Supplemental Figures

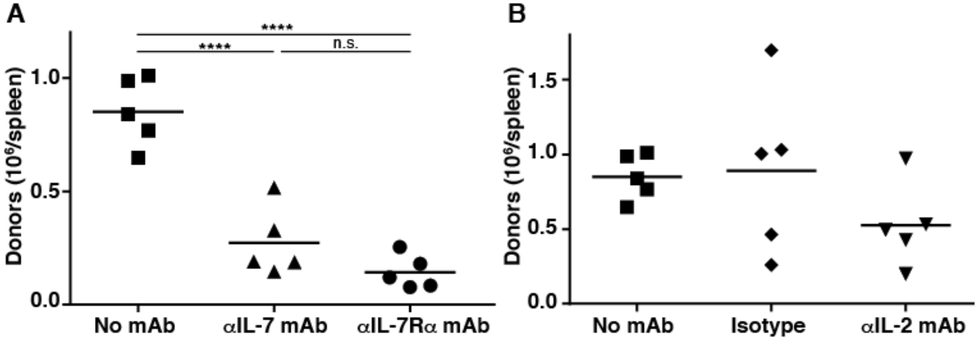

Figure S1. Blockade of IL-7 but not IL-2 reduces engraftment of IL-12 conditioned (Tc1) $CD8^+$ T cells in lymphodepleted mice. (**A-B**), 5 x 10^6 pmel-1 Tc1 cells were transferred into 6 Gy irradiated mice injected with the indicated monoclonal antibody (mAb), and donor cells were enumerated in the spleens 7 days later (**** $p < 0.0001$). Results are representative of at least 2 independent experiments.

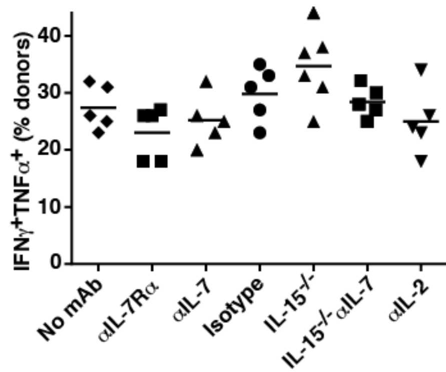

Figure S2. Blockade of cytokine signaling does not reduce the functionality of donor $CD8^+$ T cells. Pmel-1 $CD8^+$ T cells ($5x10^6$) activated with IL-12 (Tc1) were transferred into irradiated WT B6 or $IL\text{-}15^{-/-}$ hosts, which were then treated with the indicated monoclonal antibodies. Seven days later, splenocytes from recipient mice were harvested and restimulated with relevant peptide (hgp100) *ex vivo* for 5 h. The donor $CD8^+$ T cells were assessed for cytokine production via intracellular flow cytometry.

