

Supplementary Materials for
**Food-specific immunoglobulin A does not correlate with natural tolerance to
peanut or egg allergens**

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The PDF file includes:

Figs. S1 to S6
Legend for data file S1

Other Supplementary Material for this manuscript includes the following:

Data file S1
MDAR reproducibility checklist

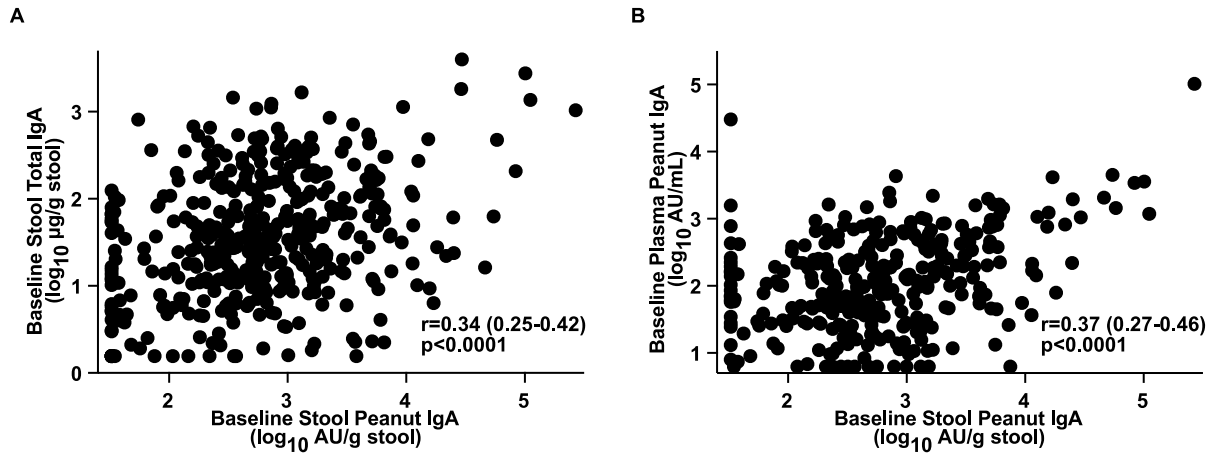


Fig. S1. Stool peanut-specific immunoglobulin A (IgA) is correlated with stool total IgA and plasma peanut-specific IgA.

All baseline samples from the Consortium of Food Allergy Research 2 (CoFAR2) cohort were analyzed. (A) The correlation of baseline stool total IgA with baseline stool peanut-specific IgA is shown (n=430 pairs). (B) The correlation of baseline plasma peanut-specific IgA with baseline stool peanut-specific IgA is shown (n=325 pairs). Correlations were assessed using two-tailed Pearson tests. Pearson's r with 95% confidence interval and p-values are displayed. AU, arbitrary units.

