

## Supplemental Figures

### **MyD88 signaling in T cells directs IgA-mediated control of the microbiota to promote health**

Jason L. Kubinak<sup>1</sup>† , Charisse Petersen<sup>1</sup>† , W. Zac Stephens<sup>1</sup>, Ray Soto<sup>1</sup>, Erin Bake<sup>1</sup>,  
Ryan M. O'Connell<sup>1</sup> and June L. Round<sup>1,2</sup>

<sup>1</sup>Department of Pathology, Division of Microbiology and Immunology, University of Utah School of Medicine, Salt Lake City, UT 84112 <sup>2</sup> To whom correspondence should be addressed [june.round@path.utah.edu](mailto:june.round@path.utah.edu)

† Co- first authors

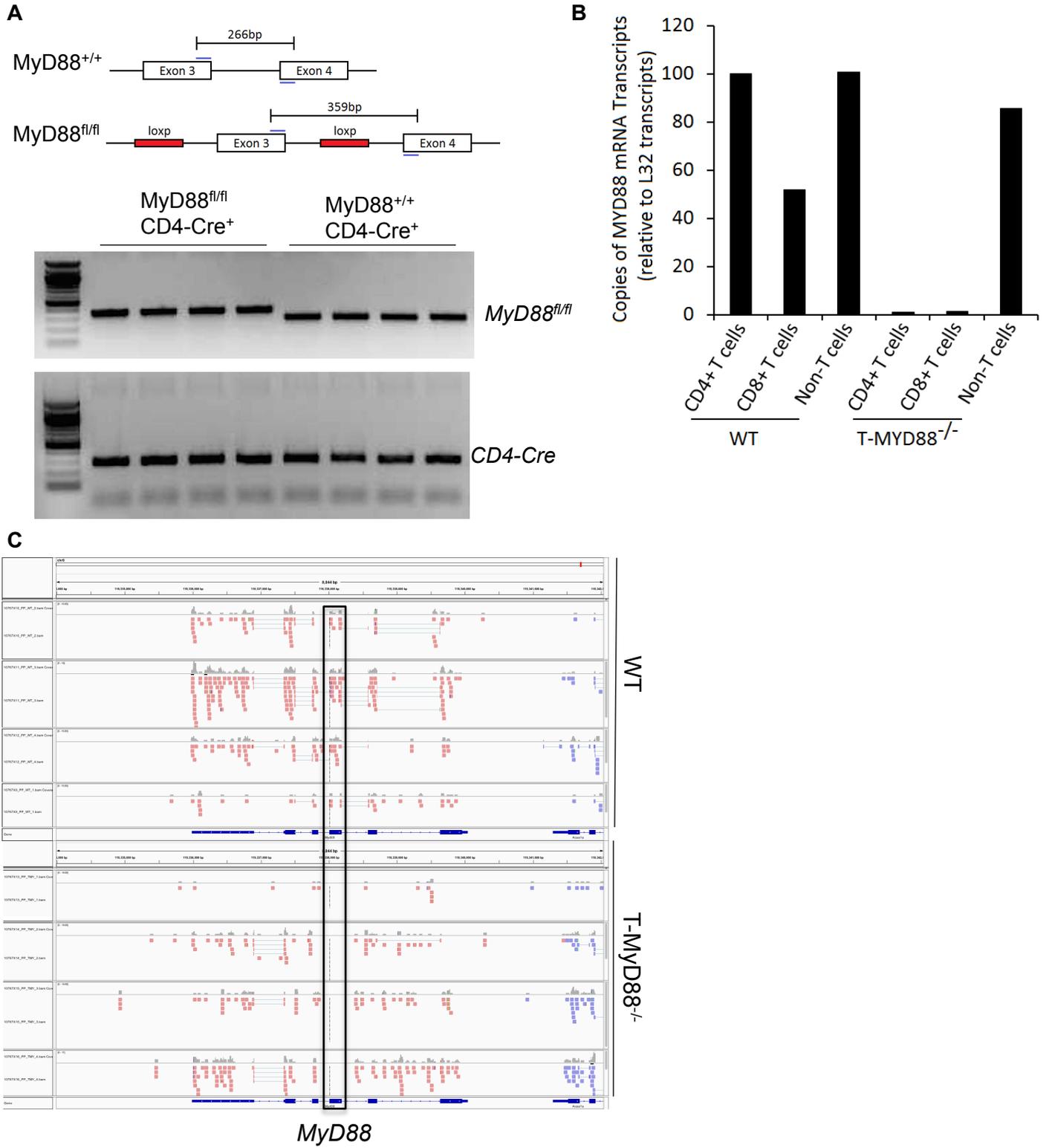


Fig. S1.

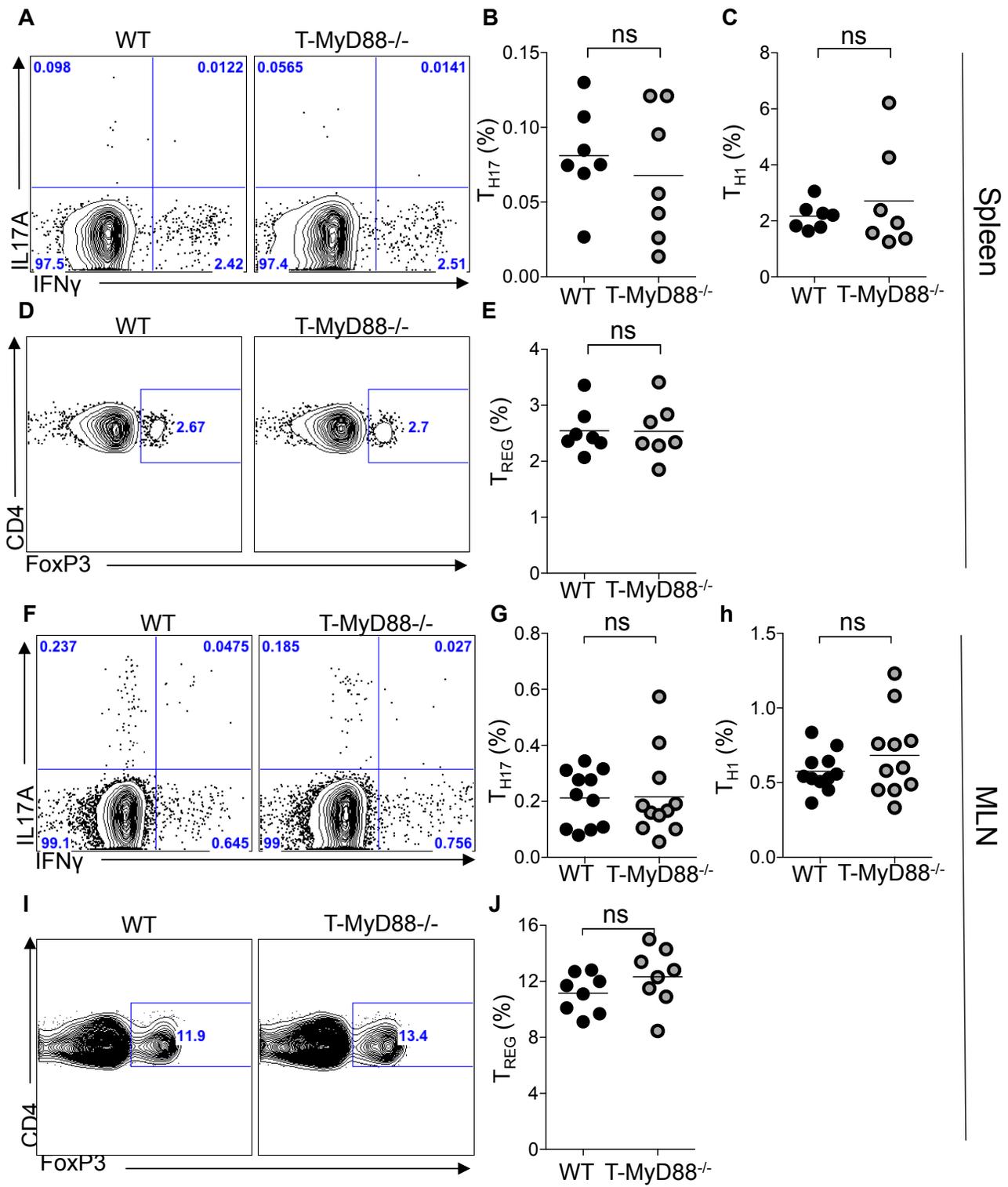


Fig. S2.

