Supplemental Figures and Legends

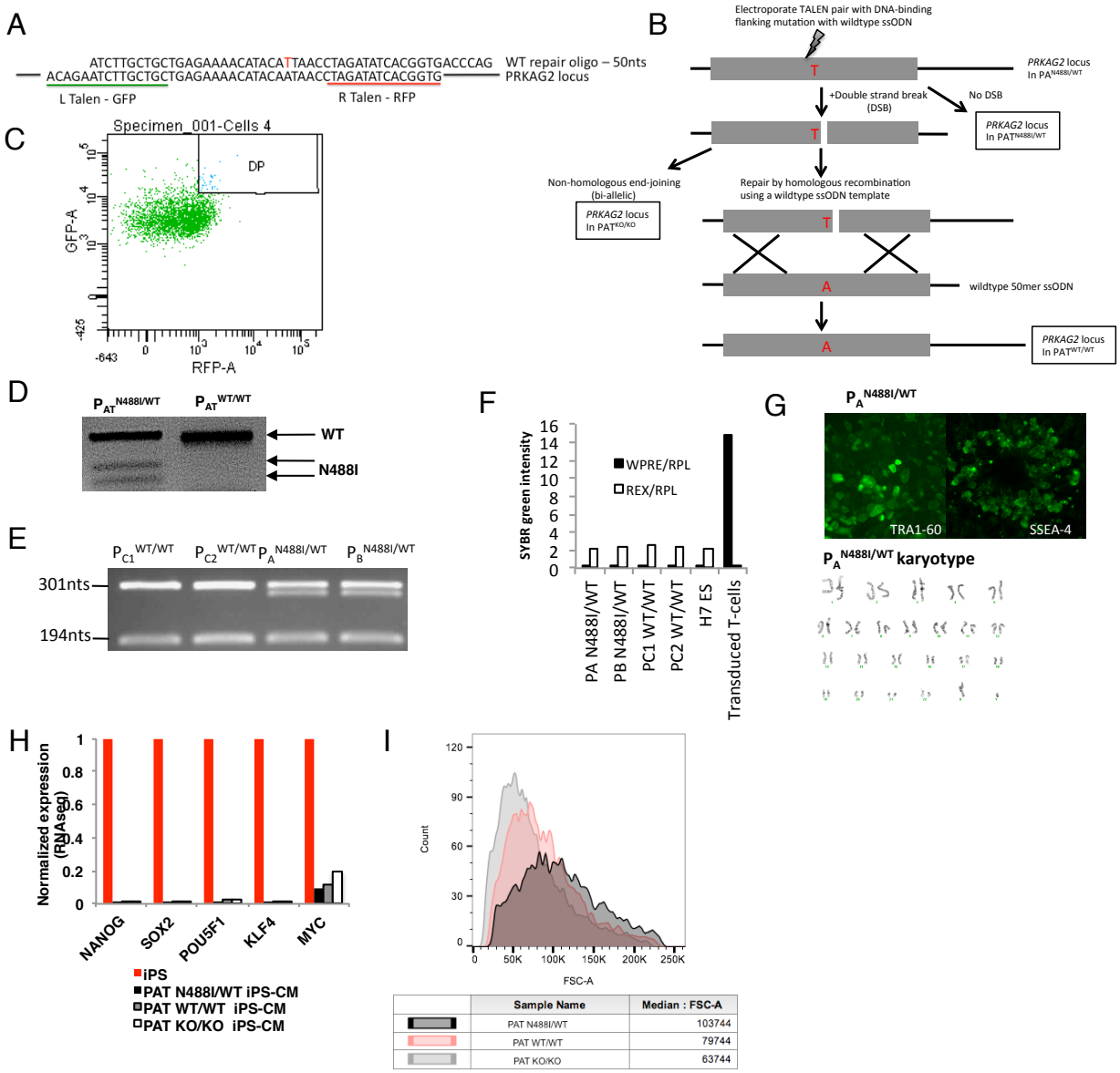

Figure S1. Related to Figure 1: TALEN isogenic iPS model of PRKAG2 cardiomyopathy. **(A)** PRKAG2 genomic region illustrates the genomic locus targeted by a pair of TALE nucleases conjugated to either green fluorescent protein (GFP) on the left (L) TALEN or red fluorescent protein (RFP) on the right (R) TALEN. WT repair locus is a 50mer ssODN (red "T" denotes corrected nucleotide). **(B)** Illustration of PRKAG2-N488I genomic locus after genome editing to create a TALEN isogenic iPSc series. **(C)** Representative FACS analysis of iPScs transfected with TALEN pair and analyzed for double positive (DP) GFP and RFP cells. DP iPScs were sorted and sequenced for evidence of gene editing at the PRKAG2 N488I locus. **(D)** Restriction fragment length polymorphism (RFLP) analysis of PCR amplicons from the PRKAG2 N488I locus of $P_{AT}^{N488I/WT}$ and $P_{AT}^{WT/WT}$ iPScs digested with MSE1 restriction enzyme (New England Biolabs). **(E)** RFLP analysis with MSE1 of cDNA from the patient-specific cohort of iPScs confirms the presence of N488I in $P_{A}^{N488I/WT}$ and $P_{B}^{N488I/WT}$ iPScs. **(F)** Quantitative PCR analysis of pluripotency gene ZFP42 (REX) and transgene silencing gene Woodchuck Hepatitis Virus

Posttranscriptional Regulatory Element (WPRE) normalized to housekeeping gene RPLP0 (RPL). Pluripotency control is an H7 ES line and transgene leak control is STEMCCA-transduced human T-cells. **(G)** (top panel) Immunostaining of pluripotency markers TRA1-60 and SSEA-4 (Alexa 488) in representative iPScs from $P_A^{N488I/WT}$ along with (bottom panel) normal karyotype. **(H)** RNAseq data of pluripotency transcripts from $P_{AT}^{N488I/WT}$ iPS cells compared to iPS-CMs differentiated from $P_{AT}^{N488I/WT}$ iPScs. **(I)** Representative FACS histogram of TALEN isogenic iPS-CMs analyzed for forward scatter (FSC) signal. Data are mean FPKM of pooled triplicates (F and H). For FACS, at least 10,000 events were analyzed.

