Matrigel Mattress

A Method for the Generation of Single Contracting Human-Induced Pluripotent Stem Cell–Derived Cardiomyocytes

Tromondae K. Feaster, Adrian G. Cadar, Lili Wang, Charles H. Williams, Young Wook Chun, Jonathan E. Hempel, Nathaniel Bloodworth, W. David Merryman, Chee Chew Lim, Joseph C. Wu, Björn C. Knollmann, Charles C. Hong

Rationale: The lack of measurable single-cell contractility of human-induced pluripotent stem cell–derived cardiac myocytes (hiPSC-CMs) currently limits the utility of hiPSC-CMs for evaluating contractile performance for both basic research and drug discovery.

Objective: To develop a culture method that rapidly generates contracting single hiPSC-CMs and allows quantification of cell shortening with standard equipment used for studying adult CMs.

Methods and Results: Single hiPSC-CMs were cultured for 5 to 7 days on a 0.4- to 0.8-mm thick mattress of undiluted Matrigel (mattress hiPSC-CMs) and compared with hiPSC-CMs maintained on a control substrate (<0.1-mm thick 1:60 diluted Matrigel, control hiPSC-CMs). Compared with control hiPSC-CMs, mattress hiPSC-CMs had more rod-shape morphology and significantly increased sarcomere length. Contractile parameters of mattress hiPSC-CMs measured with video-based edge detection were comparable with those of freshly isolated adult rabbit ventricular CMs. Morphological and contractile properties of mattress hiPSC-CMs were consistent across cryopreserved hiPSC-CMs generated independently at another institution. Unlike control hiPSC-CMs, mattress hiPSC-CMs display robust contractile responses to positive inotropic agents, such as myofilament calcium sensitizers. Mattress hiPSC-CMs exhibit molecular changes that include increased expression of the maturation marker cardiac troponin I and significantly increased action potential upstroke velocity because of a 2-fold increase in sodium current ($I_{Na}$).

Conclusions: The Matrigel mattress method enables the rapid generation of robustly contracting hiPSC-CMs and enhances maturation. This new method allows quantification of contractile performance at the single-cell level, which should be valuable to disease modeling, drug discovery, and preclinical cardiotoxicity testing. (Circ Res. 2015;117:995-1000. DOI: 10.1161/CIRCRESAHA.115.307580.)

Key Words: excitation contraction coupling • matrigel • myocytes, cardiac • pluripotent stem cells • stem cells

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Given species differences in cardiac myocyte (CM) physiology, human-induced pluripotent stem cell–derived CMs (hiPSC-CMs) are gaining recognition for their potential in human heart disease modeling, preclinical cardiotoxicity evaluation, and drug discovery.1 Realization of this potential depends on the ability to assess excitation–contraction (E–C) coupling, including contractile properties of individual hiPSC-CMs. To date, most functional evaluations of isolated hiPSC-CMs have focused on electrophysiology2 and calcium (Ca) handling3 measurements, whereas contractile properties have not been routinely evaluated because of limited cell shortening of hiPSC-CMs cultured under standard conditions. In principle, a variety of biophysical techniques, such as atomic
force microscopy, traction force microscopy, and micropost deflection, are available to assess mechanical properties of isolated hiPSC-CMs; however, because these do not address the issue of limited cell shortening, they have not been widely adopted.4–7 Alternatively, engineered heart tissues can be used to assess contractile properties of hiPSC-CMs or mixtures of hiPSC-CMs and support cells8–10; however, compared with hiPSC-CMs, engineered heart tissues are difficult and expensive to generate and do not allow simultaneous assessment of contractility and Ca handling at the single-cell level. Here, we report a simple and rapid method that generates rod-shaped hiPSC-CMs with aligned myofilaments that exhibit features of physiological and molecular maturation and robust cell shortening with each cardiac cycle, thereby enabling straightforward contractility assessment and hence E–C coupling analysis.

Methods
An expanded Methods section is provided in the Online Data Supplement.

Preparation of Matrigel Mattress Substrates
Matrigel mattress platforms were prepared the same day of CM dissociation and seeding. Briefly, mattresses were arrayed on a glass coverslip (Corning) or Delta TPG Dish (Fisher Scientific). Application of 1-μL lines of completely thawed, ice cold, undiluted growth factor-reduced Matrigel (8–12 mg/mL; Corning) was arrayed in parallel. Matrigel was evenly pipetted at an angle of 45° (Figure 1B) with a P2 pipette and corresponding 10-μL tip. The mattresses were allowed to incubate for 8 to 10 minutes at room temperature at which point 200 μL of RPMI 1640 medium, 2% B-27 supplement (Invitrogen), and 1% Pen-Strep (Life Technologies), with the addition of 40,000 CMs, were immediately added, halting mattress polymerization. Each mattress had a width of ≈0.85 mm and was ≈23-mm long. The thickness of each mattress was 0.4 to 0.8 mm.

