

## 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 [Authors & Referees](#) 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

MetaMorph 7.7.2, VisiView 2.1.4, R Studio 0.97.449, StepOnePlus RT-PCR system integrated software, COPAS BIOSORT F500 (Union Biometrica) integrated software, latest used version 5404 and Illumina HiSeq 2500 integrated softwares.

Data analysis

R Studio 0.97.449; R packages QuasR v1.22.0, EdgeR v 3.24.0, ; Fiji/ImageJ 1.52p; Excel 2013

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/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

RNA-seq data that support the findings of this study have been deposited in the Gene Expression Omnibus (GEO) under accession codes GSE92851. Previously published ChIP-seq data that were re-analysed here are available from ModEncode (<http://data.modencode.org/>), for L3\_H3K9me1/2/3 (5036, 5050, 5037, 5040), L3\_H3K27me3 (5045, 5051), L3\_H3K27ac (5054), L3\_H3K4me2/3 (5055, 3576). 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](https://www.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 methods were used to predetermine sample size. For genome-wide analyses (RNAseq) experiments were done at least in duplicates as commonly done in the field. For microscopy, every experimental repetition included parallel imaging of multiple worms, resulting in samples sizes in the range of tens of worms scored. For derepression assays with the high throughput COPAS BIOSORTER, hundreds to thousands of worms were scored. For qPCR analyses we analyzed 3 independent biological samples with three technical triplicates as commonly done in the field. In all cases we reached a high enough sample size so that robustness is ensured. |
| Data exclusions | No data exclusions were applied, except for the fluorescence/derepression quantification by the worm sorter in which samples with criteria size corresponding to the L1 larval stage were selected and EXT <5 (bacteria instead of worms) were excluded in every analysis. This exclusion criteria was pre-established for each experiment, because we often get a small bacteria contamination in the derepression quantification with the COPAS BIOSORTER.                                                                                                                                                                                                          |
| Replication     | Every experiment was reliably reproduced, with 2 to 4 biological replicates according to the experiment and as mentioned in the figure legends or methods, and 3 technical replicates were done for each biological replicate for ChIP-qPCRs and RT-qPCRs.                                                                                                                                                                                                                                                                                                                                                                                                            |
| Randomization   | No method of randomization was used as this is not relevant to the field of study. All samples were allocated to a group according to their genotype or RNAi treatment.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
| Blinding        | Investigators were not blinded to group allocation during most data collection and analysis.<br>Blinding for the key derepression experiments would not have been relevant because fluorescent and phenotypic markers already indicate the investigator the genotype he/she is looking at.                                                                                                                                                                                                                                                                                                                                                                            |

## 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                                           |
|-------------------------------------|-----------------------------------------------------------------|
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> Antibodies                  |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Eukaryotic cell lines                  |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Palaeontology                          |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> Animals and other organisms |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Human research participants            |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Clinical data                          |

### Methods

| n/a                                 | Involved in the study                              |
|-------------------------------------|----------------------------------------------------|
| <input checked="" type="checkbox"/> | <input type="checkbox"/> ChIP-seq                  |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> Flow cytometry |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> MRI-based neuroimaging    |

## Antibodies

|                 |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |
|-----------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Antibodies used | For ChIP-qPCR : H3K27me3, Millipore (07-449), Lot #2686928<br>Dynabeads M-280 Sheep Anti-Rabbit IgG, Thermo Fisher, (11203D), Lot#00331016<br>For RNA-IP-qPCR : Anti-FLAG M2 magnetic beads, Sigma-Aldrich (M8823), Lot#SLBW7376<br>There is no "dilution" information, since the beads are first saturated with 10 microgram/ml antibody in solution and then recovered and used for IP. The amount of beads used depends on the size of the sample. The anti-Flag beads are already saturated with antibody and were used as is.                                                                                                                                                                                                                  |
| Validation      | The H3K27me3 antibody is commercially available, highly published including for its use in the Modencode project in C.elegans<br>This antibody is dot blot tested for trimethylated lysine 27 specificity and validated in WB, ICC, IP by the company and has been validated in our hands by IF in mes-2 mutant worms, where there is no H3K27me3 and the signal disappeared, in contrast to WT worms.<br>The Dynabeads conjugated antibody has been validated by Thermo Fisher and widely used in the research community.<br>The antibody anti-FLAG coupled with magnetic beads from (Sigma-Aldrich) has been validated by the company for IF, WB, IP and more. In our hands we validated it by IP on worms that do not bear the FLAG tag protein. |

## Animals and other organisms

Policy information about [studies involving animals](#); [ARRIVE guidelines](#) recommended for reporting animal research

### Laboratory animals

Strain Original Name / OC Genotype Reference

GW1 N2\* wild-type, Bristol isolate

GW76 gwls4 [baf-1p::GFP-lacI::let-858 3'UTR; myo-3p::RFP] X (Meister et al., 2010)

GW306 NL2507 pkls1582[let-858p::GFP::let-858 3'UTR; rol-6(su1006)]V (Towbin et al., 2012)

GW566 gwls39 [baf-1p::GFP-LacI::let-858 3'UTR; vit-5p::GFP] III; gwls4 [baf-1p::GFP-lacI::let-858 3'UTR; myo-3p::RFP] X (Towbin et al., 2012)

GW653 YG118 yglis[baf-1p::GFP-lmn-1 Y59C; unc-119(+)]; unc-119(ed-3) (Mattout et al., 2011)

GW299 gwls25 [tbb-1p::wmCherry-LacI::tbb-2 3'UTR unc-119(+)] unc-119(ed3) This study

