

Seasonal influenza vaccination expands haemagglutinin-specific antibody breadth to older and future A/H3N2 viruses

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Supplementary file

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Supplementary Table 1: Demographics of the study population

Characteristics	Adults					Children		
	Total (n=42)	Infection (n=12)	2010 IIV (n=10)	2013 IIV (n=10)	2010 & 2013 IIV (n=10)	Total (n=42)	2012 LAIV (n=20)	2013 LAIV (n=22)
Age (median $\pm$ SD)	36.0 $\pm$ 9.6	33.5 $\pm$ 8.3	34.5 $\pm$ 7.7	46.0 $\pm$ 11.1	37 $\pm$ 9.1	5.0 $\pm$ 5.2	5.0 $\pm$ 5.5	5.0 $\pm$ 4.9
Sex (Female/Male)	32/10	9/3	7/3	9/1	7/3	20/22	8/12	12/10
High-risk condition*	1 (2.4%)	0 (0%)	0 (0%)	0 (0%)	1 (10%)	-	-	-
Previous seasonal vaccination	22 (52.4%)	3 (25%)	6 (60%)	8 (80%)	5 (50%)	-	-	-

*Pregnancy, chronic respiratory diseases, neurological diseases, immunosuppressive diseases, heart diseases and obesity.

- Not applicable for children.

A total of 42 adults and 42 children were included in the study.

Supplementary Table 2: Statistical analysis for Figure 2

Analysis		Multiple comparison		Friedman test	
		Z value	Adjusted p value	Friedman statistics	P value
PE09 infected (n=7), Fig 2a					
	Pre inf vs 6M	4.244	<0.0001	19.46	0.0002
	Pre inf vs 18M	2.484	0.0389		
TX12 infected (n=5), Fig 2b					
	Pre inf vs 6M	-	0.0625*	-	-
Single IIV 2010 (n=10), Fig 2c					
	D0 vs D21	4.850	<0.0001	27.60	<0.0001
	D0 vs 6M	3.291	0.0030		
Single IIV 2013 (n=10), Fig 2d					
	D0 vs D21	3.811	0.0004	20.01	0.0002
Double IIV 2010 (n=10), Fig 2e					
	D0 vs D21	3.897	0.0003	20.09	0.0002
	D0 vs 6M	2.425	0.0459		
Double IIV 2013 (n=10), Fig 2f					
	D0 vs D21	3.724	0.0006	22.07	<0.0001
LAIV 2012 (n=22), Fig 2g					
	D0 vs D28	5.438	<0.0001	50.35	<0.0001
	D0 vs D56	4.258	<0.0001		
	D0 vs 6M	2.616	0.0356		
LAIV 2013 (n=20), Fig 2h					
	D0 vs D28	3.855	0.0005	31.67	<0.0001
	D0 vs D56	3.025	0.0099		

*Two-tailed exact p value from Wilcoxon test.

Data was analysed using a matched, repeated measure, non-parametric Friedman's test with Dunn's multiple comparison test, except the TX12 infected group due to missing samples for long-term follow-up. The TX12 infected group was analysed using non-parametric Wilcoxon matched-pairs signed rank test for pre-infection and 6-9M post infection time points.

Supplementary Table 3: Statistical analysis for Figure 3 and 4

Analysis		Multiple comparison		Friedman test	
		Z value	Adjusted p value	Friedman statistics	P value
PE09 infected					
Fig 3a (n=7)					
Pre inf vs 6M					
	HK14	3.519	0.0013	17.21	0.0006
	TX12	4.347	<0.0001	20.45	0.0001
	PE09	4.244	<0.0001	19.46	0.0002
	WI05	3.726	0.0006	18.78	0.0003
Fig 3b (n=7)					
Pre inf vs 18M					
	TX12	2.484	0.0389	20.45	0.0001
	PE09	2.484	0.0389	19.46	0.0002
	WI05	2.588	0.0290	18.78	0.0003
TX12 infected					
Fig 3d (n=5)					
Pre inf vs 6M					
	TX12	-	0.0625*	-	-
2010 IIV (n=10), Fig 4a					
D0 vs D21					
	HK14	3.897	0.0003	21.48	<0.0001
	TX12	4.850	<0.0001	28.16	<0.0001
	PE09	4.850	<0.0001	27.60	<0.0001
	WI05	4.590	<0.0001	25.84	<0.0001
	CA04	3.811	0.0004	18.80	0.0003
	FU02	3.984	0.0002	20.84	0.0001
	SY97	2.858	0.0128	16.14	0.0011
D0 vs 6M					
	HK14	2.511	0.0361	21.48	<0.0001
	TX12	3.551	0.0012	28.16	<0.0001
	PE09	3.291	0.0030	27.60	<0.0001
	WI05	4.590	0.0361	25.84	<0.0001
2013 IIV (n=10), Fig 4b					
D0 vs D21					
	HK14	3.464	0.0016	19.64	0.0002
	TX12	3.811	0.0004	20.01	0.0002
	PE09	3.811	0.0004	20.93	0.0001
	WI05	3.118	0.0055	15.64	0.0013
	CA04	2.858	0.0128	13.88	0.0031
Double 2010 IIV (n=10), Fig 4c					
D0 vs D21					
	HK14	3.204	0.0041	16.54	0.0009
	TX12	4.070	0.0001	21.19	<0.0001
	PE09	3.897	0.0003	20.09	0.0002
	WI05	3.724	0.0006	17.23	0.0006
	CA04	2.944	0.0097	14.10	0.0028
D0 vs 6M					
	PE09	3.897	0.0459	20.09	0.0002
Double 2013 IIV (n=10), Fig 4d					
D0 vs D21					
	TX12	3.724	0.0006	22.07	<0.0001
	PE09	3.377	0.0022	18.63	0.0003
	WI05	2.944	0.0097	14.82	0.0020
	CA04	2.858	0.0128	13.70	0.0034

*Two-tailed exact p value from Wilcoxon test.

Data was analysed using a matched, repeated measure, non-parametric Friedman's test with Dunn's multiple comparison test, except the TX12 infected group due to missing samples. The TX12 infected group was analysed using non-parametric Wilcoxon matched-pairs signed rank test for pre infection and 6-9M post infection time points.

Supplementary Table 4: Statistical analysis for Figure 5

Analysis			Multiple comparison		Friedman test	
			Z value	Adjusted p value	Friedman statistics	P value
2012 LAIV (n=20), Fig 5a						
	D0 vs D28					
	HK14		4.207	0.0001	26.69	<0.0001
	TX12		5.438	<0.0001	50.39	<0.0001
	PE09		4.720	<0.0001	40.63	<0.0001
	WI05		3.796	0.0006	34.72	<0.0001
	D0 vs D56					
	HK14		4.300	<0.0001	26.69	<0.0001
	TX12		4.258	<0.0001	50.39	<0.0001
	PE09		4.001	0.0003	40.63	<0.0001
	WI05		4.617	<0.0001	34.72	<0.0001
	CA04		2.770	0.0224	24.10	<0.0001
	D0 vs 6M					
	HK14		3.642	0.0011	26.69	<0.0001
	TX12		2.565	0.0413	50.39	<0.0001
	PE09		2.565	0.0413	40.63	<0.0001
	WI05		2.924	0.0138	34.72	<0.0001
	D0 vs 12M					
	HK14		2.514	0.0478	26.69	<0.0001
2013 LAIV (n=22), Fig 5b						
	D0 vs D21					
	HK14		3.220	0.0051	16.30	0.0026
	TX12		3.318	0.0036	20.61	0.0004
	D0 vs D56					
	TX12		2.830	0.0186	20.61	0.0004
	D0 vs 6M					
	HK14		2.879	0.0160	16.30	0.0026

Data was analysed using a matched, repeated measure, non-parametric Friedman's test with Dunn's multiple comparison test.

