SUPPLEMENTAL MATERIAL

Supplemental Methods

Cardiac magnetic resonance imaging. MRI was performed on a 9.4T Bruker Biospec MRI system with a 30 cm bore, a 12 cm gradient insert, and an Autopac automated sample positioning system (Bruker Biospin Inc, Billerica, MA). Animals were anesthetized in an induction chamber with 3% isoflurane and transferred to a dedicated imaging bed with isoflurane delivered via nosecone at 1-2%. Cardiac and respiratory signals were monitored using an MR-compatible physiologic monitoring system and a feedback-controlled warm water circulating system was used to maintain body temperature (SA Instruments, Stonybrook, NY). Animals were placed in the prone position and a radiofrequency coil with inner diameter 40mm was mounted on the bed.

After acquisition of localizer images, the atria were imaged using a prospectively triggered flow compensated cine FLASH sequence (flcFLASH) with TR/TE/α = 10 ms / 2.4 ms / 15°, matrix size 192 x 192, field of view 3 cm x 3 cm, slice thickness 1 mm, and 12 frames per cardiac cycle. A series of coronally oriented slices was acquired sequentially, to create a stack fully covering right and left atria. A single sagittal slice was acquired through each of the right and left atria, as was a single short-axis slice through both atria.

Analysis was performed on the coronal images using the freely available software Segment version 2.0 R5450 (http://segment.heiberg.se) [1]. The end-systolic frame of the cine images was identified, and contours of the right and left atrium were manually traced using the interpolated spline tool. Sagittal and axial images were used to cross-check the accuracy of the segmentation. End systolic volumes were exported from Segment and analyzed using GraphPad Prism 7.

Mouse Continuous ECG Telemetry. TA11 ETA-F10 wireless radio telemeters (Data Science International, Minneapolis, MN) were implanted subcutaneously in 12-14 week old mice under isoflurane anesthesia as previously described [2]. Baseline ECG data was used from 30 minute windows of clean traces between 23:00 – 4:00, during the animals' active period. Isoproterenol was administered intraperitoneally (ip) at 4mg/kg consistently at 9:00 AM, with 30 minutes of data used following administration [3]. ECG traces were evaluated for arrhythmic events or confounding noise, and these evaluations were carried out in duplicate blinded to genotype. Representative Poincaré plots were generated using the R-R interval from each individual beat over the 30 minute period to plot RR, RR+1 values [4]. R-R variability was further visualized with a histogram of RR/RR+1 values for each group of mice from each beat over the 30-minute period. Variation in heart rate and QRS duration were obtained by averaging the values over the 30-minute windows to obtain a single average value per animal, followed by analysis between groups.

Histology. Hearts were flash frozen in liquid nitrogen and stored at -80°C until sectioned. For LacZ staining 10μm thick sections were fixed in 0.2% glutaraldehyde, washed with PBS, and stained with lacZ stain (5mM K ferrocyanide, 5mM K ferricyanide, 2mM $MgCl_2$, 0.02% NP-40, 0.01% Na Deoxycholate and 1mg/ml X-Gal dissolved in DMSO) [5]. The sections were incubated in staining solution at 37°C overnight, washed (100mM $NaPO_4$, 0.2% nonident, 0.1% Na Deoxycholate), dehydrated and mounted under cover glass. For hematoxylin and eosin staining and Masson's trichrome staining hearts were fixed in formalin, embedded in paraffin, and sectioned at 5μm. Samples were prepared for transmission electron microscopy by fixing in 2.5% glutaraldehyde, post-fixed in 2% osmium tetroxide and embedded in EMbed 812 and sectioned at ~75nm.

Mouse RNA-seq. RNA libraries were constructed using the TruSeq RNA Sample Prep Kit version 2.0 (Illumina) from quadriceps and heart tissue from 129T2/SvEmsJ mice [6]. RNA samples from each tissue were indexed with unique adapters and pooled for 100bp paired-end sequencing with an Illumina HiSeq 2000. Reads were aligned with TopHat v2.1.0 to the mouse genome (mm9) and the Ensembl *Mus Musculus* GTF file (Mus_musculus_NCBI37) [7]. The average sample alignment rate was 85.81% and each sample had an average of 40,209,436 aligned reads. Cufflinks 2.1.1 was used to assess read counts per gene and Cuffdiff from was used to determine differential gene expression between samples [8]. Genes with an adjusted p-value with a false-discovery rate < .05 were determined to be significant. Gene expression levels were reported in fragments per kilobase of exon per million fragments mapped (FPKM).

