

**Supplementary Figure 1. Frequency of CD4<sup>+</sup>Foxp3<sup>+</sup> T cells in spleen and DLN after treatment with anti-CTLA-4.** Mice inoculated with B16/BL6 tumor cells at day 0 were either untreated or treated with anti-CTLA4 therapy at days 3, 6 and 9. Six to 7 days after the last treatment, they were sacrificed and spleen and tumor draining lymph node (DLN) cells were analyzed by flow cytometry. Horizontal solid lines intersecting circular data points represent means; \*\* indicates  $p<0.01$  and ns indicates Not Statistically Significant.

**Supplementary Figure 2. Untreated ICOS<sup>-/-</sup> mice have decreased frequency of CD4<sup>+</sup>Foxp3<sup>+</sup> T cells as compared to untreated wt mice.** Mice inoculated with B16/BL6 tumor cells at day 0 were untreated and 6-7 days later, TILs were analyzed by flow cytometry. The frequency of CD4<sup>+</sup>Foxp3<sup>+</sup> T cells in TILs was significantly decreased in ICOS<sup>-/-</sup> mice as compared to untreated wt mice. Horizontal solid lines intersecting circular data points represent means; \*\*\* indicates  $p<0.001$

**Supplementary Figure 3. Minimal cytokine production by CD4<sup>+</sup>ICOS<sup>+</sup> and CD4<sup>+</sup>ICOS<sup>-</sup> T cells from untreated wt mice.** Tumor draining lymph node (DLN) Foxp3<sup>+</sup> cells from untreated wt mice were analyzed by flow cytometry. A representative experiment demonstrating minimal intracellular cytokine staining for ICOS<sup>+</sup> CD4 and ICOS<sup>-</sup> CD4 cells is shown.

**Supplementary Figure 4. Minimal cytokine production by T cells when co-cultured with B16/BL6 tumor cells in the absence of DCs.** A representative experiment demonstrating minimal IFN- $\gamma$  production is shown for ICOS<sup>-</sup> CD8 and ICOS<sup>+</sup> CD8 T cells in the absence of DCs.

**Supplementary Figure 5. Untreated wt and ICOS<sup>-/-</sup> mice have similar frequency of CD8 T cells that recognize antigen.** (A) TILs from the indicated mice were analyzed for recognition of TRP2<sub>180-188</sub>-pentamer by gating on CD8 population in a representative experiment and (B) summary data expressed as mean ± SEM from three independent experiments is presented with 5 mice/group for each experiment; (C) CD8 T cells from wt and ICOS<sup>-/-</sup> mice were cultured with melanoma peptide antigens (gp100, TRP1, TRP2) or B16/BL6 melanoma tumor cells and analyzed by ELISPOT assays for IFN- $\square$  production. Vertical bars represent means. The results shown are summary data expressed as mean ± SEM; *ns* indicates Not Statistically Significant.