Supplemental Materials

Long-term efficacy and safety of cardiac genome editing for catecholaminergic polymorphic ventricular tachycardia

Oliver M. Moore[1,2,3], Yuriana Aguilar-Sanchez[1,2], Satadru K. Lahiri[1,2], Mohit M. Hulsurkar[1,2], J. Alberto Navarro-Garcia[1,2], Tarah A. Word[1,2], Joshua A. Keefe[1,2], Dean Barazi[1,2], Elda M. Munivez[1,2], Charles T. Moore[1,2], Vaidya Parthasarathy[1,2], Jayso'n Davidson[1,2], William R. Lagor[1,2], So Hyun Park[4], Gang Bao[4], Christina Y. Miyake[5], Xander H.T. Wehrens[1,2,3,5,6,7]

[1]Cardiovascular Research Institute, Baylor College of Medicine, Houston, TX 77030, USA.

[2]Department of Integrative Physiology, Baylor College of Medicine, Houston, TX 77030, USA.

[3]Department of Neuroscience, Baylor College of Medicine, Houston, TX 77030, USA.

[4]Department of Bioengineering, Rice University, Houston, TX 77030, USA.

[5]Department of Pediatrics (Cardiology), Baylor College of Medicine, Houston, TX 77030, USA.

[6]Department of Medicine (Cardiology), Baylor College of Medicine, Houston, TX 77030, USA.

[7]Center for Space Medicine, Baylor College of Medicine, Houston, TX 77030, USA. SA

Supplemental Methods

Adeno-Associated Virus (AAV) Plasmids. The AAV-CRISPR control vector, pAAV-U6-SA-BbsI-TNT-SACas9-HA-OLLAS-spA was generated through subcloning of the human TNT promoter into plasmid 109320 (Addgene, Watertown, MA). The AAV-CRISPR vector contains a site for cloning custom gRNA. CRISPR SaCas9 guide RNA (gRNA) was designed to target the *Ryr2* g.GT528-527AA mutation site. The guide was

chosen based on its proximity to a SaCas9 recognition sequence, NNGRR, and containment of the mutation site within the 3'-end of the gRNA to minimize off target editing of the wild-type allele. Oligos for the gRNA were annealed and cloned into the BbsI restriction digest site using the following oligo sequences: 5'-CACC gCCGAAGGAGAAAAAGTGCAAG-3', and 5'-AAAC CTTGCACTTTTTCTCCTTCGGc-3'. The final guide RNA vector pAAV-U6-SA-gRNA-TNT-SACas9-HA-OLLAS-spA was Sanger sequenced at the cloning sites, and the inverted terminal repeats (ITRs) were verified by restriction digest. Sequences of plasmids used to generate the AAVs are listed in Supplemental Figure 1.

Adeno-Associated Virus type 9 (AAV9) Production and Purification. AAV9 was packaged in the HEK293T cells (ATCC) using the triple transfection method of Xiao *et al.*[1]. The Adenoviral helper plasmid pAdDeltaF6 (PL-F-PVADF6) and the AAV9 packaging vector pAAV2/9 (PL-T-P0008-R2) were obtained from the University of Pennsylvania Vector Core. Large-scale plasmid preps of these packaging vectors were generated by Puresyn, Inc (Malvern, PA). The control and guide RNA vectors described above were used for the AAV production. Briefly, 12 hours before transfection, cells were seeded in 150 mm plates (30-50% confluent) fed with DMEM (#BE15-604D, Lonza, Morristown, NJ) containing 10% fetal bovine serum (#A5670701, Gibco, Grand Island, NY) with L-Glutamine and Penicillin/Streptomycin (#10378016, Gibco, Grand Island, NY).

After 48-72 hours, cells were processed by TrypLE (#12604013, Gibco, Grand Island, NY), collected in PBS (#10010023, Corning, Corning, NY) and resuspended in

resuspension buffer (50 mM Tris–HCl pH 8.0, 150 mM NaCl, 2 mM $MgCl_2$). The cells were subjected to 3 freeze-thaw cycles at -80°C for 10 minutes and 37°C for 20 minutes. Cells were added with 3,000 U Benzonase (#9025-65-4, MilliporeSigma, Burlington, MA) and incubated at 37°C for 1 hour to digest cellular genomic DNA. The suspension was treated with $1/39^{th}$ volume of 1M $CaCl_2$ solution and 2/3 volume of 20% PEG 8000/1.25N NaCl to remove the cell debris and precipitate AAV. AAV was then resuspended in HBS and purified by $CsCl_2$ gradient. After a round of $CsCl_2$ gradient at 45,000 rpm and 60,000 rpm, fractions were collected according to the desired refractive index, respectively, 1.3680-1.3750 for the first gradient and 1.3680-1.3750 for the second gradient.

AAV was dialyzed against PBS in 10,000 MWCO Slide-A-Lyzer Cassettes (#A52971, ThermoFisher, Waltham, MA), and concentrated using Amicon 100 kDa MWCO centrifugal filtration device (# UFC910008, MilliporeSigma, Burlington, MA) prior to storage at -80°C. AAVs were tittered following DNAse digestion and using ABM's qPCR AAV Titer Kit (G931, ABM, Richmond, BC). AAV transgene plasmids were serially diluted to generate standard curves, and the genomic copy numbers per mL were calculated for each virus [2].

Next-Generation Deep Sequencing. Total genomic DNA was isolated from ventricular tissues by TRIzol (#15596, Life Technologies, Carlsbad, CA) and reverse transcribed by iScript (#1708841, Bio-Rad, Hercules, CA). *Ryr2* genomic DNA was amplified with primers targeting upstream and downstream of the mutation site by Phusion High-Fidelity DNA Polymerase (#M0530S, New England Biolabs, Ipswich, MA). Primers used: Fw: 5'-CGTCATCTGGAGAGGGAATG-3', Rev: 5'-CTCTCAGCAGAGCATCAAGC-3'.

Adapters were added through PCR. All amplicons were purified using the Wizard PCR Clean-up system (#A9282, Promega, Madison, WI). Samples were sent to Azenta (Burlington, MA) for next-generation amplicon sequencing.

Paired-end Illumina reads were merged using FLASH (Fast Length Adjustment of Short reads) [3]. WT and mutant alleles were filtered based on WT and mutant allele-specific SNPs using SeqKit [4]. WT or mutant reads were analyzed separately for editing frequency using CRISPResso2 [5]. Percentages of WT or mutant reads, and Indel frequency on WT or mutant reads were reported, respectively.

RT-PCR. Total RNA was isolated from ventricular tissues by TRIzol (#15596, Life Technologies, Carlsbad, CA) and was reverse transcribed by iScript (#1708841, Bio-Rad; Hercules, CA). The iTaq Universal SYBR Green Supermix (Thermo Fisher Scientific, Waltham, MA) was used for the quantitative polymerase chain reaction (PCR) analysis of diluted cDNA in 1:10. The $\Delta\Delta$CT method was used to calculate relative quantities normalized to L7. Primers used: RyR2 Fw: 5'-TCAAACCACGAACACATTGAGG-3', RyR2 Rev: 5'-AGGCGGTAAAACATGATGTCAG-3', Jph2 Fw: 5'-ACTCTGGCTCCTGGAACTTTG-3', Jph2 Rev: 5'-GCGCCCCTTGGTCTCTATG-3', RPL7 Fw: 5'-AAGATCAAGCGCCTGAGAAAG-3', RPL7 Rev: 5'-TGCAGGTACATAGAAGTTGCCA-3', SaCas9 Fw: 5'-GTACTACGAGGAAACCGGGAAC-3', SaCas9 Rev: 5'-GTTGTTGTAGAAGGAGGCGATAAAC-3', U6 Fw: 5'-CCTTCATATTTGCATATACGATACAAGG CTGTTAG-3'.

Western Blotting. Ventricular heart samples were crushed in liquid nitrogen and resuspended in RIPA buffer containing 1% CHAPS, Phos-STOP (#4906837001, Sigma-Aldrich; St. Louis, MO) and cOmplete Mini protease inhibitor cocktail (#4693124001, Sigma-Aldrich; St. Louis, MO), 20mM sodium fluoride (NaF), 1mM Na_3VO_4. Lysates were further homogenized with steel beads using a homogenizer (Tissue Lyser LT; Qiagen, Germantown, MD) at 50-Hz for 8 minutes. These samples were sonicated 3 times for 2 seconds each and centrifuged at 14,000 rpm for 20 mins at 4°C. Supernatants were collected as lysates, and protein concentration was measured using a NanoDrop spectrophotometer (Thermo Fisher Scientific, Waltham, MA). Human heart, mouse heart, or cell lysates (75 mg) were denatured in 2x Laemmli sample buffer (#1610737, Biorad; Hercules, CA) with 5% 2-mercaptoethanol (#M3148, Sigma-Aldrich; St. Louis, MO) for 10 mins at 70°C. SDS gel electrophoresis was done on 10-12% acrylamide gel at 100 Volts. Proteins were further transferred onto 0.45-micron polyvinylidene fluoride (PVDF) membranes for 1.5h at 100 Volts. Membranes were blocked for 1 h at room temperature and incubated overnight at 4°C with primary antibodies diluted in blocking buffer. Primary antibodies used were RYR2 (1:2000, Custom, Yenzyme, Rabbit) and GAPDH (1:10,000; mouse monoclonal; #MAB374, EMD Millipore, Burlington, MA). Membranes were washed 3 times with TBST (0.1% tween-20) for 10 minutes each and incubated with secondary antibody (1:10,000) for 1 h at room temperature. Secondary antibodies used were anti-mouse-Alexa-Fluor-680 (1:10,000, goat polyclonal; #A-21057, Thermo Fisher Scientific, Waltham, MA) and anti-rabbit-IR800 (1:10,000, goat polyclonal; #A-32735, Thermo Fisher Scientific, Waltham, MA). After washing 3x10 minutes in TBST,

membranes were developed using LICOR Odyssey infrared imager (LICOR, Lincoln, NE). Bands were quantified using the ImageJ software and normalized to GAPDH levels.

