Supplementary Figures

Figure S1. Intestinal colonization with *E. coli* 541-15 protects from colitis. Wildtype mice were colonized with *E. coli* 541-15 or gavaged with LB broth (CTRL) and 3 days later 2% DSS was provided. Before colonization and at day 7 of DSS treatment, samples were col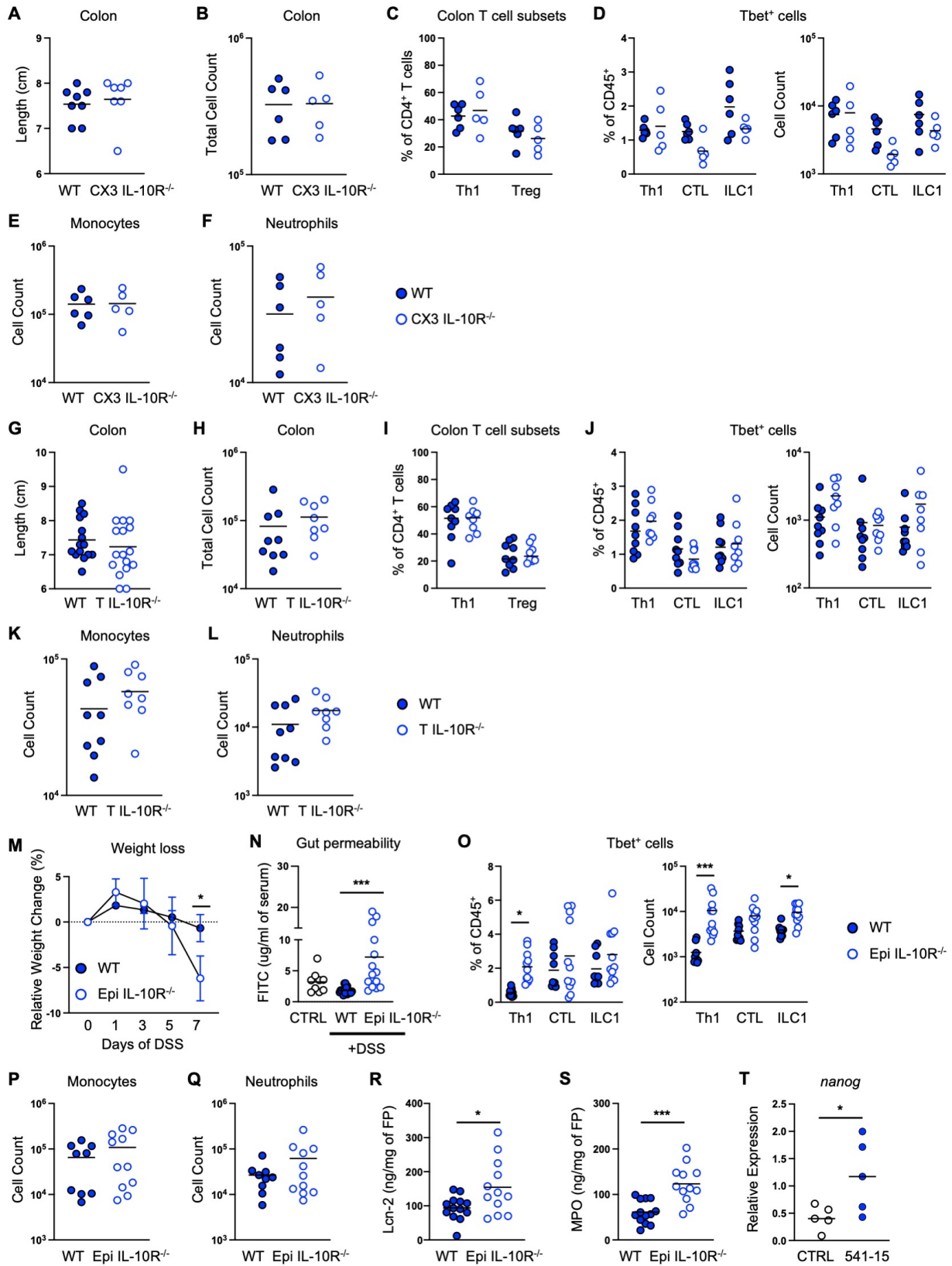lected. (A) *E. coli* relative abundance in fecal pellets. At day 7 of DSS treatment fecal pellets were collected, DNA was isolated, and 16S rDNA sequencing was performed. (B) Principal component analysis. (C) Shannon index. (D) Bacterial phyla relative abundance. At day 7 of DSS treatment distal colons and mesenteric lymph nodes were collected for gene expression analysis and cytokine assessment, respectively. (E) *tnfa* relative expression. (F) Flow cytometry and numbers of IFNγ and IL-17A producing CD4 T cells, CD8 T cells, and ILCs. Each replicate is a biologically independent sample. Individual dots represent samples from individual mice. Data are shown as individual values and mean, compared by two-tailed unpaired t-test or two-way ANOVA with Fisher's LSD post hoc test. The results are representative of at least two independent experiments. *P<0.05 was considered statistically significant; **P <0.01; ****P<0.0001.
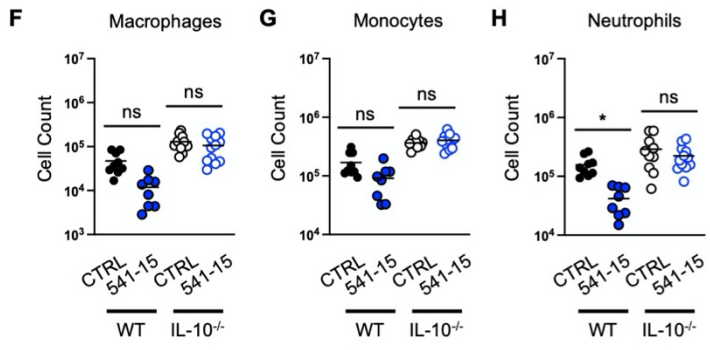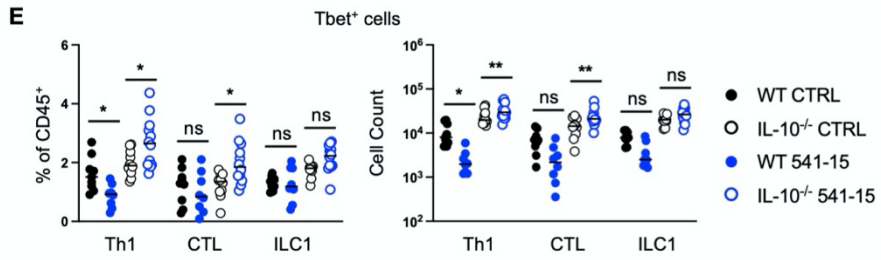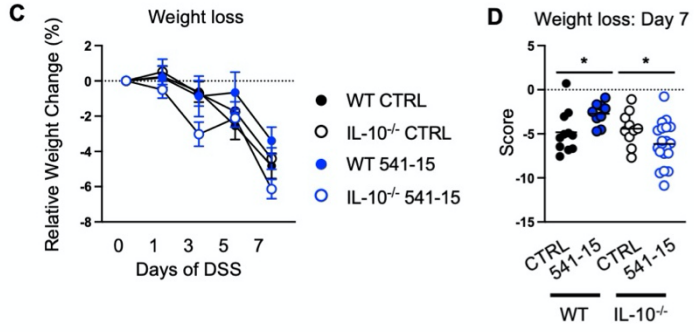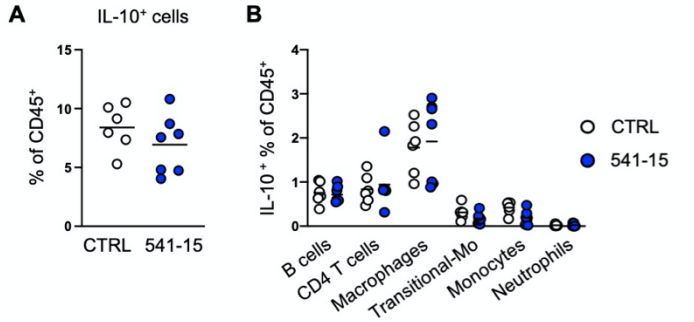
Figure S2. Intestinal colonization with *E. coli* 541-15 prevents colitis through IL-10 induction. Vert-X mice were colonized with *E. coli* 541-15 or gavaged with LB broth (CTRL). At day 7 post-colonization, samples were collected. Frequencies of (A) total IL-10$^+$ immune