Supplemental material

Hou et al., https://doi.org/10.1084/jem.20181134

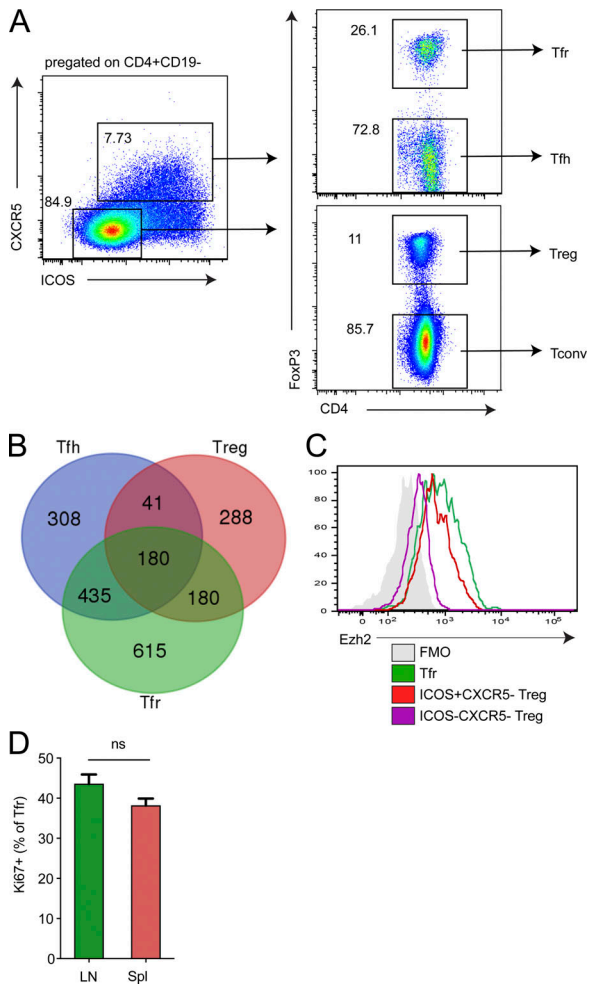

Figure S1. **Additional RNA-seq data comparing T conv, Tfh, Tfr, and T reg cells. (A)** Gating strategy for sorting of Tfr, Tfh, T reg, and T conv cells for RNA-seq experiments. Plots are pregated on $CD4^+CD19^-$ cells. **(B)** Venn diagram showing overlap of differentially expressed ($P < 0.01$) genes between indicated population and T conv cells. **(C)** Comparison of Ezh2 expression in Tfr, $ICOS^-CXCR5^-$ T regs, and $ICOS^+CXCR5^-$ T regs on day 7 after immunization with NP-OVA. FMO, full minus one stain. **(D)** Ki67 expression in Tfr cells from lymph node or spleen on day 7 after immunization, as in Fig. 1, I–K.

