

Life Sciences Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form is intended for publication with all accepted life science papers and provides structure for consistency and transparency in reporting. Every life science submission will use this form; some list items might not apply to an individual manuscript, but all fields must be completed for clarity.

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.

Conventional, power-based sample size estimation for two-tailed Student's t-test (SigmaPlot 13.0; Systat Software Inc.) confirmed our experience from previous studies, suggesting a sample size of 5 – 10 (measurements in individual cells) as sufficient to detect relevant differences in our experimental settings at α of 0.05 and a power of 0.8.

2. Data exclusions

Describe any data exclusions.

Current recordings displaying initial leak currents were excluded in patch clamp experiments based on the observation of a time and intervention-independent linear noisy current-voltage relationship.

3. Replication

Describe whether the experimental findings were reliably reproduced.

All live cell experiments were repeated and results were reproduced on several days of experimentation, with separate cell passages and transfections.

4. Randomization

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

Cells expressing either wild-type or mutant channels were selected based on the fluorescence of heterologously expressed ion-channels. Channel constructs were expressed alternating in the available passages of host cells (HEK293). Compounds were tested in multiple cell passages and transfections.

5. Blinding

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

Investigators were not blinded when performing the live cell recording experiments but in the later stage of detailed data analysis.

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

- ☐ ☒ The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)
- ☐ ☒ A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
- ☐ ☒ A statement indicating how many times each experiment was replicated
- ☒ ☐ 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)
- ☒ ☐ A description of any assumptions or corrections, such as an adjustment for multiple comparisons
- ☒ ☐ The test results (e.g. P values) given as exact values whenever possible and with confidence intervals noted
- ☒ ☐ A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)
- ☐ ☒ 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.

OriginPro 2016G and SigmaPlot 13, MODELLER (version 9v17), pClamp - Clampfit 10.4, MATLAB 2016a

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.

Unique plasmids are freely available.

9. Antibodies

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

No antibodies used in the study.

10. Eukaryotic cell lines

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

HEK293 were purchased from DSMZ (ACC305)

b. Describe the method of cell line authentication used.

authentication is provided by DSMZ

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

HEK293 are regularly 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.

No misidentified cell line used.

► 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.

12. Description of human research participants

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

No human research participants.

Life Sciences Reporting Summary

Reporting Summary – Extended Comments

General comments:

- Wherever statistics have been derived (e.g. error bars, box plots, statistical significance) the legend needs to provide and define the n number (i.e. the sample size used to derive statistics) as a precise value (not a range), using the wording “n=X biologically independent samples/animals/independent experiments” etc. as applicable.
- The figure legends must indicate the statistical test used. Where appropriate, please indicate in the figure legends whether the statistical tests were one-sided or two-sided and whether adjustments were made for multiple comparisons.
- Please provide the test results (e.g. P values) as exact values whenever possible and with confidence intervals noted.
- Please state in the legends how many times each experiment was repeated independently with similar results. This is needed for all experiments, but is particularly important wherever representative experiments are shown. If space in the legends is limiting, this information can be included in a section titled “Statistics and Reproducibility”.

Specific comments:

Figure 1b

- Please clarify what the sample size (n) represents using the wording “n=X biologically independent samples/animals/independent experiments” etc. as applicable.

Figure 1d

- Please clarify what the sample size (n) represents using the wording “n=X biologically independent samples/animals/independent experiments” etc. as applicable.
- Please indicate in the figure legend the statistical test used. If appropriate, please also state whether the tests were one- or two-sided and whether adjustments were made for multiple comparisons.
- The p value has been provided as a range; please provide the precise value if possible and appropriate.

Figure 3a (inset)

- Please clarify what the sample size (n) represents using the wording “n=X biologically independent samples/animals/independent experiments” etc. as applicable.

Figure 3b

- Error bars are defined in the figure legend but are not plotted in the figure panel. Please ensure the data are displayed as intended and that the figure is accurately described in the legend.
- N numbers are not indicated in the figure panel as stated in the legend; please provide and define the N numbers.

Figure 3c-d (left and middle panels)

- Please state in the legend how many times the experiment was repeated independently with similar results.

Figure 3c-d (right)

- Please clarify what the sample size (n) represents using the wording “n=X biologically independent samples/animals/independent experiments” etc. as applicable.

- Please indicate in the figure legend the statistical test used. If appropriate, please also state whether the tests were one-or two-sided and whether adjustments were made for multiple comparisons.
- Please either provide the exact p value of the statistical test or the threshold used to establish significance (e.g. $p > 0.05 = ns$).

Figure 4a

- Please clarify what the sample size (n) represents using the wording "n=X biologically independent samples/animals/independent experiments" etc. as applicable.
- Please indicate in the figure legend the statistical test used. If appropriate, please also state whether the tests were one-or two-sided and whether adjustments were made for multiple comparisons.
- The p value has been provided as a range; please provide the precise value if possible and appropriate.

Figure 4b

- None of the elements in the G652A panel have been plotted in red as indicated in the figure legend. Please ensure the data are displayed as intended and the figure is accurately described in the legend.

Figure 5a

- Please clarify what the sample size (n) represents using the wording "n=X biologically independent samples/animals/independent experiments" etc. as applicable.

