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Unveiling cellular mechanisms underlying MYZAP related dilated cardiomyopathy using patient specific hiPSC cardiomyocytes

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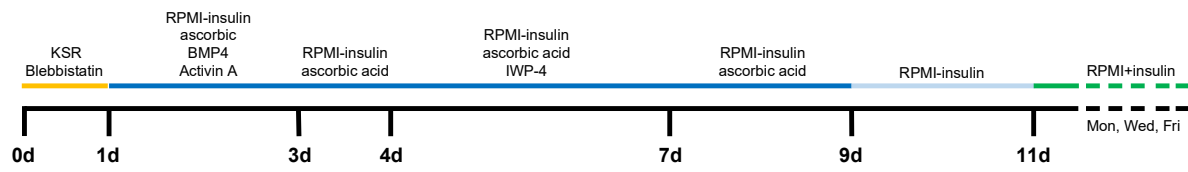
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Supplementary Figure S1



Supplementary Figure S1. Schematic diagram illustrates the differentiation protocol, d represents days

Supplementary Table S1. List of target and housekeeping genes with their qPCR TaqMan assay IDs and detailed qPCR analysis

Gene	Abbreviation	Synonyms	Assay ID
Catenin beta 1	CTNNB1	Beta-catenin	Hs00355045
Mitogen-activated protein kinase 3	MAPK3	ERK1, extracellular signal-regulated kinase, MAPK 1	Hs00385075
Vinculin	VCL		Hs00419715_m
Actinin alpha 2	ACTN2	F-actin, cross-linking protein, alpha-actinin	Hs00153809
Myosin heavy chain 7	MYH7	Beta-myosin heavy chain	Hs01110632
Caspase 3	CASP3	Apoptosis-related cysteine peptidase, apoptosis-related cysteine protease	Hs00234387
Myosin phosphatase-RhoA-interacting protein	MPRIIP		Hs00819388_m
Glyceraldehyde-3-phosphate dehydrogenase	GAPDH		Hs02758991_g1
Natriuretic peptide B	NPPB	ProBNP, BNP, brain natriuretic factor prohormone	Hs00173590
Desmoplakin	DSP	DP	Hs00950591
Tight junction protein 1	TJP1	Tight junction protein ZO-1, ZO-1, zona occludens 1	Hs01551871
Gap junction protein alpha 1	GJA1	Connexin-43, CX43	Hs00748445
Troponin T2, cardiac type	TNNT2	Cardiac muscle troponin T, cTnT	Hs00165960
Cadherin 2	CDH2	N-Cadherin	Hs00983056
Myocardium-enriched zonula occludens-1-associated protein	MYZAP	MYOZAP, GCOM1, Gup, Myocardium-Enriched Zonula Adherens Protein and GRINL1A Upstream Protein	Hs00940303 Hs00940306

