Major Resources Table

In order to allow validation and replication of experiments, all essential research materials listed in the Methods should be included in the Major Resources Table below. Authors are encouraged to use public repositories for protocols, data, code, and other materials and provide persistent identifiers and/or links to repositories when available. Authors may add or delete rows as needed.

Animals (in vivo studies)

Species	Vendor or Source	Background Strain	Sex	Persistent ID / URL
Mouse	Jackson Labs	C57BL/6J	M	https://www.jax.org/strain/000664?featured
Mouse	Prof. Lucie Carrier, University Medical Center Hamburg-Eppendorf	129/Svj/Black Swiss	M/F	
Rat	Envigo	Sprague-Dawley	M/F	https://www.inotivco.com/model/hsd-sprague-dawley-sd

Genetically Modified Animals

	Species	Vendor or Source	Background Strain	Other Information	Persistent ID / URL
Parent - Male	Mouse	Prof. Lucie Carrier, University Medical Center Hamburg-Eppendorf	129/Svj/Black Swiss	Heterozygous *Mybpc3*-targeted knock-in; originally described in PMID: 19590044	BlackSwiss;129/Svj-*Mybpc3$^{tm2.1Lcrr}$*
Parent - Female	Mouse	Prof. Lucie Carrier, University Medical Center Hamburg-Eppendorf	129/Svj/Black Swiss	Heterozygous *Mybpc3*-targeted knock-in; originally described in PMID: 19590044	BlackSwiss;129/Svj-*Mybpc3$^{tm2.1Lcrr}$*

Antibodies

Target antigen	Vendor or Source	Catalog #	Working concentration	Lot # (preferred but not required)	Persistent ID / URL
α-tubulin clone DM1A	Cell Signaling Technology	3873	1:1,000; WB		https://www.cellsignal.com/products/primary-antibodies/a-tubulin-dm1a-mouse-mab/3873
Detyrosinated α-tubulin	Abcam	ab48389	1:1,000; WB		https://www.abcam.com/en-de/products/primary-antibodies/detyrosinated-alpha-tubulin-antibody-microtubule-marker-ab48389
α-tubulin	Marie-Jo Moutin, GIN, Grenoble, France	Custom-made	1:5,000; WB		

DOI [to be added]

Detyrosinated α-tubulin	Marie-Jo Moutin, GIN, Grenoble, France	Custom-made	1:2,000; WB 1:1,000; IF		
TTL	Proteintech	13618-1-AP	1:500; WB		https://www.ptglab.com/products/TTL-Antibody-13618-1-AP.htm
α-actinin	Sigma Aldrich	A7811	1:800; IF		https://www.sigmaaldrich.com/DE/de/product/sigma/a7811
α-actinin	Sigma Aldrich	SAB2108642	1:800; IF		https://www.sigmaaldrich.com/DE/de/product/sigma/sab2108642
DsRed	Takara Clontech	632392	1:500; WB		https://www.takarabio.com/search-results?term=632392
GAPDH	HyTest	5G4	1:10,000; WB		https://shop.hytest.fi/product/glyceraldehyde-3-phosphate-dehydrogenase-gapdh-antibody
Histone H3	Abcam	ab24834	1:3,000; WB		https://www.abcam.com/en-de/products/primary-antibodies/histone-h3-antibody-mabcam-24834-nuclear-loading-control-and-chip-grade-ab24834
N-cadherin	Sigma Aldrich	C2542	1:500; IF		https://www.sigmaaldrich.com/DE/de/product/sigma/c2542
Peroxidase-conjugated anti-mouse IgG	Jackson ImmunoResearch	515-035-003	1:20,000; WB		https://www.jacksonimmuno.com/catalog/products/515-035-003
Peroxidase-conjugated anti-mouse IgG	Sigma Aldrich	A9044	1:10,000; WB		https://www.sigmaaldrich.com/DE/de/product/sigma/a9044
Peroxidase-conjugated anti-rat IgG	Jackson ImmunoResearch	112-035-003	1:10,000; WB		https://www.jacksonimmuno.com/catalog/products/112-035-003
Peroxidase-conjugated anti-rabbit IgG	Sigma Aldrich	A0545	1:6,000; WB		https://www.sigmaaldrich.com/DE/de/product/sigma/a0545
Goat anti-rabbit IgG Alexa Fluor 488	Thermo Fisher Scientific	A-11034	1:800; IF		https://www.thermofisher.com/antibody/product/Goat-anti-Rabbit-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-11034
Goat anti-mouse IgG Alexa Fluor 488	Thermo Fisher Scientific	A-11001	1:800; IF		https://www.thermofisher.com/antibody/product/Goat-anti-Mouse-IgG-H-L-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-11001
Goat anti-rabbit IgG Alexa Fluor 546	Thermo Fisher Scientific	A-11035	1:800; IF		https://www.thermofisher.com/antibody/product/Goat-anti-Rabbit-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-11035
Goat anti-mouse IgG Alexa Fluor 647	Thermo Fisher Scientific	A-21236	1:800; IF		https://www.thermofisher.com/antibody/product/Goat-anti-Mouse-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-21236