Supplementary Table 5: Statistical analysis for Figure 6

Analysis		Wilcoxon tests		Summary
		P value	Adjusted p value	
Born 2003-2009 (n=31), Fig 6a				
	HK14	0.0014	0.0042	**
	TX12	0.0001	0.0003	**
	PE09	0.0233	0.0310	*
	WI05	0.0001	0.0002	**
	CA04	0.0156	0.0310	*
Born 1995-2002 (n=11), Fig 6b				
	HK14	0.0039	0.0270	*
	TX12	0.0020	0.0155	*
	PE09	0.0039	0.0270	*
	WI05	0.0078	0.0270	*
	CA04	0.0039	0.0270	*
	FU02	0.0156	0.0310	*
	SY97	0.0039	0.0270	*
Born 1977-1987 (n=14), Fig 6c				
	HK14	0.0005	0.0024	**
	TX12	0.0001	0.0010	***
	PE09	0.0001	0.0010	***
	WI05	0.0001	0.0010	***
	CA04	0.0010	0.0029	**
	FU02	0.0005	0.0024	**
	SY97	0.0137	0.0272	*
Born 1967-1976 (n=14), Fig 6d				
	HK14	0.0002	0.0022	**
	TX12	0.0001	0.0012	**
	PE09	0.0002	0.0022	**
	WI05	0.0010	0.0068	**
	CA04	0.0020	0.0117	*
	FU02	0.0020	0.0117	*
	SY97	0.0020	0.0117	*
	BE92	0.0156	0.0461	*
	SI87	0.0547	0.0547	#
	HK68	0.0234	0.0463	*
Born 1948-1966 (n=14), Fig 6e				
	HK14	0.0040	0.0078	**
	TX12	0.0005	0.0029	**
	PE09	0.0010	0.0049	**
	WI05	0.0020	0.0078	**
	CA04	0.0020	0.0078	**
	SY97	0.0840	0.0840	#

Statistical analyses were performed with non-parametric Wilcoxon matched-pairs signed rank test with individual ranks computed for each comparison, and Holm-Šídák method for multiple comparisons.

Supplementary Table 6: Statistical analysis of group comparisons for Figure 6

Analysis	Pre-exposure		Post-exposure	
	t value	Adjusted p value	t value	Adjusted p value
HK14				
1967-1976 vs. 1948-1966	0.271	ns	2.582	0.0718
1967-1976 vs. 1995-2002	0.642	ns	3.089	0.0184
1967-1976 vs. 2003-2009	2.027	ns	3.564	0.0038
1995-2002 vs. 2003-2009	2.596	0.0914	0.276	ns
TX12				
1967-1976 vs. 1995-2002	0.243	ns	2.753	0.0528
1967-1976 vs. 2003-2009	2.048	ns	2.921	0.0351
PE09				
1967-1976 vs. 1995-2002	1.277	ns	3.547	0.0041
1977-1987 vs. 1995-2002	0.705	ns	3.483	0.0046
1967-1976 vs. 2003-2009	1.393	ns	3.325	0.0073
1977-1987 vs. 2003-2009	2.109	ns	3.245	0.0084
1995-2002 vs. 2003-2009	2.745	0.0599	1.022	ns
WI05				
1948-1966 vs. 2003-2009	1.155	ns	3.366	0.0055
1967-1976 vs. 2003-2009	1.791	ns	4.163	0.0003
1977-1987 vs. 2003-2009	0.757	ns	4.084	0.0004
1995-2002 vs. 2003-2009	2.652	0.0783	3.594	0.0027
CA04				
1948-1966 vs. 2003-2009	2.317	ns	4.482	<0.0001
1967-1976 vs. 2003-2009	1.919	ns	4.961	<0.0001
1977-1987 vs. 2003-2009	1.442	ns	4.881	<0.0001
1995-2002 vs. 2003-2009	2.080	ns	3.873	0.0008
FU02				
1948-1966 vs. 1995-2002	6.115	<0.0001	5.430	<0.0001
1967-1976 vs. 1995-2002	5.479	<0.0001	5.239	<0.0001
1977-1987 vs. 1995-2002	4.907	<0.0001	5.175	<0.0001
1948-1966 vs. 2003-2009	9.643	<0.0001	12.54	<0.0001
1967-1976 vs. 2003-2009	8.848	<0.0001	12.30	<0.0001
1977-1987 vs. 2003-2009	8.132	<0.0001	12.22	<0.0001
1995-2002 vs. 2003-2009	1.828	ns	5.270	<0.0001
SY97				
1948-1966 vs. 2003-2009	6.489	<0.0001	7.912	<0.0001
1967-1976 vs. 2003-2009	6.967	<0.0001	8.776	<0.0001
1977-1987 vs. 2003-2009	6.887	<0.0001	8.870	<0.0001
1995-2002 vs. 2003-2009	5.271	<0.0001	7.320	<0.0001
WU95				
1977-1987 vs. 1967-1976	2.982	0.0203	2.446	ns
1948-1966 vs. 1995-2002	2.820	0.0289	1.820	ns
1977-1987 vs. 1995-2002	4.410	0.0001	4.115	0.0004
1948-1966 vs. 2003-2009	3.669	0.0020	2.975	0.0237
1967-1976 vs. 2003-2009	2.158	ns	2.975	0.0237
1977-1987 vs. 2003-2009	5.658	<0.0001	5.846	<0.0001
BE92				
1967-1976 vs. 1948-1966	1.559	ns	2.379	0.0517
1977-1987 vs. 1948-1966	2.982	0.0146	2.582	0.0453
1948-1966 vs. 1995-2002	2.884	0.0159	1.936	ns
1967-1976 vs. 1995-2002	4.346	0.0001	4.167	0.0002
1977-1987 vs. 1995-2002	5.681	<0.0001	4.358	0.0001
1948-1966 vs. 2003-2009	4.000	0.0004	5.265	<0.0001
1967-1976 vs. 2003-2009	5.830	<0.0001	8.057	<0.0001
1977-1987 vs. 2003-2009	7.500	<0.0001	8.296	<0.0001