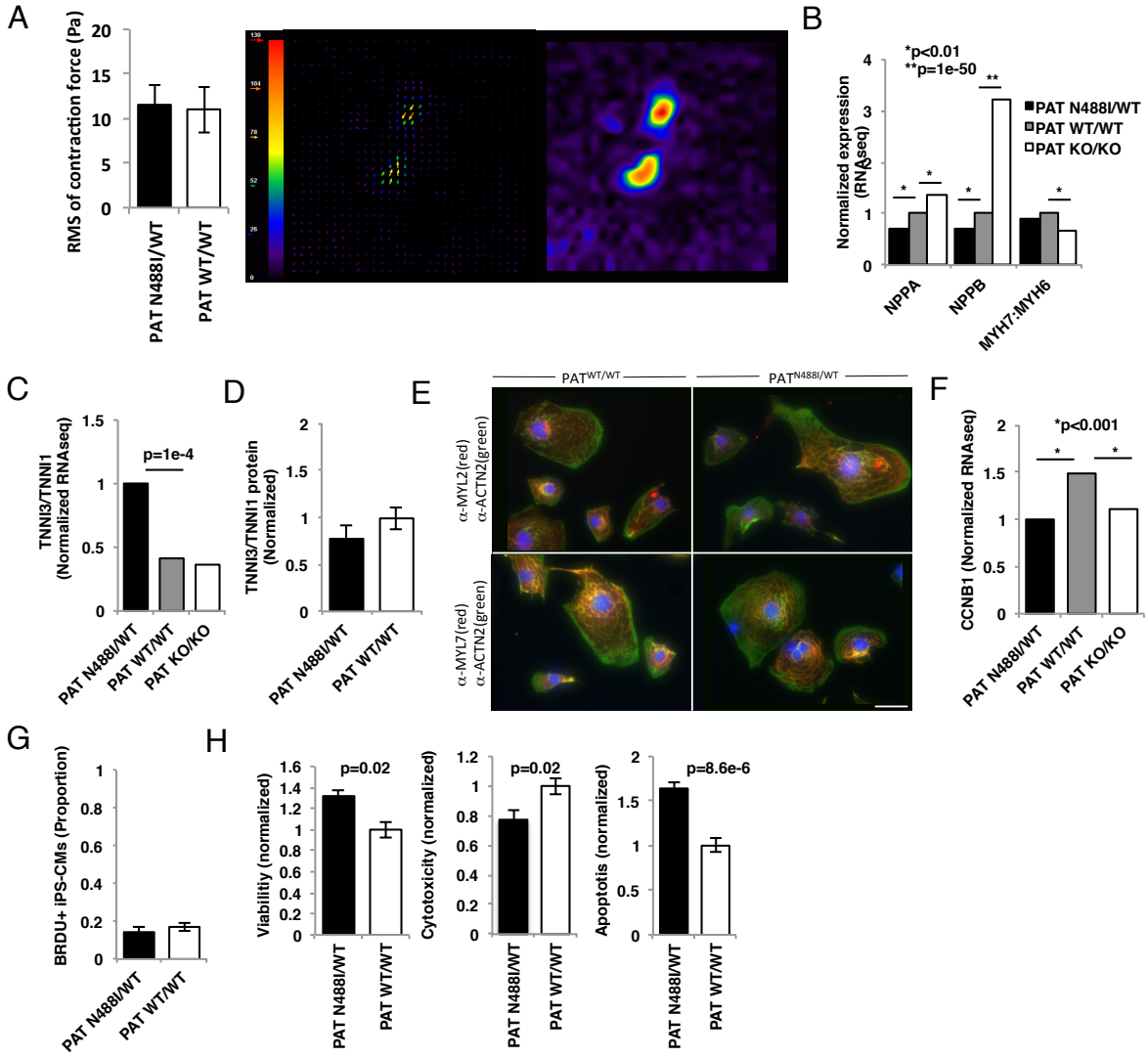

Figure S2. Related to Figure 2: Single iPS-CM contractility, gene expression and viability. **(A)** Quantification of root mean square (RMS) traction force (Pa) of single TALEN isogenic iPS-CMs on polyacrylamide gels with representative traction stress vector map (n>8 iPS-CMs per condition). **(B)** Expression of heart failure-related gene transcripts including A-type and B-type natriuretic peptides (*NPPA* and *NPPB*) and the beta myosin to alpha myosin heavy chain ratio (*MYH7*: *MYH6*) are shown. **(C)** The normalized ratio of cardiac (*TNNI3*) to skeletal (*TNNI1*) troponin I isoform transcript expression. **(D)** The normalized ratio of TNNI3 to TNNI1 protein expression in iPS-CMs by densitometry of immunoblots probed with isoform-specific antibodies (n>3 lanes each). **(E)** Immunofluorescence of iPS-CMs probed with antibodies to MYL2 (top row; ventricular-type) and MYL7 (bottom row; atrial-type) and co-stained with sarcomeric actinin A and DAPI. Scale bar=20μm. **(F)** Expression of cell cycle-dependent transcript cyclin B1 (*CCNB1*) in TALEN isogenic iPS-CMs. **(G)** Quantification of proportion of BRDU+ stained iPS-CMs after 48 hours of BRDU pulse (n>200 iPS-CMs per genotype). **(H)** IPS-CMs from $P_{AT}^{N488I/WT}$ and $P_{AT}^{WT/WT}$ were exposed to the cardiotoxic drug doxorubicin and analyzed for iPS-CM viability (left panel) and cytotoxicity (middle panel) by cell-permeant AFC and cell-impermeant R110 fluorescence, respectively, while apoptosis was measured by caspase 3/7 luciferase activity assays (right panel) (n≥6 replicates). Significance assessed by Student's t-test (A, D, G-H) and

Bayesian p-value (B, C, F) and data are normalized mean +/- SEM (A, D, G-H), normalized FPKM (B, C, F).