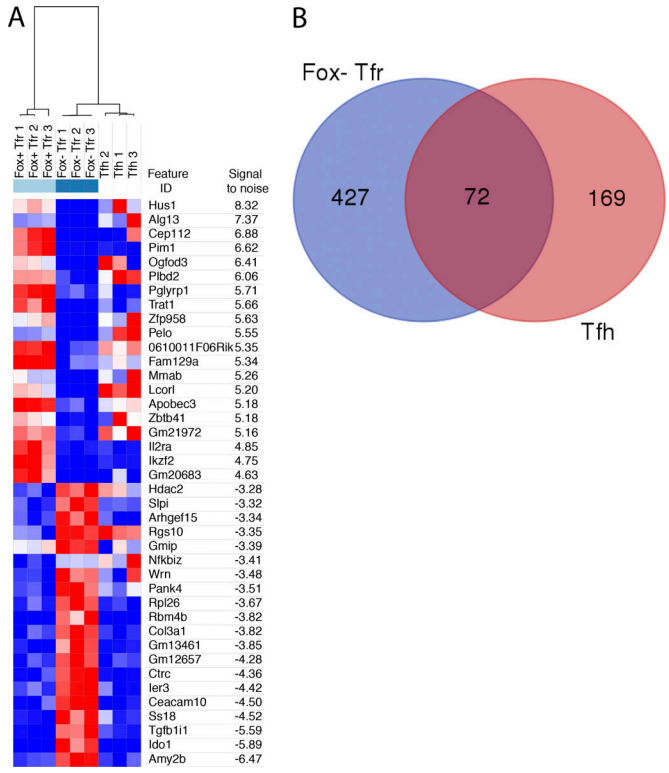

Figure S2. **Additional RNA-seq analysis for ex vivo generated ex-Tfr cells. (A)** Heat map of top 20 and bottom 20 genes (using the Morpheus marker selection tool) between FoxP3-expressing Tfr cells (Fox^+ Tfr) and ex-Tfr (Fox^- Tfr) cells. **(B)** Venn diagram comparing the overlap of differentially expressed genes ($P < 0.01$) between indicated population and $FoxP3^+$ Tfr cells.

