

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 (<i>n</i>) 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 <i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i>
☒	☐ 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
☒	☐ 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 [availability of computer code](#)

Data collection	Xia2, Agilent MassHunter
Data analysis	Xia2, Phenix, PHASER, COOT, MOLPROBITY, PyMOL 2.5.2, DIA-NN, AlphaFold 2, AlphaFold 3, Skyline, NanoAnalyze, Agilent MassHunter

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](#)

CpKc TR coordinates and associated structure factors have been deposited with the PDB under accession code 8V1X. The generated mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE partner repository with the dataset identifier PXD055149. The LC-MS data of PimS0 enzymatic assay is available on Panorama Public (https://panoramaweb.org/pks_diol_production_Dan_et_al.url). All strains created in this study are available in the Joint BioEnergy Institute's Inventory of Composable Elements (ICE, <https://public-registry.jbei.org/folders/852>). The ICE repository requires account creation. All

other data generated in this study is provided in the Supplementary Information and the Source Data file provided with this paper. Data is also available from the corresponding author upon request.

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	Not applicable.
Reporting on race, ethnicity, or other socially relevant groupings	Not applicable.
Population characteristics	Not applicable.
Recruitment	Not applicable.
Ethics oversight	Not applicable.

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 & 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](https://www.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	No statistical methods were used to pre-determine sample sizes but our sample sizes are similar to those reported in previous publications, as mentioned in the "Methods Statement" section of the manuscript. For X-ray crystal structures, the sample size (total number of reflections) was assessed based on % completeness, multiplicity and Rmerge. Data collection involved a 360-degree rotation of a single protein crystal. Resolution cutoff was determined using I/sigma and CC1/2 as evaluation criteria. Enzymatic assays, proteomics analysis and cultivation experiments were conducted in at least triplicate, unless specifically mentioned otherwise in the manuscript.
Data exclusions	Some reflections were automatically rejected during X-ray data processing and scaling. Default rejection criteria were used in the data processing software (Xia2). 5-10% of unique reflections were randomly excluded for Free-R calculation. The quantity of the targeted proteins in the cell culture samples was plotted using the average mass spectrometry signal response of the three most intense tryptic peptides of each identified protein (Top3 method).
Replication	The crystal structure was collected from a single crystal. All other replication attempts (in triplicate or quadruplicate) were successful.
Randomization	Free-R flag for crystallographic data was assigned randomly to unique reflections using Phenix.
Blinding	Blinding is not applicable to this study because the experimental design and data analysis involve objective biological measurements or computational analyses that are not subject to observer bias.

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

Involvement in the study
- ☒

☐ Antibodies
- ☒

☐ Eukaryotic cell lines
- ☒

☐ Palaeontology and archaeology
- ☒

☐ Animals and other organisms
- ☒

☐ Clinical data
- ☒

☐ Dual use research of concern
- ☒

☐ Plants

Methods

- n/a

Involvement in the study
- ☒

☐ ChIP-seq
- ☒

☐ Flow cytometry
- ☒

☐ MRI-based neuroimaging

Plants

Seed stocks

Not applicable.

Novel plant genotypes

Not applicable.

Authentication

Not applicable.