SUPPLEMENTAL MATERIAL

Yue et al., http://www.jem.org/cgi/content/full/jem.20151438/DC1

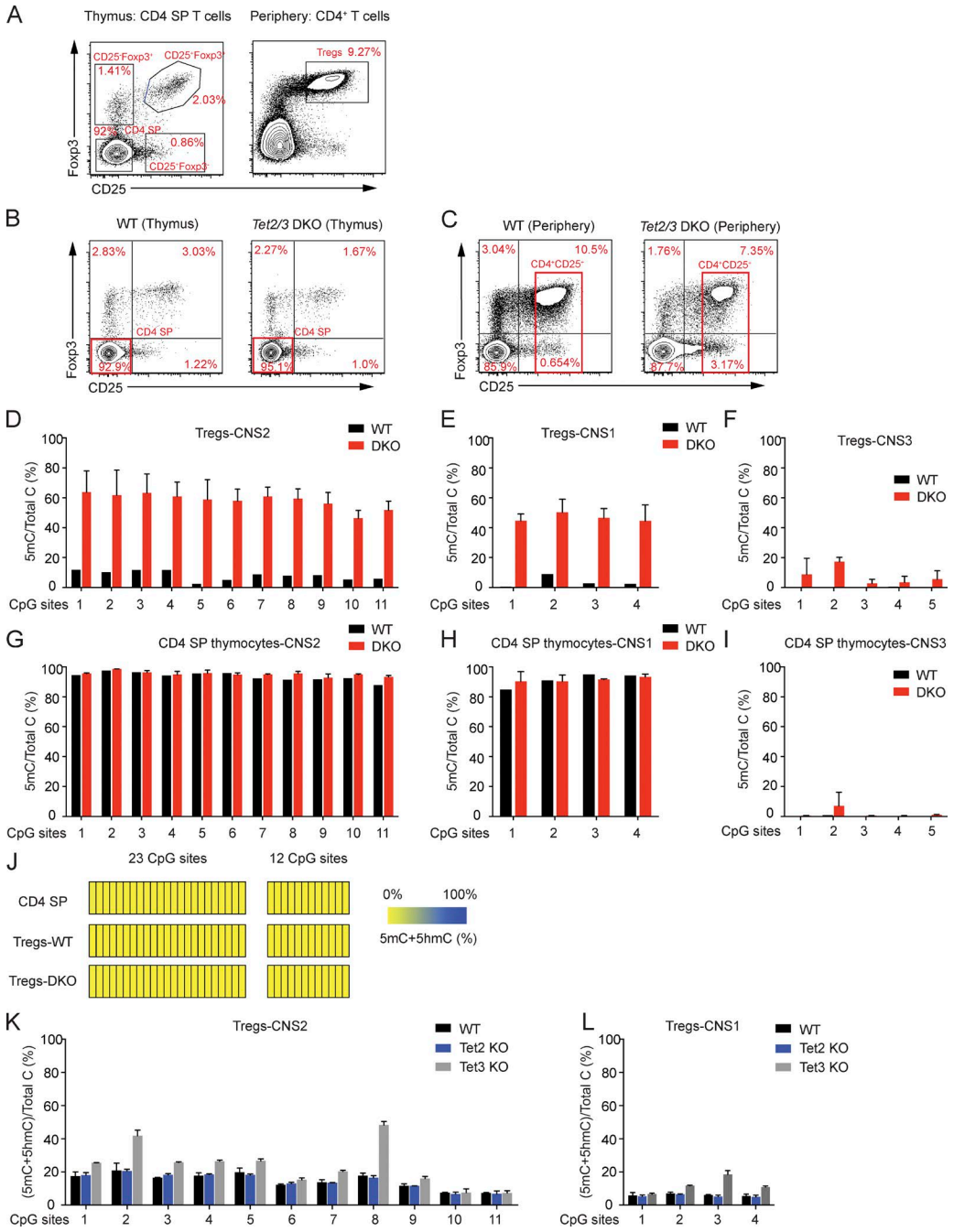

Figure S1. **Tet2 and Tet3 mediate the loss of 5mC in *Foxp3 CNS* elements.** (A, left) Sorting strategy used to isolate CD4 SP, precursor T reg cells ($CD25^+Foxp3^-$ and $CD25^-Foxp3^+$ subsets), and $CD25^+Foxp3^+$ (T reg) cells in the thymus. Right: Gating strategy for peripheral T reg cells ($CD4^+CD25^{+-}Foxp3^+$). (B) Foxp3 versus CD25 expression within CD4 SP thymocytes from WT (left) or Tet2/3 *DKO* (right) mice. The red squares indicate the CD4 SP thymocytes sorted for the methylation analysis in G–J. (C) Foxp3 versus CD25 expression within $CD4^+$ peripheral T cells from WT (left) or Tet2/3 *DKO* (right) mice. The red squares indicate the $CD4^+CD25^+$ T reg cells sorted for the methylation analysis in D–F. (D–F) Graphs depicting the percentage of 5mC determined by BS-seq and oxBS-seq in peripheral T reg cells in 11 CpGs in *Foxp3 CNS2* (D), 4 CpGs in *Foxp3 CNS1* (E), and 5 CpGs in *Foxp3 CNS3* (F). (G–I) Graphs depicting the percentage of 5mC determined by BS-seq and oxBS-seq in CD4 SP thymocytes in 11 CpGs in *Foxp3 CNS2* (G), 4 CpGs in *Foxp3 CNS1* (H), and 5 CpGs in *Foxp3 CNS3* (I). (J) Heat maps depicting the percentage of (5mC + 5hmC)/total C in CpGs within two distinct regions