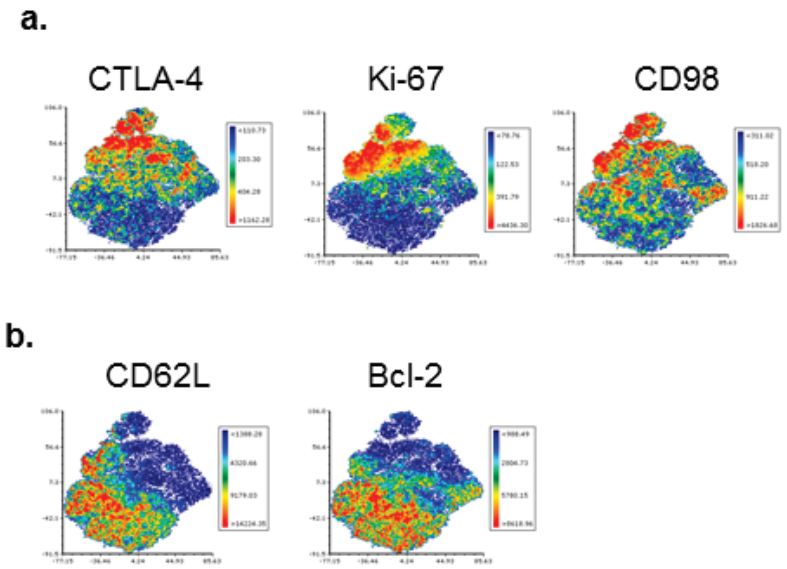
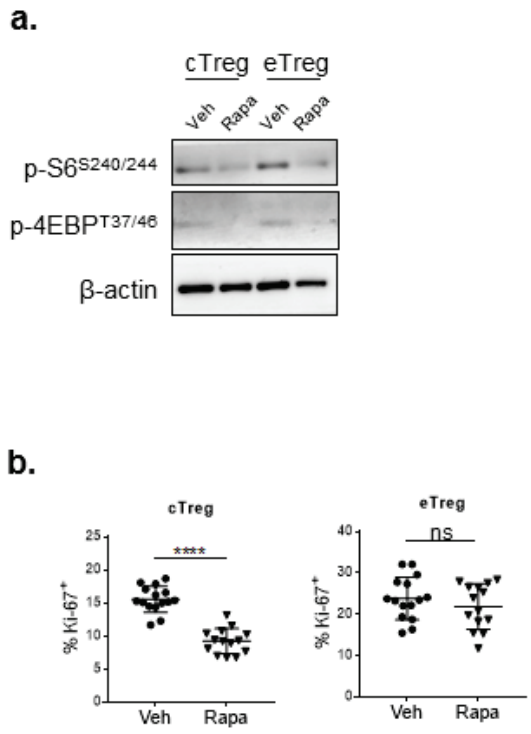
Supplemental Figure 1



Supplementary Figure 1. Effector and central Treg molecules from splenic Tregs have distinct clustering patterns

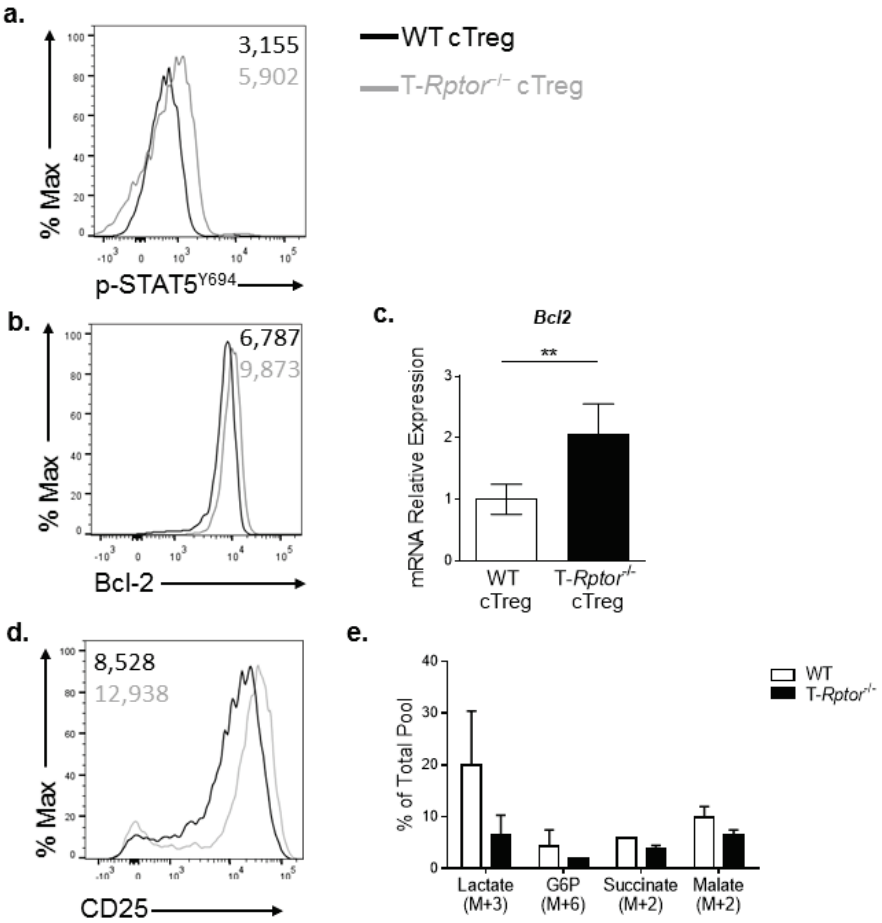
Same as in Figure 1E, the heatmap is overlaid with additional effector Treg molecules (CTLA-4, Ki-67, and CD98) and (b) central Treg molecules (CD62L and Bcl-2) from splenic Treg onto a t-SNE plot. Data are representative of at least three independent experiments.

