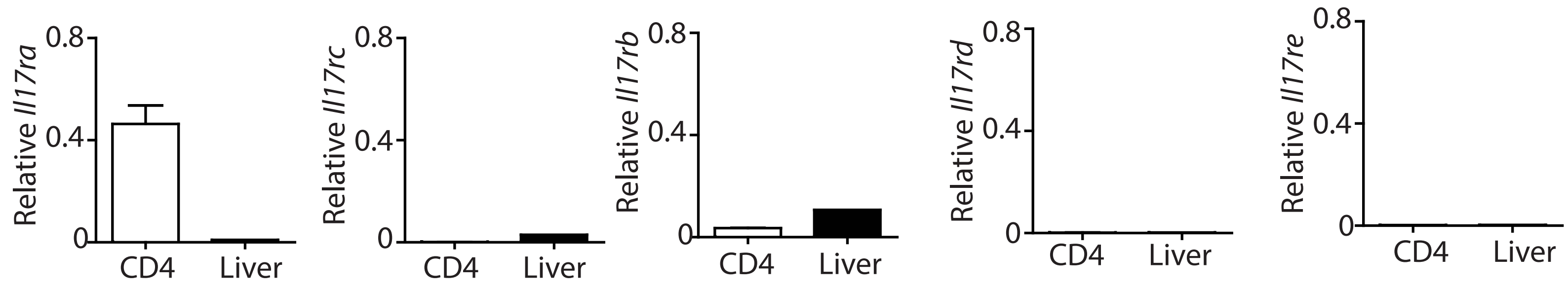
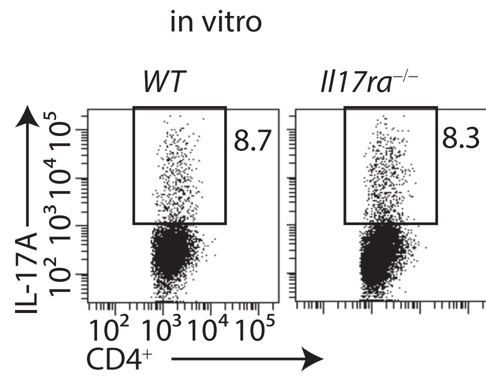
Figure S1

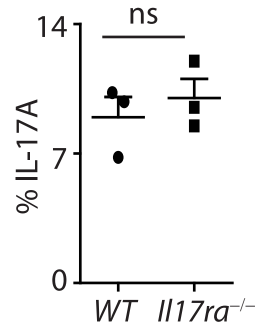
A



B

in vitro





C

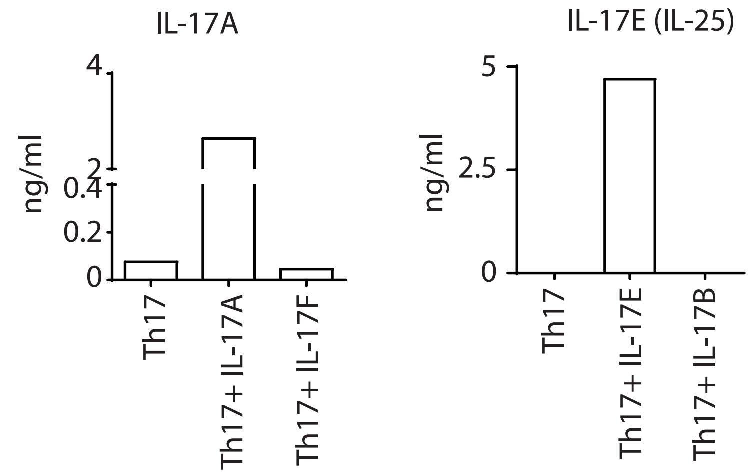
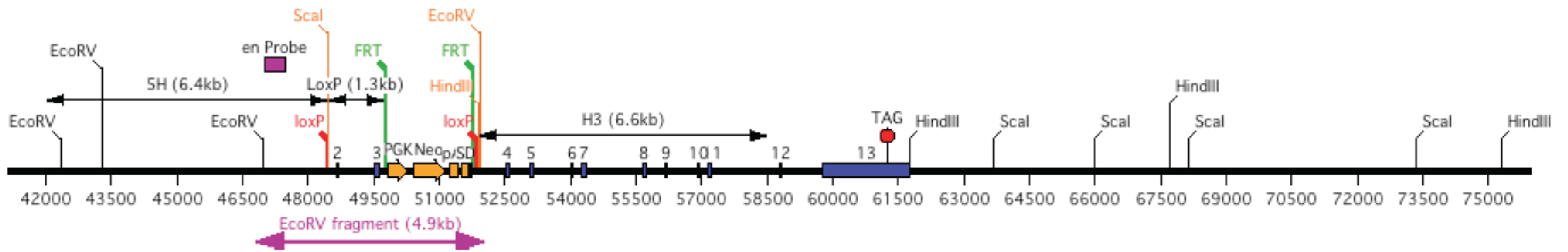


Figure S2

A

IL-17RA targeting vector-exon 3 is floxed

1170_Lewis_Targeted



B

Primers:			
IL17ra F1:	GCC TCC AAG TCT AGC TTT GCT TGG C		
IL17ra R1:	AGG CCC CTG AGA GCG GTT CA		
IL17ra F2:	CCC TTG GGA GGA GAC CAC TGC T		
IL17ra R2:	CAC CCC TAA GGA GGT CCG GAA TGT		
Annealing tem	62 C		

Primers F1&R1:	**F2 & R2**
WT: 304	298
Het: 304 and ~350	298 and 350
Homo: ~350	350

Primers F1 and R2:	
WT -cre:	~1685
Homo Flox -cre:	~1800
Het Flox -Cre:	~1685 & ~1800
Het Flox +Cre:	~450, ~1685, & ~1800
Homo Flox +cre:	~450

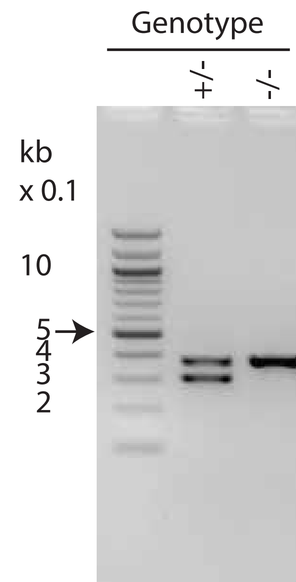
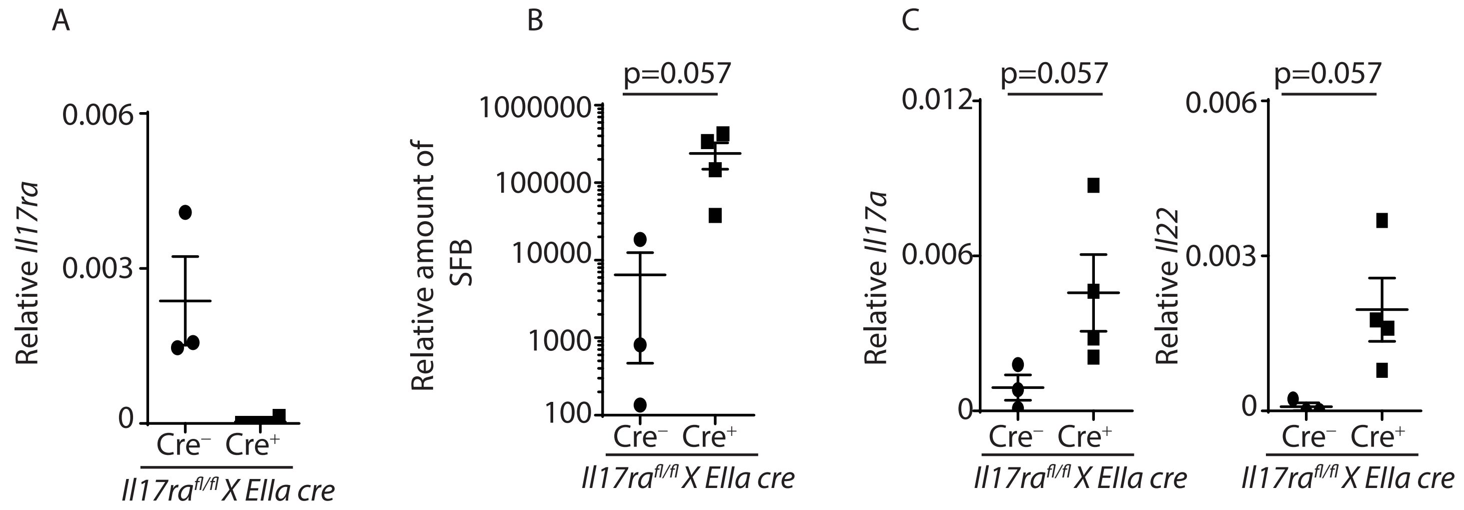
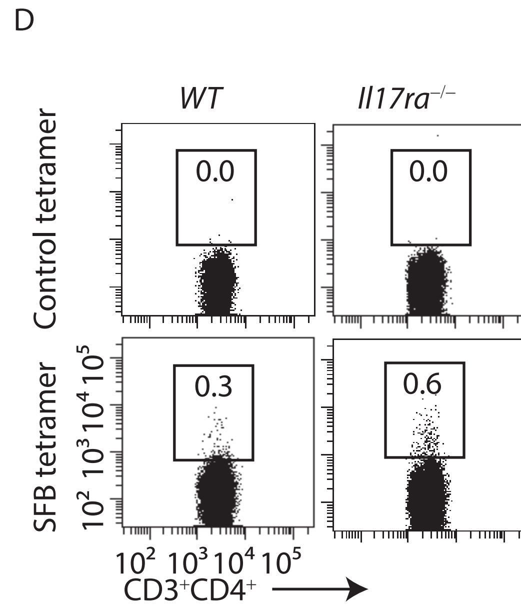


