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Supplementary Materials for

Preexisting immunity shapes distinct antibody landscapes after influenza virus infection and vaccination in humans

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Other Supplementary Material for this manuscript includes the following:

(available at stm.sciencemag.org/cgi/content/full/12/573/eabd3601/DC1)

Data file S1 (Microsoft Excel format). Raw data.

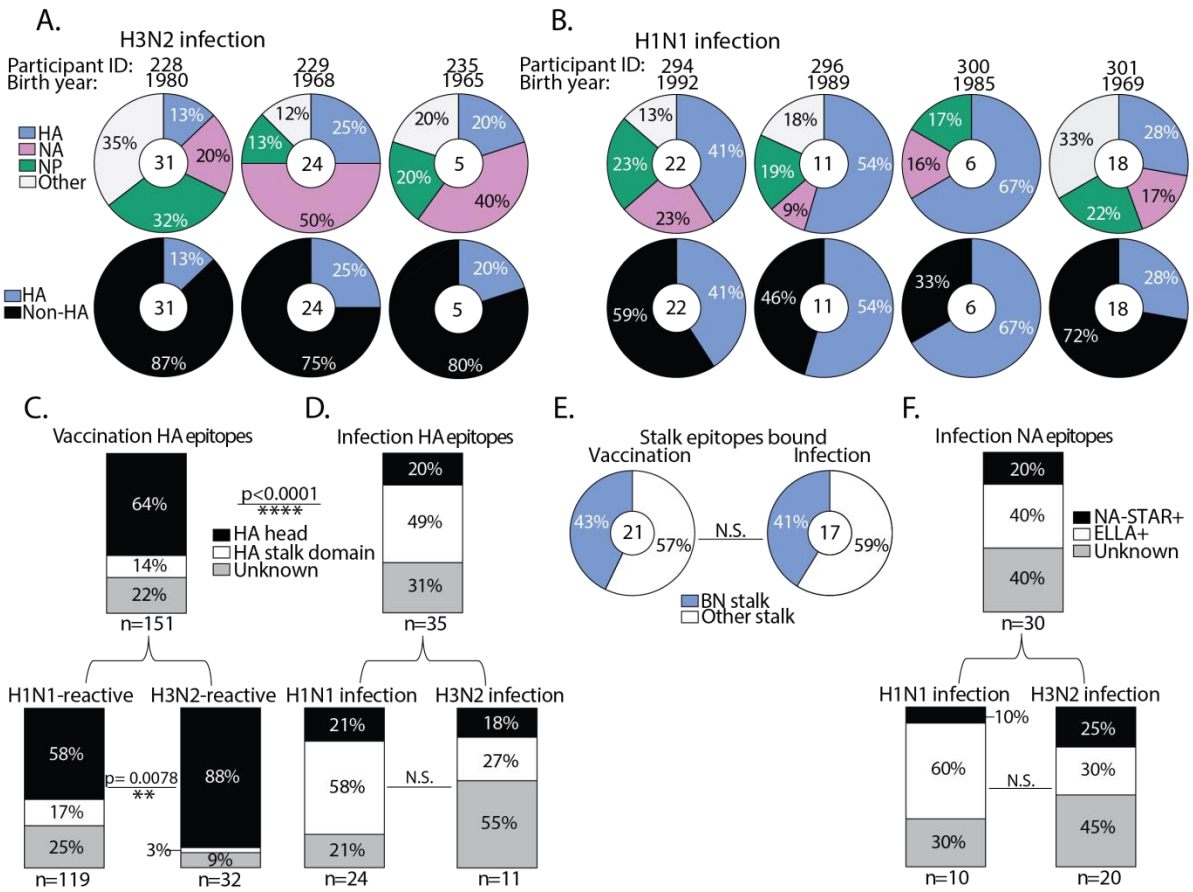

Fig. S1. Reactivity of influenza virus infection–induced antibodies from individual participants and conserved epitope reactivity. (**A, B**) Pie charts show binding of 2014–2015 H3N2 infection-induced mAbs (**A**) and 2015–2016 H1N1 infection-induced mAbs (**B**) to a panel of hemagglutinin (HA), neuraminidase (NA), and nucleoprotein (NP) recombinant proteins by ELISA. Recombinant proteins were chosen from the relevant circulating strains during the time of mAb isolation from infected individuals. (**C, D**) Bar charts demonstrate influenza virus HA epitopes bound by vaccination-induced HA-reactive mAbs (**C**) and infection-induced HA-reactive mAbs (**D**). (**E**) Pie charts demonstrate the percentage of distinct stalk epitopes bound by stalk domain-reactive mAbs. MAbs binding the broadly neutralizing stalk epitope were determined by CR9114 competition ELISA, and mAbs binding undefined stalk epitopes were determined by ELISA against a headless HA stalk construct and chimeric HA. (**F**) Bar charts display NA epitopes bound by NA-reactive infection-induced mAbs, determined by NA-STAR and ELLA assay. Numbers in the center of or below each chart indicate the number of mAbs tested. Statistical significance was determined by Fisher's exact test, ****p<0.0001; **p=0.0078 (C–F). Data are representative of 2–3 independent experiments performed in duplicate.

