

Supplementary Materials for

The Long-Term Survival Potential of Mature T Lymphocytes Is Programmed During Development in the Thymus

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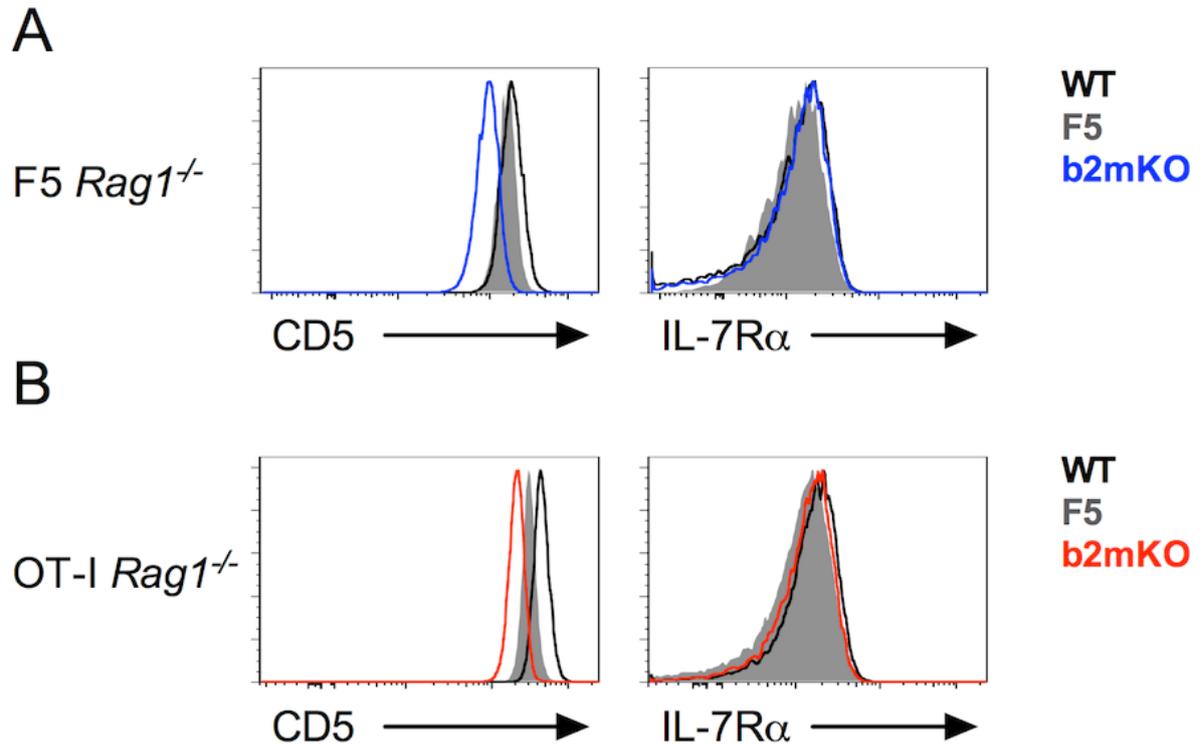


Fig. S1. IL-7R α abundance on peripheral T cells from F5 and OT-I mice is not tuned by spMHC contact in replete *b2m*^{-/-} hosts. Total lymph node cells (5×10^6 cells) from F5 or OT-I TCR transgenic mice were transferred into wild-type (WT) or *b2m*^{-/-} recipients ($n = 3$ mice) and were recovered three days later. **(A)** The left histogram shows CD5 and the right histogram shows IL-7R α on F5 *Rag1*^{-/-} donor cells recovered from WT recipients (black line), *b2m*^{-/-} recipients (blue line), or on control cells isolated from intact F5 *Rag1*^{-/-} mice (gray fill). **(B)** The left histogram shows CD5 and the right histogram shows IL-7R α on OT-I *Rag1*^{-/-} donor T cells recovered from WT recipients (black line), *b2m*^{-/-} recipients (red line), or on control cells isolated from intact OT-I *Rag1*^{-/-} mice (gray fill). Data are representative of two experiments.

