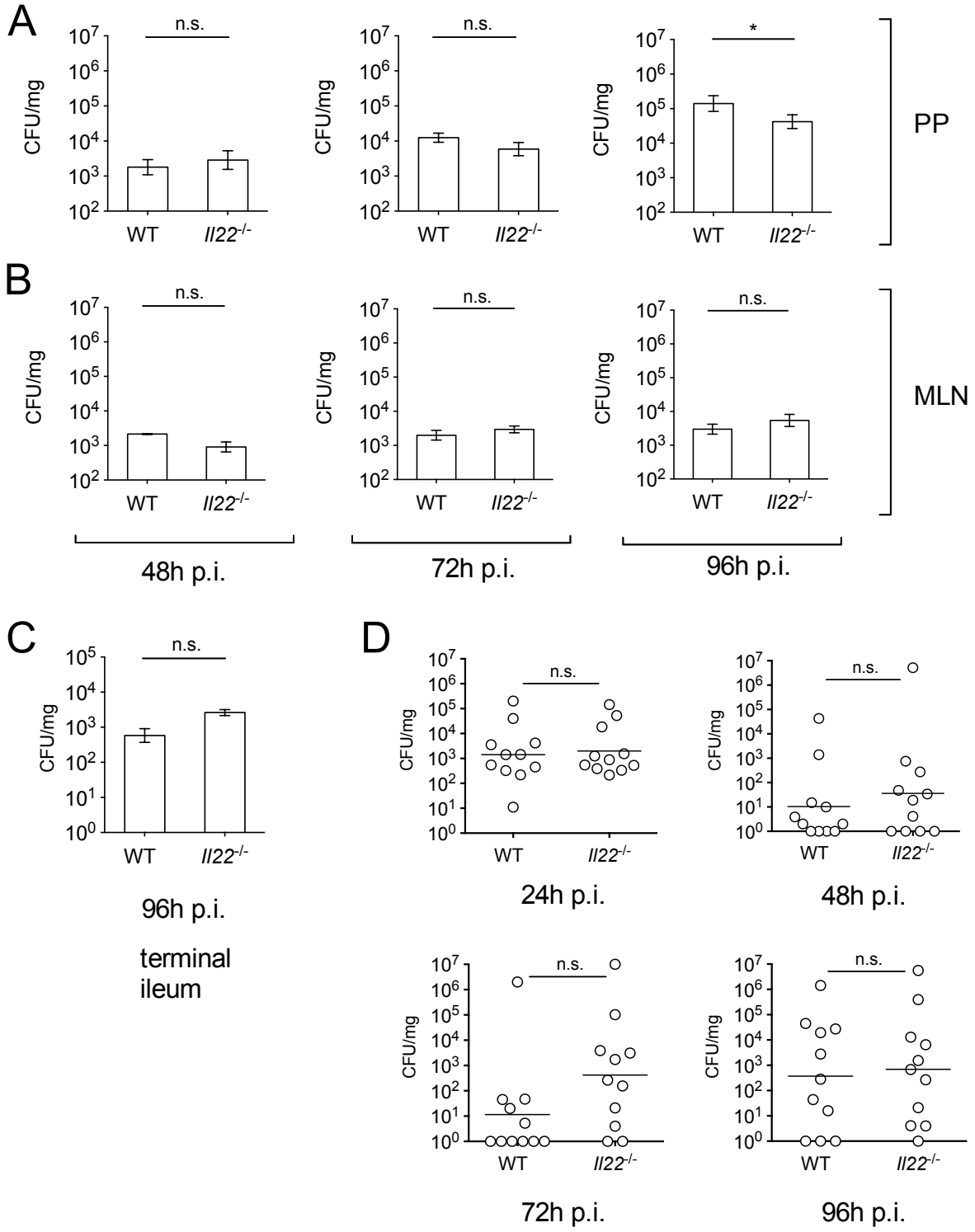
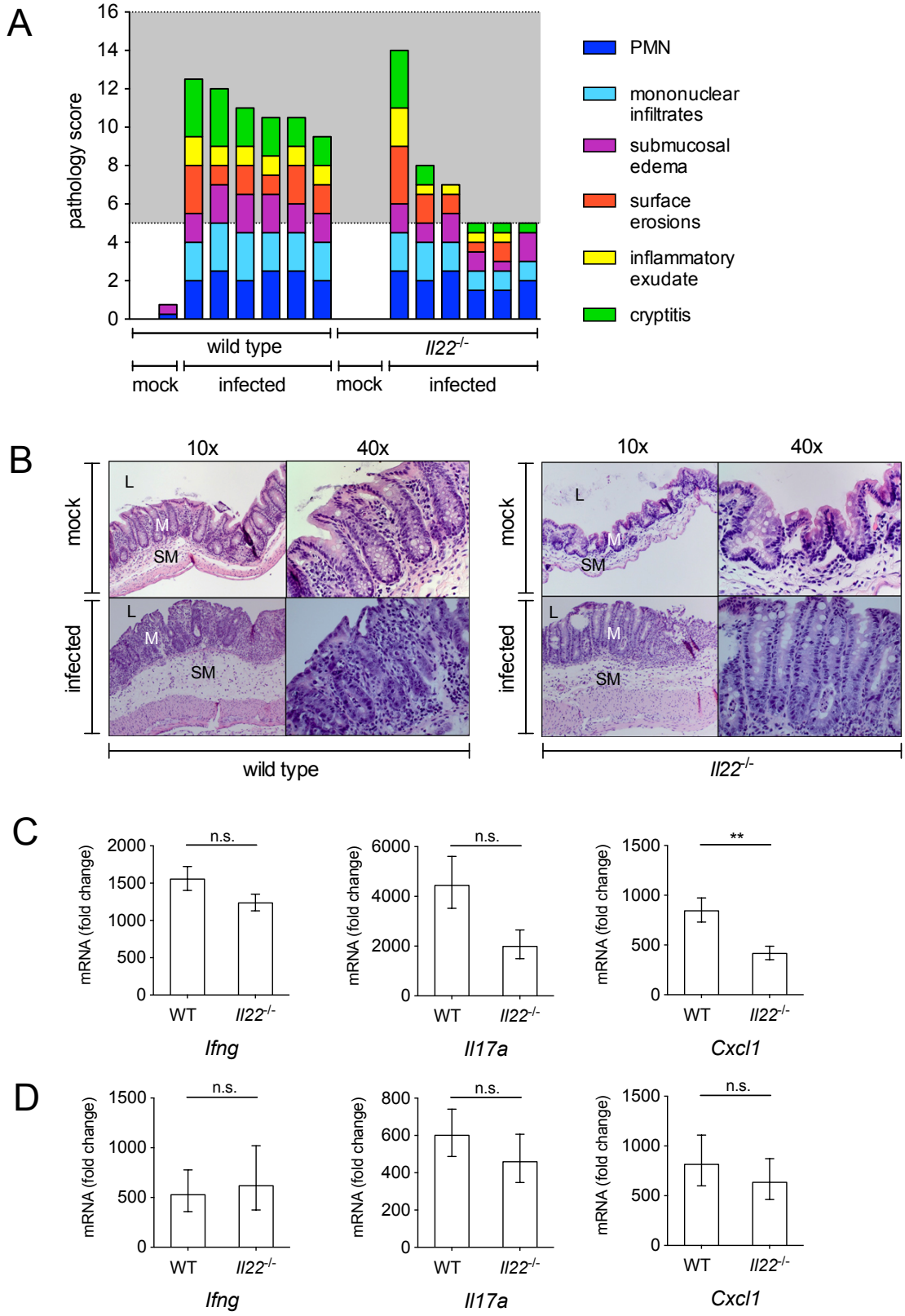
Supplementary figure 1 (Related to figure 1)



