# **Supporting Information**

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## **SI Materials and Methods**

Memory B-Cell Culture. Whole-blood samples were collected from normal donors under institutional review board-approved informed consent (Western Institutional Review Board, Olympia, WA) and peripheral blood mononuclear cells (PBMC) were purified by standard techniques. B-cell cultures were set up using PBMC, B cells enriched by selection with M2-expressing cells, or IgG<sup>+</sup> memory B cells enriched from PBMC via negative depletion of non-IgG<sup>+</sup> cells with antibodies to CD3, CD14, CD16, IgM, IgA, and IgD on magnetic beads (Miltenyi), as previously described (1). Briefly, to promote B cell activation, proliferation, terminal differentiation, and antibody secretion, cells were seeded in 384-well microtiter plates in the presence of feeder cells and conditioned media generated from mitogen-stimulated human T cells from healthy donors. The culture supernatants were collected 8 d later and screened in a high-throughput format for binding reactivity to M2 protein expressed on HEK 293 cells stably transfected with influenza virus M2 (A/Fort Worth/50 H1N1), using fluorescent imaging (FMAT system, Applied Biosystems).

Reconstitution of Recombinant mAbs from B-Cell Cultures. Messenger RNA was isolated from lysed B-cell cultures using magnetic beads (Ambion). After reverse transcription with gene-specific primers, variable domain genes were PCR-amplified using V<sub>H</sub>, V $\kappa$ , and V $\lambda$  family-specific primers with flanking restriction sites (1). PCR reactions producing an amplicon of the expected size were identified using 96-well E-gels (Invitrogen) and the variable domain amplicons were cloned into the pTT5 expression vector (National Research of Canada, Ottawa, Canada) containing human IgG1-, Ig $\kappa$ -, or Ig $\lambda$ -constant regions. Each V<sub>H</sub> pool was combined with the corresponding V $\kappa$ , or V $\lambda$  pools from individual BCC wells and was transiently transfected in 293-6E cells to generate recombinant antibody. Conditioned media was harvested 3 to 5 d after transfection and assayed for antibody binding to M2 protein expressed on HEK 293 cells. Individual clones were isolated from positive pools and unique  $V_H$  and  $V_L$ genes were identified by sequencing. From these, monoclonal antibodies were subsequently expressed and reassayed for binding activity.

**ELISA.** To detect viral antigen, either 10.2  $\mu$ g/mL UV-inactivated H1N1 A/Puerto Rico/8/34 (PR8) virus (Advanced Biotechnologies, Inc.) was passively adsorbed to 384-well plates in 25  $\mu$ L PBS/ well for 16 h at 4 °C, or PR8 inactivated by  $\beta$ -propiolactone (Advanced Biotechnologies, Inc.) was biotinylated (EZ-Link Sulfo-NHS-LC-Biotin, Pierce) and likewise adsorbed to plates coated with neutravidin (Pierce). Virus-coated and biotinylated virus-coated plates were blocked with PBS containing 1% milk or BSA, respectively. Binding of mAbs at the indicated concentrations was detected with HRP-conjugated goat anti-human Fc antibody (Pierce) and visualized with TMB substrate (Thermo-Fisher). The M2e peptide, SLLTEVETPIRNEWGCRCNDSSD (Genscript) was passively adsorbed at 1  $\mu$ g/mL and antibody binding to the peptide was detected by the same method.

**FACS Analysis of Virally Infected Cells.** To detect M2e following in vitro infection, Madin-Darby canine kidney (MDCK) cells were treated with PR8 at multiplicity of infection (MOI) of 60:1 for 1 h at 37 °C, after which the culture media was replaced. The infected MDCK cells were further cultured for 16 h before harvesting for cell staining with the indicated mAbs. Bound anti-M2 mAbs were visualized on viable cells with Alexafluor 647-conjugated goat

anti-Human IgG H&L antibody (Invitrogen). Flow cytometry was performed on FACSCanto equipped with the FACSDiva software (Becton Dickenson). For the panel of anti-M2 mAbs, 20  $\mu$ L samples of supernatant from transient transfections from each of the IgG heavy- and light-chain combinations was used to stain the 293 stable cell line expressing M2 of A/Hong Kong/483/97 FACS analysis was performed as above.

