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 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	The CFX Connect Real-Time PCR Detection System (Bio-Rad, California, USA) was used for qRT-PCR; the ImageSaver6 Version 2.0.3 software was used for western blotting; LAS AF Lite 2.6.0 and ZEN 3. 2 (blue edition) softwares were used for subcellular localization and BiFC; Illumina pipeline for the HiSeq 2500 was used for RNA-sequencing; the TECAN Infinite M200 microplate reader was used for transient dual-luciferase reporter assay.
Data analysis	Tassel 5.0 software and PLINK 2.0 software were used in the linkage disequilibrium analysis; GEMMA 0.941 software was used in the association analysis; RNA-Seq data were processed by Kallisto for gene expression abundance analysis and DESeq2 for differentially expressed genes analysis; ClustalX 1.83 software was used in the sequence alignment; MEGA 11 software was used in the phylogenetic tree construction; Prism v.6.0 (GraphPad) softwarewas used in the two-sided paired Student's t-test and one-way ANOVA with Tukey's multiple comparisons test; GraphPad Prism 6, and Adobe Illustrator were used for the graphical drawing.

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 raw sequencing data of the GWAS generated in this study have been deposited in the NCBI SRA database under the BioProject accession numbers PRJNA1278882 and PRJNA1277491. The RNA-seq raw data generated in this study have been deposited in the NCBI SRA database under the BioProject accession number PRJNA1276623. The genomic sequences of Chalk9 and OsEBP89 in cultivated rice varieties were obtained from the Rice Functional Genomics and Breeding Database (RFGB) (http://www.rmbreeding.cn/). The genomic sequences of Chalk9 in wild rice accessions were downloaded from OryzaGenome (http://viewer.shigen.info/oryzagenome2detail/index.xhtml) or Gramene (https://www.gramene.org/). All constructs and transgenic plants generated in this study are available from the corresponding author upon reasonable request. Source data are provided with this article.

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	No Reporting on sex and gender was referenced in this manuscript
Reporting on race, ethnicity, or other socially relevant groupings	No Reporting on race, ethnicity,or other socially relevant groupings
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	We typically used biological replicates for gene expression analysis and LUC/REN assays. Phenotypic analyses and physiological measurements were performed on 9 or more biological replicates. This is a commonly used sample size in this research field.
Data exclusions	No data were excluded.
Replication	All experiments were performed with at least three independent replicates and were reproducible.
Randomization	All samples were randomly allocated into experimental groups and there is no intentional choice.
Blinding	No blinding was used as it was not applicable for the nature of the experimental setup.

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-HA (1:3000, ab9110, Abcam);
Anti-GFP (1:3000, ab290, Abcam) ;
Anti-GST (1:3000, CW0084M, CWBIO) ;
Anti-MBP (1:5000, HT701, Transgene);
Anti-ubiquitin (1:1,000, RM4934; Biodragon);
Anti-Actin (1:5000, CW0264M; CWBIO);
Anti-OsEBP89 (1:1000, produced in our laboratory).

Validation

Anti-HA antibody validation could be found in the website: https://www.abcam.cn/products/primary-antibodies/ha-tag-antibody-chip-grade-ab9110.html.
Anti-GFP antibody validation could be found in the website: https://www.abcam.cn/products/primary-antibodies/gfp-antibody-ab290.html.
Anti-GST antibody validation could be found in the website: https://www.cwbio.com/product/detail/id/10104.
Anti-MBP antibody validation could be found in the website: https://www.transgen.com/antibody_tag/389.html.
Anti-ubiquitin antibody validation could be found in the website: https://www.biodragon.cn/cn/goods/goodsView?GoodsId=71863&Catalog=.
Anti-Actin antibody validation could be found in the website: https://www.cwbio.com/product/detail/id/10174.

Plants

Seed stocks

The plant materials, including near-isogenic line, Yangdao6, Guichao2, Nipponbare, and Zhonghua11 transgenic plants as well as Indica varieties, were generated in our laboratory and can obtained by contacting the corresponding author.

Novel plant genotypes

Chalk9 and OsEBP89-Overexpressing and -RNAi transgenic plants were generated using Agrobacterium-mediated transformation methods and at least two positive lines were selected for experiments. Chalk9 and OsEBP89-Gene-edited lines were obtained by Agrobacterium transformation using the CRISPR/Cas9 system (pYLCRISPR/Cas9-MH vector) to target specific genes. Homozygous transgenic plants of the T1 generation or later were used for phenotypic evaluation.

Authentication

Specific primers were designed to identify the positive transgenic materials by PCR, and the relative expression levels were determined by qRT-PCR. Hygromycin was also used to screen positive seeds and seedlings when the materials were planted. For gene-edited lines, specific primers were designed to identify the gene-edited plants by sequencing. All transgenic plants were planted, managed, and harvested in a uniform manner to avoid secondary effects.