

# Supplementary materials for

## *hoxa1a* null zebrafish as a model for studying HOXA1-associated heart malformation in Bosley- Salih-Alorainy Syndrome

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Supplemental Item	Title
Table S1	Primers for ISH probes synthesis
Table S2	Primers for qPCR
Figure S1	Cas9 mRNA dosage response.
Figure S2	18 sequenced <i>hoxa1a</i> alleles after injection.
Figure S3	Two frameshift (-7bp and +8bp) mutation types isolated from F1 progeny.
Figure S4	Survival rate of wild type siblings, <i>hoxa1a</i> heterozygotes and <i>hoxa1a</i> null zebrafish during embryos stage.
Figure S5	Quantified ventricle circularity.
Figure S6	In-situ hybridization using <i>nppb</i> probes labeling heart chambers in 3dpf embryos.
Figure S□	In-situ hybridization using <i>amhc</i> probes labeling atrium in 3dpf embryos.
Figure S8	In-situ hybridization using <i>vmhc</i> probes labeling ventricle in 3dpf embryos.
Figure S9	Longitudinal sectional images stained with H&E between WT control and <i>hoxa1a</i> <sup>-/-</sup> mutants in 1-month-old zebrafish with different slices.
Figure S10	<i>hoxa1a</i> null zebrafish exhibited abnormal tail fin phenotype and craniofacial development.
Figure S11	Phylogenetic tree showing relationships between zebrafish <i>hoxa1a</i> and homologous/paralogous genes in other species.

Table S1. Primers for ISH probes synthesis

Locus	Forward primer (5'-3')	Reverse primer (5'-3')
<i>amhc</i>	<u>TAATACGACTCACTATAGGGAG</u> <u>ATAATGCATGATAGCGCCGGT</u>	GGAGTACGTGAAGGGGCAAA
<i>vmhc</i>	<u>TAATACGACTCACTATAGGGAG</u> AAGAGTCCCACCACAGTCTCA	CCTCTCTCAGCGGAAACCTG
<i>nppa</i>	<u>TAATACGACTCACTATAGGGAG</u> AAGGGTGCTGGAAGACCCTAT	GAGACACTCAGAGATGGCCG
<i>nppb</i>	CATTCCCGTAGTCGGCCTTC	GGATCCATTAACCCTCACTAAAGG GAACCCCGACTGTGTTACATCCCA A
<i>cmlc2</i>	TTGGCTGCATAGATCAGAACC	<u>TAATACGACTCACTATAGGGAGGC</u> TGCTGATGTGAATGTTGAAC
<i>has2</i>	<u>TAATACGACTCACTATAGGGAG</u> <u>ACGCGCGGTGTATTTTCGTGGC</u>	GGCCCTATGCATCGCAGCCT
<i>hand2</i>	ACCATGGCACCTTCGTACAG	<u>TAATACGACTCACTATAGGGAGATG</u> GCCAACCAGTTCTCCCTTT
<i>notch1b</i>	GAGGTGGCCGCCAAACAGCA	CCCACATGCGTGAGCGTCGT

Table S2. Primers for qPCR

Locus	Forward primer (5'-3')	Reverse primer (5'-3')
<i>hoxa5a</i>	CGTGCTCATCCTTAGCCAACTCTC	CTGTGAAGCTGTCTGTCCACTACC
<i>hoxa9a</i>	AATGATGACGGCACGGAATA	TCGCATGAAGCCAGTTGGA
<i>hoxb1b</i>	TCGGTCTCTGCAAGCCACTATCA	TCTTGGAGTCGCTGTCACTGTCA
<i>hoxb5a</i>	AGGCGTCTCACAGAGCGAACA	TGCCGTCTGGTCCAGTCATATCAT
<i>hoxb5b</i>	AAGCAAGAATCTGTGGCGACCTC	GCGTCTGGTAGCGAGTGTAAGC
<i>hoxb8a</i>	CCATGTGCGGTAACCTGTC	GTCGCTATACTGAACCAAGTCG
<i>hoxb6a</i>	CCCTTGGATGCAACGGATGA	CGAAGACGCCGCATTGAGA
<i>hoxc1a</i>	CCTCCTTCCAAAGCTGTCGT	CCTCGTCAGAATCTCGTCCG
<i>hoxc5a</i>	TCAGTCACGGACTGTTGAGC	CGACGTGTGAGGTATCGGTT

