

Fig. S1

Shakya et al.

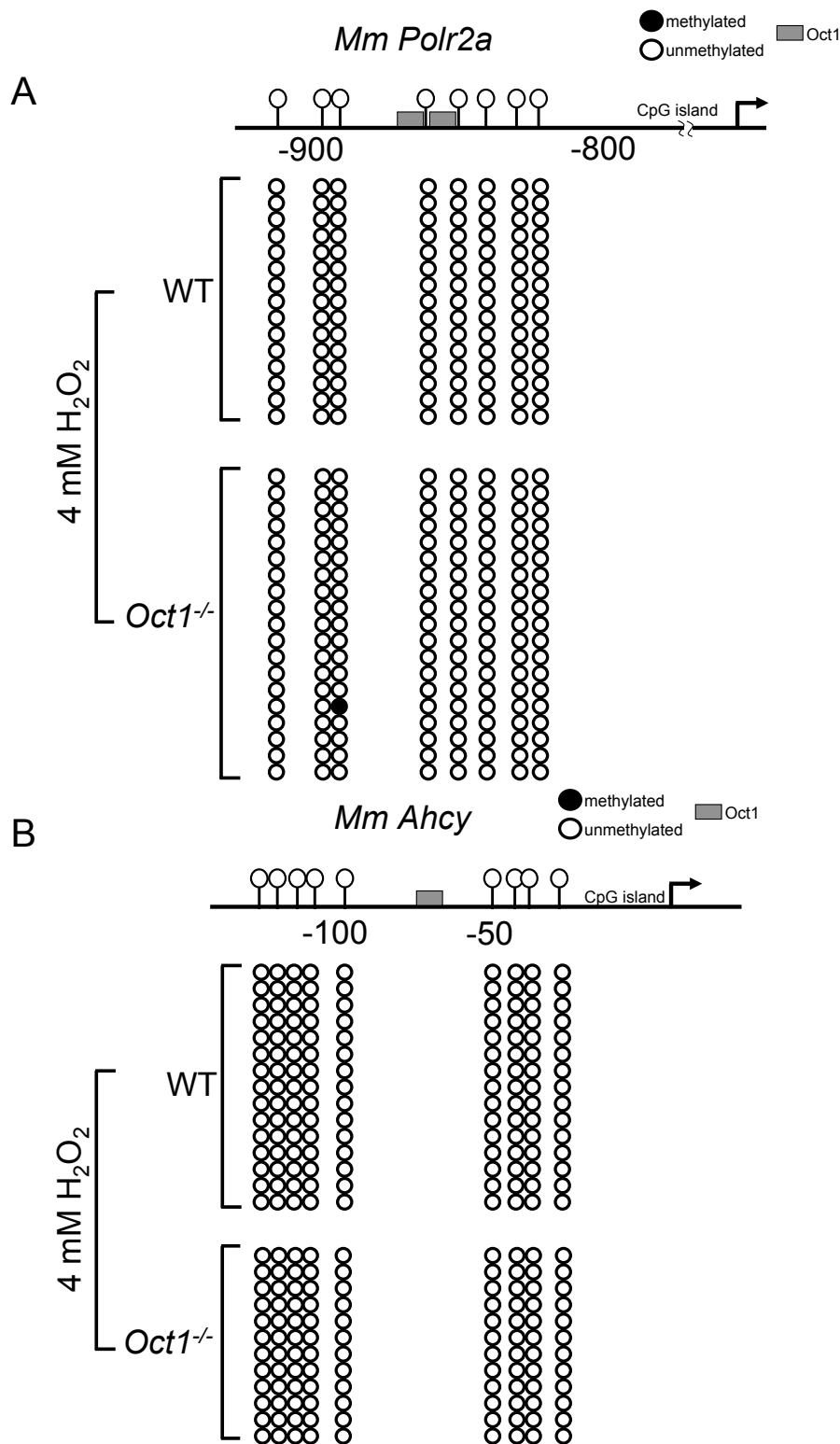