Results
HiPSC-CMs are routinely cultured on substrates such as gelatin or Matrigel, a commercially available extracellular matrix preparation11 (Figure 1A). However, under these standard culturing conditions, hiPSC-CMs typically display variable morphology without a dominant axis of myofibril alignment, which are different from freshly isolated mature adult CMs. As a result, individual hiPSC-CMs cultured under traditional methods exhibit limited cellular shortening (Online Movie I). We hypothesized that hiPSC-CMs may be induced to shorten if maintained on a culture substrate with suitable biophysical and biochemical properties.12 Of a variety of culture substrates tested,13 we discovered that culturing

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**Figure 1.** Overview of Matrigel mattress method. A, Schematic of cardiac induction and Matrigel mattress method plating protocol. B, Matrigel mattress method workflow. C, Matrigel mattresses plated on Delta TPG Dish (Fisher Scientific), white arrows indicate the edge of each mattress, and blue dye was included to visualize mattress. D, human-induced pluripotent stem cell–derived cardio myocytes (hiPSC-CMs) plated on control (left) and mattress (right). Scale bar, 50 μm. White arrows indicate the edge of mattress platform. RT indicates room temperature. CHIR indicates CHIR99021.
single hiPSC-CMs on undiluted Matrigel with a thickness of at least 0.4 mm, which we term Matrigel mattress, promoted myofibril alignment (Online Figure I), a rod-like cell shape (Online Figure II and Online time-lapse Movie), and robust hiPSC-CM shortening (Online Movie II). In this method, hiPSC-CMs are first generated by small-molecule–based cardiac differentiation and maintained on standard cardiac induction substrate. Then on days 30 to 35 of cardiac differentiation, single hiPSC-CMs are dissociated, replated on Matrigel mattress and cultured for an additional 5 to 7 days before analysis (Figure 1A and 1B). Of hiPSC-CMs cultured in the standard manner (eg, Matrigel, 1:60 dilution), only a small fraction (3.3±0.5%) of cells exhibited visible cell contractions. In contrast, essentially all (91.0±5.0%) hiPSC-CMs maintained on Matrigel mattress, henceforth referred to as mattress hiPSC-CMs, exhibited visible cellular contractions (P<0.0001; Figure 2C, bottom). Mattress hiPSC-CMs reached peak contractility after 3 days on the mattress, and these properties remained stable for ≤14 days at which point the Matrigel loses its integrity. When quantitated with video-based edge detection, spontaneously contracting mattress hiPSC-CMs exhibited ≈9.0±1.5% cell shortening compared with 0.4±0.2% for control hiPSC-CMs (P<0.0001; Online Table I). Cell shortening of this magnitude has previously been reported only for late-stage hiPSC-CMs after 80 to 120 days in culture.

We next compared mattress hiPSC-CMs to acutely isolated rabbit and mouse CMs. Strikingly, mattress hiPSC-CMs displayed comparable cell shortening (Figure 2D and 2E) despite an overall shorter resting cell length. Contractile kinetics (ie, time to peak and time to baseline) of mattress hiPSC-CMs were more similar to those of rabbit CMs, which are thought to be a closer model of human CMs than mouse CMs. These contractile properties were replicated across multiple independently generated hiPSC-CM lines, including postrecovery from cryopreservation (Online Table I). Cell shortening of mattress hiPSC-CMs was associated with acquisition of morphological features comparable with that of freshly isolated adult CMs, including cell elongation, decreased circularity (Figure 2A and 2B), and increased sarcomere length relative to control hiPSC-CMs (Figure 2B; Online Figure I). The position on the mattress (ie, center versus edge) had no significant effect on the hiPSC-CM shape. The contraction force produced by individual hiPSC-CMs on mattress can be calculated from the cell length, cell shortening, and the elastic modulus of the Matrigel mattress (5.8 kPa; Online Figure IIIE). Mattress hiPSC-CMs exhibit an average contraction force of 0.3±0.1 mN per mm² cross-sectional area. The calculated force values based on our cell shortening measurements were closely correlated with values measured independently in the

Figure 2. Matrigel mattress morphometry and contractility. A, Immunofluorescence staining of cardiac myocyte (CM) structural marker α-actinin (green) and nucleus (blue, DAPI) for control human-induced pluripotent stem cell–derived CMs (hiPSC-CMs; left), mattress hiPSC-CMs (middle), and rabbit CMs (right). Scale bar, 20 µm; magnification, ×63. B, Cell morphometry measuring circularity index (left) and sarcomere length (right) for indicated CMs; error bars, SD (n=9–48 cells per group). C, Cell morphology measuring cell shortening (middle), cell shortening kinetics (right); error bars, SD (n=6–20 cells per group). *** P<0.0001 vs hiPSC-CMs. * P<0.05 vs mouse CMs. n.s. indicates not significant.
same mattress hiPSC-CMs using traction force microscopy (Online Figure III).

We then examined whether culturing hiPSC-CMs on a Matrigel mattress affected key aspects of E–C coupling, such as intracellular Ca handling, electrophysiology, and CM-specific molecular profile. We found that Ca-handling properties were not significantly different between control and mattress hiPSC-CMs (Figure 2C, top; Online Table II), and hiPSC-CMs maintained under both conditions displayed robust caffeine-releasable Ca release from sarcoplasmic reticulum stores. However, compared with control hiPSC-CMs, mattress hiPSC-CMs displayed a modest increase in maximum return velocity of twitch Ca transient and faster caffeine-induced Ca decay. In addition, electrophysiological parameters of mattress and control hiPSC-CM were similar (Online Figure IV). For instance, the amplitude and mean diastolic potential of both control and mattress hiPSC-CMs were comparable with freshly isolated rabbit CMs. Of note, however, the upstroke velocity of mattress hiPSC-CMs was higher than that of control hiPSC-CMs (Online Table III), a finding that is consistent with the over 2-fold higher sodium current density of mattress compared with control hiPSC-CMs\(^2\) (Online Figure VD). Finally, transcriptional profiling of a subset of cardiac genes involved in E–C coupling (Online Figure VA) showed differential expression in mattress hiPSC-CMs. For instance, expression of TNNI3, encoding cardiac troponin I, a CM maturation marker, which is minimally expressed in hiPSC-CMs maintained under traditional conditions,\(^1\) was markedly elevated in mattress hiPSC-CMs. Western blot analysis of cardiac troponin I and slow skeletal troponin I, the fetal isoform predominant in traditional hiPSC-CMs, confirmed that mattress hiPSC-CMs exhibited higher cardiac troponin I levels in conjunction with a stoichiometric reduction in slow skeletal troponin I levels (Online Figure VIB and VIC).