	1995-2002 vs. 2003-2009	0.360	ns	2.608	0.0453
BE89					
	1967-1976 vs. 1948-1966	2.169	0.0882	2.311	0.0618
	1977-1987 vs. 1948-1966	3.117	0.0093	3.126	0.0091
	1948-1966 vs. 1995-2002	2.763	0.0231	2.782	0.0218
	1967-1976 vs. 1995-2002	4.797	<0.0001	4.949	<0.0001
	1977-1987 vs. 1995-2002	5.687	<0.0001	5.714	<0.0001
	1948-1966 vs. 2003-2009	3.480	0.0031	3.988	0.0004
	1967-1976 vs. 2003-2009	6.025	<0.0001	6.700	<0.0001
	1977-1987 vs. 2003-2009	7.139	<0.0001	7.658	<0.0001
	1995-2002 vs. 2003-2009	0.021	ns	0.466	0.6577
SI87					
	1948-1966 vs. 1995-2002	3.560	0.0019	3.790	0.0008
	1967-1976 vs. 1995-2002	4.450	<0.0001	5.448	<0.0001
	1977-1987 vs. 1995-2002	5.404	<0.0001	5.766	<0.0001
	1948-1966 vs. 2003-2009	3.880	0.0007	4.729	<0.0001
	1967-1976 vs. 2003-2009	4.993	<0.0001	6.803	<0.0001
	1977-1987 vs. 2003-2009	6.187	<0.0001	7.202	<0.0001
BK79					
	1967-1976 vs. 1948-1966	2.779	0.0329	2.445	0.0573
	1948-1966 vs. 1995-2002	1.971	ns	2.898	0.0190
	1967-1976 vs. 1995-2002	4.577	<0.0001	5.191	<0.0001
	1977-1987 vs. 1995-2002	2.861	0.0298	3.280	0.0064
	1948-1966 vs. 2003-2009	1.963	ns	3.685	0.0017
	1967-1976 vs. 2003-2009	5.224	<0.0001	6.555	<0.0001
	1977-1987 vs. 2003-2009	3.077	0.0170	4.163	0.0003
VI75					
	1967-1976 vs. 1977-1987	3.185	0.0118	3.330	0.0072
	1967-1976 vs. 1995-2002	3.514	0.0041	3.900	0.0009
	1948-1966 vs. 2003-2009	1.642	ns	2.892	0.0270
	1967-1976 vs. 2003-2009	4.188	0.0003	5.365	<0.0001
HK68					
	1948-1966 vs. 1967-1976	2.507	0.0600	2.650	0.0322
	1948-1966 vs. 1977-1987	4.947	<0.0001	5.844	<0.0001
	1967-1976 vs. 1977-1987	2.440	0.0600	3.194	0.0072
	1948-1966 vs. 1995-2002	5.531	<0.0001	6.213	<0.0001
	1967-1976 vs. 1995-2002	3.179	0.0091	3.726	0.0012
	1948-1966 vs. 2003-2009	5.531	<0.0001	7.905	<0.0001
	1967-1976 vs. 2003-2009	3.905	0.0007	4.794	<0.0001

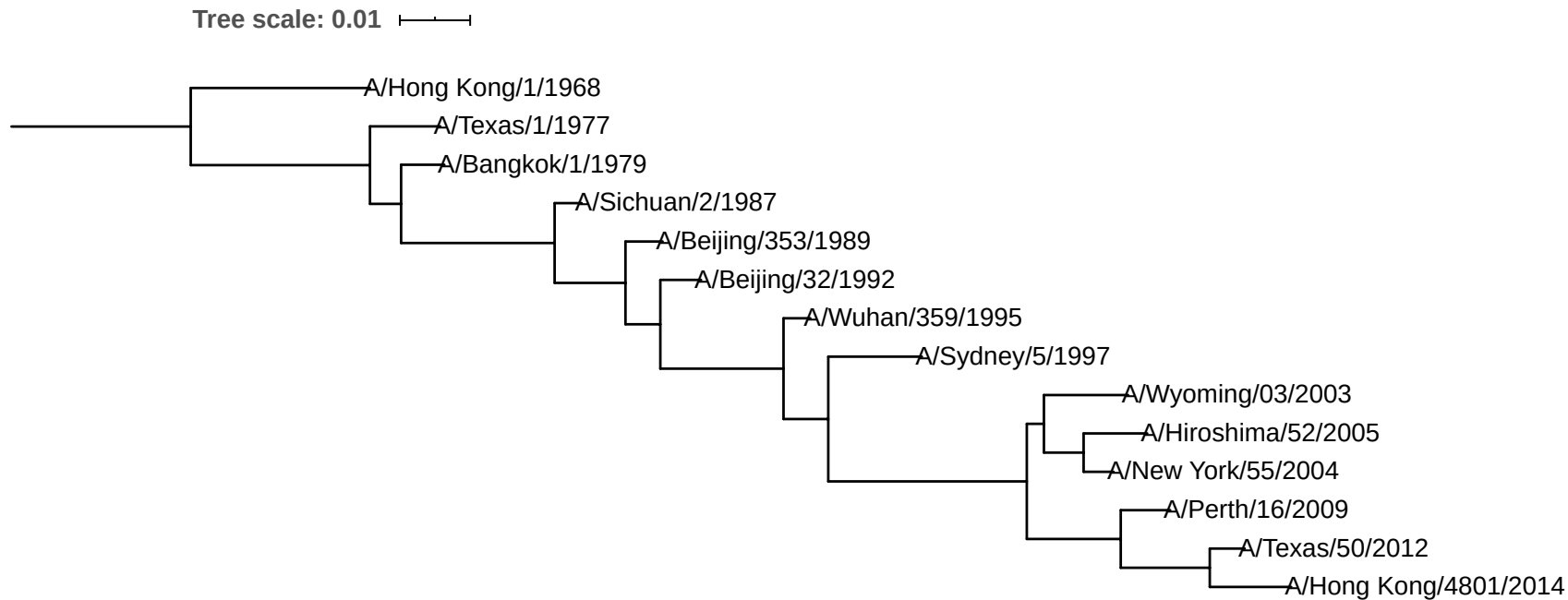
Data was analysed using two-way ANOVA and Holm-Sidak's multiple comparisons test with individual variances computed for each comparison.

ns – not significant p value > 0.05

Supplementary Table 7: Statistical analysis of seroprotection for Figure 7

Analysis	Chi-square	P value	Summary
Figure 7a – Pre-exposure			
1967-1976 vs. 1995-2002	7.189	0.0073	**
1948-1966 vs. 2003-2009	8.378	0.0038	**
1967-1976 vs. 2003-2009	11.95	0.0005	***
1977-1987 vs. 2003-2009	10.45	0.0012	**
Figure 7b – Peak post-exposure			
1967-1976 vs. 1948-1966	3.861	0.0494	*
1948-1966 vs. 1995-2002	4.616	0.0317	*
1967-1976 vs. 1995-2002	9.858	0.0017	**
1977-1987 vs. 1995-2002	6.660	0.0099	**
1948-1966 vs. 2003-2009	25.28	<0.0001	***
1967-1976 vs. 2003-2009	28.45	<0.0001	***
1977-1987 vs. 2003-2009	30.21	<0.0001	***
1995-2002 vs. 2003-2009	16.05	<0.0001	***
Figure 7c – Long-term post-exposure			
1967-1976 vs. 1948-1966	3.102	0.0782	ns
1967-1976 vs. 1995-2002	8.747	0.0031	**
1948-1966 vs. 1995-2002	3.956	0.0047	*
1977-1987 vs. 1995-2002	7.727	0.0054	**
1967-1976 vs. 2003-2009	19.59	<0.0001	***
1948-1966 vs. 2003-2009	15.21	<0.0001	***
1977-1987 vs. 2003-2009	20.61	<0.0001	***
1995-2002 vs. 2003-2009	6.972	0.0083	**
Figure 7d – Pre-exposure			
Prev vacc vs no prev vacc	8.904	0.0028	**
Prev vacc vs infected	5.526	0.0187	*
Figure 7f – Long-term post-exposure			
Prev vacc vs no prev vacc	3.339	0.0677	ns
Prev vacc vs infected	2.234	0.1350	ns