cells from the colonic lamina propria and (B) identification of IL-10$^+$ immune cells. IL-10 deficient (IL-10$^{-/-}$) and wildtype (WT) littermate mice were colonized with *E. coli* 541-15 or gavaged with LB broth (CTRL) and 3 days later 2% DSS was provided. At day 7 of DSS treatment, samples were collected. (C and D) Relative weight change. Colon lamina propria cells were isolated. (E) Frequencies and counts of total Th1 cells, CTLs, and ILC1s. Counts of (F) Macrophages, (G) Monocytes, and (H) Neutrophils. Each replicate is a biologically independent sample. Individual dots represent samples from individual mice. Data are shown as individual values and mean, compared by two-tailed unpaired t-test or two-way ANOVA with Fisher's LSD post hoc test. The results are representative of at least two independent experiments. *P<0.05 was considered statistically significant; **P <0.01; ****P<0.0001.
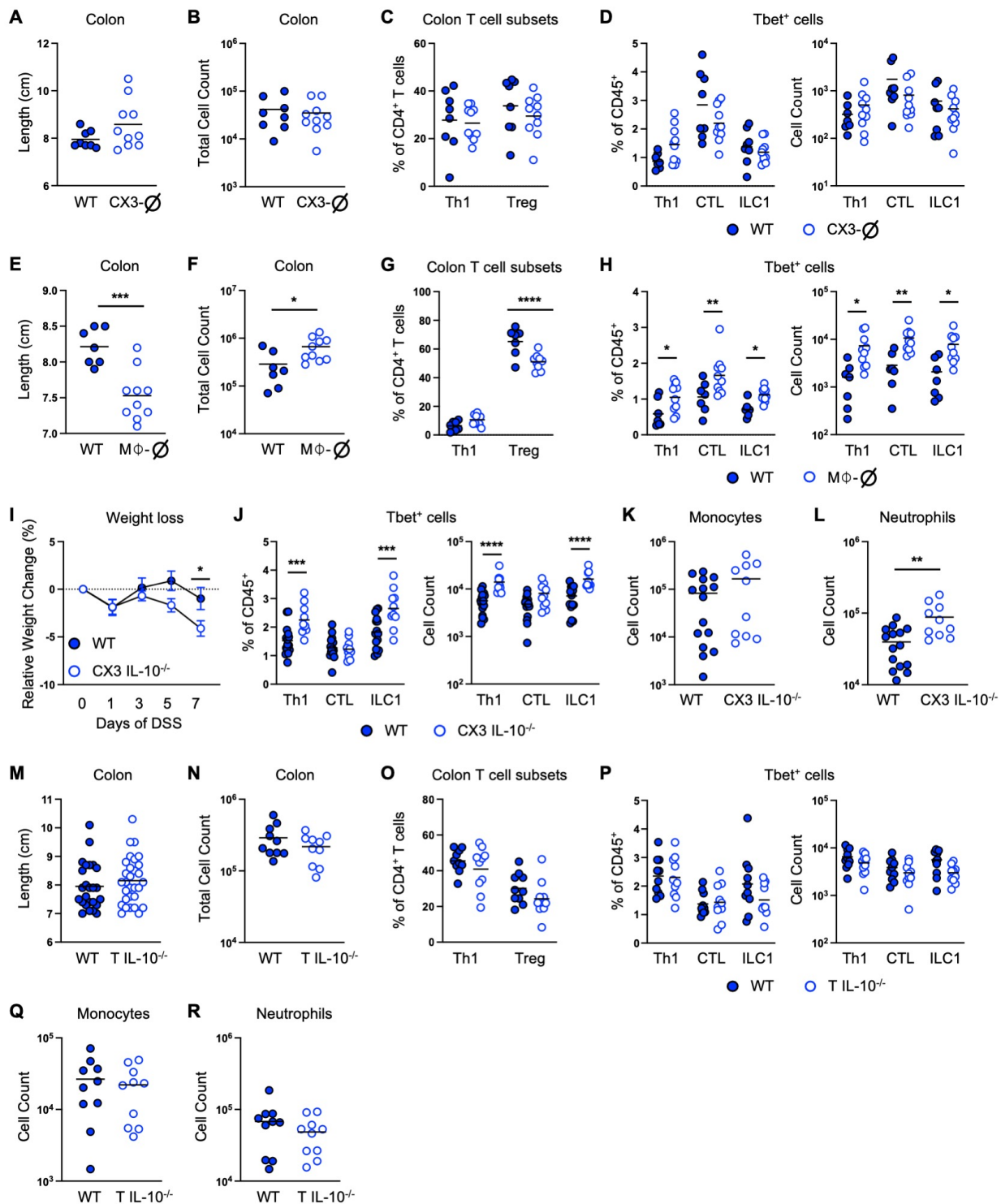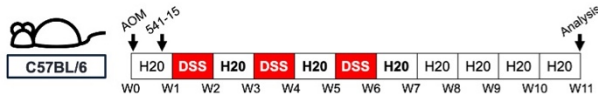
Figure S3. Intestinal colonization with *E. coli* 541-15 prevents colitis by inducing IL-10 production by CX$_3$CR1$^+$ macrophages. CX3-DTR (CX3-∅) and wildtype (WT) littermate mice were colonized with *E. coli* 541-15 and 3 days later 2% DSS was provided. At day 7 of DSS treatment, samples were collected. 3 days before colonization and every 2 days throughout the experiment DT was injected to both groups. (A) Colon lengths. Colon lamina propria cells were isolated. (B) Number of immune cells. (C) Frequencies of Th1 and Tregs. (D) Frequencies and counts of total Th1 cells, CTLs, and ILC1s. CX3-STOP-DTR (MΦ-∅) and wildtype (WT) littermate CD11c-Cre mice were colonized with *E. coli* 541-15 and 3 days later 2% DSS was provided. At day 7 of DSS treatment, samples were collected. 