Inventory of Supplemental Information

1. Supplemental text
2. Supplemental Figures 1-7
3. Supplemental Table 1
4. Supplemental Table 2

SUPPLEMENTAL INFORMATION

SUPPLEMENTAL FIGURE LEGENDS

Figure S1. Samd14-Enh Deletion Does Not Impact Steady-State Levels of Hematopoietic Progenitors *In Vivo*.

(A) Representative flow cytometric plots of $Lin^-Sca^+Kit^+$ cells, and $Lin^-Sca^-Kit^+$ CMP, GMP and MEP populations in WT and Samd14-$Enh^{-/-}$ total bone marrow. (B) Quantitation of flow cytometric analysis of R1-R5 populations using CD71 and Ter119 staining from bone marrow of WT and Samd14-$Enh^{-/-}$ mice (n = 3). (C) Quantitation of total cell number (left) and flow cytometric analysis of R1-R5 populations using CD71 and Ter119 staining (right) from WT and Samd14-$Enh^{-/-}$ cells isolated from E14.5 fetal liver and expanded *in vitro* for 3 days (n = 3). (C) Allele-specific qRT-PCR analysis of *Gata2* primary transcripts from *Gata2* $+9.5^{+/-}$ WT and Mut alleles in control (n = 3) and PHZ-treated (n = 4) spleen. (Related to Figure 1).

Figure S2. Samd14-Enh Deletion Does Not Alter Expression of Genes Flanking *Samd14*. Relative expression of *Ppp1r9b* and *Pdk2* in WT and Samd14-$Enh^{-/-}$ splenic cells from control and PHZ-treated mice (n = 3). Statistical significance represented by mean +/- SEM.; *$p<0.05$. (Related to Figure 3).

Figure S3. Samd14-$Enh^{-/-}$ Mice Have Fewer Splenic Kit^+ Cells Following PHZ Treatment.

Representative flow cytometric analysis of Ter119$^-$CD71$^+$Kit$^+$ cells in WT and Samd14-Enh$^{-/-}$ control spleen, and spleen following PHZ treatment. (Related to Figures 4 and 6).

Figure S4. Analysis of Proliferation and Apoptosis During Anemia in CD71$^+$Ter119$^-$ Kit$^+$ Splenic Cells. (A) (left) Representative flow cytometric plot of Ki-67 and 4',6-diamidino-2-phenylindole (DAPI) staining of WT and Samd14-Enh$^{-/-}$ PHZ-treated spleen. Gate represents cells in G2/S phase, (2N chromosomal DNA content and Ki67$^+$) (right) Quantitation of G2/S cells within the 4 cell populations in the spleen. Cells were bead-sorted to isolate Ter119$^+$ and Ter119$^-$ prior to analysis (n = 3). (B) (left) Representative flow cytometric plot of AnnexinV and DRAQ7^{TM} staining of WT and Samd14-Enh$^{-/-}$ spleen. (right) Quantitation of percentages of live (DRAQ7$^-$AnnexinV$^-$), early apoptotic (DRAQ7$^-$ AnnexinV$^+$), late apoptotic (DRAQ7$^+$AnnexinV$^+$), and dead (DRAQ7$^+$AnnexinV$^-$) cells within the 4 cell populations, distinguished by CD71 and Ter119 staining (n = 3). (C) Representative flow cytometric gating strategy for analysis of apoptosis in Ter119$^-$ CD71$^+$Kit$^+$ cells in WT and Samd14-Enh$^{-/-}$ spleens from vehicle or PHZ-treated mice. (D) Quantitation of percentages of live (DRAQ7$^-$AnnexinV$^-$), early apoptotic (DRAQ7$^-$ AnnexinV$^+$), late apoptotic (DRAQ7$^+$AnnexinV$^+$), and dead (DRAQ7$^+$AnnexinV$^-$) cells within the three c-Kit$^+$ cell populations in the spleen, distinguished by CD71 and Ter119 staining (n = 3). (Related to Figure 4).

