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Supplemental Information

Human Engineered Heart Tissue: Analysis of Contractile Force

Ingra Mannhardt, Kaja Breckwoldt, David Letuffe-Brenière, Sebastian Schaaf, Herbert Schulz, Christiane Neuber, Anika Benzin, Tessa Werner, Alexandra Eder, Thomas Schulze, Birgit Klampe, Torsten Christ, Marc N. Hirt, Norbert Huebner, Alessandra Moretti, Thomas Eschenhagen, and Arne Hansen

Supplemental Information

Supplemental Data

Figure S1. Related to Figure 1.

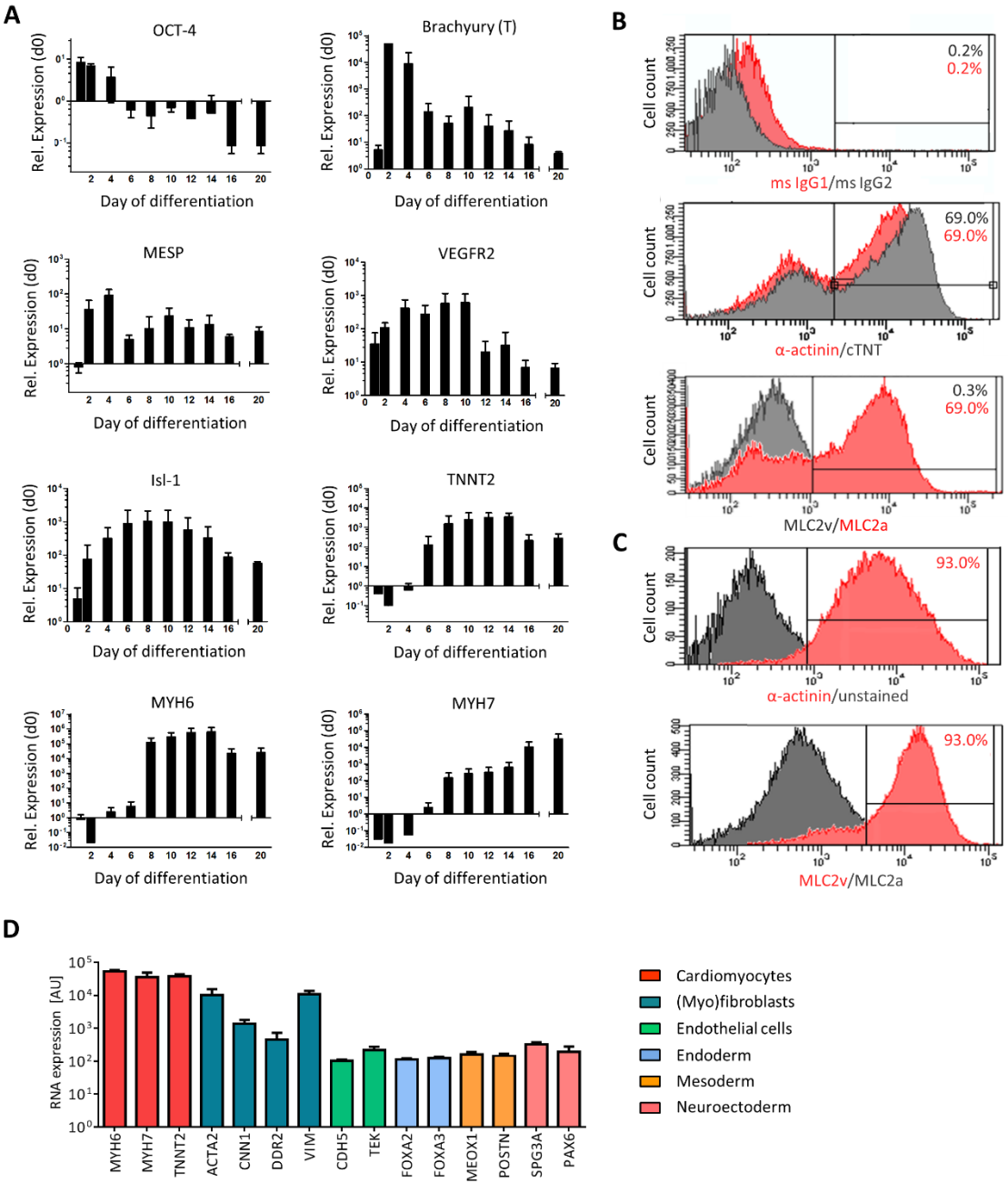


Figure S1: Cardiac differentiation. Replicates are indicated as "EHTs/number of independent experiments", depicted are mean±SEM. **A:** Quantitative RT-PCR results showing the relative expression levels of markers of pluripotency, mesoderm, early and late CM development over 20 days of differentiation (n=4/4). Expression levels are normalized to GUSB and expression levels at day 0. **B, C:** Characterization of differentiated cells by FACS analysis. **B:** Cells at the end of differentiation. **C:** Cells isolated from EHT after three weeks in culture. **D:** Characterization of cellular markers at the end (day 21) of differentiation. Relative RNA expression from whole transcriptome analysis for markers of cardiomyocytes, (myo)fibroblasts, endothelial cells, mesoderm, endoderm and neuroectoderm (n=4/1).

Figure S2. Related to Figure 2.

