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.

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	cryo-EM data was collected using EPU software (Thermofisher Scientific)
Data analysis	cryoSPARC v3.1.0, RELION 3.1, and Bsoft package v2.0.5 for cryo-EM data analysis. AlphaFold 2.2 for initial model generation. UCSF Chimera (v1.11.2), Coot (v0.9.8.1), and Phenix (1.18.2) for model building. MolProbity for validation. UCSF ChimeraX (v1.4) and Pymol (v2.4.0) for structural analysis and figure generation. AlphaFold3 for structural predictions. Origin 2019 for graph visualization. antiSMASH v7.0.0 for BGC annotation. NCBI BLAST+ v2.12.0 (BLASTP) for protein similarity network construction. BiG-SCAPE v2.0.0 for BGC similarity network analysis. Cytoscape v3.10.3 for network visualization. MEGA v11.0.13 for sequence and phylogenetic analysis. MAFFT v7.525 for multiple sequence alignment. trimAl v1.5.1 for alignment trimming. and IQ-TREE v3.1.1 for maximum-likelihood phylogenetic inference. R v4.5.3 and the PACo package v0.4.2 were used for cophylogenetic analysis. Python packages used for data processing, statistical analysis, and visualization included NumPy v2.4.4, SciPy v1.17.1, pandas v3.0.2, scikit-learn v1.8.0, Biopython v1.86, Matplotlib v3.10.8, DendroPy v5.0.8, and taxopy v0.14.0.

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

The cryo-EM maps and model coordinates have been deposited on Electron Microscopy Data Bank (EMDB) and Protein Data Bank (PDB), respectively (apo-ClbB: EMD-69442; NADPH-bound ClbB: EMD-69437 and 24CH)). Mass spectrometry and mass photometry data are presented in Supplementary Figs. 1c and 12, respectively.

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 & 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	Statistical methods were not used to determine sample size. For cryo-EM structure determination of apo-ClbB-PKS, 15,345 movies were collected. For NADPH-bound ClbB-PKS structure, 10,054 movies were collected, which provided sufficient resolution for model building. For in vitro biosynthesis and LC-MS analysis, a minimum of three biological replicates (n≥3) were performed for each condition, which is standard practice in the field.
Data exclusions	For cryo-EM data processing, particles classified as "junk" (fuzzy particles, ECA≥4) during 2D classification, were excluded for further data analysis.
Replication	All experimental results were confirmed across a minimum of 3 independent experimental replicates with consistent outcomes.
Randomization	Randomization was not used in this study.
Blinding	Blinding was not applicable to this 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

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

Plants

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