012). The sequence identity and sequence coverage threshold was set to 90% and 97% respectively. For each cluster containing two or more sequences, a consensus DNA sequence using members from the cluster was generated. When a cluster contained just two sequences and the consensus DNA failed to translate without a stop codon, the junction from each sequence was analyzed separately. For cases where there were more than two sequences in a cluster and the consensus DNA sequence failed to translate without a stop a codon, the sequence with lowest identity to all other member sequences was removed and an attempt was made to translate the consensus DNA sequence using the remaining members. This procedure was repeated until a consensus DNA sequence that translated without any stop codons was obtained. All singleton clusters were discarded. In those cases where framework primers were used in the PCR prep that resulted in the n-terminal truncation of the read, amino acids from the corresponding germline V gene were used to fill in the framework region.

To estimate the number of unique recombination events (lineages) from the consensus DNA sequences, all CDR H3 junctions were analyzed using the program JOINSOLVER (Souto-Carneiro *et al.*, 2004) (joinsolver.niaid.nih.gov/). The number of unique recombination events for each class after clustering appears in FIG. 6A. For each consensus DNA sequence, the sequence at the junction was extracted and generated an amino acid and DNA sequence alignment with the corresponding germline D-gene (FIGs. 14A-14C). The SRA project

numbers for the normal donor NGS data are SRP026397, SRP067168 and SRP073039. The SRA project number for the influenza-vaccinated donor data is SRP015957.

Sequences from supplementary data set 1 belonging to the paired heavy and light chain dataset (DeKosky *et al., Nature medicine* 21, 86-91, 2015) were screened for each of the sequence signatures. Sequences matching the correct signature were then used to identify the corresponding transcript in the SRP047462 dataset. However, since these transcripts did not cover the full HV or LV coding region, the assigned germline gene sequence was used to fill in the missing region in the variable domain of the antibody sequence.

Antibody homology model calculation. Homology models for identified antibody sequences were generated using NEST (Petrey *et al., Proteins* 53 Suppl 6, 430-435, 2003) or SWISS-MODEL (Arnold *et al., Bioinformatics* 22, 195-201, 2006) using the corresponding VRC 310 antibody crystal structure.

Frequentist probability analysis. The likelihood of observing convergence for a set of genetic elements from different *m* lineages among N total lineages was estimated based on the probability of the following: given a particular set of genetic elements were observed in the first lineage, the same set of genetic elements were observed *m*-1 times in the rest of the N-1 lineages. A uniform distribution was used for the probabilities of observing a particular HV (51 genes), HJ (6 genes), HD (27 genes), LV (77 genes), or LJ (7 genes) gene. The probability distribution of CDRH3 and CDRL3 lengths were taken from the abYsis database (bioinf.org.uk/abysis/). As an example, if 3 lineages out of the 78 lineages from the 6 subjects share the same HV, HD, HJ, CDRH3 length, LV, and CDRL3 length, then the frequentist probability can be calculated as the following:

$$P = \binom{N-1}{m-1}(p)^{m-1} * (1-p)^{N-1-(m-1)} = \binom{78-1}{3-1}(p)^{3-1} * (1-p)^{78-1-(3-1)} = 4.9E * 10^{-12}$$

where

$$p = p_{HV} * p_{HD} * p_{HJ} * p_{(CDRH3\ length=16)} * p_{LV} * p_{(CDRL3\ length=9)} = \frac{1}{51} * \frac{1}{27} * \frac{1}{6} * 0.062 * \frac{1}{77} * 0.42 = 4.1 * 10^{-8}$$

Neutralization Breadth Calculation. The percentage of human-infecting influenza HA subtypes neutralized by antibody was calculated as the minimum branch length connecting all neutralized viruses (at the specified IC50 cutoff) divided by total branch length of the phylogenetic tree generated from all tested viral strains. The sequence alignment was generated using MUSCLE and the phylogenetic tree was generated using the maximum likelihood method.

Antibody Neutralization Fingerprint. An antibody neutralization fingerprint is defined as the potency-based pattern of neutralization of a set of viral variants. In the case of HIV-1 (Georgiev *et al., Science* 340, 751-756, 2013) antibody neutralization fingerprints have been shown to be epitope-specific: antibodies targeting similar epitopes have similar fingerprints, and vice versa. The neutralization fingerprinting analysis involves two steps: (a) compute the correlations between the neutralization fingerprints of each pair of antibodies, effectively transforming an antibody-virus neutralization matrix into an antibody-antibody correlation matrix; and (b) apply hierarchical clustering to the antibody-antibody correlation matrix to obtain an antibody clustering tree, in which distances are based on the similarity of the different antibody neutralization fingerprints. One of the factors influencing the success of the clustering procedure is the number of viral variants used in the analysis: a small number of viral variants is typically associated with less accurate antibody clustering (Georgiev *et al., Science* 340, 751-756, 2013). Here, the neutralization fingerprints and antibody clustering was computed based on a set of 15 diverse influenza strains. Antibodies with less than 30% neutralization breadth on the 15-strain panel were not included in the analysis.

Table 2A. VRC 310 trial immunization scheme, related to FIG. 1.

Group	N=	Day 0	Day 28	Day 56	Day 84	Day 112	Day 168
1	9	H5N1 MIV					H5N1 MIV
2	10	H5 DNA	H5N1 MIV				
3	11	H5 DNA		H5N1 MIV			
4	11	H5 DNA			H5N1 MIV		
5	11	H5 DNA				H5N1 MIV	
6	11	H5 DNA					H5N1 MIV

Table 2B. VRC 310 select subject information, related to FIG. 1.

Subject	Age	Gender	VRC 310 vaccine arm	Pre-H5N1 H7N7 neutralization titer (reciprocal IC_{50})	Post-H5N1 H7N7 neutralization titer (reciprocal IC_{50})
1	29	Female	3	81.0	127.3
10	54	Female	6	12.1	71.8
16	21	Female	4	99.9	243
27	29	Male	3	102.7	140.4
29	29	Male	6	127.5	49.4
31	27	Female	3	301.6	129.2
36	40	Female	2	563.6	303
54	20	Female	2	602.4	326.5
56	45	Female	5	159.0	217.3
59	22	Male	3	158.9	73.3

Table 3A. 56.a.09 interface with HA of A/Hong Kong/1-4-MA21-1/1968, related to FIG. 2.

	56.a.08	A/Hong Kong/1-4-MA21-1/1968 HA	Distance (Å)
Hydrogen bonds			
	H:GLN 1[N]	A:MAN1042[O4]	3.60
	H:ARG 33[NH1]	A:NAG1039[O6]	3.73
	H:ARG 33[NH2]	A:MAN1041[O6]	3.30
	H:ARG 50[NH1]	B:ASP 19[OD1]	3.37
	H:ARG 50[NH2]	B:ASP 19[OD2]	3.40
	H:TYR 52[OH]	B:GLY 16[O]	3.01
	H:SER 53[OG]	B:GLU 15[OE1]	3.30
	H:MET 98[O]	A:NAG1038[O6]	3.55
	H:PHE 100[N]	A:NAG1038[O6]	3.48
	H:GLY 100a[N]	A:NAG1038[O5]	3.77
	H:GLY 100a[N]	A:NAG1038[O6]	3.57
	L:SER 31[O]	B:GLN 42[NE2]	2.54
	L:SER 31[OG]	B:GLN 42[OE1]	2.12
	L:SER 31[OG]	B:ASP 46[OD1]	3.90
	L:SER 32[OG]	B:GLN 42[NE2]	2.83
	L:TYR 33[OH]	B:ASP 19[O]	2.31
	L:THR 57[OG1]	A:MAN1042[O3]	3.02
	L:TYR 92[OH]	B:ASP 19[OD2]	2.21
	L:GLY 94[O]	B:ALA 36[N]	3.55
Salt bridges			
	H:ARG 50[NH1]	B:ASP 19[OD1]	3.37
	H:ARG 50[NH2]	B:ASP 19[OD1]	3.62
	H:ARG 50[NH2]	B:ASP 19[OD2]	3.40

Table 3B. Buried surface area of 56.a.09 antibody in complex with A/Hong Kong/1-4-MA21-1/1968 H3 hemagglutinin. Residues that form hydrogen bonds and salt bridges are indicated, related to FIG. 2.

56.a.09 antibody

Residue	Bond type	Accessible Surface Area (Å²)	Buried Surface Area (Å²)
H:GLN 1	H	202.4	102.2
H:GLY 26		68.0	19.0
H:ASP 27		33.7	12.6
H:ASN 32		89.4	5.7
H:ARG 33	H	185.5	102.0
H:ALA 35		2.3	1.5
H:ARG 50	HS	51.6	23.6
H:TYR 52	H	44.8	40.3
H:ARG 52b		100.4	65.8
H:SER 53	H	102.7	14.3
H:TYR 56		92.4	50.9
H:ASP 58		56.7	10.3
H:ALA 97		44.6	23.0
H:MET 98	H	103.2	40.6
H:ILE 99		85.2	11.6
H:PHE 100	H	190.3	181.3
H:GLY 100a	H	63.0	57.4
H:ILE 100b		121.1	112.2
H:VAL 100c		56.5	13.3
H:ILE 100d		81.6	46.2
L:GLU 1		150.8	20.5
L:SER 28		79.1	5.0
L:ALA 30		67.6	43.7
L:SER 31	H	64.3	30.3
L:SER 32	H	77.8	29.4
L:TYR 33	H	119.8	73.1
L:TYR 50		110.2	11.9
L:THR 57	H	134.8	30.6
L:TYR 92	H	74.0	48.4
L:ASP 93		21.9	7.2
L:GLY 94	H	74.7	56.7
L:SER 95		103.3	27.8
L:TYR 97		132.4	1.8

A/Hong Kong/1-4-MA21-1/1968 H3 hemagglutinin Residue	**Bond type**	**Accessible Surface Area (Å²)**	**Buried Surface Area (Å²)**
A:HIS 18		124.8	33.0
A:PRO 21		103.1	5.5
A:THR 37		23.9	4.7
A:ASN 38		91.3	40.4
A:NAG1038	H	356.7	75.8
A:NAG1039	H	355.5	88.5
A:BMA1040		292.8	38.0
A:MAN1041	H	293.8	119.2
A:MAN1042	H	290.4	94.5
B:GLU 15	H	169.7	52.1
B:GLY 16	H	46.1	46.0
B:MET 17		21.32	0.2
B:ILE 18		156.0	135.5
B:ASP 19	HS	116.9	116.9
B:GLY 20		18.7	13.7
B:TRP 21		163.3	51.0
B:ARG 25		115.4	12.6
B:GLN 34		81.8	34.7
B:ALA 35		21.8	17.1
B:ALA 36	H	30.4	20.2

B:LEU 38		116.6	93.4
B:LYS 39		167.9	8.6
B:THR 41		6.2	5.4
B:GLN 42	H	89.2	72.6
B:ILE 45		79.6	79.6
B:ASP 46	H	84.0	4.5
B:ILE 48		55.2	20.1
B:ASN 49		114.4	15.2
B:GLU 150		88.6	21.6
B:ARG 153		65.7	4.4

Table 3C. 31.b.09 interface with A/California/04/2009 H1 hemagglutinin, related to FIG. 3.

	31.b.09	A/California/04/2009 HA	Distance (Å)
Hydrogen bonds			
	L:VAL 27c[N]	A:ASN 382[OD1]	3.68
	L:SER 65[OG]	A:ASN 289[N]	3.72
	L:SER 65[OG]	A:THR 290[O]	3.21
	L:HIS 93[NE2]	A:ASP 375[OD2]	3.35
	H:HIS 98[NE2]	A:VAL 347[O]	2.38
Salt bridges			
	L:HIS 93[NE2]	A:ASP 375[OD1]	3.79
	L:HIS 93[NE2]	A:ASP 375[OD2]	3.35

Table 3D. Buried surface area of 31.b.09 antibody in complex with A/California/04/2009 H1 hemagglutinin. Residues that form hydrogen bonds and salt bridges are indicated, related to FIG. 3.

31.b.09 antibody Residue	**Bond type**	**Accessible Surface Area (Å²)**	**Buried Surface Area (Å²)**
H:TRP50		10.1	2.4
H:TYR53		152.0	59.3
H:GLY55		32.1	17.5
H:ASN56		136.6	50.5
H:ASN58		67.4	9.1
H:HIS98	H	108.4	73.8
H:ILE99		44.1	10.4
H:LEU100		148.9	110.4
H:THR100a		98.5	1.4
L:GLN27		106.2	30.5
L:GLY27a		47.0	25.2
L:LEU27b		14.5	6.7
L:VAL27c	H	81.1	66.9
L:TYR27d		116.6	51.2
L:ILE27e		133.1	110.2
L:ASP28		128.3	60.1
L:GLY29		43.0	10.8
L:THR31		38.1	16.6
L:ASN50		60.8	4.3
L:VAL51		20.5	10.5
L:PHE52		114.8	75.8
L:SER65		50.7	22.1
L:GLY66		31.2	13.5
L:SER67	H	105.2	47.9
L:GLY68		41.1	4.4
L:THR92		12.4	5.9
L:HIS93	HS	112.0	37.8
L:TRP94		177.8	36.0

A/California/04/2009 H1 hemagglutinin Residue	Bond type	Accessible Surface Area (Å²)	Buried Surface Area (Å²)
A:HIS 18		18.02	8.0
A:ASP 24		43.93	7.2
A:HIS 38		79.39	51.6
A:SER 39		21.73	21.1
A:VAL 40		57.31	45.8
A:GLN 282		20.28	0.61
A:LYS 285		186.53	0.37
A:GLY 286		18.22	11.7
A:ALA 287		40.10	17.6
A:ILE 288		74.82	41.8
A:ASN 289	H	115.97	75.7
A:THR 290	H	21.00	11.6
A:SER 291		98.27	48.4
A:ARG 315		49.59	1.3
A:THR 318		15.34	14.5
A:GLY 345		47.26	1.1
A:VAL 347	H	106.64	57.8
A:ASP 348		95.60	72.5
A:GLY 349		9.49	8.7
A:TRP 350		26.36	26.4
A:TYR 363		97.47	2.5
A:ALA 365		16.07	3.8
A:LEU 367		128.86	87.1
A:THR 370		9.94	3.8
A:GLN 371		92.76	56.3
A:ILE 374		68.26	66.3
A:ASP 375	HS	77.16	25.0
A:ILE 377		11.88	5.2
A:THR 378		68.77	57.0
A:ASN 379		101.07	6.4
A:VAL 381		14.89	10.0
A:ASN 382	H	80.04	66.7
A:ILE 385		67.85	26.6
A:GLU 479		87.21	7.4

H: Hydrogen bond, S: Salt bridge

Table 3E. 16.a.26 interface with A/Hong Kong/1-4-MA21-1/1968 H3 hemagglutinin, related to FIG. 4.

	16.a.26	A/Hong Kong/1-4-MA21-1/1968 HA	Distance (Å)
Hydrogen bonds			
	H:ARG 31[NH1]	B:ASP 19[O]	3.8
	H:TYR 53[OH]	B:ASP 19[O]	2.9
	H:GLN 98[OE1]	B:GLN 42[NE2]	3.5
	H:GLY 99[N]	B:GLN 42[OE1]	3.1
	H:VAL 100b[N]	B:ASN 49[OD1]	2.9
	L:GLU 68[OE2]	B:THR 59[N]	3.1
	L:ASN 92[ND2]	A:ASP 32[OD2]	3.3
Salt bridges			
	L:GLU 68[OE2]	B:LYS 58[NZ]	3.9

Table 3F. Buried surface area of 16.a.26 antibody in complex with A/Hong Kong/1-4-MA21-1/1968 H3 hemagglutinin. Residues that form hydrogen bonds and salt bridges are indicated, related to FIG. 4.

16.a.26 antibody Residue	Bond type	Accessible Surface Area (Å²)	Buried Surface Area (Å²)
H:ARG 31	H	104.7	25.8
H:TYR 53	H	111.0	103.3
H:THR 54		51.3	48.2

H:GLY 55		62.0	7.6
H:ASN 56		91.1	24.8
H:GLN 61		168.9	7.3
H:LYS 97		45.5	2.2
H:GLN 98	H	35.5	25.8
H:GLY 99	H	43.2	31.1
H:GLU 100		101.7	19.3
H:VAL 100a		148.2	77.1
H:VAL 100b	H	48.3	35.3
H:LEU 100c		142.0	28.4
H:PRO 100d		110.8	61.3
H:SER 110g		11.4	0.7
H:ARG 110i		169.2	15.8
L:GLN 27		96.8	5.9
L:SER 28		67.4	9.4
L:GLY 30		35.7	26.8
L:LYS 31		79.4	11.8
L:PHE 32		91.2	37.2
L:TYR 50		84.8	17.5
L:ARG 65		182.8	6.1
L:SER 67		49.2	27.3
L:GLU 68	HS	96.7	57.6
L:ASN 92	H	59.8	19.3
L:ASN 93		63.9	50.6
L:VAL 94		136.0	8.51
L:PRO 95		75.8	5.03
A/Hong Kong/1-4-MA21-1/1968 H3 hemagglutinin Residue	**Bond type**	**Accessible Surface Area (Å²)**	**Buried Surface Area (Å²)**
A:ASN 22		129.7	7.4
A:ASP 32	H	107.4	38.3
A:GLN 33		108.0	52.2
A:NAG1038		362.7	111.4
B:ILE 18		153.4	1.6
B:ASP 19	H	106.0	32.6
B:GLY 20		11.5	1.8
B:TRP 21		124.3	21.0
B:LEU 38		128.3	55.5
B:LYS 39		173.8	40.4
B:THR 41		14.0	13.7
B:GLN 42	H	92.3	82.8
B:ILE 45		38.3	38.3
B:ASP 46		75.3	3.1
B:ILE 48		40.6	13.1
B:ASN 49	H	66.4	45.7
B:LEU 52		112.3	53.0
B:ASN 53		99.6	75.3
B:ILE 56		133.0	61.7
B:GLU 57		104.5	34.2
B:LYS 58	S	175.8	14.5
B:THR 59	H	98.9	62.9
B:GLU 61		115.9	8.8

H: Hydrogen bond, S: Salt bridge

Table 3G. 16.g.07 interface with A/Hong Kong/1-4-MA21-1/1968 H3 hemagglutinin, related to FIG. 4.

	16.g.07	A/Hong Kong/1-4-MA21-1/1968 HA	Distance (Å)
Hydrogen bonds			

	H:THR 55[O]	B:LYS 39[NZ]	3.6
	H:TYR 54[OH]	B:ASP 19[O]	3.4
	H:ARG 100[NH1]	B:ASP 46[OD1]	3.6
	H:VAL 105[N]	A:NAG1038[O3]	3.6
	H:VAL 105[O]	A:NAG1038[O6]	2.8
	H:SER 106[N]	B:ASN 49[OD1]	2.8
	H:ALA 108[N]	B:ASN 53[OD1]	2.7
	L:ARG 31[NH1]	B:ILE 56[O]	3.2
Salt bridges			
	H:ASP 57[OD1]	B:LYS 39[NZ]	3.4
	H:ASP 57[OD2]	B:LYS 39[NZ]	3.6
	H:ARG 100[NH1]	B:ASP 46[OD1]	3.6

Table 3H. Buried surface area of 16.g.07 antibody in complex with A/Hong Kong/1-4-MA21-1/1968 H3 hemagglutinin. Residues that form hydrogen bonds and salt bridges are indicated, related to FIG. 4.

16.g.07 antibody Residue	**Bond type**	**Accessible Surface Area (Å²)**	**Buried Surface Area (Å²)**
H:ARG 31		92.0	1.6
H:TYR 54	H	123.6	107.9
H:THR 55	H	82.9	67.4
H:GLY 56		40.8	2.6
H:ASP 57	S	78.1	26.1
H:ARG 100	HS	80.9	19.5
H:VAL 101		16.9	6.9
H:GLN 102		56.2	42.3
H:MET 103		90.2	52.5
H:GLU 104		88.8	18.8
H:VAL 105		153.4	86.2
H:SER 106	H	19.6	8.1
H:PRO 107		82.5	62.8
H:ALA 108	H	42.3	13.5
H:THR 109		114.1	36.5
H:TRP 113		74.2	30.3
H:GLU 104		88.8	32.3
H:VAL 105	H	153.4	29.3
H:SER 106		19.6	0.2
H:PRO 107		82.5	1.5
L:ASP 28		100.4	1.5
L:SER 30		38.5	31.8
L:ARG 31	H	133.1	99.7
L:TRP 32		144.4	74.0
L:ARG 63		141.9	2.8
L:SER 67		78.8	5.2
L:ARG 31		133.1	6.0
L:SER 52		49.6	10.3
L:LEU 54		60.7	11.5
L:SER 60		117.6	60.1
L:ARG 63		141.9	26.0
A/Hong Kong/1-4-MA21-1/1968 H3 hemagglutinin Residue	**Bond type**	**Accessible Surface Area (Å²)**	**Buried Surface Area (Å²)**
A:ASN 54		72.9	27.1
A:PRO 55		61.9	4.4
A:LYS 264		191.4	27.3
A:ILE 278		136.7	25.7
A:GLU 280		73.7	10.8
A:ASN 290		68.2	6.3
A:NAG1038	H	361.0	83.5

B:ILE 18		169.2	9.3
B:ASP 19	H	65.3	16.4
B:GLY 20		17.8	8.0
B:TRP 21		123.2	9.7
B:ALA 36		23.3	0.2
B:LEU 38		129.1	60.1
B:LYS 39	HS	174.3	61.7
B:THR 41		16.2	15.7
B:GLN 42		86.0	78.4
B:ILE 45		46.0	45.6
B:ASP 46	HS	86.2	31.0
B:ILE 48		41.3	15.5
B:ASN 49	H	80.3	77.7
B:LEU 52		99.2	39.6
B:ASN 53	H	108.7	106.8
B:ARG 54		168.8	2.1
B:ILE 56		133.2	69.1
B:GLU 57		79.4	48.7
B:LYS 58		184.7	35.1
B:THR 59		123.8	31.4
B:GLU 61		126.6	2.6

Table 3I. Antibody 31.a.83 interface with A/Hong Kong/1-4-MA21-1/1968 H3 hemagglutinin, related to FIG. 5.

	31.a.83	A/Hong Kong/1-4-MA21-1/1968 HA	Distance (Å)
Hydrogen bonds			
	H:TYR 100a[OH]	B:ASN 53[OD1]	3.52
	H:ASN 100b[N]	B:ASN 49[OD1]	3.67
	H:TYR 111g[OH]	B:ASP 19[O]	3.02
	H:TYR 100h[N]	B:GLN 42[OE1]	2.70
	H:TYR 100k[OH]	B:GLY 50[O]	3.90
	H:TYR 100h[O]	B:GLN 42[NE2]	2.86
	H:ILE 100[O]	A:NAG1038[O6]	2.78
	H:TYR 100k[OH]	*A'*:ASP 32[OD1]	3.73
	L:SER 28[O]	*A'*:GLN 33[NE2]	3.69

Table 3J. Buried surface area of 31.a.83 antibody in complex with A/Hong Kong/1-4-MA21-1/1968 H3 hemagglutinin. Residues that form hydrogen bonds and salt bridges are indicated, related to FIG. 5.

31.a.83 antibody Residue	**Bond type**	**Accessible Surface Area (Å²)**	**Buried Surface Area (Å²)**
H:ASN 53		70.1	22.5
H:GLY 54		69.5	11.7
H:GLY 55		26.7	15.6
H:TYR 58		92.5	18.3
H:PRO 99		136.0	16.4
H:ILE 100	H	134.9	106.1
H:TYR 100a	H	86.0	69.0
H:ASN 100b	H	52.1	15.4
H:LEU 100c		169.7	169.7
H:MET 100d		124.3	114.2
H:PRO 100e		75.6	4.9
H:TYR 110g	H	131.9	128.7
H:TYR 110h	H	46.7	29.5
H:SER 110i		5.2	5.2
H:THR 110j		84.5	34.4
H:TYR 110k	H	122.9	60.5

L:ILE 2		8.4	4.7
L:GLN 27		130.1	53.7
L:SER 28	H	90.7	53.3
L:VAL 29		1.0	0.6
L:ARG 30		131.9	75.1
L:SER 31		70.1	33.1
L:SER 52		66.5	19.8
L:THR 53		76.1	0.5
L:ARG 54		92.9	23.8
L:GLY 66		22.6	2.9
L:SER 67		73.7	25.0
L:THR 69		56.3	0.1
L:TYR 91		57.0	41.9
L:ASN 92		86.3	34.2
L:HIS 93		85.2	40.8
A/Hong Kong/1-4-MA21-1/1968 H3 hemagglutinin Residue	**Bond type**	**Accessible Surface Area (Å²)**	**Buried Surface Area (Å²)**
A:HIS 18		102.5	20.2
A:ASN 38		82.9	14.7
A:THR 40		55.8	29.6
A:ILE 278		152.8	22.6
A:GLU 280		117.0	20.5
A:ASN 290		62.6	6.6
A:ALA 304		58.4	0.7
A:THR 318		79.3	33.7
A:NAG1038	H	363.7	118.4
B:MET 17		27.6	1.8
B:ILE 18		162.0	22.3
B:ASP 19	H	75.4	13.8
B:GLY 20		15.0	10.2
B:TRP 21		124.7	41.3
B:ALA 36		17.6	0.3
B:LEU 38		141.7	66.9
B:THR 41		13.6	13.6
B:GLN 42	H	91.2	77.5
B:ILE 45		50.0	48.6
B:ASP 46		76.0	32.2
B:ILE 48		42.8	12.4
B:ASN 49	H	77.8	77.8
B:GLY 50	H	43.5	13.4
B:LEU 52		98.1	53.0
B:ASN 53	H	88.5	71.9
B:ILE 56		118.0	36.2
B:GLU 57		110.9	38.6
B:LYS 58		166.6	80.1
B:THR 59		106.9	4.0
A':LEU 25		102.5	36.2
A':LYS 27		154.0	25.3
A':THR 30		128.8	2.3
A':ASP 31		53.0	5.9
A':ASP 32	H	82.5	35.6
A':GLN 33	H	121.7	105.6
A':ASN 312		95.6	0.2

Italics indicate binding interaction with a second protomer.

Example 2

Additional anti-influenza A antibodies

This example describes identification and characterization of the 3150206_1A05, 3155305P_1A05, and 3155305P _1B06 antibodies, which specifically bind to influenza HA protein and neutralize group 1 and group 2 influenza A viruses.

The 3150206_1A05, 3155305P_1A05, and 3155305P _1B06 antibodies were isolated from subjects immunized with an influenza vaccine. The heavy and light chain variable region sequences of these antibodies are provided above. Characteristics include:

Antibody name	HV gene	HD gene	HJ gene	CDR H3 length (sequence)	LV gene	LJ gene	CDR L3 length (sequence)
3150206_1A05	HV1-2	HD3-9	HJ4	18 (ARDSGMRYFDWLSGYFDF, SEQ ID NO: 103)	KV3-20	KJ4	9 (QVYDDLRVI, SEQ ID NO: 105)
3155305P_1A05	HV6-1	HD3-3	HJ4	17 (ARAGIMIFGVIVGGLDV, SEQ ID NO: 108)	KV1-39	KJ2	10(QQSSTKPGYT, SEQ ID NO: 113)
3155305P _1B06	HV6-1	HD3-3	HJ3	17 (ARAGVTVFGVVVGAMDV, SEQ ID NO: 112)	KV3-15	KJ2	5 (QQYEF, SEQ ID NO: 115)

HA antibody binding and competition assays

Binding of the 3150206_1A05, 3155305P_1A05, and 3155305P _1B06 antibodies to HA proteins from different subtypes was assayed (FIG. 18). Meso Scale Discovery (MSD) 384 well Streptavidin coated SECTOR® Imager 2400 Reader Plates (Standard) (Cat# L25SA-1) were blocked with 35 µL of 5% (W/V) MSD Blocker A (Cat # R93BA-4) for 30 to 60 min and then washed thrice with 0.05%Tween PBS (wash buffer). The plates were coated with biotinylated HA protein at 2 µg/mL concentration for 1 hour and then washed with the wash buffer. 1% MSD Blocker A was used as assay diluent in the assay. Isolated monoclonal antibodies were added to the plates at 10 µg/mL with the 1% MSD blocker A and a 3 fold serial dilution was performed. The plates were again washed with the wash buffer after an hour of incubation with the antibodies and SULFO-TAG conjugated Anti-Hu/NHP IgG secondary detection antibody (Cat# D20JL-6) was used for detection at 1 µg/mL. After an hour of incubation, the unbound secondary detection antibody was washed off the plates and the plates were read using 1X MSD Read Buffer; Cat# R92TC-2 on the MSD sector imager 2400.

Production of pseudotyped lentiviral vectors and measurement of antibody neutralizing activity

The neutralization profile of the 3150206_1A05, 3155305P_1A05, and 3155305P _1B06 antibodies was assessed by pseudovirus assay (FIG. 19).

Influenza pseudotyped lentiviral vectors expressing a luciferase reporter gene were produced as described (Yang *et al., Science* 317, 825-828, 2007). Briefly, 293T cells were co-transfected by using the following plasmids: 17.5 µg of pCMV-R8.2, 17.5 µg of pHR'CMV-Luc, 1 µg CMV/R H1 South Carolina/1/1918, H1 Puerto Rico/8/1934, H1 New Caledonia/20/1999, H1 California/04/2009-Mut, H2 Canada/720/2005, H3 Hong Kong/1/1968, H3 Perth/16/2009, H5 Indonesia/05/2005, H7 Netherlands219/2003, H7 Anhui/1/2013) or H9 Hong Kong 1074/1999, or INF-B-Brisbane/60/2008; and 0.125 µg of the corresponding NA (18 million cells in a 15cm dish). For the production of H1N1, H2N2, H3N2, H7N7, H7N9 and H9N2 pseudovirus, a human type II transmembrane serine protease TMPRSS2 gene was included in transfection for the proteolytic activation of HA(Bottcher *et al., J. Virol.,* 80, 9896-9898, 2006). Cells were transfected overnight and replenished with fresh

medium. Forty-eight hours later, supernatants were harvested, filtered through a 0.45-μm syringe filter, aliquoted, and frozen at -80°C before use.

The neutralization assays were carried out as follows: monoclonal Abs at various dilutions were mixed with pseudoviruses for 45 minutes and then added to 293A cells in 96-well dishes (10,000 cells per well). Additional fresh medium was added 2 hours later. Three days after infection, cells were lysed in 20 μl of cell culture lysis buffer (Promega, Madison, WI). Luciferase assay reagent (50 μl; Promega) was added to the cell lysate prior to measuring luciferase activity. The protocol for human sera neutralization assays is identical to that for monoclonal antibodies, with one exception. Human sera is initially pretreated with receptor destroying enzyme II (Denka Seiken, Japan) to eliminate serum nonspecific inhibitors in accordance with the manufacturer's protocol prior to beginning the neutralization assays.

