Krüppel-like transcription factors KLF1 and KLF2 have unique and coordinate roles in regulating embryonic erythroid precursor maturation

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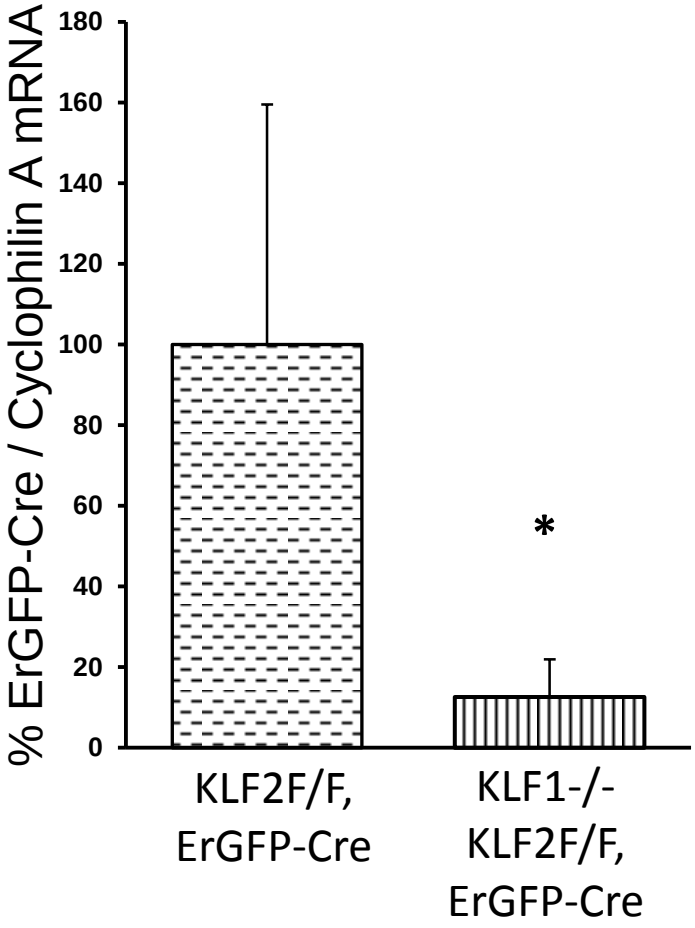
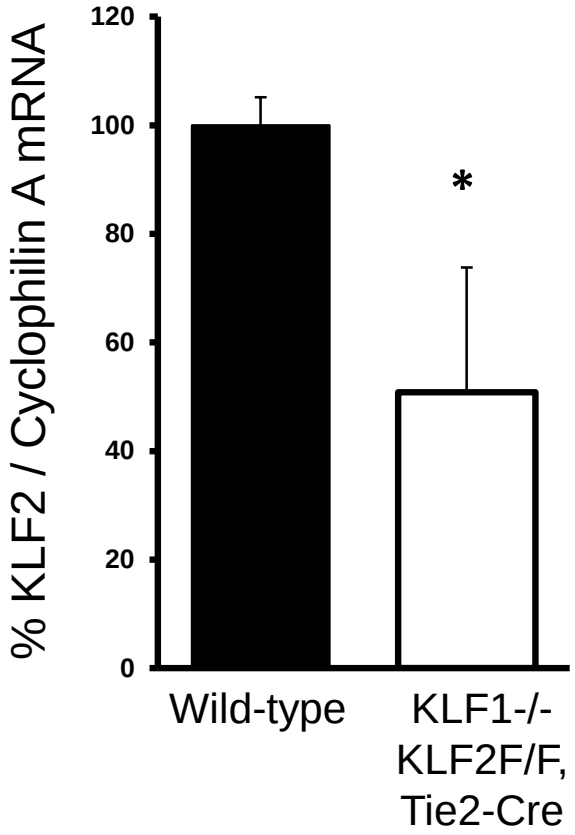
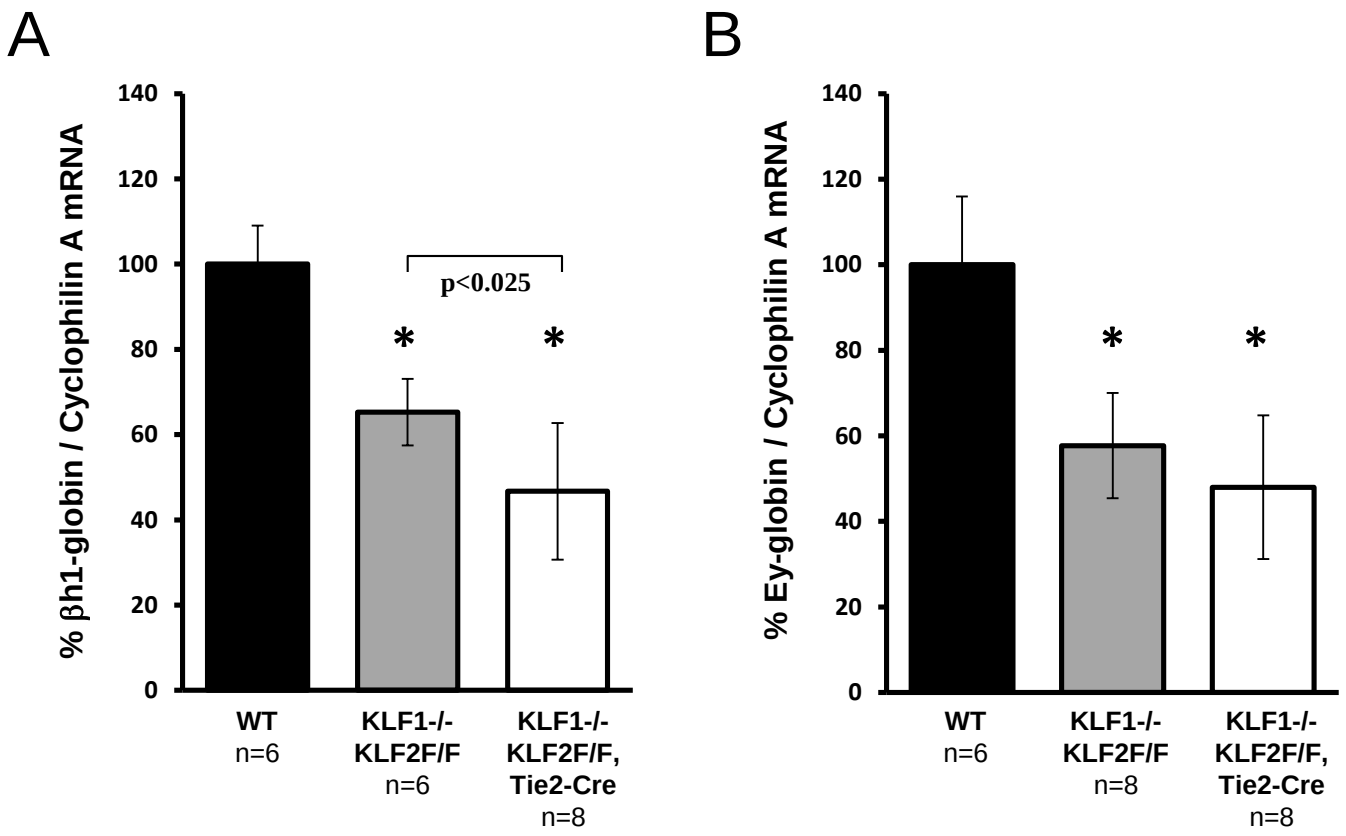


