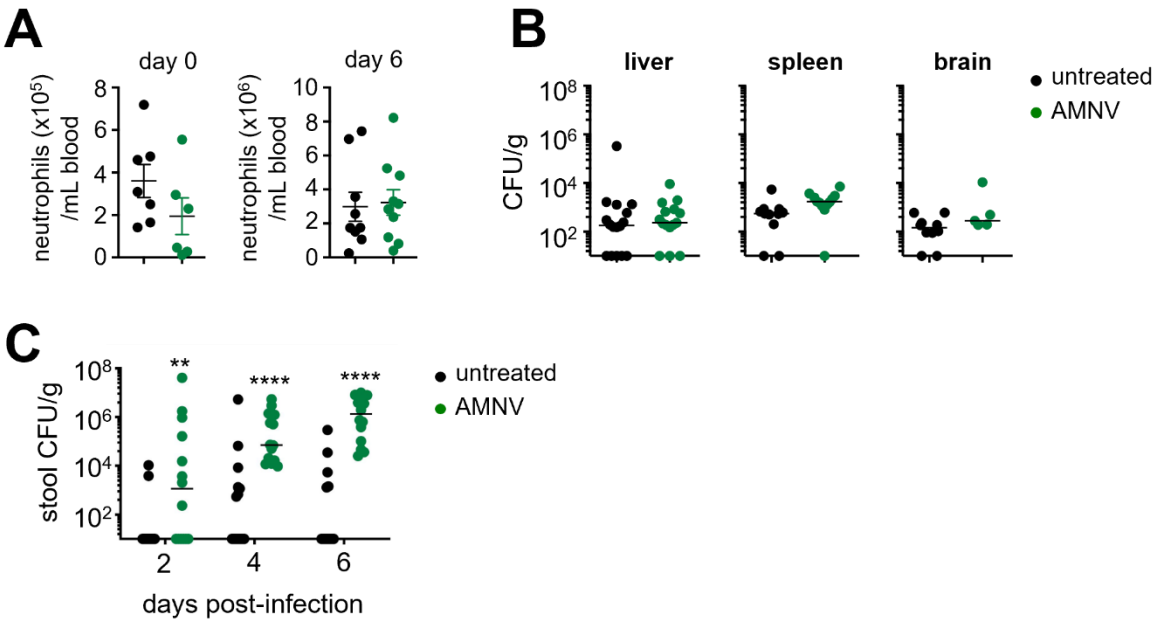

Figure S1: Effect of broad-spectrum antibiotic exposure on (A) neutrophil number in peripheral blood, (B) fungal burden in the indicated organs at day 7 post-infection and (C) stool fungal burdens. **Related to Figure 1**.

