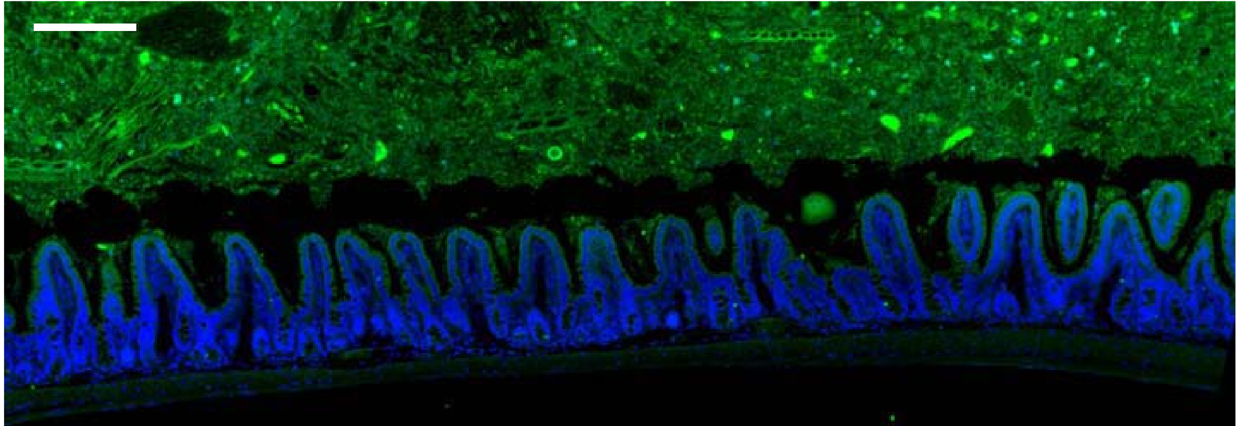
Supporting Table 1:

Primers used in this study

Gene	Forward Primer	Reverse Primer
Genotyping Primers		
FLAG-Myd88	CAGGGCCCATGGACTACAAAGAC GATGACG	CTGGTACCTCAGGGCAGGGACAA AGCCTTG
Endogenous *Myd88* gene	TGGCATGCCTCCATCATAGTTAACC	GTCAGAAACAACCACCACCATGC
Neo	ATCGCCTTCTATCGCCTTCTTGACG	
Vil-Cre	GTGTGGGACAGAGAACAAACC	ACATCTTCAGGTTCTGCGGG
$Myd88^{fl/fl}$	GTTGTGTGTGTCCGACCGT	GTCAGAAACAACCACCACCATGC
$RegIII\gamma^{+/+}$ allele	GCAGCAGGAAAGCAAGTCCATTCT	ATGCTCATGCAAGTCAGGGAGGTA
$RegIII\gamma^{-/-}$ allele (+Pgk-neo)	GCAGCAGGAAAGCAAGTCCATTCT	GAAAGCGAAGGAGCAAAGCTGCTA
$RegIII\gamma^{-/-}$ allele (-Pgk-neo)	GCAGCAGGAAAGCAAGTCCATTCT	TCAGTGAGAGACGATGCGGCTAAT
Q-PCR Primers		
18S rRNA	CATTCGAACGTCTGCCCTAT	CCTGCTGCCTTCCTTGGA
RegIIIγ	TTCCTGTCCTCCATGATCAAA	CATCCACCTCTGTTGGGTTC
Bacterial 16S rRNA gene primers		
Eubacteria (Universal)	ACTCCTACGGGAGGCAGCAGT	ATTACCGCGGCTGCTGGC
Bacteroides	GGTTCTGAGAGGAGGTCCC	CTGCCTCCCGTAGGAGT
Mouse Intestinal *Bacteroides* (MIB)	CCAGCAGCCGCGGTAATA	CGCATTCCGCATACTTCTC
Lactobacillus/Enterococcus Group (Lact)	AGCAGTAGGGAATCTTCC	CACCGCTACACATGGA
Eubacterium rectale/ *Clostridium coccoides* group (Erec)	ACTCCTACGGGAGGCAGC	GCTTCTTAGTCAGGTACCGTCA
Segmented filamentous bacteria (SFB)	GACGCTGAGGCATGAGAGCA	GACGGCACGGATTGTTATTC

