Supplemental Data

An Essential Role of the Forkhead-Box Transcription Factor Foxo1 in Control of T Cell Homeostasis and Tolerance

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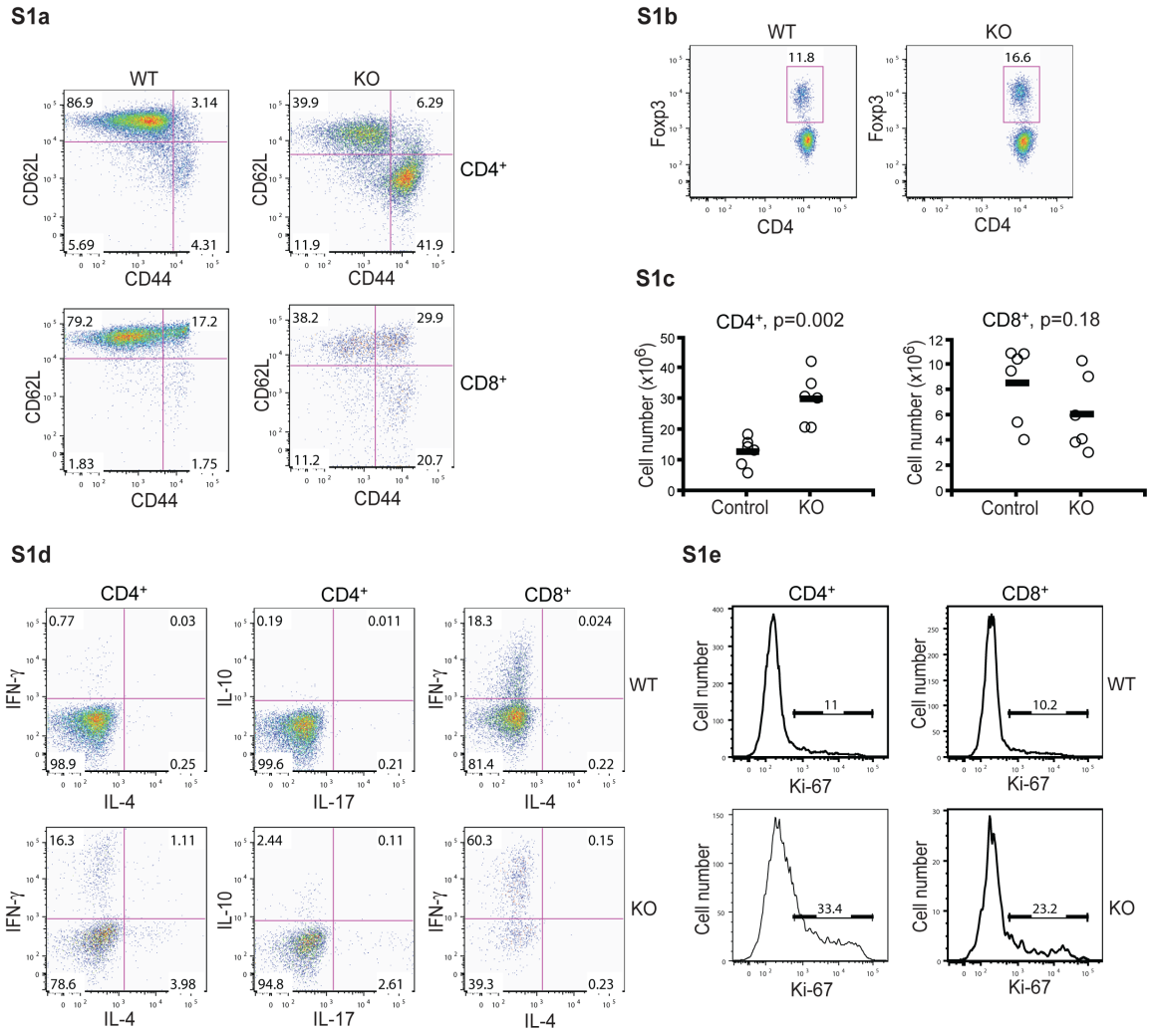

Figure S1. Analysis of Foxo1-deficient T Cells in the Mesenteric Lymph Nodes

(**a**) Expression of CD44 and CD62L in $CD4^+$ and $CD8^+$ T cells from the mesenteric lymph nodes (mLNs) of Foxo1/Foxo1 (wild-type, WT) and CD4Cre-Foxo1/Foxo1 (knockout, KO) mice at 6 weeks old. These are representative results of six independent experiments.

