Host-microbiota interactions in the pathogenesis of antibiotic-associated diseases.

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Supplementary Information

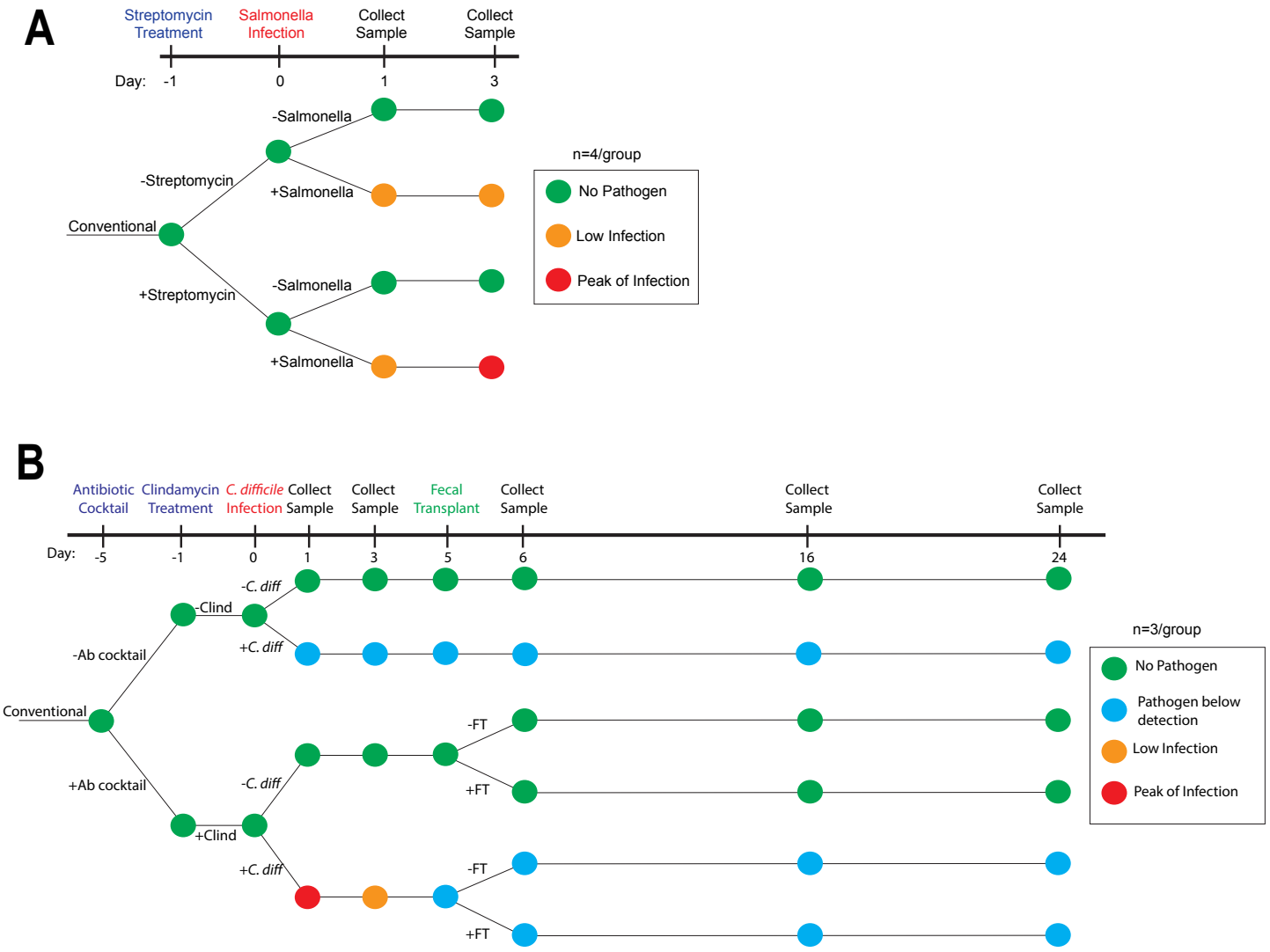

Figure S1. Antibiotic-associated Experimental Design,Related to Experimental Procedures. (a) In the Salmonella model, conventionally-raised Swiss Webster mice (n=4/group) were treated with 20 mg Streptomycin 24-hours prior to infection with 108 CFUs of Salmonella typhimurium. Mice were followed for 3 days and fecal pellets were collected prior to antibiotic treatment, prior to infection and 1 and 3 days post-infection. (b) In the C. difficile model, conventional mice were treated with an antibiotic cocktail in drinking water for 2 days, placed on regular drinking water for 2 days and finally given a single dose of 1mg clindamycin 24-hours prior to infection with 108 CFUs C. difficile. 5-days post-infection, mice that received antibiotics (with or without pathogen) received a fecal transplant from the conventional mice. All mice were followed for 24-days post-infection.

