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Last updated by author(s): May 25, 2026

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	SoftMax Pro (Molecular Devices, v7.1) for fluorescence data collection using the FlexStation 3 microplate reader. ZEN (Zeiss, v2.3) for confocal fluorescence image acquisition. Launch DSCRun (TA Instruments, v4.7.1) for DSC data collection. BD Accuri C6 Plus software (BD Biosciences) for flow-cytometry data collection. LAS X software (Leica Microsystems, v4.7.0) for FLIM-FRET data acquisition using the STELLARIS 8 FALCON system.
Data analysis	GraphPad Prism (v8.0.2) for graph generation and statistical analysis. ClustalW version 2.1 via the GenomeNet web server was used for sequence alignment. MEGA (v5.1) for phylogenetic tree construction. Launch NanoAnalyze (TA Instruments, v3.11.0) for DSC data analysis. Microsoft Excel 2019 for data processing and table preparation. Microsoft PowerPoint 2019 and Adobe Illustrator 2023 for figure preparation.

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 data supporting the findings of this study are available in this paper and its Supplementary Information. Source data underlying the graphs in Figs. 1–5 and Extended Data Figs. 1–10 are provided with this paper as Source Data Figs. 1–5 and Source Data Extended Data Figs. 1–10, respectively. Raw images of uncropped gels are provided in Supplementary Figure 1. Rice genomic variation and haplotype information used in this study were obtained from the RiceVarMap v2.0 database (https://ricevarmap.ncpgr.cn). Protein sequences used for phylogenetic analyses were obtained from Phytozome (https://phytozome.jgi.doe.gov). Arabidopsis T-DNA insertion mutant information and genotyping guidance were obtained from TAIR (https://www.arabidopsis.org).

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 method was used to predetermine sample size. Sample sizes were chosen based on established experimental protocols, previous studies in the field and our prior experience with similar assays, and were comparable to those commonly used for rice heat-tolerance phenotyping, yeast fluorescence transport assays, lipidomics, microscopy and biochemical analyses. The exact sample sizes are indicated in the corresponding figure panels and legends.
Data exclusions	No data that pass quality control were excluded from statistical analysis.
Replication	Independent biological replicates were used in all experiments and are described in the relevant figure legends. All assays were repeated independently at least twice, or as indicated in the Methods and figure legends, with consistent results.
Randomization	All sample allocation was randomised.
Blinding	In our study, blinding was not deemed necessary since we made no a priori assumptions on the response of the different samples to the experimental treatment, samples were all treated in parallel and there was no selection prior measurement since all samples treated were always measured.

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

Anti-UGPase antibody (Agrisera, AS14 2813; 1:5,000).
Anti-Histone H3 antibody (Proteintech, 17168-1-AP; 1:5,000).
Anti-BiP antibody (Agrisera, AS09 481; 1:5,000).
Anti-COXII antibody (Agrisera, AS04 053A; 1:5,000).
Anti-IDH antibody (Agrisera, AS06 203A; 1:5,000).
Anti-V-ATPase antibody (Agrisera, AS07 213; 1:5,000).
Anti-Tic40 antibody (Agrisera, AS10 709; 1:5,000).
Anti-Sec21p antibody (Agrisera, AS08 327; 1:5,000).
Anti-H^+-ATPase antibody (Agrisera, AS07 260; 1:5,000).
Anti-Actin antibody (Sangon Biotech, D191048; 1:3,000).
Anti-FLAG antibody (Proteintech, 66008; 1:3,000).
Anti-OsALA5 monoclonal antibody custom-generated by Absea Biotechnology Co., Ltd. against amino acids 116–308 of OsALA5; 1:3,000.
HRP-conjugated Goat Anti-Mouse IgG (H+L) (Proteintech, SA00001-1; 1:3,000).
HRP-conjugated Goat Anti-Rabbit IgG (H+L) (Proteintech, SA00001-2; 1:3,000).

