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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

- | | | |
|-------------------------------------|-------------------------------------|--|
| <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 type="checkbox"/> | <input checked="" type="checkbox"/> | 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> |
| <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 type="checkbox"/> | <input checked="" 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 type="checkbox"/> | <input checked="" 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 <i>Give P 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

Data analysis

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 data that support this study are available from the corresponding authors upon request. The 3D cryo-EM density maps have been deposited in the Electron Microscopy Data Bank under the accession numbers EMD-48139 [<https://www.ebi.ac.uk/pdbe/entry/emdb/EMD-48139>] (apo TRPML2 closed); EMD-48140 [<https://www.ebi.ac.uk/pdbe/entry/emdb/EMD-48140>] ((-)ML-SI3-bound TRPML2 closed); EMD-48141 [<https://www.ebi.ac.uk/pdbe/entry/emdb/EMD-48141>] ((+)ML-SI3-bound TRPML2 pre-open); EMD-48142 [<https://www.ebi.ac.uk/pdbe/entry/emdb/EMD-48142>] ((+)ML-SI3-bound TRPML2 open); EMD-48143 [<https://www.ebi.ac.uk/pdbe/entry/emdb/EMD-48143>]

www.ebi.ac.uk/pdbe/entry/emdb/EMD-48143] (ML2-SA1-bound TRPML2 pre-open); EMD-48144 [https://www.ebi.ac.uk/pdbe/entry/emdb/EMD-48144] (ML2-SA1/PI(3,5)P2-bound TRPML2 open); EMD-48135 [https://www.ebi.ac.uk/pdbe/entry/emdb/EMD-48135] (ML2-SA1-bound TRPML1VA/AG partially open); EMD-48136 [https://www.ebi.ac.uk/pdbe/entry/emdb/EMD-48136] ((+)ML-SI3-bound TRPML1VA/AG partially open); EMD-48137 [https://www.ebi.ac.uk/pdbe/entry/emdb/EMD-48137] ((+)ML-SI3-bound TRPML1VA/AG pre-open); and EMD-48138 [https://www.ebi.ac.uk/pdbe/entry/emdb/EMD-48138] (ML-SA5-bound TRPML1WT open). Atomic coordinates for the atomic model have been deposited in the Protein Data Bank under the accession number 9EKW [https://doi.org/10.2210/pdb9EKW/pdb] (apo-TRPML2 closed); 9EKX [https://doi.org/10.2210/pdb9EKX/pdb] ((-)ML-SI3-bound TRPML2 closed); 9EKY [https://doi.org/10.2210/pdb9EKY/pdb] ((+)ML-SI3-bound TRPML2 pre-open); 9EKZ [https://doi.org/10.2210/pdb9EKZ/pdb] ((+)ML-SI3-bound TRPML2 open); 9ELO [https://doi.org/10.2210/pdb9ELO/pdb] (ML2-SA1-bound TRPML2 pre-open); 9EL1 [https://doi.org/10.2210/pdb9EL1/pdb] (ML2-SA1/PI(3,5)P2-bound TRPML2 open); 9EKS [https://doi.org/10.2210/pdb9EKS/pdb] (ML2-SA1-bound TRPML1VA/AG partially open); 9EKT [https://doi.org/10.2210/pdb9EKT/pdb] ((+)ML-SI3-bound TRPML1VA/AG partially open); 9EKU [https://doi.org/10.2210/pdb9EKU/pdb] ((+)ML-SI3-bound TRPML1VA/AG pre-open); and 9EKV [https://doi.org/10.2210/pdb9EKV/pdb] (ML-SA5-bound TRPML1WT open). Initial/final PDB states for MD simulations are provided as Source data. The source data underlying Figs 4a-c, 5c, and Supplementary Fig. 8 are provided with this paper. Previously published structures referred to in this paper include: 5WJ5 [http://doi.org/10.2210/pdb5WJ5/pdb], 7DYS [http://doi.org/10.2210/pdb7DYS/pdb], 6AYE [http://doi.org/10.2210/pdb6AYE/pdb], 5T4D [http://doi.org/10.2210/pdb5T4D/pdb], 5WJ9 [http://doi.org/10.2210/pdb5WJ9/pdb], 6MHO [http://doi.org/10.2210/pdb6MHO/pdb], 6DVZ [http://doi.org/10.2210/pdb6DVZ/pdb], and 6D1W [http://doi.org/10.2210/pdb6D1W/pdb]. 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 | 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](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 | No sample size calculation was performed. The data size for cryo-EM was determined by the availability of the microscope time and the particle density on the grids. Sufficient cryo-EM data were collected to achieve the reported resolution of map, which is sufficient for model building. At least three independent experiments (n=3) were carried out for all the functional assays as indicated in the figure legends. Data were analyzed using appropriate equations in GraphPad Prism 10.2.3. |
| Data exclusions | Cryo-EM data processing involved removing poor-quality or damaged particles to achieve high resolution maps through pre-established standard data classification procedures. |
| Replication | Each experiment was reproduced at least three times on separate occasions. Experimental findings were reliably reproduced. |
| Randomization | Randomization was not necessary as the independent variables to be tested were sufficient for the functional interpretations within this study. i.e. WT vs mutant vs control conditions or dose response determination. |
| Blinding | Blinding is not necessary or valid for the purposes of structural determination. For functional analysis, blinding was not necessary due to the quantitative nature of the experiment. All experimental data acquired in included in our statistical 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 |
| <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 and archaeology |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Animals and other organisms |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Clinical data |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Dual use research of concern |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Plants |

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

anti-DDDDK-tag mAb, MBL, M185-3L, 1:3000 dilution
 anti-GFP rabbit mAb, Cell Signaling Technology, 2956S, 1:1000 dilution
 anti-Beta Actin mouse mAb (8H10D10), Cell Signaling Technology, 3700S, 1:1000 dilution
 Anti-rabbit IgG HRP-linked antibody, Cell Signaling Technology, 7074S, 1:2000 dilution
 Anti-mouse IgG HRP-linked antibody, Cell Signaling Technology, 7076S, 1:2000 dilution

Validation

Antibodies have been used and validated by the manufacturer and in other publications prior to use.

Eukaryotic cell lines

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

Cell line source(s)

HEK-293S GnTI⁻ (ATCC no. CRL-3022), HEK293 (ATCC)

Authentication

No further authentication was performed for commercially available cell lines.

Mycoplasma contamination

periodically test negative

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

None of the cell lines used is listed in the database of commonly misidentified cell lines maintained by ICLAC.

Plants

Seed stocks

Report on the source of all seed stocks or other plant material used. If applicable, state the seed stock centre and catalogue number. If plant specimens were collected from the field, describe the collection location, date and sampling procedures.

Novel plant genotypes

Describe the methods by which all novel plant genotypes were produced. This includes those generated by transgenic approaches, gene editing, chemical/radiation-based mutagenesis and hybridization. For transgenic lines, describe the transformation method, the number of independent lines analyzed and the generation upon which experiments were performed. For gene-edited lines, describe the editor used, the endogenous sequence targeted for editing, the targeting guide RNA sequence (if applicable) and how the editor was applied.

Authentication

Describe any authentication procedures for each seed stock used or novel genotype generated. Describe any experiments used to assess the effect of a mutation and, where applicable, how potential secondary effects (e.g. second site T-DNA insertions, mosaicism, off-target gene editing) were examined.