



# Influenza virus infection history shapes antibody responses to influenza vaccination

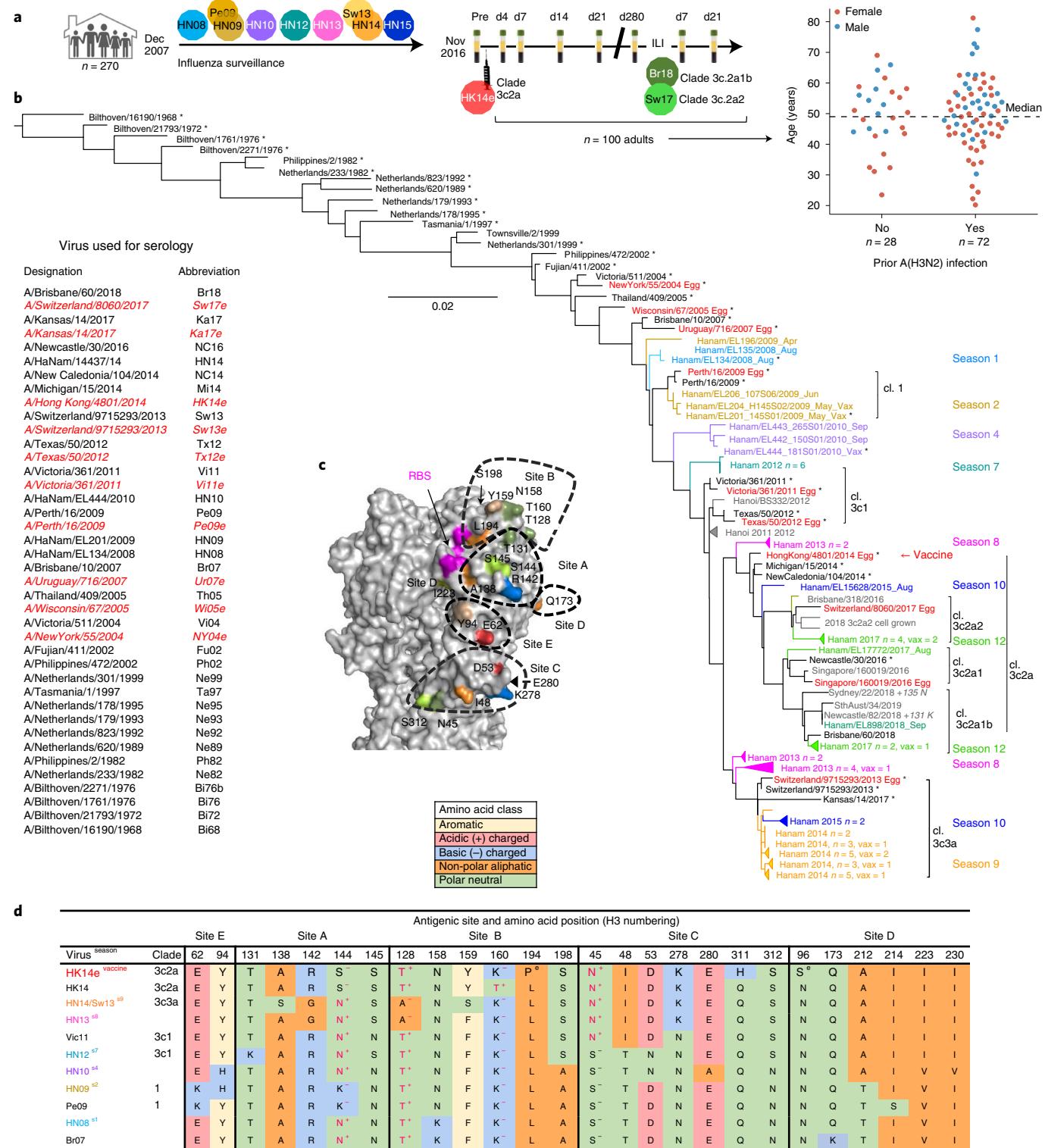
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**Studies of successive vaccination suggest that immunological memory against past influenza viruses may limit responses to vaccines containing current strains. The impact of memory induced by prior infection is rarely considered and is difficult to ascertain, because infections are often subclinical. This study investigated influenza vaccination among adults from the Ha Nam cohort (Vietnam), who were purposefully selected to include 72 with and 28 without documented influenza A(H3N2) infection during the preceding 9 years (Australian New Zealand Clinical Trials Registry 12621000110886). The primary outcome was the effect of prior influenza A(H3N2) infection on hemagglutinin-inhibiting antibody responses induced by a locally available influenza vaccine administered in November 2016. Baseline and postvaccination sera were titrated against 40 influenza A(H3N2) strains spanning 1968–2018. At each time point (baseline, day 14 and day 280), geometric mean antibody titers against 2008–2018 strains were higher among participants with recent infection (34 (29–40), 187 (154–227) and 86 (72–103)) than among participants without recent infection (19 (17–22), 91 (64–130) and 38 (30–49)). On days 14 and 280, mean titer rises against 2014–2018 strains were 6.1-fold (5.0- to 7.4-fold) and 2.6-fold (2.2- to 3.1-fold) for participants with recent infection versus 4.8-fold (3.5- to 6.7-fold) and 1.9-fold (1.5- to 2.3-fold) for those without. One of 72 vaccinees with recent infection versus 4 of 28 without developed symptomatic A(H3N2) infection in the season after vaccination ( $P = 0.021$ ). The range of A(H3N2) viruses recognized by vaccine-induced antibodies was associated with the prior infection strain. These results suggest that recall of immunological memory induced by prior infection enhances antibody responses to inactivated influenza vaccine and is important to attain protective antibody titers.**

**R**NA viruses undergo relatively rapid mutation, which can critically impact vaccination strategies<sup>1</sup>. Influenza viruses are particularly prone to substitutions within the major surface protein, hemagglutinin (HA), as a consequence of viral RNA replication without proofreading<sup>2</sup> and selection of human antibody escape mutants. This process, termed antigenic drift, facilitates recurrent influenza infection throughout life. In turn, prevention by vaccination requires repeated administration of vaccine containing regularly updated virus strains. Vaccine effectiveness (VE) has been found to be poor against A(H3N2) viruses from 2010 onward, when VE estimation by subtype became more widely implemented<sup>3</sup>. This could, in part, be due to greater mismatch between vaccine and circulating strains. A(H3N2) viruses have

undergone greater antigenic evolution compared to A(H1N1) and B influenza viruses<sup>4</sup>, and more often acquire substitutions within antigenic sites when propagated in eggs to produce vaccine<sup>4,5</sup>. It is further speculated that vaccine immunogenicity and effectiveness may be limited by recall of immunological memory against past strains, a hypothesis that was first proposed in the 1960s and termed original antigenic sin<sup>6</sup>. Interest in this phenomenon has been revived by a series of recent reports that antibody responses<sup>7</sup> and VE against A(H3N2) viruses<sup>8–11</sup> are attenuated among people who received vaccine in prior years. A meta-analysis indicates that although adverse effects of repeat vaccination are more pronounced for A(H3N2) than for other subtypes, there is substantial heterogeneity in effects<sup>12</sup>.

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**Fig. 1 | Participant selection and investigation of previously circulating A(H3N2) viruses.** **a**, Study design and timeline. **d**, day; Dec, December; Nov, November; Pre, before. **b**, Phylogenetic tree of the HA genes of viruses recovered from Ha Nam cohort ILI cases (colored by season) and viruses used to construct antibody landscapes (colored black if cell grown or red if egg grown). Viruses from participants of the vaccine study are indicated by the suffix 'vax'. Clades (cl.) and subclades are delineated using parentheses. **c**, Model of the globular head of HK14e HA (SWISS-MODEL: AOAOKOYAS1), showing amino acid positions within antigenic sites A to E that differed from at least one of the prior infecting strains and receptor binding site (RBS) residues. **d**, Antigenic site positions that varied between HK14e and at least one prior infecting strain are tabulated and shaded according to amino acid properties. Substitutions that result in gain (+) or loss (−) of glycosylation are colored in pink. Egg-adapted substitutions are indicated by a superscript e. The asterisks in **b** and **d** indicate the viruses that were used for landscape serology, and that are listed in the associated table.

The cellular and molecular mechanisms underlying the variable effects of prior vaccination and pre-existing immunity remain largely undefined. The antigenic distance hypothesis postulates that when successive vaccine strains are antigenically similar, existing antibodies or memory B cells attenuate vaccine immunogenicity by masking or clearing vaccine antigen, resulting in attenuated VE if the vaccine and epidemic strains differ, but not if they are similar<sup>13</sup>. Alternately, it is hypothesized that memory B cells induced by prior vaccination dominate and focus responses on epitopes that are conserved between prior and prevailing vaccine strains, compromising responses against epitopes that have changed<sup>14</sup>. This could enhance antibody responses and VE, if epidemic strains retain those conserved epitopes but reduce VE if these epitopes have changed<sup>14</sup>. The epitopes recognized by influenza virus neutralizing antibodies are largely located on the globular head of HA, surrounding the receptor binding site<sup>15</sup>. Up to 131 amino acid positions in the head of HA of A(H3N2) viruses have been associated with antigenic variation and assigned to one of five antigenic sites, designated A to E<sup>16,17</sup>. Antigenic sites A and B are immunodominant<sup>16</sup>, and single amino acid substitutions in these sites can result in escape from vaccine-induced immunity, particularly if glycosylation sites are introduced<sup>14,18</sup>.

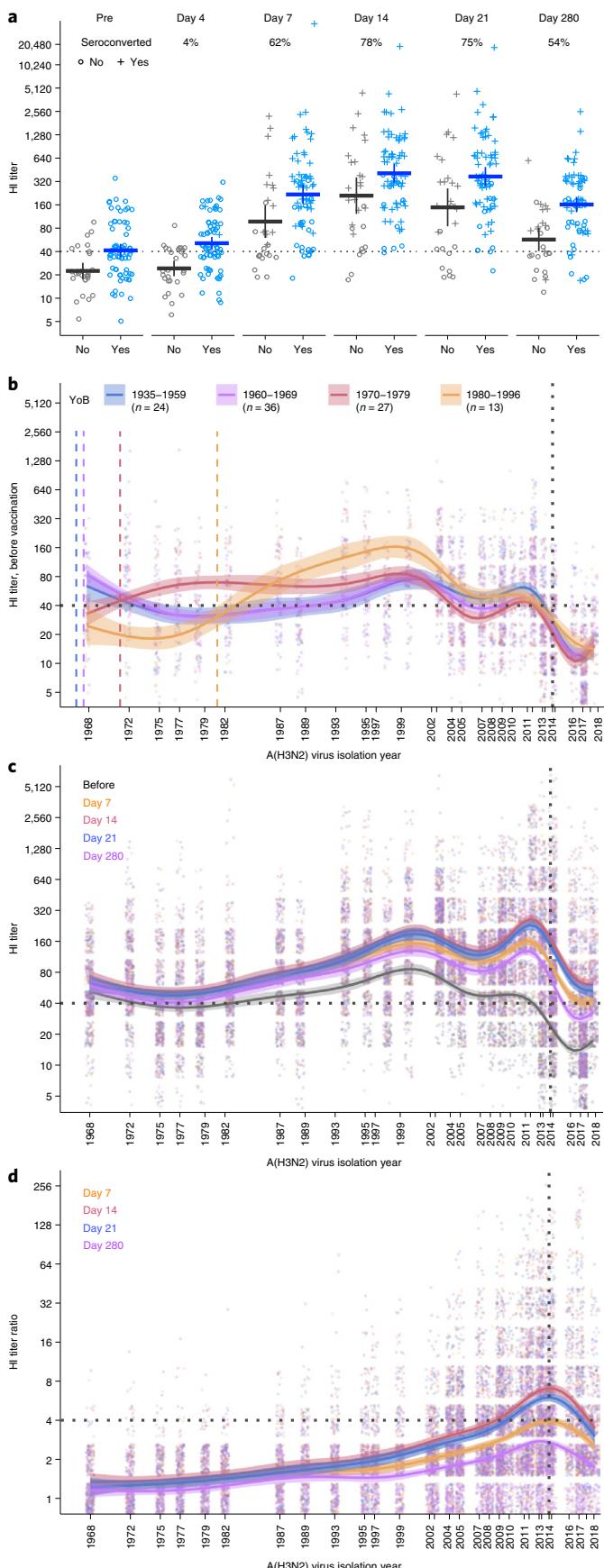
Few studies consider how prior influenza infections affect the immunogenicity and protection afforded by influenza vaccines. Understanding infection history is contingent on detecting asymptomatic or subclinical infection, which may account for up to three-quarters of influenza virus infections<sup>19,20</sup>. To this end, we investigated vaccine immunogenicity among participants of a cohort in northern Vietnam (Ha Nam cohort)<sup>19</sup> who were influenza vaccine naïve and had been monitored for clinical or subclinical influenza infection for 9 years.

## Results

**Study design.** The primary study objective was to determine the effect of recent influenza A(H3N2) infection on vaccine-induced antibody responses against A(H3N2) viruses. Therefore, adult participants of the Ha Nam cohort were purposefully selected based on influenza infection history during the preceding 9 years of cohort participation (ANZCTR 12621000110886) (Fig. 1a and Extended Data Fig. 1). The Ha Nam cohort commenced in December 2007, when 945 members of 270 randomly selected households were enrolled to participate in monitoring for influenza infection via active influenza-like illness (ILI) surveillance, RT-PCR on swabs and serology on blood samples collected annually or biannually to detect

**Fig. 2 | Kinetic and strain coverage of the A(H3N2) virus-reactive antibody response to vaccination.** **a**, Titers against the HK14e vaccine strain are shown for each participant ( $n=100$ ) by time point and prior A(H3N2) infection status. Each symbol represents a biologically independent sample. Crosses indicate titers that were at least fourfold higher than prevaccination titers (seroconverted, legend). Bars and error bars show geometric means and 95% confidence intervals, respectively. **b**, Prevaccination titer landscapes across strains spanning 1968 to 2018 were estimated using GAMs. Participants are grouped by year of birth (YoB), with dashed and color-matched vertical lines representing the earliest strain that participants could have been exposed to. Numbers of biologically independent samples tested for each age group and antigen are shown. **c**, Antibody titer landscapes for prevaccination (gray-shaded area) and postvaccination (colored lines) time points are compared for all 100 vaccinees. **d**, Fitted landscapes of postvaccination titer rise are shown for all 100 vaccinees. Shading in **b-d** indicates 95% confidence intervals for the model, and dots show individual participant titers against antigens representing each year. Dotted horizontal lines indicate thresholds for seropositivity or seroconversion. Dotted vertical lines in **b-d** indicate the position of the vaccine antigen.

seroconversion<sup>19</sup>. The vaccine study commenced November 2016. Participants were considered for inclusion if they were aged  $\geq 18$  years and had completed all investigations to detect influenza infec-



**Table 1 | Proportions of participants with and without recent A(H3N2) virus infection who were seropositive or seroconverted against vaccine and subsequently circulating strains**

Prior H3N2	Test antigen <sup>clade</sup>	Seropositive (HI ≥ 40)								Seroconverted					
		Before		Day 7		Day 14		Day 21		Day 280 <sup>a</sup>		Day 14		Day 280 <sup>a</sup>	
		n (%)	P	n (%)	P	n (%)	P	n (%)	P	n (%)	P	n (%)	P	n (%)	P
No	HK14e <sup>3c2a</sup>	8 (29)	0.000	25 (89)	0.065	26 (93)	0.076	23 (82)	0.006	21 (78)	0.026	18 (64)	0.058	12 (44)	0.180
Yes		51 (71)		71 (99)		72 (100)		71 (99)		66 (94)		60 (83)		42 (60)	
No	Mi14 <sup>3c2a</sup>	1 (4)	0.001	16 (57)	0.001	20 (71)	0.000	19 (68)	0.000	17 (63)	0.114	20 (71)	0.064	12 (44)	0.656
Yes		25 (35)		64 (89)		71 (99)		69 (96)		56 (80)		64 (89)		36 (51)	
No	NC16 <sup>3c2a1</sup>	3 (11)	0.002	17 (61)	0.003	21 (75)	0.000	19 (68)	0.000	15 (56)	0.008	17 (61)	0.140	11 (41)	0.652
Yes		31 (43)		65 (90)		72 (100)		69 (96)		58 (83)		55 (76)		33 (47)	
No	Br18 <sup>3c2a1b</sup>	5 (18)	0.020	20 (71)	0.003	25 (89)	0.065	25 (89)	0.683	15 (56)	0.008	13 (46)	0.066	7 (26)	0.805
Yes		32 (44)		68 (94)		71 (99)		67 (93)		58 (83)		49 (68)		22 (31)	
No	Ka17 <sup>3c3a</sup>	0 (0)	0.017	9 (32)	0.014	11 (39)	0.000	9 (32)	0.000	3 (11)	0.004	12 (43)	0.005	3 (11)	0.024
Yes		13 (18)		44 (61)		58 (81)		53 (74)		30 (43)		53 (74)		24 (34)	
No	Sw17 <sup>3c2a2</sup>	0 (0)	1.000	1 (4)	0.035	4 (14)	0.082	2 (7)	0.019	1 (4)	0.170	6 (21)	0.235	2 (7)	0.722
Yes		1 (1)		16 (22)		24 (33)		21 (29)		11 (16)		25 (35)		9 (13)	
No	H1N1pdm09	5 (18)	1.000					23 (82)	0.527			23 (82)	0.756		
Yes		14 (19)						63 (88)				62 (86)			

<sup>a</sup>On day 280, samples were provided by 27 of 28 participants without prior A(H3N2) infection and 70 of 72 participants with prior A(H3N2) infection. P values were determined by Fisher's exact test.

