

Supplementary

Table S1. Viruses used in HI assays

Designation	Accession ^a	Abbr	Vaccine	Clade	passage history ^b
A(H1N1)					
A/California/07/2009	EPI516528	Ca09	2010-16		C2, MDCK3
A/Michigan/45/2015	EPI4802570	Mi15	2017-19		M1, C2, MDCK3
A/Michigan/45/2015e	EPI859651	Mi15e	2017-19		X, E2
A/Brisbane/02/2018	EPI2195531	Br18	2020	1	MDCK3
A/Brisbane/02/2018e -IVR-190	EPI1322979	Br18e	2020	5a.2	E3
A/Victoria/2570/2019e- IVR	EPI1741926	Vi19e	2021	5a.2	E1
A/Sydney/5/2021	EPI2397515	Sy21	2022	5a.2a	MDCK3
A(H3N2)					
A/Switzerland/9715293/2013	EPI1868576	Sw13		3c3a	Siat, Siat9
A/New Caledonia/104/2014	EPI1868582	NC14		3c2a	MDCK1, Siat7
A/Singapore/INFIMH-16-0019/2016e	EPI1381189	Si16e	2018	3c2a1	E7
A/Newcastle/30/2016	EPI1868578			3c2a1	Siat5
A/Switzerland/8060/2017	EPI1326015	Sw17		3c2a2	Siat, Siat 2
A/Switzerland/8060/2017e	EPI1322581	Sw17e	2019	3c2a2	E7
A/Kansas/14/2017	EPI1868579	Ka17		3c3a	Siat2, Siat 3
A/Brisbane/60/2018	EPI1868580	Br18		3c2a1b +135K	Siat 3
A/Sydney/22/2018	EPI1485365	Sy18		3c2a1b +135N	Siat, Siat4 ^p
A/Newcastle/82/2018	EPI1595444	Nc18		3c2a1b.2	Siat4
A/South Australia/34/2019e	EPI1440494	SA19e	2020	3c2a1b.2	E7
A/Hong Kong/2671/2019e	EPI1694139	HK19e	2021	3c2a1b.1b	E8
B					
B/Brisbane/60/2008e (Vic)	EPI173277		2018		E2
B/Colorado/6/2017e (Vic)	EPI1126205		2019		E6, IZP
B/Washington/02/2019e (Vic)	EPI1482046		2020-21		E5
B/Phuket/3073/2013e (Yam)	EPI551297		2018-21		E7

a: accession codes for virus HA gene sequences in GISAID, <https://gisaid.org/>.

b: passaged in cells (C); embryonated hen's eggs (E); Madin-Darby canine kidney (MDCK) cell or MDCK cells stably transfected with α -2,6 sialyltransferase (Siat), undefined (X); further enrichment by gradient centrifugation to collect intrazonal pool (IZP).

Table S2. Influenza exposure and antibody titre profiles for each participant at enrolment

ID	Age Y	Prior vaccination or infection date(s)	Year(s) enrolled	Vaccination date(s) enrolment year 1	Average HI titre ^d		% viruses HI ≥40 ^d		NI titre	
					A(H3N2)	A(H1N1)	A(H3N2)	A(H1N1)	A(H3N2)	A(H1N1)
Documented prior vaccination										
8-08	2.3	30-Sep-19 ^a , 13-May-20	2021	16-Jul-21	51	5	100%	0%	12	9
9-04	2.6	25-May-18 ^a , 12-Apr-19	2020, 2021	6-May-20)	29	39	43%	43%	20	125
9-05	3.7	03-Jul-19 ^a	2020	6-May-20	1691	10	100%	0%	797	27
9-09	4.5	18-Jun-18 ^a	2020	13-May-20	331	97	100%	100%	55	2511
9-10	4.1	9-Sep-17, 6-May-18, 06-May-19	2020, 2021	25-May-20	331	11	100%	0%	94	74
9-11	3	3-Sep-18, 03-Jun-19	2020, 2021	2-Jun-20	20	97	0%	100%	9	4748
9-12	1.9	24-Apr-19	2020, 2021	1-Jun-20	26	5	29%	0%	1	1
Documented prior infection then vaccination (excluded)										
9-07	2.5	30-Dec-18; 07-May-19	2020, 2021	22-Apr-20	17	400	0%	100%	9	2488
Documented prior influenza A infection; A(H1N1) and A(H3N2) seropositive at enrolment										
8-06	4	21-Jul-19	2021	24-Jun-21, 21-Jul-21	103	40	100%	71%	114	523
Documented prior influenza A infection; A(H3N2) seropositive only at enrolment										
8-05	1.8	30-Sep-19	2021	21-May-21	21	5	14%	0%	9	48
8-07	2	25-Jun-19	2021, 2022	10-Jun-21	34	5	43%	0%	9	13
9-01	1.3	11-Aug-19	2019, 2020	22-Aug-19, 19-Sep-19 ^b	97	5	100%	0%	20	1
9-02	1.5	29-Aug-19	2019, 2020	10-Oct-19, 7-Nov-19 ^b	74	7	100%	0%	51	94
9-03	2.3	29-Aug-19	2020, 2021	21-Apr-20, 19-May-20 ^c	354	5	100%	0%	243	1
9-08	1	23-Jul-19	2020	22-Apr-20	263	5	100%	0%	158	1
9-13	1.5	02-Aug-19	2020	24-Jun-20	206	5	100%	0%	145	1
No documented prior vaccination or infection; A(H3N2) seropositive only at enrolment										
9-06	4.2	unknown	2020	21-Apr-20	149	5	100%	0%	322	8
No documented prior vaccination or infection; A(H1N1) and A(H3N2) seropositive at enrolment										
8-09	0.6	none	2021	12-Jul-21, 11-Aug-21	63	19	100%	14%	5	18
No documented prior vaccination or infection; seronegative at enrolment										
8-04	0.5	none	2019	30-Sep-19, 7-Nov-19 ^b	7	6	0%	0%	1	12

