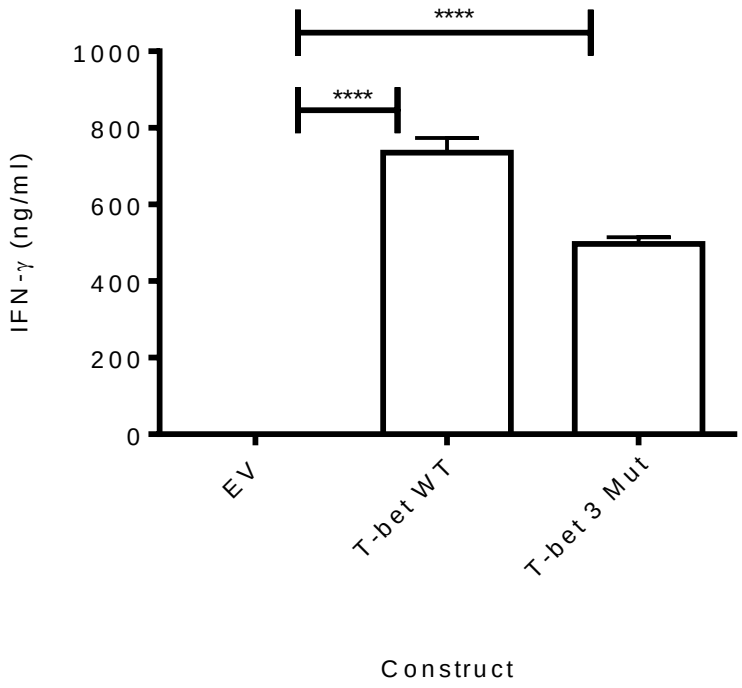
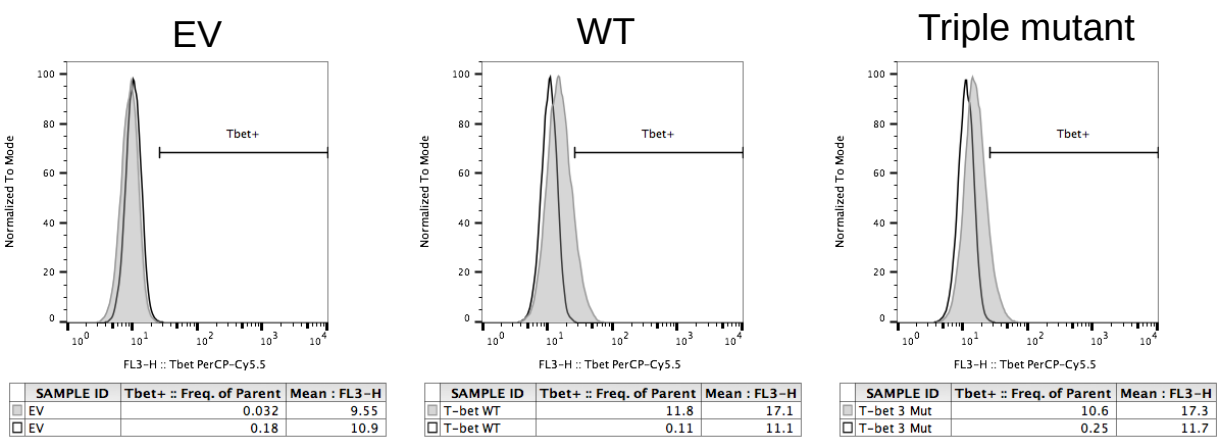
Supplemental figure 1

