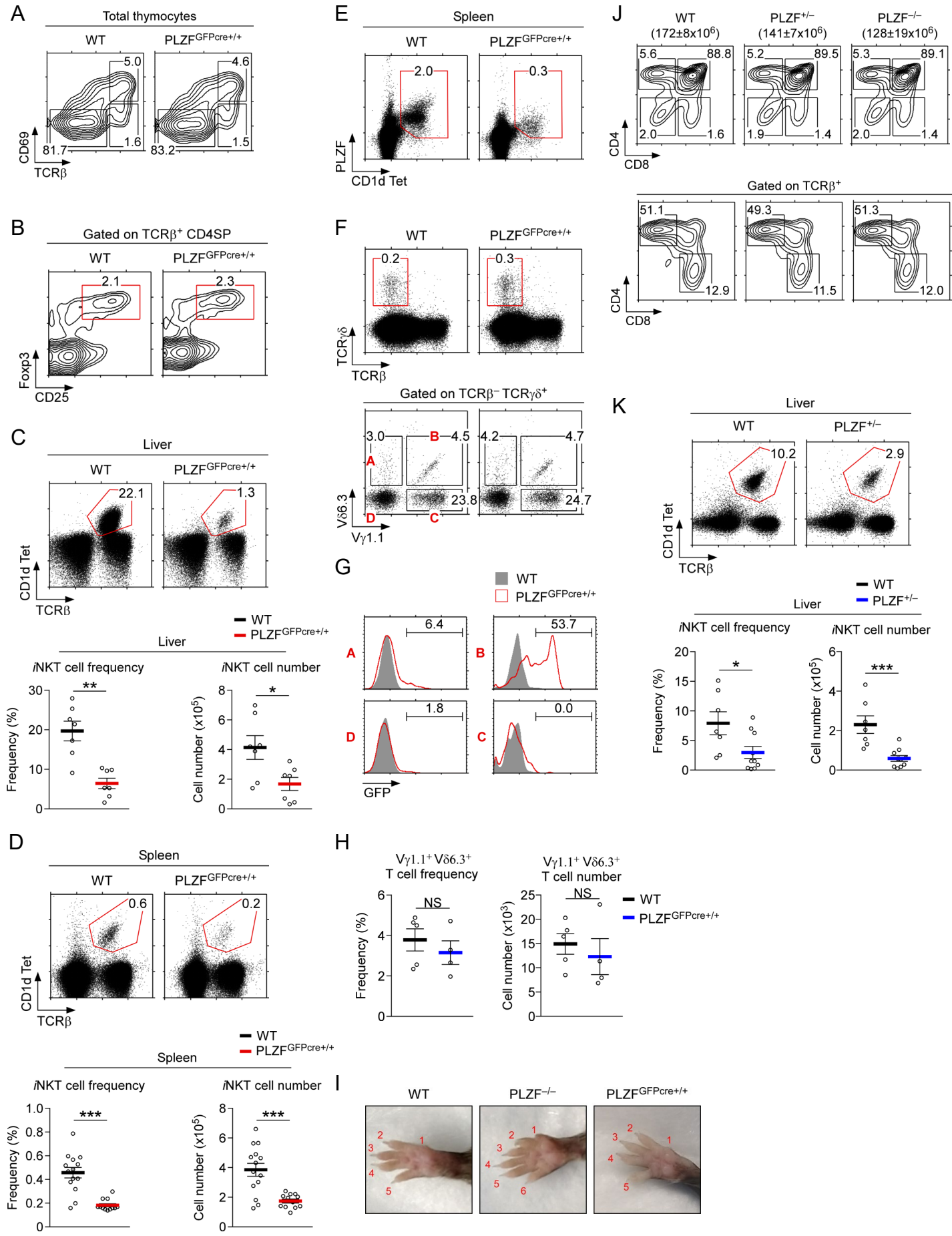
Suppl. Figure 1



Suppl. Figure 1. *i*NKT cell development in PLZF^{GFPcre} knock-in mice. Related to Figure 1 and Figure 2.

(**A**) CD69 versus TCRβ expression of WT and PLZF$^{GFPcre+/+}$ total thymocytes.

(**B**) Foxp3^{+}CD25^{+} Treg cells among CD4SP thymocytes of WT and PLZF$^{GFPcre+/+}$ mice.

(**C, D**) Liver and spleen *i*NKT cells were identified by CD1dTet and anti-TCRβ antibody staining. Graphs show frequencies (bottom, left) and numbers (bottom, right) of *i*NKT cells in C57BL/6 wildtype (WT) and PLZF$^{GFPcre+/+}$ mice. Results show summary of 7 independent experiments.

