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Supplemental Information

Monitoring Human-Induced Pluripotent Stem Cell-Derived Cardiomyocytes with Genetically Encoded Calcium and Voltage Fluorescent Reporters

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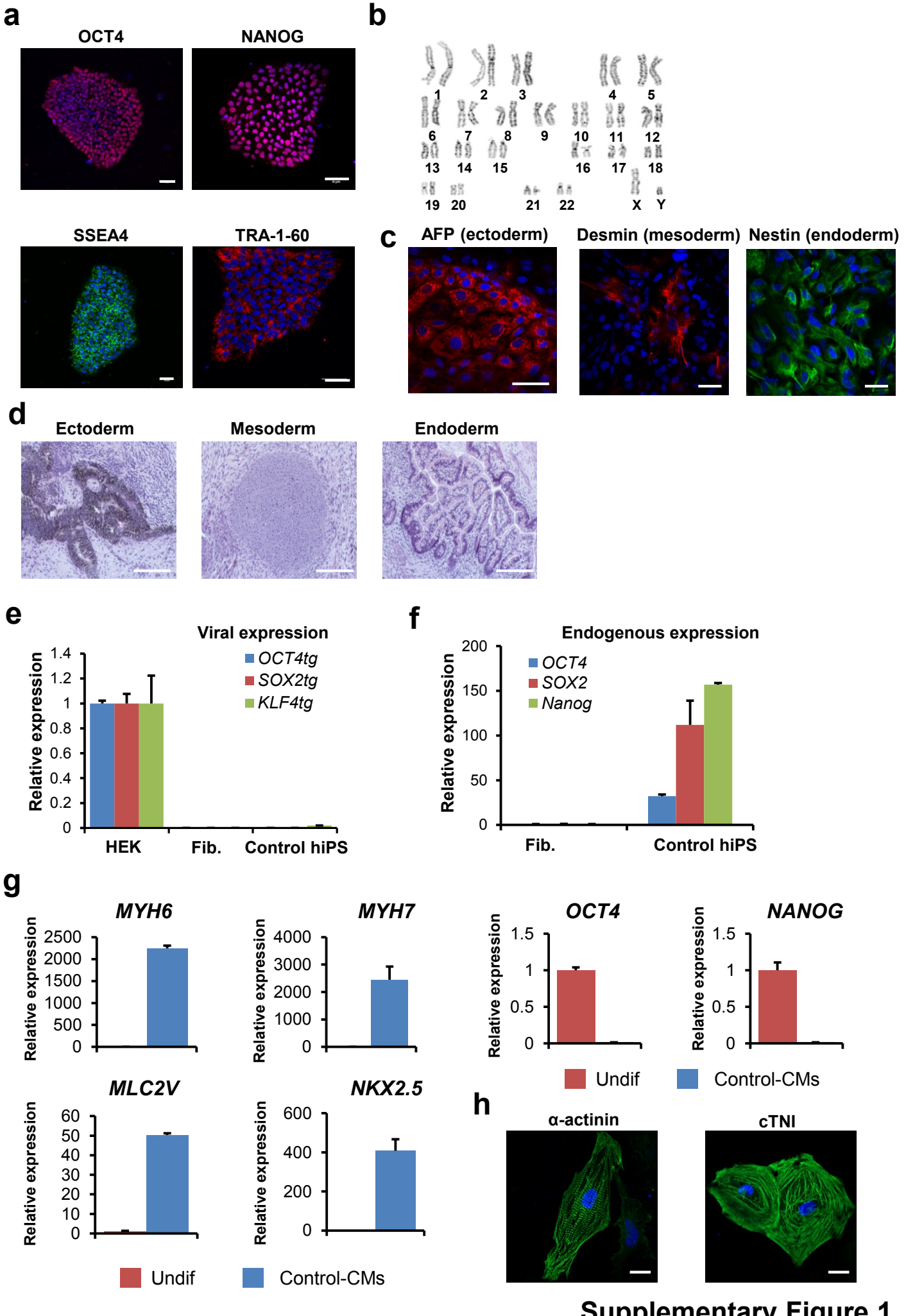
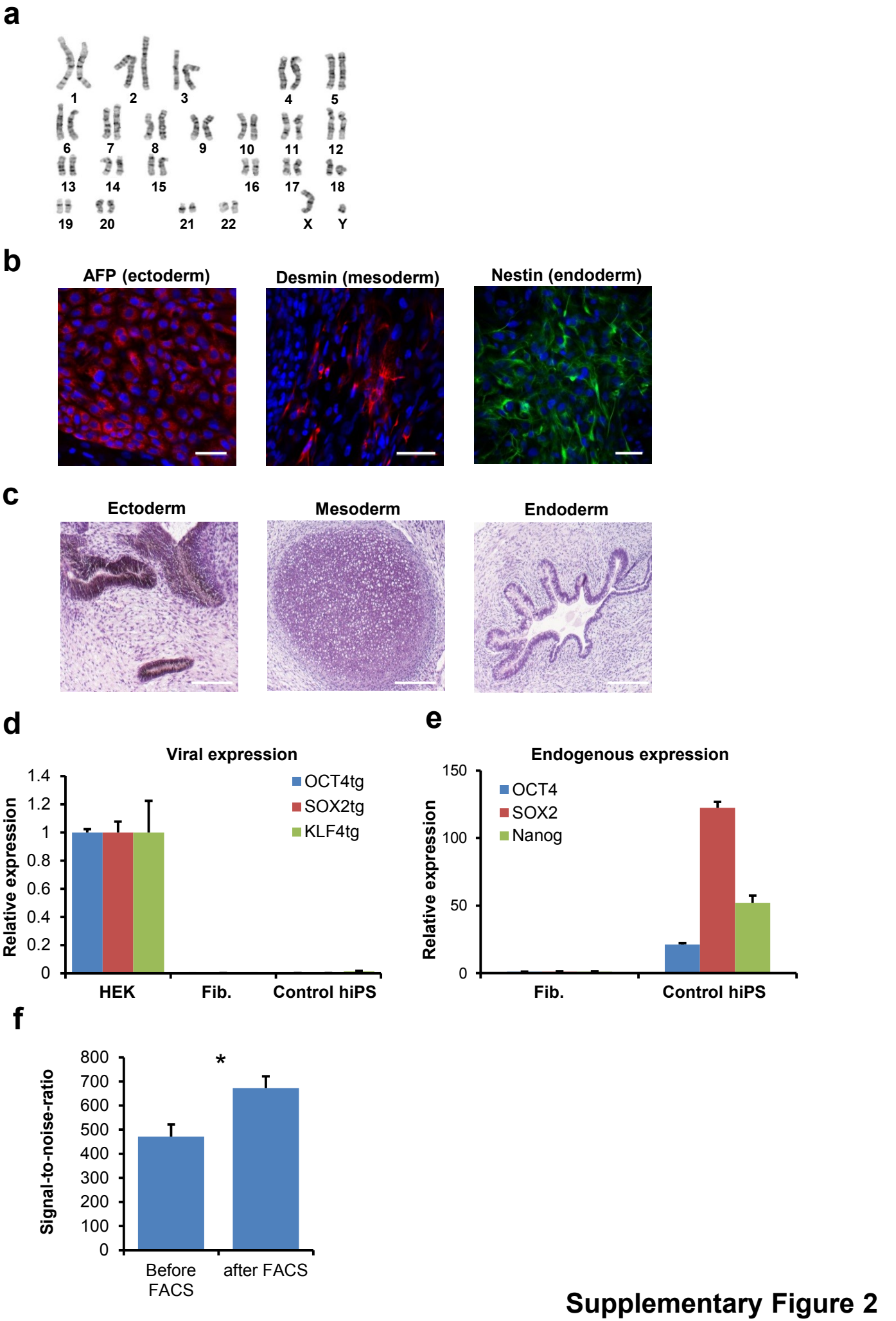
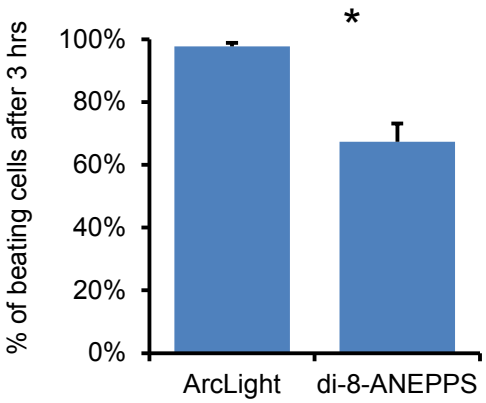


