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# RNA structural dynamics regulate early embryogenesis through controlling transcriptome fate and function

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## Abstract

**Background:** Vertebrate early embryogenesis is initially directed by a set of maternal RNAs and proteins, yet the mechanisms controlling this program remain largely unknown. Recent transcriptome-wide studies on RNA structure have revealed its pervasive and crucial roles in RNA processing and functions, but whether and how RNA structure regulates the fate of the maternal transcriptome have yet to be determined.

**Results:** Here we establish the global map of four nucleotide-based mRNA structures by icSHAPE during zebrafish early embryogenesis. Strikingly, we observe that RNA structurally variable regions are enriched in the 3' UTR and contain *cis*-regulatory elements important for maternal-to-zygotic transition (MZT). We find that the RNA-binding protein Elavl1a stabilizes maternal mRNAs by binding to the *cis*-elements. Conversely, RNA structure formation suppresses Elavl1a's binding leading to the decay of its maternal targets.

**Conclusions:** Our study finds that RNA structurally variable regions are enriched in mRNA 3' UTRs and contain *cis*-regulatory elements during zebrafish early embryogenesis. We reveal that Elavl1a regulates maternal RNA stability in an RNA structure-dependent fashion. Overall, our findings reveal a broad and fundamental role of RNA structure-based regulation in vertebrate early embryogenesis.

**Keywords:** RNA structure, Zebrafish early embryogenesis, Structure-based regulome, Elavl1a

## Background

Vertebrate early embryogenesis is initially directed by a set of maternal RNAs and proteins, yet the mechanisms controlling this program remain largely unknown. Recent transcriptome-wide studies on RNA structure have revealed its pervasive and crucial roles in RNA processing and functions, but whether and how RNA structure regulates the fate of the maternal transcriptome have yet to be determined. Here we establish the global map of four nucleotide-based mRNA structures by icSHAPE during zebrafish early embryogenesis. Strikingly, we observe that RNA structurally variable regions are enriched in the 3' UTR and contain *cis*-regulatory elements important for maternal-to-zygotic transition (MZT). We find that the RNA-binding protein Elavl1a stabilizes maternal mRNAs by binding to the *cis*-elements. Conversely, RNA structure formation suppresses Elavl1a's binding leading to the decay of its maternal targets. Our study finds that RNA structurally variable regions are enriched in mRNA 3' UTRs and contain *cis*-regulatory elements during zebrafish early embryogenesis. We reveal that Elavl1a regulates maternal RNA stability in an RNA structure-dependent fashion. Overall, our findings reveal a broad and fundamental role of RNA structure-based regulation in vertebrate early embryogenesis.



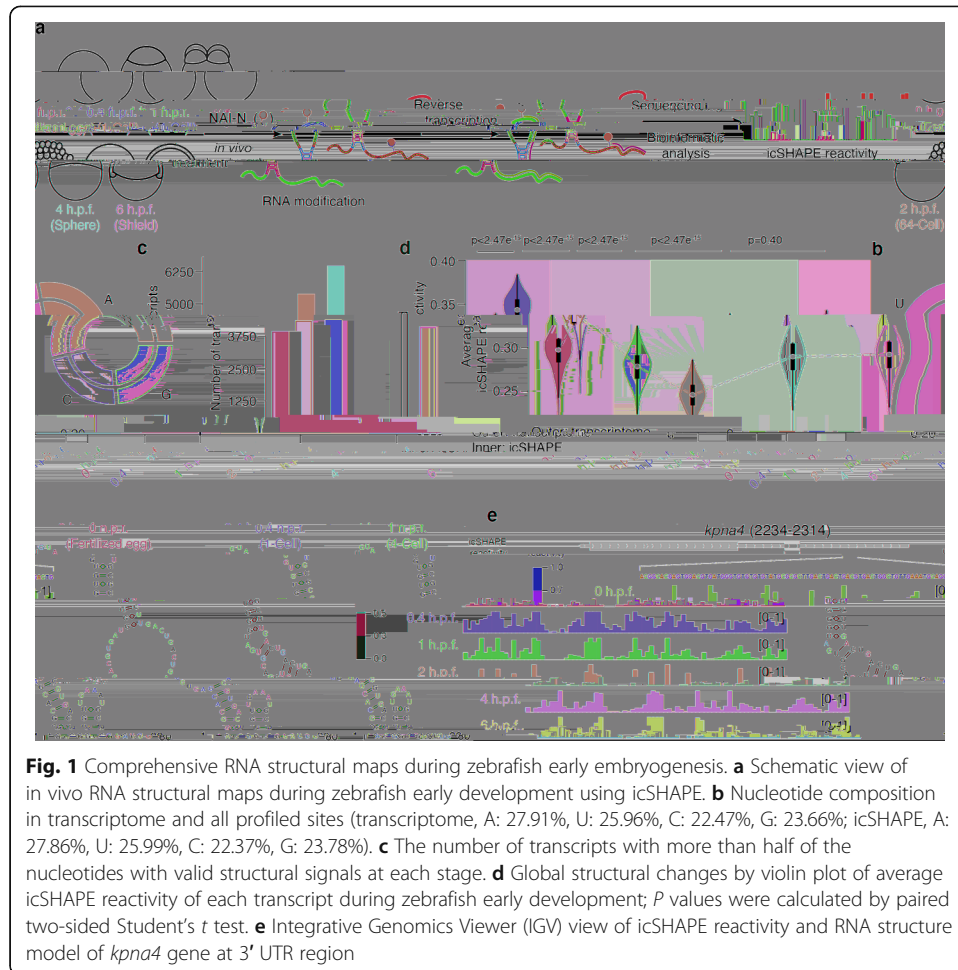
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## Results