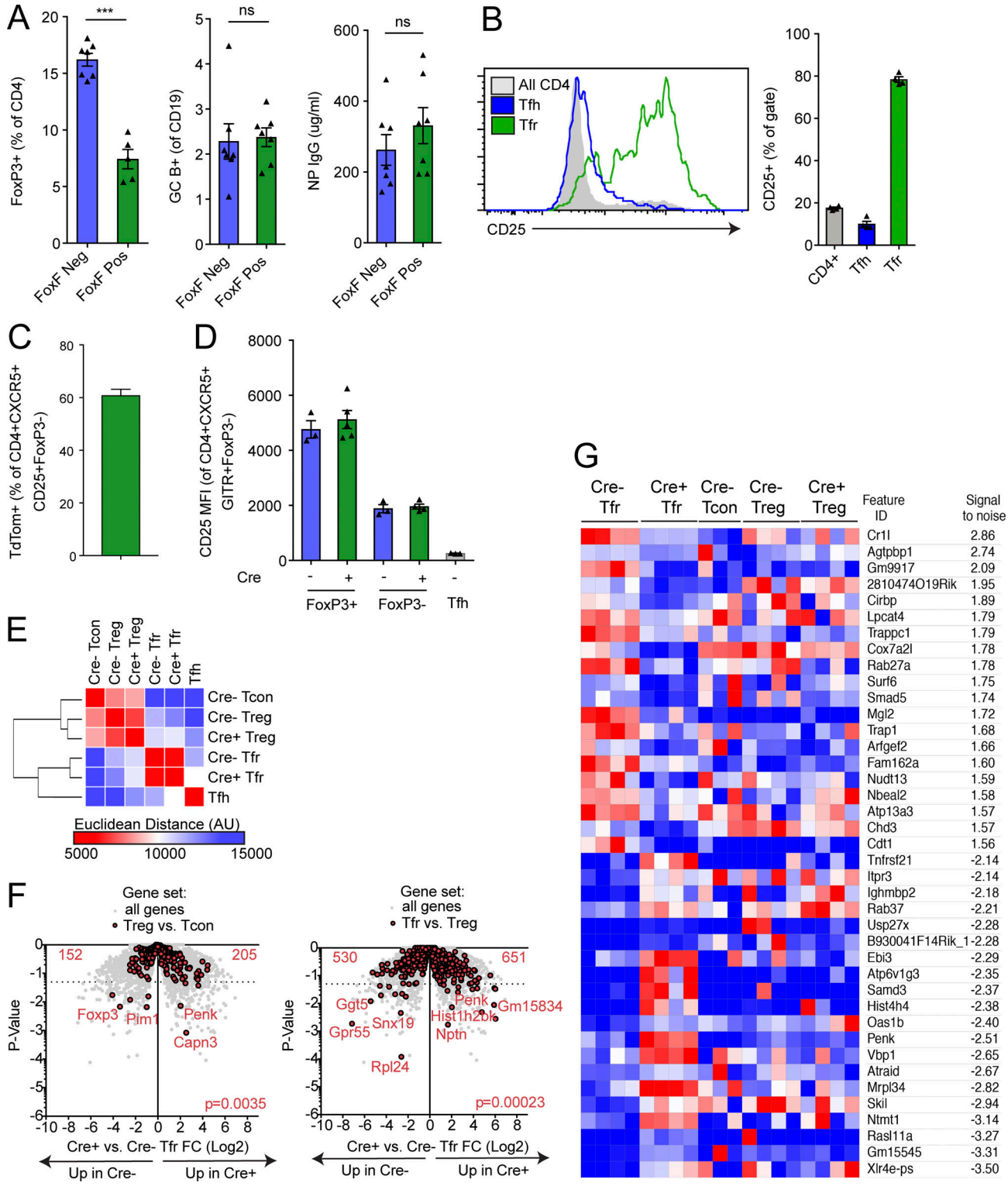

Figure S3. **Additional analysis of FoxP3-deleted mice. (A)** Analysis of FoxP3$^+$ cells, GC B cells (gated as CD19$^+$FAS$^+$GL7$^+$), and serum NP-specific IgG levels in *FoxP3$^{f/f}$* mice (FoxF Neg) or *FoxP3$^{f/f}$* UBC$^{Ert2\text{-}Cre}$ mice (FoxF Pos) immunized with NP-OVA, treated with tamoxifen (as in Fig. 4 A), and analyzed 14 d later. **(B)** Expression of CD25 in all CD4$^+$ cells, in Tfh cells, and in Tfr cells. Representative histograms (left) and column graphs (right) are shown. **(C)** Frequency of TdTomato$^+$ cells (as a percentage of CD4$^+$CXCR5$^+$CD25$^+$FoxP3$^-$) from FoxP3^{Cre} Rosa26^{LoxSTOPLoxTdTomato} mice immunized with NP-OVA 7 d previously. **(D)** Expression of CD25 on FoxP3$^+$ and FoxP3$^-$ subsets as a percentage of CD4$^+$CXCR5$^+$GITR$^+$ cells. **(E)** Similarity matrix (using Euclidean distance) of indicated populations using RNA-seq gene expression analysis. Tfr cells were sorted as CD4$^+$CD19$^-$CXCR5$^+$CD25$^+$GITR$^+$ cells from indicated mice. **(F)** Volcano plots of gene expression of Tfr cells from control (Cre$^-$) or Cre expressing (Cre$^+$) *FoxP3$^{f/f}$* mice. **(G)** Heat map of top 20 and bottom 20 genes (using the Morpheus marker selection tool) between Tfr cells from Cre$^-$ or Cre$^+$ mice (as in D). All error bars indicate standard error. ***, P < 0.001 using Student's *t* test. Data are from individual experiments and are representative of three independent experiments (A–D) or are from combined experiments (E–G).