(**E**) PLZF expression in spleen *i*NKT cells of WT and PLZF$^{GFPcre+/+}$ mice. Dot plots are representative of 3 independent experiments.

(**F**) Frequencies of γδ T cells (top) and Vγ1.1Vd6.3 γδ T cells (bottom) among thymocytes of the indicated mice. Results show summary of 3 independent experiments with total 5 WT and 4 PLZF$^{GFPcre+/+}$ mice.

(**G**) PLZF^{GFPcre} reporter expression in thymic γδ T cell subsets, identified by distinct usages of Vγ1.1 and Vδ6.3.

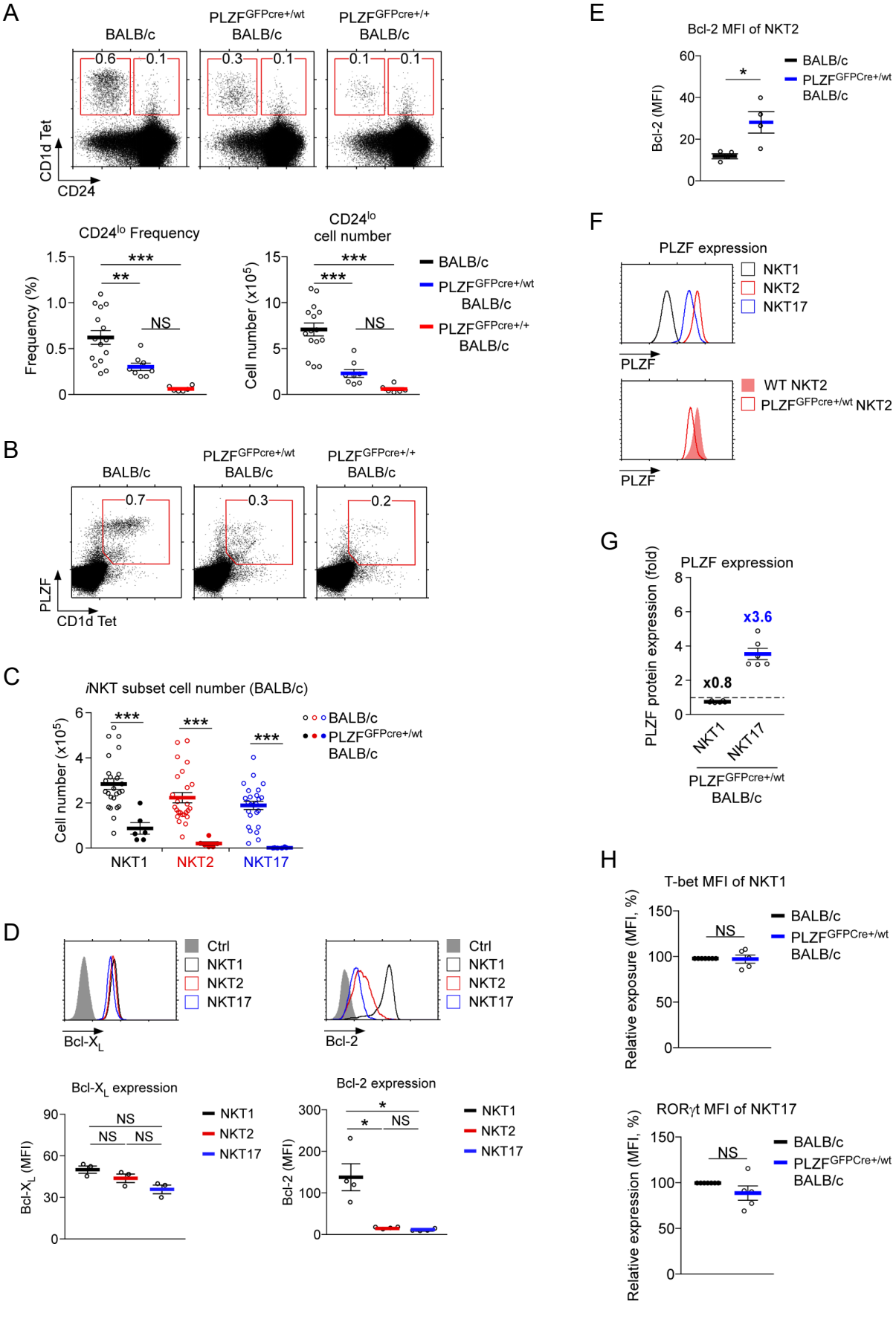
(**H**) Frequencies and cell numbers of thymic Vγ1.1Vδ6.3 γδ T cells of indicated mice. Results show summary of 3 independent experiments with total 5 WT and 4 PLZF$^{GFPcre+/+}$ mice.

