SUPPLEMENTAL DATA

Pcbp1 and Ncoa4 regulate erythroid iron storage and heme biosynthesis.

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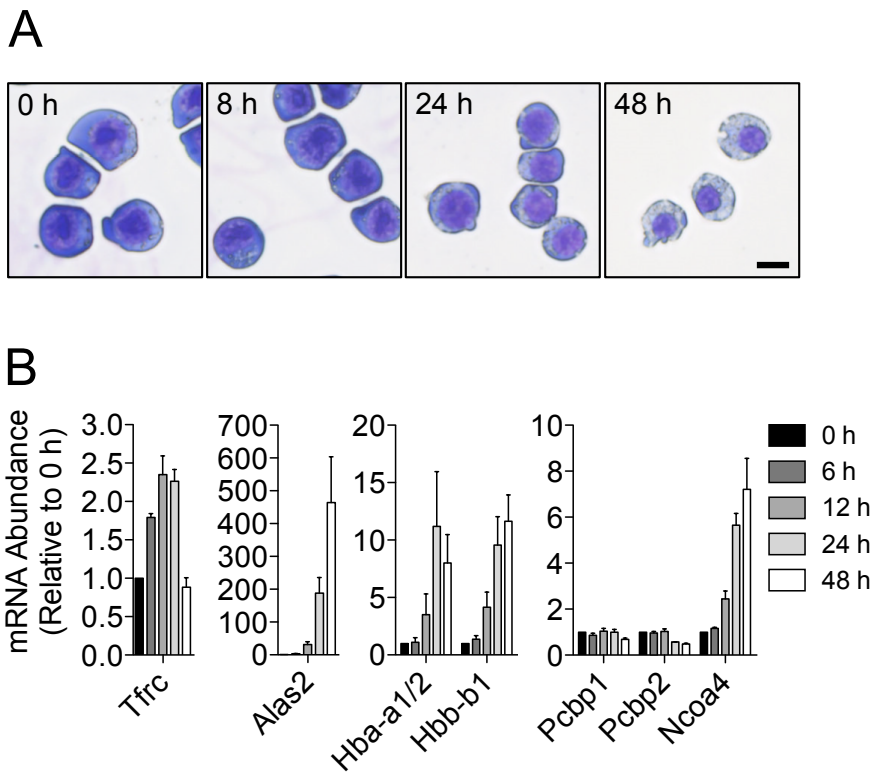
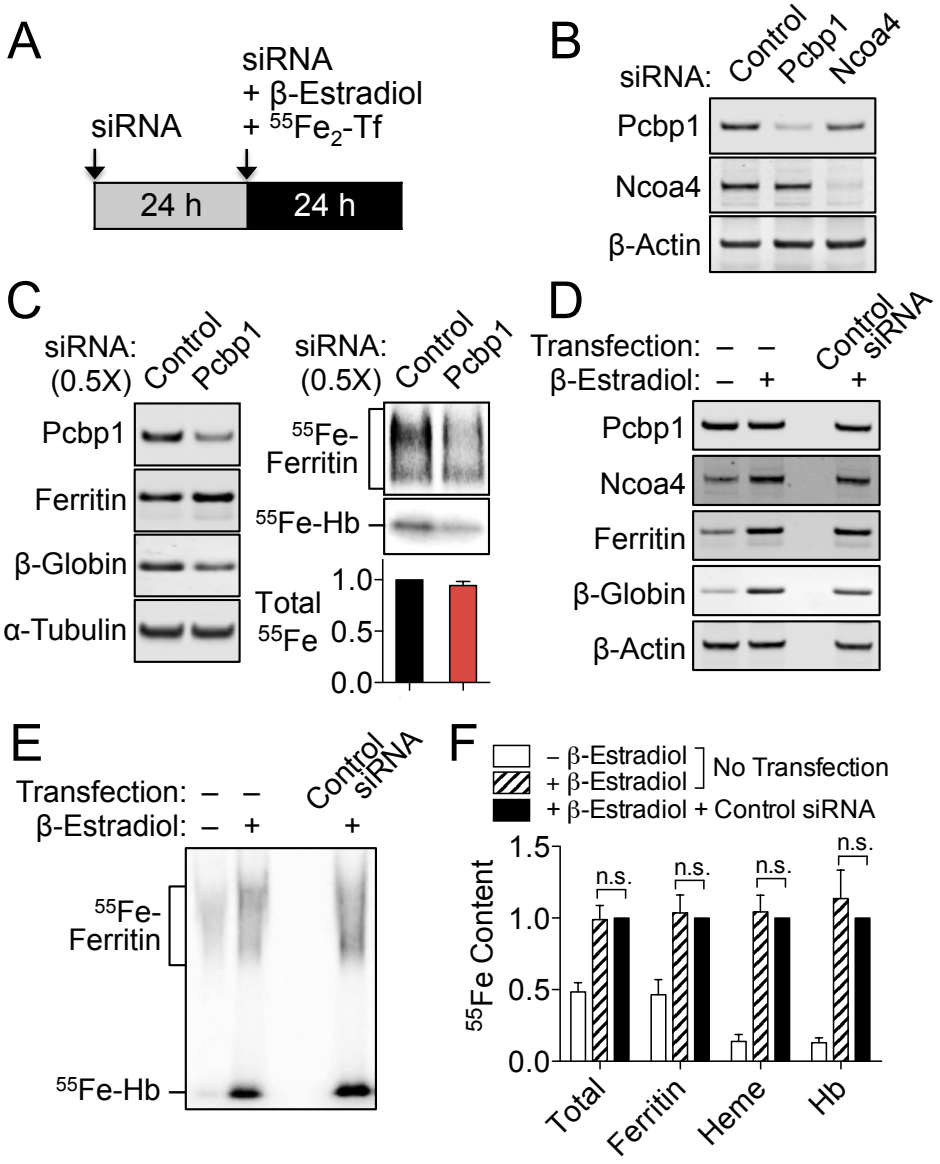
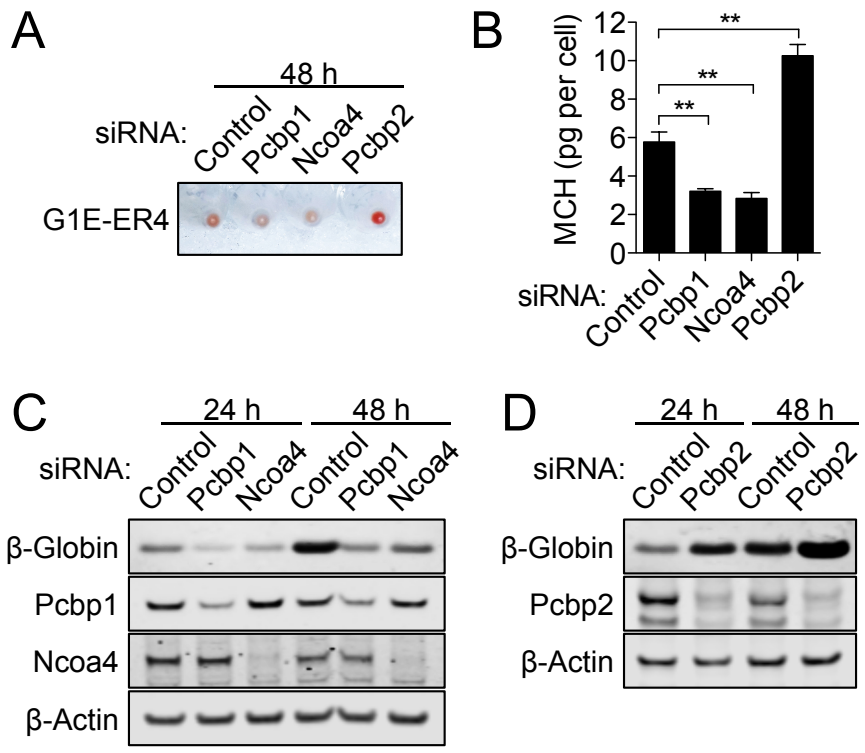


