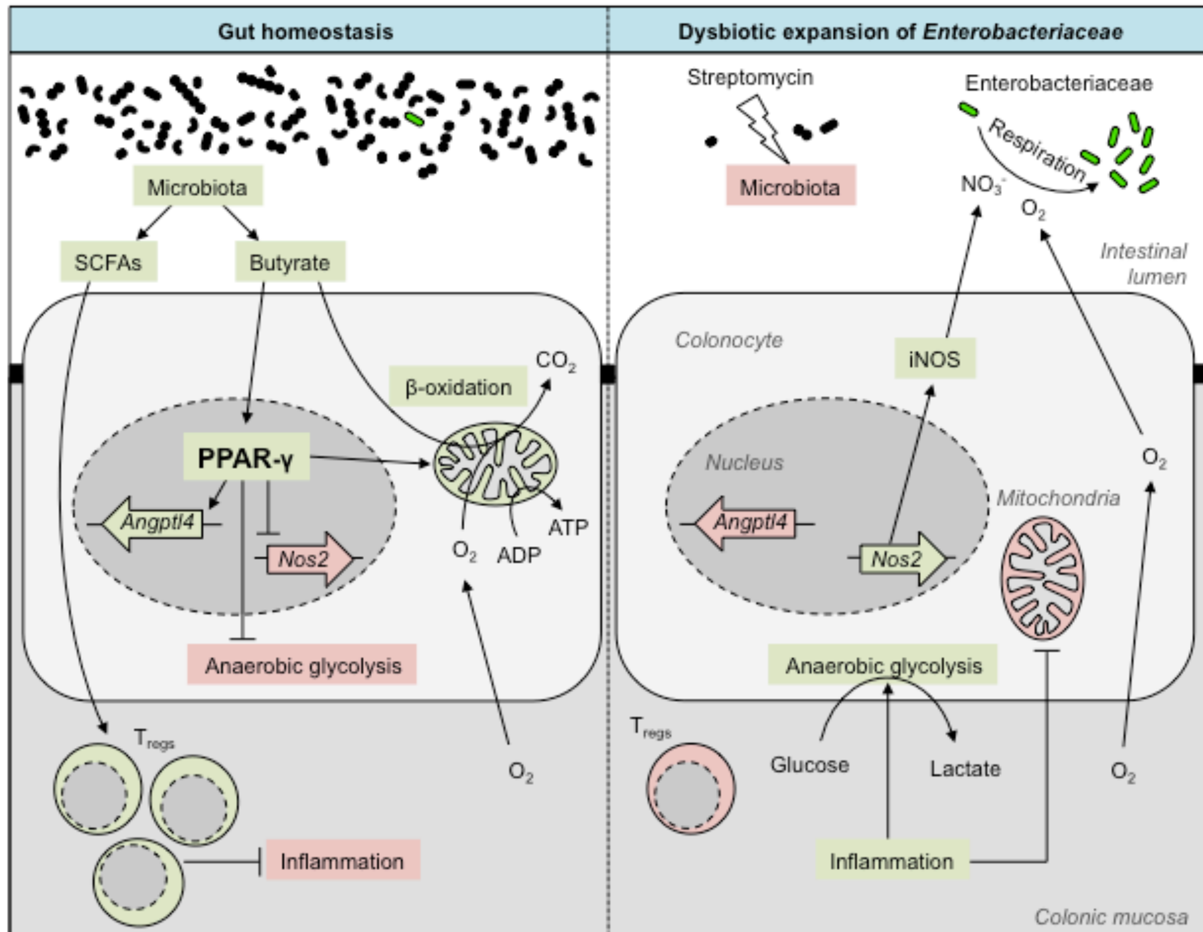


1

2 **Figure S1: Microbiota-induced epithelial PPAR-γ signaling limits a dysbiotic expansion of**

3 ***Enterobacteriaceae*.** Schematic showing how microbiota-induced epithelial PPAR-γ signaling

4 maintains gut homeostasis by limiting the luminal bioavailability of oxygen (O_2) and nitrate (NO_3^-

5). SCFAs, short-chain fatty acids; T_{regs}, regulatory T cells; *Angptl4*, the gene encoding

6 Angiopoetin-like 4; *Nos2*, the gene encoding inducible nitric oxide synthase (iNOS); NO_3^-,

7 nitrate; O_2, oxygen.

