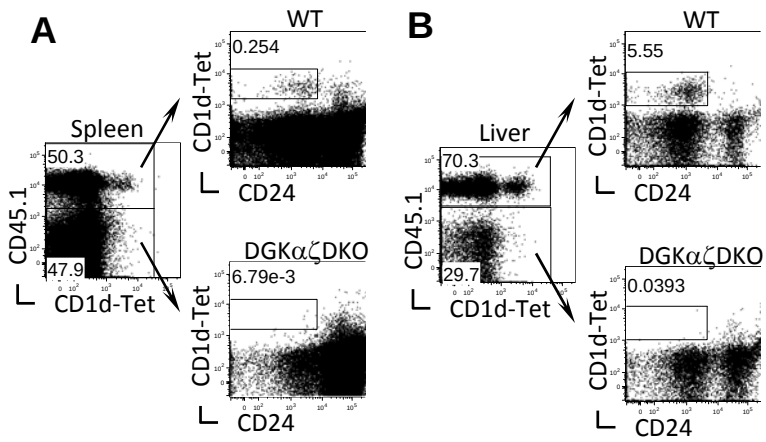

Figure S1. *i*NKT assessment in the spleen and liver of chimeric mice reconstituted with WT and DGKαζDKO bone marrow cells.
CD45.1^{+} WT and CD45.2^{+} DGKαζDKO bone marrow cells were 1:1 mixed and adoptively transferred into sublethally irradiated TCRβ$^{-/-}$δ$^{-/-}$ recipient mice. Seven to eight weeks later, splenocytes (A) and liver mononuclear cells (B) were harvested from the recipient mice and subjected to flow cytometry analysis. For both (A) and (B), left panel shows expression of CD45.1 and CD1d-Tet on total live cells; right panels show expression of CD1d-Tet and CD24 on CD45.1^{+} gated WT cells (top) and CD45.1^{-} gated DGKαζDKO cells (bottom). Numbers indicate percentage of the gated cells. Data are representative of three experiments.

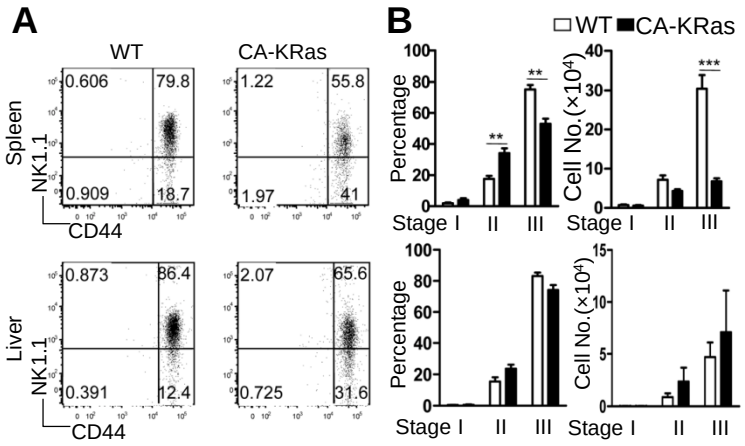

Figure S2. Defective *i*NKT terminal maturation in the periphery of CA-KRas mice. Splenocytes and liver mononuclear cells from age- and sex-matched CA-KRas mice and WT controls were isolated, counted, and subjected to flow cytometry. Data shown are representative of five mice per group. Left, flow cytometry analysis of CD4 and NK1.1 cell surface expression on live CD1d-Tet^{+}CD24^{-} gated cells in spleen (top) and liver (bottom). Right, percentage and number of stage 1, 2, and 3 CD1d-Tet^{+}CD24^{-} gated cells in spleen (top row) and liver (bottom).