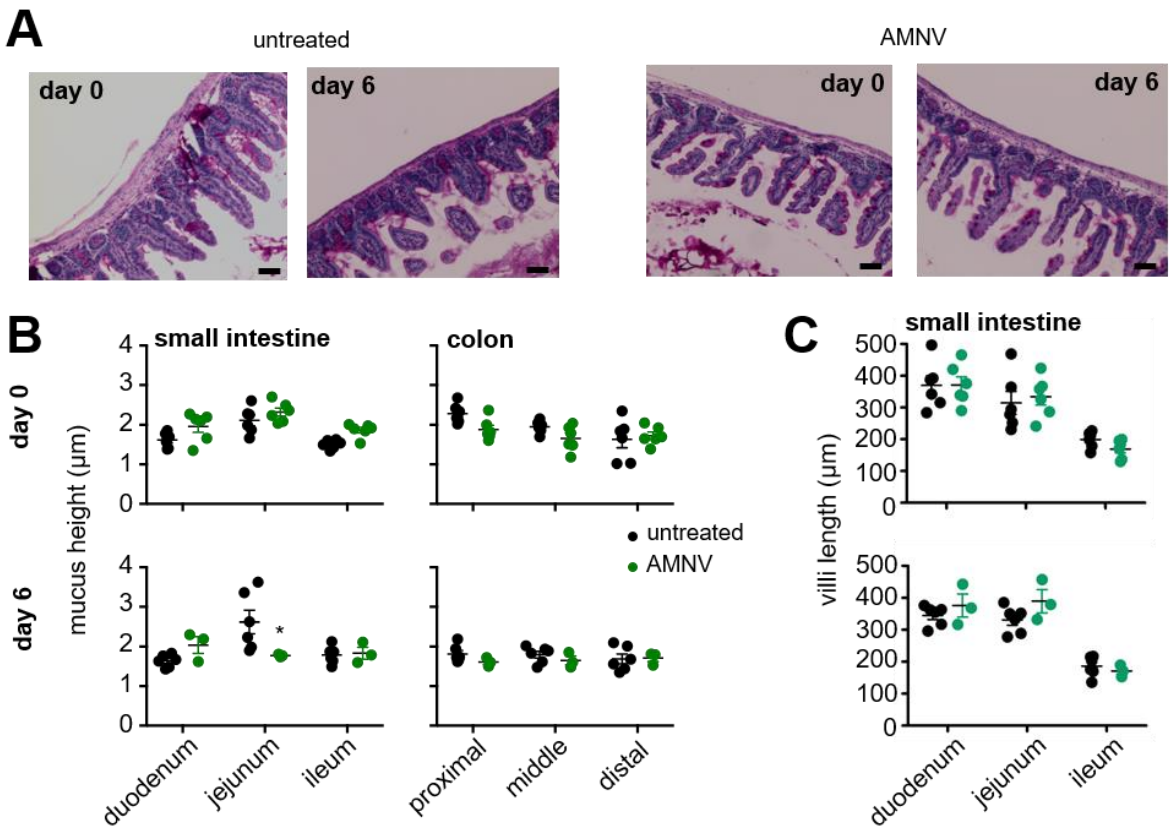

Figure S2: Escape of commensal gut bacteria during broad-spectrum antibiotic pre-exposure and subsequent invasive candidiasis is non-inflammatory. (A) Representative PAS-stained images of the ileum of untreated and AMNV pre-exposed mice, at either day 0 or day 6 relative to fungal infection. Scale bar is 50 µm. Images are representative of 6 individual animals from 2 independent experiments. (B) Quantification of mucus height and (C) villi length in the small intestine and/or colon at day or day 6 relative to fungal infection in untreated (n=6) and AMNV pre-exposed (n=6) mice. Each point represents an individual animal. Data pooled from 2 independent experiments. All measurements were done blindly to the group of mice. **Related to Figure 2.**