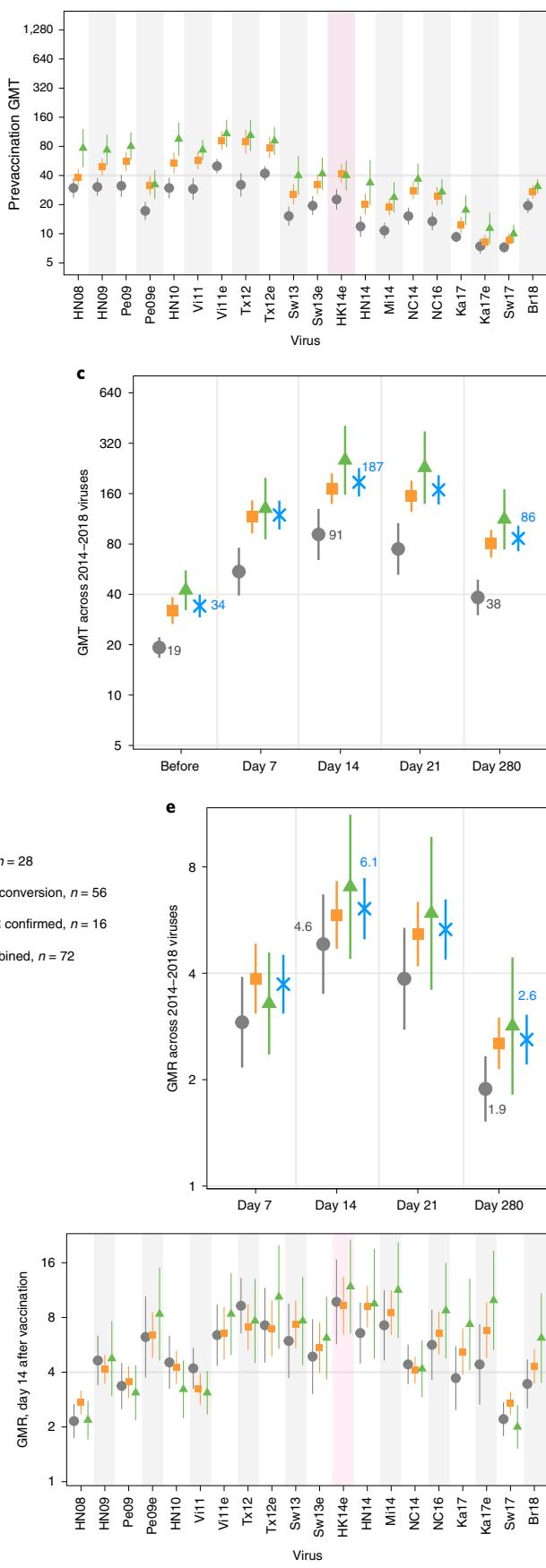
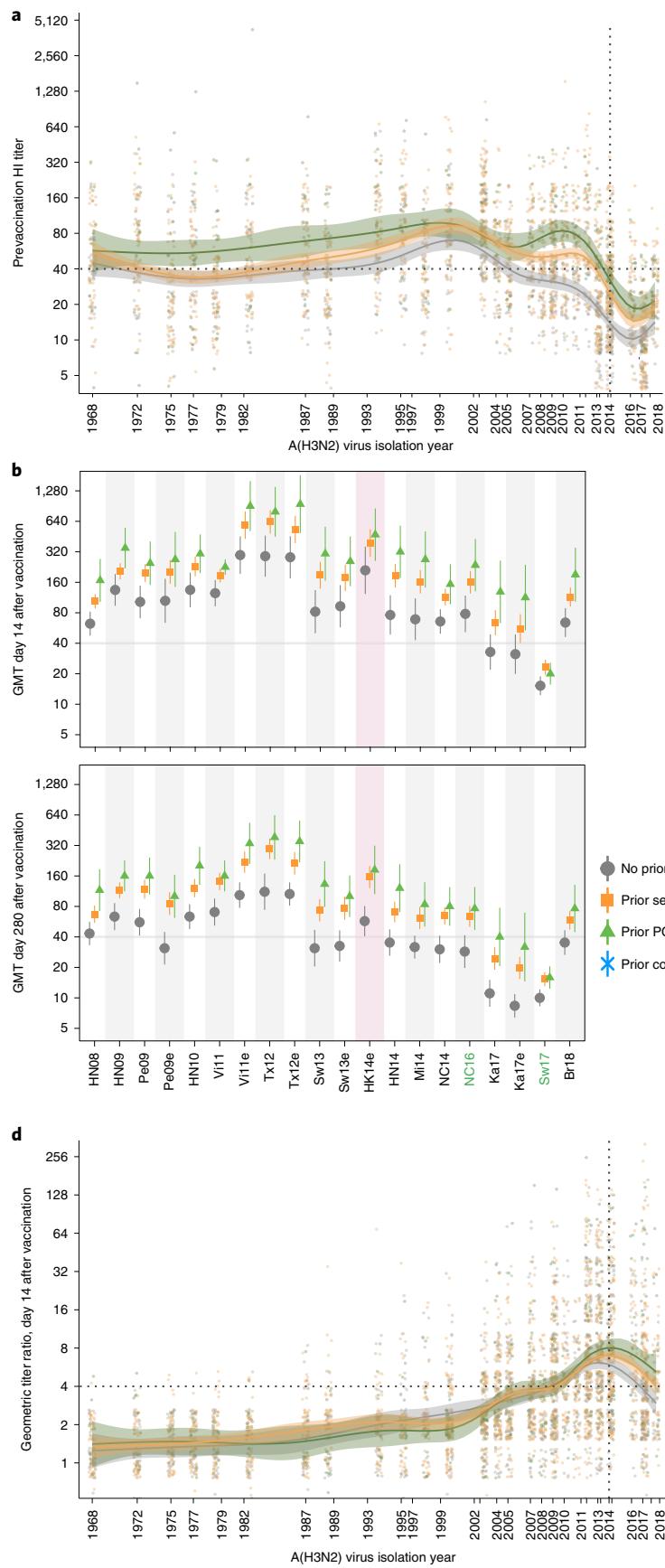
tions since December 2007. We aimed to recruit 50 participants with and 50 without documented A(H3N2) infection; however, only 32 of 161 eligible participants lacked laboratory-confirmed A(H3N2) infection during the preceding 9 years (Extended Data Fig. 1). We therefore selected 82 of 129 participants with prior A(H3N2) infection based on proximity of age to the 32 participants without prior infection and sex (Extended Data Fig. 1 and Supplementary Table 1). A total of 100 of 114 selected participants provided written informed consent to participate in the vaccine study, including 28 of 32 without prior A(H3N2) infection and 72 of 82 with prior A(H3N2) infection. Age and sex distributions were similar between participants with and without prior A(H3N2) infection (Fig. 1a), and 40 of 72 (55%) versus 14 of 28 (50%) had a prior A(H1N1) virus infection.

Participants received licensed, locally available influenza vaccine (Vaxigrip, Sanofi Pasteur) containing inactivated, chicken egg-grown A/Hong Kong/4801/2014 (H3N2), A/California/7/2009 (H1N1)pdm09 and B/Brisbane/60/2008 viruses. Blood samples were collected before and after vaccination (days 4, 7, 14, 21 and 280) and after infection, if detected in the subsequent season (Fig. 1a). Sera were tested by hemagglutination inhibition (HI) assay to determine antibody titers against 40 A(H3N2) viruses that circulated between 1968, when A(H3N2) viruses emerged in humans, and 2018, 4 years after the vaccine strain (Fig. 1b and Supplementary Table 2). The primary outcome was fold-rise in antibody titer, determined from geometric mean ratios (GMRs) on days 14 and 280 after vaccination, comparing vaccinees with and without A(H3N2) infection during the preceding 9 years.

**Description of prior A(H3N2) infections.** Prior A(H3N2) virus infection was detected as ILI, confirmed by RT-PCR, for 16 of 72 participants, and as seroconversion without ILI for 56 of 72; 51 of 72 had one recent prior A(H3N2) infection, 18 of 72 had two and 3 of 72 had three prior infections (Extended Data Table 1). The year that participants were last infected with an A(H3N2) virus ranged from 2008 to 2015. A(H3N2) viruses circulating during these years belonged to a range of genetic clades (Fig. 1b and Extended Data Table 1), and were distinct from the vaccine strain—A/Hong Kong/4801/2014 (HK14e), which belongs to clade-3c2a. Twenty-six antigenic site positions differed between at least one prior strain and HK14e (Fig. 1c,d). Viruses circulating in 2014 (HN14/Sw13-like, clade-3c3a) differed substantially from HK14e in sites A and B, where seven amino acids were substituted, including six to amino acids that had different properties or affected a glycosylation site. Viruses circulating in 2012 (HN12-like, clade-3c1) and earlier differed more from HK14e in site C, where five positions were substituted to an amino acid with different properties or affecting a glycosylation site (Fig. 1d).

**Antibody responses to the vaccine A(H3N2) strain.** Analysis of the kinetics of antibody production against the HK14e vaccine strain (Fig. 2a) showed that vaccination induced robust antibody production within 7 days. Titers were highest on day 14 and remained at least fourfold higher than prevaccination titers on day 280 for 54% of participants. Geometric mean titers (GMTs) were higher among participants who had recent A(H3N2) virus infection at all time

**Fig. 3 | Recent A(H3N2) virus infection enhances the titer and strain coverage of A(H3N2)-reactive antibodies induced by vaccination.** Titers and titer ratios are compared for vaccinees without recent A(H3N2) infection or with infection confirmed by seroconversion or RT-PCR. Numbers and symbols for each group are shown in the central legend. Numbers represent biologically independent samples tested against each antigen, with the exception that the prior combined infection group is also presented as separate prior seroconversion and prior PCR-confirmed groups. **a**, Prevaccination titers presented as GAM estimates against year of A(H3N2) virus circulation (left) or as GMTs for individual viruses (right). **b**, GMTs on days 14 (top) and 280 (bottom) after vaccination. **c**, GMTs averaged against 2008–2018 strains by time point. **d**, Postvaccination titer ratios presented as in **a**. **e**, GMRs averaged across 2014–2018 viruses by time point. Shading (**a**,**d**) or error bars (all panels) represent 95% confidence intervals. Horizontal lines indicate thresholds for seropositivity or seroconversion. Dotted vertical lines or red panels indicate the position of the vaccine antigen. GMT or GMR values are indicated for select groups in **c** and **e**.



points. GMRs for participants with and without prior infection were 9.8-fold (7.3- to 13.1-fold) versus 9.3-fold (5.8- to 14.8-fold) on day 14 and 4.0-fold (3.0- to 5.3-fold) versus 2.5-fold (1.6- to 4.0-fold) on day 280. Proportions seropositive (titer  $\geq 40$ ) and seroconverted (titer rise  $\geq 4$ -fold) were also higher among participants with recent infection (Table 1). Titers and titer rises were at least as high for older compared to younger adults, particularly for those with recent prior A(H3N2) infection (Extended Data Fig. 2). Recent A(H3N2) virus infection had little effect on the proportion of participants seropositive against A(H1N1)pdm09 in the vaccine (Table 1). These results indicate that recent A(H3N2) virus infection enhanced the capacity of the vaccine to induce and maintain A(H3N2)-reactive, but not A(H1N1)-reactive, antibodies. Therefore, effects of recent infection were likely to be mediated by subtype-specific memory B cells rather than by broadly cross-reactive B or T cells.

**Cross-reactivity of vaccine-induced antibodies.** The strain coverage of antibodies induced by vaccination was examined using generalized additive models (GAMs) to estimate titer and titer rise landscapes for viruses arranged by circulation year. Prevaccination antibody titers were relatively high against strains encountered early in life, as well as against 1993–2002 strains (Fig. 2b and Supplementary Fig. 2). Titer rise from day 7 to day 280 after vaccination diminished as virus genetic and temporal distance from HK14e increased and was negligible against the oldest strains (Fig. 2c,d and Supplementary Fig. 3). The boosting of titers against past strains, referred to as back-boosting, could reflect low-avidity antibody binding to past strains when antibody concentrations are high, as titer rise extended across more strains on day 14 after vaccination than on day 280 (Fig. 2d). However, back-boosting was largely limited to strains circulating after participant's birth years (Extended Data Fig. 3). This finding is consistent with a previous study<sup>21</sup> and suggests that back-boosting reflects recall of memory B cells induced by prior infections.

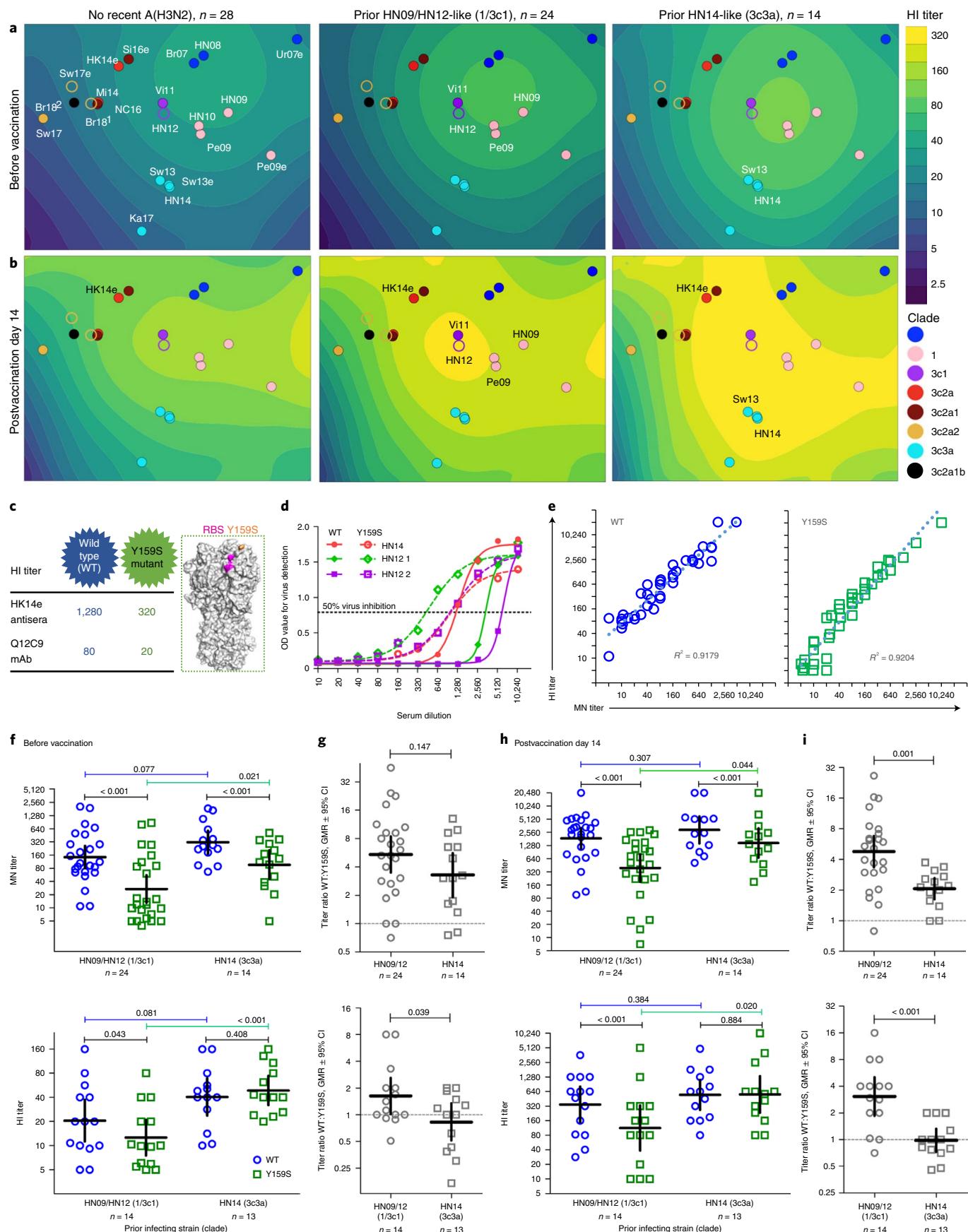
**Effect of recent infection on antibody cross-reactivity.** Antibody cross-reactivity was compared between participants with and without recent A(H3N2) infection confirmed by seroconversion, RT-PCR or the combination. Prevaccination titers differed mainly against strains circulating since 2008, corresponding with participant's infection status since December 2007 (Fig. 3a and Supplementary Tables 4 and 5). Differences increased after vaccination, most notably against strains circulating after the vaccine strain, and among participants with RT-PCR-confirmed infection (Fig. 3b,c and Extended Data Table 2). GMTs across 2008–2018 strains on days 14 and 280 were 187 (154–227) and 86 (72–103) for participants with recent infection versus 91 (64–130) and 38 (30–49) for participants without recent infection. Titer rise was similar against prior strains but was higher against vaccine and subsequently circulating strains among participants with recent A(H3N2) infection (Fig. 3d, Extended Data Table 3). GMRs averaged against vaccine

