Li et al. Elavl1a regulates zebrafish erythropoiesis via post-transcriptional control of *gata1*

Supplemental Figures

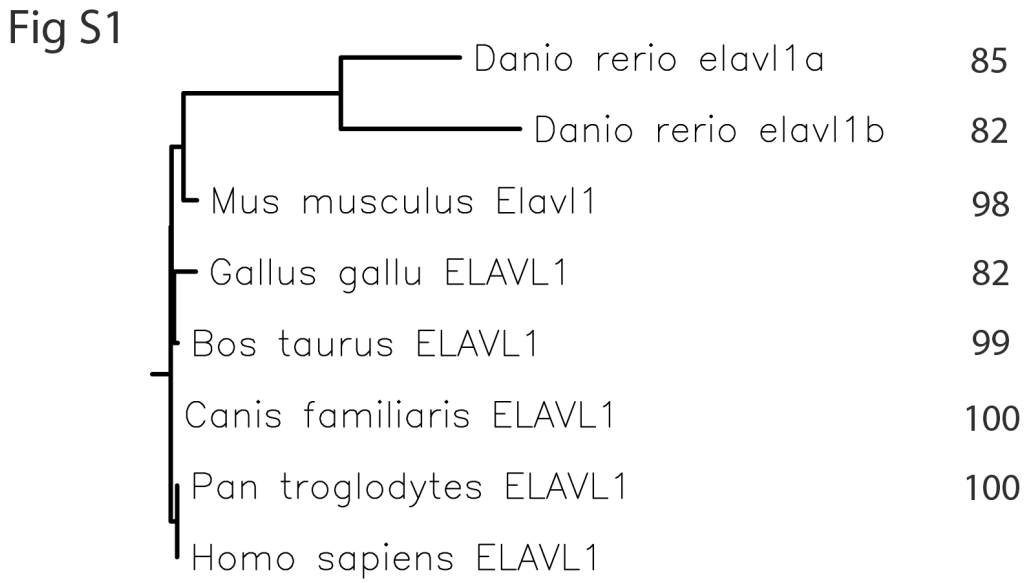
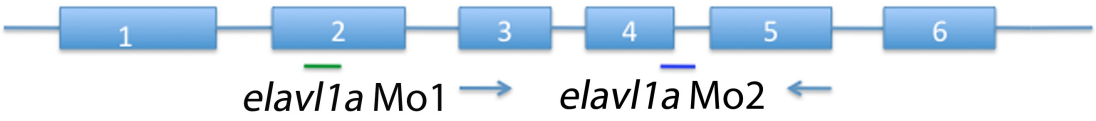


Fig. S1. Conservation of ELAVL1 genes. Homologous gene sequences from NCBI were analyzed by Clustal software through the website: http://workbench.sdsc.edu/. The rooted evolutionary tree reveals that zebrafish Elavl1a and Elavl1b are homologues of human ELAVL1. The percent similarity identified by each protein alignment relative to the human protein is indicated.

Fig S2

A



B

	Human ELAVL1	Zebrafish elavl1a	Zebrafish elavl1b
Chromosome	19 -	2 -	11 +
Genome size	47073bp	9688bp	45707bp
mRNA size	6075bp	2131bp	1759bp
Amino acid	326	324	322
Exon number	6	6	8
Starting codon in exon	2 17th bp	2 13th bp	4 16th bp
Exon 1 size	151bp	37bp	63bp
Intron 1 size	13664bp	4141bp	608bp
Exon 2 size	188bp	178bp	66bp
Intron 2 size	10462bp	451bp	11208bp
Exon 3 size	104bp	104bp	63bp
Intron 3 size	7205bp	463bp	115bp
Exon 4 size	154bp	154bp	181bp
Intron 4 size	5935bp	91bp	31141bp
Exon 5 size	226bp	226bp	104bp
Intron 5 size	3759bp	2419bp	623bp
Exon 6	5235bp	1431bp	154bp
Intron 6			111bp
Exon 7			226bp
Intron 7			155bp
Exon 8			903bp

Fig. S2. The elavl1a gene structure is similar to human ELAVL1. A) Schematic of the *elavl1a* gene structure showing the relative position of exons 1-6 (boxes) and translational (Mo1) and splice blocking (Mo2) morpholinos. Arrows indicate PCR primers used in Fig. 1. B) Comparison of human ELAVL1 with zebrafish *elavl1a* and *elavl1b* gene structures.

