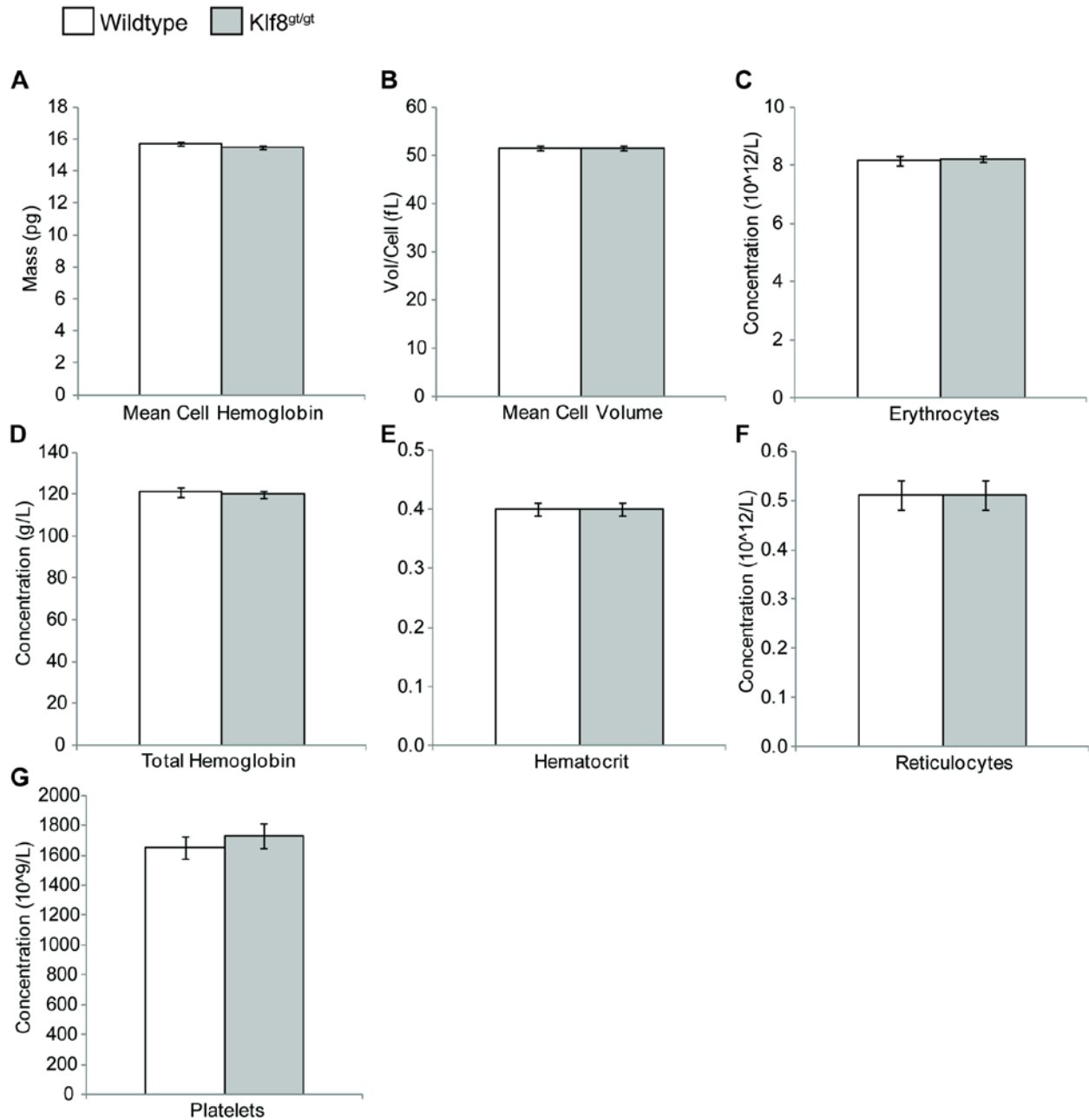
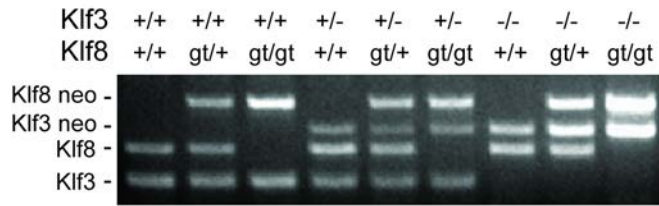


