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Supplemental Information

Effect of Antibiotic-Mediated Microbiome Modulation on Rotavirus Vaccine Immunogenicity: A Human, Randomized-Control Proof-of-Concept Trial

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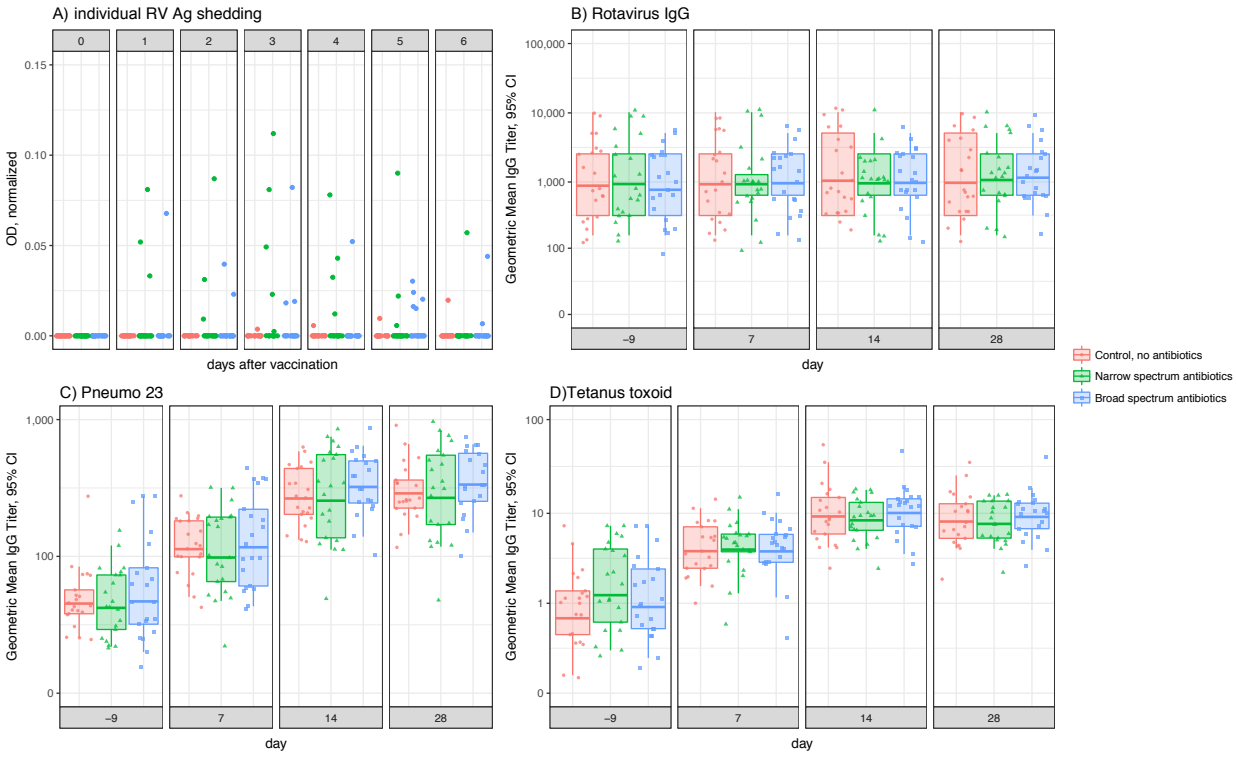
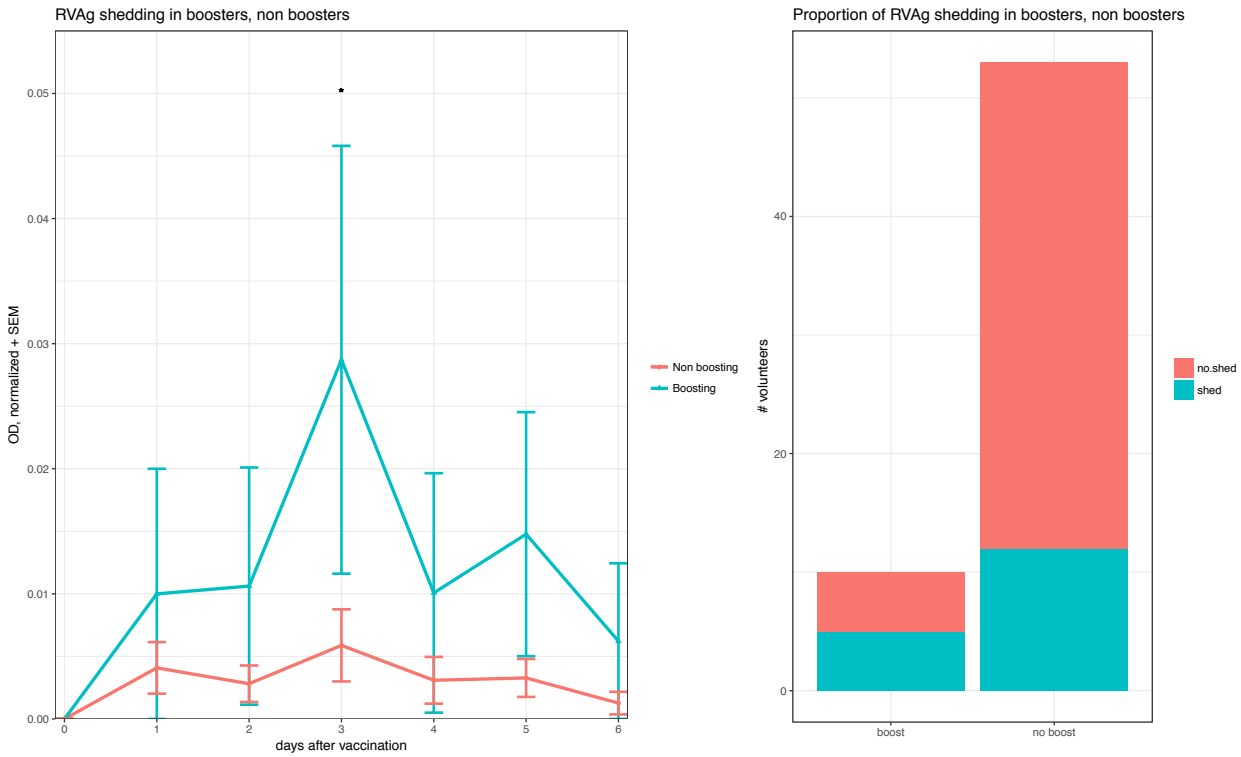
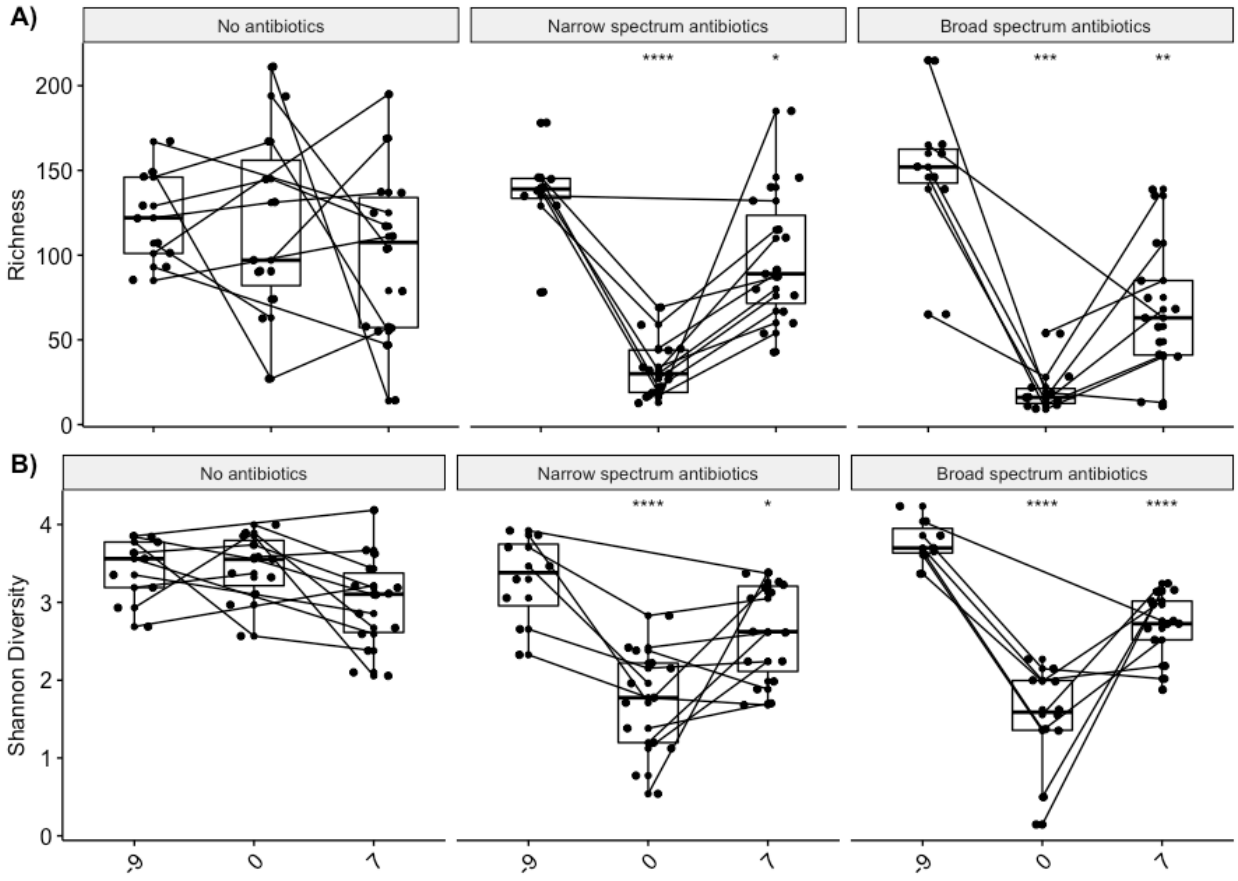
Supplemental figures and tables legends



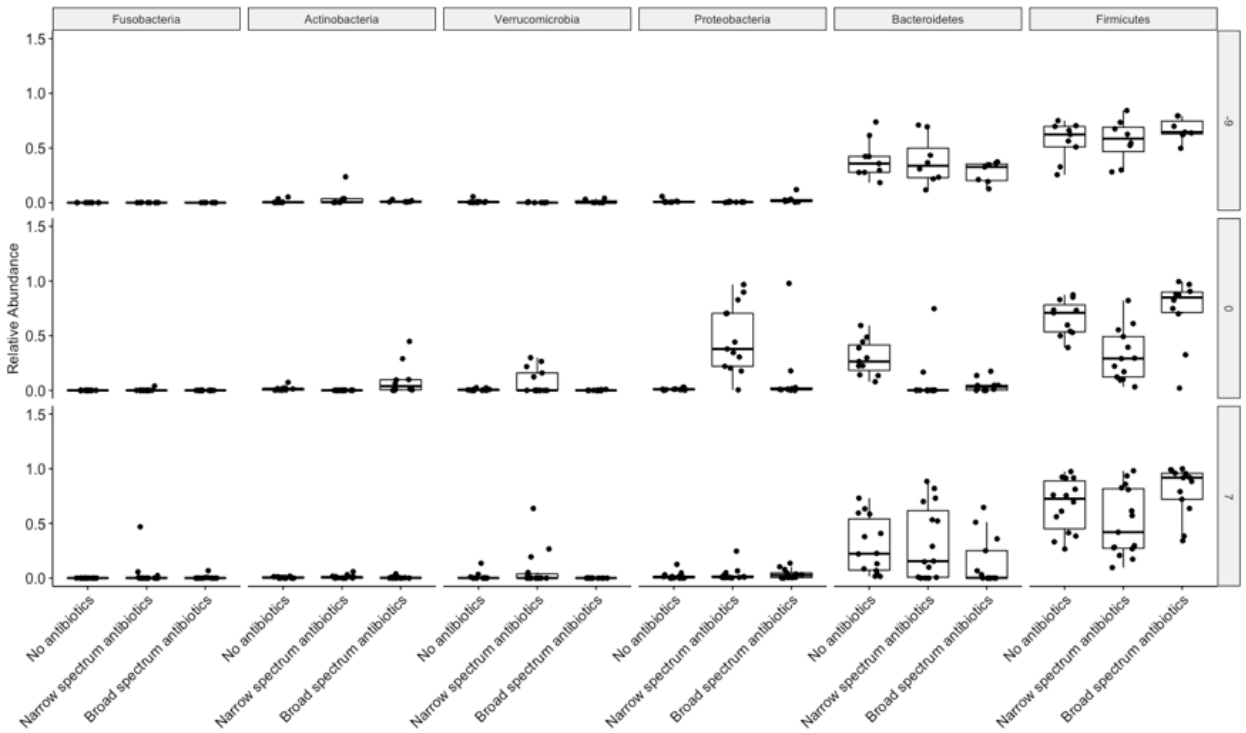
Supplemental figure S1 Vaccine antibody response and shedding over time, related to figure 2 (A) Dot plot of RVAg shedding per day. Significance was determined using Friedman's with Dunn's multiple comparison test (B)RV-specific IgG GMT per treatment arm