Programmed Electrical Stimulation (PES) Studies. Electrophysiology studies were performed in R176Q/+ mice and wild-type littermates at 6 weeks or 12 months post AAV9 injection, as described [2]. First, anesthesia was induced by placing the mouse inside an induction chamber filled with 2% isoflurane (#029405, Covetrus, Houston, TX) in 100% O_2. Following induction, the mouse was transferred to a heated platform (Indus Instruments, Houston, TX) where the body temperature was maintained at 36.5-37.0°C and anesthesia (1.5% isoflurane in 100% O2 was maintained via a nose cone). The surface ECG was monitored using electrodes embedded in the heated board, connected to the external stimulator in recording mode (STG3008, MultiChannel Systems, Reutlingen, Germany), and acquired using the IOX2.4 acquisition software (Emka Technologies, Sterling, VA). A 0.5-inch incision was created to the right of the midline at the level of the clavicle. Then, subcutaneous tissue, salivary glands, and lymphatic tissues were separated with the help of blunt forceps to expose the right jugular vein. A tunnel was made under the jugular to cross a 6-0 suture to tie the proximal end of the vein and place another suture at the distal end of the visualized edge. While pulling gently on the suture to keep it straight, a small incision was made with the help of micro-scissors in the jugular and a 1.1 F octapolar catheter (EPR-800, Millar, Houston, TX) was inserted through it. The catheter was gently pulled, and the proper position of the catheter was verified by the waveforms of the 4 intracardiac electrocardiograms, as described [6].

Baseline ECG parameters (RR, HR, PR, QRS, and QTc) were measured in R176Q/+ mice and WT littermates that were treated with either control virus (AAV9-SaCas9/Bbsl) or editing virus (AAV9-SaCas9/gRNA). To mimic adrenergic stimulation, mice were injected with isoproterenol (2 mg/kg, i.p.) and caffeine (120 mg/kg, i.p.). To determine the inducibility of ventricular arrhythmias (VA) after adrenergic stimulation, programmed intracardiac stimulation was performed 10 minutes after the injections. All mice were subjected to the S1-S2 extra stimuli pacing at a cycle length of 70 ms. In some mice, protocols were repeated at 90 ms cycle length. VA was defined as greater than 10 consecutive ventricular beats at a rate faster than 600 bpm. The incidence of inducible VA was calculated by the number of mice with reproducible VA divided by the total number of mice studied in each group.

Calcium Imaging Studies. Mouse hearts were excised, cannulated, and perfused retrogradely using a heated Langendorff system via the aorta, as described [7]. The perfusion buffer consisted of Ca^{2+}-free Tyrode solution containing (mM): 140 NaCl, 5.4 KCl, 1 $MgCl_2$, 5 HEPES, and 10 Glucose, pH 7.4, supplemented with 22µg/mL Liberase TH (#5401119001, Sigma-Aldrich, St. Louis, MO). After digesting, hearts were perfused with 5mL Kraft-Brühe (KB) solution containing (mM): 90 KCl, 30 K_2HPO_4, 5 $MgSO_4$, 5 pyruvic acid, 5 B-hydroxybutyric acid, 5 creatine, 20 taurine, 0.5 EGTA, 5 HEPES, 10 glucose, pH 7.2. Left ventricular tissue was teased apart and strained through a 250µm nylon mesh to isolate single cells. Ventricular cardiomyocytes were re-adapted to 1.8mM Ca^{2+} as described[8], and loaded for 30 min with 2µM Fluo-4-AM (F14201, Invitrogen, Carlsbad, CA) for Ca^{2+} imaging. Cells were plated onto laminin-coated (#354232, Corning,

Corning, NY) coverslips and imaged using an LSM880 confocal microscope (Carl Zeiss, Thornwood, NY, USA) in line scan mode with 1024 pixels per line at 813Hz acquisition using a 40X objective. Cardiomyocytes were paced at 1-Hz for 15s, unstimulated for the 60s, and then perfused with 10mM caffeine to assess SR load. Ca^{2+} transient amplitude and kinetics were analyzed using pCLAMP10 (Molecular Devices, San Jose, CA, USA). The Ca^{2+} spark frequency (CaSpF) was analyzed using the SparkMaster ImageJ plugin [9].

Echocardiography. Chest hair was removed using Nair cream before imaging. Mice were then anesthetized with an isoflurane concentration ranging from 1.5% to 2.0% mixed with 100% oxygen. To keep their body temperatures within a physiological range (36.5°C to 37.5°C), mice were placed on a temperature-regulated platform, and body temperature was monitored using a rectal probe [10]. Cardiac function was assessed through ultrasound echocardiography, using both the Vevo 2100 and Vevo F2 systems (Fujifilm VisualSonics, Toronto, ON, Canada) with a probe operating at 30 MHz and 57 MHz, respectively. Both B-mode and M-mode images were recorded in short and long-axis views of the hearts. Short-axis M-mode echocardiograms were subsequently analyzed with the VevoLab software to measure systolic function and cardiac chamber dimensions. Long-axis B-mode images were analyzed to assess left atrial size.

Supplemental Table 1. ECG parameters at baseline and after isoproterenol at 6 weeks post-AAV9.

Baseline	WT	RQ-Con	P (WT vs. RQ-Con)	RQ-Edited	P (RQ-Con vs. RQ-gRNA)
N (mice)	7	7		7	
HR (bpm)	524.8 ± 16.6	526.3 ± 11.9	0.999	517.9 ± 11.0	0.999
P wave (ms)	16.1 ± 0.82	17.1± 1.11	0.999	18.1 ± 0.67	0.638
PR interval (ms)	38.4 ± 0.78	36.9 ± 1.19	0.999	36.6 ± 2.55	0.999
QRS interval (ms)	13.4 ± 0.34	12.5 ± 0.52	0.601	11.2 ± 0.31	0.192
QT interval (ms)	47.8 ± 1.37	52.9 ± 2.49	0.304	57.6 ± 2.54	0.633
QTc interval (ms)	44.6 ± 1.32	49.5 ± 2.16	0.363	53.4 ± 2.16	0.589
ISO + Caffeine	**WT**	**RQ-Con**	**P (WT vs. RQ-Con)**	**RQ-Edited**	**P (RQ-Con vs. RQ-gRNA)**
N (mice)	7	7		7	
HR (bpm)	699.0 ± 9.3	588.8 ± 10.6	**>0.001**	663.0 ± 16.8	0.036
P wave (ms)	15.1 ± 0.98	15.1 ± 1.10	0.999	15.6 ± 1.33	0.999
PR interval (ms)	32.4 ± 0.54	40.1 ± 1.35	**0.001**	34.2 ± 0.66	**0.042**
QRS interval (ms)	11.8 ± 0.52	11.3 ± 0.32	0.823	12.1 ± 0.31	0.028
QT interval (ms)	40.4 ± 1.11	49.4 ± 2.23	**0.001**	45.3 ± 2.14	0.507
QTc interval (ms)	43.6 ± 0.98	48.9± 1.99	0.070	47.5 ± 1.86	0.999

HR: Heart rate; ISO: isoproterenol. The Kruskal-Wallis test, followed by Dunn's multiple comparison post hoc test, was performed to compare the WT vs RQ control (Con) or RQ control vs RQ gRNA groups. The bolded P values indicate statistical significance (P<0.05).

Supplemental Table 2. Ca^{2+} transient and spark parameters measured in isolated cells from WT and RQ-treated mice with Ctr and gRNA.

Spark Parameters	WT	RQ-Con	P (WT vs. RQ-Con)	RQ-Edited	P (WT vs. RQ-gRNA)	P (RQ-Con vs. RQ-gRNA)
N (mice)	5	6		6		
n (cells)	20	20		17		
CaSpF	1.05 ± 0.21	8.02 ± 1.59	**<0.001**	2.16 ± 0.47	0.723	**<0.001**
Ca^{2+} spark amplitude (F/F0)	0.53 ± 0.07	0.99 ± 0.08	**<0.003**	1.19 ± 0.09	**<0.001**	0.273
Spark FWHM (µm)	0.99 ± 0.06	1.17 ± 0.07	0.498	0.88 ± 0.05	0.531	0.099
Spark FDHM (ms)	13.05 ± 1.09	15.84 ± 1.33	0.468	12.16 ± 0.70	0.835	0.217
Spark Full Width (µm)	1.60 ± 0.17	2.08 ± 0.19	0.444	1.74± 0.18	0.881	0.738
Spark Full Duration (ms)	20.75 ± 2.32	31.36 ± 3.97	0.107	23.20 ± 2.27	0.879	0.264
Spark Tau (ms)	17.22 ± 2.19	23.01 ± 2.27	0.542	24.88 ± 6.74	0.377	0.943
Spark TtP (ms)	9.70 ± 1.36	11.40 ± 1.23	0.758	9.09 ± 1.04	0.929	0.554

FDHM, full duration at half-maximum; FWHM, full width at half-maximum; Tau, decay rate; TtP, time to peak. Data presented as mean ± SEM. Statistics based on Nested, one-way ANOVA. The bolded P values indicate statistical significance (P<0.05).

