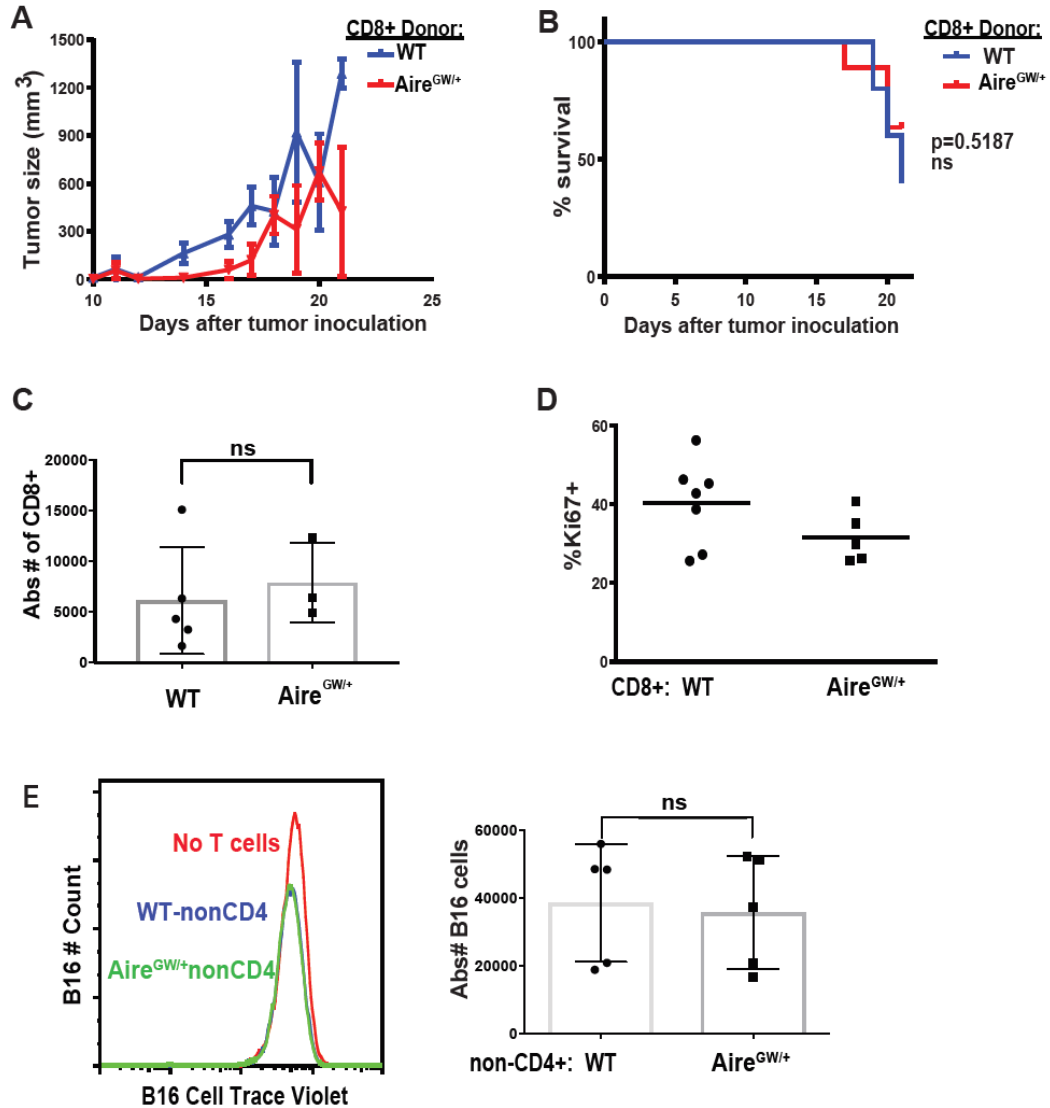


Supplementary Figures

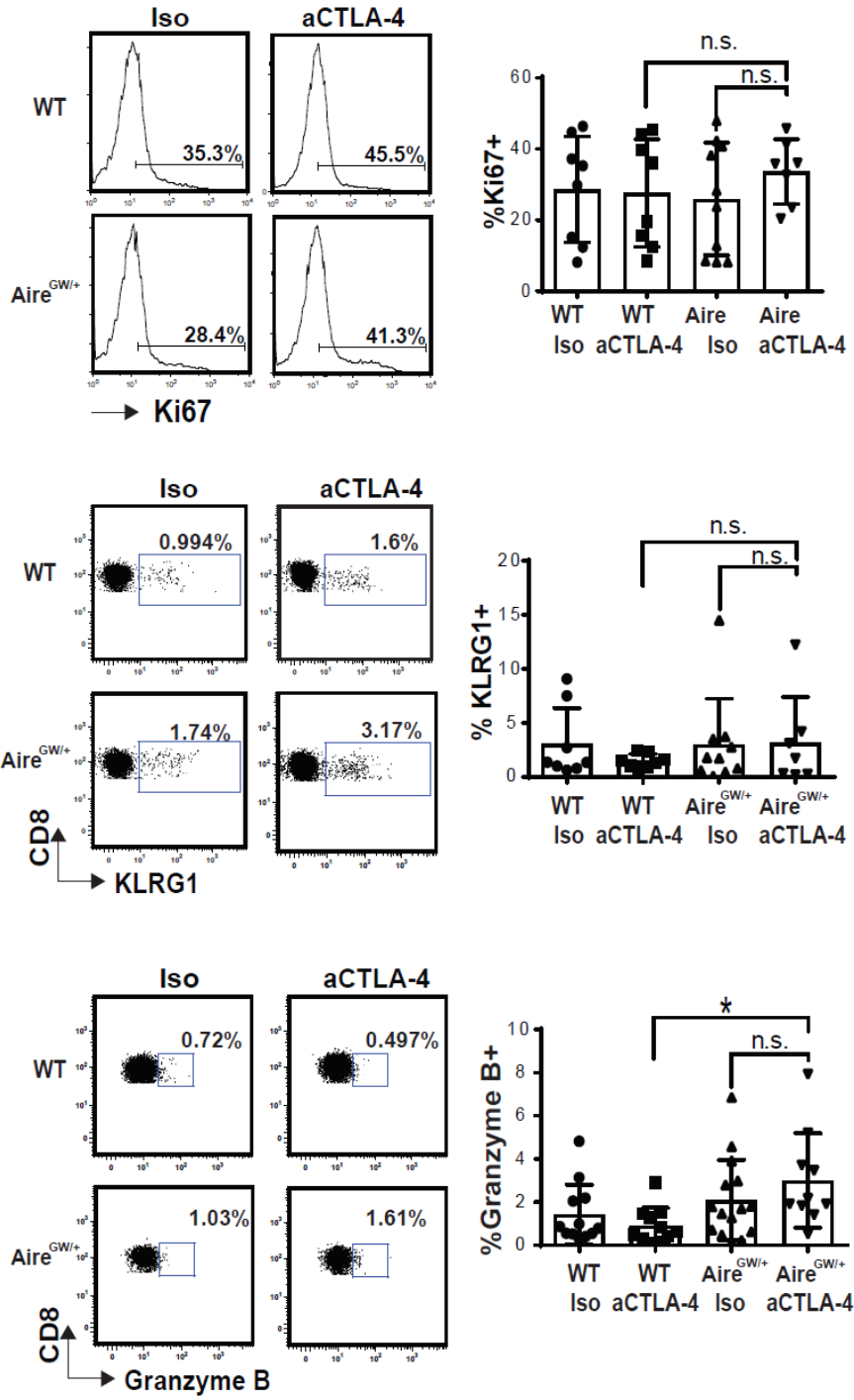
Supplementary Figure 1



Supplementary Figure 1: Aire deficiency has minimal effects on CD8⁺ T cell cytolytic function in melanoma bearing mice. A and B) RAG^{-/-} recipients of CD8⁺ T cells from WT and Aire^{GW/+} mice were followed for B16 melanoma tumor growth and survival after B16 inoculation; n=10 per group, **Mann Whitney U-test**. *p<0.05, n.s.=non-significant. C) Absolute numbers of CD8⁺ tumor-infiltrating cells. **t-test**. ns=not significant. D) Average cumulative frequencies of Ki67⁺