a: for 2-dose vaccination schedules the date of the second dose is shown

b: vaccines used for children aged < 3Y contained 7.5 µg of each HA (half-dose) according to the National Immunization Program in Australia in 2019.

c: contrary to the protocol, blood was drawn after vaccine dose 1 instead of dose 2

d: seven A(H3N2) and seven A(H1N1) viruses representing strains circulating, or in vaccines during children's lifetimes were considered as per Figure 1.

Table S3. Intervals in days between vaccination and sample collection ^a

PID	Classification	Year 1			Year 2		
		Visit 1	Visit 2	Visit 3	Visit 1	Visit 2	Visit 3
8-04	no prior	0	12	27			
8-05	prior A(H3N2)	0		18			
8-06	prior influenza A	0	7	28			
8-07	prior A(H3N2)	0	7	28	0	7	28
8-08	prior vaccination	0	7	25			
8-09	unclear prior	0	7	29			
9-01	prior A(H3N2)	0		28	0	7	28
9-02	prior A(H3N2)	0	7	29	0	7	27
9-03 ^b	prior A(H3N2)	0	7	28	0	7	
9-04	prior vaccination	0	6	30	0	8	28
9-05	prior vaccination	0	7	28			
9-06	prior A(H3N2)	0	7	28			
9-07	prior infection & vaccination	0	8	28	0		27
9-08	prior A(H3N2)	0	14	42			
9-09	prior vaccination	0	7	28			
9-10	prior vaccination	0	7	28	0	7	28
9-11	prior vaccination	0	7	28	0	7	33
9-12	prior vaccination	0	8	28	0	7	28
9-13	prior A(H3N2)	0	7	21			

a: intervals that were outside the specified ranges for post-vaccination visit 1 (7 +5 days) and post-vaccination visit 2 (d28 +/- 7)

b: contrary to the protocol, blood was drawn 7 and 28 days after vaccine dose 1 instead of dose 2