M2 Variant Analyses. Individual full-length M2 cDNA mutants were synthesized with single ala mutations at each position of the ectodomain representing A/Forth Worth/1/50 (D20), as well as were the 43 naturally occurring variants of M2 (Blue Heron Technology). The mutants were cloned into the plasmid vector pcDNA3.1. After transient transfection with Lipofectamine (Invitrogen), HEK293 cells were treated with 1 µg/mL of the indicated mAbs in PBS supplemented with 1% FBS and 0.2% NaN<sub>3</sub> (FACS buffer). Bound anti-M2 mAbs were visualized on viable cells with Alexafluor 647-conjugated goat anti-Human IgG H&L antibody (Invitrogen). Flow cytometry was performed with FACSCanto equipped with the FACSDiva software (Becton Dickenson). The relative binding to the naturally occurring variants was expressed as the percentage of the respective mAb staining of the D20 transiently transfected cells, using the formula of Normalized MFI (%) =  $100 \times (MFI_{experimental} -$ MFI<sub>mock transfected</sub>)/(MFI<sub>D20</sub> - MFI<sub>mock transfected</sub>).

**Therapeutic Efficacy Studies in Mice.** Animal studies were conducted under Institutional Animal Care and Use Committee protocols (H5N1 A/Vietnam/1203/04: University of Tokyo, H1N1 A/Puerto Rico/8/34: University of Wisconsin-Madison). We inoculated six groups of 10 mice (female 6- to 8-wk-old BALB/C) intranasally with  $5 \times LD_{50}$  of A/Vietnam/1203/04 (Fig. 2 *A* and *B*) or six groups of five mice intranasally with  $5 \times LD_{50}$  A/Puerto Rico/8/34 (Fig. 2 *C* and *D*). At 24, 72, and 120 h postinfection, the mice received i.p. injections of a 400-µg/200-µL dose of the anti-M2e mAbs TCN-031 TCN-032, control human mAb 2N9, control chimeric mAb ch14C2, PBS, or were left untreated. Mice were weighed daily for 2 wk and were killed when weight loss exceeded 20% (H5N1 study shown in Fig. 2 *A* and *B* and H1N1 study shown in Fig. 2 *C* and *D*) of the preinfection body weight.

Pathological Assessment in Lung, Liver, and Brain of Mice Treated with Anti-M2e mAbs TCN-031 and TCN-032 After Challenge with H5N1 A/Vietnam/1203/04. Lung, liver, and brain issues were collected from three animals per group on day 6 postviral challenge, formalin-fixed, and embedded in paraffin. Paraffin-embedded tissues were cut into 5-µm sections and mounted on glass slides. One replicate slide was stained with H&E and the other replicate slide was processed for immunohistological staining with an anti-VN1203 rabbit antibody using the Dako EnVision System. Histological changes were evaluated under a light microscope.

Viral Titers in Lung, Liver, and Brain of Mice Treated with Anti-M2e mAbs TCN-031 and TCN-032 After Challenge with H5N1 A/Vietnam/ 1203/04. Organs were collected from three animals per group on days 3 and 6 postviral challenge and frozen at -80 °C until use. Samples were thawed, weighed, and homogenized in 1 mL MEM containing BSA (MEM/BSA) followed by centrifugation at 1,540 × g for 5 min. Supernatants were diluted with MEM/ BSA, added to MDCK cells that had been washed twice with PBS, and incubated for 1 h at 37 °C. Virus inoculums were aspirated and cells were washed once with PBS. A monolayer of 1% Agarose in MEM/BSA containing 0.5  $\mu$ g/mL of trypsin was

added to the infected cells and the plates were incubated upsidedown for 48 h at 37  $^{\circ}$ C. The cells were then fixed with 20% methanol and the number of plaques was counted.

