

Critical influence of the thymus on peripheral T cell homeostasis

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Supporting Information

Figure S1: Peripheral frequencies of CD4⁺, Foxp3⁺/CD4⁺ and activated T cells are altered in aged mice. Frequencies of CD4⁺, CD8⁺, Foxp3⁺/CD4⁺ and CD62L⁻CD44⁺/CD4⁺ T cells were determined, by flow cytometry, in the blood, spleen and peripheral lymph nodes of young (2-3 months-old) and aged (>18 months-old) mice. (A) Representative dot-plots show the gating strategy. Scatter-plot graphs depict the percentages of peripheral populations in (B) BALB/c mice (n=6) and (C) C57BL/6 mice (n=6). Data are from three independent experiments and statistical significance was determined by Two-way ANOVA with Bonferroni's Multiple Comparison Test (B) (*p<0.05, **p<0,01***p<0,001).

Figure S2: Absolute numbers of CD4⁺ T cells are decreased, while CD4⁺Foxp3⁺ Treg cells are unchanged, in the blood of thymectomized mice. Absolute numbers of CD4⁺, CD4⁺Foxp3⁺ and CD4⁺Foxp3⁻ cells were determined by flow-cytometry. Column bar graphs show the number of (A) CD4⁺, (B) CD4⁺Foxp3⁻ and (C) CD4⁺Foxp3⁺ cells in blood samples of young mice 30 days after thymectomy (Tx, n=4). Data are from one experiment and statistical significance was determined by unpaired Student's t test (B and C) (*p<0.05, **p<0,01***p<0,001).

Figure S3: The frequency of CD4⁺CD25⁺Foxp3⁻ effector T cells is also increased in aged mice and the Treg/Teff cell ratio is not altered. (A) Column bars show the the percentage of Treg (CD4⁺CD25⁺Foxp3⁺) and Teff (CD4⁺CD25⁺Foxp3⁻) determined by flow-cytometry in the blood of young mice (2 months-old, n=4), aged mice (18 months old, n=4), aged mice 30 days after grafting with an young thymus (Grafted, n=4) and young mice 30 days after thymectomy (Tx, n=4). (B) Column bars show the ratio Treg/Teff among CD4⁺ Tcells in each group. Data are pooled from two independent experiments and statistical significance was determined by Two-way ANOVA with Bonferroni's Multiple Comparison Test (A) and One-way ANOVA with Bonferroni's Multiple Comparison Test t (B) (*p<0.05, **p<0,01***p<0,001).