### The RNA structure landscape during zebrafish early embryogenesis revealed "hot" structurally variable sites enriched with *cis*-regulatory elements

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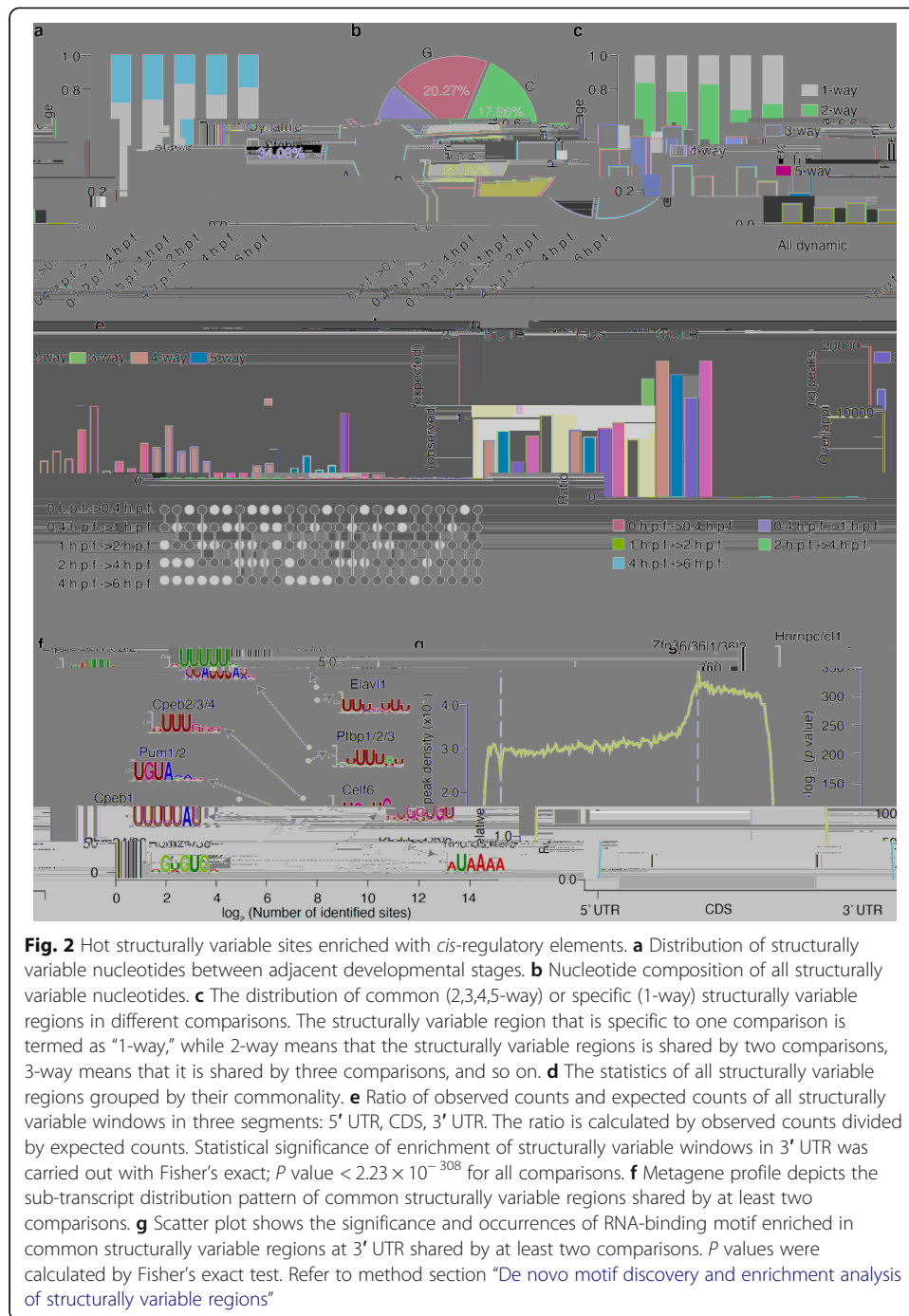
