

## 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](#).

### 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  |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> The exact sample size ( $n$ ) for each experimental group/condition, given as a discrete number and unit of measurement  |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly  |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> The statistical test(s) used AND whether they are one- or two-sided<br><i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i>   |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> A description of all covariates tested  |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons  |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> 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) |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> For null hypothesis testing, the test statistic (e.g. $F$ , $t$ , $r$ ) with confidence intervals, effect sizes, degrees of freedom and $P$ value noted<br><i>Give <math>P</math> values as exact values whenever suitable.</i>                            |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings  |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes  |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> 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

Data analysis

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

All data is available in the main text, the supplementary materials, and/or at NCBI Gene Expression Omnibus (GSE125947, GSE115511, and GSE115478).  
Figures 2, 4, 5, 6 S1, S3, S4, and S6 contain raw data.  
There are no restrictions on data availability.

## 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 sample-size calculation was performed statistically. All sample sizes are comparable or exceed current sample sizes used in the literature. For single cell RNA-Seq, three biological replicates were chosen as there was high sample correlation when one or three samples were chosen. Most of the single cell studies in literature used no replicates.
Data exclusions	We excluded protein traps that had little or no expression. This exclusion criteria was predetermined.
 Replication	Within an experiment, samples were compared using Spearman correlations. We also compared bulk RNA-Seq with scRNA-Seq to verify reproducibility across technologies.
Randomization	Randomization is not relevant to this study because there are no case/control type comparisons. All comparisons are within individual cells or within a single biological class
Blinding	Blinding was not relevant to this study because there are no case/control type comparisons.

## 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 and archaeology          |
| <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                          |
| <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

mouse Anti-RNA polymerase II, clone CTD4H8 MilliporeSigma, Burlington, MA, gift from Eduardo Gorab Millipore05-623;  
RRID:AB\_309852  
rat Anti-RNA polymerase II subunit B1 (phospho CTD Ser-2), clone 3E10 antibody MilliporeSigma, Burlington, MA Millipore:04-1571;  
RRID:AB\_11212363

rabbit Anti-RNA polymerase II CTD repeat YSPTSPS (phospho S5) Abcam, Cambridge, MA Abcam:ab5131; RRID:AB449369  
 guinea pig Anti-Asl A gift of G. Rogers, University of Arizona, Tucson, AZ  
 Mouse Anti-Lamin DmO DSHB, Iowa City, IA DSHB:ADL84.12; RRID:AB\_528338  
 Mouse Anti-Lamin C DSHB, Iowa City, IA DSHB:LC28.26; RRID:AB\_528339  
 Mouse Anti-Fascin III DSHB, Iowa City, IA DSHB:7G10; RRID:AB\_528070  
 Mouse Anti-hu-li tai shao, not hts-PC DSHB, Iowa City, IA DSHB:1B1; RRID:AB\_528070  
 Goat anti-Guinea Pig IgG (H+L) - Alexa Fluor 488 Thermo Fisher Scientific, Waltham, MA Invitrogen:A11073; RRID:AB\_2534117  
 Goat anti-Mouse IgG (H+L) - Alexa Fluor 568 Thermo Fisher Scientific, Waltham, MA Invitrogen:A11004; RRID:AB\_2534072  
 Goat anti-Rabbit IgG (H+L) - Alexa Fluor 647 Thermo Fisher Scientific, Waltham, MA Invitrogen:A21244; RRID:AB2535812  
 Goat anti-Rat IgG (H+L) - Alexa Fluor 647 Thermo Fisher Scientific, Waltham, MA Invitrogen:A21247; RRID:AB\_141778  
 Goat anti-Chicken IgY (H+L) - Alexa Fluor 488 Thermo Fisher Scientific, Waltham, MA Invitrogen:A11039; RRID:AB\_2534096  
 Goat anti-Mouse IgG (H+L) - Alexa Fluor 488 Thermo Fisher Scientific, Waltham, MA Invitrogen:A11029; RRID:AB\_2534088  
 Chicken Anti-GFP Abcam, Cambridge, MA Abcam:ab13970; RRID:AB\_300798  
 Rabbit Anti-dimethyl-Histone H3 (Lys9) MilliporeSigma, Burlington, MA Millipore:07-441; RRID:AB\_310619  
 Rabbit Anti-acetyl-Histone H4 (Lys5) MilliporeSigma, Burlington, MA Millipore:07-327; RRID:AB\_310523  
 Goat Anti-Rabbit IgG (whole molecule)-FITC MilliporeSigma, Burlington, MA Sigma:F0382; RRID:AB\_259384  
 Sheep Anti-digoxigenin-rhodamine, Fab fragments Roche, Mannheim, Germany Roche:11 207 750 910

Antibodies are validated by the supplier by either immunoprecipitation by a cell lysate and analyzed by mass spectrometry, western blot and detecting the protein in tissue or cells.

