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Supplemental Information

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Supplemental Information

Structure and function analysis of an antibody recognizing all influenza A subtypes

Nicole L. Kallewaard[1*], Davide Corti[2*], Patrick J. Collins[3*], Ursula Neu[3*], Josephine M. McAuliffe[1], Ebony Benjamin[1], Leslie Wachter-Rosati[1], Frances J. Palmer-Hill[1], Andy Q. Yuan[4], Philip A. Walker[5], Matthias K.Vorlaender[3], Siro Bianchi[2], Barbara Guarino[2], Anna De Marco[2], Fabrizia Vanzetta[2], Gloria Agatic[2], Mathilde Foglierini[6], Debora Pinna[6], Blanca Fernandez-Rodriguez[6], Alexander Fruehwirth[6], Chiara Silacci[6], Roksana W. Ogrodowicz[5], Stephen R. Martin[5], Federica Sallusto[6], JoAnn A. Suzich[1], Antonio Lanzavecchia[6,7†], Qing Zhu[1†], Steven J. Gamblin[3†] and John J. Skehel[3†]

[1]Department of Infectious Disease and Vaccines, MedImmune LLC, One MedImmune Way, Gaithersburg, MD 20878, USA
[2]Humabs BioMed SA, Via Mirasole 1, 6500, Bellinzona Switzerland
[3]Mill Hill Laboratory, The Francis Crick Institute, London NW7 1AA, United Kingdom
[4]Department of Antibody Discovery and Protein Engineering, MedImmune LLC, One MedImmune Way, Gaithersburg, MD 20878, USA
[5]Structural Biology Science Technology Platform, Mill Hill Laboratory, Francis Crick Institute, London NW7 1AA, United Kingdom
[6]Institute for Research in Biomedicine, Università della Svizzera italiana, 6500 Bellinzona, Switzerland
[7]Institute for Microbiology, ETH Zurich, Wolfgang-Pauli-Strasse 10, 8093 Zurich, Switzerland

*These authors contributed equally.
†Correspondence should be addressed to: E-mail: zhuq@MedImmune.com (QZ), lanzavecchia@irb.usi.ch (AL), steve.gamblin@crick.ac.uk (SJG), and John.Skehel@crick.ac.uk (JJS)

SUPPLEMENTAL EXPERIMENTAL PROCEDURES:

Monoclonal antibody isolation, and ex vivo affinity maturation

Monoclonal antibody isolation was perfomed as previously described (Pappas et al., 2014; Traggiai et al., 2004). In brief, peripheral blood mononuclear cell (PBMC) samples were obtained from a single healthy donor after vaccination following written consent. Memory B cells were isolated from cryopreserved or fresh PBMCs using CD22 microbeads or anti-FITC (fluorescein isothiocyanate) microbeads (Miltenyi Biotec) after staining of PBMCs with CD22-FITC, and were immortalized with Epstein–Barr virus (EBV) and CpG in multiple wells. Supernatants of immortalized B cell clones containing monoclonal antibodies were screened for binding to HA proteins representing two different HA subtypes. Using this methodology, we identified a monoclonal from primary screening in ELISA assay that showed cross-reactive binding to recombinant HA proteins of A/Vietnam/2005 H5N1 and A/ Netherlands/2003 H7N7. Variable gene sequences were isolated from this cross-reactive clone by RT-PCR, cloned and expressed as a full-length IgG transiently in 293 T cells. The lead FY1 antibody was further modified to revert the non-germline framework amino acid changes and affinity optimize the CDRs. For the affinity optimization, full-length IgG constructs containing the different variants created by CDR based parsimonious mutagenesis were expressed transiently in 293 T cells and supernatants were screened by ELISA to select clones that resulted in increased binding activity to H3 while maintaining or increasing the binding activity to H1 HA proteins. Coating concentrations of 0.15 µg/ml of rabbit anti-human IgG were used to capture and normalize variant IgG constructs from supernatants, and binding was probed using 0.5 µg/ml of biotinylated HA proteins A/Perth/2009 H3N2 and A/California/7/2009 H1N1 followed by the addition of streptavidin-HRP (1:5000). Beneficial single mutations were combined and cloned into a combinatorial library, which were expressed and screened by ELISA as described above, leading to the generation of MEDI8852.