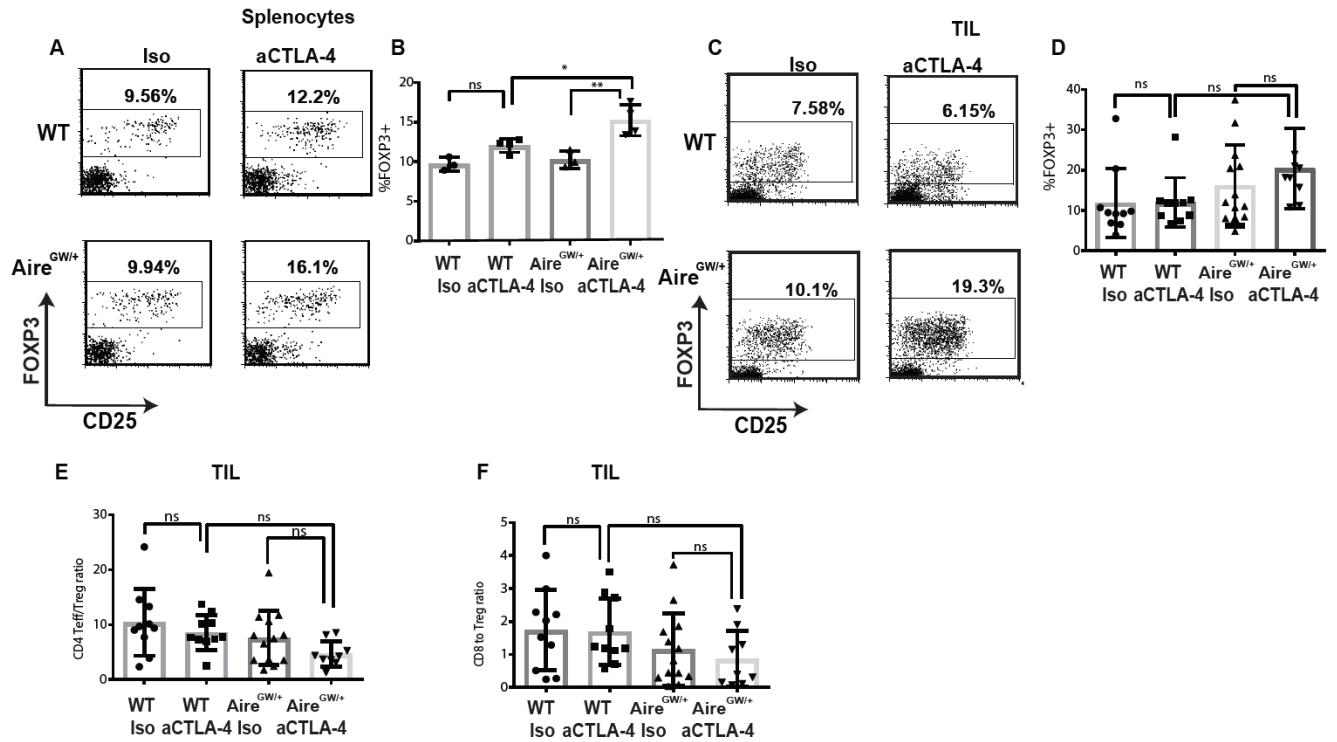
among CD8⁺ T cells. Tumor-infiltrating lymphocytes (TIL) were harvested on Day 19 following B16 melanoma inoculation in RAG^{-/-} recipients of CD8⁺ T cells from WT and Aire^{GW/+} donor mice. **t-test. ns=not significant.** E) Representative flow cytometry histogram comparison and average absolute B16 cell numbers of cell trace violet-labeled B16 cells after co-incubation with non-CD4⁺ T cells from WT and Aire^{GW/+} mice. **t-test. ns=not significant.**