3 days before colonization and every 2 days throughout the experiment DT was injected to both groups. (E) Colon lengths. Colon lamina propria cells were isolated. (F) Number of immune cells. (G) Frequencies of Th1 and Tregs. (H) Frequencies and counts of total Th1 cells, CTLs, and ILC1s. IL-10$^{flox/-}$ (CX3 IL-10$^{-/-}$) and IL-10$^{flox/+}$ (WT) littermate CX$_3$CR1-CreERT2 mice were colonized with *E. coli* 541-15 and 3 days later 2% DSS was provided. At day 7 of DSS treatment, samples were collected. 3 days before colonization and every 2 days throughout the experiment 4OHT was injected to both groups. (I) Relative weight change (X Axis is days of DSS). (J) Frequencies and counts of total Th1 cells, CTLs, and ILC1s. Counts of (K) Monocytes and (L) Neutrophils. IL-10$^{flox/-}$ (T IL-10$^{-/-}$) and IL-10$^{flox/+}$ (WT) littermate CD4-Cre mice were colonized with *E. coli* 541-15 and 3 days later 2% DSS was provided. At day 7 of DSS treatment, samples were collected. (M) Colon lengths. Colon lamina propria cells were isolated. (N) Number of immune cells. (O) Frequencies of Th1 and Tregs. (P) Frequencies and counts of total Th1 cells, CTLs, and ILC1s. Counts of (Q) Monocytes and (R) Neutrophils. Each replicate is a biologically independent sample. Individual dots represent samples from individual mice. Data are shown as individual values and mean, compared by two-tailed unpaired t-test or two-way ANOVA with Fisher's LSD post hoc test. The results are representative of at least two independent experiments. *P<0.05 was considered statistically significant; **P <0.01; ***P<0.001; ****P<0.0001.