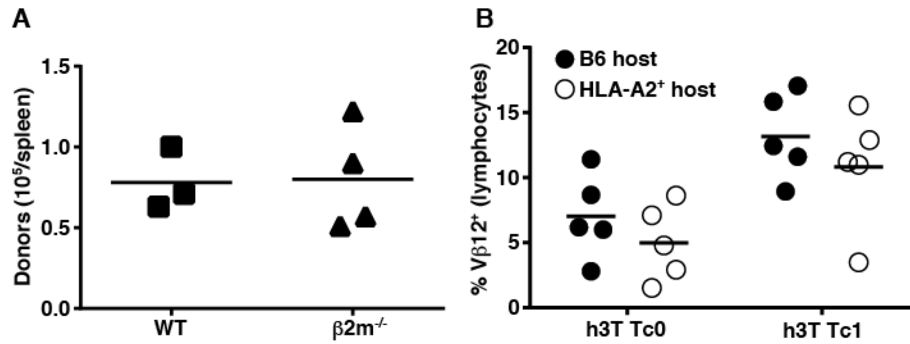

Figure S3. Activated $CD8^+$ T cells do not require TCR engagement for engraftment in an irradiated host. (**A**) 2 x 10^6 Tc1 (pmel-1) were transferred into irradiated WT or beta 2 microglobulin (β2m) knockout recipient mice, and the $Thy1.1^+$ donor cells were enumerated in the spleens 7 days later ($p > 0.05$ for all comparisons; results are representative of 2 independent experiments). (**B**) 3 x 10^6 h3T cells activated without (Tc0) or with IL-12 (Tc1) were transferred into irradiated WT (no HLA-A2) or $HLA\text{-}A2^+$ transgenic mice, and the frequency of donor T cells ($hV\beta12^+$) among the live lymphocyte gate was determined on day 7 ($n = 5$, $p > 0.05$ for both host comparisons).

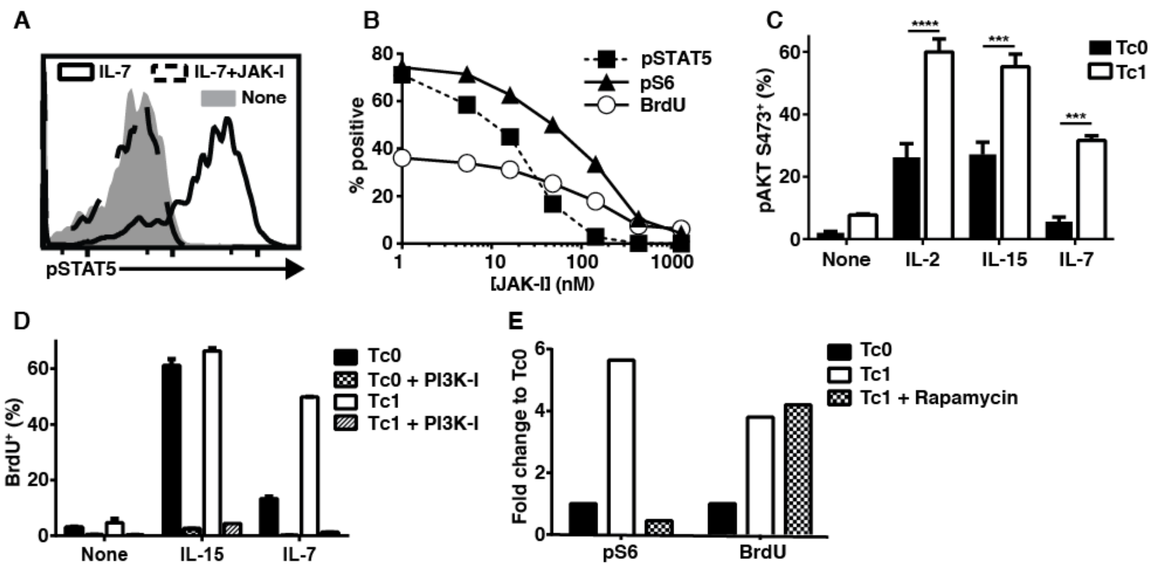

Figure S4. IL-7 mediated proliferation of Tc1 is STAT5 and PI3K dependent. (**A-D**) Day 3 activated Tc0 or Tc1 (pmel-1) cells were pretreated with inhibitors for 30 min when applicable and then cultured with 100ng/mL of the indicated cytokine. (**A**) Representative histogram of Tc1 pSTAT5 levels after indicated treatment (1.3 □M JAK-I). (**B**) Tc1 cells were pretreated with various doses of JAK-I, cultured with IL-7 and then assessed for pSTAT5, pS6 and BrdU levels as in Figure 3. (**C**) pAKT S473 levels in Tc0 or Tc1 cells after reculture in the indicated cytokine (n = 3, *** $p < 0.001$, **** $p < 0.0001$). (**D**) BrdU levels after reculture in cytokines with or without PI3K inhibitor LY294002 (50 μM). (e) pmel-1 T cells were activated with hgp100 alone (Tc0), hgp100 + IL-12 (Tc1) or hgp100 + IL-12 + 20 nM rapamycin (Tc1 + Rapamycin) for 3 days, then recultured in IL-7 and assessed for pS6 and BrdU levels. All results are representative of at least 2 independent experiments.

