SUPPLEMENTAL MATERIALS

Determining the Pathogenicity of a Genomic Variant of Uncertain Significance Using CRISPR/Cas9 and Human Induced Pluripotent Stem Cells

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SUPPLEMENTAL METHODS

The data, analytic methods, and study materials for the purposes of reproducing the results or replicating procedures can be made upon request to the corresponding author who manages the information.

Cell Culture: iPSCs were maintained under feeder-free conditions in defined E8 media (Thermo Fisher Scientific) on tissue culture plates coated with hESC-qualified Matrigel (BD Biosciences). iPSC-CMs were maintained in a RPMI 1640 medium (Thermo Fisher Scientific) supplemented with B27 supplements (Thermo Fisher Scientific) as described in published paper.[1] All cell types were maintained at 5% CO_2 and 37°C.

PCR Detection: DNA was extracted from collected cells using QuickExtract solution (Epicenter). PrimeSTAR® GXL DNA Polymerase (Clontech) was used for PCR. The sequence of primers used for identifying targeted colonies is as follows: forward primer, 5'- cccaagcaaagccagcctgact; and reverse primer, 5'- tgcccgaccttctcacttctcca.

Vector Construction: pSpCas9(BB)-2A-GFP (PX458) was a gift from Feng Zhang (Addgene plasmid # 48138). For the site-specific CRISPR/Cas9 construction, two reverse complementary guide oligos were annealed first and then ligated to the linearized PX458 vector.

Fluorescent Activated Cell Sorting (FACS): iPSC-CMs were treated with Accutase® solution (Sigma-Aldrich) at 37°C for 5 minutes. Afterwards, cells were collected for centrifugation, following which the supernatant was removed and the cell pellet was resuspended using PBS.

Fixation, permeabilization, and antibody staining were then performed following the instruction of the BD Cytofix/CytopermTM Fixation/Permeabilization Solution Kit (Thermo Fisher Scientific, BDB554714). After staining, cells were resuspended in 500 uL of FACS buffer. Cell solution was then filtered in Falcon® 5mL Round Bottom Polystyrene, 12 x 75 mm Test Tube, with Cell Strainer Snap Cap (Corning), and loaded on a BD Biosciences FACS Aria II instrument fitted with a 100 μm nozzle using FACSDiva software. For iPSC characterization, anti-OCT4 antibody (Abcam, ab181557) and anti-SSEA4 antibody (Abcam, ab16287) were used. For detection of iPSC-CMs cardiac-specific proteins, anti-cardiac troponin T antibody (Abcam, ab45932) and anti sarcomeric-α-actinin antibody (Sigma-Aldrich, A7811-100UL) were applied.

Quantitative PCR (qPCR): Total RNAs were isolated from all studied iPSC-CMs lines at day 45-50 post differentiation, using the miRNeasy Mini kit (QIAGEN). 1 μg of RNA was used for synthesizing cDNA with the iScriptTM cDNA Synthesis kit (Bio-Rad). 0.25 μL of the cDNA was used to quantify gene expression using TaqMan probes and TaqMan Universal PCR Master Mix (Thermo Fisher Scientific).

Cell Size Measurement: Cells were first fixed by 4% paraformaldehyde (PFA) for 10 minutes at room temperature. After washing twice with PBS, cells were incubated with 0.1% Triton® X-100 for 20 minutes. Cells were then stained using 0.1% Triton® X-100, 0.1% Hoechst (Thermo Fisher Scientific) and 0.2% Alexa Fluor® 488 Phalloidin (Thermo Fisher Scientific) for 1 hour at room temperature. After washing twice with PBS, cells were imaged using a Cytation 5 Imaging Reader.

Contractility Assay: iPSC-CMs were dissociated using Accutase for 5 minutes and then were collected for centrifugation. Afterwards, cells were resuspended and counted. Each well of a 24-well plate was seeded with 300k cells, forming a synchronously beating iPSC-CMs monolayer. After a week of recovery, contractility was measured using the Sony SI8000 cell motion imaging system, in the presence of 5% CO_2 and at a temperature of 37°C. Prior to initiation of recording, the cells were given time to equilibrate for 15 minutes at the given setting. Video imaging of the beating iPSC-CMs monolayers were recorded from 2 separate locations in each well (center and lateral edge), spatially adjusted in an automated recognized fashion, for 10 sec, at a frame rate of 75 fps, a resolution of 1024 × 24 pixels, and a depth of 8 bits using a 10× objective.

Optical Imaging of ASAP2: Dissociated iPSC-CMs were seeded on Matrigel-coated 35 mm glass bottom dishes with 20 mm micro-well (Cellvis). iPSC-CMs were infected with the pLV-EF1A-ASAP2 lentiviral vector (gift from Dr. Michael Z. Lin at Stanford University) with the multiplicity of infection (MOI) of 3 in RPMI-B27 medium containing polybrene (1 μg/ml) (VectorBuilder). Optical imaging of ASAP2 was performed 7 days after infection. Cells were superfused with Tyrode's solution at 37°C. The Tyrode solution consisted of NaCl (140 mmol/L), KCl (5.4 mmol/L), CaCl2 (1.8 mmol/L), MgCl2 (1 mmol/L), HEPES (10 mmol/L), and glucose (10 mmol/L); pH was adjusted to 7.4 with NaOH. ASAP2 was excited at 488 nm and emission was collected over 510 nm. Line scan images were acquired on a Zeiss LSM710 confocal microscope (Zeiss) equipped with a 40× oil lens (NA: 1.30). Data were analyzed using ImageJ.

Ca^{2+} Imaging: Dissociated iPSC-CMs were seeded on Matrigel-coated coverslips (CS-24/50,

thickness 1 mm, Warner Instruments). Cells were loaded with 5 μM Fura-2AM (Thermo Fisher Scientific) in the presence of 0.02% Pluronic F-127 (Thermo Fisher Scientific) in Tyrode's solution for 10 minutes at room temperature, followed by washing with Tyrode's solution. iPSC-CMs were field-stimulated at 0.5 Hz at 37°C. Single cell Ca^{2+} imaging was conducted on a Nikon Eclipse Ti-E inverted microscope mounted with 40× oil immersion objective (0.95 NA). Fura-2 was excited at 340 nm and 380 nm wavelength using a Lambda DG-4 ultra-high-speed wavelength switcher (Sutter Instrument), and the emission of Fura-2 was collected over 510 nm wavelength. Raw data were analyzed using customized Matlab scripts and Ca^{2+} signal was presented as 340/380 ratio.

SUPPLEMENTAL FIGURE LEGENDS

Supplemental Figure S1. Pedigree of the VUS*MYL3*$_{(170C>A)}$ carrier's family. (A) Pie chart depicting the overall annotation profile of genetic variants that were detected in 135 heart disease-related genes across 54 healthy individuals. Numbers in the pie chart present the number of variants per category. **(B)** Schematic pedigree of the proband's family. The VUS*MYL3*$_{(170C>A)}$ carrying proband (II-1) is marked by a red asterisk. Female family member are marked by circles and male by squares.. Shaded symbols indicate members carrying the VUS*MYL3*$_{(170C>A)}$, whereas open symbols represent absence of the VUS*MYL3*$_{(170C>A)}$. **(C)** Sanger sequencing results of all family members of the VUS*MYL3*$_{(170C>A)}$ carrier. Nucleotides positions of the VUS*MYL3*$_{(170C>A)}$ are depicted by framing squares.

Supplemental Figure S2. Pluripotency identification of iPSCs derived from the heterozygous VUS*MYL3*$_{(170C>A)}$ carrier. (A) Bright field image of cultured iPSC colonies. Scale bar: 100 μm. **(B)** Pluripotency related gene expression in the heterozygous VUS*MYL3*$_{(170C>A)}$ iPSCs relative to human ESC line H7. **(C)** Immunostaining of the heterozygous VUS*MYL3*$_{(170C>A)}$ iPSCs using anti-OCT4 (green) and anti-SSEA4 antibody (red). Scale bar: 200 μm. **(D)** FACS analysis of OCT4 and SSEA4 expression in the heterozygous VUS*MYL3*$_{(170C>A)}$ iPSCs. Blue, isotype control; Red, antigen staining for SSEA4 (left panel) or OCT4 (right panel). **(E)** Gene expression analysis of pluripotency related genes and differentiation related genes after embryoid body formation. **(F)** Normal karyotype of the heterozygous VUS*MYL3*$_{(170C>A)}$ iPSCs. Error bar: three replicates, mean ± SD.

Supplemental Figure S3. Identification of iPSC-CMs derived from the two healthy control

lines, isogenic corrected line and heterozygous VUS*MYL3*$_{(170C>A)}$ line. (A) Sanger sequencing confirmation of the isogenic corrected iPSC clone 2 and clone 3. **(B)** Bright field morphology of iPSC-CMs from the two healthy control lines, three isogenic corrected clones, and heterozygous VUS*MYL3*$_{(170C>A)}$ line. Scale bars, 50 μm. **(C)** Flow cytometry analyses of specific iPSC-CMs markers (TNNT and sarcomeric-α-actinin). Blue, isotype control; Red, antigen staining for TNNT (upper panel) or α-Actinin (lower panel). **(D)** Immunostaining analysis of specific iPSC-CMs markers TNNT (green) and sarcomeric-α-actinin (red) of the isogenic corrected iPSC clone 2 and clone 3 derived CMs. **(E)** Cell size measurement of three isogenic corrected iPSC clones-derived CMs. Data from ≥ 200 cells (acquired from three differentiations performed at three separate occasions) of each clone, mean ± SD. ns: non-significant, compared with isogenic corrected clone 1.