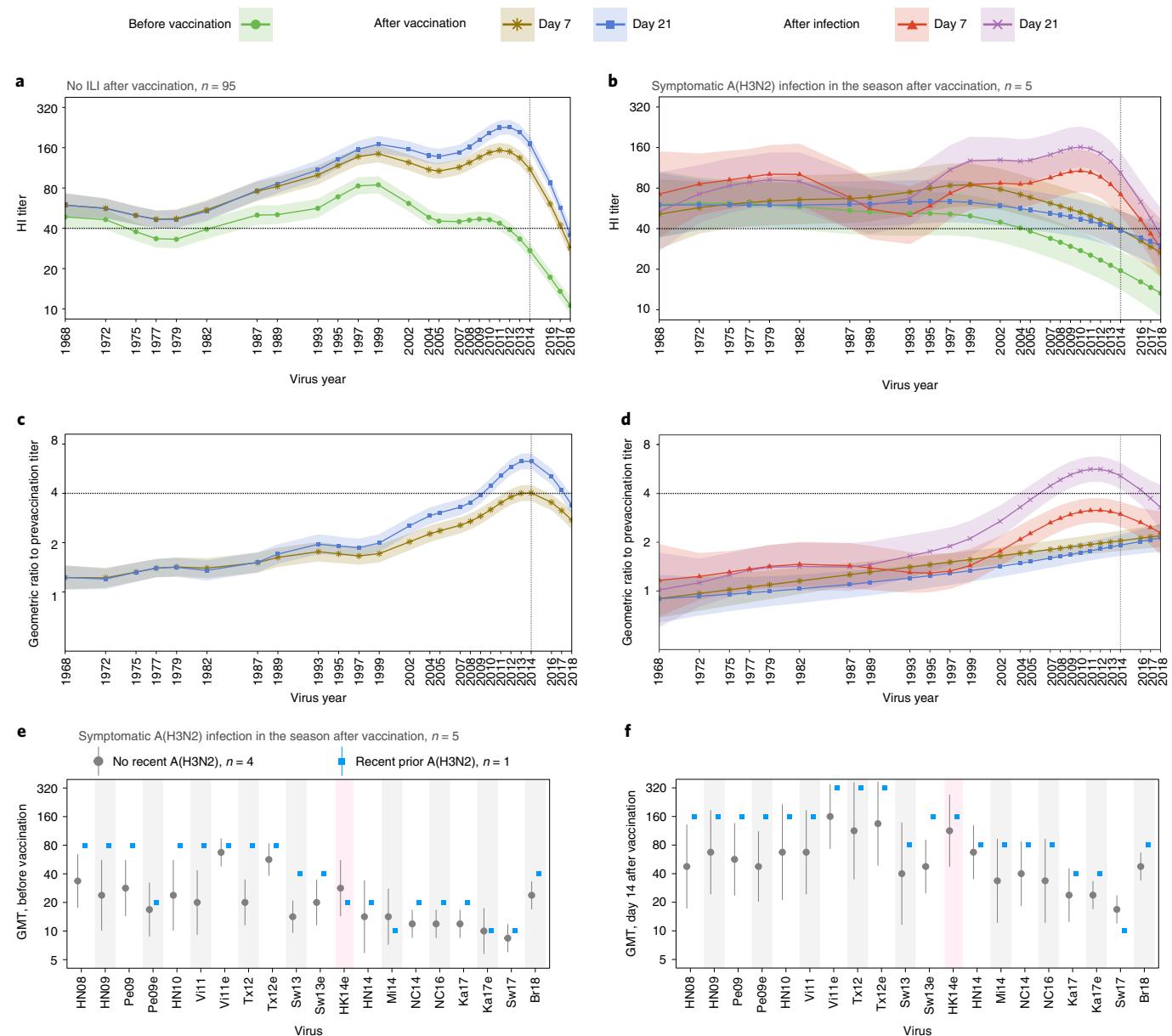
and subsequently circulating strains differed most on day 280, when titers were on average 2.6-fold (2.2- to 3.1-fold) higher than baseline titers among participants with recent infection compared to 1.9-fold (1.5- to 2.3-fold) higher among participants without recent infection (Fig. 3e). This boosting of titers against future strains was accompanied by higher rates of seroconversion against subsequent A/Kansas/14/2017 (Ka17) and A/Brisbane/60/2018 (Br18) strains (Table 1). Effects of recent infection were observed across all ages (Extended Data Fig. 3) and whether participants had one, two or three recent infections (Extended Data Fig. 4). However, titers and titer rises tended to increase with proximity of prior infection (Extended Data Fig. 5). Importantly, participants with prior infection had higher GMTs on day 280 against viruses from clades that were causing infections in the cohort by that time (Fig. 3b and Extended Data Table 2). We have shown previously that HI titers of 40 can be associated with substantial protection in this cohort<sup>22</sup>. These results indicate that memory from recent infection enhances the magnitude and breadth of A(H3N2)-reactive antibodies induced by vaccination.

**Strain-specific effects of prior infections.** As described above, amino acids that were substituted between prior A(H3N2) strains and the HK14e vaccine strain were concentrated within antigenic sites A and B for HN14-like (clade-3c3a) viruses that were detected in the cohort from 2013 to 2015 but within site C for 2009 and 2012 strains (Fig. 1d). We therefore examined whether the cross-reactivity of antibodies induced by vaccination differed between participants who had been infected with an A(H3N2) virus that was HN14-like versus HN09- or HN12-like. Participants infected multiple times between 2009 and 2012 were excluded (Supplementary Table 3). Antibody landscapes, modeled against a two-dimensional map of virus antigenic distances, differed between participants infected with HN14-like viruses versus earlier viruses (Fig. 4). Most notably, day 14 postvaccination landscapes were relatively skewed toward HN14 and other clade-3c3a viruses among the group with prior HN14-like virus infection (Fig. 4b). Similar trends were observed for landscapes on day 280 after vaccination and titer-rise landscapes (Extended Data Fig. 6a-d). These results suggest that memory recall may drive antibody production toward epitopes that are shared between the vaccine strain and prior infecting strains.

To further investigate whether the prior infecting strain affects antibody production against site B of the HK14e vaccine, sera were titrated against reverse-engineered viruses bearing wild-type HK14e HA or HA containing a substitution in site B (Fig. 4c). Y159S was chosen because substitutions at this position are known to have large antigenic effects<sup>18</sup> and because Sw13e has an S at position 159 and is antigenically distinct from HK14e (Fig. 1d and Fig. 4a). Characterization using ferret antisera indicated that the Y159S virus was antigenically distinct from HK14e, as well as from Sw13e (clade-3c3a) (Supplementary Table 4). Antibody titers against wild-type versus Y159S virus were compared using

**Fig. 4 | The strain coverage of antibodies induced by vaccination is influenced by the A(H3N2) strain that caused prior infection.** **a,b**, Pre-vaccination (a) and d14 post-vaccination (b) antibody titers of participants grouped by prior A(H3N2) infection status were modelled against a two-dimensional map of virus antigenic distances using LOWESS regression. Estimated titers are represented as contours. Viruses are colored by (sub)clade, and circles are filled if sera were titrated against that virus. Abbreviated virus designations are shown in the top left panel and only for clades that caused prior infection in the other panels. **c**, Sera were titrated against reverse-engineered viruses bearing wild-type (WT) HK14e HA or HA containing a Y159S substitution in site B, which was antigenically distinct based on titers of HK14e antisera and a site B-directed monoclonal antibody (mAb) (Q12C9). **d**, Representative MN assay data showing day 14 postvaccination sera titrated against wild-type versus Y159S virus and comparing participants with prior HN12 and HN14 infection (graph legend). **e**, Correlation of MN versus HI antibody titers of pre- and day 14 sera from 27 vaccinees. **f,g**, Pre-vaccination titers (f) and ratios of titers (g) against Y159S versus wild-type virus of participants grouped by prior infecting strain, determined by MN assay (top) or HI assay (bottom). **h,i**, d14 post-vaccination titers (h) and ratios of titers (i) against Y159S versus wild-type virus of participants grouped by prior infecting strain, determined by MN assay (top) or HI assay (bottom). Numbers of biologically independent samples tested are shown adjacent to each plot. Results for each sample are presented as symbols. Error bars represent geometric means and 95% confidence intervals (CI). P values are shown for two-sided t tests, specified as paired tests for within-group comparisons across viruses and non-paired tests for across-group comparisons within viruses.





**Fig. 5 | Antibody titer landscapes associated with infection in the season after vaccination.** **a-d**, Participants were grouped by whether they developed symptomatic A(H3N2) infection in the season after vaccination. Antibody titers modeled against virus circulation year are shown for participants without (a) and with (b) symptomatic A(H3N2) infection. Antibody titer ratios modeled against virus circulation are shown for participants without (c) and with (d) symptomatic A(H3N2) infection. GAMs were used to fit titers and titer ratios at the time-points indicated in the legend. Shading indicates 95% confidence intervals. **e,f**, Vaccinees who developed symptomatic A(H3N2) infection in the season after vaccination were grouped by whether they had A(H3N2) infection during 9 years before vaccination to compare pre-vaccination (e) and d14 post-vaccination (f) titers presented as GMTs. Error bars indicate 95% confidence intervals. Dotted horizontal lines indicate thresholds for seropositivity or seroconversion. Dotted vertical lines or red panels indicate the position of the vaccine antigen.

microneutralization (MN) and HI assays, which were strongly correlated (Fig. 4d,e). MN titers were higher against wild-type compared to Y159S virus regardless of the prior infecting strain (Fig. 4f,h), which could be in part because the infectious dose of virus in the assay was marginally higher for the Y159S virus (Supplementary Fig. 3). Nevertheless, differences between wild-type and Y159S virus titers were greater among participants with prior HN09 and/or HN12-like virus infection than among those with prior HN14-like virus infection (Fig. 4f-i and Extended Data Fig. 6e,f). HI titers at baseline were on average 1.6-fold higher against Y159S virus among participants with prior HN09/HN12-like virus infection (Fig. 4g).

This ratio increased after vaccination to around 3-fold on day 14 (Fig. 4i) and 2.6-fold on day 280 (Extended Data Fig. 6h), indicating that vaccination induced antibodies against site B of HK14e among participants with prior HN09 and/or HN12 infection. In contrast, postvaccination HI titers were equivalent against wild-type and Y159S virus among participants with prior HN14-like virus infection (Fig. 4h,i), suggesting that less of the antibody induced was directed against immunodominant site B. In turn, we speculate that there may have been better induction of antibodies against subdominant sites, such as site C, which was relatively well conserved across past and future strains (Supplementary Table 5).

**Influenza virus infection during the postvaccination season.** Five vaccinees developed symptomatic A(H3N2) virus infection 275–340 days after vaccination, coinciding with case detection in the Ha Nam cohort as a whole. Infecting strains belonged to clades 3c2a2 and 3c2a1b (Fig. 1b). Cases were disproportionately detected among vaccinees who lacked recent A(H3N2) infection (4/28, 14%) compared to vaccinees who had recent infection (1/72, 1.4%; odds ratio, 0.084, 95% confidence interval, 0.009–0.793;  $P=0.021$ ). In contrast, A(H3N2) ILI cases accounted for similar proportions of vaccinees with recent A(H1N1) virus infection (3/54, 5.6%) and without recent A(H1N1) virus infection (2/37, 5.4%). Postvaccination antibody titers were relatively low among vaccinees who developed symptomatic A(H3N2) virus infection (Fig. 5a versus Fig. 5b), and titer rises were transient and did not increase from day 7 to day 21 (Fig. 5c versus Fig. 5d). Titer rise was greater after infection than after vaccination in these participants and increased further from day 7 to day 21 (Fig. 5b,d), indicating that infection was more immunogenic than vaccination. The vaccinee who developed symptomatic infection despite prior infection with HN09-like and HN12-like viruses had relatively high postvaccination titers against the vaccine strain (Fig. 5e). However, this individual was infected with an A/Switzerland/8060/2017 (Sw17)-like (clade-3c2a2) virus and had low titers and no titer rise against Sw17 (Fig. 5e). Symptomatic A(H3N2) cases came from four households, which together contained five vaccinees who were not symptomatically infected despite possible exposure (Extended Data Table 4). Three of the five unaffected vaccinees from these households had recent prior infection compared to only one of five cases (Extended Data Table 4). Taken together, these results suggest that adults who were infected with an A(H3N2) virus up to 9 years before vaccination were better protected against antigenically drifted A(H3N2) viruses and that protection was mediated by subtype-specific rather than cross-reactive immune responses.

## Discussion

In the current study, adults who had undergone active investigation to detect influenza virus infections since December 2007 received inactivated influenza vaccine in November 2016, and antibody titers were assessed against A(H3N2) viruses spanning 1968–2018. Antibody titers against older strains were associated with year of birth, whereas titers against post-2007 strains were associated with recent A(H3N2) infection status. However, titers were also relatively high against 1993–2002 strains. This deviates from antigenic sin and seniority hypotheses, which suggest that strains encountered earlier in life are higher in the antibody hierarchy because later infections back-boost antibodies against earlier strains and/or because immune responses to earlier strains mitigate responses to later strains<sup>6,23</sup>. Antibody titers against the vaccine A(H3N2) strain, as well as recent past strains, rose substantially within 7 days of vaccination, indicating that memory B cells were recalled. In contrast, young children produce negligible antibody within 7 days of their earliest influenza infections<sup>24</sup>, and the antibody induced mostly targets the HA of the strain that caused infection<sup>25</sup>. Participants with an A(H3N2) virus infection during 9 years before vaccination had higher antibody titers and more persistent titer rise against the vaccine virus and future circulating viruses. Similarly, in this cohort, symptomatic A(H3N2) infections were predominantly detected among vaccinees who lacked prior A(H3N2) virus infection, indicating that both vaccine immunogenicity and effectiveness are enhanced by immunological memory associated with prior infection.

The boosting effect of prior infection, observed here, contrasts with reports of negative effects of prior or repeated vaccination<sup>7–11</sup>, suggesting that the type of prior exposure is highly relevant. It was also notable that vaccine responses were at least as good among older compared to younger adults, contrasting with studies in more

highly vaccinated populations<sup>26,27</sup>. The recommended annual interval between influenza vaccinations is typically shorter than that between influenza infections. However, titers and titer rises following vaccination tended to increase with proximity of prior infection, indicating that time between exposures does not directly account for the different effect of prior infection. Several groups have demonstrated that neutralizing antibodies can become focused on limited virus epitopes that have remained conserved across successively encountered strains<sup>28,29</sup>. It is hypothesized that recalled memory B cells dominate and focus responses on epitopes that are well conserved in successively encountered strains, which could either enhance or compromise protection depending upon whether these targeted epitopes undergo mutation in subsequent strains<sup>13,14</sup>. In the current study, the strain coverage of antibodies, and capacity to generate antibodies against a prominent site B epitope, were shaped by the prior infecting strain, consistent with memory B cell dominance. These findings present a paradox whereby memory B cell recall is pivotal for inactivated egg-based influenza vaccine to elicit sufficient antibody for protection but may also be problematic in terms of the capacity for vaccination to update immunity by generating memory B cells and antibodies against epitopes that have mutated in a new vaccine strain. To generate antibodies and memory B cells against variant epitopes, influenza vaccines must either induce memory B cells to undergo further affinity maturation<sup>30</sup> or induce naive B cell differentiation. Memory B cells may have a competitive advantage, because they have undergone affinity maturation and may compete more successfully for antigen to engage T cell help for further differentiation and they additionally less reliant than naive B cells on T cell help for activation<sup>31,32</sup>. Inactivated influenza vaccines deliver antigen transiently and induce minimal innate costimulation and hence may have little capacity to activate naive B cells and generate new B cell clones and antibodies in the presence of vaccine-reactive memory B cells.

Infection induced higher antibody titers against a broader antigenic range of A(H3N2) viruses than vaccination among individuals who developed A(H3N2) ILI in the season after vaccination. This suggests that infection may have greater potential to expand the antibody repertoire than vaccination. In turn, as the epitope range of the memory B cell pool increases, the potential to recognize epitopes in a new vaccine strain will also increase, providing a mechanism for the differential effects of prior infection and vaccination. Similarly, in ferrets and mice, priming with inactivated influenza vaccine induces little to no measurable antibody and no protection against variant virus strains, whereas priming by infection induces more antibody and substantial protection against variant strains<sup>33,34</sup>. These differences in antibody responses may reflect a greater capacity for influenza virus infection, as opposed to vaccination, to activate both the innate and adaptive immune systems<sup>35</sup> and in turn activate naive B cells. Additionally, antigen may be retained for longer periods after infection than vaccination and may be available to engage naive B cells after the memory B cell response starts to contract<sup>36</sup>.

The study has several limitations. The objective, to investigate the effect of prior A(H3N2) infection on vaccine immunogenicity against A(H3N2) viruses, required an observational design, and the sample size was constrained by the rarity of people who lacked A(H3N2) infection over a 9-year period. Therefore, inferences are suggestive rather than conclusive. It would be possible to perform larger studies looking at effects of infection in the prior season only; however, the results presented here indicate that it is important to consider infections over a number of years. Although we used seroconversion in addition to ILI surveillance to determine participant's prior infection status, some asymptomatic infections may be missed using a fourfold or greater titer rise as the criterion for seroconversion<sup>37</sup>. It was also clear that titers and titer rises were higher among participants with infection confirmed by RT-PCR than by

seroconversion, which could reflect the potential for serologically confirmed infections to be false positive<sup>37</sup>. Alternately, recent studies indicate that antibody titers can increase with severity of influenza infection<sup>38</sup>.