Supplementary Figures

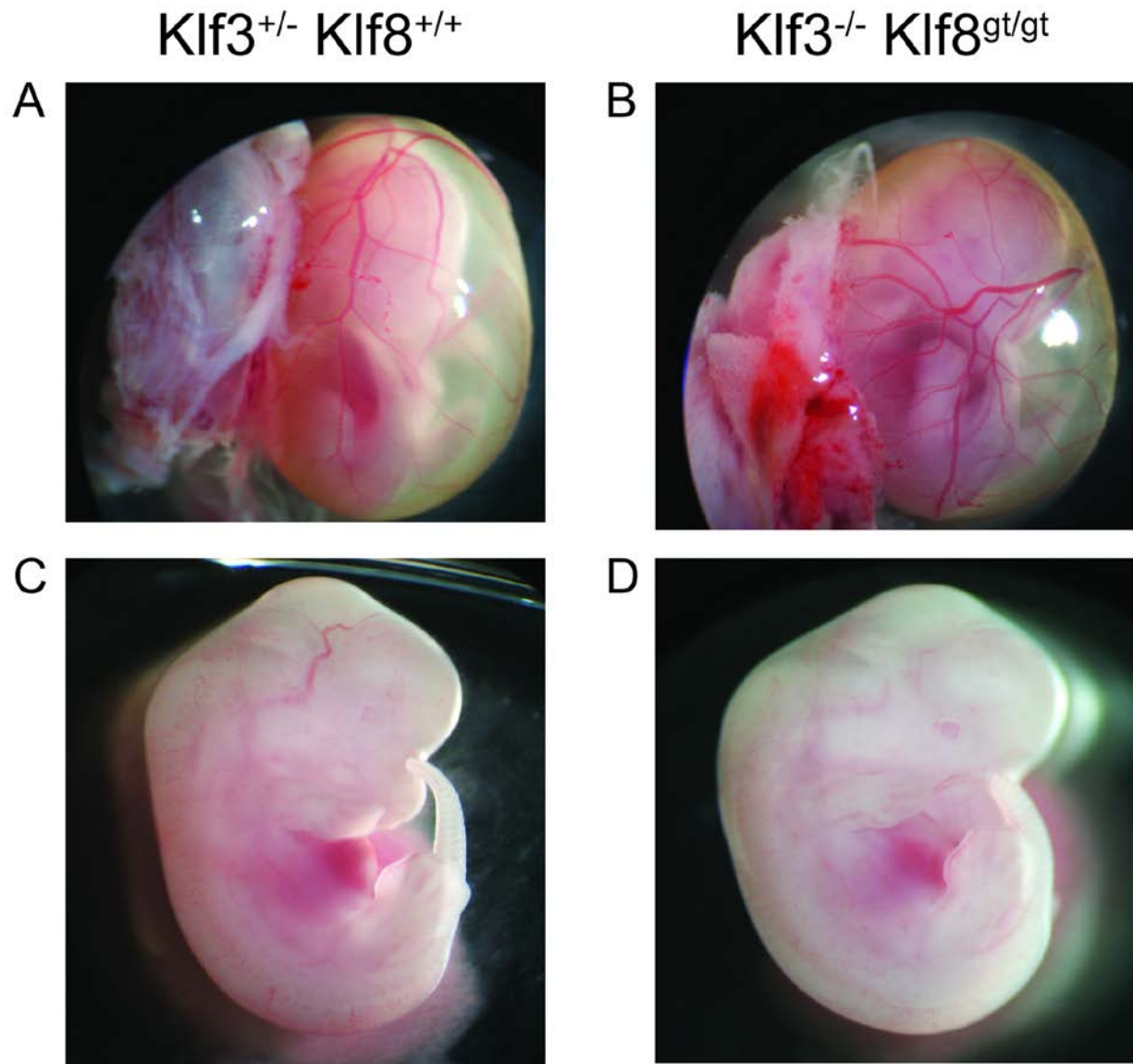


Supplementary Figure 1. Peripheral blood parameters are normal in *Klf8*^{gt/gt} mice. (A-G)

