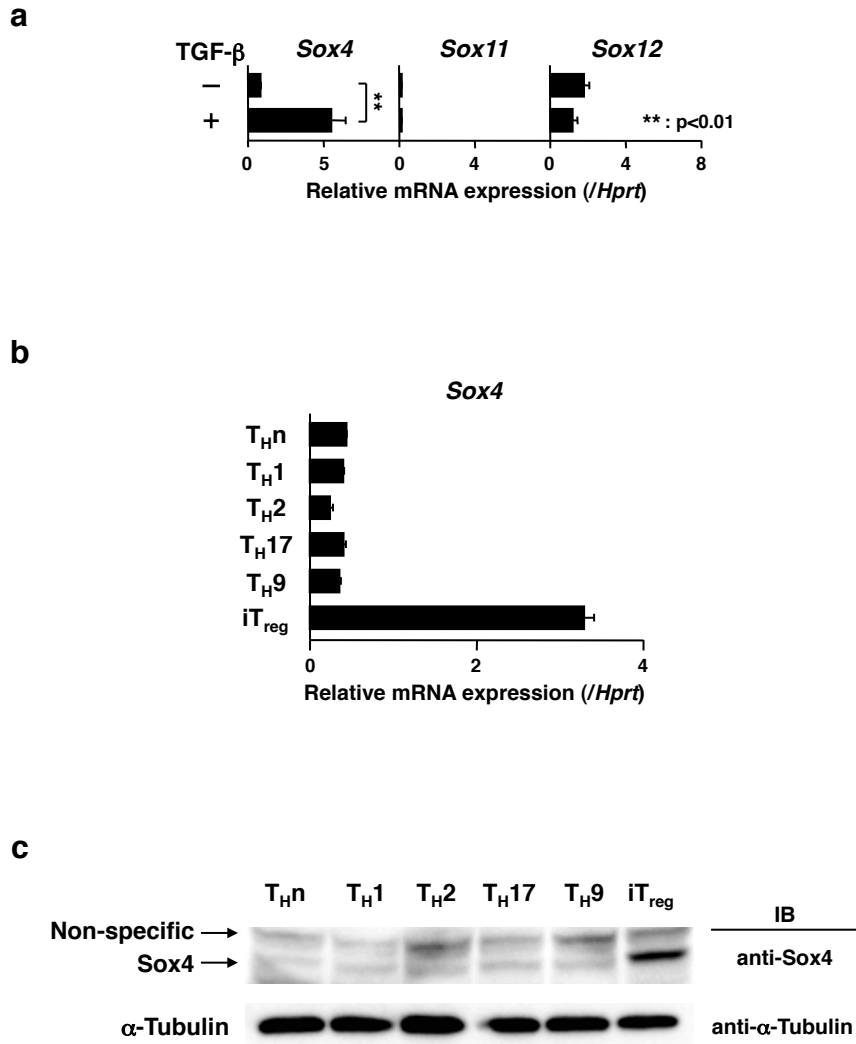


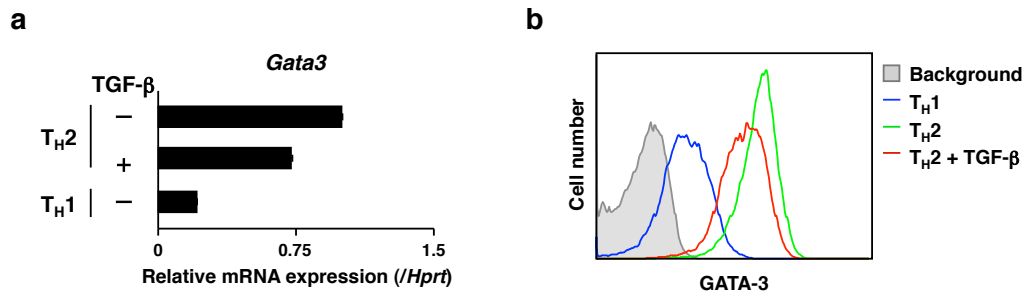
## Supplementary Information

### **Sox4 is a downstream target of TGF- $\beta$ signaling and suppresses GATA3-induced T<sub>H</sub>2 cell differentiation**

