

Reporting Summary

Nature Portfolio 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 Portfolio policies, see our [Editorial Policies](#) 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 (<i>n</i>) 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 <i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i>
☐	☒ 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. <i>F</i> , <i>t</i> , <i>r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted <i>Give P values as exact values whenever suitable.</i>
☒	☐ 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 <i>d</i> , Pearson's <i>r</i>), 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	Cell images were collected using BioTek Cytation 5 imaging reader. Tissue samples were analyzed using Zen Blue 3.3. Gel and membrane images were analyzed using BioRad Image Lab 2.0. qPCR data were collected using Roche Real Time Cyclor 480.
Data analysis	Cell images were analyzed using Gen5 Image Prime 3.11. Tissue samples were analyzed using Zen Blue 3.3 and ImageJ. Data plotting and statistical analyses were performed using GraphPad Prism 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 and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio [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 description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our [policy](#)

All normalized sequencing data and curated proteomics data are available as supplemental data tables or have original sources referenced. Fly proteomics data are available from the following

link and login information:

<https://massive.ucsd.edu/ProteoSAFe/private-dataset.jsp?task=d8f8a278868d42398c9ba8c772395c7b>

Username: MSV000088897_reviewer

Password: winter

The raw RNA sequencing data fastq files were lost during a recent server crash. This information was previously relayed to the handling editor.

Research involving human participants, their data, or biological material

Policy information about studies with [human participants or human data](#). See also policy information about [sex, gender \(identity/presentation\), and sexual orientation](#) and [race, ethnicity and racism](#).

Reporting on sex and gender N/A

Reporting on race, ethnicity, or other socially relevant groupings N/A

Population characteristics N/A

Recruitment N/A

Ethics oversight N/A

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

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

For a reference copy of the document with all sections, see [nature.com/documents/nr-reporting-summary-flat.pdf](https://www.nature.com/documents/nr-reporting-summary-flat.pdf)

Life sciences study design

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

Sample size Sample sizes were selected based on previously published standards for sizes using the described models. mtd RNAi fly lifespans were performed with smaller sample sizes due to larval lethality, but were repeated multiple times to ensure replication.

Data exclusions No data were excluded from analyses.

Replication Experiments were replicated a minimum of 3 times except where specifically stated, including multiple replicates by different researchers. Replication was consistent across repeated experiments.

Randomization For experiments with treatments, cells and flies were separated randomly into their specific conditions (dietary, genetic induction, or treatment group). Cells and flies were sorted into their respective assay conditions randomly for each condition.

Blinding Blinding was not possible in this study. It is not possible to be blinded from diet-related analyses as the color of the diet is different between conditions.

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

n/a	Involved in the study
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☐	☒ Animals and other organisms
☒	☐ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used