Figure S3





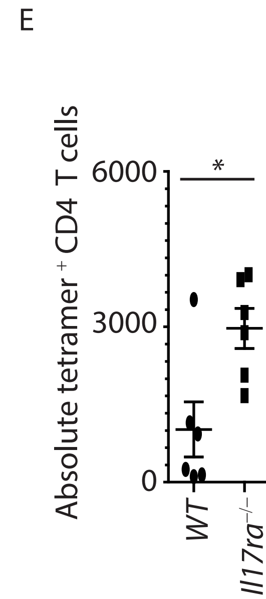
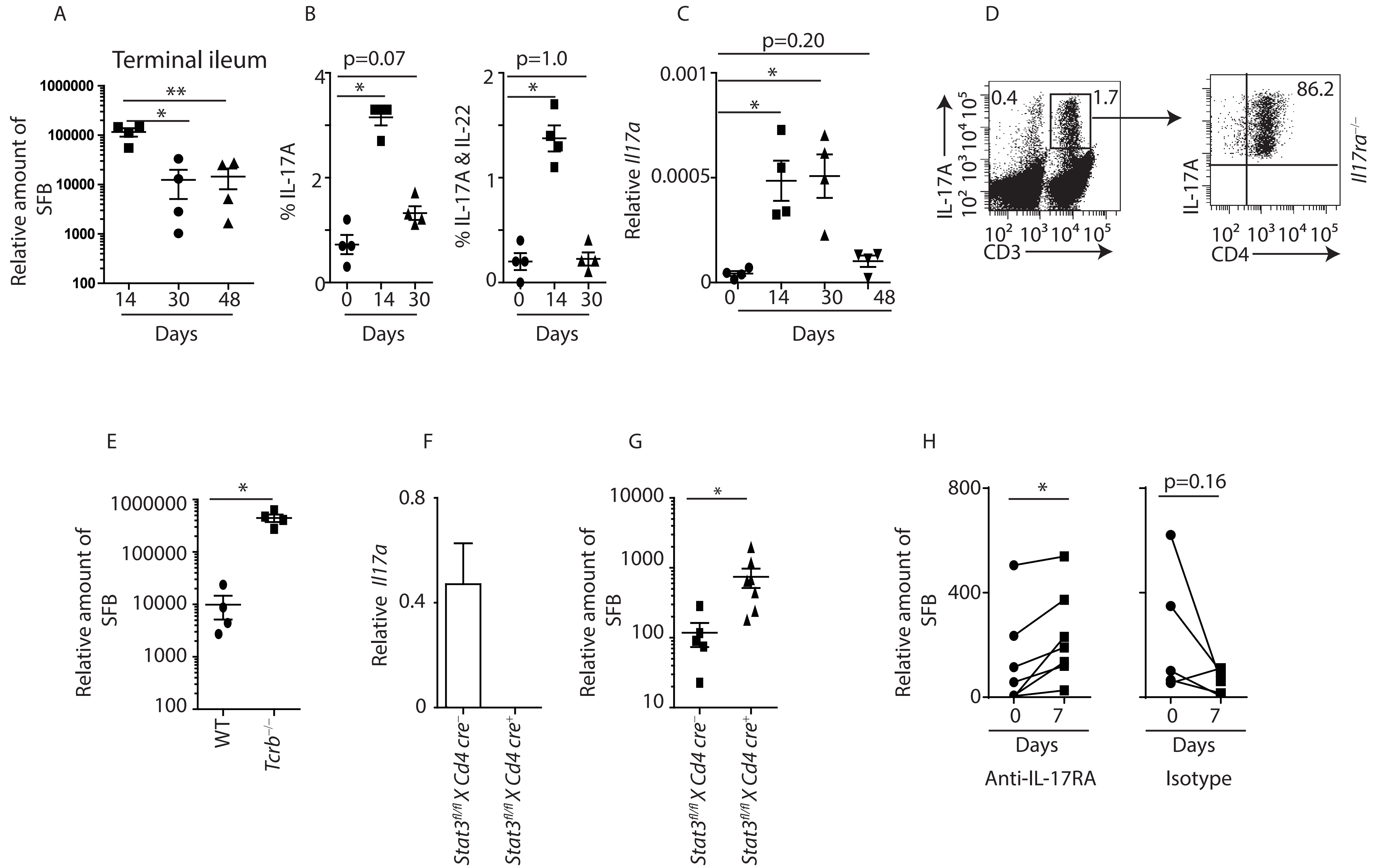
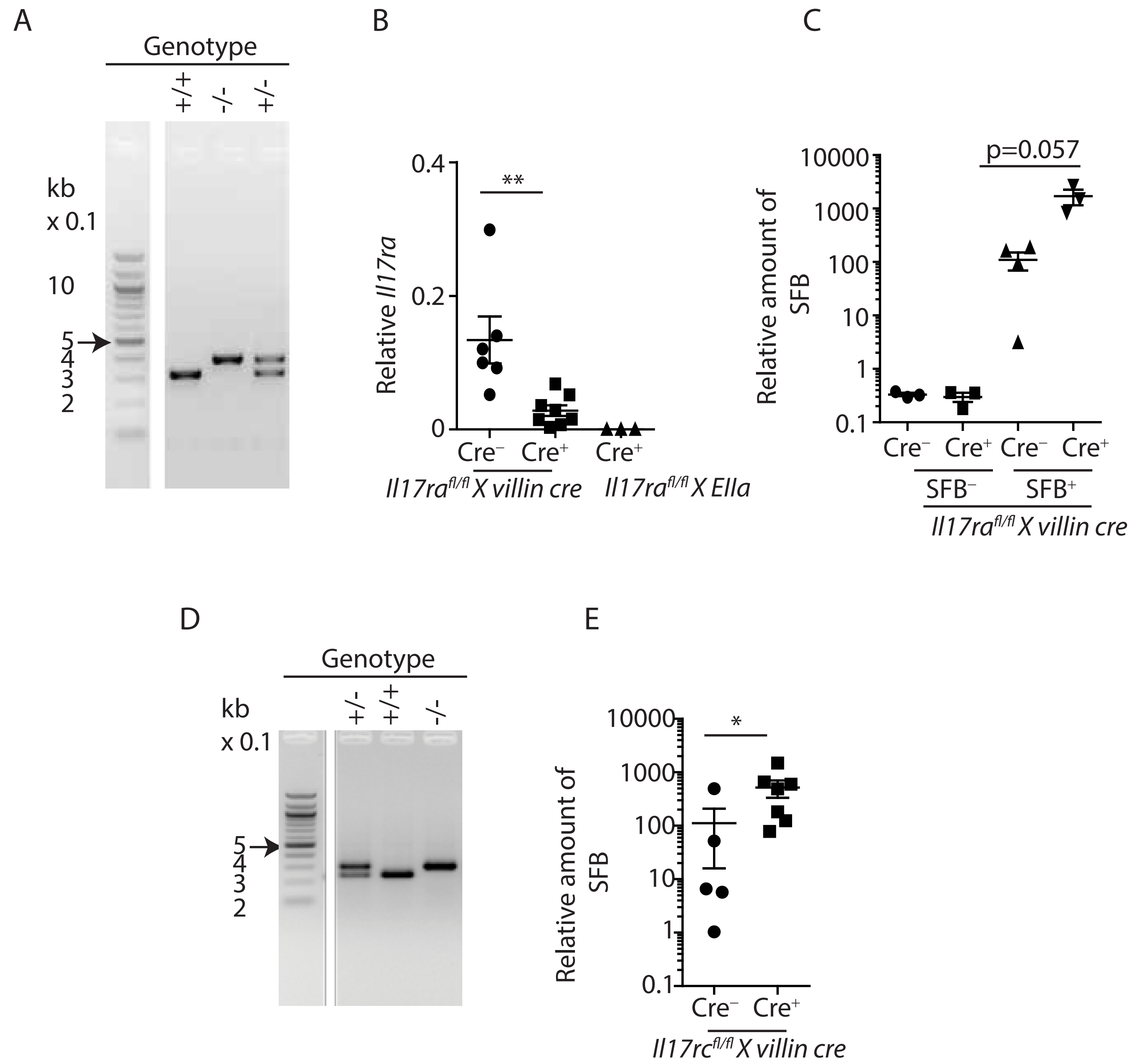