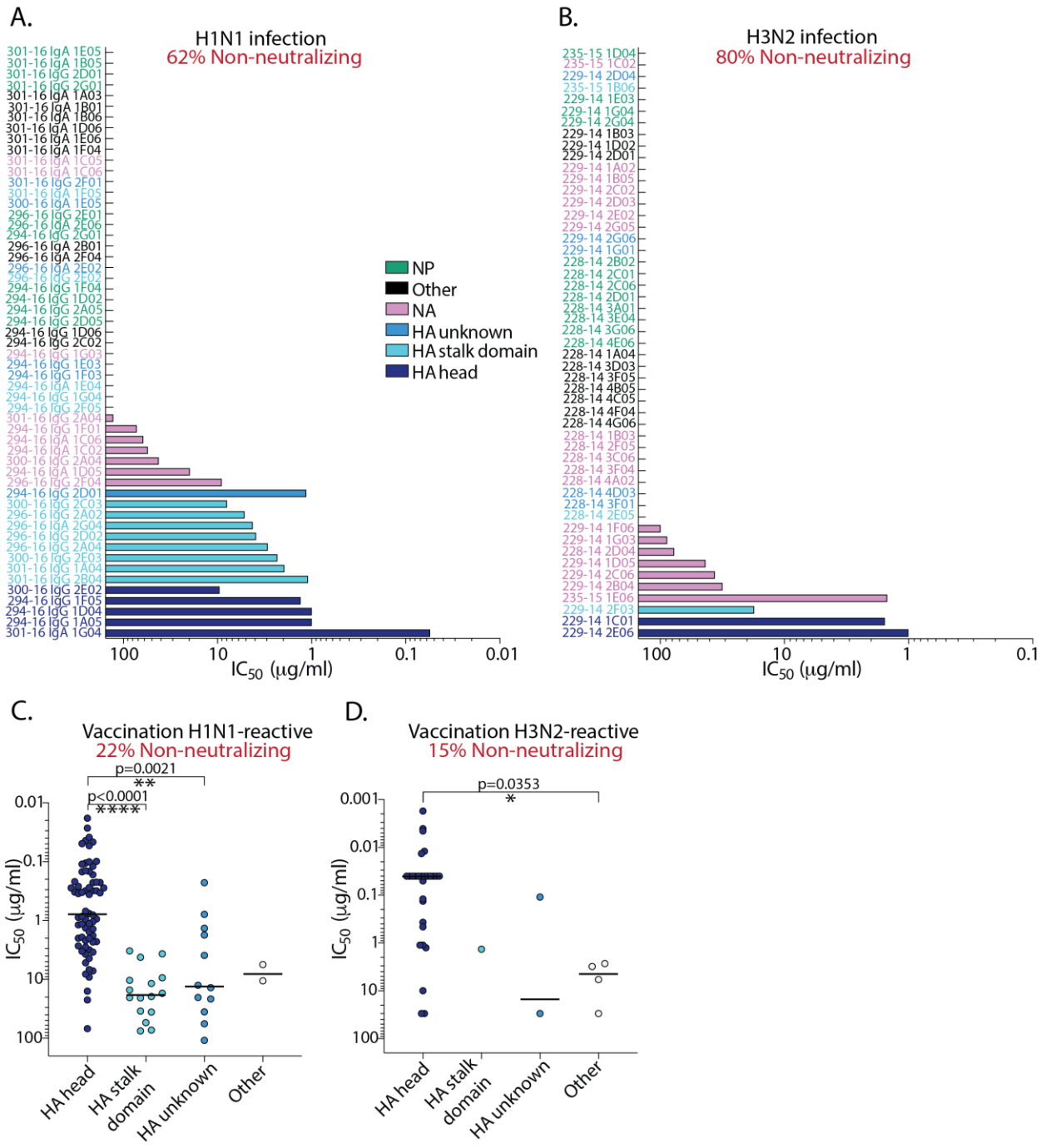

Fig. S2. Neutralization potency of influenza virus infection– and vaccination-induced antibodies stratified by subtype reactivity. (**A, B**) Bar graphs display the potency of HA-reactive and NA-reactive neutralizing mAbs induced by H1N1 infection (n= 56) (**A**) and H3N2 infection (n= 51) (**B**), expressed as microneutralization (MN) IC_{50} values. (**C, D**) The potency of vaccination-induced mAbs binding distinct epitopes was compared for H1N1-reactive (n=100) (**C**) and H3N2-reactive (n=39) mAbs (**D**), depicted as MN IC_{50} values. Statistical significance was determined using an unpaired non-parametric Kruskal-Wallis test with Dunn's correction for

multiple comparisons, ****p<0.0001; **p=0.0021; *p=0.0353 (**C**–**D**). Data are representative of 2–3 independent experiments performed in duplicate.

