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Corresponding author(s): Alexander Sobolevsky

Last updated by author(s): May 25, 2021

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 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 *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☒	☐ 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 *Give P values as exact values whenever suitable.*
☒	☐ 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	Leginon 3.5, SerialEM 3.8, EPU 2
Data analysis	Leginon 3.5, SerialEM 3.8, EPU 2, MotionCor2, gCTF 1.06, gCTF 1.18, RELION 3.1, CryoSPARC 2.14, UCSF Chimera 1.14, UCSF ChimeraX 0.9, COOT 0.9.2, PHENIX 1.18, Pymol 2.4.0, pCLAMP 10.2, pCLAMP 10.3, Clampfit 10.3, GROMACS 2020.4, Origin 9.0

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 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

All data needed to evaluate the conclusions of the paper are present in the paper and/or the Supplementary Materials. Cryo-EM density maps have been deposited to the Electron Microscopy Data Bank (EMDB) under the accession codes EMD-23853 (mTRPV3 closed, 4 °C, MSP2N2), EMD-23854 (mTRPV3 closed, 42 °C, MSP2N2), EMD-23855 (mTRPV3 sensitized, 42 °C, MSP2N2), EMD-23856 (mTRPV3 closed, 4 °C, cNW11), EMD-23857 (mTRPV3 closed, 42 °C, cNW11), and EMD-23858 (mTRPV3 open, 42 °C, cNW11) (see Extended Data Table 1). The corresponding model coordinates have been deposited to the Protein Data Bank (PDB) under accession codes 7MIJ (mTRPV3 closed, 4 °C, MSP2N2), 7MIK (mTRPV3 closed, 42 °C, MSP2N2), 7MIL (mTRPV3 sensitized, 42 °C, MSP2N2), 7MIM (mTRPV3 closed, 4 °C, cNW11), 7MIN (mTRPV3 closed, 42 °C, cNW11), and 7MIO (mTRPV3 open, 42 °C, cNW11) (see Extended Data Table 1). Structured under the PDB accession codes 6PVN and 6PVP were used as initial models for refinements.

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	Electrophysiological data sample sizes were chosen to maximize statistical significance, taking into consideration experimental setup, our own experience and published literature. Sample sizes were chosen in accordance with our previous study: Singh, A. K. et al. Structural basis of temperature sensation by the TRP channel TRPV3. Nat Struct Mol Biol 26, 994-998, doi:10.1038/s41594-019-0318-7 (2019).
Data exclusions	No data have been excluded.
Replication	All electrophysiological experiments were replicated on a different day, using a different cell line transfection. All replication attempts were successful. n = 15; n = 19.
Randomization	Cells for electrophysiological experiment were picked at random. The sequence of measurements from experimental and control groups were chosen at random.
Blinding	For electropysiological experiments, pseudonymized sample codes were used instead of plasmid names.

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
☒	☐ Human research participants
☒	☐ 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

Cell line source(s)	HEK293S GnTI-, ATCC, Cat#CRL-3022 HEK293T, Millipore Sigma, 12022001 Sf9, Gibco, Cat#12659017
Authentication	None of the cell lines used have been authenticated
Mycoplasma contamination	The cell lines used have been tested for mycoplasma contamination by the providers (negative results) but have not been retested in the lab
Commonly misidentified lines (See ICLAC register)	No commonly misidentified lines were used in this study