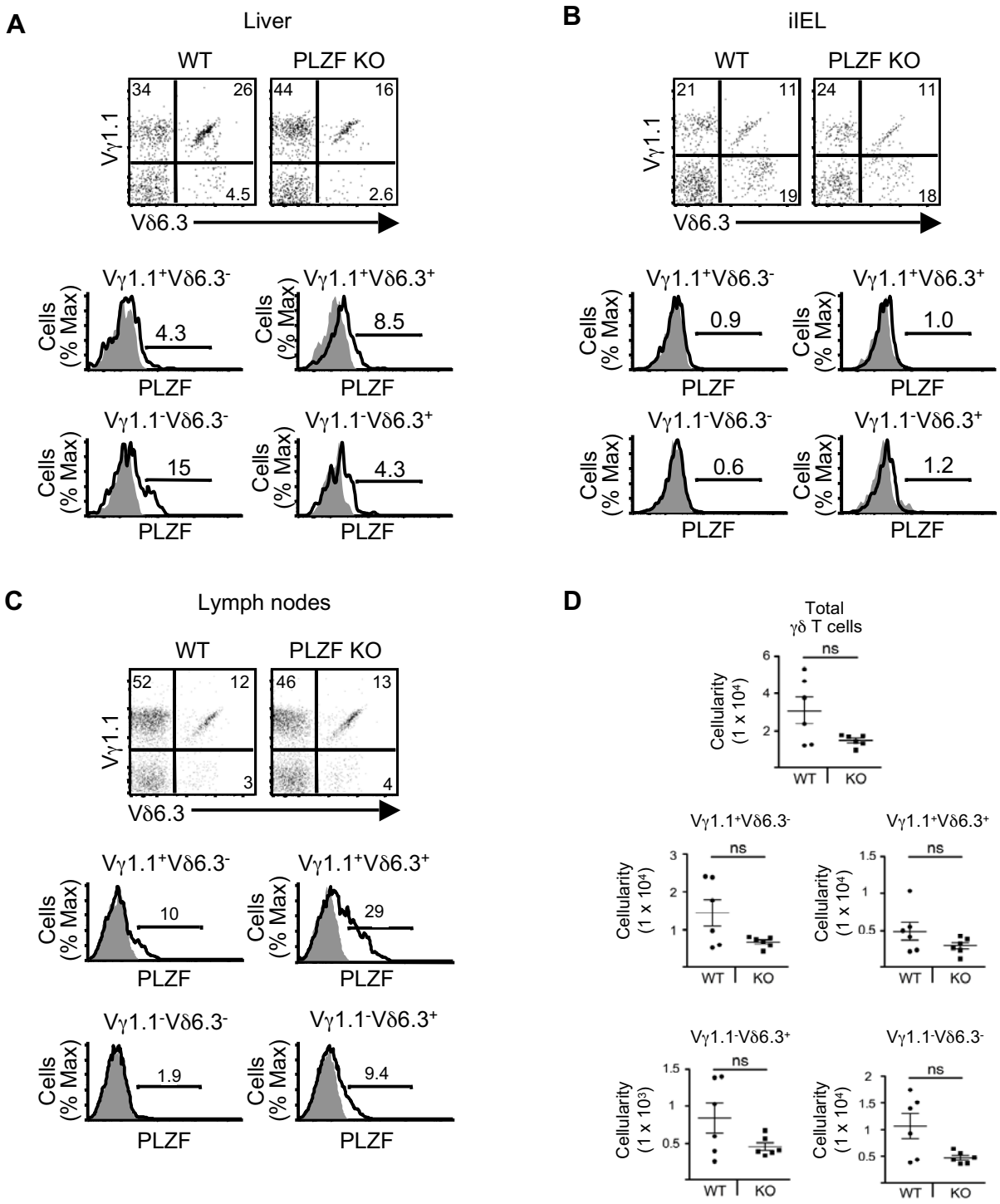



Figure S1 Alonzo et al

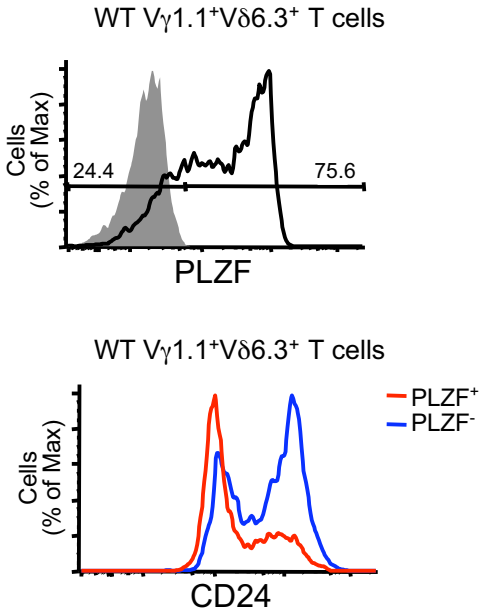
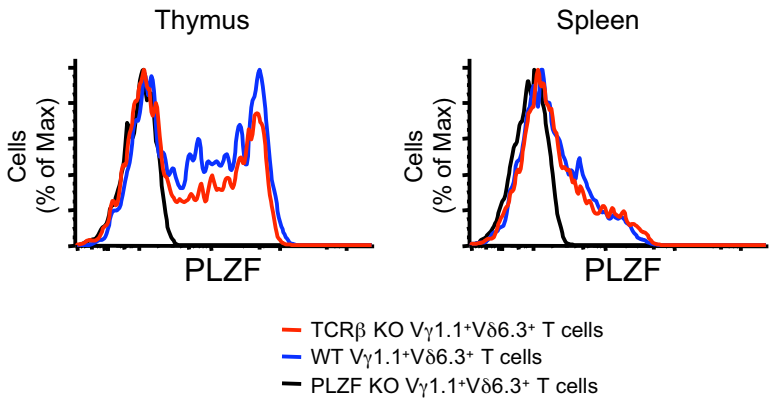