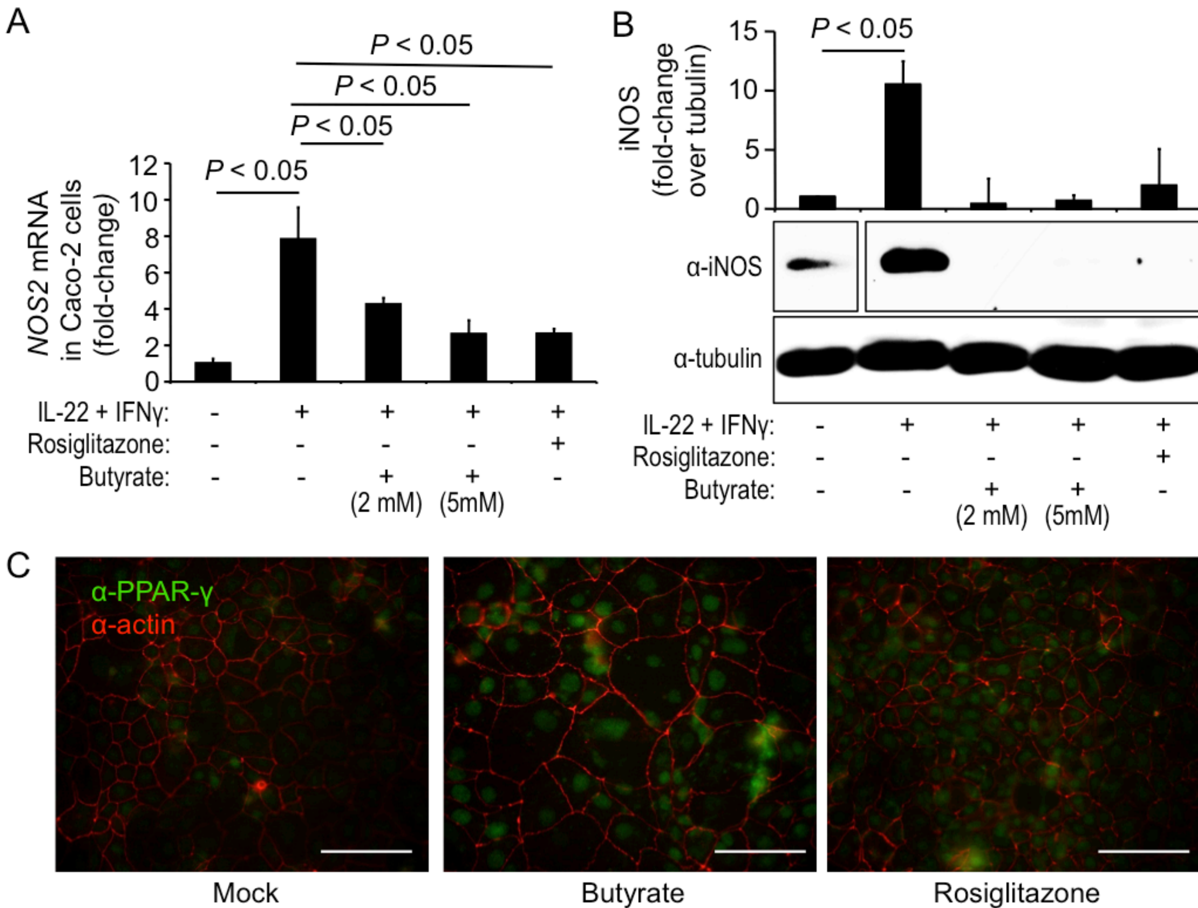