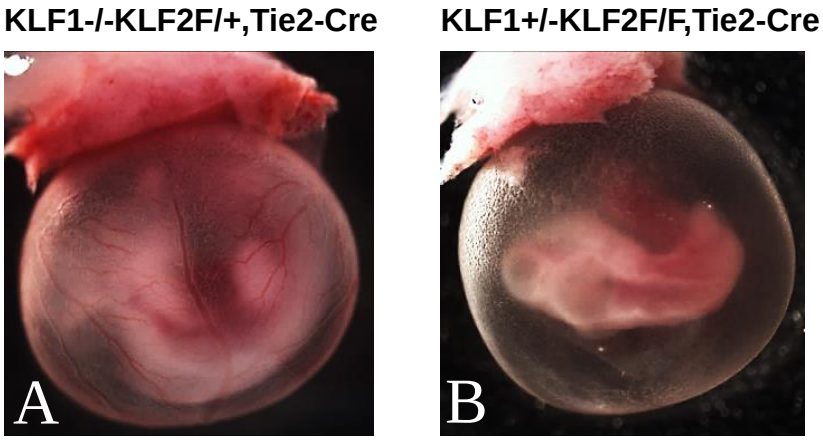
Supplementary Figure 1. ErGFP-Cre mRNA expression is regulated by KLF1 in erythroid cells. ErGFP-Cre mRNA expression in KLF2F/F,ErGFP-Cre (n=3) E10.5 peripheral blood compared to KLF1-/- KLF2F/F,ErGFP-Cre (n=5). The ErGFP-Cre-to-Cyclophilin A mRNA ratio for KLF2F/F,ErGFP-Cre was taken as 100%. Cyclophilin A mRNA was used as an internal standard for qRT-PCR. Error bars indicate standard deviation. * = $p < 0.025$



