JEM

SUPPLEMENTAL MATERIAL

Leung et al., http://www.jem.org/cgi/content/full/jem.20091033/DC1

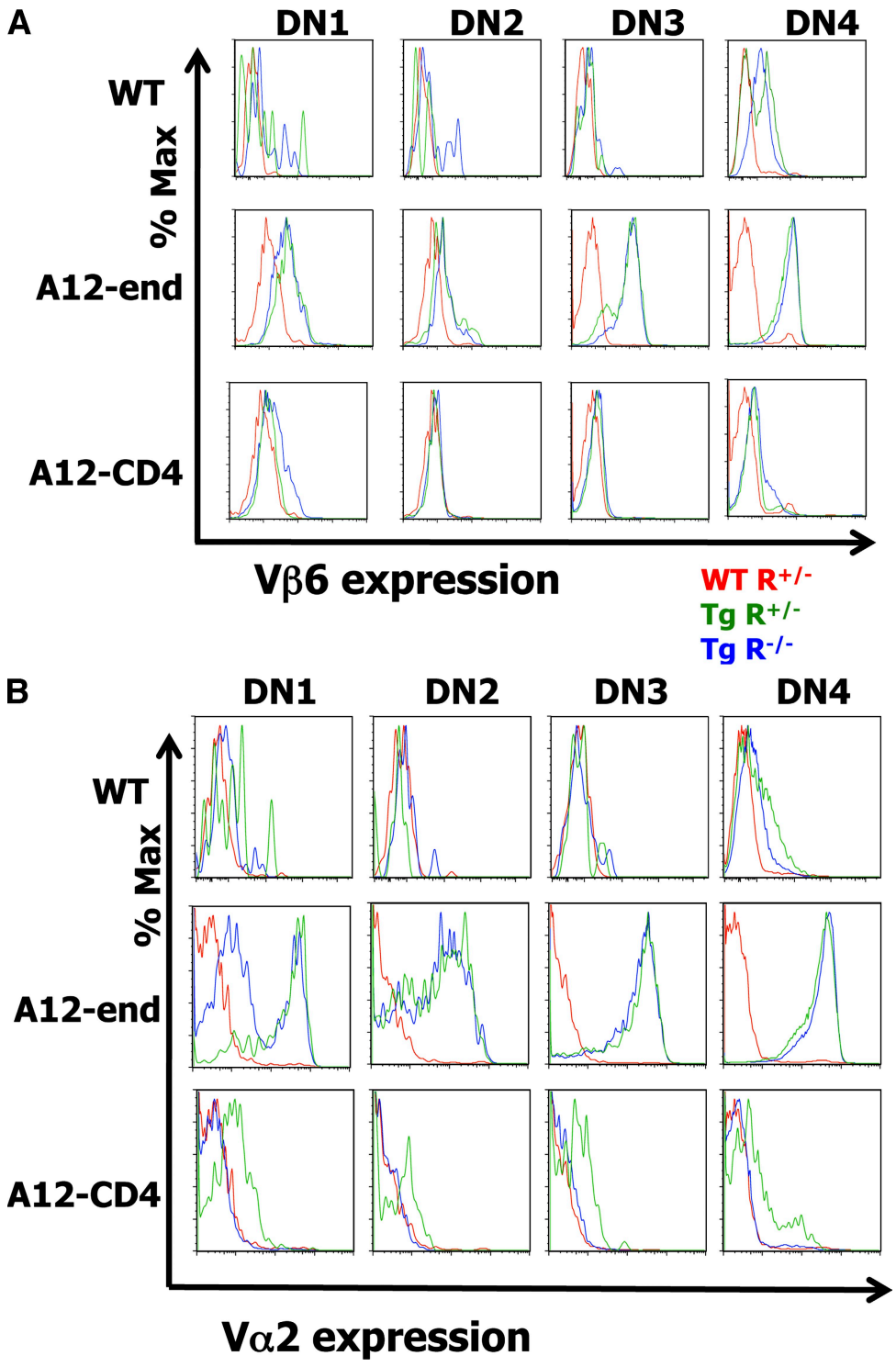

Figure S1. TCR-β and TCR-α are not expressed in DN stages of thymic development of T reg TCR Tg with TCR-α under CD4 promoter. FACS analysis of WT, A12-end and A12-CD4, and 2P-CD4 RAG$^{-/-}$. (A) Vβ6 expression in DN1 (CD44^{hi} CD25^{-}), DN2 (CD44^{hi}CD25^{hi}), DN3 (CD44^{-} CD25^{hi}), and DN4 (CD44^{-} CD25^{-}) stages. DN cells were gated on CD4^{-} CD8^{-} CD3^{-} thymocytes. (B) Vα2 expression in DN1 (CD44^{hi} CD25^{-}), DN2 (CD44^{hi}CD25^{hi}), DN3 (CD44^{-} CD25^{hi}), and DN4 (CD44^{-} CD25^{-}) stages. DN cells were gated on CD4^{-} CD8^{-} CD3^{-} thymocytes. Data are representative of three independent experiments.

