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.

Statistics

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
☐	☒	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 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. *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

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	Cryo-EM data collection: SerialEM v4.0 Immunoflouorescence images were acquired using the NIS-Elements A1R (Nikon) software Western blots were collected using the Bio-Rad Image Lab v6.1.0 Flow Cytometry data collection: SpectroFlo v3.0
Data analysis	Cryo-EM data processing: cryoSPARC v4.4.1, DeepEMhancer v20220530_cu11 Atomic model building: COOT v0.9.8.8; Phenix v1.21 Model Validation: Molprobity server; PDB Validation server Structure analysis: Pymol v2.5.4, UCSF ChimeraX v1.7.1 Structure prediction: AlphaFold v2.3.1 Fluorescence imaging analysis: Fiji v1.54f (ImageJ, NIH) Statistical analysis: Prism v9.5.1 (GraphPad) Data visualization and plotting: Prism v9.5.1 (GraphPad); BiochemPy website (https://biochempy.bb.iastate.edu/) Binding isotherm fitting: DynaFit v4.08.187 Mass spectrometry analysis: Proteome Discoverer v3.0 (Thermo Fisher); MaxQuant v.2.0.3.0 Flow Cytometry data analysis: FlowJo v10

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 maps and models have been deposited in the EMDB, with accession number EMD-43870 [https://www.ebi.ac.uk/emdb/EMD-43870], EMD-43871 [https://www.ebi.ac.uk/emdb/EMD-43871], EMD-43872 [https://www.ebi.ac.uk/emdb/EMD-43872], and EMD-43873 [https://www.ebi.ac.uk/emdb/EMD-43873], and PDB, with accession number 9AU7 [https://doi.org/10.2210/pdb9AU7/pdb]. AlphaFold Multimer-derived models are available in ModelArchive, with the accession code ma-swt4h [https://www.modelarchive.org/doi/10.5452/ma-swt4h]. Mass spectrometry data have been deposited at the MassIVE repository, with accession number MSV000095676 [https://doi.org/doi:10.25345/C5M902F39] and MSV000094101 [https://doi.org/doi:10.25345/C5BV7B635]. Source data are provided for all uncropped western blots, Coomassie-blue gels, and all quantitative datasets presented in this paper. To our knowledge, all information required to reanalyze the data reported here is publicly available. Any additional data we inadvertently missed will be shared upon reasonable request. This paper does not report original code.

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.*
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.

☒ 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 disclose on these points even when the disclosure is negative.

Sample size	We chose sample size based on the literature (Refs 4, 6).
Data exclusions	A subset of cryo-EM images of low quality or high heterogeneity were removed using standard procedures during 2D classification and 3D reconstruction.
Replication	All replication attempts were successful. Almost all experiments were performed at least 2 independent times, and when possible, by two different individuals.

Randomization	Protein interaction experiments in cells were performed after transient or stable transfection of cells. Plates of cells were randomly allocated to the transfection groups indicated in each experiment. For immunofluorescence experiments, samples differed only in the nature of the transfection, which was randomized as indicated above. All samples were thereafter stained for the same markers, across all groups. To avoid potential operator bias, random fields of view were chosen for imaging in immunofluoresence experiments.
Blinding	The investigators were not blinded during data collection as it was not practical due to the large effects of the experimental manipulations which precluded effective blinding. During quantification, whenever possible, a separate investigator handled the data in a blinded fashion. Experimental groups were only revealed during statistical analysis.

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.

Materials & experimental systems

n/a	Involved in the study
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☒	☐ Animals and other organisms
☒	☐ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☐	☒ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used	All the antibodies used are listed in Supplementary Table 4, stating source, catalog or identifying number, clone name, application, and dilution.
Validation	All custom-made antibodies used were previously validated using knockdown or knockout cell lines, and the appropriate references are noted in Supplementary Table 4. Other validation information for commercially available antibodies is noted in Supplementary Table 4.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	The source of the cell lines is included in the methods section. Standard, commercial insect cell lines used this study include Sf9 cells (Expression Systems). HEK293T (Cat # CRL-3216) and HeLa (Cat # CCL-2) cell lines were obtained from the American Type Culture Collection (Manassas, VA). Lenti-X 293T cells (Cat #632180) were obtained from Takara.
Authentication	Commercial insect cells were authenticated based on their stereotypical morphology and growth rate. Mammalian cell lines have been authenticated using STR analysis in the past 10 years. They were not re-authenticated for this study.
Mycoplasma contamination	Cell lines were periodically tested for mycoplasma and all the experiments were performed on the cells that were mycoplasma negative.
Commonly misidentified lines (See ICLAC register)	The cell lines used in this study are well established cell lines and are not listed as commonly misidentified lines.

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 was applied.*
Authentication	*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.*

Flow Cytometry

Plots

Confirm that:

☒ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

☒ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).

☒ All plots are contour plots with outliers or pseudocolor plots.

☒ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation	Details for sample preparation are included in the methods section
Instrument	The samples were run on a Cytek Aurora instrument, model number N7-00021
Software	Data was collected using the SpectroFlo v3.0 software and was analysed using FlowJo v10 software
Cell population abundance	The analysis was on a pure population of cells in culture.
Gating strategy	Gating strategy included the exclusion of the cell debris and doublets, followed by the exclusion based on the unstained and isotype control.

☒ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.