(**I**) Images of front limbs of the indicated mouse strains. Numbers indicate finger digits for each mouse.

(**J**) CD4 versus CD8 profiles of total (top) and TCRβ^{+}-gated thymocytes (bottom) of the indicated mice. Results show summary of 8 independent experiments with total 12 WT, 11 PLZF$^{+/-}$, and 5 PLZF$^{-/-}$ mice.

(**K**) Liver *i*NKT cells in PLZF$^{+/-}$ mice. *i*NKT cells were identified by CD1dTet and anti-TCRβ antibody staining (top), and frequency and number of liver *i*NKT cells were determined for the indicated mouse strains (bottom). Results are summary of 8 independent experiments.

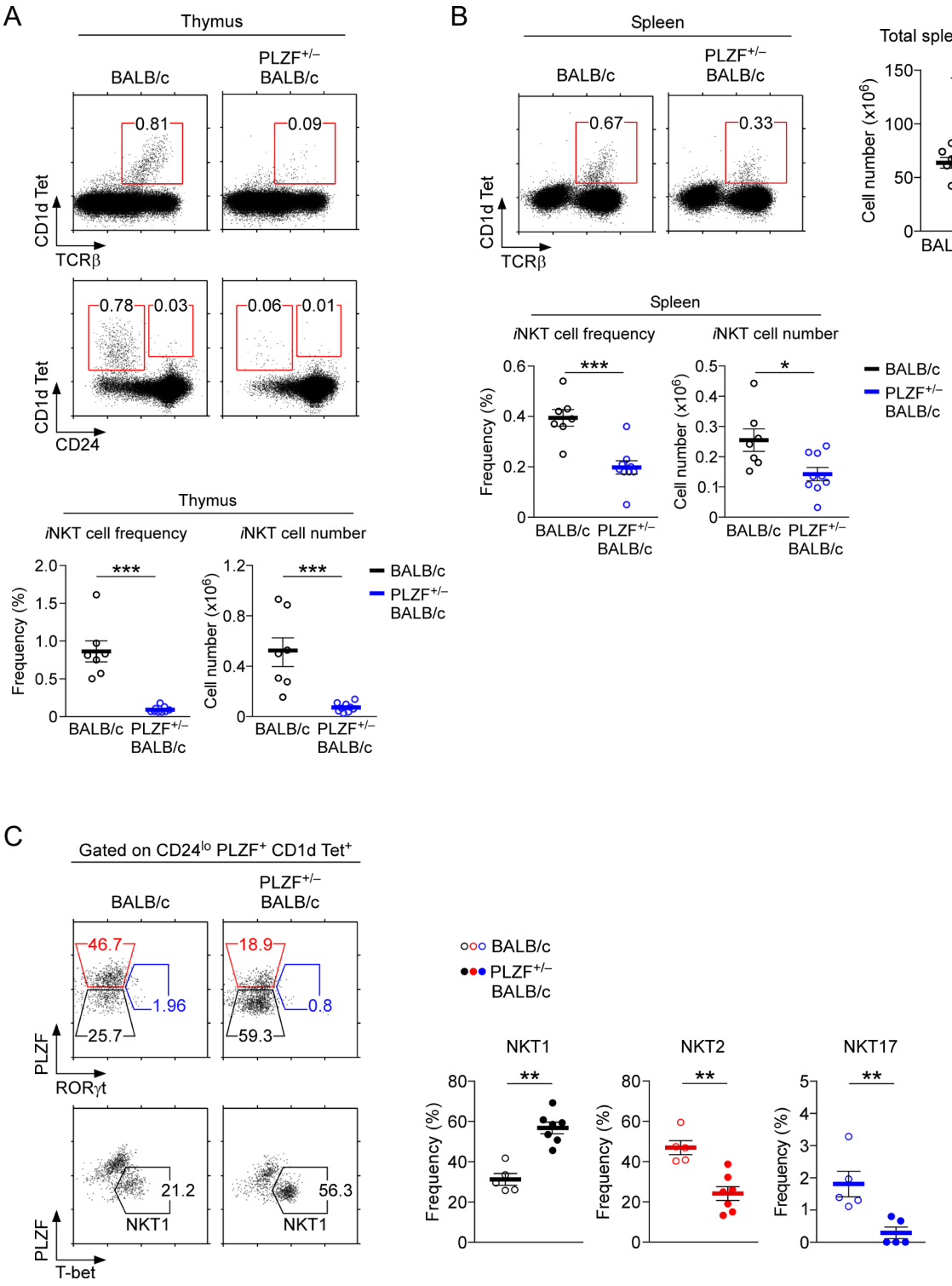
Suppl. Figure 2



Suppl. Figure 2. *i*NKT cell development in PLZF^{GFPcre} BALB/c mice. Related to Figure 3.

