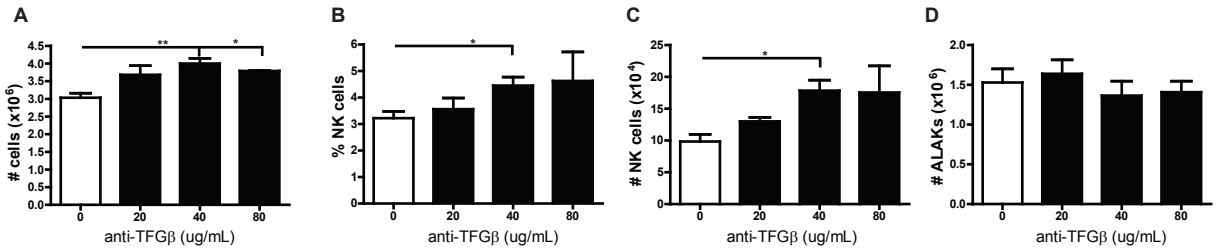
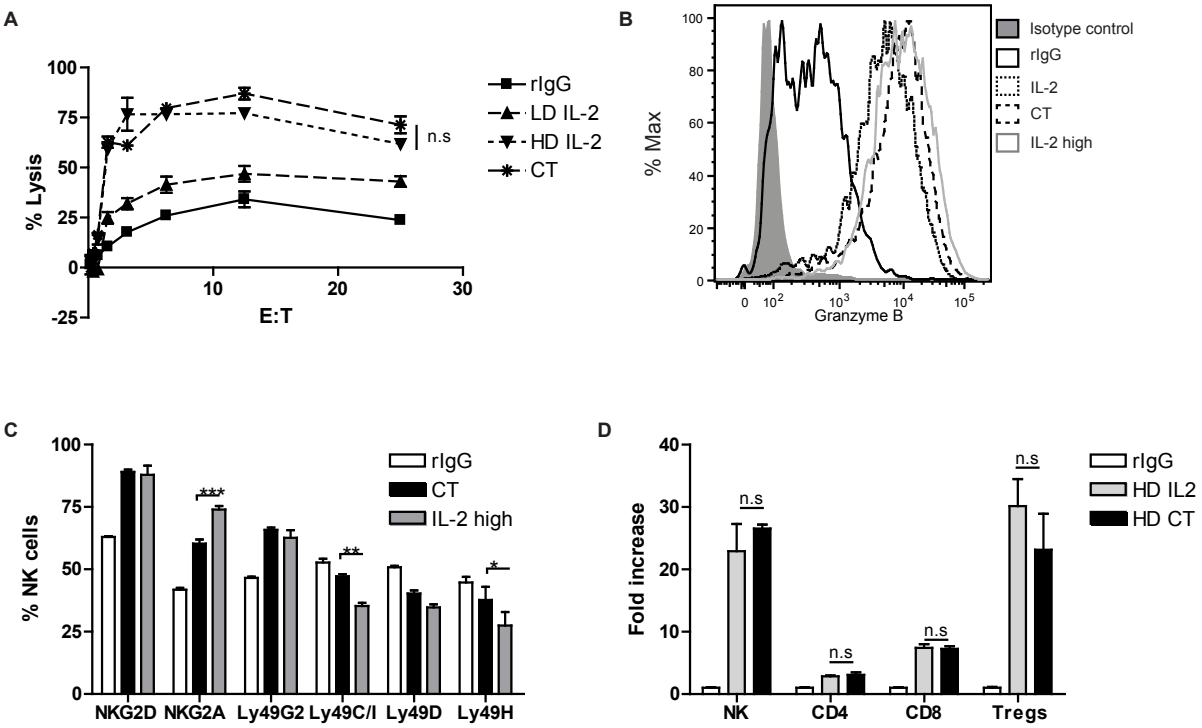
Supplemental Figure 1