Dissemination and colonization of *S.* Typhimurium in WT and *Il22*$^{-/-}$ mice

(A-C) Wild-type (WT) and *Il22*$^{-/-}$ mice were pre-treated with streptomycin, infected with *S.* Typhimurium and sacrificed two, three or four days after infection. Peyer's Patches (PP), Mesenteric Lymph Nodes (MLN), and terminal ileum were harvested, homogenized and the bacterial burden was enumerated. Data presented show colony forming units (CFU) per mg of tissue. (A+B) 48h: WT n=3, *Il22*$^{-/-}$ n=4; 72h: WT n=11, *Il22*$^{-/-}$ n=9; 96h: WT n=11, *Il22*$^{-/-}$ n=10 (C) WT n=11, *Il22*$^{-/-}$ n=14. Data represent the geometric mean ± standard error. **(D)** WT and *Il22*$^{-/-}$ mice infected with *S.* Typhimurium without previous treatment with streptomycin. Fecal samples were collected at days one, two, three and four after infection and the bacterial burden was enumerated (WT n=11, *Il22*$^{-/-}$ n=11). Each dot represents one mouse. Horizontal bar represents the geometric mean. A significant decrease over WT control is indicated by * (*P* value ≤ 0.05), n.s. = not significant.

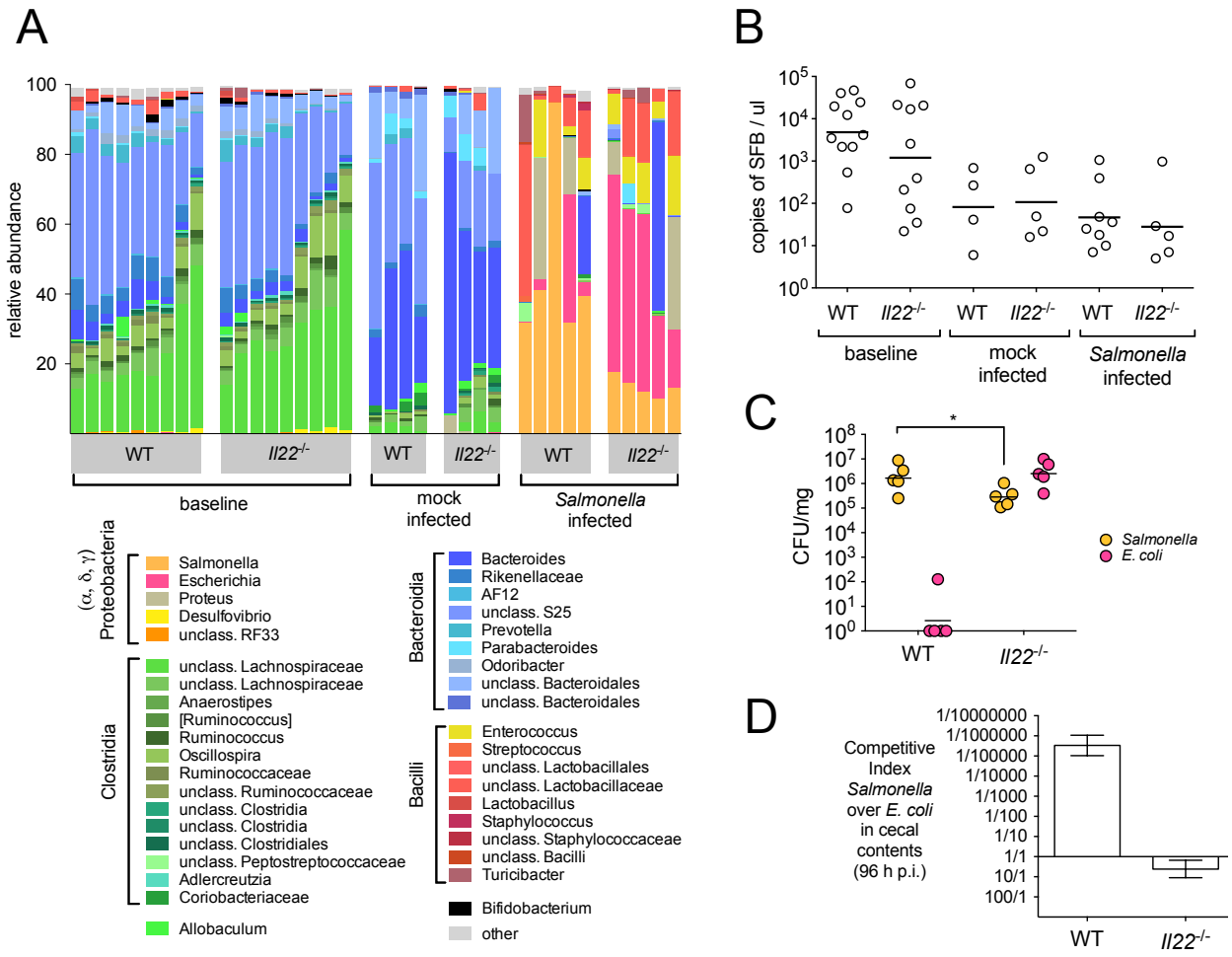
Supplementary figure 2 (related to figure 2)



