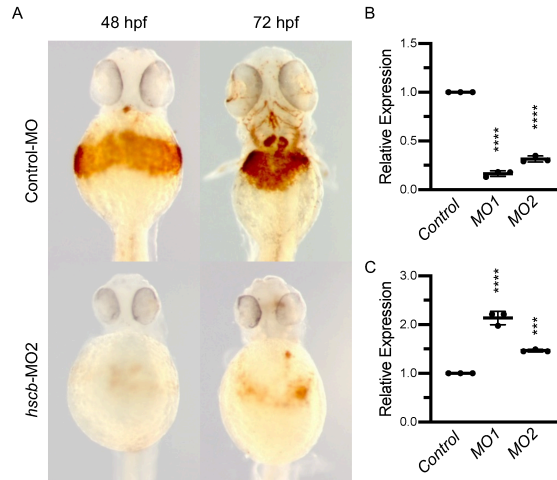
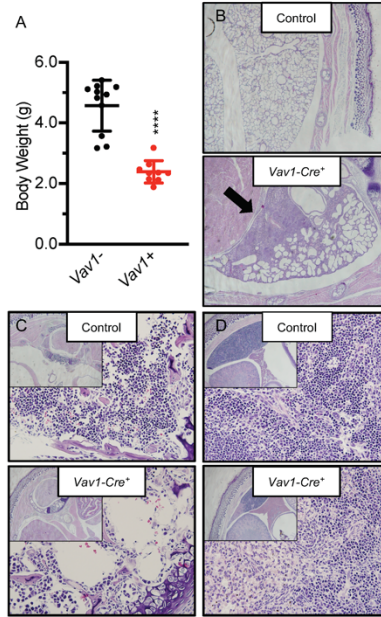


Supplemental Figure 1. HSCB depletion in K562 decreases Fe-S protein expression and impairs respiratory capacity. **A.** Diagram of K562 shRNA experimental model (inf: infection with shHSCB or shSCR; 0-15 days). **B.** Representative western blots of HSCB protein expression in K562 cells infected with various shHSCB (09, 28, 34 [sh1], 40, 87 [sh2]). **C.** Cells as in (B) blotted for mitochondrial respiratory proteins. **D.** Time course of fetal hemoglobin (HbF) accumulation in K562 cells 0-96 hrs following induction with sodium butyrate (NaB). **E.** FECH expression peaks at 24 hrs post-NaB induction, while IRP expression is stable. ACO2, aconitase 2; ACT, actin; ATP5A, ATP synthase F1 subunit alpha; CS, citrate synthase; COXII, mitochondrially encoded cytochrome C oxidase II; CS, citrate synthase (mitochondrial protein control); FECH, ferrochelatase; FTH, ferritin heavy chain FTL, ferritin light chain; HbF; hemoglobin F; HSCB, heat shock cognate B; HSC70, heat shock cognate protein 70 kDa; HSPA9, heat shock protein family A (Hsp70) member 9; IRP, iron regulatory protein; NDUFB8, NADH:ubiquinone oxidoreductase subunit B8; NI, non-infected; OXPHOS, oxidative phosphorylation; RC, respiratory complex; SC, scrambled; SDHB succinate dehydrogenase B subunit; sh; short hairpin RNA; UQCRC2, ubiquinol-cytochrome C reductase core protein 2.

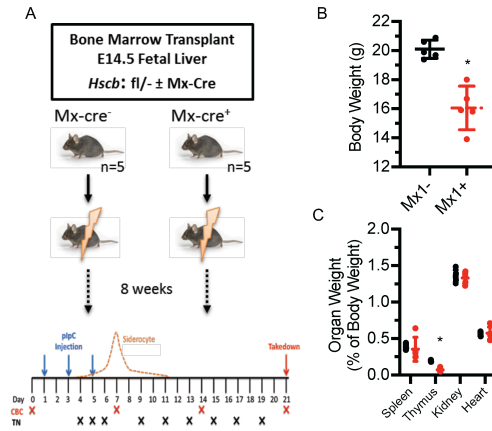


Supplemental Figure 2. HSCB depletion in zebrafish embryos impairs hemoglobinization.