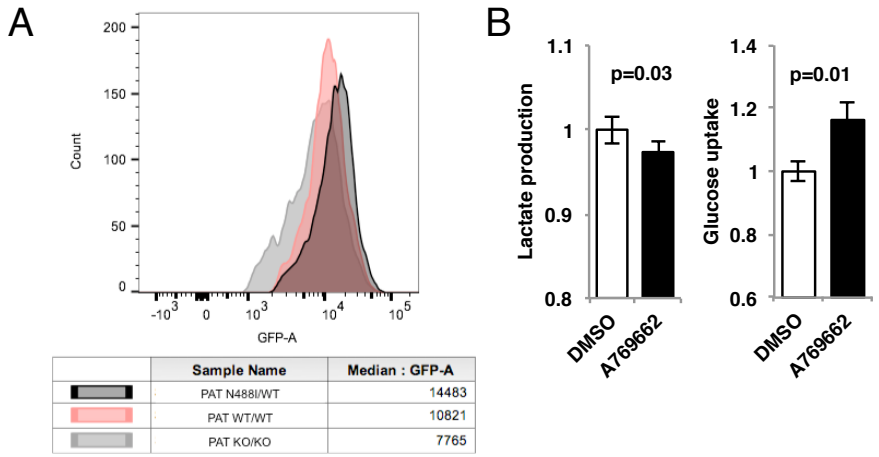

Figure S3. Related to Figure 4: Mitochondrial content and metabolic assays of iPS-CMs. **(A)** Representative FACS histogram of TALEN isogenic iPS-CMs analyzed for Mitotracker Green signal. **(B)** Biochemical assays of glucose uptake and lactate production in conditioned media from iPS-CMs during treatment with A769662 (5μm). For FACS, at least 10,000 events were analyzed. Data are mean +/- SEM (B) and significance assessed by Student's t-test (B).

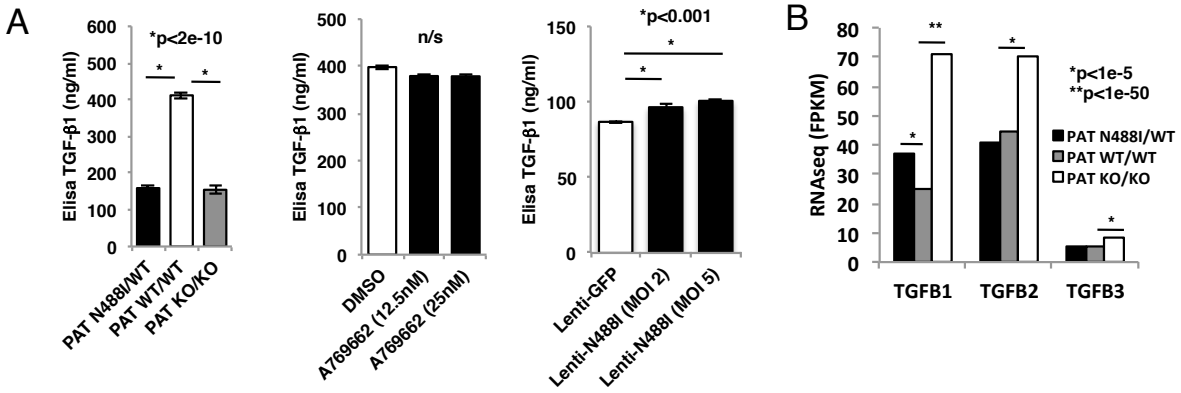

Figure S4. Related to Figure 6: Growth factor production from iPS-CMs. (A) Conditioned media analyzed for TGFβ-1 production using ELISA for AMPK activation by (left panel) TALEN isogenic iPS-CMs, (middle panel) A769662 or (right panel) lentiviral transduction. **(B)** Transcript analysis of TGFβ isoforms (1-3) from the TALEN isogenic series of iPS-CMs. Data are means +/- SEM (A) and FPKM (B). Statistical significance assessed by Student's t test (A) or Bayesian p-value (B).

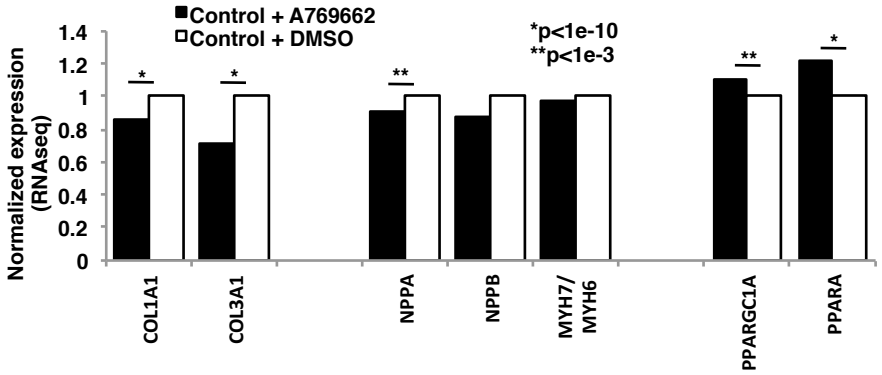

Figure S5. Related to Figure 7: Gene expression analysis of control mice with A769662 treatment. Normalized transcript analysis of extracellular matrix (*COL1A1* and *COL3A1*), pathologic hypertrophy (*NPPA*, *NPPB* and *MYH7/MYH6*) and metabolic factors (*PPARGC1A* and *PPARA*) in control mouse LV with A769662 treatment. Data are normalized FPKM and significance assessed by Bayesian p-value.

