

Fig. S1. PFOS does not alter *il17a* or *il22* expression in zebrafish larvae during TNBS-induced inflammation. (A-B) Violin plots showing relative expression of *il17a*, *il22*, *lyz*, *mpeg1.1*, *lck*, *trac*, and *tjap1* analyzed by qPCR in dissected intestines or carcasses at 120 hpf following exposure to TNBS (70 μ g/mL) and PFOS (200 nM). n = 11-12, 4 experiments. Data show transcript levels as A. U. respect to *eef11a1*. The black line represents the median. * p < 0.05, ** p < 0.01, *** p < 0.001. One-way ANOVA with Fisher's Least Significant Difference (LSD).

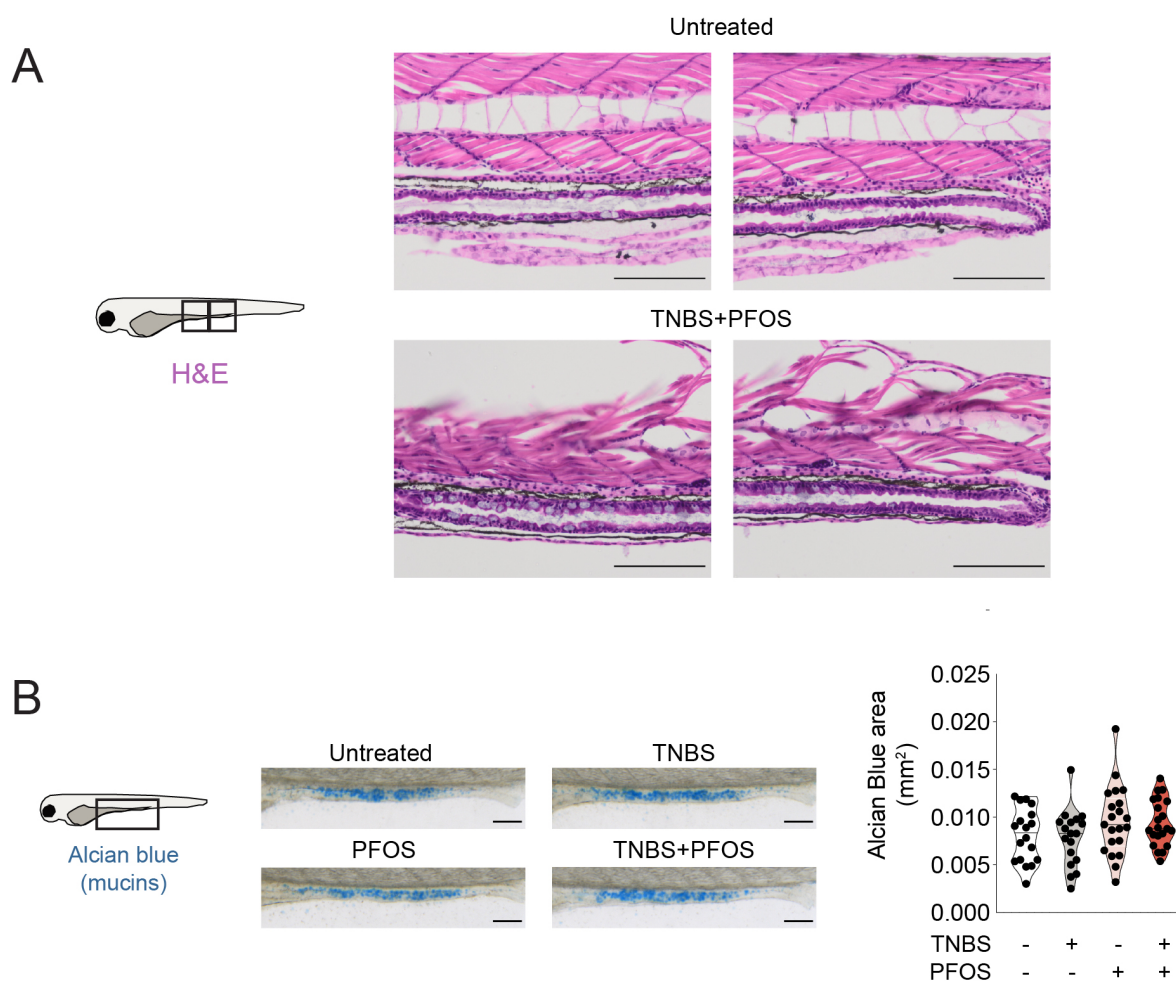


Fig. S2. PFOS exposure does not alter intestinal epithelial architecture or mucin production. (A) Representative images of hematoxylin & eosin (H&E) stained 5 days post-fertilization larvae following 48 hours exposure to TNBS (70 $\mu\text{g}/\text{mL}$) and PFOS (200 nM). (B) Whole-mount alcian blue staining of zebrafish embryos treated with TNBS+PFOS. The graph represents the quantification of the alcian blue positive area per intestinal area, as described in the cartoon. $n = 18-21$, 1 experiment. Each data point represents one 120 h post fertilization zebrafish larvae. The black line represents the median. One-way ANOVA with Fisher's Least Significant Difference (LSD).