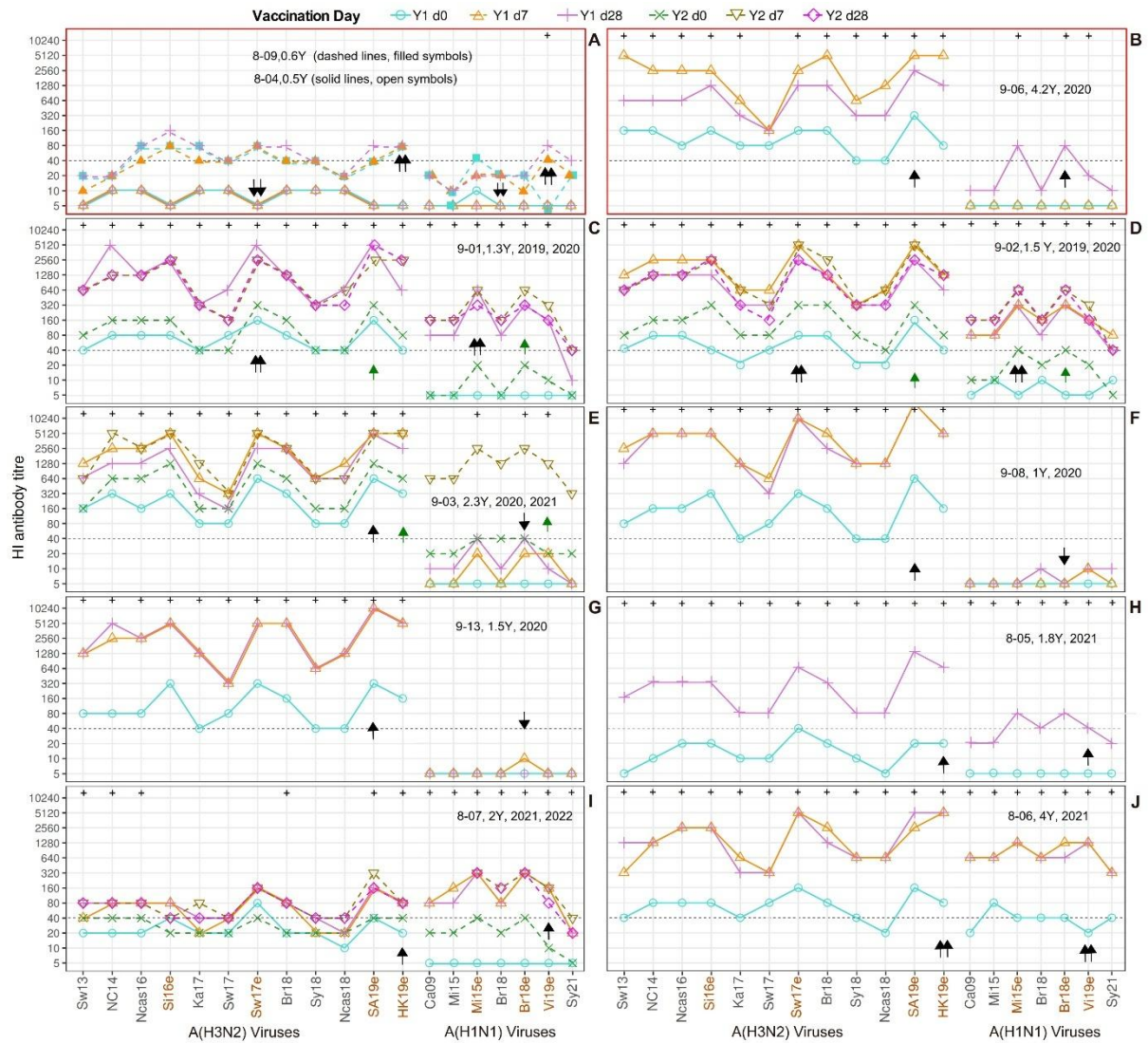


Figure S1. Antibody titre landscapes across the course of vaccination for children who lacked documented prior vaccination, including children with and without documented prior influenza A infection. Hemagglutination inhibition (HI) antibody titres are shown for a range of A(H3N2) and A(H1N1) viruses representing strains that circulated or were in vaccines during children's lifetimes. Each plot shows pre and post vaccination timepoints (legend) for an individual child, except panel A which shows two children. Panels A and B (red boxes) show children who lacked documented prior influenza A infection. Panels C-H are children with documented prior influenza A infection. Children's ID numbers, ages, and study years are shown in each panel. Abbreviated virus names (x axis) are coloured brown for egg-grown viruses and black for cell-grown viruses. Full names are given in Table S1. Arrows (▼▲) indicate the vaccine strains each child received in year 1. Double arrows (↕) indicate that two doses were given, with the exception of participant 9-03 who received two-doses but had blood-draws performed after dose 1. Green arrows (▲) indicate vaccine strains received in the second study year for children who re-enrolled. + indicates a four-fold rise in titres after vaccination (seroconversion) in the first year enrolled (Y1).