Supplemental Table Legends

Table S1. Related to Figure 1: DNA sequences for (A) TALEN experiment and (B) sequencing primers.

Table S2. Related to Figure 1: IPS genotypes for TALEN isogenic series.

Table S3. Related to Figures 3, 4 and 6: Differentially regulated transcripts identified from RNA sequencing of TALEN isogenic IPS-CMs.

Table S4. Related to Figures 3, 4 and 6: Differentially regulated transcripts identified from RNA sequencing of P-S iPS-CMs.

Table S5. Related to Figure 3: Principle components gene list.

Table S6. Related to Figure 5: Liquid chromatography – tandem mass spectroscopy (LC-MS/MS) of steady state metabolites in conditioned media from TALEN isogenic iPS-CMs correlated to AMPK activity.

Table S7. Related to Figure 7: Differentially regulated transcripts identified from RNA sequencing of mouse HCM treated with A769662 or DMSO control.

Supplemental Movie Legends

Movie S1. Related to Figure 2: $P_{AT}^{WT/WT}$ CMT paced at 1 Hz.

Movie S2. Related to Figure 2: $P_{AT}^{N488I/WT}$ CMT paced at 1 Hz.

Supplemental Experimental Procedures

LC-MS/MS metabolomics

Day 30-40 iPS-CMs differentiated from $PAT^{N488I/WT}$, $PAT^{WT/WT}$ and $PAT^{KO/KO}$ iPScs were seeded in 6 well plates and cultured in serum-free RPMI-B27 media (Invitrogen) for five days prior to processing. The media was siphoned off and the plate immersed in high performance liquid chromatography (HPLC) - grade water, the water decanted and aspirated, and then the culture plate carefully placed on the surface of liquid nitrogen in a liquid nitrogen-resistant basin for 15 seconds to quench cellular metabolic activity. Next, 2mls of extraction medium (75% 9:1 Methanol:Chloroform, 25% H_2O) were poured into each well. After 15 seconds 1.5ml was aspirated into respective labeled sample tubes, and 0.5ml was aspirated from each well into collective pooled extract tubes. Extracts were then centrifuged (14,000 rpm, 4 °C for 20 minutes), and 1.25ml of the supernatant was aliquoted into conical Eppendorf tubes and labeled isotope standards (L-Phenylalanine-d_8 and L-Valine-d_8) were added to the supernatants. Samples were then dried down on a speedvac concentrator (Thermo Scientific) and re-suspended in 100µl of acetonitrile: water (50: 50 v/v) before injection. Sample injection volume was 5 or 10µl, depending on liquid chromatography-tandem mass spectrometry (LC-MS) acquisition method, described below. LC-MS data were acquired using three methods on two LC-MS machines. The first two methods were acquired using a 4000 QTrap triple quadrupole mass spectrometer (Applied Biosystems/Sciex) that was coupled to a multiplexed LC system comprised of a 1200 Series pump (Agilent Technologies) and an HTS PAL autosampler (Leap Technologies) equipped with two injection ports and a column selection valve, as previously reported(Wang et al., 2011). For the positive ion mode (PIM) method, the pump was configured for hydrophilic interaction chromatography (HILIC) using a 150 x 2.1 mm Atlantis HILIC column (Waters) and mobile phase (mobile phase A: 10 mM ammonium formate and 0.1% formic acid, v/v; mobile phase B: acetonitrile with 0.1% formic acid, v/v). In the negative ion mode (NIM) method, the pump was configured for reverse phase (RP) chromatography using a Synergy Polar-RP 4.6 x 50 mm column (Phenomenex) and mobile phase (mobile phase A: 5 mM ammonium acetate in water: acetonitrile [95:5, v/v], mobile phase B: 5 mM ammonium acetate in acetonitrile: water [95:5, v/v]). Injection volume was 10 ul for both methods. Multiplexing was used to enable the measurement of 106 metabolite transitions, in PIM, and 67 metabolite transitions in NIM. In PIM,

