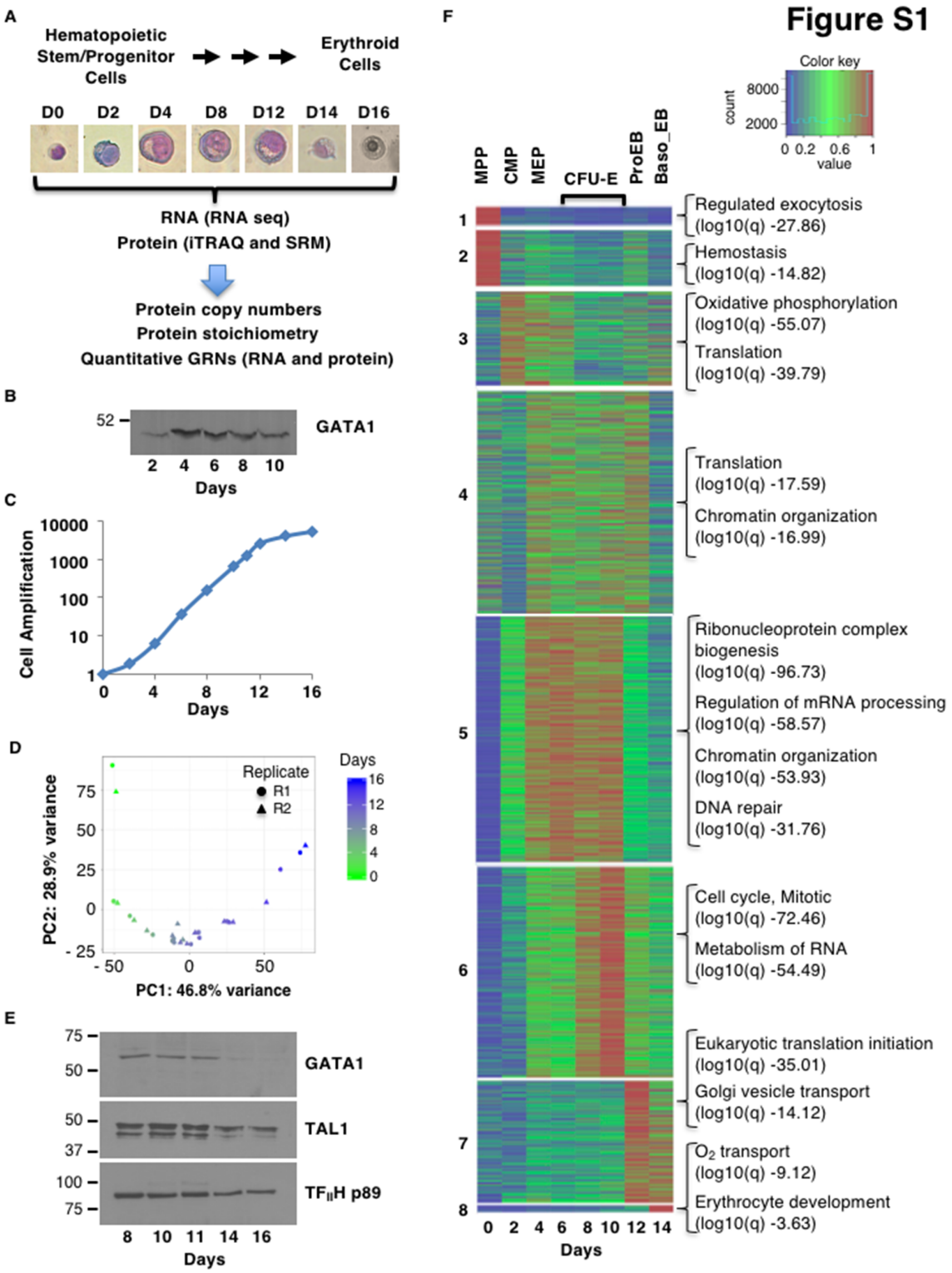

Figure S1. (Related to Figure 1) RNA and Protein Quantification of Transcription Factors and Co-factors during Ex Vivo Human Erythropoiesis.

(A) Schematic of sample collection at different time-points followed by analyses at the RNA and protein levels. Erythroid differentiation was induced ex vivo from cord blood-derived $CD34^+$ HSPCs. Giemsa-stained cells are shown at representative days (magnification 40x).

(B) Western blot analysis of GATA1 protein at the indicated days during ex vivo erythropoiesis. Molecular masses (in kDa) are indicated on the left.

