

Online Supplemental data

Title:

Diminished B-cell response after repeat influenza vaccination

Authors

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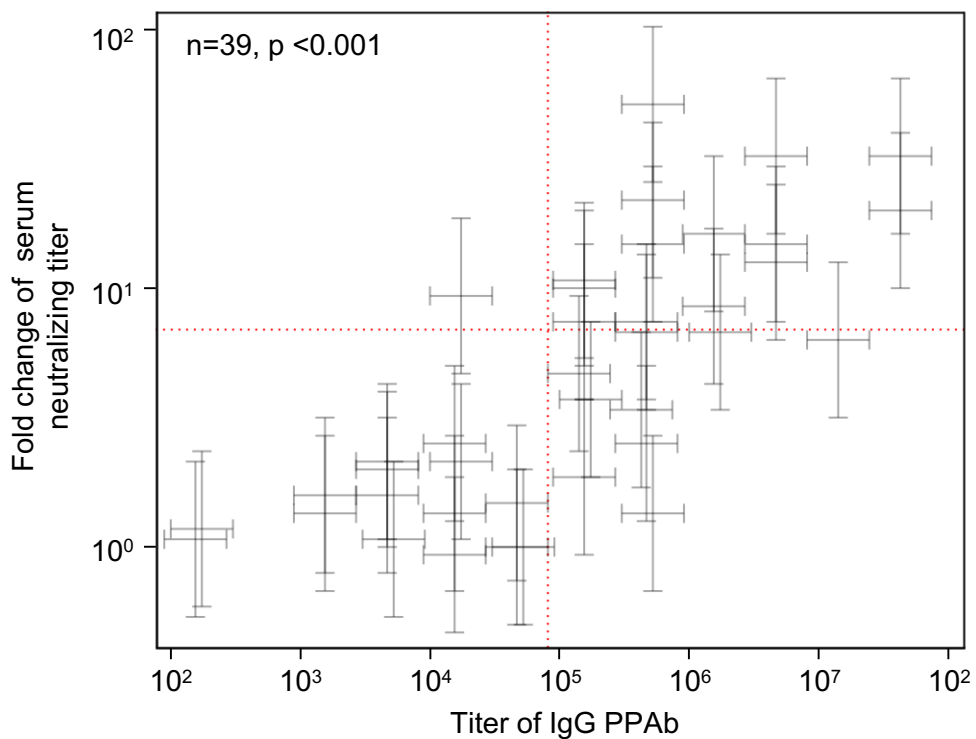
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Figure S1. Details of statistical method for Figure 1D. Censored intervals were categorized as below, above or straddling the midpoint (red dotted line) for each variable, which generated a 3×3 contingency table of counts of observations. Using this contingency table, association between fold change of serum neutralizing titer and PPAb IgG (titer against 2011 TIV) was tested using a Freeman-Halton exact test [1].

Reference:

1. G. H. Freeman and J. H. Halton. Note on an exact treatment of contingency, goodness of fit and other problems of significance. *Biometrika* **1951**; 38:141-149.



Supplemental Figures

Figures S2 – S5. Fit of shared-parameter model to longitudinal outcomes.