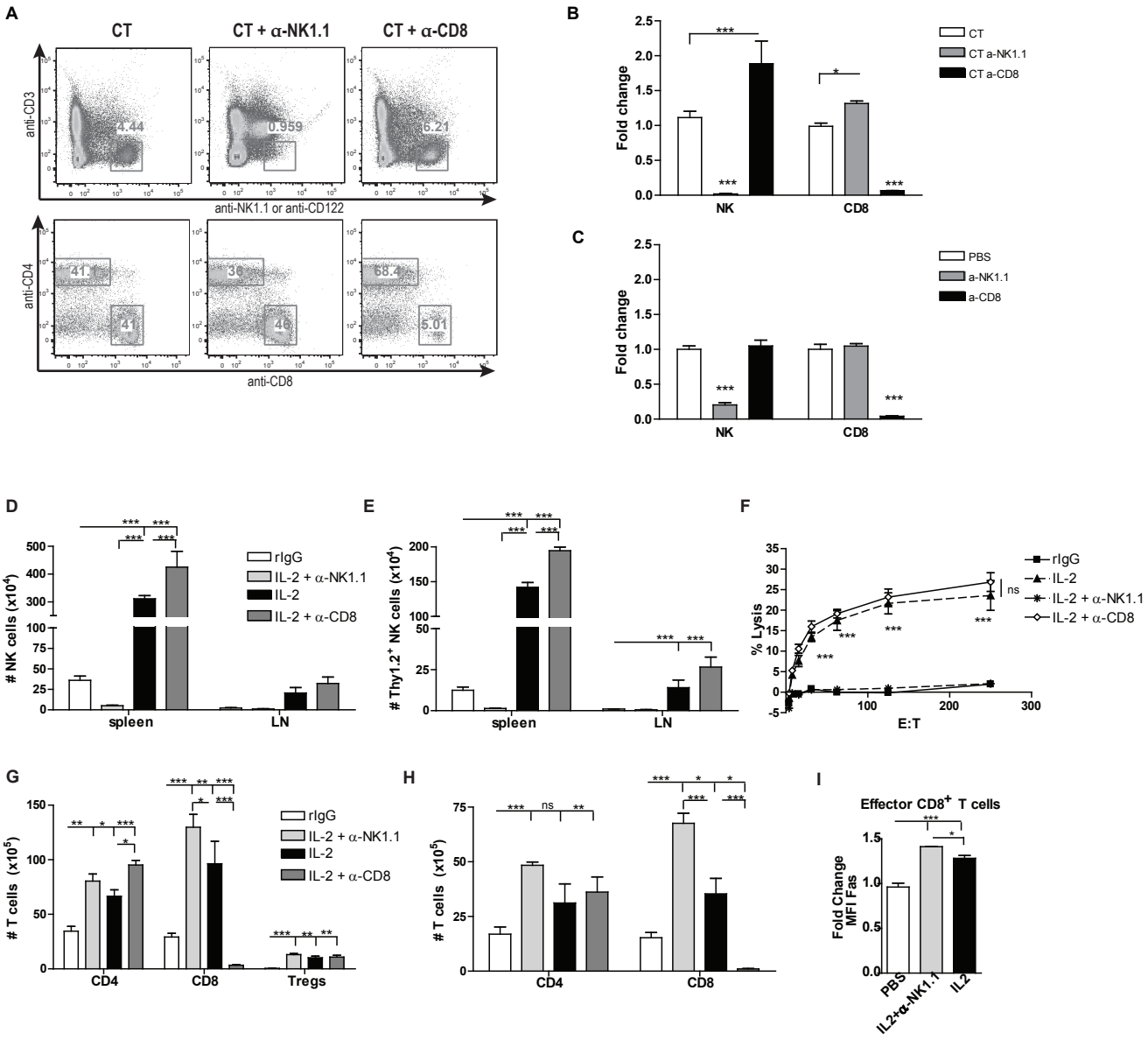
Supplemental Figure 1. In vitro IL2-stimulated splenocytes generate more NK cells in the presence of anti-TGFβ. C57BL/6 non-T-cell depleted or T-cell depleted splenocytes were cultured in the presence of IL2 and/or anti TGFβ. At day 7, cells were collected and analyzed for NK cells (CD45+CD3-NK1.1+) by flow cytometry. (A) Total cells collected in non-T-cell depleted cultures. (B) Percentage of NK cells in non-T-cell depleted cultures. (C) Total numbers of NK cells of non-T-cell depleted cultures. (D) Total numbers of adherent lymphokine-activated killer cells (ALAKs) collected from T-cell depleted cultures. Data is representative of two experiments. Significance was assessed by Student's two-tailed t-test (*$p<0.05$, **$p<0.01$).