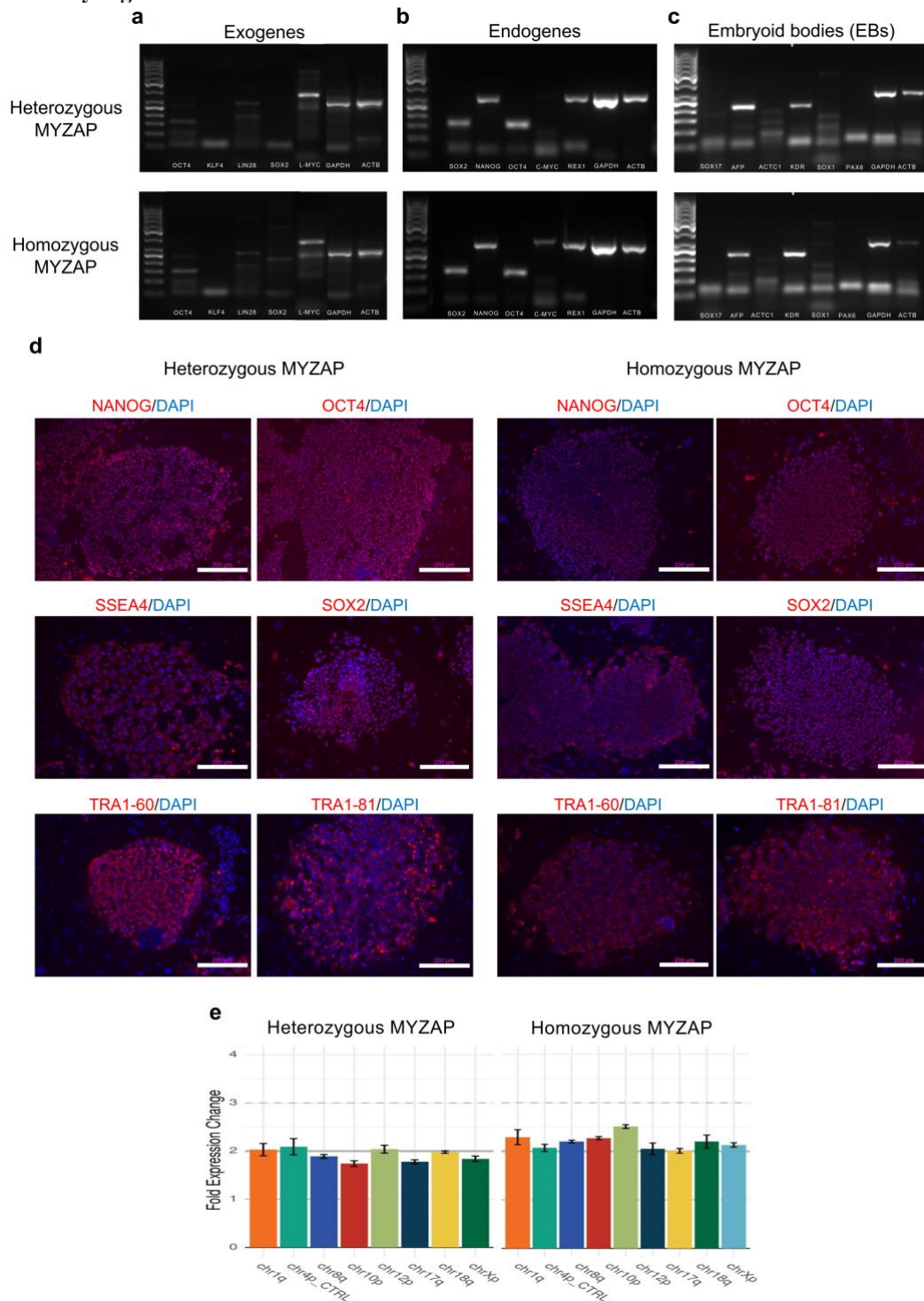
Raw Data																				
Gene	Control 1	Control 2	Control 3	Control 4	Control 5	Control 6	Control 7	Control 8	Control 9	Control 10	Homo 1	Homo 2	Homo 3	Homo 4	Homo 5	Homo 6	Homo 7	Homo 8	Homo 9	Homo 10
MYZAP 303	27.77	28.39	28.34								32.74	32.73	34.24							
MYZAP 306	27.88	28.28	29.23								33.66	33.24	33.02							
TNNT2				19.91	20.30	20.40	20.81	20.30						19.64	18.99	20.16	21.26	18.99		
MYH7				22.34	23.08	22.70	22.69		23.32					22.78	22.28	22.62	24.10		22.93	
NPPB				21.71		36.72	22.98	21.99	37.58					20.63		35.30	22.97	20.33	36.66	
VCL				23.14		24.28				24.54				23.18		23.85				23.94
CASP3				26.20	26.77	26.26	29.48							26.20	26.23	26.53	29.19			
CTNNB1				23.15		24.09		24.12		24.23				23.57		23.48		23.87		23.21
CDH2				22.78		23.70				24.68				23.01		23.47				23.99
TJP1				24.67	25.45	25.97	26.93		26.16					25.31	25.04	25.63	26.98		25.66	
DSP				24.73	25.09	25.11	27.20							24.23	24.08	24.91	26.53			
MRIP						27.08		26.78		27.73						26.83		26.83		27.62
MAPK				28.15		29.15				30.32				28.21		28.65			28.71	
GJA1				23.28		23.48		23.46	24.62					26.03		23.13		26.97	24.10	
ACTN2				21.79	23.48	22.80	23.66							21.54	21.22	22.50	24.51			
Housekeeping gene																				
GAPDH	28.39	29.27	29.79	20.14	20.31	20.34	20.84	20.92	21.58	21.09	27.64	27.47	27.28	20.29	19.84	20.40	21.48	20.50	20.99	20.98