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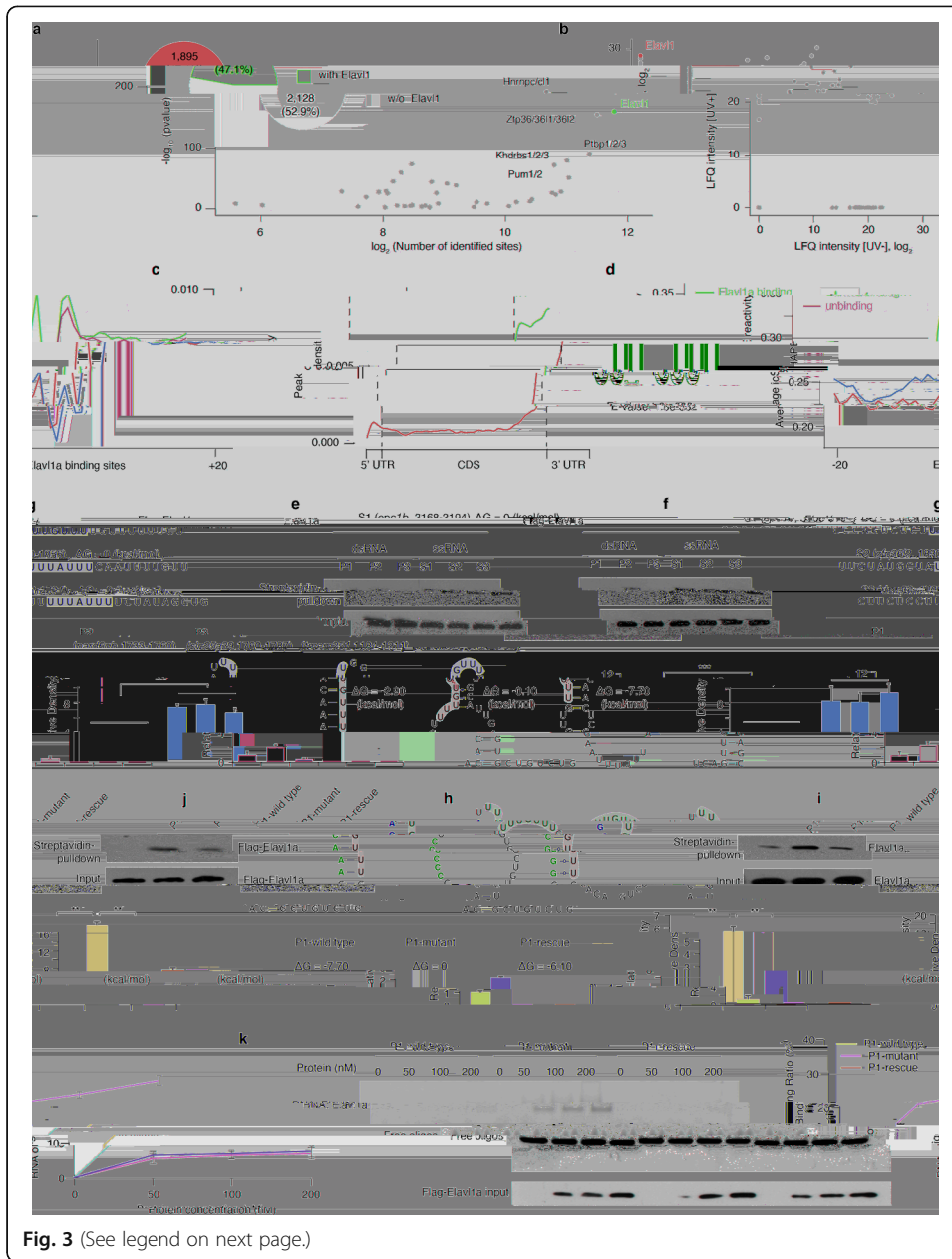
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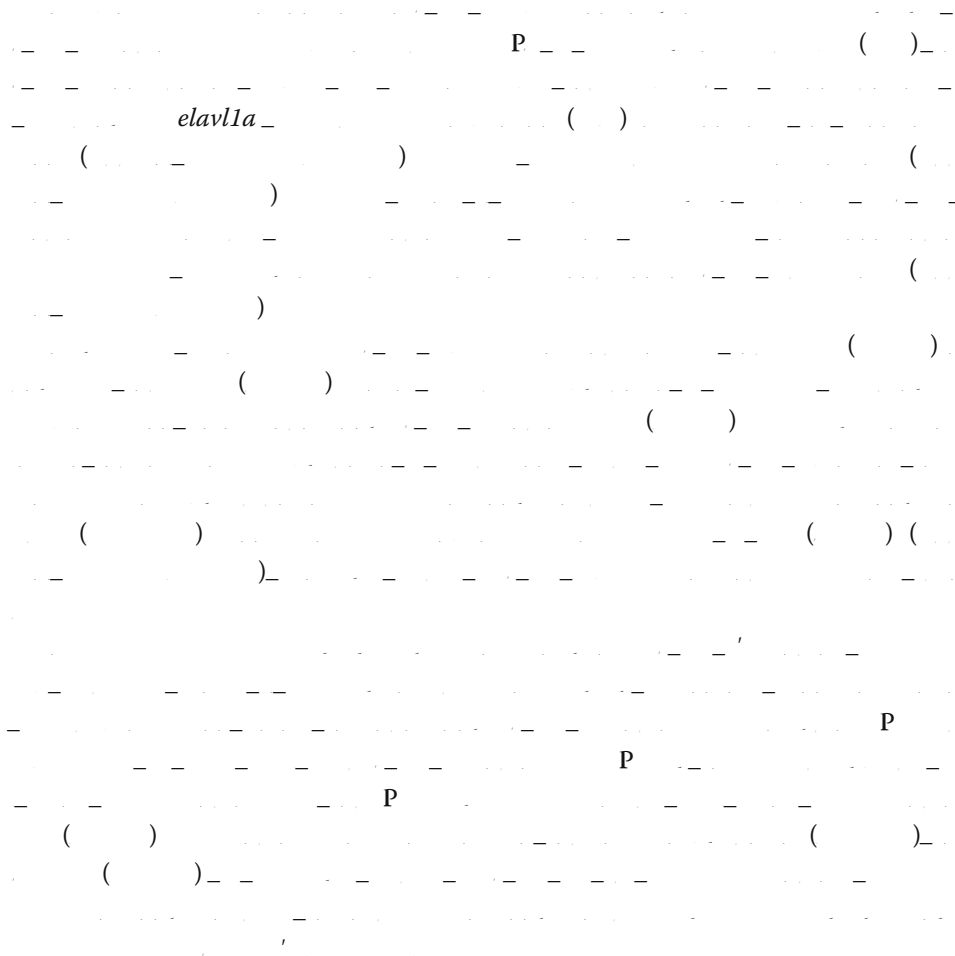




