Supplemental Figure S1

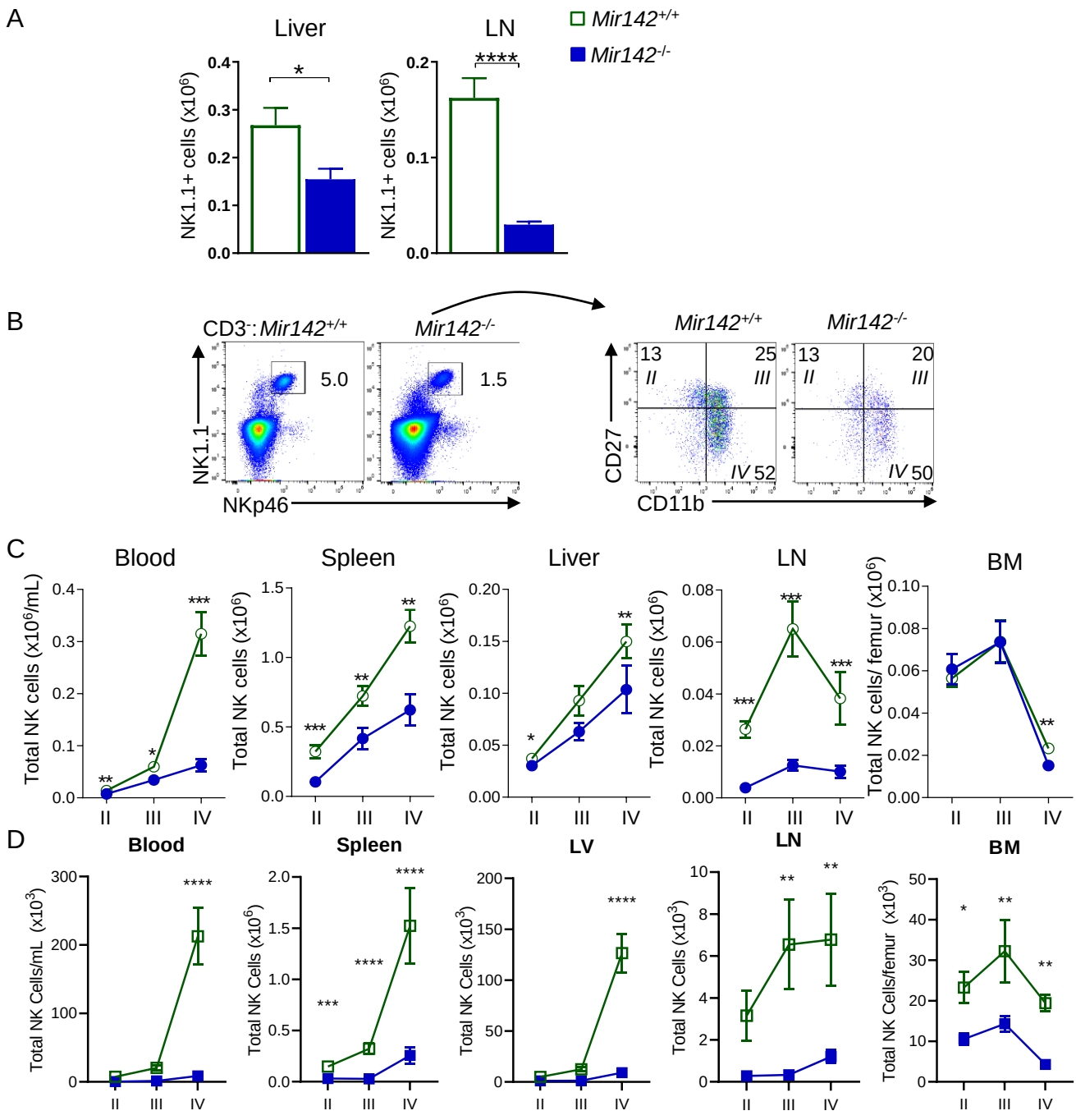

Figure S1. Mir142 deficiency reduces peripheral CD3$^-$NK1.1$^+$ numbers without altering maturation (related to Fig. 1). (**A**) Liver and lymph nodes (LN) from age- and sex-matched *Mir142*$^{+/+}$ (control, green) and *Mir142*$^{-/-}$ (blue) were harvested and assessed for the presence NK cells (CD45$^+$CD3$^-$NK1.1$^+$NKp46$^+$) by flow cytometry. Summary data from 3 independent experiments with 6-9 mice per group. Data were compared using Mann-Whitney test. (**B-D**) Blood, spleen, liver, and lymph node (LN), and bone marrow (BM) of age- and sex-matched *Mir142*$^{+/+}$ (control, green circles) and *Mir142*$^{-/-}$ (blue circles) were harvested and assessed for the presence of NK1.1$^+$ cells (CD45$^+$CD3$^-$NK1.1+) by flow cytometry. (**B**) Representative flow plot of NK1.1$^+$ cells (gated on CD3$^-$) and the stage II (CD27$^+$CD11b$^-$), III (CD27$^+$CD11b$^+$), and IV (CD27$^-$CD11b$^+$) maturation status from the spleen of the indicated mice. Numbers depict the frequency of cells within the indicated region. (C-D) Summary data showing the total stage II, III, and IV NK cell numbers (**C**) in the indicated tissues from control and *Mir142*$^{-/-}$ mice, or (**D**) *Ncr1-cre*$^+$ control (green squares) and *Ncr1-cre*$^+$ *Mir142*$^{f/f}$ (blue squares) mice. Error bars represent the SEM. Data are from at least 3 independent experiments with at least 12 mice per group. Data were compared using Student's T test; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

