SUPPLEMENTARY MATERIALS AND METHODS

ES Cell Line Derivation and Characterization

Human embryos were donated following informed consent and personal communication with MStem Cell staff to ensure all questions regarding donation were answered. Following hESC production and characterization, documents demonstrating adherence to NIH-established guidelines for embryo donation and hESC production of UM14-1, UM22-2 and UM38-2 were submitted to NIH for placement on the NIH hESC Registry and approvals were granted on 5/31/2012 (Registration #-0162), 3/26/2013 (Registration #-0209), and 5/14/2012 (Registration #-0155), respectively. Derivation of hESCs and their derivatives prior to acceptance on the NIH registry were performed with non-federal funds. Additionally, studies after placement on the NIH registry were also supported by non-federal funds. Briefly, blastocyst morphology was assessed four hours after embryo warm or thaw, and dictated the mode of hESC derivation. Laser-dissected inner cell masses (UM14-1 and UM22-2; supplemental figure 1) or whole blastocyst (UM38-2 PGD) were plated on human foreskin fibroblast (HFF) –feeders. After the appearance of the primary hESC colony (passage 0) colonies were manually split with a pulled glass rod to obtain early hESC colonies. The early colonies were cultured for 5 to 7 days on HFF and split with a pulled glass rod at least another 2 times. To secure the existence of hESC line several fragments of hESC colonies were vitrified in French straws while the remaining fragments were expanded for traditional cryopreservation or establishment of active cell cultures. Characterization of active hESC lines was performed with immunofluorescence for pluripotency markers [(Oct4, Nanog, SOX2, SSEA4 and TRA-1-60) Figure 1, supplementary figure 1]. Hypertrophic

cardiomyopathy-affected hESC and the control lines were differentiated for 21 days in culture as embryoid bodies and expressed lineage markers of endoderm (α-fetoprotein), mesoderm (brachyury) and ectoderm (tubulin βIII) were identified by PCR (Figure 1c-e; and supplementary figure 1). Finally, G-band karyotyping of UM38-2, UM14-1, and UM22-2 demonstrated euploid hESC lines (Figure 1 and supplemental figure 1).

Confirmation of splice defect resulting from the MYBPC3 mutation

RT-PCR of *MYBPC3* cDNA from UM22-2 control and UM38-2 PGD-HCM samples was performed using a forward primer in exon 25 (atgcggctgaacttcgacctgatt) and reverse primer in exon 29 (ctgtggggctgttgcggatgctc) and analyzed by gel electrophoresis. Bands corresponding to the wild-type and mutant transcript for PGD-HCM were gel-extracted and Sanger sequenced. Quantification of total *MYBPC3* mRNA was performed with qRT-PCR using the taqman gene expression assay (probe, Hs01076202_m1). The amount of wild-type *MYBCP3* was quantified using a taqman probe (Hs01076206_m1) that binds only to the wild-type transcript since the binding site spans the junction of exon 26-27, which then enabled calculation of the relative mutant transcript abundance. Pfaffl correction was performed to control for primer efficiency. Figure 1e-g shows the confirmation of the *MYBPC3* mutation.

Cardiac Directed Differentiation

hESC cardiac directed differentiation was done as previously described using the highly efficient matrix sandwich protocol relying on sequential application of cytokines and extracellular matrix at specific time points of differentiation(20) Cardiac directed differentiation was routinely performed in 24 well dish format on either plastic bottom

wells or custom made silicone coverslips to enable immunofluorescent analysis. Cardiac myocyte phenotype analysis was made between days 17 and 36, time points when there was no detectable expression of pluripotent stem cell markers (figure 2).

Western Blot Analysis

Protein expression was quantified by Western blot analysis as described recently.[22] Pluripotent stem cell protein expression of Oct3/4 (mouse monoclonal, 1:100, sc-5279 from Santa Cruz Biotechnology) was quantified and normalized to GAPDH protein expression (rabbit polyclonal, 1:5000 G9545 from Sigma) during the time course of cardiac directed differentiation. Total sarcomeric myosin (mouse monoclonal, 1:1000, MF20 from ATCC) was also normalized to GAPDH. Total cMyBP-C expression (Rabbit polyclonal antibody, 1:500 ab95200 from Abcam, or 1:250 ab171153 from Abcam) was normalized to total GAPDH protein (rabbit polyclonal, 1:5000 G9545 from Sigma) to determine the time course of protein expression during cardiac directed differentiation (figure 2). In other experiments to control for possible variation of cardiac differentiation efficiency total cMyBP-C protein expression was normalized to the myofilament protein expression of α-actinin (mouse monoclonal, 1:3000, A7811 from Sigma, figure 2). Gene transfer of full length cMyBP-C was detected by Western blotting for the unique FLAG epitope engineered into the *MYBPC3* adenovirus (anti-M2-FLAG, mouse monoclonal, Sigma).