Supplemental Figure S4. Gene expression analysis of three isogenic corrected iPSC clones-derived CMs.

Gene expression profile (*NPPA, MYH6, TNNT2, MYH7, MYL2, MYL3, MYL4, MYL6, MYL7* and *MYL9*) of three isogenic corrected iPSC clones-derived CMs. Data from three replicates (acquired from three differentiations performed on three separate occasions), mean ± SD.

ns: non-significant, compared with isogenic corrected clone 1.

Supplemental Figure S5. Functional analysis of the isogenic corrected iPSC clones-derived CMs and heterozygous VUS*MYL3*$_{(170C>A)}$ iPSC-CMs. (A) Beating rate (left panel), contraction velocity (middle panel), and relaxation velocity (right panel) analysis of three isogenic corrected iPSC clones-derived CMs. Data from three replicates, mean ± SD. ns: non-significant, compared

with isogenic corrected clone1. **(B)** Contraction distance, relaxation distance, contraction duration, and relaxation duration analysis of iPSC-CMs from the two healthy control lines, isogenic corrected clone 1, and heterozygous VUS*MYL3*$_{(170C>A)}$ line. Data from three replicates, mean ± SD. ns: non-significant, compared with two healthy control lines, and the isogenic corrected clone 1. **(C)** Number of iPSC-CMs showing proarrhythmic activity in isogenic corrected clone 2 and clone 3-derived CMs lines. **(D)** Number of iPSC-CMs showing calcium transients with a proarrhythmic phenotype in isogenic corrected clone 2 and clone 3-derived CMs lines. Three replicates means three differentiations performed on three separate occasions.

Supplemental Figure S6. Sequence validation of homozygous VUS*MYL3*$_{(170C>A)}$ and frameshift mutation *MYL3*$^{(170C>A/fs)}$ iPSC clones. (A) Sequence of gRNA and ssODN used for generating the homozygous VUS*MYL3*$_{(170C>A)}$ iPSC line. **(B)** Sanger sequencing results of homozygous VUS*MYL3*$_{(170C>A)}$ iPSC clone 2 and clone 3. **(C)** Sanger sequencing results of genomic DNA isolated from frameshift mutation *MYL3*$^{(170C>A/fs)}$ clone 2 and clone 3 showing 1bp insertion in the genome of frameshift mutation *MYL3*$^{(170C>A/fs)}$ clone2 (upper panel) and 13bp deletion in the genome of frameshift mutation *MYL3*$^{(170C>A/fs)}$ clone3 (lower panel). Red lower-case letters (tga): premature stop codon, Red capitalized letter 'A': VUS*MYL3*$_{(170C>A)}$. **(D)** Sanger sequencing results of cDNA from frameshift mutation *MYL3*$^{(170C>A/fs)}$ clone1 (top panel), clone2 (middle panel) and clone3 (bottom panel). Nucleotides positions of the VUS*MYL3*$_{(170C>A)}$ are depicted by a framing square.

Supplemental Figure S7. Characterization of homozygous VUS*MYL3*$_{(170C>A)}$ and frameshift mutation *MYL3*$^{(170C>A/fs)}$ clones-derived CMs. (A) Gene expression profile of three

homozygous VUS*MYL3*$_{(170C>A)}$ (left panel) and three frameshift mutation *MYL3*$^{(170C>A/fs)}$ clones-derived CMs (right panel). **(B)** Cell size measurement of homozygous VUS*MYL3*$_{(170C>A)}$ (left panel) and frameshift mutation *MYL3*$^{(170C>A/fs)}$ (right panel) clones-derived CMs. Data from ≥ 200 cells, mean ± SD**. (C)** Immunostaining analysis.. Scale bars: 10 μm. **(D-F)** Beating rate, contraction velocity, and relaxation velocity analysis, respectively. (**G)** Number of homozygous VUS*MYL3*$_{(170C>A)}$ clone 2- and clone 3- (left panel) and frameshift mutation *MYL3*$^{(170C>A/fs)}$ clone 2- and clone 3- (right panel) derived CMs showing normal and proarrhythmic activity. (**H)** Number of homozygous VUS*MYL3*$_{(170C>A)}$ clone 2- and clone 3- (left panel) and frameshift mutation *MYL3*$^{(170C>A/fs)}$ clone 2- and 3- (right panel) -derived CMs showing calcium transients with a proarrhythmic phenotype. Measurements were conducted in three replicates (three differentiations performed at three separate occasions). ns: non-significant, compared with either homozygous VUS*MYL3*$_{(170C>A)}$ clone 1 or frameshift mutation *MYL3*$^{(170C>A/fs)}$ clone 1. Data presented as mean ± SD.

Supplemental Figure S8. Characterization of *MYL3*$_{(170C>G)}$ and *MYBPC3*$_{(961G>A)}$ iPS-CMs. **(A)** Sequence of gRNA and ssODN used for generating the isogenic *MYL3*$_{(170C>G)}$ iPSC line. **(B)** Sanger sequencing results of *MYL3*$_{(170C>G)}$ iPSC clone 2 and clone 3. **(C)** Cell size measurement of three *MYL3*$_{(170C>G)}$ clones-derived CMs. Data from ≥ 200 cells, **(D)** Immunostaining analysis of three *MYL3*$_{(170C>G)}$ clones-derived CMs and *MYBPC3*$_{(961G>A)}$ iPSC-CMs. Scale bars: 10 μm. **(E)** HCM-related gene expression analysis of the three *MYL3*$_{(170C>G)}$ clones-derived CMs. **(F)** Beating rate (top panel), contraction velocity (middle panel), and relaxation velocity (bottom panel) analysis of three *MYL3*$_{(170C>G)}$ clones-derived CMs. (**G)** Number of *MYL3*$_{(170C>G)}$ clone 2- and clone 3-derived CMs showing proarrhythmic activity. (**H)** Number of *MYL3*$_{(170C>G)}$ clone 2- and clone 3-derived CMs showing calcium transients with a proarrhythmic phenotype. **(I)**

Diastolic calcium of the isogenic corrected, heterozygous VUS*MYL3*$_{(170C>A),}$ homozygous VUS*MYL3*$_{(170C>A)}$, frameshift *MYL3*$^{(170C>A/fs)}$, *MYL3*$_{(170C>G)}$ and *MYBPC3*$_{(961G>A)}$ iPSC-CMs. ns: non-significant, * $p<0.05$, compared with the isogenic corrected line.

Measurements in all panels were conducted in three replicates (three differentiations performed at three separate occasions). In panels C, E, F, ns: non-significant, compared with *MYL3*$_{(170C>G)}$ clone1. Data presented as mean ± SD.

Supplemental Table S1. 17 "likely pathogenic" variants detected in healthy individuals

Locus	gene	location	function	protein	coding	Polyphen-2	Associated disease
chr1:116275561	*CASQ2*	NM_001232.3	missense	p.Phe189Leu	c.567C>G	1	CPVT
chr2:220284876	*DES*	NM_001927.3	missense	p.Ala213Val	c.638C>T	0.896	ARVC/DCM
chr3:38645238	*SCN5A*	NM001160161.1	missense	p.Leu619Phe	c.1855C>T	0.975	LQT
chr3:46902303	*MYL3*	NM_000258.2	missense	p.Ala57Asp	c.170C>A	1	HCM
chr4:114288907	*ANK2*	NM_001148.4	missense	p.Leu3740Ile	c.11218C>A	0.905	VT /VF
chr4:114288920	*ANK2*	NM_001148.4	missense	p.Thr3744Asn	c.11231C>A	0.951	VT /VF
chr8:11614575	*GATA4*	NM_002052.4	missense	p.Ser377Gly	c.1129A>G	0.54	DCM
chr10:69881254	*MYPN*	NM_001256267.1	missense	p.Tyr20Cys	c.59A>G	1	HCM/DCM
chr11:123513271	*SCN3B*	NM_018400.3	missense	p.Val110Ile	c.328G>A	0.999	LQT
chr11:47359047	*MYBPC3*	NM_000256.3	missense	p.Ala833Thr	c.2497G>A	0.999	HCM
chr12:121175678	*ACADS*	NM_000017.3	missense	p.Arg171Trp	c.511C>T	0.994	SCAD
chr12:121176083	*ACADS*	NM_000017.3	missense	p.Gly209Ser	c.625G>A	0.664	SCAD
chr14:23892910	*MYH7*	NM_000257.3	missense	p.Met982Thr	c.2945T>C	0.985	HCM
chr19:49686146	*TRPM4*	NM_017636.3	missense	p.Trp525Ter	c.1575G>A	-	Brugada
chrX:31496398	*DMD*	NM_004006.2	missense	p.His2921Arg	c.8762A>G	0.83	DMD
chrX:31496426	*DMD*	NM_004006.2	missense	p.Asn2912Asp	c.8734A>G	0.08	DMD
chrX:31496431	*DMD*	NM_004006.2	missense	p.Glu2910Val	c.8729A>T	0.21	DMD

Catecholaminergic polymorphic ventricular tachycardia (CPVT); arrhythmogenic right ventricular cardiomyopathy (ARVC); hypertrophic cardiomyopathy (HCM); long QT (LQT); ventricular tachycardia/ventricular fibrillation (VT/VF); dilated cardiomyopathy (DCM); spontaneous coronary artery dissection (SCAD); duchenne muscular dystrophy (DMD).