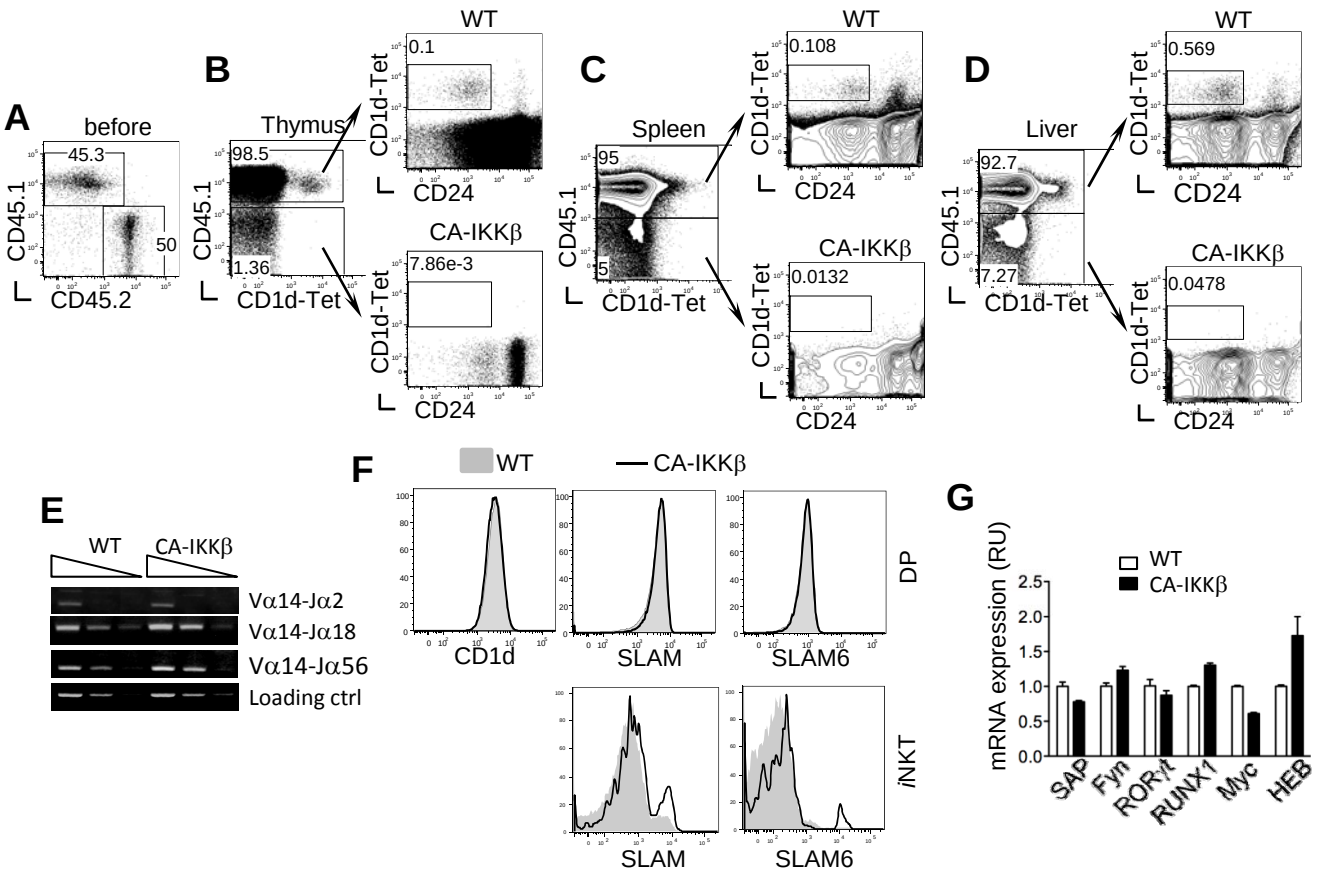

Figure S3. *i*NKT developmental defects in CA-IKKβ mice are cell-intrinsic. (A)-(D) CD45.1$^+$ WT and CD45.2$^+$ CA-IKKβ bone marrow cells were mixed in a 1:1 ratio and adoptively transferred into sublethally irradiated TCRβ$^{-/-}$δ$^{-/-}$ recipient mice. 7-8 weeks later, the development of *i*NKT cells in thymus (B), spleen (C), and liver (D) of recipient mice were examined. (A) Expression of CD45.1 and CD45.2 on mixed WT and CA-IKKβ bone marrow cells before adoptive transfer. (B)-(D) Left panel shows CD45.1 and CD1d-Tet staining on total live cells; Right panels show CD1d-Tet and CD24 staining on CD45.1$^+$ gated WT (top) and CD45.1$^-$ gated CA-IKKβ (bottom) cells. Numbers indicate percentage of the gated cells. Data are representative of three experiments. (E) Semi-quantitative PCR analysis of sorted CD4$^+$CD8$^+$ thymocytes from WT and CA-IKKβ mice with primers for Vα14-Jα2, Vα14-Jα18, Vα14-Jα56, and CD14 (loading control). (F) Expression of CD1d, SLAM (CD150), and SLAMF6 (Ly108) on live DP thymocytes and *i*NKT cells from WT and CA-IKKβ mice. Data are representative of three mice per group. (G) Realtime PCR analysis of mRNA expression of various proteins in sorted CD4$^+$CD8$^+$ thymocytes from WT and CA-IKKβ mice.