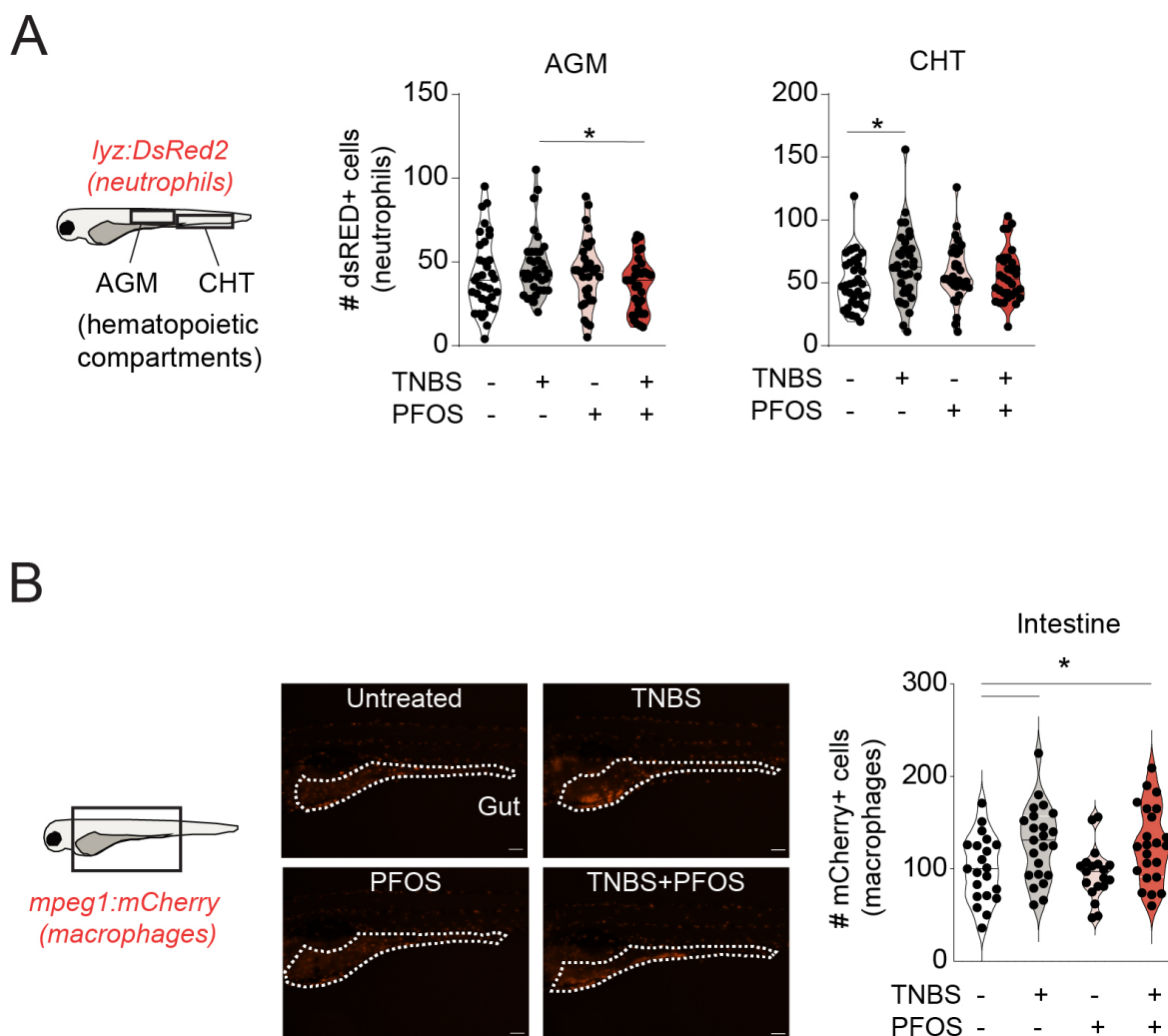


Fig. S3. PFOS does not alter neutrophils numbers at hematopoietic sites nor macrophages intestinal numbers during TNBS-induced inflammation in zebrafish larvae. (A) Quantification of DsRed2-positive cells (neutrophils) in the hematopoietic regions (AGM; aorta-gonad-mesonephros, and CHT; caudal hematopoietic tissue) as described in the cartoon. $n = 32-35$, 5 experiments. Each data point represents one 120 h post fertilization zebrafish larvae. (B) Quantification of mCherry-positive cells (macrophages) in the gastrointestinal tract of 120 h post fertilization *mpeg1:mCherry-F* larvae. Representative images are 7x magnification. The dash line demarks the gastrointestinal tract area. $n = 19-23$, 2 experiments. Each data point represents one 120 h post fertilization zebrafish larvae. The black line represents the median. * $p < 0.05$. One-way ANOVA with Fisher's Least Significant Difference (LSD).

