

## Supplemental Figure Legends

### Figure S1. Characterization of B6+*Ctla4*<sup>-/-</sup>→B6 mixed radiation bm chimeras.

(A) Donor reconstitution of B6+*Ctla4*<sup>-/-</sup>→B6 mice and the frequencies of CD4<sup>+</sup>CD25<sup>+</sup> Tregs within each donor population are shown relative to negative control staining (top and middle panels). Intracellular Foxp3 staining of gated CD4<sup>+</sup>CD25<sup>+</sup> T cells within each donor population is also shown (bottom panel).

(B) Expression of CD44 and CD62L by CD4<sup>+</sup>CD25<sup>-</sup> LNT cells from wildtype, *Ctla4*<sup>-/-</sup> and B6(CD45.1)+*Ctla4*<sup>-/-</sup>(CD45.2)→B6(CD45.1) mixed bm chimeras.

### Figure S2. Effect of CTLA-4Ig on T cell proliferation.

Resting CD4<sup>+</sup>CD25<sup>+</sup> Tregs of B6 origin and CD4<sup>+</sup>CD25<sup>-</sup> Tconv cells of *Ctla4*<sup>-/-</sup> origin from B6+*Ctla4*<sup>-/-</sup>→B6 chimeras were stimulated by anti-CD3 (1μg/ml) + APC in the presence or absence of CTLA-4Ig (10μg/ml). Proliferation was measured by <sup>3</sup>H-thymidine incorporation and mean cpm ± SD of triplicate wells are shown. Data are representative of two independent experiments.

### Figure S3. CTLA-4<sup>TgWT</sup> expression in CD4<sup>+</sup>CD25<sup>-</sup> T cells.

Top panels display CD25 surface staining of gated CD4<sup>+</sup> LNT cells and the numbers indicate the percentage of CD4<sup>+</sup> T cells that are CD4<sup>+</sup>CD25<sup>+</sup> (dashed lines represent negative control staining). Bottom panels display intracellular CTLA-4 staining of gated CD4<sup>+</sup>CD25<sup>+</sup> and CD4<sup>+</sup>CD25<sup>-</sup> T cells. MFI of CTLA-4 staining are also indicated.

**Figure S4. Characterization of Foxp3 transgenic mice.**

(A) Top panel displays the Foxp3 transgenic construct, and bottom panels display the thymus profiles of Foxp3<sup>Tg</sup> and wild type B6 littermate mice.

(B) Foxp3 expression in Foxp3<sup>Tg</sup> CD4SP thymocytes and spleen CD4<sup>+</sup> T cells. Intracellular staining for Foxp3 in CD4SP thymocytes and CD4<sup>+</sup> spleen T cells from B6 and Foxp3<sup>Tg</sup> mice are displayed along with their MFI. The numbers indicate the percentage of CD4<sup>+</sup> T cells that are Foxp3<sup>+</sup>. Data are representative of three independent experiments.

(C) Transgenic Foxp3 did not alter wildtype CTLA-4 transgenic (CTLA-4<sup>TgWT</sup>) protein expression. Intracellular CTLA-4 and Foxp3 staining of freshly isolated CD4<sup>+</sup>CD25<sup>-</sup> T cells from indicated mice were assessed. Dashed lines represent negative control staining. Data are representative of three independent experiments.