Figure S2 Alonzo et al

**A**




**B**

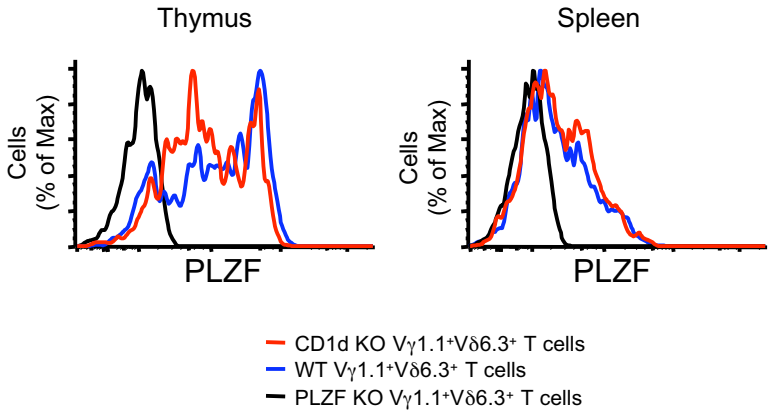



Figure S3 Alonzo et al

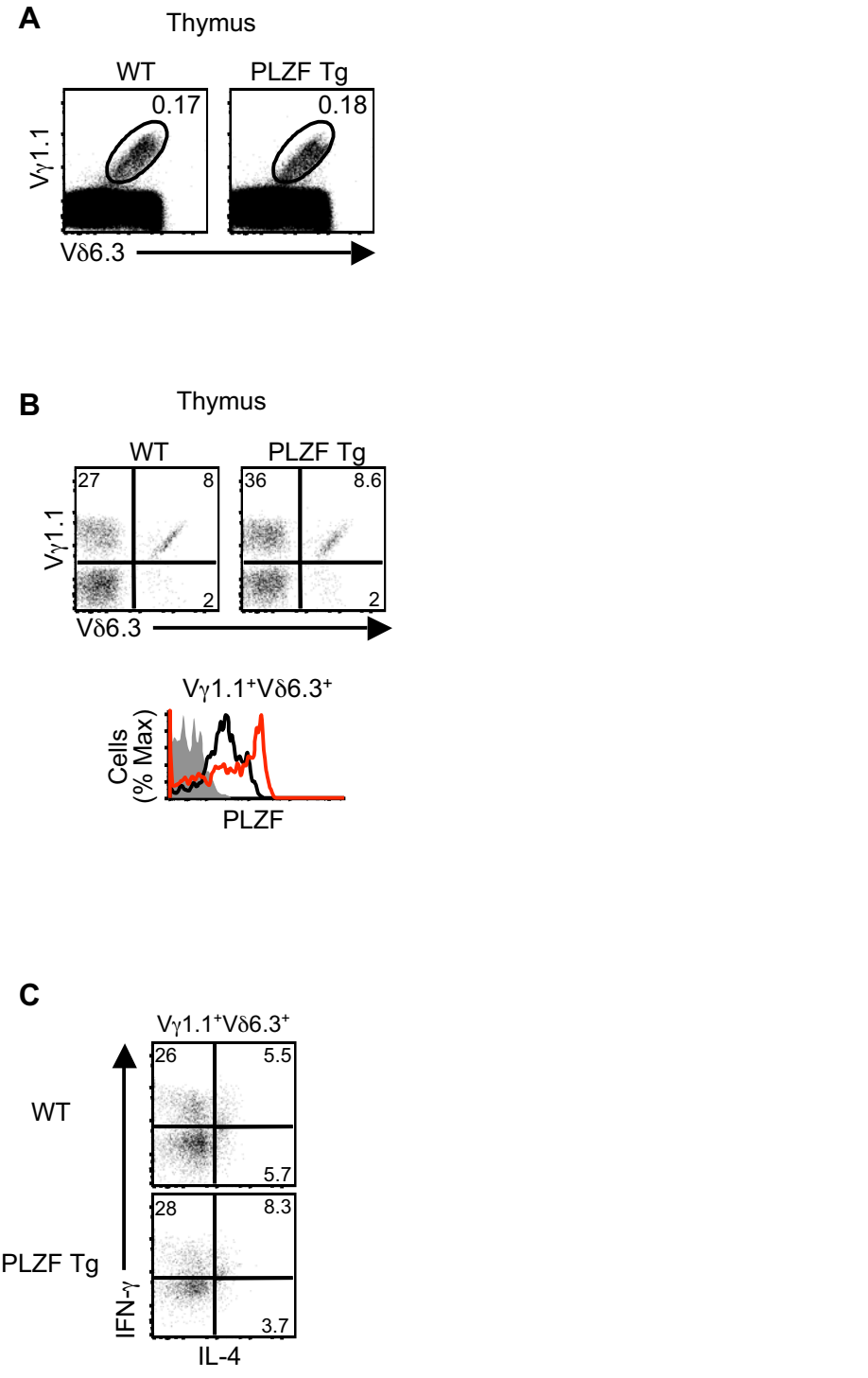



Figure S4 Alonzo et al

