SUPPLEMENTAL MATERIAL

Data S1.

Material and Methods

Ethics statement

The skin biopsies from two healthy donors and one SQTS patient were obtained with written informed consent. The study was approved by the Ethics Committee of Medical Faculty Mannheim, Heidelberg University (approval number: 2009-350N-MA) and by the Ethics Committee of University Medical Center Göttingen (approval number: 10/9/15), and carried out in accordance with the approved guidelines and conducted in accordance with the Helsinki Declaration of 1975, as revised in 1983.

Generation of human iPS cells (hiPSC)

Somatic cell isolation and primary culture

Human fibroblast cultures were derived from skin punch biopsies of the donors. Skin punch biopsy (3-4 mm) was taken aseptically by a surgeon, placed in DMEM (Thermo Fisher Scientific, #11960044) containing 200 U/ml penicillin and 200 µg/ml streptomycin (Thermo Fisher Scientific, #15140122) and transferred to the lab within 48 h. Biopsies were mechanically cut in pieces of 0.5-1 mm, were placed epidermis upside in the cell culture dish and cultured in human fibroblast medium (HFBM) composed of DMEM (Thermo Fisher Scientific, #11960044) supplemented with 10% Fetal Bovine Serum (Thermo Fisher Scientific, #10270106), 1x MEM Non-Essential Amino Acids Solution (Thermo Fisher Scientific, #11140035), 2 mM L-Glutamine (Thermo Fisher Scientific, #25030024), 50 µM β-mercaptoethanol (Serva Electrophoresis, #28625), 10 ng/ml bFGF (Peprotech, #100-18B), 100 U/ml penicillin and 100 µg/ml streptomycin (Thermo Fisher Scientific, #15140122) at

37°C with 5% CO2 atmosphere. Medium was changed every other day. Reprogramming of fibroblasts was performed before passage 3 (p3).

Generation of hiPSCs

HiPSC lines isSTQSa1.7 (GOEi091-A.7, here abbreviated as SQTS), isSQTSa1.8 (GOEi091-A.8) and isSQTSa1.15 (GOEi091-A.15) were generated from fibroblasts in feeder free culture conditions using the integration-free CytoTune-iPS 2.0 Sendai Reprogramming Kit (Thermo Fisher Scientific, #A16517) with the reprogramming factors OCT4, KLF4, SOX2, c-MYC according to manufacturer's instructions with modifications. In brief, $1.5x10^4$ early passage fibroblasts were plated in two wells of a Matrigel-coated 24-well plate in HFBM two days before transduction. Cells were transduced at 40-50% confluence with Sendai virus cocktail (hKOS: hc-Myc: hKlf4) at a MOI of 10:10:6 according to the counted cell number of extra well (typically $2.5x10^4$ cells/well) in HFBM. Virus was removed after 24 h and HFBM was changed every other day.

HiPSC lines ipWT1.1 (GOEi014-B.1), ipWT1.3 (GOEi014-B.3, here abbreviated as D2) and ipWT1.6 (GOEi014-B.6) were generated from fibroblasts in feeder free culture conditions using the integration-free episomal 4-in-1 CoMiP reprogramming plasmid (Addgene, #63726) with the reprogramming factors OCT4, KLF4, SOX2, c-MYC and short hairpin RNA against p53, as described previously with modifications [1]. In brief, $5x10^5$ early passage fibroblasts were used for electroporation with the Nucleofector 2b Device (Lonza) with program P22 or U23 by using the NHDF Nucleofector Kit (Lonza, #VPD-1001) and 2 µg of the reprogramming plasmid. Transfected cells were plated in one well of a Matrigel-coated 6-well plate in HFBM supplemented with 500 µM sodium butyrate (Sigma-Aldrich, #B5887), 2 µM Thiazovivin (Millipore, #420220), 100 U/ml penicillin and 100 µg/ml streptomycin. Medium was changed every other day with HFBM supplemented with 500 µM sodium butyrate.

At d7 post transduction/transfection, cells were replated in various dilutions in Matrigel-coated 6-well plates in HFBM supplemented with 500 µM sodium butyrate and 2 µM Thiazovivin. From d8, medium was changed to E8 medium (Thermo Fisher Scientific, #A1517001) supplemented with 500 µM sodium butyrate (d8-d11) with daily medium change. Cells were monitored for morphology change and appearance of colonies (typically after 2-3 weeks). Individual colonies with iPSC-like morphology were picked mechanically in Matrigel-coated 12-well plates in E8 medium supplemented with 2 µM Thiazovivin. Newly established iPSC lines were passaged with Versene solution (Thermo Fisher Scientific, #15040066) and cultured in E8 medium supplemented with 2 µM Thiazovivin on the first day after passaging in Matrigel-coated plates for at least ten passages before being used for pluripotency characterization and differentiation experiments.

Spontaneous in vitro differentiation of hiPSCs

For embryoid body (EB) formation, $5x10^4$ hiPSCs together with $2.5x10^4$ mouse embryonic fibroblasts were plated in each well of a 96-well U-bottom plate in hES medium composed of DMEM-F12 (Thermo Fisher Scientific, #31331028), 15% Knockout Serum Replacement (Thermo Fisher Scientific, #10828028), 1x MEM Non-Essential Amino Acids Solution (Thermo Fisher Scientific, #11140035), 50 µM β-mercaptoethanol (Serva Electrophoresis, #28625) and 2 µM Thiazovivin, the plate was centrifuged at 250 g for 5 min and co-cultures were cultivated in suspension to form multicellular EB aggregates. At d2, medium was changed to differentiation medium composed of IMDM Glutamax (Thermo Fisher Scientific, #31980022), 20% Fetal Bovine Serum (Thermo Fisher Scientific, #10270106), 1x MEM Non-Essential Amino Acids Solution and 450 µM 1-Thioglycerol (Sigma-Aldrich, #M6145) for further 6 days with medium change every other day. At d8, EBs were plated onto 0.1% gelatin-coated 6-well plates and cultured for up to one month in differentiation medium with medium change every other day.

Alkaline phosphatase staining

Alkaline phosphatase activity in hiPSC lines was detected using the Alkaline Phosphatase Kit (Sigma-Aldrich, #86R-1KT) according to the manufacturer´s instructions.

