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Reporting Summary

Nature Research 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 Research 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
	\square 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. <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
\boxtimes	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
X	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

No software was used.

Data analysis

For small RNA libraries, sequences were parsed from adapters using FASTQ/A Clipper (options: -Q33 -I 17 -c -n -a TGGAATTCTCGGGTGCCAAGG) and quality filtered using the FASTQ Quality Filter (options: -Q33 -q 27 -p 65) from the FASTX-Toolkit v. 0.0.13 (http://hannonlab.cshl.edu/fastx_toolkit/), mapped to the C. elegans genome WS258 using Bowtie2 v. 2.2.2 (default parameters), and reads were assigned to genomic features using FeatureCounts (options: -t exon -g gene_id -O --fraction —largestOverlap) which is part of the Subread v. 1.5.1 package. Differential expression analysis was done using DESeq2 v. 1.22.2. To define gene lists from IP experiments, a 2-fold-change cutoff, a DESeq2 adjusted p-value of ≤0.05, and at least 10 RPM in the IP libraries were required to identify genes with significant changes in small RNA levels. Additionally, any genes identified as having differentially enriched small RNAs from control samples (HA or FLAG immunoprecipitations from wild-type animals), were removed from further analysis.

For mRNA libraries, sequences were parsed from adapters using Trimmomatic v. 0.36 (options: PE -phred33 ILLUMINACLIP:<fasta with adaptor sequences>:2:30:10 LEADING:3 TRAILING:3 SLIDINGWINDOW:4:30 MINLEN:30) and mapped to the C. elegans genome WS258 using HISAT2 v. 2.1.0 (options: --dta-cufflinks --known-splicesite-infile <path to file of known splice sites>). Reads were assigned to transcripts using FeatureCounts (options: -t exon -g gene_id -p) which is part of the Subread v. 1.5.1 package. Differential expression analysis was performed using DESeq2 v. 1.22.2.

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 Research 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 list of figures that have associated raw data
- A description of any restrictions on data availability

The RNA sequencing data generated in this study are available through Gene Expression Omnibus (GEO) under accession code GSE151828 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE151828). The mass spectrometry proteomics data generated in this study are available through the ProteomeXchange Consortium via the PRIDE partner repository78 with the dataset identifier PXD021227 (http://www.ebi.ac.uk/pride/archive/projects/PXD021227)

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Life scier	nces study design
All studies must di	sclose on these points even when the disclosure is negative.
Sample size	small RNA and mRNA seq data analysis: 2-3 biological replicates (see Supplementary Table 6). RT-qPCR analysis: 3 biological and 3 technical replicates. Sample sizes were not predetermined by statistical methods, but by conventional requirements in the respective fields.
Data exclusions	One set of small RNA libraries was excluded because the libraries contained high levels of degraded rRNA. The samples were generated again and new libraries were constructed.
Replication	All immunofluorescence and live imaging experiments were performed at least two times, and at least 3 germlines were imaged per sample, per condition, with similar results. All western blots were performed at least twice. Additional information about number of replicates for specific experiments can be found in the figure legends.
	For all experiments, control and experimental samples were treated in parallel and animals were chosen randomly from plates for
Randomization	experiments such as brood size and imaging.

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
Antibodies	ChIP-seq
Eukaryotic cell lines	Flow cytometry
Palaeontology and archaeology	MRI-based neuroimaging
Animals and other organisms	·
Human research participants	
Clinical data	
Dual use research of concern	
·	

Antibodies

Antibodies used

Anti-FLAG M2 (Sigma Aldrich F1804, RRID:AB_262044), 1:1,000 Western, 1:500 IF Anti-HA 3F10 (Roche 11867423001, RRID:AB_390918), 1:500 IF

