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☐ Initial submission ☐ Revised version ☒ Final submission

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.

   No sample size calculations were performed

2. **Data exclusions**

   Describe any data exclusions.

   Data were excluded for failed experiments. These included mutants which presented poor labelling efficiency and were not appropriate for PELDOR. Patches, which presented unstable baseline conductance were excluded from electrophysiology analysis.

3. **Replication**

   Describe whether the experimental findings were reliably reproduced.

   Replicate experiments, where applicable, were successful.

4. **Randomization**

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

   Not applicable

5. **Blinding**

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

   Not applicable

   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.

a) DeerAnalysis, b) MMM, c) MtsslWizard, d) Gromacs, e) Patchmaster, f) OriginPro, g) VMD, h)Pymol

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.

M. tuberculosis MtbMscL WT plasmid will be available for distribution upon request

9. Antibodies

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

Not applicable. No antibodies were used

10. Eukaryotic cell lines

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

Not applicable. No eukaryotic cell lines were used.

b. Describe the method of cell line authentication used.

Not applicable

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

Not applicable

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.

Not applicable

▶ 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.

Not applicable. No animals or animal/derived materials were used in the study

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.

Not applicable.

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Flow Cytometry Reporting Summary

Form fields will expand as needed. Please do not leave fields blank.

▶ Data presentation

For all flow cytometry data, confirm that:

☐ 1. The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

☐ 2. The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).

☐ 3. All plots are contour plots with outliers or pseudocolor plots.

☐ 4. A numerical value for number of cells or percentage (with statistics) is provided.

▶ Methodological details

5.	Describe the sample preparation.	Not applicable. No flow cytometry was used in the study
6.	Identify the instrument used for data collection.	Not applicable
7.	Describe the software used to collect and analyze the flow cytometry data.	Not applicable
8.	Describe the abundance of the relevant cell populations within post-sort fractions.	Not applicable
9.	Describe the gating strategy used.	Not applicable

Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information. ☐