Generation of hiPSC-CMs

Frozen aliquots of hiPSCs were thawed and cultured without feeder cells and differentiated into hiPSC-CMs as described with some modifications [2]. Culture dishes and wells were coated with Matrigel (Corning). Culture medium of hiPSCs was TeSR-E8 (Stemcell Technologies) and for hiPSC-CMs we used RPMI 1640 Glutamax (Life Technologies) containing sodium pyruvate, Penicillin / Streptomycin, B27 (Life Technologies) and ascorbic acid (Sigma Aldrich). Adding of CHIR99021 (Stemgent), BMP-4 (R&D Systems), Activin A (R&D Systems), FGF-2 (MiltenyiBiotec) and IWP-4 (Stemgent) at different time points induced the cells to differentiate into hiPSC-CMs during 3 weeks. During the third week a lactate (Sigma Aldrich) containing RPMI-medium without glucose and glutamine (WKS, Germany) was added to select for cardiomyocyte cells. At 40 to 50 days of culture with basic culture medium, cardiomyocytes were dissociated from 24 well plates and plated on Matrigel-coated 3.5 cm petri dishes for patch-clamp measurements: The cells were incubated with 300µl (150 U) collagenase CLS I (Worthington, Germany) for 40 min at 37°C, washed with PBS and incubated with 0.05% Trypsin-EDTA (Life Technologies) for 4 min at 37°C. After adding of RPMI medium containing 10% FCS, cells were centrifuged at 250 x g for 2 min at room temperature, the supernatant discarded and the cells resuspended with basic culture medium. The cells were plated on the 3.5 cm petri dishes at a density of 2 – 4 x 10^4 cells/dish for subsequent patch-clamp experiments. A minimum of 3 differentiation experiments of all 3 different iPSC lines were used for each analysis.

Reverse transcriptase-polymerase chain reaction

To quantify the steady-state mRNA expression of the hiPSC-CMs, RNA was reverse transcribed and qPCR was performed as described [3]. Gene symbols, RefSeq No. and Cat. No. of the primers used for qPCR analyses in hiPSC-CMs characterization were listed in supplementary table 1 (Table S1). For evaluation of the characteristics of the used hiPSC lines, RT-PCR was performed as follows: Total RNA of hiPSC cultures was isolated using the SV Total RNA Isolation System (Promega, #Z3105) according to manufacturer's instructions. 100 ng RNA was used for the first-strand cDNA synthesis by using MULV Reverse Transcriptase (Thermo Fisher Scientific, #N8080018) and Oligo d(T)16 (Thermo Fisher Scientific, #N8080128). One-tenth of cDNA was used as PCR template and amplified using the GoTaq G2 DNA polymerase (Promega, #M7845) according to manufacturer's instructions. The relative expression level of the target gene compared with that of the housekeeping gene, GAPDH, was calculated by the $2^{-\Delta\Delta Ct}$ method. Primer sequences, annealing temperatures and cycles used for RT-PCR analyses of the hiPSC lines were listed in supplementary table 2 (Table S2).

Immunocytochemical staining

HiPSC cultures were fixed with Roti-Histofix 4% (Carl Roth, #P087) at RT for 20 min and blocked with 1% bovine serum albumin (BSA; Sigma Aldrich, #F7524) in PBS at 4°C overnight. Primary antibodies were applied in 1% BSA for 1 h at 37°C or overnight at 4°C. Secondary antibodies with minimal cross reactivity were administered in 1% BSA for 1 h at RT. For nuclear or cytosolic proteins (OCT4, SOX2, NANOG, LIN28), cells were permeabilized with 0.1% Triton-X100 (Carl Roth, #3051) in staining solution. Nuclei were stained with 4.8 µM DAPI (Thermo Fisher Scientific, #D1306) for 10 min at RT. Samples were mounted in Fluoromount-G (Thermo Fisher Scientific, #00-4958-02). Antibodies and dilutions used were listed in supplementary table 3 (Table S3). Images were collected using the Axio

Observer Z1 microscopy system (Carl Zeiss) with Axiocam MRm, Plan-Apochromat 20x/0.8 objective or A-Plan 10x/0.25 objective and Axiovision software.

Immunofluorescence staining for KCNH2 channel analysis was performed using appropriate primary antibodies and Alexa Fluor conjugated secondary antibodies (ThermoFisher) according to the manufacturer's instructions. The primary antibodies used in hiPSC-CMs were a-actinin (Sigma Aldrich), TNNT (Sigma Aldrich) and KCNH2 (Santa Cruz; Alomone). All the other antibodies used for characterization of hiPSC lines are listed in supplementary table 3 (Table S3). Fluorescence measurement was performed according to method described previously using ImageJ [4]. Briefly, the cell of interest was selected, and measurement parameters were set to get the area, integrated density and mean gray value. Then a region next to the cell that has no fluorescence was selected as the background. This step was repeated for other cells in the field of view that should be measured. The corrected total cell fluorescence was calculated as (CTCF)= Integrated Density – (Area of selected cell x Mean fluorescence of background readings). Mean fluorescence density was obtained by CTCF/ Area. Pictures were taken using the same exposure parameters. More than 60 cells from 5-8 pictures were selected for analysis. Given that the cells were not permeated during staining and the anti-Kv11.1 (hERG) (extracellular) antibody binding to the extracellular peptide between S1 and S2 domains was used, the staining was considered to come from surface membrane hERG channels.

Flow cytometry

HiPSC cultures between passage 10-15 were dissociated with Versene solution into single cells, fixed with Roti-Histofix 4% (Carl Roth, #P087) at RT for 20 min and blocked with 1% bovine serum albumin (BSA; Sigma Aldrich, #F7524) in PBS at 4°C for at least 2 h. Cells were permeabilized with 0.1% Triton-X100 in staining solution and coincubated with fluorescence-conjugated antibodies against OCT4 and TRA-1-60 at RT for 1 h. Nuclei were co-stained with 8.1 µM Hoechst 33342 (Thermo Fisher Scientific, # H1399). Subsequently, cells were analyzed

using the LSRII flow cytometer (BD Biosciences) and BD FACSDiva software. Gating of cells was applied based on forward scatter area (FSC-A) and sideward scatter area (SSC-A) as well as on gating of single cells (based on DNA signal width). At least 10,000 events were analyzed per sample.

Measurement of intracellular calcium concentration

To measure the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$), cells were loaded with the fluorescent Ca^{2+}-indicator Fluo-3 AM. First, 1.5 ml PSS (see below) was added into a petri dish with hiPSC-CMs cultured for 2 to 4 days. The following steps were executed under consideration of the light sensitivity of the fluorescent Ca^{2+} Indicator Fluo-3. Then, 50 µg of the membrane permeable acetoxymethyl ester derivative of Fluo-3 was dissolved in 44 µl of the Pluronic F-127 stock solution (20% w/v in DMSO) to get a 1 mM Fluo-3 AM stock solution, which can be stored at -20 °C for a maximum of 1 week. Next, 15 µl of the Fluo-3 AM stock solution were added into 1.5 ml PSS resulting in a final concentration of 10 µM Fluo-3 and the dish was agitated carefully. The cells were incubated at room temperature for 10 minutes in an optically opaque box to protect from light. Thereafter, the PSS was carefully sucked out and discarded and the cells were washed with PSS for 4-5 times. Finally, the cells in PSS were kept at room temperature for about 30 minutes for de-esterification before measurements. After de-esterification the fluorescence of the cells was measured by using Cairn Optoscan calcium imaging system (Cairn Research, UK). Fluorescence is excited by 488 nm and emitted at 520 nm. Changes in $[Ca^{2+}]_i$ were described by

$$[Ca^{2+}]_i = k_d\left(\frac{F}{F_{\max} - F}\right)$$

, where k_d=dissociation constant of Fluo-3 (400-nmol/L), F = Fluo-3 fluorescence; F_{max} = Ca^{2+}-saturated fluorescence obtained at the end of each experiment by damaging the cell with the patch pipette and measuring the increase of fluorescence to maximum [5].

