nature portfolio

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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 *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	EPU 3.3, pCLAMP ClampEx 10.7, OPUS, GageCon, PatchControl384 v. 2.3.0
Data analysis	CryoSPARC 4.4, Coot 0.9.8.9, Phenix 1.20.1, UCSF ChimeraX 1.74, PyMOL 2.5.5, APBS 3.4.1, HOLLOW 1.3, CAVER Analyst 2.0 Beta, Bridge, C-Graphs, pCLAMP ClampFit 10.7, Origin Pro 2019, PPM 3.0, GageCon, GROMACS (v. 2023.3), DataControl384 software v. 2.3.0

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 maps have been deposited in the Electron Microscopy Data Bank (EMDB) under accession codes EMD-45467 [https://www.ebi.ac.uk/emdb/EMD-45467] (HcKCR1_C110A dark-adapted state), EMD-45468 [https://www.ebi.ac.uk/emdb/EMD-45468 (HcKCR1_C110A laser-flash-illuminated state), and EMD-45469 [https://www.ebi.ac.uk/emdb/EMD-45469] (HcKCR1_C110A continuous-light-illuminated state). The coordinates have been deposited in the RCSB

Protein Data Bank (PDB) under accession codes 9CDC [http://doi.org/10.2210/pdb9cdc/pdb] (HcKCR1_C110A dark-adapted state), 9CDD [http://doi.org/10.2210/pdb9cdd/pdb] (HcKCR1_C110A laser-flash-illuminated state), and 9CDE [http://doi.org/10.2210/pdb9cde/pdb] (HcKCR1_C110A continuous-light-illuminated state). The patch clamp data generated in this study are provided in the Source Data file.

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	Not applicable, as no human research participants were used in this study.
Reporting on race, ethnicity, or other socially relevant groupings	Not applicable, as no human research participants were used in this study.
Population characteristics	Not applicable, as no human research participants were used in this study.
Recruitment	Not applicable, as no human research participants were used in this study.
Ethics oversight	Not applicable, as no human research participants were used in this study.

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

Life sciences study design

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

Sample size	No statistical methods were used to pre-determine sample sizes, but our sample sizes were as large as practical, given the technical demands of the experiments, and similar to those reported in the previous publication [Morizumi et al. Nature Communications (2023) 14:4365].
Data exclusions	For measurements of the membrane (seal) resistance in the dark in the C110 mutants, wells with photocurrents <20 pA at 0 mV (i.e., those that captured non-expressing cells) were excluded from the analysis. For an unbiased estimation of the photocurrent amplitude, the data from wells that formed seals with a resistance <500 MΩ were excluded. For a more accurate estimation of the Vr values by plotting the IV curves, wells with a seal resistance <500 MΩ and photocurrents of the absolute magnitude <20 pA at -100 mV were excluded. To estimate the decay τ values, wells with a seal resistance <500 MΩ and photocurrents of the absolute magnitude <100 pA at -100 mV were excluded. In manual patch clamp experiments, the cells were selected for patching by inspecting their tag fluorescence; non-fluorescent cells and cells in which no GΩ seal was established or lost during recording were excluded from the analysis.
Replication	In automated and manual patch clamp experiments, the photocurrent traces recorded from different cells transfected with the same construct were considered biological replicates (reported as n values). These values indicate how often the experiments were performed independently.
Randomization	Plasmids encoding different channel variants were randomly assigned to transfect identical cell batches. Each mutant was tested upon at least two independent transfections on different experimental days, and the data obtained were pooled together. In automated patch clamp studies, cells were randomly drawn into the wells. Cells transfected with the wild-type HcKCR1 were included in each experiment as a control.
Blinding	Data collection was not performed blind to the conditions of the experiments, because this was an observational study aimed at characterization of HcKCR1 mutants. Analysis of automated patch clamping data was performed automatically and identically over individual samples using DataControl software and hence was blinded.

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
☒	☐ Plants

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)	HEK293 cells were obtained from the American Type Culture Collection (ATCC; catalog #CRL-1573).
Authentication	The HEK293 cell line was authenticated by STR profiling at ATCC.
Mycoplasma contamination	The HEK293 cell line was tested negative for micoplasma contamination by a PCR test.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified lines were used in this study.

Plants

Seed stocks	Not applicable
Novel plant genotypes	Not applicable
Authentication	Not applicable