315-53-1F12, 315-09-1B12, and 315-02-1E04 Antibodies

Additional HA-specific antibodies were isolated from a human subject immunized with an influenza HA-based immunogen, including the 315-53-1F12, 315-09-1B12, and 315-02-1E04 antibodies. Relevant characteristics for these antibodies are provided below:

Name	Class		SEQ ID NO				
			A.A.	DNA	CDR1	CDR2	CDR3
315-53-1F12	VH6-1	VH	363	369	375	107	376
		VL	364	370	64	377	378
315-09-1B12	VH1-18 Q-x-x-V	VH	365	371	379	62	380
		VL	366	372	381	29	382
315-02-1E04	VH1-18+DH3-9	VH	367	373	383	384	385
		VL	368	374	386	387	388

The antibodies were assayed for HA-binding and virus neutralization as discussed above, and each showed specific binding activity and neutralization of both Group 1 and Group 2 influenza strains. To further evaluate the protective ability of these mAbs across group 1 and group 2 strains they were tested for protection of mice infected with A/California/07/2009 (H1N1) or A/Shanghai/02/2013 (H7N9) (FIG. 21). For the protection studies, mice were passively administered 5 mg/kg of the indicated antibody (10 mice/group) 24 hours before infection with A/Anhui/01/2013 (H7N9) or A/California/07/2009 (H1N1). As a control, one group received VRC01 IgG, an HIV-specific antibody. Mice were euthanized when they reached 80% of their initial weight. When mAbs were given 24 hours prior to H7N9 infection, 90-100% survival was observed in mice that received one of the mAbs, with no loss in weight in the majority of the animals (FIG. 21, top). Similarly, the mAbs provided 100% protection from H1N1 infection in mice with minimal loss in weight (FIG. 21, bottom). In contrast, all but one of the mice that received the anti-HIV mAb VRC01 had significant weight loss after infection and were euthanized.

It will be apparent that the precise details of the embodiments described may be varied or modified without departing from the spirit of the described embodiments. We claim all such modifications and variations that fall within the scope and spirit of the claims below.

Claims

1. An isolated monoclonal antibody, comprising:

a heavy chain variable region and a light chain variable region comprising a heavy chain complementarity determining region (HCDR)1, a HCDR2, and a HCDR3 and a light chain complementarity determining region (LCDR)1, a LCDR2, and a LCDR3 of the V_H and V_L set forth as one of:

(a) SEQ ID NOs: 1 and 2, respectively (54.f.01);

(b) SEQ ID NOs: 3 and 4, respectively (56.a.09);

(c) SEQ ID NOs: 5 and 6, respectively (01.k.01);

(d) SEQ ID NOs: 7 and 8, respectively (31.b.09);

(e) SEQ ID NOs: 9 and 10, respectively (16.g.07);

(f) SEQ ID NOs: 11 and 12, respectively (54.a.84);

(g) SEQ ID NOs: 13 and 14, respectively (16.a.26);

(h) SEQ ID NOs: 15 and 16, respectively (54.a.39);

(i) SEQ ID NOs: 17 and 18, respectively (31.a.83);

(j) SEQ ID NOs: 71 and 72, respectively (3150206_1A05);

(k) SEQ ID NOs: 73 and 74, respectively (3155305_1A05);

(l) SEQ ID NOs: 75 and 76, respectively (3155305_1B06);

(m) SEQ ID NOs: 363 and 364, respectively (315-53-1F12);

(n) SEQ ID NOs: 365 and 366, respectively (315-09-1B12); or

(o) SEQ ID NOs: 367 and 369, respectively (315-02-1E04); and

wherein the monoclonal antibody specifically binds to influenza A HA protein and neutralizes group 1 and group 2 influenza A viruses.

2. The isolated antibody of claim 1, wherein the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2, and the LCDR3 comprise the amino acids sequences set forth as:

(a) SEQ ID NOs: 19, 20, 21, 22, 23, and 24, respectively (54.f.01);

(b) SEQ ID NOs: 25, 26, 27, 28, 29, and 30, respectively (56.a.09);

(c) SEQ ID NOs: 31, 32, 33, 34, 35, and 36, respectively (01.k.01);

(d) SEQ ID NOs: 37, 38, 39, 40, 41, and 42, respectively (31.b.09);

(e) SEQ ID NOs: 43, 44, 45, 46, 47, and 48, respectively (16.g.07);

(f) SEQ ID NOs: 49, 50, 51, 52, 53, and 54, respectively (54.a.84);

(g) SEQ ID NOs: 55, 56, 57, 58, 59, and 60, respectively (16.a.26);

(h) SEQ ID NOs: 61, 62, 63, 64, 29, and 65, respectively (54.a.39);

(i) SEQ ID NOs: 66, 67, 68, 69, 29, and 70, respectively (31.a.83);

(j) SEQ ID NOs: 101, 102, 103, 104, 29, and 105, respectively (3150206_1A05);

(k) SEQ ID NOs: 106, 107, 108, 109, 29, and 110, respectively (3155305_1A05);

(l) SEQ ID NOs: 111, 107, 112, 113, 114, and 115, respectively (3155305_1B06);

(m) SEQ ID NOs: 375, 107, 376, 64, 377, and 378, respectively (315-53-1F12);

(n) SEQ ID NOs: 379, 62, 380, 381, 29, and 382, respectively (315-09-1B12); or

(o) SEQ ID NOs: 383, 384, 385, 386, 387, and 388, respectively (315-02-1E04).

3. The isolated antibody of claim 1 or claim 2, wherein the V_H and the V_L comprise the amino acid sequences set forth as:

(a) SEQ ID NOs: 1 and 2, respectively (54.f.01);

(b) SEQ ID NOs: 3 and 4, respectively (56.a.09);

(c) SEQ ID NOs: 5 and 6, respectively (01.k.01);

(d) SEQ ID NOs: 7 and 8, respectively (31.b.09);

(e) SEQ ID NOs: 9 and 10, respectively (16.g.07);

(f) SEQ ID NOs: 11 and 12, respectively (54.a.84);

(g) SEQ ID NOs: 13 and 14, respectively (16.a.26);

(h) SEQ ID NOs: 15 and 16, respectively (54.a.39);

(i) SEQ ID NOs: 17 and 18, respectively (31.a.83);

(j) SEQ ID NOs: 71 and 72, respectively (3150206_1A05);

(k) SEQ ID NOs: 73 and 74, respectively (3155305_1A05);

(l) SEQ ID NOs: 75 and 76, respectively (3155305_1B06);

(m) SEQ ID NOs: 363 and 364, respectively (315-53-1F12);

(n) SEQ ID NOs: 365 and 366, respectively (315-09-1B12); or

(o) SEQ ID NOs: 367 and 369, respectively (315-02-1E04).

4. An isolated monoclonal antibody, comprising:

a heavy chain variable region and a light chain variable region comprising a heavy chain complementarity determining region (HCDR)1, a HCDR2, and a HCDR3 and a light chain complementarity determining region (LCDR)1, a LCDR2, and a LCDR3 of the V_H and V_L of any one of the antibodies listed in FIGs. 20A-20G.

5. The isolated monoclonal antibody of claim 4, wherein the heavy and light chain variable regions comprise the amino acid sequences set forth as the heavy and light chain variable regions of any one of the antibodies listed in FIGs. 20A-20G.

6. An isolated antibody, comprising:

a heavy chain variable region comprising a HCDR1, a HCDR2, and a HCDR3, and a light chain variable region comprising a LCDR1, a LCDR2, and a LCDR3, wherein:

the heavy chain variable region comprises the following by kabat positioning: a Gln at position 1; a Val at position 2; a Gly at position 26; an Asp at position 27; a Asn, His or Tyr at position 32; a Ser at position 33; an Ala, Ser or Thr, or no residue at position 35; an Arg at position 50; a Tyr at position 52; an Arg at position 52b; a Ser at position 53; a Tyr at position 56; an Asp at position 58; an Arg at position 94; an Ala, Phe, Val, or Pro at position 97; a Met at position 98; an Ile at position 99; a Phe at position 100; a Gly at position 100a; an Ile or Val at position 100b; a Val, Leu, or Asp at position 100c;and a Met, Ile, or Val at position 100d;

the light chain variable region comprises the following by kabat positioning: a Glu at position 1; a Gln or Arg at position 27; a Ser at position 27a; an Ala, Ser, Gly, or Val at position 29; an Arg or Ser at position 30; a Val or Ser at position 31; a Tyr at position 32; a Phe, Tyr, or Lys at position 49; a Ser, Thr, Pro, or Asn at position 56; a

Tyr, Phe, or His at positon 91; an Asp or Gly at position 92; a Gly, Ser, or Val at position 93; a Ser at position 94; and a Phe or Tyr at position 96; and

the antibody specifically binds to influenza A HA protein and neutralizes group 1 and group 2 influenza A viruses.

7. The isolated antibody of claim 6, wherein the light chain variable region comprises the following by kabat positioning: a Glu at position 1; a Gln or Arg at position 27; a Ser at position 27a; an Ala, Ser, or Gly at position 29; a Ser at position 30; a Ser at position 31; a Tyr at position 32; a Phe or Tyr at position 49; a Ser, Thr, Pro, or Asn at position 56; a Tyr or Phe at positon 91; an Asp or Gly at position 92; a Gly or Ser at position 93; a Ser at position 94; and a Phe or Tyr at position 96.

8. The isolated antibody of claim 6 or claim 7, wherein

the HCDR1 comprises the amino acid sequence set forth as $GDX_1VSSX_2SAX_3$ (SEQ ID NO: 116);

the HCDR2 comprises the amino acid sequence set forth as $TYYRSX_4WYX_5$ (SEQ ID NO: 117);

the HCDR3 comprises the amino acid sequence set forth as $ARX_6SX_7MIFGX_8X_9X_{10}X_{11}X_{12}X_{13}X_{14}X_{15}$ (SEQ ID NO: 118);

the LCDR1 comprises the amino acid sequence set forth as $X_{16}SVX_{17}X_{18}X_{19}Y$ (SEQ ID NO: 119);

the LCDR2 comprises the amino acid sequence set forth as $GX_{20}S$ (SEQ ID NO: 120);

the LCDR3 comprises the amino acid sequence set forth as $QX_{21}X_{22}X_{23}X_{24}SX_{25}X_{26}T$ (SEQ ID NO: 121); and wherein

X_1 is Ser or Thr; X_2 is Asn, His or Tyr; X_3 is Ala, Ser, Thr, or no amino acid; X_4 is Lys or Arg; X_5 is Ser, Asn, Thr, Tyr, Gly, Ser, or Thr; X_6 is Gly, Ala, or Val; X_7 is Ala, Phe, Val, or Pro; X_8 is Ile or Val; X_9 is Val, Leu, or Asp; X_{10} is Met, Ile, or Val; X_{11} is Gly or no amino acid; X_{12} is Ala, Ile, Val, or Glu; X_{13} is Phe, Leu, or Met; X_{14} is Asp or Glu; X_{15} is Gln, Phe, Cys, Ser, Tyr, or Leu; X_{16} is Gln or Arg; X_{17} is Ala, Ser, Gly, or Val; X_{18} is Ser or Arg; X_{19} is Ser or Val; X_{20} is Ala, Val, or Thr; X_{21} is Gln or Arg; X_{22} is Tyr, Phe, or His;X_{23} is Asp or Gly; X_{24} is Gly, Ser, or Val; X_{25} is Gln, His, or Arg; and X_{26} is Tyr or Phe.

9. The isolated antibody of any of claims 6-8, wherein the heavy chain variable region comprises a germline origin of HV6-1, HD3-3, and HJ4 or HJ5 genes, and the light chain variable region comprises a germline origin of KV3-20 and KJ4 or KJ5 genes.

10. An isolated antibody, comprising:

a heavy chain variable region comprising a HCDR1, a HCDR2, and a HCDR3, and a light chain variable region comprising a LCDR1, a LCDR2, and a LCDR3, wherein:

the heavy chain variable region comprises the following by kabat positioning: a Trp at position 50; a Tyr at position 53; an Asn at position 54; an Ala or Gly at position 55; a Asn or His at position 56; an Asn or Gln at position 58; an Ala, Asp, His, Asn, Thr at position 98; an Ile at position 99; a Leu at position 100; and a Thr at position 100a;

the light chain variable region comprises the following by kabat positioning a Gln at position 27; a Gly at position 27a; a Leu at position 27b; a Leu or Val at position 27c; a Phe, His, or Tyr at position 27d; an Ile at

position 27e; a Asp at position 28; a Gly at position 29; a Thr at position 31; an Asn, Glu, His, or Lys at position 50; a Val or Ile at position 51; a Ser or Phe at position 52; a Ser at position 65; a Gly at position 66; a Ser at position 67; a Gly at position 68; a Thr at position 92; a His or Tyr at position 93; a Trp at position 94; and

wherein the antibody specifically binds to influenza A HA protein and neutralizes group 1 and group 2 influenza A viruses.

11. The isolated antibody of claim 10, wherein

the HCDR1 comprises the amino acid sequence set forth as $GYX_1FX_2X_3X_4G$ (SEQ ID NO: 122);

the HCDR2 comprises the amino acid sequence set forth as $X_5SX_6YNX_7X_8X_9$ (SEQ ID NO: 123);

the HCDR3 comprises the amino acid sequence set forth as $X_{10}RDX_{11}X_{12}X_{13}ILTGX_{14}X_{15}X_{16}DX_{17}$ (SEQ ID NO: 124);

the LCDR1 comprises the amino acid sequence set forth as $QGLX_{18}X_{19}IDGX_{20}X_{21}Y$ (SEQ ID NO: 125);

the LCDR2 comprises the amino acid sequence set forth as $X_{22}X_{23}X_{24}$ (SEQ ID NO: 126);

the LCDR3 comprises the amino acid sequence set forth as $X_{25}QGTX_{26}WPX_{27}T$ (SEQ ID NO: 127); and

wherein X_1 is Arg, Thr, Asp, or Ser; X_2 is Ser, Thr, or Asn; X_3 is Ser, Thr, or Asn; X_4 is Tyr, Ser, Phe, or His; X_5 is Ile or Val; X_6 is Ala or Gly; X_7 is Gly or Ala; X_8 is His or Asn; X_9 is Thr or Ile; X_{10} is Ala or Thr; X_{11} is Gln or Arg; X_{12} is Arg, Tyr, Gly, Phe, Ser, or Pro; X_{13} is Asp, Ala, Asn, Thr, or His; X_{14} is Gly, Pro, Ser, Tyr, Asp, Phe, or Cys; X_{15} is Leu, Asn, Ala, Arg, Asp, His, or Gln; X_{16} is Phe, Gly, Thr, Asp, or Leu; X_{17} is Cys, Tyr, His, Asp, Phe, Ser, or Ile; X_{18} is Val or Leu; X_{19} is His, Tyr, or Phe; X_{20} is Asn or Ser; X_{21} is Thr or Ile; X_{22} is Asn, His, Lys, or Glu; X_{23} is Val, or Ile; X_{24} is Phe or Ser; X_{25} is Met or Leu; X_{26} is Tyr or His; and X_{27} is Tyr, Leu, Arg, or Ile.

12. The isolated antibody of claim 10 or claim 11, wherein the heavy chain variable region comprises a germline origin of HV1-18, HD3-9, and HJ4 genes, and the light chain variable region comprises a germline origin of KV2-30 and KJ2 or KJ5 genes.

13. The isolated antibody of any of the prior claims, comprising a human framework region, and/or a human constant domain.

14. The isolated antibody of any of the prior claims, wherein the antibody is an IgG.

15. The isolated monoclonal antibody of any of the prior claims, comprising a recombinant constant domain comprising a modification that increases the half-life of the antibody, particularly wherein the modification increases binding to the neonatal Fc receptor.

16. The isolated monoclonal antibody or antigen binding fragment of claim 9, wherein the recombinant constant domain is an IgG_1 constant domain comprising M428L and N434S mutations.

17. An antigen binding fragment of the isolated monoclonal antibody of any of the prior claims that specifically binds to influenza HA protein and neutralizes Group 1 and Group 2 influenza viruses.

18. The antigen binding fragment of claim 17, wherein the antigen binding fragment is a Fv, Fab, $F(ab')_2$, scFV or a $scFV_2$ fragment.

19. The antibody or antigen binding fragment of any of the prior claims, conjugated to an effector molecule or a detectable marker.

20. An isolated nucleic acid molecule encoding the antibody or antigen binding fragment of any of the prior claims.

21. An isolated nucleic acid molecule encoding the V_H and/or the V_L of the antibody or antigen binding fragment of any of claims 1-19.

22. The isolated nucleic acid molecule of claim 20 or claim 21, wherein the nucleic acid molecule is a recombinant nucleic acid molecule.

23. The isolated nucleic acid molecule of any of claims 20-22, comprising a cDNA molecule encoding the antibody or antigen binding fragment.

24. The isolated nucleic acid molecule of any of claims 20-23, operably linked to a promoter.

25. An expression vector comprising the nucleic acid molecule of any of claims 20-24.

26. A host cell, comprising the nucleic acid molecule or vector of any of claims 20-25.

27. A method of producing an antibody or antigen binding fragment that specifically binds to influenza A HA protein, comprising:

expressing one or more nucleic acid molecules encoding the V_H and the V_L of the antibody or antigen binding fragment of any of claims 1-19 in a host cell, thereby producing the antibody or antigen binding fragment.

28. The method of claim 27, wherein the one or more nucleic acid molecules are one or more cDNA molecules encoding the V_H and the V_L of the antibody or antigen binding fragment.

29. The method of claim 27 or claim 28, further comprising purifying the antibody that specifically binds to influenza A HA.

30. A pharmaceutical composition for use in treating or preventing influenza A viral infection, comprising an effective amount of the antibody, antigen binding fragment, nucleic acid molecule, or vector, of any of claims 1-29; and

a pharmaceutically acceptable carrier.

31. The pharmaceutical composition of claim 30, wherein the composition is sterile.

32. The pharmaceutical composition of claim 30 or claim 31, wherein the composition is in unit dosage form or a multiple thereof.

33. A method of detecting the presence of an influenza A virus in a biological sample, comprising:

contacting the biological sample with an effective amount of the antibody or antigen binding fragment of any of claims 1-19 under conditions sufficient to form an immune complex; and

detecting the presence of the immune complex on the biological sample, wherein the presence of the immune complex on the biological sample indicates the presence of the influenza A virus in the sample.

34. The method of claim 33, wherein detecting the detecting the presence of the immune complex on the biological sample indicates that the subject has an influenza A viral infection.

35. A method of treating or inhibiting an influenza A viral infection in a subject, comprising administering to a subject with or at risk of an influenza A viral infection a therapeutically effective amount of the antibody, antigen binding fragment, nucleic acid molecule, vector, or pharmaceutical composition of any of claims 1-25 or 30-32.

36. A kit for detecting influenza A virus in a sample, detecting influenza A viral infection in a subject, or for treating or inhibiting influenza A viral infection in a subject, the kit comprising:

a container comprising the antibody, antigen binding fragment, nucleic acid molecule, vector, or pharmaceutical composition of any of claims 1-25 or 30-32, and instructions for using the kit.

37. Use of the antibody, antigen binding fragment, nucleic acid molecule, vector, or pharmaceutical composition of any of claims 1-25 or 30-32, to treat or inhibit influenza A viral infection in a subject, or to detect the presence of a influenza A virus in a biological sample, or to detect a influenza A viral infection in a subject.

FIG. 1A

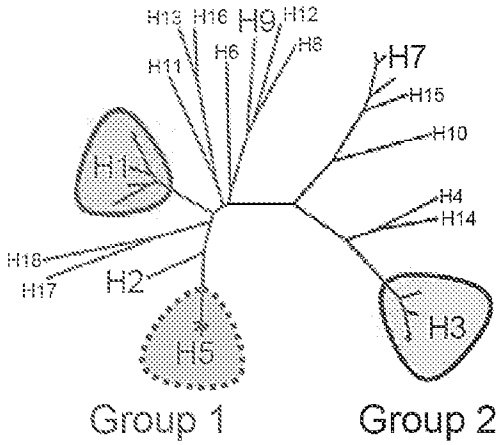


FIG. 1B

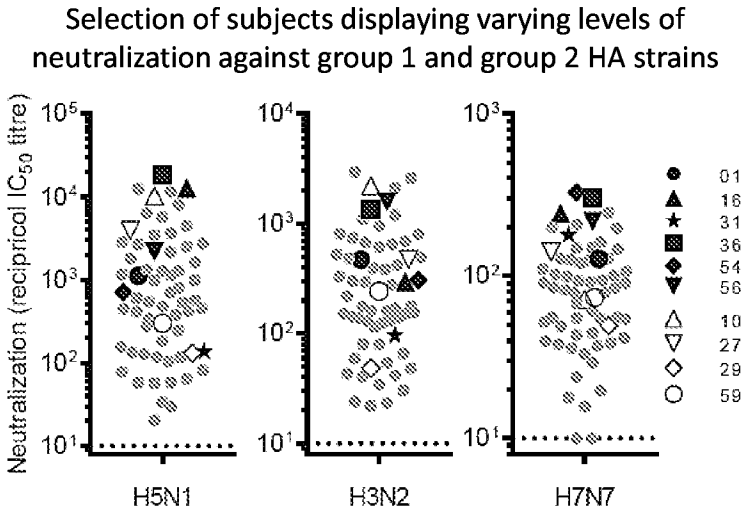


FIG. 1C

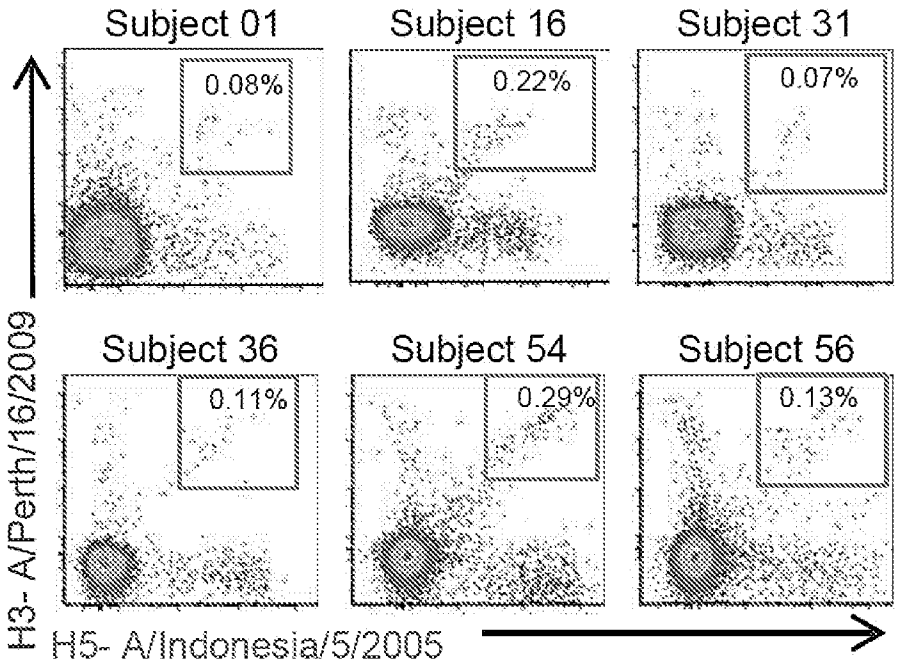


FIG. 1D

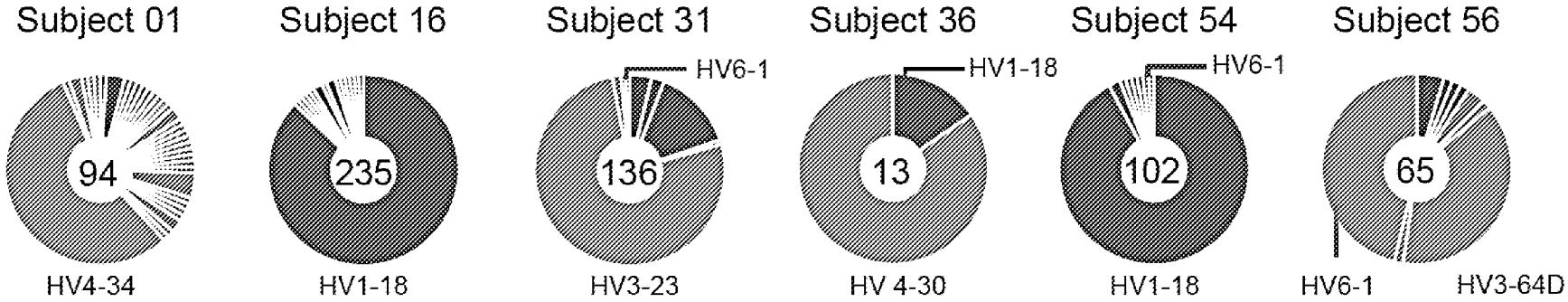


FIG. 1E

Number of genetic similarities	Antibody name (subject.lineage.clone)	Frequentist probability	Number of clones per subject per genetic cluster	HV gene	HD gene	HJ gene	CDR H3 length*	HV maturation	LV gene	LJ gene	CDR L3 length*	Binding competition	Neutralization breadth	
													Group 1	Group 2
	31.g.01		1	HV6-1	HD3-3	HJ4/5	16	8%	KV3-20	KJ2	9	n.d.	n.d.	n.d.
6	54.f.01	4.9E-12	1	HV6-1	HD3-3	HJ4/5	16	6%	KV3-20	KJ3	9	+++	H1, H2, H5	H3, H7
	56.a.09 ☼		30	HV6-1	HD3-3	HJ4/5	16	4%	KV3-20	KJ2	9	+++	H1, H5	H3, H7
6	01.k.01	3.6E-6	1	HV1-18	HD3-9	HJ4	15	9%	KV2-30	KJ5	9	+++	H1, H5	H3
	31.b.09 ☼		26	HV1-18	HD3-9	HJ4	15	5%	KV2-30	KJ2	9	+++	H1, H5	H3, H7
5	16.g.07 ☼	6.9E-5	8	HV1-18	HD2-15	HJ2	21	11%	KV1-12	KJ2	9	+++	H1, H5, H9	H3, H7
	54.a.84		92	HV1-18	HD2-15	HJ2	21	11%	KV3-11	KJ1	9	+++	H1, H5, H9	H3, H7
5	16.a.26 ☼	6.9E-5	93	HV1-18	HD2-2	HJ5	21	8%	KV1-39	KJ2	9	+++	H1, H5, H9	H3, H7
	54.a.38		92	HV1-18	HD2-2	HJ5	21	6%	KV3-11	KJ1	9	+++	H1, H9	H3, H7
4	31.a.83 ☼	0.004	104	HV3-23	HD3-9	HJ6	24	8%	KV3-15	KJ2	9	+++	H1, H2, H5, H9	H3, H7
	56.h.01		2	HV3-23	HD3-9	HJ6	28	8%	KV2-29	KJ4	9	+++	H1, H2, H9	None
4	01.s.01	0.007	1	HV1-69	HD5-18	HJ4	15	10%	KV4-1	KJ3	9	n.b.	None	None
	31.f.01		1	HV1-69	HD3-22	HJ4	15	7%	KV3-20	KJ2	9	+++	H1, H2, H5, H9	None
4	31.f.01	5.1E-5	1	HV1-69	HD3-22	HJ4	15	7%	KV3-20	KJ2	9	+++	H1, H2, H5, H9	None
	56.ND.11		1	HV1-69	HD3-22	HJ6	15	6%	KV3-20	n.r.	n.r.	n.d.	n.d.	n.d.
4	54.ND.03	0.001	1	HV1-69	HD3-22	HJ6	13	8%	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	56.g.01		1	HV1-69	HD3-22	HJ6	13	6%	KV3-20	KJ2	9	n.b.	None	None
4	54.e.01	0.001	1	HV7-4-1	HD3-9	HJ4	21	1%	KV3-15	KJ1	8	+++	H1, H5	H3
	56.k.01		1	HV3-49	HD3-9	HJ4	21	9%	KV2-30	KJ1	9	n.d.	n.d.	n.d.
3	01.i.01	0.032	1	HV3-23	HD6-13	HJ4	15	0%	KV4-1	KJ1	9	n.b.	None	None
	56.i.01		1	HV4-4	HD6-13	HJ4	15	3%	KV2-40	KJ2	7	n.d.	n.d.	n.d.
n.a.	31.d.01	n/a	2	HV3-30	HD3-9	HJ3	21	4%	KV4-1	KJ2	9	+++	H1, H2, H5, H9	None
n.a.	01.a.44	n/a	53	HV4-34	HD2-8	HJ6	24	12%	KV1-9	KJ2	9	+++	H1, H2, H5	H3, H7

*IMGT CDR 3 lengths used; n.d.: not determined; ☼: crystal structure in complex with HA determined; n.b.: no binding to HA; n.r.: not recovered; None: no pseudovirus neutralization observed; n.a.: not applicable

