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Supplemental Information

Emergent Global Contractile Force in Cardiac Tissues

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Supporting Material

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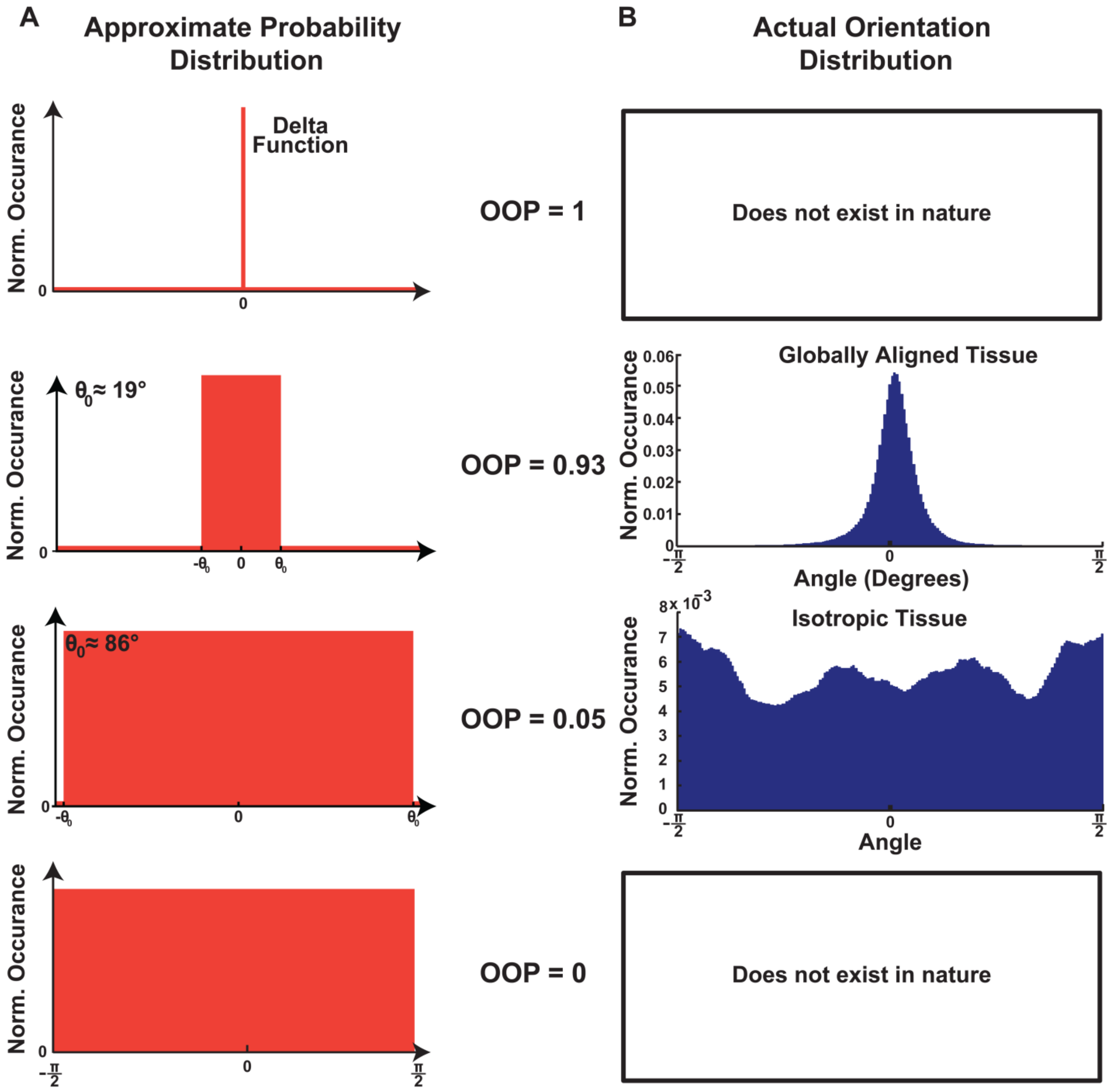

Fig. S1: Approximate probability distribution vs. actual orientation distribution. (A) Analytical representation of the approximate probability distribution of various conditions. An OOP of 1 has a delta function probability distribution. The spread of the probability distribution increases with decreasing OOP until an OOP of 0, at which point the probability is equal for all point on the interval $\left[-\frac{\pi}{2},\frac{\pi}{2}\right]$. (B) In nature, the constructs do not reach an OOP of 1 or 0, but rather some in between OOP. A well-organized globally aligned anisotropic tissue can achieve an OOP close to 1 and a disorganized isotropic tissue can reach an OOP value close to 0.