Figure S5. Samd14-Enh Is Not Required for SCF/c-Kit Signaling in Bone Marrow. (A) Flow cytometric analysis of Ter119-depleted WT and Samd14-Enh$^{-/-}$ bone marrow cells stained with CD71 and c-Kit. (B) Representative histograms of pAKT and pERK in

$CD71^{+}Kit^{+}$ WT control and PHZ-treated (top) and Samd14-$Enh^{-/-}$ (bottom) treated with vehicle or SCF. Histograms were normalized to percentage of maximum cell number. (C) Quantitation of pAKT and pERK MFI in WT and Samd14-$Enh^{-/-}$ bone marrow cells (n = 3). (Related to Figure 4).

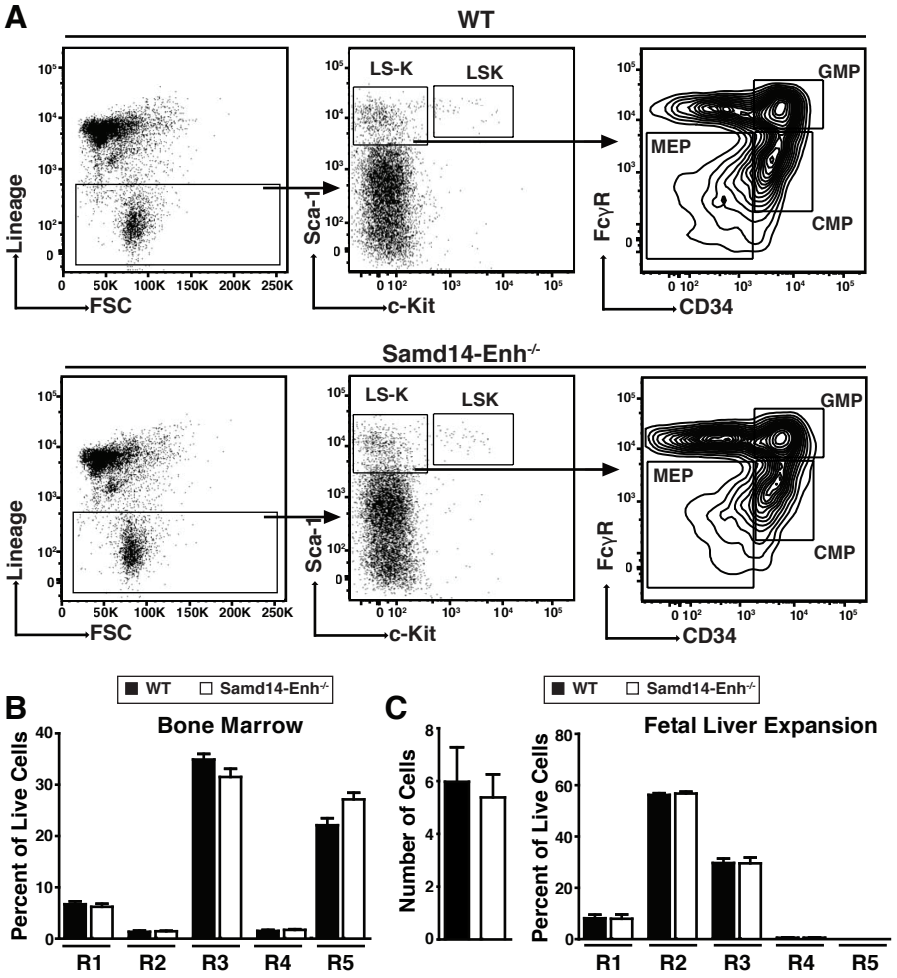
Figure S6. Occupancy of Components of an E-box-GATA Multiprotein Complex at Anemia-Sensing Enhancers.

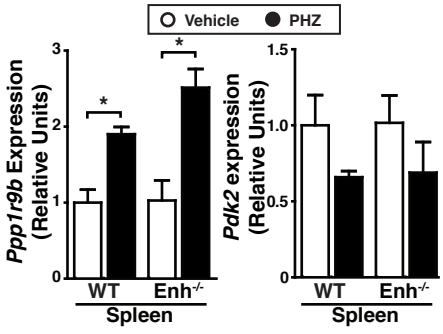
(A) Relative mRNA levels of *Ldb1* and *Lmo2* in FACS-purified $CD71^{+}Ter119^{-}Kit^{+}$ splenic cells from WT control and PHZ-treated mice (n = 3). (B) ChIP-seq of GATA-2 (GSE29193) occupancy in mouse G1E proerythroblast cells (Trompouki et al., 2011), GATA-2 (GSM641911), Scl/TAL1 (GSM641910), Ldb1 (GSM641909) in mouse Lin- hematopoietic progenitors (Li et al., 2011), and Lmo2 (GSM552237) in HPC-7 cells (Wilson et al., 2010) at indicated sites containing E-box-GATA composite elements (Related to Figure 6).

