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. |
| | 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 <i>d</i> , Pearson's <i>r</i>), 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 Microscopy data v

Microscopy data were collected using 3i SlideBook6 and ZEISS ZEN 3.4 (blue edition).

Data analysis

Microscopy data were analyzed using FIJI software (v2.1.0/1.53f; Build 5f23140693), R (version 4.1.0), RStudio (version 1.4.1717) using custom scripts. Statistics and graphs were calculated/constructed in R.

High-throughput sequencing data were analyzed on an Ubuntu 16.04.6 LTS (GNU/Linux 4.15.0-142-generic x86_64) computer using Cutadapt (version 2.10) and HISAT2 (version 2.2.1).

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

All data will be made available on request. RNA sequencing datasets have been deposited onto the NCBI Sequence Read Archive (SRA) under the BioProject accession number of PRINA819556.

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| Please select the or | ne 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 t | he document with all sections, see <u>nature.com/documents/nr-reporting-summary-flat.pdf</u> | |
| | | |
| Life scier | nces study design | |
| All studies must dis | close on these points even when the disclosure is negative. | |
| Sample size | Sample sizes were chosen based off of an estimated number of samples that appeared to reflect the variability of the population. No statistical method was used to predetermine sample size. | |
| Data exclusions | Data were only excluded from the analysis if the control feature used for normalizing between samples and conditions appeared aberrant. Such instances were infrequent. | |
| Replication | Experiments were conducted multiple times using RNAi triggers against three different genes. Similar observations were made for these three triggers. | |
| Randomization | Samples were allocated into experimental groups by genotype and RNAi condition. Covariates were controlled for by maintaining all samples in the same growth and media conditions. | |
| Blinding | Investigators were not blinded for these experiments, as the identity of each condition could always be identified through examination of the RNA level alone. | |
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| Reportin | g 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 & exp | perimental systems Methods | |
| n/a Involved in th | e study n/a Involved in the study | |
| Antibodies | ChIP-seq | |
| Eukaryotic | | |
| | ogy and archaeology MRI-based neuroimaging | |
| | d other organisms | |
| | earch participants | |
| Clinical data | | |
| Dual use re | esearch of concern | |
| Antibodies | | |
| Antibodies used | anti-FLAG M2 primary antibody (Millipore Sigma; Cat #: MF1804) Goat Anti-Mouse IgG1 HRP-conjugated secondary antibody (JacksonImmuno; Cat #: 115-035-205) | |
| Validation | Antibodies were self validated via western blot on a strain lacking the epitope, and no bands were detected (see Fig. S8A). | |
| Animals and | other organisms | |
| Policy information | about <u>studies involving animals</u> ; <u>ARRIVE guidelines</u> recommended for reporting animal research | |

Laboratory animals Experiments were conducted on adult C. elegans hermaphrodites, embryos, and L1s. All strains were of the N2 background. Wild animals No wild animals were used in this study. Field-collected samples No field-collected samples were used in this study. No ethical approval or guidance is needed for C. elegans work. Ethics oversight

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