Supplementary Figures

T Cell Factor-1 initiates T helper 2 fate by inducing GATA-3 and repressing Interferon-γ

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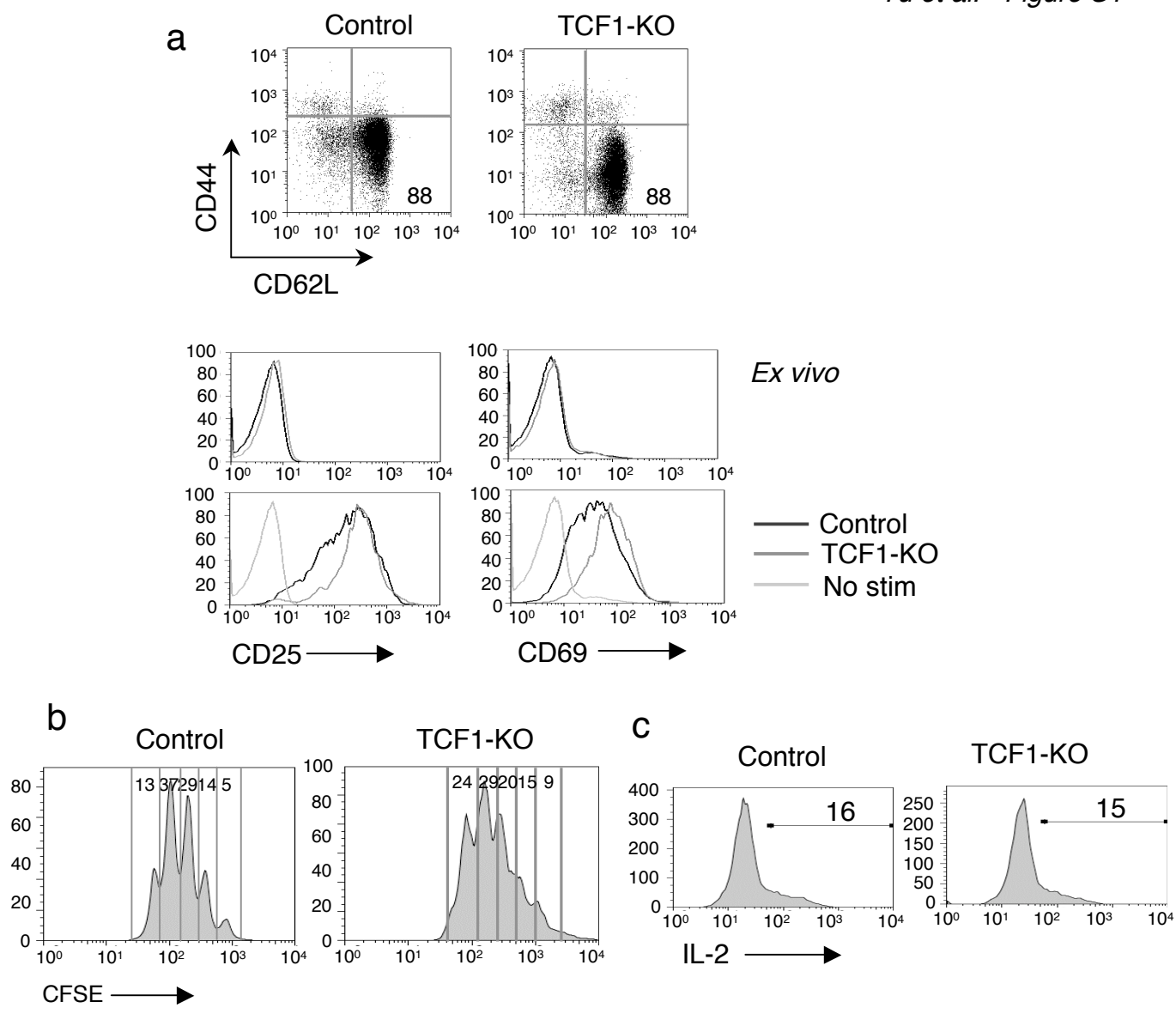


