

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	Imaging data were collected with Zeiss Zen Black and Leica LAS X software.
Data analysis	Images were analyzed using FIJI/ImageJ version 2.16. Single cell RNA-seq data were analyzed using the Seurat, Monocle3 pipelines for R. The cross-species comparison was performed using the python based SAMap pipeline. Additional scripts for clustering analysis and cross-species analyses generated by this study are available on GitHub (https://github.com/Perpag/Pm-ovary-clustering-analysis) and Zenodo (https://zenodo.org/records/20185904) as described in the Code Availability Statement. The computational and image analysis workflows utilized Cell Ranger Software Suite v7.1.0, R v4.4.1, Seurat v5.3.0, AGAT v0.7.0, GNU coreutils v8.26, SAMap v1.02, Monocle3 v1.3.7, EnhancedVolcano v1.22.0, topGO v2.56.0, clusterProfiler v4.12.6, Fiji/ImageJ v2.16.0, the Fiji plugin BigStitcher v2.3.4, Amira 2024.1, and HCR 3.0 Probe Maker v2.0.

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 scRNA-seq data generated in this study have been deposited in the NCBI Gene Expression Omnibus database under accession number GSE292963 (<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE292963>). An interactive single cell atlas for the *P. miniata* integrated dataset generated in this study is available through the Broad Institute's Single Cell Portal at https://singlecell.broadinstitute.org/single_cell/study/SCP3291.

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	not applicable (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	A sample size of 3 ovary biopsies from three different individual sea stars was determined to be sufficient to cover the range of reproductive states observed during the reproductive cycle.
Data exclusions	No data were excluded from this study.
Replication	Experiments were independently replicated multiple times across diverse biological samples. Single-cell RNA sequencing was performed on ovaries from three independent adult female sea stars, while HCR, FISH, IHC, and fluorescent staining experiments were repeated at least three times, with specific figure panels representing between 3 and 7 independent biological replicates, each using at least three ovarian lobes. Ovary LDN-193189 experiments were independently repeated at least two times using matched control and treated samples from the same female and included analysis of a minimum of 10 follicles per condition, while larval LDN-193189 experiments were independently repeated at least three times.
Randomization	This study uses wild-caught sea stars of mixed genetic backgrounds. Because there are no visual markers that allow one to select individuals with particular traits, this study randomly selected its experimental subjects.
Blinding	Much of the work in our study requires highly specialized knowledge and experimental skills, so blinding was not feasible for our lab and relevant to the descriptive work done. Blinding was applicable to Fig 6 h where images were blinded to the person doing the scoring.

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

The following antibodies were used in this study: mouse monoclonal DM1 α anti- α -tubulin (Santa Cruz Biotechnology, catalog #sc-32293, 1:100), mouse monoclonal anti-acetylated tubulin (Sigma-Aldrich, catalog #T6793, 1:100), mouse monoclonal anti- α -tubulin (Sigma-Aldrich, catalog #T5168, 1:100), anti-MHC (custom-made anti-SpMHC, PRIMM, Italy, gift from Dr. Ina Arnone, 1:50), rabbit polyclonal anti-serotonin (Sigma-Aldrich, catalog #S5545, 1:500), rabbit polyclonal anti-TH (Sigma-Aldrich, catalog #AB152, 1:100), mouse monoclonal anti-RNA polymerase II (Abcam, catalog #ab5408, 1:250), mouse monoclonal anti-Myosin II (Developmental Studies Hybridoma Bank, clone CMII 25, 1:50), mouse monoclonal anti-pSMAD1/5 (Cell Signaling Technology, clone 41D10, catalog #9516T, 1:200), rabbit monoclonal anti-HA (Cell Signaling Technology, clone C29F4, 1:300), goat anti-mouse Alexa Fluor 488 secondary antibody (Invitrogen, catalog #A-11001, 1:1000), goat anti-rabbit Alexa Fluor 546 secondary antibody (Invitrogen, catalog #A11035, 1:1000 or 1:300 for expansion microscopy), and goat anti-rabbit Alexa Fluor 488 secondary antibody (Invitrogen, catalog #A-11034, 1:200). The monoclonal antibody 1E11 was a gift from Dr. Ina Arnone and was used at a 1:20 dilution. Anti-Digoxigenin antibody (Sigma-Aldrich, catalog #11207733910) was used at 1:1000 for FISH experiments.