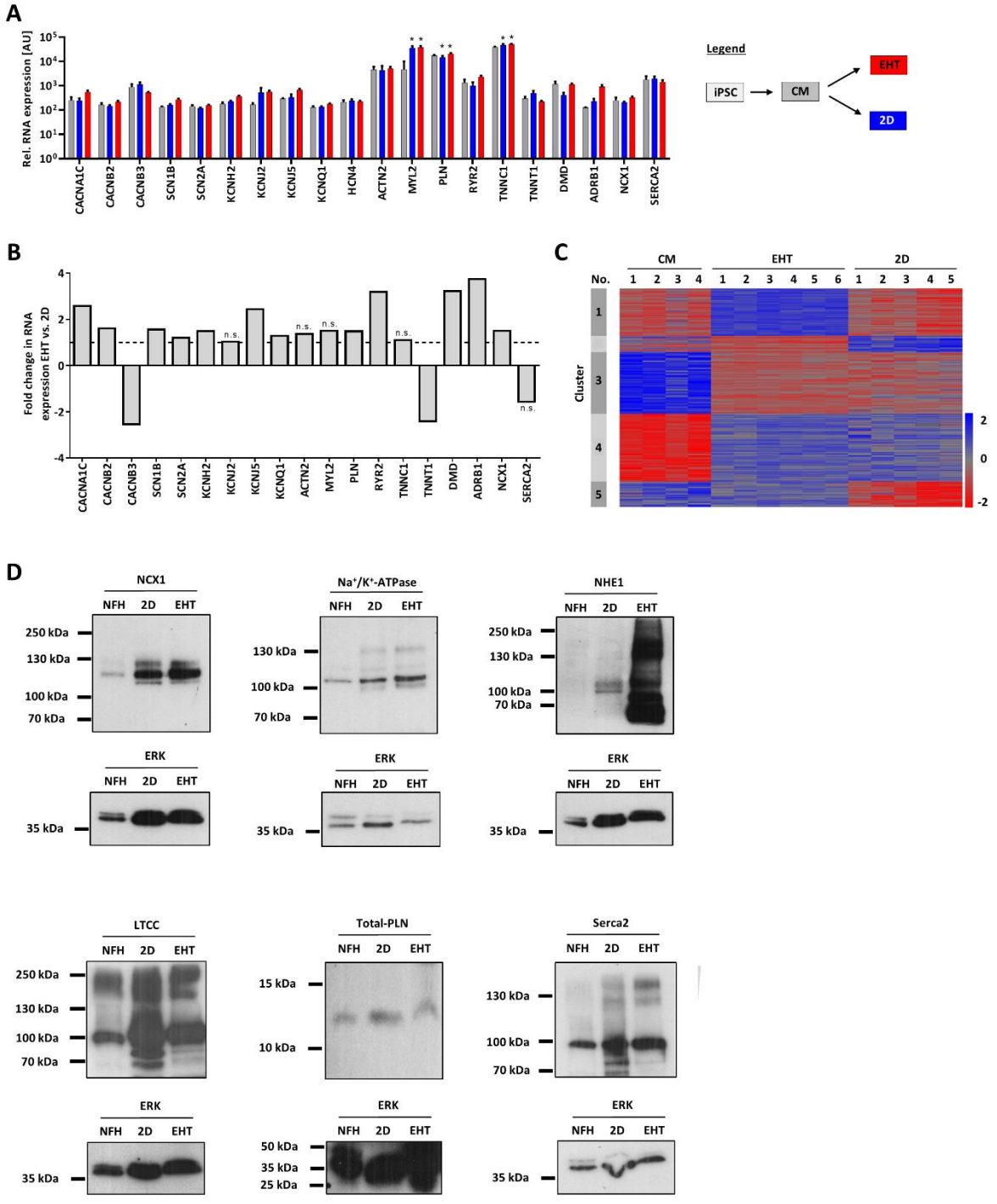


Figure S2: Expression analyses. Replicates are indicated as "EHTs/number of independent experiments". **A-D:** Gene and protein expression analysis comparing cardiomyocytes at the end of differentiation (CM; grey) with those cultivated for 21 more days in 2D (blue) or EHT (red) format (see legend). **A:** Gene expression levels comparing CM, 2D and EHT (Two-way ANOVA with Bonferroni's post-test; * $p<0.05$ vs. CM; n=4,5,6/3 for CM, 2D, EHT; depicted are mean±SEM). See also Table S1. **B:** Mean fold change in RNA expression in EHT (n=5/3) vs. 2D (n=6/3) for ion channels and cardiac genes. **C:** K-mean cluster analysis. See also Table S2 for functional aspects. **D:** Protein abundance of calcium handling proteins in 2D, EHT and non-failing human heart samples (NFH). Western blot analysis revealed expression of Na^+/Ca^{2+} exchanger-1 (NCX1, ~120 kDa), Na^+/K^+-ATPase (~112 kDa), Na^+/H^+ exchanger-1 (NHE1; ~100 kDa with an additional band at ~72 kDa 110-130 (glycosylated), 90 kDa (precursor), 210 kDa dimer), L-type calcium channel (~210 kDa), phospholamban (Total-PLN, ~12 kDa) and Serca2 ATPase (SERCA2, ~110 kDa). ERK (~40 kDa) was used as loading control.

Figure S3. Related to Figure 3.

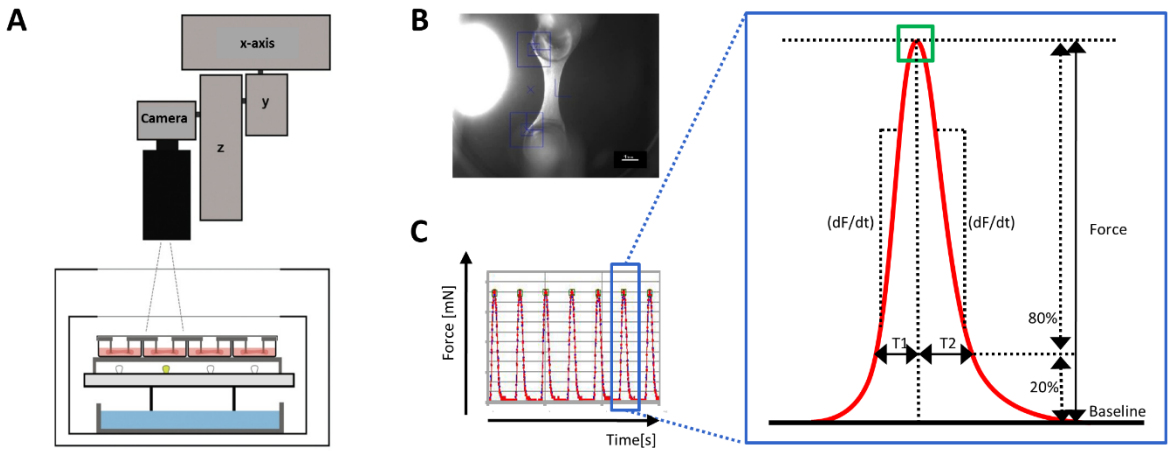


Figure S3: Contraction analysis of human EHTs. **A:** Setup with gas- and temperature-controlled incubation chamber with LEDs, glass top and camera above (adapted from Schaaf et al. 2011). **B:** Live-image of an EHT with depicted figure recognition (blue crosses) during the measurement. **C:** Force-time-diagram showing contraction peaks with contraction time (T1) and relaxation time (T2) at 20% peak height, contraction velocity (dF/dt), relaxation velocity (dF/dt) and force (green square as quality control for contraction peak identification of customized software).

Figure S4. Related to Figure 4.