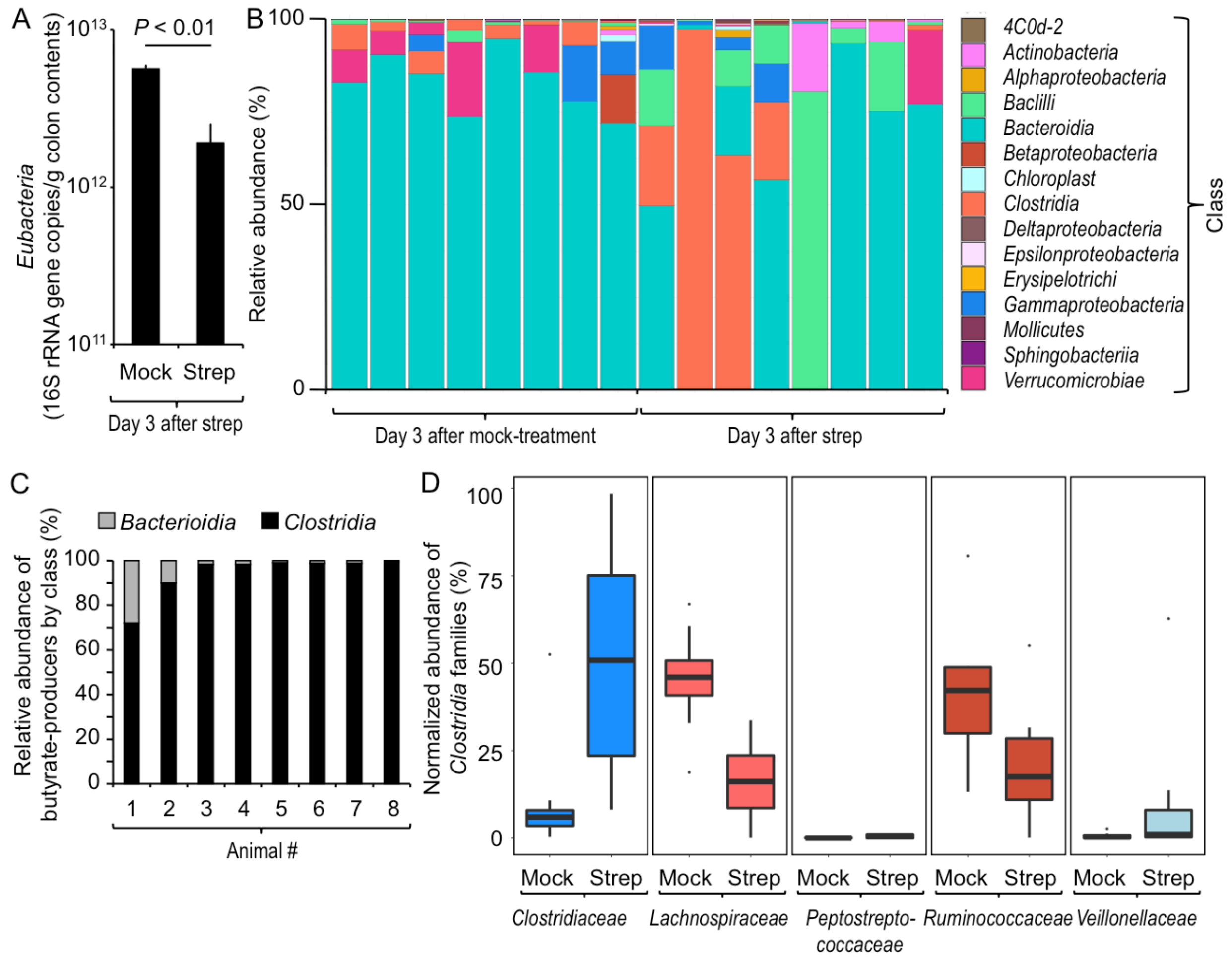