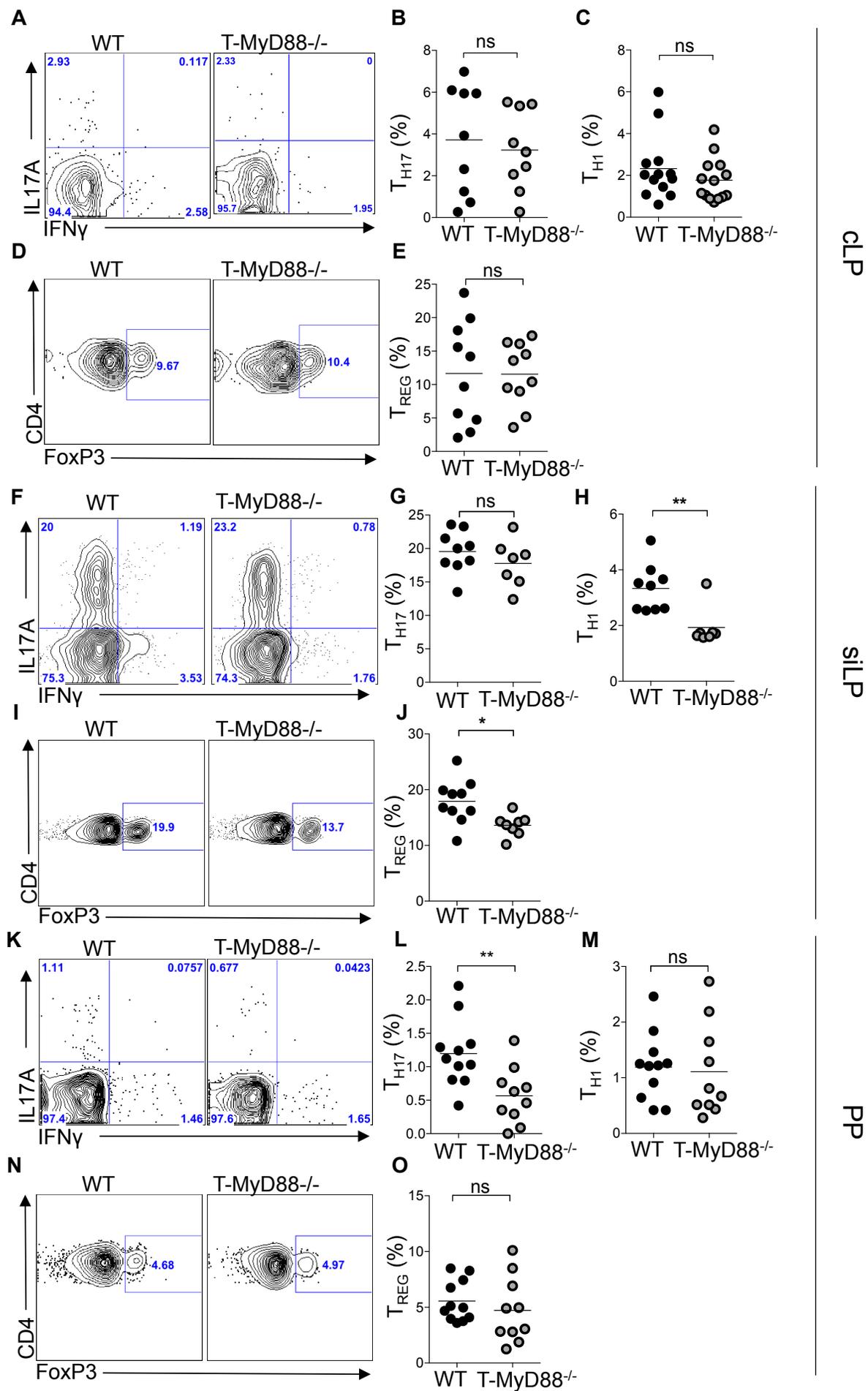
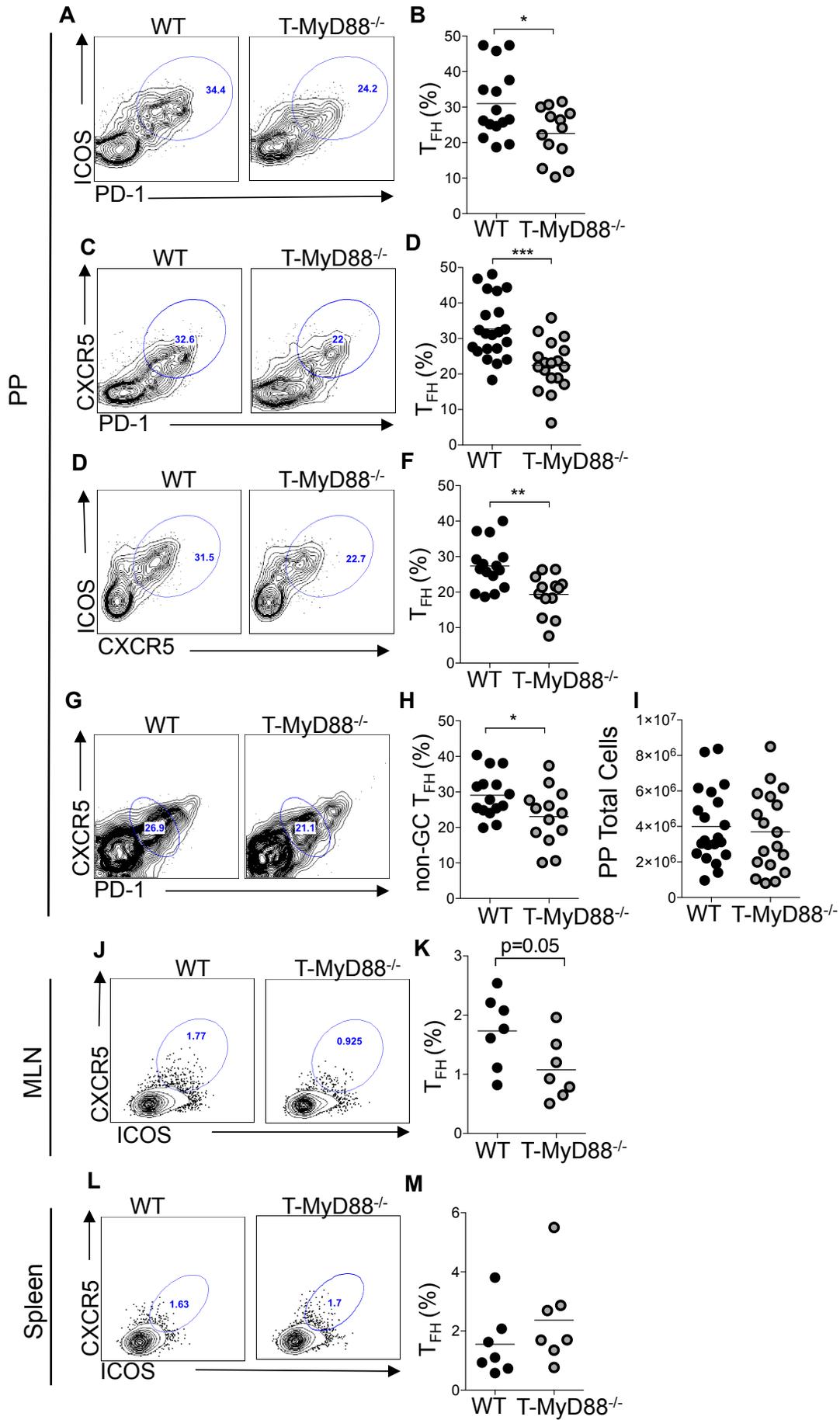
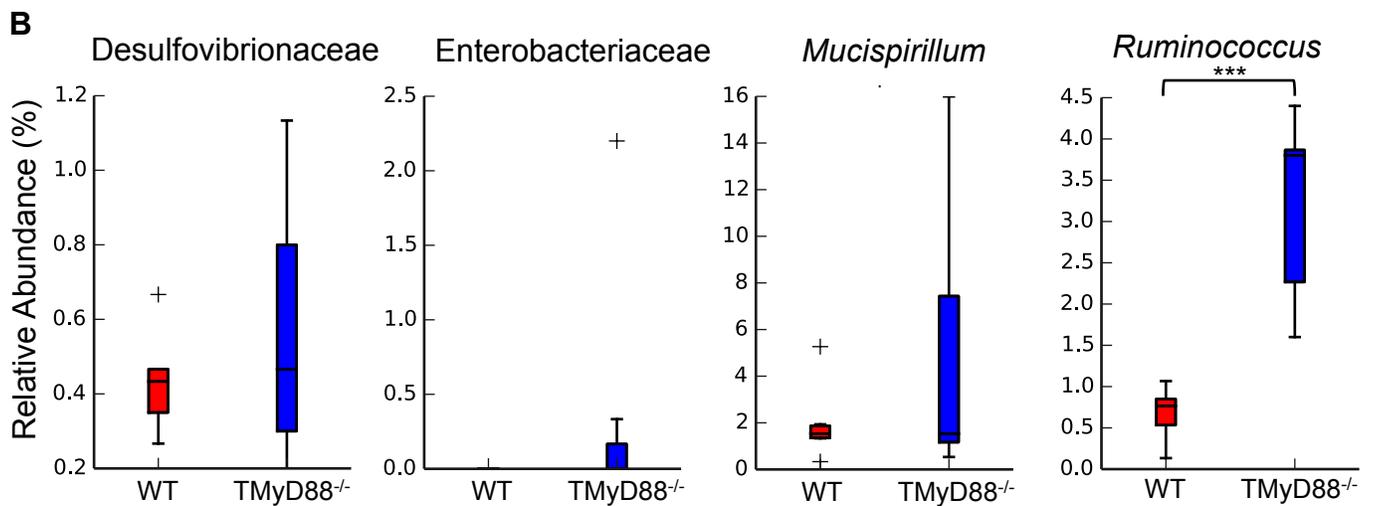
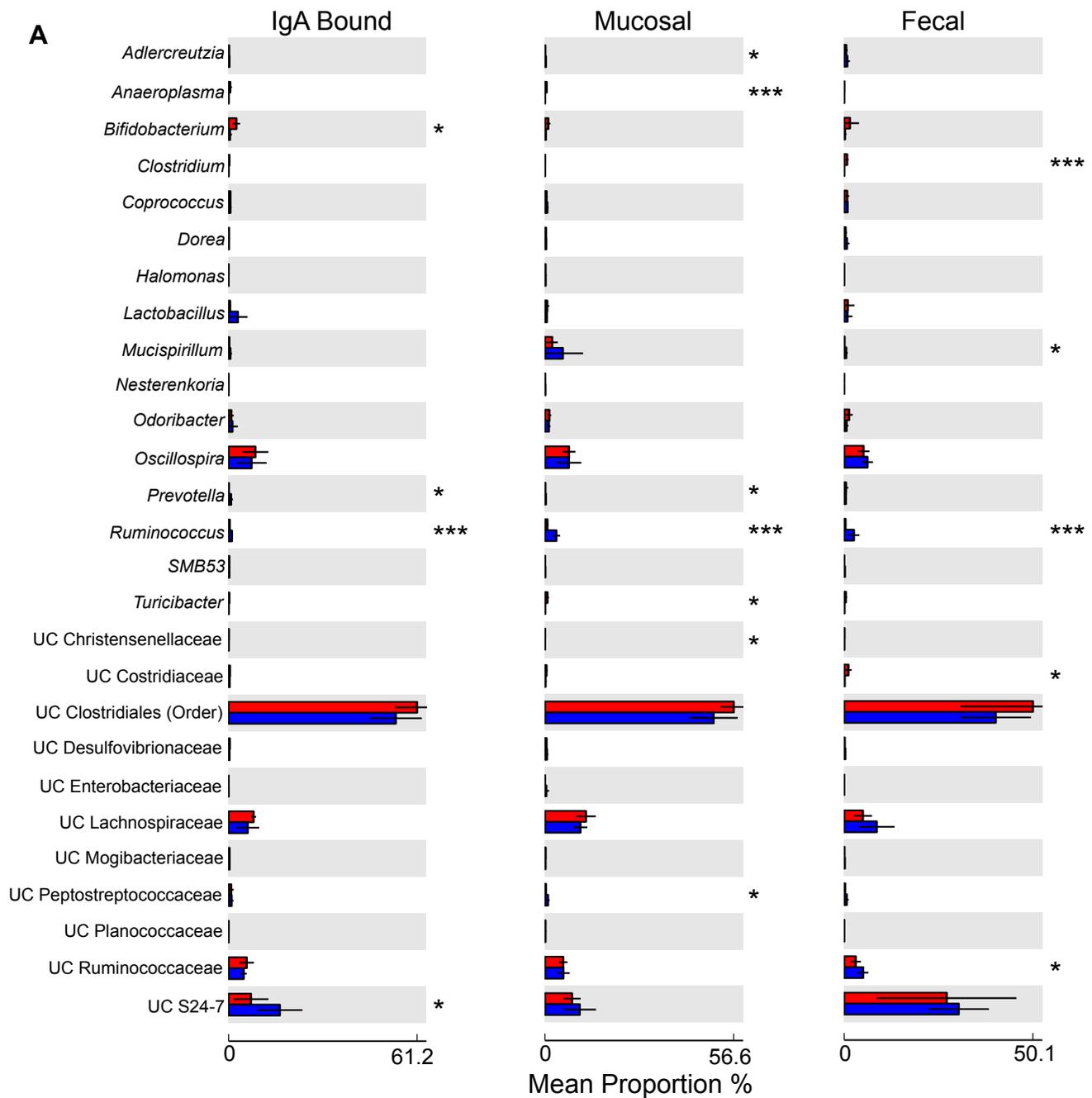


