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

Data collection is described in detail in the method section.

MaxQuant quantitative proteomics software (version 1.5.2.8) (Cox, J. & Mann, M. MaxQuant enables high peptide identification rates, individualized p.p.b.-range mass accuracies and proteome-wide protein quantification. Nat. Biotechnol. 26, 1367–1372 (2008).)

ImageJ (v1.53i)

CRISPOR (v4.99)

LAS AF (v2.7.3.9723/v3.1.0)

VisiView (v5.0.0.10)

Huygens Remote Manager (v3.6)

IMOD (Kremer, J. R., Mastronarde, D. N. & McIntosh, J. R. Computer Visualization of Three-Dimensional Image Data Using IMOD. J. Struct. Biol. 116, 71–76 (1996).)

EggNode (Huerta-Cepas, J. et al. eggNOG 4.5: a hierarchical orthology framework with improved functional annotations for eukaryotic, prokaryotic and viral sequences. Nucleic Acids Res. 44, D286–D293 (2016).)

Composition Profiler (Vacic, V., Uversky, V. N., Dunker, A. K. & Lonardi, S. Composition Profiler: a tool for discovery and visualization of amino acid composition differences. BMC Bioinformatics 8, 211 (2007).)

EggNOG (v4.5.1) (Huerta-Cepas, J. et al. eggNOG 4.5: a hierarchical orthology framework with improved functional annotations for eukaryotic, prokaryotic and viral sequences. Nucleic Acids Res. 44, D286–D293 (2016).)

Data analysis

Data analysis is described in detail in the method section.

smRNA-seq data was analyzed using the following publicly available tools and custom scripts. Comparisons between samples were done using R v3.5.1. For the full pipeline we refer to the text, code can be made available upon request.
Cutadapt v1.9
FASTX-Toolkit v0.0.14
Seqtk v1.2
Bowtie v1.2.2
BEDTools v2.27.1
HTSeq v0.9.0
SAMTools v1.5
deepTools v2.4.2
https://github.com/Tunphie/SequencingTools/blob/main/smRNA_TypeCounter.py
https://github.com/Tunphie/SequencingTools/blob/main/CoverageOnProteinCodingGenes.py

R (v3.5.2/v4.0.3), R-Studio (v1.1.453), ggplot2 (v3.2.1/v3.3.3), ggrepel (v0.8.1), stats (v3.5.2)

ImageJ (v1.53i), Icy (v2.1.4.0), IMARIS (v9.7.2)

EasyFRAP-web (Koulouras, G. et al. EasyFRAP-web: A web-based tool for the analysis of fluorescence recovery after photobleaching data. Nucleic Acids Res. 46, W467–W472 (2018).)

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 accession number for the smRNA-seq data generated in this study is PRJNA629991 (https://dataview.ncbi.nlm.nih.gov/object/PRJNA629991?reviewer=72dsklt8i261719d2vq572tcq5). The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE 37 partner repository with the dataset identifier PXD019099. CLEM data is available online: Mendeley Data, V1, doi: 10.17632/dgb8d7h2hz.1. Source data are provided with this study. All other data supporting the findings of this study are available from the corresponding author on reasonable request.

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

Sample sizes for the worm experiments were not based on statistical methods but on previously published similar experiments yielding consistent and reproducible results.

Reference for Mis phenotype: de Albuquerque, B. F. M., Placentino, M. & Ketting, R. F. Maternal piRNAs Are Essential for Germline Development following De Novo Establishment of Endo-siRNAs in Caenorhabditis elegans. Dev. Cell 34, 448–456 (2015).

Sample sizes for mass spectrometry experiments were established as quadruplicates (LFQ), as is well-accepted in the proteomics field. Results for all IP-MS/MS experiments were derived from four biological replicates (4 separate extract preparations per condition). For immunoprecipitation and Western Blot experiments, animals were prepared from bleaching 1-2 high density plates of gravid adults per sample, L4 samples, young adult samples, and gravid adult samples were prepared from a pool of synchronized animals (200 µl per sample).

