A

B

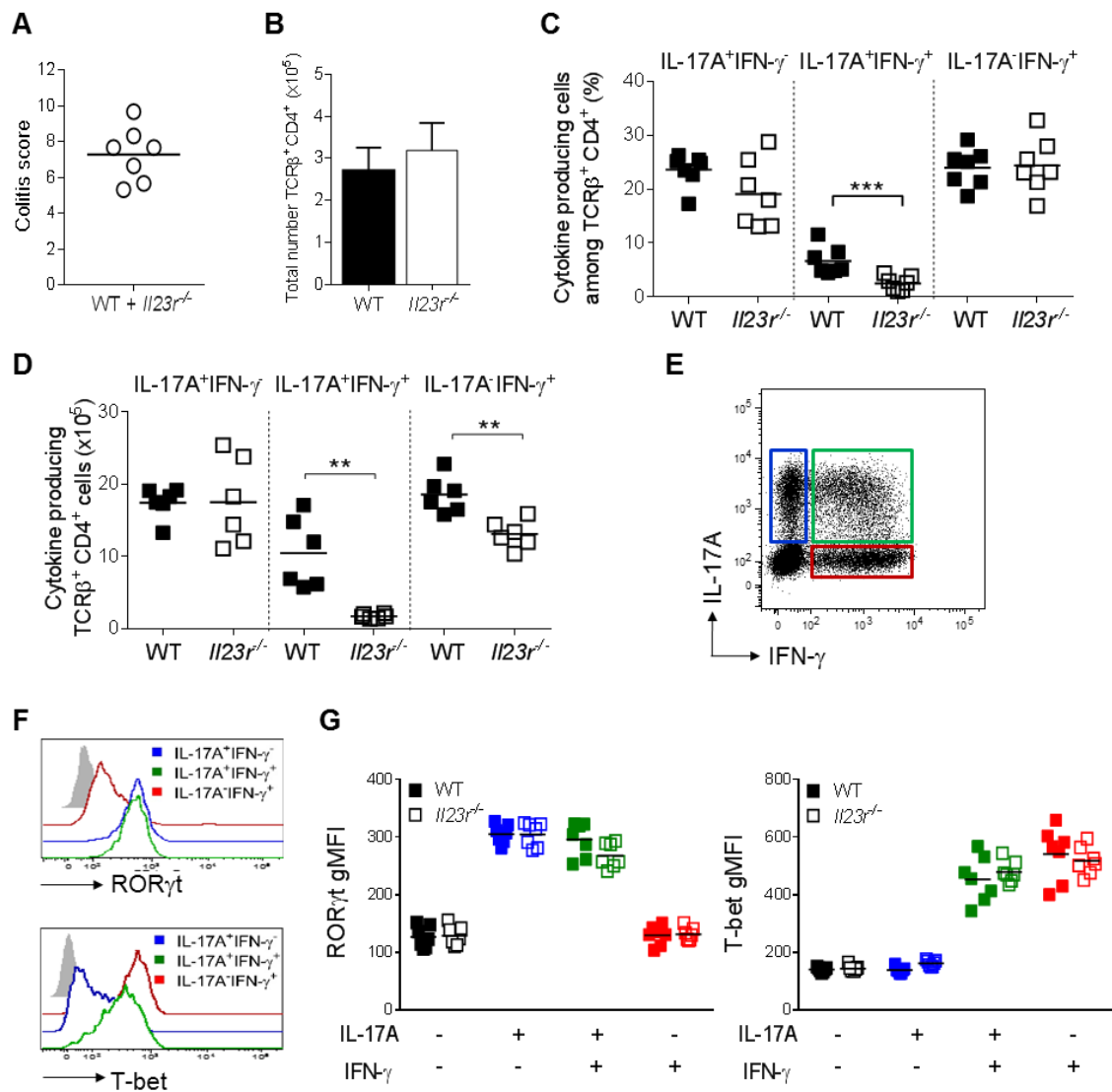**Supplementary Fig. 1**

IL-23R-expressing CD4^{+} T cells with a Th17 signature accumulate in bacteria-driven colitis

