Figure S1

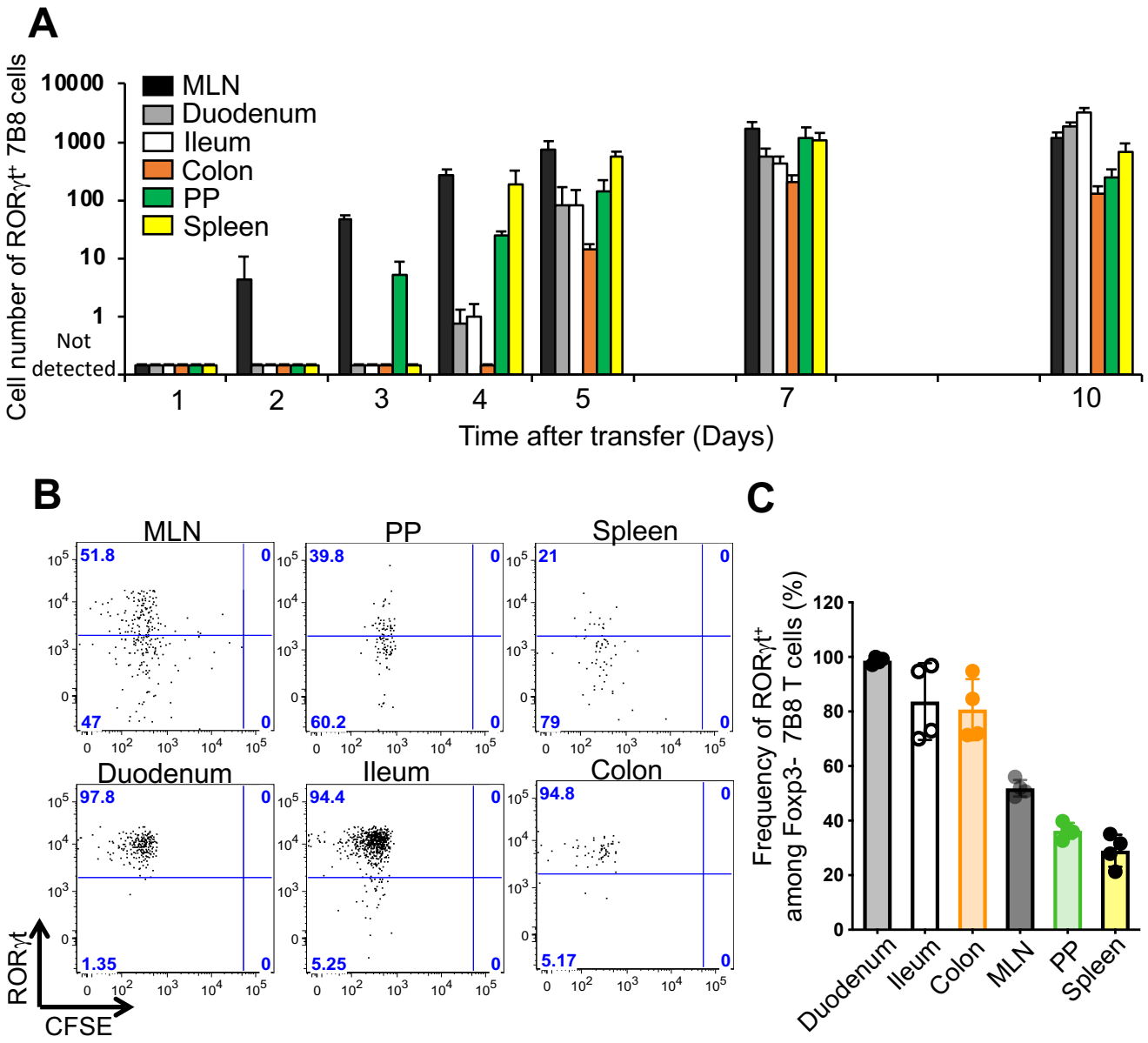

Figure S1. Kinetics of SFB-specific Th17 cell differentiation in tissues of SFB-colonized mice (related to Figure 1)

(A) Kinetics of SFB-specific Th17 cell differentiation following transfer of 5,000 naïve 7B8 T cells (*Cd45.1/ Cd45.2)* into SFB-colonized C57BL/6 (*Cd45.2/ Cd45.2)* mice Th17 cell numbers in the MLN, duodenum, ileum, colon, PPs, and spleen are shown . SFB-specific Th17 cells were defined as Foxp3$^-$ RORγt$^+$ 7B8 T cells. All data were generated from one time course experiment with 3 or 4 mice at each time point, but multiple time points were repeated independently with similar results. Data are represented as mean +/- SD. **(B)** CFSE dilution and RORγt expression among the transferred 7B8 T cells in MLN, duodenum, ileum, colon, PPs, and spleens of SFB-gavaged mice at 10 days following transfer, representative of data in Fig. S1A. **(C)** Frequency of RORγt$^+$ cells among Foxp3$^-$ 7B8 T cells in the described location.