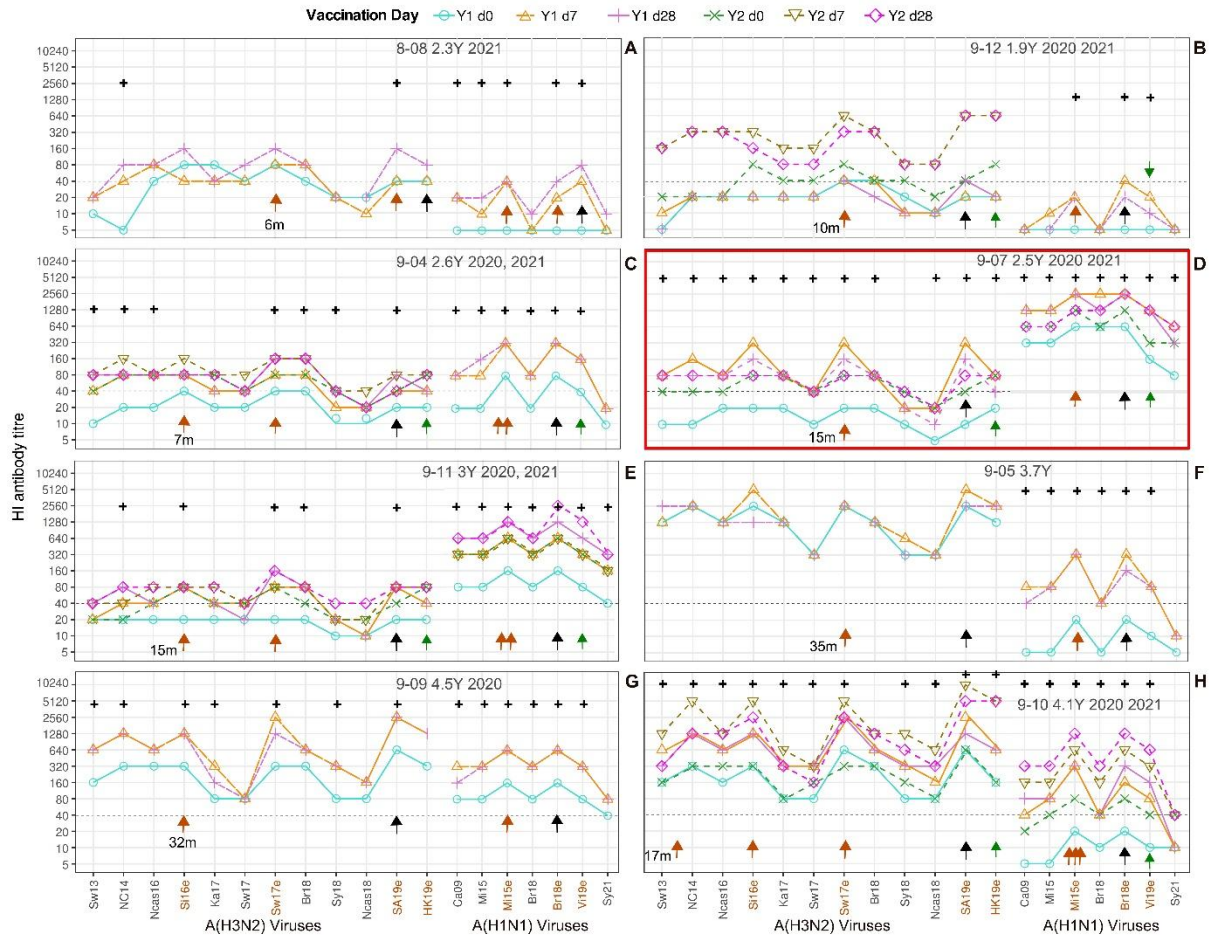


Figure S2. Antibody titre landscapes across the course of vaccination for children with documented prior influenza vaccination. Panels A-H show results for eight children with prior vaccination, of whom one (9-07, panel D, red outline) also had prior infection. Plots are constructed as described in Figure S1. Black arrows (▲) indicate strains in the vaccine each child received in the year they enrolled. Brown arrows (▲) indicate prior vaccine strains received, with age in months of children indicated next to their first documented vaccination. Green arrows (▲) indicate vaccine strains received in the second study year for children who re-enrolled. + indicates a four-fold rise in titres after vaccination (seroconversion). Participant 9-10 (panel H) received vaccine containing A/Hong Kong/4801/2014 in 2017, which was not among the viruses tested, only a cell-grown equivalent (A/New Caledonia/104/2014 (NC14)).

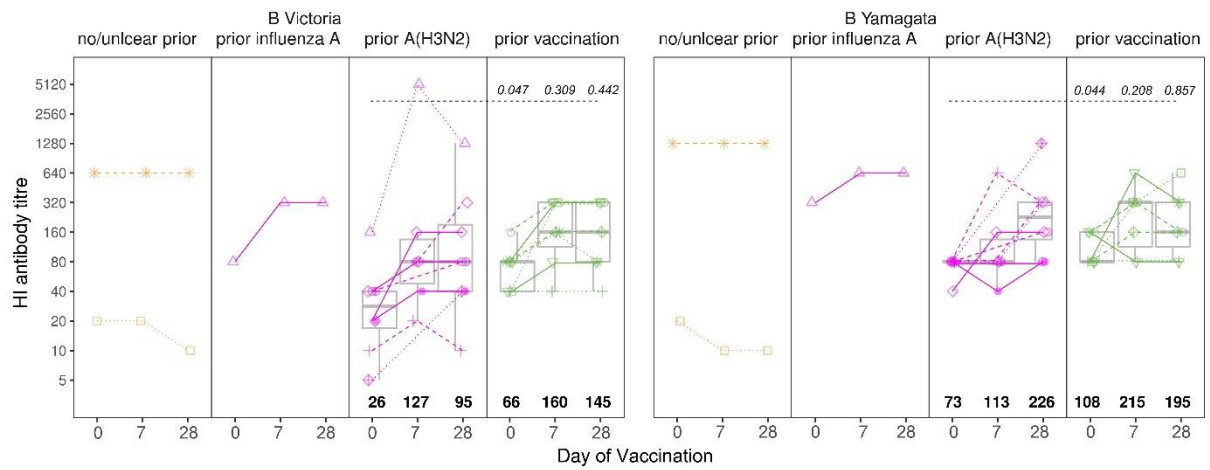


Figure S3. HI antibody titres against homologous influenza B vaccine strains before and after vaccination in the first year of enrolment. Children were classified based documented prior history of influenza A infection or vaccination and on seropositivity at enrolment. Viruses used were egg-grown and ether split B. Symbols show results for individual participants. Boxplots represent medians and interquartile ranges. Numbers in bold below box plots show geometric mean titres (GMT). Sera with HI titres below 10, the limit of detection, were assigned a value 5.

Table S4. Comparison of study year 1 post-vaccination titres against A(H1N1) versus A(H3N2) vaccine strains

