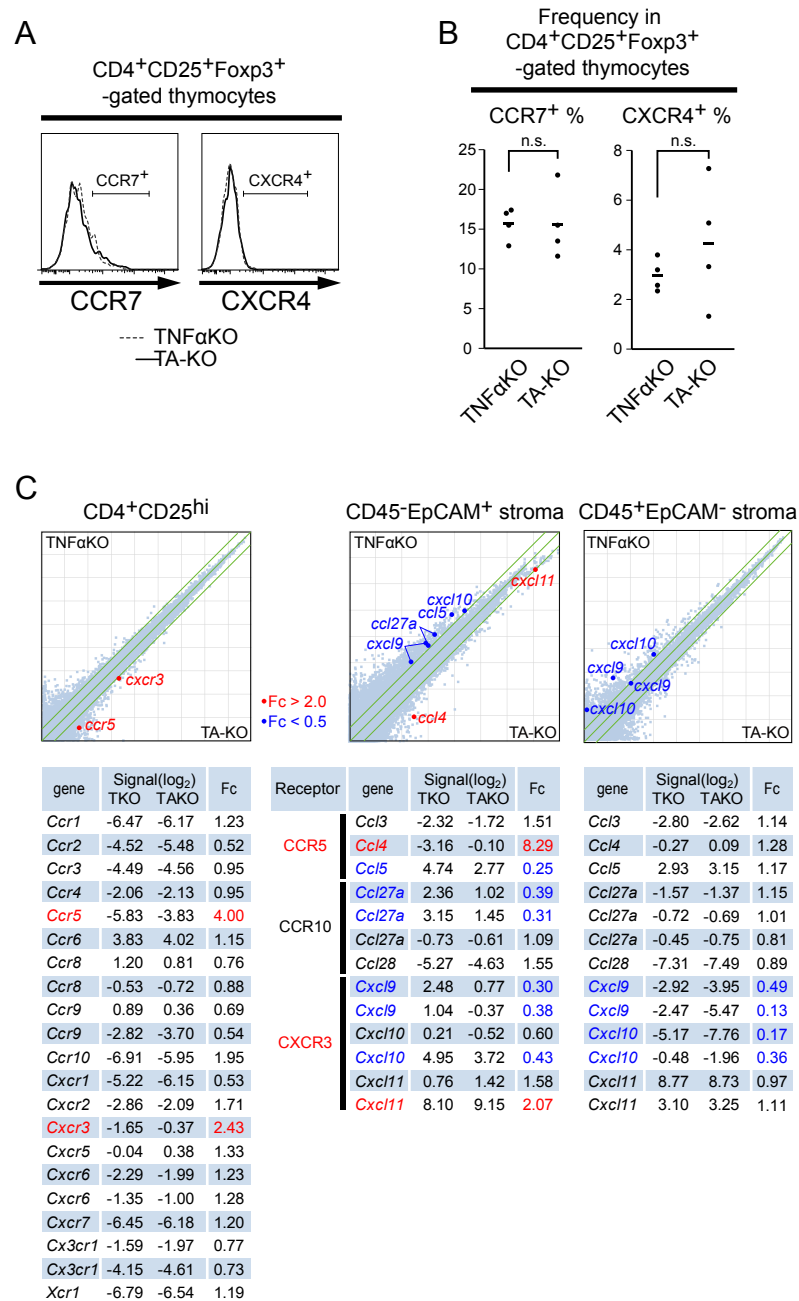