Antibody Reactivity to A/California/4/2009-Infected Cells. MDCK cells were infected with media alone or media containing A/ California/4/09 (H1N1) or A/Memphis/14/96 (H1N1) at an MOI of  $\approx$ 1 and were cultured for 24 h at 37 °C. The cells were detached from the tissue culture plates with trypsin, washed extensively, and then fixed in 2% paraformaldehyde for 15 min. The cells were incubated with 1 µg/mL of the indicated antibodies and the primary antibody binding was detected with Alexafluor 647-conjugated goat anti-Human IgG H&L antibody (Invitrogen). The cells were analyzed with a Becton Dickinson FACSCalibur and data were processed using FlowJo software.

**Peptide Competition Analysis of Antibody Binding.** Transient transfection supernatant containing antibody was screened for binding to 293 cells stably transfected with M2 from H1N1 (A/Forth Worth/1/50 H1N1), or mock-transfected cells, in the presence or absence of the M2e peptide SLLTEVETPIRNEWGCRCNDSSD (Genscript) at 5 µg/mL. Bound anti-M2 mAbs were detected with anti-huIgG Fc FMAT Blue at 700 ng/mL in DMEM with 10% FCS and visualized by fluorescent imaging (FMAT system, Applied Biosystems).

Antibody Binding-Competition Analysis. For the panel of anti-M2e MAbs, 293-6E cells were transiently transfected to generate recombinant antibody and 100 µL of conditioned media was harvested 3 to 5 d after transfection. Competition was performed by first incubating  $2.5 \times 10^6$  CHO cells expressing the M2 protein of A/Hong Kong/483/97 (HK M2) with 10 µg/mL TCN-032 Fab for 2 h at 4 °C followed by incubation with 20 µL of transient transfection supernatant containing anti-M2 antibody for 2 h at 4 °C. The anti-M2 bound to the cell surface was visualized by staining with goat anti-huIgG Fc-Alexafluor488 (Invitrogen) and quantified by flow cytometry. For the competition experiment with TCN-031 and TCN-032, the stable HK-M2 transfectant was treated with 10 µg/mL of mAb TCN-031, TCN-032, or 2N9, for 1 h at 4 °C, followed by staining with AF647 (Invitrogen) labeled TCN-031 or TCN-032 at 1 µg/mL for 1 h at 4 °C. Flow cytometry was performed on a FACSCanto equipped with the FACSDiva software program.

1. Walker L, et al. (2009) Broad and potent neutralizing antibodies from an African donor reveal a new HIV-1 vaccine target. *Science* 326:289–293.

Affinity Measurement of Anti-M2e mAbs. Fabs of mAbs TCN-031, TCN-032, and ch14C2 were used to measure the binding affinity to M2 on purified H1N1 A/Puerto Rico/8/34 influenza A virus (Advanced Biotechnologies Inc.) via Biacore analysis. Virus diluted to  $1 \times 10^9$  viral particles/mL in PBS at 20 °C was directly coupled to the sensor chip via amine linkage. Fabs were tested at 1, 3, 9, 27, and 81 nM for binding to virus at 25 °C using a Biacore T100. The results are from one experiment.

