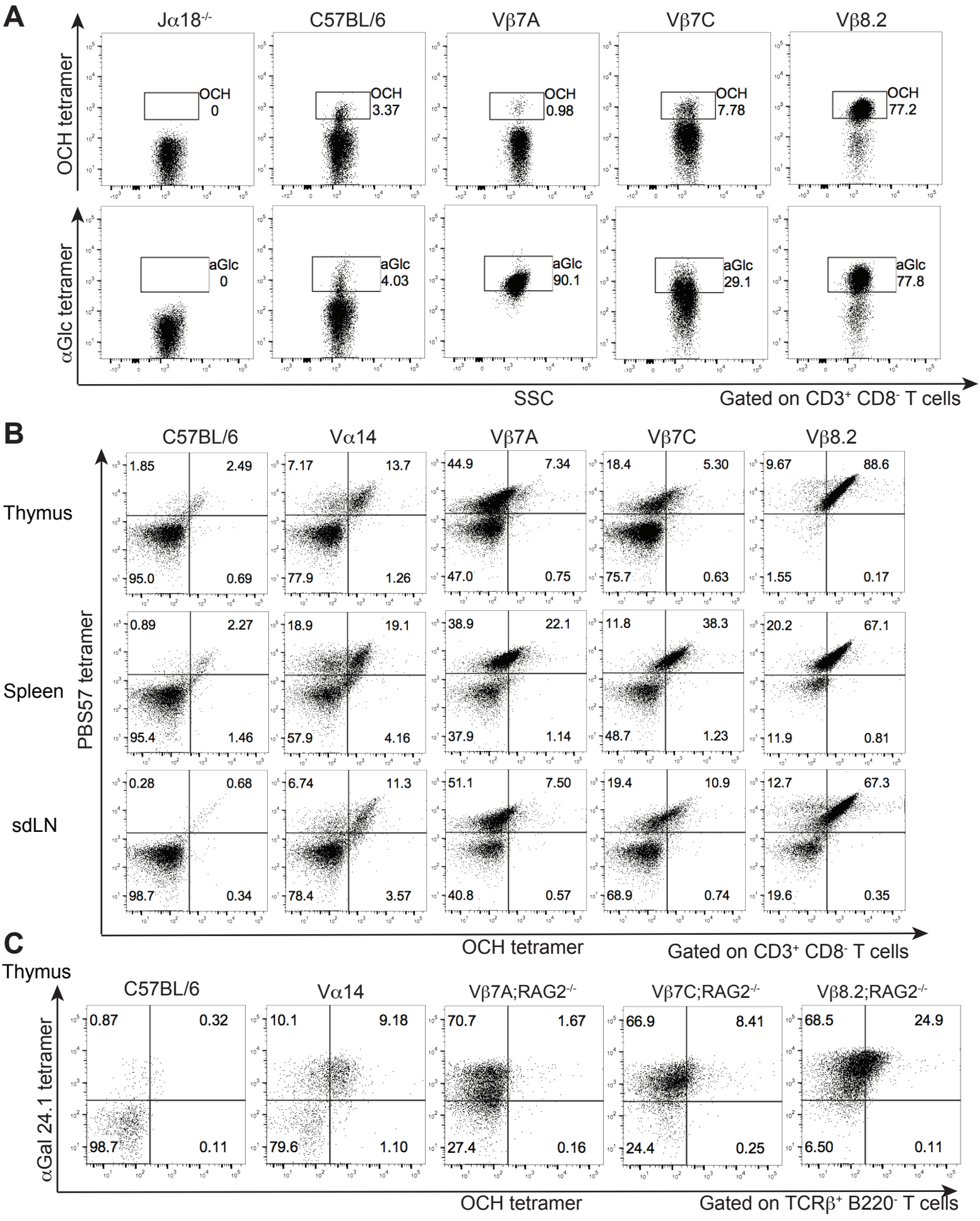
