

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 P 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	PatchMaster v2x53; Rosetta version 2016.20; Meta Image Series 7.8; Titan Krios microscope (FEI) , K2 Summit direct electron detector (Gatan)
Data analysis	Igor Pro 5.05; UCSF Chimera version 1.1219; PyMOL version 2.6.0; Coot version 0.8.9.2; cryoSPARC version 3.2.0; Phenix version 1.15.2-3472

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](#)

Structure coordinates and cryo-EM density maps have been deposited in the Protein Data Bank under accession numbers: 8JEV and EMD-36200 for mCIC-3_apo, 8JGJ and EMD-36236 for mCIC-3_ATP, 8JGK and EMD-36237 for mCIC-3_ADP, 8JGL and EMD-36238 for mCIC-3_AMP, 8JGS and EMD-36245 for mCIC-3_T607T_apo, 8JGV and EMD-36246 for mCIC-3_I607T_ATP. Source data are provided with this paper.

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

This finding has nothing to do with gender.

Reporting on race, ethnicity, or other socially relevant groupings

This finding has nothing to do with race, ethnicity, or other socially relevant groupings.

Population characteristics

This finding has nothing to do with population characteristics.

Recruitment

This finding has nothing to do with recruitment.

Ethics oversight

This finding has nothing to do with any organization.

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

Experiments were performed at least three independent times. Sample size in all experiments were estimated based in previous published experiments. In this study, the statistic analysis was obtained using student t test and the values represents means plus minus standard error of the mean. The data size for cryo-EM was determined by the availability of the microscope time and the particle density on the grids. The sample size for patch-clamp recording, which was 4-8. The sample sizes for other assays were also determined based on the reproducibility of experiments, which were usually more than 3 .

Data exclusions

No data were excluded.

Replication

Since experiments were performed at least 4 independent times, all experimental findings were reproducible.

Randomization

Randomly selected samples and organisms were allocated into experimental groups.

Blinding

Investigators were not blinded to group allocation, because no grouping was needed for this study.

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

Anti-GFP, Proteintech, 66002-1-Ig; Anti-GAPDH, Abcam, ab9485; Goat Anti-Rabbit IgG H&L (HRP), Abcam, ab6721

Eukaryotic cell lines

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

Cell line source(s)

HEK293S (ATCC, #CRL-3022); HEK293T (ATCC, #CRL-3216).

Authentication

No further authentications were performed for this study.

Mycoplasma contamination

No mycoplasma contamination tests were performed for this study.

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

None of the cell lines used are listed in the ICLAC database.