Heterozygous C57BL/6 IL-23R-GFP knock-in mice were infected orally with *Helicobacter hepaticus* and received weekly i.p. injections of IL-10R blocking antibody. Mice were sacrificed 2 weeks post infection **(A)** Representative plots of IL-23R(GFP) expression among CD4^{+} T cells from the spleen, MLN and colon of steady state or inflamed mice. GFP-negative WT mice were used as staining control. Numbers in gates represent frequencies. **(B)** qRT-PCR analysis of mRNA levels of indicated genes in FACS-purified IL-23R(GFP)[+] and IL-23R(GFP)[-] CD4^{+} T cells from the colon of steady state or inflamed mice (n = 4 per group). Data represent pooled results from two independent experiments. Bars are the mean and error bars represent the standard error of the mean (s.e.m). *P<0.05, ***P<0.001 as calculated by Mann-Whitney U test.

Supplementary Fig. 2

Expression of RORγt and T-bet in CD4$^+$ T cells is not controlled by IL-23 signalling

Mixed bone marrow chimaeras were generated by irradiation of C57BL/6 *Rag1$^{-/-}$* mice followed by i.v. injection of a 1:1 mixture of WT (CD45.1$^+$) and *Il23r$^{-/-}$* (CD45.2$^+$) bone marrow cells. After 8 weeks, mice were infected orally with *Helicobacter hepaticus* and received weekly injections of IL-10R blocking antibody. Mice were sacrificed 4 weeks post infection and assessed for intestinal inflammation. **(A)** Colitis Score. **(B)** Total numbers of WT and *Il23r$^{-/-}$* CD4$^+$ T cells present in the colon. **(C)** Frequencies and **(D)** total numbers of IL-17A$^+$ and/or IFN-γ$^+$ cells among WT or *Il23r$^{-/-}$* CD4$^+$ T cells in the colon. **(E)** Representative FACS plots showing IL-17A$^+$IFN-γ$^-$ (blue), IL-17A$^+$IFN-γ$^+$ (green) and IL-17A$^-$IFN-γ$^+$ (red) populations among WT CD4$^+$ T cells in the colon. **(F)** Representative histogram of RORγt **(top)** and T-bet **(bottom)** expression among the cytokine-expressing cells gated as described in **E**. **(G)** Geometric mean fluorescence intensity (gMFI) of RORγt **(left)** and T-bet **(right)** among cytokine-expressing CD4$^+$ T cells of WT or *Il23r$^{-/-}$* origin gated as indicated.

Data represent results from a single experiment (n = 7). Bars are the mean and each symbol represents an individual mouse. Bars are the mean and error bars represent s.e.m. ***$P<0.001$ as calculated by Mann-Whitney U test
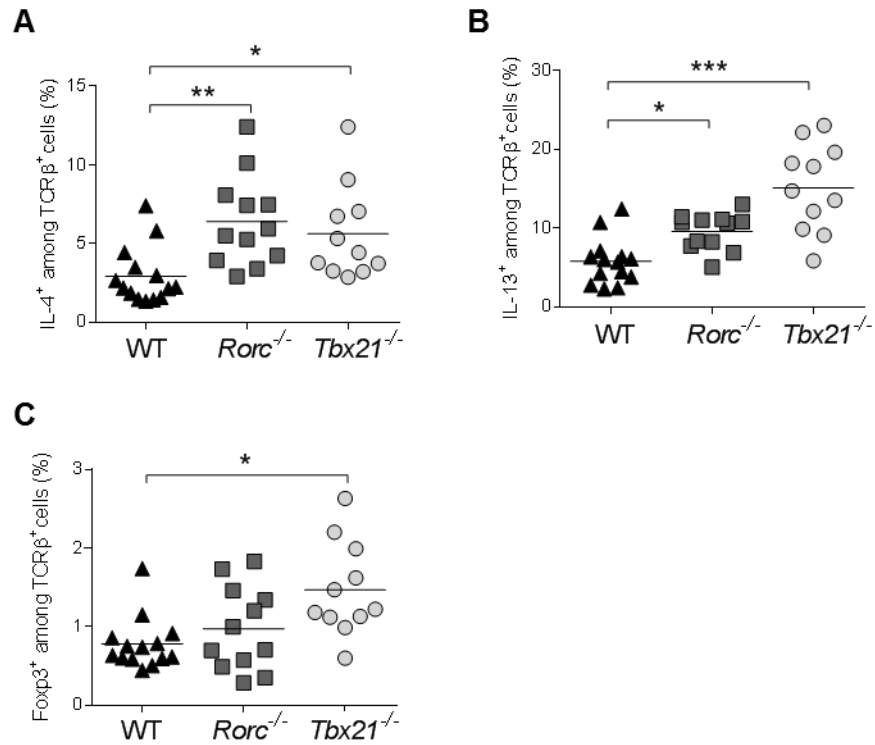
Supplementary Fig. 3

