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Supplemental Information

Comparison of 10 Control hPSC Lines for Drug Screening in an Engineered Heart Tissue Format

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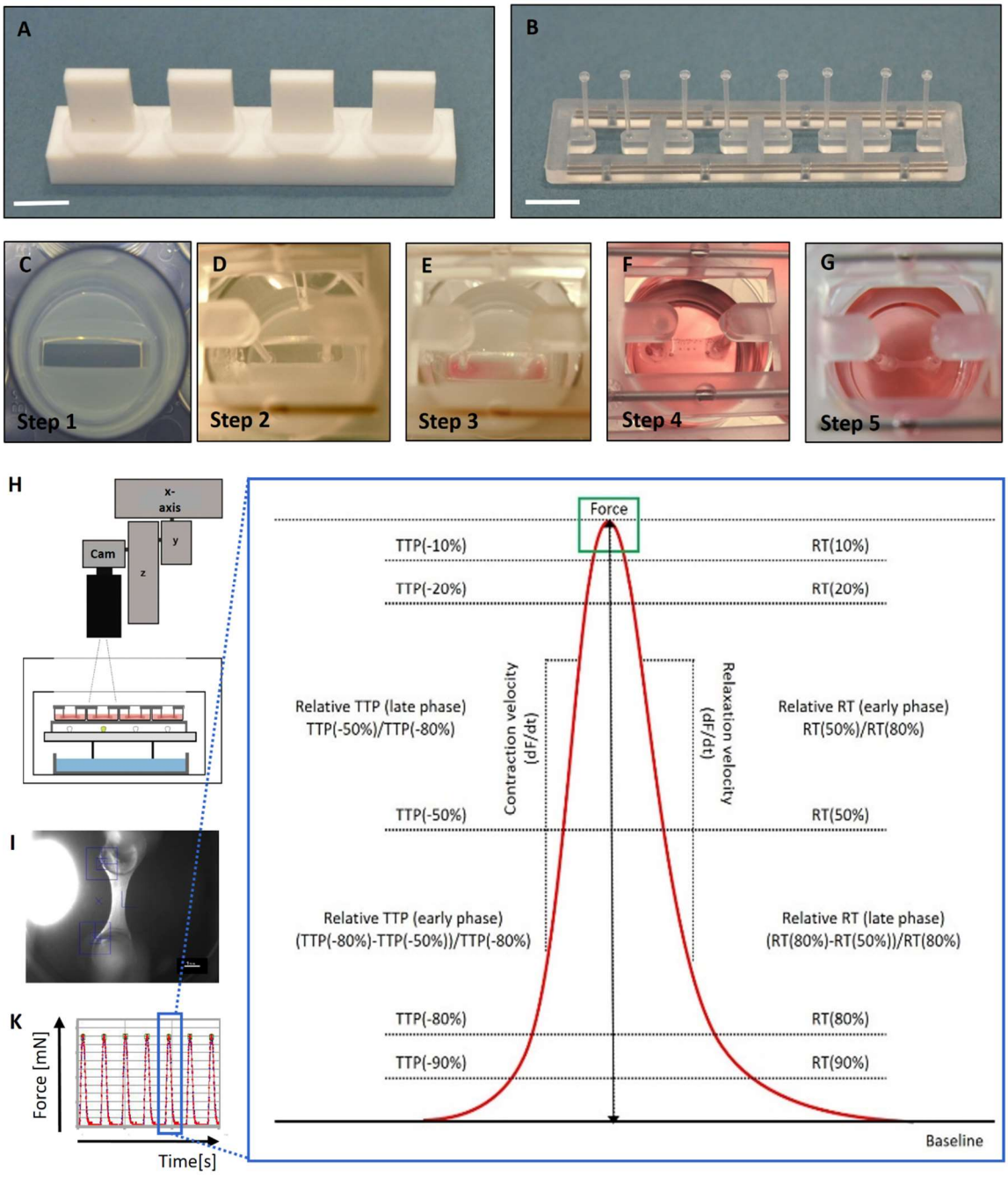
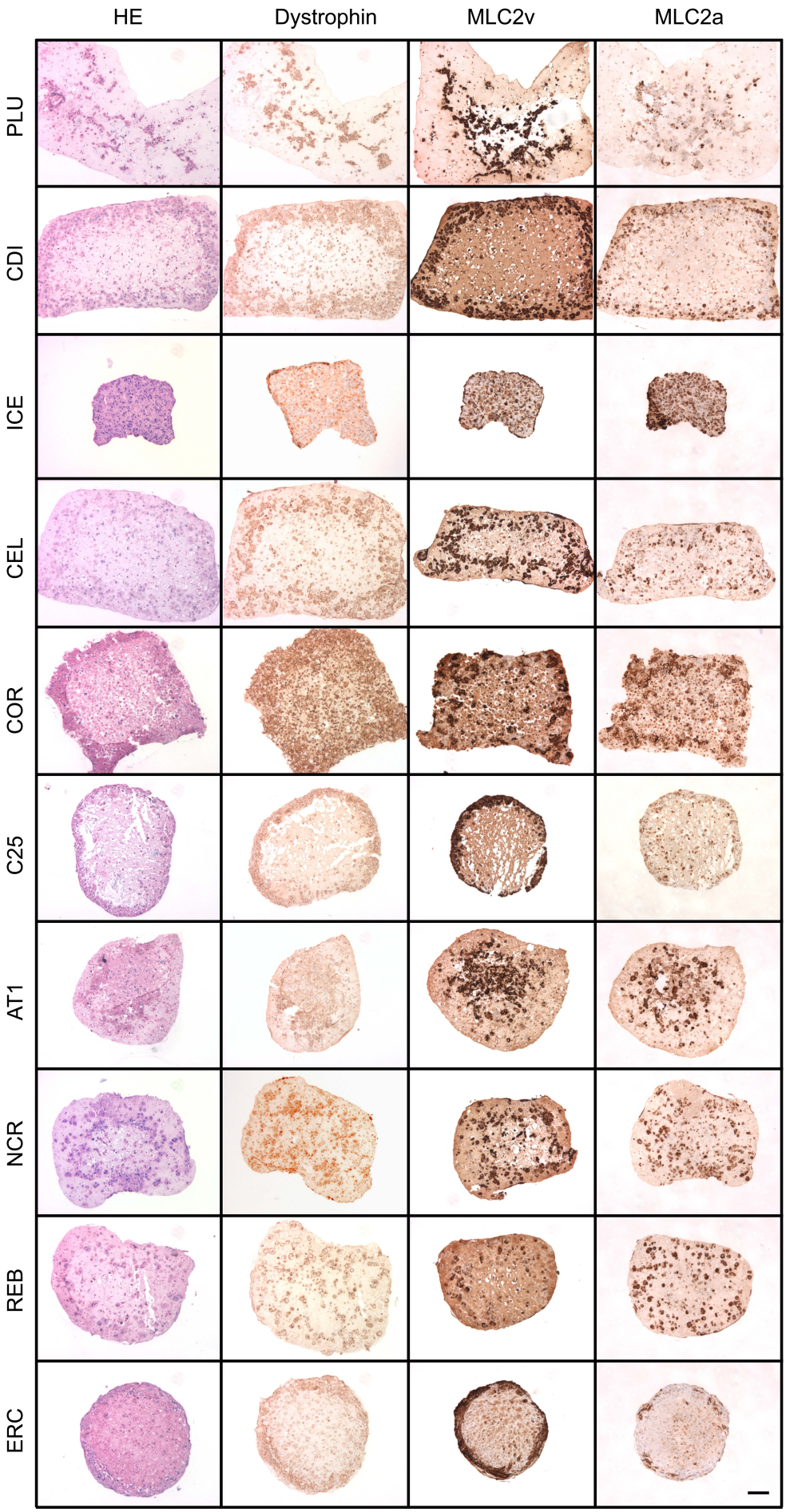
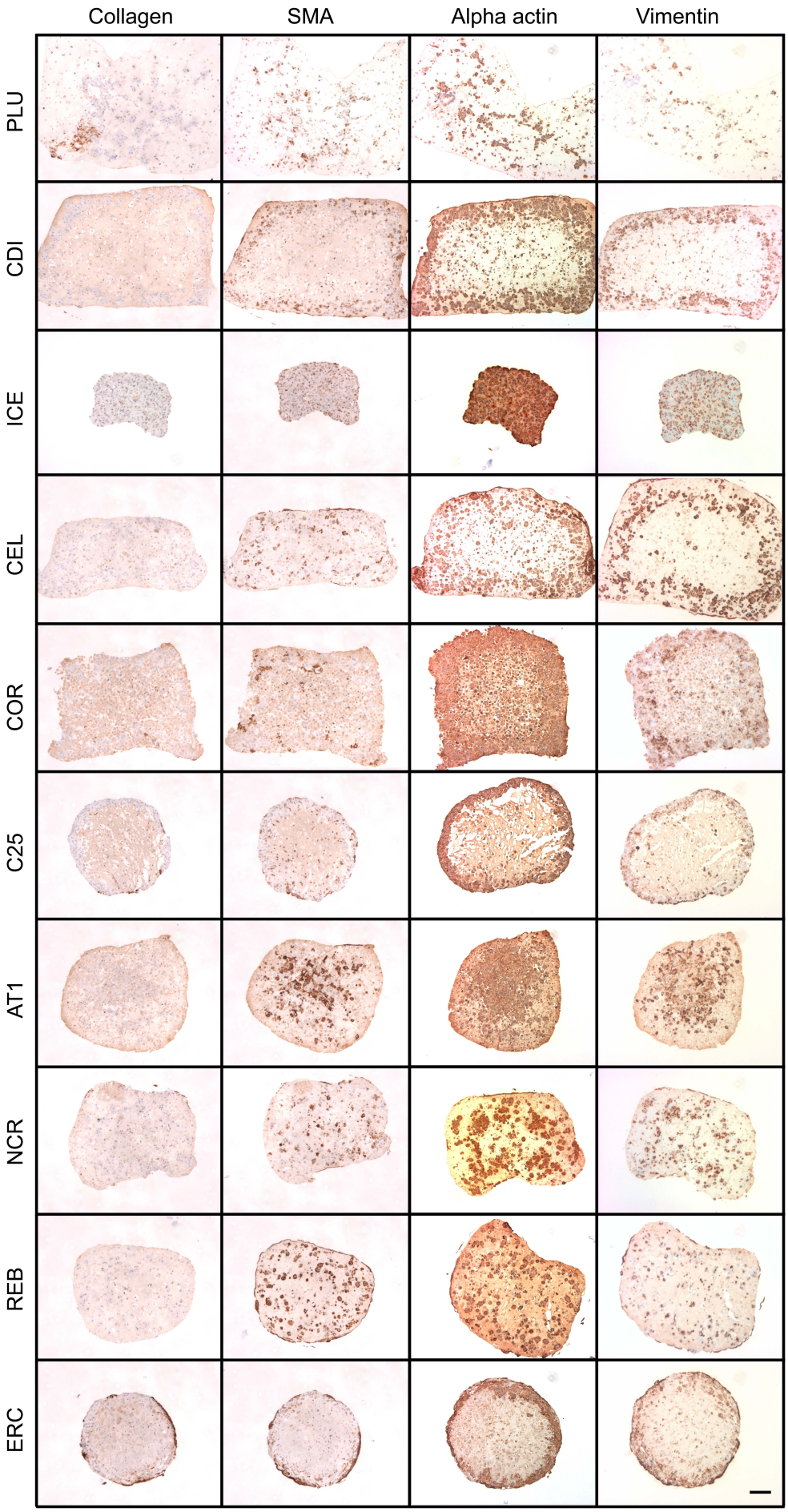