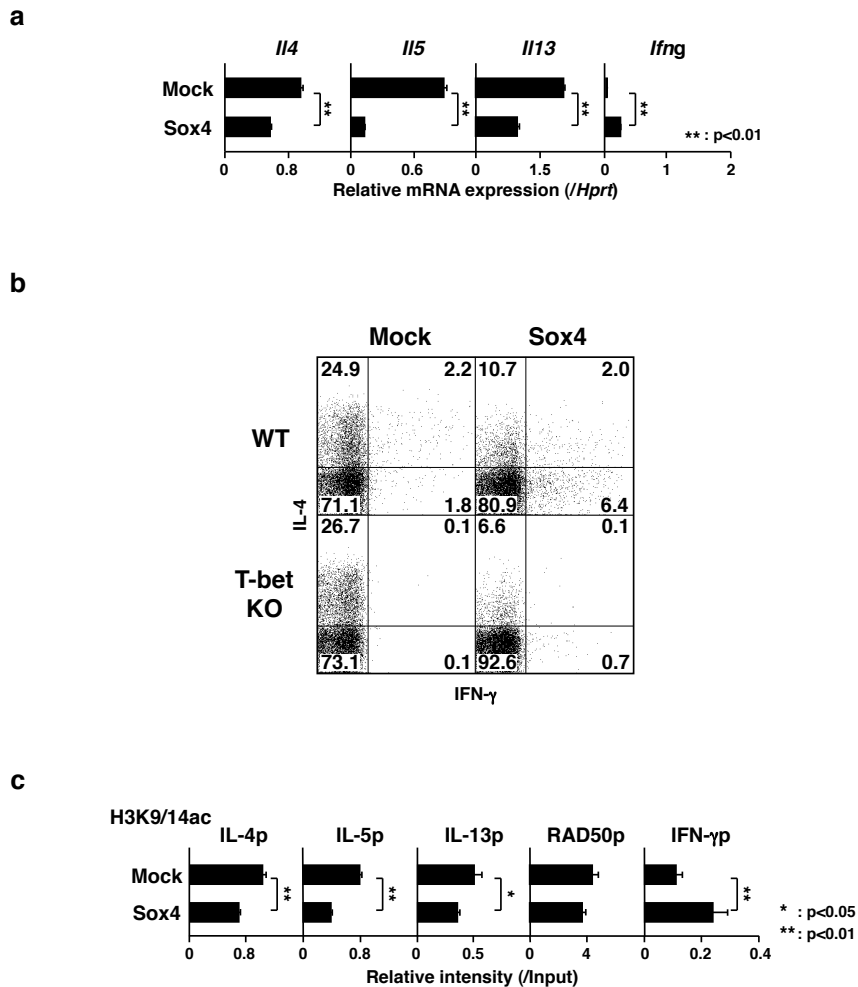
Makoto Kuwahara, Masakatsu Yamashita, Kenta Shinoda, Soichi Tofukuji, Atsushi Onodera, Ryo Shinnakasu, Shinichiro Motohashi, Hiroyuki Hosokawa, Damon Tumes, Chiaki Iwamura, Veronique Lefebvre and Toshinori Nakayama



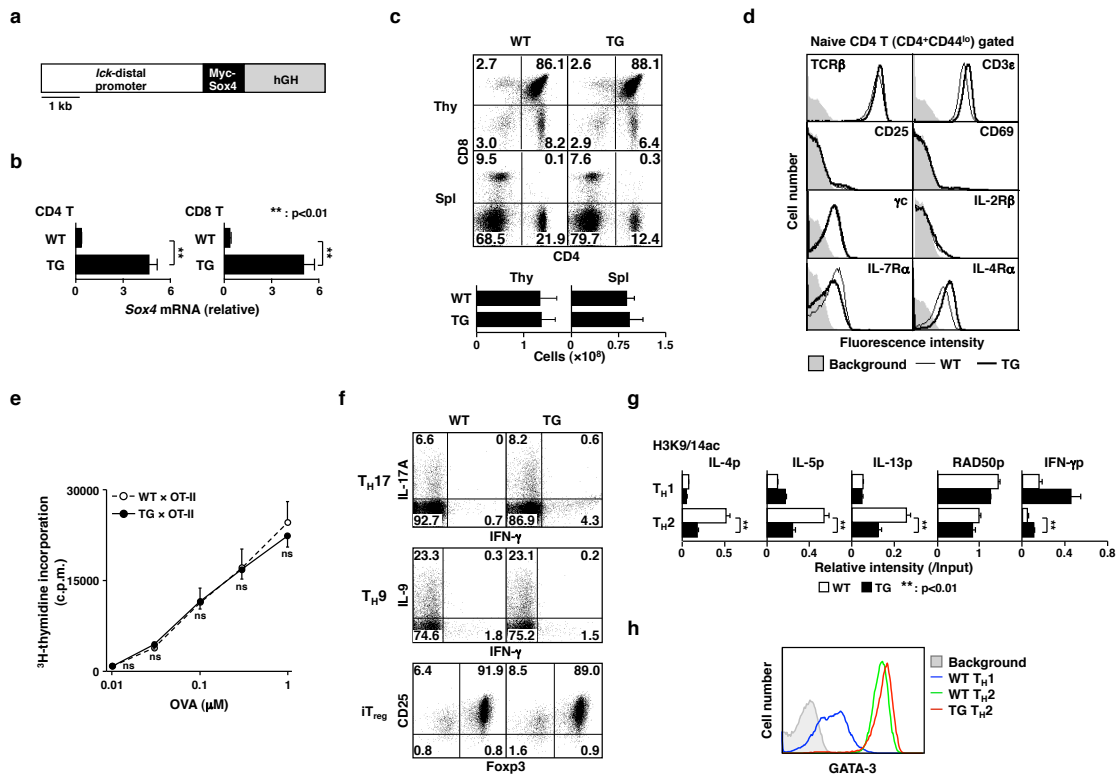
**Supplementary Figure 1. Sox4 expression in  $T_H$  subsets. (a),** Quantitative RT-PCR of *Sox11*, *Sox12* and *Hprt* mRNA expression in effector  $CD4^+$  T cells 5 days after initial stimulation. The relative intensity (/*Hprt*), the mean of three samples, is shown with standard deviation. *Sox4* mRNA (b) and protein (c) expression in *in vitro* differentiated  $T_H$  cells.