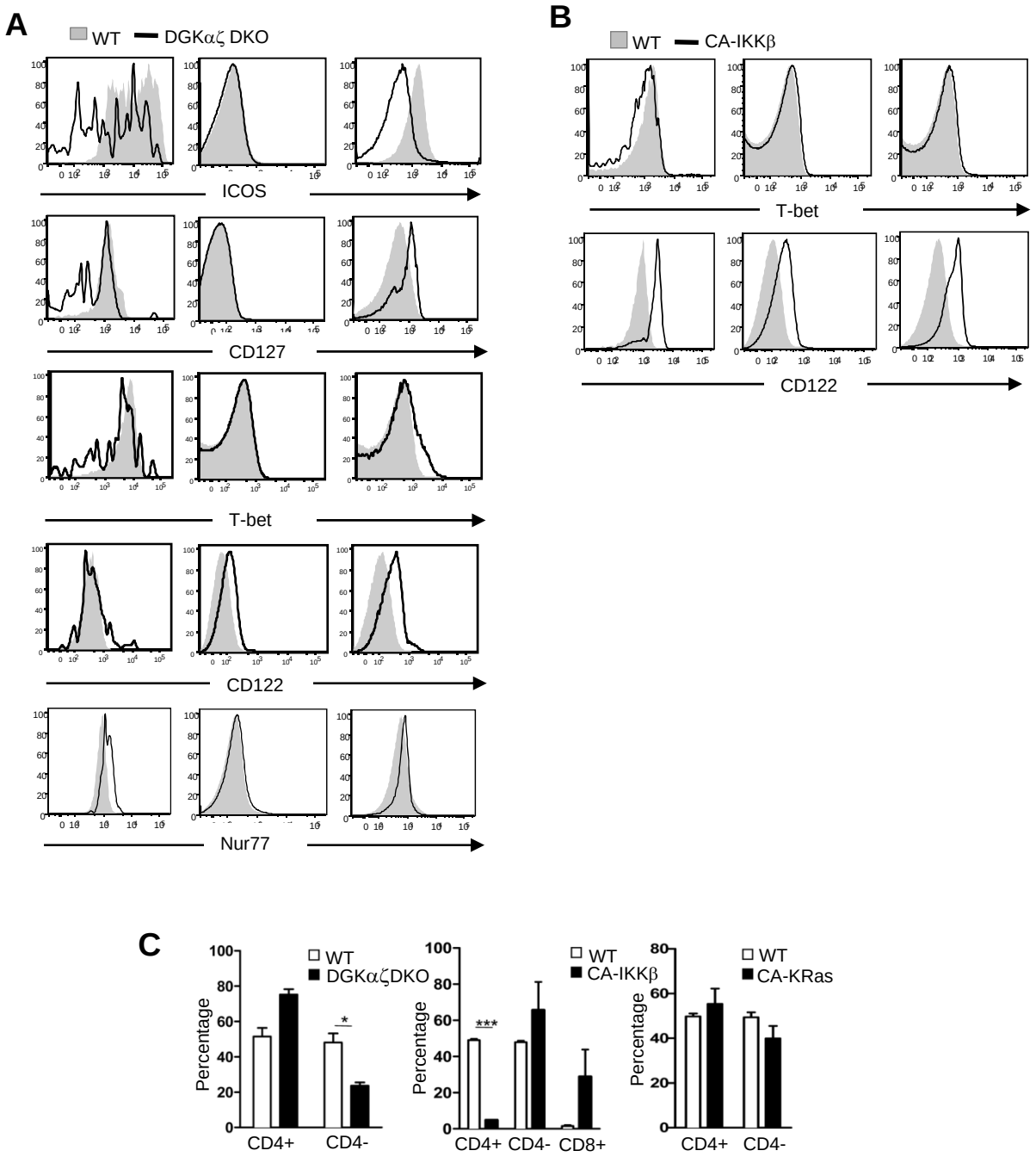

Fig S4. Expression of CD127, ICOS, CD122, T-bet, and Nur77 in *i*NKT cells, DP thymocytes, and TCRβ^{+}CD1Dtet^{-} thymocytes from WT and DGKαζDKO mice (A) or CA-IKKβ mice (B). (C) CD4^{+}, CD4^{-}, and CD8^{+} *i*NKT cells in DGKαζDKO, CA-IKKβ, and CA-KRas mice.