

## 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.

### Statistics

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

- | n/a                                 | Confirmed                                                                                                                                                                                                                                                                                      |
|-------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> The exact sample size ( $n$ ) for each experimental group/condition, given as a discrete number and unit of measurement                                                                                                                                    |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly                                                                                                                                    |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> The statistical test(s) used AND whether they are one- or two-sided<br><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 all covariates tested                                                                                                                                                                                                                                |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons                                                                                                                                                   |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> 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) |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> For null hypothesis testing, the test statistic (e.g. $F$ , $t$ , $r$ ) with confidence intervals, effect sizes, degrees of freedom and $P$ value noted<br><i>Give <math>P</math> values as exact values whenever suitable.</i>                            |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings                                                                                                                                                                      |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes                                                                                                                                                |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> 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 | Bulk RNA-seq data was obtained using the Illumina NovaSeq 6000. Images were acquired via transmitted light microscopy, fluorescence stereomicroscopy (Axion Zoom.V16, ZEISS), or confocal microscopy (SP8, Leica).                                            |
| Data analysis   | Images and statistical analysis was performed using ImageJ (version 1.8.0), Adobe Illustrator 2023, and GraphPad Prism (version 8.3.0). Data were presented as mean $\pm$ SEM, using unpaired two-tailed Student's $t$ -test or one-way analysis of variance. |

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 and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio [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 description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our [policy](#)

All data are available within the Article and Supplementary Files, or obtained from the corresponding authors on reasonable request. The sequence data that support the findings of this study will be deposited in the Genome Sequence Archive in National Genomics Data Center, China National Center for Bioinformatics/Beijing Institute of Genomics, Chinese Academy of Sciences.

# 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](https://www.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     | All samples used in this study were described in the manuscript.          |
| Data exclusions | No data were excluded from analyses.                                      |
| Replication     | At least three independent repeated experiments were carried out.         |
| Randomization   | Samples from both experimental and control groups were randomly selected. |
| Blinding        | No blinding was used in this study.                                       |

## 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

|                                     |                                                                 |
|-------------------------------------|-----------------------------------------------------------------|
| n/a                                 | Involved in the study                                           |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> Antibodies                  |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Eukaryotic cell lines                  |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Palaeontology and archaeology          |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> Animals and other organisms |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Clinical data                          |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Dual use research of concern           |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Plants                                 |

### Methods

|                                     |                                                    |
|-------------------------------------|----------------------------------------------------|
| n/a                                 | Involved in the study                              |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> ChIP-seq                  |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> Flow cytometry |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> MRI-based neuroimaging    |

### Antibodies

|                 |                                                                                                                                                                                                                                                                                                          |
|-----------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Antibodies used | The following primary antibodies were used for immunofluorescence: rabbit anti-Ddx4(1:200), rabbit anti-Pcna(1:200), rabbit anti-Sycp3(1:200), rabbit anti-Nanos2(1:200). These antibodies were generated in prior studies.                                                                              |
| Validation      | rabbit anti-Ddx4(1:200), rabbit anti-Pcna(1:200), rabbit anti-Sycp3(1:200), rabbit anti-Nanos2(1:200)<br><a href="http://doi.org/10.3389/fendo.2023.1044318">http://doi.org/10.3389/fendo.2023.1044318</a> , <a href="https://doi.org/10.1210/endocr/bqab258">https://doi.org/10.1210/endocr/bqab258</a> |

### Animals and other research organisms

Policy information about [studies involving animals](#); ARRIVE guidelines recommended for reporting animal research, and [Sex and Gender in Research](#)

|                         |                                                                                                                                                                                                                                                                                                                      |
|-------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Laboratory animals      | All experiments in this study were conducted using zebrafish obtained from the Institute of Hydrobiology, Chinese Academy of Sciences (Wuhan, China). All zebrafish were maintained and reared at the China Zebrafish Resource Center of the National Aquatic Biological Resource Center (CZRC-NABRC, Wuhan, China). |
| Wild animals            | No wild animals were involved in this study.                                                                                                                                                                                                                                                                         |
| Reporting on sex        | Donor spermatogonial stem cells were derived exclusively from male fish SSCs. Recipient fish were used without sex-based selection.                                                                                                                                                                                  |
| Field-collected samples | This study did not involve samples collected from the field.                                                                                                                                                                                                                                                         |
| Ethics oversight        | All animal experiments are supervised by the Animal Care Committee of the University of Chinese Academy of Sciences.                                                                                                                                                                                                 |

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