Immunoglobulin lineage and sequence analysis

The DNA Maximum Likelihood program (Dnaml) of the PHYLIP package, version 3.69, was used to estimate immunoglobulin phylogenies from nucleotide sequences that were first aligned using ClustalW2, as previously described12. IgHCDR3 regions were defined with their Kabat numbering using the software available on the Abnum website (http://www.bioinf.org.uk/abs/abnum/). The V, D and J genes of the IgH DNA sequences were identified using the IMGT database as a reference (Brochet et al., 2008; Ye et al., 2013). The UCA sequences were inferred with Antigen Receptor Probabilistic Parser (ARPP) UA Inference software (Kepler, 2013), and produced by gene synthesis (Genscript). Branchpoint antibodies were determined by the antibody phylogenetic tree derived by dnaml, and produced by gene synthesis (Genscript).

Recombinant HA protein and binding assays

Recombinant HA proteins were expressed and purified as previously described (Benjamin et al., 2014) ELISA binding assays were performed using 384 well plates (Maxisorp, Nunc) with 0.5 µg/ml of purified HA protein as described in **Table S2**. ELISA plates were washed, blocked 1% (w/v) casein (Thermo Scientific), and serially diluted antibodies were incubated for 1 hr at room temperature. Bound antibodies were detected using a peroxidase-conjugated mouse anti-human IgG antibody (KPL 1:10,000), followed by development with TMB solution (KPL). The absorbance at 450nm was measured, and EC_{50} values were calculated using a non-linear regression of log (inhibitor) vs response in Graph Pad Prism.
Cell surface expressed protein binding assays were conducted using flow cytometry to detect binding of antibodies to HA transfected cells. HEK 293 cells were transiently transfected with full-length wild type HA expressing plasmids. Forty-eight hours after transfection, cells were detached with trypsin, and incubated with 5 µg/ml of FY1 or MEDI8852 on ice for 1 hour. Antibody remaining bound to surface expressed HA protein after washing was then stained with a goat anti-human IgG Daylight 649 (Jackson ImmunoResearch), and detected by flow cytometry.

FACS based binding to cell surface expressed HA proteins
HA protein used as follows; subtype H1 (A/South Carolina/1/18 (H1N1)), subtype H4 A/duck/Czechoslovakia/56 (H4N6)), subtype H8 (A/turkey/Ontario/6118/68 (H8N4)), subtype H10 (A/chicken/Germany/N49 (H10N7)), subtype H11 (A/duck/Memphis/546/74 (H11N9)), subtype H12 (A/duck/Alberta/60/76 (H12N5)), subtype H13 (A/gull/Maryland/704/77 (H13N6)), subtype H14 (A/mallard/Astrakhan/263/82 (H14N5)), subtype H15 (A/shearwater/West Australia/2576/79 (H15N9)), subtype H16 (A/black-headed gull/Sweden/2/99 (H16N3)), subtype H17 (A/little yellow-shouldered bat/Guatemala/164/2009 (H17N10)), and subtype H18 (A/flat-faced bat/Peru/033/2010 (H18N11)).

Viruses and microneutralization assay
Wild-type influenza strains were obtained from the Centers for Disease Control and Prevention or purchased from the American Tissue Culture Collection. Cold adapted (ca) live-attenuated influenza vaccine viruses were generated by either classical reassortment or by reverse genetics (Jin et al., 2003). All viruses were propagated in embryonated chicken eggs, and virus titers were determined by mean 50% tissue culture infective dose (TCID50) per milliliter. Complete viral strain designations are shown in **Table S1**. The microneutralization assay was performed as described previously (Benjamin et al., 2014). Briefly, 60 TCID50 of virus/well was added to three-fold serial dilutions of antibody in a 384-well plate in complete MEM medium containing 0.75ug/ml Trypsin (Worthington) in duplicate wells. After 1 hour incubation at 33°C 5% CO2, $2x10^4$ MDCK cells/well were added to the plate. Plates were incubated at 33°C 5% CO2 incubator for approximately 40 hr, and the NA activity was measured by adding a fluorescently-labeled substrate, methylumbelliferyl-N-acetyl neuraminic acid (MU-NANA) (Sigma) to each well and incubated at 37°C for 1 hr. Virus replication represented by NA activity was quantified by reading fluorescence using the following settings: excitation 355 nm, emission 460 nm; 10 flashes per well. The concentration of antibody required for a 50% reduction in viral replication (IC_{50}) was calculated using a non-linear fit algorithm curve fit in Graph Pad Prism. If neutralization curve was not complete, then value was assigned the highest concentration of inhibitor tested.

