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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 did not determine required sample sizes a priori for either the lethality or fertility assays.

2. Data exclusions

Describe any data exclusions.

No data were excluded from any analyses.

3. Replication

Describe whether the experimental findings were reliably reproduced.

The fertility and lethality effects of gene knockouts were reproducible across each control and experimental line for each of the genes we tested.

4. Randomization

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

Samples were randomly allocated to positions in boxes and boxes were randomly assigned positions in incubators. That is, crosses were set up in unlabeled vials and placed in boxes at pre-determined positions (boxes were labeled).

5. Blinding

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

Only during data collection. Counts were performed blindly in the sense that all tubes were unlabeled and counts were collected based solely on box position. Counts from a position were then linked back to the original ID after data collection was completed.

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.

Software

Policy information about availability of computer code

7. Software

Describe the software used to analyze the data in this study.	bedtools v2.25.0 BLAT BWA MEM 0.7.12 Cufflinks v2.2.1 Genome Analysis ToolKit v3.4.0 multiz 11.2 PAML 4.7a PicardTools v1.95 R 3.2.2 samtools v0.1.18 TopHat v2.1.1 Custom perl scripts to calculate population genomic statistics, using equations from classic literature.

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 fly stocks are available upon request, as noted in the paper. There are no restrictions.

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).	Antibodies were used for staining fixed samples. They are fully explained in our Methods section. They have been validated in previous publications in the same species, D. melanogaster, and these papers are referenced in the manuscript.

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.	No eukaryotic cell lines were used in this study.
b. Describe the method of cell line authentication used.	NA
c. Report whether the cell lines were tested for mycoplasma contamination.	NA
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.	NA

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 strains used in this study are described in the text or in the supplementary material.

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.	This study did not involve any human subjects.