Because culturing hiPSC-CMs on Matrigel mattress permits simultaneous analysis of Ca handling and contractility at the single-cell level, we next examined the E–C coupling in response to pharmacological interventions (Figure 3). Mattress hiPSC-CMs increased contractility to inotropic interventions of increasing extracellular Ca, with an EC\(_{50}\) (ie, effective concentration yielding 50% of maximal response) of \(\approx 1.2\) mmol/L\(^1\) (Figure 3A–3C). Treatment with the negative inotrope verapamil, an L-type Ca-channel antagonist, led to a concentration-dependent decrease in cellular contraction with an EC\(_{50}\) of \(0.3\) mmol/L\(^1,2\) (Figure 3D–3F). Treatment with the myofilament Ca sensitizer EMD57033 led to a concentration-dependent increase in contraction with an EC\(_{50}\) of \(\approx 2.6\) mmol/L\(^1\) (Figure 3H–3I) with negligible effects on the intracellular Ca transient (Figure 3J). To our knowledge, this is the first direct demonstration that individual hiPSC-CMs are capable of altering contractility in response to changes in myofilament

| Figure 3. Pharmacological analysis. A, D, and G, Representative Ca transients (top) and contraction traces (bottom) treated with indicated compound. Contraction and Ca concentration–response curves, B and E, Extracellular Ca (Hill slope=18.7 and 2.6, respectively). E and F, Verapamil (Hill slope=−1.8 and −0.9, respectively). H and I, EMD57033 (Hill slope=1.1); n=5–20 cells per group. EC\(_{50}\) indicates effective concentration 50% of maximal response; and Veh, vehicle. |
Ca sensitivity, a finding that previously could not be observed using hiPSC-CMs maintained on traditional substrates or by evaluating changes in Ca handling alone (Figure 3J).

Discussion

Here, we demonstrate that culturing hiPSC-CMs on Matrigel mattress for 5 to 7 days leads to relative morphological, molecular, and functional maturation, enabling robust cell shortening in a much shorter time frame than traditional methods, such as engineered heart tissue or long-term culture.15,20 It is worth noting that maturing hiPSC-CMs exhibit an average contraction force per cross-sectional area that is comparable with that of engineered heart tissues made from hiPSCs.20 Because hiPSC-CMs on Matrigel mattress and standard culture substrate showed similar Ca handling (Figure 2C, top; Online Table II), our results suggest that the enhanced sarcomere alignment along the long axis of the cell (Figure 2A; Online Figure I) is a primary mechanism for the enhanced contractile response of mattress hiPSC-CMs. Other factors, such as the lower substrate stiffness of the mattress, are likely contributory and will need to be explored in future studies. Although other techniques, such as microcontact printing, have been shown to improve sarcomere alignment and direct hiPSC-CMs toward a mature-like phenotype with contractile function,20,21 our Matrigel mattress method enables rapid assessment of contractile properties and Ca handling at the single-cell level using standard equipment available in any laboratory studying adult primary CMs. The Matrigel mattress method can also serve as a foundation for development of a high-throughput multiplexed platform to simultaneously monitor Ca handling and contractility of individual patient-derived hiPSC-CMs. Because Matrigel is commercially available with little apparent lot-to-lot variability, easily prepared, and requires no additional functionalization, we anticipate that the present method can be immediately adopted for a wide spectrum of applications, including disease modeling, drug discovery, and preclinical cardiototoxicity evaluation.

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Disclosures

J.C. Wu is a cofounder of Stem Cell Theranostics. The other authors report no conflicts.

References


Novelty and Significance

**What Is Known?**

- Single human-induced pluripotent stem cell–derived cardiac myocytes (hiPSC-CMs) under standard culture conditions display limited changes in cellular geometry (ie, cell shortening) with each cardiac cycle.
- Existing techniques to assess hiPSC-CM contractility are expensive, time consuming, and technically challenging. As a result, investigations of hiPSC-CM function have primarily focused on their electrophysiological and Ca-handling properties.

**What New Information Does This Article Contribute?**

- Culturing hiPSC-CMs on commercially available Matrigel mattress rapidly (3 days) promotes cell elongation, sarcomere alignment, and single-cell contractility.
- Mattress hiPSC-CMs display single-cell shortening properties comparable with those of acutely isolated adult rabbit CMs.
- Mattress hiPSC-CMs display positive inotropic response to myofilament Ca sensitizer, EMD57033 with negligible effect on Ca handling.

