Supplementary information

A multiple redundant genetic switch locks in the transcriptional signature of T regulatory cells

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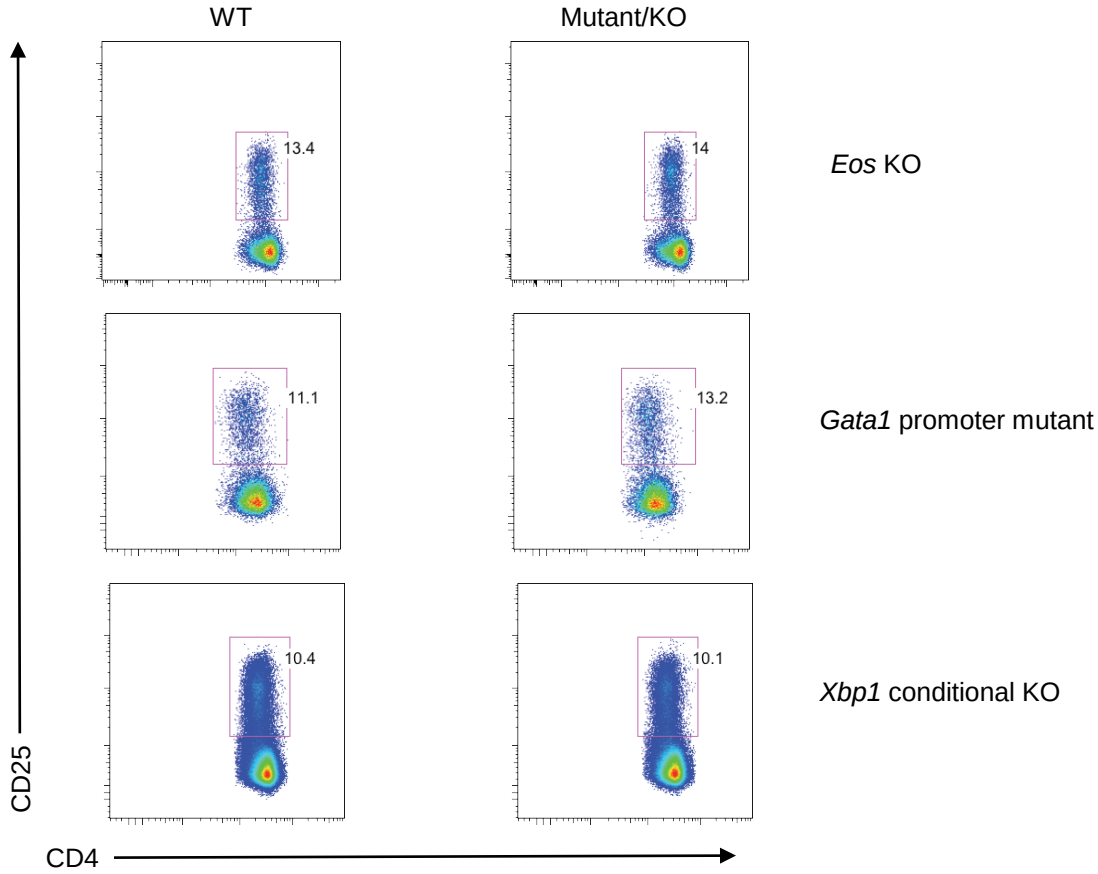
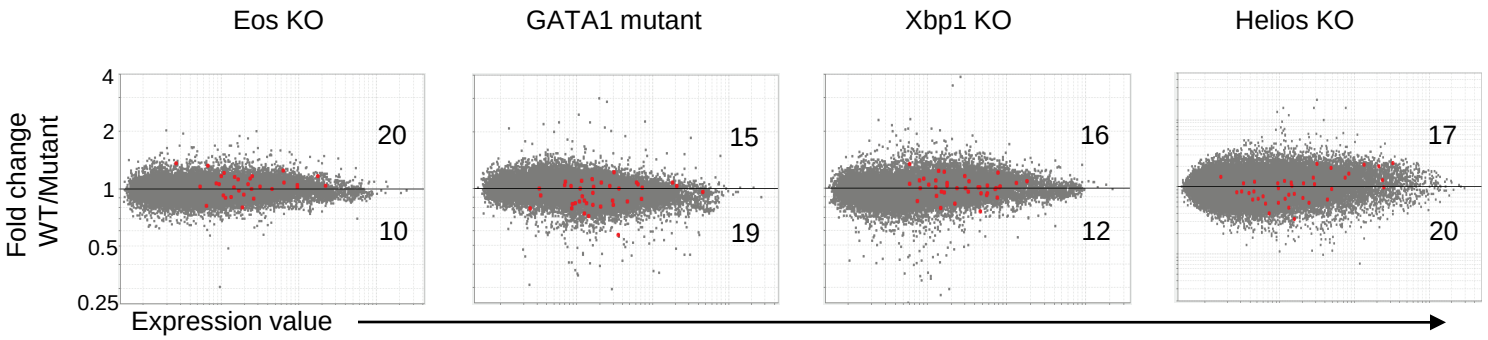