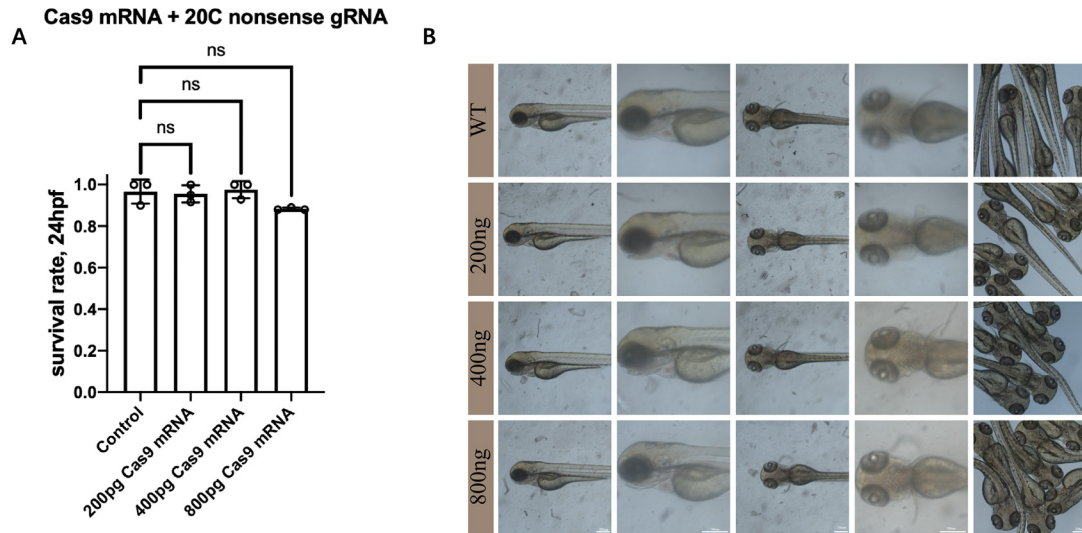


Figure S1. Cas9 mRNA dosage response. (A) Survival rate in 24hpf embryos after microinjected into 200pg/400pg/800pg Cas9 mRNA with 150pg 20C nonsense gRNA. (B) Overall view and heart imaging after co-injection of Cas9 mRNA and 20C nonsense gRNA.

18 sequenced *hoxa1a* alleles after gRNA 1 injection

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ATTTTTCGTCCATAAGTGGTGGTGGAGATGGAGGTAGGGGGCTCGTGCTCAGTCAGGGCGT (Reference)
-----CTCAGTCAGGGCGT -92*
-----CTCAGTCAGGGCGT -62*
ATTTTTCGTCCATAAGTGGTG-----GGCTCGTGCTCAGTCAGGGCGT -20*
ATTTTTCGTCCATAAGTGGTGG--AG-----GGCTCGTGCTCAGTCAGGGCGT -17*
ATTTTTCGTCCATAAGTGGTGGTGG-----GGCTCGTGCTCAGTCAGGGCGT -16*
ATTTTTCGTCCATAAATGGTGGTGGAGATGGAGGTAG-----TGCTCAGTCAGGGCGT -10*
ATTTTTCGTCCATAAGTGGTGGTGGAGATGGAGG-----TTCGTGCTCAGTCAGGGCGT -9
ATTTTTCGTCCATAAGTGGTGGTGGAGATGGAGG-----TAGCTCGTGCTCAGTCAGGGCGT -6
ATTTTTCGTCCATAAGTGGTGGTGGAGATGGAGGTAG--GGGGCTCGTGCTCAGTCAGGGCGT -2*
ATTTTTCGTCCATAAGTGGTGGTGGAGATGGAGGTAGGGGGGCTCGTGCTCAGTCAGGGCGT 0
ATTTTTCGTCCATAAGTGGTGGTGGAGATGGAGATGGAGGGGGGCTCGTGCTCAGTCAGGGCGT 0
ATTTTTCGTCCATAAGTGGTGGTGGAGATGGAGGTAGctCGGGGGCTCGTGCTCAGTCAGGGCGT +2* (x2)
ATTTTTCGTCCATAAGTGGTGGTGGAGATGGAGGTAGctCGGGGGCTCGTGCTCAGTCAGGGCGT +3
ATTTTTCGTCCATAAGTGGTGGTGGAGATGGAGGTGGagatggagaCGGGGGCTCGTGCTCAGTCAGGGCGT +9
ATTTTTCGTCCATAAGTGGTGGTGGAGATGGAGGTAGCGGGGGCTCGTGCTCAGTCAGGGCGT 0, Wild type (x3)

