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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	For live imaging data collection, we used the a Zeiss inverted laser confocal microscope (Zeiss LSM 980) with ZEN Microscopy Software; For RNA-sequencing, the RNA Library was sequenced on the DNBSEQ-T7 platform to generate 150 bp paired-end reads; For ATAC-seq data collection, the library was sequenced on the Illumina Novaseq 6000 platform to generate 150 bp paired-end reads; For CUT&Tag-seq data collection, the library was sequenced on the Illumina Nova X Plus platform to generate 150 bp paired-end reads.
Data analysis	For RNA-sequencing analysis, low-quality reads and adapter sequences were trimmed to generate clean reads using fastp (version 0.24.0). The sequences were then mapped and annotated using the Capsella rubella v1.1 genome sequence with STAR (version 2.7.11b). Gene expression levels were calculated as fragments per kilobase of transcript per million fragments (FPKM) using an R script. Differentially Expressed Genes (DEGs) were identified as those with a fold change ≥ 2 and a False Detection Rate (FDR) < 0.05 using the R package DESeq2 (version 1.42.1). GO enrichment analysis of Biological Process (BP) on the DEGs was performed using the R packages enrichplot (version 1.22.0) and clusterProfiler (version 4.10.1). Boxplots were drawn using the R package ggplot2 (version 3.5.2). For the RNA-seq experiment, at least three independent biological replicates were conducted for each genotype. For ATAC-seq data analysis, the raw reads were filtered (reads shorter than 35 bp and bases with a quality value less than Q10) using fastp (version 0.24.0) to generate clean FASTQ files. The sequences were then mapped back to the Capsella rubella v1.1 genome using Bowtie2 (version 2.5.4). Duplicate reads were removed by sambamba (version 1.0.1) and bedtools (version 2.31.1). Peaks were called using MACS2 (version 2.1.4) with a screening criterion of FDR < 0.05. The CrWT stage 12–14 fruit ATAC data were published previously. Differentially enriched peaks between CrWT and Crjag were identified using the R package DiffBind (version 3.12.0). Significant closed chromatin accessibility in Crjag was defined as fold change < 0.8 and FDR < 0.05. DeepTools (version 3.5.6) was used to map the density distribution of sequencing reads in the upstream and downstream regions of the Transcription Start Site (TSS) and the Transcription End Sits (TES) of each gene. The profile plot was generated using the plotProfile function. For visualization, datasets were converted to bigwig format using

bamCoverage in DeepTools (version 3.5.6) with a bin size of 1 bp and normalized by the RPKM method, then visualized using Integrative Genomics Viewer (IGV, version 2.4.14). Gene annotation was performed by ChIPseeker (version 1.38.0) using a 2 kb promoter-proximal window. The ATAC-seq experiments were performed with two independent biological replicates.

For CUT&Tag-seq data analysis, the raw reads were filtered using fastp (version 0.24.0) to generate clean FASTQ files. After filtering low-quality reads, the sequences were mapped back to the Capsella rubella v1.1 genome using Bowtie2 (version 2.5.4). Duplicate reads were removed by sambamba (version 1.0.1) and bedtools (version 2.31.1). Peaks were called using MACS2 (version 2.1.4) with a p value of 0.01. The bigwig files were generated by DeepTools (version 3.5.6) for visualization using IGV (version 2.16.2). Gene annotation was performed with a 2 kb promoter-proximal window using ChIPseeker (version 1.38.0). For each antibody and genotype, two biological replicates were conducted.

For data integration, Venn diagrams were generated with the R package VennDiagram (version 1.7.3).

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

All genotypes and reporter lines described here are available upon request.