**Figure S5. Regulation of CTLA-4 surface expression by TCR signaling.**

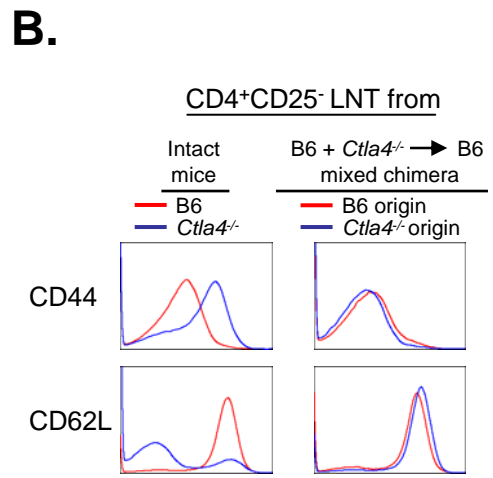
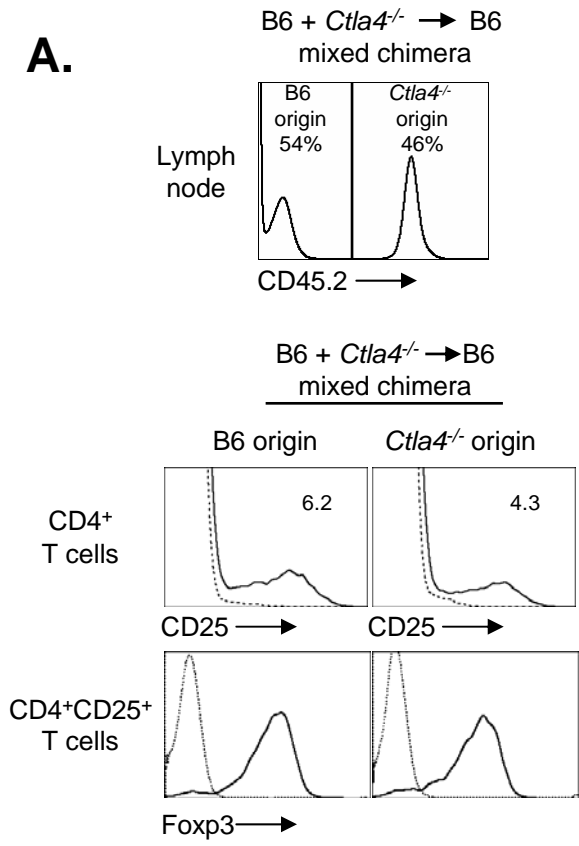
Surface and intracellular CTLA-4 staining of CD4<sup>+</sup>CD25<sup>-</sup> Tconv cells from the indicated mice are shown (left panels). To assess the effect of TCR signaling on CTLA-4 localization, resting CD4<sup>+</sup>CD25<sup>-</sup> Tconv cells of CTLA-4<sup>TgWT</sup> or CTLA-4<sup>TgΔ</sup> origin from B6+ CTLA-4<sup>TgΔ</sup> → B6 mixed chimeras were cultured at 37°C for 60 min with PE-conjugated anti-CTLA-4 mAb in plates that had been coated with anti-TCR/CD28 mAbs. Acquired fluorescence by cultured T cells (RED) was compared with that on fresh T cells (Black) (right panels). Dashed lines represent negative control staining.

**Supplemental Videos for Figure 3****Figure 3 Video 1 and Video 2. 3D visualization of CTLA-4 in Tregs and Tconv cells.**

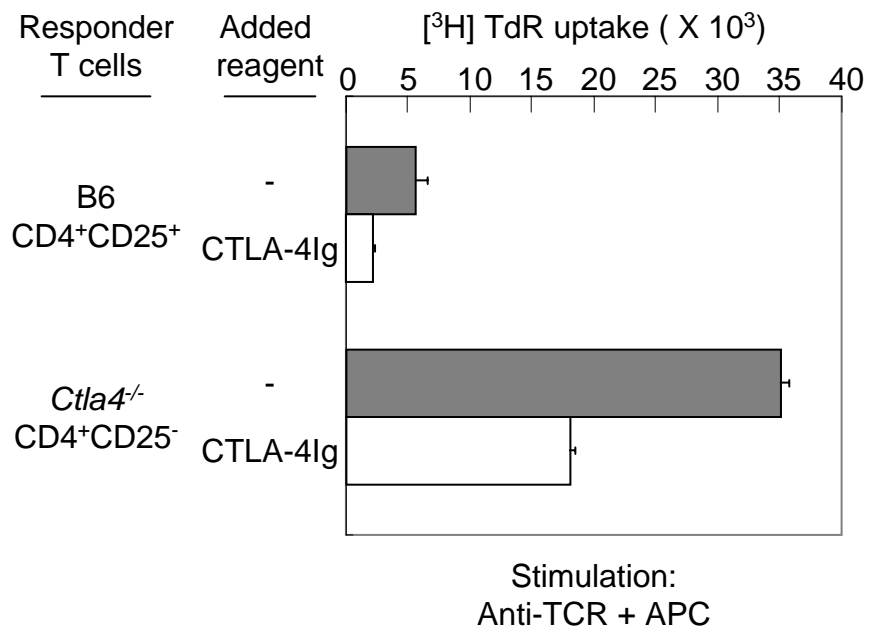
CD4<sup>+</sup>CD25<sup>+</sup> Tregs (Video 1) and CD4<sup>+</sup>CD25<sup>-</sup> Tconv cells (Video 2) from CTLA-4<sup>Tg<sup>WT</sup></sup> mice were surface stained for TCR (shown in red) and then fixed and stained for intracellular CTLA-4 (shown in green). The distribution of TCR and CTLA-4 was assessed by confocal microscopy. 3D reconstitution was accomplished with Imaris 5.5.

