Supplementary material

Table S1. Primers sequences and PCR conditions.

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Table S2. List of antibodies used for western, CHIP, RIP and immunofluorescence studies.

Antibody name	Company	Dilution	Application
Anti-α tubulin	Santa Cruz Biotechnology (sc-23948)	1:2000	Western blotting
Anti-Nkx2.5	Cell Signaling Technology (8792)	1:1000	Western blotting
Anti-Gata4	Santa Cruz Biotechnology (sc-25310)	1:1000	Western blotting
Anti-Myf5	GeneTex (GTX87746)	1:1000	Western blotting
Anti-MyoD	Santa Cruz Biotechnology (sc-377460)	1:500	Western blotting
Anti-Mef2C	Cell Signaling Technology (5030)	1:1000	Western blotting
Anti-Histone 3	Cell Signaling Technology (9715)	1:1000	Western blotting
Anti-H3K4Me1	Abcam (ab8895)	1:500	Western blotting
		2 µg for 25 µg of chromatin	CHIP
Anti-Actin	SIGMA (A2172)	1:200	Immunostaining
Anti-Myosin	DSHB (MF20)	1:200	Immunostaining
ANTI-FLAG M2 Affinity Gel	SIGMA (A2220)	N.A	RIP
Anti-MYH1A	DSHB (F59)	1:50	Immunostaining
Anti-CD166	DSHB (zn8)	1:50	Immunostaining
Anti-TNNT2	Santa Cruz Biotechnology (sc-20025)	1:50	Immunostaining
Anti-mouse IgG (H+L),-Alexa Fluor 594 Conjugate	Cell Signaling Technology (8890)	1:200	Immunostaining
Anti-Rabbit IgG (H+L)-Alexa Fluor 568 Conjugate	Thermo Fisher (A10042)	1:200	Immunostaining

Table S3. Significant different expression gene in *ddx39ab* mutant. This file includes Genes that that have FDR≤0.001 and Fold Change >2.

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Table S4. Significant alternative splicing events in *ddx39ab* mutant embryo. Four types of alternative splicing events, including exon skipping, intron retention, 5' and 3' splicing sites usage, were calculated from the RNA-seq data of control and *ddx39ab* mutant embryo (24hpf) by the OLego and Quantas software packages. The spread sheet for each type of events contains 7 columns, including gene accession number, chromosome, strand, constitutive exon, alternative events.

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Table S5. Gene Ontology (GO) enrichment analysis of significant different expression gene in *ddx39ab* mutant. $P<0.01$, $FDR<0.05$.

Click here to Download Table S5

Table S6. List of Ddx39ab-interacting RNAs identified by RIP-Seq analysis. q value≤0.1.

