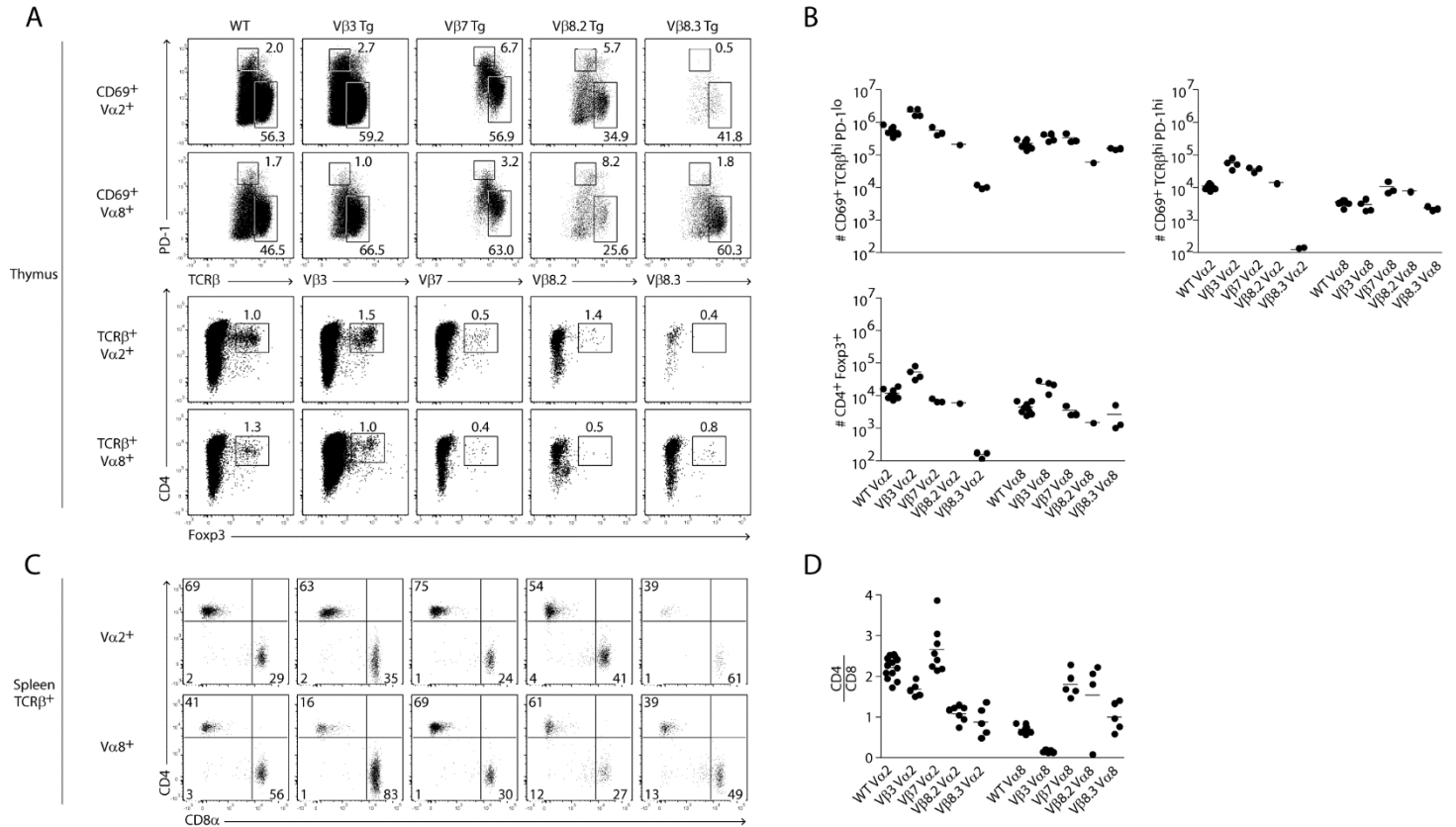
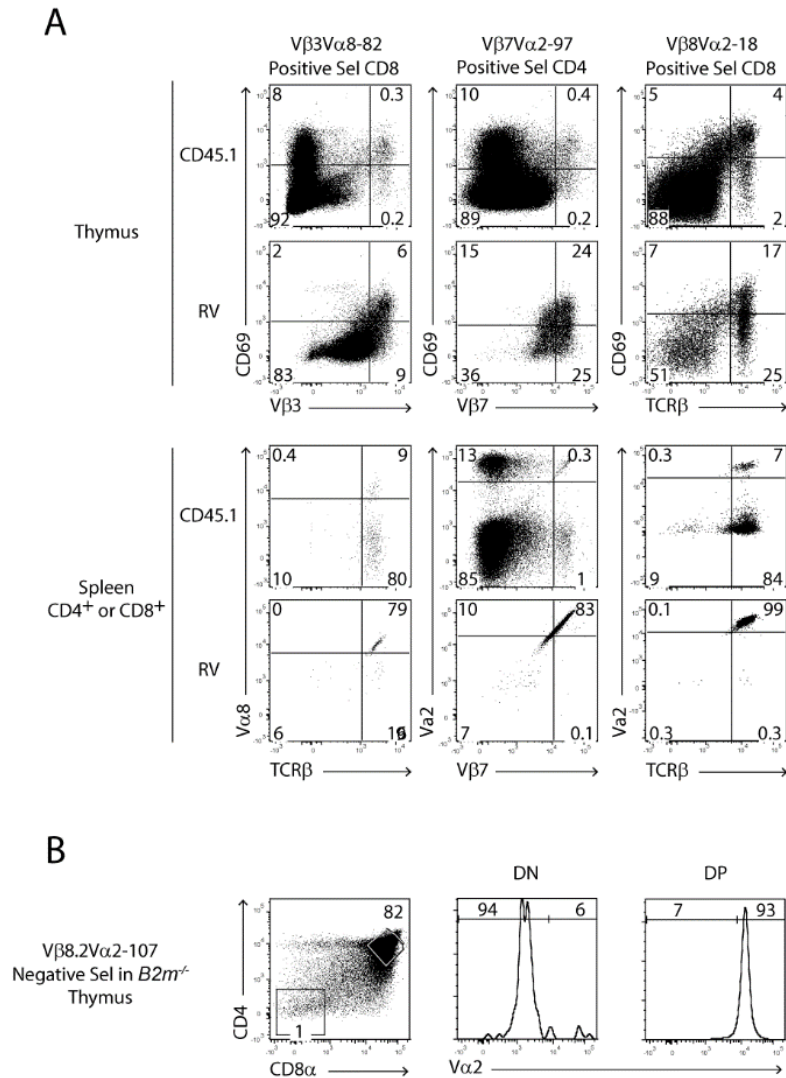