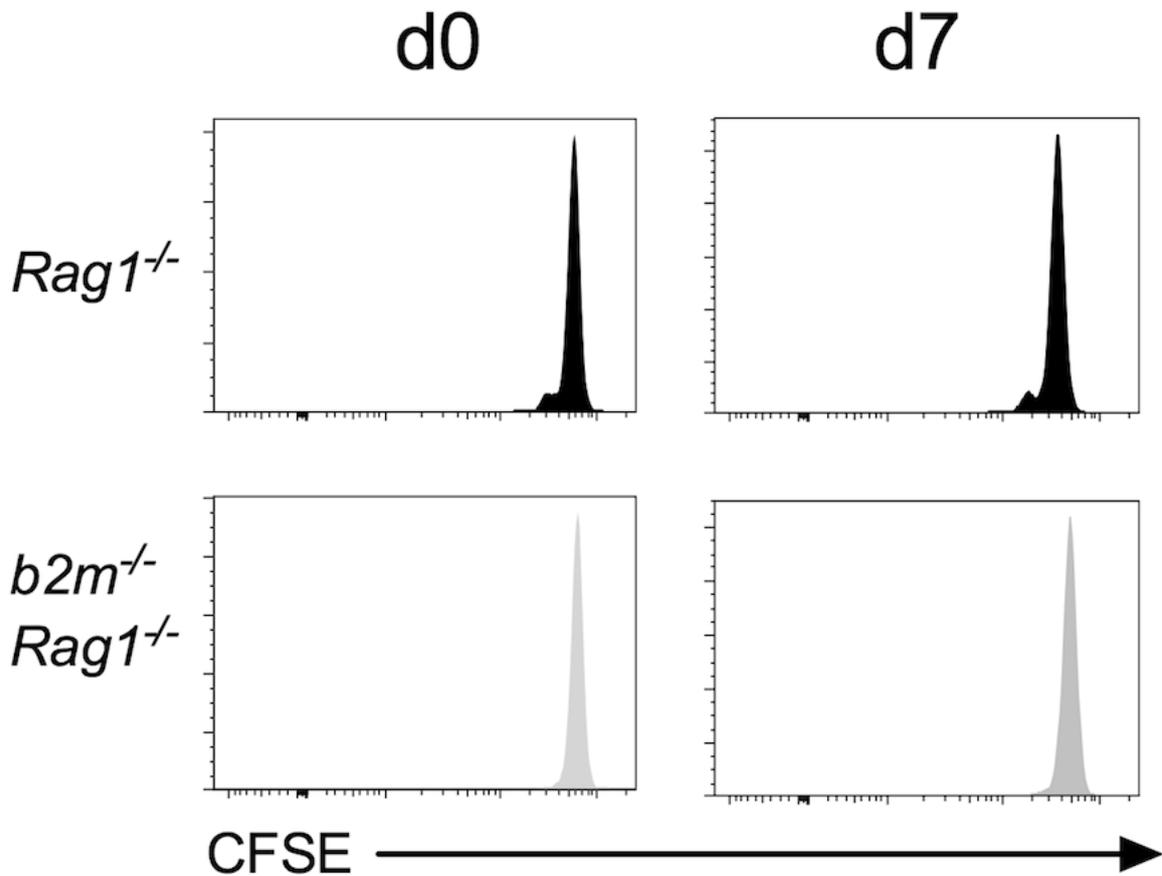


Fig. S2. Prior contact of F5 T cells with spMHC does not determine their proliferative response to IL-7 in vitro. CFSE-labelled F5 T cells were transferred (5×10^6 /mouse) to *Rag1*^{-/-} or *b2m*^{-/-} *Rag1*^{-/-} recipients (n = 3 mice). Three days later, cells were recovered and cultured in the presence of IL-7 (100 ng/ml) for seven days. Histograms are of the CFSE labelling of F5 T cells recovered from the indicated recipients ex vivo (day 0) and on day 7 of culture in vitro. Data are representative of three experiments.