In vitro fusion and HA cleavage assays
Antibody mediated fusion inhibition was tested using a low pH induced red blood cell fusion model adaptated from protocol described in (Wang et al., 2010). In brief, A/Puerto Rico/8/34 virus (10 x 106 TCID50) propagated in the presence of trypsin was incubated with human red blood cells (2% final red cell concentration) on ice for 10 minutes. Antibody dilutions were incubated with virus for 30 minutes at room temperature. Red blood cells were added to the virus-antibody mixture for 30 minutes at 37°C, then sodium acetate buffer (0.5 M pH 5.0) was added for additional 45 minutes at 37°C. Samples were centrifuged for 6 minutes at 400xg and incubated for additional 45 minutes at room temperature and then re-pelleted for 6 minutes at 400xg. Supernatants were transferred to an ELISA plate for determination of absorbance at 540 nm. To evaluate the ability of MEDI8852 to inhibit the low pH activated conformational change in HA, H5 HA (A/Vietnam/1194/2004 carrying substitution N186K) and H5-MEDI8852 Fab complex solutions (0.5 mg/ml) were incubated at various pH values (obtained by adding 0.15 M citrate buffer, pH 3.5), adjusted to neutral pH by using 1M Tris-HCl, pH 8.0, and digested with TPCK treated trypsin at a ratio of 20:1 (wt:wt) for 30 min at 37 ºC. The digestion was stopped using an equal amount, to trypsin, of soybean trypsin inhibitor. Tryptic digestion products were analyzed by SDS/PAGE. To assess the ability of antibody to block the cleavage of HA protein, baculovirus expressed recombinant HA from A/New Caledonia/20/99 (H1N1) or A/Hong Kong/8/68 (H3N2) was incubated with antibody at molar ratio of 15:1 (mAb:HA) for 40 min. The antibody-HA mixture was then exposed to 2.5 µg/ml of TPCK-treated trypsin and further incubated for 5, 10, 20, and 40 minutes at 37°C. Samples were separated on a polyacrylamide gel and then transferred to a PVDF membrane for Western

blot analysis using a biotinylated human monoclonal antibody (FO32) (Humabs BioMed) that is specific for the HA2 and HA0 of influenza A strains.

Measurement of Fc-effector function

ADCC assays were preformed using primary human NK cells as effector cells isolated from the PBMCs of healthy donors and target cells of A/Puerto Rico/8/34 H1N1 or A/Hong Kong/8/68 H3N2 influenza virus infected A549 cells at a target to effector ratio of 6:1. Antibody dependent cell killing was measured using LDH release assay after 4 hours of incubation. For ADCP activity, human monocytes were isolated from PBMCs of healthy donors and incubated with M-CSF for 6-7 days to differentiate into macrophages. Macrophages fluorescently labeled violet were incubated with MDCK target cells stably expressing either H1 or H3 HA proteins from A/South Dakota/06/2007 H1N1 or A/Hong Kong/8/68 H3N2 and fluorescently labeled with CFSE at an effector to target ratio of 6:1. Antibody-mediated phagocytosis was determined after 2 hours incubation by flow cytometry, measuring the total macrophage percentage that contained target cells (i.e., the double positive violet and CFSE stained cells). CDC activity was measured using rabbit low-tox complement that had been pre-adsorbed to infected MDCK cells. Complement was mixed with influenza A/Puerto Rico/8/34 H1N1 infected MDCK cells, and MEDI8852 dependent cell killing was measured using LDH release assay after 2 hours of incubation.

