nature portfolio

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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 <u>Editorial Policies</u> and the <u>Editorial Policy Checklist</u>.

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For	all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or inlethods section.
n/a	Confirmed
	$oxed{\boxtimes}$ 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.
\boxtimes	A description of all covariates tested
\boxtimes	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>
\boxtimes	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
\boxtimes	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
\boxtimes	\square Estimates of effect sizes (e.g. Cohen's d , Pearson's r), indicating how they were calculated
	Our web collection on <u>statistics for biologists</u> contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection

RT-qPCR data were acquired with Bio-Rax CFX v3.1

Chlorophyll fluorescence imaging data were acquired and analyzed with Walz ImagingWin v2.47 Protein abundance data were acquired and analyzed with ProteinSimple Compass v4.1.0 Bioluminescence imaging data were acquired and analyzed with Photek Image32

Data analysis

RNAseq analysis used Fastqc 0.11.3, Trimmomatic 0.33, Kallisto 0.44.0, Degust (DOI: 10.5281/zenodo.3258932; degust.erc.monash.edu). Circadian time-series analysis used the meta2d command within the MetaCycle v1.2.0 package, running in R v4.1.1. Data analysis and graphs prepared using R v4.1.1 (using ggplot2 v3.4.0, ggpubr v0.4.0 and cowplot v1.1.1); RNA sequencing data analyzed using R v3.6.1.

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 <u>availability of data</u>

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 RNASeq data for this study are available in the European Nucleotide Archive (ENA; https://www.ebi.ac.uk/ena) with the project ID PRJEB45855.

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	the document with all sections, see <u>natu</u>	ure.com/documents/nr-reporting-summary-flat.pdf					
Life scier	nces study des	ign					
All studies must dis	sclose on these points even whe	en the disclosure is negative.					
Sample size	No sample-size calculation was performed. Sample sizes were based on sample sizes used previously to investigate this signalling pathway (Noordally et al. Science 2013 (https://doi.org/10.1126/science.1230397), Belbin et al. New Phytologist 2016 (https://doi.org/10.1111/nph.14176).						
Data exclusions	No data were excluded from analyses.						
Replication	At least three completely independent experiments were conducted for each dataset, with the exception of protein abundance experiments where two completely independent experiments were conducted. All replication attempts across all experiments were successful (no data are included that did not replicate successfully).						
Randomization	Plant material was positionally-randomized within growth chambers.						
Blinding	Blinding was not used for temperature treatments (it is not possible to blind for time of day or temperature treatment, because this is obto the experimentalist). Experimentalists were blind to genotype during RNA isolation, RT-qPCR, protein isolation and immunodetection, during physiological assays (chlorophyll fluorescence analysis, freezing tolerance analysis).						
We require informati	ion from authors about some types ted is relevant to your study. If you	materials, systems and methods of materials, experimental systems and methods used in many studies. Here, indicate whether each material, are not sure if a list item applies to your research, read the appropriate section before selecting a response. Methods					
Materials & experimental systems n/a Involved in the study		n/a Involved in the study					
Antibodies	,	∑ ChIP-seq					
Eukaryotic	cell lines	Flow cytometry					
Palaeonto	logy and archaeology	MRI-based neuroimaging					
Animals ar	nd other organisms						
Human res	search participants						
Clinical da	ta						
Dual use r	esearch of concern						

Antibodies

Antibodies used

Antibodies used were for PSII D2 (Agrisera AS06146); PSI-C (Agrisera AS10939); RbcL (Agrisera AS03037).

Validation

All three antibodies are validated by Agrisera with this information provided on the supplier's website. Dilution series of antibodies were conducted before experimentation to ensure optimal specificity. PSII D2 (Agrisera ASO6146), PSI-C (PSAC) (AS10939) and RbcL (AS03037) validated by manufacturer for use with Arabidopsis thaliana.