A Colon

B Colon

C Colon T cell subsets

D Tbet+ cells

E Monocytes

F Neutrophils

WT
CX3 IL-10R−/−

G Colon

H Colon

I Colon T cell subsets

J Tbet+ cells

K Monocytes

L Neutrophils

WT
T IL-10R−/−

M Weight loss

N Gut permeability

O Tbet+ cells

WT
Epi IL-10R−/−

P Monocytes

Q Neutrophils

R

S

T *nanog*

Figure S4. Intestinal colonization with *E. coli* 541-15 suppresses colitis by modulating IL-10 signaling to intestinal epithelium. CX_3CR1-CreERT2$^+$ (CX3 IL-10R$^{-/-}$) and CX_3CR1-CreERT2$^-$ (WT) littermate IL-10R$\alpha^{flox/flox}$ mice were colonized with *E. coli* 541-15 and 3 days later 2% DSS was provided. At day 7 of DSS treatment, samples were collected. 3 days before colonization and every 2 days throughout the experiment 4OHT was injected to both groups. (A) Colon lengths. Colon lamina propria cells were isolated. (B) Number of immune cells. (C) Frequencies of Th1 and Tregs. (D) Frequencies and counts of total Th1 cells, CTLs, and ILC1s. Counts of (E) Monocytes and (F) Neutrophils. CD4-Cre$^+$ (T IL-10R$^{-/-}$) and CD4-Cre$^-$ (WT) littermate IL-10R$\alpha^{flox/flox}$ mice were colonized with *E. coli* 541-15 and 3 days later 2% DSS was provided. At day 7 of DSS treatment, samples were collected. (G) Colon lengths. Colon lamina propria cells were isolated. (H) Number of immune cells. (I) Frequencies of Th1 and Tregs. (J) Frequencies and counts of total Th1 cells, CTLs, and ILC1s. Counts of (K) Monocytes and (L) Neutrophils. LGR5-CreERT2$^+$ (Epi IL-10R$^{-/-}$) and LGR5-CreERT2$^-$ (WT) littermate IL-10R$\alpha^{flox/flox}$ mice were colonized with *E. coli* 541-15 and 3 days later 2% DSS was provided. At day 7 of DSS treatment, samples were collected. 