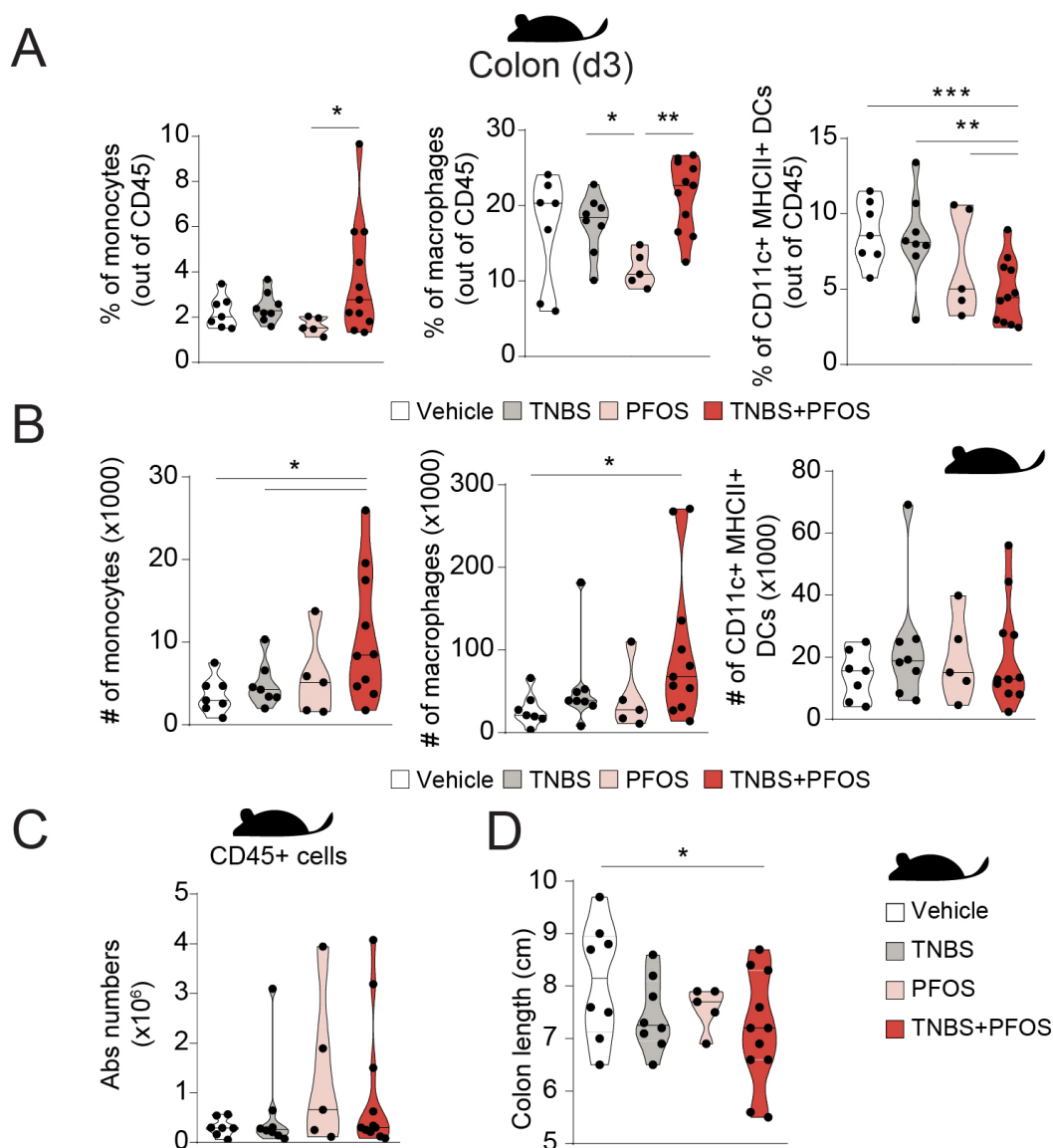


Fig. S4. Myeloid cell characterization of PFOS exacerbated colitis in the colon. (A-C) Violin plots showing frequencies out of CD45⁺ cells and absolute numbers of myeloid cells in the colon lamina propria, analyzed by flow cytometry. n = 5-11, 3 experiments. (D) Violin plots showing colon length of mice treated with PFOS and TNBS as described in Figure 3D. The black line represents the median. * p < 0.05, ** p < 0.01, *** p < 0.001. One-way ANOVA with Fisher's Least Significant Difference (LSD) test.

