## **Supplemental Figures**:



<u>Supplemental Figure 1</u>: Expression pattern of CD44 and CD25 on thymocytes from WT and DKO HLA-DR3 mice. Thymocytes extracted from 3-week-old WT and DKO HLA-DR3 mice were enumerated and analyzed by flow cytometry following staining with indicated antibodies. A total of 6-8 mice from each group were analyzed. Representative dot plots are shown.



<u>Supplemental Figure 2</u>: Distribution of FoxP3<sup>+</sup> T regulatory cells within the thymus of WT and DKO HLA-DR3 mice. Thymocytes extracted from 3-week-old WT and DKO HLA-DR3 mice were permeabilized and stained with anti-FoxP3 antibodies following the intracellular staining protocol along with anti-CD3, anti-CD25 or anti-TCR  $\beta$  antibodies. A total of 6-8 mice from each group were analyzed. Representative dot plots are shown.



<u>Supplemental Figure 3</u>: Peripheral T cell repertoire in DKO HLA-DR3 mice in comparison to WT HLA-DR3 mice. Frequency of  $CD3^+$  T cells expressing indicated TCR V $\beta$  families in the spleens of 6- to 8-week-old WT and DKO HLA-DR3 mice were analyzed by flow cytometry. A total of 6-8 mice from each group were analyzed. Representative dot plots are shown.



<u>Supplemental Figure 4</u>: Distribution of FoxP3<sup>+</sup> T regulatory cells, DNT cells and their activation profile in the spleens DKO HLA-DR3 mice. Splenocytes extracted from 6- to 8-weeks WT and DKO HLA-DR3 mice were indicated antibodies. A total of 6-8 mice from each group were analyzed. (A) FoxP3<sup>+</sup> Tregs (B) Expression of CD44 and CD62L on total CD3<sup>+</sup> splenocytes and (C) Distribution of splenic DNT cells. Representative dot plots are shown.