Validation

Anti- α tubulin (Sigma # T5168) and DM1 α (Santa Cruz Biosciences #sc-32293) were used to visualize the nervous system and microtubules (Kim N, et al., Repulsive Sema3E-Plexin-D1 signaling coordinates both axonal extension and steering via activating an autoregulatory factor, Mtss1. Elife. 2024 Mar 25;13:e96891. doi: 10.7554/eLife.96891. and Chinen T, et al., The γ -tubulin-specific inhibitor gatastatin reveals temporal requirements of microtubule nucleation during the cell cycle. Nat Commun. 2015 Oct 27;6:8722. doi: 10.1038/ncomms9722.) Anti-acetylated tubulin (Sigma # T67930) was used to label cilia (Paganos P, et al., Single-cell RNA sequencing of the Strongylocentrotus purpuratus larva reveals the blueprint of major cell types and nervous system of a non-chordate deuterostome. Elife. 2021 Nov 25;10:e70416. doi: 10.7554/eLife.70416.) The echinoderm panneuronal marker 1e11 (gift from Dr. Arnone) to label the nervous system elements in echinoderm ovaries (Burke RD, et al., A synaptotagmin gene in the sea urchin Strongylocentrotus purpuratus. J Comp Neurol. 2006 May 10;496(2):244-51. doi: 10.1002/cne.20939.), anti-serotonin (5HT) to label serotonergic neurons and serotonin in the germ lineage (Yaguchi, J., Yaguchi, S. Sea urchin larvae utilize light for regulating the pyloric opening. BMC Biol 19, 64 (2021). https://doi.org/10.1186/s12915-021-00999-1), anti-TH to label dopaminergic neurons (Paganos P, et al., Single-cell RNA sequencing of the Strongylocentrotus purpuratus larva reveals the blueprint of major cell types and nervous system of a non-chordate deuterostome. Elife. 2021 Nov 25;10:e70416. doi: 10.7554/eLife.70416.), the echinoderm specific antibody against MHC to label muscles (Andrikou C, et al., Logics and properties of a genetic regulatory program that drives embryonic muscle development in an echinoderm. Elife. 2015 Jul 28;4:e07343. doi: 10.7554/eLife.07343.), the anti-RNA polymerase II to label DNA as it recognizes RNA pol II that acts as a scaffold to recruit other proteins, essentially marking the DNA for different stages of transcription (Swartz SZ, et al., Quiescent Cells Actively Replenish CENP-A Nucleosomes to Maintain Centromere Identity and Proliferative Potential. Dev Cell. 2019 Oct 7;51(1):35-48.e7. doi: 10.1016/j.devcel.2019.07.016.). Non-muscle anti-Myosin II (Developmental Studies Hybridoma bank # CMII 25) was used to label cell membranes (Colpan M, et al., CAP2 is a regulator of actin pointed end dynamics and myofibrillogenesis in cardiac muscle. Commun Biol. 2021 Mar 19;4(1):365. doi: 10.1038/s42003-021-01893-w.). Anti-HA antibody (CST #C29F4) recognizes a widely used tag that has been extensively validated in the field and was used to recognize the commercial HAK-actin probe which carries this tag. Anti-pSMAD1/5(41D10) (Cell Signaling Technology #9516T) specifically detects endogenous levels of SMAD1 and SMAD5 only when dually phosphorylated at Ser463 and Ser465 and is also predicted to detect SMAD9 (SMAD8) when phosphorylated at Ser465 and Ser467. (Pieplow A, et al., CRISPR-Cas9 editing of non-coding genomic loci as a means of controlling gene expression in the sea urchin. Dev Biol. 2021 Apr;472:85-97. doi: 10.1016/j.ydbio.2021.01.003)

Eukaryotic cell lines

Policy information about [cell lines and Sex and Gender in Research](#)

Cell line source(s)

State the source of each cell line used and the sex of all primary cell lines and cells derived from human participants or vertebrate models.

Authentication

Describe the authentication procedures for each cell line used OR declare that none of the cell lines used were authenticated.

Mycoplasma contamination

Confirm that all cell lines tested negative for mycoplasma contamination OR describe the results of the testing for mycoplasma contamination OR declare that the cell lines were not tested for mycoplasma contamination.

Commonly misidentified lines
(See [ICLAC](#) register)

Name any commonly misidentified cell lines used in the study and provide a rationale for their use.

