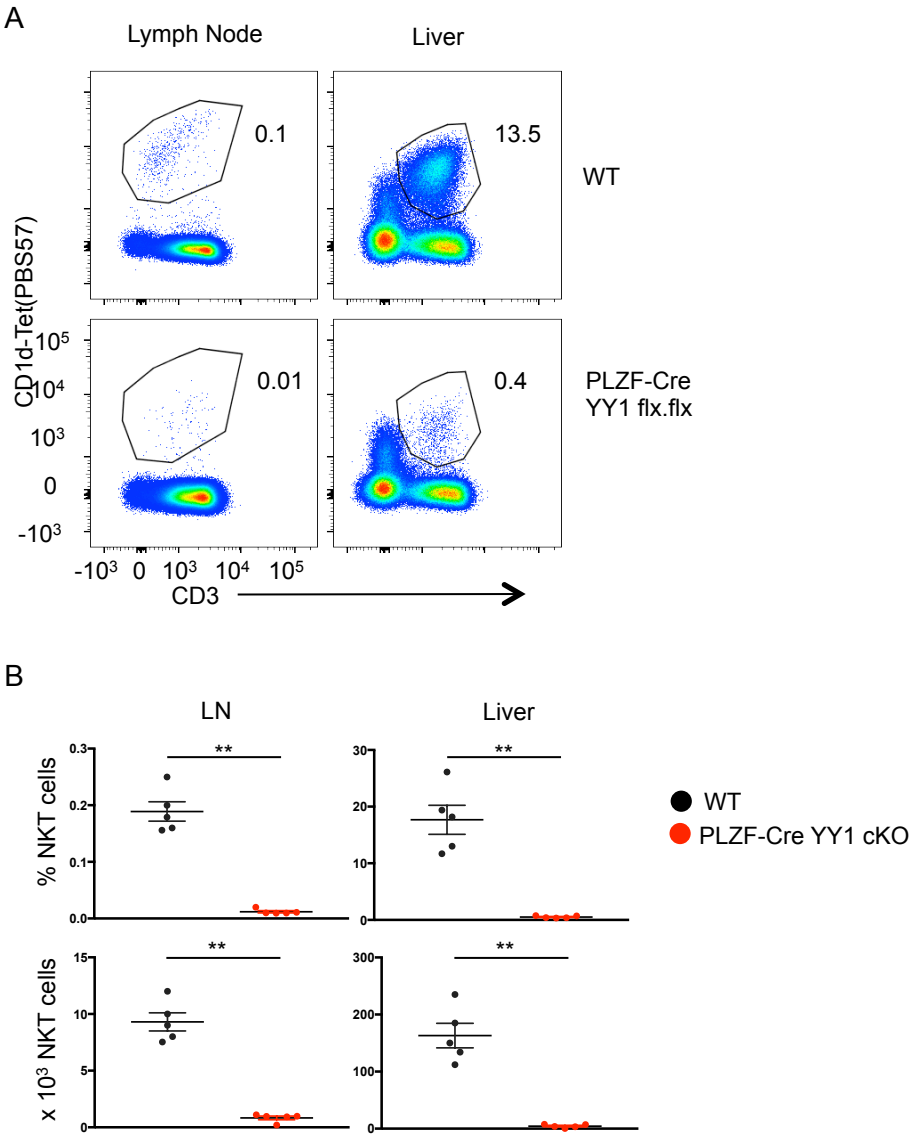

Figure S1. NKT cell frequency and absolute numbers in the liver and lymph nodes of PLZF-cre YY1 flx.flx mice. (Related to Figure 1) (**A, B**) Leukocytes were isolated from the lymph nodes (left) and livers (right) of C57BL/6 or PLZF-Cre YY1 flx.flx mice. The frequency and absolute number of NKT cells (MHCII$^-$, CD3$^+$, CD1dtet$^+$) in each tissue was determined by FACS. A representative FACs plot is shown in (A) and compiled data concerning the frequency (B, top) and absolute number (B, bottom) of NKT cells are in (B). Graphs show compiled data from 5 (B) mice, examined in 3 or more independent experiments. The horizontal lines indicate the mean (±s.e.m.). **P<0.01 determined by Mann-Whitney U Test (B).

