SUPPLEMENTARY MATERIALS AND METHODS

hPSC lines culture

Previously described NKX2-5$^{eGFP/w}$ hESCs and wild-type hiPSCs (Elliott et al., 2011; Zhang et al., 2014) were seeded on Vitronectin Recombinant Human Protein (Life technologies) and cultured in E8 medium (Life Technologies). Cells were passaged twice a week using PBS (Life Technologies) containing EDTA 0.5mM (Life Technologies). RevitaCell Supplement (Life Technologies; 1:200) was added during hiPSC passaging.

Differentiation into endothelial cells and cardiomyocytes

Cardiac differentiation was induced in a monolayer as described previously (Elliott et al., 2011; van den Berg et al., 2015). Briefly, for CM condition, 25 × 10^3/cm^2 were seeded on plates coated with 75 µg/mL (growth factor reduced) Matrigel (Corning) the day before differentiation (day -1). At day 0, cardiac mesoderm was induced by changing E8 to BPEL medium (Bovine Serum Albumin [BSA] Polyvinyl alcohol Essential Lipids; (Ng et al., 2008)), supplemented with a mixture of cytokines (20 ng/mL BMP4, R&D Systems; 20 ng/mL ACTIVIN A, Miltenyi Biotec; 1.5 µM GSK3 inhibitor CHIR99021, Axon Medchem). After 3 days, cytokines were removed and a Wnt inhibitor (5 µM, XAV939, Tocris Bioscience) was added for 3 days. BPEL medium was refreshed every 3–4 days. Alternatively, for EC and CMEC conditions, 12.5 × 10^3 cells/cm^2 were seeded on matrigel at day -1. At day 0, cardiac mesoderm was induced as described above. At day 3, cytokines were removed and VEGF (50 ng/ml, R&D Systems) alone (EC condition) or in combination with XAV939 (5 µM) (CMEC condition) was added. BPEL medium supplemented with VEGF was refreshed every 3–4 days.

FACS analysis

Staining was done in PBS containing 0.5% BSA (Sigma Aldrich) and 2 mM EDTA. Antibodies were used as follows: anti-VCAM1-PE (R&D); anti-CD34-APC (Miltenyi Biotech), anti-KDR-PE (R&D), anti-VEC-PECY7 (eBioscience), anti-CD31-APC (eBioscience), anti-CXCR4-PE (BD Biosciences); MACS Comp Bead kit antimouse IgK (Miltenyi Biotech). Samples were measured with a MACSQuant VYB (Miltenyi Biotech) equipped with a violet (405 nm), blue (488 nm) and yellow (561 nm) laser. In order to allow direct comparisons between different experimental groups, equal population gates were applied. Details of antibodies used are provided in Supplementary Table S2.

Isolation of CD34$^+$ endothelial cells

CMEC population was detached on day 6 using TrypLE 1X for 5 min at 37°C, 5% CO_2, centrifuged for 3 min at 1100 rpm, washed and re-suspended in 1 ml of EasySep buffer (PBS containing 2% FCS [Life Technologies] and 1mM EDTA) into a 5mL round-bottomed tube. Before isolation, a small aliquot was taken for anti-CD34-APC antibody staining and FACS

analysis (pre-isolation fraction). $CD34^+$ cells were isolated using a Human cord blood CD34 Positive selection kit II (Stem Cell Technologies) following the manufacturer's instructions. After isolation, an aliquot of post-isolation fraction was taken for anti-CD34-APC antibody staining and FACS analysis. $CD34^+$ cells were resuspended in BPEL medium and counted. For $CD34^+$ culture, $10 \times 10^3/cm^2$ cells were seeded on Fibronectin (Fibronectin from bovine plasma 2-5µg/ml; Sigma Aldrich) and cultured in BPEL medium supplemented with VEGF (50ng/ml). After 3-4 days, cells were confluent and cryopreserved ($30cm^2$/vial) in CryoStor® CS10 medium (0.5ml/vial; Stem Cell Technologies) or dissociated for MT formation.

Isolation of $VCAM1^+$ cardiomyocytes

CM population was detached on day 14-17 using TrypLE 2X for 5 min at 37°C, 5% CO_2, centrifuged for 3 min at 1100 rpm, washed and re-suspended in 1 ml of EasySep buffer into a 5mL round-bottom tube. Cell suspension was stained for 30 min at 4C with an anti-VCAM1-PE antibody described previously. After 30 min, a small aliquot was taken for FACS analysis (pre-isolation fraction). $VCAM1^+$ cells were isolated by using a Human PE Selection kit (Stem Cell Technologies) following the manufacturer instructions. After isolation, a small aliquot of post-isolation fraction was taken for FACS analysis. $VCAM1^+$ cells were resuspended in BPEL medium, counted and used for electrophysiology, immunofluorescence or MT formation.

Generation and cultivation of cardiac microtissues

To generate MTs from isolated $VCAM1^+$ cardiomyocytes, CM population was stained with anti-VCAM1-PE antibody and isolated as described above. Alternatively, CM population was dissociated using TrypLE 2X for 5 min at 37°C, 5% CO_2 (non-enriched $VCAM1^+$ cardiomyocytes). Endothelial cells were prepared as follow: briefly, 1 to 3 days before MT formation, a vial of cryopreserved endothelial cells was thawed and cultured in BPEL medium supplemented with VEGF (50 ng/ml) on Fibronectin-coated plates (Fibronectin from bovine plasma 2-5µg/ml; Sigma Aldrich). The day of MT formation (day 0), endothelial cells were detached using TrypLE 1X for 5 min at 37 °C, 5% CO_2, centrifuged for 3 min at 1100 rpm and resuspended in BPEL medium. For MT-CM: cardiomyocytes were diluted to 5000 cells per 50 µl BPEL medium. For MT-CMEC: cell suspensions were combined together to 5000 cells per 50 µl BPEL medium supplemented with 50 ng/ml VEGF. For both MT-CM and MT-CMEC, cell suspensions were seeded on V-bottom 96 well microplates (Greiner bio-one) and centrifuged for 10 min at 1100 rpm. MTs were incubated at 37°C, 5% CO2 for 7-20 days with media refreshed every 3 days. Analysis of MTs was performed after 7-20 days in culture.

Immunofluorescence analysis

For immunostaining of $VCAM1^+$ cardiomyocytes, approximately $200 \times 10^3/cm^2$ cells were seeded on 75 µg/mL Matrigel-coated 13 mm plastic coverslips (Sarstedt) and fixed for 20 min in 4% paraformaldehyde, permeabilized for 10 min with PBS containing 0.1% Triton-X 100 (Sigma-Aldrich) and blocked for 1h with PBS containing 5% (vol/vol) FCS and 5% goat serum

(Vector Laboratories). Samples were incubated overnight at 4°C with TNNI (Santa Cruz) and α-ACTININ (Sigma–Aldrich) antibodies. Primary antibodies were detected with Cy3- (Dianova) and Alexa Fluor 488- (Invitrogen) conjugated donkey secondary antibodies, for 1h at room temperature. Cells were washed three times with PBS, each time incubated for 20 min and stained with DAPI (Life Technologies) for 30 min at room temperature. Stained cells were mounted onto microscope slides with ProLong Gold antifade Mountant with DAPI (Life Technologies). Images were captured using Leica Microsystems LAS AF6000. Details of antibodies used are provided in Supplementary Table S2.

For whole mount microtissue immunofluorescence staining, MTs were washed in PBS on day 7 and fixed for 30 min with 4% paraformaldehyde, washed 3 times in PBS and stored at 4 °C until processing. MTs were permeabilized for 20 min with PBS containing 0.2% Triton X-100 and blocked for 2 h in PBS containing 5% FCS and 5% goat serum. All incubations were done at room temperature. Samples were then incubated overnight at 4°C with CD31 (Dako) and TNNI primary antibodies. MTs were washed 3 times with PBS at room temperature, each time incubated for 10 min. Secondary antibodies (Cy3 and Alexa Fluor 488) were added overnight at 4°C. The following day, MTs were washed 3 times with PBS at room temperature, each time incubated for 20 min and then stained with DAPI for 30 min at room temperature. MTs were mounted onto microscope slides with ProLong Gold antifade Mountant with DAPI. Images were captured using a Leica SP8WLL confocal laser-scanning microscope. Details of antibodies used are provided in Supplementary Table S2.