Prior exposure group	pid	Timepoint	A(H1N1) titre	A(H3N2) titre	H3N2/H1N1 titre ratio
no prior	8-04	d7	5	5	1
no prior	8-09	d7	40	80	2
prior A unknown	8-06	d7	1280	5120	4
prior A(H3N2)	8-07	d7	160	80	0.5
prior A(H3N2)	9-02	d7	320	5120	16
prior A(H3N2)	9-03	d7	20	5120	256
prior A(H3N2)	9-06	d7	5	5120	1024
prior A(H3N2)	9-08	d7	5	20480	4096
prior A(H3N2)	9-13	d7	10	10240	1024
Geometric Mean (+/- 95% CI)					144 (10-2098)
prior vaccination	8-08	d7	40	40	1
prior vaccination	9-04	d7	320	40	0.125
prior vaccination	9-05	d7	320	5120	16
prior vaccination	9-09	d7	640	2560	4
prior vaccination	9-10	d7	160	2560	16
prior vaccination	9-11	d7	640	80	0.125
prior vaccination	9-12	d7	40	20	0.5
Geometric Mean (+/- 95% CI)					1.3 (0.3-6.2)
no prior	8-04	d28	5	5	1
no prior	8-09	d28	80	80	1
prior A unknown	8-06	d28	1280	5120	4
prior A(H3N2)	8-05	d28	40	640	16
prior A(H3N2)	8-07	d28	160	80	0.5
prior A(H3N2)	9-01	d28	640	5120	8
prior A(H3N2)	9-02	d28	320	2560	8
prior A(H3N2)	9-03	d28	40	5120	128
prior A(H3N2)	9-06	d28	80	2560	32
prior A(H3N2)	9-08	d28	5	20480	4096
prior A(H3N2)	9-13	d28	5	10240	2048
Geometric Mean (+/- 95% CI)					45 (6-364)
prior vaccination	8-08	d28	80	80	1
prior vaccination	9-04	d28	320	80	0.25
prior vaccination	9-05	d28	160	2560	16
prior vaccination	9-09	d28	640	2560	4
prior vaccination	9-10	d28	320	1280	4
prior vaccination	9-11	d28	1280	80	0.0625
prior vaccination	9-12	d28	20	40	2
Geometric Mean (+/- 95% CI)					1.3 (0.3-5.4)

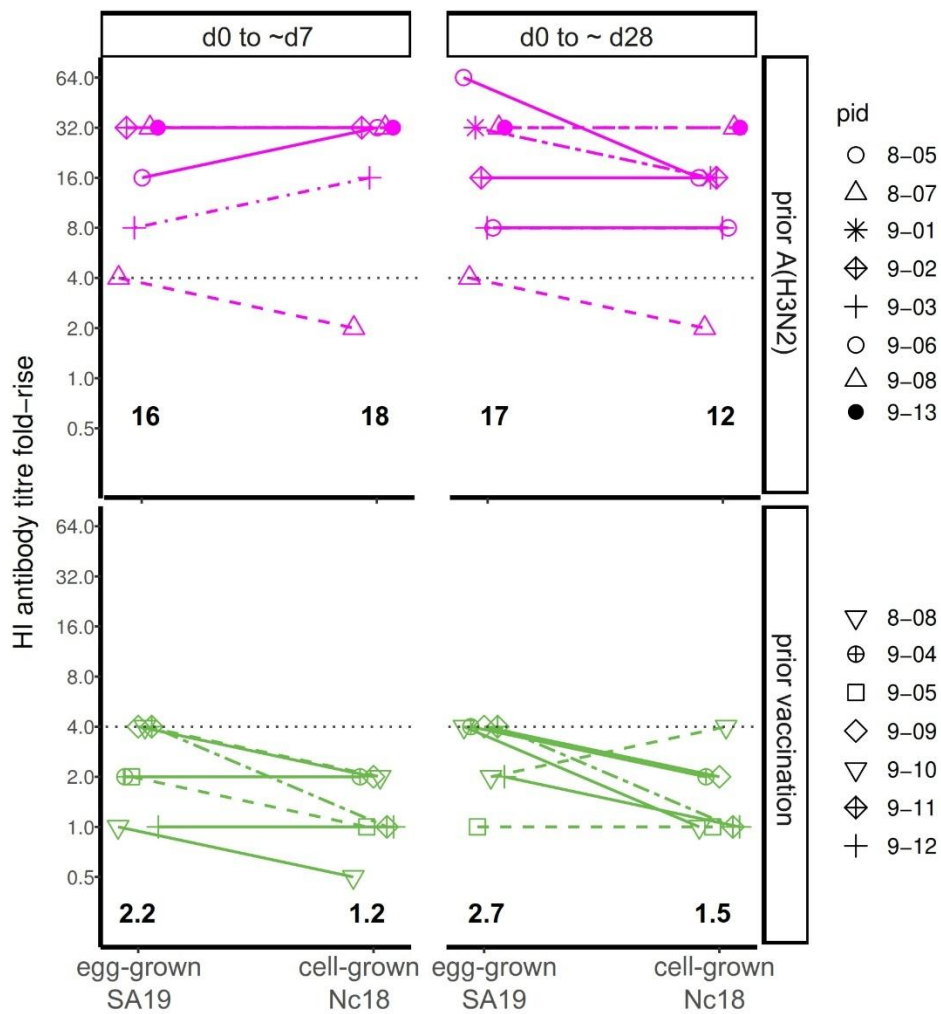


Figure S4. Vaccine-induced HI antibody titre fold-rise against egg versus cell-grown equivalents of the 2020 A(H3N2) vaccine strain among children classified as prior infection versus prior vaccination. HI antibody titre fold-rise against egg-grown A/South Australia/34/2019 (SA19) versus SIAT cell-grown A/Newcastle/82/2018 (Nc18), which differ by three amino acids within HA (K160T, I186V, F219S) due to egg-adaptation. Data are shown for ~d7 and ~d28 after vaccination. Lines connect values for the sample against egg- and cell-grown viruses. Values below symbols are geometric mean rises for each group.