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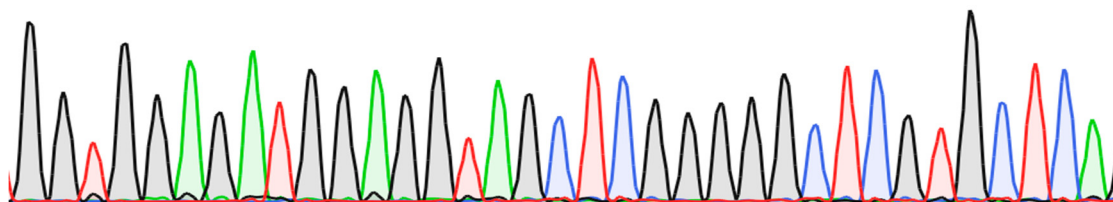
Indel mutations: 15 out of 18 (83.3%)

\*Frame-shift mutations: 9 out of 18 (50%)

Insertions: 4 out of 18

e.g., +2bp peak map

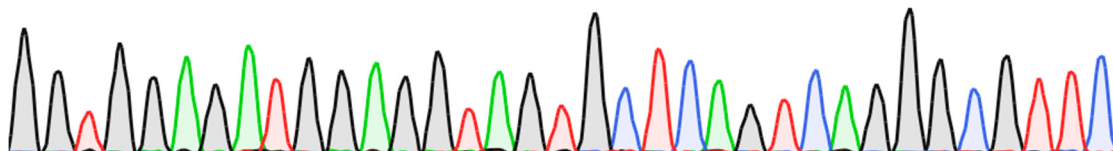
G G T G G A G A T G G A G G T A G C T C G G G G G C T C G T G C T C A



Deletions: 9 out of 18

e.g., -10bp

G G T G G A G A T G G A G G T A G T G C T C A G T C A G G G C G T T C



Single-site mutations: 4 out of 18

e.g., C to G conversion

G G T G G A G A T G G A G G T A G G G G G G C T C G T G C

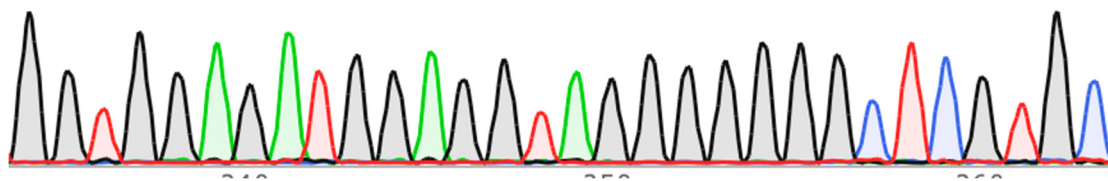


Figure S2. 18 sequenced *hoxala* alleles after injection. 15 out of 18 alleles carried mutations (including small insertion/deletion and single-site mutations). 9 out of 18 alleles carried frame-shift mutations (marked with \*).