Supplemental Table S2. Frequencies of the VUS*MYL3*$_{(170C>A)}$ in different populations

Population	Allele Count	Allele Number	Homozygous Number	Allele Frequency
European (Non-Finnish)	5	66684	0	7.498e-05
South Asian	5	16512	0	0.0003028
East Asian	3	8646	0	0.000347
Latino	1	11574	0	8.64e-05
Other	0	906	0	0
African	0	10402	0	0
Finnish	0	6614	0	0
Total	14	121338	0	0.0001154

Supplemental Table S3. No indels were found at the potential off-target loci of the gRNA for generating three independent isogenic corrected clones (C1, C2 and C3).

Sequence	Mismatches	Locus	Indels		
			C1	C2	C3
GGTCTTCCTTGCACTCTGCCGAG	2MMs [1:12]	chr14:-105241012	N	N	N
AGTTTTTATTGAACTCTGCCCAG	3MMs [4:7:8]	chr12:-45858141	N	N	N
ACTCTTCCAGGAACTCTGCCAAG	3MMs [2:9:10]	chr8:+139651269	N	N	N
AGGGCTCATTGAACTCTGCCAAG	4MMs [3:4:5:8]	chr2:-60510140	N	N	N
CTTCTTCATAGAACTCTGCCAAG	4MMs [1:2:8:10]	chr11:-77389083	N	N	N
TTTCTTCATAGAACTCTGCCTGG	4MMs [1:2:8:10]	chr14:-99019889	N	N	N
TGCTGTCCTTGAACTCTGCCGGG	4MMs [1:3:4:5]	chr20:+39783116	N	N	N
AGTGGTTGTTGAACTCTGCCTGG	4MMs [4:5:7:8]	chr18:+48814709	N	N	N
TTTCTCCTTTGAACTCTGCCCAG	4MMs [1:2:6:8]	chr9:+2359678	N	N	N
ACCCTTCTCTGAACTCTGCCCAG	4MMs [2:3:8:9]	chr13:-95203119	N	N	N

Supplemental Table S4. No indels were found at the potential off-target loci of the gRNA for generating three independent homozygous VUS*MYL3*$_{(170C>A)}$ clones (C1, C2 and C3).

Sequence	Mismatches	Locus	Indels		
			C1	C2	C3
AGTGAGGTTGAACAGCATGAGAG	3MMs [1:5:9]	chr12:-107792399	N	N	N
TGGGCAGTCAAACAGCATGAGAG	3MMs [3:6:10]	chr18:+52953785	N	N	N
TGTGCTTTCCAACAGCATGAGAG	3MMs [6:7:10]	chr10:+4729601	N	N	N
TGTCCTCTCCAACAGCATGACAG	4MMs [4:6:7:10]	chr1:+40222149	N	N	N
GATGCGATCGAACAGCATGTGAG	4MMs [1:2:7:20]	chr6:+41250150	N	N	N
TCTGTGGTAGCACAGCATGAGAG	4MMs [2:5:9:11]	chr5:-132089491	N	N	N
AGTGGGGTTGAAGAGCATGAGGG	4MMs [1:5:9:13]	chr11:+127348738	N	N	N
TGTGGGTTCCAAAAGCATGAAGG	4MMs [5:7:10:13]	chr5:-54405129	N	N	N
TGTCCAGTCCAACAGCTTGAGGG	4MMs [4:6:10:17]	chr3:+129239902	N	N	N
AGTGGGGTCGTACAGCATCAGAG	4MMs [1:5:11:19]	chr1:+156884123	N	N	N

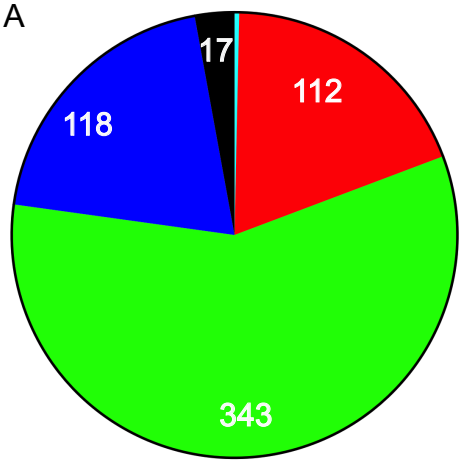
Supplemental Table S5. No indels were found at the potential off-target loci of the gRNA for generating three independent *MYL3*$_{(170C>G)}$ clones (C1, C2 and C3).

Sequence	Mismatches	Locus	Indels		
			C1	C2	C3
GGTCTTCCTTGCACTCTGCCGAG	2MMs [1:12]	chr14:-105241012	N	N	N
AGTTTTTATTGAACTCTGCCCAG	3MMs [4:7:8]	chr12:-45858141	N	N	N
ACTCTTCCAGGAACTCTGCCAAG	3MMs [2:9:10]	chr8:+139651269	N	N	N
AGGGCTCATTGAACTCTGCCAAG	4MMs [3:4:5:8]	chr2:-60510140	N	N	N
CTTCTTCATAGAACTCTGCCAAG	4MMs [1:2:8:10]	chr11:-77389083	N	N	N
TTTCTTCATAGAACTCTGCCTGG	4MMs [1:2:8:10]	chr14:-99019889	N	N	N
TGCTGTCCTTGAACTCTGCCGGG	4MMs [1:3:4:5]	chr20:+39783116	N	N	N
AGTGGTTGTTGAACTCTGCCTGG	4MMs [4:5:7:8]	chr18:+48814709	N	N	N
TTTCTCCTTTGAACTCTGCCCAG	4MMs [1:2:6:8]	chr9:+2359678	N	N	N
ACCCTTCTCTGAACTCTGCCCAG	4MMs [2:3:8:9]	chr13:-95203119	N	N	N

Reference:

1. Burridge PW, Li YF, Matsa E, Wu H, Ong SG, Sharma A, Holmstrom A, Chang AC, Coronado MJ, Ebert AD, Knowles JW, Telli ML, Witteles RM, Blau HM, Bernstein D, Altman RB and Wu JC. Human induced pluripotent stem cell-derived cardiomyocytes recapitulate the predilection of breast cancer patients to doxorubicin-induced cardiotoxicity. *Nat Med*. 2016;22:547-556.

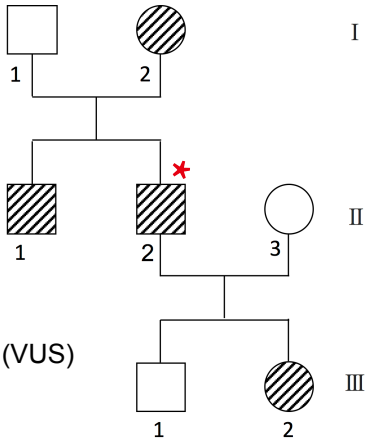
A



Drug response variants
Unknown
Benign or likely benign variants
Variants of unknow significance (VUS)
Likely pathogenic variants

Total=592 variants

B



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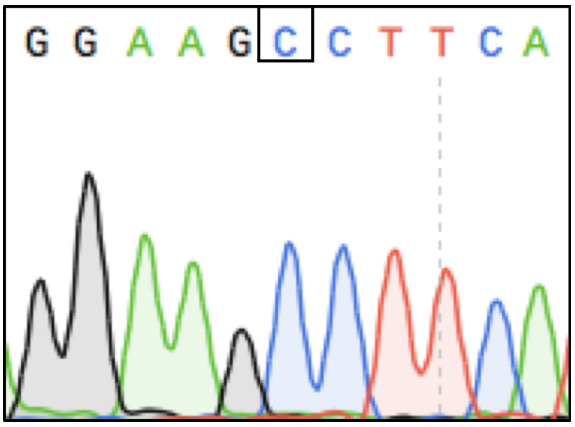

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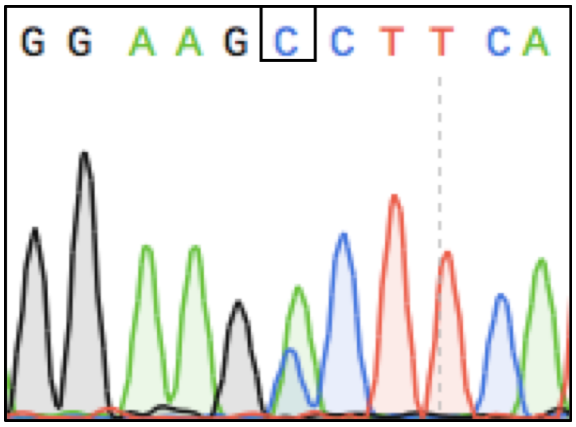

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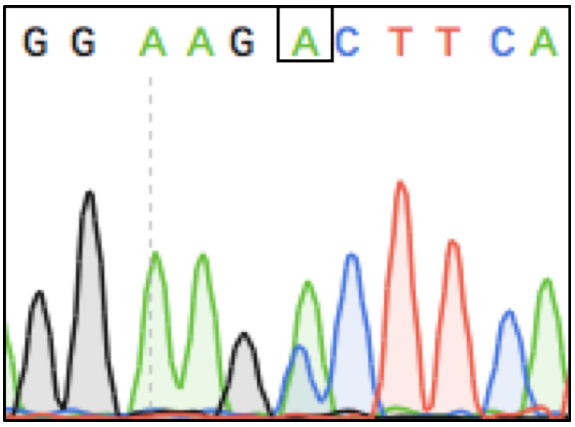

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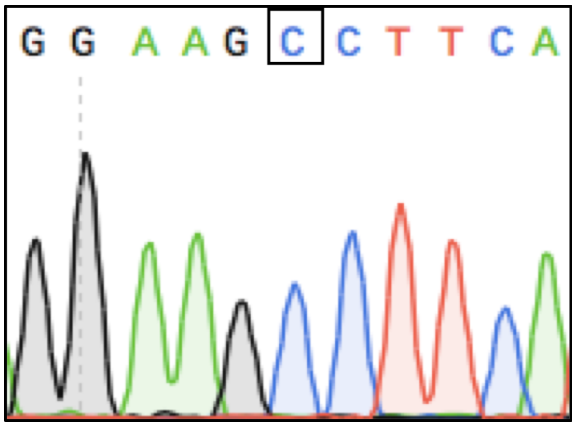