(**b**) Percentage of mLN $CD4^+Foxp3^+$ regulatory T (Treg) cells from WT and KO mice at 6 weeks old.

(**c**) Number of mLN $CD4^+$ and $CD8^+$ T cells from control (Foxo1/Foxo1) and KO mice aged between 6 and 8 weeks (n=6). The p values between the cell number of the two groups of mice are shown.

(**d**) $CD4^+$ and $CD8^+$ T cells isolated from the mLNs of WT and KO mice were stimulated with PMA and ionomycin for 4 hr and analyzed for the expression of IFN-γ, IL-4, IL-10, and IL-17 by intracellular cytokine staining. These are representative results of two independent experiments.

(**e**) Expression of Ki-67 in $CD4^+$ and $CD8^+$ T cells from the mLNs of WT and KO mice aged between 6 and 8 weeks.

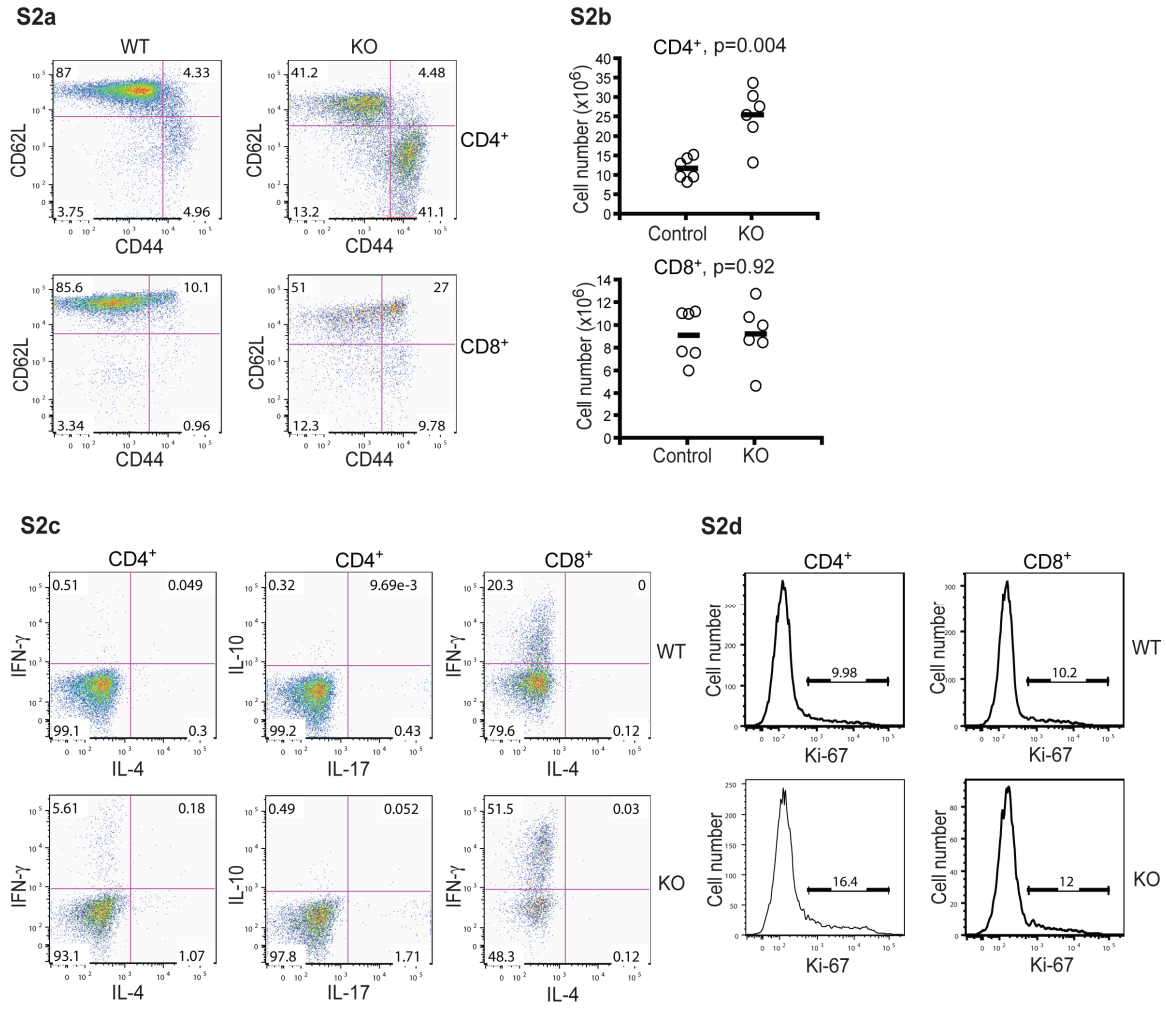

Figure S2. Analysis of Foxo1-deficient T Cells in the Peripheral Lymph Nodes

(**a**) Expression of CD44 and CD62L in $CD4^+$ and $CD8^+$ T cells from the peripheral lymph nodes (pLNs) of Foxo1/Foxo1 (wild-type, WT) and CD4Cre-Foxo1/Foxo1 (knockout, KO) mice at 6 weeks old. These are representative results of six independent experiments.