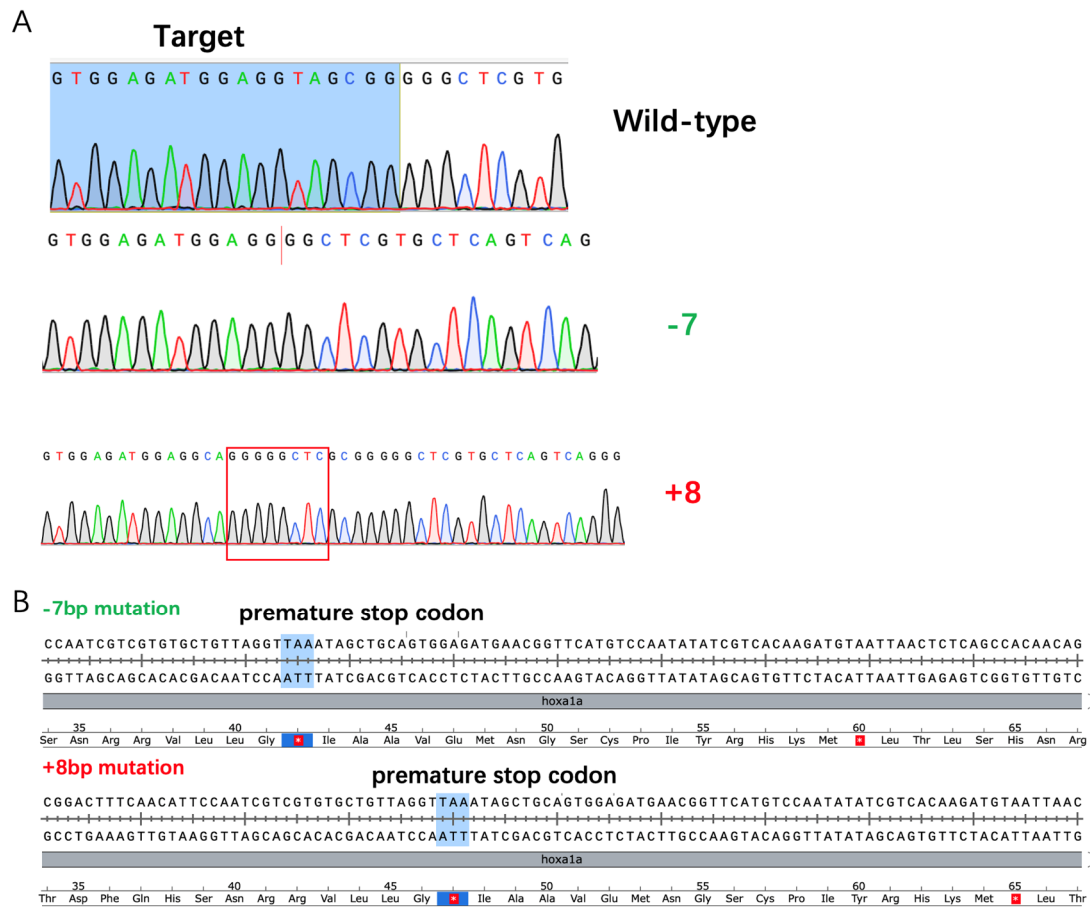


Figure S2. Two frameshift (-7bp and +8bp) mutation types isolated from F1 progeny. (A) Peak map after TA cloning. (B) Premature stop codon in -7bp mutation (42aa) and +8bp mutation (47aa).

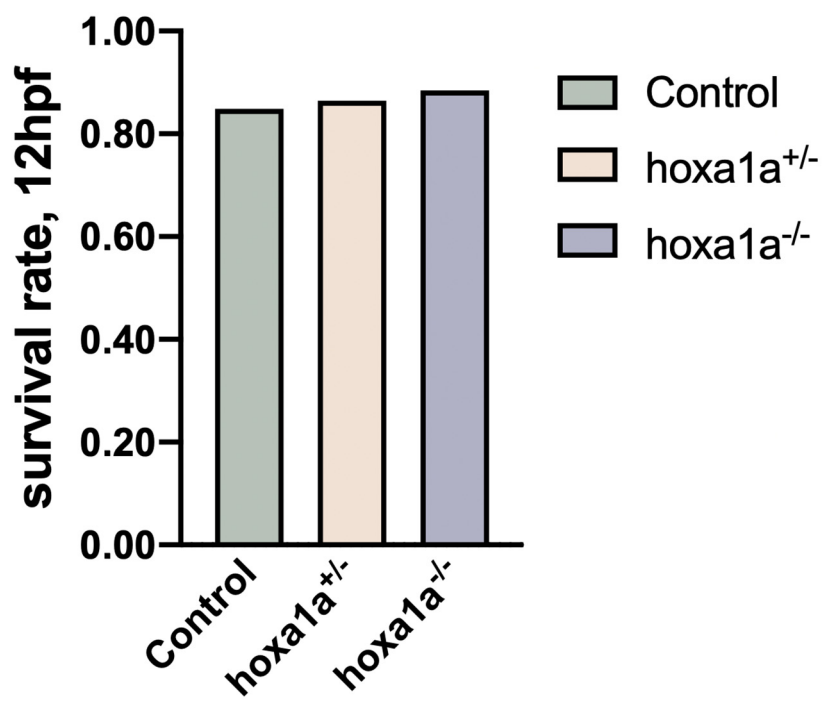


Figure S3. Survival rate of wild type siblings, *hoxa1a* heterozygotes and *hoxa1a* null zebrafish during embryos stage.