Supplementary Figure 1



Supplementary Figure 2

a



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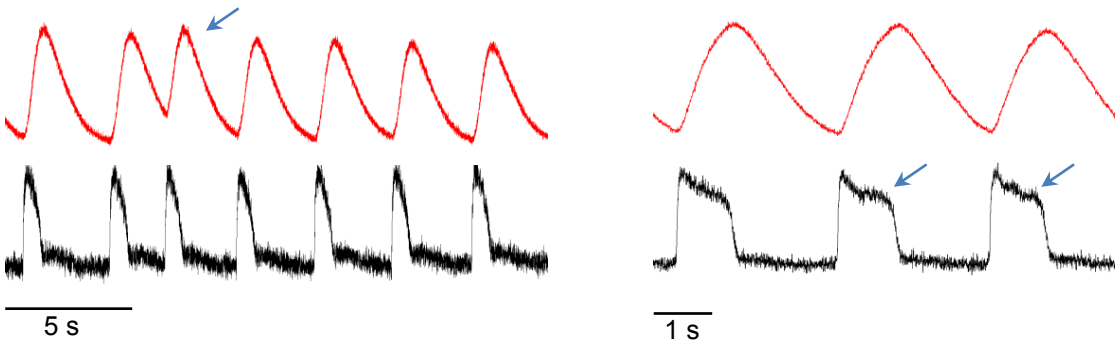
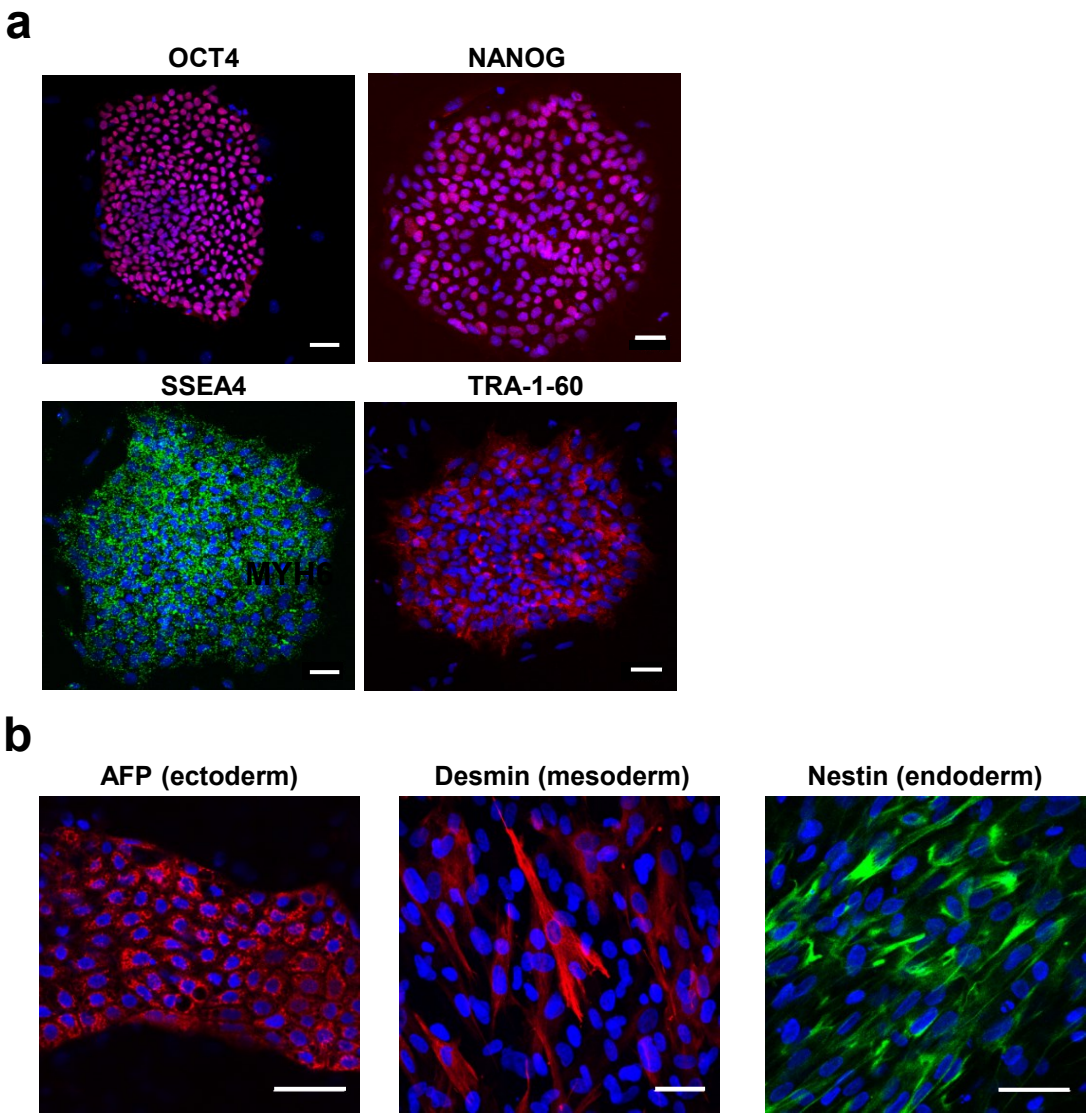