Reprogramming to induced pluripotent stem cells. Cells isolated from subjects were reprogrammed to induced pluripotent stem cells (iPSCs) by electroporating four plasmids [9]. Plasmids used for reprogramming were pCXLE-hOCT3/4-shp53-F, pCXLE-hSK, and pCXLE-hUL, with pCXLE-EGFP co-electroporated to determine successful electroporation. All plasmids were gifts from Shinya Yamanaka (Addgene plasmids # 27077, 27078, 27080, 27082, respectively). Cells were washed once in PBS and then resuspended in 200 µL of electroporation buffer (Bio-Rad, 1542677). Cell suspensions were placed in electroporation cuvette with 0.4 cm gap (Bio-Rad, 1542088). Electroporation was done using Gene Pulser Xcell Electroporation System (Bio-Rad, 1652660) at the following setting for fibroblasts: exponential decay at 300 V, 200 µFD, 100 Ω. Two different electroporation settings were tested for LCLs: 300 V, 200 µFD, 100 Ω and 300 V, 960 µFD, 200 Ω. Electroporated fibroblasts were plated onto 12-well plate pre-coated with mouse embryonic fibroblasts (MEF). Electroporated LCLs were plated onto 12-well plate without MEF and then moved onto MEF plates 7 days after electroporation. MEFs (Millipore, PMEF-CF) used as feeder cells were plated 24 hours prior to electroporation in wells pre-coated with 0.1% gelatin (Millipore, ES-006-B). Electroporated cells were placed in WiCell media with ascorbic acid on the day of electroporation. Fresh media was added the next day. On the second day, media was replaced with WiCell with 50 µg/mL ascorbic acid (Santa Cruz, sc-228390) and 0.5 mM sodium butyrate (Stemgent, 04-005). Starting day 12, cells were fed WiCell media without ascorbic acid or sodium butyrate. Media was switched every other day until colonies appeared. Colonies were picked manually and subsequently expanded on feeder plates until at least passage 5, and then adapted to feeder-free system using plates coated with hES-qualified Matrigel (Corning, 354277) and grown in mTeSR1 (Stemcell Technologies, 05875).

Cardiomyocyte differentiation. Reprogrammed human iPSCs were differentiated into cardiomyocytes following a previously published protocol with some modifications [10]. Briefly, iPSCs were plated in Matrigel-coated 12-well plates at 100,000-250,000 cells/cm^2 in mTeSR1 with 2µM thiazovivin (Stemcell Technologies, 72254). The cells were fed mTeSR1 for two days. Media was then switched to RPMI 1640 with B-27 minus insulin (Thermo Fisher Scientific, A1895601) and 9 µM CHIR99021 (Tocris, 4423). CHIR99021 was taken off 24 hours later by replacing the media with RPMI 1640 supplemented with B-27 minus insulin. 48 hours later, media was replaced with RPMI 1640 supplemented with B-27 minus insulin and 5 µM IWP2 (R&D Systems, 3533/10). IWP2 was taken off 48 hours later by replacing the media with RPMI 1640 with B-27 minus insulin. Finally, 48 hours after the end of IWP2 treatment, media was changed to RPMI 1640 with B-27 (Thermo Fisher Scientific, 17504-044). Spontaneous beating was observed as early as 12 days after initial CHIR treatment.

