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	FIJI ImageJ2 2.16.0/1.54p
Data analysis	RStudio 2024.12.1+563 (2024.12.1+563); R v4.6.0; Cellpose 3.1.0; Seurat 5.4.0; DoubletFinder 2.0.6; ggplot2 4.0.1; Stats v3.6.2; Circlize v0.6.16; Cellchat v1.4.0

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

The raw FASTQ files generated during the current study are available under GSA #CRA033265 and on SRA under #PRJNA1448102. The processed Seurat datasets are available on GEO under #GSE327206. Source Data of non scRNA-seq figure data are provided with this paper. All relevant scRNAseq code will be deposited on GitHub upon publication under https://github.com/angellswearera/scRNAseq_xenopus_regen_wills and can be accessed via release doi: 10.5281/zenodo.20549290.117 All other code is available within the Source Data folder.

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	n/a
Reporting on race, ethnicity, or other socially relevant groupings	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

Life sciences study design

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

Sample size	Samples sizes for scRNAseq experiment were determined based on animal availability then organized across timepoints by expected cell collection size. Other sample sizes were chosen either based on availability (i.e. BrdU, Dendra2) or power analysis (HCR).
Data exclusions	Cells collected via scRNAseq were excluded from data analysis based on collection quality, based on metrics described in the Methods. No other data was excluded. Data for HCR experiments was only excluded at the un7dpa timepoint, where cells were downsampled randomly to adjust for the difference in tissue size and cell count.
Replication	scRNAseq experiments were collected as bulk samples with many biological replicates, as outlined in Methods. HCR experiments were duplicated both between tadpoles and between tadpole clutches in order to control for clutch-specific effects.
Randomization	Animals were randomly assigned regeneration timepoints for both scRNAseq and other experiments.
Blinding	Blinding was not possible, due to easily identifiable phenotypes such as tadpole age and injury status.

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-BrdU (Fisher: PA5-32256) at 1:200; anti-HuC/HuD (Invitrogen: A21271/16A11) at 1:200; anti-TH (Immunostar: 22941) at 1:200
Validation	Antibodies were validated against expected patterns of localization.

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	Animals were aged as reported. Animals were either Xtr. Wildtype strain or Xtr.Tg(pax6:GFP;cryga:RFP;actc1:RFP)Grngr tadpoles, as described. All animals were mated in house from sexually mature parents bought from the National Xenopus Resource Center.
Wild animals	n/a
Reporting on sex	Tadpoles were not sorted based on sex and thus sex-specific data was not collected.
Field-collected samples	n/a
Ethics oversight	Use of Xenopus tropicalis was carried out in accordance with ethical regulations and under the approval and oversight of the IACUC committee and Office of Animal Welfare at UW, an AALAC-accredited institution

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

Plants

Seed stocks	n/a
Novel plant genotypes	n/a
Authentication	n/a

Flow Cytometry

Plots

Confirm that:

☒ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

☒ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).

☒ All plots are contour plots with outliers or pseudocolor plots.

☒ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation	Tail tissues were collected within 30 minutes of amputation and added to a vial with 72 µl trypLE (ThermoFisher Scientific:12604013) and 3 µl 100 mg/mL collagenase P (ROCHE:11213857001) for every 100 tail tissues. Samples were moved to a 28C water bath for dissociation and triturated with a p200 every 5 minutes until dissociated fully and no chunks was visible (30-60 minutes). To quench the reaction, 150 µl of ice cold 10% FBS was added for every 100 tail tissues. Samples were then spun down for 5 minutes at 1000xg. Supernatant was disposed and cells were resuspended in 300 µl 10% FBS for FACS
Instrument	BD FACSAria III
Software	FACSDiva
Cell population abundance	Cell abundance was determined with a Countess 3 FL Automated Cell Counter (ThermoFisher Scientific). Cells were not retested for GFP+ cell abundance.
Gating strategy	Cells were gated by FSC/SSC, then by assessing the GFP-/GFP+ cell boundary. This gate was more permissive (i.e. it was drawn to include cells along the boundary of these two populations) to collect as many GFP+ neural cells and slightly GFP+ neurons as possible.

☒ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.