Supplemental EXPERIMENTAL PROCEDURES

Confirmation of SFB Colonization

Genomic DNA was purified from 1-2 mm terminal ileum or fresh fecal pellets by phenol-chloroform extraction and dissolved with TE buffer containing 100 ng/ml of RNase A. SFB-specific 16S was detected by real-time qPCR. To normalize SFB copy number, universal 16S and/or host genomic DNA (*mll-23r* locus) was also detected by real-time qPCR. Copy numbers were calculated with plasmid standards.

Isolation of Lamina Propria Lymphocytes and Epithelial Cells

Mesenteric fat tissue and Peyer's patches were carefully removed from intestinal tissues. The proximal 1/3 and distal 1/3 of small intestine were designated as duodenum and ileum, respectively. Tissues were incubated in 5 mM EDTA in PBS containing 1 mM of DTT for 20 min at 37°C with rotation (200 rpm). EDTA-DTT washes were pelleted to enrich for intestinal epithelial cells. Remaining tissues were incubated in a second EDTA wash (5 mM EDTA in PBS). Tissues were then further digested (10% fetal calf serum, 1.0 mg/ml each of Collagenase D (Roche) and 100 μg/ml DNase I (Sigma), and 50 U/ml Dispase (Worthington)) at 37°C for 30 min. Digested tissues were then passed through a 70 μm cell strainer. Mononuclear cells were enriched by 40:80 Percoll gradient. Lamina propria (LP) lymphocytes were collected from the Percoll gradient interphase.

RNA was prepared using Trizole (Invitrogen) or RNA easy kit (Qiagen) following manufacturer protocols.

Cell Staining for Flow Cytometry

Live-dead labeling with DAPI (Sigma) and/or Aqua (Invitrogen) was performed and only live cells were analyzed. Fixed cells were permeabilized using Permeabilization buffer (eBiosciences) and further stained with anti-Foxp3, anti-RORγt and anti-GFP using the Foxp3 staining buffer set (eBiosciences). For analysis of ILC3s, live LP cells were stained with anti-NK1.1, anti-Klrg1, anti-Klrb1b (clone 2D12, subclone from 2D9) as previously described (Aust et al., 2009), anti-CD4, anti-Sca-1, anti-NKp46 and anti-CCR6. Anti-CD11b, anti-CD14, anti-CD19, anti-B220, anti-TCRβ, anti-TCRγδ, and anti-CD3 were used as dump gate.

To detect IL-22 from Th17 cells and ILC3s, isolated LP cells were incubated with or without 50 ng/ml phorbol myristate acetate (PMA) (Sigma), 500 ng/ml Ionomycin (Sigma) in the presence of GolgiSTOP (BD Biosciences) in complete media at 37°C for four hours. Cells were stained with anti-TCRγδ, anti-TCRβ, anti-CD3, anti-CD4, anti-NKp46 and anti-CCR6. Anti-CD11b, anti-CD14, anti-CD19, anti-B220 were also used as dump gate. Cells were further stained intracellularly with anti-Foxp3, anti-RORγt and anti-IL-22 using the Foxp3 staining buffer set. Flow cytometric analysis was performed on an LSRII (BD Biosciences) or an Aria (BD Biosciences). All data were re-analyzed using FlowJo (Tree Star). MHC class II tetramers with a bound immunodominant SFB antigen were

previously described (Yang et al., 2014). Commercially available antibodies used in flow cytometry experiments above are listed in Supplementary Table 1.

RNA FISH

Intestinal tissues were prepared as Swiss rolls, and placed directly into Methacarn fixation buffer (60% absolute methanol, 30% chloroform, 10% glacial acetic acid) overnight at 4°C. Tissues were washed 3 times in 70% ethanol. Tissues were embedded with paraffin and processed into 5 μm sections. Tissue sections were deparaffinized in 2 changes of xylene. Tissues were then rehydrated in successive ethanol washes (95% and 90%). Following the final rehydration in ddH_2O, tissues were treated with warm (56°C) hybridization buffer (0.9 M NaCl, 20 mM Tris-HCl pH 7.2, 0.1% SDS) with 500 nM SFB probe and 250 nM 16S probe (described in primer information panel). Tissues were placed into sealed, humidifying chamber and incubated in humidifier overnight at 50°C. Tissues were washed 2 times in warm (56°C) wash buffer (0.9 M NaCl, 20 mM Tris-HCl pH 7.2) for 10 min, stained with 1 mg/ml DAPI for 15 min and washed twice in wash buffer for 10 min. Slides were dried and mounted with Prolong Gold Antifade Reagent (Life technologies). Images were taken on a Zeiss 710 confocal microscope.

