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Corresponding author(s): Qin Liu

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 Authors & Referees and the Editorial Policy Checklist.

Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. 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
☐	☒	An indication of 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 statistics 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
☐	☒	Clearly defined error bars *State explicitly what error bars represent (e.g. SD, SE, CI)*

Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

Data collection	Nikon NIS Elements AR software for calcium imaging data collection pCLAMP 10.5 software (Axon Instruments, U.S.) and Patchmaster software (v2; Heka Electronic) for electrophysiology data collection
Data analysis	Nikon NIS Elements AR software for calcium imaging data analysis pCLAMP 10.5 software (Axon Instruments, U.S.) and Patchmaster software (v2; Heka Electronic) for electrophysiology data analysis GraphPad Prism5 for statistical 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/reviewers upon request. 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 generated or analysed during this study are included in this published article (and its supplementary information files).

Field-specific reporting

Please select the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences

For a reference copy of the document with all sections, see nature.com/authors/policies/ReportingSummary-flat.pdf

Life sciences

Study design

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

Sample size	The criteria for determining the number of animals used in itch and pain behavioral assays is based on the "Sample size determination" (Dell et al. 2002, ILAR J, 43(4), 207–13), and analysis of recently published papers that are relevant to our study (reference 56-58 for online method). Three factors are important to calculate sample size (Dell et al, 2002). 1. the size of the effect under study (difference between experimental groups) 2. the desired power of the experiment to detect the effect (usually 80-90%) 3. the significance level (we chose 0 .05). The animals used in our current study were all age-matched, congenic C57BL/6 inbred male mice. Animals with the same genotype are genetically identical, while the only genetic differences between genotypes are at the indicated alleles. Furthermore, all practically feasible care was observed by our researchers and animal care technicians to ensure that these mice received identical upbringing until our experiment. Hence, individual variation between animals with the same genotype is minimal. For statistical comparison of two genotypes, there is much less variability in the results and our sample sizes are sufficient.
Data exclusions	No animal or data point was excluded from analysis
Replication	All histology, calcium imaging, and electrophysiology experiments were repeated using tissues from at least 3 different mice. All attempts at replication were successful.
Randomization	Animals were placed into experimental groups based either on their genotype (no randomization) or through simple randomization.
Blinding	Itch and pain behaviors were scored by researchers blinded to mouse genotypes or treatment condition.

Materials & experimental systems

Policy information about availability of materials

n/a	Involved in the study
☒	☐ Unique materials
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☐	☒ Research animals
☒	☐ Human research participants

Antibodies

Antibodies used	Anti-GFP antibody (A11122, Lot#1925070; used at 1:1000 dilution), and Alexa Fluor® 488-conjugated goat anti-rabbit antibody (A11008, Lot#1797971; used at 1:1000 dilution), FITC-conjugated avidin (434411, Lot#1561410A; used at 1:1000 dilution), and Rhodamine-conjugated avidin (A003-00, Lot#2496; used at 1:400 dilution) were purchased from Thermo Scientific (Asheville, NC). Chicken anti-GFP (GFP-1020, Lot#GFP697986; used at 1:1000 dilution) was purchased from Aves Lab (Tigard, Oregon). Anti-DTR antibody (AF259NA, Lot#PX0911111; used at 1:200 dilution) was purchased from R&D Systems (Minneapolis, MN). Anti-CGRP antibody (T-4239, Lot#040269-6; used at 1:1000 dilution) was purchased from Peninsula Laboratories International, Inc. (San Carlos, CA). Anti-hMrgprX1 antibody (used at 1:1000 dilution) was generated by Liang Han in Dr. Xinzhong Dong's lab at the Johns Hopkins School of Medicine in Baltimore, MO. Cy5-conjugated donkey anti-goat antibody (705175147, Lot#131485; used at 1:500 dilution) was purchased from Jackson ImmunoResearch Laboratories, Inc. (West Grove, PA).

Validation	Anti-MrgprX1 antibody was validated in this study using humanized hMrgprX1 transgenic mice in which human MrgprX1 replaced mouse Mrgprs in primary sensory neurons. Validation data for other antibodies are available from the commercial providers.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	KNRK cells obtained from ATCC
Authentication	The authentication data are available from ATCC
Mycoplasma contamination	The cell line was not tested for mycoplasma contamination in the authors' laboratory.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used

Research animals

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Animals/animal-derived materials	C57BL/6J wild-type (Stock#: 000664), B6;129S6-Gt(ROSA)26Sortm14(CAG-tdTomato)Hze/J (ROSA26tdTomato; Stock#:007908), C57BL/6-Gt(ROSA)26Sortm1(HBEGF)Awai/J (ROSA26DTR; Stock#:007900), B6N.Cg-Ssttm2.1(cre)Zjh/J (Stock#: 018973) and B6N(Cg)-Nmbtm1.1(KOMP)Vlcg/J mice (025862) were ordered form the Jackson Laboratory (Bar Harbor, ME). MrgprA3EGFP-Cre, PirtGCaMP3/+ and hMrgprX1;Mrgpr-cluster-/- mice were generous gifts from Dr. Xingzhong Dong of Johns Hopkins University. MrgprDEGFP/+ mice were from Dr. David J. Anderson of the California Institute of Technology. TRPM8GFP/+ mice were from Dr. Gina Story. Nav1.8Cre, NMB-/-, NMBR-/-, and NMBReGFP transgenic mice were from Dr. Zhou-Feng Chen of Washington University in St. Louis. VGLUT3Cre/+ tissues were from Dr. Qiufu Ma of Dana-Farber Cancer Institute. Animals used for behavioral tests were backcrossed to the C57BL/6J background for at least 10 generations and maintained in the congenic background. Male mice (two to three months old) were used for behavioral tests. All animal experiments were performed under protocols approved by the Animal Care and Use Committee of Washington University School of Medicine.

Method-specific reporting

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ Magnetic resonance imaging