Current methods to evaluate contractile properties of single hiPSC-CMs are difficult because single hiPSC-CMs cultured under standard conditions largely lack contractile responses. Here, we establish a new culture method the Matrigel mattress that rapidly (3 days) generates elongated hiPSC-CMs that exhibit robust cell shortening. Using this method, we characterized the contractile properties of hiPSC-CMs and their response to positive and negative inotropic agents. We show that hiPSC-CMs from multiple laboratories have comparable contractile properties, even after recovery from cryopreservation. We further demonstrate that these contractile properties are comparable with adult rabbit ventricular CMs. The Matrigel method can be readily implemented for basic science studies, drug discovery, and preclinical cardiac safety assessment.
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SUPPLEMENTAL MATERIAL

Methods and Materials
The University Committee on Use and Care of Animals at Vanderbilt University Medical Center approves all animal protocols. All animal protocols conformed to the Guiding Principles in the Care and Use of Animals of the American Physiological Society. Data averaged from multiple days were used for analysis. All chemicals, unless otherwise specified, were obtained from Sigma (St. Louis, MO). All Protocols were approved by Vanderbilt University Medical Center Institutional Review Board.

Human iPSC Derivation and Culture
Human induced pluripotent stem cells (hiPSCs) from healthy volunteers were generated, as previously described\(^3,11\). Briefly, 3 hiPSC lines were used. Line CC2 was derived from dermal fibroblast by non-integrating episomal based reprogramming at Vanderbilt University. Line CJ1-001 was derived from peripheral blood mononuclear cells by non-integrating episomal based reprogramming at Vanderbilt University. Line SCVI13 was derived from dermal fibroblast by lentivirus based reprogramming at Stanford University. Individual colonies with typical hESC morphology were picked at day 17 to 30 and clonally expanded. HiPSCs were maintained on growth factor-reduced Matrigel (Corning) coated plates (1:200 dilution, DMEM/F12) in mTeSR1 medium (Stemcell Technologies). Cells were passaged every 4 days using 0.5 mmol/L EDTA (Life Technologies) in D-PBS without CaCl\(_2\) or MgCl\(_2\) (Life Technologies). 10 \(\mu\)mol/L Rho kinase inhibitor Y-27632 (CalBiochem) was added for the first 24 hours after passaging. Cells were maintained at 37 ºC, with 5 % CO\(_2\) and 5 % O\(_2\).

Small Molecule Cardiac Differentiation
Human induced pluripotent stem cell derived cardiomyocytes (hiPSC-CMs) were generated by small molecule based cardiac differentiation\(^2\). Briefly, hiPSCs (> p20) were split at 1:12 ratio with 0.5 mmol/L EDTA (Life Technologies) in D-PBS without CaCl\(_2\) or MgCl\(_2\) (Life Technologies), as above. Cells were grown for 4 days at which time they reached ~ 80 % confluence. This is referred to as day 0; medium was changed to basal medium RPMI 1640 (11875, Life Technologies) plus B27 without insulin supplement (A1895601, Life Technologies), supplemented with 6 \(\mu\)mol/L CHIR99021 (Selleck Chemicals). On day 2 medium was changed to RPMI 1640 plus B27 without insulin supplement without CHIR99021. On day 3 medium was changed to basal medium RPMI 1640 plus B27 without insulin supplement, supplemented with 5 \(\mu\)mol/L IWR-1 (Sigma). Medium was changed on day 5 to RPMI 1640 plus B27 without insulin, and every other day until day 10. At day 10 medium was changed to metabolic selection medium consisting of RPMI 1640 without glucose (11879, Life Technologies) plus B27 without insulin. On day 12 medium was changed to RPMI 1640 medium (11875, Life Technologies), 2 % B-27 supplement (Invitrogen), and 1 % Pen-Strep (Life Technologies) until dissociation. Spontaneously contracting cells were observed from day 7.

hiPSC-CM Dissociation and Plating
HiPSC-CMs were dissociated and plated as described\(^3\). Briefly hiPSC-CMs were washed with D-PBS without CaCl\(_2\) or MgCl\(_2\) (Life Technologies) and incubated with TrypLE Express (Life Technologies) for 15 minutes at 37 ºC to dissociate cells, at day 30 - 35 post cardiac differentiation. HiPSC-CMs were filtered (100 \(\mu\)m) (Falcon) and plated at low density (40,000 cells) on either control (Matrigel growth factor-reduced 1:60 in DMEM/F12) or Matrigel mattress coated dish, as described below) in approximately 200 \(\mu\)L solution containing: RPMI 1640 medium (11875, Life Technologies), 2 % B-27 supplement (Invitrogen), 1 % Pen-Strep (Life
Technologies) and 10 µmol/L Rho kinase inhibitor Y-27632 (CalBiochem) was added for the first 24 hours and after several hours additional media was added. hiPSC-CMs were maintained at 5 % CO₂ and 21 % O₂. Medium was changed every day and experiments were performed 5 - 7 days after plating. Cryopreserved hiPSC-CMs, SCVI13 (Stanford), were thawed and plated on Matrigel (1:200 dilution) for 4 days to allow CM contractile machinery to recover from cryopreservation prior to plating, as described above.

**Preparation of Matrigel Mattress Substrates**
Matrigel mattress platforms were prepared the same day of CM dissociation and seeding. Briefly, mattresses were arrayed on a glass coverslip (Corning) or Delta TPG Dish (Fisher Scientific). Application of 1 µL lines of completely thawed, ice cold, undiluted growth factor-reduced Matrigel [8 - 12 mg/mL] (Corning) were arrayed in parallel. Matrigel was evenly pipetted at a 45 degree angle (Figure 1B) with a P2 pipet and corresponding tip. The mattresses were allowed to incubate for 8 - 10 minutes at room temperature at which point 200 µL of RPMI 1640 medium, 2 % B-27 supplement (Invitrogen), and 1 % Pen-Strep (Life Technologies), with the addition of 40,000 CMs were immediately added, halting mattress polymerization. Each mattress had a width of approximately 0.85 mm. The thickness of each mattress was found to be in the range of 0.40 - 0.88 mm and each line was approximately 23 mm long.

