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Corresponding author(s): Ben Shen

Life Sciences Reporting Summary

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Please do not complete any field with "not applicable" or n/a. Refer to the help text for what text to use if an item is not relevant to your study.
For final submission: please carefully check your responses for accuracy; you will not be able to make changes later.

Experimental design

1. Sample size

   Describe how sample size was determined.

   Sample sizes were determined according to the field standards. In general, all experiments were run at least three independent times with representative data shown (see Figures for exact n values). Where appropriate, error bars represent standard deviations. X-ray data collected were from single crystals.

2. Data exclusions

   Describe any data exclusions.

   No data was excluded. Amino acid sequences from public databases that contained His6 tags were removed prior to sequence alignment and phylogenetic analysis.

3. Replication

   Describe the measures taken to verify the reproducibility of the experimental findings.

   All experiments were successfully replicated (at least 3 independent experiments). However, PtmA2 kinetics in borate buffer, pH 9.0, were unsuccessful due to poor stability of the protein. Southern analysis and size exclusion chromatography were only performed once; however, the result corresponded to complementary experiments (see Supplementary Information).

4. Randomization

   Describe how samples/organisms/participants were allocated into experimental groups.

   Randomization is not relevant to our study because this study does not involve group allocation.

5. Blinding

   Describe whether the investigators were blinded to group allocation during data collection and/or analysis.

   Blinding is not applicable to this study because this study does not involve group allocation.

Note: all in vivo studies must report how sample size was determined and whether blinding and randomization were used.

6. Statistical parameters

For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

n/a	Confirmed
☐	☒ The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)
☐	☒ A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒ A statement indicating how many times each experiment was replicated
☒	☐ 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 any assumptions or corrections, such as an adjustment for multiple comparisons
☒	☐ Test values indicating whether an effect is present *Provide confidence intervals or give results of significance tests (e.g. P values) as exact values whenever appropriate and with effect sizes noted.*
☐	☒ A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)
☐	☒ Clearly defined error bars in all relevant figure captions (with explicit mention of central tendency and variation)

See the web collection on statistics for biologists for further resources and guidance.

Software

Policy information about availability of computer code

7. Software

Describe the software used to analyze the data in this study.

Agilent Mass Hunter Qualitative Analysis B.06.00 was used for chromatography and mass spectrometry. Clustal OMEGA, MUSCLE, MEGA 5.2, and ESPript were used for sequence alignments and phylogeny. Unicorn 7.0.2 was used for protein chromatography. The Km Vmax Tool Kit from www.IC50.tk was used for kinetic analysis. HKL-3000 suite, Phenix, CCP4, Coot, MOLREP, MolProbity, Autosol/AutoBuild, Refmac, PISA, and Pymol were used for structure modeling and analysis.

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). *Nature Methods* guidance for providing algorithms and software for publication provides further information on this topic.

Materials and reagents

Policy information about availability of materials

8. Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a third party.

All materials are available.

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).

This study did not involve antibodies.

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.

This study did not involve eukaryotic cell lines.

b. Describe the method of cell line authentication used.

This study did not involve eukaryotic cell lines.

c. Report whether the cell lines were tested for mycoplasma contamination.

This study did not involve eukaryotic cell lines.

d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by ICLAC, provide a scientific rationale for their use.

This study did not involve eukaryotic cell lines.

Animals and human research participants

Policy information about studies involving animals; when reporting animal research, follow the ARRIVE guidelines

11. Description of research animals

Provide all relevant details on animals and/or animal-derived materials used in the study.

This study did not involve animals.

Policy information about studies involving human research participants

12. Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants.

This study did not involve human research participants.