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 |
| | 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> |
| \boxtimes | For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings |
| \boxtimes | 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 <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 was used for data collection

Data analysis

Code: https://github.com/scbe-lab/pristina-cell-type-atlas [DOI: 10.5281/zenodo.10671442]. EvidentialGene tr2aacds4.pl approach (March 2020 v4 version)

Transdecoder v5.5 diamond v2.0.8.146

EggNOG mapper v2 FastQC v0.11.9

CutAdapt v2.8 PairFQv0.17

SPLiTseq toolbox vl.0

Dropseq_tools v2.3.0 STAR v2.7.3a

Picard v2.21.1

Scanpy vl.9.3 Solo v0.l

Python v3.8.10 DESeq2 vl.30.0

R v4.0.3

OrthoFinder v2.3.8 BLAST+ v2.10.0

| ComplexHeatmap v2.II.I |
|------------------------|
| ggplot2 v3.3.3 |
| WGCNA v1.69 |
| edgeR v3.32.1 |
| limma v3.46.0 |
| topGO v2.42.0 |
| HCRProbeMaker v0.3.2 |
| Fiji v2.9.0 |
| bamtools v2.5.1 |
| samtools v1.10 |

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 <u>availability of data</u>

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 datasets supporting the conclusions of this article are available in: |
|---|
| Bioproject: PFUNA961657 |
| GEO (scRNA-seq reads): GSE230505 |
| BioSample (Iso-seq reads): SAMN34360745 |
| |
| BUSCO database https://busco.ezlab.org/ |
| nr database https://www.ncbi.nlm.nih.gov/refseq/about/nonredundantproteins/ |
| Pfam database http://pfam-legacy.xfam.org/ |
| PANTHER database https://pantherdb.org/ |
| SUPERFAMILY database https://supfam.org/SUPERFAMILY/ |
| AnimalTFDB v3.0 https://guolab.wchscu.cn/AnimalTFDB |
| SwissProt https://www.uniprot.org/ |
| EggNOG database http://eggnog5.embl.de |
| EpiFactors database https://epifactors.autosome.org/ |

Research involving human participants, their data, or biological material

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

| Reporting on sex and gender | This study did not involve research in humans. |
|--|--|
| Reporting on race, ethnicity, or other socially relevant groupings | This study did not involve research in humans. |
| Population characteristics | This study did not involve research in humans. |
| Recruitment | This study did not involve research in humans. |
| Ethics oversight | This study did not involve research in humans. |

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

Field-specific reporting

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|-----------------------------------|----------------------------|--|
| Please select the one belo | w that is the best fi | it for your research. If you are not sure, read the appropriate sections before making your selection. |
| ∑ Life sciences | Behavioural | & social sciences |
| For a reference copy of the docum | nent with all sections, se | e 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

No statistical method was used for determining sample size. The sample size for single cell experiments was calculated by performing pilot

| Sample size | studies with different amounts of animals and evaluating the cell content using cell cytometry. The minimum required was determined in previous literature (Garcia-Castro et al Genome Biology 2021) |
|-----------------|--|
| Data exclusions | We removed doublet detected by Solo and Scrublet from the dataset, eliminating 4,966 cells (6.1%). |
| Replication | Our dataset includes 3 biological replicates. All attempts at replication were successful. |
| Randomization | This study does not require randomization. Covariates (feeding conditions) are stated in the dataset. Our analysis does not study the effects of feeding but aims at generating a cell type atlas of wild type conditions. Therefore, this covariate is not relevant to our study. |
| Blinding | Blinding is not relevant for this study. The statistical analysis of our dataset does not have any variable sensitive to researcher blinding. |

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 | | Methods | |
|----------------------------------|-------------------------------|-------------|------------------------|
| n/a | Involved in the study | n/a | Involved in the study |
| \boxtimes | Antibodies | \times | ChIP-seq |
| \boxtimes | Eukaryotic cell lines | \boxtimes | Flow cytometry |
| \boxtimes | Palaeontology and archaeology | \boxtimes | MRI-based neuroimaging |
| | Animals and other organisms | | |
| \boxtimes | Clinical data | | |
| \boxtimes | Dual use research of concern | | |
| \boxtimes | Plants | | |
| | | | |

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

Pristina leidyi worms were originally obtained from Carolina Biological Supply Company and established as a culture by Alexandra E. Bely around 2001 (doi:10.1242/dev.128.14.2781). Part of this culture was transfered and established at Oxford Brookes University in 2019.

Wild animals

This study did not involve wild animals.

Sex can not be determined, as the model organism does not reproduce sexually in lab conditions. Generally, annelids from this group are hermaphrodite when sexualised.

Field-collected samples

The study did not involve samples collected from the field.

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

No ethical approval is required to work with annelids.

Plants

Seed stocks

Ethics oversight

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.