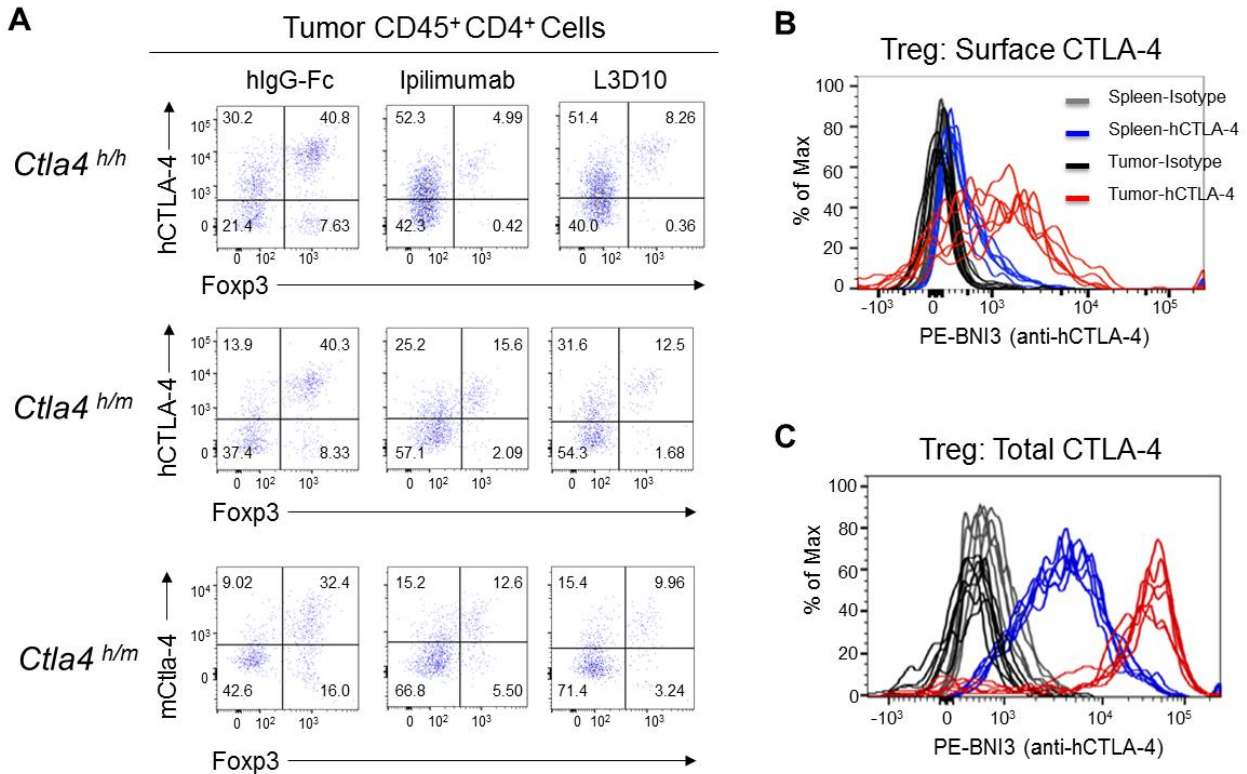


Figure S6



Supplementary information, Figure S6 CTLA-4 was preferentially highly expressed in tumor-infiltrated Tregs. **(A)** Tumor-derived Tregs had higher expression of CTLA-4 than Foxp3-negative CD4 T cells. As in Figure 7, MC38 tumor cells were injected into *Ctla4*^{h/h} or *Ctla4*^{h/m} mice and mice were treated with 100µg control hlgG-Fc or anti-CTLA-4 mAbs. Five days after the third antibody treatment, mice were sacrificed and tumor cells were subjected to flow cytometric analysis on human CTLA-4 or mouse Ctla-4 expression in tumor-infiltrated CD45⁺CD4⁺Foxp3⁺ Tregs and CD45⁺CD4⁺Foxp3⁻ T cells. Data represent the results from one of three independent experiments. **(B, C)** Tregs from tumor had higher expression of both surface CTLA-4 and total CTLA-4 than that from spleen. As in Figure 7, 14 days after MC38 tumor inoculation, *Ctla4*^{h/h} mice were sacrificed for flow cytometric analysis on surface CTLA-4 **(B)** and total CTLA-4 **(C)** expression in spleen and tumor derived CD4⁺Foxp3⁺ Tregs. Each line of the histogram

plots indicates one individual mouse. $n=6$ mice and data shown represent the results from one of at least three independent experiments.