

Expanded View Figures

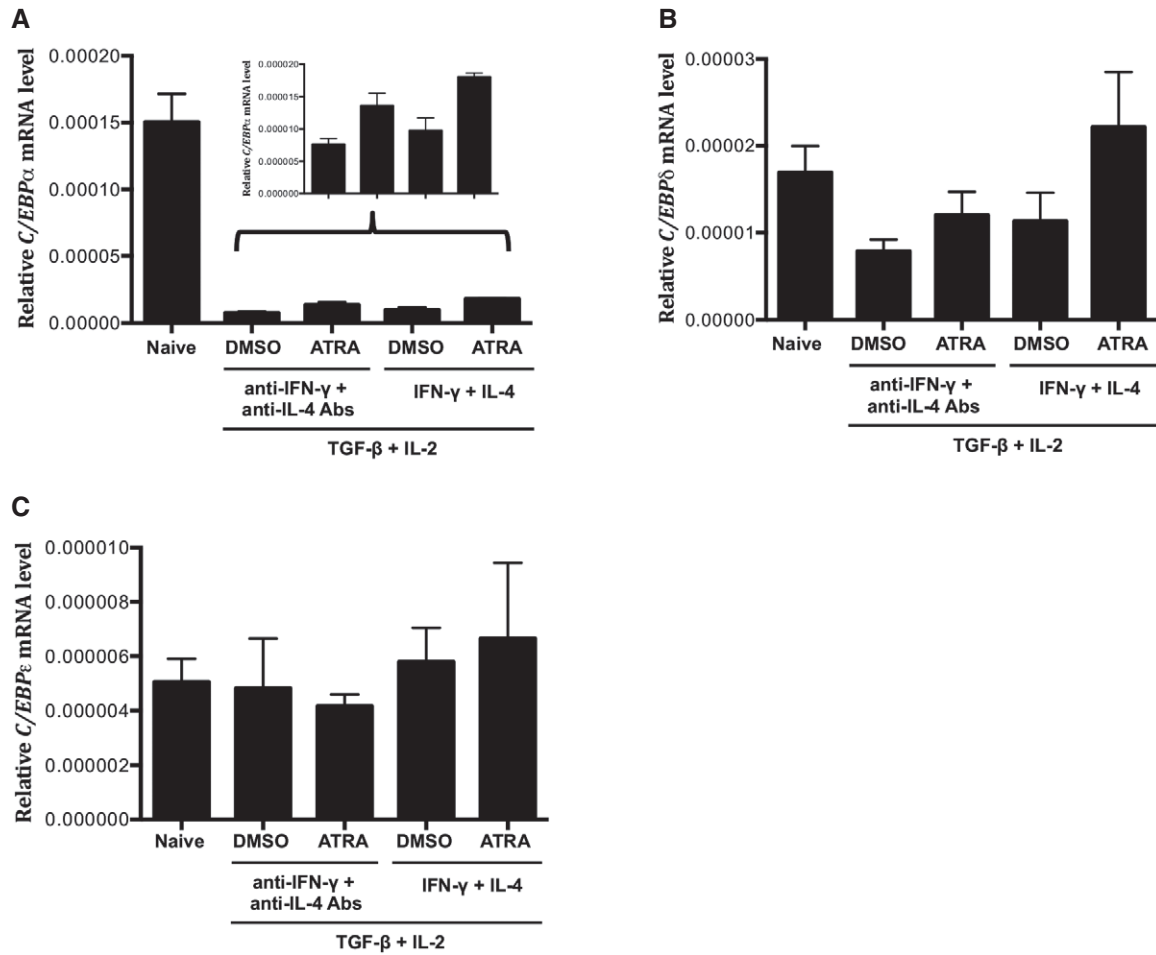


Figure EV1. C/EBP α and C/EBP δ are also upregulated by RA.