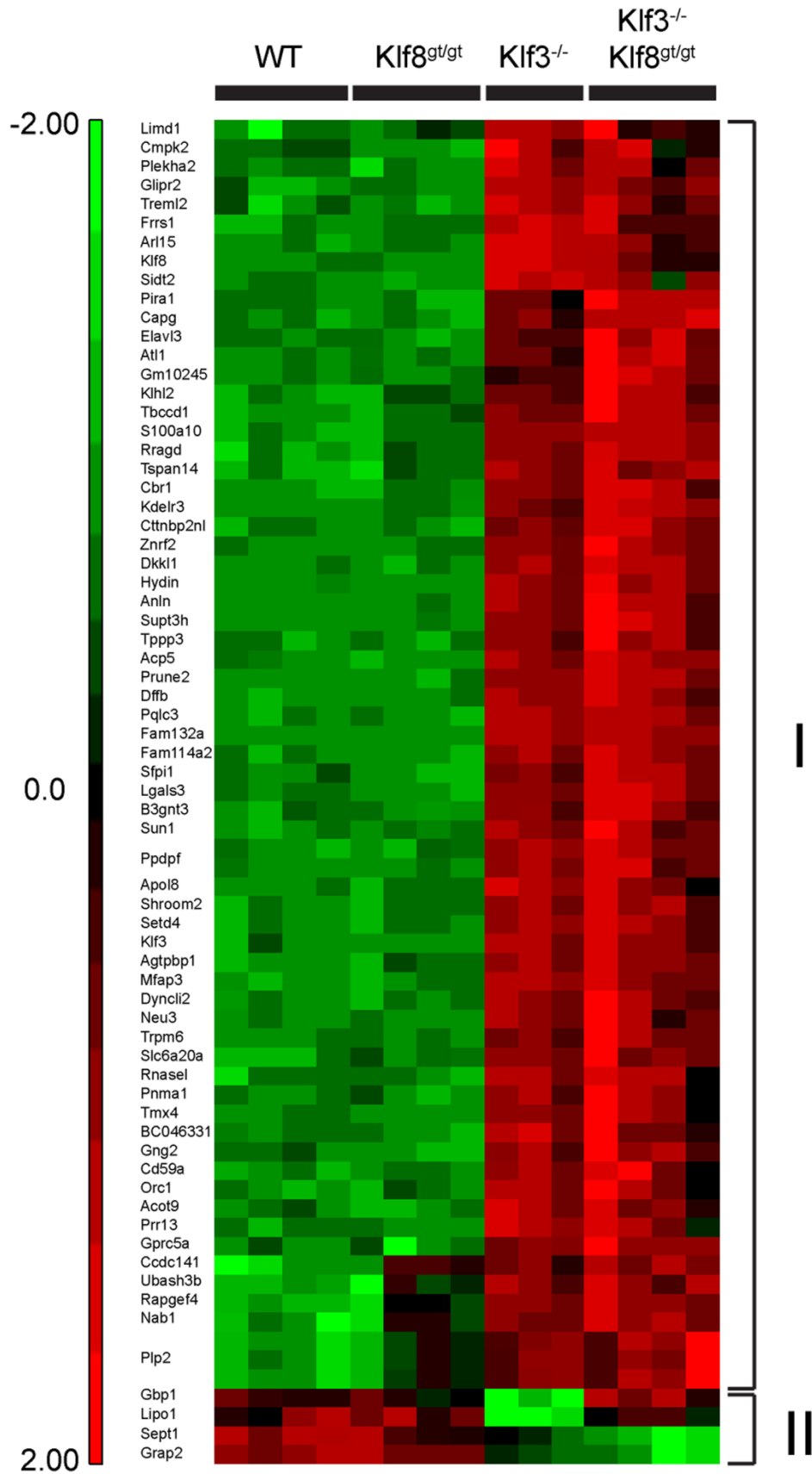
Blood counts were performed for 21 *Klf8* mutant mice (*Klf8*^{gt} males or *Klf8*^{gt/gt} females) and 18 wildtype mice at 11-12 weeks of age. Error bars represent standard error of the mean.



