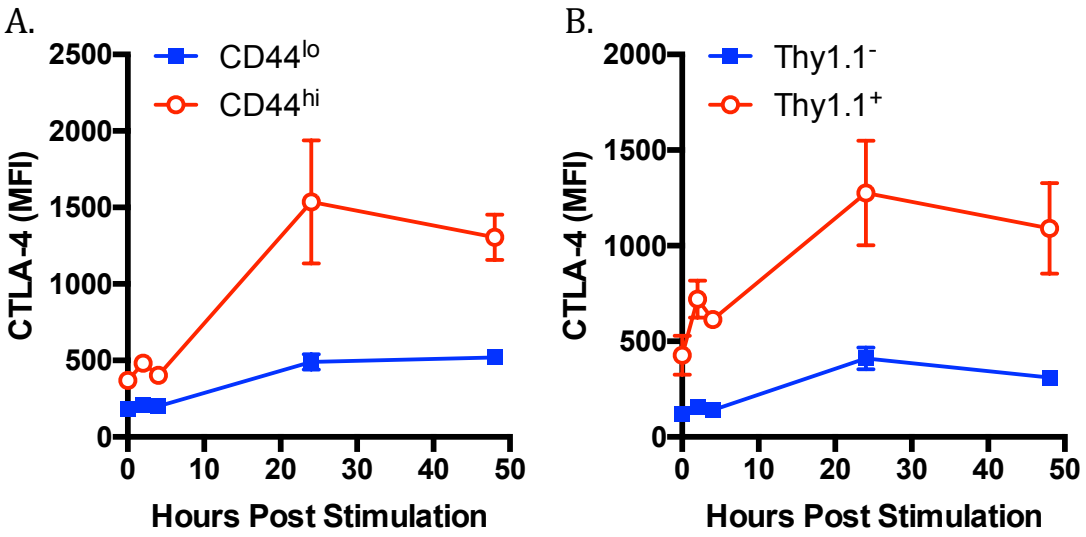
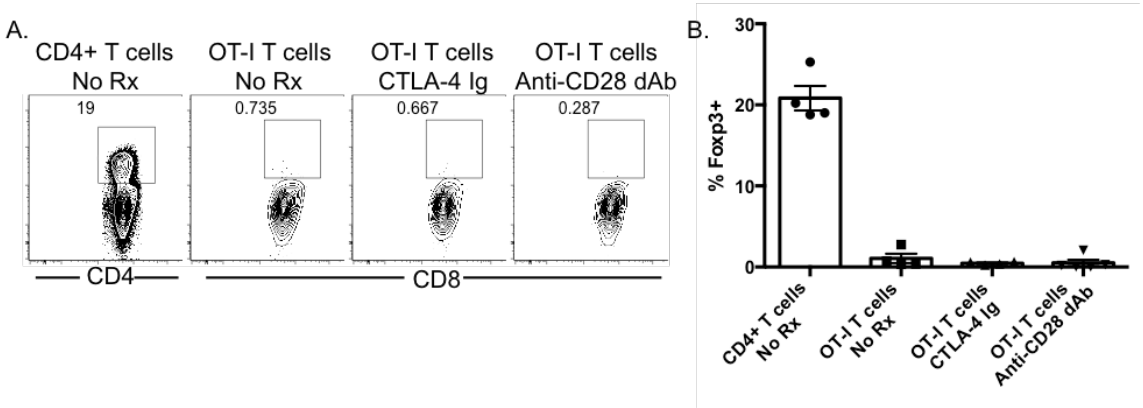
Supplemental Figures

