Supplementary Information Inventory

RUNNING TITLE: *Bacteroides* induce disease in mice with IBD (Bloom et al)

Supplementary Info

Figure S1. Scoring of intestinal gross pathology, related to Figure 1
Representative cecal whole mount samples imaged at 7X, 20X, 40X, and 90X magnification (see Methods) and scored by an anatomic pathologist (TSS) blinded to the identity of the samples according to a previously validated scoring system: 0, normal; 1, focal ulcers present; 2, ulcers and diffuse, mild mucosal thickening; and 3, ulcers and diffuse, severe mucosal thickening (Kang et al., 2008). Samples in which different areas of the image most closely matched different scoring criteria were assigned the average of the two scores. 7X scale bar = 6 mm; 20X scale bar = 2 mm; 40X scale bar = 1 mm; 90X scale bar = 0.44 mm.

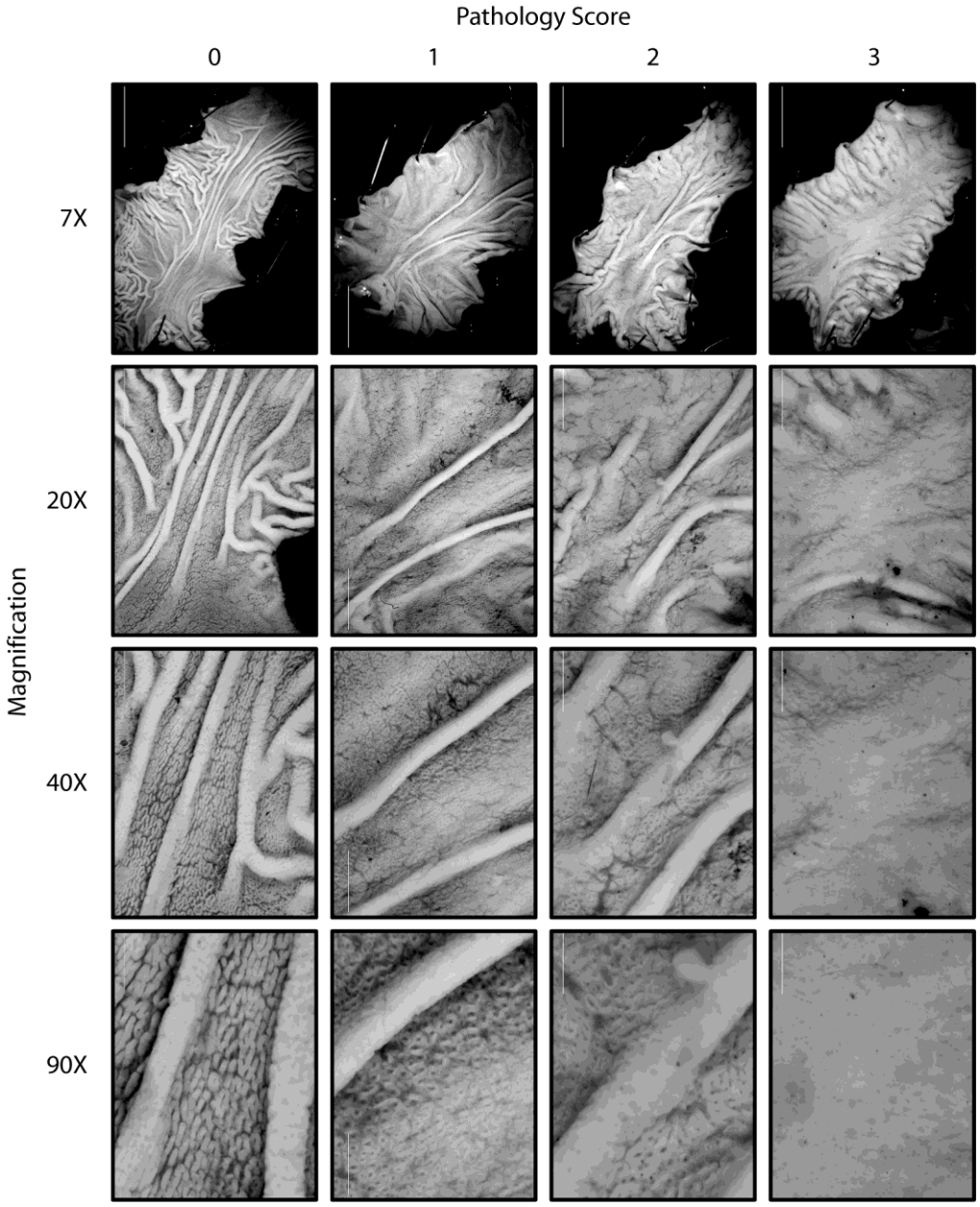

Figure S2. Analysis of antibiotic-induced microbiota changes by taxon-specific 16S rRNA gene qPCR, related to Figure 2

(A-D) Plots of fecal samples from untreated and antibiotic-treated dnKO and *Il10r2*$^{+/-}$ mice (n = 6 per group) analyzed by quantitative PCR using validated primer sets specific for the 16S rRNA gene of bacteria from the Bacteroidetes families Bacteroidaceae-Porphyromonadaceae-Prevotellaceae and from the Firmicutes families Lachnospiraceae-Ruminococcaceae, Enterococcaceae, and Lactobacillaceae (Nava et al., 2010). *Il10r2*$^{+/-}$ and dnKO mice within each treatment group were co-housed; data are compiled from ≥3 cages/group. Results of qPCR assays are normalized to sample weight and shown as box plots with five-number summaries (smallest observation, lower quartile, median, upper quartile and largest observation). Kruskal-Wallis test with post-hoc Dunn's test: (A) H_3 = 6.583, p = 0.0767; (B) H_3 = 9.436, p = 0.0240; (C) H_3 = 17.85, p = 0.0005; (D) H_3 = 16.26, p = 0.0010. All significant pairwise comparisons are displayed: *, p < 0.05; ** < 0.01. When data from mice of both genotypes was compiled by treatment group (n = 12 mice per group consisting of 6 dnKO and 6 *Il10r2*$^{+/-}$ mice), analysis using Mann-Whitney U-test found that antibiotic treatment was associated with significant depletion of BPP (p = 0.0304) and Lachnospiraceae-Ruminococcaceae (p = 0.0226) and significant enrichment of Enterococcaceae and Lactobacillaceae were significantly enriched (p < 0.0001).