Figure S2: PPAR-γ agonists repress iNOS synthesis in human colonic cancer epithelial (Caco-2) cells. Caco-2 cells ($N = 4$) were treated with the indicated cytokines and/or the PPAR-γ agonists butyrate or rosiglitazone. (A) *NOS2* expression was determined by real-time PCR. (B) Synthesis of iNOS was detected by Western blot (a representative image is shown in the middle panel) using tubulin as a loading control (a representative image is shown in the bottom panel). Quantification of the results is shown in the top panel. (C) Images showing expression of actin (red fluorescence) and PPAR-γ (green fluorescence) in Caco-2 cells grown in a microfluidic chamber. Scale bars represent 50 μm.



1

2 **Figure S3: Effect of streptomycin treatment on the gut microbiota.**

3 (A-D) Mice (*N* = 8) were mock-treated or treated with streptomycin. (A) The copy number of 16S
4 rRNA genes was determined in DNA isolated from colon contents. Bars represent geometric
5 means ± standard error. (B) Community composition at the class level was determined by 16S
6 profiling of DNA isolated from colon contents. (C) Relative abundances of OTUs from butyrate-
7 producing families, plotted according to their class affiliation. (D) Normalized abundance of
8 families belonging to the class Clostridia in colon contents was determined by 16S profiling.
9 Boxes in whisker plots represent the second and third quartiles, while lines indicate the first and
10 fourth quartiles.

11

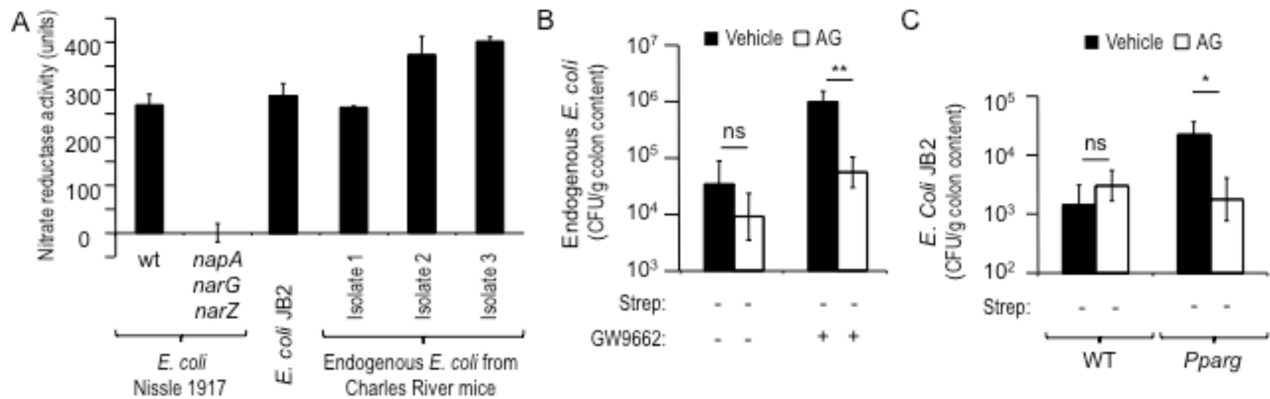
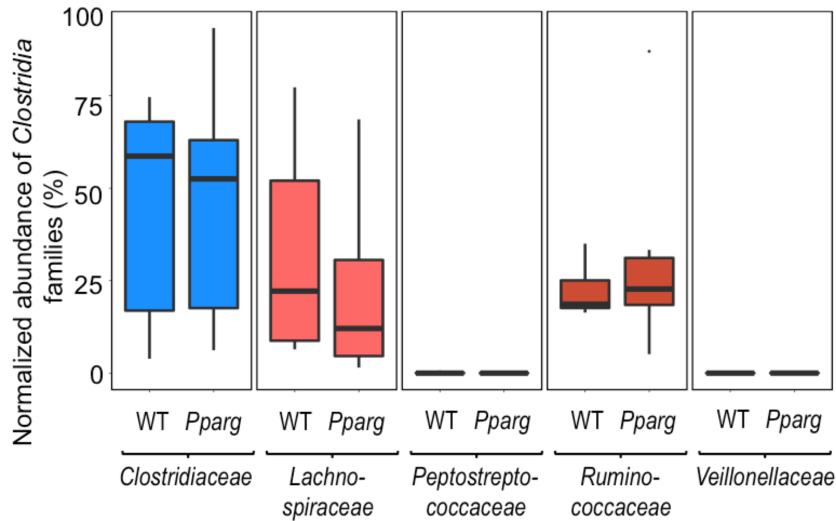