Antibody-Dependent Cell-Mediated Cytotoxicity. MDCK cells were infected with A/Solomon Island/3/06 (H1N1) influenza virus (Virapur) at a MOI of 3 for 4 h, after which the media was replaced. Cells were harvested the next day and treated with 2 µg/mL of either TCN-031, TCN-032, or 2N9 for 1 h, washed, and resuspended in phenol red-free RPMI supplemented with 5% FBS, penicillin/streptomycin, glutamax, and ß-mercaptoethanol (complete RPMI). Human natural killer (NK) cells were purified by depletion of non-NK PBMC using the MACS NK Cell Isolation Kit (Miltenyi Biotec). After washing once with PBS, the NK cells were resuspended in complete RPMI. The infected MDCK cells were plated at  $10^4$  cells per well and 50 µL per well in 96-well Vbottom plates. NK cells were added in 50 µL/well at the indicated effector:target ratios. Alternatively, as controls, complete RPMI or 10% TritonX-100 lysis buffer was added to a total of 100  $\mu$ L/ well. The plates were incubated at 37 °C for 4 h. Samples were analyzed in triplicate. Supernatants (50 µL per well) were harvested and delivered to a blackwall 96-well flatbottom plate. Cytolysis was quantified by the detection of released lactate dehydrogenase using the CytoTox 96 Nonradioactive Cytotoxicity Assay kit (Promega). Cytolysis values were derived by the formula of % Specific Release =  $100 \times (experimental release - spontane$ ous release)/(detergent release - spontaneous release).

**Complement-Dependent Cytolysis.** The stable CHO transfectant cell lines expressing full length HK-M2 or a mock control were treated with 5  $\mu$ g/mL of mAbs TCN-031, TCN-032, ch14C2, or negative control 5J12, followed by washing with PBS containing 1% FBS. The cells were then incubated with 25% human complement (Sigma) in PBS containing 1% FBS at 37 °C for 1 h. Cells were again washed, and subsequently stained with propidium iodide followed by analysis by FACS.

## Heavy chain

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Fig. S1. (Continued)

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Germline Viapps 1-39 "01 TCH-031 43/7 59 F10 4410 59/6 50/6 50/6 39 F23 39 F23 59 F23 50 F23	F S (5 S	G S	GT			5 3	R3 T	G N I			D F	AT	Y Y	c		5	CDR3 ( S 7 N N N N N N F		L .	T 1 1 1	F	G G C C C C C C C C C C C C C C C C C C		FR	R 1 К		M	K	104*1 102*1 102*1 102*1 103*1 103*1 103*1 104*1 104*1	Ka 01 or 01 or 01 or 01 01 01 01 01 01	0210 0210 0210	coinin Coor Coor Coor Coor	KU2*0 KU2*0 KU2*0	gme 28 or 28 or	nt KJ2** KJ2**	222		
Germine Viappa 1-39"01 TCIH-031 4917 39 Pu0 44110 5526 5526 5526 59 Pu3 96 5 43 Pu5 99 21 20 23		G S	G T			5	R3 T	5 L 6 1	Q P		D F	AT		<b>c</b>			CDR3  N  N  N  N  N  N  F	9 P P V V V	L 1	T 1 1		G G C C C C C C C C C C C C C C C C C C		FR	24 R 1 K 1 K 1 K 1 K 1 K 1 K 1 K 1 K		1 M V	K	104 102 102 105 105 105 105 105 105 104 104 104 104 105 104 105 105 105 105 105 105 105 105 105 105	Kaj 01 or 01 or 01 or 01 01 01 01 01 01 01	0210 0210 0210	cinin Cor Cor Cor	KU2*0 KU2*0 KU2*0	gme 98 or 98 or 98 or	nt 10210 10210	222		
Germline Wispps 1-85"01 TCH-081 4817 58 PL0 4410 556 526 526 526 539 P23 566 5 48 PL8 59)21 2002 62 B11 497 2		R	GT		T I	S	R3 T	5 L 6 N			D F	AT						P P P P P P P P P P P P P P P P P P P				G G C C P C C C P C C C C P C C C C P C		FR	R 1 K		M V	K	10410 10210 10210 10210 10310 10310 10310 10310 10310 10310 10310	Kaj 01 or 01 or 01 or 01 01 01 01 01 01 01 01	0210 0210 0210	©inin © or © or	N2*0 N2*0 N2*0	gme 28 or 28 or 28 or	kU2*1 KU2*1 KU2*1	2 2 2 2		
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Germline Vitappa 1-35"01 143/7 159 Pu0 14110 7506 1503 39 Pt3 39 Pt3 39 Pt3 39 Pt3 20	F         5         6         5           I         I         I         I           I         I	R	G			F		S L G . N I	Q P		5 S		Y. Y				CDR3 N N N N N N F F 1 1 1	P P V V V M						FR	R 1 K 1 K 1 K 1 K 1 K 1 K 1 K 1 K		I I	K	N411 N211 N211 N211 N211 N211 N311 N311 N3	Kai 01 or 01 or 01 or 01 01 01 01 01 01 01 01 01 01	0210 0210 0210 0210	ce or ce or ce or ce or	N2*0 N2*0 N2*0	gme 28 or 28 or 28 or	1021 1021 1021	2222		
Germline Wispps 1-85 "01 TCH-081 4817 55 40 55 46 52 CL3 89 P23 86 45 48 P18 59 51 49 P18 59 51 49 P18 59 51 41 62 8 62 5 11 41 62 8 62 5 11 41 62 8 63 5 64 6 7 8 1 8 1 8 1 8 1 8 1 8 1 8 1 8 1 8 1 8 1		R	G					S L G G N I G	CL P		5 S	A T	Y Y					3 P P V V V P M I	L 1 1 1 1 1 1 1 4	T 1 1 1				FR	R 1 K		I I	K	N41 N21 N21 N21 N31 N31 N41 N31 N41 N31 N31 N31 N31 N31 N31 N31 N31	Kai 01 or 01 or 01 or 01 01 01 01 01 01 01 01 01 01 01 01 01	0210 0210 0210 0210	az or az or az or az or	N2*0 N2*0 N2*0	gme 28 or 28 or 28 or	10210 10210 10210	222		
Germine Viapps 1-99"01 TCH-031 4917 39 PLO 44110 536 505 505 505 3665 48 PLS 99723 20023 62 BL1 41623 23 SL2 44 HL 45019		R	G T					S L G 1 N 1	Q P		5 5 5 5 5	A T						3 P P P V V V V V V V V V V V V V		T 1 1 1				FR	R         1           K         1		I I	K	N41 N21 N21 N21 N21 N21 N21 N21 N21 N21 N2	Kai 01 01 01 07 01 07 01 01 01 01 01 01 01 01 01 01	- KU2 * C KU2 * C KU2 * C		N2*0 N2*0 N2*0	gme 28 or 28 or 28 or	102 1 102 1 102 1		<u>6</u>	