Figure S4





A
Genotype
+/+
-/-
+/-
kb
x 0.1
10
5
4
3
2
B
Relative Il17ra
0.4
0.2
0
**
Cre⁻
Cre⁺
Cre⁺
Il17ra fl/fl X villin cre
Il17ra fl/fl X EIIa
C
Relative amount of SFB
10000
1000
100
10
1
0.1
p=0.057
Cre⁻
Cre⁺
Cre⁻
Cre⁺
SFB⁻
SFB⁺
Il17ra fl/fl X villin cre
D
Genotype
+/-
+/+
-/-
kb
x 0.1
5
4
3
2
E
Relative amount of SFB
10000
1000
100
10
1
0.1
*
Cre⁻
Cre⁺
Il17rc fl/fl X villin cre

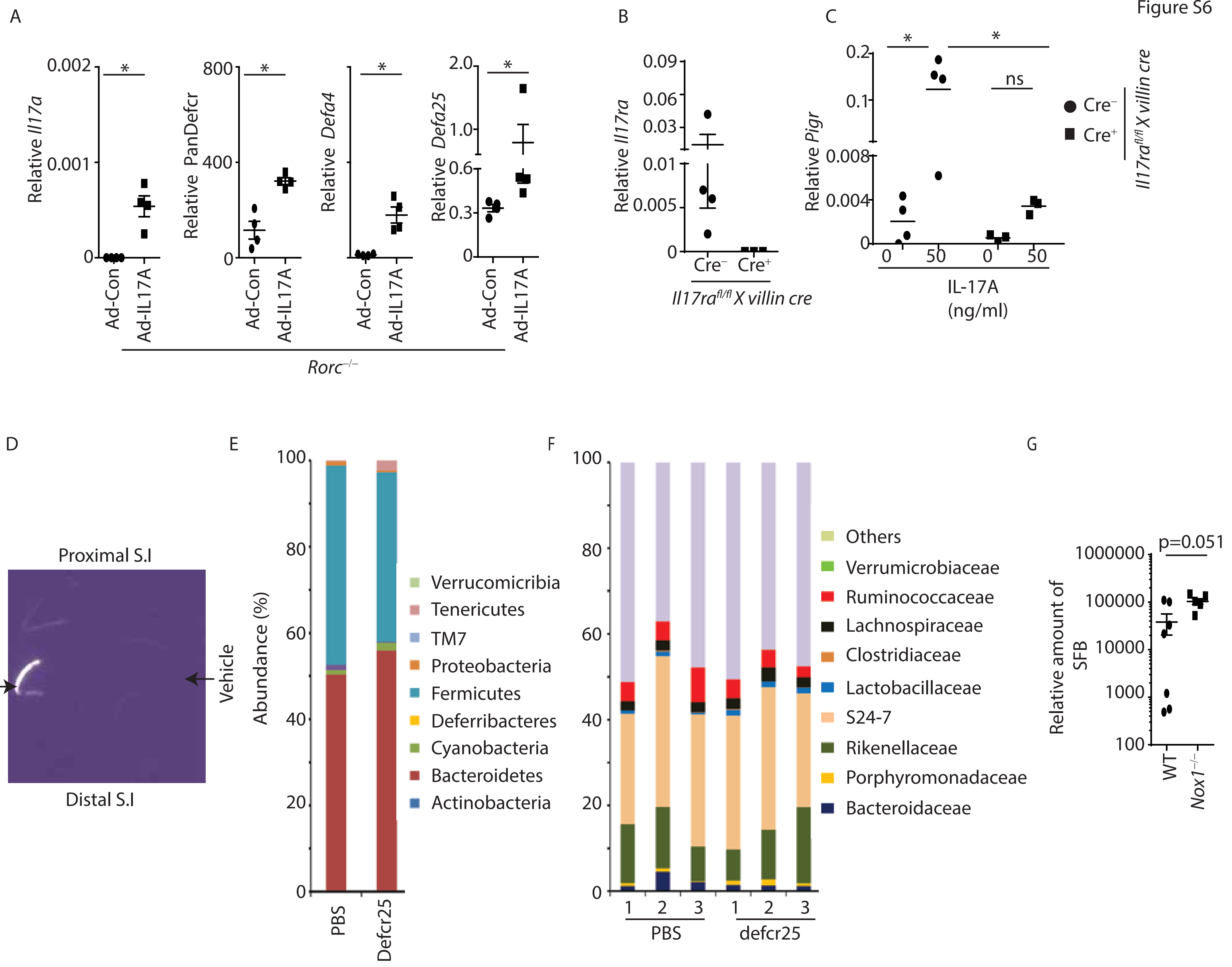
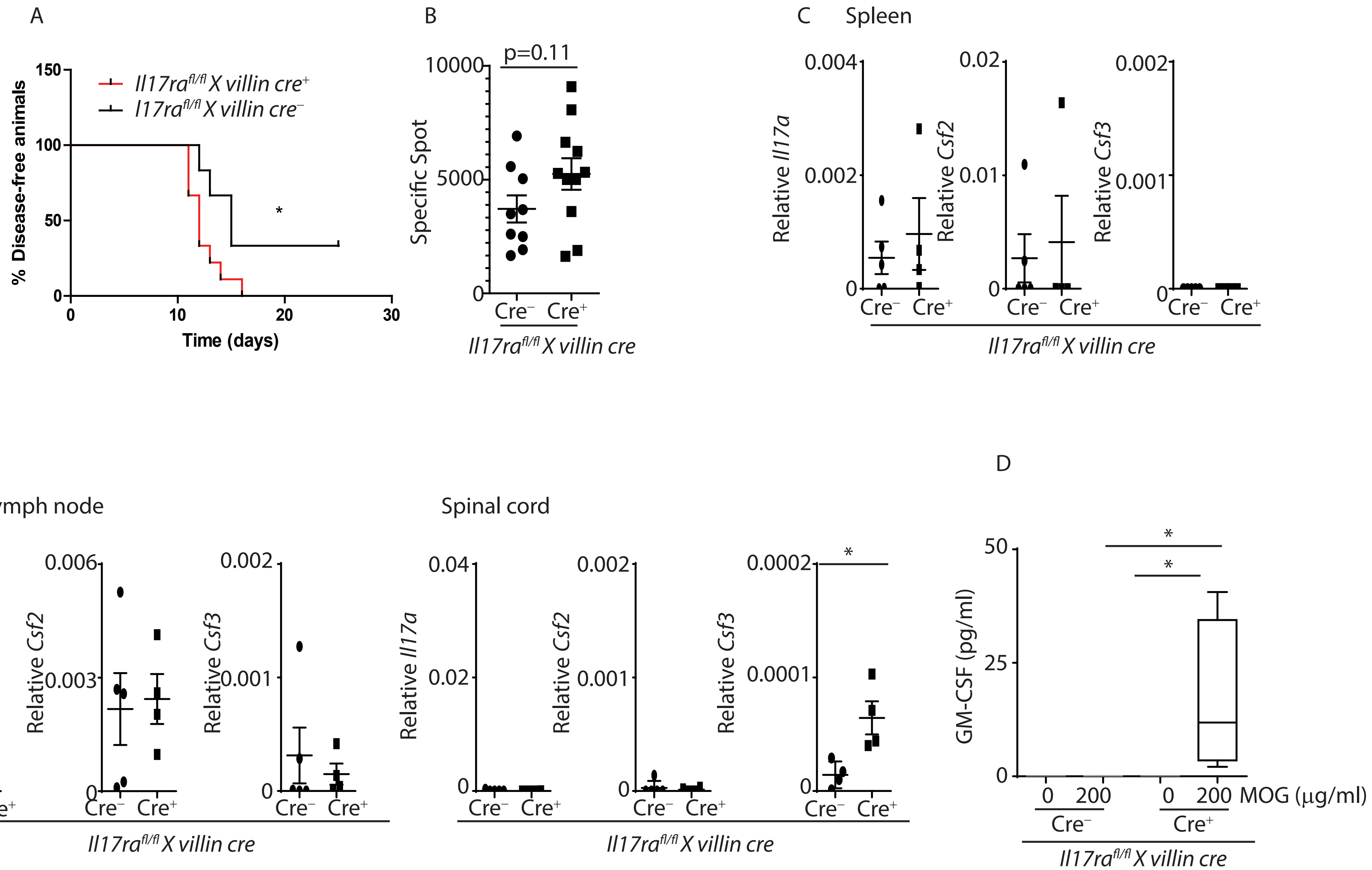