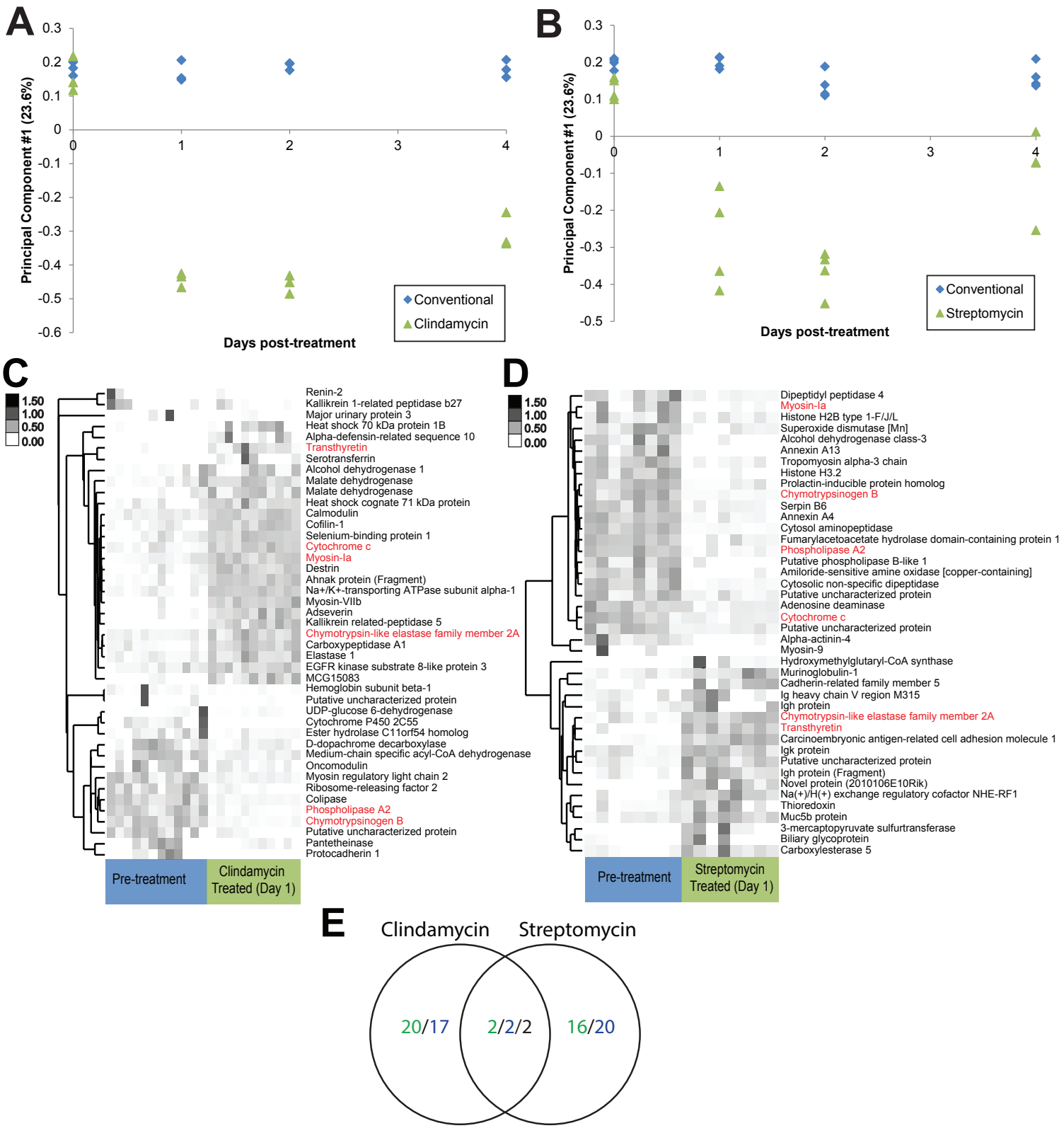

Figure S2. Antibiotic Treatment Causes Perturbations to Microbiota Composition and Host Proteome, Related to Figure 1. PCA analysis of the microbiota upon treatment with (a) Clindamycin and (b) Streptomycin, compared to untreated mice over the 4 days after antibiotic treatment. (c,d) Cluster analyses of proteins significantly changed in expression (FDR<0.05, >1 ln fold change) when comparing (c) clindamycin or (d) streptomycin treated mice 1-day after treatment with the same mice prior treatment. Each column represents a single mouse. Red protein names indicates their significant regulation in both treatment conditions. (e) Venn Diagram comparing the proteins identified in the above analysis. Green= up-regulated in antibiotic-treated condition, Blue= down-regulated in the antibiotic-treated condition, black= up-regulated in clindamycin treatment but down-regulated in streptomycin treatment.