Patch Clamp

Electrical signals were recorded with an Axopatch 200B Amplifier (Molecular Devices) and digitized with a Digidata 1440A (Molecular Devices) connected to an x86 Windows PC running pClamp 10.4. All measurements were performed at 37 °C. Data were analyzed with ClampFit 10.4 (Molecular Devices) and Prism 7.0a (Graphpad Software) for Mac. Current-clamp experiments were performed in the perforated patch configuration. Cells were perfused with Tyrode's solution containing (mM): 154 NaCl, 5.4 KCl, 1.8 $CaCl_2$, 1 $MgCl_2$, 5 HEPES-NaOH, 5.5 D-Glucose; pH was adjusted to 7.35 with NaOH. Glass capillaries (2-3.5 MΩ) were filled with an intracellular solution containing (mM): 125 K-Gluconate, 20 KCl, 10 NaCl, 10 HEPES; pH was adjusted to 7.2 with KOH. Amphotericin B (Sigma Aldrich) was dissolved in DMSO just before the experiments and added to the intracellular solution to reach a final concentration of 0.22 mM.

Multielectrode array (MEA)

MEA experiments were performed using a 64 electrodes USB-MEA system (Multichannel Systems). All the experiments were performed at 37 °C in BPEL medium. MEA chambers were coated with human Fibronectin (40 µg/ml, Alfa Aesar) before MT seeding. Acute dose–response curves were generated by adding aliquots at fixed 1:100 dilutions every 10 min

(Navarrete et al., 2013). Traces were analyzed with a custom-made protocol to quantify both QT and RR intervals.

Contraction analysis

Movies of paced MTs were acquired with a ThorLabs DCC3240M camera at 100 frames per second with the ThorLabs uc480 software (v 4.20). Contraction and contraction velocity profiles were obtained by analysing movies with a custom-made ImageJ macro (ImageJ v. 2.0.0-rc-49).

Gene expression analysis

For RT–qPCR, total RNA was purified using the RNeasy Mini Kit (Qiagen) according to the manufacturer's protocol. 1µg of RNA was reverse transcribed by using the iScript-cDNA Synthesis kit (Bio-Rad). Expression profiles of genes of interest were determined by qPCR using 6ng/µl of cDNA and the iTaq Universal SYBR Green Supermixes (Bio-Rad). Gene expression was assessed by a Bio-Rad CFX384 real time system. The expression of two reference genes *(RPL37A* and *HARP*) was stable in our samples and not affected by experimental conditions, therefore only *RPL37A* was used for normalization. Data were analyzed by using the ΔΔCt method (Livak and Schmittgen, 2001; Pfaffl, 2001). Further normalization to TNNT2 or *VEC* is specified in figure legends. Primer sequences are provided in Supplementary Table S1.

Cell pellets of primary Human Umbilical Artery Endothelial Cell (HUAEC), Human Umbilical Vein Endothelial Cell (HUVEC), Human Dermal Blood Endothelial Cell (HDBEC) and Human Cardiac Microvascular Endothelial Cell (HCMEC) from PromoCell were used to extract RNA as described above.

Statistics

Ordinary one-way, two-way ANOVA or Mann-Whitney test for paired or unpaired measurements were applied as appropriate to test for differences in means between groups/conditions. Post hoc comparison between individual means or medians was performed by Tukey's method, and P-values have been corrected for multiple testing using the Holm–Sidak or Dunn's method. Detailed statistics are indicated in each figure legend. Data are expressed and plotted as the Mean ± SEM. Statistical significance was defined as $P < 0.05$. Statistical analysis was performed with GraphPad 7.0b for Mac.

REFERENCES

Elliott, D. A., Braam, S. R., Koutsis, K., Ng, E. S., Jenny, R., Lagerqvist, E. L., Biben, C., Hatzistavrou, T., Hirst, C. E., Yu, Q. C., et al. (2011). NKX2-5eGFP/w hESCs for isolation of human cardiac progenitors and cardiomyocytes. *Nat Meth* **8**, 1037–1040.

Livak, K. J. and Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods* **25**, 402–408.

Navarrete, E. G., Liang, P., Lan, F., Sanchez-Freire, V., Simmons, C., Gong, T., Sharma, A., Burridge, P. W., Patlolla, B., Lee, A. S., et al. (2013). Screening Drug-Induced Arrhythmia Using Human Induced Pluripotent Stem Cell-Derived Cardiomyocytes and Low-Impedance Microelectrode Arrays. *Circulation* **128**, S3–S13.

Ng, E. S., Davis, R., Stanley, E. G. and Elefanty, A. G. (2008). A protocol describing the use of a recombinant protein-based, animal product-free medium (APEL) for human embryonic stem cell differentiation as spin embryoid bodies. *Nat Protoc* **3**, 768–776.

Pfaffl, M. W. (2001). A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic Acids Research* **29**, 45e–45.

van den Berg, C. W., Elliott, D. A., Braam, S. R., Mummery, C. L. and Davis, R. P. (2015). Differentiation of Human Pluripotent Stem Cells to Cardiomyocytes Under Defined Conditions. In *Patient-Specific Induced Pluripotent Stem Cell Models*, pp. 163–180. New York, NY: Springer New York.

Zhang, M., D'Aniello, C., Verkerk, A. O., Wrobel, E., Frank, S., Ward-van Oostwaard, D., Piccini, I., Freund, C., Rao, J., Seebohm, G., et al. (2014). Recessive cardiac phenotypes in induced pluripotent stem cell models of Jervell and Lange-Nielsen syndrome: Disease mechanisms and pharmacological rescue. *Proc Natl Acad Sci USA* **111**, E5383–E5392.

Table S1. Primer sequences for qRT-PCR.

Primer	Forward	Reverse
RPL37A	GTGGTTCCTGCATGAAGACAGTG	TTCTGATGGCGGACTTTACCG
MESP1	GTGCTGGCTCTGTTGGAGA	CAGAGACGGCGTCAGTTGT
TBX5	GGGCAGTGATGACATGGAG	GCTGCTGAAAGGACTGTGGT
ISL1	AAAGTTACCAGCCACCTTGGA	ATTAGAGCCCGGTCCTCCTT
NKX2.5	CAAGTGTGCGTCTGCCTTT	TTGTCCGCCTCTGTCTTCTC
TNNT2	AGCATCTATAACTTGGAGGCAGAG	TGGAGACTTTCTGGTTATCGTTG
ETV2	CAGCTCTCACCGTTTGCTC	AGGAACTGCCACAGCTGAAT
KDR	CCATCTCAATGTGGTCAACCTTCT	TCCTCAGGTAAGTGGACAGGTTTC
VEC	GGCATCATCAAGCCCATGAA	TCATGTATCGGAGGTCGATGGT
CD31	GCATCGTGGTCAACATAACAGAA	GATGGAGCAGGACAGGTTCAG
MEOX2	CCAAGGATGCACAGTCTGG	AGGAGGAAAACCTTCGTGCTG
GATA4	GACAATCTGGTTAGGGGAAGC	GAGAGATGCAGTGTGCTCGT
GATA6	TCCAACTTCCACCTCTTCTAAC	TCTCCCGCACCAGTCATC
MYL2	TACGTTCGGGAAATGCTGAC	TTCTCCGTGGGTGATGATG
MYL7	CCGTCTTCCTCACGCTCTT	TGAACTCATCCTTGTTCACCAC
MYL4	AAGCCTTTGTCAAGCACATCA	AGGACTCCATCTCAGCTCACC
MYL3	AAGGAGGTCGAGTTTGATGCT	TCCTTGAACTCTTCAATCTGCTC
MYH6	CCAGGTCAACAAGCTTCGAG	TGTCACTCCTCATCGTGCAT
MYH7	AGTCCCAGGTCAACAAGCTG	GGGCTGAGCAGATCAAGATG
TNNI1	GTGGGTGACTGGAGGAAGAA	GTGAGCTGGGTTGGAGAAGA
TNNI3	CACCTCAAGCAGGTGAAGAAG	CAGGAAGGCTCAGCTCTCAA
ACTN2	GATGGAGCACATTCGTGTTG	TGATCCATCAGGCCATTCTT
TCAP	GGCAGAATGGAAGGATCTGAC	TGTCTCTGGGTGTCCTCCTC
SCN5A	GAGCTCTGTCACGATTTGAGG	GAAGATGAGGCAGACGAGGA
CACNA1C	CAATCTCCGAAGAGGGGTTT	TCGCTTCAGACATTCCAGGT
KCNQ1	TCCTGGTCTGCCTCATCTTC	AAGAACACCACCAGCACGAT
KCNE1	TCTCTGGCCAGTTTCACACA	CTCAAACTTCCCAGGCACAC
KCNJ12	TGGATCCTTTCCAGTTGGTG	CGGCTCCTCTTGAGTTCTATCTT
KCNJ2	CGCTTTTTACAAACCACTGGA	TGGGAGCCTTGTGGTTCTAC
HCN4	CAATGAGGTGCTGGAGGAGT	GGTCGTGCTGGACTTTGTG
NCX1	ACATCTGGAGCTCGAGGAAA	CTGGAATTCGAGCTCTCCAC
SERCA2A	ACAATGGCGCTCTCTGTTCT	ATCCTCAGCAAGGACTGGTTT
PLN	TCCCATAAACTGGGTGACAGA	TGATACCAGCAGGACAGGAAG
RYR2	GCTATTCTGCACACGGTCATT	ATTTCCGTGCCACTTCCTTT
CASQ2	CCGGGACAATACTGACAACC	CTTCTCCCAGTAGGCAACGA
S100A1	CTGAGCAAGAAGGAGCTGAAAG	ACCTTGTCCACAGCATCCAC