A



B

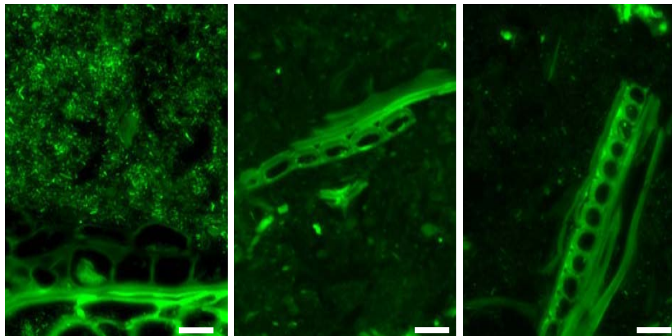


Figure S1: Physical separation between host and microbiota in the small intestine. (A) Small intestines of wild-type C57BL/6 mice were prepared for fluorescence *in situ* hybridization by fixation in Carnoy's fixative, which preserves the mucus layer (*7*). Tissue sections were hybridized to a probe that recognizes the 16S rRNA genes of all bacteria (green), and counterstained with DAPI to visualize nuclei (blue). Scale bar=200 μm. **(B)** FISH analysis of luminal contents using the 16S rDNA universal probe used in (A), a non-specific scrambled probe, or no probe (probe-negative control). The probe-negative control demonstrates autofluorescence of plant materials in the intestinal lumen when visualized in the FITC channel. Scale bars=50 μm.

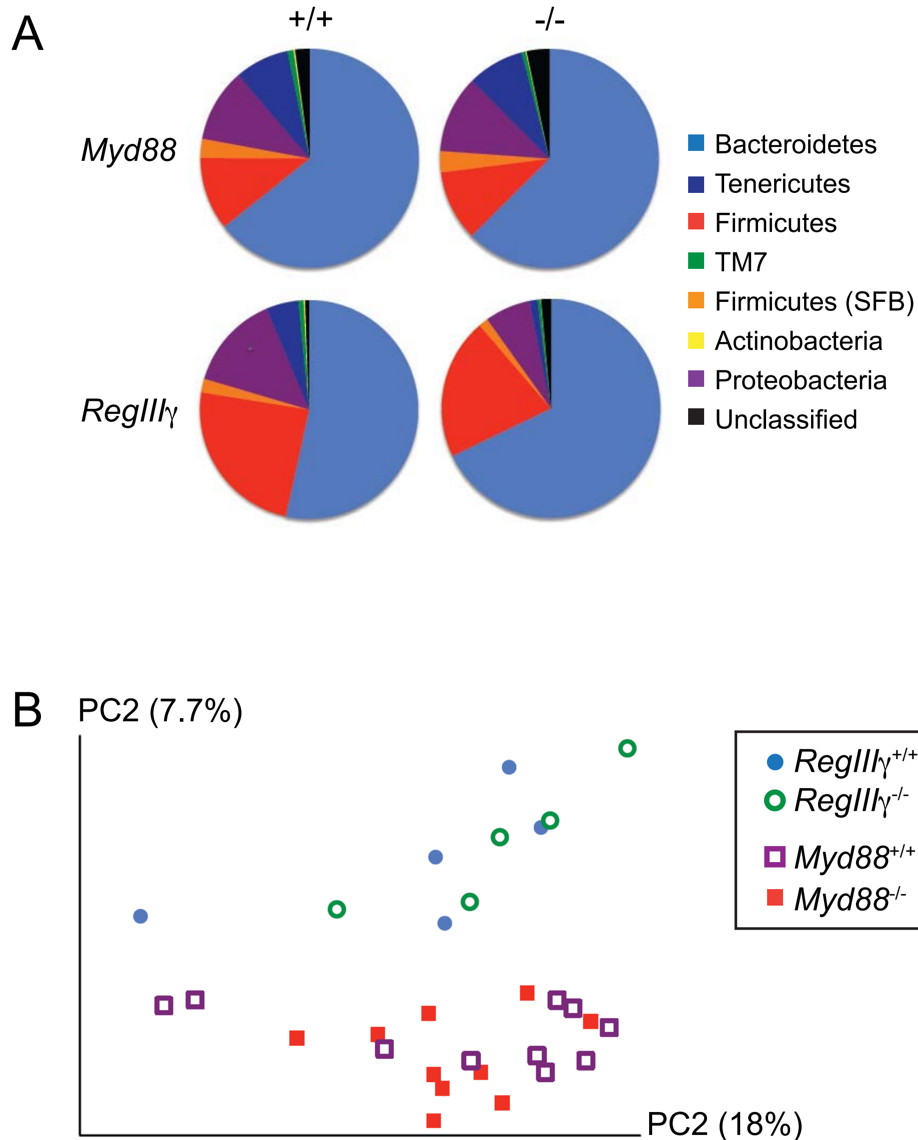
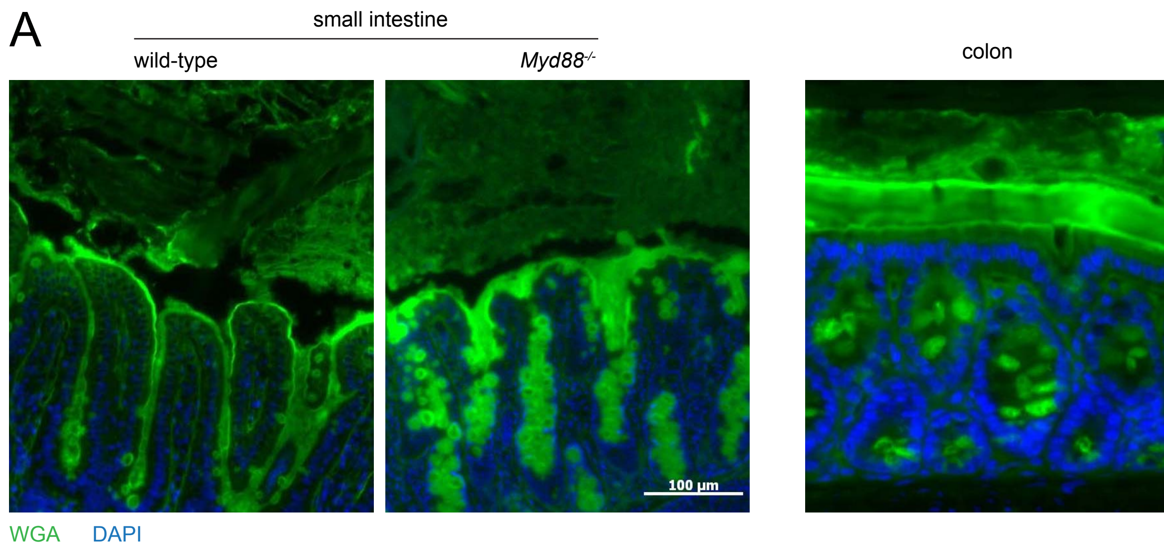