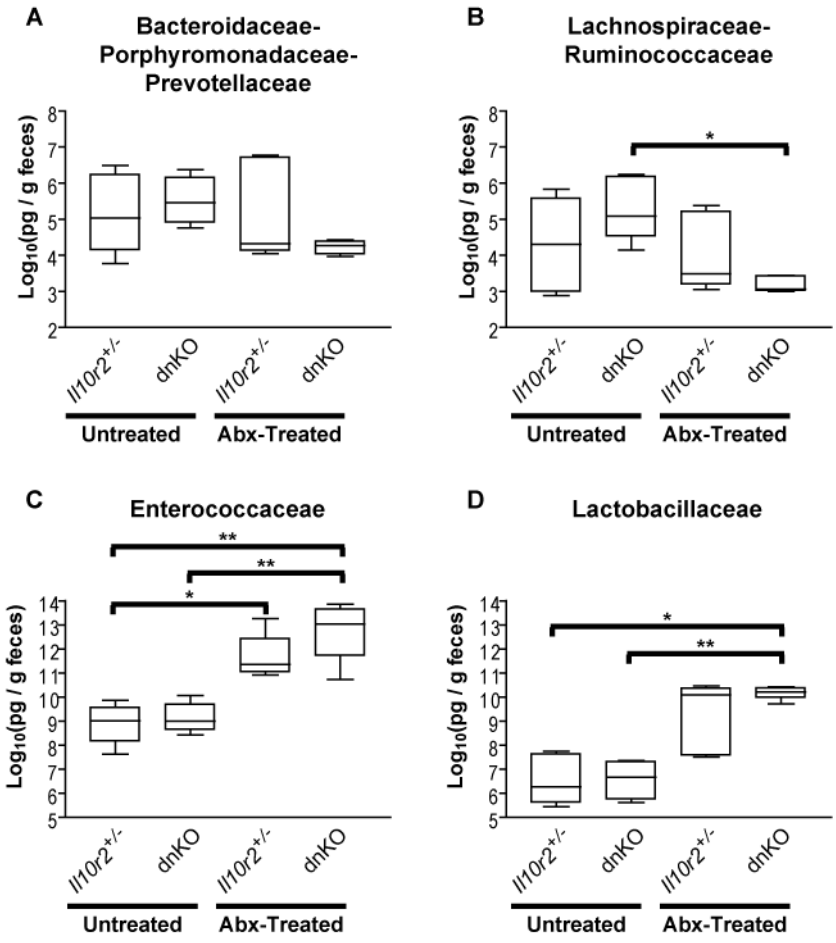

Figure S3. Disease induction by commensal *Bacteroides* species, related to Figure 4

(A) Representative rectal histology of antibiotic-pretreated mice of the indicated genotypes gavaged with PBS or a pure culture of the *Bacteroides thetaiotaomicron* isolate ($7x10^7$ cfu/mouse) as described in Figures 4C to 4H. Scale bar = 50 µm.

(B) Immunoblots of *B. vulgatus* lysate probed with a 1:200 dilution of pooled serum collected from antibiotic-pretreated *Il10r2*$^{+/-}$ (n=3) or dnKO (n=6) mice immediately prior to gavage with 10^8 cfu/mouse of *B. vulgatus* (pre-gavage) or at sacrifice 3 weeks later (post-gavage). Secondary antibody is α-mouse IgG. Equal amounts of bacterial lysate were separated by SDS-PAGE, transferred to nitrocellulose membranes, blotted, and exposed in parallel for all groups.

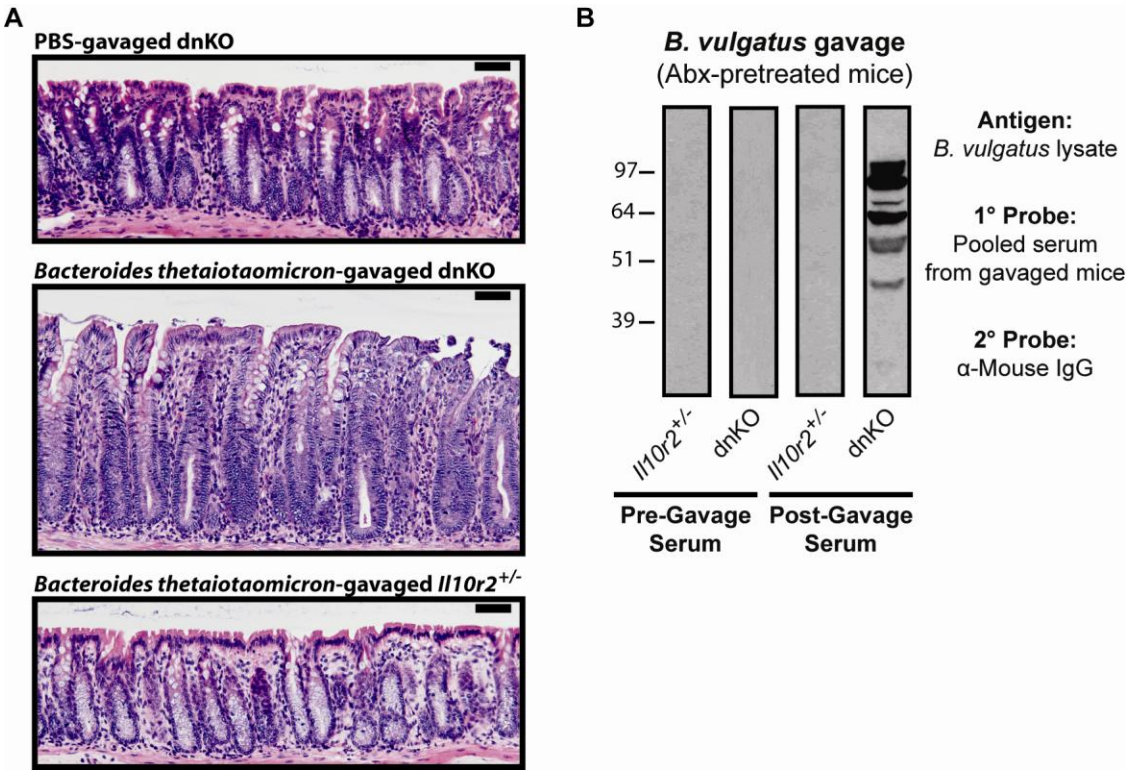

Figure S4. Bacterial load and *Bacteroides* culture characteristics on BBE agar, related to Figure 5

