naturereseach

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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 followed a published temperature preference assay protocol (doi:10.1038/ng1513) and used the largest sample size (100) from the protocol.

2. **Data exclusions**

   Describe any data exclusions.

   > No data was excluded

3. **Replication**

   Describe whether the experimental findings were reliably reproduced.

   > We performed the temperature preference assay six times independently using flies from different generations.

4. **Randomization**

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

   > We collected 100 randomly chosen flies from each strain for each experiment. These flies were collected from a pool of >500 files originating from 10 vials of adult flies from each strain. We switched the sides of the fly strains on the aluminum block to ensure a balanced design (A4 on the top panel 3/6 times and on the bottom panel 3/6 times and vice versa for w1118).

5. **Blinding**

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

   > The flies at the end of the temperature preference assays were counted independently by at least two persons. The genotype was withheld to the counters.

   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 exact sample size (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 central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)
☒	☐	Clearly defined error bars

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

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▸ Software

Policy information about availability of computer code

7. Software

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

> All custom software used in this study have been deposited in GitHub. The software used in this study include:
> PBcR-MHAP v8.3rc1
> DBG2OLC v1.0
> IrysSolve 2.1
> mscaffolder
> bedtools v2.25.0
> Repeatmasker v4.0.6
> BUSCO v1.22
> MUMmer v3.23
> SVMU 0.1beta
> smrtanalysis v2.3
> quickmerge v0.1
> Pilon v1.3
> CNVnatorv0.3
> Pindel v0.2.4
> Pecnv 0.1.8
> SweepFinder2

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 sequence data have been deposited to NCBI and are publicly available. The fly strains used in this study are available from Bloomington Stock Center and the transgenic fly strains we generated for the p24-2 RNAi experiments are available upon request.

9. Antibodies

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

> N/A

10. Eukaryotic cell lines

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

> N/A

b. Describe the method of cell line authentication used.

> N/A

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

> N/A

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.

> N/A

▸ 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.

> We used fruit fly D. melanogaster strains for our experiments.

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.

> N/A