

Supplementary Figure 1

ETBF activate Stat3 in B6 and Min mice colons

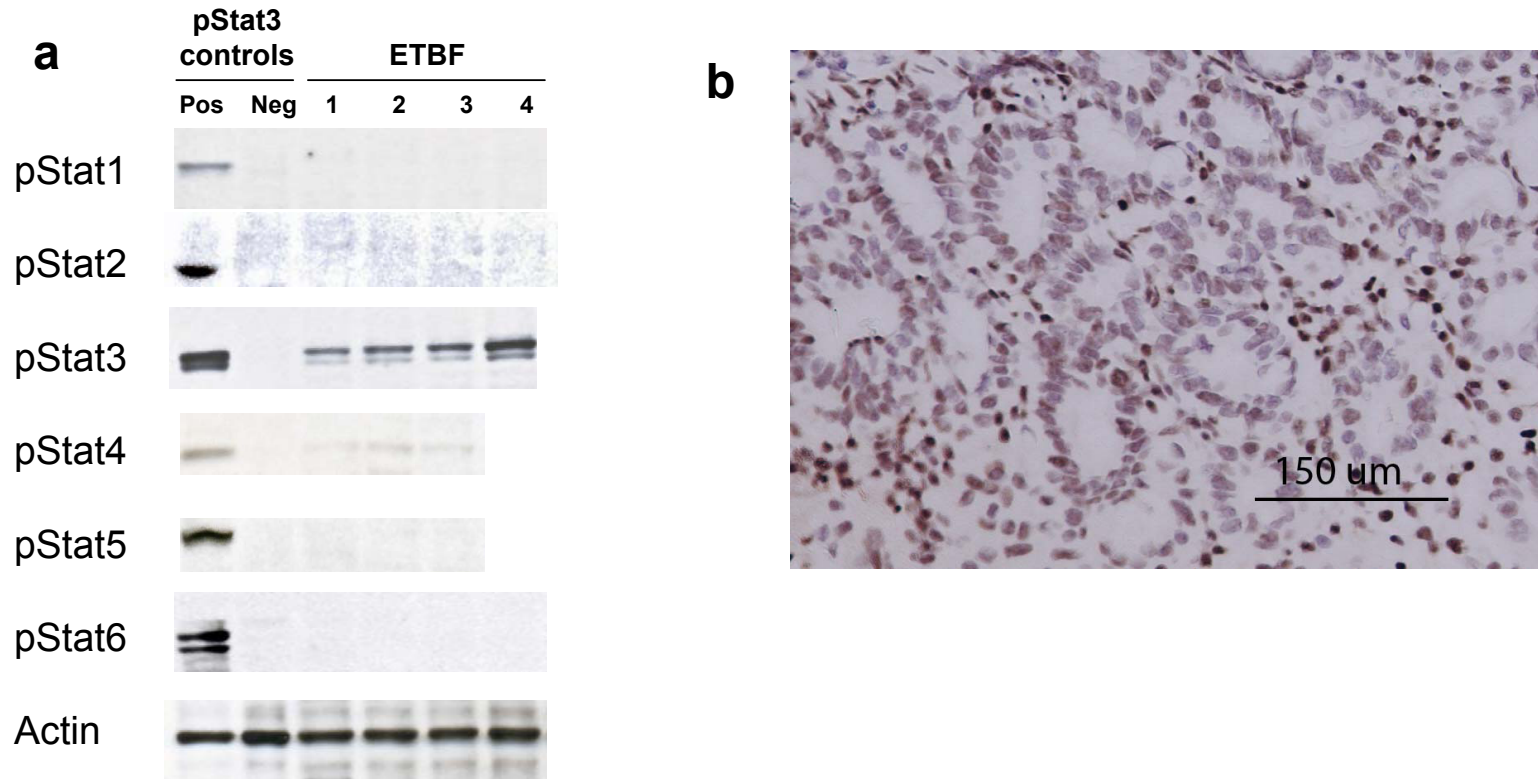


Figure Legend: (a) ETBF induce predominantly pStat3 in colons of C57Bl/6 mice. pStat nuclear proteins were evaluated by western blot on nuclear extracts of colons of 3-4 ETBF-colonized C57Bl/6 mice. Positive and negative controls for each pStat protein are included for comparison. Equal amounts of nuclear protein were loaded in each lane. Actin further serves as a loading control. (b) pStat3 staining in infiltrating leukocytes of the colonic mucosa of an ETBF-colonized Min mouse. An example of ETBF-colonized mucosa in which marked inflammatory infiltrates demonstrated pStat3 staining by IHC.