RNAseq Analysis

Naïve sorted $CD4^+$ T cells were cultured in 20 ng/ml IL-6 and 0.1 ng/ml TGF-β for 48h in the presence or absence of 2 μg/ml rmSAA1 before collection of RNA.

RNAseq libraries were prepared and sequenced by the Illumina RapidRun at the Genome Services Laboratory, HudsonAlpha. RNAseq data from two biological replicates were mapped to mm10 genome by Tophat2.0 and compared using CuffDiff with biased correction. Genes significantly up or downregulated more than two-fold with p-value<0.05 were considered significantly affected by mSAA1 treatment in vitro. Ingenuity Pathway Analysis was used to identify upstream regulators for the SAA-affected gene set.

For comparison of Th17 cells and ILC3 expression profiles, *Il17a*$^{gfp/gfp}$ and *Il23r*$^{gfp/+}$ reporter animals stably colonized with SFB were used to sort CD3^{+}CD4^{+}IL-17AGFP^{+} Th17 cells and CD3^{-}CD4^{-}IL-23RGFP^{+} ILC3 respectively. RNAseq libraries were prepared and sequenced. Normalized DESeq reads were used for data analysis. RNAseq datasets are available on GEO (GSE70599).

Ribosome TRAP Analysis

EF1-lox-stop-lox-GFP-L10 animals were crossed to epithelial specific (*Villin*-Cre), T cell specific (*Cd4*-Cre), myeloid cell specific (*Cd11c*-Cre), or liver cell specific (*Albumin-Cre*) transgenic lines to express the EGFP-L10a protein in the respective cell populations (Stanley et al., 2013). Epithelial or lamina propria cell extracts were subject to anti-ribosome IP using 2 μg anti-GFP antibodies (Invitrogen) and harvested in Protein G magnetic Dyna beads (Invitrogen) as described previously (Heiman et al., 2014). Complementary DNAs (cDNAs) were synthesized from TRIzol (Invitrogen)-isolated RNA, using Superscript III (Invitrogen). RNAseq libraries were prepared and sequenced by the Illumina

RapidRun at the Genome Services Laboratory, HudsonAlpha. Sequencing reads were mapped by Tophat and transcript levels were determined by Cufflinks. Ingenuity Pathway Analysis was used to identify the enriched upstream signaling pathways for mRNAs with increased ribosomal association in response to SFB colonization. TRAPseq datasets are available on GEO (GSE70599).

IL-22 Blockade

Mice were injected with mIL-22-specific mAb (clone 8E11; Genentech) at a dose of 150 μg per mouse intraperitoneally every other day starting at day one prior to introduction of SFB, as previously reported (Zheng et al., 2008). Control groups received isotype control IgG1 mAb (Genentech). Four days after gavage, total RNA from the ileum epithelial fraction was purified to evaluate SAA1/2 mRNA transcripts by qRT-PCR. Three days after SFB^e gavage, 5,000 naïve T cells from IL-17A-GFP reporter 7B8 Tg mice were adoptively transferred and IL-17A producing cells were detected among $Foxp3^-$ $ROR\gamma t^+$ transferred 7B8 T cells.

Organoid Culture

Organoid isolation was performed as described (Sato and Clevers, 2013). Briefly, crypts were collected by incubation with 5 mM EDTA in PBS. Isolated crypts were re-suspended with Matrigel (Invitrogen) and plated onto 24-well plates with Advanced DMEM/F12 media (Invitrogen) with 1x N2 supplement (Invitrogen), 1x B27 supplement (Invitrogen), 1 mM N-Acetylcycteine (Sigma), 100 ng/ml of mNoggin (Peprotech), 50 ng/ml mEGF (Invitrogen), and 1 μg/ml hR-Spondin

(R&D) (complete organoid culture medium). Five or seven days after plating, budding organoids were treated with various concentrations of rIL-22 (Peprotech) or PBS in complete crypt culture medium for two or six hours. Total RNA was purified to evaluate SAA1/2 mRNA transcripts by real-time qPCR.

SFB-specific Transgenic T Cell Transfer

Five thousand naïve ($CD4^+$, $CD62L^{hi}$, $CD44^{low}$, $CD25^-$, $TCRV\beta14^+$ T cells were isolated from murine spleen and lymph nodes of 7B8 TCR Tg mice on the Ly5.1 background by FACS sorting. Sorted naïve T cells were stained with CellTrace CFSE Cell Proliferation kit (Life Technology) to follow cell division, and administered into each congenic Ly5.2 recipient mouse by retro-orbital injection. Animals were sacrificed on day four or seven, and lamina propria mononuclear cells from MLN and ileum were harvested as described (Yang et al., 2014). Ex vivo restimulation was performed for five hours in the presence of 500 ng/mL PMA, 500 ng/mL Ionomycin and GolgiStop. For 7B8-IL17A-IRES-GFP transfer experiments, cells were fixed in Foxp3 staining buffer from eBioscience and stained for CD45.1, $TCRV\beta14$, $ROR\gamma t$, Foxp3, and anti-GFP-Alexa488 antibodies.