GW886 yglis[baf-1p::GFP-lmn-1 Y59C; unc-119(+)]; set-25(n5021) III. met-2(n4256) III This study

GW638 met-2(n4256) set-25(n5021) III (Towbin et al., 2012)

GW637 met-2(n4256) set-25(n5021) III; gwls4 [baf-1p::GFP-lacI::let-858 3'UTR; myo-3p::RFP] X (Towbin et al., 2012)

GW214 hpl-2(tm1489) III; gwls4 [baf-1p::GFP-lacI::let-858 3'UTR; myo-3p::RFP] X (Gonzalez et al., 2015)

GW468 mes-2(bn11) unc-4(e120)/mnC1 dpy-10(e128) unc-52(e444)II; gwls4[myo-3::RFP baf-1::GFP lacI let-858] X. (Towbin et al., 2012)

GW849 gws17 [cec-4p::cec-4::WmCherry::cec-4 3'UTR] II (Gonzalez et al., 2015)

GW1108 EG6070 oxc2 oxSi221 [eft-3p::GFP + Cbr-unc-119(+)] II (in ttTi5605); unc-119(ed3) III (Frøkjær-Jensen et al., 2012)

GW638 met-2(n4256) set-25(n5021) III (Towbin et al., 2012)

GW1109 HW1390 oxc4 lsm-8 (xe17 [myo2p::mcherry::unc54 3'UTR])IV This study

GW1125 oxc6 lsm-8 (xe17 [myo2p::mcherry::unc54 3'UTR])IV/nT1[qls51](IV;V) This study

GW1119 oxc6 lsm-8 (xe17 [myo2p::mcherry::unc54 3'UTR])IV/nT1[qls51](IV;V); pkls1582[let-858::GFP rol-6(su1006)]V This study

GW1120 oxc6 lsm-8 (xe17 [myo2p::mcherry::unc54 3'UTR])IV ; pkls1582[let-858::GFP rol-6(su1006)]V This study

GW1148 oxc6 met-2(n4256) set-25(n5021) III; lsm-8 (xe17 [myo2p::mcherry::unc54 3'UTR])IV/nT1[qls51](IV;V) This study

GW923 VC2663\* oxc5 lsm-5(ok3431) V/nT1[qls51](IV;V) This study

GW925/ GW1082 VC904\* oxc5 eea-1&gut-2(gk407) V/nT1[qls51](IV;V) This study

GW931 oxc6 eea-1&gut-2(gk407) V/nT1[qls51](IV;V) ; gwls4 [ myo-3::RFP baf-1::GFP lacI let-858] X This study

GW933 oxc6 lsm-5(ok3431) V/nT1[qls51](IV;V); gwls4 [ myo-3::RFP baf-1::GFP lacI let-858] X This study

GW1080 VC2785\* oxc5 lsm-4&ada-2(ok3151)/mln1[mls14 dpy-10(e128)]II. This study

GW1004 gwEx81[WRM062D\_B06::gfp::3xFlag] This study

GW1420 dcap-2 (tm2470)/ nT1 IV; pkls1582[let-858::GFP rol-6(su1006)]V This study

GW1500 lsm-1(tm3585)/mln1[mls14 dpy-10(e128)]II; pkls1582[let-858::GFP rol-6(su1006)]V This study

GW1613 EM599 him-5(e1490) V; lin-15B&lin-15A(n765) X; bxIs13 CGC

\*CGC: Caenorhabditis Genetics Center

OCx4 : out-crossed 4 times

### Wild animals

the study did not involve wild animals

### Field-collected samples

The study did not involve samples collected from the field.

### Ethics oversight

The transgenic C elegans are swiss biosafety level 1, and are duly reported and registered as such.

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

## Flow Cytometry

### Plots

Confirm that:

- ☒ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
- ☒ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).
- ☒ All plots are contour plots with outliers or pseudocolor plots.
- ☒ A numerical value for number of cells or percentage (with statistics) is provided.

### Methodology

#### Sample preparation

The flow cytometry- based method used in the study quantified the GFP or mCherry fluorescence intensity of described reporters in RNAi treated or untreated worms (C.elegans), not in cells. The flow cytometry-based method was also used in order to sort and select the homozygous deletion mutant worms for RNA extraction for the RNA-seq, ChIP-qPCR and RNA-decay experiments. The worms were collected from plates and washed with M9 buffer to get rid of bacteria before they are assessed for the fluorescence intensity or sorted according to their genotype, according to their size.

#### Instrument

The worm sorter: COPAS BIOSORT F-500 (Union Biometra).

|                           |                                                                                                                                                                                                                                                                                                                                                                                                                                                                |
|---------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Software                  | The COPAS BIOSORT associated software version 5404 and previous version was used to collect the data, and analysis was done using Excel and R-studio.                                                                                                                                                                                                                                                                                                          |
| Cell population abundance | About 5-15% of the worm population was selected for sorting homozygous worms at the desired developmental stage (L3 ).<br>About 20-40% of the worm population was quantified for fluorescence intensity at the desired developmental stage (L1).                                                                                                                                                                                                               |
| Gating strategy           | The gating strategy was determined empirically by verifying the size, shape gonad and vulva developmental stage by microscopic examination. Morphological validations during the sorting process were also performed.<br>Sorting of the homozygous animals was done by selecting non-GFP pharynx animals, and the gating was also determined stringently by examining the two populations and by verifying the different criteria with fluorescent microscopy. |

☒ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.