A



B

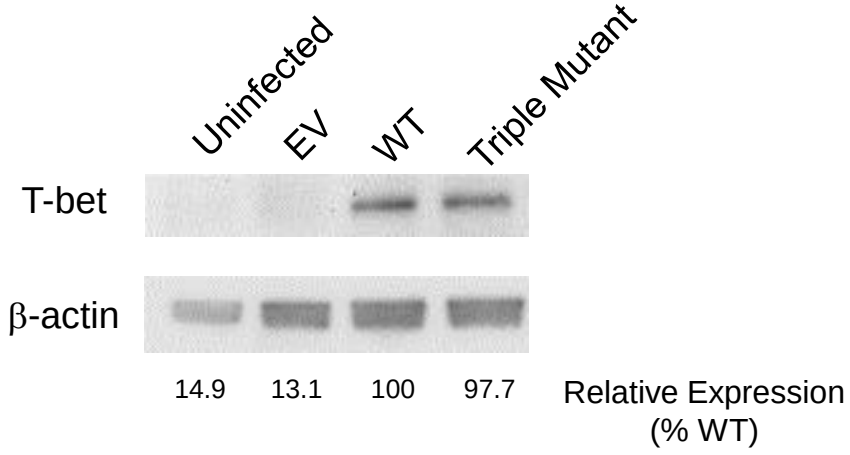


SAMPLE ID	Tbet+ :: Freq. of Parent	Mean : FL3-H
EV	0.032	9.55
EV	0.18	10.9

SAMPLE ID	Tbet+ :: Freq. of Parent	Mean : FL3-H
T-bet WT	11.8	17.1
T-bet WT	0.11	11.1

SAMPLE ID	Tbet+ :: Freq. of Parent	Mean : FL3-H
T-bet 3 Mut	10.6	17.3
T-bet 3 Mut	0.25	11.7