Supplementary Figure 2

Splenic cells display intracellular IFN- γ but minimal IL-17 in ETBF-colonized Min mice.

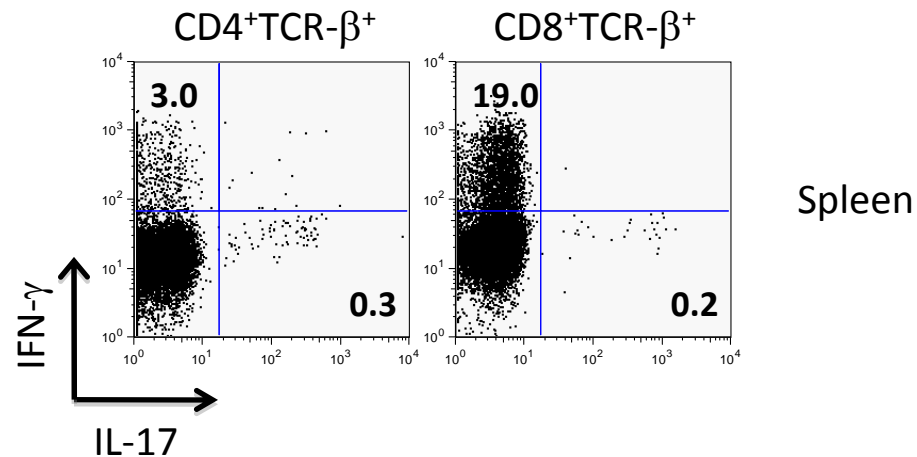


Figure Legend: Splenic cells isolated from Min mice colonized with ETBF for 1 wk and activated with PMA, ionomycin and GolgiplugTM for 5 hr were first stained for surface markers followed by intracellular staining for IFN- γ and IL-17.

Supplementary Figure 3

CD3⁻ and CD3⁺CD8⁺ leukocytes are not responsible for IL-17 production in ETBF-colonized Min mice.