7 days before colonization 4OHT was injected on two consecutive days to both groups. (M) Relative weight change (X Axis is days of DSS). At day 7 mice were gavaged with FITC-dextran and 3 hours later blood was collected. (N) FITC concentration in serum. Colon lamina propria cells were isolated. (O) Frequencies and counts of total Th1 cells, CTLs, and ILC1s. Counts of (P) Monocytes and (Q) Neutrophils. (R) Lipocalin-2 (Lcn-2) and (S) Myeloperoxidase (MPO) concentration in fecal pellets. Wildtype mice were colonized with *E. coli* 541-15 or gavaged with LB broth (CTRL) and 3 days later 2% DSS was provided. At day 7 of DSS treatment distal colons were collected for gene expression analysis. (T) *nanog* relative expression. Each replicate is a biologically independent sample. Individual dots represent samples from individual mice. Data are shown as individual values and mean, compared by two-tailed unpaired t-test or two-way ANOVA with Fisher's LSD post hoc test. The results are representative of at least two independent experiments. *$P<0.05$ was considered statistically significant; ***$P<0.001$.
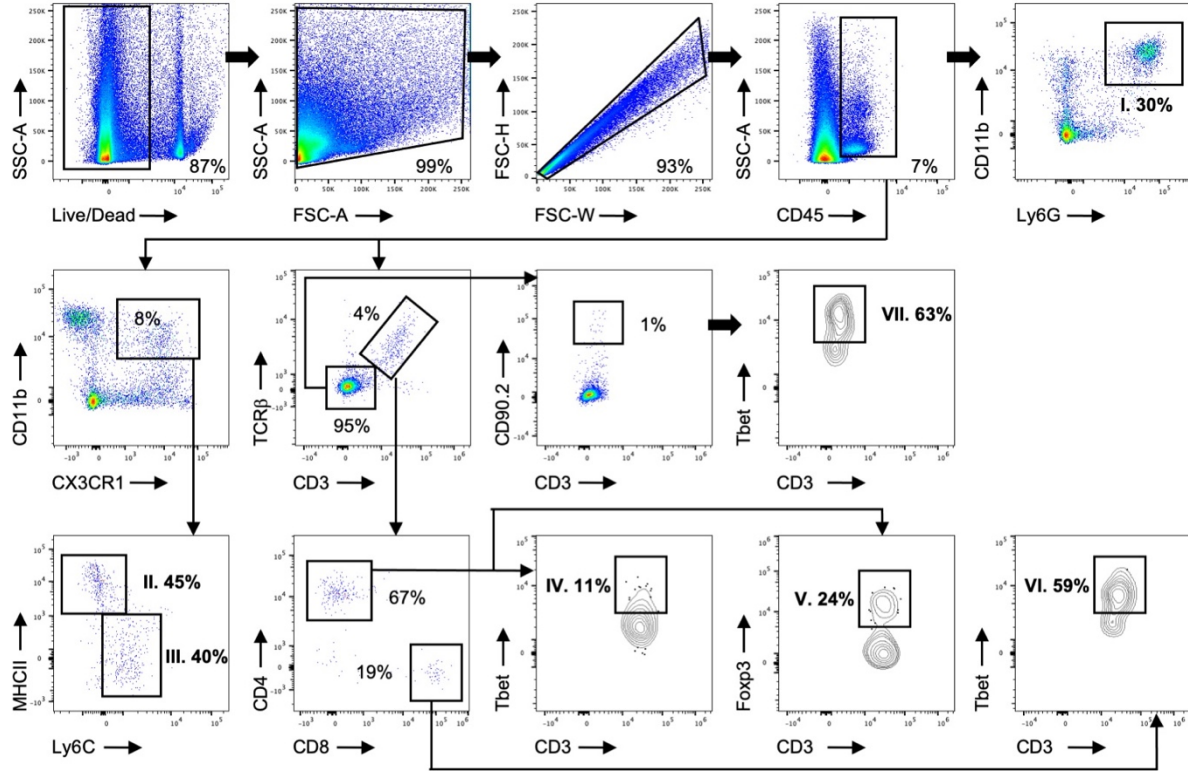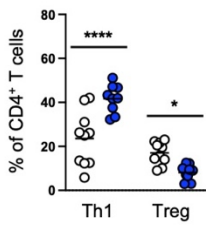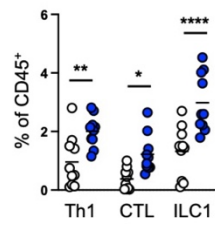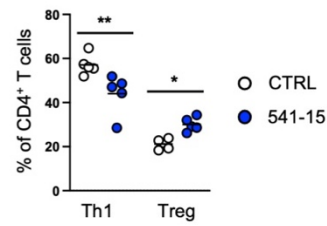
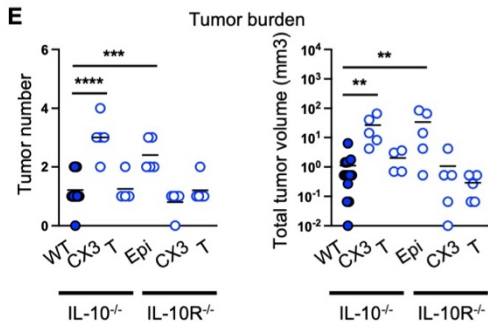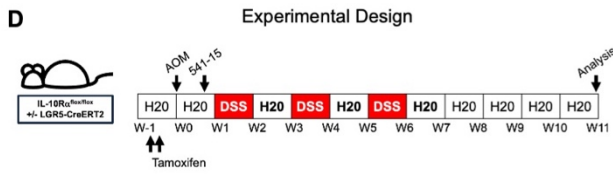**A** Experimental Design