Taken together, the results of this study indicate that prior A(H3N2) virus infection may increase the titer and breadth of antibody responses induced by a new A(H3N2) vaccine strain, and thereby enhance protection despite antigenic drift. However, the range of strains against which antibodies are induced may be dictated by the prior infecting strain, consistent with a memory-dominated response. Such memory dominance may need to be overcome in future vaccine strategies to increase protection against drifted A(H3N2) viruses.

### Online content

Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at <https://doi.org/10.1038/s41591-022-01690-w>.

Received: 28 May 2021; Accepted: 10 January 2022;

Published online: 17 February 2022

### References

- Sanjuan, R., Nebot, M. R., Chirico, N., Mansky, L. M. & Belshaw, R. Viral mutation rates. *J. Virol.* **84**, 9733–9748 (2010).
- Taubenberger, J. K. & Morens, D. M. The pathology of influenza virus infections. *Annu. Rev. Pathol.* **3**, 499–522 (2008).
- Belongia, E. A. et al. Variable influenza vaccine effectiveness by subtype: a systematic review and meta-analysis of test-negative design studies. *Lancet Infect. Dis.* **16**, 942–951 (2016).
- Zost, S. J. et al. Contemporary H3N2 influenza viruses have a glycosylation site that alters binding of antibodies elicited by egg-adapted vaccine strains. *Proc. Natl Acad. Sci. USA* **114**, 12578–12583 (2017).
- Wu, N. C. et al. Preventing an antigenically disruptive mutation in egg-based H3N2 seasonal influenza vaccines by mutational incompatibility. *Cell Host Microbe* **25**, 836–844 e835 (2019).
- Francis, T. On the doctrine of original antigenic sin. *Proc. Am. Phil. Soc.* **104**, 572–578 (1960).
- Thompson, M. et al. Effects of repeated annual inactivated influenza vaccination among healthcare personnel on serum hemagglutinin inhibition antibody response to A/Perth/16/2009 (H3N2)-like virus during 2010–11. *Vaccine* **34**, 981–988 (2016).
- Ohmit, S. E. et al. Influenza vaccine effectiveness in the community and the household. *Clin. Infect. Dis.* **56**, 1363–1369 (2013).
- McLean, H. Q. et al. Impact of repeated vaccination on vaccine effectiveness against influenza A(H3N2) and B during 8 seasons. *Clin. Infect. Dis.* **59**, 1375–1385 (2014).
- Skowronski, D. M. et al. A perfect storm: impact of genomic variation and serial vaccination on low influenza vaccine effectiveness during the 2014–2015 season. *Clin. Infect. Dis.* **63**, 21–32 (2016).
- Sullivan, S. G. et al. Low interim influenza vaccine effectiveness, Australia, 1 May to 24 September 2017. *Euro. Surveill.* **22**, 17-00707 (2017).
- Belongia, E. A. et al. Repeated annual influenza vaccination and vaccine effectiveness: review of evidence. *Expert Rev. Vaccines* **16**, 1–14 (2017).
- Smith, D. J., Forrest, S., Ackley, D. H. & Perelson, A. S. Variable efficacy of repeated annual influenza vaccination. *Proc. Natl Acad. Sci. USA* **96**, 14001–14006 (1999).
- Cobey, S. & Hensley, S. E. Immune history and influenza virus susceptibility. *Curr. Opin. Virol.* **22**, 105–111 (2017).
- Zost, S. J. et al. Identification of antibodies targeting the H3N2 hemagglutinin receptor binding site following vaccination of humans. *Cell Rep.* **29**, 4460–4470 (2019).
- Wiley, D. C., Wilson, I. A. & Skehel, J. J. Structural identification of the antibody-binding sites of Hong Kong influenza haemagglutinin and their involvement in antigenic variation. *Nature* **289**, 373–378 (1981).
- Lee, M. S. & Chen, J. S. Predicting antigenic variants of influenza A/H3N2 viruses. *Emerg. Infect. Dis.* **10**, 1385–1390 (2004).
- Koel, B. F. et al. Substitutions near the receptor binding site determine major antigenic change during influenza virus evolution. *Science* **342**, 976–979 (2013).
- Horby, P. et al. The epidemiology of interpandemic and pandemic influenza in Vietnam, 2007–2010: the Ha Nam household cohort study I. *Am. J. Epidemiol.* **175**, 1062–1074 (2012).
- Hayward, A. C. et al. Comparative community burden and severity of seasonal and pandemic influenza: results of the Flu Watch cohort study. *Lancet Respir. Med.* **2**, 445–454 (2014).
- Fonville, J. M. et al. Antibody landscapes after influenza virus infection or vaccination. *Science* **346**, 996–1000 (2014).
- Hoa, L. N. M. et al. Influenza A(H1N1)pdm09 but not A(H3N2) virus infection induces durable seroprotection: results from the Ha Nam cohort. *J. Infect. Dis.* <https://doi.org/10.1093/infdis/jiaa293> (2020).
- Lessler, J. et al. Evidence for antigenic seniority in influenza A (H3N2) antibody responses in southern China. *PLoS Pathog.* **8**, e1002802 (2012).
- Murphy, B. R. et al. Secretory and systemic immunological response in children infected with live attenuated influenza A virus vaccines. *Infect. Immun.* **36**, 1102–1108 (1982).
- Meade, P. et al. Influenza virus infection induces a narrow antibody response in children but a broad recall response in adults. *mBio* **11**, e03243-19 (2020).
- Henry, C. et al. Influenza virus vaccination elicits poorly adapted B cell responses in elderly individuals. *Cell Host Microbe* **25**, 357–366 (2019).
- Ranjeva, S. et al. Age-specific differences in the dynamics of protective immunity to influenza. *Nat. Commun.* **10**, 1660 (2019).
- Li, Y. et al. Immune history shapes specificity of pandemic H1N1 influenza antibody responses. *J. Exp. Med.* **210**, 1493–1500 (2013).
- Linderman, S. L. et al. Potential antigenic explanation for atypical H1N1 infections among middle-aged adults during the 2013–2014 influenza season. *Proc. Natl Acad. Sci. USA* **111**, 15798–15803 (2014).
- Wrammert, J. et al. Rapid cloning of high-affinity human monoclonal antibodies against influenza virus. *Nature* **453**, 667–671 (2008).
- Good, K. L., Avery, D. T. & Tangye, S. G. Resting human memory B cells are intrinsically programmed for enhanced survival and responsiveness to diverse stimuli compared to naive B cells. *J. Immunol.* **182**, 890–901 (2009).
- Auladell, M. et al. Distinguishing naive- from memory-derived human B cells during acute responses. *Clin. Transl. Immunol.* **8**, e01090 (2019).
- Houser, K. V., Pearce, M. B., Katz, J. M. & Tumpey, T. M. Impact of prior seasonal H3N2 influenza vaccination or infection on protection and transmission of emerging variants of influenza A(H3N2)v virus in ferrets. *J. Virol.* **87**, 13480–13489 (2013).
- Kim, J. et al. Prior infection with influenza virus but not vaccination leaves a long-term immunological imprint that intensifies the protective efficacy of antigenically drifted vaccine strains. *Vaccine* **34**, 495–502 (2016).
- Kreijtz, J. H. C. M., Fouchier, R. A. M. & Rimmelzwaan, G. F. Immune responses to influenza virus infection. *Virus Res.* **162**, 19–30 (2011).
- Sangster, M. Y., Nguyen, P. Q. T. & Topham, D. J. Role of memory B cells in hemagglutinin-specific antibody production following human influenza A virus infection. *Pathogens* **8**, 167 (2019).
- Cauchemez, S. et al. Influenza infection rates, measurement errors and the interpretation of paired serology. *PLoS Pathog.* **8**, e1003061 (2012).
- Tricoche, A. D. et al. Symptoms, infection duration, and hemagglutinin inhibition antibody response in influenza A infections. *J. Infect. Dis.* **223**, 838–842 (2021).

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## Methods

**Study design and participants.** Participants were purposefully selected from a household-based influenza cohort established in Thanh Ha Commune, Thanh Liem District, Ha Nam Province, northern Vietnam (Extended Data Fig. 1). The cohort has been described previously<sup>19</sup>. In brief, the Ha Nam cohort enrolled 270 randomly selected households between 17 November and 7 December 2007. Household members were asked to participate in active ILI surveillance, provide swabs if they developed ILI and provide blood samples annually or biannually, at times spanning influenza transmission peaks<sup>19</sup>. Swabs were assessed by RT-PCR to detect influenza virus RNA, and sera were assessed to determine HI antibody titers against circulating strains. Infection was defined as detection of RT-PCR-confirmed ILI or a fourfold or greater rise in antibody titer (seroconversion).

The primary objective was to determine whether vaccine-induced antibody titer rises against A(H3N2) viruses differ between participants with and without documented prior A(H3N2) infection. Sample size was based on the assumption that GMRs of post- to prevaccination antibody titers would differ by 0.7 with a standard deviation of 1.0, giving an effect size of 0.7. It was estimated that 33 participants per group would provide 80% power to detect this effect with 95% confidence. This was inflated to 50 participants per group to account for loss to follow-up and facilitate comparison of people infected with A(H3N2) in different years since 2007.

Inclusion criteria were age  $\geq 18$  years and continued participation in cohort investigations to ascertain prior infection status. Participants with a history of allergic reactions were excluded. A total of 371 of 556 adults registered interest in participating in a vaccine study at the time of reconsent for the Ha Nam cohort in July 2016 (Extended Data Fig. 1), and 161 had participated continually in ILI surveillance and all blood sample collections, including 32 without and 129 with A(H3N2) infection since December 2007 (Extended Data Fig. 1). For each of the 32 participants without recent A(H3N2) infection, two or three participants with prior infection were selected based on proximity of their ages and sex to obtain a similar ratio of males to females (Supplementary Table 1). A total of 100 of 114 selected participants consented to the vaccine study between 1 October and 6 November 2016. This included 28 of 32 without recent infection and 72 of 82 with recent A(H3N2) infection. Ages and proportions female were similar among nonselected, selected and consenting participants (Extended Data Fig. 1). Selected participants were from 79 of 210 household remaining in the cohort, with three households contributing three participants each and 16 households contributing two participants each.

Participants received licensed, locally available Trivalent inactivated influenza vaccine (TIV; Vaxigrip, Sanofi Pasteur) in November 2016. Blood samples were collected before and 4, 7, 14, 21 and 280 days after vaccination. Blood samples were also collected 7 and 21 days after confirmed influenza illness occurring in the season after vaccination.

Study protocols were approved by ethics committees of the University of Melbourne (1646470), the National Institute of Hygiene and Epidemiology in Vietnam (IRB-VN01057 – 08/2016) and the Oxford Tropical Medicine Research Unit (30–16). All participants provided written informed consent (conducted in Vietnamese). Participants were compensated financially for each investigation commensurate with the time required. The study was not prospectively registered as a clinical trial, because participants were not assigned to intervention versus control groups. However, study protocols were retrospectively included on the Australian New Zealand Clinical Trials Registry (12621000110886).

**Virus propagation and characterization.** Viruses were propagated in mammalian cell lines and/or in 10- to 12-day-old embryonated chicken eggs (Supplementary Table 2). Madin–Darby canine kidney (MDCK) cells and MDCK cells transfected with 2,6-sialtransferase (SIAT) were grown in DMEM (Gibco) containing penicillin/streptomycin and 10% fetal bovine serum (Bovagen). A number of viruses acquired neuraminidase (NA) substitutions, which that have been associated with erythrocyte agglutination via NA<sup>39</sup>, when propagated in MDCK cells (Supplementary Table 2). HA titers of most of these viruses decreased when oseltamivir was added to inhibit NA, but HI titers did not uniformly increase in the presence of oseltamivir. Therefore, viruses were plaque-selected on SIAT cells to produce stocks that lacked NA T148X or D151X substitutions and were more sensitive to detect HI antibodies (Supplementary Table 6 and Supplementary Fig. 4).

Reverse genetics viruses were produced using the eight plasmid system based on A/Puerto Rico/8/1934 (PR8) (ref. <sup>40</sup>). The Y159S substitution was introduced into the HA of HK14e using the following primers: forward, 5'-CTTAAACAGCAATACCCAGCATGGAACGTGACT-3'; reverse, 5'-TATTGCTGTTAAGGGTCAACCAATTT-3'. Wild-type and Y159S HA were cloned into the vector PHW2000 (ref. <sup>40</sup>). The 7:1 reassortant viruses were generated using plasmids encoding PR8 internal and NA genes, and HA of HK14e or HK14e-Y159S. Plasmids were transfected into cocultured 293T/SIAT cells, and then recovered viruses were propagated in eggs. Reverse genetics viruses were assessed by HI assay using antisera raised against HK14e and Sw13e and a human mAb (Q129C) that recognizes site B of A/Victoria/361/2011 (generously provided by A. Townsend, MRC Weatherall Institute of Molecular Medicine).

HA and NA genes of viruses used for serology and/or from swabs of Ha Nam cohort participants (isolates or clinical specimens) were sequenced via Sanger sequencing and aligned using the multiple alignment using fast Fourier transform algorithm in MegAlign Pro 13 (DNASTAR Lasergene 13). Phylogenetic trees were edited in FigTree version 1.4.4 (2006–2018, A. Rambaut, Institute of Evolutionary Biology, University of Edinburgh; <http://tree.bio.ed.ac.uk/>). HA antigenic site positions (Fig. 1c), defined by Lee et al.<sup>17</sup>, that varied between HK14e and at least one recent prior strain were tabulated to determine whether antigenic variation from HK14e was clustered within particular sites and if this varied between prior infecting strains (Fig. 1d).

Viruses circulating since 2007 were antigenically characterized by HI assay using ferret antisera generated for routine virus characterization by the WHO Collaborating Center for Reference and Research on Influenza, Melbourne (Supplementary Table 7). A two-dimensional map of virus antigenic distances was generated from the matrix of two-way titers of each sera against each virus using antigenic cartography software (Racmacs, <https://acorg.github.io/Racmacs/>).

**Serological assays.** Sera were assessed by HI assay to determine antibody titers against influenza viruses. Assays were performed according to WHO Global Influenza Surveillance Network protocols<sup>41</sup> with the exception that volumes were reduced to 25  $\mu$ l each of diluted sera, virus and 1% erythrocytes (0.33% final). Guinea pig erythrocytes were used for titration of antibodies against all A(H3N2) viruses, based on initial comparisons of titers obtained using guinea pig versus turkey erythrocytes (Supplementary Fig. 5). Sera were treated with receptor destroying enzyme (Denka Sieken), adsorbed with 5% erythrocytes, and then tested over twofold serial dilutions from 1:10 to 1:10,240. Each individual's complete set of sera were tested against all viruses using the same batch of erythrocytes. Quality control viruses and sera were run with each new batch of samples/erythrocytes and were accepted if HA and HI titers were within twofold of initial values. HI titers were read using an automated reader (CypherOne, InDevR). Instrument settings for plate reading were determined by comparison with manual titer reads (Supplementary Fig. 6) and then applied to all plates. Antibody titers against reverse engineered viruses were validated by MN assay, conducted according to World Health Organization protocols<sup>41</sup> using SIAT cells, and plasma treated as per the HI assay protocol above.

**Outcomes.** The primary outcome was vaccine immunogenicity, comparing GMRs of antibody titers among participants with and without recent A(H3N2) virus infection. GMTs and proportions seropositive (defined as a titer of 40 or more) or seroconverting (defined as a fourfold or greater titer rise) were also compared. The strain coverage of antibodies induced by vaccination was further compared by fitting antibody titer landscapes across all A(H3N2) viruses tested<sup>41</sup>. Titers were determined at a range of time points, but comparison focused on day 14 after vaccination, when titer peaks were detected, and on day 280, when titer decay plateaus<sup>42</sup>.

Post-hoc comparisons of participants who had been infected with viruses from distinct genetic clades, and participants who did or did not develop A(H3N2) ILI in the season after vaccination, were also performed.

**Statistical analysis.** GMTs and GMRs were estimated from  $\log_2$  HI titers and from differences of  $\log_2$  titers at post- minus prevaccination time points. GMTs and GMRs were calculated for individual viruses ( $n=40$ ) and groups of viruses representing prior exposure and postvaccination periods, which were averaged for each person. To estimate the size of the effect of recent infection on GMTs and GMRs, a mixed-effects linear regression model was used, which included a random effects term to account for within-person correlations of antibody titers over time and an interaction term for time of serum collection by recent infection status. Fisher's exact test was used to compare proportions with and without prior infection who seroconverted on day 14, maintained a fourfold titer rise on day 280 or became infected after vaccination.

To construct and compare antibody landscapes across strains, we used either GAMs or regression models with locally weighted scatterplot smoothing<sup>21</sup> to fit  $\log_2$  titers against A(H3N2) viruses organized temporally or antigenically, respectively. We used the GAM function from the R package mgcv and accounted for repeated measurements on each individual through specification of a random effect<sup>43</sup>. Plots were generated with ggplot2 (ref. <sup>44</sup>). Previously published code, available in GitHub, was used to generate two-dimensional maps of virus antigenic distances (<https://acorg.github.io/Racmacs/>, version 1.1.4) and fit antibody landscapes using the locally weighted scatterplot smoothing regression model (<https://github.com/acorg/ablandscapes>, version 1.0.2).

**Reporting Summary.** Further information on research design is available in the Nature Research Reporting Summary linked to this article.

