Supporting Information

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SI Materials and Methods

Patients and Vaccines. All studies were approved by the Emory University institutional review board (IRB no. 03027 and 555-2000). Clinical information is detailed in Table S1. Twenty-four healthy adult volunteers were vaccinated with the monovalent pandemic (H1N1) 2009 vaccine. Twenty-two had been previously vaccinated with the 2009/10 seasonal TIV 4–160 d before receiving the pandemic (H1N1) 2009 vaccine. Subject 2, who was given the seasonal 2009/10 TIV only 4 d before receiving pandemic (H1N1) 2009 vaccine, was excluded from all cross-reactivity assays due to concerns about the generation of plasmablasts against the three seasonal strains (including H1N1 Brisbane/59/07), which would confound our data. Data obtained from 2008/09 and 2009/10 season TIV vaccine studies were used for comparison. All vaccines were obtained from Sanofi Pasteur. PBMCs were isolated, washed, and resuspended in RPMI with 10% (vol/vol) FCS for immediate use or frozen for subsequent analysis. Plasma samples were saved at −80 °C for subsequent analysis.

Viruses and Antigens. The pandemic (H1N1) 2009 influenza virus (A/California/04/09) was provided by R. J. Webby (St. Jude Children's Hospital). Other influenza virus stocks used for the assays were obtained from the Centers for Disease Control (CDC) and freshly grown in eggs, prepared, and purified as described (1). They included the following: A/Fort Monmouth/1/47 (H1N1), A/New Jersey/11/76, A/New Caledonia/20/99 (H1N1), A/Solomon Island/3/06, A/Brisbane/59/07 (H1N1), and A/Brisbane/10/07 (H3N2). Recombinant HA proteins were provided by the influenza reagent resource (www.influenzareagentresource.org) of the CDC [recombinant HA from A/California/04/2009 (H1N1; #FR-180), A/Brisbane/10/2007 (H1N1; #FR-61), A/Brisbane/59/2007 (H3N2; #FR-65)] or by Biodefense and Emerging Infections research repository [www.beiresources.org; recombinant HA from A/Indonesia/05/2005 (H5N1)]. A/Brevig Mission/1/1918 (H1N1) was obtained from the CDC.

ELISPOT and Memory B-Cell Assay. Direct ELISPOT to enumerate the number of either total IgG-secreting, pandemic H1N1 influenza-virus-specific, vaccine-specific, and recombinant HA-specific plasmablasts present in the PBMC samples was performed as described (2). In brief, 96-well ELISPOT filter plates (Millipore) were coated overnight purified influenza virions, recombinant HAs, the 08/09 influenza vaccine diluted 1/20 in PBS, or goat anti-human Ig (Invitrogen). Plates were washed and blocked; then, antibody-secreting cells were detected with biotinylated anti-human IgG, IgM, or IgA antibody (Invitrogen) followed by avidin-D-HRP conjugate (Vector Laboratories) and then developed using AEC substrate (3 amino-9 ethyl-carbazole; Sigma-Aldrich). Memory cells were detected, as described (3), by incubating PBMCs at 5×10^5 cells per mL in R-10 supplemented with pokeweed mitogen extract (PWM), phosphothiolated CpG ODN-200626, and *Staphylococcus aureus* Cowan (SAC) (Sigma) for 6 d; total and virus-specific IgG-secreting plasmablasts were quantified by ELISPOT assay.

Flow Cytometry Analysis and Cell Sorting. Analytical flow cytometry analysis was performed after fixing in 2% (vol/vol) PFA. Cell sorting on purified PBMCs used either a FACSVantage or ARIAII cell sorter and analysis by FlowJo. Antibodies used include: anti-CD27(eBioscience) and anti–CD3-PECy7 or PerCP, anti–CD20-PECy7 or PerCP, anti–CD38-PE, anti–CD27-APC, and anti–CD19-FITC (BD). Antibody-secreting cells (ASCs) were gated and isolated as $CD19^+CD3^-CD20^{lo/-}CD27^{high}$ $CD38^{high}$ cells.

Generation of mAbs and Variable Gene Repertoire Analysis. As detailed (1, 4, 5), VH and Vκ genes were PCR-amplified from the transcripts of single ASCs and then sequenced. These variable genes were then cloned into IgG1 or Igκ expression vectors and cotransfected into the 293A cell line for expression. Variable genes were analyzed for identity and mutations using in-house analysis software and the IMGT search engine (6, 7). Background mutation rate by this method is ∼1 base exchange per 1,000 bases sequenced (based on sequences of constant region gene segments). Comparisons were made to published data (1, 8–10). Antibody sequences are deposited in the GenBank database.