Rabbit polyclonal anti-OXR1 Invitrogen Cat#PA5-72405
 Rabbit polyclonal anti-OXR1 abcam Cat#ab103042
 Goat polyclonal anti-VPS35 abcam Cat#ab10099
 Rabbit polyclonal anti-VPS26A Proteintech Cat#12804-1-AP
 Rabbit polyclonal anti-VPS26B Proteintech Cat#15915-1-AP
 Rabbit polyclonal anti-VPS29 Abcam Cat#ab236796
 Mouse monoclonal anti- β -Galactosidase (LacZ) Promega Cat#Z3781
 Rabbit polyclonal anti-LC3B Novus Biologicals Cat#NB100-2220
 Rabbit polyclonal anti-Atg8 Sigma-Aldrich Cat#ABC974
 Mouse monoclonal anti-Cathepsin B [CA10] abcam Cat#ab58802
 Rabbit polyclonal anti-GAPDH abcam Cat#ab9485
 Rabbit polyclonal anti- β -actin Cell Signaling Cat#4967
 Mouse monoclonal anti- α -Tubulin [DM1A] Sigma-Aldrich Cat#T6199
 Mouse monoclonal anti-Rab7 [EPR7589] abcam Cat#ab50533
 Mouse monoclonal anti-elav DSHB Cat#Elav-9F8A9
 Mouse monoclonal anti-EEA1 [G-4] Santa Cruz Biotechnology Cat#sc-137130
 Mouse monoclonal anti-ATP5A [15H4C4] abcam Cat#ab14748
 Mouse monoclonal anti-LAMP1 [H4A3] abcam Cat#ab25630
 Mouse monoclonal anti-Calreticulin [FMC 75] abcam Cat#ab22683
 Mouse polyclonal anti-GM130 abcam Cat#ab169276
 Mouse monoclonal anti-GFP [B-2] Santa Cruz Biotech Cat#sc-9996
 Mouse monoclonal anti-Tau [HT7] Invitrogen Cat#MN1000
 Sheep anti-Mouse IgG HRP-linked secondary Sigma Aldrich Cat#NXA931V
 Donkey anti-Rabbit IgG HRP-linked secondary Sigma Aldrich Cat#NA934V
 Mouse anti-Goat IgG HRP-conjugated secondary Santa Cruz Biotech Cat#sc-2354
 Alexa Fluor 488 donkey anti-rabbit IgG (H+L) secondary Invitrogen Cat#A21206
 Alexa Fluor 647 donkey anti-goat IgG (H+L) secondary Invitrogen Cat#A21447
 Alexa Fluor 647 donkey anti-mouse IgG (H+L) secondary Invitrogen Cat#A21238

Validation

Rabbit polyclonal anti-OXR1 Invitrogen Cat#PA5-72405 WB concentration: 1:500 Validated by RNAi in manuscript.
 Rabbit polyclonal anti-OXR1 abcam Cat#ab103042 ICC concentration: 1:100 Validated against human OXR1 mutation in manuscript.
 Goat polyclonal anti-VPS35 abcam Cat#ab10099 WB concentration: 1:500
 ICC concentration: 1:100 Validated by KO (abcam).
 Rabbit polyclonal anti-VPS26A Proteintech Cat#12804-1-AP WB concentration: 1:500 Previously validated (Hou et al., referenced in manuscript).
 Rabbit polyclonal anti-VPS26B Proteintech Cat#15915-1-AP WB concentration: 1:500 Validated by molecular weight in manuscript.
 Rabbit polyclonal anti-VPS29 Abcam Cat#ab236796 WB concentration: 1:500 Validated by KO (abcam).
 Mouse monoclonal anti- β -Galactosidase (LacZ) Promega Cat#Z3781 WB concentration: 1:500
 IHC concentration: 1:100 Validated by molecular weight in manuscript.
 Rabbit polyclonal anti-LC3B Novus Biologicals Cat#NB100-2220 WB concentration: 1:150 Validated by KO (Novus Biologicals).
 Rabbit polyclonal anti-Atg8 Sigma-Aldrich Cat#ABC974 WB concentration: 1:500 Validated by molecular weight in manuscript.
 Mouse monoclonal anti-Cathepsin B [CA10] abcam Cat#ab58802 WB concentration: 1:500 Validated by molecular weight in manuscript.
 Rabbit polyclonal anti-GAPDH abcam Cat#ab9485 WB concentration: 1:500 Validated by molecular weight in manuscript.
 Rabbit polyclonal anti- β -actin Cell Signaling Cat#4967 WB concentration: 1:500 Validated by company (Cell Signaling).
 Mouse monoclonal anti- α -Tubulin [DM1A] Sigma-Aldrich Cat#T6199 WB concentration: 1:500 Validated by company (Sigma-Aldrich).
 Mouse monoclonal anti-Rab7 [EPR7589] abcam Cat#ab50533 WB concentration: 1:500
 ICC concentration: 1:100 Validated by company (abcam).
 Mouse monoclonal anti-elav DSHB Cat#Elav-9F8A9 IHC concentration: 1:100 Validated by neuronal specificity in manuscript
 Mouse monoclonal anti-EEA1 [G-4] Santa Cruz Biotechnology Cat#sc-137130 ICC concentration: 1:100 Validated by company (Santa Cruz Biotechnology).
 Mouse monoclonal anti-ATP5A [15H4C4] abcam Cat#ab14748 ICC concentration: 1:100 Validated by company (abcam).
 Mouse monoclonal anti-LAMP1 [H4A3] abcam Cat#ab25630 ICC concentration: 1:100 Validated by company (abcam).
 Mouse monoclonal anti-Calreticulin [FMC 75] abcam Cat#ab22683 ICC concentration: 1:100 Validated by company (abcam).
 Mouse polyclonal anti-GM130 abcam Cat#ab169276 ICC concentration: 1:100 Validated by company (abcam).
 Mouse monoclonal anti-GFP [B-2] Santa Cruz Biotech Cat#sc-9996 WB concentration: 1:500 Validated by molecular weight in