Supplemental Figure S2

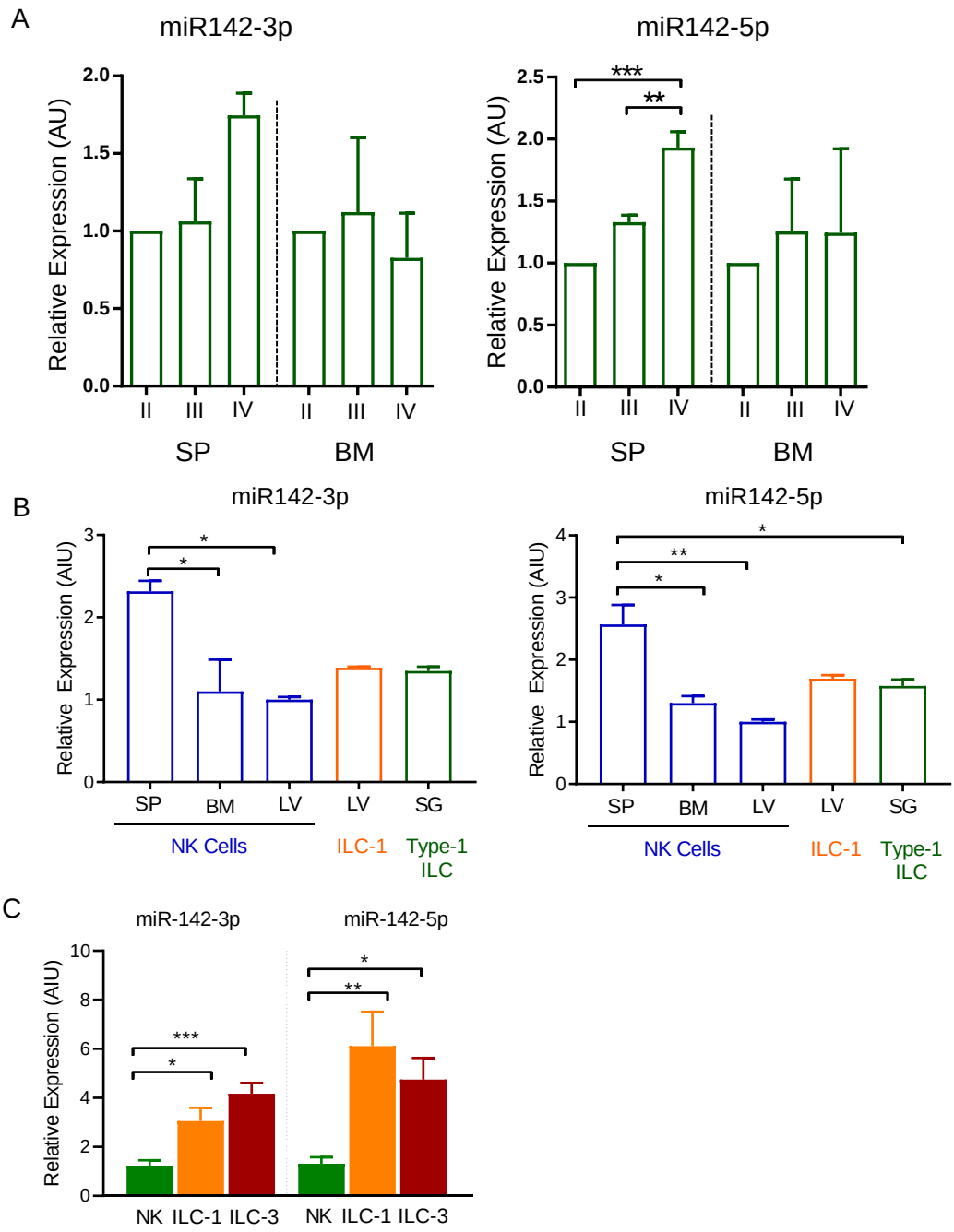

Figure S2. Mir142-3p and -5p are expressed throughout NK cell maturation and type-1 ILC compartment (related to Fig 1 and 2). (**A**) Cells were isolated from the spleen and bone marrow of C57BL/6J mice and NK cells ($CD3^-$ $NK1.1^+$) sorted by stage: $CD27^+CD11b^-$ (II), $CD27^+CD11b^+$ (III), and $CD27^-CD11b^+$ (IV) to >96% purity. (**B**) Cells were isolated from SP, BM, LV, and SG of C57BL/6J mice. From these tissues NK (CD3-NK1.1+CD49a-CD49b+), ILC-1 (CD3- NK1.1+ CD49a+ CD49b-) and type-1 ILC (CD3- NK1.1+) were sorted to > 96% purity. (**C**) Human subsets were flow sorted from tonsils. NK (CD3-CD56+NKp44-CD103-CD94+), ILC-1(CD3-CD56+NKp44+CD103+), and ILC-3 (CD3-CD56+NKp44+CCR6+CD103-). Cells were lysed in TRIzol and RNA isolated according to manufacturer's instructions (Invitrogen). cDNA was generated using the high-capacity cDNA reverse transcription kit (Life Technologies). Mir142-3p (**A**) and -5p (**B**) were detected using TaqMan primer/probes and normalized to sno-135 by the $\Delta\Delta C_t$ method. The assay was performed on an ABI StepOne machine (Applied Biosystems). Mir142-3p and -5p expression were normalized to stage II NK cells. Data are summary of 2-3 independent experiments. Error bars represent the SEM. Significance was determined using an ANOVA; ** $p < 0.01$, *** $p < 0.001$.

