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.

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St	at	ict	100

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. <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>
×	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
×	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

Microscopic images were collected using Carl Zeiss Zen software. The luminescence was recorded using a FlexStation3 (Molecular Devices). The phosphorylation signals were detected by a Typhoon 9410 phosphor imager. Peptide identification and phosphosites assignments were performed with the Proteome Discoverer software (version 1.4, Thermo Fisher). TIRF images were collected at 1- to 2-s intervals using ELYRA 7 (Carl Zeiss).

Data analysis

Images were analyzed using ImageJ/Fiji software (version 2.0.0-rc-68/1.52h). Data were analyzed using graphpad prism software.For correlation coefficient analyses, time-lapse VAEM series were cropped and analyzed using the built-in MATLAB function corr2 defined by Vidali et al. (Vidali, L. et al. Myosin XI is essential for tip growth in Physcomitrella patens. Plant Cell 22, 1868-1882 (2010)).

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 <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 description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

The authors declare that all data supporting the findings of this study are included in the manuscript and its supplementary files are available from the corresponding author upon request. Source data are provided with this paper.

Field-spe	cific reporting		
Please select the or	be below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.		
X Life sciences	Behavioural & social sciences Ecological, evolutionary & environmental sciences		
For a reference copy of the	ne document with all sections, see <u>nature.com/documents/nr-reporting-summary-flat.pdf</u>		
Life scien	ices study design		
All studies must disc	close on these points even when the disclosure is negative.		
Sample size	Sample size was determined for each experiment based on similar data reported in the scientific literature.		
Data exclusions	No data were excluded from the analyses.		
Replication	At least two or three independent biological repeats were done for all experiments.		
Randomization	Data were randomly collected.		
Blinding	All data collection and analysis were performed as double-blind experiments.		
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	g for specific materials, systems and methods		
	on from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, ed 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 & exp	perimental systems Methods		
n/a Involved in the	e study n/a Involved in the study		
Antibodies	ChIP-seq		
Eukaryotic			
Palaeontology and archaeology MRI-based neuroimaging Animals and other organisms			
Human research participants			
Clinical data	3		
Dual use re	search of concern		
Antibodies			
Antibodies used	Anti-FLAG (RM1002) (Beijing Ray Antibody Biotech), dilution factor: 1:2000 Anti-ACTIN (AC009) (ABclonal), dilution factor: 1:2000		
	Anti-GFP (RM1008) (Beijing Ray Antibody Biotech), dilution factor: 1:2000		
	Anti-MPK6 (A7104) (Sigma-Aldrich), dilution factor: 1:2000 Anti-p44/42 MAPK (4370) (Cell Signaling Technology), dilution factor: 1:2000		
	Anti-GST (RM1005) (Beijing Ray Antibody Biotech), dilution factor: 1:2000		
	Anti-HA (RM1004) (Beijing Ray Antibody Biotech), dilution factor: 1:2000 Anti-pS779 (Abmart), dilution factor: 1:1000		
Validation Anti-FLAG:http://www.rayantibody.com/index.php?c=product&a=type&tid=12			
	Anti-ACTIN:https://abclonal.com.cn/catalog/AC009		
	Anti-GFP:http://www.rayantibody.com/index.php?c=product&a=type&tid=12		

Anti-MPK6: https://www.sigmaaldrich.com/catalog/product/roche/11814460001? lang=zh®ion=CN-11814460001? lang=zh®ion=CN-1181460001? lang=zh®ion=CN-11814600

Anti-p44/42 MAPK:https://www.cellsignal.cn/products/primary-antibodies/phospho-p44-42-mapk-erk1-2-thr202-tyr204-d13-14-4e-

Anti-HA:http://www.rayantibody.com/index.php?c=product&a=type&tid=12

Anti-pS779:http://www.ab-mart.com