doi:10.1038/nature07231

Supplementary Table 1. Serologic testing of volunteers of varying ages

(Reciprocal titers are shown, for the indicated test)

Donor birthdate category	Subject #	HAI, 1918 VLPs	Neut, 1918 virus	Neut, Sw/30 virus
1915 or earlier	1	640	640	320
	2	80	80	80
	3	160	320	80
	4	1280	320	640
	5	640	1280	640
	6	640	640	640
	7	80	20	80
	9	160	160	80
	10	320	320	320
	11	160	640	320
	12	160	320	320
	13	80	40	80
	14	160	640	160
	16	160	320	160
	17	320	1280	320
	18	320	1280	320
	19	640	2560	640
	20	< 20	40	20
	21	320	640	320
	22	320	320	320
	23	320	320	320
	25	320	640	320
	26	20	40	20
	27	80	320	80
	29	320	160	80
	30	320	320	80
	31	320	320	80
	32	160	80	20
	34	320	80	40
	35	>1280	640	80
	36	>1280	2560	640

	38	>1280	640	320
1926-35	37	20	10	<20
	39	320	80	40
	40	20	10	<20
	41	20	<10	<20
	42	<20	20	<20
	43	<20	<10	<20
	46	20	20	40
	55	20	20	20
	62	80	40	80
	64	40	320	<20
1936-45	8	<20	<10	<20
	15	<20	20	20
	51	~20	20	<20
	53	<20	10	<20
	54	<20	<10	<20
	59	<20	<10	<20
	60	80	40	20
	61	40	40	<20
	63	640	2560	640
	65	<20	20	<20
1946-55	44	<20	<10	<20
	45	20	<10	<20
	47	<20	<10	<20
	48	<20	<10	<20
	49	<20	20	<20
	50	80	20	<20
	52	<20	20	<20
	56	160	160	40
	57	<20	<20	<10.
	58	640	40	640

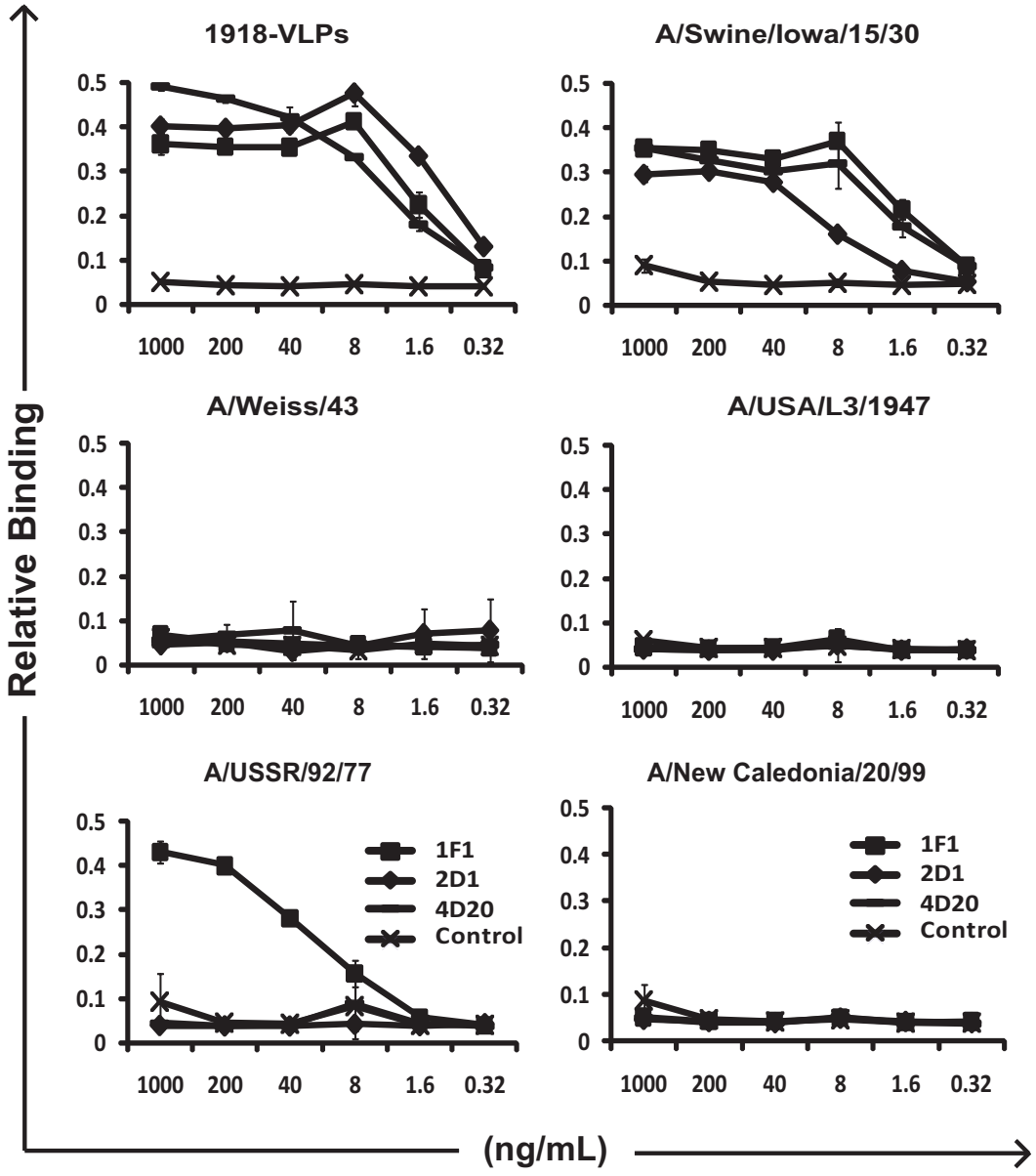
Supplementary Table 2. Neutralization or HAI specific activity (μg/mL) against representative H1N1 viruses

