Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Research policies, see Authors & Referees and the Editorial Policy Checklist.

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

- The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
- A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
- The statistical test(s) used AND whether they are one- or two-sided

Only common tests should be described solely by name; describe more complex techniques in the Methods section.

- A description of all covariates tested

- A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons

- A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) and variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)

- For null hypothesis testing, the test statistic (e.g. F, t, r) with confidence intervals, effect sizes, degrees of freedom and P value noted. Give P values as exact values wherever suitable.

- For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings

- For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes

- Estimates of effect sizes (e.g. Cohen's d, Pearson's r), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection

NIK Elements (Version 5.20.2, Nikon) and LAS X (Version 3.5.2, Leica) software packages were used for data collection.

Data analysis

All images were analyzed using Fiji (Image J v1.0 and v2.0], Matlab R2018b (Version 9.5.94444, Mathworks), R version [3.3.0], Python Version 2.7] and Imaris (Version 7.0, Oxford Instruments]. Custom codes were written and are included in this article. All statistical analyses were performed using GraphPad Prism (Version 7.0e, and 8.1.2, Graphpad) or estimationstats.com.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

All animals and data generated and analyzed are available upon request from the corresponding authors. Custom codes used in this study are included in the article.
Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

- Life sciences
- Behavioural & social sciences
- Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see [nature.com/documents/nr-reporting-summary-flat.pdf](http://nature.com/documents/nr-reporting-summary-flat.pdf)

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

- Sample size: No sample size calculation was performed. The sample size is chosen based on work of other groups using zebrafish embryos as a model system.
- Data exclusions: No data were excluded from the analysis.
- Replication: All attempts at replication were successful. Experiments were performed at least three times unless otherwise noted in the manuscript.
- Randomization: The experiments with embryos were allocate randomly into experimental groups that were treated differently. For many genetic experiments, embryos were randomly assigned to experiments because the genotype of the embryos was not known until after data collection and genotyping.
- Blinding: Blinding is not relevant to this study. The experiments require the investigators to group the data between control and testing condition to quantify differences. However, embryos of different genotypes were imaged and grouped based on genotype after image collection for data analysis. Thus, at the time of data collection the genotype was unknown.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

| Materials & experimental systems | Methods |
|----------------------------------|---------|
| n/a | Involved in the study |
| ☐ Antibodies | ☑ | Involved in the study |
| ☑ Eukaryotic cell lines | ☑ | ChIP-seq |
| ☒ Palaeontology | ☐ | Flow cytometry |
| ☐ Animals and other organisms | ☐ | MRI-based neuroimaging |
| ☑ Human research participants | ☐ | |
| ☒ Clinical data | ☐ | |

Antibodies

- Antibodies used: anti-Digoxigenin-Alkaline Phosphatase antibody (dilution: 1:5000) from Millipore Sigma, catalog number:11093274910
- Validation: Use of the anti-DIG-AP antibody for in situ hybridization against DIG-labeled anti-sense RNA probes has been reported in zebrafish (Thissie C et al. Nature Protocols 3, 59-69(2008))

Eukaryotic cell lines

- Policy information about [cell lines](#)
- Cell line source(s): Flp-In™ 1-Rex™-293 cells are derived from 293 HEK cells from ATCC and purchased from Invitrogen Catalog number R78007
- Authentication: The cell lines were not tested for authentication in our lab.
- Mycoplasma contamination: The cell lines were not tested for mycoplasma contamination in our lab.
- Commonly misidentified lines (See [ICLAC](#) register): No commonly misidentified lines were used.
### Animals and other organisms

Policy information about studies involving animals: ARRIVE guidelines recommended for reporting animal research.

| Category                  | Description                                                                                                                                 |
|---------------------------|--------------------------------------------------------------------------------------------------------------------------------------------|
| Laboratory animals        | Danio rerio zebrafish strains at adult (3-20 months) and embryonic stages (until 48 hours post fertilization) were used. Females and male adult fish were used for natural mating. Sex of embryos is undetermined. Sex is determined by 75 dpf in zebrafish. |
| Wild animals              | This study did not involve wild animals.                                                                                                                                                               |
| Field-collected samples   | This study did not involve samples collected from the field.                                                                                                                                            |
| Ethics oversight          | Zebrafish care and use of live fish for experiments were approved and overseen by the New York University School of Medicine Institutional Animal Care and Use Committee.                                     |

Note that full information on the approval of the study protocol must also be provided in the manuscript.