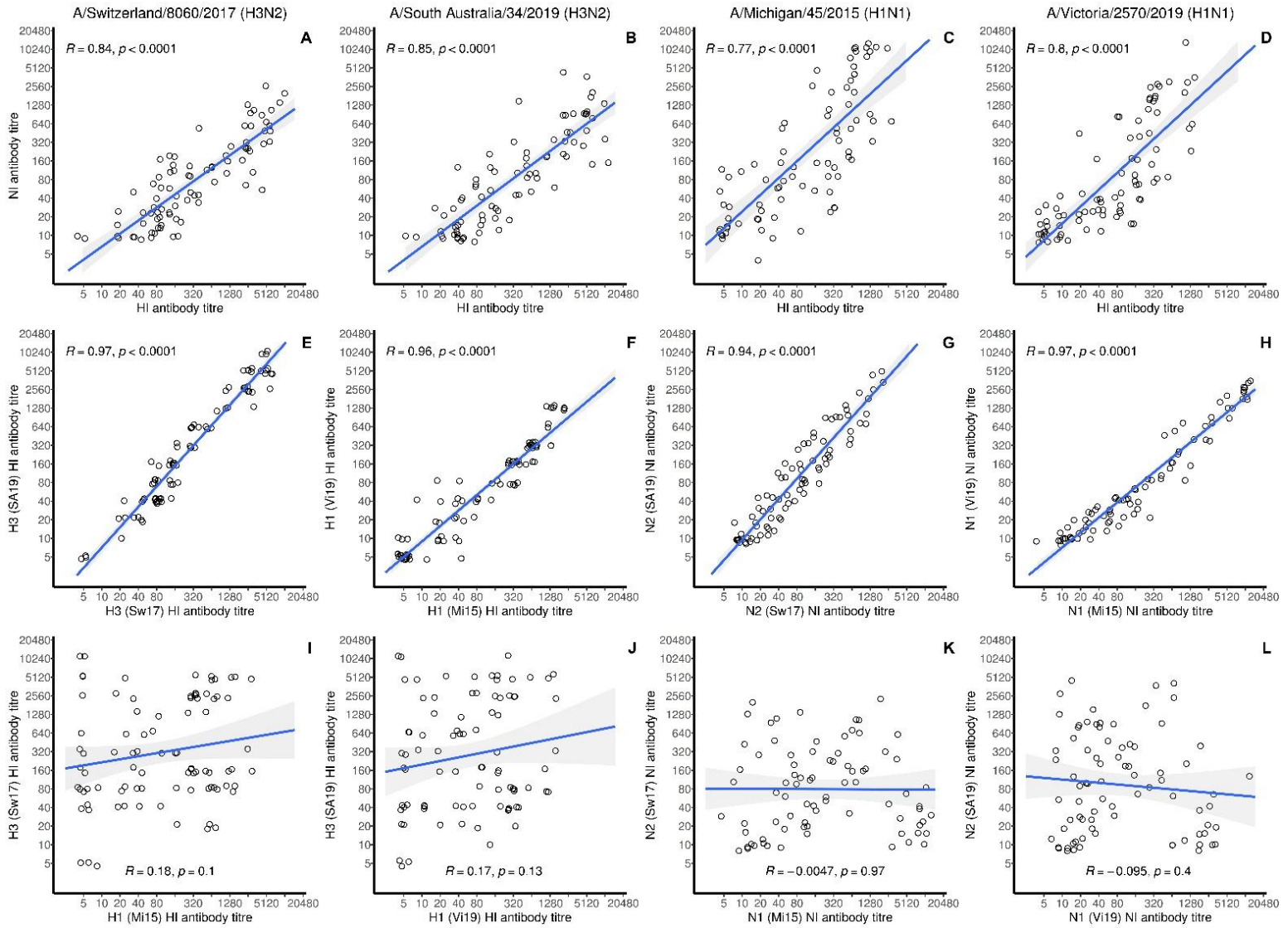


Figure S5. HI and NI antibody titres correlate well within strains and subtypes but not across A(H3N2) and A(H1N1) subtypes. (A-D) HI versus NI titres against equivalent strains including two A(H3N2) and two A(H1N1) strains. **(E-F)** HI titres against unique strains belonging to the same subtype. **(G-H)** NI titres against unique strains belonging to the same subtype. **(I-J)** HI titres against A(H1N1) versus A(H3N2) strain in 2019 and 2020 vaccines, respectively. **(K-L)** NI titres against A(H1N1) versus A(H3N2) strain in 2019 and 2020 vaccines, respectively. Each dot represents a unique sample collected before or after vaccination in year 1.

