

Life Sciences Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form is intended for publication with all accepted life science papers and provides structure for consistency and transparency in reporting. Every life science submission will use this form; some list items might not apply to an individual manuscript, but all fields must be completed for clarity.

For further information on the points included in this form, see [Reporting Life Sciences Research](#). For further information on Nature Research policies, including our [data availability policy](#), see [Authors & Referees](#) and the [Editorial Policy Checklist](#).

► Experimental design

1. Sample size

Describe how sample size was determined.

No sample size calculation was performed.

For each type of experiment, we performed the number of replicates necessary to ensure that each result is reproducible (this is detailed for each experiment in the appropriate section of Methods).

For enhancer reporter assays at least 3 independent transgenic lines derived from different founders were generated to confirm the specificity of the observed expression.

2. Data exclusions

Describe any data exclusions.

NA (no samples were excluded).

3. Replication

Describe whether the experimental findings were reliably reproduced.

All attempts at replication were successful.

4. Randomization

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

This study does not include experimental groups and therefore it does not require randomization.

5. Blinding

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

No experimental groups were allocated in this study

Note: all studies involving animals and/or human research participants must disclose 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 |
| ☐ | ☒ 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.) |
| ☒ | ☐ A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly |
| ☐ | ☒ A statement indicating how many times each experiment was replicated |
| ☐ | ☒ The statistical test(s) used and whether they are one- or two-sided (note: only common tests should be described solely by name; more complex techniques should be described in the Methods section) |
| ☒ | ☐ A description of any assumptions or corrections, such as an adjustment for multiple comparisons |
| ☐ | ☒ The test results (e.g. <i>P</i> values) given as exact values whenever possible and with confidence intervals noted |
| ☐ | ☒ A clear description of statistics including <u>central tendency</u> (e.g. median, mean) and <u>variation</u> (e.g. standard deviation, interquartile range) |
| ☐ | ☒ Clearly defined error bars |

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.

IMAGEJ was used to analyze fin phenotypes. Custom software was used for the 4C-seq experiments. Amira 3D Software 6.0 was used for analysis and segmentation of scanned images. Graph Pad Prism software was used for statistical analysis.

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 for-profit company.

There is no restriction

9. Antibodies

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

No antibodies were used

10. Eukaryotic cell lines

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

No eukaryotic cell lines were used.

b. Describe the method of cell line authentication used.

NA

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

NA

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.

NA

► 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 details on animals and/or animal-derived materials used in the study.

For functional experiments, we employed zebrafish (*Danio rerio*, strain AB/Tübingen) and medaka (*Oryzias latipes*, strain iCab). Adult animals were used for embryo production. Zebrafish and medaka embryos and larvae from both sexes were fixed for analysis from 72 hours to 3 weeks post fertilization. Medaka male and female adult fish were also used for bone staining procedures. DNA was obtained from mouse (*Mus musculus*), anole (*Anolis carolinensis*), coelacanth (*Latimeria menadoensis*), zebrafish (*Danio rerio*), gar (*Lepisosteus oculatus*), skate (*Leucoraja erinacea*), and sea lamprey (*Petromyzon marinus*) adult animals from both sexes.

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 human research participants.