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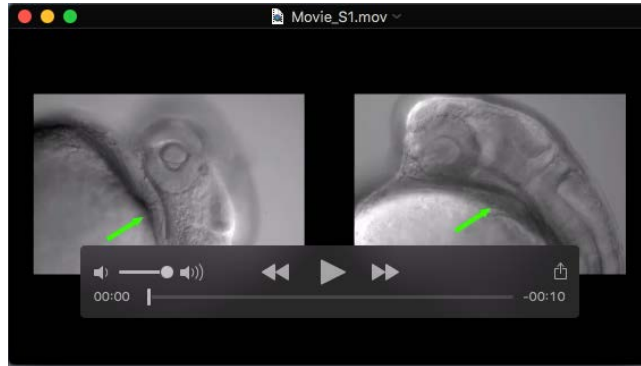
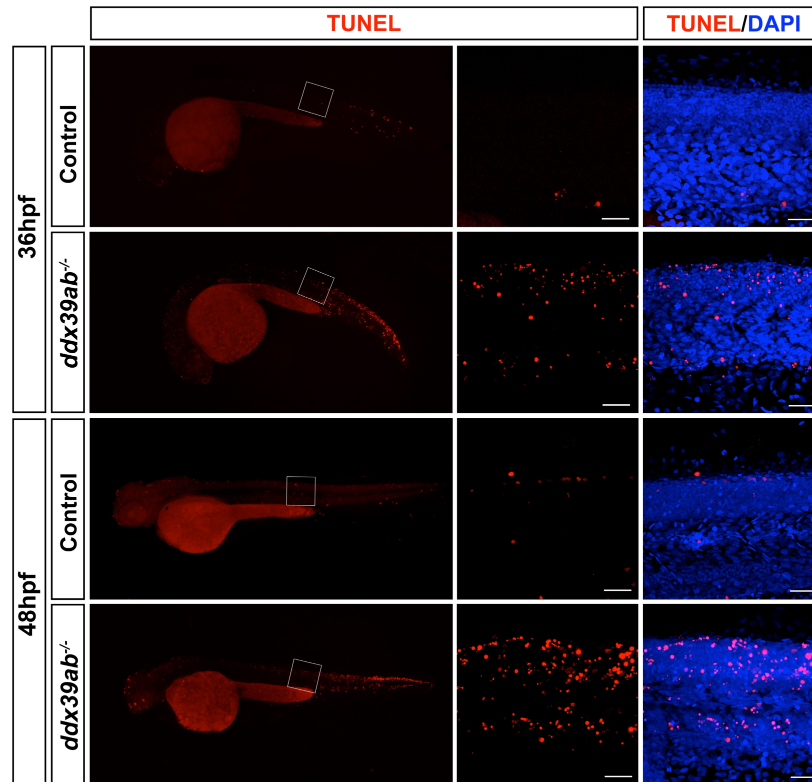


Movie 1. Contraction of the definitive heart tube in RP -011 homozygous and control embryo.

Movie 2. Spontaneous tail movements in RP -011 homozygous and control embryo.

Supplementary figures



Supplemental Figure 1. **Cell death in wild type or *ddx39ab* mutant embryo.** Right panels, magnified view of the boxed region. Scale bars, 20um. For each stage, at least 10 embryos for each genotype were analyzed and representative samples are shown.

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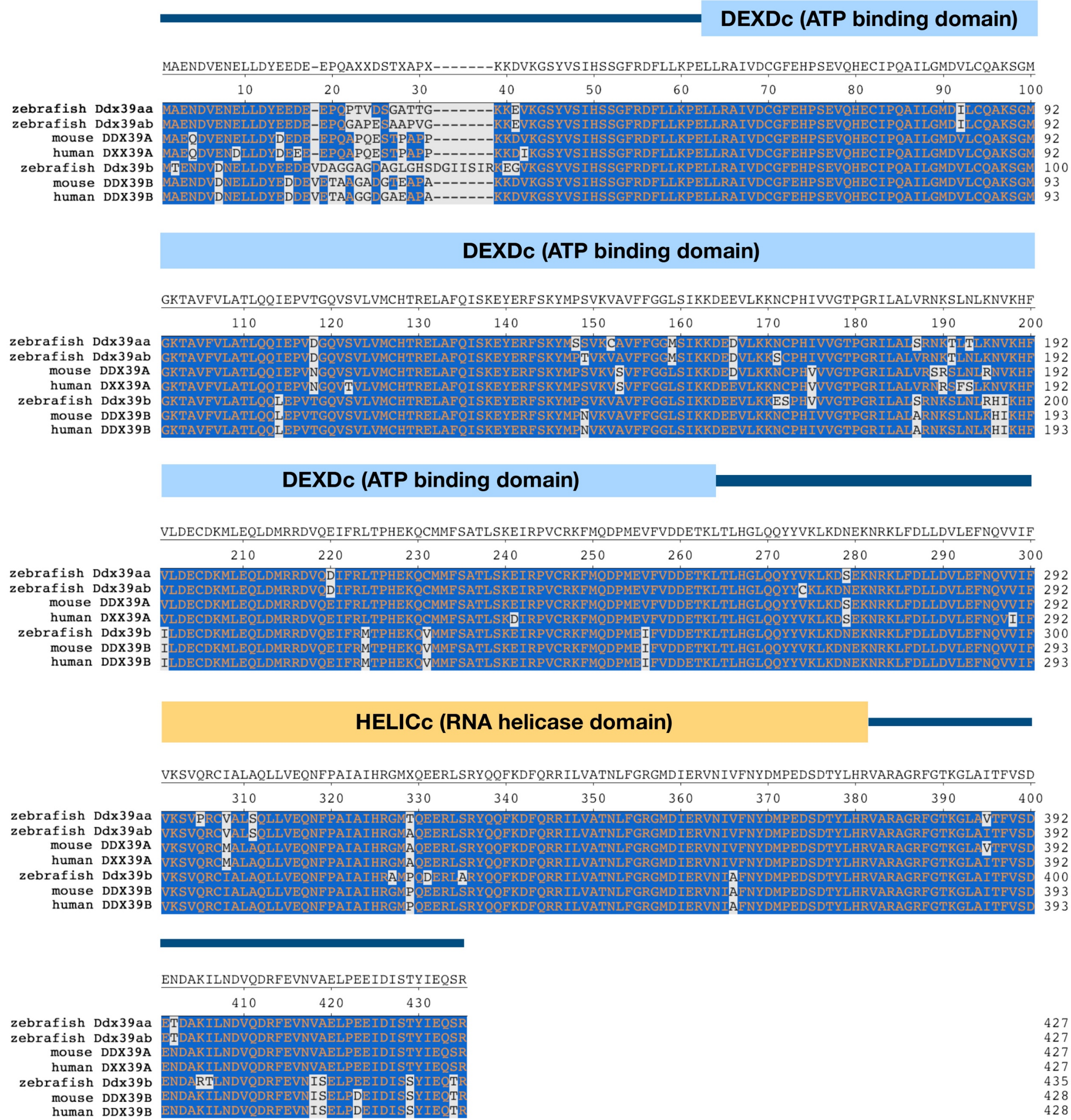

