

## Reporting Summary

Nature Research 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 Research policies, see [Authors & Referees](#) 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 ( $n$ ) 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 checked="" type="checkbox"/> | <input type="checkbox"/> The statistical test(s) used AND whether they are one- or two-sided<br><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 checked="" type="checkbox"/> | <input 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. $F$ , $t$ , $r$ ) with confidence intervals, effect sizes, degrees of freedom and $P$ value noted<br><i>Give <math>P</math> 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 $d$ , 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 | SerialEM was used to collect all of the cryo-EM data. Clampex 11.0 software (Axon Instruments) was used for collecting patch-clamp electrophysiology data. Molecular dynamics simulation was conducted using GROMACS 5.1 and CHAP.                                         |
| Data analysis   | WARP and cisTEM were used for image processing, CTF estimation, and particle extraction. Single particle analysis including 2D classification, ab-initio 3D reconstruction, 3D refinement, 3D classification, and per particle CTF refinement were performed using cisTEM. |

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/reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research [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 list of figures that have associated raw data
- A description of any restrictions on data availability

Cryo-EM maps and structural coordinates generated during this study will be deposited in the Electron Microscopy Data Bank with accession codes: ###, ###, and ###. We will have the accession codes as soon as we complete the submission.

## 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     | Cryo-EM images were collected until the resolution and 3D reconstruction converges.                                                               |
| Data exclusions | Some patch recordings were exclude when there are artifacts associated with sealing of membranes of HEK293 cells associated with health of cells. |
| Replication     | Patch clamp electrophysiology recordings were done from at least six individual patches.                                                          |
| Randomization   | N.A.                                                                                                                                              |
| Blinding        | N.A.                                                                                                                                              |

## 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 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                    |
| <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                    |

### 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 |

## Antibodies

|                 |                                                                                                                                                                                 |
|-----------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Antibodies used | 1D4 antibodies                                                                                                                                                                  |
| Validation      | 1D4 antibodies are validated by the manufacturer at the University of British Columbia. <a href="https://uilo.ubc.ca/rho-1d4-antibody">https://uilo.ubc.ca/rho-1d4-antibody</a> |

## Eukaryotic cell lines

Policy information about [cell lines](#)

|                                                                      |                                                                                                                                                  |
|----------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------|
| Cell line source(s)                                                  | HEK293, Sf9                                                                                                                                      |
| Authentication                                                       | All of the cell-lines used at Cold Spring Harbor Laboratory (CSHL) are going through the "Cell Authentication Service" at University of Arizona. |
| Mycoplasma contamination                                             | The above celllines are tested for potential contamination with micoplasm.                                                                       |
| Commonly misidentified lines<br>(See <a href="#">ICLAC</a> register) | N.A.                                                                                                                                             |