FIG. 2A

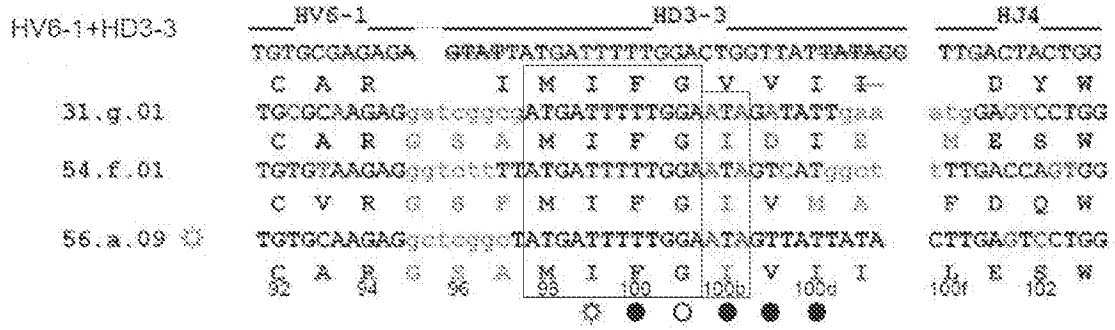


FIG. 2B

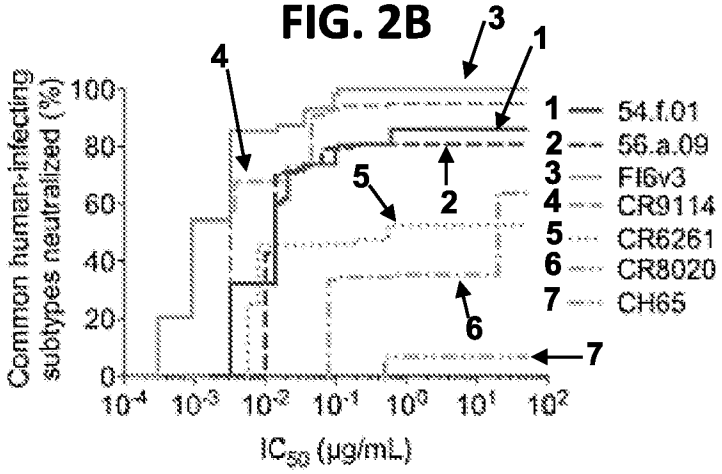
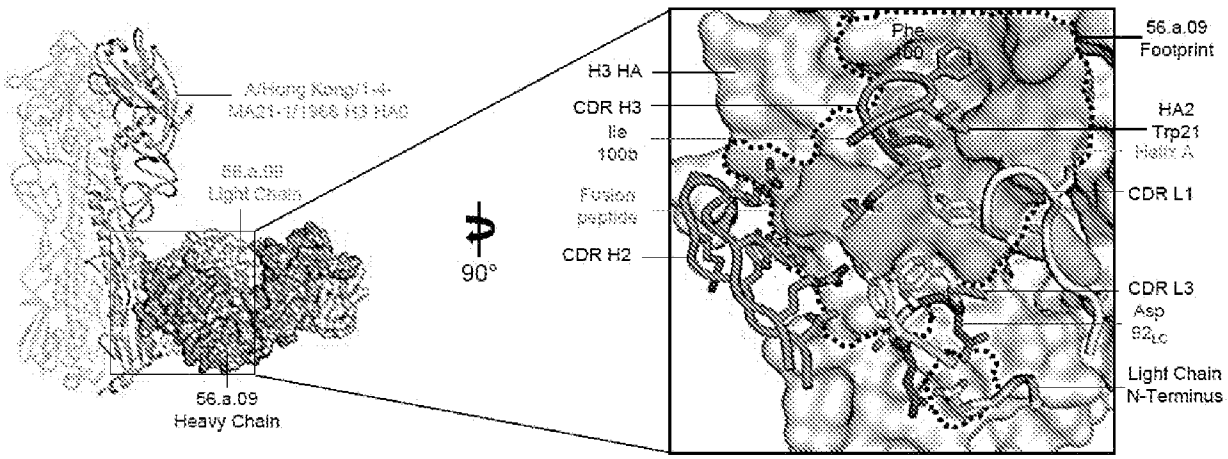


FIG. 2C



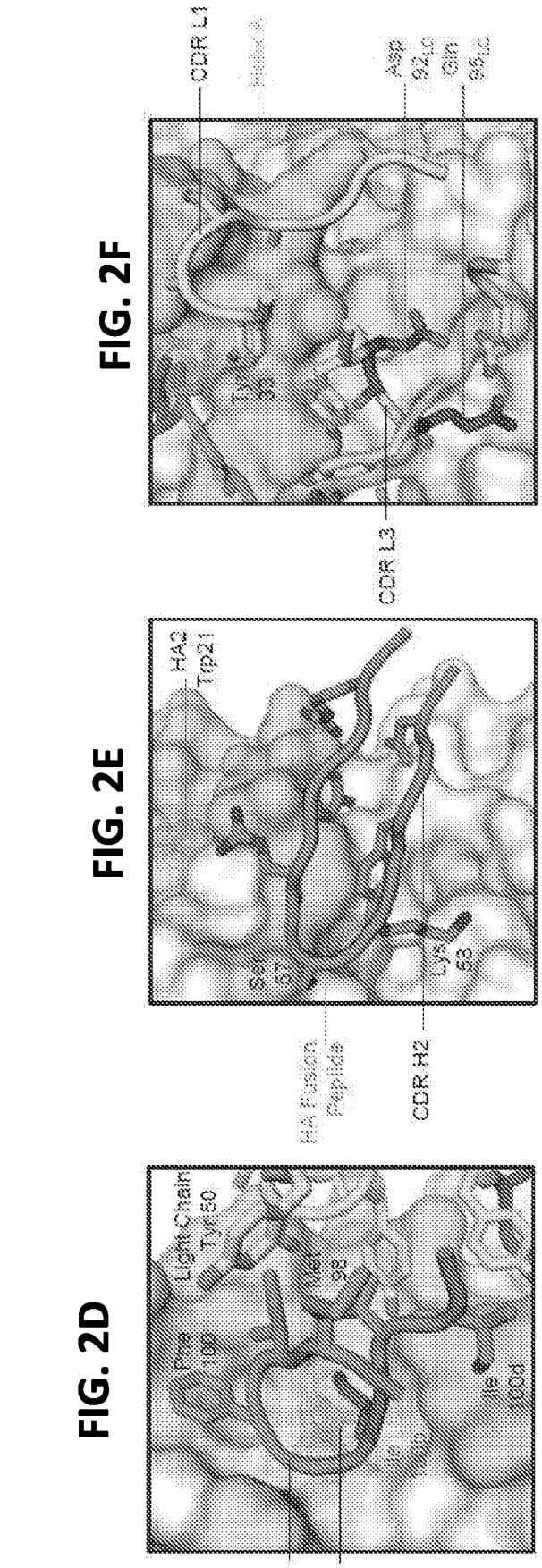


FIG. 2D

FIG. 2E

FIG. 2F

Longer HD motif: ¥ x ϰ X ϰ
Compatible HD genes:
HD3-3 with compatible alleles
HD3-3*01 and HD3-3*02
Shorter HD motif: ¥ x ϰ
Compatible HD genes:
HD1-1, HD1-14, HD1-20, HD2-2, HD2-8, HD2-15, HD3-3, HD3-9, HD3-10, and HD6-13

HV motif: 9 residue CDR H2
Compatible HV genes:
HV6-1 with
compatible alleles
HV6-1*01 and HV6-1*02

LV motif: ¥ at residue 33
Compatible LV genes:
34 genes compatible

¥ = F, Y or W
x = G, A or S
ϰ = I, V, L, or M
X = any residue

FIG. 2G

FIG. 3A

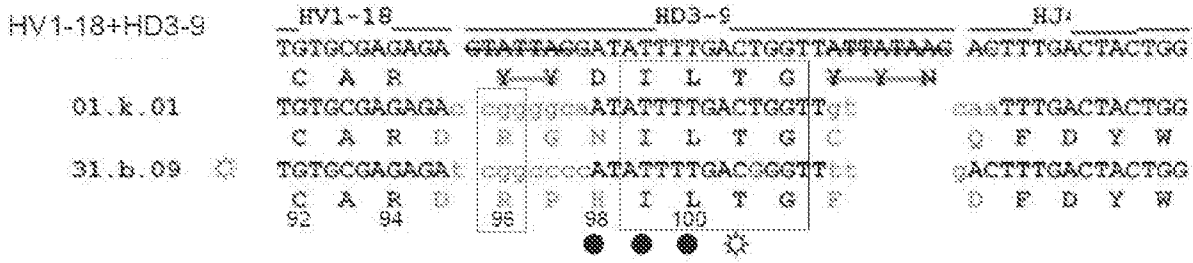


FIG. 3B

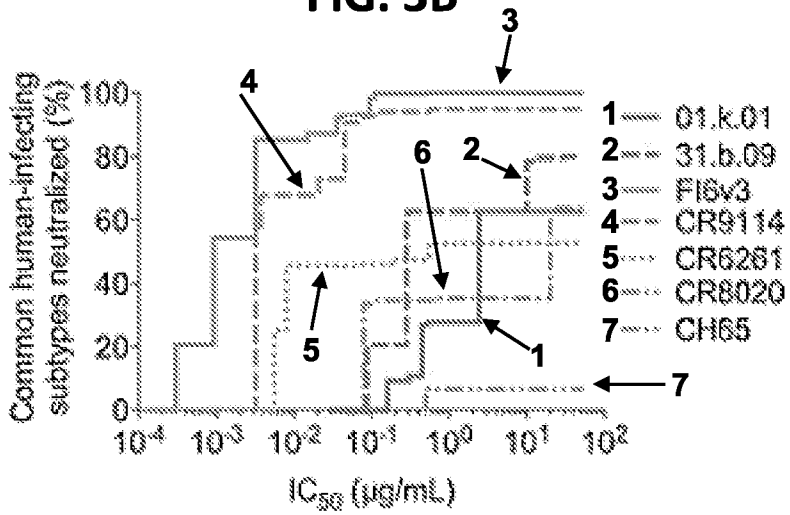


FIG. 3C

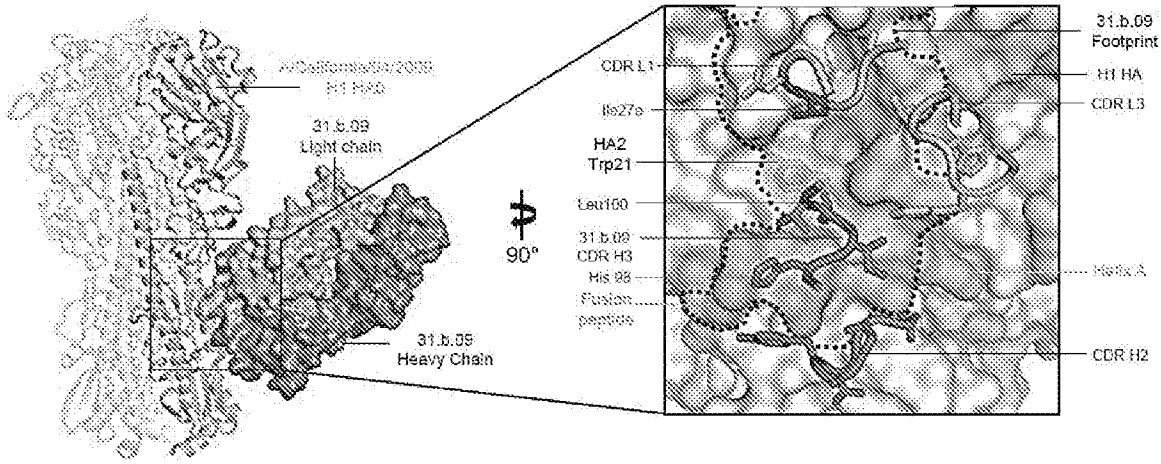
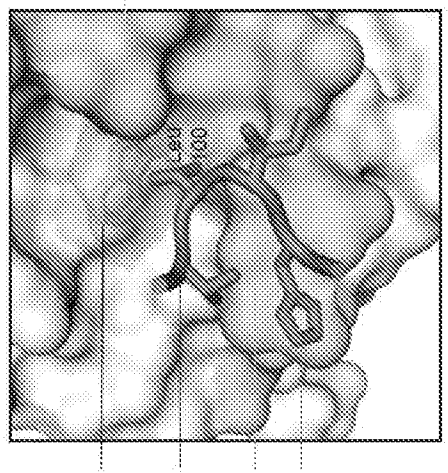
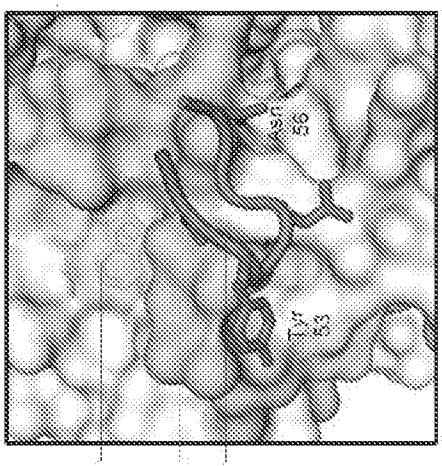


FIG. 3D



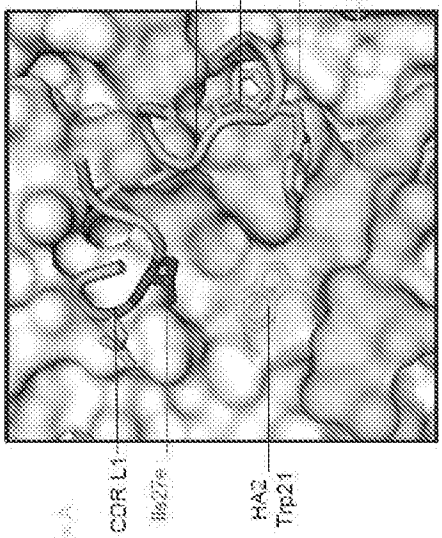
HD Motif: Leu100
Compatible HD genes:
All 34 D genes compatible

FIG. 3E



CDR H2 motif: Trp at residue 50
Tyr at residue 53, Asn at 56
Compatible HV genes:
HV1-18
HV1-69
HV7-34-1
HV7-81

FIG. 3F



LV motif: CDR L1=16 aa
Compatible LV genes:

KV2-4	KV2D-18
KV2-18	KV2D-24
KV2-24	KV2D-26
KV2-28	KV2D-28
KV2-29	KV2D-29
KV2-30	KV2D-30

FIG. 3G

FIG. 4A

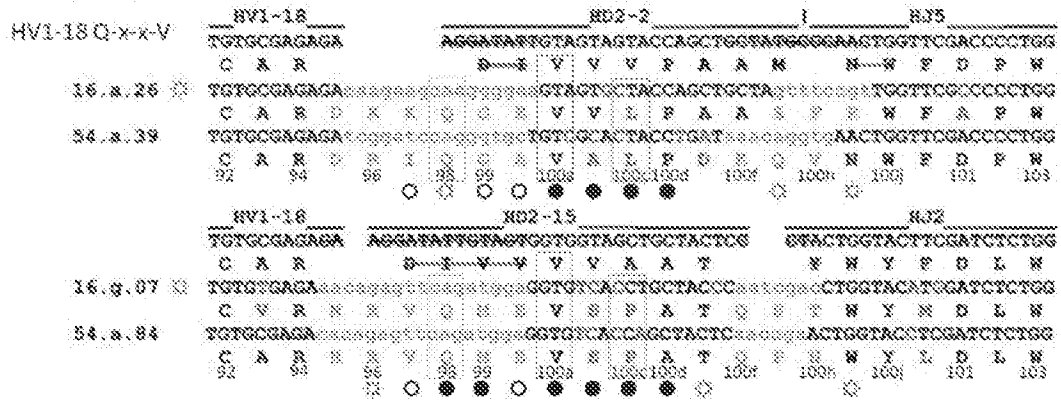
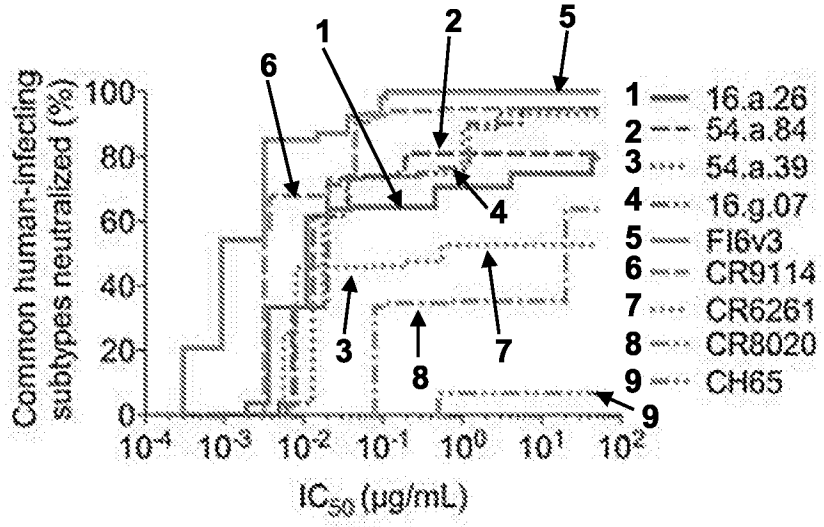


FIG. 4B


Common human-infecting subtypes neutralized (%)
100
80
60
40
20
0
10^{-4} 10^{-3} 10^{-2} 10^{-1} 10^{0} 10^{1} 10^{2}
IC_{50} (µg/mL)
1 16.a.26
2 54.a.84
3 54.a.39
4 16.g.07
5 FI6v3
6 CR9114
7 CR6261
8 CR8020
9 CH65

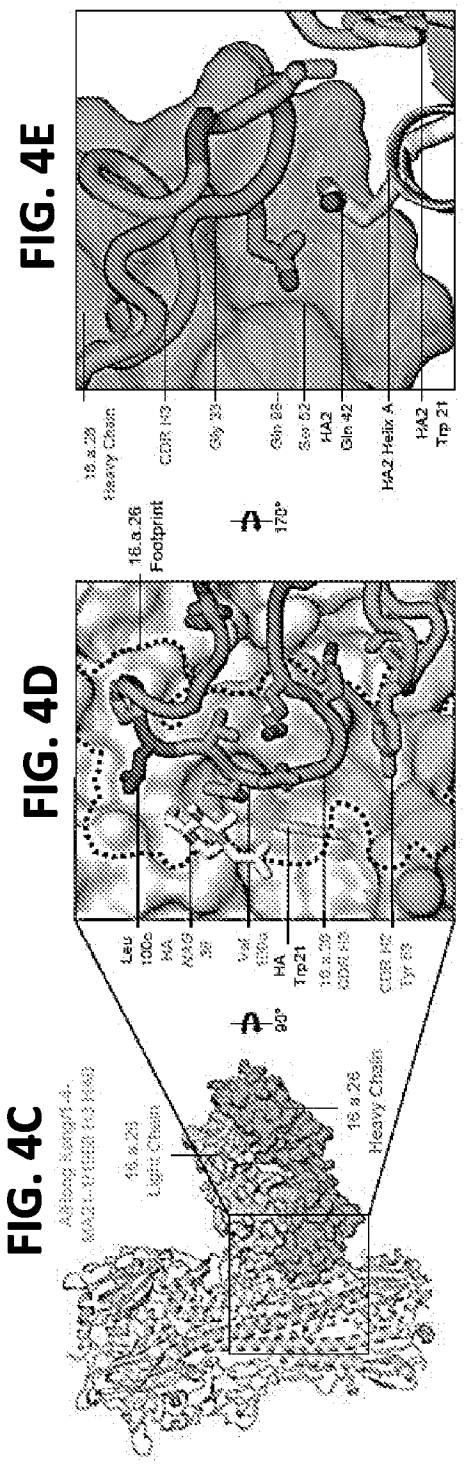


FIG. 4C

FIG. 4D

FIG. 4E

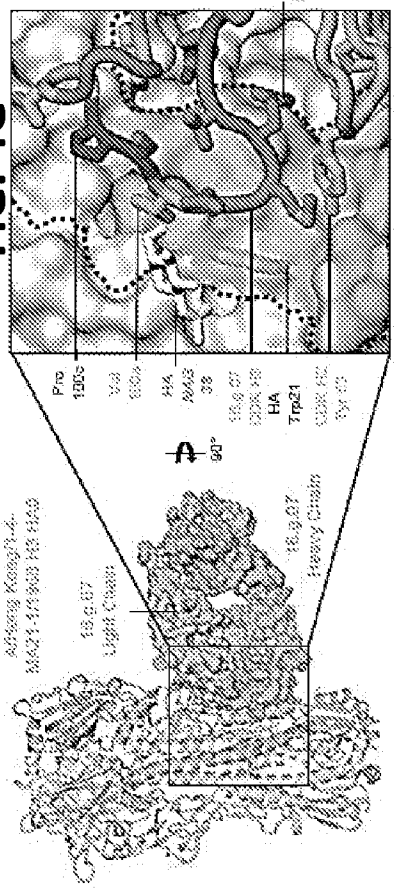


FIG. 4F

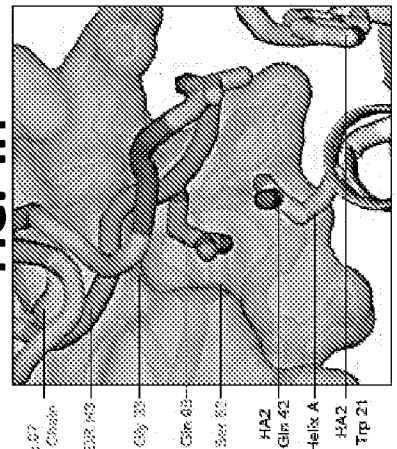


FIG. 4G

FIG. 4H

HD Motif: Val100a
Compatible HD genes:
All 34 D genes compatible

FIG. 4I

HV Contact Residue Motif : Tyr 53
Compatible HV genes:
HV1-18, HV1-45, HV1-68,
HV1-NL1, HV7-34-1, and HV7-81
HV Structural Motif:
Gly33 and Ser52/Thr52
Compatible HV genes:
HV1-18 with compatible alleles
HV1-18*01, HV1-18*02,
HV1-18*03, and HV1-18*04

FIG. 4J

FIG. 5A

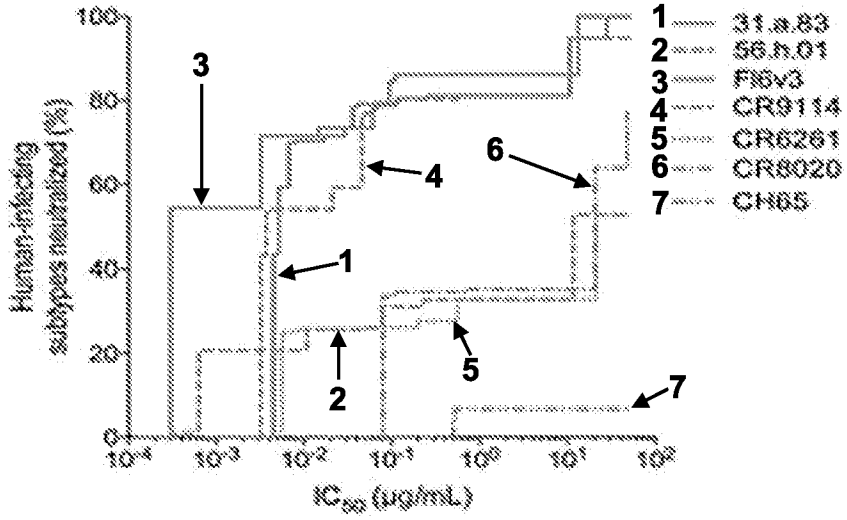


FIG. 5B

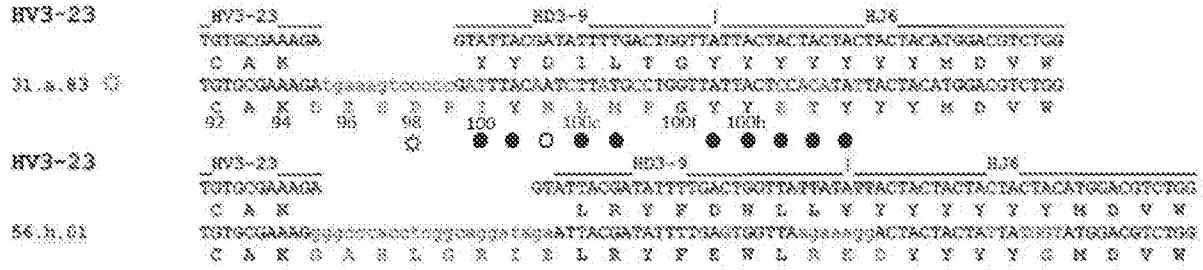


FIG. 5C

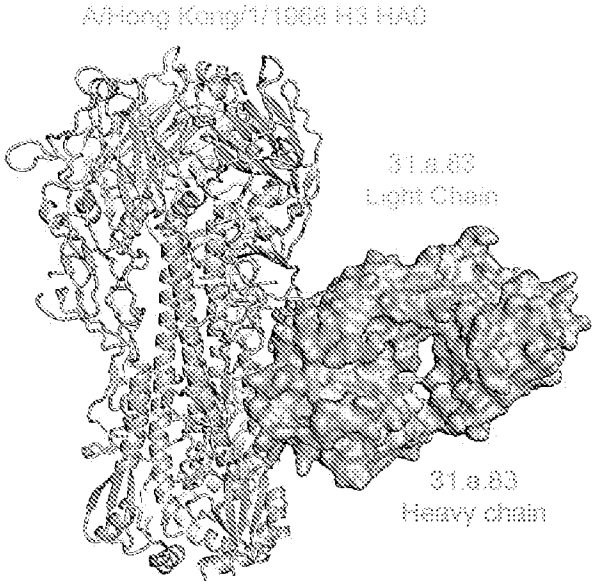


FIG. 5D

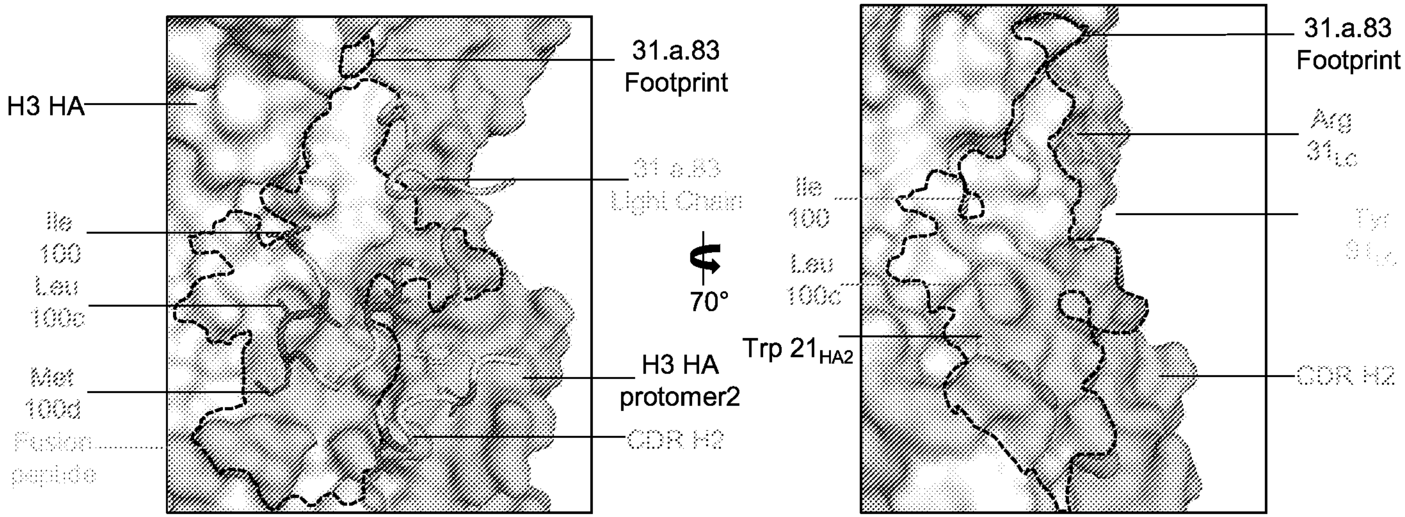


FIG. 5E Lineage-unique group 1-2 neutralizing antibody epitopes

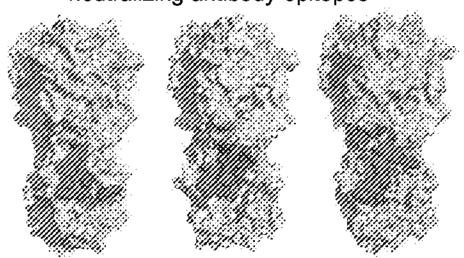


FIG. 5F Multidonor group 1-2 neutralizing antibody epitopes

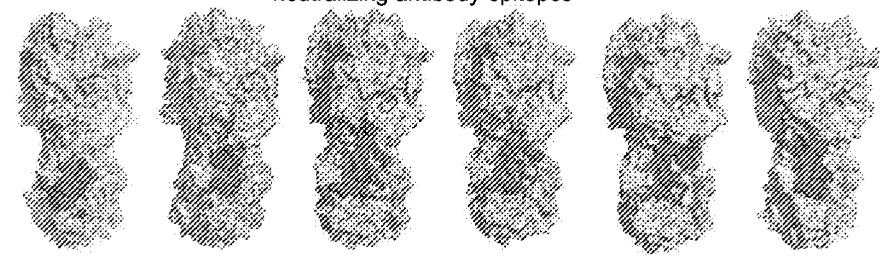


FIG. 5G

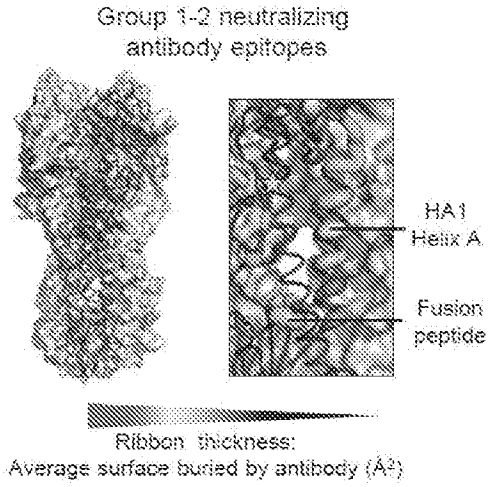
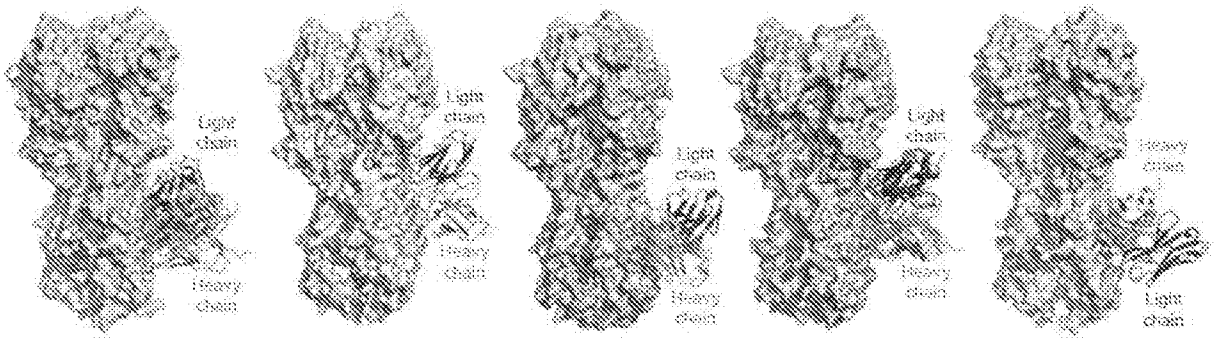


FIG. 5H

Antibody recognition of a group 1-2 supersite



Non-convergent HV3-30 | Non-convergent HV3-23 | Convergent HV6-1 | Convergent HV1-18 | Convergent HV1-69

