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OBSERVATION
Novel Systems Biology Techniques

Evaluating Organism-Wide Changes in the Metabolome and Microbiome following a Single Dose of Antibiotic

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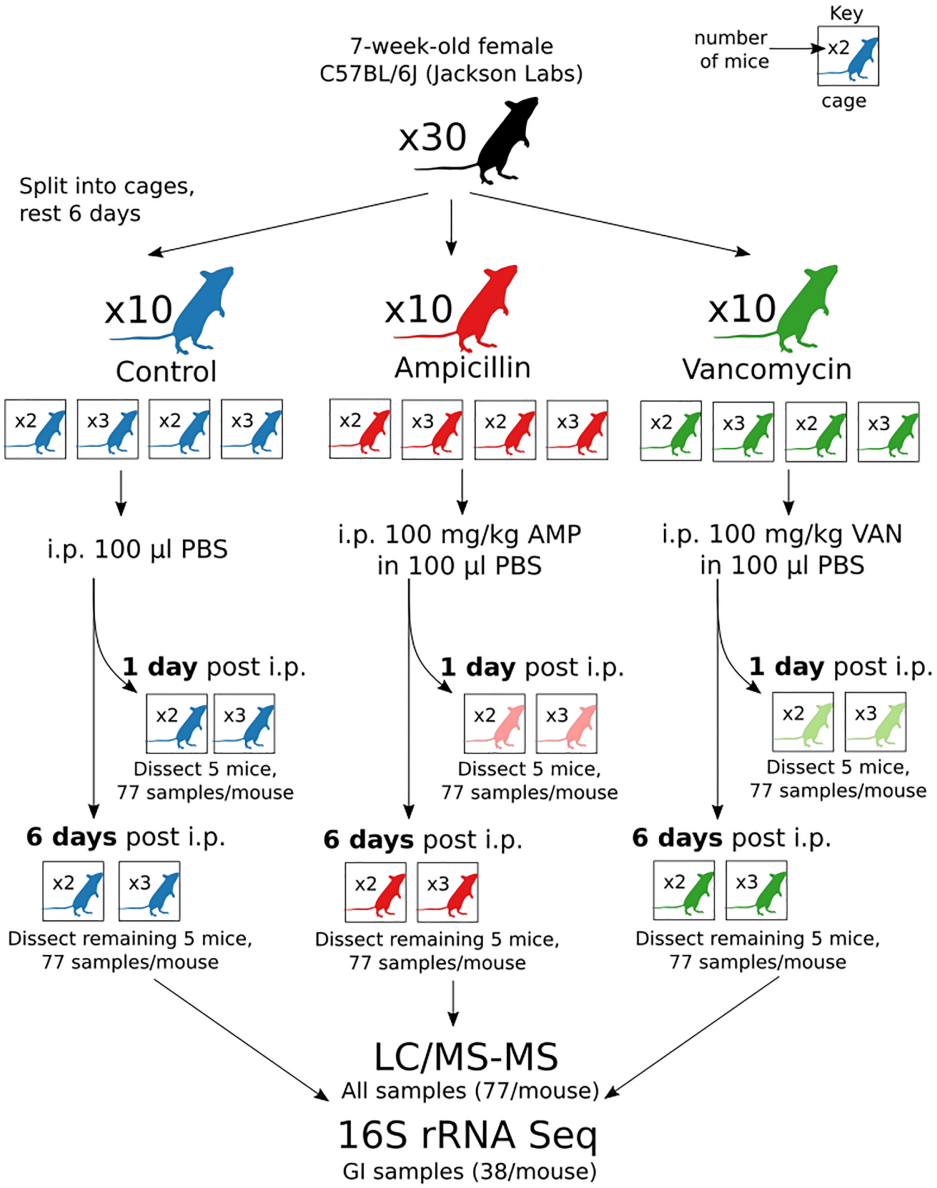

Figure S1: Overview of study design. Thirty-seven-week-old mice were ordered from Jackson Laboratories and randomly housed 2 or 3 mice per cage. After a 6-day rest period, mice were administered either 100 µl PBS, 100 mg/kg AMP in 100 µl PBS, or 100 mg/kg VAN in 100 µl PBS via intraperitoneal injection. Twenty-four hours later, 5 mice per group were dissected by following the protocol detailed in the methods. Six days postantibiotic administration, the remaining 5 mice per group were dissected by following the protocol detailed in the methods.