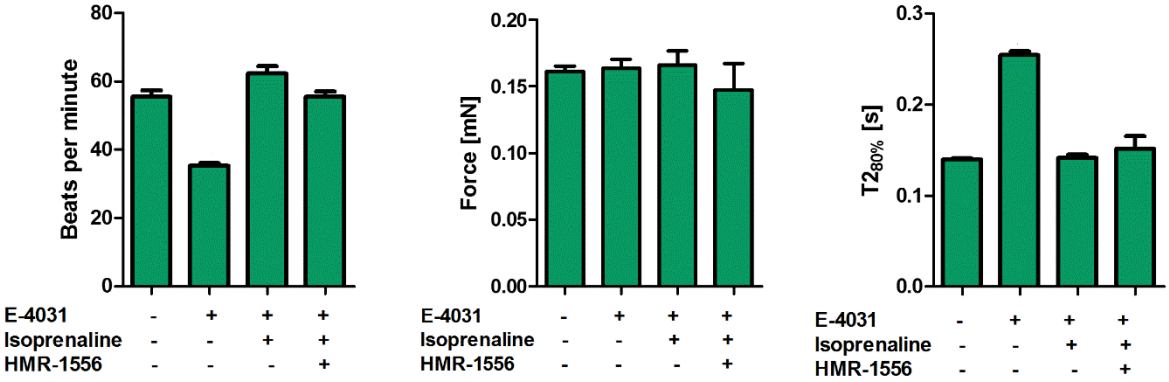
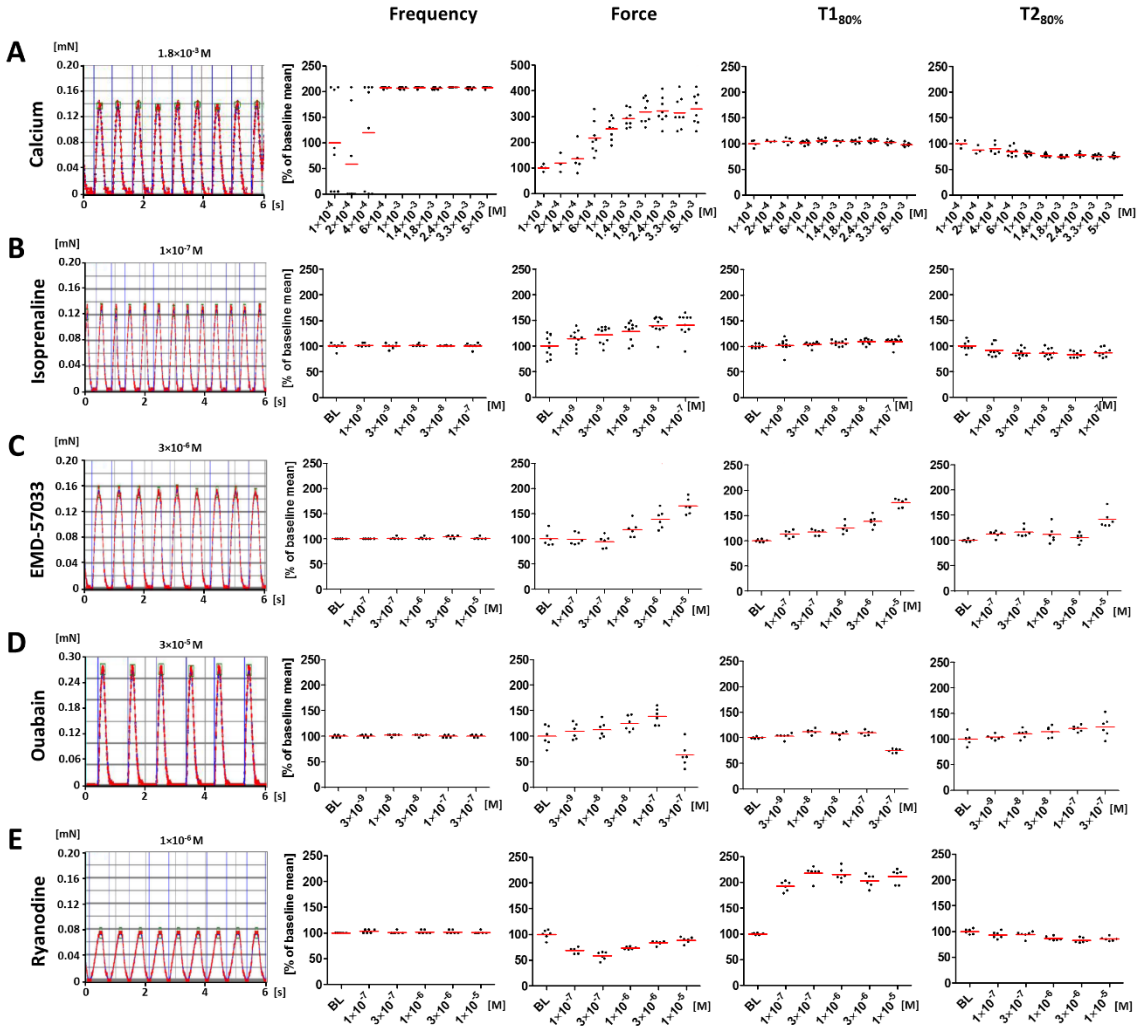


Figure S4: **I_{Ks}.** Response of spontaneous hiPSC-EHT contractions to I_{Ks}-inhibitor HMR-1556 (1 µM) after I_{Kr}-inhibition with E-4031 (100 nM) and ß-adrenergic stimulation with isoprenaline (10 nM). Change in beating frequency, contraction force and relaxation time T2 in modified Tyrode's solution with 1 mM calcium. Replicates are indicated as "EHTs/number of independent experiments"; n=4/1, depicted are mean±SEM.

Figure S5. Related to Figure 3 and Figure 5.



F

	hiPSC-EHT	NFH
Isoprenaline	+41% (peak force)	+300 – 800% (peak force) (Schotten et al. 2001, Böhm et al. 1991)
Calcium concentration response curve	EC_{50} 0.6 mM	EC_{50} of 5.6 mM (Böhm et al. 1991)
Calcium concentration response curve (skinned preparations)	EC_{50} of 2.4 µM (Stoehr et al. 2014)	EC_{50} of 1.06 µM (Hajjar & Gwathmey 1992)
Post-rest-potentiation	+17%	+76% (Pieske et al. 1996)
Frequency-dependent acceleration of relaxation	-14% (1-2 Hz)	-33% (1-2 Hz) (Mulieri et al. 1992)
Force-frequency-relationship	-/-	+68% (Pieske et al. 1996)
Frank-Starling law	+57%	+200% (Schwinger et al. 1994)

Figure S5: Pharmacological characterization of hiPSC-EHTs. A-E: Concentration response curves in modified Tyrode's solution (0.5-0.6 mM Ca^{2+}). Electrical stimulation: 1-2 Hz (blue lines). **Left:** Exemplary original recording of EHT contraction pattern at the indicated concentration. **Right:** Scatter-plot depiction of the concentration-response curve for frequency, force, contraction time (T1) and relaxation time (T2). Replicates are indicated as "EHTs/number of independent experiments"; n=6-10/1-2; data are depicted as scatter plot with mean. **F:** Comparison of effects in hiPSC-EHTs with published values in non-failing heart (NFH) tissue.