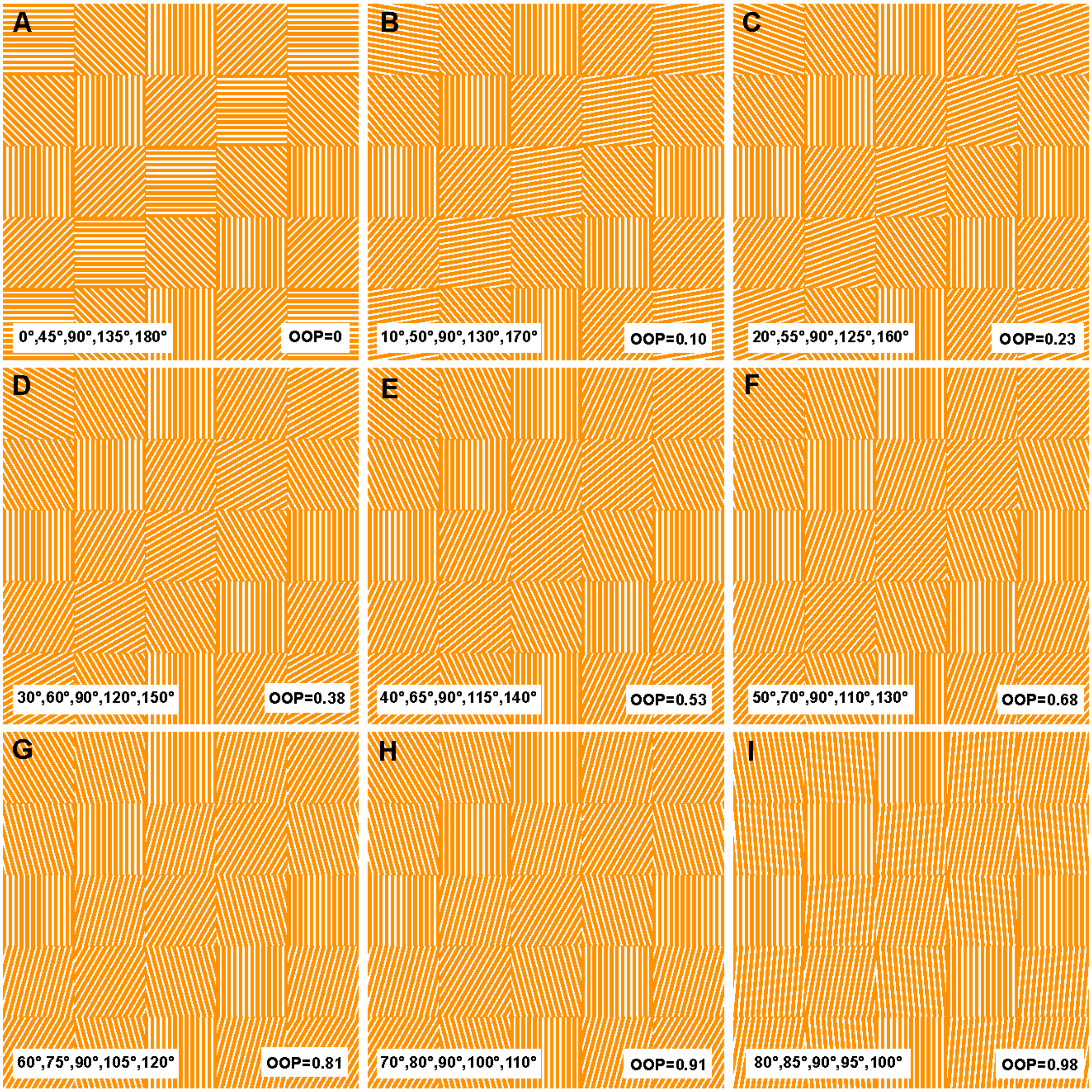

Fig. S2: Parquet patterns. Sample schematic of parquet variations from least organized to most organized (A-I).