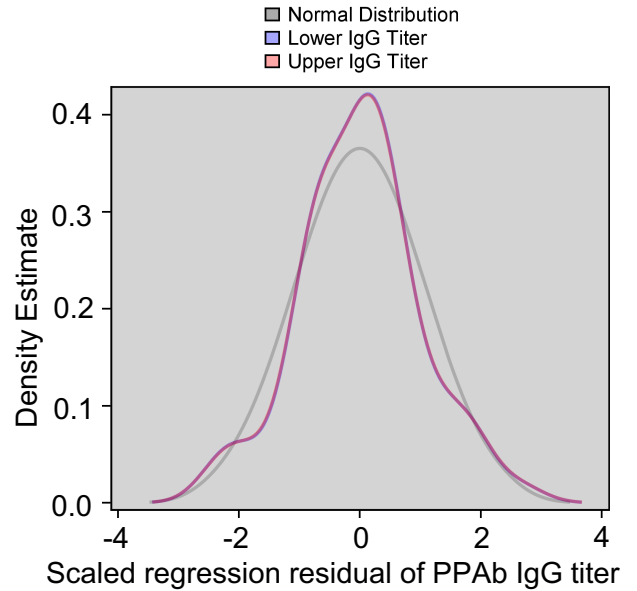
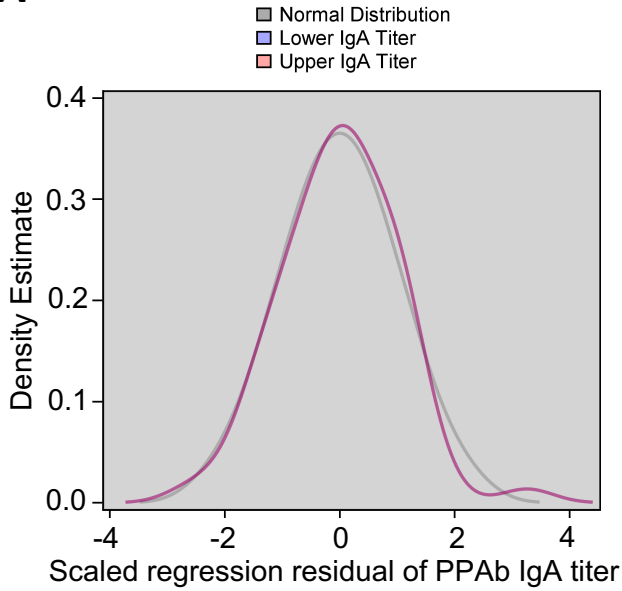
All figures compare the kernel density estimate [1] of the distribution of the observed data to the parametric distribution assumed for that model. Presented observed data are residuals (unexplained variation) from the fit of the regression model. Data were logarithm transformed prior to regression analysis for IgA PPAbs and IgG PPAbs titers (Figure S2A), numbers of IgA and IgG plasmablasts per 10^5 B cells (Figure S2B), and fold change of HAI titer (Figure S4). Percentages of IgA and IgG plasmablasts (Figure S2C) were arcsine square root transformed [2] prior to analysis. To limit the effect of larger relative errors of the smaller AUC values on comparison between years (Figure 3), the HA-specific binding activity data were not transformed for results presented in main text (see also Figure S3). For comparison, results for transformed HA-specific binding activity data are shown in Figures S12 and S13.

References:

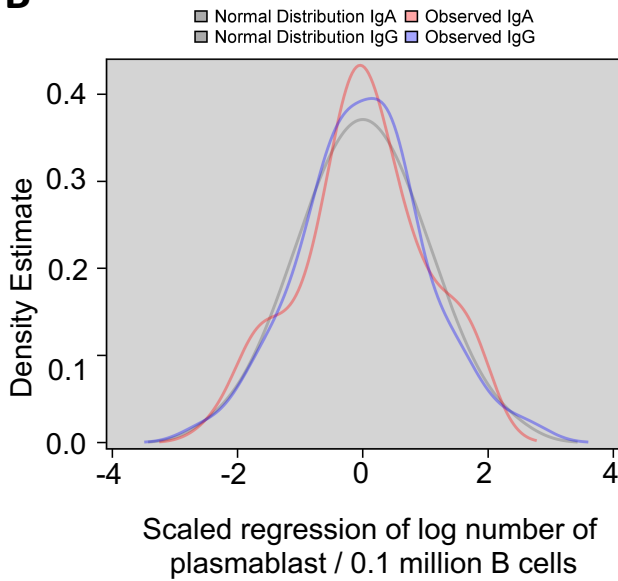
1. Silverman BW. Density estimation for statistics and data analysis. London; New York: Chapman and Hall, **1986**.
2. Sokal RR. Biometry: the principles and practice of statistics in biological research. In: Rohlf FJ, ed. 2nd ed. San Francisco: W.H. Freeman, **1981**:427-8.