Supplemental Table 3. Overview of echocardiography parameters.

4-months	WT	RQ-Con	P (WT vs. RQ-Con)	RQ-Edited	P (RQ-Con vs. RQ-gRNA)
N (mice)	9	7		7	
Heart Rate (bpm)	528.4 ± 13.3	486.4 ± 18.8	0.205	470.3 ± 32.6	0.905
LVESD (mm)	2.36 ± 0.10	2.59 ± 0.08	0.181	2.47 ± 0.12	0.661
LVEDD (mm)	3.80 ± 0.09	3.96 ± 0.09	0.462	3.84 ± 0.12	0.692
LVESV (uL)	19.8 ± 2.09	24.7 ± 1.73	0.206	22.2 ± 2.65	0.709
LVEDV (uL)	62.4 ± 3.74	68.8 ± 3.84	0.480	63.9 ± 4.83	0.721
SV (uL)	42.6 ± 2.04	44.1 ± 2.38	0.884	41.8 ± 2.54	0.792
LVEF (%)	68.7 ± 1.70	64.16 ± 1.24	0.113	65.9 ± 1.98	0.752
LVFS (%)	38.0 ± 1.30	34.7 ± 0.91	0.134	35.8 ± 1.53	0.816
LV Mass (mg)	22.5 ± 1.21	21.4 ± 1.30	0.059	19.5 ± 1.47	0.051
LV Mass Corrected (mg)	91.3 ± 4.07	109.5 ± 5.70	0.059	87.7 ± 5.92	0.054
CO (mL/min)	73.1 ± 3.25	87.6 ± 4.56	0.807	70.3 ± 4.81	0.602
LVAW;s (mm)	0.74 ± 0.02	0.87 ± 0.03	**0.016**	0.75 ± 0.04	0.108
LVAW;d (mm)	0.67 ± 0.03	0.79 ± 0.05	0.156	0.71 ± 0.03	0.438
LVPW;s (mm)	0.97 ± 0.06	0.97 ± 0.04	0.988	0.91 ± 0.05	0.609
LVPW;d (mm)	0.74 ± 0.02	0.75 ± 0.05	0.980	0.64 ± 0.04	0.275
8-months	**WT**	**RQ-Con**	**P (WT vs. RQ-Con)**	**RQ-Edited**	**P (RQ-Con vs. RQ-gRNA)**
N (mice)	9	7		7	
Heart Rate (bpm)	507.1 ± 15.2	541.3 ± 7.4	0.151	487.3 ± 24.0	0.147
LVESD (mm)	2.42 ± 0.09	2.5 ± 0.13	0.875	2.36 ± 0.09	0.691
LVEDD (mm)	3.88 ± 0.14	3.93 ± 0.17	0.966	3.72 ± 0.16	0.649

LVESV (uL)	21.0 ± 1.95	23.0 ± 2.99	0.844	19.7 ± 1.98	0.649
LVEDV (uL)	66.1 ± 5.48	68.6 ± 6.87	0.955	60.0 ± 6.16	0.630
SV (uL)	45.1 ± 3.70	45.5 ± 4.43	0.997	40.3 ± 4.48	0.691
LVEF (%)	68.3 ± 0.91	66.9 ± 1.77	0.758	67.0 ± 1.26	0.999
LVFS (%)	37.6 ± 0.72	36.6 ± 1.26	0.758	36.6 ± 1.01	>0.999
LV Mass (mg)	22.5 ± 1.35	24.7 ± 2.52	0.965	19.9 ± 2.80	>0.999
LV Mass Corrected (mg)	109.5 ± 9.73	113.2 ± 11.2	0.966	113.3 ± 11.7	>0.999
CO (mL/min)	87.6 ± 7.78	90.6 ± 8.98	0.730	90.7 ± 9.34	0.435
LVAW;s (mm)	0.79 ± 0.04	0.89 ± 0.07	0.424	0.84 ± 0.05	0.823
LVAW;d (mm)	0.80 ± 0.03	0.82 ± 0.05	0.917	0.80 ± 0.04	0.951
LVPW;s (mm)	0.97 ± 0.05	0.96 ± 0.04	0.986	0.99 ± 0.04	0.791
LVPW;d (mm)	0.76 ± 0.05	0.75 ± 0.05	0.988	0.87 ± 0.06	0.271
12-months	**WT**	**RQ-Con**	**P (WT vs. RQ-Con)**	**RQ-Edited**	**P (RQ-Con vs. RQ-gRNA)**
N (mice)	9	7		7	
Heart Rate (bpm)	500.8 ± 16.3	502.3 ± 14.9	0.997	514.4 ± 9.7	0.778
LVESD (mm)	2.33 ± 0.15	2.58 ± 0.13	0.440	2.33 ± 0.11	0.370
LVEDD (mm)	3.68 ± 0.18	4.02 ± 0.16	0.358	3.76 ± 0.20	0.587
LVESV (uL)	19.8 ± 3.18	24.8 ± 2.93	0.497	19.3 ± 2.19	0.327
LVEDV (uL)	59.1 ± 6.83	71.7 ± 6.61	0.406	62.1 ± 7.62	0.621
SV (uL)	39.3 ± 3.80	46.9 ± 4.23	0.402	42.8 ± 5.48	0.828
LVEF (%)	67.6 ± 1.58	65.9 ± 1.99	0.780	68.8 ± 0.63	0.393
LVFS (%)	36.9 ± 1.14	36.0 ± 1.47	0.875	37.8 ± 0.60	0.522
LV Mass (mg)	19.4 ± 1.66	23.4 ± 1.79	0.150	21.9 ± 2.60	0.611
LV Mass Corrected (mg)	100.8 ± 9.13	124.4 ± 7.58	**0.015**	110.9 ± 11.7	0.611

CO (mL/min)	69.5 ± 6.92	99.5 ± 6.06	0.273	88.8 ± 9.32	0.890
LVAW;s (mm)	0.75 ± 0.02	0.94 ± 0.05	**0.027**	1.02 ± 0.05	0.563
LVAW;d (mm)	0.78 ± 0.03	0.87 ± 0.06	0.496	0.83 ± 0.02	0.877
LVPW;s (mm)	1.00 ± 0.04	0.97 ± 0.03	0.882	0.98 ± 0.07	>0.999
LVPW;d (mm)	0.78 ± 0.02	0.79 ± 0.02	0.926	0.79 ± 0.04	1.000

Bpm, beats per minute; LVESD, left ventricular end-systolic diameter; LVEDD, left ventricular end-diastolic diameter; LVESV, left ventricular end-systolic volume; LVEDV, left ventricular end-diastolic volume; SV, stroke volume; LVEF, left ventricular ejection fraction; LVFS, left ventricular fractional shortening; CO, cardiac output; LVAW;s, left ventricular anterior wall diameter in systole; LVAW;d, left ventricular anterior wall diameter in diastole; LVPW;s, left ventricular posterior wall diameter in systole; LVPW;d, left ventricular posterior wall diameter in diastole. One-way ANOVA with Tukey's post hoc test was performed to compare the WT vs RQ control (Con) or RQ control vs RQ gRNA groups. The bolded P values indicate statistical significance ($P<0.05$).

Supplemental Table 4. ECG parameters at baseline and after isoproterenol at 12 months post-AAV9.

Baseline	WT	RQ-Con	P (WT vs. RQ-Con)	RQ-Edited	P (RQ-Con vs. RQ-gRNA)
N (mice)	6	7		5	
HR (bpm)	550.8 ± 5.9	503.1 ± 15.2	**0.040**	524.6 ± 16.8	0.977
P wave (ms)	16.1 ± 0.82	17.1 ± 1.11	0.999	18.1 ± 0.67	0.638
PR interval (ms)	49.2 ± 2.52	44.8 ± 1.61	0.490	50.7 ± 1.86	0.119
QRS interval (ms)	20.5 ± 0.98	21.8 ± 1.05	0.912	24.6 ± 0.92	0.159
QT interval (ms)	49.0 ± 1.13	49.9 ± 2.38	0.999	49.2 ± 1.98	0.999
QTc interval (ms)	45.9 ± 1.85	46.7 ± 2.21	0.999	49.9 ± 2.94	0.999
ISO + Caffeine	**WT**	**RQ-Con**	**P (WT vs. RQ-Con)**	**RQ-Edited**	**P (RQ-Con vs. RQ-gRNA)**
N (mice)	6	7		5	
HR (bpm)	640.2 ± 9.9	610.4 ± 14.9	0.269	668.8 ± 9.8	**0.012**
P wave (ms)	15.1 ± 0.98	15.1 ± 1.10	0.999	15.6 ± 1.33	0.999
PR interval (ms)	24.3 ± 4.67	27.2 ± 4.40	0.999	29.0 ± 5.63	0.999
QRS interval (ms)	20.0 ± 0.90	22.5 ± 1.55	0.847	22.1 ± 2.61	0.999
QT interval (ms)	44.7 ± 1.82	49.5 ± 2.39	0.847	46.7 ± 2.38	0.904
QTc interval (ms)	46.7 ± 0.66	48.6 ± 2.07	0.999	45.9 ± 1.25	0.998

HR: Heart rate; ISO: isoproterenol. The Kruskal-Wallis test, followed by Dunn's multiple comparison post hoc test, was performed to compare the WT vs RQ control (Con) or RQ control vs RQ gRNA groups. The bolded P values indicate statistical significance ($P<0.05$).

