Supporting information

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SI Materials and Methods

Bone Marrow Chimeric Mice. For bone marrow (BM) transplantation experiments, the recipient mice were lethally irradiated with 960 rads 24 h before receiving BM transfers. Total BM cells were harvested from the femurs and tibias of donor mice (2–3 mo of age) and depleted of mature T cells, B cells, and MHC class II^+ lymphocytes by using a mixture of antibodies containing anti-CD4 (RL172), anti-CD8 (TIB105 and TIB210), anti-CD19 (1D3), and anti-MHC class II (M5/114) followed by complement-mediated lysis. BM cells from two different types of donor mice were mixed at the indicated ratio. Each recipient mouse received 4–6 $\times$ 10^6 cells in 200 μL of 1× PBS via tail vein injection. All BM chimeras were reconstituted for 4–6 wk before the analysis of T-cell development and function.

Flow Cytometry. Cells were preincubated with the anti-FcγR mAb 2.4G2 to block nonspecific antibody binding before they were stained with the FITC-, PE-, PerCP-, PE-Cy7-, APC-, APC-Cy7- or biotin-conjugated antibodies as listed in *Materials and Methods*. Events were acquired on a FACSCanto (BD) flow cytometer, and the data were analyzed with the FlowJo software (Tree Star).

Th1 Differentiation. CD4 cells were purified from single-cell suspensions of splenocytes from chimeric mice with antimouse CD4 microbeads (Miltenyi Biotec). CD4 T cells (1 $\times$ 10^6/mL) were stimulated with 5 μg/mL plate-bound anti-CD3ε (145-2C11) for 5 d with 1 μg/mL anti-CD28 (37.51), 3.5 ng/mL IL-12 (Roche), 10 μg/mL anti–IL-4 (11B11) and 50 units of IL-2 (Roche).

Cytokine Intracellular Staining. Differentiated Th1 CD4 T cells were stimulated with 50 ng/mL phorbol myristyl acetate and 1.5 μM ionomycin (Calbiochem) for 5 h. Monensin (Sigma) at 3 μM was added during the last 3 h of stimulation. Activated Th1 cells were stained with anti-CD45.1 and anti-CD45.2 antibodies. Cells were then fixed in 2% (wt/vol) paraformaldehyde for 30 min at room temperature and permeabilized with 0.2% (wt/vol) saponin (Sigma), followed by staining with anti–IL-4 and anti–IFN-γ for flow cytometry.

PLZF Intracellular Staining. PLZF staining was done using the Foxp3 staining buffer set (eBioscience) and following the protocol according to the company, except that anti-Foxp3 mAb was replaced by antimouse PLZF mAb.

Statistical Analysis. The two-tailed Student *t* test was used to analyze the statistical significance of the difference between groups as indicated. A *P* value smaller than 0.05 was considered statistically significant.