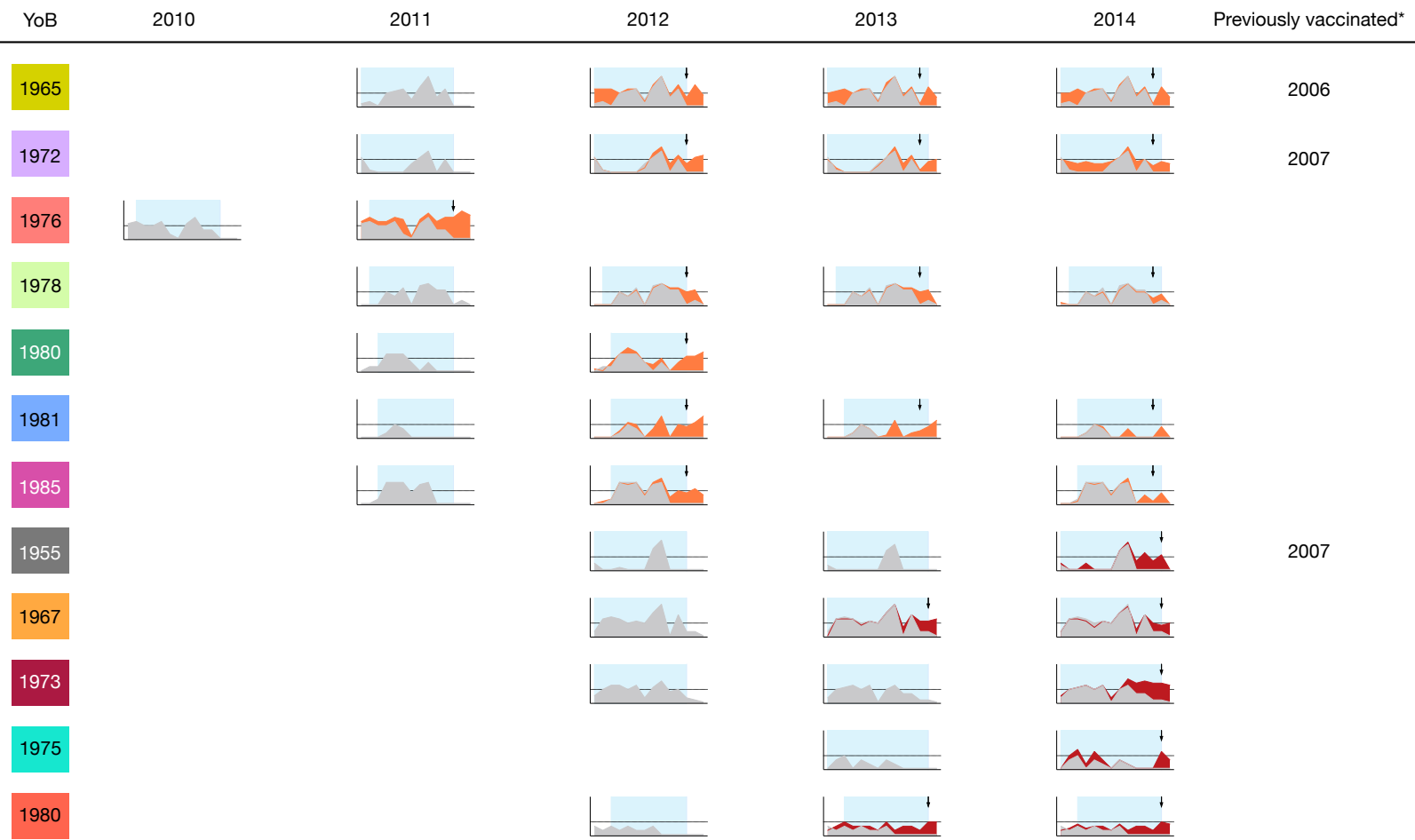
Data was analysed using log-rank Mantel-Cox test.
ns – not significant p value > 0.05



Supplementary Figure 1: Phylogenetic tree of influenza A/H3N2 viruses based on the hemagglutinin (HA) sequence. The HA sequences of 14 influenza A/H3N2 strains circulated from 1968 up until 2018 were obtained from National Center for Biotechnology Information (NCBI, USA, <https://www.ncbi.nlm.nih.gov>) and Global initiative on sharing all influenza data (GISAID, <https://www.gisaid.org>). The phylogenetic tree was generated using NGphylogeny.fr, a free online tool from NCBI. HA distances of 0.01 is indicated in the upper left corner.

Supplementary Figure 2: Adult individual landscapes

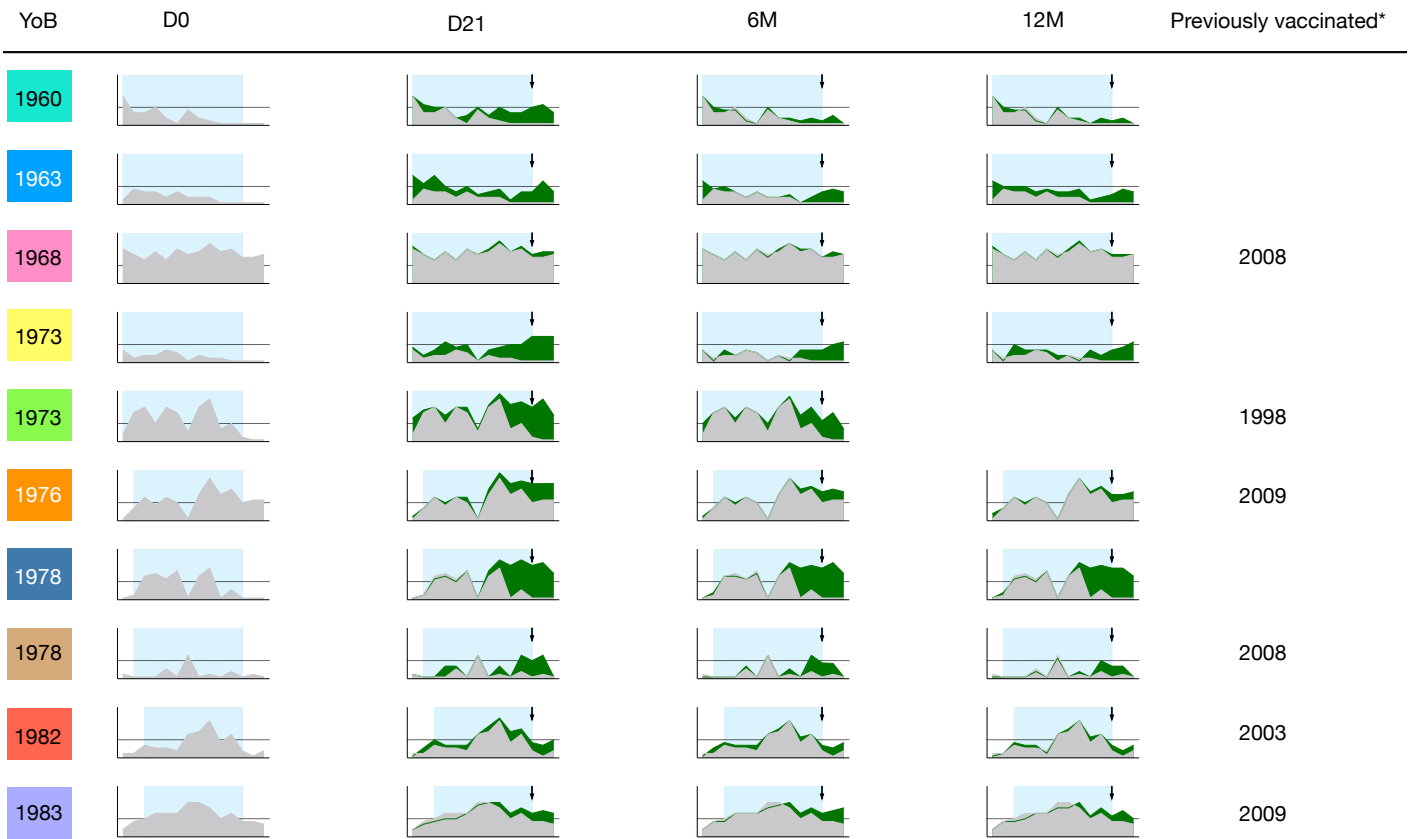
a) Infection group



*Last previous seasonal vaccination.