**Traction Force Microscopy**
Traction force maps were generated using the traction force microscopy method. Briefly, the contraction cycle of isolated hiPSC-CMs maintained on Matrigel mattress containing 0.75 µm (Polysciences) fluorescence beads at 1:50 dilution were tracked by phase-contrast for contractile motion and fluorescence microscopy for substrate deformation; videos of individual hiPSC-CMs, cultured on mattress containing beads, were captured and particle image velocimetry (PIV) used to assess displacement deformation of hiPSC-CMs. Fourier transform traction cytometry (FTTC) was used to access force using ImageJ plugin. For traction force microscopy experiments spontaneously beating hiPSC-CMs in 2 mmol/L Ca Tyrode were evaluated at room temperature.

**Primary Myocyte Isolation**
Mouse and rabbit ventricular cardiomyocytes (CMs) were isolated by a modified collagenase/protease method as previously described. All experiments were conducted in Tyrode’s solution containing (in mmol/L): CaCl₂, NaCl 134, KCl 5.4, MgCl₂ 1, glucose 10, and HEPES 10, pH adjusted to 7.4 with NaOH. Final concentration of Ca is 2 mmol/L.

**Video Based Edge Detection**
Video edge detection was used to assess cellular contraction (i.e., cell shortening) of contracting CMs. Briefly, CMs were visualized using a Nikon Ellipse T5100 coupled to IonOptix video microscopy system (IonOptix). Spontaneous or field stimulated contraction traces were recorded in 2 mmol/L Ca Tyrode solution. For each cell and each experimental condition typical contraction parameters including percent cell shortening (i.e., percent of resting cell length) and contractile kinetics (i.e., time to peak 90% and time to baseline 90%) values were averaged. Contraction traces were recorded and analyzed using commercially available data analysis software (IonOptix, IonWizard™ Milton, MA). Only isolated hiPSC-CMs with aspect ratio greater than or equal to 4 were used, and non-shortening cells were excluded from analysis.

To assess contractility we used on changes in cell length; instead of sarcomere length. We found, unlike with video based edge detection, not every hiPSC-CM could be measured using the sarcomere length detection software. This likely due to the significantly smaller, shorter and
thinner morphology of hiPSC-CMs compared to adult CMs and hence fewer sarcomeres available for the analysis software to visualize. However, it seems likely that once software is optimized for hiPSC-CM morphology and size, sarcomere shortening measurements will become routine as well.

**Measurement of Intracellular Ca**

CMs were loaded with Fura-2 acetoxymethyl ester, Fura-2 AM (Molecular Probes Inc, Eugene, OR) as described previously. Briefly, CMs were incubated with 2 μmol/L Fura-2 AM for 8 minutes at room temperature to load the indicator in the cytosol. CMs were washed twice for 10 minutes with Tyrode’s solution containing 250 μmol/L probenecid to retain the indicator in the cytosol. A minimum of 30 min were allowed for de-esterification before imaging the cells. Fura-2 AM loaded Ca transients were recorded during spontaneous beating or 0.2 Hz field stimulation in 2 mmol/L Ca Tyrode’s solution. For experiments assessing SR content after 20 seconds or recording stimulation was switched off followed by application of caffeine 10 mmol/L for 5 seconds to estimate SR Ca content. For each cell and each experimental condition, τ, amplitude and baseline values were averaged. Ca transients were recorded and analyzed using commercially available data analysis software (IonOptix, IonWizardTM Milton, MA). All experiments were conducted at room temperature. Tyrode’s solution containing (in mmol/L): CaCl2, NaCl 134, KCl 5.4, MgCl2 1, glucose 10, and HEPES 10, pH adjusted to 7.4 with NaOH.

**Immunohistochemistry**

Immunostaining of CMs was carried out as before. Briefly, CMs were fixed in 2 % paraformaldehyde for 5 minutes at room temperature, permeabilized with 0.2 % Triton X-100 (Sigma) for 10 minutes at 4 °C. Samples were blocked with 1 % BSA in PBS solution and incubated for 1 hour at room temperature. Primary antibody, α-actinin (mouse monoclonal, Sigma), was added in 0.1 % Triton X-100 1 % BSA in PBS solution and incubated overnight at 4 °C. Samples were washed with 0.2 % Tween 20 in PBS, secondary antibodies specific to the primary IgG isotype were diluted (1:1000) in the same solution as the primary antibodies and incubated at room temperature for 1 hour. Samples were washed with 0.2 % Tween 20 in PBS twice and mounted. Slides were examined with Olympus IX81 microscope coupled to Slidebook software. For morphometric analysis images were imported into Image J and analyzed using standard plugins.

**Measurement of cell volume**

Measurements of hiPSC-CM volume were obtained as previously described. Z-stacks were obtained using a confocal microscope (LSM 510, 25X oil immersion Plan-Neofluar lens) as described. Briefly, hiPSC-CMs were loaded with 5 μmol/L Calcein AM (Molecular Probes, Eugene, OR), for 30 min at room temperature; to label intracellular space. HiPSC-CMs were washed twice to remove extracellular Calcein with no Ca Tyrode solution. Fluorescence was excited at 488 nm with 1 % argon laser power. 25-46 images (1.5 μm sections) were obtained for each hiPSC-CM. Images were reconstructed in three-dimensions and surface area and volume analyzed using the three-dimensional image analysis program IMARIS (Bitplane, South Windsor, CT).

