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 <u>Editorial Policies</u> and the <u>Editorial Policy Checklist</u>.

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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
\boxtimes	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.
X	A description of all covariates tested
X	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)
\boxtimes	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>
\boxtimes	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
\boxtimes	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
\boxtimes	Estimates of effect sizes (e.g. Cohen's <i>d</i> , Pearson's <i>r</i>), 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 availability of computer code

Data collection

Data was collected on an FEI Titan Krios cryo-electron microscope equipped with a K3 Summit direct electron detector (Gatan, Pleasanton, CA) operating in super-resolution counting mode. Images were recorded with SerialEM v3.8 5.

Data analysis

Data was analyzed, processed, and visualized using WARP v1.09, cryoSPARC v3.2, Relion v3.1, ChimeraX v1.0, trRosetta server, Namdinator v20191016-5814c947, Isolde v1.2, Phenix v1.18rc5, ConSurf sever, and PyMol v2.5.

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 $\underline{\text{policy}}$

The cryo-EM structure and associated atomic model of DrmAB-ADP have been deposited into the Electron Microscopy Data Bank and the Protein Data Bank with accession codes EMD-24938 [https://www.ebi.ac.uk/pdbe/entry/emdb/EMD-24938] and PDB 7S9V [http://doi.org/10.2210/pdb7S9V/pdb], respectively. The cryo-EM structure of DrmAB-ADP-DNA and associated atomic model have been deposited with accession codes EMD-24939 [https://www.ebi.ac.uk/pdbe/entry/emdb/EMD-24939] and PDB 7S9W [http://doi.org/10.2210/pdb7S9W/pdb], respectively. Source Data are provided with this paper. Materials and correspondence requests should be addressed to Stan J.J. Brouns (stanbrouns@gmail.com) David W. Taylor (dtaylor@utexas.edu).

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\times Life sciences	Behavioural & social sciences Ecological, evolutionary & environmental sciences
For a reference copy of t	he document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf
Life scier	nces study design
All studies must dis	close on these points even when the disclosure is negative.
Sample size	The dataset contained 5 million particles, and at least 144,000 particles were used for the final reconstruction. These are typical particle image numbers for cryo-EM datasets.
Data exclusions	2D and 3D classification procedures were used to exclude damaged and 'bad' particles. This is standard practice in cryo-EM and is necessary in order to obtain homogeneous high-resolution cryo-EM structures. No data was excluded from the in vivo or in vitro experiments.
Replication	Cryo-EM datasets were collected with multiples samples in separate imaging sessions. The majority of the components of the protein complex were nearly identical among structures from all datasets. Three independent replicates were performed for biochemical and EOP assays. All replication experiments were successful.
Randomization	No randomization was performed. To accurately build a model, it is necessary to know the identify of the complex.
Blinding	Particle classification and resolution estimation were performed automatically from particle processing software packages.

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.

Ma	terials & experimental systems	Me	thods
n/a	Involved in the study	n/a	Involved in the study
\boxtimes	Antibodies	\boxtimes	ChIP-seq
\boxtimes	Eukaryotic cell lines	\boxtimes	Flow cytometry
\boxtimes	Palaeontology and archaeology	\boxtimes	MRI-based neuroimaging
\boxtimes	Animals and other organisms		
\boxtimes	Human research participants		
\boxtimes	Clinical data		
\boxtimes	Dual use research of concern		