SUPPLEMENTARY MATERIAL

Gut microbiota derived propionate regulates the expression of Reg3 mucosal lectins and ameliorates experimental colitis in mice

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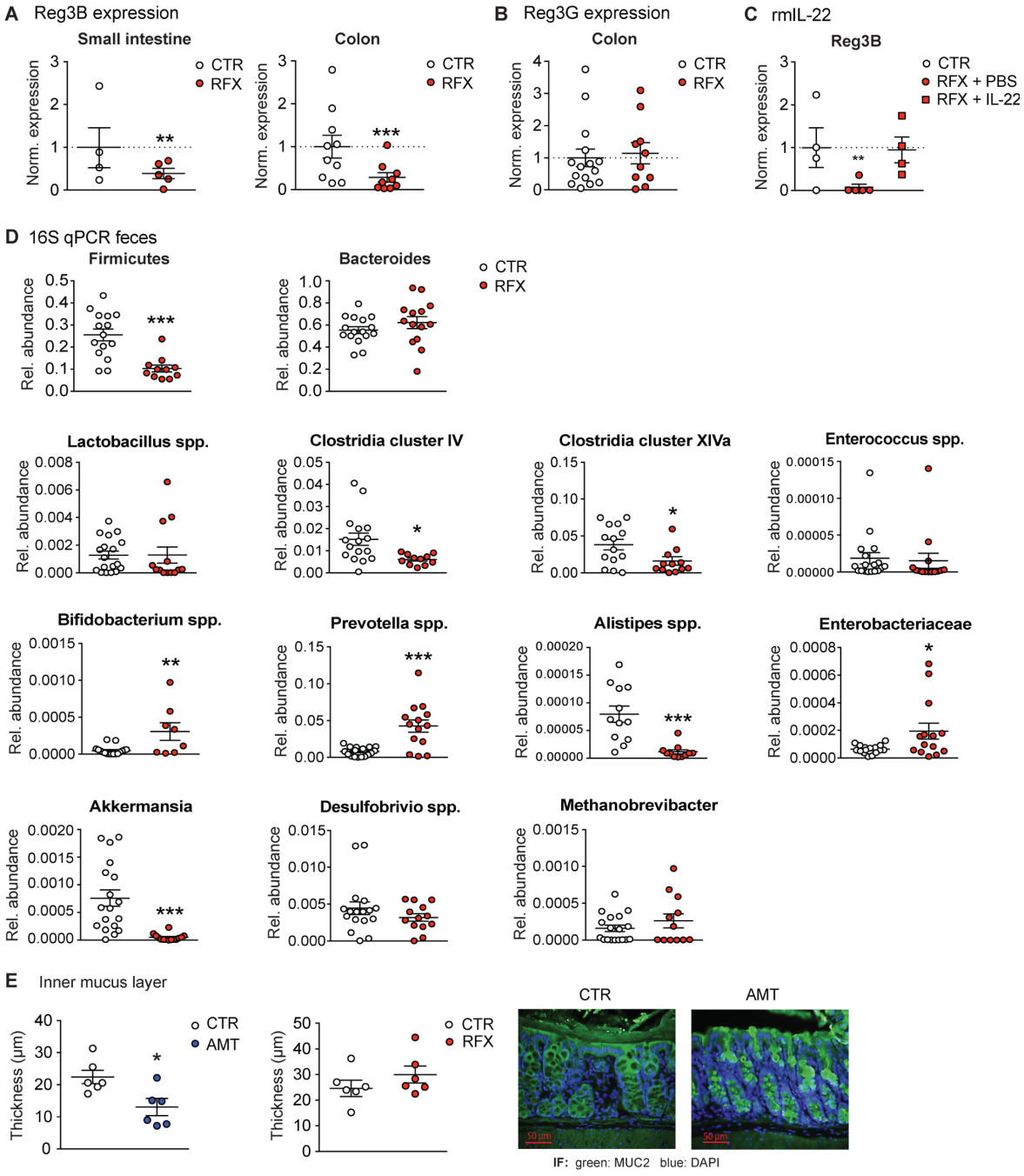
Supplementary Figure 1.