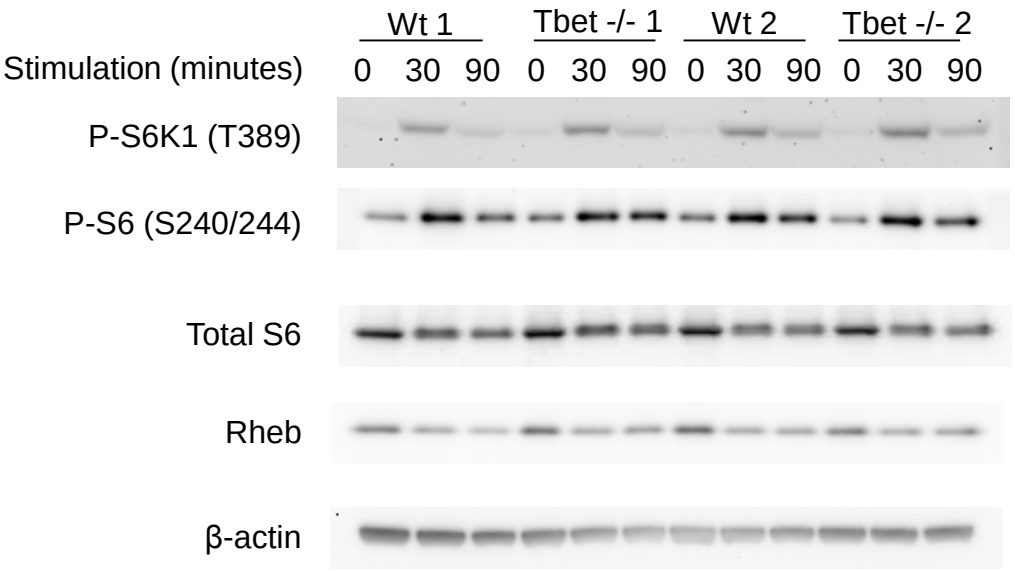
C



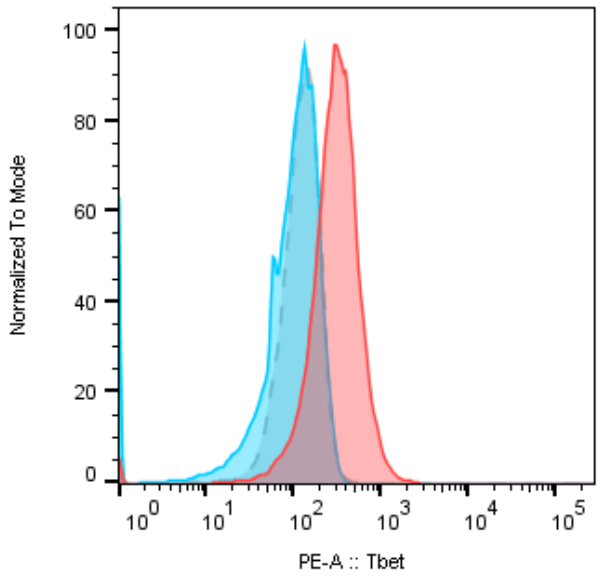
Supplemental Fig 1. A. Equal amounts of GFP+ EL4 cells stably transduced with either WT/triple mutant T-bet or empty vector were plated and stimulated with PMA/Ionomycin overnight. Supernatants were harvested and analyzed for IFNγ production using ELISA. B. The same transduced EL4 cells that were used for ELISA were stained for T-bet. C. EL4 cells stably transduced with either WT/triple mutant T-bet or empty vector were lysed in RIPA buffer and 30 µg of protein was separated by SDS-PAGE and transferred to PVDF membrane. Western blot was performed with anti T-bet (eBioscience, clone eBio4B10) or anti b-actin (Cell Signaling, clone D6A8) and anti-mouse IgG-HRP or anti-rabbit IgG-HRP, respectively (GE Healthcare). Protein expression was detected using ECL-Prime reagent (GE Healthcare) and quantified on a UVP Biochemi 500 imaging system. Relative expression is T-bet area density/b-actin area density with WT T-bet expression normalized to 100. Results shown are representative of 3 independent experiments.