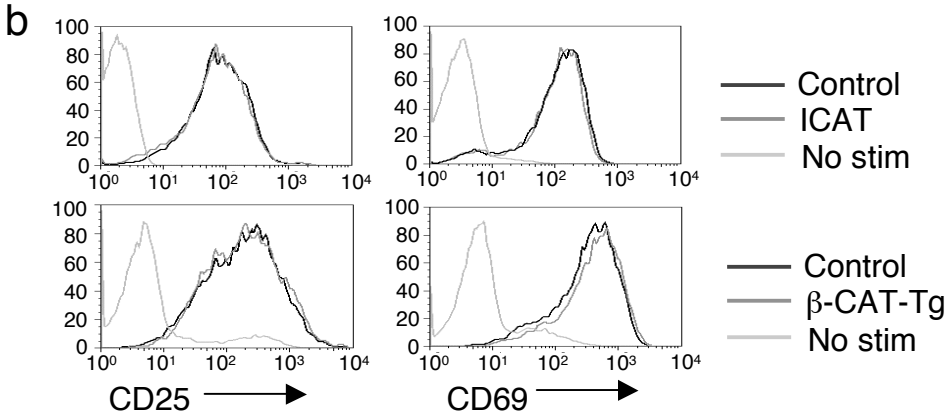
Supplementary Figure 1. Naïve-enriched TCF1-KO CD25$^-$ CD4$^+$ T cells are activated normally upon TCR-stimulation. (a) Early activation of TCF1-KO CD4$^+$ T cells upon TCR stimulation is normal. Purified CD25$^-$ CD4$^+$ T cells from control and TCF1-KO mice were stained with anti-CD44 and anti-CD62L and analyzed by flow cytometry (upper panels). CD25$^-$ CD4$^+$ T cells were activated with anti-CD3 and anti-CD28 and early activation was measured by induction of CD25 and CD69 (lower panels). Data are representative of 6 independent plots. (b) TCF1-KO CD4$^+$ T cells proliferate normally upon TCR stimulation. CFSE labeled CD25$^-$ CD4$^+$ T cells from control and TCF1-KO mice were stimulated with plate-bound anti-CD3 plus anti-CD28 antibodies for 3 days. CFSE intensity on gated CD4$^+$ T cells is shown and numbers show the percentage of cells in each cell division. Data are representative of 6 independent plots. (c) IL-2 production is unaffected in TCR-stimulated TCF1-KO CD4$^+$ T cells. CD25$^-$ CD4$^+$ T cells from control and TCF1-KO mice were stimulated with APC plus anti-CD3 for 2 days and IL-2 production was analyzed by intracellular staining. Date are representative of 6 independent plots.

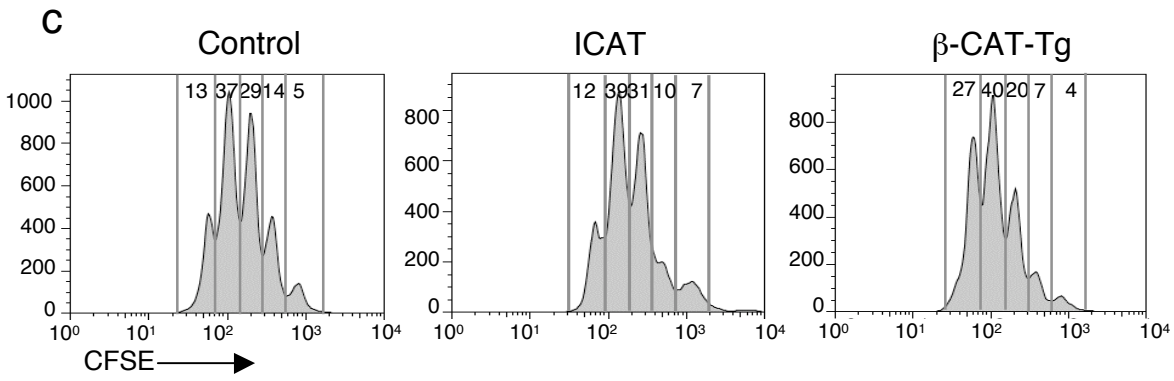
a ICAT - Inhibitor of β-catenin and TCF (ICAT)
binds β-catenin and prevents its interaction with TCF
REFS: # 45 to 48 in the text.