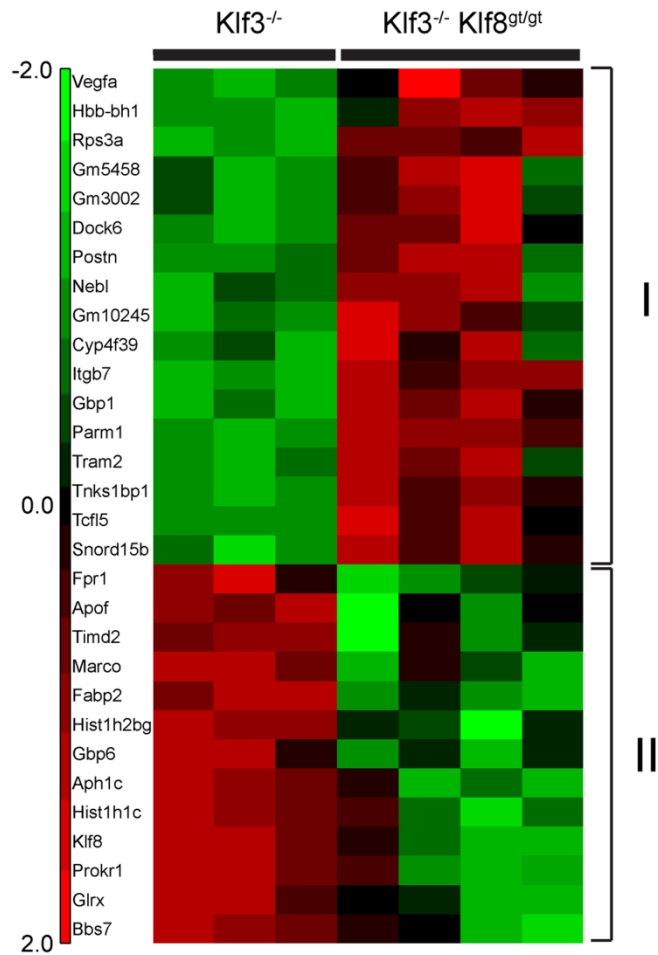
Supplementary Figure 2. Multiplex PCRs demonstrating the nine possible genotypes resulting from crossing the *Klf3* and *Klf8* mutant lines. PCRs were performed on genomic DNA extracted from embryonic tissue and products were resolved on a 2% agarose gel stained with ethidium bromide.



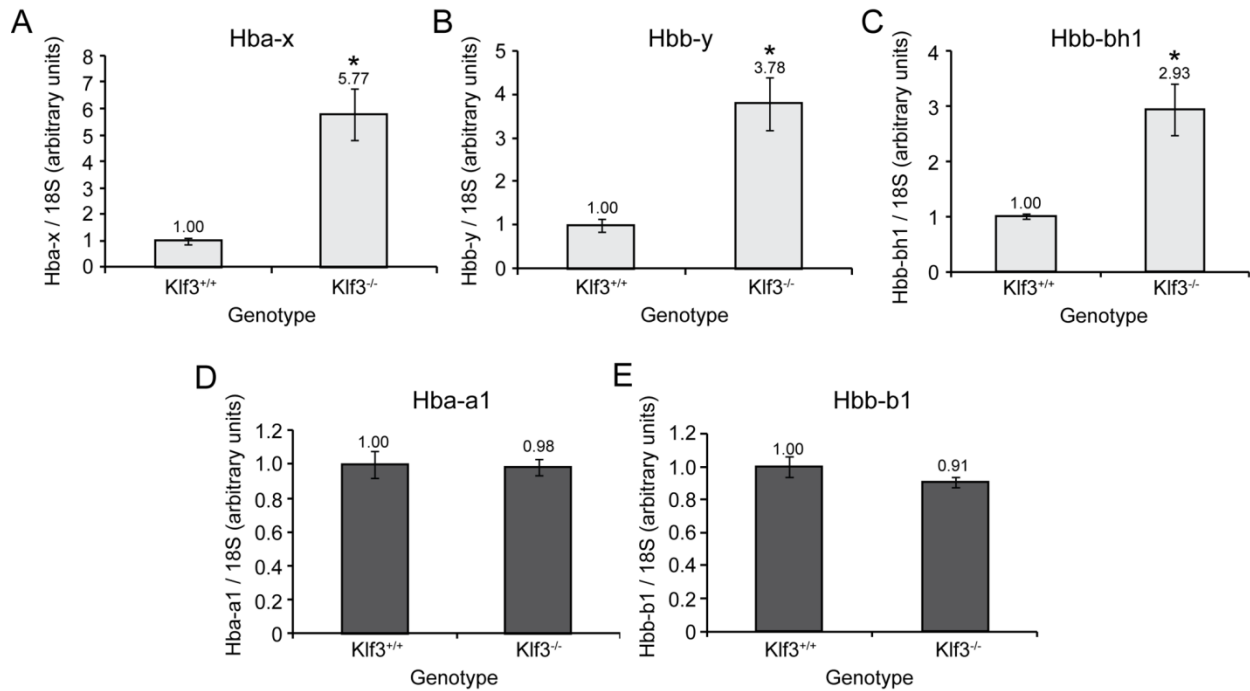
Supplementary Figure 3. *Klf3*^{-/-} *Klf8*^{gt/gt} embryos are largely phenotypically normal at embryonic day E13.5. (A and C) *Klf3*^{+/-} *Klf8*^{+/+} embryo with placenta and yolk sac attached (A) and removed (C). (B and D) *Klf3*^{-/-} *Klf8*^{gt/gt} embryo with placenta and yolk sac attached (B) and removed (D).