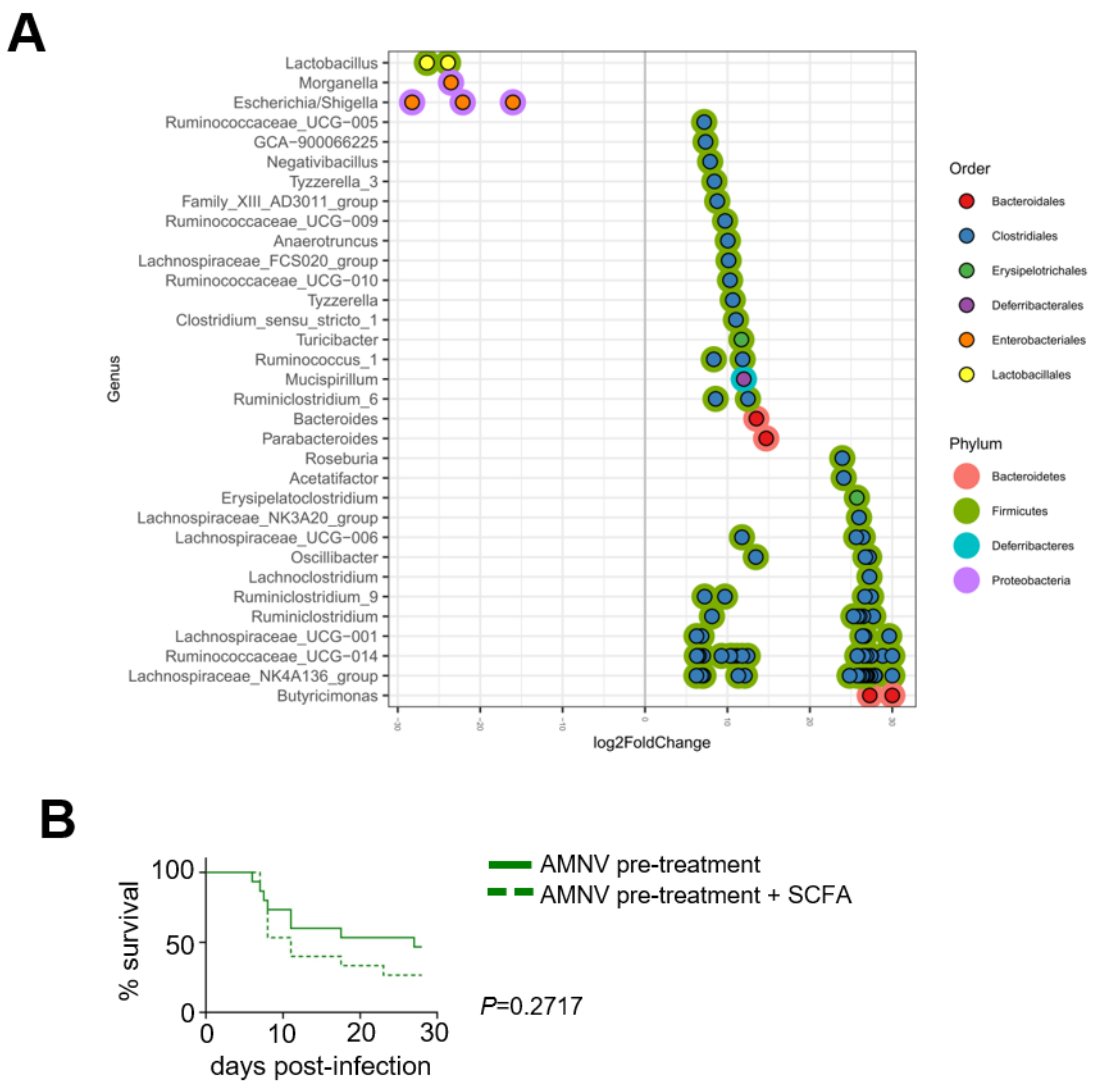

Figure S3: Broad-spectrum antibiotic pre-exposure depletes SCFA-producing bacteria but SCFA treatment does not provide a protective benefit. (A) DESeq2 differential abundance testing of ASVs between untreated and AMNV pre-exposed samples. Data is presented as the main log2 fold change between both tested groups with respect to all significant ASVs. A negative log-fold change implies a higher abundance in the AMNV treated group whereas a positive log2 fold change implies a higher abundance in the Control group. Significant differences were established at an FDR < 0.05. Legend represents the Phylum (outside circle) and Order (inner circle) to which each significant genera belongs. (B) Survival curve of AMNV pre-exposed mice with or without supplementation of SCFAs in the drinking water (treatment started 1 week prior to infection and continued throughout the infection period). Data is pooled from 2 independent experiments with n=15 mice per group, and analyzed by Log-rank Mantel-Cox test. **Related to Figure 2.**