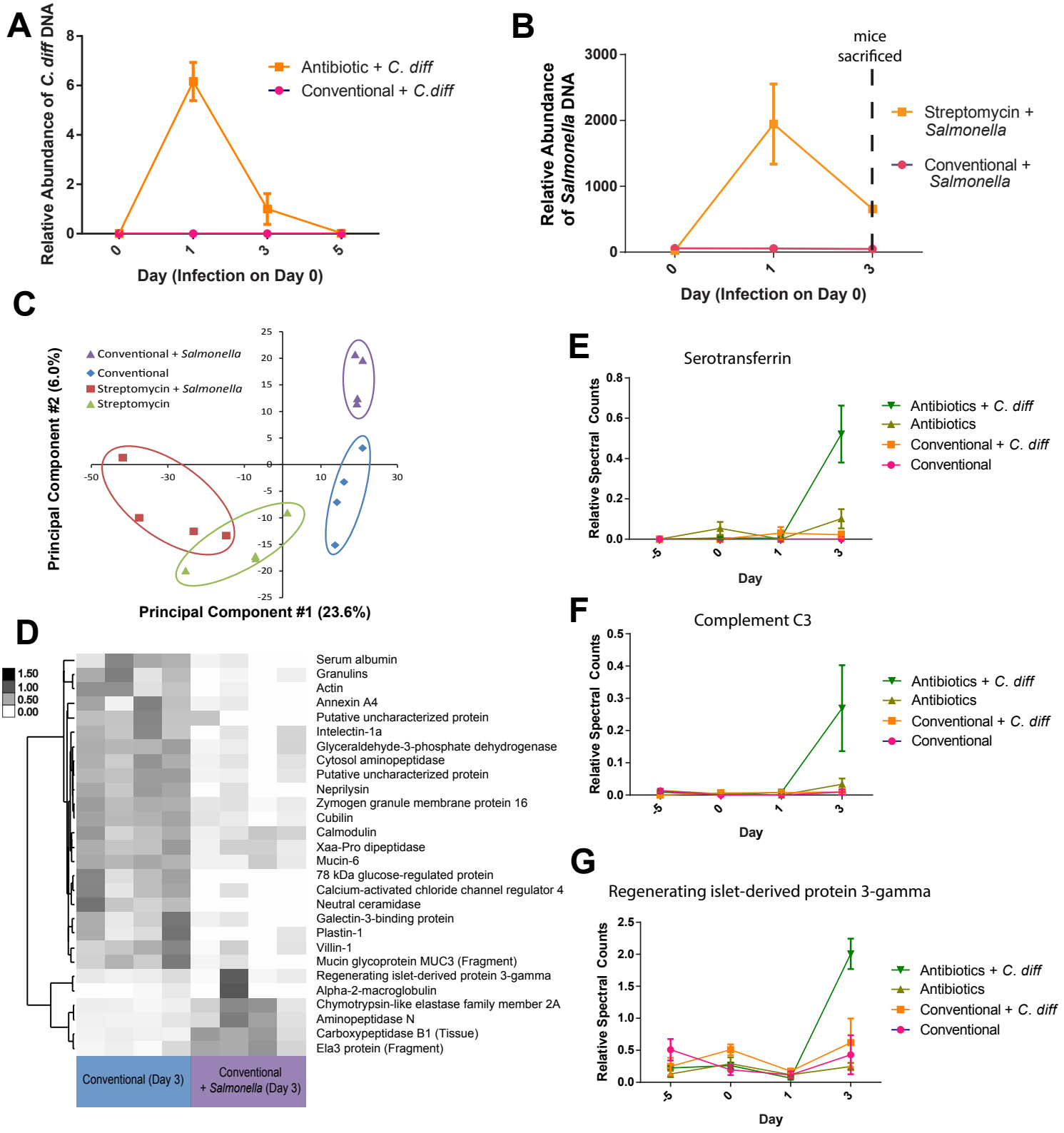

Figure S3. Host-Microbiota Kinetics During Pathogen Infection, Related to Figure 2. (a,b) Pathogen load was measured using qPCR assays for (a) C. difficile and (b) Salmonella in mice treated with antibiotics or vehicle controls (mean +/- SEM). (c) PCA of the microbial community composition was conducted on all mice in the Salmonella experiment, 3 days post-infection. (d) Cluster analysis of the proteins significantly (FDR<.05, ln fold change>1) regulated in conventional mice infected with Salmonella 3-days post-infection. (e-g) From mice in the C. difficile experiment, normalized spectral counts were plotted over time for the innate-immune proteins (e) Serotransferrin, (f) Complement C3, and (g) the anti-microbial protein regenerating islet-derived protein 3-gamma (REG3 γ) (mean +/- SEM).