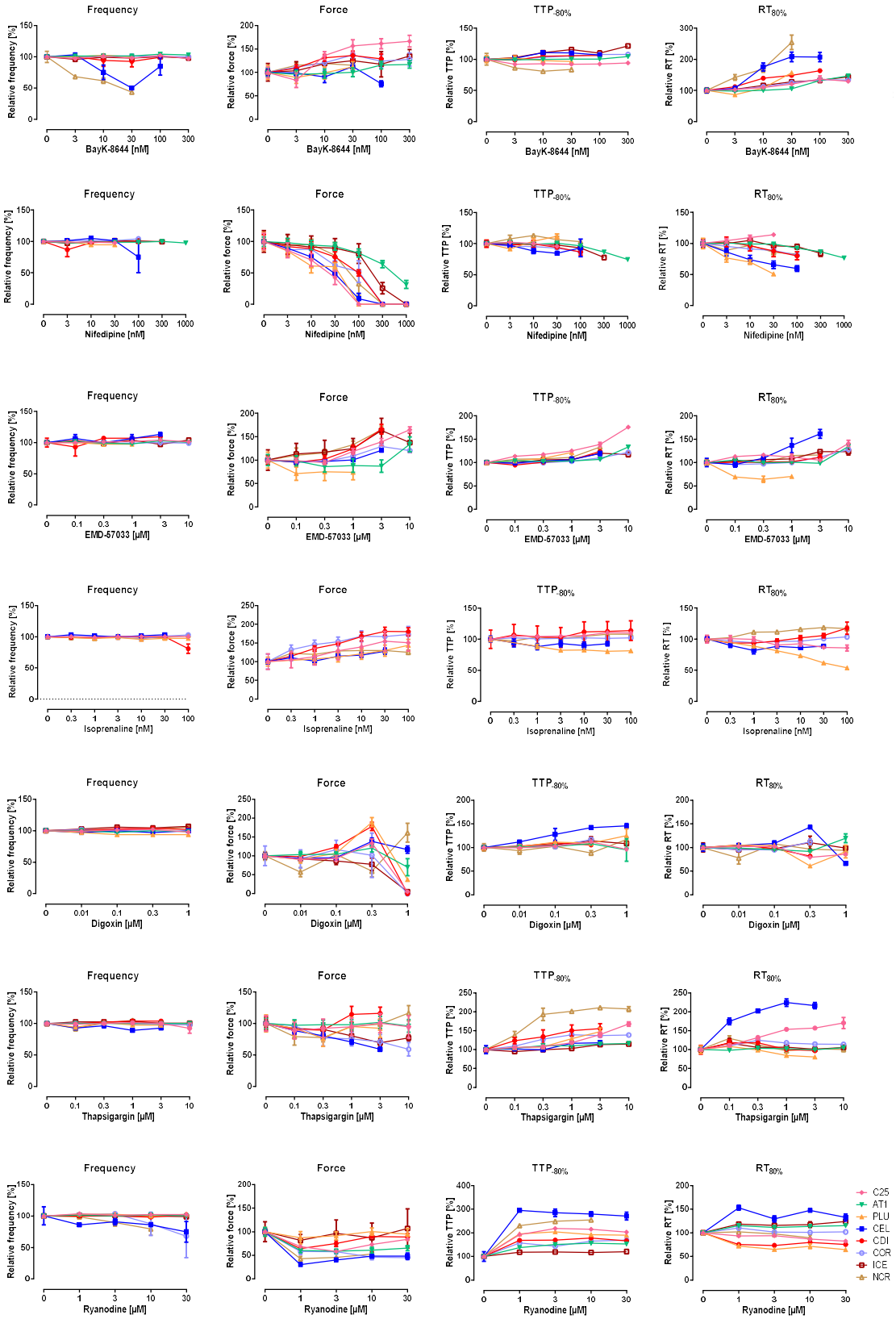
Supplementary Figure 1. Principle of engineered heart tissue (EHT), Related to Figure 1. (A-G) Casting process of EHT. **(A)** Teflon® spacer. **(B)** Silicone rack. **(C-G)** Graphical display of EHT generation step by step. **(C)** Upper view on a well of a 24-well-plate with casting mold in agarose after removal of the PTFE spacer. **(D)** Pair of posts from the PDMS rack positioned in the casting mold. **(E)** Reconstitutionmix pipetted into the casting mold and around the silicone posts. **(F)** Freshly generated EHT at day 0, transferred to a new culture dish with medium. **(G)** Remodeled EHT in medium at day 15. **(H-K)** Contraction analysis of EHT. **(H)** EHT analysis instrument with computer-controlled camera above the gas- and temperature controlled incubation chamber with EHTs in 24-well-culture dish on top of a LED panel. **(I)** Live view of an EHT during analysis with the automated contraction analysis software. **(K)** Exemplary contraction pattern displaying contraction force over time and enlarged schematic contraction peak, displaying the analysis parameter force, time to peak (TTP), relaxation time (RT), contraction velocity (CV), relaxation velocity (RV) as well as relative TTP and RT phases (modified from Mannhardt et al. 2017).