Supplementary Figure 3



Supplementary Figure 4

Supplementary Table 1

Gene	Forward primer (5'-3')	Reverse primer (5'-3')	Annealing temperature
GAPDH	ACCCACTCCTCCACCTTTGAC	ACCCTGTTGCTGTAGCCAAATT	60C
SOX2tg	ATCCCAGTGTGGTGGTACG	AAGGCATTCATGGGCCGCTTG	63C
OCT4tg	TGTACTCCTCGGTCCCTTTC	CAGGTGGGGTCTTTCATTC	63C
KLF4tg	CTGCGGCAAAACCTACACAAA	TTATCGTCGACCACTGTGCTGG	63C
OCT4	TGGTTGGAGGGAAGGTGAAG	TGTCTATCTACTGTGTCCCAG	60C
NANOG	AGAATAGCAATGGTGTGACGCAG	TGGATGTTCTGGGTCTGGTTGC	60C
SOX2	ACCAATCCCATCCACACTCAC	TCTATACAAGGTCCATTCCCCC	63C
MYH6	TACAGGACCTGGTGGACAAGC	TTGCGGAACTTGGACAGGTTG	60C
MYH7	AGAGTCGGTGAAGGGCATGAG	AGCTTGTCTACCAGGTCCTG	60C
NKX2.5	TCTATCCACGTGCCTACAGC	AGAAAGTCAGGCTGGCTCAAG	63C
MLC2V	ACAACTGACACCAACACCTGC	AGTCCAAGTTGCCAGTCACGTC	63C
CASQ2	AGAAGCTGTGTATGTGCAGG	CACTGGGTTTCTTCAAGTGC	55 C

Supplementary Figure Legends

Supplementary Figure 1: Derivation and characterization of the hiPSCs line. (Related to the "Expression of ArcLight in hiPSC-CMs" section in the Results).

[a] Immunofluorescent staining of the hiPSCs colonies for the pluripotency markers OCT4, NANOG, SSEA4, and TRA-1-60. Scale-bars: 50µm. **[b]** Karyotype analysis of the generated hiPSCs. **[c]** Immunostaining of *in vitro* differentiating EBs for Desmin (mesoderm), alpha-Fetoprotein (AFP, endoderm) and Nestin (ectoderm). Scale-bars: 50µm. **[d]** Hematoxilin and Eosin staining of teratomas formed following injection of the undifferentiated into SCID mice. Note the development of pigmented neural epithelium (ectoderm), hyaline cartilage (mesoderm), and gastrointestinal epithelium (endoderm). Scale-bars: 200µm. **[e]** Real-time quantitative PCR showing the down-regulation of *OCT4*, *SOX2* and *KLF4* transgenes in the generated hiPSCs. Values are normalized to the house-keeping gene *GAPDH* and expressed as mean±SEM. Expression values (RQ- Relative Quantification) are relative to HEK293T cells (HEK) transiently transfected with the three plasmids to produce reprogramming virions. Fib-fibroblasts used for reprogramming**. [f]** Real-time quantitative PCR evaluating the endogenous levels of the pluripotency genes *NANOG*, *SOX2*, and *OCT 4* in healthy-control fibroblasts and the generated hiPSCs. Values are normalized to the house-keeping gene *GAPDH* and expressed as mean±SEM. Expression values (RQ) are relative to levels in the dermal fibroblasts.
[g] Quantitative-PCR analysis of the differentiating hiPSCs-CM revealing the expression of cardiac-specific genes (*NKX2.5, MLC-2V, MYH-6, MYH-7*) and down-regulation of the pluripotent genes (*OCT4*, *NANOG*). All qPCR experiments included 3 biological replicates measured as 2 technical replicates. **[h]** Positive immunostaining of the hiPSC-CMs for sarcomeric α-actinin and cardiac troponin-I (cTnI).