Figure S6. Related to Figure 6.

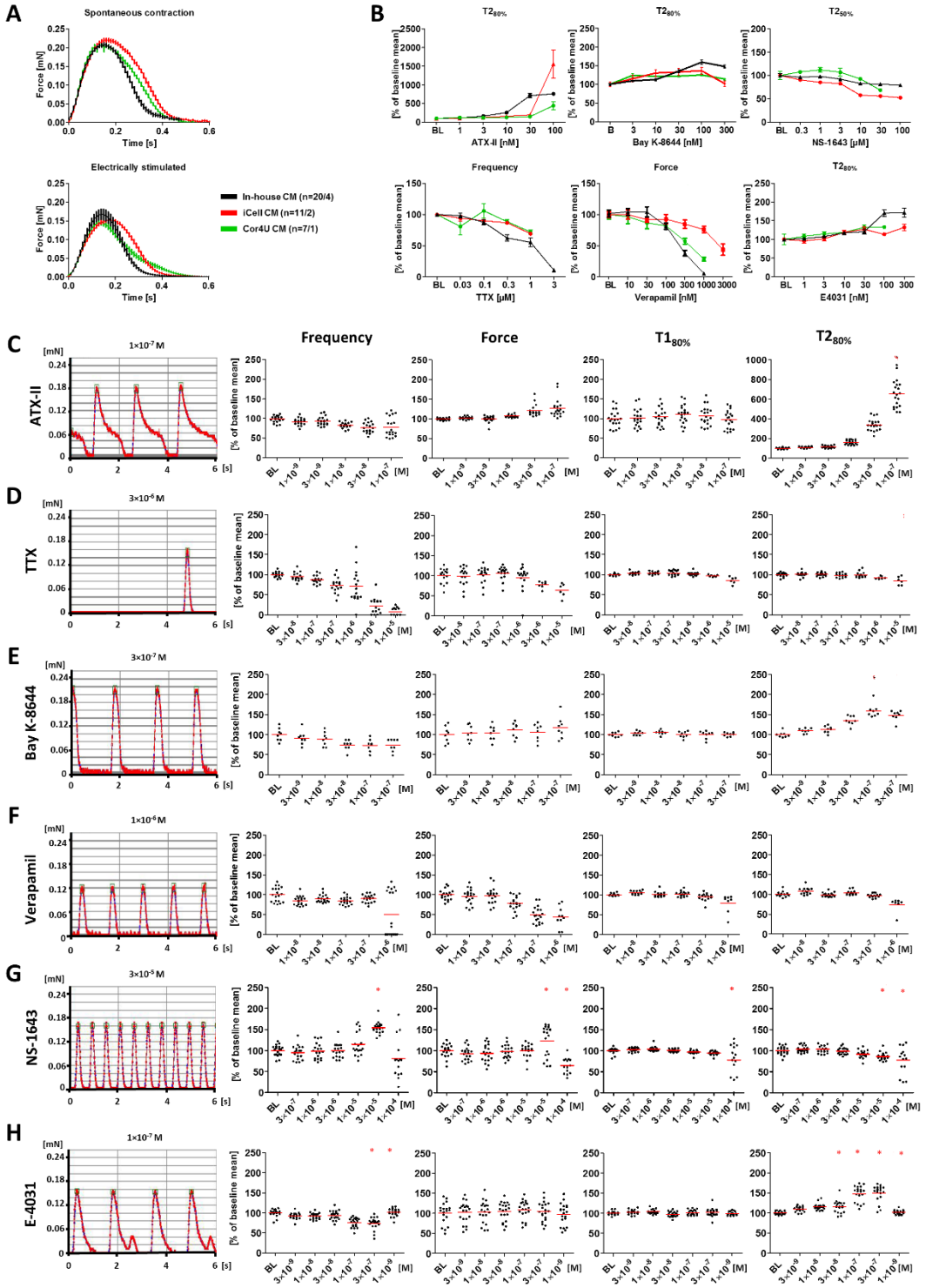


Figure S6: Pharmacological characterization of hiPSC-EHTs. **A:** Average contraction peaks (mean±SEM) of spontaneous contracting (top) and electrically stimulated (1.5 Hz; bottom) EHTs. Black: in-house reference CM (n=20/3), red: iCell® CM (n=11/2), green: Cor4U® CM (n=7/1), depicted are mean±SEM. **B:** Concentration response curves in modified Tyrode's solution (1.8 mM Ca^{2+}) for the ion channel modulators introduced in Figure 6 and their effect on the most prominently changed parameter of EHT contraction; depicted are mean±SEM. **C-H:** Concentration response curves in modified Tyrode's solution (1.8 mM Ca^{2+}) as partially displayed in Figure 6. **Left:** Exemplary original recording of EHT contraction pattern at the indicated concentration. **Right:** Scatter-plot depiction of the concentration-response curve for frequency, force, contraction time (T1) and relaxation time (T2). Replicates are indicated as "EHTs/number of independent experiments; n=8-20/2-3; data are depicted as scatter plot with mean. Statistical analysis for experiments with ≥3 independent experiments: One-way ANOVA with Dunnett's post-test vs. baseline conditions (BL); *p<0.05.

Figure S7. Related to Figure 3 and Figure 7.