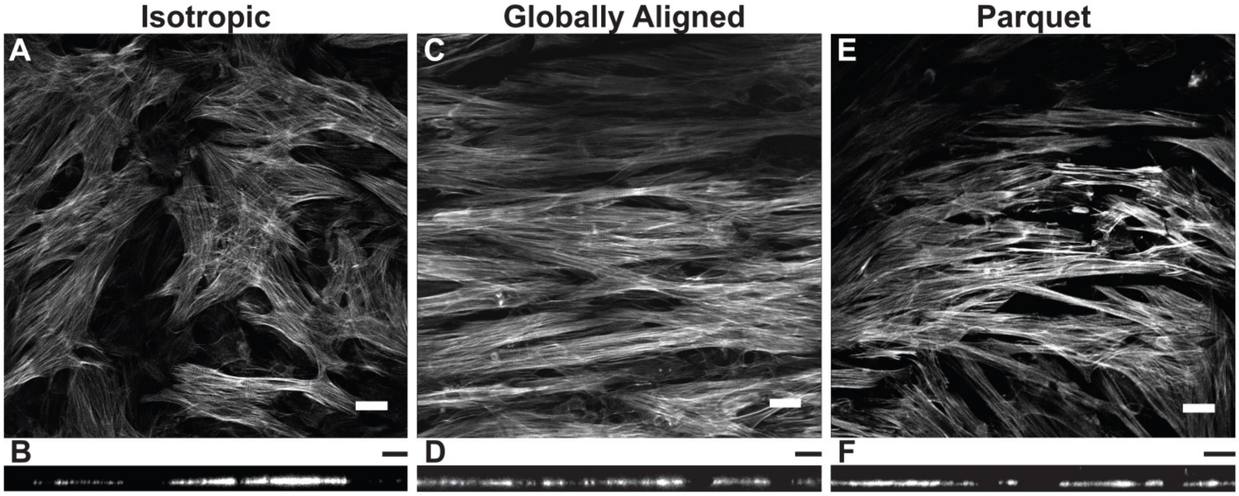

Fig. S3: Confocal images of cell thickness. (A,C,E) Z-stacks were obtained for isotropic (N=4), globally aligned (N=4), and parquet (N=3) samples. (B,D,F) Orthogonal views were used to determine cell thickness (Table S6). Scale bars: 25 µm all panels.

Table S1: List of Local and Global OOP data and statistics for each tissue represented in Fig. 2K. Overall significance level is set to 0.050. (Values are Mean±Standard Deviation).

Tissue Type	Actin Local OOP	Actin Global OOP	Sample Size
Globally aligned	0.92 ± 0.01	0.92 ± 0.01	3
Parquet Images Excluding Borders	0.88 ± 0.01	0.04 ± 0.03	3
Parquet Images Including Borders	0.81 ± 0.05	0.11 ± 0.09	3
Isotropic	0.45 ± 0.04	0.07 ± 0.03	3

Comparison of Global OOP	Unadjusted p-value	Critical Level	Significant?
Globally aligned vs. Parquet w/o borders	<0.001	0.009	Yes
Globally aligned vs. Isotropic	<0.001	0.010	Yes
Globally aligned vs. Parquet w/ borders	<0.001	0.013	Yes
Parquet w/o borders vs. Parquet w/ borders	0.119	0.017	No
Parquet w/o borders vs. Isotropic	0.405	0.025	No
Parquet w/ borders vs. Isotropic	0.411	0.050	No

Comparison of Local OOP	Unadjusted p-value	Critical Level	Significant?
Globally aligned vs. Isotropic	<0.001	0.009	Yes
Parquet w/o borders vs. Isotropic	<0.001	0.010	Yes
Parquet w/ borders vs. Isotropic	<0.001	0.013	Yes
Globally aligned vs. Parquet w/o borders	0.002	0.017	Yes
Parquet w/o borders vs. Parquet w/ borders	0.008	0.025	Yes
Globally aligned vs. Parquet w/ borders	0.299	0.050	No

Table S2: List of genes tested in Rat Cardiovascular Disease Array. Genes in red were significantly different between isotropic and globally aligned tissues. P-values in blue indicate genes for which the expression was undetected and thus the fold-change result was erroneous or un-interpretable as determined by the online software.