Figure S2

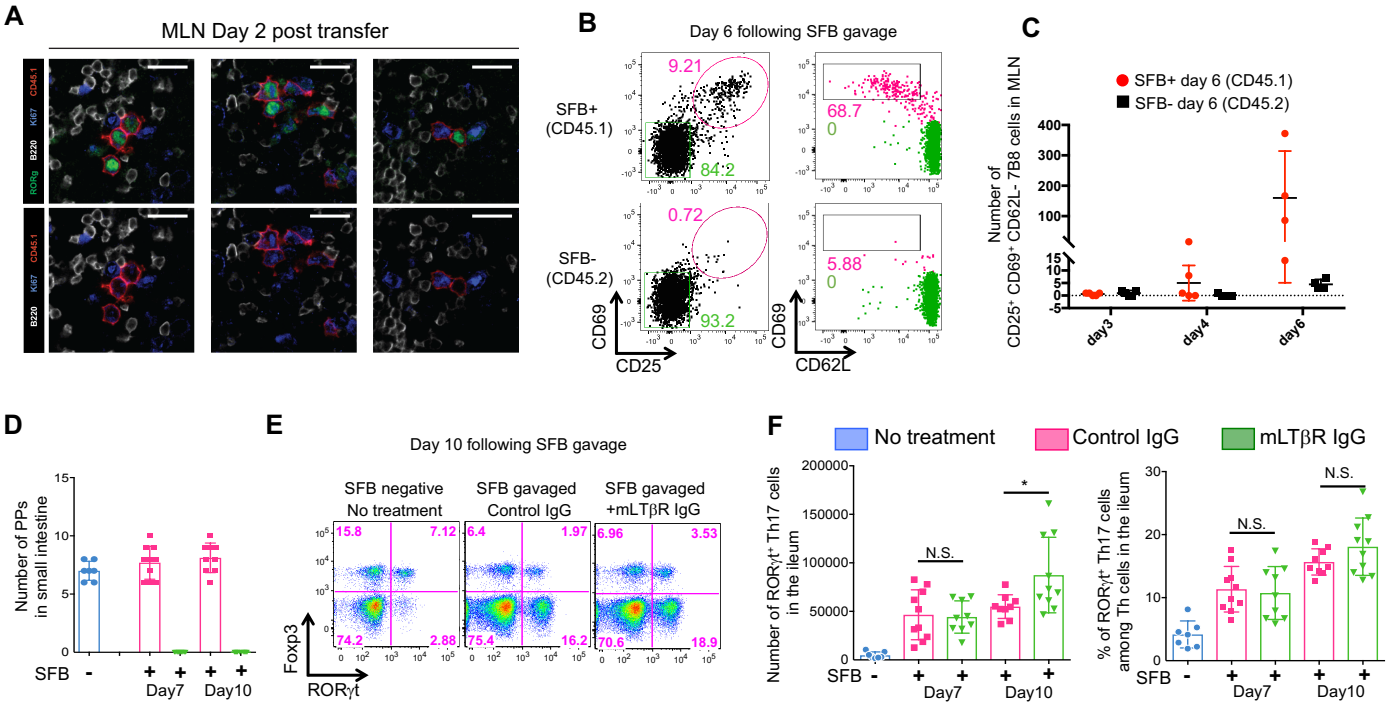

Figure S2. Role of MLN and PPs in Th17 cell induction following SFB gavage (related to Figure 2)
(A) Proliferation of SFB-specific T cells in cell clusters within the MLN T cell zone and follicular border at 2 days following transfer of 50,000 naïve 7B8 T cells. Sections were stained with anti-RORγt$^+$, anti-CD45.1, anti-B220, and anti-Ki67, and were analyzed by confocal microscopy. Representative of 5 mice from 2 experiments. The scale bars in the pictures correspond to 20 μm. **(B and C)** Ex vivo priming of naïve SFB-specific T cells by purified APCs (CD11b$^+$ and CD11c$^+$ cells). APCs from MLN were purified at several time points (Day3: n=5, Day4: n=5, Day6: n=4) after SFB or saline gavage. All the sorted APCs from MLNs of each mouse (about 3,000-5,000 APCs) were co-cultured with 10,000 sorted naïve T cells from SFB-specific TCR Tg mice for 24 h. Activation was assessed by expression of CD25 and CD69 and loss of CD62L on cells gated for TCRvβ14 and CD4 expression. Gated cells by the green rectangles as CD25 and CD69 double negative T cells and magenta circles as CD25 and CD69 double positive T cells in the left panel were further analyzed by CD62L gate in the right panel (B). (C) Kinetics of antigen presentation activity by APCs from MLNs following SFB gavage. Activated SFB-specific T cells were defined as TCRvβ14$^+$, CD4$^+$, CD25$^+$, CD69$^+$, and CD62L$^-$. **(D-F)** Th17 cell differentiation in PP-depleted mice gavaged with SFB. 150 μg LTβR-Ig was intravenously injected in SFB-free pregnant B6 mice on gestational days 16.5 and 18.5 (Rennert et al., 1996). Number of PPs in the small intestine after LTβR-IgG or control IgG administration during gestation is shown in (D). The frequency and total cell number of RORγt$^+$ Foxp3$^-$ Th17 cells in the ileum at day 10 after SFB gavage, with representative FACS panels (E) and cumulative data (F). Each symbol represents a single mouse, and the color bar indicates average +/- SD. Results from two independent experiments were combined (SFB negative: n=7, control IgG injected SFB-gavaged mice: n=10 (Day7) and 9 (Day10), LTβR-Ig injected SFB gavaged mice n=9 (Day7) and 10 (Day10). Two-tailed unpaired Student's t-tests were performed. Less than 0.05 of P-value was treated as significant difference. * $p<0.05$. N.S.: Not significant.