TRDN	GTGTCTCCCACAAAGCAGAAA	GGTCTGCAGGAGTGAAAGGA
NPPA	TGATCGATCTGCCCTCCTAA	TCCTCCCTGGCTGTTATCTTC
NPPB	GCTTTGGGAGGAAGATGGAC	TGTGGAATCAGAAGCAGGTGT
ACTA1	AAGAGCTACGAGCTGCCAGA	ACAGGTCCTTCCTGATGTCG
CKMT2	CAAGGACCCACGCTTTTCTA	TCCACCAGGTAATTGACTCCA
ITGA7	CATCCTCCTGGCTGTACTGG	GGAATCTTCACCGCATGGTA
COUPTFII	GCTTTCCACATGGGCTACAT	CAAGTGGAGAAGCTCAAGGC
FOXC2	GAGCCGTCTCGGAAGCAG	CCGCAGCCCGGTAGTAATTC
NRP1	AACACCAACCCCACAGATG	AAGTTGCAGGCTTGATTCG
NRP2	CTGGAAGCAGCATTGTGTG	TAACTCGCTGATGGGGAGA
HEY2	TCATGAAGTCCATGGCAAGA	TTGTGCCAACTGCTTTTGAA
NFATC1	GCCCCTATTCCTGTAACGGT	ATGTGGCAACTAGGAGTGGG
CX40	AATCAGTGCCTGGAGAATGG	CGAACCTGGATGAAACCTTC
β_1*AR*	AAGAGAAAGGATGGAGGCAAA	GCCCTACACAAGGAAAGCAA
β_2*AR*	TGGTGATCATGGTCTTCGTCT	TCCACCTGGCTAAGGTTCTG

Table S2. Antibodies used for FACS and immunofluorescence analyses.

	Antibody	**Company**	**Catalogue number**	**Dilution**
FACS	Anti-VCAM1-PE	R&D	FAB5649P	1:20
	Anti-CD34-APC	Miltenyi Biotech	130-090-954	1:20
	Anti-KDR-PE	R&D	FAB357P	1:20
	Anti-VEC-PECY7	eBioscience	25-1449-42	1:100
	Anti-CXCR4-PE	BD Pharmigen	555974	1:20
	Anti-CD31-APC	eBioscience	17-0319-42	1:100
	Mouse IgG2a-APC	Miltenyi Biotech	130-098-850	1:20
	Mouse IgG2A PE	R&D	IC003P	1:20
	Mouse IgG1 PE	R&D	IC002P	1:20
	Mouse IgG1 K APC	eBioscience	17-4714-41	1:100
Immunofluorescence	TNNI	Santa Cruz	Sc-15368	1:500
	α-ACTININ	Sigma–Aldrich	A7811	1:800
	CD31	Dako	M0823	1:200
	Cy3	Dianova	715-165-150	1:100
	AF488	Invitrogen	A21206	1:200

Study	Cardiac tissue given name	Number of cells per cardiac tissue	CM source	EC source	CM culture medium	EC culture medium	Cardiac tissue medium (in the presence of ECs)	Substrate/scaffold	Assays performed	Purpose of the study
Narmoneva et al., 2004	Three-Dimensional culture	0.7 x 10^6 cells/cm2 (CMs alone) or 1.4 x 10^6 cells/cm2 (coculture)	Neonatal mouse CMs (1-2 days old)	Mouse ECs from heart and lungs (6-8 weeks old)	DMEM + 7% fetal calf serum	DMEM + 20% fetal calf serum + porcine heparin + endothelial cell growth stimulant	DMEM + 10% fetal calf serum	1% peptide hydrogel scaffolds	Immunofluorescence; cell death assay; evaluation of contractile areas	Transplantation
Caspi et al., 2007	Engineered cardiac tissue	4 x 10^5 cells per tissue	hESCs	hESCs or HUVEC	Knockout DMEM + 20% fetal bovine serum	hESC-ECs: Endothelial cell growth medium HUVEC: EGM-2 medium + 2% FBS	50% EGM-2 and 50% standard ES cell culture medium	Porous sponges composed of 50% poly-L-lactic acid (PLLA) and 50% polylactic-glycolic acid (PLGA); Matrigel	Immunofluorescence; cell viability assay; laser scanning confocal Ca^{2+} imaging	Transplantation
Stevens et al., 2009	Cardiac tissue patch	3 x 10^6 cells per patch	hESCs	hESCs or HUVEC	RPMI + B27	hESC-ECs: huEB medium (80% KO-DMEM + 20% FBS) + VEGF g 50 ng/mL HUVEC: EGM-2 medium	huEB medium or huEB medium + M199 medium or RPMI-B27 medium	X	Immunofluorescence; Passive mechanical measurements; *in vivo* transplantation into skeletal muscle; engraftment in heart	Transplantation
Tulloch et al., 2011	Engineered Myocardium/ cardiac muscle	2 x 10^6 CMs or 2 x 10^6 CMs + 1 x 10^6 HUVEC	hESCs or hiPSCs	HUVEC	RPMI + B27 followed by 80% Knockout-DMEM + 20% fetal bovine serum	EBM2	80% Knockout-DMEM and 20% fetal bovine serum	Collagen type I, 11% mouse basement membrane extract	Immunofluorescence; transmission electron microscopy; gene expression profile; cardiac engraftment	Transplantation
Ravenscroft et al., 2016	Cardiac Microtissue	500 cells per 100 µl	hESCs or hiPSCs	Primary human coronary artery endothelial cells (HCAEC)	hESC-CM: RPMI + B27 hiPSC-CM: iCell CM maintenance media	Endothelial basal medium MV2 + 5% FCS + supplements(EGF, bFGF, IGF, VEGF, Ascorbic Acid, Hydrocortisone)	50% RPMI + B27 (or iCell CM medium) + 50% MV2 medium + supplements	X	Immunofluorescence; gene expression profile; video-based edge detection of contraction; Ca^{2+} transient measurements	Drug toxicity tests