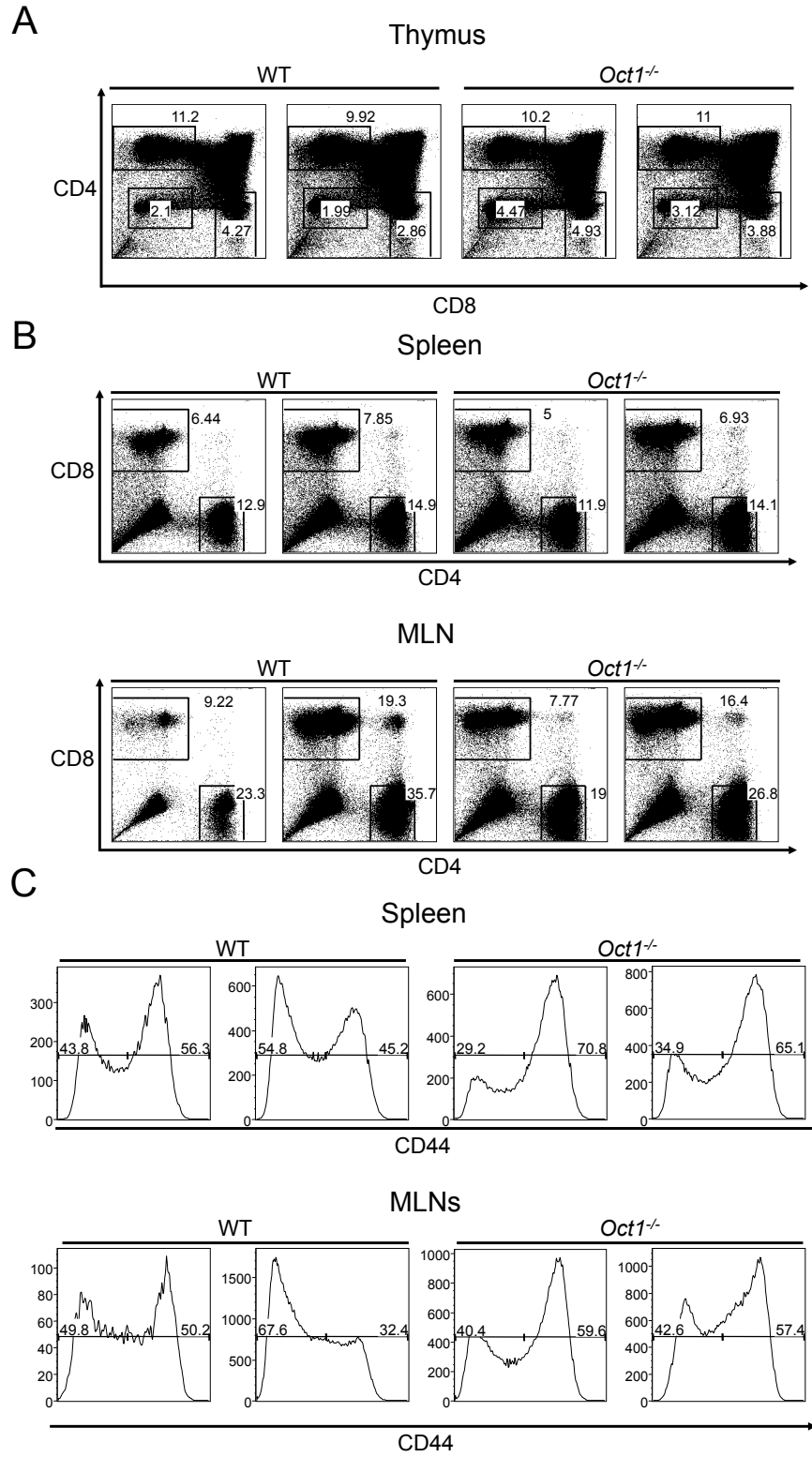