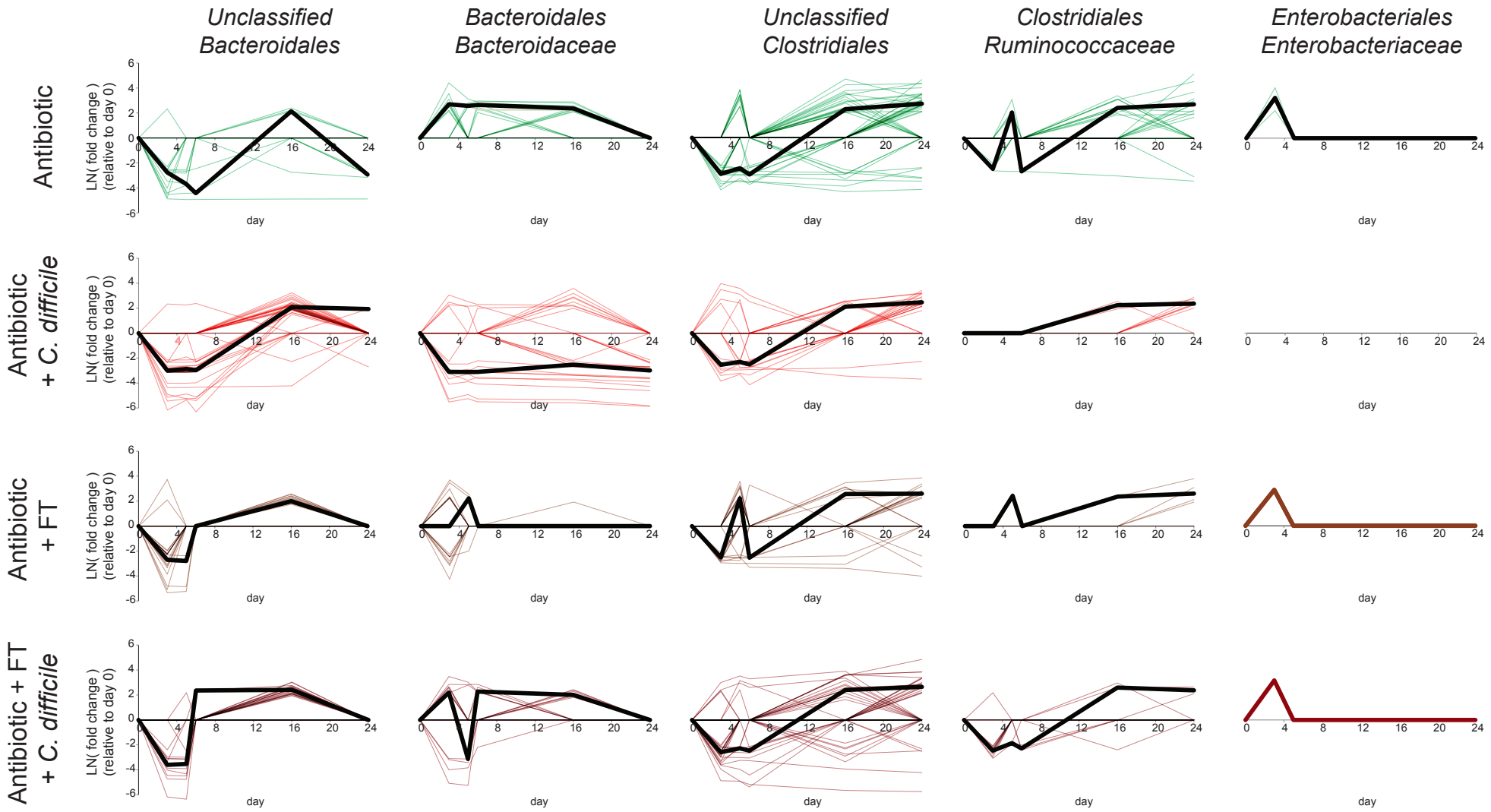

Figure S4. Recovery Dynamics of Individual OTUs Over 24 Day Fecal Transplant Comparison, Related to Figure 4. Five of the six taxa described in Figure 1C are represented, as distinct plots for each of the four conditions represented in Figure 4. As described in Figure 1C, each colored line represents significant fold changes (natural log, LN) of a single OTU with respect to the same mouse's OUT levels at day zero. Black lines line represents the median fold change of OTUs that significantly deviated from their day zero levels. No significantly changed OTUs were found for the taxon Lactobacilliales Enterococcaceae (not shown). Numerical fold change values for all taxa can be found in Table S5.