Media compositions. Growth media for lymphoblastoid cell lines were composed of RPMI 1640 (Thermo Fisher Scientific, 11875-119), 20% fetal bovine serum (Thermo Fisher Scientific, 16000-044), and 1% penicillin/streptomycin (Thermo Fisher Scientific, 15070-063). Growth

media for primary dermal fibroblasts were composed of DMEM (Thermo Fisher Scientific, 11995-073) and 15% FBS. MEFs were plated in DMEM, 10% FBS, and 1% penicillin/streptomycin. Incomplete WiCell consisted of DMEM/F-12 50/50 mix (Corning, 10-092-CV) with 20% KnockOut Serum Replacement (Thermo Fisher Scientific, 10828-028), 1% non-essential amino acids (Thermo Fisher Scientific, 11140-050), and 1% GlutaMax (Thermo Fisher Scientific, 35050-061). Beta-mercaptoethanol (Thermo Fisher Scientific, 21985-023) and fibroblast growth factor (Miltenyi Biotec, 130-093-842) were added to incomplete WiCell at final concentrations of 0.1 mM and 12.5 ng/mL, respectively, to make WiCell. Cardiomyocyte differentiation media were RPMI 1640 supplemented with 1% B-27 or B-27 minus insulin.

Supplemental Tables

Table S1. Population frequencies of *MYBPHL* variants from the ExAc database.

Type	Position	Frequency
Splice Donor	c. 33+1G>A	0.000008238
	c.730+1G>A	0.000008237
	c.430+1G>A	0.00001706
Stop Gained	p.Tyr307Ter	0.000008268
	p.Arg255Ter	0.00145
	p.Gln241Ter	0.000008237
	p.Trp192Ter	0.000008239
	p.Trp158Ter	0.00002471
	p.Arg113Ter	0.0001156
Frameshift	p.Thr271AsnfsTer6	0.00003295
	p.Thr249GlyfsTer9	0.000008237
	p.Ser214ProfsTer82	0.00001647
	p.Leu191ProfsTer19	0.00001648
	p.Gly131TrpfsTer11	0.000008293
	p.Ile53AspfsTer38	0.000008237

Table S2. *Mybphl* null mice are born in Mendelian ratios.

	WT	Het	Null
Observed	24	48	25
Expected	24.25	48.5	24.25

Supplemental Figures

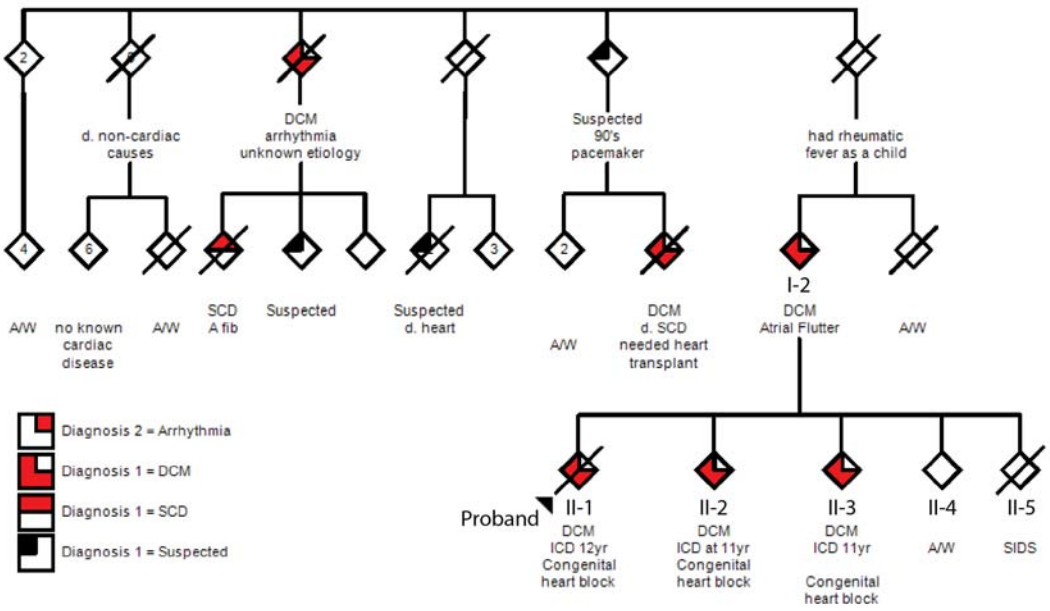

Figure S1. Expanded pedigree of affected family shows DCM and arrhythmia extending to the family of I-2.

