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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 Editorial Policies and the Editorial Policy Checklist.

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

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n/a	Confirmed
	$oxed{\boxtimes}$ 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.
\boxtimes	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
\boxtimes	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

All microscopy images were acquired using the Zen software (Carl Zeiss).

Data analysis

Statistical analysis of the automated worm tracking videos was performed as previously described. Briefly, statistical significance between each group was blindly calculated using Wilcoxon rank-sum test and correcting for false-discovery rate. Statistical analysis of RNA-seq comparison was performed using DESeq2 as previously described. Statistical analysis for various aspects of ChIP-seq was done using MACS2, MEME-ChIP and Diffbind. All microscopy fluorescence quantifications were done in the Zen software (Carl Zeiss). All statistical tests for fluorescence quantifications and behavior assays were conducted using Prism (Graphpad) and Excel as described in figure legends.

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

Raw and processed RNA-seq data is available at GEO accession #GSE158274. Raw and processed ChIP-seq data is available at GEO accession #GSE181288.

Field-spe	cific reporting			
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 scien	ices study design			
All studies must dis	close on these points even when the disclosure is negative.			
Sample size	No sample size calculation was performed. Standard number of replicates/animals from previous experience were used.			
Data exclusions	For all experiment, a single outlier per experimental group was removed only if it is statistically significant in the Grubb's test (p<0.05).			
Replication	All experiments were done with multiple biological replicates and all effects were shown with all replicates. All experiments were repeated at least once independently. At each repeat, all control and experimental conditions were included, and the results of all independent experiments were combined. Whenever representative microscopy images were shown without any quantification, the exact same results were observed in at least 10 animals unless variability is stated.			
Randomization	Whenever possible, all samples/animals were randomized.			
Blinding	Whenever possible for behavioral analysis, the scoring was done blindly. For all other molecular and microscopy experiments, experimenters were not blind during data collection/analysis.			
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.				
	perimental systems Methods			
n/a Involved in the				
Antibodies	ChIP-seq			
Eukaryotic	cell lines Flow cytometry			
Palaeontolo	ogy and archaeology MRI-based neuroimaging			
Animals and	d other organisms			
Human research participants				
Clinical data				
Dual use re	search of concern			
Antibodies				
Antibodies used	Sigma-Aldrich Mouse monoclonal anti-FLAG M2 Cat# F1804 for INTACT nuclei isolation (3ul/reaction); Chromotek GFP-Trap Magnetic Beads (Cat# gtd) for ChIP-seq (25ul/reaction).			
Validation	Validation is confirmed as the pulldown of FLAG-tagged nuclei has fluorescent protein at the nuclear membrane that can be visualized as well as by functional validation of the NGS data. LIN-14 ChIP by Chromotek beads were validated with functional changes in gene expression.			
Animals and	other organisms			

Policy information about <u>studies involving animals</u>; <u>ARRIVE guidelines</u> recommended for reporting animal research

Laboratory animals

 $\hbox{C. elegans. Whenever synchronization of developmental stages was necessary, animals were egg prepped according to standard}\\$ $protocol\ and\ synchronized\ at\ the\ L1\ stage.\ They\ were\ then\ plated\ on\ food\ and\ collected\ after\ 8\ +/-1\ hrs,\ 21\ +/-1\ hrs,\ 30\ +/-2\ hr,\ 40\ hrs,\ 40\ hrs,\$ +/- 2hrs, and 53 +/- 2hrs for L1, L2, L3, L4, and adult stages, respectively for either molecular or behavioral analysis. These time points were chosen such that the animals were in the middle of each larval stage or relatively early in adulthood for the analysis. Dauer animals were obtained using standard crowding, starvation and high temperature conditions.

Wild animals

No wild animals used.

Field-collected samples

No field-collected samples were used.

Ethics oversight

No ethics approval was necessary.

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

ChIP-seq

Data deposition

Confirm that both raw and final processed data have been deposited in a public database such as GEO.

Confirm that you have deposited or provided access to graph files (e.g. BED files) for the called peaks.

Data access links

May remain private before publication.

Raw and processed ChIP-seq data is available at GEO accession #GSE181288.

Files in database submission

Raw fastq sequencing files, bed/tabular files for peak calling/differential binding, and all annotated files for peak and differential binding sites

Genome browser session (e.g. <u>UCSC</u>)

not applicable

Methodology

Replicates

5 replicates for each developmental stage (L1 and L2) and type (IP and Input)

Sequencing depth

The libraries were sequenced on Illumina NextSeq 500 machines with 75bp single-end reads.

Antibodies

Chromotek GFP-Trap Magnetic Beads (Cat# gtd)

Peak calling parameters

MAC2 default parameters

Data quality

BAM alignment files were filtered for 20+ MAPQ scores using SAMtools. Correlation among samples was done using DeepTools to ensure separate clustering of ChIP and Input samples. Signal strength of ChIP enrichment using signal extaction scaling (SES) was done using DeepTools. Overlap of ChIP-seq was also done with RNA-seq results, and functional validation using a few gene expression reporters were done.

Software

After initial quality check, the reads were mapped to WS220 using BWA (Li and Durbin, 2009) and filtered using SAMtools (Li et al., 2009). Peaks were called using MACS2 (Feng et al., 2012). The ChIP-seq peak distribution was calculated and plotted using ChIPseeker (Yu et al., 2015). The consensus binding motif was obtained using MEME-ChIP (Machanick and Bailey, 2011). Differential binding analysis between L1 and L2 was done using Diffbind (Ross-Innes et al., 2012). All peaks and differential binding sites were annotated and assigned to the nearest gene using ChIPseeker (Yu et al., 2015).