(A) Plot of fecal samples from untreated and antibiotic-treated dnKO and *Il10r2*$^{+/-}$ mice (n = 6 per group) analyzed by quantitative PCR using primers for the bacterial *rpoB* gene (Nava et al., 2010). *Il10r2*$^{+/-}$ and dnKO mice within each treatment group were co-housed; data are compiled from ≥3 cages/group. Results are normalized to sample weight and shown as a box plot with five-number summaries (smallest observation, lower quartile, median, upper quartile and largest observation). Kruskal-Wallis test with post-hoc Dunn's test: $H_3 = 16.82$, $p = 0.0008$. All significant pairwise comparisons are displayed: *, $p < 0.05$.

(B) To confirm the long-term stability of *Bacteroides* elimination by antibiotics, cages of antibiotic-pretreated non-dnKO mice (2-5 mice per cage) were gavaged with sterile PBS (n = 5 cages) or with 2.9×10^8 cfu *B. thetaiotaomicron* per mouse (n = 5 cages) and maintained using rigorous animal husbandry practices as described in the Experimental Procedures. 28 weeks post-gavage, fecal samples from mice in each cage were collected, pooled by cage, and quantitatively titered on BBE agar. Titers from individual cages are displayed. The presence of BBE-cultivable bacteria in a single PBS-gavaged cage is presumed due to accidental contamination during cage-changing and animal-handling procedures.

(C) Pure cultures of (1.) *B. vulgatus*, (2.) *Bacteroides* sp. TP5, and (3.) *B. thetaiotaomicron* isolates (see Table S2) streaked on BBE media and incubated anaerobically for 48 hrs. Insets show representative colonies of each isolate (indicated by black squares in the low-power image) were photographed on a dissecting microscope at 12.5X magnification images with both backlighting and reflected lighting at an exposure time of 1/500 s. The isolates grow with characteristic size, morphology, and pigmentation on BBE agar. *B. vulgatus* forms non-pigmented, circular, convex colonies with entire edges that are 1-2 cm in diameter after 48 hrs growth. *B. thetaiotaomicron* forms colonies that are similar in size and morphology to *B. vulgatus* but strongly pigmented. *Bacteroides* sp. TP5 forms circular, convex colonies with entire edges that are 0.25-1 cm in diameter and non-pigmented or very lightly pigmented after 48 hrs. Culture plate diameter = ~8.6 cm; scale bars = 1 mm.

(D) Representative BBE fecal titer plate from a *B. thetaiotaomicron*-gavaged mouse exhibiting characteristic *B. thetaiotaomicron* colony appearance (photographed after 72-hr anaerobic incubation). Bacterial identity was confirmed as *B. thetaiotaomicron* by 16S rRNA gene sequencing of representative colonies.

