

## 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](#).

### Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. 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
- ☐ ☒ An indication of 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 statistics 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
- ☐ ☒ Clearly defined error bars  
*State explicitly what error bars represent (e.g. SD, SE, CI)*

Our web collection on [statistics for biologists](#) may be useful.

### 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<sup>31,62</sup>. 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 upon request. 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 DNA paths from all cells are available upon request as indicated in the Supplementary materials (the files are too large to append as Extended Data Tables). Image data is available upon request. No datasets requiring public deposition were produced for this study.

## Field-specific reporting

Please select 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/authors/policies/ReportingSummary-flat.pdf](https://www.nature.com/authors/policies/ReportingSummary-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

### Materials & experimental systems

|                                     |                                                                 |
|-------------------------------------|-----------------------------------------------------------------|
| n/a                                 | Involved in the study                                           |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Unique biological materials            |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Antibodies                             |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> Eukaryotic cell lines       |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Palaeontology                          |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> Animals and other organisms |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Human research participants            |

### 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 |

## Eukaryotic cell lines

Policy information about [cell lines](#)

|                                                                      |                                                                                                                                                                                                                                                             |
|----------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Cell line source(s)                                                  | mES R1 cells were a generous gift from the Wysocka lab.                                                                                                                                                                                                     |
| Authentication                                                       | These cells were authenticated by colleagues in the Wysocka lab by karyotyping, RNA-seq, ChIP-seq, and in vitro differentiation all of which gave results consistent with the pluripotent stem cell identity. Additional chimera assays were not performed. |
| Mycoplasma contamination                                             | The cells tested negative for mycoplasma contamination prior to our experiments.                                                                                                                                                                            |
| Commonly misidentified lines<br>(See <a href="#">ICLAC</a> register) | N/A                                                                                                                                                                                                                                                         |

## Animals and other organisms

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

### Laboratory animals

Wild type and mutant 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), mutants used were Fab-7 and Fub, which were gifted from Welcome Bender. Embryo from age 8 hours post fertilization through 12 hours post fertilization were analyzed as indicated.

### Wild animals

No wild animals were used in these studies.

### Field-collected samples

No field collected samples were used in these studies.