Supplemental Figure 2



Supplemental Figure 2. Both central and effector Tregs are sensitive to rapamycin treatment Same as Figure 3, mice were treated with either vehicle or rapamycin (300 μg/kg) for 6 days. (a) Immunoblot analysis of mTOR signaling pathway from sorted splenic cTregs and eTregs from either vehicle- or rapamycin-treated mice. β-Actin served as a loading control. (b) Flow cytometry analysis of Ki-67 from splenic cT_R and eT_R. ****$P < 0.0001$; ns, no significance (Mann-Whitney *t* test). Data are representative of three independent experiment.

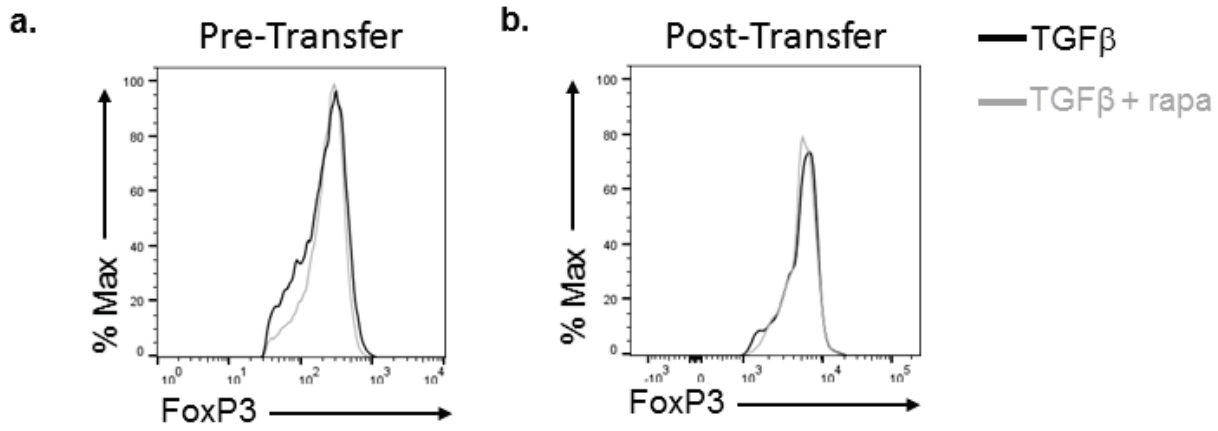
Supplemental Figure 3



Supplementary Figure 3. *Rptor*-deficient cTregs have enhanced central Treg phenotype

Same as in Figure 4, (a) flow cytometry analysis of p-STAT5 Y694 and (b) Bcl2 expression between WT and T-*Rptor*$^{-/-}$ cTregs. (c) Bcl2 mRNA expression was assessed between sorted eTregs and cTregs from WT mice. (d) Flow cytometry analysis of CD25 on WT and T-*Rptor*$^{-/-}$ cTregs. (e) WT and T-*Rptor*$^{-/-}$ mice were injected intravenously with [U-^{13}C] glucose. Glucose tracing study was performed on glycolysis and TCA cycle metabolites in splenic Treg cells isolated from WT or T-*Rptor*$^{-/-}$ mice. $^{**}P < 0.005$ (Mann-Whitney *t* test). Data are representative of at least three independent experiments (a-d) or one experiment composed of three mice (e).

Supplemental Figure 4



Supplementary Figure 4. Tregs subsets with different mTOR activity have same FoxP3 expression

Same as in Figure 6 and 7, Tregs were generated under Treg-polarizing condition in the presence or absence of rapamycin and were subsequently adoptively transferred into WT hosts. Flow cytometry analysis examining FoxP3 expression (a) pre-transfer and (b) post-transfer.

Data are representative of three independent experiments.