**Fig. S1.** Amino acid sequences of the variable regions of anti-M2e mAbs. Framework regions 1 to 4 (FR 1–4) and complementarity-determining regions 1 to 3 (CDR 1–3) for  $V_H$  and  $V_K$  are shown. FR, CDR, and gene names are defined using the nomenclature in the IMGT database (International ImMunoGeneTics Information system, http://www.imgt.org). Gray boxes denote identity with the germ-line sequence, which is shown in light-blue boxes; hyphens denote gaps; white boxes are amino acid replacement mutations from the germ line.



Fig. S2. Competition binding analysis of a panel of anti-M2e mAbs with TCN-032 Fab. The indicated anti-M2e mAbs were used to bind to the stable CHO transfectant expressing M2 of A/Hong Kong/483/97 that had previously been treated with or without 10 µg/mLTCN-032 Fab fragment. The anti-M2e mAb bound to the cell surface was detected with goat anti-hulgG Fc-Alexafluor488 FACS and analyzed by flow cytometry. The results are derived from one experiment.



Fig. S3. Monoclonal antibodies TCN-031 and TCN-032 recognize the same region on M2e. The CHO transfectant stably expressing M2 for A/Hong Kong/483/97 as stained with 10  $\mu$ g/mL TCN-031, TCN-032, or 2N9, followed by detection with Alexafluor647-labeled TCN-031 (TCN-031AF647) or TCN-032 (TCN-032AF647) and analysis by flow cytometry. The results are representative of three experiments.



