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Supplementary information

Influenza virus infection history shapes antibody responses to influenza vaccination

In the format provided by the authors and unedited

Supplementary information: Influenza virus infection history drives and shapes antibody responses to influenza vaccination

Contents

Supplementary Table 1: List of eligible and selected participants

Prior A(H3N2)	Age	Sex	Selected	Consent
yes	19	Male	no	
yes	19	Male	yes	no
yes	20	Female	yes	yes
yes	22	Female	no	
yes	21	Female	yes	yes
no	23	Female	yes	yes
yes	23	Male	no	
yes	23	Female	yes	yes
yes	25	Female	yes	yes
yes	54	Female	no	
yes	30	Male	yes	yes
no	31	Female	yes	yes
yes	30	Female	no	
yes	32	Female	no	
yes	32	Male	no	
no	32	Female	yes	yes
no	34	Female	yes	yes
yes	32	Male	no	
yes	34	Male	no	
yes	33	Female	yes	yes
yes	36	Female	yes	yes
yes	33	Female	yes	yes
yes	36	Female	yes	no
yes	35	Male	no	
no	36	Female	yes	yes
yes	37	Male	no	
yes	37	Female	yes	yes
yes	37	Female	yes	no
yes	37	Female	yes	yes
yes	39	Female	no	
yes	39	Female	yes	yes
yes	40	Female	no	
yes	40	Female	yes	yes
yes	40	Female	yes	yes
yes	41	Male	yes	yes
yes	41	Female	no	
yes	50	Male	no	
yes	42	Female	yes	no
yes	42	Male	no	
yes	42	Female	yes	yes
no	42	Female	yes	yes
yes	43	Female	no	
yes	43	Female	yes	yes
yes	43	Female	yes	yes
yes	43	Male	yes	yes
yes	43	Female	yes	yes
no	42	Female	yes	yes
yes	43	Female	yes	yes
yes	43	Male	yes	yes
no	44	Male	yes	yes
no	44	Male	yes	yes
yes	44	Female	no	
yes	44	Female	no	
no	44	Male	yes	yes
yes	45	Female	yes	yes
yes	45	Female	yes	no
no	45	Female	yes	yes
yes	45	Female	yes	yes
yes	44	Female	no	
yes	46	Female	yes	yes
yes	46	Female	yes	yes
yes	46	Female	yes	yes
yes	46	Female	no	
yes	46	Female	yes	yes
yes	47	Male	yes	yes
yes	46	Male	yes	yes
yes	44	Male	yes	yes
yes	47	Male	yes	yes
yes	47	Male	no	
yes	47	Male	yes	yes
yes	49	Female	yes	yes
no	48	Female	yes	yes
no	48	Female	yes	no
no	48	Female	yes	yes
yes	48	Male	yes	no
yes	48	Female	yes	yes
yes	30	Male	yes	yes
yes	48	Female	no	
yes	48	Male	yes	yes
yes	46	Female	yes	yes
yes	50	Female	yes	yes
no	50	Male	yes	yes
yes	50	Female	no	
no	49	Female	yes	yes
yes	50	Female	yes	yes

Blue = selected no prior; Green = selected with prior

Contd. p 4

Prior A(H3N2)	Age	Sex	Selected	Consent
no	51	Female	yes	yes
yes	52	Male	no	
yes	50	Female	yes	yes
yes	50	Female	no	
yes	51	Female	no	
yes	51	Male	yes	yes
yes	52	Male	yes	yes
yes	52	Male	yes	yes
yes	49	Female	yes	yes
no	53	Male	yes	yes
yes	53	Male	no	
yes	53	Male	yes	no
yes	52	Male	yes	yes
yes	54	Male	no	
yes	53	Male	yes	yes
yes	53	Male	yes	yes
yes	53	Female	yes	yes
yes	53	Female	no	
yes	54	Male	yes	yes
no	54	Male	yes	yes
yes	54	Female	no	
yes	53	Female	no	
yes	53	Male	no	
yes	54	Female	yes	yes
no	55	Female	yes	yes
yes	55	Female	yes	yes
no	56	Male	yes	yes
yes	53	Male	yes	yes
yes	56	Male	yes	yes
yes	56	Female	yes	yes
yes	56	Male	yes	yes
yes	56	Female	yes	yes
no	57	Female	yes	yes
no	58	Male	yes	yes
yes	58	Female	yes	yes
yes	58	Female	yes	no
yes	58	Female	no	
no	58	Female	yes	yes
yes	60	Male	yes	yes
yes	57	Female	yes	yes
no	57	Female	yes	yes
yes	58	Male	no	
no	57	Male	yes	no
yes	59	Male	yes	yes

Prior A(H3N2)	Age	Sex	Selected	Consent
yes	62	Male	yes	no
yes	60	Female	no	
yes	60	Female	yes	yes
yes	61	Female	no	
yes	61	Female	no	
yes	61	Female	yes	yes
yes	61	Female	no	
no	61	Female	yes	yes
yes	61	Female	yes	yes
yes	62	Male	no	
yes	62	Male	yes	no
yes	62	Female	yes	yes
yes	62	Male	yes	yes
yes	62	Male	yes	yes
yes	62	Female	yes	yes
yes	63	Male	no	
no	65	Male	yes	yes
no	66	Male	yes	yes
no	67	Male	yes	no
yes	66	Male	no	
yes	63	Female	no	
yes	66	Female	no	
no	70	Female	yes	yes
yes	69	Male	yes	yes
yes	70	Male	yes	yes
yes	72	Male	yes	yes
yes	74	Male	no	
no	86	Female	yes	no
yes	86	Male	yes	yes
yes	81	Female	yes	yes
yes	88	Male	no	

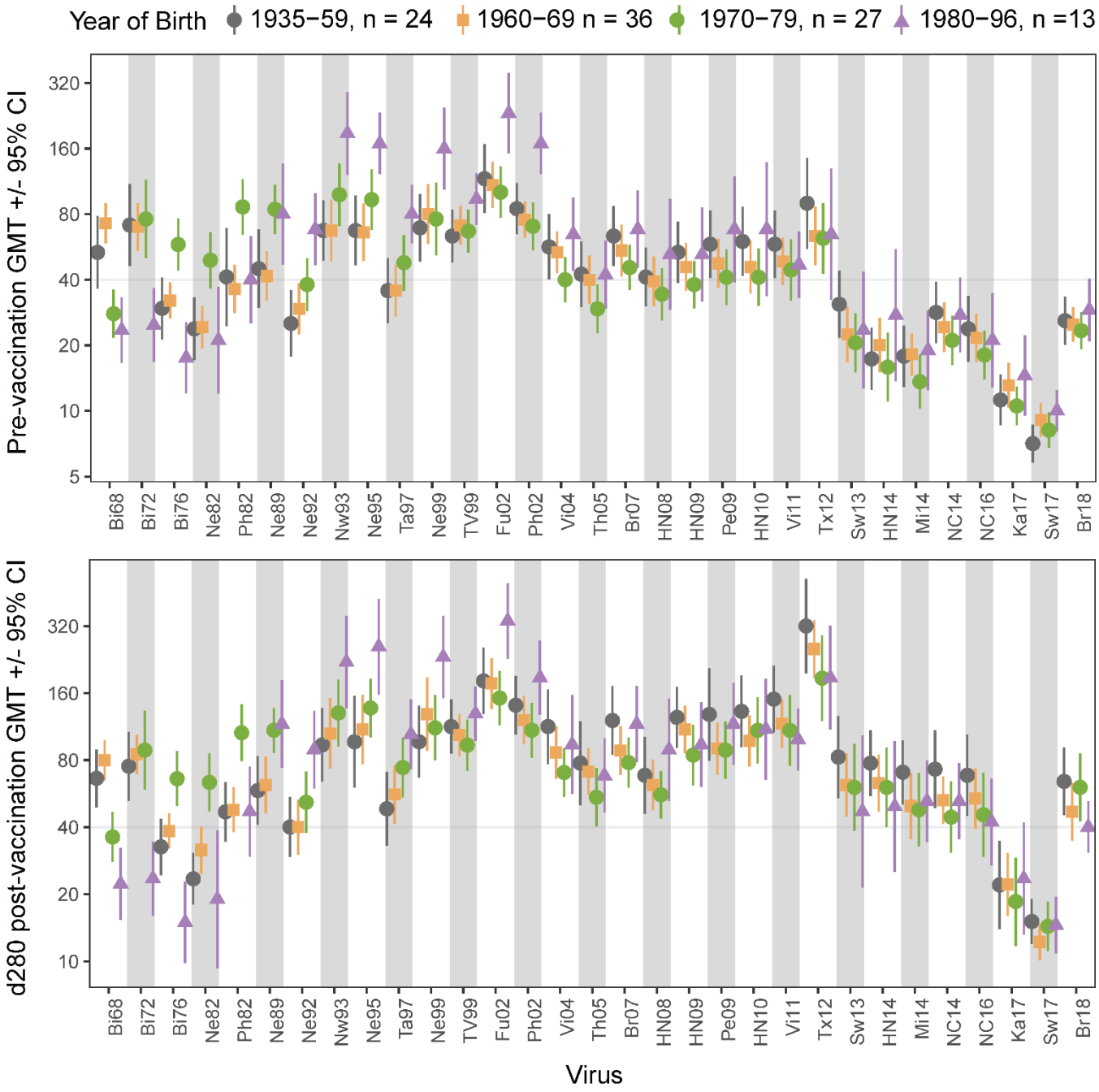
Blue = selected no prior; Green = selected with prior

Supplementary Table 2: A(H3N2) viruses used for serology, showing passage history and assessment of NA mediated agglutination

