

Supplemental Data

Table S1 Total Thymocytes Number from *Mekk3* T-KO Mice

Data represents the mean \pm s.d. of total thymocytes from 12 pairs of 6-10 week old NCL (f/+) and *Mekk3* T-KO (f/-) littermates.

Figure S1. Expression of YFP Positive Lymphocyte in Lck-Cre-*Mekk3*^{fl/+}-ROSA26-EYFP Mice

Thymocytes and splenocytes harvested from Lck-Cre-*Mekk3*^{fl/+}-ROSA26-EYFP mice were stained with anti-CD4-PE and anti-CD8-APC. Histograms represent the expression of YFP on DN (CD4⁻CD8⁻), DP (CD4⁺CD8⁺), CD4SP (CD4⁺CD8⁻) and C8SP (CD4⁻CD8⁺) subsets of thymocytes, and on CD4⁺, CD8⁺ and CD4⁻CD8⁻ (non-T) subsets of splenocytes.

Figure S2. Analyses of CD3 Expression in *Mekk3* T-KO Thymocytes

Total thymocytes prepared from a pair of 8-week old NCL (f/+) and *Mekk3* T-KO (f/-) mice, and analyzed by multi-color flow cytometry using anti-CD4, anti-CD8, and anti-CD3 ϵ antibodies. The CD3 expression on gated double negative (DN), double positive (DP), and CD4 and CD8 single positive (SP) thymocytes is presented as histograms. Data shown is a representative of more than four pairs of mice.

Figure S3. Development of Treg Cell in Thymus

Detection of CD4⁺CD25⁺Foxp3⁺ T regulatory cells in NCL (f/+) and *Mekk3* T-KO (f/-) mice. Total thymocytes were first surface stained with PerCP-anti-CD4, FITC-anti-CD8, and APC-anti-CD25 antibodies, then followed by intracellular staining for Foxp3 using a PE-anti-Foxp3 antibody. The staining was analyzed by multi-color flow cytometry. The CD4 single positive (CD4⁺CD8⁻) cells were gated and analyzed further for CD25 and Foxp3 positive cells. The numbers shown in the figure next to the gated cells are percentages of CD4⁺CD25⁺Foxp3⁺ T regulatory cells in WT and *Mekk3* T-KO mice respectively.

Figure S4. Analysis of CFSE Dilution of Donor CD4 T cells under Lymphopenic Condition.

CFSE labeled Thy1.1 lymph nodes T cells were mixed with *Mekk3* KO T cells (f/-) at a roughly 1:1 ratio and transferred into sub-lethally irradiated C57BL/6-Ly5.2 mice as described in **Figure 3**. Recipient mice were analyzed at day 3 or day 5 as indicated after transfer by flow cytometry.

Figure S5. Expression of T cell Activation Markers CD69 and CD25 in *Mekk3* KO T cells.

Splenocytes CD4 T cells (top panel) and CD8 T cells (bottom panel) from NCL (f/+) and *Mekk3* T-KO mice (f/-) were analyzed for their CD69 and CD25 expression by multi-color flow cytometry.

Figure S6. Expression of CD62L, IL-7R, CD5 and Bcl-2 in *Mekk3* KO T Cells.

Histograms show the expression of CD62L, IL-7R, CD5, and Bcl-2 in spleen CD4 and CD8 T cells from NCL (f/+) and *Mekk3* T-KO (f/-) mice. Data shown is a representative of more than 3 pairs of mice.

