SUPPLEMENTARY INFORMATION

Cell Culture and Reagents

Neonatal Rat Ventricular Myocyte (NRVM) Isolation

Neonatal rat ventricular myocytes (NRVM) were isolated from day 0-1 Sprague Dawley rat pups as previously described using repeated rounds of trypsin (Worthington Biochemical) digestion. To enrich for cardiomyocytes, cells were plated on tissue culture plastic for 1hr at 37º C and 5% CO_2. The resulting supernatant was collected and plated on fibronectin (Sigma Aldrich) coated dishes overnight. These cells were used the following day for microtissue seeding.

Human Pluripotent Stem Cell Culture and Cardiac Differentiation

The human induced pluripotent stem (iPS) cell line SV20 was provided by the iPS Core lab at the University of Pennsylvania Cardiovascular Institute (http://web.expasy.org/cellosaurus/CVCL_EL23). Undifferentiated colonies were maintained on Matrigel™ (Beckman Dickinson) coated plates (0.5mg Matrigel™ per 6 -well plate) in mTESR™ medium (Stem Cell Technologies) at 37º C, 5% CO_2, and 21% O_2. Colonies were passaged every 3 to 5 days using accutase (Sigma Aldrich).

Cardiac differentiation was performed as previously described (ref). Differentiation was performed on cells dissociated with accutase and re-plated in Matrigel™ coated 12-well plates (2mg per 12-well plate) at a density of 10^6cells/well in mTeSR media supplemented with 5µM Rock Inhibitor (Y27632; Enzo) overnight. Media was exchanged every 2 days to RPMI 1640 (Gibco) + B27 supplement (minus insulin; Gibco) supplemented with either 1uM Chiron 99021 (LC Laboratories) or 2uM Wnt-C59 (Tocris Bioscience). Day 6 and beyond, cells were maintained in RPMI+B27 (plus insulin) media; initial beating was seen starting at day 7-12. Metabolic selection to enrich for cardiomyocytes was performed after day 10. In brief, media was exchanged to RPMI (without glucose; Gibco) + 5mM sodium DL-lactate (Sigma Aldrich) for 6-10 days with media changes every 2 days. Cells were given several days to recover after metabolic selection before performing any experiments. After metabolic selection, cardiomyocyte purity was in the range of 80-90% (data not shown).

Human Mesenchymal Stem Cell Culture

Human Mesenchymal Stem Cells (hMSCs) were purchased from Lonza and maintained in alpha MEM media supplemented with 2mM (1%) Glutamax and 100U/mL Penicillin/Streptomycin solution (all from Gibco). Cells were passaged every 3-4 days with 0.05% Trypsin EDTA (Gibco) up until passage 10 where cells became senescent. Cells from passages 7-10 were used for experiments involving human CMTs to minimize hMSC proliferation within microtissues.

Cardiac Microtissue (CMT) Platform

Multilayer silicon master templates were created by photo-patterning SU-8 (Microchem) photoresist onto silicon wafers (Single-sided polished, Prime grade) using successive spin coats (CEE 200X, Brewer Science, Inc.), alignment (ABM 3000 HR Mask Aligner, ABM Inc.), UV exposure (360nm), and baking steps. Negatives of silicon wafer, were cast by pouring a pre-polymer (10:1 base to curing agent ratio) of poly-dimethyl siloxane (PDMS, Sylgard 184; Dow

Corning) onto wafers. Devices were cast using negatives that were plasma cleaned under air and silanized with trichloro -(1H, 1H, 2H, 2H-perfluorooctyl) silane (Sigma Aldrich) overnight. For active tension measurements, fluorescent beads (Fluoresbrite® YG Carboxylate Microspheres 3.00µm) diluted 1:3000 in ethanol were centrifuged into silanized negatives to embed beads in final device. PDMS pre-polymer (5:1 to 15:1 base to curing agent ratio) was then poured onto silanized negatives, which were then inverted onto a hardened layer of PDMS in a 35 mm dish. These substrates were baked at 60º C for 24hr. Stamps were removed from the molds carefully using ethanol as a lubricant to avoid damaging tops of pillars.