Palaeontology and Archaeology

Specimen provenance	<i>Provide provenance information for specimens and describe permits that were obtained for the work (including the name of the issuing authority, the date of issue, and any identifying information). Permits should encompass collection and, where applicable, export.</i>
Specimen deposition	<i>Indicate where the specimens have been deposited to permit free access by other researchers.</i>
Dating methods	<i>If new dates are provided, describe how they were obtained (e.g. collection, storage, sample pretreatment and measurement), where they were obtained (i.e. lab name), the calibration program and the protocol for quality assurance OR state that no new dates are provided.</i>
☐ Tick this box to confirm that the raw and calibrated dates are available in the paper or in Supplementary Information.	
Ethics oversight	<i>Identify the organization(s) that approved or provided guidance on the study protocol, OR state that no ethical approval or guidance was required and explain why not.</i>

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

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	Species: adult <i>Patiria miniata</i> sea stars. Strain: not applicable, as these were wild-caught marine invertebrates of mixed genetic backgrounds. Sex: females were used for ovary isolation and ovarian experiments; larvae were generated from fertilized embryos. Age: exact age was unknown because the animals were wild-caught adults.
Wild animals	Animals were obtained from South Coast Bio, San Diego, CA, USA, and Monterey Abalone Company, Monterey, CA, USA. Capture and transport were performed by the suppliers according to their standard collection and shipping procedures. After arrival, animals were maintained in temperature-controlled circulating seawater aquaria at 15°C at the Marine Resources Center, Marine Biological Laboratory, Woods Hole, MA, USA. For ovarian experiments, ovarian fragments were surgically isolated from adult females through the oral side of the arm using minimally invasive procedures. No animals were killed as part of this study, and animals were maintained alive in aquarium systems following tissue collection procedures. Sea stars are marine invertebrates and are not subject to IACUC regulation
Reporting on sex	As our study focuses on oocyte development, only females were used in this study. Sex determination is made by gonad biopsy.
Field-collected samples	Field collected samples were not used.
Ethics oversight	No ethical approval was required for this study as it only used marine invertebrate animals, which are not subject to IACUC regulation. Nonetheless, the animals were treated with the utmost respect and as humanely as possible.

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

Clinical data

Policy information about [clinical studies](#)

All manuscripts should comply with the ICMJE [guidelines for publication of clinical research](#) and a completed [CONSORT checklist](#) must be included with all submissions.

Clinical trial registration	<i>Provide the trial registration number from ClinicalTrials.gov or an equivalent agency.</i>
Study protocol	<i>Note where the full trial protocol can be accessed OR if not available, explain why.</i>
Data collection	<i>Describe the settings and locales of data collection, noting the time periods of recruitment and data collection.</i>
Outcomes	<i>Describe how you pre-defined primary and secondary outcome measures and how you assessed these measures.</i>

Dual use research of concern

Policy information about [dual use research of concern](#)

Hazards

Could the accidental, deliberate or reckless misuse of agents or technologies generated in the work, or the application of information presented in the manuscript, pose a threat to:

- | | |
|-------------------------------------|---|
| No | Yes |
| ☒ | ☐ Public health |
| ☒ | ☐ National security |
| ☒ | ☐ Crops and/or livestock |
| ☒ | ☐ Ecosystems |
| ☒ | ☐ Any other significant area |

Experiments of concern

Does the work involve any of these experiments of concern:

- | | |
|-------------------------------------|--|
| No | Yes |
| ☒ | ☐ Demonstrate how to render a vaccine ineffective |
| ☒ | ☐ Confer resistance to therapeutically useful antibiotics or antiviral agents |
| ☒ | ☐ Enhance the virulence of a pathogen or render a nonpathogen virulent |
| ☒ | ☐ Increase transmissibility of a pathogen |
| ☒ | ☐ Alter the host range of a pathogen |
| ☒ | ☐ Enable evasion of diagnostic/detection modalities |
| ☒ | ☐ Enable the weaponization of a biological agent or toxin |
| ☒ | ☐ Any other potentially harmful combination of experiments and agents |

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, mosaicism, off-target gene editing) were examined.

ChIP-seq

Data deposition

- ☐ Confirm that both raw and final processed data have been deposited in a public database such as [GEO](#).
- ☐ Confirm that you have deposited or provided access to graph files (e.g. BED files) for the called peaks.

Data access links

May remain private before publication.

For "Initial submission" or "Revised version" documents, provide reviewer access links. For your "Final submission" document, provide a link to the deposited data.

Files in database submission

Provide a list of all files available in the database submission.