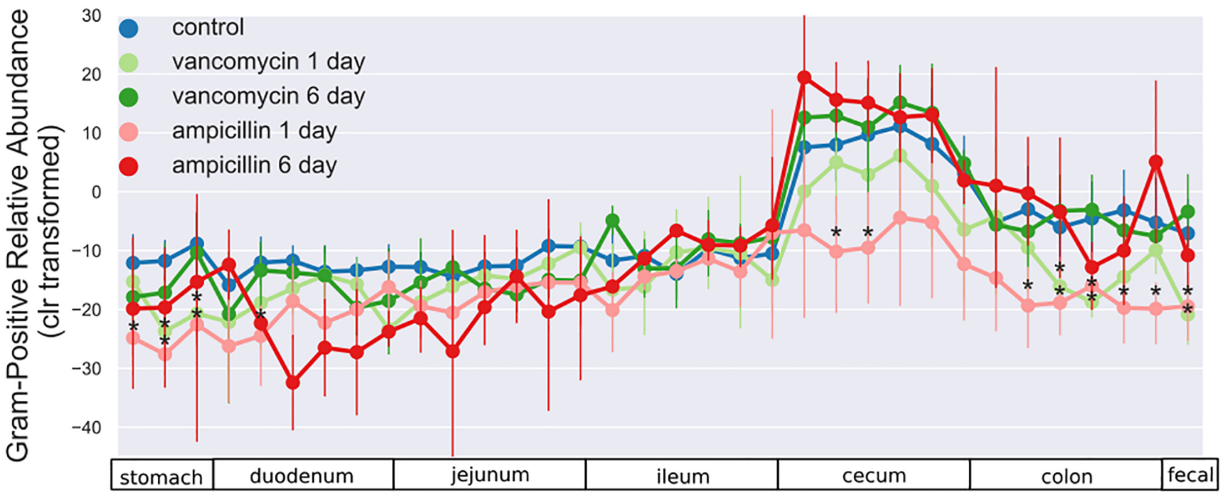

Figure S2: Antibiotics reduce levels of Gram-positive bacteria. Asterisks indicate a significant difference in the centered log ratio (clr)-transformed proportion of Gram-positive bacteria compared to the control (Mann-Whitney U test, $P < 0.05$ after Benjamini-Hochberg correction for multiple comparisons). Error bars represent the 95% confidence interval (CI).