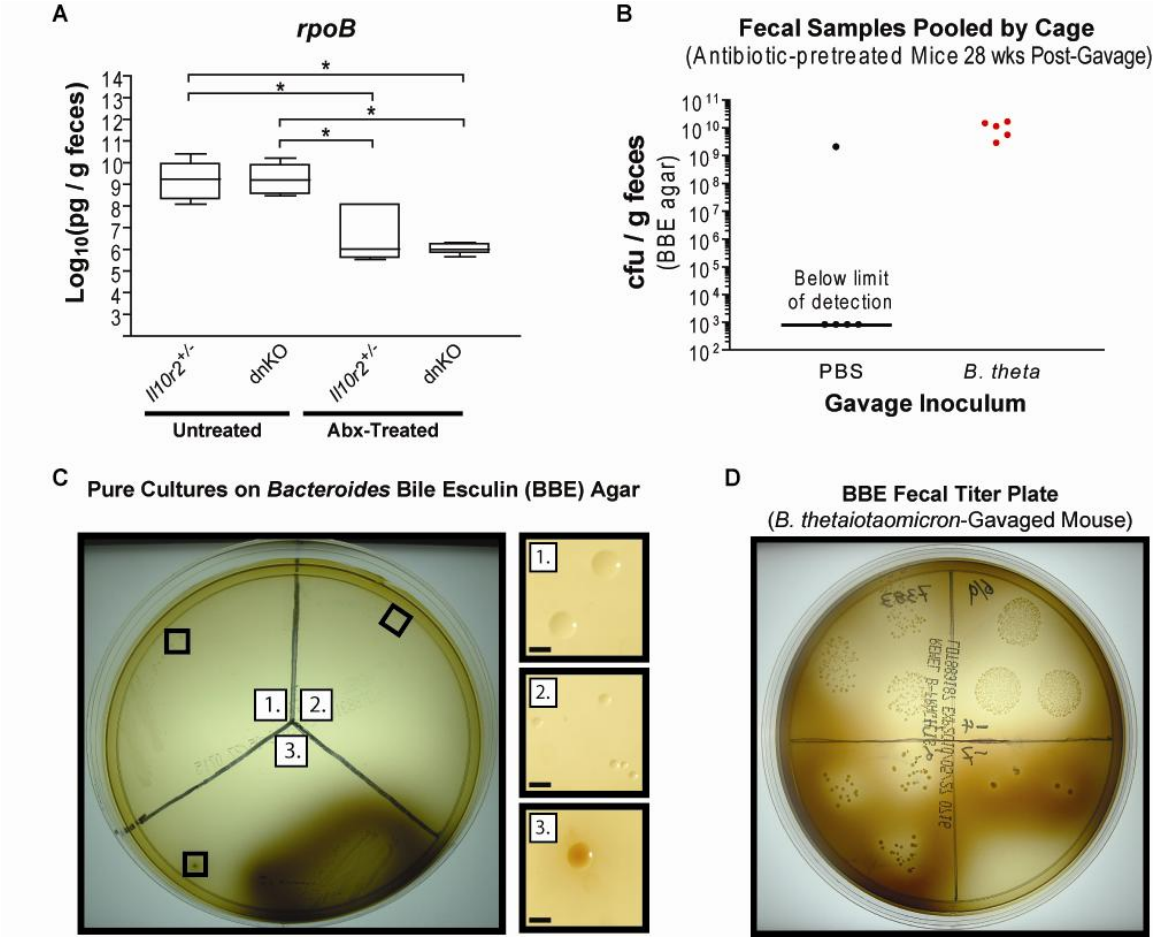
A
rpoB
Log10(pg / g feces)
Il10r2+/-
dnKO
Il10r2+/-
dnKO
Untreated
Abx-Treated
B
Fecal Samples Pooled by Cage
(Antibiotic-pretreated Mice 28 wks Post-Gavage)
cfu / g feces
(BBE agar)
Below limit
of detection
PBS
B. theta
Gavage Inoculum
C
Pure Cultures on Bacteroides Bile Esculin (BBE) Agar
1.
2.
3.
D
BBE Fecal Titer Plate
(B. thetaiotaomicron-Gavaged Mouse)

Figure S5. Approximate percentages of BBE- and MacConkey-cultivable fecal bacteria in *Il10r2*$^{+/-}$ and dnKO mice, related to Figure 6

Approximate percentages of fecal bacteria on *Bacteroides* bile esculin (BBE) agar and MacConkey agar (selective for Enterobacteriaceae) were calculated by dividing BBE titers and MacConkey titers of by the corresponding titers of total cultivable bacteria (on non-selective ANB agar) in samples from untreated *Il10r2*$^{+/-}$ and dnKO mice (see Figure 6A to 6C). Percentages of "Other" were calculated by subtracting BBE and MacConkey percentages from 100%. Genotypes and housing arrangements of the mice are shown.