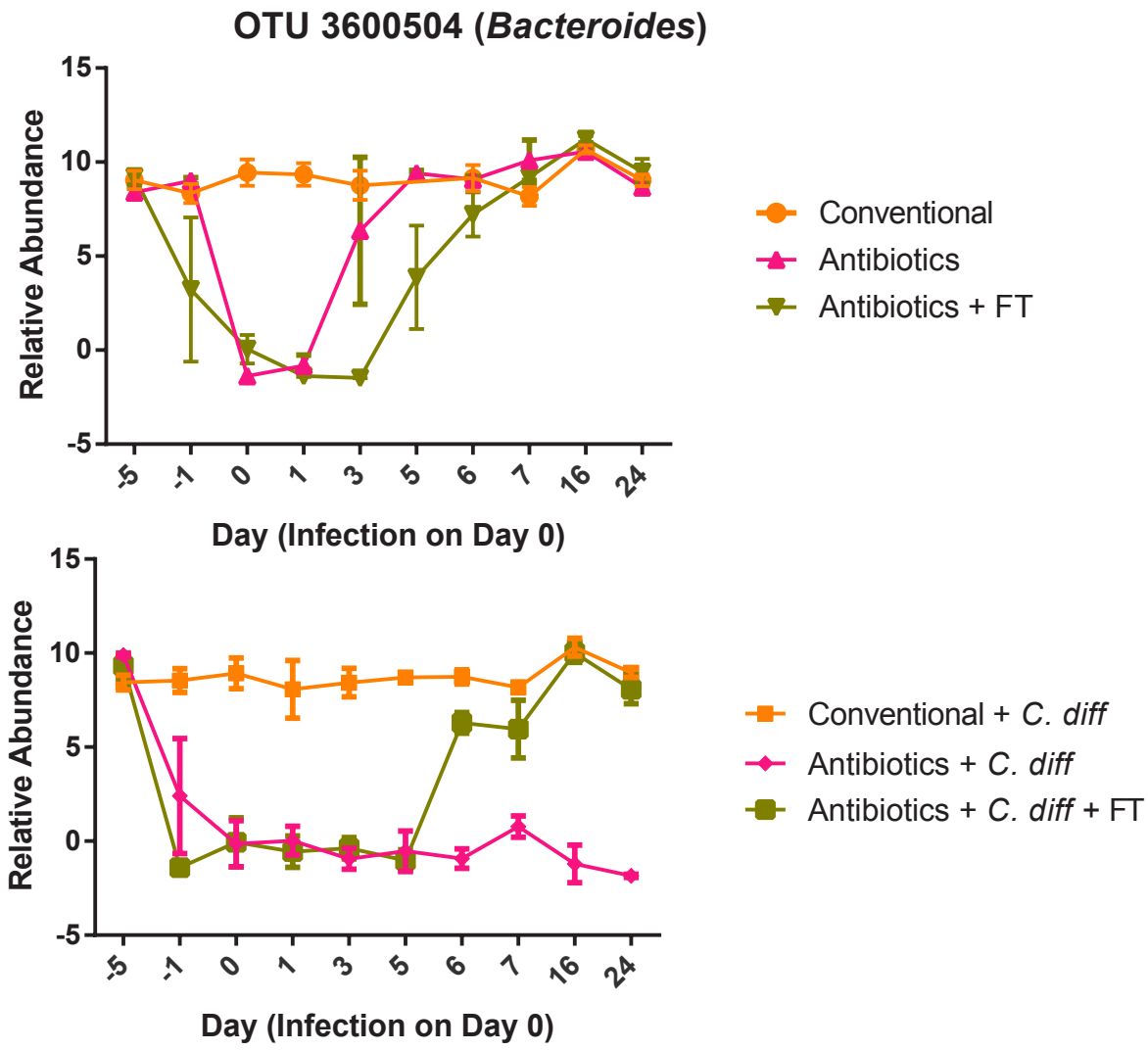

Figure S5. C. difficile Infection Prevents the Recovery of a Commensal Microbe, Related to Figure 4. Relative abundance of OTU 3600504, a member of the Bacteroides (mean +/- SEM).

Table S1. Antibiotic Treatment Perturbs the Gastrointestinal Microbiota, Related to Figure 1. Fold-change for all significantly-different OTUs 1 and 4 days post-antibiotic treatment as measured by DESeq ($p<0.05$, >1 ln fold-change).

Table S2. *Salmonella* Infection Perturbs the Microbiota in an Antibiotic-Dependent Manner, Related to Figure 2. Fold change for all significantly-different OTUs on the day of *Salmonella* infection in conventional and streptomycin treated mice, and for the following 4 days, as measured by DESeq ($p<0.05$, >1 ln fold-change).

Table S3. Host Proteins Regulated During Antibiotic-Associated *Salmonella* Infection, Related to Figure 2. The fold-change for all significantly-different host proteins when antibiotic-treated, *Salmonella* infected mice are compared to conventional controls as measured by QSpec ($p<0.05$, >1 ln fold-change).

Table S4. Host Proteins Regulated During DSS Colitis, Related to Figure 3. The fold-change for all significantly-different host proteins when DSS-treated mice are compared to the same mice prior to treatment, as measured by QSpec ($p<0.05$, >1 ln fold-change).

Table S5. Recovery of the Microbiota After Clindamycin, *C. difficile* Infection and Fecal Transplant, Related to Figure 4. The significantly-different OTUs from the 4 groups of mice that received clindamyicn treatment throughout the last 21 days of the experiment as measured by DESeq ($p<0.05$, >1 ln fold-change).

Table S6. Protein abundance (spectral counts) corresponding with all mice and time points, Related to Figures 1-4.

Table S7. Taxon abundance (normalized 16S rDNA sequence reads) corresponding with all mice and time points, Related to Figures 1, 2 and 4.