nature research

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Reporting Summary

Nature Research 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 Research policies, see our Editorial Policies and the Editorial Policy Checklist.

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section

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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. <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>
x	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
x	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
x	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 <u>availability of computer code</u>

Data collection

Leica TCS SP8 confocal laser scanning microscope (Leica, Germany) was used for confocal imaging and data collection. Bio-Rad CFX Manager software (version 3.1) was used to collect qPCR raw data.

Data analysis

AS X software (version 3.4.2.18368) was used to analyze confocal microscope Images and FLIM/FRET (https://www.leica-microsystems.com/products/microscope-software/p/leica-las-x-ls/)

The Microsoft Excel 2016 and Graphpad Prism 8 (Graphpad Prism software, version 8.0.1)) were used for statistics and bar graphs overlaid with dot plots.

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 Research guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a <u>data availability statement</u>. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

Original data that support the findings of this study are available from the corresponding author upon request.

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 you	r selection.
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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 chose at least two individual transgenic lines and more than three individual plants of each lines to observe the phenotype, gene transcription and the protein expression. For protoplast protein expression, about 2×10^{4} cells were used for Co-IP and 2×10^{4} cells for protein detection. Fifteen protoplasts were used to determine the average value of τ for each pair of proteins analyzed. There were more than 3 replications showing similar results for RT-PCR and qRT-PCR experiments. For quantification of Western band intensity, 3 replications were used in the statistical analysis showing similar pattern. Statistics were indicated in the figure legends.

Data exclusions

No data were excluded from analyses in the experiments.

Replication

All experiments in main figures were repeated at least three times and reliably produced.

Randomization

For all Arabidopsis transgenic plants, protoplast cells, N. benthamiana and bacterial strains, all samples were randomly selected throughout this study.

Blinding

The purpose of research is to observe a phenotype of gene knockout or over-expression, thus, the blinding design is not applicable to this study.

Behavioural & social sciences study design

All studies must disclose on these points even when the disclosure is negative.

Study description

Briefly describe the study type including whether data are quantitative, qualitative, or mixed-methods (e.g. qualitative cross-sectional, quantitative experimental, mixed-methods case study).

Research sample

State the research sample (e.g. Harvard university undergraduates, villagers in rural India) and provide relevant demographic information (e.g. age, sex) and indicate whether the sample is representative. Provide a rationale for the study sample chosen. For studies involving existing datasets, please describe the dataset and source.

Sampling strategy

Describe the sampling procedure (e.g. random, snowball, stratified, convenience). Describe the statistical methods that were used to predetermine sample size OR if no sample-size calculation was performed, describe how sample sizes were chosen and provide a rationale for why these sample sizes are sufficient. For qualitative data, please indicate whether data saturation was considered, and what criteria were used to decide that no further sampling was needed.

Data collection

Provide details about the data collection procedure, including the instruments or devices used to record the data (e.g. pen and paper, computer, eye tracker, video or audio equipment) whether anyone was present besides the participant(s) and the researcher, and whether the researcher was blind to experimental condition and/or the study hypothesis during data collection.

Timing

Indicate the start and stop dates of data collection. If there is a gap between collection periods, state the dates for each sample cohort.

Data exclusions

If no data were excluded from the analyses, state so OR if data were excluded, provide the exact number of exclusions and the rationale behind them, indicating whether exclusion criteria were pre-established.

Non-participation

State how many participants dropped out/declined participation and the reason(s) given OR provide response rate OR state that no participants dropped out/declined participation.

Randomization

If participants were not allocated into experimental groups, state so OR describe how participants were allocated to groups, and if allocation was not random, describe how covariates were controlled.

Ecological, evolutionary & environmental sciences study design

All studies must disclose on these points even when the disclosure is negative.

Study description

Briefly describe the study. For quantitative data include treatment factors and interactions, design structure (e.g. factorial, nested, hierarchical), nature and number of experimental units and replicates.

Research sample	Describe the research sample (e.g. a group of tagged Passer domesticus, all Stenocereus thurberi within Organ Pipe Cactus National Monument), and provide a rationale for the sample choice. When relevant, describe the organism taxa, source, sex, age range and any manipulations. State what population the sample is meant to represent when applicable. For studies involving existing datasets, describe the data and its source.
Sampling strategy	Note the sampling procedure. Describe the statistical methods that were used to predetermine sample size OR if no sample-size calculation was performed, describe how sample sizes were chosen and provide a rationale for why these sample sizes are sufficient.
Data collection	Describe the data collection procedure, including who recorded the data and how.
Timing and spatial scale	Indicate the start and stop dates of data collection, noting the frequency and periodicity of sampling and providing a rationale for these choices. If there is a gap between collection periods, state the dates for each sample cohort. Specify the spatial scale from which the data are taken
Data exclusions	If no data were excluded from the analyses, state so OR if data were excluded, describe the exclusions and the rationale behind them, indicating whether exclusion criteria were pre-established.
Reproducibility	Describe the measures taken to verify the reproducibility of experimental findings. For each experiment, note whether any attempts to repeat the experiment failed OR state that all attempts to repeat the experiment were successful.
Randomization	Describe how samples/organisms/participants were allocated into groups. If allocation was not random, describe how covariates were controlled. If this is not relevant to your study, explain why.
Blinding	Describe the extent of blinding used during data acquisition and analysis. If blinding was not possible, describe why OR explain why blinding was not relevant to your study.
Did the study involve fiel	d work? Yes No

