

Reporting Summary

Nature Portfolio 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 Portfolio policies, see our [Editorial Policies](#) 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.

n/a	Confirmed
☐	☒ The exact sample size (<i>n</i>) 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 <i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i>
☒	☐ 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. <i>F</i> , <i>t</i> , <i>r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted <i>Give P values as exact values whenever suitable.</i>
☒	☐ 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 <i>d</i> , Pearson's <i>r</i>), 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	QCapture software, Olympus SZX12 microscope equipped with an Olympus QColor3 camera
Data analysis	QCapture software, FASTQC (v0.11.5), STAR (v2.6.0), HTSeq (v1.99.2), DESeq2 (v3.14), R (v4.0.3), RSEM (v1.2.28), MAFFT (v7.490), RAxML (v8), MrBayes (v3.2.7a), SUMOplot, GPS-SUMO

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 and methods used during this study are summarized in this article. The raw data and images are available from the corresponding author upon reasonable request.

Human research participants

Policy information about [studies involving human research participants and Sex and Gender in Research](#).

Reporting on sex and gender	N/A
Population characteristics	N/A
Recruitment	N/A
Ethics oversight	N/A

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

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	Data from <i>Xenopus</i> and lamprey were obtained from $n \geq 3$ biological replicates for each experimental condition with a minimum of $n = 30$ embryos per replicate. Consistency of phenotypes across each replicates (loss, gain, no change in gene expression) were based on internal control experiments in which the unmanipulated side of the embryo serves as the control condition.
Data exclusions	Dead embryos from both lamprey and <i>Xenopus</i> were discarded and not included in the sample sizes for replicate experiments.
Replication	All gene expression and RNA-Seq analyses for both lamprey and <i>Xenopus</i> were performed in triplicate.
Randomization	Lamprey and <i>Xenopus</i> adults were chosen based on overall health, fertility, and quality of eggs and gametes.
Blinding	Blinding was not necessary for our experiments because experimental and control conditions were performed in parallel. Details are provided in Methods.

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
☐	☒ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☐	☒ Animals and other organisms
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used	Anti-Digoxigenin-AP, polyclonal, diluted 1:2000 (Sigma SKU 11093274910), anti-PAX3 (DSHB)
Validation	Anti-Digoxigenin-AP: validated by the manufacturer: "The polyclonal antibody from sheep is specific to digoxigenin and digoxin and shows no cross-reactivity with other steroids, such as human estrogens and androgens." (https://www.sigmaaldrich.com/US/en/product/roche/11093274910)

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	Xenopus laevis males and females of unknown age
Wild animals	Live lamprey were caught in streams connected with Lake Michigan. Animals were transported to the laboratory in chilled water.
Reporting on sex	N/A
Field-collected samples	N/A
Ethics oversight	All procedures were approved by Northwestern University’s Institutional Animal Care and Use Committee (IACUC A3283-01)

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