Virus	mAb 1F1		mAb 1I20		mAb 2B12		mAb 2D1		mAb 4D20		Human IgG	
	Neut	HAI	Neut	HAI	Neut	HAI	Neut	HAI	Neut	HAI	Neut	HAI
A/South Carolina/1/1918	0.57	0.37	0.69	0.18	0.97	0.47	0.64	0.32	0.32	0.21	>	>
A/Swine/Iowa/15/30	0.094	0.04	0.22	0.08	0.63	1.3	ND	0.08	ND	0.04	>	>
A/Weiss/43	1.8	0.31	>	>	>	>	ND	>	ND	>	>	>
A/USA/L3/1947	>	>	>	>	>	>	ND	>	ND	>	>	>
A/USSR/92/77	0.88	0.16	>	>	>	>	ND	>	ND	>	>	>
A/New Caledonia/20/99	>	>	>	>	>	>	ND	>	ND	>	>	>

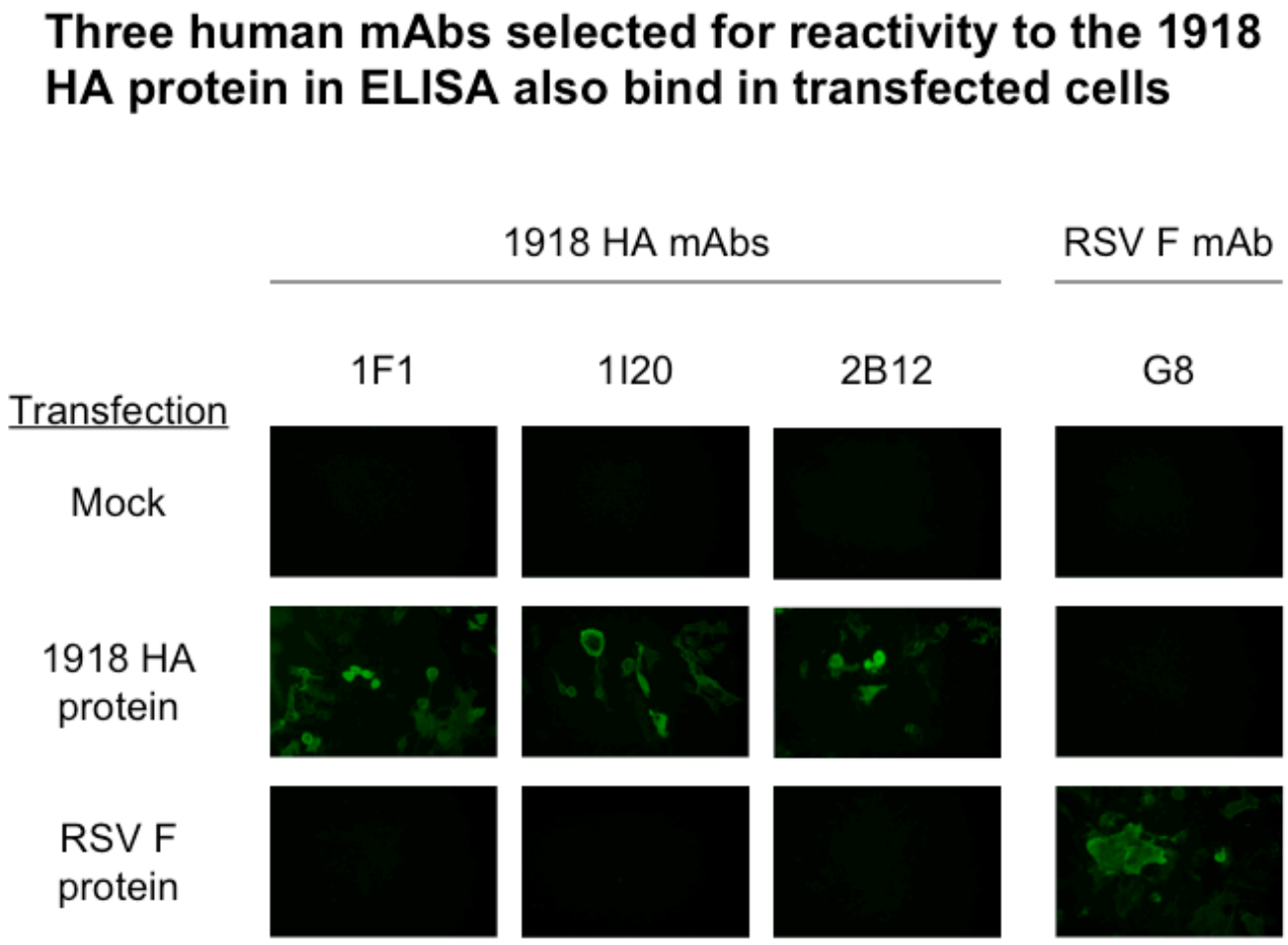
Specific neutralization or HAI activity of mAbs was calculated as the lowest concentration of mAb that displayed activity in the respective assays.