Supplementary Figure 2: Characterization of the transgenic ArcLight-hiPSCs line. (Related to Figure 2).

[a] Karyotype analysis of the ArcLight-hiPSCs. **[b]** Immunostaining of *in vitro* differentiating EBs for Desmin (mesoderm), alpha-Fetoprotein (AFP, endoderm) and Nestin (ectoderm). Scale bars: 50μm. **[c]** Hematoxilin and Eosin staining of teratomas formed following injection of the undifferentiated into SCID mice. Note the development of pigmented neural epithelium (ectoderm), hyaline cartilage (mesoderm), and gastrointestinal epithelium (endoderm). Scale-bars: 200μm. **[d]** Real-time quantitative PCR showing the down-regulation of *OCT4*, *SOX2* and *KLF4* transgenes in the generated hiPSCs. Values are normalized to the house-keeping gene *GAPDH* and expressed as mean±SEM. Expression values (RQ) are relative to HEK293T cells (HEK) transiently transfected with the three plasmids to produce reprogramming virions. Fib-fibroblasts used for reprogramming**.** All qPCR experiments included 3 biological replicates measured as 2 technical replicates**. [e]** Real-time quantitative PCR evaluating the endogenous levels of the pluripotency genes *NANOG*, *SOX2*, and *OCT 4* in healthy-control fibroblasts and the generated hiPSCs. Values are normalized to the house-keeping gene *GAPDH* and expressed as mean±SEM. Expression values (RQ) are relative to levels in the dermal fibroblasts. All qPCR experiments included 3 biological replicates measured as 2 technical replicates
[f] Comparing ArcLight fluorescence signal-to-noise ratio (SNR) values in cardiomyocytes derived from hiPSCs before (proviral integration of 9.72 copies/cell) and after (copy number: 12.7 copies/cell) FACS enrichment. SNR increased from 470.9±50.9 (n=20 in 3 independent experiments) in the hiPSC-CMs derived from the line before FACS enrichment to 672.8±48.1 after FACS enrichment (n=17 in 3 independent experiments; *$p<0.01$).

Supplementary Figure 3: Dual fluorescent imaging. [Related to Fig.3c (a) and Fig.6j (b)].
[a] Percentage of hiPSC-CMs continuing to beat 3h following fluorescent imaging of ArcLight-hiPSC-CMs (n=121 in 10 independent experiments) or hiPSC-CMs loaded with the voltage-sensitive dye di-8-ANEPPS (n=70 in 7 independent experiments).

[b] Combined voltage and calcium imaging in hiPSC-CMs, transduced to express two genetically-encoded indicators: Arch(D95N) to derive optical action-potentials (black-tracing) and GCamP5G to acquire intracellular calcium transients (red-tracing). Shown are tracings acquired following application of ATX-II (30nM) known to augment I_{NaL}. Note the significantly prolonged action potential duration and calcium transient. Also note the development of a premature action potential (left-panel, arrows) manifested in both the optical calcium and voltage signals as well as small EADs (right-panel, arrows) identified only in the voltage recordings.

Supplementary Figure 4: Characterization of the patient-specific CPVT2-hiPSCs line. (Related to Fig.7)

[a] Immunofluorescent staining of the hiPSCs colonies for the pluripotency markers OCT4, NANOG, SSEA4, and TRA-1-60. Scale-bars: 50μm. **[b]** Immunostaining of *in-vitro* differentiating EBs for Desmin (mesoderm), alpha-Fetoprotein (AFP, endoderm) and Nestin (ectoderm). Scale-bars: 50μm. **[c]** Real-time quantitative PCR showing the down-regulation of *OCT4*, *SOX2* and *KLF4* transgenes in the generated hiPSCs. Values are normalized to the house-keeping gene *GAPDH* and expressed as mean±SEM. Expression values (RQ) are relative to HEK293T cells (HEK) transiently transfected with the three plasmids to produce reprogramming virions. Fib-fibroblasts used for reprogramming**.** All qPCR experiments included 3 biological replicates measured as 2 technical replicates. **[d]** Real-time quantitative PCR evaluating the endogenous levels of the pluripotency genes *NANOG*, *SOX2*, and *OCT 4* in healthy-control fibroblasts and the generated hiPSCs. Values are normalized to the house-keeping gene *GAPDH* and expressed as mean±SEM. Expression values (RQ) are relative to levels in the dermal fibroblasts. All qPCR experiments included 3 biological replicates measured as 2 technical replicates**. [e]** Sequencing of the *CASQ2* gene identifying the presence of the homozygous D307H point mutation in the CPVT2-hiPSCs.

Supplementary Movie Legends

Supplementary Movie 1: Dynamic display showing the cyclic changes in the fluorescence levels of dispersed single-cells ArcLight-expressing hiPSC-CMs during the cardiac-cycle. The movie shows a pair of coupled hiPSC-CMs. Note the reduction in fluorescence intensity concomitant with membrane depolarization (during the development and course of the action-potential) followed by an increase in fluorescent intensity during repolarization and resting membrane state.

Supplementary Movie 2: Efficient cardiomyocyte differentiation of the transgenic ArcLight-hiPSCs into cardiomyocytes using the directed monolayer differentiation system. Note the development of a large-scale beating monolayer that may be visual to the naked eye as well as during microscopic examination.

Supplementary Movie 3: Dynamic display depicting the cyclic changes in the fluorescence levels of the cardiomyocyte monolayer, which was derived from the stable transgenic ArcLight-hiPSCs line. Note the reduction in fluorescent intensity concomitant with the rapid depolarization of phase 0 of the action-potential, just prior to cell contraction.

Supplementary Movie 4: Dynamic display showing the cyclic changes in the fluorescence levels of single-cell dispersed GCaMP5-expressing hiPSC-CMs. Note the marked increase in the fluorescence intensity concomitant with the rise in intracellular calcium levels (following the development of an action-potential) followed by a decrease in fluorescent levels during relaxation.