**Supplementary Figure 2. GATA-3 expression in TGF- $\beta$ -treated  $T_H2$  cells. *Gata3* mRNA (a) and protein (b) expression in TGF- $\beta$ -treated developing  $T_H2$  cells.**

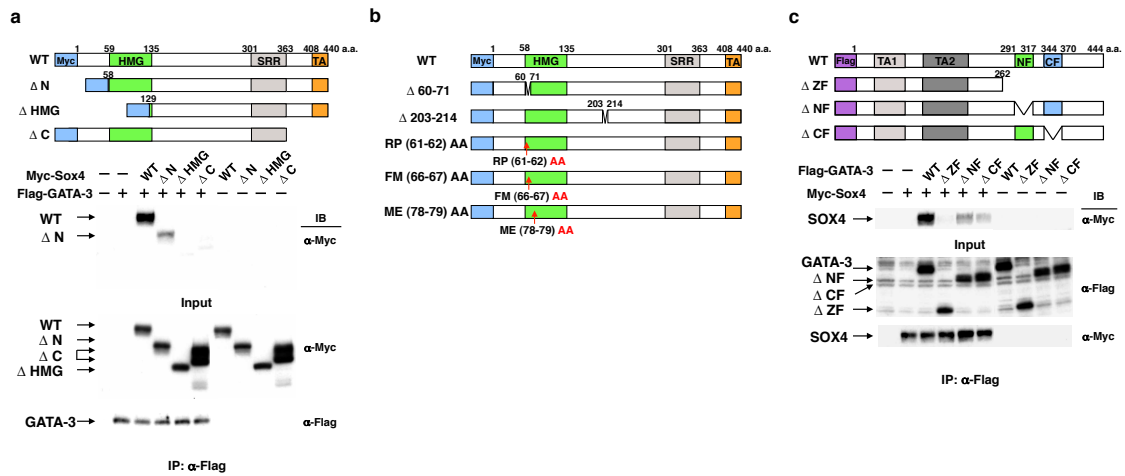


**Supplementary Figure 3. Effect of Sox4 introduction into developing T<sub>H</sub>2 cells.** (a), Quantitative RT-PCR of indicated cytokine mRNA in Sox4-transduced T<sub>H</sub>2 cells. (b), Effect of T-bet-deficiency on Sox4-mediated inhibition of T<sub>H</sub>2 cell differentiation. IFN $\gamma$ /IL-4 staining profiles of Sox4-introduced cells are shown. The percentages of cells in each quadrant are indicated. (c), The levels of acetylation of H3-K9/14 at the IL-4, IL-5, RAD50 and IFN $\gamma$  promoter regions in Sox4-transduced T<sub>H</sub>2 cells were determined by ChIP assay followed by quantitative PCR.