> Indicates activity was not detected at any concentration tested, up to 2.5 μg/mL.

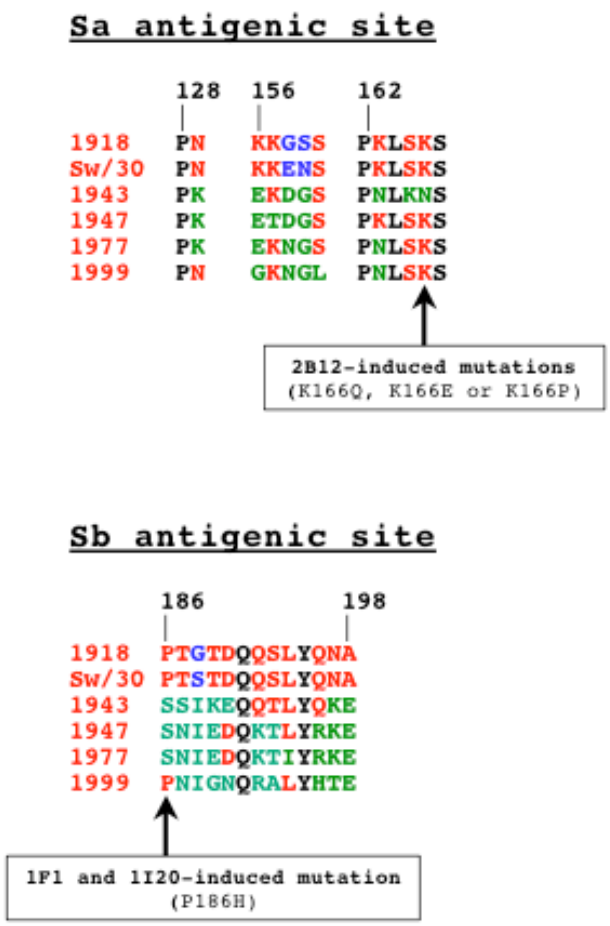
ND indicates not determined.



Supplementary Figure 1. Binding of human mAbs to representative 20th century H1N1 viruses. Equivalent HA units of 1918 virus-like particles, A/Iowa/Swine /30, A/Weiss/43, A/USA/L3/1947, A/USSR/92/77 and A/New Caledonia/20/99 influenza viruses were absorbed onto ELISA plates. An ELISA was performed using serial 1:5 dilutions of mAbs 1F1, 2D1, 4D20 or an H5-specific control mAb. Relative binding (y-axis) indicates optical density in ELISA binding assay to absorbed VLPs or virus.



Supplementary Figure 2. Immunofluorescence detection of binding of 1918 HA-specific mAbs to cultured cells transfected with 1918 HA. HEp-2 cells were transfected with cDNA encoding 1918 HA or respiratory syncytial virus (RSV) or mock transfected, then antigen was detected by staining with one of three mAbs to 1918 influenza HA at a concentration of 50 ng/mL or a similarly prepared human mAb to RSV designated clone G8, followed by staining with Alexa 488 conjugated goat anti-human IgG antibodies. Digital photomicrographs were obtained using epifluorescence on a Nikon TE300 microscope.



Supplementary Figure 3. Alignments of the Sa and Sb antigenic sites from the HA proteins of select H1N1 viruses. The viruses included are: influenza A/South Carolina/1/18 (H1N1); influenza A/Swine/Iowa/15/30 (H1N1) virus; influenza A/Weiss/43 (H1N1) virus; influenza A/FM/1/47 (H1N1) virus; influenza A/USSR/92/77 (H1N1) virus; influenza A/New Caledonia/20/99 (H1N1) virus; respectively labeled: 1918, Sw/30, 1943, 1947, 1977 or 1999. The alignments are adapted from [13], and the numbering is based upon alignment to the H3 HA of influenza A/Aichi/2/68 (H3N2) virus, as described [11]. Black text indicates residues conserved among all six HAs. Blue text highlights differences between 1918 and Sw/30 viruses. Red text indicates residues conserved between 1918 and later viruses. Green residues indicate residues that differ from the 1918 HA. Arrows indicate residues that were changed following selection of mAb neutralization resistant mutants.