Figure S3

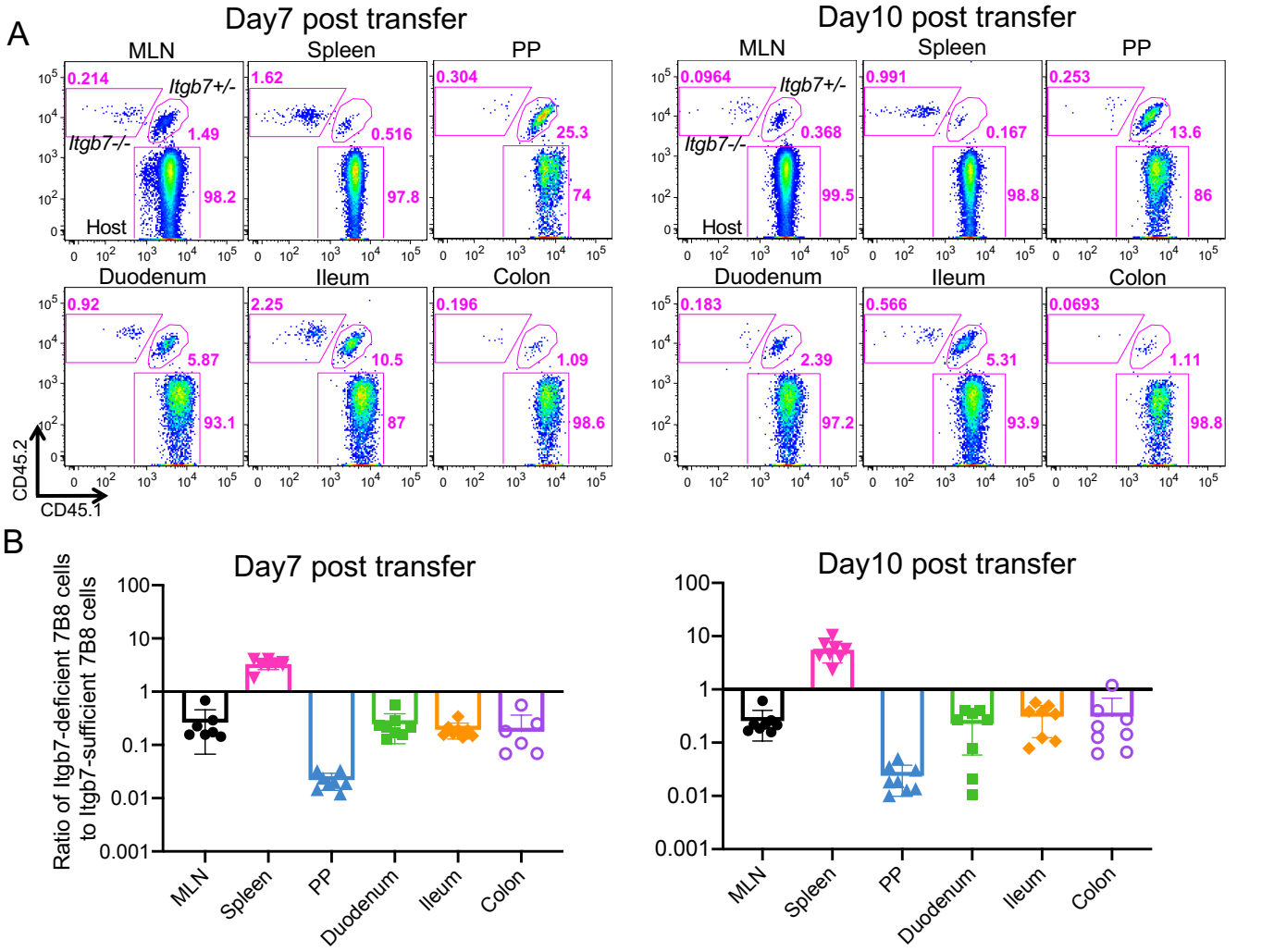

Figure S3. The role of a4b7 integrin in migration of SFB-specific Th17 cells from MLN to local tissues (related to Figure 3)

(A and B) SFB-specific T cells in different tissues at 7 and 10 days following co-transfer of 5,000 naïve integrin β7 (*Itgb7*)-sufficient (*Cd45.1/Cd45.2*) and 5,000 *Itgb7*-deficient (*Cd45.2/Cd45.2*) 7B8 T cells into SFB gavaged mice (*Cd45.1/Cd45.1*). In (B), each symbol represents tissue cell ratio in a single mouse, and the color bar indicates average +/- SD. Two experiments were performed and results were combined (n=8 for each time point).