Geometric mean (GM) haemagglutination inhibition (HI) titers are shown on the Y-axis and the 14 antigenically distinct influenza A/H3N2 viruses from old (1968) to new (2018) along the X-axis. The grey shaded area represents pre-infection titers, orange and red represents post-infection titres at up to three different years. Adults were divided into two groups based on the infecting virus: the first 7 individuals were infected with a PE09-like virus in either 2011 or 2012 (orange post-infection titres), the remaining 5 adults were infected with TX12-like virus in 2013 or 2014 (red post-infection titres). The black arrow indicates the infecting virus, and the dotted line indicates the HI titre of 40. The period of viral exposure is highlighted by a light blue background. Adults were arranged according to birth year (left hand side) within each group.

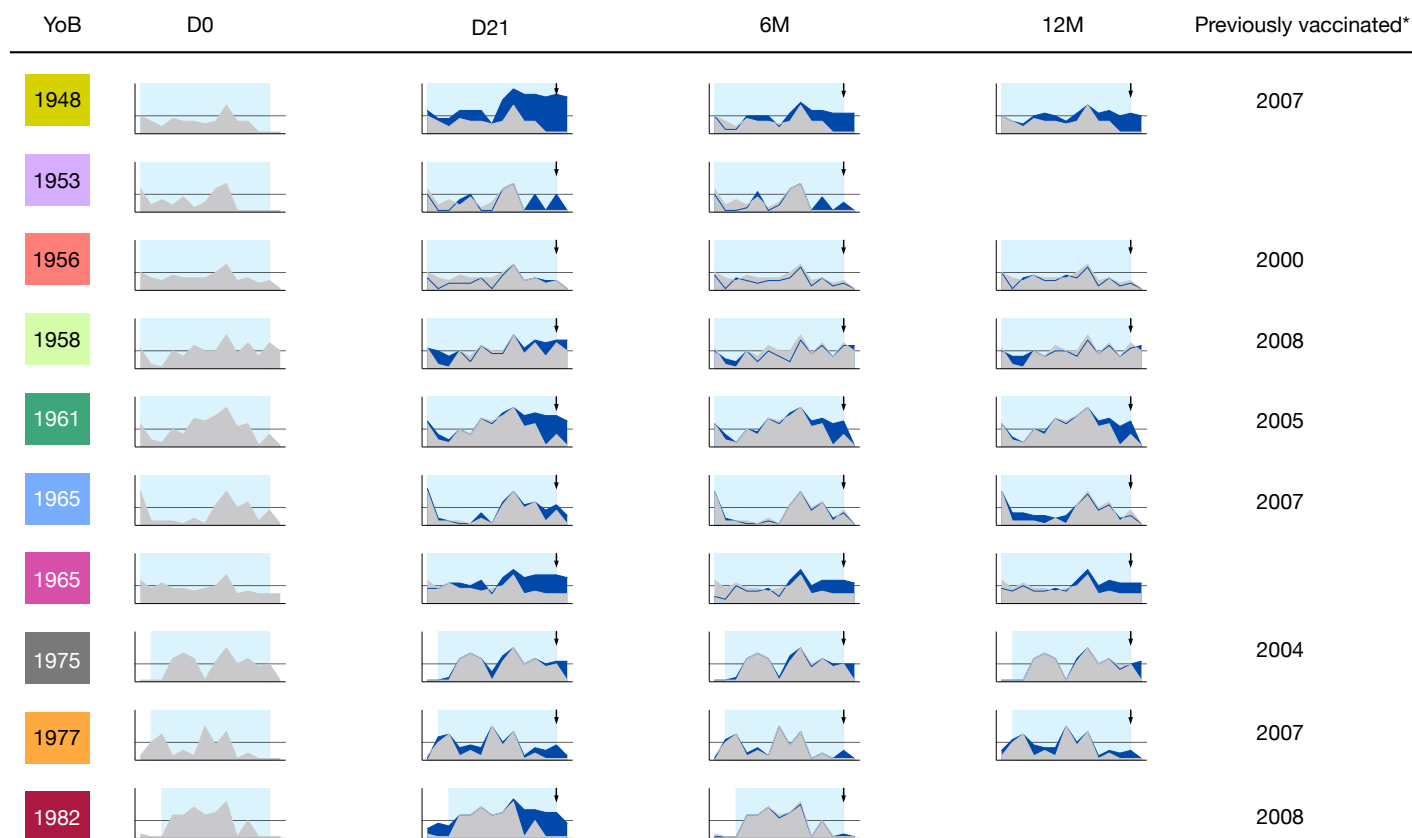
b) Single 2010 IIV



*Last previous seasonal vaccination.

Geometric mean (GM) haemagglutination inhibition (HI) titers are shown on the Y-axis and the 14 antigenically distinct Influenza A/H3N2 viruses from old (1968) to new (2018) along the X-axis. The grey area represents pre-IIV titers at Day 0 (D0), dark green represents post-IIV titres at 21 days (21D), 6 months (6M) and 12 months (12M). Ten adults were included in this group, arranged according to their birth year (left hand side). The black arrow indicates the vaccination virus, and the dotted line indicates the HI titre of 40. The period of viral exposure is highlighted by a light blue background.