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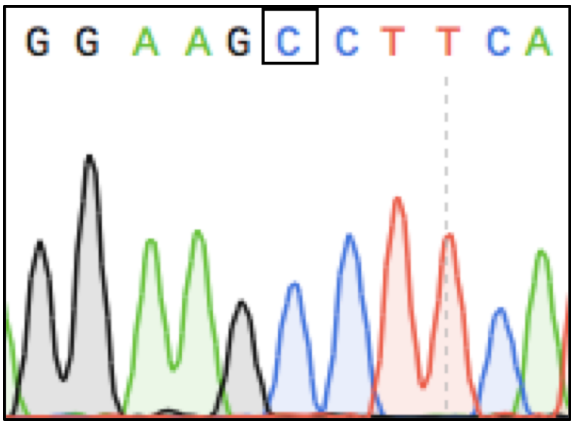
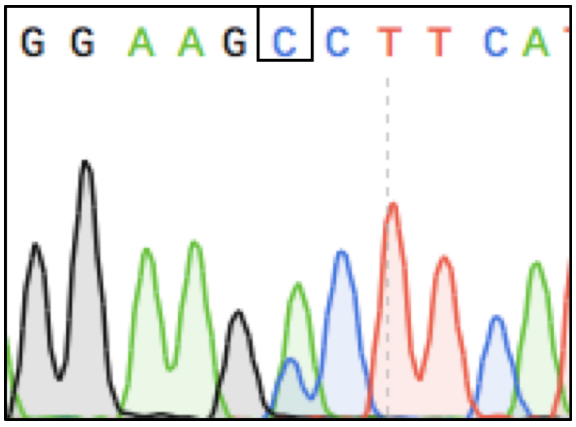
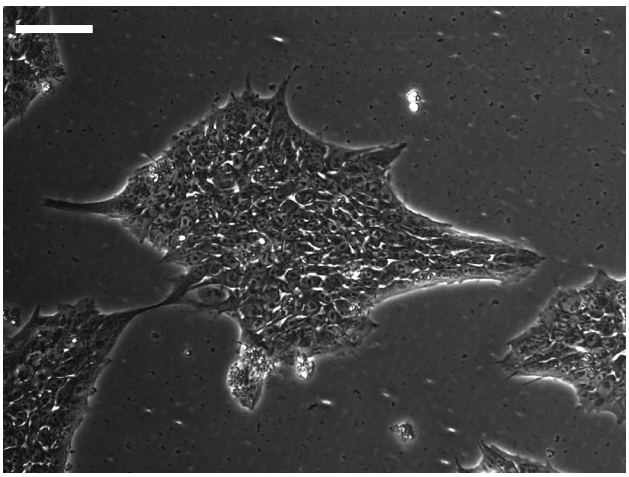


III.1



III.2

A



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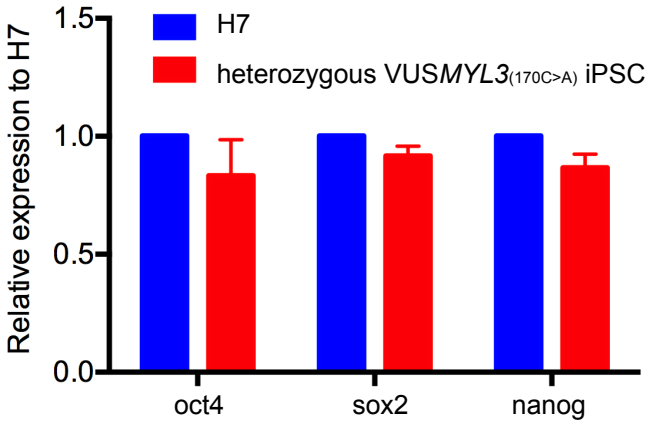
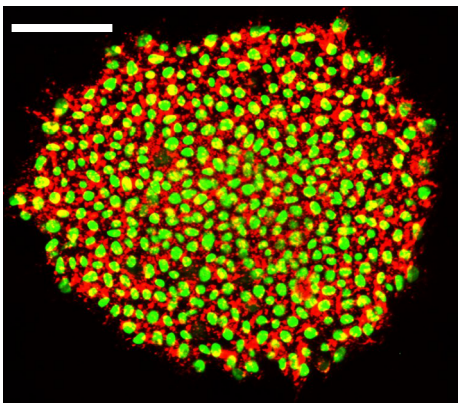
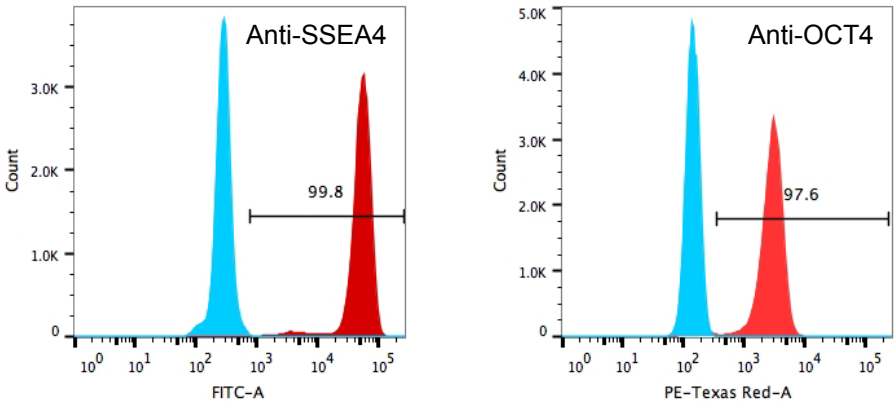

H7
heterozygous VUS*MYL3*(170C>A) iPSC
Relative expression to H7
1.5
1.0
0.5
0.0
oct4
sox2
nanog

