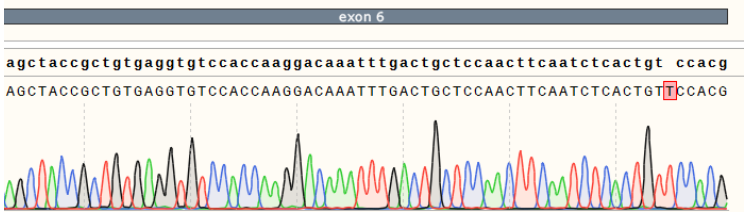
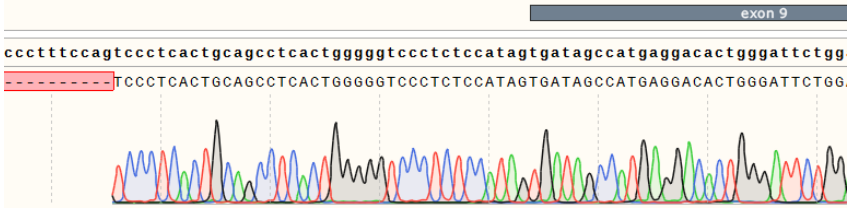
A

Mutant 1



Mutant 2



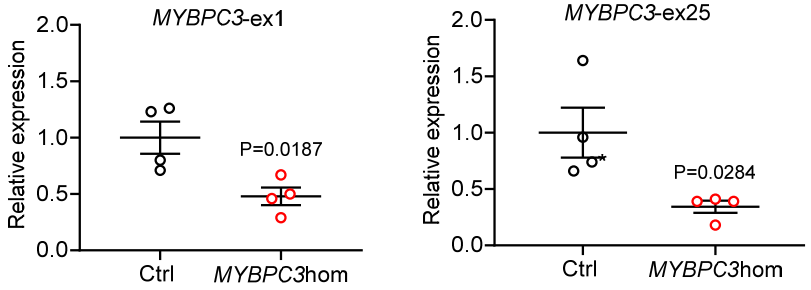
Mutant 1: c.768insT; p.Val256fsX28, Truncation at the end of the C1 domain, 284 aa, 34-kDa protein:

MPEPGKKPVSAFSKKPRSVEVAAGSPAVFEAETERAGVKV
RWQRGGSDISASNKYGLATEGTRHTLTVREVGPADQGSYA
VIAGSSKVKFDLKVIEAEKAEPMLAPAPAPAEATGAPGEA
PAPAAELGESAPSPKGSSSAALNGPTPGAPDDPIGLFVMR
PQDGEVTVGGSITFSARVAGASLLKPPVVKWFKGKWVDLS
SKVGQHLQLHDSYDRASKVYLFELHITDAQPAFTGSYRCE
VSTKDKFDCSNFNLTVPRGHGHRRPGPPISLPPHEPGWRW
SADQ*

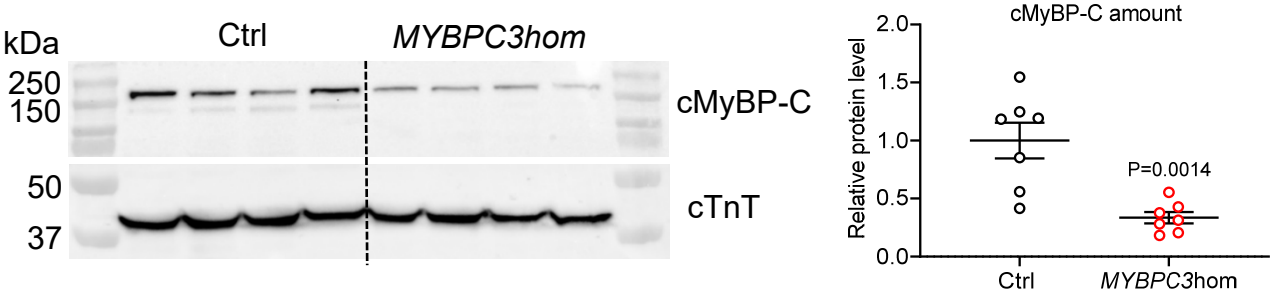
Mutant 2: c.768insT/c.852-1Ins38 nt; p.Val256 + 40 new aa in the M-motif, 1286 aa, 156-kDa protein:

MPEPGKKPVSAFSKKPRSVEVAAGSPAVFEAETERAGVKVRWQRG
GSDISASNKYGLATEGTRHTLTVREVGPADQGSYAVIAGSSKVKF
DLKVIEAEKAEPMLAPAPAPAEATGAPGEAPAPAAELGESAPSPK
GSSSAALNGPTPGAPDDPIGLFVMRPQDGEVTVGGSITFSARVAG
ASLLKPPVVKWFKGKWVDLSSKVGQHLQLHDSYDRASKVYLFELH
ITDAQPAFTGSYRCEVSTKDKFDCSNFNLTVPRGHGHRRPGPPIS
LPPHEPGWRWSADQSLTAASLGVPLHSDSHEDTGILDFSSLLKKS
SSFRTPRDSKLEAPAEEDVWEILRQAPPSEYERIAFQYGVTDLRG
MLKRLKGMRRDEKKSTAFQKKLEPAYQVSKGHKIRLTVELADHDA
EVKWLKNGQEIQMSGRYIFESIGAKRTLTISQCSLADDAAYQCVV
GGEKCSTELFVKEPPVLITRPLEDQLVMVGQRVEFECEVSEEGAQ
VKWLKDGVELTREETFKYRFKKDGQRHHLIINEAMLEDAGHYALC
TSGGQALAELIVQEKKLEVYQSIADLMVGAKDQAVFKCEVSDENV
RGVWLKNGKELVPDSRIKVSHIGRVHKLTIDDVTPADEADYSFVP
EGFACNLSAKLHFMEVKIDFVPRQEPPKIHLDCPGRIPDTIVVVA
GNKLRLDVPISGDPAPTVIWQKAITQGNKAPARPAPDAPEDTGDS
DEWVFDKKLLCETEGRVRVETTKDRSIFTVEGAEKEDEGVYTVTV
KNPVGEDQVNLTVKVIDVPDAPAAPKISNVGEDSCTVQWEPPAYD
GGQPILGYILERKKKKSYRWMRLNFDLIQELSHEARRMIEGVVYE
MRVYAVNAIGMSRPSPASQPFMPIGPPSEPTHLAVEDVSDTTVSL
KWRPPERVGAGGLDGYSVEYCPEGCSEWVAALQGLTEHTSILVKD
LPTGARLLFRVRAHNMAGPGAPVTTTEPVTVQEILQRPRLQLPRH
LRQTIQKKVGEPVNLLIPFQGKPRPQVTWTKEGQPLAGEEVSIRN
SPTDTILFIRAARRVHSGTYQVTVRIENMEDKATLVLQVVDKPSP
PQDLRVTDAWGLNVALEWKPPQDVGNTELWGYTVQKADKKTMEWF
TVLEHYRRTHCVVPELIIGNGYYFRVFSQNMVGFSDRAATTKEPV
FIPRPGITYEPPNYKALDFSEAPSFTQPLVNRSVIAGYTAMLCCA
VRGSPKPKISWFKNGLDLGEDARFRMFSKQGVLTLEIRKPCPFDG
GIYVCRATNLQGEARCECRLEVRVPQ*

B



C



Supplemental Fig. 1: Additional information on hiPSC-CM model *MYBPC3*hom.