Validation

Commercial primary antibodies used in this study were validated by the suppliers for the indicated target proteins and applications according to the corresponding product information. Manufacturer product profiles and validation/application information are provided on the corresponding product pages for the antibodies used in this study:

anti-UGPase antibody (Agrisera, AS14 2813):Rabbit polyclonal antibody raised against His-tagged full-length Hordeum vulgare UGPase. Manufacturer-tested for WB; recommended dilution 1:10,000; expected/apparent MW approximately 52 kDa. Confirmed reactivity: Arabidopsis thaliana, Hordeum vulgare, Zea mays; predicted reactivity: Oryza sativa. Manufacturer-listed selected references include Kleczkowski LA & Decker DD, 2015. Product profile/validation information: https://www.agrisera.com/en/artiklar/ugpase-udp-glucose-pyrophosphorylase-cytoplasm-marker-hordeum-vulgare.html.

anti-Histone H3 antibody (Proteintech, 17168-1-AP): Rabbit polyclonal antibody against recombinant human Histone H3 protein. Manufacturer-tested for WB, IHC, IF/ICC, FC, IP and ELISA; observed MW 15–17 kDa. Tested reactivity: human, mouse and rat; literature-cited reactivity includes Arabidopsis. RRID: AB_2716755. Product profile/validation information: https://www.ptgcn.com/products/Histone-H3-Antibody-17168-1-AP.htm.

anti-BiP antibody (Agrisera, AS09 481):Rabbit polyclonal antibody raised against a KLH-conjugated synthetic peptide derived from Arabidopsis thaliana BiP1/BiP2/BiP3. Manufacturer-tested for WB; recommended WB dilution 1:2,000; expected/apparent MW 73.5/80 kDa. Confirmed reactivity includes Arabidopsis thaliana, Oryza sativa, Solanum lycopersicum, Triticum aestivum and Zea mays. Manufacturer-listed WB reference: Sikorskaite et al., Plant Methods, 2013, Fig. 2A, PMID: 23886449. Product profile/validation information: https://www.agrisera.com/en/artiklar/bip2-luminal-binding-protein-2.html.

anti-COXII antibody (Agrisera, AS04 053A):Rabbit polyclonal antibody raised against a KLH-conjugated synthetic peptide conserved in eudicots, monocots and Physcomitrella patens. Manufacturer-tested for BN-PAGE and WB; recommended WB dilution 1:1,000; expected/apparent MW approximately 29.4/30 kDa in Arabidopsis thaliana. Confirmed reactivity includes Arabidopsis thaliana, Hordeum vulgare, Nicotiana tabacum, Physcomitrium patens, Triticum aestivum and Zea mays; predicted reactivity includes Oryza sativa. Manufacturer-listed WB references include Yang and Wang, BMC Plant Biology, 2017, Fig. 2A, PMID: 28056797. Product profile/validation information: https://www.agrisera.com/en/artiklar/coxii-cytochrome-oxidase-subunit-ii-marker-of-mitochondrial-inner-membrane.html.

anti-IDH antibody (Agrisera, AS06 203A): Rabbit polyclonal antibody raised against KLH-conjugated peptides conserved in higher-plant mitochondrial NAD-dependent isocitrate dehydrogenase subunits. Manufacturer-tested for IF and WB; recommended WB dilution 1:5,000; expected/apparent MW approximately 39/45 kDa in Arabidopsis thaliana. Confirmed reactivity includes Arabidopsis thaliana, Oryza sativa, Solanum lycopersicum, Pisum sativum, Solanum tuberosum and Zea mays. Manufacturer-listed WB reference: Yin et al., PLoS One, 2016, Fig. 6A, PMID: 27124767. Product profile/validation information: https://www.agrisera.com/en/artiklar/idh-isocitrate-dehydrogenase.html.

anti-V-ATPase antibody (Agrisera, AS07 213): Rabbit polyclonal antibody raised against a KLH-conjugated synthetic peptide from the plant V-ATPase subunit E. Manufacturer-tested for IF, IHC and WB; recommended WB dilution 1:2,000–1:5,000; expected/apparent MW approximately 26/31 kDa in Arabidopsis thaliana. Confirmed reactivity includes Arabidopsis thaliana, Hordeum vulgare, Nicotiana tabacum, Oryza sativa, Triticum aestivum and Zea mays. Manufacturer-provided application examples include WB and IF, including Oryza sativa WB in Chen et al., J. Exp. Bot., 2013, Fig. 3B,C and 4C,D, PMID: 23963678. Product profile/validation

information: https://www.agrisera.com/en/artiklar/v-atpase-epsilon-subunit-of-tonoplast-hatpase.html.