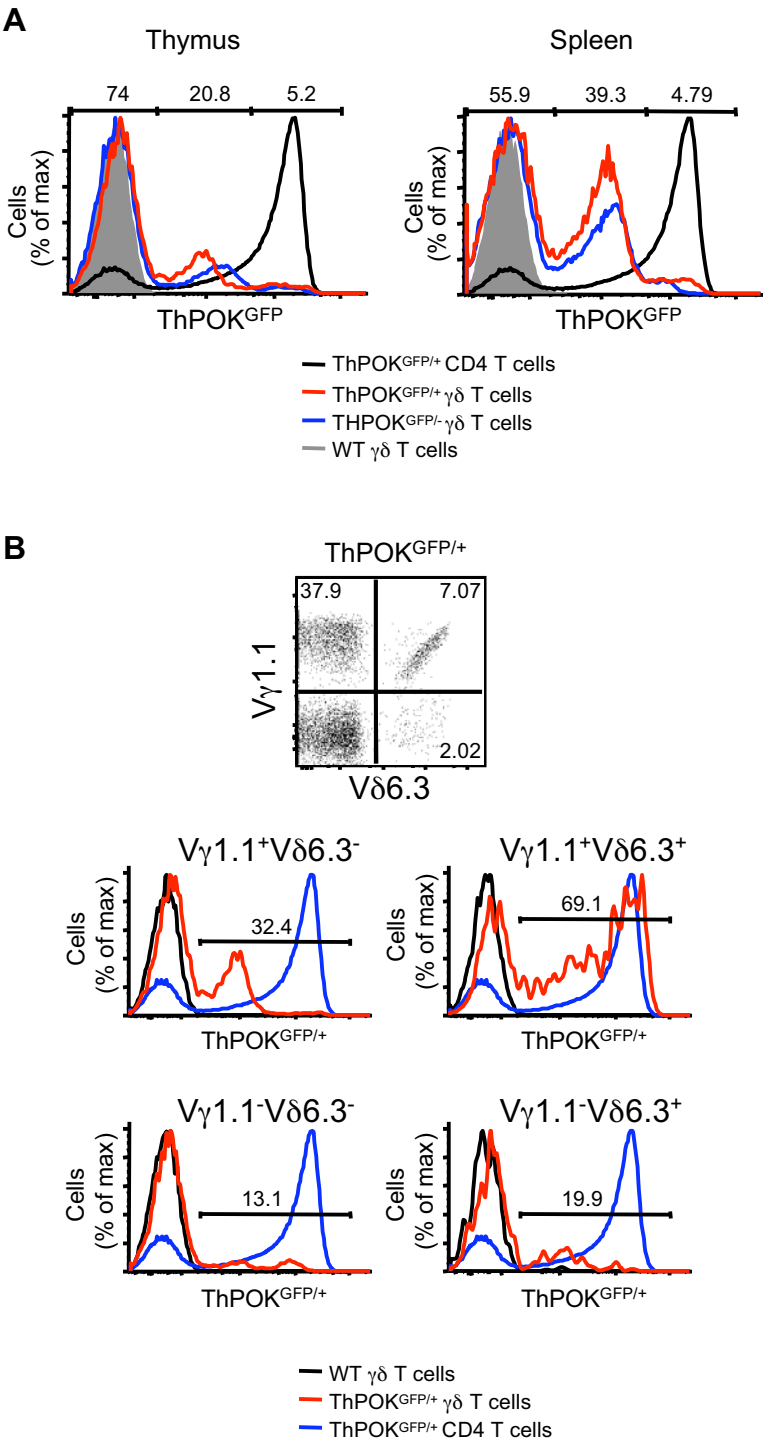



Figure S5 Alonzo et al

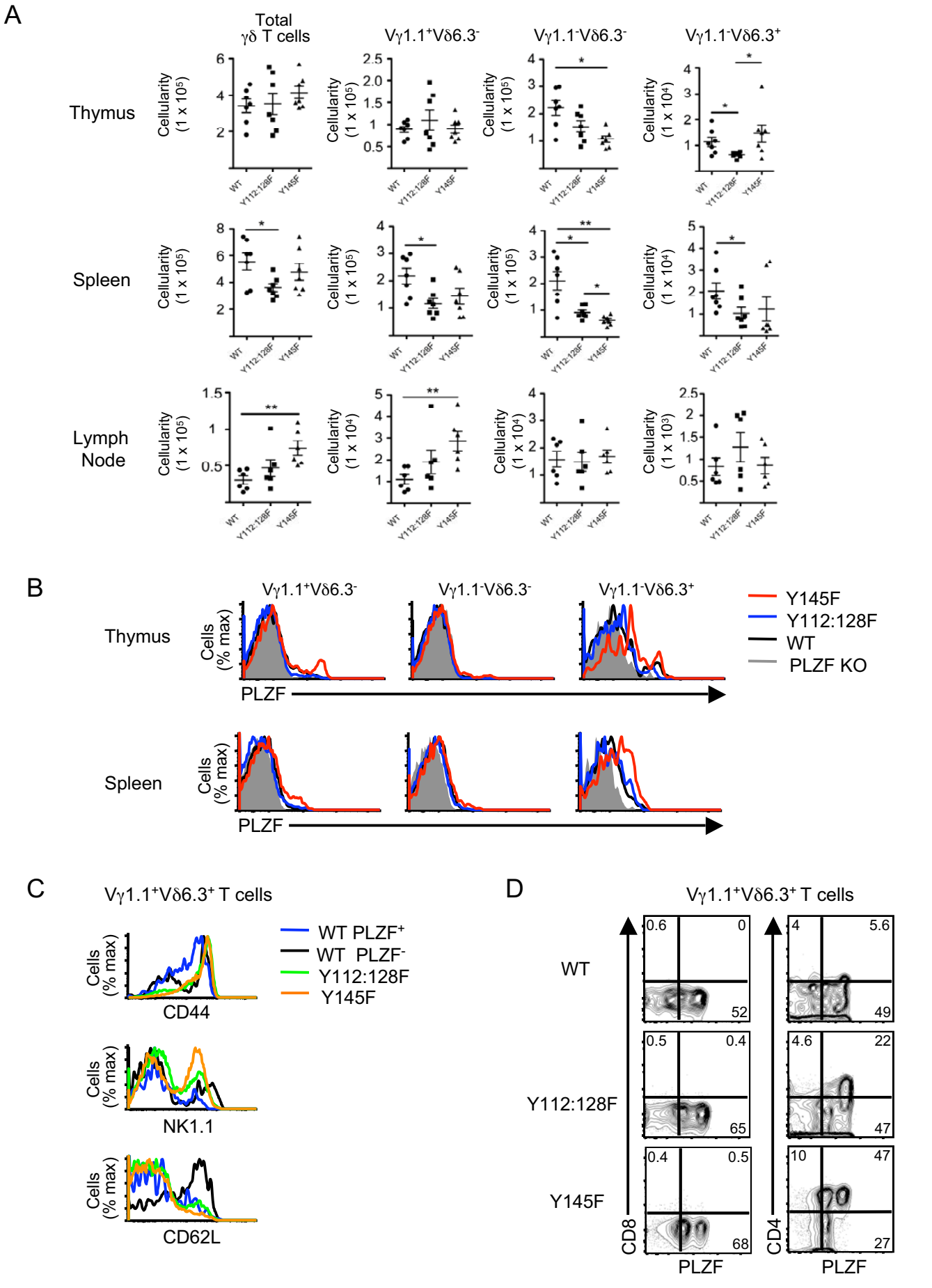