Therapeutic efficacy studies in mice and ferrets

All murine study protocols were approved and conducted in accordance with MedImmune's Institutional Animal Care and Use Committee and subsequently performed in an Association for the Assessment and Accreditation of Laboratory Animal Care (AAALAC)-certified facility. Six- to eight-week-old BALB/c mice (Harlan Laboratories) were used in these studies. Mice were weighed on the day of or 1 day before virus challenge and monitored daily for 14 days for weight loss and survival (mice with body weight loss of $\geq$25% were euthanized). For the study of prophylactic and therapeutic efficacy, mice were infected intranasal with 3 x MLD50 of CA/2009 (9.5×10^4 TCID50/mouse); 5x MLD50 of WSN/33 (6×10^2 TCID50/mouse); 7 x MLD50 of rHK/68 (3.6×10^4 TCID50/mouse) on Day 0. Animals administered a single IP dose of 10 mg/kg, 3 mg/kg, or 1 mg/kg of MEDI8852 on Day 0, Day 1, Day 2, Day 3, or Day 4 post-infection, depending on the virus strain. For oseltamivir comparison studies, mice were administered 25 mg/kg oseltamivir PO BID for 5 days, or a single dose of MEDI8852 10mg/kg IV with equivalent volume of vehicle given PO BID for 5 days to mimic the hydration of oseltamivir treated animals. To assess virus load in the lungs, four mice from each group were euthanized on Day 5 post-infection. Whole lungs were homogenized in 10% (wt/vol) sterile L15 medium (Invitrogen) and titrated on MDCK cells to determine the $TCID_{50}$/gram of tissue. Lung function was measured using the MouseOx™ Pulse-oximeter infrared sensor collar clip (Starr Life Sciences, Oakmont PA) to determine the percent blood SpO2 of mice on day 6 post infection. The ferret H5N1 study was completed under contract at Southern Research Institute (Birmingham, Alabama). Five-to-six months' old influenza sero-negative ferrets (Triple F Farms) were challenged intranasally with 1 LD90 of A/Vietnam/1203/04 (H5N1) highly pathogenic avian influenza virus in 1.0 ml (approximately 0.5 ml/nare). Infected animals were treated with either a single IV dose of MEDI8852 at 25 mg/kg, oseltamivir at 25 mg/kg BID for 5 days initiated at 1, 2, or 3 days post infection. Control-treated animals received a 25 mg/kg IV dose of isotype control monoclonal antibody on Day 1 post-infection. Bio-metric data systems chip was implanted between the shoulder blade for identification and temperature monitoring. Animals were monitored for weight loss, fever, clinical signs, and survival.

MEDI8852 Fab and complex crystallization.
A/Vietnam/1194/2004 recombinant virus containing substitution Asn186Lys and H7 A/Turkey/Italy/214845/2002, Group 1 and Group 2 viruses respectively, Figure 2. The viruses were grown in hens eggs and purified by sucrose density gradient centrifugation, according to standard protocols (Skehel and Schild, 1971). H5 and H7 HAs were purified from the virus membrane by detergent extraction followed by trypsin digestion (H5) or bromelain digestion (H7). The digestion was then further purified by ion exchange chromatography using a Q FF Sepharose column and finally by gel filtration chromatography, on a superdex-200 16/60 column (GE) in 20mM Tris/HCl pH 8.0, 150 mM NaCl. The Fab fragments from MEDI8852 IgG were prepared by Endoproteinase Lys-C digestion. The Fab fragments were purified using Protein A sepharose affinity chromatography (HiTrap Protein A, 1 ml column) followed by gel filtration chromatography using a superdex-200 16/60 column (GE) in 20 mM Tris/HCl pH 8.0, 150 mM NaCl. Excess MEDI8852 Fab was added to purified H5 and H7 HA and incubated overnight at 4^{o}C for complex formation in 20 mM Tris/HCl pH 8.0, 150 mM NaCl. The complex was then loaded onto a superdex-200 gel filtration column to purify the predicted 3Fab-HA complex from any excess Fab. Peak fractions corresponding to the Fab-HA complex were pooled and concentrated for crystallization. MEDI8855_H5 complex crystals were obtained from 16% PEG 3350, 0.1 M Pipes pH 7.0, cryoprotected with 25% ethylene glycol prior to freezing. MEDI8852_H7 complex crystals were obtained from 45% pentaerythritol propoxylate (PEP) 15/4, 0.1 M Tris, pH 8.5. MEDI8852 Fab crystals were obtained from 20% PEG 3350, 0.2 M Ammonium Dihydrogen Phosphate after seeding with crystals obtained from 18% PEG8000, 0.1 M Sodium Cacodylate pH 6.5 and 0.2 M Calcium Acetate Hydrate. Crystals were cryoprotected with 25% ethylene glycol prior to freezing.