Supplementary Figure 2



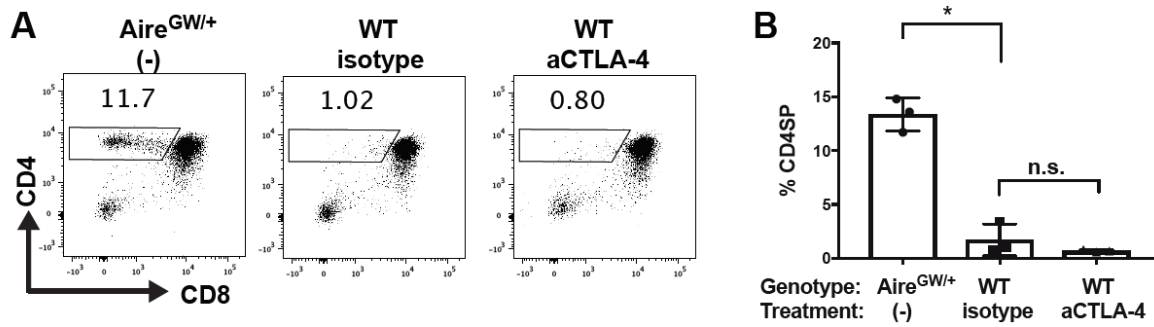
Supplementary Figure 2: Modest effect of Aire deficiency and anti-CTLA-4 antibody on CD8+ T cell responses. Tumor-infiltrating lymphocytes (TIL) were harvested on Day 19 following B16 melanoma inoculation in WT and Aire^{GW/+} mice treated with aCTLA-4 or iso antibody. Representative flow cytometry plots and average cumulative frequencies of Ki67⁺, KLRG1⁺, and Granzyme B⁺ among CD8⁺ T cells. n=9-12 in each group. **One-way ANOVA with Tukey's multiple comparisons test.** ns=not significant, *p<0.05.

Supplementary Figure 3



Supplementary Figure 3: Anti-CTLA-4 antibody treatment in Aire deficient mice does not decrease Treg population. Splenocytes and tumor-infiltrating lymphocytes (TIL) were harvested on Day 19 following B16 melanoma inoculation in WT and Aire^{GW/+} mice treated with anti-CTLA-4 antibody (aCTLA-4) or isotype control antibody (Iso). A) Representative flow cytometry plots of FOXP3 and CD25 expression and (B) average cumulative frequencies of FOXP3⁺ Tregs within CD4⁺ splenocyte population. C) Representative flow cytometry plots of FOXP3 and CD25 expression and (D) average cumulative frequencies of FOXP3⁺ Tregs within CD4⁺ TIL population. E and F) Average TIL cumulative ratio of CD4⁺ Teffs : FOXP3⁺ Tregs (E) and CD8⁺ T cells : FOXP3⁺ Tregs (F). **One-way ANOVA with Tukey's multiple comparisons test.** *p<0.05, **p<0.01, n.s= not significant.

Supplementary Figure 4

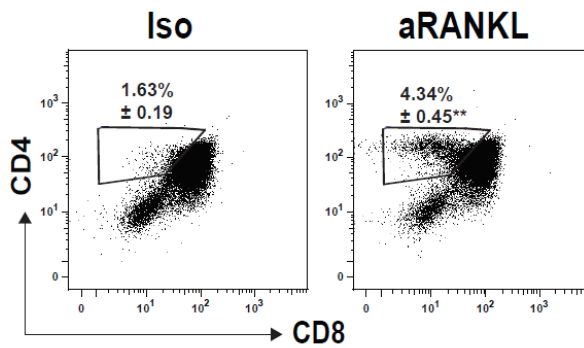


Supplementary Figure 4: anti-CTLA-4 antibody does not impair negative selection of TRP-1 specific CD4+ T cells.