Histopathology and expression of inflammatory genes in WT and *Il22*$^{-/-}$ mice after infection with *S*. Typhimurium.

(A) Blinded histopathology score indicating the score of individual mice 72h after either mock infection or infection with *S.* Typhimurium. The grey region includes scores indicative of moderate to severe inflammation. (B) H&E stained cecal sections from representative animals in each group at 72h post infection. An image at lower magnification (10x) and one at higher magnification (40x) from the same section are shown. L=lumen; M=mucosa; SM=submucosa. Note marked edema in the submucosa and inflammation in mice infected with *S.* Typhimurium. (C+D) *Ifng, Il17a* and *Cxcl1* were detected by quantitative real time PCR at 72h (C) and 96h (D) in the cecum of WT mice and *Il22*$^{-/-}$ mice after infection with WT *S.* Typhimurium. (C+D) infected WT n=6, infected *Il22*$^{-/-}$ n=6, mock n=2. Data are expressed as fold increase over mock-infected WT mice. n.d. = not detected. Data represent the geometric mean ± standard error. A significant increase over mock control is indicated by ** (*P* value ≤ 0.01), n.s. = not significant.

Supplementary figure 3 (related to figure 3)

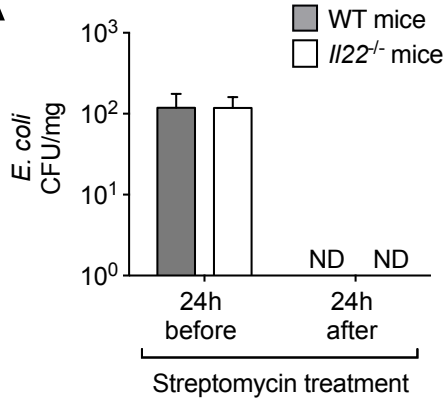


Microbiota analysis of WT and *Il22*$^{-/-}$ mice.

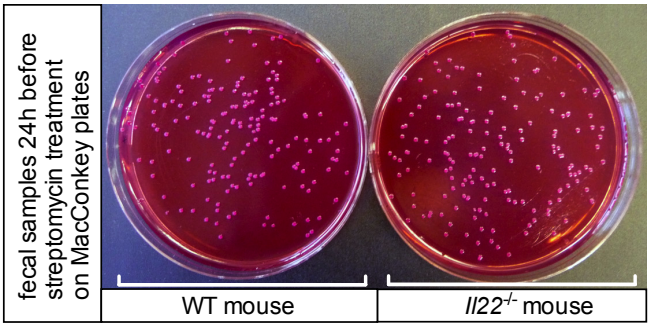
(A) MiSeq analysis of the microbiota. Each bar represents the microbial composition of one mouse (baseline n=9/group, mock infected n=4/group, *S*. Typhimurium infected n=5/group). **(B)** Analysis of the segmented filamentous bacteria (SFB) in the fecal microbiota using 16S rRNA gene qRT-PCR (baseline n=8/group, mock infected n=4-5/group, *S*. Typhimurium infected n=5-8/group). Samples were either taken before streptomycin treatment (baseline) or from mock-infected animals or *S*. Typhimurium-infected animals at 96h post infection. Graphed are the copy numbers of SFB per µl of DNA. **(C+D)** Colonization level (C) and competitive index (D) of mice included in the microbiota analysis depicted in **A**.