Effects of T-bet or RORγt deficiency on Th2 and Treg differentiation

Colitis was induced in C57BL/6 *Rag1*^{-/-} mice as described in Figure 3. **(A)** Frequency of IL-4 producing cells, **(B)** IL-13 producing cells and **(C)** Foxp3^{+} cells among CD4^{+} T cells in the colon. Data represent pooled results from two independent experiments (n=14 for WT, n=11 for *Tbx21*^{-/-}, n=12 for *Rorc*^{-/-}). Bars are the mean and each point represents an individual mouse. *P<0.05, **P<0.01, ***P<0.001 as calculated by Kruskal-Wallis one-way ANOVA with Dunn's post-test.
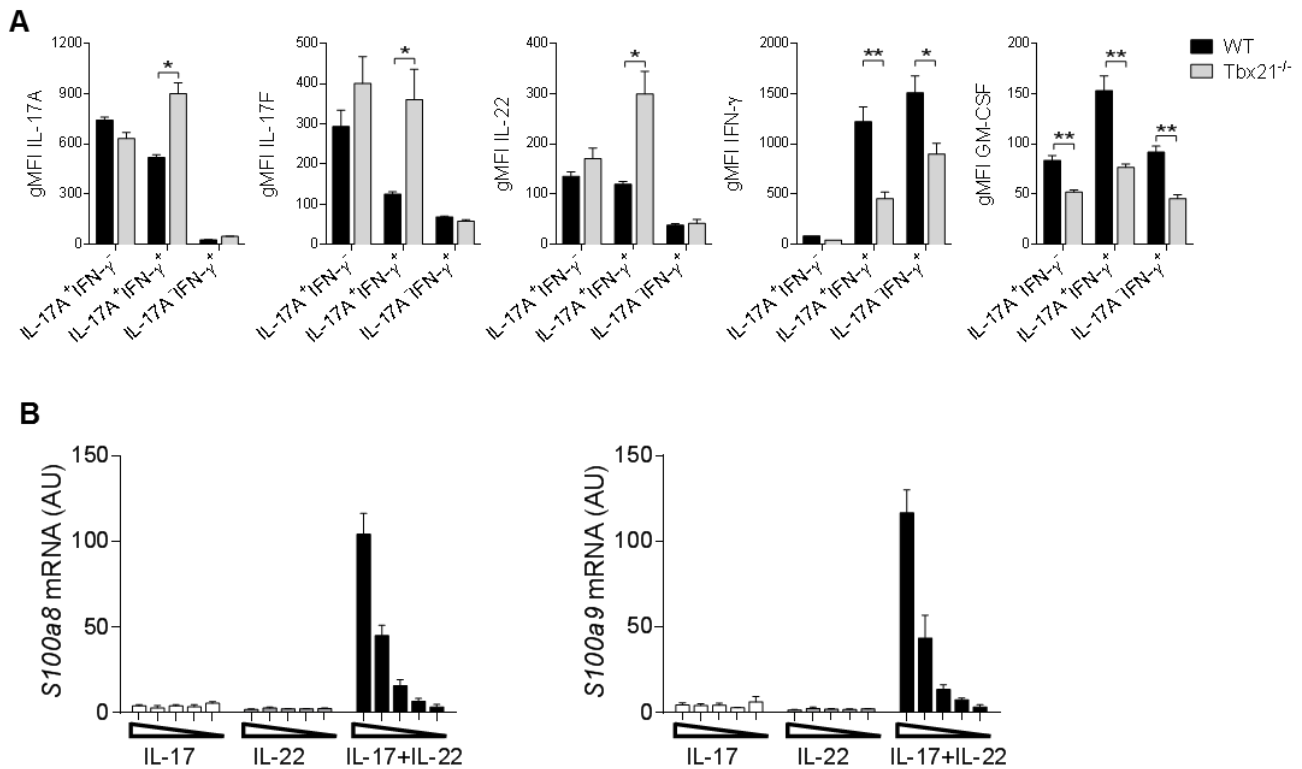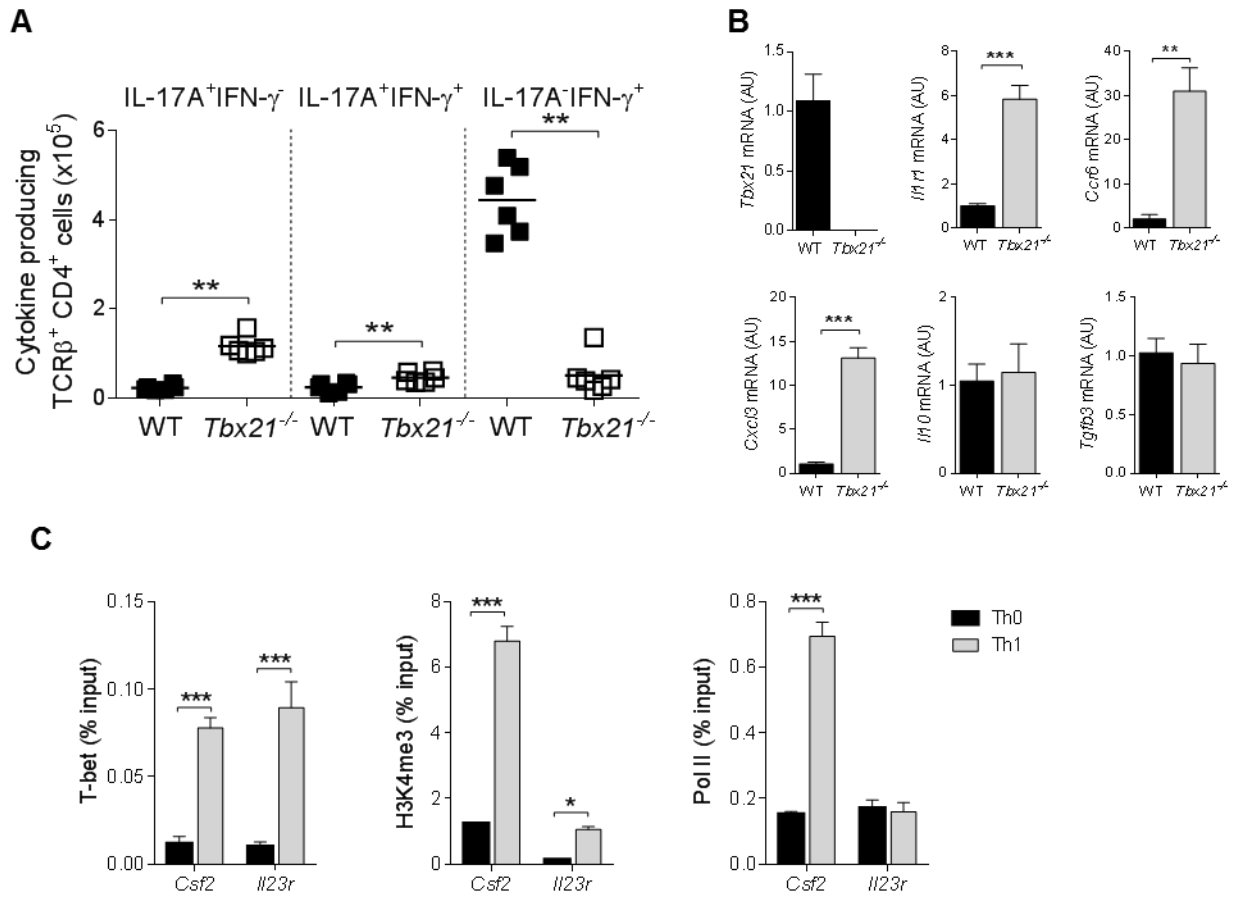
Supplementary Fig. 4

