Supplemental Information:

The differentiation of ROR-γt expressing iNKT17 cells is orchestrated by Runx1

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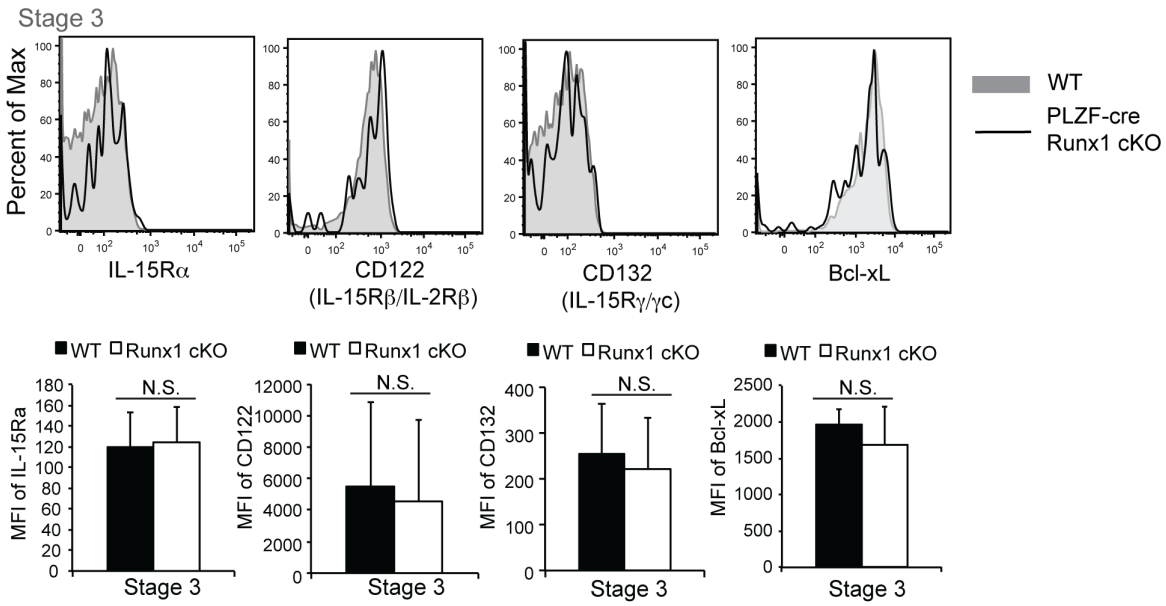
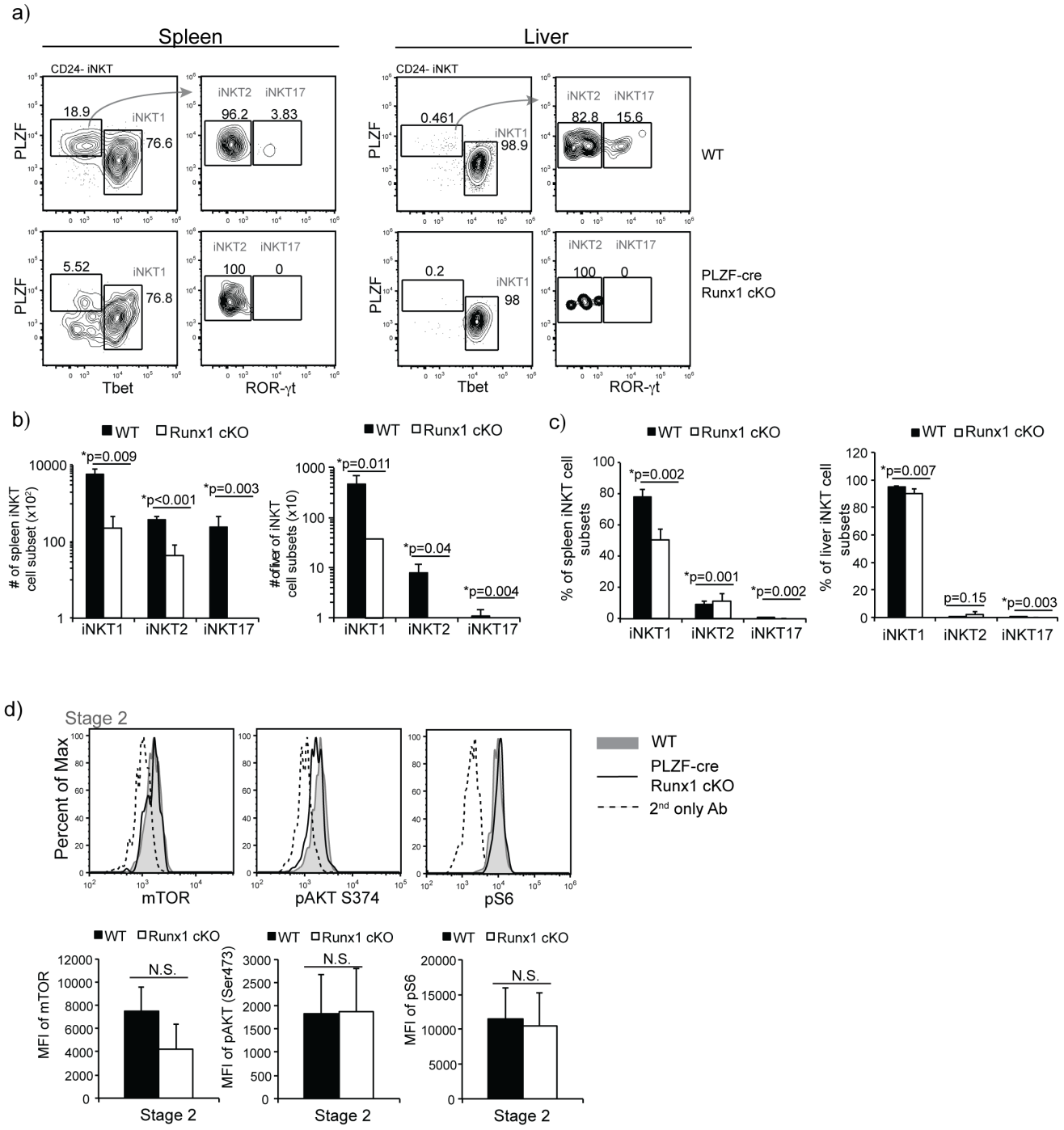