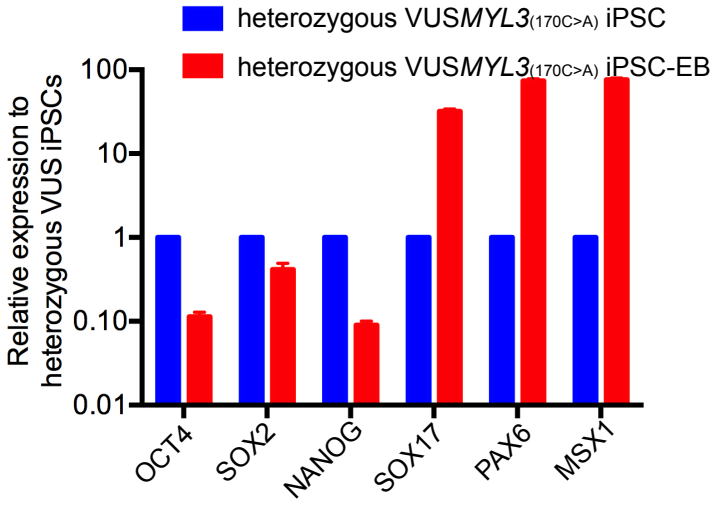
C



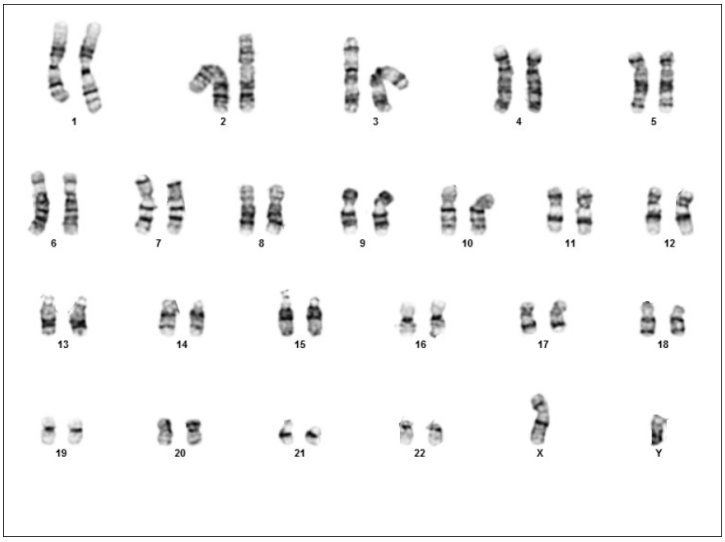
D

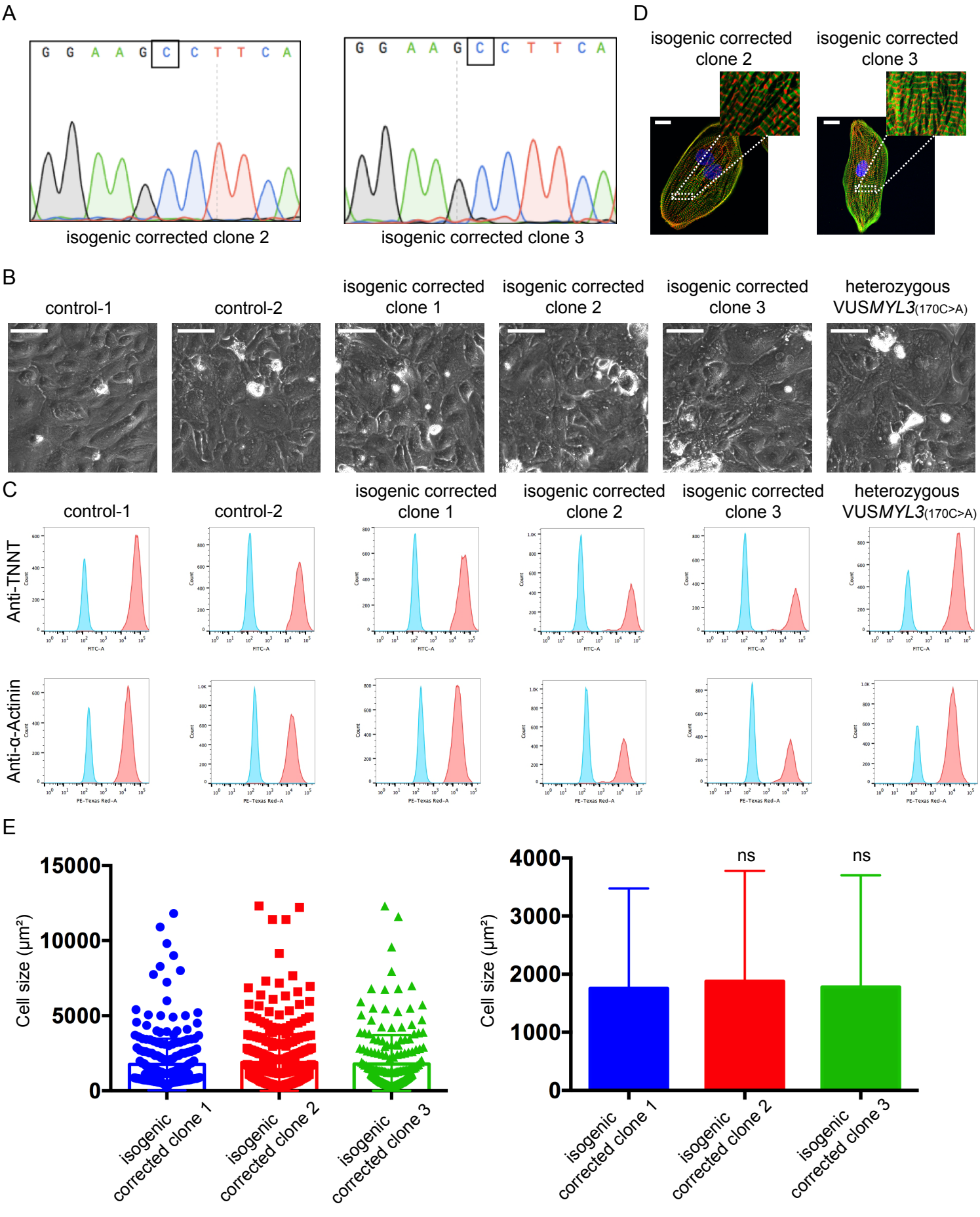

Anti-SSEA4
Count
3.0K
2.0K
1.0K
0
99.8
FITC-A
Anti-OCT4
5.0K
4.0K
3.0K
2.0K
1.0K
0
97.6
PE-Texas Red-A

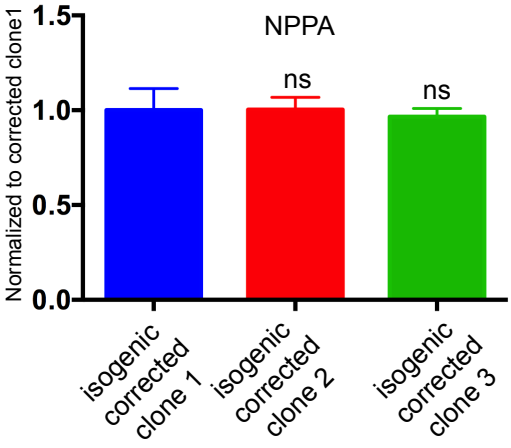
E

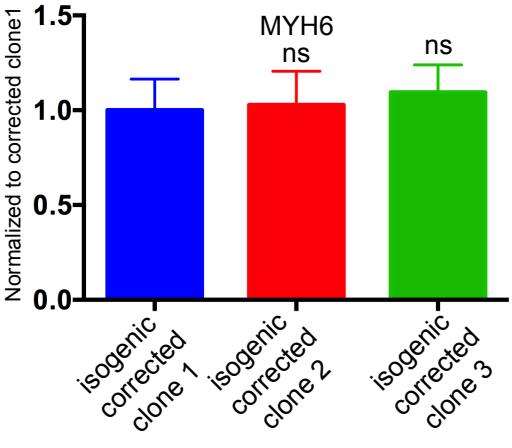

heterozygous VUS*MYL3*(170C>A) iPSC
heterozygous VUS*MYL3*(170C>A) iPSC-EB
Relative expression to heterozygous VUS iPSCs
100
10
1
0.10
0.01
OCT4
SOX2
NANOG
SOX17
PAX6
MSX1

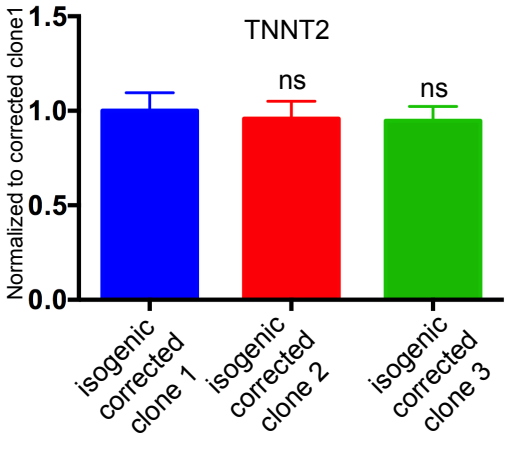
F

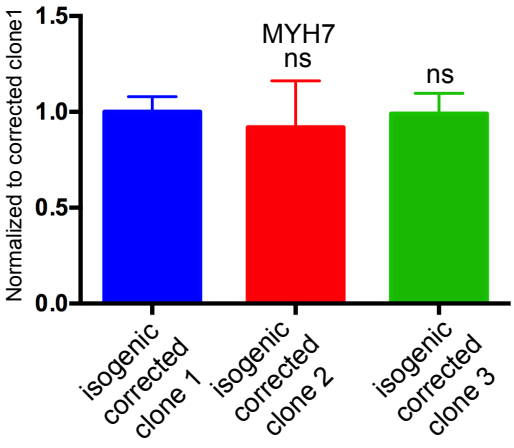


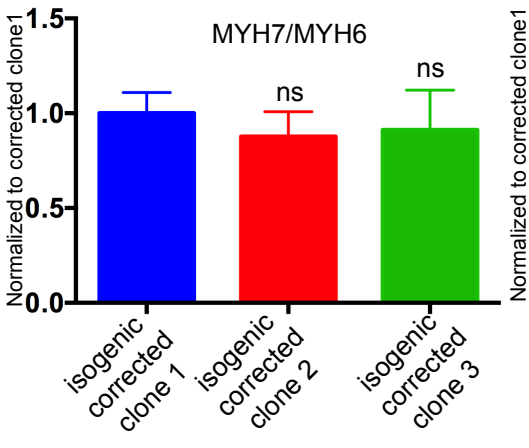

A
G G A A G C C T T C A
isogenic corrected clone 2
G G A A G C C T T C A
isogenic corrected clone 3
D
isogenic corrected clone 2
isogenic corrected clone 3
B
control-1
control-2
isogenic corrected clone 1
isogenic corrected clone 2
isogenic corrected clone 3
heterozygous VUS MYL3 (170C>A)
C
control-1
control-2
isogenic corrected clone 1
isogenic corrected clone 2
isogenic corrected clone 3
heterozygous VUS MYL3 (170C>A)
Anti-TNNT
Anti-α-Actinin
FITC-A
PE-Texas Red-A
Count
E
Cell size (μm²)
15000
10000
5000
0
isogenic corrected clone 1
isogenic corrected clone 2
isogenic corrected clone 3
4000
3000
2000
1000
0
ns
ns

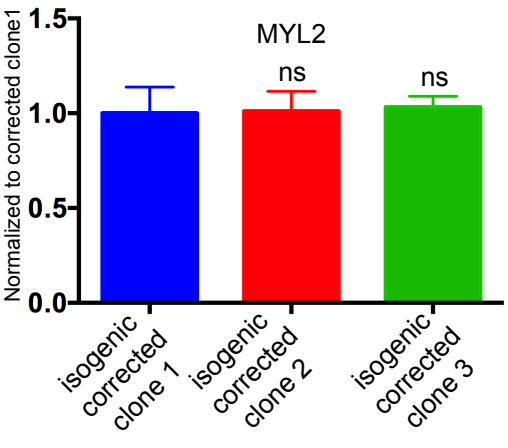

NPPA
Normalized to corrected clone1
1.5
1.0
0.5
0.0
ns
ns
isogenic corrected clone 1
isogenic corrected clone 2
isogenic corrected clone 3

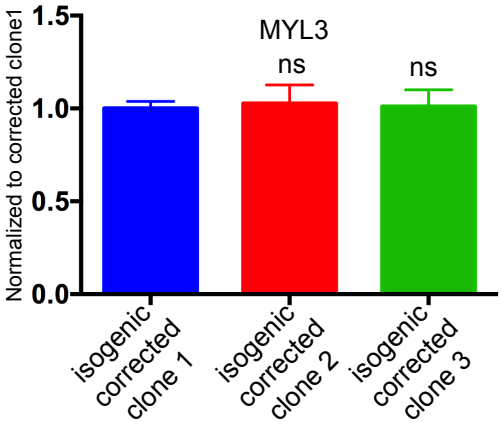

MYH6
Normalized to corrected clone1
1.5
1.0
0.5
0.0
ns
ns
isogenic corrected clone 1
isogenic corrected clone 2
isogenic corrected clone 3