B Spleen
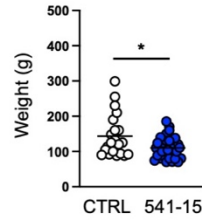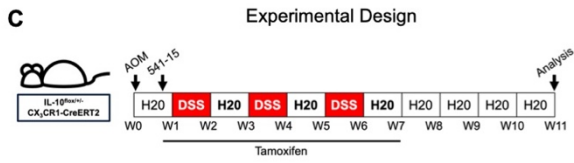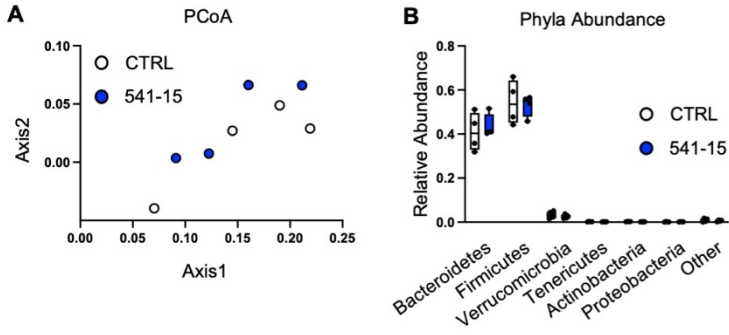
C