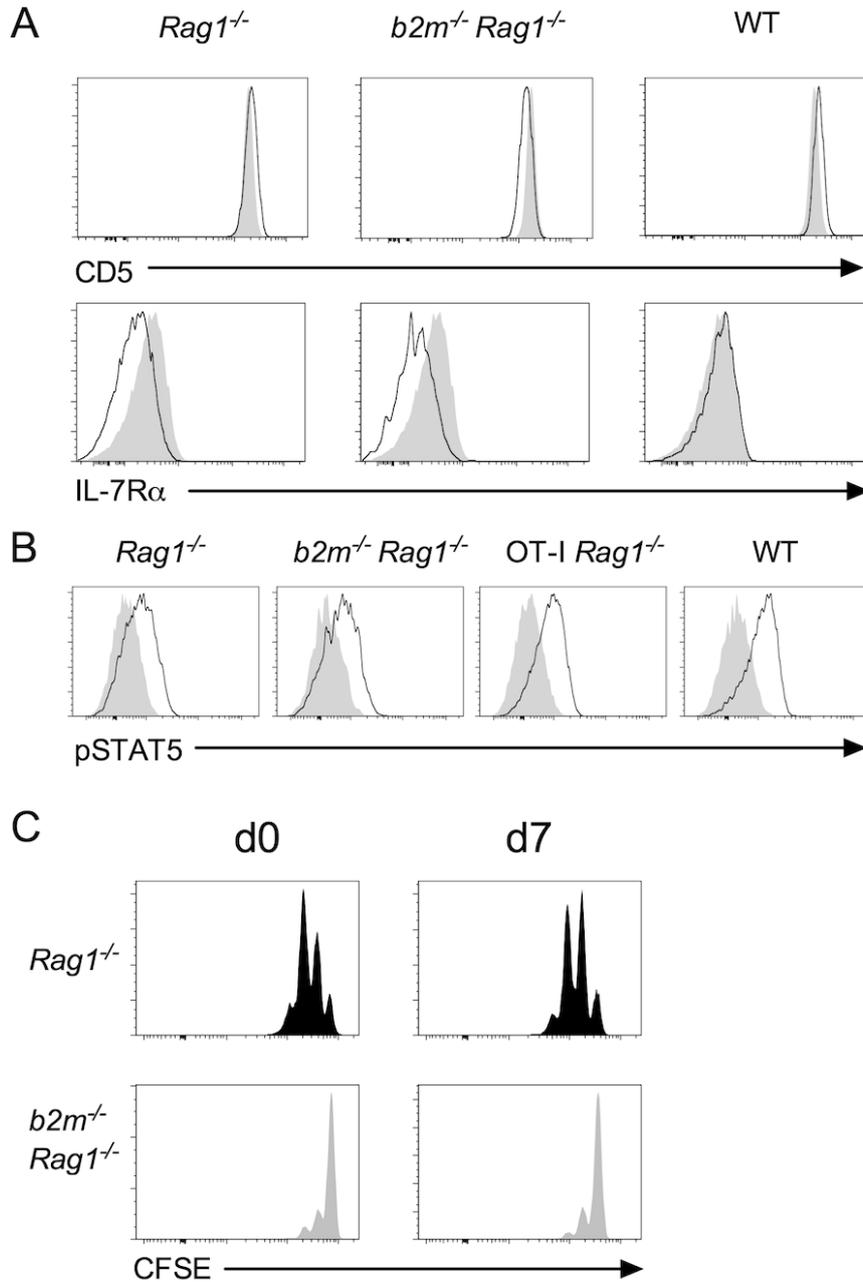


Fig. S3. IL-7R α abundance and function in OT-I T cells in the absence of TCR ligands are normal. OT-I T cells were transferred (5×10^6 /mouse) to *Rag1*^{-/-}, *b2m*^{-/-} *Rag1*^{-/-}, or CD45.1 WT recipient mice (n = 3 mice each). Histograms show CD5 and IL-7R α on donor CD45.2⁺TCR^{hi} CD8⁺ OT-I T cells (black line) recovered from the indicated recipients as compared with CD5 and IL-7R α on OT-I T cells from control OT-I mice (gray fill). **(B)** Histograms show pSTAT5 in donor OT-I T cells recovered from *Rag1*^{-/-}, *b2m*^{-/-} *Rag1*^{-/-}, and CD45.1 WT recipient mice or on T cells from control OT-I mice that were either unstimulated (gray fill) or were stimulated with IL-7 (5 ng/ml, black line) for 30 min at 37°C. Data are representative of two independent experiments. **(C)** CFSE-labelled OT-I T cells were transferred (5×10^6 cells per mouse) to *Rag1*^{-/-} or *b2m*^{-/-} *Rag1*^{-/-} recipients. Three days later, cells were recovered and cultured in the presence of IL-7 (100 ng/ml) for seven days. Histograms are of the CFSE-labelling of OT-I T cells recovered from the indicated recipients ex vivo (day 0) and on day 7 of culture in vitro. Data are representative of three or more experiments.