anti-Tic40 antibody (Agrisera, AS10 709): Rabbit polyclonal antibody raised against a KLH-conjugated synthetic peptide derived from plant Tic40 sequences including Arabidopsis thaliana Tic40. Manufacturer-tested for IF and WB; recommended WB dilution 1:2,500; expected/apparent MW 48/45 kDa in Arabidopsis thaliana. Confirmed reactivity includes Arabidopsis thaliana, Nicotiana benthamiana, Nicotiana tabacum, Oryza sativa, Physcomitrium patens, Solanum lycopersicum, Triticum aestivum, Zea mays and Vitis vinifera. Manufacturer-listed WB references include Wang et al., Nature Communications, 2020, Fig. 1F, PMID: 32198392, and Loudya et al., Genome Biology, 2021, Fig. 5F, PMID: 33975629. Product profile/validation information: https://www.agrisera.com/en/artiklar/tic40-chloroplast-inner-envelope-membrane-translocon-complex-protein.html.

anti-Sec21p antibody (Agrisera, AS08 327): Rabbit polyclonal antibody raised against a GST-fusion fragment of recombinant Arabidopsis thaliana Sec21. Manufacturer-tested for IF and WB; recommended dilution 1:1,000 for both IF and WB; expected/apparent MW 98 kDa. Confirmed reactivity: Arabidopsis thaliana, Phaeodactylum tricornutum and Zea mays; predicted reactivity includes Oryza sativa. Manufacturer-provided WB and IF application examples are available, and selected references include Vogel et al., Nature Communications, 2022, Fig. 1D, PMID: 36371501. Product profile/validation information: https://www.agrisera.com/en/artiklar/sec21p-gamma-subunit-cop-vesicles.html.

anti-H^+-ATPase antibody (Agrisera, AS07 260): Rabbit polyclonal antibody raised against a KLH-conjugated synthetic peptide derived from plant plasma membrane H^+-ATPase sequences. Manufacturer-tested for IF, immunolocalization and WB; recommended WB dilution 1:1,000–1:10,000; expected/apparent MW approximately 90–95 kDa in Arabidopsis thaliana. Confirmed reactivity includes Oryza sativa, Arabidopsis thaliana, Hordeum vulgare, Nicotiana benthamiana, Nicotiana tabacum, Zea mays and other plant species. Manufacturer-provided WB application examples and published-use information include Yang et al., Nature Communications, 2016, Fig. 6A, PMID: 27109828. Product profile/validation information: https://www.agrisera.com/en/artiklar/hatpase-plasma-membrane-hatpase.html.

anti-Actin antibody (Sangon Biotech, D191048): Mouse monoclonal anti-plant-actin antibody raised against recombinant/fusion protein of Nicotiana tabacum ACTIN. Manufacturer-recommended for WB; dilution 1:1,000–1:5,000; predicted band size 42 kDa; reactivity: plants. WB validation was performed by the supplier using soybean, Arabidopsis and Nicotiana tabacum lysates. Supplier-listed related reference: Yuan et al., Nucleic Acids Research, 2021, PMID: 33270882. Product profile/validation information: https://store.sangon.com/productDetail?productInfo.code=D191048&productInfo.signature=4enAt7.

anti-FLAG antibody (Proteintech, 66008-4-Ig): Mouse monoclonal antibody, clone 8H6A10, against the DYKDDDDK/FLAG tag. Manufacturer-tested for WB, IF/ICC, FC (Intra), IP and ELISA; recommended WB dilution 1:5,000–1:50,000. Tested reactivity: recombinant protein. RRID: AB_2918475. Manufacturer-listed citations include applications in WB, IP, CoIP, ChIP and other assays. Product profile/validation information: https://www.ptgcn.com/products/Flag-tag-Antibody-66008-4-Ig.htm.