Supplemental figure 2

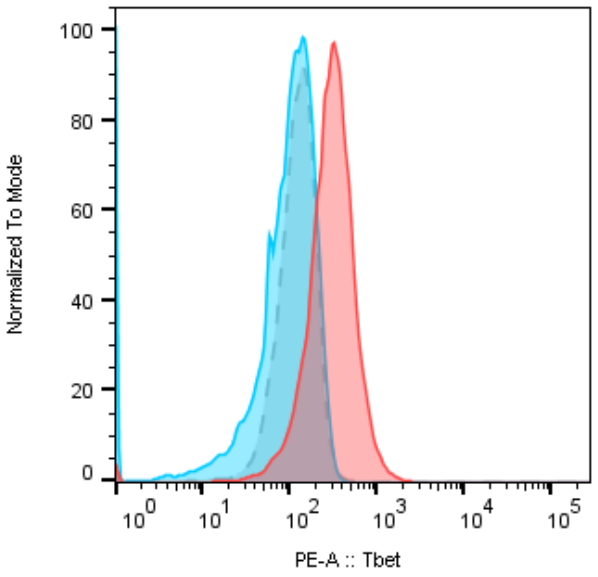
A



B



Wt 1
Tbet KO 1
No Stain Control

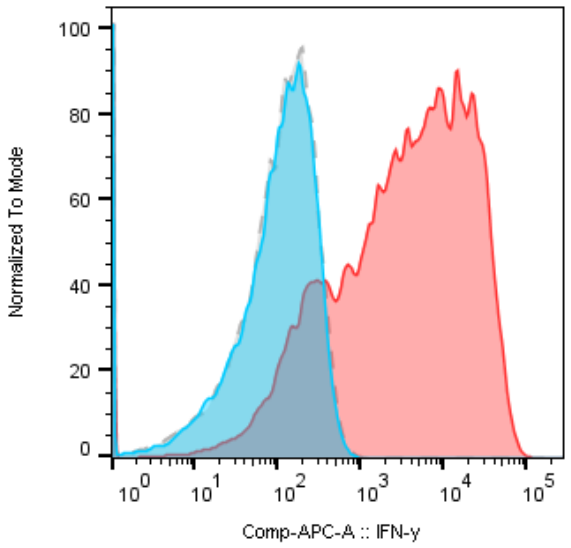


Wt 2
Tbet KO 2
No Stain Control

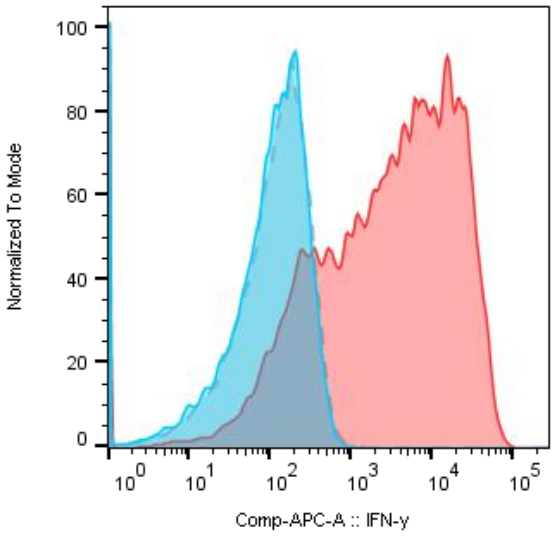
Supplemental Figure 2. A. CD4+ T cells were isolated from either WT or T-bet KO mice and stimulated with 3 μg/ml plate-bound anti-CD3 and 2 μg/ml of soluble anti-CD28 antibody. Stimulated cells were harvested at indicated time points and pS6K1, pS6, total S6, Rheb and β-actin expression were measured by Western blot. B. CD4+ T cells from WT and T-bet KO mice were stimulated as in A in the presence of IL-12 and T-bet expression was measured after 72 hours by flow cytometry. Results shown are representative of 2 independent experiments with 2 biological replicates of each genotype per experiment.