Mouse and Human T Helper Cell Culture

Mouse naïve T cells were purified from lymph nodes and spleens of six to eight week old C57BL/6 mice by sorting $DAPI^-$, $CD8^-$, $CD19^-$, $CD4^+$, $CD25^-$, $CD62L^+$, $CD44^{low/Int}$ on a FACSAria (BD Biosciences). Cells were cultured in IMDM (Sigma) supplemented with 10% heat-inactivated FBS (Hyclone), 50 U penicillin-

streptomycin (Invitrogen), 2 mM glutamine, and 50 µM β-mercaptoethanol. For T cell polarization, 200 µl cells were seeded at $0.3x10^5$ cells per mL in 96-well plates pre-coated with 5 µg/mL anti-CD3ε (eBioscience, 145-2C11) and 10 µg/mL anti-CD28 (eBioscience, clone 37.51). Cells were cultured for five days in either suboptimal Th17 conditions (0 - 0.1 ng/mL TGF-β (Peprotech), 20 ng/mL IL-6 (Peprotech)), Th1 conditions (10 ng/mL IL-12 (Peprotech), 10 U/mL IL-2 (Peprotech), and 2 ng/mL anti-IL-4 (eBiosciences)), or Th0 conditions (10 U/mL IL-2). For cytokine analysis, cells were incubated for five hours with 50 ng/mL PMA, 500 ng/mL ionomycin and GolgiStop. LSR II (BD Biosciences) and FlowJo software were used for flow cytometry and analysis. Dead cells were excluded using the Live/Dead fixable aqua dead cell stain kit (Invitrogen). Human T cells were isolated from peripheral blood of healthy donors using anti-human CD4 MACS beads (Miltenyi). Th17 polarizing conditions were previously described (Manel et al., 2008). Human CD4 T cells were cultured in 96-well U bottom plates in 10 U/ml of IL-2, 10 ng/mL of IL-1β, 10 ng/ml of IL-23, 1 µg/ml of anti-IL-4, 1 µg/mL of anti-IFNγ and anti-CD3/CD28 activation beads (LifeTechnologies) at a ratio of 1 bead per cell. Recombinant mouse SAA1 and human SAA1 were purchased from R&D and Peprotech, respectively, and resuspended in 0.1% BSA PBS and were used in in vitro T helper cell cultures. Anti-human SAA antibodies were purchased from Abcam (Clone 115) and used at 5 µg/ml in human Th17 in vitro culture assays. Wildtype naïve mouse $CD4^+$ T cells were cultured in Th17 polarizing condition with the addition of 2 µg/ml rmSAA1 at 0h or

24h in the presence or absence of 2 μg/ml monoclonal rabbit anti-mouse SAA antibodies (LifeTechnology, 700830).

RORγt Transcriptional Activity in Polarized Th17 Cells

Murine Th17 cells were cultured for 48h in the presence or absence of 2 μg/ml rmSAA. One μg RORE-Firefly luciferase reporter construct and one μg control renilla construct were introduced into cultured Th17 cells using the Amaxa Nucleofector 4D (Lonza) according to the manufacturer's instructions. Luciferase activity was measured using the dual luciferase reporter kit (Promega) at 24h after transfection. Relative luciferase units (RLU) were calculated as a function of Firefly luciferase reads over those of renilla luciferase.

Immunofluorescence Microscopy

Terminal ileum from SFB^{e}- or JAX-gavaged mice were Swiss-rolled, fixed for four hours in 4% paraformaldehyde, incubated overnight in 30% sucrose, and frozen in OCT. Tissue was cut into five μM sections, blocked in TBS-T+G (0.3% Triton X-100, 5% Normal goat serum), and incubated overnight in primary antibody (anti–mouse CD326/EpCAM-APC, clone G8.8; eBioscience, and anti–mouse P-Stat3 (Y705), clone D3A7; Cell Signaling) in TBS-T+G. Tissue was washed and incubated with Alexa Fluor 488 Goat anti-Rabbit IgG (H+L) (Molecular Probe) for 1h before DAPI staining. Tissue was imaged using an LSM 710 confocal (Carl Zeiss) and images were processed using ImageJ.

qRT-PCR

Quantitative RT- PCR was performed using the Hot Start-IT SYBR Green (Afymetrix) on the Roche real-time PCR system (Roche 480). Serial dilutions of plasmid standards were included for each gene to generate a standard curve to allow for calculation of the input amount of cDNA for each gene. For analysis of mRNA transcripts, RNA samples were treated with DNase (Roche) prior to cDNA synthesis to avoid effect of DNA contamination. Gene-specific primers spanning exons were used (table below). Values were normalized to GAPDH for each sample.

