

Thpok-independent repression of *Runx3* by *Gata3* during CD4⁺ T-cell differentiation in the thymus.

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Supporting Information

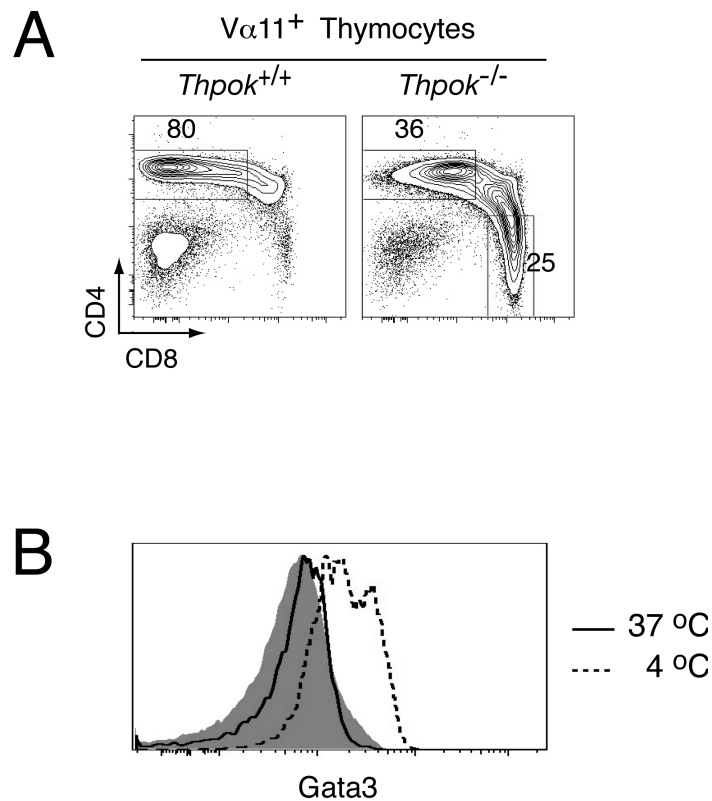


Figure 1. Characterization of *Runx3*-expressing thymocyte subsets. (A) Contour plots of CD4 vs. CD8 expression in V α 11⁺ thymocytes from *Thpok*^{+/+} and *Thpok*^{-/-} AND TCR transgenic mice. (B) Expression of intra-cellular *Gata3* in wild-type CD4 SP thymocytes after overnight single-cell suspension culture (37 °C, solid line histogram) or kept at 4 °C as a control (dashed line histogram). *Gata3* expression in DP thymocytes is shown as a reference (gray-shaded histogram).