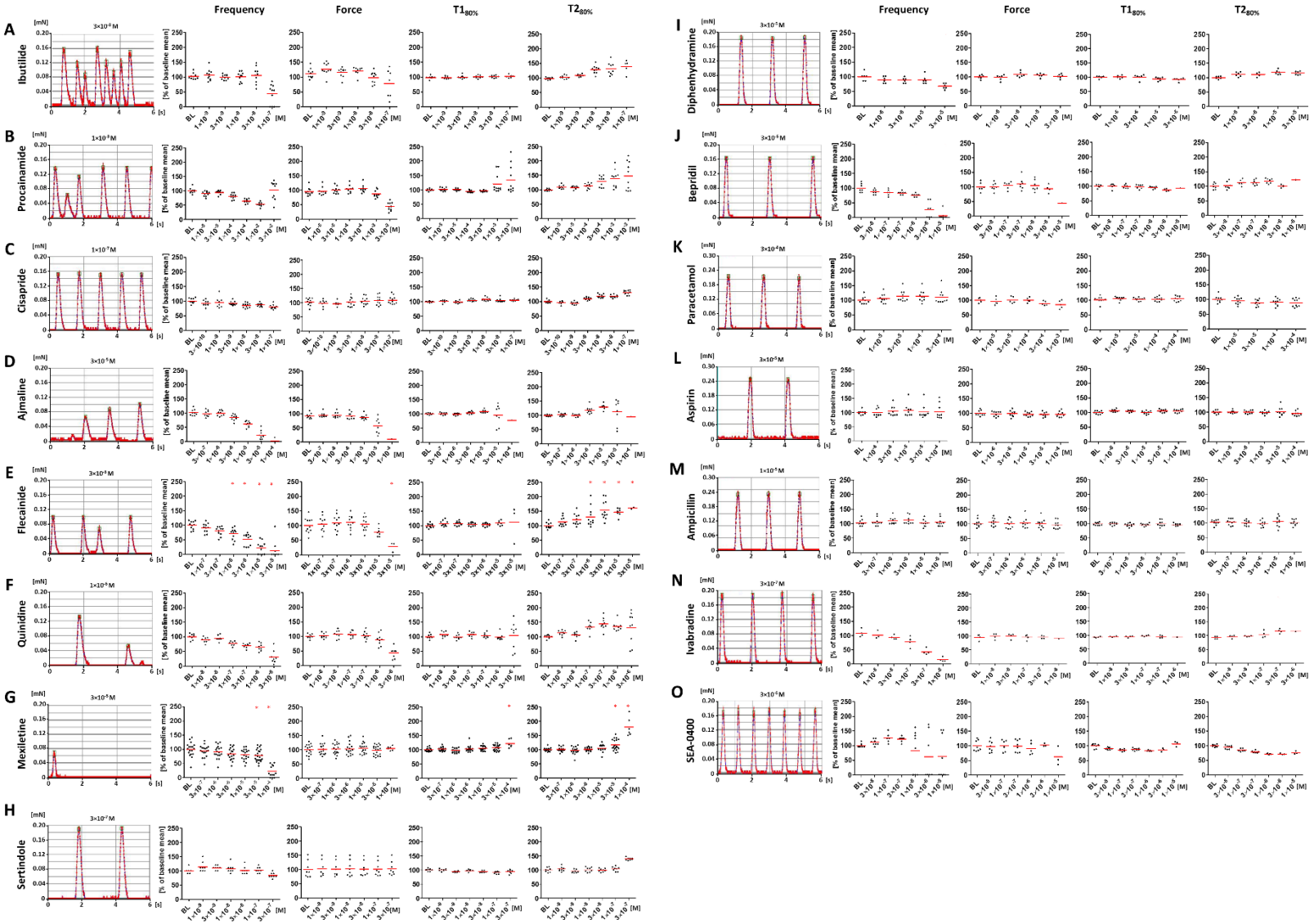


Figure S7: Pharmacological characterization of hiPSC-EHTs. Concentration response curves in modified Tyrode's solution (**A-N:** 1.8 mM Ca^{2+}, **O:** 1 mM Ca^{2+}). **Left:** Exemplary original recording of EHT contraction pattern at the indicated concentration. **Right:** Scatter-plot depiction of the concentration-response curve for frequency, force, contraction time (T1) and relaxation time (T2). Replicates are indicated as "EHTs/number of independent experiments"; n=4-23/1-3; data are depicted as scatter plot with mean. Statistical analysis for experiments with ≥3 independent experiments: One-way ANOVA with Dunnett's post-test vs. baseline conditions (BL); *p<0.05.

Table S1 (see external excel file). Related to Figure 2 and Figure S2.
Results of transcriptome analysis comparing CM with 21-days older time matched 2D and EHT culture.

Table S2. Related to Figure 2, Figure S2 and Table S1.
Enrichment analysis using G:Profiler, with a simulation based analytical threshold for significance estimation. BP = GeneOntology Biological Process; CC = GeneOntology Cellular Component; MF = GeneOntology Molecular Function; ke = KEGG database; mi = miRBase; tf = TransFac; a = annotation; q = query; inters. = intersection.

Source: Cluster 1 of 5: Genes differentially expressed in ANOVA (0.1% FDR; N=57)

p-value	Genes in annotation (a)	Genes in query (q)	a inters. q	(a inters. q) / q	(a inters. q) / a	Annotation-id	Database	Title
5.00e-02	1	10	1	0.100	1.000	OMIM:615219	omi	HYDROCEPHALUS, NONSYNDROMIC, AUTOSOMAL RECESSIVE 2; HYC2
5.00e-02	1	10	1	0.100	1.000	OMIM:201300	omi	NEUROPATHY, HEREDITARY SENSORY AND AUTONOMIC, TYPE IIA; HSAN2A;HSAN IIA;NEUROPATHY, HEREDITARY SENSORY, TYPE IIA; HSN2A;HSN IIA;ACROOSTEOLYSIS, NEUROGENIC;ACROOSTEOLYSIS, GIACCAI TYPE;NEUROPATHY, HEREDITARY SENSORY RADICULAR, AUTOSOMAL RECESSIVE;MORVAN DISEASE;NEUROPATHY, PROGRESSIVE SENSORY, OF CHILDREN;NEUROPATHY, CONGENITAL SENSORY
5.00e-02	1	10	1	0.100	1.000	OMIM:614492	omi	PSEUDOHYPOALDOSTERONISM, TYPE IIC; PHA2C
5.00e-02	1	10	1	0.100	1.000	OMIM:612313	omi	GLASS SYNDROME; GLASS;CHROMOSOME 2q32-q33 DELETION SYNDROME
5.00e-02	1	10	1	0.100	1.000	OMIM:615493	omi	MENTAL RETARDATION, AUTOSOMAL RECESSIVE 37; MRT37
5.00e-02	1	10	1	0.100	1.000	OMIM:614831	omi	SPINOCEREBELLAR ATAXIA, AUTOSOMAL RECESSIVE 13; SCAR13
5.00e-02	1	10	1	0.100	1.000	OMIM:258501	omi	3-@METHYLGLUTACONIC ACIDURIA, TYPE III; MGCA3;MGA, TYPE III; MGA3;OPTIC ATROPHY PLUS SYNDROME;OPTIC ATROPHY, INFANTILE, WITH CHOREA AND SPASTIC PARAPLEGIA;IRAQI-JEWISH 'OPTIC ATROPHY PLUS';COSTEFF SYNDROME;OPTIC ATROPHY 3, AUTOSOMAL RECESSIVE;OPA3, AUTOSOMAL RECESSIVE
5.00e-02	1	10	1	0.100	1.000	OMIM:165300	omi	OPTIC ATROPHY 3, AUTOSOMAL DOMINANT; OPA3;OPTIC ATROPHY AND CATARACT, AUTOSOMAL DOMINANT
5.00e-02	1	10	1	0.100	1.000	OMIM:607276	omi	RESTING HEART RATE, VARIATION IN;RHR

Source: Cluster 2 of 5: Genes differentially expressed in ANOVA (0.1% FDR; N=20)

p-value	Genes in annotation (a)	Genes in query (q)	a inters. q	(a inters. q) / q	(a inters. q) / a	Annotation-id	Database	Title
5.00e-02	1	2	1	0.500	1.000	CORUM:2003	cor	COX1 HOMODIMER COMPLEX