Supplemental Figure 1. Sequence of CRISPR/Cas9 genome editing and control plasmids used to generate adeno-associated viral vectors.

A. pAAV-U6-BbsI-gRNA-TNT-SACas9-HA-OLLAS-spA (control plasmid)

Legend: ITR, U6 Promoter, BbsI cloning site, Guide RNA Scaffold, Human Troponin T Promoter, NLS, SaCas9

cagctgcgcgctcgctcgctcactgaggccgcccgggcaaagcccgggcgtcgggcgacctttggtcgcccggcctcagtgagcgagcgagcgcgcagaga
gggagtggccaactccatcactaggggttccttgtagttaatgattaacccgccatgctacttatctacgtagccatgctctggTGTACAAAAAAGCAG
GCTTTAAAGGAACCAATTCAGTCGACTGGATCCGGTACCAAGGTCGGGCAGGAAGAGGGCCTATT
TCCCATGATTCCTTCATATTTGCATATACGATACAAGGCTGTTAGAGAGATAATTAGAATTAATTT
GACTGTAAACACAAAGATATTAGTACAAAATACGTGACGTAGAAAGTAATAATTTCTTGGGTAGT
TTGCAGTTTTAAAATTATGTTTTAAAATGGACTATCATATGCTTACCGTAACTTGAAAGTATTTCGA
TTTCTTGGCTTTATATATCTTGTGGAAAGGACGAAACACCgggtcttcacgcgcgtgaagacCagttttagtactctggaaaca
gaatctactaaaacaaggcaaaatgccgtgtttatctcgtcaacttgttggcgagattttttTCTAGACTCAGTCCATTAGGAGCCAGTAG
CCTGGAAGATGTCTTTACCCCCAGCATCAGTTCAAGTGGAGCAGCACATAACTCTTGCCCTCTGCC
TTCCAAGATTCTGGTGCTGAGACTTATGGAGTGTCTTGGAGGTTGCCTTCTGCCCCCCAACCCTGCT
CCCAGCTGGCCCTCCCAGGCCTGGGTTGCTGGCCTCTGCTTTATCAGGATTCTCAAGAGGGACAGC
TGGTTTATGTTGCATGACTGTTCCCTGCATATCTGCTCTGGTTTTAAATAGCTTATCTGAGCAGCTG
GAGGACCACATGGGCTTATATGGCGTGGGGTACATGTTCCTGTAGCCTTGTCCCTGGCACCTGCCA
AAATAGCAGCCAACACCCCCCACCCCCACCGCCATCCCCCTGCCCCACCCGTCCCCTGTCGCACAT
TCCTCCCTCCGCAGGGCTGGCTCACCAGGCCCCAGCCCACATGCCTGCTTAAAGCCCTCTCCATCC
TCTGCCTCACCCAGTCCCCGCTGAGACTGAGCAGACGCCTCCAGGATCTGTCGGCAGccATGGCCC
CAAAGAAGAAGCGGAAGGTCGGTATCCACGGAGTCCCAGCAGCCAAGCGGAACTACATCCTGGG
CCTGGACATCGGCATCACCAGCGTGGGCTACGGCATCATCGACTACGAGACACGGGACGTGATCG
ATGCCGGCGTGCGGCTGTTCAAAGAGGCCAACGTGGAAAACAACGAGGGCAGGCGGAGCAAGAG
AGGCGCCAGAAGGCTGAAGCGGCGGAGGCGGCATAGAATCCAGAGAGTGAAGAAGCTGCTGTTC
GACTACAACCTGCTGACCGACCACAGCGAGCTGAGCGGCATCAACCCCTACGAGGCCAGAGTGAA
GGGCCTGAGCCAGAAGCTGAGCGAGGAAGAGTTCTCTGCCGCCCTGCTGCACCTGGCCAAGAGAA
GAGGCGTGCACAACGTGAACGAGGTGGAAGAGGACACCGGCAACGAGCTGTCCACCAAAGAGCA
GATCAGCCGGAACAGCAAGGCCCTGGAAGAGAAATACGTGGCCGAACTGCAGCTGGAACGGCTG
AAGAAAGACGGCGAAGTGCGGGGCAGCATCAACAGATTCAAGACCAGCGACTACGTGAAAGAAG
CCAAACAGCTGCTGAAGGTGCAGAAGGCCTACCACCAGCTGGACCAGAGCTTCATCGACACCTAC
ATCGACCTGCTGGAAACCCGGCGGACCTACTATGAGGGACCTGGCGAGGGCAGCCCCTTCGGCTG
GAAGGACATCAAAGAATGGTACGAGATGCTGATGGGCCACTGCACCTACTTCCCCGAGGAACTGC
GGAGCGTGAAGTACGCCTACAACGCCGACCTGTACAACGCCCTGAACGACCTGAACAATCTCGTG
ATCACCAGGGACGAGAACGAGAAGCTGGAATATTACGAGAAGTTCCAGATCATCGAGAACGTGTT
CAAGCAGAAGAAGAAGCCCACCCTGAAGCAGATCGCCAAAGAAATCCTCGTGAACGAAGAGGAT
ATTAAGGGCTACAGAGTGACCAGCACCGGCAAGCCCGAGTTCACCAACCTGAAGGTGTACCACGA
CATCAAGGACATTACCGCCCGGAAAGAGATTATTGAGAACGCCGAGCTGCTGGATCAGATTGCCA
AGATCCTGACCATCTACCAGAGCAGCGAGGACATCCAGGAAGAACTGACCAATCTGAACTCCGAG
CTGACCCAGGAAGAGATCGAGCAGATCTCTAATCTGAAGGGCTATACCGGCACCCACAACCTGAG
CCTGAAGGCCATCAACCTGATCCTGGACGAGCTGTGGCACACCAACGACAACCAGATCGCTATCT
TCAACCGGCTGAAGCTGGTGCCCAAGAAGGTGGACCTGTCCCAGCAGAAAGAGATCCCCACCACC
CTGGTGGACGACTTCATCCTGAGCCCCGTCGTGAAGAGAAGCTTCATCCAGAGCATCAAAGTGAT
CAACGCCATCATCAAGAAGTACGGCCTGCCCAACGACATCATTATCGAGCTGGCCCGCGAGAAGA
ACTCCAAGGACGCCCAGAAAATGATCAACGAGATGCAGAAGCGGAACCGGCAGACCAACGAGCG
GATCGAGGAAATCATCCGGACCACCGGCAAAGAGAACGCCAAGTACCTGATCGAGAAGATCAAG
CTGCACGACATGCAGGAAGGCAAGTGCCTGTACAGCCTGGAAGCCATCCCTCTGGAAGATCTGCT
GAACAACCCCTTCAACTATGAGGTGGACCACATCATCCCCAGAAGCGTGTCCTTCGACAACAGCT