the samples were injected directly onto the HILIC column that was eluted at a flow rate of 250 μL/min with initial conditions of 5% mobile phase A and 95% mobile phase B followed by a 10.5 min linear gradient to 60% mobile phase A. In NIM, samples were injected directly onto the RP column, that was eluted at a flow rate of 250 μL/min with initial conditions of 95% mobile phase A and 5% mobile phase B followed by a 6 minute linear gradient to 100% mobile phase B. MS analyses were carried out using electrospray ionization (ESI) and multiple reaction monitoring (MRM) scans in both ion modes. Declustering potentials and collision energies were optimized for each metabolite by infusion of reference standards prior to sample analyses. The dwell time for each transition was 30ms, the ion spray voltage was 5kV, and the source temperature was 450ºC for both methods. The third acquisition method was optimized for nucleotide triphosphates, high-energy intermediates, organic acids, TCA cycle intermediates, and glycolytic intermediates. This method used HILIC chromatography on a 2.1×100mm 3.5 μm Xbridge Amide column (Waters) in negative ion mode. Mobile phase A was 95:5 (v/v) water: acetonitrile with 20mM ammonium acetate and 20mM ammonium hydroxide (pH 9.5). Mobile phase B was acetonitrile. The chromatography system consisted of a 1260 Infinity autosampler (Agilent) connected to a 1290 Infinity HLPC binary pump system (Agilent). Injection volume was 5 ul. The initial conditions were 0.25 mL/min of 85% mobile phase B followed by a linear gradient to 35% mobile phase B over 6 minutes. This was followed by a linear gradient to 2% mobile phase B over 0.5 minutes held for an additional 0.5 minutes, then a 0.5 minute gradient return to 85% mobile phase B. Column equilibration was continued for 4.5 minutes at 0.5 mL/min for a total cycle time of 12 minutes. The column compartment was maintained at 30°C. The HPLC pump was connected to a 6490 QQQ (Agilent) triple quadrupole mass spectrometer equipped with an electrospray ionization source, on which 157 metabolites were optimized for negative mode detection. 73 of the metabolites were reproducibly detected in cell extract and were selected for dynamic multiple reaction monitoring (MRM) that had a minimum dwell time of 30ms for each metabolite. Final mass spectrometry settings for the QQQ 6490 were sheath gas temperature 400°C, sheath gas flow 12 L/min, drying gas temperature 290°C, drying gas flow 15 L/min, capillary voltage 4000V, nozzle pressure 30 psi, nozzle voltage 500V, and delta EMV 200V. Metabolite quantification was determined by integrating peak areas using MassHunter QQQ Quant (Agilent). All metabolite peaks were manually reviewed for peak quality in a blinded manner. In addition, pooled cellular extract samples were interspersed within each analytical run at standardized intervals every 10 injections, enabling the monitoring and correction for temporal drift in mass spectrometry performance. The nearest neighbor flanking pair of pooled plasma was used to normalize samples in a metabolite-by-metabolite manner. Internal standard peak areas were monitored for quality control and individual samples with peak areas differing from the group mean by more than two standard deviations were reanalyzed. Formic acid, methanol, chloroform, LC-MS–grade solvents and L-valine-d8 were purchased from Sigma-Aldrich. L-phenylalanine-d8 was purchased from Cambridge Isotope Laboratories. Ammonium acetate, ammonium hydroxide, and HPLC Optima grade acetonitrile, methanol, and water were purchased from Fisher Scientific.

Cardiac microtissue (CMT) platform

Polydimethylsiloxane (PDMS) micro-fabricated tissue gauges (μTUG) substrates were molded from the SU-8 masters, with embedding fluorescent microbeads (Fluoresbrite 17147; Polysciences, Inc.) onto the cantilevers. μTUG substrates were treated with 0.2% pluronic F127 for 30 minutes to prevent cell-extracellular matrix interactions. iPS-CMs were dissociated after trypsin digestion and mixed with stromal cells (human mesenchymal stem cells, hMSCs) and extracellular matrix slurry to enable tissue compaction. Stromal cells were pretreated with 10μg/ml Mitomycin C (Sigma) to prevent proliferation. The amount of stromal cells added was