Supplemental Figure S3

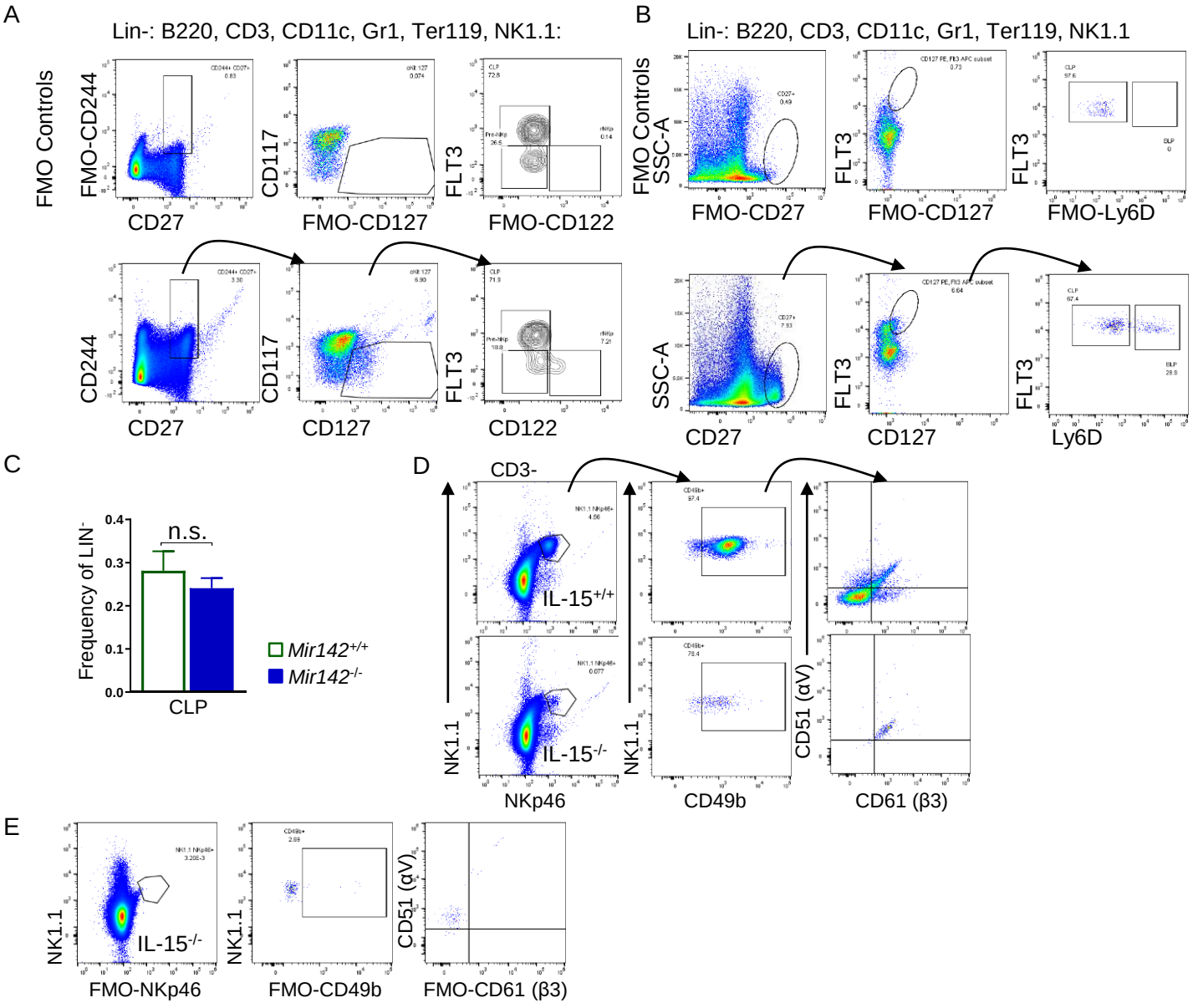

Figure S3. NK cell precursor and IL-15$^{-/-}$ gating strategy (related to Fig. 1 and 6). Gating strategy used to assess (**A**) pre-NKp (Lin$^-$CD27$^+$CD244$^+$CD122$^-$CD127$^+$FLT3$^-$), rNKp (Lin$^-$CD27$^+$CD244$^+$CD122$^+$CD127$^+$FLT3$^-$), and (**B**) CLP (Lin$^-$CD27$^+$FLT3$^+$CD127$^+$Ly6D$^-$) in the bone marrow. Fluorescence minus one (FMO) controls are indicated in the top row. Staining for each marker is shown in the bottom row. (**C**) Summary data of total CLP from control (green) and *Mir142*$^{-/-}$ mice. Data from 12-15 mice from 3 independent experiments. Data were compared using Student T-test; see Figure 1. (**D-E**) Control and *Il15*$^{-/-}$ spleens were assessed for NK cells (CD3- NK1.1+ NKp46+ CD49b+). (**D**). Representative gating strategy from CD3- lymphocytes from *Il15*$^{+/+}$ and *Il15*$^{-/-}$ mice. (**E**) Fluorescence-Minus-One (FMO) of the indicated marker from *Il15*$^{-/-}$ splenocytes from (D) See Fig. 6.