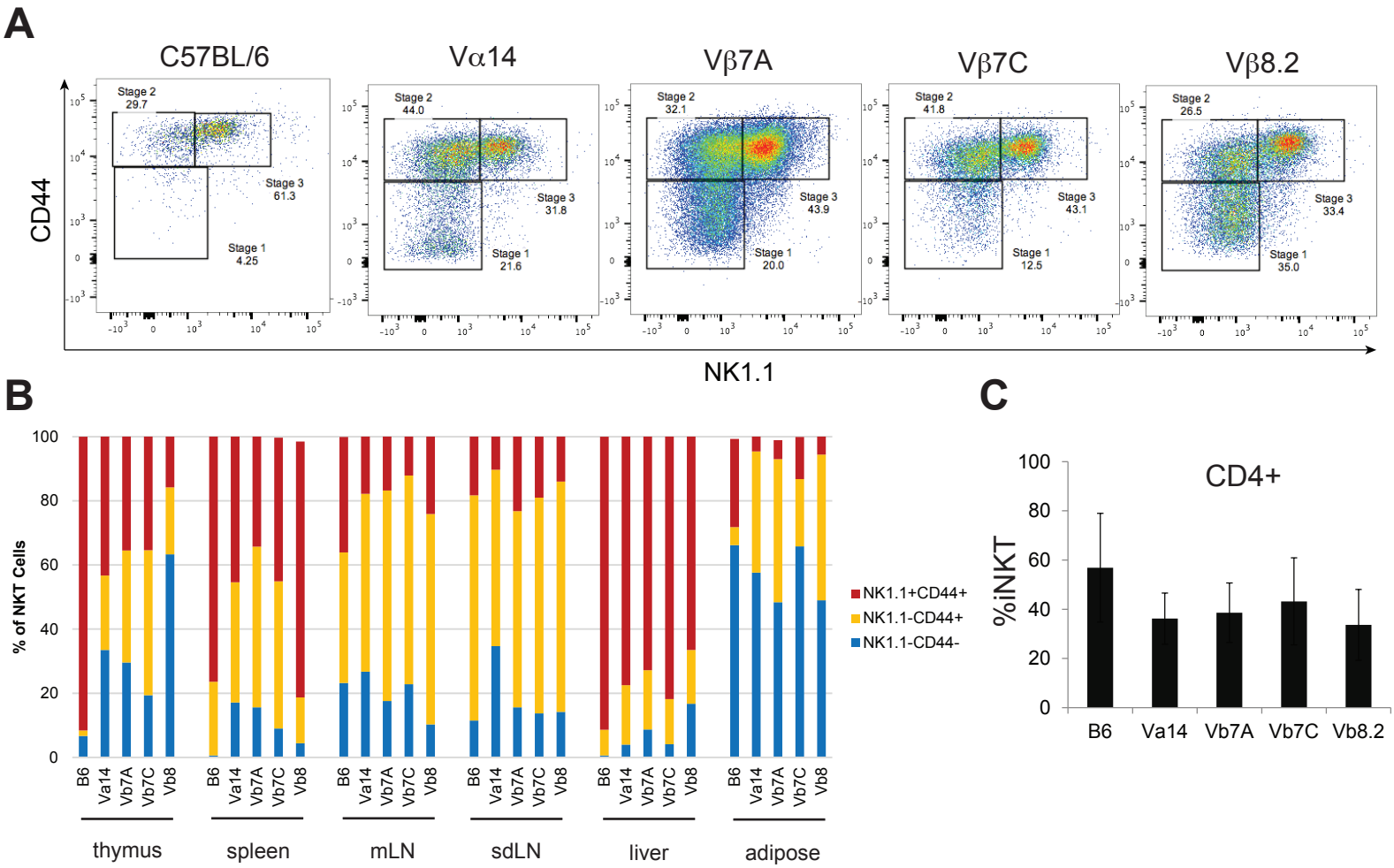