Reference for worm growing for mass spectrometry: Cordeiro Rodrigues RJ, de Jesus Domingues AM, Hellmann S, Dietz S, de Albuquerque BFM, Renz C, Ulrich HD, Sarkies P, Butter F, Ketting RF. PETISCO is a novel protein complex required for 21U RNA biogenesis and embryonic viability. Genes Dev. 2019 Jul 1;33(13-14):857-870. doi: 10.1101/gad.322446.118. Epub 2019 May 30. PMID: 31147388; PMCID: PMC6601512.
The Western Blot of Figure 5h-i was prepared by picking 100 L4 stage animals for each sample.

For each microscopy experiment, 30-50 animals per condition were used to image gonads. For imaging isolated, male-derived germ cells, 10 cells per cell-type and condition were used for image analysis/quantification.

	Reference for RNAe scoring: Luteijn, M. J. et al. Extremely stable Piwi-induced gene silencing in Caenorhabditis elegans. EMBO J. 31, 3422–3430 (2012). The mortal germline experiments, 90 L2 or L3 animals were distributed to 15 NGM plates (90 mm diameter), resulting in six larvae per plate. Reference for Mrt phenotype: Dietz S, Almeida MV, Nischwitz E, Schreier J, Viceconte N, Fradera-Sola A, Renz C, Ceron-Noriega A, Ulrich HD, Kappei D, Ketting RF, Butter F. The double-stranded DNA-binding proteins TEBP-1 and TEBP-2 form a telomeric complex with POT-1. Nat Commun. 2021 May 11;12(1):2668. doi: 10.1038/s41467-021-22861-2. PMID: 33976151; PMCID: PMC8113555.
Data exclusions	No data were excluded from the analyses
Replication	Results from IP-MS/MS experiments were verified by co-immunoprecipitation experiments followed by Western Blot, as well as co-localization studies. The IP-MS/MS and RIP-seq experiments were not repeated as the significant enrichments stem from four and three biological replicates, respectively. For each microscopy experiment, 30-50 animals per condition were used to image gonads. For imaging isolated, male-derived germ cells, 10 cells per cell-type and condition were used for image analysis/quantification. Co-localization analyses of proximal gonads was performed with multiple animals and yielded similar results. Mutator-induced sterility was scored using at least 40 F1 animals, which were randomly picked from 5 cross-fertilized hermaphrodites. Five precise CLEM overlays of either spermatocytes or spermatids were used to assess PEI granule positioning within the cytoplasm. FRAP experiments were performed on four or five foci. We confirm that all attempts at replication were successful.
Randomization	Mutator-induced sterility was scored using at least 40 F1 animals, which were randomly picked from 5 cross-fertilized hermaphrodites. Further randomization is not relevant to this study, as distinct genotypes had to be generated before each experiment.
Blinding	Blinding was not applicable for this study, because the genotype of the strains had to be assigned before experiments could be performed.