Microtissue Seeding Procedure

Briefly, CMT arrays were sterilized with 70% ethanol and treated with 0.02% w/v Pluronic F127 (Sigma Aldrich) to prevent cell attachment against the walls of the wells on the surface. A liquid neutralized collagen I solution (1 mg/ml) was created by mixing collagen I (rat tail, Corning), fibrinogen (0.5 mg/mL final concentration; Sigma Aldrich), HEPES (10mM final conc.), $NaHCO_3$ (4.17mM final conc.; Sigma), 1M NaOH (neutralization ratio: 0.5; Sigma), M199 (10x) (1x final conc.; Sigma Aldrich, cat no. M0650) in sterile dH_2O on ice. 10^6- $1.2x10^6$ NRVMs were mixed with collagen solution and centrifuged into a single array. Excess collagen in between wells was removed using a vacuum aspirator. Collagen was polymerized inside an incubator at 37º C, 5%CO_2, 21% O_2 for 15min. Culture media consisting of DMEM high glucose + 10% Horse Serum + 2% Chicken Embryo Extract (Charles River Laboratories) + 1% Antibiotic/Anti-mycotic solution (Gibco) was added to arrays.

Electrical Stimulation of Microtissues

Microtissue arrays were field stimulated by placing 2 carbon electrodes into either side of the array via drilling holes into the lid of a 35mm dish. Electrical stimulation parameters were: biphasic, rectangular pulse, 15V/cm, 1ms duration, 1ms delay, and 1Hz frequency. Microtissues were analyzed with respect to spontaneous beating rate (number of contractions counted within 60 sec interval), excitation threshold (ET, minimum stimulation voltage required for synchronous contractions), and maximum capture rate (MCR, maximum beating frequency that can be captured). These parameters were assessed starting on microtissue day 2, before and after treatment with sunitinib using a Nikon TE2000U inverted microscope (Nikon Corporation) equipped with temperature and CO_2 control (37º C, 5% CO_2)