Patch-clamp

Standard patch-clamp recording techniques were used to measure the I_{Na}, $I_{CaL,}$ I_{to}, I_{Kr}, I_{Ks}, I_{K1} and action potential (AP) in the whole-cell configuration at room temperature. Patch electrodes were pulled from borosilicate glass capillaries (MTW 150F; world Precision Instruments, Inc., Sarasota, FL) using a DMZ-Universal Puller (Zeitz-InstrumenteVertriebs GmbH, Martinsried, Germany) and filled with pre-filtered pipette solution (see below). Pipette resistance ranged from 1-2 MΩ. Electrode offset potentials were zero-adjusted before a Giga-seal was formed. After a Giga-seal was obtained, fast capacitance was first compensated and then the membrane under the pipette tip was disrupted by negative pressure to establish the whole-cell configuration. To determine the cell capacitance, a voltage pulse from -80 to -85 was given to record the cell capacitance transient current. Then the area under transient current curve was integrated and divided by 5 mV to get the whole cell capacitance in pF. Thereafter the membrane capacitance (Cm) and series resistance (Rs) were compensated (60-80%). Liquid junction potentials were not corrected. Signals were acquired at 10 kHz and filtered at 2 kHz with the Axon 200B amplifier and Digidata 1440A digitizer hardware as well as pClamp10.2 software (Molecular Devices, Sunnyvale, CA). Myocytes were held at −80 mV for I_{Na}, I_{to}, I_{CaL} and at -40 mV for I_{Kr}, I_{Ks}, and I_{K1} measurements. For I_{Na} measurements, currents were elicited by using depolarizing pulses of 400 ms from -100 mV to +50 mV with 10 mV increments at 0.5 Hz. For measurements of $I_{Ca\text{-}L}$, cells were depolarized from -80 mV to -40 mV for 500 ms and then to different potentials (-100 to +60 mV) for 500 ms. For I_{Kr} recordings, cells were depolarized from -20 to +80 mV for 2s and repolarized to -30 mV. For I_{Ks} recordings, cells were depolarized from -40 mV to +50 mV for 4s and repolarized to -40 mV. For I_{to} recordings, currents were evoked by pulses from -80 mV to +80 mV for 500 ms. For IK1, currents were elicited by pulses from -120 mV to +60 mV for 500 ms. Measured currents were normalized to the membrane capacitance. Current-voltage (I-V) relationships were generated by plotting the current density against voltages. The TTX sensitive late I_{Na} was measured as the area under

the current curve integrated from 50 to 350 ms after the beginning of the depolarization pulse. E-4031 (3 µM) was used for isolating I_{Kr}, chromanol 293 B (10 µM) for I_{Ks}, 4-AP (5 mM) for I_{to}, $BaCl_2$ (100 µM) for I_{K1}. The blocker-sensitive currents were used for comparison between donor- and sQT-cells. To minimize the effects of run-down of recorded currents on the results of experiments, we carefully monitored the time-dependent change of currents. Recordings were started after the current reached a steady state, normally within 3 to 5 minutes.

The bath solution for late sodium current contained (mmol/l): 135 NaCl, 20 CsCl, 1.8 $CaCl_2$, 1 $MgCl_2$, 10 Hepes, 10 glucose, 0.001 nifedipine, pH 7.4 (CsOH). Microelectrodes were filled with (mmol/l): 10 NaCl, 135 CsCl, 2 $CaCl_2$, 3 MgATP, 2 TEA-Cl, 5 EGTA and 10 HEPES (pH7.2 CsOH).

The bath solution for I_{CaL} recordings contained (mmol/l): 140 TEA-Cl, 5 $CaCl_2$, 1 $MgCl_2$, 10 Hepes, 0.01 TTX, 2 4-AP, pH 7.4 (CsOH). Microelectrodes were filled with (mmol/l): 10 NaCl, 135 CsCl, 2 $CaCl_2$, 3 MgATP, 2 TEA-Cl, 5 EGTA and 10 HEPES (pH7.2 CsOH).

The bath solution (PSS) for I_{to}, I_{Ks}, I_{K1} and AP measurements contained (mmol/l): 130 NaCl, 5.9 KCl, 2.4 $CaCl_2$, 1.2 $MgCl_2$, 11 glucose, 10 HEPES, pH 7.4 (NaOH). For the I_{to} measurements, 10 µM nifedipine, 10 µM TTX and 1 µM E-4031 were added in the bath solution to block I_{CaL}, I_{Na} and I_{Kr}. For I_{Ks} measurements, 2 mM 4-AP and 1 µM E-4031 were added. For I_{K1} measurements the concentration of KCl was increased to 20 mM. The pipette solution contains 10 mM HEPES, 126 mM KCl, 6 mM NaCl, 1.2 mM $MgCl_2$, 5 mM EGTA, 11 mM glucose and 1 mM MgATP, pH 7.4 (KOH).

To improve I_{Kr} measurements, the Cs^{2+} currents conducted by the KCNH2 channels were measured. External solution for Cs^{2+} currents (mmol/L): 140 CsCl, 2 $MgCl_2$, 10 HEPES, 10 Glucose, pH=7.4 (CsOH). Pipette solution: 140 CsCl, 2 $MgCl_2$, 10 HEPES, 10 EGTA, pH=7.2 (CsOH).

Statistics

If not otherwise indicated data are shown as mean ± SEM and were analyzed using InStat© (GraphPad, San Diego, USA) and SigmaPlot 11.0 (Systat GmbH, Germany). By analyzing the data with the Kolmogorov Smirnov test it was decided whether parametric or non-parametric tests were used for analysis. Student's t-test and the Mann–Whitney U-test were used to compare continuous variables with normal and non-normal distributions, respectively. The chi-squared-test for independence was used to compare categorical variables. For parametric data one-way ANOVA with Bonferroni post-test for multiple comparisons was performed. For non-parametric data the Kruskal-Wallis test with Dunn's multiple comparisons post-test was used. Paired t-test was used for comparisons of data before and after application of a drug. $p<0.05$ (two-tailed) was considered significant.

Table S1. Gene symbols, RefSeq No. and Cat. No. of the primers used for qPCR analyses in hiPSC-CMs characterization.

Gene symbol	RefSeq No.	Cat. No. Primers
CACNA1C (L-type Ca2+ channel)	NM_000719	PPH01378G
KCND3 (Ito, Kv4.3)	NM_004980	PPH06923A
KCNH2 (IKr, Kv11.1)	NM_000238	PPH01660A
KCNJ2	NM_000891	PPH01618E
KCNQ1 (I_{Ks}, Kv7.1)	NM_000218	PPH01419A
SCN5A (Na+ channel, Nav1.5)	NM_000335	PPH01671F

RefSeq No. : GenBank NCBI Reference Sequences

Cat. No. Primers: Qiagen RT² qPCR Primer Assays

Table S2. Primer sequences, annealing temperatures and cycles used for RT-PCR analyses in hiPSC pluripotency characterization.