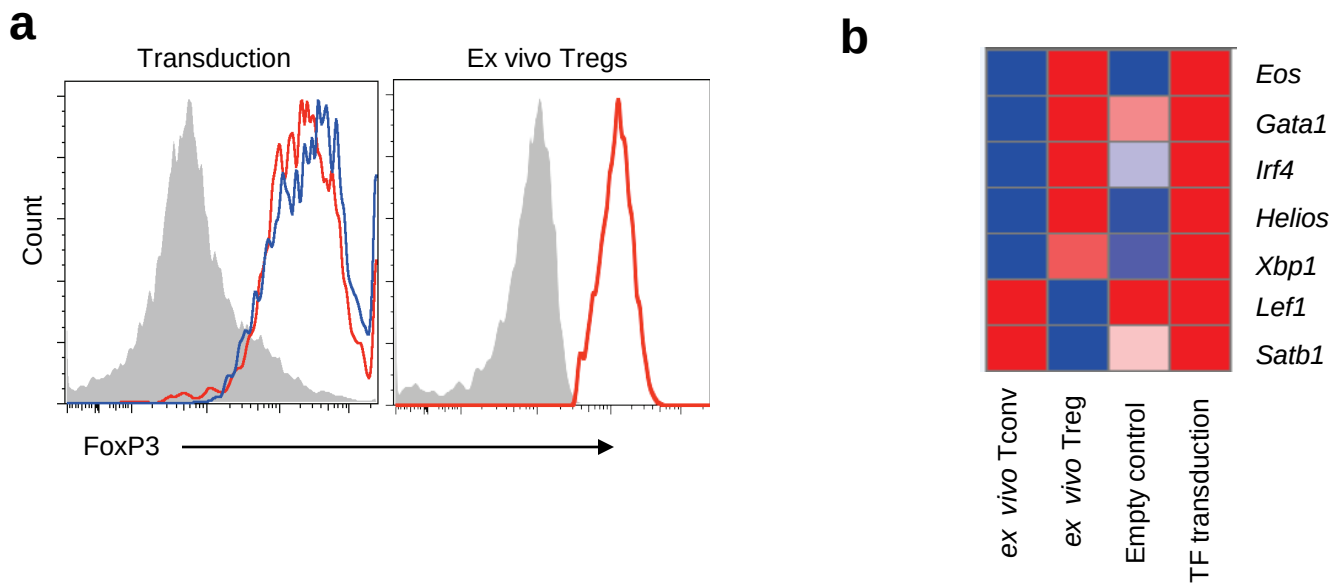


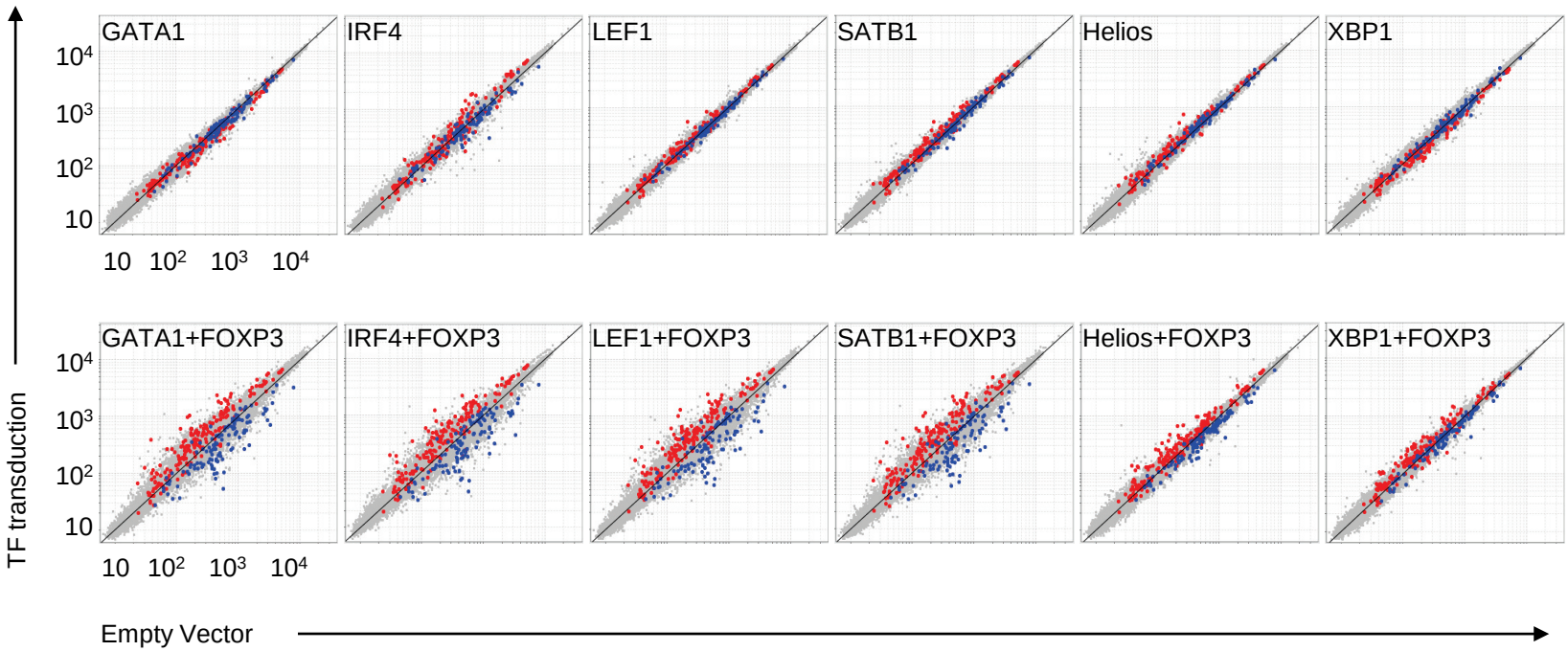
Supplementary Figure 1. Treg fraction in mice bearing deficiencies in Treg signature-determining factors.
Representative flow cytometric analysis of splenocytes from the mutant mice or their WT littermates. Cells were pre-gated as $CD4^{+}CD8a^{-}CD19^{-}CD11b^{-}CD11c^{-}$. The gate shown was used to sort cells for gene expression profiling.
Data are representative of two experiments.