SUPPLEMENTARY FIGURES

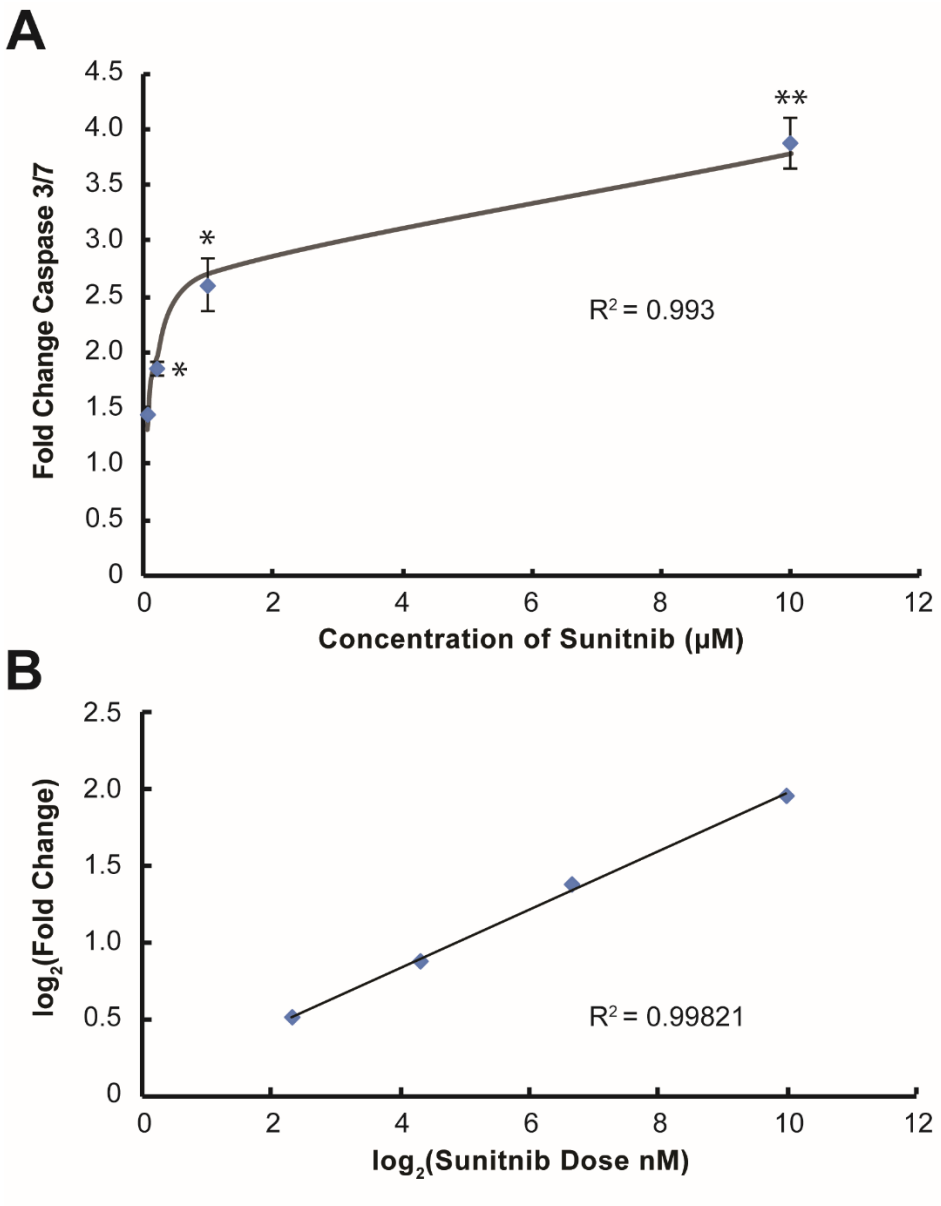


Figure S1: Modeling dose dependence of sunitinib induced caspase 3/7 activation- **A)** Non-linear curve fitting of data from Figure 1C using a $\log_2$ function R^2=0.993. **B)** $\log_2$-$\log_2$ plot of data from Figure 1C fit with linear curve yielded similar results R^2=0.9982. See figure 1C for statistics.

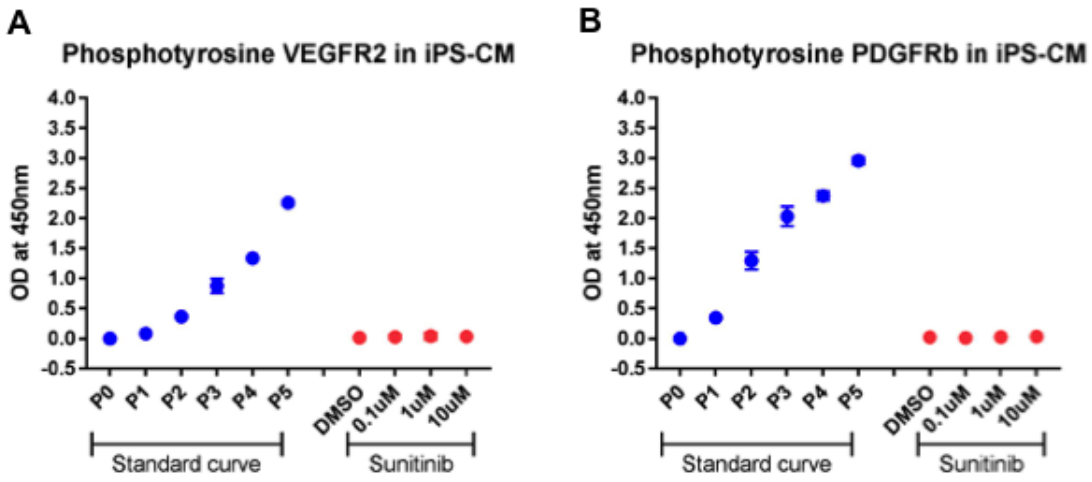
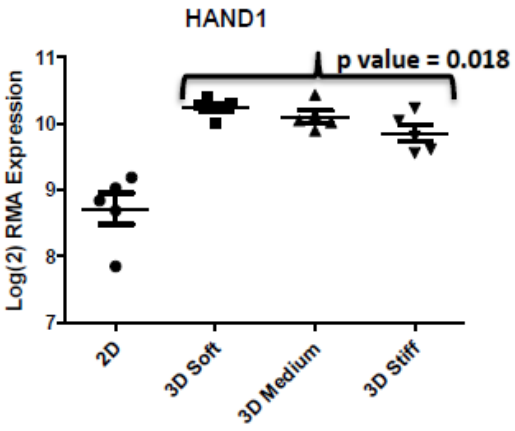
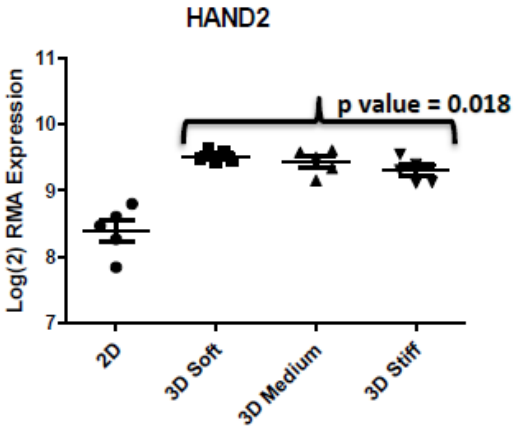
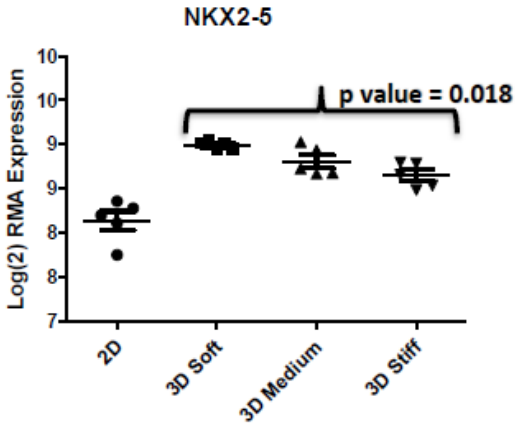
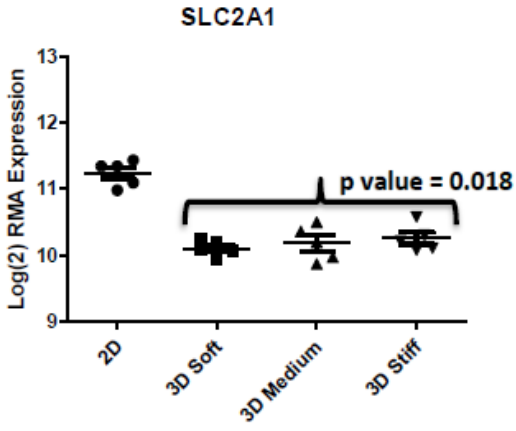