FIG. 6A

Class	Sequence signature*	Post-VRC310 Heavy-Light (515,594 #)	DeKosky et al. Heavy-Light partial sequences (3,019,679)	Pre-TIV Jiang et al. Heavy-only (759,337)	Post-TIV Jiang et al. Heavy-only (3,045,513)	Healthy normal donors Heavy-only (1,739,891)
HV6-1 +HD3-3	VH6-1 + D3-3 ^{98}MIFGI CDR H3 = 16	21 (0.004%) 3	0 (0%) 0	0 (0%) 0	13 (0.0004%) 1	0 (0%) 0
HV1-18 +HD3-9	VH1-18 ^{96}RxxILTG CDR H3 = 15	16 (0.003%) 3	17 (0.0006%) 1	0 (0%) 0	123 (0.004%) 3 (2)	64 (0.0036%) 2 (1)
HV1-18 (Q-x-x-V)	VH1-18 ^{53}Y^{54}T ^{98}QxxV CDR H3 = 17-21	309 (0.06%) 14	0 (0%) 0	0 (0%) 0 (1)	242 (0.008%) 3	2 (0.00011%) 1

FIG. 6B

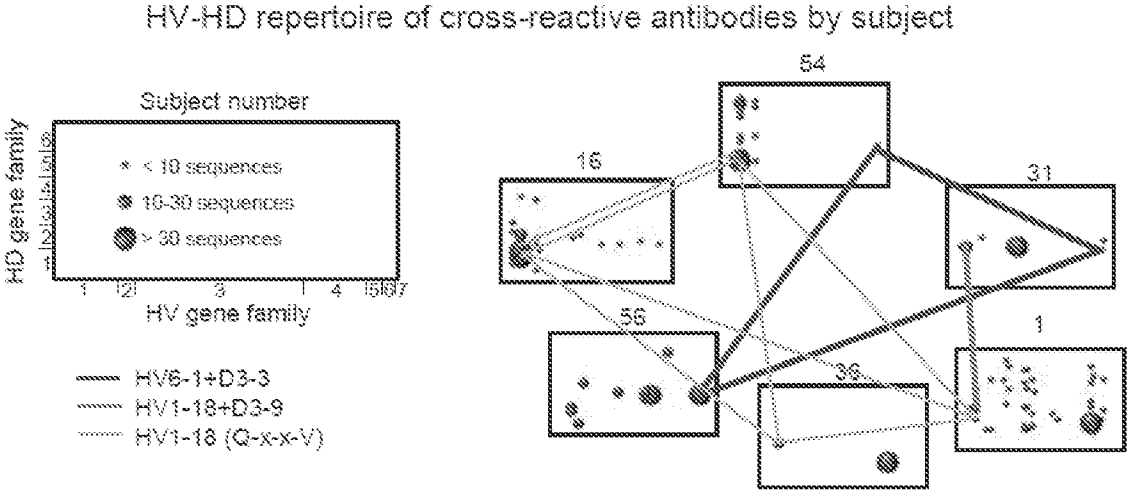


FIG. 6C

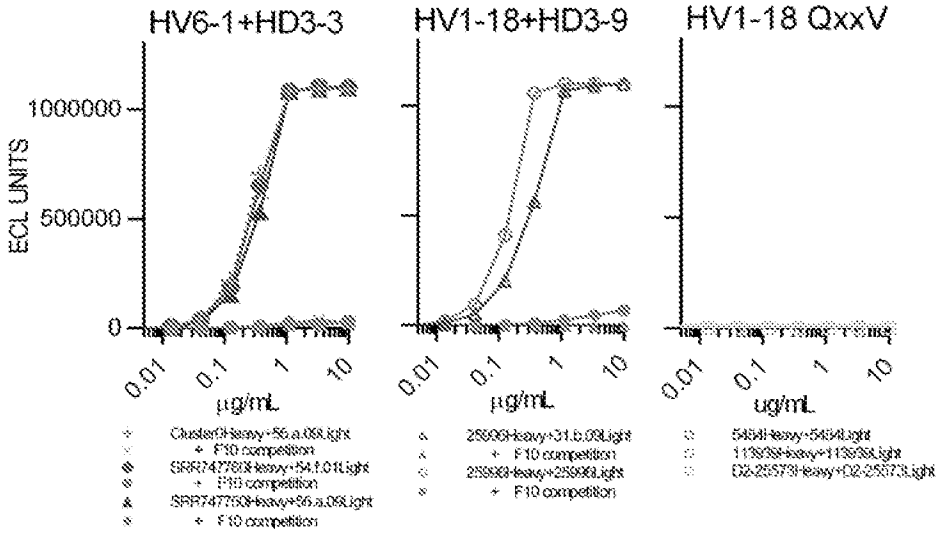


FIG. 6D

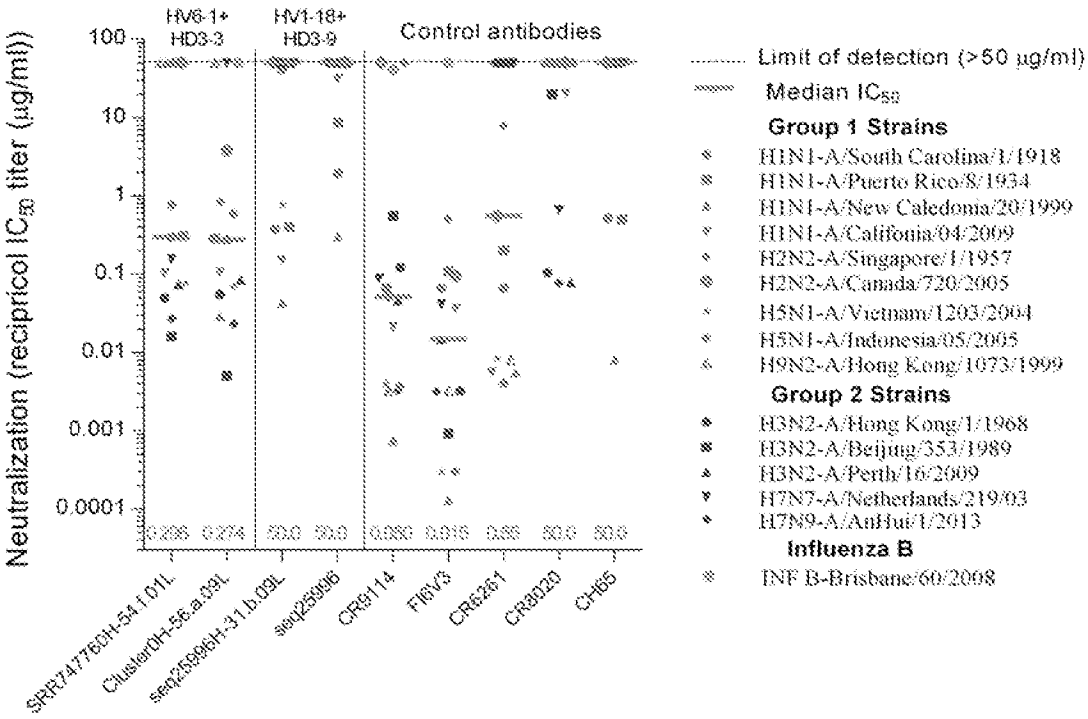

HV6-1+ HD3-3
HV1-18+ HD3-9
Control antibodies
Neutralization (recipricol IC50 titer (µg/ml))
100
10
1
0.1
0.01
0.001
0.0001
Limit of detection (>50 µg/ml)
Median IC50
Group 1 Strains
H1N1-A/South Carolina/1/1918
H1N1-A/Puerto Rico/8/1934
H1N1-A/New Caledonia/20/1999
H1N1-A/California/04/2009
H2N2-A/Singapore/1/1957
H2N2-A/Canada/720/2005
H5N1-A/Vietnam/1203/2004
H5N1-A/Indonesia/05/2005
H9N2-A/Hong Kong/1073/1999
Group 2 Strains
H3N2-A/Hong Kong/1/1968
H3N2-A/Beijing/353/1989
H3N2-A/Perth/16/2009
H7N7-A/Netherlands/219/03
H7N9-A/AnHui/1/2013
Influenza B
INF B-Brisbane/60/2008
SRR747760H-54.f.01L
Cluster0H-56.a.09L
seq259986H-31.b.09L
seq259986
CR9114
FI6v3
CR6261
CR8020
CH65

FIG. 6E

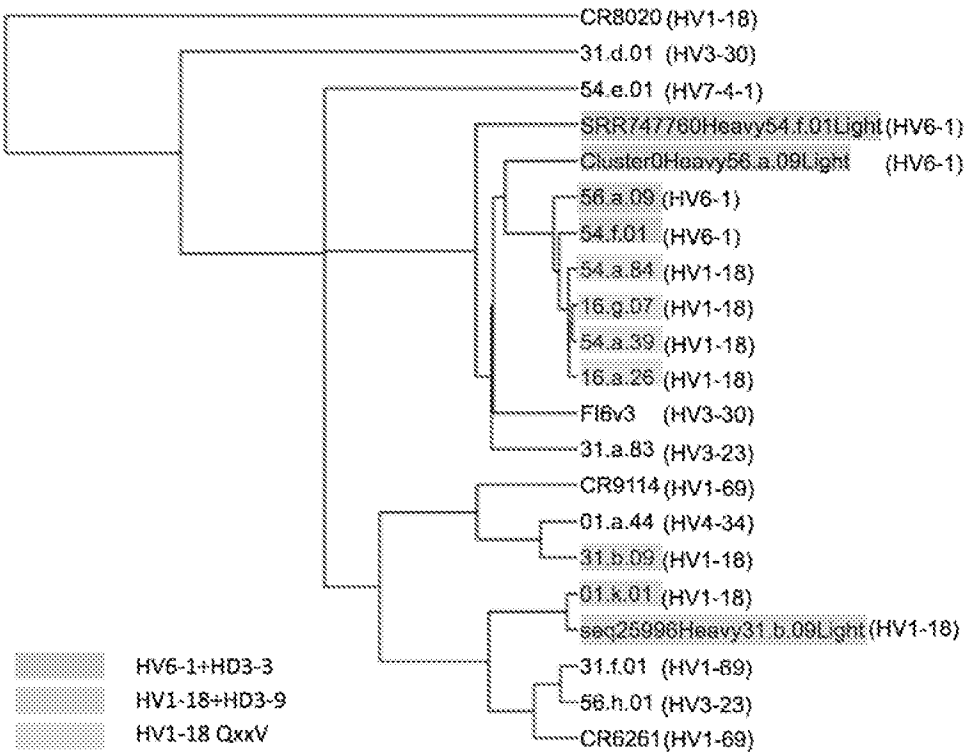

CR8020 (HV1-18)
31.d.01 (HV3-30)
54.e.01 (HV7-4-1)
SRR747760Heavy54.f.01Light (HV6-1)
Cluster0Heavy56.a.09Light (HV6-1)
56.a.09 (HV6-1)
54.f.01 (HV6-1)
54.a.84 (HV1-18)
16.g.07 (HV1-18)
54.a.39 (HV1-18)
16.a.26 (HV1-18)
FI6v3 (HV3-30)
31.a.83 (HV3-23)
CR9114 (HV1-69)
01.a.44 (HV4-34)
31.b.09 (HV1-18)
01.k.01 (HV1-18)
seq259986Heavy31.b.09Light (HV1-18)
31.f.01 (HV1-69)
56.h.01 (HV3-23)
CR6261 (HV1-69)
HV6-1+HD3-3
HV1-18+HD3-9
HV1-18 QxxV

FIG. 7A

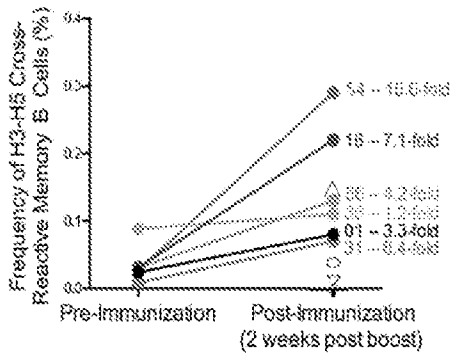


FIG. 7B

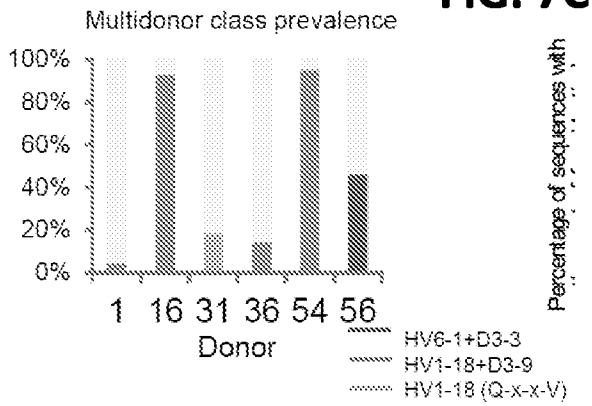


FIG. 7C

FIG. 7D

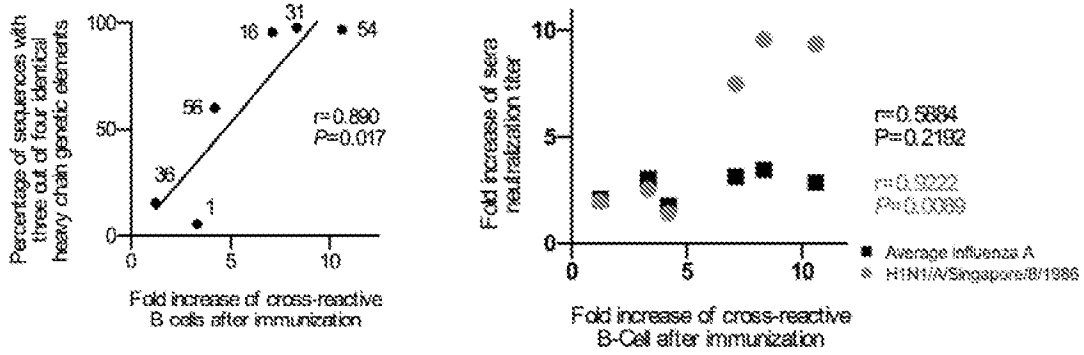


FIG. 7E

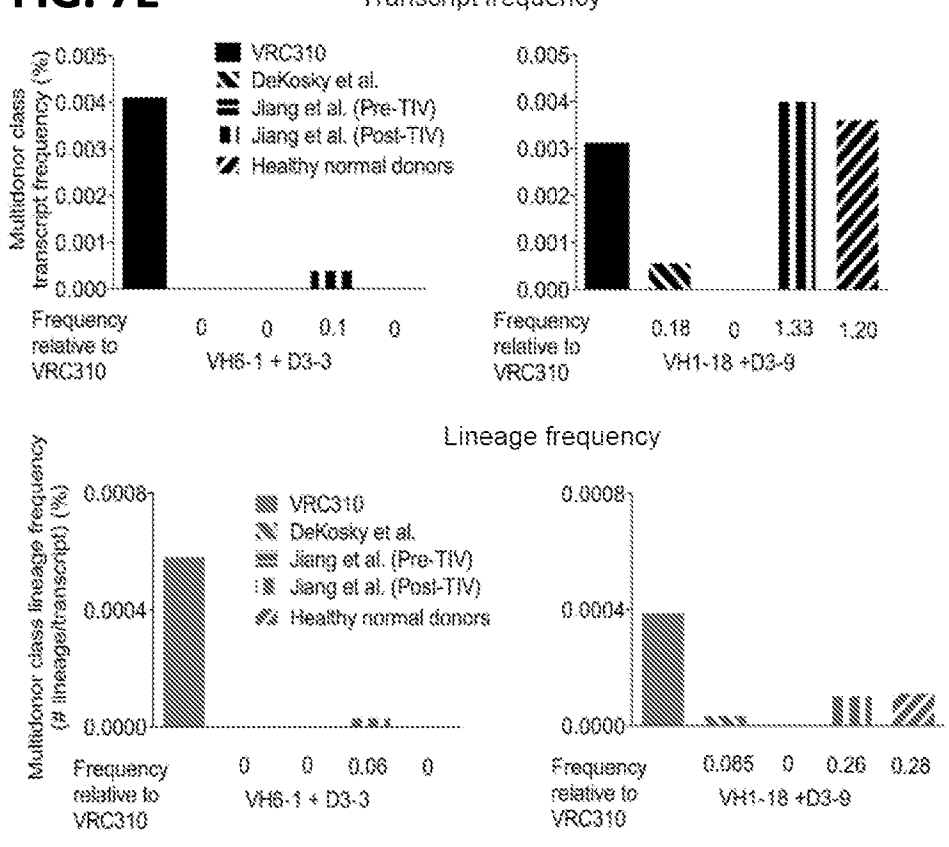


FIG. 7F

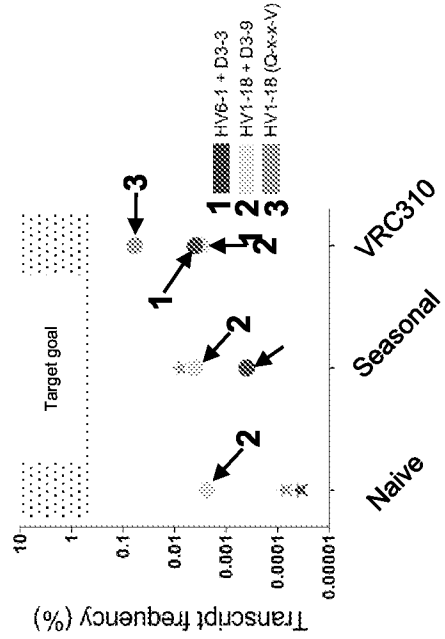

Transcript frequency (%)
10
1
0.1
0.01
0.001
0.0001
0.00001
Target goal
HV6-1 + D3-3
HV1-18 + D3-9
HV1-18 (Q-x-V)
1
2
3
VRC310
Seasonal
Naive

FIG. 7G

Influenza HA
HA stem region
PDB ID: 4FNK
Variable glycan
CR6261 Antibody footprint
Conserved glycan
CR8020 Antibody footprint
HV1-18 (Q-x-V) epitope
HV6-1/HD3-3 epitope

FIG. 8A

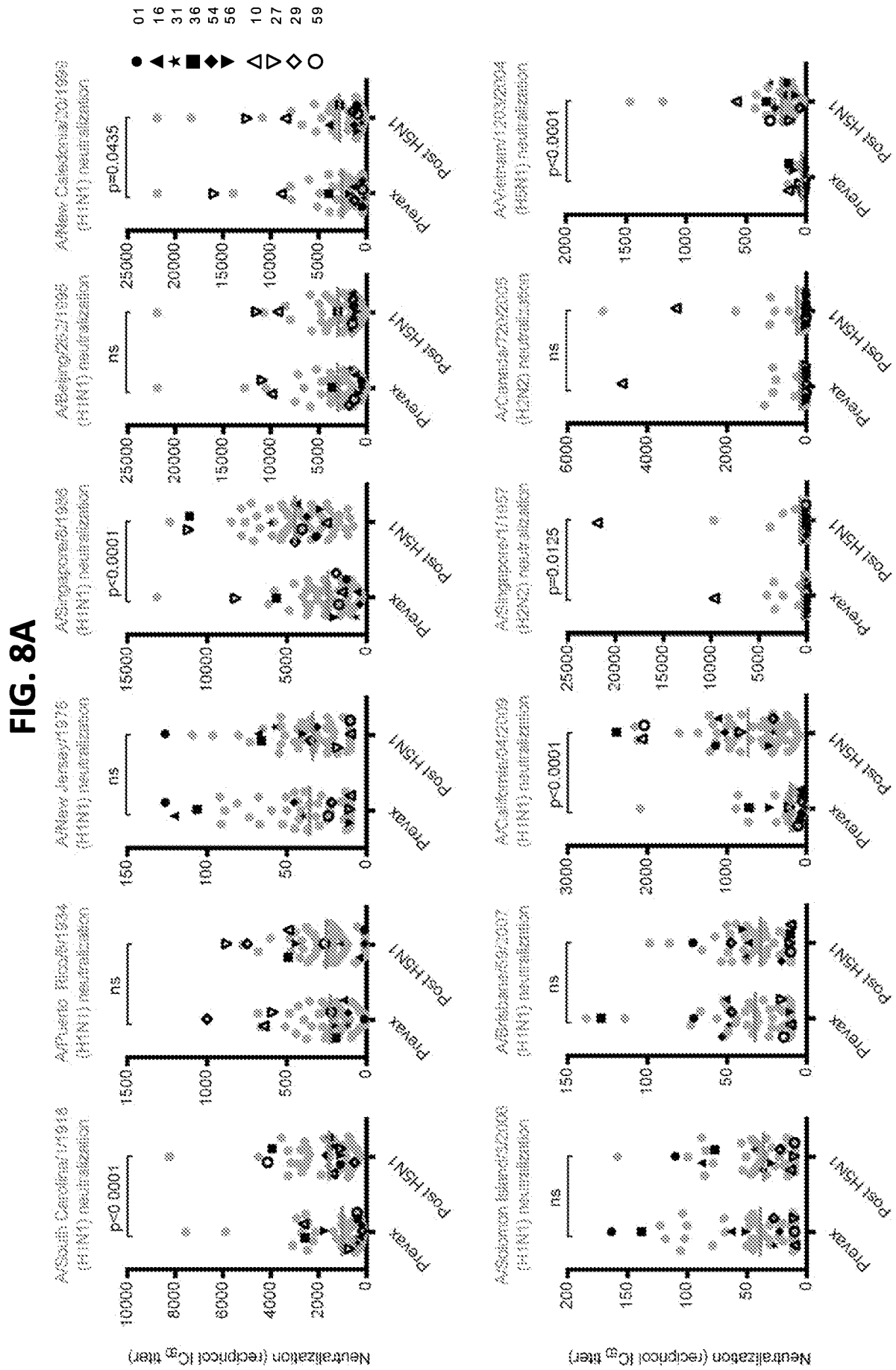
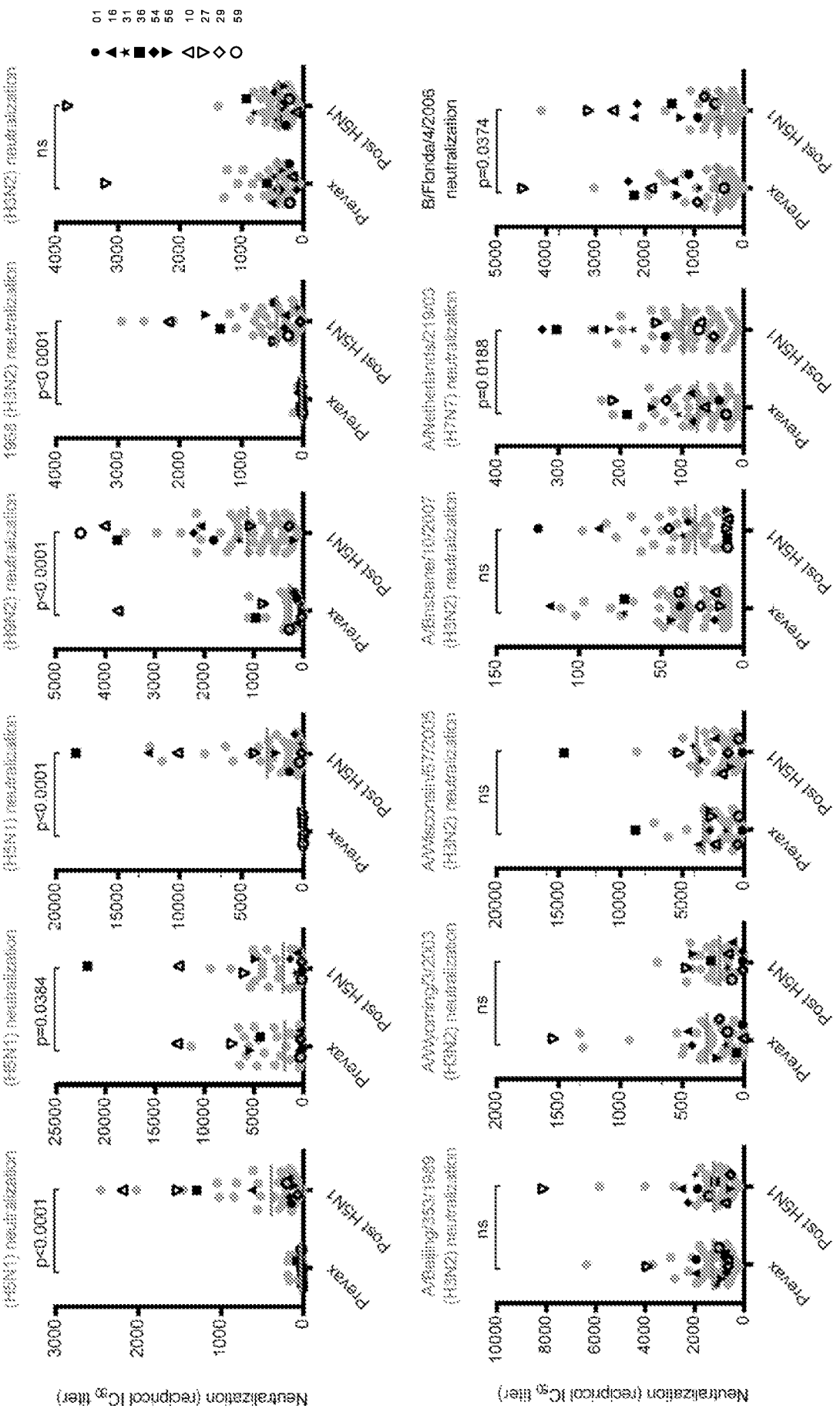

A/South Carolina/1/1918 (H1N1) neutralization
A/Puerto Rico/8/1934 (H1N1) neutralization
A/New Jersey/1976 (H1N1) neutralization
A/Singapore/6/1986 (H1N1) neutralization
A/Beijing/262/1995 (H1N1) neutralization
A/New Caledonia/20/1999 (H1N1) neutralization
A/Solomon Islands/3/2006 (H1N1) neutralization
A/Brisbane/59/2007 (H1N1) neutralization
A/California/04/2009 (H1N1) neutralization
A/Singapore/1/1957 (H2N2) neutralization
A/Canada/720/2005 (H3N2) neutralization
A/Vietnam/1203/2004 (H5N1) neutralization
p<0.0001
ns
p<0.0001
p=0.0435
p<0.0001
p=0.0125
p<0.0001
Pre-vax
Post H5N1
Neutralization (reciprocal IC50 titer)
01
16
31
36
54
56
10
27
29
59

FIG. 8B


A/Anhui/1/2005 (H5N1) neutralization
A/Turkey/1/2005 (H5N1) neutralization
A/Indonesia/05/2005 (H5N1) neutralization
A/Hong Kong/1073/1999 (H9N2) neutralization
A/Hong Kong/1-4-MA21-1/ 1968 (H3N2) neutralization
A/Bangkok/1/1979 (H3N2) neutralization
A/Beijing/353/1989 (H3N2) neutralization
A/Wyoming/3/2003 (H3N2) neutralization
A/Wisconsin/67/2005 (H3N2) neutralization
A/Brisbane/10/2007 (H3N2) neutralization
A/Wetherlands/219/03 (H7N7) neutralization
B/Florida/4/2006 neutralization
p<0.0001
p=0.0384
p<0.0001
p<0.0001
p<0.0001
ns
p=0.0188
p=0.0374
Prevax
Post HSN1
Neutralization (reciprocal IC50 titer)
01 16 31 36 54 56 10 27 29 59