**Fig. 3** (See legend on next page.)

(See figure on previous page.)

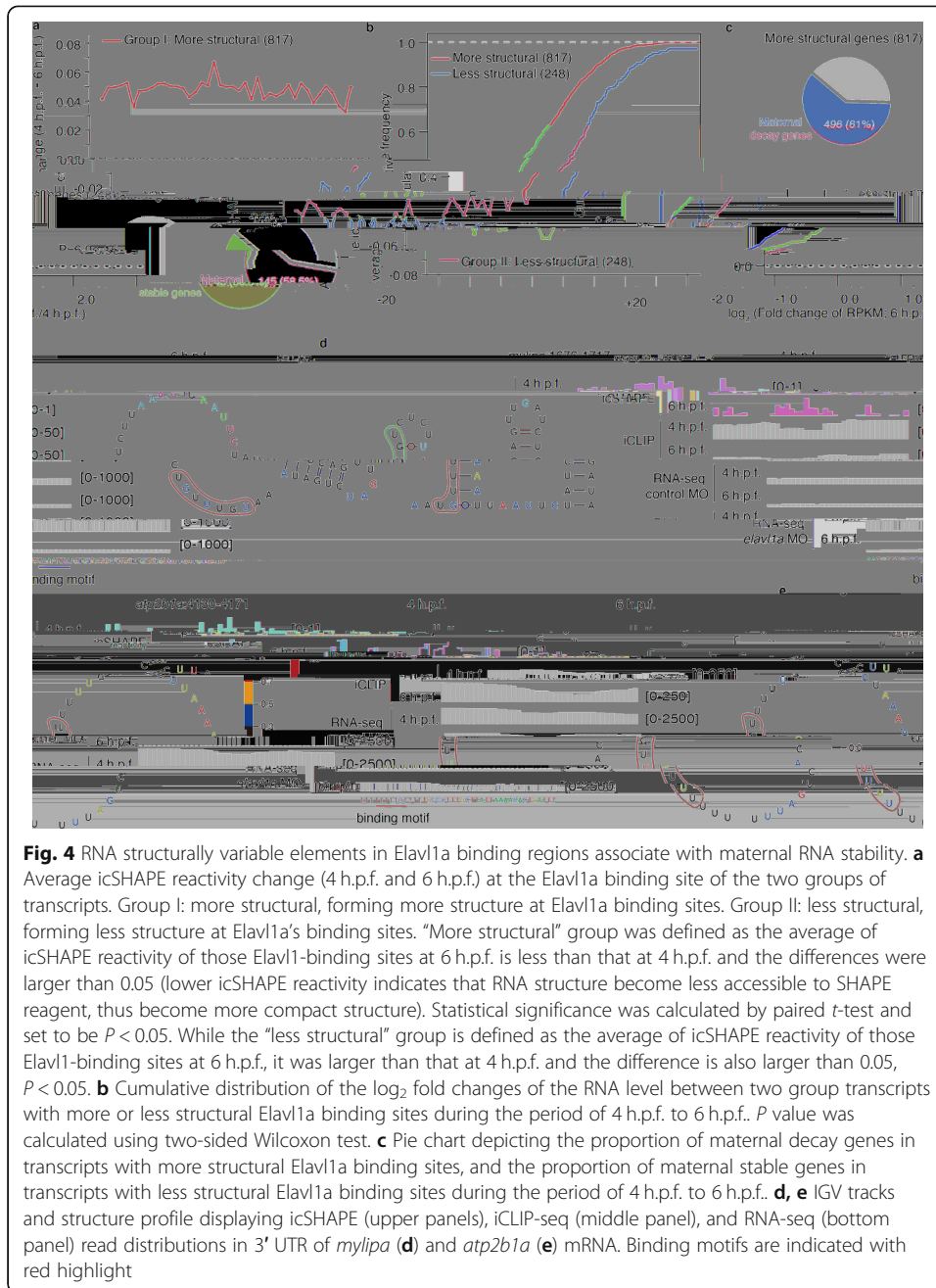
**Fig. 3** Elavl1a prefer to bind single-stranded RNA in vivo and in vitro which enriched in structurally variable regions in 3' UTRs. **a** Scatter plot shows the significance and occurrence of RNA-binding motif enriched in structurally variable windows at 3' UTR between 4 h.p.f. and 6 h.p.f.; *P* values were calculated by Fisher's exact test. Inner pie chart shows 47.1% of transcripts with structurally variable regions at their 3' UTR containing Elavl1 binding motif. **b** Scatter plot shows Elavl1a's enrichment in UV (+) sample at 4 h.p.f.. LFQ, label free quantitation. **c** Distribution of Elavl1a peaks across the length of mRNA and binding motif identified by Dreme (MEME suite) with Elavl1a-binding peaks in 3' UTR (*E*-value =  $1.8 \times 10^{-332}$ ). **d** icSHAPE metaprofile around Elavl1a binding sites and unbound sites with the same motif shows that Elavl1a tend to bind ssRNA in vivo. **e** The structure models of six endogenous RNA probes containing Elavl1a binding sites. Elavl1a binding sites were colored in red background. **f** Demonstration of endogenous Elavl1a pulled down by endogenous RNA probes containing Elavl1a binding sites. Upper, western blotting; lower, quantification level. Error bars, mean  $\pm$  s.d., *n* = 3. *P* values were calculated using Student's *t* test. **g** Demonstration of purified Flag-Elavl1a pulled down by endogenous RNA probes containing Elavl1a binding sites. Upper, western blotting; lower, quantification level. Error bars, mean  $\pm$  s.d., *n* = 3. *P* values were calculated using Student's *t* test. **h** The structure models of designed P1 wild-type, P1 mutant, and P1 rescue RNA probes containing Elavl1a binding sites and flanking regions. **i** Demonstration of endogenous Elavl1a pulled down by designed endogenous RNA probes containing Elavl1a binding sites. Upper, western blotting; lower, quantification level. Error bars, mean  $\pm$  s.d., *n* = 3. *P* values were calculated using Student's *t* test. **j** Demonstration of purified Flag-Elavl1a pulled down by designed endogenous RNA probes containing Elavl1a binding sites. Upper, western blotting; lower, quantification level. Error bars, mean  $\pm$  s.d., *n* = 3. *P* values were calculated using Student's *t* test. **k** EMSA (left) and line graph quantification (right) showing the binding ability of purified Flag-Elavl1a with designed P1 wild-type, P1 mutant, and P1 rescue RNA probes containing Elavl1a binding sites. In total, 100 nM of RNA probes was incubated with different concentrations of Flag-Elavl1a protein. The RNA binding ratio was calculated by (RNA protein) / ((free RNA) + (RNA protein)). Error bars, mean  $\pm$  s.d., *n* = 3