Source: Cluster 3 of 5: Genes differentially expressed in ANOVA (0.1% FDR; N=68)

p-value	Genes in annotation (a)	Genes in query (q)	a inters. q	(a inters. q) / q	(a inters. q) / a	Annotation-id	Database	Title
5.83e-05	181	51	8	0.157	0.044	GO:0035966	BP	RESPONSE TO TOPOLOGICALLY INCORRECT PROTEIN
3.57e-05	170	51	8	0.157	0.047	GO:0006986	BP	RESPONSE TO UNFOLDED PROTEIN
9.19e-04	259	51	8	0.157	0.031	GO:0034976	BP	RESPONSE TO ENDOPLASMIC RETICULUM STRESS
8.19e-06	141	51	8	0.157	0.057	GO:0035967	BP	CELLULAR RESPONSE TO TOPOLOGICALLY INCORRECT PROTEIN
4.85e-06	132	51	8	0.157	0.061	GO:0034620	BP	CELLULAR RESPONSE TO UNFOLDED PROTEIN
4.04e-06	129	51	8	0.157	0.062	GO:0030968	BP	ENDOPLASMIC RETICULUM UNFOLDED PROTEIN RESPONSE
3.98e-02	19	51	3	0.059	0.158	GO:0036499	BP	PERK-MEDIATED UNFOLDED PROTEIN RESPONSE
1.33e-02	169	24	5	0.208	0.030	KEGG:04141	keg	PROTEIN PROCESSING IN ENDOPLASMIC RETICULUM
2.57e-02	532	48	8	0.167	0.015	MI:hsa-miR-130a*	mi	MI:HSA-miR-130a*
4.12e-04	87	21	5	0.238	0.057	REAC:381119	rea	UNFOLDED PROTEIN RESPONSE (UPR)
9.69e-03	28	21	3	0.143	0.107	REAC:381042	rea	PERK REGULATES GENE EXPRESSION
4.00e-02	10054	54	38	0.704	0.004	TF:M00932_1	tf	FACTOR: SP1; MOTIF: NNGGGGCGGGGNN; MATCH CLASS: 1
3.05e-02	11319	54	41	0.759	0.004	TF:M07354_0	tf	FACTOR: EGR-1; MOTIF: GCGGGGGCGG; MATCH CLASS: 0
4.56e-02	5051	54	25	0.463	0.005	TF:M03567_1	tf	FACTOR: SP2; MOTIF: NYSGCCCCGCCCCCY; MATCH CLASS: 1

Source: Cluster 4 of 5: Genes differentially expressed in ANOVA (0.1% FDR; N=82)

p-value	Genes in annotation (a)	Genes in query (q)	a inters. q	(a inters. q) / q	(a inters. q) / a	Annotation-id	Database	Title
4.27e-02	6363	78	38	0.487	0.006	TF:M01072_1	tf	FACTOR: HIC1; MOTIF: NSNNNNTGCCCSSNN; MATCH CLASS: 1

Source: Cluster 5 of 5: Genes differentially expressed in ANOVA (0.1% FDR; N=32)

p-value	Genes in annotation (a)	Genes in query (q)	a inters. q	(a inters. q) / q	(a inters. q) / a	Annotation-id	Database	Title
5.00e-02	1	2	1	0.500	1.000	OMIM:300376	omi	MUSCULAR DYSTROPHY, BECKER TYPE; BMD;BECKER MUSCULAR DYSTROPHY;MUSCULAR DYSTROPHY, PSEUDOHYPERTROPHIC PROGRESSIVE, BECKER TYPE
5.00e-02	1	2	1	0.500	1.000	OMIM:613152	omi	MUSCULAR DYSTROPHY-DYSTROGLYCANOPATHY (CONGENITAL WITHOUT MENTAL RETARDATION),TYPE B, 4; MDDGB4;MUSCULAR DYSTROPHY, CONGENITAL, FKTN-RELATED
5.00e-02	1	2	1	0.500	1.000	OMIM:302045	omi	CARDIOMYOPATHY, DILATED, 3B; CMD3B;CARDIOMYOPATHY, DILATED, X-LINKED; XLCM
5.00e-02	1	2	1	0.500	1.000	OMIM:253800	omi	MUSCULAR DYSTROPHY-DYSTROGLYCANOPATHY (CONGENITAL WITH BRAIN AND EYEANOMALIES), TYPE A, 4; MDDGA4;FUKUYAMA CONGENITAL MUSCULAR DYSTROPHY; FCMD;WALKER-WARBURG SYNDROME OR MUSCLE-EYE-BRAIN DISEASE, FKTN-RELATED
5.00e-02	1	2	1	0.500	1.000	OMIM:611615	omi	CARDIOMYOPATHY, DILATED, 1X; CMD1X;CARDIOMYOPATHY, DILATED, WITH MILD OR NO PROXIMAL MUSCLE WEAKNESS
5.00e-02	1	2	1	0.500	1.000	OMIM:611588	omi	MUSCULAR DYSTROPHY-DYSTROGLYCANOPATHY (LIMB-GIRDLE), TYPE C, 4; MDDGC4;MUSCULAR DYSTROPHY, LIMB-GIRDLE, TYPE 2M; LGMD2M
5.00e-02	1	2	1	0.500	1.000	OMIM:310200	omi	MUSCULAR DYSTROPHY, DUCHENNE TYPE; DMD;DUCHENNE MUSCULAR DYSTROPHY;MUSCULAR DYSTROPHY, PSEUDOHYPERTROPHIC PROGRESSIVE, DUCHENNE TYPE

