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recent with Country	Corresponding author(s): Fang Tian & Hong-Gang Wang
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Reporting Summary	
Nature Research wishes to improve the reproducibility of the work that we p In reporting. For further information on Nature Research policies, see our <u>Edi</u>	, , , , , , , , , , , , , , , , , , , ,
Statistics	
or all statistical analyses, confirm that the following items are present in the	figure legend, table legend, main text, or Methods section.
/a Confirmed	
The exact sample size (n) for each experimental group/condition, given	ven as a discrete number and unit of measurement
A statement on whether measurements were taken from distinct sa	imples 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 comp.	lex techniques in the Methods section.
A description of all covariates tested	
A description of any assumptions or corrections, such as tests of no	mality and adjustment for multiple comparisons
A full description of the statistical parameters including central tend AND variation (e.g. standard deviation) or associated estimates of u	lency (e.g. means) or other basic estimates (e.g. regression coefficient) ncertainty (e.g. confidence intervals)
For null hypothesis testing, the test statistic (e.g. F , t , r) with confide Give P values as exact values whenever suitable.	ence intervals, effect sizes, degrees of freedom and P value noted
For Bayesian analysis, information on the choice of priors and Mark	ov chain Monte Carlo settings
igwedge For hierarchical and complex designs, identification of the appropria	ate level for tests and full reporting of outcomes
\square Estimates of effect sizes (e.g. Cohen's d , Pearson's r), indicating how	they were calculated
Our web collection on statistics for biologists cont	ains articles on many of the paints above.
Software and code	
olicy information about availability of computer code	
Data collection Bruker Topspin3.2	
Data analysis (GraphPad Prism7.0, NMRView5.2.2, ImageJ, NMRPipe3.0	
or manuscripts utilizing custom algorithms or software that are central to the research but not yer eviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the	
Data	
olicy information about <u>availability of data</u>	
All manuscripts must include a <u>data availability statement</u> . This statement sh - Accession codes, unique identifiers, or web links for publicly available datasets	nould provide the following information, where applicable:
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- A list of figures that have associated raw data
- A description of any restrictions on data availability

NMR resonance assignments have been deposited with the BMRB with accession numbers 50479 (hAtgΔ90-190 in aqueous solution), 50480 (hAtgΔ90-190 in bicelles), 50481 (hAtg3∆90-190_P21A in bicelles), and 50470 (hAtg3∆1-25 in aqueous solution). PDBs of 3VX8, 6OJJ, and 2DYT are available for download at www.rcsb.org.

Field-spe	cific re	porting		
Please select the on	e below that is	the best fit for your research. If you are not sure, read the appropriate sections before making your selection.		
Life sciences	□ 8	ehavioural & social sciences		
For a reference copy of th	re document with	all sections, see <u>nature.com/documents/nr-reporting-summary-flat.pdf</u>		
Life scien	ices stu	ıdy design		
All studies must disc	close on these	points even when the disclosure is negative.		
Sample size	n=6, based on p	pased on prior research		
Data exclusions	No data were e	excluded from analysis		
Replication	6 replicates; all	ates; all attempts at replication were successful		
Randomization	N/A, as westerr	ss western blot analysis for controlled samples		
Blinding	N/A, as western blot analysis for controlled samples			
Reporting	g for sp	pecific materials, systems and methods		
•		about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.		
Materials & exp				
n/a Involved in the		n/a Involved in the study		
Antibodies		ChIP-seq		
Eukaryotic e		Flow cytometry		
	ogy and archaeol			
	d other organism earch participant			
Human research participants Clinical data				
Dual use research of concern				
Antibodies				
Antibodies used	mCher	ry antibody (Abcam, ab125096); b-actin antibody (Sigma, A5441-100ut), LC3 antibody (Novus Biologicals, NB100-2220		
Validation	Antibo	dies were validated by overexpression and knockdown or knockout approaches.		
e- 1	E			
Eukaryotic ce				
Policy information a		UEV200T cells users purchased from ATCC and Atg2 / MEEs users provided by Dr. Shangkan (Victor) lin / Dutroys University		
Cell line source(s)		HEK293T cells were purchased from ATCC and Atg3-/- MEFs were provided by Dr. Shengkan (Victor) Jin (Rutgers University - Robert Wood Johnson Medical School, NJ)		
Authentication		All cell lines in the lab are passaged for less than 6 months before use and periodically authenticated by mycoplasma testing, morphologic inspection and STR analysis.		
Mycoplasma cont	amination	Mycoplasma contamination was periodically tested and the results were negative for the contamination		
Commonly miside (See <u>ICLAC</u> register)		No commonly misidentified cell lines were used in the study		