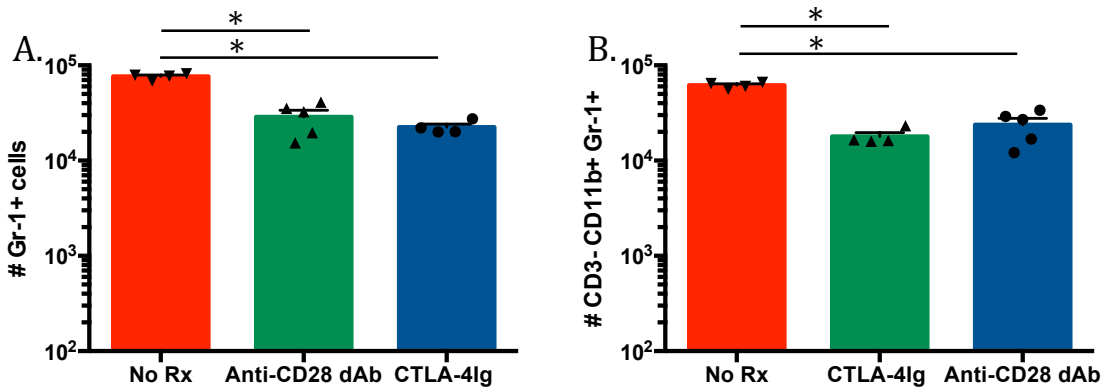


Supplemental Figure 1. CTLA-4 is increased and sustained on in vivo generated and endogenous polyclonal CD8⁺ memory T cells relative to naïve CD8⁺ T cells. A, OVA-*Listeria*-immunized animals were sacrificed on day 25 post-infection and splenocytes were restimulated with PMA and ionomycin ex vivo. At 0, 2, 4, 24, and 48 hours post-stimulation, CD44^{hi} CD8⁺ memory T cells vs. CD44^{lo} CD8⁺ naïve T cells were assessed for CTLA-4 expression. B, OVA-*Listeria*-immunized animals were sacrificed on day 25 post-infection and Thy1.1⁺ CD8⁺ memory T cells were stimulated with PMA and ionomycin ex vivo. CTLA-4 expression was assessed at 0, 2, 4, 24, and 48 hours post-stimulation. Data shown are representative of two independent experiments with a total of 6-8 animals per group.



Supplemental Figure 2. Graft-specific CD8⁺ T cells do not differentiate into regulatory T cells in the presence of selective CD28 blockade. 10^4 Thy1.1⁺ OT-I T cells were adoptively transferred into naïve B6 Thy1.2 hosts and infected with

Listeria-OVA to generate recipients containing memory OT-I T cells. On day 30 post-infection, mice received an OVA-expressing skin graft and were treated with 200ug CTLA-4 Ig or 100ug anti-CD28 dAb on days 0, 2, and 4 post transplant. Animals were sacrificed on 10 post-transplant and graft-draining LN were harvested. The frequency of Foxp3$^+$ T cells among Thy1.1$^+$ CD8$^+$ T cells was quantified in (A) representative flow cytometry plots and (B) summary data of 4-5 animals per group. Foxp3 expression among total polyclonal CD4$^+$ T cells is shown as a positive control.



Supplemental Figure 3. Frequencies of Gr-1$^+$ neutrophils and CD3$^-$CD11b$^+$Gr-1$^+$ MDSCs were not different between CTLA-4 Ig treated and anti-CD28 dAb treated animals. 10^4 Thy1.1$^+$ OT-I T cells were adoptively transferred into naïve B6 Thy1.2 hosts and infected with *Listeria*-OVA to generate recipients containing memory OT-I T cells. On day 30 post-infection, mice received an OVA-expressing skin graft and were treated with 200ug CTLA-4 Ig or 100ug anti-CD28 dAb on days 0, 2, and 4 post transplant. Animals were sacrificed on 10 post-transplant and skin grafts were harvested and processed for graft-infiltrating leukocytes. The frequencies of Gr-1$^+$ neutrophils (A) and CD3$^-$CD11b$^+$Gr-1$^+$ MDSCs (B) were not different between CTLA-4 Ig treated and anti-CD28 dAb treated animals. Data shown are representative of two independent experiments with a total of 8-10 animals per group. *$p<0.05$.