Supplementary Movie 5: Dual fluorescence imaging in the same hiPSC-CMs of the genetically-encoded calcium indicator GCaMP5 (green) and the calcium-sensitive dye Rhod-3 (red). Note the temporal correlation between the changes observed in both fluorescent signals.

Supplemental Extended Experimental Procedures

Human induced pluripotent cells derivation

Dermal fibroblasts were obtained from a healthy individual and from a patient diagnosed with catecholaminergic polymorphic ventricular tachycardia type II (CPVT-2). All studies were approved by the IRB committee (Helsinki committee) of Rambam Medical Center. The fibroblasts were reprogrammed to generate the patient-specific human induced pluripotent stem cells (hiPSCs) clones by retroviral delivery of three reprogramming factors (*SOX2, KLF4 and OCT4*), followed by application of the Histone-Deacetylase inhibitor, valproic acid (VPA) as previously described (Itzhaki et al., 2012; Itzhaki et al., 2011).

Briefly, Moloney-based retroviral vectors (pMXs) containing human complementary DNAs (cDNAs) of *OCT4, SOX2 and KLF4* (Addgene plasmids 17964, 17218 and 17219, respectively) were used for retrovirus particle production. These plasmids were co-transfected with the helper plasmid encoding VSVG into HEK-293GP cells for virus production. Virus-containing media were collected at 48 and 72h after transfection and used for two 24h rounds of infection of the fibroblasts. Cells were then re-plated at a density of $1–2\times10^5$ cells per well on a MEF feeder layer, cultured in ES medium and treated with 0.9mM VPA for 14d. The hiPSC clones, which morphologically resembled hESCs and were positively stained with vital TRA-1-81 or TRA-1-60 staining, were selected and expanded for further characterization. For the long QT syndrome studies a hiPSC line previously derived in our lab using the same method was utilized (Itzhaki et al., 2011).

Propagation of hiPSCs and cardiomyocyte differentiation

Colonies of hiPSCs were cultured on 1:200 growth factor–reduced Matrigel (Corning; FAL356231) or Culltrex-coated plates (Trevigen; 3433-005-01) using hESC mTeSR-1 cell culture medium (StemCell Technologies; 05852). Cells were passaged via disassociation with

0.5mM EDTA (Invitrogen; 15575-038) in D-PBS without $CaCl_2$ or $MgCl_2$ (Life-Technologies; 10977-035) for 7 minutes at room temperature every 4-6 days, and replated in mTeSR medium supplemented with 2μM of the ROCK inhibitor Thiazovivin (Selleck Chemicals; S1459) for the first day following passaging.

To induce differentiation, three to five days after passaging or when the cells reached 80-90% confluence (day 0 of differentiation), the culture-medium was switched to a differentiation medium containing RPMI-1640, 2% B27 (Life Technologies; 21875-034), 2% B27 supplement minus insulin (Life Technologies; 05-0129SA), 1% penicillin/streptomycin (100 U/ml and 100 g/ml, respectively; Biological Industries; 03-031-1B) , supplemented with 6μM CHIR99021 (selective inhibitor of glycogen synthase kinase 3β (GSK-3β) leading to canonical Wnt pathway activation; Stemgent; 04-0004-10) for two days. On day two, medium was replaced by RPMI/B27 medium (without CHIR), and on day three medium was replaced to RPBMI/B27 medium supplemented with 5μM IWR-1 (Sigma-Aldrich; I0161) for another two days. From day 5 onwards, the cells were cultured with RPMI/B27 every other day.

On day 8-10, spontaneous contraction could be identified in the differentiating monolayers. Beating monolayers were enzymatically dissociated into small clusters and single cardiomyocytes by applying TrypLE Express (Life-Technologies) for 3 minutes at 37℃, and plated on matrigel-coated coverslips for further characterization. Studies were performed on 30-50d differentiated hiPSC-CMs.

Karyotype analysis: Karyotype analysis was performed using standard G-banding chromosome analysis according to standard procedures.

Teratoma analysis: Undifferentiated hiPSCs were injected subcutaneously to immunodeficient 5-7 week old male SCID-beige mice. Teratomas, developing 8 weeks after injection, were harvested and fixed in 4% formaldehyde, embedded in paraffin, cryosectioned (10μm) and

stained with hematoxylin and eosin (H&E). Sections were imaged using a Nikon Eclipse Ti inverted microscope equipped with a Nikon DS-Ri1 color digital camera.

In vitro differentiation into embryoid body (EB) formation: Undifferentiated hiPSCs were grown on mitomycin C treated murine embryonic fibroblasts (MEF) and dispersed into small clumps using Collagenase IV (300 U/mL; Life Technologies) for 45minutes at 37°C and cultivated for 10 days as EBs in suspension medium consisting of 80% knockout DMEM (Life Technologies) supplemented with 20% defined fetal bovine serum (HyClone), L-glutamine 1 mM (Life Technologies), mercaptoethano l 0.1 mM (Life Technologies), and 1% nonessential amino acids (Life Technologies). The EBs were plated on 0.1% gelatin-coated 18mm x18mm glass coverslips for an additional two weeks, supplemented with EBs medium composed of 80% DMEM (Life Technologies), supplemented with 20% defined fetal bovine serum, L-glutamine 1mM, mercaptoethanol 0.1 mM, 1% nonessential amino acids and 1% penicillin/streptomycin, followed by fixation and immunostaining to confirm the presence of cell-derivatives of the three embryonic germ layers.