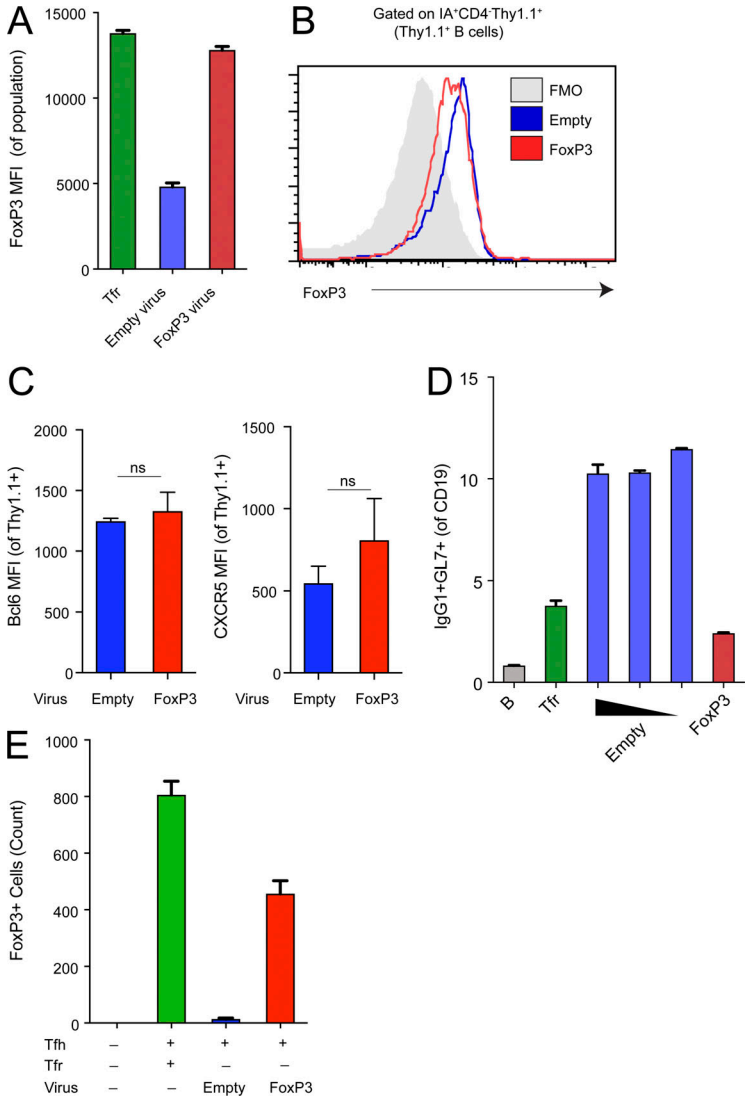

Figure S4. **Additional analysis of FoxP3 overexpression in Tfh cells. (A)** Analysis of FoxP3 expression on Tfr cells and Thy1.1+ (transduced) Tfh cells from cultures as in Fig. 5. MFI, mean fluorescence intensity. **(B)** Analysis of FoxP3 expression in B cells infected with FoxP3-encoding retrovirus as in Fig. 5, A–G. FMO, full minus one stain. **(C)** Analysis of Bcl6 and CXCR5 expression on Thy1.1+ (transduced) Tfh cells from cultures as in Fig. 5. **(D)** Analysis of class-switched B cells in cultures in which different amounts of empty virus were added (100, 30, or 10 µl). **(E)** Relative counts per well for Tfr cells and FoxP3+Thy1.1+ cells from cultures as in Fig. 5. All error bars indicate standard error. Data are from individual experiments of triplicate wells and are representative of three (A, C, and D) or two (B) individual experiments.

