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☐ Initial submission   ☐ Revised version   ☒ Final submission

Life Sciences Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form is intended for publication with all accepted life science papers and provides structure for consistency and transparency in reporting. Every life science submission will use this form; some list items might not apply to an individual manuscript, but all fields must be completed for clarity.

For further information on the points included in this form, see Reporting Life Sciences Research. For further information on Nature Research policies, including our data availability policy, see Authors & Referees and the Editorial Policy Checklist.

▶ Experimental design

1. **Sample size**

   Describe how sample size was determined.

   > We performed initial experiments to evaluate the variance of the population, and chose the sample size so that we have a 80% power of detecting a mean difference of 20% from the controls, with a significance level of 5% (two sided). GraphPad StatMate software was used for these estimates.

2. **Data exclusions**

   Describe any data exclusions.

   > No samples were excluded. Within each fixed sample, dead cells and mitotic cells (identified by DAPI staining) were excluded. Cells that display excessive rotational/ translational motions that could not be corrected for with our registration method, were excluded from the analysis of focus dynamics in movies.

3. **Replication**

   Describe whether the experimental findings were reliably reproduced.

   > All the described findings were reliably reproduced.

4. **Randomization**

   Describe how samples/organisms/participants were allocated into experimental groups.

   > The cells quantified in each experiment were randomly sampled from the total population of cells.

5. **Blinding**

   Describe whether the investigators were blinded to group allocation during data collection and/or analysis.

   > We assured reliable and unbiased data quantification by having different investigators quantify each experiment independently. Analyses were done on randomized samples. No animals or human research participants were involved. Other levels of blinding are not relevant to this study.

   Note: all studies involving animals and/or human research participants must disclose whether blinding and randomization were used.

6. Statistical parameters

For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

n/a | Confirmed

☐ ☒ The <u>exact sample size</u> (*n*) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)

☐ ☒ A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly

☐ ☒ A statement indicating how many times each experiment was replicated

☐ ☒ The statistical test(s) used and whether they are one- or two-sided (note: only common tests should be described solely by name; more complex techniques should be described in the Methods section)

☐ ☒ A description of any assumptions or corrections, such as an adjustment for multiple comparisons

☐ ☒ The test results (e.g. *P* values) given as exact values whenever possible and with confidence intervals noted

☐ ☒ A clear description of statistics including <u>central tendency</u> (e.g. median, mean) and <u>variation</u> (e.g. standard deviation, interquartile range)

☐ ☒ Clearly defined error bars

See the web collection on statistics for biologists for further resources and guidance.

▸ Software

Policy information about availability of computer code

7. Software

Describe the software used to analyze the data in this study.

> Details are specified in the methods sections. The main software used for data analyses are SoftWorX (6.0), Matlab (R2013a), R (Version 0.98.978), and Imaris x64 (7.7.1). Two custom algorithms used for this study are now published in 'Methods in Enzymology' (Caridi et al., https://doi.org/10.1016/bs.mie.2017.11.033), and references have been updated accordingly.

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). *Nature Methods* guidance for providing algorithms and software for publication provides further information on this topic.

▸ Materials and reagents

Policy information about availability of materials

8. Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a for-profit company.

> All materials generated for this study are readily available from the authors or commercial sources specified in the Methods session.

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).