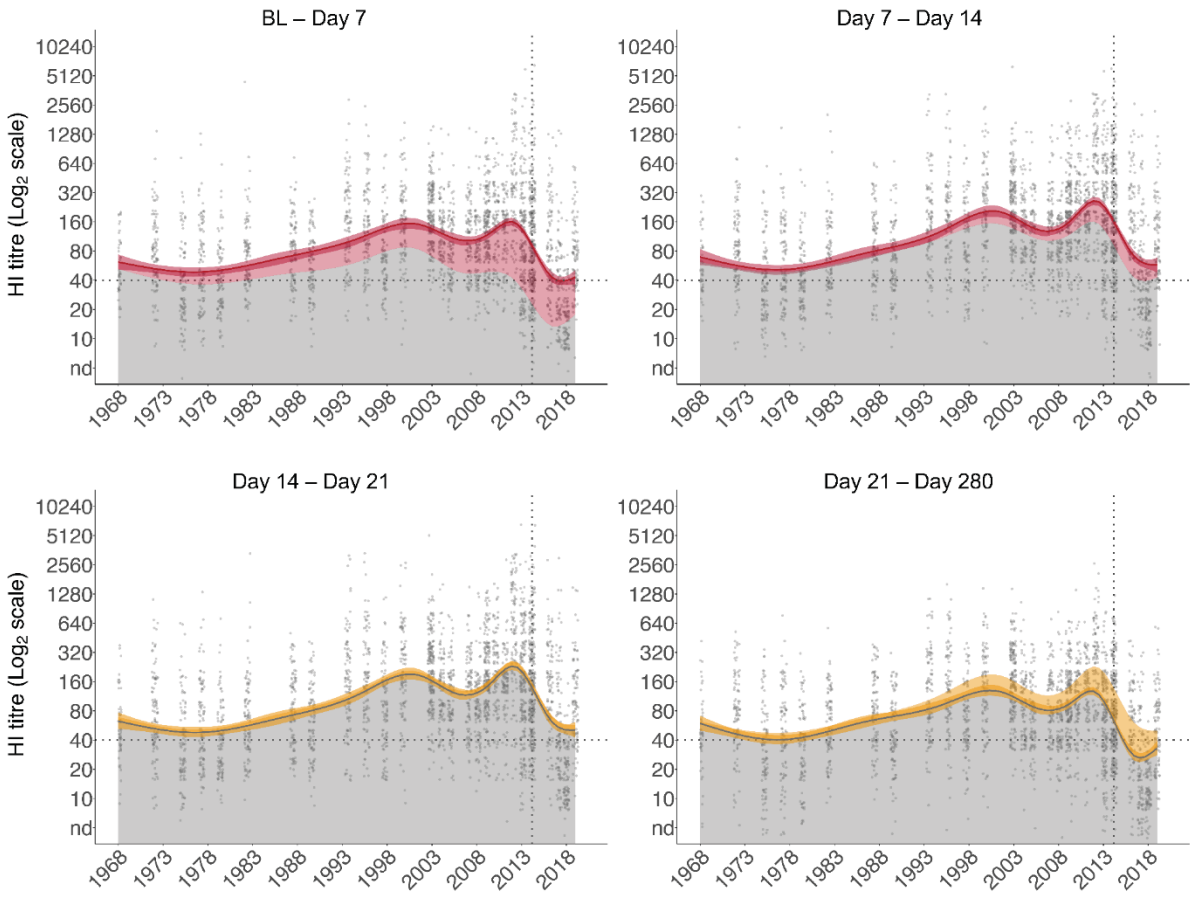
Year	Designation	Passage [a]	NA aa sequence 147-151 (X mix)	HA Titre [b,c] T	HA Titre [b,c] GP	HA Titre [b,c] GP^{OST}	HI, GMT [c] PBS	HI, GMT [c] OST	sera n [d]	p [e]
1968	A/Bilthoven/16190/68	X,MDCK3	DTIHD	16	32	64	47	42	45	·233
1972	A/Bilthoven/21793/72	MDCK3	DTIHD	64	128	128	**44**	31	20	·002
1975	A/Bilthoven/1761/76	MDCK3	DTIHD	32	64	64	**44**	33	29	·005
1977	A/Bilthoven/2271/76	X,MDCK3	DTIHD	32	128	128	**67**	51	20	·002
1979	A/Netherlands/233/82	tMK1,MDCK4	DTIHD	16	32	32	36	33	44	·199
1982	A/Philippines/2/82	MDCKX,2	DTIHD	16	16	32	62	44	20	·163
1987	A/Netherlands/620/89	X,tMK1,MDCK3	DTVHD	32	32	64	59	58	31	·768
1989	A/Netherlands/823/92	X,MDCK3	DTVHD	32	32	32	56	52	20	·606
1993	A/Netherlands/179/93	X,MDCK3	DTVHD	32	32	32	**74**	57	43	·000
1995	A/Netherlands/178/95	293T,MDCK4	DTVHD	16	32	32	**88**	61	15	·001
1997	A/Tasmania/1/97	MDCK7	DTVHD	64	32	32	40	34	20	·259
1999	A/Netherlands/301/99	MDCK5	DTVHD	16	16	32	140	120	31	·090
1999	A/Townsville/2/99	MDCK2, SIAT1[p]	DTVHX(D|G)	*16*	128	64	57	**293**	8	·005
2002	A/Philippines/472/02	MDCK6[p]	DTVHX(D|N)	4	16	8	58	**101**	20	·009
2002	A/Fujian/411/02	X,MDCK9, SIAT1	DTVHD	8	32	24	113	109	43	·594
2004	A/Victoria/511/04	MDCKx,2[p]	DTVHX(D|N|S|G)	32	64	16	45	**69**	18	·011
2005	A/Thailand/409/05	P2,MDCK2[p]	DTVHX(D|G)	32	64	32	34	32	18	·331
2007	A/Brisbane/10/07	MDCKX,5,SIAT1[p]	DxVRX(k|i)(N|E|K)	24	128	32	75	78	18	·298
2008	A/HaNam/EL134/08	MDCK4, SIAT1[p]	NxVRD(T|K)	16	32	16	73	**135**	8	.064
2009	A/HaNam/EL201/09	MDCKX,3,SIAT1	NTVRD	na	8	8	80	70	5	.621
2009	A/Perth/16/09	MDCKX,5	NIVRD	32	64	4	50	43	19	·333
2010	A/HaNam/EL444/10	MDCK3,SIAT1[p]	NxVRD(t|i)	16	64	32	93	**166**	17	·008
2011	A/Victoria/361/11	MDCK2,SIAT1[p]	NTVRX(D|G)	32	64	32	25	**67**	20	·000
2012	A/Texas/50/12	C2,MDCK6,SIAT1	NTVHX(D|G)	16	64	32	65	**133**	44	·000
2013	A/Switzerland/9715293/13	SIAT, SIAT8	NTVRD	32	64	64	39	34	21	·132
2014	A/Michigan/15/14	MDCK1, SIAT6	NTVRD	16	40	40	**40**	30	47	·000
2014	A/New Caledonia/104/14	MDCK1, SIAT4[p]	NTVRX(D|G)	0	64	32	38	36	23	·549
2014	A/HaNam/EL437/14	X,SIAT2	NTVRD	4	32	16	-	-	-	-
2016	A/Newcastle/30/16	SIAT1,SIAT4	NTVRD	8	32	32	47	41	19	·333
2017	A/Kansas/14/17	SIAT3,SIAT1	NTARD	8	32	64	127	101	9	.080
2017	A/Switzerland/8060/17	SIAT2,SIAT2	NTVRD	8	96	9	17	22	5	.373
2018	A/Brisbane/60/18	SIAT3	NTVRD	1	128	128	137	132	18	.331
2004	A/New York/55/04[egg]	SPFCK3,Egg6	DTVHD	256	256	512	**48**	36	21	·000
2005	A/Wisconsin/67/05[egg]	SPFCK3, Egg8	DTVHD	64	32	64	**34**	25	20	·001
2007	A/Uruguay/716/07[egg]	SPFCK1,Egg5	DTVHD	256	128	128	**55**	39	18	·001
2009	A/Perth/16/09[egg]	Egg6	NTVRD	256	32	32	**56**	24	22	·000
2011	A/Victoria/361/11[egg]	Egg6	NTVRD	256	256	256	-	-	-	-
2012	A/Texas/50/12[egg]	Egg5,Egg2	NTVHD	na	128	128	-	-	-	-
2013	A/Switzerland/9715293/13[egg]	Egg6	NTVRD	512	256	256	37	39	21	·835
2014	A/Hong Kong/4801/14[egg]	Egg7	NTVRD	128	128	128	69	62	45	·260
2017	A/Kansas/14/17[egg]	Egg9	NTARD	-	256	-	-	-	-	-

a: passage cell type and number of passages (C/P/X = passage undefined; MDCK = Madin Darby Canine Kidney cells; SIAT = human 2,6-sialtransferase transfected MDCK cells; SPFCK = chicken kidney cell).
b: HA titre determined against red blood cells from turkeys (T) or guinea pigs (GP)
c: HA and HI titres were determined with and without Osletamivir (OST) at a final concentration of 20 nM
d: Homologous strain ferret anti-sera and human sera were used
e: Two-tailed, paired t-test for the comparison of HI titres obtained with versus without OST
p: Viruses were subsequently plaqued on SIAT cells (S. Table 11, p. 22) before use in serology

Classic NA-mediated agglutination = NA 148/151 substitutions + OST reduces HA titres + OST increases HI titre
NA-mediated agglutination is apparent from the effect of OST on HA titres, but OST had minimal effect on HI titre
Effects of OST were apparent in the absence of NA substitutions



Supplementary. Fig. 1: Antibody titres across A(H3N2) strains for participants stratified by age group.
The strain reactivity of sera was assessed pre-vaccination, and 280d post vaccination, when there was limited acute exposure to influenza virus. Participants are stratified by year of birth (YOB, legend). Comparison across strains shows that GMTs were relatively high against strains encountered early in life, as well as against 1993-2002 strains, regardless of year of birth. By d280 post-vaccination, GMTs were also relatively high against a 2012 strain regardless of year of birth, potentially indicating that strains can emerge that escape effects of prior strain immunity and/or that are intrinsically more immunogenic.



Supplementary. Fig 2: Incremental change in antibody landscapes at each study time point.
Lines represent titre landscapes across strains estimated using GAMs. Titre landscapes at the first time point in each panel are shown in grey and landscapes at the subsequent time point are shown in red if higher and in yellow if lower than at the earlier time point. Dark shading either side of the lines indicates the 95% CIs for the model, and dots show individual participant titres against each antigen. Light shaded areas reflect the difference in the landscapes between the time points. BL: baseline.

Supplementary. Table 3. Participants inclusion in analysis of effects of the prior infecting strain (HN14- versus HN09- or HN12-like)

HN14-like, clade-3c3a seasons					HN09- or HN12-like, clade-1/3c1 seasons [2]				
Seasons infected	Last strain[1]	Assays [3]	Birth Year	Sex	Seasons infected	Last strain	Assays [3]	Birth Year	Sex
2,9	HN14-like (3c3a)	MN, HI	1945	M	7	HN12-like (3c1)	MN, HI	1954	M
2,9	HN14-like (3c3a)	MN, HI	1954	F	7	HN12-like (3c1)	MN, HI	1955	F
1,10	HN14-like (3c3a) [1]	MN, HI	1957	M	2	HN09-like (1)	MN, HI	1955	F
9	HN14-like (3c3a)	MN, HI	1958	M	2	HN09-like (1)	MN, HI	1958	F
4,9	HN14-like (3c3a)	MN, HI	1962	F	2	HN09-like (1)	MN, HI	1960	F
9	HN14-like (3c3a)	MN, HI	1964	M	1,7	HN12-like (3c1)	MN, HI	1960	F
9	HN14-like (3c3a)	MN, HI	1965	M	2	HN09-like (1)	MN, HI	1966	F
9	HN14-like (3c3a)	MN, HI	1970	F	2	HN09-like (1)	MN, HI	1967	M
9	HN14-like (3c3a)	MN, HI	1973	F	7	HN12-like (3c1)	MN, HI	1969	M
8	HN14-like (3c3a) [1]	MN, HI	1973	F	2	HN09-like (1)	MN, HI	1972	F
9	HN14-like (3c3a)	MN, HI	1976	F	7	HN12-like (3c1)	MN, HI	1975	M
9	HN14-like (3c3a)	MN, HI	1982	F	1,7	HN12-like (3c1)	MN, HI	1977	F
9	HN14-like (3c3a)	MN, HI	1986	M	7	HN12-like (3c1)	MN, HI	1992	F
9	HN14-like (3c3a)	MN only	1962	M	2	HN09-like (1)	MN, HI	1994	F
11	HK14-like (3c2a) [1]	na	1958	F	7	HN12-like (3c1)	MN only	1935	F
8	indeterminate	na	1971	F	2	HN09-like (1)	MN only	1954	M
8	indeterminate	na	1978	F	2	HN09-like (1)	MN only	1956	F
					7	HN12-like (3c1)	MN only	1960	M
					7	HN12-like (3c1)	MN only	1963	M
					7	HN12-like (3c1)	MN only	1966	F
					2	HN09-like (1)	MN only	1970	F
					7	HN12-like (3c1)	MN only	1970	M
					7	HN12-like (3c1)	MN only	1973	M
					7	HN12-like (3c1)	MN only	1978	F
					2,7	HN12-like (3c1)	not tested	1960	M
					2,7	HN12-like (3c1)	not tested	1963	M
					2,5,7	HN12-like (3c1)	not tested	1967	F
					4,7	HN12-like (3c1)	not tested	1971	F
					4,7	HN12-like (3c1)	not tested	1983	F

1: All viruses detected in the cohort in season 9 were HN14-like (clade-3c3a), whereas viruses detected in seasons 8, 10, and 11 were only classified as HN14-like if infection caused ILI and virus in swabs could be sequenced to confirm.

2: To minimize complexity, participants with prior clade 1/3c1 infection were limited to those infected in season 2 only, season 7 only, or season 1 and 7. This excluded 6 participants who were infected in multiple seasons.

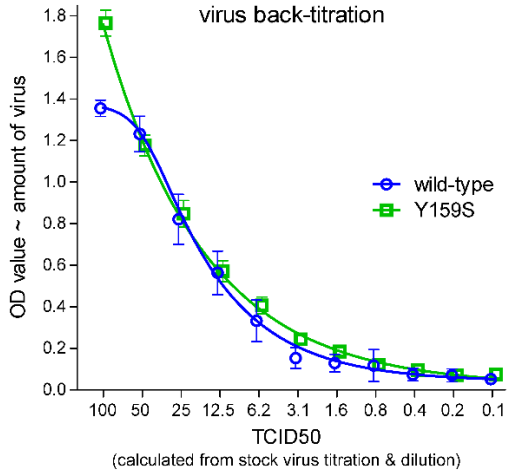
3: 14 of 24 participants infected in season 2, 7 or 1 and 7 were also tested by HI assay. Their age and sex distribution was similar to that for participants with prior 3c3a infection.

Supplementary. Table 4. Antigenic characterization of RG viruses bearing wild-type and Y159S HA of HK14e

	Virus			
Antisera/mAb	HK14e	RG HK14e HA	RG Y159S HA	Sw13e
Anti-HK14e	**1280** [1]	1280	320	80
Anti-Sw13e	80	80	160	**640** [1]
Q12C9 [2]	80	80	20	20

1: Antisera was raised in a single ferret for each virus. Titers of homologous antisera are in bold.

2: Q129C mAb was generated from B cells of a person exposed to A/Victoria/361/2011, and selects for substitutions at positions 160 and 194 in site B. Generously provided by Alain Townsend (MRC Weatherall Institute of Molecular Medicine, UK).



Supplementary Fig. 3: Back titration of RG viruses diluted to 100x the 50% tissue culture infectious dose (TCID50) based on stock virus titration. Back titrations were performed according to WHO protocols (online methods). In brief, two-fold dilutions of the 100 TCID50 stock were incubated overnight on SIAT cells before detecting virus using anti-NP mAb. Each virus was titrated in replicates of 8, Symbols indicate means and error bars indicate 95% confidence intervals for 8 replicate titrations of each virus.