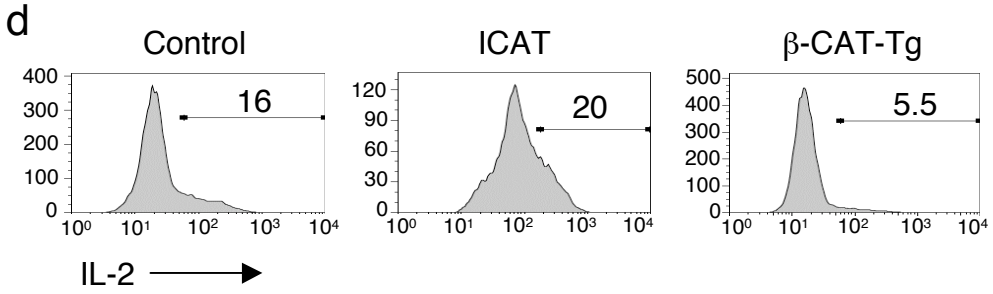
Enforced expression from the proximal *Lck* promoter
results in expression of ICAT in thymocytes and T cells in ICAT mice
REF: # 49 in the text (Int. Imm. 20:925, 2008).

Stabilized β-catenin is a mutant form in which the GSK-3β phosphorylation sites have been deleted. This mutant form, expressed from the proximal *Lck* Promoter, is expressed in thymocytes and T cells in β-CAT-Tg mice.
REF: #50 in the text (Int. Imm. 15:1485, 2003).

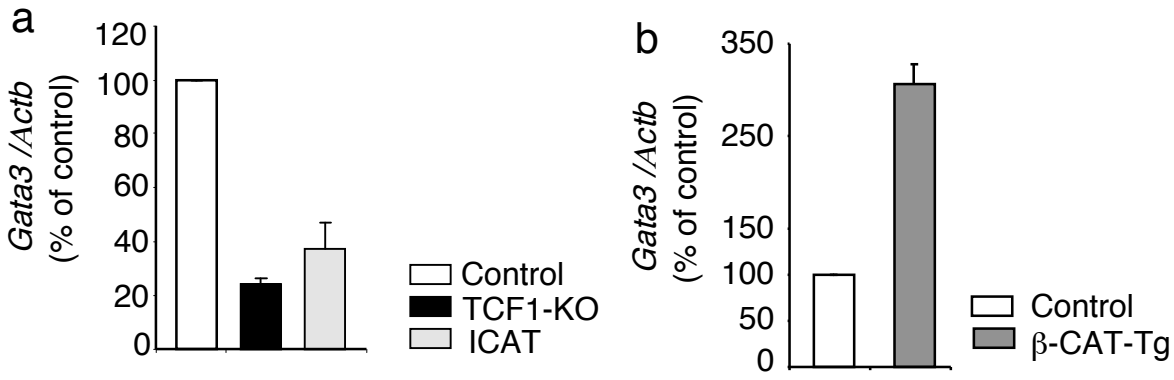
TCR-activation of $CD4^+$ T cells from β-CAT-Tg and ICAT mice is shown below.



