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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	More than 2,000,000 bacteria genome and 30,000 metagenome assemblies were collected from NCBI and JGI databases; Illumina NovaSeq 6000 platform (targeted deep sequencing and RNA-seq)
Data analysis	FlowJo X v10.0.7 (flow cytometry); CRISPResso2 v2.0.20b with a custom barcode-demultiplexing script (deep-sequencing analysis); Cas-OFFinder (in silico off-target prediction); Olympus FV3000 inverted confocal microscope (immunofluorescence imaging); Medusa Small Animal Electrophysiology Recording System with Athena online acquisition software (24-h video-ECoG); Trimmomatic v0.39-2, HISAT2 v2.2.1 and DESeq2 v3.5 (RNA-seq filtering, alignment and differential expression); TBLASTN and Prodigal (metagenomic mining of Cas12m orthologs); MEGA X and MEGA 12 (phylogenetic tree construction and multiple sequence alignment); ESPript 3.0 (alignment visualization); AlphaFold3 and PyMOL v3.1.6.1 (structure prediction, analysis and visualization); GraphPad Prism v8.2.1 (statistical analysis).

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 the sequencing data have been deposited in the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) under BioProject ID PRJNA1492244 and PRJNA1524337. All plasmids generated in this study are available from the corresponding author upon request, and some have been deposited with Addgene under accession numbers 262479-262495. Source data are provided with this paper.

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	N/A
Reporting on race, ethnicity, or other socially relevant groupings	N/A
Population characteristics	N/A
Recruitment	N/A
Ethics oversight	N/A

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	No statistical analysis were performed to predetermine sample size in this study. But sample sizes in this study are in accordance to those generaly used in genome editing experiments. All cellular experiments were conducted with two to three biologically independent replicates. For all animal experiments, between three and thirty two mice were used.
Data exclusions	No data were excluded.
Replication	All main text figure experiments were repeated at least once and attempts at replication were successful.
Randomization	Human cells were grown at identical condition, after seeding cells into 24-wells plates, we randomly selected cells for test group and control group. Mice used for gene editing therapy were allocated to control or AAV treated group randomly.
Blinding	For molecular, cell and animals experiments, no blinding was applied.

Behavioural & social sciences study design

All studies must disclose on these points even when the disclosure is negative.

Study description	*Briefly describe the study type including whether data are quantitative, qualitative, or mixed-methods (e.g. qualitative cross-sectional, quantitative experimental, mixed-methods case study).*
Research sample	*State the research sample (e.g. Harvard university undergraduates, villagers in rural India) and provide relevant demographic*

Research sample	*information (e.g. age, sex) and indicate whether the sample is representative. Provide a rationale for the study sample chosen. For studies involving existing datasets, please describe the dataset and source.*
Sampling strategy	*Describe the sampling procedure (e.g. random, snowball, stratified, convenience). Describe the statistical methods that were used to predetermine sample size OR if no sample-size calculation was performed, describe how sample sizes were chosen and provide a rationale for why these sample sizes are sufficient. For qualitative data, please indicate whether data saturation was considered, and what criteria were used to decide that no further sampling was needed.*
Data collection	*Provide details about the data collection procedure, including the instruments or devices used to record the data (e.g. pen and paper, computer, eye tracker, video or audio equipment) whether anyone was present besides the participant(s) and the researcher, and whether the researcher was blind to experimental condition and/or the study hypothesis during data collection.*
Timing	*Indicate the start and stop dates of data collection. If there is a gap between collection periods, state the dates for each sample cohort.*
Data exclusions	*If no data were excluded from the analyses, state so OR if data were excluded, provide the exact number of exclusions and the rationale behind them, indicating whether exclusion criteria were pre-established.*
Non-participation	*State how many participants dropped out/declined participation and the reason(s) given OR provide response rate OR state that no participants dropped out/declined participation.*
Randomization	*If participants were not allocated into experimental groups, state so OR describe how participants were allocated to groups, and if allocation was not random, describe how covariates were controlled.*

Ecological, evolutionary & environmental sciences study design

All studies must disclose on these points even when the disclosure is negative.