Immunostaining studies: Undifferentiated hiPSCs colonies, differentiating EBs, and dispersed hiPSC-CMs were plated on 12mm matrigel-coated glass coverslips (Warner instruments), fixed with 4% paraformaldehyde (Bio-Lab) for 20 minutes at room temperature, washed three times in D-PBS, permeabilized with 1% Triton (Sigma-Aldrich) for 10 minutes at room temperature, blocked with 5% horse serum (Gibco) for 1 hour in room temperature, and incubated for overnight at 4°C with the primary antibodies. The antibodies used targeted: Oct-3/4 (1:150; goat; Santa Cruz Biotechnology, sc-8629), TRA-1-60 (1:200; mouse; Santa Cruz Biotechnology, sc-21705), NANOG (1:300; rabbit; Peprotech, 500-P236), SSEA-4 (1:200; mouse; R&D Systems, MAB1435), Nestin (1:200; mouse; Chemicon, Merck Millipore, MAB5326), Desmin (1:200; rabbit; Neomarkers, Thermos Scientific, RB-9014-P0), α-

fetoprotein (AFP) (1:50; mouse; Cell Marque, 203A-15), sarcomeric α-actinin (1:200; mouse; Sigma-Aldrich, A7811), and cardiac troponin I (cTNI) (1:200; mouse; Chemicon, Merck Millipore, MAB1691).

The specimens were washed three times (each for 5 minutes) with D-PBS and incubated for 1 hour at room temperature in the dark with the following 1:200 diluted secondary antibodies: Cy5 donkey anti-goat IgG (705-175-147), Cy5 donkey anti-rabbit IgG (711-175-152), Cy5 donkey anti-mouse IgG (715-175-150), Alexa Fluor 647 goat anti-mouse IgM (115-605-075), Alexa Fluor 488 donkey anti-mouse IgG (715-545-150), Cy3 donkey anti-rabbit IgG (711-165-152), Cy3 donkey anti-mouse IgM (715-165-140), Cy3 donkey anti-goat IgG (705-165-147) (All from Jackson Immunoresearch laboratories) and Cy3 goat anti-mouse IgG (Chemicon, AP124C). Primary and secondary antibodies were diluted in PBS containing 3% horse-serum and 0.1% Triton. Nuclei were counterstained with DAPI (1:500, Sigma-Aldrich, D9564). The preparations were examined using a Zeiss LSM-700 laser-scanning confocal microscope (Zeiss).

For evaluating the presence of ArcLight in ArcLight-hiPSCs and control hiPSCs, colonies were plated on 35mm imaging micro- dishes (Ibidi) and live imaging was performed using a Zeiss LSM510 META (Zeiss) laser-scanning confocal microscope. ArcLight was excited by a 488nm line of a 30mW multiline aragon laser, and emission was filtered through 505-550 nm band pass filter. For the Immunostaining studies involving undifferentiated ArcLight-hiPSC colonies and ArcLight-hiPSC-CMs, Cy5 or Alexa Fluor 647 based secondary antibodies were used to minimize cross talk with ArcLight Fluorescence.

Genomic sequencing of the CPVT2-hiPSCs: Genomic DNA was isolated from the patient-specific CPVT2-hiPSCs colonies using the high-pure polymerase chain reaction kit (Roche). The relevant DNA fragment of the *CASQ2* gene was amplified by PCR using 100ng of genomic DNA. Primers sequenced are indicated in Table S1. PCR products were then sequenced.

Real-time quantitative PCR: Relevant HEK-cells, undifferentiated hiPSCs and hiPSCs-CMs were frozen in liquid-nitrogen. Total RNA was isolated using TRIzol (Invitrogen; 15596-026) according to the manufacturer's protocol. 1µg of total RNA was DNAse treated (DNAse I, Invitrogen) and reverse transcribed using superscript II Reverse transcriptase (Invitrogen) with oligo-dT primers according to the manufacturer's protocol.

Real-time PCR was performed using LightCycler 480 SYBR green master I (Roche) on a StepOnePlus Real-time PCR system (Applied Biosystems) using the following program: five minutes pre-incubation at 95ºC and then 40 cycles of 10 seconds denaturation at 95ºC, 20 seconds annealing (temperatures are provided in Supplementary Table 1), and 20 seconds elongation at 72ºC. Data were analyzed using LinRegPCR quantitative PCR data analysis software. The starting concentration of transcripts estimated by this software was corrected for the estimated starting concentration of the housekeeping gene *GAPDH*. Primer sequences are included in Supplementary Table 1.

Lentiviral transduction of the ArcLight and GCaMp5 transgenes

Transient expression in hiPSCs-CMs: Lentiviral transduction was used for ArcLight and GCaMP5 transgene delivery to the hiPSC-CMs. To this end, HEK293T packaging cells were grown at 37°C, 5% CO_2, in DMEM supplemented with 10% FBS, glutamine and penicillin-streptomycin. A day before transfection, HEK293T cells were dissociated using 0.25% trypsin for 1 min at 37 ºC, and re-plated on 10cm poly-L-lysine 0.01% coated plates, to reach 70-80% confluence after 24 hours. pLV-CAG-*ArcLight* was kindly provided by David Milan (Massachusetts General Hospital). pLV-TroponinT-*GCaMP5* plasmid was kindly provided by John Epstein (University of Pennsylvania). pLV-GCaMP5G-*ArchD95N* was obtained from Addgene (plasmid 42168). All aforementioned plasmids and the lentiviral backbone plasmids were amplified in bacteria.

At day 0, HEK293T cells were transfected with 4.5µg of the vector, 3µg of NRF packaging plasmid and 1.5µg of the VSVG plasmid using jetPEI reagent (Polyplus; 101-10) according to the manufacturer's instructions. At day 1, HEK293T cell's medium was replaced by RPMI/B27 medium (6 ml medium per 10cm plate). Fresh virus-containing media were collected at 48 and 72 h (days 2 and 3) after transfection and used for two rounds of infection of the dissociated hiPSC-CMs. The viral dosages for the ArcLight, GCaMP5 and GCaMP5+D95N plasmids were $7x10^5$ TU/ml, $1.3x10^6$ TU/ml, and $3.4x10^6$ TU/ml respectively. No cellular adverse effects were found following transduction.