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
☒	☐ Human research participants
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used	Monoclonal ANTI-FLAG® (clone M2), Art. No. F3165, Sigma-Aldrich Myc-Tag (clone 9B11) Mouse mAb, Art. No. 2276, Cell Signaling Technology Monoclonal anti-HA (clone 12CA5) mouse antibody, in-house production anti-Histone H3, Art. No. H0164, Sigma-Aldrich polyclonal ANTI-FLAG® antibody produced in rabbit, Art. No. F7425, Lot. No. 086M4803V, Sigma-Aldrich Myc-Tag (clone 71D10) Rabbit mAb, Art. No. 2278, Cell Signaling Technology polyclonal anti-HA-Tag antibody produced in rabbit, Art. No. SAB4300603, Lot. No. 5117T501, Sigma-Aldrich β-Actin (clone D6A8) Rabbit mAb, Art. No. 8457, Cell Signaling Technology Monoclonal Anti-α-Tubulin antibody produced in mouse (clone B-5-1-2), Art. No. T6074, Sigma-Aldrich Polyclonal Anti-RFP Antibody Pre-adsorbed, produced in rabbit, Lot. #33754, Art. No. 600-4001-379, ROCKLAND Monoclonal Anti-GFP Antibody (clone B-2), Santa Cruz, Cat. #sc-9996, Lot.#K1115 anti-mouse IgG, HRP-linked antibody, Art. No. 7076, Cell Signaling Technology anti-rabbit IgG, HRP-linked antibody, Art. No. 7074, Cell Signaling Technology
Validation	Antibodies were used according to manufacturer's instructions. Every immuno-based approach included appropriate negative controls, e.g. biological samples that lack the epitope, which is recognized by a specific antibody. Western blot and IP functionality were confirmed using C. elegans extract containing a tagged fusion protein with the appropriate untagged control protein.

anti-FLAG (clone M2): no validation statement on manufacturer's website, western blot and IP functionality were confirmed using C. elegans extract containing a FLAG fusion protein.
anti-MYC (clone 9B11): Myc-Tag (9B11) Mouse mAb detects recombinant proteins containing the Myc epitope tag. The antibody recognizes the Myc-tag fused to either the amino or carboxy terminus of targeted proteins in transfected cells. The antibody may cross-react with c-myc protein. The antibody may weakly cross-react with a protein of unknown origin ~90kDa. Species Reactivity: All Species Expected.
Monoclonal anti-HA (clone 12CA5) antibody: Soluble lysate from exponentially-growing yeast cultures expressing HA-tagged proteins were separated on 4-15% gradient gel (BioRad). Proteins were blotted an a nitrocellulose membrane using semi-dry transfer (10 min High MW program on TransBlot Turbo blotter) and the membrane was blocked 1h in 5% skim milk/PBS/0.1% Tween-20. Primary antibodies: mouse anti-HA (in-house or Covance, 1:1000 o/N in blocking solution) at 4°C Secondary antibody: goat anti-mouse HRP-coupled (BIORAD 170-5047) 1:3000 1h at RT in blocking solution. The western blot was developed using ECL substrate Dura (Thermo, #34076). Exposure times as indicated in the figure. The experiment was performed by Katharina Bender from Brian Luke's group, IMB, Mainz, Germany.
anti-Histone H3 antibody: By immunoblotting, a working antibody dilution of 1:5,000-1:10,000 is recommended using a whole cell extract of the A431 human epidermoid carcinoma cell line, and a whole cell extract of the mouse fibroblast NIH3T3 cell line. By immunoblotting, a working antibody dilution of 1:2,500-1:5,000 is recommended using a whole cell extract of the rat pheochromocytoma PC12 cell line.
polyclonal ANTI-FLAG® antibody: Western Blot: Cell Line/Tissue Extract of E. coli expressed FLAG-tagged protein. Specification: PRE.2.ZQ5.10000015515.
MYC-Tag (clone 71D10) antibody: c-Myc Antibody detects endogenous levels of total c-Myc protein. This antibody is not recommended for detection of Myc-tagged fusion proteins (use Cell Signaling Technology cat. #2276 or #2278). Species Reactivity: Human, Mouse, Rat. Species predicted to react based on 100% sequence homology: Pig.
polyclonal anti-HA-Tag antibody: western blot and IP functionality were confirmed using C. elegans extract containing a HA fusion protein.
anti-β-Actin (clone D6A8): quality control by Sigma-Aldrich: working dilutions for western blot of at least 1:100 were determined using chicken gizzard extract.
Monoclonal Anti-α-Tubulin antibody (clone B-5-1-2): WB: Cell Line/Tissue Extract: HS-68 Human Foreskin Fibroblasts Cells
Polyclonal Anti-RFP Antibody: This product was prepared from monospecific antiserum by immunoaffinity chromatography using Red Fluorescent Protein (Discosoma) coupled to agarose beads followed by solid phase adsorption(s) to remove any unwanted reactivities.   Expect reactivity against RFP and its variants: mCherry, tdTomato, mBanana, mOrange, mPlum, mOrange and mStrawberry. Assay by immunoelectrophoresis resulted in a single precipitin arc against anti-Rabbit Serum and purified and partially purified Red Fluorescent Protein (Discosoma). No reaction was observed against Human, Mouse or Rat serum proteins.
Monoclonal Anti-GFP Antibody (clone B-2): no validation statement on manufacturer's website, western blot and IP functionality were confirmed using C. elegans extract containing a GFP fusion protein.
anti-mouse IgG, HRP-linked antibody: Application Key: WB-Western Blot IP-Immunoprecipitation IHC-Immunohistochemistry ChIP-Chromatin Immunoprecipitation IF-Immunofluorescence F-Flow Cytometry E-P-ELISA-Peptide. Species Cross-Reactivity Key: H-Human M-Mouse R-Rat Hm-Hamster Mk-Monkey Vir-Virus Mi-Mink C-Chicken Dm-D. melanogaster X-Xenopus Z-Zebrafish B-Bovine Dg-Dog Pg-Pig Sc-S. cerevisiae Ce-C. elegans Hr-Horse All-All Species Expected
anti-rabbit IgG, HRP-linked antibody: Application Key: WB-Western Blot IP-Immunoprecipitation IHC-Immunohistochemistry ChIP-Chromatin Immunoprecipitation IF-Immunofluorescence F-Flow Cytometry E-P-ELISA-Peptide. Species Cross-Reactivity Key: H-Human M-Mouse R-Rat Hm-Hamster Mk-Monkey Vir-Virus Mi-Mink C-Chicken Dm-D. melanogaster X-Xenopus Z-Zebrafish B-Bovine Dg-Dog Pg-Pig Sc-S. cerevisiae Ce-C. elegans Hr-Horse All-All Species Expected