(**b**) Number of pLN $CD4^+$ and $CD8^+$ T cells from control (Foxo1/Foxo1) and KO mice aged between 6 and 8 weeks (n=6). The p values between the cell number of the two groups of mice are shown.

(**c**) $CD4^+$ and $CD8^+$ T cells isolated from the pLNs of WT and KO mice were stimulated with PMA and ionomycin for 4 hr and analyzed for the expression of IFN-γ, IL-4, IL-10, and IL-17 by intracellular cytokine staining. These are representative results of two independent experiments.

(**d**) Expression of Ki-67 in $CD4^+$ and $CD8^+$ T cells from the pLNs of WT and KO mice aged between 6 and 8 weeks.

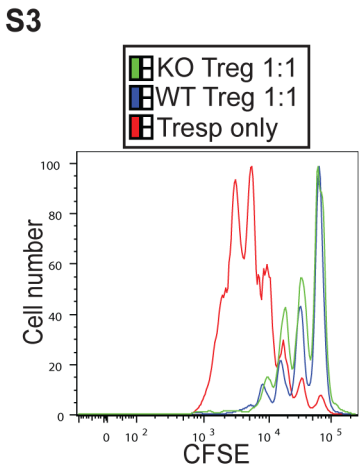

Figure S3. Suppressive Function of Foxo1-deficient Treg Cells

$CD4^+Foxp3^+$ regulatory T cells (Treg) were isolated from Foxo1/Foxo1 (wild-type, WT) and CD4Cre-Foxo1/Foxo1 (knockout, KO) mice. The $CD44^{lo}CD4^+$ cells from WT mice were labeled with CFSE and used as responder T cells (Tresp). The suppression was shown by comparison of CFSE dilution in the Tresp cells cultured with or without 1:1 Treg cells.

