

Life Sciences Reporting Summary

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Please do not complete any field with "not applicable" or n/a. Refer to the help text for what text to use if an item is not relevant to your study. [For final submission](#): please carefully check your responses for accuracy; you will not be able to make changes later.

▶ Experimental design

1. Sample size

Describe how sample size was determined.

Whole genome bisulfite and RNA seq sample sizes were determined based on previous published literature and biostatistician's recommendation.

2. Data exclusions

Describe any data exclusions.

We excluded non-viable and abnormally developed embryos from our analyses.

3. Replication

Describe the measures taken to verify the reproducibility of the experimental findings.

Two biological replicates were performed for RNA seq. For RT-qPCRs, at least three biological replicates were performed to confirm the consistency and successful reproduction of the results.

4. Randomization

Describe how samples/organisms/participants were allocated into experimental groups.

After selecting surviving embryos, controls, mutants and treated animals were scored as described in methods section and figure legends.

5. Blinding

Describe whether the investigators were blinded to group allocation during data collection and/or analysis.

Authors were not blinded because of the obvious morphological phenotypes. Authors were aware of the embryos injected with control DMSO or Aza or AzaD. Quantitative measurements were performed to verify subjectively observed results.

Note: all in vivo studies must report how sample size was determined and whether blinding and randomization were used.

6. Statistical parameters

For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

- | | |
|-------------------------------------|--|
| n/a | Confirmed |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> The <u>exact sample size</u> (<i>n</i>) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.) |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> A statement indicating how many times each experiment was replicated |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> The statistical test(s) used and whether they are one- or two-sided
<i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i> |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> A description of any assumptions or corrections, such as an adjustment for multiple comparisons |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> Test values indicating whether an effect is present
<i>Provide confidence intervals or give results of significance tests (e.g. P values) as exact values whenever appropriate and with effect sizes noted.</i> |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> A clear description of statistics including <u>central tendency</u> (e.g. median, mean) and <u>variation</u> (e.g. standard deviation, interquartile range) |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> Clearly defined error bars in <u>all</u> relevant figure captions (with explicit mention of central tendency and variation) |

See the web collection on [statistics for biologists](#) for further resources and guidance.

► Software

Policy information about [availability of computer code](#)

7. Software

Describe the software used to analyze the data in this study.

Trimmomatic, Bismark, RNA-STAR, DESeq2, Microsoft Excel, Microsoft Powerpoint, and Photoshop.

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). *Nature Methods* [guidance for providing algorithms and software for publication](#) provides further information on this topic.

► Materials and reagents

Policy information about [availability of materials](#)

8. Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a third party.

No unique materials were used.

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).

Sheep Anti-Digoxigenin-AP (11 093 274 910) Roche used for developing in situ hybridization stainings, validated in Thisse and Thisse, 2008 Nat. Protoc.

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.

No eukaryotic cell lines were used in this study.

b. Describe the method of cell line authentication used.

No eukaryotic cell lines were used in this study.

c. Report whether the cell lines were tested for mycoplasma contamination.

No eukaryotic cell lines were used in this study.

d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by [ICLAC](#), provide a scientific rationale for their use.

No eukaryotic cell lines were used in this study.

► Animals and human research participants

Policy information about [studies involving animals](#); when reporting animal research, follow the [ARRIVE guidelines](#)

11. Description of research animals

Provide all relevant details on animals and/or animal-derived materials used in the study.

Adult Zebrafish (*Danio rerio*) and *Astyanax mexicanus* were used to obtain embryos by natural matings. Embryos and larvae up to day 5 were used in different analyses.

Policy information about [studies involving human research participants](#)

12. Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants.

This study did not involve the use of human research participants