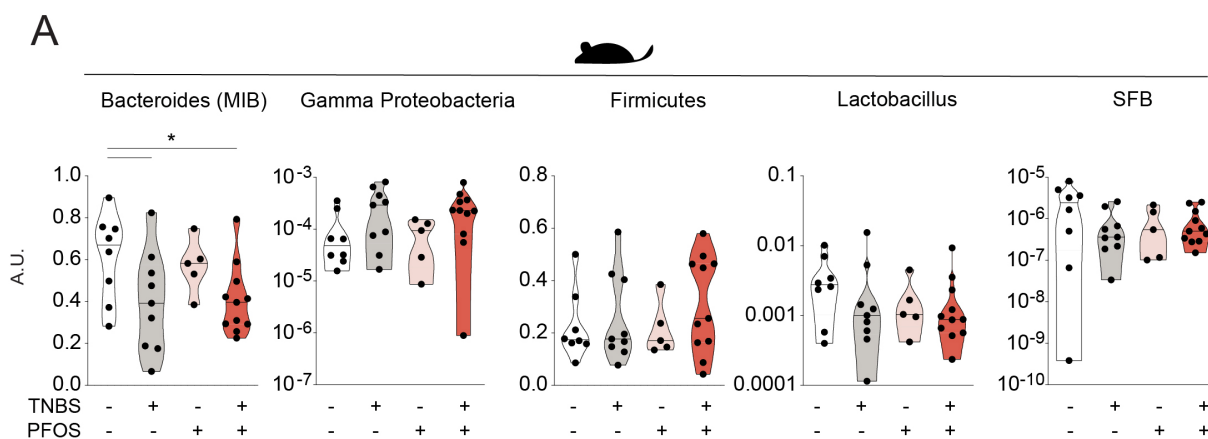


Fig. S5. PFOS exposure results in reduced abundance of Bacteroides. (A) Violin plots showing relative abundance of Bacteroides (Mouse Intestinal Bacteroides, MIB), Gamma Proteobacteria, Firmicutes, *Lactobacillus*, and segmented filamentous bacteria (SFB) relative to universal 16S levels analyzed by qPCR. Bacterial DNA was isolated from stool obtained from the colon. n = 5-11, 3 experiments. The black line represents the median. * p < 0.05. One-way ANOVA with Fisher's Least Significant Difference (LSD) test.