Study description	*Briefly describe the study. For quantitative data include treatment factors and interactions, design structure (e.g. factorial, nested, hierarchical), nature and number of experimental units and replicates.*
Research sample	*Describe the research sample (e.g. a group of tagged Passer domesticus, all Stenocereus thurberi within Organ Pipe Cactus National Monument), and provide a rationale for the sample choice. When relevant, describe the organism taxa, source, sex, age range and any manipulations. State what population the sample is meant to represent when applicable. For studies involving existing datasets, describe the data and its source.*
Sampling strategy	*Note the sampling procedure. Describe the statistical methods that were used to predetermine sample size OR if no sample-size calculation was performed, describe how sample sizes were chosen and provide a rationale for why these sample sizes are sufficient.*
Data collection	*Describe the data collection procedure, including who recorded the data and how.*
Timing and spatial scale	*Indicate the start and stop dates of data collection, noting the frequency and periodicity of sampling and providing a rationale for these choices. If there is a gap between collection periods, state the dates for each sample cohort. Specify the spatial scale from which the data are taken*
Data exclusions	*If no data were excluded from the analyses, state so OR if data were excluded, describe the exclusions and the rationale behind them, indicating whether exclusion criteria were pre-established.*
Reproducibility	*Describe the measures taken to verify the reproducibility of experimental findings. For each experiment, note whether any attempts to repeat the experiment failed OR state that all attempts to repeat the experiment were successful.*
Randomization	*Describe how samples/organisms/participants were allocated into groups. If allocation was not random, describe how covariates were controlled. If this is not relevant to your study, explain why.*
Blinding	*Describe the extent of blinding used during data acquisition and analysis. If blinding was not possible, describe why OR explain why blinding was not relevant to your study.*

Did the study involve field work? ☐ Yes ☐ No

Field work, collection and transport

Field conditions	*Describe the study conditions for field work, providing relevant parameters (e.g. temperature, rainfall).*
Location	*State the location of the sampling or experiment, providing relevant parameters (e.g. latitude and longitude, elevation, water depth).*
Access & import/export	*Describe the efforts you have made to access habitats and to collect and import/export your samples in a responsible manner and in compliance with local, national and international laws, noting any permits that were obtained (give the name of the issuing authority, the date of issue, and any identifying information).*

Disturbance | *Describe any disturbance caused by the study and how it was minimized.*

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

Antibodies

Antibodies used: Anti-dystrophin (Sigma, D8168, 1:500), anti-vinculin (CST, 13901S, 1:1000), anti-utrophin (Santa Cruz, sc-33700, 1:200 or 1:500), anti-follistatin (Invitrogen, PA5-102043, 1:500), anti-calnexin (Abclonal, A24433, 1:500), anti-NaV1.1 (Alomone, ASC-001, 1:500), anti-laminin (Sigma, L0663, 1:200), anti-HA tag (Roche, 11867423001, 1:200), anti-Oct4 (BD bioscience, 611203, 1:200), anti-Sox2 (CST, 14962, 1:200), anti-Klf4 (R&D, AF3158, 1:200), anti-rabbit IgG(H+L)-HRP (Epizyme, LF102, 1:1000), Alexa Fluor® 488 AffiniPure donkey anti-rabbit IgG (Jackson ImmunoResearch labs, 711-545-152, 1:1000) and Alexa Fluor 647 AffiniPure donkey anti-mouse IgG (Jackson ImmunoResearch labs, 715-605-151, 1:1000) were used in this study.

Validation:
https://www.sigmaaldrich.cn/CN/zh/product/sigma/d8168
https://www.cellsignal.cn/products/primary-antibodies/vinculin-e1e9v-rabbit-monoclonal-antibody/13901
https://www.scbt.com/p/utrophin-antibody-8a4
https://www.thermofisher.cn/cn/zh/antibody/product/Follistatin-Antibody-Polyclonal/PA5-102043
https://www.sigmaaldrich.cn/CN/zh/product/sigma/l0663
https://www.alomone.com/products/asc-001-anti-scn1a-na-sub-v-sub-1-1-antibody
https://www.sigmaaldrich.cn/CN/zh/product/roche/roahaha
https://www.bdbiosciences.com/zh-cn/products/reagents/microscopy-imaging-reagents/immunofluorescence-reagents/purified-mouse-anti-oct3-4.611203?tab=product_details
https://www.cellsignal.cn/products/primary-antibodies/sox2-d1c7j-rabbit-monoclonal-antibody/14962
https://www.rndsystems.com/products/mouse-klf4-antibody_af3158
https://www.epizyme.cn/Products/Details/LF102
https://www.jacksonimmuno.com/catalog/products/711-545-152
https://www.jacksonimmuno.com/catalog/products/715-605-151

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s): HEK293T, N2a and Peripheral blood mononuclear cells (PBMCs)

Authentication: HEK293T and N2a (ATCC) cells were validated by supplier. Peripheral blood mononuclear cells (PBMCs) from healthy donors (Hycells) were used to isolate primary human CD3* T cells with the EasySep Human T Cell Isolation Kit (STEMCELL Technologies).

Mycoplasma contamination: All cell lines tested negative for mycoplasma contamination.

Commonly misidentified lines (See ICLAC register): None misidentified lines were used.

Palaeontology and Archaeology

Specimen provenance: N/A

Specimen deposition: N/A

Dating methods	N/A

☒ Tick this box to confirm that the raw and calibrated dates are available in the paper or in Supplementary Information.