Structure determination
Crystals were frozen by direct immersion in liquid nitrogen and diffraction datasets were collected at 100K at the IO2 and IO4 beamlines at the diamond light source (Harwell, UK). Diffraction datasets were indexed and integrated with XDS (Kabsch, 2010) and scaled with XSCALE. . For solving the MEDI8852 Fab fragment, a search model was generated by blasting the sequences of its heavy and light chains and using the structures with the highest sequence homology. The search models were generated in chainsaw from CCP4 (Collaborative Computational Project, Number 4, 1994). Molecular replacement was carried out with Phaser (McCoy et al., 2007), which found two constant domains and two variable domains corresponding to two Fab fragments. The structure was refined by rigid body and simulated annealing refinement in Phenix (Adams et al., 2010), then completely rebuilt into the electron density, and then refined by alternating cycles of model building in Coot (Emsley and Cowtan, 2004) and TLS, restrained coordinate and B factor refinement in Refmac (Murshudov et al., 2011). The complex structures were solved by molecular replacement using the cognate HA and MEDI8852 Fab structures as search models. For the H5 complex, Phaser found solutions for two HA trimers as well as for two variable domains of MEDI8852. Electron density was visible for the other 4 variable domains as well and atomic models were placed in the appropriate positions. After rigid body refinement in Phenix, electron density appeared for the constant domains, which were manually placed in the structure. There was clear difference density for HCDR3 and LCDR1 of MEDI8852 in the complex. To build the model of the complex, an unbiased omit map was calculated for each domain (HA1 fusion, HA1 esterase, HA1 head, HA2, MEDI8852 heavy chain variable, MEDI8852 heavy chain constant, MEDI8852 light chain variable, MEDI8852 light chain constant) in Phenix. Figures of merit were calculated for these Fourier maps using SigmaA (Collaborative Computational Project, Number 4, 1994) and the maps were subjected to twofold and six fold non-crystallographic symmetry averaging in DM. A sharpening B factor of 75 A^2 was applied to the structure factors before averaging. HCDR3 and LCDR1 were completely rebuilt into the averaged maps, and other residues were rebuilt according to the averaged maps as well as difference Fourier maps. The H7 structure was solved by molecular replacement in Phaser which found the one HA monomer as well as one variable and one constant domain of MEDI8852 in the asymmetric unit. The structure was refined by restrained coordinate refinement with global NCS restraints and TLS refinement in Refmac, and TLS and group B

factor refinement in Phenix with one TLS group per domain (see above). Crystallographic statistics are summarized in **Table S4**.

