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Supplemental Information

Mitochondrial Dysfunctions Contribute to Hypertrophic Cardiomyopathy in Patient iPSC-Derived Cardiomyocytes with *MT-RNR2* Mutation

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SUPPLEMENTAL FIGURE LEGENDS

Figure S1. Generation and characterization of induced pluripotent stem cells. (A) Phase contrast photographs of H1 embryonic stem cells (ESCs), iPSCs derived from control participants (Con-iPSCs), and iPSCs derived from HCM patients (HCM-iPSCs), all grown on feeder layers. iPSCs at passage 10 or more are shown. Scale bar, 100 μm. The same cell lines were stained for alkaline phosphatase (AP). Scale bar, 100 μm. Immunofluorescence analysis for the presence of the pluripotency markers (TRA-1-60, TRA-1-81, Nanog, SSEA-3 and SSEA-4) are indicated; nuclei are stained in blue with DAPI. Scale bar, 50 μm. See also Figures S2A-S2E. (B) Quantitative PCR was performed to detect the expression of endogenous pluripotent genes and marker genes in three germ layers from *in vitro* HCM-iPSC embryoid bodies (HCM-iPSC-EBs) and Ctrl-iPSC-EBs. H1 ESCs were used as a control. Values refer to the respective undifferentiated pluripotent colonies.*OCT4* and *NANOG* were used as markers of pluripotency, whereas *AFP*, *GATA4*, and *SOX17* (endoderm), *TBX1* (mesoderm), and *PAX6* and *SOX1* (ectoderm) were used as markers of differentiation. (C) Teratoma produced by HCM-iPSCs and Con-iPSCs showing derivatives of ectoderm (pigmented epithelium), mesoderm (smooth muscle) and endoderm (gut epithelium). Scale bar, 20 μm.

Figure S2. Generation and characterization of induced pluripotent stem cells. (A) The expression of endogenous pluripotent genes of iPSCs. UCs are negative controls and H1 is positive control. (B) The silencing of exogenous transgenes of

iPSCs. UCs are negative controls and H1 is positive control. (C) Integration of the exogenous transgenes by semi-quantitative PCR. (D) The methylation of promoter regions in the *Oct4* and *Nanog* genes of iPSCs by bisulfite sequencing analysis. UCs are negative controls and H1 is positive control. (E) Karyotype analysis of HCM-iPSCs and Ctrl-iPSCs.

Figure S3. Identification of m.2336T>C mutation in the *MT-RNR2* gene. Partial sequence chromatograms of the 16S rRNA gene from Con-UCs (A), Con-iPSCs (B), HCM-UCs (C) and HCM-iPSCs (D), respectively. The arrows indicate the location of the base change at position 2336.

Figure S4. Representative immunostaining and quantification analyses of MLC2v and MLC2a. (A) Representative immunostaining for MLC2v and MLC2a in HCM-iPSC-CMs and Con-iPSC-CMs. Nuclei are stained in blue with DAPI. (B) Quantification of single and double positive cells in HCM-iPSC-CMs (n=41) compared to Con-iPSC-CMs (n=65). Data represent three control individuals (3 clones) and one patient (3 clones) with three independent experiments. The n is the total replicates of each Group. Data are represented as mean ± SEM.

Figure S5. Western blotting and quantification analyses of mitochondrial calcium uniporter (MCU). VDAC is shown as a loading control. Data represent three control individuals (3 clones) and one patient (3 clones) with three independent experiments. Data are represented as mean ± SEM. ***P<0.001.

Figure S6. Steady-state level of SR Ca^{2+} transport marker proteins. (A) Western blotting and quantification analysis of SERCA, NCX, PLB, PLB-p. β-ACTIN is

shown as a loading control. (B) The PLB-p/PLB ratio in Con-iPSC-CMs and HCM-iPSC-CMs. Data represent three control individuals (3 clones) and one patient (3 clones) with three independent experiments. Data are represented as mean ± SEM. *P<0.05.

Figure S7. Properties of K^+ current (I_K) in HCM-iPSC-CMs. (A) Representative traces for I_K current using whole cell patch-clamp from HCM-iPSC-CMs (n=28) and Con-iPSC-CMs (n=30). (B) Current-voltage (I-V) relationship of I_K was determined from -60 to +60 mV in 10 mV increments from HCM-iPSC-CMs (n=28) and Con-iPSC-CMs (n=30). (C) Mean peak of I_K in HCM-iPSC-CMs (n=28) and Con-iPSC-CMs (n=30). (D) I_K density-voltage relationship was determined from -60 to +60 mV in 10 mV increments from HCM-iPSC-CMs (n=28) and Con-iPSC-CMs (n=30). (E) Peak I_K density in HCM-iPSC-CMs (n=28) and Con-iPSC-CMs (n=30). Data represent three control individuals (3 clones) and one patient (3 clones) with three independent experiments. The n is the total replicates of each Group. Data are represented as mean ± SEM. *P<0.05, **P<0.01, ***P<0.001.

EXPERIMENTAL PROCEDURES

Ethical Statement

The clinical evaluations and urine cells (epithelial-like cells detached from tubules) were obtained from HCM family members under informed consent and using procedures approved by the Ethics Committee of the First Affiliated Hospital, Zhejiang University School of Medicine, China.

Generation of HCM-iPSCs

The clinical evaluations and urine cells (epithelial-like cells detached from tubules) were obtained from HCM family members under informed consent and using procedures approved by the Ethics Committee of the First Affiliated Hospital, Zhejiang University School of Medicine, China. The generation and characterization of patient-specific induced pluripotent stem cells (iPSCs) from urine samples were performed as previously reported (Zhou et al., 2012; Zhang et al., 2016). Urine cells were cultured in REBM (Renal Epithelial Basal Medium, Lonza) medium containing SingleQuot Kit CC-4127 supplements (Lonza). pMX-based retroviruses (Addgene) with the reprogramming factors Oct-4, Sox-2, Klf-4, and c-Myc were infected into 5-10×10^4 cells in a 6-well culture dish. After 3 or 4 days of transfection, 5×10^4 cells were split onto a layer of feeder cells using half human embryonic stem cell (ESC) medium with 10 ng/mL basic fibroblast growth factors (Invitrogen) and half dFBS medium [(DMEM high glucose medium (Invitrogen) with 20% human defined fetal bovine serum (Hyclone)]. On day 5, 50 μg/ml Vitamin C (Sigma) and 1 mmol/L valproic acid (Merck)

were added; Vitamin C was maintained in the medium but valproic acid was removed on day 12. The medium was replaced with mTesR1 medium (StemCell) on day 12 to the end of the experiment. Colonies with an ESC-like appearance were picked up and expanded on feeders or mTesR1 medium with Matrigel (BD Biosciences). We defined this stage as passage one.