ELISA and HAI Assays. Whole virus, recombinant HA, or vaccine-specific ELISA was performed as described (1). In brief, microtiter plates were coated with purified virus at 10 μg/mL, 1:20 dilution of vaccine, or with 0.5 μg/mL of recombinant HA protein. Goat anti-human IgG-HRP (Jackson ImmunoResearch Laboratories) was used to detect binding and developed using *o*-Phenylenediamine dihydrochloride substrate solution. Absorbencies were measured at OD490 on a microplate reader (Bio-Rad). Estimates of binding were calculated using area under the curve from eight dilutions of antibody (10–0.125 μg/mL) using GraphPad Prism. The HAI titers were determined as described (1). In brief, the serum or mAb samples were serially diluted with PBS in 96-well v-bottom plates and 4 HAU of live, egg-grown virus for 60 min before incubation with 0.5% turkey RBCs (Rockland Immunochemicals) for an additional 30 min. The serum titers or minimum effective concentrations were read based on the final dilution at which hemagglutination was inhibited. For competition ELISA, an additional preincubation with unlabeled competitor antibodies to the HA-stalk epitope at a 10-fold molar excess was then performed before application of the mAbs to the plate. Competitors consisted of one of two known stem-binding mAbs (70-1F02 or 70-5B03) or a negative control antibody specific for the HA globular head (EM-4C04) (11). Competition level was calculated as the percentage inhibition of the half-maximal binding concentration of test antibody relative to the absorbance without added competitor.

Neutralization Assays. For micronutralization assays, 100 $TCID_{50}$ of virus in 50 μL of DMEM were incubated with 50 μL of twofold-diluted antibodies (20–0.15625 μg/mL) at 37 °C for 1 h. Cells were washed and incubated in the media [supplemented with antibiotics, 0.5% BSA, and 0.5 μg/mL tosyl phenylalanyl chloromethyl ketone (TPCK)-Trypsin] for 60 h. The MN titer was the concentration of mAbs that completely inhibited infection. Stem-binding antibodies were tested for neutralizing capacity using the $PRNT_{50}$ assay. PBS-washed Madin–Darby canine kidney (MDCK) cells were grown in six-well plates at a density of 8×10^5 per well for 1 d then combined with 10-fold dilutions of virus in 500 μL of DMEM at 37 °C for 1 h with mixing every 10 min. Cells were washed with PBS and overlaid with 199 media (supplemented with antibiotics, 0.2% BSA, and 0.5 μg/mL TPCK-Trypsin) containing 0.5% agarose (Seakem), incubated for 36–40 h and fixed with 2% PFA for 10 min. Agarose plugs were removed, and cells were stained with 0.1% crystal violet in 25% EtOH for 1 min; then, the plates were dried and used to count plaques to calculate the virus titer by PFU. For the $PRNT_{50}$ assay, threefold-diluted mAbs (30–0.12 μg/mL) were combined with 100 PFU of virus in 250 μL of DMEM and incubated at 37 °C for 1 h before the plaque assay as above. The concentration of antibodies that reduced plaques to <50 PFU were scored as the $PRNT_{50}$.

1. Wrammert J, et al. (2008) Rapid cloning of high-affinity human monoclonal antibodies against influenza virus. *Nature* 453:667–671.
2. Crotty S, et al. (2003) Cutting edge: Long-term B cell memory in humans after smallpox vaccination. *J Immunol* 171:4969–4973.
3. Crotty S, Aubert RD, Glidewell J, Ahmed R (2004) Tracking human antigen-specific memory B cells: A sensitive and generalized ELISPOT system. *J Immunol Methods* 286:111–122.
4. Smith K, et al. (2009) Rapid generation of fully human monoclonal antibodies specific to a vaccinating antigen. *Nat Protoc* 4:372–384.
5. Wardemann H, et al. (2003) Predominant autoantibody production by early human B cell precursors. *Science* 301:1374–1377.
6. Ehrenmann F, Kaas Q, Lefranc MP (2010) IMGT/3Dstructure-DB and IMGT/DomainGapAlign: A database and a tool for immunoglobulins or antibodies, T cell receptors, MHC, IgSF and MhcSF. *Nucleic Acids Res* 38(Database issue):D301–D307.
7. Lefranc MP, et al. (2009) IMGT, the international ImMunoGeneTics information system. *Nucleic Acids Res* 37(Database issue):D1006–D1012.
8. Zheng NY, et al. (2004) Human immunoglobulin selection associated with class switch and possible tolerogenic origins for C delta class-switched B cells. *J Clin Invest* 113: 1188–1201.
9. Zheng NY, Wilson K, Jared M, Wilson PC (2005) Intricate targeting of immunoglobulin somatic hypermutation maximizes the efficiency of affinity maturation. *J Exp Med* 201:1467–1478.
10. Koelsch K, et al. (2007) Mature B cells class switched to IgD are autoreactive in healthy individuals. *J Clin Invest* 117:1558–1565.
11. Wrammert J, et al. (2011) Broadly cross-reactive antibodies dominate the human B cell response against 2009 pandemic H1N1 influenza virus infection. *J Exp Med* 208: 181–193.

