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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	SEC measurements were gathered with Unicorn Software (v 5.20). UV-Vis datasets were gathered with Cary WinUV software (v 5.0.0.999). The cryo-EM dataset was collected automatically using the EPU software (v2.3) in a Titan Krios microscope (ThermoFisher Scientific) operated at 300 kV.
Data analysis	(Cryo-EM) Acquired movies were motion corrected with MotionCor2 (v1.1.0), and their contrast transfer function (CTF) effects corrected in CtfFind (v4.1.8). Using RELION-2, we manually picked approximately 1,000 particles to generate several 2D averages, which were used as templates for the subsequent automatic particle picking. particles were selected and further refined to 3.3-Å resolution by 3D refinement, CTF refinement, and Bayesian polishing in RELION-3.0. To improve the PAS1 domain density, we down-sampled the particle images by a factor of 2 (to 2.058 Å/pixel) and first subjected the particles to a consensus refinement. We then performed 3D variability analysis in cryoSPARC-3.2 with a resolution filter (cutoff) set to 10 Å. Modeling, refinement, and validation were performed with Coot (v0.9.5), PHENIX (v 1.19.2-4158-000), and MolProbity (v4.5), respectively. Structural visualizations and figure generation were performed with USCF Chimera (v 1.15), UCSF ChimeraX (v1.2.5) and PyMOL (v2.5). (UV-Vis kinetic measurements) Data were extracted and processed using R Studio version 1.0.136. Data were fit using non-linear least squares regression using R Studio or Microsoft Excel for Mac version 16.49. Mass spectrometric data were processed with Proteome Discoverer version 2.0.0.802 (ThermoFisher Scientific). FPLC profiles were collected using the Unicorn software (Cytiva).

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

Data Availability. Full versions of all SDS-PAGE gels and blots are provided in Supplementary Fig. 1. Source data for all the graphs in Figures 3 and 4 can be found in Supplementary Tables 1 and 2, respectively. The 3D cryo-EM map of the full-length Arabidopsis PhyB at 3.3-Å resolution has been deposited in the EMDB database with accession code EMD-24780. The corresponding atomic model has been deposited in the RCSB database with accession code 7RZW. This study made use of several publicly available protein structures obtained from the RCSB database (http://www.rcsb.org) under accession codes 4OUR, 6TC5, 3DGE, 4GCZ, 4U7O and 4I5S.
We added that statement "Normalized data points and fit lines from reactions representative of three technical replicates are shown for reversion measurements at 725 nm. (see Extended Data Table 3 for rate constants and standard deviations.)" to the legends of both Figures 3 and 4 as recommended by Reviewer #2

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	Spectroscopic data (spectra and kinetics) and autophosphorylation experiments were repeated in triplicate. Concentration dependence of thermal reversion were collected as singleton measurements at a given concentration with the total number of concentrations, n = 16 for wt and n = 3 for the F420E mutant. All datapoints for SEC measurement are shown in Figures 3b and 4d. Ultimately the final Cryo-EM map was derived from 154,901 particles. While no sample size calculation was performed, appropriate sample sizes were chosen to ensure reproducibility while managing limited material.
Data exclusions	There were no data exclusions, except for omission of particle images during cryo-EM map refinement as a matter of course.
Replication	All attempts at data reproducibility were successful. In general data were collected in triplicate or with a number of independent samples sufficient to see the appropriate trend, e.g., sufficient data points in Fig. 3b,d and 4d to show concentration dependent effects on assembly or thermal reversion properties
Randomization	In general, randomization of samples was not applicable to these studies. Solution conditions and temperature were tightly controlled, all proteins used in this study were expressed, purified and analyzed in the same manner.
Blinding	Blinding could not be used for the cryo-EM experiment as the sequence must be known for the model to be determined. Blinding was not relevant as all data were collected and processed in an automated fashion and fit when necessary by automated programs.

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
☒	☐ Human research participants
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

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