Primer information

Name	Forward	Reverse
bUniF340 (Universal 16S)	ACTCCTACGGGAGGCAGCAGT	ATTACCGCGGCTGCTGGC
bSFB736F (SFB 16S)	GACGCTGAGGCATGAGAGCAT	GACGGCACGAATTGTTATTCA
genomic IL23R	GCAAATGGAAATGTCAGCAGAGCC	GCAGCTCACTTTCAGTAATCTGGG
mIl17a (mRNA)	TTTAACTCCCTTGGCGCAAAA	CTTTCCCTCCGCATTGACAC
mIl17f (mRNA)	TCCCCTGGAGGATAACACTG	GGGGTCTCGAGTGATGTTGT
mIl22 (mRNA)	CCGAGGAGTCAGTGCTAAGG	CATGTAGGGCTGGAACCTGT

mIl23R (mRNA)	AGAGACACTGATTTGTGGGAAAG	GTTCCAGGTGCATGTCATGTT
mRORγt (mRNA)	GACAGGGAGCCAAGTTCTCA	CTTGTCCCCACAGATCTTGCA
mSaa1/2 (mRNA)[#]	AGTGGCAAAGACCCCAATTA	GGCAGTCCAGGAGGTCTGTA
mGAPDH (mRNA)	AATGTGTCCGTCGTGGATCT	CATCGAAGGTGGAAGAGTGG
mIfnγ (mRNA)	ACAGCAAGGCGAAAAAGGATG	TGGTGGACCACTCGGATGA

[#] Both SAA1 and 2 mRNAs were detected by the same primer set.

Name	Sequence	Modification
SFB FISH probe	GCGAGCTTCCCTCATTACAAGG	5Alex546N/

Antibody Information

For flow cytometry and cell sorting		
Name	**Clone number**	**Company**
Anti-Mouse CD3e	145-2C11	eBioscience
Anti-Mouse CD4	RM4-5	eBioscience
Anti-Mouse CD8	53-6.7	eBioscience
Anti-Mouse CD11b	M1/70	eBioscience
Anti-Mouse CD14	5a2-8	eBioscience
Anti-Mouse CD16/32 Fc Block	2.4G2	Tonbo
Anti-Mouse CD19	1D3	Tonbo
Anti-Mouse CD25	PC61.5	eBioscience
Anti-Human/Mouse CD44	1M7	eBioscience
Anti-Mouse CD45.1	A20	eBioscience
Anti-Mouse CD45.2	104	eBioscience
Anti-Mouse CD196 (CCR6)	140706	BD
Anti-Mouse/Rat IL-17A	eBio17B7	eBioscience
Anti-Human IL-17A	eBio64DEC17	eBioscience
Anti-Human CD4	OKT4	eBioscience
Anti-Human IL-17F	SHLR17	eBioscience
Anti-Human/Mouse RORγt	AFKJS-9	eBioscience
Anti-Human/Mouse IL-22	IL22JOP	eBioscience
Anti-GFP Alexa 488	(Polyclonal)	Molecular Probe
Anti-GFP Biotin	5F12.4	eBioscience
Anti-Mouse TCRb	H57-597	eBioscience
Anti-Mouse gd TCR	eBioGL3	eBioscience
Anti-Mouse Vb14 TCR	14.2	BD
Anti-Mouse NK1.1	PK136	eBioscience
Anti-Mouse CD335 (NKp46)	29A1.4	eBioscience
Anti-Mouse KLRG1	2F1	eBioscience
Anti-Mouse Ly-6A/E (Sca-1)	D7	BioLegend/ebioscience
Anti-Mouse MHCII (I-A/I-E)	M5/114.15.2	eBioscience
Streptavidin		eBioscience
Anti-Mouse RORγt	B2D	eBioscience
Anti-Mouse/Rat Foxp3	FJK-16s	eBioscience
Anti-Mouse B220	RA3-6B2	eBioscience
Anti-Human/Mouse T-bet	eBio4B10	eBioscience
Anti-Human/Mouse GATA-3	TXAJ	eBioscience
Anti-Mouse IL-13	eBio13A	eBioscience
Anti-Mouse IL17-F	9D3.1C8	BioLegend
Anti-Mouse/Rat Ki-67	SolA15	eBioscience

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