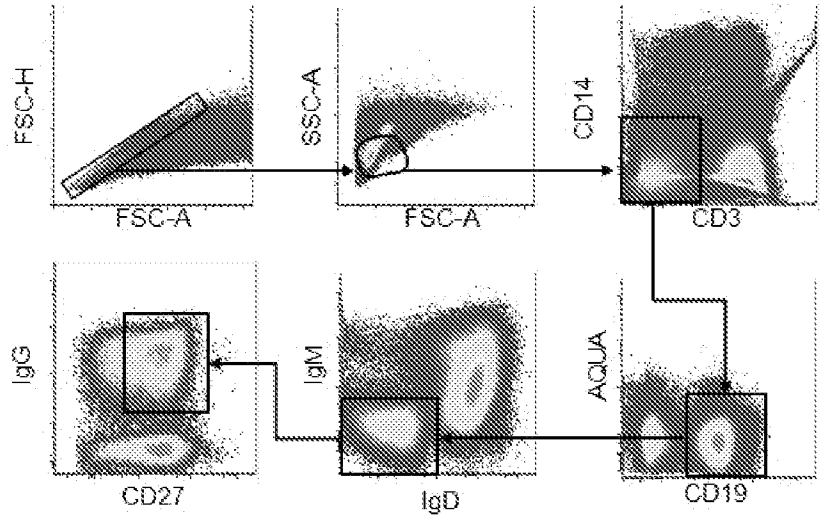

FSC-H
FSC-A
SSC-A
FSC-A
CD14
CD3
IgG
CD27
IgM
IgD
AQUA
CD19

FIG. 9A

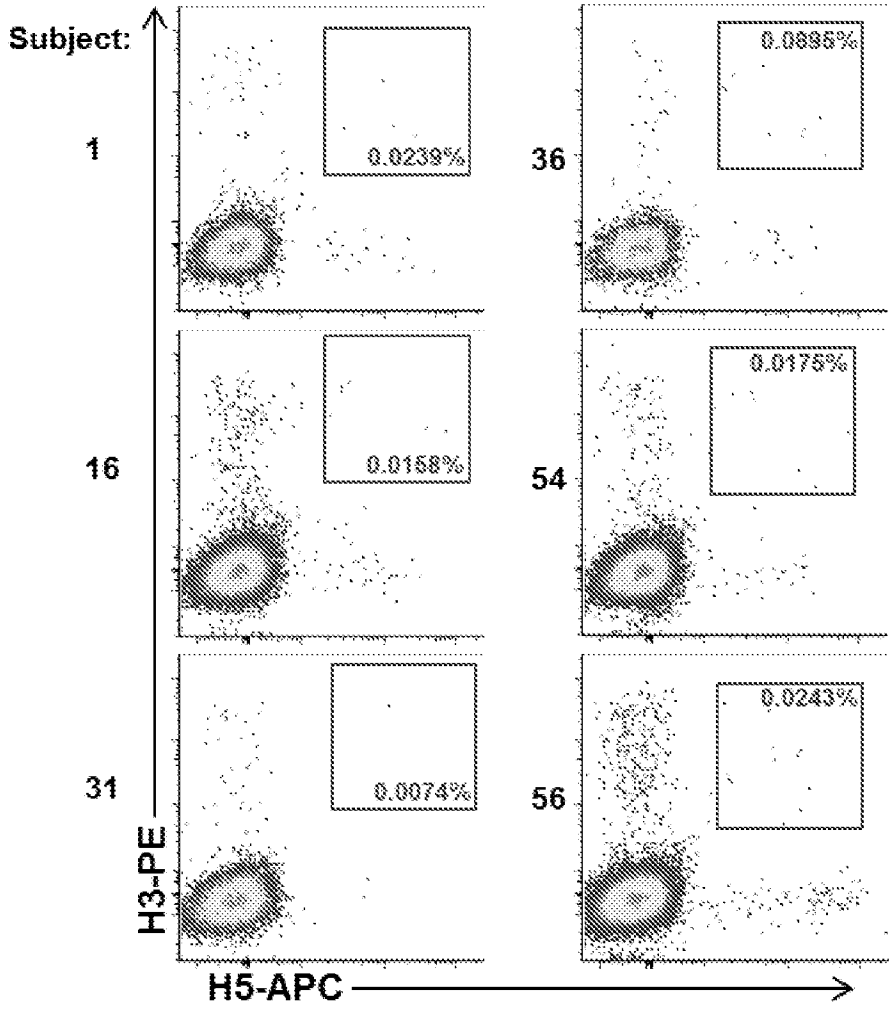
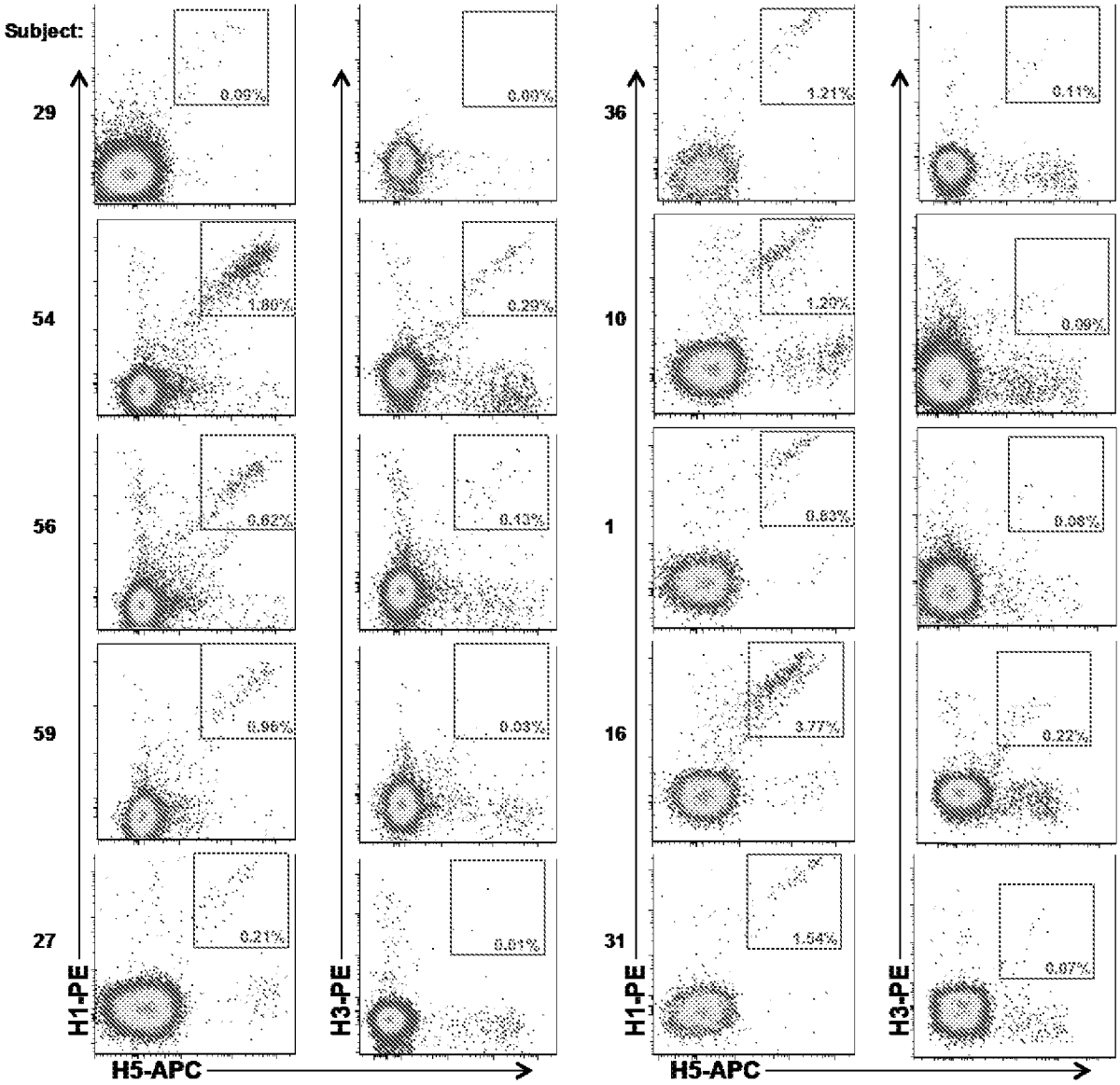

Subject:
1
0.0239%
36
0.0895%
16
0.0158%
54
0.0175%
31
0.0074%
56
0.0243%
H3-PE
H5-APC

FIG. 9B

FIG. 9C


Subject:
29
54
56
59
27
36
10
1
16
31
0.09%
0.03%
1.80%
0.29%
0.62%
0.13%
0.96%
0.03%
0.21%
0.01%
1.21%
0.11%
1.20%
0.09%
0.83%
0.08%
3.77%
0.22%
1.54%
0.07%
H1-PE
H3-PE
H5-APC

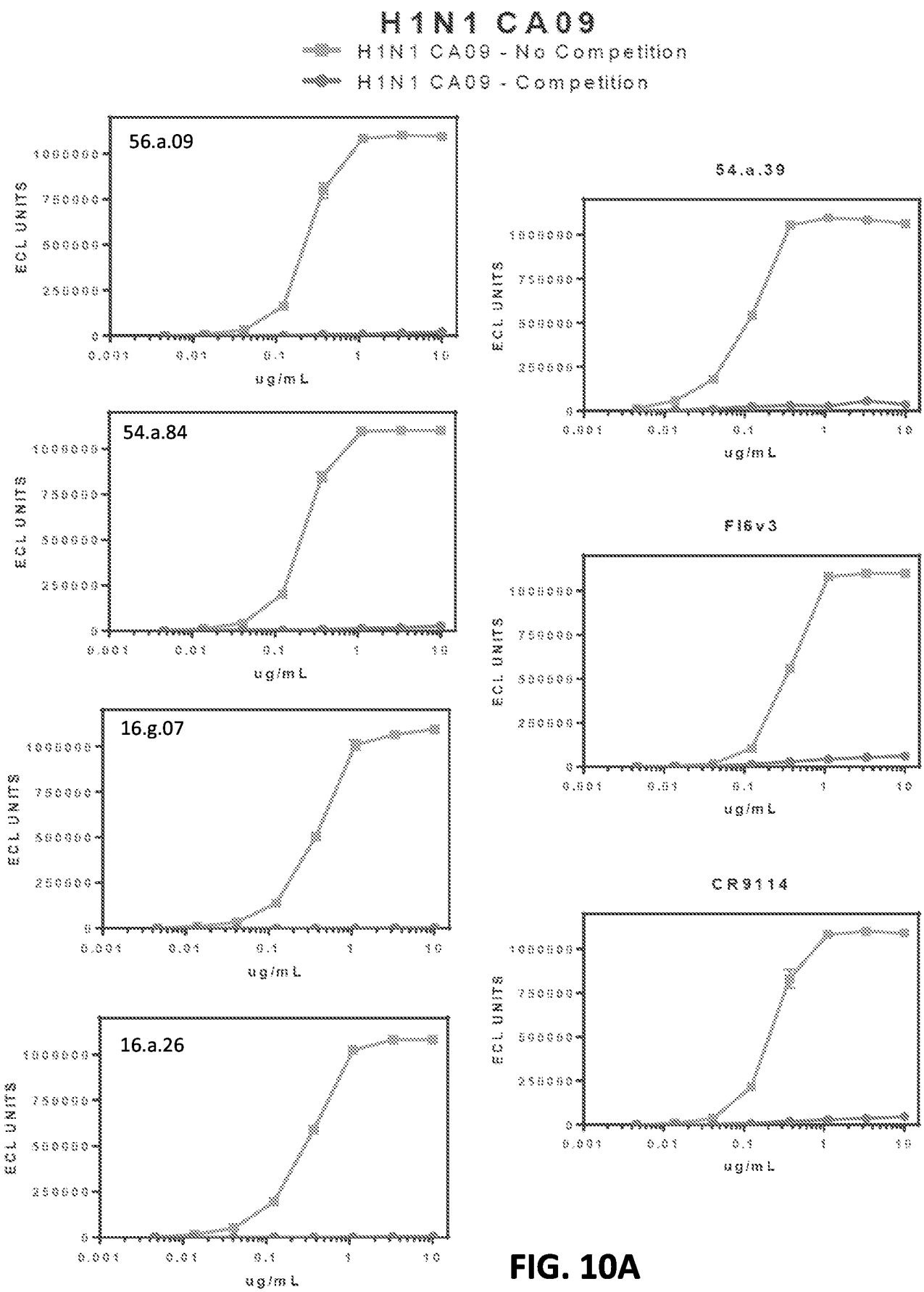

H1N1 CA09
H1N1 CA09 - No Competition
H1N1 CA09 - Competition
56.a.09
54.a.84
16.g.07
16.a.26
54.a.39
FI6v3
CR9114
ECL UNITS
ug/mL
0.001
0.01
0.1
1
10

FIG. 10A

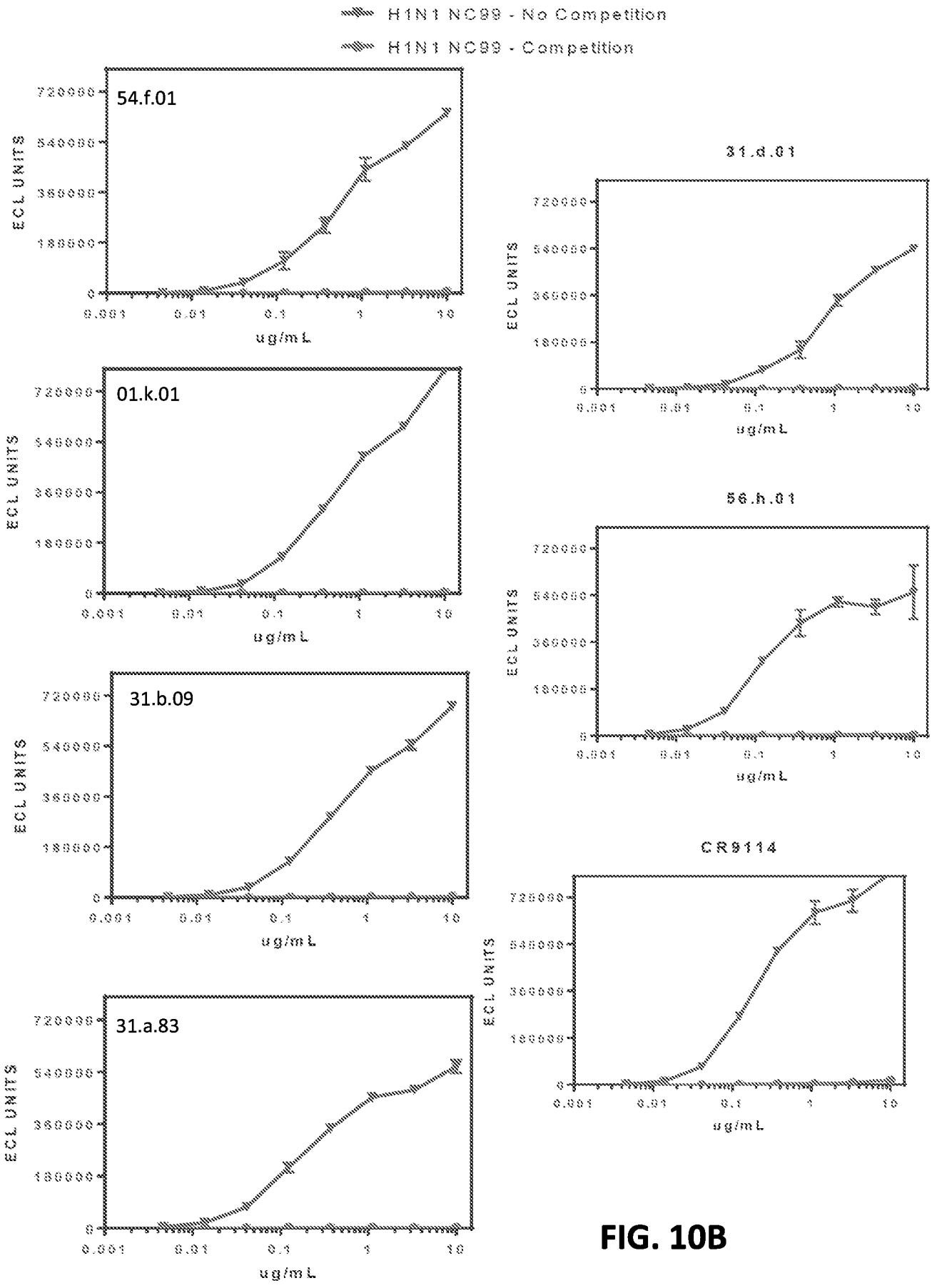

H1N1 NC99
H1N1 NC99 - No Competition
H1N1 NC99 - Competition
54.f.01
31.d.01
01.k.01
56.h.01
31.b.09
CR9114
31.a.83
ECL UNITS
ug/mL
0.001
0.01
0.1
1
10

FIG. 10B

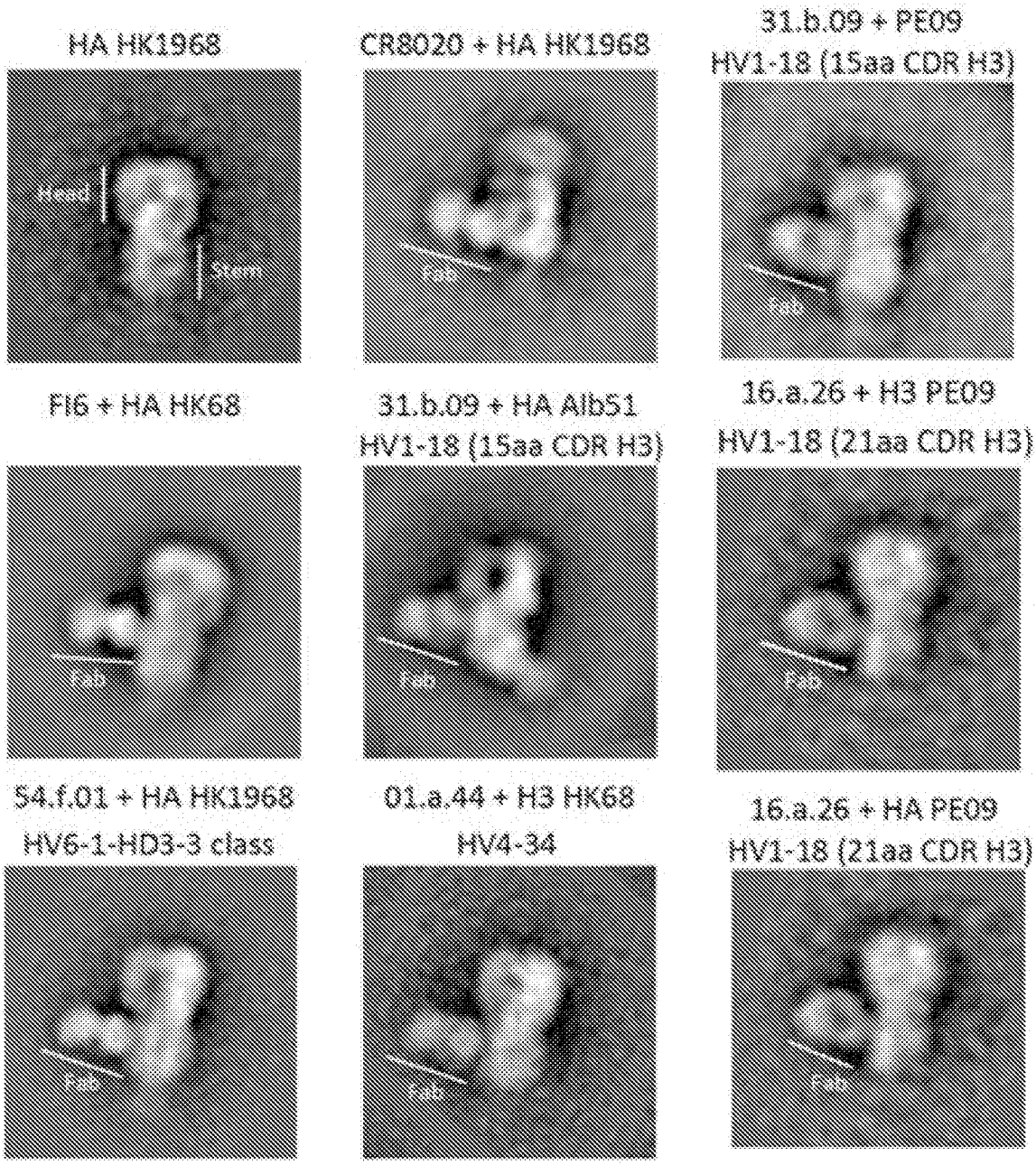

HA HK1968
CR8020 + HA HK1968
31.b.09 + PE09
HV1-18 (15aa CDR H3)
FI6 + HA HK68
31.b.09 + HA Alb51
HV1-18 (15aa CDR H3)
16.a.26 + H3 PE09
HV1-18 (21aa CDR H3)
54.f.01 + HA HK1968
HV6-1-HD3-3 class
01.a.44 + H3 HK68
HV4-34
16.a.26 + HA PE09
HV1-18 (21aa CDR H3)

FIG. 10C

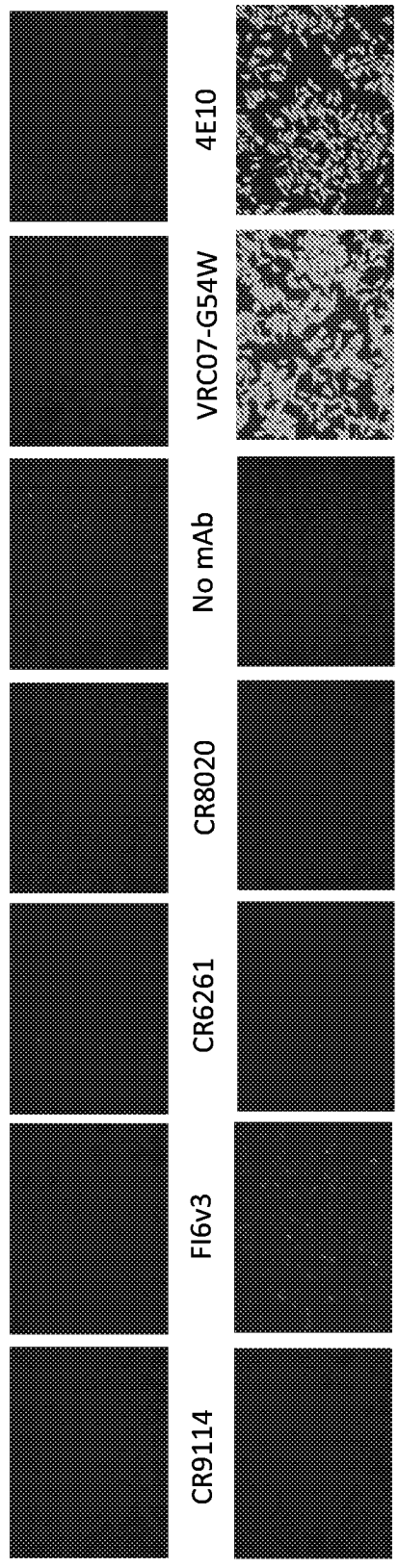


FIG. 10D

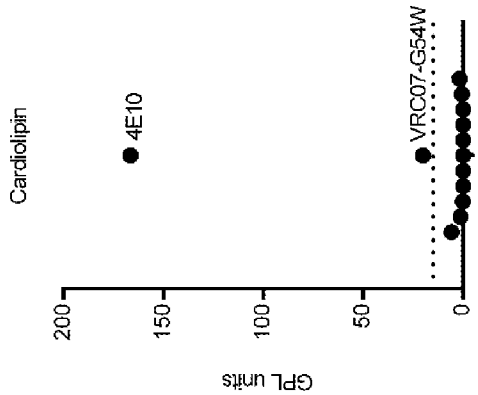


FIG. 10E

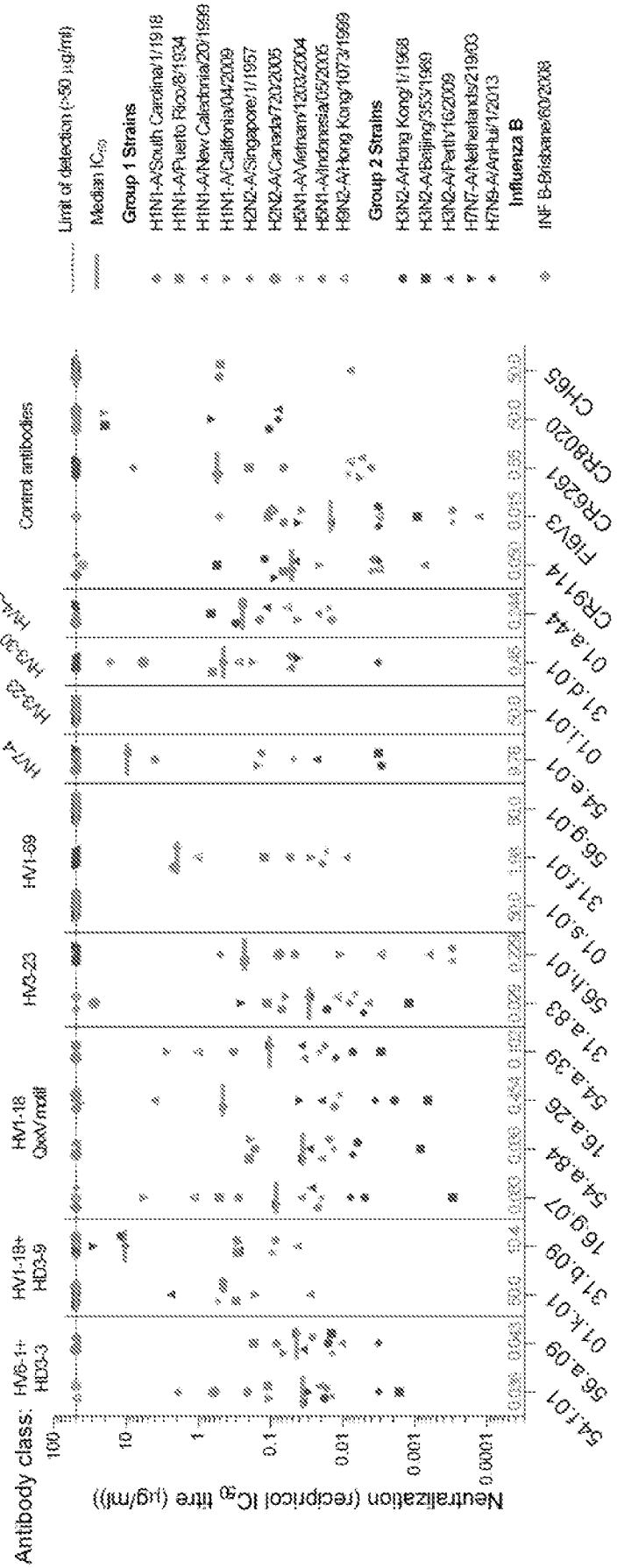

Limit of detection (>50 µg/ml)
Median IC50
Group 1 Strains
H1N1-A/South Carolina/1/1918
H1N1-A/Puerto Rico/8/1934
H1N1-A/New Caledonia/20/1999
H1N1-A/California/04/2009
H2N2-A/Singapore/1/1957
H2N2-A/Canada/720/2005
H5N1-A/Vietnam/1203/2004
H5N1-A/Indonesia/05/2005
H5N2-A/Hong Kong/1073/1999
Group 2 Strains
H3N2-A/Hong Kong/1/1968
H3N2-A/Beijing/353/1989
H3N2-A/Perth/16/2009
H7N7-A/Netherlands/219/03
H7N9-A/Anhui/1/2013
Influenza B
INF B-Brisbane/60/2008
Antibody class:
Neutralization (reciprocal IC50 titre (µg/ml))
Control antibodies

FIG. 11

FIG. 12A

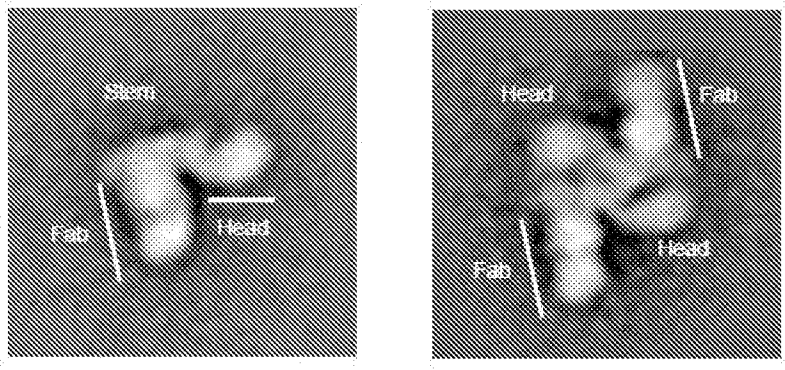


FIG. 12B

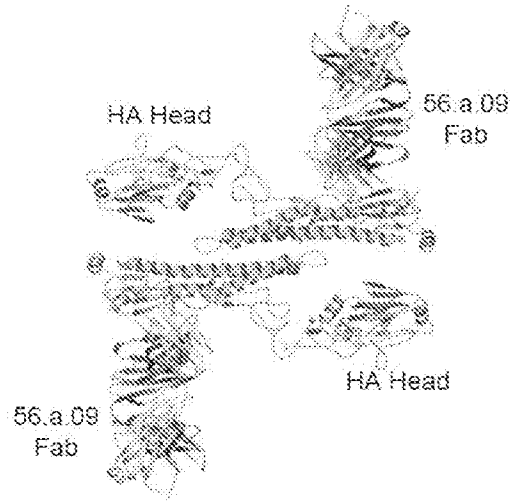


FIG. 12C

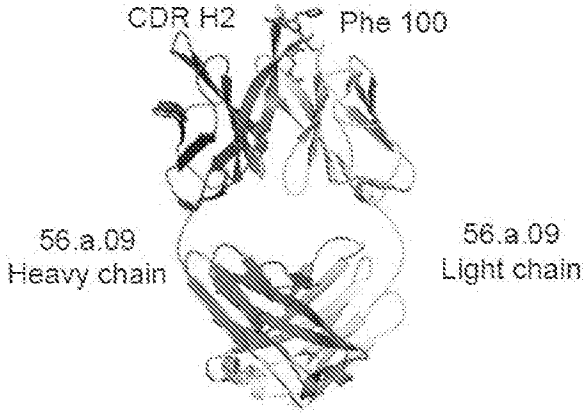


FIG. 12D

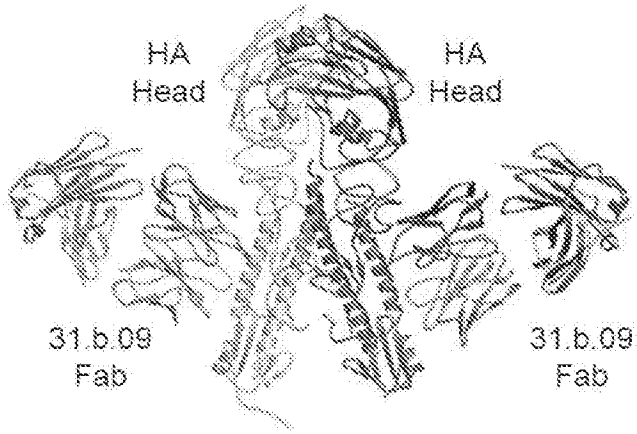


FIG. 13A


CDRH1 __CDRH2__ SEQ ID
HV6-1*01 QVQLQQSGPGLVKPSQTLSLTCAISGDSVSSNSAAWNWIRQSPSRGLEWLGRTYYRSKWYNDYAVSVKSRITINPDTSKNQFSLQLNSVTPEDTAVYYCAR NO: 324
31.g.01
54.f.01
56.a.09
_CDRH_1_ _CDRH_2_ SEQ ID
HV1-18*01 QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYGISWVRQAPGQGLEWMGWISAYNGNTNYAQKLQGRVTMTTDTSTSTAYMELRSLRSDDTAVYYCAR NO: 325
01.k.01
31.b.09
_CDRH_1_ _CDRH_2_ SEQ ID
HV1-18*01 QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYGISWVRQAPGQGLEWMGWISAYNGNTNYAQKLQGRVTMTTDTSTSTAYMELRSLRSDDTAVYYCAR NO: 325
16.g.07
54.a.84
16.a.26
54.a.39
_CDRH_1_ _CDRH_2_ SEQ ID
HV3-23 EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAISGSGGSTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAK NO: 326
31.a.83