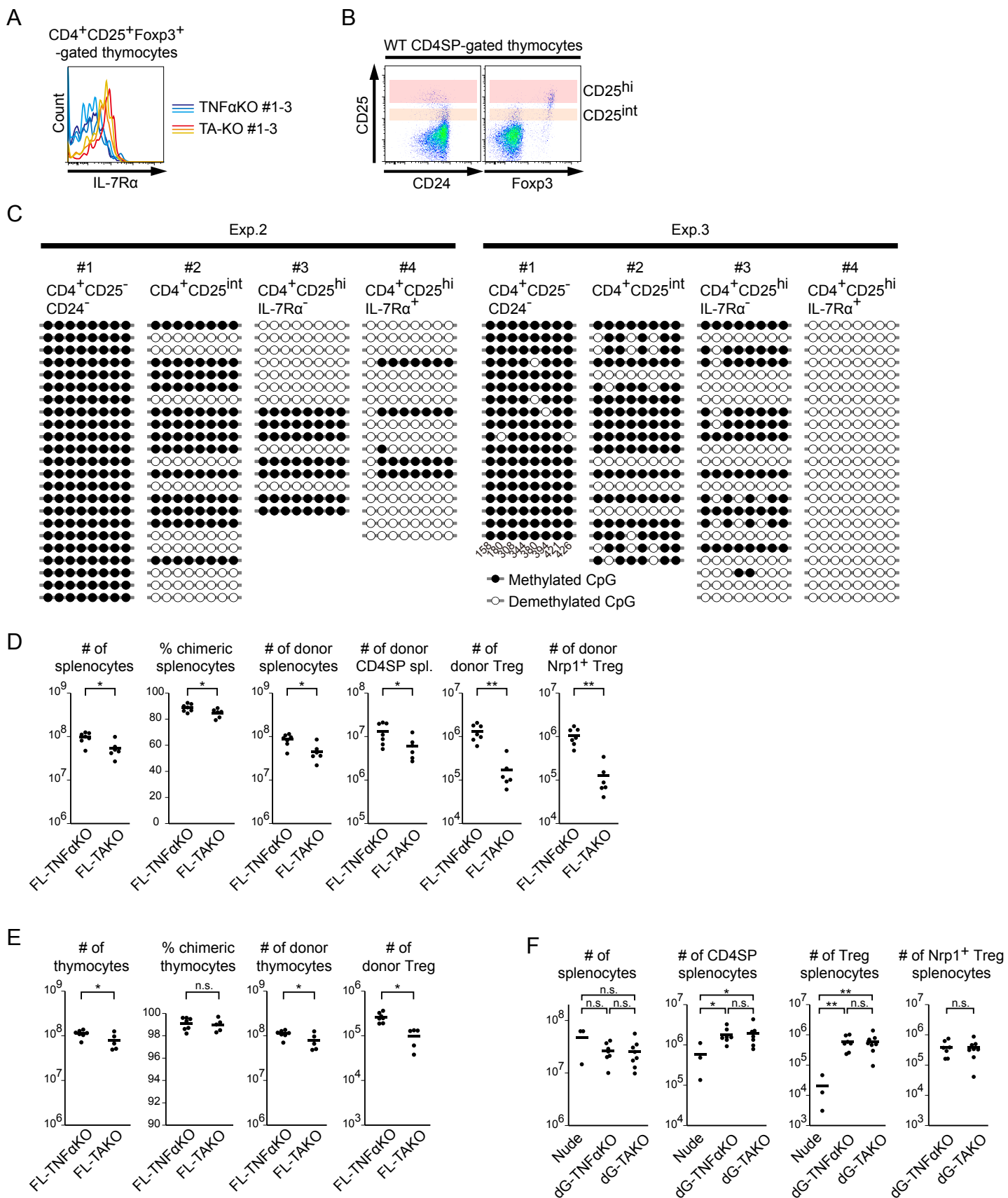
Supplemental Fig. 1.