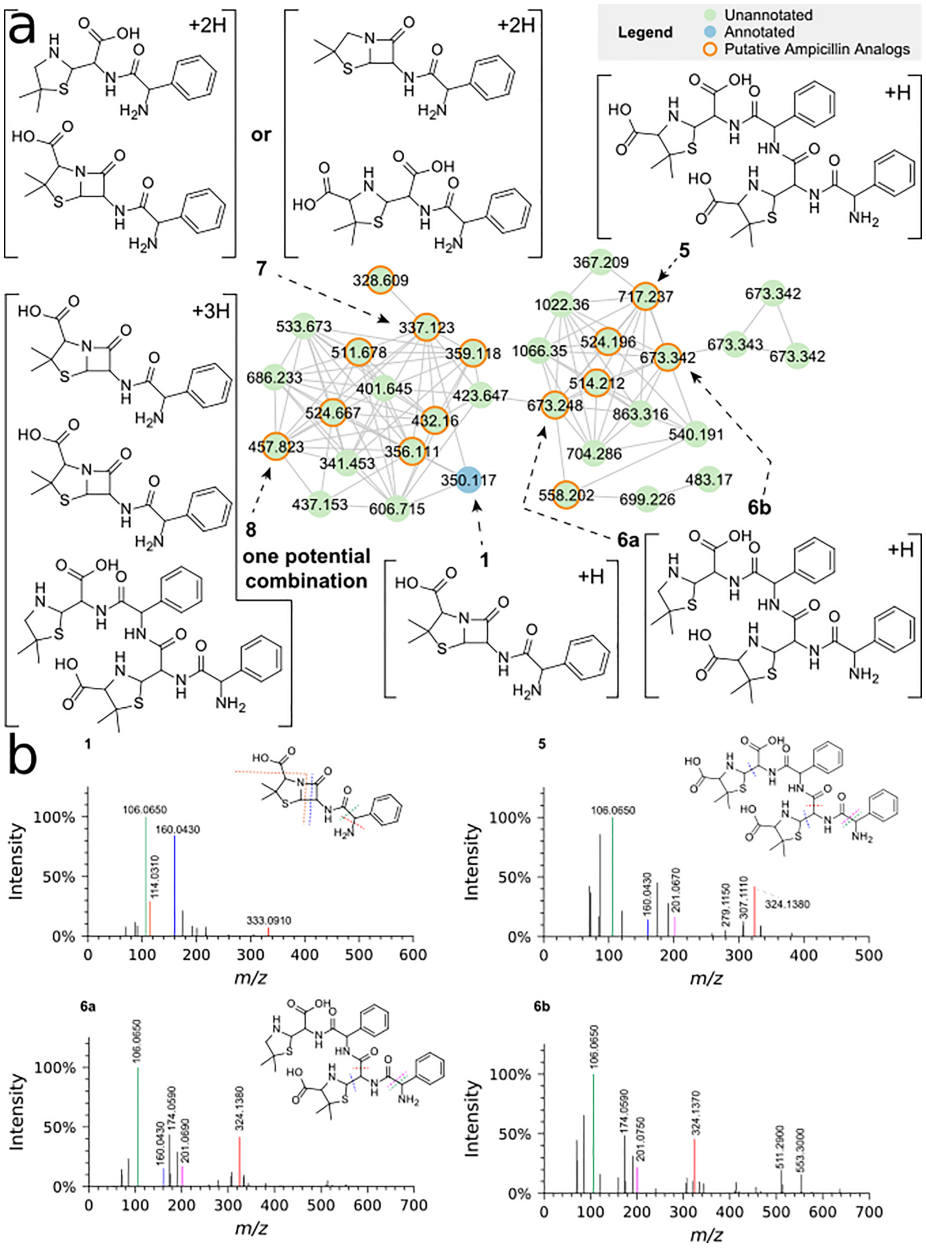

Figure S3: Molecular network of ampicillin and ampicillin network features. (a) GNPS molecular network displaying the molecular family in which ampicillin was annotated based on spectral library matching (blue) with unannotated analogs (green). Circles outlined in orange indicate a putative ampicillin analog. Neutral-charge chemical structures proposed are indicated. (b) MS/MS spectra for AMP. Putative fragments ions resulting from fragmentation of the proposed neutral-charge chemical structures are indicated using color.

