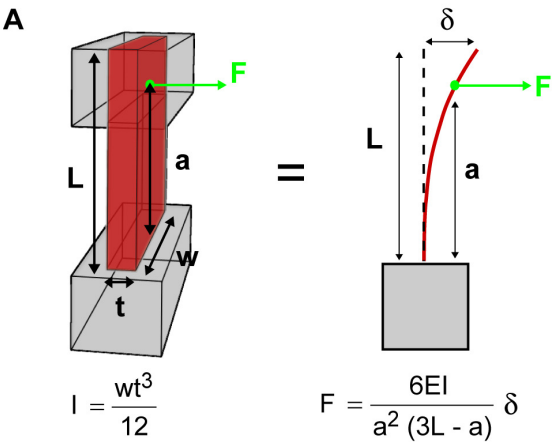
Supporting Information

Legant et al. 10.1073/pnas.0900174106

A



B

	Geometry 1 (rigid)	Geometry 2 (flexible)
t (μm)	33.2 ± 2.1	19.6 ± 1.0
w (μm)	163.0 ± 2.4	157.6 ± 2.7
L (μm)	199.3 ± 3.7	188.4 ± 4.6
a (μm)	163.0 ± 2.4	151.9 ± 4.2
E (MPa)	1.61 ± 0.16	1.61 ± 0.16
k (μN/μm)	**0.42 ± 0.07**	**0.10 ± 0.02**

C

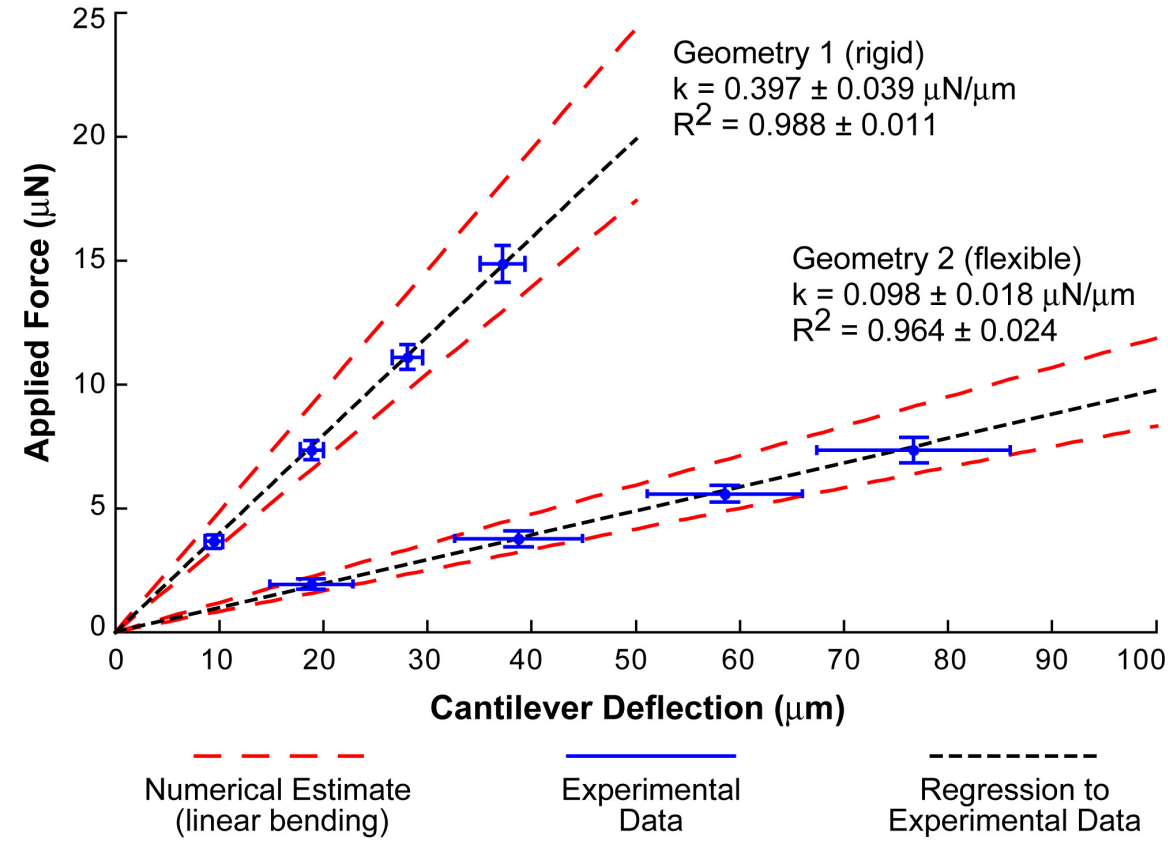


Fig. S1. Characterization of cantilever mechanics. (*A*) CAD images depicting linear modeling of cantilever deflections. Cantilever deflection is modeled as 1D beam bending due to a load applied at the centroid of the top cap. (*B*) Experimental measurements of cantilever dimensions. Spring constant (*k*) is calculated using the formulas presented in *A*. (*C*) Plot of force vs. cantilever deflection showing the numerical estimates from (*B*, dashed red lines), the experimental data using calibrated micropipettes (blue cross-hairs) and the average linear regression to the experimental data (dashed black lines). Data from (*B*) are the average ± standard deviation from 40 measurements (40 cantilevers of each geometry, 20 measurements each from 2 separate substrates). Data from (*C*) are the average of data points (blue cross-hairs) ± standard deviation from 3 sequential deflections each of 20 cantilevers from each geometry (10 each from 2 separate substrates). Dashed black lines are linear plots of the average spring constant for each geometry based on linear regressions to the experimental data from each individual cantilever measurement.