Gene Symbol	Description	Function	p-value (compared to globally aligned)	p-value (compared to isotropic)	
			Parquet	Parquet	Globally aligned
Pde7a	Phosphodiesterase 7A	Signal Transduction	0.024	0.034	0.011
Ubb	Ubiquitin B	Apoptosis	0.485	0.011	0.015
Hmgcl	3-hydroxymethyl-3-methylglutaryl-Coenzyme A lyase	Signal Transduction	0.287	0.032	0.047
Crem	CAMP responsive element modulator	Transcriptional Regulation	0.283	0.045	0.002
Zyx	Zyxin	Apoptosis	0.192	0.054	0.042
Nr3c2	Nuclear receptor subfamily 3, group C, member 2	Signal Transduction	0.969	0.064	0.031
Rassf1	Ras association (RalGDS/AF-6) domain family member 1	Signal Transduction	0.785	0.067	0.046
Cdkn1b	Cyclin-dependent kinas inhibitor 1B	Cell Cycle	0.974	0.074	0.050
Adrb1	Adrenergic, beta-1-, receptor	Signal Transduction	0.631	0.086	0.050
Ren	Renin	Cardiac Remodeling	0.697	0.097	0.015
Stat1	Signal transducer and activator of transcription 1	Transcriptional Regulation	0.854	0.118	0.019
S100a1	S100 calcium binding protein A1	Stress & Immune Response	0.487	0.275	0.049
Klhl3	Kelch-like family member 3	Transcriptional Regulation	0.109	0.479	0.015

Gene Symbol	Description	Function	p-value (compared to globally aligned)	p-value (compared to isotropic)	
			Parquet	Parquet	Globally aligned
Actc1	Actin, alpha, cardiac muscle 1	Sarcomere Structural Proteins	0.887	0.068	0.241
Adra1a	Adrenergic, alpha-1A-, receptor	Signal Transduction	0.214	0.262	0.173
Adra1b	Adrenergic, alpha-1B-, receptor	Signal Transduction	0.908	0.568	0.514
Adra1b	Adrenergic, alpha-1B-, receptor	Signal Transduction	0.908	0.568	0.514
Adra1d	Adrenergic, alpha-1D-, receptor	Signal Transduction	0.837	0.193	0.075
Adrb2	Adrenergic, beta-2-, receptor,surface	Signal Transduction	0.754	0.689	0.230
Adrb3	Adrenergic, beta-3-, receptor	Signal Transduction	0.542	0.542	0.217
Aebp1	AE binding protein 1	Cardiac Remodeling	0.855	0.945	0.860
Agtr1a	Angiotensin II receptor, type 1a	Signal Transduction	0.389	0.270	0.081
Anxa4	Annexin A4	Cardiac Remodeling	0.490	0.339	0.177
Ar	Androgen receptor	Signal Transduction	0.995	0.193	0.170
Atp2a2	ATPase, Ca++ transporting, cardiac muscle, slow twitch 2	Transporters	0.780	0.096	0.086
Atp5a1	ATP synthase, H+ transporting, mitochondrial F1 complex, alpha subunit 1, cardiac muscle	Transporters	0.396	0.412	0.934
C6	Complement component 6	Stress & Immune Response	0.475	0.504	0.852
Ccl11	Chemokine (C-C motif) ligand 11	Stress & Immune Response	0.235	0.233	0.184

