# 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.

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

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n/a	Cor	nfirmed
	X	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.
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)
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>
×		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
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 about availability of computer code

Data collection

No custom code was used to collect data. Cryo EM data was collected using EPU (v 3.2) software (Thermo Fisher Scientific). Gel images were taken using the Image Lab Touch v3.0.0.07 (Biorad) and GDS Touch 2 v.2.1.1.6 (Instas) softwares. UNICORN 7.8 (GE) was used for recording size exclusion traces. Fluorescent gels were imaged using Amersham Typhoon v.2.0.0.6 (GE) and Image reader FLA-5000 Series V1.0 (Fujifilm) Softwares. Plate reader data was recorded using i-control v 1.10.4 (Thermo Fisher Scientific). Mass photometry data was recorded using AcquireMP v.2023 R1.1 (Refeyn Ltd.). Mass spectrometry data was collected using Xcalibur v.4.7 (Thermo Fisher Scientific).

Data analysis

Cryosparc v.4.2-4.5 (Structura Biotech. Inc.), EMReady v.1.0, Phenix v.1.19, WinCoot v.0.98, ISOLDE v.1.4-1.8, Modelangelo and ChimeraX v.1.4-1.8 were used for cryo-EM data processing, map improvement, model building and visualization/analysis. For the lacZ repression assay, OpenCFU v.3.9.0 was used for quantification. For GFP-fluorescence assays, R-studio v.2023.03.0+286 (Posit PBC) was used for analysis. Mass photometry was analyzed using DiscoverMP v.2023 R1.2 (Refeyn Ltd.). For EMSAs analysis, fluorescence intensities were quantified in ImageJ v.1.53k (NIH). Prism v. 9.4.1 (Graphpad) was used to plot data and fit curves. Mass spectrometry data was processed with MSFragger pipeline (MS Fragger v3.8, Fragpipe v 20, IonQuant v1.9.8, Philosopher v5.0.0)

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 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 policy

Cryo-EM model coordinates have been deposited in the PDB under accession codes 8RC2 [https://doi.org/10.2210/pdb8rc2/pdb], 8RC3 [https://doi.org/10.2210/ pdb8rc3/pdb], 8RFJ [https://doi.org/10.2210/pdb8rfj/pdb], 8S35 [https://doi.org/10.2210/pdb8s35/pdb], 8S36 [https://doi.org/10.2210/pdb8s36/pdb] and 8S37 [https://doi.org/10.2210/pdb8s37/pdb]. The final cryo-EM maps, as well as consensus and focused maps, have been deposited in the EMDB under accession codes EMD-19045 [https://www.ebi.ac.uk/emdb/EMD-19045], EMD-19046 [https://www.ebi.ac.uk/emdb/EMD-19046], EMD-19125 [https://www.ebi.ac.uk/emdb/EMD-19046], EMD-19046], EMD-19046 [https://www.ebi.ac.uk/emdb/EMD-19046], EMD-19046 [https://www.ebi.ac.uk/e EMD-19125], EMD-19688 [https://www.ebi.ac.uk/emdb/EMD-19688], EMD-19689 [https://www.ebi.ac.uk/emdb/EMD-19689] and EMD-19690 [https:// www.ebi.ac.uk/emdb/EMD-19690], as well as EMD-19120 [https://www.ebi.ac.uk/emdb/EMD-19120], EMD-19124 [https://www.ebi.ac.uk/emdb/EMD-19124], EMD-19126 [https://www.ebi.ac.uk/emdb/EMD-19126], EMD-19127 [https://www.ebi.ac.uk/emdb/EMD-19127] and EMD-51026 [https://www.ebi.ac.uk/emdb/EMD-19127] EMD-51026], respectively. Previously published model coordinates used in this study are available at the PDB under accession codes 7XG2 [https://doi.org/10.2210/ pdb7xg2/pdb], 7JHY [https://doi.org/10.2210/pdb7jhy/pdb], 6H66 [https://doi.org/10.2210/pdb6h66/pdb], 7TRA [https://doi.org/10.2210/pdb7tra/pdb], 7XEX [https://doi.org/10.2210/pdb7xex/pdb], 7XF0 [https://doi.org/10.2210/pdb7xf0/pdb], 7XF1 [https://doi.org/10.2210/pdb7xf1/pdb], 7XG3 [https://doi.org/10.22 pdb7xg3/pdb], 7XG4 [https://doi.org/10.2210/pdb7xg4/pdb], 7XG1 [https://doi.org/10.2210/pdb7xg1/pdb] and 7XG0 [https://doi.org/10.2210/pdb7xg0/pdb]. Protein mass spectrometry raw data have been deposited in the ProteomeXchange repository under accession code PXD056399 [https:// proteomecentral.proteomexchange.org/cgi/GetDataset?ID=PXD056399]. Source data are provided with this paper.

