

## 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 ( $n$ ) 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

Zeiss ZEN software (version 3.1) for Zeiss LSM900 confocal microscope  
LightCycler 480 Software (release 1.5.1.62) for RT-qPCR and ChIP-qPCR  
Stellaris RNA FISH probe designer tool for designing smiFISH probes

Data analysis

Fiji (v1.53q) for image analysis  
MATLAB (R2021b) for image analysis  
R (v4.1.2) for statistical analysis  
IGV (v2.12.3) for visualization of genome profile  
HISAT2 (version 2.2.0) for mapping of public CAGE-seq data  
Samtools (version 1.10) for conversion of analyzed data format  
bedtools (version 2.29.2) for editing and analysis of bed files.

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](#)

Previously published sequence data are available under accession number GSE30757, GSE86966, GSE55306, GSE36212 and E-MTAB-4787.

The original live-imaging data used for the analysis shown in main and supplementary figures have been deposited to Zenodo database (<https://doi.org/10.5281/zenodo.7545929>).

The MATLAB codes for nuclei tracking and MS2/PP7 signal recording are available at the Zenodo database (<https://doi.org/10.5281/zenodo.7545929>).

## Human research participants

Policy information about [studies involving human research participants and Sex and Gender in Research](#).

Reporting on sex and gender

Not applicable.

Population characteristics

Not applicable.

Recruitment

Not applicable.

Ethics oversight

Not applicable.

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

The sample size was not statistically predetermined.  
Samples used for RT-qPCR and ChIP-qPCR were harvested from independent three biological replicates, based on standard in the field.  
We typically observed >150 nuclei at single live-imaging experiment, which is sufficient for the following statistical analysis. Sample sizes are indicated in the manuscript.

Data exclusions

No data was excluded from analysis.

Replication

Most of experiments were repeated multiple times.  
In Supplementary Figure 7, 9, and 16, sufficient number of nuclei were obtained from a single embryo for further computational analysis.

Randomization

Not applicable. Used embryos were categorized by genotype before experiments.

Blinding

Not applicable. Quantitative analysis was not manually performed.

## 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 &amp; 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 and archaeology          |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> Animals and other organisms |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Clinical data                          |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Dual use research of concern           |

## Methods

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

## Antibodies

Antibodies used

Sheep polyclonal anti-digoxigenin antibody (Roche, 11093274910)  
 Mouse monoclonal anti-biotin (invitrogen, 03-3700, clone Z021)  
 Mouse monoclonal anti-dorsal (DSHB, AB\_528204, clone 7A4)  
 Mouse monoclonal anti-RNA polymerase II (Millipore, 05-623, clone CTD4H8)  
 Alexa Fluor 555 donkey anti-sheep antibody (invitrogen, A-21436)  
 Alexa Fluor 488 donkey anti-mouse antibody (invitrogen, A-21202)

Validation

For both of anti-digoxigenin and -biotin antibody, application for FISH experiments was confirmed by previous publications (e.g., Yokoshi, M. et al., 2022). For anti-dorsal antibody, application for immunoprecipitation assay was confirmed by previous publications (e.g., Whalen, A. M. et al., 1993). For anti-RNA polymerase II antibody, application for immunoprecipitation assay was confirmed by previous publications (e.g., Lagha, M. et al., 2013).

## 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

Species: *Drosophila melanogaster*  
 Strain: For production of transgenic strains, vk00033 line (BDSC #9750) was used.  
 For production of genome-edited strains, nanos-Cas9 strain was used.  
 Age: embryos

Wild animals

We did not use wild animals.

Reporting on sex

Sex of embryos was not discriminated.

Field-collected samples

We did not use field-collected samples.

Ethics oversight

No ethical approval was required for the research using *D.melanogaster*.

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