TCAACAACAAGGTGCTCGTGAAGCAGGAAGAAAACAGCAAGAAGGGCAACCGGACCCCATTCCA
GTACCTGAGCAGCAGCGACAGCAAGATCAGCTACGAAACCTTCAAGAAGCACATCCTGAATCTGG
CCAAGGGCAAGGGCAGAATCAGCAAGACCAAGAAAGAGTATCTGCTGGAAGAACGGGACATCAA
CAGGTTCTCCGTGCAGAAAGACTTCATCAACCGGAACCTGGTGGATACCAGATACGCCACCAGAG
GCCTGATGAACCTGCTGCGGAGCTACTTCAGAGTGAACAACCTGGACGTGAAAGTGAAGTCCATC
AATGGCGGCTTCACCAGCTTTCTGCGGCGGAAGTGGAAGTTTAAGAAAGAGCGGAACAAGGGGTA
CAAGCACCACGCCGAGGACGCCCTGATCATTGCCAACGCCGATTTCATCTTCAAAGAGTGGAAGA
AACTGGACAAGGCCAAAAAAGTGATGGAAAACCAGATGTTCGAGGAAAAGCAGGCCGAGAGCAT
GCCCGAGATCGAAACCGAGCAGGAGTACAAAGAGATCTTCATCACCCCCCCACCAGATCAAGCACA
TTAAGGACTTCAAGGACTACAAGTACAGCCACCGGGTGGACAAGAAGCCTAATAGAGAGCTGATT
AACGACACCCTGTACTCCACCCGGAAGGACGACAAGGGCAACACCCTGATCGTGAACAATCTGAA
CGGCCTGTACGACAAGGACAATGACAAGCTGAAAAAGCTGATCAACAAGAGCCCCGAAAAGCTG
CTGATGTACCACCACGACCCCCAGACCTACCAGAAACTGAAGCTGATTATGGAACAGTACGGCGA
CGAGAAGAATCCCCTGTACAAGTACTACGAGGAAACCGGGAACTACCTGACCAAGTACTCCAAAA
AGGACAACGGCCCCGTGATCAAGAAGATTAAGTATTACGGCAACAAACTGAACGCCCATCTGGAC
ATCACCGACGACTACCCCAACAGCAGAAACAAGGTCGTGAAGCTGTCCCTGAAGCCCTACAGATT
CGACGTGTACCTGGACAATGGCGTGTACAAGTTCGTGACCGTGAAGAATCTGGATGTGATCAAAA
AAGAAAACTACTACGAAGTGAATAGCAAGTGCTATGAGGAAGCTAAGAAGCTGAAGAAGATCAG
CAACCAGGCCGAGTTTATCGCCTCCTTCTACAACAACGATCTGATCAAGATCAACGGCGAGCTGT
ATAGAGTGATCGGCGTGAACAACGACCTGCTGAACCGGATCGAAGTGAACATGATCGACATCACC
TACCGCGAGTACCTGGAAAACATGAACGACAAGAGGCCCCCCAGGATCATTAAGACAATCGCCTC
CAAGACCCAGAGCATTAAGAAGTACAGCACAGACATTCTGGGCAACCTGTATGAAGTGAAATCTA
AGAAGCACCCTCAGATCATCAAAAAGGGCAAAAGGCCGGCGGCCACGAAAAAGGCCGGCCAGGC
AAAAAAGAAAAAGggatcctacccatacgatgttccagattacgctagcggcttcgccaacgagcttggacccaggttgatgggaaagtaagaattc
ctagagcgaataaaagatctttattttcattagatctgtgtgttggtttttttgtgtgatgcagctacgtagataagtagcatggcgggttaatcattaactacaaggaaccc
ctagtgatggagttggccactccctctctgcgcgctcgctcgctcactgaggccgggcgaccaaaggtcgcccgacgcccgggctttgcccgggcggcctcagt
gagcgagcgagcgcgcagctgcattaatgaatcggccaacgcgcggggagaggcggtttgcgtattgggcgctcttccgcttcctcgctcactgactcgctgcg
ctcggtcgttcggctgcggcgagcggtatcagctcactcaaaggcggtaatacggttatccacagaatcaggggataacgcaggaaagaacatgtgagcaaaac
cgcagcaaaaggccaggaaccgtaaaaaggccgcgttgctggcgtttttccataggctccgcccccctgacgagcatcacaaaaatcgacgctcaagtcagag
gtggcgaaacccgacaggactataaagataccaggcgtttccccctggaagctccctcgtgcgctctcctgttccgaccctgccgcttaccggatacctgtccgcc
tttctcccttcgggaagcgtggcgctttctcatagctcacgctgtaggtatctcagttcggtgtaggtcgttcgctccaagctgggctgtgtgcacgaaccccccgttc
agcccgaccgctgcgccttatccggtaactatcgtcttgagtccaacccggtaagacacgacttatcgccactggcagcagccactggtaacaggattagcagag
cgaggtatgtaggcggtgctacagagttcttgaagtggtggcctaactacggctacactagaaggacagtatttggtatctgcgctctgctgaagccagttaccttcg
gaaaaagagttggtagctcttgatccggcaaacaaaccaccgctggtagcggtggtttttttgtttgcaagcagcagattacgcgcagaaaaaaaggatctcaaga
agatcctttgatcttttctacggggtctgacgctcagtggaacgaaaactcacgttaagggattttggtcatgagattatcaaaaaggatcttcacctagatccttttaaa
ttaaaaatgaagttttaaatcaatctaaagtatatatgagtaaacttggtctgacagttaccaatgcttaatcagtgaggcacctatctcagcgatctgtctatttcgttcat
ccatagttgcctgactccccgtcgtgtagataactacgatacgggagggcttaccatctggccccagtgctgcaatgataccgcgagacccacgctcaccggctc
cagatttatcagcaataaaccagccagccggaagggccgagcgcagaagtggtcctgcaactttatccgcctccatccagtctattaattgttgccgggaagctag
agtaagtagttcgccagttaatagtttgcgcaacgttgttaccattactacaggcatcgtggtgtcacgctcgtcgtttggtatggcttcattcagctccggttcccaacg
atcaaggcgagttacatgatcccccatgttgtgcaaaaaagcggttagctccttcggtcctccgatcgttgtcagaagtaagttggccgcagtgttatcactcatggtt
atggcagcactgcataattctcttactgtcatgccatccgtaagatgctttttctgtgactggtgagtactcaaccaagtcattctgagaatagtgtatgcggcgaccgag
ttgctcttgcccggcgtcaatacgggataataccgcgccacatagcagaactttaaaagtgctcatcattggaaaacgttcttcggggcgaaaactctcaaggatctt
accactattgagatccagttcgatgtaacccactcgtgcacccaactgatcttcagcatcttttactttcaccagcgtttctgggtgagcaaaaacaggaaggcaaaat
gccgcaaaaaagggaataagggcgacacggaaatgttgaatactcatactcttcctttttcaatattattgaagcatttatcaggttattgtctcatgagcggatacat
atttgaatgtatttagaaaataaacaaataggggttccgcgcacatttccccgaaaagtgccacctgaaattataaacgttaatattttgttaaaattcgcgttaaatttt
gttaaatcagctcatttttaaccaataggccgaaatcggcaaaaatcccttataaatcaaaagaatagaccgagatagggttgagtgttgttccagtttggaacaagag
tccactattgaggaacgtgaactccagcgtcaaagggcgaaaaaccgtctatcggggcgatggcccactacgtgaaccatcaccctaatcaagttttttggggtcg
aggtgccgtaaagcactaaatcggaaccctaaagggagcccccgatttagagcttgacggggaaagccggcgaacgtggcgagaaaggaagggaagaaagc
gaaaggagcgggcgctagggcgctggcaagtgtagcggtcacgctgcgcgtaaccaccacacccgccgcgcttaatgcgccgctacagggcgcgtcccattc
gccattcaggctgcgcaactgttgggaagggcgatcggtgcgggcctcttcgctattacgc

