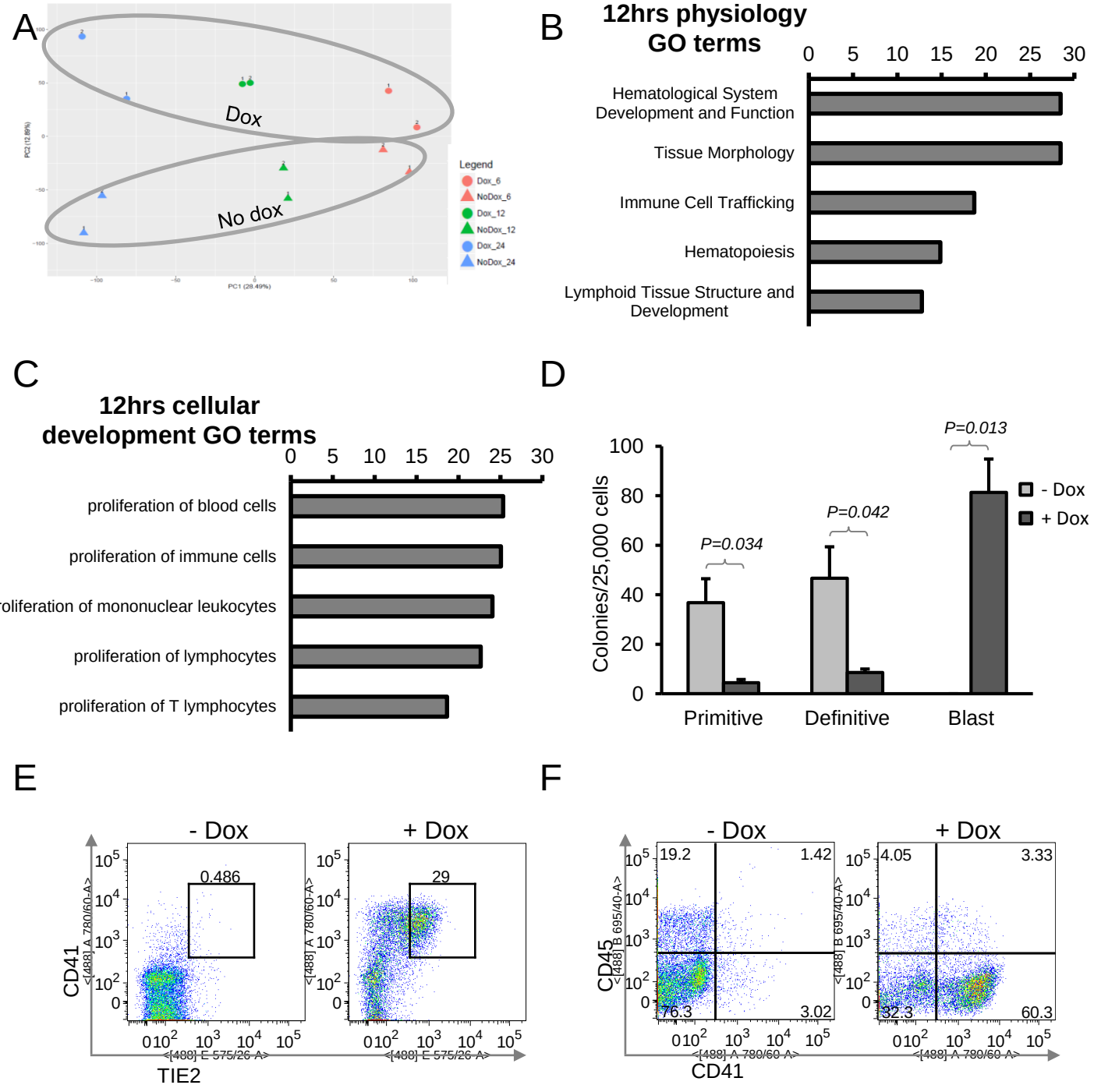

Figure S1. SOX7 inhibits a hematopoietic transcriptional program. FLK1^{+} cells were sorted from day 3.5 *iSox7* EBs and cultured in HE differentiation media. Doxycycline was added after 48 hours, and RNAseq was performed 6, 12 and 24 hours post doxycycline induction. Cells were replated in a hematopoietic progenitor cell CFU assay 24 hours post doxycycline induction. (A) PCA plot of RNAseq dataset at 6, 12, and 24 hours post doxycycline induction. (B & C) Ingenuity Pathway Analysis of the genes inhibited by SOX7 at 12 hours. (D) Hematopoietic progenitor cell CFU assay with and without doxycycline (n=3 biological repeats, Student's one-tailed paired t-test). (E & F) Cells were harvested from the CFU assay after 8 days and analysed by flow cytometry.