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	*Use the terms sex (biological attribute) and gender (shaped by social and cultural circumstances) carefully in order to avoid confusing both terms. Indicate if findings apply to only one sex or gender; describe whether sex and gender were considered in study design; whether sex and/or gender was determined based on self-reporting or assigned and methods used.* *Provide in the source data disaggregated sex and gender data, where this information has been collected, and if consent has been obtained for sharing of individual-level data; provide overall numbers in this Reporting Summary. Please state if this information has not been collected.* *Report sex- and gender-based analyses where performed, justify reasons for lack of sex- and gender-based analysis.*
Reporting on race, ethnicity, or other socially relevant groupings	*Please specify the socially constructed or socially relevant categorization variable(s) used in your manuscript and explain why they were used. Please note that such variables should not be used as proxies for other socially constructed/relevant variables (for example, race or ethnicity should not be used as a proxy for socioeconomic status).* *Provide clear definitions of the relevant terms used, how they were provided (by the participants/respondents, the researchers, or third parties), and the method(s) used to classify people into the different categories (e.g. self-report, census or administrative data, social media data, etc.)* *Please provide details about how you controlled for confounding variables in your analyses.*
Population characteristics	*Describe the covariate-relevant population characteristics of the human research participants (e.g. age, genotypic information, past and current diagnosis and treatment categories). If you filled out the behavioural & social sciences study design questions and have nothing to add here, write "See above."*
Recruitment	*Describe how participants were recruited. Outline any potential self-selection bias or other biases that may be present and how these are likely to impact results.*
Ethics oversight	*Identify the organization(s) that approved the study protocol.*

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	Sample-size calculation was not performed. Sample size was considered sufficient according to statistical significance of the test obtained.

Data exclusions	No data were excluded from the analyses.
Replication	A number between 2 and 4 biological replicates were performed and all showed reproducible results.
Randomization	The plants used in his study were grown in controlled environment rooms in random positions. Analysed samples for e.g. ChIP-PCR or qPCR were made of of tissue from different plants grown in random position.
Blinding	Blinding was not performed due to geographical distance between participants. Instead we carried out biological reps in both labs.

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	Guinea pig anti-rabbit IgG (Heavy & Light Chain) antibody, Easybio, ABIN101961, Polyclonal, NE-200-032405; Anti-GFP antibody, abcam, ab290, Polyclonal, 1037873-10; Normal rabbit IgG antibody, Merck, 12-370, Polyclonal, 4072474; Anti-HA-tag antibody, MBL, M180-3MS, TANA2; Anti-DDDDK-tag antibody, MBL, M185-3MS, FLA-1; Anti-FLAG® M2 antibody, Sigma-Aldrich, F3165, M2, SLCC4005; Anti-trimethyl-Histone H3 (Lys27) antibody, Millipore, 07-449, Polyclonal, 3449045; Anti-Histone H3 (mono methyl K4) antibody, abcam, ab8895, Polyclonal, GR3312607-2; Anti-Histone H3 (tri methyl K4) antibody, abcam, ab8580, Polyclonal, GR3425199-1; Acetyl-Histone H3 (Lys9) (C5B11) rabbit monoclonal antibody, CST, 9649T, ,13; Anti-acetyl-Histone H3 (Lys14) antibody, Millipore, 07-353, Polyclonal, 4101335; Anti-acetyl-Histone H3 (Lys18) antibody, Millipore, 07-354, Polyclonal, 4086374; Anti-Histone H3 (acetyl K27) antibody, abcam, ab4729, Polyclonal, GR3442884-1; Anti-acetyl-Histone H3 (Lys23) Antibody, Millipore, 07-355, Polyclonal, 4130116;
Validation	All these antibodies are commercially available under the catalog number

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 Capsella rubella plants used in this study are in ecotype Cr22.5
Novel plant genotypes	CrJAG:GUS (CrWT background), CrMSI2:GUS (CrWT background), CrMSI3:GUS (CrWT background), CrMSI4:GUS (CrWT background), CrAUR2:GUS (CrWT/Crjag background); pCrJAG:CrJAG:GFP (Crjag background); pCrJAG:CrJAG(DBD):GFP (Crjag background), pCrJAG:CrJAG(DBD)-CrMSI2:GFP (Crjag background), pCrJAG:CrJAG(DBD)-CrMSI3:GFP (Crjag background), Crjag-ge , Atjag-ge, Crmsi2-ge, Crmsi3-ge and Crmsi2/3-ge double mutant.
Authentication	All Capsella lines were verified by genotyping.