nature portfolio

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Last updated by author(s): Nov 8, 2024

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 (*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	Bio-Rad ChemiDoc Touch Imaging Software V1.2, ZEISS ZEN software 2.5, Hitachi H-7650 transmission electron microscope
Data analysis	GraphPad Prism V8.0.1 , image J

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

The main data supporting the findings of this study are available within the article and its Supplementary Figures. The source data underlying Figs. 1–6, Supplementary Figs. 4, 8, 13, 14, 16 are provided as a Source Data file. Source data are provided with this paper.

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

Life sciences study design

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

Sample size	No statistical methods were used to predetermine the sample sizes. Sample sizes were chosen as large as possible while still practically doable in terms of data collection and as sufficient when results could be reliably reproduced. For confocal imaging, at least three biological replicates were used. More than 5 individual seedlings, 20 individual root cells, or 10 protoplast cells were observed for each biological replicate. For Co-IP or WB, three biological replicates were performed. Each replicate contained a group of 5×10^5 Arabidopsis protoplast cells or 15 Arabidopsis seedlings. For ubiquitination protein detection, three biological replicates were performed. Each replicate contained a group of 0.3 g Arabidopsis seedlings. For TEM assay, 10 Arabidopsis root tips were randomly selected for high pressure freezing. A minimum of three blocks (each block contains 1 root tip) from each sample were randomly selected for sectioning and TEM imaging.
Data exclusions	No samples or recordings were excluded.
Replication	All experiments contain at least three biological replicates and were replicated independently and showed similar results.
Randomization	The Arabidopsis seedlings with the same genetic background were randomly assigned to normal conditions or heat stress treatment groups for subsequent analysis.
Blinding	The investigators were blinded to group allocation during data collection and analysis.

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

All the antibodies are commercially sourced antibodies.
Anti-actin Rabbit polyclonal antibody (1:5000) (Sangon Biotech; Cat# D110007)
Anti-GFP Mouse monoclonal antibody(1:5000) (Transgene; Cat# HT801-01)
Anti-FLAG Mouse monoclonal antibody(1:5000) (Transgene; Cat# HT201-01)
Anti-mCherry Mouse monoclonal antibody (1:5000) (HUABIO; Cat# HA601186)
Anti-GFP Rabbit polyclonal antibody (1:1000) (abcam; Cat# ab290)
Anti-Ubiquitin11 Rabbit polyclonal antibody (1:5000) (Agrisera; Cat# AS08307A)
Anti-ATG8 Rabbit polyclonal antibody (1:3000)(Agrisera; Cat# AS14 2769)
Anti-NBR1 Rabbit polyclonal antibody (1:3000)(Agrisera; Cat# AS14 2805A)

Validation

Validation statement for actin antibody (Sangon Biotech; Cat# D110007) can be found at the product website.<https://store.sangon.com/productDetail?productInfo.code=D110007>
Validation statement for GFP antibody (Transgene; Cat# HT801-01) can be found at the product website.<https://www.transgen.com.cn/antibody_tag/390.html>
Validation statement for FLAG antibody (Transgene; Cat# HT801-01) <https://www.transgen.com/antibody_tag/371.html>
Validation statement for mCherry antibody (HUABIO; Cat# HA601186) can be found at the product website.<http://www.huabio.cn/product/mCherry-antibody-HA601186>
Validation statement for GFP antibody (Abcam; Cat# ab290) can be found at the product website.<https://www.abcam.cn/products/primary-antibodies/gfp-antibody-ab290.html>
Validation statement for Ubiquitin11 antibody (Agrisera; Cat# AS08307A) can be found at the product website.<https://www.agrisera.com/en/artiklar/ubq11-ubiquitin-.html>
Validation statement for ATG8 antibody (Agrisera; Cat# AS14 2769) can be found at the product website.<https://www.agrisera.com/en/artiklar/ubq11-ubiquitin-.html>
Validation statement for NBR1 antibody (Agrisera; Cat# AS14 2805A) can be found at the product website.<https://www.agrisera.com/en/artiklar/nbr1-autophagy-substrate-nbr1.html>

Dual use research of concern

Policy information about dual use research of concern

Hazards

Could the accidental, deliberate or reckless misuse of agents or technologies generated in the work, or the application of information presented in the manuscript, pose a threat to:

No	Yes	
☒	☐	Public health
☒	☐	National security
☒	☐	Crops and/or livestock
☒	☐	Ecosystems
☒	☐	Any other significant area

Experiments of concern

Does the work involve any of these experiments of concern:

No	Yes	
☒	☐	Demonstrate how to render a vaccine ineffective
☒	☐	Confer resistance to therapeutically useful antibiotics or antiviral agents
☒	☐	Enhance the virulence of a pathogen or render a nonpathogen virulent
☒	☐	Increase transmissibility of a pathogen
☒	☐	Alter the host range of a pathogen
☒	☐	Enable evasion of diagnostic/detection modalities
☒	☐	Enable the weaponization of a biological agent or toxin
☒	☐	Any other potentially harmful combination of experiments and agents

Plants

Seed stocks

T-DNA insert mutant ubp1a-1 (GK-625F11-022320) and ubp1b-1 (GK-262E01-014951) were obtained from the Arabidopsis Biological Resource Center (ABRC). The pUBQ10::ATG5-GFP, pUBQ10::ATG9-GFP, pUBQ10::EYFP-ATG8f, pUBQ10::ATG18a-GFP, pUBQ10::mCherry-ATG8f× pUBQ10::NBR1-GFP, pUBQ10::mCherry-ATG8f× pUBQ10::GFP-SYP32, and atg5-1 mutants have been described previously. The pUBQ10::ATG1a-GFP, pUBQ10::ATG13a-GFP, pUBQ10::ATG6-GFP, pUBQ10::GFP-VPS34, pUBQ10::ATG3-GFP, pUBQ10::ATG7-GFP, pUBQ10::UBP1c-mCherry, pUBQ10::UBP1c-GFP, pUBQ10::ATG13a-GFP/ubp1abc, pUBQ10::ATG6-GFP/ubp1abc, pUBQ10::ATG5-GFP/ubp1abc, pUBQ10::EYFP-ATG8f/ubp1abc were generated using the floral dip method with Agrobacterium tumefaciens GV3101. The marker lines VHAa1-mRFP, mRFP-SYP32, Mito-mRFP and mRFP-Rha1 have been described previously.

Novel plant genotypes

The Knockout of UBP1c mutant(ubp1c-cas9) was generated using an egg-specific promoter-driven CRISPR-Cas9-edited system using targeting guide RNA sequence (GTTTAGCATCCTGCTGTTT and CAAGTCGAAAGCTCCTCCC) followed by segregation of the Cas9 transgene byback crossing to the WT. As a result, a 427 bp region of the UBP1c gene region was deleted and this was a null mutant. The triple loss of function mutant ubp1abc was generated by pollen crossing ubp1a-1, ubp1b-1, and ubp1c-cas9, which was verified by genotyping.

The pUBQ10::ATG1a-GFP, pUBQ10::ATG13a-GFP, pUBQ10::ATG6-GFP, pUBQ10::GFP-VPS34, pUBQ10::ATG3-GFP, pUBQ10::ATG7-GFP, pUBQ10::UBP1c-mCherry, pUBQ10::UBP1c-GFP, pUBQ10::ATG13a-GFP/ubp1abc, pUBQ10::ATG6-GFP/ubp1abc, pUBQ10::ATG5-GFP/ubp1abc, pUBQ10::EYFP-ATG8f/ubp1abc, pUBQ10::mCherry-RBP47b, pUBQ10::mCherry-RBP47b/ubp1abc were generated using the floral dip method with Agrobacterium tumefaciens GV3101.

The pUBQ10::ATG13a-GFP× pUBQ10::UBP1c-mCherry, pUBQ10::ATG6-GFP× pUBQ10::UBP1c-mCherry, pUBQ10::ATG5-GFP× pUBQ10::UBP1c-mCherry, pUBQ10::ATG9-GFP× pUBQ10::UBP1c-mCherry, pUBQ10::UBP1c-GFP× pUBQ10::mCherry-ATG8f, pUBQ10::ATG13a-GFP× mRFP-Rha1, pUBQ10::ATG6-GFP× mRFP-Rha1, pUBQ10::ATG5-GFP× mRFP-Rha1, pUBQ10::ATG13a-GFP× Mito-mRFP, pUBQ10::ATG6-GFP× Mito-mRFP, pUBQ10::ATG5-GFP× Mito-mRFP, pUBQ10::ATG13a-GFP× mRFP-SYP32, pUBQ10::ATG6-GFP× mRFP-SYP32, pUBQ10::ATG5-GFP× mRFP-SYP32, pUBQ10::ATG13a-GFP× VHAa1-mRFP, pUBQ10::ATG6-GFP× VHAa1-mRFP, pUBQ10::ATG5-GFP× VHAa1-mRFP, pUBQ10::UBP1c-GFP/atg5-1 plants were generated by pollen crossing.

Authentication

Describe any authentication procedures for each seed stock used or novel genotype generated. Describe any experiments used to assess the effect of a mutation and, where applicable, how potential callus growth was confirmed to be the result of transformation.