manuscript.

Mouse monoclonal anti-Tau [HT7] Invitrogen Cat#MN1000 WB concentration: 1:1000 Validated by molecular weight in manuscript.
 Sheep anti-Mouse IgG HRP-linked secondary Sigma Aldrich Cat#NNA931V WB concentration: 1:3000
 Donkey anti-Rabbit IgG HRP-linked secondary Sigma Aldrich Cat#NA934V WB concentration: 1:3000
 Mouse anti-Goat IgG HRP-conjugated secondary Santa Cruz Biotech Cat#sc-2354 WB concentration: 1:3000
 Alexa Fluor 488 donkey anti-rabbit IgG (H+L) secondary Invitrogen Cat#A21206 ICC concentration: 1:500
 Alexa Fluor 647 donkey anti-goat IgG (H+L) secondary Invitrogen Cat#A21447 ICC concentration: 1:500
 Alexa Fluor 647 donkey anti-mouse IgG (H+L) secondary Invitrogen Cat#A21238 ICC concentration: 1:500

Eukaryotic cell lines

Policy information about [cell lines and Sex and Gender in Research](#)

Cell line source(s)	Fibroblasts were generated from skin biopsies, as previously described (Wang et al, 2019). Sex was not considered in the study design. Cell lines used are as follows: F0062.1 Male human healthy skin fibroblasts provided by laboratory of Dr. Philippe Campeau, CHU Sainte Justine Research Center, Montreal, QC, Canada. F0342.1 Male human skin fibroblasts with OXR1 mutation (c.1324delA; p.Ser44Valfs*2) provided by laboratory of Dr. Philippe Campeau, CHU Sainte Justine Research Center, Montreal, QC, Canada.
Authentication	Cell lines were authenticated at CHU Sainte-Justine, Montreal, as previously described (Wang et al, 2019). Cell lines were authenticated at sample collecting by Sanger sequencing.
Mycoplasma contamination	All cell lines tested negative for mycoplasma contamination.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified lines were used in this study.

Animals and other research organisms

Policy information about [studies involving animals](#); [ARRIVE guidelines](#) recommended for reporting animal research, and [Sex and Gender in Research](#)