Figure S2

A



B



C

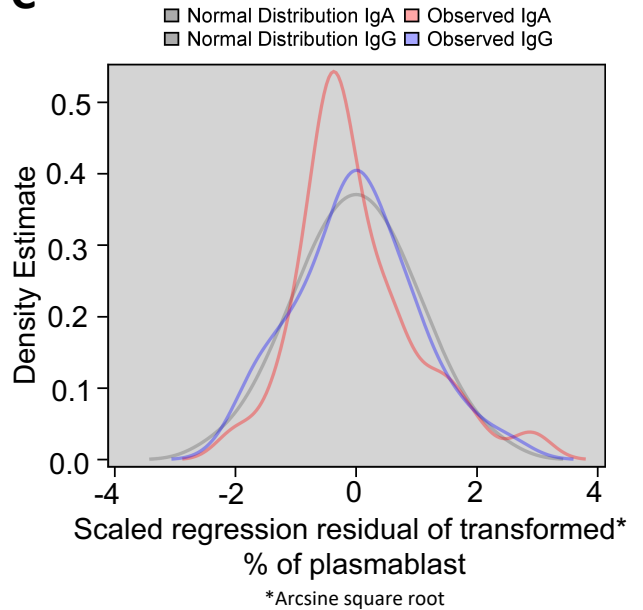


Figure S3

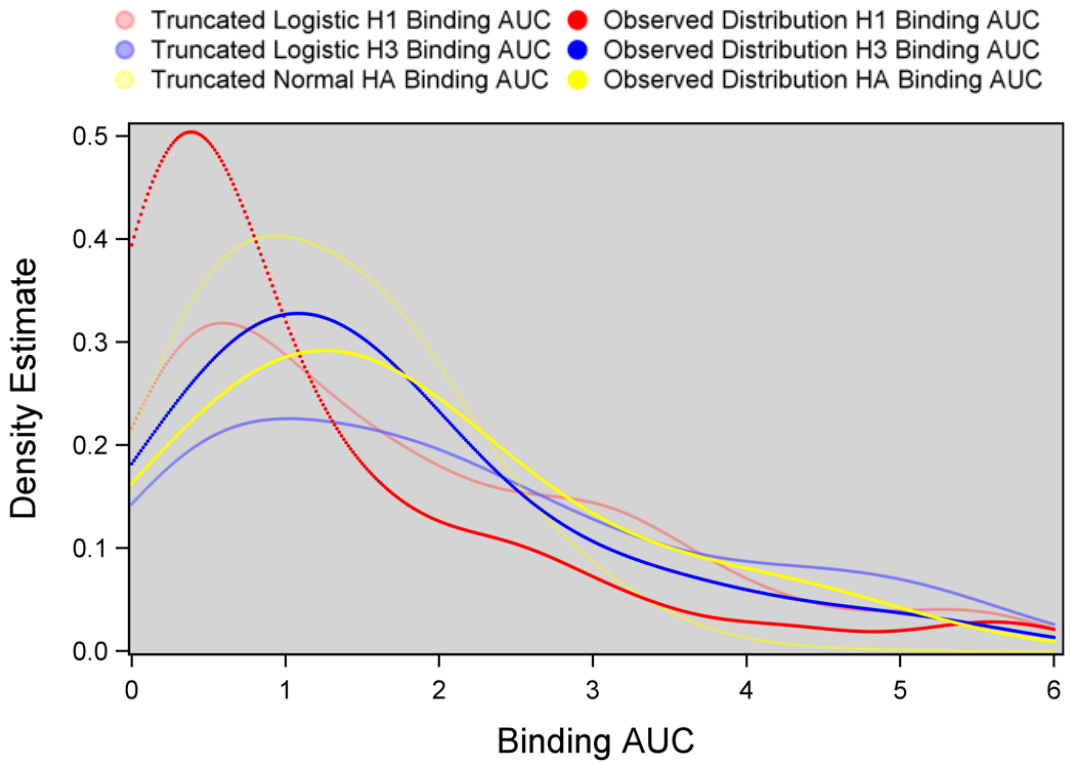


Figure S4

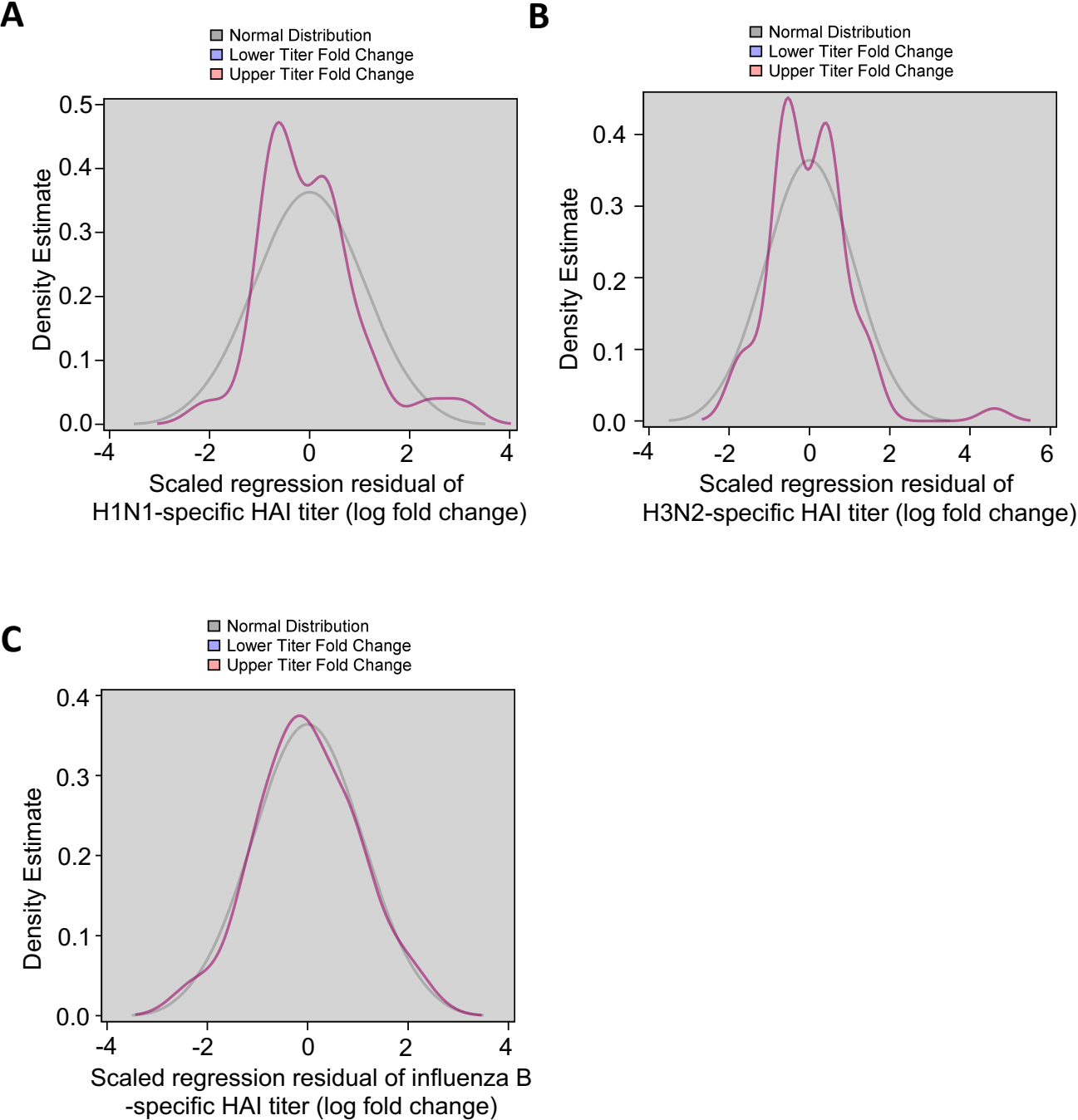
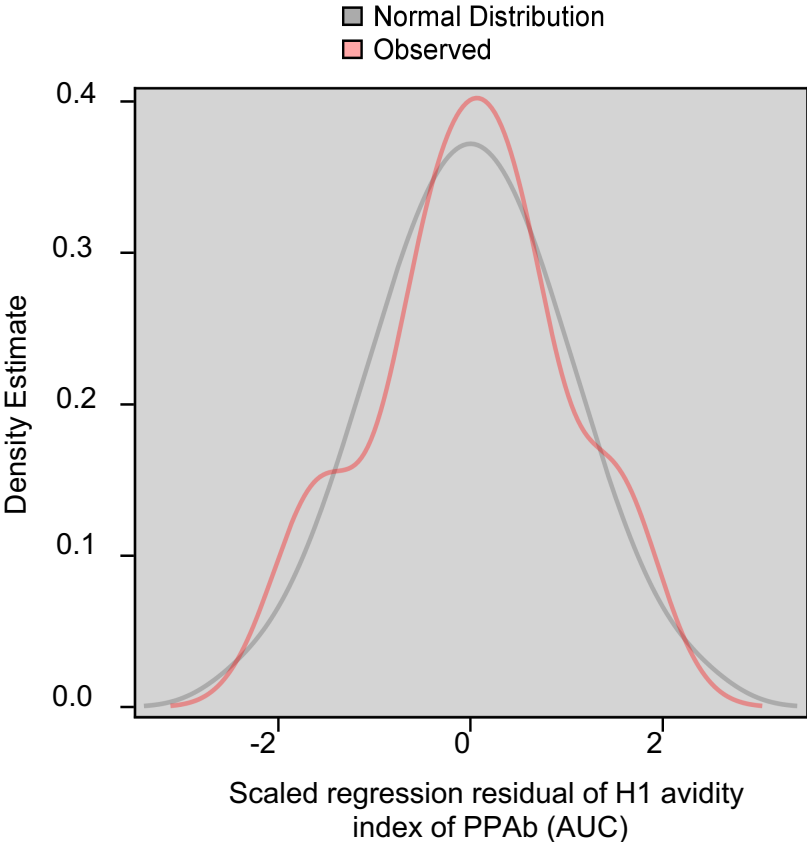


Figure S5



Figures S6 – S9. Vaccine-specific B cell/antibody response in 2010/2011 is positively associated with participant duration on study. The shared-parameter model permitted estimation of association between the 2010/2011 level of each outcome and participant duration on study (2010/2011 through 2014). This association was detected ($p < 0.05$) at various estimated strengths in the four outcomes shown (Figures S6 – S9). Positive association indicates that higher levels of immune response in 2010/2011 were associated with longer duration on study. Participants who leave the study in third or fourth year are estimated to have had lower levels of the immune response after their first vaccination. The shared-parameter model may at least partially correct [1] bias in estimates of 2013-2014 seasonal means due to any dropout that depends upon observed and unobserved (latent) baseline characteristics of the participant. In Figure 2A, the regression model's estimates of the means in 2013 and 2014 (black circles) sit low in the observed data indicating that the regression model is adjusting those means downward to account for low responders in 2010/2011 who dropped from the study.