iPSCs characterization

Characterization of iPSCs, such as alkaline phosphatase (AP) staining, expression of endogenous pluripotent genes, silencing of exogenous transgenes, transgene integration, immunocytochemistry, karyotyping and DNA methylation, were performed as previously reported (Zhang et al., 2016). DNA was extracted using a DNeasy Tissue kit (Qiagen), and total RNA was extracted using Trizol (Invitrogen). Quantitative real-time PCR (qPCR) was performed using an ABI PRISM 7900HT Sequence Detection System (Applied Biosystems) and SYBR Premix EX Taq^{TM} (Takara). The samples were analyzed in triplicate and β-actin values were used for normalization. Bisulfite sequencing technology was used to detect the methylation pattern of the promoter region of *Oct4* and *Nanog*. Immunofluorescence staining confirmed the expression of pluripotency markers in these iPSCs. Primary antibodies included Nanog (1:200, R&D), SSEA-3 (1:400, Developmental Studies Hybridoma Bank), SSEA-4 (1:100, Abcam), TRA-1-60 (1:100, Millipore), and TRA-1-81 (1:100, Millipore). The secondary antibodies included Alexa Fluor 488 goat anti-mouse IgM, Alexa Fluor 594 goat anti-rat IgM, Alexa Fluor 594 donkey anti-mouse IgG, and Alexa Fluor 594 donkey anti-

goat IgG (all from Invitrogen). A laser scanning confocal microscope LSM710 (Zeiss) was used to capture immunofluorescence staining images.

In vitro and *in vivo* differentiation

To determine the differentiation ability of human iPSCs *in vitro*, we used floating cultivation to form embryoid bodies (EBs), as previously reported (Zhang et al., 2016). iPSCs on feeders were treated with dispase (Invitrogen) and then collected by scraping. After centrifugation, cell pellets were re-suspended in human ESC medium without bFGF and grown for 8 days in non-adherent dishes. The formed EBs were then transferred to Matrigel-coated dishes to allow differentiation for another 8 days before RNA extraction. To test iPSC pluripotency *in vivo*, we injected 2×10^6 iPSCs subcutaneously or intramuscularly into immunocompromised non-obese diabetic–severe combined immunodeficient mice (NOD-SCID) using procedures approved by the experimental animal welfare ethics committee of Zhejiang University, China. The tumors were sectioned after 8-10 weeks, fixed with PBS containing 4% paraformaldehyde and stained with hematoxylin/eosin (Zhang et al., 2016).

Differentiation of iPSCs into cardiomyocytes

Established iPSC lines from HCM patients and the control group were differentiated into cardiomyocyte lineages (iPSC-CMs) using monolayer differentiation protocols as described previously (Lian et al., 2012). iPSCs were seeded onto a Matrigel-coated cell-culture dish at $1\text{-}2\times10^5$ cells/cm^2 in mTeSR1 supplemented with 5μmol/L ROCK

inhibitor (Y-27632, Sigma) (day-5) for 24h. Then, the cells were cultured in mTeSR1 medium, which was changed daily (day-4~day-1). At day 0, the cells were treated with CHIR99021 (12μmol/L, Selleck) in RPMI1640/B27-insulin for 24h. The medium was then changed to RPMI1640/B27-insulin (day1). IWP2 (5μmol/L, Tocris) was added at day 3 and then removed during the medium change on day 5. The cells were maintained in RPMI1640/B27 starting on day 7, and were subcultured every 3 days. The mature beating CMs were picked up, dissociated and seeded on coverslips for functional assays, such as calcium imaging, patch-clamp, immunostaining and more.

Measurement of cell size

Cell size measurements were performed as previously described (Parra et al., 2014). Cells were cultured on coverslips for 72 h, incubated for 1 h with Mitotracker Red (200 nmol/L) and maintained in RPMI 1640 medium. Then cells were fixed with RPMI 1640 medium containing 4% paraformaldehyde for 15 min, and 0.4% Triton X-100 for permeabilization for 15min. Nonspecific sites were blocked with 5% skimmed milk in PBS for 1 h and then the cells were incubated withanti-TNNI3 at 4℃ overnight. The secondary antibody was Alexa-Fluor-488-conjugated anti-mouse-IgG. Confocal image stacks were captured with a Zeiss LSM710 laser scanning confocal microscope and the analysis software was Zen2011.Cell size was analyzed using the Image J-3D Object counter plug-in. Each experiment was performed at least three times and 30-50 cells per condition were quantified.

ATP/ADP measurements

The ATP/ADP level in cells was measured using bioluminescent detection (ADP/ATP Ratio Assay Kit, Abcam) according to the manufacturer's instructions. The cells were plated in 96well microplates (Corning) with RPMI1640 medium containing 20% FBS for 72 hrs. The bioluminescent intensities were measured on multi-mode microplate reader (Synergy H1 Hybrid, BioTek). The ATP test mixture (100μL/well) was added to obtain Data A. Next, 50μL/well of Nucleotide Releasing Buffer was added to release ATP and ADP and obtain Data B and Data C. Finally, Data D was determined by adding the ADP test mixture. The ATP/ADP ratio = (Data B-Data A) / (Data D-Data C). Each experiment was repeated at least three times.

Mitochondrial membrane potential assay

The mitochondrial membrane potential was measured using fluorescence detection (JC-10, Abcam) according to the manufacturer's instructions. iPSC-CMs were cultured on 96-well black-walled clear-bottom plates for 48 hrs in mTeSR medium and RPMI1640 medium with 20% FBS, respectively. After the above cells were incubated with 100 μL fresh medium for 30 min and dyed with 50 μL JC-10 solution for 30min, the fluorescence intensities (Ex/Em=485/525nm and Ex/Em=540/590nm) were measured on multi-mode microplate reader (Synergy H1 Hybrid, BioTek). The ratio between monomeric (Em=590 nm) and aggregate (Em=525 nm) forms of JC-10 represents the change in the mitochondrial membrane potential. A high ratio indicates a high mitochondrial membrane potential.

Measurement of intracellular Ca^{2+}

The intracellular Ca^{2+} concentrations were measured as described previously with slight modifications (Liao et al., 2016). iPSC-CMs were dissociated and loaded in a culture medium containing 5 μmol/L Fura-2 acetoxymethylester (Fura-2AM, Dojindo Laboratories) at 37°C for 30min and then washed to remove extracellular Fura-2AM. They were then resuspended in Hanks' balanced salt solution (140 mmol/L NaCl, 5 mmol/L KCl, 10 mmol/L HEPES, 1 mmol/L $CaCl_2$, 1 mmol/L $MgCl_2$, 1 mg/ml glucose, pH7.4) at a concentration of 3×10^7 cells/mL. The calcium flux was measured using excitation at 340 nm and 380 nm in a fluorescence spectrometer (LS55, PerkinElmer Life Sciences). Calibration was performed using 0.1% Triton X-100 for total fluorophore release and 10 mmol/L EGTA to chelate the free Ca^{2+}. The intracellular Ca^{2+} concentrations were calculated using a fluorescence spectrometer measurement program.

