Cytotoxicity assay

WT and Elf-1$^{-/-}$ mice were given (i.p.) 100 μg of poly(I:C), and the splenic NK activity was assessed after 16 hr using the standard ^{51}Cr-release assay with Yac-1 target cells. Briefly, a single-cell suspension of splenic effector cells was prepared. Cytotoxic killing was determined by measuring ^{51}Cr release from ^{51}Cr-labeled YAC-1 cells (10^4/well) that had been incubated with effector cells for 4 hr at 37 °C. The percentage specific lysis was calculated as s follows: 100 × (experimental release - spontaneous release) / (maximum release - spontaneous release).

Cytokine production by NK cells and conventional T cells

To examine the ability of NK cells and conventional T cells to secrete cytokine, hepatic leukocytes were stimulated with PMA/ionomycin for 5 hr. Monensin (10 μM) was added 2 hr before harvest. Cells were stained with CD1d/αGalCer tetramer, anti-TCRβ, and anti-NK1.1 antibody, followed by intracellular staining for IFN-γ.

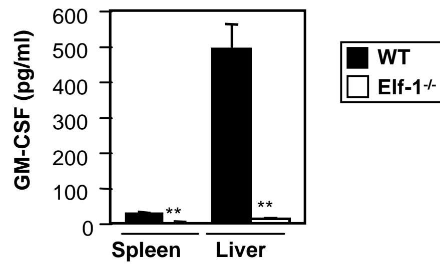

Figure S1. Elf-1$^{-/-}$ mice exhibit reduced amounts of GM-CSF production. Splenocytes and hepatic leukocytes were isolated from WT and Elf-1$^{-/-}$ mice and stimulated with αGalCer. After 48 h, levels of GM-CSF in the supernatant were detected by ELISA. Error bars represent the SD of triplicate wells. Data shown are representative of 2 independent experiments.

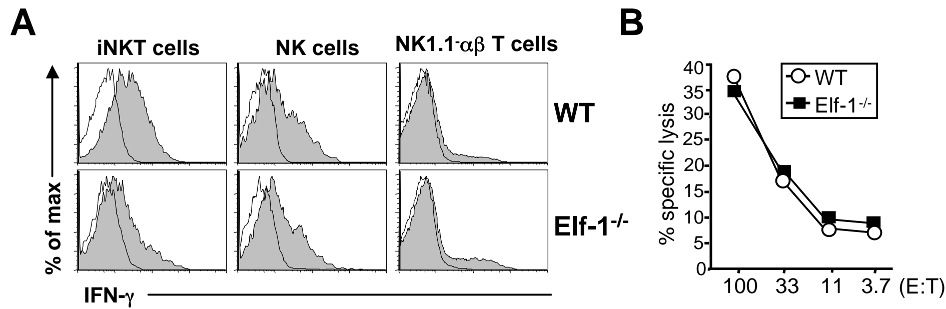

Figure S2. NK cells and conventional T cells found in Elf-1$^{-/-}$ mice exhibit normal function. (A) Hepatic leukocytes were isolated from Elf-1$^{-/-}$ and WT mice and stimulated with PMA/ionomycin. Cells were then stained with mAb against various cell surface markers, stained intracellularly for IFN-γ, and analyzed by flow cytometry. Histograms depict staining for IFN-γ on iNKT cells (CD1d/αGalCer tetramer^{+}TCRβ^{+} gate), NK cells (NK1.1^{+}TCRβ^{-} gate), and conventional T cells (NK1.1^{-} CD1d/ αGalCer tetramer^{-}TCRβ^{+} gate). Results are representative of 2 independent experiments. (B) NK cytolytic activity of Elf-1-deficient splenocytes. Splenocytes from the polyI:C treated Elf-1$^{-/-}$ and WT mice were used as effectors in a ^{51}Cr release assay. ^{51}Cr-labeled YAC-1 cells were used as target cells at the indicated effector:target (E:T) ratio. Results are representative of 2 independent experiments.

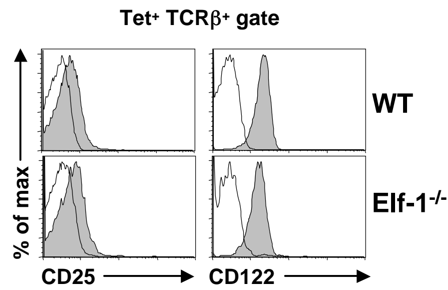

Figure S3. Residual NKT cells in Elf-1$^{-/-}$ mice have normal IL-2 receptor chain expression. Hepatic leukocytes were isolated from Elf-1$^{-/-}$ and WT mice and stained with CD1d/αGalCer tetramer and mAb against TCRβ, CD25 (IL-2Rα), and CD122 (IL-2Rβ), then analyzed by flow cytometry. Histograms depict CD25 and CD122 expression within the tetramer^{+}TCRβ^{+} population. Results are representative of 2 individual experiments.