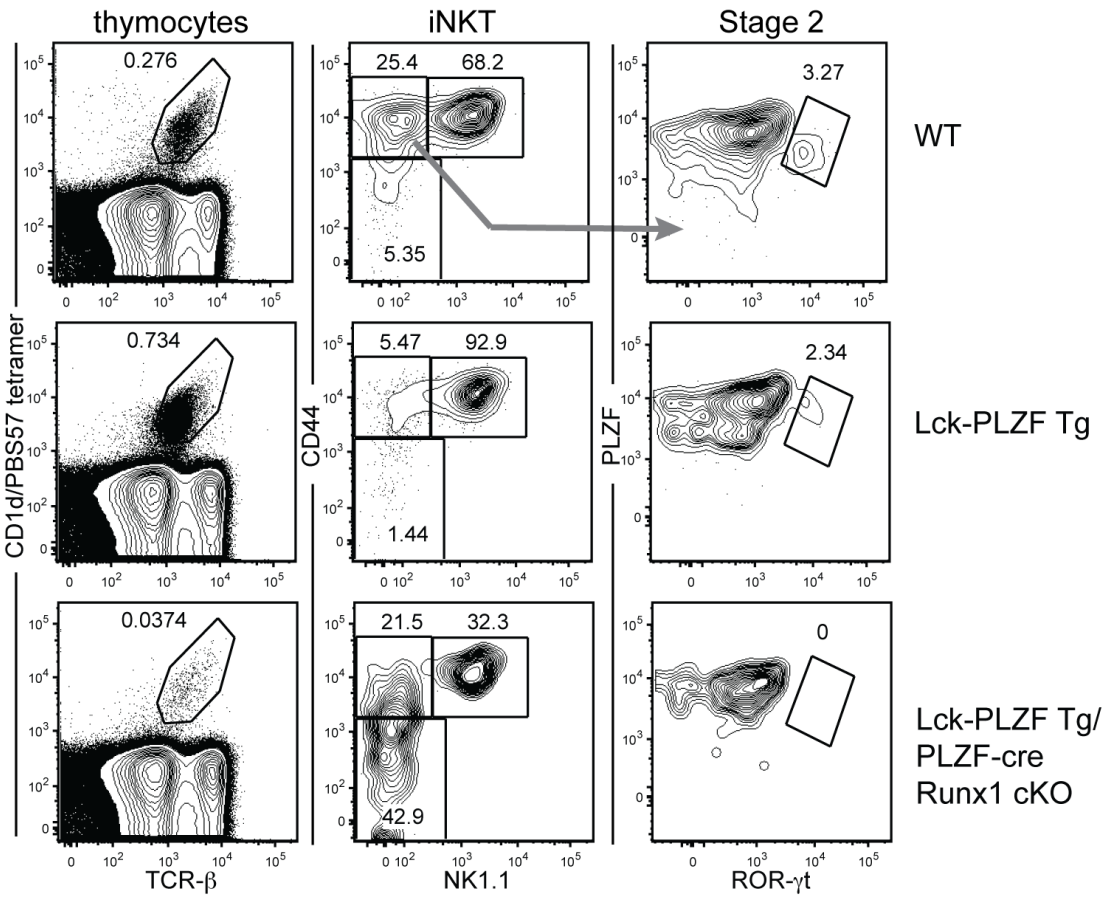
Supplemental Figure 1: Runx1-deficient Stage 3 iNKT cells have normal expression of IL-15R and Bcl-xL.