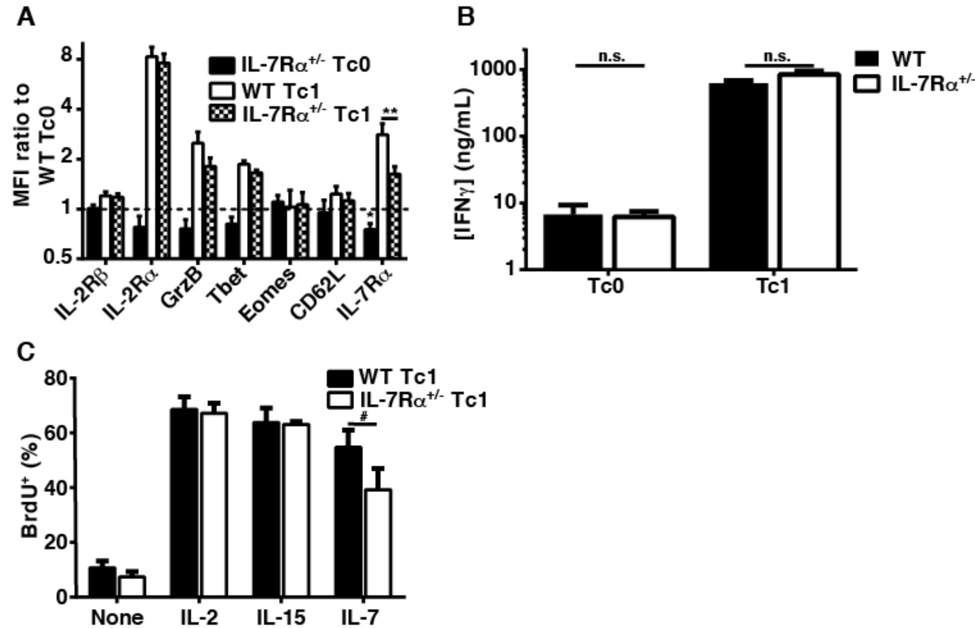

Figure S5. Tc1 cells generated from IL-7Rα$^{+/-}$ and wildtype mice exhibit a similar profile except for IL-7Rα expression. (**A**) Mean fluorescence intensity (MFI) ratios of pmel-1 IL-7Rα$^{+/-}$ Tc0, wildtype Tc1 or IL-7Rα$^{+/-}$ Tc1 relative to wildtype Tc0 cells for the markers indicated (n = 5-7; asterisks indicate significantly different from Tc0, which is represented by the dashed line). (**B**) IFNγ levels in the supernatant of day 3 cultures as measured by ELISA (n = 3). (**C**) Tc1 or IL-7R□$^{+/-}$ Tc1 cells were recultured with media only (None) or 100ng/mL mIL-2, mIL-15 or mIL-7 overnight then assessed for BrdU positivity (n = 4-6). ** $p < 0.01$, * $p < 0.05$, # $p < 0.1$).

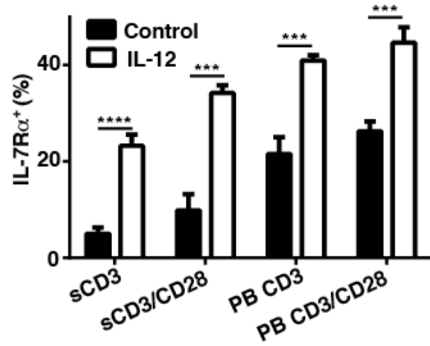

Figure S6. The level of TCR stimulation, costimulation, and IL-12 drive IL-7Rα expression after CD8$^+$ T cell activation. Polyclonal CD8$^+$ T cells from B6 mice were stimulated as indicated and IL-7Rα levels were assessed 3 days later (combined data from 3 independent experiments, *** $p < 0.001$, **** $p < 0.0001$).