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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 <u>Editorial Policies</u> and the <u>Editorial Policy Checklist</u>.

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	Cor	firmed
	\boxtimes	The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
	\boxtimes	A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
	\boxtimes	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.
	\square	A description of all covariates tested
	\boxtimes	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
	\boxtimes	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)
	\boxtimes	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.
\boxtimes		For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
\boxtimes		For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
\boxtimes		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	Xcalibur ver2.2 SP1.48; EPU 2.9		
Data analysis	Image J (version 2, NIH, USA); GraphPad Prism 9 (GraphPad Software, LLC, USA); Proteome discoverer 2.5, MASCOT 2.6; RELION 3.0; Coot 0.9.4; UCSF Chimera 1.15		

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 data are available in the manuscript or as supplementary information. The cryo-EM map and coordinates were deposited to the Electron Microscopy Database (EMDB) and to the PDB under the following accession codes: EMD-34022 and PDB 7YQ8. The mass spectrometry data were deposited to the accession numbers are PXD040977 for ProteomeXchange and JPST002096 for jPOST.

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 & soc	al sciences 🛛 Ecological, evolutionary & environmental sciences
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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	Sample sizes in the study were determined to ensure data validation and reproducibility. For qRT-PCR, ChIP-qPCR, immobilized template and in vitro transcription, in vitro kinase-mass spectrometry, and relaxation assays, the sample sizes were > 2.
Data exclusions	Exclusion criteria were pre-established. In multiple measurements, some data, which were too high or low, compared to other ones, could be excluded in the data collection. The data without properly-working controls within were excluded.
Replication	The experimental findings in the study were validated to be reproducible.
Randomization	In the study, the samples in any experimental group were generated side-by-side and randomly to prevent any bias in sample preparation and to minimize the technical and background variables.
Blinding	Experimenters and investigators were blinded to group allocation during data analysis in the study.

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	\boxtimes	ChIP-seq
	Eukaryotic cell lines	\boxtimes	Flow cytometry
\boxtimes	Palaeontology and archaeology	\boxtimes	MRI-based neuroimaging
\boxtimes	Animals and other organisms		
\boxtimes	Clinical data		
\boxtimes	Dual use research of concern		
\boxtimes	Plants		

Antibodies

Antibodies used

Pol II (ab817, Abcam; #2629, Cell Signaling Technology; A304-405A, Bethyl Laboratories), CDK9 (sc-13130, Santa Cruz Biotechnology), MED23 (A300-425A, Bethyl Laboratories), ELK1 (sc-365876, Santa Cruz Biotechnology; #91825, Cell Signaling Technology), ERK1/2

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(ab17942, Abcam), ERK1 (sc-271269, Santa Cruz Biotechnology), ERK2 (ab32081, Abcam), TOP2B (A300-949A, Bethyl Laboratories; sc-25330, Santa Cruz Biotechnology), and S2 Pol II (ab5095, Abcam)

Validation

All antibodies used for the study were validated by their vendors (Abcam, Cell signaling Technology, Bethyl Laboratories, Santa Cruz Biotechnology) for the conducted assays. All antibodies used for the study were referenced by multiple papers according to the vendors' websites.

Eukaryotic cell lines

Policy information about <u>cell lines and Sex and Gender in Research</u>			
Cell line source(s)	HEK293 cells purchased from ATCC were used for this study		
Authentication	The cell line used for the study was authenticated by the vendor		
Mycoplasma contamination	The cell line used in the study is mycoplasma-negative		
Commonly misidentified lines (See <u>ICLAC</u> register)	N/A		

Plants

Seed stocks	N/A
Novel plant genotypes	N/A
Authentication	N/A