Primer	Primer for	Primer rev	Length bps	Temp /Cycles
OCT4	GACAACAATGAAAATCTTCAGGAGA	TTCTGGCGCCGGTTACAGAACCA	218	58°C / 36 cycles
SOX2	ATGCACCGCTACGACGTGA	CTTTTGCACCCCTCCCATTT	437	58°C / 30 cycles
NANOG	AGTCCCAAAGGCAAACAACCCACTTC	ATCTGCTGGAGGCTGAGGTATTTCTGTCTC	164	64°C / 36 cycles
LIN28	AGTAAGCTGCACATGGAAGG	ATTGTGGCTCAATTCTGTGC	410	58°C / 36 cycles
FOXD3	GTGAAGCCGCCTTACTCGTAC	CCGAAGCTCTGCATCATGAG	353	58°C / 38 cycles
GDF3	TTCGCTTTCTCCCAGACCAAGGTTTC	TACATCCAGCAGGTTGAAGTGAACAGCACC	311	58°C / 32 cycles
AFP	ACTCCAGTAAACCCTGGTGTTG	GAAATCTGCAATGACAGCCTCA	255	58°C / 30 cycles
ALB	CCTTTGGCACAATGAAGTGGGTAACC	CAGCAGTCAGCCATTTCACCATAGG	355	62°C / 35 cycles
TNNT2	GACAGAGCGGAAAAGTGGGA	TGAAGGAGGCCAGGCTCTAT	305	58°C / 30 cycles
MYH6	GTCATTGCTGAAACCGAGAATG	GCAAAGTACTGGATGACACGCT	413	58°C / 38 cycles
TH	GCGGTTCATTGGGCGCAGG	CAAACACCTTCACAGCTCG	215	56°C / 34 cycles
MAP2	CCACCTGAGATTAAGGATCA	GGCTTACTTTGCTTCTCTGA	482	55°C / 32 cycles
GAPDH	AGAGGCAGGGATGATGTTCT	TCTGCTGATGCCCCCATGTT	258	58°C / 30 cycles

Table S3. Antibodies and dilutions used for immunocytochemistry of iPSC pluripotency characterization.

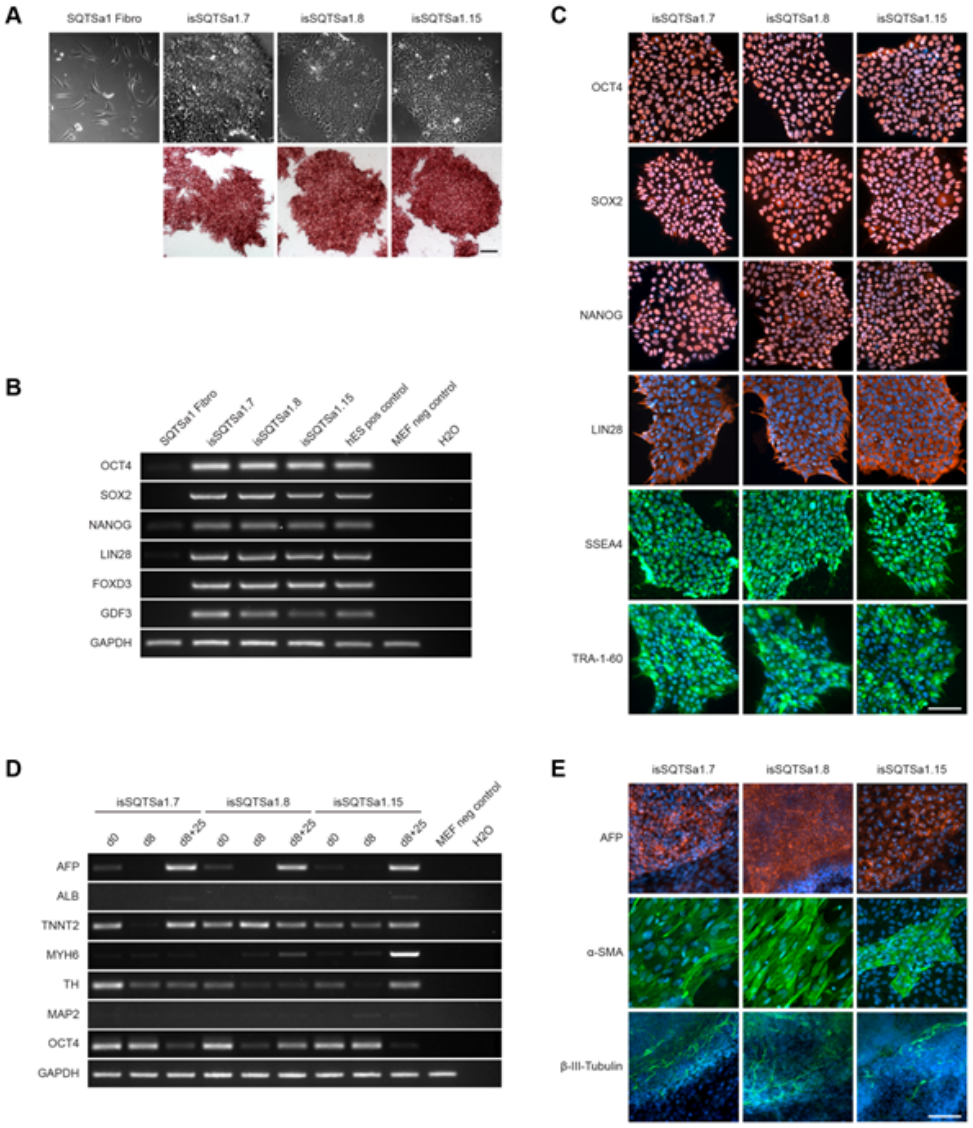
Primary antibody	**Type**	**Dilution**	**Supplier**
hOCT3/4	polyclonal goat IgG	1:40	R&D Systems, #AF1759
hSOX2	monoclonal mouse IgG1	1:200	Thermo Fisher Scientific, #MA1-014
hNANOG	polyclonal rabbit IgG	1:100	Thermo Fisher Scientific, #PA1-097
hLIN28	polyclonal goat IgG	1:300	R&D Systems, #AF3757
hSSEA4	monoclonal mouse IgG3	1:100	Thermo Fisher Scientific, #MA1-021
hTRA-1-60	monoclonal mouse IgM	1:200	Abcam, #ab16288
hAFP	polyclonal rabbit IgG	1:100	DAKO, #A0008
α-SMA	monoclonal mouse IgG2a	1:3000	Sigma-Aldrich, #A2547
β-III-Tubulin	monoclonal mouse IgG2A	1:2000	Covance, #MMS-435P
Secondary antibody	**Type**	**Dilution**	**Supplier**
Alexa Fluor 488	polyclonal donkey α-mouse IgG	1:1000	Thermo Fisher Scientific, #A21202
Alexa Fluor 555	polyclonal donkey α-goat IgG	1:1000	Thermo Fisher Scientific, #A21432
Alexa Fluor 555	polyclonal donkey α-mouse IgG	1:1000	Thermo Fisher Scientific, #A31570
Alexa Fluor 555	polyclonal donkey α-rabbit IgG	1:1000	Thermo Fisher Scientific, #A31572
FITC	polyclonal goat α-mouse IgM	1:200	Jackson Immuno, #115-097-020
Flow Cytometry		**Dilution**	**Supplier**
Alexa Fluor 488 mouse anti-human TRA-1-60		1:50	BD Biosciences, #560173
Alexa Fluor 647 mouse anti-OCT3/4		1:50	BD Biosciences, #560329