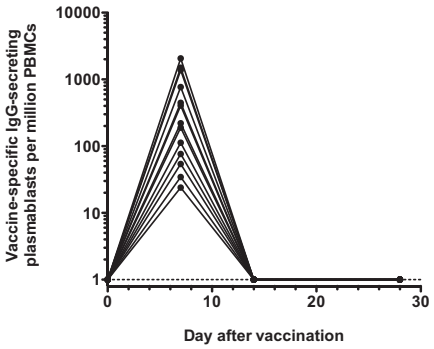

Fig. S1. The 2008/09 trivalent inactivated influenza vaccine induces a rapid plasmablast response. Healthy adult volunteers were vaccinated with the 2008/09 TIV. PBMCs were taken at 0, 7, 14, and 28 d postvaccination, and the number of vaccine-specific IgG-producing plasmablasts was determined by ELISPOT. Dotted lines represent the limits of detection for each assay.

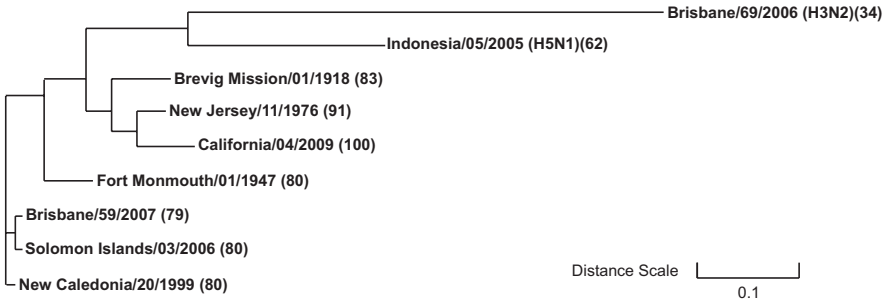

Fig. S2. Sequence homology of HAs from H1N1 strains. HA sequences were obtained from GenBank (http://www.ncbi.nlm.nih.gov/protein/). Sequences were aligned using ClustalW2 and displayed as a phylogenetic tree. Numbers in brackets represent pairwise alignment scores. Correlation analysis was done using Spearman's rank correlation, and comparison between groups was done using Student *t* test.