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	Methods
n/a Involved in the study	n/a Involved in the study
Antibodies	X ChIP-seq
Eukaryotic cell lines	Flow cytometry
Palaeontology and archaeology	MRI-based neuroimaging
X Animals and other organisms	·
Human research participants	
✗ ☐ Clinical data	
Dual use research of concern	

Antibodies

Anti-HA-Peroxidase, Roche, Cat # 12013819001, 1:2000 dilution
Anti-FLAG-Peroxidase, Sigma-Aldrich, Cat # A8592, 1:2000 dilution
Anti-GFP, Roche, Cat # 11814460001, 1:2000 dilution
Anti-Mouse IgG, HRP-linked antibody, Cell Signaling, Cat # 7076, 1:5000 dilution
Anti-Rabbit IgG, HRP-linked antibody, Cell Signaling, Cat # 7074S, 1:5000 dilution
Anti-GST-Peroxidase, Millipore Sigma, Cat # 16209, 1:2000 dilution
Anti-MBP,Biolegend, Cat # 906901, 1:2000 dilution
Anti-FLAG M2 Affinity gel, Sigma-Aldrich, Cat # 2220

Validation Antibodies were validated by the manufacturers and further evaluated using the proper negative controls.

Eukaryotic cell lines

Policy information about <u>cell lines</u>	
Cell line source(s)	Insect sf9 cells were stocked in Pingwei Li's lab at Texas A&M university.
Authentication	Insect sf9 cells used were authenticated by the provider.
Mycoplasma contamination	Insect sf9 cells were tested negative for mycoplasma contamination.

Commonly misidentified	line
(See ICLAC register)	

none			

Palaeontology and Archaeology

Specimen provenance

Provide provenance information for specimens and describe permits that were obtained for the work (including the name of the issuing authority, the date of issue, and any identifying information).

Specimen deposition

Indicate where the specimens have been deposited to permit free access by other researchers.

Dating methods

If new dates are provided, describe how they were obtained (e.g. collection, storage, sample pretreatment and measurement), where they were obtained (i.e. lab name), the calibration program and the protocol for quality assurance OR state that no new dates are provided.

Tick this box to confirm that the raw and calibrated dates are available in the paper or in Supplementary Information.

Ethics oversight

Identify the organization(s) that approved or provided guidance on the study protocol, OR state that no ethical approval or guidance was required and explain why not.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals

For laboratory animals, report species, strain, sex and age OR state that the study did not involve laboratory animals.

Wild animals

Provide details on animals observed in or captured in the field; report species, sex and age where possible. Describe how animals were caught and transported and what happened to captive animals after the study (if killed, explain why and describe method; if released, say where and when) OR state that the study did not involve wild animals.

Field-collected samples

For laboratory work with field-collected samples, describe all relevant parameters such as housing, maintenance, temperature, photoperiod and end-of-experiment protocol OR state that the study did not involve samples collected from the field.

Ethics oversight

Identify the organization(s) that approved or provided guidance on the study protocol, OR state that no ethical approval or guidance was required and explain why not.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Human research participants

Policy information about <u>studies involving human research participants</u>

Population characteristics

Describe the covariate-relevant population characteristics of the human research participants (e.g. age, gender, 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.

Clinical data

Policy information about clinical studies

All manuscripts should comply with the ICMJEguidelines for publication of clinical research and a completed CONSORT checklist must be included with all submissions.

Clinical trial registration

Provide the trial registration number from ClinicalTrials.gov or an equivalent agency.

Study protocol

Note where the full trial protocol can be accessed OR if not available, explain why.

Data collection

Describe the settings and locales of data collection, noting the time periods of recruitment and data collection.

Outcomes

Describe how you pre-defined primary and secondary outcome measures and how you assessed these measures.