**Action potential measurement**

Cardiac action potentials were measured from single cardiomyocytes with a ruptured whole cell patch-clamp technique. Briefly, cells were superfused with Tyrode’s solution containing (in mmol/L) 137 NaCl, 5.4 KCl, 10 HEPES, 10 Glucose, 1 MgCl2, 1.8 CaCl2; pH was adjusted to 7.4 with NaOH. The glass pipette had access resistance of 3-6 MQ after filling with the internal pipette solution containing (in mmol/L) 110 KCl, 5 Mg-ATP, 0.5 MgCl2, 5 EGTA, 10 HEPES, pH
adjusted to 7.2 with KOH. Action potential was recorded using Axopatch 200B, Digidata 1322A and pClamp 8.0 software (Axon Instruments, Foster City, CA, USA) for data amplification and acquisition. A 2 ms current pulse at approximately 20% above threshold was provided to evoke action potentials at a cycle length of 2 s (0.5 Hz). Electrophysiological data were analyzed using Clampfit 9.0 and were prepared by using Origin 7.2.1. All experiments were performed at room temperature.

**Measurements of sodium current**
Sodium currents ($I_{Na}$) were measured using external solution contained (in mmol/L): 135 CsCl, 10 NaCl, 10 HEPES, 10 Glucose, 1.8 CaCl$_2$, 1 MgCl$_2$, pH 7.4 (CsOH). To eliminate L- and T-type calcium currents, 1 µmol/L nisoldipine and 200 µmol/L NiCl$_2$ were added to the external solution. The internal pipette solution contained (in mmol/L): 5 NaCl, 135 CsCl, 5 EGTA, 10 HEPES, 5 Mg-ATP, 2 CaCl$_2$, pH 7.2 (CsOH). $I_{Na}$ densities were measured by applying 40 ms test pulse between -80 mV and 60 mV in 10 mV increments from a holding potential of -120 mV. Steady-state inactivation was measured by varying the conditioning holding potential from -140 mV to -20 mV, followed by a 40 ms test pulse to -20 mV.

**Contraction Force Calculation from cell length and cell shortening measurements**
Contraction force was calculated based on the following equation.

$$F = \left( \frac{E A_0 \Delta L}{L_0} \right)$$

Where, $F$ is the force exerted on an object
- $E$ is the Young's modulus (modulus of elasticity) of the substrate
- $A_0$ mean hiPSC-CM cross-sectional area
- $\Delta L$ is the amount by which the length of the hiPSC-CM changes
- $L_0$ is the resting length of the hiPSC-CM

Briefly, the resting cell length, $L_0$, was determined by video based edge detection (IonOptix). Mean modulus, $E$, of the Matrigel mattress, 5.8 kPa, was determined by Atomic Force Microscopy. $\Delta L$, the change in cell length (i.e., peak cell shortening amplitude), of each cell was determined using video based edge detection (IonOptix). The mean cross sectional area, $A_0$, was calculated from Z stack images as described. Assumptions for this analysis: as hiPSC-CMs shorten equal force is applied to the Matrigel mattress (i.e., that Matrigel mattress moves as CM contracts). This is shown in our traction force analysis, by displacement of embedded florescent beads (Online Figure III).

**Compounds and pharmacological assay**
All compounds were re-suspended based on manufactures recommendations. Briefly, EMD57033 (Sigma) was prepared in DMSO and Verapamil (Sigma) was prepared in distilled water.

Before experiments, individual hiPSC-CMs were selected based on good contraction and morphology. HiPSC-CMs with no obvious baseline, irregular contractions or aspect ratio less than 4 were not selected for analysis. The position of suitable hiPSC-CMs was recorded. Each experiment typically measured pharmacological response for 5 to 20 hiPSC-CMs taken from independent cardiac differentiations. HiPSC-CMs were perfused with Tyrode’s solution with vehicle and allowed to equilibrate for approximately 5 minutes before experiments. The positive inotrope EMD57033 was used with external Ca concentration 1 mmol/L Tyrode around the
EC$_{50}$. HiPSC-CMs were exposed to increasing concentration of compound for 250 seconds or until steady state was reached$^{20}$.

**Cardiac gene quantitative PCR screen**

A panel of cardiac genes were screened by qPCR. Briefly, total RNA was isolated from Day 30-35 mattress and control hiPSC-CMs via TRizol Reagent and DNase treated via DNA-free DNA removal kit per manufacturer’s instructions (Ambion). cDNA was generated using high-capacity cDNA reverse transcription Kit (Life Technologies). qPCR arrays were performed using Taqman Viia7 Fast Real-Time RT-PCR System according to the manufacturer’s instructions (Applied Biosystems, Foster City, CA, USA). All TaqMan probes were obtained from Applied Biosystems (Online Table IV). Transcripts were normalized to housekeeping gene 18S rRNA and differential fold changes were presented as heat map.

