

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 (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 Serial EM

Data analysis GraphPad Prism 6.0, Gctf software, RELION 2.01b1, USCF Chimera 1.14, PyMOL Molecular Graphics System 2.4.1, PHENIX 1.19.2, COOT 0.8.9.2, Motioncor2, MolProbity, Gautomatch, EMAN2, CCPEM, Refmac, AutoDockTools package and Autodock4.2.6, VMD, CHARMM ParamChem web server, NAMD2.12, AMBER18, CPPTRAJ, PyReweight toolkit, Axograph X, version 1.7.4

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](#)

All relevant data are available from the authors and/or included in the manuscript or Supplementary Information.

The electron density map has been deposited to EMD (Accession code EMD-23280 (MIPS521-ADO-A1R-Gi2) & EMD-23281(ADO-A1R-Gi2))
The models have been deposited in the PDB (Accession code 7LD3 (MIPS521-ADO-A1R-Gi2) & 7LD4(ADO-A1R-Gi2)).
Raw gel images used for Extended Data Figure 3b & 3c have been provided as Supplementary Figure 1.

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	Number of technical replicates and biological replicates are reported in the figure legends. Sample size was determined based on variability of the response deviating from the mean as indicated by the standard error of the mean (SEM), which is also represented in the figures. Group sizes were based on previous similar studies by the investigators.
Data exclusions	Behavioural studies: Intrathecal catheter patency and placement was confirmed after each experiment by administration of intrathecal lignocaine (20 ul, 1%) which causes brief bilateral hind limb paralysis if the catheter is functional. Rats were excluded if complete hind limb paralysis was not achieved (n=8 animals were excluded, n=168 animals included). For conditioned place preference experiments, rats that spent more than 80% of their time in one chamber during the pre-conditioning recording were eliminated from the study (n=5 animals were excluded, n=48 animals included). Electrophysiology: Neurons with unstable baseline measurements (that had not been recognised by the experimenter during recording), were excluded. This included neurons that had post-synaptic current amplitudes that steadily decreased or increased over the 10-15 minute baseline recording period.
Replication	All data was replicable, with the number of replicates provided in text and/ or figure legends. Behavioural experiments: Experiments were repeated multiple times on different days by two different investigators. All attempts at replication were successful. Electrophysiology experiments: Experiments were repeated multiple times on different days using rats that had undergone surgery by two different investigators. All attempts at replication were successful. Atrial beat rate experiments: Experiments were repeated multiple times on different days. All attempts at replication were successful.
Randomization	Behaviour and electrophysiology experiments: Adult rats were randomly assigned to treatment groups. Test compounds and concentrations were randomly assigned over multiple testing days. Atrial beat rate experiments: Adult rat atria were randomly assigned to treatment groups Other experiments: Samples were not randomized for MD, pharmacology or structural biology experiments as this was not applicable for the experiments described.
Blinding	Behavioural studies: The test compound (or vehicle) and dose of compound were blinded to the experimenter. Electrophysiology experiments: Rats with neuropathic pain and controls were blinded to the experimenter. Other experiments: no blinding was applied to the MD, pharmacology, or structural biology data generated in our study, as this is not applicable to the types of biochemical or structural data generated.

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

Antibodies

Antibodies used	Mouse anti-His antibody (cat. no. 564 34660, QIAGEN) 1:5000 Goat anti-mouse Alex647 (cat. no. A-21235, ThermoScientific) 1:1000 AlexaFluor®488 conjugated Gai2 mouse monoclonal antibody (cat. no. sc13534, Santa 567 Cruz) 1:1000
Validation	All antibodies have been commercially obtained & validated, and are described as required in Methods. Mouse anti-His antibody (cat. no. 564 34660, QIAGEN) has been validated for Western blot, with specificity demonstrated by detection of different targets with a His tag. Further information available at https://www.qiagen.com/us/products/discovery-and-translational-research/protein-purification/tagged-protein-expression-purification-detection/anti-his-antibodies-bsa-free/ Goat anti-mouse Alex647 (cat. no. A-21235, ThermoScientific) has been validated for Western blot by the manufacturer. Further information available at https://www.thermofisher.com/antibody/product/Goat-anti-Mouse-IgG-H-L-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-21235 AlexaFluor®488 conjugated Gai2 mouse monoclonal antibody (cat. no. sc13534, Santa 567 Cruz) has been validated for Western blot, with specificity demonstrated for Gai2. Further information is available at https://www.scbt.com/p/galpha-i-2-antibody-l5

Eukaryotic cell lines

Policy information about [cell lines](#)

Cell line source(s)	All cell lines were originally obtained from Invitrogen or Expression Systems. Flp-In™-CHO Cell Line (R75807; Invitrogen) Sf9 Insect Cells (Expression Systems) Tni Insect Cells (Expression Systems)
Authentication	None of the cell line have been authenticated.
Mycoplasma contamination	All cell lines tested negative for mycoplasma contamination
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used.

Animals and other organisms

Policy information about [studies involving animals](#); [ARRIVE guidelines](#) recommended for reporting animal research

Laboratory animals	Neuropathic pain model: Male and female Sprague-Dawley rats, 7-12 weeks old Intrathecal drug administration and in vivo pain behavioural assays: Male and female Sprague-Dawley rats, 9-16 weeks Rotarod: Male and female Sprague Dawley rats (n=35, groups of 3-4), 7-9 weeks of age Spinal cord slice electrophysiology: Male and female rats Sprague-Dawley (9-15 weeks) Atrial beat rate experiments: Sprague-Dawley male rats (7-8 weeks)
Wild animals	The study did not involve wild animals
Field-collected samples	The study did not contain field-collected samples
Ethics oversight	All experiments involving animals were approved by the Monash University Animal Ethics Committee and every precaution was taken to prevent animal suffering during these experiments.

Note that full information on the approval of the study protocol must also be provided in the manuscript.