Supplemental Figure S4

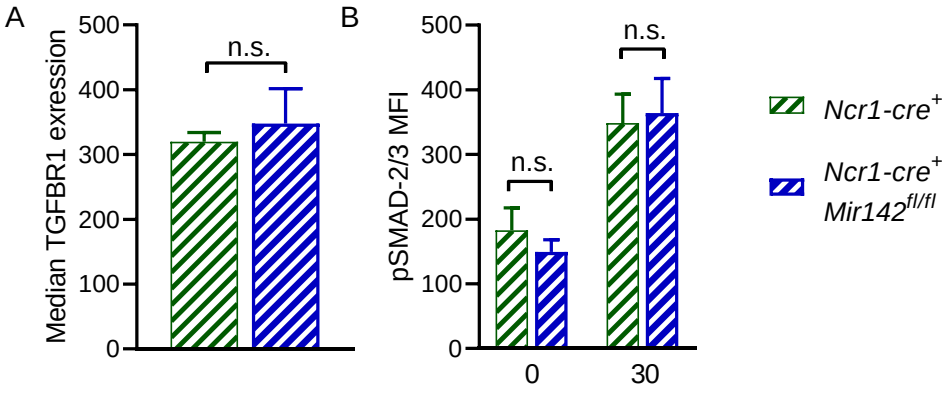

Figure S4. TGF-β signaling, cell cycling, and cell death in control and Mir142-deficient type 1-ILC (related to Fig. 4). (**A**) Summary TGFBR1 expression on type-1 ILC1 ($CD3^{-}NK1.1^{+}NKp46^{+}$) from SP of indicated mice; data from > 3 independent experiments were compared with T test (n=6-8 mice per group) (**B**) SP cells were stimulated with 10 ng/mL TGF-β1 for 30 minutes or left unstimulated (0 minutes) and assessed for phosphorylated SMAD-2/3 (p-SMAD-2/3). Data were compared using Mann-Whitney (A) or 2-Way ANOVA (B), see Fig. 4 Error bars represent SEM. Student's T test did not reveal any significant differences.