Fig. S3.





**Fig. S5.**

	Sample Subset:	Mucosa	IgA Bound	Fecal	WT Only	TMyD88-/- Only	WT Only	TMyD88-/- Only
	Test	WT vs. TMyD88	WT vs. TMyD88	WT vs. TMyD88	Mucosal vs. IgA Bound	Mucosal vs. IgA Bound	Fecal vs. Mucosal	Fecal vs. Mucosal
Separately Housed Genotypes	Unweighted UniFrac	0.0005	0.0013	0.0005	0.0023	0.0004	0.0016	0.0123
	Weighted UniFrac	0.0014	0.0087	0.1245	0.0143	0.0087	0.0094	0.0004
	Bray-Curtis	0.0007	0.0011	0.0005	0.0021	0.0004	0.0024	0.0022
Cohoused Genotypes	Unweighted UniFrac	0.0111	0.1267	0.1000	0.0002	0.0001	0.0006	0.0001
	Weighted UniFrac	0.4578	0.4100	0.6234	0.0007	0.0020	0.0053	0.0020
	Bray-Curtis	0.0681	0.0519	0.0827	0.0002	0.0001	0.0138	0.0002

**Table S1.**

Reference OTU ID *	WT Mean †	T-MyD88-/- Mean †	P-value	P-value (FDR)	Order	Family	Genus
263907	0.90	4.45	0.0005	0.0233	Clostridiales	Lachnospiraceae	UNCLASSIFIED
274021	2.90	11.55	0.0005	0.0233	Clostridiales	Lachnospiraceae	UNCLASSIFIED
262104	4.10	0.82	0.0034	0.1054	Clostridiales	Lachnospiraceae	UNCLASSIFIED
327900	0.50	4.09	0.0118	0.2434	Clostridiales	Ruminococcus	UNCLASSIFIED
214116	13.40	2.45	0.0143	0.2434	Clostridiales	UNCLASSIFIED	UNCLASSIFIED
276312	4.80	16.91	0.0157	0.2434	Clostridiales	UNCLASSIFIED	UNCLASSIFIED
311695	18.70	56.91	0.0207	0.2581	Clostridiales	UNCLASSIFIED	UNCLASSIFIED
<i>de novo</i>	2.40	8.18	0.0222	0.2581	Clostridiales	Lachnospiraceae	UNCLASSIFIED
209030	8.20	14.09	0.0459	0.4650	Bacteroidales	UNCLASSIFIED	UNCLASSIFIED

\* Greengenes 13\_8 Reference OTU IDs, except as noted for single *de novo* OTU.