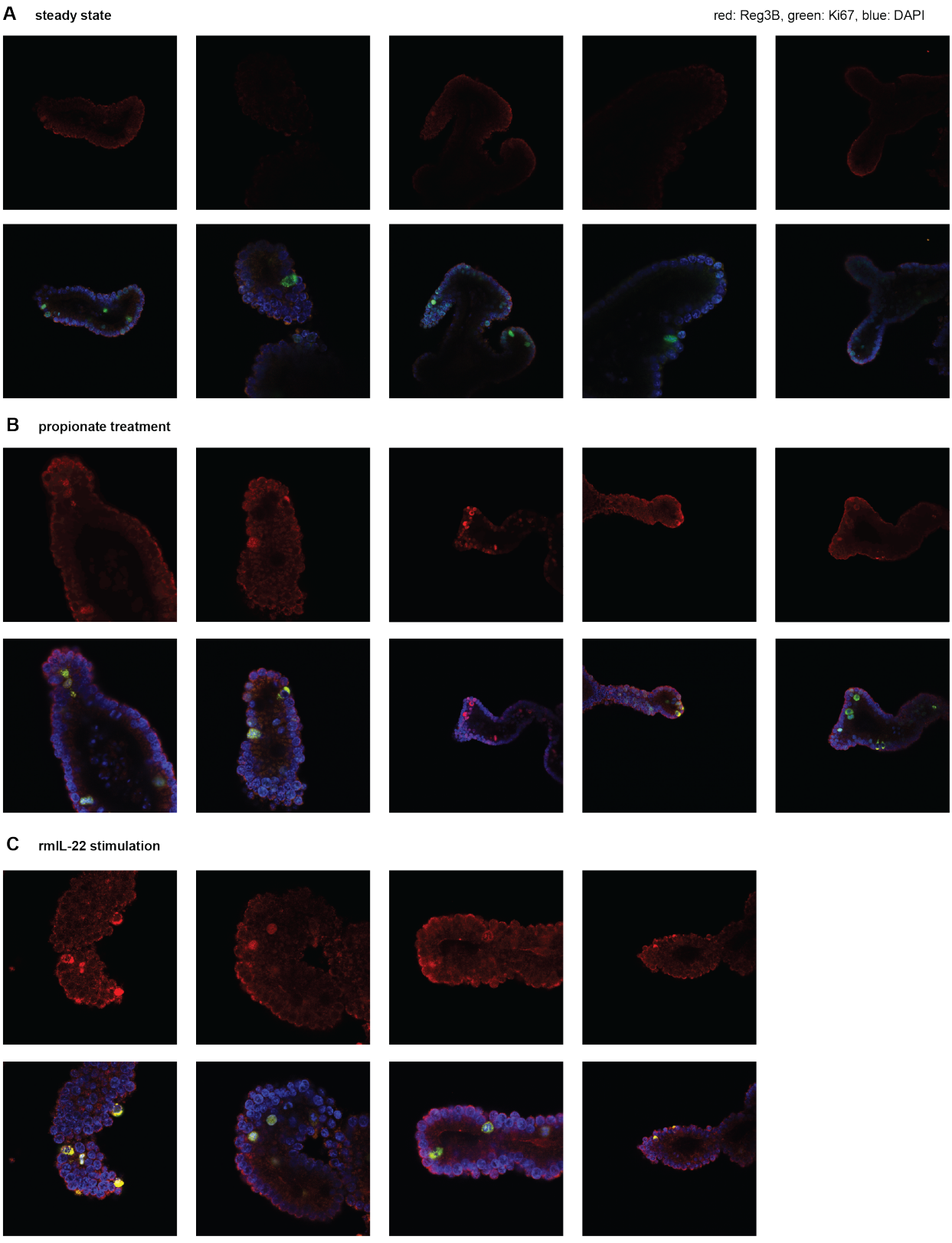


Antibiotic-induced Reg3 modulation and microbiota changes. (A) Reduced expression of RegB after treatment with RFX in the small intestine (*left*) and in the colon (*right*) compared to untreated SPF mice (CTR); (B) No change of RegG expression in the colon after RFX administration. (C) Three injections (i.p., every 12h) of rmIL-22 (4µg; according to Lindemans et al., Nature 2015) rescued the expression of Reg3B in the cecum of RFX-treated mice. (D) GULDA-based 16S rRNA qPCR approach to