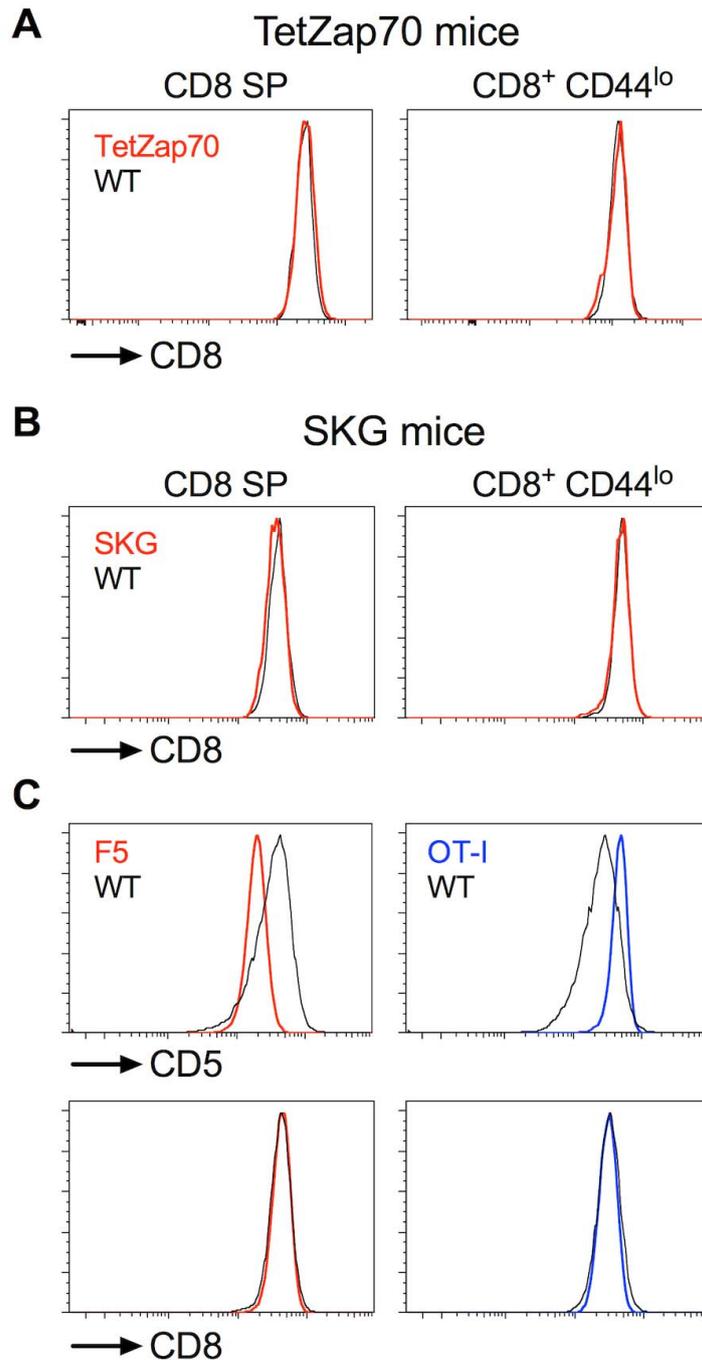


Fig. S4. CD8 abundance on CD8⁺ cells from TCR transgenic and *Zap70* mutant mice is unchanged. (A) Histograms show CD8 on CD8⁺ SP thymocytes and CD44^{lo} naïve CD8⁺ lymph node T cells from TetZap70 mice continuously fed doxycycline (red lines) and from WT control (black lines) mice depicted in Fig. 2E. (B) Histograms show CD8 on CD8⁺ SP thymocytes and on CD44^{lo} naïve CD8⁺ lymph node T cells from SKG mice (red lines) and WT control mice (black lines) depicted in Fig. 2C. (C) Histograms show CD5 and CD8 on F5 T cells (red lines) or OT-I T cells (blue) in the mixed bone marrow chimeras depicted in Fig. 3D. The abundances of CD5 and CD8 on WT CD44^{lo} CD8⁺ T cells from the corresponding chimeric mice are shown as controls (black lines). Data are representative of two (C) or three (A) and (B) experiments.