10% of the total myocyte population. A suspension of ~$1.1x10^6$ cells within reconstitution mixture, consisting of 2.25mg/mL liquid neutralized collagen I (BD Biosciences) and 0.5mg/mL human fibrinogen (Sigma-Aldrich), was added to the substrate. The device was centrifuged to drive the cells into the micro-patterned wells. For quantifying stimulation (Boudou et al., 2012), forces, bright-field and fluorescence images were taken at 30Hz (Photometrics Evolve EMCCD camera (Photometrics), and an A-Plan 10X objective on a Nikon Eclipse Ti (Nikon Instruments, Inc.) microscope which was equipped with a live cell incubator. Only tissues that were uniformly anchored to the tips of the cantilevers were included in the analysis. The displacement of fluorescent microbeads at the top of the cantilevers was then tracked using the SpotTracker plug-in in ImageJ (National Institutes of Health). Displacement values were run through a custom MATLAB script to compute twitch force (dynamic tension) and resting tension. Electrical field stimulation of biphasic square pulses of 1ms was given by placing two carbon electrodes (Ladd Research laboratories) separated by 2cms on the sides of the samples connected through platinum wires to a stimulator. Cantilever spring constants were computed utilizing a capacitive MEMS force sensor mounted on a micromanipulator as described previously.

Mouse protocols, cardiac imaging, and fibrosis quantification

Hearts were excised from isoflurane- euthanized mice, washed in PBS, fixed overnight in 4% paraformaldehyde, and embedded in paraffin. After serial sectioning of hearts (apex to base), 13 evenly distributed 5μm sections were stained with Masson trichrome. Heart sections were scanned by BZ-9000 Generation II (Keyence). Fibrosis areas within sections were measured by software BZ-II Analyzer (Keyence). The percentage of total fibrosis area was calculated as the summed blue-stained areas divided by total ventricular area. Echocardiogram data were obtained using Vevo 770 High-Resolution In Vivo Micro-Imaging System and RMV 707B scan-head (VisualSonics Inc.) as previously described (Teekakirikul et al., 2010). Ultrasonographer was blinded to the treatment arms of the study during imaging. The images were acquired as 2D and M-mode (left parasternal long and short axes) and measurements were averaged from 3 consecutive heartbeats of M-mode tracings as recommended by the American Society of Echocardiography's Guidelines (Lang et al., 2005). LV wall thickness (LVWT) was measured by short axis image.

Traction force microscopy

Polyacrylamide gels were made by creating a solution of acrylamide containing 7.5% acrylamide and 4% bis-acrylamide along with 0.2μm fluorescently labeled polystyrene beads (Invitrogen) that was polymerized using ammonium persulfate and TEMED between a hydrophilic glass coverslip and quartz glass. The polymerized gel was subjected to deep UV in a UV ozone cleaner for 2 minutes and then a solution of 17 mg/ml N-hydroxysuccinimide (NHS; Fluka) and 10 mg/ml EDC (1-ethyl-3-[3- dimethylaminopropyl]carbodiimide hydrochloride; Pierce)was deposited onto the surface of the gel for 15minutes.The gels were then incubated with 50μg/ml fibronectin for 2 hours at 37°C. Gels were washed 3 times with PBS before seeding cells. The contractile traction stresses exerted by myocytes on Fn coated PAA substrates of E~7.9kPa rigidity were computed after 48 hours of plating by measuring the displacement of 0.2 μm fluorescent beads (Invitrogen) embedded within the gels. Briefly, images of bead motion near the cell- surface interface on the substrate surface (before and after cell detachment with 10%SDS), were acquired, aligned using Image J (NIH) and converted into displacement vectors using the particle image velocimetry program implemented through the Image J plugin. An estimate of cell traction stresses was computed from the substrate displacement fields using the Fourier transform traction cytometry (FTTC) method.

BRDU assay

Day 30 TALEN isogenic IPS-CMs were cultured in the presence of bromodeoxyuridine (BRDU; Invitrogen) for 48 hours. Cells were then fixed with 4% PFA and stained with antibodies to BRDU (Alexa-488), Troponin I (Santa Cruz) and DAPI. BRDU+ nuclei were quantified using fluorescent microscopy and proportion BRDU+ was determined by counting BRDU+ and Troponin I+ co-stained nuclei.

Supplemental References

Wang, T.J., Larson, M.G., Vasan, R.S., Cheng, S., Rhee, E.P., McCabe, E., Lewis, G.D., Fox, C.S., Jacques, P.F., Fernandez, C.*, et al.* (2011). Metabolite profiles and the risk of developing diabetes. Nat Med *17*, 448-453.