Hemagglutinin sequences analysis and phylogenic tree

Hemagglutinin (HA) full-length gene sequences for all subtypes (H1 to H18) were obtained from NCBI Influenza Virus Resource (IVR) database (Bao et al., 2008) and the Global Initiative on Sharing Avian Influenza Database (GISAID) EpiFlu™ database as of July 20, 2015. For each HA subtype, after purging sequences that were identical or contained ambiguous nucleotide identities, the protein coding sequences were pairwise aligned with their respective HA subtype reference protein sequence. Sequences with different length than the reference were removed. In total, 12'472 H1, 404 H2, 12'263 H3, 1'005 H4, 694 H5, 1'152 H6, 119 H7, 119 H8, 1'838 H9, 590 H10, 487 H11, 153 H12, 67 H13, 16 H14, 10 H15, 7 H16, 2 H17 and 1 H18 isolates were analyzed respectively. The resulting protein datasets from IVR and GISAID were merged for each HA subtype and only the sequences not in IVR were added from GISAID. Finally, the occurrence of different amino acid identities was analyzed at HA residues 8 (HA1), 15, 16, 18, 19, 20, 21, 38, 42, 45, 48, 49 and 52 (HA2). The HA subtype reference protein sequences for HA subtypes 1 to 16 were aligned with Clustal Ω (Sievers et al., 2011) and a phylogenic tree was derived by neighbor-joining method (bootstrapped 1,000 times) using MEGA 7 software (Kumar et al., 2016) **(Figure 2A**).

Affinity measurements using surface plasmon resonance and biolayer interferometry.

Purified H1 and H3 HA proteins binding affinity to MEDI8852 Fab was measured using a ProteOn 3000 instrument. Briefly, anti-His-tag monoclonal antibody was amine-coupled to a GLC sensor chip with final anti-His-tag capture surface densities of ~2000 resonance units (RUs). Kinetic measurements were preformed by injecting a 100 nM solution of each HA protein, and then bound with three-fold serial dilutions of MEDI8852 Fab. Binding data for all concentrations of each HA protein, plus buffer alone, were collected, and dissociation data were collected for 30 min. Binding data was globally fit to a 1:1 binding model (ProteOn Manager software) that included a term to correct for mass transport-limited binding, should it be detected. This analysis determined the kinetic rate constants (k_{on}, k_{off}), from which the apparent K_D was then calculated as k_{off}/k_{on}. Purified H5 and H7 binding to immobilized MEDI8852 was measured on an Octet RED biolayer interferometer (Pall ForteBio Corp., Menlo Park, CA, USA). Biotinylated anti-IgG-CH1 conjugate (Thermo Fisher Scientific) was first immobilized on streptavidin biosensors (Pall ForteBio Corp., Menlo Park, CA, USA) at a concentration of approximately 1 µg/ml in 10 mM HEPES (pH 7.4), 150 mM NaCl, 3 mM EDTA and 0.005% Tween-20. MEDI8852 Fabs were then bound to the anti-IgG-CH1 conjugate and the binding of HA (at 0.2 – 200 nM) to the immobilized Fabs was measured at 25^{o}C in a 400 second association step.

SUPPLEMENTAL TABLES

Table S1: Viral Strain Designations, Related to Figure 2.

Abbreviation	Isolate Name	HA Subtype Group
H1 SC/18	A/South Carolina/1/18 (H1N1)	Group 1
WSN/33	A/Wilson Smith N/33 (H1N1)	Group 1
PR/34	A/Puerto Rico/8/34 (H1N1)	Group 1
FM/47	A/Fort Monmouth/1/47 (H1N1)	Group 1
NJ/76	A/New Jersey/8/76 (H1N1)	Group 1
KW/86	A/Kawasaki/9/86 (H1N1)	Group 1
TX/91	*ca* A/Texas/36/91 (H1N1)	Group 1
BJ/95	*ca* A/Beijing/262/95 (H1N1)	Group 1
SZ/95	*ca* A/Shenzhen/227/95 (H1N1)	Group 1
NC/99	*ca* A/New Caledonia/20/99 (H1N1)	Group 1
SI/2006	A/Solomon Island/3/2006 (H1N1)	Group 1
SD/2007	*ca* A/South Dakota/06/2007 (H1N1)	Group 1
CA/2009 (H1)	A/California/7/2009 (H1N1)	Group 1
BR/2010	A/Brisbane/10/2010 (H1N1)	Group 1
HK/2010	A/Hong Kong/2212/2010 (H1N1)	Group 1
NH/2010	A/New Hampshire/04/2010 H1N1	Group 1
NY/2012	A/NewYork/36/2012 (H1N1)	Group 1
WA/2012	A/Washington/24/2012 (H1N1)	Group 1
BO/2013	A/Bolivia/559/2013 (H1N1)	Group 1
JP/57	*ca* A/Japan/305/57 (H2N2)	Group 1
MO/2006 (H2)	*ca* A/swine/Missouri/4296424/2006 (H2N3)	Group 1
HK/2003	*ca* A/Hong Kong/213/2003 (H5N1)	Group 1
VT/2004 (H5)	ca A/Vietnam/1203/2004 (H5N1)	Group 1
AB/85	*ca* A/mallard/Alberta/89/85 (H6N2)	Group 1
HK/97 (H6)	*ca* A/teal/Hong Kong/W312/97 (H6N1)	Group 1
H12 ON/68	A/turkey/Ontario/6118/68 (H8N4)	Group 1
HK/97 (H9)	*ca* A/chicken/Hong Kong/G9/97 (H9N2)	Group 1
HK/99	*ca* A/Hong Kong/1073/99 (H9N2)	Group 1
H11 ME/74	A/duck/Memphis/546/74 (H11N9)	Group 1
H12 AL/76	A/duck/Alberta/60/76 (H12N5)	Group 1

