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Reporting Summary

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Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. 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
☐	☒	An indication of 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 statistics 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
☐	☒	Clearly defined error bars *State explicitly what error bars represent (e.g. SD, SE, CI)*

Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

Data collection	Fluorescence polarization data were collected on Gen5 1.10.8. Matrix-assisted laser desorption/ionization-time of flight mass spectrometry data were collected on Bruker Daltonics Compass 1.4 for flexSeries. Liquid chromatography–mass spectrometry data were collected on MassLynx V4.1 SCN639. Differential Scanning Fluorimetry data were collected with Bio-Rad CFX Manager 3.1. Size-exclusion chromatograms were collected and analyzed using PrimeView and PrimeView Evaluation. X-ray diffraction data were collected using software developed by the staff at LS-CAT (https://ls-cat.org/index.html) . MD simulations were carried out using GPU accelerated code (pmemd) of the Amber 16 and AmberTools 17 package using the Gaussian 16 package for charge calculations. Melting curves were obtained using a CFX Connect Real-Time PCR Detection System (Bio-Rad Laboratories, Inc.).
Data analysis	Fluorescence polarization data were analyzed by OriginPro 2017. 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. Gaussian 16 was used for the Quantum Mechanical calculations (RESP partial charges and structure minimization).

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/reviewers upon request. 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 except for Scheme 1 have associated raw data which can be provided upon request. PDB accession codes are provided in the Author Information section

Field-specific reporting

Please select the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences

For a reference copy of the document with all sections, see nature.com/authors/policies/ReportingSummary-flat.pdf

Life sciences

Study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	The number of DurN site-directed variants was selected according to multi-sequence alignments and structural observations. Ten total variants were generated. We believe this sample size to be sufficiently large as residues that are highly conserved and/or involved in substrate/product recognition are most likely to be important for catalysis, and the sample size we selected encompasses all of the relevant residues based on the above considerations.
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

Materials & experimental systems

Policy information about availability of materials

n/a	Involved in the study
☒	☐ Unique materials
☒	☐ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Research animals
☒	☐ Human research participants

Method-specific reporting

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ Magnetic resonance imaging