Normalized Data																				
Gene	Control 1	Control 2	Control 3	Control 4	Control 5	Control 6	Control 7	Control 8	Control 9	Control 10	Homo 1	Homo 2	Homo 3	Homo 4	Homo 5	Homo 6	Homo 7	Homo 8	Homo 9	Homo 10
MYZAP 303	0.78	0.93	1.38								0.015	0.013	0.004							
MYZAP 306	0.88	1.23	0.92								0.009	0.011	0.011							
TNNT2				1.02	1.15	1.16	1.03	1.16						1.34	1.81	1.23	1.15	1.87		
MYH7				1.00	1.17	1.18	1.07		1.01					0.82	1.26	1.10	0.59		0.87	
NPPb				1.04		1.11	1.04	1.18	1.09					2.37		2.81	1.57	2.38	1.29	
VCL				0.99		1.17				1.01				1.03		1.44				1.42
CASP3				0.95	1.19	1.18	1.06							1.11	1.05	0.87	1.91			
CTNNB1				1.07		1.16		1.18		1.02				0.83		1.59		0.90		1.89
CDH2				0.95		1.16				1.02				1.02		1.23				1.51
TJP1				0.94	1.19	1.14	1.07		1.01					0.72	0.96	1.31	1.50		0.94	
DSP				0.97	1.15	1.20	1.05							1.58	1.45	1.16	2.55			
MRIP						1.18		1.21		1.00						1.25		0.73		1.00
MAPK				1.00		1.17				1.01				1.08		1.47				2.87
GJA1				1.02		1.15		1.17	1.01					0.17		1.34		0.07	0.96	
ACTN2				0.95	1.17	1.18	1.05							1.32	3.47	1.30	0.88			

Fold Change																				
Gene	H1/C1	H2/C2	H3/C3	H4/C4	H5/C5	H6/C6	H7/C7	H8/C8	H9/C9	H10/C10										
MYZAP 303	0.02	0.01	0.00																	
MYZAP 306	0.01	0.01	0.01																	
TNNT2				1.32	1.57	1.06	1.12	1.61												
MYH7				0.82	1.08	0.93	0.55		0.86											
NPPb				2.28		2.54	1.52	2.02	1.19											
VCL				1.03		1.23				1.41										
CASP3				1.17	0.89	0.73	1.80													
CTNNB1				0.78		1.37		0.76		1.86										
CDH2				1.08		1.06				1.48										
TJP1				0.76	0.81	1.14	1.41		0.93											
DSP				1.62	1.26	0.97	2.43													
MRIP						1.05		0.60		1.00										
MAPK				1.08		1.26				2.85										
GJA1				0.16		1.17		0.06	0.94											
ACTN2				1.39	2.96	1.10	0.84													

H1 H10 represent homozygous 1 through homozygous 10
C1 C10 represent control 1 through control 10