Supplementary. Table 5. Antigenic site positions that vary between HK14e and strains circulating before and after vaccination

	Antigenic Site and Amino Acid Position (H3 numbering) [a]																									
	Site E					Site A						Site B						Site C						Site D			
Virus (clade)	**62**	**91**	**92**	**94**	**261**	**131**	**135**	**138**	**142**	**144**	**145**	**128**	**159**	**160**	**193**	**194**	**198**	**45**	**48**	**53**	**278**	**311**	**312**	**121**	**171**	**212**	**223**
HN09 (1)	K	S	K	H	R	T	T	A	R	K^{-}	N	T^{+}	F	K^{-}	F	L	A	S^{-}	T	D	N	Q	N	N	N	T	V
HN12 (3c1)	E	S	K	Y	R	K	T	A	R	N^{+}	S	T^{+}	F	K^{-}	F	L	S	S^{-}	T	N	N	Q	S	N	N	A	I
HN14 (3c3a)	E	S	K	Y	R	T	T	S	G	N^{+}	S	A^{-}	S	K^{-}	F	L	S	N^{+}	I	D	K	Q	S	N	N	A	I
HK14e (3c2a)	**E**	**S**	**K**	**Y**	**R**	**T**	**T**	**A**	**R**	**S**$^{-}$	**S**	**T**$^{+}$	**Y**	**K**$^{-}$	**F**	**P**[e]	**S**	**N**$^{+}$	**I**	**D**	**K**	**H**	**S**	**N**	**N**	**A**	**I**
HK14 (3c2a)	E	S	K	Y	R	T	T	A	R	S^{-}	S	T^{+}	Y	T^{+}	F	L	S	N^{+}	I	D	K	Q	S	N	N	A	I
HN17[b] (3c2a1)	E	S	K	Y	R	T	T	A	R	S^{-}	S	T^{+}	Y	T^{+}	F	L	S	N^{+}	I	D	N	H	S	K	K	A	I
HN17[c] (3c2a1b)	G	S	R	Y	R	T	K	A	G	S^{-}	S	A^{-}	Y	T^{+}	F	L	S	N^{+}	I	D	K	Q	S	K	K	A	I
HN17[d] (3c2a2)	E	S	K	H	Q	K	T	A	K	R^{-}	S	T^{+}	Y	T^{+}	F	L	S	N^{+}	I	D	K	H	S	N	N	A	I
Ka17 (3c3a)	E	N	K	Y	R	T	T	S	G	K^{-}	S	A^{-}	S	K^{-}	S	L	S	N^{+}	I	D	K	Q	S	N	N	A	I

a: residues are coloured according to amino acid properties as in Figure 1 c,d

b: A/Ha Nam/EL177772/17 ~ Si16, NC16

c: A/Ha Nam/EL17804/17, A/Ha Nam/EL17805/17 ~ Br18

d: A/Ha Nam/EL17762/17, A/Ha Nam/EL17795/17~ Sw17

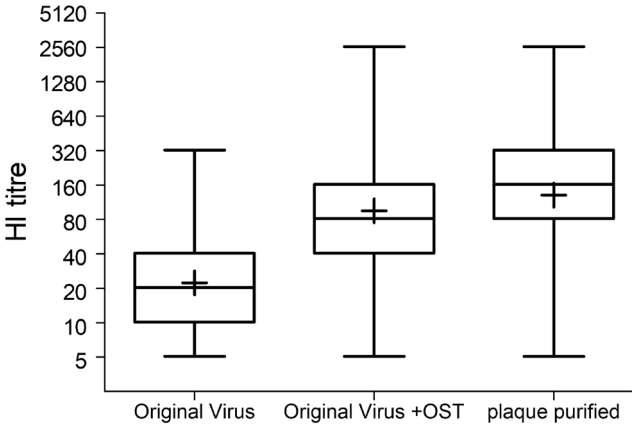
e: egg adaptive substitution

+/-: Amino acid substitutions that result in the gain (+) or loss (-) of a glycosylation site are coloured in pink.

Supplementary. Table 6: NA sequences and titres of viruses after plaque selection on SIAT cells

Virus	NA 147-151 sequence	HA PBS	HA OST	HI PBS	HI OST
A/Townsville/2/99	DTVHD	64	64	123	147
A/Philippines/472/02	DTVHD	64	64	123	113
A/Victoria/511/04	DTVHD	48	48	67	73
A/Thailand/409/05	DTVHD	128	128	52	48
A/Brisbane/10/07	DTVRD	128	128	147	160
A/Hanoi/EL134/08	NTVRD	24	24	na	na
A/Perth/16/09	NTVRD	64	64	135	147
A/Victoria/361/11*	NTVRD	96	96	*	*
A/New Caledonia/104/14	NTVRD	64	64	87	80

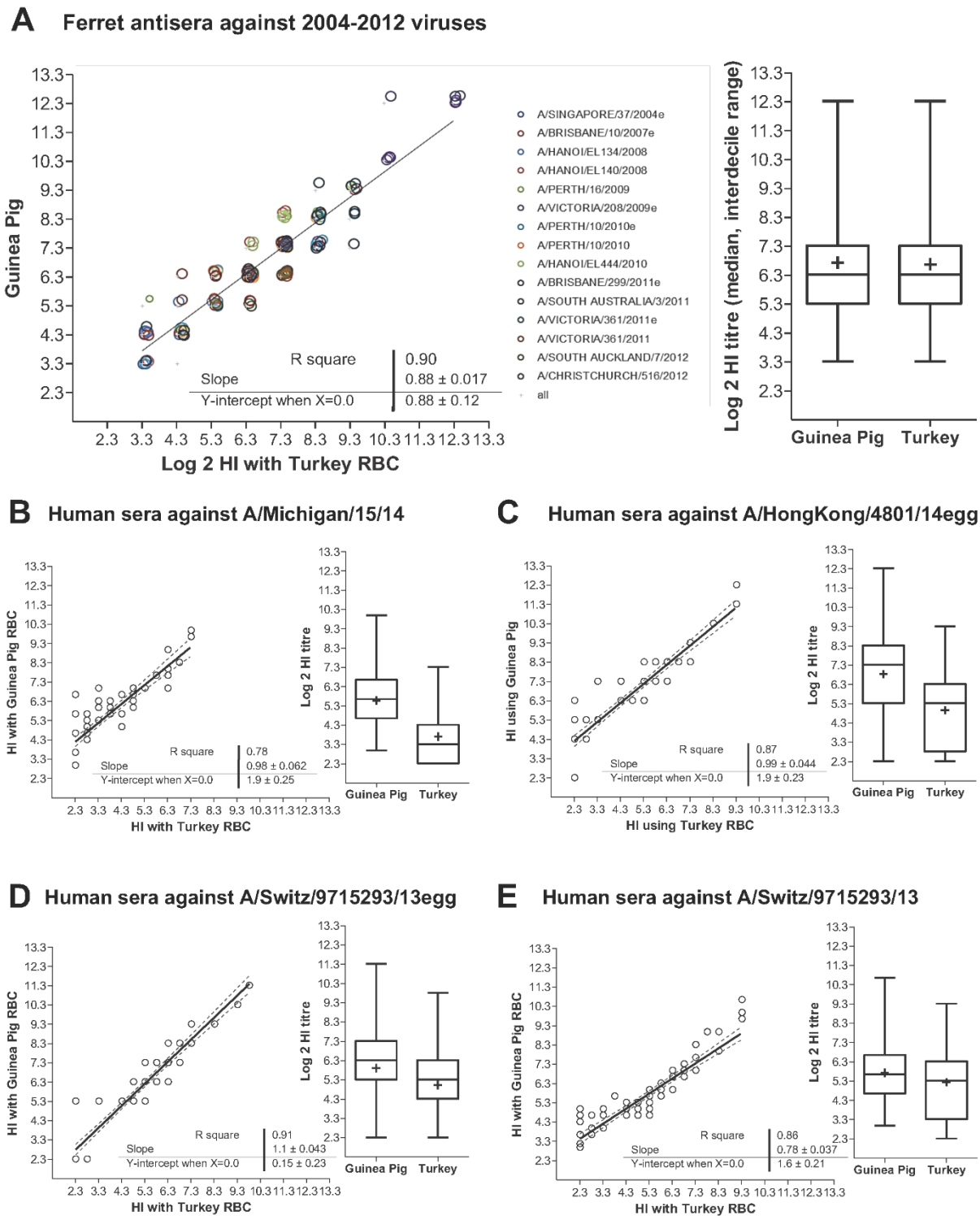
*Titres of all study sera with and without Oseltamivir are summarized in Figure S4 (below).



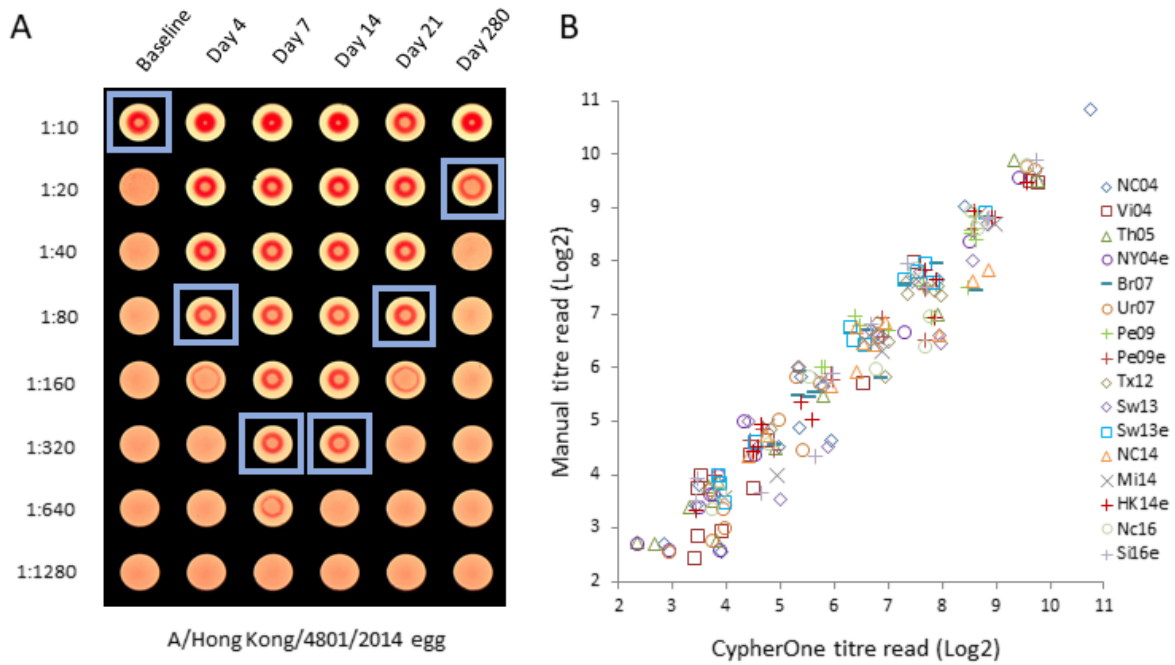
Supplementray. Fig. 4: Serum HI titres against A/Victoria/361/2011 are improved by adding Oseltamivir or by plaque selection on SIAT cells. Box and whisker plots show medians, interquartile ranges and ranges. GMTs are indicated by the + symbol. 600 sera were tested, from100 vaccinees at 6 time points (pre-vaccination and days 4, 7, 14, 21 and 280 post-vaccination). OST: oseltamivir.

Supplementary. Table 7: Viruses used to generate antisera for antigenic characterization and cartography

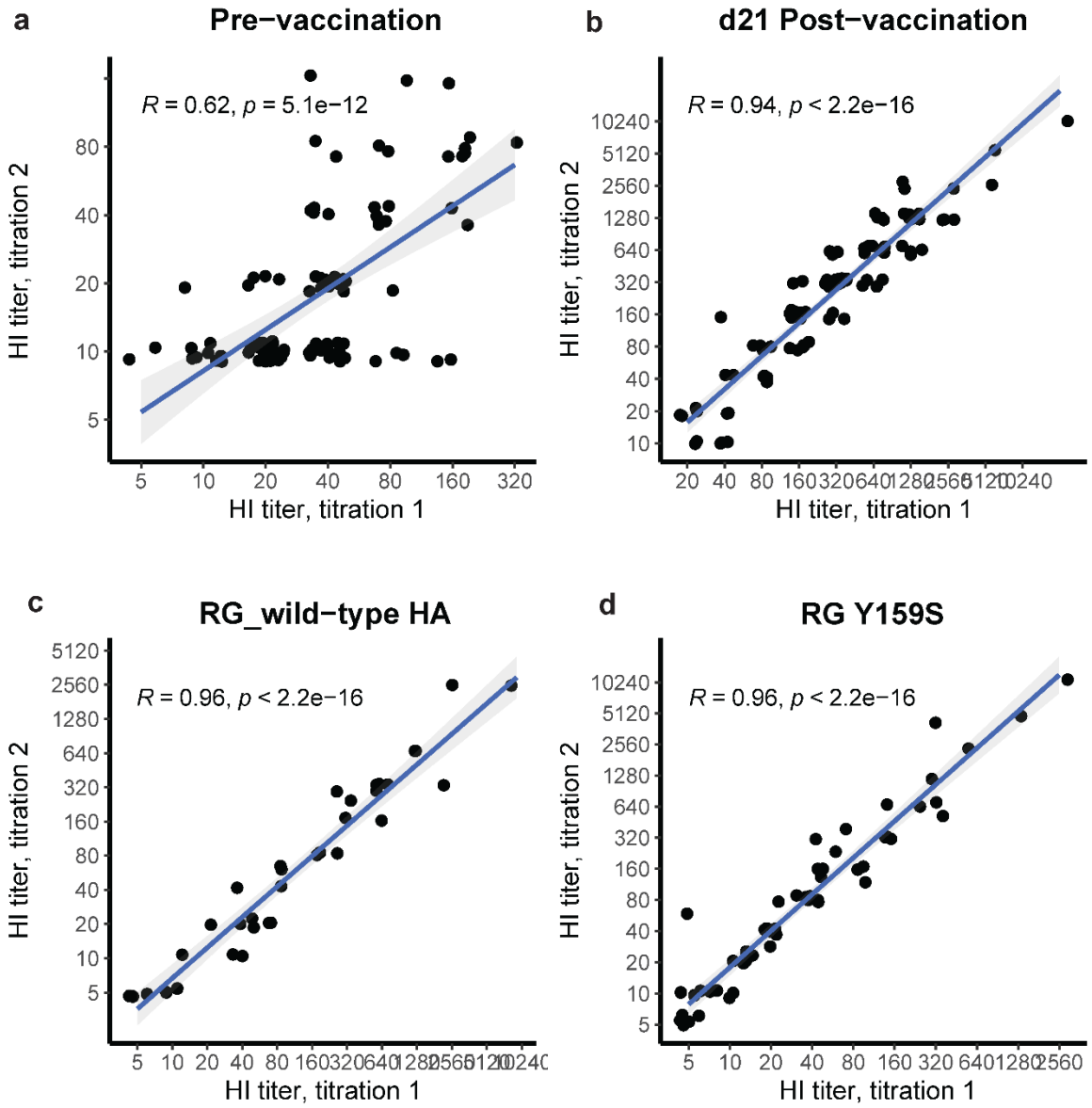
Virus	Clade
A/Brisbane/10/2007	
A/Victoria/8/10	1
A/Perth/16/09	1
A/Victoria/8/10	1
A/Victoria/361/11	3c1
A/Texas/50/12	3c1
A/Switz/9715293/13	3c3a
A/HongKong/4801/14e	3c2a
A/Michigan/15/14	3c2a
A/Newcastle/30/16	3c2a1
A/Victoria/653/17	3c2a1b 135K
A/Brisbane/318/16	3c2a2
A/SthAustralia/10/18	3c2a2
A/Brisbane/34/18	3c3a



Supplementary. Fig. 5: Impact of erythrocyte type on HI titres of ferret first-infection antisera or human sera against A(H3N2) viruses that circulated between 2004 and 2014. Log_2 titres are shown for HI assays performed with Turkey versus Guinea Pig erythrocytes. Left panels show linear regression analysis with 95% confidence intervals. Right panels show summary statistics including median, inter-decile range and range (box and whiskers), and means (+). Panel A shows titers of nine different ferret anti-sera (raised against A/Singapore/37/2004^{egg}, A/Brisbane/10/2007^{egg}, A/Perth/16/2009, A/Victoria/208/2009^{egg}, A/Perth/10/2010, A/Perth/10/2010^{egg}, A/Brisbane/299/2011^{egg}, A/South Australia/3/2011, A/South Auckland/7/2012, A/Christchurch/516/2012, A/Victoria/361/2011^{egg}, and A/Victoria/361/2011) assessed against 15 viruses listed in the graph legend. Panels B-E show titers of pre-vaccination, post-vaccination and post-season sera from 25 individuals (75 sera in total).