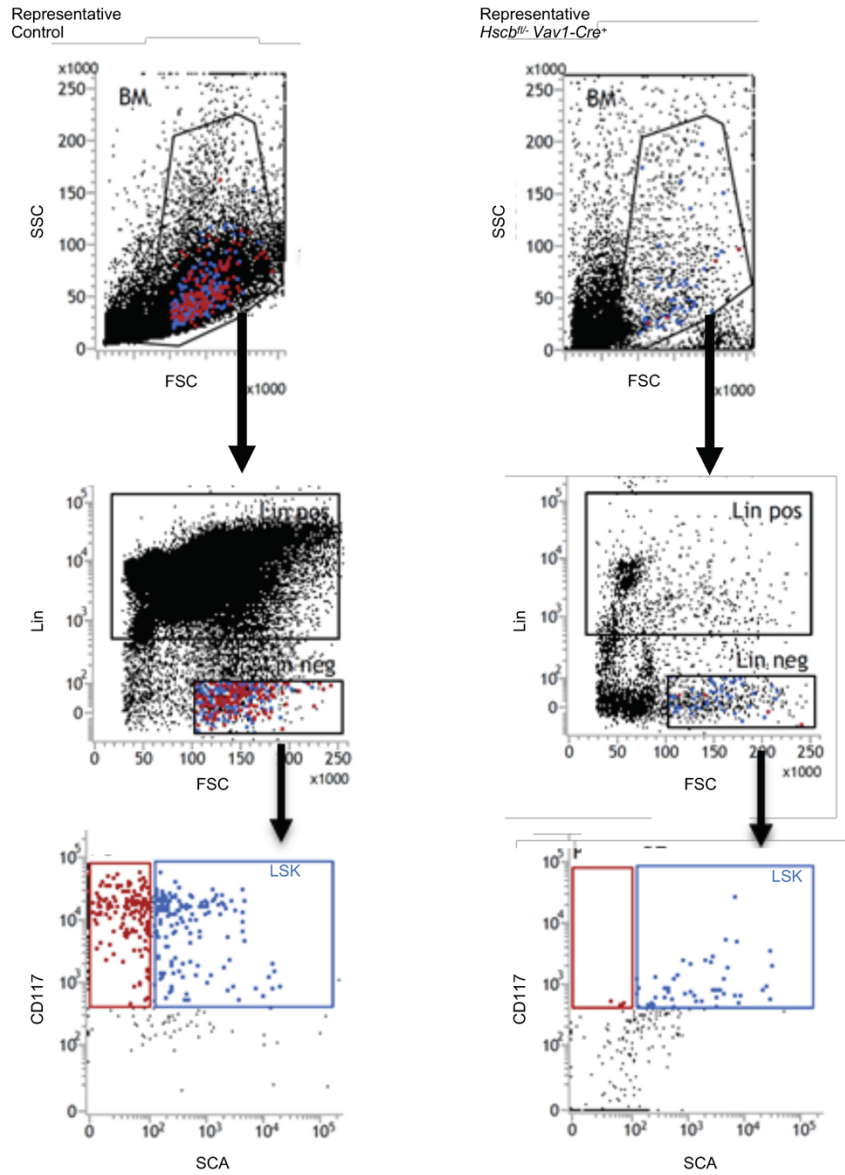
A. O-dianisidine staining in *hscb* morpholino (*hscb*-MO2)-treated embryos demonstrates reduced hemoglobinization compared to control embryos at 48- and 72-hrs post-fertilization (hpf). Control embryos are the same as in Figure 4B. **B.** *hscb* mRNA is decreased ~85% and 75% in *hscb*-MO1 and -MO2 treated embryos, respectively. **C.** *tfr1* mRNA is upregulated in *hscb*-MO1 and -MO2 treated embryos. Data are \pm S.D. *** $P < 0.001$, **** $P < 0.0001$.



Supplemental Figure 3. *Hscb* deletion in hematopoietic lineages by *Vav1-Cre* results in pancytopenia, growth failure and lethality. A. P7 *Hscb-Vav1*⁺ animals are runted compared to their littermates. t test ****P<0.0001. **B.** *Hscb-Vav1*⁺ animals typically die of pneumonia. Alveoli packed with bacteria, lacking an inflammatory infiltrate are indicated by the arrow. **C.** Vertebral marrow space and **D.** Spleen are depleted of hematopoietic cells in the *Hscb-Vav1*⁺ animals.



