nature portfolio

Corresponding author(s): Lijun Wang

Last updated by author(s): May 7, 2025

# 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<br>*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<br>*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 Bio-Rad C1000 Touch Thermal Cycler was used for qRT-PCR; the Tanon-5200 Chemiluminescent Imaging System was used for western blotting and EMSA; the Leica TCS SP5 Confocal Scanning Microscope was used for subcellular localization; the Illumina NovaSeq 6000 system (Illumina, USA) was used for DAP-seq; and the Promega Luminometer (GloMax™ 20/20) was used for the transient dual-luciferase reporter assay. |
| Data analysis | The TASSEL software was used for GWAS analysis, while PLINK was employed to identify independent markers for GWAS. The MEGA X software was utilized for phylogenetic tree construction. TASSEL was also used to calculate Tajima's D and π values. Arlequin was applied to compute the genetic distance among haplotypes, which was subsequently converted into a minimum spanning tree (MST) using Hapstar. SPSS 26.0 software was used to perform the two-sided paired Student's t-test and one-way ANOVA with Duncan's test. GraphPad Prism 8.0.1 was 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

Data supporting the findings of this work are available within this paper and its Supplementary Information files. The materials used in this study are available from the corresponding authors upon request. GWAS data of 121 accessions, resequencing data of 'Guipu 1', 'Cabernet Sauvignon', and 118 hybrid progenies derived from their cross, as well as the DAP-seq data for TTC4 generated in this study, have been deposited in National Genomics Data Center (https://ngdc.cncb.ac.cn/) under accession CRA025447. Gene sequence and annotation information is available from the Phytozome v13 (https://phytozome-next.jgi.doe.gov/). Source data are provided in 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 | The sample size used in our study is determined based on the appropriate sample size in relevant papers within the field (Mu et al., 2023, Plant Cell, https://doi.org/10.1093/plcell/koac308, Li et al., 2023, Plant Cell, https://doi.org:10.1093/plcell/koad210, Zhou et al., 2019, Genome Biology, https://doi.org:10.1186/s13059-019-1771-7). |
| Data exclusions | No data were excluded. |
| Replication | All experiments were performed with at least three independent replicates. |
| Randomization | Measurements and sample collections were carried out on plants randomly selected under uniform growth conditions. All samples were randomly assigned to experimental groups. |
| Blinding | Data collection and analysis were performed in a blinded manner. |

# 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: The information of the antibodies used in this study was shown below:
Mouse monoclonal anti HIS (CWBIO Company, cat#tcw0286m, 1:2000);
Mouse monoclonal anti MBP (ThermoFisher Company, cat#MA1-10837, 1:2000)

Validation: https://cwbio.com/product/detail/id/10177
https://www.thermofisher.cn/antibody/primary/target/mbp

# 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 | The plant materials used in this study, including TW plants, JX plants and plantlets, Y2 plants, 41B grape suspension cells, as well as plants with transient overexpression or RNAi (targeting TTC4, HSP18.1, APX3, and SPL13) and grape suspension cells with stable overexpression of TTC4, were generated in this study. |
| Novel plant genotypes | Transient overexpression plants, as well as stable grape suspension cells, were obtained using the Agrobacterium-mediated transformation method. |
| Authentication | Specific primers were designed to identify transient overexpression and RNAi plants, as well as stable overexpression suspension cells, and to measure the expression levels of the relevant genes using RT-qPCR. |