## Data availability

The protocol is available at <https://www.anzctr.org.au/Trial/Registration/TrialReview.aspx?id=380758&isReview=true>. Participant data were recorded into a secure, auditable online database called CliRes, which was developed by the Oxford University Clinical Research Unit, Vietnam (<https://clires.oucru.org/>). Serological

data were linked to participant data using Microsoft Access version 15.0.5349.1000. The dataset used for analysis will be made available on request and will be publicly available at <https://melbourne.figshare.com/> within one year of this publication. Plots showing titers for each antigen and time point for each individual are also presented in Supplementary Fig. 8. HA ( $\pm$ NA) sequences of influenza viruses included in the analyses are available on GISAID. GISAID accession codes are listed in Supplementary Table 8.

## References

39. Lin, Y. P. et al. Neuraminidase receptor binding variants of human influenza A(H3N2) viruses resulting from substitution of aspartic acid 151 in the catalytic site: a role in virus attachment? *J. Virol.* **84**, 6769–6781 (2010).
40. Hoffmann, E., Neumann, G., Kawaoka, Y., Hobom, G. & Webster, R. G. A DNA transfection system for generation of influenza A virus from eight plasmids. *Proc. Natl Acad. Sci. USA* **97**, 6108–6113 (2000).
41. WHO Global Influenza Surveillance Network *WHO Global Influenza Surveillance Network: Manual for the Laboratory Diagnosis and Virological Surveillance of Influenza* (WHO Press, 2011).
42. Andraud, M. et al. Living on three time scales: the dynamics of plasma cell and antibody populations illustrated for hepatitis A virus. *PLoS Comput. Biol.* **8**, e1002418 (2012).
43. Wood, S. N. Fast stable restricted maximum likelihood and marginal likelihood estimation of semiparametric generalized linear models. *J. R. Stat. Soc. B* **73**, 3–36 (2011).
44. R Core Team *R: A Language and Environment for Statistical Computing* (R Foundation for Statistical Computing, 2017).

## Acknowledgements

Funding for this study was provided by the National Health and Medical Research Council, Australia (grant 1103367 to A.F.) and National Foundation for Science and Technology Development (NAFOSTED 108.04-2019.08, L.T.Q.M.). The WHO Collaborating Centre for Reference and Research on Influenza is funded by the Australian Government Department of Health. The Oxford University Clinical Research Unit – Hanoi and H.R.v.D. are funded through Wellcome Africa Asia program grants (089276/Z/09/Z and 106680/Z/14/Z). We are grateful to the Ha Nam Preventive Medicine Centre and People's Committees of Thanh Liem District for their support and the people of Thanh Ha Commune for participating in this study. We would like to thank the Thanh Ha Commune health workers for their dedication to conducting active surveillance and cross-sectional surveys. We also wish to thank the Ministry of Health of Vietnam for their continuing support of the research collaboration between the Oxford University Clinical Research Unit and the National Institute for Hygiene and Epidemiology. We are grateful to members of the Oxford University Clinical Research Unit, including P. Horby for his role in establishing the Ha Nam cohort, N. Nguyen Minh Trang for project coordination and B. Huyen Trang for administrative support. A. Malet, H. Peck and Y.-M. Deng and their staff at Melbourne WHO Collaborating Centre for Reference and Research performed initial isolation and characterization of many of the influenza viruses used. We thank S. Sanchez for assisting with microneutralization assays. Thanks also to K. Subbarao and N. Thi Hoang Oanh for helpful comments on the manuscript. K.K. was supported by the Australian National Health and Medical Research Council (Leadership Investigator Fellowship 1173871). M.A. and L.H. were supported by the Melbourne International Research Scholarship and the Melbourne International Fee Remission Scholarship from the University of Melbourne. The funders had no role in the conduct of the study.

## Author contributions

M.A. assisted with virus propagation, performed serology, assisted with data analysis and codrafted the manuscript. H.V.M.P. comanaged the Ha Nam cohort, including sample collection and processing and diagnostic testing over the course of the vaccination study, and critically reviewed the manuscript. L.C. assisted with virus propagation and serology, sequenced virus HA and NA genes, performed microneutralization antibody assays, plaqued viruses and critically reviewed the manuscript. L.T.Q.M. coconceived and codesigned the study, comanaged the Ha Nam cohort sample collection and processing and diagnostic testing over the 9-year course of cohort investigation and vaccination study and critically reviewed the manuscript. Y.Y.T. constructed reverse genetics viruses and performed HI with these viruses, performed components of the data analysis, and critically reviewed the manuscript. S.W. performed components of the data analysis and critically reviewed the manuscript. P.Q.T. codesigned the study, comanaged Ha Nam cohort field work and data collection over the 9-year course of cohort investigation and over the vaccination study and critically reviewed the manuscript. D.P. assisted with data analysis and critically reviewed the manuscript. N.T.D. assisted with study design, managed all activities of the health care workers to collect samples and data, managed communication with participants and critically reviewed the manuscript. N.L.K.H., L.T.T., N.T.H.T., T.T.K.H., N.T.N.D. and V.T.N.B. processed samples, performed influenza diagnostic testing and virus isolation over 9 years of cohort investigation (between 2007 and 2016) and over the course of vaccination and subsequent follow-up, assisted with data cleaning, and critically reviewed the manuscript. A.K. assisted with data analysis and critically reviewed the manuscript. L.H. assisted with virus propagation and critically reviewed the manuscript. T.N.D. and D.D.A. comanaged the Ha Nam cohort over the 9-year course of cohort investigation and over the vaccination study and critically reviewed the manuscript. K.K. contributed to data interpretation and critical review of the manuscript. S.D.B., K.L.G.-J., D.S., I.B. and H.W. codesigned the study and critically reviewed the manuscript. S.S. assisted with data analysis and critically reviewed the manuscript. H.R.v.D. coconceived and codesigned the study, comanaged the Ha Nam cohort over the course of the vaccination study and critically reviewed the manuscript. A.F. conceived the study; comanaged Ha Nam cohort sample collection and processing and diagnostic testing over the 9-year course of cohort investigation and over the vaccination study; assisted with sample processing, virus propagation and serology; managed data and data analysis; and codrafted the manuscript.

## Competing interests

H.R.v.D. was funded by Sanofi (travel and consultancy fees) to present at, and attend a meeting about, the potential role of influenza vaccination in antimicrobial resistance in 2019. S.D.B. reports grants from NIH during the conduct of the study. All other authors declare no competing interests.

## Additional information

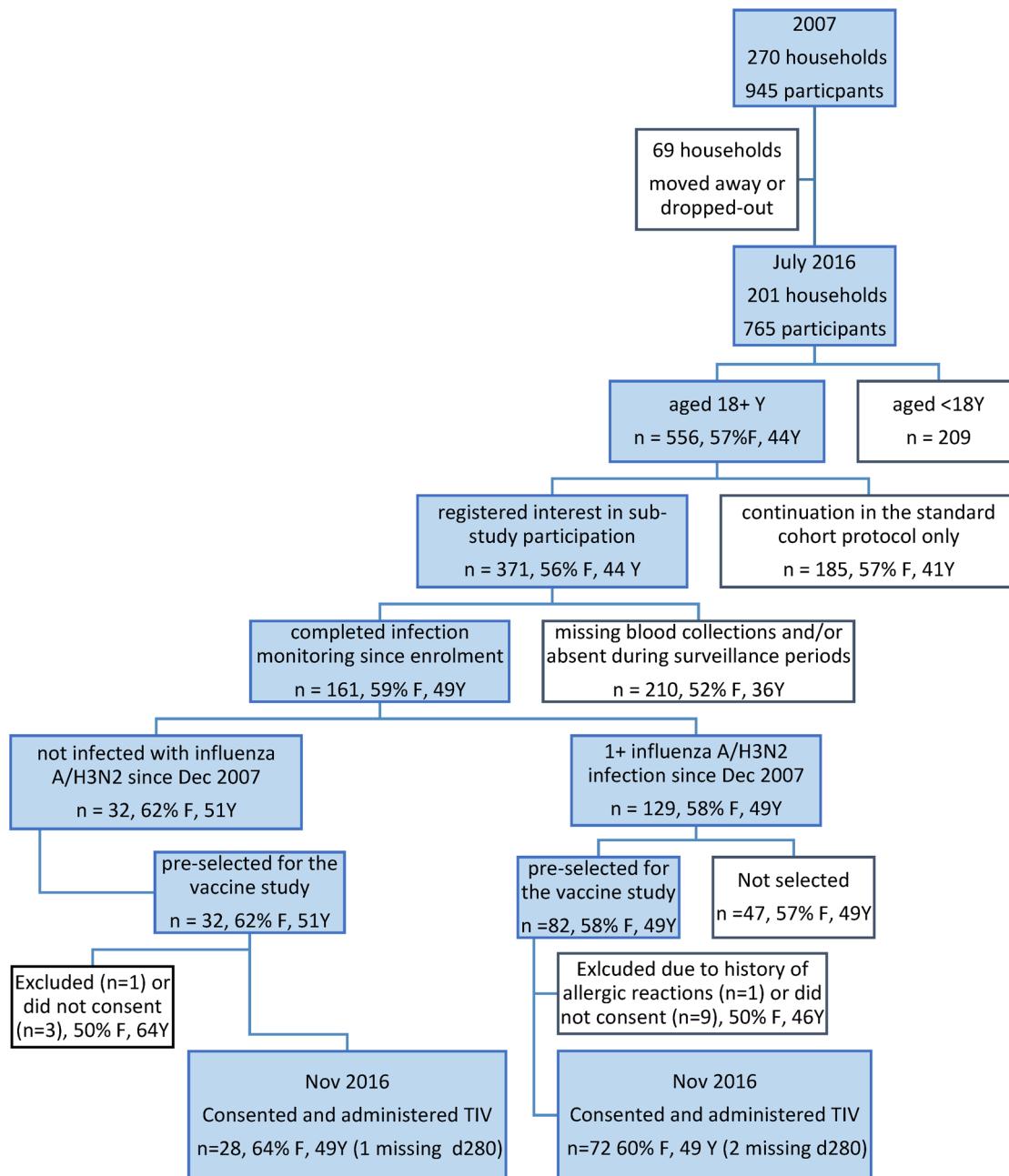
**Extended data** is available for this paper at <https://doi.org/10.1038/s41591-022-01690-w>.

**Supplementary information** The online version contains supplementary material available at <https://doi.org/10.1038/s41591-022-01690-w>.

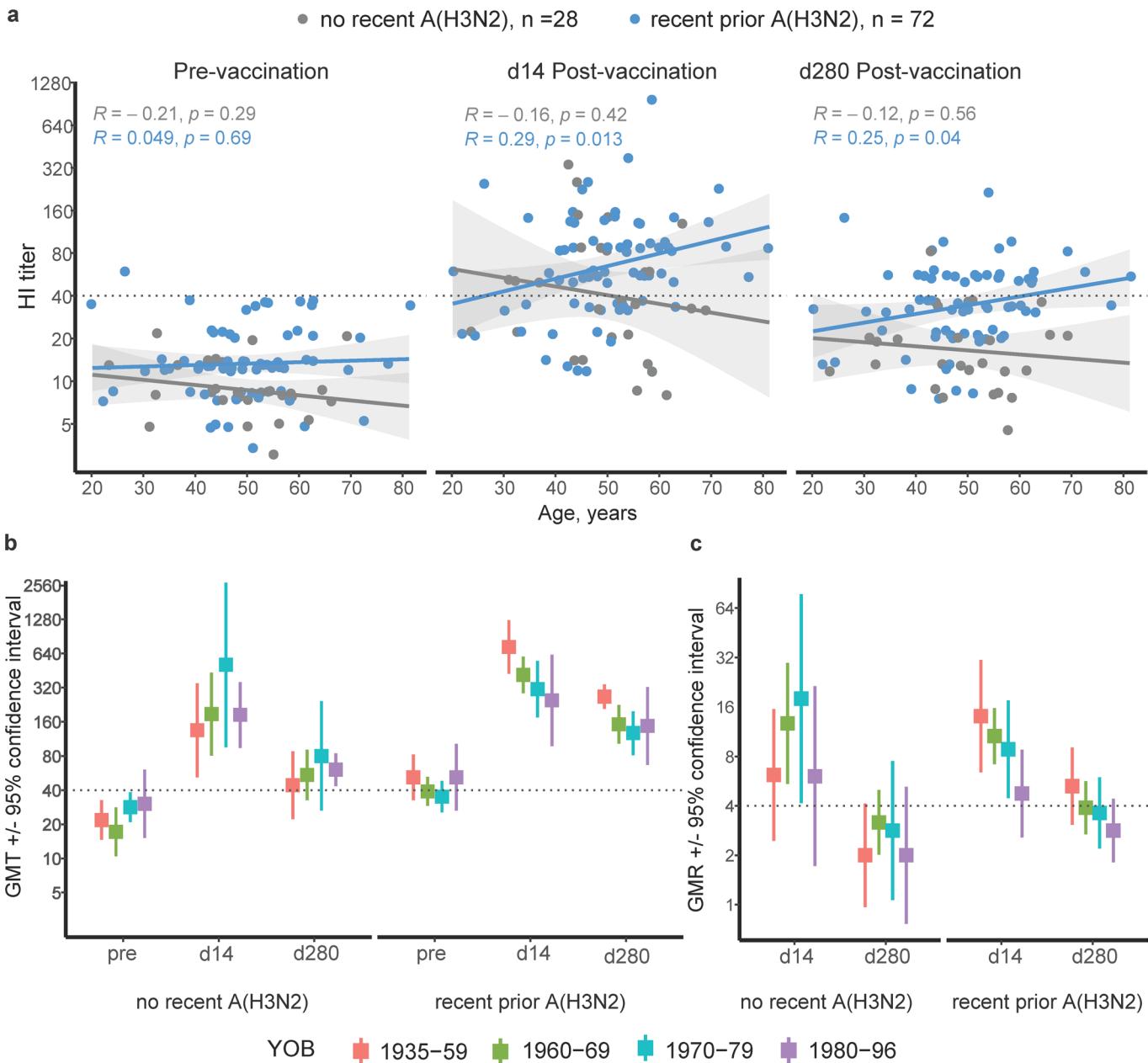
**Correspondence and requests for materials** should be addressed to Annette Fox.

**Peer review information** *Nature Medicine* thanks Gail Potter and the other, anonymous, reviewer(s) for their contribution to the peer review of this work. Alison Farrell was the primary editor on this article and managed its editorial process and peer review in collaboration with the rest of the editorial team.

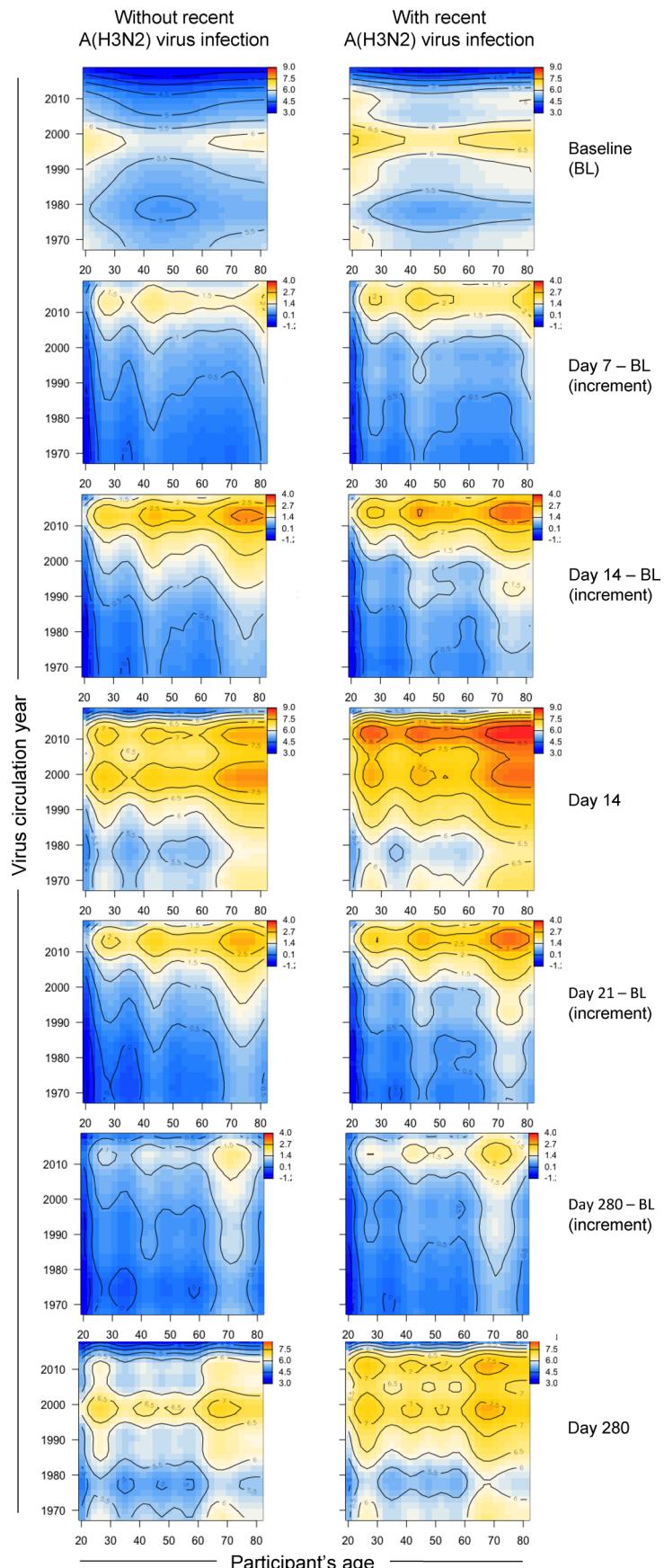
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**Extended Data Fig. 1 | Flowchart depicting participant selection for the vaccine study.** Participants included and excluded at each step are indicated by blue and white text boxes, respectively. Percentages female (F) and median age in years (Y) are shown at each step. Selection for the vaccine study was limited to participants aged  $\geq 18$  years ( $n=556$ ), who registered interest in participating in sub-studies involving vaccination and/or additional blood samples when re-consented in July 2016 ( $n=371$ ). Selection was further limited participants who had provided blood samples at all 12 time points since Dec-2007, and who had been present during all influenza transmission periods ( $n=161$ ). Only 32 participants lacked a detectable A(H3N2) infection, and all were selected. 82/129 who had an A(H3N2) infection were then selected based on similarity of age to participants without prior infection, and sex. For each participant lacking recent infection, we selected two to three recently infected participants who were nearest in age, while selecting 3 females selected for every 2 males (S. Table 1). 14 of 114 selected participants were excluded or did not consent. Age and proportion female were similar among non-selected, selected and consenting participants, with the exception that participants with missing blood collections and/or absent during surveillance periods were younger and fewer were female.