RNA structurally variable elements in Elavl1a binding regions correlate with maternal RNA stability

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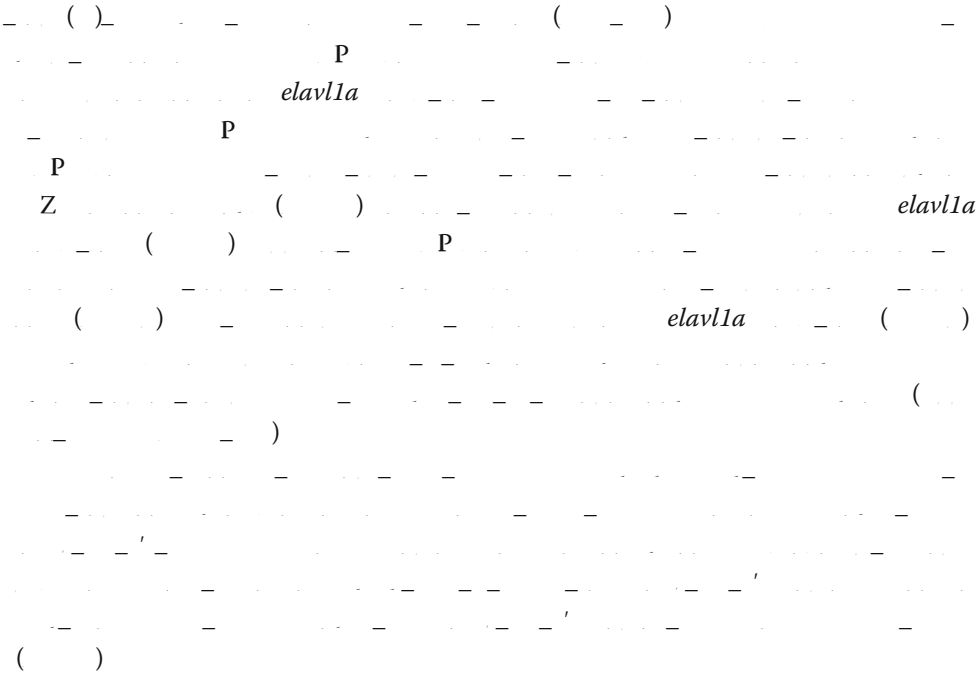
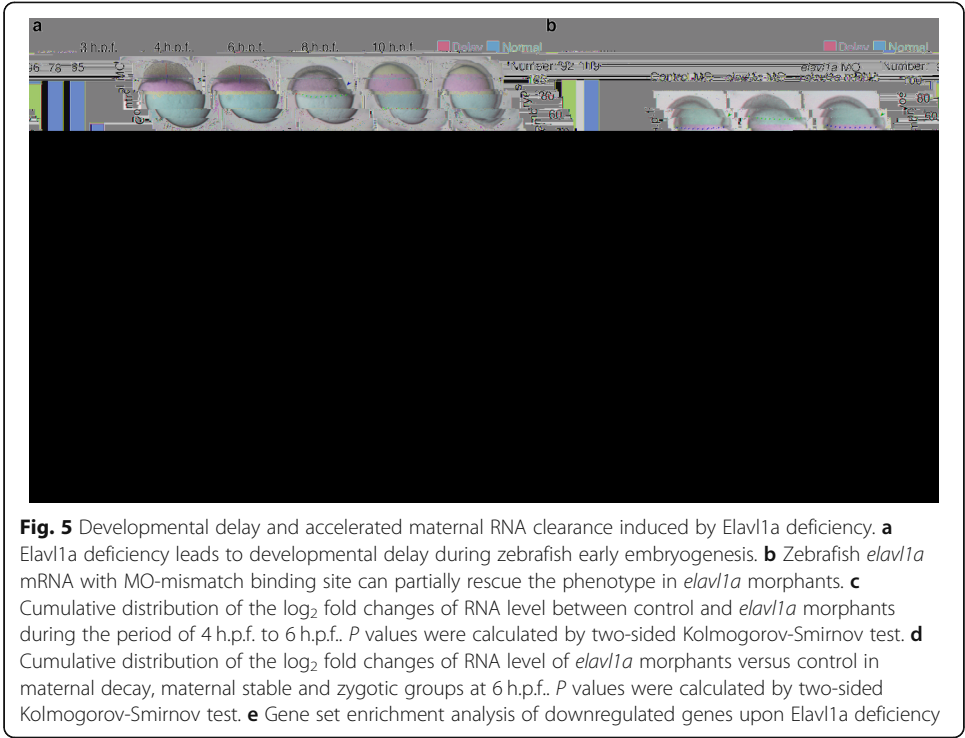