Figure S2: Luminal community structure in *Myd88*$^{-/-}$ and *RegIIIγ*$^{-/-}$ mice.
(A) Mean relative abundances of bacterial phyla from the luminal communities of *Myd88*$^{-/-}$, *RegIIIγ*$^{-/-}$, and wild-type mice. The phylum Firmicutes was divided into SFB and all other Firmicutes. All mice analyzed were the offspring of heterozygous crosses that remained co-housed. Thus, *Myd88*$^{-/-}$ and *RegIIIγ*$^{-/-}$ mice each have distinct wild-type controls. Plotted values are relative mean sequence abundances of each phylum (6,000 sequences/sample). The mean differences between all phyla in both cohousing groups did not attain statistical significance by t-test, indicating that *Myd88*$^{-/-}$ and *RegIIIγ*$^{-/-}$ luminal communities are similar to their co-housed wild-type littermates at the phylum level. **(B)** Comparisons of small intestinal microbiota composition of *RegIIIγ*$^{-/-}$, *Myd88*$^{-/-}$ and wild-type mice. Principal Coordinates 1 and 2, from the unweighted UniFrac analyses, are plotted for each sample. Each symbol represents a sample colored by mouse type. The percent variance explained by the principal coordinates is indicated in parentheses. The results show that both *Myd88*$^{-/-}$ and *RegIIIγ*$^{-/-}$ harbor luminal communities that are similar to their co-housed wild-type littermates. However, there are significant differences between the co-housed groups that are depending on caging but independent of genotype.