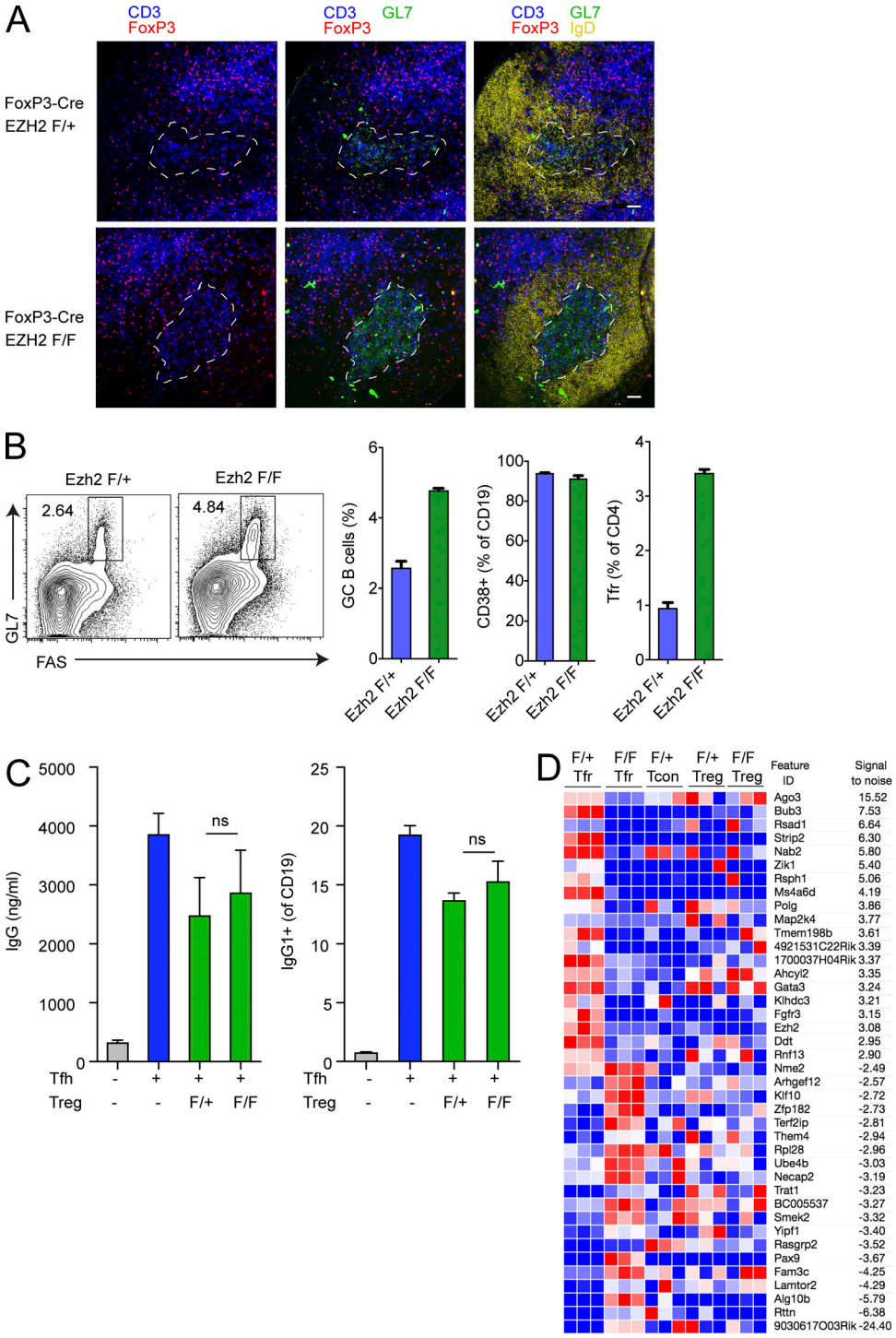

Figure S5. **Additional analysis of Ezh2 conditional knockout mice. (A)** Immunofluorescence micrographs of draining lymph nodes of indicated mice 10 d after immunization with NP-OVA. Bars, 50 µm. **(B)** Analysis of GC B cells (left), CD38$^+$ naive B cells (middle), or Tfr cells (right) in indicated mice 9 d after immunization with NP-OVA. **(C)** In vitro suppression assay in which B and Tfh cells from control mice were cultured with CD4$^+$ICOS$^-$CXCR5$^-$FoxP3$^+$ T reg cells from control (F/+) or Ezh2-deleted (F/F) mice as in Fig. 6 C. **(D)** Top 20 and bottom 20 genes (using Morpheus marker selection) differentially expressed between Tfr cells as in Fig. 6 E. All error bars indicate standard error. Data are from individual experiments and are representative of two independent experiments (A and B) or are from individual experiments of triplicate wells and are representative of two experiments (C).