Fig. 54. Viral titers in lung, liver, and brain of mice treated with anti-M2e mAbs TCN-031 and TCN-032 after challenge with H5N1 A/Vietnam/1203/04. BALB/C mice (*n* = 19) were treated by i.p. injection of a 400-µg/200-µL dose of TCN-031, TCN-032, control human mAb 2N9, control chimeric mAb ch14C2, PBS, or left untreated. Tissue viral titers were determined from three mice per group at 3 and 6 d postinfection in the lungs (as an indicator of local replication) and in liver and brain (as an indicator of the systemic spread which is characteristic of H5N1 infection).



Fig. S5. TCN-031 and TCN-032 can potentiate cytolysis by NK cells. MDCK cells were infected with A/Solomon Island/3/2006 (H1N1) virus, and were treated with mAbs TCN-031, TCN-032, or the subclass-matched negative control mAb 2N9. The cells were then challenged with purified human NK cells, and the lactate dehydrogenase released as a result of cell lysis was measured through light absorbance. The results are representative of two separate experiments with two different normal human donors.



Fig. S6. Complement-dependent cytolysis of M2-expressing cells bound with anti-M2 mAb. The stable transfectant expressing M2 of A/Hong Kong/483/97 and a mock control were treated with the indicated mAbs and subsequently challenged with human complement. Lysed cells were visualized by propidium iodide staining followed by FACS analysis. The data are representative of two experiments.

Table S1. Ig gene segment use of	human anti-M2e mAb
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		Heavy-chair	n germ-line gen	e segments	Light-chain germ-line gene segme							
Group	mAb	Variable	Diversity	Joining	Variable	Joining						
A	TCN-032	IGHV4-59*01	IGHD2-15*01	IGHJ4*02	IGKV1-39*01, or IGKV1D-39*01	IGKJ4*01						
	43J7	IGHV4-59*07	IGHD1-26*01	IGHJ4*02	IGKV1-39*01, or IGKV1D-39*01	IGKJ2*01						
	53P10	IGHV4-59*07	IGHD1-26*01	IGHJ4*02	IGKV1-39*01, or IGKV1D-39*01	IGKJ2*01						
	44110	IGHV4-59*07	IGHD1-26*01	IGHJ4*02	IGKV1-39*01, or IGKV1D-39*01	IGKJ2*01						
	55J6	IGHV4-59*01	IGHD5-18*01	IGHJ4*02	IGKV1-39*01, or IGKV1D-39*01	IGKJ5*01						
	52C13	IGHV4-59*01	IGHD5-18*01	IGHJ4*02	IGKV1-39*01, or IGKV1D-39*01	IGKJ5*01						
	39P23	IGHV4-59*01	IGHD4-23*01	IGHJ4*01	IGKV1-39*01, or IGKV1D-39*01	IGKJ1*01						
	36G5	IGHV4-59*01	IGHD2-8*01	IGHJ6*04	IGKV1-39*01, or IGKV1D-39*01	IGKJ3*01						
	48P18	IGHV4-59*01	IGHD2-15*01	IGHJ6*02	IGKV1-39*01, or IGKV1D-39*01	IGKJ4*01						
	59J21	IGHV4-59*01	IGHD2-15*01	IGHJ6*02	IGKV1-39*01, or IGKV1D-39*01	IGKJ4*01						
	20123	IGHV4-59*01	IGHD6-6*01	IGHJ6*02	IGKV1-39*01, or IGKV1D-39*01	IGKJ5*01						
В	TCN-031	IGHV3-66*01	IGHD3-10*01	IGHJ3*01	IGKV1-39*01, or IGKV1D-39*01	IGKJ2*01						
	44H4	IGHV3-66*01	Cannot assign	IGHJ6*02	IGKV1-39*01, or IGKV1D-39*01	IGKJ5*01						
	45019	IGHV3-66*01	Cannot assign	IGHJ6*02	IGKV1-39*01, or IGKV1D-39*01	IGKJ5*01						
	60D19	IGHV3-66*01	Cannot assign	IGHJ6*02	IGKV1-39*01, or IGKV1D-39*01	IGKJ2*01						
С	62B11	IGHV4-31*03	IGHD4-23*01	IGHJ6*02 (a)	IGKV1-39*01, or IGKV1D-39*01	IGKJ5*01						
	41G23	IGHV4-31*03	IGHD3-16*01	IGHJ6*02	IGKV1-39*01, or IGKV1D-39*01	IGKJ5*01						

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Reference sequences for each mAb heavy and light chain were analyzed using IMGT/V-QUEST to determine gene use.