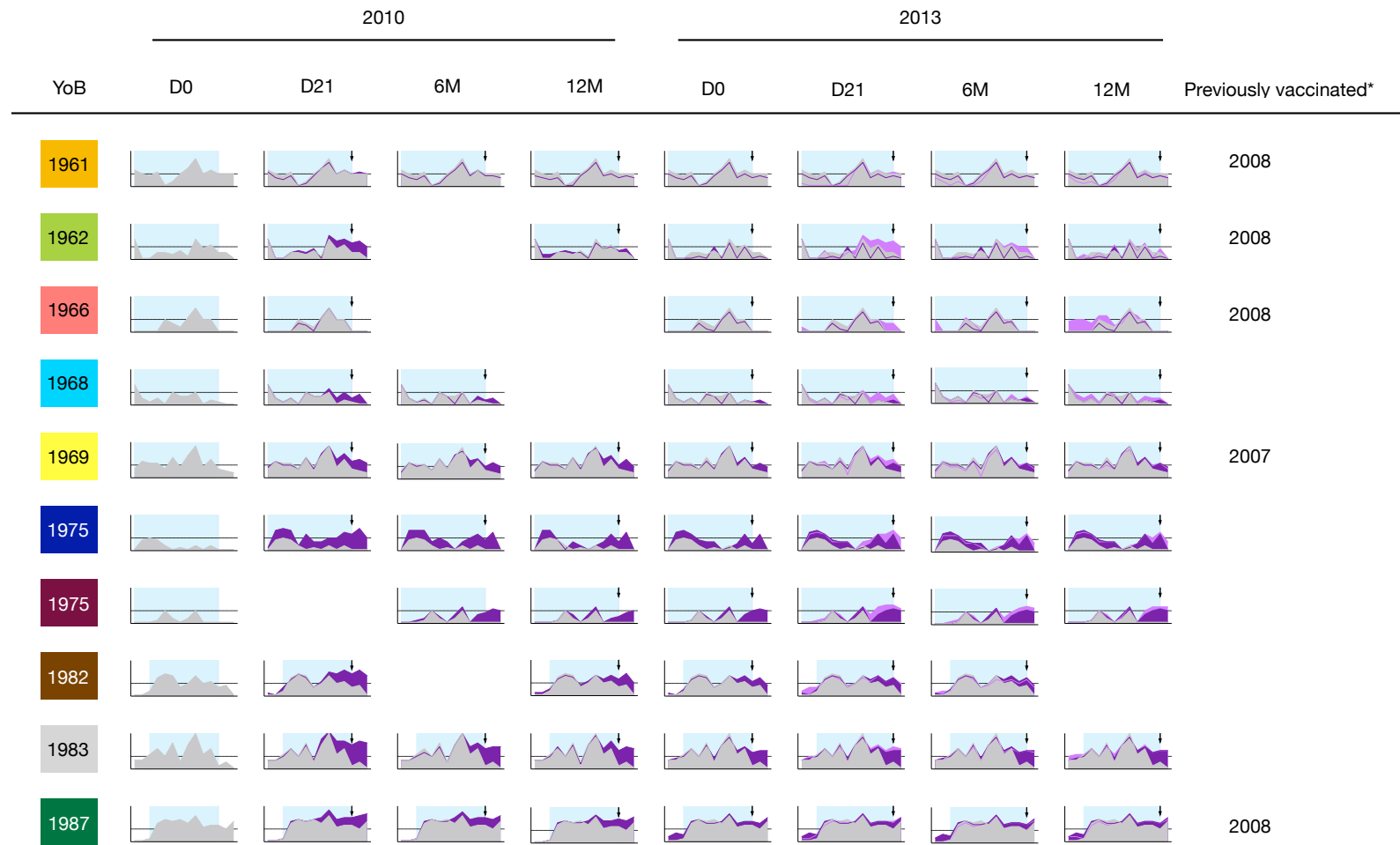
c) Single 2013 IIV



*Last previous seasonal vaccination.

Geometric mean (GM) haemagglutination inhibition (HI) titers are shown on the Y-axis and the 14 antigenically distinct Influenza A/H3N2 viruses from old (1968) to new (2018) along the X-axis. The grey area represents pre-IIV titers, dark blue represents post-IIV titres at 21 days (21D), 6 months (6M) and 12 months (12M). Ten adults were included in this group, arranged according to their birth year (left hand side). The black arrow indicates the vaccination virus, and the dotted line indicates the HI titre of 40. The period of viral exposure is highlighted by a light blue background.

d) Double 2010 & 2013 IIV



*Last previous seasonal vaccination.

Geometric mean (GM) haemagglutination inhibition (HI) titers are shown on the Y-axis and the 14 antigenically distinct Influenza A/H3N2 viruses from old (1968) to new (2018) along the X-axis. The grey area represents pre-IIV titers at Day 0 (D0) 2010, post-IIV titres in dark purple after 1st vaccination (PE09) 21 days (21D), 6 months (6M) and 12 months (12M).and in light purple after 2nd vaccination (TX12) at D21, 6 and 12M. Ten adults were included in this group, arranged according to birth year (left hand side). The black arrow indicates the vaccination virus, and the dotted line indicates the HI titre of 40. The period of viral exposure is highlighted by a light blue background.

Supplementary Figure 3: Individual landscapes after live attenuated influenza vaccine (LAIV) in children

a) LAIV 2012



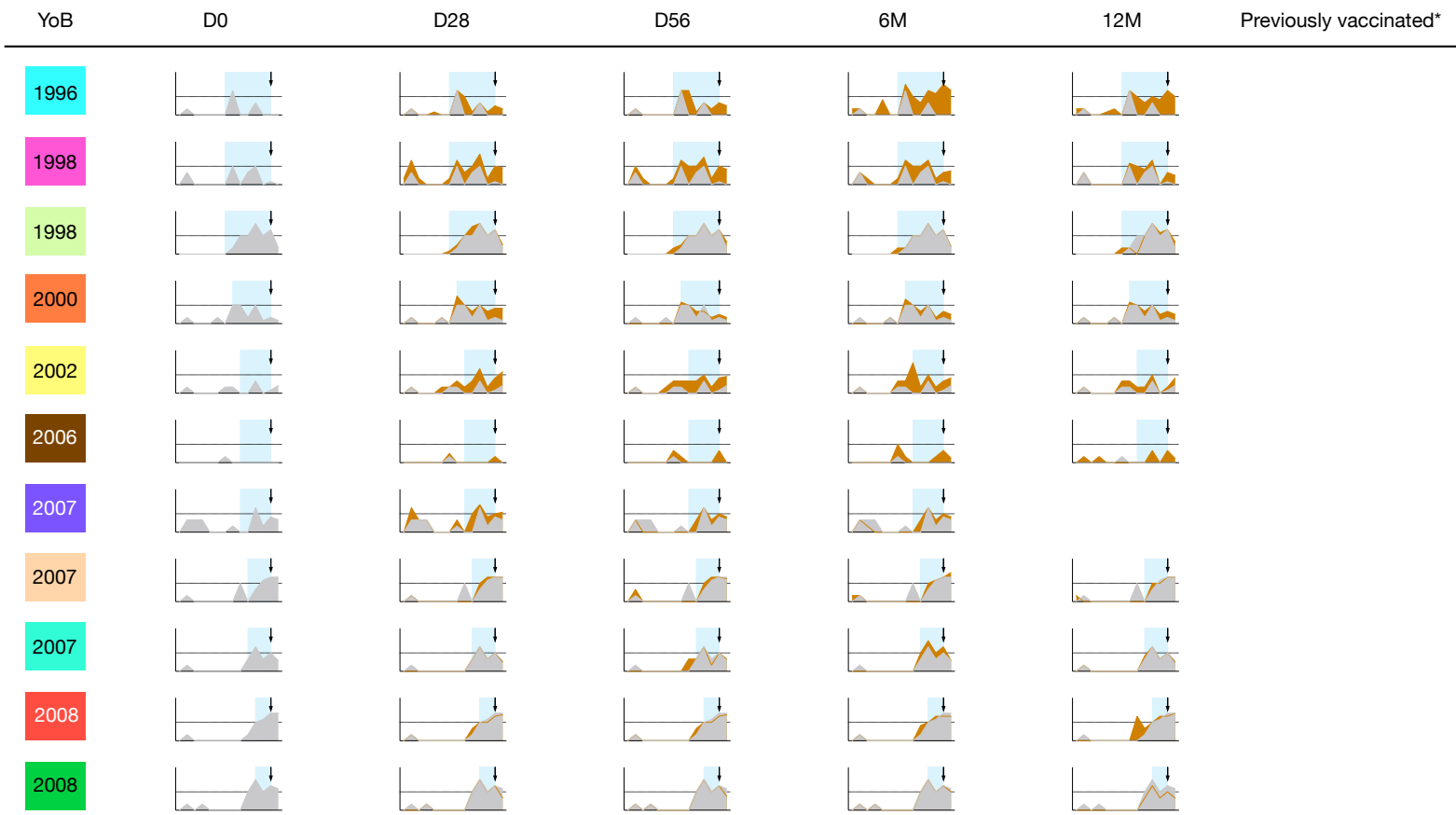
*Previous seasonal vaccination.



*Previous seasonal vaccination.