Supplementary figure 4 (related to figure 4)

A



B



C

Sample	description	O type	H type	LT	Sta	STb	STX1	STX2	eae	cnf1	cnf2	papG3	sfa	fyuA	iroN
Isolate 1	*Il22*$^{-/-}$ mouse, uninfected	166	6 or 41	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	POS	NEG
Isolate 2	*Il22*$^{-/-}$ mouse, uninfected	166	6 or 41	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	POS	NEG
Isolate 3	*Il22*$^{-/-}$ mouse, infected	166	6 or 41	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	POS	NEG
Isolate 4	WT mouse, uninfected	166	6 or 41	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	POS	NEG
Isolate 5	WT mouse, uninfected	166	6 or 41	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	POS	NEG
Isolate 6	WT mouse, uninfected	166	6 or 41	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	POS	NEG
Isolate 7	WT mouse, infected	166	6 or 41	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	POS	NEG

D

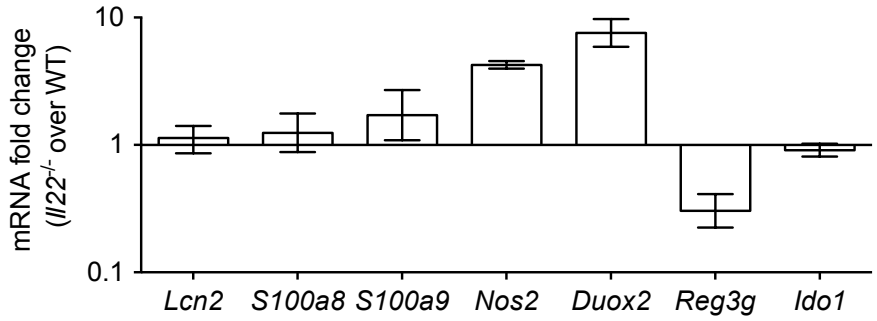
	representative of all *E. coli* colonies isolated	
Antibiotic	**MIC**	**Interpretation**
Amikacin	<=4.0000	Susceptible
Amoxicillin/ Clavulanic Acid	>1.0000	No Interpretation
Ampicillin	>1.0000	No Interpretation
Cefazolin	2	Susceptible
Cefovecin	1	Susceptible
Cefoxitin	4	Susceptible
Cefpodoxime	<=2.0000	Susceptible
Ceftiofur	0.5	Susceptible
Cephalothin	8	Susceptible
Chloramphenicol	<=4.0000	Susceptible
Clindamycin	>4.0000	**Resistant**
Doxycycline	<=2.0000	Susceptible
Enrofloxacin	<=0.2500	Susceptible
Erythromycin	>4.0000	**Resistant**
Gentamicin	<=1.0000	Susceptible
Imipenem	<=1.0000	Susceptible
Marbofloxacin	<=0.2500	Susceptible
Oxacillin + 2 NaCl	>4.0000	**Resistant**
Penicillin	>8.0000	**Resistant**
Rifampin	>2.0000	No Interpretation
Ticarcillin	<=8.0000	Susceptible
Ticarcillin/ Clavulanic Acid	<=8.0000	Susceptible
Trimethoprim/ Sulphamethoxazole	<=0.5000	Susceptible

Characterization of *E. coli* strains from WT and *Il22*$^{-/-}$ mice.

(A+B) Fecal samples were collected from WT and *Il22*$^{-/-}$ mice 24h before and 24h after treatment with streptomycin, then plated on MacConkey agar. (A) *E. coli* CFU (n=6 mice per

group 24h before, n=6 mice per group 24h after). Data represent the geometric mean ± standard error; ND = not detected. (B) Representative pictures of MacConkey plates with fecal samples from mice 24h before streptomycin treatment. **(C+D)** *E. coli* was isolated from WT and *Il22*$^{-/-}$ mice before and after infection with *S.* Typhimurium. Pure cultures were analyzed by the *E. coli* Reference Center for serotype, toxins, adhesion factors (C) and antibiotic susceptibility (D).

Supplementary figure 5 (related to figure 5)



Baseline expression of genes coding for antimicrobial proteins.

Lcn2, S100a8, *S100a9*, *Duox2, Nos2, Reg3g, and Ido1* were detected by quantitative real-time PCR in the cecum of mock-infected WT mice and *Il22*$^{-/-}$ mice 72h after mock infection. WT n=6, *Il22*$^{-/-}$ n=6. Data are expressed as fold increase of the geometric mean of mock-infected *Il22*$^{-/-}$ mice over WT mice. Data represent the geometric mean ± standard error.

