

(A) Schematic diagram showing *gata2b* gene structure and sgRNA target site near the translation start site on the antisense strand. (B-F) Generation of the *gata2b* mutant line. Mutations at the *gata2b* locus were analyzed by restriction enzyme digestion (B) and DNA sequencing (C and D), and this deletion resulted in premature translation termination (E). The *gata2b* mRNA level was significantly down-regulated in the mutant (F). Deletions are indicated by dashes. The numbers in brackets represent the bases deleted/inserted from each allele. the frameshift-altered amino acid sequence for the mutated *gata2b* is indicated in red. M, marker; PAM, protospacer adjacent motif.

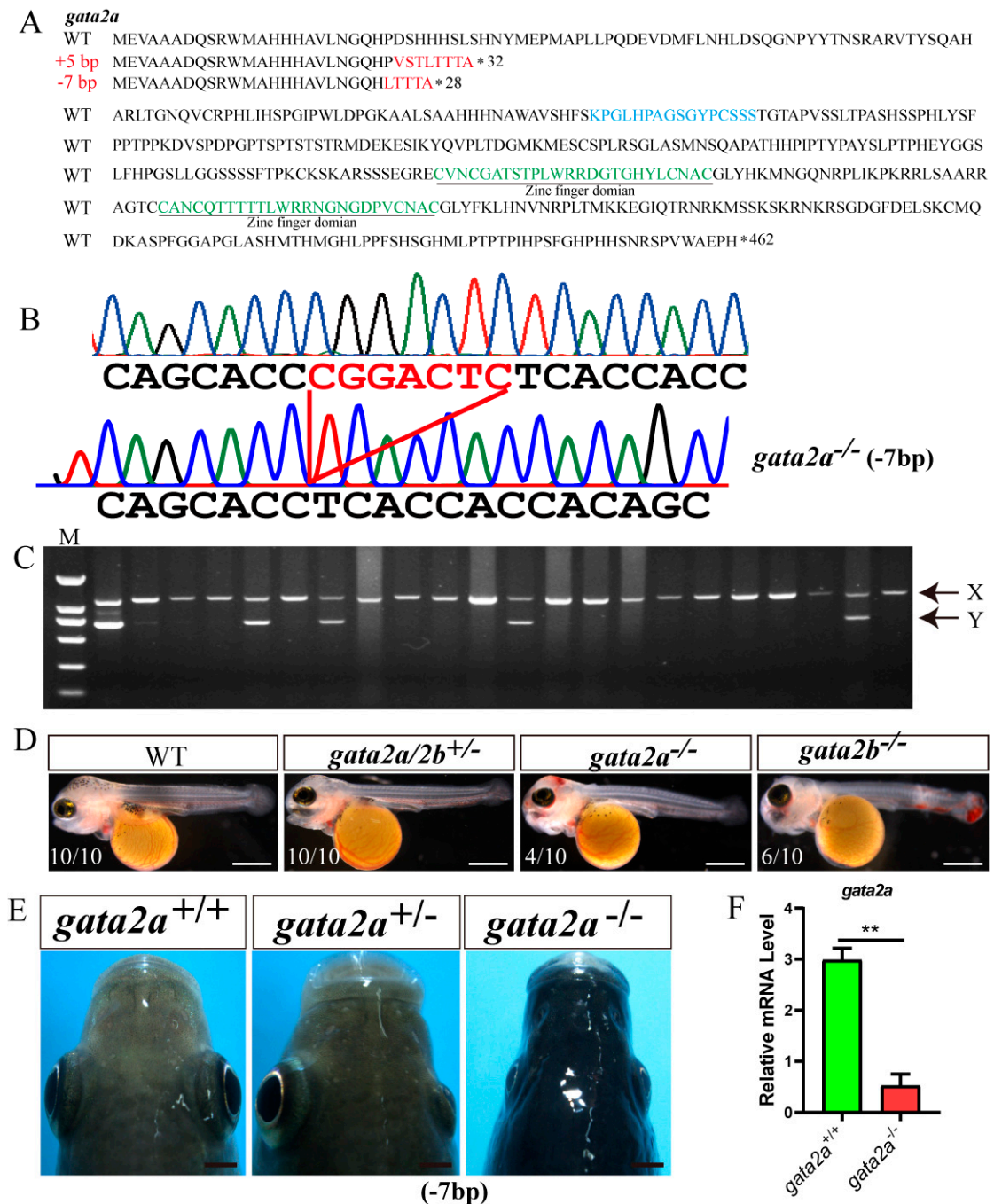


Figure S3 Homozygous mutants of *gata2a* with 7-bp deletion

(A) Amino acid sequences of WT and frameshift-altered *gata2a* mutants. (B) Sequencing results of *gata2a* gene from WT and homozygous mutants (-7 bp). (C) Example of sex genotyping for the F2 generation. (D) Mutants with hematopoiesis defects. About half of the *gata2a* (4/10) and *gata2b* (6/10) homozygous mutants with severe hematopoiesis defects during hatching and died after hatching. (E) Eyes morphology of the *gata2a* mutants (-7 bp). (F) The *gata2a* mRNA level was significantly down-regulated in the mutant. WT: wild type. M: marker.