Gut epithelial cells are synergistically activated by IL-17A and IL-22

C57BL/6 *Rag1*^{-/-} mice were injected i.p. with 4×10^5 CD4^{+}CD25^{-}CD45RB^{hi} T cells from C57BL/6 WT or *Tbx21*^{-/-} donors. Mice were sacrificed when recipients of *Tbx21*^{-/-} T cells developed clinical signs of disease (4-6 weeks). **(A)** gMFI of IL-17A, IL-17F, IL-22, IFN-γ or GM-CSF among IL-17A^{+} and/or IFN-γ[+] colonic T cells. **(B)** Primary epithelial cells were isolated form the colon of steady state C57BL/6 *Rag1*^{-/-} mice. Cells were stimulated with IL-17A, IL-22 or combination of both (5 point dose response ranging from 10 ng/ml, 1 ng/ml, 100 pg/ml, 10 pg/ml to 1pg/ml) for 4 hrs after which cells were harvested and analysed by qRT-PCR for the indicated genes.

Data in A represent pooled results from two independent experiments (n=14 for WT, n=11 for *Tbx21*^{-/-}).

Bars are the mean and error bars represent s.e.m. Data in B represent results from one of three independent experiments, bars are the mean and error bars represent s.d. *P<0.05, **P<0.01, as calculated by Mann-Whitney U test
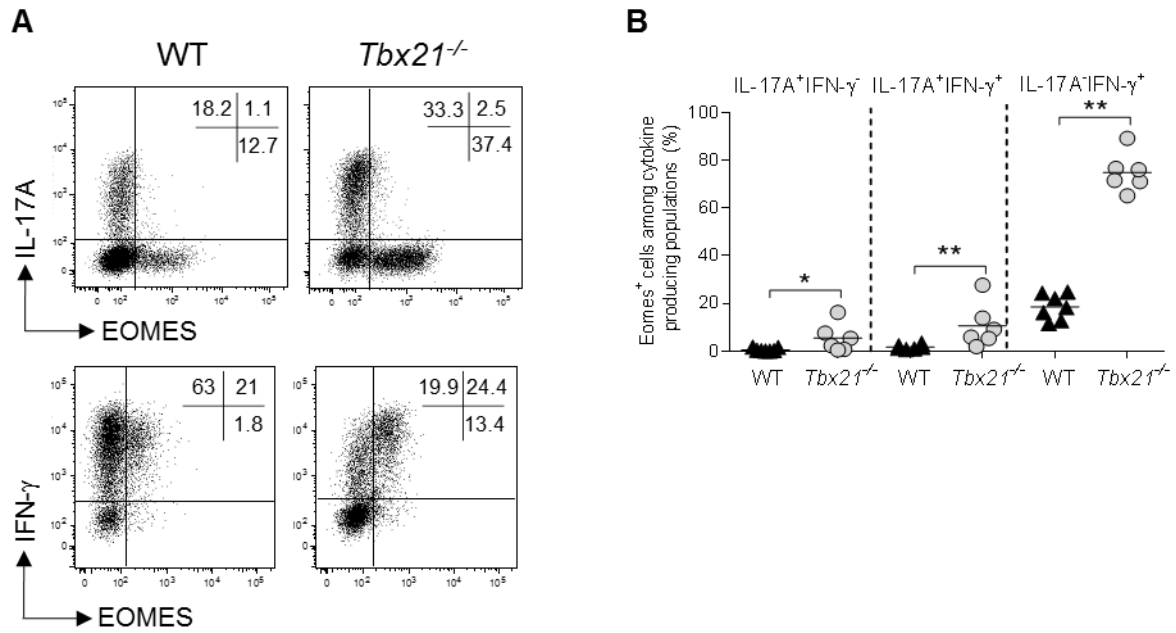
Supplementary Fig. 5