FIG. 13B

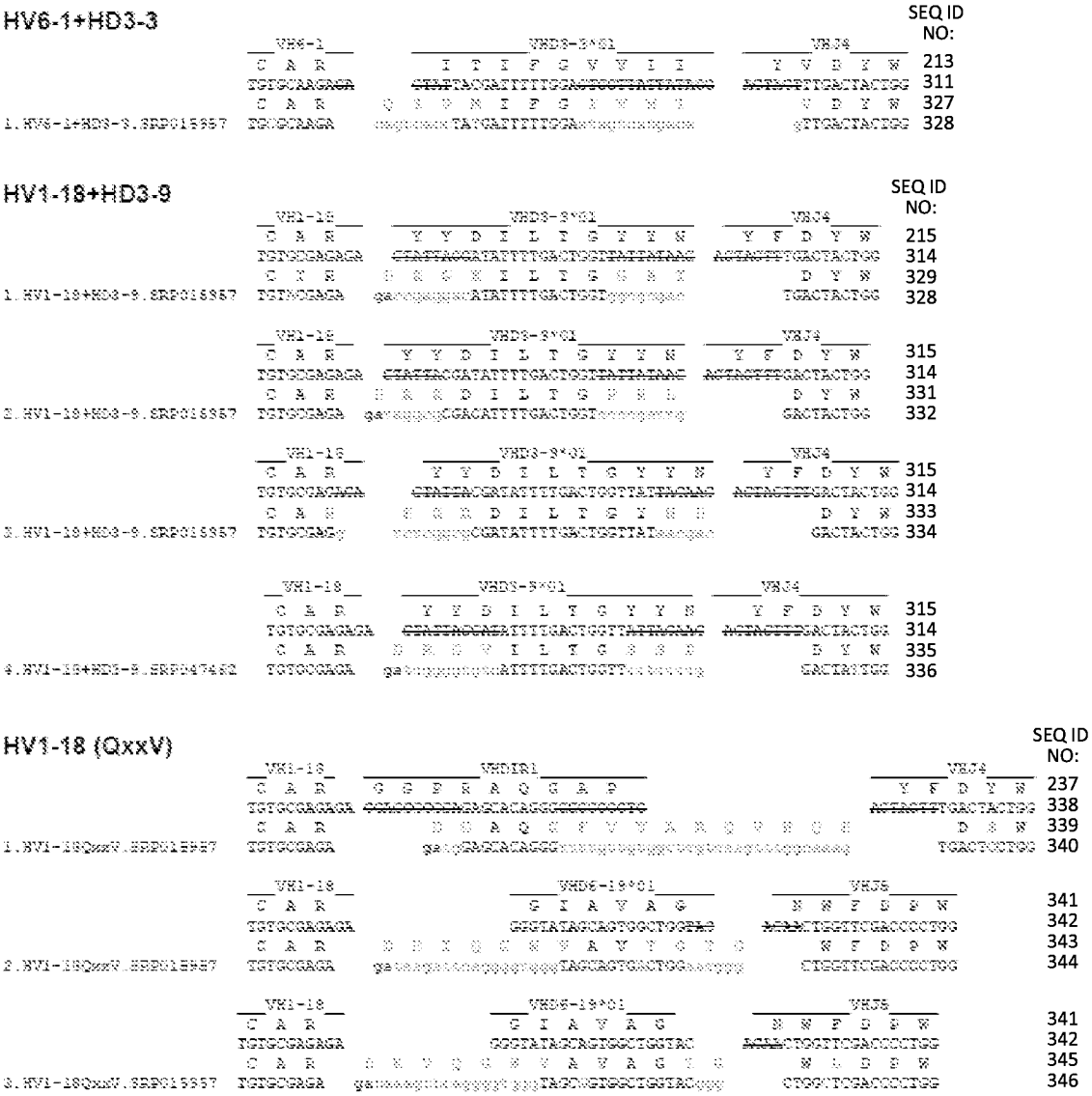


FIG. 13C

HV1-18+HD3-9

	VH1-18	VHD3-9*01	VHJ3	SEQ ID NO:
	C A R	Y Y D I L T G Y Y N	D A F D I W	
	TGTGCGAGA	GTATTACGATATTTTGACTGGTTATTATAAC	TGATGCTTTTGATATCTGG	247
	C A R	X X Y D I L T G	D A F D I W	348
1.HV1-18+HD3-9.SRP026397	TGTGCGAGA	gaaccgTTACGATATTTTGACTGG	TGATGCTTTTGATATCTGG	349
				350

	VH1-18	VHD3-9*01	VHJ4	SEQ ID NO:
	C A R	Y Y D I L T G Y Y N	Y F D Y W	315
	TGTGCGAGA	GTATTACGATATTTTGACTGGTTATTATAAC	ACTACTTTGACTACTGG	314
	C A R	D R G D I L T G P S	L D Y W	351
2.HV1-18+HD3-9.SRP073039	TGTGCGAGA	gatcgtgggCGATATTGACTGGccaagcc	cTTGACTACTGG	352

HV1-18 (QxxV)

	VH1-18	VHD4-4*01R	VHJ6	SEQ ID NO:
	C A R	V V T V V	Y Y Y Y Y G M D V W	
	TGTGCGAGA	GTAGTTACTGTAGTAGTGCA	ATTACTACTACTACGGTATGGACGTCTGG	253
	C A R	D K V Q G I V T V V H K A	L D V W	354
1.HV1-18QxxV.SRP026397	TGTGCGAGA	gacaaggtccagggaactgtaACTGTAGTagtgcaaagcc	cTGGACGTTTGG	355
				356

FIG. 13D

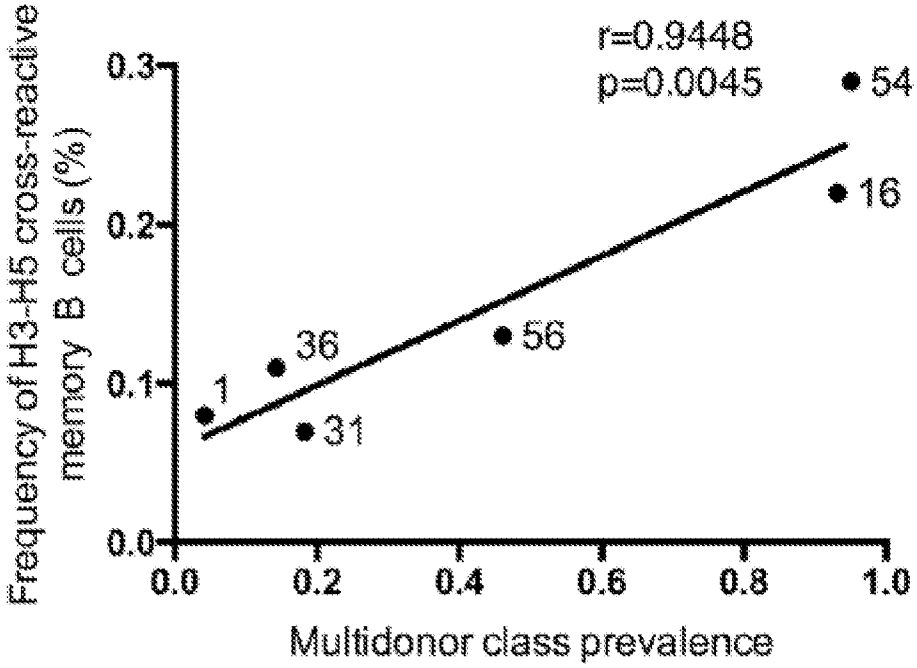

r=0.9448
p=0.0045
54
16
56
36
1
31
0.3
0.2
0.1
0.0
0.0
0.2
0.4
0.6
0.8
1.0
Frequency of H3-H5 cross-reactive memory B cells (%)
Multidonor class prevalence

FIG. 14A

	H3-Brisbane 2007	H3-California 2004	H3-Moscow 1999	H3-Perth 2009	H3-Sydney 1997
56.a.09	2968000	3037000	2898000	2975000	3010000
M98A	3068000	3088000	2780000	3041000	3042000
F100A	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
M98A-F100A	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

	H1-California 2009	H3-Hong Kong 1968		H1-California 2009	H3-Hong Kong 1968
56.a.09	3033000	2967000	54.f.01	3138000	3097000
R52bA	3159000	3185000	R52bA	3196000	3164000
W55A	3235000	3203000	W55A	3175000	3133000
R52bA-W55A	3134000	3157000	R52bA-W55A	3167000	3140000
56.a.09	3033000	2967000	54.f.01	3138000	3097000
g6-1CDRH2	3125000	3060000	g6-1CDRH2	3237000	3187000
4-39CDRH2	[illegible]	[illegible]	4-59*05CDRH2	3151000	3081000
4-61CDRH2	[illegible]	[illegible]	4-61CDRH2	3191000	1932000
4-30-2*05CDRH2	[illegible]	[illegible]	4-30-2*05CDRH2	2691000	[illegible]
4-30-4CDRH2	[illegible]	[illegible]	4-31-CDRH2	3139000	[illegible]
gD3-3*01CDRH3	3158000	3038000	gD3-3*01CDRH3	2794000	3078000
D3-9fr1CDRH3	[illegible]	[illegible]			
D3-10fr3CDRH3	[illegible]	[illegible]			
D3-10CDRH3-A	[illegible]	2612000			
D6-13invfr2CDRH3	[illegible]	2938000			

	H1-California 2009	H3-Hong Kong 1968		H1-California 2009	H3-Hong Kong 1968
56.a.09 gVDJ	3152000	3108000	54.f.01 gVDJ	1926000	[illegible]
R52bA	2785000	[illegible]	R52bA	[illegible]	[illegible]
W55A	3187000	3143000	W55A	[illegible]	[illegible]
R52bA-W55A	1748000	[illegible]	R52bA-W55A	[illegible]	[illegible]
4-30-4CDRH2	[illegible]	[illegible]	4-30-2CDRH2	[illegible]	[illegible]
4-30-2*05CDRH2	[illegible]	[illegible]	4-31CDRH2	[illegible]	[illegible]
4-39CDRH2	[illegible]	[illegible]	4-59*05CDRH2	[illegible]	[illegible]
4-61CDRH2	[illegible]	[illegible]	4-61CDRH2	[illegible]	[illegible]

FIG. 14B

	H3-Brisbane 2007	H3-California 2004	H3-Moscow 1999	H3-Perth 2009	H3-Sydney 1997
16.a.26	2911000	2867000	2808000	2865000	2797000
Q98A	3305000	3273000	3217000	3304000	3198000
V100aA	3458000	3389000	3354000	3439000	3330000
Q98A-V100aA	3180000	2945000	2512000	3069000	2623000

	H1-California 2009	H3-Hong Kong 1968		H1-California 2009	H3-Hong Kong 1968
16.g.07	2865000	3100000	16.a.26	3027000	3092000
16.g.07-S52N	[illegible]	3065000	16.a.26-S52N	2878000	3105000
16.g.07-S52I	[illegible]	3116000	16.a.26-S52I	2773000	3010000
16.g.07-G33Y	[illegible]	[illegible]	16.a.26-G33Y	3171000	3165000
16.g.07-G33S	2707000	1819000	16.a.26-G33S	3182000	3147000
16.g.07-HV1-45	[illegible]	[illegible]	16.a.26-HV1-45	no expression	no expression
16.g.07-HV1-68	no expression	no expression	16.a.26-HV1-68	no expression	no expression

	H3-Brisbane 2007	H3-California 2004	H3-Moscow 1999	H3-Perth 2009	H3-Sydney 1997
H-mature/L-UCA	3033000	2702000	2408000	2808000	2584000
H-UCA/L-mature	[illegible]	[illegible]	[illegible]	[illegible]	2128000
H-UCA/L-UCA	1618000	[illegible]	[illegible]	[illegible]	[illegible]

	H1-California 2009	H3-Hong Kong 1968		H1-California 2009	H3-Hong Kong 1968
16.g.07-gVDJ	[illegible]	[illegible]	16.a.26-gVDJ	[illegible]	3104000

FIG. 14C

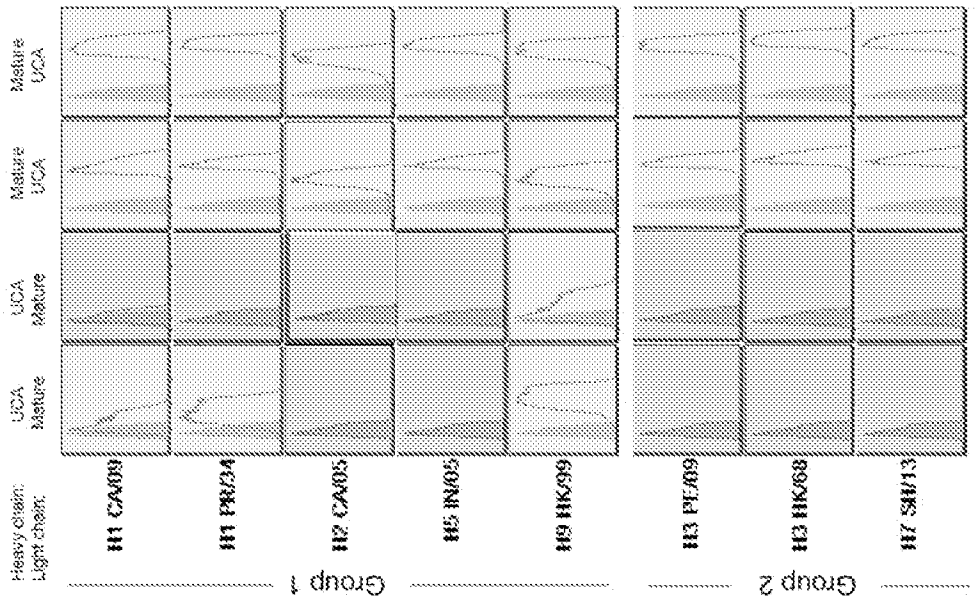


FIG. 14D

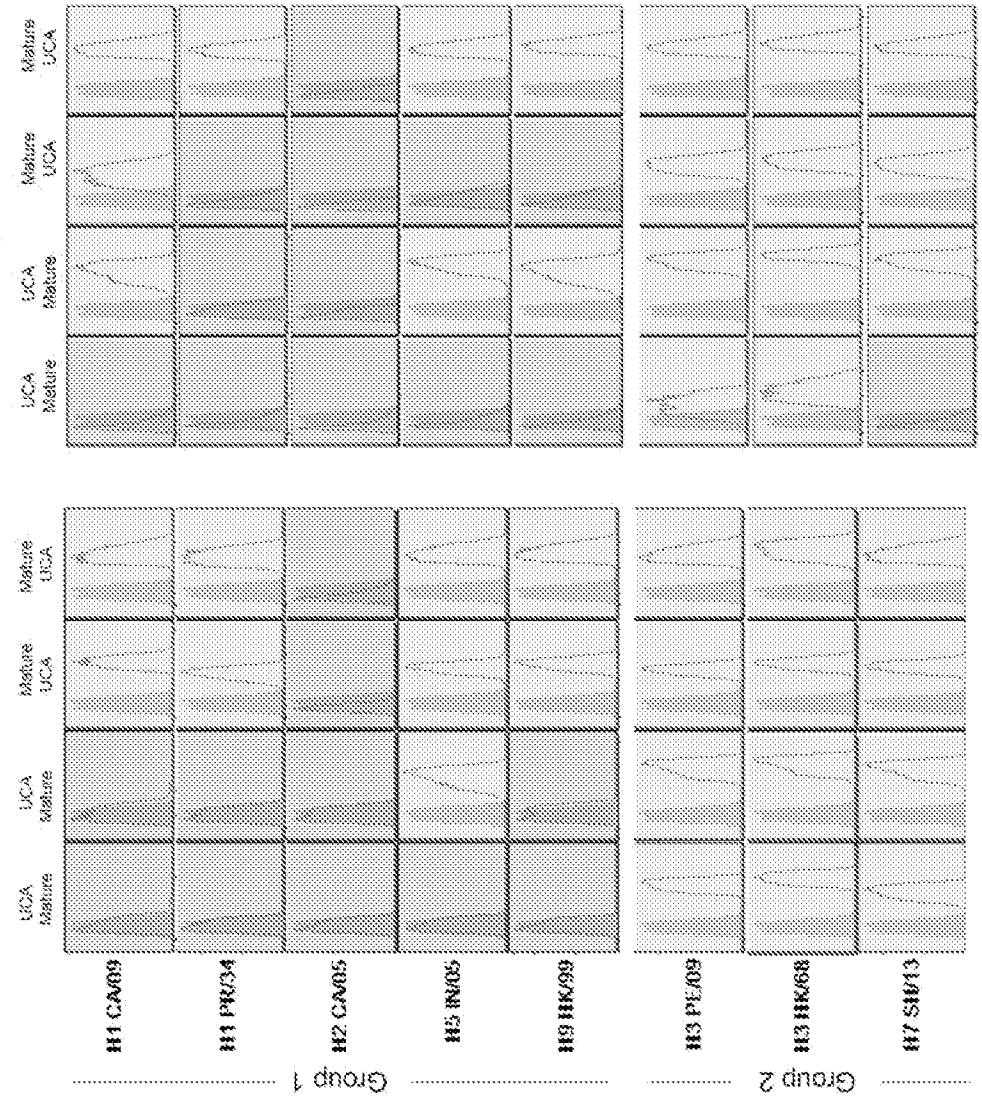


FIG. 15A

IC_{50} neutralization titers assessed using pseudovirus entry inhibition assay.

Class	HV gene	Antibody name	H1N1-A/South Carolina/1/1918	H1N1-A/Puerto Rico/8/1934	H1N1-A/New Caledonia/20/1999	H1N1-A/Califonia/04/2009	H2N2-A/Singapore/1/1957	H2N2-A/Canada/720/2005	H5N1-A/Vietnam/1203/2004	H5N1-A/Indonesia/05/2005	H9N2-A/Hong Kong/1073/1999	H3N2-A/Hong Kong/1/1968	H3N2-A/Beijing/353/1989	H3N2-A/Perth/16/2009	H7N7-A/Netherlands/219/03	H7N9-A/AnHui/1/2013	INF B-Brisbane/60/2008
HV6-1-HD3-3	HV6-1	54.f.01	0.21	0.11	0.021	0.1	1.9	0.61	0.014	0.017	>50	0.018	0.0017	0.036	0.029	0.0032	>50
HV6-1-HD3-3	HV6-1	56.a.09	0.083	0.17	0.01	0.064	>50	>50	0.014	0.026	>50	0.016	0.014	0.035	0.043	0.0032	>50
HV1-18-HD3-9	HV1-18	01.k.01	0.46	0.3	0.029	0.16	>50	>50	0.54	0.44	>50	>50	>50	2.4	>50	>50	>50
HV1-18-HD3-9	HV1-18	31.b.09	0.29	0.29	0.043	0.28	>50	>50	0.095	0.096	>50	12	>50	0.28	29	10	>50
HV1-18 (Q-x-x-V)	HV1-18	16.g.07	0.28	0.51	0.037	5.7	>50	>50	0.02	0.022	1.2	0.0049	0.0003	0.027	0.083	0.0078	>50
HV1-18 (Q-x-x-V)	HV1-18	54.a.84	0.16	0.2	0.02	0.19	>50	>50	0.013	0.015	>50	0.0062	0.00064	0.029	0.036	0.0073	>50
HV1-18 (Q-x-x-V)	HV1-18	16.a.26	0.45	>50	4.1	43	>50	>50	0.011	0.013	>50	0.0019	0.00066	0.02	0.04	0.0036	>50
HV1-18 (Q-x-x-V)	HV1-18	54.a.39	0.33	>50	0.035	2.7	>50	>50	0.021	0.018	1	0.0075	0.003	0.037	0.1	0.013	>50
	HV3-23	31.a.83	0.069	0.11	0.0045	0.061	>50	28	0.0067	0.008	0.012	0.017	0.0012	0.029	0.25	0.0061	>50
	HV3-23	56.h.01	0.045	0.23	0.00063	0.011	0.49	0.077	0.0003	0.0003	0.003	>50	>50	>50	>50	>50	>50
	HV1-69	01.s.01	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
	HV1-69	31.f.01	0.054	0.12	0.009	0.027	2	2.1	0.017	0.015	1	>50	>50	>50	>50	>50	>50
	HV1-69	56.g.01	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
	HV7-4	54.e.01	4	>50	9.8	0.15	>50	>50	0.047	0.13	>50	0.0029	0.0032	0.023	>50	>50	>50
	HV3-23	01.i.01	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
	HV3-30	31.d.01	0.45	0.64	0.054	0.17	17	5.8	0.05	0.052	0.28	>50	>50	>50	0.041	0.0032	>50
	HV4-34	01.a.44	0.14	0.24	0.022	0.041	>50	>50	0.018	0.014	0.059	0.3	0.67	0.11	>50	>50	>50
Control antibodies	HV1-69	CR9114	0.05	0.066	0.00075	0.021	>50	>50	0.004	0.0037	0.0032	0.12	0.55	0.045	0.068	0.0032	41
	HV3-30	FI6v3	0.065	0.11	0.00013	0.036	0.51	0.094	0.0003	0.0003	0.0032	0.0032	0.00092	0.015	0.041	0.0032	>50
	HV1-69	CR6261	0.066	0.2	0.0056	0.0056	7.9	0.55	0.0084	0.004	0.008	>50	>50	>50	>50	>50	>50
	HV1-18	CR8020	>50	>50	>50	20	>50	>50	>50	>50	>50	0.1	20	0.079	0.66	0.078	>50
	HV1-2	CH65	0.52	0.5	0.008	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
HV6-1-HD3-3	SRR747760 Heavy 54.f.01 Light		0.3	0.31	>50	0.1	>50	>50	0.075	0.76	>50	0.05	0.016	0.074	0.16	0.027	>50
HV6-1-HD3-3	NGS Heavy 56.a.09 Light		0.27	0.29	0.029	0.11	0.83	3.8	0.069	0.59	>50	0.055	0.005	0.065	>50	0.023	>50
2	seq25996 Heavy 31.b.09 Light		0.37	0.4	0.043	0.15	>50	>50	0.78	40	>50	>50	>50	>50	>50	>50	>50
2	seq25996 Heavy and Light		1.9	8.5	0.3	31	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50

FIG. 15B

IC_{80} neutralization titers assessed using pseudovirus entry inhibition assay.

Signature	HV gene	Antibody name	H1N1-A/South Carolina/1/1918	H1N1-A/Puerto Rico/8/1934	H1N1-A/New Caledonia/20/1999	H1N1-A/Califonia/04/2009	H2N2-A/Singapore/1/1957	H2N2-A/Canada/720/2005	H5N1-A/Vietnam/1203/2004	H5N1-A/Indonesia/05/2005	H9N2-A/Hong Kong/1073/1999	H3N2-A/Hong Kong/1/1968	H3N2-A/Beijing/353/1989	H3N2-A/Perth/16/2009	H7N7-A/Netherlands/219/03	H7N9-A/AnHui/1/2013	INF B-Brisbane/60/2008
HV6-1-HD3-3	HV6-1	54.f.01	0.63	3	0.11	0.48	>50	>50	0.089	0.081	>50	0.22	0.0057	0.096	29	0.016	>50
HV6-1-HD3-3	HV6-1	56.a.09	1	1.9	0.074	0.24	>50	>50	0.033	0.17	>50	0.15	0.026	0.1	>50	0.036	>50
HV1-18-HD3-9	HV1-18	01.k.01	2.9	13	0.15	0.75	>50	>50	6.4	8.5	>50	>50	>50	19	>50	>50	>50
HV1-18-HD3-9	HV1-18	31.b.09	1.9	3.2	0.22	1.2	>50	>50	0.92	0.95	>50	>50	>50	1.2	>50	>50	>50
HV1-18 (Q-x-x-V)	HV1-18	16.g.07	2.1	14	0.2	17	>50	>50	0.14	0.14	>50	0.052	0.0016	0.068	>50	0.036	>50
HV1-18 (Q-x-x-V)	HV1-18	54.a.84	0.78	22	0.12	1	>50	>50	0.15	0.14	>50	0.055	0.0023	0.081	4.1	0.035	>50
HV1-18 (Q-x-x-V)	HV1-18	16.a.26	4.6	>50	>50	>50	>50	>50	0.082	0.14	>50	0.062	0.0021	0.052	>50	0.044	>50
HV1-18 (Q-x-x-V)	HV1-18	54.a.39	2.1	>50	0.2	11	>50	>50	0.2	0.21	>50	0.11	0.011	0.096	>50	0.074	>50
	HV3-23	31.a.83	0.45	1.1	0.062	0.25	>50	>50	0.11	0.22	0.31	0.38	0.0064	0.09	>50	0.069	>50
	HV3-23	56.h.01	0.14	1.7	0.059	0.039	>50	5.6	0.062	0.05	0.51	>50	>50	>50	>50	>50	>50
	HV1-69	01.s.01	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
	HV1-69	31.f.01	0.27	1.3	0.062	0.098	>50	>50	0.17	0.33	>50	>50	>50	>50	>50	>50	>50
	HV1-69	56.g.01	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
	HV7-4	54.e.01	>50	>50	>50	1.5	>50	>50	2.4	12	>50	0.14	0.011	0.089	>50	>50	>50
	HV3-23	01.i.01	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
	HV3-30	31.d.01	2.9	20	0.26	0.54	>50	>50	0.52	0.47	3.6	>50	>50	>50	>50	>50	>50
	HV4-34	01.a.44	0.95	4.4	0.088	0.19	>50	>50	0.075	0.16	0.44	6.2	16	0.53	>50	>50	>50
Control antibodies	HV1-69	CR9114	0.19	0.74	0.024	0.096	>50	>50	0.069	0.07	0.12	0.98	6.2	0.16	>50	0.034	>50
	HV3-30	FI6v3	0.49	0.47	0.015	0.15	6.6	6.3	0.084	0.029	1.48	0.016	0.0026	0.047	>50	0.05	>50
	HV1-69	CR6261	0.31	1.6	0.033	0.064	>50	>50	0.13	0.072	0.20	>50	>50	>50	>50	>50	>50
	HV1-18	CR8020	>50	>50	>50	>50	>50	>50	>50	>50	>50	0.81	>50	0.29	>50	0.42	>50
	HV1-2	CH65	1.8	1.7	0.012	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
HV6-1-HD3-3	SRR747760 Heavy 54.f.01 Light		1.4	10	>50	0.47	>50	>50	0.84	20	>50	0.48	0.054	0.3	>50	0.2	>50
HV6-1-HD3-3	NGS Heavy 56.a.09 Light		2.1	25	0.18	0.45	>50	>50	0.7	10	>50	0.4	0.022	0.25	>50	0.14	>50
HV1-18-HD3-9	seq25996 Heavy 31.b.09 Light		2.2	32	0.3	0.92	>50	>50	18	>50	>50	>50	>50	>50	>50	>50	>50
HV1-18-HD3-9	seq25996 Heavy and Light		30	>50	1.9	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50

FIG. 15C

IC_{50} neutralization titers assessed using micro-neutralization assay.

			Group 1 strains				Group 2 strains				
Signature	HV gene	Antibody name	A/ Kawasaki /86(H1N1)	A/Solomon Islands/3/2006 (H1N1)	A/Brisbane/59 /2007 (H1N1)	A/California/04 /2009 (H1N1)	A/Hong Kong/8/68 (H3N2)	A/Christchurch/313/2003 (H3N2)	A/Brisbane/10 /2007 (H3N2)	A/Perth/16 /2009 (H3N2)	A/chicken/Germany /n/1949 (H10N7)
HV6-1-HD3-3	HV6-1	54.f.01	9.64	54.55	63.00	13.30	1.86	1.05	5.04	24.03	>100
HV6-1-HD3-3	HV6-1	56.a.09	19.87	36.81	>100	7.60	1.67	0.13	7.16	14.94	63.00
HV1-18-HD3-9	HV1-18	01.k.01	32.40	>100	>100	40.90	>34.0	0.74	>100	>34.0	>100
HV1-18-HD3-9	HV1-18	31.b.09	91.85	>100	>100	>101.0	>101.0	1.67	>100	>101.0	>100
HV1-18 (Q-x-x-V)	HV1-18	16.g.07	88.96	>100	>100	>105.5	1.75	5.05	1.36	12.90	39.70
HV1-18 (Q-x-x-V)	HV1-18	54.a.84	37.20	>100	>100	>23.5	1.60	61.64	1.88	9.70	31.50
HV1-18 (Q-x-x-V)	HV1-18	16.a.26	>100	>100	>100	>90.5	6.00	>100	0.79	5.48	50.00
HV1-18 (Q-x-x-V)	HV1-18	54.a.39	>100	>100	>100	>179.5	1.56	>100	1.52	3.93	31.50
	HV3-23	31.a.83	31.05	>100	>100	86.60	10.39	5.15	2.52	23.30	50.00
	HV3-23	56.h.01	>100	>100	NT	6.76	>28.5	>100	>100	>28.5	NT
	HV1-69	01.s.01	>100	>100	NT	NT	NT	>100	>100	NT	NT
	HV1-69	31.f.01	14.60	79.99	NT	NT	NT	>100	>100	NT	NT
	HV1-69	56.g.01	>100	>100	NT	NT	NT	>100	>100	NT	NT
	HV7-4	54.e.01	>100	>100	NT	NT	NT	>100	>100	NT	NT
	HV3-23	01.i.01	>100	>100	NT	NT	NT	>100	>100	NT	NT
	HV3-30	31.d.01	>100	>100	NT	1.96	>31.0	>100	>100	>31.0	NT
	HV4-34	01.a.44	>100	>100	NT	35.45	>178.0	>100	>100	>178.0	NT
Control antibodies	HV1-69	CR9114	6.58	7.31	39.70	3.00	9.45	0.78	12.45	NT	63.00
	HV3-30	FI6v3	29.13	75.65	17.70	7.36	1.61	3.62	0.91	NT	>100
	HV1-69	CR6261	9.54	18.06	NT	4.01	>174.5	1.10	>100	NT	NT
	HV1-18	CR8020	>100	>100	NT	>48.5	6.33	>100	2.11	NT	NT
	HV1-2	CH65	0.01	0.02	NT	NT	NT	0.04	>100	NT	NT
HV6-1-HD3-3	HV6-1	NGS-Heavy 56.a.09 Light	22.57	>100	39.70	NT	NT	1.99	7.96	NT	NT

FIG. 15D

IC_{80} neutralization titers assessed using micro-neutralization assay.

			Group 1 strains				Group 2 strains				
gnature	HV gene	Antibody name	A /Kawasaki/ 86 (H1N1)	A/Solomon Islands/3/2006 (H1N1)	A/Brisbane/59 /2007 (H1N1)	A/California/04 /2009 (H1N1)	A/Hong Kong/8/68 (H3N2)	A/Christchurc h/313/2003 (H3N2)	A/Brisbane/10 /2007 (H3N2)	A/Perth/16 /2009 (H3N2)	A/chicken/Germany /n/1949 (H10N7)
V6-1-D3-3	HV6-1	54.f.01	19.66	>100	>100	>87.0	15.1	>100	>100	>87	>100
V6-1-D3-3	HV6-1	56.a.09	23.06	>100	>100	>55.5	41.7	>100	>100	>55.5	>100
/1-18-D3-9	HV1-18	01.k.01	43.18	>100	>100	>50.0	>34.0	>100	>100	>34.0	>100
/1-18-D3-9	HV1-18	31.b.09	98.94	>100	>100	>101.0	>101.0	>100	>100	>101.0	>100
/1-18 -x-x-V)	HV1-18	16.g.07	>100	>100	>100	>105.5	37.15	>100	>100	>105.0	>100
/1-18 -x-x-V)	HV1-18	54.a.84	89.91	>100	>100	>23.5	23.00	>100	>100	>23.5	>100
/1-18 -x-x-V)	HV1-18	16.a.26	>100	>100	>100	>90.5	89.00	>100	31.41	>90.0	>100
/1-18 -x-x-V)	HV1-18	54.a.39	>100	>100	>100	>179.5	7.24	>100	16.76	>100	>100
	HV3-23	31.a.83	47.01	>100	>100	>111.0	111.00	>100	27.62	>111.0	>100
	HV3-23	56.h.01	>100	>100	NT	>28.5	>28.5	>100	>100	>28.5	NT
	HV1-69	01.s.01	>100	>100	NT	NT	NT	>100	>100	NT	NT
	HV1-69	31.f.01	29.76	>100	NT	NT	NT	>100	>100	NT	NT
	HV1-69	56.g.01	>100	>100	NT	NT	NT	>100	>100	NT	NT
	HV7-4	54.e.01	>100	>100	NT	NT	NT	>100	>100	NT	NT
	HV3-23	01.i.01	>100	>100	NT	NT	NT	>100	>100	NT	NT
	HV3-30	31.d.01	>100	>100	NT	>31.0	>31.0	>100	>100	>31.0	NT
	HV4-34	01.a.44	>100	>100	NT	>100.0	>178.0	>100	>100	>178.0	NT
	HV1-69	CR9114	12.18	19.66	>50	48.9	>75	>100	>100	NT	NT
	HV3-30	FI6v3	66.06	>100	>50	>50.0	28.2	>100	6.79	NT	NT
Control ıtibodies	HV1-69	CR6261	16.06	53.40	NT	43.6	>174.5	>100	>100	NT	NT
	HV1-18	CR8020	>100	>100	NT	>48.5	>48.5	>100	61.32	NT	NT
	HV1-2	CH65	0.01	0.03	NT	NT	NT	>100	>100	NT	NT
V6-1-D3-3	HV6-1	NGS-Heavy 56.a.09 Light	61.32	>100	>100	NT	NT	>100	34.17	NT	NT

FIG. 16

Crystallographic data collection and refinement statistics.

