

Supplementary Information

Cross-reactive human antibody responses to H5N1 influenza virus neuraminidase are shaped by immune history

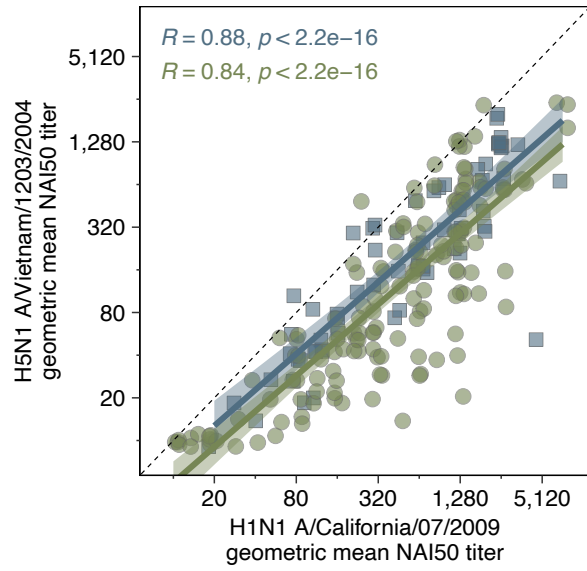
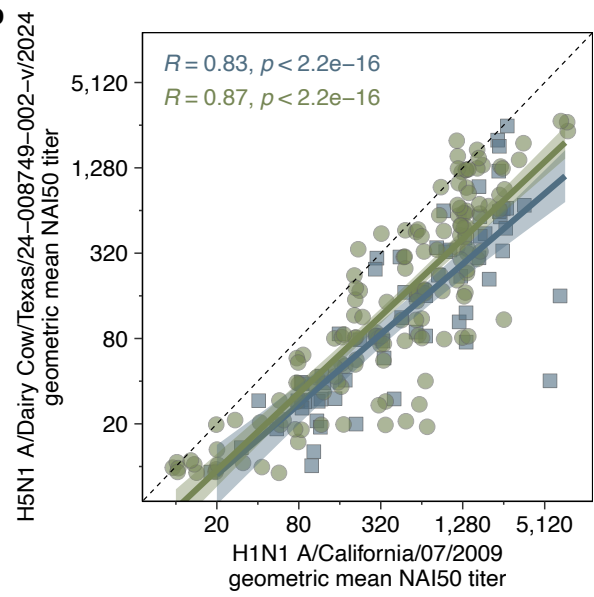
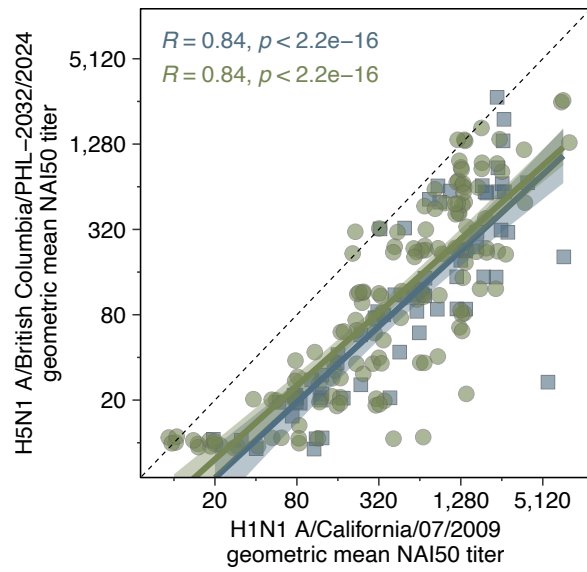