Masumoto et al., 2016	Engineered cardiac tissue	3 x 10^6 cells per tissue	hiPSCs	hiPSCs	RPMI+ B27	RPMI+ B27 + VEGF	High glucose-modified Dulbecco's essential medium + 20% fetal bovine serum	Acid-soluble rat-tail collagen type I neutralized with alkali Buffer; Matrigel	Contractile force measurements; implantation in rats; next-generation RNA sequencing	Transplantation
Mannhardt et al., 2016	Engineered heart tissue	1 x 10^6 cells per tissue	hiPSCs	X	DMEM/F-12 followed by RPMI	X	DMEM + 10% horse serum + 10 µg/ml insulin + 33 µg/ml aprotinin (No endothelial cells)	Agarose and custom-made Teflon spacers casting molds with solid silicone racks; Matrigel	Contraction analysis	Transplantation
Huebsch et al., 2016	Micro-Heart muscle	2 x 10^3 cells per tissue (+ 2 x 10^3 stromal cells)	hiPSCs	X	RPMI+ B27	X	Knockout Dulbecco's Modified Eagle Medium + 20% fetal bovine serum following RPMI+ B27 medium	PDMS stencils	Immunofluorescence; Scanning Electron Microscopy; Video Microscopy based drug response studies	Drug response analysis
Present study	Cardiac microtissue	5 x 10^3 cells per tissue (5000 cells per 50 µl)	hESCs or hiPSCs	hESC or hiPSC	BPEL	BPEL + VEGF	BPEL + VEGF	X	Immunofluorescence; in depth gene expression profile; MEA analysis; contraction analysis	Cardiac disease modeling Drug screening Drug discovery

TABLE S3. Comparison between existing cardiac tissue models.

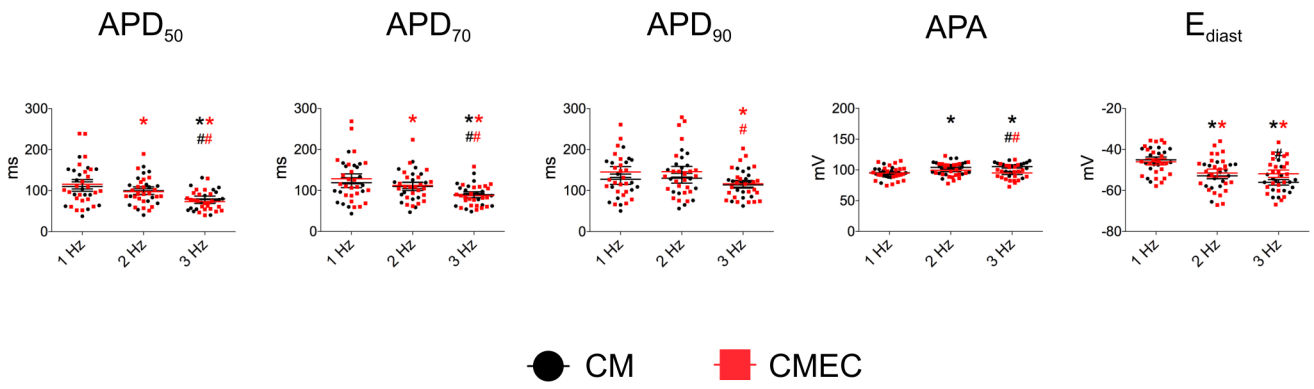


FIGURE S1

Figure S1. Rate dependency of cardiomyocytes differentiated upon CM and CMEC condition. Electrophysiological analysis of day 21 *NKX2-5*$^{eGFP/w}$ hESC cardiomyocytes differentiated under CM (black) and CMEC (red) conditions. * = $p < 0.05$ vs. 1 Hz. # = $p < 0.05$ vs. 2 Hz. Data were analyzed with repeated measurement. Two-way ANOVA with Sidak's multiple comparisons test. N = 16-24 from three independent differentiation experiments.

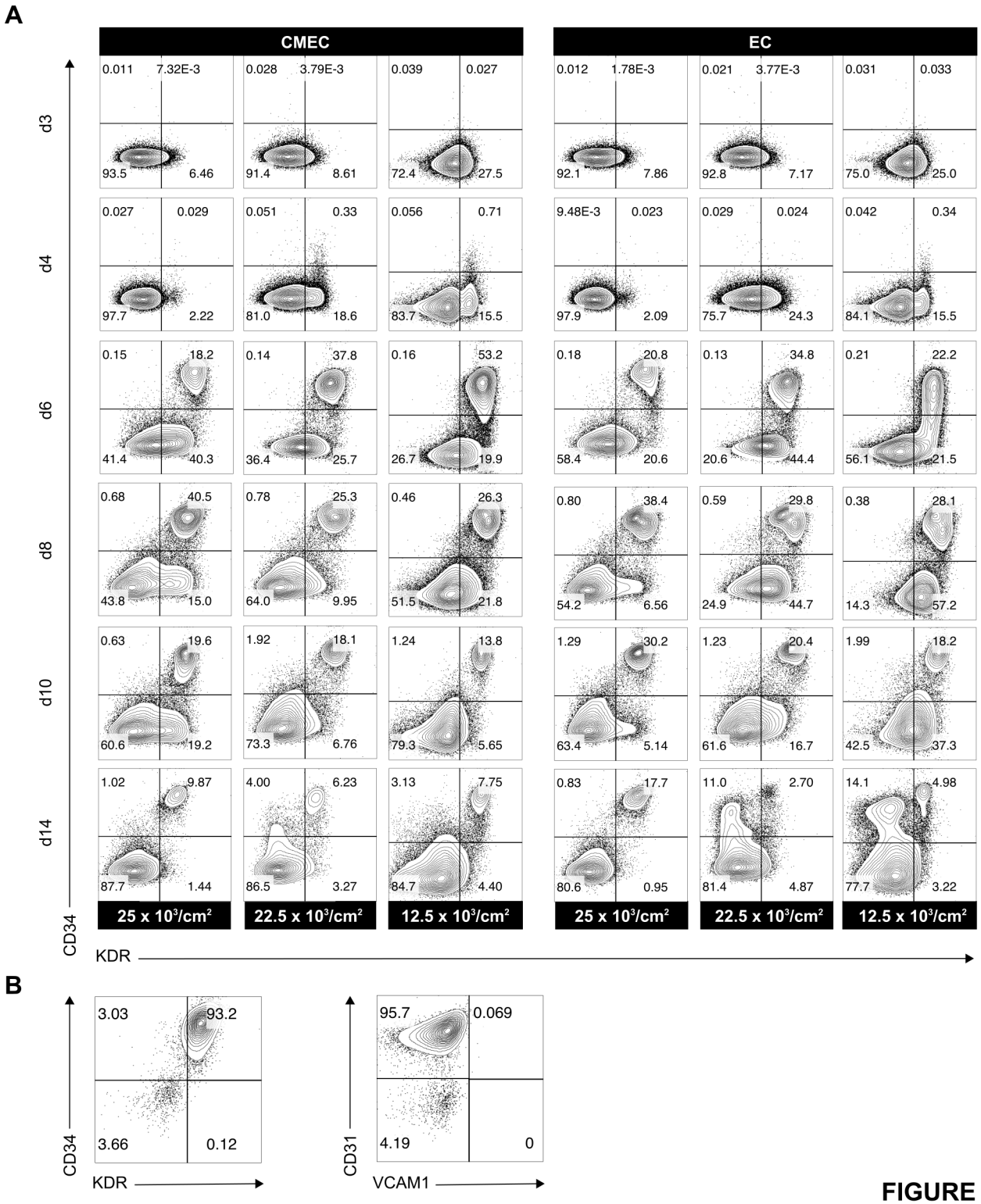


FIGURE S2

Figure S2. Isolation of CD34^{+} endothelial cells. (A) FACS plots for CD34 together with KDR of CMEC and EC populations measured in the NKX2.5$^{eGFP/w}$ hESCs at the indicated time points (d=days) of differentiation at different cell-seeding densities. Numbers in the quadrants represent the respective percentage of cells. N = 1. (B) FACS plots for CD34 together with KDR (left panel) and for CD31 together with VCAM1 (right panel) of enriched CD34^{+} endothelial cells measured in the NKX2.5$^{eGFP/w}$ hESCs after cryopreservation and re-plating. Numbers in the quadrants represent the respective percentage of cells. N = 1.

