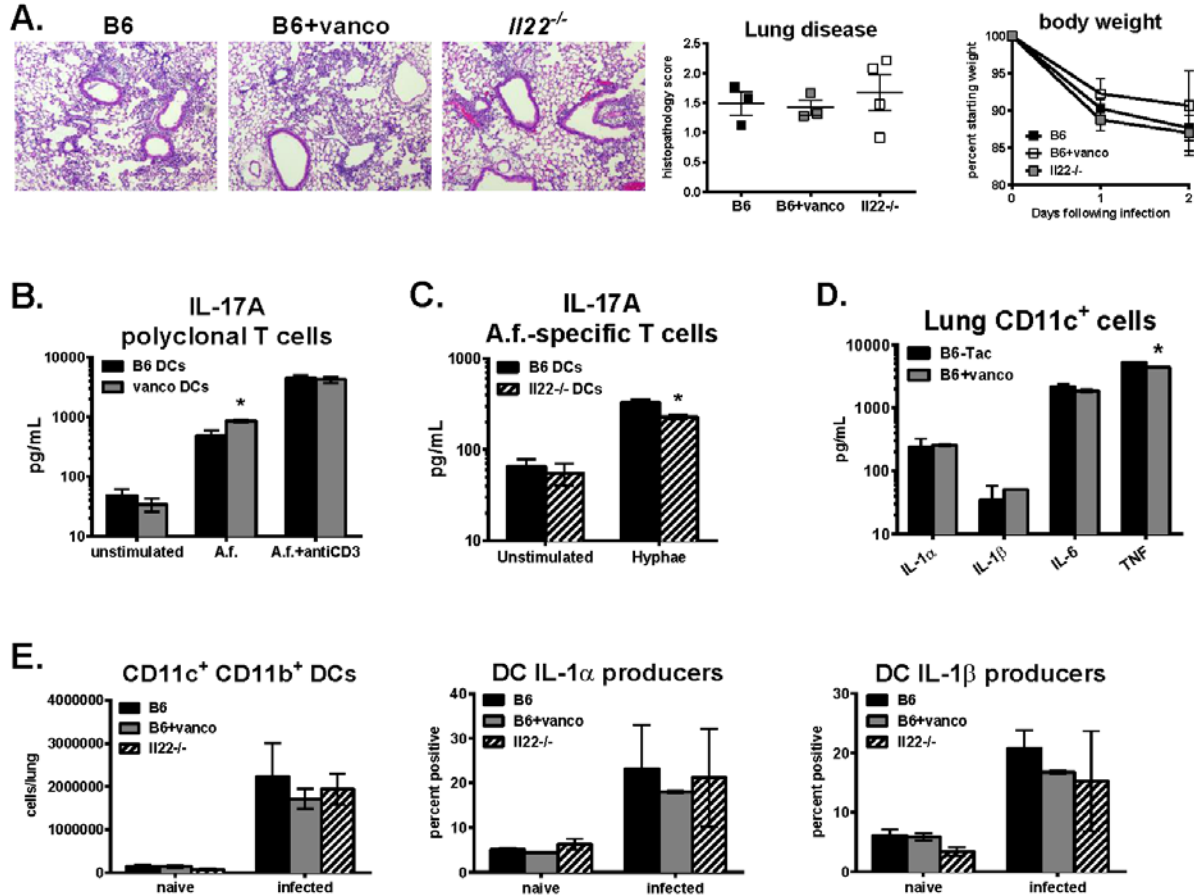


## Supplementary Figures

S1

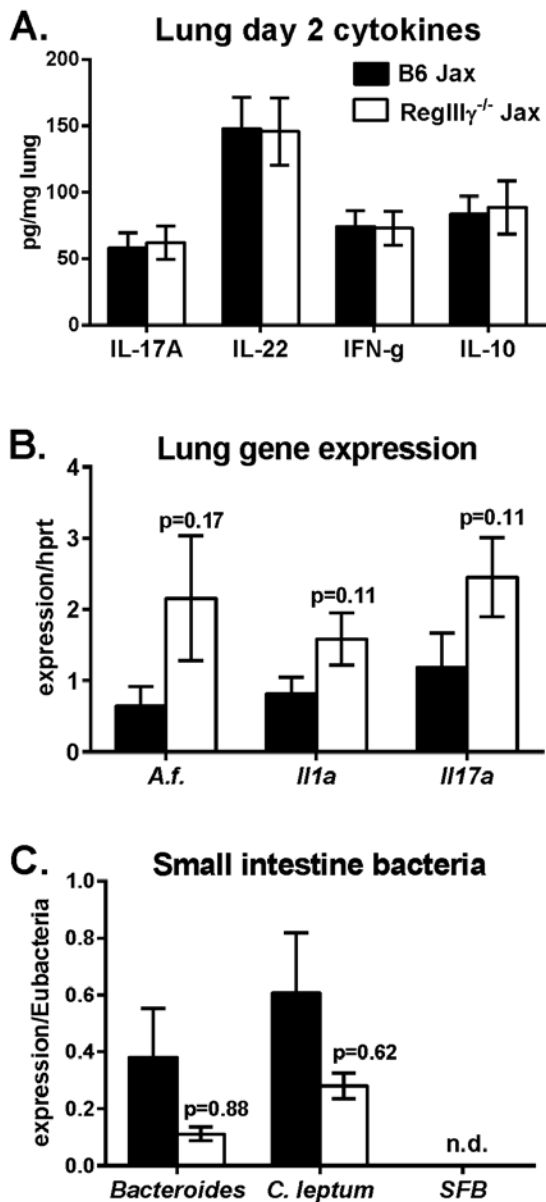


**S1. A. Disease severity following *A. fumigatus* infection.** B6 mice on normal water, vancomycin water, or *II22*<sup>-/-</sup> mice were neutrophil-depleted and infected with *A. fumigatus*. Two days later, lungs were formalin-fixed and stained with hematoxylin and eosin. Representative lung images, histopathology scores from individual mice, and body weight are shown. **B and C. Interleukin-17 production *in vitro*.** B. Splenic CD4<sup>+</sup> CD62L<sup>+</sup> cells were incubated with lung CD11c<sup>+</sup> cells isolated from B6-Tac mice maintained on normal water versus vancomycin water. Cells were cultured in the presence of media (unstimulated), heat-killed *A. fumigatus*, or anti-CD3 plus *A. fumigatus*. Interleukin-17 levels in supernatants were measured on day 3. C. Splenic CD4<sup>+</sup> CD62L<sup>+</sup> cells from *A. fumigatus*-specific TCR transgenic Af3.16 mice were incubated with lung CD11c<sup>+</sup> cells from B6 or *II22*<sup>-/-</sup> mice, as indicated. Cells were cultured for three days with media (unstimulated) or hyphae, and IL-17 was measured in supernatants. Assay was performed in triplicate. **D. *In vitro* DC cytokine production**

**in response to *A. fumigatus*.** CD11c<sup>+</sup> cells were purified from lungs of naïve B6-Taconic mice on normal water versus vancomycin. Cells were incubated overnight with heat-killed swollen *A. fumigatus*, and cytokines were measured in supernatants, as indicated. Wells were treated in duplicate (lungs). **E. Lung DC accumulation and ex vivo IL-1 $\alpha$ / $\beta$  production following fungal infection.** Mice (n=2) were infected with *A. fumigatus* or remained uninfected (naïve). Two days later, lung single cell suspensions were incubated with Golgiplug for 5h and stained for CD11c, CD11b, MHC class II and IL-1 $\alpha$  or IL-1 $\beta$ . Data show the total number of CD11c<sup>+</sup> CD11b<sup>+</sup> DCs in naïve and infected mice (left), percent of DCs staining positive for IL-1 $\alpha$  (middle) and percent of DCs staining positive for IL-1 $\beta$  (right).

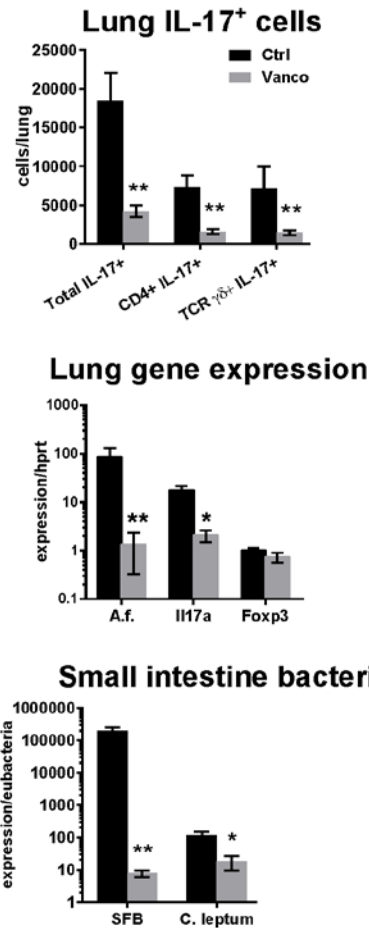
## S2

## SFB-negative mice

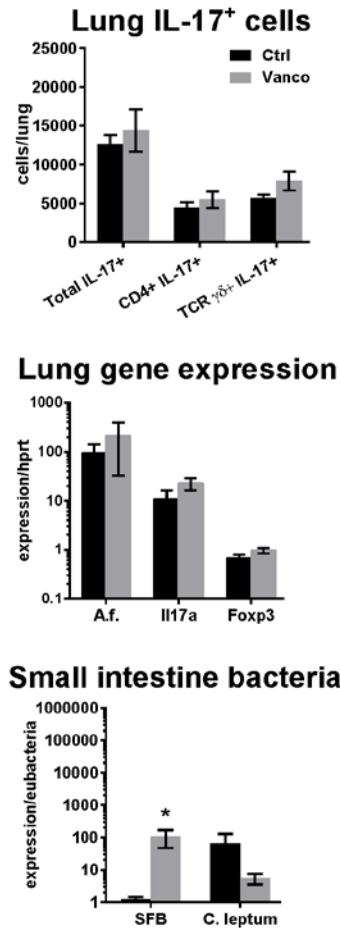


**S2. Pulmonary immune response in SFB-deficient *RegIII $\gamma$ <sup>-/-</sup>* strain.** *RegIII $\gamma$ <sup>-/-</sup>* mice and B6 controls purchased from Jackson Labs were neutrophil depleted and infected with *A. fumigatus*. Two days later, lung cytokines were measured by ELISA (top), genes measured by PCR (middle) and intestinal microbiota measured by PCR (bottom). Data are shown as Mean  $\pm$  SEM and combined from two experiments with n=8-10.

### S3 A. Taconic mice



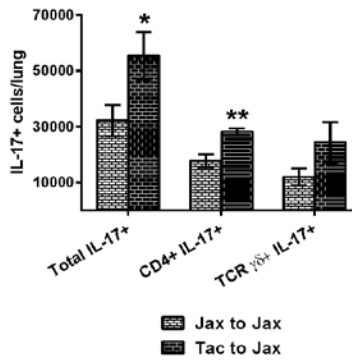
### B. Jackson mice



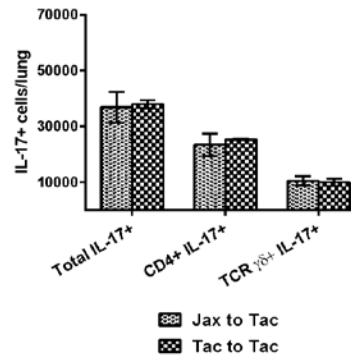
**S3. Vancomycin abrogates pulmonary Th17 immunity in SFB-colonized mice.** A. An SFB-negative cohort of B6 Taconic mice was gavaged with fecal suspensions from SFB-high Taconic donors. Mice were then placed on vancomycin water for two weeks, or maintained on normal water, neutrophil-depleted and infected with *A. fumigatus* as described in Materials and Methods. Two days following infection, lung single cell suspensions were restimulated with PMA plus ionomycin, and stained for intracellular IL-17A. Shown are the total numbers of IL-17<sup>+</sup>, CD4<sup>+</sup> IL-17<sup>+</sup> and TCR  $\gamma\delta$ <sup>+</sup> IL-17<sup>+</sup> cells, as indicated (top). cDNA from lung (middle) or distal small intestine (bottom) was used as a template for real-time PCR to amplify *A. fumigatus* 18S rDNA, *Il17a*, *Foxp3* or bacteria 16S genes, as indicated. Gene expression is normalized to *Hprt* (lung; middle) or *Eubacteria* (small intestine; bottom). B. Data for B6 Jackson mice, shown as Mean +/- SEM (n=4-5).

## S4

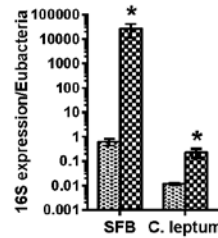
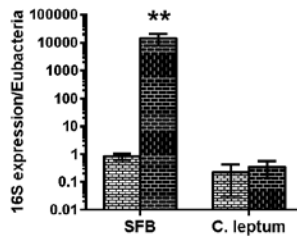
### A. Jackson recipients



### B. Taconic recipients



Lung IL-17<sup>+</sup> cells



Microbiota

**S4. Transferring SFB-enriched microbiota to the intestine augments pulmonary Th17 immunity.** B6 Jackson (A; Jax) or Taconic (B; Tac) mice were placed on vancomycin water for three weeks, then placed on normal water and gavaged with fecal suspensions from Jax or Tac donors, and rested for an additional three weeks. Mice were neutrophil-depleted and infected, as described in Materials and Methods, with tissues analyzed two days following infection. Data show the total numbers of lung T cell subsets producing IL-17 (top) and microbiota colonization in the small intestine (bottom), represented as Mean +/- SEM (n=4-5).