Geometric mean (GM) haemagglutination inhibition (HI) titers are shown on the Y-axis and the 14 antigenically distinct Influenza A/H3N2 viruses from old (1968) to new (2018) along the X-axis. The grey area represents pre-LAIV titers (Day 0, D0), pink represents post-IIV titres at 28 days (28D) and 56D, 6 months (6M) and 12 months (12M). Twenty children were included in this group (a) 10 and (b) 10, arranged according to birth year (left hand side). The black arrow indicates the vaccination virus, and the dotted line indicates the HI titre of 40. The period of viral exposure is highlighted by a light blue background.

b) LAIV 2012



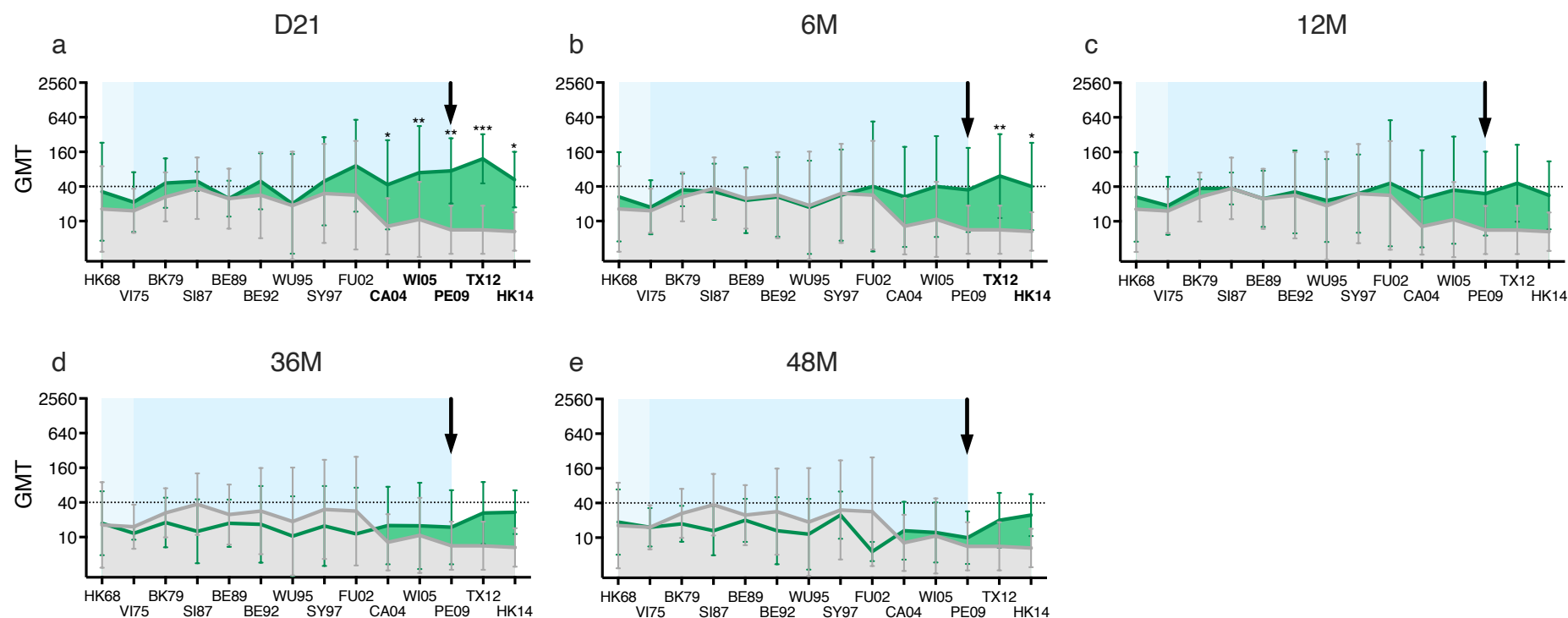
*Previous seasonal vaccination.



*Previous seasonal vaccination.

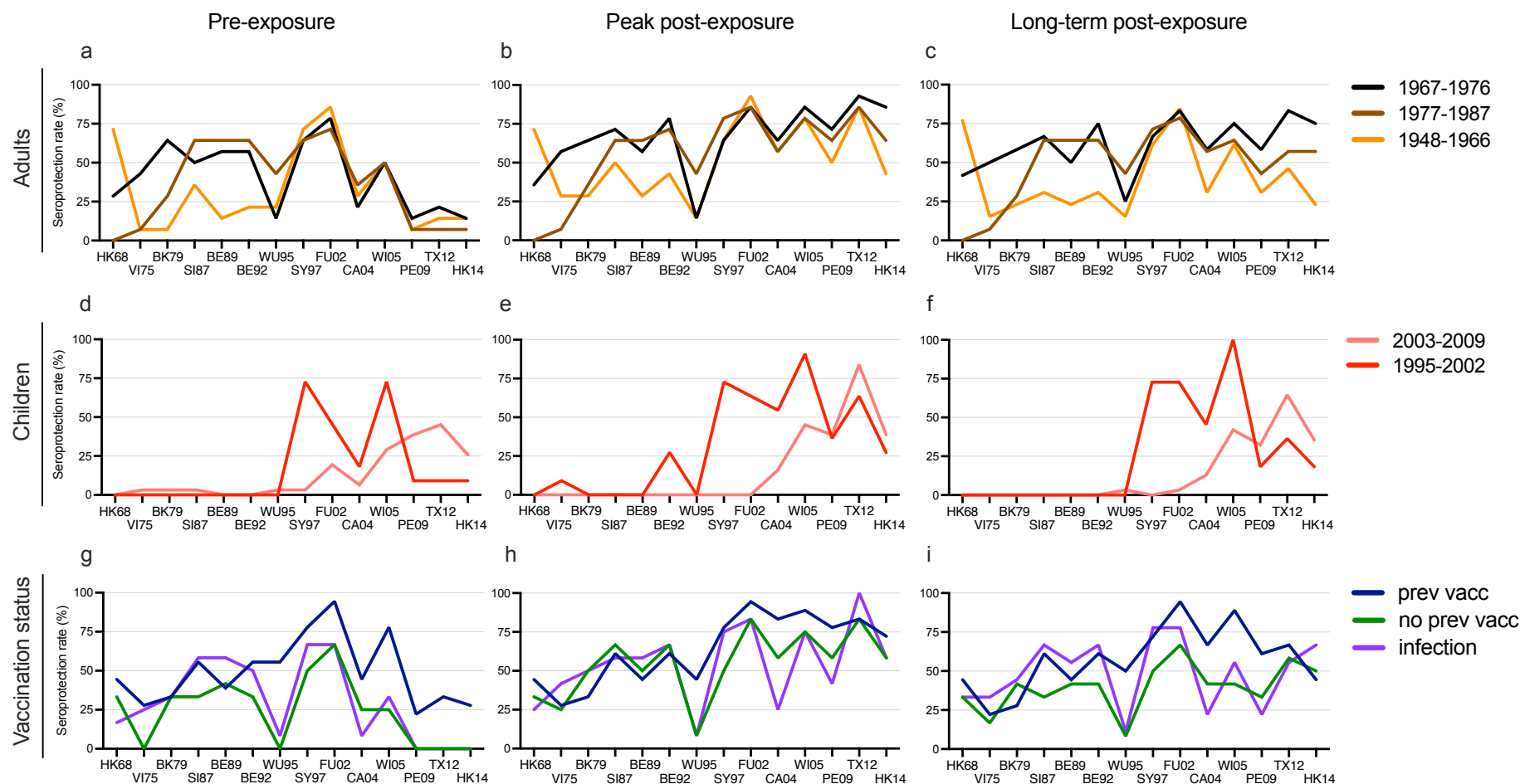
Geometric mean (GM) hemagglutination inhibition (HI) titers are shown on the Y-axis and the 14 antigenically distinct Influenza A/H3N2 viruses from old (1968) to new (2018) along the X-axis. The grey area represents pre-LAIV titers Day 0 (D0), pink represents post-IIV titres at 28 days (28D) and 56D, 6 months (6M) and 12 months (12M). Twenty-two children were included in this group (a) 11 and (b) 11, arranged according to birth year (left hand side). The black arrow indicates the vaccination virus, and the dotted line indicates the HI titre of 40. The period of viral exposure is highlighted by a light blue background.