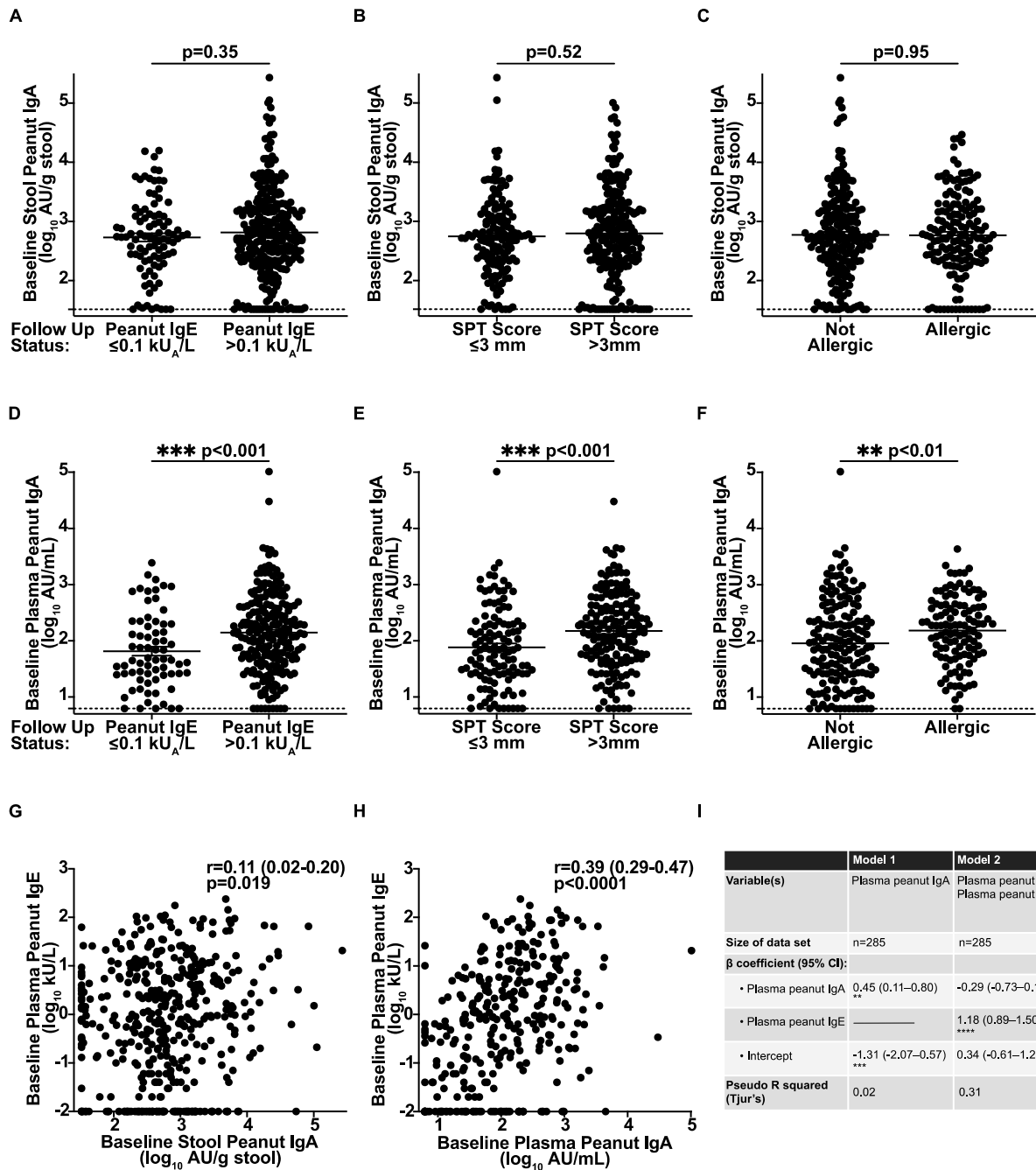
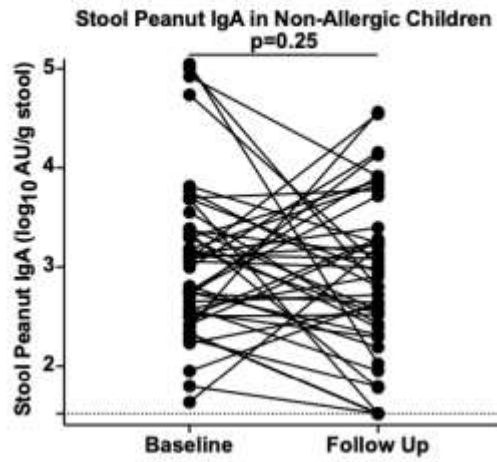


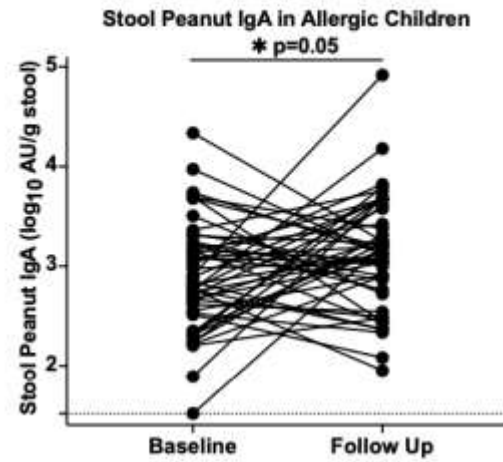
Fig. S2. Peanut-specific IgA does not predict protection from future peanut sensitization or allergy in an atopic cohort.