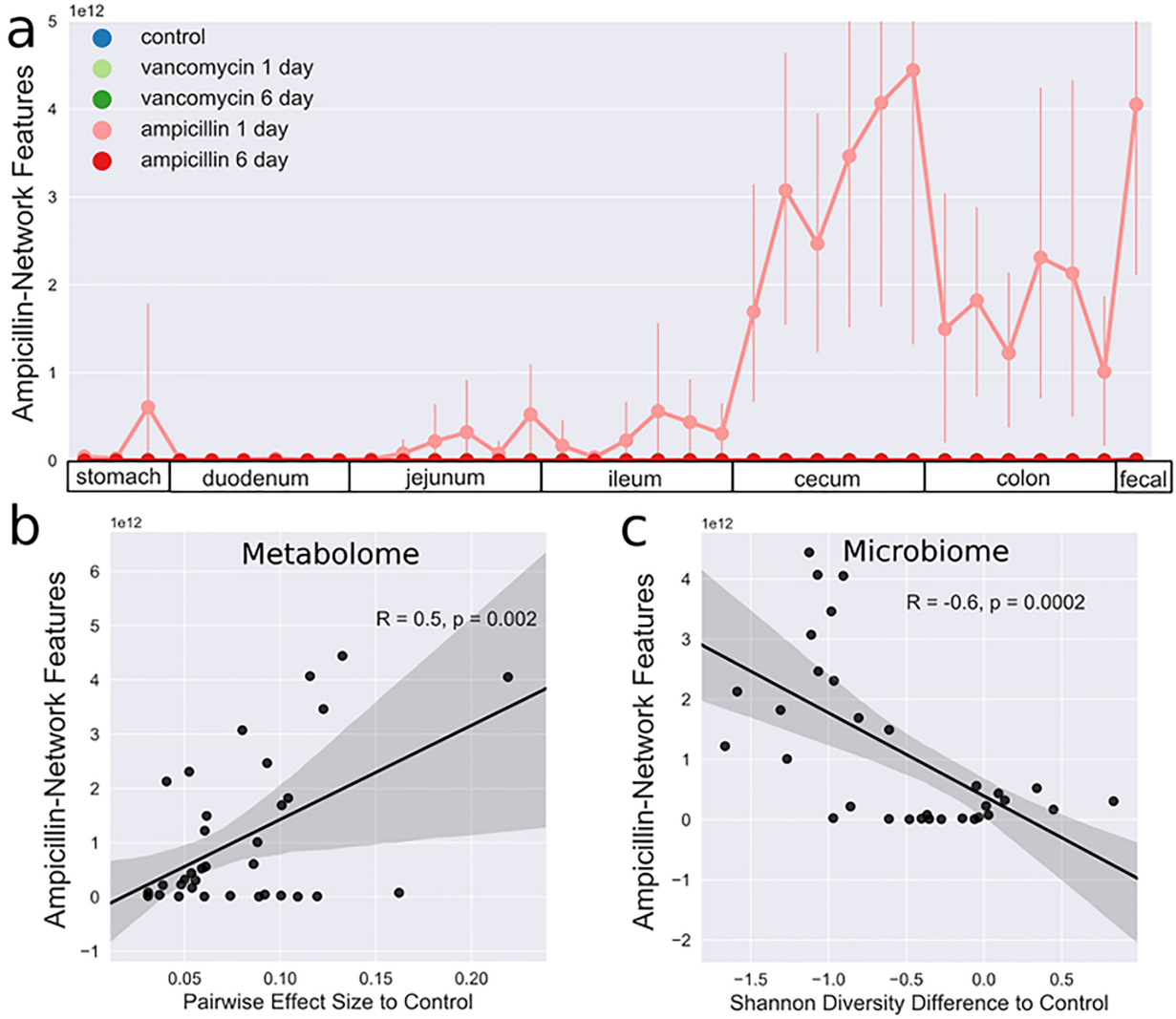

Figure S4: Ampicillin network features correlate with changes in the metabolome and microbiome. (a) The sum of the peak area of putative ampicillin network features moving down the GI tract; error bars represent the 95% CI. (b) Pearson correlation of ampicillin network features with metabolome pairwise effect size to control (Bray-Curtis) for gut samples (stomach to fecal, down the GI) only from the ampicillin 1-day group. (c) Pearson correlation of ampicillin network features with the change in Shannon diversity (alpha diversity) relative to control samples for gut samples (stomach to fecal, down the GI) only from the AMP.d1 group.