Primary antibodies used in Drosophila cells were: anti-actin (1:1000, Abcam, ab8224); anti-γH2Av (1:1000, Rockland, 600-401-914); anti-Rad51 (1:1000, gift from J. Kadonaga); anti-HA (1:1000, Abcam, ab9134 for Western blot; 1:1000; Covance, 16B12 for IF); anti-FLAG (1:1000, Sigma, F1804); anti-GFP (1:1000, Invitrogen, AP11122 for Western blot; 1:1000 Aves Lab, GFP-1020 for IF; Rockland, 600-101-215 for Ip); anti-H3K9me2 (1:750, Wako Chemicals, MABI0307, 302-32369); anti-Wash (1:10, Developmental Studies Hybridoma Bank, P3H3); anti-Wasp (1:5, Developmental Studies Hybridoma Bank, P5E1); anti-Scar (1:10, Developmental Studies Hybridoma Bank, P1C1); anti-Whamy (1:10, Developmental Studies Hybridoma Bank, P1D1); Phalloidin (1:500 AlexaFluor488, Invitrogen, A12379), anti-Arpc3A (1:10,000, for Wb kind gift from L. Cooley), anti-Arpc3B (1:500 for IF, kind gift from L. Cooley), anti-MyoV (head) (1:500, kind gift from A. Eprussi), anti-Unc45 (1:500, kind gift from S. Bernstein), anti-Smc5 (SDI, 1:800)3; anti-Smc6; (SDI, 1:800)3; anti-Nup62 (1:1000, kind gift from H. Ohkura); anti-Rad50 (1:1000, kind gift from M. Gatti), anti-dPIAS (1:1000, kind gift from G. Karpen), anti-TopBP1 (1:1000, kind gift from M. Michael36); anti-HP1a (1:500, Developmental Studies Hybridoma Bank, C1A9). Primary antibodies used in NIH3T3 cells were: anti-H3K9me3 (1:2000, Abcam, ab8898); anti pH3S10 (1:4000, Milipore, 06-570); anti-gamma H2A.X (phospho-S139; 1:2000, Abcam, ab26350). Secondary antibodies for IF were from Life Technologies and Jackson Immunoresearch. Those used for Western blot were from Pierce and Santa Cruz Biotech. Antibodies were previously validated3,4,14 or validated by comparing signals in the presence of the protein of interest with signals after RNAi depletions or IF signals in the absence of primary antibodies.

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.

Kc167 (Kc) cells were used for most experiments and were purchased from the Drosophila Genomic Resource Center (DGRC). Mouse NIH3T3 cells were obtained from ATCC.

b. Describe the method of cell line authentication used.

Kc167 (Kc) cells were authenticated by DGRC, and mouse NIH3T3 cells by ATCC.

c. Report whether the cell lines were tested for mycoplasma contamination.

Cell lines were tested for micoplasma contamination and no contamination was found.

d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by ICLAC, provide a scientific rationale for their use.

No commonly misidentified cell lines were used in this study.

▶ Animals and human research participants

Policy information about studies involving animals; when reporting animal research, follow the ARRIVE guidelines

11. Description of research animals

Provide details on animals and/or animal-derived materials used in the study.

Fly stocks were obtained from BDSC (http://fly.bio.indiana.edu) or VDRC (www.vdrc.at) and are: Myo1A (BDSC #33971) y[1] sc[*] v[1]; P{y[+t7.7] v[+t1.8]=TRiP.HMS00298}attP2; Myo1B (BDSC #41689) y[1] v[1]; P{y[+t7.7] v[+t1.8]=TRiP.HMS02253}attP2; Arp3 (BDSC #32921) y[1] sc[*] v[1]; P{y[+t7.7] v[+t1.8]=TRiP.HMS00711}attP2; Wash (BDSC #62866) y[1] sc[*] v[1]; P{y[+t7.7] v[+t1.8]=TRiP.HMC05339}attP40; Scar, (BDSC #31126) y[1]v[1];P{y[+t7.7] v[+t1.8]=TRiP.JF01599}attP2; Act5c-GAL4 (BDSC #4414) y[1] w[*]; P{w[+mC]=Act5C-GAL4}25FO1/CyO, y[+]; Unc45 (VDRC #v108868) P{KK101311}VIE-260B. Smc5 trans-heterozygous mutant was smc57/19 previously described in Chiolo et al, Cell, 2011 (doi:10.1016/j.cell.2011.02.012). The WT control was w1118 (http://flybase.org/reports/FBal0018186.html). To obtain 3rd instar larvae for karyotyping of neuroblast metaphase spread, RNAi lines were crossed to the Act5c-GAL4 line (rebalanced with CyO-GFP) and non-GFP larvae were picked for karyotyping as described in Chiolo et al, Cell, 2011 (doi:10.1016/j.cell.2011.02.012).

Policy information about studies involving human research participants

12. Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants.

The study did not involve human research participants.