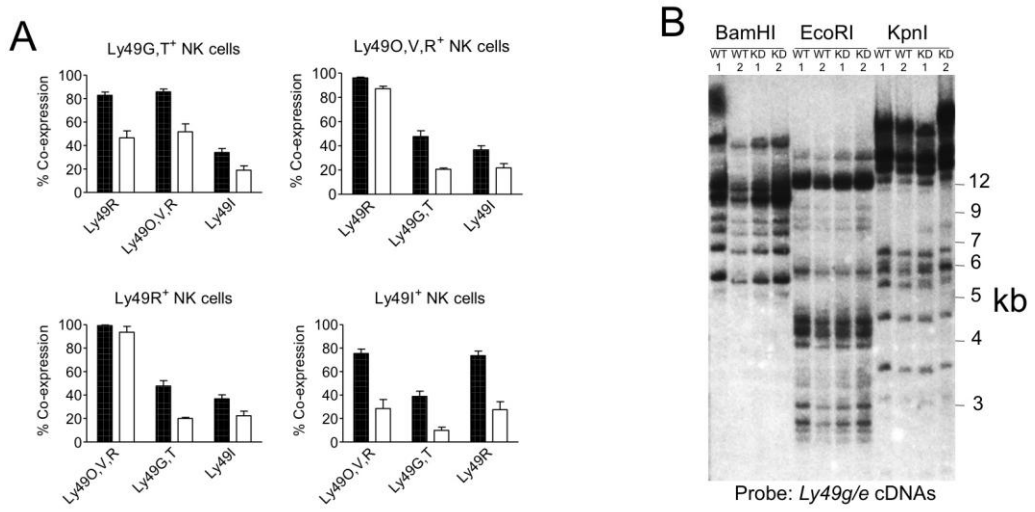

Figure S1. Analysis of Ly49 expression and gene organization in NKC^{KD} mice. (A) Ly49 co-expression is greatly reduced on NKC^{KD} NK cells. Ly49 co-expression on NK cells from WT (black bars) or NKC^{KD} (white bars) mice was analyzed by multi-color flow cytometry using the indicated Ly49-specific mAbs. The data are presented as the mean percentage of Ly49 co-expression when gated on a particular Ly49-positive subset (n = 6 mice for both genotypes) ±SD. The NK subset being analyzed for co-expression is indicated above the graph. (B) Restriction fragment length polymorphism analysis reveals normal *Klra* gene content in NKC^{KD} mice. A Southern blot was performed on thymus DNA obtained from two WT and two NKC^{KD} mice. DNA was digested with EcoRI, KpnI, or BamHI and after transfer, probed with *Klra7* (Ly49g) and *Klra5* (Ly49e) cDNAs. These experiments were performed with mice on the 129S1 background.