T-bet controls *Csf2* and *Il23r* expression in *in vitro* differentiated Th1 cells

C57BL/6 *Rag1*$^{-/-}$ mice were injected i.p. with 2×10^5 CD4$^+$CD25$^-$CD45RB^{hi} T cells from C57BL/6 WT (CD45.1$^+$) and *Tbx21*$^{-/-}$ (CD45.2$^+$) donors. Mice were sacrificed when recipients of *Tbx21*$^{-/-}$ T cells developed clinical signs of disease (~5 weeks). **(A)** Total numbers of WT and *Tbx21*$^{-/-}$ CD4$^+$ T cells present in the colon. **(B)** CD4$^+$ T cells were purified by flow cytometry from the inflamed colon and mRNA levels of indicated genes analysed by qRT-PCR. **(C)** Naïve CD4$^+$CD25$^-$CD44$^-$CD62L$^+$ T cells were cultured under Th0 or Th1 conditions for 36h and stimulated with fresh IL-12 (10 ng/ml) for the last 4h. ChIP-qRT-PCR for the occupancy of T-bet (TSS of *Csf2*, locus encoding part of intron 3 of *Il23r*), H3K4me3 or RNA Pol II around the TSS of indicated genes was analysed.

Data in A-B represent results from one of two independent experiments (n=6 for WT+*Tbx21*$^{-/-}$). Bars are the mean and each symbol represents an individual mouse. Bars are the mean and error bars represent s.e.m. Data in C are pooled results from three independent experiments, bars are the mean and error bars represent s.e.m. *P<0.05, **P<0.01, ***P<0.001 as calculated by Mann-Whitney U test.
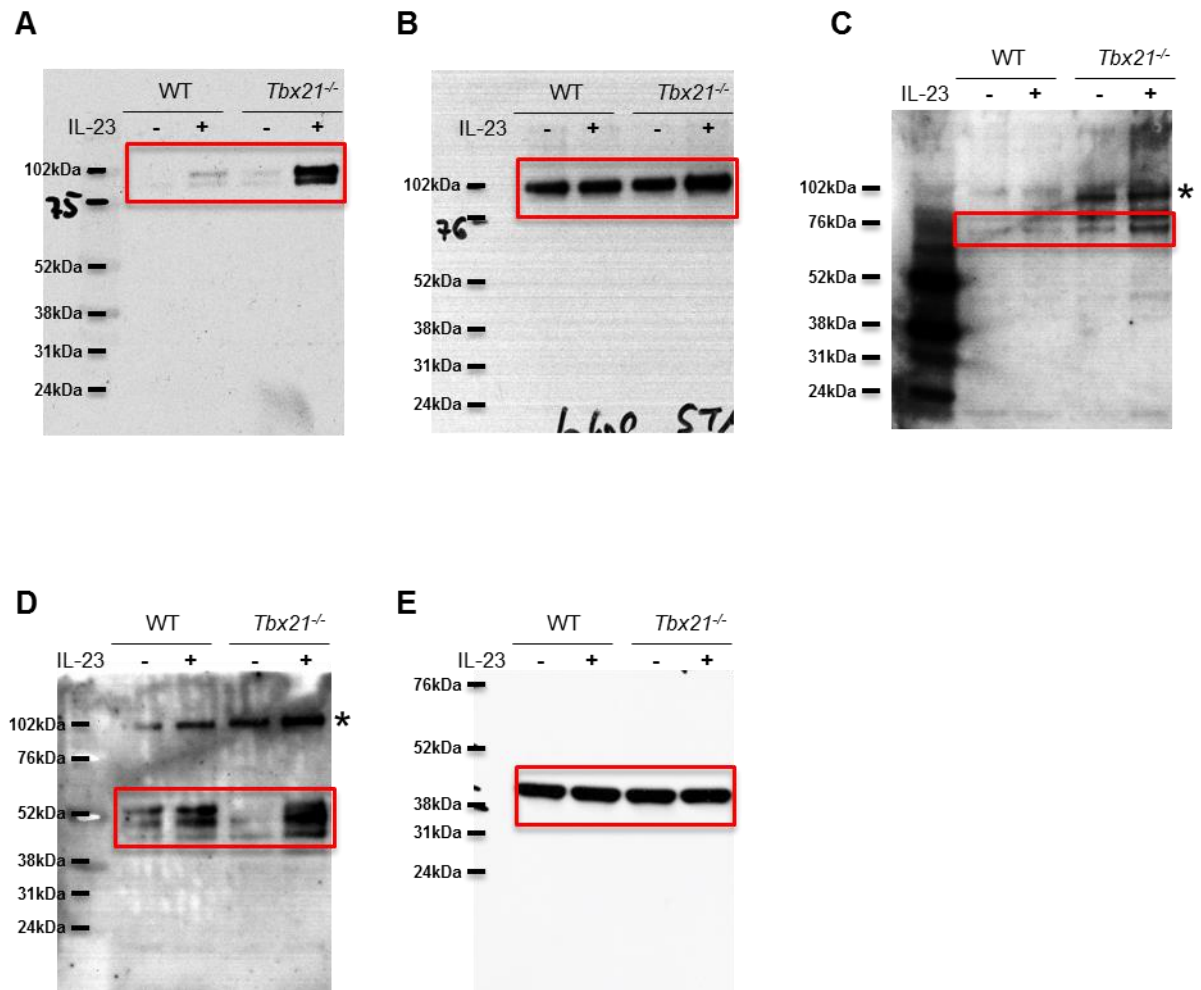