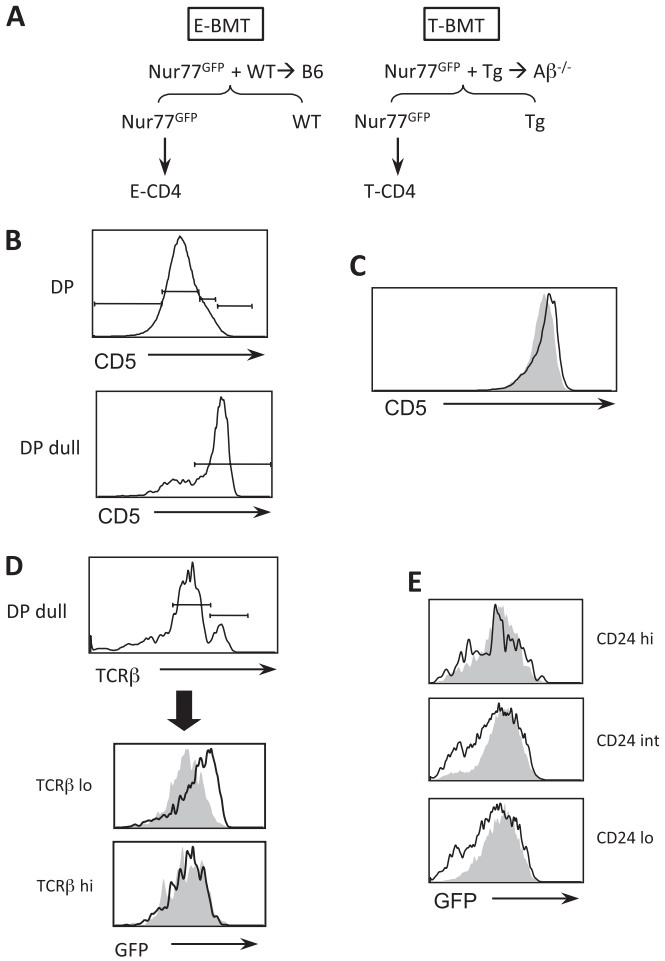

Fig. S1. Nur77^{GFP} expression in T- and E-CD4 T cells. (*A*) Generation of Nur77^{GFP} E- and T-CD4 T cells. To produce Nur77^{GFP} E-CD4 T cells, Nur77^{GFP} BM cells (CD45.2^{+}) were cotransferred with WT BM (CD45.1^{+}CD45.2^{+}) into B6.SJL hosts (CD45.1^{+}) [Nur77^{GFP}+WT→B6] (E-BMT). For Nur77^{GFP} T-CD4 T cells, Nur77^{GFP} and Tg BM cells were cotransferred into lethally irradiated MHC class II-deficient hosts resulting in [Nur77^{GFP}+Tg→Aβ$^{-/-}$] T-BMT. E- and T-CD4 T cells that were originated from Nur77^{GFP} BM were compared for their GFP expression. (*B*) CD5 expression of cells analyzed in Fig. 1*C*. (*C*) GFP^{+} DP dull cells were gated from E- (shaded) and T-BMT (line) and CD5 levels were compared. (*D*) TCRβ lo but not TCRβ hi DP thymocytes from T-BMT mice express higher GFP than that of E-BMT mice. Shaded and line histograms indicate cells from E- and T-BMT mice. (*E*) CD4 SP thymocytes were divided on the basis of the level of CD24 expression in E- and T-CD4 T cells and GFP expression of less mature to more mature cells was compared. Shaded and line histograms indicate cells from E- and T-BMT mice.