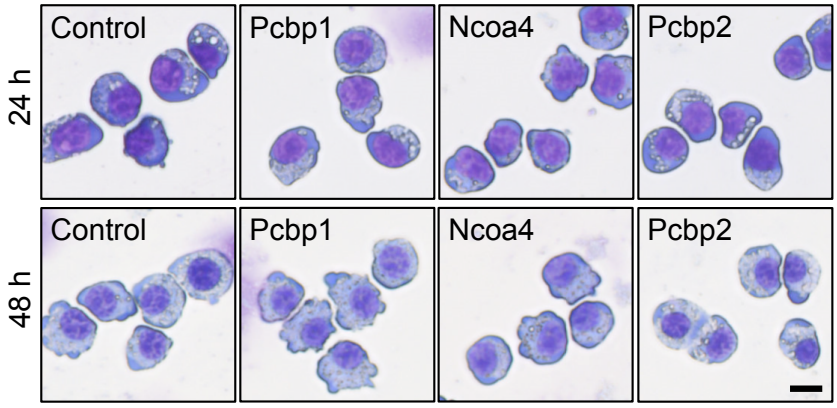
Supplemental Figure 1. **Differentiation of G1E-ER4 cells induced by β-estradiol treatment. A.** Morphological changes of G1E-ER4 cells during β-estradiol-induced differentiation. Cytospin samples were stained by Wright-Giemsa method. Scale bar = 10 μm. **B.** Temporal expression trend of transcripts during G1E-ER4 development. Relative mRNA abundance of erythroid marker genes and ferritin iron regulators at differing developmental stages of G1E-ER4.



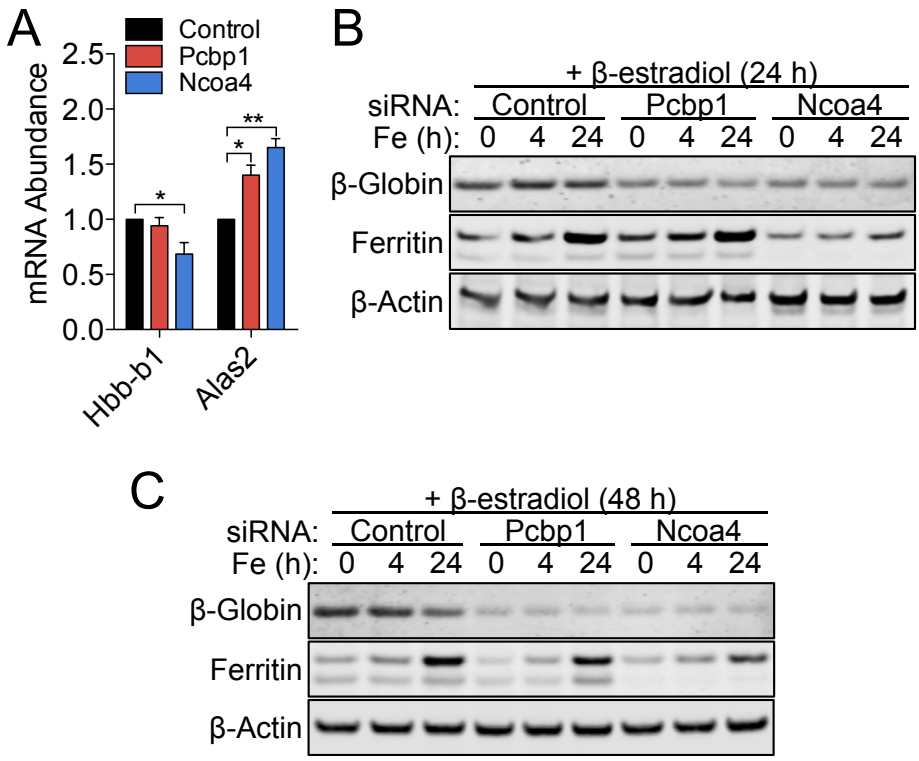
Supplemental Figure 2. Depletion of Pcbp1 and Ncoa4 in G1E-ER4 cell model of erythroid differentiation. A. Schematic of experimental design. Cell were subjected to two sequential transfections of non-targeting siRNA or siRNA against Pcbp1 or Ncoa4. Cells were induced to differentiate with β-estradiol after the second siRNA treatment. Labeling with $^{55}Fe_2$-Tf occurred during differentiation for 24 h. **B.** Efficient depletion of Pcbp1 and Ncoa4. Cells were analyzed by immunoblot. **C**. Impaired iron transfer without change in iron accumulation with partial depletion of Pcbp1. Cells were transfected with lower amouts (0.5x) of siRNA, producing partial depletion of Pcbp1. Iron labeling and analysis was performed as in Fig. 2. **D-F.** Lack of effect of control siRNA on protein expression **(D)**, iron flux **(E)**, or iron accumulation **(F)**.



