

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 (<i>n</i>) 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
<i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i> |
| ☒ | ☐ 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> |
| ☒ | ☐ 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 <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 for data collection.
Data analysis	ChIP-seq, RNA-seq, PIRCh-seq and ChIRP-seq were processed with Bowtie2 v2.4.5, SAMtools v1.13, CrossMap v0.6.4, Trimmomatic v0.39, HISAT2 v2.2.1, HTSeq v2.0.2, Picard tools, and MACS2 v2.2.7.1. Differential gene expression analysis was performed with R package DESeq2 v1.38.3. Functional motif and pathway enrichment analysis was performed with STREME and AME in MEME Suite v5.5.0, and PANTHER. R packages ggpubr v0.6.0 and ggplot2 v3.4.4 were used to perform statistical analyses and data visualization. Bigwig tracks were generated with deepTools v3.5.1 and visualized with IGV v2.14.1.

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 NGS data have been deposited in GEO database under the accession number GSE248701. The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE partner repository with the dataset identifier PXD047183.

Research involving human participants, their data, or biological material

Policy information about studies with [human participants or human data](#). See also policy information about [sex, gender \(identity/presentation\), and sexual orientation](#) and [race, ethnicity and racism](#).

Reporting on sex and gender

Reporting on race, ethnicity, or other socially relevant groupings

Population characteristics

Recruitment

Ethics oversight

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

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

Data exclusions

Replication

Randomization

Blinding

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
☒	☐ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

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

Antibodies

Antibodies used

anti-H3K27me3 (C36B11) (#9733, Cell Signaling Technology): ChIP-seq
 anti-H3K27me3 (#39155, Active Motif): PIRCh-seq
 anti-H4K16ac (#39167, Active Motif): PIRCh-seq
 anti-His (HT501-01, Transgen): western blot (1:5,000)
 Goat Anti-Mouse IgG (H+L), HRP Conjugate (HS201-01, Transgen): western blot,(1:10,000)

Validation

anti-H3K27me3 (C36B11) (#9733, Cell Signaling Technology):
<https://www.cellsignal.com/products/primary-antibodies/tri-methyl-histone-h3-lys27-c36b11-rabbit-mab/9733>

anti-H3K27me3 (#39155, Active Motif):
<https://www.activemotif.com/catalog/details/39155/histone-h3-trimethyl-lys27-antibody-pab>

anti-H4K16ac (#39167, Active Motif):
<https://www.activemotif.com/catalog/details/39167/histone-h4-acetyl-lys16-antibody-pab>

anti-His (HT501-01, Transgen):
https://www.transgenbiotech.com/tag_antibody/proteinfind_anti_his_mouse_monoclonal_antibody.html

Goat Anti-Mouse IgG (H+L), HRP Conjugate (HS201-01, Transgen):
https://www.transgenbiotech.com/secondary_antibody/proteinfind_goat_anti_mouse_igg_h_l_hrp_conjugate.html

Eukaryotic cell lines

Policy information about [cell lines and Sex and Gender in Research](#)

Cell line source(s)

Drosophila S2 cells were originated from Thermo Fisher (R69007).

Authentication

RNA-seq confirmed the identity of S2 cells.

Mycoplasma contamination

Not tested.

Commonly misidentified lines
(See [ICLAC](#) register)

No commonly misidentified lines were used in this study.

Animals and other research organisms

Policy information about [studies involving animals](#); [ARRIVE guidelines](#) recommended for reporting animal research, and [Sex and Gender in Research](#)

Laboratory animals

Drosophila roX1 roX2 double knockout lines: y[1] w[1118] v[1] roX1[ex6] roX2[9-4]/FM7i, P{w[+mC] =ActGFP} JMR3 stock (PMID: 30532158). The samples were collected at larval stage.

Wild animals

No wild animals were involved.

Reporting on sex

Male larvae were used in this study, with females as siblings control.

Field-collected samples

No field-collected samples were involved.

Ethics oversight

No ethical approval was required for studies using Drosophila as it is invertebrate.

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

Plants

Seed stocks	N/A
Novel plant genotypes	N/A
Authentication	N/A

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 <i>May remain private before publication.</i>	https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE248701
Files in database submission	<i>Provide a list of all files available in the database submission.</i>
Genome browser session (e.g. UCSC)	<i>Provide a link to an anonymized genome browser session for "Initial submission" and "Revised version" documents only, to enable peer review. Write "no longer applicable" for "Final submission" documents.</i>

Methodology

Replicates	Two replicates
Sequencing depth	10-20 million reads per replicate was obtained for ChIP-seq. Paired-end, 150-bp 35-40 million reads per replicate was obtained for RNA-seq. Paired-end, 100-bp 20-30 million reads per replicate was obtained for PIRCh-seq. Paired-end, 75-bp
Antibodies	anti-H3K27me3 (C36B11) (#9733, Cell Signaling Technology)
Peak calling parameters	macs2 callpeak -t -c -g dm --broad-cutoff 0.1 --broad --nomodel -f BAMPE
Data quality	name number of peaks M-WT-H3K27me3-rep1_peaks.broadPeak 5407 M-WT-H3K27me3-rep2_peaks.broadPeak 4785 M-roXKO-H3K27me3-rep1_peaks.broadPeak 6328 M-roXKO-H3K27me3-rep2_peaks.broadPeak 6190 F-WT-H3K27me3-rep1_peaks.broadPeak 8897 F-WT-H3K27me3-rep2_peaks.broadPeak 10620
Software	Bowtie2 v2.4.5, SAMtools v1.13, Trimmomatic v0.39, Picard tools, and MACS2 v2.2.7.1.