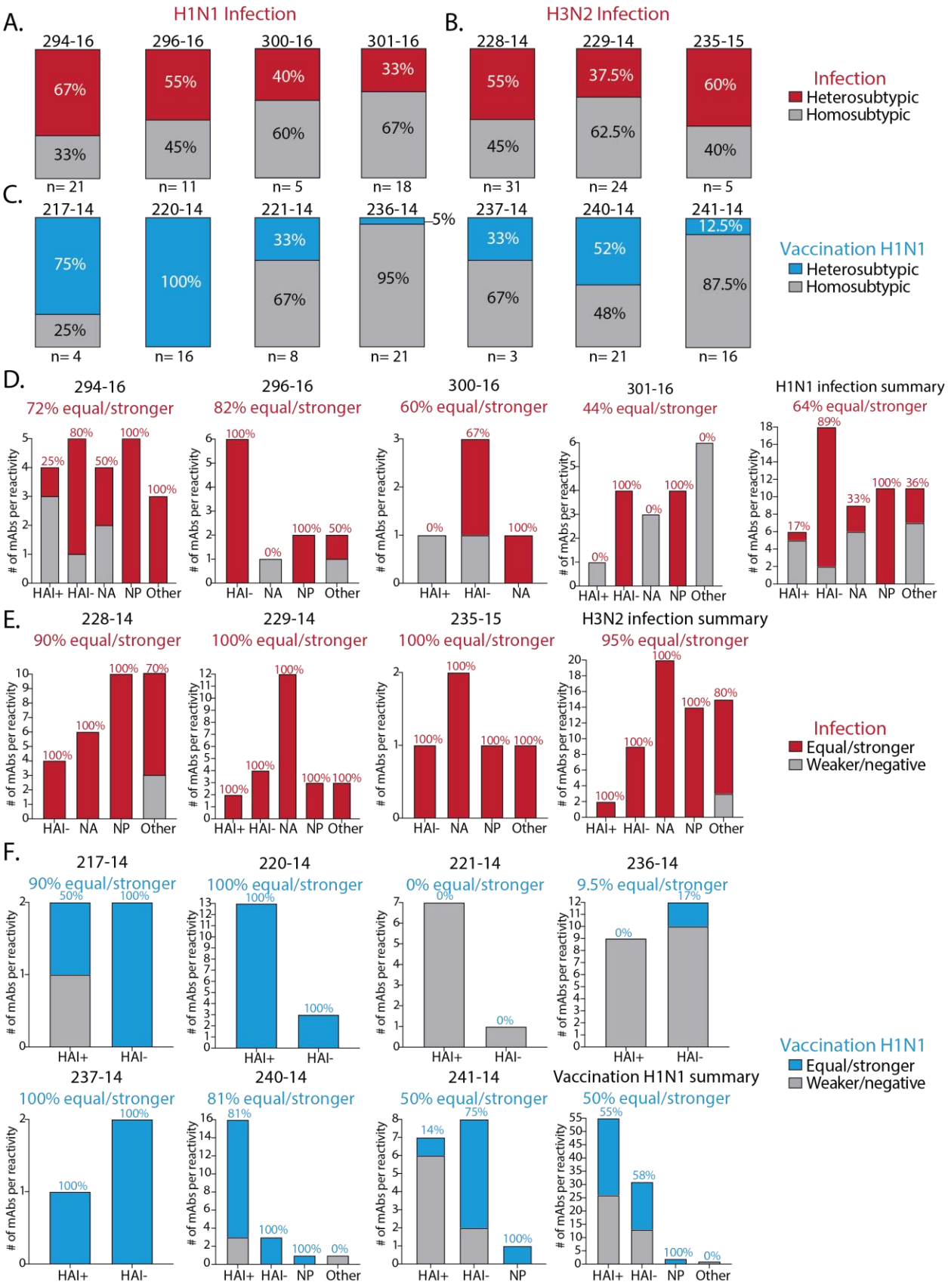

Fig. S3. Antibody cross-reactivity and affinity toward past strains by individual participants. (**A–C**) Bar charts display heterosubtypic cross-reactivity for H1N1 infection-

induced mAbs tested against H3N2 strains (**A**), H3N2 infection-induced mAbs tested against H1N1 strains (**B**), and H1N1-reactive quadrivalent influenza vaccine-induced mAbs tested against H3N2 strains (**C**). Heterosubtypic cross-reactivity was defined based on the ability of a mAb to bind to at least one or more strains opposite of the inducing subtype. (**D**–**F**) Bar graphs display mAbs from H1N1-infected individuals (**D**), H3N2-infected individuals (**E**), and vaccinated individuals (**F**; quadrivalent influenza vaccine, H1N1-reactive) exhibiting equal or stronger affinity to past viral strains relative to inducing strains. Data are broken down by individual mAb reactivities and summarized for each cohort in the last graph of each panel. Numbers below each bar chart indicate the number of mAbs tested per individual, and data are representative of 2–3 independent experiments performed in duplicate.