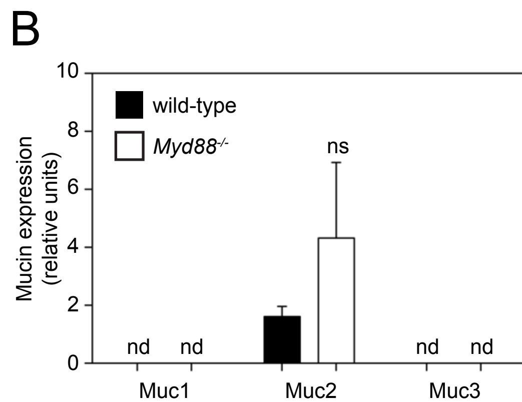


Figure S3: Small intestinal mucus production in *Myd88*$^{-/-}$ mice.

(A) Small intestinal sections were fixed in Carnoy's fixative, which preserves the mucus layer (*7*). The sections were stained with wheat germ agglutinin (WGA; green), which recognizes glycan structures on mucin glycoproteins (*23*). Nuclei were detected with a DAPI counterstain (blue). Colon is shown for comparison, and exhibits a characteristic dense inner mucus layer (*5*). Scale bars=100 μm.

(B) Q-PCR analysis of small intestinal mucin 2 (*Muc2*) expression. Small intestines were first surveyed for expression of *Muc1*, *Muc2*, and *Muc3*, major mucin components of large intestinal mucus. *Muc1* and *Muc3* mRNAs were not detectable, regardless of mouse genotype, suggesting that they are not major mucus components in the small intestine. *Muc2* was expressed in both wild-type and *Myd88*$^{-/-}$ mice (N=3 mice of each genotype). ns, not significant; Error bars, ±SEM; nd, not detected.

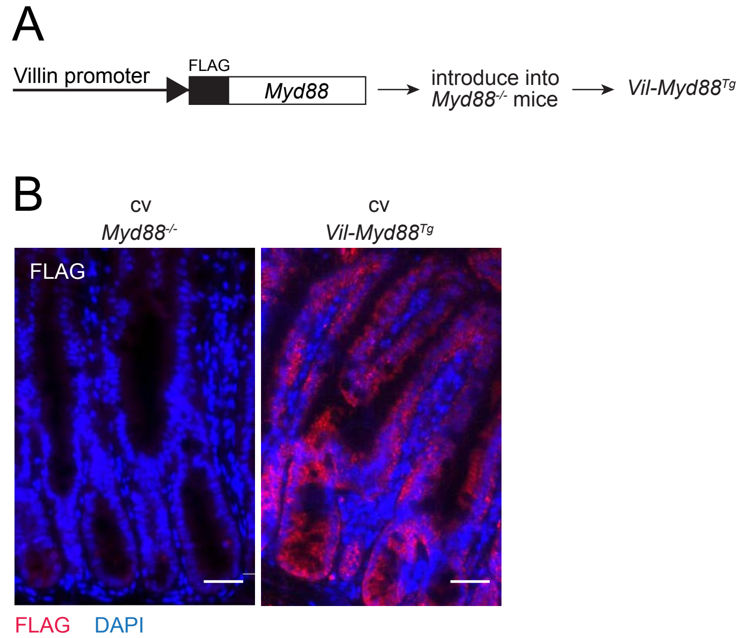


Figure S4: Generation and analysis of *Vil-Myd88^{Tg}* mice.
(A) A *Myd88* transgene with an N-terminal FLAG epitope fusion was cloned downstream of the epithelial cell-specific *Villin* promoter (*8*). Transgenic lines were generated and crossed onto *Myd88$^{-/-}$* mice. *Vil-Myd88^{Tg}* denotes mice harboring the *Vil-FLAG-Myd88* transgene on a *Myd88$^{-/-}$* background. **(B)** To detect transgene expression, small intestinal tissues were probed with an anti-FLAG antibody (red), which specifically labeled epithelial cells, and counterstained with DAPI to visualize nuclei (blue). Mice were further analyzed for expression of the MyD88-dependent antibacterial protein RegIIIγ in Figure 2. Scale bars=50 μm. Experimental animals were generated from crosses of *Vil-Myd88^{Tg}* animals with *Myd88$^{-/-}$* animals, and co-housed *Vil-Myd88^{Tg}* and *Myd88$^{-/-}$* littermates were analyzed.

