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 Methods section.
n/a	Confirmed
	\square 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	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

Bio-Rad ChemiDocTM XRS+(western blotting); Bio-Rad CFX Manger v3.1(qPCR data); Zeiss LSM710 (subcellular location).

Data analysis

Microsoft Excel 2010, GraphPad Prism 6 and Spss v17.0 (one-way ANOVA, Duncan's multiple range testwere used for statistical analysis. Quantitative analysis of immunoblots and fluorescence intensity is performed using Quantity Tools of Image Lab software (Bio-Rad). MEGA7 is used for constructing phylogenetic tree of OsGLS1s.

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

Data supporting the findings of this work are available within the paper and its supplementary information files. A reporting summary for this article is available as a supplementary information file. The datasets and genetic materials generated and analyzed during the current study are available from the corresponding author

upon request. Sequence of genes used in this study can be found in the MSU database (http://rice.plantbiology.msu.edu/) under the following accession numbers: OsGLS1 (LOC_Os04g01160), OsPIN2 (LOC_Os06g44970) OsAUX1 (LOC_Os05g37470) OsPIN1a (LOC_Os11g04190) and OsPIN1b (LOC_Os02g50960). Sequence for constructing the phylogenetic tree of GLS1s can be found in The Phytozome v13 database (https://phytozome-next.jgi.doe.gov/). The source data are provided with this paper.

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	ut studies with <u>human participants or human data</u> . See also policy information about <u>sex, gender (identity/presentation)</u> , and <u>race</u> , ethnicity and racism.		
Reporting on sex and	gender No human participants involved.		
Reporting on race, et other socially relevan groupings			
Population character	Stics No human participants involved.		
Recruitment	No human participants involved.		
Ethics oversight	No human participants involved.		
Note that full information	on the approval of the study protocol must also be provided in the manuscript.		
ield-speci	fic reporting		
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Life sciences	Behavioural & social sciences		
	ocument with all sections, see <u>nature.com/documents/nr-reporting-summary-flat.pdf</u>		
Life science	es study design		
II studies must disclos	e on these points even when the disclosure is negative.		
Sample size San	nples sizes for each experiment is indicated in legends. Each experiment contains at least three biological replicates.		
Data exclusions No	data is excluded from the analyses.		
use	All experiments in this study are repeated independently at least three times. For RT-qPCR at least three biologically independent samples are used each time. For subcellular location, field and physiological and biochemical experiments, the results representative of three independent experiments. The number of biological replicates in each experiment is indicated in the legends.		
Randomization For	field experiments, the mutant and wild type plants were grown in a completely-randomized block design with three replicates.		
Blinding The	The investigators were blinded to group allocation during data collection.		
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Reporting	for specific materials, systems and methods		
,	om authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each materi		
ystem 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 & experi	mental systems Methods		
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Antibodies	ChIP-seq		
Eukaryotic cell I	ines		
Palaeontology a	Palaeontology and archaeology MRI-based neuroimaging		
Animals and oth	ner organisms		
Clinical data			
Dual use resear	ch of concern		
☐ ☐ Plants			

Antibodies

Antibodies used

OsPIN1a, OsPIN1b and OsPIN2 were customized by ABclonal Technology Co.,Ltd.; anti-GFP, Sigma-Aldrich, G1544; anti-FLAG, Sigma-Aldrich, F1804; Anti-His, Abclonal, AE003; anti-actin, Sigma-Aldrich, A3853; anti-ubiquitin, Santa Cruz Biotechnology, sc-8017; anti-GST, TransGen Biotechnology, HT601; anti-MBP, Sigma-Aldrich, A4213; rabbit anti-mouse IgG secondary antibody conjugated to peroxidase, Sigma-Aldrich, A9044; Alexa Fluor 555, Invitrogen, A-21428;

Validation

The antibodies of OsPIN1a,OsPIN1b and OsPIN2 have been reported in previous paper; Other antibodies can be found at their respective company websites.

Dual use research of concern

Policy information about <u>dual use research of concern</u>

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
\boxtimes	Public health
\boxtimes	National security
X	Crops and/or livestock
X	Ecosystems
\boxtimes	Any other significant area

Experiments of concern

Does the work involve any of these experiments of concern:

Vo	Yes
X	Demonstrate how to render a vaccine ineffective
X	Confer resistance to therapeutically useful antibiotics or antiviral agents
X	Enhance the virulence of a pathogen or render a nonpathogen virulent
\boxtimes	Increase transmissibility of a pathogen
\boxtimes	Alter the host range of a pathogen
X	Enable evasion of diagnostic/detection modalities
\boxtimes	Enable the weaponization of a biological agent or toxin
\times	Any other potentially harmful combination of experiments and agents

Plants

Seed stocks

The seeds were stored in the State Key Laboratory of Plant Environmental Resilience, College of Life Sciences, Zhejiang University.

Novel plant genotypes

The gls1 and pin2 single mutants were generated by ethyl methanesulfonate and CRISPR/Cas9-mediated gene editing; gls1 pin2 double mutants were generated by hybridization; Agrobacterium-based transformation was used to generate the transgenic lines and two independent lines were analyzed.

Authentication

PCR and Sanger sequencing were used to verify the genome editing generated at the designated genomic targets. RT-qPCR and western blotting were used to verify the expression of the transgenes.