	56.a.08 Fab	56.a.08 Fab with A/Hong Kong/1-4-MA21-1/1968 (H3N2)	31.b.09 Fab with A/California/04/2009 (H1N1)	16.a.16 Fab with A/Hong Kong/1-4-MA21-1/1968 (H3N2)	16.g.07 Fab with A/Hong Kong/1-4-MA21-1/1968 (H3N2)	31.a.83 Fab with A/Hong Kong/1-4-MA21-1/1968 (H3N2)
Data collection						
Growth condition	0.1M Na acetate, pH 5.5, 25% (w/v) PEG-400, 11% (w/v) PEG-8000	0.28M ammonium citrate, pH 8.5 12.5% (w/v) PEG-8000	15% PEG-3350, 0.1M $MgCl_2$, 0.1M imidazole, pH 6.5, 1.75M NaCl	0.89M Na cacodylate, pH 6.5, 0.18M Mg acetate, 7.9% (w/v) PEG-8000, 0.01M L-cysteine	0.09 M MES, pH 6.0, 12% (w/v) PEG-6000, 0.018M TCEP HCl	0.2M Tris, pH 8.5, 5% (w/v) PEG-8000 20% (v/v) PEG-300 10% glycerol
Space group	$P2_13_12$	$C222_1$	P622	$P3_12_12_6$	$P2_12_12_1$	C2
Cell Constants						
a, *b*, *c* (Å)	78.8, 103.8, 60.0	123.9, 136.6, 311.4	208.65 208.65 252.03	121.81. 233.91. 302.45	128.85, 147.56, 209.79	279.44 154.28 157.94
α, β, γ (°)	90.0, 90.0, 90.0	90.0, 90.0, 90.0	90.0 90.0 120.0	90.0, 90.0, 90.0	90.0, 90.0, 90.0	90.0 116.9 90.0
Wavelength (Å)	1.000	1.000	1.000	1.000	1.000	1.000
Resolution (Å)	50.0 - 2.16 (2.54 - 2.16)	50.0 – 2.97 (3.07 – 2.97)	50.0 – 3.52 (3.65 – 3.52)	50.0 - 2.5 (2.59 - 2.50)	50.0 – 2.79 (2.88 - 2.79)	50.0 – 3.51 (3.64 – 3.51)
R_{merge}	0.0712 (0.2186)	0.1617 (0.4148)	0.156 (0.486)	0.1179 (0.3704)	0.2231 (0.5166)	0.127 (0.589)
$CC_{1/2}$	0.996 (0.972)	0.995 (0.734)	0.995 (0.625)	0.992 (0.756)	0.929 (0.540)	--
No. reflections	325361 (9282)	196829 (3758)	192537 (1263)	994583 (23786)	371042 (3214)	678687
No. unique reflections	18309 (1610)	38347 (2147)	28978 (635)	245485 (13867)	83716 (2466)	58736 (5630)
$I/\sigma I$	18.29 (7.41)	13.74 (2.06)	11.75 (2.38)	14.05 (2.07)	4.69 (1.26)	13.0 (1.5)
Completeness (%)	99.0 (89.0)	91.40 (52.16)	71.0 (18.0)	82.55 (47.16)	83.27 (24.86)	78.94 (30.26)
Redundancy	6.8 (5.8)	5.2 (1.7)	6.6 (1.7)	4.1 (1.7)	4.4 (1.3)	2.1 (1.6)
Refinement						
Resolution (Å)	32.93 - 2.46 (2.54 - 2.46)	46.82 – 2.97 (3.07 – 2.97)	38.70 – 3.52 (3.65 – 3.52)	49.28 - 2.5 (2.582 - 2.503)	40.67 – 2.79 (2.88-2.79)	41.88 - 3.51 (3.64 – 3.51)
CC*	0.999 (0.993)	0.999 (0.917)	0.999 (0.877)	0.998 (0.926)	0.981 (0.837)	--
R_{work} / R_{free}	0.225 (0.288)/ 0.265 (0.336)	0.243 (0.357)/ 0.277 (0.480)	0.281 (0.348)/0.336 (0.448)	0.188 (0.277)/ 0.220 (0.312)	0.219 (0.345)/ 0.243 (0.389)	0.272 (0.354)/ 0.324 (0.363)
No. atoms	3389	14928	14057	46342	22144	21918
Protein	3319	14363	14018	43123	21758	21666
Ligand/ion	4	555	39	504	238	252
Water	134	0	0	1715	148	0
B-factors						
Protein	53.5	108.40	102.80	62.50	71.60	158.9
Ligand/ion	55.4	181.50	67.22	105.10	94.70	195.5
Water	45.9	N/A	N/A	45.50	45.30	N/A
R.m.s. deviations						
Bond lengths (Å)	0.004	0.011	0.004	0.006	0.004	0.003
Bond angles (°)	0.817	1.72	0.841	1.05	0.973	0.797
Ramachandran						
Favored (%)	97.5	91.6	94.3	94.7	94.9	91.1
Allowed (%)	2.5	4.2	3.6	4.2	4.4	7.2
Disallowed (%)	0.0	2.2	2.1	1.1	0.7	1.7

Values in parentheses are for highest-resolution shell

FIG. 17A

Light chain

Heavy chain	Donor 31	Donor 54	Donor 56
	31.g.01	54.f.01	56.a.09
31.g.01	No expression	No expression	No expression
54.f.01	Binding and competition	Cross-neutralization	Binding and competition
56.a.09	Binding and competition	Binding and competition	Cross-neutralization

Light chain

Heavy chain	Donor 1	Donor 31
	01.k.01	31.b.09
01.k.01	Cross neutralization	Binding and competition
54.f.01	Binding and competition	Cross-neutralization

Light chain

Heavy chain	Donor 16		Donor 54		SH-K1
	16.a.26	16.g.07	54.a.39	54.a.84	CT149
16.a.26	Cross-neutralization	Binding and competition	No binding	Binding and competition	No binding
16.g.07	No binding	Cross-neutralization	No binding	No binding	No binding
54.a.39	Binding and competition	No binding	Cross-neutralization	Binding and competition	No binding
54.a.84	Binding and competition	No binding	Binding and competition	Cross-neutralization	No binding
CT149	No binding	No binding	No binding	No binding	Cross-neutralization

Donor SH-K1 was from Severance Hospital, Korea (Wu et al., 2015)

FIG. 17B

	16.a.26 (21)	16.g.07 (21)	54.a.39 (21)	54.a.84 (21)	CT149 (19)
16.a.26 (21)	1	0.711	0.781	0.781	-
16.g.07 (21)	0.711	1	0.664	0.672	-
54.a.39 (21)	0.781	0.664	1	0.805	-
54.a.84 (21)	0.781	0.672	0.805	1	-
CT149 (19)	-	-	-	-	1

FIG. 17C

	16.a.26	16.g.07	54.a.39	54.a.84	CT149
HV1-18+QxxV.1.SRP026397	No binding	No binding	No binding	No binding	No binding
HV1-18+QxxV.1.SRP015957	No expression	No binding	No binding	No binding	No binding
HV1-18+QxxV.2.SRP015957	No binding	No binding	No binding	No binding	No binding
HV1-18+QxxV.3.SRP015957	No expression	No binding	No binding	No binding	No binding

FIG. 17D

	16.a.26 (21)	16.g.07 (21)	54.a.39 (21)	54.a.84 (21)	CT149 (19)
HV1-18+QxxV.1.SRP026397 (18)	-	-	-	-	-
HV1-18+QxxV.1.SRP015957 (19)	-	-	-	-	0.758
HV1-18+QxxV.2.SRP015957 (19)	-	-	-	-	0.773
HV1-18+QxxV.3.SRP015957 (19)	-	-	-	-	0.773

FIG. 18

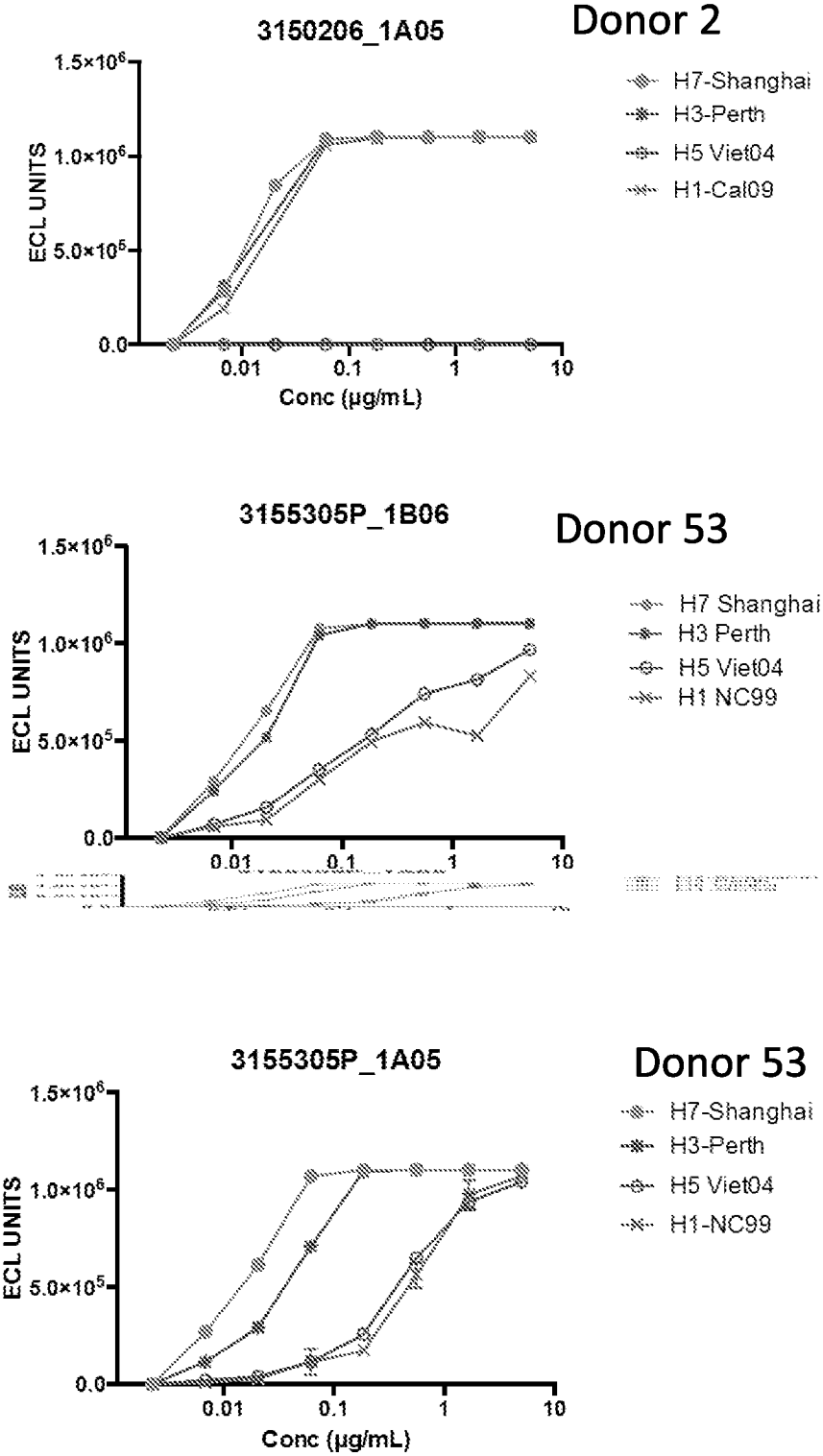

3150206_1A05
Donor 2
ECL UNITS
1.5×10^6
1.0×10^6
5.0×10^5
0.0
0.01
0.1
1
10
Conc (µg/mL)
H7-Shanghai
H3-Perth
H5 Viet04
H1-Cal09
3155305P_1B06
Donor 53
ECL UNITS
H7 Shanghai
H3 Perth
H5 Viet04
H1 NC99
3155305P_1A05
Donor 53
ECL UNITS
Conc (µg/mL)
H7-Shanghai
H3-Perth
H5 Viet04
H1-NC99

FIG. 19

	Group 1									
	H1					H2		H5		H9
mAbs	SC1918	PR8	NC99	SI06	CA09	SG57	CAN05	VN04	IND05	HK99
3155305P_1A05	0.395	0.783	1.225	0.66	0.281	0.463	2.449	0.064	1.327	0.23
3155305P_1B06	0.36	0.626	0.171	0.076	0.055	0.184	0.407	0.013	0.492	0.007
CR9114	0.045	0.017	0.046	0.029	0.05	>50	>50	<0.0032	0.17	<0.0032
FI6V3	0.116	0.032	0.141	0.243	0.176	0.249	1.382	<0.0032	0.123	<0.0032
CR6261	0.035	0.096	0.07	0.099	0.061	0.429	2.528	<0.0032	0.081	<0.0032
CR8020	>50	>50	>50	>50	>50	>50	>50	7.52	>50	10.366

	Group 2							B
	H3					H7		
mAbs	HK68	Beijing89	Perth09	Texas12	SW13	Nether03	Anhui13	Bris08
3155305P_1A05	0.087	>50	0.057	0.014	0.025	4.033	0.181	>50
3155305P_1B06	0.016	0.007	0.032	0.016	0.013	3.744	0.095	>50
CR9114	0.082	0.771	0.019	0.023	0.137	1.371	0.006	12.046
FI6V3	<0.0032	<.0032	0.004	<0.0032	0.008	>50	<0.0032	>50
CR6261	>50	>50	>50	>50	>50	>50	>50	>50
CR8020	0.164	4.157	0.039	0.022	0.17	>50	0.052	>50

CR9114	anti-stem_Gr1+2 and B
FI6V3	anti-stem_Gr1+2
CR6261	anti-stem_Gr1
CR8020	anti-stem_Gr2

FIG. 20A

6-1/3-3 class VH sequences

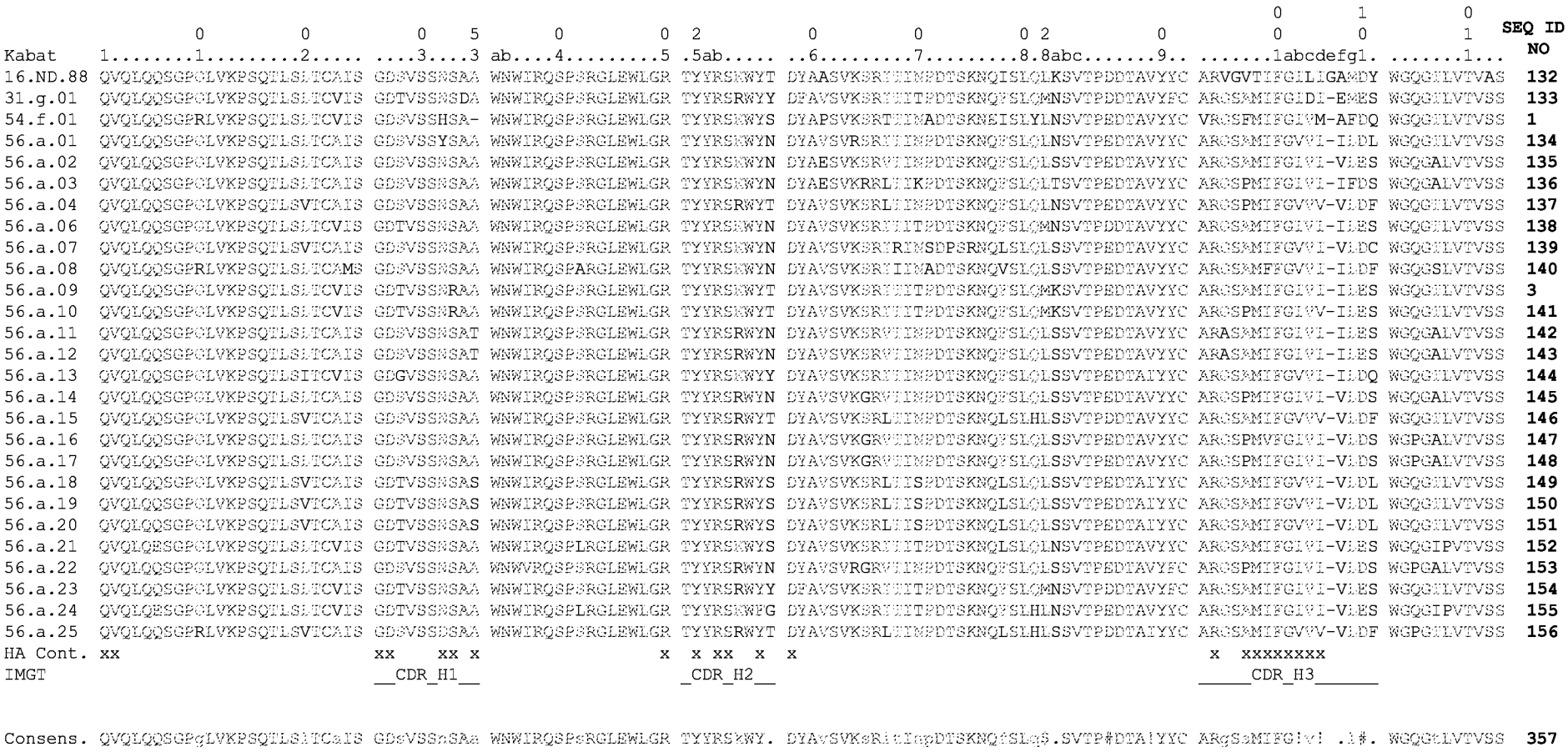


FIG. 20B

6-1/3-3 class VL sequences

	0 0	7 0	0	0	0 0 0	0	0 0	SEQ ID
Kabat	1........1.........2......	2a..3..	.......4.........	5..	.......6.........7.........8........	.9.......	..1........	NO
31.g.01	EIVMTQSSNTLSLSPGERTGISCRVS	QSVVRVY	LDWYRKEPGLATGFFIK	GVS	NRASDVPDRFSGRGFGTEYIFTISRVEQGDFAVYYC	QQYDVSRYT	FGQGTKVEIK	157
54.f.01	EIVLTQSPGTLSLSPGERATLSCRAS	QSVSSSY	LAWYQQKPGQSPRLLIY	GTS	TRATGIPDRFSGSGSGTEFTLTITRLEPEDFAVYYC	QQFDGSHFT	FGPGTKVDIK	2
56.a.01	EIVLTQSPVSLSLSPGERATLSCRAS	QSVSSSY	LAWYQQKPGQAPRLLIY	GTS	YRATGIPDRFSGSGSGTDFTLTISRLEPSDFAVYYC	QQFGSSQYT	FGQGTKLEIK	158
56.a.02	EIVLTQSPVSLSLSPGERATLSCRAS	RSVGSSY	LAWYQQKPGQAPRLLIF	GAS	SRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYC	QQFDGSHYT	FGRGTKLEIK	159
56.a.03	EIVLTQSPVSLSLSPGERATLSCRAS	QSVSSSY	LAWYQQKPGQAPRLLIF	GAS	TRANGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYC	QQFDGSHYT	FGRGTKLEIK	160
56.a.04	EIVLTQSPVSLSLSPGERATLSCRAS	QSVSSSY	LAWYQQRPGQAPRLLIF	GAS	NRATGIPDRFSGSGSGTDFTLTISRLEPGDFAVYYC	QQFTGSHYT	FGRGTKLEIK	161
56.a.05	EIVLTQSPVSLSLSPGERATLSCRAS	QSVASSY	LAWYQQKPGQAPRLLIY	GAS	NRATGVPDRFSGSGSGTDFILTISRLEPEDFAVYYC	QQYDGSQYT	FGQGTKLEIK	162
56.a.06	EIVLTQSPVSLSLSPGERATLSCRAS	QSVASSY	LAWYQQKPGQAPRLLIY	GAS	NRATGVPDRFSGSGSGTDFILTISRLEPEDFAVYYC	QQHDGSQYT	FGQGTKLEIK	163
56.a.07	EIVLTQSPVSLSLSPGERATLSCRAS	QSVSSSY	LTWYQQKPGQAPRLLIF	GAS	SRASGVPDRFSGSGSGTDFTLTISRLEPSDFAVYYC	QQFDGSHYT	FGRGTKLEIK	164
56.a.08	EIVLTQSPVSLSLSPGERATLSCRAS	QSVSSSY	LAWYQQKPGQAPRLLIY	GAS	SRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYC	QQFGGSHYT	FGRGTKLEIK	165
56.a.09	EIVLTQSPGTLSLSPGERATLSCRAS	QSVASSY	LAWYQQKPGQAPRLLIY	GAS	SRATGVPDRFSGSGSGTDFILTISRLEPEDFAVYYC	QQYDGSQYT	FGQGTKLEIK	166
56.a.10	EIVLTQAPVTLSLSPGERATLSCRAS	QSVASSY	LAWYQQKPGQAPRLLIY	GAS	SRATGVPDRFSGSASGTDFILTISRLEPSDFAVYYC	QQYDGSQYT	FGQGTKLEIK	167
56.a.11	EIVLTQSPVSLSLSPGERATLSCRAS	QSVSSSY	LAWYQQKPGQAPRLLIF	GAS	SRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYC	QQFDGSHYT	FGRGTKLEIK	168
56.a.12	EIVLTQSPVTLSLSPGERATLSCRAS	QSVSSSY	LAWYQQKPGQAPRLLIF	GAS	SRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYC	QQFTGSHYT	FGRGTKLEIK	169
56.a.13	EIVLTQSPVSLSLSPGERATLSCRAS	QSVSSSY	LAWYQQKPGQAPRLLIY	GAS	TRATGIPDRFSGSGSGTDFTLTISRLEPSDFAMYYC	QQFGGSHYT	FGRGTKLEIK	170
56.a.14	EIVLTQSPVTLSLSPGERATLSCRAS	QSVSSSY	LAWYQQKPGQAPRLLIF	GAS	SRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYC	QQFDGSHYT	FGRGTKLEIK	171
56.a.15	EIVLTQSPVTLSLSPGERATLSCRAS	QSVSSSY	LAWYQQRPGQAPRLLIF	GAS	SRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYC	QQFTGSQYT	FGRGTKLEIK	172
56.a.16	EIVLTQSPVSLSLSPGERATLSCRAS	QSVSSSY	LAWYQQKPGQAPRLLIF	GAS	SRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYC	QQFDGSHYT	FGRGTKLEIK	173
56.a.17	EIVLTQSPVSLSLSPGERATLSCRAS	QSVSSSY	LAWYQQKPGQAPRLLIF	GAS	SRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYC	QQFDGSHYT	FGRGTKLEIK	174
56.a.18	EIVLTQSPVSLSLSPGERATLSCRAS	QSVSSSY	LAWYQQRPGQAPRLLIF	GAS	SRAPGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYC	QQFTGSHYT	FGRGTKLEIK	175
56.a.19	EIVLTQSPVSLSLSPGERATLSCRAS	QSVSSSY	LAWYQQRPGQAPRLLIF	GAS	SRAPGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYC	QQFDGSHYT	FGRGTKLEIK	176
56.a.20	EIVLTQPPVSLSLSPGERATLSCRAS	QSVSSSY	LAWYQQRPGQAPRLLIF	GAS	SRAPGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYC	QQFDGSHYT	FGRGTKLEIK	177
56.a.21	EIVLTQSPVSLSLSPGERATLSCRAS	QSVASSY	LAWYQQKPGQAPRLLIY	GAS	SRATGVPDRFSGSGSGTDFILTISRLEPSDFAVYYC	QQYTGSQYT	FGQGTKLEIK	178
56.a.22	EIVMTQSSVSLSLSPGERATLSCRAS	QSVSSSY	LAWYQQKPGQAPRLLIF	GAS	SRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYC	QQFDGSHYT	FGRGTKLEIK	179
56.a.23	EIVLTQSPVSLSLSPGERATLSCRAS	QSVASSY	LAWYQQKPGQAPRLLIY	GVS	NRATGVPDRFSGSGSGTDFILTISRLEPGDFAVYYC	QQYDGSQYT	FGQGTKLEIK	180
56.a.24	EIVLTQSPVSLSLSPGERATLSCRAS	QSVASSY	LAWYQQKPGQAPRLLIY	GAS	FRATGVPDRFSGSGSGTDFILTISRLEPSDFAVYYC	QQFTGSQYT	FGQGTKLEIK	181
56.a.25	EIVLTQSPVSLSLSPGERATLSCRAS	QSVSSSY	VAWYQQRPGQAPRLLIF	GAS	SRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYC	QRFDGSQYT	FGRGTKLEIK	182
HA Cont.	x	xx xxxx	x		x	xxxx x		
IMGT		_CDRH1_		H2_		_CDR_H3__		
Consens.	EIV$TQSpvsLSLSPGERatlSCRaS	QSVsssY	LaWYqqkPGqAprllI.	GaS	.RAtg!PDRFSGsGsGT#%tlTISRlEpeDFAVYYC	QQ%dgS.YT	FGqGTKlEIK	358

FIG. 20C

1-18/3-9 class VH sequences

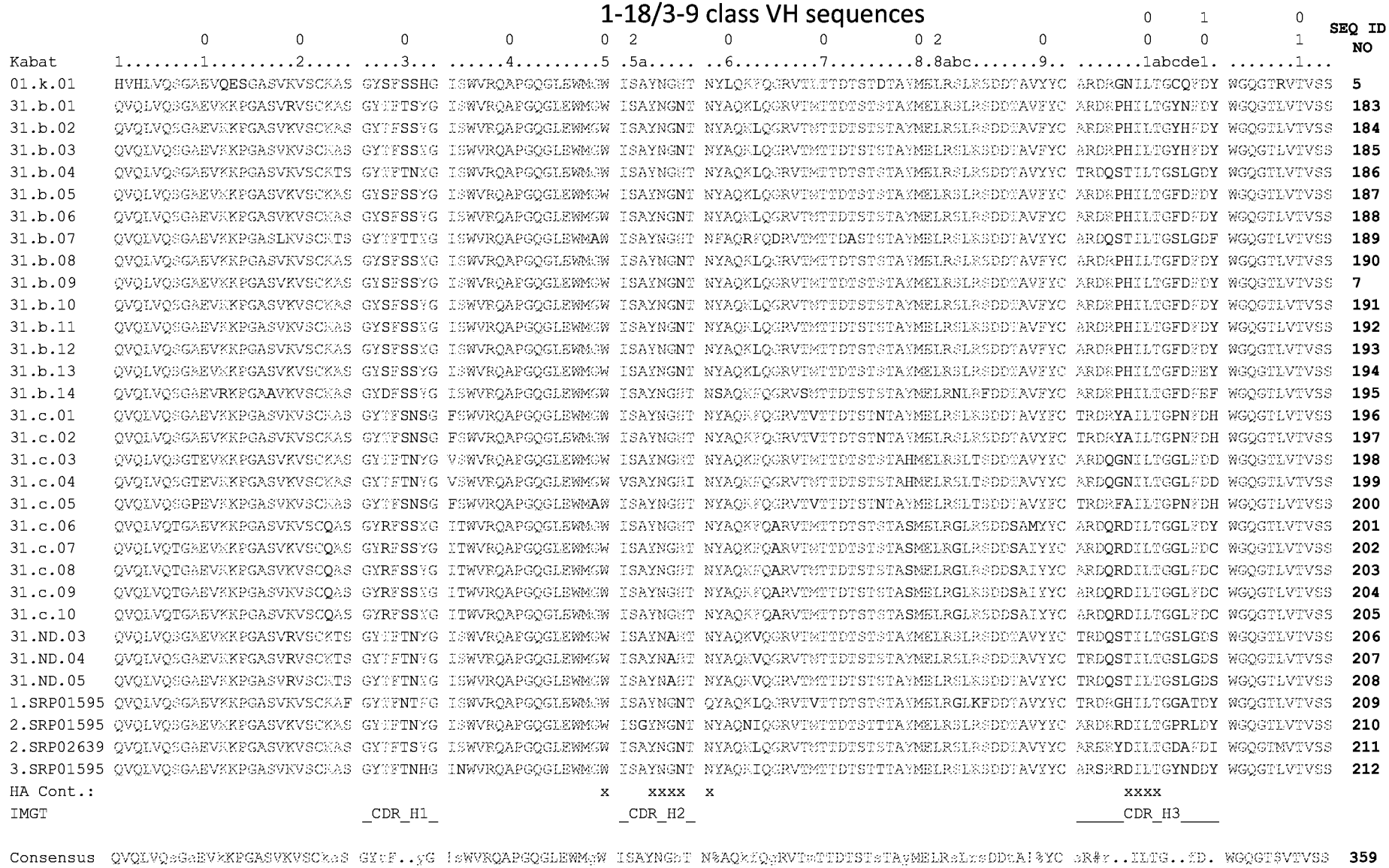
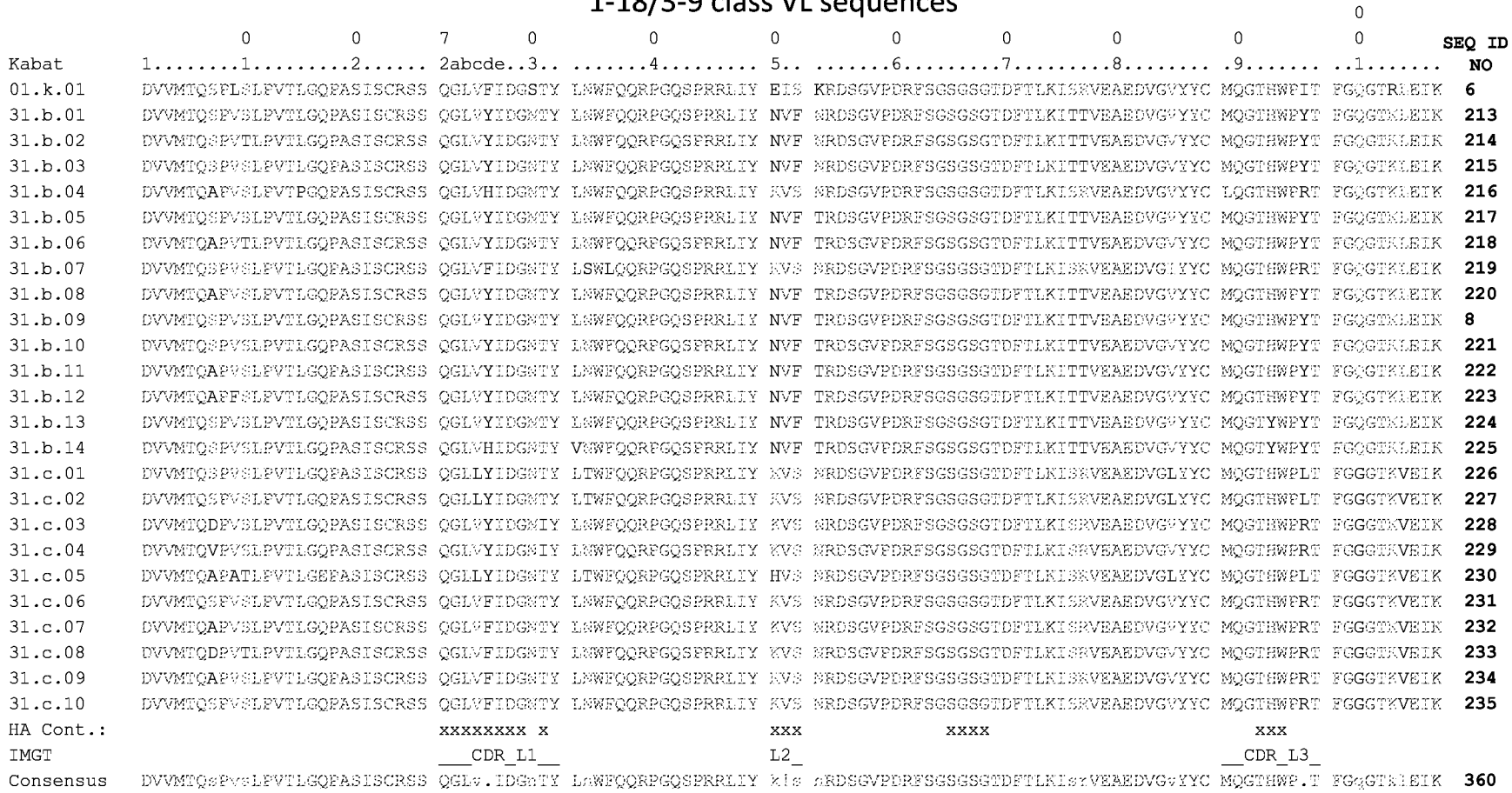