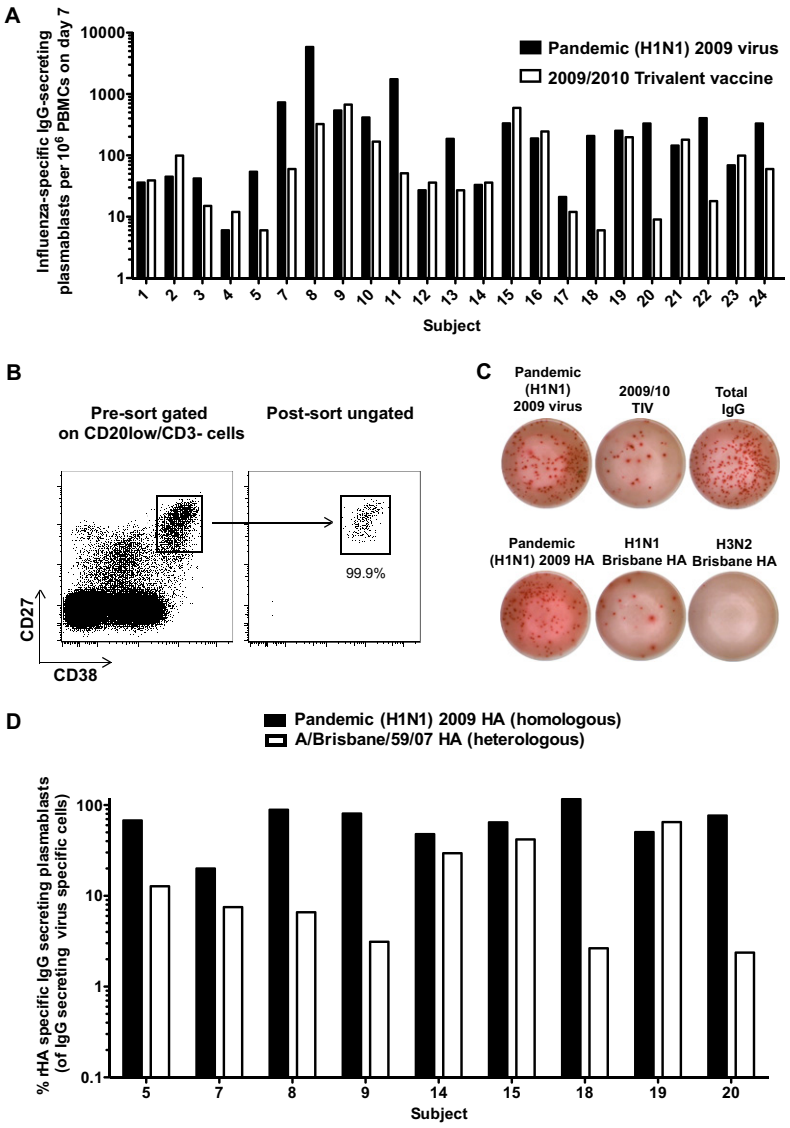

Fig. S3. Plasmablasts induced by the monovalent (H1N1) 2009 vaccine cross-react with the 2009/10 seasonal TIV. Healthy adult volunteers were vaccinated with pandemic (H1N1) 2009 vaccine. (*A*) The numbers of IgG-producing plasmablasts in day 7 PBMCs that reacted against pandemic (H1N1) 2009 virus or the 2009/10 TIV (which contained the A/Brisbane/59/07 H1N1 strain) were determined by ELISPOT. (*B*) Example of plasmablast isolation by flow cytometry. (*C*) Representative ELISPOT images showing total IgG-producing plasmablasts and those reactive against indicated HA proteins. (*D*) ELISPOT scoring of sorted plasmablasts reactive against HA derived from the indicated viruses. ELISPOT for one donor is not shown due to insufficient plasmablast numbers postsort.

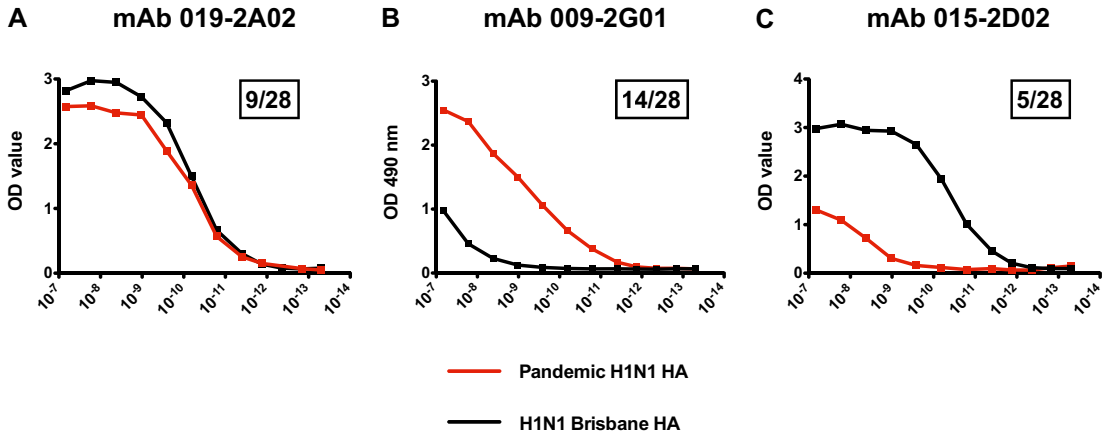

Fig. S4. Patterns of cross-reactivity among HA-specific vaccine-induced monoclonal antibodies. The 28 HA-specific monoclonal antibodies were analyzed by ELISA for their binding to HA proteins derived from either the pandemic H1N1 2009 or the Brisbane H1N1 [A/Brisbane/59/07 (H1N1)] influenza strains. The antibodies showed binding patterns that conformed to three distinct categories. (*A*) One category (9 of 28 antibodies) showed very similar binding to both HAs. (*B* and *C*) Another category (14 of 28 antibodies) showed better binding to the pandemic H1N1 HA, likely representing ongoing adaptation through affinity maturation (*B*), whereas the last category (5 of 28 antibodies) bound better to the Brisbane HA (*C*), consistent with OAS (original antigenic sin).