Calcium Imaging

Calcium imaging were performed as described previously with slight modifications (Zou et al., 2017). iPSC-CMs were seeded on gelatin-coated glass coverslips for 2 days. Cells were dissociated and loaded with 5 μmol/L Fura-2 AM (Dojindo Laboratories) in bath solution containing 0.01% pluronic F127 for 30 min at room temperature, and washed 5 times with bath solution. Imaging was acquired in an Olympus microscope (BX51WI) with a 40 × objective lens on an Andor DL-604M EMCCD camera. Data

was collected using the Macro-manager software. Fura-2 was excited by a Lambda XL light source and fluorescent signals were collected at a rate of 1 Hz. The fluorescence ratio F(λex340 nm)/F(λex380 nm) was calculated and imaging data were analyzed using Imaging J. The bath solution contained: 140 mmol/L NaCl, 5 mmol/L KCl, 2 mmol/L $MgCl_2$, 1.8 mmol/L $CaCl_2$, 10 mmol/L HEPES, 10 mmol/L glucose.

Caffeine treatment of iPSC-CMs

Cells were perfused with PBS containing 1.8 mmol/L Ca^{2+} and 2 mmol/L Mg^{2+} and paced at 1Hz to view regular transients. A pulse of 20 mmol/L stock caffeine solution was added after 1 minute recording.

Measurement of L-type Ca^{2+} currents

The L-type Ca^{2+} currents (I_{CaL}) were recorded using whole-cell patch-clamp (Axopatch-700A and MultiClamp 700B amplifier, Axon Instruments, Sunnyvale, CA), as previously described (Hamill et al., 1981). The pipette electrode (2.5-4.5 MΩ) was filled with a solution containing 120 mmol/L CsCl, 5 mmol/L Na_2-ATP, 5 mmol/L $MgCl_2$, 10 mmol/L EGTA, and 5 mmol/L HEPES, pH 7.2. The external solution contained 140 mmol/L NaCl, 1 mmol/L $MgCl_2$, 1.8 mmol/L $CaCl_2$, 5 mmol/L CsCl and 10 mmol/L HEPES (pH 7.4). I_{CaL} was elicited by a single 200 ms voltage pulse to various test potentials from the holding potential of -40 mV at a frequency of 0.1Hz. The recordings were initiated 5 min after the patch break, to allow for equilibration of the patch pipette solution with the intracellular milieu. The current-voltage (I/V)

relationship of I_{CaL} was obtained by plotting the peak current amplitude in response to voltage pulses to potentials between -40 mV and +60 mV from the holding potential in 10 mV increments. The data was then analyzed with Lab Chart8 (AD Instruments).

Current-voltage (*I*-*V*) relationship of I_{CaL}

The current-voltage (*I*-*V*) relationship of I_{CaL} was determined by applying test pulses (100 ms) from -60 to +50 mV in 10 mV increments at a frequency of 0.2 Hz. Prepulses from a holding potential of -80 to -40 mV (P, duration: 50 ms) along with TTX (10 mmol/L) were applied to inactivate Na^+ currents. The prestep to -40 mV was also applied to inactivate the T-type Ca^{2+} current.

Measurement of K^+ currents

For recording K^+ currents the extracellular solution contained: 135 mmol/L N-methyl-D-glucamine (NMDG) chloride, 5 mmol/L KCl, 3.6 mmol/L CaCl2, 1 mmol/L MgCl2, 3 mmol/L NiCl2, and 10 mmol/L HEPES, pH adjusted to 7.40 at 37°C with NMG. The intracellular solution contained: 50 mmol/L KCl, 80 mmol/L K-aspartate, 1 mmol/L $MgCl_2$, 3 mmol/L MgATP, 10 mmol/L EGTA, and 10 mmol/L HEPES, pH adjusted to 7.40 with NMDG. Depolarization-activated outward K^+ currents were elicited by a family of 500 ms depolarizations from a –80 mV holding potential to voltages ranging from –60 to +60 mV (10 mV steps).

Steady-state activation and inactivation of I_{CaL}

Steady-state activation was determined by stepping to various prepulse voltages (10 mV increments) levels from -60 to +20 mV for 100 ms from a holding potential of -80 mV before repolarizing to a fixed test pulse at -50 mV. Steady-state inactivation was determined by stepping to various prepulse voltages (-60 to +40 mV, 10 mV increments) for 5 s before depolarization to a fixed test potential of + 10mV for 100 ms to evoke channel opening. There was a 5 ms interpulse interval that enabled the resetting of the activation gate between the end of the prepulse and the beginning of the test pulse. Both steady-state activation and inactivation curves were fitted with a Boltzmann function (G=A2+(A1-A2)/(1+exp((V1/2-x)/k) that describes conductance (G) as a function of the potential (x). V1/2 is half-activating or half inactivating potential, and k is the slope factor.

Action potential analysis

For single-cell action potential analysis, the whole-cell patch clamp configuration (Axopatch-700A and MultiClamp 700B amplifier, Axon Instruments, Sunnyvale, CA) (Hamill et al., 1981) was used. Cells were digested at 37℃ for 30 min with 1 mg/ml collagenase B (Roche) and seeded for 2 days on gelatin-coated glass coverslips. For action potential recording, patch pipettes (3-5 MΩ) were filled with solutions containing 10 mmol/L HEPES, 120 mmol/L KCl, 1 mmol/L $MgCl_2$, 3 mmol/L Mg-ATP and 10 mmol/L EGTA, (pH 7.3), and a bath solution containing10 mmol/L HEPES, 140 mmol/L NaCl, 5.4 mmol/L KCl, 1.8 mmol/L $CaCl_2$, 10 mmol/L glucose, and 1 mmol/L $MgCl_2$ (pH 7.4). The MultiClamp 700B Amplifier, Digidata 1440A analog-to-digital

converter and pClamp10.2 software (Axon Instruments/ Molecular Devices) were used for data amplification, acquisition and analysis.

Western blot analysis

Twenty micrograms of proteins obtained from iPSCs and iPSC-CMs were denatured and loaded on 12% SDS-PAGE (sodium dodecyl sulfate polyacrylamide gels). Afterward, the proteins were electro-transferred to polyvinylidene difluoride (PVDF) membrane and subjected to Western blotting. Membranes were blocked in Tris-Buffered Saline and Tween20 (TBST) (150 mmol/L NaCl, 10 mmol/L Tris–HCl, pH 7.5 and 0.1% (v/v) Tween 20) containing 5% (w/v) milk, then incubated with the corresponding primary and secondary antibodies. The primary antibodies used for this experiment were the ND5 (1:1000, Abcam), COII (1:2000, Abcam), CYTB (1:1000, Sigma), ATP8 (1:200, Santa Cruz), ANF (1:2000, Abcam), BNP (1:2000, Abcam), TNNNI3 (1:2000, Abcam), α-ACTIN (1:500, Immunoway), MLC2v (1:1000, Abcam), MLV2a (1:500, Proteintech), RyR (1:1000, Abcam), PLB (1: 1000, Abcam), PLB-p (1:500, Abcam), SERCA2 (1:500, Abcam), MCU (1:1000, Abcam), NCX (1:1000, Abcam), β-ACTIN (1:1000, Proteintech) and VDAC (1:1000, Abcam). Peroxidase Affini Puregoatanti-mouse IgG and goatanti-rabbit IgG (Jackson) were used as a secondary antibody and protein signals were detected using the CLINX chemiscope and ECL system (CWBIO).