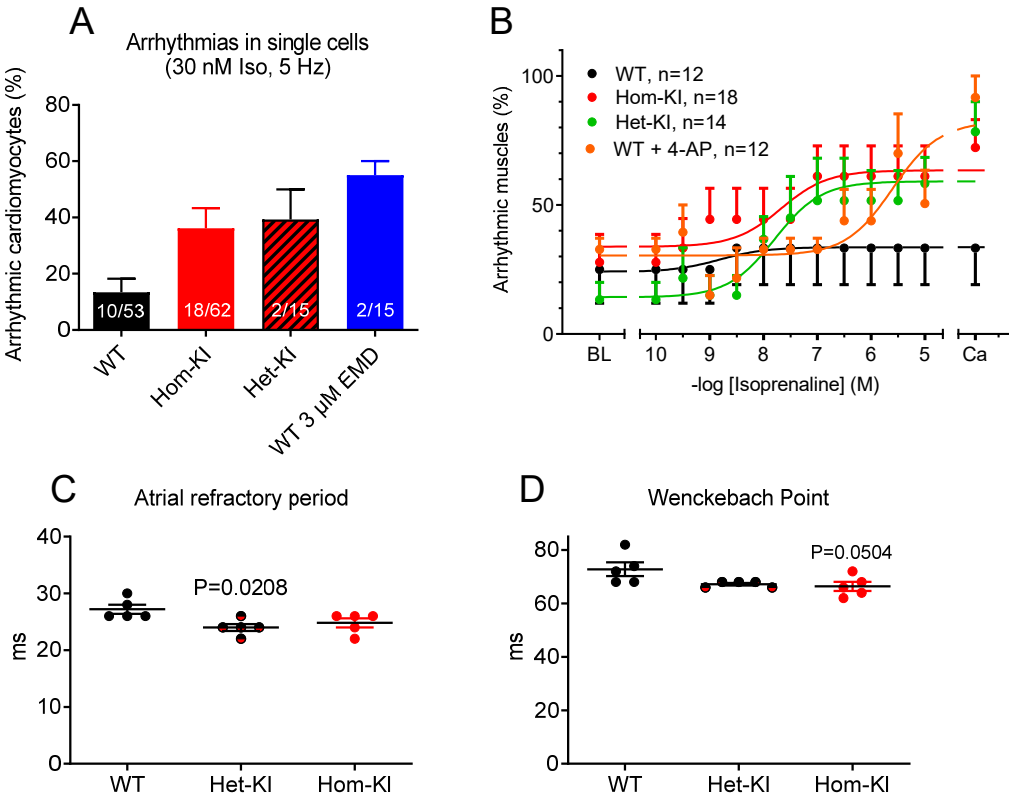
A, Sequencing of RT-PCR in *MYBPC3*hom EHTs. Source: SnapGene® screenshots. Top: T-insertion at the end of exon 6 of *MYBPC3*, which leads to a stop codon in exon 9 (=mutant 1). Bottom: additional intron retention (38 bp) at the end of intron 8, which, combined with the T-insertion, leads to the insertion of 40 new amino acids and restoration of the reading frame (=mutant 2). Right: protein consequence of both mutants. New amino acids are indicated in red.

B: RT-qPCR analysis of *MYBPC3* mRNA levels with primer pairs located around exon 1 (left) or exon 25 (right) and normalized to *TNNT2* (cardiac troponin T) in isogenic control and *MYBPC3*hom EHTs (n=4/group).

C: left, Western Blot stained for cardiac myosin-binding protein c (cMyBP-C) and cardiac troponin T (cTnT) of total protein extracts from control and *MYBPC3hom* EHTs. Separation is indicated by the dotted line. Right, Densitometric quantification of cMyBP-C level, normalized to cTnT loading signal and related to cMyBP-C average of isogenic controls, n=7/group).

Data are expressed as mean±SEM, with P values vs. Ctrl obtained with the unpaired Student's t-test.

Abbreviations: aa, amino acids; Ctrl, isogenic control; EHTs, engineered heart tissues; kDa, kilodalton; nt, nucleotides.



Supplemental Fig. 2: Arrhythmia incidence in mouse ventricular cardiomyocytes and muscle strips, additional data form Langendorff-perfused hearts.