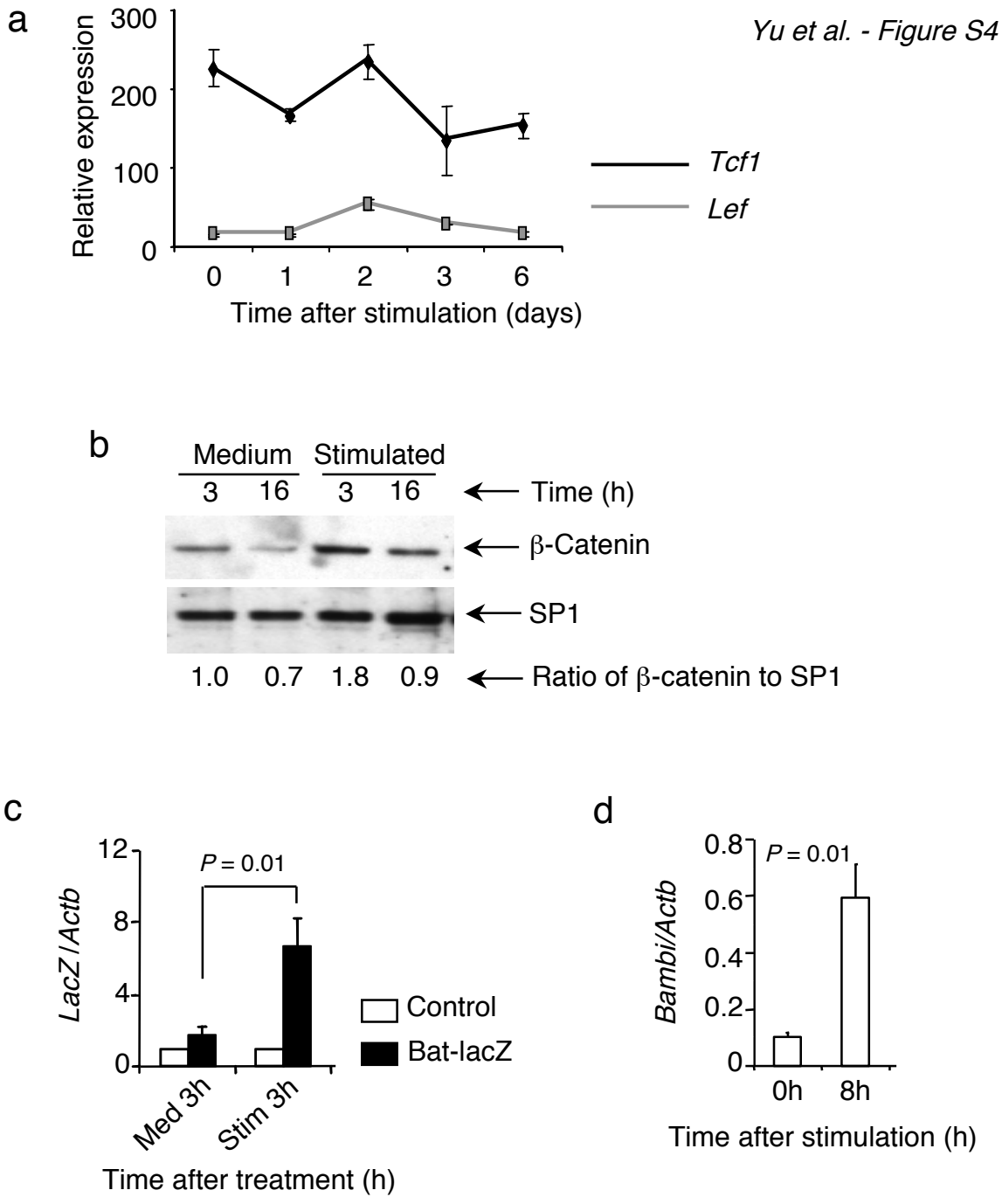




Supplementary Figure 2. Activation of ICAT and β-CAT-Tg $CD4^+$ T cells upon TCR-stimulation. (a) Description of ICAT and β-CAT-Tg mice. ICAT is a naturally occurring small protein that binds β-catenin and prevents its interaction with TCF1. ICAT gene and stabilized mutant of β-catenin gene were engineered upstream of the proximal *Lck* promoter to generate ICAT and β-CAT-Tg mice, respectively. (b) ICAT and β-CAT-Tg $CD4^+$ T cells are normally activated upon TCR-stimulation. $CD25^-$ $CD4^+$ T cells were purified from control, ICAT and β-CAT-Tg mice and stimulated with plate bound anti-CD3 plus anti-CD28 followed by surface staining with control antibody, anti-CD25 and anti-CD69. Data are representative of 6 independent plots. (c) ICAT and β-CAT-Tg $CD4^+$ T cells proliferate normally upon TCR-stimulation. $CD25^-$ $CD4^+$ T cells were purified from control, ICAT and β-CAT-Tg mice and stimulated as in (b) and proliferation was assessed by CFSE dilution as described in S1b. Data are representative