Quantitative real-time PCR

Total RNA preparations were obtained by using Trizol reagent (Invitrogen) from 1×10^7 cells as described elsewhere. One microgram of total RNA was reverse transcribed to cDNA using PrimeScript RT reagent Kit (Takara). The reaction system was the followed SYBR Premix EX TaqTM (Takara) instructions. The forward and reverse primers for PCR amplification are shown in supplemental table 1. *GAPDH* or *VDAC* served as an endogenous control. The quantitative real-time PCR was performed on an ABI PRISM 7900HT Sequence Detection System (Applied Biosystems). The relative amount of mRNA to endogenous control was calculated using the Ct cycle method as following formula: ΔΔCt=[Ct(target gene)−Ct(control gene)]target cell − [Ct(target gene)−Ct(control gene)]reference cell.

Quantification of mtDNA copy number

The mtDNA copy number was quantified as described previously (Venegas et al., 2012). A 1241bp section of the human β-globin gene and a 1724bp sequence of mtDNA located within the 16S rRNA gene was inserted into the cleaved blunt-ended *Hind* Ⅲ and *Ava* Ⅰ restriction sites of the pCR plasmid, respectively. The resultant plasmid pCR Ⅱ (6073bp) was verified as having only one copy of each insert by restriction enzyme digestion and DNA sequencing. The relative mtDNA copy numbers were measured by quantitative real-time PCR and corrected by measurement of the nuclear DNA. The primers for the *MT-RNR2* gene were 5'-GCACACCCGTCTATGTAGCAAA -3' and 5'-GATTTAGAGGGTTCTGTGGGCA-3'. The primers for the nuclear β-globin gene were 5'-

AGAAACTGGGCATGTGGAGACA-3' and 5'-ATGAGCCTTCACCTTAGGGTTG-3'. The quantitative real-time PCR was performed using an ABI PRISM 7900HT Sequence Detection System (Applied Biosystems) and SYBR Premix EX Taq™ (Takara). Sample mtDNA content (mtDNA copies per cell) were calculated using the following formula:

mtDNA copies per cell = (*MT-RNR2* gene copies/β-globin gene copies) × 2

Mutational analysis of mitochondrial genome

Genomic DNA was isolated from the iPSCs of HCM patients and healthy unrelated individuals using DNeasy Tissue kit (Qiagen). The entire mtDNA were PCR amplified in 24 overlapping fragments by use of sets of the light-strand and heavy-strand oligonucleotide primers, as described previously (Liu et al., 2014). Each fragment was purified and subsequently analyzed by direct sequencing in an ABI 3730xl automated DNA sequencer using the Big Dye Terminator Cycle sequencing reaction kit. The resultant sequence data was compared with the revised Cambridge reference sequence (GenBank accession No. NC_001807) (Andrews et al., 1999). The published data on http://www.mtdb.igp.uu.se/ was used to determine the allelic frequency of the identified variants.

Statistical analysis

Statistical analysis was carried out using the Student's unpaired, two-tailed t-test contained in the Microsoft-Excel program. In Figure Legends, all data represent three

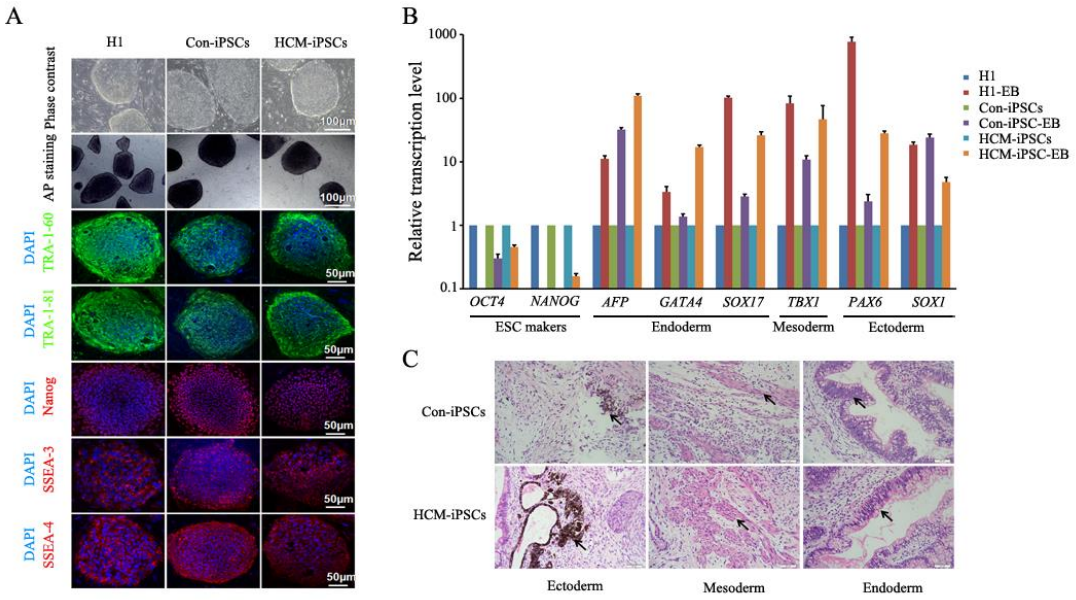
control individuals (3 clones) and one patient (3 clones) with at least three independent experiments. Several replicates for each clone were performed in every independent experiment. The n is the total replicates of each Group. Data are represented as mean ± SEM. *P<0.05, **P<0.01, ***P<0.001.

Supplemental References

Andrews RM, Kubacka I, Chinnery PF, Lightowlers RN, Turnbull DM, Howell N. (1999). Reanalysis and revision of the Cambridge reference sequence for human mitochondrial DNA. Nat Genet. 23:147.

Venegas V and Halberg MC. (2012). Measurement of mitochondrial DNA copy number. Methods Mol Biol. 837:327-35.