assess microbiota changes after RFX treatment in SPF mice. (E) *Left*, thickness of inner mucus layer in AMT or RFX treated mice compared to controls measured in colonic sections by immunofluorescence

anti-MUC2 antibody staining; *right*, representative photomicrographs for MUC2 immunofluorescence of colon sections from AMT mice vs. controls (MUC2, green; DAPI, blue: scale bars 50μm). Data are means ± S.E.M.. *p<0.05, **p<0.01, ***p<0.001; n = 4 – 16 mice / group.

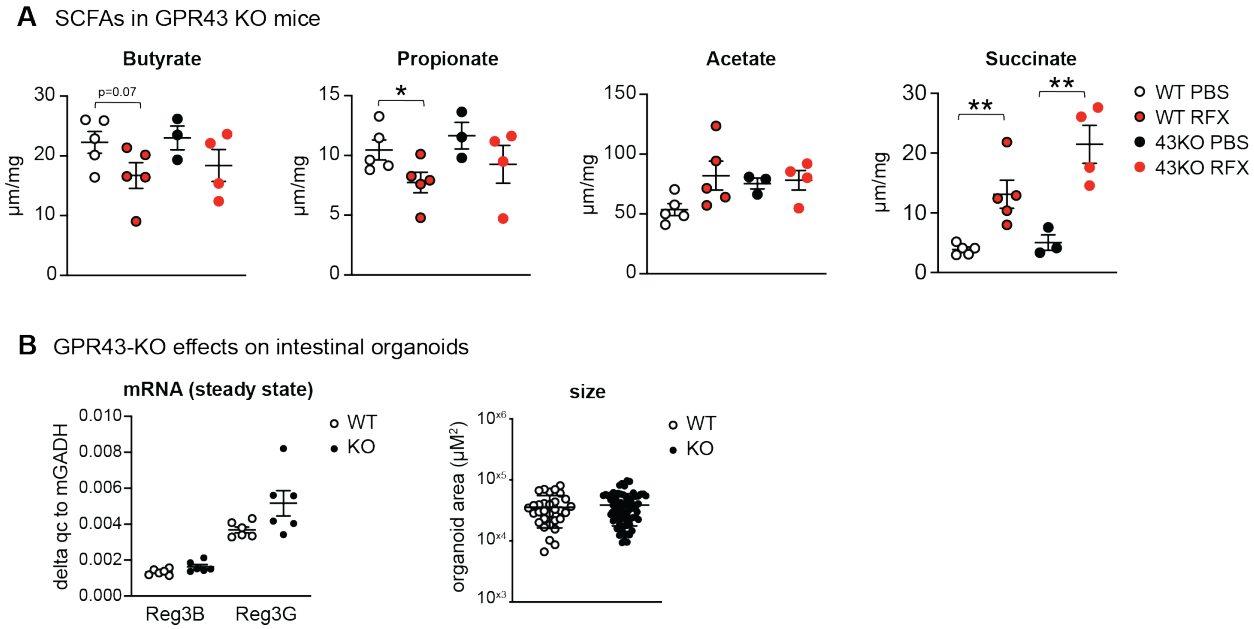
Supplementary Figure 2.