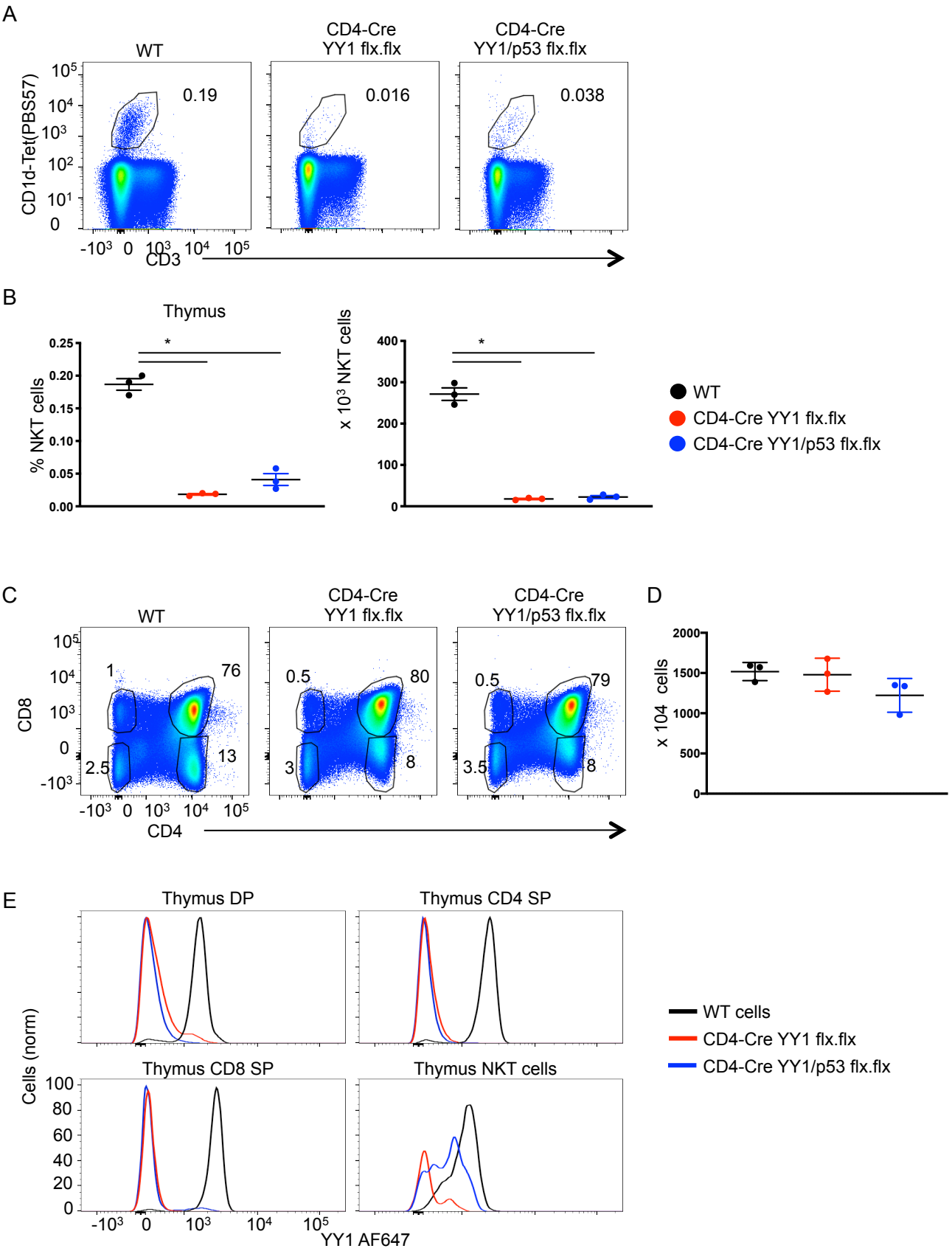

Figure S2. NKT cell frequency and absolute numbers in CD4-Cre YY1 flx.flx mice. (Related to Fig. 1)
(A-E) Thymocytes were isolated from C57BL/6 mice, CD4-Cre YY1 flx.flx mice or CD4-Cre YY1/p53 flx.flx mice and analyzed by FACS with the indicated antibodies. Representative FACS plots showing the frequency of NKT cells (MHCII$^-$, CD3$^+$, CD1dtet$^+$,) in each mouse strain are shown in (A). The frequency (B, left) and absolute number of NKT cells (B, right) are shown in (B). Expression of CD4 and CD8 by thymocytes (MHCII-) isolated from the indicated mouse strains is depicted in (C). The total number of cells in the thymus of each indicated mouse strain is displayed in (D). The expression of YY1 in thymocytes at different stages of development in each mouse strain is shown in (E). Graphs show compiled data from 3 mice, examined in 3 independent experiments. The horizontal lines indicate the mean (±s.e.m.). *P<0.05 determined by One way anova (B, D).