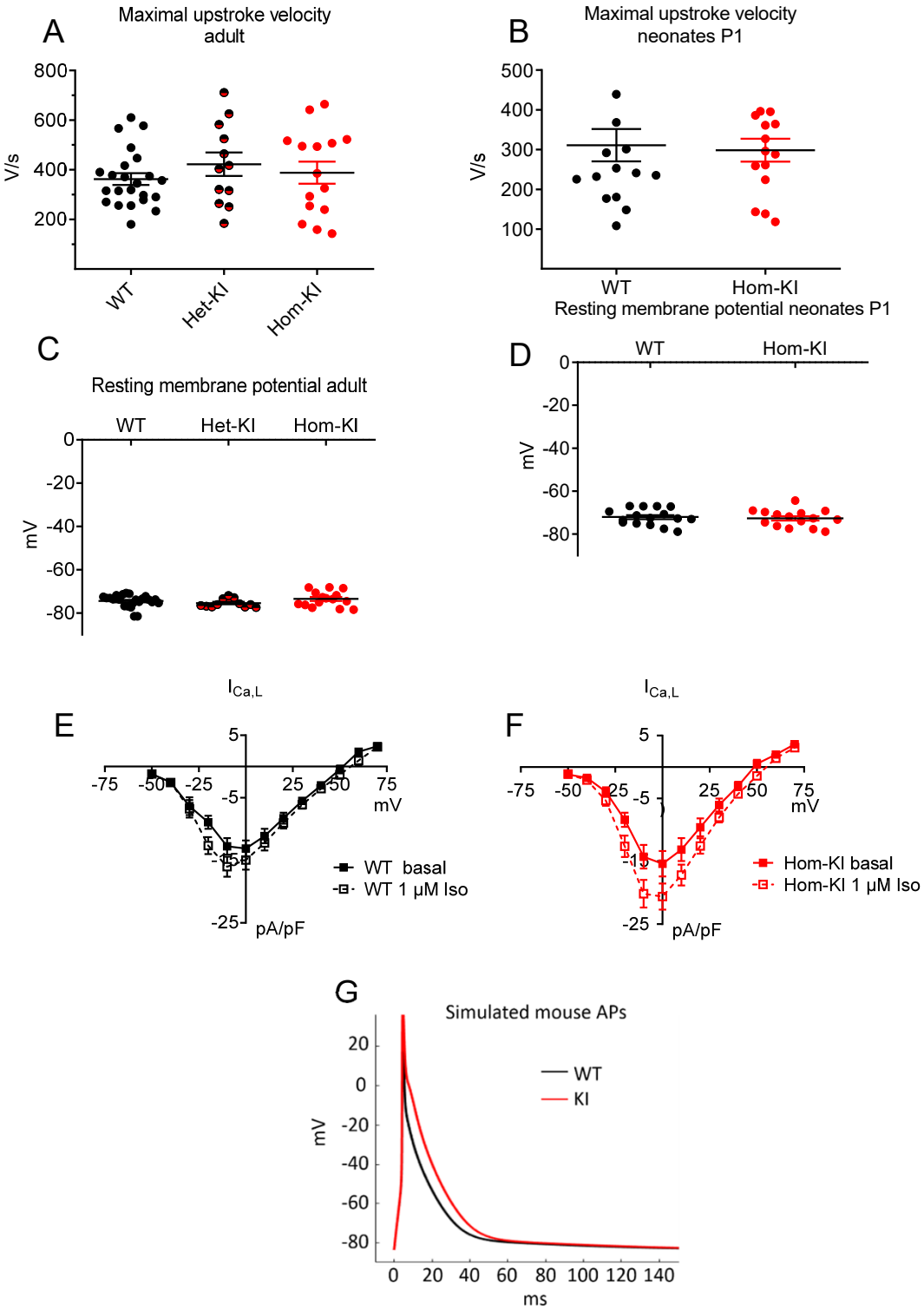
A: Quantification of arrhythmically beating ventricular mouse cardiomyocytes stimulated with 30 nM isoprenaline at 5 Hz pacing rate (n-numbers (preparations/cells) indicated in bar graphs).

B: Quantification of arrhythmically beating ventricular muscle strips stimulated with cumulatively increased concentrations of isoprenaline at 2 Hz pacing rate (n-numbers (muscle strips) indicated in graph, 4 muscles per heart). 4-AP was administered at 1 mM.

C: Quantification of atrial refractory period times in Langendorff-perfused hearts (n=5-6). Stimulation protocol: S1: 12 x 100 ms, S2.