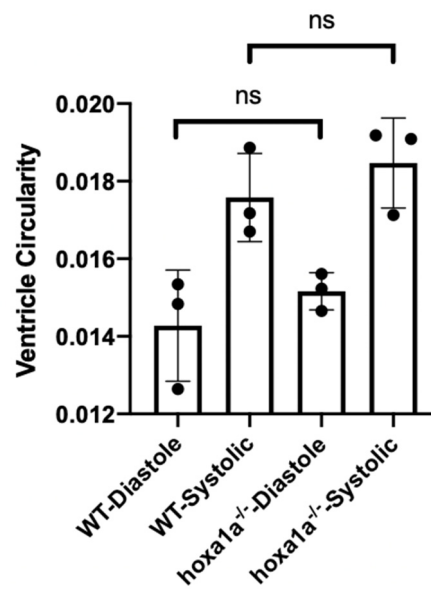
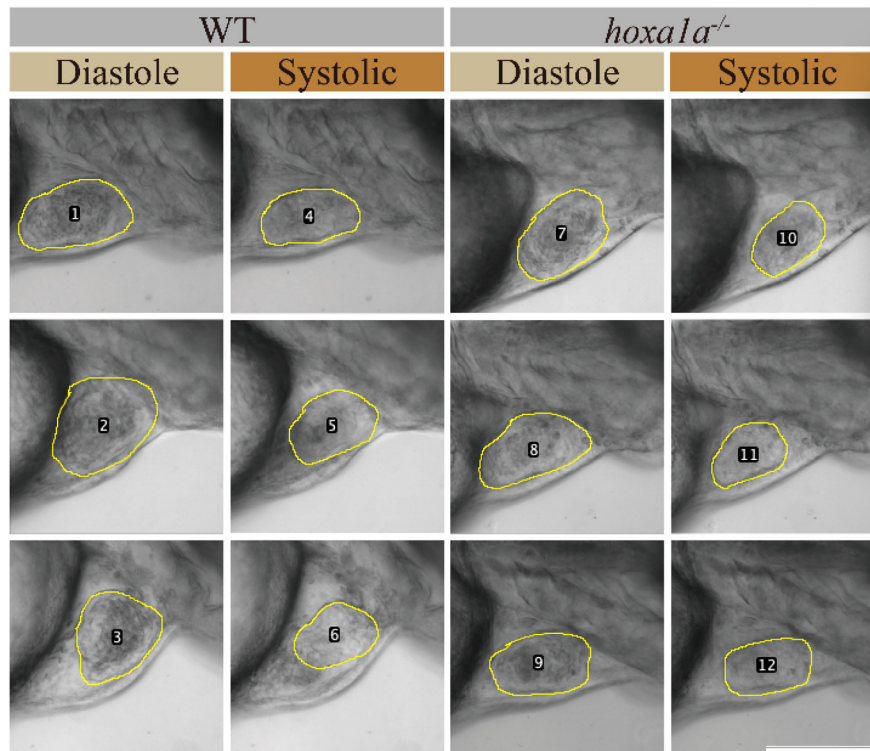


Figure S4. Quantified ventricle circularity.  $\text{Circularity} = \frac{4\pi \times \text{area}}{\text{perimeter}^2}$ . There is no significant difference between WT and *hoxa1a* homozygotes in diastole and systolic.

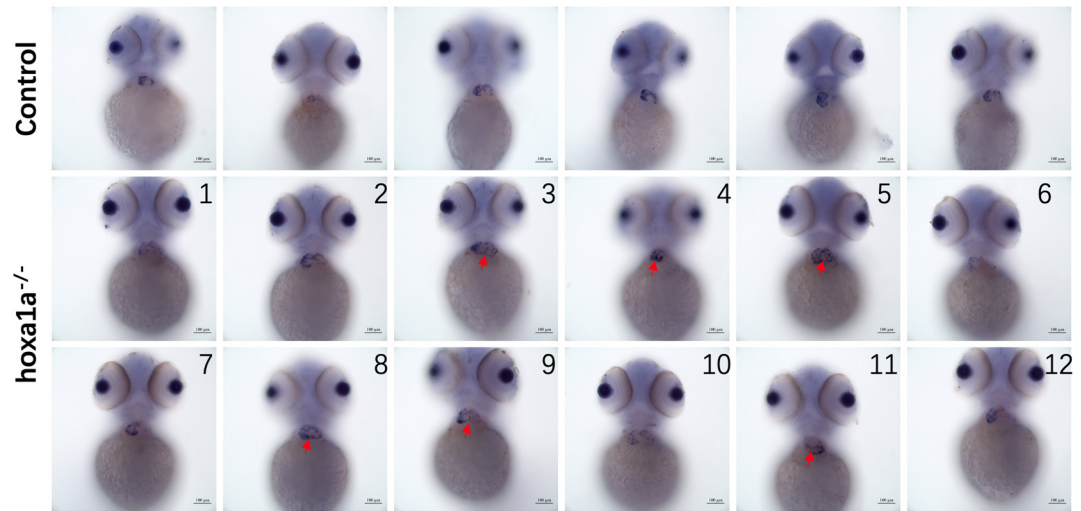
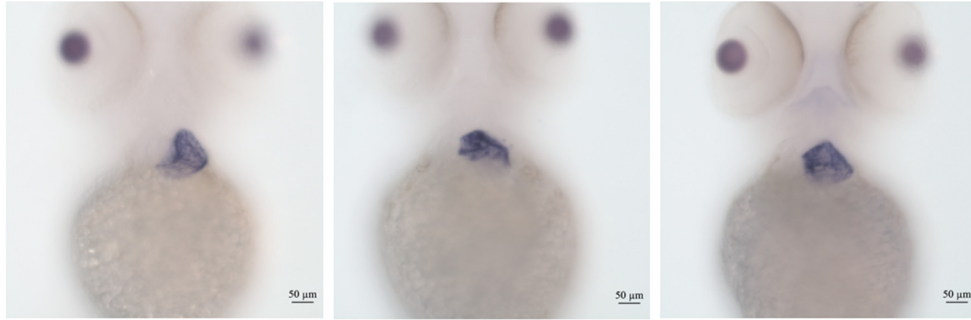