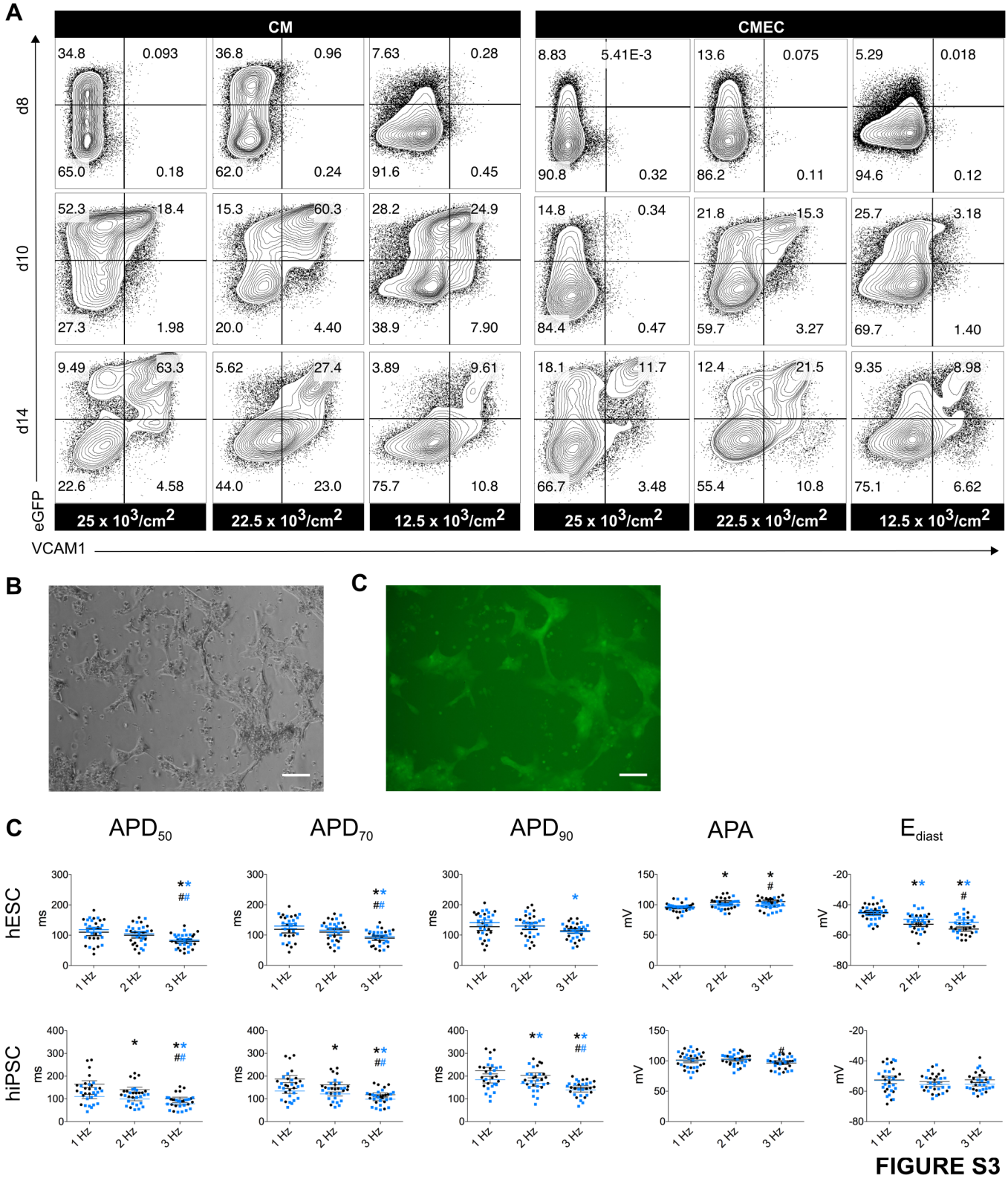


FIGURE S3

Figure S3. Isolation of VCAM1$^+$ cardiomyocytes. (A) FACS plots for VCAM1 together with eGFP of CM and CMEC populations measured in the NKX2.5$^{eGFP/w}$ hESCs at the indicated time points (d=days) of differentiation at different cell-seeding densities. Numbers in the quadrants represent the respective percentage of cells. N = 1. (B) Representative bright field and (C) GFP fluorescence images of the morphological appearance of CM-derived VCAM1$^+$ cardiomyocytes from NKX2.5$^{eGFP/w}$ hESCs after isolation and re-plating. Scale bar: 100 µm.
(D) Rate dependency of non-enriched (black) and enriched VCAM1$^+$ (blue) cardiomyocytes. Action potential parameters from cardiomyocytes differentiated from *NKX2-5*$^{eGFP/w}$ and hiPSCs. * = $p < 0.05$ vs. 1 Hz. # = $p < 0.05$ vs. 2 Hz. Data were analyzed with repeated measurement Two-way ANOVA with Sidak's multiple comparisons test. N = 16-16 for hESCs and N=15-18 for hiPSCs, each from three independent differentiation experiments.