(**A**) Mature (CD24^{lo}) thymic *i*NKT cells were identified in BALB/c, PLZF$^{GFPcre+/wt}$ BALB/c and PLZF$^{GFPcre+/+}$ BALB/c mice (top). Frequencies (bottom left) and numbers (bottom right) of CD24^{lo} thymic *i*NKT cells were determined from at least 6 independent experiments with 15 BALB/c, 8 PLZF$^{GFPcre+/wt}$ BALB/c and 6 PLZF$^{GFPcre+/+}$ BALB/c mice.
(**B**) Intracellular PLZF expression in thymic *i*NKT cells of indicated mice. Dot plots are representative of 3 independent experiments.
(**C**) Cell numbers of individual *i*NKT subsets in BALB/c and PLZF$^{GFPcre+/wt}$ BALB/c thymocytes. Results show summary of 6 independent experiments with 25 BALB/c and 6 PLZF$^{GFPcre+/wt}$ BALB/c mice.
(**D**) Intracellular staining for Bcl-x_L and Bcl-2 in individual *i*NKT subsets of BALB/c thymocytes (top). Graphs show summary of 3 independent experiments with total 4 BALB/c and 4 PLZF$^{GFPcre+/wt}$ BALB/c mice
(**E**) Relative abundance of Bcl-2 proteins in thymic NKT2 cells of BALB/c and PLZF$^{GFPcre+/wt}$ BALB/c mice. Graph is summary of 3 independent experiments with total 4 BALB/c and 4 PLZF$^{GFPcre+/wt}$ BALB/c mice.
(**F**) Intracellular staining for PLZF in *i*NKT subsets of BALB/c thymocytes (top) and in thymic NKT2 cells from WT BALB/c and PLZF$^{GFPcre+/wt}$ BALB/c mice (bottom). Histograms are representative of 6 independent experiments.
(**G**) Relative PLZF expression in NKT1 and NKT17 subsets of PLZF$^{GFPcre+/wt}$ BALB/c thymocytes, which was determined as fold difference to PLZF expression in NKT1 cells of WT BALB/c. Results show summary of 6 independent experiments with 25 BALB/c and 6 PLZF$^{GFPcre+/wt}$ BALB/c mice.
(**H**) Relative abundance of T-bet (top) and RORγt proteins (bottom) in NKT1 and NKT17 cells of BALB/c and PLZF$^{GFPcre+/wt}$ BALB/c thymocytes, respectively. Data are summary of 5 independent experiments with total 7 BALB/c and 5 PLZF$^{GFPcre+/wt}$ BALB/c mice.