of 6 independent plots. (d) IL-2 production by activated ICAT and β-CAT-Tg $CD4^+$ T cells. $CD25^-$ $CD4^+$ T cells were purified from control, ICAT and β-CAT-Tg mice and stimulated as in (b) and IL-2 production was assessed by intracellular staining. Data are representative of 4 independent plots.



Supplementary Figure 3. TCF1 and β-catenin cooperatively regulate total *Gata3* expression. Purified $CD25^-$ $CD4^+$ T cells from control (n=6), TCF1-KO (n=2), ICAT (n=6)(a) or β-CAT-Tg mice (n=6) (b) were stimulated by APCs plus anti-CD3. On day 6 after stimulation the amount of total *Gata3* mRNA in stimulated cells was examined by real-time RT-PCR and is shown relative to control cells (control = 100%).


a
Relative expression
300
200
100
0
0 1 2 3 6
Time after stimulation (days)
Tcf1
Lef
b
Medium Stimulated
3 16 3 16
Time (h)
β-Catenin
SP1
1.0 0.7 1.8 0.9
Ratio of β-catenin to SP1
c
LacZ/Actb
12
8
4
0
P = 0.01
Control
Bat-lacZ
Med 3h
Stim 3h
Time after treatment (h)
d
Bambi/Actb
0.8
0.6
0.4
0.2
0
P = 0.01
0h 8h
Time after stimulation (h)