Supplemental Figure 2. DDX39 protein sequences alignment. Functional domains are labeled. The orthologs and paralogs of DDX39 in zebrafish, mouse and human share 94% amino acid identity, differing in only 21 residues.

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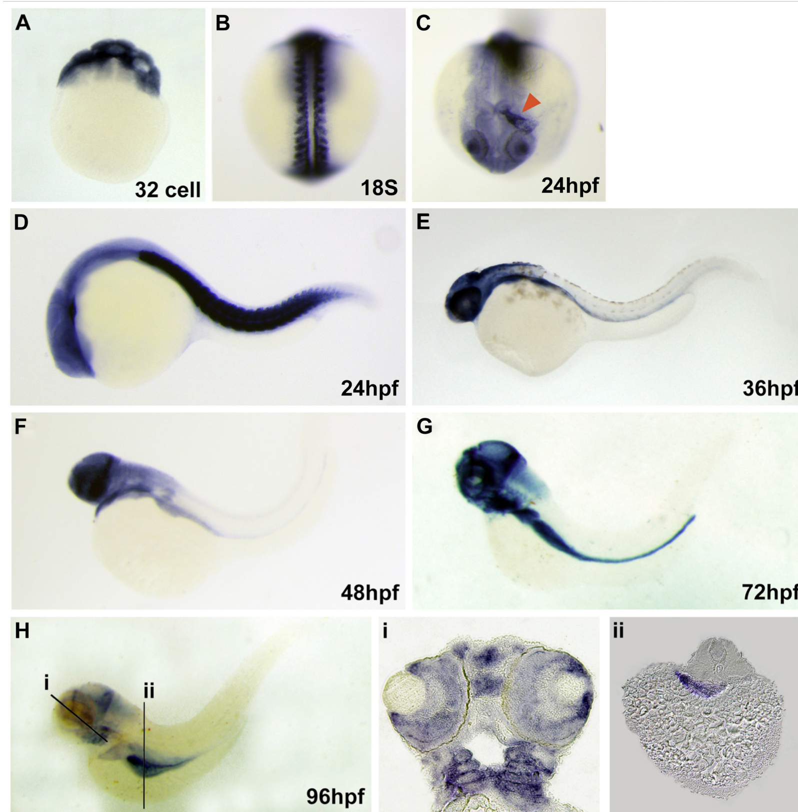