## B. pAAV-U6-gRNA-TNT-SACas9-HA-OLLAS-spA (gRNA plasmid)

Legend: ITR, U6 Promoter, Guide RNA , Human Troponin T Promoter, NLS, SaCas9

cagctgcgcgctcgctcgctcactgaggccgcccgggcaaagcccgggcgtcgggcgacctttggtcgcccggcctcagtgagcgagcgagcgcgcagaga
gggagtggccaactccatcactagggggttccttgtagttaatgattaacccgccatgctacttatctacgtagccatgctctggTGTACAAAAAAGCAG
GCTTTAAAGGAACCAATTCAGTCGACTGGATCCGGTACCAAGGTCGGGCAGGAAGAGGGCCTATT
TCCCATGATTCCTTCATATTTGCATATACGATACAAGGCTGTTAGAGAGATAATTAGAATTAATTT
GACTGTAAACACAAAGATATTAGTACAAAATACGTGACGTAGAAAGTAATAATTTCTTGGGTAGT
TTGCAGTTTTAAAATTATGTTTTAAAATGGACTATCATATGCTTACCGTAACTTGAAAGTATTTCGA
TTTCTTGGCTTTATATATCTTGTGGAAAGGACGAAACACCgCCGAAGGAGAAAAAGTGCAAGgttttag
tactctggaaacagaatctactaaaacaaggcaaaatgccgtgtttatctcgtcaacttgttggcgagatttttTCTAGACTCAGTCCATTAGGA
GCCAGTAGCCTGGAAGATGTCTTTACCCCCAGCATCAGTTCAAGTGGAGCAGCACATAACTCTTGC
CCTCTGCCTTCCAAGATTCTGGTGCTGAGACTTATGGAGTGTCTTGGAGGTTGCCTTCTGCCCCCCA
ACCCTGCTCCCAGCTGGCCCTCCCAGGCCTGGGTTGCTGGCCTCTGCTTTATCAGGATTCTCAAGA
GGGACAGCTGGTTTATGTTGCATGACTGTTCCCTGCATATCTGCTCTGGTTTTAAATAGCTTATCTG
AGCAGCTGGAGGACCACATGGGCTTATATGGCGTGGGGTACATGTTCCTGTAGCCTTGTCCCTGGC
ACCTGCCAAAATAGCAGCCAACACCCCCCACCCCCACCGCCATCCCCCTGCCCCACCCGTCCCCTG
TCGCACATTCCTCCCTCCGCAGGGCTGGCTCACCAGGCCCCAGCCCACATGCCTGCTTAAAGCCCT
CTCCATCCTCTGCCTCACCCAGTCCCCGCTGAGACTGAGCAGACGCCTCCAGGATCTGTCGGCAGc
cATGGCCCCAAAGAAGAAGCGGAAGGTCGGTATCCACGGAGTCCCAGCAGCCAAGCGGAACTAC
ATCCTGGGCCTGGACATCGGCATCACCAGCGTGGGCTACGGCATCATCGACTACGAGACACGGGA
CGTGATCGATGCCGGCGTGCGGCTGTTCAAAGAGGCCAACGTGGAAAACAACGAGGGCAGGCGG
AGCAAGAGAGGCGCCAGAAGGCTGAAGCGGCGGAGGCGGCATAGAATCCAGAGAGTGAAGAAG
CTGCTGTTCGACTACAACCTGCTGACCGACCACAGCGAGCTGAGCGGCATCAACCCCTACGAGGC
CAGAGTGAAGGGCCTGAGCCAGAAGCTGAGCGAGGAAGAGTTCTCTGCCGCCCTGCTGCACCTGG
CCAAGAGAAGAGGCGTGCACAACGTGAACGAGGTGGAAGAGGACACCGGCAACGAGCTGTCCAC
CAAAGAGCAGATCAGCCGGAACAGCAAGGCCCTGGAAGAGAAATACGTGGCCGAACTGCAGCTG
GAACGGCTGAAGAAGACGGCGAAGTGCGGGGCAGCATCAACAGATTCAAGACCAGCGACTACG
TGAAAGAAGCCAAACAGCTGCTGAAGGTGCAGAAGGCCTACCACCAGCTGGACCAGAGCTTCATC
GACACCTACATCGACCTGCTGGAAACCCGGCGGACCTACTATGAGGGACCTGGCGAGGGCAGCCC
CTTCGGCTGGAAGGACATCAAAGAATGGTACGAGATGCTGATGGGCCACTGCACCTACTTCCCCG
AGGAACTGCGGAGCGTGAAGTACGCCTACAACGCCGACCTGTACAACGCCCTGAACGACCTGAAC
AATCTCGTGATCACCAGGGACGAGAACGAGAAGCTGGAATATTACGAGAAGTTCCAGATCATCGA
GAACGTGTTCAAGCAGAAGAAGAAGCCCACCCTGAAGCAGATCGCCAAAGAAATCCTCGTGAAC
GAAGAGGATATTAAGGGCTACAGAGTGACCAGCACCGGCAAGCCCGAGTTCACCAACCTGAAGG
TGTACCACGACATCAAGGACATTACCGCCCGGAAAGAGATTATTGAGAACGCCGAGCTGCTGGAT
CAGATTGCCAAGATCCTGACCATCTACCAGAGCAGCGAGGACATCCAGGAAGAACTGACCAATCT
GAACTCCGAGCTGACCCAGGAAGAGATCGAGCAGATCTCTAATCTGAAGGGCTATACCGGCACCC
ACAACCTGAGCCTGAAGGCCATCAACCTGATCCTGGACGAGCTGTGGCACACCAACGACAACCAG
ATCGCTATCTTCAACCGGCTGAAGCTGGTGCCCAAGAAGGTGGACCTGTCCCAGCAGAAAGAGAT
CCCCACCACCCTGGTGGACGACTTCATCCTGAGCCCCGTCGTGAAGAGAAGCTTCATCCAGAGCA
TCAAAGTGATCAACGCCATCATCAAGAAGTACGGCCTGCCCAACGACATCATTATCGAGCTGGCC
CGCGAGAAGAACTCCAAGGACGCCCAGAAAATGATCAACGAGATGCAGAAGCGGAACCGGCAGA
CCAACGAGCGGATCGAGGAAATCATCCGGACCACCGGCAAAGAGAACGCCAAGTACCTGATCGA
GAAGATCAAGCTGCACGACATGCAGGAAGGCAAGTGCCTGTACAGCCTGGAAGCCATCCCTCTGG
AAGATCTGCTGAACAACCCCTTCAACTATGAGGTGGACCACATCATCCCCAGAAGCGTGTCCTTCG
ACAACAGCTTCAACAACAAGGTGCTCGTGAAGCAGGAAGAAAACAGCAAGAAGGGCAACCGGAC
CCCATTCCAGTACCTGAGCAGCAGCGACAGCAAGATCAGCTACGAAACCTTCAAGAAGCACATCC
TGAATCTGGCCAAGGGCAAGGGCAGAATCAGCAAGACCAAGAAAGAGTATCTGCTGGAAGAACG
GGACATCAACAGGTTCTCCGTGCAGAAAGACTTCATCAACCGGAACCTGGTGGATACCAGATACG
CCACCAGAGGCCTGATGAACCTGCTGCGGAGCTACTTCAGAGTGAACAACCTGGACGTGAAAGTG

AAGTCCATCAATGGCGGCTTCACCAGCTTTCTGCGGCGGAAGTGGAAGTTTAAGAAAGAGCGGAA
CAAGGGGTACAAGCACCACGCCGAGGACGCCCTGATCATTGCCAACGCCGATTTCATCTTCAAAG
AGTGGAAGAAACTGGACAAGGCCAAAAAAGTGATGGAAAACCAGATGTTCGAGGAAAAGCAGGC
CGAGAGCATGCCCGAGATCGAAACCGAGCAGGAGTACAAAGAGATCTTCATCACCCCCCACCAG
ATCAAGCACATTAAGGACTTCAAGGACTACAAGTACAGCCACCGGGTGGACAAGAAGCCTAATAG
AGAGCTGATTAACGACACCCTGTACTCCACCCGGAAGGACGACAAGGGCAACACCCTGATCGTGA
ACAATCTGAACGGCCTGTACGACAAGGACAATGACAAGCTGAAAAAGCTGATCAACAAGAGCCC
CGAAAAGCTGCTGATGTACCACCACGACCCCCAGACCTACCAGAAACTGAAGCTGATTATGGAAC
AGTACGGCGACGAGAAGAATCCCCTGTACAAGTACTACGAGGAAACCGGGAACTACCTGACCAA
GTACTCCAAAAAGGACAACGGCCCCGTGATCAAGAAGATTAAGTATTACGGCAACAAACTGAACG
CCCATCTGGACATCACCGACGACTACCCCAACAGCAGAAACAAGGTCGTGAAGCTGTCCCTGAAG
CCCTACAGATTCGACGTGTACCTGGACAATGGCGTGTACAAGTTCGTGACCGTGAAGAATCTGGA
TGTGATCAAAAAAGAAAACTACTACGAAGTGAATAGCAAGTGCTATGAGGAAGCTAAGAAGCTG
AAGAAGATCAGCAACCAGGCCGAGTTTATCGCCTCCTTCTACAACAACGATCTGATCAAGATCAA
CGGCGAGCTGTATAGAGTGATCGGCGTGAACAACGACCTGCTGAACCGGATCGAAGTGAACATGA
TCGACATCACCTACCGCGAGTACCTGGAAAACATGAACGACAAGAGGCCCCCCAGGATCATTAAG
ACAATCGCCTCCAAGACCCAGAGCATTAAGAAGTACAGCACAGACATTCTGGGCAACCTGTATGA
AGTGAAATCTAAGAAGCACCCTCAGATCATCAAAAAGGGCAAAAGGCCGGCGGCCACGAAAAAG
GCCGGCCAGGCAAAAAAGAAAAAGggatcctaccatacgatgttccagattacgctagcggcttcgccaacgagcttggacccaggttg
atgggaaagtaagaattcctagagcgaataaaagatctttattttcattagatctgtgtgttggttttttgtgtgatgcagctacgtagataagtagcatggcgggttaatc
attaactacaaggaacccctagtgatggagttggccactccctctctgcgcgctcgctcgctcactgaggccgggcgaccaaaggtcgcccgacgcccgggcttt
gcccgggcggcctcagtgagcgagcgagcgcgcagctgcattaatgaatcggccaacgcgcggggagaggcggtttgcgtattgggcgctcttccgcttcctc
gctcactgactcgctgcgctcggtcgttcggctgcggcgagcggtatcagctcactcaaaggcggtaatacggttatccacagaatcaggggataacgcaggaa
agaacatgtgagcaaaaccgcagcaaaaggccaggaaccgtaaaaaggccgcgttgctggcgtttttccataggctccgcccccctgacgagcatcacaaaaat
cgacgctcaagtcagaggtggcgaaacccgacaggactataaagataccaggcgtttccccctggaagctccctcgtgcgctctcctgttccgaccctgccgctt
accggatacctgtccgcctttctcccttcgggaagcgtggcgctttctcatagctcacgctgtaggtatctcagttcggtgtaggtcgttcgctccaagctgggctgtg
tgcacgaaccccccgttcagcccgaccgctgcgccttatccggtaactatcgtcttgagtccaacccggtaagacacgacttatcgccactggcagcagccactg
gtaacaggattagcagagcgaggtatgtaggcggtgctacagagttcttgaagtggtggcctaactacggctacactagaaggacagtatttggtatctgcgctctg
ctgaagccagttaccttcggaaaaagagttggtagctcttgatccggcaaacaaaccaccgctggtagcggtggtttttttgtttgcaagcagcagattacgcgcag
aaaaaaaggatctcaagaagatcctttgatctttctacggggtctgacgctcagtggaacgaaaactcacgttaagggattttggtcatgagattatcaaaaaggatc
ttcacctagatcctttttaaattaaaaatgaagttttaaatcaatctaaagtatatatgagtaaacttggtctgacagttaccaatgcttaatcagtgaggcacctatctcagc
gatctgtctatttcgttcatccatagttgcctgactccccgtcgtgtagataactacgatacgggagggcttaccatctggccccagtgctgcaatgataccgcgaga
cccacgctcaccggctccagatttatcagcaataaaccagccagccggaagggccgagcgcagaagtggtcctgcaactttatccgcctccatccagtctattaat
tgttgccgggaagctagagtaagtagttcgccagttaatagtttgcgcaacgttgttaccattactacaggcatcgtggtgtcacgctcgtcgtttggtatggcttcattc
agctccggttcccaacgatcaaggcgagttacatgatcccccatgttgtgcaaaaaagcggttagctccttcggtcctccgatcgttgtcagaagtaagttggccgc
agtgttatcactcatggttatggcagcactgcataattctcttactgtcatgccatccgtaagatgcttttctgtgactggtgagtactcaaccaagtcattctgagaatag
tgtatgcggcgaccgagttgctcttgcccggcgtcaatacgggataataccgcgccacatagcagaactttaaaagtgctcatcattggaaaacgttcttcggggcg
aaaactctcaaggatcttaccactattgagatccagttcgatgtaacccactcgtgcacccaactgatcttcagcatcttttactttcaccagcgtttctgggtgagcaaa
aacaggaaggcaaaatgccgcaaaaaagggaataagggcgacacggaaatgttgaatactcatactcttcctttttcaatattattgaagcatttatcagggttattgt
ctcatgagcggatacatatttgaatgtatttagaaaaataaacaaataggggttccgcgcacatttccccgaaaagtgccacctgaaattataaacgttaatattttgtta
aaattcgcgttaaatttttgttaaatcagctcatttttaaccaataggccgaaatcggaaaaatcccttataaatcaaaagaatagaccgagatagggttgagtgttgtt
ccagtttggaacaagagtccactattgaggaacgtgaactccagcgtcaaagggcgaaaaaccgtctatcggggcgatggcccactacgtgaaccatcacccta
atcaagttttttggggtcgaggtgccgtaaagcactaaatcggaaccctaaagggagcccccgatttagagcttgacggggaaagccggcgaacgtggcgagaa
aggaagggaagaaagcgaaaggagcgggcgctagggcgctggcaagtgtagcggtcacgctgcgcgtaaccaccacacccgccgcgcttaatgcgccgct
acagggcgcgtcccattcgccattcaggctgcgcaactgttgggaagggcgatcggtgcgggcctcttcgctattacgc