## SUPPLEMENTAL INFORMATION

**Table S1, related to Figure 1: Description of TCRs examined in this study.** The list of TCRs includes TCR $\beta$  (top) and TCR $\alpha$  (bottom) with the source of the TCR, the V $\beta$ , D $\beta$ , and J $\beta$  or V $\alpha$  and J $\alpha$  usage, and the sequence of the junctional segments. Several TCRs derived from DP<sup>lo</sup> PD-1<sup>hi</sup> thymocytes or iIEL (107,108, 109, U1, and U4) were previously described (McDonald et al., 2014). For each TCR $\alpha\beta$  pair, the selection phenotype is listed. No Sel, no selection; PS4 or 8, positive selection as CD4 or CD8; NS, negative selection.



**Figure S1, related to Figure 1: Mice with restricted TCRVβ repertoires generate all TCRαβ<sup>+</sup> cell lineages at expected frequencies.** Flow cytometry analysis of the thymus (A,B) and spleen (C,D) of wild-type littermate and Vβ transgenic mice gated as indicated. CD69<sup>+</sup> TCRβ<sup>hi</sup> PD-1<sup>lo</sup> and CD69<sup>+</sup> TCRβ<sup>hi</sup> PD-1<sup>hi</sup> cells represent positively and negatively selected thymocytes, respectively. (A,C) Representative analysis. (B,D) Summary of results.



**Figure S2, related to Figure 1: Levels and kinetics of expression of TCR $\alpha$  in the RV<sup>cond</sup> system, A) Flow cytometry analysis of the thymus and spleen from 3 TCRs, showing cells expressing the indicated RV-TCRs and control CD45.1 cells in the same chimera. B) Flow cytometry analysis of the thymus of a RV<sup>cond</sup> chimera expressing an MHC I-reactive TCR on a *B2m*<sup>-/-</sup> background (to avoid the presence of DP<sup>lo</sup> or DN TCR<sup>+</sup> post-negative selection thymocytes) showing the frequency of V $\alpha$ 2-expressing cells among DN and DP thymocytes.**