(**a**) Expression of IL-15 receptor subunits: IL-15Rα, IL-15Rβ (IL-2Rβ, CD122), IL-15Rγ (γc, CD132), and pro-survival gene Bcl-xL in Stage 3 iNKT cells of WT (grey filled) and PLZF-cre Hdac3 cKO (black line) mice. Data is representative of at least 3 mice/genotype from 3 independent experiments. Quantification of MFI for IL-15Rα, IL-15Rβ (IL-2Rβ, CD122), IL-15Rγ (γc, CD132), and Bcl-xL in Stage 3 iNKT cells of WT (black bar) and PLZF-cre Hdac3 cKO (white bar) mice. Data is calculated from at least 3 mice/genotype from 3 independent experiments. All statistical analysis was done using Student's *t-test.* Means ± S.E.M.



Supplemental Figure 2: Absence of peripheral iNKT17 cells in the spleen and liver of PLZF-cre Runx1 cKO mice.

(**a**) FACS analysis of splenic and liver iNKT1, iNKT2 and iNKT17 cells of WT (top) and PLZF-cre Runx1 cKO (bottom) mice. Gating for functional subsets were done as defined in Figure 4, using PLZF, Tbet and ROR-γt. Data is representative of at least 11 mice/genotype for spleen and 9 mice/genotype for liver. (**b**) Absolute number of iNKT cell subsets (iNKT1, iNKT2 and iNKT17) in spleen and liver of WT (black bars) and PLZF-cre Runx1 cKO (white bars) mice. Data is calculated from at least 11 mice/genotype for spleen and 9 mice/genotype for liver. (**c**) Frequency of iNKT cell subsets (iNKT1, iNKT2 and iNKT17) in spleen and liver of WT (black bars) and PLZF-cre Runx1 cKO (white bars) mice. Data is calculated from at least 11 mice/genotype for spleen and 9 mice/genotype for liver. (**d**) Expression of mTOR, pAKT (Ser374), and pS6 in Stage 2 iNKT cells of WT (grey filled), PLZF-cre Hdac3 cKO (black line) mice and Secondary antibody only control (dashed line). Data is representative of at least 4 mice/genotype from 4 independent experiments. Quantification of MFI for mTOR, pAKT (Ser374), and pS6 in Stage 2 iNKT cells of WT (black bar) and PLZF-cre Hdac3 cKO (white bar) mice. Data is calculated from at least 4 mice/genotype from 4 independent experiments. All statistical analysis was done using Student's *t-test.* Means ± S.E.M.



Supplemental Figure 3: Ectopic expression of PLZF in Runx1-deficient iNKT cells does not rescue block in iNKT17 differentiation. FACS analysis of iNKT cell development and ROR-γt expression in Stage 2 iNKT cells in WT, Lck-PLZF Tg and Lck-PLZF Tg/PLZF-cre Runx1 cKO mice. Analysis of iNKT cells was performed as described in Fig 1. Data is representative of 3 mice/genotype from 3 independent experiments.