Figure S4

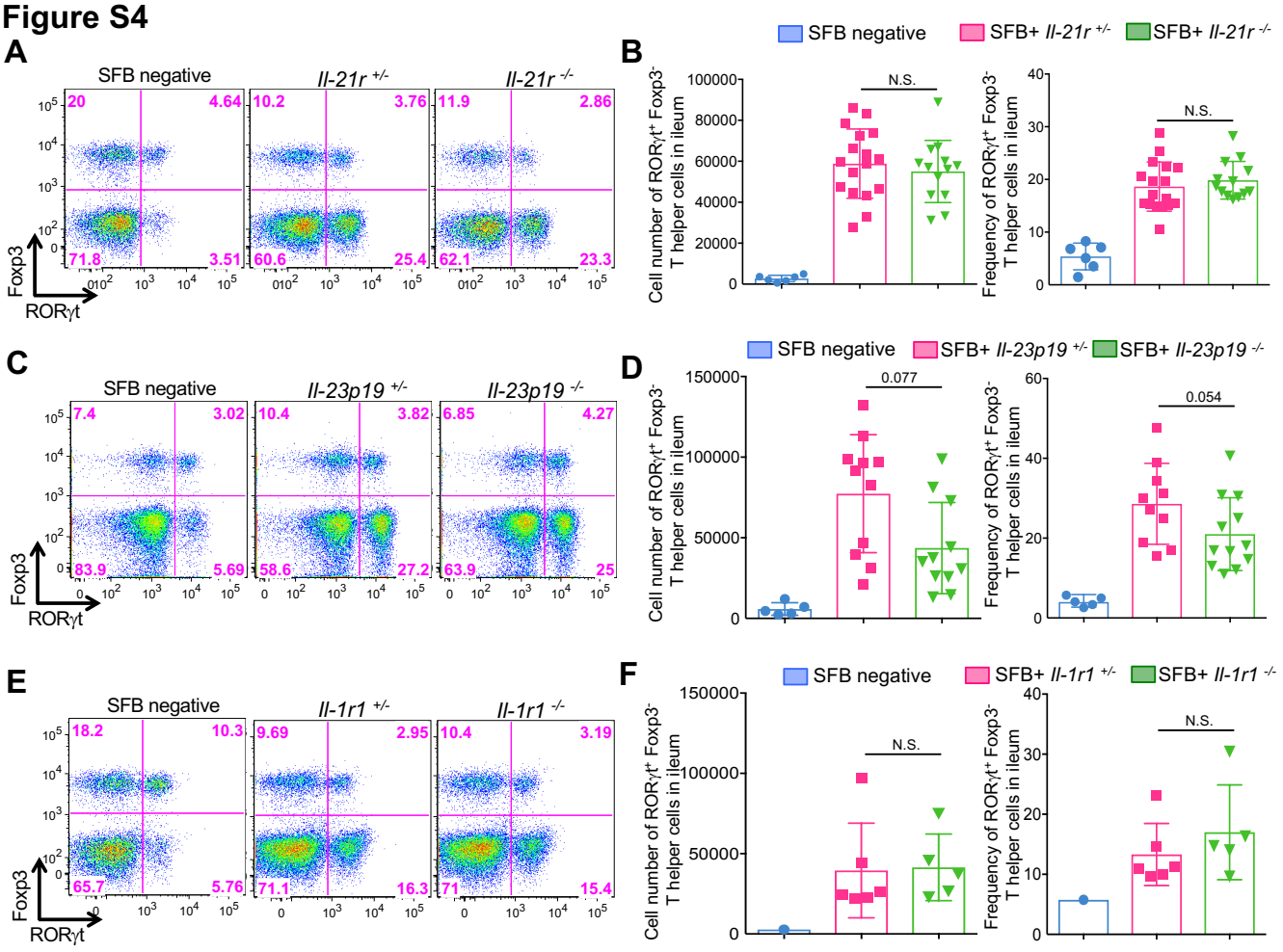

Figure S4. Cytokine requirements for SFB-induced Th17 cell differentiation in vivo (related to Figure 4)

(A-F) Th17 cell differentiation in response to SFB gavage in mice deficient for IL-21R (A and B), IL-23 p19 (C and D), and IL-1R1 (E and F). SFB was gavaged into *Il-21r*, *Il-23 p19*, and *Il1r1* sufficient and deficient co-housed littermates and at days 7 (IL-21R and IL-1R1) or 10 (IL-23 p19) ileal LP RORγt^{+} Foxp3^{-} Th17 cells were quantified by FACS. (A), (C), and (E) are representative FACS panels; (B), (D), and (F) show aggregate Th17 cell numbers. Experiments were done at least twice with several mutant and littermate controls. Results were combined and each dot shows an individual mouse, with color bars indicating average +/- SD. Two-tailed unpaired Student's t-tests were performed. Less than 0.05 of P-value was treated as significant difference. N.S.: not significant.