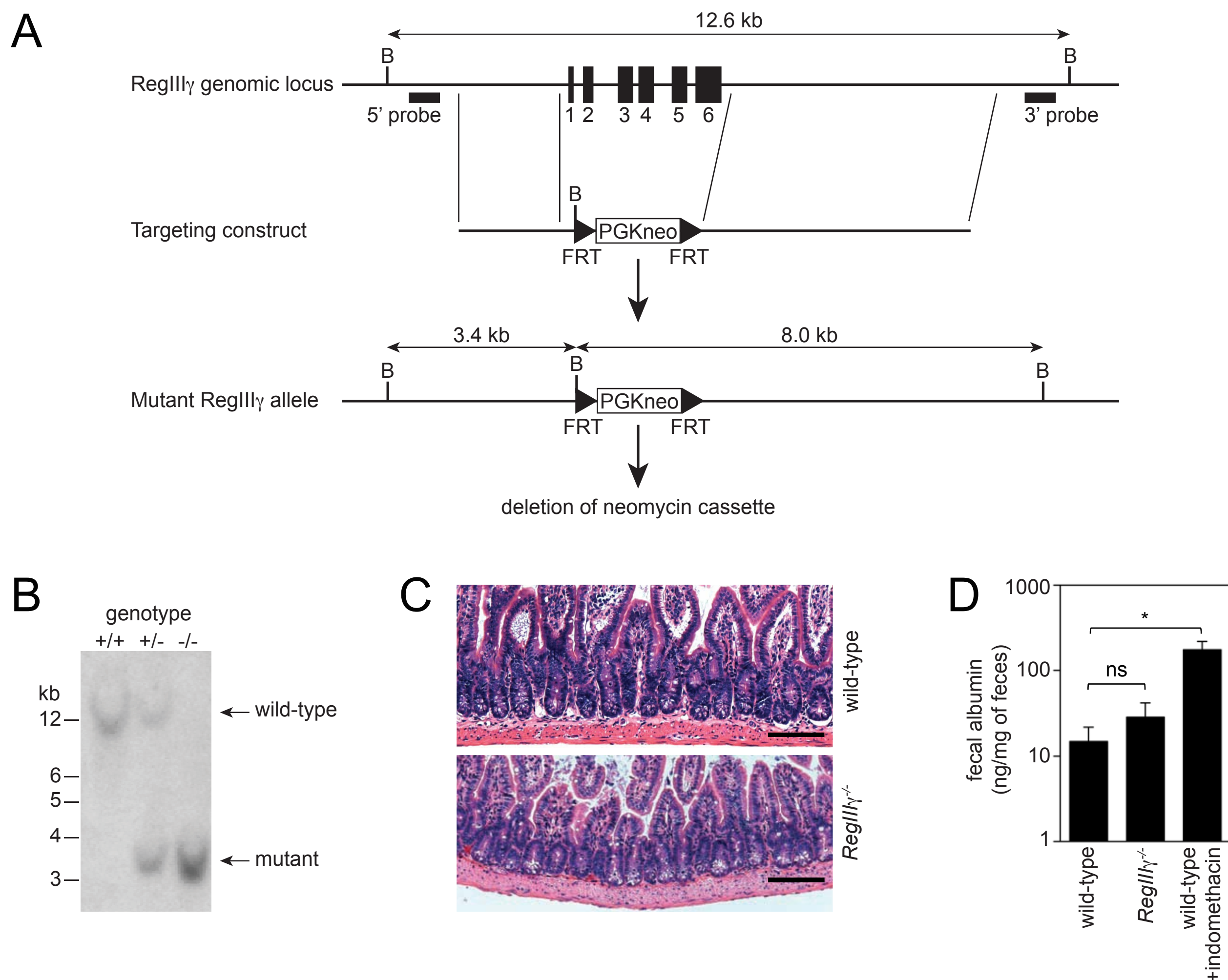


Figure S5: Targeted disruption of the RegIIIγ gene by homologous recombination.