**Western Blot analysis**

Western blot analysis was carried out as before$^{13}$. Briefly, hiPSC-CMs were seeded on mattress or control (i.e., Matrigel 1:60 dilution) for 5 - 7 days. hiPSC-CMs were lysed with RIPA buffer supplemented with protease inhibitor and phosphatase inhibitor cocktail (Sigma). To remove excess Matrigel from mattress lysates, all lysates were centrifuged at 16,000 rpm for 15 minutes at 4 °C. Protein was quantified using Bio-Rad DC Protein Assay. 5 µg of protein lysates were resolved on 12 % mini-Protean TGX gels (Bio-rad) and transferred onto nitrocellulose membranes for immunoblotting. Membranes were incubated with primary antibodies α-tubulin (rabbit monoclonal, Abcam), ssTnI (rabbit polyclonal, Sigma), cTnI (rabbit polyclonal, Abcam) overnight at 4 °C. LI-COR secondary antibody were used and detected by Odyssey CLX (LI-COR) per manufacturer’s protocol. Protein band intensities were quantified and normalized to alpha-tubulin band intensities using LI-COR Image Studio software.

**Time-lapse microscopy**

Differential Interface Contrast (DIC) time lapse images were acquired using a Zeiss LSM Meta 710 confocal microscope. Briefly, day 30 hiPSC-CMs were dissociated and re-plated on Matrigel mattress on MatTek 35 mm petri dishes (P35G-1.5-14-C). hiPSC-CMs were maintained at 37 °C with 5 % CO$_2$ and imaged every 15 minutes for 10 hours. Images were exported into ImageJ for processing.

**Statistical analysis**

Data are mean ± SD unless indicate otherwise. Statistical differences among two groups were tested with two-tailed Student’s $t$ test. Statistical differences among more than two groups were assessed using one-way ANOVA followed by tukey correction. Results were considered statistically significant if the $p$-value was less than 0.05. Statistical analysis was performed using Graphpad Prism 6.
### Online Table I. hiPSC-CM Contractile Properties

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Mattress (CC2)</th>
<th>Mattress (CJ1 – 001)</th>
<th>Mattress (Stanford)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak Cell Shortening Amplitude (µm)</td>
<td>0.2 ± 0.1</td>
<td>5.1 ± 1.0**</td>
<td>7.0 ± 1.0***</td>
<td>7.0 ± 1.0***</td>
</tr>
<tr>
<td>Cell Shortening (% resting length)</td>
<td>0.4 ± 0.2</td>
<td>9.0 ± 1.5***</td>
<td>9.4 ± 1.0**</td>
<td>8.7 ± 1.0**</td>
</tr>
<tr>
<td>t to peak 50% (s)</td>
<td>n.m.</td>
<td>0.2 ± 0.02</td>
<td>0.3 ± 0.05</td>
<td>0.2 ± 0.02</td>
</tr>
<tr>
<td>t to peak 90% (s)</td>
<td>n.m.</td>
<td>0.4 ± 0.03</td>
<td>0.6 ± 0.1</td>
<td>0.4 ± 0.04</td>
</tr>
<tr>
<td>t to baseline 50% (s)</td>
<td>n.m.</td>
<td>0.3 ± 0.02</td>
<td>0.3 ± 0.03</td>
<td>0.3 ± 0.04</td>
</tr>
<tr>
<td>t to baseline 90% (s)</td>
<td>n.m.</td>
<td>0.4 ± 0.02</td>
<td>0.6 ± 0.05</td>
<td>0.6 ± 0.1</td>
</tr>
<tr>
<td>Beats per minute (bpm)</td>
<td>23.0 ± 4.2</td>
<td>24.0 ± 2.0</td>
<td>15.0 ± 2.0</td>
<td>18.7 ± 2.0</td>
</tr>
</tbody>
</table>

n 9 17 6 9

**Online Table I.** hiPSC-CM Contractile Properties (non-paced). Stanford hiPSC-CM line, SCVI13. Data are mean ± SEM ***$P < 0.0001$ vs control, **$P < 0.0043$ vs control. n.m., not measurable.
### Online Table II. Matrigel Mattress Calcium Handling Properties

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Mattress</th>
<th>P – value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diastolic Ca ($F_{ratio}$)</td>
<td>1.0 ± 0.02</td>
<td>1.0 ± 0.02</td>
<td>0.6</td>
</tr>
<tr>
<td>CaT Amplitude ($F_{ratio}$)</td>
<td>0.5 ± 0.04</td>
<td>0.6 ± 0.03</td>
<td>0.1</td>
</tr>
<tr>
<td>CaT Decay ($t_{twitch}$ s)</td>
<td>0.9 ± 0.06</td>
<td>0.9 ± 0.04</td>
<td>1.0</td>
</tr>
<tr>
<td>Time to Peak 90% (s)</td>
<td>0.3 ± 0.03</td>
<td>0.4 ± 0.04</td>
<td>0.2</td>
</tr>
<tr>
<td>Time to Baseline 90% (s)</td>
<td>2.0 ± 0.2</td>
<td>1.9 ± 0.1</td>
<td>0.8</td>
</tr>
<tr>
<td>SR Ca Content ($F_{ratio}$)</td>
<td>0.7 ± 0.04</td>
<td>0.8 ± 0.04</td>
<td>0.09</td>
</tr>
<tr>
<td>Caff Tau ($t_{Caff}$ s)</td>
<td>3.0 ± 0.1</td>
<td>2.7 ± 0.1*</td>
<td>0.04</td>
</tr>
<tr>
<td>Vmax Upstroke ($F_{ratio}$/s)</td>
<td>4.1 ± 0.6</td>
<td>5.5 ± 0.7</td>
<td>0.2</td>
</tr>
<tr>
<td>Vmax Decay ($F_{ratio}$/s)</td>
<td>0.5 ± 0.4</td>
<td>0.7 ± 0.1*</td>
<td>0.03</td>
</tr>
</tbody>
</table>

n 25 35

**Online Table II.** Mattress Ca handling Properties (non-paced). Data are mean ± SEM. *P<0.05 vs control.
Online Table III. Electrophysiological Characterization