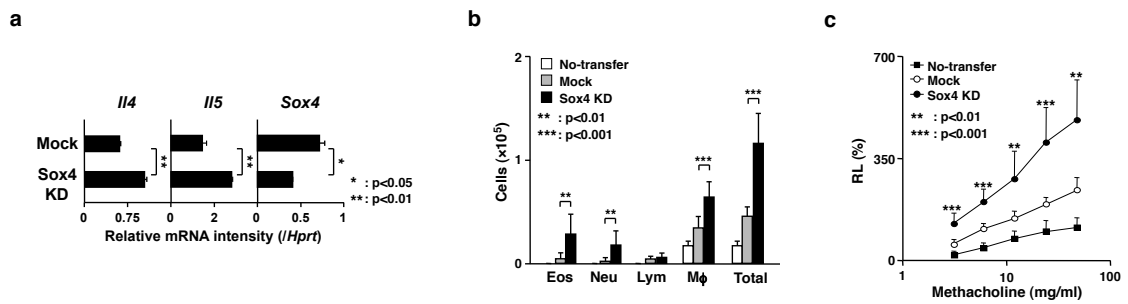


**Supplementary Figure 4. Phenotypic characterization of Sox4 TG CD4<sup>+</sup> T cells.** (a), Schematic representations of the construct used for the generation of T cell-specific Sox4 TG mice. The expression of Sox is controlled by the *lck*-distal promoter. (b), *Sox4* mRNA levels in naïve CD4/CD8 T cells from Sox4 TG mice were determined by quantitative RT-PCR. The relative intensity (*/Hprt*) is shown with standard deviation. WT; wild-type, TG; Sox4 TG. (c), Representative CD4/CD8 profiles of thymocytes and splenocytes of Sox4 TG mice are shown (upper panel). The number of thymocytes and splenocytes in Sox4 TG mice are also shown with standard deviation (n=11; lower panel). (d), The phenotypic features of splenic CD4<sup>+</sup> T cells from Sox4 TG mice. (e), Antigenic OVA peptide-induced proliferative responses of Sox4 x OT-II (OVA-specific TCRαβ TG mice) double-TG naïve CD4<sup>+</sup> T cells in the presence of irradiated C57BL/6 APCs (Thy1.2-negative splenocytes). The mean values of <sup>3</sup>H-thymidine incorporation in each culture 2 days after the initial stimulation are shown with standard deviation. No significant difference (n.s.) was noted. (f), T<sub>H</sub>17, T<sub>H</sub>9 and iT<sub>reg</sub> cell differentiation in Sox4 TG

naïve CD4<sup>+</sup> T cells. The IFN $\gamma$ /IL-17, IFN $\gamma$ /IL-9 or Foxp3/CD25 staining profiles were determined by intracellular staining. The percentages of cells in each quadrant are indicated. (g), The levels of histone H3-K9/14 acetylation at the IL-4, IL-5, RAD50 and IFN $\gamma$  promoter regions in Sox4 TG T<sub>H</sub>2 cells were determined by ChIP assay followed by quantitative PCR. (h), Intracellular staining of GATA-3 protein in Sox4 TG T<sub>H</sub>2 cells.