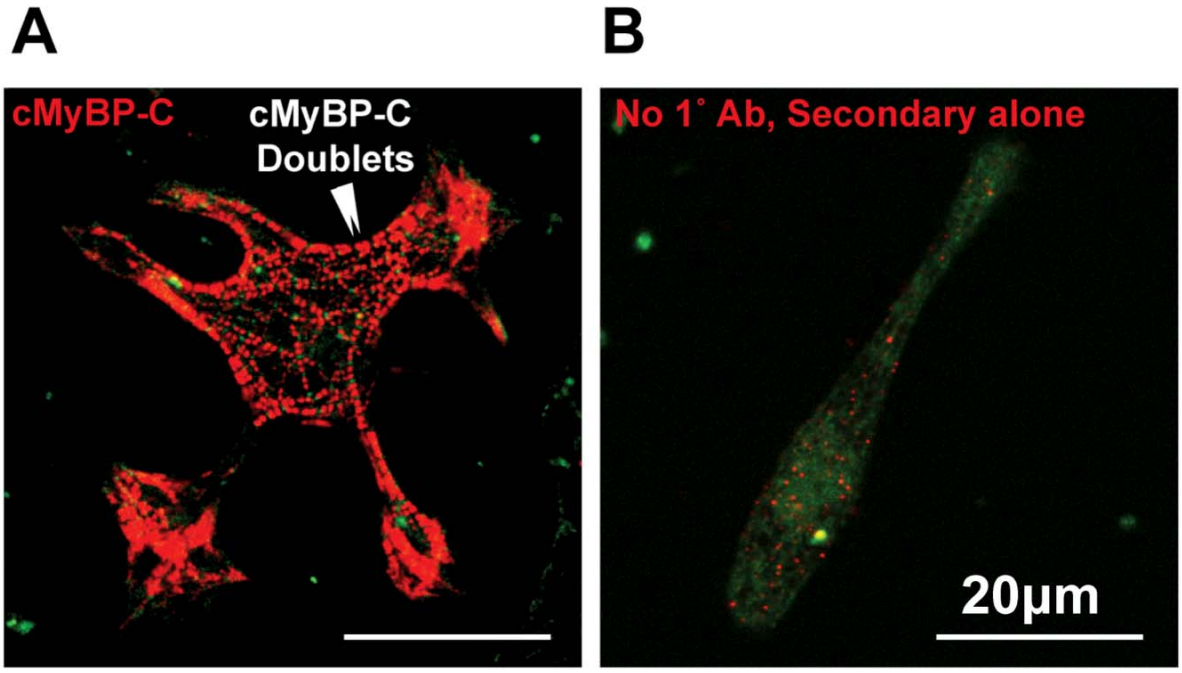

Figure S2. cMyBP-C immunofluorescence microscopy shows doublet patterning. A. Immunofluorescence microscopy with an anti-cMyBP-C antibody in neonatal mouse cardiomyocytes showing the classic MyBP-C-protein doublets along each myofilament. **B.** No primary antibody control for the donkey anti-mouse Alexa594 secondary antibody used for both anti-Myc and anti-cMyBP-C primary antibodies.