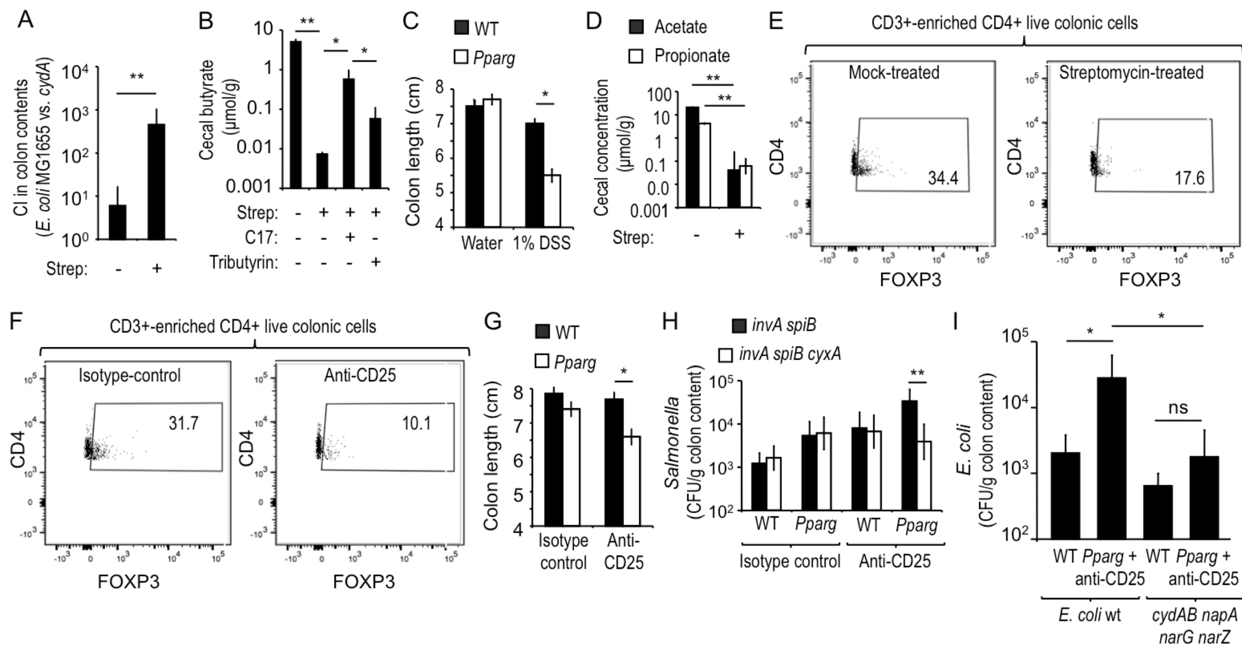
Figure S4: Treatment with a PPAR-γ antagonist drives an iNOS-dependent expansion of endogenous *E. coli* in mice from Charles River. (A) Nitrate reductase activity was determined (N = 3) in *E. coli* Nissle 1917 wild type (wt), an isogenic *napA narG narZ* mutant, *E. coli* JB2 and endogenous *E. coli* isolates from C57BL/6 mice purchased from Charles River. (B) Mice from Charles River (*N* = 6) were mock-treated or inoculated with the PPAR-γ antagonist GW9662 and received aminoguanidine (AG) supplementation or vehicle control. (C) Groups (*N* = 5) of mice lacking epithelial *Pparg* expression (*Pparg*) or littermate controls (WT) were infected with *E. coli* strain JB2 and received either AG supplementation or vehicle control. (B and C) The abundance of endogenous *E. coli* was determined by spreading dilutions on McConkey agar. Bars represent geometric means ± standard error. *, $P < 0.05$; **, $P < 0.01$; ns, not statistically significantly different.

1



2 **Figure S5: Abundance of Clostridia families in mice lacking epithelial PPAR-γ signaling**
3 **and littermate control animals.** Comparison of mice (*N* = 6) lacking epithelial PPAR-γ
4 signaling (*Pparg*$^{fl/fl}$*Villin*$^{cre/-}$) with their littermate controls (*Pparg*$^{fl/fl}$*Villin*$^{-/-}$). Normalized abundance
5 of families belonging to the class Clostridia in colon contents was determined by 16S profiling.