† Mean abundance per 1500 sequences per sample

**Table S2.**

## METHODS

### Mice

*Conventionally colonized (SPF) mice.* C57Bl/6 MyD88<sup>LoxP/LoxP</sup> mice (Jackson Laboratories) were crossed to C57Bl/6 CD4-Cre animals (Taconic) to produce MyD88<sup>WT/WT</sup> CD4-Cre<sup>+</sup> mice (WT) and MyD88<sup>LoxP/LoxP</sup> CD4-Cre<sup>+</sup> (T-MyD88<sup>-/-</sup>) animals. Age matched male and female animals were used to compare phenotypic differences between genotypes as well as severity of TNBS induced colitis. For the antibiotics experiment, age matched FoxP3-GFP<sup>+</sup> mice (Jackson Laboratories) were compared for phenotypic differences in T<sub>FH</sub> cells within gut associated lymphoid tissues. Aged matched female C57Bl/6 WT and IL1R<sup>-/-</sup> mice (Jackson Laboratories) were used to compare T<sub>FH</sub> development and germinal center responses in this absence of IL1 signaling. The use of animals in all experiments was in strict adherence to federal regulations as well as the guidelines for animal use set forth by the University of Utah Institutional Animal Care and Use Committee.

*Germ-free (GF) mice.* GF mice were maintained in sterile isolators and verified monthly for GF status by plating and PCR of feces. GF BALB/c, GF C57Bl/6, and GF RAG1<sup>-/-</sup> animals were used in this study. Age matched GF BALB/c mice were compared to SPF BALB/c mice to compare phenotypic differences in T<sub>FH</sub> cells within gut associated lymphoid tissues. The TLR-ligand experiment: GF C57Bl/6 mice were born GF and then given *ad libitum* access to drinking water containing 10µg/mL of Pam3CSK4 (Invivogen) for two weeks. Bone marrow (BM) reconstitutions of the T and B cell compartments: GF Rag1<sup>-/-</sup> mice were reconstituted with bone marrow (~2.5x10<sup>6</sup> cells) from WT and T-MyD88<sup>-/-</sup> mice via retro-orbital injection. Mono-association experiments: BM reconstituted GF Rag1<sup>-/-</sup> mice were colonized with an erythromycin/gentamicin resistant strain of *Bacteroides fragilis* that was engineered to express OVA (*B.fragilis*-OVA; kindly provided by Dr. Sarkis Mazmanian (California Institute of Technology)). Animals were maintained for two months with 1mg/mL of both erythromycin and gentamicin in drinking water under SPF housing conditions and were validated to be correctly colonized by aerobic & anaerobic plating as well as PCR of feces. BM reconstituted GF Rag1<sup>-/-</sup> mice were analyzed two-months post-colonization. TLR-ligand feeding experiment: reconstituted GF Rag1<sup>-/-</sup> mice were maintained on 1mg/mL of ampicillin (Fisher Scientific), neomycin (Fisher Scientific), erythromycin (Fisher Scientific), and gentamicin (GoldBio) for two months. During the last two weeks, 10µg/mL of Pam3CSK4 (Invivogen) was added to antibiotic cocktails. For IL1β blocking experiments, GF Rag1<sup>-/-</sup> mice were reconstituted with WT bone marrow (~2.5x10<sup>6</sup> cells). Reconstituted mice were maintained on 1mg/mL of ampicillin (Fisher Scientific), neomycin (Fisher Scientific), erythromycin (Fisher Scientific) and gentamicin (GoldBio) for two months. 10µg/mL of Pam3CSK4 (Invivogen) was added to antibiotic cocktails during the final two weeks of the experiment. During this two-week period, 50µg of anti-IL1β neutralizing antibody (Thermo Scientific Cat#MM425B) or 50µg of IgG control antibody (Thermo Scientific Cat#31903) were administered to mice every other day via retro-orbital injection<sup>1</sup>. Mice were sacrificed less than 24 hours after the final injection of antibody.

## Enzyme-linked immunosorbant assay (ELISA)

To quantify luminal IgA, colons were cut open longitudinally and feces and mucus were scraped out and placed into 1.5mL Eppendorf tubes. Luminal contents were re-suspended in 500 $\mu$ L of sterile 1X HBSS and spun at 400 x g for 5 minutes to remove coarse materials. Supernatants were then placed in a new 1.5mL Eppendorf tube and spun at 8000 x g for 5 minutes to pellet bacteria. This step was repeated until samples were clear of bacterial pellets. Supernatants (containing IgA) were then placed in a new 1.5mL Eppendorf tube and used as samples (1/10 and 1/100 (v/v) dilutions) for an IgA-specific ELISA kit (eBioscience; performed per kit instructions). Absorbance was read at 450nm and concentrations of IgA were calculated with a standard curve. Concentrations were normalized to fecal weight. For quantification of OVA specific IgA, supernatants containing IgA were collected as above and IgA quantification was performed with the same IgA-specific ELISA kit with slight modification. Instead of coating plates with capture antibody specific for IgA, plates were coated overnight at 4°C with 4 $\mu$ g/mL ovalbumin in 1X PBS. Absorbance was read at 450nm and normalized to fecal weight.

## T cell isolation

Lymphocytes were isolated from spleens and the resulting cells were sorted through MACS columns with either positive (CD4 microbeads (Miltenyi)) or negative selection (CD4+ T Cell Isolation Kit II (Miltenyi)). Lymphocyte enrichment was performed following kit instructions. Isolated T cells were further purified via FACS with a BD FACSAria Cell Sorter. For Naïve T cells, CD3<sup>+</sup>CD4<sup>+</sup>CD62L<sup>+</sup>CD25<sup>-</sup> cells were collected into RPMI media (Corning) supplemented with 10% fetal bovine serum (v/v) (Gibco BRL), 50 U/mL penicillin, 50  $\mu$ g/mL streptomycin (Cellgro), 5 $\mu$ M 2-Mercaptoethanol (CalBiochem), 1 $\mu$ M sodium pyruvate (Cellgro), 1X MEM Nonessential Amino Acids (Cellgro), and 2.05mM L-glutamine (Cellgro). Purified T<sub>FH</sub> cells (CD3<sup>+</sup>CD4<sup>+</sup>B220<sup>-</sup>CXCR5<sup>+</sup>PD1<sup>+</sup>) and T<sub>FH</sub>-depleted T cells (CD3<sup>+</sup>CD4<sup>+</sup>B220<sup>-</sup>CXCR5<sup>-</sup>PD1<sup>-</sup>) were collected in supplemented RPMI media.