Gene Symbol	Description	Function	p-value (compared to globally aligned)	p-value (compared to isotropic)	
			Parquet	Parquet	Globally aligned
Ccl2	Chemokine (C-C motif) ligand 2	Stress & Immune Response	0.892	0.870	0.787
Ccnd1	Cyclin D1	Cell Cycle	0.214	0.262	0.173
Col11a1	Collagen, type XI, alpha 1	Cardiac Remodeling	0.908	0.568	0.514
Col1a1	Collagen, type I, alpha 1	Cardiac Remodeling	0.424	0.664	0.294
Col3a1	Collagen, type III, alpha 1	Cardiac Remodeling	0.838	0.619	0.640
Creb5	CAMP responsive element binding protein 5	Transcriptional Regulation	0.068	0.200	0.132
Cryab	Crystallin, alpha B	Sarcomere Structural Proteins	0.487	0.450	0.118
Crym	Crystallin, mu	Sarcomere Structural Proteins	0.581	0.275	0.249
Ctgf	Connective tissue growth factor	Cell Growth	0.735	0.197	0.214
Cxcl12	Chemokine (C-X-C motif) ligand 12 (stromal cell-derived factor 1)	Stress & Immune Response	0.864	0.312	0.270
Dcn	Decorin	Cardiac Remodeling	0.829	0.488	0.373
Dmd	Dystrophin	Cardiac Remodeling	0.943	0.378	0.195
Dusp6	Dual specificity phosphatase 6	Signal Transduction	0.933	0.900	0.949
Enah	Enabled homolog (Drosophila)	Transcriptional Regulation	0.454	0.124	0.185
Epor	Erythropoietin receptor	Signal Transduction	0.481	0.734	0.668

Gene Symbol	Description	Function	p-value (compared to globally aligned)	p-value (compared to isotropic)	
			Parquet	Parquet	Globally aligned
F2r	**Coagulation factor II (thrombin) receptor**	**Cardiac Remodeling**	**0.256**	**0.607**	**0.182**
Fn1	**Fibronectin 1**	**Cardiac Remodeling**	**0.878**	**0.089**	**0.051**
Frzb	**Frizzled-related protein**	**Signal Transduction**	**0.732**	**0.464**	**0.717**
G0s2	**G0/G1 switch 2**	**Cell Cycle**	**0.739**	**0.652**	**0.976**
Gja1	**Gap junction protein, alpha 1**	**Cardiac Remodeling**	**0.385**	**0.126**	**0.750**
Hmgcr	**3-hyroxy-3-methylglutaryl-Coenzyme A reductase**	**Signal Transduction**	**0.890**	**0.053**	**0.064**
Hmgn2	**High mobility group nucleosomal binding domain 2**	**Transcriptional Regulation**	**0.149**	**0.796**	**0.242**
Maoa	**Monoamine oxidase A**	**Apoptosis**	**0.412**	**0.257**	**0.591**
Mapk1	**Mitogen activated protein kinase 1**	**Signal Transduction**	**0.746**	**0.133**	**0.099**
Mapk8	**Mitogen activated protein kinase 8**	**Signal Transduction**	**0.866**	**0.509**	**0.429**
Msi2	**Musashi RNA-binding protein 2**	**Transcriptional Regulation**	**0.775**	**0.067**	**0.202**
Myh10	**Myosin, heavy chain 10, non-muscle**	**Sarcomere Structural Proteins**	**0.671**	**0.357**	**0.243**
Myh6	**Myosin, heavy chain 6, cardiac muscle, alpha**	**Sarcomere Structural Proteins**	**0.593**	**0.641**	**0.975**
Ndufb5	**NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 5**	**Apoptosis**	**0.894**	**0.205**	**0.274**
Nebl	**Nebulette**	**Sarcomere Structural Proteins**	**0.917**	**0.263**	**0.352**
Nfia	**Nuclear factor I/A**	**Transcriptional Regulation**	**0.667**	**0.627**	**0.453**