Figure S6
A
Relative Il17a
Relative PanDefcr
Relative Defa4
Relative Defa25
Ad-Con
Ad-IL17A
Rorc−/−
B
Relative Il17ra
Cre−
Cre+
Il17ra fl/fl X villin cre
C
Relative Pigr
ns
IL-17A
(ng/ml)
Cre−
Cre+
D
Proximal S.I
Distal S.I
Rhodamine-Calmodulin
Vehicle
E
Abundance (%)
Verrucomicribia
Tenericutes
TM7
Proteobacteria
Fermicutes
Deferribacteres
Cyanobacteria
Bacteroidetes
Actinobacteria
PBS
Defcr25
F
Others
Verrumicrobiaceae
Ruminococcaceae
Lachnospiraceae
Clostridiaceae
Lactobacillaceae
S24-7
Rikenellaceae
Porphyromonadaceae
Bacteroidaceae
PBS
defcr25
G
p=0.051
Relative amount of SFB
WT
Nox1−/−


A
% Disease-free animals
Il17ra fl/fl X villin cre+
l17ra fl/fl X villin cre−
Time (days)
B
p=0.11
Specific Spot
Cre− Cre+
Il17ra fl/fl X villin cre
C Spleen
Relative Il17a
Relative Csf2
Relative Csf3
Inguinal lymph node
Spinal cord
D
GM-CSF (pg/ml)
MOG (μg/ml)

Lumen		**Il17ra fl/fl x villin**		
OTU	**P**	**Cre+**	**Cre-**	**taxonomy**
207749	0.03389485	12	50.3333333	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; S24-7; ;
185550	0.03389485	159.5	80	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; S24-7; ;
376862	**0.04974599**	**27.25**	**1.66666667**	**Bacteria; Firmicutes; Clostridia; Clostridiales; Clostridiaceae; Candidatus Arthromitus;**
162639	0.06658004	5.5	2.33333333	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; S24-7; ;
331878	0.07363827	0	0.66666667	Bacteria; Firmicutes; Clostridia; Clostridiales; ; ;
186777	0.07592696	1.5	0	Bacteria; Firmicutes; Clostridia; Clostridiales; Lachnospiraceae; ;
4306262	0.07709987	46	16.6666667	Bacteria; Verrucomicrobia; Verrucomicrobiae; Verrucomicrobiales; Verrucomicrobiaceae; Akkermansia; muciniphila
203713	0.07709987	17.25	82	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; S24-7; ;
328143	0.0777599	0	3.66666667	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; S24-7; ;
339905	0.0777599	0	1	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; S24-7; ;
207284	0.0777599	0	1	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; S24-7; ;
324943	0.0777599	0	1.33333333	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; S24-7; ;
134065	0.0777599	0	3	Bacteria; Firmicutes; Clostridia; Clostridiales; Lachnospiraceae; ;
174791	0.0777599	0	1.33333333	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; S24-7; ;
265052	0.0791864	195.25	0	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; S24-7; ;
4449518	0.0791864	3	0	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; Rikenellaceae; ;
198339	0.13836234	2.25	4	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; S24-7; ;
190673	0.14221324	1.5	4.33333333	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; S24-7; ;
177414	0.14603295	5.75	3.33333333	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; S24-7; ;
196578	0.14982084	6.25	2.66666667	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; S24-7; ;

Stool		**Il17ra fl/fl x villin**		
OTU	**P**	**Cre+**	**Cre-**	**taxonomy**
228043	0.01141204	1.25	0	Bacteria; Firmicutes; Clostridia; Clostridiales; ; ;
264155	0.0151862	2.25	0.25	Bacteria; Firmicutes; Clostridia; Clostridiales; ; ;
262166	0.01654894	0.5	2.25	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; S24-7; ;
376862	**0.01724874**	**5.75**	**0.25**	**Bacteria; Firmicutes; Clostridia; Clostridiales; Clostridiaceae; Candidatus Arthromitus;**
174805	0.01941866	0.5	3.5	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; S24-7; ;
175712	0.03599301	2.25	0.5	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; S24-7; ;
299171	0.04042398	0	0.75	Bacteria; Firmicutes; Clostridia; Clostridiales; ; ;
185550	0.04330814	57.5	77	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; S24-7; ;
258559	0.04550026	0	1.75	Bacteria; Firmicutes; Clostridia; Clostridiales; Lachnospiraceae; ;
258550	0.04550026	1	0	Bacteria; Firmicutes; Clostridia; Clostridiales; Ruminococcaceae; ;
199307	0.0472209	1.75	0	Bacteria; Firmicutes; Clostridia; Clostridiales; Ruminococcaceae; ;
191558	0.0472209	1.75	0	Bacteria; Firmicutes; Clostridia; Clostridiales; Ruminococcaceae; Oscillospira;
273967	0.0472209	0	1.5	Bacteria; Firmicutes; Clostridia; Clostridiales; Lachnospiraceae; ;
263687	0.0472209	1.5	0	Bacteria; Firmicutes; Clostridia; Clostridiales; Lachnospiraceae; ;
2897332	0.0472209	1.75	0	Bacteria; Firmicutes; Clostridia; Clostridiales; Lachnospiraceae; [Ruminococcus]; gnavus
185162	0.05300787	2.75	0.5	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; S24-7; ;
186777	0.05754695	6.25	1.5	Bacteria; Firmicutes; Clostridia; Clostridiales; Lachnospiraceae; ;
851733	0.08854192	0.25	1.75	Bacteria; Firmicutes; Bacilli; Lactobacillales; Lactobacillaceae; Lactobacillus;
176118	0.09942495	2.75	0.5	Bacteria; Firmicutes; Clostridia; Clostridiales; Ruminococcaceae; Oscillospira;
276509	0.10806337	2.75	6.25	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; S24-7; ;

Supplemental Figures legends

Figure S1, related to Figure 1. IL-17 receptor expression in CD4 T cells.

(A) Splenic $CD3^+CD4^+$ T cells were sorted and analyzed for the expression of *Il17ra, Il17rb, Il17rc, Il17rd* and *Il17re* by qPCR (n=2). Liver was used as a positive control.

(B) Naïve CD4 T cells from WT (n=3) and *Il17ra*$^{-/-}$ (n=3) mice were polarized to Th17 cells. After 4 days culture, cells were stained with anti-CD3, anti-CD4 and anti-IL-17A antibodies. CD4 T cells (gated on $CD3^+CD4^+$) producing IL-17A were analyzed by flow cytometer (left panel). Percentages of IL-17 producing CD4 T cells from multiple mice are shown in right panel.

(C) Naïve CD4 T cells from *Thy1.1-Il17F* reporter mice were polarized to Th17 cells in the presence of indicated IL-17RA ligands. ELISA data shows recovery of IL-17A and IL-25 in the culture supernatant Th17 differentiated cells.

Figure S1A was generated from n=2 mice in each group. Flow cytometry data are representative plots of n=3 mice in each group. Each symbol represents a separate animal. Data presented as mean ± SEM (Mann-Whitney test).

Figure S2, related to Figure 1. Schematic targeting strategy for the *Il17ra* allele.