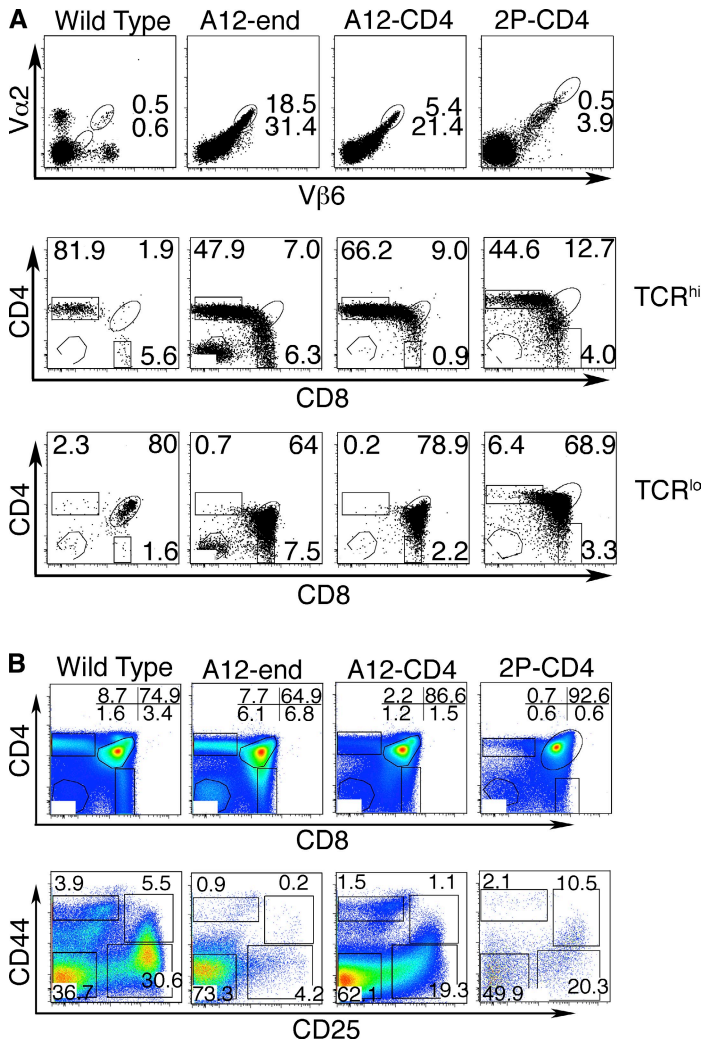

Figure S2. T reg TCR Tg mice with TCR-α under CD4 promoter have dominant DN3 population and TCR^{hi}-expressing cells in CD4^{+} SP but not in CD4^{-} CD8^{-} DN cells. (A) FACS analysis of WT, A12-end RAG$^{-/-}$, A12-CD4 RAG$^{-/-}$, and 2P-CD4 RAG$^{-/-}$ thymocytes. Analysis was gated on total live lymphocytes with Vβ6^{hi} Vα2^{hi} and Vβ6^{int} Vα2^{int}. (B) FACS analysis of WT, A12-end and A12-CD4, and 2P-CD4 RAG$^{-/-}$ thymocytes. DN cells were gated on CD4^{-} CD8^{-} CD3^{-} thymocytes. Data are representative of at least four independent experiments.