Supplemental Figure 4. *Hscb* deletion in hematopoietic lineages by *Mx1-Cre* in the adult animal results in pancytopenia and lethality. A. Schema of *Hscb* *Mx1-Cre* transplant experiments. **B.** Weight loss observed in *Hscb* *Mx1-Cre*⁺ stem cell recipients. **C.** Organ weights normalized to body weight showing thymic atrophy in *Hscb* *Mx1-Cre*⁺ stem cell recipients. t test *P<0.05, ****P<0.0001



Supplemental Figure 5. Flow cytometry gating strategy for lineage negative, Sca1⁺, Kit⁺ (Lin⁻Sca⁺Kit⁺).

Supplemental Table 1. *HSCB* genomic DNA primers

Forward primer	Sequence	Reverse primer	Sequence
HSCB ex1F	TGCCTCCTGGGAGTTGTAGT	HSCB ex1R	AGATCCGTCCTCCCCTCTC
HSCB ex2F	CCCCATAAGAGCAAGAACCA	HSCB ex2R	AAGGCTACTGCCACCCAGT
HSCB ex3F	AGCCTGGATGACTCAACGTC	HSCB ex3R	TGGCTGTCCTACGCCATAAT
HSCB ex4F	CATTACTCTGCCATAGCTTCTT	HSCB ex4R	CCCGGGCCATTATATACTCT
HSCB ex5F	TCCTTTGTTGTGTTCAAACCAG	HSCB ex5R	TGGCTATGCTTCAGTGCTTG
HSCB ex6F	ATCGCTGCCCTAGTCATCAG	HSCB ex6R	CCCAATTACCAGAAGCCAGA
HSCB pro1F	TTCCAGAGGTCTTTCAAGTGAA	HSCB pro1R	GTTTAGCGCCACTCTGCTG
HSCB pro2F	TTCCCATATGACTCACCGC	HSCB pro2R	ATCAGTCCCGCCTTCTCTTC
HSCB pro3F	TATGGACTGGTACGAGCGC	HSCB pro3R	CCTTCATCCCTGTCTCTCCC
HSCB pro4F	TGGTAAGGAAATAGGACAGACTC	HSCB pro4R	AGTGACTGGTGGGGAAGATC

Supplemental Table 2. Primary antibodies

Name	Protein	Supplier	Reference
ACO2	Mitochondrial aconitase	Abcam	ab129069
ACT	Actin	Cell Signaling	13E5
ATP5A*	Mitochondrial membrane ATP synthase (F(1)F(0) ATP synthase	Abcam	MS601/ab110411
COX2*	Cyclooxygenase 2	Abcam	MS601/ab110411
CS	Citrate synthase	Cell Signaling	D7V8B
FECH	Ferrochelatase	Proteintech	14466-1-AP
FTH	Ferritin subunit H	Cell Signaling	4393S
FTL	Ferritin subunit L	Sigma	HPA041602
HBF	Hemoglobin fetal	Abcam	ab137096
HSC70	Heat shock cognate 71 kDa protein	Santa Cruz	sc-7298
HSCB	HSC20/heat shock cognate B	Origene	TA507274
HSPA9	GRP75, heat shock protein A9	Abcam	ab2799
IMMT	Inner Membrane Mitochondrial Protein/ Mitofilin	Abcam	ab137057
IRP1/ACO1	Iron regulatory protein 1	Cell Signaling	20272
IRP2	Iron regulatory protein 2	Cell Signaling	37135
Lipoate	Lipoic acid	Abcam	ab58724
MTCYB	Mitochondrially Encoded Cytochrome B	Abcam	ab81215
NDUFB8	NADH:Ubiquinone Oxidoreductase Subunit B8	Abcam	ab110242
SDHA	Succinate Dehydrogenase Complex Flavoprotein Subunit A	Abcam	ab14715
SDHB	Succinate Dehydrogenase Complex Iron Sulfur Subunit B	Abcam	ab175225
tOXPHOS	Human total OXPHOS	Abcam	MS601/ab110411
UQCRC2	Ubiquinol-Cytochrome C Reductase Core Protein 2	Abcam	ab14745

Supplemental Table 3. Secondary antibodies

Supplier	Name	Reference
GE Healthcare	Ecl Anti Mouse	NA931V
GE Healthcare	Ecl Anti Rabbit	NA9340V