Supporting Information

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SI Materials and Methods

Cell Culture. All animal protocols were approved by the Harvard University Animal Care and Use Committee. Ventricles from 2-d-old Sprague–Dawley rats were harvested, minced, and incubated with 1 mg/mL trypsin for 12 h at 4 °C. Single cells were isolated by incubating the tissue with 1 mg/mL collagenase (Worthington Biochemical) for 2 min at 37 °C, agitating the tissue several times with a pipette, collecting the supernatant, and repeating this process three more times until no tissue remained. To reduce nonmyocyte cell populations, cell suspensions were incubated twice in tissue culture flasks for 45 min and the supernatant was collected. Myocytes were suspended in M199 media supplemented with 10% FBS, 20 mM glucose, 2 mM L-glutamine, 1.5 μM vitamin B12, 50 U/mL penicillin, 10 mM Hepes, and 0.1 mM MEM nonessential amino acids. Samples were seeded with 0.67 to 1.0×10^6 cells. Stretched samples were seeded with a higher cell density to ensure even tissue confluence in all samples because mechanical agitation from the stretching device interfered with initial cell adhesion. Myocytes were maintained in supplemented M199 media with 10% FBS for the first 48 h and were then switched to supplemented M199 media with 2% FBS.

Microcontact Printing. An array of 80×12 μm^2 rectangles arranged into a "brick wall" pattern with a "sawtooth" interface at their longitudinal borders was designed in AutoCAD and etched onto a photolithographic mask. Silicon wafers were spin-coated with SU-8 2002 photoresist (Microchem), aligned under the photolithographic mask, exposed to UV light for 5 s, and submerged in SU-8 developer to dissolve unmasked regions. After silanizing the wafer, polydimethylsiloxane (PDMS; Sylgard 184; Dow Corning) was poured over the wafer and allowed to polymerize at 65 °C for at least 4 h. The PDMS block was then peeled off the wafer, trimmed, and used as a stamp. Multiple stamps were fabricated from the same wafer.

Elastic silicone membranes (Specialty Manufacturing) with a thickness of 0.01 inches were clamped into stainless steel brackets, as previously described (1). A 25-mm-diameter ring of silicon tubing was affixed to the center of the membrane by using PDMS. Immediately before fibronectin (FN) application, membranes were treated in a UV-ozone cleaner (Jelight) to sterilize and activate the surface for FN binding. The PDMS stamps described earlier were coated with 50 μg/mL FN (BD Biosciences) for 1 h, dried, and inverted onto the center of the membranes within the silicon ring. Separate membranes were coated uniformly with 50 μg/mL FN added directly to the membrane for 20 min immediately following UV-ozone treatment.

Gene Expression Analysis. RNA was isolated from tissues after 6, 24, and 96 h in culture by using Stratagene Absolutely RNA Miniprep Kit (Agilent Technologies). To normalize across cell harvests, RNA was collected from myocytes cultured on substrates coated with isotropic FN for 1 h for each harvest. RNA quality was validated with a Nanodrop spectrophotometer (Thermo Scientific) and an Agilent 2100 Bioanalyzer (Agilent Technologies). mRNA was amplified and hybridized to Affymetrix GeneChip Rat Gene 1.0 ST Arrays in accordance with the manufacturer's instructions, and scanned with an Affymetrix GeneChip Scanner 3000 7G. Probe cell intensity data files were loaded into Affymetrix Expression Console Software and normalized by using the robust multichip average method (2). Signal values were log2-transformed and analyzed with Bioconductor open-source software and the limma package in R (3). Expression values for each condition were averaged, fit to a linear model, and compared by using Bayes statistics.

Genes were filtered to include only those which showed a statistically different expression value in any experimental condition at any time point compared with the expression value at 1 h after seeding ($P < 0.05$), resulting in 19,179 genes (Tables S1–S3). Gene expression values were analyzed with the Gene Expression Dynamics Inspector bioinformatics software package (4, 5), which uses unsupervised machine learning algorithms to organize genes with highly similar behavior into clusters. Each cluster is represented by a tile and tiles are arranged into mosaics, with clusters of similar behavior located in close proximity. Each tile represents the same genes for each mosaic. The centroid of each cluster is encoded with a color, indicating up- and down-regulated genes.