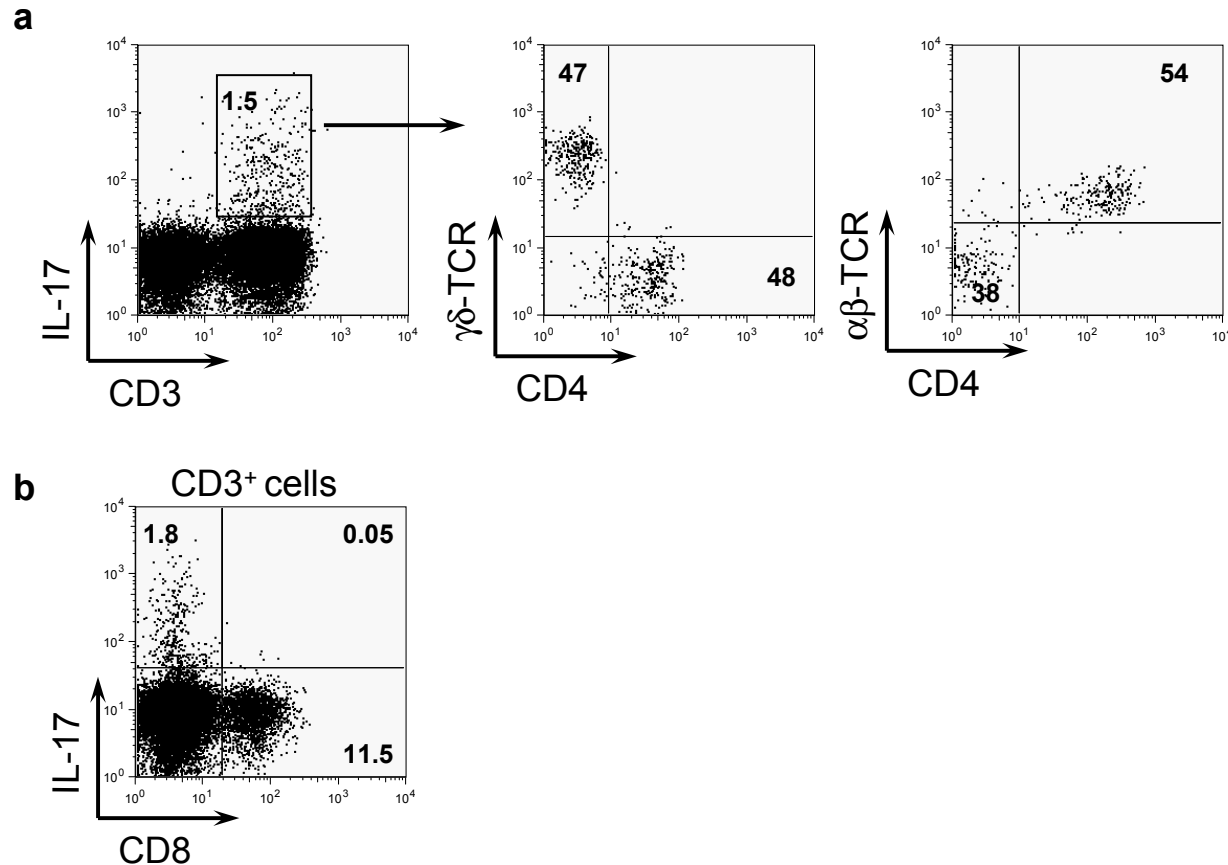


Figure Legend: Isolated colonic lymphocytes from Min mice colonized with ETBF for 1 wk and activated 5 hrs *in vitro* with PMA and ionomycin in the presence of GolgiplugTM. **(a)** CD3⁻ leukocytes do not display ICS for IL-17. Two right dot plots show the expression of $\gamma\delta$ -TCR, $\alpha\beta$ -TCR and CD4⁺ on the CD3⁺IL-17⁺ lymphocytes. **(b)** CD3⁺CD8⁺ lymphocytes do not display ICS for IL-17. The dot plot is derived from CD3⁺ cells. The figure is representative of at least 3 independent experiments.

Supplementary Figure 4

Mucosal cytokine expression in NTBF- and ETBF-colonized Min mice.

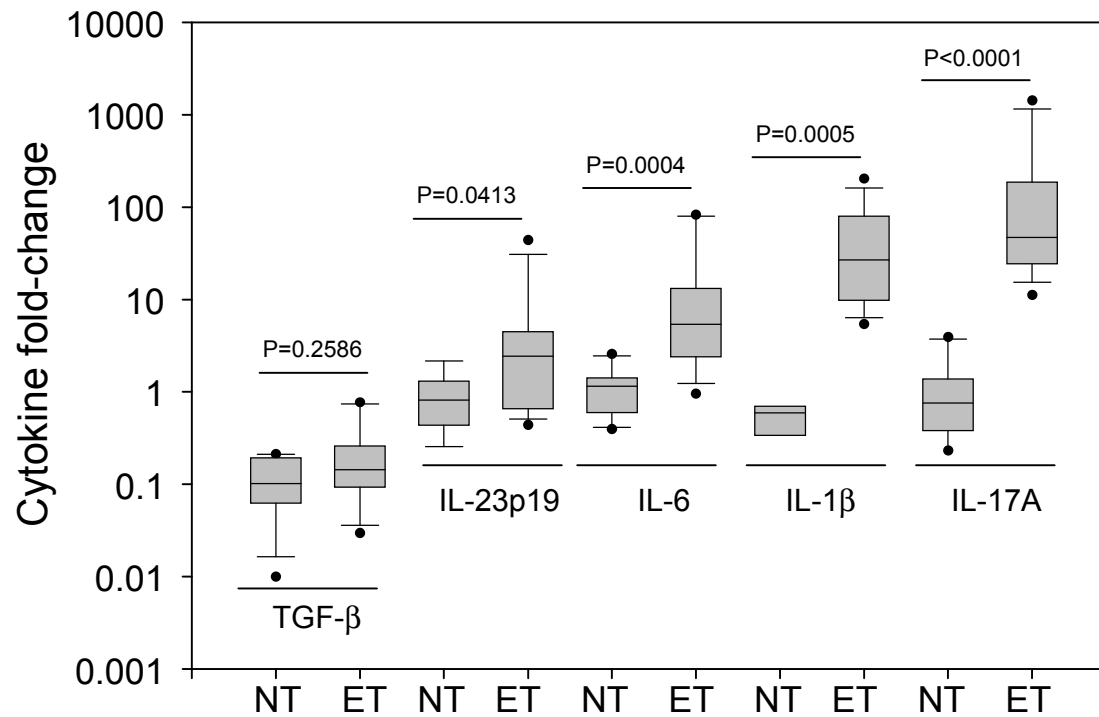


Figure Legend: qPCR analysis of Th17 pathway cytokines in the colonic mucosa of Min mice colonized with NTBF or ETBF for 1 wk. The distribution of the cytokine data are shown as box and whisker plots. NT = NTBF-colonized and ET = ETBF-colonized mice. $n = 10-15$ per condition except NT, IL-1 β ($n = 4$).