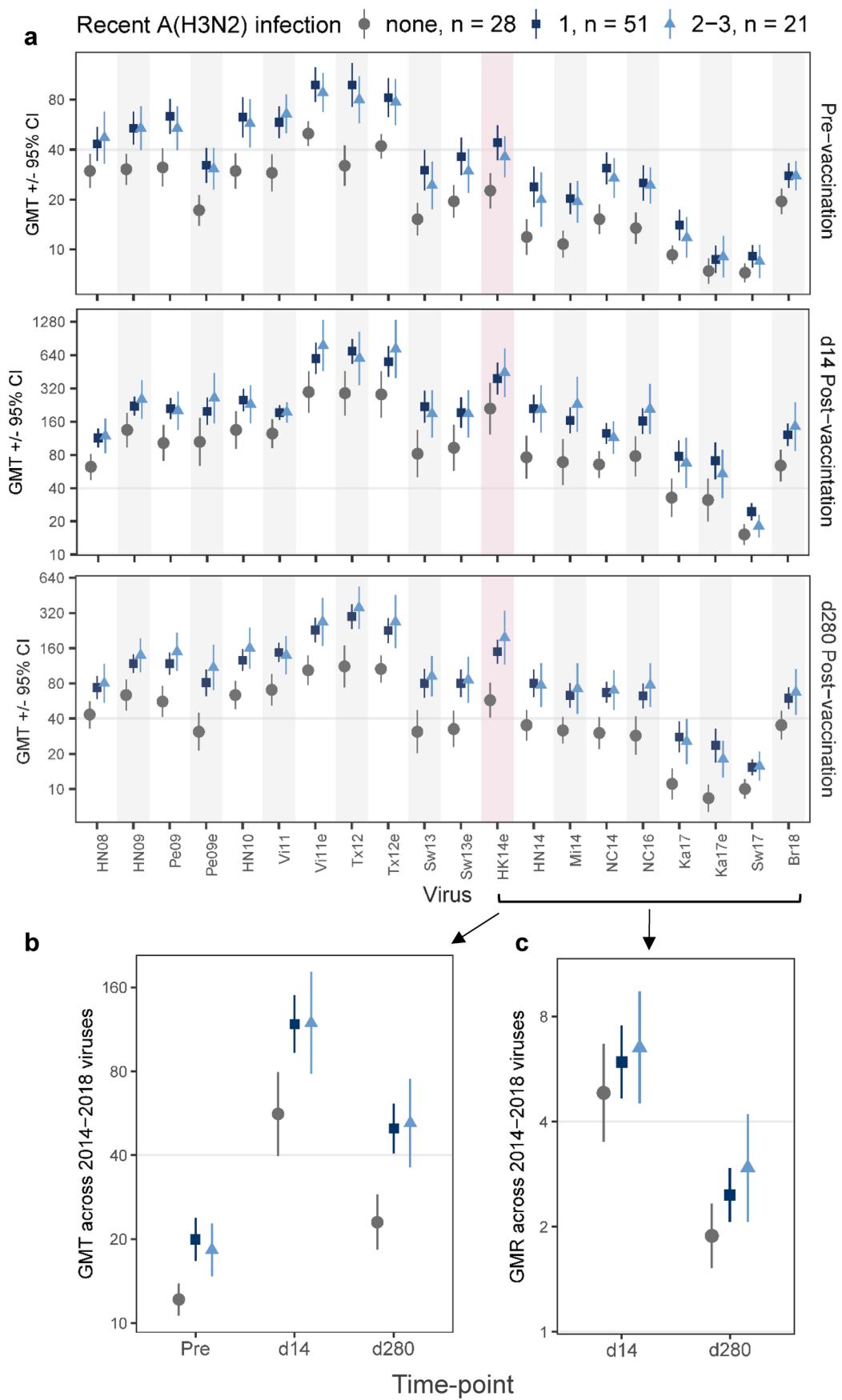


**Extended Data Fig. 2 | Antibody responses to the vaccine A(H3N2) strain, by age and prior A(H3N2) infection status and age group.** a, Titers against egg-grown A/Hong Kong/4801/2014 are plotted against participant age and prior infection status (color coded, legend) for the time points indicated. Linear regression lines are shown for each group with 95% confidence intervals. Pearson's correlation coefficients are shown with 2-sided p values. b, GMTs and c, GMRs are shown for participants stratified by prior infection and age group (color coded, legend) at the indicated time points. Symbols indicate mean values and error bars indicate 95% confidence intervals. Numbers per age group with no recent A(H3N2) are: 1935–59, n = 8; 1960–60, n = 9; 1970–79, n = 6; 1980–86, n = 5. Numbers per age group with recent A(H3N2) are: 1935–59, n = 16; 1960–60, n = 27; 1970–79, n = 21; 1980–86, n = 8. Horizontal lines indicate cut-offs for seropositivity or seroconversion.



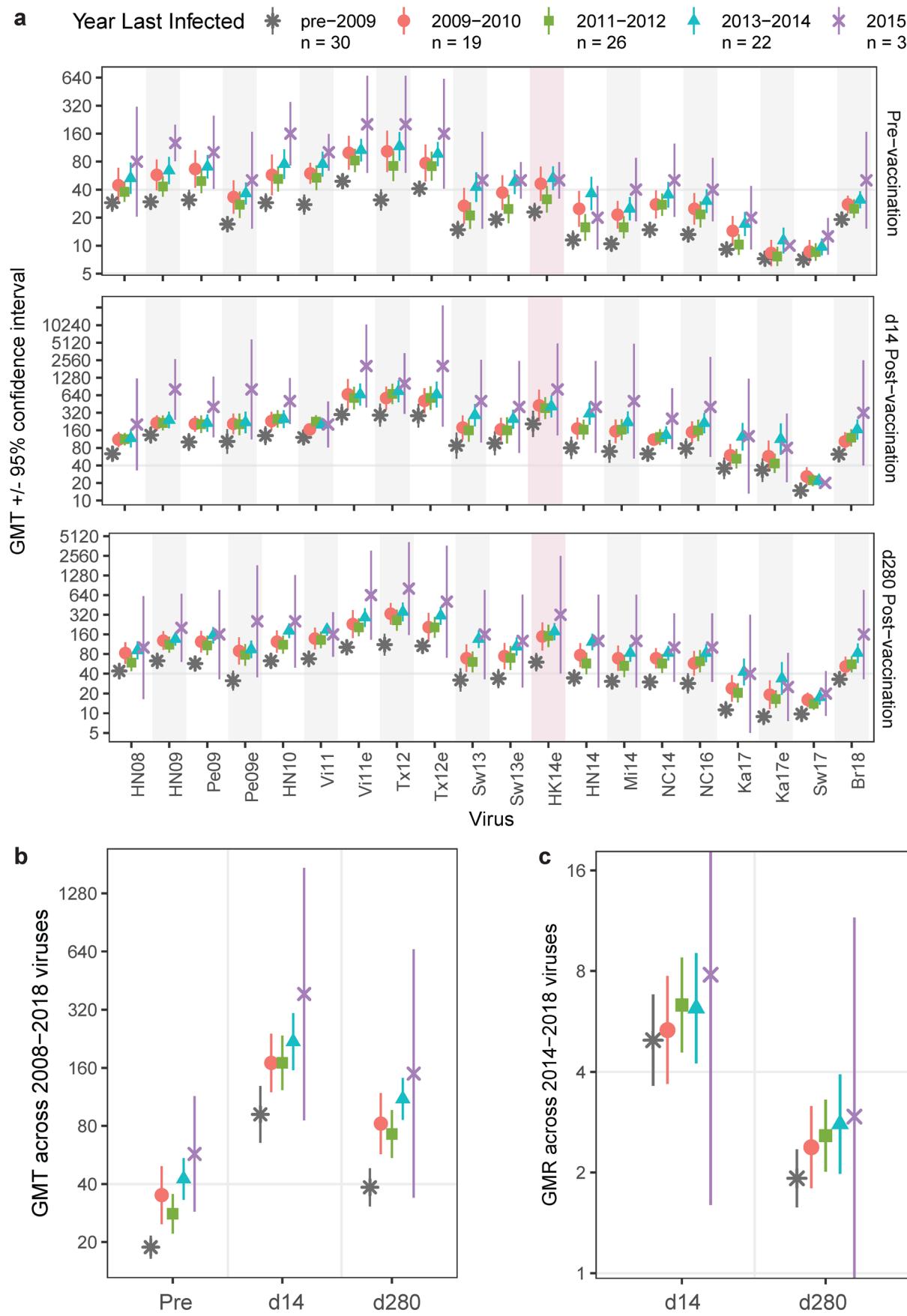
Extended Data Fig. 3 | See next page for caption.

**Extended Data Fig. 3 | Antibody titers across age groups in participants with and without A(H3N2) virus infection since 2007.** GAMs were used to fit titres and titre increments by virus circulation year and participant age. Participants with ( $n=72$ ) and without ( $n=28$ ) recent A(H3N2) virus infection are compared. Titre rise against the oldest strains was limited among the youngest participants and increased with increasing participant age. Titres and titre rises across strains were greater among participants with prior infection irrespective of age, indicating that effects of recent infection were not strongly age dependent.



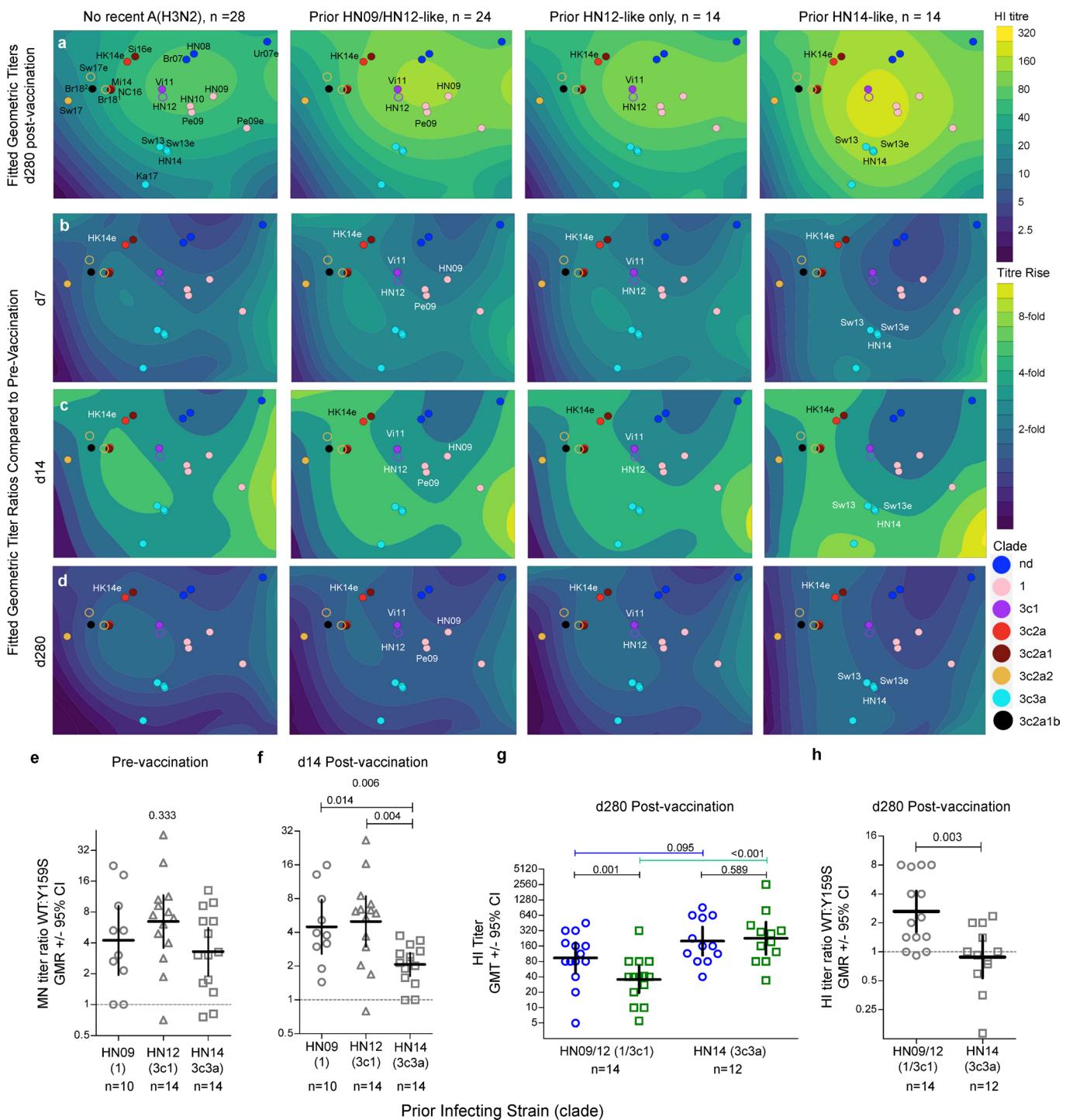
Extended Data Fig. 4 | See next page for caption.

**Extended Data Fig. 4 | Antibody titers and titer rises by number of recent A(H3N2) infections.** a, GMTs against individual strains spanning 2008 to 2018 are compared for participants with 0, 1 or 2–3 recent infections (legend). b, GMTs and c, GMRs averaged across 2014 to 2018 strains were compared. a–c, symbols indicate mean values and error bars indicate 95% confidence intervals. Horizontal lines represent cut-offs for seropositivity or seroconversion. Red panels in a highlight the vaccine strain. Samples sizes for all plots are shown in panel a.



Extended Data Fig. 5 | See next page for caption.

**Extended Data Fig. 5 | Antibody titers and titer rises by year of last A(H3N2) infection.** a, GMTs against individual strains spanning 2008 to 2018 are compared by year of last infection (legend). b, GMTs and c, GMRs averaged across 2014 to 2018 strains are compared. a-c, symbols indicate mean values and error bars indicate 95% confidence intervals. Horizontal lines represent cut-offs for seropositivity or seroconversion. Red panels in a highlight the vaccine strain. Sample sizes for all plots are shown in panel a.



**Extended Data Fig. 6 | Effects of prior A(H3N2) strain on the strain coverage of antibodies induced by vaccination.** a, antibody titre landscape on d280 post-vaccination, constructed as in Fig. 4. b-d, antibody titre rise landscapes on days 7, 14, and 280 post-vaccination. Effects of prior infection, and of the clade causing infection, can be detected by day 7, and are maintained until day 280 after vaccination. Sample sizes are shown in panel a. e, f, ratios of MN titres against Y159S versus wild-type virus. Data presented in Fig. 4g & i, are re-analysed to show participants infected HN09-like and HN12-like viruses separately. P values are shown for two-sided ANOVA, or if significant for post-hoc comparisons between the group infected with HN14-like viruses and either of the earlier viruses using Bonferroni's Multiple Comparison Test. g, h, HI titers and ratios of titers, against Y159S versus wild-type virus on day 280 post-vaccination. P values are shown for two-sided t-tests, specified as paired tested for within group comparisons across viruses, and as nonpaired tests for across group comparisons within viruses.