Dual use research of concern

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

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Could the accidental, deli in the manuscript, pose a	berate or reckless misuse of agents or technologies generated in the work, or the application of information presented threat to:		
No Yes Public health National security Crops and/or livest Ecosystems Any other significa			
Experiments of concer	n		
Does the work involve an	y of these experiments of concern:		
Confer resistance t Enhance the virule Increase transmiss Alter the host rang Enable evasion of o Enable the weapor	to render a vaccine ineffective to therapeutically useful antibiotics or antiviral agents there of a pathogen or render a nonpathogen virulent bility of a pathogen e of a pathogen liagnostic/detection modalities tization of a biological agent or toxin lly harmful combination of experiments and agents		
	and final processed data have been deposited in a public database such as <u>GEO</u> . edeposited or provided access to graph files (e.g. BED files) for the called peaks.		
Data access links May remain private before public	For "Initial submission" or "Revised version" documents, provide reviewer access links. For your "Final submission" document, provide a link to the deposited data.		
Files in database submiss	on Provide a list of all files available in the database submission.		
Genome browser session (e.g. <u>UCSC</u>) Provide a link to an anonymized genome browser session for "Initial submission" and "Revised version" documents only, to enable peer review. Write "no longer applicable" for "Final submission" documents.			
Methodology			
Replicates	Describe the experimental replicates, specifying number, type and replicate agreement.		
Sequencing depth	Describe the sequencing depth for each experiment, providing the total number of reads, uniquely mapped reads, length of reads and whether they were paired- or single-end.		
Antibodies	Describe the antibodies used for the ChIP-seq experiments; as applicable, provide supplier name, catalog number, clone name, and lot number.		
Peak calling parameters	Specify the command line program and parameters used for read mapping and peak calling, including the ChIP, control and index files used.		
Data quality	Describe the methods used to ensure data quality in full detail, including how many peaks are at FDR 5% and above 5-fold enrichment.		
Software	Describe the software used to collect and analyze the ChIP-seq data. For custom code that has been deposited into a community		

repository, provide accession details.

Flow Cytometry

Plots			
Confirm that:			
The axis labels state the mark	er and fluorochrome used (e.g. CD4-FITC).		
The axis scales are clearly visib	ole. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).		
All plots are contour plots with	n outliers or pseudocolor plots.		
A numerical value for number	of cells or percentage (with statistics) is provided.		
Methodology			
	Describe the sample preparation, detailing the biological source of the cells and any tissue processing steps used.		
Instrument	Identify the instrument used for data collection, specifying make and model number.		
	Describe the software used to collect and analyze the flow cytometry data. For custom code that has been deposited into a community repository, provide accession details.		
	Describe the abundance of the relevant cell populations within post-sort fractions, providing details on the purity of the samples and how it was determined.		
	Describe the gating strategy used for all relevant experiments, specifying the preliminary FSC/SSC gates of the starting cell population, indicating where boundaries between "positive" and "negative" staining cell populations are defined.		
Tick this box to confirm that a	figure exemplifying the gating strategy is provided in the Supplementary Information.		
Magnetic resonance in	naging		
Experimental design			
Design type	Indicate task or resting state; event-related or block design.		
Design specifications	Specify the number of blocks, trials or experimental units per session and/or subject, and specify the length of each trial or block (if trials are blocked) and interval between trials.		
Behavioral performance measure	State number and/or type of variables recorded (e.g. correct button press, response time) and what statistics were used to establish that the subjects were performing the task as expected (e.g. mean, range, and/or standard deviation across subjects).		
Acquisition			
Imaging type(s)	Specify: functional, structural, diffusion, perfusion.		
Field strength	Specify in Tesla		
Sequence & imaging parameters	Specify the pulse sequence type (gradient echo, spin echo, etc.), imaging type (EPI, spiral, etc.), field of view, matrix size, slice thickness, orientation and TE/TR/flip angle.		
Area of acquisition	State whether a whole brain scan was used OR define the area of acquisition, describing how the region was determined		
Diffusion MRI Used	Not used		
Preprocessing			

Preprocessing software Provide detail on software version and revision number and on specific parameters (model/functions, brain extraction, segmentation, smoothing kernel size, etc.).

physiological signals (heart rate, respiration).

If data were normalized/standardized, describe the approach(es): specify linear or non-linear and define image types used for transformation OR indicate that data were not normalized and explain rationale for lack of normalization.

Describe the template used for normalization/transformation, specifying subject space or group standardized space (e.g. original Talairach, MNI305, ICBM152) OR indicate that the data were not normalized.

Describe your procedure(s) for artifact and structured noise removal, specifying motion parameters, tissue signals and

Noise and artifact removal

Normalization template

Normalization

Volume censoring	Define your software and/or method and criteria for volume censoring, and state the extent of such censoring.

Statistical modeling & infere	ence	
Model type and settings	Specify type (mass univariate, multivariate, RSA, predictive, etc.) and describe essential details of the model at the first and second levels (e.g. fixed, random or mixed effects; drift or auto-correlation).	
Effect(s) tested	Define precise effect in terms of the task or stimulus conditions instead of psychological concepts and indicate whether ANOVA or factorial designs were used.	
Specify type of analysis: W	/hole brain	
Statistic type for inference (See Eklund et al. 2016)	Specify voxel-wise or cluster-wise and report all relevant parameters for cluster-wise methods.	
Correction	Describe the type of correction and how it is obtained for multiple comparisons (e.g. FWE, FDR, permutation or Monte Carlo).	
Vodels & analysis n/a Involved in the study Functional and/or effectiv Graph analysis Multivariate modeling or particular		
Functional and/or effective con	Report the measures of dependence used and the model details (e.g. Pearson correlation, partial correlation, mutual information).	
Graph analysis	Report the dependent variable and connectivity measure, specifying weighted graph or binarized graph, subject- or group-level, and the global and/or node summaries used (e.g. clustering coefficient, efficiency, etc.).	

Multivariate modeling and predictive analysis

Specify independent variables, features extraction and dimension reduction, model, training and evaluation metrics.