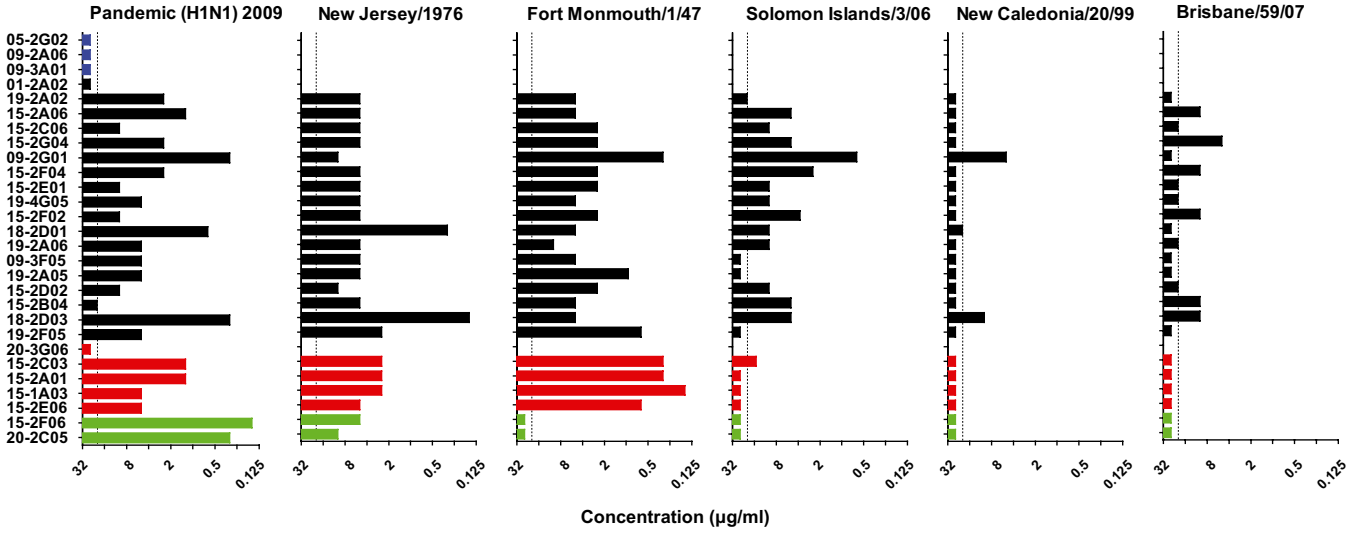

Fig. S5. Cross-reactivity of HA-specific monoclonal antibodies by HAI. Twenty-eight pandemic (H1N1) HA-binding mAbs were tested for HAI activity against a panel of H1N1 virus strains. Influenza strains are arranged in order of sequence similarity to the pandemic (H1N1) 2009, and mAbs are arranged according to cross-reactivity and degree of binding to pandemic (H1N1) 2009 HA. Dotted lines represent limits of detection. Data are representative of two to four repeat experiments.

Table S1. Clinical characteristics of study and control groups

Vaccine	No.	Age, years (range)	Female, %	Interval between seasonal and pandemic H1N1 2009 vaccines, days (range)
Pandemic H1N1 2009 vaccine*	24	39.5 (26–64)	79.2	77 (4–160)
2008/09 TIV	31	31 (21–46)	67.7	NA
2009/10 TIV	27	29 (21–47)	74.1	NA

Number of subjects, age, sex, and time interval between receiving pandemic (H1N1) 2009 vaccine and 2009/10 TIV are shown. Age and interval between vaccinations are expressed as median and range. NA, not applicable.

*Pandemic H1N1 2009 influenza emerged well after the components of the seasonal vaccine had been confirmed. The monovalent vaccine was therefore administered after the seasonal vaccine. However, other than one subject who was later excluded from the plasmablast analysis, all vaccinees were given the pandemic vaccine at least 2 wk after the seasonal vaccine. Because plasmablasts generated in response to influenza vaccine disappear from the blood within 14 d, those induced by the seasonal influenza vaccine would not have directly contributed to the analysis. Furthermore, although the seasonal vaccine may have boosted memory B cells and influenced subsequent humoral responses, most subjects had also been vaccinated the previous year. Because the vaccine formulation was identical in the 2 years (A/Brisbane/59/2007 and A/Brisbane/10/2007), a substantial alteration in the memory B-cell repertoire would have been unlikely.

Table S2. Sequence, mutation, and V gene rearrangement data for pandemic (H1N1) 2009 virus-specific mAbs