## TNBS-Induced Colitis and Microbial Rescues

Age matched WT and T-MyD88<sup>-/-</sup> mice were challenged with TNBS as previously described<sup>4</sup> with a few modifications. Briefly, 100 $\mu$ L per mouse of 2.5% (v/v) Picrylsulfonic acid (Sigma) in 100% EtOH was administered intra-rectally with a silicone catheter (Solomon Scientific). Weights were collected just prior to challenge and for five days following challenge to quantify weight loss. On the fifth day after challenge a second dose of TNBS was administered and tissues were collected for histology two days later. Histology was performed blindly according to previously published criteria (9). Briefly, both crypt loss and inflammation was scored according to the following guidelines. The entire length of the colon from just under the cecum to the rectum was analyzed and percent of crypt loss and percent of colon affected by inflammation was also taken into consideration. Therefore each animal received a score for crypt loss and inflammation severity as well as the percent of the colon affected. For crypt loss severity

a score of 0-3 was given (0=no crypt loss; 1=mild crypt loss, most crypts still visible with a few areas effected; 2=medium severity, greater crypt loss, fewer crypts visible in large areas; 3=very large areas of total crypt loss, places where crypts are completely gone). For inflammation a score was given from 0-3 (0=no evidence of inflammatory infiltrate; 1=very low level of cells infiltrating into the tissue; 2=thickening of lamina propria, and clear infiltrating lymphocytes into epithelial tissue; 3=thickening of lamina propria and large boluses of inflammatory infiltrates that correspond with areas a crypt loss). The percentage scoring was as follows: 0=no area affected; 0.5=1-5%; 1=5-20%; 1.5=20-30%; 2=30-45%; 2.5=45-60%; 3=60-70%; 3.5=70-80%; 4=>80%. This was the same scoring system used for crypt loss and inflammation.

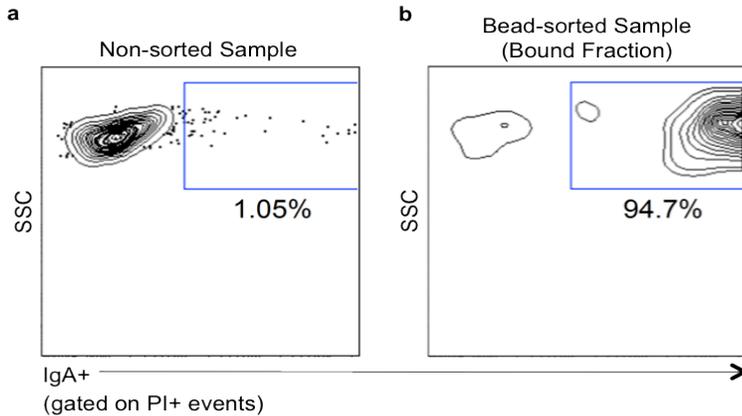
For microbiota-rescue experiments, T-MyD88<sup>-/-</sup> mice were treated with 0.5mg/mL of ampicillin (Fisher Scientific), neomycin (Fisher Scientific), erythromycin (Fisher Scientific), and gentamicin (GoldBio) for one week. Antibiotic treatment was then terminated and mice were orally gavaged daily for one week with 100uL of microbiota stock derived from either WT or T-MyD88<sup>-/-</sup> mice. Microbiota stocks were prepared from luminal contents (fecal material and mucus scrapings) that were suspended in sterile 1X HBSS (1mL/gram materials) and then gently spun down at 400 x g for 5 minutes to remove course materials. Mice were administered TNBS as described above one week after the last gavage.

#### **qPCR measurement of total bacterial load in feces and mucosa.**

Animals were sacrificed and a single fecal pellet as well as a 1cm length of colon (immediately distal to the cecum) were collected, placed in 1X cell lysis solution (sterile water containing 10mM TRIS HCL-pH8.0, 25mM sterile EDTA, and 1% (v/v) sterile SDS), and stored at -20°C until DNA extraction. Prior to storage, colon sections were gently flushed of residual feces with sterile 1X HBSS buffer. A two-day DNA isolation protocol (with a 24 hour proteinase K lysis step) was followed. Briefly, one mouse fecal pellet (or a 1cm snippet of colon) was placed in 500µl 1X cell lysis. 3µl of proteinase K (20mg/mL (Thermo Scientific)) was added and then samples were placed in a thermal block for 24 hours at 55°C. Samples were then cooled to RT, then 3µl of RNase A (10mg/mL (Thermo Scientific)) were added and samples were incubated at 37°C for 30 minutes. Samples were then cooled to RT and 200µl of 5M ammonium acetate was. To precipitate out proteins, samples were subsequently vortexed for 10 seconds, placed in an ice bath for 10 minutes, and then centrifuged at 20000 x g for 6 minutes. Supernatants (excluding pelleted proteins) were then placed in a new 1.5mL Eppendorf tube and mixed with 600µl of 100% isopropanol containing glycogen (20mg/mL (Thermo Scientific)). Samples were gently inverted 50 times to mix and then left to sit at RT for 10 minutes. Samples were spun at 20000 x g for 6 minutes. Two washes of the DNA pellet were then performed. For each wash, DNA pellets were re-suspended in 600µl of 70% (v/v) EtOH and spun at 20,000 x g for 6 minutes. After the second wash, tubes were inverted onto a sterile tissue and allowed to air-dry for 15 minutes. DNA pellets were then re-suspended in 100µl sterile H<sub>2</sub>O and stored at -20°C. Sample DNA concentrations were quantified with a Nanodrop spectrophotometer (Thermo Scientific). The total amounts of bacteria within the feces and mucosa was then quantified with Eubacteria-specific 16S primers (see Supplementary Table 1). All qPCR reactions were conducted in 12.5µl volumes with the GoTaq qPCR Master Mix (Promega). qPCR experiments were conducted on a Lightcycler LC480 instrument (Roche) with the parameters described above.

#### **Flow cytometric analysis of IgA-bound Bacteria.**

Fecal pellets were collected from animals and homogenized in 500µl of sterile 1X HBSS buffer. Samples were spun once at 400 x g to remove course materials from the fecal suspension. Supernatants (containing bacteria) were collected and placed in a new 1.5mL tube. Samples were spun at 8000 x g for 5 minutes to pellet bacteria. Bacterial pellets were washed twice by re-suspending pellet in 500µl of sterile 1X HBSS and



spinning for 5 minutes at 8000 x g. Bacterial suspensions were then blocked on ice for 15 minutes in 500µl sterile 1X HBSS containing 1% (v/v) BSA. Samples were spun at 8000 x g for 5 minutes. Bacterial pellets were then stained for 20 minutes at 4°C in the dark in 500µl of sterile 1X HBSS containing a

1/250 (v/v) dilution of a rat anti-mouse IgA antibody conjugated to a PE fluorochrome (Southern Biotech, cat#1165-09L). Pellets were washed twice as above in sterile 1X HBSS containing 1% (v/v) BSA. Pellets were then stained for 20 minutes at 4°C in 500µl of sterile 1X HBSS containing SYBR green I (1/10000 (v/v) dilution, Life Technologies). After SYBR staining samples were analyzed on an LSRFortessa flow cytometer (BD Biosciences). A representative flow cytometry plot is provided.

### Isolation and 16S rRNA sequencing of IgA-bound, fecal and mucosal bacteria.

Animals were sacrificed and their entire lower digestive tract (from duodenum to rectum) was removed and longitudinally sectioned. One fecal pellet and a 1cm section of the distal colon were collected from each animal to characterize the fecal and mucosal microbiota communities, respectively. The colon section was scraped and rinsed in sterile 1X HBSS during collection. Fecal and mucosa samples were immediately frozen at -80°C in 2mL screw cap tubes containing ~250 mg of 0.15 mm garnet beads (MoBio, cat# 13122-500) for down-stream DNA extraction. For IgA-bound sample collection the luminal contents, including mucosal scrapings, from distal ileum to rectum were collected with forceps, placed in a 15mL conical tube, and spun briefly to sediment materials. 2mL of sterile 1X HBSS (without Ca<sup>2+</sup>) was then added to each sample. Samples were vortexed at medium speed for 20 seconds and then spun for 5 minutes at 60 x g at 4°C to collect coarse material. 1.5 mL of the supernatant was collected in a 1.5 mL Eppendorf tube and kept on ice while 2mL of new sterile 1X HBSS was added to the sample. This process was repeated 4 times to collect 4-1.5ml Eppendorf tubes per animal sample containing bacteria separated from the most coarse fecal material. Tubes were then spun at 5000 x g for 5 minutes and supernatant was discarded to remove unbound Ig. Pellets were washed with 600µl sterile 1X HBSS and 2 sets of 2-1.5mL Eppendorf tubes from each sample were combined. Tubes for each sample were then spun again at 5000 x g for 5 minutes. Supernatants were discarded and the

