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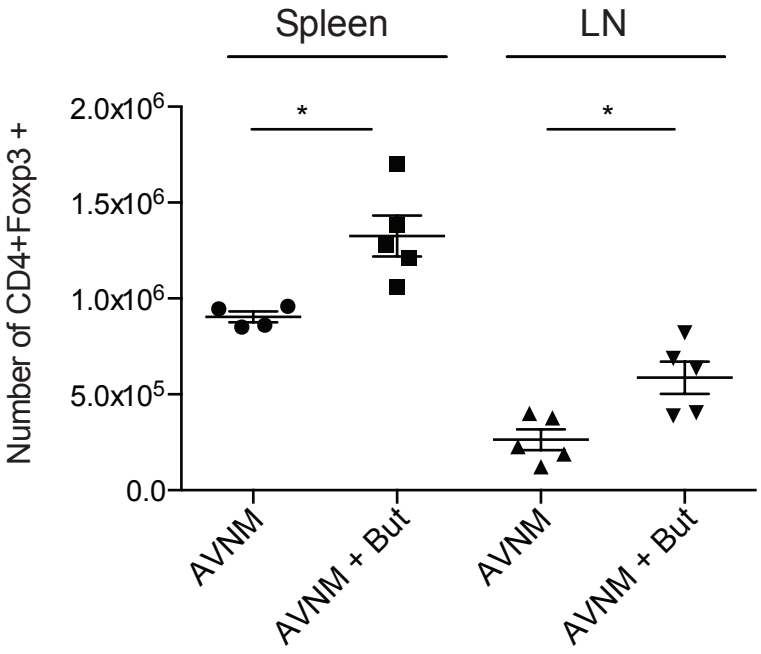
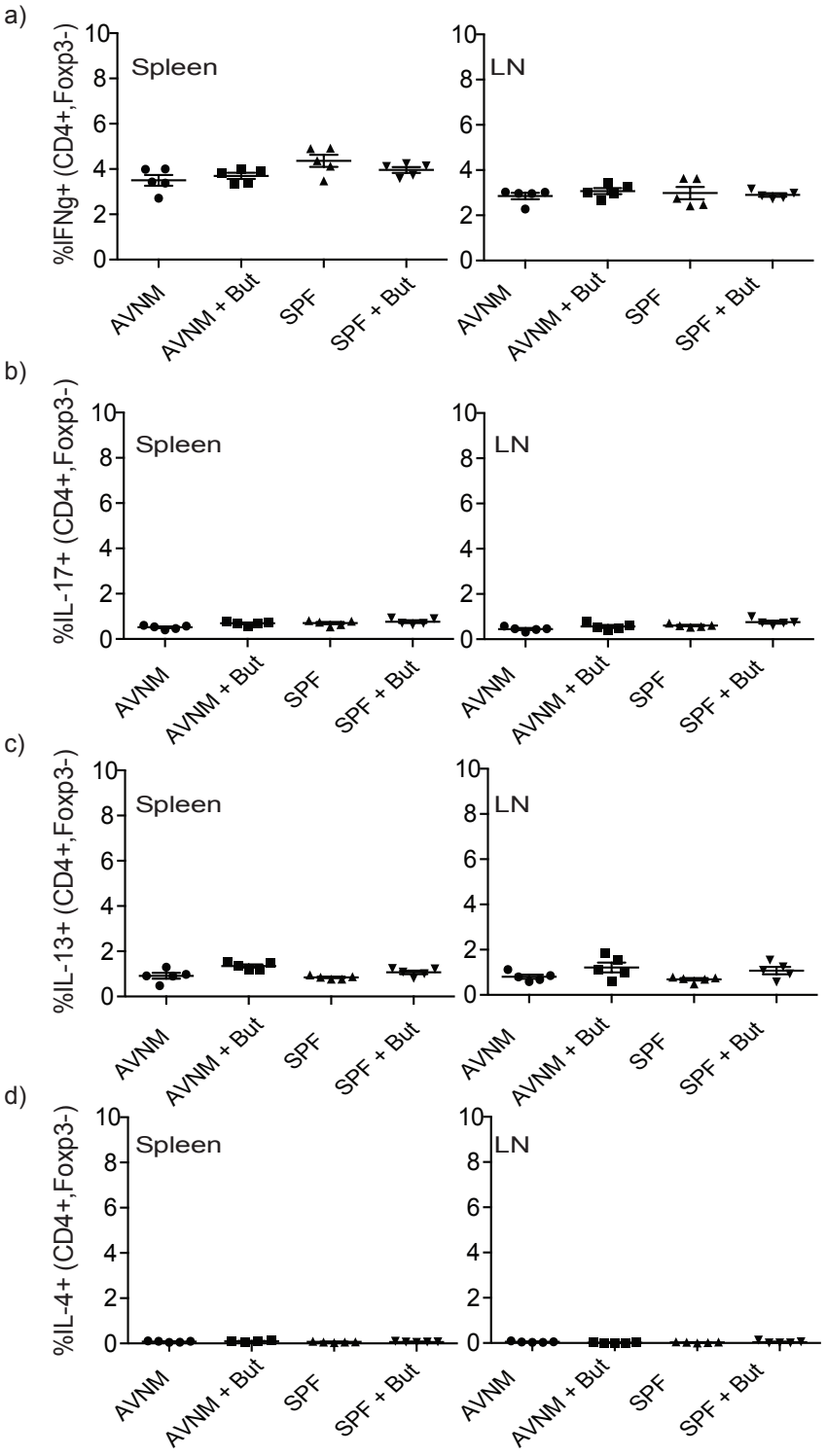
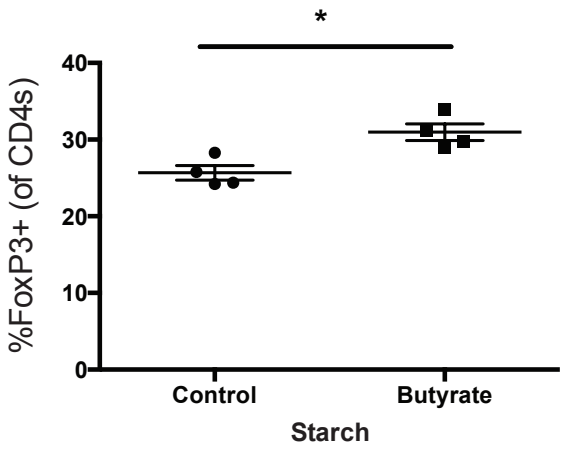