Figure S5

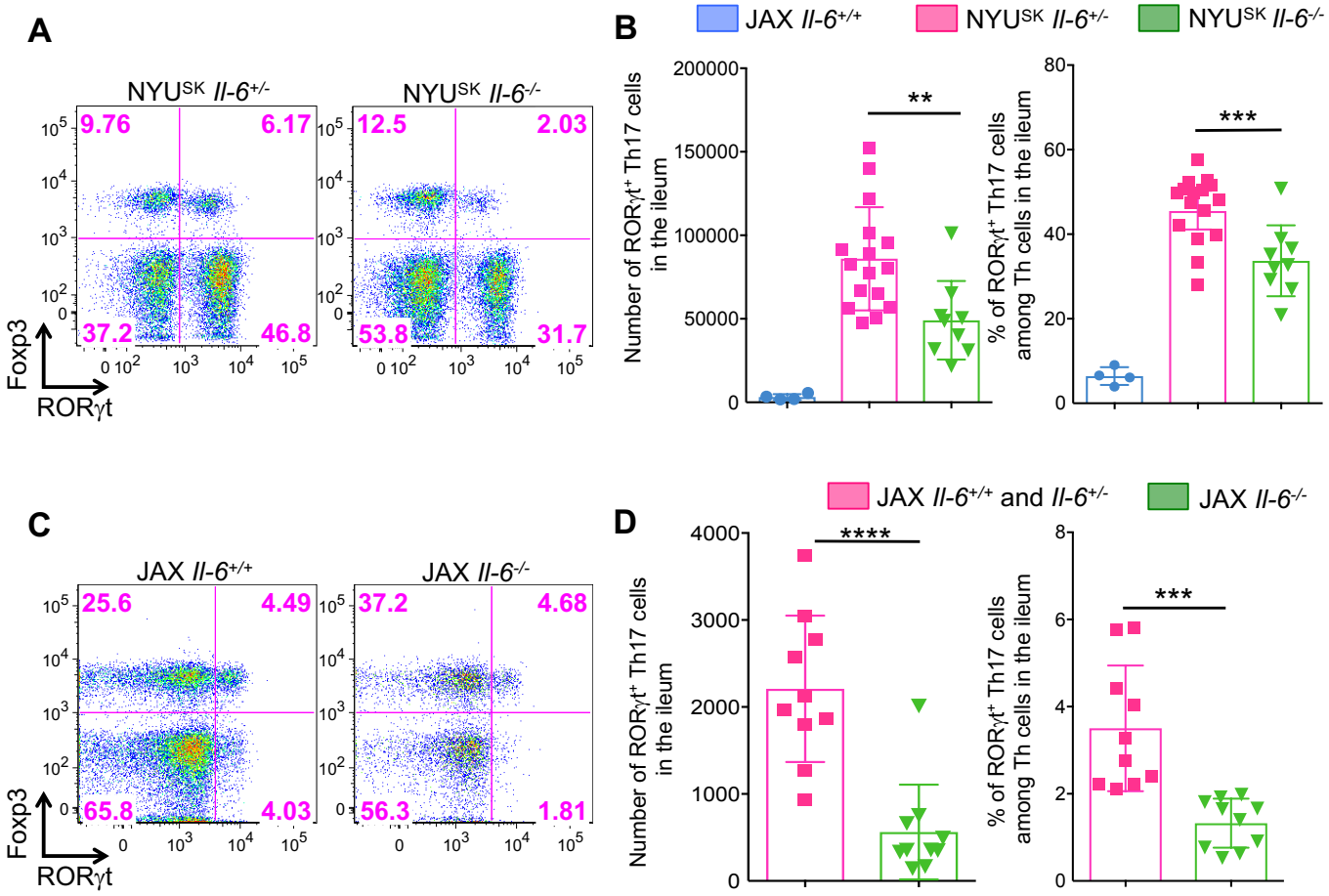

Figure S5. Comparison of IL-6 requirements for Th17 cell differentiation in mice housed in vivaria with and without SFB (Related to Figure 4)

(A and B) Th17 cells in ileum of stably SFB-colonized *Il-6* heterozygous or homozygous null C57BL/6 mice housed in the Skirball animal facility at New York University (NYU^{SK}). Mice were confirmed as SFB^{+} by qPCR. Representative flow cytometry analysis (A) and statistics of number (B, left) and frequency (B, right) of RORγt^{+} Foxp3^{-} Th17 cells in the ileum. Experiments were done three times with biological replicates (SFB negative *Il-6*$^{+/-}$*:* n=4, *Il-6*$^{+/-}$*:* n=16, and *Il-6*$^{-/-}$*:* n=9) and the results were combined. Littermates were cohoused from birth. **(C and D)** IL-6 requirement for Th17 cell differentiation in mice housed at Jackson Laboratory (JAX) in SFB-free conditions. Data shown as in (A and B). Experiments were done twice with 10 mice total in each group, and combined results are shown. Dots show individual mice and color bars indicate average +/- SD. Two-tailed unpaired Student's t-tests were performed. Less than 0.05 of P-value was treated as significant difference. ** p<0.01, *** p<0.001, and **** p<0.0001. N.S.: not significant.