(C) Cell amplification during ex vivo erythropoiesis.

(D) Principal component analysis (PCA) monitoring gene expression changes over time as measured by RNAseq.

(E) Western blot analysis of GATA1, TAL1 and $TF_{II}Hp89$ proteins at the indicated days during ex vivo erythropoiesis. Molecular masses (in kDa) are indicated on the left.

(F) k-means clustering analysis of iTRAQ data at different time-points. The top enriched Gene Ontology terms for each cluster are indicated.

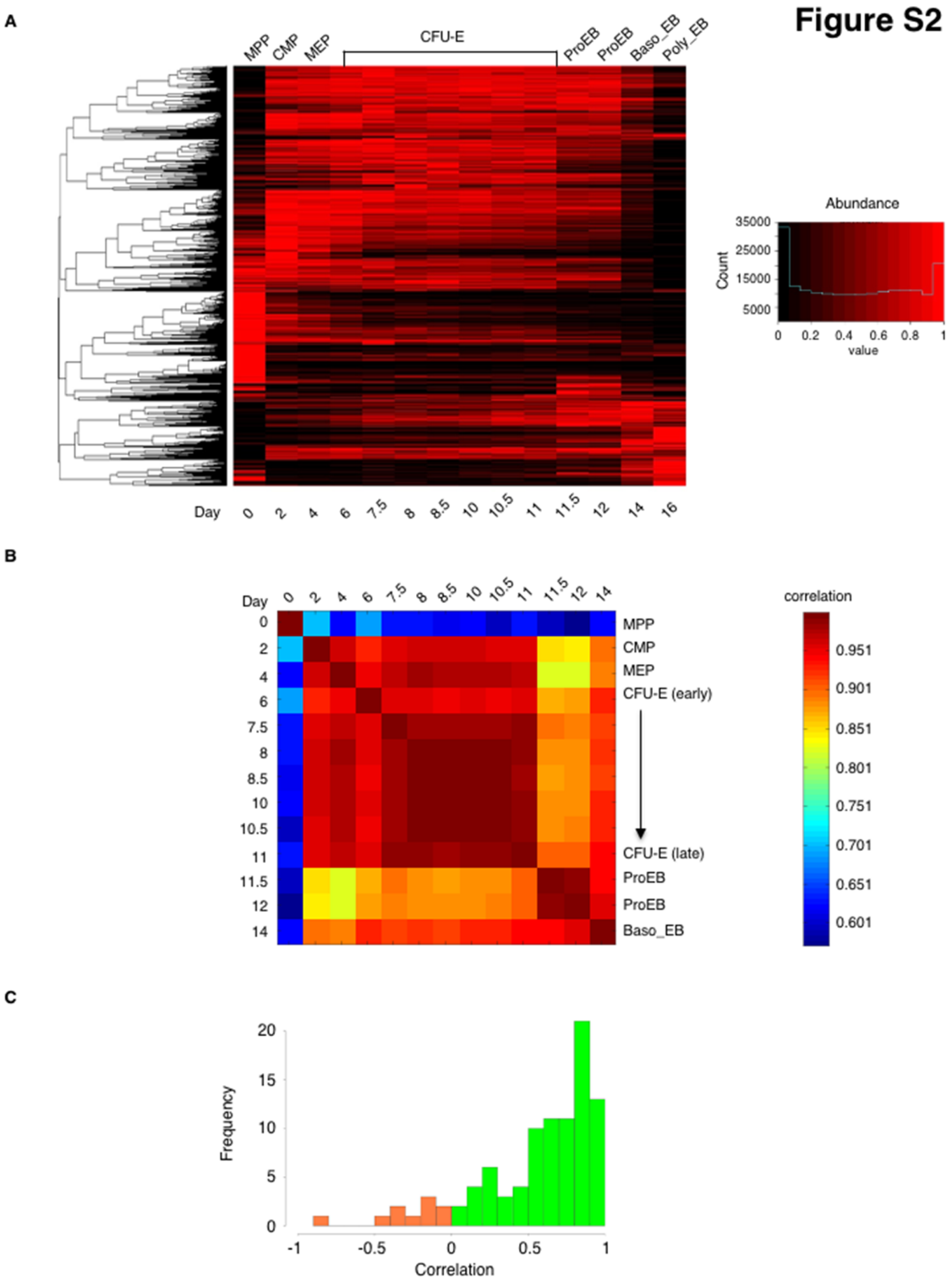

Figure S2. (Related to Figure 1) RNA and Protein Quantification of Transcription Factors and Co-factors during Ex Vivo Human Erythropoiesis.