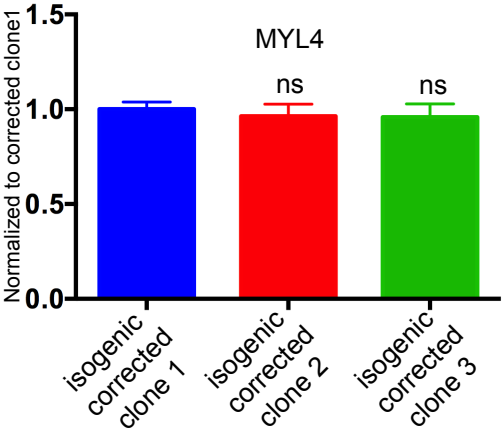

TNNT2
Normalized to corrected clone1
1.5
1.0
0.5
0.0
ns
ns
isogenic corrected clone 1
isogenic corrected clone 2
isogenic corrected clone 3

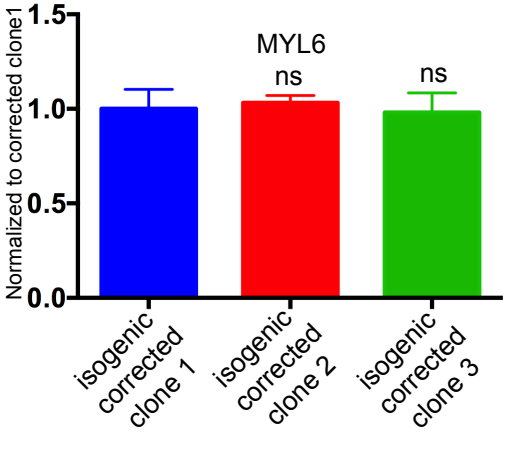

MYH7
Normalized to corrected clone1
1.5
1.0
0.5
0.0
ns
ns
isogenic corrected clone 1
isogenic corrected clone 2
isogenic corrected clone 3

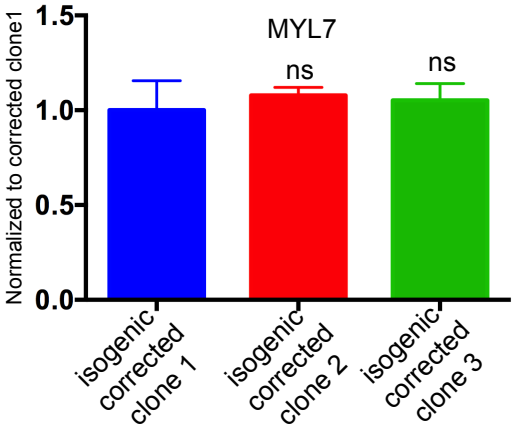

MYH7/MYH6
Normalized to corrected clone1
1.5
1.0
0.5
0.0
ns
ns
isogenic corrected clone 1
isogenic corrected clone 2
isogenic corrected clone 3

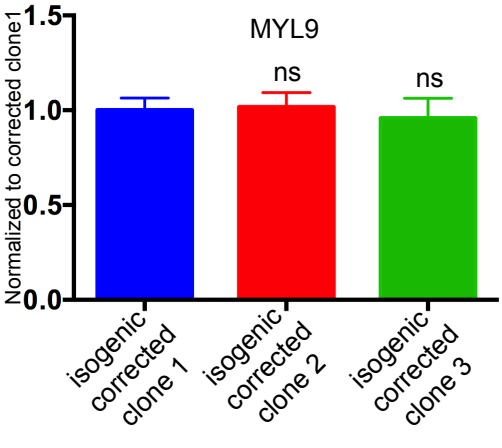

MYL2
Normalized to corrected clone1
1.5
1.0
0.5
0.0
ns
ns
isogenic corrected clone 1
isogenic corrected clone 2
isogenic corrected clone 3

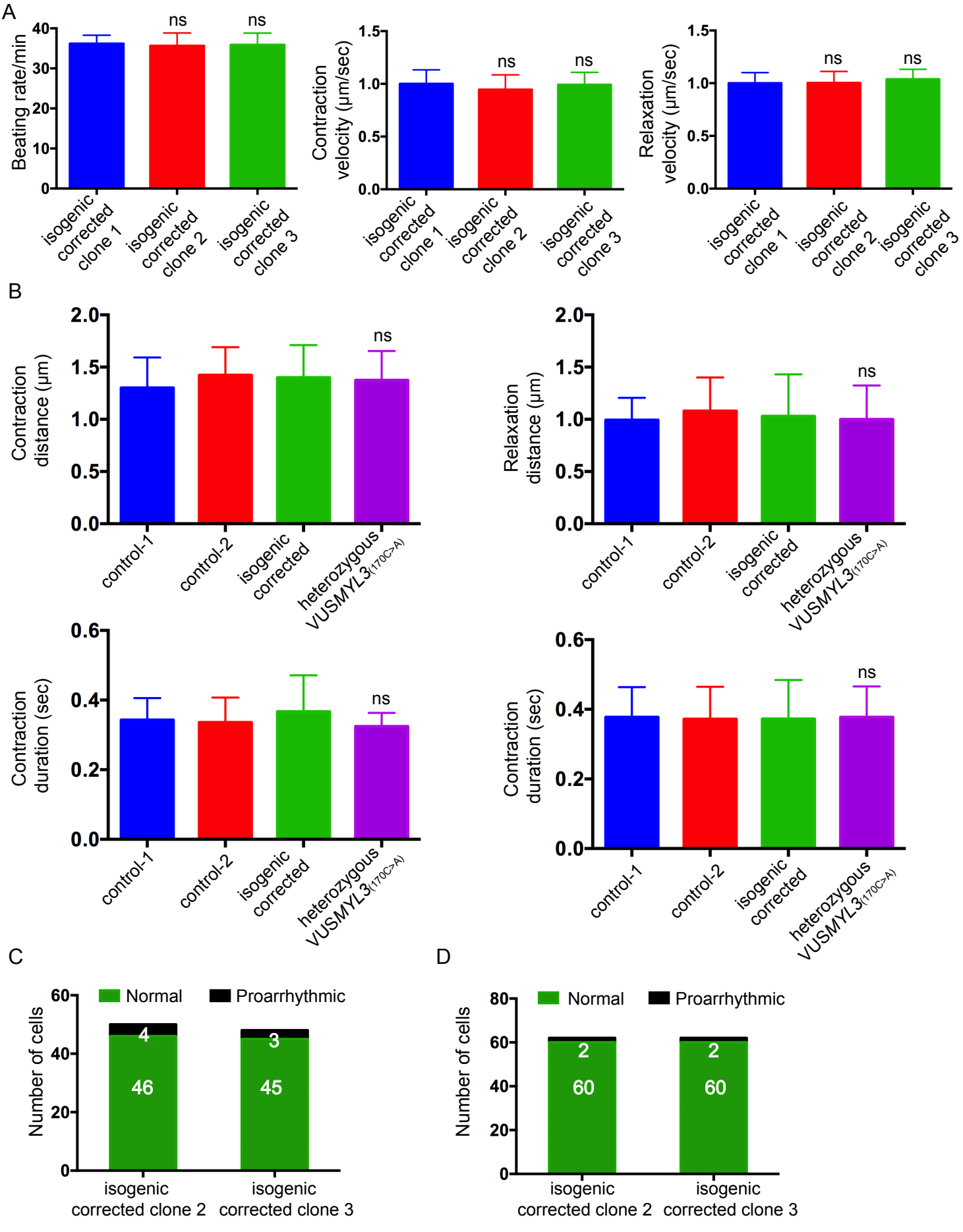

MYL3
Normalized to corrected clone1
1.5
1.0
0.5
0.0
ns
ns
isogenic corrected clone 1
isogenic corrected clone 2
isogenic corrected clone 3


MYL4
Normalized to corrected clone1
1.5
1.0
0.5
0.0
ns
ns
isogenic corrected clone 1
isogenic corrected clone 2
isogenic corrected clone 3


MYL6
Normalized to corrected clone1
1.5
1.0
0.5
0.0
ns
ns
isogenic corrected clone 1
isogenic corrected clone 2
isogenic corrected clone 3


MYL7
Normalized to corrected clone1
1.5
1.0
0.5
0.0
ns
ns
isogenic corrected clone 1
isogenic corrected clone 2
isogenic corrected clone 3


MYL9
Normalized to corrected clone1
1.5
1.0
0.5
0.0
ns
ns
isogenic corrected clone 1
isogenic corrected clone 2
isogenic corrected clone 3


A
Beating rate/min
40
30
20
10
0
ns
ns
isogenic corrected clone 1
isogenic corrected clone 2
isogenic corrected clone 3
Contraction velocity (µm/sec)
1.5
1.0
0.5
0.0
ns
ns
isogenic corrected clone 1
isogenic corrected clone 2
isogenic corrected clone 3
Relaxation velocity (µm/sec)
1.5
1.0
0.5
0.0
ns
ns
isogenic corrected clone 1
isogenic corrected clone 2
isogenic corrected clone 3
B
Contraction distance (µm)
2.0
1.5
1.0
0.5
0.0
ns
control-1
control-2
isogenic corrected
heterozygous VUSMYL3(170C>A)
Relaxation distance (µm)
2.0
1.5
1.0
0.5
0.0
ns
control-1
control-2
isogenic corrected
heterozygous VUSMYL3(170C>A)
Contraction duration (sec)
0.6
0.4
0.2
0.0
ns
control-1
control-2
isogenic corrected
heterozygous VUSMYL3(170C>A)
Contraction duration (sec)
0.6
0.4
0.2
0.0
ns
control-1
control-2
isogenic corrected
heterozygous VUSMYL3(170C>A)
C
Number of cells
60
40
20
0
Normal
Proarrhythmic
4
46
3
45
isogenic corrected clone 2
isogenic corrected clone 3
D
Number of cells
80
60
40
20
0
Normal
Proarrhythmic
2
60
2
60
isogenic corrected clone 2
isogenic corrected clone 3

