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Reporting Summary

Nature Research 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 Research 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	Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) was carried out on Bruker UltraFlextreme. Liquid chromatography electrospray ionization tandem mass spectrometry (LC/ESI-MS/MS) was carried out and processed using a Triple TOF 4600 System (AB Sciex) equipped with a Prominence Ultra-Fast Liquid Chromatography (UFLC) system (Shimadzu). UV-Vis spectrometry was recorded by Cary 300 (Agilent Technologies). Conditions for all ESI-MS and MS/MS were set as follows: nebulizer gas: 55 psi; heater gas: 55 psi; curtain gas: 35 psi; drying temperature: 550 °C; ion spray voltage: 5500 V; declustering potential: 100 V; collision energy: 35 V (positive); collision energy spread: 10 V. The mass range and accumulation time are 400-4000 m/z, 250 ms for ESI-MS and 100-2000 m/z, 100 ms for MS/MS, respectively. Collision-induced dissociation (CID) was performed for fragmentation of the respective peptide ions. Calibration solutions purchased from AB SCIEX were used for instrument calibration, and high resolution was chosen in the ESI+ mode. X-ray data were collected at BL-17U1 or BL-18U1 station of the Shanghai Synchrotron Radiation Facility (SSRF), China. PROPKA was used for pKa calculations. Gaussian 16 was used for structure optimization and RESP partial charges calculation. AutoDock 4.2 and AutoDockTools-1.5.6 are used for molecular docking. Amber 16 and AmberTools 16 are used for molecular dynamics simulations.
Data analysis	Matrix-assisted laser desorption/ionization-time of flight mass spectrometry data were analyzed by Bruker Daltonics Compass 1.4 for flexSeries. Liquid chromatography–mass spectrometry data were analyzed on MassLynx V4.1 SCN639. X-ray diffraction data were processed using autoPROC, XDS, autoSHARP, Buccaneer, REFMAC5, CCP4, Phaser MR, eLBOW, Phenix Refine, and Coot. Modeling and figure generation was performed using PyMOL 1.8 and Chimera 1.10.2. Bio-Rad CFX Manager 3.1 Data Analysis tool was used to analyze DSF melt curves and the data plotted in OriginPro 2016 (OriginLab). All software packages and code used are commercially available or available in the literature.

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 Research 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 list of figures that have associated raw data
- A description of any restrictions on data availability

All figures have associated raw data which can be provided upon request. All crystal structures have been deposited in the PDB database under accession number 7V9N for EryP_closed,7V9P for EryP_intermediate, 7V9Q for EryP_open and 7V9O for EryP_E802R.

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	The number of EryP site-directed variants was selected according to multi-sequence alignments and structural observations. Two EryP mutants with active site residue mutations were generated, and we believe this sample size to be sufficiently large as residues are highly conserved. For engineering efforts in this study, EryP_E802R and AplP_R98E-A368E-A779R were generated, and we believe that this sample size is sufficient to demonstrate the correlation between domain interactions and catalytic activities of these enzymes.
Data exclusions	Before structural refinement in REFMAC5, a random 5% of the diffraction data were removed to calculate R-free values.
Replication	All attempts at replication were successful. We state the number of replicates for each experiment in the paper. We performed each replicate under consistent experimental conditions to the best of our ability, to ensure replication of our findings
Randomization	n/a
Blinding	n/a

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