Supplemental figure 3

A

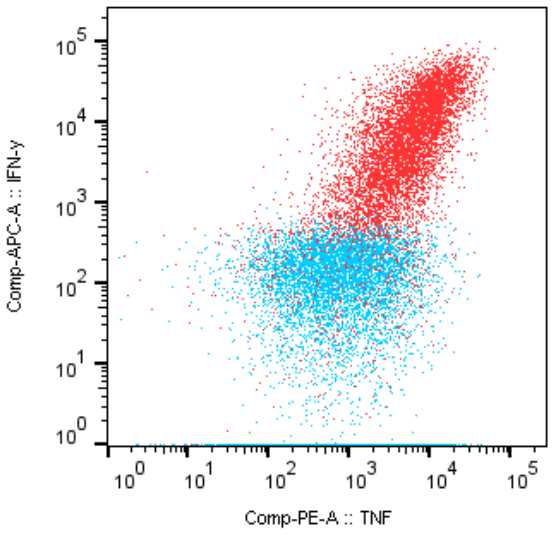


Wt 1 Stim
Tbet KO 1 Stim
No Stim Control

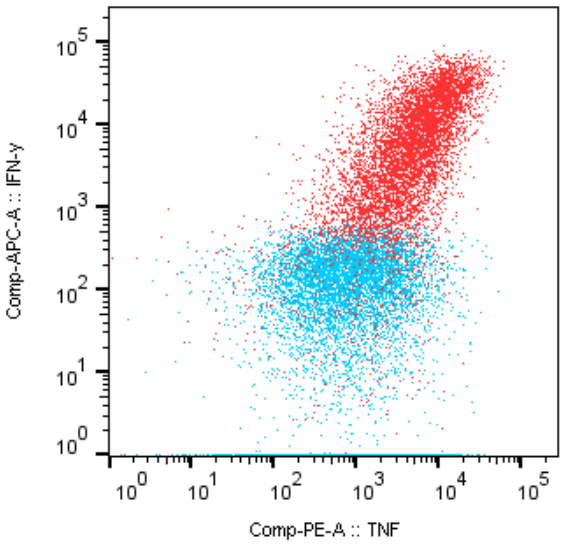


Wt 2 Stim
Tbet KO 2 Stim
No Stim Control

B



Wt 1 Stim
Tbet KO 1 Stim



Wt 2 Stim
Tbet KO 2 Stim

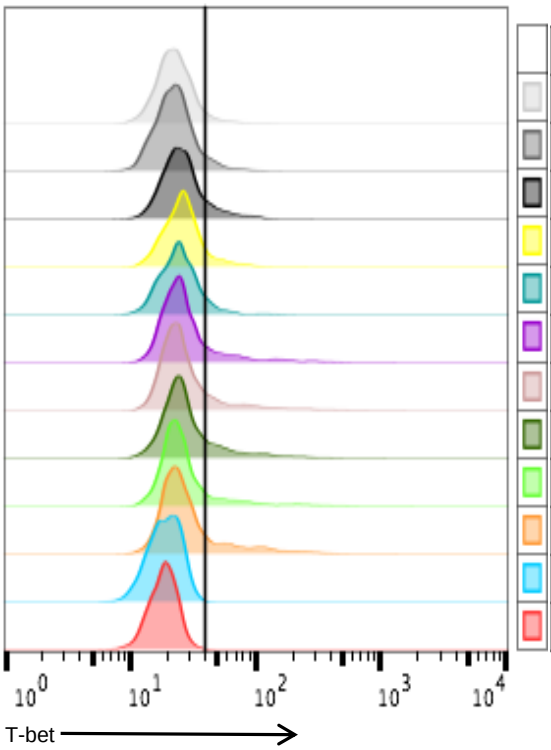
IFN-y

TNFα

Supplemental Figure 3. CD4+ T cells cultured as in Supplemental Figure 1B were stimulated with PMA/ionomycin in the presence of Golgi transport inhibitor for 6 hours IFNγ (A) and TNFα (B) expression was measured by intracellular cytokine staining. Data are representative of staining of cells from 4 WT and 4 KO mice.

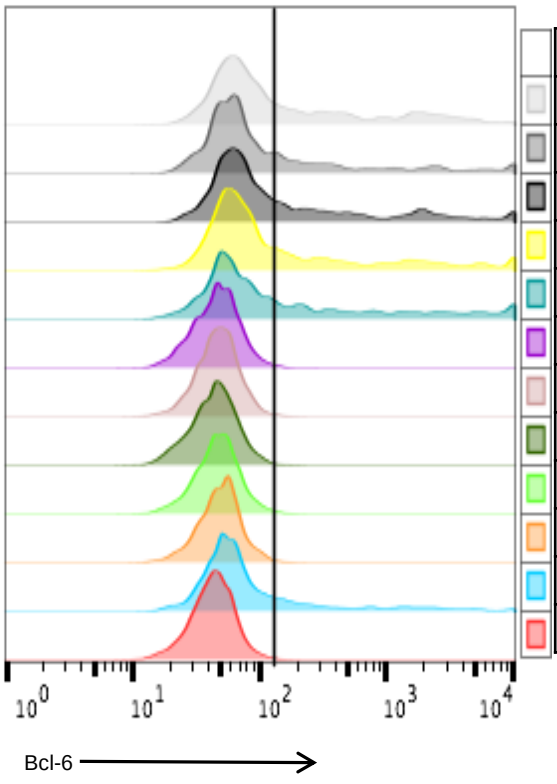
Supplemental figure 4

A



Sample	%T-bet positive	MCF
Bcl-6 + T-bet S224A	3.8	25.1
Bcl-6 + T-bet Y76A	5.36	28.9
Bcl-6 + T-bet S52A	10.1	31.5
Bcl-6 + T-bet triple	8.96	34.3
Bcl-6 + T-bet WT	6.8	31.6
T-bet S224A	16.1	50.2
T-bet Y76A	15.6	52.5
T-bet S52A	17.9	54.7
T-bet triple	15.7	56.1
T-bet WT	18.7	58.2
Bcl-6	0.26	20.7
EV	0.21	19.1

B



Sample	%Bcl-6 positive	MCF
Bcl-6 + T-bet S224A	34.1	622
Bcl-6 + T-bet Y76A	26.4	604
Bcl-6 + T-bet S52A	31.3	680
Bcl-6 + T-bet triple	31.0	718
Bcl-6 + T-bet WT	35.1	885
T-bet S224A	0.40	50.0
T-bet Y76A	0.25	50.3
T-bet S52A	0.26	47.5
T-bet triple	0.28	50.6
T-bet WT	0.34	52.4
Bcl-6	20.0	393
EV	0.14	45.9

Supplemental Fig 4. EL4 cells were co-transfected with either WT/mutant T-bet or empty vector (EV) and SOCS3 luciferase reporter plasmid in the absence or presence of Bcl-6, as in Figure 8C. A. T-bet expression levels were measured by intracellular protein staining detected by flow cytometry and are depicted as % T-bet positive cells and mean channel fluorescence (MCF), gated on live, GFP+ transfected cells. B. Bcl-6 levels were measured in the same manner as in A. Results shown are representative of 3 independent experiments.