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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.

Please do not complete any field with "not applicable" or n/a. Refer to the help text for what text to use if an item is not relevant to your study.

For final submission: please carefully check your responses for accuracy; you will not be able to make changes later.

Experimental design

1. Sample size

Describe how sample size was determined.

No statistical methods were used to determine sample size. Sample sizes were typical for probe development studies (eg. Hochbaum et al, Nat Methods, 2014; Gong et al, Science, 2015; Abdelfattah et al, J Neurosci, 2016)

2. Data exclusions

Describe any data exclusions.

No data were excluded from the analyses.

3. Replication

Describe the measures taken to verify the reproducibility of the experimental findings.

Attempts at replication were successful.

4. Randomization

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

Randomization was not applicable as there was no sample allocation into groups. Experiments involved characterization of individual voltage probes.

5. Blinding

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

Blinding was used where relevant using a transfection marker.

Note: all in vivo studies must report how sample size was determined and 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 *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☐	☒ A description of any assumptions or corrections, such as an adjustment for multiple comparisons
☐	☒ Test values indicating whether an effect is present *Provide confidence intervals or give results of significance tests (e.g. P values) as exact values whenever appropriate and with effect sizes 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 in all relevant figure captions (with explicit mention of central tendency and variation)

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.	LabView 2016 and MATLAB 2015 versions were used to analyze data from the high-throughput platform and optical recordings in patch clamp and TEMPO experiments, respectively. Custom-written LabView code is available on Dropbox (link provided in manuscript). NeuroPlex (Redshirt Imaging) and pClamp10 were used for data acquisition for imaging and electrophysiology, respectively.

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 third party.	Plasmids generated in this work will be available from commercial sources (Addgene) prior to publication. Viruses and fly stocks will be provided by authors 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).	No antibodies were used in this study.

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.	HEK293 cells (CRL-1573) and excitable HEK cells (CRL-3269) were obtained from ATCC (Manassas, VA).
b. Describe the method of cell line authentication used.	Authenticated by ATCC using STR analysis
c. Report whether the cell lines were tested for mycoplasma contamination.	All cell lines were tested negative 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.	Yes; our cell lines were acquired from ATCC, which regularly performs STR analysis to avoid distribution of misidentified lines. In our experiments, HEK293 cells served solely as a system for expression and characterization of voltage indicators rather than the subject of investigation.

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 all relevant details on animals and/or animal-derived materials used in the study.	C57BL/6, CD-1 wildtype, PV-Cre and Thy1-YFP transgenic mice were used. Female mice were used for in utero electroporation, male mice were used for in vivo imaging. In all other experiments, mice were used without regard to sex. The mice aged between 3 weeks and 4 months (the exact ages are mentioned in the manuscript text). In experiments involving flies, we used adult female transgenic flies (2 days old).

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.	The study did not involve human research participants.