H13 MA/77	A/gull/Maryland/704/77 (H13N6)	Group 1
H16 SW/99	A/black-headed gull/Sweden/2/99 (H16N3)	Group 1
H17 GU/09	A/little yellow-shouldered bat/Guatemala/164/2009	Group 1
H18 PU/10	A/flat-faced bat/Peru/033/2010 (H18N11)	Group 1
rHK/68	7:1 A/Puerto Rico/8/34, A/HK/68 HA reassortant	Group 2
HK/68	A/Hong Kong/8/68 (H3N2)	Group 2
VC/75	A/Victoria/3/75 (H3N2)	Group 2
LA/87	A/Los Angeles/2/87 (H3N2)	Group 2
SG/93	A/Shangdong/9/93 (H3N2)	Group 2
WH/95	7:1 *ca* A/WH/95 (H3N1)	Group 2
SY/97	*ca* A/Sydney/5/97 (H3N2)	Group 2
PA/99	*ca* A/Panama/2007/99 (H3N2)	Group 2
CA/2004	*ca* A/California/7/2004 (H3N2)	Group 2
WI/2005	A/Wisconsin/67/2005 (H3N2)	Group 2
PT/2009 (H3)	*ca* A/Perth/16/2009 (H3N2)	Group 2
VC/2011	A/Victoria/361/2011 (H3N2)	Group 2
BR/2011	A/Berlin/93/2011 (H3N2)	Group 2
NY/2012	A/New York/39/2012 (H3N2)	Group 2
TX/2012	A/Texas/50/2012 (H3N2)	Group 2
AM/2013	A/American Samoa/4786/2013 (H3N2)	Group 2
SW/2013	A/Switzerland/9715293/2013 (H3N2)	Group 2
NC/2014	A/New Caledonia/71/2014 (H3N2)	Group 2
PU/14	A/Palau/6759/2014 (H3N2)	Group 2
MN/2010	A/Minnesota/11/2010 (H3N2v)	Group 2
IN/2011	A/Indiana/10/2011 (H3N2v)	Group 2
H4 CZ/56	A/duck/Czechoslovakia/56 (H4N6)	Group 2
NT/2003	*ca* A/Netherlands/219/2003 (H7N7)	Group 2
BC/2004	*ca* A/British Columbia/CN-6/2004 (H7N3-LP)	Group 2
AN/2013	*ca* A/Anhui/1/2013 (H7N9)	Group 2
H10 GE/49	A/chicken/Germany/N49 (H10N7)	Group 2
H14 AS/82	A/mallard/Astrakhan/263/82 (H14N5)	Group 2
H15 AU/79	A/shearwater/West Australia/2576/79 (H15N9)	Group 2

Table S2. Binding Activity and Affinity Measurements of FY1 and MEDI8852, Related to Figure 2.