Figure S5. In-situ hybridization using *nppb* probes labeling heart chambers in 3dpf embryos. *nppb* mis-expressed in 3, 4, 5, 8, 9 and 11 in *hoxa1a* homozygotes.

Control



*hoxa1a*<sup>-/-</sup>

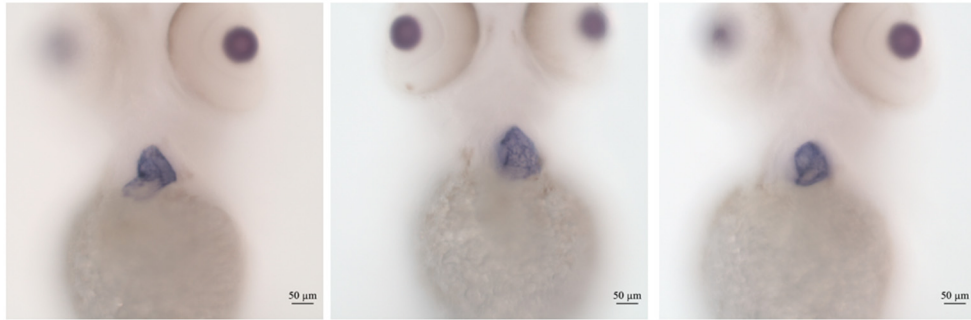


Figure S6. In-situ hybridization using *amhc* probes labeling atrium in 3dpf embryos.

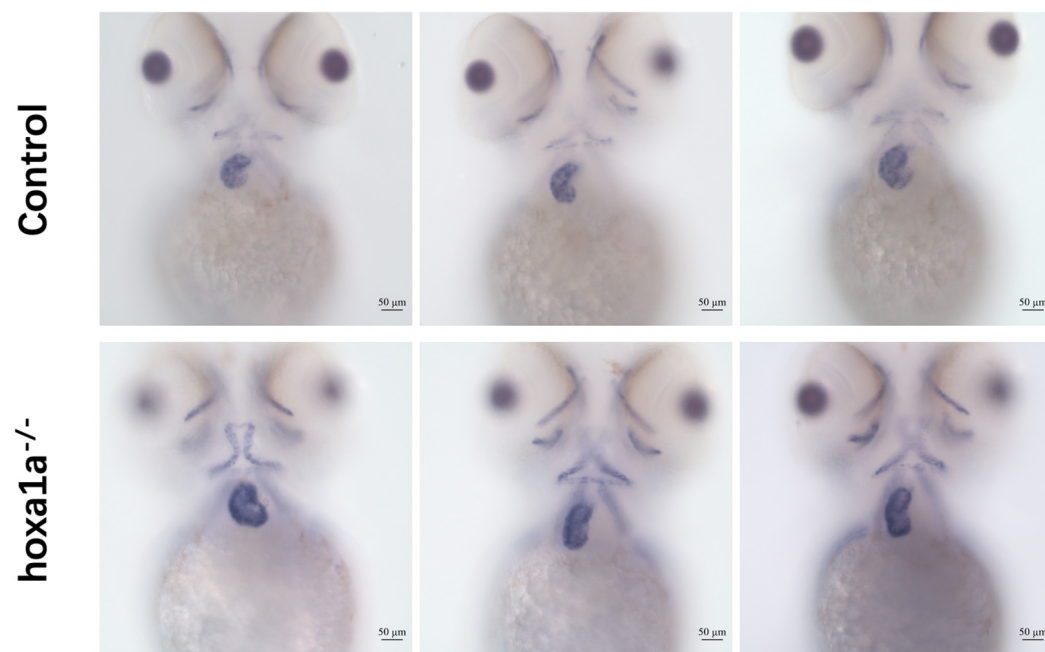


Figure S□ In-situ hybridization using *vmhc* probes labeling ventricle in 3dpf embryos.