**Figure 3 Video 3 and Video 4. 3D visualization of Golgi retention of CTLA-4 in Tconv cells but not Tregs.**

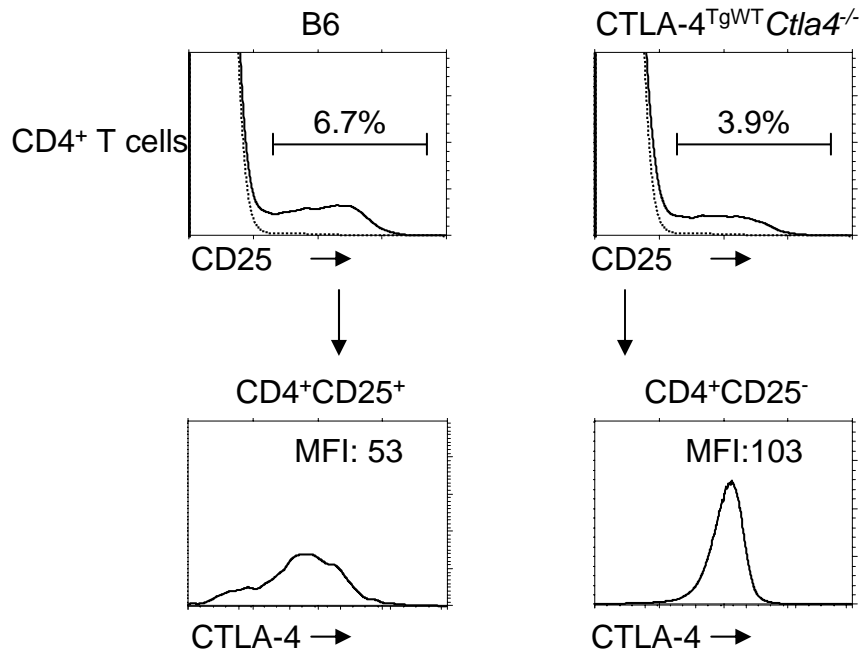
CD4<sup>+</sup>CD25<sup>+</sup> Tregs (Video 3) and CD4<sup>+</sup>CD25<sup>-</sup> Tconv cells (Video 4) from CTLA-4<sup>Tg<sup>WT</sup></sup> mice were fixed and stained for intracellular CTLA-4 (shown in green) and GM130 (shown in red). 3D reconstitution was accomplished with Imaris 5.5.