Creating a stable transgenic ArcLight-expressing hiPSCs clone. To create a transgenic hiPSCs clone stably expressing the ArcLight transgene (ArcLight-hiPSC), undifferentiated hiPSCs colonies were transduced in the same manner with pLV-CAG-*ArcLight* lentivirus. A week after transduction, positive ArcLight expressing colonies were identified based on their green-fluorescence, isolated by microdissection, and further propagated. This process was repeated for further enrichment for another two isolation rounds, performed after 3 and 4 weeks after transduction. In addition, we performed two rounds of FACS-based enrichment of the ArcLight-expressing cells based on sorting undifferentiated hiPSCs with high-levels of ArcLight green fluorescence. To determine provirus copy number, we isolated genomic DNA from undifferentiated ArcLight-hiPSCs before and after enrichment using FACS selection. Provirus copy numbers were detected by qPCR using the Lenti-X Provirus Quantitation kit (Clontech) according to manufacturer's protocol.

Flow cytometry and cell sorting. For cell sorting, undifferentiated ArcLight-hiPSC cultures were passaged via disassociation with 0.5mM EDTA in D-PBS for 7 minutes at room temperature, filtered through 40 µm cell strainer (Corning), and sorted using BD FACSAria I (BD Biosciences) based on the degree of eGFP fluorescence selecting the 10% of cells demonstrating the highest fluorescence. For differentiation efficiency evaluation, representative

hiPSC-CM differentiated cultures, from healthy control and LQT2-hiPSC-CMs, CPVT-hiPSC-CMs, and ArcLight-hiPSC-CMs were dissociated using TrypLE at 37°C, incubated with the fixable viability dye eFluor 450 (eBiosciences), fixated and permeabilized using the Fix & Perm kit (Invitrogen), and stained with the primary antibody mouse anti-cardiac troponin I (Millipore), and the secondary antibody goat anti-mouse IgG-Cy3 at 4°C, and filtered using 40μm cell strainer before flow cytometry analysis. Negative controls were samples derived from each hiPSC-CM line incubated with only the secondary antibody. Analytical flow cytometry was performed using LSR Fortessa II flow cytometer (BD Biosciences), and analysis was carried out through BD FACSDiva software (BD Biosciences).

Pharmacological studies:

E-4031 (500nM, Alomone Labs, E-500), ATX-II (30nM, Alomone labs, STA-700), ouabain (500nM, Sigma-Aldrich, O3125), sotalol (20μM, Sigma-Aldrich, S0278), isoproterenol (1μM , Sigma-Aldrich, I6504), and erythromycin (30μM, Sigma-Aldrich, E5389) were dissolved in H_2O, while chromanol 293B (Sigma-Aldrich, C2615), cisapride (Sigma-Aldrich, C4740), and nilotinib (Adooq Bioscience, A10644-25) were dissolved in DMSO. Identical DMSO amounts (0.1%) were present at final dilution and used as vehicle controls.

Optical imaging of the hiPSCs-CMs expressing the genetically-encoded indicators

A Zeiss LSM 700 laser-scanning confocal microscope equipped with a 1.0 NA water immersion x40 water objective (Zeiss W Plan-Apo) was used to measure the fluorescence intensity of ArcLight, GCaMP5G or Rhod-3-AM. The 12mm glass coverslips containing the hiPSC-CMs were placed on a 35mm dish (Corning; 430165) perfused with Tyrode's solution including (in mM): NaCl-140; KCl-5.4; $CaCl_2$-1.8; $MgCl_2$-1; HEPES-10; and glucose-10 (pH 7.4; NaOH). The Zeiss TempModule S system was used to control temperature (30-32°C). In experiments including pacing, cells were plated on 35mm optical plates (Matek) with a field

stimulation electrodes (RC-37FS; Warner instruments), and paced using stimulus isolation unit (SIU-102; Warner instruments), by applying 5-ms suprathreshold bipolar stimulation pulses of up to 50mA.

The hiPSCs-CMs expressing either ArcLight-A242 or GCaMP5G were excited using a 10mW 488nm solid-state laser and emission was split by a variable secondary dichroic beam-splitter set at 493nm collecting the high wavelengths part of the spectrum (λ>493nm) into the photomultiplier detector. The images and fluorescence data were acquired through Zen 2010 software (Carl Zeiss MicroImaging GmbH). To derive a dynamic display depicting the changes in fluorescence intensity in of the ArcLight- or GCaMP5-expressing hiPSCs-CMs (for Supplementary Movies 1, 4 and 6) the frame-acquisition mode was used. To perform morphological and quantitative analysis of the optically-derived APs and intracellular calcium transients, changes in ArcLight or GCaMP5 fluorescence were recorded using the line-scan mode (512 pixels per line) at a rate of 530 lines per second, and dynamic range of 8 bit (per pixel).

In experiments assessing drug effects, following acquisition of baseline recording the different drug solutions were added directly into the 35mm dish to achieve the required drug concentrations studied. Recording were then performed after a 15-minute stabilizing period for all of drugs tested besides nilotinib, where recording were performed after 60 minutes.

For ArcLight fluorescent studies in the hiPSCs-CMs monolayer (Supplementary Movie 3) a Zeiss Axio Observer Z1 inverted microscope with 0.3 NA x10 objective was used. The X-cite Series 120Q metal-halide arc lamp (Lumen Dynamics Group) provided high illumination of the cells, conditioned by eGFP filter set 38HE (Zeiss; 489038-0000-000) composed of 470/40-nm excitation bandpass filter, 495nm long pass dichroic and 525/50-nm emission band pass filter, imaging onto a high speed digital camera (AxioCam HSm; Zeiss) using Axiovision program (Zeiss).

