nature portfolio

Corresponding author(s): Albertha J.M. Walhout

Last updated by author(s): 4/17/2025

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	Most Worm Perturb-Seq (WPS) libraries were sequenced on the BGISEQ-500 platform with 100-bp paired-end reads. A subset of the libraries was sequenced on the Illumina NextSeq 500 with NextSeq 500/550 High Output Kit v2.5 (75 Cycles), 14 cycles for read 1 and 75 cycles for read 2.
Data analysis	Raw reads were processed using an in-house dolphinNext pipeline to generate a gene-by-sample read count matrix (https://github.com/XuhangLi/WPS). Differential expression analysis was performed following the established EmpirDE framework (https://github.com/XuhangLi/EmpirDE) to identify differentially expressed genes. The WPS data analysis pipeline is available at https://github.com/XuhangLi/WPS including detailed procedures of raw data processing, quality control and EmpirDE analysis. A walkthrough of the pipeline can be found in the repository. A standalone R package for the EmpirDE analysis can be found in https://github.com/XuhangLi/EmpirDE.

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

Source codes and data for reproducing all results related to core computational analyses (simulation, benchmarking and DE analysis) have been deposited in Zenodo for full reproducibility [10.5281/zenodo.15223779]. Other source data are provided with this paper. Raw and processed data in this study are available in Gene Expression Omnibus (GEO) session GSE255865. Downloadable read count data are also available at the WPS portal hosted in our WormFlux website https://wormflux.umassmed.edu/WPS.

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	No human participants were used in this study
Reporting on race, ethnicity, or other socially relevant groupings	No human participants were used in this study
Population characteristics	No human participants were used in this study
Recruitment	No human participants were used in this study
Ethics oversight	No human participants were used in this study

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	For RNA-seq experiments, approximately ~2,500 L2 C. elegans, ~1000 L3 C. elegans, ~500 L4 C. elegans or 200 adult C. elegans were utilized for each sample. All experiments were conducted with three or more biological replicates to ensure robustness and reproducibility of the results.
Data exclusions	No data is excluded.
Replication	Three or more biological replicates for each experiment were done as mentioned in the figure legends
Randomization	In each experiment, C. elegans were randomly assigned to various experimental groups. For each biological replicate, a control experiment was concurrently performed alongside the test groups to maintain consistent experimental conditions and allow for direct comparisons.
Blinding	Blinding was not implemented in this study. WPS is based on arrayed libraries of RNAi bacteria. All samples were manipulated identically in parallel and blinding does not apply to this situation.

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

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	Caenorhabditis elegans N2 strain was used for this study
Wild animals	No wild animals were used in this study
Reporting on sex	No sex biased data is reported
Field-collected samples	No field collected samples were used in this study
Ethics oversight	No ethical approval was needed for C. elegans or bacterial studies

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.*