(A) A schematic of the targeting strategy for the *Il17ra* allele.

(B) Primers used (left panel) to characterize *Il17ra* floxed mice by PCR (right panel).

Figure S3, related to Figure 2. SFB inoculation into SFB-free *Il17ra*$^{fl/fl}$ x *EIIa* cre$^+$ mice resulted into SFB overgrowth.

(A) Terminal ileum of *Il17ra*$^{fl/fl}$ x *EIIa* cre$^+$ (n=4) and littermate control cre$^-$ (n=3) mice were analyzed for the expression of *Il17ra* (primers span exon 1-2 of *Il17ra*) by qPCR.

(B) SFB-free *Il17ra*$^{fl/fl}$ x *EIIa* cre$^+$ (n=4) and littermate control cre$^-$ (n=3) mice were gavaged with SFB containing *Rorc*$^{-/-}$ mice fecal contents. After 30 days of fecal transplant, terminal ileum of *Il17ra*$^{fl/fl}$ x *EIIa* cre$^+$ (n=4) and littermate control cre$^-$ (n=3) mice were analyzed for SFB colonization by qPCR.

(C) Terminal ileum of *Il17ra*$^{fl/fl}$ x *EIIa* cre$^+$ (n=4) and littermate control cre$^-$ (n=3) mice were analyzed for the expression of *Il17a* and *Il22* by qPCR.

(D) Lymphocytes were isolated from lamina propria of WT (n=6) and *Il17ra*$^{-/-}$ (n=6) mice, and stained for CD3, CD4 and SFB or control tetramer. SFB (n=6) or control tetramer (n=3) stained CD4 T (gated on $CD3^+CD4^+$) cells were analyzed by flow cytometry. Data are representative of two independent experiments.

(E) Data shown are absolute number of SFB-specific CD4 T cells in the lamina propria of WT (n=6) and *Il17ra*$^{-/-}$ (n=6) mice. Data are generated from two independent experiments.

Flow cytometry data are representative plots from multiple mice (n=3-6). Each symbol indicates data from single mouse. $^*P \leq 0.05$ (Mann-Whitney test, Two-tailed).

Figure S4, related to Figure 3. Th17 cells regulate SFB colonization.

(A) SFB-free C57BL/6 Jackson mice were transplanted with SFB colonized *Rorc*$^{-/-}$ mice fecal contents. Terminal ileum of these mice were analyzed for SFB colonization at various time points (n=4 mice in each groups) by qPCR.

(B) Lymphocytes were isolated from lamina propria of SFB-transplanted C57BL/6 Jackson mice at various time points, and stained with anti-CD3, anti-CD4, anti-IL-17A and anti-IL-22 antibodies. Percentages of IL-17A or both IL-17A^{+} and IL-22-producing CD4 T (gated on CD3^{+}CD4^{+}) cells at various time points (n=4 mice in each group) were shown.

(C) Terminal ileum of SFB-transplanted C57BL/6 Jackson mice were analyzed for the expression of *Il17a* by qPCR.

(D) Lamina propria lymphocytes of SFB-colonized *Il17ra*$^{-/-}$ mice were stained with anti-CD3, anti-CD4 and anti-IL-17A antibodies, and analyzed for T and non-T cells-specific IL-17A generation.

(E) SFB-free age and gender (male) matched C57BL/6 WT (n=4) and *Tcrb*$^{-/-}$ (n=4) Jackson mice were transplanted with SFB-containing *Rorc*$^{-/-}$ mice fecal contents. These mice were co-housed for 2 weeks, and separated (based on genotype) for additional 4 weeks. After 6 weeks, terminal ileums of WT and *Tcrb*$^{-/-}$ mice were analyzed for SFB colonization by qPCR.

(F) Naïve CD4 T cells from *Stat3*$^{fl/fl}$ x *Cd4* cre^{+} (n=2) and littermate control cre^{-} (n=2) mice were polarized to Th17 cells. After 4 days culture, cells were analyzed for the expression of *Il17a* by qPCR.

(G) Fecal contents of *Stat3*$^{fl/fl}$ x *Cd4* cre^{+} (n=5) and littermate control cre^{-} (n=7) mice were analyzed for SFB abundance by qPCR.

(H) SFB-colonized WT mice injected with anti-IL-17RA or isotype antibodies. Fecal contents of WT mice, before (day 0) and after (day 7) anti-IL-17RA or isotype antibody injection, were analyzed for SFB abundance by qPCR.

Each symbol indicates data from a single mouse. Flow cytometry plots are representative of multiple mice (n=3-4). Data presented as mean ± SEM on relevant figures. $*P \leq 0.05$; $**P \leq 0.01$ (Mann-Whitney test, Two tailed for figure S4E and S4G or One-Way ANOVA for figure S4A, S4B and S4C, Paired T-test for figure S4H).

Figure S5, related to Figure 3. Intestinal IL-17RA and IL-17RC signaling regulates SFB colonization.

(A) Genotype of *Il17ra*$^{fl/fl}$ x *villin* cre floxed mice were determined by PCR using specific primer pairs as described in figure S2B.

(B) *In vivo* characterization of *Il17ra*$^{fl/fl}$ x *villin* cre^{+} floxed mice. Terminal ileum of *Il17ra*$^{fl/fl}$ x *villin* cre^{+} (n=8) and littermate control cre^{-} (n=6) mice as well as positive control *Il17ra*$^{fl/fl}$ x *EIIa* cre^{+} (n=3) mice were analyzed for the expression of *Il17ra* (primers span exon 1-2) by qPCR. Data are representative of two independent experiments.

(C) SFB-free *Il17ra*$^{fl/fl}$ x *villin* cre^{+} (n=3) and littermate cre^{-} (n=3) mice were transplanted with SFB containing *Rorc*$^{-/-}$ mice fecal contents. Data demonstrate SFB abundance before and 15 days (n=3-4) after SFB transplantation.

(D) Genotype of *Il17rc*$^{fl/fl}$ x *villin* cre^{+} floxed mice were determined by PCR using specific primer pairs as described in material and methods.

(E) Fecal contents of *Il17rc*$^{fl/fl}$ x *villin* cre^{+} (n=7) and littermate control cre^{-} (n=5) mice were analyzed for SFB colonization by qPCR.

Data presented as mean ± SEM for B, C and E. $*P \leq 0.05$; $**P \leq 0.01$ (Mann-Whitney test, Two-tailed).

Figure S6, related to Figure 5 and 6. α-defensin expression and its impact in microbiome.

(A) *Rorc*$^{-/-}$ mice (n=4) were injected with recombinant adenovirus expressing murine IL-17A (Ad-17A) or control vector (Ad-Con). After 7 days, terminal ileum were analyzed for the expression of *Il17a*, pan-Defensin, *Defa4* and *Defa25* by qPCR.

(B) Enteroids from *Il17ra*$^{fl/fl}$ x *villin* cre^{+} (n=3) and littermate control cre^{-} mice were cultured, and stimulated with recombinant IL-17A. qPCR data confirmed lack of *Il17ra* expression in *Il17ra*$^{fl/fl}$ x *villin* cre^{+} (n=3) mice enteroid. Data are representative of two independent experiments.

(C) IL-17A stimulated *Il17ra*$^{fl/fl}$ x *villin* cre^{+} (n=3) and littermate control cre^{-} (n=4) mice enteroids were analyzed for the expression of *Pigr* by qPCR. Data are representative of two independent experiments.

(D) Rhodamine-conjugated calmodulin (molecular weight 16kD, similar to α-defensin) was detected in the lumen of the small intestine within 20 minutes. Small intestines were scanned using Bio-rad Chemidoc imaging system. One representative image demonstrated out of two independent experiments.

(E and F) SFB-colonized *Il17ra*$^{-/-}$ mice were treated with vehicle (PBS) or defcr25 peptide. On 12-post vehicle (n=3) or defcr25 (n=3) treatment, fecal contents of *Il17ra*$^{-/-}$ mice were analyzed for commensal diversity at E) phyla and F) selected family level by 16S microbial sequencing.

(G) Terminal ileum of co-housed WT (n=7) and *Nox1*$^{-/-}$ (n=6) mice were analyzed for SFB colonization by qPCR.

Data presented as mean ± SEM on relevant figures. $*P \leq 0.05$. (Mann-Whitney test, Two-tailed and ANOVA).

Figure S7, related to Figure 7. Enteric IL-17R-signaling regulates EAE incidence.