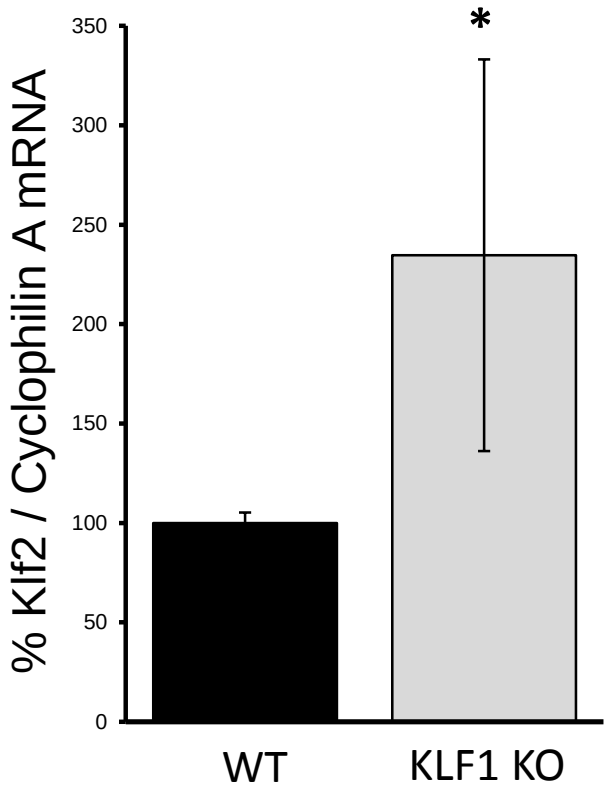
Supplementary Figure 2. Tie2-Cre expression produces an approximately 50% reduction in KLF2 mRNA. KLF2 mRNA expression in KLF1-/-KLF2F/F, Tie2-Cre (n=7) E10.5 peripheral blood compared to wild-type (n=4). The Tie2-Cre-to-Cyclophilin A mRNA ratio for wild-type was taken as 100%. Cyclophilin A mRNA was used as an internal standard for qRT-PCR. Error bars indicate standard deviation. * = $p < 0.01$



