

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 (<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

Image Lab 6.0 (BioRad)
Odyssey Li-cor application software V3.0
Zen 2012 SP1 (black edition)

Data analysis

GraphPad Prism 7.0 for statistics
Image Lab 6.0 (BioRad)
Odyssey Li-cor application software V3.0
PyMOL 1.8.4.2
CellProfiler 3.1.5
KNIME 3.4.1
Fiji (ImageJ 2.0)

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 Fig. 1e and f, 2d and h, 3c and f, 4b,c,f and h, 5b-g, 6c and e, and 7b,c,e and h and Supplementary Fig. 1b and f, and 2e have been provided as Supplementary Table 1. All other data supporting the findings of this study are available from the corresponding authors upon 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

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	No statistical method was used to determine the correct sample size but these were instead determined based on our experiences from previous studies using similar methodologies.
Data exclusions	No data was excluded from the study.
Replication	All experimental replicates were performed independent of each other and all replication attempts were successful.
Randomization	n/a
Blinding	n/a

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
☒	☐ Animals and other organisms
☒	☐ Human research participants
☒	☐ Clinical data

Methods

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

Antibodies

Antibodies used

anti-ATG16L1 mouse mAb, MBL M150-3, 1:1000
 anti-ATG5 rabbit pAb, CST 2630, 1:1000
 anti-ATG3 rabbit pAb, CST 3415, 1:1000
 anti-Beta-ACTIN (8H10D10) mouse mAb, CST 3700, 1:5000
 anti-GAPDH rabbit mAb, CST 5174, 1:5000
 anti-GABARAP (E1J4E) rabbit mAb, CST 13733, 1:1000
 anti-LC3B (D11) XP rabbit mAb, CST 3868, 1:1000
 anti-LC3B rabbit pAb, MBL PM036, 1:500
 anti-WIP12 mouse mAb [2A2], Abcam ab105459, 1:2000
 anti-FLAG rabbit pAb, Sigma F7425, 1:1000
 anti-Myc rabbit pAb, CST 2272, 1:1000
 DyLight-680 anti-mouse pAb, Invitrogen SA5-10170, 1:5000
 DyLight-800 anti-rabbit pAb, Invitrogen SA5-10044, 1:5000
 Starbright Blue 700 anti-mouse Bio-Rad 12004158, 1:10000
 Starbright Blue 700 anti-rabbit Bio-Rad 12004161, 1:10000
 HRP-conjugated anti-mouse pAb, Jackson ImmunoResearch 115-035-003, 1:5000
 HRP-conjugated anti-rabbit pAb, Jackson ImmunoResearch 111-035-144, 1:5000
 Cy3 anti-mouse pAb, Jackson ImmunoResearch 715-165-151, 1:500
 Cy3 anti-rabbit pAb, Jackson ImmunoResearch 711-165-152, 1:500
 Cy5 anti-rabbit pAb, Jackson ImmunoResearch 711-175-152, 1:500
 DyLight649 anti-rabbit, pAb Jackson ImmunoResearch 711-495-152, 1:500

Validation

anti-ATG16L1 mouse mAb (MBL, M150-3). Validation: tested in CRISPR Cas9 knock-out cell lines in this study and in <https://www.mblintl.com/products/m150-3>
 anti-ATG5 rabbit PoAb (CST, 2630). Validation:

<https://www.cellsignal.com/products/primary-antibodies/atg5-antibody/2630>
 anti-ATG3 rabbit pAB (CST, 3415). Validation: <https://www.cellsignal.com/products/primary-antibodies/atg3-antibody/3415>
 anti-Beta-ACTIN (8H10D10) mouse mAb (CST, 3700). Validation: <https://www.cellsignal.com/products/primary-antibodies/b-actin-8h10d10-mouse-mab/3700>
 anti-GAPDH rabbit mAb (CST, 5174). Validation: <https://www.cellsignal.com/products/primary-antibodies/gapdh-d16h11-xp-rabbit-mab/5174>
 anti-LC3B (D11) XP rabbit mAb (CST, 3868). Validation: <https://www.cellsignal.com/products/primary-antibodies/lc3b-d11-xp-rabbit-mab/3868>
 anti-LC3B rabbit pAB (MBL, PM036). Validation: <http://ruo.mbl.co.jp/bio/e/dtl/A/?pcd=PM036>
 anti-WIP1 mouse mAb [2A2] (Abcam, ab105459). Validation: tested in CRISPR Cas9 knock-out cell lines in this study and in <https://www.abcam.com/wip1-antibody-2a2-ab105459.html>
 anti-FLAG rabbit pAB (Sigma, F7425). Validation: <https://www.sigmaaldrich.com/catalog/product/sigma/f7425?lang=en®ion=NO>
 anti-Myc rabbit pAB (CST, 2272). Validation: <https://www.cellsignal.com/products/primary-antibodies/myc-tag-antibody/2272>

Eukaryotic cell lines

Policy information about [cell lines](#)

Cell line source(s)	HEK293, U2OS and RAW264.7 were obtained from ATCC. HEK293FT and HEK-F were obtained from Invitrogen.
Authentication	The cell lines were not authenticated.
Mycoplasma contamination	All cell lines were tested for mycoplasma using the MycoAlert™ Mycoplasma Detection Kit (LONZA)
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.