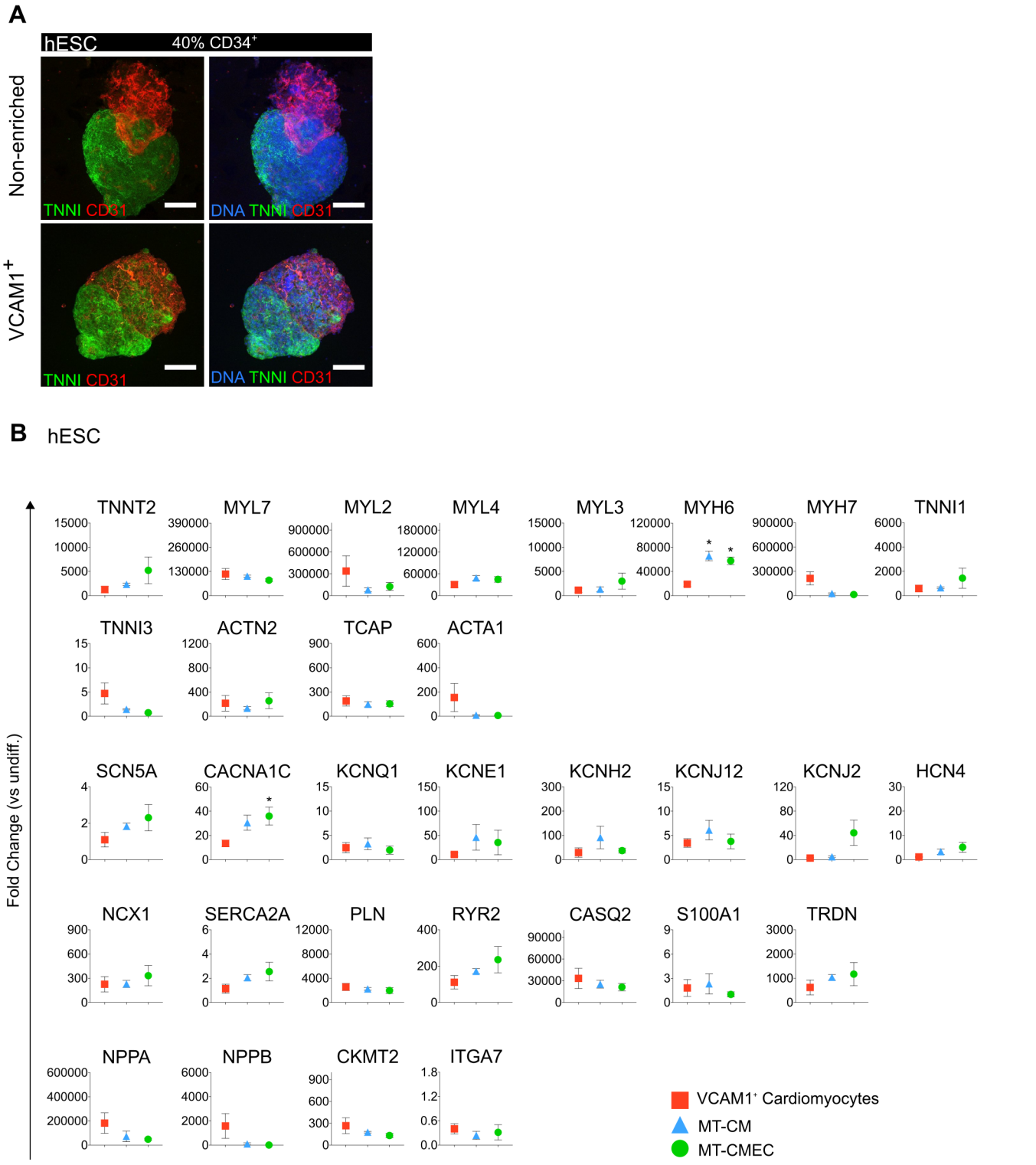
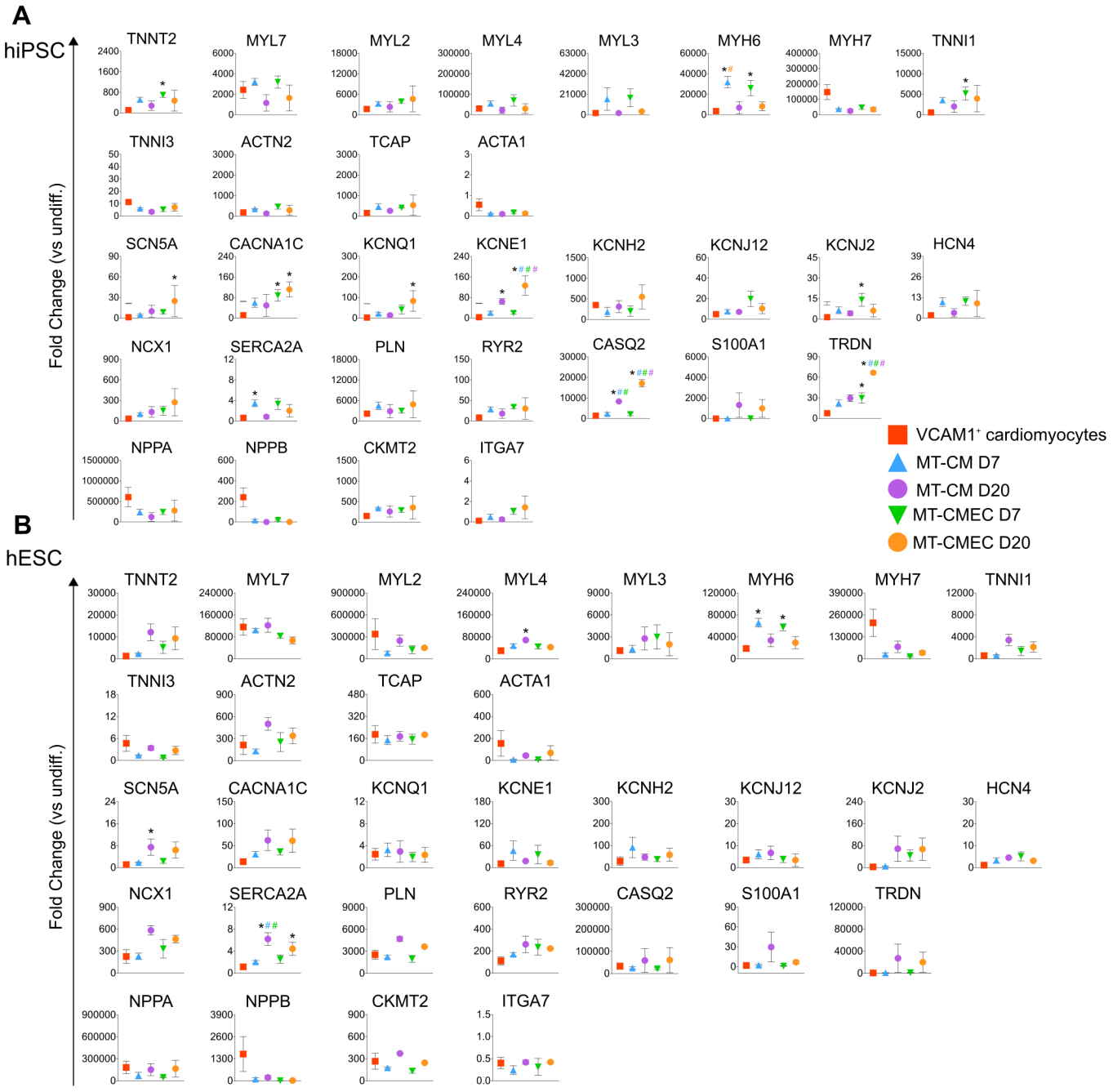


FIGURE S4

Figure S4. Characterization of day 7-MTs from hESCs. (A) Immunofluorescence analysis of sarcomeric cardiomyocyte TNNI (green) and endothelial cell surface marker CD31 (red) of cardiac MTs from non-enriched (upper panel) and enriched VCAM1$^+$ (lower panel) cardiomyocytes. Immunofluorescence data refer to day 7-MTs generated from *NKX2-5*$^{eGFP/w}$ hESCs. Percentages of CD34$^+$ cells are shown at the top. Nuclei are stained in blue with DAPI. Scale bar: 100 μm. (B) qRT-PCR analysis for key sarcomeric genes, ion channels and calcium-handling genes, as well as other cardiac genes of interest on day 7 MT-CM, MT-CMEC and on day 21-age-matched VCAM1$^+$ cardiomyocytes from hESCs. All values are normalized to *RPL37A* and are relative to undifferentiated hESCs. Ordinary one-way ANOVA with Tukey's multiple comparisons test. * = $P < 0.05$ vs. VCAM1$^+$ cardiomyocytes. $N \geq 3$. Data are shown as mean ± SEM.


A
hiPSC
Fold Change (vs undiff.)
TNNT2
MYL7
MYL2
MYL4
MYL3
MYH6
MYH7
TNNI1
TNNI3
ACTN2
TCAP
ACTA1
SCN5A
CACNA1C
KCNQ1
KCNE1
KCNH2
KCNJ12
KCNJ2
HCN4
NCX1
SERCA2A
PLN
RYR2
CASQ2
S100A1
TRDN
NPPA
NPPB
CKMT2
ITGA7
VCAM1+ cardiomyocytes
MT-CM D7
MT-CM D20
MT-CMEC D7
MT-CMEC D20
B
hESC
Fold Change (vs undiff.)
TNNT2
MYL7
MYL2
MYL4
MYL3
MYH6
MYH7
TNNI1
TNNI3
ACTN2
TCAP
ACTA1
SCN5A
CACNA1C
KCNQ1
KCNE1
KCNH2
KCNJ12
KCNJ2
HCN4
NCX1
SERCA2A
PLN
RYR2
CASQ2
S100A1
TRDN
NPPA
NPPB
CKMT2
ITGA7

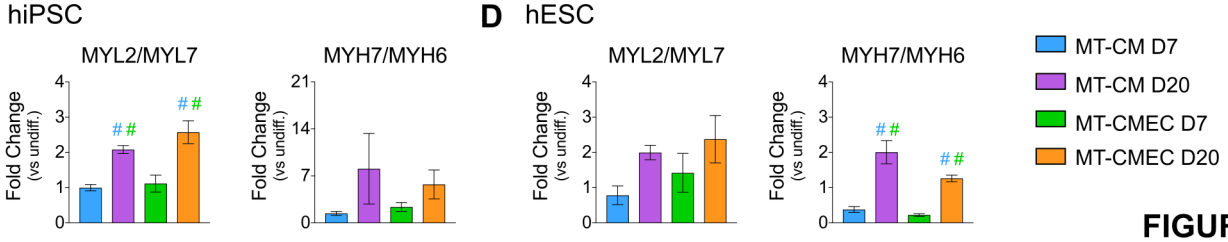

C hiPSC
MYL2/MYL7
MYH7/MYH6
Fold Change (vs undiff.)
D hESC
MYL2/MYL7
MYH7/MYH6
Fold Change (vs undiff.)
MT-CM D7
MT-CM D20
MT-CMEC D7
MT-CMEC D20