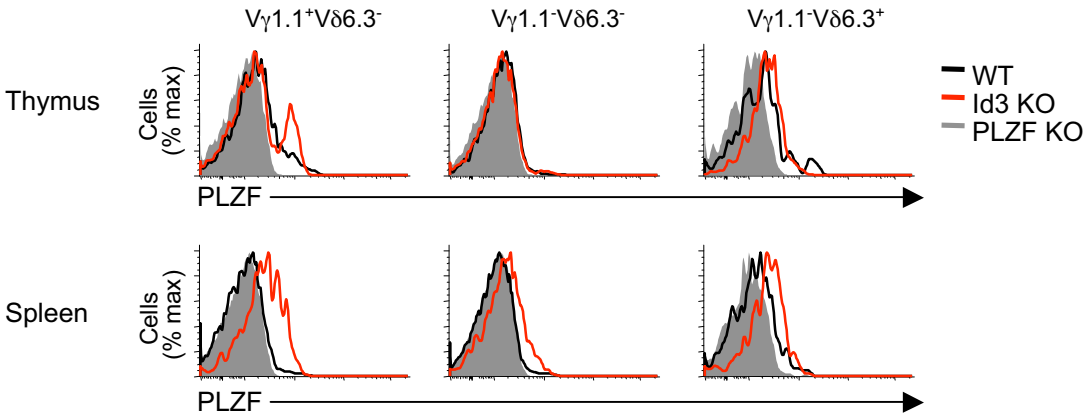
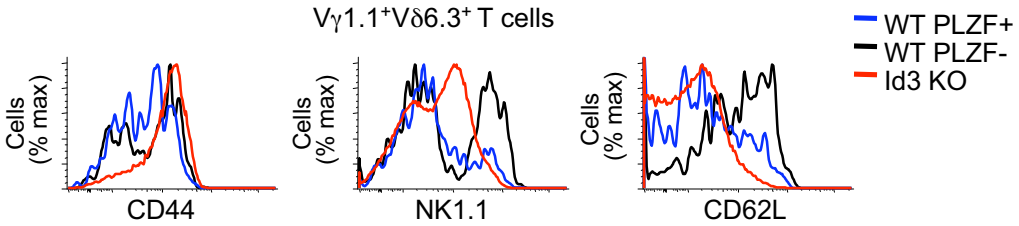