Supplementary. Fig. 6: HI assay reading. (A) example of images captured and titres determined using the CypherOne reader, showing sera collected at all time points from one participant against HK14e. (B) Comparison of titres read via CypherOne versus manually. 12 sera were titrated against the 16 viruses shown in the legend. The sera included 10 ferret antisera (raised against A/Malaysia/1/2004, A/Brisbane/10/2007, A/Victoria/8/2010, A/Perth/16/2009, A/Texas/50/2012, A/Switzerland/9715293/2013, A/New Caledonia/104/2014, A/Michigan/15/2014, A/Newcastle/30/2016 and A/Singapore/INIFMH-16-0019/2016) and pre and post-vaccination sera from a single individual.



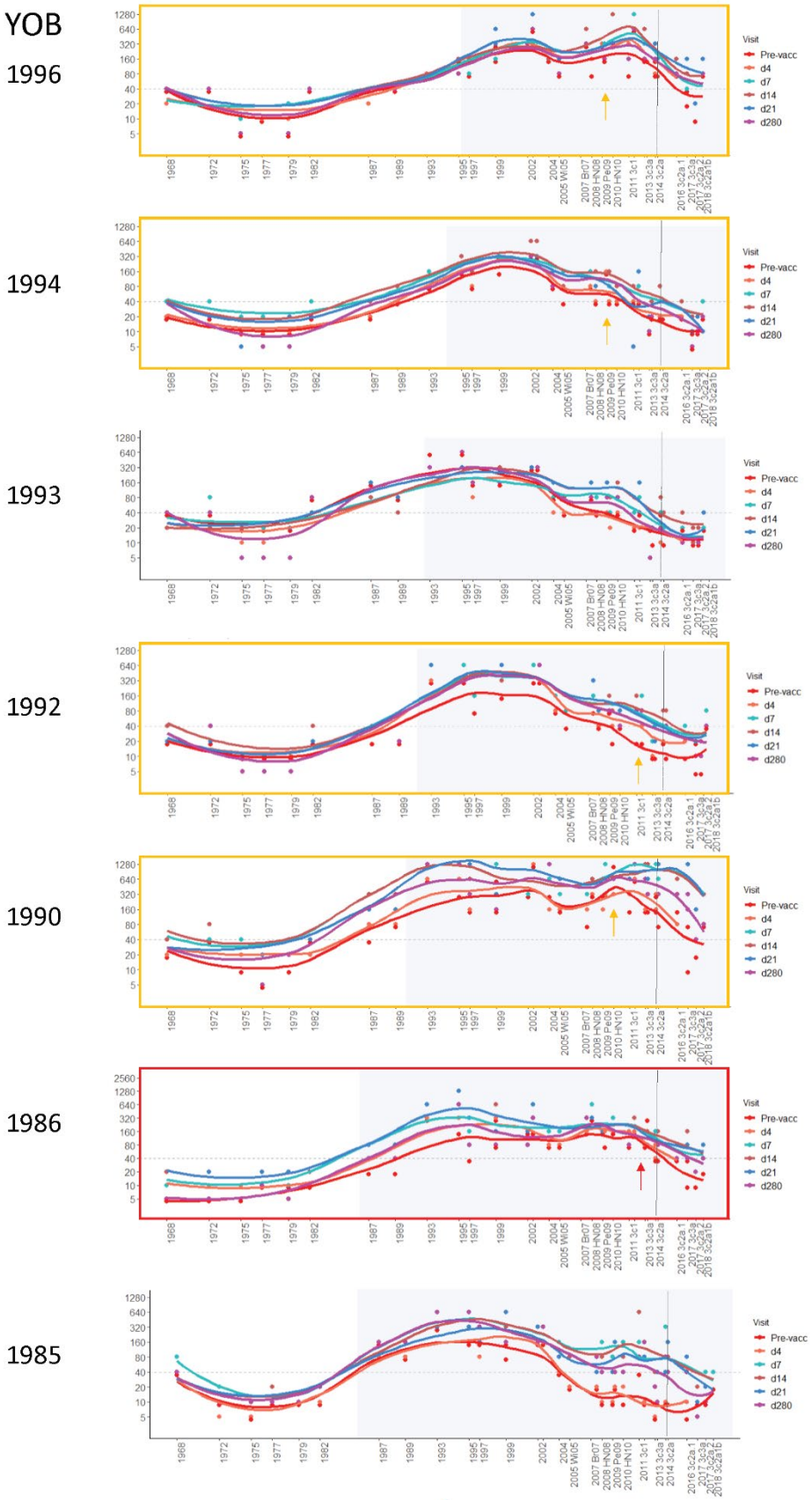
Supplementary. Fig. 7: Replication of HI titers. HI titers against HK14e (**a, b**) or against reverse engineered (RG) viruses **(c, d**) were measured in two independent experiments by different researchers. (**a, b**) 100 sera collected pre- and d21 post-vaccination were measured twice. (**c, d**) Pre and post-vaccination sera from 27 participants (54 sera in total) were measured twice against each virus. Selection of the 27 participants is described in Supplementary Table 3. R and p values are shown for linear regression analysis. Shading represents the 95% confidence interval for the linear regression model.

Supplementary. Table 8: Virus Sequence GISAID Accession Numbers

Application	Virus_Name	Passage History	GISAID Accession	
			HA	NA
Serology, Phylogenetics	A/Bilthoven/16190/1968	X,MDCK3	EPI1868399	EPI1868400
Serology, Phylogenetics	A/Bilthoven/21793/1972	MDCK3	EPI1868401	EPI1868410
Serology, Phylogenetics	A/Bilthoven/1761/1976	MDCK3	EPI1868402	EPI1868409
Serology, Phylogenetics	A/Bilthoven/2271/1976	X,MDCK3	EPI1868403	EPI1868408
Serology, Phylogenetics	A/Netherlands/233/1982	tMK1,MDCK4	EPI1868404	EPI1868407
Serology, Phylogenetics	A/PHILIPPINES/2/1982	MDCKX,MDCK2	EPI1868405	EPI1868406
Serology, Phylogenetics	A/Netherlands/620/1989	X,tMK1,MDCK3	EPI1868433	EPI1868445
Serology, Phylogenetics	A/Netherlands/823/1992	X,MDCK3	EPI1868434	EPI1868446
Serology, Phylogenetics	A/Netherlands/179/1993	X, MDCK3	EPI1868435	EPI1868447
Serology, Phylogenetics	A/Netherlands/178/1995	293T,MDCK4	EPI1868436	EPI1868448
Serology, Phylogenetics	A/Tasmania/1/1997	MDCK7	EPI1868440	EPI1868476
Serology, Phylogenetics	A/Netherlands/301/1999	MDCK5	EPI1868437	EPI1868473
Serology, Phylogenetics	A/Philippines/472/2002	MDCK6, Siat3	EPI1868438	EPI1868474
Serology, Phylogenetics	A/Fujian/411/2002	XC2,MDCK4,SIAT1	EPI1868441	EPI1868477
Serology, Phylogenetics	A/Victoria/511/2004	MDCKx, MDCK2, SIAT3	EPI1868443	EPI1868479
Serology, Phylogenetics	A/New York/55/2004	SPFCK3,E6	EPI1868442	EPI1868478
Serology, Phylogenetics	A/Thailand/409/2005	P2,MDCK2, SIAT3	EPI1868439	EPI1868475
Serology, Phylogenetics	A/Wisconsin/67/2005	CK3, E9	EPI1868444	EPI1868480
Serology, Phylogenetics	A/Brisbane/10/2007	MDCKX,MDCK5, SIAT4	EPI1868482	EPI1868546
Serology, Phylogenetics	A/Uruguay/716/2007	SPFCK1,E5	EPI1868481	EPI1868489
Serology, Phylogenetics	A/HaNam/EL134/2008	MDCK4, SIAT4	EPI1868483	EPI1868547
Serology, Phylogenetics	A/Perth/16/2009	MDCKx,MDCK1,Siat2	EPI1868486	EPI1868488
Serology, Phylogenetics	A/Perth/16/2009	E7	EPI1868485	EPI1868487
Serology, Phylogenetics	A/HaNam/EL201/2009	MDCKX,MDCK3, SIAT1	EPI1868484	EPI1868548
Serology, Phylogenetics	A/HaNam/EL444/2010	MDCK3,Siat4	EPI1868549	EPI1868554
Serology, Phylogenetics	A/Victoria/361/2011	MDCK2,Siat3,plaque,Siat2	EPI1868575	EPI1868583
Serology, Phylogenetics	A/Victoria/361/2011	E6	EPI1868550	EPI1868555
Serology, Phylogenetics	A/Texas/50/2012	MDCK1,C2,MDCK6,Siat4	EPI1868581	EPI1868584
Serology, Phylogenetics	A/Texas/50/2012	E5,E2	EPI1868551	EPI1868556
Serology, Phylogenetics	A/Switzerland/9715293/2013	SIAT, SIAT8	EPI1868576	EPI1868585
Serology, Phylogenetics	A/Switzerland/9715293/2013	E7	EPI1868552	EPI1868573
Serology, Phylogenetics	A/Michigan/15/2014	MDCK1, SIAT1,SIAT5	EPI1868577	EPI1868586
Serology, Phylogenetics	A/New Caledonia/104/2014	MDCK1, Siat7	EPI1868582	EPI1868587
Serology, Phylogenetics	A/Hong Kong/4801/2014	E8	EPI1868553	EPI1868574
Serology, Phylogenetics	A/HaNam/EL14437/2014	X,SIAT2	EPI1868591	
Serology, Phylogenetics	A/Newcastle/30/2016	Siat1,Siat4	EPI1868578	EPI1868588
Serology, Phylogenetics	A/Kansas/14/2017	E9	EPI1318832	EPI1318831
Serology, Phylogenetics	A/Kansas/14/2017	SIAT3,SIAT1	EPI1868579	EPI1868589
Serology, Phylogenetics	A/Switzerland/8060/2017	E7	EPI1322581	EPI1322580
Serology, Phylogenetics	A/Brisbane/60/2018	SIAT3	EPI1868580	EPI1868590
Phylogenetics	A/Singapore/INFIMH-16-0019/2016	E5+1+E1	EPI1381189	
Phylogenetics	A/Brisbane/318/2016	SIAT4	EPI919300	
Phylogenetics	A/Sydney/22/2018	MDCK-SIAT, SIAT4	EPI1485365	
Phylogenetics	A/Newcastle/82/2018	MDCK33016PF p2	EPI1610368	
Phylogenetics	A/Brisbane/112/2018	MDCK1SIAT1	EPI1281484	
Phylogenetics	A/South Australia/34/2019	SIAT1	EPI1387331	
Phylogenetics	A/South Australia/10/2018	SIAT1	EPI1251122	
Phylogenetics	A/Hanam/EL135/2008_Aug	MDCK2	EPI1921266	
Phylogenetics	A/Hanam/EL196/2009_Apr	MDCK1	EPI1921267	
Phylogenetics	A/Hanam/EL204_H145S02/2009_May	MDCK2	EPI1921268	
Phylogenetics	A/Hanam/EL206_107S06/2009_Jun	MDCK1	EPI1921269	
Phylogenetics	A/Hanam/EL442_150S01/2010_Sep	MDCK2	EPI1921270	
Phylogenetics	A/Hanam/EL443_265S01/2010_Sep	MDCK2	EPI1921271	
Continued next page				