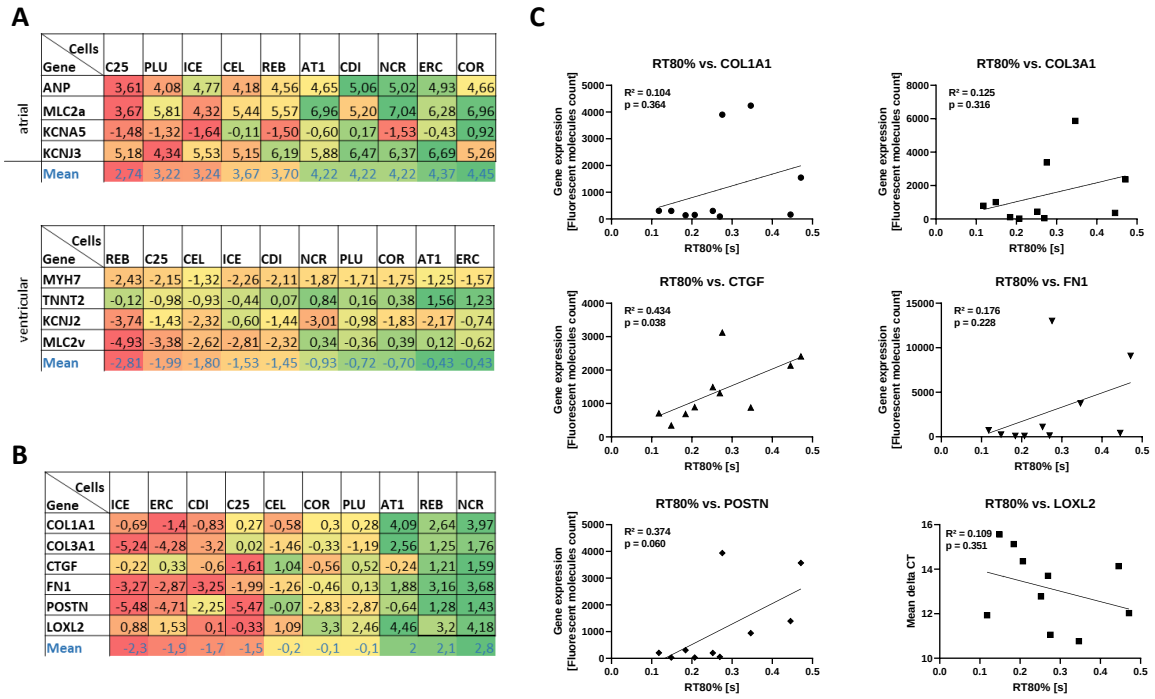
Supplementary Figure 2. Histo-logical analysis of transversally cut EHTs. Haematoxylin and eosin (HE), Anti-dystrophin, anti-MLC2v, anti-MLC2a stained slides showing overviews of the EHTs. Scale bar 100 µm. Three-digit code for cell lines: PLU = pluricyte cardiomyocytes, CDI = iCell cardiomyo-cytes, ICE = iCell² cardiomyocytes, CEL = cellartis cardiomyocytes, COR = Cor4U cardiomyo-cytes, C25/AT1/NCR/REB/ERC = in-house differentiated hiPSC-derived cardio-myocytes. Related to Figure 3.