(A) Data shown are EAE disease incidence in *Il17ra*$^{fl/fl}$ x *villin* cre^{+} (n=8) as well as littermate control cre^{-} (n=6) mice. Data generated from two independent experiments.

(B) Lymphocytes were harvested from the spleen of *Il17ra*$^{fl/fl}$ x *villin* cre^{+} (n=11) and littermate control cre^{-} mice (n=8) on day 9 post EAE induction, and re-stimulated with MOG peptide. ELISPOT was performed to detect MOG-specific IL-17A response. Data were generated from three independent experiments.

(C) Inguinal lymph node (ILN), spleen and spinal cord were harvested from *Il17ra*$^{fl/fl}$ x *villin* cre^{+} (n=4) and littermate control cre^{-} mice (n=5) on day 9 post EAE induction. These organs were analyzed for the expression of *Il17a, Csf2* and *Csf3* by qPCR. Data shown are representative of two independent experiments.

(D) Lymphocytes were harvested from the lamina propria of *Il17ra*$^{fl/fl}$ x *villin* cre^{+} (n=4) and littermate control cre^{-} mice (n=5) on day 9 post EAE induction, and re-stimulated with MOG peptide. ELISA was performed to detect GM-CSF in the culture supernatant of MOG re-stimulated (24 hours) cells.

Percentage incidence graphs were analyzed using Log-rank test. Data presented as mean ± SEM on relevant figures. $P \leq 0.05$. (Mann-Whitney test, Two-tailed and One-way ANOVA).

Supplemental Table S1 legend

Data shown are top 20 differentially regulated OTUs for terminal ileum luminal contents (top panel) and stool (bottom panel) of co-housed *Il17ra*$^{fl/fl}$ *x villin* cre^{+} and littermate control cre^{-} mice. Table 1, related to Figure 4.

Supplemental Experimental Procedures

Methods

Mice: Age matched 6-12 weeks old mice were used in all experiments. All of the animal studies were conducted with the approval of the University of Pittsburgh Institutional Animal Care and Use Committee. *Stat3* floxed (*Stat3$^{fl/fl}$*) mice on C57BL/6 background were obtained from Mark H. Kaplan. 6-week-old wild-type (WT) C57BL/6 mice were also purchased from Taconic (Germantown, NY) or Jackson laboratory. SFB-free WT, *Nox1$^{-/-}$* and *Tcrb$^{-/-}$* were purchased from Jackson and used immediately for experiment. SFB-free WT and *Nox1$^{-/-}$* mice were inoculated with SFB and housed at University of Pittsburgh animal facility. WT and *Igha$^{-/-}$* mice were housed in pathogen-free conditions at Albany Medical College, NY. *Igha$^{-/-}$* mice were backcrossed a minimum of eight times onto the C57BL/6 background and the absence of unrelated 129 genes was confirmed by SNP analysis.

Generation of *Il17ra$^{fl/fl}$*, *ll17ra$^{fl/fl}$ x Cd4* cre, *ll17ra$^{fl/fl}$* x *villin* cre^{+} and *ll17rc$^{fl/fl}$* x *villin* cre^{+} mice: A schematic of the targeting strategy for the IL17RA allele with PCR primers are shown (Figure S2A). *Il17ra$^{fl/fl}$* mice in C57BL/6 background were generated and tested for SFB. *Cd4* cre and villin cre mice were obtained from Jackson. *Cd4* cre and villin cre mice were SFB-free as revealed by RT-PCR. To determine functional deletion of the IL-17RA in *Il17ra$^{fl/fl}$* mice, we crossed this line to *EIIa* cre, and performed flow cytometry for IL-17RA in peripheral blood (data not shown). In addition, we confirmed recombination at the DNA level by allele specific PCR (data not shown). *Il17ra$^{fl/fl}$* mice were bred to *Cd4* cre, v*illin* cre and *EIIa* cre to generate knockout mice. To generate *Il17ra$^{fl/fl}$ x villin* cre^{+} and *Il17rc$^{fl/fl}$ x villin* cre^{+} conditional knockout mice, we bred heterozygous mice to get mutant as shown in supplemental figure S2A and S5A. We further bred *Il17ra$^{fl/fl}$ x villin* cre^{+} male mice to *Il17ra$^{fl/fl}$ x villin* cre^{-} female mice to get 1:1 cre^{+} and cre^{-} mice. A schematic of the targeting strategy for the *Il17ra* allele with PCR primers are shown in figure S2A and S2B. *Il17rc$^{fl/fl}$* mice were bred to villin cre mice to generate gut epithelial specific conditional knockout mice. A schematic of the targeting strategy for the *Il17rc* allele with PCR primers are shown below.

Genotype/	Primers pair 5'-3'
Il17rc_flox_FP:	GGA AGG CAT GAG GAT TGC AGA CT
Il17rc_flox_RP:	CAG ACT TTC CAG CTT CTT CAG GCT
Il17rc_postCRE_FOR:	TTC TTC AGG GAA GGG CTT AGG ACA
Product size – Genotype (using FP and RP)	WT- 229bp, Het – 229 & 287, Homo - 287
Recombination product size (Using postCRE and RP)	WT-cre: 705, Homo Flox-cre: 897, Het Flox-Cre: 705 & 897, Het Flox Cre^{+}: 297, 705, &897, Homo Flox cre^{+}: 297

SFB-screening and colonization: Bacterial genomic DNA was isolated from the fecal pellet of mice using QiAMP stool DNA extraction kit as per manufacturer's instruction (QIAGEN). qPCR was performed to quantitate relative abundance of SFB and total bacteria (Eubacteria) using group specific rDNA primers SFB736F – GACGCTGAGGCATGAGAGCAT, SFB844R - GACGGCACGGATTGTTATTCA, Eubact Uni340FP - ACTCCTACGGGAGGCAGCAGT, Eubact Uni514RP – ATTACCGCGGCTGCTGGC as described before (Croswell et al., 2009; Salzman et al., 2010). Trizol RNA extraction protocol (Life Technologies) was used to isolate mouse terminal ileum RNA. Mouse terminal ileum RNA was reverse transcribed to cDNA and relative abundance of SFB was determined using SYBR-green based standard qPCR technique. Relative gene expression for SFB was normalized to total bacteria (Eubacteria).

16S rRNA microbial community analysis: Fecal or terminal ileum luminal DNA from *ll17ra$^{fl/fl}$* x *villin* cre^{+} mice as well as littermate control cre^{-} mice (co-housed and 4 weeks after separation = 6 weeks and 10 weeks age) were isolated using QiAMP stool DNA extraction kit. Fecal DNA on day 12-post vehicle or defcr25 inoculated *Il17ra$^{-/-}$* mice were also utilized for microbial community analysis. Microbial community analysis utilized PCR amplification of the V4 region of 16S rRNA followed by sequencing on an Illumina MiSeq as previously described (Caporaso et al., 2012). Sequences were analyzed using QIIME 1.7.0 with default quality trimming except for a minimum q-score of 20 (Caporaso et al., 2010). Taxonomy was assigned utilizing UCLUST alignment to the Greengenes 13.5 taxonomy at 97% similarity (DeSantis et al., 2006; Edgar, 2010). Following taxonomy assignment, 2000 sequences from each sample were chosen to enable even cross-sample comparison.

Differentially abundant OTUs were determined utilizing the group significance script within QIIME. 16S microbial sequencing data was deposited under SRA accession 4677462.3 to 4677494.3 on MG-RAST.

Animal treatments: To assay the effect of anti-microbial peptides on SFB colonization, mice were gavaged with PBS or 10 μg/mouse of active defcr25 (H_2N-CEDLICYCRTRGCKRRERLNGTCRKGHLMYMLWCC-COOH) peptide (Peptide Synthesis Facility, University of Pittsburgh) on days 0, 2, 4, 6, 8, 10. Terminal ileum on day 12-post vehicle or defcr25 treatment was analyzed for SFB colonization by qPCR. To demonstrate feasibility of *per os* approach, we administered a Rhodamine dextran peptide (70kd) or 50 μg Rhodamine-conjugated calmodulin peptide (molecular weight 16 kD, similar to α-defensin) or vehicle by gavage in total volume of 200 μl, and imaged delivery to the lumen of the small intestine within 20 minutes using Bio-rad ChemiDoc.MP imaging system. Both peptides were successfully delivered to the small intestine (Figure S6D and data not shown). Mice were given vancomycin (1 g/L) in drinking water for 30 days to deplete SFB colonization. Mice were intraperitoneally injected with anti-IL-17RA (Kind gift from Amgen) or isotype antibodies (500 μg/mouse) on day 0, 3 and 6. Before and after antibody treatment, relative expression of SFB was determined by qPCR. Mice were injected intraperitoneally with adenovirus expressing IL-17A ($1X10^{10}$ PFU) or empty vector. Terminal ileum on day 7 post adenovirus injection was analyzed for the expression of *Il17a* and several α-defensin transcripts. Construction, generation and quality control of the adenovirus expressing murine IL-17A and control virus has been described (Schwarzenberger et al., 1998).