Reg3B secretion from enterocytes in intestinal organoids



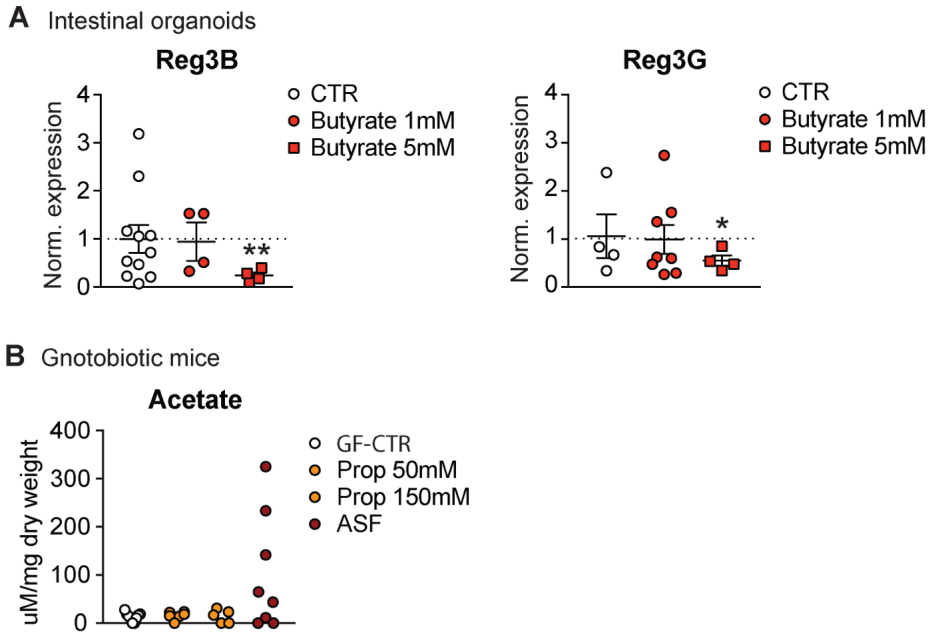
Reg3B in intestinal organoids from naïve C57BL/6 mice. Confocal images with immunohistochemical staining of Reg3B, Ki-67 or a nucleus staining with DAPI at (A) steady state, or (B) after propionate treatment (1mM, incubation for 12h) or (C) after stimulation with rmIL-22 (1ng/ml media; incubation for 12h). Top rows are 40x confocal images of anti-Reg3B immunofluorescence (red), and bottom rows merging of anti-Reg3B (red), -Ki67 (green) and DAPI staining (blue). For the organoid experiments, technical triplicates and 1 biological replicate were performed with images taken from different technical and biological replicates.

Supplementary Figure 3.