Supplementary Figure 1. Butyrate increases numbers of Treg cells *in vivo*. B6 mice were treated for one week with antibiotics (AVNM) in the absence or presence of butyrate (+But) in drinking water for 2 weeks. The absolute numbers of splenic and lymph node Treg cells are shown as mean +/- SEM. * $P \leq 0.05$ (Student's *t*-test). Data in this figure are representative of at least 3 independent experiments.

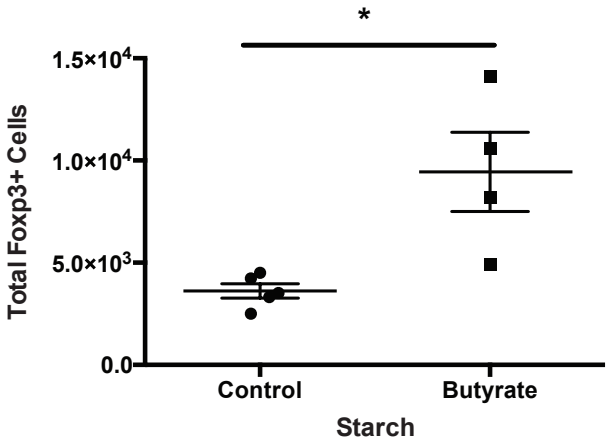


Supplementary Figure 2. Cytokine production by *ex vivo* isolated T effector cells is not increased in the presence of butyrate.
a-d) B6 mice were treated with antibiotics (AVNM) in the absence or presence of butyrate (+But) in drinking water for 2 weeks. Cytokine production by splenic and lymph node Foxp3$^-$ CD4$^+$ T cells was assessed by intracellular flow cytometry upon stimulation for 5 h with CD3 and CD28 (5 μg mL^{-1} each) in the presence of brefeldin A. Proportion of IFN-γ (a), IL-17 (b), IL-13 (c) and IL-4 (d) producing cells within Foxp3$^-$CD4$^+$ T cell population are shown as mean percent +/- SD. * P ≤ 0.05 (Student's *t*-test). Data in this figure are representative of at least 3 independent experiments.