D: Quantification of Wenckebach point times in Langendorff-perfused hearts (n=5-6). Data are expressed as mean±SEM, with P values vs WT in panels A, C and D obtained with the Kruskal-Wallis test, followed by Dunn‘s multiple comparison test. In panel B, F-test for maximal values indicated P=0.0470 between WT vs. Hom-KI and Het-KI, P=0.0053 between WT vs. Hom-KI, P=0.0291 between WT vs. Het-KI, and P=0.0586 between Hom-KI and WT 4-AP. F-test for EC50 of isoprenaline indicated P=0.0044 between WT 4-AP vs Hom-KI.

Abbreviations: 4-AP, 4-aminopyridine; EMD, EMD57033; Iso, isoprenaline; Het-KI, heterozygous *Mybpc3*-targeted knock-in; Hom-KI, homozygous *Mybpc3*-targeted knock-in; WT, wild-type.



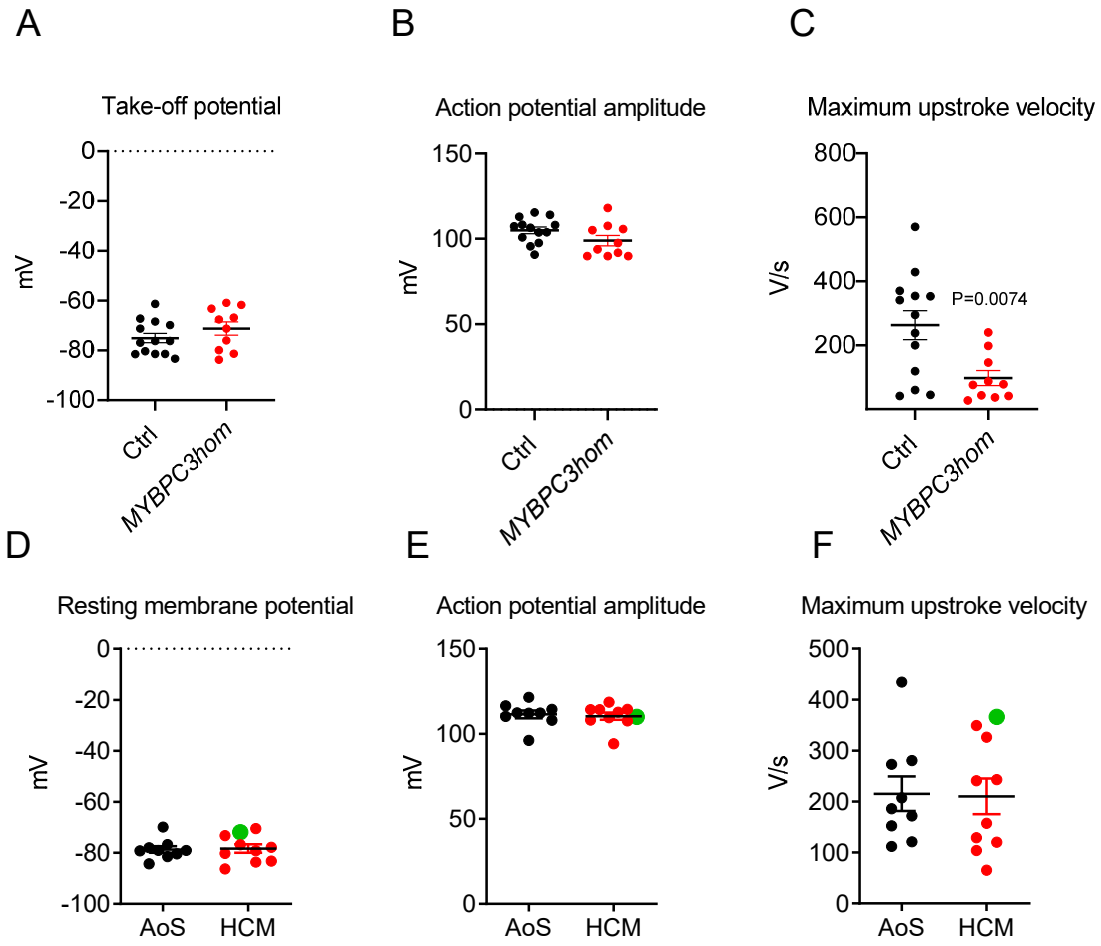
Supplemental Fig. 3: Additional electrophysiological data.

