

Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure 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
<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. F , t , r) with confidence intervals, effect sizes, degrees of freedom and P 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 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 EPU 2 (ThermoFischer Scientific), Axon 700B amplifier, Digidata 1440A and Clampex 10.4 (Molecular Devices)

Data analysis RELION 3.1.2, cryosparc V3.3, ChimeraX 1.3, Phenix 1.16, COOT 0.8.9.2, MolProbity 4.02, HOLE 2.2, Clampex 10.4, GraphPad prism 8.0

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 map of hPANX2 has been deposited to Electron Microscopy Data Bank with accession code EMD-28902 [<https://www.ebi.ac.uk/pdbe/entry/emdb/EMD-28902>]. Atomic coordinates for hPANX2 have been deposited to the Protein Data Bank (PDB) with accession code 8F7C [<https://www.rcsb.org/structure/8F7C>]. Previously published PDB codes that are referred to include 6UZY [<https://www.rcsb.org/structure/6UZY>] and 6NZW [<https://www.rcsb.org/structure/6NZW>]. The source data underlying Fig. 1c, 5c, and 5f, and Supplementary Fig. 4b, 4c, 5, and 6 are provided as a Source Data file.

Human research participants

Policy information about [studies involving human research participants and Sex and Gender in Research.](#)

Reporting on sex and gender

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](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	Electrophysiological recordings were repeated at least five times. The sample size is determined based on data consistency indicated by standard errors of the mean. The number of cryo-EM images was determined by available microscope time.
Data exclusions	For cryo-EM reconstructions of the hPANX2 channel, 123, 155 particles were included for 3D classification. Three of the four classes resulted in poor transmembrane density and were discarded (97,964). 25,191 particles were used in the final reconstruction.
Replication	Electrophysiological experiments were duplicated. Results are reproducible. Cryo-EM experiments were reproducible with independent datasets.
Randomization	In cryo-EM experiments, particles for reconstruction were automatically picked by the software. For electrophysiology, cells were randomly selected (each patched only once).
Blinding	The investigators were blinded to group allocation during data collection and analysis.

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

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Eukaryotic cell lines

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

Cell line source(s)	Pichia pastoris (Invitrogen,C17500), HEK293T (Thermo Fisher Scientific,R79007).
Authentication	The cell lines were not authenticated.
Mycoplasma contamination	Cell lines were not tested for mycoplasma contamination.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used in the study.