Rhod-3AM loading: In experiments evolving the calcium-sensitive dye Rhod-3AM, cells were loaded with fresh loading buffer containing 10μM Rhod-3 AM (Molecular Probes; R10145), 1X PowerLoad concentrate and 2.5 mM Probenecid at room temperature in the dark for 45 minutes, washed twice with DPBS, incubated with incubation buffer containing 2.5mM Probenecid for 30 minutes, then washed and perfused with Tyrode's solution.

***Dual calcium indicator imaging*:** For the dual calcium indicator experiments, GCaMP5G and Rhod-3-AM were excited simultaneously by the 10mW (488nm) and 10mW (555nm) solid-state lasers respectively. Emission was split by a variable secondary dichroic beamsplitter set at 555nm into two photomultipliers detectors each preceded by an emission filter. For GCaMP5, fluorescence emission reaching the first photomultiplier (λ<555 nm) was short-pass filtered at 555nm; while for Rhod-3 fluorescence, emission reaching the second photomultiplier (λ>555nm) was long-pass filtered at 560nm. Fluorescent changes were acquired in the line-scan mode at a rate of 530 lines per second for optical-signal analysis and in the frame-mode for acquiring dynamic displays depicting changes in cell-fluorescence (supplementary movie 5).

Di-8-ANEPPS loading: In experiments evolving the voltage-sensitive dye di-8-ANEPPS, cells were loaded with fresh loading buffer containing 2 μM Rhod-3 AM (Molecular Probes; D-3167 and incubated at 37°C for 15 minutes, then washed and perfused with Tyrode's solution.

***Dual voltage indicator imaging*:** For the dual voltage indicator experiments, ArcLight and di-8-ANEPPS were excited simultaneously by the 10mW (488nm) solid-state laser. Emission was split by a variable secondary dichroic beamsplitter set at 555nm into two photomultipliers detectors each preceded by an emission filter. For ArcLight signal acquisition, fluorescence emission reaching the first photomultiplier (λ<555 nm) was filtered to include 490nm-520nm of emission spectrum. For di-8-ANEPPS signal acquisition, fluorescence reaching the second photomultiplier was filtered to include 615nm-800nm of emission spectrum.

Combined calcium and voltage imaging. For the combined calcium and voltage imaging involving simultaneous imaging of ArcLight and Rhod-3-AM fluorescence, a similar setup to that used for the dual calcium indicator imaging experiments was utilized.

For the dual calcium and voltage imaging of the hiPSCs-CMs using the dual genetically-encoded reporters, GCaMP5G and Arch(D95N) were excited simultaneously by 10mW 488nm solid and 10mW 555nm solid-state lasers respectively. Emission was split by a variable secondary dichroic beam-splitter set at 550nm into two photomultipliers detectors each preceded by an emission filter. For GCaMP5, fluorescence emission reaching the first photomultiplier (λ<555 nm) was short-passed filtered at 555nm; while for Arch(D95N) fluorescence, emission reaching the second photomultiplier (λ>555nm) was long-passed filtered at 640nm.

Data analysis:

ArcLight optically-derived action-potentials. ArcLight recordings were analyzed using a custom-written Maltab program (The MathWorks Inc.) in which photobleaching correction was applied by subtracting fourth polynomial function resulting from fitting the indicator photobleaching. Furthermore, 100Hz low pass filtering was applied, and APD_{90} was calculated as the average time interval required reaching 90% of repolarization starting from 50% maximal upstroke height. Average peak to peak interval was used to determine contraction-rate. For displaying the ArcLight optical signals, the fluorescence axis was inverted.

***GCaMP5G and Rhod-3 AM optically-derived intracellular calcium transient analysis*:**

GCaMP5G and Rhod-3 AM recordings were analyzed using the Clampfit 10 program (Molecular Devices) in order to measure: (1) the rise-time interval, defined as the time interval from the timing of the signal at 10% of its maximal amplitude to the timing of its peak; (2) the decay-time defined as time interval from the intracellular calcium peak to the time point in which calcium level is reduced back to 10% of its peak levels; and (3) beating rate as defined

by the average peak to peak interval.. The calcium level was indicated as F/F_0, where F_0 is the resting diastolic GCaMP5G or Rhod-3 AM fluorescence.

Arch(D95N) recordings analysis. Arch(D95N) recordings were analyzed using a custom-written Maltab program (The MathWorks Inc.) in which photobleaching correction was applied similar to the correction applied for the ArcLight recordings. For the dual indicator experiments, Fluorescence intensity of both indicators was normalized for display purposes.

Signal-to-noise-ratio analysis: To calculate the signal-to-noise-ratio we calculated both the noise power and the effective signal power. The noise power was calculated as the mean squared value of 100-ms interval at the end of phase 4 of the AP or before the beginning of the next calcium transient. The effective signal power was calculated as the mean squared values of 40-ms interval at the maximum of the AP/calcium transient minus the noise power. The signal-to-noise-ratio was calculated as the ratio between the effective signal power to the noise power. For each cell 10 consecutive APs or calcium transients were used to calculate the signal-to-noise-ratio. For comparing signal-to-noise-ratio of ArcLight and di-8-ANEPPS recordings only single indicator experiments were used, due to the fact that in dual-indicator experiments part of the emission fluorescence was filtered to minimize signal crosstalk.

Statistical analysis: Continuous variables are reported as mean ± SEM. Categorical variables are expressed as frequencies. Categorical differences between groups were evaluated by using the chi-square test. Differences between group means were compared using the unpaired student *t* test. For the drug studies, differences between baseline and post-drug application values were compared using paired student t test. A value of $p<0.05$ was considered statistically significant.

References:

Itzhaki, I., Maizels, L., Huber, I., Gepstein, A., Arbel, G., Caspi, O., Miller, L., Belhassen, B., Nof, E., Glikson, M.*, et al.* (2012). Modeling of catecholaminergic polymorphic ventricular tachycardia with patient-specific human-induced pluripotent stem cells. J Am Coll Cardiol *60*, 990-1000.

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