Figure S1.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H1
Con-iPSCs
HCM-iPSCs
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DAPI Nanog
DAPI SSEA-3
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100μm
50μm
B
Relative transcription level
1000
100
10
1
0.1
H1
H1-EB
Con-iPSCs
Con-iPSC-EB
HCM-iPSCs
HCM-iPSC-EB
OCT4
NANOG
AFP
GATA4
SOX17
TBX1
PAX6
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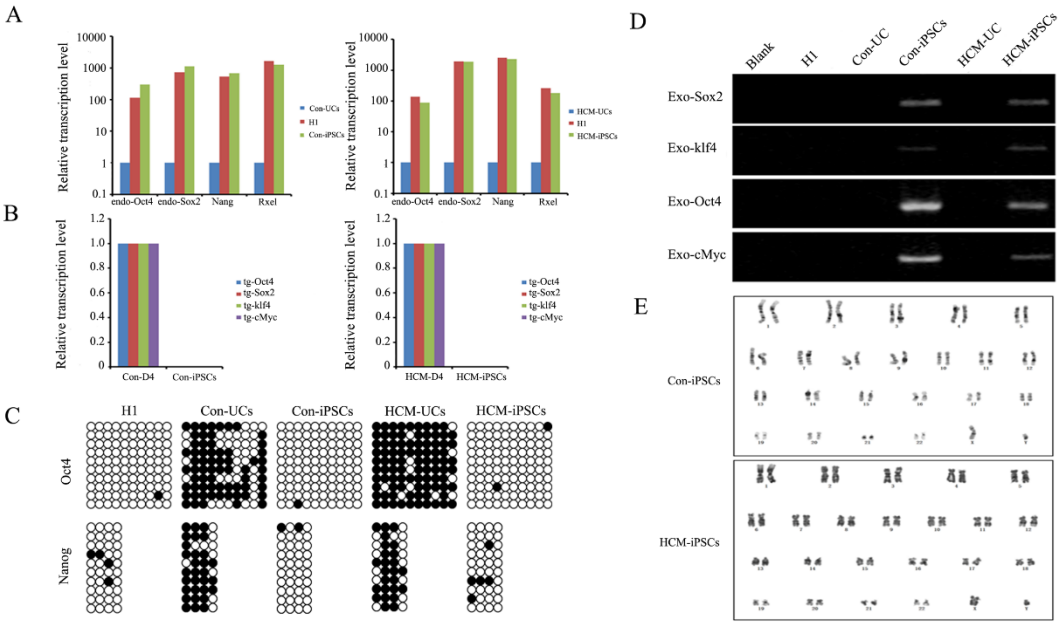
Endoderm

Figure S2.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Rxel
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Con-iPSCs
HCM-UCs
HCM-iPSCs
B
1.2
1.0
0.8
0.6
0.4
0.2
0
Con-D4
HCM-D4
tg-Oct4
tg-Sox2
tg-klf4
tg-cMyc
C
H1
Con-UCs
Con-iPSCs
HCM-UCs
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Figure S3.

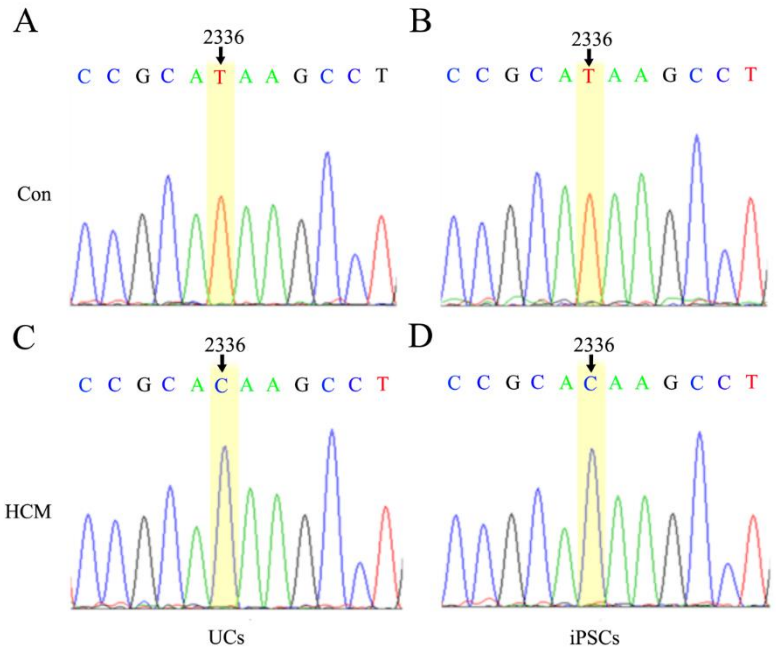
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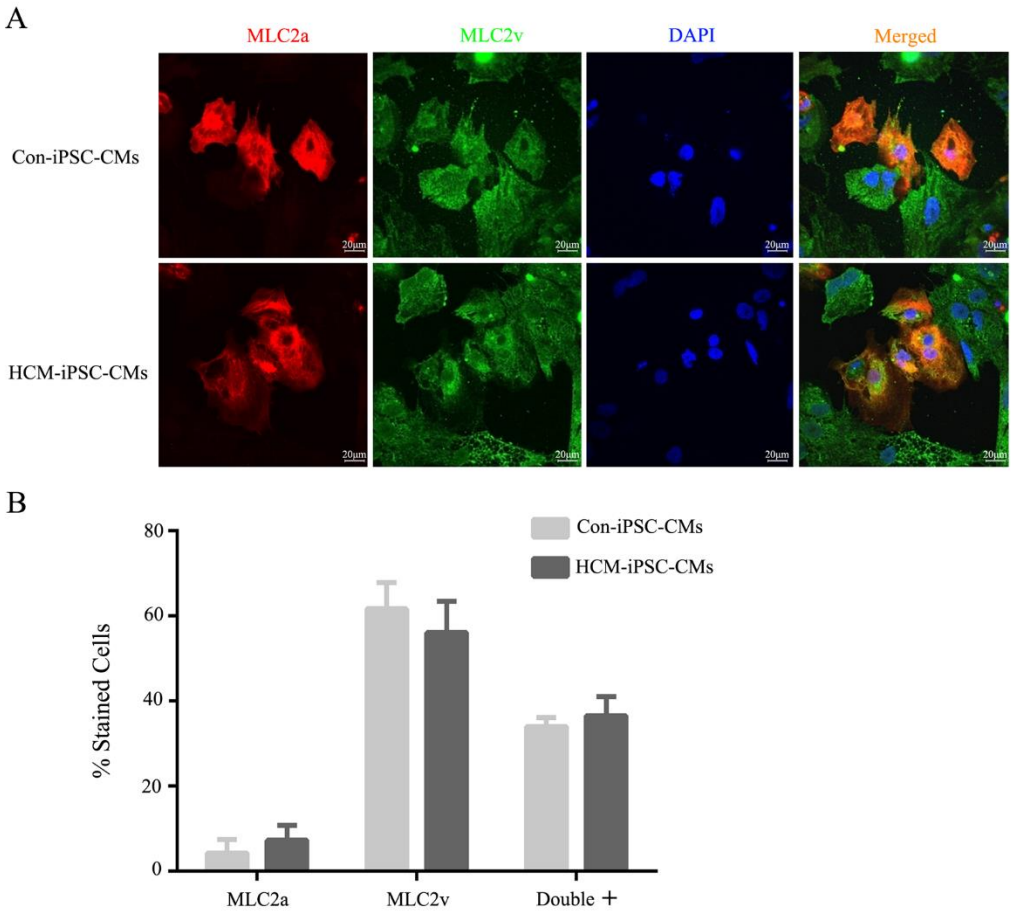
Figure S4.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MLC2a
MLC2v
DAPI
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HCM-iPSC-CMs
20μm
B
% Stained Cells
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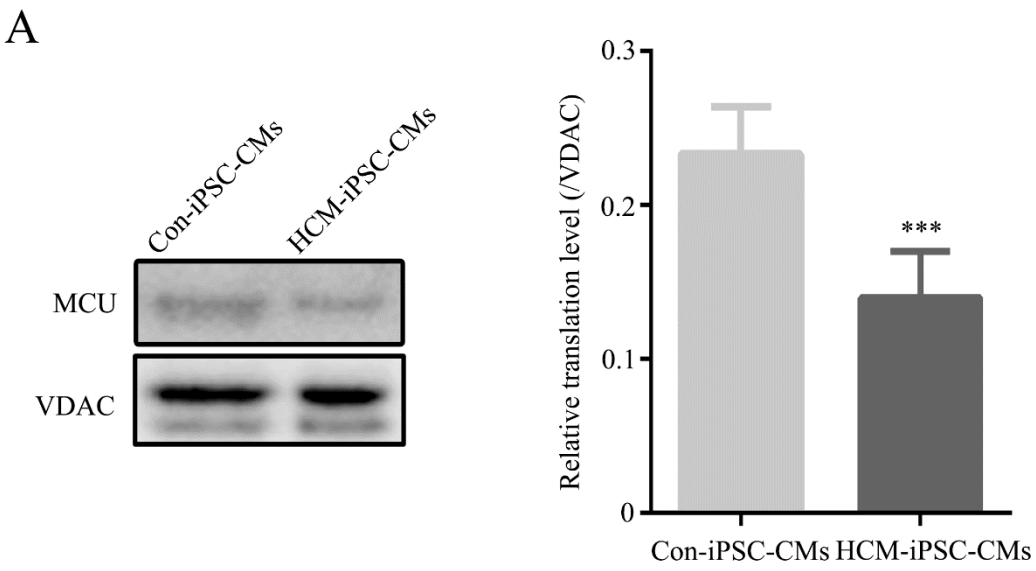
60
40
20
0
Con-iPSC-CMs
HCM-iPSC-CMs
MLC2a
MLC2v
Double +