Supplemental Figure 2



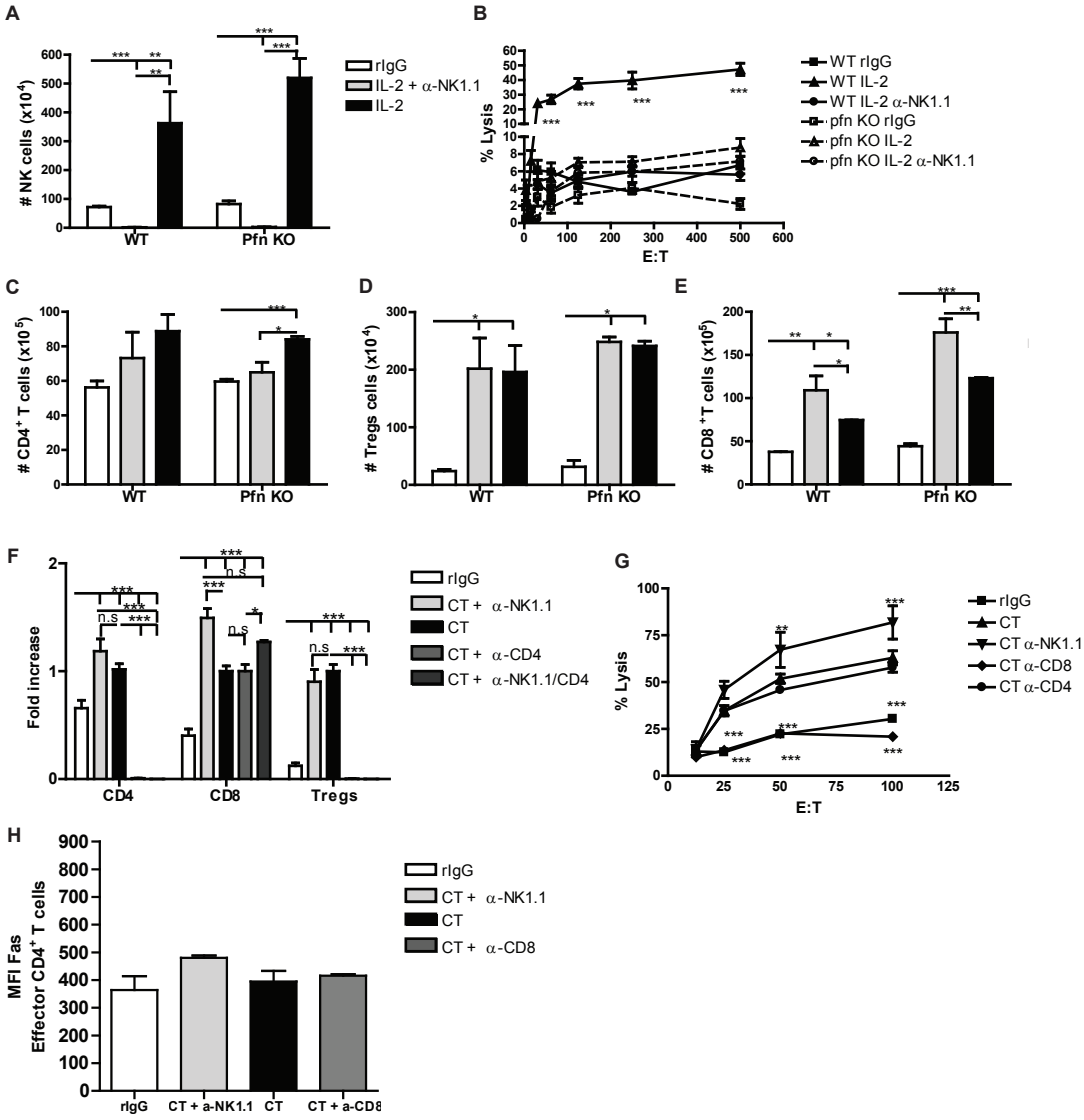
S**upplemental Figure 2. Functional and phenotypical similarities of NK cells expanded after high dose of IL2 and CT treatment.** 24h after end of treatment spleens were collected and analyzed for NK cell function and phenotype. (A) Cr-release assay of purified NK cells after receiving LD IL2, HD IL2 and CT (with LD IL2). (C) Representative histogram of Granzyme B expression (GranB) on gated NK cells is shown. (D) NK cell receptor distribution 24h after end of treatment. (E) Fold increase of NK, CD4, CD8 T cells and Tregs after receiving HD IL2 with or without anti-TGFβ. Data is representative of three independent experiments (A-C) or one experiment (D) with 3 mice per group (mean ± SEM). One-Way Anova or Two-Way Anova was used to assess significance (*p<0.05, **p<0.01, ***p<0.001). It is only displayed significance between HD IL2 and CT.