All baseline samples from CoFAR2 with at least 2 years of follow up were analyzed. Baseline stool peanut-specific IgA concentrations are shown, stratified by follow up (A) peanut-specific immunoglobulin E (IgE) abundance (n=93 ≤ 0.1 kilounits of allergen-specific IgE per liter (kU_A/L), n=287 > 0.1 kU_A/L), (B) peanut skin prick test (SPT) score (n=147 ≤ 3 mm, n=234 > 3 mm), and (C) clinical peanut allergy (n=213 not allergic, n=155 peanut allergic; n=55 equivocal peanut allergy samples excluded). Baseline plasma peanut-specific IgA concentrations are shown, stratified by follow up (D) peanut-specific IgE (n=70 ≤ 0.1 kU_A/L, n=218 > 0.1 kU_A/L), (E) peanut SPT score (n=117 ≤ 3 mm, n=172 > 3 mm), and (F) clinical peanut allergy (n=169 not allergic, n=116 peanut allergic; n=40 equivocal peanut allergy samples excluded). Shown are correlations of baseline plasma peanut-specific IgE abundance and (G) baseline stool peanut-specific IgA concentrations (n=432 pairs), and (H) baseline plasma peanut-specific IgA concentrations (n=330 pairs), with r and 95% confidence interval denoted. (I) Shown is a summary of univariate and multivariate regression of effect of plasma peanut-specific IgA +/- plasma peanut-specific IgE on allergy outcome. Dotted lines indicate limit of detection of assay. Data are shown with mean and SEM. Means were compared using unpaired t-tests. Correlations were measured with two-tailed Pearson tests. P-values are as labeled. ** and *** indicate p<0.01 and p<0.001, respectively.

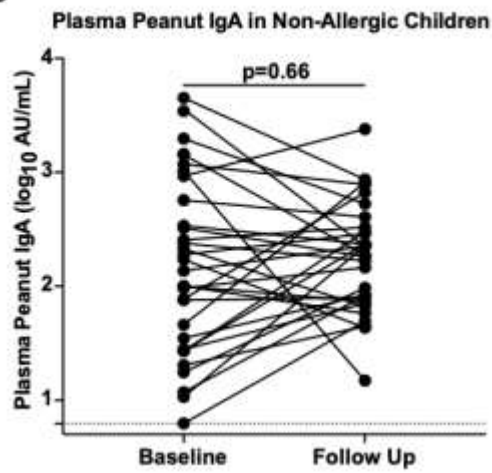
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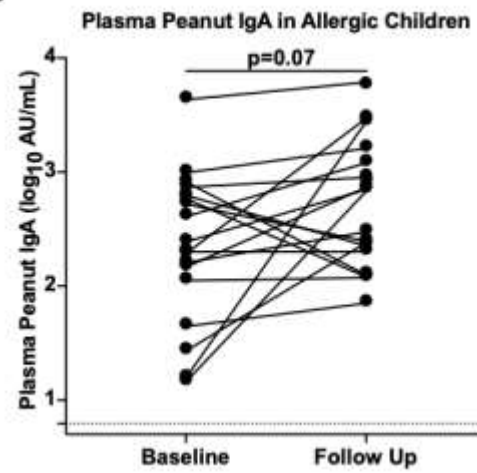
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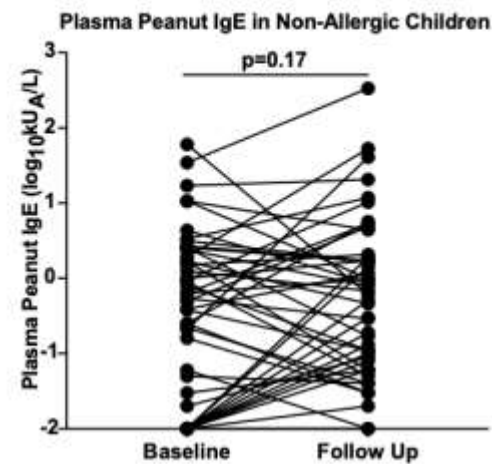
C