A–C Real-time qRT–PCR analysis of (A) C/EBP α , (B) C/EBP δ , (C) C/EBP ϵ mRNA in CD4⁺ naive T cells inactivated (naive) or cultured for 24 h under conditions as indicated. Data are representative of two independent experiments with consistent results and normalized with β -actin (mean and s.e.m. of quadruplicates).

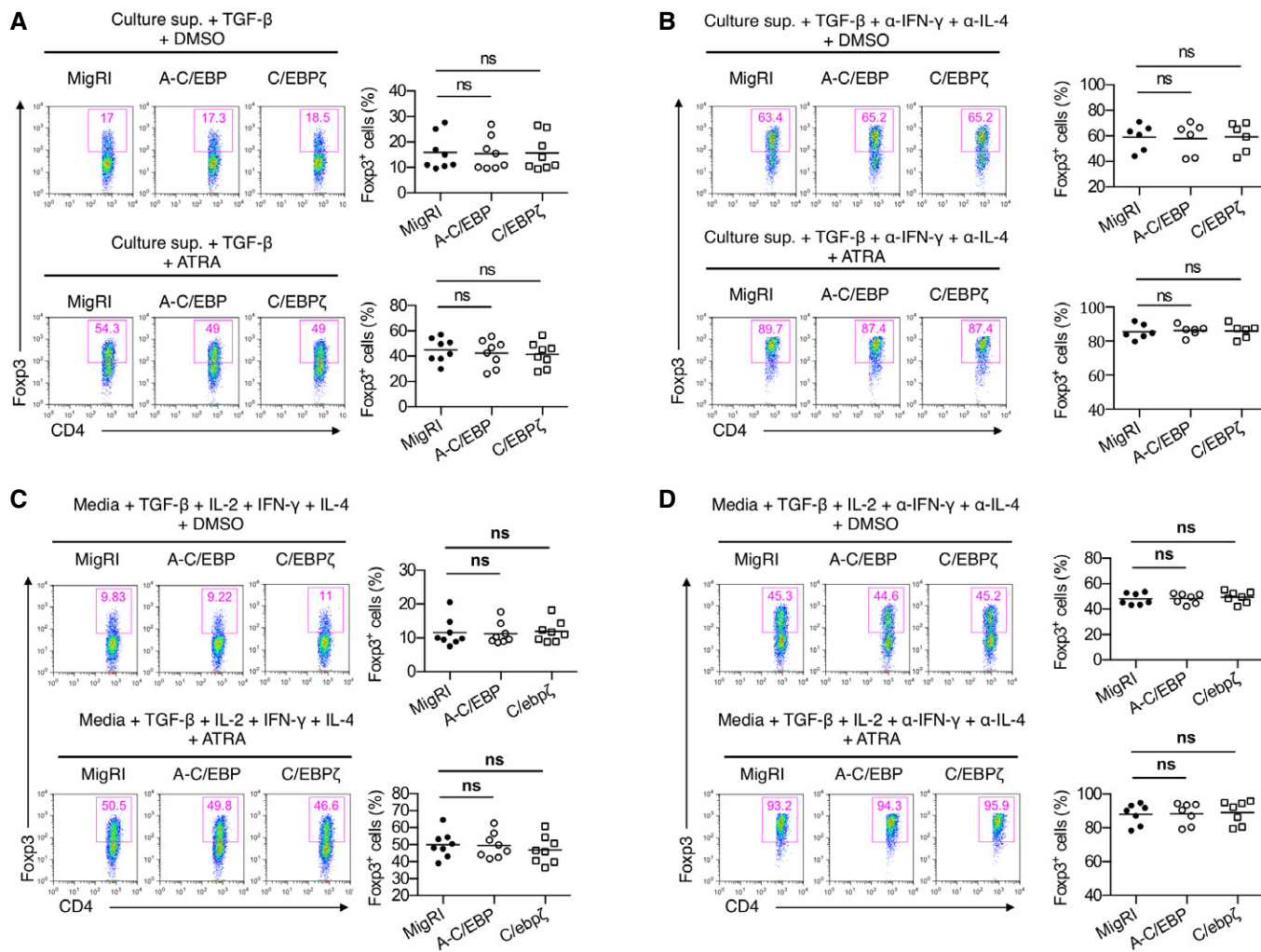


Figure EV2. GFP-negative cell fraction of Fig 1C-F.