Fig S3

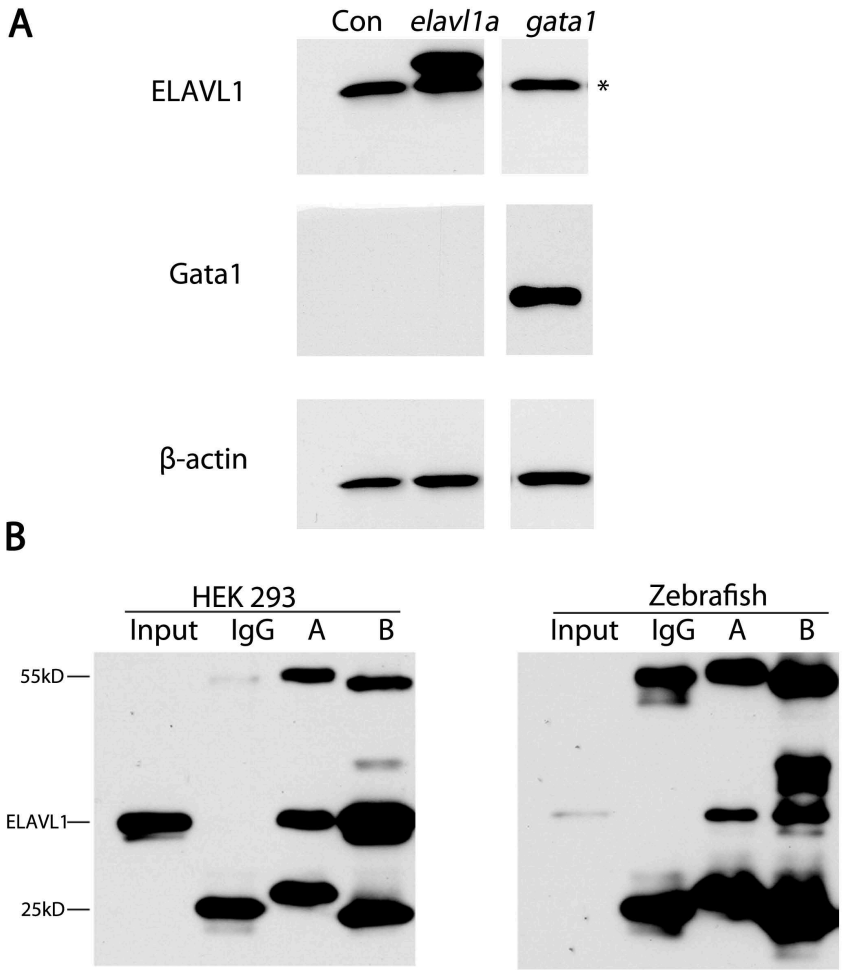


Fig. S3. Recognition of zebrafish Elavl1a and Gata1 protein by anti-human ELAVL1 and GATA1 antibodies. A) Zebrafish *elavl1a* or *gata1* expression constructs were transfected into HEK293 cells, and lysates were probed by western blotting using ELAVL1 monoclonal antibody or GATA1 polyclonal antibody. * indicates the endogenous ELAVL1 protein in HEK293 cells. B) Two distinct ELAVL1 antibodies (A and B) precipitate endogenous human ELAVL1 (left panel) and zebrafish Elavl1 (right panel). Lysates were derived from HEK293 cells (left) or 24 hpf zebrafish embryos (right). Antibody A is from Clonegene, B from Santa Cruz. 55 kD indicates the position of the IgG heavy chain and 25 kD the position of the IgG light chain.