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control hiPSC-CM</th>
<th>Mattress hiPSC-CM</th>
<th>Rabbit-CM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amplitude (mV)</td>
<td>118.0 ± 3.0</td>
<td>124.0 ± 4.0</td>
<td>114.0 ± 4.0</td>
</tr>
<tr>
<td>dV/dt max (V/s)</td>
<td>64.0 ± 4.0</td>
<td>84.0 ± 8.0*</td>
<td>121.0 ± 10.0****</td>
</tr>
<tr>
<td>MDP (mV)</td>
<td>-74.0 ± 1.0</td>
<td>-74.0 ± 2.0</td>
<td>-66.0 ± 1.0</td>
</tr>
<tr>
<td>APD50 (ms)</td>
<td>326.0 ± 13.0</td>
<td>318.0 ± 15.0</td>
<td>345.0 ± 33.0</td>
</tr>
<tr>
<td>APD90 (ms)</td>
<td>378.0 ± 14.0</td>
<td>376.0 ± 16.0</td>
<td>404.0 ± 33.0</td>
</tr>
<tr>
<td>Cm (pF)</td>
<td>17.0 ± 1.0</td>
<td>17.0 ± 1.0</td>
<td>97.0 ± 7.0****</td>
</tr>
<tr>
<td>n</td>
<td>53</td>
<td>46</td>
<td>11</td>
</tr>
</tbody>
</table>

Online Table III. Electrophysiological Characterization (non-paced). Data are mean ± SEM. *P < 0.05 vs control, ****P < 0.0001 vs control.
<table>
<thead>
<tr>
<th>Primers</th>
<th>Description for Primers</th>
</tr>
</thead>
<tbody>
<tr>
<td>CASQ2-Hs00154286_m1</td>
<td>calsequestrin 2 (cardiac muscle)</td>
</tr>
<tr>
<td>KCNQ1-Hs00923522_m1</td>
<td>potassium voltage-gated channel, KQT-like subfamily, member 1</td>
</tr>
<tr>
<td>KCNE4-Hs01851577_s1</td>
<td>potassium voltage-gated channel, Isk-related family, member 4</td>
</tr>
<tr>
<td>SCN5A-Hs00165693_m1</td>
<td>sodium channel, voltage-gated, type V, alpha subunit ATPase, Ca++ transporting, cardiac muscle, slow twitch 2</td>
</tr>
<tr>
<td>ATP2A2-Hs00544877_m1</td>
<td>ryanodine receptor 2 (cardiac)</td>
</tr>
<tr>
<td>TNNI3-Hs00165957_m1</td>
<td>troponin I type 3 (cardiac)</td>
</tr>
<tr>
<td>TNNI1-Hs00913333_m1</td>
<td>troponin I type 1 (skeletal, slow)</td>
</tr>
<tr>
<td>18S-Hs99999901_s1</td>
<td>Eukaryotic 18S rRNA</td>
</tr>
</tbody>
</table>
Online Figure I. Matrigel mattress sarcomere architecture. hiPSC-CMs display organized sarcomere architecture which is aligned along the longitudinal axis of the cell. A and B control hiPSC-CM. C and D Mattress hiPSC-CM. Scale bar 10 µm. 63X.
Online Figure II. Sequential montage of hiPSC-CMs on Matrigel mattress. Mattress hiPSC-CMs adopt elongated morphology at ~ 10 hours post plating. Scale bar, 20 µm.
Online Figure III. Traction force correlation. A, Phase contrast image of hiPSC-CMs on Matrigel mattress with embedded fluorescence beads (0.75 µm diameter). B, Fluorescent image of A. C, Representative displacement field image of fluorescent beads calculated with particle image velocimetry (PIV) algorithm. D, Fourier transform traction cytometry (FTTC). (A – D) Scale bar, 20 µm. E, Correlation between Matrigel mattress platform contraction force calculated from cell shortening measurements and traction force estimated with the FTTC algorithm (n = 15 cells).
Online Figure IV. Electrophysiological Characterization of CMs. Representative action potential recordings of indicated CMs (non-paced). Dotted line indicates 0 mV.
Online Figure V. Characteristics of mattress sodium ($I_{Na}$) current. A, Representative $I_{Na}$ current of control hiPSC-CMs and B, mattress hiPSC-CMs. Traces were elicited by voltage protocol shown below current recording. C, Current-voltage relationship of peak $I_{Na}$ current from control hiPSC-CMs and Mattress hiPSC-CMs. D, $I_{Na}$ steady-state inactivation for control hiPSC-CMs and mattress (hiPSC-CMs). (Control hiPSC-CMs, black, n = 13) and (mattress hiPSC-CMs, grey, n = 15). $V_{1/2}$ half activation voltage.
Online Figure VI. Molecular characterization. A, Heat map from cardiac gene expression screen, control and mattress hiPSC-CMs, measured by real-time qPCR. Black represents low expression, and yellow represents high expression. B, Western blot confirmation for indicated proteins (n = 2 independent biological replicates). C, Summary bar graphs of relative ssTnI and cTnI expression.