Figure S6

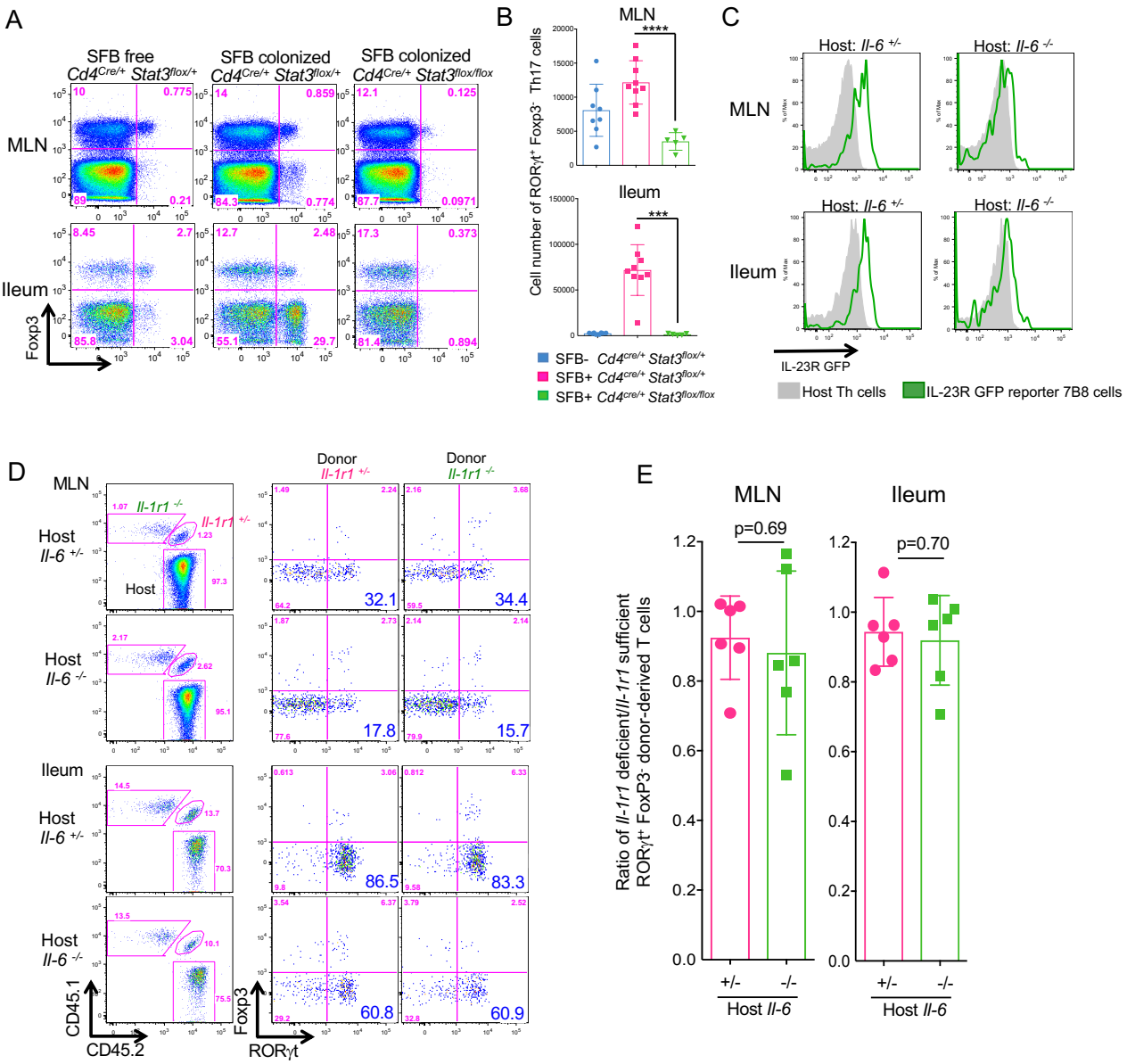

Figure S6. Stat3-dependent cytokine redundancy in SFB-specific Th17 cell differentiation in the MLN and ileum (related to figure 5)

(A-B) Th17 cells from the MLN and ileum of *Cd4*$^{cre/+}$ *Stat3*$^{flox/+}$ and *Cd4*$^{cre/+}$ *Stat3*$^{flox/flox}$ mice stably colonized with SFB or SFB-free. Representative FACS plots (A) and numbers of RORγt^{+}Foxp3^{-} CD4^{+} T cells (B). Experiments were repeated three times with several mice and combined data are shown. Dots represent individual mice and the color bars indicate average +/- SD. (SFB negative control: n=9, *Cd4*$^{cre/+}$ *Stat3*$^{flox/+}$*:* n=9, *Cd4*$^{cre/+}$ *Stat3*$^{flox/flox}$: n=5). **(C)** Expression of *Il-23r*^{gfp} reporter in SFB-specific 7B8 TCR transgenic Th17 cells in the MLN and the ileum of *Il-6* sufficient and deficient mice at 7 days following naïve T cell transfer. **(D and E)** SFB-specific Th17 cell differentiation in the presence or absence of IL-1R1 signaling in *Il-6* sufficient and deficient mice. 5000 naïve T cells from cytokine receptor sufficient and deficient 7B8 Tg mice, with indicated isotypes, were co-transferred into SFB-gavaged *Il-6* sufficient and deficient mice (*Cd45.2/Cd45.2*). Representative FACS plots of ileal T cells at 7 days following naïve T cell transfer (D). Ratios of RORγt^{+} Foxp3^{-} Th17 cells (*Il1r1* sufficient vs deficient T cells) in *Il-6* sufficient (n=6) and deficient (n=6) mice (E). Data from two experiments with similar results were combined. Dots represent individual mice and color bars indicates average +/- SD . Two-tailed unpaired Student's t-tests were performed. Less than 0.05 of P-value was treated as significant difference. *** p<0.001 and **** p<0.0001.