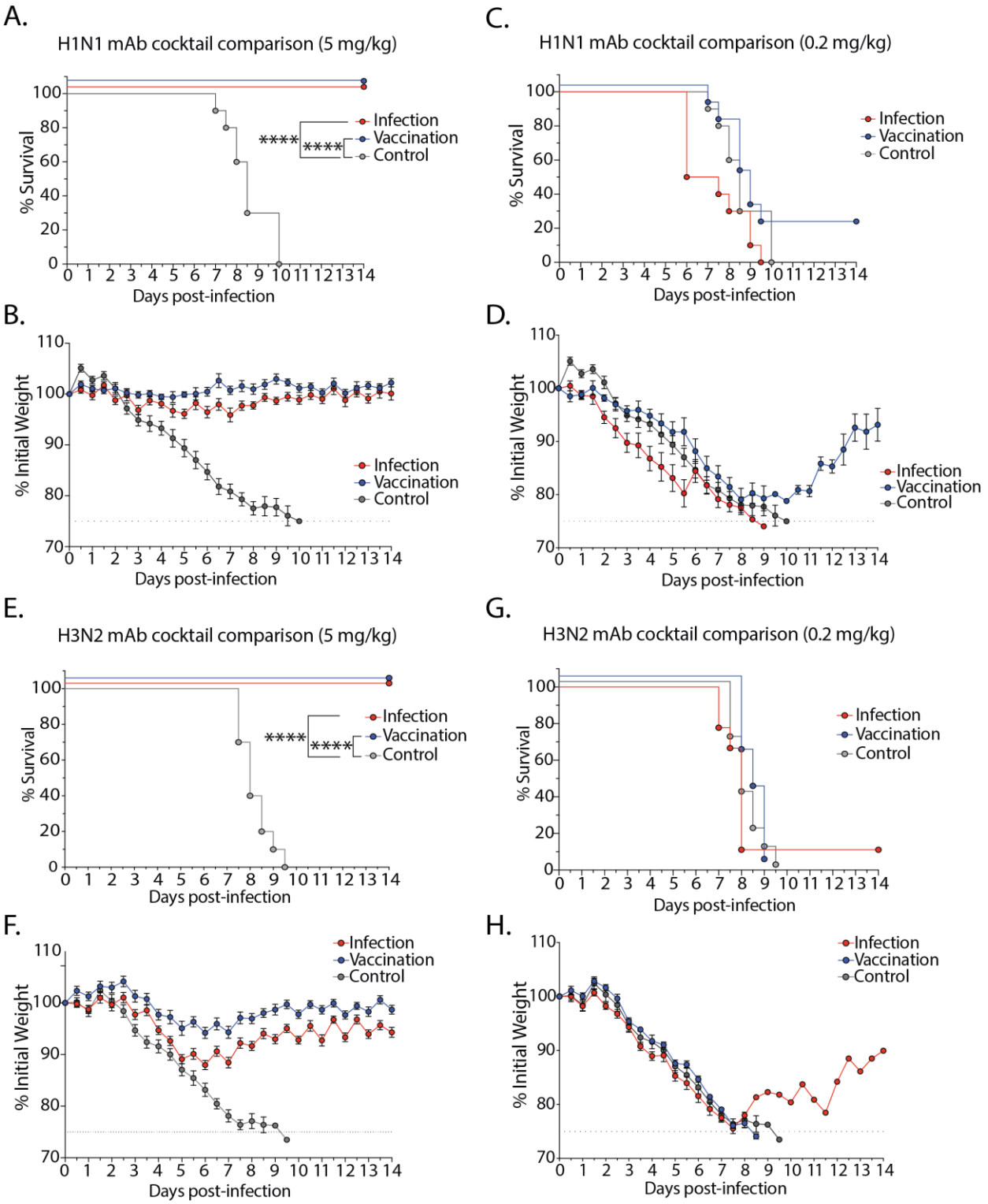

Fig. S4. Prophylactic protection of influenza virus infection– and vaccination-induced antibodies at 0.2 and 5 mg/kg. (**A**–**D**) Weight loss and survival curves display in vivo prophylactic protective ability of infection- and vaccination-induced mAb cocktails administered intraperitoneally at 5 mg/kg (**A**–**B**) or 0.2 mg/kg (**C**–**D**) to 6–8-week-old female BALB/C mice challenged with 10 LD_{50} mouse-adapted A/Netherlands/602/2009 H1N1 virus. (**E**–**H**) Weight

loss and survival curves display in vivo prophylactic protective ability of infection and vaccination-induced mAb cocktails administered intraperitoneally at 5 mg/kg (**E**–**F**) or 0.2 mg/kg (**G**–**H**) to 6–8-week-old female BALB/C mice challenged with 10 LD_{50} mouse-adapted A/Philippines/2/1982 H3N2 virus. Data are representative of two independent experiments and depicted as survival (**A, C, E, G**) and weight loss (**B, D, F, H**) curves. Statistical significance for survival curves was determined using a Mantel-Cox log-rank test, ****$p<0.0001$. Weight loss is presented as mean±SEM (n=9–10 mice per group).