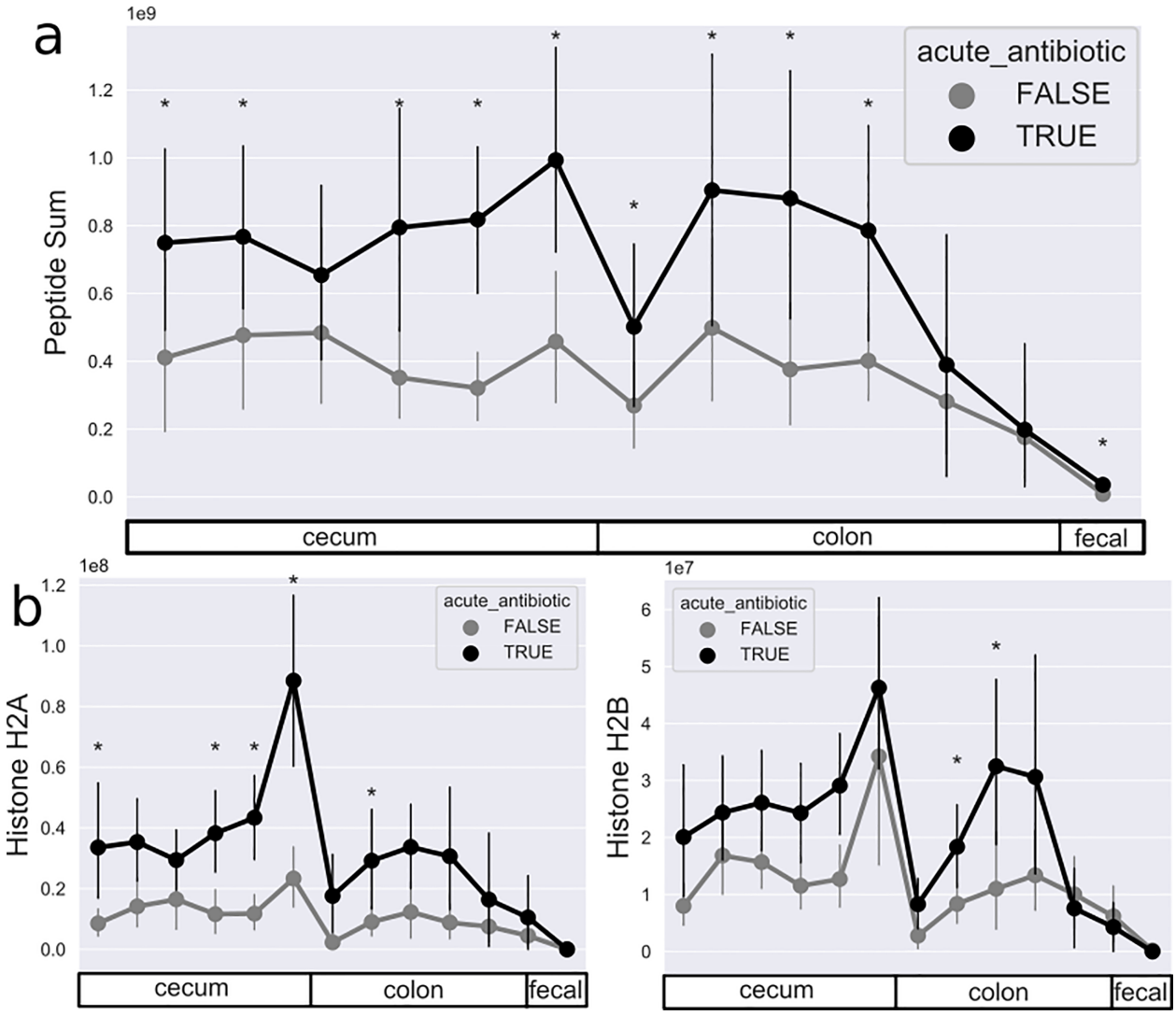

Figure S5: Small peptides are elevated in the lower GI tract day 1 postantibiotic exposure. (a) Sum of peptide spectral abundances along the lower GI tract comparing AMP.d1- and VAN.d1 (acute antibiotic)-treated mice to control mice and mice at day 6 posttreatment (nonacute). Significance indicates a *P* value of <0.05 by Mann-Whitney U test. (b) Abundances of peptides from histones, both H2A and H2B, were elevated in the lower GI tract of acute antibiotic-treated mice (AMP.d1 and VAN.d1) compared to other groups; significance testing was by dsFDR. Error bars represent the 95% CI.