To generate heat maps for specific Gene Ontology terms, genes were filtered to include only those that showed statistically different expression levels at 96 h between tissues on isotropic FN with and without stretch and/or tissues on patterned FN with and without longitudinal stretch ($P < 0.05$). Differentially regulated genes were categorized by selected Gene Ontology terms by inputting filtered probe set IDs into the AmiGO! Slimmer from the Gene Ontology project (http://amigo.geneontology.org/cgi-bin/amigo/slimmer).

Sarcomere Alignment. After 4 d in culture, tissues were fixed with 4% paraformaldehyde and 0.15% Triton X-100 in PBS solution for 10 min at room temperature. Tissues were incubated with the following primary antibodies: polyclonal rabbit anti-FN (F3648; Sigma-Aldrich) and monoclonal mouse anti-sarcomeric α-actinin (A7811; Sigma-Aldrich) for 80 min at room temperature. Following multiple PBS solution rinses, tissues were incubated with Alexa Fluor 488 goat anti-mouse IgG, Alexa Fluor 546 goat anti-rabbit IgG, Alexa Fluor 633 phalloidin, and DAPI (Invitrogen) for 1 h at room temperature. All antibodies and chemical stains were diluted 1:200 in PBS solution. Samples were rinsed with PBS solution, and the piece of membrane with the tissue was excised, mounted on a glass slide, coated with ProLong Gold Anti-Fade reagent (Invitrogen), covered with a glass coverslip, and sealed with nail polish. Ten images per coverslip were captured with a CCD camera (CoolSnap; Photometrics) mounted on an inverted fluorescent microscope (DMI 6000B; Leica Microsystems) using a 40× objective lens. Custom MATLAB software (MathWorks) was used to threshold α-actinin immmunosignals and detect the orientation angles of continuous pixel segments (6, 7). All orientation angles from a single tissue were consolidated and used to calculate the orientational order parameter (8). Orientational order parameters from each tissue for a single condition were averaged and statistically compared by Student *t* test.

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4. Sheehy SP, Huang S, Parker KK (2009) Time-warped comparison of gene expression in adaptive and maladaptive cardiac hypertrophy. *Circ Cardiovasc Genet* 2(2):116–124.
5. Eichler GS, Huang S, Ingber DE (2003) Gene Expression Dynamics Inspector (GEDI): For integrative analysis of expression profiles. *Bioinformatics* 19(17):2321–2322.
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7. Feinberg AW, et al. (2012) Controlling the contractile strength of engineered cardiac muscle by hierarchal tissue architecture. *Biomaterials* 33(23):5732–5741.
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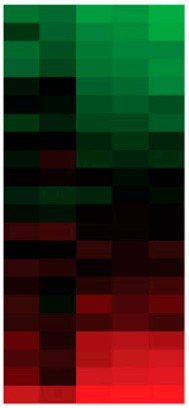
Myofibril

Abcc9	ATP-binding cassette, sub-family C (CFTR/MRP), member 9
Lrrc10	Leucin rich repeat containing 10
Myh6	Myosin, heavy chain 6, cardiac muscle, alpha
Hspb1	Heat shock 27kDa protein 1
Fhl2	Four and a half LIM domains 2
Abra	Actin-binding Rho activating protein
Pdlim3	PDZ and LIM domain 3
Cryab	Crystallin, alpha B
Nexn	Nexilin (F actin binding protein)
Nrap	Nebulin-related anchoring protein
Trpc1	Transient receptor potential cation channel, subfamily C, member 1
Myh7	Myosin, heavy chain 7, cardiac muscle, beta
Smpx	Small muscle protein, X-linked
Obscn	Obscurin, cytoskeletal calmodulin and titin-interacting RhoGEF
Ilk	Integrin-linked kinase
Svil	Supervillin
Pdlim5	PDZ and LIM domain 5
Mmp2	Matrix metallopeptidase 2 (gelatinase A, 72kDa gelatinase, 72kDa type IV collagenase)
Ankrd1	Ankyrin repeat domain 1 (cardiac muscle)
Trim32	Tripartite motif containing 32
Dst	Dystonin
Glrx3	Glutaredoxin 3
Myh3	Myosin, heavy chain 3, skeletal muscle, embryonic
Trim54	Tripartite motif containing 54
Actn1	Actinin, alpha 1