(A) The endogenous *RegIIIγ* locus, targeting construct, and targeted locus are shown. Exons are represented by numbered black boxes. The targeting construct was designed to replace all 6 exons with a neomycin resistance gene (PGKneo) flanked by FRT sites. Bam HI (B) digestion of the endogenous locus generates a 12.6 kb fragment that is detected by the 5' probe. In correctly targeted clones, this probe detects a 3.4 kb fragment. PGKneo, neomycin resistance cassette. The PGKneo cassette, flanked by FRT sites, was deleted by intercrossing *RegIIIγ*$^{+/-}$ mice with transgenic "deletor" mice that constitutively express FLP recombinase. Following neomycin cassette deletion, *RegIIIγ*$^{+/-}$ mice were backcrossed for 8 generations to C57BL/6 mice. Experimental animals were generated from *RegIIIγ*$^{+/-}$ intercrosses and all experiments were performed on co-housed *RegIIIγ*$^{-/-}$ and *RegIIIγ*$^{+/+}$ littermates.

(B) Southern blot analysis of the targeted *RegIIIγ* locus. The 5' probe shown in (A) was hybridized to BamHI-digested genomic DNA from the offspring of intercrossed *RegIIIγ*$^{+/-}$ mice. Wild-type and mutant alleles are marked by 12.6 and 3.4 kb bands, respectively.

(C) *RegIIIγ*$^{-/-}$ mice do not show signs of overt intestinal pathology. Small intestines from *RegIIIγ*$^{-/-}$ mice and their co-housed wild-type littermates were prepared in Bouin's fixative, paraffin-embedded, sectioned, and stained with hematoxylin and eosin. Scale bar=100 μm.

(D) Intestinal permeability of *RegIIIγ*$^{-/-}$ mice was measured by measuring serum protein loss into the intestinal lumen. Wild-type and *RegIIIγ*$^{-/-}$ littermates were analyzed by enzyme-linked immunosorbent assay (ELISA) for fecal albumin. As a positive control, one group of wild-type mice was pre-treated with indomethacin (7.5 mg/kg) for 24 hours to increase intestinal permeability. N=3-4 mice per group; *, $p<0.05$; Error bars, ±SEM; ns, not significant.