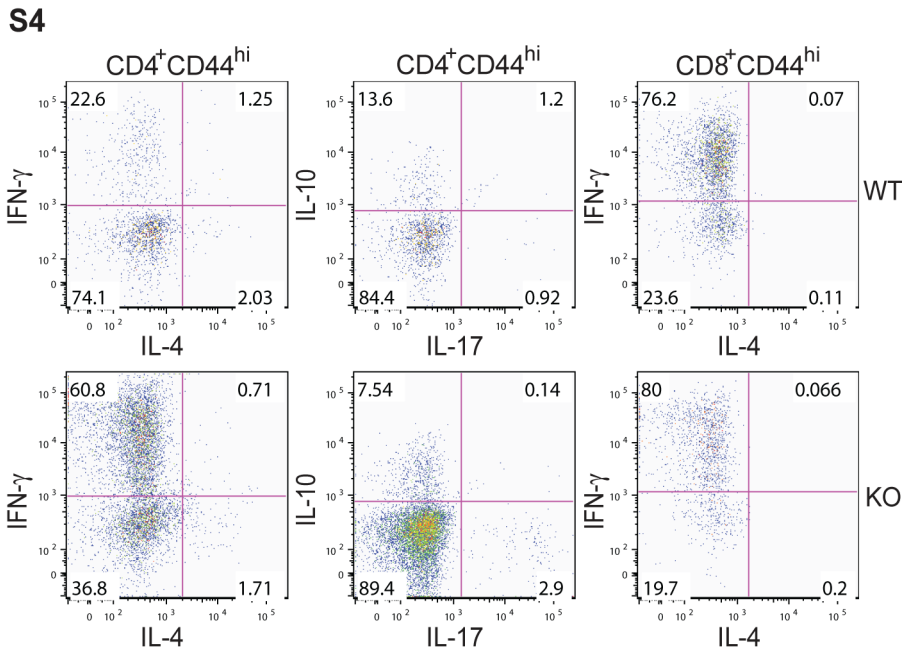

Figure S4. Cytokine Production by CD44^{hi} T cells

T cells isolated from the spleens of Foxo1/Foxo1 (wild-type, WT) and CD4Cre-Foxo1/Foxo1 (knockout, KO) mice were stimulated with PMA and ionomycin for 4 hr and analyzed for the expression of IFN-γ, IL-4, IL-10, and IL-17 in CD44^{hi}CD4^{+} and CD44^{hi}CD8^{+} T cells. These are representative results of two independent experiments.

S5

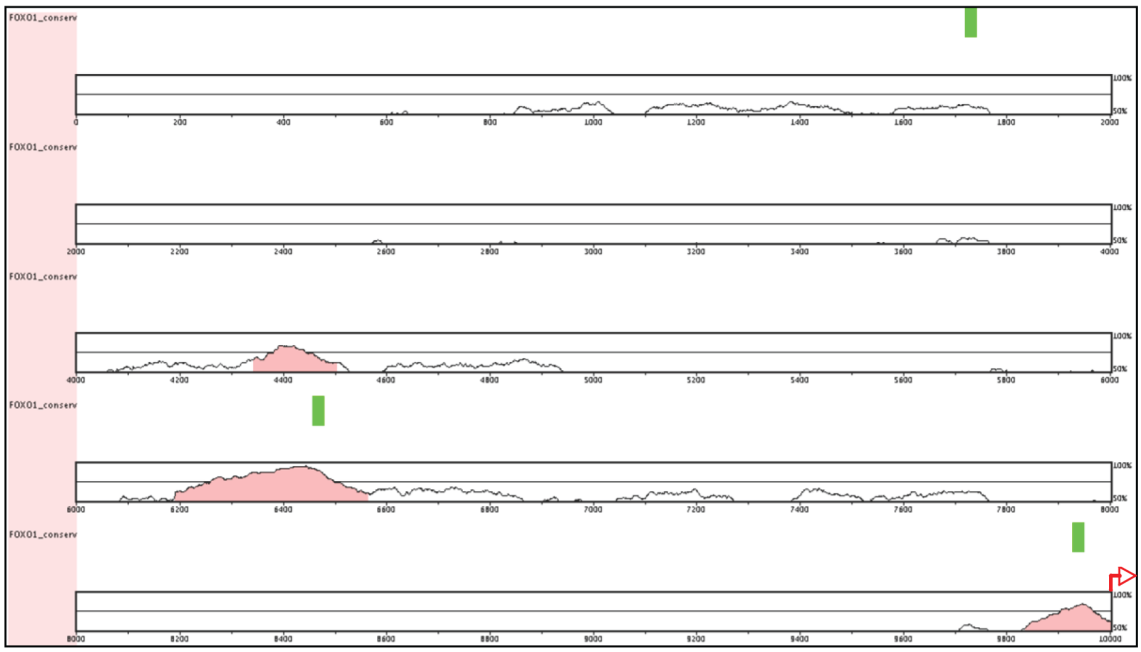

Figure S5. Potential Foxo1-Binding Sites in the Promoter Region of *Il7r* Gene

The conserved putative Foxo1-binding sites in the *Il7r* locus were analyzed by rVISTA online (http://genome.lbl.gov/vista/rvista/submit.shtml). Ten kilobase pairs (10 kb) of nucleotides upstream of transcription start site (indicated by red arrow) from mouse and human *Il7r* genes were used for rVISTA analysis. Three conserved non-coding sequences (CNSs, marked by pink color), and three conserved Foxo1 binding sites (shown as green bars) were depicted.