A

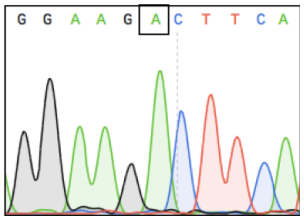
5'-g g a a g c c t t c a t g c t g t t c g a c c g c a c a c c c a a g t-3' myl3 wild type allele

3'-c c t t c g g a a g t a c g a c a a g c t g g c g t g t g g g t t c a-5'

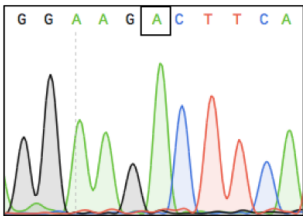
g g a a g t a c g a c a a g c t g g c g t g t - 5' homozygous-VUS*myl3*$_{(C>A)}$ gRNA

ggggttccctggtctgagggtcctgccaggggtgtggggagtggggtaggctgaccctgggagtgtctgtggctcact
gccctctcctggcagagttcaaggaagActtcatgctgttcgaccgcac -3' **homozygous-VUS ssODN**

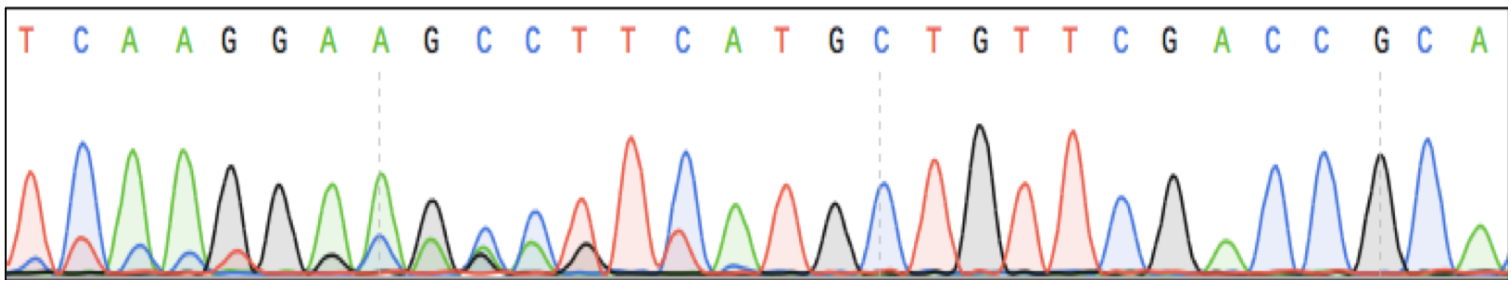
B homozygous clone 2



homozygous clone 3

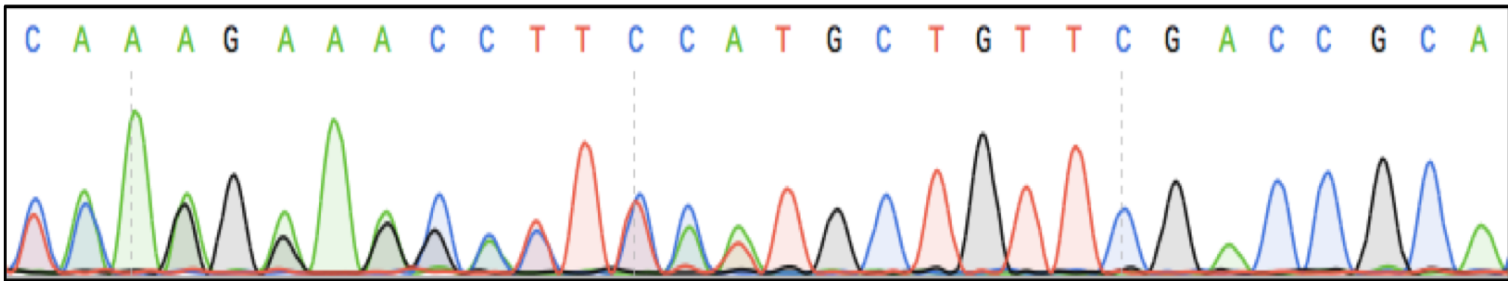


C



VUS*MYL3*$_{(170C>A)}$ allele Ag ttc aag gaa gAc ttc atg ctg ttc gac cgc aca ccc aag tgt gag atg aag atc acc t

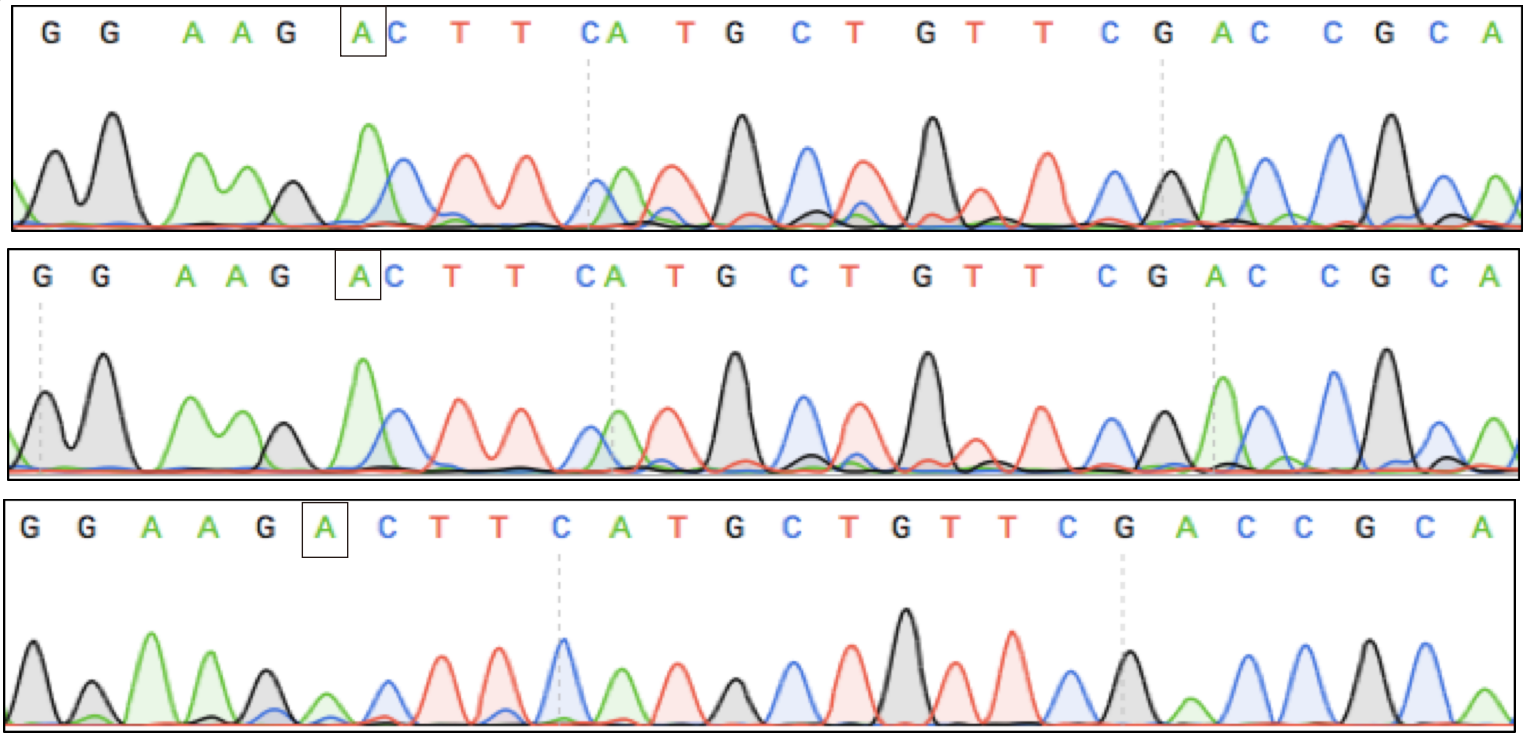
frameshift mutation allele Ag ttc aag gaa gCc ttc att gct gtt cga ccg cac acc caa gtg **tga** gat gaa gat cac c

