

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
<input type="checkbox"/>	<input checked="" type="checkbox"/> The exact sample size ( <i>n</i> ) for each experimental group/condition, given as a discrete number and unit of measurement
<input type="checkbox"/>	<input checked="" type="checkbox"/> A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
<input type="checkbox"/>	<input checked="" type="checkbox"/> The statistical test(s) used AND whether they are one- or two-sided <i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i>
<input type="checkbox"/>	<input checked="" type="checkbox"/> A description of all covariates tested
<input type="checkbox"/>	<input checked="" type="checkbox"/> A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
<input type="checkbox"/>	<input checked="" type="checkbox"/> 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)
<input type="checkbox"/>	<input checked="" type="checkbox"/> 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>
<input checked="" type="checkbox"/>	<input type="checkbox"/> For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
<input checked="" type="checkbox"/>	<input type="checkbox"/> For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
<input checked="" type="checkbox"/>	<input type="checkbox"/> 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 [statistics for biologists](#) contains articles on many of the points above.

Software and code

Policy information about [availability of computer code](#)

Data collection	Latitude, Leica LAS X, Nikon Elements AR v 4.5, Berthold ICE software
Data analysis	Snappene 8.0, Unicorn 7.6, ASTRA 7, BioXTAS RAW 2.2.1, ATSAS 3.2.1-1, RELION 4.0, cryoSPARC 4.1.2, Phenix 1.21.1, COOT 0.9.8.92, PyMOL 2.4.1, ChimeraX 1.6.1, GROMACS 2022.1, ImageJ 1.52e, Nikon Elements, Graphpad Prism 10

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](#)

All data are available in the main text or the supplementary materials or source data. The final model and cryo-EM data have been deposited into the Protein Data Bank under PDB ID 8VY2 and EMDB ID EMD-43644. Small angle X-ray scattering data are available at the SASBDB under accession codes SASDVS7 and SASDVT7

Molecular dynamics simulations are available as Supplementary Data 3. Plasmids and other reagents are available upon reasonable request to the corresponding authors, Demet Araç and Richard C. Sando.

## 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](https://www.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

No sample size calculation was performed. A sample size of N=3 independent experiments is commonly used in biological studies using cell cultures.

### Data exclusions

No Data were excluded

### Replication

All experiments were repeated in part or in whole at least three times to ensure reproducibility. All imaging and signaling assays were performed in at least triplicate, and all attempts at replication were successful. Image analyses were done using at least three independent replicates with quantifications from randomly selected imaging fields per replicate.

### Randomization

Quantifications result from randomly selected imaging fields per replicate. Cells were placed into different positions for signaling assay plates randomly.

### Blinding

Blinding is not relevant to this study, all experiments were performed based on standardized protocols and readouts, and are not influenced by the investigator.

## 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 &amp; experimental systems

## Methods

n/a	Involved in the study
<input type="checkbox"/>	<input checked="" type="checkbox"/> Antibodies
<input type="checkbox"/>	<input checked="" type="checkbox"/> Eukaryotic cell lines
<input checked="" type="checkbox"/>	<input type="checkbox"/> Palaeontology and archaeology
<input checked="" type="checkbox"/>	<input type="checkbox"/> Animals and other organisms
<input checked="" type="checkbox"/>	<input type="checkbox"/> Clinical data
<input checked="" type="checkbox"/>	<input type="checkbox"/> Dual use research of concern
<input checked="" type="checkbox"/>	<input type="checkbox"/> Plants

n/a	Involved in the study
<input checked="" type="checkbox"/>	<input type="checkbox"/> ChIP-seq
<input checked="" type="checkbox"/>	<input type="checkbox"/> Flow cytometry
<input checked="" type="checkbox"/>	<input type="checkbox"/> MRI-based neuroimaging

## Antibodies

Antibodies used

anti-HA mouse, Covance Cat# MMS101R; 1:2,000  
 ZO-1 polyclonal rabbit antibody, Invitrogen #61-7300, 1:2,000  
 goat anti-mouse Alexa Fluor 488, ThermoFisher #A11001, 1:1,000  
 goat anti-rabbit Alexa Fluor 546, ThermoFisher #A11010, 1:1,000

Validation

Commercially available antibodies used in our studies have been validated by the manufacturer and our lab and others through comparison of obtained immunohistochemistry and/or western blot results between labs, and confirmation of specificity. We validate each new lot of antibody by reproducing results obtained with previous batches of antibody.

## Eukaryotic cell lines

Policy information about [cell lines and Sex and Gender in Research](#)

Cell line source(s)

Sf9 cells (Thermo Fisher, 12659017), High Five cells (Trichoplusia ni, female, ovarian ThermoFisher, B85502), HEK293T mammalian cells (ATCC, CRL-3216), HEK293 mammalian cells (ATCC, CRL-1573).

Authentication

HEK293 cell lines from ATCC have been validated by the supplier through STR analysis and cytogenetic studies. Sf9 and High Five cell lines were not authenticated.

Mycoplasma contamination

Cell lines tested negative for mycoplasma contamination (ATCC).

Commonly misidentified lines  
 (See [ICLAC](#) register)

No commonly misidentified cell lines were used in this study.

## Plants

Seed stocks

N/A

Novel plant genotypes

N/A

Authentication

N/A