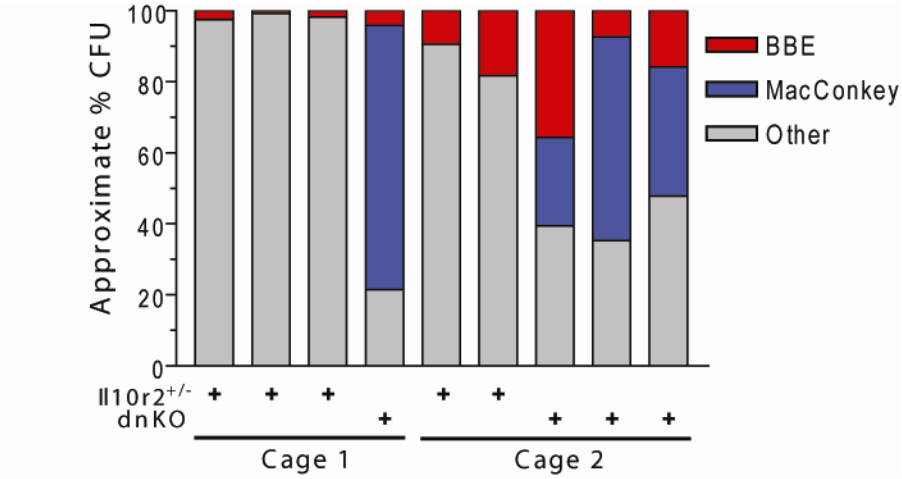

Figure S6. Isolation of a colitis-enriched *Escherichia coli* from feces of an untreated dnKO mouse, related to Figure 7

A prominent, distinctive bacterial colony type (convex, circular, white-yellow, entire margins, strongly β-hemolytic, 2-3 mm diameter after 48 hrs incubation) was isolated from the feces of an untreated (spontaneously colitic) dnKO mouse cultured on non-selective ANB agar. The colony type comprised 33% of all colonies observed on the titer plate ($2.0x10^9$ cfu/g out of $6.0x10^9$ total cfu/g) and was the single most abundant single colony type present in the fecal sample; it did not grow on either LKV or CNA agar (data not shown).

(A) The isolate was identified by 16S rRNA sequencing as *Escherichia coli*, a member of the family Enterobacteriaceae. Table lists isolate ID, most similar bacterial type strain, percent sequence identity, and accession number of type strain sequence. Classification as *E. coli* was confirmed with 99% confidence using phenotypic methods (see Experimental Procedures).

(B) Descending colon histology of the untreated dnKO mouse from which *E. coli* was isolated and of a co-housed untreated *Il10r2*$^{+/-}$ mouse. Scale bar = 200 μm.

A

Isolate ID	Closest Type Strain (Closest Cultured Isolate)	% Identity	Accession Number of Closest Type Strain
dn15.6244.1	*Escherichia fergusonii* (*Escherichia coli* ED1a)	99.8% (100.0%)	AF530475 (CU928162)

B

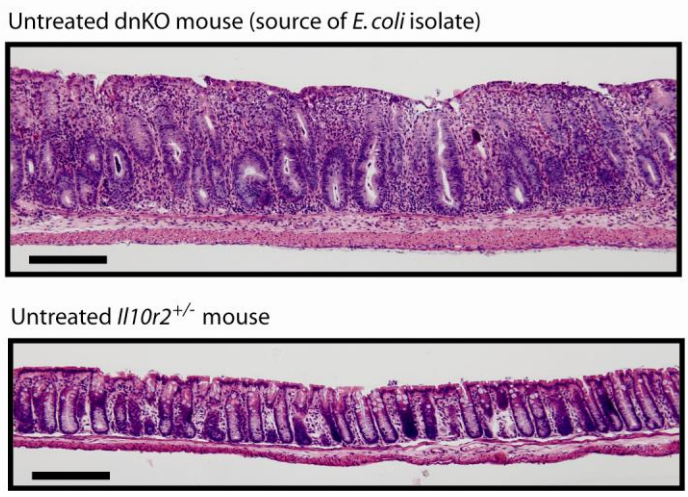

Table S1. Preparation of bacterial mixed cultures to screen for colitis induction, related to Figure 3

Media Type	Culture Conditions	10-Fold Dilution Factor of Intestinal Contents from Untreated Mice	# of Colonies on Culture Plates
ANB	Anaerobic	4.5	1.4×10^3
CNA		4.5	1.6×10^3
LKV		4	1.4×10^3
Chocolate	Aerobic	0	3.9×10^5