## Research involving human participants, their data, or biological material

Policy information about studies with human participants or human data. See also policy information about sex, gender (identity/presentation), and sexual orientation and race, ethnicity and racism.

Reporting on sex and gender

Use the terms sex (biological attribute) and gender (shaped by social and cultural circumstances) carefully in order to avoid confusing both terms. Indicate if findings apply to only one sex or gender; describe whether sex and gender were considered in study design; whether sex and/or gender was determined based on self-reporting or assigned and methods used. Provide in the source data disaggregated sex and gender data, where this information has been collected, and if consent has been obtained for sharing of individual-level data; provide overall numbers in this Reporting Summary. Please state if this information has not been collected.

Report sex- and gender-based analyses where performed, justify reasons for lack of sex- and gender-based analysis.

Reporting on race, ethnicity, or other socially relevant groupings

Please specify the socially constructed or socially relevant categorization variable(s) used in your manuscript and explain why they were used. Please note that such variables should not be used as proxies for other socially constructed/relevant variables (for example, race or ethnicity should not be used as a proxy for socioeconomic status).

Provide clear definitions of the relevant terms used, how they were provided (by the participants/respondents, the researchers, or third parties), and the method(s) used to classify people into the different categories (e.g. self-report, census or administrative data, social media data, etc.)

Please provide details about how you controlled for confounding variables in your analyses.

Population characteristics

Describe the covariate-relevant population characteristics of the human research participants (e.g. age, genotypic information, past and current diagnosis and treatment categories). If you filled out the behavioural & social sciences study design questions and have nothing to add here, write "See above."

Recruitment

Describe how participants were recruited. Outline any potential self-selection bias or other biases that may be present and how these are likely to impact results.

Ecological, evolutionary & environmental sciences

Ethics oversight

Identify the organization(s) that approved the study protocol.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

# 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.

Behavioural & social sciences For a reference copy of the document with all sections, see <u>nature.com/documents/nr-reporting-summary-flat.pdf</u>

## Life sciences study design

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

Sample size

Life sciences

No sample size calculation was performed. Experiments were replicated at least three times, where feasible, and showed consistent results.

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## 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
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×	Eukaryotic cell lines	x	☐ Flow cytometry
×	Palaeontology and archaeology	x	MRI-based neuroimaging
×	Animals and other organisms		
×	Clinical data		
x	Dual use research of concern		
×	Plants		

### **Plants**

Seed stocks

Report on the source of all seed stocks or other plant material used. If applicable, state the seed stock centre and catalogue number. If plant specimens were collected from the field, describe the collection location, date and sampling procedures.

Novel plant genotypes

Describe the methods by which all novel plant genotypes were produced. This includes those generated by transgenic approaches, gene editing, chemical/radiation-based mutagenesis and hybridization. For transgenic lines, describe the transformation method, the number of independent lines analyzed and the generation upon which experiments were performed. For gene-edited lines, describe the editor used, the endogenous sequence targeted for editing, the targeting guide RNA sequence (if applicable) and how the editor

Authentication

was applied. Describe any authentication procedures for each seed stock used or novel genotype generated. Describe any experiments used to assess the effect of a mutation and, where applicable, how potential secondary effects (e.g. second site T-DNA insertions, mosiacism, off-target gene editing) were examined.