A - D: Maximum upstroke velocities (A, B) and resting membrane potentials (C, D) of action potentials measured in subendocardial layers of the left ventricle of mouse hearts. n adult: WT=16, Het-KI=15, Hom-KI=12, n neonatal: WT=16, Hom-KI=15. Means±SEM are shown.

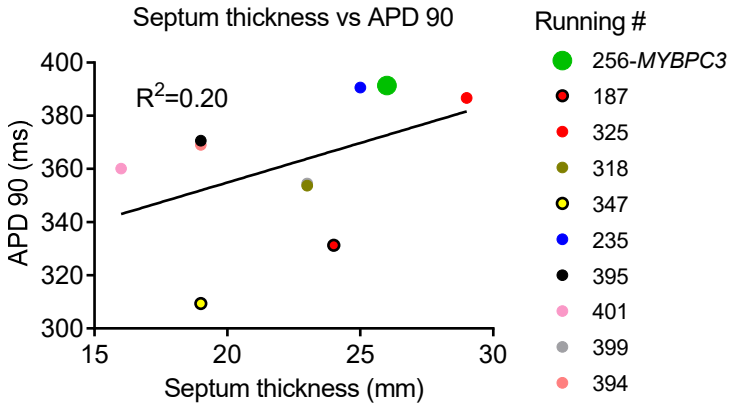
E, F: L-Type Ca^{2+} currents ($I_{Ca,L}$)measured in voltage-clamped adult ventricular mouse cardiomyocytes at different voltages (n=8/group).

G: Simulated ventricular mouse action potentials based on all electrophysiological parameters (K^+, Ca^{2+} current measurements and Na^+ current indirectly measured by upstroke velocity) obtained for this study.

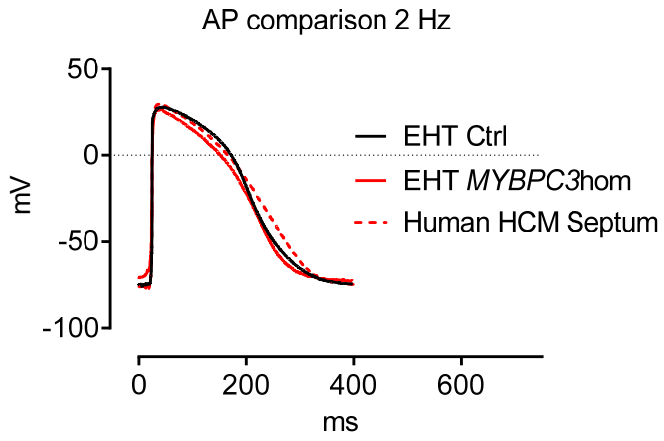
Abbreviations: APs, action potentials; Iso, isoprenaline; Het-KI, heterozygous *Mybpc3*-targeted knock-in; Hom-KI, homozygous *Mybpc3*-targeted knock-in; WT, wild-type.



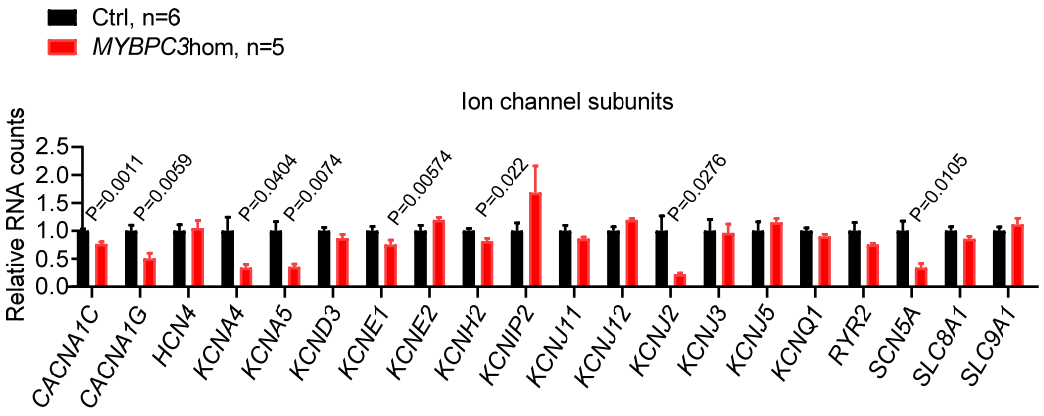
Supplemental Fig. 4: Additional data from action potential measurements in human engineered heart tissue and septum specimen.
Quantification of take-off / resting membrane potential, action potential amplitudes and maximum upstroke velocities of measured action potentials in EHTs (A-C, n=13 for Ctrl and 10 for *MYBPC3*hom) and septum specimen (D-F, n=9 for AoS, n=10 for HCM, including in green the *MYBPC3* mutation).
Data are expressed as mean±SEM. P value vs. Ctrl were obtained with the unpaired Student's t-test.
Abbreviations used: AoS, aortic stenosis; Ctrl, isogenic control; *MYBPC3*hom, homozygous *MYBPC3*.