Supplemental Figure 2. Baseline ECG tracings and intracardiac ventricular electrograms obtained in mice 6 weeks after AAV9 injections.
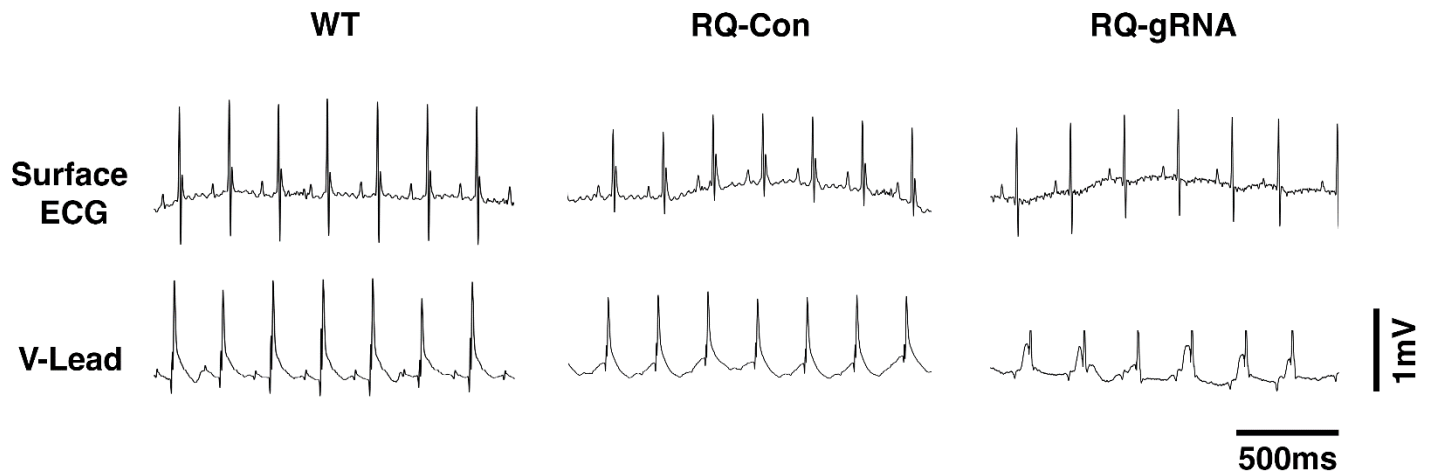