Serial $10^{0.5}$-fold dilutions of intestinal contents (see Methods) were plated in parallel on solid media and incubated either anaerobically or aerobically. From each anaerobic media type, we selected a dilution at which ~1500 colonies grew. Each culture was harvested from the plate and frozen at -80˚C in single-use aliquots with 20% pre-reduced glycerol. The aerobic culture was grown on chocolate agar and harvested by the same method. The table lists culture conditions, media types, 10-fold dilution factors, and numbers of cfu on the plates from which cultured bacteria were harvested.

Table S2. Fecal bacterial isolates from antibiotic-treated dnKO mice, related to Figure 3

Isolate ID	Closest Type Strain	16S rRNA Percent Identity	Accession Number of Closest Type Strain
AbxANB1	Clostridium clostridioforme	99.1%	M59089
AbxANB2	Enterococcus gallinarum	99.8%	AF039900
AbxANB3	Lactobacillus intestinalis	98.1%	AJ306299
AbxANB4	Lactobacillus gasseri	98.9%	AF519171
AbxANB5	Staphylococcus epidermidis	100.0%	D83363

Fecal samples from dnKO mice treated with antibiotics for ≥3 weeks were transferred to an anaerobic chamber, suspended in sterile, pre-reduced PBS, serially diluted, and cultured anaerobically on non-selective Anaerobic Reducible Blood (ANB) agar. Unique-appearing, abundant isolates were picked. Genomic DNA extracted from individual isolates was PCR-amplified using broad-range bacterial 16S rRNA gene primers. PCR products were directly sequenced using the original PCR primers. The most similar bacterial type strain sequences were identified using the Ribosomal Database Project's SeqMatch program supplemented by BLAST analysis. The table shows the isolate ID and the name, percent sequence identity, and accession number of the most similar bacterial type strain. Species that were isolated independently from mice in at least two different cages are listed. All isolates listed belonged to the phylum Firmicutes with >99% similarity to sequences identified in metagenomic sequencing of rodent commensal microbiota (not shown).

Table S3. Gram-negative obligate anaerobes isolated from LKV agar, related to Figure 4

Isolate ID	Closest Type Strain (Closest Cultured Isolate)	16S rRNA Percent Identity	Accession Number of Closest Type Strain (Closest Cultured Isolate)
dnLKV2	*Bacteroides uniformis*	99%	AB050110
dnLKV3	*Bacteroides massiliensis* (Bacteroides sp. TP-5)	94% (99%)	AY126616 (AB499846)
dnLKV7	*Bacteroides dorei* (*Bacteroides vulgatus*)	97% (99%)	AB242142 (AB510712)
dnLKV8	*Parabacteroides distasonis*	97%	AB238922
dnLKV9	*Bacteroides thetaiotaomicron*	99%	AE015928
dnLKV18	*Parabacteroides goldsteinii*	99%	AY974070

Genomic DNA extracted from individual isolates was PCR-amplified using broad-range bacterial 16S rRNA gene primers. PCR products were directly sequenced using the original PCR primers. The most similar bacterial type strain sequences were identified using the Ribosomal Database Project's SeqMatch program supplemented by BLAST analysis. Table lists unique species of Gram-negative obligate anaerobes isolated by this method, showing the isolate ID and the name, percent sequence identity, and accession number of the most similar bacterial type strain. For isolates with <98% identity to the most similar type strain, the most similar cultured isolate in the RDP database was also identified. All isolates belonged to the phylum Bacteroidetes with >99% similarity to sequences identified in metagenomic sequencing of rodent commensal microbiota (not shown).

SUPPLEMENTARY REFERENCES

Kang, S. S., Bloom, S. M., Norian, L. A., Geske, M. J., Flavell, R. A., Stappenbeck, T. S., and Allen, P. M. (2008). An antibiotic-responsive mouse model of fulminant ulcerative colitis. PLoS Med *5*, e41.
Nava, G. M., Friedrichsen, H. J., and Stappenbeck, T. S. (2010). Spatial organization of intestinal microbiota in the mouse ascending colon.