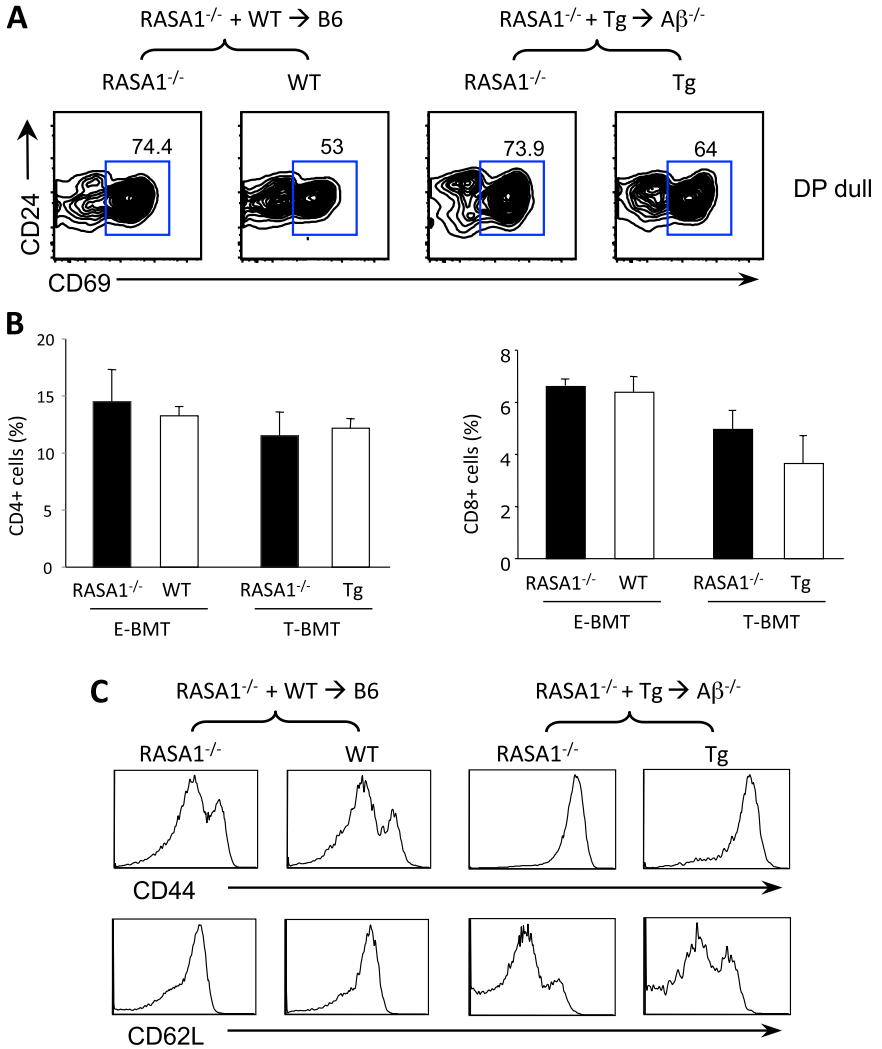

Fig. S2. The development of T-CD4 T cells with RASA1 deficiency. (*A*) RASA1 deficiency induces CD69^{hi} cells. [RASA1$^{-/-}$+WT→B6] E-BMT and [RASA1$^{-/-}$+Tg→Aβ$^{-/-}$] T-BMT were constructed and DP dull populations were gated and compared for CD69 and CD24 expression. Numbers show the percentage of gated CD69^{hi} cells in the DP dull population. (*B*) Splenic cells from the same chimeras in *A* were used to assess peripheral CD4 and CD8 T-cell percentages. Shown are mean ± SD. (*C*) Representative CD44 and CD62L expression of splenic CD4 T cells from RASA1$^{-/-}$ BM chimeras.

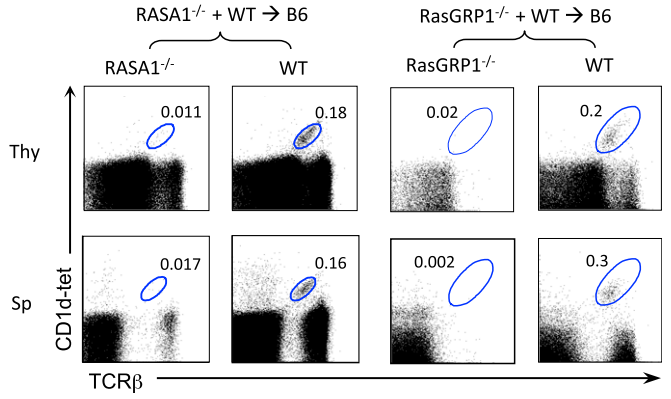

Fig. S3. iNKT cell development was severely reduced in the absence of RASA1 or RasGRP1. Total thymocytes and splenocytes from indicated mice were analyzed to examine iNKT cells using TCRβ and PBS-57-loaded CD1d-tetramers. Numbers indicate the percentages of iNKT cells among total thymocytes or splenocytes. Data are representative of five pairs of mice.