pellets were re-suspended in 500µl of sterile 1X HBSS with 0.1% (v/v) BSA. Pairs of tubes for each sample were then combined into a single 1.5mL Eppendorf tube to obtain ~1 mL of bacterial suspension depleted of unbound Ig. This suspension contained Ig-bound and unbound bacteria. 125µl of streptavidin-coated magnetic beads (CELLlection Biotin Binder Kit, Life Technologies cat. # 11533D) per sample were washed and pre-incubated with 10µg of biotinylated anti-mouse IgA (Biolegends, cat. # 407004) for 30 minutes at room-temperature (with gentle rocking) according to the manufacturer's specifications. The pre-incubated bead-antibody mixture was then added to the 1mL of bacterial sample and incubated for 30 minutes at 4°C with gentle shaking. After incubation, 1mL of sterile 1X HBSS supplemented with 0.1% (v/v) BSA and 2 mM EDTA was added. Samples were transferred to a sterile 5mL Falcon tube (BD Biosciences) and placed on a magnet. The solution contained unbound bacteria was removed by pipette and the tube was then removed from the magnet. Magnetic beads (with IgA-bound bacteria) were re-suspended in 1X HBSS supplemented with 0.1% (v/v) BSA to wash. This was done three times to thoroughly wash beads containing IgA-bound bacteria. Buffer was removed and samples were washed three then washed three times with HBSS with 0.1% (v/v) BSA. After the final wash was removed, samples were removed from the magnet and 200 µl of sterile TE (pH 8.0) with 0.1% (v/v) Tx-100 (filter sterilized through 0.22 µm filter) was added. The suspended magnetic bead and IgA-bound bacteria mixture was added to 2 mL screw cap tubes containing ~250 mg of 0.1 mm zirconia/silica beads (Biospec, cat. # 11079101z) and immediately frozen and stored at -80°C for down-stream DNA extraction.

IgA-bound, fecal and mucosal samples were all processed similarly for DNA extraction and sequencing. First, each sample that already contained beads and 200µl TE with 0.1% (v/v) Tx-100 was thawed at 75°C for 5 minutes and then chilled on ice. 200µl lysis buffer AL was added (Qiagen, cat. # 19075) and bead-beating for 1 minutes with a Mini-Beadbeater-16 (Biospec, cat. # 607) was performed. Mucosal tissue samples were bead-beat for an additional minute to complete homogenization, with 5 minutes on ice in between beatings to prevent sample heating. Samples were then spun down for 1 minute at 8,000 x g to avoid foaming prior to adding 200µl of 100% ethanol and vortexing. Samples were spun down again to avoid transfer of any remaining coarse material and supernatant was added to a Qiagen DNeasy (Qiagen, cat. # 69504) (fecal and mucosal samples) or Qiagen DNA micro (Qiagen, cat. # 56304)(IgA-bound) column. IgA-bound samples were first placed against a magnet to remove the magnetic beads prior to loading on columns. All samples were then further processed according to Qiagen's specifications after loading on columns. DNA was eluted in 65µl of Qiagen's buffer AE.

In order to amplify the bacterial 16S rRNA and obtain high-quality, long reads, we targeted the hypervariable regions 3 and 4, initially primer sequences based off the S-D-Bact-0341-b-S-17/S-D-Bact-0785-a-A-21 pair identified by Klindworth, et al 2013<sup>5</sup>. However, empirically, we determined these primers to have strong amplification efficiency of host genomic and mitochondrial DNA in addition to the bacterial 16S rRNA gene. We modified them to avoid human and mouse amplification while having similar *in silico* predicted coverage of bacterial taxa. Then, in a strategy based off that developed by Kozich, et al . 2013<sup>6</sup>, we added 2 nucleotide linkers non-complementary to most bacteria sequences followed by Illumina adapter sequences with different 8

nucleotide barcodes on forward and reverse primers. We differ from Kozich's strategy in that we used Illumina's sequencing primers to initiate reads and subsequently trimmed our primer sequences from the raw reads, instead of using the 16S primers themselves to initiate Illumina sequencing reads. The modified primers (Supplemental Table 1) resulted in less than 0.06% of our quality-filtered reads from either end aligning to the host (mouse) genome (data not shown). PCR cycling conditions were as follows: 98°C initial denaturation for 2 minutes; 26 cycles of 98°C for 30 sec seconds, 53.6°C anneal for 20 seconds, 72°C extension for 30 seconds; final single extension at 72°C for 2 minutes. Each PCR was done in triplicate with Phusion HotStart II (Thermo Scientific, cat. # F-549L) with the supplied GC buffer and 200nM of each primer in 25µl reaction volumes, then combined and cleaned up with ZR-96 DNA cleanup-kit (Zymogen, cat. #D4017). Cleaned, barcoded PCR amplicons from each sample were multiplexed then mixed with PhiX control (5% of final library)(Illumina, cat. #FC-110-3001) to increase base diversity, and sequenced on an Illumina MiSeq at the University of Utah's high-throughput sequencing core with paired-end 300 cycle sequencing.

De-multiplexed sequences were processed with mothur<sup>7</sup>, Qiime<sup>8</sup>, and custom perl scripts. Briefly, mothur's make.contigs was first used to trim primer and linker sequences and combine each set of paired-end reads into a single long contig, requiring a minimum of 20 nucleotides overlap (thereby discarding contigs greater than 536 nucleotides after primer and linker trimming) and a maximum quality score difference of 6 between overlapping bases. We then discarded sequences that had any 'N' nucleotides (initially uncalled by MiSeq or introduced by mothur due to difference in pairs of overlapping reads), homopolymer stretches greater than 15, or those that did not align to the region targeted by our primers. Finally, quality-filtered long contigs were used in Qiime 1.8.0 to pick open-reference 97% OTUs with uclust<sup>9</sup> and make taxonomic calls against the Greengenes 13\_8<sup>10</sup> reference set and taxonomy, requiring a minimum OTU cluster size containing 10 sequences. Chimeric sequences were subsequently screened out with ChimeraSlayer<sup>11</sup> and a phylogeny made with the program FastTree 2<sup>12</sup>. All analyses involving fecal and mucosal samples were based off an OTU table rarefied to 1,500 sequences per sample allowing us to maximize retention of biological replicates, whereas analyses involving comparisons within only IgA-bound samples were rarefied to 5,100 sequences per sample due to a more even sequencing depth among these samples.

### qPCR validation of conditional MyD88 expression knockout.

CD4<sup>+</sup>, CD8<sup>+</sup>, and non T cells were sort purified via FACS on a FACS ARIA instrument (BD) from the spleens of a WT and T-MyD88<sup>-/-</sup> animal and qPCR was used to determine the fold reduction in MyD88 expression. Briefly, cellular RNA was extracted via phenol-chloroform precipitation and cDNA was created with the qScript cDNA synthesis kit (Quanta Biosciences). qPCR was conducted as described above with the GoTaq qPCR Master Mix (Promeg

