nature portfolio

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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 (*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 whenever 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	Image acquisition: Live images were obtained using a Zeiss Discovery microscope and an AxioVision v4.7.1 camera. Fluorescent images were acquired using a Leica SP6 or a Leica STELLARIS 5 confocal microscopes
Data analysis	MAFFT v7, IQTree v1.6.4, Mr Bayes v3.2, Geneious v8.1.7, and Figtree v1.4.4., software were used for phylogenetic analyses. FastQC v0.12, Rcorrector v1.0.4, TrimGalore 3.7, Trinity-v2.14.0, Bowtie2 v2.5.4, OrthoFinder v. 2.5.5, DESeq2 v1.44.0, and Pheatmap v1.0.12 were used for transcriptome assembly and differential gene expression. ImageJ v1.55, Imaris v10, Adobe Illustrator v26, and Biorender, were used for image processing and making figures. GraphPad Prism v10.3.1 was used for graphs and statistical analyses .

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

Data for gene sequences and primers are provided in Supplementary Table 1, Supplementary Table 2, Supplementary Table 4, Supplementary Table 5, and Supplementary Data 1.
HCR Fish probe design was made through custom Python and R scripts available at https://github.com/cooketho/make_hcr_probes

Research involving human participants, their data, or biological material

Policy information about studies with human participants or human data. See also policy information about sex, gender (identity/presentation), and sexual orientation and race, ethnicity and racism.

Reporting on sex and gender	*Use the terms sex (biological attribute) and gender (shaped by social and cultural circumstances) carefully in order to avoid confusing both terms. Indicate if findings apply to only one sex or gender; describe whether sex and gender were considered in study design; whether sex and/or gender was determined based on self-reporting or assigned and methods used. Provide in the source data disaggregated sex and gender data, where this information has been collected, and if consent has been obtained for sharing of individual-level data; provide overall numbers in this Reporting Summary. Please state if this information has not been collected. Report sex- and gender-based analyses where performed, justify reasons for lack of sex- and gender-based analysis.*
Reporting on race, ethnicity, or other socially relevant groupings	*Please specify the socially constructed or socially relevant categorization variable(s) used in your manuscript and explain why they were used. Please note that such variables should not be used as proxies for other socially constructed/relevant variables (for example, race or ethnicity should not be used as a proxy for socioeconomic status). Provide clear definitions of the relevant terms used, how they were provided (by the participants/respondents, the researchers, or third parties), and the method(s) used to classify people into the different categories (e.g. self-report, census or administrative data, social media data, etc.) Please provide details about how you controlled for confounding variables in your analyses.*
Population characteristics	*Describe the covariate-relevant population characteristics of the human research participants (e.g. age, genotypic information, past and current diagnosis and treatment categories). If you filled out the behavioural & social sciences study design questions and have nothing to add here, write "See above."*
Recruitment	*Describe how participants were recruited. Outline any potential self-selection bias or other biases that may be present and how these are likely to impact results.*
Ethics oversight	*Identify the organization(s) that approved the study protocol.*

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

Life sciences study design

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

Sample size	Critical sample sizes were chosen according to researcher maximum effort and are similar to previously planarian published data (5-10 animals) for highly penetrant phenotypes.
Data exclusions	There were no data exclusions in any of the analyses.
Replication	All experimental findings were reproduced in at least 2 independent experiments. For single-eye RNA sequencing experiment we performed individual RNA libraries for each sample, resulting in a total of 12 libraries for G. multidiverticulata (discernible), 12 for G. multidiverticulata (non-discernible), 11 for G. dorotocephala, 11 for S. mediterranea, and 13 for D. japonica.
Randomization	Animals for all experiments were randomly selected from a large collection of animals.
Blinding	Investigators were not blinded during data collection, but majority of the analyses (specified in the text) were analyzed blindly, except when it

Blinding | was not feasible

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
☒	☐ Plants

Methods

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

Antibodies

Antibodies used: anti-rabbit-HRP (Thermo Fisher 65-6120)
anti-mouse IgG (H + L) antibody Alexa conjugated (Invitrogen A-10029, A-11031, A-21235)
anti-arrestin (VC1, gift from K. Agata)
anti-H3P (Millipore 05-817R-I, clone 63-1C-8)
Goat anti-Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 647 (Thermo Fisher)

Validation: No validation of these antibodies was performed. Literature describing their common use in planarian research is cited.

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: Girardia dorotocephala (commercially purchased by Carolina Biological Supply Company, Burlington, NC; Item #132970), Schmidtea mediterranea strain (CIW4), and Dugesia japonica (kindly provided by Agata Lab)

Wild animals: Girardia multidiverticulata were collected from the cave Buraco do Bicho, located in Serra da Bodoquena, Mato Grosso do Sul, Brazil (20°33'50"S and 56°43'50"W), under the ICMBio SISBIO permit 93921-1. This species is currently classified as Critically Endangered according to the Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio), and a culture of this species is maintained in the Brown Lab in the Department of Zoologyy at the University of São Paulo

Reporting on sex: *Indicate if findings apply to only one sex; describe whether sex was considered in study design, methods used for assigning sex. Provide data disaggregated for sex where this information has been collected in the source data as appropriate; provide overall numbers in this Reporting Summary. Please state if this information has not been collected. Report sex-based analyses where performed, justify reasons for lack of sex-based analysis.*

Field-collected samples: Girardia multidiverticulata were maintained in room temperature, in small tanks, in the dark, with freshwater or Montjuic planarian water, and were fed weekly with pieces of liver. Animals were starved 1–2 weeks prior to experiments.

Ethics oversight: No ethical oversight or protocols were required as all animals used in this study were invertebrates.

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

Plants

Seed stocks	*Report on the source of all seed stocks or other plant material used. If applicable, state the seed stock centre and catalogue number. If plant specimens were collected from the field, describe the collection location, date and sampling procedures.*
Novel plant genotypes	*Describe the methods by which all novel plant genotypes were produced. This includes those generated by transgenic approaches, gene editing, chemical/radiation-based mutagenesis and hybridization. For transgenic lines, describe the transformation method, the number of independent lines analyzed and the generation upon which experiments were performed. For gene-edited lines, describe the editor used, the endogenous sequence targeted for editing, the targeting guide RNA sequence (if applicable) and how the editor was applied.*
Authentication	*Describe any authentication procedures for each seed stock used or novel genotype generated. Describe any experiments used to assess the effect of a mutation and, where applicable, how potential secondary effects (e.g. second site T-DNA insertions, mosiacism, off-target gene editing) were examined.*