Figure S5.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Con-iPSC-CMs
HCM-iPSC-CMs
MCU
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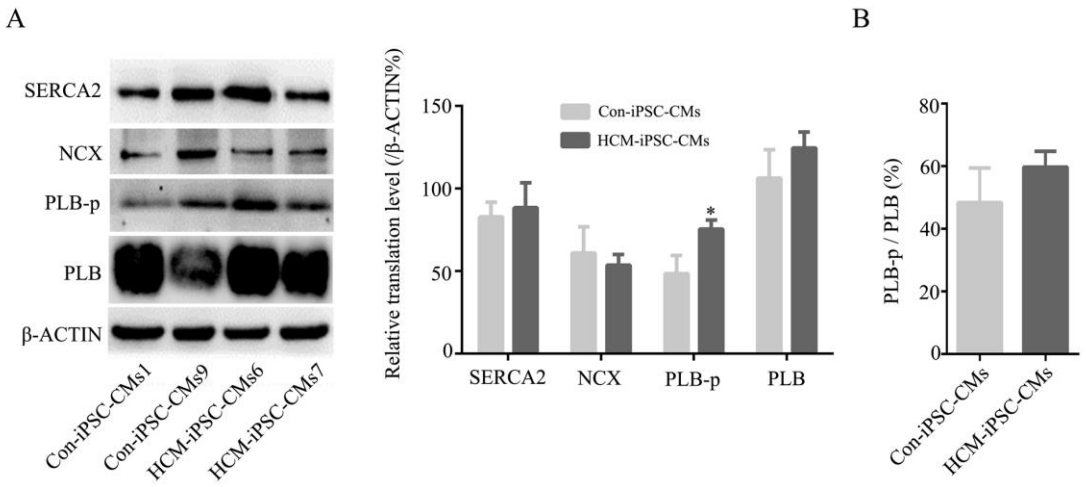
Con-iPSC-CMs
HCM-iPSC-CMs

Figure S6.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HCM-iPSC-CMs6
HCM-iPSC-CMs7
Relative translation level (/β-ACTIN%)
150
100
50
0
Con-iPSC-CMs
HCM-iPSC-CMs
*
SERCA2
NCX
PLB-p
PLB
B
PLB-p / PLB (%)
80
60
40
20
0
Con-iPSC-CMs
HCM-iPSC-CMs

Figure S7.

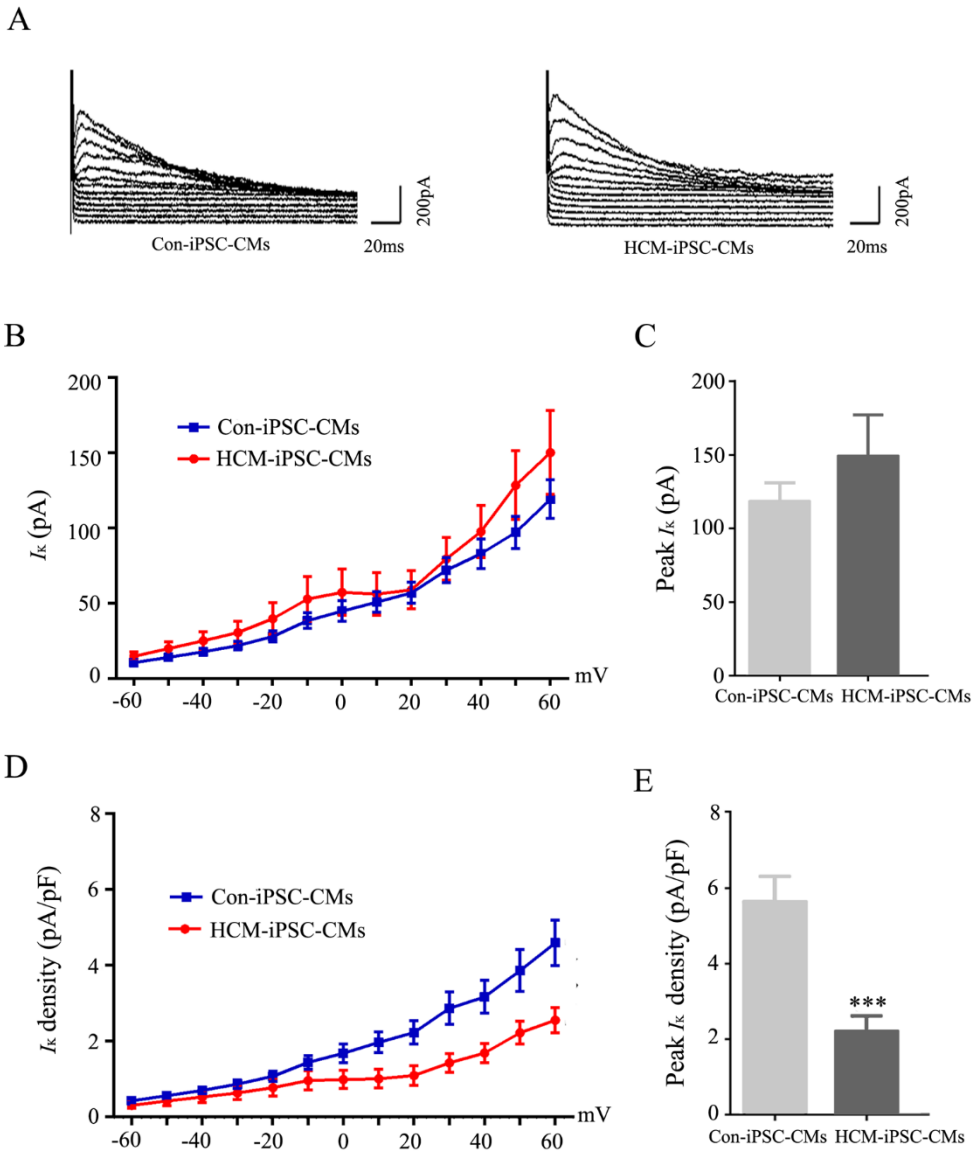
A
200pA
Con-iPSC-CMs
20ms
200pA
HCM-iPSC-CMs
20ms
B
Con-iPSC-CMs
HCM-iPSC-CMs
I_K (pA)
mV
C
Peak I_K (pA)
Con-iPSC-CMs
HCM-iPSC-CMs
D
Con-iPSC-CMs
HCM-iPSC-CMs
I_K density (pA/pF)
mV
E
Peak I_K density (pA/pF)
***
Con-iPSC-CMs
HCM-iPSC-CMs