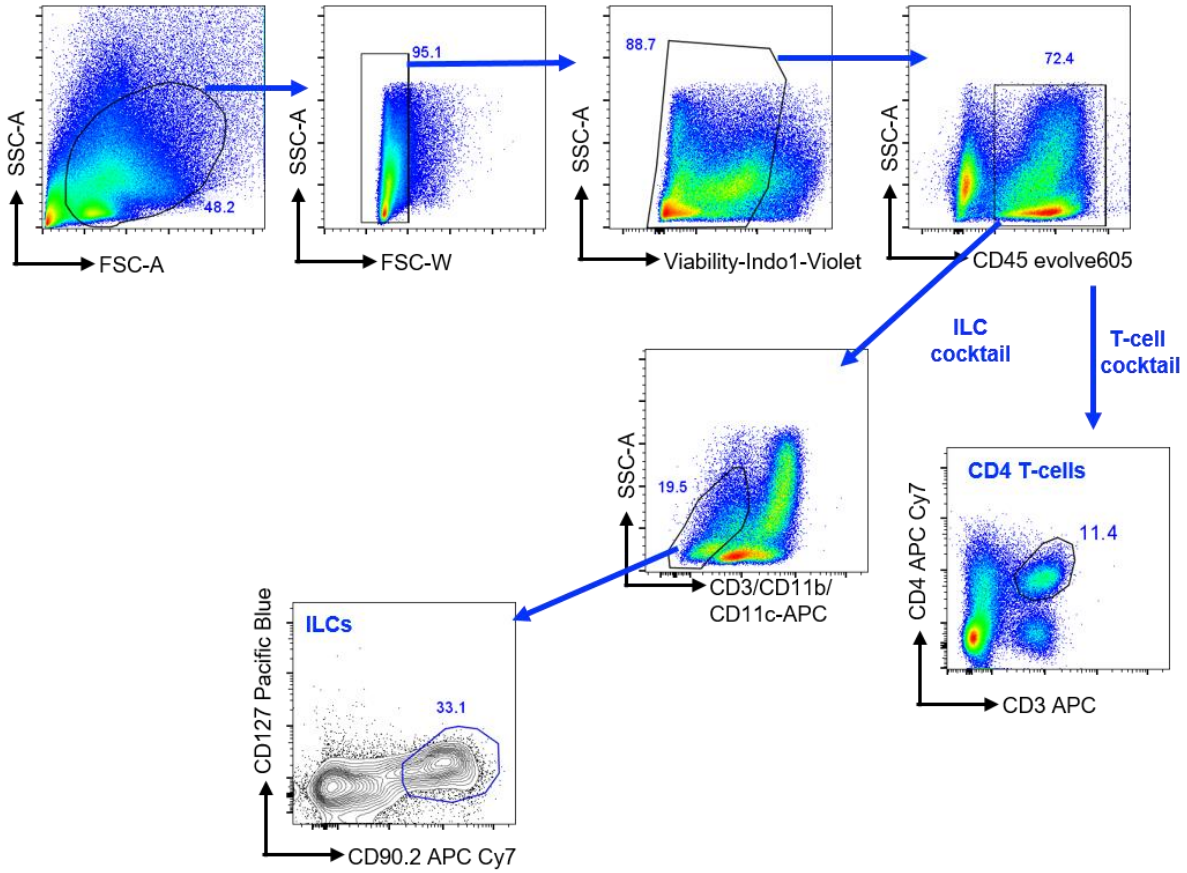

Figure S4: Lymphocyte gating strategy used in the FACS experiments within this study. All plots are shown using intestinal leukocytes as an example. The same gating strategy was employed on spleen samples where appropriate. **Related to Figure 3.**