Microbiota transplantation and co-housing: Four fecal pellets were dissolved in 2 ml sterile PBS and filtered through 100 micron cells strainer. 200 μl of fecal suspension were gavaged into mice. Fecal content was screened by qPCR at various time points for SFB colonization. SFB-free WT and *Tcrb*$^{-/-}$ (Jackson Laboratories) mice were gavaged with SFB-containing feces as described above and co-housed for 2 weeks. After 2 weeks of co-housing, mice were separated based on genotype for additional 30 days. The SFB colonization was estimated on day 30 post separation. WT and *Il17ra*$^{-/-}$ or WT and *Nox1*$^{-/-}$ mice were co-housed for 2 weeks immediately after weaning. The SFB colonization was estimated on day 14 post cohousing.

Enteroid Culture: Approximately 5 cm of mouse proximal jejunal tissue was resected and rinsed with cold PBS to remove debris. The tissue was then transferred to PBS containing 5 mM EDTA and incubated for 30 minutes with gentle shaking at 4°C. After incubation, the tissue was removed, placed into cold PBS, and gently shaken for 30 seconds. The tissue was then transferred to fresh PBS and again shaken. This was repeated 3 times to ensure a high yield of viable crypts for culture. Each fraction was spot-checked, and any with a high amount of debris or low crypt yield was discarded. Fractions that had high yields with low single cell debris were filtered through a 70 μm nylon filter (BD Biosciences). Filtered crypts were pelleted via centrifugation and re-suspended in Matrigel hESC-qualified Matrix (Corning) at a concentration of 40 crypts per 10 μL Matrigel. 10 μL of this suspension was then dispensed per well in 48-well plates. After polymerization of Matrigel for 30 minutes at 37°C and 5% CO_2, the solid matrix was overlaid with 200 μL medium containing advanced Dulbecco's modified Eagle medium (DMEM)/F12, supplemented with 1-X gentamycin/kanamycin, 10 mM Hepes, 1-X GlutaMAX, 1-X N2, 1-X B27 (all from Invitrogen). The following growth factors were also added to the media prior to plating: murine EGF 100 μg/mL, murine noggin100 μg/mL, human R-spondin-1 250 μg/mL (R&D Systems). Media was changed every 4-6 days, and the enteroids were passaged at day 7. Passaging was accomplished by replacing the media with 200 □L cold PBS containing 10 μM Y27632 (Sigma-Aldrich). The Matrigel was then scraped to dissociate the enteroids from the plate, and the entire contents of the well were aspirated using a 30 ½ G needle and dispensed into a new 15 mL conical tube. This was then centrifuged, and the contents re-plated using primary culture concentrations. Passage was performed every 1-2 weeks with a 1:4 split ratio. Enteroids underwent two rounds of passaging before stimulation experiments, thus removing any non-epithelial contaminants in the culture.

Enteroid Stimulation and RNA Isolation: After the final round of passaging, enteroids from three biological replicates were allowed to grow for two days, then stimulated with rIL-17A (0 and 50 ng/mL); Five wells, with a concentration of 40 enteroids per well, were collected per condition at 24 hours, lysed with Qiagen RLT buffer containing β-mercaptoethanol, vortexed, and then immediately stored at -80°C. RNA was subsequently isolated using Qiagen Qiashredder columns for homogenization, followed by an RNeasy Mini Kit preparation, as per manufactures instructions.

RNA sequencing: Total RNA from terminal ileum (1-4 μg) of 6 weeks old WT, *Il17a*$^{-/-}$, *Il17ra*$^{-/-}$, *ll17ra*$^{fl/fl}$ x *villin* cre^{+} and littermate control cre^{-} mice or IL-17A stimulated enteroids were used as starting material for deep sequencing using the in-house Illumina TrueSeq RNA Sample Preparation v2 Guide. Briefly, mRNA was purified with oligo-dT beads, fragmented with magnesium and heat-cataylzed hyrdolysis, and used as a template for first- and second-strand cDNA synthesis with random primers. The cDNA 3' ends were adenylated, followed by adaptor ligation and a 15-cycle PCR to enrich DNA fragments. Quantification of cDNA libraries were performed by using Kapa Biosystems primer premix kit with Illumina-compatible DNA primers. The cDNA libraries were pooled at a final concentration 1.8 pM. Single-readsequencing was performed on Illumina Genome Analyzer IIx and NextSeq 500.

Analysis of gene expression: Raw reads from Illumina NextSeq500's in fastq format were trimmed to remove adaptor/primer sequences. Trimmed reads were then aligned using BWA (version 0.5.9, settings aln -o 1 -e 10 -i 5 -k 2 -t 8) against the mouse genome build 37.2 in geneSifter Analysis Edition for Next Generation Sequencing (Geospiza, Seattle, WA). Additional alignment and post-processing were done with Picard tools (version 1.58) including local realignment and score recalibration to generate a finale genomic aligned set of reads. Reads mapping to the genome were characterized as exon, intron, or intergenic using the matched annotation for the genomic reference sequence. The remaining unmapped reads from the genomic alignment were then aligned to a splice reference created using all possible combinations of known exons and then categorizing these as known or novel splice events. This aligned data was then used to calculate gene expression by taking the total of exon and known splice reads for each annotated gene to generate a count value per gene. For each gene there was also a normalized expression value generated in two ways: 1) Reads per Mapped Million (RPM), which was calculated by taking the count value and dividing it by the number of million mapped reads, 2) Reads per Mapped Million per Kilobase (RPKM), which was calculated by taking the RPM vale and dividing it by the kilobase length of the longest transcript for each gene. The RPM values were subsequently used for comparing gene expression across samples to remove the bias of different numbers of reads mapped per sample. RPKM values were subsequently used for comparing relative expression of genes to one another to remove the bias of different numbers of mapped reads and different transcript lengths.

EAE: *ll17ra*$^{fl/fl}$ x *villin* cre^{+} and littermate control cre^{-} mice were used for EAE experiments. Co-housed cre^{+} and cre^{-} mice were separated and kept in separate cage 1 week before EAE induction. These mice were immunized at both sites on the hind flank with 100 μg peptide corresponding to the immunodominant epitope of myelin oligodendrocytes glycoprotein (MOG_{35-55}) (Bio-Synthesis, Inc.) in 200 μl CFA containing 100 μg *M. tuberculosis* strain H37Ra (Difco) as described previously (McGeachy et al., 2009). EAE was also induced after 2 weeks of vancomycin (1 g/L) or control water treatment. Vancomycin or control water-treated SFB^{+} *ll17ra*$^{fl/fl}$ x *villin* cre^{+} mice were immunized as above. Mice were maintained on antibiotic water throughout EAE time course. All immunized mice also received 200 ng of pertussis toxin (Sigma-Aldrich) intraperitoneally on days 0 and 2. Mice severity scores for EAE were evaluated blindly according to the following scale: 1: flaccid tail; 2: impaired righting reflex and hind limb weakness; 3: partial hind limb paralysis; 4: complete hind limb paralysis; 5: hind limb paralysis with partial fore limb paralysis; 6: moribund.

Scanning Electron Microscopy: 1 cm pieces of 6 week old mouse distal small intestine were cut open and fixed in 2.5% glutaraldehyde and 2% paraformaldehyde in PBS (pH 7.2) for 4 hours and processed for standard SEM at Center of Biologic Imaging, University of Pittsburgh. Processing of tissue included incubation of tissue in 2.5% glutaraldehyde for 1 hour followed by three washes with PBS. Tissues then stained with 1% OsO_4 for 1 hour followed by 3 washes with 1x PBS. After PBS, tissues were then dehydrated through a series of ethanol washes (30%, 50%, 70%, 90% and 100%) followed by three changes with 100% hexamethyldisilazane. Finally, tissues were mounted on SEM stubs. Images were taken on a JEOL 6550 FEGSEM microscope. SEM images of SFB were taken at 0.5K and 2K magnification.