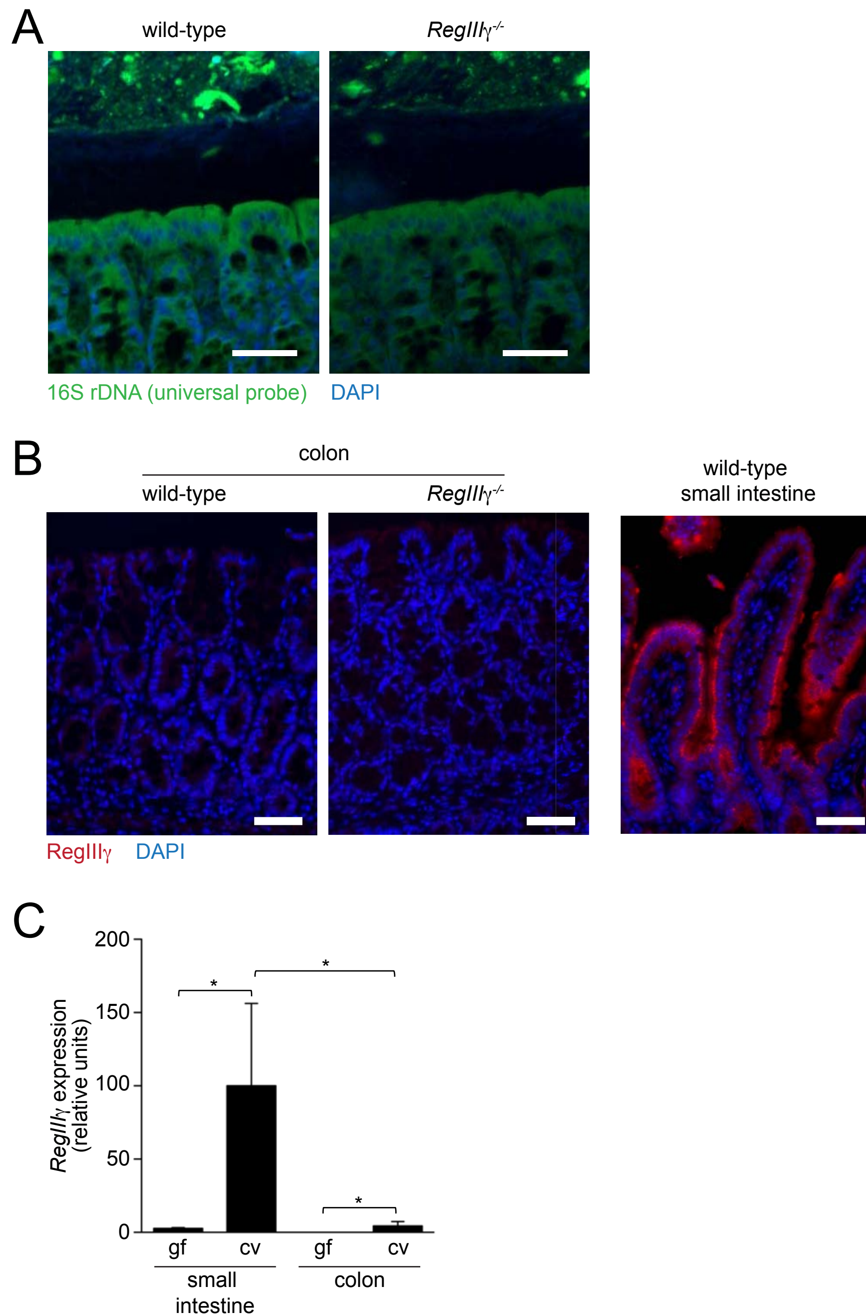


Figure S6: Analysis of spatial relationships between host and microbiota in the colons of *RegIIIγ*$^{-/-}$ mice.

(A) FISH analysis of microbiota localization in colon using a universal bacterial 16S rRNA gene probe. Scale bars=50 μm. **(B)** RegIIIγ was detected in colon with anti-RegIIIγ antiserum (*13*)(red). Nuclei were counterstained with DAPI (blue). A section of wild-type small intestine is stained for comparison. Scale bars=50 μm. **(C)** Q-PCR quantification of *RegIIIγ* transcripts in small intestine and colon. Expression levels in conventionally-raised (cv) mice are compared to those in germ-free (gf) mice, which lack a microbiota. N=4 mice per group. Statistical significance was determined by the Mann-Whitney test. *, $p<0.05$; error bars, ±SEM.

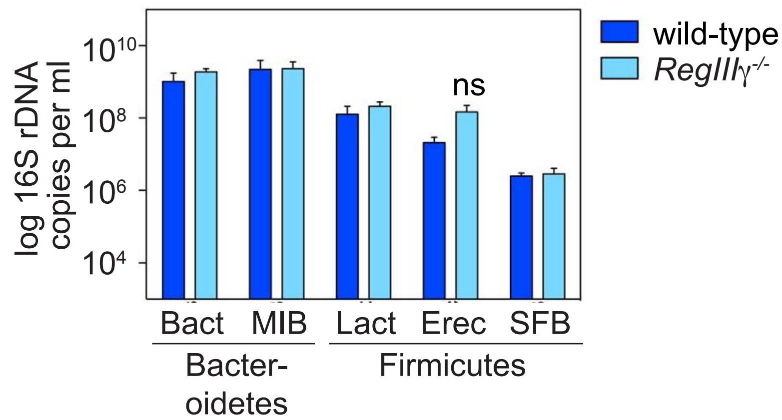


Figure S7: Analysis of luminal microbiota in *RegIIIγ$^{-/-}$* mice.
Quantification of total ileal luminal bacteria by Q-PCR determination of 16S rRNA gene copy number. Mice were co-housed littermates from heterozygous crosses. N=5-7 mice/genotype from five littermate groups. ns, not significant.