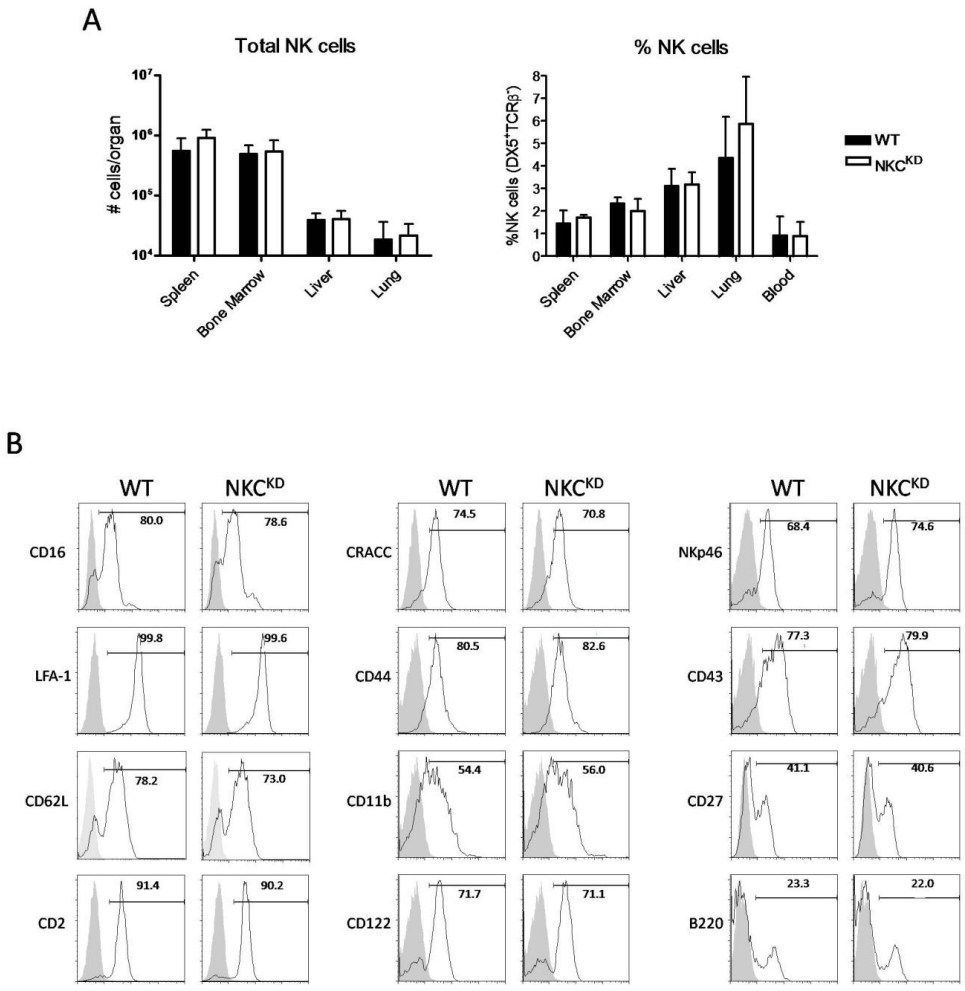

Figure S2. NKC^{KD} NK cells differentiate and seed peripheral organs normally. (A) Single cell suspensions from various organs of the indicated mouse strains were prepared, counted, stained with anti-CD49b (DX5) and anti-TCRβ mAb, and analyzed by flow cytometry. The results are shown as the number (left) or percentage (right) of DX5^{+}TCRβ^{-} cells among the whole population. The combined data of two independent experiments is shown (n = 6). (B) Flow cytometric analysis of the indicated cell surface markers (open peaks) was performed on splenocytes from WT and NKC^{KD} mouse littermates. The histograms indicate fluorescence intensity from DX5^{+}TCRβ^{-}-gated cells. The percentage of positively staining cells relative to the isotype control staining (grey peak) is indicated in the top right. These experiments were performed with mice on the B6 background.

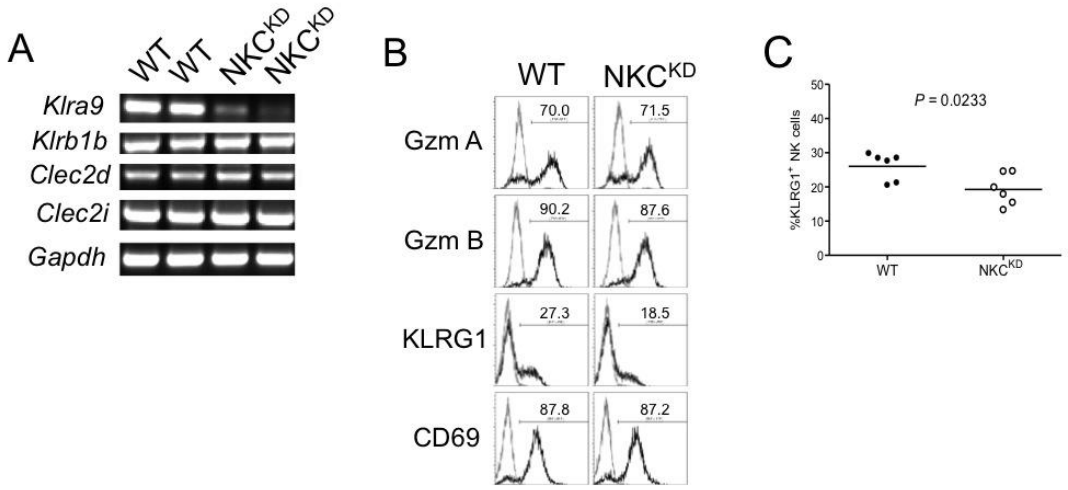

Figure S3. Surface marker and granule-associated protein expression on NKC^{KD} NK cells. (A) RT-PCR for the indicated NKC-resident genes and *Gapdh* was performed on total RNA isolated from WT and NKC^{KD} LAK cells. The RT-PCR result of two independent NK cell RNA isolations is shown. (B) Intracellular or cell surface mAb staining followed by flow cytometry was performed on splenocytes cultured with IL-2 for three days to determine NK cell activation marker expression. Analysis was performed on DX5^{+}TCRβ^{-}-gated cells. The percent of positive staining for the indicated marker (dark line) is indicated at the top right of the histogram. The isotype control staining is shown as a light grey line. Data are representative of at least three similar experiments. (C) Staining for KLRG1 on IL-2 activated NK cells was performed as described in (B) but for multiple individual mice of each strain. The horizontal bar indicates the mean and each symbol represents a different mouse. The statistical significance is indicated. This experiment was performed three times with two individual WT and NKC^{KD} mice per experiment. Pooled data are shown. <u>These experiments were performed with mice on the 129S1 background.</u>