Supplemental Figure 3. Baseline ECG tracings and intracardiac ventricular electrograms obtained in mice 12 months after AAV9 injections. * indicates amplifier artefact.
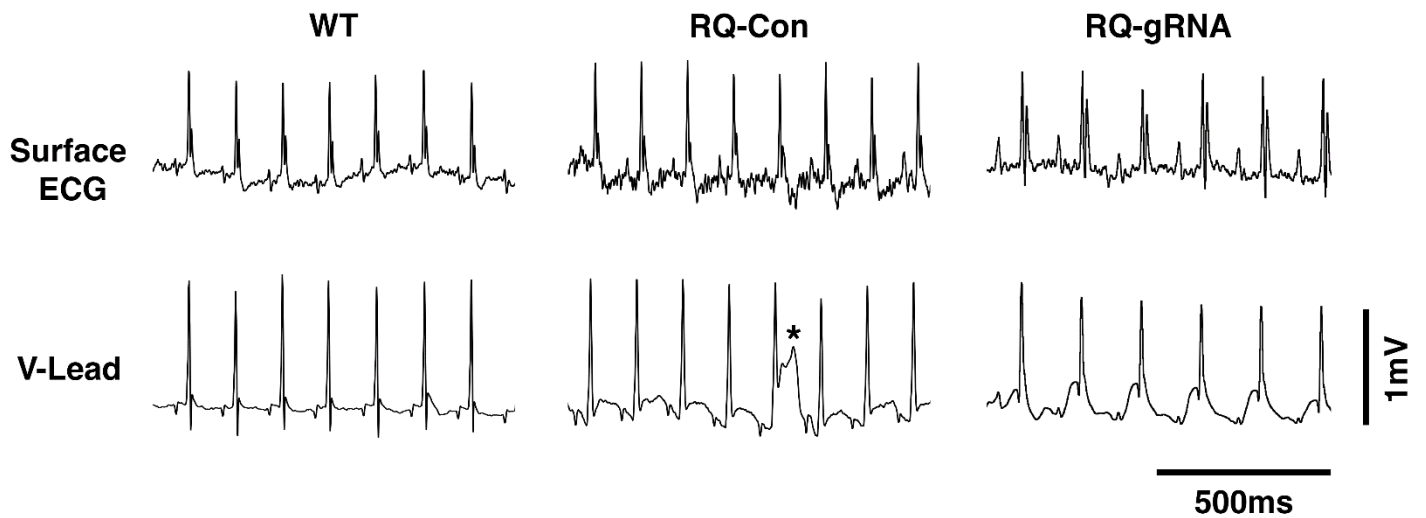
Supplemental Figure 4. H&E Staining of Heart and Liver Sections after Long-Term Genome Editing. (**A**) Hematoxylin and eosin (H&E) staining of transverse cardiac sections showing normal cardiac structure and dimensions at 1x magnification and normal tissue organization. (**B**) H&E staining of liver sections showing normal tissue structure and absence of immune cell infiltration.
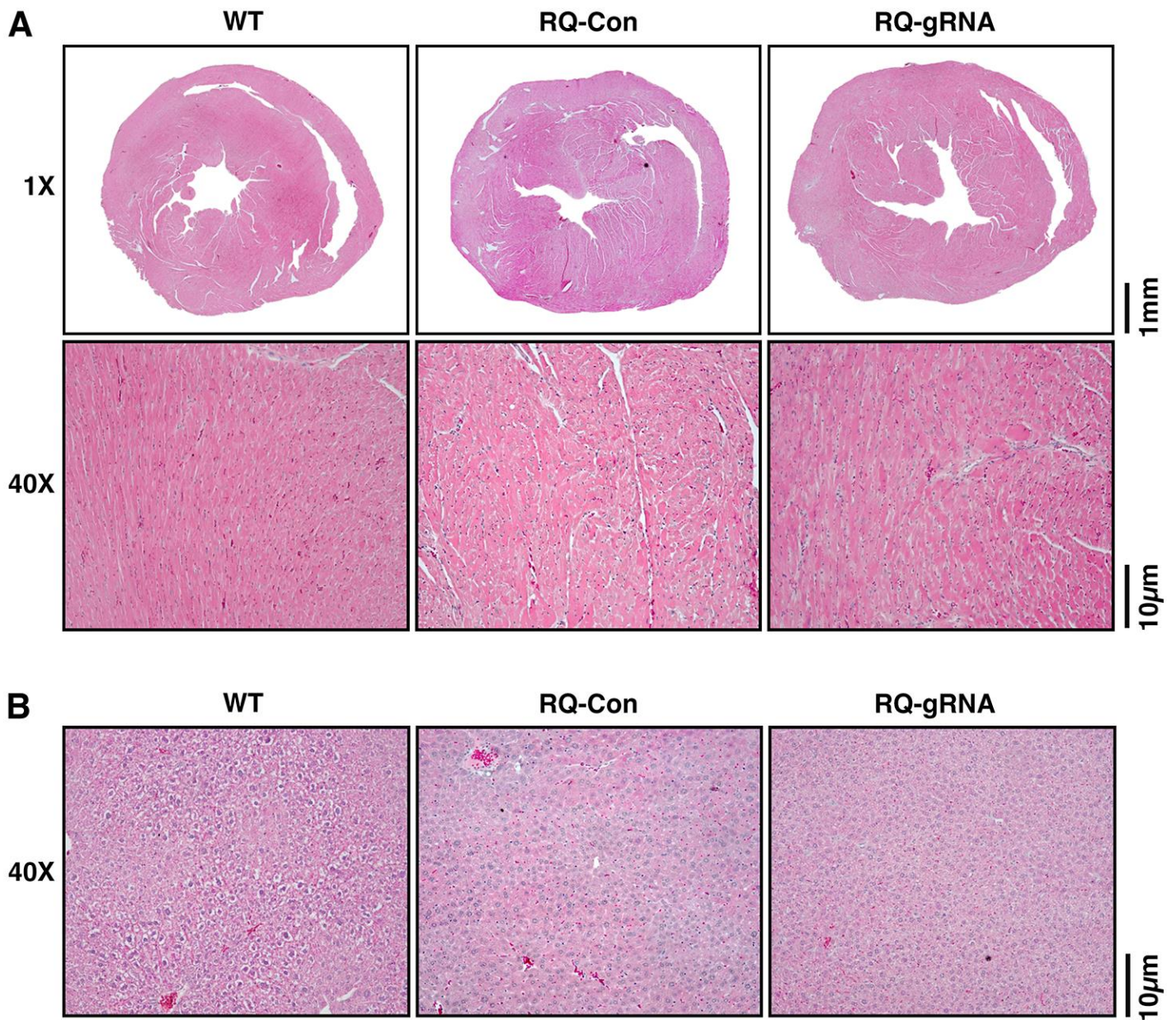
Supplemental Figure 5. Persistent SaCas9 expression after genome editing. (**A**) Schematic of AAV genome with U6 genomic DNA (gDNA) and SaCas9 cDNA primers shown below. (**B**) PCR amplification of AAV genome in ventricles of R176Q/+ mice at 12 months after CRISPR/Cas9 AAV9 injection (RQ-gRNA) compared with non-injected control mice (RQ-Con). (**C**) cDNA qRT-PCR quantification of SaCas9 mRNA levels relative to GAPDH mRNA. Data is shown as mean ± SEM. After confirming normality, an unpaired student t-test was performed.
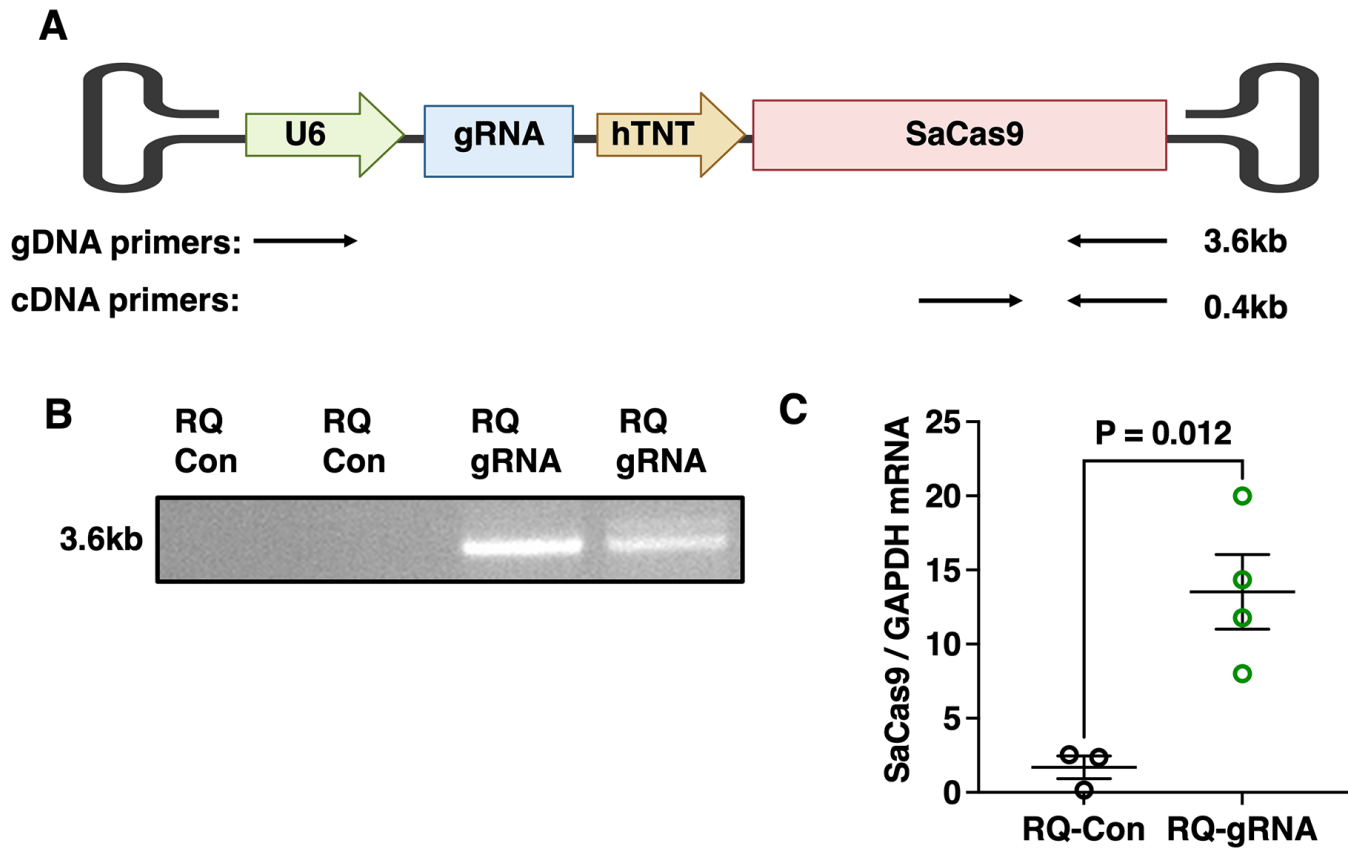
Supplemental References

1. Xiao X, Li J, Samulski RJ. Production of high-titer recombinant adeno-associated virus vectors in the absence of helper adenovirus. *J Virol* 1998;72:2224-32.10.1128/JVI.72.3.2224-2232.1998.

2. Pan X, Philippen L, Lahiri SK, et al. In Vivo Ryr2 Editing Corrects Catecholaminergic Polymorphic Ventricular Tachycardia. *Circ Res* 2018;123:953-63.10.1161/CIRCRESAHA.118.313369.

3. Magoc T, Salzberg SL. FLASH: fast length adjustment of short reads to improve genome assemblies. *Bioinformatics* 2011;27:2957-63.10.1093/bioinformatics/btr507.

4. Shen W, Le S, Li Y, Hu F. SeqKit: A Cross-Platform and Ultrafast Toolkit for FASTA/Q File Manipulation. *PLoS One* 2016;11:e0163962.10.1371/journal.pone.0163962.

5.   Clement K, Rees H, Canver MC, et al. CRISPResso2 provides accurate and rapid genome editing sequence analysis. *Nat Biotechnol* 2019;37:224-6.10.1038/s41587-019-0032-3.

6.   Li N, Wehrens XH. Programmed electrical stimulation in mice. *J Vis Exp* 201010.3791/1730.10.3791/1730.

7.   Quick AP, Wang Q, Philippen LE, et al. SPEG (Striated Muscle Preferentially Expressed Protein Kinase) Is Essential for Cardiac Function by Regulating Junctional Membrane Complex Activity. *Circ Res* 2017;120:110-9.10.1161/CIRCRESAHA.116.309977.

8.   Campbell HM, Quick AP, Abu-Taha I, et al. Loss of SPEG Inhibitory Phosphorylation of Ryanodine Receptor Type-2 Promotes Atrial Fibrillation. *Circulation* 2020;142:1159-72.10.1161/CIRCULATIONAHA.120.045791.

9.   Picht E, Zima AV, Blatter LA, Bers DM. SparkMaster: automated calcium spark analysis with ImageJ. *Am J Physiol Cell Physiol* 2007;293:C1073-81.10.1152/ajpcell.00586.2006.

10.   Respress JL, van Oort RJ, Li N, et al. Role of RyR2 phosphorylation at S2814 during heart failure progression. *Circ Res* 2012;110:1474-83.10.1161/CIRCRESAHA.112.268094.