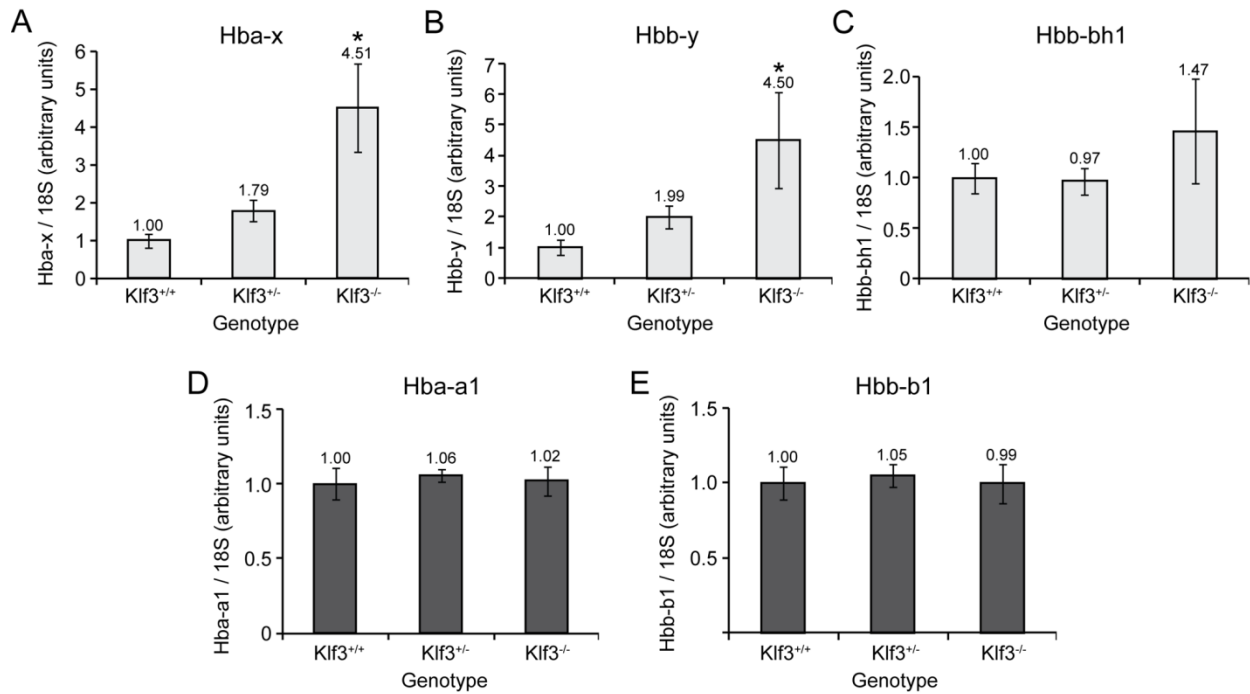
Supplementary Figure 4. Genes that are derepressed in *Klf3*^{-/-} E13.5 Ter119⁺ fetal liver cells are also derepressed in *Klf3*^{-/-} *Klf8*^{tg/tg} cells. The heat map represents the list of genes that are significantly deregulated in *Klf3*^{-/-} cells compared to wildtype (WT). The majority of genes are upregulated (group I) in the absence of KLF3 rather than downregulated (group II).