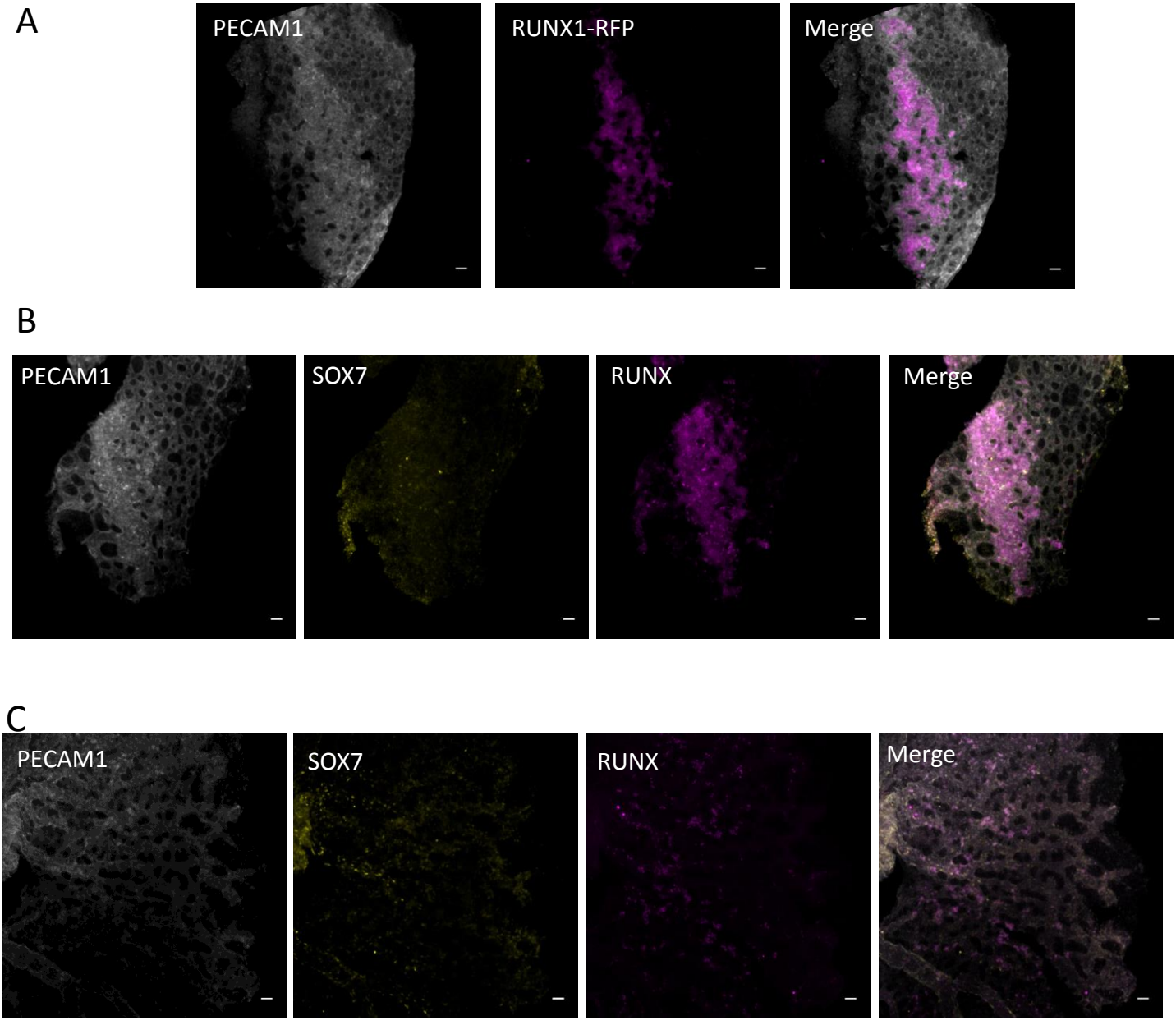

Figure S2. SOX7 and RUNX expression in the yolk sac at E8.5 and E9.5 (A) Wholemount staining for PECAM1 together with proximal RUNX1-RFP expression at E8.5 from RUNX1$^{RFP/WT}$ embryos. (B) Wholemount staining for SOX7, RUNX and PECAM1 in yolk sacs at