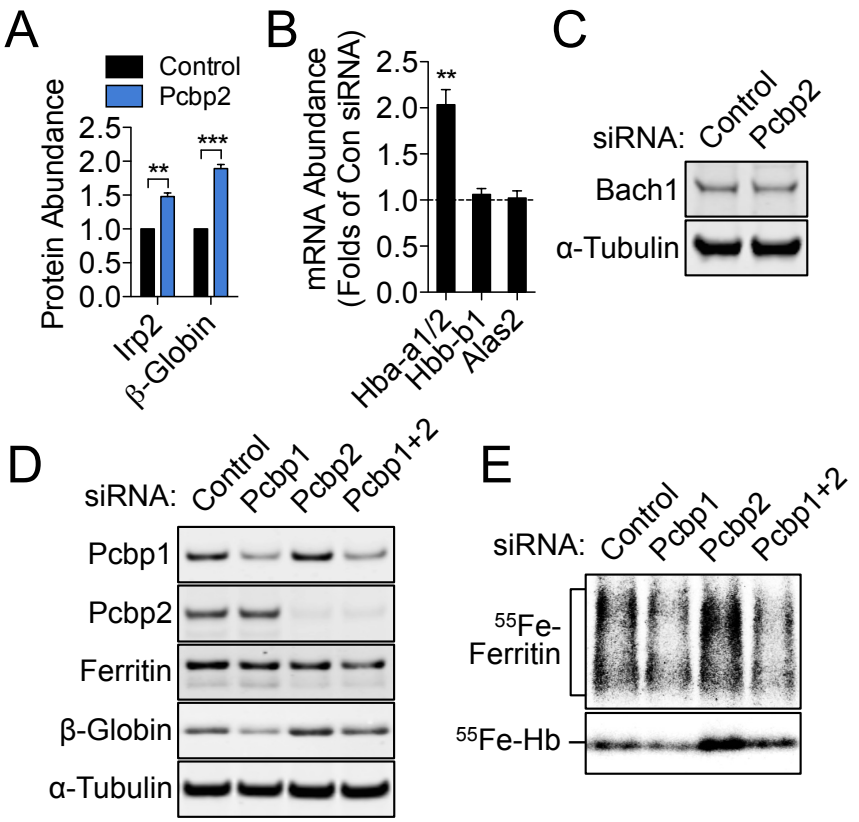
Supplemental Figure 3. Dysregulated heme production by Pcbp or Ncoa4 knockdown after 48 h of G1E-ER4 development. Cell pellets **(A)** and quantitative measures of mean cellular Hb **(B)** after 48 h of differentiation. **C.** β-Globin protein abundance reflects the cellular heme status of Pcbp1- or Ncoa4-deficient G1E-ER4 cells after 48 h of development. **D.** Elevated β-Globin protein abundance in Pcbp2-deficient cells at 24 and 48 h of differentiation.