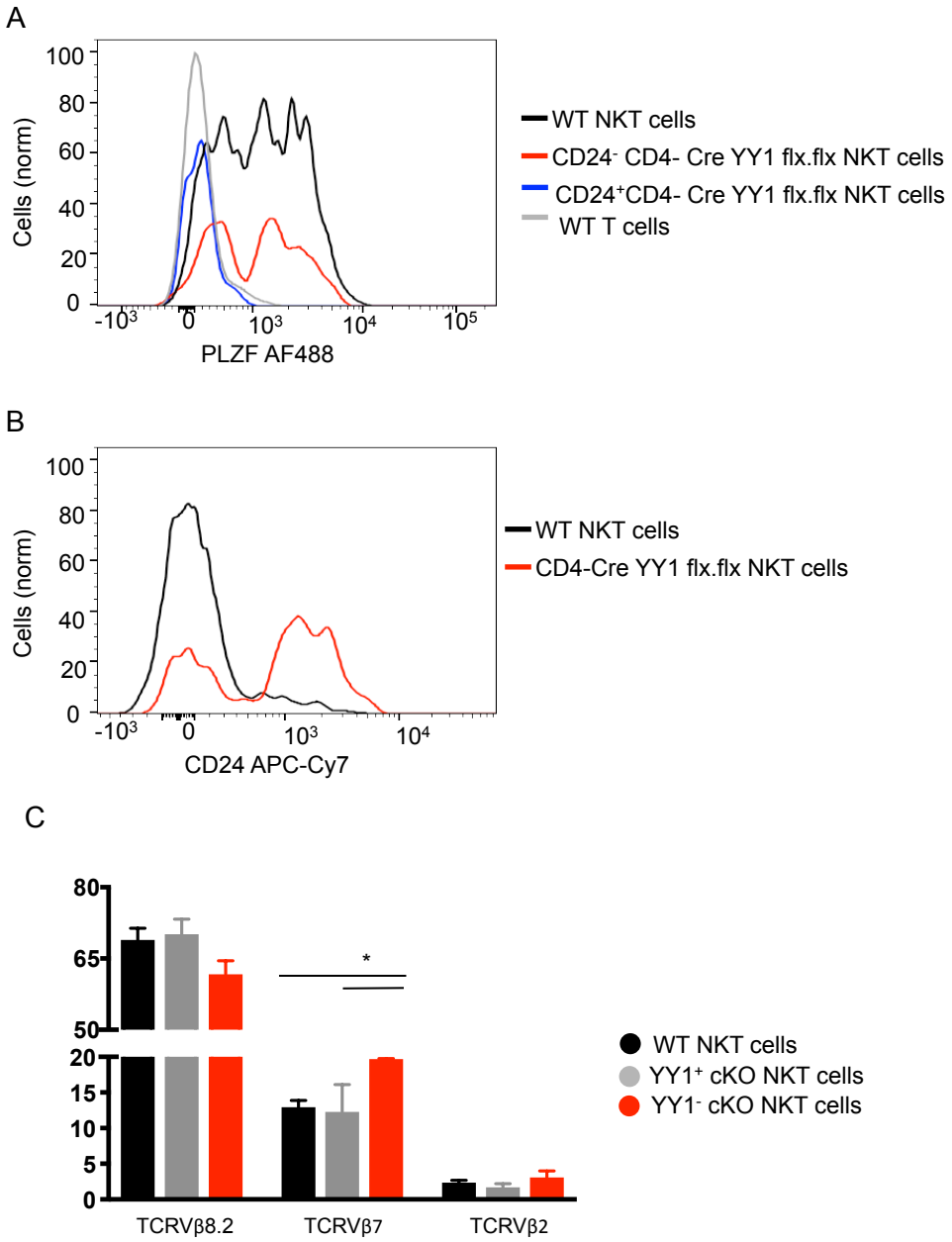

Figure S3. YY1 deficient NKT cells isolated from CD4-Cre YY1 flx.flx mice express PLZF (Related to Figure 3) (A-B) Thymocytes were isolated from C57BL/6 (WT) mice or CD4-Cre YY1 flx.flx mice and analyzed by FACS with the indicated antibodies. (A) A representative histogram of PLZF expression in WT NKT cells (MHCII$^-$, CD3$^+$, CD1dtet$^+$, CD24$^-$), YY1 deficient, CD24$^-$ NKT cells (MHCII$^-$, CD3$^+$, CD1dtet$^+$, CD24$^-$, YY1$^-$) from CD4-Cre YY1 flx.flx mice, YY1 deficient, CD24$^+$ NKT cells (MHCII$^-$, CD3$^+$, CD1dtet$^+$, CD24$^+$, YY1$^-$) from CD4-Cre YY1 flx.flx mice, and WT T cells (MHCII$^-$). (B) A representative histogram comparing CD24 expression on WT NKT cells (MHCII$^-$, CD3$^+$, CD1dtet$^+$) and YY1 deficient NKT cells (MHCII$^-$, CD3$^+$, CD1dtet$^+$, YY1$^-$) isolated from CD4-Cre YY1 flx.flx mice. (C) TCRVβ usage on wildtype NKT cells, YY1$^+$ cKO NKT cells, and YY1$^-$ cKO NKT cells isolated from the thymus was measured by FACs. Data in (A-C) are representative of 3 mice from 3 independent experiments. The horizontal lines indicate the mean (±s.e.m.). *P<0.05 determined by One way Anova (C).

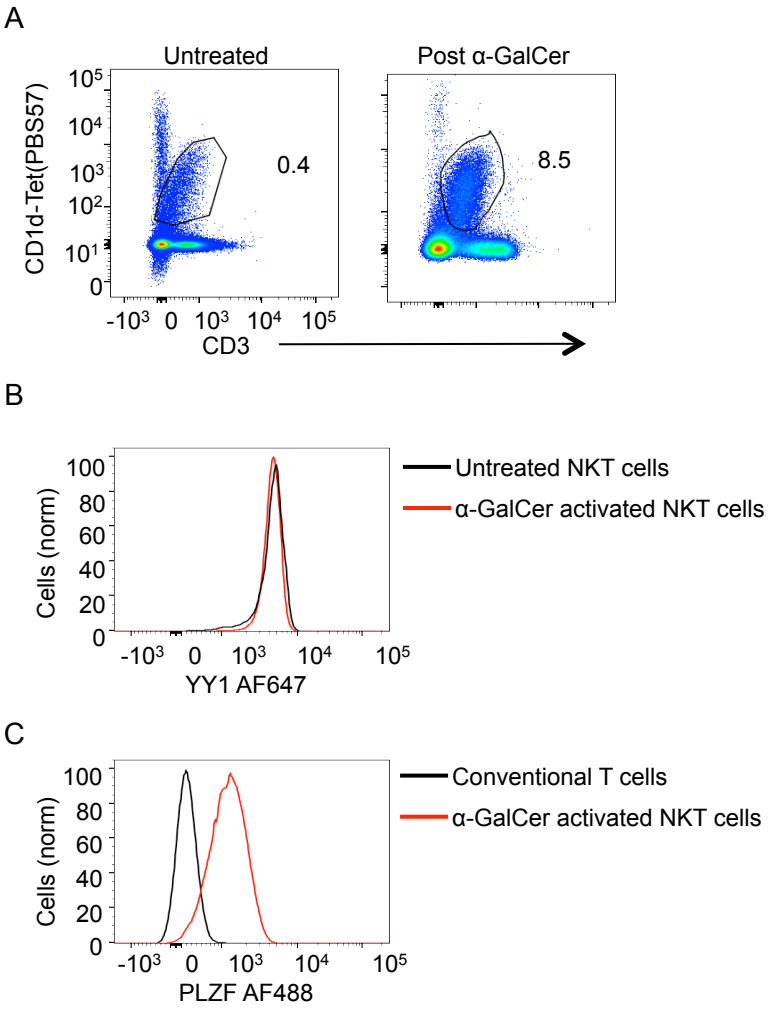

Figure S4. NKT cells expand in response to α-GalCer treatment and do not lose expression of YY1 or PLZF. (Related to Fig. 7) C57BL/6 (WT) mice were injected intravenously with α-GalCer. Mice were sacrificed 72 hours after injection. Spleens were analyzed by FACS using the indicated antibodies. (A) The frequency of NKT cells ($CD3^+$, $CD1dtet^+$) increased 20 fold in response to α-GalCer treatment. (B) YY1 expression was indistinguishable between untreated and α-GalCer treated NKT cells. (C) α-GalCer treated NKT cells maintain PLZF expression.