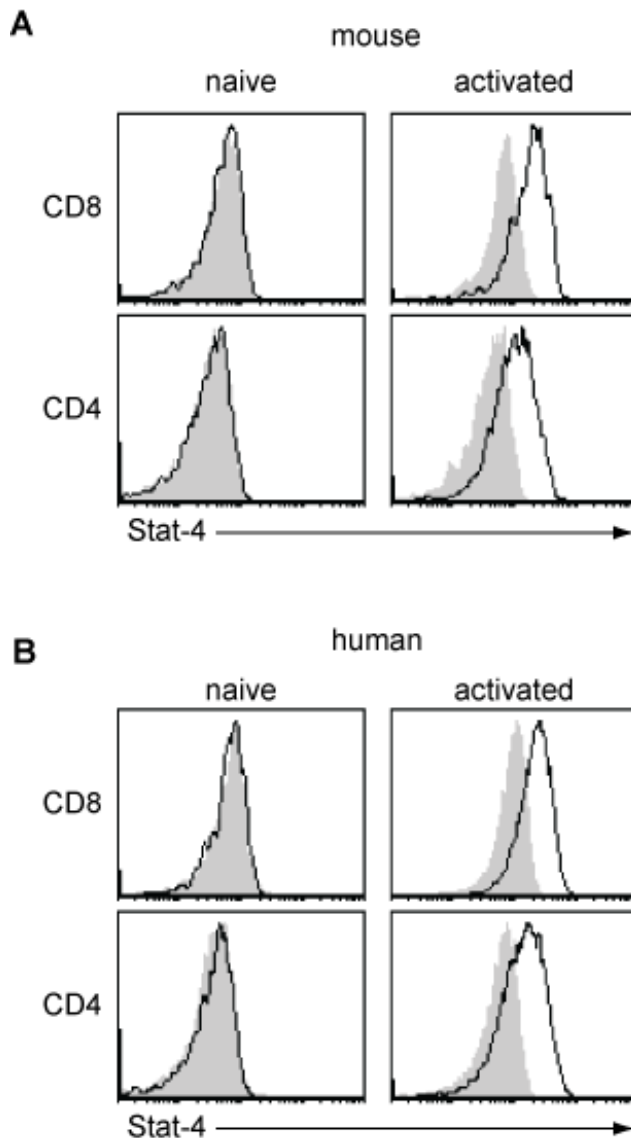
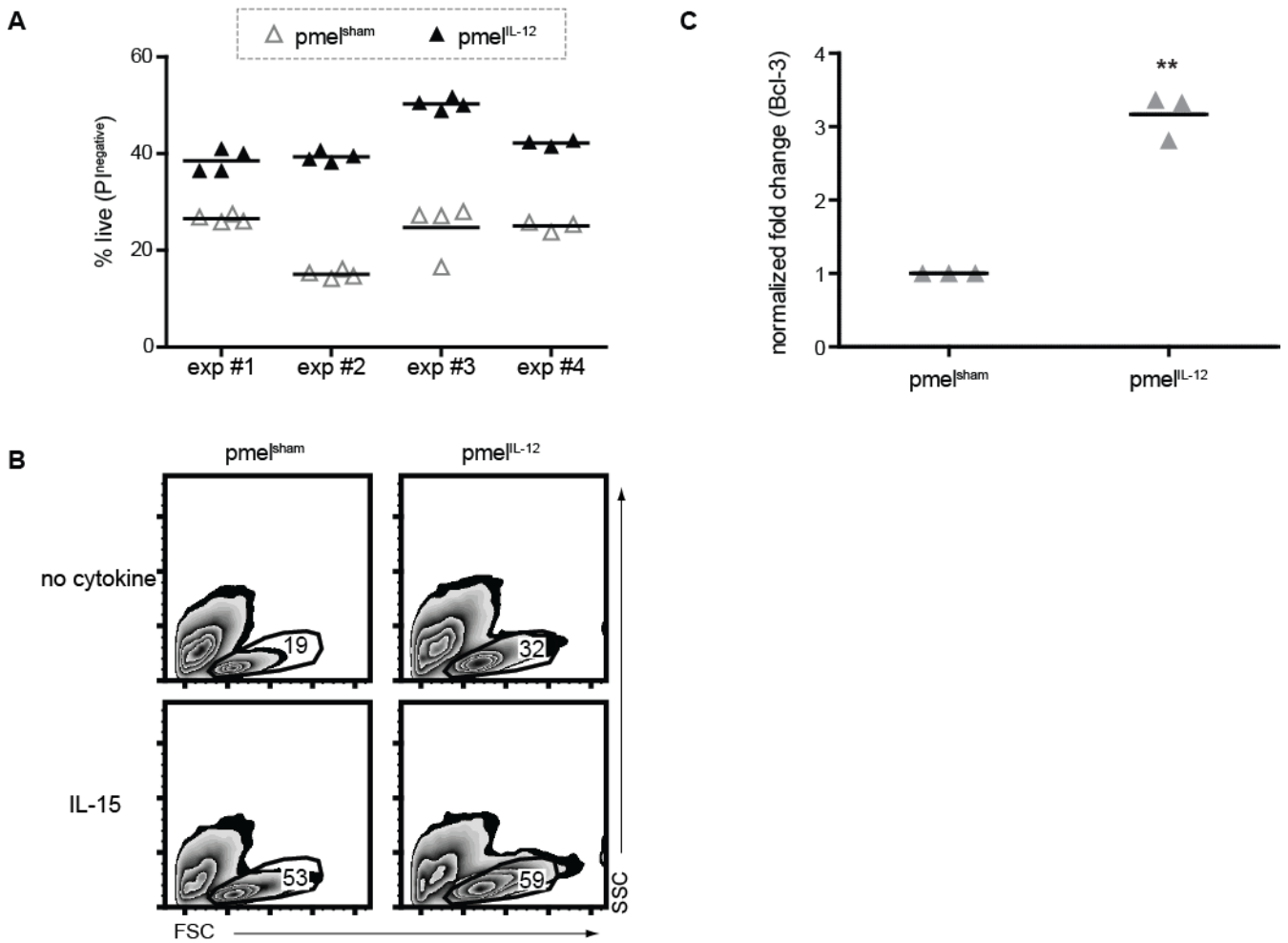