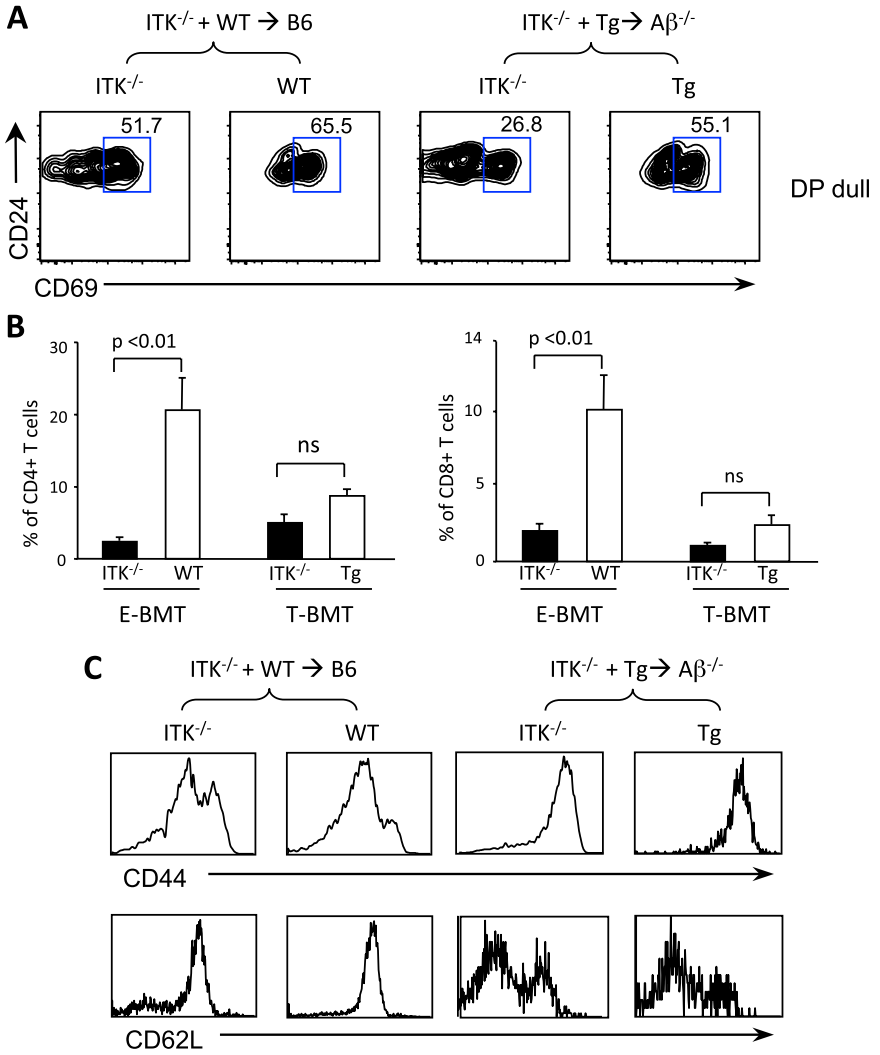

Fig. S4. Deficiency of ITK did not affect effector phenotype of T-CD4 T cells. (*A*) ITK deficiency reduces CD69^{hi} cells. [ITK$^{-/-}$+WT→B6] E-BMT and [ITK$^{-/-}$+Tg→Aβ$^{-/-}$] T-BMT were constructed and DP dull populations were gated and compared for CD69 and CD24 expression. Numbers show the percentage of gated CD69^{hi} cells in the DP dull population. (*B*) Summary of splenic CD4 and CD8 T-cell percentages. Shown are mean ± SD. (*C*) Representative CD44 and CD62L expression of splenic CD4 T cells from ITK$^{-/-}$ BM chimeras.

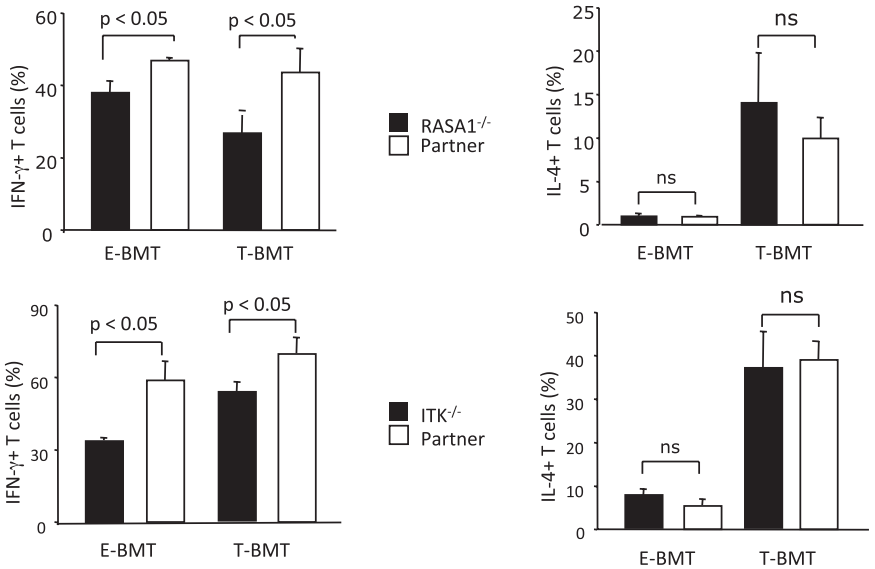

Fig. S5. Effector function of T-CD4 T cells is not affected by the strength of TCR signaling. Summary of the percentages of IFN-γ– or IL-4–producing cells in the experiments of Fig. 5. Shown are mean ± SD.