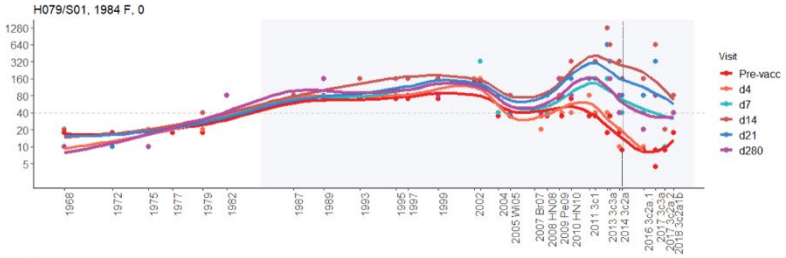
Application	Virus_Name	Passage History	GISAID Accession	
			HA	NA
Phylogenetics	A/Hanam/EL12112_145S04/2012_Dec	MDCK2	EPI1921272	
Phylogenetics	A/Hanam/EL12113_171S03/2012_Dec	MDCK2	EPI1921273	
Phylogenetics	A/Hanam/EL12114_153S02/2012_Dec	MDCK2	EPI1921274	
Phylogenetics	A/Hanam/ELI12116/2012_Dec	MDCK2	EPI1921275	
Phylogenetics	A/Hanam/EL13124_150S04/2013_Jan	MDCK2	EPI1921276	
Phylogenetics	A/Hanam/EL13142_283S04/2013_Feb	MDCK2	EPI1921277	
Phylogenetics	A/Hanam/EL13232_145S03/2013_Sep	MDCK2	EPI1921278	
Phylogenetics	A/Hanam/EL13234_202S04/2013_Sep	MDCK2	EPI1921279	
Phylogenetics	A/Hanam/EL13237_275S01/2013_Sep	MDCK2	EPI1921280	
Phylogenetics	A/Hanam/EL13239_103S01/2013_Oct	MDCK2	EPI1921281	
Phylogenetics	A/Hanam/EL13240_093S06/2013_Oct	MDCK2	EPI1921282	
Phylogenetics	A/Hanam/EL13246_284S06/2013_Oct	MDCK2	EPI1921283	
Phylogenetics	A/Hanam/EL13255_171S02/2013_Nov	MDCK2	EPI1921284	
Phylogenetics	A/Hanam/EL14302_193S03/2014_Jan	clinical specimen	EPI1921285	
Phylogenetics	A/Hanam/EL14420_321S03/2014_May	MDCK2	EPI1921286	
Phylogenetics	A/Hanam/EL14405_149S03/2014_Apr	clinical specimen	EPI1921287	
Phylogenetics	A/Hanam/EL14427_151S01/2014_May	MDCK3	EPI1921288	
Phylogenetics	A/Hanam/EL14428_194S01/2014_May	MDCK2	EPI1921289	
Phylogenetics	A/Hanam/EL14423_251S02/2014_May	MDCK3	EPI1921290	
Phylogenetics	A/Hanam/EL14431_H194S03/2014_May	MDCK2	EPI1921291	
Phylogenetics	A/Hanam/EL14432_194S02/2014_May	MDCK2	EPI1921292	
Phylogenetics	A/Hanam/EL14435_263S03/2014_May	MDCK2	EPI1921293	
Phylogenetics	A/Hanam/EL14443_138S02/2014_May	MDCK2	EPI1921294	
Phylogenetics	A/Hanam/EL14441_165S02/2014_May	MDCK2	EPI1921295	
Phylogenetics	A/Hanam/EL14445_063S02/2014_Jun	MDCK2	EPI1921296	
Phylogenetics	A/Hanam/EL14446_018S02/2014_Jun	MDCK2	EPI1921297	
Phylogenetics	A/Hanam/EL14448_108S01/2014_Jun	MDCK2	EPI1921298	
Phylogenetics	A/Hanam/EL14449_178S01/2014_Jun	MDCK2	EPI1921299	
Phylogenetics	A/Hanam/EL14452_152S03/2014_Jun	MDCK2	EPI1921300	
Phylogenetics	A/Hanam/EL14457_280S01/2014_Jun	MDCK2	EPI1921301	
Phylogenetics	A/Hanam/EL14459_280S03/2014_Jun	MDCK2	EPI1921302	
Phylogenetics	A/Hanam/EL14460_280S04/2014_Jun	MDCK2	EPI1921303	
Phylogenetics	A/Hanam/EL15593_321S05/2015_Feb	clinical specimen	EPI956449	EPI956448
Phylogenetics	A/Hanam/EL15597_192S08/2015_Apr	clinical specimen	EPI956451	EPI956450
Phylogenetics	A/Hanam/EL15628_252S05/2015_Aug	clinical specimen	EPI956407	EPI956406
Phylogenetics	A/Hanam/EL17762_045S02/2017_Aug	SIAT1	EPI1328398	EPI1328397
Phylogenetics	A/Hanam/EL17767_025S01/2017_Aug	clinical specimen	EPI1921304	
Phylogenetics	A/Hanam/EL17772_155S01/2017_Aug	clinical specimen	EPI1921305	
Phylogenetics	A/Hanam/EL17773_280S01/2017_Aug	clinical specimen	EPI1921306	
Phylogenetics	A/HaNam/ELI17781/2017_Aug	clinical specimen	EPI1921307	
Phylogenetics	A/Hanam/EL17795_053S02/2017_Sep	SIAT1	EPI1921308	
Phylogenetics	A/Hanam/EL17804_045S01/2017_Oct	clinical specimen	EPI1921309	
Phylogenetics	A/Hanam/EL17805_155S05/2017_Oct	clinical specimen	EPI1921310	
Phylogenetics	A/HaNam/ELI17807/2017_Oct	clinical specimen	EPI1921311	
Phylogenetics	A/Hanam/EL898_238S12/2018_Sep	SIAT1	EPI1328403	EPI1328402

Supplementary. Fig. 8: Individual Antibody Landscapes. Titres across strains at each time-point are presented for 100 participants, using loess to fit titres across strains (colour-coded lines). Participants are arranged from most recent YOB. Blue shading indicates the viruses that circulated during that participant's lifetime. Participants who had infection detected by seroconversion or RT PCR are indicated by yellow and red borders, respectively, with yellow and red arrows indicating the infecting strain(s).



YOB

1984



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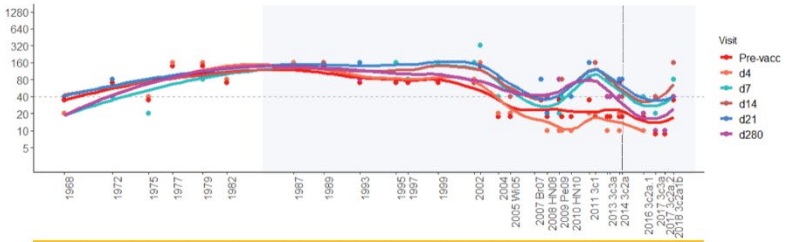
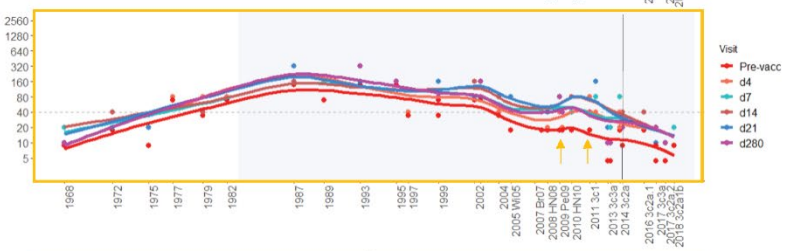
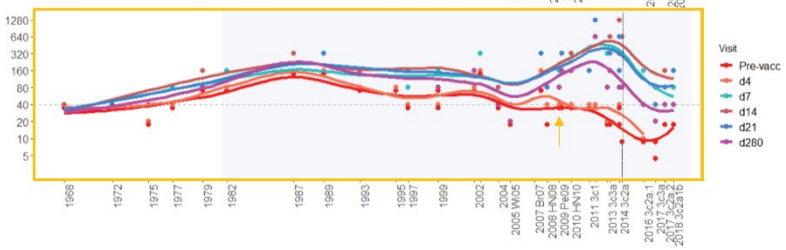


1983



1982

1982



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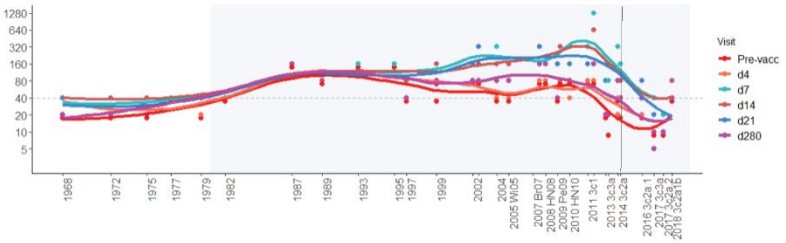
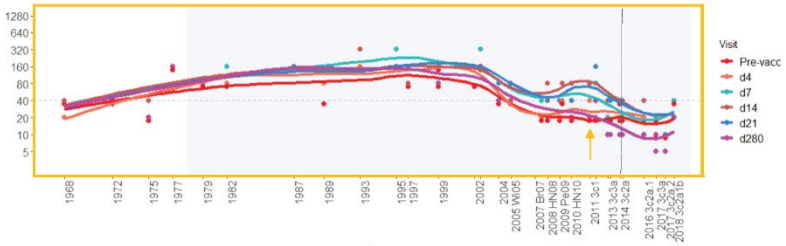
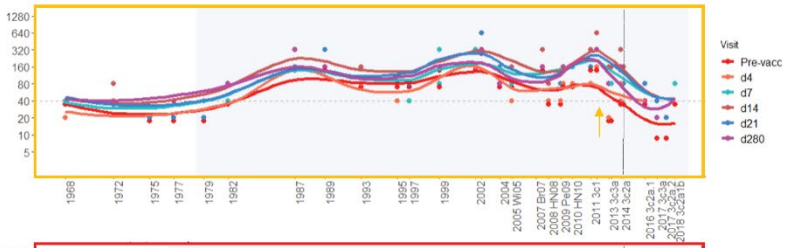


1978

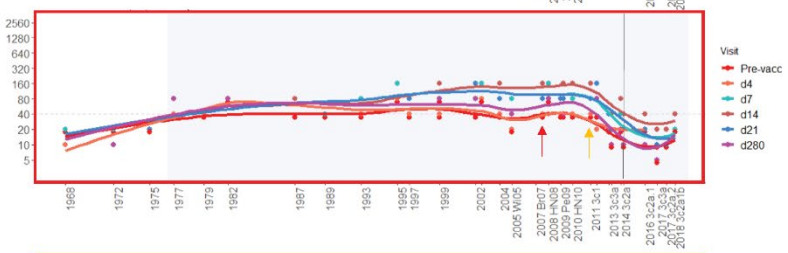


YOB

1978



1977



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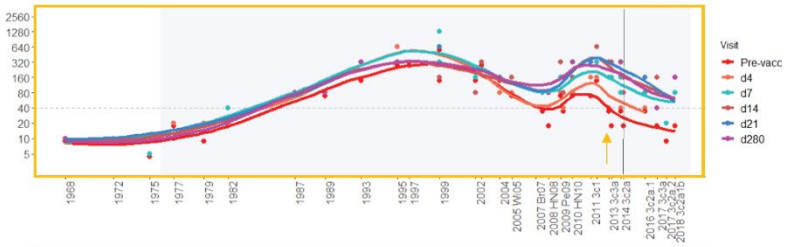

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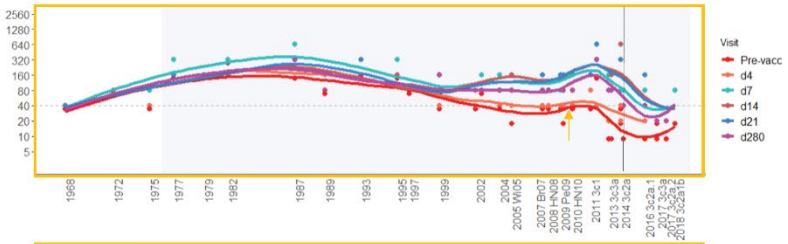
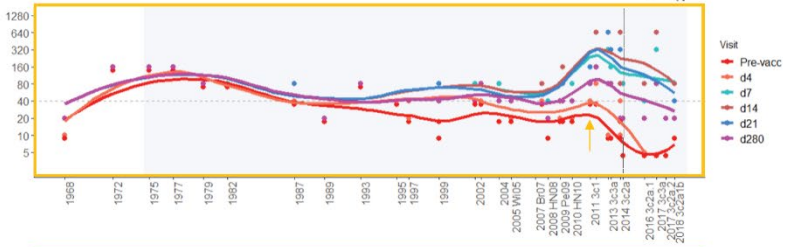


1975



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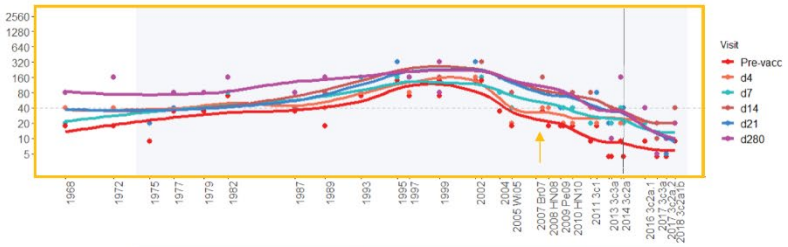