**Elavl1a-mediated mRNA stability is required for early development**

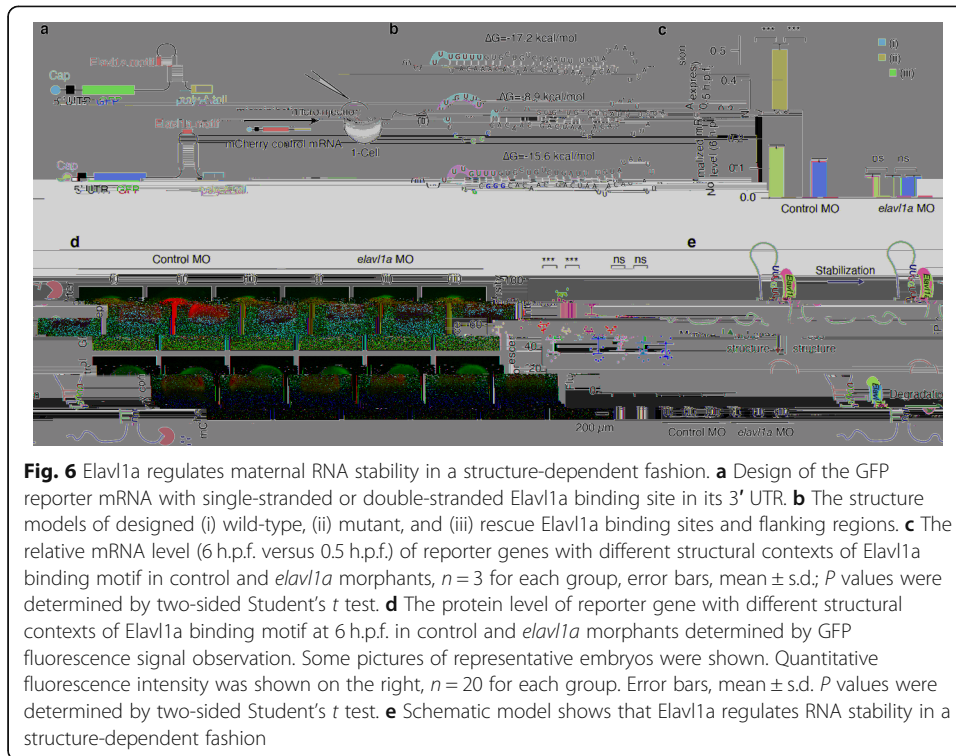
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**Elavl1a regulates maternal RNA stability in a structure-dependent fashion**