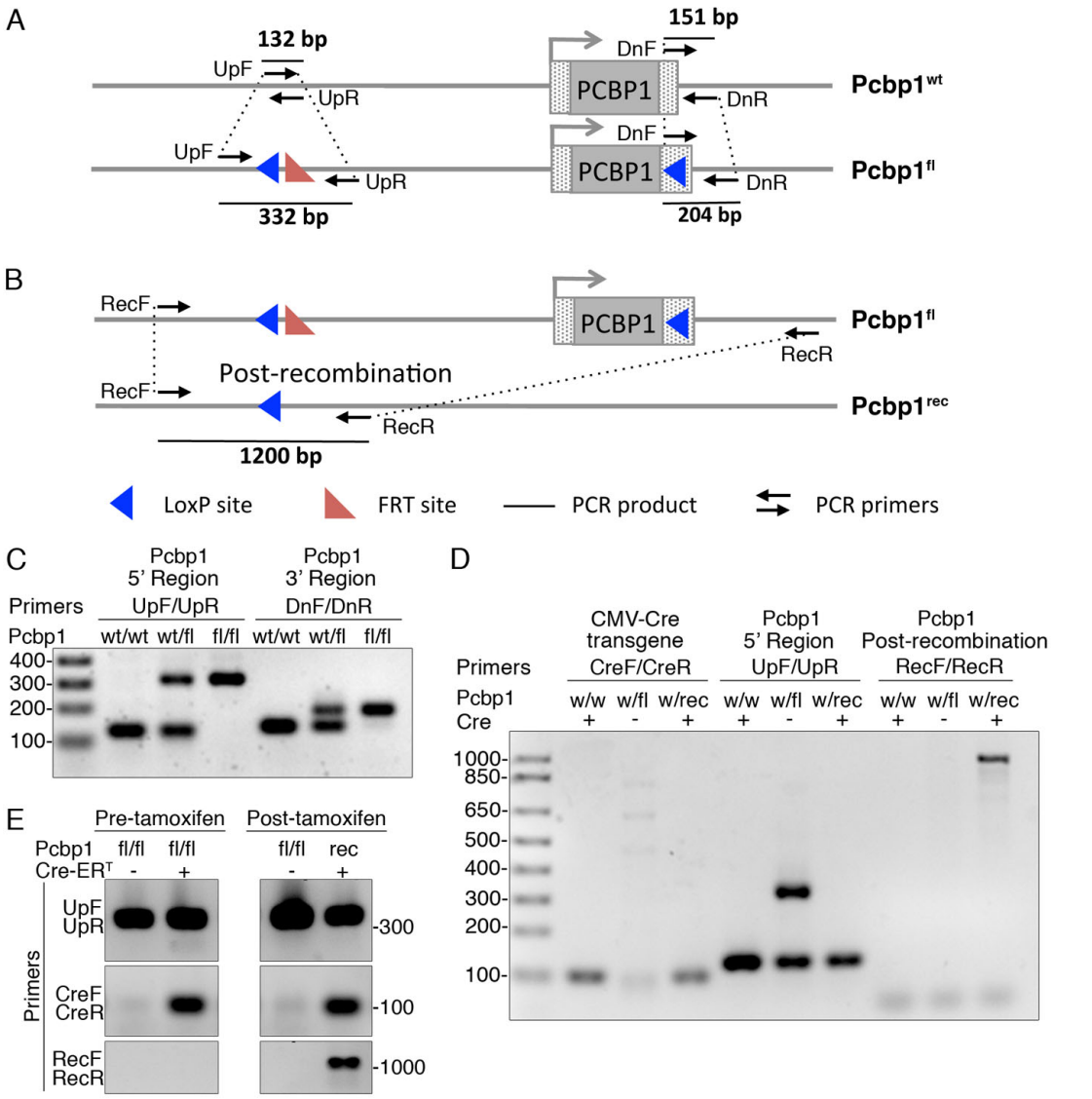
Supplemental Figure 4. Similar morphological changes in siRNA-treated G1E-ER4 cells during differentiation. Cytospin samples of transfected G1E-ER4 cells were stained by the Wright-Giemsa method after each indicated time of development. Scale bar = 10 μm.



