

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	EPU (Thermo Fisher Scientific), an iBright FL1500 Imaging System (Thermo Fisher Scientific), Unicorn V7.1
Data analysis	CryoSPARC v3.3, Coot v 0.9.4 , Phenix v 1.19.2-4158 USCF Chimera , USCF Chimera X, Pymolv1.8.2.0, Adobe Illustrator, Geneious prime (v2022.2), ImageJ (v1.53k), Clustal Omega, Jalview, GraphpadPrism

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 reconstructed maps are available from the EMDB database under access codes EMDB-19057, EMD-19061, EMD-19069, EMD-19060, EMDB-19072, EMD-19073 and EMD-19074. The atomic models are available in the PDB database, access codes PDB-ID 8RCR, PDB-8RCU, PDB-8RD9. micrograph images are deposited as accession codes EMPIAR-12492, EMPIAR-12483, EMPIAR-12491.

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 n/a

Reporting on race, ethnicity, or other socially relevant groupings n/a

Population characteristics n/a

Recruitment n/a

Ethics oversight n/a

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

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size Sample size for Cryo-EM data was determined by collecting data in the form of movies containing particle images to obtain a sufficient number of particles images that would provide high resolution 3D reconstruction following refinement based on the FSC threshold of 0.143.

Data exclusions For electrophysiology experiments no data was excluded. For cryoEM Images suffering image drift, ice contamination, and/or cubic ice format were excluded during image processing. Particles in 2D classes showing no secondary structural features and in 3D classes showing unsatisfactory structural features were excluded from the final reconstructions in all datasets analyzed.

Replication Electrophysiology experiments were carried out in at least 6 replicates

Randomization No randomization was involved as this study did not involve use of sample collection from different experimental groups involving participants. No multi-group comparison has been performed in this study. Western blots were done in triplicate, and at least 6 cells were used for different doses in electrophysiology experiments to obtain data for relevant statistical analysis

Blinding Blinding is not applicable as this study did not involve sampling different experimental groups involving participants. We used at least 6 cells for each experiment of the same commercial cell line therefore randomization is not applicable in this case

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

Involvement 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

Involvement in the study

☒ ☐ ChIP-seq

☒ ☐ Flow cytometry

☒ ☐ MRI-based neuroimaging

Antibodies

Antibodies used	rabbit anti-human TRPM4 antibody (epitope: 1137CRDKRESDSERLKRTSQKV1155, Pineda, Berlin, Germany) and mouse anti-Na ⁺ /K ⁺ ATPase antibody (Abcam ab 7671)
Validation	validation was carried out by western blot experiment as detailed on the manufacturer details of the product and confirmed in our hands

Eukaryotic cell lines

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

Cell line source(s)	TsA-201 cells from ECACC ref 96121229
Authentication	Commercial cell line was not further authenticated
Mycoplasma contamination	Yes
Commonly misidentified lines (See ICLAC register)	n/a

Plants

Seed stocks	n/a
Novel plant genotypes	n/a
Authentication	n/a