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### Discussion



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**Methods**

**Animal models**

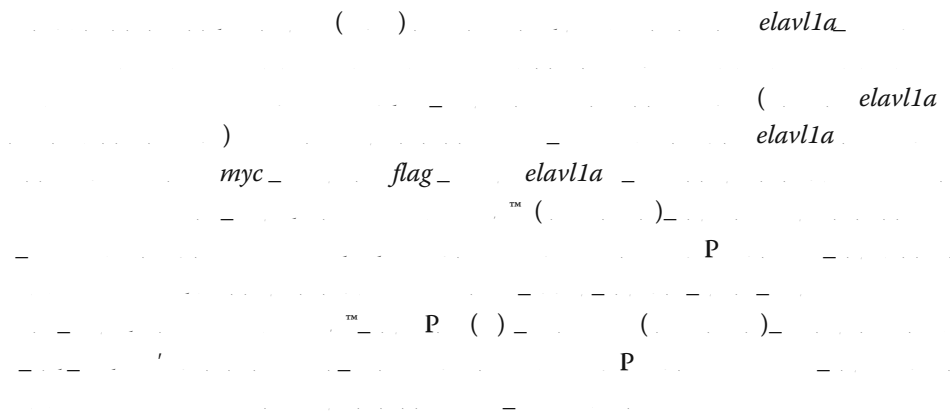
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**Cell lines**

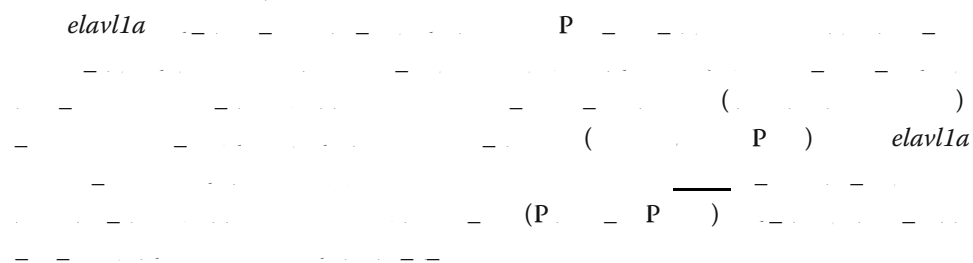
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Morpholinos, vector construction, mRNA synthesis, injection



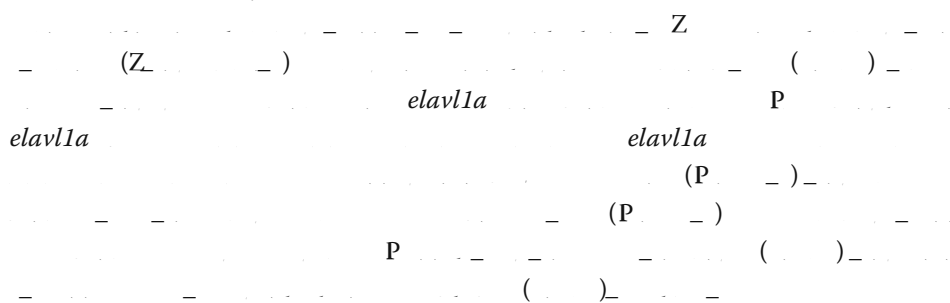
Generation of mutant by CRISPR/Cas9



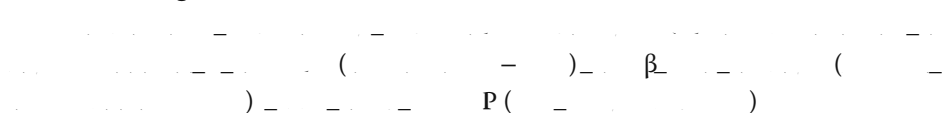
Microscopy



Whole-mount in situ hybridization



Western blotting



**Manual SHAPE analysis**

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**In vivo SHAPE modification**

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**icSHAPE deep-sequencing library preparation**

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**Elavl1a iCLIP**

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**Elavl1a RIP**

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**In vivo isolation of mRBPs from zebrafish embryos**

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**Protein purification in mammalian cells**

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**In vivo RNA pulldown assay**

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**In vitro RNA pulldown assay**

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### Electrophoretic mobility shift assay (EMSA)

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### Identification of structurally variable nucleotides and regions and “hot” structurally variable sites

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### Enrichment of structurally variable regions in different parts of transcripts

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### De novo motif discovery and enrichment analysis of structurally variable regions

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#### De novo motif discovery in structurally variable regions

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#### RBP binding motif enrichment analysis

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**Data processing and peak calling of iCLIP**