(A) k-means clustering analysis of normalized mRNA expression (measured by RNA-seq) at the indicated days.

(B) Correlation matrix of normalized protein expression (measured by SRM) at the indicated days.

(C) Correlation of protein changes over time as measured by iTRAQ and SRM. Positive correlations are in green. Negative correlations are in orange.

Figure S3

A

	SRM Conc	WB Conc
TRIM28	46.8 fmol/ug	49.4 fmol/ug
PU.1	2.2 fmol/ug	2.7 fmol/ug
GFI1B	1.02 fmol/ug	3.9 fmol/ug

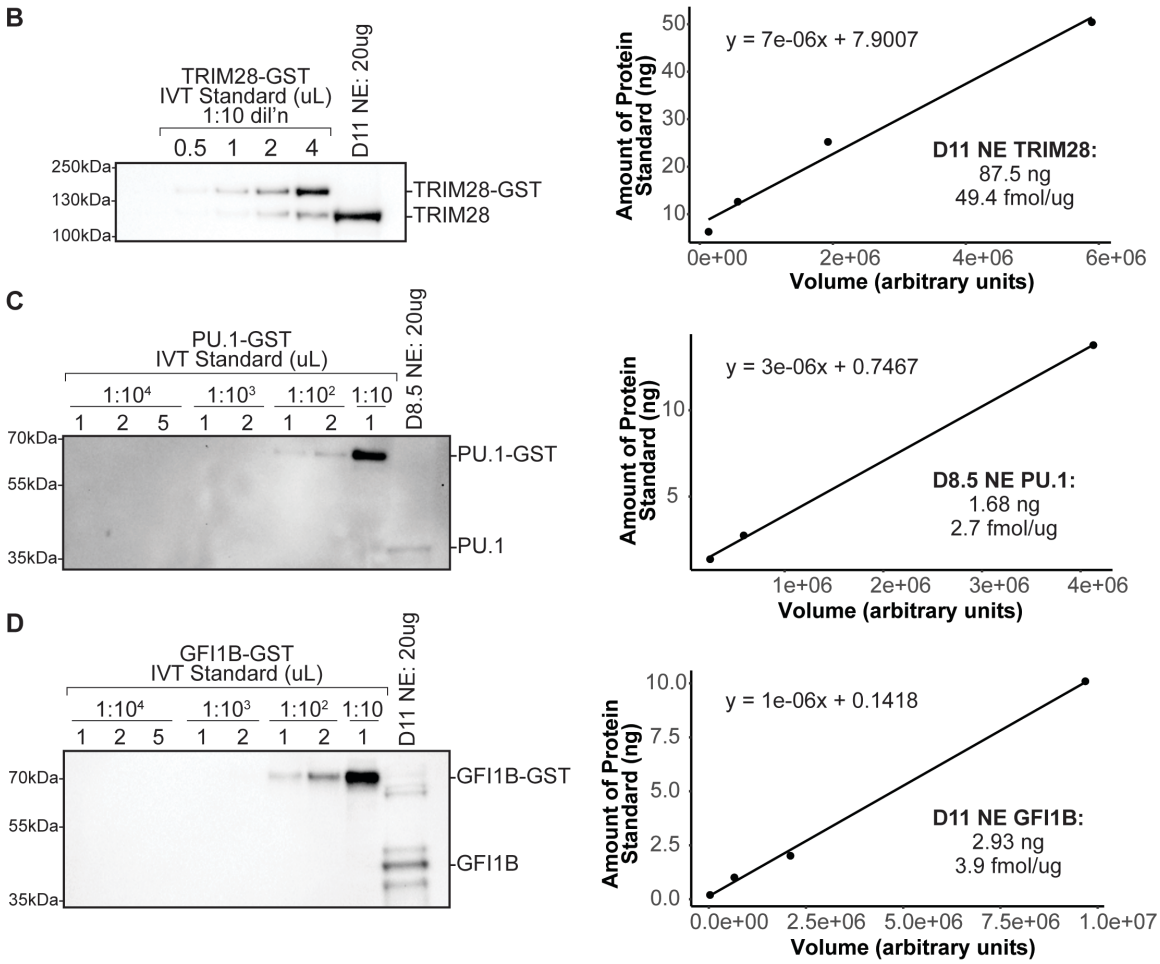

Figure S3. (Related to Figure 1) Validation of SRM-based protein quantification with quantitative Western blots.