Validation

## Animals and other organisms

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

Laboratory animals

All strains used L3 larval or adult males. We also use data from adult w[1118] females.

y w  
 Tempe-T  
 w[1118]  
 w[1118] P{w[+mC]=PTT-GA}Nrg[G00305]  
 w[1118]; Mi{ET1}fIn[MB03038]/TM3, Sb[1] Ser[1]  
 "w[1] w[\*]; Mi{y[+mDint2]=MIC}rdo[MIO2219]"  
 "w[\*]; P{w[+mC]=PTT-un}Fs(2)Ket[GFP]/CyO; P{w[+mC]=mRFP-Nup107.K}7.1"  
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 "y[1] w[\*]; Mi{y[+mDint2]=MIC}Nlg3[MIO2443]/TM3, Sb[1] Ser[1]"  
 "y[1] w[\*]; Mi{y[+mDint2]=MIC}Cht5[MIO3431]/TM3, Sb[1] Ser[1]"  
 w[1118] P{w[+mC]=PTT-GB}Pdc4[G93]  
 "y[1] w[\*]; Mi{PT-GFSTF.1}tutl[MIO0290-GFSTF.1]/SM6a"  
 "y[1] w[\*]; Mi{y[+mDint2]=MIC}Syn[MIO5723]"  
 y[1] w[\*]; Mi{y[+mDint2]=MIC}RunxA[MIO7404]  
 "y[1] w[\*]; P{w[+mC]=PTT-GA}cindr[CA06686]"  
 w[\*]; P{w[+mC]=PTT-un}Dek[G00131]  
 "w[\*]; P{w[+mC]=PTT-GA}Fas3[G00258]"  
 "y[1] w[\*]; Mi{y[+mDint2]=MIC}rdo[MIO8797]"  
 w[\*]; P{w[+mC]=PTT-GC}e(y)3[CC01368]  
 "w[\*]; P{w[+mC]=PTT-GC}Mapmodulin[CC01398]"  
 "w[\*]; P{w[+mC]=PTT-GC}osa[CC00445]"  
 y[1] w[\*]; Mi{y[+mDint2]=MIC}rdo[MIO10011]"  
 "y[1] w[\*]; Mi{y[+mDint2]=MIC}Syn[MIO11740]"  
 "y[1] w[\*]; Mi{y[+mDint2]=MIC}Nlg3[MIO8924]"  
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 "y[1] w[67c23]; Mi{PT-GFSTF.0}spir[MIO5646-GFSTF.0]/CyO"  
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 "y[1] w[\*]; Mi{PT-GFSTF.0}p53[MIO1307-GFSTF.0]/TM6C, Sb[1] Tb[1]"  
 "y[1] w[\*]; Mi{PT-GFSTF.1}tok[MIO6118-GFSTF.1]/TM6C, Sb[1] Tb[1]"  
 "y[1] w[67c23]; Mi{PT-GFSTF.1}ADD1[MIO9552-GFSTF.1]/CyO"  
 "w[\*]; Tl{Tl}Efa6[GFP-C]"  
 "y[1] w[\*]; Mi{y[+mDint2]=MIC}kkv[MIO15197] CG14668[MIO15197]/TM3, Sb[1] Ser[1]"  
 "y[1] w[\*]; Mi{PT-GFSTF.1}dpr17[MIO8707-GFSTF.1]"  
 "y[1] w[\*]; Mi{PT-GFSTF.1}twin[MIO7336-GFSTF.1]/TM6C, Sb[1] Tb[1]"  
 "y[1] w[\*]; Mi{PT-GFSTF.2}Sap-r[MIO11015-GFSTF.2]/TM6C, Sb[1] Tb[1]"  
 "y[1] w[\*]; Mi{PT-GFSTF.1}bol[MIO0386-GFSTF.1]/TM6C, Sb[1] Tb[1]"  
 "y[1] w[\*]; Mi{PT-GFSTF.0}CG17646[MIO4004-GFSTF.0]"  
 "y[1] w[\*]; Mi{PT-GFSTF.1}SRPK[MIO6550-GFSTF.1]"

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"y[1] w[*]; Mi{PT-GFSTF.1}stai[Mi07398-GFSTF.1]"  
"P{GawB}E132; ; P{w[+mC]=tubP-GAL80[ts]}2"  
"y[*] w[*]; P{w[+mC]=UAS-2xEGFP}AH3"  
"y[*] w[*]; P{GawB}NP1624 / CyO, P{UAS-lacZ.UW14}UW14"  
"w[*]; P{vas.EGFP.HA}"
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Wild animals

This study did not involve wild animals.

Field-collected samples

Tempe-T flies are collected from the field

Ethics oversight

This study only used *Drosophila melanogaster*, which does not require ethics oversight.

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