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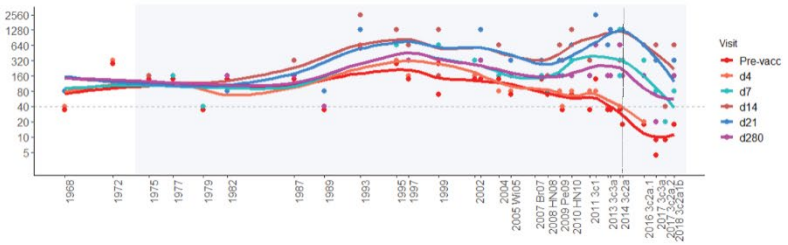
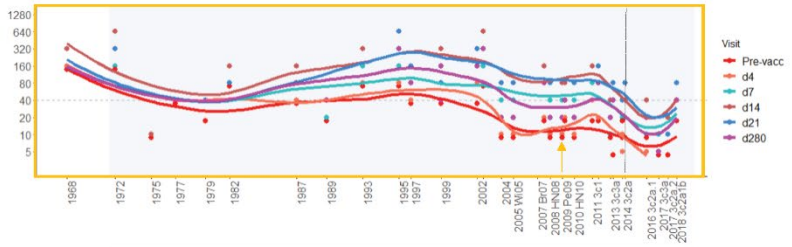
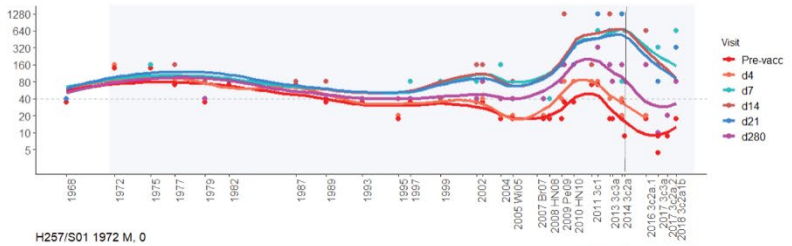
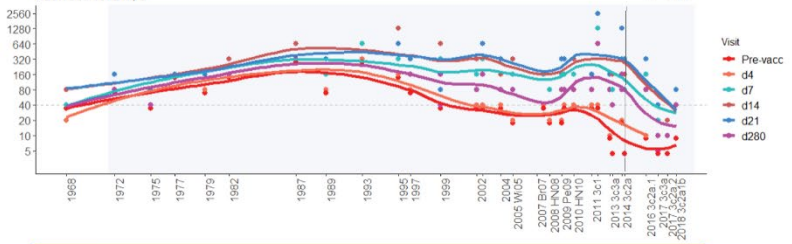


YOB

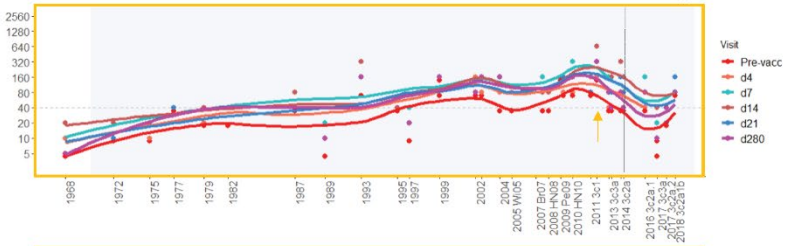
1972

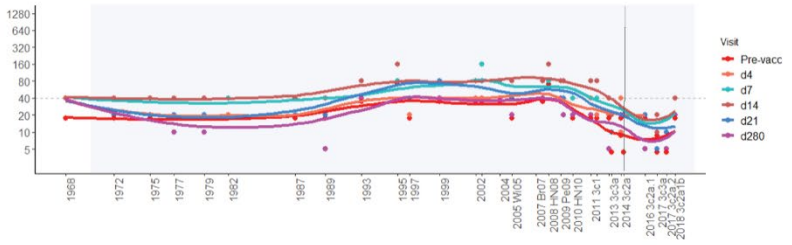


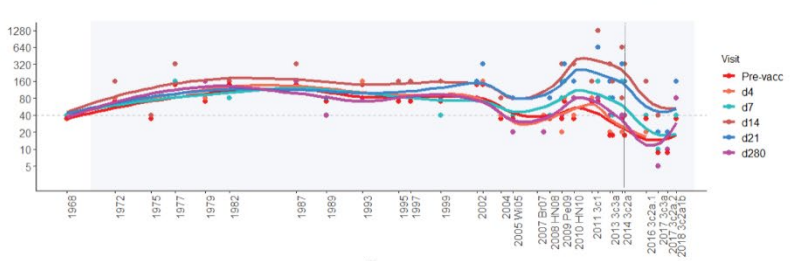


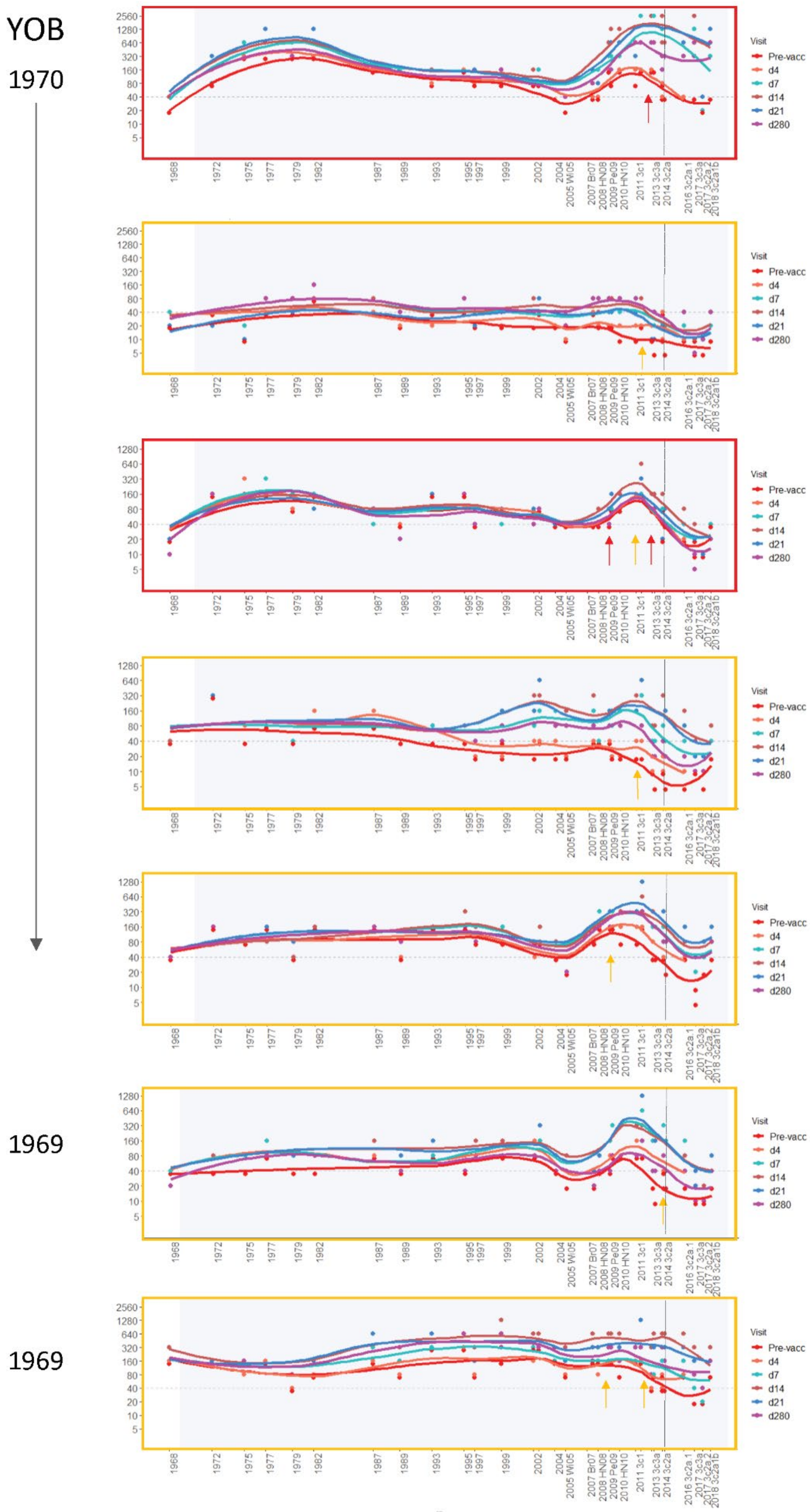


1971





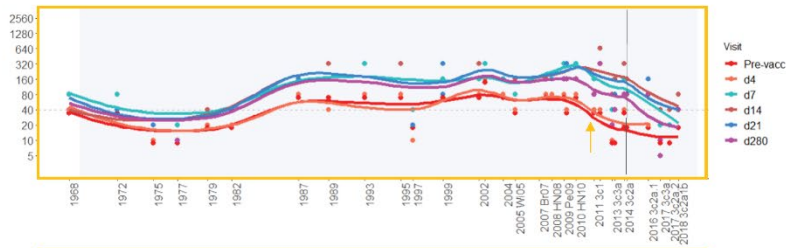




YOB
1970
1969
1969
Visit
Pre-vacc
d4
d7
d14
d21
d280

YOB

1969



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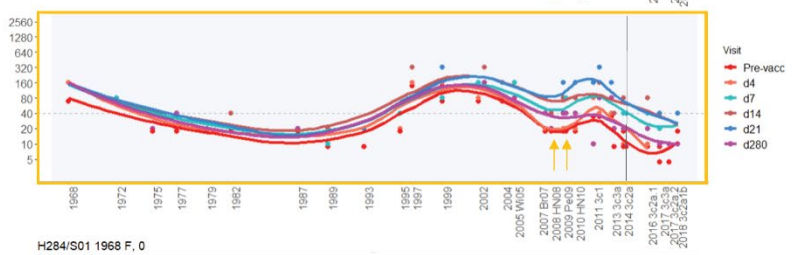

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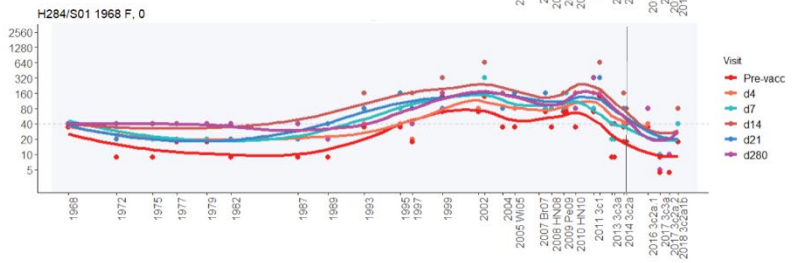

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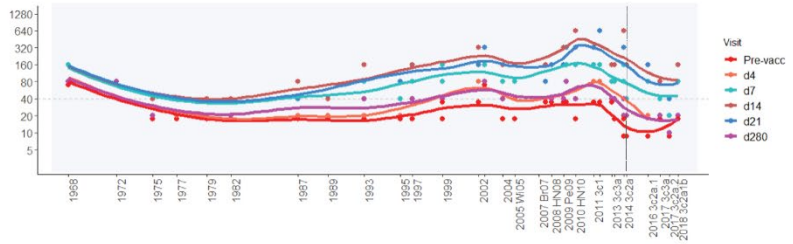
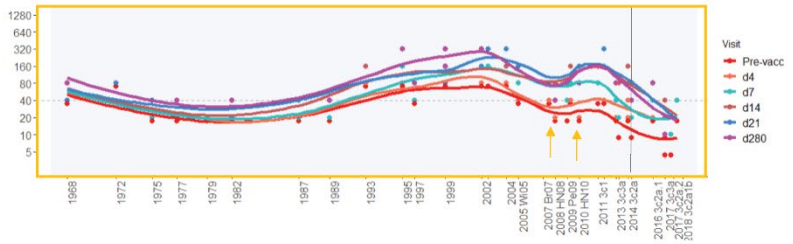
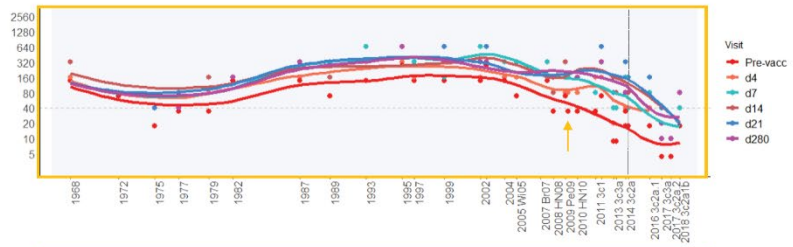
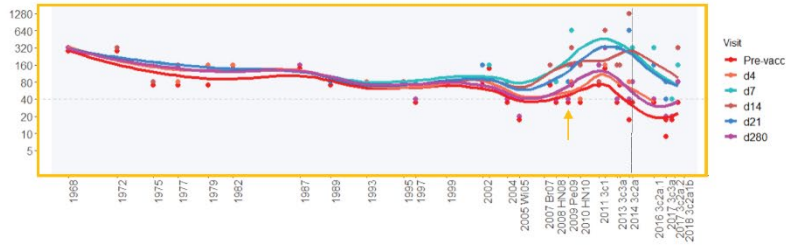