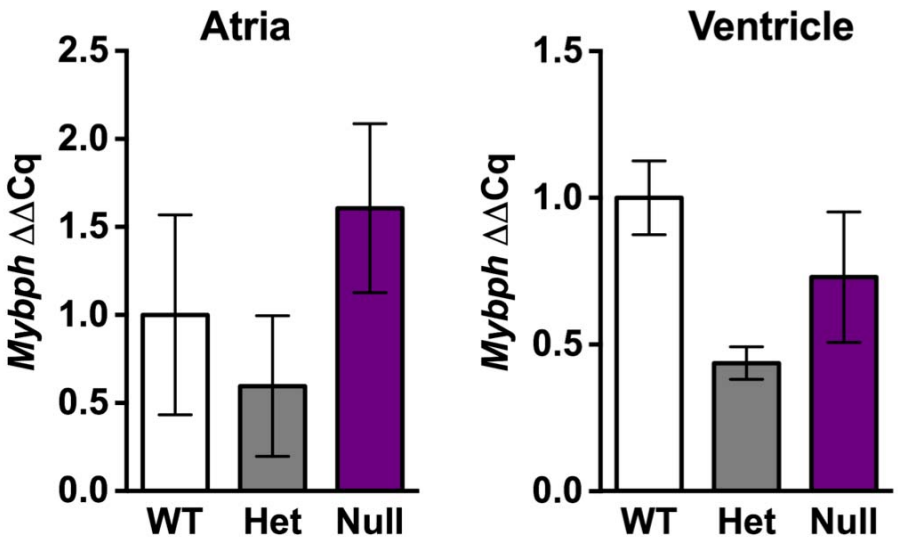

Figure S3. *Mybph* expression is not significantly altered in the atria or ventricle in *Mybphl* null or heterozygous mice. (n=5) Analyzed by One-Way ANOVA with a Tukey's multiple comparison test. ANOVA atria p value = 0.3679; ventricle p = 0.0641.

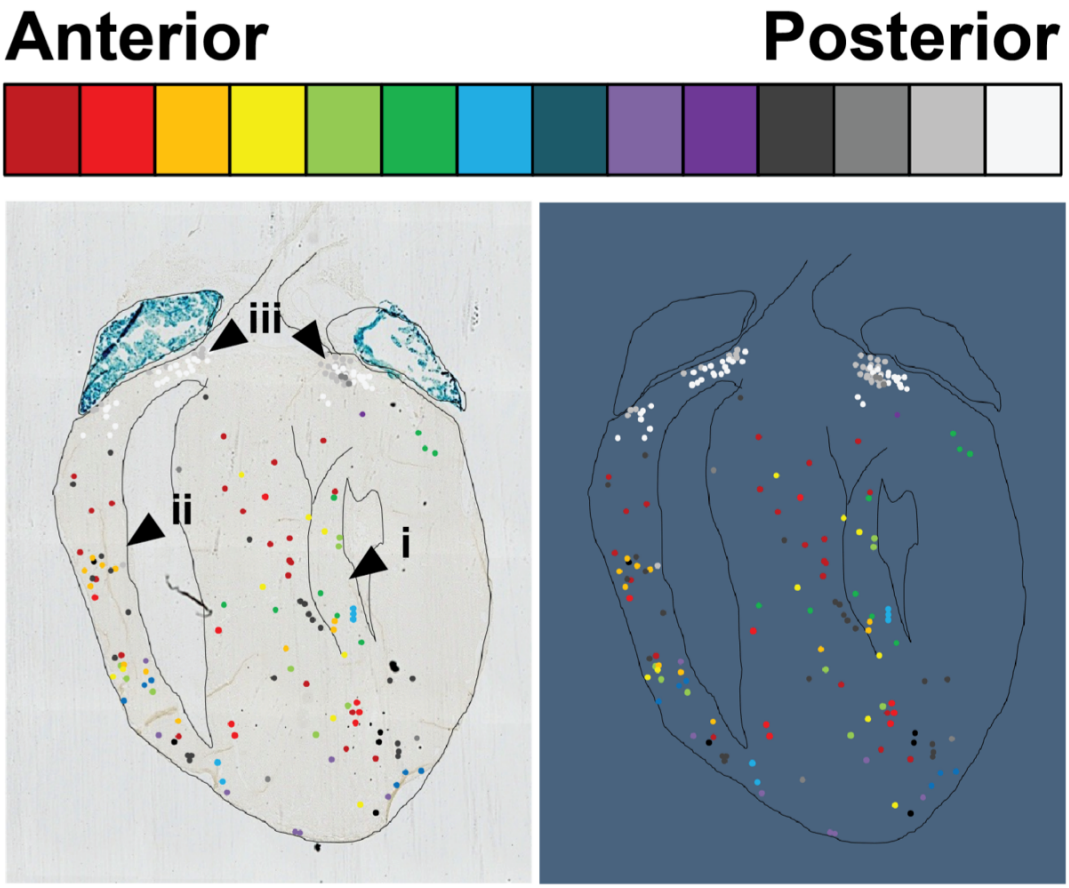

Figure S4. *Mybphl*-driven LacZ positive cells in the ventricle enrich in the septum and right ventricular free wall. This composite map illustrates the approximate locations of LacZ positive ventricular cells representing expression from the Mybphl locus. Serial sections were analyzed over a 700µm region of the heart (78 sections X 10µm each). Sections progressed through the anterior endocardium. Dots represent a single focus or a cluster of foci of LacZ positivity, illustrated in **Figure 4**. Localization shows LacZ puncta highly represented along the ventricular septum (i), right ventricular free wall (ii), and in the atrioventricular border (iii).