Technical Note: We employed these shared-parameter regression models specifically because they could be applied to the range of longitudinal outcomes examined, including interval-censored longitudinal outcomes (titer and titer fold change). We appreciate that model assumptions can impact findings; so we were careful to examine the distributional assumptions of the longitudinal outcomes (Figures S2A through S5). Further, the fitting algorithm was supplied with a grid of starting values to ensure identification of the global optimum. To further assess robustness of findings, in separate analyses, we transformed HA-specific binding activity data prior to analysis (Figures S12 and S13). Finally, we employed a robust estimator of the covariance matrix of the parameter estimates [2] as an additional safeguard against departures from regression model assumptions.

References:

1. McCulloch CE. Joint modelling of mixed outcome types using latent variables. *Statistical methods in medical research*. **2016**; 17:53-73.
2. White H. Maximum Likelihood Estimation of Misspecified Models. *Econometrica* **1982**; 50:1-25.

Figure S7

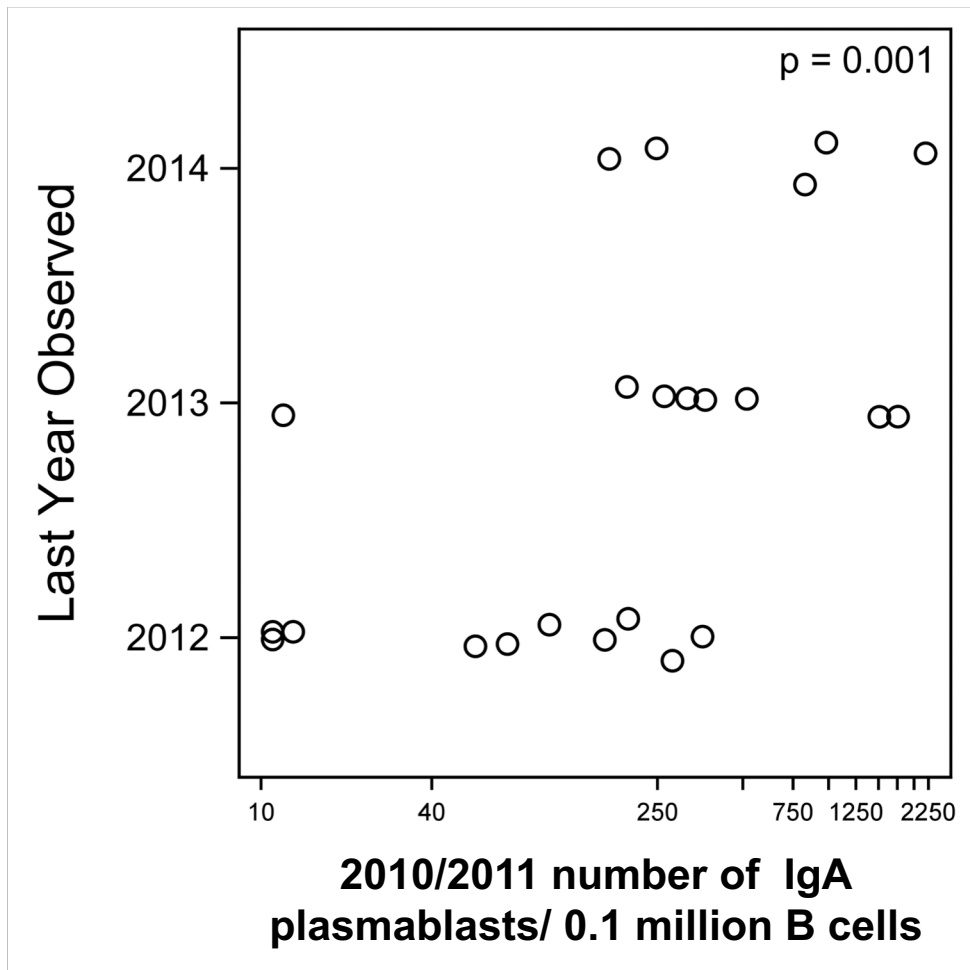


Figure S8

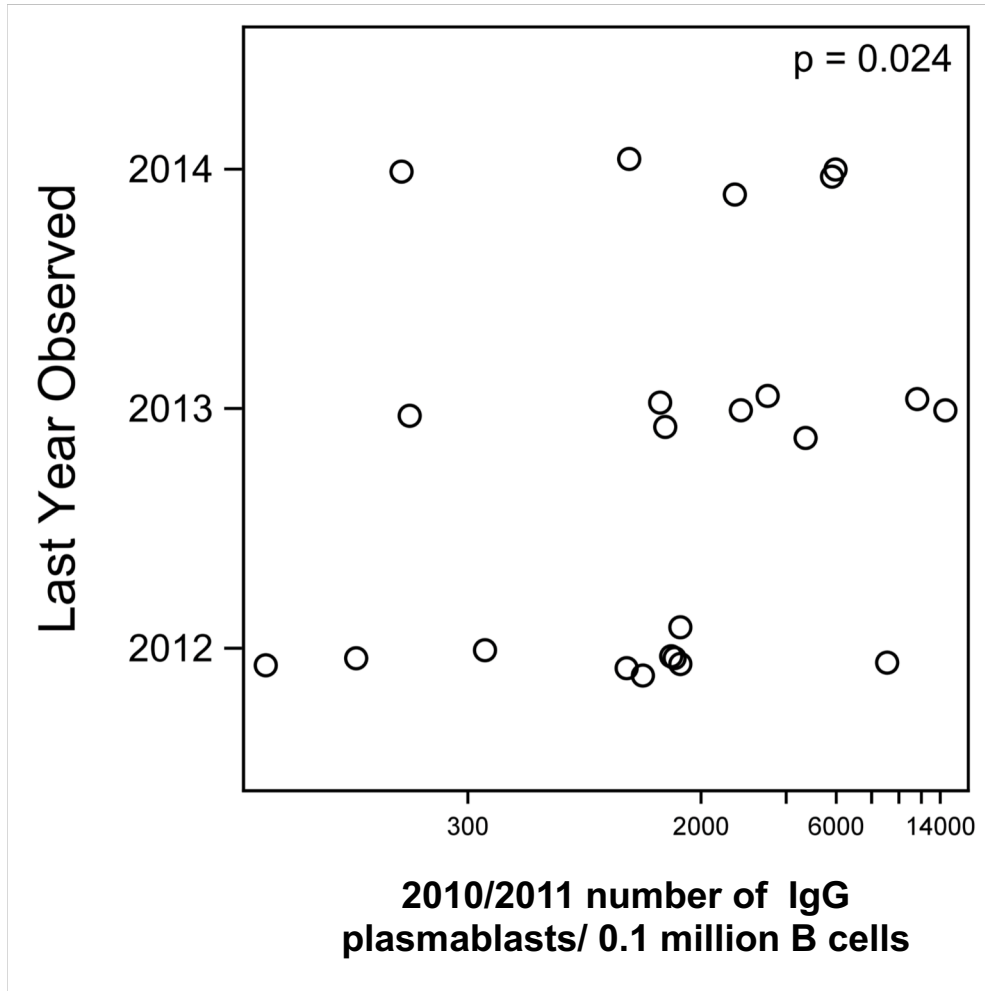


Figure S9

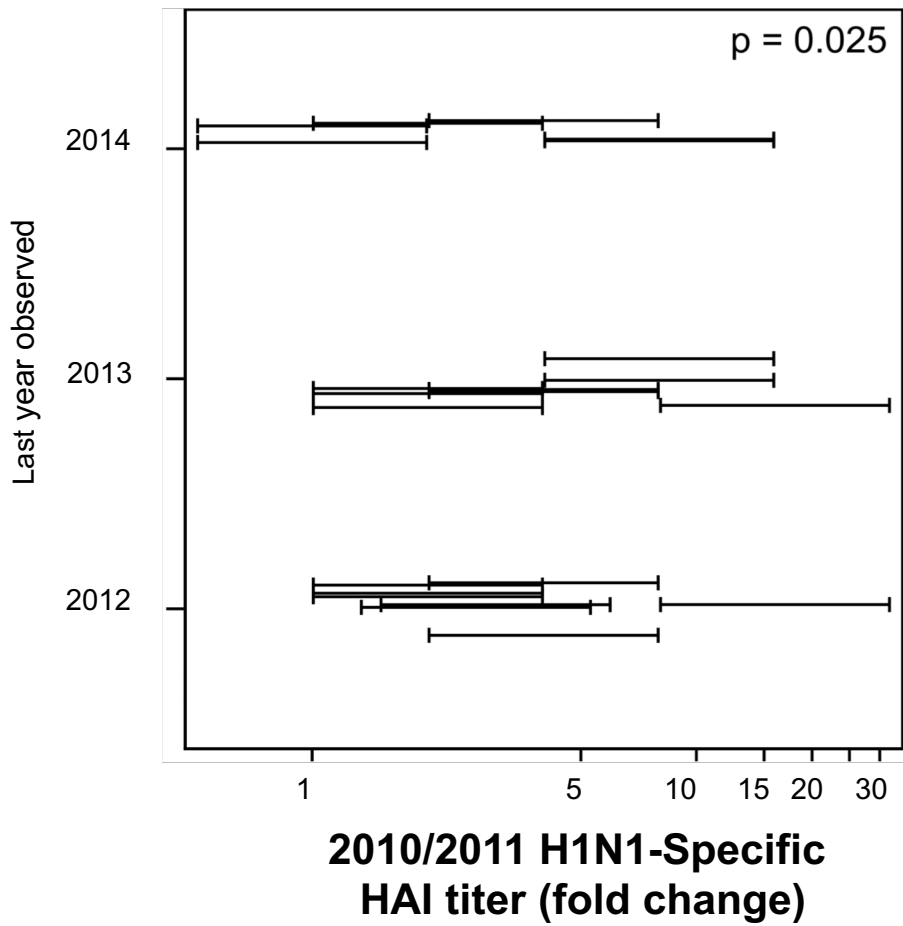


Figure S10. Serum HAI titers before (day 0) and after (day 28) each TIV immunization. Titers were measured with 2x serially diluted samples. Each vertical bracket denotes the interval-censored titer. Data are randomly jittered in horizontal to facilitate viewing of individual titer intervals.

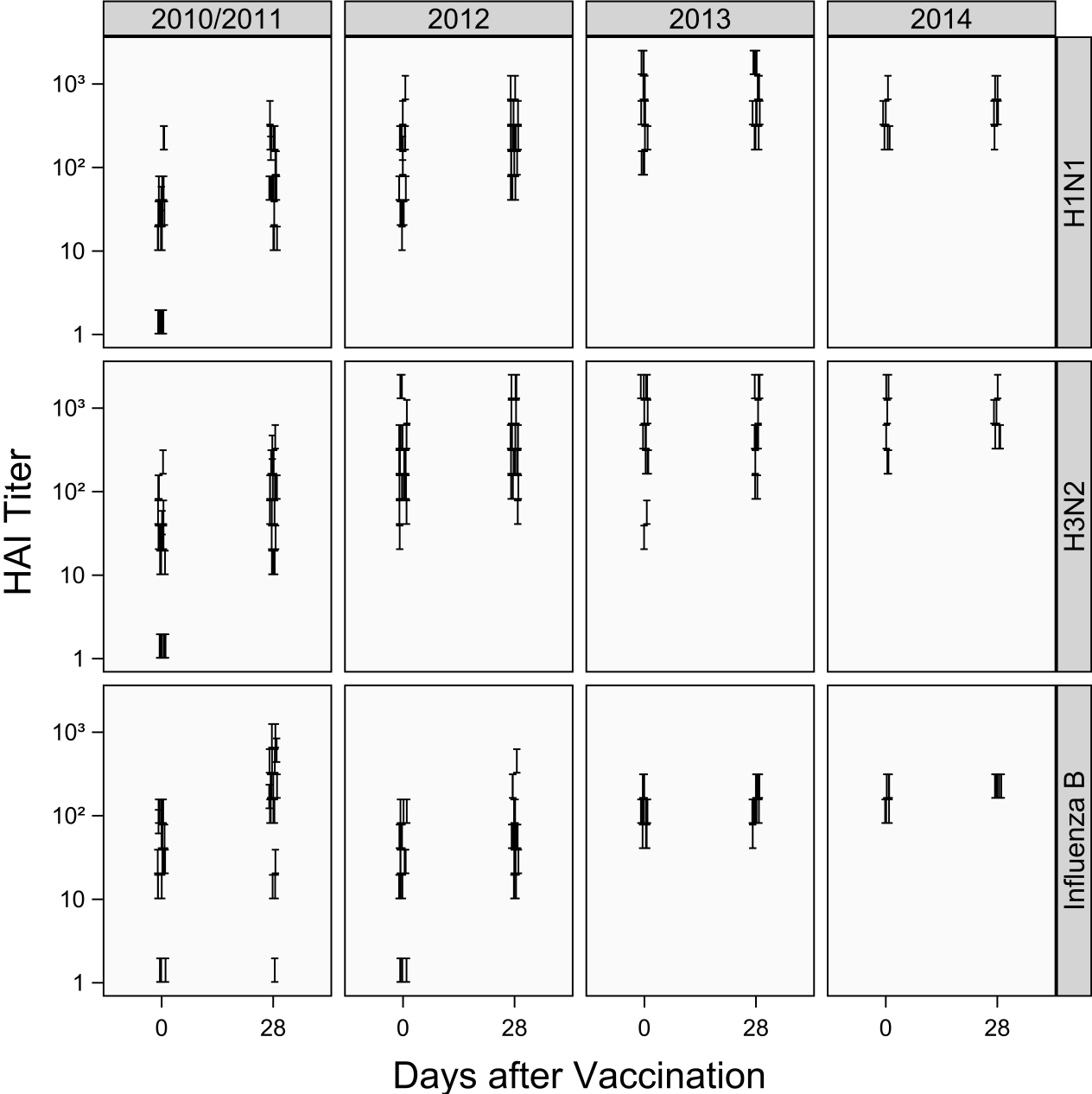
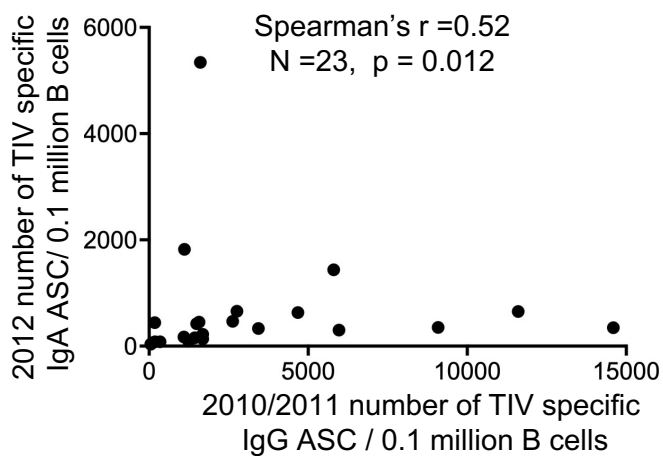
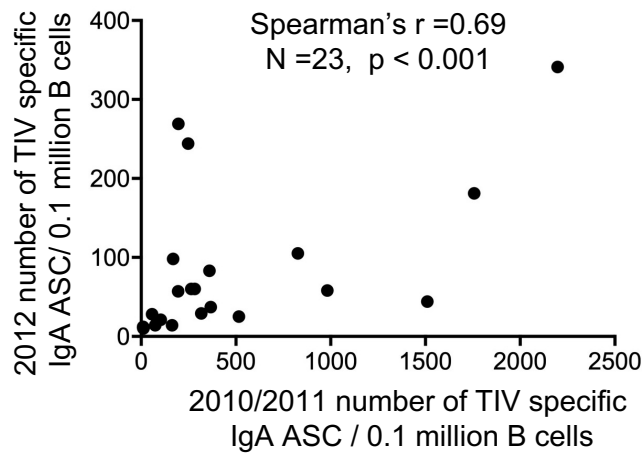