Supplementary Figure 5

Distribution of colon tumor sizes in ETBF-colonized Min mice treated with Th17 pathway neutralizing antibodies.

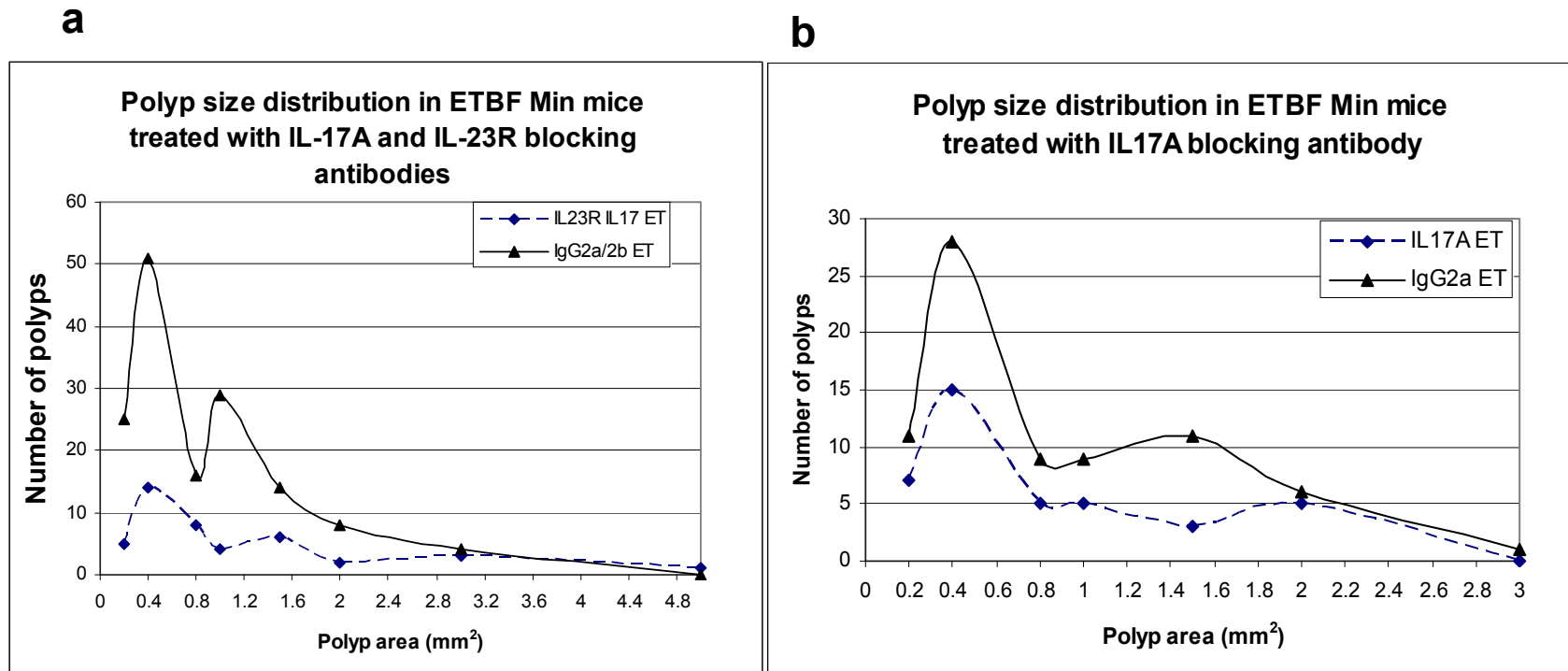


Figure Legend: Methylene blue-stained colon tumors were counted at 5 wks after bacterial inoculation and sized by microscopy. In panel (a), ETBF-colonized Min mice were treated with IL-17A and IL-23R neutralizing antibodies or isotype control antibodies. In panel (b), ETBF-colonized Min mice were treated with IL-17A neutralizing antibodies or isotype control antibodies.