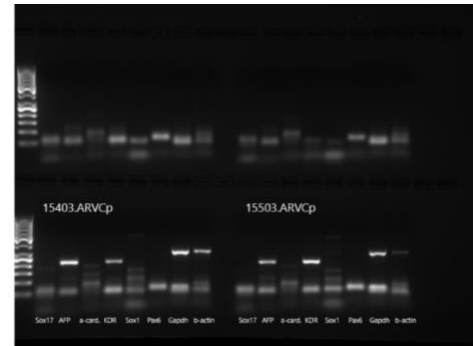
Supplementary Figure S2



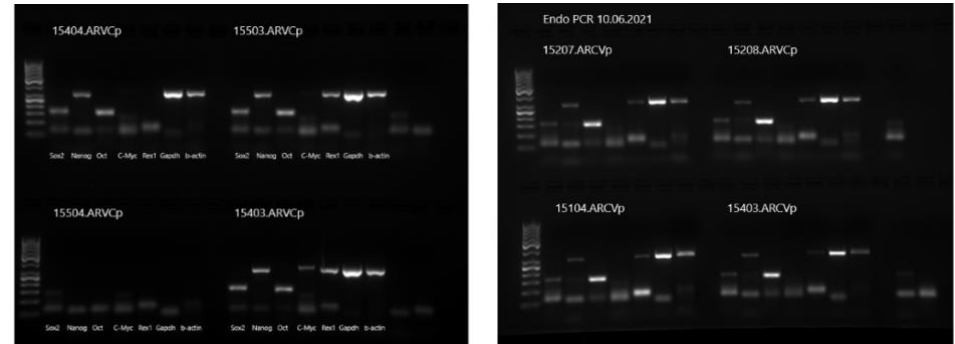
Supplementary Figure S2. UTA. 15403 and UTA.15503 hiPSC-lines pluripotency characterization by gene and protein expression, and karyotype analysis. **a** The virally transferred Sendai exogenes OCT4, L-MYC, SOX2, Lin-28 and KLF4 were silenced in the hiPSC-lines. **b** The endogenous pluripotency genes OCT4, REX1, SOX2, NANOG, and c-MYC were expressed in the hiPSC-lines. **c** The hiPSC-lines formed embryoid bodies (EBs) which expressed at least one marker from each of the germ layers: endoderm (AFP, SOX17), mesoderm (KDR, ACTC1), and ectoderm (SOX1, PAX6). **d** The protein level expression of the pluripotency markers was confirmed by immunocytochemistry. The red color indicates NANOG, OCT4, SOX2, SSEA4, TRA1-60 or TRA1-81, whereas the blue color indicates the nuclei stained using DAPI. **e** Karyotype analysis of the hiPSC-lines. The presented gel electrophoresis images are cropped and compiled from a routinely performed broader panel of cell lines characterization.

The original uncropped and full-length images for the cell lines characterization:

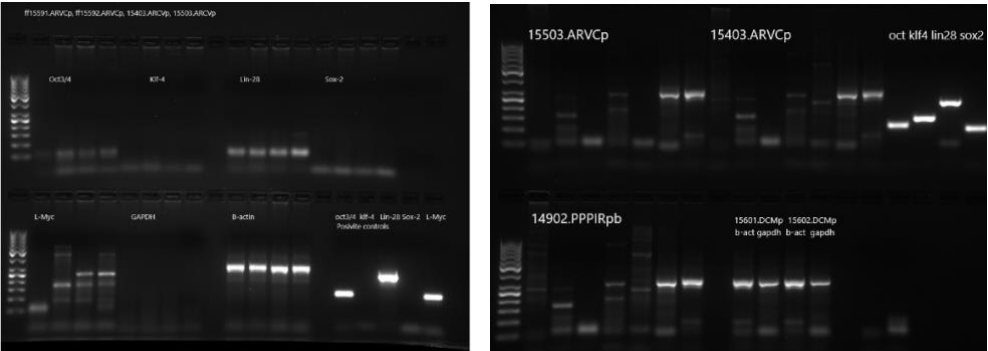
EB PCR



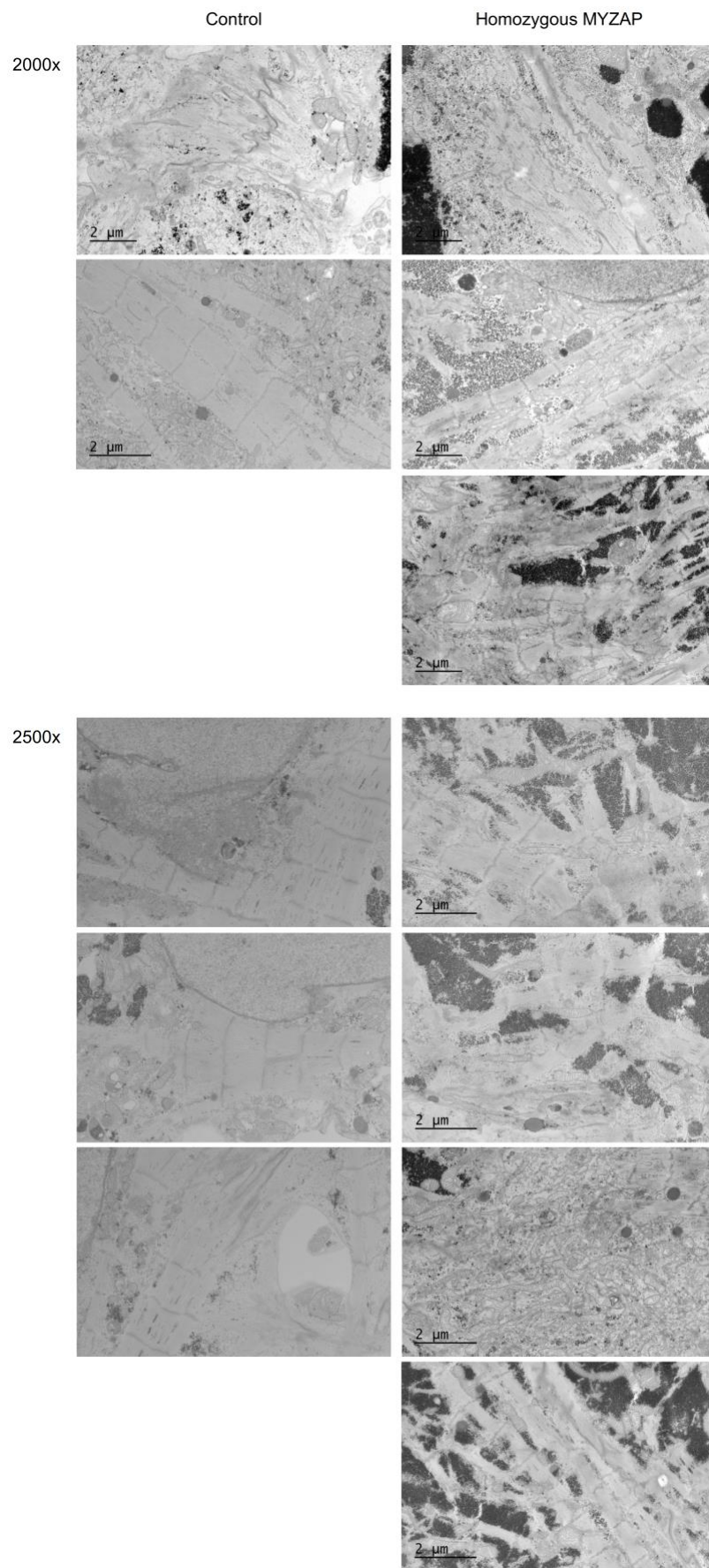
Endo PCR

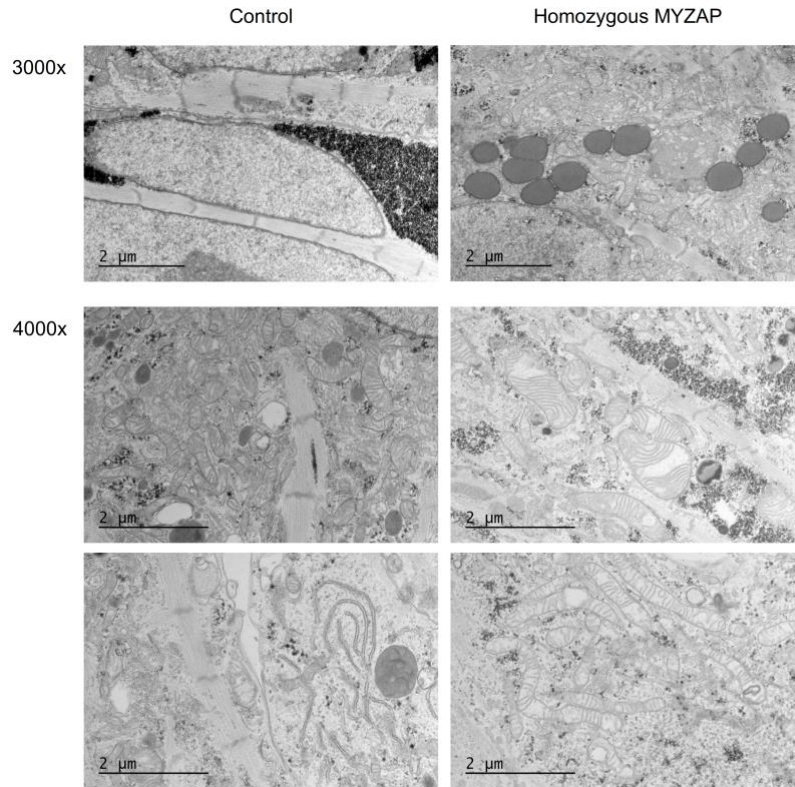


Exo PCR



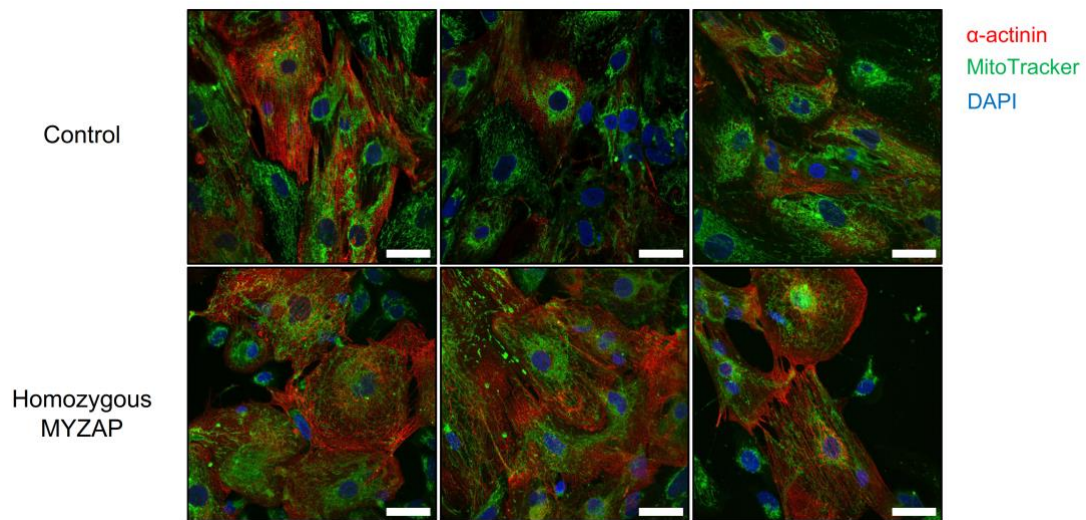
Supplementary Figure S3





Supplementary Figure S3. Representative transmission electron microscopy (TEM) images of control and homozygous MYZAP cell lines are presented across a range of magnifications (2,000 $\times$, 2,500 $\times$, 3,000 $\times$, and 4,000 $\times$). Alongside the TEM images featured in Figure 2 of the manuscript, these micrographs served as the basis for a suite of quantitative ultrastructural analyses, encompassing mitochondrial number, mitochondrial area, mitochondrial length, glycogen area, sarcomere length, and cristae area.

Supplementary Figure S4



Supplementary Figure S4. Representative confocal images of hiPSC-CMs stained with α -actinin (red), MitoTracker Deep Red (green), and DAPI (blue) in control and homozygous *MYZAP* hiPSC-CMs. Scale bars: 40 μ m.

Supplementary Table S2. Detailed quantitative analysis of delayed afterdepolarizations (DADs)

Cell line	Spontaneous beating		Stimulation at 1 Hz
	DADs Occurrence	DADs rate	DADs Occurrence
Control	5/14 (35.7%)	6.7±2.9 DADs/min	3/19 (15.8%)
Homozygous	7/11 (63.6%)	12.5±3.7 DADs/min	6/13 (46.2%)