Supplemental Figure 3. Zebrafish *ddx39ab* embryonic expression pattern. (A): In situ hybridization experiment revealed there is maternal deposit ddx39ab mRNA in early stage embryo. (B): Strong expression of *ddx39ab* could be observed in myotome at 18 somite stage. (C-D): At 24hpf enriched *ddx39ab* mRNA could be observed in lens, heart tube and trunk muscle. Triangle indicated expression in heart tube. (E-H): Later on, the expression was restrained to specific regions in brain, retina (i), pharyngeal arches (i) and endoderm derived organs (ii). A, lateral view. B, dorsal view. C, frontal view. D to H, head to left. i and ii are sections of H as indicated. hpf, hour past fertilization.

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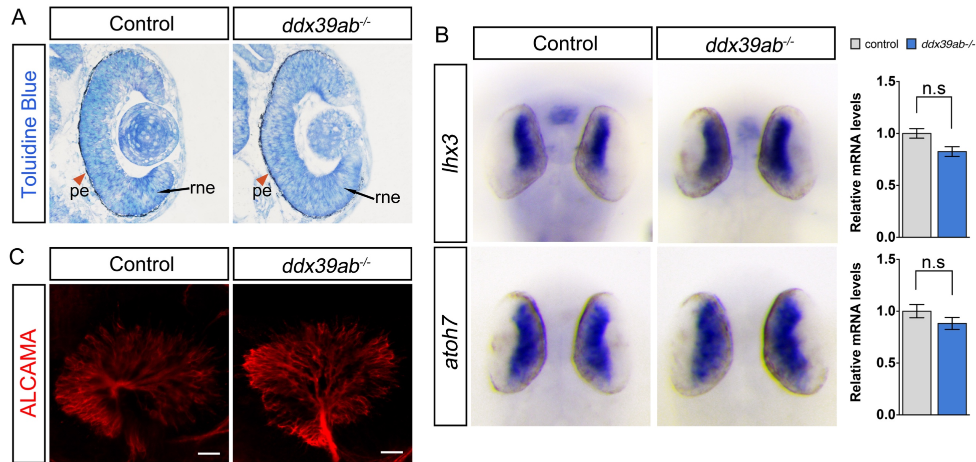

Supplemental Figure 4. Retina development in *ddx39ab* mutants. (A): Frontal sections of retina at 32 hpf. (B): RNA *in situ* hybridization and qPCR results are shown for retinal ganglion marker gene expression in control and *ddx39a* mutant zebrafish embryos. Dorsal views with anterior to the top. For qPCR results, data are mean ± SEM. ns: not significant. (C): Confocal images of retinal ganglion cell (labeled with zn8 antibody) from wild type and *ddx39ab* mutant embryo at 48 hpf. Scale bars, 20um. A, B and C, at least 15 embryos for each genotype were analyzed and representative samples are shown.