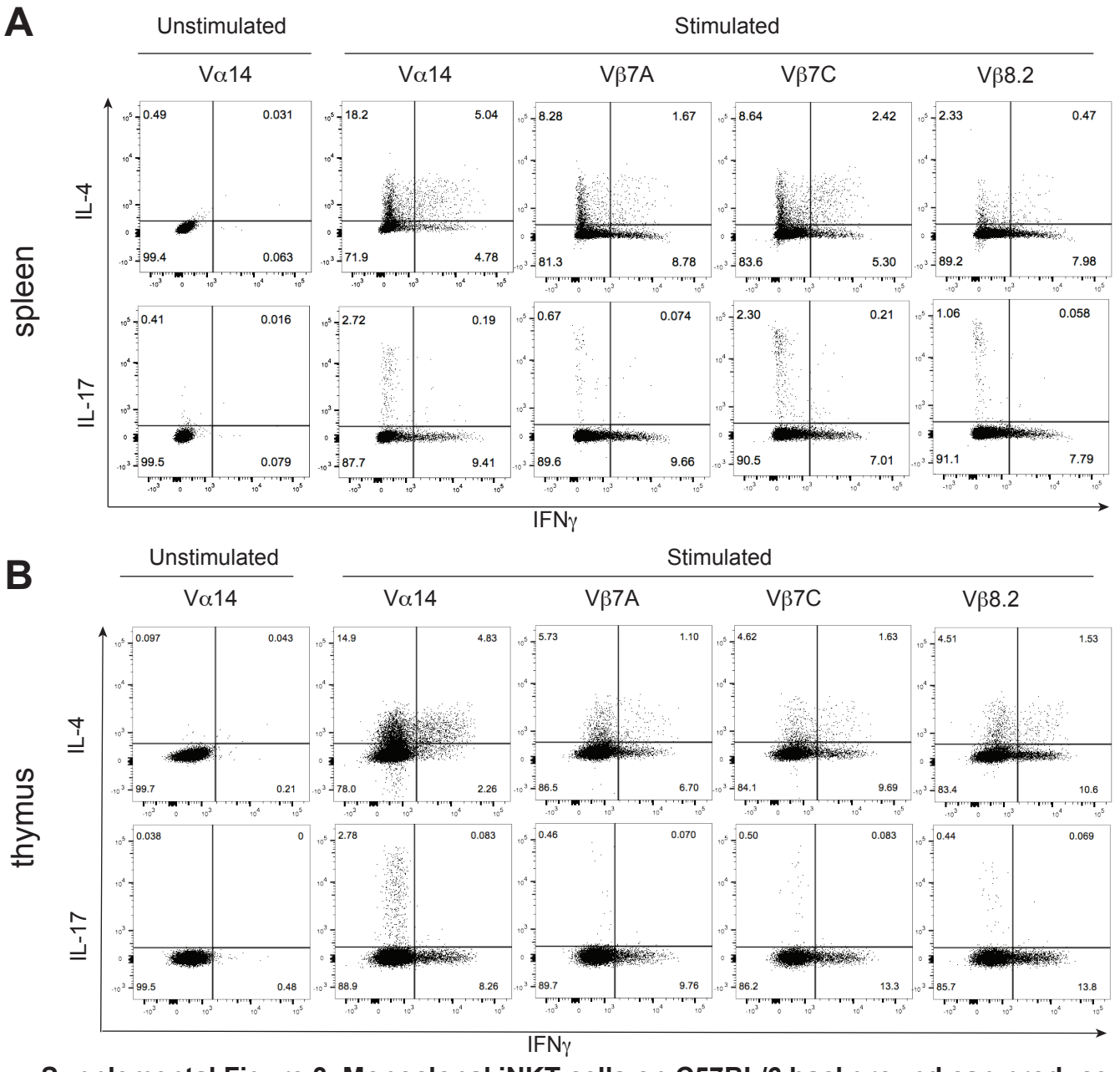
Supplemental Figure 1. Monoclonal iNKT cell mice have differing antigen preference. (A) Splenocytes from Jα18$^{-/-}$, C57BL/6, and indicated TN lines were harvested and stained with anti-CD3, anti-CD8, and either OCH tetramer or α-GlcCer tetramer, as indicated. **(B)** Thymus, spleen, and sdLN were harvested from C57BL/6 and indicated TN lines and stained with anti-CD3, anti-CD8, OCH, and PBS57 tetramers simultaneously. **(C)** Thymii from C57BL/6, Vα14, and monoclonal TN lines crossed onto a RAG2$^{-/-}$ background were harvested and stained with TCRβ, B220, OCH tetramer, and α-GalCer (24.1) simultaneously. 24.1 represents a naturally occurring lipid tail length.