Figure S6 Alonzo et al

A




B




C

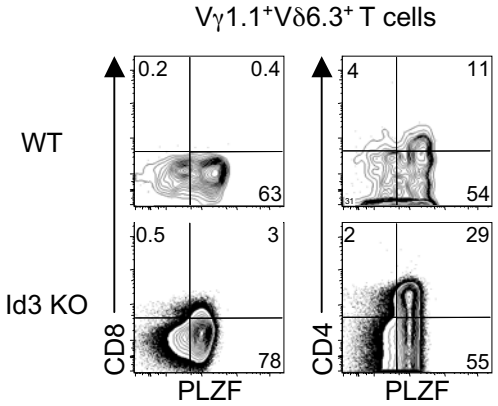



Figure S7 Alonzo et al

**Supplementary material.**

**Figure S1. PLZF expression in γδ T cells subsets.** Intracellular staining for PLZF in subpopulations of γδ T cells in (A) liver lymphocytes (liver), (B) intraepithelial lymphocytes (iIELs), (C) Lymph nodes from wild-type (WT) and PLZF-deficient (PLZF KO) mice. (D) Absolute numbers of total γδ T cells, and γδ T cell subsets from the lymph nodes of wild-type and PLZF-deficient mice. γδ T cells from PLZF-deficient mice were used as a negative control for PLZF staining. Numbers adjacent to outlined areas indicate percent of γδ T cell subsets (A-C) or bracketed lines in histograms specify percent total PLZF positive cells (A-C). Data are representative of more than four independent experiments. All flow cytometry plots are quantified in log10 fluorescence.

**Figure S2. Analysis of CD24 expression on developing Vγ1.1$^+$Vδ6.3$^+$ T cells.** γδ T cells enriched from the thymuses of 10-day-old wild-type were stained with anti-γδ TCR, anti-Vγ1.1, anti-Vδ6.3 TCR, anti-CD24 and anti-PLZF antibodies. Numbers above each bracket in the histogram indicate percent PLZF-positive Vγ1.1$^+$Vδ6.3$^+$ T cells. Vγ1.1$^+$Vδ6.3$^+$ T cells from PLZF-deficient mice served as a negative control for PLZF staining. Data are representative of three independent experiments. All flow cytometry plots are quantified in log10 fluorescence.

**Figure S3. PLZF expression in Vγ1.1$^+$Vδ6.3$^+$ T cells from TCRβ and CD1d-deficient mice**. (A) PLZF expression analysis in Vγ1.1$^+$Vδ6.3$^+$ T cells from wild-type (blue), TCRβ-deficient (red) and (B) CD1d-deficient (red) thymocytes and lymphocytes. Vγ1.1$^+$Vδ6.3$^+$ T cells from PLZF-deficient mice served as a negative control for PLZF staining. Data are

representative of three independent experiments. All flow cytometry plots are quantified in log10 fluorescence.

**Figure S4. Ectopic expression of PLZF has no effect on γδ T cell effector functions.** (A) Intracelluar staining for PLZF in γδ T cells as described, or (B) $V\gamma1.1^{+}V\delta6.3^{+}$ T cells in WT, PLZF-deficient and Lck-PLZF transgenic thymocytes. (C) Intracellular staining for IFN-γ and IL-4 from pooled splenocytes and lymphocytes in WT (top) and Lck-PLZF transgenic (bottom). Data are representative of at least three independent experiments. All flow cytometry plots are quantified in log10 fluorescence.