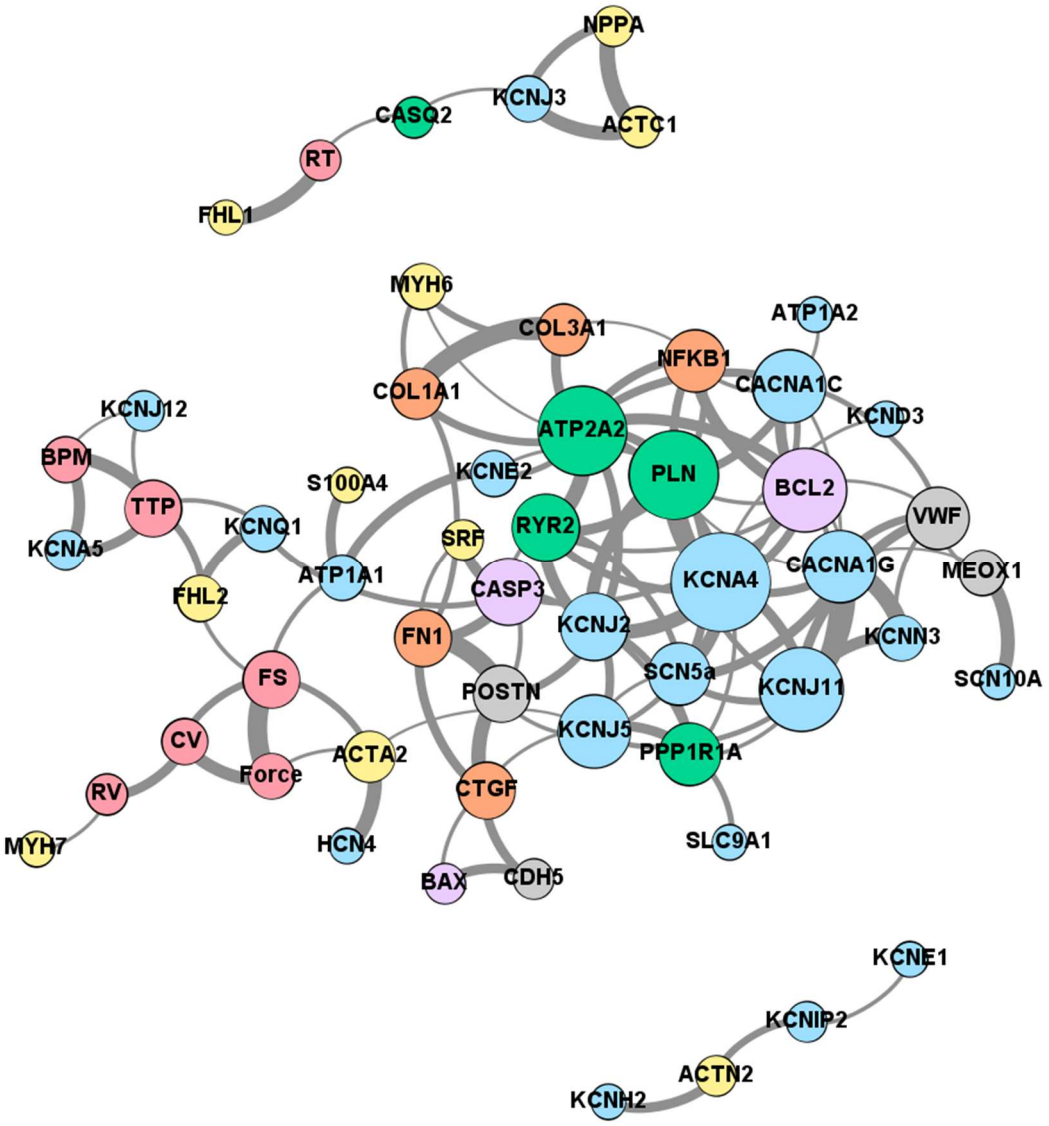
Supplementary Figure 3. Histological analysis of transversally cut EHTs. Anti collagen, anti-smooth muscle actin (SMA), anti-alpha actin, anti-vimentin stained slides showing overviews of the EHTs. Scale bar 100 µm. Three-digit code for cell lines: PLU = pluricyte cardiomyocytes, CDI = iCell cardiomyo-cytes, ICE = iCell² cardiomyocytes, CEL = cellartis cardio-myocytes, COR = Cor4U cardiomyo-cytes, C25/AT1/ NCR/REB/ERC = in-house differentiated hiPSC-derived cardiomyocytes. Related to Figure 3.



Supplementary Figure 4. Full concentration response curves, Related to Figure 5. Concentration response curves indicating changes in contraction data frequency, force, time to peak (TTP) and relaxation time (RT) for the different cell lines (colour coding see figure legend at bottom right corner; n=2-6 EHTs per drug see Supplementary Table 2).



Supplementary Figure 5. Gene expression analysis expressed as log2 (fold change of non-failing human heart), Related to Figure 7. Heat map coloring indicating lower expression than NFH in red and higher expression in green with cell lines sorted respectively. **(A)** Atrial and ventricular genes in the ten different cell lines. Linear regression analysis of these genes and relaxation time of the EHT did not detect any significant correlation. **(B)** Markers of fibrosis and extracellular matrix proteins. **(C)** Correlation of fibrotic transcripts and relaxation time RT80%. Linear regression analysis indicated overall poor correlation with Pearson's correlation coefficients R^2 <0.4. Slope was significantly different from zero only for CTGF (see p values indicated in each graph).



Supplementary Figure 6. Network analysis of correlating factors, Related to Figure 7. Graphic illustration of contraction force parameters and genes where signal amplitude or gene expression levels correlate with a Pearson's correlation coefficient R^2>0.7. Correlation of mean values of the ten different hiPSC-EHT for contraction parameters was performed with Microsoft excel and network analysis visualized in Gephi (version 0.9.2) force atlas-2 layout with color coding for different groups as undirected interaction (red: contraction parameters; yellow: cardiomyopathy genes; blue: ion channels and pumps genes; orange: growth related genes; green: adrenergic signaling genes; purple: apoptosis genes; grey: non-cardiomyocytes genes). The size of the nodes corresponds to the amount of correlating partners, the thickness of the edges indicates level of correlation (thicker line = higher R^2), distances are chosen at random.