Ethics oversight	N/A

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Animals and other research organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research, and Sex and Gender in Research

Laboratory animals	C57BL/6J mice were obtained from Shanghai SLAC Laboratory Animal Co.,Ltd, and 129S-Scn1atm1Kea/Mmjax mice (JAX#024761) were obtained from Jackson Laboratory. For all experiments, heterozygous 129S-Scn1atm1Kea/Mmjax males were crossed to C57BL/6J females to generated F1 offspring. DMDΔmE50E51, hE50KI and DMDΔmE23, hE23KI was generated on a C57BL/6J background via CRISPR-Cas9-mediated homology-directed repair.
Wild animals	This study did not involve wild animals.
Reporting on sex	Given that DMD is the most common sex-linked lethal disease in males, male mice were selected for this study. In contrast, no specific sex was selected for the Dravet animal model.
Field-collected samples	No field-collected samples were used in this study.
Ethics oversight	All animal experiments were conducted in accordance with the protocols approved by the Animal Care and Use Committees of both the Institute of Neuroscience and the Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Clinical data

Policy information about clinical studies

All manuscripts should comply with the ICMJE guidelines for publication of clinical research and a completed CONSORT checklist must be included with all submissions.

Clinical trial registration	N/A
Study protocol	N/A
Data collection	N/A
Outcomes	N/A

Dual use research of concern

Policy information about dual use research of concern

Hazards

Could the accidental, deliberate or reckless misuse of agents or technologies generated in the work, or the application of information presented in the manuscript, pose a threat to:

No	Yes	
☒	☐	Public health
☒	☐	National security
☒	☐	Crops and/or livestock
☒	☐	Ecosystems
☒	☐	Any other significant area

Experiments of concern

Does the work involve any of these experiments of concern:

No	Yes	
☒	☐	Demonstrate how to render a vaccine ineffective
☒	☐	Confer resistance to therapeutically useful antibiotics or antiviral agents
☒	☐	Enhance the virulence of a pathogen or render a nonpathogen virulent
☒	☐	Increase transmissibility of a pathogen
☒	☐	Alter the host range of a pathogen
☒	☐	Enable evasion of diagnostic/detection modalities
☒	☐	Enable the weaponization of a biological agent or toxin
☒	☐	Any other potentially harmful combination of experiments and agents

Plants

Seed stocks	N/A
Novel plant genotypes	N/A
Authentication	N/A

ChIP-seq

Data deposition

☐ Confirm that both raw and final processed data have been deposited in a public database such as GEO.

☐ Confirm that you have deposited or provided access to graph files (e.g. BED files) for the called peaks.

Data access links *May remain private before publication.*	N/A
Files in database submission	N/A
Genome browser session (e.g. UCSC)	N/A

Methodology

Replicates	N/A
Sequencing depth	N/A
Antibodies	N/A
Peak calling parameters	N/A
Data quality	N/A
Software	N/A

Flow Cytometry

Plots

Confirm that:

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

☒ 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).

☒ All plots are contour plots with outliers or pseudocolor plots.

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

Methodology

Sample preparation	Post-transfected cells were culture medium, and analyzed by flow cytometry.
Instrument	For EGFP activation assay, Beckman CytoFlex was used, and BD FACSAria III was used for cell sorting.
Software	FACS data were analyzed with FlowJo X (v10.0.7)
Cell population abundance	EGFP/mCherry activation rates = Mean EGFP or mCherry Intensity in BFP positive cells.
Gating strategy	Gating strategy for EGFP activation assay was provided at Supplementary Figure 16 and for indel analysis, the mCherry positive cells were sorted.

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

Magnetic resonance imaging

Experimental design

Design type	N/A
Design specifications	N/A
Behavioral performance measures	N/A

Acquisition

Imaging type(s)	N/A
Field strength	N/A
Sequence & imaging parameters	N/A
Area of acquisition	N/A

Diffusion MRI ☐ Used ☒ Not used

Preprocessing

Preprocessing software	N/A
Normalization	N/A
Normalization template	N/A
Noise and artifact removal	N/A
Volume censoring	N/A

Statistical modeling & inference

Model type and settings	N/A
Effect(s) tested	N/A

Specify type of analysis: ☐ Whole brain ☐ ROI-based ☐ Both

Statistic type for inference (See Eklund et al. 2016)

N/A

Correction

N/A

Models & analysis

n/a	Involved in the study
☒	☐ Functional and/or effective connectivity
☒	☐ Graph analysis
☒	☐ Multivariate modeling or predictive analysis

Functional and/or effective connectivity

Report the measures of dependence used and the model details (e.g. Pearson correlation, partial correlation, mutual information).

Graph analysis

Report the dependent variable and connectivity measure, specifying weighted graph or binarized graph, subject- or group-level, and the global and/or node summaries used (e.g. clustering coefficient, efficiency, etc.).

Multivariate modeling and predictive analysis

Specify independent variables, features extraction and dimension reduction, model, training and evaluation metrics.