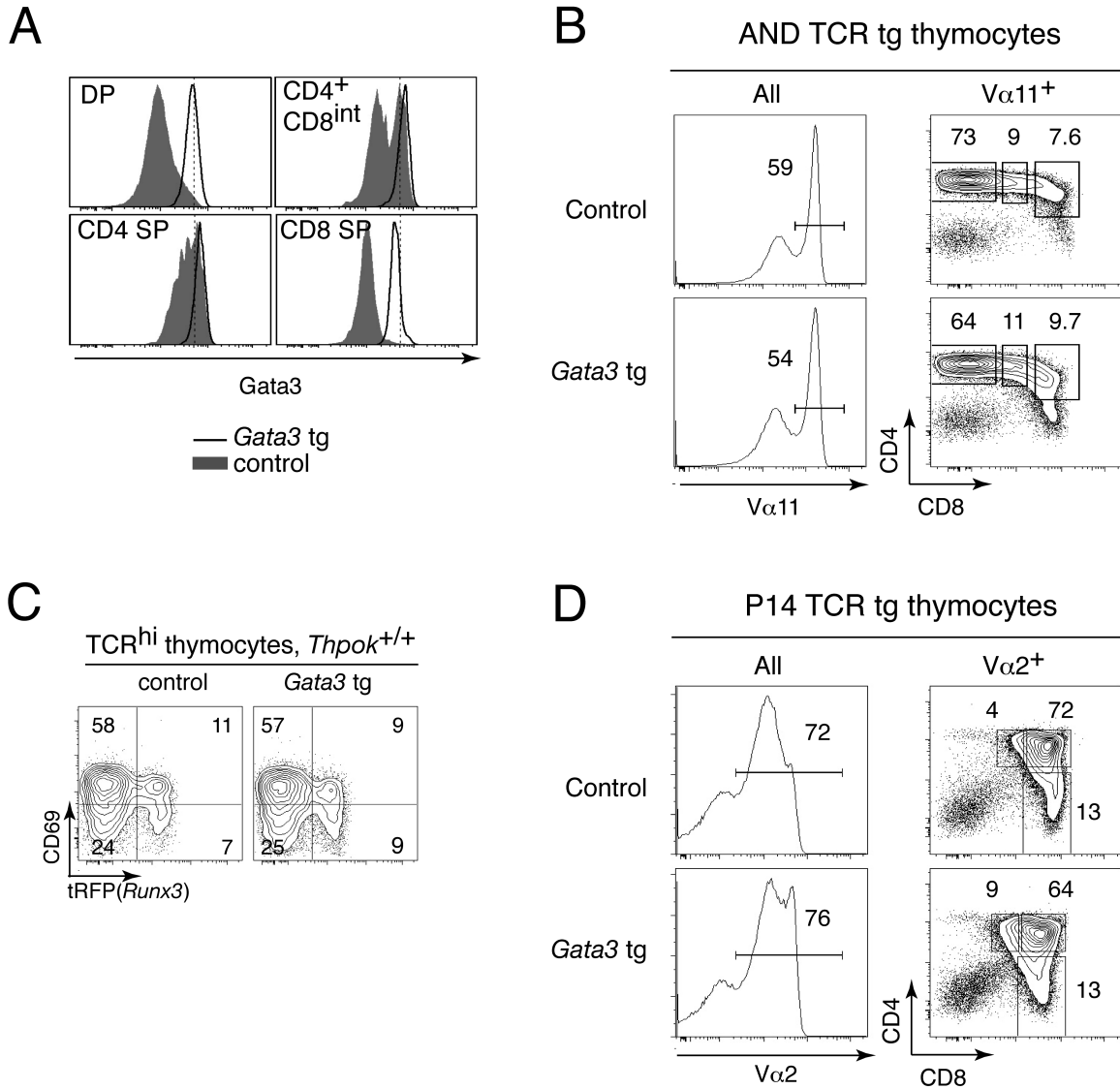


Figure 2. Development of *Gata3*-transgenic thymocytes. (A) Expression of intracellular *Gata3* in *Thpok*^{-/-} *Gata3* transgenic thymocyte subsets (solid line) or their non-transgenic counterparts (gray-shaded histograms) is displayed as in Fig. 2A. (B, D) Single-color histograms (left) show expression of the transgenic TCR α chains in thymocytes from *Gata3*-transgenic or control mice carrying either the MHC II-restricted AND (A) or MHC I-restricted P14 (C) TCR transgene ($V\alpha 11$ and $V\alpha 2$ respectively). Contour plots (right) show expression of CD4 and CD8 on $V\alpha 11^{\text{hi}}$ (A) and $V\alpha 2^{\text{hi}}$ (C) thymocytes, gated as indicated on the left. (C) Plots depict expression of *Runx3*^{tRFP} vs. CD69 in post-selection (TCR^{hi}) thymocytes from *Gata3* transgenic and control *Thpok*^{+/+} mice. Note that only MHC I-restricted cells express *Runx3* in *Thpok*^{+/+} mice, so that these experiments explore *Gata3*-mediated repression of *Runx3* in MHC I-restricted cells.

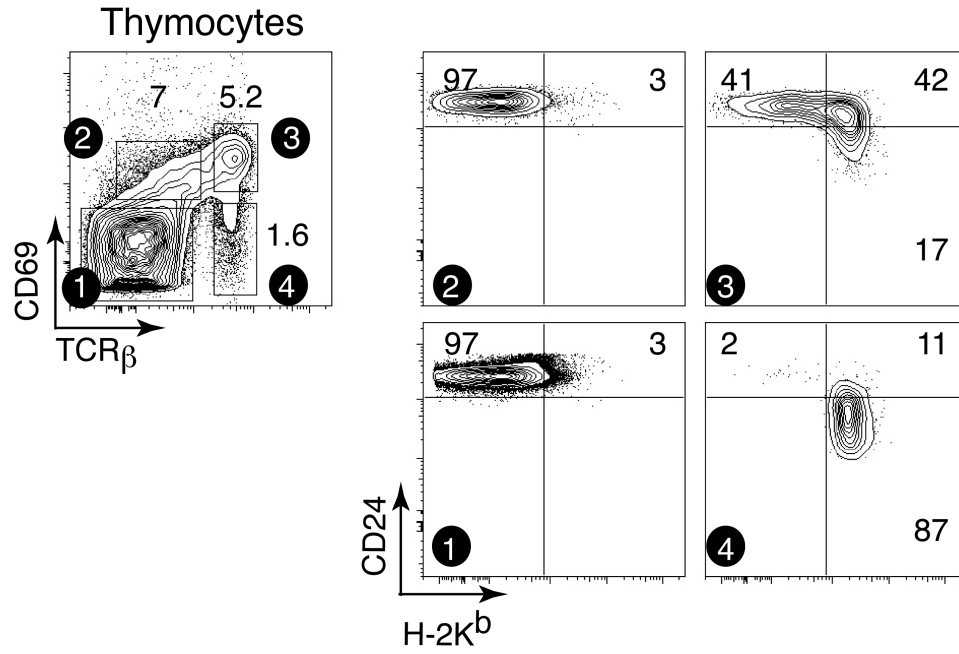


Figure 3. Comparison of CD69, CD24 and H-2K^b expression during positive selection. H-2K^b and CD24 expression (right plots) are displayed in thymocytes subsets defined by TCR and CD69 expression (left plot). White numbers on a black background indicate the normal developmental progression.