E8.5. (C) Wholemount staining for SOX7, RUNX and PECAM1 in yolk sacs at E9.5. Scale bars 50µm (A & B), 20µm (C).

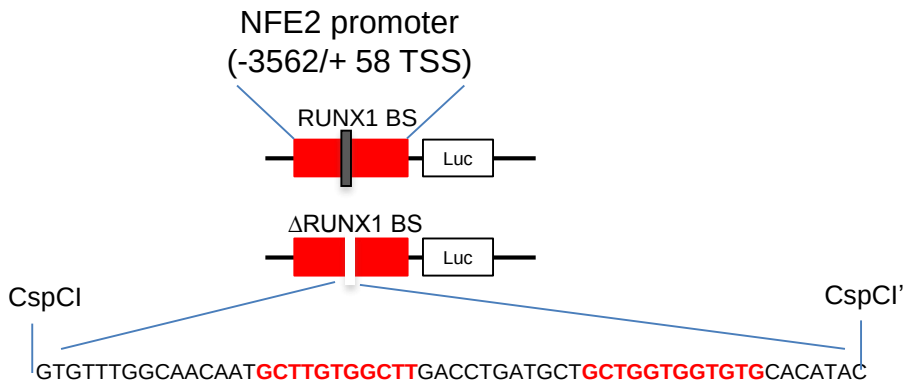

Figure S3. RUNX1 binds the NFE2 promoter region. (A) Deletion of two RUNX1 binding sites (red) in the NFE2 promoter using CspCI restriction enzyme.