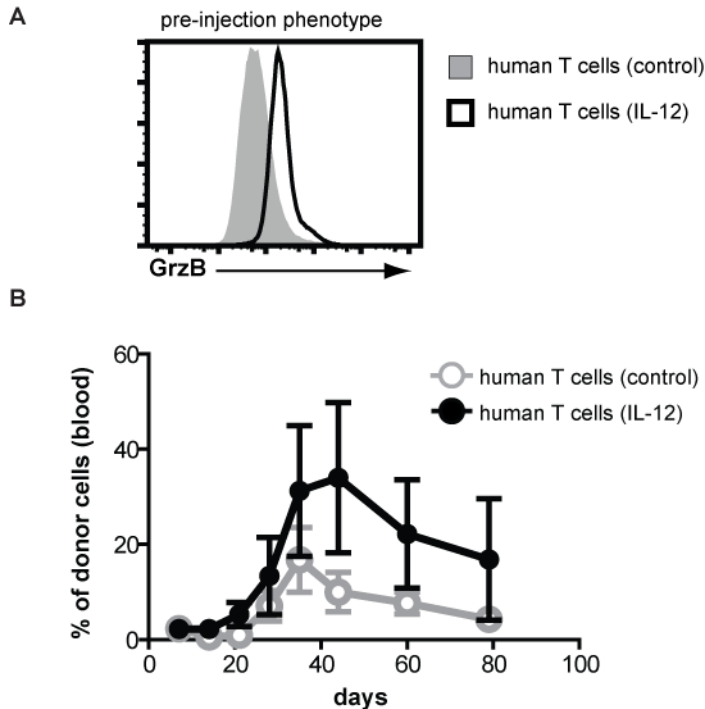
Supplemental figure 1. IL-12-conditioning improves CD8 T cells survival at least 10-fold in tumor-bearing mice. Mice with s.c. B16 tumors were treated on day 13 with cyclophosphamide (CTX). On day 14, pmel^{IL12} or pmel^{sham} cells were adoptively transferred into mice. Three million cells were injected except 3x10⁵ in the 1:10 condition. Mice were monitored at least twice per week and sacrificed when tumor volume exceeded 400mm². Shown are the percentage of live mice at each time point. Using a Gehan-Wilcoxon test, time to sacrifice was compared across groups using data from this figure and figure 2. There were significant differences between certain groups, and notably between pmel^{sham} + CTX and pmel^{IL-12} (1:10) + CTX (p=0.004).



Supplemental Figure 2: IL-12 induces phosphorylation of Stat-4 in activated but not naïve T cells. (A) Unstimulated splenocytes or day 3 CD3/CD28-activated splenocytes were stimulated for 1 hour with or without mIL-12 (200ng/ml) and then assessed for pSTAT4 by flow cytometry. (B) Resting human PBMCs or CD3-activated PBMCs were stimulated for 1 hour with or without hIL-12 (200ng/ml) and then assessed for pSTAT4 by flow cytometry. Results are representative of at least 2 independent experiments.



Supplemental Figure 3: IL-12-conditioning enhances CD8⁺ T cell survival in the absence of T cell growth factors. (A) Pmel^{IL-12} or pmel^{sham} cells were plated without addition of T cell growth factors overnight and viability was assessed by staining cells with propidium iodide (PI). Each triangle represents one well and the bar indicates the mean. Four independent experiments are shown. There was a significant difference ($p < 0.00001$) between IL-12 and sham conditions. **(B)** Representative staining of cells from ‘A’ using forward scatter (FSC) and side scatter (SSC) to determine viability. The bottom row shows the impact of mIL-15 (1ng/ml) during the overnight culture. **(C)** RNA was isolated from pmel^{IL-12} or pmel^{sham} cells, and mRNA expression levels were determined by real-time PCR. There was a significant difference (** $p < 0.01$) comparing IL-12 and sham conditions.



Supplemental Figure 4. IL-12-conditioned human T cells persist in immunodeficient NSG mice. (A) Human PBMCs were stimulated with anti-CD3 for 2 days and maintained in IL-2 and IL-15 for 5 days. Cells were cultured throughout with or without IL-12 and then phenotyped by flow cytometry for granzyme B expression. **(B)** Cells (10^7) from 'A' were adoptively transferred into NSG mice. The line indicates the mean ($n=5$) and the bars show standard error. In two similar experiments, we failed to achieve engraftment of human T cells.