Supplemental Experimental Procedures

Expansion and differentiation of human induced pluripotent stem cells

Undifferentiated hiPSC control cell line (Moretti et al. 2010; in the following named "in-house reference") was expanded on Geltrex® (Gibco, A1413302; 1:200 in DMEM, 37 °C, 30 min) coated 6-well-dishes and T80-flasks in FTDA medium (37 °C, 5% CO_2, 5% O_2) as recently published (Frank et al., 2012) with 30 ng/ml FGF2: DMEM/F-12 (Gibco, 21331), 2 mM L-Glutamine (Gibco, 25030), 0.5% penicillin/streptomycin (Gibco, 15140), 5 mg/l transferrin (Sigma, T8158), 5 µg/l selenium (Sigma, S5261), 0.1% human serum albumin (Biopur, 05-720-1B), 1x Lipid-Mix (Sigma-Aldrich, L5146), 5 mg/l insulin (Sigma-Aldrich, I9278), 50 nM dorsomorphin (Tocris, 3093), 2.5 ng/ml activin A (R&D Systems, 338-AC), 0.5 ng/ml human TGFß1 (PeproTech, 100-21C), 30 ng/ml FGF2 (PeproTech, 100-18B). Cardiomyocytes were differentiated from hiPSC in a three-stage protocol (Figure 1). Confluent hiPSC were incubated with Rho kinase inhibitor Y-27632 (Biaffin, PKI-Y27632-010; 10 µM in FTDA medium) for 1 hour, washed with PBS (Gibco, 14190) twice and dissociated with 0.5 mM EDTA (10 minutes; Roth, 80432). After automated cell counting (CASY® cell counter, OMNI Life Science), cells were transferred to spinner flasks. Embryoid bodies (EBs) were generated during culture of single cell suspension (30×10^6 cells/100 ml) in 250 and 500 ml spinner flasks (FTDA medium with 4 mg/ml polyvinyl alcohol [PVA; Sigma-Aldrich, P8136], 10 µM Y-27632) by stirring at 40 rpm in a gas and temperature controlled incubator (37 °C, 5% CO_2, 5% O_2) for 24 h (Zweigerdt et al., 2011). An aliquot of EBs was transferred into a 15 ml falcon tube and EBs were sedimented (10 min). EB volume of the aliquot was estimated according to falcon tube scale and EB volume of the entire preparation was calculated. Mesodermal differentiation was induced in suspension culture on Pluronic® F-127 (Sigma-Aldrich, P2443; 1% in PBS, 37 °C, overnight) coated T175 flasks (200 µl EBs/40 ml medium) for three days with daily medium change of 50% of the volume (37 °C, 5% CO_2, 5% O_2). The medium consisted of RPMI 1640 (Gibco, 21875), 4 mg/ml PVA, 0.5% penicillin/streptomycin, 10 mM HEPES (pH 7.4; Roth, 9105.4), 0.05% human serum albumin, 5 mg/l transferrin, 5 µg/l selenium, 10 µM Y-27632, 0.1% Lipid-Mix, 250 µM 2-phospho-L-ascorbic acid trisodium salt (Sigma-Aldrich, 49752), 10 ng/ml BMP-4 (R&D Systems, 314-BP), 3 ng/ml activin-A, 5 ng/ml FGF2. Cardiac differentiation was performed either in suspension culture or in adhesion culture (Figure 1) with a medium consisting of RPMI 1640, 0.5% penicillin/streptomycin, 10 mM HEPES, 1% transferrin-selenium, 1 µM Y-27632, 0.05% Lipid-Mix, 250 µM 2-phospho-L-ascorbic acid trisodium salt, 0.1% human serum albumin, 100 nM Wnt-inhibitor DS-07 (4-(*cis-endo*-1,3-dioxooctahydro-*2H*-4,7-methanoisoindol-2-yl)-*N*-(quinolin-8-yl)-*trans*-cyclo-hexylcarboxamide; (Lanier et al., 2012); 37 °C, 5% CO_2, 21% O_2). For adhesion culture EBs were transferred to Geltrex® coated vessels and evenly distributed (100 µl EBs/T75 flask). For suspension culture the EBs were left in the Pluronic® coated vessels. The attached or suspended colonies were cultured for three days with medium change only on day 2 and 3. For the next four days the cells were cultured in RPMI 1640 supplemented with 0.5% penicillin/streptomycin, 10 mM HEPES, 500 µM 1-thioglycerol (Sigma, M6145), 1 µM Y-27632, 2% B-27 supplement (Gibco, 17504-044), 100 nM DS-07 (37 °C, 5% CO_2, 21% O_2). Spontaneous beating usually occurred around day 9-11 of cardiac differentiation. For the following 4-5 days the beating colonies were cultured in the same medium composition without the addition of the WNT-inhibitor DS-07.

Flow cytometry (FACS) analysis

HiPSC-cardiomyocytes were dissociated to single-cell suspensions with collagenase 2 (200 U/ml, Worthington, LS004176 in HBSS minus Ca^{2+}/Mg^{2+}, Gibco, 14175-053) for 4 h at 37 °C. Cells were fixed with ice-cold methanol for 20 min at 4 °C and the cell membrane permeabilized in FACS buffer containing 0.5% saponine. Intracellular proteins were stained successively with primary and secondary antibodies in FACS buffer containing 0.5% saponine for 30 min at 4 °C. The following antibodies were used: alpha-actinin (Sigma-Aldrich, A7811, 1:800), MLC2a (Synaptic Systems, 311011, 1:100), MLC2v (Proteintech, 10906-1-AP, 1:100), troponin T (Abcam, ab45932, 1:800), Alexa Fluor® 488 anti-rabbit (Life technologies, A-11034, 1:800), Alexa Fluor® 488 anti-mouse (Life technologies, A-11001, 1:800). For negative control we used isotype antibodies (Figure S1B): anti-mouse IgG1 for alpha-actinin and troponin T and anti-mouse IgG2b for MLC2a. Samples were analyzed with a BD FACSCanto II Flow Cytometer and the BD FACSDiva Software 6.0.

Quantitative PCR

Samples were taken on day 0, between day 2 and day 16 every other day and on day 20 of differentiation. For each time point, four differentiation runs were analysed. RNA was isolated after proteinase K digestion with the RNeasy Kit (Qiagen) according to manufacturer's instructions. The conversion to cDNA was performed with the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems). QPCR was performed with SYBR-Green

(Fermentas) according to manufacturer's instructions in technical triplicates. Glucuronidase-beta (GUSB) was used as reference transcript for normalization. The target sequences were amplified during 40 cycles in an AbiPrism7000HT cycler.

Primer sequences (5´→ 3´):

Name	Gene	Primer (forward)	Primer (reverse)
Alpha MHC	MYH6	TTCATTGACTTTGGCATGGA	GGCTTCTGGAAATTGTTGGA
Beta MHC	MYH7	CACAGCTCTGTCCTGCTCTG	TTCTAGCCGCTCCTTCTCTG
Cardiac troponin T (total)	TNNT2	TTCGACCTGCAGGAGAAGTT	GAGCGAGGAGCAGATCTTTG
Glucoronidase beta	GUSB	AAACGATTGCAGGGTTTCAC	CTCTCGTCGGTGACTGTTCA
Brachyury homolog	T	GCAAAAGCTTTCCTTGATGC	ATGAGGATTTGCAGGTGGAC
Islet	ISL1	GTTACCAGCCACCTTGGAAA	GGACTGGCTACCATGCTGTT
Mesoderm posterior 1 homolog	MESP	GAAGTGGTTCCTTGGCAGAC	TCCTGCTTGCCTCAAAGTGT
Kinase insert domain receptor, VEGFR2	KDR/ FLK1	GCGATGGCCTCTTCTGTAAG	ACACGACTCCATGTTGGTCA
Oct4 POU class 5 homeobox 1	POU5F1	CGAAAGAGAAAGCGAACCAG	GCCGGTTACAGAACCACACT