Custom antibody: The anti-OsALA5 monoclonal antibody was custom-generated by Absea Biotechnology Co., Ltd. against OsALA5 amino acids 116–308 and was validated in this study by immunoblotting. An OsALA5-specific band was detected at the expected molecular weight in OsALA5-expressing samples but was absent in OsALA5 knockout samples. The validation result is shown in Extended Data Fig. 3a, with corresponding raw western blot images provided in Supplementary Figure 1.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	HEK293F cells were purchased from Sino Biological Inc. and cultured in SMM 293T-II medium (M293TII, Sino Biological Inc.). Yeast strain ZHY709 (MATa his3 leu2 ura3 met15 dnf1Δ dnf2Δ drs2::LEU2), which lacks endogenous plasma-membrane P4-ATPase activity, was used for co-immunoprecipitation, lipid uptake and FLIM-FRET assays. BY4741 (MATa his3 leu2 ura3 met15; EUROSCARF) served as the wild-type control. Single-knockout yeast strains dnf1Δ (YDR093W, 6162) and dnf2Δ (YDR093W, 4028) were obtained from the Yeast Knock-Out (YKO) Collection of the Saccharomyces Genome Deletion Project.
Authentication	No further authentication of HEK293F cells was done after purchasing. The yeast cells were verified with the primers listed in Supplementary Table 1 after purchasing and the double mutant (dnf1Δ dnf2Δ) was generated using a PCR-based strategy.
Mycoplasma contamination	HEK293F cells were obtained from a commercial supplier and were not further tested for mycoplasma contamination in our laboratory.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used in this study.

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 insertion mutants of ALA9, ALA10, ALA11 and ALA12 were obtained from the Nottingham Arabidopsis Stock Centre NASC .
Novel plant genotypes	The hot1 mutant was isolated from an EMS-mutagenized library in the elite indica rice R527 background. OsALA5 knockout (OsALA5-KO), complementation (OsALA5-C and CL), overexpression (OsALA5-OE) lines, and NIL-OsALA5Hap7 were generated as described in the Methods. Homozygous Arabidopsis T-DNA insertion lines were verified by PCR genotyping, and double mutants were produced by crossing. Additional CRISPR/Cas9 knockout alleles (ala10-1, ala11-1, and ala10-1/11-1) were validated by sequencing.
Authentication	The genotypes of mutants were verified by PCR with the primers listed in Supplementary Table 1.

Flow Cytometry

Plots

Confirm that:

☒ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

☒ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).

☒ All plots are contour plots with outliers or pseudocolor plots.

☒ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation	Yeast cells (BY4741, dnf1Δ, dnf2Δ and dnf1Δ dnf2Δ) were grown overnight in YPD at 30°C (200 rpm) to OD600 ≈ 0.3, diluted to OD600 = 0.3 in fresh YPD, and subjected to heat stress at 55°C for 1h (controls maintained at 28°C). After treatment, cells were resuspended in PBS (pH 7.4) containing propidium iodide (PI; 5μgmL^{-1}) and incubated for 10min in the dark prior to flow-cytometric analysis. Four independent biological replicates were performed.
Instrument	PI fluorescence was acquired using a BD Accuri C6 Plus flow cytometer (BD Biosciences) and recorded in the FL2-A channel.
Software	BD Accuri C6 Plus software (BD Biosciences) was used for data acquisition and export. Data processing, statistics and plotting were performed using GraphPad Prism 8.0.2.
Cell population abundance	Membrane-integrity loss was quantified as the mean PI fluorescence intensity (FL2-A) of the acquired yeast events for each sample. Results were obtained from four independent biological replicates.
Gating strategy	No post-acquisition gating was performed. All acquired yeast events were analyzed under identical acquisition settings.

Gating strategy

Representative raw FSC-A/SSC-A plots and PI fluorescence (FL2-A) histograms illustrating the no-gating flow-cytometry analysis are provided in Supplementary Figure 2.

☐ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.