Supplemental Figure 1

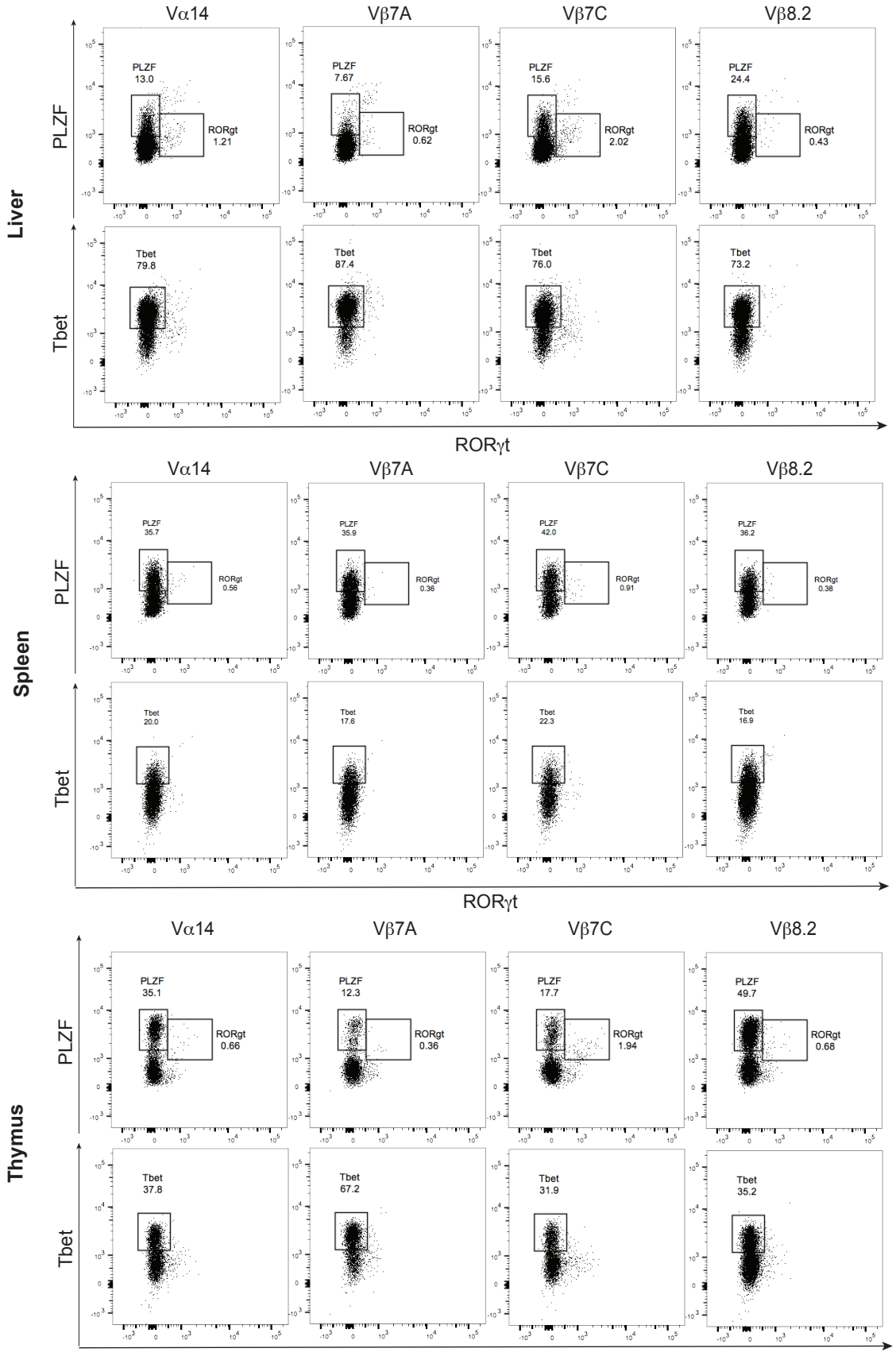


Supplemental Figure 2. CD44 and NK1.1 distribution is influenced by tissue of origin. (A) Thymocytes from each of the indicated mouse strains were stained with CD1d (PBS57) tetramer and antibodies to NK1.1, CD44, and CD4, and analyzed by flow cytometry. Representative flow plots are shown. n=3 mice per group. (B) iNKT cells from the indicated tissues analyzed as in (A) were quantified. Graph shows the percent iNKT cells in each gate, averaged from 3 mice per group. (C) Pooled spleen and lymph nodes from the indicated mouse strains were stained with CD1d (PBS57) tetramer and antibodies to CD3 and CD4, and analyzed by flow cytometry. Shown is the percent of iNKT cells that are $CD4^+$, averaged over 5 mice per group. Error bars are SD.

Supplemental Figure 2



Supplemental Figure 3. Monoclonal iNKT cells on C57BL/6 background can produce all major cytokines. Cells from (A) spleen or (B) thymus of all TN lines were stimulated *in vitro* with PMA/Ionomycin for 4 hours, in the presence of GolgiStop. Cells were stained with anti-CD3 and CD1d (PBS57) tetramer, before being fixed, permeabilized, and stained with antibodies to IFNγ, IL-4, and IL-17. Results shown are gated on $CD3^{+}CD1d\text{-}tetramer^{+}$ cells.



Supplemental Figure 4. Distribution of iNKT cell subsets in liver of limited dilution bone marrow chimeras (BMC). Jα18$^{-/-}$ mice were lethally irradiated, reconstituted with 95% Jα18$^{-/-}$ bone marrow and 5% bone marrow of the indicated TN lines, and analyzed 8 weeks later. n=4 mice per group. Liver, spleen, and thymus cells from all BMC mice were stained with anti-CD3 and CD1d (PBS57) tetramer, before being fixed, permeabilized, and stained with antibodies to PLZF, Tbet, and RORγt. Results shown are gated on CD3^{+}CD1d-tetramer^{+} cells.

Supplemental Figure 4