Supplementary Figure 5. Heat map showing the relative expression of the genes that are deregulated in *Klf3*^{-/-} *Klf8*^{gt/gt} Ter119⁺ E13.5 fetal liver cells compared to *Klf3*^{-/-}. These genes represent potential targets of KLF8 and also KLF3. Genes that are significantly upregulated in *Klf3*^{-/-} *Klf8*^{gt/gt} cells are classified as group I and downregulated genes constitute group II.

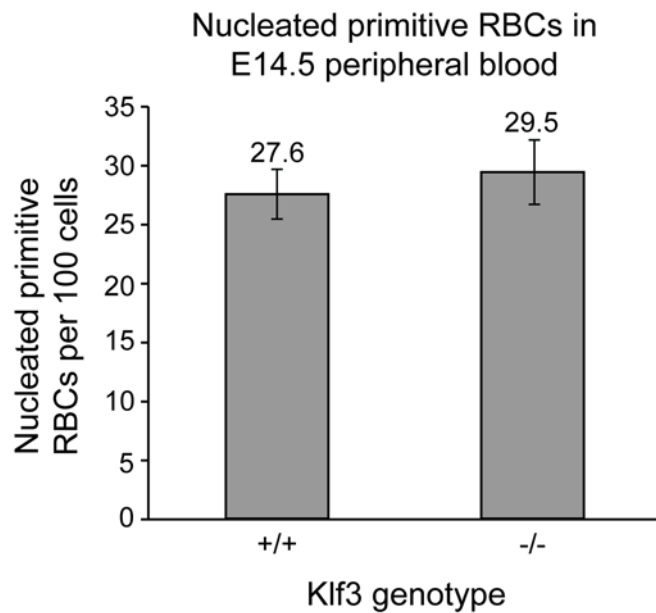


Supplementary Figure 6. Embryonic globin genes are derepressed in E14.5 *Klf3*^{-/-} Ter119⁺ fetal liver cells. Transcript levels for *Hba-x* (A), *Hbb-y* (B), *Hbb-bh1* (C), *Hba-a1* (D) and *Hbb-b1* (E) were determined by qRT-PCR analysis of total RNA. Data represent the average of five *Klf3*^{+/+} and seven *Klf3*^{-/-} embryos. Embryonic globin genes are depicted in light grey whilst adult globin genes are dark grey. Expression levels have been normalized to *18S* rRNA and wildtype samples have been set to 1.0 for each gene. Error bars indicate standard error of the mean. *, $P \leq 0.02$ (two-tailed t test compared to wildtype).