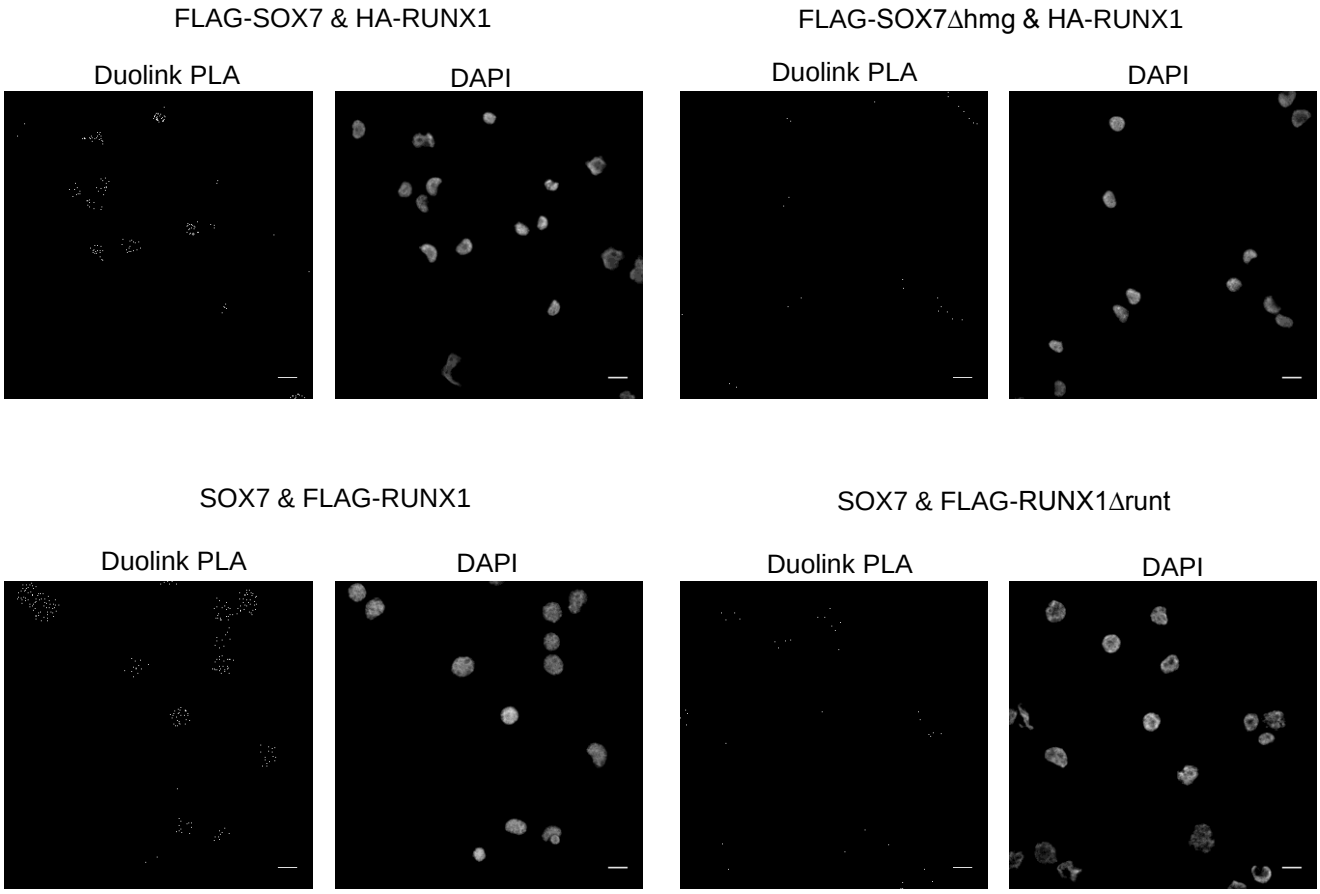

Figure S4. Single channel images of the representative Duolink PLA in Figure 5. HEK293T cells were transfected with the indicated pcDNA3 plasmids before Duolink PLA. Scale bars: 20μm.

