

Life Sciences Reporting Summary

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For further information on the points included in this form, see [Reporting Life Sciences Research](#). For further information on Nature Research policies, including our [data availability policy](#), see [Authors & Referees](#) and the [Editorial Policy Checklist](#).

► Experimental design

1. Sample size

Describe how sample size was determined.

For most functional studies, the sample sizes was >5 to ensure adequate power.

2. Data exclusions

Describe any data exclusions.

No data were excluded from the analysis.

3. Replication

Describe whether the experimental findings were reliably reproduced.

We did to replicate the structural experiments, which is standard practice. The electrophysiological experiments were readily and reliably reproduced.

4. Randomization

Describe how samples/organisms/participants were allocated into experimental groups.

Randomization is not relevant to the structural experiments. For electrophysiological recordings, experiments with different conditions (e.g., WT vs. mutant channels) were performed in an alternating order on the same day.

5. Blinding

Describe whether the investigators were blinded to group allocation during data collection and/or analysis.

Blinding is not relevant to the structural experiments. Blinding was not done for the electrophysiological experiments because we needed to design specific protocols for each experiment and to record from different types of channels (i.e., WT vs. mutant) in an alternating fashion.

Note: all studies involving animals and/or human research participants must disclose whether blinding and randomization were used.

6. Statistical parameters

For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

- | n/a | Confirmed |
|-------------------------------------|--|
| <input type="checkbox"/> | <input checked="" type="checkbox"/> The <u>exact sample size</u> (n) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.) |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> A statement indicating how many times each experiment was replicated |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> The statistical test(s) used and whether they are one- or two-sided (note: only common tests should be described solely by name; more complex techniques should be described in the Methods section) |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> A description of any assumptions or corrections, such as an adjustment for multiple comparisons |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> The test results (e.g. P values) given as exact values whenever possible and with confidence intervals noted |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> A clear description of statistics including <u>central tendency</u> (e.g. median, mean) and <u>variation</u> (e.g. standard deviation, interquartile range) |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> Clearly defined error bars |

See the web collection on [statistics for biologists](#) for further resources and guidance.

► Software

Policy information about [availability of computer code](#)

7. Software

Describe the software used to analyze the data in this study.

pCLAMP 8.2, MotionCorr2, CTFFIND3, RELION 1.4, ResMap, Coot, USCF chimera, Phenix, MolProbity, PyMOL, HOLE, and THUNDER (developed by Mingxu Hu, Hongkun Yu and colleagues at Xueming Li Lab)

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). *Nature Methods* [guidance for providing algorithms and software for publication](#) provides further information on this topic.

► Materials and reagents

Policy information about [availability of materials](#)

8. Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a for-profit company.

All materials are available from the authors or commercial sources described in the paper.

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).

N/A

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.

HEK 293T from American Type Culture Collection (ATCC), Sf9 cells were from Invitrogen, and Tni cells were from Expression Systems.

b. Describe the method of cell line authentication used.

None of the cell lines used have been authenticated.

c. Report whether the cell lines were tested for mycoplasma contamination.

The cells lines were not tested for mycoplasma contamination.

d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by [ICLAC](#), provide a scientific rationale for their use.

N/A

► Animals and human research participants

Policy information about [studies involving animals](#); when reporting animal research, follow the [ARRIVE guidelines](#)

11. Description of research animals

Provide details on animals and/or animal-derived materials used in the study.

No animals were used.

Policy information about [studies involving human research participants](#)

12. Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants.

This study did not involve human subjects.