### Primers used in this study.

Primer Name	Use	Sequence (5' - 3') *	Reference
-------------	-----	----------------------	-----------

ill_S-D-Bact-0346-a-S-17 †	Microbiota Sequencing	AATGATACGGCGACCACCGAGATCTACACXXXXXXXXXACACTCTTT CCCTACACGACGCTCTTCCGATCT <b>T</b> AGGGRGGCWGCAGTRRGG	
ill_S-D-Bact-0781-b-A-23 †	Microbiota Sequencing	CAAGCAGAAGACGGCATAACGAGATXXXXXXXXXGTGACTGGAGTTCA GACGTGTGCTCTTCCGATCT <b>TT</b> CTACHVGGGTATCTAATCCTGTT	
UniF340	Total Bacteria qPCR	ACTCCTACGGGAGGCAGCAGT	Barman, et al. 2008 <sup>13</sup>
UniR514	Total Bacteria qPCR	ATTACCGCGCTGCTGGC	Barman, et al. 2008 <sup>13</sup>
OVAqPCR F	<i>B. fragilis</i> OVA qPCR	AGAAATGTCTTCAGCCAAGCTC	
OVAqPCR R	<i>B. fragilis</i> OVA qPCR	GCCCATAGCCATTAAGACAGATGTG	
MyD88-F	MyD88 qPCR KO validation	CCCCTCGCAGTTTGTG	Ling, et al. 2013 <sup>14</sup>
MyD88-R	MyD88 qPCR KO validation	TGCCTCCAGTTCCTTTG	Ling, et al. 2013 <sup>14</sup>

\* **Bold** nucleotides indicate linker sequence, underlined nucleotides indicate the 16S rRNA gene targeting sequences, the 8 nucleotide barcode position is indicated by 'X'

† Naming after "ill\_" by convention in (Alm., 1996.<sup>15</sup>)

## Statistics

Pair-wise comparison of experimental groups in Figures 1, 2, 3, and 5, as well as their respective Extended Data Figures, were performed with an unpaired two-tailed Student's t-test. A Welch's correction was used for data sets with unequal variance (Figure 1b, 1c, 1f, 2g, 3a, 3g). For microbial community similarity comparisons, pairwise comparisons of phylogenetic similarity (Figure 4b, 4d, 5b, 5e) was performed with an unpaired two-tailed non-parametric t test with 9999 Monte Carlo simulations. Significance testing of genotype and sample type effects in 4a, 4c and 5a were performed with a PERMANOVA incorporating 9,999 permutations on relevant distance matrices as noted. A Mantel's test was used to test for significant correlations between distance matrices summarized in Figure 6b. Estimates of dispersion in all figures represent standard deviation around the mean with the exception of Figure 7a which represents standard error.

## REFERENCES

- 1 Cecic, I. & Korbelik, M. Mediators of peripheral blood neutrophilia induced by photodynamic therapy of solid tumors. *Cancer Lett* **183**, 43-51 (2002).
- 2 Atarashi, K. *et al.* Induction of colonic regulatory T cells by indigenous *Clostridium* species. *Science* **331**, 337-341, doi:10.1126/science.1198469 (2011).
- 3 Round, J. L. *et al.* The Toll-like receptor 2 pathway establishes colonization by a commensal of the human microbiota. *Science* **332**, 974-977, doi:10.1126/science.1206095 (2011).
- 4 Round, J. L. & Mazmanian, S. K. Inducible Foxp3+ regulatory T-cell development by a commensal bacterium of the intestinal microbiota. *Proc Natl Acad Sci U S A* **107**, 12204-12209, doi:10.1073/pnas.0909122107 (2010).
- 5 Klindworth, A. *et al.* Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. *Nucleic Acids Res* **41**, e1, doi:10.1093/nar/gks808 (2013).

- 6 Kozich, J. J., Westcott, S. L., Baxter, N. T., Highlander, S. K. & Schloss, P. D. Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the MiSeq Illumina sequencing platform. *Appl Environ Microbiol* **79**, 5112-5120, doi:10.1128/AEM.01043-13 (2013).
- 7 Schloss, P. D. *et al.* Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Appl Environ Microbiol* **75**, 7537-7541, doi:10.1128/AEM.01541-09 (2009).
- 8 Caporaso, J. G. *et al.* QIIME allows analysis of high-throughput community sequencing data. *Nat Methods* **7**, 335-336, doi:10.1038/nmeth.f.303 (2010).
- 9 Edgar, R. C. Search and clustering orders of magnitude faster than BLAST. *Bioinformatics* **26**, 2460-2461, doi:10.1093/bioinformatics/btq461 (2010).
- 10 McDonald, D. *et al.* An improved Greengenes taxonomy with explicit ranks for ecological and evolutionary analyses of bacteria and archaea. *Isme J* **6**, 610-618, doi:10.1038/ismej.2011.139 (2012).
- 11 Haas, B. J. *et al.* Chimeric 16S rRNA sequence formation and detection in Sanger and 454-pyrosequenced PCR amplicons. *Genome Res* **21**, 494-504, doi:10.1101/gr.112730.110 (2011).
- 12 Price, M. N., Dehal, P. S. & Arkin, A. P. FastTree 2--approximately maximum-likelihood trees for large alignments. *PLoS One* **5**, e9490, doi:10.1371/journal.pone.0009490 (2010).
- 13 Barman, M. *et al.* Enteric salmonellosis disrupts the microbial ecology of the murine gastrointestinal tract. *Infect Immun* **76**, 907-915, doi:10.1128/IAI.01432-07 (2008).
- 14 Ling, H. P. *et al.* Expression of intestinal myeloid differentiation primary response protein 88 (MyD88) following experimental traumatic brain injury in a mouse model. *J Surg Res* **179**, e227-234, doi:10.1016/j.jss.2012.03.030 (2013).
- 15 Alm, E. W., Oerther, D. B., Larsen, N., Stahl, D. A. & Raskin, L. The oligonucleotide probe database. *Appl Environ Microbiol* **62**, 3557-3559 (1996).

## Supplementary Figure Legends

**Fig. S1. T-MyD88<sup>-/-</sup> mice lack expression of MyD88 specifically within T cells (CD4+ and CD8+), Related to Figure 1. (A)** A schematic representing the insertion of loxP sites flanking exon 3 in MyD88<sup>fl/fl</sup> animals. Primer locations (shown in blue) and expected PCR product lengths shown on the representative gel. DNA bands from CD4-Cre specific primers are also shown on a representative gel. **(B)** MyD88 mRNA expression was measured by qPCR as described in the methods section. WT, defined by MyD88<sup>+/+</sup> CD4-Cre<sup>+</sup> were compared to T-MyD88<sup>-/-</sup>, defined as MyD88<sup>fl/fl</sup> CD4-Cre<sup>+</sup> **(C)** RNA-sequencing of samples from genotypes revealed a specific loss of RNA from the third exon of the MyD88 gene within T-MyD88<sup>-/-</sup> T cells.

**Fig. S2. Analysis of T<sub>H1</sub>, T<sub>H17</sub>, and T<sub>REG</sub> population frequencies within spleens and mesenteric lymph nodes (MLNs) of WT and T-MyD88<sup>-/-</sup> mice, Related to Figure 1. (A-E)** T cell subsets within the spleen were measured by flow cytometry. Representative plots were

initially gated on CD3<sup>+</sup> CD4<sup>+</sup> cells; **(A)** T<sub>H1</sub> cells are defined by CD3<sup>+</sup> CD4<sup>+</sup> IFNγ<sup>+</sup>, and T<sub>H17</sub> cells are defined by CD3<sup>+</sup> CD4<sup>+</sup> IL-17<sup>+</sup>; Data are compiled for **(B)** T<sub>H17</sub> frequencies, **(C)** T<sub>H1</sub> frequencies (n=7 for each group). **(D)** T<sub>REG</sub> cells are defined by CD3<sup>+</sup> CD4<sup>+</sup> FoxP3<sup>+</sup>; data are compiled for **(E)** T<sub>REG</sub> frequencies (n=7 for each group). **(F-J)** T cell subsets within the MLNs were measured by flow cytometry. Representative plots were initially gated on CD3<sup>+</sup> CD4<sup>+</sup> cells; **(F)** T<sub>H1</sub> cells are defined by CD3<sup>+</sup> CD4<sup>+</sup> IFNγ<sup>+</sup> and T<sub>H17</sub> cells are defined by CD3<sup>+</sup> CD4<sup>+</sup> IL-17<sup>+</sup>; Data are compiled for **(G)** T<sub>H17</sub> frequencies, **(H)** T<sub>H1</sub> frequencies (n=11 for each group). **(I)** T<sub>REG</sub> cells are defined by CD3<sup>+</sup> CD4<sup>+</sup> FoxP3<sup>+</sup>; data are compiled for **(J)** T<sub>REG</sub> frequencies (n=8 for each group). P-value<0.05 (\*); P-value<0.01 (\*\*); P-value<0.001 (\*\*\*) using an unpaired, two-tailed t test. Related to Figure 1

