

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 (<i>n</i>) 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
<i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i> |
| ☒ | ☐ 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. <i>F</i> , <i>t</i> , <i>r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted
<i>Give P values as exact values whenever suitable.</i> |
| ☒ | ☐ 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 <i>d</i> , Pearson's <i>r</i>), 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	1) Cardiac function was assessed by transthoracic echocardiography using high resolution micro-imaging systems (Vevo 3100 Preclinical Imaging System, VisualSonics). 2) ECG signals recorded with ADInstruments Powerlab 3) Li-Cor Odyssey software was utilized for immunoblot detection 4) Nautilai (CuriBio) fluorescence imager for calcium transients data collection 5) Quant Studio 7 (Applied Biosystems) was used for all qPCR data collection
Data analysis	1) Echocardiography analysis was performed using Vevo Lab version 5.6.1 2) LabChart 8 software was used to assess QT intervals 3) ImageJ (NIH) densitometry analysis was utilized for immunoblot detection 4) CuriBio Pulse cloud analysis software for calcium transients data analysis 5) Statistical analyses were performed using GraphPad Prism 9

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 data supporting the findings described in this manuscript are available in the article and in the Supplementary Information. The source data are provided with this paper in the "Source Data" file. Sequences are available in the Supplemental Sequence tab of the Source Data file.

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](https://www.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	For animal studies, ejection fraction was the primary outcome measure used to determine sample size. In addition, the animal sample size per treatment group was determined based on our in-house experience of conducting efficacy, dose-ranging, and long-term survival benefit studies using AAV9 as test article.
Data exclusions	Animals found dead were excluded from tissue DNA, RNA, or protein analyses.
Replication	Wherever possible, experiments were independently replicated to confirm reported results. All attempts at replication were successful.
Randomization	Mice were randomized before being assigned to either vehicle or treatment groups based on a minimum of sex, age, and body weight. Ejection fraction was also used whenever possible.
Blinding	An individual blinded to the treatment performed and analyzed the echocardiography data and functional analysis.

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	1) Western blots: mouse anti-MYBPC3 antibody (E-7) (sc-137180, Santa Cruz Biotechnology); rabbit anti-GAPDH antibody [EPR16891] (ab181602, Abcam), mouse anti-alpha Tubulin antibody [DM1A] (ab7291, Abcam); rabbit anti-alpha Actinin 2 Polyclonal antibody (PA5-27863, Thermo Fisher); rabbit anti-Phospho-PKA Substrate (RRXS*/T*) antibody (100G7E) (# 9624, Cell Signlaing Technologies) 2) ICC/IHC: mouse anti-MYBPC3 antibody (E-7) (sc-137180, Santa Cruz Biotechnology); rabbit anti-alpha Actinin 2 Polyclonal antibody (PA5-27863, Thermo Fisher) 3) Flow cytometry detection: mouse anti-Cardiac Troponin T Monoclonal Antibody (13-11) (MA5-12960, Thermo Fisher)
Validation	All antibodies were used based on the manufacturer's recommend dilutions. Antibodies were validated whenever possible on KO mouse tissue or KO human iPSC derived cardiomyocytes to assess specificity.

Eukaryotic cell lines

Policy information about [cell lines](#) and [Sex and Gender in Research](#)

Cell line source(s)	Tenaya's Stem Cell Research Oversight Committee approved the study protocol (TT-2501) for iPSCs. The human iPSC lines used in this study were generated from a healthy male patient, WTC-11 (Coriell Institute: # GM25256; 30 y.o. Japanese male, deposited by Dr. Bruce R. Conklin (Gladstone Institute of Cardiovascular Disease, UCSF)). Informed consent was obtained for this procedure. MYBPC3 ^{-/-} and MYBPC3 ^{+/-} iPSCs were generated by a CRISPR-Cas9 paired gRNA approach to remove exons one and two in WTC-11 line which was modified with a blasticidin resistance gene at the MYH6 locus. Human cardiac fibroblasts: PromoCell Cat.No.C-12375. No cells were generated de novo from patients for this study.
Authentication	All cell lines were karyotyped and sequence confirmed.
Mycoplasma contamination	All cell lines tested negative for mycoplasma.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used in this study.

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	Applied Stemcell Inc. (Milpitas, CA) was contracted for creation of Mybpc3 Exon1-Exon2 knockout mice, including generation of paired guide RNAs (GGCATCAAGCAGGCCACCCA and AAAGGTCAAGTTTGACCTCA) and utilization of the CRISPR/Cas9 system on a C57BL/6 background. CD-1 [®] mice were used for a pilot safety study.
Wild animals	N/A
Reporting on sex	Mice were randomized before being assigned to either vehicle or treatment groups based on a minimum of sex, age, and body weight. Whenever possible, every attempt was made to have equal representation of males and females per group. We have provided in the "Source Data" file an Excel tab delineating the precise animal number by sex for each study. Our research findings apply to both sexes.
Field-collected samples	N/A
Ethics oversight	Animal experiments were approved by the IACUC committee of Tenaya Therapeutics and were performed in compliance with the animal use protocol (AUP: 2020.007 and 2023.007) and the National Institutes of Health.

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

Plants

Seed stocks	N/A
Novel plant genotypes	N/A
Authentication	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	1) For live-cell analysis of %GFP positive cells: cells were dissociated with TrypLE (Gibco), quenched, and assessed on a SH800 cell sorter (Sony Biotechnology). 2) For Fixed iPSC-CM analysis: A vial of differentiated iPSC-CMs are thawed from LN2 storage, counted, and aliquoted into four samples of 1e6 total live cells. The four samples are: no antibody control, secondary only control, and two tested samples. Cells are spun down and resuspended in 4% PFA (diluted from 20% PFA, Electron Microscopy Sciences) to fix for 15 minutes. Cells are next blocked and permeabilized in 4% BSA (Gibco) with 0.1% Triton-X (Sigma Aldrich) in DPBS(-/-) (Gibco). Cells are then washed 3 times with DPBS(-/-) and resuspended in primary antibody, Cardiac Troponin T Monoclonal Antibody (Invitrogen), and incubated at room temperature for one to two hours. No antibody and secondary only controls are resuspended in 4% BSA during this step. Cells are once again washed three times, resuspended in secondary antibody, Alexa Fluor 488 (Invitrogen), and left at room temperature for 30-45 minutes. No antibody control is resuspended in 4% BSA during this step. Cells are then washed three times and resuspended in 4% BSA. Finally, cells are moved to the Sony SH8000 Flow Cytometer for count. Cells are selected through live cells, single cells, and then selected for presence of GFP or not.
Instrument	Sony SH800
Software	Sony SH800
Cell population abundance	No sorting was performed.
Gating strategy	As per Fig. S9, FITC/cTnT+ cells were gated at 5000.

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