Focal Adhesion

Cav1	Caveolin 1, caveolae protein, 22kDa
Sh3kbp1	SH3-domain kinase binding protein 1
Myh6	Myosin, heavy chain 6, cardiac muscle, alpha
Lims1	LIM and senescent cell antigen-like domains 1
Ptprc	Protein tyrosine phosphatase, receptor type, C
Itga5	Integrin, alpha 5 (fibronectin receptor, alpha polypeptide)
Fhl2	Four and a half LIM domains 2
Sorbs3	Sorbin and SH3 domain containing 3
Mdc1	Mediator of DNA-damage checkpoint 1
Tes	Testis derived transcript (3 LIM domains)
Tenc1	Tensin like C1 domain containing phosphatase (tensin 2)
Epb4.1l5	Eryhtrocyte membrane protein band 4.1 like 5
Myh7	Myosin, heavy chain 7, cardiac muscle, beta
Arhgap24	Rho GTPase activating protein 24
Arhgef7	Rho guanine nucelotide exchange factor (GEF) 7
Pdlim2	PDZ and LIM domain 2 (mystique)
Ebag9	Estrogen receptor binding site associated, antigen, 9
Ptk2	Protein tyrosine kinase 2
Epha2	EPH receptor A2
Ilk	Integrin-linked kinase
Pdlim7	PDZ and LIM domain 7 (enigma)
Zyx	Zyxin
Enah	Enabled homolog (Drosophila)
Dst	Dystonin
Msn	Moesin
Jub	Jub, ajuba homolog (Xenopus laevis)
Aif1l	Allograft inflammatory factor 1-like
Itgb5	Integrin, beta 5
Actn1	Actinin, alpha 1

Heart Contraction



Rps6ka2	Ribosomal protein S6 kinase, 90kDa, polypeptide 2
Rgs2	Regulator of G-protein signaling 2, 24kDa
Map2k6	Mitogen-activated protein kinase kinase 6
Hopx	HOP homeobox
Adm	Adrenomedullin
Sema3a	Sema domain, immunoglobulin domain (Ig), short basic domain, secreted, (semaphorin) 3A
Myh6	Myosin, heavy chain 6, cardiac muscle, alpha
Ace	Angiotensin 1 converting enzyme (peptidyl-dipeptidase A) 1
Adra1b	Adrenergic, alpha-1B-, receptor
Hcn2	Hyperpolarization activated cyclic nucleotide-gated potassium channel 2
Map2k3	Mitogen-activated protein kinase kinase 3
Myh7	Myosin, heavy chain 7, cardiac muscle, beta
P2rx4	Purinergic receptor P2X, ligand-gated ion channel, 4
Cacna1g	Calcium channel, voltage-dependent, T type, alpha 1G subunit
Avpr1a	Arginine vasopressin receptor 1A
Nkx2-5	NK2 transcription factor related, locus 5 (Drosophila)
Hbegf	Heparin-binding EGF-like growth factor
Glrx3	Glutaredoxin 3
Ctgf	Connective tissue growth factor
Adra1a	Adrenergic, alpha-1A-, receptor
Tgfb2	Transforming growth factor, beta 2
Atp1a2	ATPase, Na+/K+ transporting, alpha 2 polypeptide

Fig. S1. Heat maps for Gene Ontology terms "myofibril," "focal adhesions," and "heart contraction." Genes that showed a significant difference between isotropic and isotropic plus stretch and/or patterned and patterned plus longitudinal stretch are displayed. Color bar indicates fold change differences in gene expression relative to samples collected 1 h after seeding.