Supplemental Figure S5

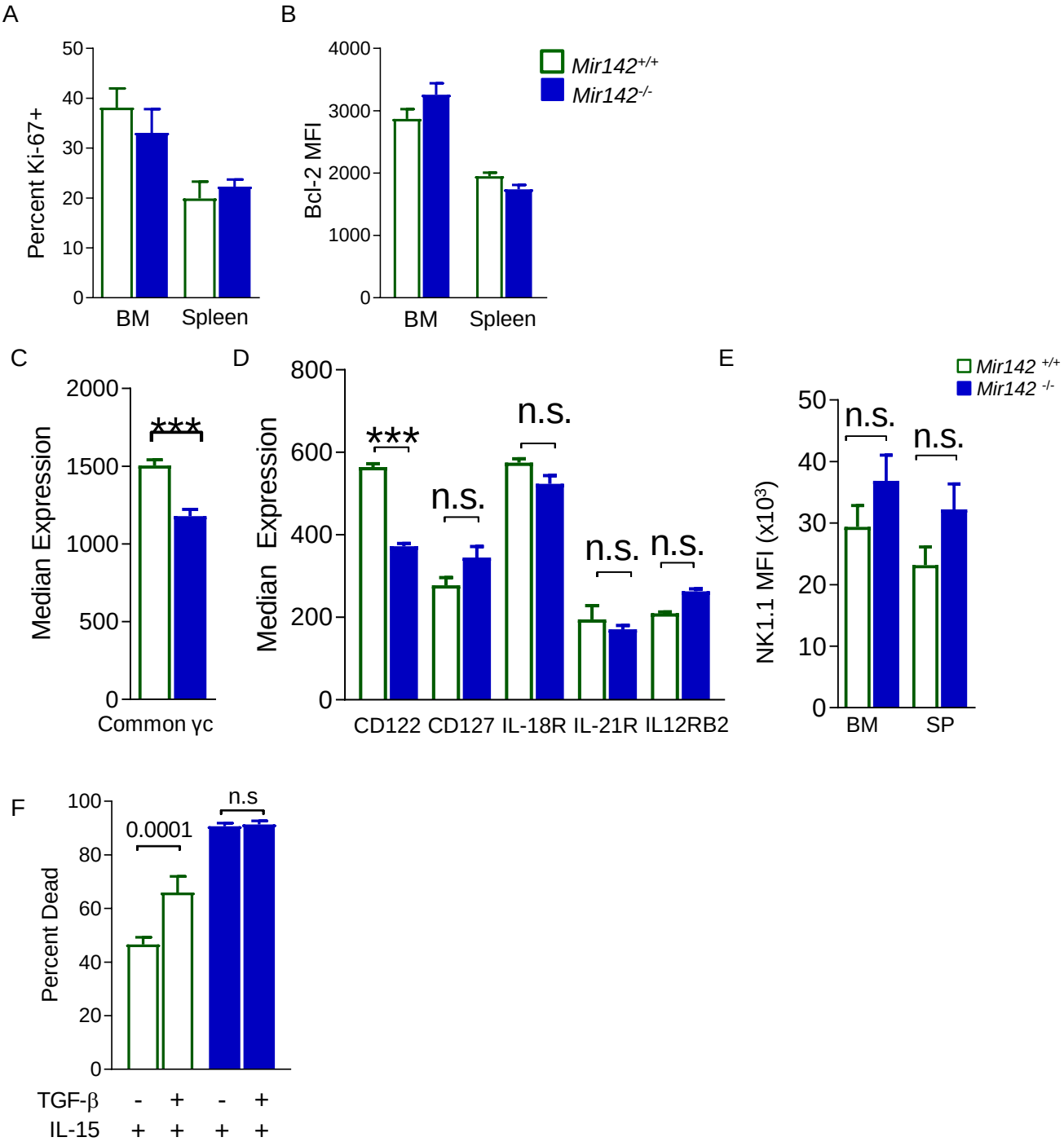

Figure S5. Median cytokine receptor expression and impacts of TGF-β on cell survival (related to Fig. 5). NK cells were harvested from *Mir142*$^{+/+}$ or *Mir142*$^{-/-}$ bone marrow (BM) or spleen and assessed by flow cytometry for (**A**) Ki-67, a nuclear protein present in proliferating cells, from CD45^{+}CD3^{-}NK1.1^{+} NK cells. (**B**) Bcl-2, an anti-apoptotic molecule. Cells were harvested from *Mir142*$^{+/+}$ (green) or *Mir142*$^{-/-}$ (blue) spleen and assessed for by flow cytometry for (**C**) CD132, (**D**) CD122, CD127, IL-18R, IL-21R, IL12RB2, (**E**) and NK1.1 as a control. Summary data from 2-3 independent experiments with N= 6-11 mice. (**F**) Splenocytes from *Mir142*$^{+/+}$ (green) or *Mir142*$^{-/-}$ (blue) mice were incubated with 10 ng/mL IL-15 +/- 10 ng/mL TGF-β for 48 hours. CD45^{+}CD3^{-}NK1.1^{+} cells were assessed for 7AAD and Annexin V by flow cytometry. Summary data showing percent dead: 7-AAD and/or Annexin V positive. MFI: Median fluorescence intensity. Summary data from 3-4 independent experiments with 15-20 mice are depicted, Mean values are shown with error bars depicting SEM. Data were compared using paired T test. Summary data from 2 independent experiments with 6-7 mice. Data are represented as mean ± SEM. Comparisons made using Ordinary one-way ANOVA; ***p<0.001, n.s. not significant.