Supplemental Materials and Methods

Bacterial Strains and Growth Conditions

Bacterial strains and plasmids used in this study are listed in **supplementary table 1**. Cultures of *S.* Typhimurium and *E. coli* were routinely incubated overnight either aerobically at 37°C in Luria-Bertani (LB) broth (per liter: 10 g tryptone, 5 g yeast extract, 10 g NaCl), on LB agar plates (1.5% Difco agar) or on MacConkey agar plates (per liter: 17 g pancreatic digest of gelatin, 3 g peptone, 10 g lactose, 1.5 g bile salts No. 3, 5 g NaCl, 0.03 g Neutral Red, 0.001 g Crystal Violet and 13.5 g agar). Antibiotics and other chemicals were added at the following concentrations (mg/L) as needed: carbenicillin (Carb), 100; chloramphenicol (Cm), 30; kanamycin (Km), 100; nalidixic acid (Nal), 50. Red colonies on MacConkey plates were analyzed using a Vitek II system for species identification. Confirmed *E. coli* strains were analyzed by the *E. coli* Reference Center at The Pennsylvania State University for serotype, adhesion factors, toxin production and antibiotic susceptibility.

Mouse Experiments

C57BL/6 mice and *Il22*$^{-/-}$ mice were used in our study. For initial experiments, C57BL/6 mice were purchased from Taconic Farms and *Il22*$^{-/-}$ mice were generated as previously described (Zheng et al., 2007) and raised under specific pathogen free conditions. Findings were confirmed with mice that were rederived in a Norovirus-free C57BL/6 background as heterozygotes with ICR (DF-1) mice from Harlan Laboratories as surrogate females. The mice were kept under specific pathogen free conditions in a Barrier facility. We kept separate C57BL/6 and *Il22*$^{-/-}$ mouse lines but both lines originated from the same heterozygote breeding pair. All experiments except experiments in which the microbiota was analyzed were performed with mice from this colony. For experiments in which the microbiota was analyzed, WT and *Il22*$^{-/-}$ littermate mice were derived from three heterozygote breeding pairs. For experiments, mice

were gavaged with 0.1 ml of a 200 mg/ml streptomycin/sterile water solution one day prior to mock infection with LB or oral infection with 1×10^9 CFU of *S*. Typhimurium in LB. For the typhoid model, mice were infected orally with 1×10^9 CFU of *S*. Typhimurium in LB without streptomycin pre-treatment. The cecum was harvested for mRNA, protein, and histopathology at 48-96h post-infection. The colon contents were collected, serially diluted, and plated on appropriate antibiotic LB agar plates to determine bacterial counts. In mixed infection experiments, data were normalized by dividing the output CFU ratio (WT / mutant) by the input CFU ratio (WT / mutant). Groups of 5-6 mice were used for each experiment. To reconstitute IL-22 in *Il22*$^{-/-}$ mice, mice were administered 30 μg of recombinant IL-22 (IL22-Fc, Genentech PRO312045) every other day starting the day of streptomycin treatment. Control mice were administered 30 μg of an isotype control antibody to ragweed (Genentech 10D9.1E11.1F12). To induce colitis the drinking water was replaced with a filter-sterilized solution of dextran sulfate sodium (DSS; relative molecular mass 36,000 – 50,000; MP Biomedicals, Santa Ana, CA) in water (Barman et al., 2008; Winter et al., 2010). The drinking water was switched for 24h to regular water 3 days prior to the end of the experiment.

Western Blots

Total protein was extracted from mouse cecum tissue using Tri-Reagent (Molecular Research Center). 15 μg of total protein were analyzed using 15% SDS-PAGE gels and transferred to PVDF membranes. The membranes were blocked with 2% nonfat dried milk and incubated at 4°C with polyclonal goat anti-mouse S100A8, polyclonal goat anti-mouse S100A9, polyclonal goat anti-mouse lipocalin-2, polyclonal goat anti-human or anti-mouse myeloperoxidase (all from R&D Systems), or polyclonal rabbit anti-mouse α/β-tubulin (Cell Signaling Technology). After overnight incubation the blots were washed and then incubated with horseradish peroxidase-conjugated anti-rabbit or anti-goat secondary antibodies (Jackson Immuno

Research) for 1 hour. After washing, bands were developed using the Immobilon Western Luminol Reagent and Peroxide Solution (Millipore) as per manufacturer's instructions and visualized using a Fujifilm LAS 4000.