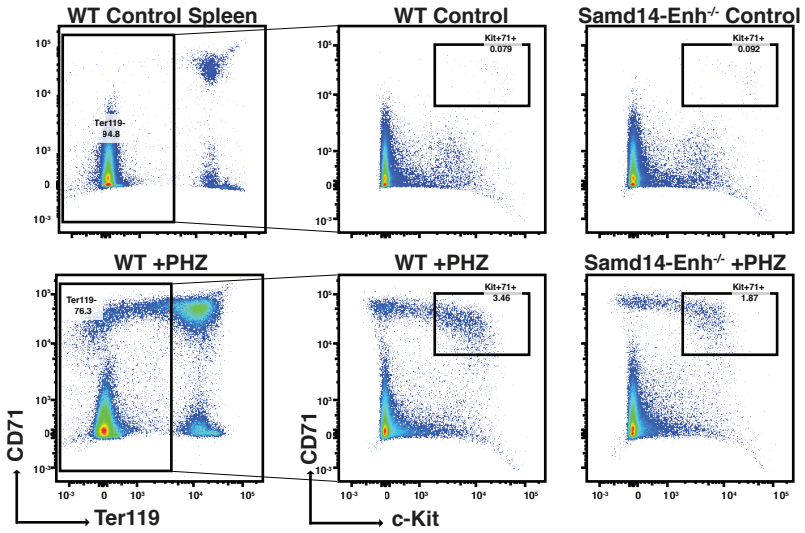
Figure S7. Epo Stimulates Expression at Anemia-Regulated Enhancer Loci. (A) Transcripts Per Million (TPM) at +9.5-like loci in G1E proerythroblast cells (GEO:GSE74371) (Tanimura et al., 2015) (n = 3). (B) Relative mRNA expression at loci containing +9.5-like elements in WT PHZ-treated splenic cells treated with Epo (2U/mL) for 0, 2 or 4 hours (n = 6). Statistical significance represented by mean +/- SEM.; **p<0.01, ***p<0.001. (Related to Figure 6).