	KD (nM)		EC_{50} (μg/ml)	
HA Protein (strain)	MEDI8852	FY1	MEDI8852	FY1
H1 (A/California/07/2009 H1N1)	0.34	1.73	-	-
H3 (A/Perth/16/2009 H3N2)	0.30	4.19	-	-
H5 (A/Vietnam/1194/2004 H5N1)	1.34	ND	-	-
H7 (A/Turkey/Italy/214845/2002	0.48	ND	-	-
H1 (A/California/7/2009 H1N1)	-	-	0.048	0.072
H2 (A/Swine/MO/2006 H2N3)	-	-	0.079	0.171
H3 (A/Perth/16/2009 H3N2)	-	-	0.030	0.095
H5 (A/Vietnam/1203/2004 H5N1)	-	-	0.061	0.099
H6 (A/teal/HK/W312/97 H6N1)	-	-	0.073	0.258
H7 (A/Netherlands/219/2003	-	-	0.064	0.045
H9 (A/chicken/HK/G9/97 H9N2)	-	-	0.095	0.129

Table S3. Survival Rates and Lung Viral Titer Reductions after MEDI8852 Treatment, Related to Figure 4.

Virus	Dose (mg/kg)	Time of MEDI8852 Administration Post Infection							
		Survival Rate (%)[a]				Log Reduction in Viral Titer[b]			
		D1	D2	D3	D4	D1	D2	D3	D4
WSN/33 H1	10	88*	100*	100*	100*	5.25*	4.59*	3.76*	2.62*
	3	100*	100*	100*	63*	3.55*	2.98*	2.86*	1.46*
	1	88*	88*	50*	63	1.79*	1.76*	1.52*	1.49*
rHK/68 H3	10	100*	100*	100*	38	4.26*	3.82*	3.61*	1.25*
	3	100*	50*	88*	38	3.07*	2.00*	2.09*	0.75*
	1	88*	63*	63*	25	1.10*	0.72*	0.42*	0.21

[a]Irrelevant isotype control mAb, R347 resulted in 13% survival for WSN/33 H1 and 0% survival for rHK/68 H3 infection

[b]Log viral titer reduction was calculated by comparing to R347 treated animals with lung viral titers of 9.51 $\log_{10}TCID_{50}$/g for WSN/33 H1 and 7.99 $\log_{10}TCID_{50}$/g for rHK/68 H3

*$p<0.05$ using the Log Rank Mantel-Cox test for survival, and students t-test for lung viral titers of MEDI8852 treated animals verses R347 control treated animals

Table S4. Data collection and refinement statistics, Related to Figure 5.

	H5/MEDI8852		H7/MEDI8852		MEDI8852	
Data collection						
Wavelength [Å]	0.9795		0.97949		0.97941	
Space group	C2		P321		$P2_12_12_1$	
Unit cell [Å]	151.26, 386.36, 165.86		144.0, 144.0, 130.92		65.2, 109.9, 139.9	
Unit cell [˚]	90, 90.44, 90		90, 90, 120		90, 90, 90	
Resolution [Å]	30 – 3.7	(3.8 –	30 – 3.75	(3.85 –	86.4 – 1.9	(1.95 –
Total reflections	214845	(15631)	54954	(4149)	320540	(23640)
Unique reflections	98353	(7327)	15424	(1155)	77785	(5822)
Completeness [%]	97.4	98.3	93.5	95.3	97.3	(98.4)
R_{meas} [%]	16.2	120.0	14.3	148.7	11.2	(85.0)
CC1/2 [%]	99.3	28.7	99.7	37.8	99.5	(68.4)
I/σI	7.05	0.99	9.89	1.10	10.0	(2.41)
Refinement						
R_{work} [%]	27.4		22.4		18.7	
R_{free} [%]	28.5		26.3		21.7	
Macromolecule	42466		7143		6759	
Ave. B-factor [$Å^2$]	152.9		151.4		29.5	
Solvent atoms	---		---		573	
Ave. B-factor [$Å^2$]	---		---		34.3	
r.m.s.d.						
Bond lengths [Å]	0.018		0.007		0.013	
Bond angles [˚]	1.47		1.23		1.53	
B-factor Wilson [$Å^2$]	136.3		137.0		24.2	
Ramachandran						
Favored [%]	97		97		98	
Outliers [%]	0.13		0.0		0.0	

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