**Fig. S3. Analysis of T<sub>H1</sub>, T<sub>H17</sub>, and T<sub>REG</sub> population frequencies within cLP, siLP, and PPs of WT and T-MyD88<sup>-/-</sup> mice, Related to Figure 1.** **(A-E)** T cell subsets within the cLP were measured by flow cytometry. Representative plots were initially gated on CD3<sup>+</sup> CD4<sup>+</sup> cells; **(A)** T<sub>H1</sub> cells are defined by CD3<sup>+</sup> CD4<sup>+</sup> IFNγ<sup>+</sup> and T<sub>H17</sub> cells are defined by CD3<sup>+</sup> CD4<sup>+</sup> IL-17<sup>+</sup>; Data are compiled for **B** T<sub>H17</sub> frequencies, **c**, T<sub>H1</sub> frequencies (n=9 WT and n=14 T-MyD88<sup>-/-</sup>). **(D)** T<sub>REG</sub> cells are defined by CD3<sup>+</sup> CD4<sup>+</sup> FoxP3<sup>+</sup>; data are compiled for **(E)** T<sub>REG</sub> frequencies (n=10 for each group). **f-j**, T cell subset within the siLP were measured by flow cytometry. Representative plots were initially gated on CD3<sup>+</sup> CD4<sup>+</sup> cells; **(F)** T<sub>H1</sub> cells are defined by CD3<sup>+</sup> CD4<sup>+</sup> IFNγ<sup>+</sup> and T<sub>H17</sub> cells are defined by CD3<sup>+</sup> CD4<sup>+</sup> IL-17<sup>+</sup>; Data are compiled for **(G)** T<sub>H17</sub> frequencies, **(H)** T<sub>H1</sub> frequencies (n=9 WT and n=7 T-MyD88<sup>-/-</sup>). **i**, T<sub>REG</sub> cells are defined by CD3<sup>+</sup> CD4<sup>+</sup> FoxP3<sup>+</sup>; data are compiled for **j**, T<sub>REG</sub> frequencies (n= 10 WT and n=8 T-MyD88<sup>-/-</sup>). **(K-O)** T cell subset within PPs were measured by flow cytometry. Representative plots were initially gated on CD3<sup>+</sup> CD4<sup>+</sup> cells; **(K)** T<sub>H1</sub> cells are defined by CD3<sup>+</sup> CD4<sup>+</sup> IFNγ<sup>+</sup> and T<sub>H17</sub> cells are defined by CD3<sup>+</sup> CD4<sup>+</sup> IL-17<sup>+</sup>; Data are compiled for **(L)** T<sub>H17</sub> frequencies, **(M)** T<sub>H1</sub> frequencies (n=10-11). **(N)** T<sub>REG</sub> cells are defined by CD3<sup>+</sup> CD4<sup>+</sup> FoxP3<sup>+</sup>; data are compiled for **o**, T<sub>REG</sub> frequencies (n=10-11). P-value<0.05 (\*); P-value<0.01 (\*\*); P-value<0.001 (\*\*\*) using an two-tailed, unpaired t test. Related to Figure 1

**Fig. S4. Analysis of PP cellularity and T<sub>FH</sub> populations within PPs, spleen, and MLNs, Related to Figure 1.** **(A-F)** T<sub>FH</sub> populations within PPs were measured by flow cytometry. Representative plots were initially gated on CD3<sup>+</sup> CD4<sup>+</sup> B220<sup>-</sup> cells **(A)** T<sub>FH</sub> populations were defined as CD3<sup>+</sup> CD4<sup>+</sup> B220<sup>-</sup> PD-1<sup>+</sup> ICOS<sup>+</sup>. Data are compiled for **(B)** T<sub>FH</sub> frequencies (n=15 WT and n=13 T-MyD88<sup>-/-</sup>). **(C)** T<sub>FH</sub> populations were defined as CD3<sup>+</sup> CD4<sup>+</sup> B220<sup>-</sup> PD-1<sup>+</sup> CXCR5<sup>+</sup>. Data are compiled for **d**, T<sub>FH</sub> frequencies (n=21 WT and n=18 T-MyD88<sup>-/-</sup>). **(E)** T<sub>FH</sub> populations were defined as CD3<sup>+</sup> CD4<sup>+</sup> B220<sup>-</sup> ICOS<sup>+</sup> CXCR5<sup>+</sup>. Data are compiled for **f**, T<sub>FH</sub> frequencies (n=15 WT and n=13 T-MyD88<sup>-/-</sup>). **(G,H)** Gating strategy for non-GC TFH cells as defined by

CD3<sup>+</sup>CD4<sup>+</sup>B220<sup>-</sup>CXCR5<sup>int</sup>PD-1<sup>int</sup> representative facs plot and **h**, data compiled (n=15 WT and n=13 T-MyD88<sup>-/-</sup>). **i**, Data are compiled from the quantification of Trypan Blue (HyClone) resistant cells from single cell suspensions of PPs. **(J,K)** T<sub>FH</sub> populations within MLNs were measured by flow cytometry. Representative plots were initially gated on CD3<sup>+</sup>CD4<sup>+</sup>B220<sup>-</sup> cells **(G)** T<sub>FH</sub> populations were defined as CD3<sup>+</sup>CD4<sup>+</sup>B220<sup>-</sup>ICOS<sup>+</sup>CXCR5<sup>+</sup>. Data are compiled for **(H)** T<sub>FH</sub> frequencies (n=7 for each group). **(L,M)** T<sub>FH</sub> populations within the spleen were measured by flow cytometry. Representative plots were initially gated on CD3<sup>+</sup>CD4<sup>+</sup>B220<sup>-</sup> cells **I**, T<sub>FH</sub> populations were defined as CD3<sup>+</sup>CD4<sup>+</sup>B220<sup>-</sup>ICOS<sup>+</sup>CXCR5<sup>+</sup>. Data are compiled for **m**, T<sub>FH</sub> frequencies (n=7 for each group). P-value<0.05 (\*); P-value<0.01 (\*\*); P-value<0.001 (\*\*\*) using an unpaired, two-tailed t test. Related to Figure 1.

**Fig. S5. Most significant taxonomic differences in mucosa, IgA bound and fecal communities between WT and T-MyD88<sup>-/-</sup>. Related to Figure 4 and 5.** **(A)** Genera mean abundances (± SD) detected in IgA bound, mucosal and fecal fractions of intestinal microbial communities. Only genera detected as > 0.1% average relative abundance within a sample type group are shown. UC, Unclassified. A large number of OTUs can only be classified to the order level within the Clostridiales. **(B)** Boxplots showing the abundance within the mucosal fraction of four taxonomic groups containing known mucolytic bacteria are shown. Despite the trend towards increased mucolytic bacteria in the mucosal fraction of T-MyD88 animals, only the genus *Ruminococcus* attains significance due to large variability between animals. (\* P-value<0.05, \*\*\* P-value<0.001. Welch's t-test). Related to Figure 4 and 5.

**Table S1. Table of P-values for microbial community dissimilarities, related to Figures 4 and 5.** Values are based on PERMANOVA with 9999 permutations shows that most observed patterns are robust to weighted, unweighted UniFrac as well as the non-phylogenetic Bray-Curtis. Related to Figure 4 and 5

**Table S2. 9 OTUs identified as significantly different in separately housed animals as well as cohoused animals, related to Figure 5D.** P-values (non-parametric t-test with 9,999 Monte Carlo simulations) are shown for differences between genotypes in cohoused animals, illustrating the conservation of patterns among some OTUs between different housing conditions, as well as different direction of shifts in abundance of OTUs identified as belonging to the *Lachnospiraceae* genus. Related to Figure 4 and 5.