Supplementary Table 1: Cell line information and quality control (QC) parameters checked prior to drug screening, Related to Figure 1. Cardiomyocyte (CM) purity was evaluated by FACS with antibodies against either cardiac troponin T (cTNT) or alpha actinin (α-act). (1) Mosqueira et al. 2018 Eur Heart J. Please note that CDI and PLU-based EHT seized spontaneous beating at submaximal external Calcium, but could be paced. *Please note that cardiomyocyte purity of commercial cell lines was reported to be lower than the producer provided numbers listed in this table (see discussion section of the manuscript; Huo et al. 2016 Tox Sci). Three-digit code for cell lines: PLU = pluricyte cardiomyocytes, CDI = iCell cardiomyocytes, ICE = iCell² cardiomyocytes, CEL = cellartis cardiomyocytes, COR = Cor4U cardiomyocytes, C25/AT1/ERC/NCR/REB = inhouse differentiated hiPSC-derived cardiomyocytes.

Cell line	**Somatic cell origin**	**Donor gender**	**Reprogramming**	**Frozen / direct**	**Origin**	**CM Purity**	**EHT in-process QC parameter (complied by n/n tested EHT)**			
							Spontaneous force (>0.1 mN)	**Regular spontaneous beating (RR' <0.5)**	**Following electrical stimulation**	**Paced force decrease at sub-max. Ca^{2+} (>-20%)**
C25	Dermal fibroblast	f	Lentivirus	Direct	University	84±8% $cTNT^{+}$; n=5	7/7	7/7	7/7	7/7
CEL	Dermal fibroblast	m	Retrovirus	Frozen	Commercial	>70% $cTNT^{+}$	12/ 12	12/12	7/7	7/7
CDI	Fibroblast	f	Retrovirus	Frozen	Commercial	98%*	10/10	10/10	10/10	9/10
PLU	Urinary tract epithelial cells	f	Non-integrative	Frozen	Commercial	>70% $cTNT^{+}$	7/7	7/7	10/10	10/10
iCE²	(Dermal) fibroblast	f	Retrovirus	Frozen	Commercial	99%	15/15	13/15	13/13	13/13
AT1	Dental pulp	f	Lentivirus	Direct	University	>80% $cTNT^{+(1)}$	23/23	23/23	12/12	12/12
COR	hESC (RUES2)	f	-	Direct	Commercial	100%*	4/8	8/8	8/8	6/8
ERC	Dermal fibroblast	f	Sendai virus	Direct & Frozen	University	86±9% $cTNT^{+}$;n=5	5/18	18/18	12/12	5/12
REB	Dermal fibroblast	m	Sendai virus	Direct & Frozen	University	83±5% $cTNT^{+}$;n=5	14/14	14/14	9/9	9/9
NCR	CD34+ cord blood	m	Episome	Frozen	University	81±2% α-act^{+};n=2	10/23	23/23	21/21	21/21

Supplementary Table 2: Results of post-test for differences in sarcomere length, Related to Figure 2. * p<0.05, ** p<0.005, *** p<0.001, ns = not significant. Three-digit code for cell lines: PLU = pluricyte cardiomyocytes, CDI = iCell cardiomyocytes, ICE = iCell² cardiomyocytes, CEL = cellartis cardiomyocytes, COR = Cor4U cardiomyocytes, C25/AT1/NCR/REB = inhouse differentiated hiPSC-derived cardiomyocytes.

Tukey's multiple comparisons test	Mean Diff.	95% CI of diff.	Significant?	Summary
PLU vs. CDI	0.1007	0.04058 to 0.1607	Yes	***
PLU vs. ICE	0.02984	-0.02426 to 0.08394	No	ns
PLU vs. CEL	-0.03541	-0.09414 to 0.02332	No	ns
PLU vs. COR	0.1432	0.07758 to 0.2088	Yes	***
PLU vs. C25	-0.02088	-0.08037 to 0.03862	No	ns
PLU vs. AT1	-0.2438	-0.2983 to -0.1893	Yes	***
PLU vs. NCR	-0.05314	-0.1124 to 0.006114	No	ns
PLU vs. REB	-0.03404	-0.09114 to 0.02306	No	ns
PLU vs. ERC	0.01325	-0.04664 to 0.07313	No	ns
CDI vs. ICE	-0.07083	-0.1243 to -0.01735	Yes	**
CDI vs. CEL	-0.1361	-0.1942 to -0.07792	Yes	***
CDI vs. COR	0.04253	-0.02257 to 0.1076	No	ns
CDI vs. C25	-0.1215	-0.1805 to -0.06261	Yes	***
CDI vs. AT1	-0.3445	-0.3983 to -0.2906	Yes	***
CDI vs. NCR	-0.1538	-0.2125 to -0.09512	Yes	***
CDI vs. REB	-0.1347	-0.1912 to -0.07819	Yes	***
CDI vs. ERC	-0.08742	-0.1467 to -0.02810	Yes	***
ICE vs. CEL	-0.06525	-0.1172 to -0.01330	Yes	**
ICE vs. COR	0.1134	0.05373 to 0.1730	Yes	***
ICE vs. C25	-0.05071	-0.1035 to 0.002110	No	ns
ICE vs. AT1	-0.2736	-0.3208 to -0.2265	Yes	***
ICE vs. NCR	-0.08298	-0.1355 to -0.03043	Yes	***
ICE vs. REB	-0.06388	-0.1140 to -0.01377	Yes	**
ICE vs. ERC	-0.01659	-0.06985 to 0.03666	No	ns
CEL vs. COR	0.1786	0.1148 to 0.2425	Yes	***
CEL vs. C25	0.01453	-0.04302 to 0.07209	No	ns
CEL vs. AT1	-0.2084	-0.2608 to -0.1560	Yes	***
CEL vs. NCR	-0.01773	-0.07503 to 0.03957	No	ns
CEL vs. REB	0.001368	-0.05370 to 0.05644	No	ns
CEL vs. ERC	0.04866	-0.009292 to 0.1066	No	ns
COR vs. C25	-0.1641	-0.2286 to -0.09951	Yes	***
COR vs. AT1	-0.3870	-0.4470 to -0.3270	Yes	***
COR vs. NCR	-0.1963	-0.2607 to -0.1320	Yes	***
COR vs. REB	-0.1772	-0.2396 to -0.1149	Yes	***
COR vs. ERC	-0.1299	-0.1949 to -0.06503	Yes	***
C25 vs. AT1	-0.2229	-0.2762 to -0.1697	Yes	***
C25 vs. NCR	-0.03226	-0.09035 to 0.02583	No	ns
C25 vs. REB	-0.01317	-0.06906 to 0.04273	No	ns
C25 vs. ERC	0.03412	-0.02461 to 0.09286	No	ns
AT1 vs. NCR	0.1906	0.1377 to 0.2436	Yes	***
AT1 vs. REB	0.2097	0.1592 to 0.2603	Yes	***
AT1 vs. ERC	0.2570	0.2034 to 0.3107	Yes	***
NCR vs. REB	0.01910	-0.03654 to 0.07473	No	ns
NCR vs. ERC	0.06639	0.007904 to 0.1249	Yes	*
REB vs. ERC	0.04729	-0.009013 to 0.1036	No	ns

Supplementary Table 3. Statistical analysis of drug screening, Related to Figure 5 and Supplementary Figure 4. Repeated measures ANOVA with Dunnett's multiple comparisons post-test. Significant differences at different drug concentrations compared to baseline level are indicated as * p<0.05, ** p<0.005, *** p<0.001, ns = not significant. Conc. = concentration, n = number of paced EHTs included in the statistical analysis, LOC = loss of capture after drug administration, † = EHT ceased beating, three-digit code for cell lines same as in Supplementary Table 1.

