

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 (<i>n</i>) 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 <i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i>
☒	☐ 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. <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 <i>P</i> 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
☒	☐ 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	For cryoEM studies: EPU v2 For cell-based studies data were collected using the following software (most recent version listed): microscopy (Leica Application Suite X v3.4.2.18368), FRET biosensors (Harmony v4.8), BRET (CLARIOstar SMART Control v6.20 and LUMIstar Omega v6.20), scanning of membranes for western blots (Amersham Typhoon control software v3.0.0.2). For mass photometry data were collected using Refeyn AquireMP (v2.5)
Data analysis	cryoSPARC v4.1.1, MotionCor2 v1.6.4, CTFFIND v4.1.14, TOPAZ 0.2.5a, RELION v4.0b, EMAN v2.2, Coot v0.9.5, Isolde v1.1, Phenix v1.20.1, AlphaFold v2, UCSF ChimeraX v1.6, pyem v0.5, python v3.9.12, matplotlib v3.6.2, GraphPad Prism v10.0.3, Fiji ImageJ2 v2.14.0/1.54f, Microsoft Excel for Mac v16.45, Refeyn DiscoverMP v2.5. For analysis of FRET biosensor data we used an in-house custom analysis pipeline for Fiji which has been previously published (Halls et al 2015 Meth Mol Biol).

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](#)

The three-dimensional cryo-EM density maps are deposited into the Electron Microscopy Data Bank (<https://www.ebi.ac.uk/pdbe/emdb/>) under accession numbers EMD-41913, 41912, 41916, 41929, 41930, 41934, 41935, and 41914. The coordinates are deposited in the Protein Data Bank (<https://www.rcsb.org>) with accession numbers 8U56, 8U54, 8U5C, 8U5N, 8U5Q, 8U5V, 8U5X, and 8U58.

All other data supporting the findings of this study are included as figures in the main text, its Extended Data, or as Supplementary Information.

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	Not applicable.
Reporting on race, ethnicity, or other socially relevant groupings	Not applicable.
Population characteristics	Not applicable.
Recruitment	Not applicable.
Ethics oversight	Not applicable.

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. n=3 independent experiments were chosen as this is standard in the field and enables statistical analysis
Data exclusions	Data were only excluded if experiment was deemed to have failed due to instrumentation error or if cells were poorly transfected or over/under confluent. For AuxSen IAA efflux assays, individual cells were not included if the influx of IAA did not cause a FRET change of >10% of the baseline.
Replication	All attempts at replication are included in the study. N represents independent biological replicates from different transfections with experiments performed on different days.
Randomization	No randomization was included in the study as it is not routine in the field/experiment
Blinding	No blinding was included in the study as it is not routine in the field/experiment

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 following details for antibodies are included in the materials and methods: mouse anti-FLAG M2 monoclonal antibody, Merck F3165, 1:5,000 dilution for westerns and 1:1,000 dilution for immunostaining rabbit anti-beta-tubulin, Cell Signaling Technology 2146, 1:1,000 dilution IRDye 680RD goat anti-mouse secondary antibody, LICOR 926-68070, 1:15,000 dilution IRDye 800CW goat anti-rabbit secondary antibody, LICOR 926-32211, 1:15,000 dilution goat anti-mouse Alexa Fluor 488 secondary antibody, Abcam ab150113, 1:1,000 dilution
Validation	1. mouse anti-FLAG M2 monoclonal antibody. Specificity information from product data sheet: Monoclonal ANTI-FLAG® M2 detects a single band of protein on a Western blot from an E. coli crude cell lysate. Species reactivity: all. Techniques: includes western blot, immunocytochemistry. 2. rabbit anti-beta-tubulin. Specificity information from supplier website: The β-Tubulin Antibody detects endogenous levels of total β-tubulin protein, and does not cross-react with recombinant α-tubulin. Species Reactivity: Human, Mouse, Rat, Monkey, Zebrafish, Bovine. Uses: includes western blotting. 3. IRDye 680RD goat anti-mouse secondary antibody. Specificity information from product insert: Based on ELISA and flow cytometry, this antibody reacts with the heavy and light chains of mouse IgG1, IgG2a, IgG2b and IgG3, and with the light chains of mouse IgM and IgA. This antibody was tested by Dot Blot and/or solid-phase adsorbed for minimal cross-reactivity with human, rabbit, goat, rat, and horse serum proteins, but may cross-react with immunoglobulins from other species. The conjugate has been specifically tested and qualified for Western blot and In-Cell Western Assay applications. 4. IRDye 800CW goat anti-rabbit secondary antibody. Specificity information from product insert: Based on ELISA and flow cytometry, this antibody reacts with the heavy and light chains of rabbit IgG, and with the light chains of rabbit IgM and IgA. This antibody was tested by Dot Blot and/or solid-phase adsorbed for minimal cross-reactivity with human, mouse, rat, sheep, and chicken serum proteins, but may cross-react with immuno-globulins from other species. The conjugate has been specifically tested and qualified for Western blot and In-Cell Western Assay applications. 5. goat anti-mouse Alexa Fluor 488 secondary antibody. From supplier website: Suitable for ELISA, IHC-Fr, IHC-P, Flow Cyt, ICC/IF.

Eukaryotic cell lines

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

Cell line source(s)	Spodoptera frugiperda 9 cells (Thermo Fisher, 11496-015), Expi293F cells (Thermo Fisher, A14527), HEK293 cells (ATCC, CRL-1573)
Authentication	Not authenticated
Mycoplasma contamination	Not tested: Sf9 and Expi293F. HEK293 cells were routinely tested for mycoplasma contamination and found negative.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used in this study