S6

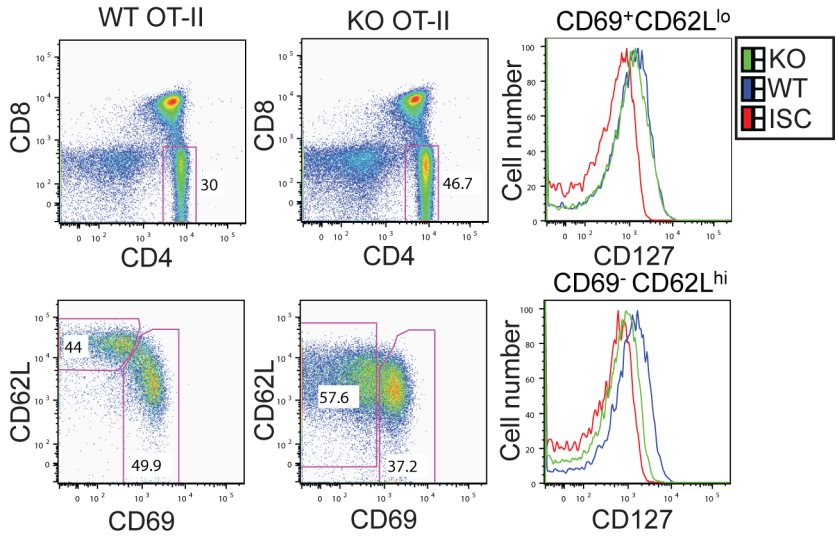

Figure S6. Development of Foxo1-deficient OT-II T cells

Thymic CD4 and CD8 profile of Foxo1/Foxo1 (wild-type, WT) and CD4Cre-Foxo1/Foxo1 (knockout, KO) OT-II mice at 8 weeks old (top left). Expression of CD69 and CD62L in $CD4^+$ OT-II thymocytes from WT and KO mice (bottom left). Expression of CD127 (IL-7R) in immature ($CD69^+CD62L^{lo}$) and mature ($CD69^-CD62L^{hi}$) $CD4^+$ OT-II thymocytes from WT and KO mice. ISC stands for the iso-type control antibody. These are representative results of four mice per group analyzed.

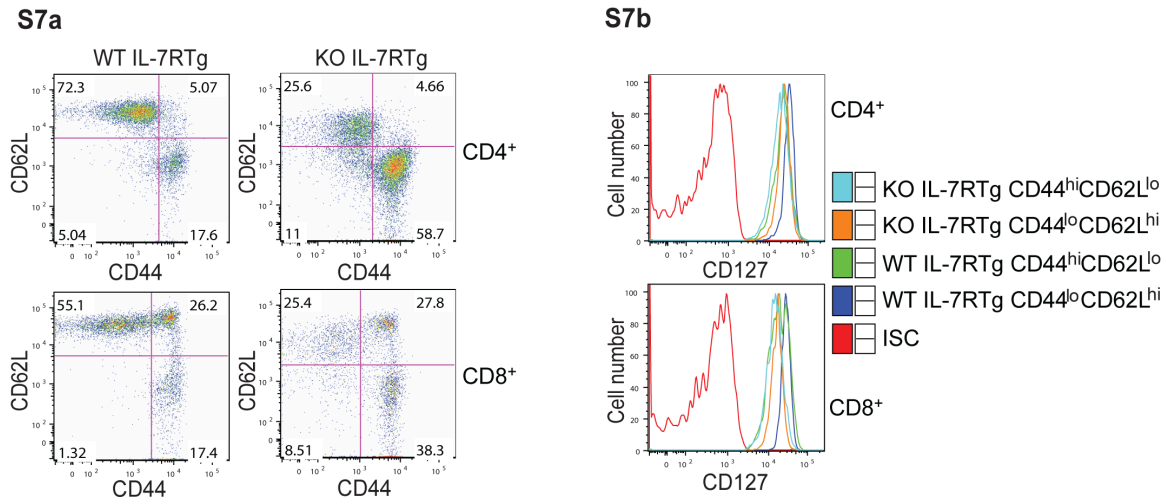

Figure S7. Expression of CD44, CD62L, and CD127 in T cells on IL-7R Transgenic Background

(a) $CD4^+$ and $CD8^+$ T cells from the spleens of Foxo1/Foxo1 (wild-type, WT) IL-7RTg and CD4Cre-Foxo1/Foxo1 (knockout, KO) IL-7RTg mice at 8 weeks old were stained with CD44 and CD62L antibodies and analyzed by flow cytometry.

(b) Expression of CD127 in splenic $CD44^{lo}CD62L^{hi}$ and $CD44^{hi}CD62L^{lo}$ T cells from WT IL-7RTg and KO IL-7RTg mice. These are representative results of two independent experiments.