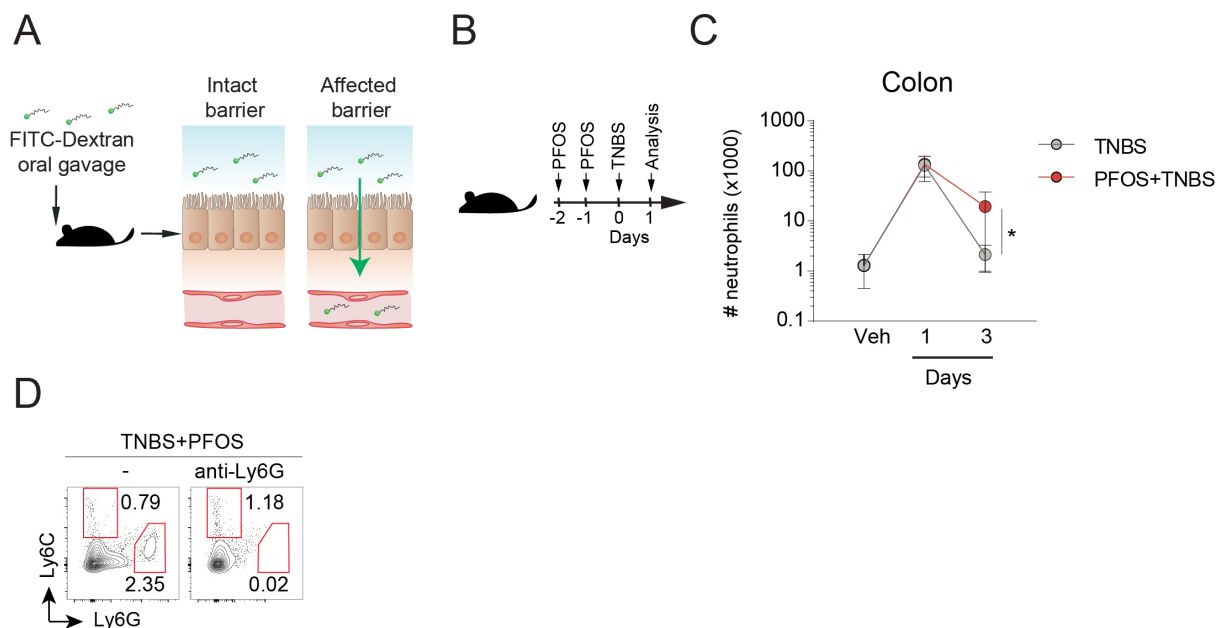


Fig. S6. PFOS does not alter neutrophil numbers or frequencies in the colon 1 day after TNBS-induced colitis and these are efficiently depleted with anti-Ly6G antibody treatment. (A) Experimental scheme of the FITC-Dextran permeability assay. (B) Experimental outline. (C) Absolute numbers of neutrophils isolated from the colon lamina propria at day 1 and 3 following TNBS administration. $n = 7-9$, 3 experiments for day 1. Data for day 3 corresponds to the data shown in Figure 3F, $n = 5-11$, 3 experiments. Veh; vehicle group from the experiment performed at day 1, thus the same values are used for both TNBS and TNBS+PFOS. (D) Representative dot plots of flow cytometry analysis of neutrophils isolated from the colon lamina propria at day 3 following neutrophil depletion in TNBS+PFOS treated mice. Neutrophils are gated as Ly6C^{int} and Ly6G⁺. Monocytes are gated as Ly6C⁺ and Ly6G⁻. * $p < 0.05$. One-way ANOVA with Fisher's Least Significant Difference (LSD) test.