1967







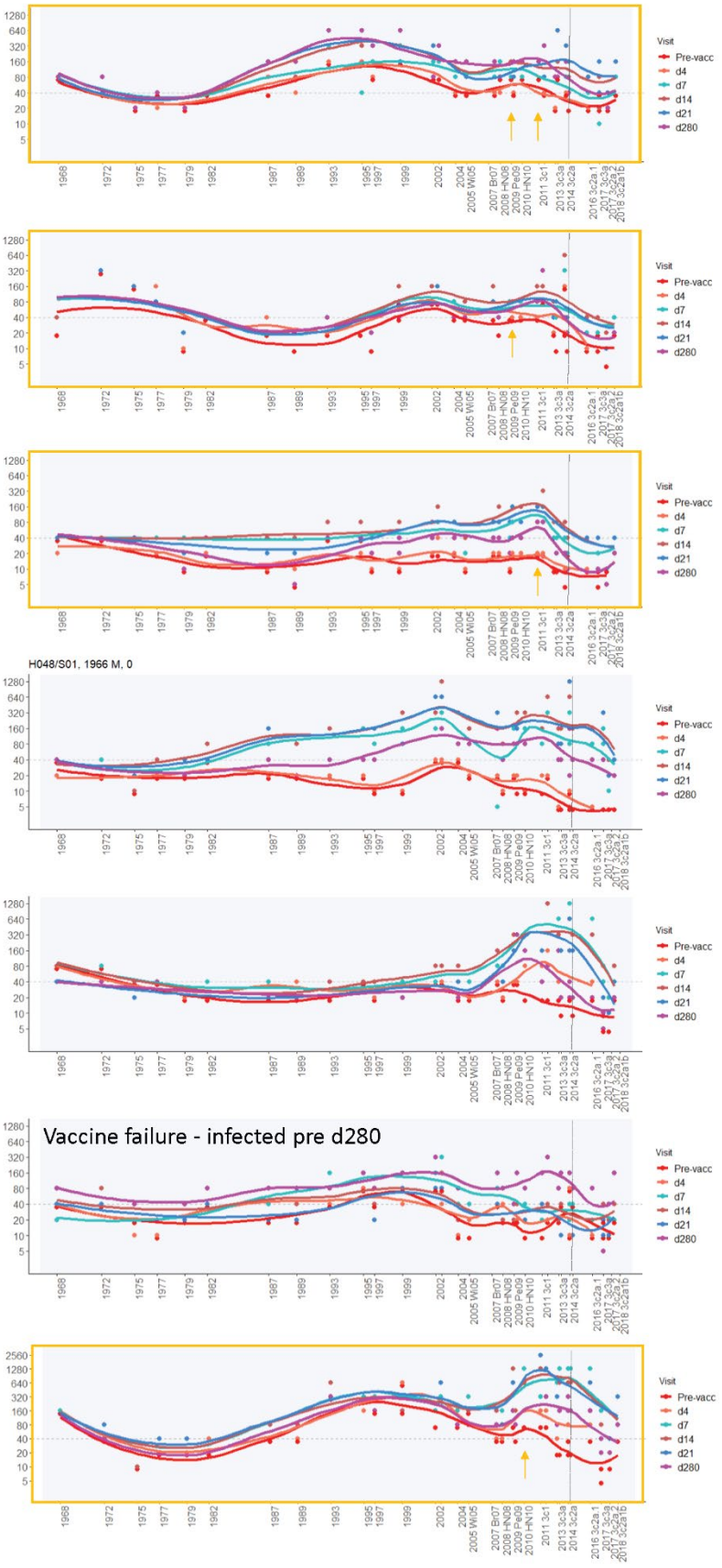
YOB

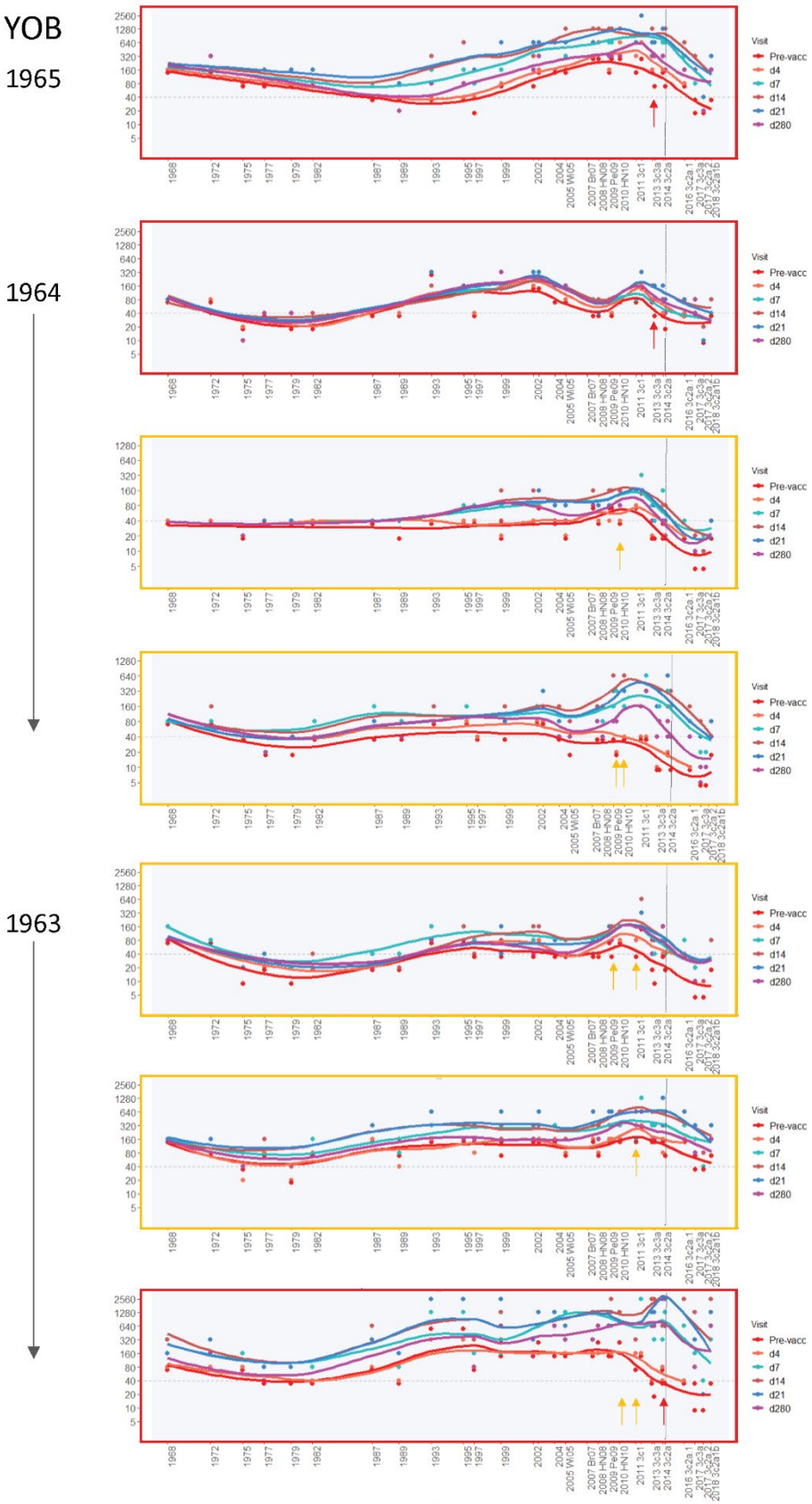
1967

1966

1965

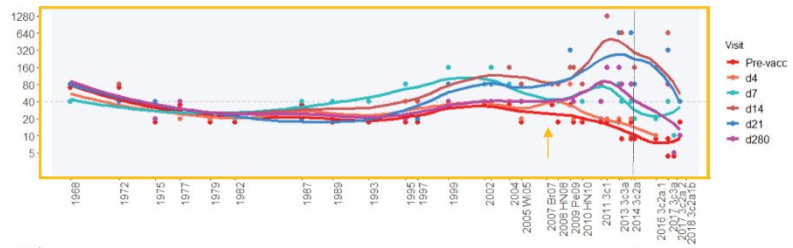
1965



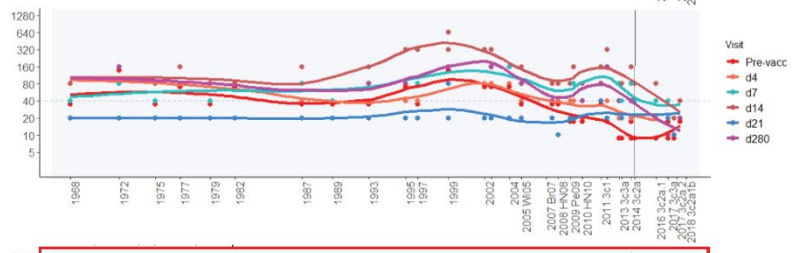

YOB
1965
1964
1963
Visit
Pre-vacc
d4
d7
d14
d21
d280

YOB

1963



1963



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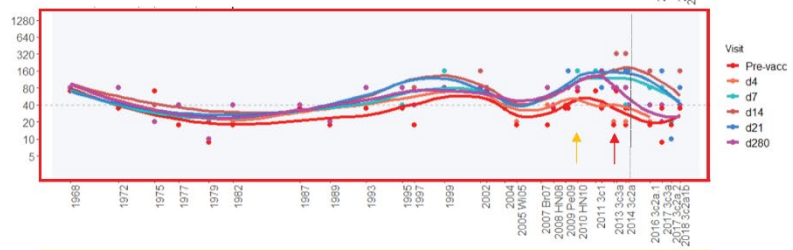
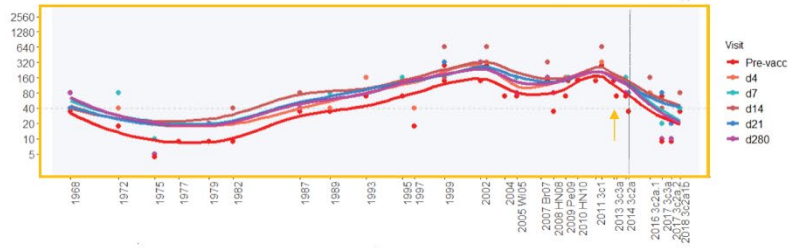
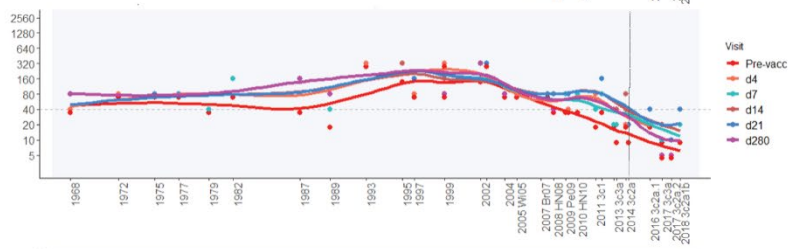
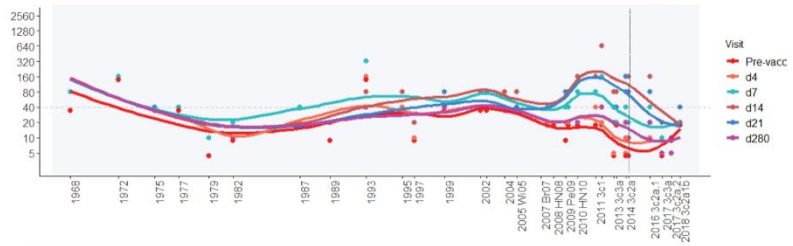






1961



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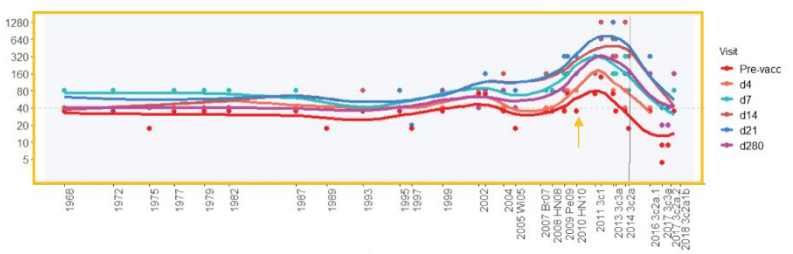
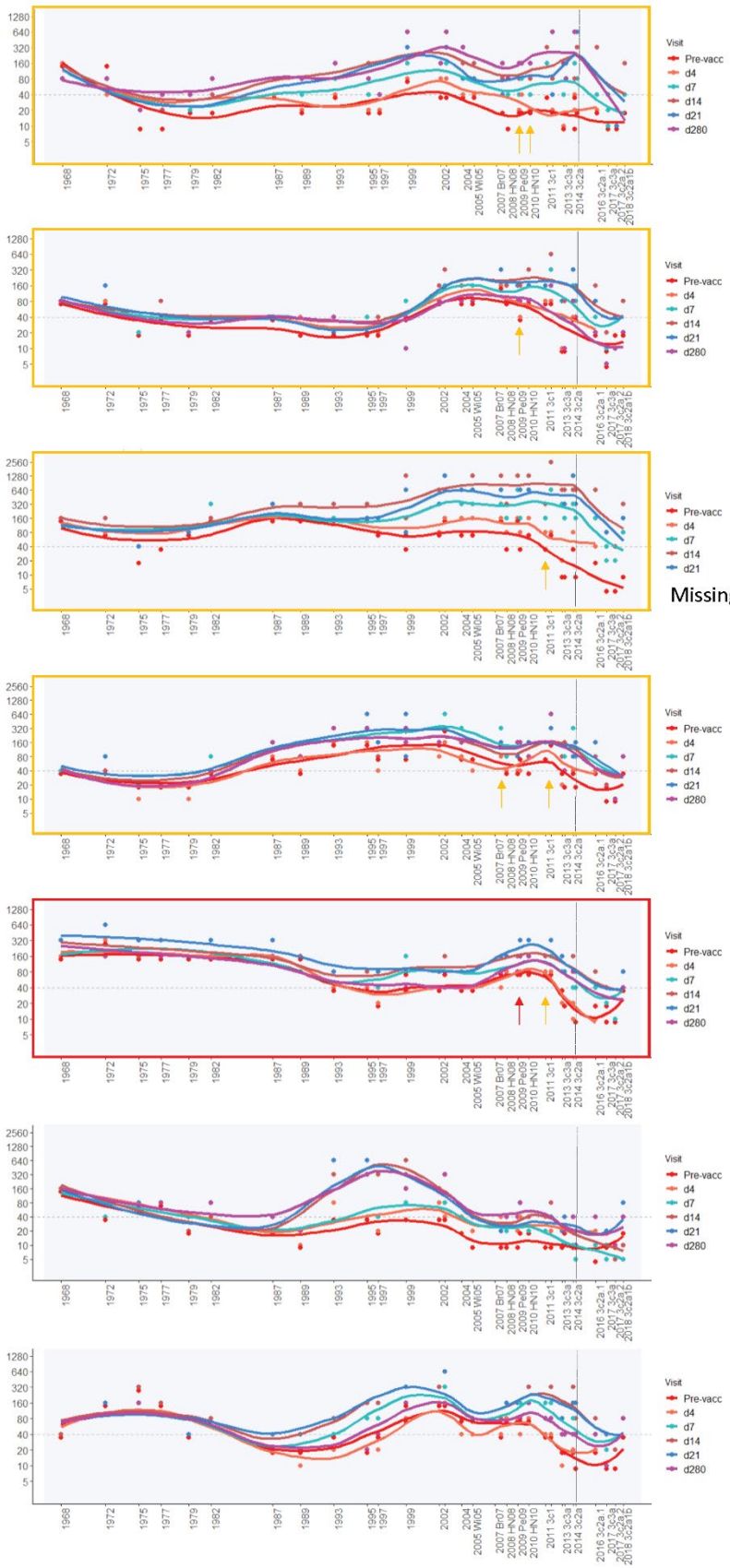