Drug		Force								TTP								RT							
	Conc./n	CDI	CEL	PLU	AT1	C25	COR	ICE	NCR	CDI	CEL	PLU	AT1	C25	COR	ICE	NCR	CDI	CEL	PLU	AT1	C25	COR	ICE	NCR
BayK-8644	n	4	4	4	6	5	4	6	4	4	4	4	6	5	4	6	4	4	4	4	6	5	4	6	4
	3 nM	*	ns	ns	ns	ns	ns	ns	LOC	ns	ns	ns	ns	ns	ns	ns	LOC	ns	ns	ns	ns	ns	ns	ns	LOC
	10 nM	**	LOC	ns	ns	**	ns	*	LOC	ns	LOC	ns	ns	ns	ns	*	LOC	**	LOC	ns	ns	ns	*	*	LOC
	30 nM	**	LOC	ns	ns	***	**	***	LOC	ns	LOC	ns	ns	ns	***	**	LOC	***	LOC	***	ns	*	**	**	LOC
	100 nM	*	LOC		*	**	ns	ns		***	LOC		ns	ns	ns	ns		***	LOC		**	*	*	*	
	300 nM				***	ns	ns	**					ns	ns	ns	**					****	ns	**	***	
Nifedipine	n	4	4	4	5	5	4	6	4	4	4	4	5	5	4	6	4	4	4	4	5	5	4	6	4
	3 nM	*	ns	ns	ns	ns	*	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	**	ns	ns	*	ns	ns
	10 nM	*	ns	*	*	**	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	*	ns	ns	*	ns	*	ns	ns	ns
	30 nM	*	***	ns	*	**	*	ns	ns	ns	**	ns	ns	ns	ns	ns	ns	*	ns	*	ns	ns	ns	ns	ns
	100 nM	*	ns	*	**	**	ns	ns	ns	ns	ns	†	ns	†	ns	ns	ns	**	ns	†	ns	†	*	ns	ns
	300 nM	*	***		*		**	**	***	†	†		*		†	**	†	†	†		ns		†	ns	†
	1000 nM				**			**					**			†					*			†	
EMD-57033	n	3	2	4	5	6	4	5	4	3	2	4	5	6	4	5	4	3	2	4	5	6	4	5	4
	0.1 µM	ns	ns	*	ns	ns	ns	ns	ns	ns	ns	ns	ns	*	ns	ns	ns	ns	ns	*	*	**	ns	ns	ns
	0.3 µM	ns	ns	**	**	ns	*	ns	**	ns	ns	ns	ns	***	ns	ns	ns	ns	ns	*	ns	*	ns	ns	ns
	1 µM	ns	ns	*	ns	ns	ns	*	**	ns	ns	**	ns	**	ns	ns	ns	ns	ns	**	ns	ns	ns	*	ns
	3 µM	**	ns		ns	*	**	*	***	ns	ns		ns	***	*	*	**	ns	ns		ns	ns	*	**	ns
	10 µM				*	**	**	**					***	****	**	ns					**	**	**	*	
Isoprenaline	n	6	6	6	0	5	4	0	5	6	6	6	0	5	4	0	5	6	6	6	0	5	4	0	5
	0.3 nM	*	ns	*		ns	ns		**	ns	ns	ns		ns	ns		ns	ns	ns	**		ns	ns		ns
	1 nM	***	ns			ns	ns		*	ns	ns	**		ns	ns		ns	*	ns	***		ns	*		**
	3 nM	***	ns	**		ns	*		**	ns	**	**		*	ns		ns	ns	ns	****		ns	ns		***
	10 nM	***	ns	**		*	**		**	**	*	**		ns	ns		ns	ns	ns	****		ns	ns		**
	30 nM	***	*	***		*	*		**	**	ns	**		*	ns		ns	ns	ns	****		ns	ns		***
	100 nM	LOC		***		**	***		**	LOC				*	ns		ns	LOC				ns	ns		***
Digoxin	n	4	4	3	5	6	4	5	4	4	4	3	5	6	4	5	4	4	4	3	5	6	4	5	4
	0.01 µM	ns	***	ns	ns	*	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	0.1 µM	***	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	*	ns	ns	ns	**	ns	ns	ns	ns	ns	ns	ns
	0.3 µM	**	**	**	ns	*	ns	ns	ns	*	**	ns	ns	**	ns	ns	ns	**	*	*	ns	***	ns	ns	ns
	1 µM	**	ns	**	ns	***	ns	**	ns	†	*	ns	ns	†	†	†	ns	†	ns	ns	ns	†	†	†	ns
Thapsigargin	n	3	4	4	6	6	3	5	4	3	4	4	6	6	3	5	4	3	4	4	6	6	3	5	4
	3 µM	ns	ns	ns	ns	*	ns	ns	*	ns	ns	ns	ns	ns	ns	ns	ns	ns	**	ns	ns	ns	ns	ns	*
	10 µM	**	*	ns	ns	ns	*	ns	*	ns	ns	ns	*	ns	ns	ns	ns	ns	***	ns	ns	**	ns	ns	ns
	30 µM	ns	**	ns	ns	ns	ns	ns	ns	ns	ns	ns	*	**	**	ns	**	ns	**	ns	ns	***	ns	ns	ns
	100 µM	*	***	ns	ns	ns	ns	*	ns	ns	ns	ns	**	**	**	ns	**	ns	**	ns	ns	***	ns	ns	ns
	300 µM				ns	ns	*	*	ns				**	***	**	ns	*				ns	*	ns	ns	ns
Ryanodine	n	3	3	3	5	6	3	5	5	3	3	3	5	6	3	5	5	3	3	3	5	6	3	5	5
	1 µM	*	*	ns	**	**	***	ns	***	**	*	*	**	***	*	ns	***	*	*	*	**	ns	ns	ns	ns
	3 µM	ns	ns	ns	**	**	*	ns	***	**	ns	**	***	***	*	*	***	**	ns	*	*	ns	ns	ns	ns
	10 µM	ns	*	ns	**	**	ns	ns	**	**	**	*	***	***	ns	ns	**	**	*	*	ns	***	ns	ns	ns
	30 µM	ns	ns	ns	**	*		ns		***	ns	***	***	***		ns		**	ns	*	*	*		ns	

Supplementary Table 4. Correlation analysis between EHT contraction parameter and levels of gene expression for indicator genes, Related to Figure 7 and Supplementary Figure 6. R^2 = Pearson's correlation coefficient indicating linear correlation between parameter A and parameter B listed in the columns in front.