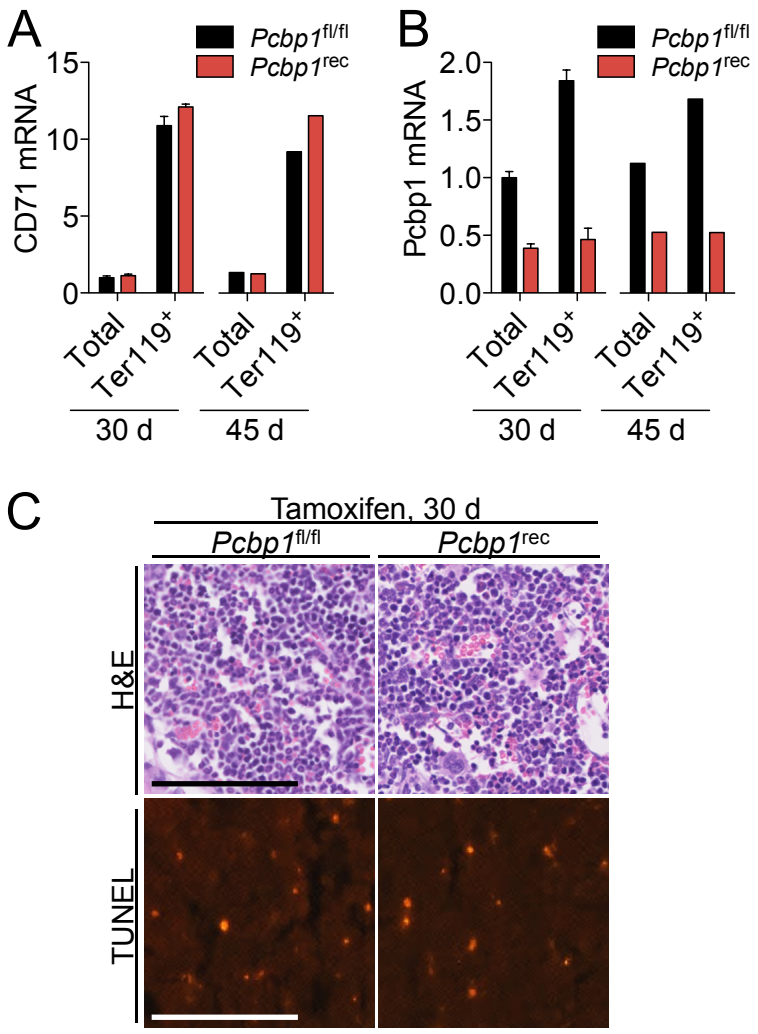
Supplemental Figure 5. Effects of Pcbp1 and Ncoa4 depletion on erythroid transcripts and failure of iron supplementation to rescue erythroid development in Pcbp1 and Ncoa4 depletion. G1E-ER4 cells were depleted of Pcbp1 and Ncoa4 and induced to differentiate for 24 or 48 hr as in Figure 2. **A.** Alas2 transcript up-regulation with Pcbp1 and Ncoa4 depletion. **B and C.** Failure of supplemental iron to reverse β-globin deficiency in Pcbp1- and Ncoa4-depleted cells after 24 **(B)** and 48 h **(C)** of differentiation. Ferric ammonium citrate was added to cells for the final 4 h and 24 h of differentiation. Increased ferritin levels indicate uptake of iron and sensing by iron regulatory proteins.



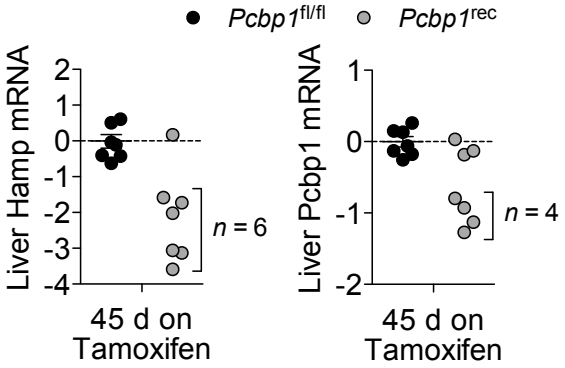
Supplemental Figure 6. Opposing effects of Pcbp2 on iron flux through ferritin. G1E-ER4 cells depleted of Pcbp1, Pcbp2, or both Pcbp1 and Pcbp2 were treated as in Figure 3. **A.** Increases in Irp2 and β-globin protein abundance by Pcbp2 depletion. Proteins were quantified by western (Figure 3) and normalized to α-tubulin. **B.** Increased α-globin transcripts and unchanged β-globin and Alas2 transcripts in Pcbp2-depleted cells. **C**. Bach1 protein levels unchanged by Pcbp2 depletion. **C** and **D.** Pcbp2 effects on iron, heme, and Hb are mediated through Pcbp1. **C**. Individual and co-depletion of Pcbp1 and Pcbp2. **D**. Defects in iron flux in Pcbp1 and Pcbp2 co-depleted cells resemble those of Pcbp1-depleted cells.