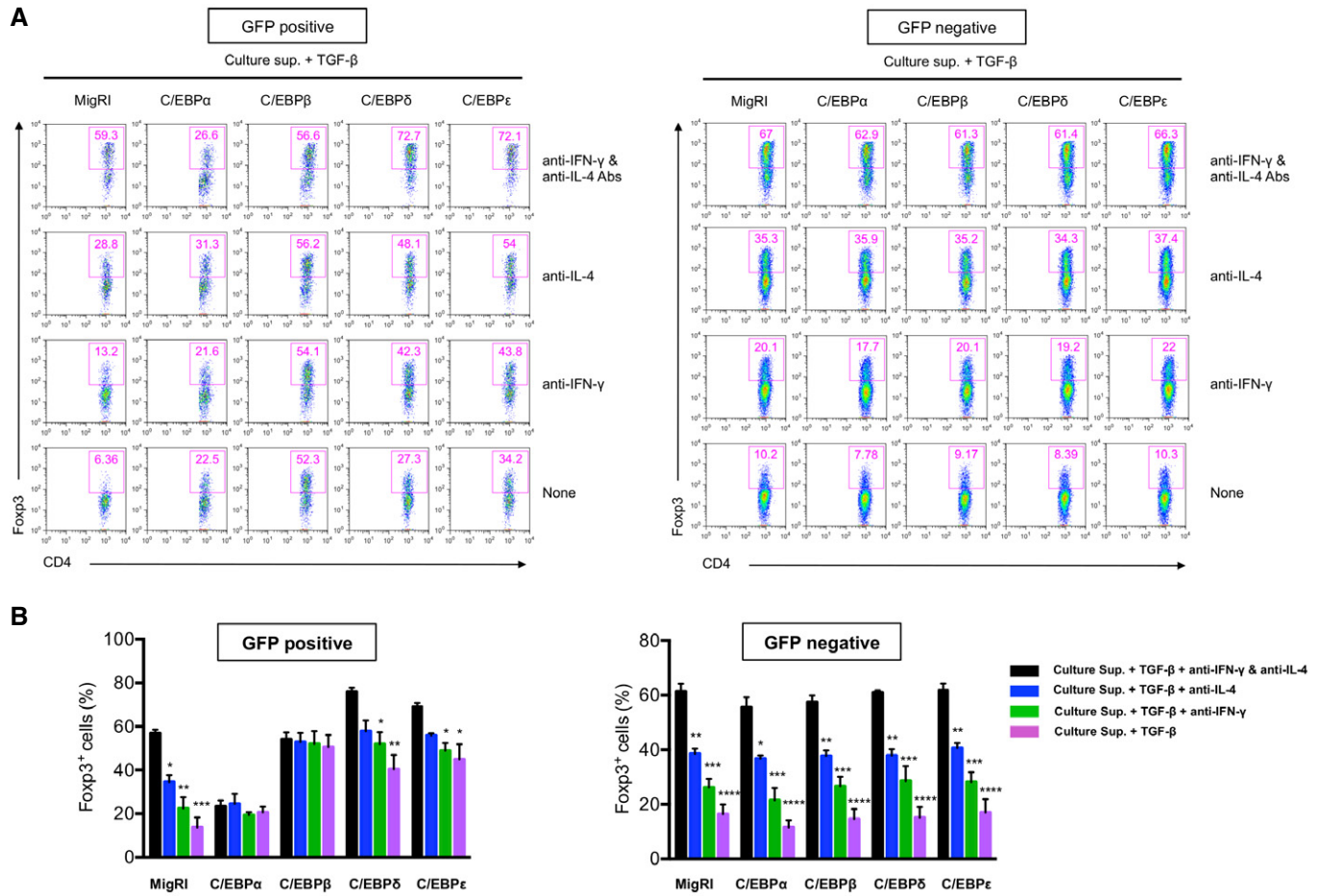


Figure EV3. C/EBP α , δ , and ϵ also counteract the effect of inhibitory cytokines on Foxp3 induction.