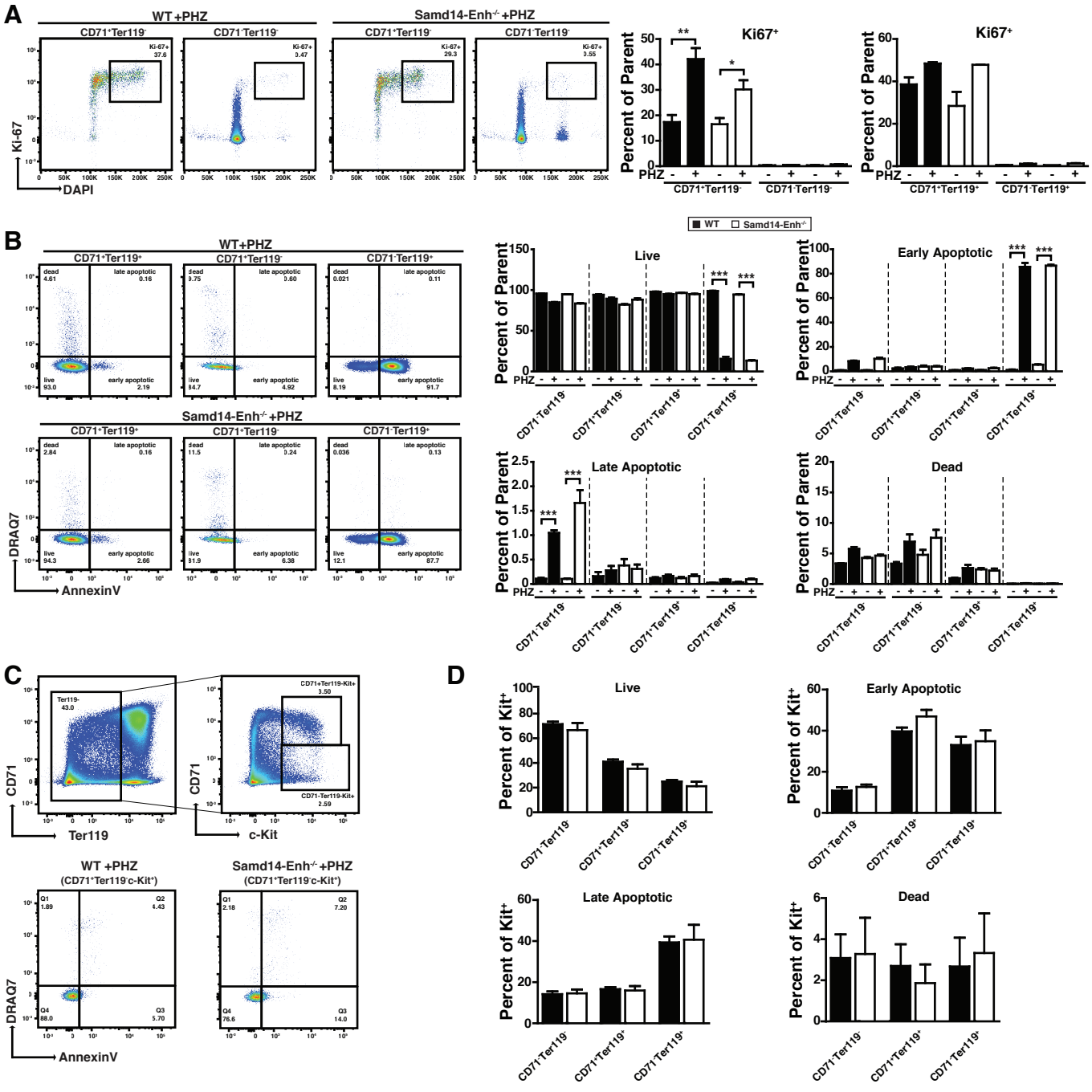
Table S1. Complete Blood Count Hematologic Parameters of WT and Samd14-Enh$^{-/-}$ Animals. Hematologic parameters (white blood cells (WBC), neutrophils (NE), lymphocytes (LY), monocytes (MO), eosinophils (EO), basophils (BA), red blood cells (RBC), hemoglobin (Hb), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular height (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDW), platelets (PLT) and mean platelet volume (MPV)) from WT (n = 8) and Samd14-Enh$^{-/-}$ (n = 10) mice (Related to Figure 1).