Supplemental Figure 7. Genotyping of *Pcbp1* conditional deletion mice. A. PCR strategy for detecting *Pcbp1* wild type (*Pcbp1*^{wt}) and floxed (*Pcbp1*^{fl}) alleles in 5' and 3' regions of the locus. *Pcbp1* is an intronless gene and sites for insertion of recombination cassettes were chosen to avoid regulatory regions. The 5' *loxP* site is 2.8 kb upstream of the start codon and the distal *loxP* site is within the 3' UTR of the transcript. Selection cassette was removed through recombination at FRT sites. **B.** PCR detection of recombination at floxed allele of *Pcbp1* (*Pcbp1*^{rec}). **C.** PCR genotyping of *Pcbp1*$^{fl/fl}$ and *Pcbp1*^{rec} mice using primers specific to 5' region or 3' region. **D.** Detection of post-recombination allele of *Pcbp1*$^{fl/fl}$. Parental female (*Tg CMV-Cre, Pcbp1*$^{w/w}$), male (*Pcbp1*$^{w/fl}$), and offspring (*Tg CMV-Cre, Pcbp1*$^{w/rec}$) were genotyped by PCR. Note that 332 bp product specific for *Pcbp1*$^{fl/fl}$ allele is not detected in offspring but the 1.2 Kbp product specific for recombination is detected.



Supplemental Figure 8. Depletion of Pcbp1 in erythropoietic cells of bone marrow and absence of bone marrow failure or increased apoptosis in Pcbp1-deficient bone marrow. A and B. Tamoxifen-induced *Pcbp1* recombination in bone marrow erythroid cells. Erythroid cells were isolated from single cell suspensions of bone marrow (Total) via MACS of Ter119-positive cells (Ter119^{+}). **A.** Higher CD71 (Tfrc) transcript abundance in the magnetically captured Ter119^{+} cells. **B.** Lower Pcbp1 mRNA levels in Ter119^{+} bone marrow cells of *Pcbp1*^{rec} mice. Transcript abundance was determined by qPCR, and normalized to Actb levels. Means ± SEM of n = 5 *Pcbp1*$^{fl/fl}$ and n = 3 *Pcbp1*^{rec} for 30 d; and n = 1 of each genotype for 45 d of tamoxifen treatments. **C.** Femoral bone sections from *Pcbp1*$^{fl/fl}$ and *Pcbp1*^{rec} mice after 30 d of tamoxifen treatments were fixed and stained by hematoxylin and eosin, *upper* panels, or assayed for apoptotic cells (Red TUNEL), *lower* panels, and imaged by light microscopy or epifluorescence. Scale bar = 100 µm.



Supplemental Figure 9. Suppressed liver *Hamp* expression is not due to hepatic Pcbp1 deficiency. Individual animals without liver *Pcbp1* recombination yet express lower levels of *Hamp* mRNA than control Pcbp1$^{fl/fl}$ animals. Hamp transcript abundance was measured by RT-PCR, and normalized to Actb mRNA. Levels relative to the mean of Pcbp1$^{fl/fl}$ are presented in a Log_2-scale. Total n = 7 per genotype.

Supplemental Table 1. Effects of *Pcbp1* deficiency on red cell indices of peripheral blood determined by CBC analyses.