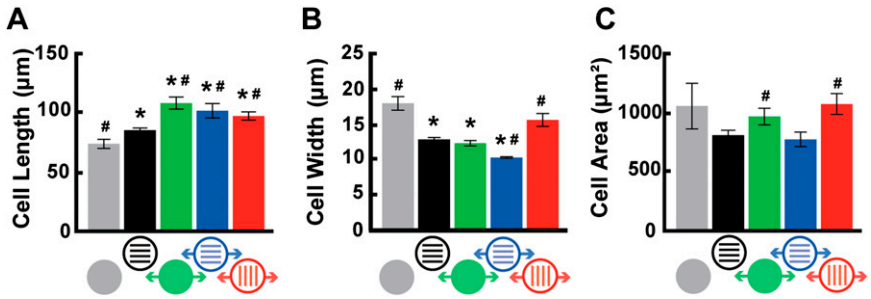
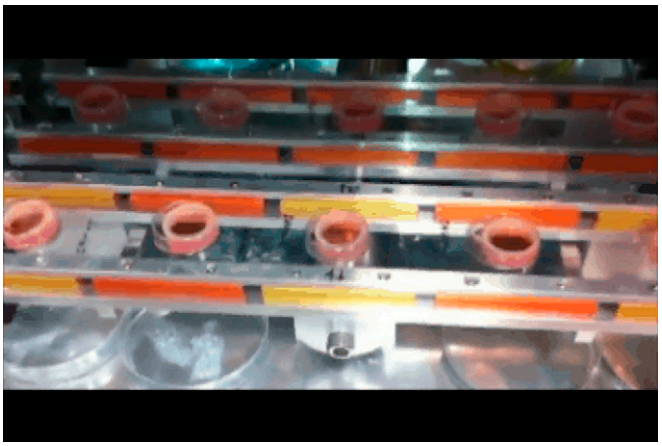
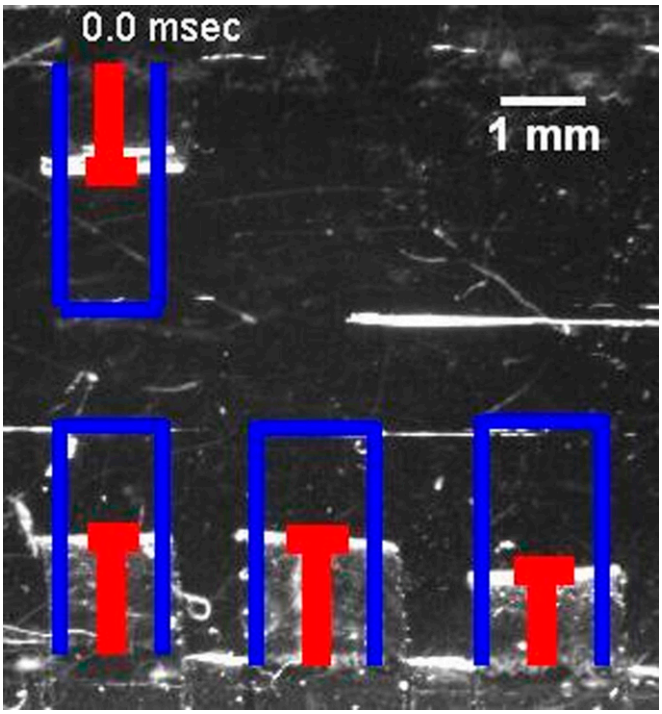


Fig. S2. Myocyte shape changes in conditioned tissues: (*A*) cell length, (*B*) cell width, and (*C*) cell area in tissues stained with di-8-ANEPPS membrane dye (mean ± SE; $n \geq 4$ tissues; $^{*}P < 0.05$ vs. static, isotropic tissues; $^{\#}P < 0.05$ vs. static, patterned tissues).



Movie S1. Cyclic stretch bioreactor. Movie of the custom-built bioreactor used to apply 10%, 3 Hz cyclic strain to engineered monolayers of cardiac tissue cultured on elastic silicone membranes.

Movie S1



Movie S2. Stretchable muscular thin films. Movie of muscular thin films cultured on stretcher membranes and subsequently released to measure stress generation in engineered cardiac tissues.

Movie S2

Dataset S1. Significant changes in gene expression at 6 h

Dataset S1

Genes are listed that showed a significant change in gene expression ($P < 0.05$) in patterned (P-), isotropic plus stretch (I+S), patterned plus longitudinal stretch (P+LS), and/or patterned plus transverse stretch (P+TS) relative to isotropic tissues (I-) after 1 h of static culture and 6 h with or without stretch. Log fold change (FC) of expression values and *P* values are listed.

Dataset S2. Significant changes in gene expression at 24 h

Dataset S2

Genes are listed that showed a significant change in gene expression ($P < 0.05$) in patterned (P-), isotropic plus stretch (I+S), patterned plus longitudinal stretch (P+LS), and/or patterned plus transverse stretch (P+TS) relative to isotropic tissues (I-) after 1 h of static culture and 24 h with or without stretch. Log fold change (FC) of expression values and *P* values are listed.

Dataset S3. Significant changes in gene expression at 96 h

Dataset S3

Genes are listed that showed a significant change in gene expression ($P < 0.05$) in patterned (P-), isotropic plus stretch (I+S), patterned plus longitudinal stretch (P+LS), and/or patterned plus transverse stretch (P+TS) relative to isotropic tissues (I-) after 1 h of static culture and 96 h with or without stretch. Log fold change (FC) of expression values and *P* values are listed.