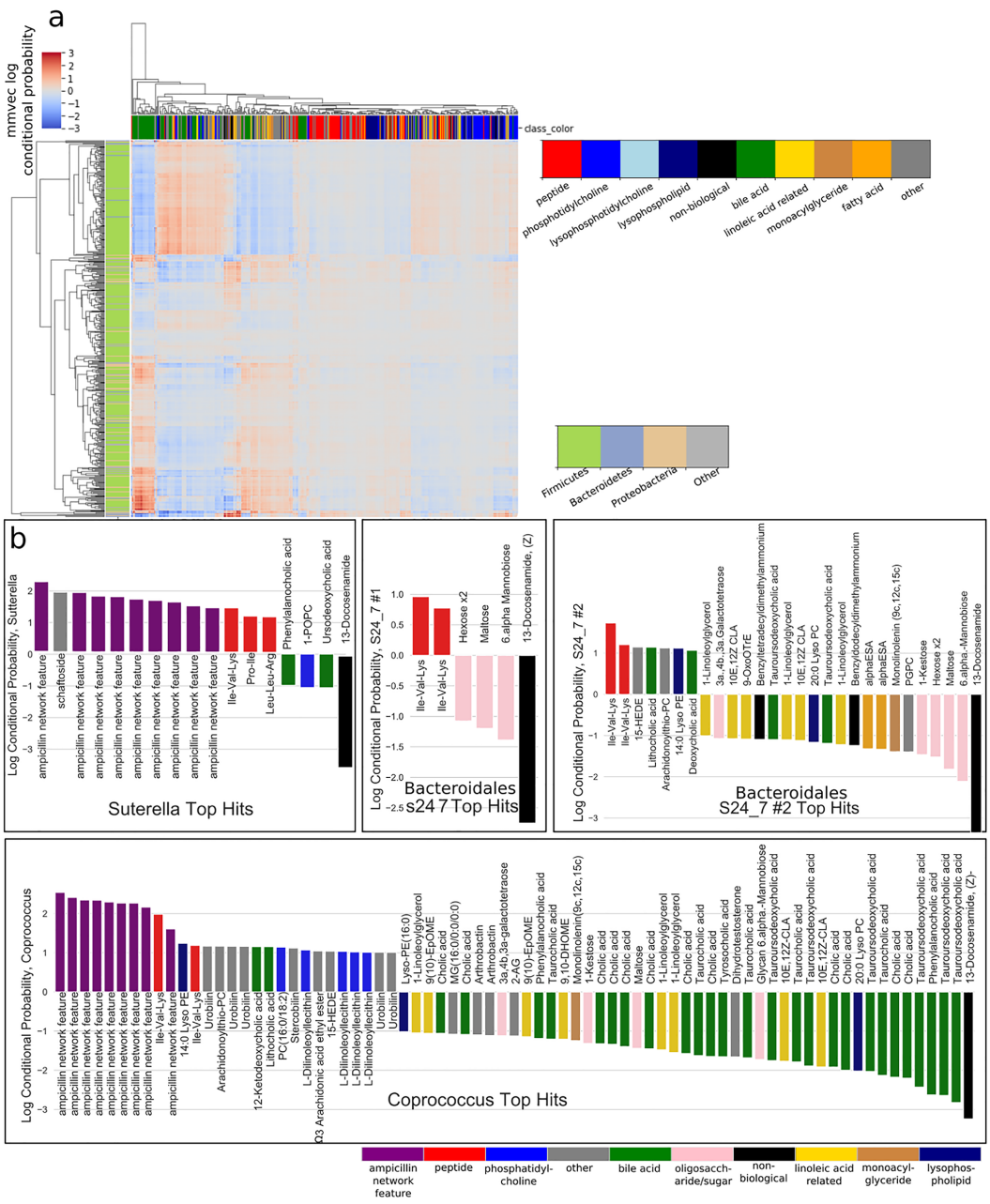

Figure S6. Cooccurrence probabilities for microbes and metabolites cluster by metabolite class. (a) Log conditional cooccurrence probabilities for all microbes and putatively annotated metabolites. The microbial phylum is indicated on the left by row, and general metabolite class is indicated on the top by column (nonbiological metabolites are those that were highly abundant in blanks). (b) A multinomial regression model was used to find sOTUs that were most associated with the AMP.d1 mice (*Coprococcus* and *Sutterella*) and VAN.d1 mice (both family S24-7) compared to the control. Annotated metabolites that had the highest and lowest cooccurrence probabilities with these sOTUs are displayed.