Parameter A	Parameter B	R^2
Force	FS	0.96
CACNA1G	KCNJ11	0.96
COL1A1	COL3A1	0.95
FN1	POSTN	0.95
KCNA4	PLN	0.93
CACNA1G	KCNN3	0.92
ACTC1	NPPA	0.90
ATP2A2	RYR2	0.89
ACTA2	HCN4	0.88
CTGF	POSTN	0.88
KCNA4	KCNJ2	0.88
T2	FHL1	0.87
ACTC1	KCNJ3	0.87
MEOX1	SCN10A	0.87
BPM	T1	0.86
KCNJ2	PLN	0.86
Force	CV	0.85
BCL2	NFKB1	0.84
KCNJ11	KCNN3	0.84
KCNJ2	RYR2	0.84
BPM	KCNA5	0.83
ATP2A2	BCL2	0.83
BCL2	CACNA1C	0.83
CASP3	SRF	0.83
CASP3	FN1	0.82
KCNA4	KCNJ11	0.82
PLN	RYR2	0.82
T1	KCNA5	0.81
CV	RV	0.81
ACTN2	KCNH2	0.81
ATP1A1	KCNE2	0.81
ATP1A1	S100A4	0.81
ATP2A2	COL3A1	0.81
BAX	CDH5	0.81
CACNA1G	SCN5a	0.81
FHL2	KCNQ1	0.81
KCNA4	SCN5a	0.81
KCNJ11	VWF	0.81
KCNJ5	PPP1R1A	0.81
ATP2A2	KCNJ2	0.80
ATP2A2	PLN	0.80
CDH5	CTGF	0.80
CTGF	FN1	0.80
KCNJ3	NPPA	0.80
ATP2A2	CACNA1C	0.79
ATP2A2	NFKB1	0.79
BCL2	KCNA4	0.79
CACNA1C	PLN	0.79
CACNA1G	KCNA4	0.79
KCNA4	NFKB1	0.79
KCNJ2	KCNJ5	0.79
PLN	SCN5a	0.79
ATP2A2	COL1A1	0.78
CACNA1G	VWF	0.78
KCNA4	RYR2	0.78
KCNJ2	POSTN	0.78
T1	FHL2	0.77
CV	FS	0.77
ACTN2	KCNIP2	0.77
ATP2A2	KCNA4	0.77
KCNJ11	SCN5a	0.77
KCNJ2	PPP1R1A	0.77
FS	ACTA2	0.76
CACNA1C	KCNA4	0.76
COL3A1	MYH6	0.76
NFKB1	PLN	0.76
RYR2	SCN5a	0.76
ATP2A2	KCNE2	0.75
CASP3	KCNQ1	0.75
COL1A1	FN1	0.75
KCNJ11	PLN	0.75
PPP1R1A	SLC9A1	0.75
BCL2	CASP3	0.74
BCL2	KCNJ11	0.74
CACNA1C	VWF	0.74
CASP3	PPP1R1A	0.74
COL1A1	MYH6	0.74
KCNA4	KCNJ5	0.74
KCNJ11	KCNJ5	0.74
T1	KCNQ1	0.73
FS	ATP1A1	0.73
BCL2	RYR2	0.73
BCL2	RYR2	0.73
CASP3	POSTN	0.73
KCNN3	VWF	0.73
T1	KCNJ12	0.72
FS	FHL2	0.72
BAX	CTGF	0.72
BCL2	PLN	0.72
CACNA1C	NFKB1	0.72
CASP3	RYR2	0.72
FN1	SRF	0.72
KCNA4	PPP1R1A	0.72
KCNE1	KCNIP2	0.72
KCNJ11	PPP1R1A	0.72
KCNJ5	POSTN	0.72
MEOX1	VWF	0.72
Force	ACTA2	0.71
T2	CASQ2	0.71
RV	MYH7	0.71
ATP1A2	CACNA1C	0.71
BCL2	CACNA1G	0.71
BCL2	VWF	0.71
CACNA1C	KCNJ11	0.71
CASQ2	KCNJ3	0.71
CTGF	KCNJ5	0.71
KCNA4	KCND3	0.71
KCNE2	PLN	0.71
KCNJ5	SCN5a	0.71
BPM	KCNJ12	0.70
ACTA2	KCNJ5	0.70
ATP2A2	MYH6	0.70
CACNA1G	MEOX1	0.70
CACNA1G	PLN	0.70
COL3A1	NFKB1	0.70

Supplemental Experimental Procedures

Generation of EHT

Human PSC-derived cardiomyocytes were obtained from 4 commercial hiPS cell lines (PLU = Pluricyte cardiomyocytes from Pluriomics (now Ncardia); CDI = iCell cardiomyocytes, and ICE = iCell² cardiomyocytes both Cellular Dynamics International; CEL = Cellartis cardiomyocytes from Takara Bio), 1 commercial hES cell line (COR = Cor4U cardiomyocytes from Axiogenesis) and differentiated from 3 Hamburg hiPS cell lines including 1 NIH-registered iPS cell line NCRM5 (C25, ERC = UKEi003-C, NCR = ND50031), and 2 Nottingham hiPS cell lines (AT1, REB = REBL-PAT).

Frozen cardiomyocytes from commercial suppliers (PLU, CDI, CEL) or Hamburg or Nottingham cell banks were stored at -150°C and cryotubes quickly thawed in a water bath (37 °C) for 3 min. A maximum of four cryotubes was handled in parallel. Cells were transferred to 50 ml falcon tubes and diluted to 10 ml per cryotube by drop-wise addition of warm (37 °C) DMEM (Biochrom F0415) or commercial thawing medium of the respective supplier (Ncardia, CDI, Cellartis).