Participant ID	Season	Age	Sex	Influenza A Strain	Comorbidities	Vaccination History	Sampling time
228-14	2014–2015	34	M	S H3N2 A/Switzerland/9715293/2013	Asthma	2011–2012 2012–2013 2013–2014	D7
229-14	2014–2015	46	F	S H3N2 A/Switzerland/9715293/2013	COPD Asthma	2011–2012 2012–2013	D7
235-15	2014–2015	49	M	S H3N2 A/Switzerland/9715293/2013	Asthma	2009–2014	D7
294-16	2015–2016	23	M	Pan H1N1 A/California/7/2009	None	2015–2016	D7
296-16	2015–2016	26	M	Pan H1N1 A/California/7/2009	None	No History	D7
300-16	2015–2016	30	M	Pan H1N1 A/California/7/2009	None	No History	D11
301-16	2015–2016	46	F	Pan H1N1 A/California/7/2009	Asthma	2014–2015	D8

Table S1. Participant characteristics for influenza virus infection cohorts. Likely infecting strains for the specified influenza seasons are referenced (*63, 64*). Vaccination history represents any known history within three years prior to the current sampling time. Sampling time is estimated based on the total number of days participants had been experiencing symptoms of influenza illness at the time of the study visit. All participants were PCR-confirmed influenza positive. S: seasonal; Pan: pandemic; COPD: chronic obstructive pulmonary disease.

