

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 Zeiss ZEN microscope software (black and blue editions)

Data analysis GraphPad Prism v6.0, Image J v2.0.0-rc-689/1.52e, Imaris v8.4

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

Source data for main and supplementary figures have been provided as Supplementary Table 2. All other data supporting the findings of this study are available from the corresponding author on reasonable request.

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

Life sciences study design

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

Sample size	For GSC counts, n>15 testes per genotype was used per biological replicate. Rationale: Variability between replicates was not often observed; in cases it was, sample size was increased. For LD counts and p4E-BP stains, n>10 testes per genotype was used. Rationale: individual GSCs would not vary LD counts or p4E-BP stains dramatically (each testis provides 5-10 GSCs). For EM (Figure 1B; Figure 2A,D; Figure 4A), number of biological replicates is >6. In all cases analyzed, no variance in the morphology of mitochondria (i.e., aberrant vs normal), presence of autophagosomes or of lipid droplets was observed within each genotype analyzed. For MitoTracker® (Figure 2B) and DHE (Figure S3A) stains, n>10 testes per genotype used. Rationale: phenotypes observed did not vary dramatically within each analyzed group.
Data exclusions	No data were excluded from the analyses.
Replication	Experimental findings were reliably reproduced within our lab, based on at least 3 biological replicates for each experiment. qPCR experiments were performed in quintuplicates, and for each experiment, n=3 of biological and technical replicates.
Randomization	Experimental groups were formed based on genotypes and genetically related cohorts, when possible.
Blinding	Blinding was not possible in our studies, as changes to mitochondrial morphology were obvious in the presence of mitochondrial markers (often used to validate the knockdowns and/or for the purposes of understanding the phenotype).

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	n/a
Involvement in the study	Involvement in the study
☐ ☒ Antibodies	☒ ☐ ChIP-seq
☒ ☐ Eukaryotic cell lines	☒ ☐ Flow cytometry
☒ ☐ Palaeontology	☒ ☐ MRI-based neuroimaging
☐ ☒ Animals and other organisms	
☒ ☐ Human research participants	
☒ ☐ Clinical data	

Antibodies

Antibodies used	rabbit anti-Vasa (d-260) (Santa Cruz, Cat# sc30210, Lot#K1715, 1:100); mouse anti-Fas3 (DSHB, clone 7G10,1:50); guinea pig anti-traffic jam (TJ) (a gift from D. Godt, 1:3,000), chicken anti-GFP (Aves, Cat#GFP-1020, Lot#GFP697986, 1:1000), rabbit anti-Ref(2)P (a gift from D. Walker, 1:250); rabbit anti-P-4E-BP1 (T37/46) (236B4) (Cell Signaling, Cat#2855S, Lot#20, 1:500); mouse anti-HA.11 (Covance, Cat#MMS-101P, Lot#, 1:800); rabbit anti-AMPK phospho-T172 (40H9) (Cell Signaling, Cat#2535S, Lot#16, 1:1000); mouse anti-Actin (Ab-5) (BD Transduction lab, Cat#612657, Lot#7159886, 1:5000); goat anti-Chicken IgG Alexa 488 (Molecular probes, Cat#A-11039, Lot# 1356650, 1:500); goat anti-Guinea Pig IgG Alexa 647 (Molecular probes, Cat#A-21450, Lot#735365, 1:500); donkey anti-Rabbit IgG Alexa 488 (Molecular probes, Cat#A-21206, Lot#1275888, 1:500); goat anti-Rabbit IgG Alexa 568 (Molecular probes, Cat#A-11036, Lot#1504529, 1:500); donkey anti-Mouse IgG Alexa 647 (Molecular probes, Cat#31571, Lot#1984047, 1:500); donkey Alexa 488 anti-mouse IgG (H+L) Highly Cross-Adsorbed, Molecular Probes, Cat# A-21202, Lot#1644644, 1:500).
Validation	For rabbit anti-Vasa, please see Lasko and Ashburner, Genes Dev 1990 and https://www.scdb.com/scdb/product/vasa-antibody-d-260 for relevant website. For mouse anti-Fas3, please see Patel et al., Cell 1987 for validation. For guinea pig anti-TJ, please see Gunawan et al., Development 2003 for validation. For chicken anti-GFP, please see Koehler et al., LCB 2017 for use in Drosophila encoded GFP and http://www.aveslab.com/products/epitope-tag-and-gfp-antibodies/anti-gfp-green-fluorescent-protein-antibodies-2/ for relevant website. For rabbit anti-Ref(2)P antibody, please see Rana et al., Nat Comm 2017 for validation. For p4E-BP1, please see Amoyel et al., Dev 2016 for validation in Drosophila testis and https://www.cellsignal.com/products/primary-antibodies/phospho-4e-bp1-thr37-46-236b4-rabbit-mab/2855 for relevant website. For mouse anti-HA, please see this publication for validation in Drosophila tissue (clonal analysis with or without dMfn:HA, Figure S1E) and https://www.biolegend.com/fr-fr/global-elements/pdf-popup/anti-ha-11-epitope-tag-antibody-11071?filename=Anti-

HA11%20Epitope%20Tag%20Antibody.pdf&pdfgen=true for relevant website.
For rabbit anti-pAMPK alfa and mouse anti-Actin, please see Ulgherait et al., Cell Rep 2015 for validation.

Animals and other organisms

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

Laboratory animals

The strains used were obtained from the following sources: Bloomington Drosophila Stock Center - P14coltG2414 ('coltOE') (BL26993), UAS-Opa1RNAi-TRiP (BL 32358), UAS-dMfnRNAi-TRiP (BL55189), UAS-dMfnmiRNA.CDS.S('dMfnRNAi-Bellen') (BL67158), UAS-CPT2RNAi (BL62455), Stat92E::GFP (BL38670), nos-Gal4 (BL4442), UAS-Tor.TED ('TorDN') (BL7013), UAS-SREBP(1-452) ('SREBP-PCA') (BL41018), UAS-SREBP.P450 ('SREBP-DN') (BL8246). Vienna Drosophila RNAi Center - UAS-dMfnRNAi-KK (v105261), UAS-coltRNAi (v106089), UAS-bmmRNAi (v37877), UAS-Drp1RNAi-GD (v44156), UAS-Tsc1RNAi (v22252), UAS-Tsc2RNAi (v6313). Personal gifts - UAS-dMfnRNAi2-4 (dMfnRNAi-Guo) and UAS-Opa1RNAi ('Opa1RNAi-Guo') (gifts from M. Guo), pCasper4-Marf-HA (Genomic)(dMfnHA') and MarfB P{neoFRT}19A (dMfnB') (gifts from H. Bellen), UAS-bmm (UAS-bmmOE) (gift from R. Kühnlein), UAS-Drp1K38A.HA (UAS-Drp1DN) (gift from J. Chung), Drp-1KG03815 FRT40A (gift from K. Mitra), GstD1-GFP (gift from U. Banerjee), nos-Gal4::VP16 (gift from M. Van Doren), sa-GFP (gift from M. Fuller), UAS-LPP (gift from R. Thakur). Lines not described in the text can be found in Flybase and/or are listed in Supplemental Table 1.

Wild animals

The study did not involve wild animals

Field-collected samples

The study did not involve samples collected from the field

Ethics oversight

Drosophila strains were raised and handled according to standard institutional regulations.

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