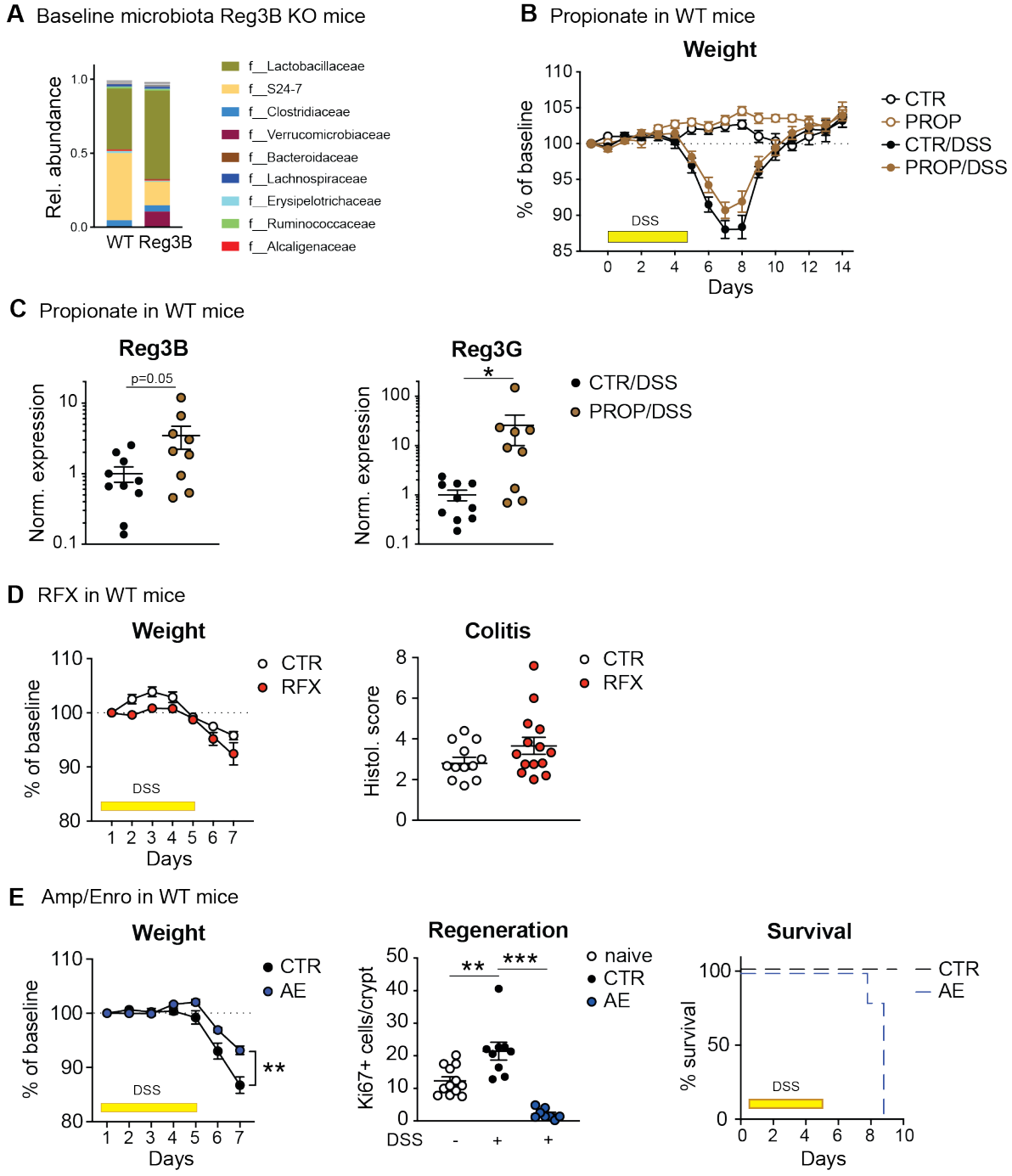
SCFA in GPR43-KO mice and intestinal organoid growth. (A) SCFA levels in cecal contents of wildtype littermate (WT) and GPR43 KO (43KO) mice receiving either PBS or rifaximin (RFX, 150mg/kg) for 10 days. (B) *Left*, non-different steady state expression levels of Reg3B and -G in WT and GPR43 KO mice without stimulation; *right*, area of cultured primary intestinal organoids of WT vs. GPR43 KO mice on day 5 after seeding organoids as a measure of growth kinetics.

Supplementary Figure 4.



SCFA experiments in organoids and in gnotobiotic mice. (A) Butyrate administration to small intestine organoids reduced expression of Reg3B and Reg3G at high doses. (B) Cecum luminal contents of acetate after propionate treatment of germ-free (GF-CTR) mice vs. mice colonized with ASF. Data are means ± S.E.M.. *p<0.05, **p<0.01; n = 4 – 8 organoid biological replicates or mice / group.

Supplementary Figure 5.