over time with 95% confidence intervals. Time-series two-way ANOVA, both with Dunnett's multiple comparison correction (C) Pneumococcal and (D) tetanus toxoid GMT antibody response with 95% confidence intervals per treatment arm over time.



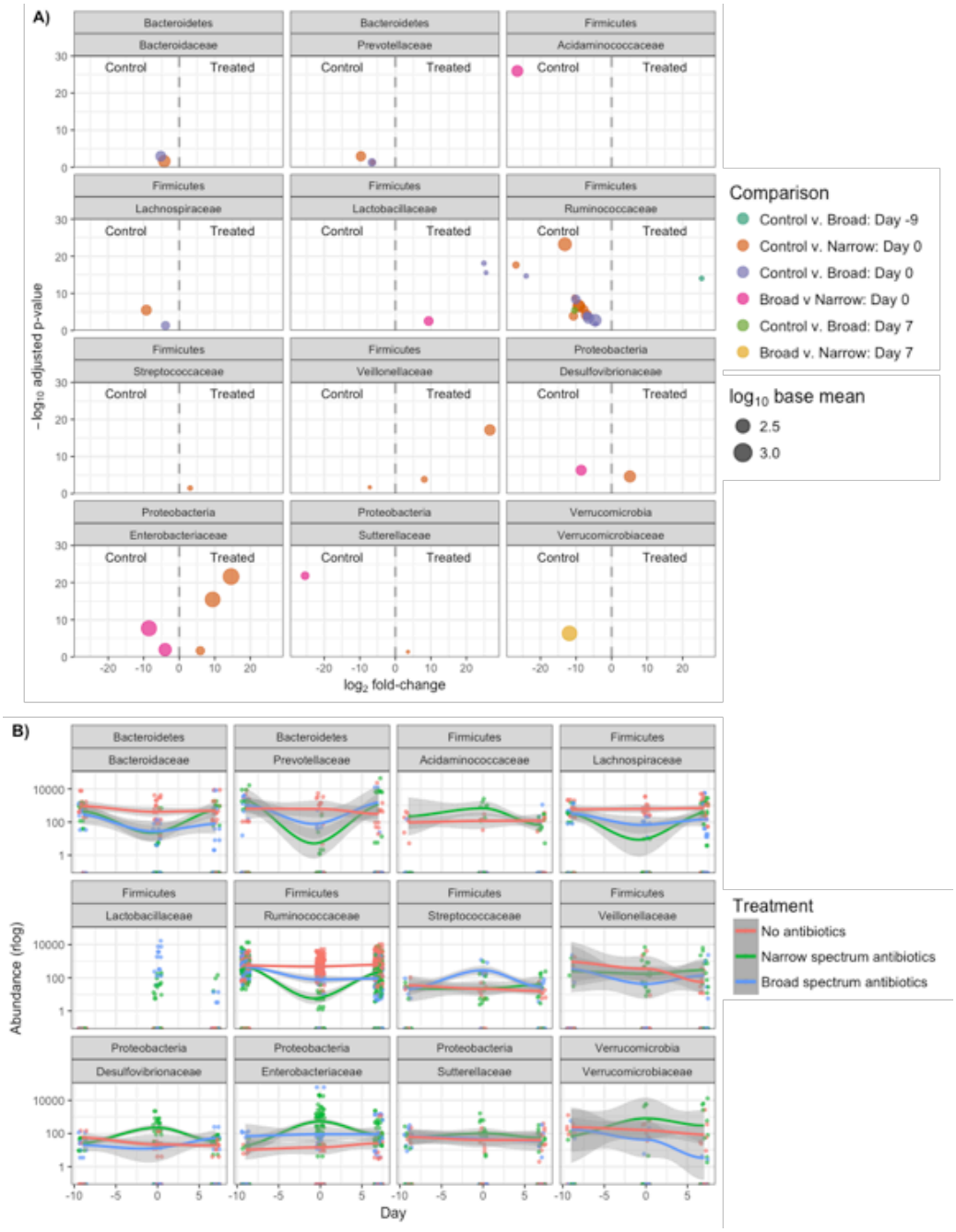
Supplemental figure S2 Correlation between RVV boosting and RV shedding, related to figure 2 (A) mean normalized OD + SEM of RV antigen shedding for boosters and non-boosters over time as determined by RV Ag ELISA. Significance was determined using Wilcoxon matched-pairs signed rank test. (B) proportion of subjects with and without boost that ever shed. Fisher's exact test.



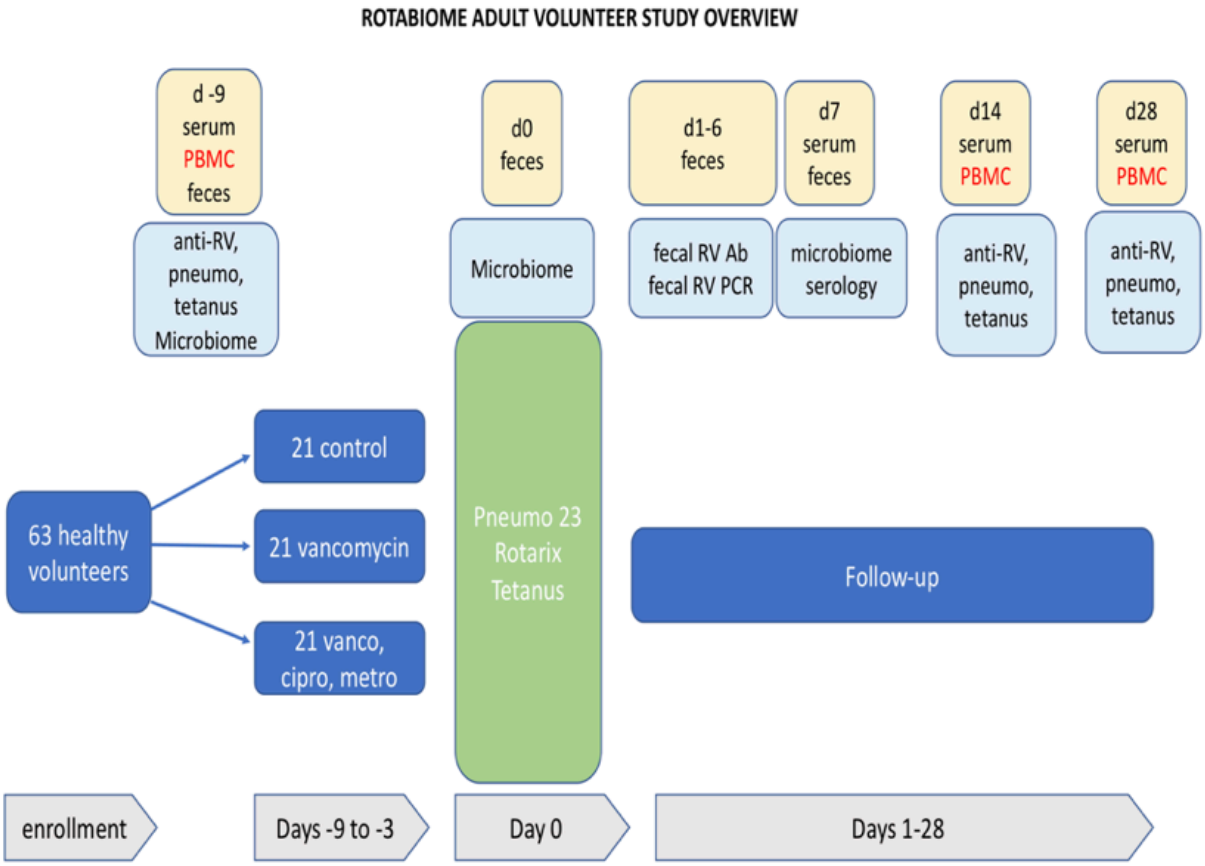