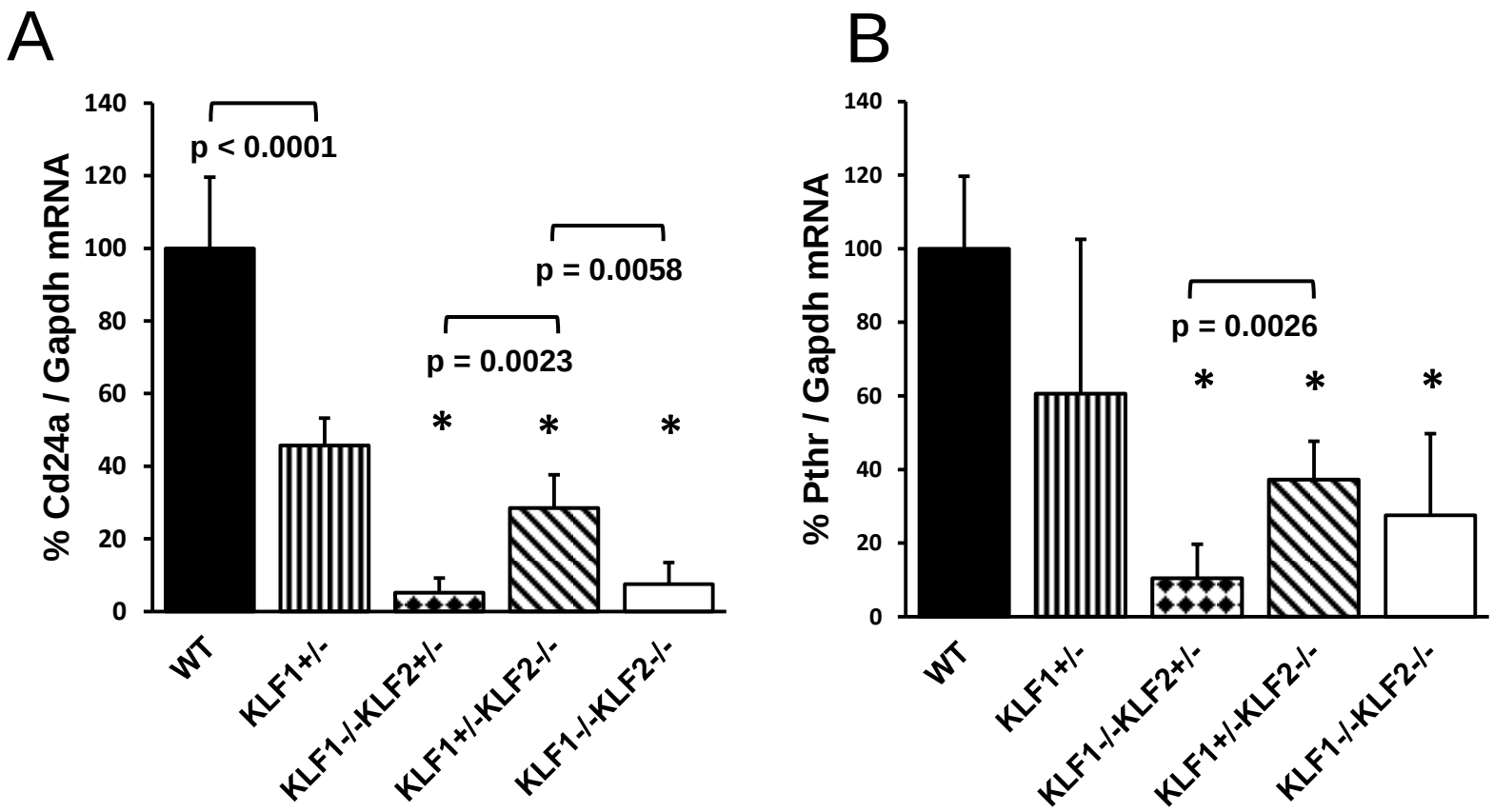
Supplementary Figure 3. KLF1/KLF2 double conditional KO erythroid cells have reduced embryonic βh1- and Ey-globin mRNA. Mouse embryonic (A) βh1-globin and (B) Ey-globin mRNA expression in E10.5 erythroid cells from wild-type (WT), KLF1-/-KLF2F/F and KLF1-/-KLF2F/F,Tie2-Cre. * = p<0.001 compared to wild-type. Other significant p-values are shown using brackets. Cyclophilin A mRNA was used as an internal standard for qRT-PCR. The globin-to-cyclophilin A mRNA ratio for WT was taken as 100%. n=6-8 for each genotype. Error bars indicate standard deviation.



Supplementary Figure 4. Gene dosage of KLF1 and KLF2 impacts the anemia phenotype. E10.5 whole-mount embryos surrounded by yolk sacs. Embryos with one normal KLF2 gene, (A) KLF1-/-KLF2F/+, Tie2-Cre (n=2) appear grossly normal; whereas a (B) KLF1+/-KLF2F/F, Tie2-Cre (n=1) embryo, with one functional KLF1 gene, appears anemic. Photographs were taken at 15X magnification.