Figure S11. Estimates of Spearman's correlation coefficient provide evidence of moderate association in vaccine-specific IgA and IgG plasmablast responses between 2010/2011 and 2012.

Figure S11



Figures S12 – S13. Robustness Assessment: Results for transformed HA-specific binding activity data. Logarithm (H3 and influenza B-HA) and square-root (H1) transformations were applied prior to shared-parameter regression analysis. Overall findings were similar to results for untransformed data shown in Figure 3. Compare untransformed data of Figures 3A – 3C of main text vs. transformed data of Figure S12. Fit of regression model is shown in Figure S13.

Figure S12

Marker	Means Comparison	Estimated Difference	Standard Error	P-value	P-value Adjusted for Multiple Comparisons
H1 Binding AUC	2012 minus 2010/2011	-0.6980	0.1473	<.0001	<.0001
H1 Binding AUC	2013 minus 2010/2011	-0.9979	0.1187	<.0001	<.0001
H1 Binding AUC	2014 minus 2010/2011	-0.7793	0.1614	<.0001	<.0001
H1 Binding AUC	2013 minus 2012	-0.2999	0.1203	0.0173	0.0346
H1 Binding AUC	2014 minus 2013	0.2186	0.1454	0.1412	0.1412
H3 Binding AUC	2012 minus 2010/2011	-0.3348	0.1097	0.0042	0.0210
H3 Binding AUC	2013 minus 2010/2011	-0.2654	0.1310	0.0501	0.2004
H3 Binding AUC	2014 minus 2010/2011	-0.3239	0.1950	0.1051	0.3153
H3 Binding AUC	2013 minus 2012	0.06942	0.1371	0.6155	1.0000
H3 Binding AUC	2014 minus 2013	-0.05850	0.2260	0.7972	1.0000
Influenza B HA Binding AUC	2012 minus 2010/2011	0.1498	0.09156	0.1104	0.5520
Influenza B HA Binding AUC	2013 minus 2010/2011	0.08412	0.1177	0.4794	1.0000
Influenza B HA Binding AUC	2014 minus 2010/2011	0.1466	0.1301	0.2671	1.0000
Influenza B HA Binding AUC	2013 minus 2012	-0.06564	0.09502	0.4940	1.0000
Influenza B HA Binding AUC	2014 minus 2013	0.06251	0.1355	0.6473	1.0000

Figure S13