Supplemental Figure S6

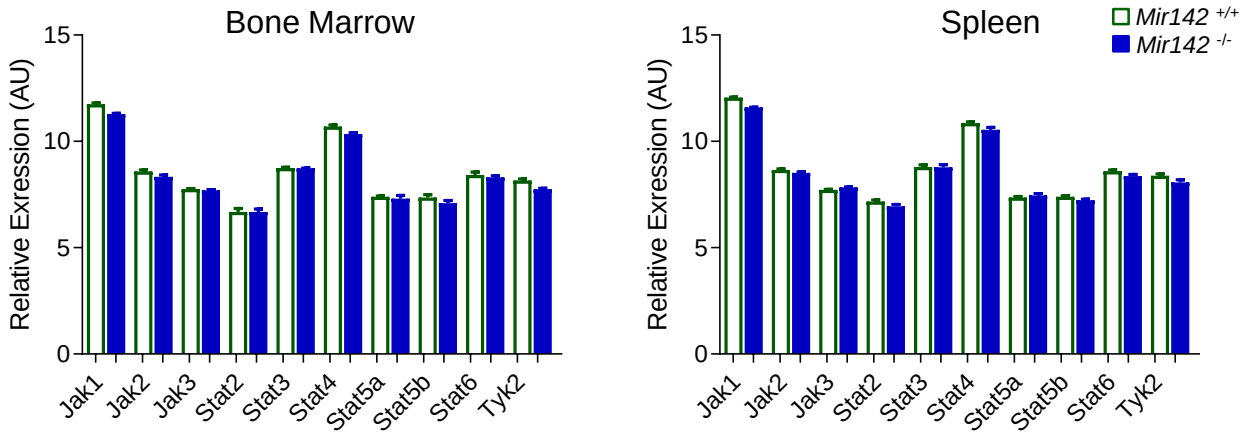

Figure S6. JAK/STAT mRNA are not affected by Mir142-deficiency (related to Fig. 5). $CD3^{-}NK1.1^{+}$ cells were sorted from *Mir142*$^{+/+}$ (green) or *Mir142*$^{-/-}$ (blue) bone marrow or spleen. RNA was isolated and converted to cDNA and analyzed on the Affymetric MoGene1.0 gene chip. Graphs summarize relative expression of the indicated molecules (3 biological replicates). Data are represented as mean ± SEM. Comparisons made using Ordinary one-way ANOVA; ***p<0.001, n.s. not significant.