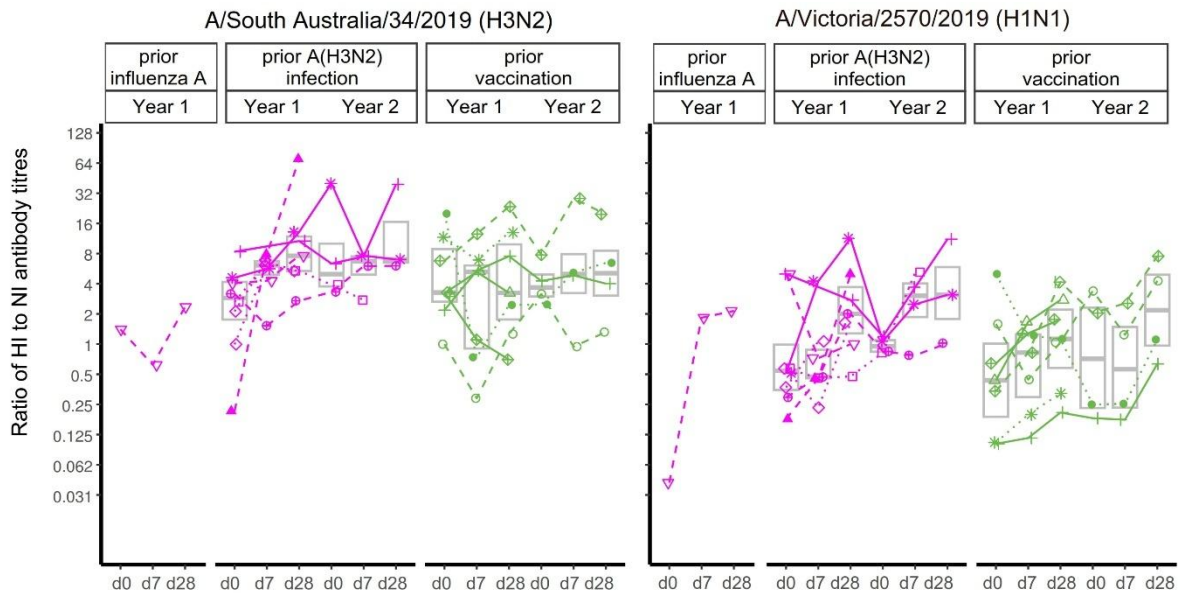


Figure S6. HI to NI antibody titre ratios. Ratios comparing antibody titres measured by HI versus NI assay against A(H3N2) and A(H1N1) strains in the 2020 vaccine. Results are shown for children with prior influenza A infection of vaccination. Children with prior infection are further divided by seropositive (SP) status at enrolment: A(H3N2) only or both A(H3N2) and A(H1N1) referred to as prior influenza A. Symbols represent results for individual participants, and boxplots represent medians and interquartile ranges. Geometric mean ratios shown in bold below each box.