Fig. S1. (A) Bisulfite sequencing of the *Polr2a* regulatory region encompassing the Oct1 binding site in wild-type and Oct1 deficient MEFs exposed to 4 mM H₂O₂ for 1 hr, and then incubated without H₂O₂ for an additional 3 hr. METHODS: *Polr2a* forward primer: GGGAAAAGTTTGGGTTT GAATAT. *Polr2a* reverse primer: CAACTAAAATCTCTCATTAAATATCCCC. (B) Similar experiment using murine *Ahcy*. Sequences *Ahcy* primers were: *Ahcy* forward, TGGTTTTTGGTGGTGTGGAT; *Ahcy* reverse, AAAACAAA AATCAACCTTACTC.



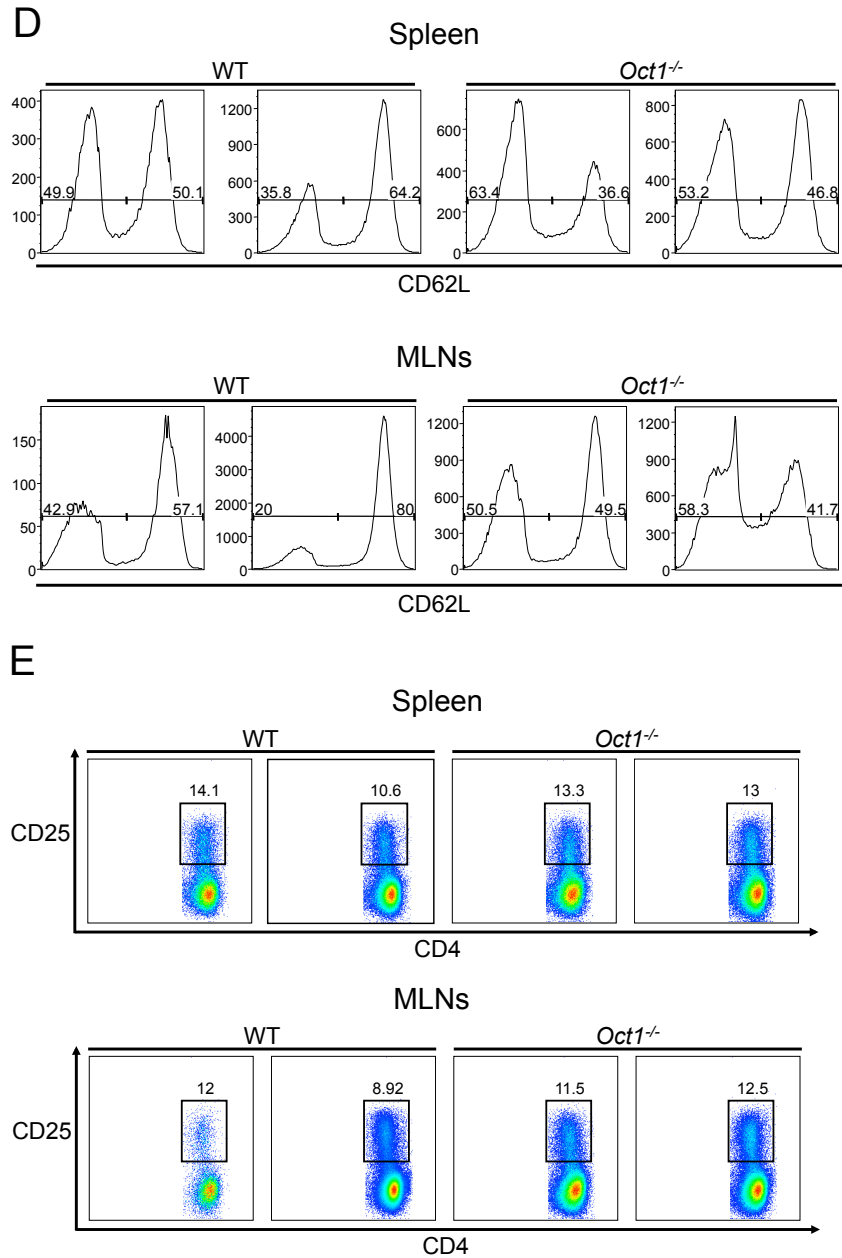


Fig. S2. T cell development and T cell subset distributions in *Oct1* deficient transplant *Rag-1^{-/-}* recipient animals. Four separate animals are shown, two transplanted with *Oct1^{-/-}* fetal liver and two with wild-type littermate controls. (A) CD4/CD8 subsets in the thymus in transplant recipient mice are shown. (B) CD4/CD8 T cell populations in spleens and lymph nodes of recipient mice. MLN: mesenteric lymph nodes. (C) CD4⁺ T cell activation state. Cells were gated for CD4. (D) CD4⁺ T memory populations. Cells were gated for CD4. (E) Levels of CD4⁺ CD25⁺ subsets. Cells were gated for CD4. METHODS: Data were collected using a FACSCanto II (BD Biosciences) and analyzed with Flowjo software. T cells were stained with anti-CD4 (Alexaflour 700), anti-CD8 (PE-Cy7), anti-CD44 (PerCP), anti-CD62L (APC) and anti-CD25 (FITC) antibodies (eBioscience).

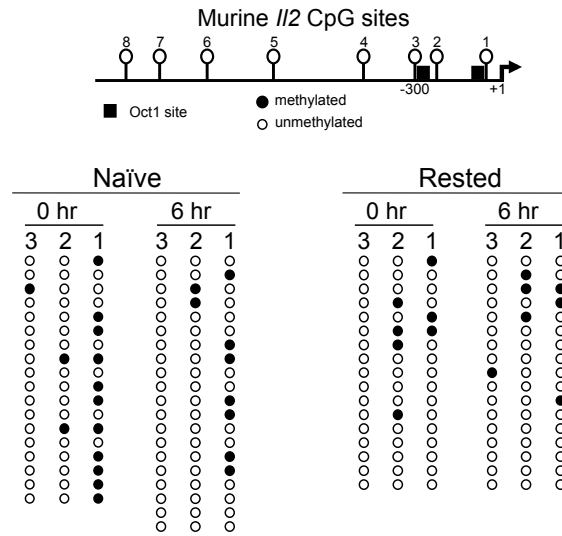


Fig. S3. *//2* promoter bisulfite sequencing using purified naïve ($CD44^{low}$) helper T cells from spleens of wild-type C57BL/6 mice prior to CD3/CD28 stimulation, or 6 hr following stimulation. The right panel shows cells that were stimulated for two days, followed by an 8 day resting period in culture without stimulation. Cells were then restimulated for 6 hr.