Suppl. Figure 3



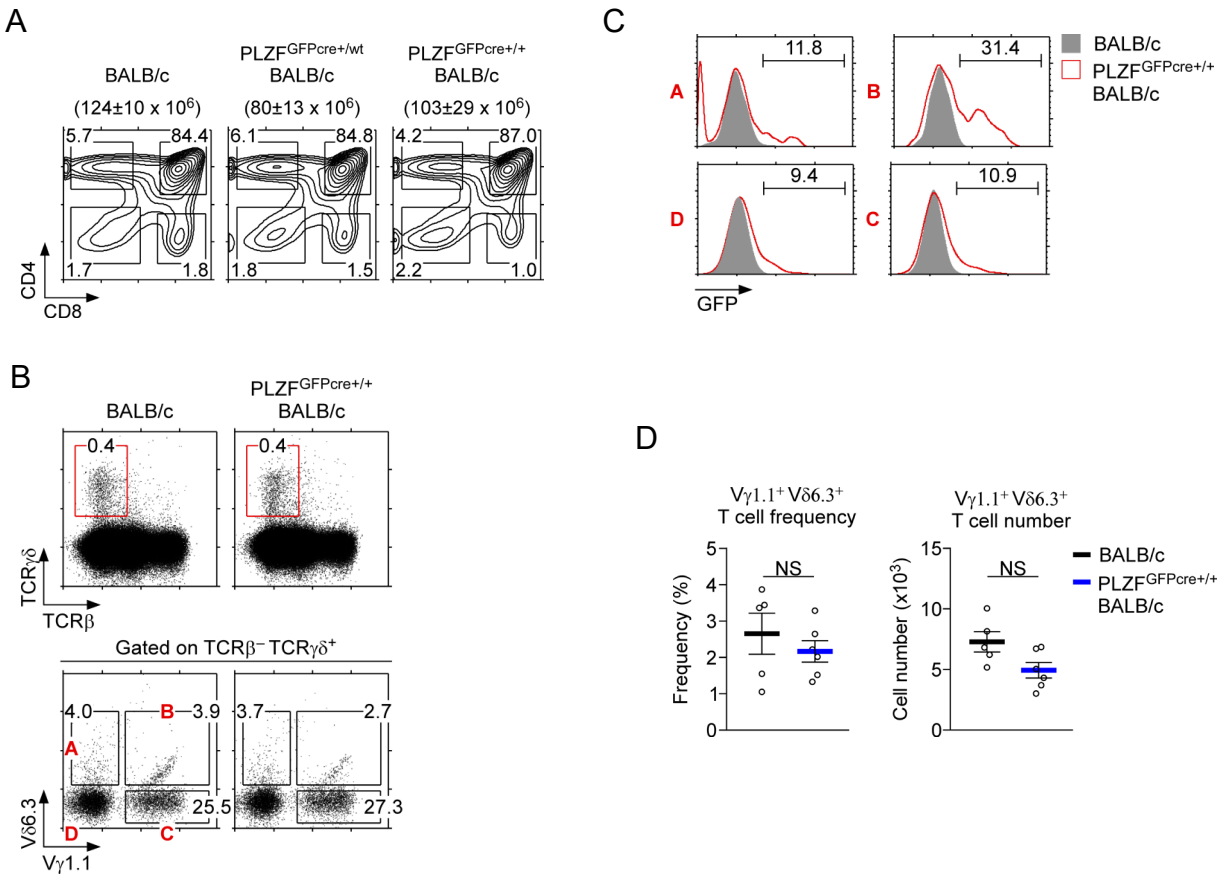
Suppl. Figure 3. *i*NKT cells in PLZF$^{+/-}$ BALB/c thymocytes and spleen cells. Related to Figure 3.

(**A**) Development of total (top) and mature (CD24^{lo}) thymic *i*NKT cells (middle) was assessed from thymocytes of PLZF$^{+/-}$ BALB/c and littermate WT BALB/c mice (top). Frequencies (bottom left) and numbers (bottom right) of thymic *i*NKT cells were determined from 4 independent experiments with total 7 BALB/c and 9 PLZF$^{+/-}$ BALB/c mice.

(**B**) Frequency and number of spleen *i*NKT cells were determined from total splenocytes of PLZF$^{+/-}$ BALB/c and littermate WT BALB/c mice. Results show summary of 4 independent experiments with total 7 BALB/c and 9 PLZF$^{+/-}$ BALB/c mice.

(**C**) *i*NKT subset composition of splenic *i*NKT cells of PLZF$^{+/-}$ BALB/c and littermate WT BALB/c mice. Dot plots are representative (left) and graphs (right) show summary of 3 independent experiments with total 5 BALB/c and 7 PLZF$^{+/-}$ BALB/c mice.

Suppl. Figure 4



Suppl. Figure 4. Thymocyte development in PLZF^{GFPcre} BALB/c mice. Related to Figure 4.

(**A**) CD4 versus CD8 profiles and total thymocyte numbers of the indicated mice. Results show summary of 3 independent experiments with total 13 BALB/c, 9 PLZF$^{GFPcre+/wt}$ BALB/c, and 3 PLZF$^{GFPcre+/+}$ BALB/c mice.

(**B**) Frequencies of γδ T cells (top) and Vγ1.1Vδ6.3 γδ T cells (bottom) among thymocytes of the indicated mice. Results show summary of 3 independent experiments with total 5 BALB/c and 6 PLZF$^{GFPcre+/wt}$ BALB/c mice.

(**C**) PLZF^{GFPcre} reporter expression in thymic γδ T cell subsets, as identified by distinct usages of Vγ1.1 and Vδ6.3.

(**D**) Frequencies and cell numbers of thymic Vγ1.1Vδ6.3 γδ T cells of indicated mice. Results show summary of 3 independent experiments with total 5 BALB/c and 6 PLZF$^{GFPcre+/+}$ BALB/c mice.