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 possible.
- 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
No customized software was used

Data analysis
No customized software was used

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

All data generated by this study is available in the main text, Methods and Supplementary data, or cited by accession codes to publicly-accessible databases. All materials, including vector plasmids and Drosophila strains, will be made available by contacting the corresponding author.

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.

- X Life sciences
- Behavioural & social sciences
- Ecological, evolutionary & environmental sciences
**Life sciences study design**

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

**Sample size**
The estimated sample size was determined based on previous studies of spontaneous mutation frequencies per gene locus for a variety of gene products in the same insect species (i.e. Drosophila melanogaster).

**Data exclusions**
No data was excluded from analyses.

**Replication**
Reproducibility of results in the experimental group was internally replicated by the discovery of spontaneous mutations and modifiers of gene function in identical independent mating groups. Second-site survivor lines were tested for putative maternal effect modifiers by three replicate single pair backcrosses of F2 double-heterozygous males and females to F1 [n].

**Randomization**
A single experimental group was tested which was comprised of 80 identical independent mating groups in addition to 6 independent control mating groups. Randomization was not required since differing experimental groups were not compared.

**Blinding**
Investigators were not blinded since control mating group survival data (zygotes reared on tetracycline diet) informed the number of experimental mating groups necessary to achieve the required experimental sample size (>10^6 zygotes reared on tetracycline-free diet).

**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 | Methods |
|----------------------------------|---------|
| n/a |
| ☒ Antibodies |
| ☒ Eukaryotic cell lines |
| ☒ Paleontology |
| ☒ Animals and other organisms |
| ☒ Human research participants |
| ☒ Clinical data |
| n/a |
| ☒ CHIP-seq |
| ☒ Flow cytometry |
| ☒ MRI-based neuroimaging |

**Animals and other organisms**

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

**Laboratory animals**
The study did not involve laboratory animals

**Wild animals**
The study did not involve wild animals

**Field-collected samples**
The study did not include organisms collected from the field

**Ethics oversight**
The University of Florida Institutional Biosafety Committee provided oversight and regulatory approval for the creation and rearing of genetically modified Drosophila melanogaster used in this study covered under sections II-D-4-a [for the creation of rDNA-modified arthropods] and III-E-2-b-(5) [for experiments with rDNA-modified arthropods associated with plants] of the NIH Guidelines.

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