Figure S7

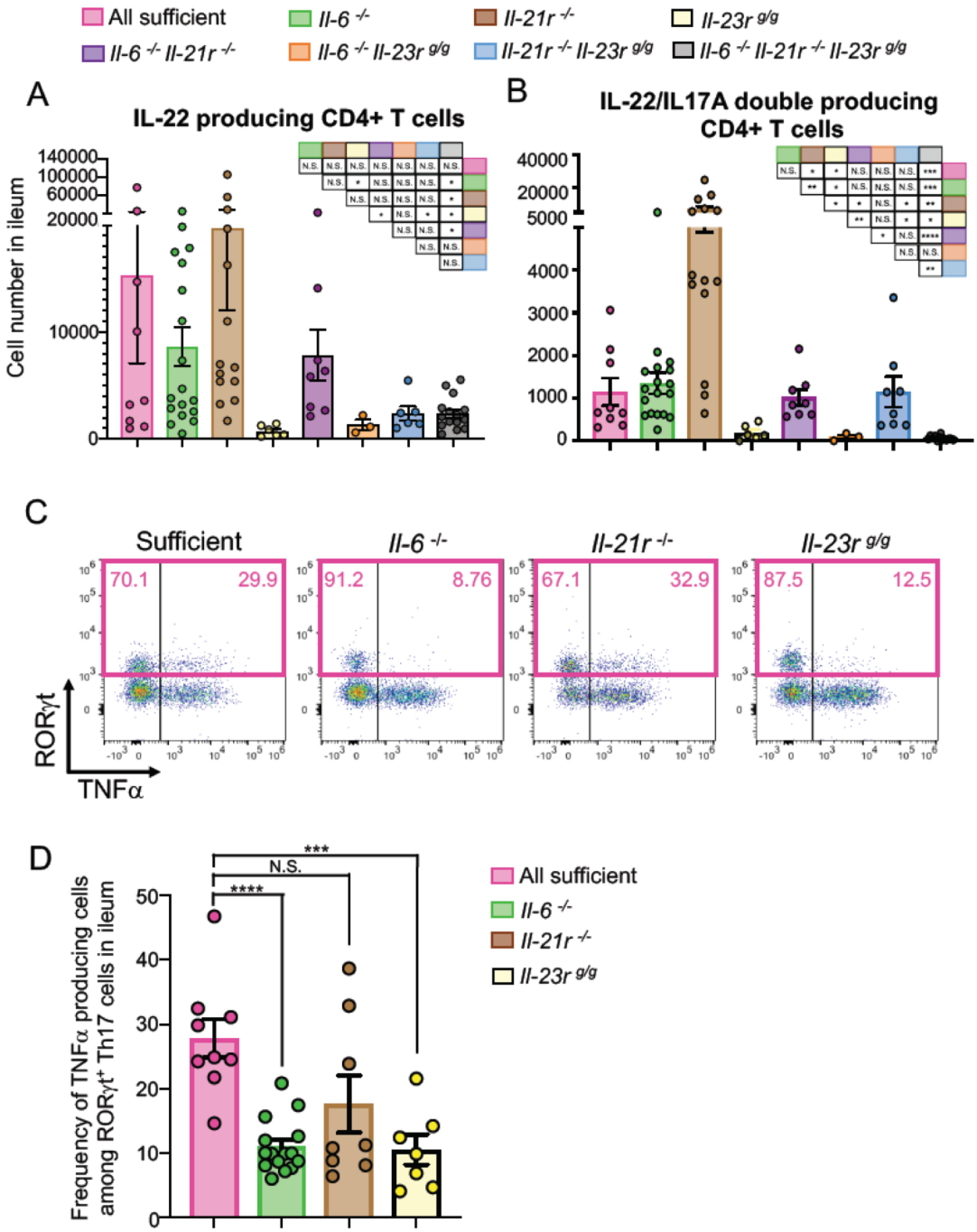

Figure S7. Contribution of IL-6, IL21R, and IL-23R to the production of cytokines by ileal Th17 cells (related to Figure 7)

(A) Cell numbers of IL-22-producing ileal CD4⁺ T cells shown in Figure 7A. (B) Cell numbers of IL-22 and IL-17A double producing ileal CD4⁺ T cells shown in Figure 7A. **(C)** Representative FACS plots of ileal Th cells stained for RORγt and TNFα. Ileal LP cells were re-stimulated ex-vivo for 4 hours. The cells were gated for MHCII⁻, TCRβ⁺, CD4⁺, CD8⁻, and Foxp3⁻ phenotype. The gated cells were further analyzed for expression of RORγt and TNFα. **(D)** Frequency of TNFα producing cells among RORγt⁺ Th17 cells shown in (C). Experiments were repeated with indicated genotypes at least three times and results are combined. Littermates were used in most cases. *p*-values for differences between each group of mice are shown in the tables. Dots represent individual mice and color bars indicate average +/- SEM. Two-tailed unpaired Student's t-tests were performed. Less than 0.05 of P-value was treated as significant difference. * p<0.05, ** p<0.01, *** p<0.001, and **** p<0.0001. N.S.: not significant.