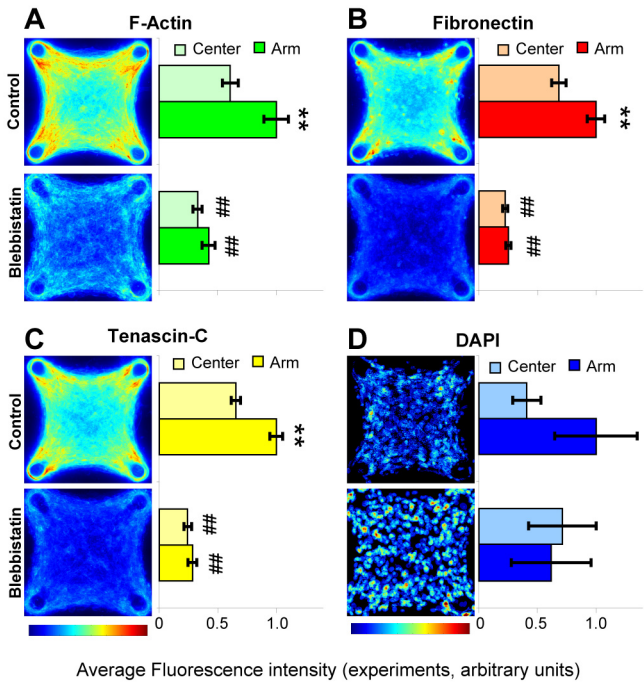
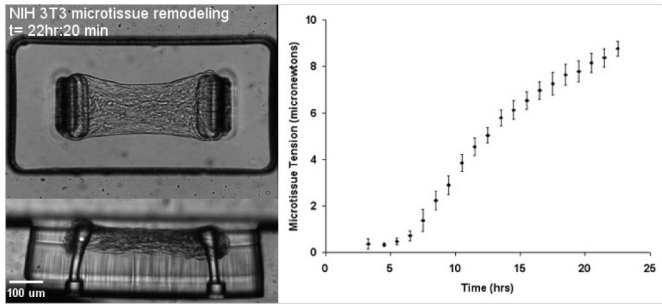
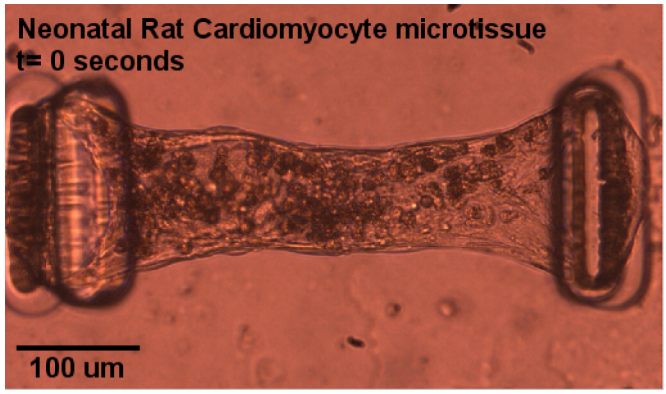


Fig. S2. Patterned protein levels within aphidicolin treated microtissues. Shown are heat maps showing distribution of filamentous actin, fibronectin, tenascin C and DAPI constructed from optical sections of microtissues under control and blebbistatin (50 μM) conditions. To normalize cell density between control and blebbistatin conditions, microtissues were cultured for 3 days after seeding in the presence of 1 μM aphidicolin before fixing. Experimental heat maps are 2D projections of immunofluorescence staining from 160 optical sections ($\approx$10 sections per microtissue for 16 different microtissues in each condition). **, $P < 0.01$ for arm vs. center; ##, $P < 0.01$ for blebbistatin vs. control.



Movie S1. NIH 3T3 microtissue remodeling and force generation. Time lapse images and corresponding force plot for microtissues constructed from 2.5 mg/mL collagen tethered to 0.397 μN/μm cantilevers. Top down and cross section perspectives represent images from 2 separate time synchronized microtissues. Force plot data points represent the average force for 5 microtissues $\pm$ SEM.

Movie S1 (MOV)



Movie S2. Spontaneously beating cardiomyocyte microtissue. Real time movie of a spontaneously beating microtissue constructed from primary neonatal rat cardiomyocytes after 1 week in culture. Microtissue was constructed from 1.75 mg/mL collagen and tethered to 0.098 μN/μm cantilevers.

Movie S2 (MOV)

Other Supporting Information Files

Table S1
Table S2
SI Appendix