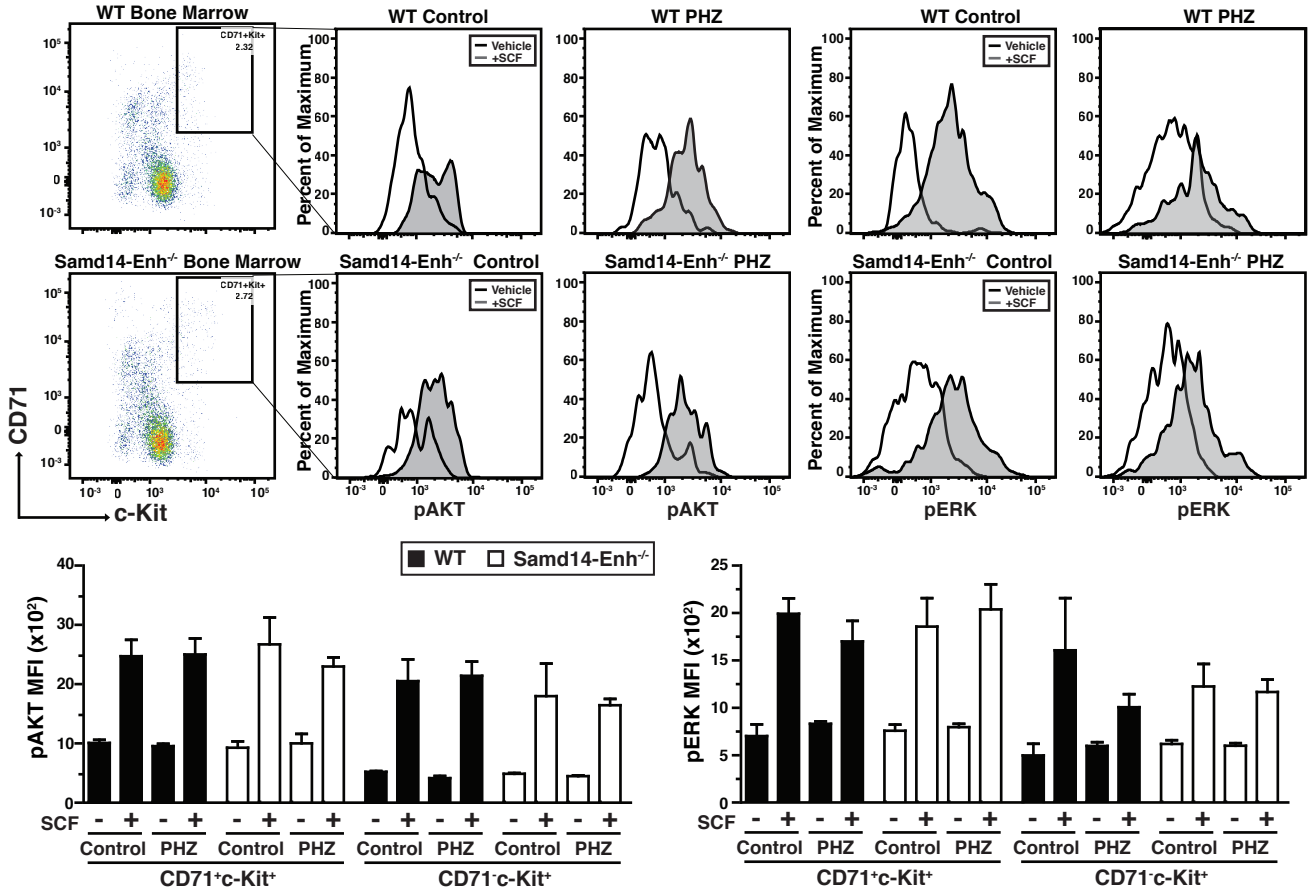
Table S2. List of qRT-PCR Primers Used. (Related to STAR Methods and the Key Resources Table)