of an upstream CpG island in *Foxp3* locus in CD4 SP thymocytes, WT, or *Tet2/3 DKO* peripheral T reg cells as determined by BS-seq. (K and L) BS-seq of the 11 CpG sites in *Foxp3 CNS2* (K) and the 4 CpG sites in *Foxp3 CNS1* (L). Graphs depict the percentage of (5mC + 5hmC)/total C in peripheral T reg cells isolated from WT, *Tet2 KO* ($Tet2^{-/-}$), and *Tet3 KO* ($Tet3^{fl/fl}$ CD4-Cre) mice. Error bars show mean ± SD of thousands of sequencing reads from at least two independent experiments.

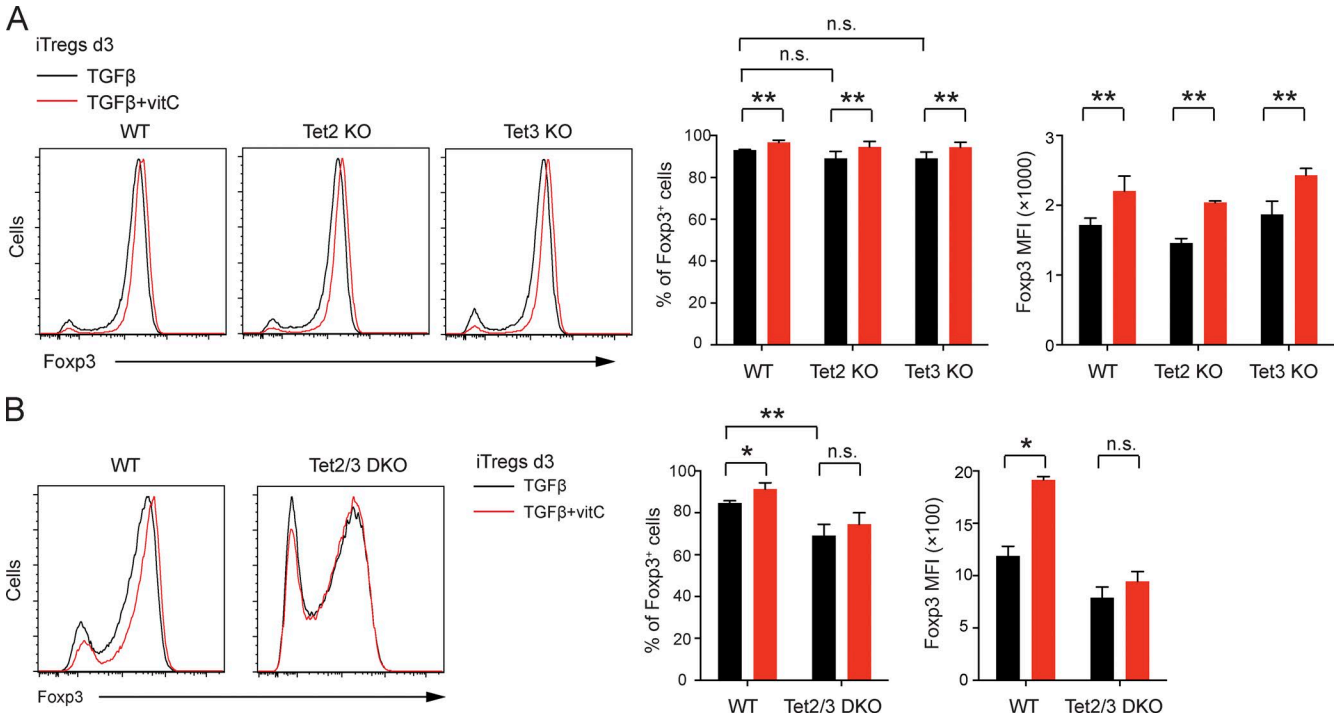

Figure S2. **Addition of vitamin C during iT reg cell differentiation enhances the percentage and MFI of Foxp3 expression via TET proteins.** (A, left) Representative histogram overlay. Right: Graphs for the percentage and MFI of Foxp3$^+$ cells and for iT reg cells differentiated for 3 d in the presence of TGF-β and TGF-β + vitamin C from WT, *Tet2*$^{-/-}$, and *Tet3*$^{fl/fl}$ CD4-Cre naive T cells. (B, left) Representative histogram overlay. Right: Graphs for the percentage and MFI of Foxp3$^+$ cells in iT reg cells differentiated from either WT or *Tet2*$^{-/-}$*Tet3*$^{fl/fl}$ ERT2-Cre cells in the presence of TGF-β and TGF-β + vitamin C for 3 d. Error bars show mean ± SD from at least three independent experiments. *, P < 0.05; **, P < 0.01 by Student's *t* test. n.s., not significant.