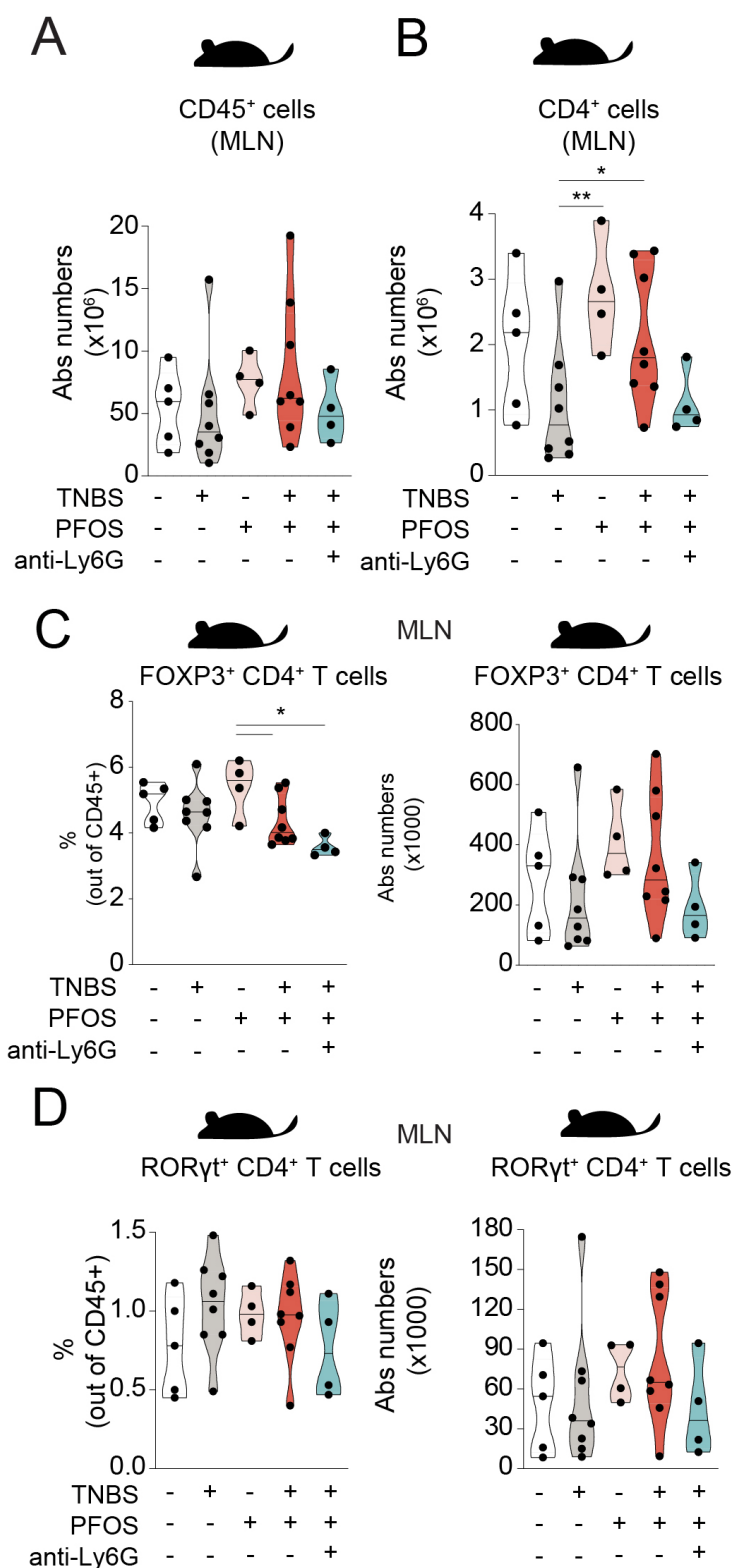


Fig. S7. PFOS exposure after TNBS-induced colitis results in increased CD4⁺ T cells in the MLN. (A-D) Flow cytometry analysis of MLN FOXP3⁺ and RORγt⁺ CD4⁺ T cells, following TNBS, PFOS and/or neutrophil depletion as in the scheme shown in Figure 5A. Violin plots represent the absolute numbers and frequencies of these populations out of CD45⁺ cells. n = 4-8, 4 experiments. The black line represents the median. * p < 0.05, ** p < 0.01, *** p < 0.001. One-way ANOVA with Fisher's Least Significant Difference (LSD) test.

Table S1. List of primers used in this study for zebrafish genes

Target gene	ENSEMBL accession number	Forward sequence (5' -> 3')	Reverse sequence (5' -> 3')
<i>il1b</i>	ENSDARG00000098700	ATCAAACCCCAATC CACAGAGT	GGCACTGAAGACAC CACGTT
<i>tnfa</i>	ENSDARG0000009511	GGAGAGTTGCCTTT ACCGCT	TTGCCCTGGGTCTT ATGGAG
<i>eef1a11</i>	ENSDARG00000020850	ACCTACCCTCCTCT TGGTCG	GGAACGGTGTGATT GAGGGAA
<i>il17a/f3</i>	ENSDARG00000041976	CGCCTTGGACATAC ACAACCTT	AGTAAATGGGTGG GACTCCA