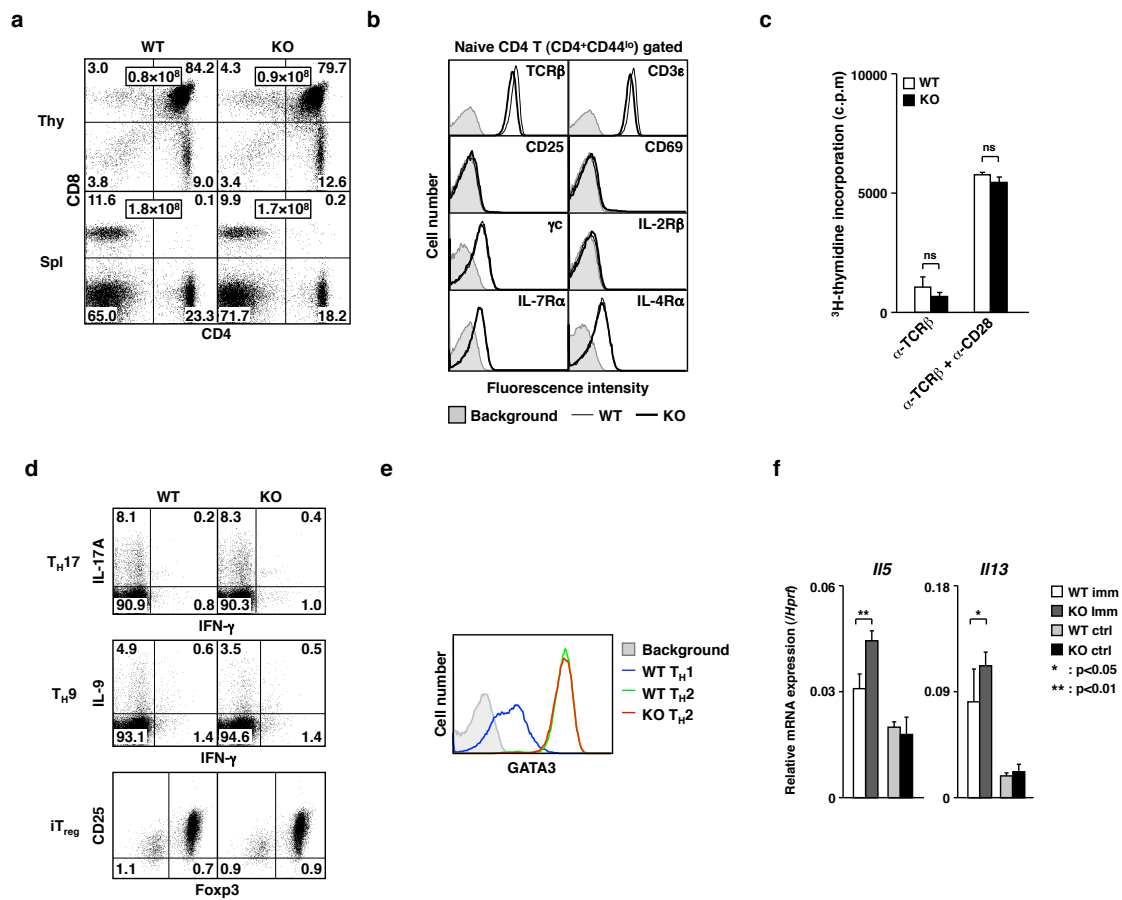


**Supplementary Figure 5. Generation and association of Sox4 and GATA-3 mutants.** (a), Association of Myc-tagged mutants Sox4 with the Flag-tagged GATA-3 in transfected 293 T cells. Schematic representation of Myc-tagged Sox4 mutants are indicated (upper panel). (b), Schematic representation of Myc-tagged Sox4 HMG box mutants. (c), Association of Flag-tagged mutants GATA-3 with the Myc-tagged Sox4 in transfected 293 T cells. Schematic representation of Flag-tagged GATA-3 mutants are indicated (upper panel). IP: immunoprecipitation, IB: immunoblotting.



**Supplementary Figure 6. Airway inflammation in mice transferred with Sox4 knockdown (Sox4 KD) T<sub>H</sub>2 cells. (a),** Quantitative PT-PCR of *Il4*, *Il5* and *Il13* mRNA expression in Sox4-knockdown T<sub>H</sub>2 cells. **(b),** Enhanced infiltration of eosinophils in the BAL fluid in mice transferred with Sox4 KD T<sub>H</sub>2 cells compared to control T<sub>H</sub>2 cells following inhalation with OVA. The absolute cell numbers of infiltrated cells are shown. **(c),** Airway hyperresponsiveness of challenged mice transferred with Sox4 KD T<sub>H</sub>2 cells. The results are presented as airway resistance (RL). The mean values (5 mice per group) are shown with standard deviation. \*\*p<0.01, \*\*\*p<0.001 by ANOVA and Bonferroni-test.





**Supplementary Figure 7. Phenotypic characterization of Sox4-deficient (*Sox4*<sup>fl/fl</sup> x CD4-Cre TG) CD4<sup>+</sup> T cells. (a), Representative CD4/CD8 profiles of thymocytes and splenocytes of Sox4-deficient mice. (b), The phenotypic features of splenic CD4<sup>+</sup>CD44<sup>low</sup> T cells from Sox4-deficient mice. WT; wild-type, KO; Sox4-deficient. (c), Proliferative responses of Sox4-deficient naïve CD4<sup>+</sup> T cells stimulated with a combination of immobilized anti-TCR $\beta$  mAb and soluble anti-CD28 mAb. The mean values of <sup>3</sup>H-thymidine incorporation in each culture are shown with standard deviation. No significant difference (n.s.) was noted. (d), T<sub>H</sub>17, T<sub>H</sub>9 and iT<sub>reg</sub> cell differentiation in Sox4-deficient naïve CD4<sup>+</sup> T cells. Intracellular staining profiles of the IFN $\gamma$ /IL-17, IFN $\gamma$ /IL-9 or Fc $\gamma$ 3/CD25 are shown. The percentages of cells in each quadrant are indicated. (e), Intracellular staining of GATA-3 protein in Sox4-deficient T<sub>H</sub>2 cells. (f), Quantitative RT-PCR of *Il5* and**

//13 mRNA in BAL fluid cells from OVA-immunized Sox4-deficient mice.