Supplementary Figure 6

IL-17 blockade does not discernibly modify Stat3 activation by ETBF.

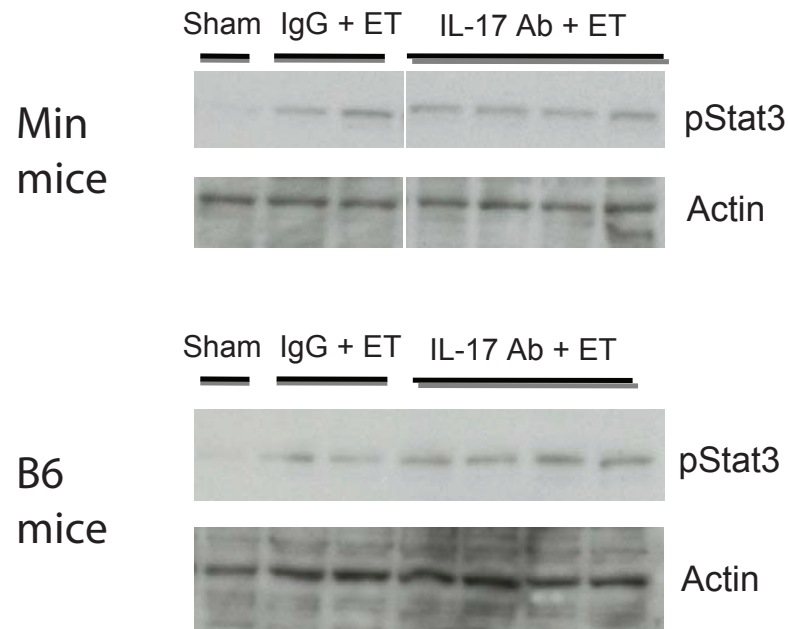


Figure Legend: Sham and ETBF-colonized Min or C57Bl/6 mice were treated with IL-17 blocking antibody (IL-17 Ab + ET) or isotype control IgG antibody (IgG + ET) for 3 d followed by extraction of mucosal nuclear proteins and western blot for activated Stat3 (pStat3). Actin serves as a loading control.

Supplementary Table 1

Mouse colon histology scores 1 wk after NTBF or ETBF colonization in wild-type (WT) or CD4 Stat3-KO C57Bl/6 mice.

	Median (range)	
	Inflammation	Hyperplasia
Sham WT (<i>n</i> = 3)	0 (0–0)	0 (0–0)
Sham CD4 Stat3-KO (<i>n</i> = 4)	0 (0–1)	0.5 (0–1)
NTBF WT (<i>n</i> = 4)	0 (0–0)	0.5 (0–1)
NTBF CD4 Stat3-KO (<i>n</i> = 4)	0 (0–1)	0.5 (0–1)
ETBF WT (<i>n</i> = 13)	3 (1–4) ^{a,b}	2 (2–3) ^{a,d}
ETBF CD4 Stat3-KO (<i>n</i> = 18)	2 (1–3) ^c	2 (1–3) ^e

^a $P \leq 0.004$ vs sham and NTBF WT, independently

^b $P \leq 0.001$ vs ETBF CD4 Stat3-KO

^c $P \leq 0.005$ vs sham and NTBF CD4 Stat3-KO, independently

^d $P \leq 0.03$ vs ETBF CD4 Stat3-KO

^e $P \leq 0.009$ vs sham and NTBF CD4 Stat3-KO, independently

Supplementary Table 2

Min mouse colon histology scores 4–5 wks after ETBF colonization
in mice treated with isotype antibodies or IL-17 and IL-23R neutralizing
antibodies or IL-17 neutralizing antibodies alone

	Median (range)			
	Inflammation	Hyperplasia	GIN	Gross Tumors
ETBF (isotype Abs) (<i>n</i> = 24)	2 (1–3)	2 (2–3)	2 (0–6)	10 (2–32)
ETBF (IL-17/IL-23R Abs) (<i>n</i> = 14)	1 (0–1) ^a	2 (1–2) ^b	0 (0–1) ^b	5 (1–9) ^c
ETBF (IL-17 Abs) (<i>n</i> = 8)	1 (1–1) ^d	1 (0–2) ^b	0.5 (0–2) ^e	3.5 (1–13) ^e

All comparisons are to ETBF (isotype antibodies).

^a *P* < 0.004

^b *P* < 0.0007

^c *P* < 0.002

^d *P* = 0.043

^e *P* < 0.02