Quantitative real-time PCR

Total RNA was extracted from mouse cecal tissue using Tri-Reagent (Molecular Research Center). Reverse transcription of 1 µg of total RNA was performed using the Transcriptor First Strand cDNA Synthesis kit (Roche). Quantitative real-time PCR (qRT-PCR) for the expression of *Actb*, *Il17a*, *Il22*, *S100a8*, *S100a9*, *Duox2, Nos2, Reg3g, Ido1, Cxcl1* and *Ifng* (**supplementary table 2**) was performed using the LightCycler 480 SYBR Green Master on the LightCycler 480 II (Roche). Conditions for qRT-PCR were 95°C for 5 minutes, then 45 cycles of 95°C for 10 seconds, 60°C for 10 seconds and 72°C for 15 seconds. Gene expression was normalized to *Actb* (β-actin) and fold changes in gene expression were relative to uninfected controls and calculated using the *ΔΔ* Ct method.

Analysis of the microbiota

Composition of the bacterial microbiota was analyzed as previously described (Barman et al., 2008; Winter et al., 2010). Briefly, the colon content was collected from mice 96h post-infection and snap frozen in liquid nitrogen. The DNA was subsequently extracted using the QIAamp DNA stool kit (Qiagen) according to the manufacturer's instruction with the following modifications. In a 1.5 ml reaction tube, a fecal sample was added to 300 mg of 0.1 mm diameter glass beads in 700 µl buffer ASL and the tube was vortexed for 20 min, then shaken vigorously for 10 min at 25 Hz in a Retsch Cryomill (without the use of liquid nitrogen). The sample was boiled for 5 min at 95°C, centrifuged, and the supernatant was collected. 700 µl buffer ASL was added to the pellet of glass beads and fecal material for a second round of extraction. Supernatant from this step was combined with supernatant from the first extraction

and was processed according to the QIAamp DNA stool kit instructions. With this method we were able to extract DNA from low abundant species like *Bifidobacterium*. Two µl of extracted bacterial DNA was used as a template for the q-PCR reaction with the primer pairs developed by (Barman et al., 2008) and presented in **supplementary table 3**. The 16S gene copy numbers per µl of DNA from each sample (one fecal pellet collected from each colon) was determined using the plasmids described in **supplementary table 1**.

Isolation of colon crypts

Streptomycin-treated C57BL/6 mice were infected with *S.* Typhimurium IR715 as detailed above and sacrificed at 48h post-infection. Crypt isolation from colon and cecum was performed as described (Whitehead et al., 1993). Briefly, cecum plus colon tissue was collected, flushed, and opened to expose mucosa. Tissue was then incubated at room temperature with a 3 mM EDTA and 0.5 mM DTT solution. After 90 minutes of incubation the tissue was transferred into PBS, shaken, and the detached crypts were decanted into a 15 ml falcon tube and spun down at 4°C, 200 x g for 5 minutes. RNA and protein were isolated using Tri-Reagent (Molecular Research Center) as per the manufacturer's instructions.

Supplemental table 1. Bacterial strains and plasmids used in this study.

Bacterial Strains

Designation	Genotype	Source or Reference
Salmonella enterica serovar Typhimurium strains		
IR715	ATCC 14028, spontaneous Nal^R derivative	Stojiljkovic et al., 1995
JZL3	IR715, Δ*znuA*::Cm	Liu et al., 2012
AJB52	*iroN*::pGP704 ($Carb^R$)	Bäumler et al. 1998
Escherichia coli strains		
K-12 substr. MG1655	ATCC 700926	ATCC
JB2	Wild type (commensal isolated from mouse gut)	This study

Plasmids

Designation	Genotype	Source or Reference
pSW191	pCR2.1::*Eubacteria* 16S rRNA	Winter et al., 2010
SFB	pCR2.1::*Segmented Filamentous Bacteria* 16S rRNA	Barman et al., 2008

Supplemental table 2. Real-time PCR primers used in this study.