(A) Comparison of protein quantification using SRM and Western blot (WB) for the indicated proteins. Potential reasons for the quantitative difference between the SRM and western blotting abundances for GFI1B could be the presence of

isoforms or post translationally modified forms of GFI1B, and/or inefficient trypsin digestion.

(B) Left: Western blot analysis of recombinant GST-tagged TRIM28 (TRIM28-GST) and endogenous TRIM28 in day 11 erythroid nuclear extract. Molecular masses (in kDa) are indicated on the left. Right: Standard curve used to quantify the amount of TRIM28 protein.

(C) Left: Western blot analysis of recombinant GST-tagged PU.1 (PU.1-GST) and endogenous PU.1 in day 11 erythroid nuclear extract. Molecular masses (in kDa) are indicated on the left. Right: Standard curve used to quantify the amount of PU.1 protein.

(D) Left: Western blot analysis of recombinant GST-tagged GFI1B (GFI1B-GST) and endogenous GFI1B in day 11 erythroid nuclear extract. Molecular masses (in kDa) are indicated on the left. Right: Standard curve used to quantify the amount of GFI1B protein.

Figure S4

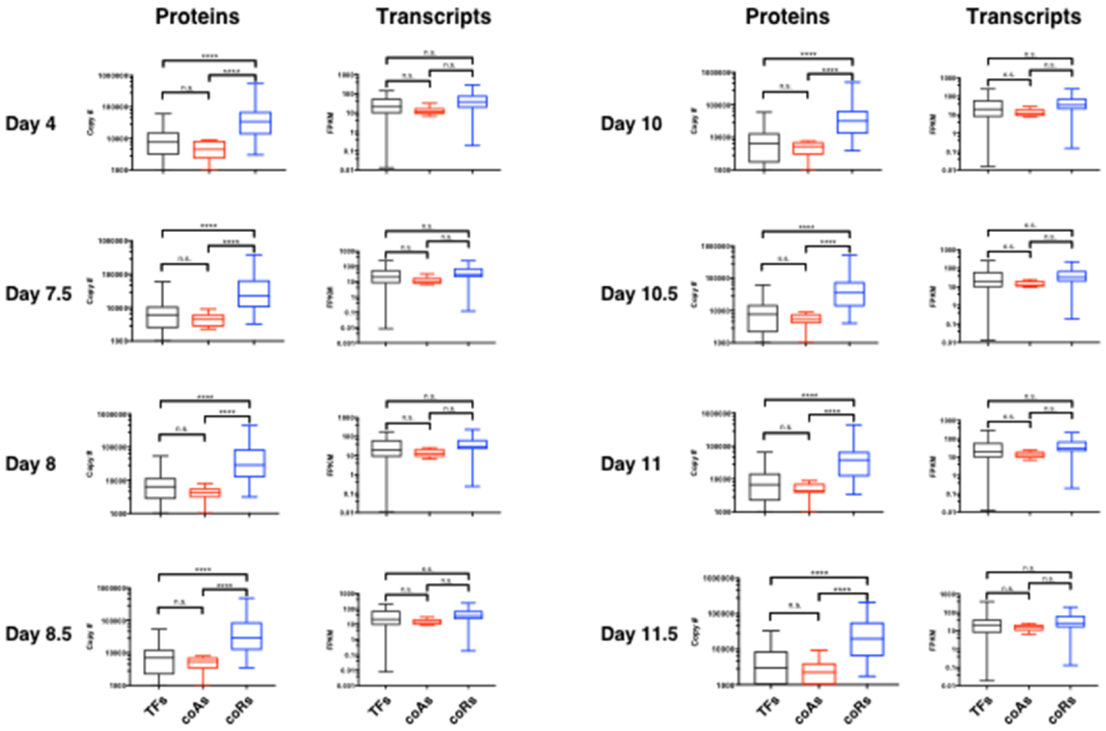

Figure S4. (Related to Figure 5) Co-activators are Less Abundant than Co-Repressors in the Nucleus.

Left panels: Box plots depicting protein abundances (in copy numbers) of TFs (black), coAs (red) and coRs (blue) at the indicated days. Right panels: Box plots depicting the mRNA abundances (in FPKM) of TFs, coAs and coRs at the indicated days. Two-tailed t-test: n.s. (non-significant), * $p < 0.05$. **$p < 0.01$. *** $p < 0.001$. **** $p < 0.0001$. For a list of TFs, coAs and coRs, see Table S4.