Supplementary Figure 4: Landscapes of vaccination long-term responses after inactivated influenza vaccine in 2010 (IIV 2010 group)



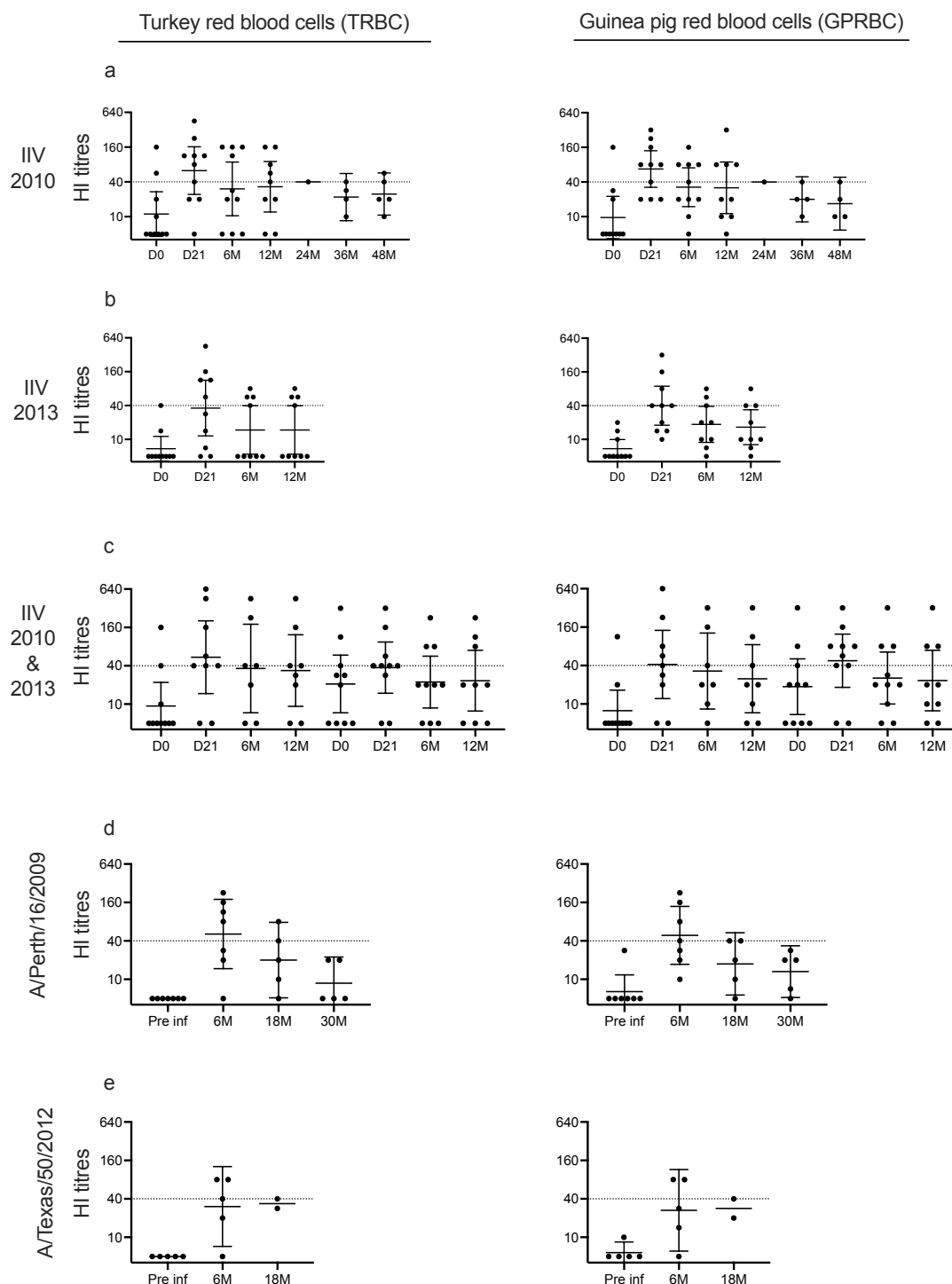
Selected subjects (n=5) from the 2010 IIV group provided blood samples for a long-term evaluation of antibody responses. The figures show geometric mean HI titres (GMTs) against 14 antigenically distinct influenza A/H3N2 viruses. Error bars represent the 95% confidence intervals of the GMTs. Grey pre-IIV titres Day 0 (D0, post-IIV titres in green at time-points day 21 (D21) (a), 6 months (6M) (b), 12 months (12M) (c), 36 months (36M) (d) and 48 months (48M) (e). The black arrow indicates the vaccination virus. The period of viral exposure is highlighted by a light blue background. Pre and post IIV HI titres were compared using non-parametric repeated measure Friedman test with Dunn's multiple comparison correction. *P < 0.05, **P < 0.01, ***P < 0.001.

Supplementary Figure 5: The impact of age and previous vaccination on the breadth of haemagglutination inhibition (HI) A/H3N2-specific antibody responses.



The seropositivity (percentages of individuals with HI titres ≥ 40) against the different influenza A/H3N2 viruses pre-exposure (pre-vaccination or pre-infection) (**a, d, g**), peak post-exposure (day 21/28 post-vaccination or 6 months post-infection) (**b, e, h**), and long-term post-exposure (6 months post-vaccination or 18 months post-infection) (**c, f, i**). Subjects were stratified by their birth year (**a-f**) or vaccination status (**g-i**). Adults were divided into 3 groups: born 1977-1987 (H1/H3 primed), 1967-1976 (H3 primed) and 1948-1966 (H1/H2 primed) (**a-c**) and children were divided into two groups: born 2003-2009 and 1995-2002 (both H1/H3 primed) (**d-f**). Adults were divided by previous vaccination history and compared to the infection group (**g-i**).

Supplementary Figure 6: Comparison of turkey and guinea pig blood in the haemagglutination inhibition (HI) assay against A/Hong Kong/4801/2014 (HK14)



HI antibodies against HK14 were tested using turkey red blood cells (TRBC) or guinea pig red blood cells (GPRBC) in the different groups of adults, (a) inactivated influenza vaccine (IIV) in 2010 (single 2010 IIV group), (b) single 2013 IIV group, (c) double 2010 and 2013 IIV group, (d) infection group infected with A/Perth/16/2009 (H3N2), (e) infection group infected with A/Texas/50/2012 (H3N2). The procedures of HI assay using TRBC were described in the Methods. Briefly, serum samples were treated with receptor-destroying enzyme (Seiken, Japan) and pre-adsorbed with packed TRBC before serial dilution from 1/10 in duplicates and incubated with 4 hemagglutinating units of virus for 1 hour. The serum-virus mixture was further incubated with 0.5% (volume/volume) TRBC for 30 minutes and the HI titre was read as the reciprocal of the highest dilution of the test sample where complete inhibition of agglutination occurs. The procedures of HI assay using GPRBC were similar to TRBC, except that the serum-virus mixture was incubated with 0.7% GPRBC for 1 hour.