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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 Authors & Referees 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	All code used in this study is available online at https://github.com/BoettigerLab, except where prohibited by copyright pertaining to derivative works as described below. Software for the control of the automated fluid handling system is available at: https://github.com/BoettigerLab/fluidics-control. Software for the control of the microscope components and for integration of liquid handling and microscope imaging routines is available at: https://github.com/BoettigerLab/storm-control. Code pertaining to the construction of Oligopaint library design used in this study was modified from routines developed in the Zhuang lab at Harvard University. The license for those original routines prohibits distribution of derivative works except to other institutions for academic use, precluding the use of publically-accessible code-repositories such as Github. Consequently, these codes will be made available upon request as permitted by the license. Descriptions of all code are provided in the Readme files in the indicated repositories.
Data analysis	Additional code used in the analysis of the ORCA data is available at https://github.com/BoettigerLab/ORCA-public. A description of the code is provided in the Readme included.

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/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

We have included a smaller version of our typical dataset that is public at https://bit.ly/2S6eCjk. Images for an entire dataset are too large to append and, thus, image data is available upon request. All DNA paths from all cells are available upon request. No datasets requiring public deposition were produced for this study.

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

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	Sample size was determined by experimental feasibility and by any associated statistics used in comparisons. All sample sizes are indicated and meet or exceed standard practice for single cell data.
Data exclusions	No data were excluded.
Replication	All results were reproduced as described and indicated in the corresponding data panels. No data failed to reproduce.
Randomization	Embryos were selected at random prior to cryosectioning and labeling.
Blinding	No blinding was used in these experiments.

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
☐	☒	Animals and other organisms
☒	☐	Human research participants
☒	☐	Clinical data

Methods

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

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	IMR90 cells and K562 cells are from ATCC.
Authentication	These cells were authenticated by the company.
Mycoplasma contamination	The cells tested negative for mycoplasma contamination prior to our experiments.
Commonly misidentified lines (See ICLAC register)	N/A

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals	Wild type flies and embryos (Drosophila melanogaster), both male and female, were used in this study as described in the methods. Wildtype strain was CantonS (Stanford Fly Core Facility). Embryo from age 10 hours post fertilization through 12 hours post fertilization were analyzed as indicated.
Wild animals	N/A
Field-collected samples	N/A
Ethics oversight	No ethical approval needed as there is no issue surrounding use of Drosophila.

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