D T cell subsets

E Tbet+ cells

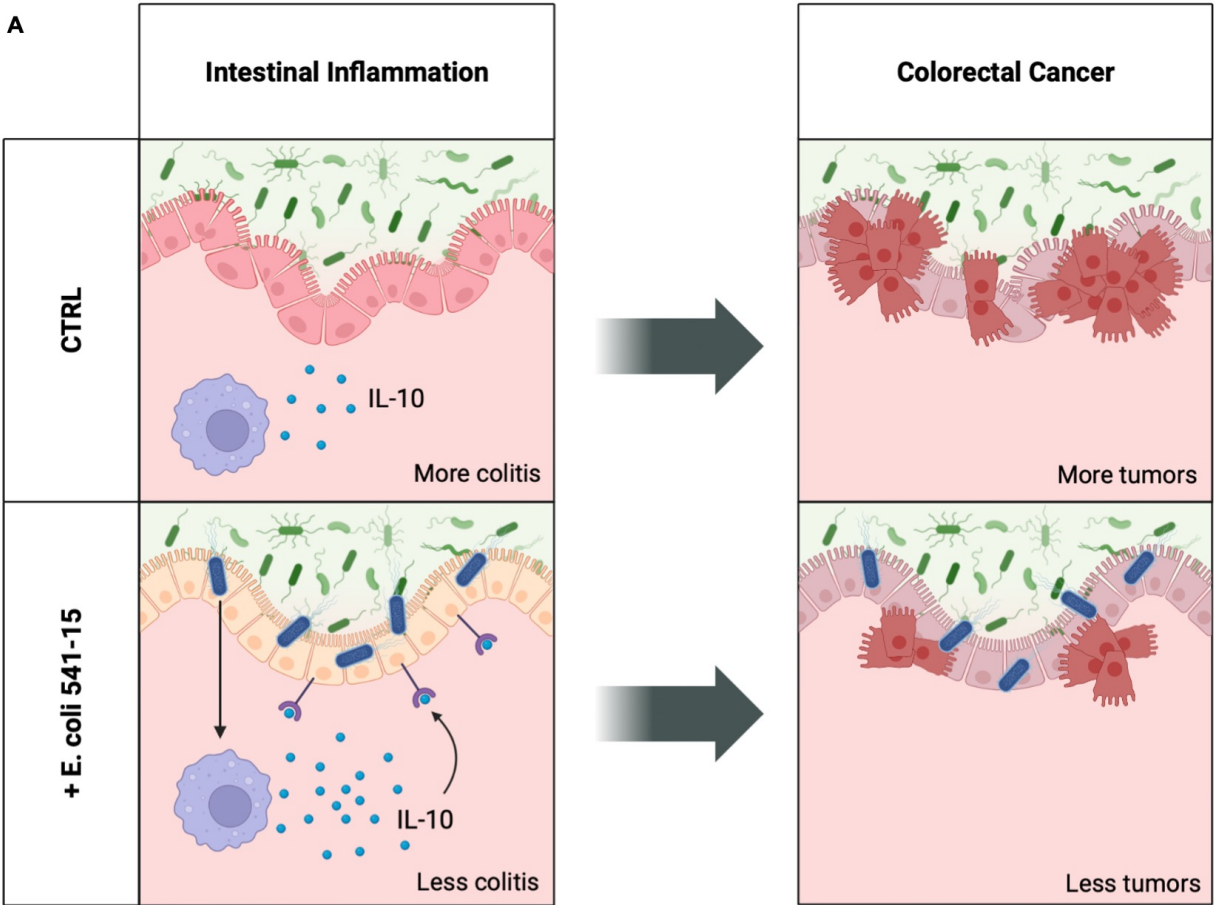**F** Colon T cell subsets

○ CTRL
● 541-15

Figure S5. Intestinal colonization with _E. coli_ 541-15 protects from colitis-associated colorectal cancer. Wildtype or CX3CR1-GFP mice were administered AOM followed by 3 cycles of 2% DSS. Before administration of DSS mice were colonized with _E. coli_ 541-15 or gavaged with LB broth (CTRL) and samples were collected 5 weeks after the last DSS treatment. (A) Experimental design. (B) Spleen weight. Tumors were individually collected, digested, and the tumor microenvironment was analyzed by flow cytometry. (C) Gating strategy to characterize: I. PMN-MDSCs, II. TAMs, III. M-MDSCs, IV. Th1s, V. Tregs, VI. CTLs, VII. ILC1s. Frequency of (D) Th1 and Tregs and (E) Total Th1 cells, CTLs, and ILC1s. Colonic tissue without tumors were collected, digested, and the lamina propria cells were analyzed by flow cytometry. (F) Frequency of Th1 and Tregs. Each replicate is a biologically independent sample. Individual dots represent samples from individual mice. Data are shown as individual values and mean, compared by two-tailed unpaired t-test or two-way ANOVA with Fisher's LSD post hoc test. The results are representative of at least two independent experiments. $*P<0.05$ was considered statistically significant; $**P <0.01$; $****P<0.0001$.