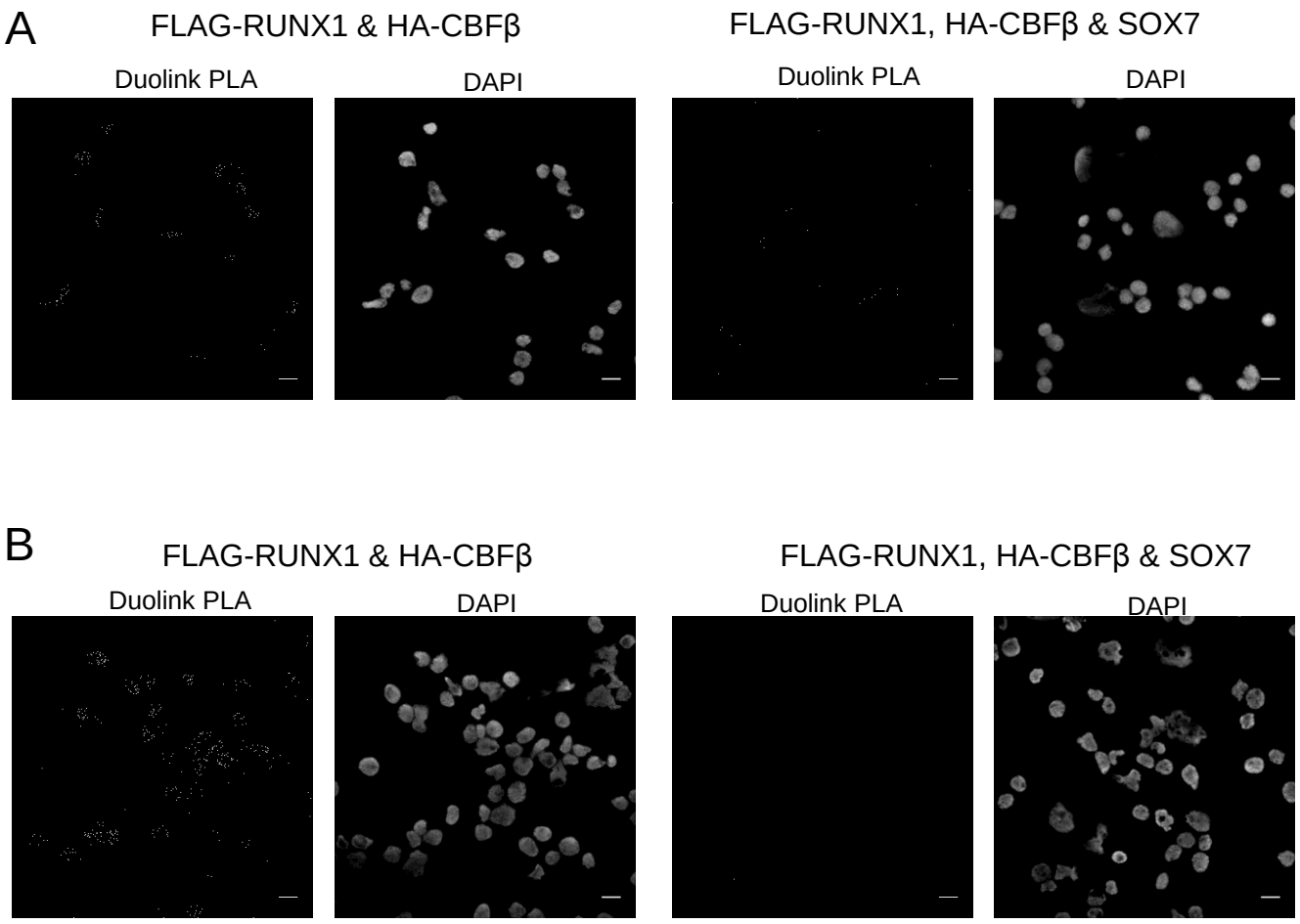

Figure S5. Single channel images of representative Duolink PLA in Figure 6. (A) RUNX1, CBFβ and SOX7 plasmids were transfected at equal concentration (1µg each). (B) SOX7 was transfected at 3 times greater concentration (3µg) than RUNX1 and CBFβ plasmids (1µg each). Scale bars: 20µm.