In vivo colitis experiments. (A) Relative abundances of fecal microbes at the family level averaged over 4 WT and 4 Reg3B KO mice (2 cages each) at steady state. (B) Weight changes of mice receiving water +/- propionate (50mM) or DSS +/- propionate (50mM) with DSS administered at 3% for 5 days. (C) Normalized expression of Reg3B/G mRNA in the colon of mice treated with 3% DSS plus propionate (PROP/DSS, n=9 mice) via drinking water or DSS alone (CTR/DSS, n=10 mice). (D) Body weight changes (*left*) and histological scores (*right*) in WT mice pretreated with RFX

vs. control for 10 days and followed by administering 2% DSS for 5 days (histologic score: p=0.11 by T-test). (E) Changes in weight during 5 days of DSS treatment (*left*) and Ki67 staining of colonic sections of tissue harvested at day 7 after DSS start (vs. mice at steady state; *middle*) in mice treated with ampicillin/enrofloxacin (AE) 7 days before and during DSS; *right*, survival analysis in a separate batch of mice treated with AE and afterwards exposed to 4% DSS compared to control mice. Data are means ± S.E.M.. *p<0.05, **p<0.01, ***p<0.001 (independent T-tests); n = 9 – 15 mice / group.

Supplementary Table 1.

Primers used for qPCR.

	Murine	
Primer	*forward*	*reverse*
GAPDH	GCCTTCTCCATGGTGGTGAA	GCACAGTCAAGGCCGAGAAT
Reg3B	TCCCAGGCTTATGGCTCCTA	GCAGGCCAGTTCTGCATCA
Reg3G	TTCCTGTCCTCCATGATCAAAA	CATCCACCTCTGTTGGGTTCA
Cryptidin-4	GCTGTGTCTATCTCCTTTGGAGG	CGTATTCCACAAGTCCCACGAAC
Lysozyme	GATGGCTACCGTGGTGT	CACCCATGCTCGAATG
IL-22	CGCTGCCCGTCAACACCCGG	CTGATCTTTAGCACTGACTCCTCG
	Bacterial	
Primer	*forward*	*reverse*
U1 universal	ACTCCTACGGGAGGCAGCAGT	GTATTACCGCGGCTGCTGGCAC
F1 Firmicutes	TGAAACTYAAAGGAATTGACG	ACCATGCACCACCTGTC
F2 Lactobac. spp.	AGCAGTAGGGAATCTTCCA	CACCGCTACACATGGAG
F6 Clostridia IV	GCACAAGCAGTGGAGT	CTTCCTCCGTTTTGTCAA
F7 Clostridia XIV	AAATGACGGTACCTGACTAA	CTTTGAGTTTCATTCTTGCGAA
F10 Enterococc. spp.	CCCTTATTGTTAGTTGCCATCATT	ACTCGTTGTACTTCCCATTGT
B1 Bacteroidetes	GGARCATGTGGTTTAATTCGATGAT	AGCTGACGACAACCATGCAG
B3 Bacteroid. spp.	CGATGGATAGGGGTTCTGAGAGGA	GCTGGCACGGAGTTAGCCGA
B9 Prevotella spp.	CACCAAGGCGACGATCA	GGATAACGCCYGGACCT
B10 Alistipes spp.	TTAGAGATGGGCATGCGTTGT	TGAATCCTCCGTATT
A1 Bifidobact. spp.	GCGTGCTTAACACATGCAAGTC	CACCCGTTTCCAGGAGCTATT
V1 Akkermansia	CAGCACGTGAAGGTGGGGAC	CCTTGCGGTTGGCTTCAGAT
E1 Methanobrevibac.	CCGGGTATCTAATCCGGTTC	CTCCCAGGGTAGAGGTGAAA
P1 Enterobacteriac.	CATTGACGTTACCCGCAGAAGAAGC	CTCTACGAGACTCAAGCTTGC
P3 Desulfovibrio spp.	CCGTAGATATCTGGAGGAACATCAG	ACATCTAGCATCCATCGTTTACAGC