Participant ID	Season	Age	Sex	Vaccine	Influenza A Vaccine Strains	Vaccination History	Sampling time
008-10	2010–2011	26	F	TIV	A/California/7/2009 (H1N1) A/Perth/16/2009 (H3N2)	2009–2010	D7
009-10	2010–2011	25	F	TIV	A/California/7/2009 (H1N1) A/Perth/16/2009 (H3N2)	2009–2010	D7
011-10	2010–2011	30	---	TIV	A/California/7/2009 (H1N1) A/Perth/16/2009 (H3N2)	2009–2010	D7
014-10	2010–2011	27	M	TIV	A/California/7/2009 (H1N1) A/Perth/16/2009 (H3N2)	2009–2010	D7
017-10	2010–2011	24	M	TIV	A/California/7/2009 (H1N1) A/Perth/16/2009 (H3N2)	2009–2010	D7
019-10	2010–2011	23	F	TIV	A/California/7/2009 (H1N1) A/Perth/16/2009 (H3N2)	2009–2010	D7
028-10	2010–2011	32	---	TIV	A/California/7/2009 (H1N1) A/Perth/16/2009 (H3N2)	2009–2010	D7
034-10	2010–2011	40	F	TIV	A/California/7/2009 (H1N1) A/Perth/16/2009 (H3N2)	2009–2010	D7
039-10	2010–2011	25	M	TIV	A/California/7/2009 (H1N1) A/Perth/16/2009 (H3N2)	2009–2010	D7
051-10	2010–2011	43	M	TIV	A/California/7/2009 (H1N1) A/Perth/16/2009 (H3N2)	2009–2010	D7
217-14	2014–2015	31	M	QIV	A/California/7/2009 (H1N1) A/Texas/50/2012 (H3N2)	2013–2014	D7
220-14	2014–2015	24	F	QIV	A/California/7/2009 (H1N1) A/Texas/50/2012 (H3N2)	Unknown	D7
221-14	2014–2015	34	F	QIV	A/California/7/2009 (H1N1) A/Texas/50/2012 (H3N2)	No History	D7
236-15	2014–2015	32	F	QIV	A/California/7/2009 (H1N1) A/Texas/50/2012 (H3N2)	No History	D7
237-14	2014–2015	32	F	QIV	A/California/7/2009 (H1N1) A/Texas/50/2012 (H3N2)	No History	D7
240-15	2014–2015	28	M	QIV	A/California/7/2009 (H1N1) A/Texas/50/2012 (H3N2)	No History	D7
241-15	2014–2015	29	F	QIV	A/California/7/2009 (H1N1) A/Texas/50/2012 (H3N2)	No History	D7

244-15	2014–2015	29	M	QIV	A/California/7/2009 (H1N1) A/Texas/50/2012 (H3N2)	2013–2014	D7

Table S2. Participant characteristics for influenza virus vaccination cohorts. Sampling time refers to day post vaccination with either the trivalent influenza vaccine (TIV) or quadrivalent influenza vaccine (QIV).

Participant ID	Cohort	Year of Birth	Reference Childhood Strains
228-14	2014–2015 H3N2 Infection	1980	A/Philippines/2/1982 H3N2
229-14	2014–2015 H3N2 Infection	1968	A/Hong Kong/1/1968 H3N2; A/Philippines/2/1982 H3N2
235-15	2014–2015 H3N2 Infection	1965	A/Hong Kong/1/1968 H3N2; A/Philippines/1982 H3N2
294-16	2015–2016 H1N1 Infection	1992	A/Texas/36/1991 H1N1; A/New Caledonia/20/1999, A/Solomon Islands/2006
296-16	2015–2016 H1N1 Infection	1989	A/Texas/36/1991 H1N1; A/New Caledonia/20/1999
300-16	2015–2016 H1N1 Infection	1985	A/Texas/36/1991 H1N1; A/New Caledonia/20/1999
301-16	2015 H1N1 Infection	1969	A/Chile/1/1983
217-14	2014–2015 QIV	1983	A/Texas/36/1991 H1N1; A Philippines/2/1982 H3N2
220-14	2014–2015 QIV	1990	A/Texas/36/1991 H1N1; A/New Caledonia/20/1999; A Philippines/2/1982 H3N2
221-14	2014–2015 QIV	1980	A/Texas/36/1991 H1N1; A Philippines/2/1982 H3N2
236-15	2014–2015 QIV	1982	A/Texas/36/1991 H1N1; A Philippines/2/1982 H3N2
237-14	2014–2015 QIV	1982	A/Texas/36/1991 H1N1; A Philippines/2/1982 H3N2
240-15	2014–2015 QIV	1986	A/Texas/36/1991 H1N1; A/New Caledonia/20/1999 H1N1; A Philippines/2/1982 H3N2
241-15	2014–2015 QIV	1985	A/Texas/36/1991 H1N1; A/New Caledonia/20/1999 H1N1, A Philippines/2/1982 H3N2