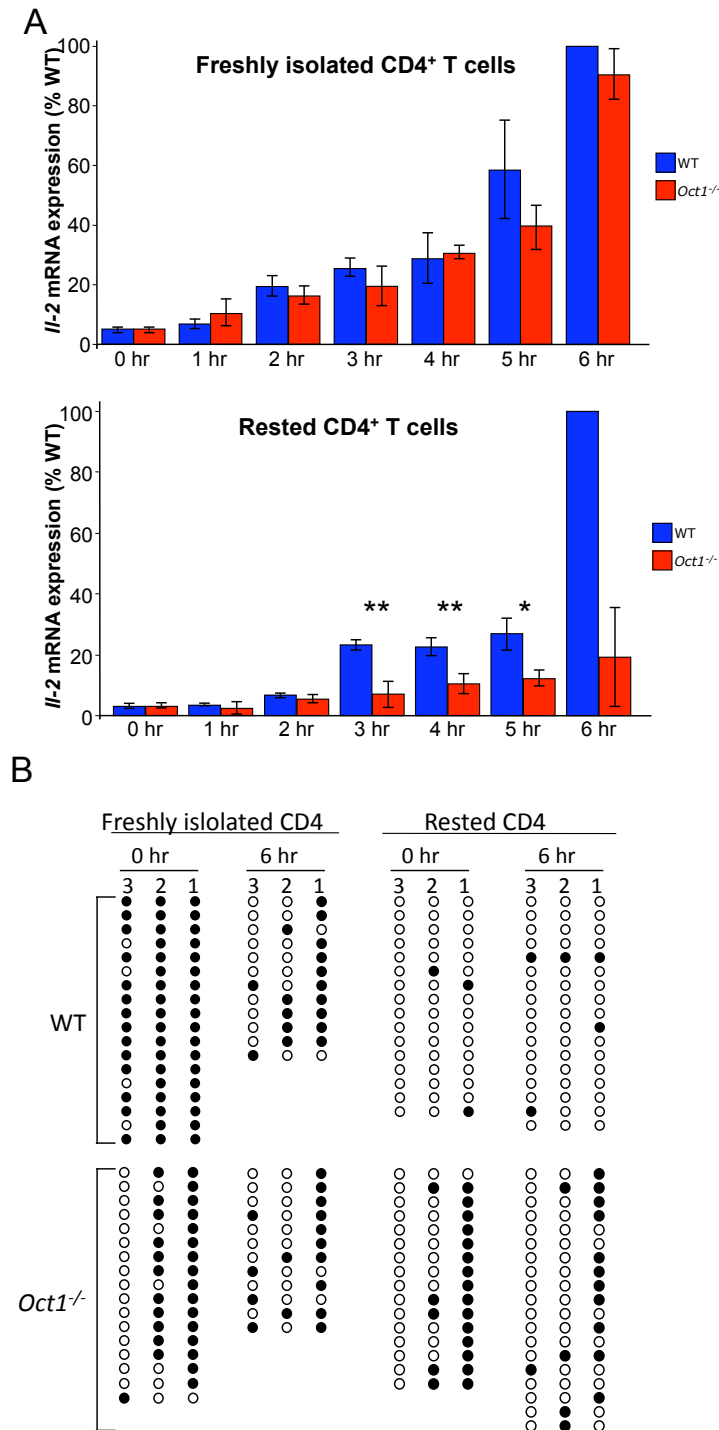


Fig. S4. (A) RT-PCR showing *Il2* mRNA expression in freshly isolated and rested, but previously stimulated, total CD4 splenic T cells following CD3/CD28 ligation. METHODS: CD4⁺ T cells were isolated using a MACS column and CD4 microbeads (Miltenyi). A time course is shown in which expression plotted as a percentage of the maximum signal (wild-type T cells, 6 hr). Values represent an average of three biological replicates. Error bars depict s.e.m. The unpaired Student's t test was used to generate *p* values for the data sets (*, *p*<0.05; **, *p*<0.01 and ***, *p*<0.001). (B) *Il2* promoter bisulfite sequencing using total CD4 splenic T cells.