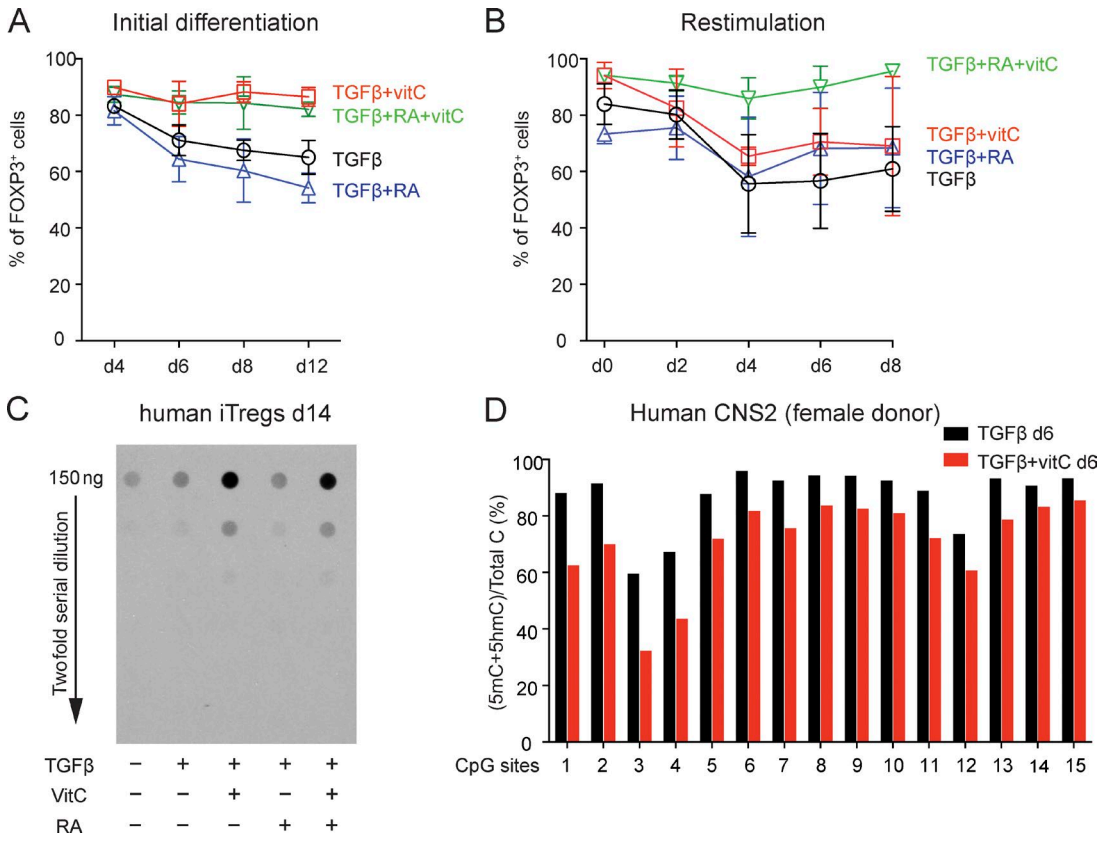

Figure S3. **Vitamin C enhances the stability of human iT reg cells and promotes the loss of 5mC in human *FOXP3 CNS2*.** (A) Percentage of FOXP3$^+$ cells in human iT reg cells differentiated from naive CD4$^+$ T cells in the presence of TGF-β, TGF-β + vitamin C, TGF-β + RA, and TGF-β + RA + vitamin C for 4, 6, 8, and 12 d. Error bars show mean ± SD from three to four independent donors. (B) Time course analysis of FOXP3 expression in human iT reg cells generated as indicated in the presence of TGF-β, TGF-β + vitamin C, TGF-β + RA, and TGF-β + RA + vitamin C and restimulated with anti-CD3 and anti-CD28 antibodies for 2, 4, 6, and 8 d. Graphs show results from three independent donors. (C) Genomic DNA from human effector cells and iT reg cells differentiated for 14 d in the presence of TGF-β, TGF-β + vitamin C, TGF-β + RA, and TGF-β + RA + vitamin C was treated with sodium BS to convert 5hmC to CMS. The relative intensity of CMS was quantified by anti-CMS dot blot assay. Data show results from two independent experiments. (D) BS-seq of 15 CpGs in human *FOXP3 CNS2*. The graph depicts the percentage of (5mC + 5hmC)/total C in human iT reg cells differentiated for 6 d without or with vitamin C from a female donor.

Table S1 is available as an Excel file and lists primer sequences used for amplicon sequencing in this study.

Table S2 is available as an Excel file and lists BS conversion efficiency assessed by spike-in control for BS or oxBS in this study.