A Flow cytometry of intracellular staining for Foxp3 in CD4⁺ naive T cells transduced with control retrovirus (MigRI) or retrovirus encoding C/EBP α , C/EBP β , C/EBP δ , or C/EBP ϵ and stimulated with anti-CD3 and anti-CD28 for 2 days in the presence of TGF- β and culture supernatant with anti-IFN- γ and anti-IL-4 Abs, anti-IL-4 Ab, anti-IFN- γ Ab, or none. Histograms are gated for GFP⁺ (left panel) and GFP⁻ (right panel) cells. Numbers indicate percent Foxp3⁺.

B Frequency of Foxp3⁺ cells from independent three experiments (mean and s.e.m.). Statistical analysis was performed using one-way ANOVA (* P < 0.05, ** P < 0.01, *** P < 0.001, **** P < 0.0001).

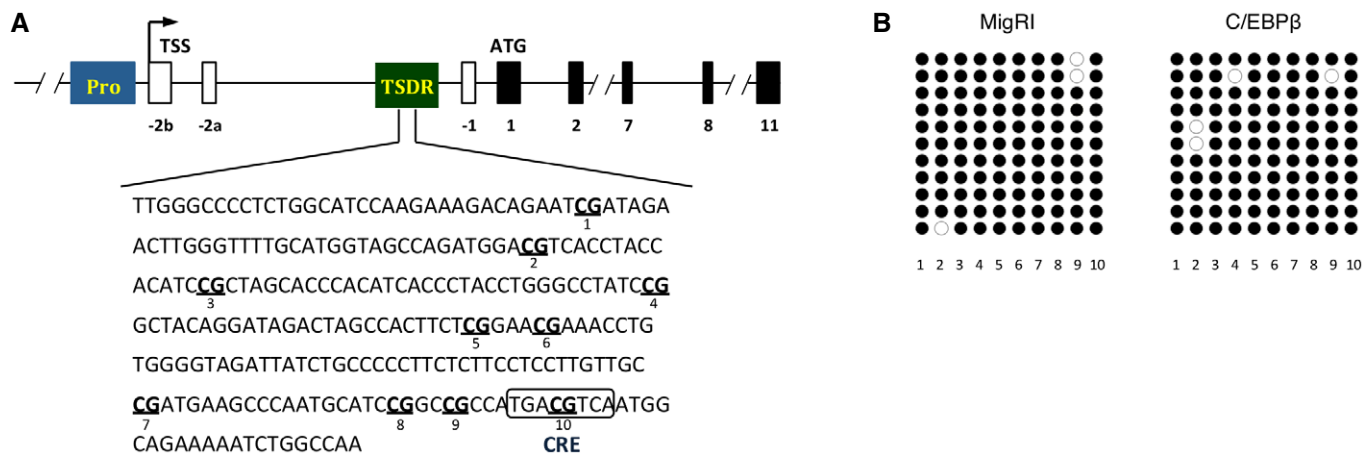


Figure EV4. C/EBPβ does not alter the methylation status of the TSDR.

A The *Foxp3* gene. Exon–intron structure and the TSDR are depicted (top). The position of 10 CpG motifs and CRE sequence within the TSDR is shown (bottom).
 B Bisulfite sequencing of FACS-sorted GFP⁺ cells from CD4⁺ naïve T cells transduced with control retrovirus (MigRI) or retrovirus encoding C/EBPβ and cultured for 2 days in the presence of TGF-β and culture supernatant.