D



E



F

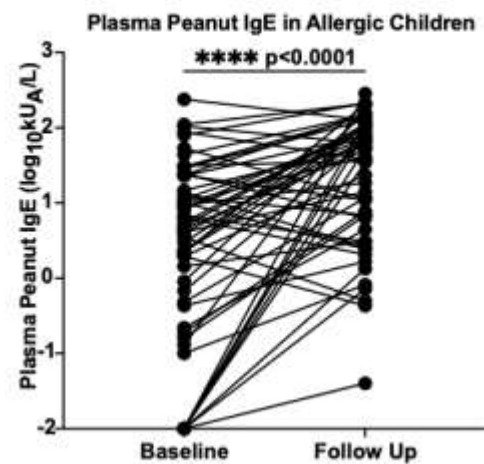


Fig. S3. Gut peanut-specific IgA increases over time in peanut allergic children.

Children with matching data from baseline and follow up timepoints in CoFAR2 were analyzed. Baseline and follow up stool peanut-specific IgA concentrations were measured in **(A)** children who were clinically not allergic to peanut at follow up (n=45) and **(B)** children who were clinically peanut allergic at follow up (n=48). Baseline and follow up plasma peanut-specific IgA concentrations were measured in **(C)** children who were clinically not allergic to peanut at follow up (n=30) and **(D)** children who were clinically peanut allergic at follow up (n=18). Baseline and follow up plasma peanut-specific IgE concentrations were measured in **(E)** children who were clinically not allergic to peanut at follow up (n=47) and **(F)** children who were clinically allergic to peanut at follow up (n=55). Dotted lines in each graph indicate limit of detection of assay. Baseline and follow up values were compared using paired t-tests, with p-values as labeled. * indicates $p < 0.05$ and **** indicates $p < 0.0001$.

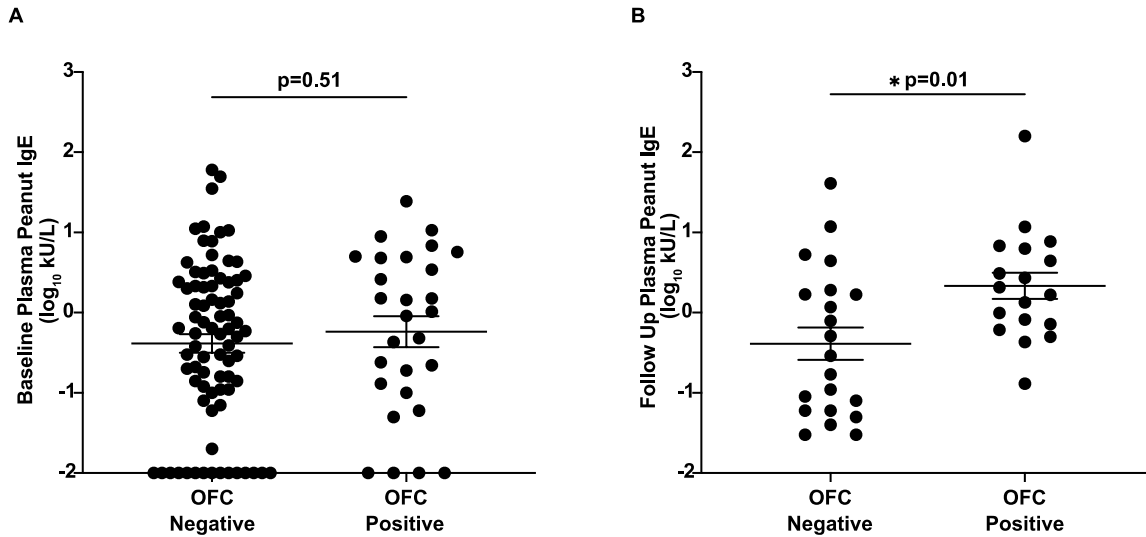


Fig. S4. Plasma peanut-specific IgE is higher in children with a positive peanut oral food challenge.

The subset of children in CoFAR2 who had a peanut oral food challenge (OFC) and accompanying plasma peanut-specific IgE data were analyzed. **(A)** Baseline plasma peanut-specific IgE concentrations are shown, stratified by OFC outcome (n=80 OFC negative and n=28 OFC positive). **(B)** Follow up plasma peanut-specific IgE concentrations are shown, stratified by OFC outcome (n=21 OFC negative and n=18 OFC positive). Data are shown with mean and SEM. Means were compared using unpaired t-tests, with p-values as labeled. * denotes p<0.05.