Fig. S4

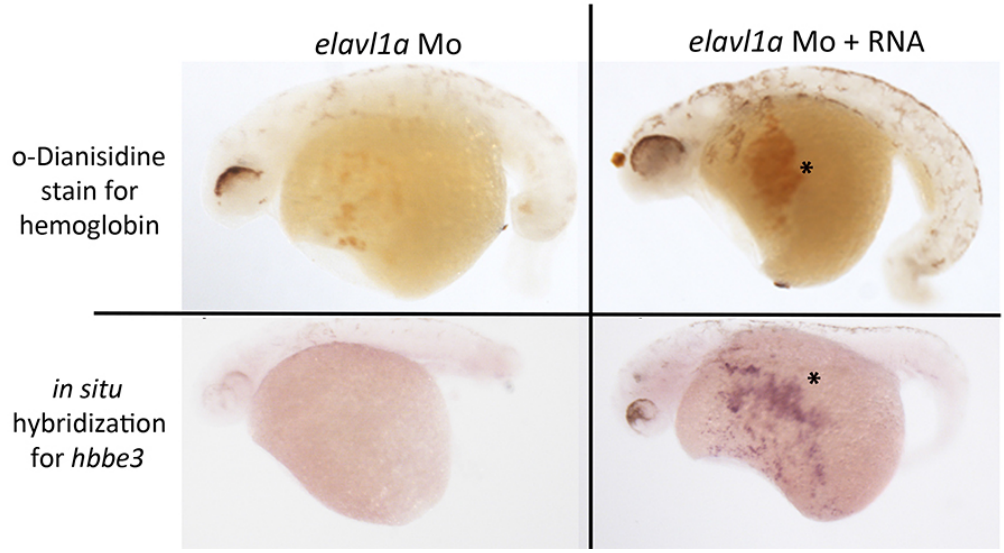


Fig. S4. Co-expression of Elavl1a can partially rescue the erythropoiesis defect in *elval1a* morphants. Shown are representative *elavl1a* morphants (left panels) or embryos that had been co-injected with 75 pg of *in vitro* generated mRNA encoding murine Elavl1 (right panels). Embryos were stained for hemoglobin (top panels) or for embryonic globin transcripts (bottom panels). Essentially 100% of the morphant embryos failed to stain for either, while the images shown on the right are representative of 56/101 (55%) and 98/118 (83%) co-injected embryos stained for o-Dianisidine or *hbbe3* RNA, respectively.

Fig. S5

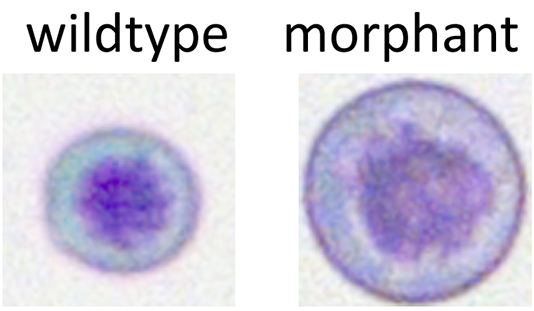


Fig. S5. The few red blood cells that form in the elavl1a morphant appear immature. Shown are representative images of the majority of red cells isolated at 48 hpf from control injected (wildtype) or morphant embryos. Cells were isolated by flow cytometry from whole dissociated *gata1:dsRed* transgenic embryos, followed by cytospin and staining with Geimsa. Magnification is 100x.

