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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<u>Authors & Referees</u> and the<u>Editorial Policy Checklist</u>.

Statistics

Fora	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					
x		A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly					
	x	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					
x		A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons					
	x	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)					
	x	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> .					
X		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					
x		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 a	bout <u>availability of computer code</u>	
Data collection	The X-ray diffraction data were collected at the beamline NE-3A at the Photon Factory, Japan.	
Data analysis	X-ray diffraction data were processed with HKL2000 suite, and the structure was determined by using PHENIX(ver 1.10.1), COOT(ver 0.8.9.2) and CNS suites. Bio-layer interferometry data were analyzed by using Blitz Pro 1.1 software. CyaA translocation assay data were analyzed with Prism 7.0.	

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/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 <u>data availability statement</u>. This statement should provide the following information, where applicable: - Accession codes, unique identifiers, or web links for publicly available datasets

code for the coordinates used for phase determination is 5X90. N-terminal portion of the VipD structure (PDB code: 4AKF) was used for homology modeling.

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

The coordinates of the structure of DotL(656-783)–IcmSW–LvgA–VpdB(461-590) are deposited in the Protein Data Bank (PDB code: 7BWK). The PDB accession

Field-specific reporting

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.							
Sample size	The sample-size was determined regarding the same experiments previously reported in the field.						
Data exclusions	No data was excluded.						
Replication	All the presented experimental results were reliably reproduced, repeated more than at least two times independently.						
Randomization	Random allocation was not relevant to the described experiments since no animal nor human studies are involved.						
Blinding	Blinding group allocation was not relevant to the study since no animal nor human studies are involved.						

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

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.

	iental systems	Methods			
n/a Involved in the stud	у	n/a Involved in the study			
Antibodies		K ChIP-seq			
Eukaryotic cell line	es	Flow cytometry			
🗴 🗌 Palaeontology		🕱 🔲 MRI-based neuroimaging			
Animals and othe	rorganisms				
Human research p	participants				
Image: Clinical data					
Antibodies used	Anti-CyaA mouse	monoclonal IgG (sc-13582, Santa Cruz Biotechnology, Inc.)			
Antibodies		monoclonal IgG (sc-13582, Santa Cruz Biotechnology, Inc.) gG (#31430, Invitrogen)			
Antibodies	goat anti-mouse l				

Eukaryotic cell lines

Policy information about <u>cell lines</u>	
Cell line source(s)	CHO-FcR cell line was obtained from Craig Roy's Lab (Nagai et al. PNAS 2004, www.pnas.org/cgi/doi/10.1073/ pnas.0406239101)
Authentication	The cell line was not authenticated.
Mycoplasma contamination	The cell line was not Mycoplasma tested.
Commonly misidentified lines (See <u>ICLAC</u> register)	No commonly misidentified cell lines was used in this study.