Supplemental Fig. 5: Relation between septum thickness measured in echocardiography in patients and action potential duration at 90% repolarization measured in septal myectomies of the same patients *ex vivo*.
Patient #256 with a *MYBPC3* mutation is indicated in green.
Abbreviation: APD, action potential duration.



Supplemental Fig. 6: Comparison of action potentials measured in human engineered heart tissue and septum specimen, paced at 2 Hz, recorded with the sharp microelectrode technique.
EHT Ctrl (isogenic control EHTs): n=13, *MYBPC3*hom: n=10, Human HCM septum: n=8



CACNA1C	Cav1.2
CACNA1G	Cav3.1
HCN4	HCN4
KCNA4	Kv1.4
KCNA5	Kv1.5
KCND3	Kv4.3
KCNE1	KCNE1 (MinK)
KCNE2	KCNE2 (MiRP1)
KCNH2	hERG
KCNIP2	KCNIP2 (KChIP2)
KCNJ11	Kir6.2
KCNJ12	Kir2.2
KCNJ2	Kir2.1
KCNJ3	Kir3.1
KCNJ5	Kir3.4
KCNQ1	Kv7.1 (Iks)
RYR2	RyR
SCN5A	Nav1.5
SLC8A1	NCX1
SLC9A1	NHE-1

Supplemental Fig. 7: Quantification of mRNAs of ion channel subunits in human engineered heart tissues.
Data were obtained with a customized Nanostring panel.
Corresponding channels to genes are given as abbreviations in the inset table.
Data are expressed as mean±SEM, and P values vs. ctrl were obtained with the unpaired Student's t-test (n=6 in Ctrl and 5 in *MYBPC3*hom).
Abbreviations: Ctrl, isogenic control; *MYBPC3*hom, homozygous *MYBPC3* mutant.

Running #	Septum thickness (mm)	LVOT gradient (mmHg)	Syncope	VTs	AF	TNT (µg/l)	NT-proBNP (pg/ml)	Medication
256	26	110	no	no	no	< 3	1517	Verapamil, BB*
187	24	85	no	yes	no	24	3386	BB
325	29	120	no	no	no	7	1667	BB
318	23	82	no	no	no	11	1519	BB
347	19	102	no	no	no	36	11269	BB, Moxonidine, Phenprocoumon
235	25	60	no	yes	yes	9	1811	BB, ACE inhibitor, Torasemide, ASA
395	19	133	no	no	no	n.d.	n.d.	BB
401	16	55	no	no	no	13	n.d.	BB, ACE inhibitor, ASA
399	23	87	no	no	no	290	2425	BB, Moxonidine, Diuretic, L-Thyroxine
394	19	64	no	no	no	12	1047	BB, Diuretic, ASA, Moxonidine

Supplemental Table 1: HCM patient data.
Abbreviations: AF, atrial fibrillation; ASA, acetylsalicylic acid; BB, beta blocker, f, female; LVOT, left ventricular outflow tract; m, male; n.d., not determined; NT-proBNP, brain natriuretic peptide; QTc, frequency-corrected QT time; TNT, cardiac troponin T; VT, ventricular tachycardia; *, patient also carried an implanted cardioverter-defibrillator.