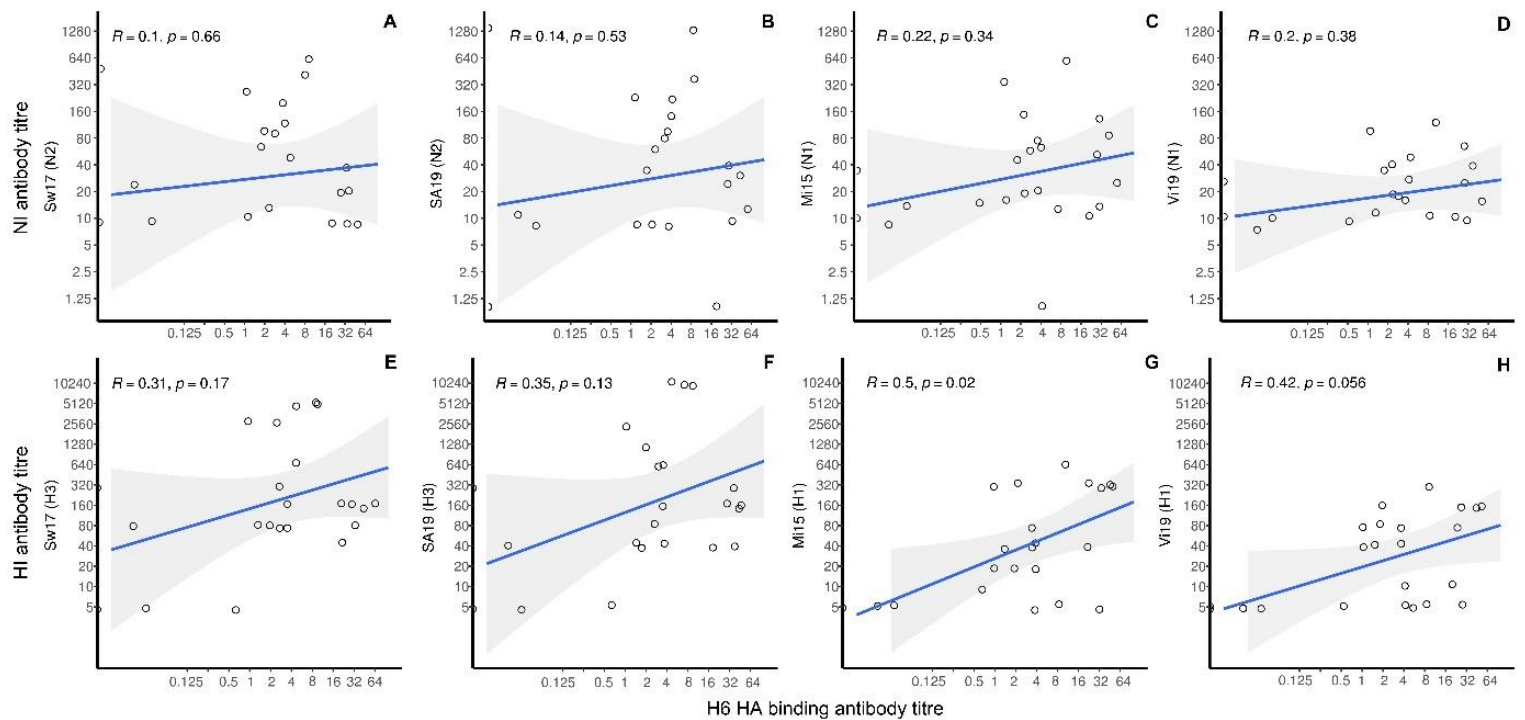


Figure S7. NI versus H6 HA binding antibody titres. (A-D) H6 HA binding antibody titres (x-axis) are compared with NI antibody titres against each strain tested including A/Switzerland/8060/2017(Sw17) and A/South Australia/34/2019 (SA19) H3N2 strains and A/Michigan/45/2015 (Mi15) and A/Victoria/2570/2019 (Vi19) H1N1 strains. (E-H) H6 HA binding antibody titres (x-axis) are compared with HI antibody titres for each strain. A subset of samples were tested.

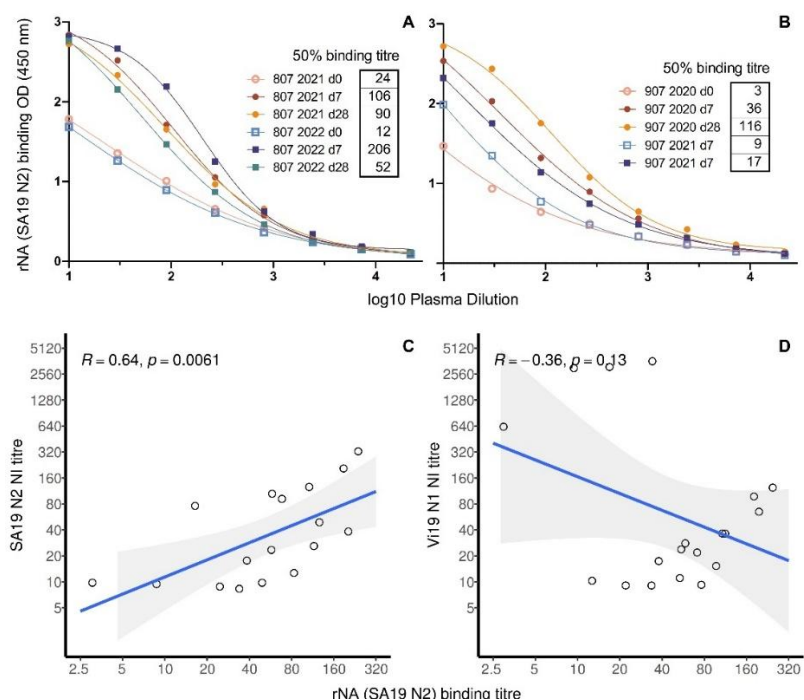


Figure S8. N2 binding antibody titres increase after vaccination and correlate with NI titres against the same virus but not against an A(H1N1) virus. Plasma dilution versus OD450 nm for antibody binding to recombinant NA of A/South Australia/34/2019 (H3N2) (SA19) are shown for plasma collected across two years of vaccination for a participant with prior infection (**A**) and prior vaccination (**B**). N2 NA binding titres for all plasma tested are correlated with NI antibody titres against virus containing the same N2 NA (**C**) or heterosubtypic N1 NA of A/Victoria/2570/2019 (H1N1) (Vi19) (**D**).

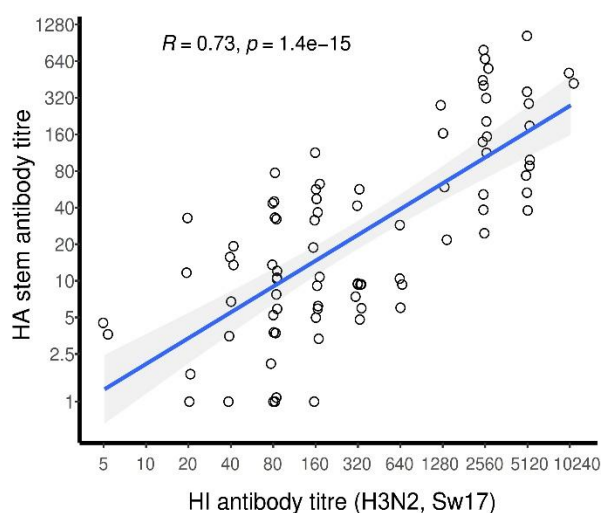


Figure S9. H3N2 HA stem binding antibody titres correlate with HI titres against A/Switzerland/8060/2017 (H3N2) virus (Sw17). Shading indicates 95% confidence intervals for the regression. P values are shown for two-sided test for association between paired samples, using Pearson's product moment correlation coefficient.