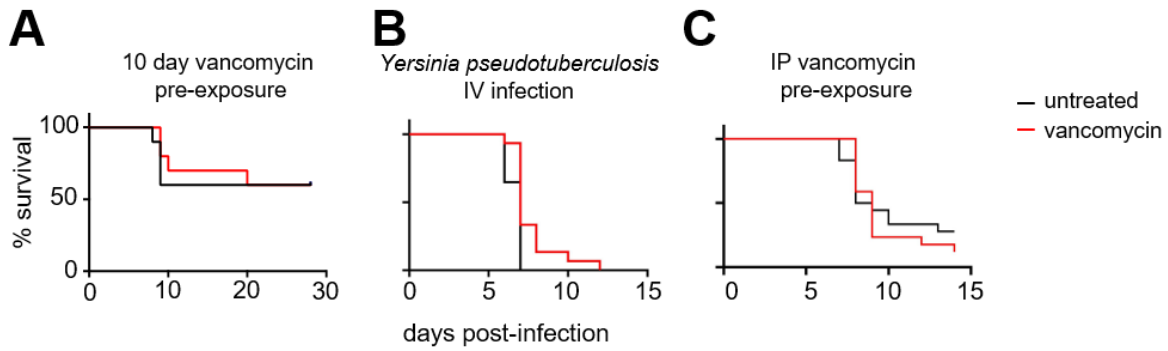

Figure S5: Long-term oral vancomycin pre-exposure selectively promotes susceptibility to invasive fungal infection. (A) Wild-type mice were pre-exposed to vancomycin in the drinking water for 10 days (or left untreated, n=10 per group) and then challenged with *C. albicans* intravenously, as before. Mortality was monitored for 28 days post-infection. Data shown is from 1 experiment. (B) Wild-type mice were pre-exposed to vancomycin (n=17) in the drinking water for 4 weeks (or left untreated, n=15) and then challenged with *Yersinia pseudotuberculosis* intravenously. Data is pooled from two independent experiments. (C) Wild-type mice were given intraperitoneal injections of vancomycin for 4 weeks (n=17) or PBS as control (n=18) prior to systemic challenge with *C. albicans*, as before. Data is pooled from two independent experiments. **Related to Figure 5.**

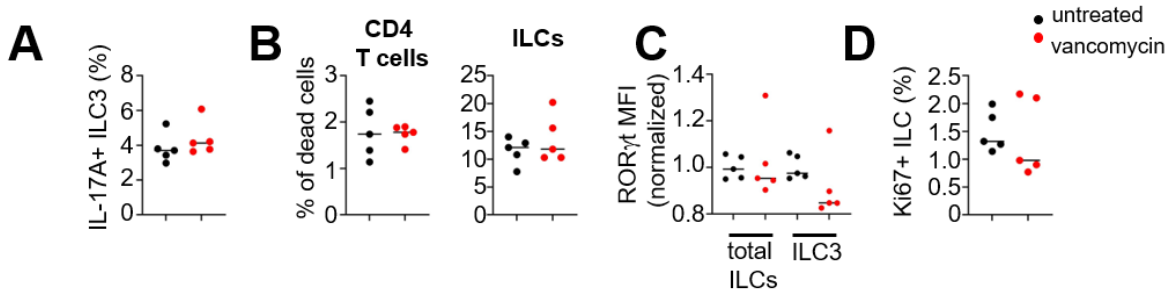

Figure S6: Vancomycin pre-exposure does not impair CD4 T cell survival or ILC responses. Frequency of (A) IL-17A$^+$ ILC3 (defined as CCR6$^+$KLRG1$^-$ within total ILCs, see Fig S6) and (B) dead CD4 T cells or ILCs within the CD45$^+$ population, in the small intestine lamina propria of untreated (black dots) and vancomycin pre-exposed mice (red dots). (C) Mean fluorescence intensity (MFI) of RORγt in total ILCs (left panel) or ILC3s (right panel), normalized to the mean of untreated control mice. (D) Frequency of Ki67+ ILCs in the small intestine lamina propria of untreated (black dots) and vancomycin pre-exposed mice (red dots).

Data in panels A-D are pooled from two independent experiments (n=5 mice per group; each point represents an individual animal) and analyzed using unpaired t-tests.

Related to Figure 6.