Species	Target	Primer pairs
Mus musculus	*Actb*	5'-GGCTGTATTCCCCTCCATCG-3' 5'-CCAGTTGGTAACAATGCCATGT-3'
Mus musculus	*Il17a*	5'-GCTCCAGAAGGCCCTCAGA-3' 5'-AGCTTTCCCTCCGCATTGA-3'
Mus musculus	*Lcn2*	5'-ACATTTGTTCCAAGCTCCAGGGC-3' 5'-CATGGCGAACTGGTTGTAGTCCG-3'
Mus musculus	*Il22*	5'-GGCCAGCCTTGCAGATAACA-3' 5'-GCTGATGTGACAGGAGCTGA-3'
Mus musculus	*S100a8*	5'-TGTCCTCAGTTTGTGCAGAATATAAA-3' 5'-TCACCATCGCAAGGAACTCC-3'
Mus musculus	*S100a9*	5'-GGTGGAAGCACAGTTGGCA-3' 5'-GTGTCCAGGTCCTCCATGATG-3'
Mus musculus	*Nos2*	5'-TTGGGTCTTGTTCACTCCACGG-3' 5'-CCTCTTTCAGGTCACTTTGGTAGG-3'

Mus musculus	*Reg3g*	5’-ATGGCTCCTATTGCTATGCC-3’ 5’-GATGTCCTGAGGGCCTCTT-3’
Mus musculus	*Ifng*	5’-TCAAGTGGCATAGATGTGGAAGAA-3’ 5’-TGGCTCTGCAGGATTTTCATG-3’
Mus musculus	*Ido1*	5'-CGACAAGGGCTTCTTCCTCGTC-3' 5'-TGGGTCCACAAAGTCACGCATC-3'
Mus musculus	*Cxcl1*	5’-TGCACCCAAACCGAAGTCAT-3’ 5’-TTGTCAGAAGCCAGCGTTCAC-3’
Mus musculus	*Duox2*	5'-GCACTGTGCAGAACAGCTAGGACAAC-3' 5'-ACCTCATCACCTTCTTGCGGGAG-3'

Supplemental table 3. Composition of mouse groups used for MiSeq microbiota analysis.

Group	Sex	Breeding pair	Litter	Age (weeks)
WT baseline	M	Z	Z1	24
	F	BB	BB1	22
	F	BB	BB2	16
	F	Z	Z2	18
	F	BB	BB1	22
	M	Z	Z2	18
	F	Z	Z3	14
	F	AA	AA1	26
	M	BB	BB1	22
***Il22*$^{-/-}$ baseline**	M	BB	BB1	22
	M	Z	Z2	18
	F	BB	BB2	16
	M	BB	BB3	13
	M	Z	Z1	24
	F	BB	BB2	16
	F	Z	Z3	14
	M	BB	BB3	13
	M	BB	BB2	16
WT mock infected	M	Z	Z1	24
	F	BB	BB1	22
	F	BB	BB2	16
	F	Z	Z2	18
***Il22*$^{-/-}$ mock infected**	M	BB	BB1	22
	M	Z	Z2	18
	F	BB	BB2	16
	M	BB	BB3	13

WT *Salmonella* infected	F	BB	BB1	22
	M	Z	Z2	18
	F	Z	Z3	14
	F	AA	AA1	26
	M	BB	BB1	22
***Il22*$^{-/-}$ *Salmonella* infected**	M	Z	Z1	24
	F	BB	BB2	16
	F	Z	Z3	14
	M	BB	BB3	13
	M	BB	BB2	16

Supplemental table 4. Real-time PCR primers used for microbiota analysis.

Species	Target	Primer pairs
Eubacteria **(Barman et al.)**	*16S rRNA*	UniF340 5'-ACTCCTACGGGAGGCAGCAGT-3' UniR514 5'-ATTACCGCGGCTGCTGGC-3'
Segmented Filamentous Bacteria (SFB) **(Barman et al.)**	*16S rRNA*	SFBF 5'- GACGCTGAGGCATGAGAGCAT -3' SFBR - 5'- GACGGCACGGATTGTTATTCA -3'