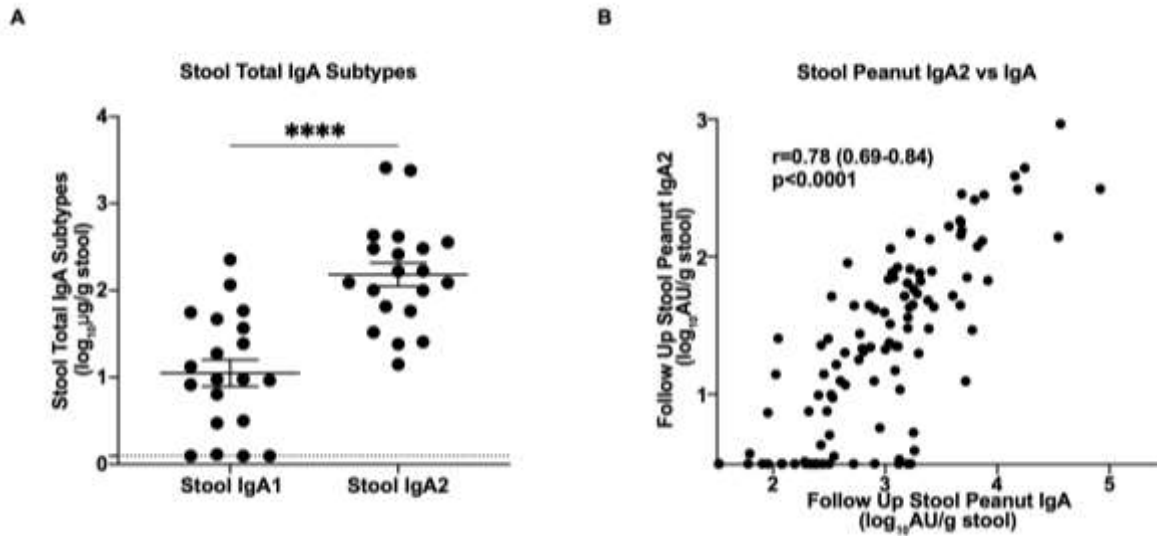


Fig. S5. Stool IgA is predominantly IgA2.

Follow up samples from the CoFAR2 cohort were analyzed in this figure. **(A)** Total IgA1 and IgA2 were measured in randomly selected matched stool samples (n=20). **(B)** Shown is the correlation of stool peanut-specific IgA2 and stool peanut-specific IgA in all available CoFAR2 follow up samples (n=109 pairs displayed, 1 pair excluded because stool peanut-specific IgA2 did not dilute well; 14 additional follow up stool samples were not included because they were completely depleted by other analyses). The dotted line indicates limit of detection of assay. Data are shown with mean and SEM. Means were compared using unpaired t-test. Correlation was measured with two-tailed Pearson test. p-values are as labeled. **** indicates p<0.0001.