Figure 5b

- Please clarify what the sample size (n) represents using the wording "n=X biologically independent samples/animals/independent experiments" etc. as applicable.
- Please indicate in the figure legend the statistical test used. If appropriate, please also state whether the tests were one-or two-sided and whether adjustments were made for multiple comparisons.
- The p value has been provided as a range; please provide the precise value if possible and appropriate.

Figure 5c

- Please state in the legend how many times the experiment was repeated independently with similar results.

Figure 5d

- Please clarify what the sample size (n) represents using the wording "n=X biologically independent samples/animals/independent experiments" etc. as applicable.
- Please indicate in the figure legend the statistical test used. If appropriate, please also state whether the tests were one-or two-sided and whether adjustments were made for multiple comparisons.
- The p value has been provided as a range; please provide the precise value if possible and appropriate.

Figure 6 (upper left)

- Please state in the legend how many times the experiment was repeated independently with similar results.

Figure 6 (upper right)

- Please clarify what the sample size (n) represents using the wording "n=X biologically independent samples/animals/independent experiments" etc. as applicable.
- Please indicate in the figure legend the statistical test used. If appropriate, please also state whether the tests were one-or two-sided and whether adjustments were made for multiple comparisons.
- The p value has been provided as a range; please provide the precise value if possible and appropriate.

Figure 6 (lower right)

- Please clarify what the sample size (n) represents using the wording "n=X biologically independent samples/animals/independent experiments" etc. as applicable.
- Please indicate in the figure legend the statistical test used. If appropriate, please also state whether the tests were one-or two-sided and whether adjustments were made for multiple comparisons.

- The p value has been provided as a range; please provide the precise value if possible and appropriate.

Figure S1

- Please clarify what the sample size (n) represents using the wording "n=X biologically independent samples/animals/independent experiments" etc. as applicable.
- Please indicate in the figure legend the statistical test used. If appropriate, please also state whether the tests were one-or two-sided and whether adjustments were made for multiple comparisons.
- Please either provide the exact p value of the statistical test or the threshold used to establish significance (e.g. $p > 0.05 = \text{ns}$).

Figure S2 (top)

- Please state in the legend how many times the experiment was repeated independently with similar results.

Figure S2 (bottom)

- Please clarify what the sample size (n) represents using the wording "n=X biologically independent samples/animals/independent experiments" etc. as applicable.

Figure S3a

- Please state in the legend how many times the experiment was repeated independently with similar results.

Figure S3c

- Please clarify what the sample size (n) represents using the wording "n=X biologically independent samples/animals/independent experiments" etc. as applicable.
- Please indicate in the figure legend the statistical test used. If appropriate, please also state whether the tests were one-or two-sided and whether adjustments were made for multiple comparisons.
- The p value has been provided as a range; please provide the precise value if possible and appropriate.

Figure S4

- Please clarify what the sample size (n) represents using the wording "n=X biologically independent samples/animals/independent experiments" etc. as applicable.
- Please indicate in the figure legend the statistical test used. If appropriate, please also state whether the tests were one-or two-sided and whether adjustments were made for multiple comparisons.
- Please either provide the exact p value of the statistical test or the threshold used to establish significance (e.g. $p > 0.05 = \text{ns}$).

Figure S5a

- In the figure legend please define the n number as a precise value not a range.
- Please clarify what the sample size (n) represents using the wording "n=X biologically independent samples/animals/independent experiments" etc. as applicable.
- Error bars are defined in the figure legend but are not plotted in the figure panel. Please ensure the data are displayed as intended and that the figure is accurately described in the legend.

Figure S6 (lower panel)

- Please clarify what the sample size (n) represents using the wording "n=X biologically independent samples/animals/independent experiments" etc. as applicable.
- Please indicate in the figure legend the statistical test used. If appropriate, please also state whether the tests were one-or two-sided and whether adjustments were made for multiple comparisons.
- Please either provide the exact p value of the statistical test or the threshold used to establish significance (e.g. $p > 0.05 = \text{ns}$).

Figure S7

- Please clarify what the sample size (n) represents using the wording "n=X biologically independent samples/animals/independent experiments" etc. as applicable.
- Please indicate in the figure legend the statistical test used. If appropriate, please also state whether the tests were one-or two-sided and whether adjustments were made for multiple comparisons.
- Please either provide the exact p value of the statistical test or the threshold used to establish significance (e.g. $p > 0.05 = \text{ns}$).

Figure S9 (left)

- Please clarify what the sample size (n) represents using the wording "n=X biologically independent samples/animals/independent experiments" etc. as applicable.
- Please indicate in the figure legend the statistical test used. If appropriate, please also state whether the tests were one-or two-sided and whether adjustments were made for multiple comparisons.
- The p value has been provided as a range; please provide the precise value if possible and appropriate.

Figure S10 (upper panel)

- Please provide the n number as stated in the figure legend.
- Please clarify what the sample size (n) represents using the wording "n=X biologically independent samples/animals/independent experiments" etc. as applicable.
- Error bars are defined in the figure legend but are not plotted in the figure panel. Please ensure the data are displayed as intended and that the figure is accurately described in the legend.

Figure S10 (lower panel)

- Please clarify what the sample size (n) represents using the wording "n=X biologically independent samples/animals/independent experiments" etc. as applicable.
- Please indicate in the figure legend the statistical test used. If appropriate, please also state whether the tests were one-or two-sided and whether adjustments were made for multiple comparisons.
- The p value has been provided as a range; please provide the precise value if possible and appropriate.

Editorial Policy Checklist – Extended Comments

No additional comments.