FIGURE S5

Figure S5. Gene expression profile of day 20-MTs. (A) qRT-PCR analysis for key sarcomeric genes, ion channels involved in AP shaping and calcium regulatory genes, as well as other cardiac genes of interest on day 7 and day 20 MT-CM and MT-CMEC together with $VCAM1^+$ cardiomyocytes generated from hiPSCs and (B) hESCs. All values are normalized to *RPL37A* and are relative to undifferentiated hiPSCs or hESCs. Ordinary one-way ANOVA with Tukey's multiple comparisons test. * = P < 0.05 vs. $VCAM1^+$ cardiomyocytes. # = P < 0.05 vs. corresponding colour coding group. N ≥ 2. Data are shown as mean ± SEM. (C) qRT-PCR analysis for *MYL2/MYL7* ratio (left panel) and *MYH7/MYH6* ratio (right panel) on day 7 and day 20 MT-CM from hiPSCs and (D) hESCs. Values are normalized to *RPL37A* and relative to undifferentiated hiPSCs or hESCs. Ordinary one-way ANOVA with Tukey's multiple comparisons test. # = P < 0.05 vs. corresponding colour coding group. N ≥ 2. Data are show as mean ± SEM.

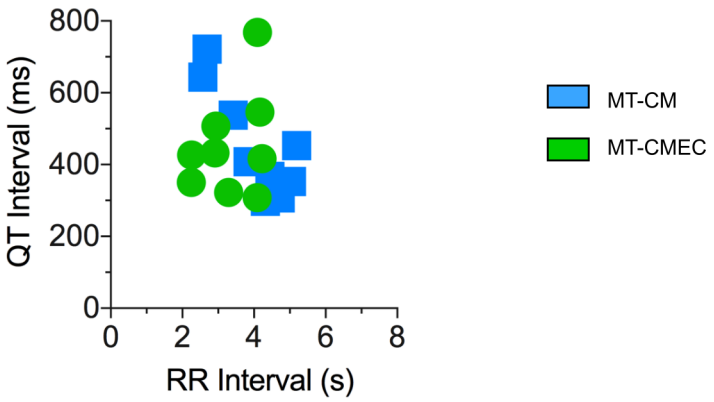


FIGURE S6

Figure S6. Rate-dependency of MTs by MEA. Correlation between QT-interval and RR-interval from MT-CM (blue) and MT-CMEC (green) from hiPSCs. A comparable electrical phenotype was exhibited by both MT-CM and MT-CMEC, indicating that the presence of endothelial cells did not affect the QT-RR relationship.

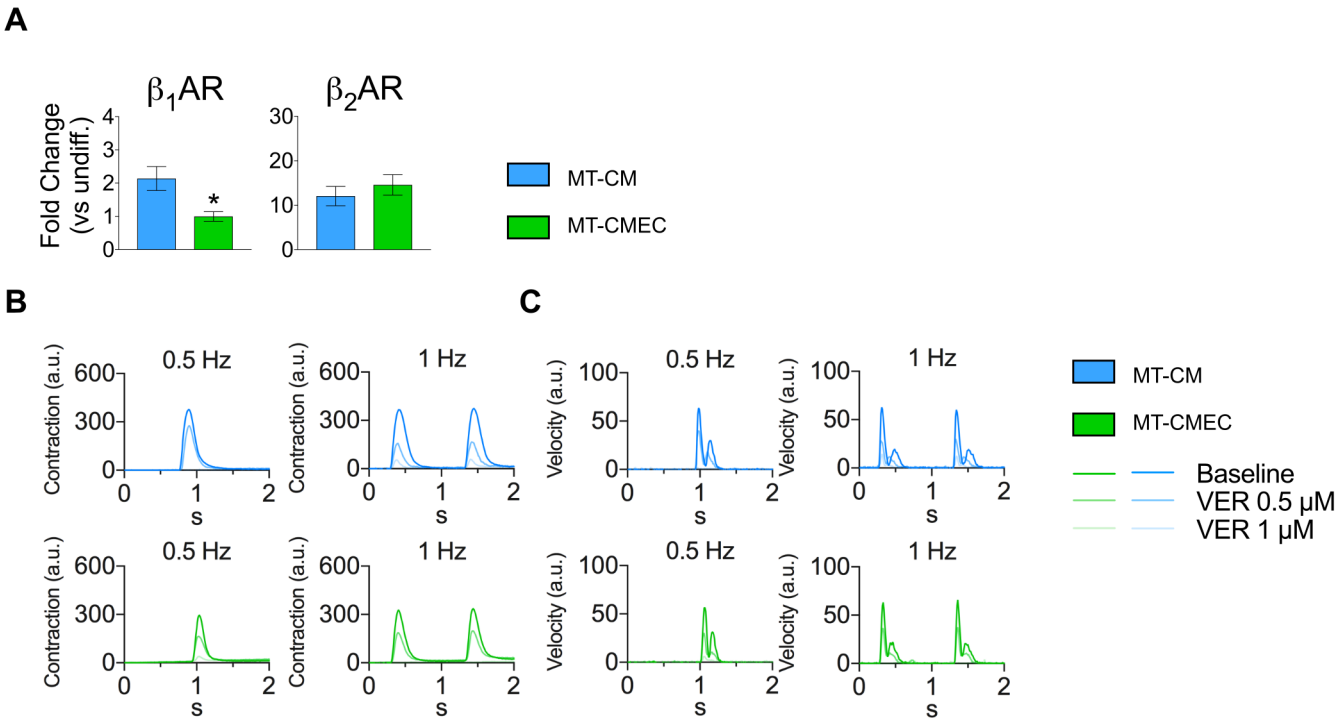
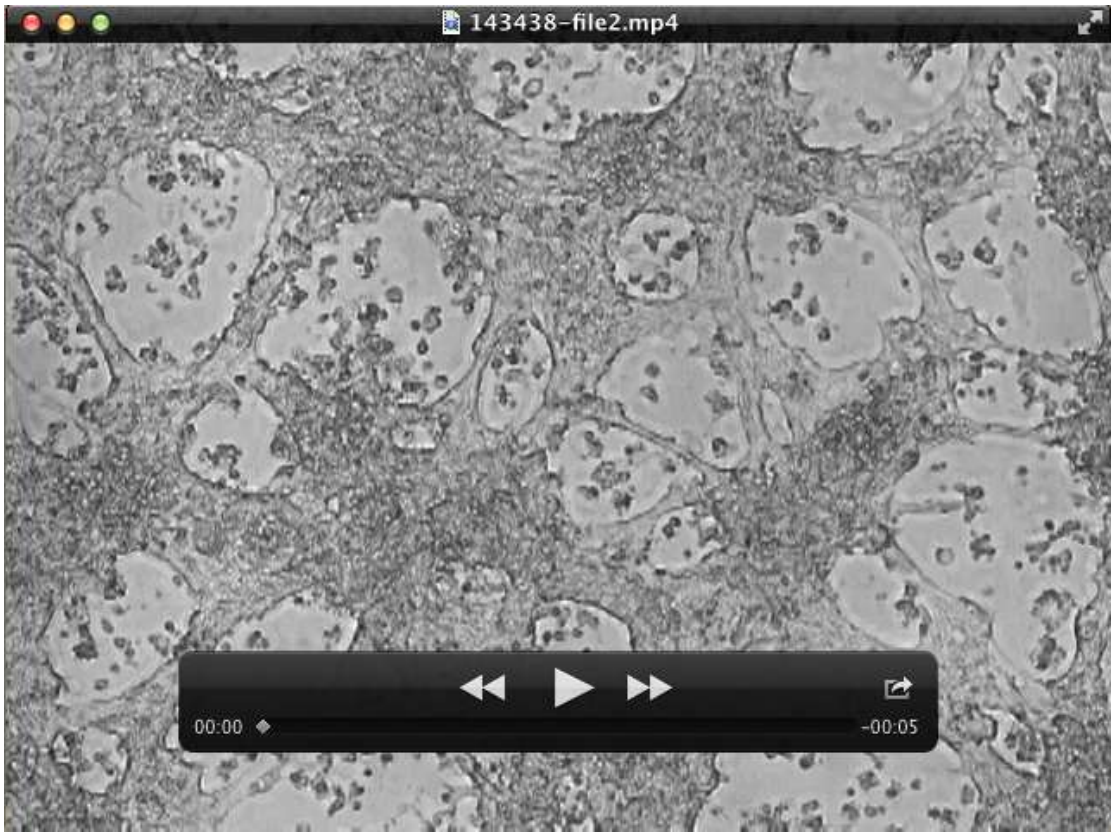