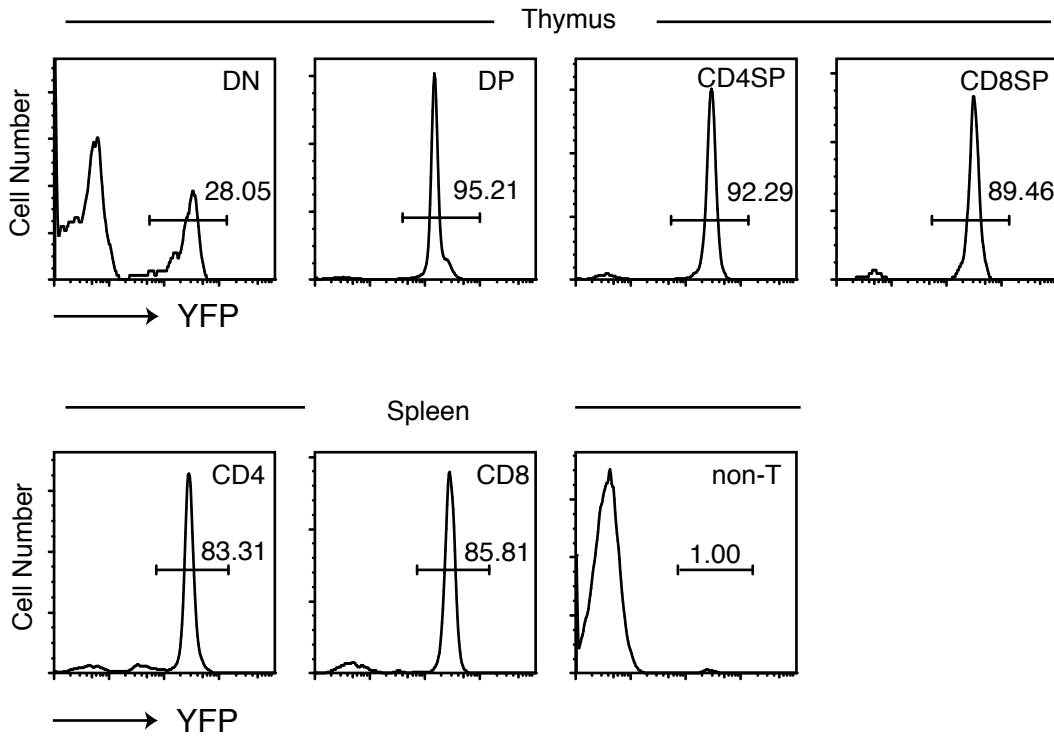


Figure S1

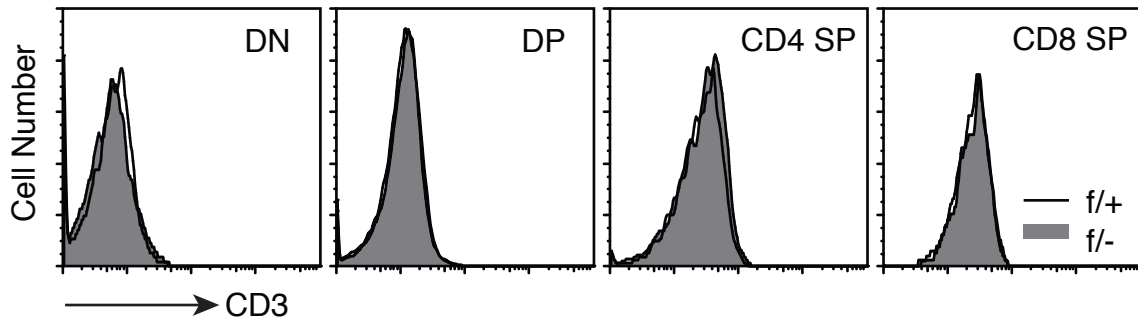


Figure S2

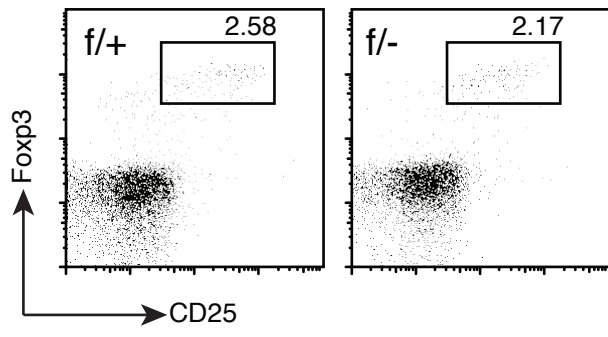


Figure S3