Th17 differentiation: Naïve CD4 (CD4^{+}CD62L^{+}) cells from WT and *Il17ra*$^{-/-}$ or Thy1.1 reporter (IL-17F) mice were isolated from spleen using STEMCELL Technologies T cell isolation kit, and polarized under Th17 differentiating condition. Briefly, cells were culture in plate bound anti-CD3 (2.5 μg/ml) and anti-CD28 (2 μg/ml) in the presence of IL-6, TGF-β, IL-23, anti-IFNγ (Ebiosciences) and anti-IL-4 (Ebiosciences) antibodies for 4 days plus 10 ng/ml of recombinant IL-17A, IL-17B, IL-17C, IL-25, or IL-17F for 4 days. All recombinant cytokines were purchased from R&D system.

Lamina propria lymphocyte isolation: Briefly, 10 cm pieces of terminal small intestine were separated from mesentery and Peyer's patches were carefully excised. Tissues were opened longitudinally and washed with HBSS. Epithelial cells were separated from lamina propria by incubating 1 cm pieces of small intestine in 5 mM EDTA on a shaker (100 rpm) for 10 minutes. Tissue were washed with HBSS without EDTA until supernatant was not cloudy. Tissues were cut into small pieces and incubated for 10 minutes at 37^0C in HBSS containing 0.3 mg/ml collagenase, 0.1 mg/ml DNase, 1 mM $CaCl_2$ and 1 mM $MgCl_2$. 10% FBS was added and digested tissue suspension was filtered using 70 micron cell strainer. After centrifugation, digested tissue pellet was re-suspended with 44% percoll and layered over 67% percoll. Mononuclear cells were isolated from an interphase of percoll gradients.

Flow cytometry: Lymphocytes were isolated from lamina propria and spleen of co-housed gender matched WT and *Il17ra*$^{-/-}$ mice or intestinal IL-17R knockout mice. WT and *Il17ra*$^{-/-}$ mice mice were co-housed for 2 weeks immediately after weaning. Single cell suspension from splenocytes or lamina propria and peripheral blood lymphocytes from these mice or vancomycin (1 g/L drinking water for 4 weeks) treated mice were re-stimulated with phorbol 12-myristate 13-acetate (50 ng/ml) and ionomycin (750 ng/ml) in the presence of brefeldin A (5 µg/ml) for 4 hours. After stimulation, cells were stained intracellularly with Ebiosciences antibodies against CD3 (17A2), CD4 (RMA4-5), IL-17 (ebio17b7), IL-22 (IL22JOP). For SFB-tetramer staining lamina propria lymphocytes were first stained with SFB-tetramer in the presence of 2% rat serum, 2% mouse serum, and CD16/CD32.Fc block in flow cytometry buffer (1% BSA, 0.05% sodium azide in PBS). Lymphocytes were washed with flow cytometry buffer and surface stained with anti-CD3 and anti-CD4 antibodies. Stained lymphocytes were acquired using BD LSR II flow cytometer and flow cytometric data was analyzed with the flow cytometry Diva software.

ELISPOT: MOG-specific IL-17A-producing T cells from the spleen of day 9 EAE induced mice were enumerated using peptide-driven ELIS POT. Briefly, 96-wells ELISPOT plates were coated with monoclonal anti-mouse IL-17, blocked with media containing 10% FBS. Cells from spleen were seeded at an initial concentration of 5×10^5 cells/well and subsequently diluted two fold. Irradiated B6 splenocytes were used as APCs at a concentration of 1×10^6 cells/well in the presence of MOG (10 µg/ml) peptide. After 24 hours, plates were washed and probed with biotinylated anti-mouse IL-17. Spots were visualized and enumerated using a CTL-Immunospot S5 MicroAnalyzer and corrected with CD4 T cells number. Absolute $CD4^+$ T cells percentage and number were determined by using flow cytometry and cells count. No spots were detected in cultures lacking antigen or when using cells from uninfected mice.

Luminex: Blood was collected on day 9 post EAE induction and centrifuged to isolate serum. We used a Bio-Plex Pro Assay (4-plex; Bio-Rad) according to the manufacturer's recommendations. Briefly, the plate was treated with Bio-Plex assay buffer, followed by vortexing and two washes. Serum (undiluted), standards, and blanks were then added to the plate and incubated at room temperature for 1 hour, at 850 rpm, covered. Following three washes, detection Abs were then diluted and added to each well. The plate was incubated as above. Following three washes, diluted streptavidin-PE was added to each well and incubated at room temperature for 20 minutes on shaker. The plate was then washed three times and resuspended in assay buffer, and beads were quantified using a Bio-Plex MAGPIX (Bio-Rad).

RT-PCR: Small pieces (3 cm) of terminal ileum were excised and stored in RNA later until use. Tissues were homogenized and RNA was isolated using Trizol RNA isolation techniques as per manufacturer's instruction. CD4 T-cells were sorted from spleen and RNA were isolated using RNeasy kit per manufacturer's instruction. Isolated RNA were reverse transcribed into cDNA using Biorad IScript kit. Biorad Sso advanced supermix were used for qPCR. Applied Biosystem primer-probe for *Il17a* (Mm00439618_m1), *Il22* (Mm00444241_m1), *Il17ra* (Mm00434214_m1), *Il17rb* (Mm00444709_m1), *Il17rc* (Mm00661861_m1), *Il17rd* (Mm00460340_m1) *Il17re* (Mm01189488_m1), *Pigr* (Mm00465049_m1), *Nox1* (Mm00549170_m1), *Csf2* (Mm01290062_m1), *Csf3* (Mm00438335_g1) and *Hprt* (Mm00446968_m1) or IDT primer-probe for defa25 (Forward Primer-CCA AGC TGT TTC TGT CTC CTT, Reverse Primer - CCA TTC AGG CGT TCT CTT CTT and probe- FAM/TG GAG ACC C/Zen/A GAA GGC TCT TCT CT/3IABkFQ) were used for RT-PCR. Gene expression were quantified and normalized to *Hprt*. RT-PCR was also performed using Bio-rad SYBR-green supermix and qPCR primer for C57BL/6.Defa4 (Forward P- CCAGGGGAAGATGACCAGGCTG and Reverse P – TGCAGCGACGATTTCTACAAAGGC) and pan-defensin (Forward P- GGTGATCATCAGACCCCAGCATCAGT and Reverse P - AAGAGACTAAAACTGAGGA GCAGC). Gene

expression was quantified and normalized to *Gapdh* (Forward P – TCATCAACGGGAAGCCCATCAC and Reverse P – AGACTCCACGACATACTCAGCACCG).

ELISA: G-CSF ELISA was performed using serum of mice. Blood was collected on day 9 post EAE induction and centrifuged to isolate serum. ELISA was performed as per manufacturer's instructions (Ray Biotech). GM-CSF ELISA was performed using cell culture supernatant. Lamina propria lymphocytes were isolated from day 9 MOG immunized *Il17ra$^{fl/fl}$* x *villin* cre^{+} and littermate control *Il17ra$^{fl/fl}$* control mice, and re-stimulated with MOG peptide (200 μg/ml) for 24 hour. Cell culture supernatants were analyzed for GM-CSF using BioLegend mouse GM-CSF ELISA kit as per manufacturer's instruction (BioLegend).

IgA western: Fecal pellets (1-2 pellets) were collected from different mouse strains (littermate controls) and dissolved in 400 μl PBS containing protease inhibitors (Roche Diagnostic). BCA assay was performed to quantify protein and 20 μg protein used for western blot. Western blot were performed in non-reducing condition using 4-10% SDS-PAGE gels (Bio-Rad system) and transferred to nitrocellulose membranes. The blot was probed with goat anti-mouse IgA-HRP (Southern Biotech). After incubation with IgA-HRP-conjugated anti-mouse antibody, membranes were washed and incubated with ECL kit (GE Healthcare, Piscataway, NJ). Signal was detected using Bio-Rad ChemiDoc.MP imaging system.

Amplex red assay: 3 cm pieces of mouse distal small intestine were collected, and fecal contents were gently removed. 200 μl of PBS were used to lavage intestinal loop. This step was repeated thrice using the same flow through PBS. Luminal lavages were centrifuge, and supernatant were collected for the assay. 50 μl of intestinal lavage were subjected to Amplex red assay according to manufacturer's instructions (Life Technologies). Different known concentrations of H_2O_2 were used as standards. Plate reading was taken at 560 nm absorbance.

Supplemental references

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