Supplementary Fig. 6
EOMES is upregulated in IFN-γ-producing T cells in the absence of T-bet
C57BL/6 *Rag1*^{-/-} mice were injected i.p. with 4×10^5 CD4^{+}CD25^{-}CD45RB^{hi} T cells from C57BL/6 WT or *Tbx21*^{-/-} donors. Mice were sacrificed when recipients of *Tbx21*^{-/-} T cells developed clinical signs of disease (4-6 weeks). **(A)** Representative plots of cytokine-producing EOMES^{+} colonic CD4^{+} T cells. **(B)** Frequencies of EOMES-expressing cells among IL-17A^{+} and/or IFN-γ^{+} CD4^{+} T cells in the colon.
Data represents results from one of two independent experiments (n=7 for WT, n=6 for *Tbx21*^{-/-}). Bars are the mean and each point represents an individual mouse. *P<0.05, **P<0.01, ***P<0.001 as calculated by Mann-Whitney U test.

Supplementary Fig. 7
Full scans of immunoblots shown in Figure 5E
CD4$^+$ T cells were purified by flow cytometry from the inflamed colon of mice receiving mix of WT and *Tbx21*$^{-/-}$ CD4$^+$ T cells. After sorting, cells were left unstimulated or stimulated with IL-23 for 12 hrs. Immunoblot for **(A)** pY705-STAT3, **(B)** total STAT3, **(C)** EOMES, **(D)** Runx3 and **(E)** Actin on whole cell lysates. Cropped immunoblots presented in Figure 5E are indicated as read squares. * depicts unspecific binding based on predicted molecular weight and information on splice variants.