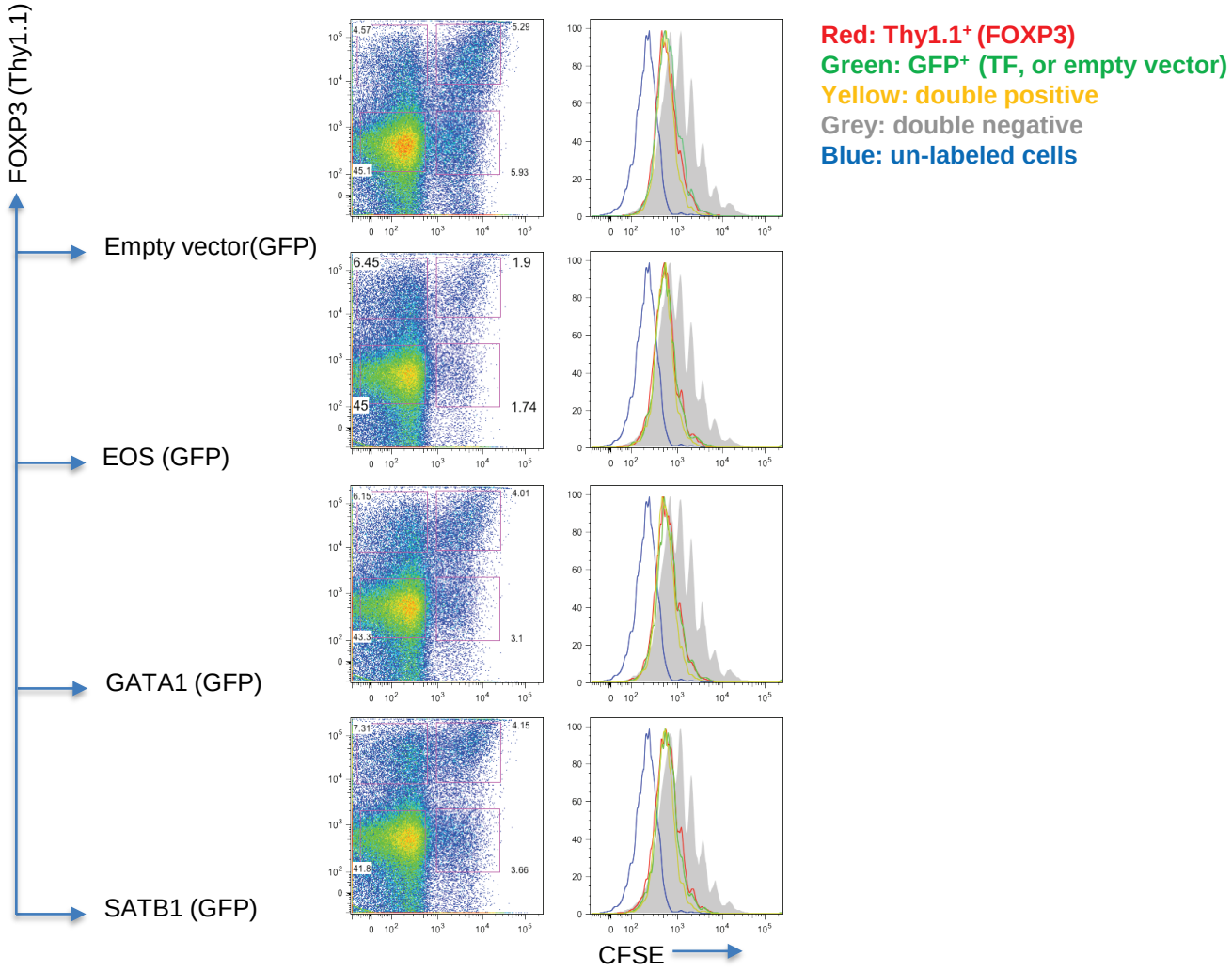
Supplementary Figure 2. Mutations in Treg signature-determining factors do not significantly affect their CLR-predicted target genes.