Living cells from commercial suppliers (Axiogenesis) and cardiomyocytes differentiated from academic hiPS lines were dissociated with a collagenase-based digestion protocol (200 U/ml in HBSS with 1 mM HEPES; 3.5 h) as previously published (Breckwoldt et al. 2017). After centrifugation (100 g; 10 min), freshly dissociated or thawed cells were resuspended in DMEM and counted manually with a Neubauer chamber and trypan blue solution (0.4%; Gibco 15250061).

EHTs were generated from fresh or frozen human PS-derived cardiomyocytes as previously published (Mannhardt et al., 2017a) using $1x10^6$ cells per 100 µl tissue (see also Supplementary Figure 1). There were no additional non-CM added to the master mix to test the hiPSC-CM alone as "of the shelf"-product. EHTs were cultivated at 40% O_2, 7% CO_2, 98% RH, 37 °C and showed spontaneous macroscopic contractions, deflecting the silicone posts, after 7-14 days.

Contraction analysis

Contraction analysis of coherently beating EHT was performed with a video-optical analysis system (Hansen et al., 2010; EHT Technologies GmbH A0001; Supplementary Figure 1). Tissue contractility was regularly monitored in serum-supplemented EHT maintenance medium. For drug screening, 1000x stock solutions of the compounds were prepared with DMSO and small aliquots for one-time use frozen at -20 °C to avoid repeated freeze-thaw cycles of the drugs. When EHT contraction force reached its plateau (usually day 15-25), drug screening was performed in protein-free Tyrode's solution at submaximal calcium and cumulative concentration-response curves under electrical stimulation (Mannhardt et al., 2017b).

Sharp microelectrode measurement

Action potentials of whole EHTs were recorded with sharp microelectrode measurements in protein-free Tyrode's solution at 36.5±0.5 °C as described previously (Lemoine et al., 2018).

Histological analysis

For whole mount immunofluorescence analysis and measurement of sarcomere length, relaxed EHTs (2-butandionemonoxime, Sigma B0753; 30 mM, 10 min, 37 °C) were fixed in p-formaldehyde (Roti®-Histofix 4%, Carl Roth, P087.3) at 4 °C overnight. After 6 h incubation in blocking solution (TBS 0.05 M pH 7.4, 10% FCS, 1% BSA, 0.5% Triton X-100), EHTs were incubated with the primary antibodies (monoclonal mouse anti-alpha actinin, 1:800, Sigma A7811; monoclonal rabbit anti-MLC2v, 1:200, Proteintec™ 10906) in antibody solution (TBS 0.05 M pH 7.4, 1% BSA, 0.5% Triton X-100) overnight. After repeated washing in PBS EHTs were exposed to secondary antibodies (Alexa Fluor® 488 goat-anti-mouse, 1:800, Invitrogen; Alexa Fluor® 546 goat-anti-rabbit, 1:800, Invitrogen) and nuclear counterstaining dye (DRAQ5™, 1:1000, Biostatus Ltd. BOS-889-001-R050) in antibody solution for at least 3 h. Finally, whole EHTs were rinsed in PBS 3-4 times and embedded in Fluoromount-G® (SouthernBiotech, 0100-01) in dented microscope slides (Carl Roth, H884.1). Sarcomere length was measured based on Z-bands of the alpha-actinin signal with a Zeiss LSM 800 microscope and respective ZEN software.

RNA isolation and expression analysis

Total RNA was extracted from EHT and native human heart tissue with the RNeasy kit (Qiagen 74104) according to manufacturer's instructions as previously published (Mannhardt et al., 2016). Nonfailing human heart samples, unsuitable for transplantation, were obtained from the University Heart Center with approval of the University of Hamburg's ethical board (reference number 532/116/9.7.1991). After fluorimetric quantitation of RNA concentration with Qubit™ according to manufacturer's instructions, transcriptome analysis was performed with the nanoString nCounter Elements technology as described previously (Prondzynski et al., 2017). In brief, 50 ng of sample RNA were used for gene expression analysis of 57 genes coding for proteins involved in cardiac excitation-contraction coupling or dysregulated in heart failure. Analysis with nCounter Sprint Profiler included normalization of mRNA levels to five housekeeping genes (*ABCF1, CLTC, GAPDH, PGK1, TUBB*).

Statistical analysis

Data in the text are presented as mean±SD. Replicate numbers described as n indicate number of EHTs or trabeculae, n/N indicate e.g. n impalements from N EHTs or n sarcomeres from N cells. Data in the graphs are presented as desribed in the respective figure legend. Statistical tests were performed with the GraphPad Prism 6.0 software. A p-value <0.05 was considered significant.

Histological analysis of engineered heart tissue

For immunohistochemical analysis of transversal sections, fixed EHTs were embedded in paraffin and consecutive 4 µm sections stained with a Ventana benchmark system (UKE Hext mouse pathology core facility) with haematoxylin and eosin, anti-dystrophin (Millipore MAB1645, 1:200, antigen retrieval with EDTA for 60 min), anti-MLC2v (SY310111, 1:300, antigen retrieval with citrate buffer for 30 min), anti-MLC2a (SY311011, 1:75, antigen retrieval with citrate buffer for 30 min), anti-collagen (Abcam Ab138492, 1:1500, antigen retrieval with citrate buffer for 30 min), anti-smooth muscle actin (Dako

M0851, 1:100, antigen retrieval with citrate buffer for 30 min), anti-alpha actin (Dako M0874, 1:200, antigen retrieval with citrate buffer for 30 min), anti-vimentin (Dako M0725, 1:200, antigen retrieval with citrate buffer for 30 min).

Quantitative real-time PCR

200 ng RNA per cell line or non-failing human heart (same samples used for Nanostring analysis; see main manuscript experimental procedures) were transcribed into cDNA with the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems™; 4368814). Quantitative RT-PCR was performed with Maxima™ SYBR™ Green/ROX 2x qPCR Master Mix (Thermo Scientific™; K0221) according to manufacturer's instructions using the ABI PRISM 7900HT Sequence detection system (Applied Biosystems) with SDS software (version 2.4) and the following primer pairs: GAPDH (for: TCGGAGTCAACGGATTTGGT; rev: TCGCCCCACTTGATTTTGGA), TNNT2 (for: AGACAGAGCGGAAAAGTGGG; rev: GTCGAACTTCTCTGCCTCCAA), MLC2a (for: AAGGTGAGTGTCCCAGAGGA; rev: CGAACATCTGCTCCACCTCAG), MLC2v (for: AGGCGGAGAGGTTTTCCAAG; rev: GGACCACTCTGCAAAGACGA), LOXL2 (for: CCCTGGGGAGAGGACATACA; rev: CCCATTCTCGCAGGTGACAT). Normalization was performed against GAPDH as housekeeping gene.

Supplemental References

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