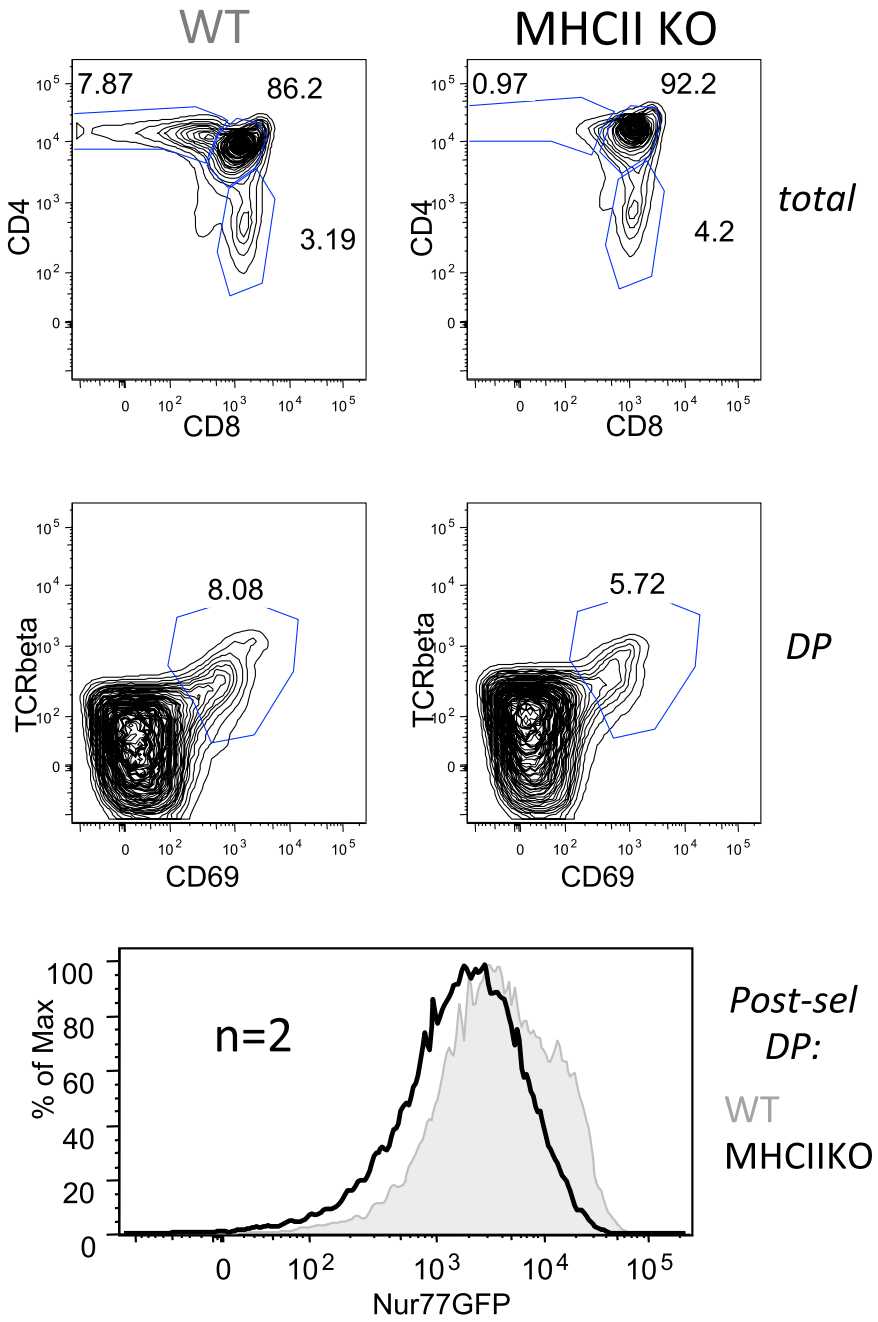

Fig. S6. TCRβ^{+} CD69^{hi} DP from wild-type Nur77^{GFP} (WT) and Nur77^{GFP}/Aβ$^{-/-}$ (MHCIIKO) mice were compared for Nur77 levels. Histograms show postselected TCRβ^{+} CD69^{hi} DP cells. Two pairs of mice are represented.