Name	V gene	V mutations	V %ID	J gene	D gene	CDR lengths	AA junction
SFV005-1C01H	IGHV1-69*01	24	92	IGHJ4*02	IGHD3-10*01	8.8.13	CAGGSDDHAWGSFYW
SFV005-1C01K	IGKV2or 2D-40*01	6	98	IGKJ2*01		12.3.9	CMQRIAFPFTF
SFV005-1D06H	IGHV4-31*06	35	88	IGHJ4*02	IGHD6-19*01	10.7.16	CARGLEGITVGAYYFDFW
SFV005-1D06K	IGKV1-13*02	26	91	IGKJ4*01		6.3.9	CQQFNSFPLTF
SFV005-2G02H	IGHV1-18*01	22	92	IGHJ4*02	IGHD3-9*01	8.8.15	CARDRRDLLTGSLGDYW
SFV005-2G02K	IGKV2 or 2D-30*01	13	96	IGKJ2*01		11.3.9	CMQGTYWPFTF
SFV009-2A04H	IGHV1-69*06	36	88	IGHJ1*01	IGHD5-18*01	8.8.13	CASPAYNSGFALLHW
SFV009-2A04K	IGKV4-1*01	7	98	IGKJ2*01		12.3.10	CQQYYSNSMYTF
SFV009-2A06H	IGHV1-69*06	20	93	IGHJ4*02	IGHD3-22*01	8.8.18	CASPDLTMVFVPHTGPLDFW
SFV009-2A06K	IGKV1-5*03	17	94	IGKJ1*01		6.3.9	CQHYDTYSGTF
SFV009-2G01H	IGHV4-59*03	15	95	IGHJ6*03	IGHD5-12*01	8.7.19	CARDCSGFEDMDSFYYFMDVW
SFV009-2G01K	IGKV3-11*01	17	94	IGKJ4*01		6.3.11	CQYRSHWPPAVTF
SFV009-3A01H	IGHV4-39*06	13	96	IGHJ4*02	IGHD2-8*01	10.7.19	CARQLTGMVYAILLPSYFDFW
SFV009-3A01K	IGKV1-5*03	6	98	IGKJ1*01		6.3.9	CQQHNSYSGAF
SFV009-3A02H	IGHV3-23*01	20	93	IGHJ3*02	IGHD3-3*01	8.8.15	CAKDRILPYDTDAFDIW
SFV009-3A02K	IGKV1-5*03	25	91	IGKJ3*01		6.3.10	CQEYHTSSRVTF
SFV009-3D04H	IGHV3-23*01	21	93	IGHJ4*02	IGHD6-6*01	8.8.16	CAKDRVVGRPWEYSLDFW
SFV009-3D04K	IGKV3-15*01	16	94	IGKJ4*01		6.3.10	CQQYNNWPPLTF
SFV009-3E06H	IGHV3-66*01	12	96	IGHJ4*02	IGHD4-11*01	8.7.11	CASRHYNYDDDYG
SFV009-3E06K	IGKV2-30*02	2	99	IGKJ5*01		11.3.8	CMQGTHWPTF
SFV009-3F05H	IGHV3-7*01	22	92	IGHJ5*02	IGHD3-10*01	8.8.18	CARAGSYGDYRPINNWFDPW
SFV009-3F05K	IGKV1-5*03	23	92	IGKJ2*01		6.3.9	CQHYSNYSYTF
SFV009-3G01H	IGHV3-30*04	13	95	IGHJ4*02	IGHD3-16*01	8.8.15	CARDPSNPPHWGNFDSW
SFV009-3G01K	IGKV3-11*01	8	97	IGKJ5*01		6.3.10	CQQRSNWPPITF
SFV009-3G03H	IGHV3-23*01	16	94	IGHJ4*02	IGHD4-17*01	8.8.16	CAKDLAVTPPAQGYLDRW
SFV009-3G03K	IGKV3-11*01	5	98	IGKJ5*01		6.3.10	CQQRSNWPPITF
SFV014-2A04H	IGHV4-61*02	8	97	IGHJ5*02	IGHD4-23*01	10.7.17	CARGIKGDYGGGANWFDPW
SFV014-2A04K	IGKV3-15*01	2	100	IGKJ2*01		6.3.10	CQQYNNWPPYTF
SFV014-2B03H	IGHV4-61*02	11	96	IGHJ5*02	IGHD3-16*02	10.7.14	CARARFFGISNWFDPW
SFV014-2B03K	IGKV1 or 1D-39*01	5	99	IGKJ1*01		6.3.9	CQQSYSAPLTF
SFV014-2B06H	IGHV1-69*01	17	94	IGHJ4*02	IGHD3-10*01	8.8.15	CARVGGALIRSSGSDYW
SFV014-2B06K	IGKV1D-17*02	2	99	IGKJ4*01		6.3.9	CLQHNSYPLTF
SFV015-1A01H	IGHV1-69*04	26	91	IGHJ6*03	IGHD6-19*01	8.8.17	CARDDYMTVDRDYYYMDVW