	15 d			30 d			45 d		
	Pcbp1$^{fl/fl}$ (n = 5)	*Pcbp1*^{rec} (n = 3)		*Pcbp1*$^{fl/fl}$ (n = 5)	*Pcbp1*^{rec} (n = 6)		*Pcbp1*$^{fl/fl}$ (n = 7)	*Pcbp1*^{rec} (n = 7)	
CBC index	Mean ± SEM	Mean ± SEM	*p*-value[a]	Mean ± SEM	Mean ± SEM	*p*-value	Mean ± SEM	Mean ± SEM	*p*-value
RBC(M/uL)	9.66 ± 0.27	9.14 ± 0.07	0.2018	9.04 ± 0.18	9.78 ± 0.30	0.0732	9.53 ± 0.18	9.59 ± 0.27	0.8583
HGB(g/dL)	13.94 ± 0.38	13.07 ± 0.07	0.1266[b]	13.70 ± 0.28	14.00 ± 0.45	0.6038	**14.11 ± 0.26**	**13.20 ± 0.27**	**0.0294**
HCT(%)	49.56 ± 1.22	47.97 ± 0.38	0.3722	48.54 ± 1.51	49.68 ± 1.74	0.6392	**50.20 ± 1.01**	**46.24 ± 0.94**	**0.0139**
MCV(fL)	51.32 ± 0.45	52.47 ± 0.26	0.1176	**53.66 ± 1.00**	**50.80 ± 0.47**	**0.0226**	**52.66 ± 0.32**	**48.30 ± 0.74**	**0.0002**
MCH(pg)	14.46 ± 0.09	14.30 ± 0.06	0.2432	**15.16 ± 0.17**	**14.32 ± 0.08**	**0.0011**	**14.80 ± 0.09**	**13.79 ± 0.15**	**< 0.0001**
MCHC(g/dL)	28.10 ± 0.33	27.23 ± 0.09	0.0969	28.24 ± 0.31	28.18 ± 0.21	0.8791	28.13 ± 0.11	28.56 ± 0.21	0.1014
RDW-CV(%)	23.60 ± 0.34	24.07 ± 1.57	0.5714[b]	22.16 ± 0.43	22.78 ± 0.37	0.2934	**22.26 ± 0.25**	**23.91 ± 0.38**	**0.0035**
RET#(K/uL)	381.82 ± 42.32	419.50 ± 33.10	0.5619	**410.86 ± 26.80**	**249.43 ± 14.90**	**0.0004**	377.86 ± 12.69	388.71 ± 28.64	0.7349
RET%(%)	4.01 ± 0.51	4.59 ± 0.37	0.4579	**4.56 ± 0.34**	**2.56 ± 0.16**	**0.0003**	3.96 ± 0.08	4.04 ± 0.24	0.9015[b]

[a]Statistically significant effects of genotype at each time-point were identified using two-tailed Student's t-test. Measures with significance in bold. Levels of significance were set at P < 0.05. [b]*P*-value from *Mann-Whitney test* due to unequal variances.

Supplemental Table 2. Primer sets for PCR-based genotyping.

Primer name	Primer sequence	Target	PCR products
UpF	5'-ATAGATGATTGGAACCAGGTAAAGT-3'	LoxP upstream of *Pcbp1*	*Pcbp1*^{wt} (132 bp) *Pcbp1*^{fl} (332 bp)
UpR	5'-GCTCCAAGGGAGCAAGCGAGCAGT-3'		
DnF	5'-CGGTAATTCCAGGTTTTAAAT-3'	LoxP downstream of *Pcbp1*	*Pcbp1*^{wt} (151 bp) *Pcbp1*^{fl} (204 bp)
DnR	5'-ACGAAACTAAAAACAGGGAAAAGCT-3'		
RecF	5'-TGCGCTATCCACTACCTACATC -3'	*Pcbp1* recombination	*Pcbp1*^{rec} (1200 bp)
RecR	5'-AGCTTATAAAACTAAATATGGAT-3'		
CreF	5-'GCGGTCTGGCAGTAAAAACTATC-3'	*Cre* allele	*Cre*$^{+}$ (~100 bp)
CreR	5'-GTGAAACAGCATTGCTGTCACTT-3'		