Gene Symbol	Description	Function	p-value (compared to globally aligned)	p-value (compared to isotropic)	
			Parquet	Parquet	Globally aligned
Nkx2-5	NK2 transcription factor related, locus 5 (dDrosophila)	Transcriptional Regulation	0.115	0.092	0.069
Nppa	Natriuretic peptide precursor A	Apoptosis	0.222	0.136	0.126
Nppb	Natriuretic peptide precursor B	Apoptosis	0.347	0.307	0.260
Npr1	Natriuretic peptide receptor A/guanylate cyclase A (atrionatriuretic peptide receptor A)	Apoptosis	0.958	0.321	0.123
Npr2	Natriuretic peptide receptor B/guanylate cyclase (atrionatriuretic peptide receptor B)	Signal Transduction	0.643	0.187	0.051
Npr3	Natriuretic peptide receptor A/guanylate cyclase C (atrionatriuretic peptide receptor C)	Signal Transduction	0.879	0.799	0.652
Nr3c1	Nuclear receptor subfamily 3, group C, member 1	Signal Transduction	0.655	0.071	0.161
Pde3a	Phosphodiesterase 3A, cGMP inhibited	Apoptosis	0.964	0.166	0.199
Pde3b	Phosphodiesterase 3B, cGMP inhibited	Signal Transduction	0.642	0.119	0.211
Pde5a	Phosphodiesterase 5A, cGMP inhibited	Signal Transduction	0.625	0.237	0.481
Postn	Periostin, osteoblast specific factor	Sarcomere Structural Proteins	0.239	0.897	0.328
Ptn	Pleiotrophin	Cell Growth	0.820	0.846	0.939
Rarres1	Retinoic acid receptor responder (tazarotene induced) 1	Cell Cycle	0.807	0.999	0.419

Gene Symbol	Description	Function	p-value (compared to globally aligned)	p-value (compared to isotropic)	
			Parquet	Parquet	Globally aligned
Rtn4	Reticulon 4	Cardiac Remodeling	0.436	0.060	0.110
S100a8	S100 calcium binding protein A8	Stress & Immune Response	0.794	0.718	0.863
Serpina3n	Serin (or cysteine) peptidase inhibitor, clase A, member 3N	Cardiac Remodeling	0.837	0.950	0.756
Sfrp4	Secreted frizzled-related protein 4	Cell Growth	0.445	0.731	0.387
Snca	Synuclein, alpha (non A4 component of amyloid precursor)	Apoptosis	0.828	0.531	0.534
Spock1	Sparc/osteonectin, cwcv and kazal-like domains proteoglycan (testican) 1	Cell Growth	0.768	0.954	0.594
Tcf4	Transcription factor 4	Transcriptional Regulation	0.698	0.137	0.089
Thbs2	Thrombospondin 2	Apoptosis	0.964	0.917	0.963
Tnni3	Troponin I type 3 (cardiac)	Cardiac Remodeling	0.837	0.219	0.125
Tnnt2	Troponin T type 2 (cardiac)	Cardiac Remodeling	0.604	0.159	0.144
Ace	Angiotensin I converting enzyme (peptidyl-dipeptidase A) 1	Cell Growth	0.700	0.288	0.015
Mmp13	Matrix metallopeptidase 13	Cardiac Remodeling	0.700	0.288	0.015
Slc12a1	Solute carrier family 12 (sodium/potassium/chloride transporters), member 1	Signal Transduction	0.700	0.288	0.015

Table S3: List of experimental data and statistics for all tissue types for each respective stress as represented in Fig. 3D. Overall significance level is set to 0.050. (Values are Mean±Standard Deviation).

Tissue Type	**Systolic Stress (kPa)**	**Diastolic Stress (kPa)**	**Active Stress (kPa)**	**Sample Size**
Globally aligned	**12.8 ± 4.87**	**6.46 ± 1.78**	**6.36 ± 4.63**	**28**
Model	**9.10 ±3.46**	**4.58 ± 1.26**	**4.51 ± 3.29**	**22**
Parquet	**7.20 ± 2.99**	**4.56 ± 1.04**	**2.64 ± 2.63**	**28**
Isotropic	**4.53 ± 1.46**	**2.56 ± 1.27**	**0.91 ± 0.59**	**27**

Comparison of Systolic Stress	**Unadjusted p-value**	**Critical Level**	**Significant?**
Globally aligned vs. Isotropic	**<0.001**	**0.009**	**Yes**
Globally aligned vs. Parquet	**<0.001**	**0.010**	**Yes**
Model vs. Isotropic	**<0.001**	**0.013**	**Yes**
Globally aligned vs. Model	**<0.001**	**0.017**	**Yes**
Parquet vs. Isotropic	**0.008**	**0.025**	**Yes**
Model vs. Parquet	**0.057**	**0.050**	**No**