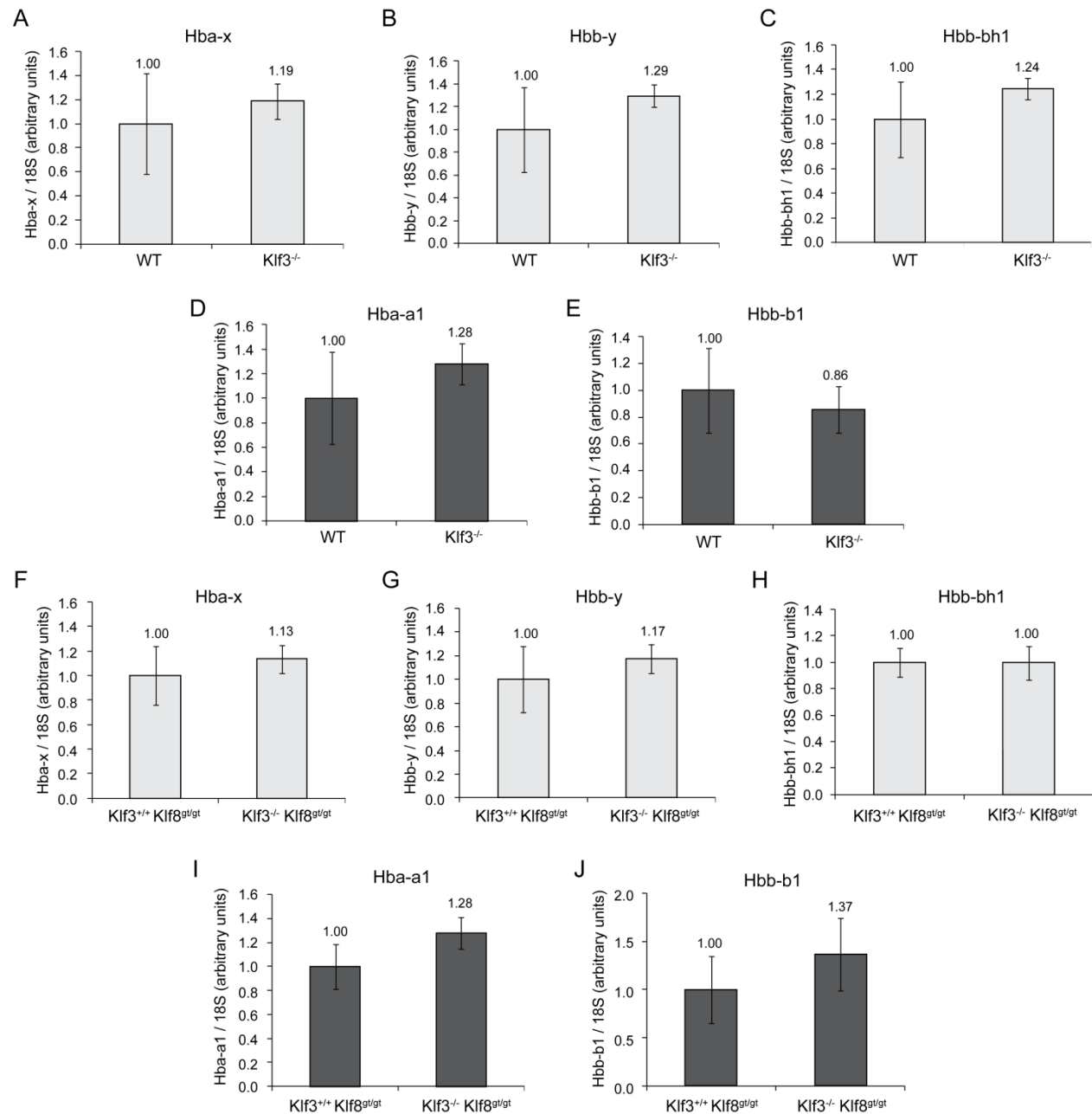


Supplementary Figure 7. Expression of globin genes in Ter119⁺ E16.5 *Klf3*^{-/-} fetal liver cells.

Transcript levels were determined by qRT-PCR for *Hba-x* (A), *Hbb-y* (B), *Hbb-bh1* (C), *Hba-a1* (D) and *Hbb-b1* (E). Data represent the average of four *Klf3*^{+/+}, thirteen *Klf3*^{+/-} and four *Klf3*^{-/-} embryos. Embryonic globin genes are depicted in light grey whilst adult globin genes are dark grey. Expression levels have been normalized to 18S rRNA and wildtype samples have been set to 1.0 for each gene. Error bars indicate standard error of the mean. *, $P \leq 0.04$ (Student's *t* test compared to wildtype).



Supplementary Figure 8. The number of nucleated, primitive red blood cells is normal in *Klf3*^{-/-} peripheral blood at E14.5. Nucleated, primitive cells were counted in triplicate cytopsin preparations of peripheral blood from three *Klf3*^{-/-} and four wildtype litter-matched E14.5 embryos.



Supplementary Figure 9. Expression of the embryonic and adult globin genes is not altered in *Klf3*^{-/-} or *Klf3*^{-/-} *Klf8*^{gt/gt} E10.5 yolk sac cells. Transcript levels for *Hba-x* (A and F), *Hbb-y* (B and G), *Hbb-bh1* (C and H), *Hba-a1* (D and I) and *Hbb-b1* (E and J) were determined by qRT-PCR analysis of total RNA. Data represent the average of five wildtype, six *Klf3*^{-/-}, three *Klf3*^{+/-} *Klf8*^{gt/gt} and four *Klf3*^{-/-} *Klf8*^{gt/gt} samples. Wildtype and *Klf3*^{-/-} embryos were derived from the

same litters whilst $Klf3^{-/-} Klf8^{gt/gt}$ embryos were compared with litter-matched $Klf3^{+/+} Klf8^{gt/gt}$ controls. Expression levels have been normalized to 18S rRNA and wildtype samples have been set to 1.0 for each gene. Error bars indicate standard error of the mean.