Genome browser session

(e.g. [UCSC](#))

Provide a link to an anonymized genome browser session for "Initial submission" and "Revised version" documents only, to enable peer review. Write "no longer applicable" for "Final submission" documents.

Methodology

Replicates

Describe the experimental replicates, specifying number, type and replicate agreement.

Sequencing depth

Describe the sequencing depth for each experiment, providing the total number of reads, uniquely mapped reads, length of reads and whether they were paired- or single-end.

Antibodies

Describe the antibodies used for the ChIP-seq experiments; as applicable, provide supplier name, catalog number, clone name, and lot number.

Peak calling parameters

Specify the command line program and parameters used for read mapping and peak calling, including the ChIP, control and index files used.

Data quality

Describe the methods used to ensure data quality in full detail, including how many peaks are at FDR 5% and above 5-fold enrichment.

Software

Describe the software used to collect and analyze the ChIP-seq data. For custom code that has been deposited into a community repository, provide accession details.

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

Describe the sample preparation, detailing the biological source of the cells and any tissue processing steps used.

Instrument

Identify the instrument used for data collection, specifying make and model number.

Software

Describe the software used to collect and analyze the flow cytometry data. For custom code that has been deposited into a community repository, provide accession details.

Cell population abundance

Describe the abundance of the relevant cell populations within post-sort fractions, providing details on the purity of the samples and how it was determined.

Gating strategy

Describe the gating strategy used for all relevant experiments, specifying the preliminary FSC/SSC gates of the starting cell population, indicating where boundaries between "positive" and "negative" staining cell populations are defined.

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

Magnetic resonance imaging

Experimental design

Design type

Indicate task or resting state; event-related or block design.

Design specifications

Specify the number of blocks, trials or experimental units per session and/or subject, and specify the length of each trial or block (if trials are blocked) and interval between trials.

Behavioral performance measures

State number and/or type of variables recorded (e.g. correct button press, response time) and what statistics were used to establish that the subjects were performing the task as expected (e.g. mean, range, and/or standard deviation across subjects).

Acquisition

Imaging type(s)

Specify: functional, structural, diffusion, perfusion.

Field strength

Specify in Tesla

Sequence & imaging parameters

Specify the pulse sequence type (gradient echo, spin echo, etc.), imaging type (EPI, spiral, etc.), field of view, matrix size, slice thickness, orientation and TE/TR/flip angle.

Area of acquisition

State whether a whole brain scan was used OR define the area of acquisition, describing how the region was determined.

Diffusion MRI

☐ Used☐ Not used

Preprocessing

Preprocessing software

Provide detail on software version and revision number and on specific parameters (model/functions, brain extraction, segmentation, smoothing kernel size, etc.).

Normalization

If data were normalized/standardized, describe the approach(es): specify linear or non-linear and define image types used for transformation OR indicate that data were not normalized and explain rationale for lack of normalization.

Normalization template	Describe the template used for normalization/transformation, specifying subject space or group standardized space (e.g. original Talairach, MNI305, ICBM152) OR indicate that the data were not normalized.
Noise and artifact removal	Describe your procedure(s) for artifact and structured noise removal, specifying motion parameters, tissue signals and physiological signals (heart rate, respiration).
Volume censoring	Define your software and/or method and criteria for volume censoring, and state the extent of such censoring.

Statistical modeling & inference

Model type and settings	Specify type (mass univariate, multivariate, RSA, predictive, etc.) and describe essential details of the model at the first and second levels (e.g. fixed, random or mixed effects; drift or auto-correlation).
Effect(s) tested	Define precise effect in terms of the task or stimulus conditions instead of psychological concepts and indicate whether ANOVA or factorial designs were used.
Specify type of analysis:	☐ Whole brain ☐ ROI-based ☐ Both
Statistic type for inference	Specify voxel-wise or cluster-wise and report all relevant parameters for cluster-wise methods.
(See Eklund et al. 2016)	
Correction	

Models & analysis

n/a	Involvement in the study
☐	☐ Functional and/or effective connectivity
☐	☐ Graph analysis
☐	☐ Multivariate modeling or predictive analysis
Functional and/or effective connectivity	Report the measures of dependence used and the model details (e.g. Pearson correlation, partial correlation, mutual information).
Graph analysis	Report the dependent variable and connectivity measure, specifying weighted graph or binarized graph, subject- or group-level, and the global and/or node summaries used (e.g. clustering coefficient, efficiency, etc.).
Multivariate modeling and predictive analysis	Specify independent variables, features extraction and dimension reduction, model, training and evaluation metrics.