A PCoA

○ CTRL
● 541-15

Axis2 / Axis1

B Phyla Abundance

Relative Abundance

○ CTRL
● 541-15

Bacteroidetes, Firmicutes, Verrucomicrobia, Tenericutes, Actinobacteria, Proteobacteria, Other

C Experimental Design

IL-10flox+/− CX₃CR1-CreERT2

AOM / 541-15

H20 | DSS | H20 | DSS | H20 | DSS | H20 | H20 | H20 | H20 | H20
W0 | W1 | W2 | W3 | W4 | W5 | W6 | W7 | W8 | W9 | W10 | W11

Analysis

Tamoxifen

D Experimental Design

IL-10Rα flox/flox +/− LGR5-CreERT2

AOM / 541-15

H20 | H20 | DSS | H20 | DSS | H20 | DSS | H20 | H20 | H20 | H20 | H20
W-1 | W0 | W1 | W2 | W3 | W4 | W5 | W6 | W7 | W8 | W9 | W10 | W11

Analysis

Tamoxifen

E Tumor burden

Tumor number

WT | CX3 | T | Epi | CX3 | T
IL-10⁻/⁻ | IL-10R⁻/⁻

Total tumor volume (mm3)

WT | CX3 | T | Epi | CX3 | T
IL-10⁻/⁻ | IL-10R⁻/⁻

Figure S6. Intestinal colonization with *E. coli* 541-15 prevents from colitis-associated colorectal cancer through IL-10 production from CX$_3$CR1$^+$ macrophages targeting the intestinal epithelium. Wildtype mice were administered AOM followed by 3 cycles of 2% DSS. Before administration of DSS mice were colonized with *E. coli* 541-15 or gavaged with LB broth (CTRL) and fecal pellets were collected as indicated in Figure S5A. DNA was isolated, and 16S rDNA sequencing was performed. (A) Principal component analysis. (B) Bacterial phyla relative abundance. IL-10$^{flox/-}$ (CX3 IL-10$^{-/-}$) and IL-10$^{flox/+}$ (WT) littermate CX$_3$CR1-CreERT2 mice; IL-10$^{flox/-}$ (T IL-10$^{-/-}$) and IL-10$^{flox/+}$ (WT) littermate CD4-Cre mice; LGR5-CreERT2$^+$ (Epi IL-10R$^{-/-}$) and LGR5-CreERT2$^-$ (WT) littermate IL-10R$\alpha^{flox/flox}$ mice; CX$_3$CR1-CreERT2$^+$ (CX3 IL-10R$^{-/-}$) and CX$_3$CR1-CreERT2$^-$ (WT) littermate IL-10R$\alpha^{flox/flox}$ mice; as well as CD4-Cre$^+$ (T IL-10R$^{-/-}$) and CD4-Cre$^-$ (WT) littermate IL-10R$\alpha^{flox/flox}$ mice were administered AOM followed by 3 cycles of 2% DSS. Before administration of DSS mice were colonized with *E. coli* 541-15 and simultaneously 4OHT was injected to all groups. Samples were collected as indicated in Figures S6C or S6D. (C, D) Experimental design. (E) Number and size of colonic tumors. Each replicate is a biologically independent sample. Individual dots represent samples from individual mice. Data are shown as individual values and mean, compared by two-tailed unpaired t-test or two-way ANOVA with Fisher's LSD post hoc test. The results are representative of at least two independent experiments. *P<0.05 was considered statistically significant; **P <0.01; ***P<0.001; ****P<0.0001.

A

	Intestinal Inflammation	Colorectal Cancer

CTRL

IL-10

More colitis

More tumors

+ E. coli 541-15

IL-10

Less colitis

Less tumors

E. coli 541-15   CX3CR1+ Macrophage   IL-10 receptor   tumor cell

Figure S7. Graphical abstract. *E. coli* 541-15 increases IL-10 producing macrophages that together with IL-10 signaling to the intestinal epithelium ameliorates intestinal inflammation and limits tumor development.