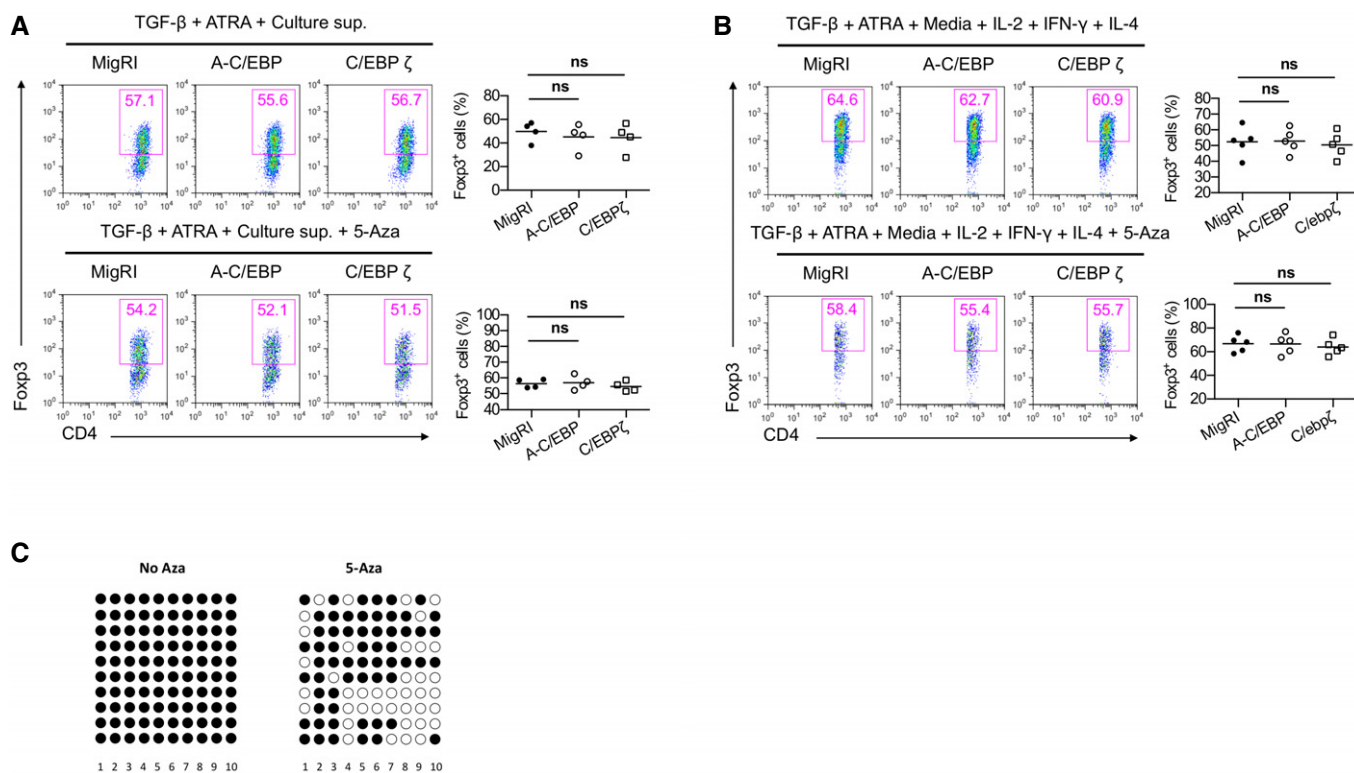


Figure EV5. GFP-negative cell fraction of Fig 4C and D, and bisulfite sequencing after 5-aza treatment.

A, B GFP-negative cell fraction of Fig 4C and D.
 C Bisulfite sequencing of TSDR of FACS-sorted GFP⁺ cells from CD4⁺ naïve T cells transduced with control retrovirus (MigRI) and cultured for 2 days in the presence of TGF-β, ATRA, 5-aza, and culture supernatant.