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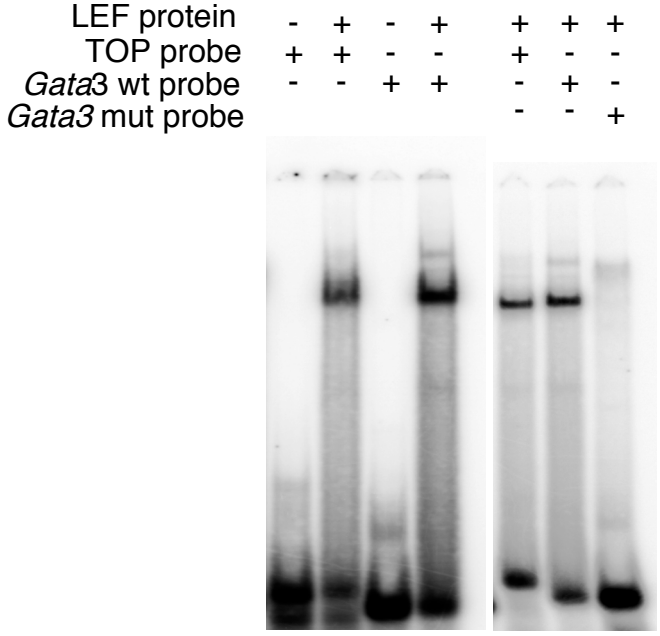
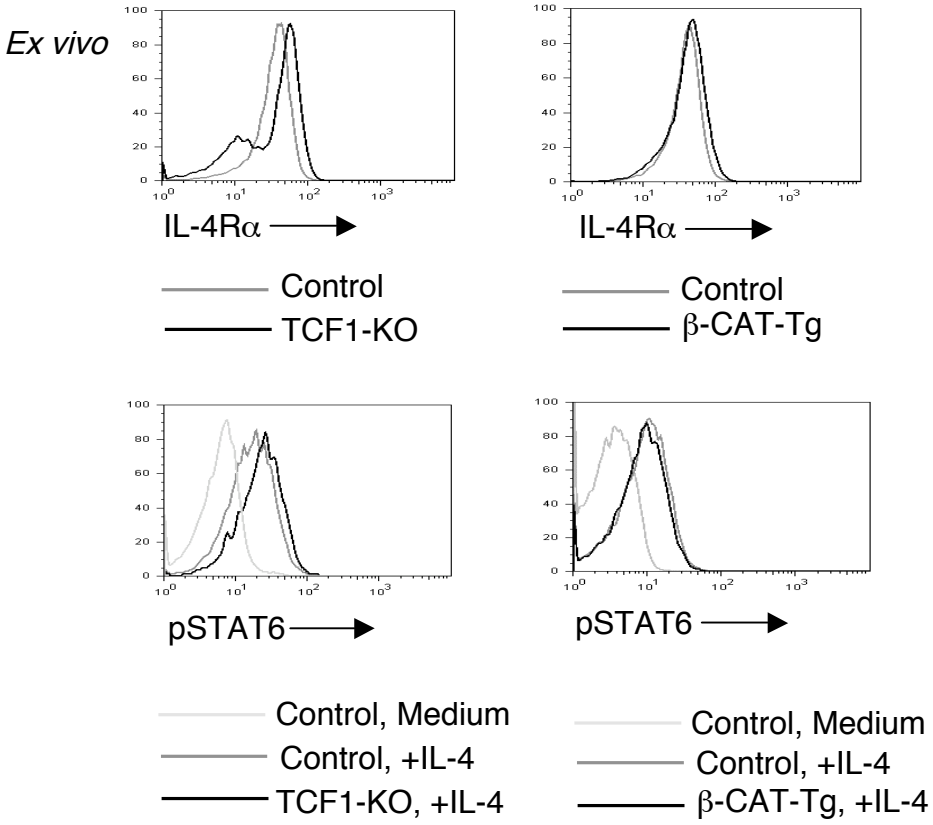
Supplementary Figure 4. TCF1 and β-catenin dependent transcription is active in stimulated $CD4^+$ T cells. (a) *Tcf1* is highly expressed in resting and activated $CD4^+$ T cells. $CD25^-$ $CD4^+$ T cells from C57BL/6 mice were stimulated with anti-CD3 plus anti-CD28 and the amount of mRNA for *Tcf1* and *Lef1* was measured at various time points by real-time RT-PCR. n = 5 independent samples. (b) TCR stimulation increases nuclear β-catenin level. Purified $CD4^+$ T cells from control mice were cultured in medium or stimulated by plate-bound anti-CD3 plus anti-CD28 antibodies for indicated amount of time. Nuclear extracts were made from the cells and analyzed by immunoblot for β-catenin protein. Lack of cytosolic contamination was confirmed by absence of PKCμ protein in the extracts. SP1 was used as a loading control. Ratio of β-catenin to SP1 protein in cells cultured in medium for 3 h is set as 1 and the relative ratio of β-catenin to SP1 proteins under other conditions are shown. Data are representative of 3 independent experiments. (c) TCR stimulation increases TCF1 and β-catenin dependent transcriptional activity. $CD4^+$ T cells from control mice or TCF1-reporter BAT-*LacZ* mice were activated with plate-bound anti-CD3 plus anti-CD28 for indicated amount of time and examined for *LacZ* expression by real-time RT-PCR (n = 3 independent samples). (d) TCR stimulation induces TCF1 and β-catenin target gene expression. TCR-stimulated control $CD4^+$ T cells were assayed for the expression of *Bambi* mRNA (n = 3 independent samples).