Table S4. Action potential parameters.

group	RP (mV)	APA (mV)	Vmax (V/s)	APD50 (ms)	APD90 (ms)	n
D1	-79.07±1.17	135.60±2.63	32.29±2.67	148.07±14.19	320.17±19.08	28
D2	-80.17±0.73	126.66±3.08	28.52±2.34	149.34±9.68	289.21±13.77	29
SQTS	-78.07±0.76	127.31±2.74	30.86±2.28	*93.86±5.15	*187.90±6.91	29
SQTS (Ctr)	-79.92±0.51	131.00±4.47	32.83±3.97	91.75±10.57	203.17±15.76	12
Quinidine	-80.42±0.70	127.00±3.63	#28.17±3.22	#130.83±16.49	#324.17±42.69	12
SQTS (Ctr)	-81.58±0.67	122.25±4.39	26.00±3.58	94.92±15.30	204.83±31.11	12
Metoprolol	-81.83±0.69	123.33±4.18	25.17±3.24	91.08±13.14	218.33±36.14	12
SQTS (Ctr)	-79.09±0.79	133.36±4.96	35.45±3.94	99.27±9.88	219.36±16.45	11
Sotalol	-78.64±0.66	133.18±4.68	35.18±3.81	93.73±9.38	230.45±18.25	11

*$p<0.05$ versus D1; #$p<0.05$ versus Ctr

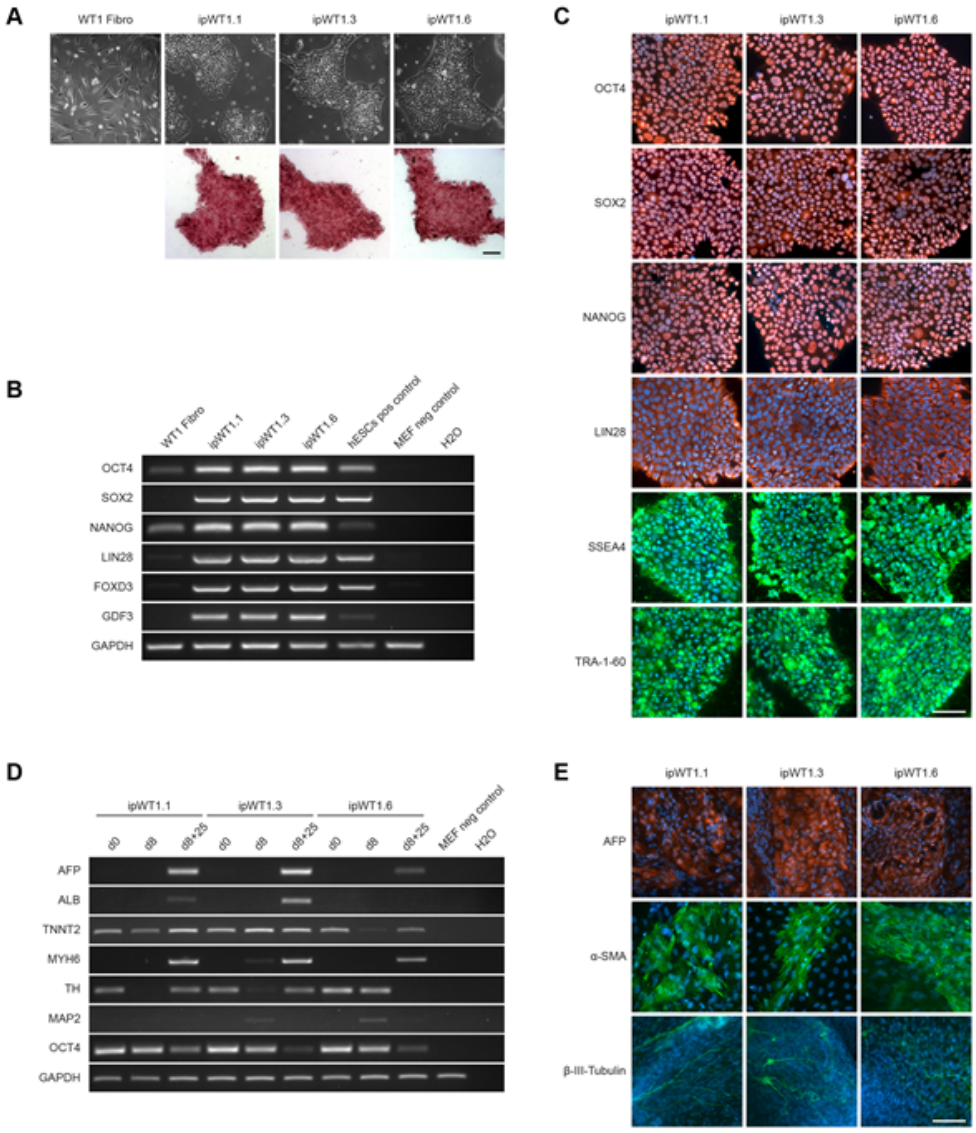
Figure S1. The generated hiPSC lines from the patient with short QT syndrome (SQTS) display pluripotent characteristics.



(A) The hiPSC (human induced pluripotent stem cell) lines isSQTSa1.7, isSQTSa1.8 and isSQTSa1.15 generated from skin fibroblasts of the SQTS-patient display a typical morphology for human pluripotent stem cells (upper panel) and are positive for alkaline phosphatase (lower panel). (B) In comparison to fibroblasts, generated hiPSC lines show expression of endogenous pluripotency markers SOX2 (sex determining region Y- box 2), OCT4 (octamer-binding transcription factor 4), NANOG (pron. nanOg, homeobox protein), LIN28 (lin-28 homolog A), FOXD3 (Forkhead Box D3) and GDF3 (Growth differentiation factor-3) at mRNA level proven

by RT-PCR (reverse transcription-polymerase chain reaction). Human embryonic stem cells (hESCs) were used as positive control, mouse embryonic fibroblasts (MEFs) were used as negative control. (C) Generated iPSC lines express pluripotency markers OCT4, SOX2, NANOG, LIN28, SSEA4 (stage-specific embryonic antigen 4) and TRA-1-60 as shown by immunofluorescence staining. Nuclei are co-stained with DAPI (4',6-diamidino-2-phenylindole). (D) Spontaneous differentiation potential of generated iPSC lines was analysed by embryoid body (EB) formation. Germ layer-specific genes like α-fetoprotein (AFP) and albumin (ALB) (endoderm), cTNT (cardiac troponin T) and α-MHC (mesoderm), and tyrosine hydroxylase (TH) and MAP2 (ectoderm) are expressed in a developmentally controlled manner during differentiation of EBs (days 0, 8, or 8+25), whereas endogenous OCT4 expression is decreased during spontaneous differentiation. MEFs were used as negative control. (E) Immunocytochemical staining of spontaneously differentiated hiPSC lines shows expression of endodermal marker AFP, mesodermal-specific α-SMA (α-smooth muscle actin) and ectodermal βIII-tubulin. Nuclei are co-stained with DAPI. Scale bars: 100 µm.