Table S1. Summary for clinical features of HCM family members

Ground	Family Member	Gender (M/F)	Age (years)	*MT-RNR2* Genotype	Symptoms	MLVWT (mm)	IVS/LPW
Maternal members	II-1	F	59	m.2336T>C	HCM	26.8	3.21
	II-3	M	49	m.2336T>C	HCM	38.7	2.93
	III-1	M	38	m.2336T>C	HCM	46.6	2.86
	III-3	M	32	m.2336T>C	HCM	37.4	3.99
Non-maternal members	II-2	M	57	Wild type	Normal	10.2	1.17
	II-4	F	48	Wild type	Normal	9.6	1.22
	III-2	F	30	Wild type	Normal	9.5	1.19
	III-4	F	31	Wild type	Normal	8.5	1.23
	Ⅳ-1	F	13	Wild type	Normal	8.2	1.28
	Ⅳ-2	M	8	Wild type	Normal	8.2	1.12

* MLVWT, maximum left ventricular wall thickness; IVS, interventricular septum thickness; LPW, left posterior wall thickness.

Table S2. mtDNA variants of UCs and iPSCs from HCM patient

Gene	Position	HCM-UC	HCM-iPSC7P10	HCM-iPSC7P23	HCM-iPSC7P33
D-loop	16223	C > T	C > T	C > T	C > T
	16295	C > T	C > T	C > T	C > T
	16519	T > C	T > C	T > C	T > C
	73	A > G	A > G	A > G	A > G
	146	T > C	T > C	T > C	T > C
	199	T > C	T > C	T > C	T > C
	489	T > C	T > C	T > C	T > C
12S rRNA	750	A > G	A > G	A > G	A > G
16S rRNA	1438	A > G	A > G	A > G	A > G
	2336	T > C	T > C	T > C	T > C
	2706	A >G	A >G	A >G	A >G
ND1	3882	G > A (syn)	G > A (syn)	G > A (syn)	G > A (syn)
	4071	C > T (syn)	C > T (syn)	C > T (syn)	C > T (syn)
ND2	4769	A > G (syn)	A > G (syn)	A > G (syn)	A > G (syn)
	4850	C > T (syn)	C > T (syn)	C > T (syn)	C > T (syn)
	5442	T > C (Phe-Leu)	T > C (Phe-Leu)	T > C (Phe-Leu)	T > C (Phe-Leu)
NC5(non-coding)	5894	A>C	A>C	A>C	A>C
CO1	6455	C > T (syn)	C > T (syn)	C > T (syn)	C > T (syn)
	7028	C > T (syn)	C > T (syn)	C > T (syn)	C > T (syn)
ATP6	8701	A > G (Thr-Ala)	A > G (Thr-Ala)	A > G (Thr-Ala)	A > G (Thr-Ala)
	8860	A > G (Thr-Ala)	A > G (Thr-Ala)	A > G (Thr-Ala)	A > G (Thr-Ala)
COIII	9266	G > A (syn)	G > A (syn)	G > A (syn)	G > A (syn)
	9540	T > C (syn)	T > C (syn)	T > C (syn)	T > C (syn)
	9824	T> C (syn)	T> C (syn)	T> C (syn)	T> C (syn)
ND3	10398	A > G (Thr-Ala)	A > G (Thr-Ala)	A > G (Thr-Ala)	A > G (Thr-Ala)
	10400	C > T (syn)	C > T (syn)	C > T (syn)	C > T (syn)
ND4	10873	T > C (syn)	T > C (syn)	T > C (syn)	T > C (syn)
	11665	C > T (syn)	C > T (syn)	C > T (syn)	C > T (syn)
	11719	G > A (syn)	G > A (syn)	G > A (syn)	G > A (syn)
	12091	T > C (syn)	T > C (syn)	T > C (syn)	T > C (syn)
ND5	12372	G > A (syn)	G > A (syn)	G > A (syn)	G > A (syn)
	12705	C > T (syn)	C > T (syn)	C > T (syn)	C > T (syn)
	12810	A > G (syn)	A > G (syn)	A > G (syn)	A > G (syn)
	13759	G > A (Ala-Thr)	G > A (Ala-Thr)	G > A (Ala-Thr)	G > A (Ala-Thr)
CYTB	14766	C > T (Thr-Ile)	C > T (Thr-Ile)	C > T (Thr-Ile)	C > T (Thr-Ile)
	14783	T > C (syn)	T > C (syn)	T > C (syn)	T > C (syn)
	15043	G > A (syn)	G > A (syn)	G > A (syn)	G > A (syn)
	15301	G > A (syn)	G > A (syn)	G > A (syn)	G > A (syn)
	15326	A > G (Thr-Ala)	A > G (Thr-Ala)	A > G (Thr-Ala)	A > G (Thr-Ala)
	15364	C > T (syn)	C > T (syn)	C > T (syn)	C > T (syn)

Table S3. Steady-state activation and inactivation parameters of I_{CaL} recorded in Con-iPSC-CMs or HCM-iPSC-CMs

	Steady-state activation			Steady-state inactivation		
	$V_{1/2}$ (mV)	k	n	$V_{1/2}$ (mV)	k	n
Con-iPSC-CMs	-28.6±1.5	6.0±0.7	25	-41.0±0.8	5.8±0.2	25
HCM-iPSC-CMs	-23.6±1.6*	7.3±0.5	23	-40.1±0.6	5.6±0.2	23

Table S4. Electrophysiological parameters of iPSC-CMs

	Con-iPSC-CMs	HCM-iPSC-CMs	*p*-Value
Beating rate (BPM)	68.4±2.1	54.0±2.8	0.0062
Cm (pF)	21.9±1.6 (n=39)	46.4±4.9 (n=34)	0.0005
Tau (μs)	190.0±17.4 (n=39)	437.6±76.2 (n=34)	0.0008
RMP (mV)	-50.2±1.3 (n=40)	-55.5±0.8 (n=43)	0.0806
APA (mV)	90.2±1.4 (n=40)	92.7±2.5 (n=43)	0.3846
Max dV/dt (mV/s)	12501±764.9 (n=40)	11917±597.8 (n=43)	0.5480
APD30 (ms)	394.2±18.7 (n=40)	496.4±24.3 (n=43)	0.0012
APD50 (ms)	484.5±19.6 (n=40)	577.7±30.1 (n=43)	0.0051
APD90 (ms)	582.3±19.6 (n=40)	669.3±30.4 (n=43)	0.0084

C_m, Cell electric capacitance; RMP, resting membrane potential; APA, action potential amplitude; Max dV/ds, maximal rate of depolarization; APD30, APD at 30% of repolarization; APD50, APD at 50% of repolarization;APD90, APD at 90% of repolarization. Values are the means±SEM.

