# 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 <u>Editorial Policies</u> and the <u>Editorial Policy Checklist</u>.

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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.
X	A description of all covariates tested
$\boxtimes$	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>
$\times$	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
X	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 <u>statistics for biologists</u> contains articles on many of the points above.

#### Software and code

Policy information about availability of computer code

Data collection

No software used for data collection.

Data analysis

R 3.4.1, Seurat v3.1.4, Bowtie v1.1,1, RSEMv1.2.19, Python 2.7, samtools 1.3.1, pysam 0.9.1, bcl2fastq v2.18, URD v1.0.1, BLAST website

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 generated in this study are included in this published article and supplementary files. Sequencing raw reads and processed counts matrices associated with this study will be deposited as NCBI BioProjects PRJNA888438 (postembryonic development) and PRJNA908236 (regeneration time course). Processed count matrices and R scripts are also available on Github with the zenodo DOI: 10.5281/zenodo.7700424. An online resource was generated to access and visualize the scRNA-seq, found at https://dredge.ptgolden.org/sc/?page=home.

Human rese	arch parti	cipants		
Policy information	about <u>studies ir</u>	nvolving human research participants and Sex and Gender in Research.		
Reporting on sex	and gender	Not applicable to this study.		
Population chara	cteristics	Not applicable to this study.		
Recruitment		Not applicable to this study.		
Ethics oversight		Not applicable to this study.		
Note that full informa	te that full information on the approval of the study protocol must also be provided in the manuscript.			
Field-spe	ecific re	porting		
Please select the or	ne below that is	the best fit for your research. If you are not sure, read the appropriate sections before making your selection.		
X Life sciences	В	ehavioural & social sciences		
For a reference copy of t	the document with	all sections, see <u>nature.com/documents/nr-reporting-summary-flat.pdf</u>		
Life scier	nces stu	udy design		
All studies must dis	sclose on these	points even when the disclosure is negative.		
Sample size	Determined by the limits of the inDrops assay which states 3000 cells/library. Each experiment was done in triplicate i.e. three libraries were made per sample. For FISH experiments, experiments were done in at least triplicate with at least 5 animals per condition and repeated successfully.			
Data exclusions	No data were e	xcluded from this study.		
Replication	At least 3 libraries per sample were successfully prepared for the single-cell data. For FISH experiments, experiments were done in at least triplicate with at least 5 animals per condition and repeated successfully.			
Randomization	Cell clustering v	vas performed using unbiased statistical methods and then fine-tuned based on FISH corroboration		
Blinding	Blinding is not relevant because single-cell data analysis was performed using an unbiased pipeline and then corroborated by FISH.			
<del> </del>	<u> </u>	pecific materials, systems and methods		
		about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, 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 Methods				
n/a   Involved in the study		n/a   Involved in the study   ☐ ChIP-seq		
Eukaryotic cell lines		Flow cytometry		
	ogy and archaeol			
Animals an	id other organism :a			
Dual use research of concern				
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### Animals and other research organisms

Policy information about <u>studies involving animals</u>; <u>ARRIVE guidelines</u> recommended for reporting animal research, and <u>Sex and Gender in Research</u>

Laboratory animals

Hofstenia miamia; Ages include hatchling juveniles (14 days post lay) through late adult (>90 days post lay). Single cell data were

Laboratory animals	collected from animals from 4 different ages (as outlined in the methods). Most FISH were performed in 2-3 week old animals. Germ line FISH were performed in adult animals.
Wild animals	This study did not include wild caught animals.
Reporting on sex	The animals are hermaphrodites and sampled at random.
Field-collected samples	No samples were collected in the field for this study.
Ethics oversight	No ethical approval required because these are unregulated marine invertebrate animals.

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