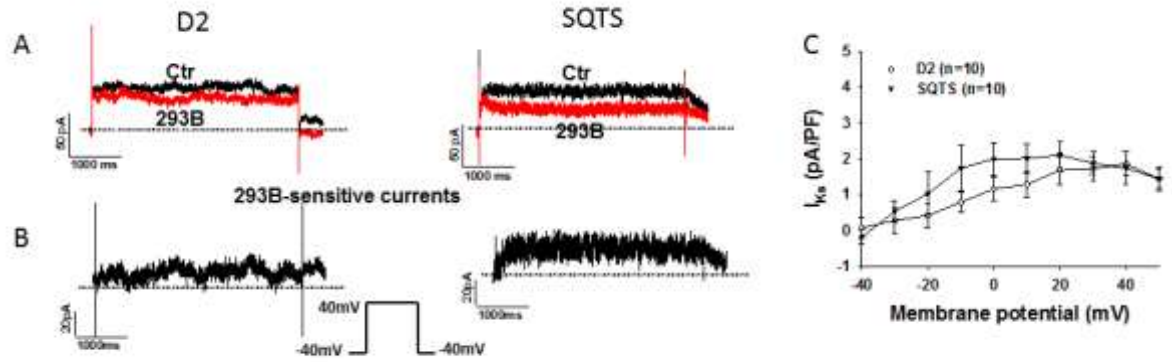
Figure S2. The generated hiPSC lines from a healthy donor display pluripotent characteristics.



(A) The hiPSC (human induced pluripotent stem cell) lines ipWT1.1, ipWT1.3 and ipWT1.6 generated from skin fibroblasts of a healthy donor (D2) display a typical morphology for human pluripotent stem cells (upper panel) and are positive for alkaline phosphatase (lower panel). (B) In comparison to fibroblasts, generated hiPSC lines show expression of endogenous pluripotency markers SOX2 (sex determining region Y- box 2), OCT4 OCT4 (octamer-binding transcription factor 4), NANOG (pron. nanOg, homeobox protein), LIN28 (lin-28 homolog A),

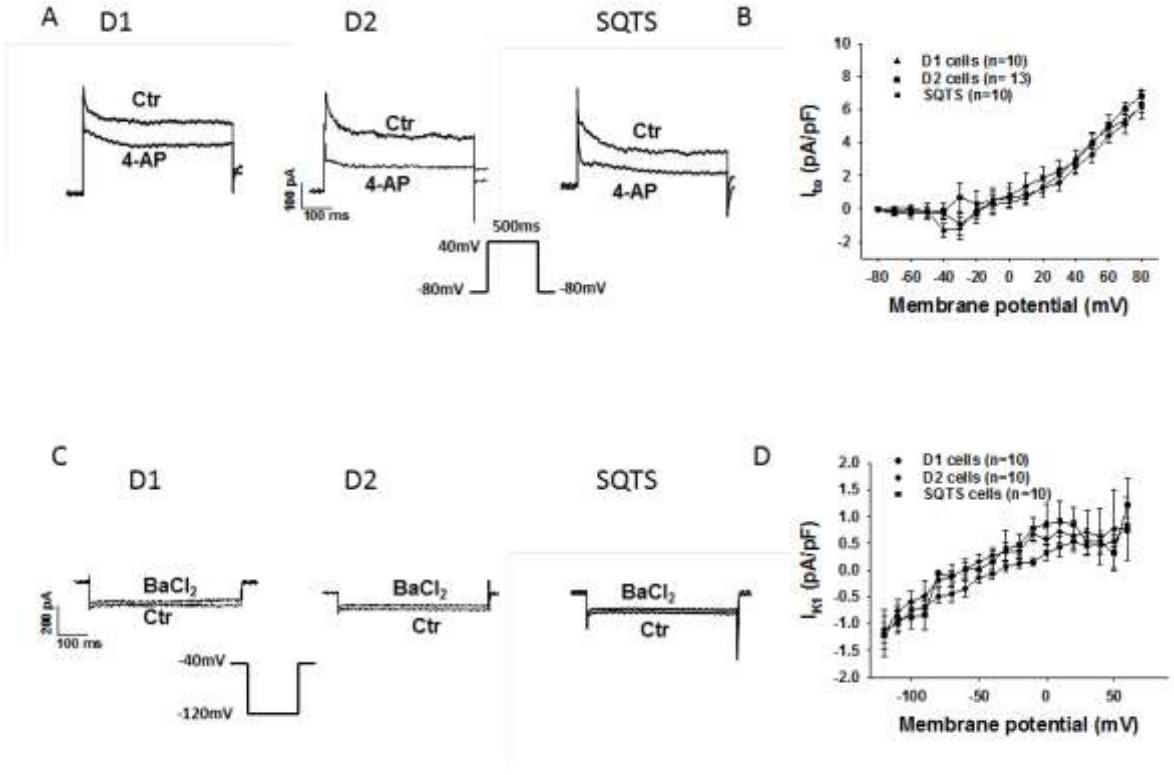
FOXD3 (Forkhead Box D3) and GDF3 (Growth differentiation factor-3) at mRNA level proven by RT-PCR (reverse transcription-polymerase chain reaction). Human embryonic stem cells (hESCs) were used as positive control, mouse embryonic fibroblasts (MEFs) were used as negative control. (C) Generated hiPSC lines express pluripotency markers OCT4, SOX2, NANOG, LIN28, SSEA4 (stage-specific embryonic antigen 4) and TRA-1-60 as shown by immunofluorescence staining. Nuclei are co-stained with DAPI (4',6-diamidino-2-phenylindole). (D) Spontaneous differentiation potential of generated hiPSC lines was analysed by embryoid body (EB) formation. Germ layer-specific genes like α-fetoprotein (AFP) and albumin (ALB) (endoderm), cTNT (cardiac troponin T) and α-MHC (mesoderm), and tyrosine hydroxylase (TH) and MAP2 (ectoderm) are expressed in a developmentally controlled manner during differentiation of EBs (days 0, 8, or 8+25), whereas endogenous OCT4 expression is decreased during spontaneous differentiation. MEFs were used as negative control. (E) Immunocytochemical staining of spontaneously differentiated hiPSC lines shows expression of endodermal marker AFP, mesodermal-specific α-SMA (α-smooth muscle actin) and ectodermal βIII-tubulin. Nuclei are co-stained with DAPI. Scale bars: 100 µm.