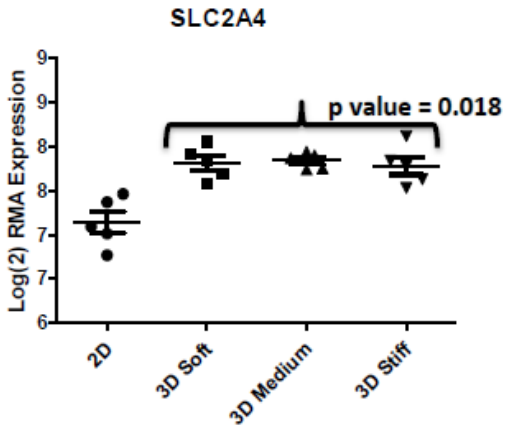
Figure S2: Human Phosphotyrosine ELISAs. Phosphotyrosine PDGFRB and VEGFR2 were measured in iPS-derived cardiomyocytes using the RayBiotech's ELISA kits (ref: PEL-PDGFRB-Y and PEL-VEGFR2-Y, RayBiotech, GA, USA), according to manufacturer's instructions. Briefly, iPS-CMs were solubilized in the Cell Lysate Buffer and incubated 30min on ice. After centrifugation at 13,000 rpm for 10min, the proteins were incubated in triplicate into the well strips for 2h at room temperature. 100ul of the 1X biotinylated anti-phosphotyrosine antibodies was incubated for 1h at room temperature followed by 45min incubation with the HRP-Streptavidin solution. The reaction was revealed with TMB One-Step Substrate Reagent for 30min. Finally, the reaction was terminated by adding 50ul of Stop Solution and read immediately by spectrophotometer at 450nm.


HAND1
p value = 0.018
Log(2) RMA Expression
11
10
9
8
7
2D
3D Soft
3D Medium
3D Stiff


HAND2
p value = 0.018
Log(2) RMA Expression
11
10
9
8
7
2D
3D Soft
3D Medium
3D Stiff


NKX2-5
p value = 0.018
Log(2) RMA Expression
10
9
8
7
2D
3D Soft
3D Medium
3D Stiff


SLC2A1
p value = 0.018
Log(2) RMA Expression
13
12
11
10
9
2D
3D Soft
3D Medium
3D Stiff


SLC2A4
p value = 0.018
Log(2) RMA Expression
9
8
7
6
2D
3D Soft
3D Medium
3D Stiff