Comparison of Diastolic Stress	**Unadjusted p-value**	**Critical Level**	**Significant?**
Globally aligned vs. Isotropic	**<0.001**	**0.009**	**Yes**
Model vs. Isotropic	**<0.001**	**0.010**	**Yes**
Globally aligned vs. Model	**<0.001**	**0.013**	**Yes**
Parquet vs. Isotropic	**<0.001**	**0.017**	**Yes**
Globally aligned vs. Parquet	**<0.001**	**0.025**	**Yes**
Model vs. Parquet	**0.953**	**0.050**	**No**

Table S3 (cont'd):

Comparison of Active Stress	Unadjusted p-value	Critical Level	Significant?
Globally aligned vs. Isotropic	**<0.001**	**0.009**	**Yes**
Model vs. Isotropic	**<0.001**	**0.010**	**Yes**
Globally aligned vs. Parquet	**<0.001**	**0.013**	**Yes**
Globally aligned vs. Model	**0.033**	**0.017**	**No**
Model vs. Parquet	**0.041**	**0.025**	**No**
Parquet vs. Isotropic	**0.061**	**0.050**	**No**

Table S4: List of experimental actin OOP values for each tissue type including number of samples. (Values are Mean±Standard Deviation).

Tissue Type	Pattern OOP	Actin Global OOP	No. of Chips
Isotropic	**0.00**	**0.09 ± 0.03**	**3**
Parquet (0,45,90,135,180)	**0.00**	**0.05 ± 0.00**	**3**
Parquet (10,50,90,130,170)	**0.10**	**0.12 ± 0.05**	**6**
Parquet (20,55,90,125,160)	**0.23**	**0.19 ± 0.10**	**5**
Parquet (30,60,90,120,150)	**0.38**	**0.27 ± 0.04**	**6**
Parquet (40,65,90,115,140)	**0.53**	**0.43 ± 0.07**	**4**
Parquet (50,70,90,110,130)	**0.68**	**0.55 ± 0.05**	**4**
Parquet (60,75,90,105,120)	**0.81**	**0.66 ± 0.02**	**3**
Parquet (70,80,90,100,110)	**0.91**	**0.72 ± 0.02**	**9**
Parquet (80,85,90,95,100)	**0.98**	**0.83 ± 0.07**	**8**
Globally aligned = (90,90,90,90,90)	**1.00**	**0.92 ± 0.01**	**3**

Table S5: List of experimental sample sizes for each tissue type including average measured systolic stress, number of samples, number of chips, and number of harvests across which each pattern was tests. Error represented as standard deviation.

Tissue Type	**Average Systolic Stress (kPa)**	**No. of Samples**	**No. of Chips**	**Across No. of Harvests**
Isotropic	**4.53 ± 1.46**	**27**	**8**	**3**
Parquet (0,45,90,135,180)	**7.20 ± 2.99**	**22**	**6**	**2**
Parquet (10,50,90,130,170)	**6.55 ± 2.62**	**26**	**6**	**4**
Parquet (20,55,90,125,160)	**8.75 ± 4.92**	**19**	**5**	**3**
Parquet (30,60,90,120,150)	**8.73 ± 3.62**	**30**	**6**	**5**
Parquet (40,65,90,115,140)	**10.0 ± 4.16**	**20**	**4**	**3**
Parquet (50,70,90,110,130)	**9.54 ± 4.37**	**22**	**4**	**3**
Parquet (60,75,90,105,120)	**9.59 ± 4.45**	**24**	**4**	**3**
Parquet (70,80,90,100,110)	**10.4 ± 5.43**	**56**	**9**	**3**
Parquet (80,85,90,95,100)	**12.4 ± 4.01**	**50**	**8**	**3**
Globally aligned = (90,90,90,90,90)	**12.8 ± 4.87**	**28**	**7**	**3**

Table S6: List of tissue thicknesses measured for each tissue type with corresponding samples sizes. Error represented as standard deviation.

Tissue Type	Measured Cell Thickness (μm)	No. of Samples
Globally aligned	**4.9 ± 0.9**	**4**
Isotropic	**6.6 ± 1.4**	**4**
Parquet (0,45,90,135,180)	**5.4 ± 0.5**	**3**

Video Captions

Video S1. Analyzed video of isotropic muscular thin films during contractility experiments.

Video S2. Analyzed video of parquet patterned muscular thin films during contractility experiments.

Video S3. Analyzed video of globally aligned muscular thin films during contractility experiments.