Supplementary Tables

Supplementary Table 1. Genes that are significantly deregulated in *Klf3*^{-/-} Ter119⁺ fetal liver at E13.5 are also deregulated in *Klf3*^{-/-} *Klf8*^{gt/gt} cells at E13.5 and in *Klf3*^{-/-} cells at E14.5. Shown are the list of genes that are significantly deregulated (>2-fold, FDR < 0.3) in *Klf3*^{-/-} Ter119⁺ fetal liver cells at E13.5 compared to wildtype. The fold changes observed in previously published microarrays (8) using tissue from E14.5 embryos are shown. The fold changes observed for *Klf3*^{-/-} *Klf8*^{gt/gt} compared to wildtype cells in this study are also shown. Genes that have been found to be significantly deregulated are shaded.

Gene	E13.5 fold change (<i>Klf3</i> ^{-/-} /WT)	E14.5 fold change (<i>Klf3</i> ^{-/-} /WT)	E13.5 fold change (<i>Klf3</i> ^{-/-} <i>Klf8</i> ^{gt/gt} versus WT)
Pqlc3	16.51	17.01	16.14
Rragd	14.64	27.19	22.56
Klf8	10.20	8.34	3.80
Fam132a	9.81	10.13	9.88
Supt3h	5.86	7.16	9.05
Kdelr3	5.79	9.08	10.98
Prune2	5.19	5.36	5.87
Arl15	5.08	5.04	3.23
S100a10	5.03	2.95	5.68
Anln	4.88	5.23	6.09
Dffb	4.84	5.53	5.07
Lgals3	4.52	7.70	6.89
Dkk1	4.51	3.05	5.45
Sidt2	4.36	5.39	2.71
Setd4	4.30	4.43	4.12
Hydin	4.23	5.79	5.17
Cttnbp2nl	3.84	6.44	5.90
Cbr1	3.71	5.37	4.77
Plekha2	3.55	2.78	2.79
Tppp3	3.31	3.60	4.08
Glpr2	3.29	2.57	2.81
Tbccd1	3.28	5.20	4.48
Shroom2	3.28	3.72	3.27
Sfpi1	3.22	5.13	4.54
Rapgef4	3.18	4.55	3.52
Klhl2	3.13	4.51	4.59

Gm10245	3.00	5.65	8.81
Slc6a20a	3.00	4.24	3.34
Atl1	2.80	4.18	5.35
Znrf2	2.76	2.79	3.53
Rnase1	2.70	2.75	2.84
Prr13	2.69	3.41	2.15
Fam114a2	2.68	3.96	2.90
Apol8	2.63	4.77	2.23
B3gnt3	2.62	3.67	3.26
Trpm6	2.59	1.80	3.30
Cd59a	2.58	2.59	2.58
Neu3	2.51	2.80	2.61
Ubash3b	2.50	1.49	2.59
Capg	2.49	3.08	3.87
Limd1	2.41	3.61	2.07
Frrs1	2.38	2.31	1.91
Tspan14	2.27	2.33	2.54
Mfap3	2.26	2.50	2.21
BC046331	2.26	3.43	2.09
Cmpk2	2.19	1.93	1.81
Plp2	2.19	2.21	2.52
Ppdpf	2.18	3.02	2.18
Acot9	2.16	2.35	1.86
Pira1	2.15	-	3.62
Gprc5a	2.14	2.51	2.56
Acp5	2.14	2.89	2.28
Elavl3	2.14	2.84	3.42
Gng2	2.13	2.64	2.47
Trem12	2.13	2.20	1.80
Agtppb1	2.11	3.57	2.16
Orc1	2.11	3.20	2.09
Tmx4	2.09	2.63	2.42
Nab1	2.09	1.81	2.36
Dync1li2	2.07	3.03	2.09
Klf3	2.06	1.94	2.14
Ccdc141	2.06	-	2.56
Sun1	2.04	2.66	2.17
Pnma1	2.02	1.54	2.22
Grap2	0.45	0.40	0.25
Gbp1	0.47	1.09	1.22
Lipo1	0.50	-	0.89
Sept1	0.50	0.36	0.28