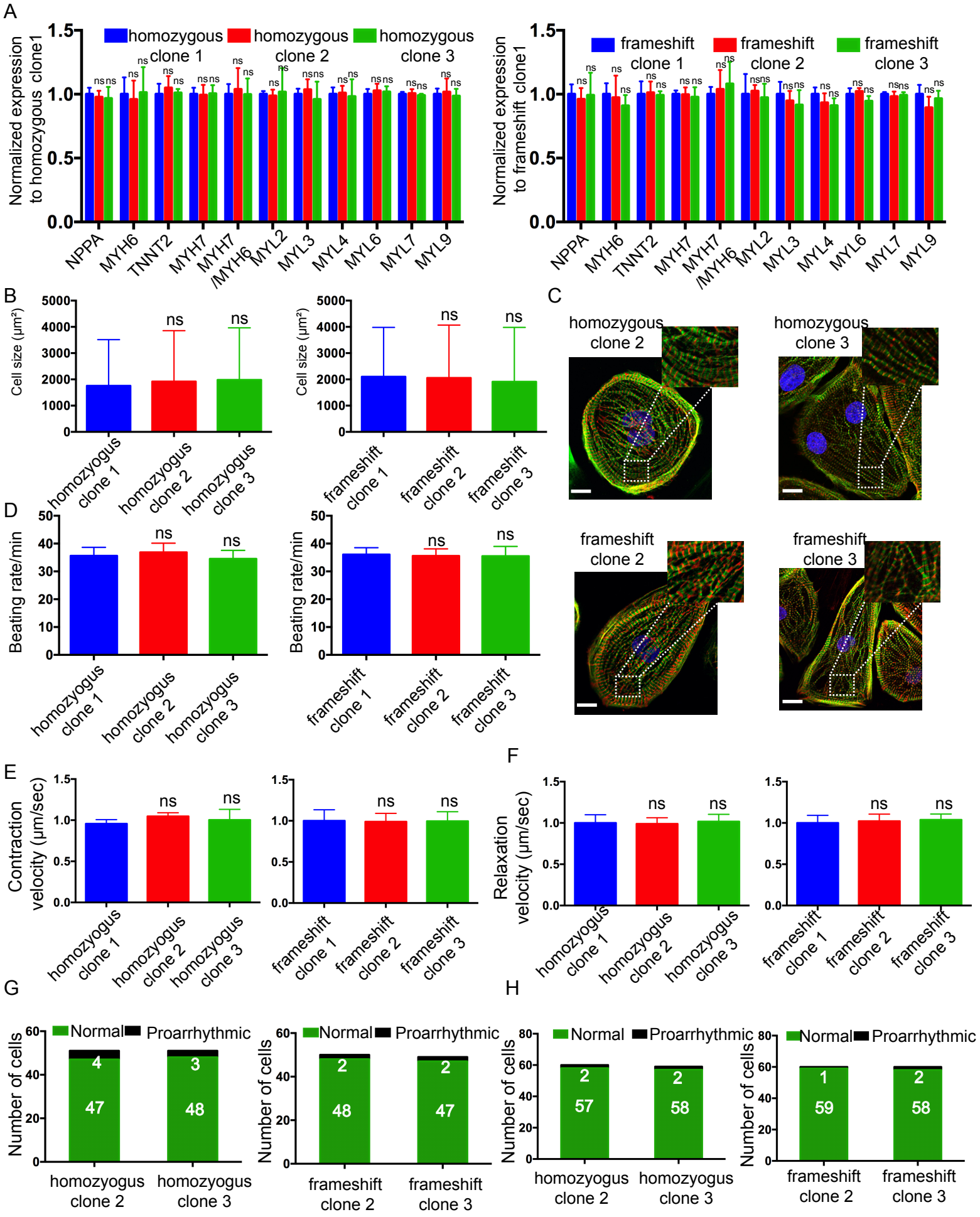


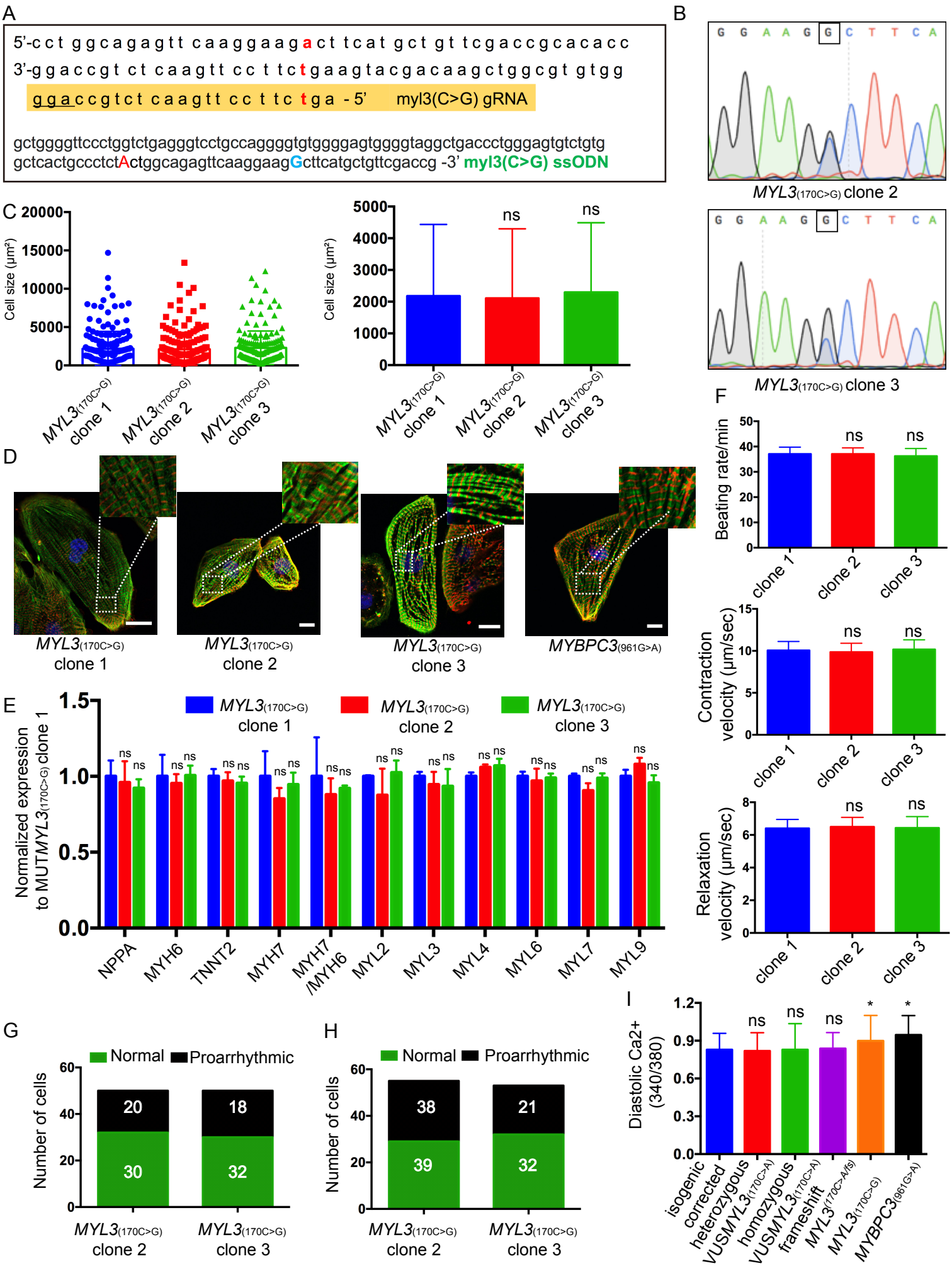
VUS*MYL3*$_{(170C>A)}$ allele Ag ttc aag gaa gAc ttc atg ctg ttc gac cgc aca ccc aag tgt gag atg aag atc acc t

frameshift mutation allele Ag tt- --- --- --- ---a tgc tgt tcg acc gca cac cca agt gtg aga **tga** aga tca cct

D




A
Normalized expression to homozygous clone1
homozygous clone 1
homozygous clone 2
homozygous clone 3
Normalized expression to frameshift clone1
frameshift clone 1
frameshift clone 2
frameshift clone 3
NPPA
MYH6
TNNT2
MYH7
MYH7 /MYH6
MYL2
MYL3
MYL4
MYL6
MYL7
MYL9
B
Cell size (μm²)
homozygous clone 1
homozygous clone 2
homozygous clone 3
frameshift clone 1
frameshift clone 2
frameshift clone 3
C
homozygous clone 2
homozygous clone 3
frameshift clone 2
frameshift clone 3
D
Beating rate/min
E
Contraction velocity (μm/sec)
F
Relaxation velocity (μm/sec)
G
Number of cells
Normal
Proarrhythmic
4
47
3
48
2
48
2
47
H
2
57
2
58
1
59
2
58


A
5'-cct ggcagagtt caaggaagact tcat gct gt tcgaccgcacacc
3'-ggaccgt ct caagtt cc t tctgaagtacgacaagctggcgt gtgg
ggaccgtct caagtt cc t tc tga - 5' myl3(C>G) gRNA
gctggggttccctggtctgagggtcctgccaggggtgtggggagtggggtaggctgaccctgggagtgtctgtg
gctcactgccctctActggcagagttcaaggaagGcttcatgctgttcgaccg -3' myl3(C>G) ssODN
B
MYL3(170C>G) clone 2
MYL3(170C>G) clone 3
C
Cell size (µm²)
MYL3(170C>G) clone 1
MYL3(170C>G) clone 2
MYL3(170C>G) clone 3
ns
D
MYL3(170C>G) clone 1
MYL3(170C>G) clone 2
MYL3(170C>G) clone 3
MYBPC3(961G>A)
E
Normalized expression to MUTMYL3(170C>G) clone 1
MYL3(170C>G) clone 1
MYL3(170C>G) clone 2
MYL3(170C>G) clone 3
NPPA
MYH6
TNNT2
MYH7
MYH7 /MYH6
MYL2
MYL3
MYL4
MYL6
MYL7
MYL9
F
Beating rate/min
Contraction velocity (µm/sec)
Relaxation velocity (µm/sec)
clone 1
clone 2
clone 3
G
Normal
Proarrhythmic
Number of cells
20
30
18
32
MYL3(170C>G) clone 2
MYL3(170C>G) clone 3
H
38
39
21
32
I
Diastolic Ca2+ (340/380)
isogenic
corrected
heterozygous VUSMYL3(170C>A)
homozygous VUSMYL3(170C>A)
frameshift MYL3(170C>A/fs)
MYL3(170C>G)
MYBPC3(961G>A)