**Figure S5. ThPOK expression in γδ T cells.** (A) GFP expression analysis in total γδ T cells from the thymus and spleen from $ThPOK^{GFP/+}$ and $ThPOK^{GFP/-}$ mice. ThPOK expression in CD4 T cells from $ThPOK^{GFP/+}$ mice is shown as a control. Numbers above bracketed lines in histograms specify the percent of γδ T cells from the $ThPOK^{GFP/+}$ in each population as defined by GFP expression levels (negative, intermediate, or high). (B) $ThPOK^{GFP/+}$ reporter expression in subpopulations of γδ T cells in splenocytes that are $V\gamma1.1^{+}$ $V\delta6.3^{-}$, $V\gamma1.1^{+}V\delta6.3^{+}$, $V\gamma1.1^{-}V\delta6.3^{+}$, or $V\gamma1.1^{-}V\delta6.3^{-}$. Numbers in quadrants indicate the percent of $V\gamma1.1^{+}V\delta6.3^{-}$ (top left), $V\gamma1.1^{+}V\delta6.3^{+}$ (top right), $V\gamma1.1^{-}V\delta6.3^{+}$ (bottom right), and $V\gamma1.1^{-}V\delta6.3^{-}$ (bottom left) or above bracketed lines in histograms specify the percent total $ThPOK^{GFP/+}$ positive cells. Wild-type γδ T cells were used as a negative control for GFP expression (A and B). All flow cytometry plots are quantified in log10 fluorescence.

**Figure S6. Phenotypic analysis of non-$V\gamma1.1^{+}V\delta6.3^{+}$ T cells in SLP76 mutant mice.** (A) Absolute numbers of total γδ T cells and $V\gamma1.1^{+}$ $V\delta6.3^{-}$, $V\gamma1.1^{-}V\delta6.3^{-}$ and $V\gamma1.1^{-}V\delta6.3^{+}$ T

cells subsets in the thymus (top), spleen (middle) and lymph nodes (bottom) in wild-type (WT), Y112:128F and Y145F mutant mice. (B) Intracellular staining for PLZF in $V\gamma1.1^+$ $V\delta6.3^-$ (left), $V\gamma1.1^-V\delta6.3^-$ (middle) and $V\gamma1.1^-V\delta6.3^+$ (right) T cells in thymocytes (top) and splenocytes (bottom) in wild-type (WT; black), Y112:128F (blue) and Y145F (red) mutant mice. (**C**) Expression of CD44, NK1.1 and CD62L by PLZF-positive (blue) and PLZF-negative (black) wild-type (WT), Y112:128F (green), and Y145F (orange) $V\gamma1.1^+V\delta6.3^+$ thymocytes. (**D**) CD4 and CD8 expression on $V\gamma1.1^+V\delta6.3^+$ thymocytes in wild-type (WT; top), Y112:128F (middle) and Y145F (bottom) as compared to intracellular staining for PLZF. PLZF-deficient γδ T cells (gray) served as a negative control for PLZF staining in histograms (B). Each symbol represents an individual mouse (* $P = 0.05$). Error bars represent the SD. Data are representative of at least six experiments. All flow cytometry plots are quantified in log10 fluorescence.

**Figure S7. Phenotypic analysis of non-$V\gamma1.1^+V\delta6.3^+$ T cells in Id3-deficient mice.** (A) (A) Intracellular staining for PLZF in $V\gamma1.1^+$ $V\delta6.3^-$ (left), $V\gamma1.1^-V\delta6.3^-$ (middle) and $V\gamma1.1^-$ $V\delta6.3^+$ (right) T cells in thymocytes (top) and splenocytes (bottom) in wild-type (WT; black), Id3-deficient (Id3 KO) mice. (B) Expression of CD44, NK1.1 and CD62L by PLZF-positive (blue) and PLZF-negative (black) wild-type (WT) and Id3-deficient (Id3 KO; red) $V\gamma1.1^+V\delta6.3^+$ thymocytes. (D) CD4 and CD8 expression on $V\gamma1.1^+V\delta6.3^+$ thymocytes in wild-type (WT; top) and Id3-deficient (Id3 KO; red) as compared to intracellular staining for PLZF. PLZF-deficient γδ T cells (gray) served as a negative control for PLZF staining in histograms (B). Data are representative of at least four experiments. All flow cytometry plots are quantified in log10 fluorescence.