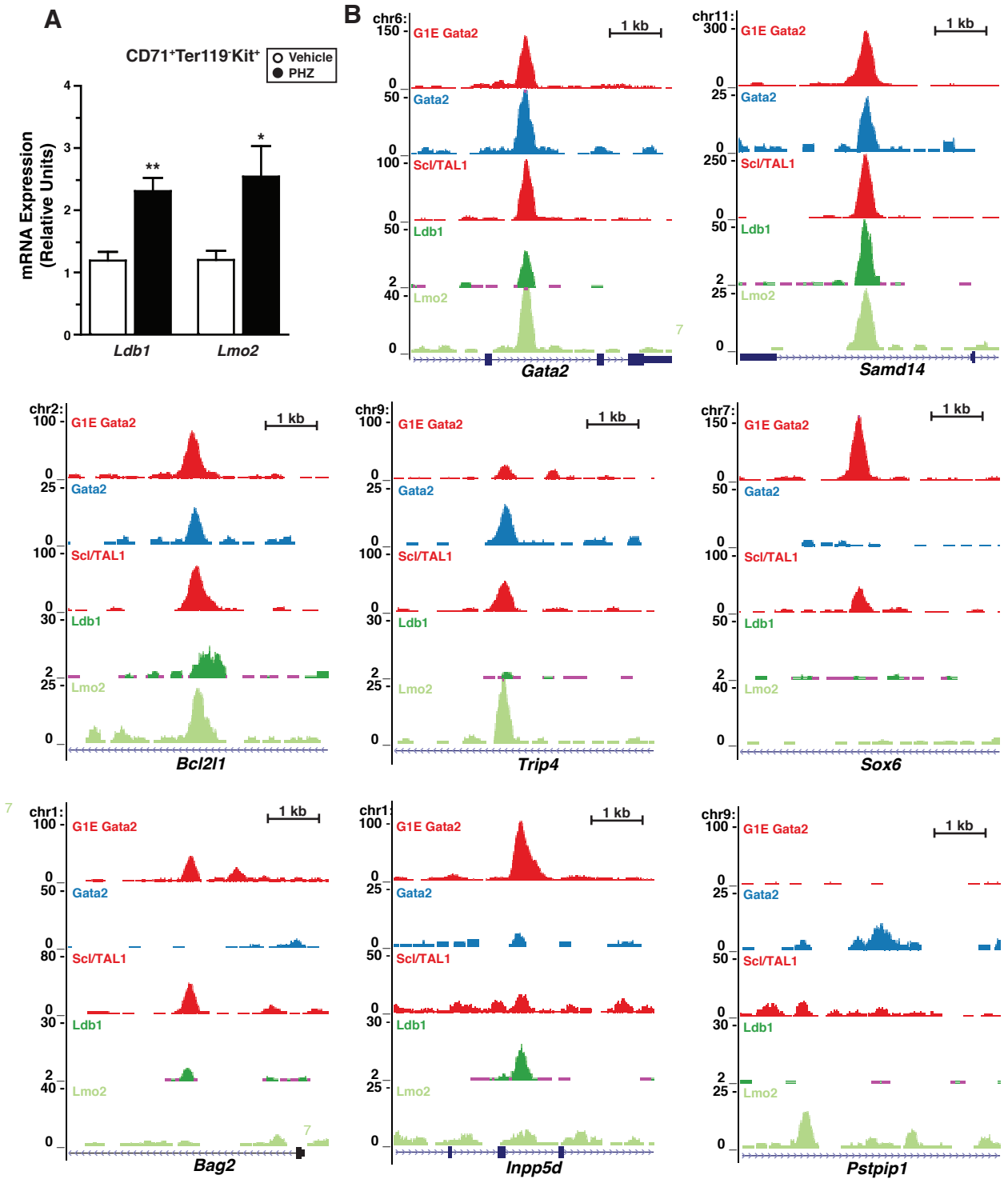

A
WT
Lineage
FSC
LS-K
LSK
Sca-1
c-Kit
GMP
MEP
CMP
FcγR
CD34
Samd14-Enh-/-
B
WT
Samd14-Enh-/-
Bone Marrow
Percent of Live Cells
R1
R2
R3
R4
R5
C
Number of Cells
Fetal Liver Expansion
Percent of Live Cells

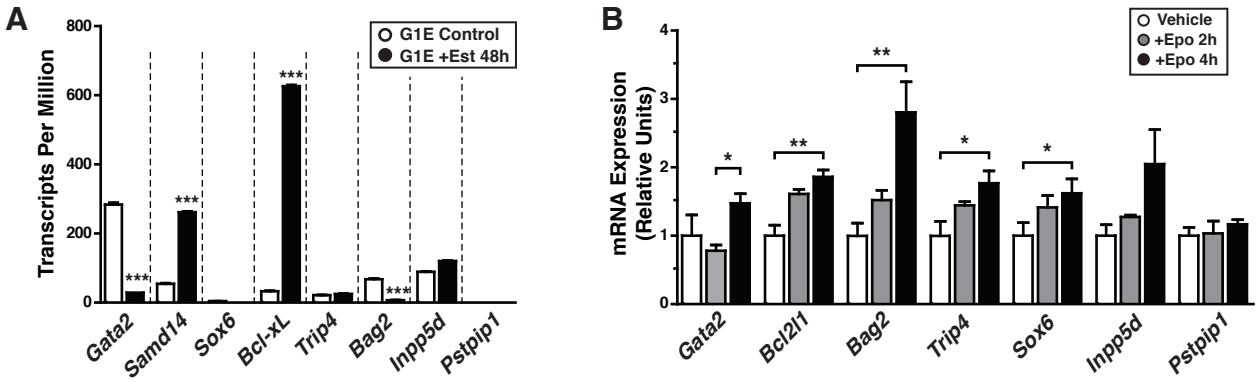

Vehicle
PHZ
*
*
Ppp1r9b Expression
(Relative Units)
3
2
1
0
WT
Enh-/-
Spleen
Pdk2 expression
(Relative Units)
1.5
1.0
0.5
0
WT
Enh-/-
Spleen