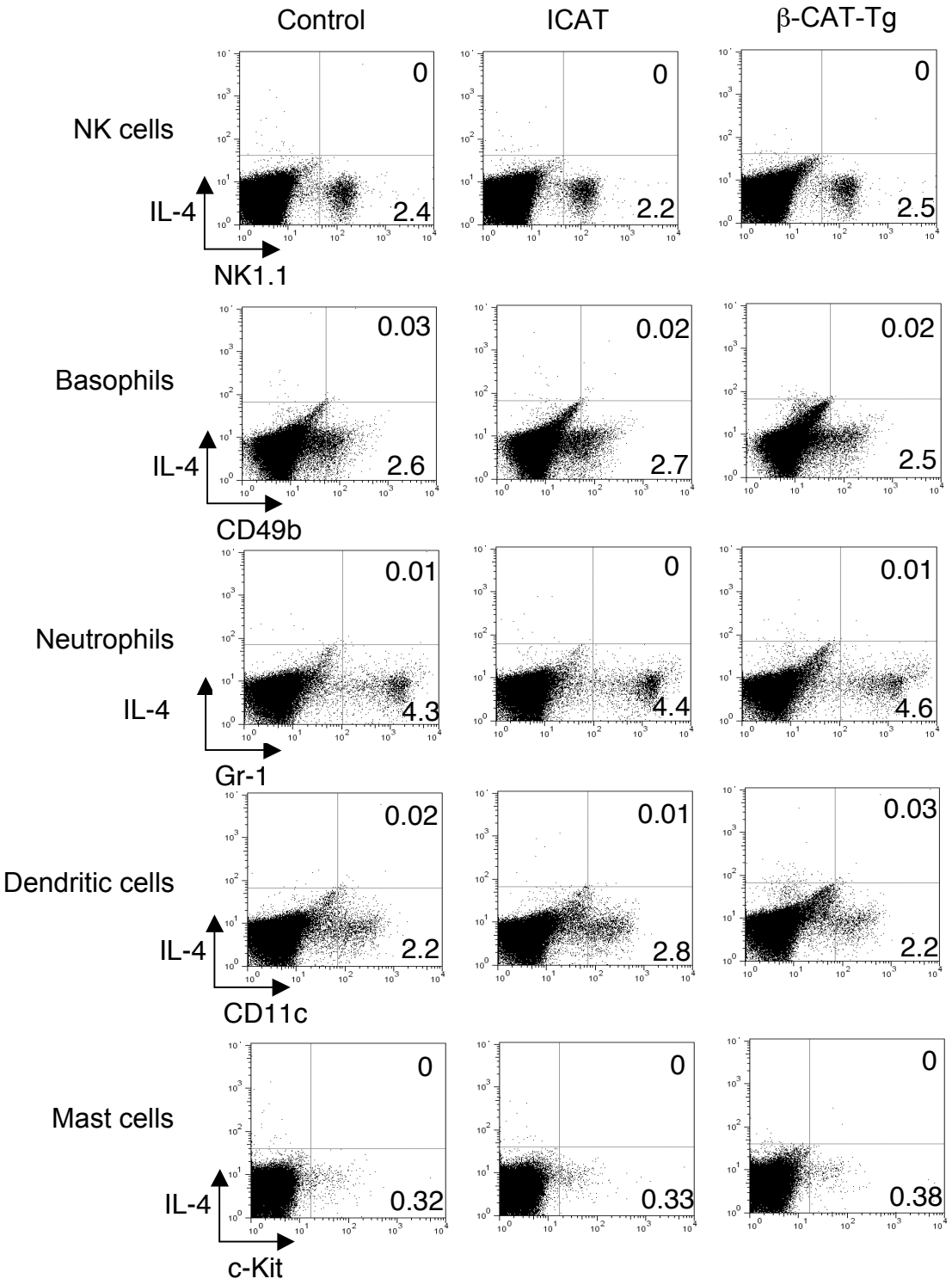


TOP probe: 5'-GATCTAGGGCACCCTTTGAAGCTCT-3'
Gata3 wt probe: 5'-GCGGGCGTCCGAATCAAAGCCCAGGTCCTC-3'
Gata3 mut probe: 5'-GCGGGCGTCCGAATGAAATTCCAGGTCCTC-3'

