nature research

Corresponding author(s): Ning Zheng & Keiko Torii

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 Authors & Referees and the Editorial Policy Checklist.

Statistical Parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. 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
☐	☒	An indication of 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 statistics 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
☐	☒	Clearly defined error bars *State explicitly what error bars represent (e.g. SD, SE, CI)*

Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

Data collection	Diffraction data for MPK6ΔNt was collected remotely from the Advanced Light Source at Lawrence Berkeley National Laboratory on the BL8.2.1 beamline
Data analysis	1. Diffraction data was indexed, integrated and scaled with HKL2000 (Otwinowski and Minor, 1997) 2. MPK6ΔNt structure was solved by molecular replacement using the Phaser-MR v2.1 (McCoy et al., 2007) program in the Phenix software suite v1.10.1-2155 (Adams et al., 2010) and PDB 5ci6 as a search model. 3. The initial MPK6ΔNt structure was created with AutoBuild was refined over successive cycles with Coot v0.8.6.1 (Emsley et al., 2010). 4. All structure figures were created using PyMOL v0.99 (DeLano Scientific LLC (Schrödinger, LLC)) 5. The non-terminal missing regions of the MPK6 structure (chain B) were modeled using Modeler (Sali and Blundell, 1993). 6. Thie modeled MPK6 structure was used as input for Rosetta flexpepdock program (Raveh et al., 2011) along with the 35 residue long amino-acid sequence of the SCRM peptide for ab initio flexible docking. 7. Initial structure of peptide sequence was modeled using the ab-initio modeling protocol of Rosetta Software Suite(Leaver-Fay et al., 2011; Simons et al., 1999). 8. FASTA alignments of A. thaliana MPK6 and H. sapiens ERK1, ERK2, ERK5, p38, p38, p38, and p38 were generated with Qiagen Bioinformatics CLC Sequence Viewer v8.0.0. 9. The alignments were exported into UCSF Chimera v1.11 (Pettersen et al., 2004), developed by the Resource for Biocomputing, Visualization, and Informatics at the University of California, San Francisco, with support from NIH P41-GM103311. 10. The Chimera MSMS package (Sanner et al., 1996) and POV-Ray v3.6 (Persistence of Vision Pty. Ltd. (2004)) were used to model solvent-excluded molecular surfaces and generate raytraced images. 11. R version 3.3.1 was used to generate Box plots, dot plots and performing statstical analysis. R is available from the website:https://www.r-project.org/ 12. Imaris 8.3.1. was used to quantify nuclear GFP signals. Sofware is commercially available from BitPlane (https://imaris.oxinst.com/)

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/reviewers upon request. 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 data availability statement. 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

MPK6 ΔN, PDB ID: 6DTL. X-ray crystallography data collection and refinement statistics supplied as Table S2. Raw data for all BLI experiments supplied as Table S4

Field-specific reporting

Please select 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/authors/policies/ReportingSummary-flat.pdf

