

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 checked="" type="checkbox"/>	<input type="checkbox"/> A description of all covariates tested
<input checked="" type="checkbox"/>	<input 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 checked="" type="checkbox"/>	<input 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	CroEM: EPU (Thermo Fisher Scientific) Molecular Dynamics: NAMD2.13/2.14 (Freeware, University of Illinois at Urbana-Champaign); Electrophysiology: pClamp 10.6 (Molecular Devices)
Data analysis	Data processing: RELION 3.1.0 (Freeware, https://www3.mrc-lmb.cam.ac.uk/relion/index.php?title=Main_Page); CTFFIND4 4.1.14 (Freeware, https://grigoriefflab.umassmed.edu/ctffind4/); DeepEMhancer 0.13 (Freeware, https://github.com/rsanchezgarc/deepEMhancer) Model building: COOT 0.9.6 (Freeware, https://www2.mrc-lmb.cam.ac.uk/personal/pemsley/coot/); ChimeraX 1.2.5 (Freeware, https://www.rbvi.ucsf.edu/chimera/); PHENIX 1.19.1-4122 (Freeware, https://phenix-online.org/); ISOLDE 1.2.2 (Freeware, https://isolde.cimr.cam.ac.uk/static/isolde/doc/isolde.html); HOLE 2.2.005 (Freeware, http://www.holeprogram.org/) Data visualisation: Chimera 1.15 (Freeware, https://www.cgl.ucsf.edu/chimera/) Analysis and plotting of MD data: Matlab 2020a (Mathworks), Electrophysiology Data analysis: Clampfit 10.6 (Molecular Devices), Excel software (Microsoft Corporation)

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 cryo-EM maps have been deposited in the Electron Microscopy Data Bank (EMDB) under accession codes

EMDB-45597 [<https://www.ebi.ac.uk/pdbe/entry/emdb/EMD-45597>] (High-K+; C4);

EMDB-45598 [<https://www.ebi.ac.uk/pdbe/entry/emdb/EMD-45598>] (Low-K+; C4);

EMDB-45599 [<https://www.ebi.ac.uk/pdbe/entry/emdb/EMD-45599>] (High-K+; C1); and

EMDB-45600 [<https://www.ebi.ac.uk/pdbe/entry/emdb/EMD-45600>] (Low-K+; C1).

The atomic coordinates have been deposited in the Protein Data Bank (PDB) under accession codes

PDB 9CHP [<https://doi.org/10.2210/pdb9CHP/pdb>] (High-K+; C4);

PDB 9CHQ [<https://doi.org/10.2210/pdb9CHQ/pdb>] (Low-K+; C4);

PDB 9CHR [<https://doi.org/10.2210/pdb9CHR/pdb>] (High-K+; C1); and

PDB 9CHS [<https://doi.org/10.2210/pdb9CHS/pdb>] (Low-K+; C1).

Previously published PDB used in this study: 5VA1 [<http://doi.org/10.2210/pdb5VA1/pdb>]; and 5VA3 [<http://doi.org/10.2210/pdb5VA3/pdb>].

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

Reporting on race, ethnicity, or other socially relevant groupings

Population characteristics

Recruitment

Ethics oversight

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

Data exclusions

Replication

Randomization

Blinding

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 type="checkbox"/>	<input checked="" 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

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 and Sex and Gender in Research](#)

Cell line source(s)	ATCC
Authentication	No specific authentication was undertaken
Mycoplasma contamination	Cell lines are tested for mycoplasma contamination on an annual basis
Commonly misidentified lines (See ICLAC register)	N/A

Animals and other research organisms

Policy information about [studies involving animals](#); [ARRIVE guidelines](#) recommended for reporting animal research, and [Sex and Gender in Research](#)

Laboratory animals	Xenopus laevis frogs (2-7 years of age; purchased from Nasco, Fort Atkinson, WI, USA)
Wild animals	N/A
Reporting on sex	N/A
Field-collected samples	N/A
Ethics oversight	The Garvan/St Vincent's Animal Ethics Committee approved all experiments

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

Plants

Seed stocks	N/A
Novel plant genotypes	N/A
Authentication	N/A