Table S3. Reference childhood strains used to characterize infection-induced mAbs.
Reference childhood strains were chosen based on available virus strains circulating within the first 10–15 years of the individual's life. A mAb was chosen as having equal or greater reactivity toward the reference viral strain if the affinity toward one or more of the indicated reference strains was within the same log molar range or greater than the affinity toward the contemporary inducing strain. QIV: Quadrivalent influenza vaccine.

Infection-induced mAb cocktails			
H1N1 Infection	**Antigen Reactivity**	**Heterosubtypic**	**Neutralizing (NL09)**
294 1A05	HA head	No	Yes
294 1E03	HA unknown	Yes	No
296 2A04	HA stalk/unknown	No	Yes
296 2E02	HA stalk/unknown	Yes	No
296 2G04	HA stalk/CR9114 epitope	Yes	Yes
301 1A04	HA stalk/CR9114 epitope	No	Yes
294 1C02	NA/near active site	No	Yes
294 1G03	NA/unknown	Yes	No
296 2E01	NP	Yes	No
301 1E05	NP	Yes	No
H3N2 Infection	**Antigen Reactivity**	**Heterosubtypic**	**Neutralizing (Phil82)**
229 1G01	HA unknown	Yes	No
229 2G06	HA unknown	Yes	No
228 3F01	HA unknown	Yes	No
229 1F06	NA/active site	No	No
229 1G03	NA/active site	No	Yes
229 1B05	NA/near active site	No	No
229 2C06	NA/near active site	No	Yes
228 2C06	NP	Yes	No
228 3A01	NP	Yes	No
235 1D04	NP	Yes	No
Vaccination-induced mAb cocktails			
H1N1-reactive	**Antigen Reactivity**	**Heterosubtypic**	**Neutralizing (NL09)**
220 2F06	HA head	No	Yes
236 IgA 1A02	HA head	No	No
236 IgG 1A02	HA stalk/unknown	No	Yes
236 1C04	HA head	No	Yes
236 1D01	Other	No	No
236 2A04	HA head	No	Yes
240 1A01	HA head	Yes	Yes
241 2F01	HA head	No	Yes
240 1C04	HA stalk/CR9114 epitope	No	Yes
240 1E01	HA head	Yes	Yes
H3N2-reactive	**Antigen Reactivity**	**Heterosubtypic**	**Neutralizing (Phil82)**
220 1E03	HA head	No	Yes
220 1G04	HA head	No	No
240 1A06	HA head	Yes	Yes
217 1D05	HA/unknown	Yes	No
008-10 5E04	HA head	Yes	No
217 1H02	HA/unknown	Yes	Yes
240 2F02	HA stalk	No	No
034 3E01	HA head	Yes	Yes
DR2A02	HA head	No	Yes
041 1C04	HA head	Not tested	No

Table S4. mAbs used for the infection- and vaccination-induced antibody cocktails. NL09: A/Netherlands/602/2009 H1N1 virus; Phil82: A/Philippines/2/1982 H3N2 virus.

HA Head	Heterosubtypic	Neutralizing (H1)	Source
294 1A05	No	Yes	Infection
236 1C04	No	Yes	Vaccination
014 3A02	Yes	Yes	Vaccination
019 3E05	Yes	Yes	Vaccination
039 5E03	No	Yes	Vaccination
HA Stalk			
296 2A04	No	Yes	Infection
301 1A04	No	Yes	Infection
296 2D02	Yes	Yes	Infection
296 2G04	Yes	Yes	Infection
294 1G04	Yes	No	Infection
NA			
300 2A04	Yes	Yes	Infection
301 1C05	No	No	Infection
296 2F04	No	No	Infection
294 1C02	No	Yes	Infection
294 1G03	Yes	No	Infection
NP			
296 2E01	Yes	No	Infection
294 1F04	Yes	No	Infection
301 1B05	Yes	No	Infection
301 1E05	Yes	No	Infection
294 1D02	Yes	No	Infection

Table S5. mAbs used in the antigen comparison cocktails.