SFV015-1A01K	IGKV3-15*01	11	96	IGKJ3*01		6.3.11	CQQYNNWPPLFSF
SFV015-1A03H	IGHV3-7*01	27	91	IGHJ6*03	IGHD5-24*01	8.8.22	CARVSREEWATVDDPHDYYYMDVW
SFV015-1A03K	IGKV1 or 1D-39*01	24	92	IGKJ4*01		6.3.9	CQQNYNPLFTF
SFV015-1A04H	IGHV3-7*01	32	89	IGHJ6*03	IGHD5-24*01	8.8.22	CVRVSREEWATVDDPHDYYYMDVW
SFV015-1A04K	IGKV1 or 1D-39*01	26	91	IGKJ4*01		6.3.9	CQQSYNSLFTF
SFV015-2A01H	IGHV3-7*01	19	93	IGHJ6*03	IGHD5-24*01	8.8.22	CARVSREEWATVDDPHDYYYMDVW
SFV015-2A01K	IGKV1 or 1D-39*01	24	91	IGKJ4*01		6.3.9	CQQSYNRLFTF
SFV015-2A06H	IGHV1-2*02	21	93	IGHJ3*02	IGHD4-17*01	8.8.16	CARDFDYGDYRGSAFDIW
SFV015-2A06K	IGKV1 or 1D-33*01	13	94	IGKJ3*01		6.3.5	CQQLNTF
SFV015-2B04H	IGHV1-2*02	39	87	IGHJ3*02	IGHD4-17*01	8.8.17	CARDIDTGDYRGADVLQMW
SFV015-2B04K	IGKV1 or 1D-33*01	24	90	IGKJ3*01		6.3.5	CQQLYTF
SFV015-2C03H	IGHV3-7*01	22	92	IGHJ6*03	IGHD5-24*01	8.8.22	CARVSREEWATVDDPHDYYYMDVW
SFV015-2C03K	IGKV1 or 1D-39*01	19	93	IGKJ4*01		6.3.9	CQQSYITLFTF
SFV015-2C04H	IGHV3-23*01	21	93	IGHJ4*02	IGHD5-24*01	8.8.19	CAREEFTDTEMTITQGDFGYW
SFV015-2C04K	IGKV1 or 1D-39*01	15	95	IGKJ3*01		6.3.9	CQRSYITPFTF
SFV015-2C06H	IGHV1-2*02	34	88	IGHJ3*02	IGHD4-17*01	8.8.17	CARDIDSGDYRAADVFQIW
SFV015-2C06K	IGKV1 or 1D-33*01	10	95	IGKJ3*01		6.3.5	CQQLTTF
SFV015-2D02H	IGHV1-2*02	35	88	IGHJ3*02	IGHD4-17*01	8.8.17	CARDIDSGDYRAADVFQIW
SFV015-2D02K	IGKV1 or 1D-33*01	8	96	IGKJ3*01		6.3.5	CQQLATF
SFV015-2E01H	IGHV1-2*02	33	89	IGHJ3*02	IGHD4-17*01	8.8.17	CARDIDSGDYRAADVFQIW
SFV015-2E01K	IGKV1 or 1D-33*01	10	95	IGKJ3*01		6.3.5	CQQLTTF
SFV015-2E06H	IGHV3-7*01	20	93	IGHJ6*03	IGHD5-24*01	8.8.22	CARVSREEWATVDDPHDYYYMDVW
SFV015-2E06K	IGKV1 or 1D-39*01	24	92	IGKJ4*01		6.3.9	CQQSYNSLFTF
SFV015-2F01H	IGHV1-18*01	14	95	IGHJ6*03	IGHD3-16*02	8.8.24	CAREGYDHLWGTYRFEAIDYYYTDVW
SFV015-2F01K	IGKV3-20*01	10	96	IGKJ1*01		7.3.9	CHQYGSSTGTF
SFV015-2F02H	IGHV1-2*02	42	86	IGHJ3*02	IGHD4-17*01	8.8.17	CARDIDFGDYRAADVFHIW
SFV015-2F02K	IGKV1 or 1D-33*01	22	91	IGKJ3*01		6.3.5	CQQLDTF
SFV015-2F03H	IGHV4-b*02	9	97	IGHJ4*02	IGHD5-12*01	9.7.13	CARYIVSTINYFDDW
SFV015-2F03K	IGKV3-20*01	8	97	IGKJ3*01		7.3.10	CQLYGGSPLFAF
SFV015-2F04H	IGHV1-2*02	19	93	IGHJ3*02	IGHD4-17*01	8.8.16	CARDFDYGDYRGSAFDIW
SFV015-2F04K	IGKV1 or 1D-33*01	23	90	IGKJ3*01		6.3.5	CQQLNTF
SFV015-2F06H	IGHV3-23*01	28	91	IGHJ4*03	IGHD5-24*01	8.8.19	CAREEFTDTEMTINQGDFAYW
SFV015-2F06K	IGKV1 or 1D-39*01	18	94	IGKJ3*01		6.3.9	CQRSFITPFTF