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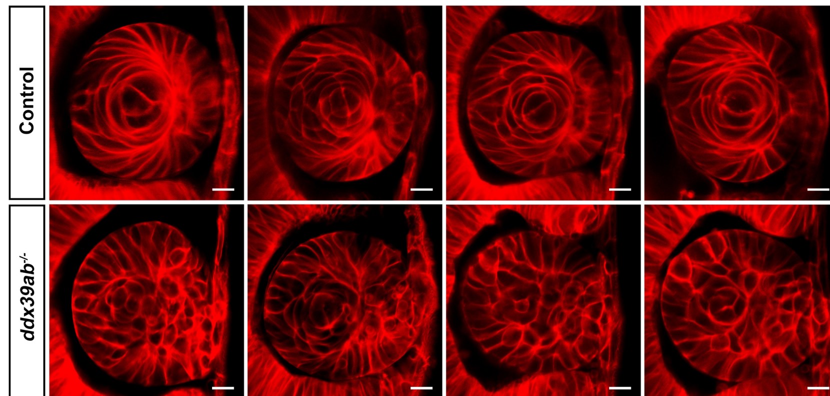
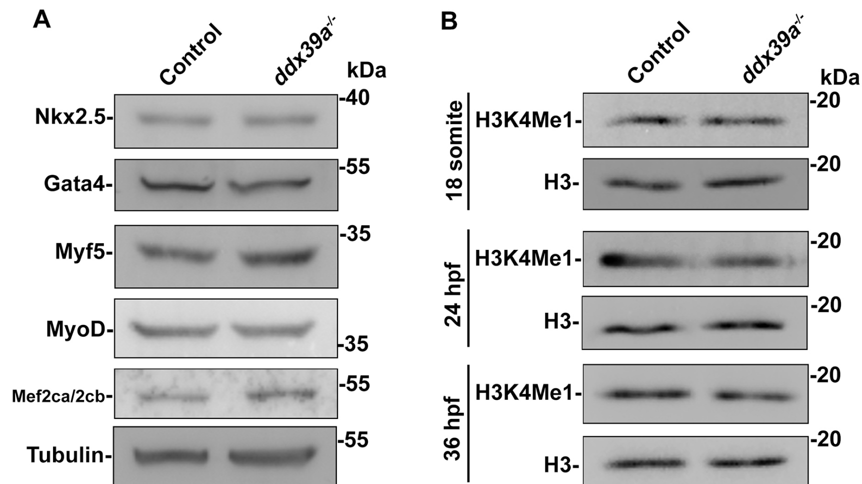


Supplemental Figure 5. Disorganization of primary lens fiber cell in *ddx39a* embryo. Cell membrane was labeled with mRFP; living images were taken at 28 hpf. Scale bars, 25 μM.



Supplemental Figure 6. Western blot analysis of the indicated proteins and histone PTMs in control and *ddx39ab* mutant embryo. (A): protein level of myocyte or cardiomyocyte key transcription regulators showed no evident change between control and *ddx39a* mutant at 24 hpf. (B): In *ddx39ab* mutant, minor decrease on H3K4Me1 level could be detected from 24 hpf.

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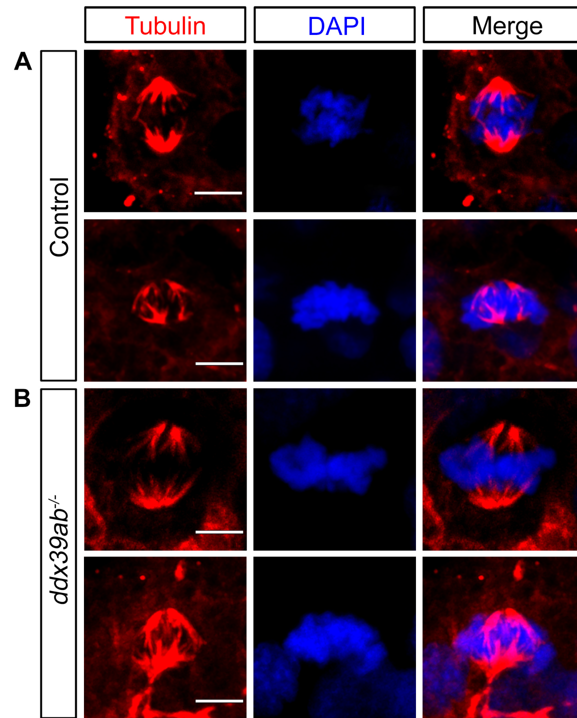

Supplemental Figure 7. *Ddx39ab* mutant embryos exhibit normal mitoses. Mitotic cells stained with DAPI (blue) and α-tubulin (red). Scale bars, 5um.

Supplementary Materials and Methods

Western blotting

Proteins were isolated by homogenizing embryos in RIPA buffer containing protease inhibitor (Sigma). Proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). For immunoblotting, proteins were transferred to polyvinylidene fluoride (PVDF) membrane using an electrophoretic transfer apparatus (Bio-Rad). The membrane was blocked with 1% non-fat milk (Bio-Rad) and incubated with primary antibody followed by 1:10,000 HRP-conjugated secondary antibodies (Sigma). Signal detection was performed using Pico West Chemiluminescent Substrate (Thermo Scientific). Information for primary antibodies are listed in Supplementary Table 2.