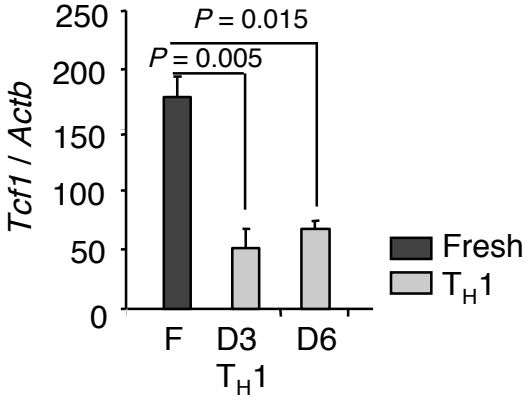
Supplementary Figure 5. Purified LEF protein binds to the TCF1 binding sites upstream of *Gata3* exon-1b. EMSA was performed using purified LEF protein and ^{32}P labeled oligonucleotide probe corresponding to a portion of *Gata3* regulatory region upstream of exon-1b containing TCF1/LEF binding site (probe and mutant probe sequences shown below the gels). Oligonucleotide probe containing the TOP sequence was used as positive control. Data are representative of 2 independent experiments.



Supplementary Figure 6. IL-4 receptor expression and signaling is normal in $CD4^+$ T cells from TCF1-KO and β-CAT-Tg mice. Freshly isolated control, TCF1-KO or β-CAT-Tg $CD25^-$ $CD4^+$ T cells were stained for surface IL-4Rα (upper panels). Purified $CD4^+$ T cells were stimulated with IL-4 for 30 min and phospho-STAT6 (pSTAT-6) level was measured by intracellular staining. Data are representative of 3 independent plots.



Supplementary Figure 7. SEB injection does not induce IL-4 production in non-T cells *in vivo*. SEB was injected and cells were removed and re-stimulated as described in Fig. 4e. *Ex vivo* cells were stained with antibodies to lineage markers followed by intracellular staining for IL-4. Dot plots are gated on TCRβ$^-$ cells. NK cells: NK1.1$^+$ TCRβ$^-$; basophils: CD49b$^+$ TCRβ$^-$; neutrophils: Gr-1$^+$ TCRβ$^-$; dendritic cells: CD11c$^+$ TCRβ$^-$ and mast cells: c-kit$^+$ TCRβ$^-$. Data are representative of 4 – 5 independent plots.



Supplementary Figure 8. *Tcf1* mRNA is down-regulated in differentiated T_H1 cells. *Tcf1* mRNA level was analyzed by real-time RT-PCR in freshly isolated $CD4^+$ T cells and $CD4^+$ T cells cultured under T_H1 skewing conditions for indicated amount of time (n = 4 independent samples).

Supplemenatry Table 1. Primer sequences

Gene name	Forward primer	Reverse primer
Gata3	5'-AGAACCGGCCCCTTATCAA -3'	5'-AGTTCGCGCAGGATGTCC-3'
Gata3-1a	5'-CTGGCTGAGATGCAGTGAAG-3'	5'-CCGCCTTGCTGGAGATCTGA-3'
Gata3-1b	5'-AGCTGTCTGCGAACACTGAG-3'	5'-TTTCTCCTCTCCCTCTCTCA-3'
Actb	5'-TGGATGACGATATCGCTGCG-3'	5'-AGGGTCAGGATACCTCTCTT-3'
Il4	5'-TCATCGGCATTTTGAACGAGG-3'	5'-CTCACTCTCTGTGGTGTTCTT-3'
Il2	5'-TGGAGCAGCTGTTGATGGACC-3'	5'-TGGCCTGCTTGGGCAAGTAA-3'
Lef1	5'-GAGGACATCAAATAAAGTGCC CG-3'	5'-AAAGTGCTCGTCGCTGTAGG TG-3'
Tcf1	5'-CATCAGCCAGAAGCAAGGAG-3'	5'-GGTCAGAGAATAAAATCCAGA GAG-3'
LacZ	5'-TCGGCGGTGAAATTATCGATG-3'	5'-ACCACCGCACGATAGAGAT TC-3'
Bambi	5'-AAACCGGTATCAGCATGACA-3'	5'-TGCACTCCAAGTCCAAGTTT-3'
Ifnγ	5'-GGATGCATTCATGAGTATTGC-3'	5'-CCTTTTCCGCTTCCTGAGG-3'