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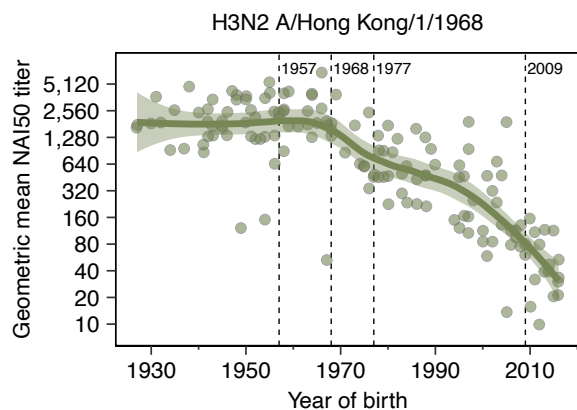
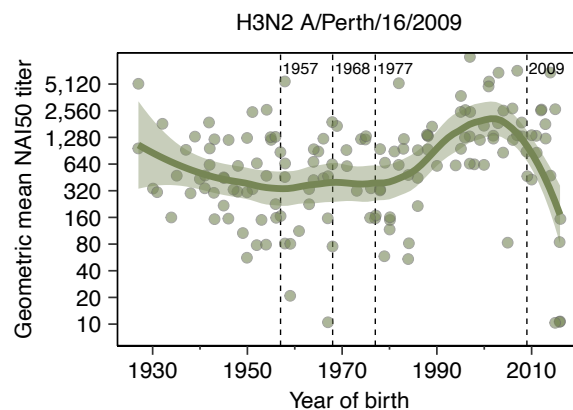
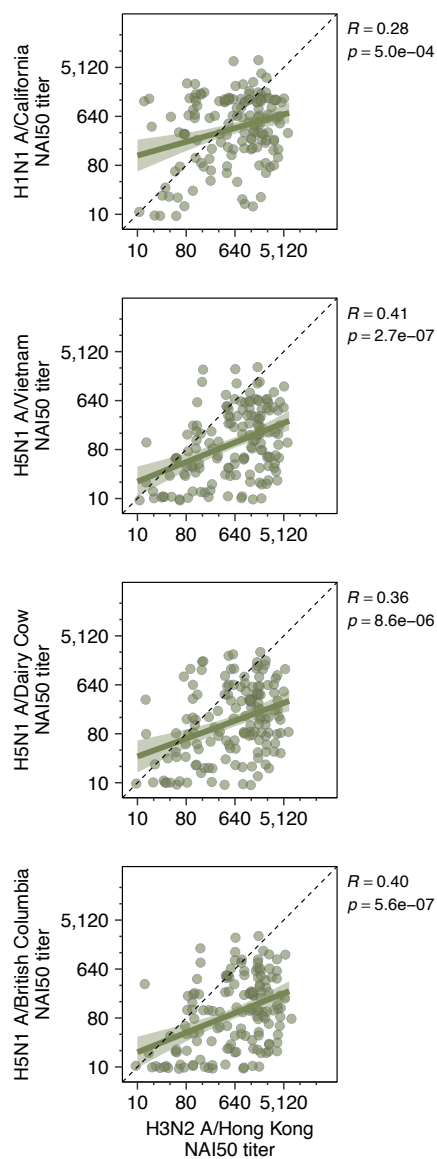
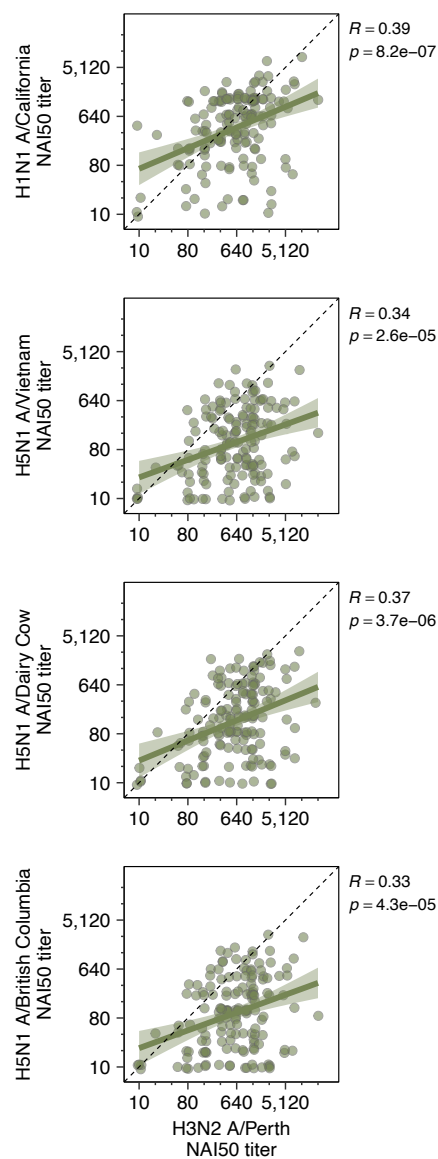
Supplementary Figures 1–2
Supplementary Tables 1–5