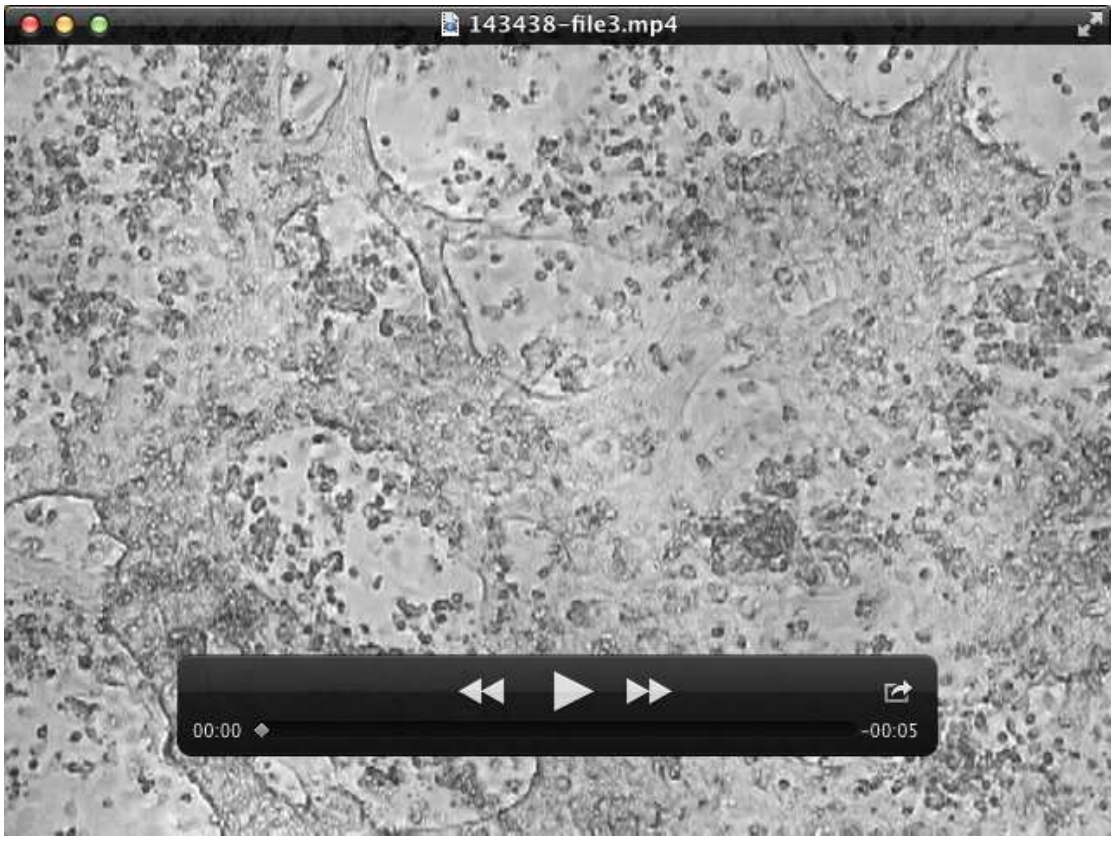
FIGURE S7

Figure S7. β-adrenoreceptors and contraction profile of MTs from hESCs. (A) qRT-PCR analysis of β-adrenoreceptors (β_1 AR, left panel; β_2 AR, right panel) in day-7 MT-CM and MT-CMEC from hESCs. Values are normalized to *RPL37A* and relative to undifferentiated hESCs. Mann-Whitney test. * = P < 0.05 vs. MT-CM. N = 3. Data are show as mean ± SEM (B) Representative traces of contraction and contraction velocity (C) profiles of MT-CM (blue) and MT-CMEC (green) generated from hESCs and paced at 0.5 and 1 Hz, in baseline and after superfusion of 500 nM and 1 µM VER.



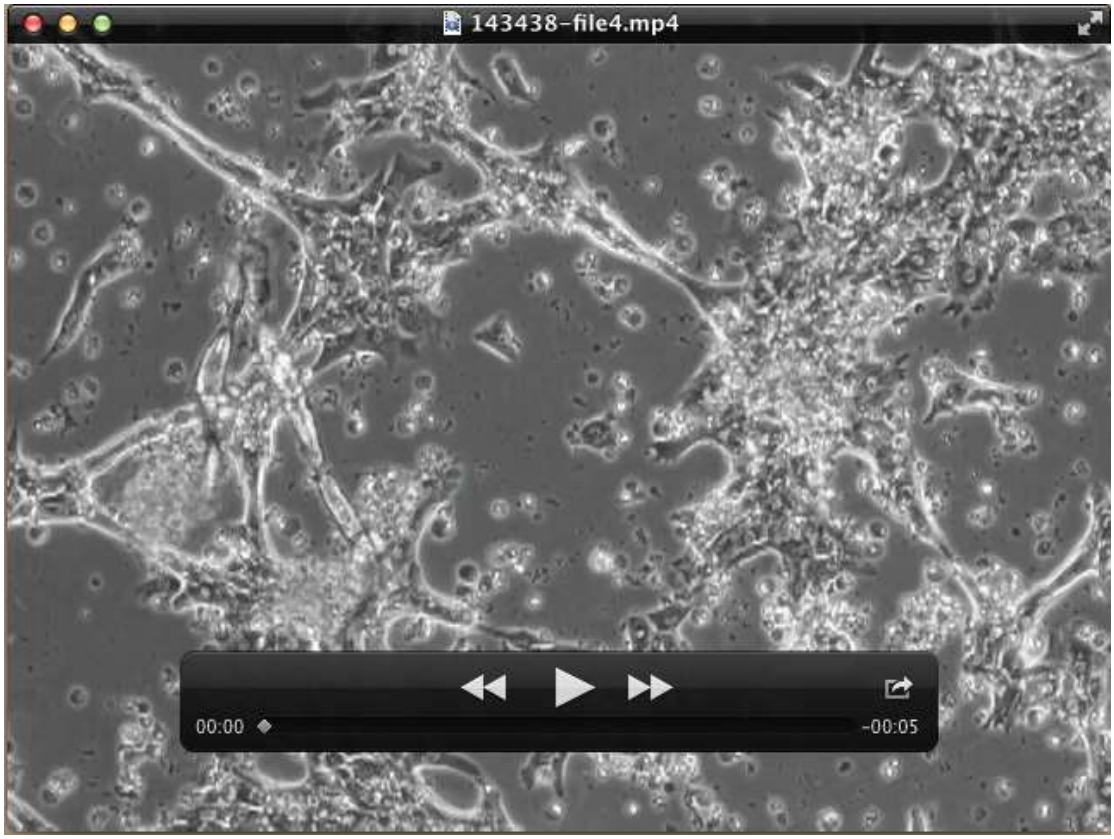
Movie 1

Beating monolayers of day 10-differentiated NKX2.5$^{eGFP/w}$ hESCs upon CM condition.



Movie 2

Beating monolayers of day 10-differentiated NKX2.5$^{eGFP/w}$ hESCs upon CMEC condition.



Movie 3

Beating monolayers of hiPSC-VCAM1^{+} cardiomyocytes after isolation and re-plating.