Supplementary materials

Table SI PCR primers for ChIP assay shown in Fig.3A

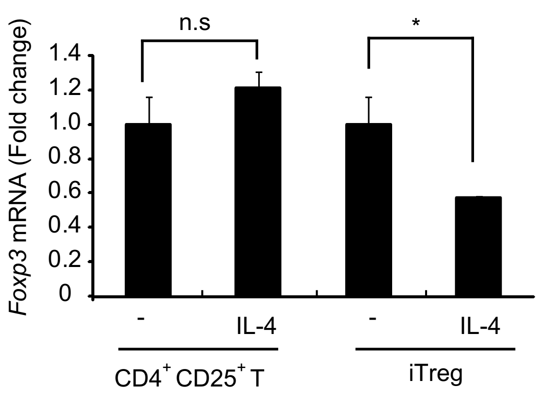
Figure S1 The effects of IL-4 on *Foxp3* levels in $CD4^{+}CD25^{+}$ nTregs and iTregs. Sorted $CD4^{+}CD25^{+}$ nTregs and *in-vitro*-differentiated iTregs were cultured under the Th0 conditions in the presence or absence of IL-4 for 12 h. After incubation, *Foxp3* mRNA levels were determined by real-time PCR and expressed as fold change to untreated cells defined as 1.0. Fold change are shown as the mean ± SD of three experiments. *, $p < 0.05$.

Figure S2 (A) Time course of *Foxp3* mRNA (left) and *GATA3* mRNA (right) induction by anti-TCR Ab in the presence or absence of TGF-β1. Naïve T cells were cultured under the iTreg condition in the presence or absence of IL-4 for the indicated period. *Foxp3* and *GATA3* levels were determined by real-time PCR. Data shows mean ± SD of triplicate samples in one representative experiment out of three experiments. *, $p < 0.01$. (B) IL-4 did not affect TGF-β1-induced Smad2 activation. Naïve T cells were cultured with IL-4 for 15 min and then stimulated with TGF-β1 for indicated periods. The cell lysate was subjected to Western blotting with indicated antibodies.

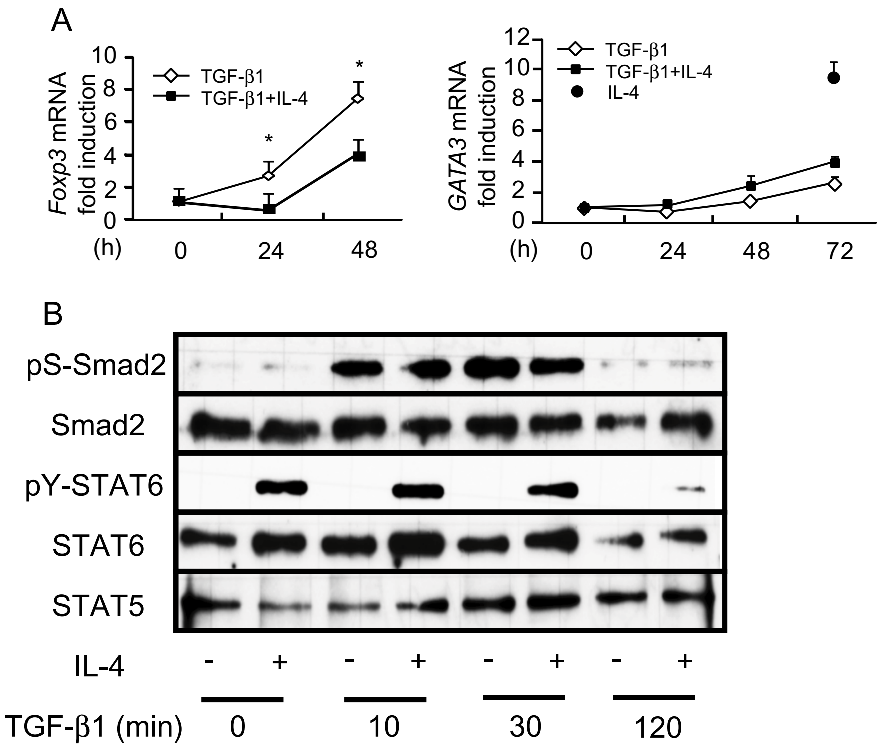
Figure S3 Stably expression of Foxp3 in EL4 cells. The empty MIGR1 plasmid and Foxp3-IRES-GFP-MIGR1 plasmid were transfected into a packaging cell line, 293GPG. EL4 cells were retrovirally transduced with the virus supernatants. Naive T cells were cultured under the Th0 or iTrg conditions for 3 days. Cells were stained with anti-Foxp3 antibody and analyzed by a flow cytyometry.

PCR product	primer sequence (5'-3') Forward	Reverse
1	cta gaa acc atg ctg caa aga c	gta ctc att ttc tca ggg tcc atg g
2	cgc agc tgc cag atc ttg aat ac	cac tcc cgt ttg caa agg ttt agg
3	gcc cca agc aac ctt aaa ctc ttg	ctg tta tag cag cca gca tca cc
4	ggg cac tca gca caa aca tga tg	gag gct tcc ttc tgc tcc aaa c
5	cct ttt acc tct gtg gtg agg g	tat acc gag aag aaa aac cac ggc g
6	gat aat gtg gca gtt tcc cac aag c	ttt ttg ccc ctg tct aag gac caa c
7	gtc tat caa ctg ctg gtc tcc ag	gtg caa aag cag ctg aag gaa gat g
8	gcc ctc tct aca aat tgc ttt tcc a	cat gca gga aga aca tgc atg ctc
9	cca gaa gat ggt gtg gga tct c	cag tga gtt tta ggc cag tca gg
10	cag gct gac ctc aaa ctc aca aag	cat acc cac act ttt gac ctc tgc
11	gtg ggc tat cta cgc agt cac tt	gag aca gtg aga gca gtt tag agg
12	ctc cat aag att tac ccc agc cac	cat gct atg gtt atg gac tgg atc c
13	ctc ttg tcc ctg tata ctg gaa gaa tg	cag agg att gga aaa ccc tct act g
14	ctc tgt cat aat gga gct cag gac	ggt tgg atg ctt ggt gag tct tag g
15	caa tat cca tga ggc ctg cct aat ac	ctt ggc cag att ttt ctg cca ttg ac
16	cta ctc acc aaa cct gat ccg cat	ggt att aag act agt gtg tca cgg g
17	tct ctc aac tct gat aag ccc cag	ggg ttt ctt gta gtc tgg aac tcc
18	gtc cca ggc cat gag aag act a	gga tag ggt tgc ttg att gag gg
19	cca cgc caa ttc caa gac aga ag	tga ctg tct tcc aag tct cgt ctg

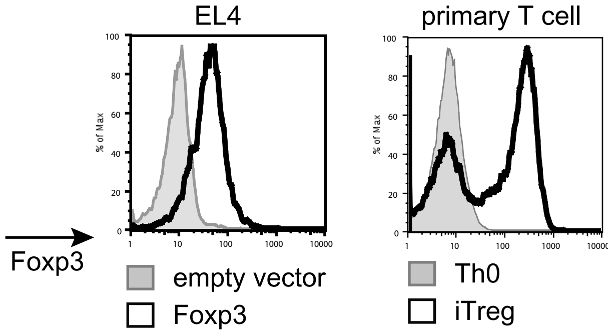
Takaki et. al., Table SI



Takaki et. al., Supplementary Figure 1



Takaki et. al., Supplementary Figure 2



Takaki et. al., Supplementary Figure 3