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)

BmN4 cells are provided from Dr. Ramesh Pillai, University of Geneva

Authentication

BmN4 cells have not been authenticated.

Mycoplasma contamination

Not tested.

Commonly misidentified lines
(See ICLAC register)

No commonly misidentified cell lines were used.

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals

Caenorhabditis elegans N2 Bristol; all mutants were derived from this strain.
Hermaphrodites and males, and the following developmental stages were used: embryo, larval stage L4, young adult, gravid adult.
The study involved the following Caenorhabditis elegans strains:
N2
USC1092
RFK956
USC1137
RFK1252
DG3226
RFK1151
YY538
SX2078
RFK1358

RFK578
RFK1357
NL1810
RFK691
RFK936
NL917
RFK652
OH441
RB1083
RFK1364
RFK750
RFK1412
RFK1413
RFK848
RFK921
RFK1366
RFK963
RFK962
RFK1032
RFK1065
RFK1027
RFK1132
RFK1356
RFK1342
RFK1350
RFK1415
RFK1416
RFK1352
RFK1355
RFK926
RFK1086
SX922
RFK233
RFK237
RFK1033
RFK832
RFK1064
RFK870
RFK1361
RFK1360
RFK788
RFK820
BA782
DU23
USC988
RFK945
NL5117
RFK935
RFK561
RFK560
RFK1148
RFK1336
RFK778
RFK817
RFK1365
RFK933
RFK1209
RFK1359
RFK1149
RFK1017
RFK1018
RFK1030
RFK1362
RFK1062
RFK1066
RFK1131
RFK1353
RFK1354
RFK1080
RFK1081
RFK1228
RFK1084

Wild animals

the study did not involve wild animals

Field-collected samples	the study did not involve samples collected from the field
Ethics oversight	no ethical approval or guidance was required

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