Supplemental Figure S7

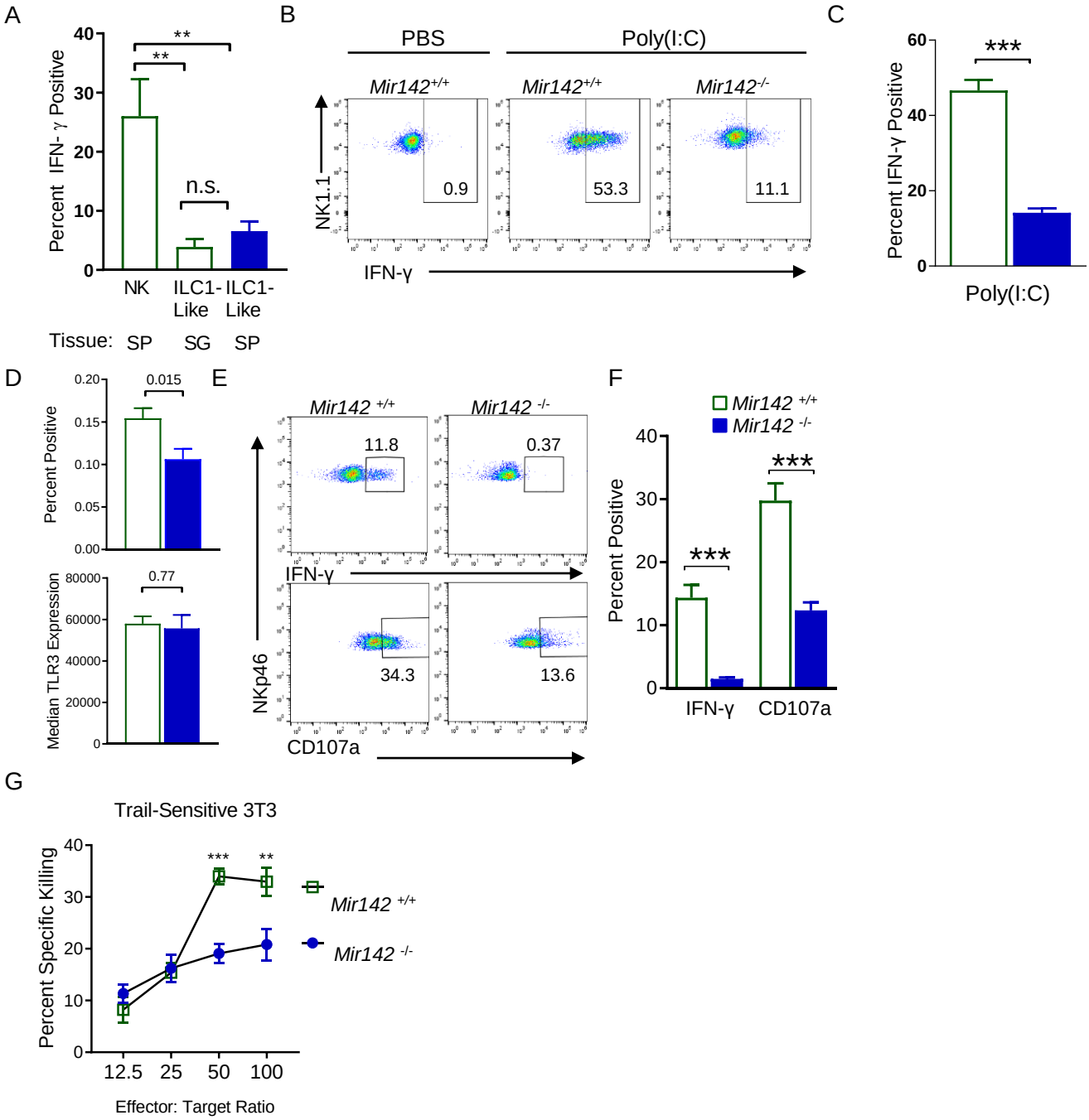

Figure S7. Mir142 deficient NK1.1⁺ cells have reduced effector responses (related to Fig. 7). (**A**) Lymphocytes were isolated from *Mir142*$^{+/+}$ (control, green bars) and *Mir142*$^{-/-}$ (blue bars) mice and stimulated for 6 hours with IL-12 + IL-15. Summary data showing IFN-γ production from each population from the indicated tissues. NK: CD3^{-} NK1.1^{+}NKp46^{+}CD49a^{-}CD49b^{+},ILC1-like:CD3^{-}NK1.1^{+}NKp46^{+}CD49a^{+}CD49b^{+}. (**B-C**) *Mir142*$^{+/+}$ (green) or *Mir142*$^{-/-}$ (blue) mice were treated with PBS or poly(I:C) intraperitoneally (ip.). After 4 hours, mice were sacrificed and the splenic NK1.1^{+} cells were assessed for intracellular IFN-γ protein by flow cytometry. (**B**) Representative flow plots showing IFN-γ staining in CD45^{+}CD3^{-}NK1.1^{+} cells. Numbers indicate the frequency of cells that fall within the indicated gate. (**C**) Summary from (B). (**D**) Splenocytes from *Mir142*$^{+/+}$ (green) or *Mir142*$^{-/-}$ (blue) mice were assessed for TLR3 in CD3-B220-NK1.1-CD11c+CD8+ cells. (**E**) Splenocytes from untreated mice were incubated with or without plate-bound anti-NK1.1 (PK136) in the presence of anti-CD107a for 6 hours. Representative flow data depicting staining of IFN-γ (top) and CD107a (bottom). (**F**) Summary data from (E). (**G**) Mice were treated with poly(I:C) i.p. and spleens harvested 24 hours later. Bulk splenocytes were placed in a standard flow-based killing assay against CFSE-labeled 3T3 cells at the indicated effector to target ratios. Mean is displayed ± SEM. Data were compared using Student's T test or RM-ANOVA (F). ** p <0.01, *** p < 0.001.