Table 52. Aminity of anti-Mize rap fragments for influenza viru	Table S2.	Affinity of anti-M2e	e Fab fragments for	<sup>,</sup> influenza virus
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Fab	$K_{\rm a}  ({\rm M}^{-1} * {\rm s}^{-1})$	$K_{\rm d}~({\rm s}^{-1})$	KD
TCN-031	1.0 x 10 <sup>6</sup>	1.4 x 10 <sup>-2</sup>	14.0 nM
TCN-032	7.4 x 10 <sup>5</sup>	2.3 x 10 <sup>-3</sup>	3.2 nM
ch14C2	5.0 x 10 <sup>2</sup>	1.8 x 10 <sup>-3</sup>	4.0 μM

Table S3. Pathological assessment of lung, liver, and brain of mice treated with anti-M2e mAbs TCN-031 and TCN-032 after challenge with H5N1 A/Vietnam/1203/04

Organs	Mouse	TCN-031	TCN-032	2N9	Ch14C2	PBS	UT/C
Lung	1	++/++	++/++	++/++	++/++	++/++	++/+++
	2	++/++	++/++	++/+++	++/++	++/++	++/++
	3	++/++	++/++	++/++	++/++	++/+++	++/++
Brain	1	_/_	-/-	+/+	_/_	+/+++	++/+++
	2	_/_	<u>+</u> /+	+/+++	+/+	_/_	+/+
	3	_/_	-/-	+/+	+/++	+/+++	++/+++
Liver	1	_/_	_/_	+/+	+/-	+/+	+/+
	2	_/_	_/_	+/+	+/-	+/-	+/-
	3	_/_	+/-	+/+	+/+	+/+	+/+

Pathological changes and viral antigens were detected in the lungs of all virus-challenged mice. The mice had similar lung lesions across all groups, although mice in the TCN-031 and TCN-032 groups had a tendency toward less viral antigen expression in the lung. In the brain and liver, lesions were not detected in mice in the TCN-31 group and only one of three mice in the TCN-032 group showed some evidence of viral antigens in the brain. Pathological changes/viral antigens: +++, severe/many; ++, moderate/moderate; +, mild/few;  $\pm$  scant/rare; –, not observed/negative.