Figure S3. Slowly activating delayed rectifier currents (I_{Ks}) in donor-and SQTS (short QT syndrome)-cells.



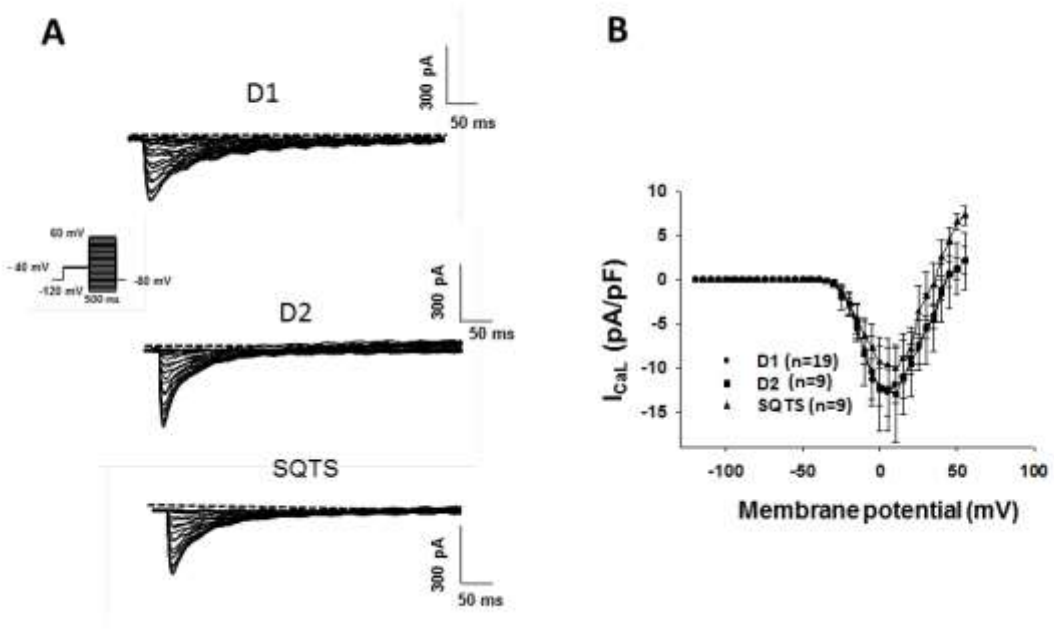
I_{Ks} (slowly activating delayed rectifier potassium channel) were evoked by the indicated protocol (B) in absence (control) and presence of a channel blocker. Chromanol 293B (10µM) was used to isolate I_{Ks} from other currents. (A) Representative I_{Ks} at +40 mV recorded in a cell from a donor (D2) and the patient (SQTS) with and without (Ctr) 293B. (B) 293B-sensitive currents. (C) I-V curves of I_{Ks} from the donor- and SQTS-cells. n, number of cells. *$p<0.05$ versus donor cells.

Figure S4. The transient outward currents (I_{to}) and inward rectifier currents (I_{K1}) in donor-and SQTS (short QT syndrome)-cells.



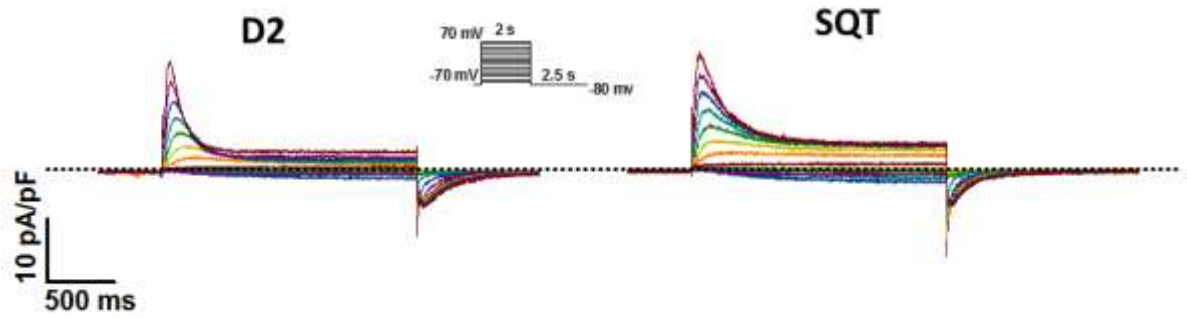
I_{to} and I_{K1} were evoked by the indicated protocol (A and C) in absence (Ctr) and presence of a channel blocker. 4-aminopyridine (4-AP, 5mM) and $BaCl_2$ (0.1 mM) was used to isolate I_{to} and I_{K1} from other currents. (A) Representative I_{to} at +40 mV recorded in a cell from the donors (D1 and D2) and the patient (SQTS). (B) I-V curves of I_{to} from the donor- and SQTS-cells. (C) Representative I_{K1} at -120 mV recorded in a cell from the donors (D1 and D2) and the patient (SQTS). (D) I-V curves of I_{K1} from the donor- and SQTS-cells. n, number of cells.

Figure S5. The L-type calcium channel currents (I_{CaL}) in donor-and SQTS (short QT syndrome)-cells.



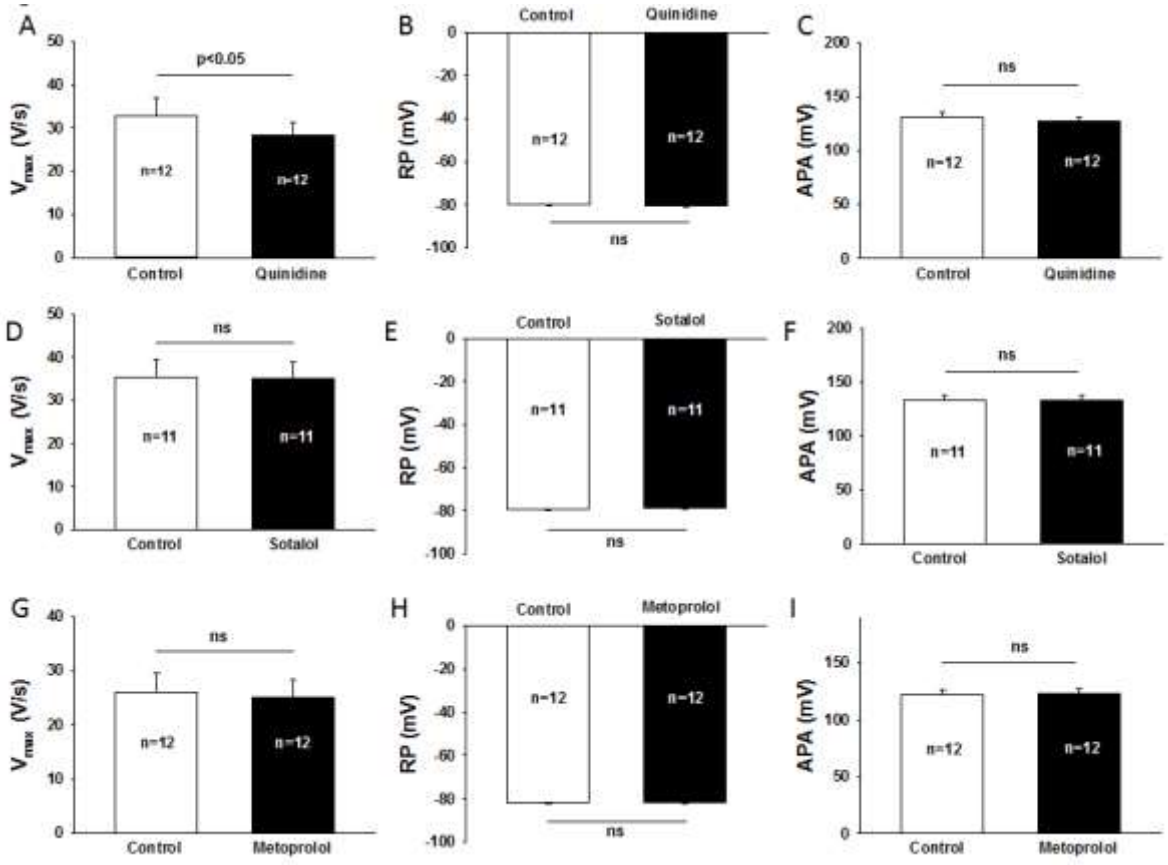
I_{CaL} was evoked by the indicated protocol (A). (A) Representative I_{CaL} at different potentials recorded in a cell from the donors (D1 and D2) and the patient (SQTS). (B) I-V (current-voltage relationship) curves of I_{CaL} from the donor- and SQTS-cells.