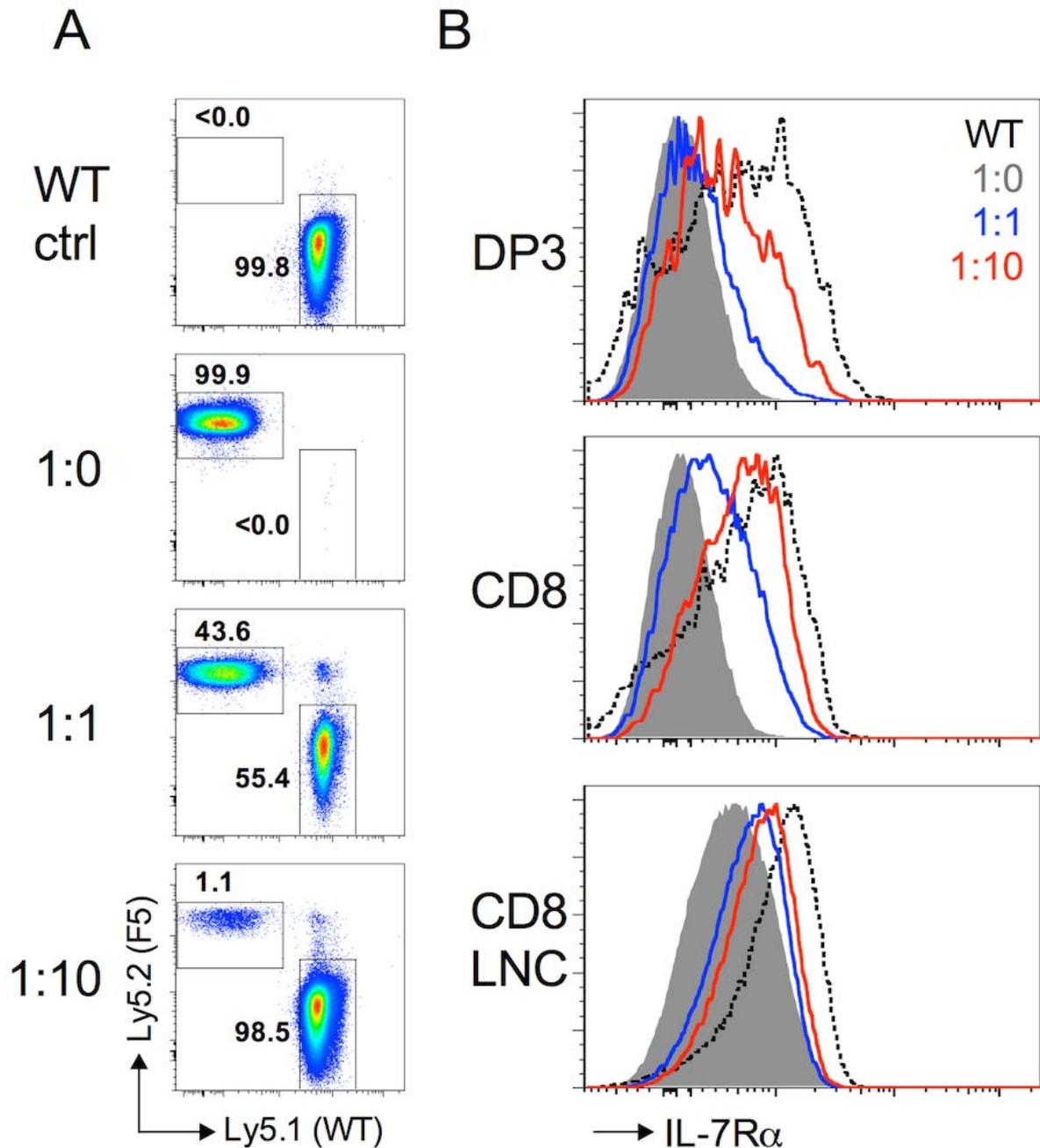


Fig. S5. IL-7R α abundance inversely correlates with clonal competition in mixed bone marrow chimeric mice. **(A)** Density plots show reconstitution of sub-lethally irradiated *Rag1*^{-/-} recipient mice eight weeks after transfer of different ratios of F5 *Rag1*^{-/-} (CD45.2) and WT (CD45.1) bone marrow cells as compared with nonirradiated WT control mice. **(B)** Histograms show IL-7R α on CD45.2⁺ DP3 and CD8⁺ SP thymocytes, as well as on CD44^{lo} naïve CD8⁺ lymphocytes from mixed bone marrow chimeric mice. Mice ($n > 2$ for each group) were reconstituted with a ratio of F5 *Rag1*^{-/-} bone marrow cells to WT bone marrow cells of 1:0 (gray