*Preprocessing and peak calling*

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*Binding motif identification*

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Gene ontology analysis

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Analysis of the icSHAPE reactivity at zebrafish RBP binding sites

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Quantification and statistical analysis

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Gene	3' UTR	5' UTR	ICSHAPE	RNA-seq	iCLIP
Gene 1	+	+	+	+	+
Gene 2	+	+	+	+	+
Gene 3	+	+	+	+	+
Gene 4	+	+	+	+	+
Gene 5	+	+	+	+	+
Gene 6	+	+	+	+	+
Gene 7	+	+	+	+	+
Gene 8	+	+	+	+	+
Gene 9	+	+	+	+	+
Gene 10	+	+	+	+	+
Gene 11	+	+	+	+	+
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Gene 100	+	+	+	+	+

## Supplementary information

Supplementary information accompanies this paper at <https://doi.org/10.1186/s13059-020-02022-2>.

**Additional file 1:** Supplementary Figures S1-S7.

**Additional file 2: Table S1.** Summary and statistics of icSHAPE, RNA-seq and iCLIP.

**Additional file 3: Table S2.** LFQ intensity of proteins in UV+ and UV- samples at 0, 0.4 and 4 h.p.f.

**Additional file 4: Table S3.** Structurally variable regions between neighboring stages and hot structurally variable regions.

**Additional file 5: Table S4.** Results of motif enrichment analysis in 3' UTR structurally variable regions.

**Additional file 6: Table S5.** Summary of DLE element in structurally variable regions during early development and its associated biological function.

**Additional file 7: Table S6.** Elavl1a binding sites at 4 h.p.f. Elavl1a binding sites identified by Flag-Elavl1a iCLIP at 4 h.p.f. and 6 h.p.f.

**Additional file 8: Table S7.** Maternal and zygotic gene sets categorized by gene expression and SNP.

**Additional file 9: Table S8.** GO term enrichment analysis of down-regulated genes upon elavl1a knockdown at 6 h.p.f.

**Additional file 10: Table S9.** List of oligos used for this Study.

**Additional file 11:** Review history.

## Acknowledgements

We thank Jifeng Wang and Mengmeng Zhang at laboratory of Proteomics, core facility in the Institute of Biophysics, CAS for their technical support of LC-MS analysis, and BIG CAS genomic platform for sequencing.

## Review history

The review history is available as Additional file 11.

## Peer review history

Barbara Cheifet was the primary editor on this article and managed its editorial process and peer review in collaboration with the rest of the editorial team.

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Development Program of China (2018YFA0800200), the National Basic Research Program of China (2019YFA0110002) and the NSFC consulting grant (91940000).

#### Availability of data and materials

The RNA-Seq, iCLIP, and icSHAPE data supporting the conclusions of this article has been deposited in the Gene Expression Omnibus database under accession number GSE120724 [64], and also the Genome Sequence Archive [65] under accession number CRA001139 [66] linked to the project PRJCA001046.

The ribosome profiling data for zebrafish embryos at 2 and 6 h.p.f. was obtained from Gene Expression Omnibus database under accession number GSE52809 (<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE52809>) (Subtelny et al., 2014) [67].

The human ELAVL1 binding sites was obtained from (<https://www.cell.com/cms/10.1016/j.molcel.2011.06.008/attachment/51bc4461-fc31-4e4d-9b6d-c0db20a7e62b/mmc3.xls>) (Lebedeva et al., 2011) [43] and (<https://www.cell.com/cms/10.1016/j.molcel.2011.06.007/attachment/ed673aa9-bc87-4a4e-94b9-64fbaa1a6f61/mmc3.zip>) (Mukherjee et al., 2011) [39].

The zebrafish iCLIP dataset for 23 RBPs was obtained from ([https://track.giraldezlab.org/vejnar\\_et\\_al\\_2019\\_genome\\_research\\_iclip/danRer11/](https://track.giraldezlab.org/vejnar_et_al_2019_genome_research_iclip/danRer11/)) (Vejnar et al., 2019) [32].

The gene set with maternal and paternal SNP information was collected from (<http://dev.biologists.org/lookup/suppl/doi:10.1242/dev.095091/-/DC1>, Harvey et al. 2013) [44].

The source code to reproduce all figures in this study are available on Github repository at site [68] and Zenodo [69].

#### Authors' contributions

B.Y.S. and J.S.Z. performed most of the experiments with assistance from Y.Y., N.Z., and H.L.W.; J.G. and T. Z performed bioinformatics analysis with help from P.L. and B.F.S.; J.H. performed experiments in zebrafish; Y.G.Y., Q.C.Z., and F.L. conceived this project, supervised the study and interpreted the data, and wrote the manuscript with assistance from Z.Y.L., J.S.Z., J.H., J.G., and B.Y.S. The authors read and approved the final manuscript.

#### Ethics approval and consent to participate

Animal experimentation: This study was approved by the Ethical Review Committee in the Institute of Zoology, Chinese Academy of Sciences, China.

#### Competing interests

The authors declare that they have no competing interests.

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