Transcriptome analysis

For transcriptome analysis we isolated RNA from cardiomyocytes at the end of differentiation and matched cultures (21 days) of hiPSC-CM in either 2D or EHT format. Cells and EHTs were washed twice in PBS, EHTs stripped from the silicone racks, and homogenized by trituration through small steel cannulas (Braun Sterican; 21G then 27G) in lysis buffer (RNeasy® mini kit , Qiagen 74104) and digested with Proteinase K (Qiagen 19133; 56 °C, 10 min). RNA was isolated with the RNeasy® mini kit (Qiagen 74104) according to standard protocol. Array data (Illumina Human HT-12 v2) were processed on the Illumina GenomeStudio V2011.1 Platform (Gene Expression Module 1.9.0), two slides, five conditions with four to five biological replicates each (cardiomyocytes, n=4/3; cardiomyocytes 2D, n=5/3; cardiomyocytes EHT, n=6/3; NCBI GEO accession number GSE80390). The array data have been quantile normalized on probe level (47,310 probes) without background correction. Probes absent in all samples (minimum detection p-value >0.05) were rejected. The remaining set of 29,258 probes underwent parametric ANOVA statistic using Partek Genomic Suite 6.7 (Table S1). ANOVA was done by taking the batch differences into account. Multiple testing correction was performed using false discovery rate (FDR) statistic (Benjamini & Hochberg, 1995). Probes underwent 0.1% FDR (n=277) were further investigated with clustering and functional enrichment approaches. General differentiation of the cell-type specific expression profiles was performed using hierarchical clustering. The K-mean clustering with Euclidean distance function was done using standardized values. K=5 was selected according to the result of the Davies Bouldin K estimation procedure (data not shown here). Functional aspects of the differential expression was analyzed using the G:Profiler interface (Reimand et al., 2007) using g:SCS threshold as significance criterion.

Histological analysis

30-35 days old EHTs were fixed in formaldehyde (Roti®-Histofix 4%, Carl Roth, P087.3) overnight at 4 °C. After embedding in paraffin, 4 µm thick longitudinal or cross sections were processed for immunohistochemical staining (monoclonal mouse anti-MLC2v 1:200, Synaptic systems 310111; monoclonal mouse anti-dystrophin 1:200, Millipore MAB1645). For whole mount immunofluorescence staining, fixed EHTs were blocked (6 h in TBS 0.05 M pH 7.4, 10% FCS, 1% BSA, 0.5% Triton X-100), incubated in antibody solution (TBS 0.05 M pH 7.4, 1% BSA, 0.5% Triton X-100) with primary antibodies (monoclonal mouse anti-α-actinin 1:800, Sigma A7811; monoclonal

rabbit anti-MLC2v 1:200, Proteintec™, 10906-1-AP; polyclonal rabbit anti-caveolin-3 1:1000, Novus Biologicals NB110-5029; polyclonal rabbit anti-junctophilin-2 1:100, Santa Cruz sc-134875), washed repeatedly with PBS, incubated in antibody solution with secondary antibodies and other stainings (Alexa Fluor® 488 goat-anti-mouse 1:800, Invitrogen; Alexa Fluor® 488 goat-anti-rabbit 1:800, Invitrogen; Alexa Fluor® 546 goat-anti-rabbit 1:200; DRAQ5™ 1:1000, Biostatus Ltd. BOS-889-001-R050; Phalloidin Alexa Fluor® 488 1:60; Invitrogen A-12379), rinsed 3-4 times in PBS and embedded in Fluoromount-G® (SouthernBiotech, 0100-01) in dented microscope slides (Carl Roth, H884.1). 2D cultures were cultivated on coated (0.1% gelatin) glas cover slips and handled accordingly.

Transmission electron microscopy

For transmission electron microscopy (TEM), EHTs were washed twice in PBS and incubated in 2-butandionemonoxime (Sigma, B0753; 30 mM in PBS, 10 min, 37 °C) to relax sarcomeres and fixed overnight in glutaraldehyde (0.36%, pH 7.0-7.5, 4 °C). Fixed EHTs were removed from silicone racks and subjected to post-fixation in osmium tetroxide solution (1%, 2 h; Science Services, 19110), dehydration and embedding in a glycidether-based resin. Ultra-thin sections (50 nm) were prepared and analyzed on a Zeiss LEO 912AB.

Western blot

21 days old hiPSC-EHTs were frozen in liquid nitrogen and stored at -80 °C. 70 µl of 1x M-PER™ Mammalian Protein Extraction Reagent (Thermo Scientific) supplemented with protease and phosphatase inhibitor (Roche) was used for the lysis per EHT. Age-matched hiPSC-CM 2D cultures or samples of non-failing human heart (NFH) were handled accordingly. After homogenization, 1x Laemmli buffer was added to the samples before heating at 95 °C for 5 minutes. SDS polyacrylamide gels (8-15%) were loaded with 4 µl of NFH lysate, 10 µl of 2D lysate and 20 µl of EHT lysate per lane. After separation, proteins were blotted onto PVDF or nitrocellulose membranes using the wet blot method. Membranes were cut, blocked in 5-10% low fat milk powder solution in TBS-Tween 0.1% and incubated over night at 4 °C with primary antibodies against ERK (1:1000, mAB#4695/Cell Signaling), L-type calcium channel (1:100, ACC-013/Alomone), Na^+/Ca^{2+} exchanger-1 (1:500, ab6495/abcam), Na^+/K^+-ATPase (1:500, #05-369/Millipore), total phospholamban (1:5000, A010-14/Badrilla), Serca2 ATPase (1:1000, MA3-919/ Thermo Scientific), Na^+/H^+ exchanger-1 (1:500, sc-28758/SantaCruz). After washing, membranes were incubated either with anti-rabbit IgG peroxidase-conjugated secondary antibody (1:5000, A0545/Sigma) or with anti-mouse IgG peroxidase-conjugated secondary antibody (1:5000, A3682/Sigma) in 5% low fat milk powder solution in TBS-Tween 0.1% for one hour at room temperature. Pierce® ECL Western Blotting Substrate (Thermo Scientific) was used for the visualization of the bands (Figure S2).

Contractile analysis of human EHTs with increase in preload

Inotropic responses to increase in preload (Frank-Starling law) of hiPSC-EHTs were determined under electrical stimulation (1-2 Hz) and perfusion in a temperature controlled (37 °C) organ bath filled with Tyrode's solution (see above, without HEPES, plus 0.3 mM ascorbic acid). Data collection and evaluation were performed with a custom-made software (BMON, G. Jäckel, Hanau) as previously described (Zimmermann et al., 2000). Preload was increased in 40 µm intervals until the force reached a plateau.

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