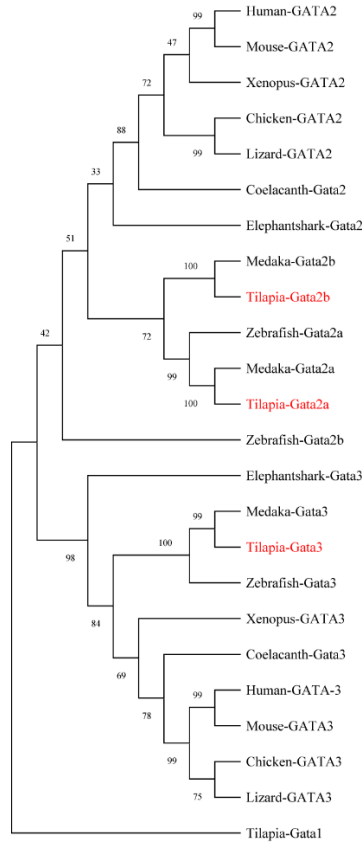


Figure S4 Phylogenetic analyses of vertebrate GATA2 and GATA3 gene

Phylogenetic tree constructed using MEGA11 with the Neighbor-Joining Algorithm method.

Table S1 Primers used in present study

Primer	Sequence (5'-3')	Purpose
<i>gata2a</i> -gRNA-F1	TAATACGACTCACTATAGGCTGTGGTGGTGAGAGTCCGTTTTAGAGCTAGAAATAGC	CRISPR/Cas9
<i>gata2b</i> -gRNA-F1	TAATACGACTCACTATAGGGCTTACTGAAGGGGCTCAGTTTTAGAGCTAGAAATAGC	
gRNA-R1	AGCACCGACTCGGTGCCAC	Mutants screening
<i>gata2a</i> -T-F1	GACTTCCACTTGGAGGCAAA	
<i>gata2a</i> -T-R1	GGTTCAGAAACATATCAACC	
<i>gata2b</i> -T-F1	CCTCATCTCCTTCACAGCCC	
<i>gata2b</i> -T-R1	TCCTTCTCGTCGATTCTGGTAG	
<i>gata2b</i> -page-F1	GGATTTCGTGGCTGGACG	PAGE for <i>gata2b</i>
<i>gata2b</i> -page-R1	ACTGCTGCTGCACGGGTAA	
F5	ATGGCTCCGAGACCTTGACTG	Sex genotyping
R3	CAGAAATGTAGACGCCAGGTAT	
<i>gata2a</i> -rt-F	AGAGAGACTGTCAGCTCA	Quantification of <i>gata2a</i> and <i>gata2b</i>
<i>gata2a</i> -rt-R	GTGCTGACCGGGTGCTGA	
<i>gata2b</i> -rt-F	TCTGACGGGGACTCAGGTCT	
<i>gata2b</i> -rt-R	TTACTGAAGGGGCCCTCA	
β -actin-rt-F	CATCCCGTCTCTGCTCACA	Internal control
β -actin-Q-R	AGGCGTACAGGGACAGCA	