Life sciences study design

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

Sample size	Sample size was determined based on similar studies in this field.
Data Exclusion	For Octet data: The K.D. value was deemed Not Fittable (N.F.) when any of following conditions were met: 1. The response values of atleast 4 of the 6 concentration points used for the protein sample were less than 1nm 2. The intercept value from the equation obtained after curve fitting was negative (Equation of the form: y=mx+c where m is the slope and c is the intercept, c/m gives the KD Value of a particular sample.) 3. The R2 value is <0.9 The K.D. value was deemed Not Determined (N.D.) when any of following conditions were met: 1. The response value of the highest concentration point is < 0.5nm. 2. The intercept value from the equation obtained after curve fitting was negative (Equation of the form: y=mx+c where m is the slope and c is the intercept, c/m gives the KD Value of a particular sample.) 3. The R2 value is <0.9 For quantitative analysis of GFP signals, Imaris ver 8.1.3 was used to analyze the maximal projection of extensive Z-stack images with the following conditions: 1. Cut off value for sphericity of 0.7 has been applied to remove non nuclear signals (e.g. background autofluorescence of cell walls and chloroplasts). 2. Unlike SPCH-GFP, GFP-SCRM and GFP-scrm-D signals persist even in the differentiated guard cells, which escape the MAPK-mediated inhibition. These cell types were hence masked from the quantitative time-course analysis.
Replication	To ensure robust reproducibility: All Octet data presented in this manuscript were repeated three times. All Y2H, BiFC, immunoblot assays were repeated atleast three times. All confocal images presented were imaged at least thrice for a single data point. For transgenic lines: atleast three individual lines for each transgenic plant were analyzed before carrying out further experiments with the lines. For quantification of stomata precursor index of transgenic and mutant lines used in the manuscript, six independent replicates were used for each genotype tested. Results from all technical- and biological replicates were consistent among them.
Randomaization	Plants for all phenotypic characterizations were randomly chosen among each genotype population.
Blinding	No blinding was performed in this study. This is because blinding requires mixing seeds of different genotypes (mutants and transgenic lines), which could lead to a risk of mislabeling.

Reporting for specific materials, systems and methods

Materials & experimental systems

n/a	Involved in the study
☐	☒ Unique biological materials
☐	☒ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology
☒	☐ Animals and other organisms
☒	☐ Human research participants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Unique biological materials

Policy information about availability of materials

Obtaining unique materials

All materials used in this study will be made available through the lead contact on the manuscript, Keiko U. Torii (ktorii@u.washington.edu)

Antibodies

Antibodies used

1. Mouse monoclonal anti-HA, Abcam, Cat# ab18181 RRID: AB_444303, Lot#GR291879-2; 1:1000 dilution; clone HA.C5
2. Mouse monoclonal anti-Myc, Abcam, Cat# ab32 RRID: AB_303599, Lot#GR206680-8; 1:2000 dilution; clone 9E10
3. Rabbit polyclonal anti-HA, Abcam, Cat# ab9110 RRID: AB_307019, Lot#288587-4, 1:5000 dilution
4. Rabbit polyclonal anti-Myc, Abcam, Cat# ab9106 RRID: AB_307014, Lot#GR130480-27, 1:2000 dilution
5. Mouse monoclonal anti-FLAG, Sigma Aldrich, Cat# F3165 RRID: AB_259529, Lot#065K6236, 1:5000 dilution; clone M2
6. Rabbit polyclonal anti-GFP, Abcam, Cat# ab290 also ENCAB615WUN RRID: AB_303395, Lot#GR278073-1, 1:2000 dilution
7. Mouse monoclonal anti-GFP, Thermo Scientific Fisher, Cat# 33-2600 RRID: AB_2533111, Lot#QC215114, 1:1000 dilution; cloneC163
8. Anti-Mouse HRP conjugated secondary antibody, ECL, Cat#NA931VS Lot#9708060, 1:50000 dilution
9. Anti-Rabbit HRP conjugated secondary antibody, Cell-Signaling, Cat#7074S Ref# 07/2014, 1:2000 dilution

Valication

1. https://www.abcam.com/ha-tag-antibody-hac5-ab18181-references.html#top-232
2. https://www.abcam.com/myc-tag-antibody-9e10-chip-grade-ab32-references.html#top-0
3. https://www.abcam.com/ha-tag-antibody-chip-grade-ab9110-references.html#top-176
4. https://www.abcam.com/myc-tag-antibody-ab9106-references.html#top-16
5. https://www.sigmaaldrich.com/catalog/product/sigma/f3165?lang=en&region=US
6. https://www.abcam.com/gfp-antibody-chip-grade-ab290-references.html#top-343
7. https://www.thermofisher.com/antibody/product/GFP-Antibody-clone-C163-Monoclonal/33-2600
8. https://www.sigmaaldrich.com/catalog/product/sigma/gena93101ml?lang=en&region=US
9. https://media.cellsignal.com/coa/7074/28/7074-lot-28-coa.pdf