FIG. 20D

1-18/3-9 class VL sequences



		SEQ ID NO
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31.b.01	DVVMTQSPVSLPVTLGQPASISCRSS QGLVYIDGNTY LNWFQQRPGQSPRRLIY NVF NRDSGVPDRFSGSGSGTDFTLKITTVEAEDVGVYYC MQGIHWPYT FGQGTKLEIK	213
31.b.02	DVVMTQSPVTLPVTLGQPASISCRSS QGLVYIDGNTY LNWFQQRPGQSPRRLIY NVF NRDSGVPDRFSGSGSGTDFTLKITTVEAEDVGVYYC MQGIHWPYT FGQGTKLEIK	214
31.b.03	DVVMTQSPVSLPVTLGQPASISCRSS QGLVYIDGNTY LNWFQQRPGQSPRRLIY NVF NRDSGVPDRFSGSGSGIDFTLKITTVEAEDVGVYYC MQGTHWPYT FGQGTKLEIK	215
31.b.04	DVVMTQAPVSLPVTPGQPASISCRSS QGLVHIDGNTY LNWFQQRPGQSPRRLIY KVS NRDSGVPDRFSGSGSGIDFTLKISRVEAEDVGVYYC LQGTHWPRT FGQGTKLEIK	216
31.b.05	DVVMIQSPVSLPVTLGQPASISCRSS QGLVYIDGNTY LNWFQQRPGQSPRRLIY NVF TRDSGVPDRFSGSGSGTDFTLKITTVEAEDVGVYYC MQGIHWPYT FGQGTKLEIK	217
31.b.06	DVVMTQAPVTLPVTLGQPASISCRSS QGLVYIDGNTY LNWFQQRPGQSPRRLIY NVF TRDSGVPDRFSGSGSGTDFTLKITTVEAEDVGVYYC MQGIHWPYT FGQGTKLEIK	218
31.b.07	DVVMTQSPVSLPVTLGQPASISCRSS QGLVFIDGNTY LSWLQQRPGQSPRRLIY KVS NRDSGVPDRFSGSGSGIDFTLKISRVEAEDVGIYYC MQGTHWPRT FGQGTKLEIK	219
31.b.08	DVVMTQAPVSLPVTLGQPASISCRSS QGLVYIDGNTY LNWFQQRPGQSPRRLIY NVF TRDSGVPDRFSGSGSGIDFTLKITTVEAEDVGVYYC MQGTHWPYT FGQGTKLEIK	220
31.b.09	DVVMIQSPVSLPVTLGQPASISCRSS QGLVYIDGNTY LNWFQQRPGQSPRRLIY NVF TRDSGVPDRFSGSGSGTDFTLKITTVEAEDVGVYYC MQGIHWPYT FGQGTKLEIK	8
31.b.10	DVVMTQSPVSLPVTLGQPASISCRSS QGLVYIDGNTY LNWFQQRPGQSPRRLIY NVF TRDSGVPDRFSGSGSGTDFTLKITTVEAEDVGVYYC MQGIHWPYT FGQGTKLEIK	221
31.b.11	DVVMTQAPVSLPVTLGQPASISCRSS QGLVYIDGNTY LNWFQQRPGQSPRRLIY NVF TRDSGVPDRFSGSGSGIDFTLKITTVEAEDVGVYYC MQGTHWPYT FGQGTKLEIK	222
31.b.12	DVVMTQAPFSLPVTLGQPASISCRSS QGLVYIDGNTY LNWFQQRPGQSPRRLIY NVF TRDSGVPDRFSGSGSGIDFTLKITTVEAEDVGVYYC MQGTHWPYT FGQGTKLEIK	223
31.b.13	DVVMIQSPVSLPVTLGQPASISCRSS QGLVYIDGMTY LNWFQQRPGQSPRRLIY NVF TRDSGVPDRFSGSGSGTDFTLKITTVEAEDVGVYYC MQGIYWPYT FGQGTKLEIK	224
31.b.14	DVVMIQSPVSLPVILGQPASISCRSS QGLVHIDGNTY VNWFQQRPGQSPRRLIY NVF TRDSGVPDRFSGSGSGTDFTLKITTVEAEDVGVYYC MQGIYWPYT FGQGTKLEIK	225
31.c.01	DVVMTQSPVSLPVTLGQPASISCRSS QGLLYIDGNTY LTWFQQRPGQSPRRLIY KVS NRDSGVPDRFSGSGSGIDFTLKISRVEAEDVGLYYC MQGTHWPLT FGGGTKVEIK	226
31.c.02	DVVMTQSPVSLPVTLGQPASISCRSS QGLLYIDGNTY LTWFQQRPGQSPRRLIY KVS NRDSGVPDRFSGSGSGIDFTLKISRVEAEDVGLYYC MQGTHWPLT FGGGTKVEIK	227
31.c.03	DVVMTQDPVSLPVTLGQPASISCRSS QGLVYIDGMIY LNWFQQRPGQSPRRLIY KVS NRDSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYC MQGIHWPRT FGGGTKVEIK	228
31.c.04	DVVMIQVPVSLPVTLGQPASISCRSS QGLVYIDGNIY LNWFQQRPGQSPRRLIY KVS NRDSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYC MQGTHWPRT FGGGTKVEIK	229
31.c.05	DVVMTQAPATLPVTLGEPASISCRSS QGLLYIDGNTY LTWFQQRPGQSPRRLIY HVS NRDSGVPDRFSGSGSGIDFTLKISRVEAEDVGLYYC MQGTHWPLT FGGGTKVEIK	230
31.c.06	DVVMTQSPVSLPVTLGQPASISCRSS QGLVFIDGNTY LNWFQQRPGQSPRRLIY KVS NRDSGVPDRFSGSGSGIDFTLKISRVEAEDVGVYYC MQGTHWPRT FGGGTKVEIK	231
31.c.07	DVVMTQAPVSLPVTLGQPASISCRSS QGLVFIDGNTY LNWFQQRPGQSPRRLIY KVS NRDSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYC MQGIHWPRT FGGGTKVEIK	232
31.c.08	DVVMTQDPVTLPVTLGQPASISCRSS QGLVFIDGNTY LNWFQQRPGQSPRRLIY KVS NRDSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYC MQGTHWPRT FGGGTKVEIK	233
31.c.09	DVVMTQAPVSLPVTLGQPASISCRSS QGLVFIDGNTY LNWFQQRPGQSPRRLIY KVS NRDSGVPDRFSGSGSGIDFTLKISRVEAEDVGVYYC MQGTHWPRT FGGGTKVEIK	234
31.c.10	DVVMTQSPVSLPVTLGQPASISCRSS QGLVFIDGNTY LNWFQQRPGQSPRRLIY KVS NRDSGVPDRFSGSGSGIDFTLKISRVEAEDVGVYYC MQGTHWPRT FGGGTKVEIK	235
HA Cont.:	xxxxxxxx x xxx xxxx xxx	
IMGT	___CDR_L1__ L2_ __CDR_L3_	
Consensus	DVVMTQsPvsLPVTLGQPASISCRSS QGLv.IDGnTY LnWFQQRPGQSPRRLIY k!s nRDSGVPDRFSGSGSGIDFTLKIsrVEAEDVGvYYC MQGTHWP.T FGqGTk!EIK	360

FIG. 20E - 1-18 (QxxV) class VH sequences

```
                                                                                                       0          1         0
                   0        0         0         0         0 2        0         0        0 2         0         0          0         1   SEQ ID
Kabat     1........1.........2.....  ....3...  ......4.........5  .5a.....  ..6.........7.........8.8abc.......9..  .......1abcdefghijk1.  .......1...   NO
01.j.01   QVQLVQSGAEVKKPGASVKVSCKPS GYTFTNYG ISWVRQAPGQGLEWMGW ISAYTGNT NYAQKFQGRVTMSTDTSTSTAYMELRSLRSDDTAVYYC ARDKVQGFV---SYYYG-LDV WGQGTTVTVSS 236
01.j.02   QVQLVQSGAEVKKPGASVKVSCKAS GYSFNTYG ISWVRQAPGQGLEWMGW ISAYTGYT KYAQKFQDRVTMTTDTSTSTAYMEVRSLRSDDSAVYYC ARDEVQGFV---SRYYG-LDV WGQGTTVTVSS 237
01.j.03   QVQLVQSGAEVKKPGASVKVSCKAS GYSLNTYG ISWVRQAPGQGLEWMGW ISAYTGNT KYAQKFQDRVTMTTDTSTNTAYMELRSLRSDDSAVYYC ARDEVQGYV---SRYYG-MDV WGQGTTVTVSS 238
16.g.07   QVQLAQSENELKKPGASVKVSCKTS GYTFTRFG MSWVRQAPGQGLEWMGW ISGYTGDT KYARSFQGRLTLTTDTSTGTAYMELRSLRSDDTAIYYC VRSRVQMEVSPATQSTWYMDL WGRGTLVSVSS 9
16.a.26   QVQLVQSGPEVKKPGASVKVSCKAS GYSFSRYG ISWVRQAPGQGLEWLGW ISGYTGNT NYAQKFQGRVTMTTDTSTSTASMELRSLRSDDTAVYYC ARDKKQGEVVLPAASFRWFAP WGQGTLVTVSS 13
16.a.27   QVQLVQSGAEVKRPGASVKVSCKAS GYTFSSFG ISWVRQAPGQGLEWLGW ISGYTGNT DYAQKFQGRVTMTTDTSTNTAYMELRSLRSDDTAVYYC ARDKKQGEVVLPAASFRWFAP WGQGTLVTVSS 239
16.a.28   QVQLVQSGAEVKRPGASVKVSCKAS GYTFSSFG ISWVRQAPGQGLEWLGW ISGYTGNT DYAQKFQGRVTMTTDTSTNTAYMELRSLRSDDTAVYYC ARDKKQGEVVLPAASFRWFAP WGQGTLVTVSS 240
16.a.29   QVQLVQSGAEVKRPGASVKVSCKAS GYTFSSFG ISWVRQAPGQGLEWLGW ISGYTGNT DYAQKFQGRVTMTTDTSTNTAYMELRSLRSDDTAVYYC ARDKKQGEVVLPAASFRWFAP WGQGTLVTVSS 241
16.a.30   QVQLVQSGAEVKRPGASVKVSCKAS GYTFSSFG ISWVRQAPGQGLEWLGW ISGYTGNT DYAQKFQGRVTMTTDTSTNTAYMELRSLRSDDTAVYYC ARDKKQGEVVLPAASFRWFAP WGQGTLVTVSS 242
16.a.31   QVQLVQSGAEVKRPGASVKVSCKAS GYTFSSFG ISWVRQAPGQGLEWLGW ISGYTGNT DYAQKFQGRVTMTTDTSTNTAYMELRSLRSDDTAVYYC ARDKKQGEVVLPAASFRWFAP WGQGTLVTVSS 243
16.a.32   QVQLVQSGAEVKKPGASVKVSCKAS SYSFTSYG ISWVRQVPGQGLEWLGW ISAYTGNT EYAQKFQGRVTMTTDTSTNTAYMELRSLRSDDTAVYYC ARDKKQGEVVLPAASFRWFAP WGQGTLVTVSS 244
16.a.33   QVQLVQSGAEVKRPGASVKVSCKAS GYTFSSFG ISWVRQAPGQGLEWLGW ISGYTGNT DYAQKFQGRVTMTTDTSTNTAYMELRSLRSDDTAVYYC ARDKKQGEVVLPAASFRWFAP WGQGTLVTVSS 245
16.a.34   QVQLVQSGAEVKRPGASVKVSCKAS GYTFSSFG ISWVRQAPGQGLEWLGW ISGYTGNT DYAQKFQGRVTMTTDTSTNTAYMELRSLRSDDTAVYYC ARDKKQGEVVLPAASFRWFAP WGQGTLVTVSS 246
16.a.35   QVQLVQSGAEVKRPGASVKVSCKAS GYTFSSFG ISWVRQAPGQGLEWLGW ISGYTGNT DYAQKFQGRVTMTTDTSTNTAYMELRSLRSDDTAVYYC ARDKKQGEVVLPAASFRWFAP WGQGTLVTVSS 247
16.a.36   QVQLVQSGAEVKRPGASVKVSCKAS GYTFTSFG ISWVRQAPGQGLEWLGW ISGYTGNT DYAQKFQGRVTMTTDTSTNTAYMELRSLRSDDTAVYYC ARDRKQGEVVLPAASFRWFAP WGQGTLVTVSS 248
16.a.37   QVQLVQSGAEVKKPGASVKVSCKAS GYTFTRFG ITWVRQAPGQGLEWMGW ISAYTGNT DYSQKFQGRVTMSTDTSTNTAYMELRSLTADDTAVYYC ARDKKQGEVVLPAASFRWFAP WGQGTLVTVSS 249
16.a.38   QVQLVQSGAEVKRPGASVKVSCKAS GYTFTSFG ISWVRQAPGQGLEWLGW ISGYTGNT DYAQNFQGRVTMTTDTSTNTAYMELRSLKSDDTAVYYC ARDKKQGEVVLPAASFRWFAP WGQGTLVTVSS 250
16.a.39   QVQLVQSGAEVKRPGASVKVSCKAT GYTFTSFG ISWVRQAPGQGLEWLGW ISGYTGNT DYAQKFQGRVTMTTDTSTNTAYMELRSLRSDDTAVYYC ARDKKQGEVVLPAASFRWFAP WGQGTLVTVSS 251
16.a.40   QGQLAQSGAEVKRPGASVKVSCKAS GYTFTSFG ISWVRQAPGQGFEWLGW ISGYTGNT DYAQKFQGRVTMTTDTSTNTAYMELRSLRSDDTAVYYC ARDKKQGEVVLPAASFRWFAP WGQGTLVTVSS 252
16.a.41   QVQLVQSGAEVKRPGASVKVSCKAS GYTFTSFG ISWVRQAPGQGLEWLGW ISGYTGNT NYAQKFQGRLTMTTDTSTNTAYMELRSLRYDDTALYYC ARDKKQGEVVLPAASFRWLAP WGQGTLVTVSS 253
16.a.42   QGQLAQSGAEVKRPGASVKVSCKAS GYTFTSFG ISWVRQAPGQGFEWLGW ISGYTGNT DYAQKFQGRVTMTTDTSTNTAYMELRSLRSDDTAVYYC ARDKKQGEVVLPAASFRWFAP WGQGTLVTVSS 254
16.a.43   QVQLVQSGGEVKRPGASVKVSCKAS GYTFTSFG ISWVRQAPGQGLEWLGW ISGYTGNT DYAQKFQGRVTMTTDTSTKTAYMELRSLRSDDTAVYYC ARDKKQGEVVLPAASFRWFAP WGQGTLVTVSS 255
16.a.44   QVQLVQSGADVKRPGASVKVSCKAS GYSFTRFG ISWVRQAPGQGLEWLGW ISGYTGNT DYAQKFQGRVTMTTDTSTNTAYMELRSLRSDDTAVYYC ARDKKQGEVVLPAASFRWFAP WGQGTLVTVSS 256
16.a.45   QVQLVQSGAEVKKPGASVKVACKAS TYSFTSYG ISWVRQVPGQGLEWLGW ISAYTGNT EYAQKFQGRVTMTTDTSTNTAYMELRSLRSDDTAVYYC ARDKKQGEVVLPAASFRWFAP WGQGTLVTVSS 257
36.g.01   QVQLVQSEAEVKKPGASVKVSCKAS GYTFTSYG ISWVRQAPGQGLEWMGW ISAYTGNT NSAQKLQGRVTMTTDTSTSTAYMELRSLRPDDTAVYYC ARGLLQGVVIYDYSYYT-MDV WGQGTTVTVSS 258
36.g.02   QVQLVQSGAEVKRPGALVKVSCKAS GYNFASHG ISWVRQAPGQGLEWMAW ISVYTGDT SFAQKFKGRVTVTTDPSTNTAFMEMRSLRSDDTAVYFC ARGLLQGAVMVDSLYYA-LDV WGQGTTVIVTS 259
54.a.39   QVHLVQSGAEVKKPGASVKVSCKVS GYTFTSYG ISWVRQAPGQGLEWMAW ISAYTGNT NFAQKFKDRVTVSTDTSTTTAYMELRGLRYDDTAVYYC ARDRIQGAVALPDKQVNWFDP WGQGTLVTVSS 260
54.a.44   QVQLVQSGAEVKKPGASVKVSCKAS GYTFTSYG ISWLRQAPGQGPEWMAW ISAYTGNT NYAQKFQGRVTVSTETSTGTAYMELRSLTSDDTAVYYC ARDRIQGAVVLPNEQVNWFDP WGQGTLVTVSS 261
54.a.45   QVQLVQSGTEVKKPGASVKVSCKAS GYTFTSYG ISWLRQAPGQGLEWMAW ISAYTGNT NYAQKFQGRVTVSTDTSTTTAYMELRSLRFDDTAVYYC ARDRIQSVVALPAQQVNWFDP WGQGTLVAVSS 262
HA Cont.:                               x                     xxxx    x                                          xxxxxxxx  x x
IMGT                             _CDR_H1_                   _CDR_H2_                                          ________CDR_H3_________
ConsensusQVQLVQSgaEVKkPGASVkVSCKaS GYtFts%G !SWVRQAPGQGlEW$aW ISaYTGnT .%AqkFqgRvT.sT#tST.TA%MESRSLrsDDTA!Y%C ARdr.Qg.V.lp.....wfdp WGQGTlVtVsS 361
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FIG. 20F - 1-18 (QxxV) class VH sequences

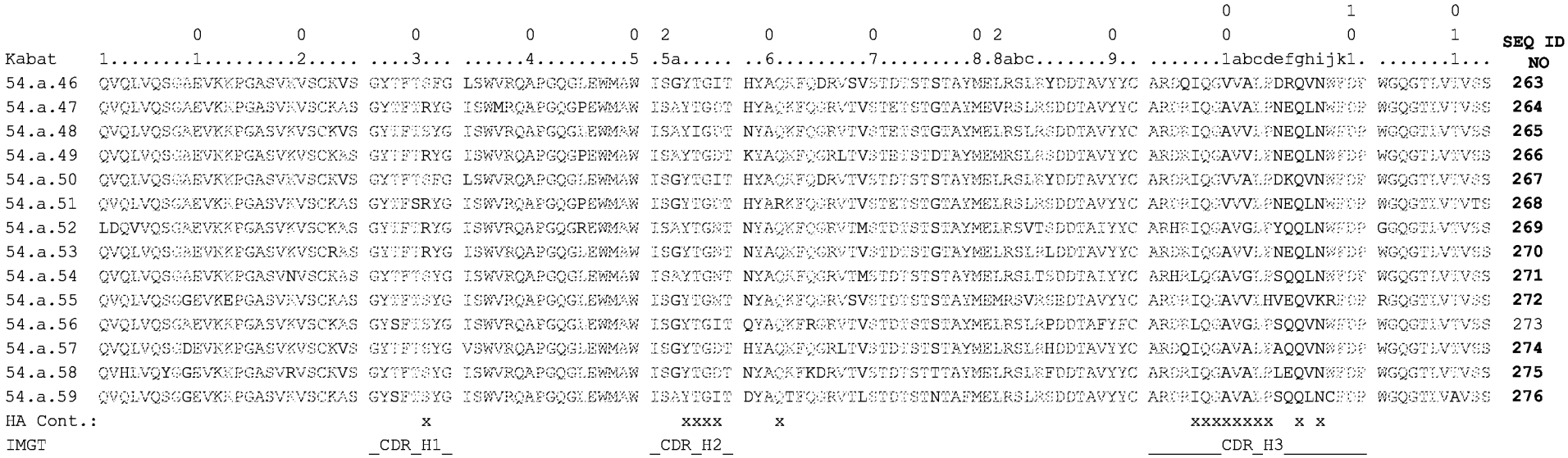


FIG. 20G - 1-18 (QxxV) class VL sequences

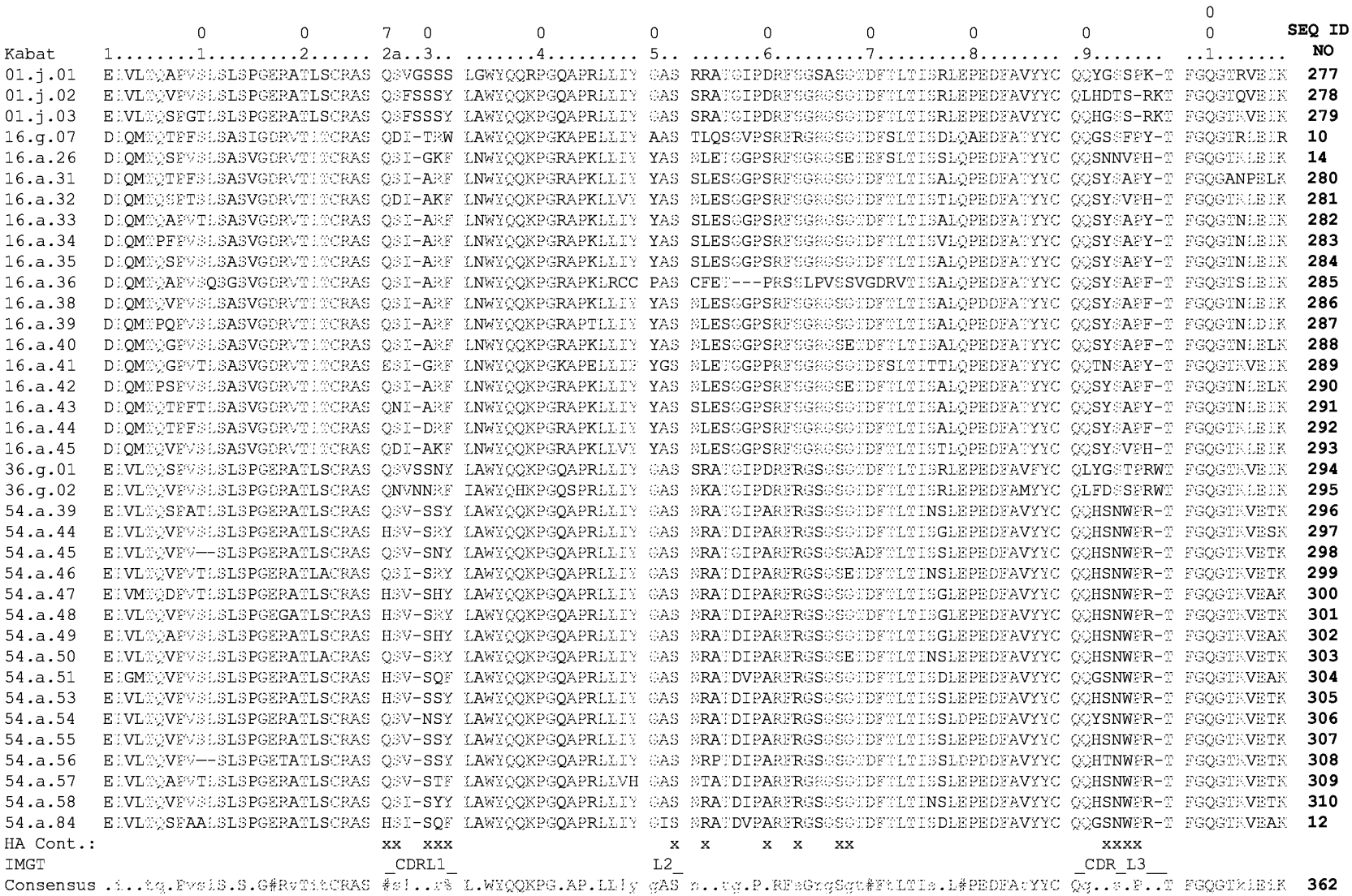
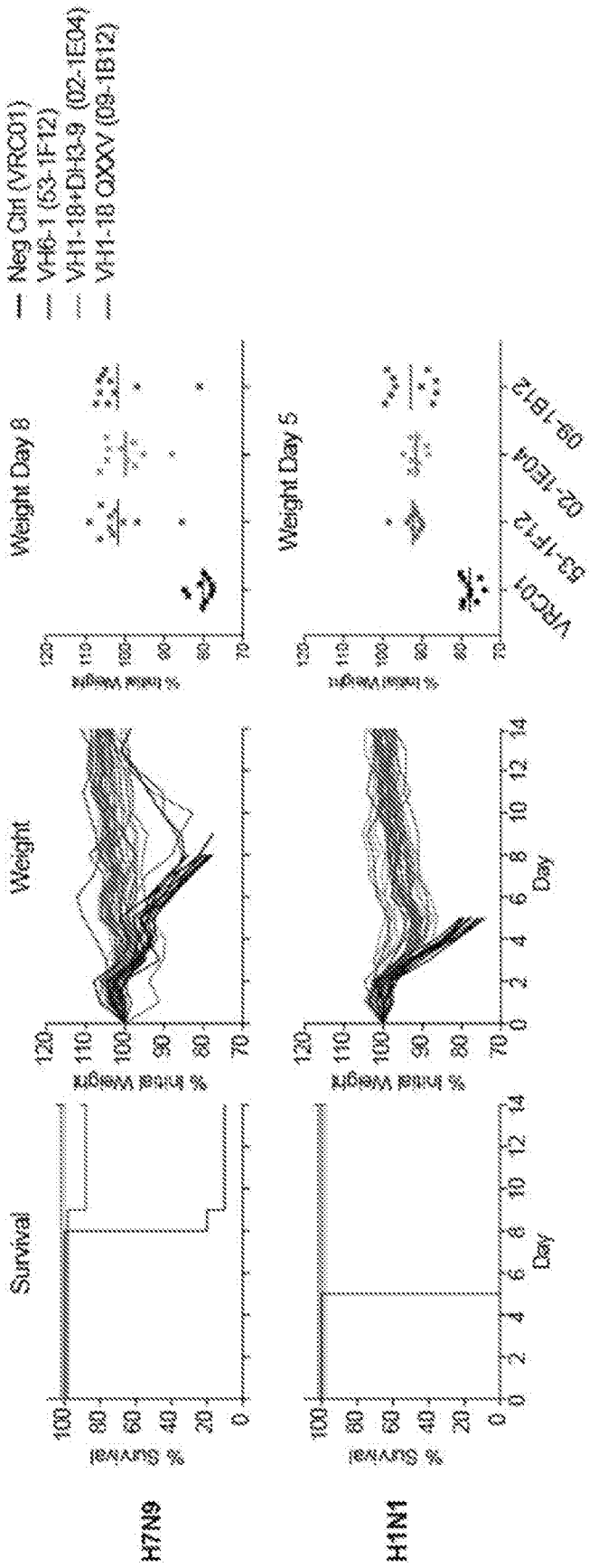


FIG. 21


Neg Ctrl (VRC01)
VH6-1 (63-1F12)
VH1-18+DH3-9 (02-1E04)
VH1-18 OXXV (09-1B12)
Weight Day 8
Weight Day 5
% Initial Weight
VRC01
63-1F12
02-1E04
09-1B12
Survival
Weight
% Survival
% Initial Weight
Day
H7N9
H1N1

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2017/030641

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K16/10
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, Sequence Search, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2015/051010 A1 (MEDIMMUNE LLC [US]; HUMABS BIOMED SA [CH]) 9 April 2015 (2015-04-09) example 1 table 4	1-27
X	WO 2014/078268 A2 (GENENTECH INC [US]; HOFFMANN LA ROCHE [CH]) 22 May 2014 (2014-05-22) figures 4A, 4B, 5A, 5B, 11	1-27
X	WO 2013/007770 A1 (CRUCELL HOLLAND BV [NL]; KWAKS THEODORUS HENDRIKUS JACOBUS [NL]; ZUIJD) 17 January 2013 (2013-01-17) page 42 - page 43	1-27

-/--

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

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"A" document defining the general state of the art which is not considered to be of particular relevance

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"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
7 July 2017	17/07/2017

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Wagner, René

1

Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	KANNAN THARAKARAMAN ET AL: "A broadly neutralizing human monoclonal antibody is effective against H7N9", PROCEEDINGS NATIONAL ACADEMY OF SCIENCES PNAS, vol. 112, no. 35, 17 August 2015 (2015-08-17), pages 10890-10895, XP55336241, US ISSN: 0027-8424, DOI: 10.1073/pnas.1502374112 page 10891	1-27
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