YOB

1960

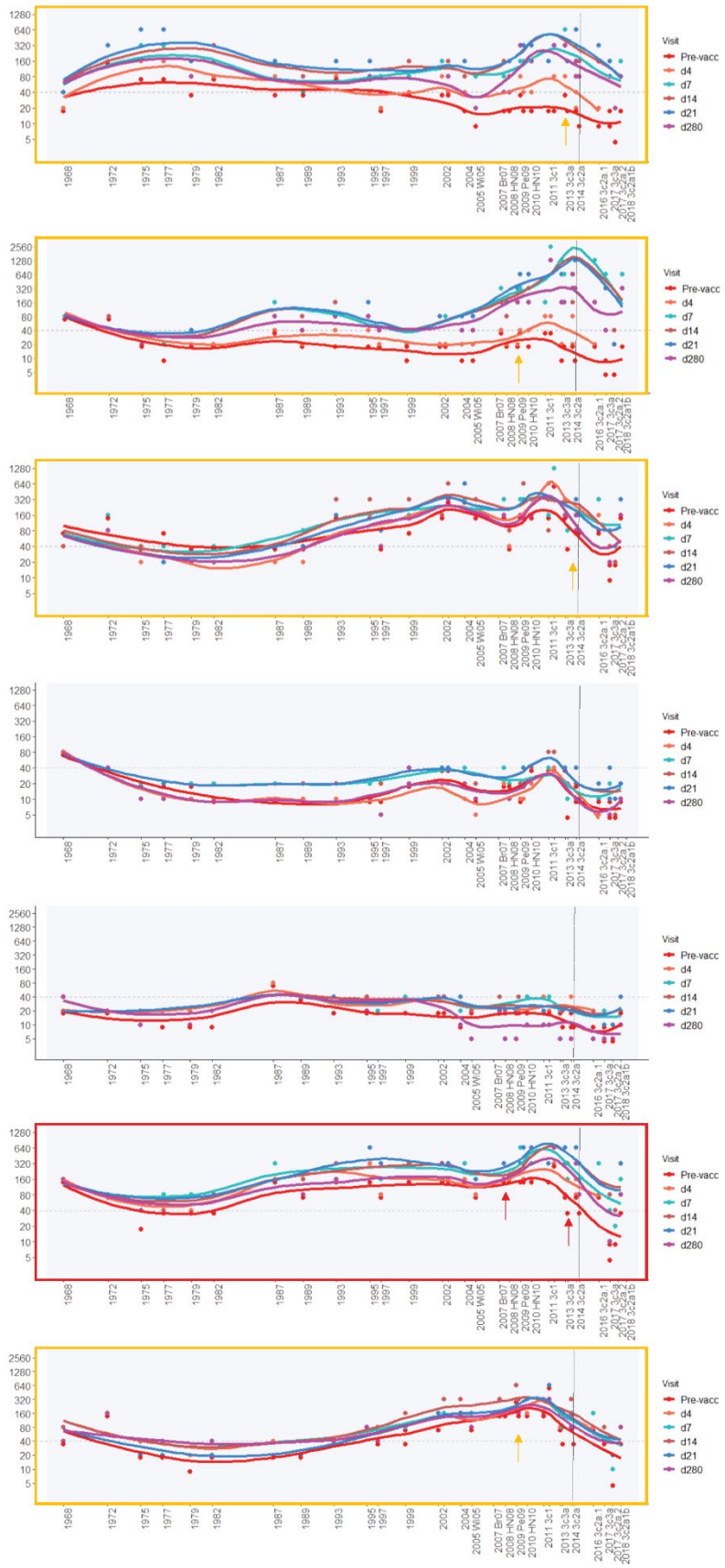


Missing d280

1959

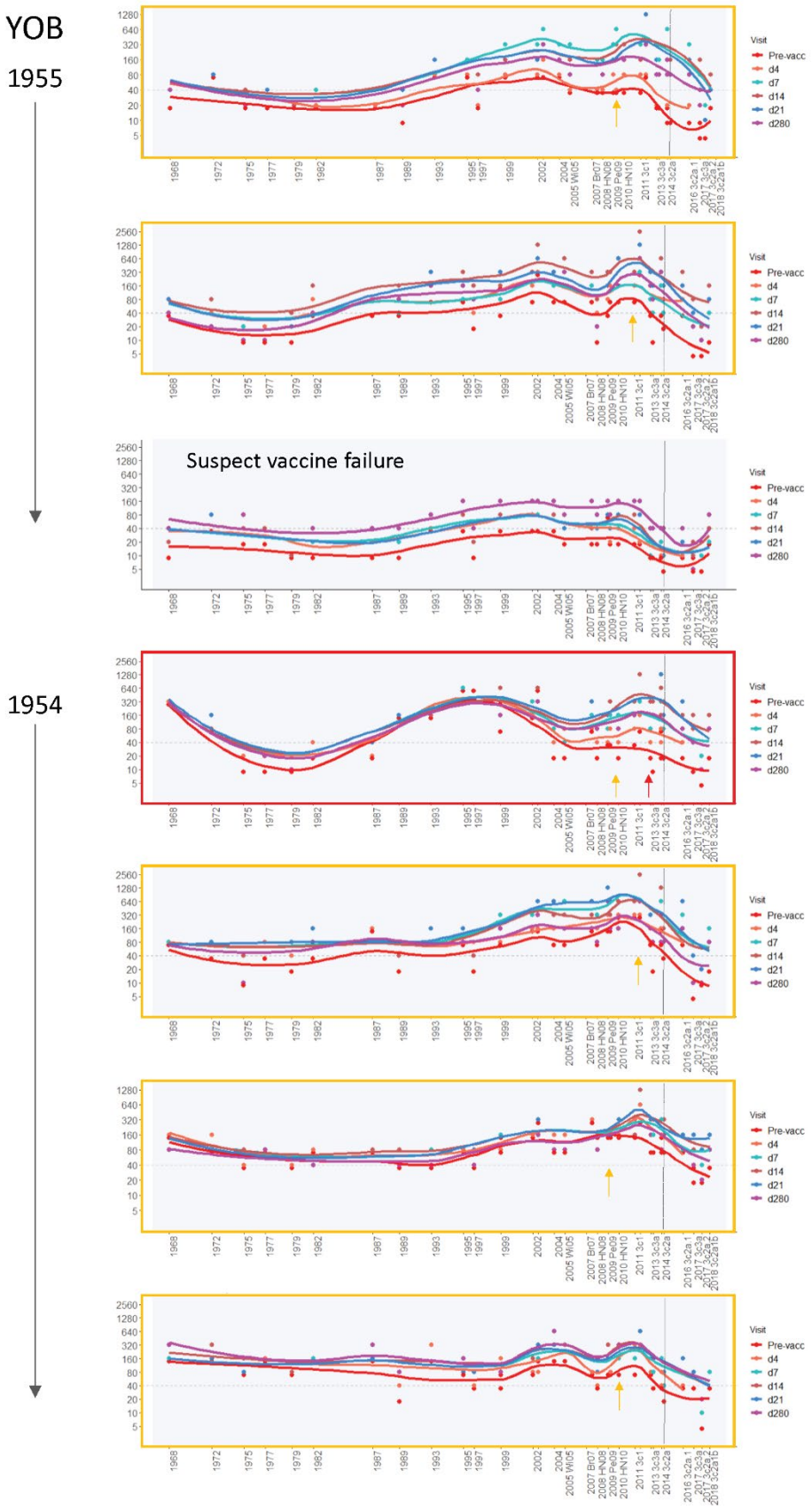
YOB

1958



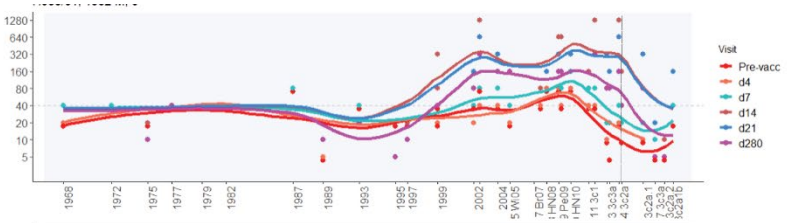
1957

1956

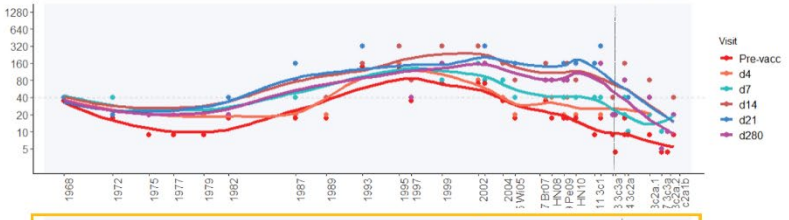

YOB
1955
1954
Suspect vaccine failure
Visit
Pre-vacc
d4
d7
d14
d21
d280

YOB

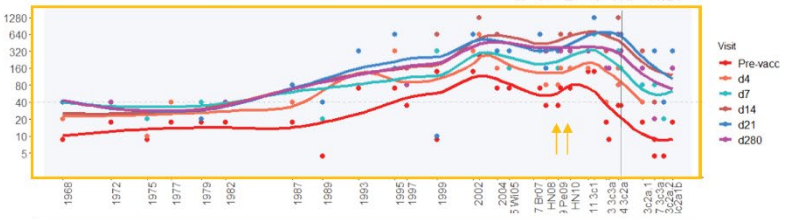
1952



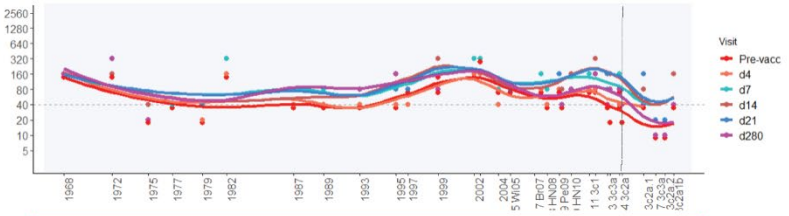
1950



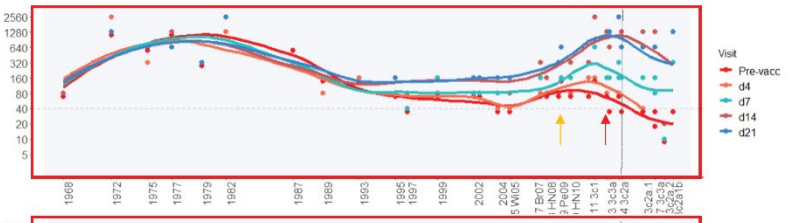
1947



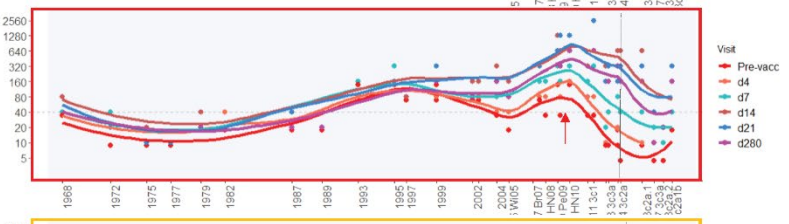
1947



1945



1944



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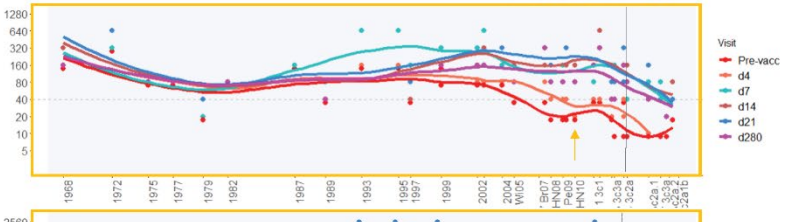
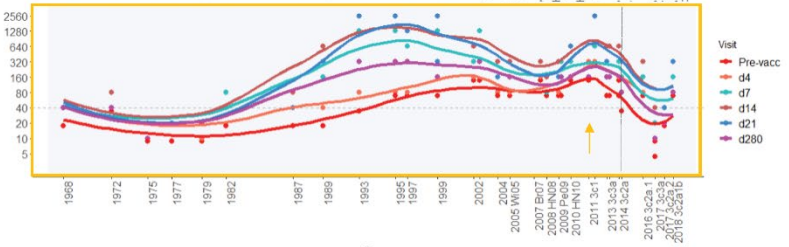


1935



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2. Beyer WE, Palache AM, Luchters G, Nauta J, Osterhaus AD. Seroprotection rate, mean fold increase, seroconversion rate: which parameter adequately expresses seroresponse to influenza vaccination? *Virus Res* 2004; **103**(1-2): 125-32.