Figure S6. Ikr recorded in hiPSC (short QT syndrome)-CMs with a holding potential of -80 mV.



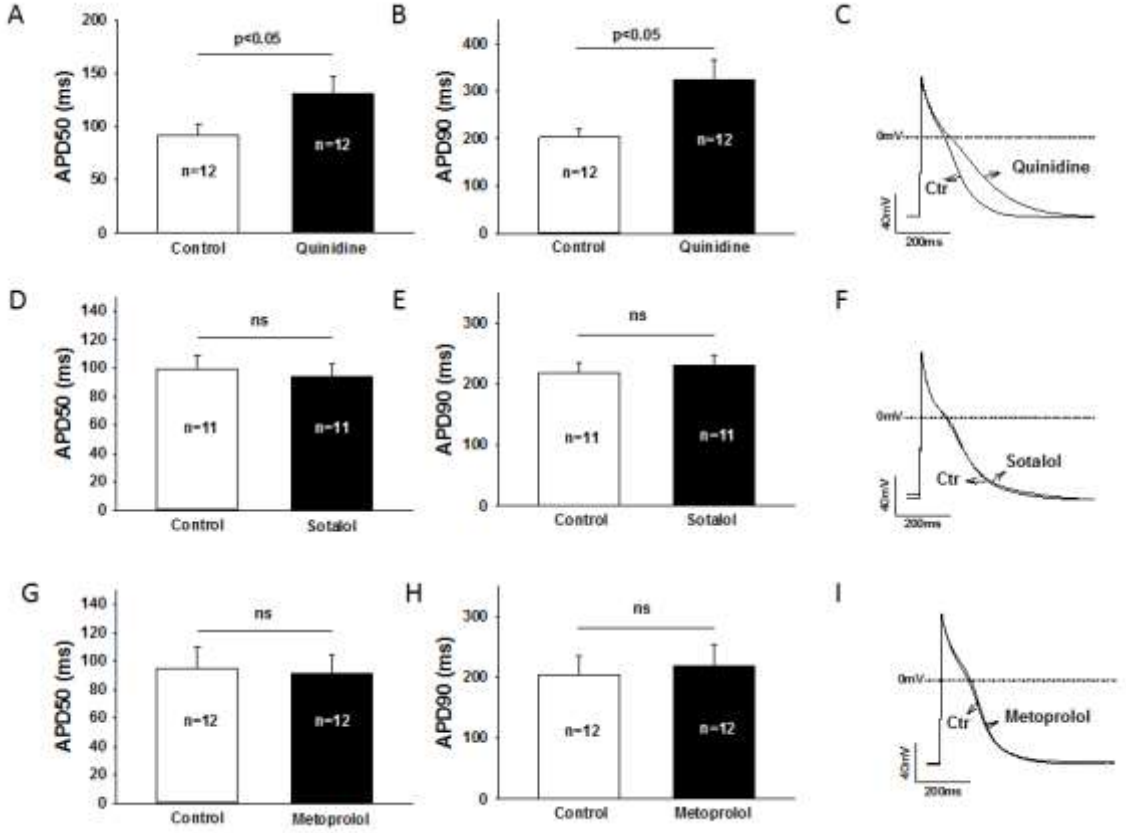
Ikr (rapidly activating delayed rectifier potassium channel) was evoked by 2 s depolarizing pulses from -70 to +70 mV with 10 mV increaments and repolarized to the holding potential of -80 mV. (A) Representative traces of I_{Kr} in a donor (D2) cell. (B) Representative traces of I_{Kr} in a SQTS (SQT) cell.

Figure S7. Effects of quinidine, sotalol and metoprolol on AP-parameters.



APs (action potentials) were recorded in a cell before (control) and after application of a drug. (A)-(C) Mean values of maximal depolarization speed (Vmax), resting potential (RP) and amplitude of AP (APA) in absence and presence of quinidine. (D)-(F) Mean values of maximal depolarization speed (Vmax), resting potential (RP) and amplitude of AP (APA) in absence and presence of sotalol. (G)-(I) Mean values of maximal depolarization speed (Vmax), resting potential (RP) and amplitude of AP (APA) in absence and presence of metoprolol. n, number of cells.

Figure S8. Effects of antiarrhythmic drugs on action potential duration (**APD) in SQTS (short QT syndrome)-cardiomyocytes.**



(A) Mean values of APD at 50% repolarization (APD50) in absence (control) and presence of 10 µM quinidine. (B) Mean values of APD at 90% repolarization (APD90) in absence (control) and presence of 10 µM quinidine. (C) Representative traces of action potentials in absence (control) and presence of 10 µM quinidine. (D) Mean values of APD at 50% repolarization (APD50) in absence (control) and presence of 100 µM sotalol. (E) Mean values of APD at 90% repolarization (APD90) in absence (control) and presence of 100 µM sotalol. (F) Representative traces of action potentials in absence (control) and presence of 100 µM sotalol. (G) Mean values of APD at 50% repolarization (APD50) in absence (control) and presence of 10 µM metoprolol. (H) Mean values of APD at 90% repolarization (APD90) in absence (control) and presence of 10 µM metoprolol. (I) Representative traces of action potentials in absence (control) and presence of 10 µM metoprolol. Values given are mean ± SEM (standard error of the mean). n, number of cells. ns, $p>0.05$.

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