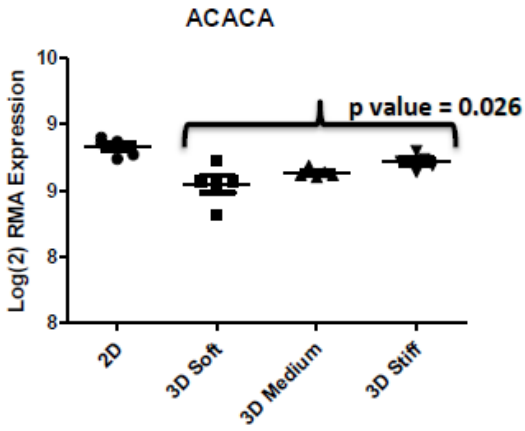

ACACA
p value = 0.026
Log(2) RMA Expression
10
9
8
2D
3D Soft
3D Medium
3D Stiff

Figure S3: Fetal Gene Expression: RNA from human embryonic stem cells (ES) grown in 2D tissue culture and 3D tissue culture, at three levels of physical stiffness, were collected 6 days after the formation of microtissues. Once five sets of samples were collected RNA was extracted using RNeasy kit and treated with DNase. RNA was hybridized with Affymetrix ST2.0 gene arrays using manufacturer instructions. All CEL files were normalized with the use of robust multi-array analysis (RMA)[1] using Bioconductor. To remove residual batch effect, expression values were further adjusted using ComBat[2]. Probe sets were removed if they displayed low expression in all samples (RMA < 5) leaving 37,161 RMA expression levels in five sets of biological replicates. Mann-Whitney U test with Holm-Bonferroni Correction (p-value cutoff of 0.05) was used to determine differential expression on a subset of 21 fetal cardiac specific genes[3,4] comparing 2D and 3D culture.

1. Irizarry, R.A. et al. Exploration, normalization, and summaries of high density oligonucleotide array probe level data. Biostatistics 4, 249-64 (2003).
2. Johnson, W.E., Li, C. & Rabinovic, A. Adjusting batch effects in microarray expression data using empirical Bayes methods. Biostatistics 8, 118-27 (2007).
3. Cox EJ, Marsh SA. A Systematic Review of Fetal Genes as Biomarkers of Cardiac Hypertrophy in Rodent Models of Diabetes. PlosOne 2014; 9(3):e92903.
4. Taegtmeyer H, Sen S, Vela D. Return to the fetal gene program: A suggested metabolic link to gene expression in the heart. Ann NY Acac Sci 2010; 1188:191-8.

SUPPLEMENTAL TABLE 1

Gene Name	2D	Soft	Medium	Stiff	Fold Change From 2D	2D vs 3D p-Values	2D vs 3D Adjusted p-Values
HAND1	8.7200	10.2480	10.1100	9.8560	0.3919	0.0012	0.018435
HAND2	8.4000	9.5140	9.4380	9.3020	0.4938	0.0012	0.018435
NKX2-5	8.1400	8.9960	8.8100	8.6560	0.6239	0.0012	0.018435
SLC2A1	11.2360	10.1000	10.1800	10.2560	2.0811	0.0012	0.018435
SLC2A4	7.1400	7.8140	7.8400	7.7800	0.6279	0.0012	0.018435
ACACA	8.8280	8.5500	8.6340	8.7180	1.1439	0.0026	0.025522
MYH6	11.9940	12.8300	12.7180	12.7240	0.5891	0.0040	0.0356139
NPPB	10.0980	8.6500	8.8220	8.9240	2.4612	0.0040	0.0356139
MEF2C	7.1740	7.5740	7.5880	7.5160	0.7656	0.0204	0.142681
ACADM	11.4260	11.5660	11.5760	11.5620	0.9063	0.0323	0.194058
ATP2A2	11.3720	11.5180	11.5740	11.6560	0.8641	0.0444	0.222225
ACTA1	9.2340	8.7860	8.7640	8.8220	1.3597	0.0444	0.222225
TTN	11.5720	11.8020	11.9980	12.0280	0.7734	0.0983	0.294891
SP1	9.3740	9.4480	9.4560	9.4000	0.9588	0.2945	0.58906
NFATC1	7.3640	7.4820	7.4680	7.2860	0.9673	0.4057	0.58906
SMAD2	10.1720	10.0580	10.1080	10.1900	1.0377	0.6622	0.58906
GYS1	9.0700	9.1020	9.0100	8.9760	1.0286	0.7267	0.58906
MYH7	10.2420	10.1960	10.3160	10.2940	0.9817	0.7933	0.58906
NFATC2	8.0080	8.0000	7.9800	8.0240	1.0046	0.7934	0.58906
NPPA	10.1860	10.4320	10.3440	10.2740	0.8925	0.8958	0.58906
MAP2K3	7.3500	7.3780	7.2780	7.3940	1.0000	0.9652	0.58906
GATA4	9.6780	9.7700	9.8420	9.7620	0.9244	1.0000	0.58906