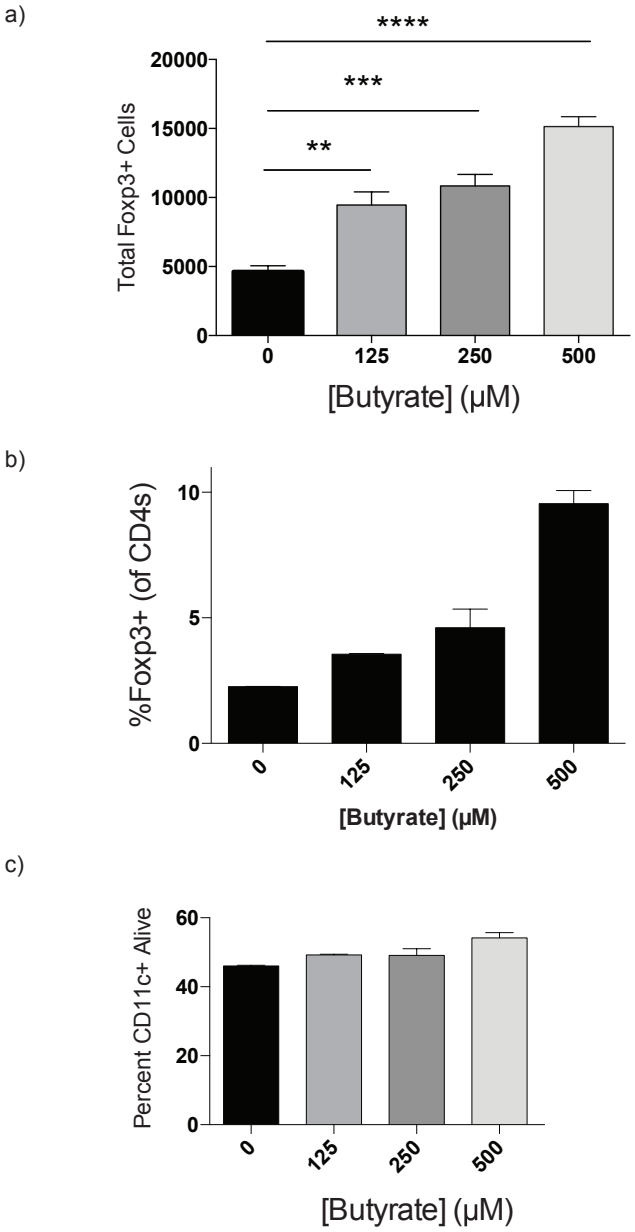
a)



b)



Supplementary Figure 3. Provision of butyrate to the colon via butyrate starch diet increases Tregs in colonic lamina propria.
a, b) Flow cytometric analysis of Foxp3$^+$ Treg cell subsets isolated from the colonic lamina propria of mice fed *ad libitum* for 3 weeks with food formulated with control or butyrate starch. The percent of total CD4$^+$ T cells (a) and absolute numbers (b) of Treg cells are shown as mean +/- SEM. * $P \leq 0.05$ (Student's *t*-test). Data are representative of 2 independent experiments.