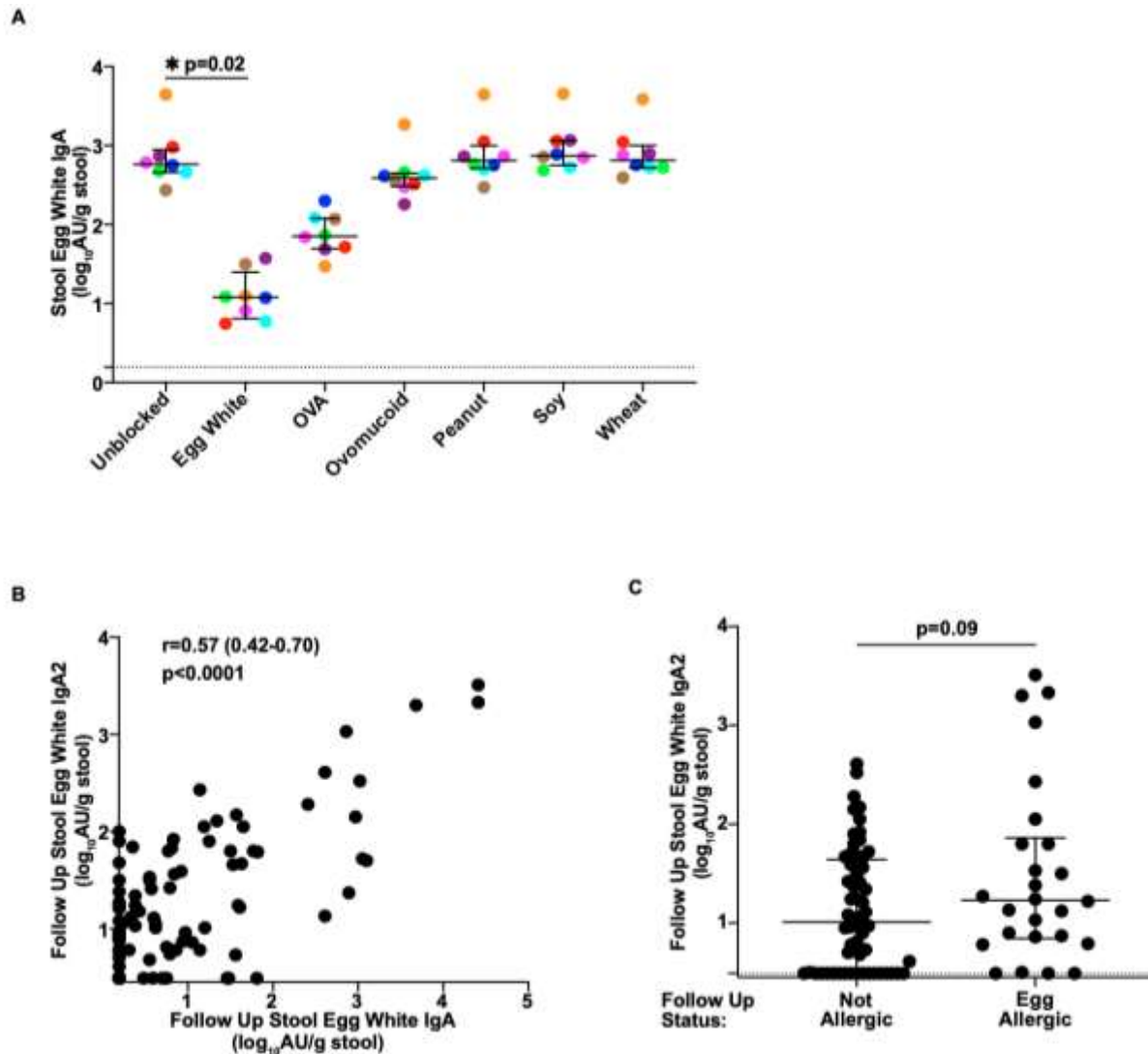


Fig. S6. Stool egg white-specific IgA2 does not correlate with egg allergy.

Samples from children in the CoFAR2 cohort at the follow up timepoint were examined.

(A) Stool egg white-specific IgA was determined using a competitive enzyme-linked immunosorbent assay using blocking with 200 µg/mL of egg white, ovalbumin (OVA), ovomucoid, peanut, soy, or wheat protein (n=8). Samples are color-coded by individual.

(B) Shown is the correlation of follow up stool egg white IgA2 with stool egg white IgA (n=99 pairs, 7 pairs excluded due to poor dilution of stool egg white IgA2; 18 additional follow up stool samples were not included because they were completely depleted by

other analyses). **(C)** Follow up stool egg white IgA2 concentrations were measured, stratified by clinical allergy status (n=60 not allergic, n=26 egg allergic, n=13 equivocal egg allergy samples excluded). Dotted lines indicate limit of detection of assay. Data are shown with median and interquartile range and were compared using Friedman test with Dunn's multiple comparisons in (A) and Mann-Whitney test in (C), with p-values as labeled. Correlation was assessed in (B) using Spearman correlation, with Spearman r, 95% confidence interval, and p value displayed. * denotes $p < 0.05$.

Data file S1. Raw, individual-level data for experiments where $n < 20$.