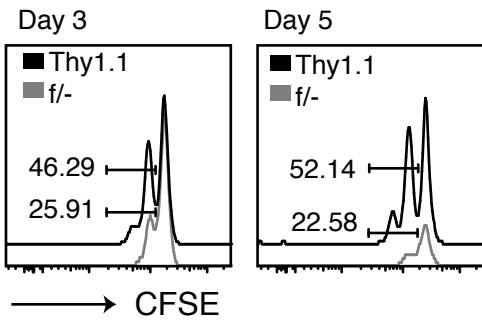


Figure S4

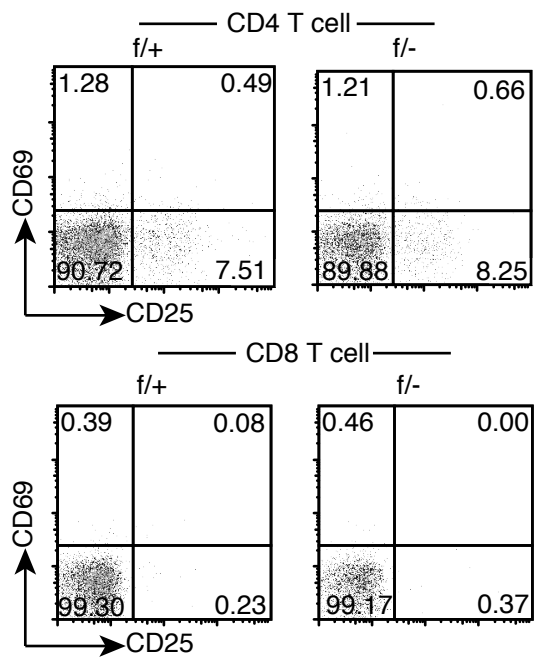


Figure S5

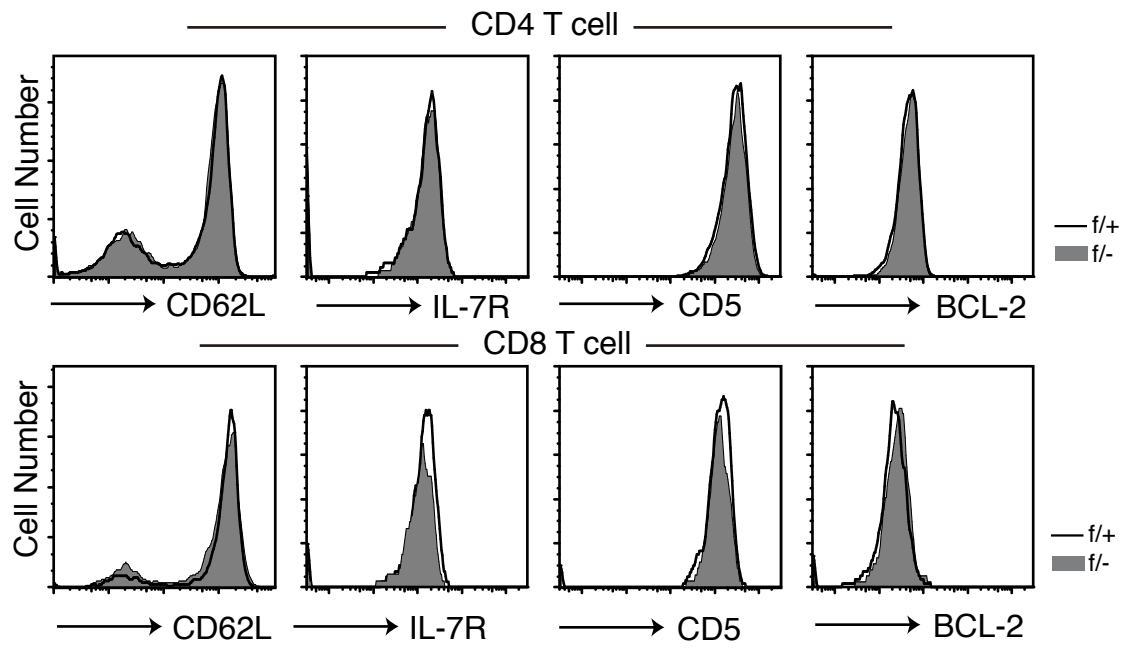


Figure S6