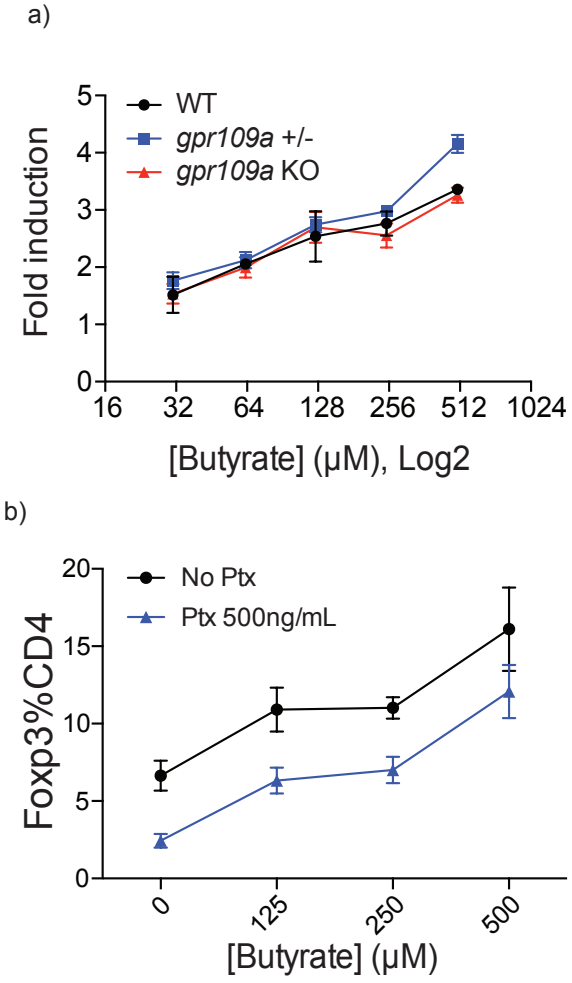


Supplementary Figure 4. Pretreatment of splenic DC with butyrate is sufficient to increase total numbers of Treg and maintains DC viability during Treg induction.

a) Induction of Foxp3 expression upon stimulation of naïve $CD4^+$ T cells by CD3 antibody in the presence of butyrate-treated or untreated Flt3L-elicited DC and TGF-β. DC were cultured with titrated amounts of butyrate or medium alone for 6 h, washed and co-cultured with FACS-purified naïve $CD4^+$ T cells in the presence of CD3 antibody and TGF-β. The data are shown as total number of $CD4^+$ cells expressing Foxp3 after 4 days of culture

b) FACS-sorted $CD11c^+MHCII^{hi}$ DC isolated from the spleen of unperturbed B6 mice were treated with butyrate at the indicated concentrations for 6 h, washed and co-cultured with FACS purified naïve $CD4^+$ T cells under Treg-inducing conditions. The data are shown as total number of $CD4^+$ cells expressing Foxp3 after 4 days of culture +/- SEM. Data are representative of at least 2 independent experiments.

c) As in (a), shown are the percent of surviving Flt3L-elicited CD11c$^+$ dendritic cells in cultures as determined by live/dead staining and flow cytometry. Data are representative of at least 2 independent experiments. The data represent mean +/- SEM. * P ≤ 0.05, ** P ≤ 0.01, *** P ≤ 0.001, **** P ≤ 0.0001, as determined by Student's *t*-test.