Supplemental Figure 3



Supplemental Fig3. NK and CD8 T cell dual regulation is TGFβ independent and requires IL2 stimulation. C57BL/6 mice were treated with anti-NK1.1 or anti-CD8 two days prior to the start rIgG, LD IL2 (0.2 million IU) or CT treatment for seven days. Single cell-suspension were stained for NK and T-cells markers 24h after end of treatment. (A) Dot-plots representing the distribution of NK, CD4 and CD8 T cells after NK and CD8 T cell depletion with mAb at 24h post-CT treatment from the spleen are shown. (B-C) Fold change in NK and CD8 T cell numbers after NK or CD8 T cell depletion that received CT (B) or rIgG treatment (C). (D) Total number of NK cells (CD45+CD3-NK1.1+) at 24h of LD single IL-2 treatment. (E) Total number of Thy1.2+ NK cells after LD IL2 treatment. (F) NK cell-dependent tumor lysis measured by 51Cr release-assay of total splenocytes as effector cells after LD IL2 treatment. (G-H) Proportion of CD4+ T-cells, CD8+ T-cells, and Tregs at 24h in the spleen (G) and LN (H) after LD IL2 treatment. (I) Fold change in MFI Fas expression on effector CD8 T cells (CD44high) after IL2 treatment. Data are representative of two independent experiments with 3 mice per group (mean ± SEM). One-Way Anova or Two-Way Anova was used to assess significance (*p<0.05, **p<0.01, ***p<0.001).

Supplemental Figure 4



Supplemental Figure 4. NK cell regulation of CD8+ T-cells is not mediated by perforin or CD4+ T-cells. Perforin-deficient (Pfn) or wild-type (WT) C57BL/6 mice were treated as previously described and spleens collected 24h post-treatment. (A) Total number of NK cells. (B) NK function was assessed by a standard 51Cr-release assay and percentage of tumor lysis against Yac-1 cells. (C-E) Total number of CD4+ T-cells, Tregs and CD8+ T-cells. Data represents one experiment with 3 mice per group (mean ± SEM). (F-G) C57BL/6 mice were treated with rIgG, IL-2 and anti-TGFβ as previously describes, some groups received anti-NK1.1 and/or anti-CD4 two days prior immunotherapy and organs were collected 24h after end of treatment. (F) Total number of CD8+ T-cells in the spleen. (G) CD8+ T-cell-dependent lysis. (H) Fas MFI of effector CD4 T cells (CD44-CD62L+) 24h after end of CT treatment with or without NK or CD8 depletion is shown. Data is representative of two experiments with 3 mice per group (mean ± SEM). One-Way Anova or Two-Way Anova was used to assess significance (*p<0.05, **p<0.01, ***p<0.001).