Supplemental figure S3 Antibiotic treatment reduces alpha diversity over time, related to figure 3 . Bacterial (A) richness and (B) Shannon diversity over time by treatment arm. Significance was calculated using a non-parametric Kruskal-Wallis test within each group at each independent time. *p≤0.05, **p≤0·01, ***p≤0·001, ****p≤0·0001.



Supplemental figure S4 Phylum changes over time and treatment arm, related to figure 4
Comparison of the relative abundance of phyla by treatment arm at each time point. Only phyla found to be significantly altered by treatment at one or more time-points were included. Members of the Elusimicrobia, Lentisphaerae, Spirochaetes, Synergistetes and Tenericutes were also identified but not found to be significantly altered by treatment at any time-point. Significance was calculated using a non-parametric Kruskal-Wallis test within each group at each independent time. *p≤0.05, **p≤0·01, ***p≤0·001, ****p≤0·0001.



Supplemental figure S5 Differentially abundant taxa by treatment arm over time, related to figure 5 (A) Volcano plots of bacterial taxa significantly ($p<0.05$, DESeq2 Wald test with a base mean > 100) differentially abundant between treatment arm at each individual time point. (B) Temporal trajectories of the normalized (rlog) abundances of all differentially abundant taxa in A). Lines indicate the loess smoothed fit across the three time points and shaded by the confidence interval around the mean. Each panel header top refers to the bacterial Phyla and bottom refers to Family. See also Table S1.