Supplementary Figure 5. Compensatory increase in KLF2 mRNA in KLF1-/- blood cells. KLF2 mRNA expression in KLF1 KO (KLF1-/-KLF2F/F, n=9) E10.5 peripheral blood compared to wild type (n=4). The KLF2-to-Cyclophilin A mRNA ratio for wild type was taken as 100%. Cyclophilin A mRNA was used as an internal standard for qRT-PCR. Error bars indicate standard deviation. * = $p < 0.025$



Supplementary Figure 6. KLF1 and KLF2 modulate the expression of the proliferation-associated genes Cd24a and Pthr. qRT-PCR was used to determine the amount of (A) Cd24a and (B) Pthr mRNA in wild-type (WT), KLF1+/-, KLF1-/-KLF2+/-, KLF1+/-KLF2-/- and KLF1-/-KLF2-/- E10.5 blood cells. Gapdh mRNA was used as an internal standard. Wild-type was taken as 100%. n= 4-10 for each genotype. All of the genotypes (KLF1-/-KLF2+/-, KLF1+/-KLF2-/- and KLF1-/-KLF2-/-) were significantly different from wild-type and KLF1+/- (* = p< 0.0001). Other significant p-values are shown using brackets. Error bars indicate standard deviation.

	Gene	Primer sequence for qRT-PCR
1.	FoxM1	FP: GGCAAAGACAGGAGAGCTATG
		RP: TCTTCCAGTTCCTGCTTAACG
2.	Cd24a	FP: CTTAGCAGATCTCCACTTACCG
		RP: GTAAATCTGCGTGGGTAGGAG
3.	Sphk1	FP: TGAATGGGCTAATGGAACGG
		RP: GTCTTCATTAGTCACCTGCTCG
4.	Pthr	FP: CTAAGCTTCGGGAGACCAATG
		RP: ACCGAAGAGTGGCACAAG
5.	E2f2	FP: CCCCAAAACCCCCAAGTCT
		RP: ACTCGCTCAGGAGGTAAATGAACT
6.	E2f4	FP: GCAGATGCTTTGCTGGAGAT
		RP: TCTGGTACTTCTTCTGGCCATTGA
7.	p18	FP: CGTCAACGCTCAAAATGGATT
		RP: GACAGCAAAACCAGTTCCATC
8.	p27	FP: ACCAAATGCCTGACTCGTC
		RP: GTTCTGTTGGCCCTTTTGTTT

Supplementary Table 1. Primer sequences used in qRT-PCR to determine amount of mRNA of respective gene. Sequences are 5' to 3'. FP – forward primer, RP – reverse primer. See text for abbreviations.

	Gene	Primer sequence for qPCR
1.	FoxM1	FP: CACGTAACCGCAAGTCTAGG
		RP: ACTCGGTTACCCCTGGG
2.	Necdin	FP: TTCGTCCAGCAGAATTACCTGAAG
		RP: GGACCCCCAGAAGAACTCGTA

Supplementary Table 2. Primer sequences used to quantitate enrichment using qPCR for ChIP assays. Sequences are 5’ to 3’. FP – forward primer, RP – reverse primer. See text for abbreviations.

Genotype	Wild type	KLF1-/-KLF2+/-	KLF1+/-KLF2-/-	KLF1-/-KLF2-/-
Globin mRNA per embryo (%)	100±27	25.1±11	10.4±13	6.4±5

Supplementary Table 3. A combined reduction in globin mRNA per cell and in number of peripheral blood cells contributes to the anemia phenotype of E10.5 KLF1+/-KLF2-/- and KLF1-/-KLF2-/- embryos. Values in the table represent an estimate of total amount of globin mRNA per embryo (± standard deviation) for each genotype arrived at by combining data for number of blood cells per embryo and total embryonic β-like globin mRNA per cell from Figures 3 C,D and E. The amount of globin mRNA per embryo for wild-type was set to 100. n = 4 to 10 for each genotype.