Laboratory animals	No animals relevant for reporting were used in this study. Only flies were used in this study. The analyses for this manuscript utilized longitudinal analyses across life. The strains used are listed here Drosophila Genetic Reference Panel strains Bloomington Drosophila Stock Center All DGRP strains Act5C-GS-Gal4 Driver (inducible, whole body) P{w[+mC]=Act5C(FRT.y[+])GAL4.Switch.PR}X, y[1] w[*] Bloomington Drosophila Stock Center #9431 Elav-GS-Gal4 Driver (inducible, neuronal) w1118 ; P{w[+mC]=elav-Switch.O}GSG301 Bloomington Drosophila Stock Center #43642 Elav-Gal4 Driver (non-inducible, neuronal) P{w[+mW.hs]=GawB}elav[C155] Bloomington Drosophila Stock Center #458 5966-GS-Gal4 (inducible, intestinal) +; 5966-GS Provided by lab of Dr. David Walker, University of California, Los Angeles repo-Gal4 (non-inducible, glial) w[1118]; P{w[+m*]=GAL4}repo/TM3, Sb[1] Bloomington Drosophila Stock Center #7415 GMR-driven mutant Tau (non-inducible, eye), Elav-Gal4 (non-inducible, neuronal) P{w[+mW.hs]=GawB}elav[C155]; P{w[+mC]=GMR-htau/Ex}1.1 Bloomington Drosophila Stock Center #51360 w1118 control strain Bloomington Drosophila Stock Center #5905 Transgenic RNAi Project (TRiP) empty vector control strain y[1] sc[*] v[1]; P{y[+t7.7] v[+t1.8]=VALIUM20-mCherry}attP2 Bloomington Drosophila Stock Center #35785 mtd RNAi y[1] sc[*] v[1]; P{TRiP.HMS01666}attP2/TM3, Sb[1] Bloomington Drosophila Stock Center #38519 mtd mutant y[1] w[*]; Mi{y[+mDint2]=MIC}mtd[MIO2920]/TM3, Sb[1] Ser[1] Bloomington Drosophila Stock Center #76158 hOXR1 overexpression w1118; P{UAS-OXR1.HA}1 Bloomington Drosophila Stock Center #64104 hOXR1 overexpression w1118; P{UAS-OXR1.HA}4/TM3, Sb1 Bloomington Drosophila Stock Center #64105 Fdxh RNAi w1118; P{GD1274}v24497 Vienna Drosophila Resource Center #24497 CG15515 RNAi w1118; P{GD8577}v39872 Vienna Drosophila Resource Center #39872 tj RNAi y[1] sc[*] v[1]; P{TRiP.HMS01069}attP2 Bloomington Drosophila Stock Center #34595 TJ-GFP w[1118]; P33657Bac{y[+mDint2] w[+mC]=tj-GFP.FPTB}VK00033 Bloomington Drosophila Stock Center #66391 ctcf RNAi y[1] sc[*] v[1]; P{TRiP.GL00266}attP2 Bloomington Drosophila Stock Center #35354 ctcf RNAi y[1] v[1]; P{TRiP.HMS02017}attP40 Bloomington Drosophila Stock Center #40850 vps26 RNAi y[1] v[1]; P{TRiP.HMS01769}attP40 Bloomington Drosophila Stock Center #38937 vps29 RNAi
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y[1] v[1]; P{TriP.HMJ21316}attP40 Bloomington Drosophila Stock Center #53951
 vps35 RNAi
 y[1] sc[*] v[1] sev2[1]; P{TriP.HMS01858}attP40 Bloomington Drosophila Stock Center #38944
 Atg8 overexpression
 y[1] w[1118]; P{w[+mC]=UASp-GFP-mCherry-Atg8a}2 Bloomington Drosophila Stock Center #37749
 hLAMP1 overexpression
 y[1] w[*]; PBac{y[+mDint2] w[+mC]=UAS-hLAMP1.HA}VK00033 Bloomington Drosophila Stock Center #86301
 Atg1 overexpression
 y[1] w[*]; P{w[+mC]=UAS-Atg1.S}6A Bloomington Drosophila Stock Center #51654
 mitoGFP overexpression
 w[1118]; P{w[+mC]=UAS-mito-HA-GFP.AP}2/CyO Bloomington Drosophila Stock Center #8442

Wild animals

No wild animals were used in this study.

Reporting on sex

Females were primarily used due to previous research indicating that dietary restriction primarily affects female flies (Spencer et al, 2003; Magwere et al, 2004), though males were used in some studies to distinguish any sex-specific phenotypes. Sex is specified in these cases.

Field-collected samples

No field-collected samples were used.

Ethics oversight

No ethical oversight was required, as no animals relevant for ethical oversight were used in this study.

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