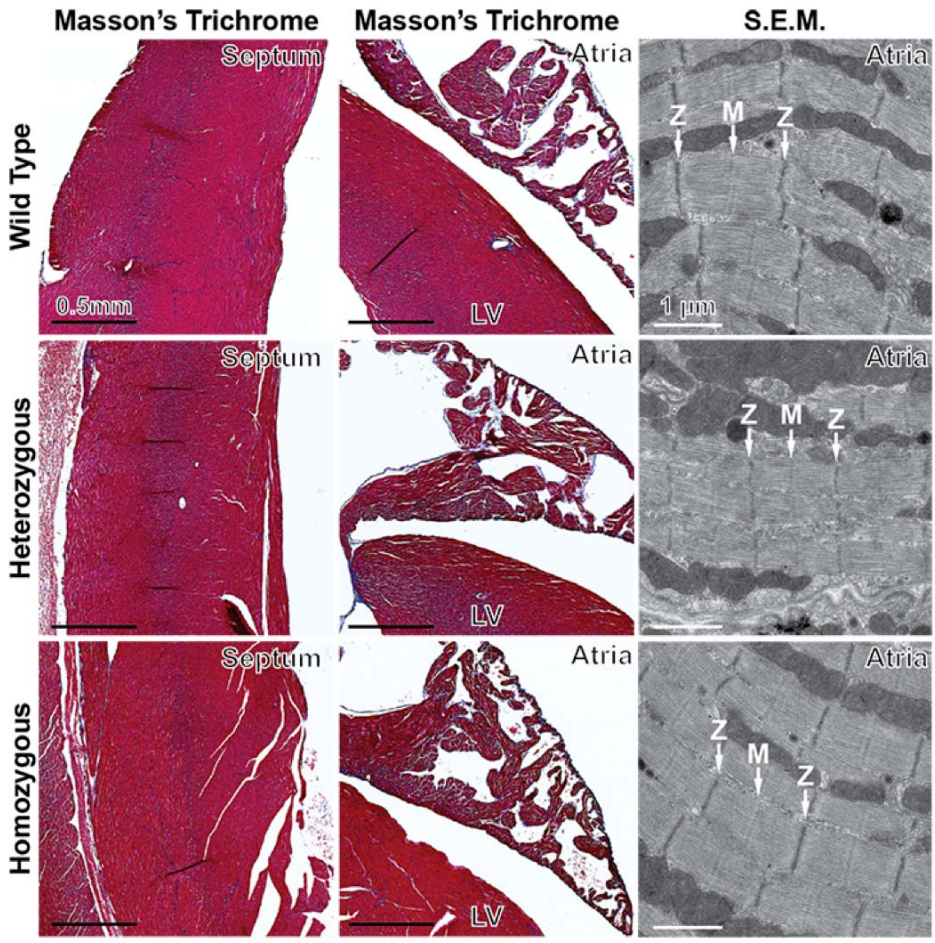

Figure S5. Loss of *Mybphl* does not lead to gross fibrosis or sarcomere disarray in 12-week old mice. Masson's Trichrome staining did not reveal obvious fibrosis in the atria or ventricle in *Mybphl* het or null mice. Transmission electron micrographs revealed normal sarcomere organization in het and null atria, with clear Z and M lines (arrows).