A) Representative flow cytometric plots of CD4⁺ and CD8⁺ thymocytes from Aire^{GW/+} or WT TRP-1 TCR Tg mice. WT TRP-1 TCR Tg were treated with either isotype control antibody (iso) or anti-CTLA-4 antibody (aCTLA-4). B) Cumulative frequency (mean \pm SD) of CD4 single positive (SP) cells. **One-way ANOVA with Tukey's multiple comparisons test.**

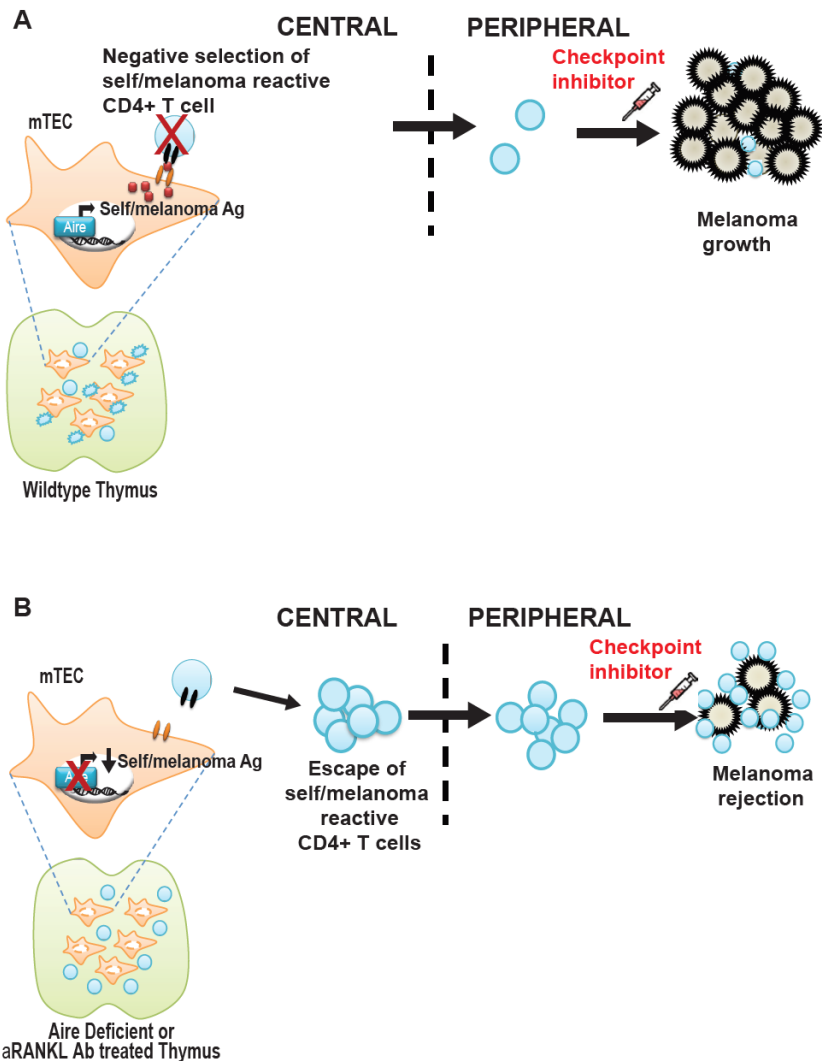
* $p < .05$. n.s.=not significant.

Supplementary Figure 5



Supplementary Figure 5: anti-RANKL antibody impairs negative selection of TRP-1 specific CD4⁺ single positive T cells. Representative flow cytometric plots of CD4⁺ and CD8⁺ thymocytes from TRP-1 TCR Tg mice treated with isotype control antibody (iso) or anti-RANKL antibody (aRANKL). Mean +/- SD shown. **t-test.** **p<.01.

Supplementary Figure 6



Supplementary Figure 6: Model of mechanism underlying additive anti-melanoma effects of combination central and peripheral tolerance blockade. A) In the thymus, Aire in mTECs promotes self/melanoma antigen expression, which results in negative selection of self/melanoma-reactive CD4⁺ T cells. As a result, the efficacy of checkpoint inhibition is limited by the scarcity of melanoma-reactive T cells in the periphery. B) Aire deficiency or transient depletion of Aire-expressing mTECs with anti-RANKL (aRANKL) antibody rescues self/melanoma antigen-reactive effector CD4⁺ T cells from negative selection. This expansion of the melanoma-reactive T cell pool available for activation by checkpoint inhibitors enhances anti-melanoma immunity.