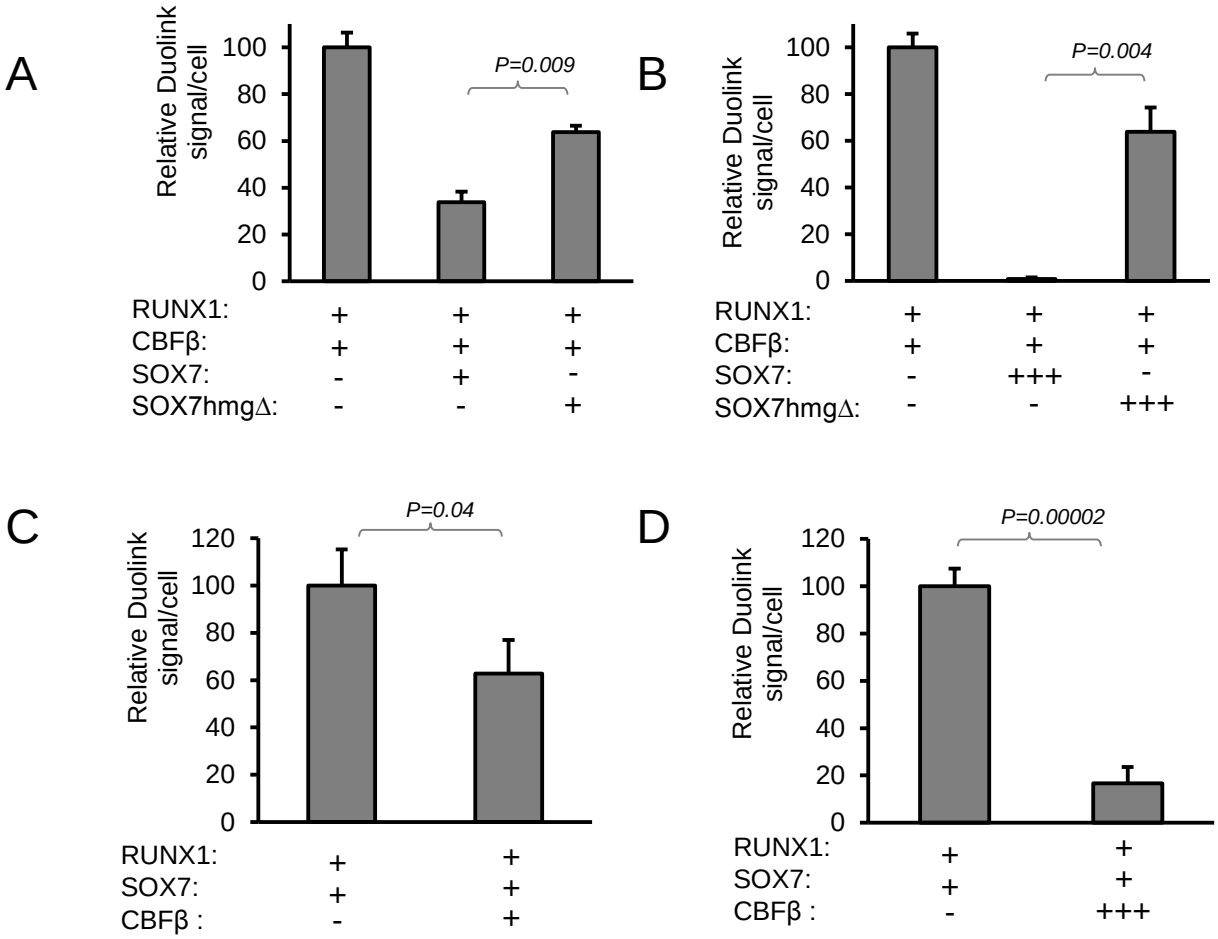

Figure S6. Competition between SOX7 and CBFβ for RUNX1 binding. HEK293T cells were transfected with pcDNA3 RUNX1, CBFβ, SOX7 and SOX7hmgΔ plasmids as indicated, together with a pcDNA3-GFP plasmid. Transfected cells were enriched by sorting for GFP before subsequent Duolink PLA analysis. (A & B) Duolink PLA analysis between RUNX1 and CBFβ, with SOX7/SOX7hmgΔ added as competitor. (A) plasmids were transfected at equal concentration (1µg each plasmid). (B) SOX7/SOX7hmgΔ plasmids were transfected at a 3 times greater concentration (3µg) than RUNX1 and CBFβ plasmids (1µg each). (C & D) Duolink PLA analysis between RUNX1 and SOX7, with CBFβ added as a competitor. (C) RUNX1, SOX7 and CBFβ plasmids were transfected at equal concentration (1µg each). (D) CBFβ plasmid was transfected at 3 times greater concentration (3µg) than RUNX1 and SOX7 plasmids (1µg each). All experiments are n=5, Student's two-tailed t-test.

Table S1. Differentially expressed genes at 6 hours, at least 1.5-fold change

Click here to Download Table S1

Table S2. Differentially expressed genes at 12 hours, at least 1.5-fold change

Click here to Download Table S2

Table S3. Differentially expressed genes at 24 hours, at least 1.5 fold change

Click here to Download Table S3

Table S4. List of PCR primers

Click here to Download Table S4