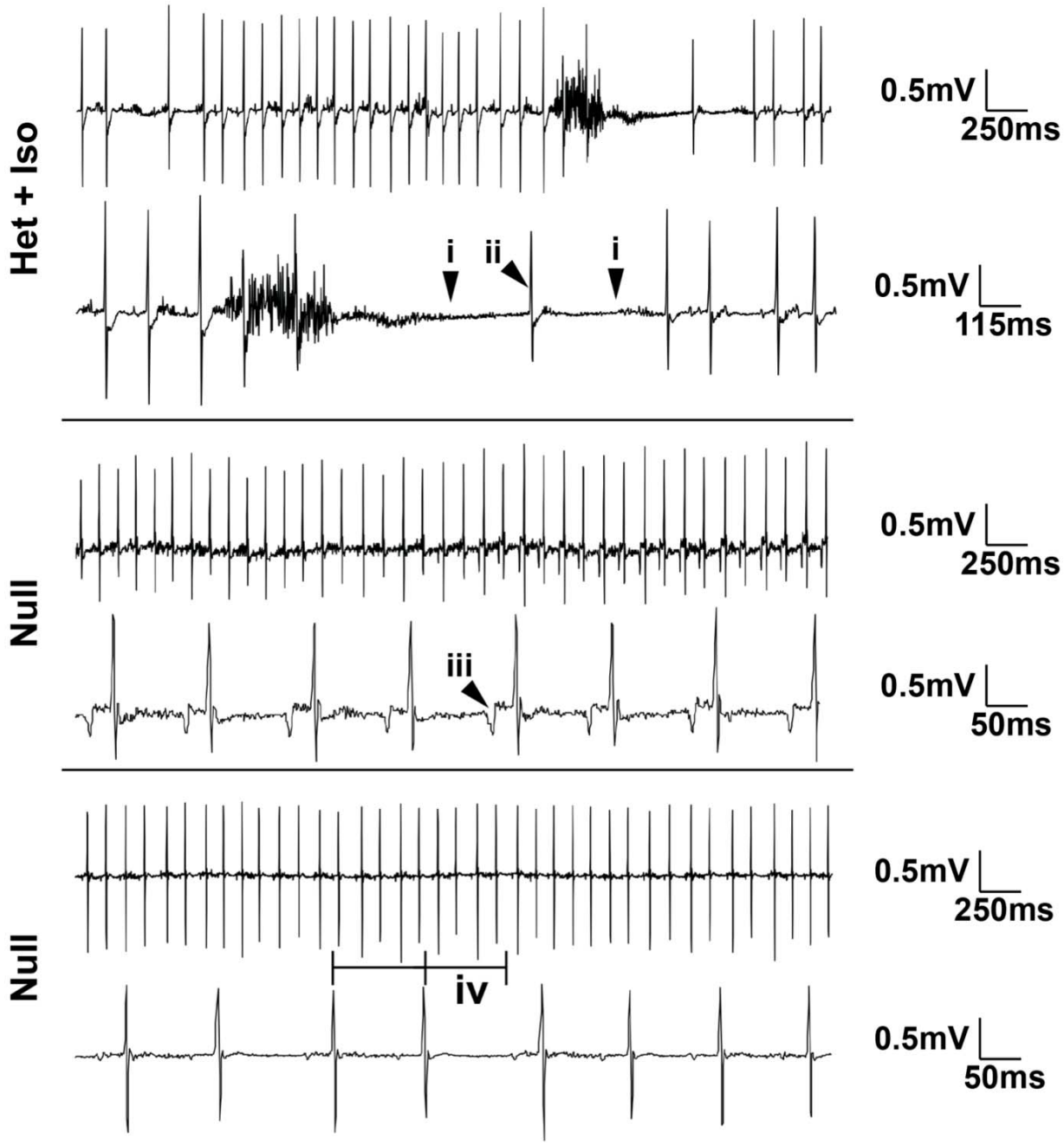

Figure S6. Examples of observed arrhythmic events. Heterozygous *Mybphl* mice following acute isoproterenol treatment exhibited periods of sinus arrest (i), with rescue beats from the ventricle (ii). P waves were observed inverting over short periods of time (iii), indicating a shifting origin of atrial depolarization consistent with ectopic atrial activation. Ventricular bigeminy was commonly observed in het and null mice (iv).

Supplemental References

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