WT Control Spleen
WT Control
Samd14-Enh-/- Control
Ter119-
94.8
Kit+71+
0.079
Kit+71+
0.092
WT +PHZ
WT +PHZ
Samd14-Enh-/- +PHZ
Ter119-
76.3
Kit+71+
3.46
Kit+71+
1.87
CD71
Ter119
CD71
c-Kit


A
WT +PHZ
Samd14-Enh-/- +PHZ
CD71+Ter119-
CD71-Ter119-
Ki-67
DAPI
Ki67+
Percent of Parent
PHZ
CD71+Ter119+
CD71-Ter119+
B
WT+PHZ
Samd14-Enh-/-+PHZ
DRAQ7
AnnexinV
dead
late apoptotic
live
early apoptotic
WT
Samd14-Enh-/-
Live
Early Apoptotic
Late Apoptotic
Dead
C
CD71
Ter119
c-Kit
WT +PHZ
(CD71+Ter119-c-Kit+)
Samd14-Enh-/- +PHZ
D
Percent of Kit+


WT Bone Marrow
CD71+Kit+
2.32
WT Control
WT PHZ
WT Control
WT PHZ
Vehicle
+SCF
Percent of Maximum
Samd14-Enh-/- Bone Marrow
CD71+Kit+
2.72
Samd14-Enh-/- Control
Samd14-Enh-/- PHZ
Samd14-Enh-/- Control
Samd14-Enh-/- PHZ
CD71
c-Kit
pAKT
pERK
WT
Samd14-Enh-/-
pAKT MFI (x10²)
pERK MFI (x10²)
SCF
Control
PHZ
CD71+c-Kit+
CD71-c-Kit+


A
CD71+Ter119-Kit+
Vehicle
PHZ
mRNA Expression (Relative Units)
Ldb1
Lmo2
B
G1E Gata2
Gata2
Scl/TAL1
Ldb1
Lmo2
1 kb
Gata2
Samd14
Bcl2l1
Trip4
Sox6
Bag2
Inpp5d
Pstpip1


A
Transcripts Per Million
800
600
400
200
0
G1E Control
G1E +Est 48h
***
***
***
***
Gata2
Samd14
Sox6
Bcl-xL
Trip4
Bag2
Inpp5d
Pstpip1
B
mRNA Expression (Relative Units)
4
3
2
1
0
Vehicle
+Epo 2h
+Epo 4h
*
**
**
*
*
Gata2
Bcl2l1
Bag2
Trip4
Sox6
Inpp5d
Pstpip1

	WT (n=8)	Samd14-Enh$^{-/-}$ (n=10)	P-value
WBC	11.94 ± 1.053	10.17 ± 1.414	0.38
NE	2.376 ± 0.330	2.139 ± 0.450	0.70
LY	8.846 ± 0.633	7.520 ± 0.950	0.32
MO	0.37 ± 0.075	0.2661 ± .28	0.17
EO	0.254 ± 0.098	0.2 ± 0.067	0.64
BA	0.1075 ± 0.041	0.05833 ± 0.026	0.31
RBC	8.696 ± 0.133	7.11 ± 0.748	0.09
Hb	12.4 ± 0.200	10.25 ± 1.052	0.13
HCT	43.2 ± 0.766	35.1 ± 3.862	0.09
MCV	49.66 ± 0.157	49.57 ± 0.584	0.90
MCH	14.26 ± 0.081	14.4 ± 0.191	0.57
MCHC	28.72 ± 0.198	29.09 ± 0.161	0.18
RDW	20.02 ± 0.097	20.07 ± 0.400	0.92
PLT	832.8 ± 33.22	753.7 ± 78.250	0.44
MPV	4.82 ± 0.102	4.771 ± 0.078	0.71

