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.

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For	all statistical ar	nalyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.			
n/a	Confirmed				
	\blacksquare 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 statis	stical test(s) used AND whether they are one- or two-sided non tests should be described solely by name; describe more complex techniques in the Methods section.			
x	A descrip	tion of all covariates tested			
	🗶 A descrip	tion 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 Give <i>P</i> values as exact values whenever suitable.				
×	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings				
X	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes				
\blacksquare Estimates of effect sizes (e.g. Cohen's d , Pearson's r), indicating how they were calculated					
Our web collection on <u>statistics for biologists</u> contains articles on many of the points above.					
Software and code					
Policy information about <u>availability of computer code</u>					
Da	ta collection	Nikon NIS Elements software version AR 5.11.02 64 bit, Simple PCI software version 6.0.			
Da	ta analysis	Fiji version 1.52n, MicrobeJ version 5.13j, GraphPad Prism 8.0, Microsoft Excel 2016			
For m	nanuscripts utilizin	g 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			

Data

Policy information about availability of data

All manuscripts must include a <u>data availability statement</u>. This statement should provide the following information, where applicable:

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.

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- $\hbox{-} For \ clinical \ datasets \ or \ third \ party \ data, \ please \ ensure \ that \ the \ statement \ adheres \ to \ our \ \underline{policy}$

Coordinates and structure factors for the Bd1075 crystal structure have been deposited in the PDB under the accession code 7O21 (https://www.rcsb.org/structure/7O21). Comparisons were made to the structures of Csd6 (PDB: 4XZZ: https://www.rcsb.org/structure/4XZZ) and Pgp2 (PDB: 6XJ6: https://www.rcsb.org/structure/6XJ6). Source data is provided with this manuscript.

Field-specific reporting					
Please select the o	ne 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 dis	sclose on these points even when the disclosure is negative.				
Sample size	No calculation was performed to predetermine sample size. Sample sizes were chosen based on methods that have produced reliable and statistically-robust data previously (Harding et al. 2020, Nat Comms; Lerner et al., 2012, PLOS Pathogens; Kuru et al., 2017, Nat Micro). Exact sample sizes (n) are given for every experiment (please refer to the manuscript figure legends).				
Data exclusions	No data exclusions				
Replication	At least three independent biological repeats carried out for experiments, consistently reproducing findings				
Randomization	Colonies or plaques of bacteria were selected at random from plates for culture for experiments, grown separately and on different days for each biological repeat.				
Blinding	Not possible due to wild-type and mutant shape difference				
Reportin	g 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 & ex	perimental systems Methods				
n/a Involved in th	ne study n/a Involved in the study				
Antibodies	ChIP-seq				
Eukaryotic cell lines					
Palaeontology and archaeology MRI-based neuroimaging					
Animals and other organisms					
Human research participants					
Clinical data					
▼ Dual use research of concern					

Antibodies

Antibodies used

anti-mCherry polyclonal antibody (Invitrogen), product no: PA5-34974, lot no: TH26110628. 1:4000 dilution

Validation

Application for western blot - 15 publications listed on manufacturer's website:

https://www.thermofisher.com/antibody/product/mCherry-Antibody-Polyclonal/PA5-34974