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	Sequencing data were collected using Illumina Nextseq 500 system, Illumina MiSeq, or ABI 3730XL Genetic Analyzers; echo data were collected using VisualSonics Vevo2100 imaging system; Contractile force measurements data were collected using Nikon A1R+ confocal system; Confocal images were acquired using a Zeiss LSM 800 and Zeiss Zen-2; Seahorse data was collected using a Seahorse XFe96 Analyzer; Histology images were acquired using Keyence BZ-X800 All-in-One Microscope; qPCR data was collected using QuantStudio 5.
Data analysis	Software used for data analysis include EditR (v1.0.10), GraphPad Prism (v.9.4.0), MATLAB (v.9.10.0.1710957), Fiji (ImageJ, Version 2.1.0), Metascape (v3.5), WAVE Software (v.2.4.3), CRISPOR (v4.99), CRISPResso2 (v.2.2.8), FastQC tool (v0.11.8), Trimmomatic (v0.39), RSeQC (v4.0.0), HiSAT2 (v2.1.0), featureCounts (v1.6.2), DESeq (1.38.0), REDItools2, FLOWJO (v10) and Adobe Photoshop (v23.2.1).

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

Raw and analyzed RNA-sequencing data generated during this study are available in the Gene Expression Omnibus (GEO) repository (http://www.ncbi.nlm.nih.gov/geo/) and are accessible through GEO series accession number GSE201755. DNA-sequencing files can be accessed at the NCBI SRA with accession code PRJNA902011. The mm10 reference genome is available at https://www.ncbi.nlm.nih.gov/assembly/GCF_000001635.20/.

Human research participants

Policy information about studies involving human research participants and Sex and Gender in Research.

Reporting on sex and gender	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	Sample sizes were not pre-determined based on statistical power calculations, but were based on our previous experience, experimental approach, and literature (PMID: 33931459, 30166439) conducting similar experimentation. For assays in which variability is commonly high (such as animal studies), we typically used an n of at least 5. For assays in which variability is commonly low (such cell culture experiments), we typically used n<5. Sample sizes for next generation sequencing data were determined following ENCODE consortium guidelines (https://www.encodeproject.org/documents/cede0cbe-d324-4ce7-ace4-f0c3eddf5972/@@download/attachment/ENCODE%20Best%20Practices%20for%20RNA_v2.pdf).
Data exclusions	No data were excluded.
Replication	All attempts at replication for standard assays (base editing, transfection/nucleofection, Seahorse metabolic assays, qPCR, immunohistochemistry, echocardiography) were successful. A majority of experiments were performed in three or more biological replicates or for iPSC-CMs, across three separate differentiations. For RNA-sequencing and amplicon sequencing of mice, three biologically independent animals were used.
Randomization	Samples were allocated randomly to experiments and processed in an arbitrary order while maintaining genotype and gender balance, as appropriate.
Blinding	Investigators were blinded to group allocation during data collection and data analysis for echocardiography analyses, measurements of contractile force of iPSC-CMs, and measurements of histology slides and stainings. For other experiments, investigators were not blinded to group allocation during data collection due to the necessary prior knowledge of sample identity for sample pooling and collection. However, investigators were blinded to group allocation during data analysis in most of cases.

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

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☐	☒ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used: rabbit anti-troponin-I (H-170 sc-15368, Santa Cruz Biotechnology, 1:200)
fluorescein-conjugated donkey anti-rabbit IgG (711-095-152, Jackson ImmunoResearch, 1:50)

Validation: All antibodies are commercially available and have been validated in previously published studies, e.g. PMID 33931459
rabbit anti-troponin-I (H-170 sc-15368, Santa Cruz Biotechnology, 1:200, https://www.scbt.com/p/troponin-i-antibody-h-170)
fluorescein-conjugated donkey anti-rabbit IgG (711-095-152, Jackson ImmunoResearch, 1:50, https://www.jacksonimmuno.com/catalog/products/711-095-152)

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s): iPSCs were derived from peripheral blood mononuclear cells (PBMCs) of a male healthy donor (annotated as HD in the manuscript), PBMCs of a female patient with HCM (annotated as HCM1 in the manuscript), and PBMCs of a male patient with HCM (annotated as HCM2 in the manuscript).

Authentication: All iPSCs readily differentiated into cardiomyocytes as evidenced by immunostaining.

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

Commonly misidentified lines (See ICLAC register): No commonly misidentified cell lines were used.

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: All mouse experiments complied with all relevant ethical regulations and were performed according to protocols approved by the Institutional Animal Care and Use Committees at University of Texas Southwestern Medical Center. UT Southwestern uses the "Guide for the Care and Use of Laboratory Animals" when establishing animal research standards. All mice used in this study were housed at the pathogen-free Animal Resource Center at the University of Texas Southwestern Medical Center. All animals were bred inside a specific pathogen free facility with 12h light:dark cycles with a temperature of 18–24 °C and humidity of 35–60% and monitored daily with no health problems. All animals were housed in groups of maximum five per cage with ad libitum access to food and water. All animals used in this study are in the C57BL/6 background. Male mice were used for heterozygous experiments as female mice have better cardio-protection than male mice (PMID: 17466956), and did not display the phenotype as readily as males, which has been described in other mice studies of HCM (PMID: 24092743, PMID: 34525843). Both male and female mice were used for homozygous experiments as the phenotype is highly penetrant for both sexes. Mice used in this study range in age from 1 day to 9 months, equivalent numbers of male/female.

Wild animals: The study did not involve wild animals.

Reporting on sex: Male animals were used for heterozygous experiments. The reason for this is that females animals have better cardio-protection than males (PMID: 17466956), and did not display the phenotype as readily as males, which has been described in other studies of HCM (PMID: 24092743, PMID: 34525843). Both male and female animals were used for homozygous experiments as the phenotype is highly penetrant for both sexes.

Field-collected samples: The study did not involve field-collected samples.

Ethics oversight	All experiments involving animals were approved by the Institutional Animal Care and Use Committee at the University of Texas Southwestern Medical Center.

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

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	iPSCs were detached from cell culture plates using Accutase, resuspended as a single-cell suspension in mTesR media with ROCK inhibitor, and filtered through a 35 micron nylon mesh filter into polypropylene test tubes before being sorted on a FACSAria II SORP using 488 nm filter.
Instrument	iPSCs were sorted using a FACSAria II SORP using 488 nm filter
Software	Flow cytometry data was collected and analyzed using FlowJo analysis software.
Cell population abundance	Initial gate contained about 50% cells, second gate contained >90% singlet cells, and third gate contained ~30% GFP positive cells.
Gating strategy	The first gate differentiated cells from debris based on SSC and FSC, followed by a second gate differentiating doublets from singlets based on SSC-H vs SSC-A, followed by the final gate based on GFP signal vs SSC. Gating was based on separation into two distinct populations compared to a non-transfected cell line as determined by an experienced user

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