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■ Pre-pdm09 sera (2005)
● Post-pdm09 sera (2017)

Supplementary Figure 1. Cross-reactive N1 antibody levels are highly correlated with those against H1N1(pdm09).

NAI titers against **(a)** H5N1 A/Vietnam/1203/2004, **(b)** A/Dairy Cow/Texas/24-008749-002-v/2024, and **(c)** A/British Columbia/PHL-2032/2024 were plotted versus NAI titers against H1N1(pdm09) A/California/07/2009. Sera samples collected in 2005 (n = 139), pre-H1N1(pdm09) emergence, are shown in blue squares and those collected in 2017 (n = 65) are shown in green circles. Correlations between titers for each cohort were determined using Pearson's correlation coefficient, with the line of best fit shown and the 95% confidence interval shaded. Source data are provided as a Source Data file.

a**b****c****d**

Supplementary Figure 2. NAI titers against seasonal H3N2 viruses are strain-specific and correlate poorly with anti-N1 titers.

Serum samples collected in 2017 were assayed for NAI antibodies against H6N2 viruses expressing the NA of **(a)** H3N2 A/Hong Kong/1/1968 (n = 146) and **(b)** H3N2 A/Perth/16/2009 (n = 151). Vertical dashed lines represent the emergence of H2N2, H3N2, and H1N1, and H1N1(pdm09) in humans in 1957, 1968, 1977, and 2009, respectively. Each circle represents the geometric mean serum NAI50 titer for a single individual from two to three independent replicates, with locally estimated scatterplot smoothing curves (smoothing parameter = 0.5) and the associated 95% CIs. Correlations between titers against **(c)** A/Hong Kong/1/1968 or **(d)** A/Perth/16/2009 and each tested N1 virus were determined using Pearson's correlation coefficient, with the line of best fit shown and the 95% CI shaded. Source data are provided as a Source Data file.

Supplementary Table 1. Correlations between NAI titers against each virus and birth year, HA group 1 imprinting probabilities, and N1 imprinting probabilities. The correlation between titer and each predictor was assessed using a two-sided Spearman's correlation test and the 95% confidence intervals were computed using Fisher's transformation. Source data are provided as a Source Data file.

Correlation			
NAI titers against	Correlate	Coefficient (95% CI)	P value
A/California/07/2009 (H1N1)	Birth year	-0.07 (-0.25, 0.11)	0.435
	HA group 1 imprinting	0.16 (-0.02, 0.33)	0.077
	N1 imprinting	0.35 (0.18, 0.5)	8.70E-05
A/Vietnam/1203/2004 (H5N1)	Birth year	-0.12 (-0.29, 0.06)	0.189
	HA group 1 imprinting	0.22 (0.04, 0.39)	0.016
	N1 imprinting	0.35 (0.18, 0.5)	1.13E-04
A/Dairy Cow/Texas/24-008749-002-v/2024 (H5N1)	Birth year	-0.10 (-0.27, 0.08)	0.296
	HA group 1 imprinting	0.19 (0.01, 0.36)	0.037
	N1 imprinting	0.32 (0.15, 0.47)	3.74E-04
A/British Columbia/PHL-2032/2024 (H5N1)	Birth year	-0.12 (-0.29, 0.06)	0.197
	HA group 1 imprinting	0.19 (0.01, 0.36)	0.035
	N1 imprinting	0.33 (0.16, 0.48)	2.81E-04
A/Washington/2148/2025 (H5N5)	Birth year	-0.41 (-0.55, -0.25)	1.49E-05
	HA group 1 imprinting	0.45 (0.3, 0.58)	1.70E-06
	N1 imprinting	0.41 (0.25, 0.55)	1.88E-05

Supplementary Table 2. Bootstrap analysis comparing the strengths of the correlations between NAI titers against each virus and birth year, HA group 1 imprinting probabilities, and N1

imprinting probabilities. Two-sided Spearman's correlation coefficients are shown in absolute value.

Boldface values indicate that predictor 1 had a stronger correlation with titers than predictor 2 did,

based on the 95% bootstrap confidence interval. Source data are provided as a Source Data file.

Virus	Predictor 1	Predictor 2	Predictor 1 titer correlation	Predictor 2 titer correlation	Observed difference	Bootstrap difference (95% CI)
A/California/07/2009 (H1N1)	N1 imprinting	HA group 1 imprinting	0.352	0.163	0.189	0.182 (0.058, 0.307)
	N1 imprinting	Birth year	0.352	0.072	0.280	0.249 (0.077, 0.385)
	HA group 1 imprinting	Birth year	0.163	0.072	0.091	0.065 (-0.095, 0.172)
A/Vietnam/1203/2004 (H5N1)	N1 imprinting	HA group 1 imprinting	0.346	0.221	0.126	0.124 (0.004, 0.255)
	N1 imprinting	Birth year	0.346	0.121	0.225	0.212 (0.057, 0.364)
	HA group 1 imprinting	Birth year	0.221	0.121	0.099	0.090 (-0.033, 0.203)
A/Dairy Cow/Texas/24-008749-002-v/2024 (H5N1)	N1 imprinting	HA group 1 imprinting	0.321	0.191	0.129	0.126 (0.007, 0.253)
	N1 imprinting	Birth year	0.321	0.097	0.224	0.206 (0.038, 0.361)
	HA group 1 imprinting	Birth year	0.191	0.097	0.095	0.077 (-0.061, 0.189)
A/British Columbia/PHL-2032/2024 (H5N1)	N1 imprinting	HA group 1 imprinting	0.327	0.193	0.134	0.130 (0.003, 0.25)
	N1 imprinting	Birth year	0.327	0.119	0.208	0.197 (0.038, 0.344)
	HA group 1 imprinting	Birth year	0.193	0.119	0.074	0.067 (-0.048, 0.173)
A/Washington/2148/2025 (H5N5)	N1 imprinting	HA group 1 imprinting	0.408	0.451	-0.043	-0.047 (-0.173, 0.083)
	N1 imprinting	Birth year	0.408	0.413	-0.004	-0.003 (-0.142, 0.144)
	HA group 1 imprinting	Birth year	0.451	0.413	0.039	0.039 (-0.053, 0.13)