(A) Absolute population cell numbers in WT, TNF α -KO, and TA-KO mice. #, number; thy., thymocytes; spl., splenocytes. (B) Representative histogram of Nrp1 expression in CD4⁺CD25⁺Foxp3⁺-gated thymocytes (left), and aggregate data for Nrp1⁺ frequencies in CD4⁺CD25⁺Foxp3⁺-gated thymocytes (right, n = 4). (C) Absolute population cell numbers in Rag1 null mice that underwent TNF α -KO or TA-KO thymus transplantation (n = 3). (D) Intact thymi of TNF α -KO or TA-KO mice were transplanted under the kidney capsules of BALB/c nude mice (TpTKO and TpTAKO, respectively), and 1 or 2 months after the transplant splenocytes were harvested from the recipients and analyzed (n = 2). Left four panels show plots of H-2K^b⁺ (donor thymus-derived) CD4SP-gated splenocytes. TpTKO mice had a definitive donor-derived CD25⁺Foxp3⁺ population, whereas TpTAKO mice showed no, or only a trace, population. Right four panels show plots of H-2K^b⁻ CD4SP-gated splenocytes. (The H-2K^b⁻ cells are host-derived cells that mature through the transplanted thymus.) The host-derived CD4⁺CD25⁺Foxp3⁺ population of TpTAKO mice was smaller than that of TpTKO mice. (E) Histograms of CD24, CD62L, and CD69 expression gated on CD4⁺CD25⁺Foxp3⁺ thymocytes from three TNF α -KO (navy, blue, and aquamarine lines) and three TA-KO (red, orange, and yellow lines) mice in the same experiment. (F) Additional results of the methylation analysis shown in Fig. 2C. (G) Expression analysis of *s1pr1* (left) and *klf2* (right) transcripts determined by quantitative real-time polymerase chain reaction (qRT-PCR). Thymocytes from 1.5-week-old WT, TNF α -KO, or TA-KO mice were sorted into CD4⁺CD8 α ⁺ (DP), CD4⁺CD8 α ⁻CD25^{hi}, and CD4⁺CD8 α ⁻CD25^{int} populations and qRT-PCR was performed. Transcript levels are shown as values normalized relative to *hypoxanthine phosphoribosyltransferase* transcript levels. Data are representative of two independent experiments. (H) Representative histogram of S1PR1 expression in CD4⁺CD25⁺Foxp3⁺-gated (Treg) and CD4⁺CD8⁺-gated (DP) thymocytes of TNF α -KO and TA-KO mice. (I) Representative plots of S1PR1 expression in CD4SP-gated thymocytes of TNF α -KO and TA-KO mice. n.s., not significant; **P* < 0.05, ***P* < 0.01, ****P* < 0.001.



Supplemental Fig. 2.

(A) Representative histograms of CCR7 and CXCR4 expression in CD4⁺CD25⁺Foxp3⁺-gated thymocytes. (B) Aggregate data for CCR7⁺ or CXCR4⁺ frequencies in CD4⁺CD25⁺Foxp3⁺-gated thymocytes (n = 4). (C) (Top) Gene expression profiles of sorted CD4⁺CD25^{hi} thymocytes (left), TECs (center), and CD45⁺EpCAM⁻ thymic stromal cells (right) from TNFα-KO or TA-KO thymi. Green lines indicate 2-fold change. (Bottom) Logarithmically transformed signal intensities of transcripts. Fc, fold change of linearized signal intensity between TA-KO and TNFα-KO. n.s., not significant.



Supplemental Fig. 3.

(A) Histograms of IL-7Rα expression gated on CD4⁺CD25⁺Foxp3⁺ thymocytes from three TNFα-KO (navy, blue, and aquamarine lines) and three TA-KO (red, orange, and yellow lines) mice in the same experiment. (B) Gating criteria of CD25^{int} and CD25^{hi} populations. Plots are gated on CD4SP thymocytes from WT mouse. CD4SP CD25^{int} cells are immature and express high level of CD24 (shaded orange). As the cells upregulate CD25 expression and gain Foxp3 expression, their expressions of CD24 diverge into low to high levels (shaded red). We utilized these CD24 expression profiles for gating CD25^{int} and CD25^{hi} populations. Under this condition, most cells in the CD4SP CD25^{hi} population expressed Foxp3. (C) Additional results of the methylation analysis shown in Fig. 4D. (D) Absolute population cell numbers in the spleens of FL-TNFαKO or FL-TAKO mice (n = 6 or 7). Chimerism refers to the rate of donor-derived cells in the whole splenocyte population; spl., splenocytes. (E) Absolute cell numbers of each population in the thymi of FL-TNFαKO or FL-TAKO mice (n = 6 or 7). Chimerism refers to the rate of donor-derived cells in the whole thymocyte population. (F) Absolute population cell numbers in the spleens of intact nude, dG-TNFαKO, or dG-TAKO mice (n = 3 to 8). n.s., not significant; *P < 0.05, **P < 0.001.