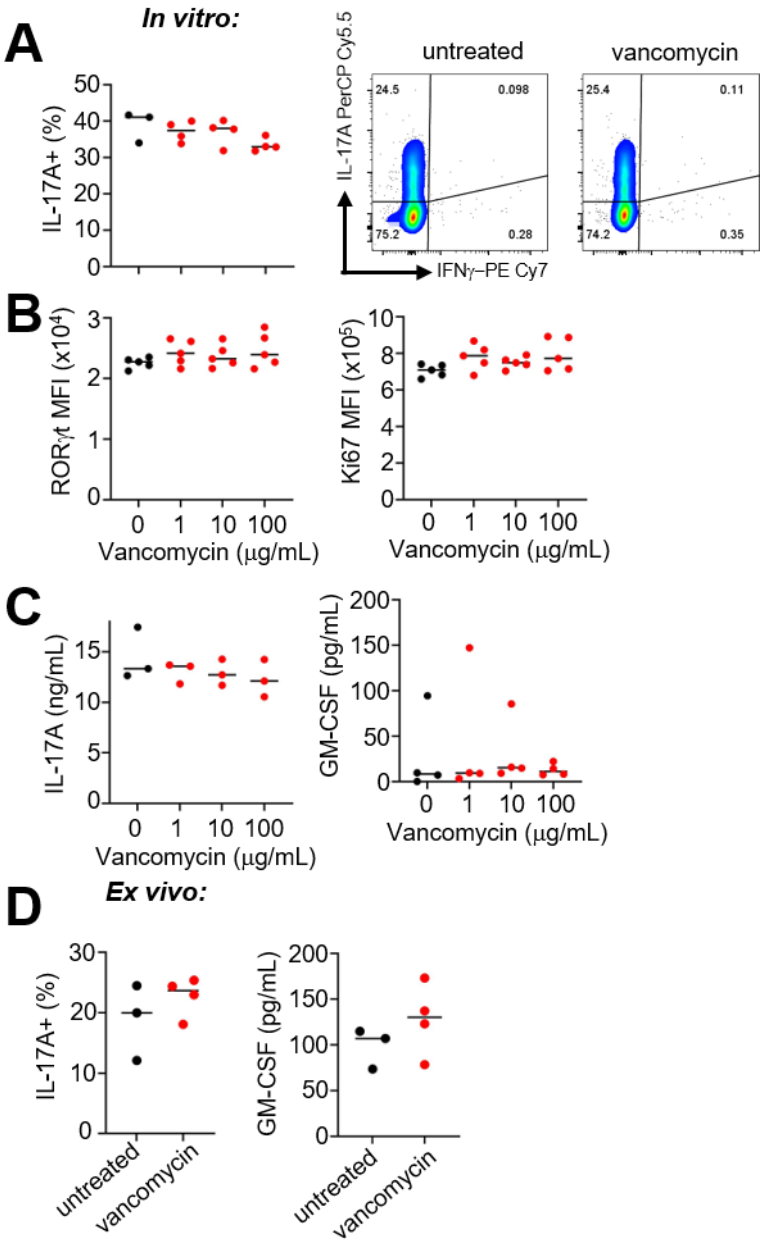

Figure S7: Vancomycin does not directly affect Th17 development. (A) Naïve CD4 T cells were cultured *in vitro* for 72 hours under Th17 inducing conditions in the presence or absence of the indicated concentrations of vancomycin. IL-17A (A), RORγt and Ki67 (B) was measured by FACS and (C) secretion of IL-17A or GM-CSF into the supernatant was measured by ELISA. FACS plots shown are representative of 2 independent experiments and are gated on live $CD4^+$ T cell singlets. (D) Naïve CD4 T cells were isolated from the spleens of wild-type mice that were untreated or pre-exposed to vancomycin for 4 weeks and cultured under Th17 inducing conditions as in (A). IL-17A production was measured by intracellular FACS and GM-CSF production was measured by ELISA. In all graphs, each dot represents a cells cultured from a single animal. **Related to Figure 6.**