<i>il22</i>	ENSDARG00000045673	CGATGACTGATACA GCACGA	TGTGCTCGTCTGAT TCCAAG
<i>cldn15la</i>	ENSDARG00000016081	TGTTTGATCGGCTG GGTTCT	AGCCTGAATGTGAA CTGGCAA
<i>lyz</i>	ENSDARG00000114098	AGCAGTTTAAAGAC CCACCG	AGCAAGTCTGAACA GGCCAC
<i>mpeg1.1</i>	ENSDARG00000055290	ATGTCGTGGCTGGA ACAGAA	ATGGTTACGGACTT GAACCC
<i>lck</i>	ENSDARG00000102525	GCCGAAGAAGATCT CGATGGT	TCCCCATGTTTACG TATTTTGTCG
<i>trac</i>	ENSDARG00000104132	CTGCGAGGAGACAG GCGTTT	GCCAGAAGATGCC AGTGAC
<i>tjap1</i>	ENSDARG00000010681	CTCACCAATAAACT TGTGGACGC	TGTCGATAGCGTTC GTTTCAGC

Table S2. List of primers used in this study to analyze bacterial abundance

Target group	Forward sequence (5' -> 3')	Reverse sequence (5' -> 3')
Universal 16S rRNA	ACTCCTACGGGAGGCAGCAGT	ATTACCGCGGCTGCTGGC
Bacteroides (MIB)	CCAGCAGCCGCGGTAATA	CGCATTCGCGATACTTCTC
Gamma Proteobacteria	GCTAACGCATTAAGTRYCCG	GCCATGCRGCACCTGTCT
Firmicutes	GGAGYATGTGGTTTAATTCGA AGCA	AGCTGACGACAACCATGCAC
<i>Lactobacillus</i>	AGCAGTAGGGAATCTTCCA	CACCGCTACACATGGAG
Segmented filamentous bacteria (SFB)	GACGCTGAGGCATGAGAGCAT	GACGGCACGGATTGTTATTCA