Table S5. Information of antibodies in the study

Antibody	Antigen	Company	Cat. No.
Anti-ANF	Atrial natriuretic factor	Abcam	ab191398
Anti-BNP	Brain natriuretic peptide	Abcam	ab92500
Anti-TNNI3	Troponin I type 3 cardiac	Abcam	ab52862
Anti-α-ACTIN	alpha-cardiac muscle actin	Immunoway	YT5110
Anti-MLC2v	ventricular myosin light chain	Abcam	ab92721
Anti-MLC2a	atrial myosin light chain 2	Proteintech	17283-1-AP
Anti-NCX	Na(+)/Ca(2+)-Exchange Protein	Abcam	ab177952
Anti-RyR	Ryanodine receptor	Abcam	ab2868
Anti-PLB	Phospholamban	Abcam	ab2865
Anti-PLB-p	Phospholamban (phospho S16)	Abcam	ab15000
Anti-SERCA2	Sarcoplasmic/endoplasmic reticulum calcium ATPase 2	Abcam	ab150435
Anti-VDAC	Voltage dependent anion channel	Abcam	ab14734
Anti-MRPL19	Mitochondrial ribosomal protein L19	Abcam	ab218978
Anti-MRPL23	Mitochondrial ribosomal protein L23	Abcam	ab174791
Anti-MT-ND5	Mitochondrial NADH dehydrogenase 5	Abcam	ab92624
Anti-MT-CYB	Mitochondrial cytochrome b	Abcam	ab81215
Anti-MT-COX2	Mitochondrial cytochrome c oxidase 2	Abcam	ab79393
Anti-MT-ATP8	Mitochondrial ATP synthase 8	Santa Cruz	SC-84231
Anti-MCU	Mitochondrial calcium uniporter	Abcam	ab45167
Anti-β-ACTIN	Beta cytoskeletal actin	Proteintech	66009-1-Ig

Table S6. Primers for quantitative PCR

Gene		Sequence (5'→3')
GAPDH	Forward	CAGGGCTGCTTTTAACTCTGGTA
	Reverse	CATGGGTGGAATCATATTGGAAC
16S rRNA	Forward	GCACACCCGTCTATGTAGCAAA
	Reverse	GATTTAGAGGGTTCTGTGGGCA
DN5	Forward	CGCTATGACATCGACATGACCAAGT
	Reverse	ACCGATACAAGTAGTCAGCCTGGAT
CYTB	Forward	AGCAACACTCCACCTCCTATTCTTG
	Reverse	GCTTACTGGTTGTCCTCCGATTCA
COX2	Forward	CCGCCATCATCCTAGTCCTCATC
	Reverse	AGTTGAAGATTAGTCCGCCGTAGTC
ATP8	Forward	CTACCACCTACCTCCCTCACCAAA
	Reverse	GGGCAATGAATGAAGCGAACAGATT
MRPL19	Forward	CCAGGAGATTCAGGTGGTCAA
	Reverse	GGACGTTCCCAGCGTTTAGA
MRPL23	Forward	GGTGCAGCATGGCTCTAACA
	Reverse	GATCTGGGAACGTGAAGGTCT
RPLP0	Forward	GATGTGCAGCTGATCAAGACTG
	Reverse	TATCAAGCACTTCAGGGTTGTAGAT
12S rRNA	Forward	ATTCGTGGATTATCAATT
	Reverse	GGATTAGATACCCTATTA
VDAC	Forward	GGTACAAGCGGGAGCACA
	Reverse	TCTGGGTCACTCGGGATT
ACTB	Forward	CCCAGAGCAAGAGAGG
	Reverse	GTCCAGACGCAGGATG
OCT4	Forward	CCTCACTTCACTGCACTGTA
	Reverse	CAGGTTTTCTTTCCCTAGCT
SOX2	Forward	CCCAGCAGACTTCACATGT
	Reverse	CCTCCCATTTCCCTCGTTTT
NANOG	Forward	TGAACCTCAGCTACAAACAG
	Reverse	TGGTGGTAGGAAGAGTAAAG
REX1	Forward	TCGCTGAGCTGAAACAAATG
	Reverse	CCCTTCTTGAAGGTTTACAC
Tg-OCT4	Forward	GGGTGGACCATCCTCTAGAC
	Reverse	CCAGGTCCGAGGATCAAC
Tg-SOX2	Forward	GGGTGGACCATCCTCTAGAC
	Reverse	GGGCTGTTTTTCTGGTTG
Tg-KLF4	Forward	GGGTGGACCATCCTCTAGAC
	Reverse	GGAAGTCGCTTCATGTGG
Tg-C-MYC	Forward	GGGTGGACCATCCTCTAGAC
	Reverse	CCTCGTCGCAGTAGAAATAC

Table S7. Information of key reagents in the study

Reagents	Company	Cat. No.
pMX-h-Oct4	Addgene	17217
pMX-h-Sox2	Addgene	17218
pMX-h-Klf4	Addgene	17219
pMX-h-c-Myc	Addgene	17220
pMX-GFP	Addgene	14567
PCL-ECO	Addgene	12371
REGM™ Renal Epithelial Cell Growth Medium	Lonza	CC-3191
REGM SingleQuot Kit Suppl. & Growth Factors	Lonza	CC-4127
Primocin	Invivogen	ant-pm-1
DMEM/HG	Gibco	C11995500BT
DMEM/F12	Gibco	C11330500BT
FBS	PAA	A15-101
Knockout serum replacement	Gibco	10828-028
NEAA	Gibco	11140-050
GlutaMAX	Gibco	35050-061
0.1% Gelatin	millipore	ES-006-B
MycoAlert®Mycoplasma Detection Kit	Lonza	LT07-218
2-Mercaptoethanol	Gibco	21985-023
bFGF	Invitrogen	13256-029
Dispase	Gibco	17105-041
Collagenase IV	Gibco	17104-019
Vc	Sigma	49752-10G
VPA	CALBIOCHEM	676380
mTeSR1	STEMCELL	#05850
Matrigel	BD	354277
REGM™ Renal Epithelial Cell Growth Medium	Lonza	CC-3191
Knockout serum replacement	Gibco	10828-028
B27-insulin	Gibco	A1895601
B27	Gibco	17504044
CHIR99021	Selleck	CT99021
IWP2	Tocris	3533
Y-27632	Sigma	Y0503
Accutase	STEMCELL	#07920
Collagenase B	Roche	11088815001