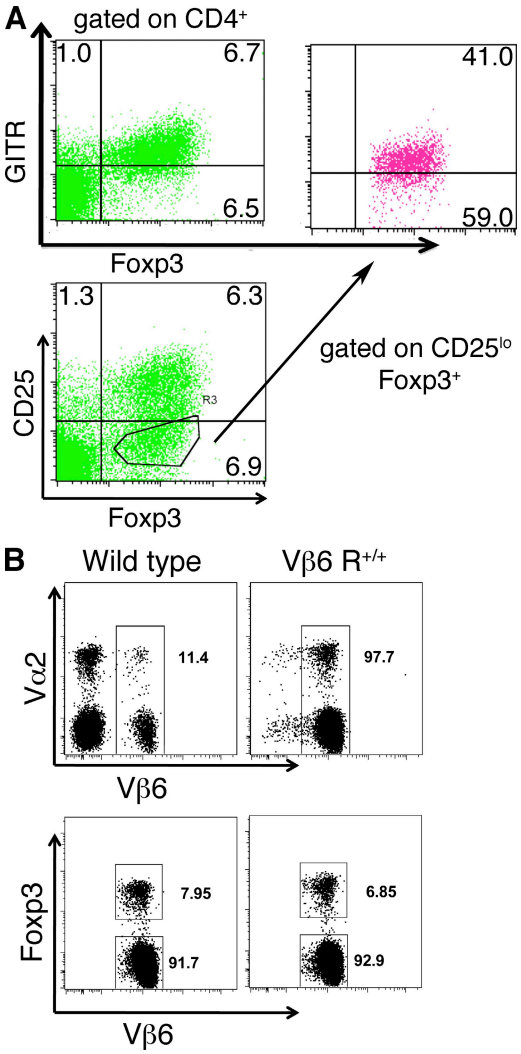

Figure S3. Foxp3 expression in Vβ6 Tg R+ mice is comparable to WT and correlates well with GITR. (A) Vβ6 Tg RAG$^{+/-}$ spleens were stained with CD4, Vβ6, Vα2, and Foxp3 and analyzed by flow cytometry. Foxp3 analysis was gated on CD4$^+$ cells. (B) Vβ6 Tg RAG$^{+/-}$ spleens were stained with CD4, CD25, GITR, and Foxp3 and analyzed by flow cytometry. Foxp3 analysis was gated on CD4$^+$ cells. Data are representative of two and three independent experiments in A and B, respectively.

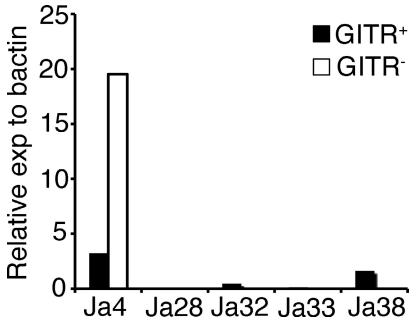

Figure S4. Verification of Jα usage in GITR$^+$ and GITR$^-$ population by real-time PCR. Vβ6^{hi} Vα2^{hi} CD4$^+$ GITR^{hi} and GITR$^-$ cells were sorted from Vβ6 Tg mice. cDNA was synthesized for real-time PCR analysis. Sequencing analysis showed Jα4 was preferentially used in GITR$^-$ population, whereas Jα38 (2P) was preferentially used in GITR$^+$ population. Data are representative of two independent experiments.