### Table S4. Amino acid sequences of ectodomains of wild-type M2 variants

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V	anant							Ami	no a	cias	1-2	3 01	thein	viz ex	xtrace	ellula	ar do	mair	1					
1	A/Brevig Mission/1/1918 H1N1	S	L	L	Т	Е	V	Е	Т	Ρ	Т	R	Ν	Е	W	G	С	R	С	Ν	D	S	S	D
2	A/Fort Monmouth/1/1947 H1N1											К				Е								
3	A/ Singapore/02/2005 H3N2										1					Е								
4	A/Wisconsin/10/1998 H1N1										Т			G		Е								
5	A/Wisconsin/301/1976 H1N1										1		S											
6	A/Panama/1/1966 H2N2		F		Ρ						Т													
7	A/New York/321/1999 H3N2										Т													Ν
8	A/Caracas/1/1971 H3N2										Т		Κ											
9	A/Taiwan/3/1971 H3N2		F								Т		S											
10	A/Wuhan/359/1995 H3N2			Р							Т		S											
11	A/Hong Kong/1144/1999 H3N2				Ρ						Т													
12	A/Hong Kong/1180/1999 H3N2				Ρ						Т			G										
13	A/Hong Kong/1774/1999 H3N2													G		Е				S	G			
14	A/New York/217/2002 H1N2										1					Е	Υ							
15	A/New York/300/2003 H1N2										1					Е	Υ			S				
16	A/swine /Spain/54008/2004 H3N2													G		Е			Υ	s				
17	A/Guangzhou/333/99 H9N2		F							L				G		Е				S				
18	A/Hong Kong/1073/1999 H9N2									L				G		Е		К		R				
19	A/Hong Kong/1/1968 H3N2																							
20	A/swine /Hong Kong/126/1982 H3N2										Т		S										G	
21	A/New York/703/1995 H3N2										Т					Е					G			
22	A/swine/Quebec/192/1981 H1N1			Р							Т													
23	A/Puerto Rico/8/1934 H1N1										Т										G			
24	A/ Hong Kong/485/1997 H5N1							D		L				G						S				
25	A/Hong Kong.542/1997 H5N1									L		К		G						S				
26	A/silky chicken/Shantou /1826/2004 H9N2													G		Е		К		S				
27	A/chicken/Taiwan/0305/2004 H6N1									н				G		Е		К		S				
28	A/Quail/Arkansas/16309–7/1994 H7N3							К						G		Е		К		S				
29	A/Hong Kong/486/1997 H5N1									L				G						S				
30	A/chicken/Pennsylvania/13552-												D	G		Е		к		S				
31	A/chicken/Heilongjiang/48/2001													G						s				
32	A/swine/Korea/S5/2005 H1N2													G		Е		К						
33	A/Hong Kong/ 1073/1999 H9N2									L				G		Е		к		s				
34	A/Wisconsin/3523/1988 H1N1										Т							к						
35	A/X-31 Vaccine strain H3N2		F								Т										G			
36	A/Chicken/Rostock/8/1934 H7N1													G		Е								
37	A/envrnmt/New York/16326–1/2005										Т		К	G		Е		Ν		S				
38	A/Indonesia/ 560H/2006 H5N1									Н				G				к		S				
39	A/chicken/Hong Kong/SF1/2003 H9N2					G				н				G						s				
40	A/chicken/Hong Kong/ YU427/2003 H9N2				Ρ											Е								
D20	A/FW/1/1950 H1N1									L				G						S				
ΗК	A/Hong Kong/483/1997 H5N1															Е				s				
VN	A/Vietnam/1203/2004 H5N1										Т													

Amino acids 1–23 of the M2 extracellular domair

The M2e sequence at the top is from A/Brevig Mission/1/18 (H1N1) and is used as the reference sequence for alignment of the M2 ectodomain amino acids 1 to 23 of 43 wild-type variants. Gray boxes denote amino acid identity with the reference sequence and white boxes are amino acid replacement mutations. This list of nonidentical sequences, except for HK, VN, and D20, was derived from M2 sequences used in refs. 2 and 3. Sequence data are from The Influenza Virus Resource at the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/genomes/FLU/FLU.html).

### Table S5. Conservation of the viral binding site for human anti-M2e mAbs compared with those for mAbs derived from immunized mice, in influenza A

mAb	Human ( <i>n</i> = 506)	Swine (n = 193)	Avian ( <i>n</i> = 665)	All (n = 1,364)
TCN-031, TCN-032 [1-SLLTE-5]	97	98	98	98
Z3G1 [2-LLTEVETPIR-11] (3)	79	39	7	38
14C2 [5-EVETPIRNEW-14] (3)	75	19	2	31

Unique M2e sequences used to calculate percent conservation were obtained from The Influenza Virus Resource at the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/genomes/FLU/FLU.html). The reported epitope for each mAb is shown in brackets.

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2. Zharikova D, Mozdzanowska K, Feng J, Zhang M, Gerhard W (2005) Influenza type A virus escape mutants emerge in vivo in the presence of antibodies to the ectodomain of matrix protein 2. *J Virol* 79:6644–6654. 3. Walker L, et al. (2009) Broad and potent neutralizing antibodies from an African donor reveal a new HIV-1 vaccine target. *Science* 326:289–293.

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