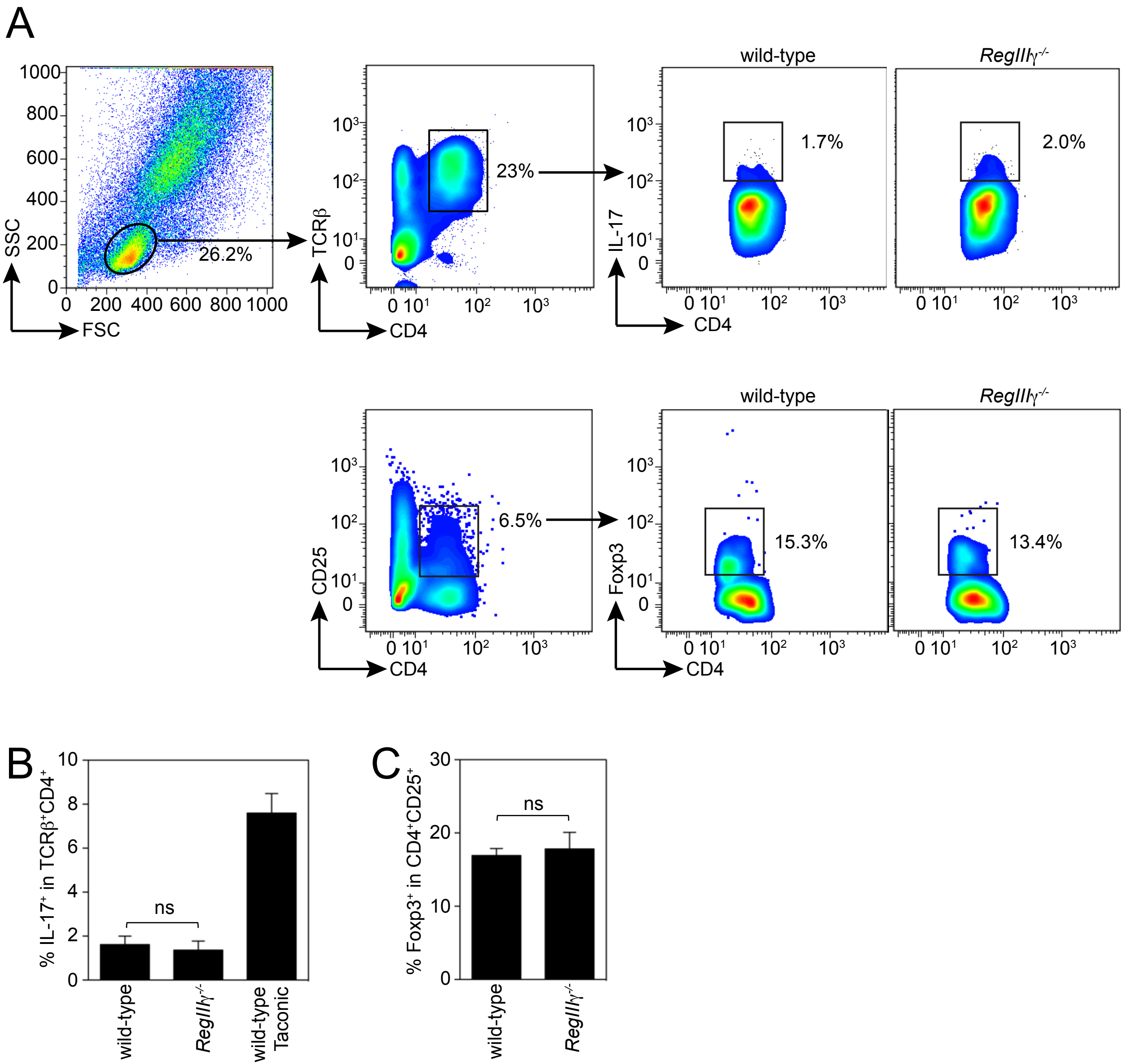


Figure S8: Enumeration of Th17 and Treg cells in *RegIIIγ*$^{-/-}$ mice.
(A) Small intestinal lamina propria cells were isolated from *RegIIIγ*$^{-/-}$ mice and their co-housed wild-type littermates at 4-5 weeks of age. For enumeration of Th17 cells, the cells were gated on TCRβ and CD4, and IL-17^{+} cells were quantified as a percentage of this population. Wild-type mice from Taconic Farms were included as a positive control for Th17 cell isolations (*2*). Treg cells were enumerated by gating on CD25 and CD4, and Foxp3^{+} cells were quantified as a percentage of this population. The IL-17 and Foxp3 gates were determined based on isotype controls. Representative FACS plots are shown. **(B)** IL-17^{+} cells as a percentage of the TCRβ^{+}CD4^{+} cell population. N=4-6 mice per group from 2-3 littermate groups. (C) Foxp3^{+} cells as a percentage of the CD4^{+}CD25^{+} cell population. N=3 mice per group from two littermate groups. ns, not significant.