Supplementary Table 3. Comparison of linear models between NAI titers against each virus and birth year, HA group 1 imprinting probabilities, and N1 imprinting probabilities. *k* indicates the number of parameters. Source data are provided as a Source Data file.

Virus	Model	<i>k</i>	loglik	AIC	ΔAIC
A/California/07/2009 (H1N1)	N1 imprinting	3	-239.97	485.95	0
	HA group 1 imprinting	3	-247.06	500.12	14.18
	Birth year	3	-247.23	500.46	14.51
A/Vietnam/1203/2004 (H5N1)	N1 imprinting	3	-248.3	502.61	0
	HA group 1 imprinting	3	-254.63	515.27	12.66
	Birth year	3	-255.46	516.91	14.3
A/Dairy Cow/Texas/24-008749-002-v/2024 (H5N1)	N1 imprinting	3	-250.58	507.16	0
	HA group 1 imprinting	3	-256.26	518.51	11.35
	Birth year	3	-256.87	519.75	12.59
A/British Columbia/PHL-2032/2024 (H5N1)	N1 imprinting	3	-250.38	506.76	0
	HA group 1 imprinting	3	-256.36	518.72	11.95
	Birth year	3	-256.85	519.7	12.94
A/Washington/2148/2025 (H5N5)	N1 imprinting	3	-152.49	310.97	0
	HA group 1 imprinting	3	-157.66	321.32	10.35
	Birth year	3	-158.36	322.73	11.76

Supplementary Table 4. Bootstrap analysis comparing the strengths of the correlations between NAI titers against each virus and birth year, age, and N1 imprinting probabilities for combined data from 2005 and 2017 samples. Correlation coefficients are shown in absolute value. Boldface values indicate that predictor 1 had a stronger correlation with titers than predictor 2 did, based on the 95% bootstrap confidence interval. Source data are provided as a Source Data file.

Virus	Predictor 1	Predictor 2	Predictor 1 titer correlation	Predictor 2 titer correlation	Observed difference	Bootstrap difference (95% CI)
A/California/07/2009	N1 imprinting	Age	0.425	0.307	0.118	0.117 (0.027, 0.218)
	N1 imprinting	Birth year	0.425	0.288	0.137	0.135 (0.037, 0.235)
	Age	Birth year	0.307	0.288	0.019	0.019 (-0.017, 0.054)
A/Vietnam/1203/2004	N1 imprinting	Age	0.421	0.317	0.104	0.103 (0.002, 0.216)
	N1 imprinting	Birth year	0.421	0.320	0.101	0.102 (0.002, 0.214)
	Age	Birth year	0.317	0.320	-0.003	0.004 (-0.037, 0.031)
A/Dairy Cow/Texas/24-008749-002-v/2024	N1 imprinting	Age	0.376	0.302	0.074	0.075 (-0.032, 0.186)
	N1 imprinting	Birth year	0.376	0.250	0.126	0.129 (0.022, 0.236)
	Age	Birth year	0.302	0.250	0.052	0.052 (0.02, 0.086)
A/British Columbia/PHL-2032/2024	N1 imprinting	Age	0.408	0.329	0.079	0.079 (-0.024, 0.183)
	N1 imprinting	Birth year	0.408	0.277	0.13	0.129 (0.025, 0.232)
	Age	Birth year	0.329	0.277	0.052	0.051 (0.019, 0.086)

Supplementary Table 5. Details of human serum samples used in this study. Additional participant metadata are provided as a Source Data file.

Cohort	Sample Type	Source	Sample Size	Birth Years
2017	Adults	Hospital of the University of Pennsylvania	119	1927–1999
	Children	Children’s Hospital of Philadelphia	36	2000–2016
2005	Adults (pre-vaccination)	Clinical Trial #NCT00230750	31	1918–1940
		Clinical Trial #NCT00115986	34	1942–1983
H1N1 infection	Children	Children’s Hospital of Philadelphia	13	2006–2023
H3N2 infection			12	2006–2022