**Extended Data Table 1 | Distribution of prior A(H3N2) virus infections by year and strain**

Season	Year	Circulating Strain <sup>a</sup> , equivalent vaccine strain <sup>b</sup> , (clade) <sup>c</sup>	Infected once n	Infected twice, n, first (last)	Infected 3 times, n, first (last)	RTPCR + ILI
1	2008	A/Ha Nam/EL134/08-like ~ Br07 + I361R	2 <sup>d</sup>	5		2 <sup>e</sup>
2	2009	A/Ha Nam/EL201/09-like ~ Pe09 + D225N ( <b>1</b> )	10 <sup>d</sup>	8 (1) <sup>d</sup>	2	2 <sup>e</sup>
3	2009	A(H1N1)pdm09 pandemic, A(H3N2) transmission was not detected				
4	2010	A/Ha Nam/EL444/10-like ~ Pe09 + K144N D53N K62E T212A I230V E280A	6 <sup>d</sup>	5 (2) <sup>d</sup>		2 <sup>e</sup>
5	2011	No sequences available	2 <sup>d</sup>	(2) <sup>d</sup>	1	0
6	2012	No sequences available	2 <sup>d</sup>	(1) <sup>d</sup>		0
7	2012/13	A/Ha Nam/EL12112/12-like ~ Vi11 + D53N T131K N145S A280E ( <b>3c1</b> )	12 <sup>d</sup>	(6) <sup>d</sup>	(1) <sup>d</sup>	1
8	2013	A/Ha Nam/EL13132/13-like ~ Sw13 + N124S S138A S159F D225N R326K A/Ha Nam/EL13237/13-like ~ HK14e + S144N Y159F D225N	4 <sup>d</sup>	(1) <sup>d</sup>		1
9	2014	A/Ha Nam/EL14443/14-like ~ Sw13 ( <b>3c3a</b> )	9 <sup>d</sup>	(3) <sup>d</sup>		7 <sup>e</sup>
10	2015	A/Ha Nam/EL15597/15-like ~ Sw13 + E62K F193S ( <b>3c3a</b> ) A/Ha Nam/EL15628/15-like ~ HK14e ( <b>3c2a</b> )	2 <sup>d</sup>	(2) <sup>d</sup>	(1) <sup>d</sup>	<sup>e</sup>
11	2015	No sequences available	2 <sup>d</sup>		(1) <sup>d</sup>	1
<b>Total</b>			<b>51<sup>d</sup></b>	<b>(18)<sup>d</sup></b>	<b>(3)<sup>d</sup></b>	

<sup>a</sup>Representative of viruses sequenced from swabs of RT-PCR-confirmed ILI cases from the Ha Nam cohort. When more than one clade circulated, the most common clade is listed first and the least common is listed last. <sup>b</sup>Vaccine strains with the highest genetic homology to circulating strains are listed followed by any amino acid positions that differed. <sup>c</sup>Clade, shown in bold, is only indicated for viruses that fall within previously defined clades as indicated on the phylogenetic tree of HA genes shown in Fig. 2a. <sup>d</sup>Number of participants who were last infected during the indicated season. For columns showing numbers of participants who were infected more than once, the numbers whose last infection was in the indicated season are in parentheses. For example, 11 participants were last infected in 2009 with A/Ha Nam/EL201/09-like virus, including 10 who were infected once, and 1 who was infected twice. <sup>e</sup>One participant had RT-PCR-confirmed A(H3N2) virus infection again in season 9 or 10.

Extended Data Table 2 | GMTs against 2004–2018 A(H3N2) virus strains

Virus	GMT (95% CI)								Effect Size of Prior H3N2			
	Pre-Vaccine		Day 7		Day 14		Day 280		Pre-vaccine	d7	d14	d280
	No Prior	Prior H3N2	No Prior	Prior H3N2	No Prior	Prior H3N2	No Prior	Prior H3N2				
Br18	20 (15, 26)	28 (23, 33)	43 (32, 58)	86 (71, 103)	64 (48, 86)	128 (106, 154)	35 (26, 47)	63 (52, 76)	1.4 (1.0, 2.0)	2.0 (1.0, 2.8)	2.0 (1.4, 2.8)	1.9 (1.2, 2.6)
Ka17	9 (7, 13)	14 (11, 17)	21 (15, 30)	45 (36, 56)	33 (23, 47)	77 (61, 97)	11 (8, 16)	28 (22, 36)	1.4 (0.9, 2.3)	2.1 (0.9, 3.2)	2.3 (1.5, 3.7)	2.6 (1.6, 4.0)
Ka17e	7 (5, 11)	9 (7, 11)	22 (15, 32)	39 (31, 50)	31 (21, 46)	65 (51, 83)	8 (6, 12)	22 (17, 28)	1.2 (0.8, 1.9)	1.9 (0.8, 2.8)	2.1 (1.3, 3.2)	2.6 (1.7, 4.3)
Sw17	7 (6, 9)	9 (8, 10)	13 (10, 16)	19 (16, 21)	15 (12, 19)	22 (20, 26)	10 (8, 12)	15 (14, 18)	1.2 (0.9, 1.6)	1.5 (0.9, 1.9)	1.5 (1.1, 1.9)	1.5 (1.2, 2.0)
NC16	13 (9, 19)	25 (20, 31)	45 (32, 65)	98 (78, 122)	78 (54, 112)	174 (139, 218)	28 (20, 41)	67 (54, 84)	1.9 (1.2, 2.8)	2.1 (1.2, 3.2)	2.3 (1.5, 3.5)	2.5 (1.5, 3.7)
NC14	15 (11, 20)	30 (25, 36)	39 (29, 52)	87 (73, 104)	66 (49, 88)	122 (102, 146)	30 (22, 40)	69 (58, 83)	2.0 (1.4, 2.8)	2.3 (1.4, 3.2)	1.9 (1.3, 2.6)	2.3 (1.6, 3.2)
Mi14	11 (8, 16)	20 (16, 25)	37 (26, 54)	95 (76, 119)	69 (48, 99)	181 (144, 228)	32 (22, 46)	67 (53, 84)	1.9 (1.2, 2.8)	2.6 (1.2, 4.0)	2.6 (1.7, 4.0)	2.1 (1.4, 3.2)
HK14e	23 (15, 34)	42 (32, 53)	97 (65, 146)	217 (169, 280)	210 (140, 314)	407 (316, 523)	57 (38, 86)	164 (127, 211)	1.9 (1.1, 3.0)	2.3 (1.1, 3.7)	2.0 (1.2, 3.0)	2.8 (1.7, 4.6)
HN14	12 (8, 17)	23 (18, 29)	41 (28, 60)	114 (90, 144)	76 (52, 111)	209 (166, 264)	35 (24, 51)	80 (63, 101)	1.9 (1.2, 3.0)	2.6 (1.2, 4.3)	2.8 (1.7, 4.3)	2.3 (1.5, 3.5)
Sw13	15 (10, 23)	28 (22, 36)	45 (30, 68)	122 (95, 157)	82 (54, 123)	209 (162, 270)	30 (20, 46)	85 (66, 109)	1.9 (1.1, 3.0)	2.6 (1.1, 4.0)	2.6 (1.6, 4.0)	2.8 (1.7, 4.6)
Sw13e	20 (13, 29)	34 (27, 44)	59 (40, 88)	122 (96, 155)	93 (63, 137)	192 (151, 244)	32 (22, 47)	83 (65, 106)	1.7 (1.1, 2.8)	2.0 (1.1, 3.2)	2.1 (1.3, 3.2)	2.6 (1.6, 4.0)
Tx12e	42 (28, 63)	79 (62, 102)	145 (97, 217)	335 (261, 431)	282 (189, 422)	603 (469, 776)	105 (70, 157)	239 (186, 308)	1.9 (1.1, 3.0)	2.3 (1.1, 3.7)	2.1 (1.3, 3.5)	2.3 (1.4, 3.7)
Tx12	32 (22, 47)	92 (72, 118)	138 (93, 204)	418 (328, 534)	289 (196, 428)	664 (520, 848)	111 (75, 164)	318 (249, 407)	2.8 (1.9, 4.6)	3.0 (1.9, 4.9)	2.3 (1.4, 3.7)	2.8 (1.9, 4.6)
Vi11	29 (22, 38)	60 (51, 71)	74 (57, 96)	155 (132, 183)	125 (96, 162)	194 (165, 228)	70 (54, 91)	146 (124, 172)	2.1 (1.5, 2.8)	2.1 (1.5, 2.8)	1.5 (1.1, 2.1)	2.1 (1.5, 2.8)
Vi11e	50 (34, 73)	94 (74, 119)	152 (104, 223)	342 (269, 434)	297 (202,435)	645 (509, 819)	103 (70, 151)	241 (190, 307)	1.9 (1.2, 3.0)	2.3 (1.2, 3.5)	2.1 (1.4, 3.5)	2.3 (1.5, 3.7)
HN10	30 (22, 41)	61 (50, 75)	82 (59, 113)	181 (148, 222)	134 (97, 186)	244 (199, 299)	63 (45, 87)	137 (112, 168)	2.0 (1.4, 3.0)	2.1 (1.4, 3.2)	1.9 (1.2, 2.6)	2.1 (1.5, 3.2)
HN09	30 (23, 41)	54 (45, 65)	80 (60, 107)	160 (133, 192)	134 (100, 180)	230 (192, 277)	63 (47, 84)	125 (104, 150)	1.7 (1.2, 2.5)	2.0 (1.2, 2.8)	1.7 (1.2, 2.5)	2.0 (1.4, 2.8)
Pe09e	17 (12, 25)	32 (25, 40)	55 (38, 81)	127 (100, 161)	105 (71, 154)	215 (169, 274)	31 (21, 45)	90 (71, 115)	1.9 (1.1, 2.8)	2.3 (1.1, 3.7)	2.0 (1.3, 3.2)	3.0 (1.9, 4.6)
Pe09	31 (23, 43)	60 (50, 73)	78 (57, 106)	149 (123, 181)	102 (75, 140)	207 (171, 251)	55 (40, 75)	128 (106, 156)	2.0 (1.3, 2.8)	1.9 (1.3, 2.8)	2.0 (1.4, 2.8)	2.3 (1.6, 3.5)
HN08	30 (22, 39)	44 (37, 53)	51 (39, 68)	96 (80, 115)	62 (47, 83)	115 (97, 138)	43 (32, 57)	76 (64, 91)	1.5 (1.1, 2.1)	1.9 (1.1, 2.6)	1.9 (1.3, 2.6)	1.7 (1.2, 2.5)
Br07	37 (28, 49)	65 (55, 77)	57 (43, 74)	138 (117, 164)	82 (62, 108)	173 (145, 205)	58 (44, 76)	118 (99, 140)	1.7 (1.2, 2.5)	2.5 (1.2, 3.5)	2.1 (1.5, 2.8)	2.0 (1.5, 2.8)
Ur07e	21 (14, 31)	36 (27, 46)	66 (43, 100)	127 (98, 165)	119 (78, 180)	201 (155, 261)	47 (31, 71)	93 (71, 120)	1.7 (1.1, 2.8)	2.0 (1.1, 3.2)	1.7 (1.1, 2.8)	2.0 (1.2, 3.2)
Th05	28 (21, 38)	42 (35, 50)	57 (42, 76)	101 (84, 121)	80 (60, 107)	124 (104, 149)	50 (37, 67)	76 (64, 92)	1.5 (1.1, 2.1)	1.7 (1.1, 2.5)	1.5 (1.1, 2.1)	1.5 (1.1, 2.1)
Wi05e	24 (16, 35)	42 (33, 53)	58 (39, 86)	116 (91, 149)	102 (69, 152)	183 (143, 234)	50 (33, 74)	91 (71, 117)	1.7 (1.1, 2.8)	2.0 (1.1, 3.2)	1.7 (1.1, 2.8)	1.9 (1.1, 3.0)
Vi04	38 (29, 50)	58 (49, 68)	84 (64, 110)	129 (109, 153)	100 (76, 131)	158 (133, 188)	65 (49, 85)	100 (84, 119)	1.5 (1.1, 2.1)	1.5 (1.1, 2.1)	1.6 (1.1, 2.1)	1.5 (1.1, 2.3)
NY04e	26 (18, 37)	43 (34, 54)	64 (44, 93)	110 (87, 139)	102 (70, 149)	163 (129, 206)	46 (32, 67)	86 (68, 108)	1.6 (1.1, 2.6)	1.6 (1.1, 2.6)	1.6 (1.0, 2.5)	1.9 (1.2, 2.8)
Fu02	88 (66,119)	134(112,162)	181 (139, 235)	237 (200, 282)	256 (190,344)	316 (263, 381)	146(109,197)	204 (170, 246)	1.5 (1.1, 2.2)	1.3 (0.9, 1.9)	1.2 (0.9, 1.8)	1.4 (1.0, 2.0)
Ph02	74 (58, 95)	89 (76, 104)	122 (96, 154)	161 (140, 187)	160 (124, 205)	201 (172, 235)	116 (90, 149)	135 (116, 159)	1.2 (0.9, 1.6)	1.3 (1.0, 1.8)	1.3 (0.9, 1.7)	1.2 (0.9, 1.6)
Ne99	69 (47,100)	90 (71,113)	108 (79, 147)	165 (131, 207)	190 (131,276)	209 (166, 264)	114 (78, 165)	131 (104, 166)	1.3 (0.8, 2.0)	1.5 (1.0, 2.4)	1.1 (0.7, 1.7)	1.2 (0.7, 1.8)
TV99	55 (44, 69)	77 (67, 88)	88 (71, 110)	126 (110, 143)	113 (90, 141)	137 (119, 157)	80 (64, 101)	117 (102, 135)	1.4 (1.1, 1.8)	1.4 (1.1, 1.9)	1.2 (0.9, 1.6)	1.5 (1.1, 1.9)
Ta97	35 (25, 49)	46 (38, 57)	61 (45, 82)	86 (70, 104)	86 (62, 119)	99 (80, 121)	51 (37, 72)	68 (56, 84)	1.3 (0.9, 1.9)	1.4 (1.0, 2.1)	1.1 (0.8, 1.7)	1.3 (0.9, 2.0)
Ne95	56 (39, 81)	95 (76, 119)	95 (67, 135)	165 (131, 207)	141 (98, 203)	186 (149, 234)	87 (61, 126)	146 (116, 183)	1.7 (1.1, 2.6)	1.7 (1.1, 2.7)	1.3 (0.9, 2.0)	1.7 (1.1, 2.6)
Ne93	69 (48,100)	92 (73,116)	105 (74, 149)	165 (131, 208)	122 (84, 176)	179 (142, 226)	79 (55, 115)	141 (112, 178)	1.3 (0.9, 2.1)	1.6 (1.0, 2.4)	1.5 (1.0, 2.3)	1.8 (1.1, 2.8)
Ne92	28 (21, 38)	36 (30, 44)	41 (31, 54)	62 (52, 73)	45 (33, 61)	71 (59, 86)	39 (29, 52)	53 (44, 64)	1.3 (0.9, 1.8)	1.5 (1.1, 2.1)	1.6 (1.1, 2.3)	1.4 (1.0, 2.0)
Ne89	46 (34, 64)	60 (49, 73)	64 (48, 85)	89 (74, 106)	76 (55, 104)	100 (82, 121)	69 (50, 95)	84 (69, 103)	1.3 (0.9, 1.9)	1.4 (1.0, 2.0)	1.3 (0.9, 1.9)	1.2 (0.8, 1.8)
Ph82	39 (28, 55)	52 (42, 64)	53 (40, 70)	76 (62, 94)	55 (39, 78)	79 (64, 98)	52 (37, 74)	67 (54, 83)	1.3 (0.9, 2.0)	1.5 (1.0, 2.2)	1.4 (1.0, 2.2)	1.3 (0.9, 1.9)
Ne82	26 (19, 35)	30 (24, 36)	31 (24, 40)	44 (36, 53)	34 (25, 47)	46 (37, 56)	29 (21, 40)	37 (30, 45)	1.2 (0.8, 1.7)	1.4 (1.0, 2.0)	1.3 (0.9, 1.9)	1.3 (0.9, 1.9)
Bi76	33 (23, 47)	35 (27, 43)	38 (30, 47)	48 (41, 57)	44 (31, 64)	49 (39, 62)	34 (23, 48)	41 (33, 51)	1.1 (0.7, 1.6)	1.3 (0.8, 2.0)	1.1 (0.7, 1.7)	1.2 (0.8, 1.9)
Bi72	50 (35, 71)	68 (55, 85)	58 (44, 77)	86 (70, 105)	64 (45, 90)	94 (76, 117)	55 (39, 78)	81 (65, 100)	1.4 (0.9, 2.1)	1.5 (1.0, 2.2)	1.5 (1.0, 2.2)	1.5 (1.0, 2.2)
Bi68	40 (30, 54)	47 (39, 57)	44 (35, 56)	58 (48, 70)	50 (37, 67)	64 (53, 77)	49 (36, 66)	54 (45, 66)	1.2 (0.8, 1.7)	1.3 (0.9, 1.9)	1.3 (0.9, 1.8)	1.1 (0.8, 1.6)

GMT estimates are shaded when confidence intervals for participants with prior A(H3N2) infection did not overlap with those of participants with no recent prior A(H3N2) infection. Effect size estimates are shaded when confidence intervals did not include 1.0. Boxes indicate viruses against which titers were highest at that time point.

