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	We used the electrophysiology tool Clampex 10.9 for data collection.
Data analysis	We used publicly available software for data analysis including R v.4.1.2, python v3.6.8, SpliceAI API, ABSplice precomputed scores, and Pangolin source code. For patch-clamp analysis, we used ClampFit 10. All custom code used for data analysis is available at https://github.com/GlazerLab/ParSE-seq with permanent DOI 10.5281/zenodo.13170912.

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

All DNA and RNA sequencing data is available at the NCBI Sequence Read Archive (NIH BioProject accession #1106089; https://www.ncbi.nlm.nih.gov/bioproject/1106089). Processed data are available on GitHub (https://github.com/GlazerLab/ParSE-seq; permanently copied at Zenodo79). All processed data are also available in the accompanying Supplementary Information/Source Data file. We have deposited variant classifications on ClinVar, and will supply the ascension

ID prior to live publication.

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	We performed variant-level analyses in iPSC-derived CMs from an established cell line (PMID: 36524479). The participant (male age 30-40) from which these cells were derived provided Informed Consent. As we were interested in variant-level effects, we did not consider sex/gender or genetic ancestry in the selection of this cell line. The Vanderbilt University Medical Center IRB (#9047) approved the use of the induced pluripotent stem cells used in this study.
Reporting on race, ethnicity, or other socially relevant groupings	N/A
Population characteristics	N/A
Recruitment	N/A
Ethics oversight	The Vanderbilt University Medical Center IRB (#9047) approved the use of the induced pluripotent stem cells 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	3 replicates of each splicing experiment in each cell type were performed. No power calculations were performed.
Data exclusions	Barcodes with high variability across replicates (standard error of mean delta PSI greater than 0.15 were excluded).
Replication	We performed 3 replicates of each splicing experiment in each cell type and compared results across replicates. We did not encounter failed replications that were excluded from the analysis.
Randomization	Our primary ParSE-seq analysis was methodological, rather than hypothesis-testing. We included many variants in SCN5A that were assessed for impact on splicing in a 'bulk' library format. Comparisons were made between variant and WT construct with randomization based on barcode blinding, but subsequent hypothesis tests were not applied amongst experimental outcomes of variants themselves.
Blinding	ParSE-seq experiments were carried out with every WT and variant construct present in a single library, therefore blinding was carried out at the level of the bulk experiment and random barcode identifier. For patch-clamp data, the experimentalists were blinded to the variant.

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 ATCC (CRL-3216). The C2 induced pluripotent stem cell line was derived from a male patient as previously reported [PMID 36524479].
Authentication	The C2 iPSC line was derived from a male donor (age 25-35) and was characterized by karotyping to ensure normal ploidy and by immunofluorescence for pluripotency markers as previously reported [PMID 36524479]. HEK cells were professionally authenticated by ATCC with bacterial/fungal culturing and STR testing.
Mycoplasma contamination	HEK cells and iPSC-CMs were negative for mycoplasma by PCR.
Commonly misidentified lines (See ICLAC register)	HEK cells are rarely misidentified according to the ICLAC registry. Our goal in using HEK cells was to enable a high-throughput splicing assay that would complement similar conditions done in low-throughput in this commonly available cell type.