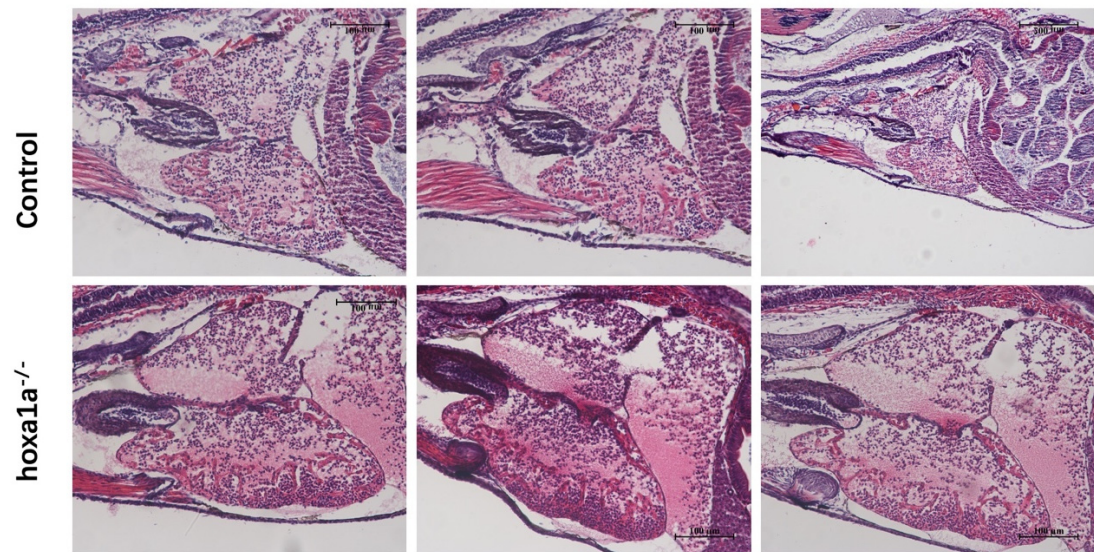


Figure S8. Longitudinal sectional images stained with H&E between WT control and *hoxa1a*<sup>-/-</sup> mutants in 1-month-old zebrafish with different slices.