DOI [to be added]

DNA/cDNA Clones

Clone Name	Sequence	Source / Repository	Persistent ID / URL

Cultured Cells

Name	Vendor or Source	Sex (F, M, or unknown)	Persistent ID / URL
Primary mouse cardiomyocytes	Jackson Labs	M	https://www.jax.org/strain/000664?featured
Primary rat cardiomyocytes	Envigo	M	https://www.inotivco.com/model/hsd-sprague-dawley-sd
hiPSC WT mTagRFP-T-TUBA1B	Allen Institute for Cell Science	M	AICS-0031 cl.35; https://www.allencell.org/cell-catalog.html
hiPSC SVBP-KO	In-house genome-editing of WT hiPSC (AICS-0031 cl.35)	M	
hiPSC TTL-KO	In-house genome-editing of WT hiPSC (AICS-0031 cl.35)	M	
hiPSC MYBPC3het	Patient derived cell line, in-house reprogramming	F	UKEi070-A
hiPSC MYBPC3ic	In-house genome-editing of MYBPC3het hiPSCs (UKEi070-A)	F	UKEi070-A1
hiPSC-derived cardiomyocytes	In-house production	M/F	

Data & Code Availability

Description	Source / Repository	Persistent ID / URL
RNA-seq data	NCBI's Gene Expression Omnibus (GEO) of the European Nucleotide Archive (ENA) at EMBL-EBI	GSE233851 (mice) GSE233852 for EHTs
Mass spectrometry proteomic data	ProteomeXchange Consortium via the PRIDE partner repository	PXD042418

Other

Description	Source / Repository	Persistent ID / URL
Endothelin-1	Sigma Aldrich	https://www.sigmaaldrich.com/DE/de/product/mm/05233800
Hoechst 33342	Thermo Fisher	https://www.thermofisher.com/order/catalog/product/de/de/H3570

DOI [to be added]