6



1

2 **Figure S6: Depletion of regulatory T cells in mice lacking epithelial PPAR-γ signaling**
3 **increases growth of *Salmonella* by aerobic respiration.** (A) Groups (*N* = 6) of streptomycin-
4 treated or mock-treated mice were inoculated with a 1:1 mixture of *E. coli* strain MG1655 and an
5 isogenic *cydA* mutant. The competitive index (CI) was determined 2 days after inoculation. (B
6 and D) Levels of butyrate (B), acetate and propionate (D) were measured by gas
7 chromatography in the cecum of mice (*N* = 6) three days after treatment with streptomycin or
8 mock treatment. (C) Groups of mice (*N* = 6) receiving no supplementation or water
9 supplemented with 1% dextran sulfate sodium (DSS) were inoculated with *E. coli* indicator
10 strains five days later (see Fig. 2D) and colon length was determined after 8 days of DSS
11 treatment. (E and F) Groups of mice (*N* = 4) were treated with streptomycin (E) or with anti-
12 CD25 antibody (F) and $CD3^+$-enriched live colonic cells analyzed for expression of CD4 and
13 FOXP3 by flow cytometry. Representative flow panels are shown. Boxes indicate the gates set
14 for the detection of T_{regs} using fluorescence-minus-one controls. (G) Groups of mice (*N* = 6)
15 were treated with anti-CD25 antibody or isotype control and colon length was determined 14
16 days later. (H) Groups of mice (*N* = 6) were treated with anti-CD25 antibody or isotype control

and 10 days later inoculated with the indicated *Salmonella* indicator strains. Bacterial numbers were determined 4 days after infection. (I) Mice were infected with either the *E. coli* Nissle 1917 wild-type (wt) or an isogenic *cydA napA narG narZ* mutant and bacterial numbers recovered from colon contents four days after infection. *, $P < 0.05$; **, $P < 0.01$; ns, not statistically significantly different.

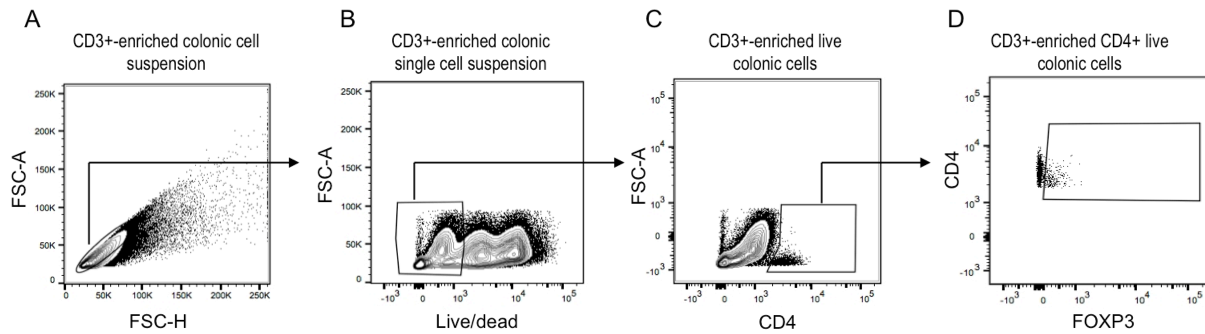


Figure S7: Gating strategy for analyzing colonic regulatory T cells. A cell suspension of the colon was enriched for T cells by high-affinity negative selection using a T cell enrichment column. (A) The resulting CD3+-enriched colonic cell suspension was gated for single cells using forward scatter pulse area (FSC-A) versus forward scatter pulse height (FSC-H). The $CD3^+$-enriched colonic single cell suspension was gated for live cells (B), followed by gating for $CD4^+$ cells (C). (D) Finally, FOXP3 expression was detected in $CD3^+$-enriched $CD4^+$ live colonic cells to identify regulatory T cells. (B-D) Gates were set using fluorescence-minus-one controls.