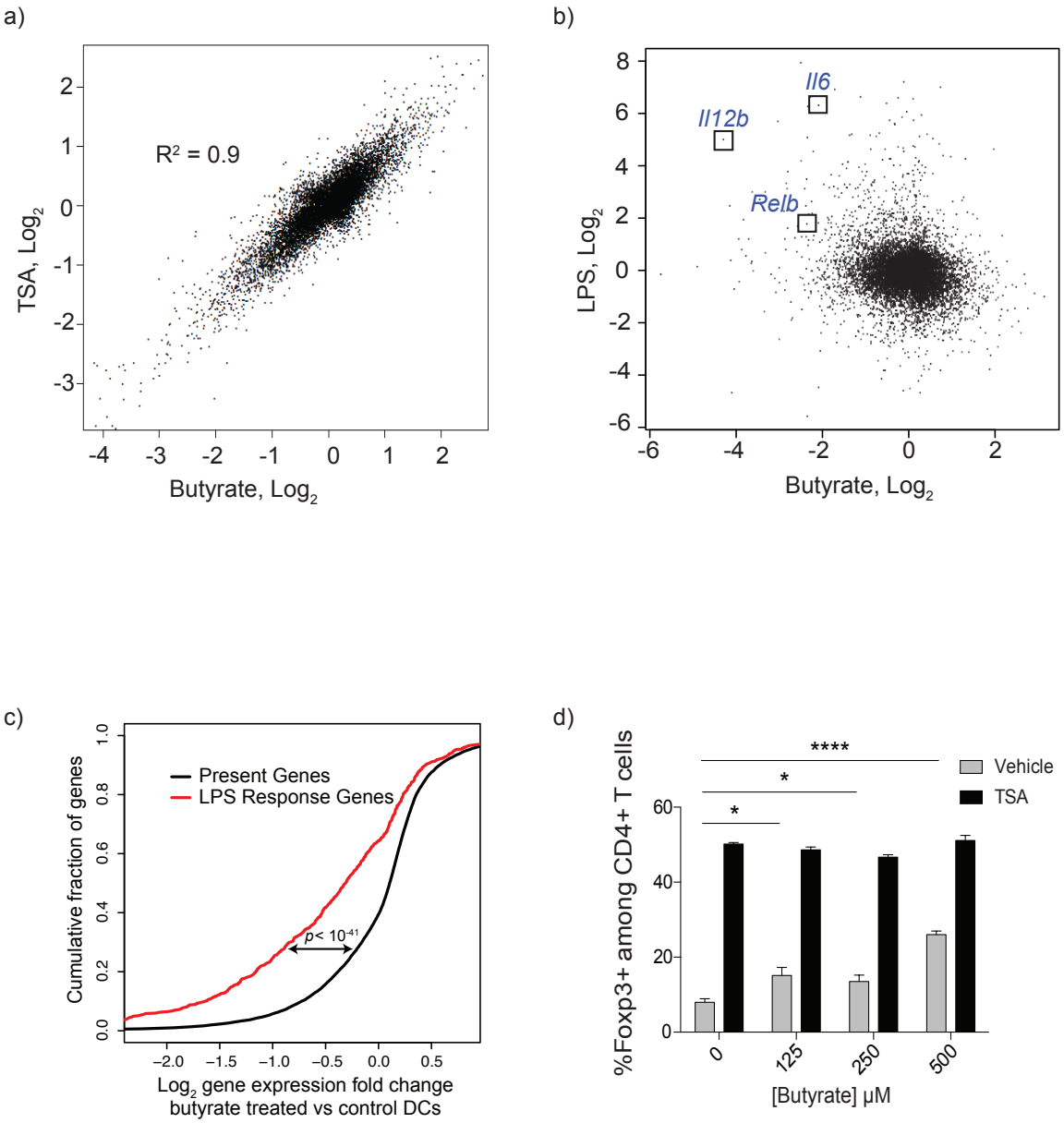


Supplementary Figure 5. GPCR sensing and butyrate transporters are not required for butyrate-dependent increase in Treg cell induction by dendritic cells.

a) Analysis of the ability of DC from *Gpr109a*$^{+/+}$ (WT), *Gpr109a*$^{+/-}$, or *Gpr109a*$^{-/-}$ (KO) mice to generate Treg cells in the presence of butyrate. DC were cultured with FACS-purified naïve CD4$^+$ T cells in the presence of CD3 antibody, TGF-β and titrated amounts of butyrate. The data are shown as fold Foxp3 induction over butyrate-untreated samples; error bars denote SEM.

b) DC were cultured for 30 minutes without or with pertussis toxin (Ptx) followed by addition of butyrate at the indicated concentrations for a total of 6 h. After washes, DC were co-cultured with FACS-purified naïve CD4$^+$ T cells under Treg cell-inducing conditions.

The data are shown as mean percent +/- SEM of Foxp3$^+$ cells within CD4$^+$ T cell population on day 4 of culture. The data in this Figure are representative of at least 2 independent experiments.



Supplementary Figure 6. Butyrate and TSA treated dendritic cells exhibit similar gene induction profiles and act via redundant pathways.
a) Microarray expression analysis of DC treated for 6 h with butyrate or TSA. Data are represented as fold induction of each indicated treatment over untreated control cells.
b) Data from LPS-stimulated DC (GSE15759) were meta-analzyed and compared to DC treated with butyrate for 6 h. Data are represented as fold induction of each treatment over untreated control cells.
c) Cumulative distribution function plot of the fold expression of LPS response genes in butyrate treated DC for 6 h over untreated control DC.
d) DC were pre-treated with trichostatin A (TSA) without or in combination with butyrate at the indicated concentrations for 6 h, washed and co-cultured with FACS-purified naïve $CD4^+$ T cells under Treg-inducing conditions. The data are shown as percent of $CD4^+$ cells expressing Foxp3 after 4 days of culture. Data are representative of 2 independent experiments; error bars denote SEM. * $P \leq 0.05$, **** $P \leq 0.0001$, as determined by Student's *t*-test