Target	Forward	Reverse
Samd14-Enh genotyping	CAGTCTGAAGGGAGGGCAC	GTCTAAAGCTACTCCAATTCTGAG
18s rRNA	CGCCGCTAGAGGTGAAATTCT	CGAACCTCCGACTTTCGTTCT
Samd14 mRNA ex4/5	GTCGTTACCCTCGAGCAGAG	ACCCCCAGGTCCAGGAATTT
Samd14_ex1/3	GACGGATGCTGGAGCTCTCT	CTGGTGGTCTCTGGTACAGC
Samd14_exon6/7	GCAGCAGGGAGTCTGTAGAAG	TCCGTGAACCAGGAAAAGGG
Samd14_intron10	CACTTTCTTGGCCAGCTCCA	GTCCACTTAGCACGCCAGAG
Samd14-Mut allele	CTGAAGGGAGGGCACATAG	GTCTAAAGCTACTCCAATTCTGAG
Samd14-WT allele	GATAAAACCCACATAGGAAACC	GTCTAAAGCTACTCCAATTCTGAG
Ptpn11 mRNA	AGTGGAGAGAGGGAAGAGCA	GCCAGACGGTTCTCTCTGTG
Rab1 mRNA	TGTCCAGCATGAATCCCGAA	AAGGCAGGACTTTCCAACCC
Bcl2l1 mRNA	GACAAGGAGATGCAGGTATTGG	TCCCGTAGAGATCCACAAAAGT
Tal1 mRNA	CGAGCGCTGCTCTATAGCCTT	TCACCCGGTTGTTGTTGGT
Gata2 mRNA	GCAGAGAAGCAAGGCTCGC	CAGTTGACACACTCCCGGC
Gata1 mRNA	GGCCCAAGAAGCGAATGATT	GGTTCACCTGATGGAGCTTGA
Kit mRNA	AGCAATGGCCTCACGAGTTCTA	CCAGGAAAAGTTTGGCAGGAT
Gata2 +9.5 site	GACATCTGCAGCCGGTAGATAAG	CATTATTTGCAGAGTGGAGGGTATTAG
Gata2 -77 site	CTTTACCACATCAGGATACAGAGCA	CACCGCACAGCAGTGATAGATAGT
Trip4 mRNA	GGCAAAAGACCTTCCCCTCA	TGGAATCCAGTTTCCCTGCTC
SOX6 mRNA	GGAAAGTCAAGTGAAGATGGAAAACT	GTTTAGCTGCAGAGCCATTCATT
Bag2 mRNA	CTTCTGCCGCTCGTCTTCCAT	TCTTTCTCCATCGCTAATCTGCC
Inpp5d mRNA	GGAGTGTCCGTCCTGGGAG	AATTCCGGAACAGCACGCAG
Pstpip1 mRNA	CAGGAAGATGTGCAAGGATGTG	TTCATCTCTGTCTGGCCACCAG
Samd14-Enh-450bp	GTGTGAGCCAAAGAGGGAAGA	TGCCCCTGTCACTGAACTCT
Samd14-Enh-200bp	CCTTAGTGCACAGGCTCTCA	TCTCCTATCAGAGTGGCTGCT
Samd14-Enh+200bp	ATGTTGAGGACCGACCTACC	AGGGACCCCTTTGCTGTTTT
Samd14-Enh+450bp	TCCAAGCATCCCTCTAGCCT	GCTGCTCATGAGGAAAGGGT
Sox6 Intron 2	CCAGCTCCCCATACACGTC	GCAGTCCCGACTTTCGTCTAA
Bag2 Intron 2	CGTGTTCCTAGGCGAAGTCC	GTGTCTCTGTGGGAGTTACCG
Bcl2l1 Intron 1	CTGAGGTCTCCCATTTCCTC	CATCTGTAAAGTGAAGATAATGG
Trip4 Intron 11	CCACAGCCTTGATGCATTGT	TCTGCCAGCAGCAGATGTAA
Inpp5d +92kb	TTTCTCCATCTGCCAAGTAGA	TCATCTCATTTATCTCTCACAACCG
Pstpip1 Intron 1	AGTTTGGTTCGGGTCTCAGC	CAAGGCACCAGGGCTTTACT