Anti-HA 3F10 Peroxidase (Roche 12013819001, RRID:AB_390917), 1:1,000 Western

Anti-PGL-1 (DSHB K76, RRID:AB_531836), 1:100 for IF

Anti-CSR-1 (Claycomb Lab), 1:2,000 Western

Anti-actin IgG (Abcam ab3280, RRID:AB_303668), 1:10,000 Western

Goat anti-mouse IgG Alexa Fluor 488 (Thermo Fisher A-11029, RRID:AB_138404), 1:1,000 IF

Goat anti-Rat IgG Alexa Fluor 555 (Thermo Fisher A-21434, RRID:AB_2535855), 1:1,000 IF Goat anti-mouse IgM Alexa Fluor 647 (Thermo Fisher A21238, RRID:AB_1500930), 1:500 IF Goat anti-mouse IgG Secondary HRP (Thermo Fisher A16078, RRID:AB_2534751), 1:5,000 Western Goat anti-rat IgG Secondary HRP (Thermo Fisher A18871, RRID:AB_2535648), 1:5,000 Western Goat anti-rabbit Secondary HRP (Thermo Fisher A16110, RRID: AB_2534782), 1:5,000 Western

Validation

Anti-PGL-1 has been validated in multiple publications, including Strome and Wood (1983) and anti-CSR-1 has been validated by Claycomb et al (2009). Anti-FLAG and anti-HA have been validated by the manufacturer. Specifically -

Anti-PGL-1 - Validated by Immunofluorescence detecting for a 40 kDa P-granules polypeptide in C. elegans embryos (Strome and Wood 1983)

Anti-CSR-1 - Validated by Immunofluorescence and Western Blot detecting for CSR-1 polypeptide from amino acid E462 to E987 in C. elegans dissected adult germlines and whole cell lysate (Claycomb et al, 2009)

Anti-FLAG M2 - Validated by Western Blot detecting for the N-term FLAG-BAP Fusion Protein in either bacterial, mammalian, or plant extract, then indirectly detected using Anti-Mouse IgG Peroxidase, and visualized using HRP chemiluminescent substrates (https://www.sigmaaldrich.com/deepweb/assets/sigmaaldrich/product/documents/754/849/anti-flag-2poster.pdf)

Anti-HA 3F10 - Validated by Western Blot detecting for purified HA-tagged Glutathione-S-transferase in eukaryotic cell extract, then indirectly detected using Anti-Rat-Ig-Biotin and Streptavidin-POD* using BM Chemiluminescence Western Blotting substrate (POD) (https://www.sigmaaldrich.com/deepweb/assets/sigmaaldrich/product/documents/248/175/roahahabul.pdf)

Anti-HA 3F10 Peroxidase - Validated by Western Blot detecting for purified HA-tagged Glutathione-S-transferase in eukaryotic cell extract, visualized by BM Chemiluminescence Blotting Substrate (POD) (https://www.sigmaaldrich.com/deepweb/assets/sigmaaldrich/product/documents/760/007/12013819001bul.pdf)

Anti-actin IgG - Validated by Western Blot in mammalian cell lines (NIH 3T3, MDA-MB-231, HeLa) whole cell lysates, and mouse liver whole tissue lysate, then indirectly detected by goat anti-mouse IgG polyclonal. Developed by ECL technique (https://www.abcam.com/actin-antibody-actn05-c4-ab3280.html)

Animals and other organisms

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

Laboratory animals

C. elegans strains (all animals used are hermaphrodites unless otherwise noted in the figure legends):

N2 (wild-type)

GE24

CB151

WM300

USC868

USC896

USC1066

USC988

USC1137

USC1092

USC1139 USC1110

USC1110

USC1112

USC1065 USC1074

USC1258

USC1259

USC1260

USC1159

USC1262

USC1263

USC1264

USC1284

USC1348

USC1326 USC1343

USC1127

USC1128

Wild animals

Wild animals were not used

Field-collected samples

Field-collected samples were not used

Ethics oversight

No ethical approval is required for C. elegans.

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