Fig. S6

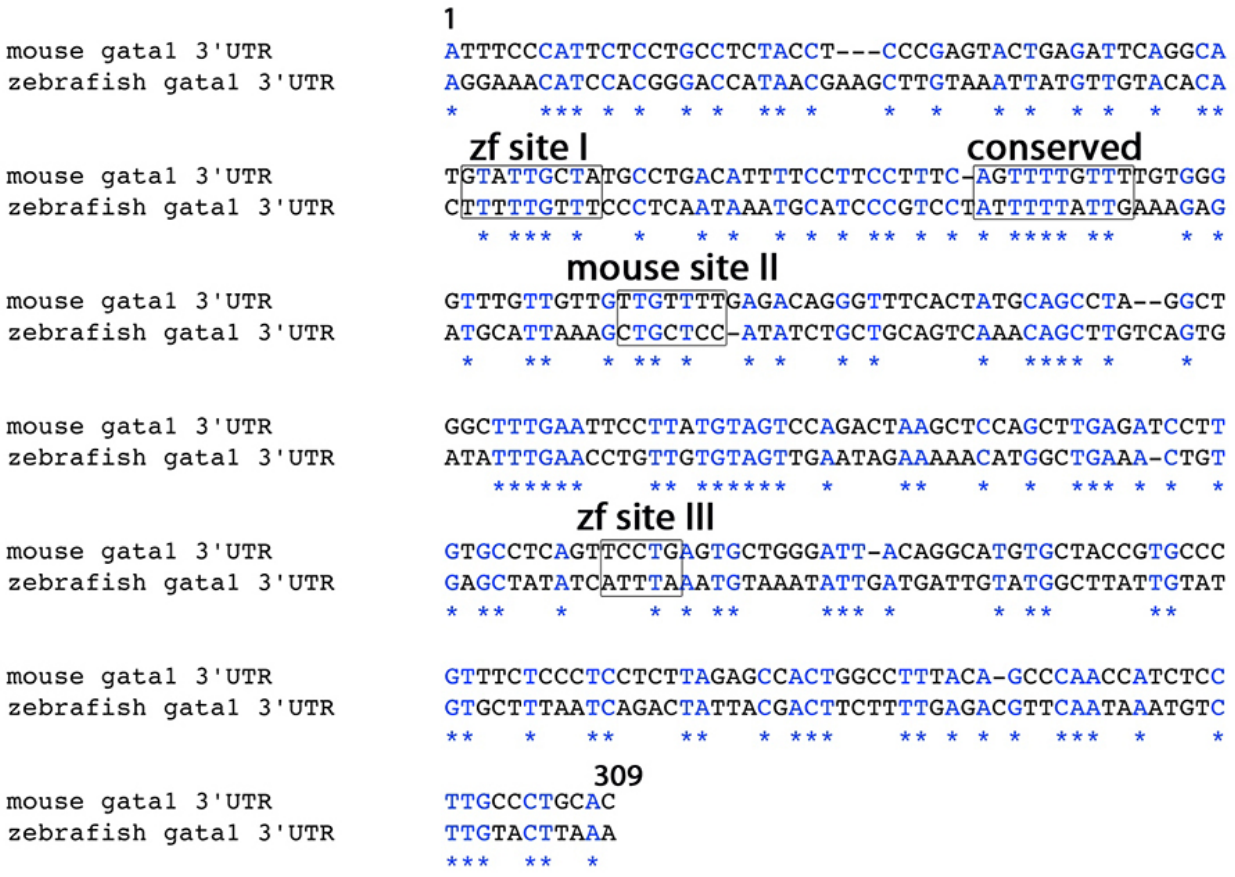


Fig. S6. Comparison of zebrafish *gata1* and mouse *Gata1* 3'-UTR sequences. The predicted Elavl1 binding sites are boxed and indicated. The zebrafish site II is conserved with murine site I. For reporter assays, zebrafish site I (TTTTTGTTT, 52-60) was mutated to GCGCCGGCG, site II (ATTTTTA, 85-91) was mutated to GCGCGCG, site III (ATTTA, 209-213) was mutated to GCGCG.