Fold change (WT/Mutant) versus expression value plots, generated from the data of **Fig. 2b**. Genes predicted by the CLR and optimization analyses to be influenced by each factor are highlighted by red dots. Numbers indicate genes differentially expressed in WT versus mutant.



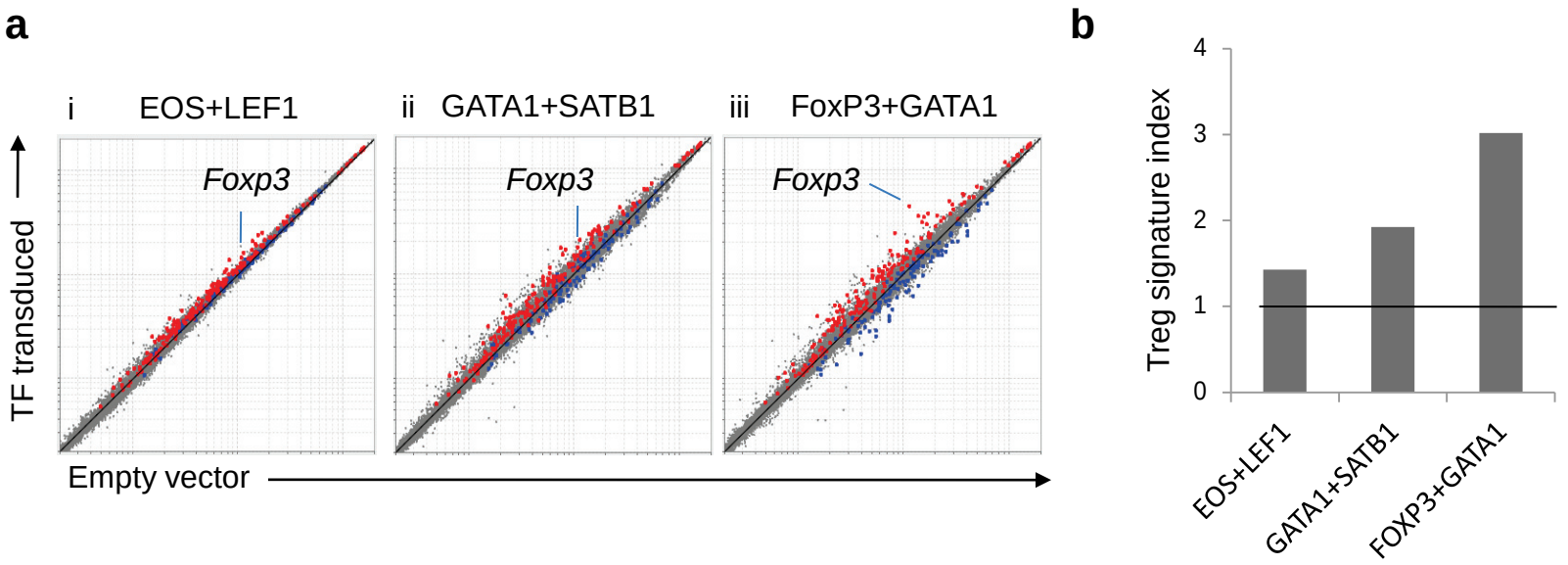
Supplementary Figure 3. Expression levels of exogenously transduced TFs.
(a) Flow cytometric quantitation of FoxP3 after intracellular staining. Left, $CD4^+$ Tconv cells transduced with retroviral vectors encoding FOXP3 (red line) or FOXP3 plus GATA1 (blue line); right, ex vivo splenic Treg cells (gated on $CD4^+CD25^+$). Anti-FoxP3 antibody (eBioscience JFK-16s), which recognizes both human and mouse FoxP3, was used. Gray histograms represent negative controls. **(b)** Expression of predicted cofactors. Cells were transduced with control retrovirus, or the corresponding TF-expressing retrovirus, as indicated by the gene symbol to the right. The affymetrix feature probes with sequences identical between human and mouse (homologous probes) were used to quantify the expression levels of exogenously transduced TFs to those in *ex vivo* isolated Treg and Tconv cells. Data are representative of three experiments.