Supplemental figure S6 Visual Study overview, related to STAR Methods: Pictorial overview of study design and methodology. Yellow is time point and type of sample collection. Light blue is how sample was used; Dark blue is patient allocation and follow-up; green is time of vaccination; light grey is time line. Enrollment continued till 21 subjects per treatment arm completed the study per-protocol. Abbreviations (vanco: vancomycin; Cipro: ciprofloxacin; metro: metronidazole; Ab: antibody; pneumo: anti-pneumococcal antibodies; tetanus: tetanus: anti-pneumococcal antibodies; RV: rotavirus; anti-RV: anti-RV IgA and IgG antibodies; PBMC peripheral blood mononuclear cells.

Supplemental table S1, related to figure 5 and figure S5: Summary of the pairwise comparison of taxa found to be differentially abundant between treatment arms, shedders, and boosters at days 0 and 7 as determined by DESeq2. Each sequence variant and it's rlog normalized abundance was assessed for differential abundance between groups at day 0 and day 7. Table gives overview of those ASV that were significantly different ($p<0.05$, DeSeq2 Wald test) between Treatment arms (Narrow vs. broad, control vs. broad, control vs. narrow); RV Shedders: Shed vs. no Shed; and RVV Boosters: Boost (day 7 $\geq$ 2-fold anti-RV IgA titer rise) vs no-boost). ASV, amplicon sequence variant; lfcSE, $\log_2$ fold change Standard Error.