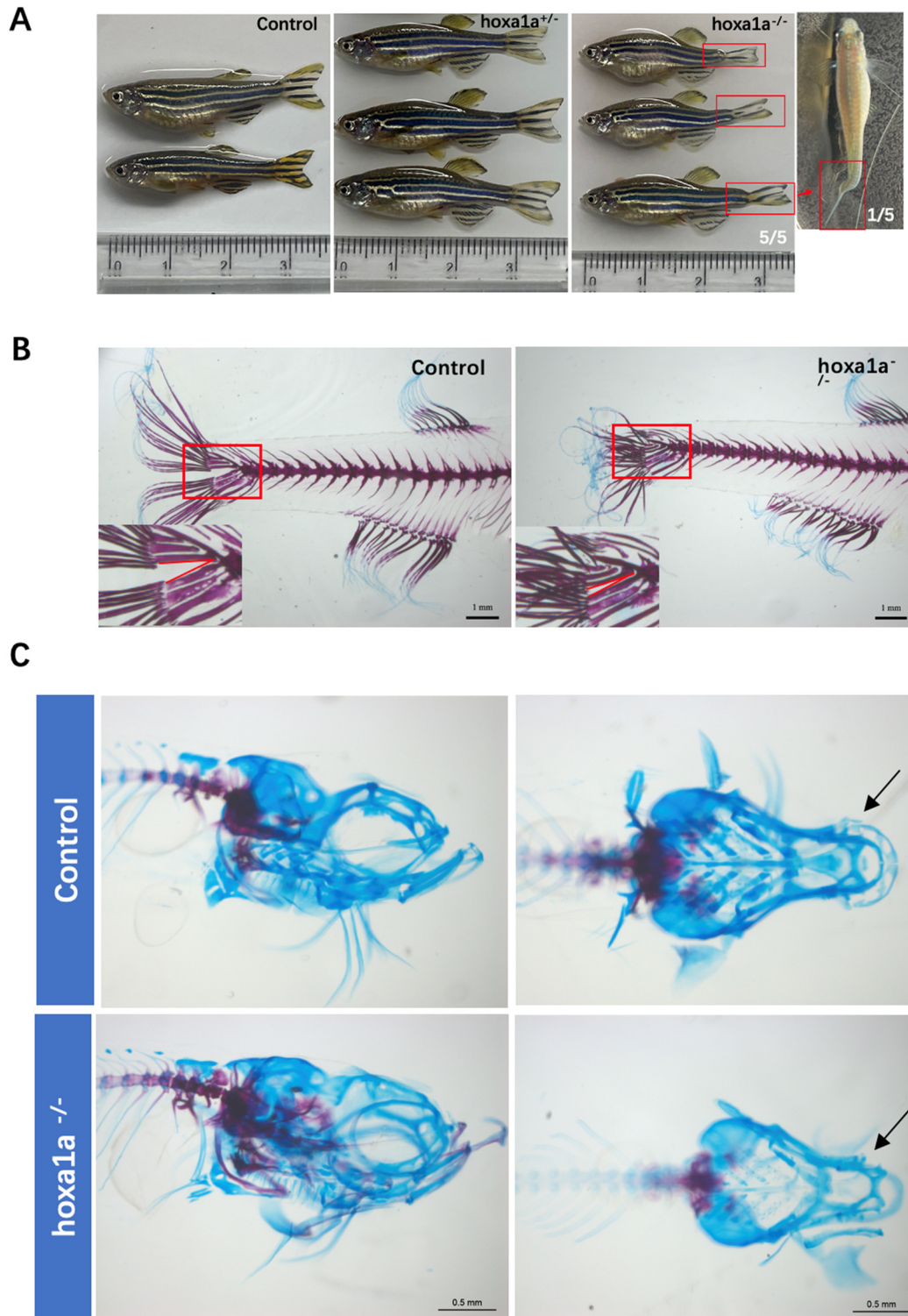


Figure S9. *hoxa1a* null zebrafish exhibited abnormal tail fin phenotype and craniofacial development. (A) *hoxa1a* homozygotes exhibited smaller and insufficiently spread tail fin compared to wild type and heterozygotes. (B) Skeleton and cartilage staining in one month fish. Tail bones of *hoxa1a* homozygotes had a smaller spread angle (marked with red lines). Scale bar: 1mm. (C) Cartilage staining of wild-type and *hoxa1a* homozygous juvenile zebrafish at 1 month. *hoxa1a* homozygote at 1 month showed a loss of cartilage

at the anterior end of the first pharyngeal arch (black arrow). Scale bar: 0.5mm.

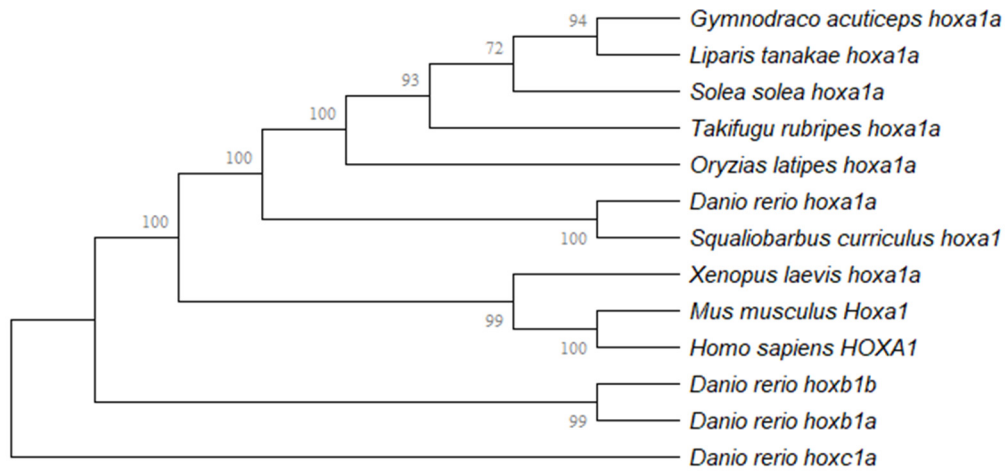


Figure S10. Phylogenetic tree showing relationships between zebrafish *hoxa1a* and homologous/paralogous genes in other species.