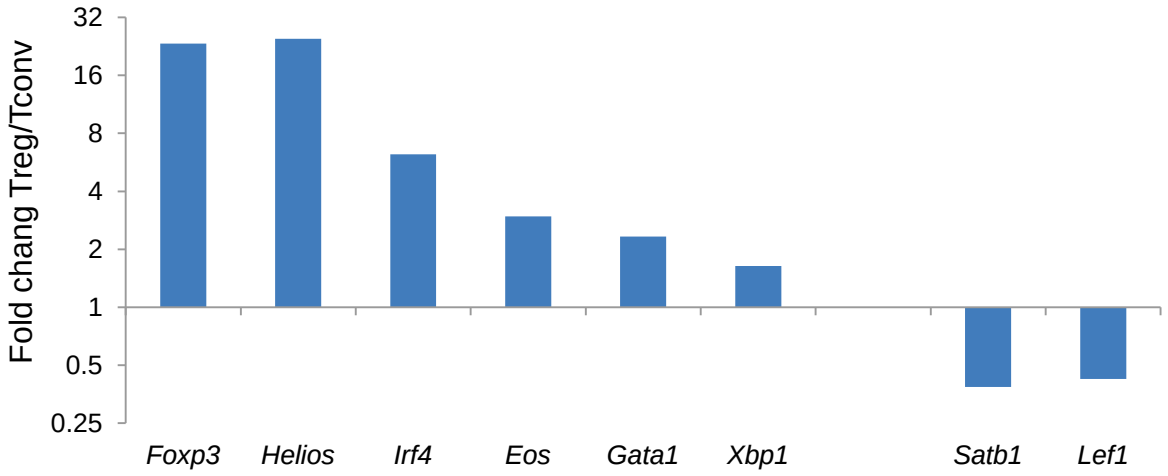
Supplementary Figure 4. Gene expression profiles of TF transduction.
Expression profiles of Tconv cells transduced with FOXP3 and predicted cofactors, alone or together. Controls were cells transduced with empty vectors. Red, Treg Up signature; Blue, Treg Down signature. Values averaged from independent triplicates.



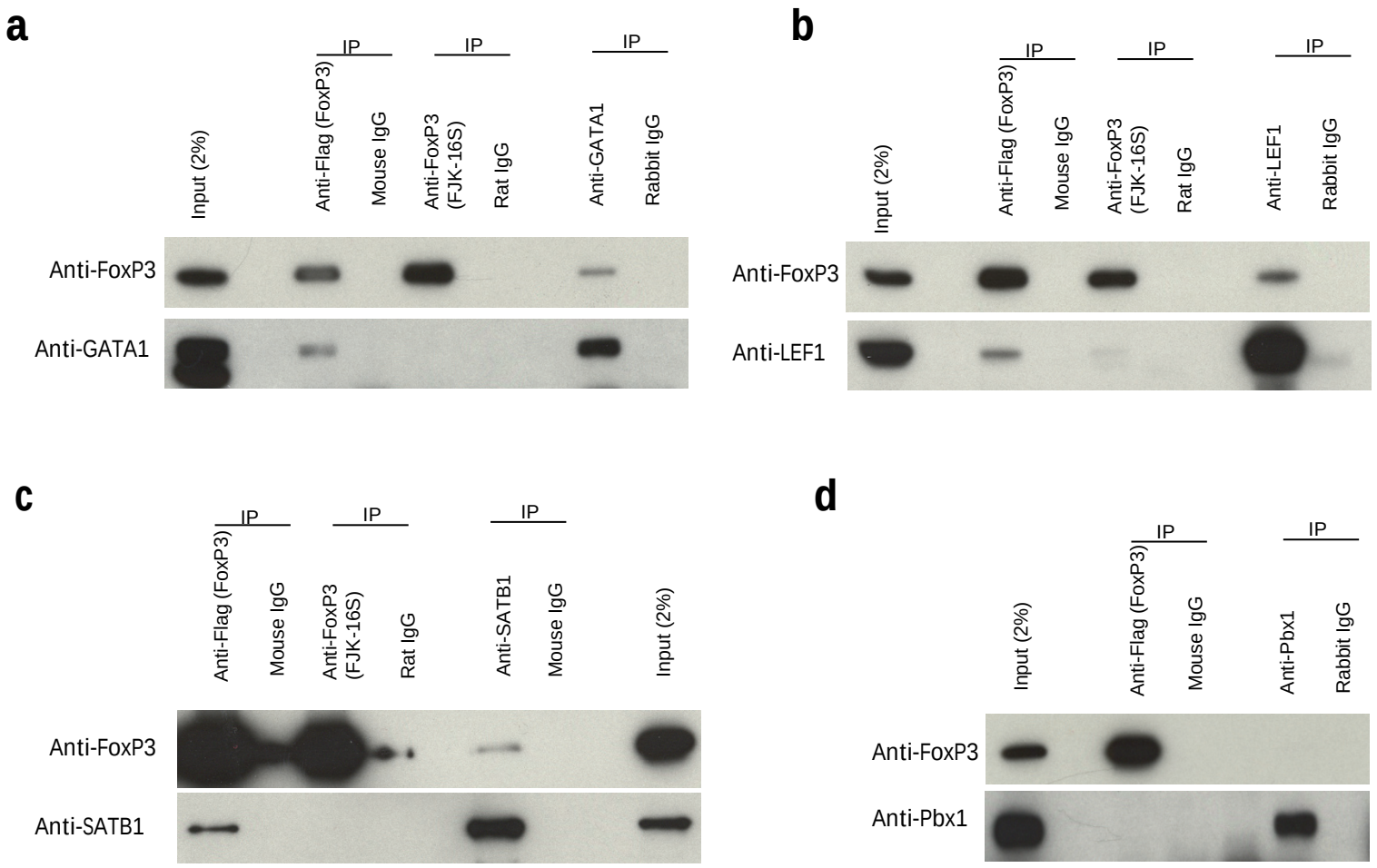
Supplementary Figure 5. Proliferation of singly or doubly transduced cells.
Tconv cells were prepared as described in Methods and labeled with CFSE before culture, retroviral transduction were done 24 hr later, and CFSE dilution was analyzed 72 hr later. Left column: dot plots of GFP (empty vector, or indicated TFs) versus Thy1.1 (FOXP3); Right column: CFSE histograms, with different colors represent the corresponding population in the left dot plots. Data are representative of two independent experiments.



Supplementary Figure 6. Effect of combination of two quintet TFs in Treg gene expression.
(a) Tconv cells were prepared as described in Methods and retrovirally transduced with EOS+LEF1 (i); GATA1+SATB1 (ii); and FoxP3+GATA1 (iii, positive controls). Controls were cells transduced with empty vectors. Red, Treg Up signature; Blue, Treg Down signature. **(b)** Treg signature indexes of the datasets in **(a)**. Values averaged from independent triplicates.



Supplementary Figure 7. Expression of *Foxp3* and cofactors in *ex vivo* Treg and Tconv cells.
Fold changes of the transcripts of *Foxp*3 and cofactors in *ex vivo* Treg compared to Tconv cells, from triplicate microarray data from Immunological Genome database (www.immgen.org).



Supplementary Figure 8. Association of FoxP3 with cofactors.
Lysates of 293 cells transfected with FoxP3 plus each individual TF: GATA1 **(a)** , LEF1 **(b)**, SATB1 **(c)** or Pbx1 **(d)**, were immunoprecipitated (IP), and subsequently immunoblotted (IB), as indicated. Data are representative of two experiments.