fill), 1:1 (blue lines), and 1:10 (red lines). Plots of IL-7R α on the corresponding populations from WT mice are shown as controls (dotted black lines). Data are representative of two independent experiments.

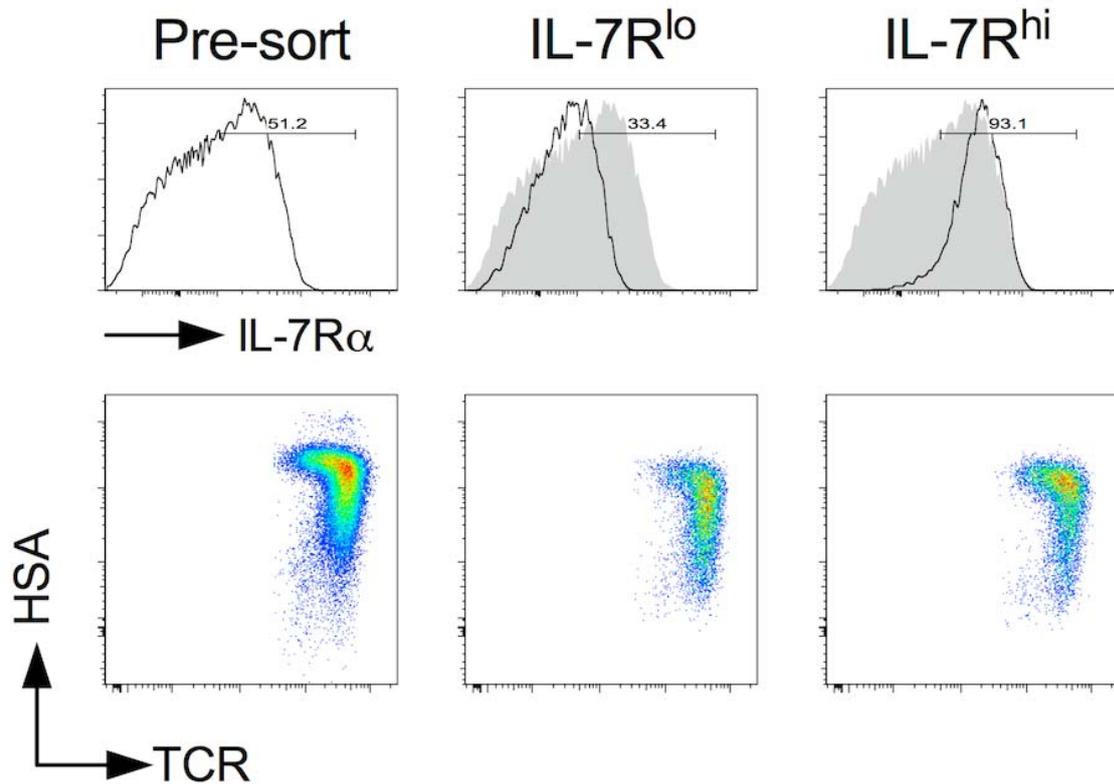


Fig. S6. IL-7R α^{hi} and IL-7R α^{lo} CD4⁺ SP thymocytes have equivalent maturation phenotypes. CD4⁺ SP thymocytes from WT mice were sorted into IL-7R α^{hi} and IL-7R α^{lo} populations. Histograms show IL-7R α on CD4⁺ SP thymocytes before sorting (left histogram and gray fills) and after sorting into the indicated populations. Density plots show HSA (CD24) against TCR on the indicated populations. Data are representative of three experiments.