Table S2. Cont.

Name	V gene	V mutations	V %ID	J gene	D gene	CDR lengths	AA junction
SFV015-2G04H	IGHV1-2*02	23	92	IGHJ3*02	IGHD4-17*01	8.8.16	CARDFDYGDYRGSAFDIW
SFV015-2G04K	IGKV1 or 1D-33*01	23	90	IGKJ3*01		6.3.5	CQQLNTF
SFV018-2D01H	IGHV5-a*01	16	95	IGHJ6*03	IGHD3-10*02	8.8.17	CTRDSFYDVDLSSFYMDVW
SFV018-2D01K	IGKV3-20*01	10	96	IGKJ2*01		7.3.9	CQQYGSSRHTF
SFV018-2D03H	IGHV1-18*01	17	94	IGHJ3*02	IGHD5-24*01	8.8.15	CARDRIDYVVYDAFDIW
SFV018-2D03K	IGKV1-5*03	18	94	IGKJ1*01		6.3.8	CQLYDDFRTF
SFV019-2A02H	IGHV1-18*01	21	93	IGHJ3*02	IGHD4-17*01	8.8.16	CARRGDYGDYRGDAFDIW
SFV019-2A02K	IGKV1 or 1D-33*01	9	95	IGKJ3*01		6.3.5	CQQVFTF
SFV019-2A05H	IGHV3-21*01	24	92	IGHJ3*02	IGHD5-24*01	8.8.27	CAKDRVRDGDNDWDSVDATYWGYGVFDTS
SFV019-2A05K	IGKV4-1*01	7	98	IGKJ1*01		12.3.9	CQQHYRIPQTF
SFV019-2A06H	IGHV3-74*01	28	91	IGHJ3*02	IGHD4-17*01	8.8.16	CVRDNDYGDYRGNAFDIW
SFV019-2A06K	IGKV1 or 1D-33*01	22	91	IGKJ4*01		6.3.5	CEQLHTF
SFV019-2F05H	IGHV3-21*01	14	95	IGHJ3*02	IGHD5-24*01	8.8.27	CARDRVRDGDNYWDSVDATYWGYGAFDIC
SFV019-2F05K	IGKV4-1*01	9	97	IGKJ1*01		12.3.9	CQQHFTTPQTF
SFV019-4D01H	IGHV4-59*01	19	93	IGHJ6*03	IGHD3-3*01	8.7.19	CARAVSTLVSVDYYFYYIDVW
SFV019-4D01L	IGLV3-21*01	24	92	IGLJ1*01		6.3.10	CQVWDRNNDPLF
SFV019-4G05H	IGHV3-74*01	24	92	IGHJ3*02	IGHD4-17*01	8.8.16	CARDHDYGDYRGNAYDIW
SFV019-4G05K	IGKV1 or 1D-33*01	14	95	IGKJ4*01		6.3.5	CQQLDSF
SFV020-2A04H	IGHV3-23*01	9	97	IGHJ4*02	IGHD2-2*01	8.8.13	CAKDPRSSVPWVAYW
SFV020-2A04K	IGKV2-30*02	2	99	IGKJ2*01		11.3.10	CMQGTHWPPYTF
SFV020-2B03H	IGHV3-23*01	13	95	IGHJ4*02	IGHD4-17*01	8.8.13	CANRMGLRPDYFDYW
SFV020-2B03K	IGKV3-20*01	5	99	IGKJ2*01		7.3.9	CQQYGTSAKTF
SFV020-2B05H	IGHV3-23*01	4	99	IGHJ4*02	IGHD6-13*01	8.8.12	CAKSPASSWYFDHW
SFV020-2B05K	IGKV3-15*01	3	99	IGKJ1*01		6.3.10	CQQDNNWPTWTF
SFV020-2C05H	IGHV4-39*01	9	97	IGHJ5*02	IGHD4-23*01	10.7.17	CARHRVGTGPEVGDWFDPW
SFV020-2C05K	IGKV3-15*01	3	99	IGKJ2*01		6.3.11	CQQYNSWPPMYTF
SFV020-2D03H	IGHV3-23*01	26	91	IGHJ6*02	IGHD3-10*01	8.8.13	CRGWFGEGINGWDVW
SFV020-2D03K	IGKV2 or 2D-28*01	11	96	IGKJ2*01		11.3.9	CMQALQTPYNF
SFV020-3F04H	IGHV3-30*03	14	95	IGHJ6*02	IGHD2-2*01	8.8.23	CATLGGDIVLEPGTRSDYYYGLDVW
SFV020-3F04K	IGKV1-5*03	13	96	IGKJ1*01		6.3.9	CQQYYTNSRMF
SFV020-3G06H	IGHV3-11*01	18	94	IGHJ3*02	IGHD3-22*01	8.8.19	CARASAYYYDSSGRAAAFDIW
SFV020-3G06K	IGKV2-28*01	6	98	IGKJ3*01		11.3.10	CMQVLQTPLFTF

Variable genes were amplified from plasmablasts stimulated by pandemic (H1N1) 2009 vaccine by single-cell RT-PCR and then determined using in-house analysis software compared with the Immunogentics V gene dataset and the IMGT search engine.