Extended Data Table 3 | Geometric mean ratios against A(H3N2) viruses circulating between 1968 and 2018

Virus	Day 7		Day 14		Day 280	
	No Prior H3	Prior H3	No Prior H3	Prior H3	No Prior H3	Prior H3
Br18	2.2 (1.6, 3.0)	3.1 (2.6, 3.8)	3.3 (2.4, 4.5)	4.7 (3.9, 5.7)	1.8 (1.3, 2.4)	2.3 (1.9, 2.8)
Ka17e	2.9 (1.9, 4.5)	4.5 (3.4, 5.8)	4.2 (2.7, 6.5)	7.4 (5.6, 9.7)	1.1 (0.7, 1.7)	2.5 (1.9, 3.3)
Ka17	2.3 (1.6, 3.3)	3.3 (2.6, 4.2)	3.5 (2.4, 5.1)	5.7 (4.5, 7.2)	1.2 (0.8, 1.7)	2.1 (1.6, 2.6)
Sw17	1.8 (1.4, 2.2)	2.1 (1.9, 2.4)	2.1 (1.7, 2.6)	2.5 (2.2, 2.9)	1.4 (1.1, 1.7)	1.7 (1.5, 2.0)
NC16	3.4 (2.3, 4.9)	3.9 (3.1, 5.0)	5.8 (4.0, 8.5)	7.0 (5.5, 8.9)	2.1 (1.4, 3.1)	2.7 (2.2, 3.5)
NC14	2.6 (2.0, 3.3)	2.9 (2.5, 3.5)	4.3 (3.3, 5.6)	4.1 (3.5, 4.9)	2.0 (1.5, 2.6)	2.4 (2.0, 2.8)
Mi14	3.5 (2.3, 5.1)	4.8 (3.7, 6.1)	6.4 (4.3, 9.5)	9.1 (7.1, 11.6)	2.9 (2.0, 4.3)	3.4 (2.6, 4.3)
HK14e	4.3 (2.7, 6.9)	5.2 (3.9, 7.0)	9.3 (5.8, 14.8)	9.8 (7.3, 13.1)	2.5 (1.6, 4.0)	4.0 (3.0, 5.3)
HN14	3.5 (2.4, 5.0)	5.0 (4.0, 6.4)	6.4 (4.4, 9.3)	9.2 (7.3, 11.7)	2.9 (2.0, 4.2)	3.6 (2.8, 4.5)
Sw13	3.0 (2.0, 4.5)	4.3 (3.4, 5.6)	5.4 (3.6, 8.1)	7.4 (5.8, 9.5)	2.0 (1.3, 3.0)	3.0 (2.3, 3.9)
Tx12e	3.5 (2.2, 5.4)	4.2 (3.2, 5.6)	6.7 (4.3, 10.5)	7.6 (5.8, 10.1)	2.5 (1.6, 3.9)	3.1 (2.3, 4.0)
Sw13e	3.1 (2.0, 4.5)	3.6 (2.8, 4.6)	4.8 (3.2, 7.1)	5.6 (4.4, 7.2)	1.6 (1.1, 2.4)	2.4 (1.9, 3.1)
Tx12	4.3 (2.9, 6.3)	4.5 (3.6, 5.7)	9.1 (6.2, 13.2)	7.2 (5.7, 9.1)	3.4 (2.4, 5.1)	3.5 (2.7, 4.4)
Vi11	2.6 (2.0, 3.3)	2.6 (2.2, 3.0)	4.3 (3.4, 5.5)	3.2 (2.7, 3.7)	2.4 (1.9, 3.1)	2.4 (2.1, 2.8)
Vi11e	3.1 (2.1, 4.5)	3.7 (2.9, 4.7)	5.9 (4.0, 8.8)	6.9 (5.4, 8.9)	2.0 (1.4, 3.0)	2.6 (2.0, 3.3)
HN10	2.8 (2.1, 3.7)	3.0 (2.5, 3.6)	4.5 (3.4, 6.1)	4.0 (3.3, 4.8)	2.1 (1.6, 2.8)	2.3 (1.9, 2.7)
HN09	2.6 (2.0, 3.5)	3.0 (2.5, 3.5)	4.4 (3.4, 5.8)	4.3 (3.6, 5.1)	2.1 (1.6, 2.7)	2.3 (1.9, 2.7)
Pe09	2.5 (1.9, 3.3)	2.5 (2.1, 2.9)	3.3 (2.5, 4.3)	3.4 (2.9, 4.0)	1.8 (1.3, 2.3)	2.1 (1.8, 2.5)
Pe09e	3.2 (2.1, 4.8)	4.0 (3.1, 5.2)	6.1 (4.0, 9.2)	6.8 (5.3, 8.8)	1.8 (1.2, 2.7)	2.9 (2.2, 3.7)
HN08	1.7 (1.4, 2.2)	2.2 (1.9, 2.5)	2.1 (1.7, 2.6)	2.6 (2.3, 3.0)	1.4 (1.2, 1.8)	1.7 (1.5, 2.0)
Ur07e	3.2 (2.2, 4.6)	3.6 (2.8, 4.5)	5.8 (4.0, 8.3)	5.7 (4.5, 7.1)	2.3 (1.6, 3.3)	2.6 (2.1, 3.3)
Br07	1.5 (1.2, 1.9)	2.1 (1.9, 2.4)	2.2 (1.8, 2.7)	2.7 (2.4, 3.0)	1.6 (1.3, 1.9)	1.8 (1.6, 2.1)
Th05	2.0 (1.6, 2.5)	2.4 (2.1, 2.8)	2.8 (2.3, 3.5)	3.0 (2.6, 3.4)	1.8 (1.4, 2.2)	1.8 (1.6, 2.1)
Wi05e	2.4 (1.8, 3.3)	2.8 (2.3, 3.4)	4.3 (3.2, 5.9)	4.4 (3.6, 5.3)	2.1 (1.5, 2.9)	2.2 (1.8, 2.7)
Vi04	2.2 (1.8, 2.7)	2.2 (2.0, 2.6)	2.6 (2.1, 3.3)	2.8 (2.4, 3.1)	1.7 (1.4, 2.1)	1.7 (1.5, 2.0)
NY04e	2.5 (1.9, 3.3)	2.6 (2.2, 3.1)	4.0 (3.0, 5.3)	3.8 (3.2, 4.6)	1.8 (1.4, 2.4)	2.0 (1.7, 2.4)
Fu02	2.1 (1.6, 2.6)	1.8 (1.5, 2.1)	2.9 (2.3, 3.7)	2.4 (2.0, 2.7)	1.7 (1.3, 2.1)	1.5 (1.3, 1.8)
Ph02	1.6 (1.3, 2.0)	1.8 (1.6, 2.1)	2.2 (1.8, 2.7)	2.3 (2.0, 2.6)	1.6 (1.3, 1.9)	1.5 (1.3, 1.7)
Ne99	1.6 (1.2, 2.0)	1.8 (1.6, 2.2)	2.8 (2.1, 3.6)	2.3 (2.0, 2.7)	1.6 (1.3, 2.1)	1.5 (1.3, 1.7)
TV99	1.6 (1.3, 1.9)	1.6 (1.5, 1.8)	2.1 (1.7, 2.5)	1.8 (1.6, 2.0)	1.5 (1.2, 1.7)	1.5 (1.4, 1.7)
Ta97	1.7 (1.4, 2.2)	1.9 (1.6, 2.1)	2.4 (1.9, 3.1)	2.1 (1.9, 2.5)	1.5 (1.2, 1.8)	1.5 (1.3, 1.7)
Ne95	1.7 (1.3, 2.1)	1.7 (1.5, 2.0)	2.5 (2.0, 3.2)	2.0 (1.7, 2.3)	1.6 (1.2, 2.0)	1.5 (1.3, 1.8)
Ne93	1.5 (1.2, 1.9)	1.8 (1.5, 2.1)	1.8 (1.4, 2.2)	1.9 (1.7, 2.3)	1.2 (0.9, 1.5)	1.5 (1.3, 1.8)
Ne92	1.5 (1.2, 1.8)	1.7 (1.5, 2.0)	1.6 (1.3, 2.0)	2.0 (1.7, 2.3)	1.4 (1.1, 1.7)	1.5 (1.3, 1.7)
Ne89	1.4 (1.1, 1.7)	1.5 (1.3, 1.7)	1.6 (1.3, 2.0)	1.7 (1.5, 1.9)	1.5 (1.2, 1.8)	1.4 (1.2, 1.6)
Ph82	1.4 (1.1, 1.6)	1.5 (1.3, 1.6)	1.4 (1.2, 1.7)	1.5 (1.4, 1.7)	1.3 (1.1, 1.6)	1.3 (1.2, 1.4)
Ne82	1.2 (1.0, 1.4)	1.5 (1.3, 1.6)	1.4 (1.1, 1.6)	1.5 (1.4, 1.7)	1.1 (1.0, 1.3)	1.2 (1.1, 1.4)
Bi76	1.3 (1.1, 1.6)	1.5 (1.4, 1.7)	1.2 (1.0, 1.4)	1.3 (1.2, 1.5)	1.2 (1.0, 1.4)	1.3 (1.2, 1.5)
Bi72	1.2 (1.0, 1.4)	1.3 (1.1, 1.4)	1.3 (1.1, 1.5)	1.4 (1.2, 1.5)	1.1 (0.9, 1.3)	1.2 (1.1, 1.3)
Bi68	1.1 (0.9, 1.3)	1.2 (1.1, 1.4)	1.3 (1.1, 1.5)	1.4 (1.2, 1.5)	1.2 (1.0, 1.4)	1.2 (1.1, 1.3)

**Extended Data Table 4 | Comparison of vaccinees who developed symptomatic A(H3N2) virus infection with unaffected vaccinees from their households**

		Participant Number	Recent A(H3N2) infection (% yes)			HI Titer against HK14e (GMT)			Fold-change (GMR)	
Household	¹			Sex (%F)	Age (median)	pre	d14	d280	d14	d280
<b>Symptomatic A(H3N2) virus infection</b>	A	1	no	M	53	20	160	40	8	2
	A	2	no	F	51	80	80	160 <sup>²</sup>	1	2 <sup>²</sup>
	B	2	no	F	43	20	40	20	2	1
	C	2	no	F	57	20	320	40	16	2
	D	1	yes <sup>²</sup>	M	57	20	160	80	8	4
			(20)	(60)	(53)	(26)	(121)	(40)	(4.6)	(2.0)
<b>Unaffected</b>	A	4	no	F	23	40	80	40	2	1
	B	1	yes	M	49	40	1280	80	32	2
	B	3	yes	F	24	20	80	40	4	2
	C	1	no	M	64	20	1280	160	64	8
	D	2	yes <sup>²</sup>	F	46	80	160	80	2	1
	E <sup>³</sup>	1	yes <sup>²</sup>	M	54	40	5120	2560	128	64
			(60)	(60)	(46)	(35)	(279)	(70)	(8.0)	(2.0)

<sup>¹</sup>Four households, referred to as A-D, had 1+ member who was vaccinated then developed symptomatic A(H3N2) infection. <sup>²</sup>Prior A(H3N2) infection was symptomatic and confirmed via RT-PCR. <sup>³</sup>ILI onset occurred before d280 serum was collected so data is excluded from the summary statistics. <sup>⁴</sup>A nonvaccinated member of household E had symptomatic A(H3N2) infection during August 2017. Data for this household was excluded from summary statistics.

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Last updated by author(s): 2022-01-06

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### Software and code

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Data collection

Participant data was recorded into a secure, auditable online database called CliRes, developed by the Oxford University Clinical Research Unit, Viet Nam <https://clires.oucru.org/>. Serological data was linked to participant data using Microsoft Access Version 15.0.5349.1000.

Data analysis

Data was analysed in R using code that is available as part of existing packages (mgcv, ggplot2). R Code used to produce Figures has been uploaded to GitHub ([https://github.com/afooxmarsh/FluVax\\_prior\\_infection\\_study](https://github.com/afooxmarsh/FluVax_prior_infection_study)). Previously published code, available in GitHub was used for antigenic cartography (<https://acorg.github.io/Racmacs>, version 1.1.4) and antibody landscapes (<https://github.com/acorg/ablandscapes>, version 1.0.2)

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The data set used for analysis (wide-format "HI\_timecourse.csv", long format "HI\_long\_diff.csv") will be made available on request and will be publicly available at <https://melbourne.figshare.com/> within one year of this publication. HA (+/- NA) sequences of influenza viruses included in the analyses are available on GISAID. GISAID accession codes are listed in the Supplementary Table 8. Plots showing titres for each antigen and time-point for each individual are also presented in the Supplementary Information.

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## Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

### Sample size

Sample size was based on a sample size calculation for the difference in geometric mean titre ratio (GMR, mean post - pre log 2 titer). We assumed that GMRs would differ by at least 0.7 between the groups with and without recent prior A(H3N2) infection, and that the standard deviation will be up to 1 for each group, based on our previous study comparing responses to antigenically similar versus distant vaccines [ref # 21, main article]. The calculated sample size was 33 per group, and we planned to recruit 50 per group, 100 in total to account for loss to follow-up. Only 32 adult cohort participants lacked A(H3N2) infection during the preceding 9 years. We therefore increased the number of participants with prior infection to 72 to allow for additional comparisons of people infected with A/H3N2 in different years since 2007, and with viruses from different antigenic clusters (i.e A/Brisbane/2007 in 2008; A/Perth/16/2009 from~ 2009-2011; A/Victoria/361/2011 from~ 2011-2013). 100/114 selected participants consented. This sample size is similar to previous studies investigating repeat vaccination.

### Data exclusions

No data were excluded

### Replication

It is not possible to replicate the longitudinal cohort. Antibody titres in pre-vaccine and d21 post-vaccine sera of all participants were measured against the A(H3N2) vaccine strain in two independent experiments by different researchers. HI titers against reverse engineered viruses were also measured in two independent experiments. Titers reproducibility was generally high (Supp Fig. 7)

### Randomization

Participants were not randomly selected. Rather participants were purposefully selected based on their history of prior A(H3N2) virus infection. Participants who lacked any confirmed A(H3N2) infection during 9 prior years of cohort participation were all selected, then 82 participants who had an infection were selected based on proximity of age and sex distribution of the non-infected participants. The final sample included 28 participants without and 72 with prior A(H3N2) infection.

### Blinding

Serology was performed using lab numbers to identify samples so that researchers were blinded to participant information.

## Reporting for specific materials, systems and methods

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|-----|---|

## Eukaryotic cell lines

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MDCK were obtained from ATCC (CCL-34). MDCK-SIAT cells were obtained from Dr Hans-Dieter Klenk (University of Marburg) whose lab developed this cell-line by stable transfection of MDCK cells with cDNA of human α-2,6-sialyltransferase (Matrosovich, M., T. Matrosovich, J. Carr, N. A. Roberts, and H.-D. Klenk. 2003. J. Virol. 77:8418-8425.)

### Cell line source(s)

None of the cell lines used were authenticated

### Authentication

None of the cell lines used were authenticated

### Mycoplasma contamination

Cells are tested monthly for mycoplasma, and are discarded if mycoplasma positive. Cells used were mycoplasma negative

### Commonly misidentified lines (See [ICLAC](#) register)

No commonly misidentified cell lines were used

## Human research participants

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### Population characteristics

This study was nested into the Ha Nam Cohort, an ongoing, prospective study of influenza infection within households. 100 Cohort Participants were included, and received influenza vaccine. 62 were female, 38 were male, and ages ranged from 20 to 81 years. A(H3N2) infection was detected during the preceding 9-years among 72 of 100. The median age of participants with and without prior A(H3N2) infection was the same (49Y), and 64% and 60% were female (Fig. 1a, Extended Fig.1)

### Recruitment

Participants aged at least 18 years, who had participated in all investigations to detect influenza infection since the

Recruitment	commencement of the cohort, and who had no history of reactions to vaccination or condition that rendered them ineligible for influenza vaccine as listed in the summary of product characteristics were asked to participate. Participants who lacked A(H3N2) virus infection since 2007 were recruited first, then participants of similar age and gender distribution who had at least one A(H3N2) virus infection detected were recruited until the sample size was met. Participants were compensated financially for each investigation commensurate with the time required. The requirement to recruit participants purposefully from an existing cohort based on prior infection status, rather than randomly, and the rarity of people who lacked A(H3N2) infection over 9 years meant that the population was biased towards females and older adults. These factors limit interpretation, and inferences are suggestive rather than conclusive. The majority of selected participants consented, and age and sex were similar among selected versus consenting participants (Extended Data Fig 1) indicating that there was little self-selection bias.
Ethics oversight	The study was approved by ethics committees of the University of Melbourne, the National Institute of Hygiene and Epidemiology in Viet Nam, and the Oxford Tropical Medicine Research Unit. All participants provided written informed consent.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

## Clinical data

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Clinical trial registration	ACTRN: ACTRN12621000110886
Study protocol	The study protocol is available on the Australian New Zealand Clinical Trials Registry ACTRN12621000110886
Data collection	The vaccine study was conducted in Ha Nam, Viet Nam. Participant samples were processed at the National Institute of Hygiene and Epidemiology (NIHE), Viet Nam. Virus isolation and sequencing were performed at NIHE and at the WHO Collaborating Centre for Reference and Research in Influenza (WHOCCRRI) in Melbourne, Australia. Serology to detect infections during 9 years prior to the vaccine study was conducted at NIHE. Serology on vaccine sera was conducted at WHOCCRRI.
Outcomes	<p>The primary outcome was vaccine immunogenicity, comparing geometric mean ratios (GMRs) of antibody titres among participants with and without recent A(H3N2) virus infection. Geometric mean titres (GMTs), and proportions seropositive (defined as a titre of 40 or more) or seroconverting (defined as a four-fold or greater titre rise) were also compared. The strain-coverage of antibodies induced by vaccination was further compared by fitting antibody titre landscapes across all A(H3N2) viruses tested<sup>21</sup>. Titres were determined at a range of time points, but comparison focused on day 14 post-vaccination, when titre peaks were detected, and on day 280, when titre decay plateaus<sup>42</sup>.</p> <p>Post hoc comparisons of participants who had been infected with viruses from distinct genetic clades, and of participants who did or did not develop A(H3N2) ILI in the season after vaccination were performed. Within participant comparisons of log<sub>2</sub> transformed titres against reverse genetics viruses bearing wild-type or Y159S HA were compared using paired t-Test. GMRs of wild-type to Y159S virus titres were compared across groups infected with different prior strains were using non-paired t-Test following verification that data passed the Shapiro Wilks Normality test.</p>