

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 The electrophysiology data was collected using pCLAMP and DATAQ instruments hardware manager, CryoEM grids were prepared frozen with Vitrobot Mark IV, CryoEM data was collected using Leginon, single molecule data was collected using Labview, Gromacs 2020.2 and Plumed 2.7 were used for all molecular dynamics simulations.

Data analysis SPARTAN, pCLAMP 10, CryoSPARC 2, Chimera 1.16, Phenix 1.14, Coot 0.8.9.1, GraphPad 9, Microsoft excel V16.44, Sigmaplot, Matlab 2020, Pymol 2.3.1, Hole 2.2.005, Vmd 1.9.3, Labview, CHARMM-GUI, Gromacs 2020.2, PLUMED 2.7, PropKa 3.1,

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](#)

CryoEM maps have been deposited in the EMDB database under accession codes: 24241, 24612, 24263, 24629, 24613, 24668, 24582, 24584, 29884, 29885, 29890, 29899, 29883. Atomic coordinates have been deposited in the PDB database under accession codes: 7N8P, 7RP5, 7N0W, 7RQ7, 7RP6, 7RSB, 7RNX, 7ROO, 8GA1, 8GA3, 8GA5, 8GAH, 8GAO

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	Sample size ranged from n=6-12 for bulk assays, and >1000 for single molecule assays. The size was chosen according to the field standards. For cryoEM experiments ~6000- ~12000 micrograph movies were recorded (exact numbers are indicated in Table 1 and 2 and in the Methods section). In all cases these numbers are sufficient to yield high resolution cryoEM structures. The number of micrograph movies used in the present study is typical for cryoEM sessions using the standard acquisition scheme and set-up described in the Methods section.
Data exclusions	Exclusion criteria was pre-established for single molecule assays as explained in the methods section. The sample size of the cryoEM experiments was reduced by excluding from processing unsuitable micrographs and particles using the following criteria: micrographs with low detected fit resolution estimation and those whose CTF failed to be fitted; false positive particles (i.e. ice images, contaminants and empty micelles) were excluded using 2D classification. Exact numbers of initial and final particles acquired during processing and used for 3D reconstruction for each structural experiment are indicated in Table 1 and 2 and in the respective Supp. Figure legends.
Replication	All studies were performed with at least 3 independent biological replicates to ensure reproducibility. For each cryoEM structural experiment 4-8 grids were prepared and screened. The best grid for each condition was used for cryoEM data acquisition and structural determination.
Randomization	Randomization was not relevant to the design of cryoEM experiments. Randomization was used by analysis programs for cryoEM image processing. RELION internally splits particles in two random half sets to calculate the Fourier shell correlation for validating the resolution during 3D refinement. Randomization was not relevant to the design of the transport, smFRET, MD simulations, or single molecule photo-bleaching measurements as no grouping was involved in the experiments.
Blinding	The experimental design did not require blinding for structural and biochemical assays. In all cases a defined system was used.

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
<input checked="" type="checkbox"/>	<input 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"/> Human research participants
<input checked="" type="checkbox"/>	<input type="checkbox"/> Clinical data
<input checked="" type="checkbox"/>	<input type="checkbox"/> Dual use research of concern

Methods

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

Eukaryotic cell lines

Policy information about [cell lines](#)

Cell line source(s)	HEK293T from ATCC
Authentication	Not authenticated
Mycoplasma contamination	Not tested
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used.