Immunofluorescent Staining and Laser Scanning Confocal Microscopy

Cardiomyocyte protein localization and spatial organization was determined by immunocytochemistry using fluorescent antibodies with subsequent high resolution laser scanning confocal microscopy as described before.[21-23] Proteins probed for include: α-actinin (mouse monoclonal, 1:3000, A7811 from Sigma), β-MyHC (mouse monoclonal, 1:500, A4.951 from ATCC), actin (1:1000, Sigma), N-cadherin (mouse monoclonal, 1:100, 610920 from BD Transduction Laboratories) and connexin 43 (rabbit polyclonal, 1:100, ab1728 from Millipore). Laser scanning confocal microscopy of fixed slides was done using a Nikon A1R system using the appropriate laser/emission spectra and optimal Z-sectioning resolution. Magnification ranged from 60X for high resolution imaging of sarcomere structure to 4X for examination of large fields of view-scale bars are included in each image.

Cardiomyocyte purification and monolayer optical mapping

On day 25 of the cardiac directed differentiation protocol cells were enzymatically dissociated using trypsin and mechanical agitation. Trypsin was neutralized using EB20 media as described before.[22, 23] Following centrifugation cells were re-suspended in MACS buffer (Miltenyi Biotec) and washed twice before incubation with SIRPA antibody (CD172a-biotin, human, Miltenyi Biotec) for 15 minutes. Cells were washed for another 3 times with MACS buffer and incubated with anti-biotin microbeads (Miltenyi Biotec). Cardiomyocytes (SIRPA+ cells) were selected using anti-biotin microbeads (Miltenyi Biotec) with flow through a magnetic field (magnetic activated cell sorting, MACS). This technique for cardiomyocyte MACS has been described before.[24] Purified cardiomyocytes were collected in EB20 media and re-plated to form ~1cm diameter monolayers (100,000 CMs per monolayer) on matrigel coated silicone coverslips. [28]

Cardiomyocytes were cultured in EB20 for four days and subsequently maintained in RPMI supplemented with B27 with insulin. Ten days after purification monolayers were loaded with the cell permeant intracellular calcium indicator rhod 2AM (10µmol L^{-1}, 10min) for optical mapping of pacemaker calcium impulses through the monolayers (HBSS, Gibco, 37°C). Configuration of the optical mapping set up has been described before and is based on light emitting diode (LED) illumination.[25] Activation maps were generated (figure 7, isochronal lines are 10ms apart) and calcium impulse conduction velocity was calculated as described before.[23]

Introduction of wild-type human cMyBP-C into hESC-CMs by adenoviral transduction

Wild-type *MYBPC3* cDNA was cloned into the Gateway® entry clone, pENTR™1a plasmid using PCR to add an N-terminal FLAG epitope sequence (ATGGATTACAAGGATGACGACGATAAG). The FLAG-*MYBPC3* construct was then moved by recombination using the Gateway® LR Clonase® enzyme to the pAd/CMV/V5-DEST™ Gateway® Vector (Life Technologies). Virus was produced in 293A cells following Lipofectamine® transfection of the expression plasmid (Life Technologies). Viral lysate was purified, expanded, and titered by the University of Michigan Vector Core. A multiplicity of infection (moi) of 250 was used to introduce *MYBPC3* into differentiating cardiomyocytes on day 12 of directed differentiation. The virus was left on the cardiomyocytes overnight.

Statistical analysis

Statistical analysis was performed with Sigmaplot (versions 10 and/or 13) or Graphpad Prisma (version 6). Normal distribution of data was checked and pairwise comparisons of 2 groups was performed with unpaired Student's t test. Multiple group comparison's was performed with ANOVA, followed by a post-test to determine differences between groups (Tukey's test for means, LSD or Newman-Keuls multiple comparisons test). Data were expressed as mean ± standard deviation. Contingency tables were submitted to Chi-square analysis.