	Scientific	
SV Total RNA isolation kit	Promega	https://www.promega.de/products/nucleic-acid-extraction/rna/sv-total-rna-isolation-system/?catNum=Z3105
TRIzol	ThermoFisher Scientific	https://www.thermofisher.com/order/catalog/product/de/de/15596018
Clarity Western ECL substrate	Bio-Rad	https://www.bio-rad.com/de-de/sku/1705061-clarity-western-ecl-substrate-500-ml?ID=1705061
Direct-zol RNA Microprep Kit	Zymo Research	https://www.zymoresearch.de/products/direct-zol-rna-microprep-kits
NEBNext Ultra II RNA Directional Library Prep Kit for Illumina	New England BioLabs	https://www.neb-online.de/art/E7760
pAAV-GFP plasmid	Cellbiolabs	https://www.cellbiolabs.com/paav-gfp-control-plasmid
InFusion cloning kit	Takara Clontech	https://www.takarabio.com/learning-centers/cloning/in-fusion-cloning-general-information/in-fusion-cloning-overview
PrimeStar GLX Polymerase	Takara Clontech	https://www.takarabio.com/products/pcr/gc-rich-pcr/primestar-gxl-dna-polymerase
pHelper plasmid	Cellbiolabs	https://www.cellbiolabs.com/aav-2-packaging-system
SYPRO Ruby protein gel stain	ThermoFisher Scientific	https://www.thermofisher.com/order/catalog/product/S12000
Sodium deoxycholate	Sigma-Aldrich	https://www.sigmaaldrich.com/DE/de/product/sigma/30970
Trifluoroacetic acid	Sigma-Aldrich	https://www.sigmaaldrich.com/DE/de/product/sial/80457
Pierce BCA protein assay kit	Thermo Fisher Scientific	https://www.thermofisher.com/order/catalog/product/de/en/23227
96-well LoBind plate	Eppendorf SE	https://www.eppendorf.com/de-de/Produkte/Spitzen-Reaktionsgefäße-und-Platten/Platten/Eppendorf-twintec-PCR-Plates-LoBind-p-0030129512
LC-MS grade water	CHEMSOLUTE	https://shop-lolab.de/wasser/cso.455.1000.gf
Sera-Mag™ Carboxylate-Modified Magnetic Speed-Beads E3	Cytiva	https://www.cytivalifesciences.com/en/us/shop/molecular-and-immunodiagnostics/magnetic-beads-and-kits/sera-mag-speedbeads-and-sera-mag-carboxylate-modified-magnetic-particles-p-05936
Sera-Mag™ Carboxylate-Modified Magnetic Speed-Beads E7	Cytiva	https://www.cytivalifesciences.com/en/us/shop/molecular-and-immunodiagnostics/magnetic-beads-and-kits/sera-mag-speedbeads-and-sera-mag-carboxylate-modified-magnetic-particles-p-05936
Acetonitrile	CHEMSOLUTE	https://www.huberlab.ch/huberlab/de/CHF/Produkte/Acetonitril-gradient-grade-für-die-HPLC-%28min-99%2C9%25%29/p/2653.2500
Ethanol	Supelco	https://de.vwr.com/store/product/733083/ethanol-99-9-durch-gc-lichrosolv-gradienten-qualitat-fur-die-flussigchromatographie-supelco
Ammonium bicarbonate	Sigma-Aldrich	https://www.sigmaaldrich.com/DE/de/product/sial/09830
Sequencing grade modified trypsin	Promega	https://www.promega.de/products/mass-spectrometry/trypsin/sequencing-grade-modified-trypsin/?catNum=V5111
Iodoacetamide	Sigma-Aldrich	https://www.sigmaaldrich.com/DE/de/product/sigma/i6125
Dithiothreitol	Roche	https://www.sigmaaldrich.com/DE/de/product/roche/dttro
Formic acid	Fisher Chemical	https://www.fishersci.de/shop/products/formic-acid-99-0-optima-lc-ms-grade-fisher-chemical/10780320
PepMap™ Neo Trap Cartridge	Thermo Fisher	https://www.thermofisher.com/order/catalog/product/de/de/174500
peptide BEH C18, nanoEase	Waters	https://www.waters.com/nextgen/de/de/shop/columns/186008795-nanoease-m-z-peptide-beh-c18-column-130a-17--m-75--m-x-250-mm-1-.html

DOI [to be added]

Andrew+ Pipetting Robot	Waters	https://www.waters.com/waters/library.htm?locale=en_US&lid=135049871
Exploris 480	Thermo Fisher	https://www.thermofisher.com/order/catalog/product/de/de/BRE725533
VanquishTM neo UHPLC system	Thermo Fisher	https://www.thermofisher.com/order/catalog/product/de/de/VN-S10-A-01

DOI [to be added]

ARRIVE GUIDELINES

The ARRIVE guidelines (https://arriveguidelines.org/) are a checklist of recommendations to improve the reporting of research involving animals. Key elements of the study design should be included below to better enable readers to scrutinize the research adequately, evaluate its methodological rigor, and reproduce the methods or findings.

Study Design

Groups	Sex	Age	Number (prior to experiment)	Number (after termination)	Littermates (Yes/No)	Other description
WT-AAV9-Empty	M/F	3-week-old	12	11	Yes	
KI-AAV9-Empty	M/F	3-week-old	12	11	Yes	
KI-AAV9-TTL	M/F	3-week-old	12	11	Yes	

Sample Size: Please explain how the sample size was decided. Please provide details of any a *prior* sample size calculation, if done.

The experimental procedures performed in KI and WT mice and the sample size were in accordance with the German Law for the Protection of Animals and accepted by the Ministry of Science and Public Health of the City State of Hamburg, Germany (Nr. 074-19). The number of required animals was based on assuming a variance of 20% (measurement inaccuracy and biological variability within a group) and an impairment of the main echocardiographic parameter, which is left ventricular fractional area shortening to 65% of the initial value of the WT-Empty group in the KI-Empty, as well as an improvement through treatment to 85 % of the initial value in the KI-TTL group, a significance level of 0.05 and a statistical power of 0.95. This power calculation resulted in the required number of animals of 36. These 36 animals are distributed evenly among the three groups mentioned. The calculation was carried out using the G-Power 3.1.7 software (University of Düsseldorf).

Inclusion Criteria

This study included any male and female mice available at the beginning of the study.

Exclusion Criteria

None.

Randomization

None.

Blinding

Yes. Hemodynamic measurements were blinded for the groups.