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Reporting Summary

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Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. 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
☐	☒	An indication of 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 statistics 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
☐	☒	Clearly defined error bars *State explicitly what error bars represent (e.g. SD, SE, CI)*

Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

Data collection	Fluorescent images were acquired with Zeiss LSM710 or Zeiss LSM880 confocal microscopes and Zen software (Zeiss).
Data analysis	For image analysis, Image J or LI-COR Image Studio softwares were used. Quantitative data were analyzed and processed using MS Excel and GraphPad Prism 7.04. Xcalibur software (Thermo Scientific) and Proteome Discoverer 1.4 interface (Thermo Scientific) with Mascot algorithm (Mascot 2.4, Matrix Science) were used for mass spectrometry analysis.

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 upon request. 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

The MS proteomics data can be accessed from the ProteomeXchange Consortium via the MassIVE repository (MSV000083259) under accession code PXD012104.

The authors declare that the main data supporting the findings of this study are available within the article and its Supplementary Information files. Source data for Figures 1c, 1f, 2c-e, 3b-f, 4a, 4d-f, 5e, 6a, 6c-d, 6g, 7a-d, S1f, S2a, S2d-e, S3a-c, S4b-c, S4g, S4j, S5a, S5e-f, S7b-c, and S8b-d have been provided as Supplementary Table 9. All other data that support the findings of this study are available from the corresponding author upon reasonable request.

Field-specific reporting

Please select 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/authors/policies/ReportingSummary-flat.pdf

Life sciences study design

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

Sample size	Sample sizes were determined based on published studies in the field or our previous experiences. No statistics was used to predetermine the sample size.
Data exclusions	No data were excluded.
Replication	Experiments in the manuscript were performed at least three times except for Figures 1e, 3b, 5c, 6e-g, S1c, S1f, S3d-f, S4d, S4e-f, S5f, S5h, S6c, S7g, S8d, and larval IP/MS experiments (in Supplementary Tables 3-4) which were performed twice and adult head IP/MS experiment (in Supplementary Tables 3-4) which was performed once. All attemps at replication were successful.
Randomization	Samples are defined by their unique genotypes. Therefore, no sample randomization was performed.
Blinding	The investigators were not blinded for group allocation as the mutants displayed obvious phenotypes and clonal analysis marks mutants.

Reporting for specific materials, systems and methods

Materials & experimental systems

n/a	Involved in the study
☐	☒ Unique biological materials
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology
☐	☒ Animals and other organisms
☒	☐ Human research participants

Methods

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

Unique biological materials

Policy information about availability of materials

Obtaining unique materials	All unique materials used are readily available from the authors upon reasonable request. Fly stocks generated in this work will be deposited in BDSC upon publication.

Antibodies

Antibodies used	Primary antibodies used in fly experiments: Rabbit anti-p62/ref(2)p (gift from Dr. Sheng Zhang); Chicken anti-GFP (Abcam, ab13970), Rat anti-ELAV (DSHB: 7E8A10); Mouse anti-Chaoptin (DSHB: 24B10); Rabbit anti-Ubqn (gift from Dr. Ming Guo); Mouse anti-actin (ICN Biomedicals: C4); Mouse anti-α-Tubulin (Sigma T9026); Rabbit anti-P-eIF2α (S51) (CST #9721); Rabbit anti-eIF2α (eIF2S1) (Abcam ab26197); Rabbit anti-GFP (Invitrogen #A-11122); Rabbit anti-Phospho-Drosophila-Akt (Ser505) (CST #4054); Rabbit Akt (pan) (CST #4691); Rabbit anti-Phospho-Drosophila-S6K (Thr398) (CST #9209); Rabbit anti-S6K (SCBT sc-9027); Rabbit anti-Phospho-4E-BP (Thr37/46) (CST #2855); Mouse anti-CTSL (R&D Systems #MAB22591); Guinea pig anti-Bip/Hsc3 (gift from Dr. Don Ryoo); Rabbit anti-Drosophila Atg8 (gift from Dr. Linda Partridge); Mouse anti-Lamin C (DSHB, LC28.26); Guinea pig anti-Vha100 (generated in the Bellen Lab). Primary antibodies used in human cell culture experiments:

Rabbit anti-p62 (MBL: PM045); Rabbit anti-GAPDH (CST #2118); Mouse anti-actin (ICN Biomedicals: C4); Rabbit anti-UBQLN1 (CST #14526); Rabbit anti-UBQLN2 (CST #85509); Rabbit anti-UBQLN4 (Abcam ab106443); Rabbit anti-Phospho S6K (T389) (CST #9205); Rabbit anti-S6K (SCBT sc-9027); Rabbit anti-Phospho ULK1 (S757) (CST #6888); Rabbit anti-LAMP1 (CST #9091); Mouse anti-LAMP2 (SCBT: H4B4); Rabbit anti-LC3B (CST: D11); Rabbit anti-ATP6V0A1 (Novus Biologicals: NBP1-89342); Rabbit anti-ATP6V1B2 (CST #14617); Mouse anti-ATP6V1D1 (SCBT: sc-393322); Mouse anti-ATP6V1H (SCBT: sc-166227); Rabbit anti-Renin R/M8.9 (Novus Biologicals: NBP1-90820); Mouse anti-FLAG M2 (Sigma: F1804)

Secondary antibodies used:
For IF: Alexa 488, and Cy3 or Cy5 conjugated secondary antibodies (Jackson ImmunoResearch, West Grove, PA) and Alexa 488-conjugated Phalloidin (Invitrogen)
For WB: IRdye 680RD and IRdye 800CW (Li-COR Biosciences) and HRP-conjugated secondary antibodies (Jackson ImmunoResearch)

All antibody dilutions are listed in Methods.

Validation

All antibodies were validated by the manufacturer or by previously published studies to be suitable for immunofluoresence and/or western blotting in flies or human cell lines. Antibody validation information is listed in Supplementary Table 7.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)

Daoy and HEK293T cell lines were from Dr. Huda Zoghbi and HEK293 UBQLN1+2+4 triple knockout cell line was from Dr. Ramanujan S. Hedge.

Authentication

Cell lines were authenticated based on their morphology and growth.

Mycoplasma contamination

Cell lines were not tested for mycoplasma contamination.

Commonly misidentified lines
(See ICLAC register)

No cell lines used in this study were found in the database of commonly misidentified cell lines that is maintained by ICLAC and NCBI Biosample.

Animals and other organisms

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

Laboratory animals

Drosophila melanogaster adult flies or larvae were used depending on each experimental design.
L2 larvae: S4h-i
Early L3 larvae: 3b-c, 4a, S4a, and S4g
Wandering L3 larvae: 1b-d, 3a, 3e, 4b, 4d-f, 5b-e, 6a, 6c-g, 8a-e, S1a-c, S3a, S3c-f, S4j, S5a-h, and S6c
Pre-pupae: 1e, S1e-f, and S4f
Adult flies:
1b: 2d old adult female
1f: 15d and 45d old females
2a-c, S2a-d, and S4b: 1d, 15d, and 30d old females
2d: 1d old females
3d, 4c: 15d old females
3f: 2d old adult females
S2e: 45d old females
S2f: 30d old females
S3b: 2-3d old adult females

Following strains are used in this study:
y w ubqn[1] FRT19A, y w ubqn[1] FRT19A; 20kb P[acman], ubqn[ywing2+], UAS-dUbqn, UAS-FLAG-dUbqn, y w;UAS-UBQLN2[WT], y w;UAS-UBQLN2[P497H], y w; FRT82B v100[3], and UAS-V100 were generated in the Bellen lab.
y1 w* P{nos-phiC31\int.NLS}X; PBac{y+-attP-3B}VK00033, Df(1)BSC871, Cg-Gal4, Act-Gal4, da-GAL4, nSyb-Gal4, y[1] w[1118] P{neoFRT}19A; P{eyFLP}, P{Ubi-mRFP.nls}, w* P{hsFLP}, P{neoFRT}19A; P{UASp-mCherry-Atg8a}2, UAS-Luciferase RNAi, UAS-Luciferase, y1 v1; UAS-V100 RNAi, w*; P{w[+mC]=sqh-EYFP-ER}3, y[1] w[1118]; P{UASp-GFP-mCherry-Atg8a}2, y[1] w*; Mi{PT-GFSTF.0}Atg1MI07056-GFSTF.0, w[1118];P{PTT-GA}VhaSFDG00259, y[1] w[67c23]; P{EPgy2}VhaSFDEY04644, w[1118];P{EP}Vha68-2EP2364, and y[1]w[67c23]; P{EPgy2}VhaM8.9EY03616 were obtained from Bloomington Drosophila Stock Center. w[1118]; P{RS5}VhaM8.95-HA-1890 was obtained from Kyoto Stock Center.
The following lines were obtained from Vienna Drosophila Research Center: P{KK107676}VIE-260B (RNAi against VhaM8.9), UAS-Ubqn RNAi was a gift from Dr. Ming Guo, UAS-LAMP1-GFP was a gift from Dr. Helmut Kramer, and cl(1), P{neoFRT}19A/Dp(1;Y) y + v+ ; eyFLP was a gift from Drs. John Olson and Utpal Banerjee. Xbp1-EGFP was a gift from Dr. Don Ryoo.

Wild animals

This study did not involve wild animals.

Field-collected samples

This study did not involve samples collected from the field.