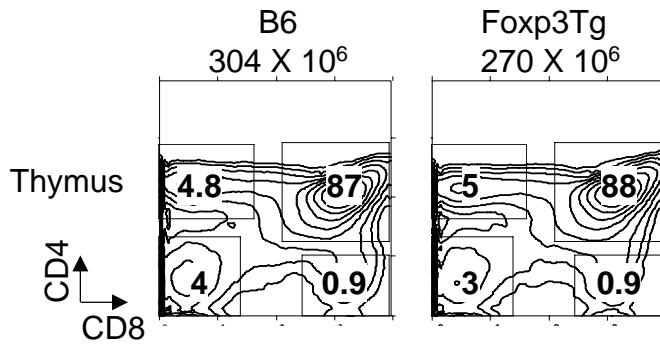
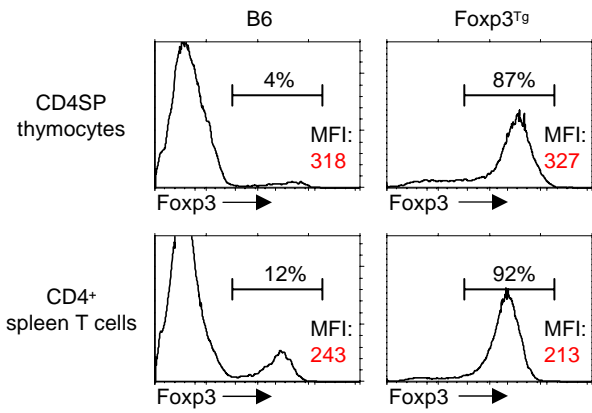
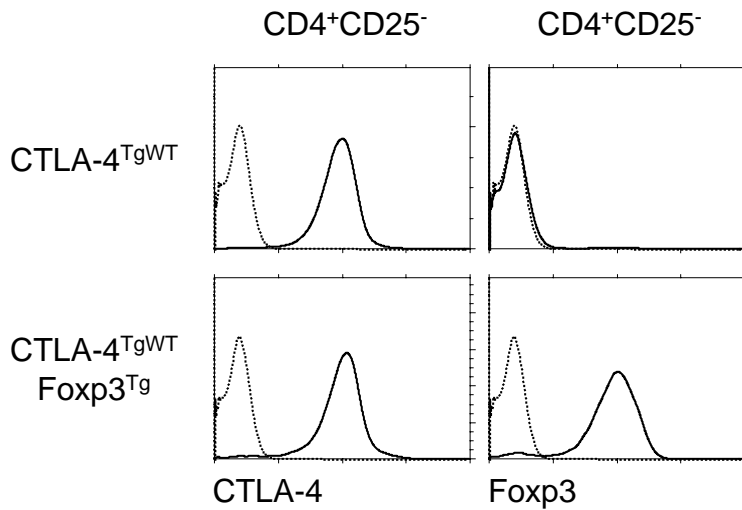
**Fig. S1**

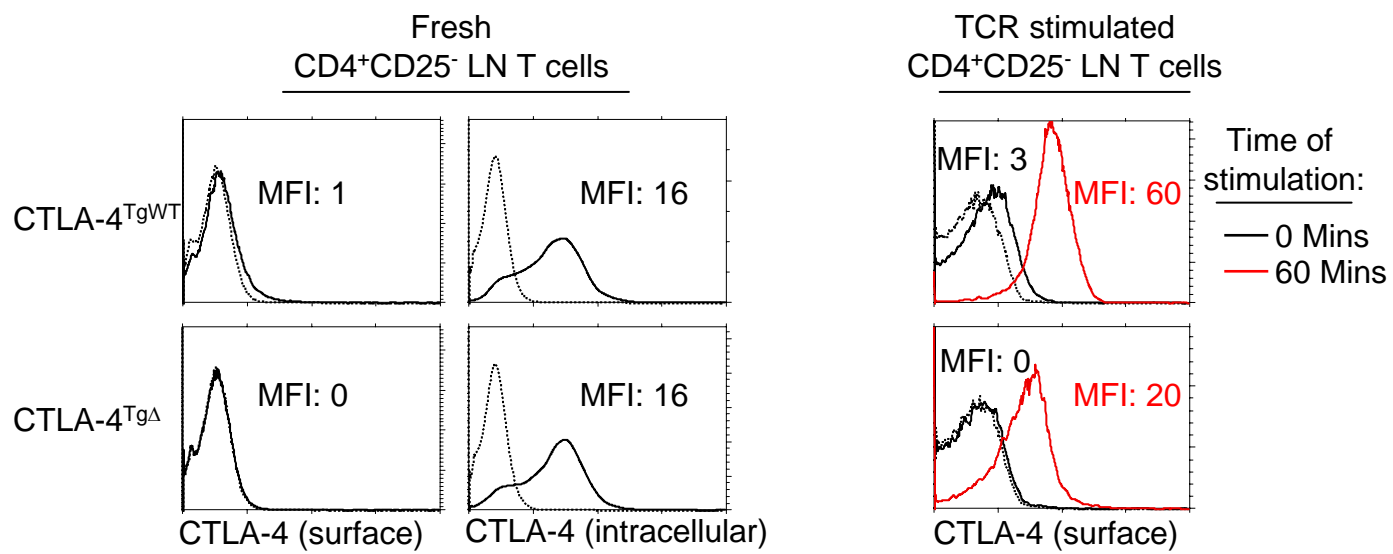


**Fig. S2**



**Fig. S3**

**A.**Construct of Foxp3<sup>Tg</sup>**B.****C.**LN CD4<sup>+</sup> T cells**Fig. S4**



**Fig. S5**