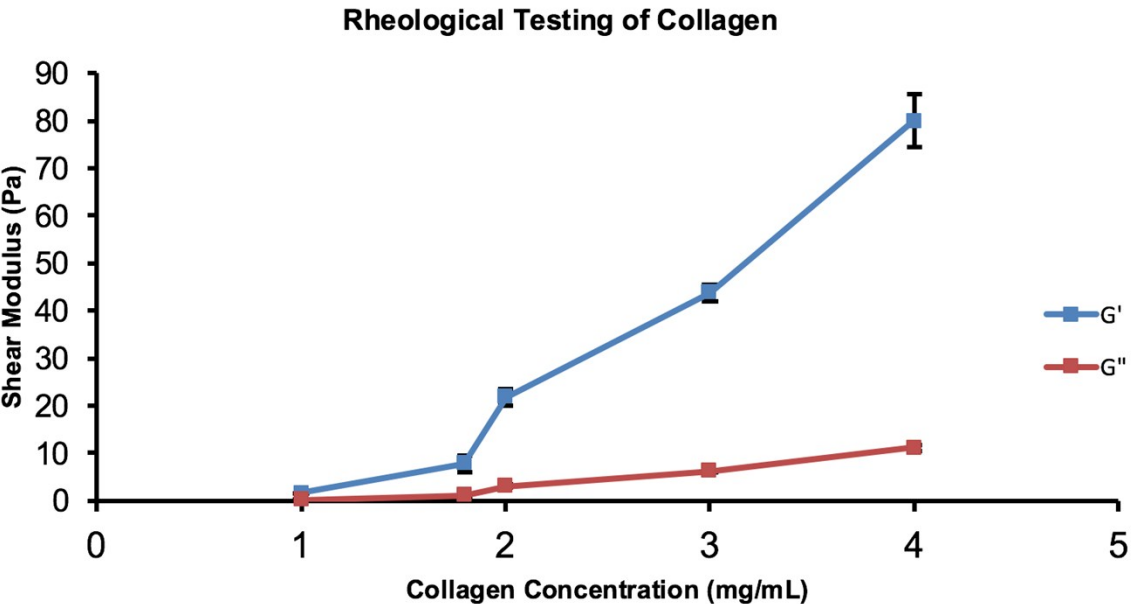


Supplementary Material

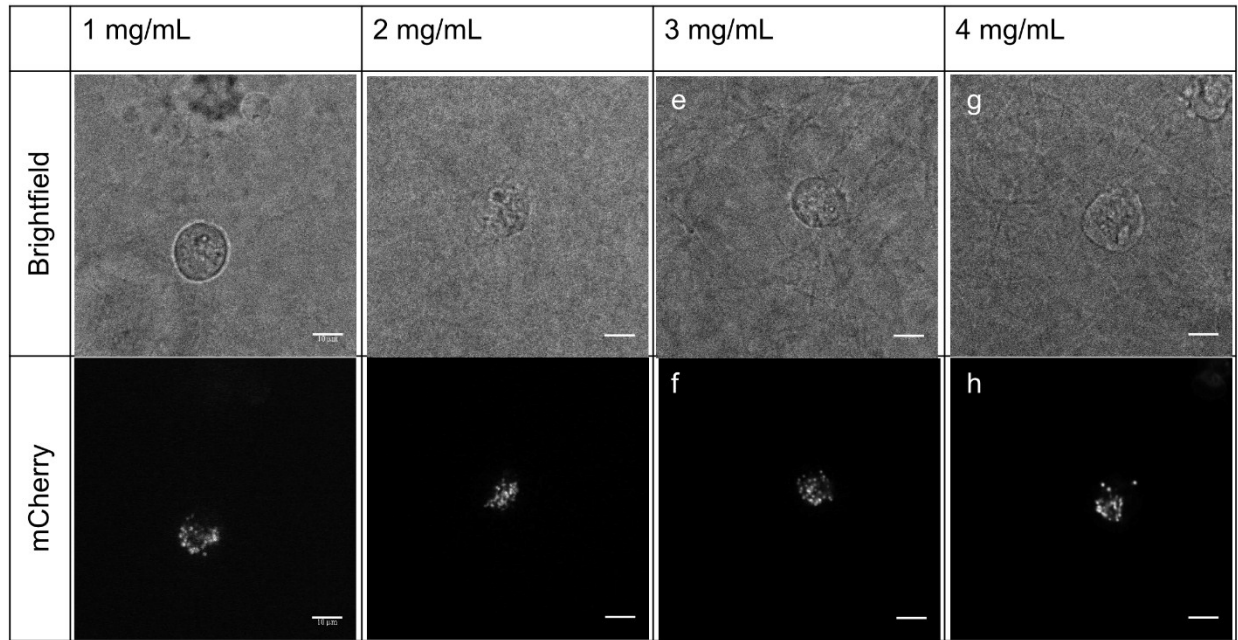
Supplemental Table S1. Shear modulus values of collagen gels.

	1.0 mg/mL	2.0 mg/mL	3.0 mg/mL	4.0 mg/mL
Average	1.639466667	21.823333333	43.765566667	80.026133333
Standard deviation	0.040002667	1.803642765	1.763220197	5.638667171

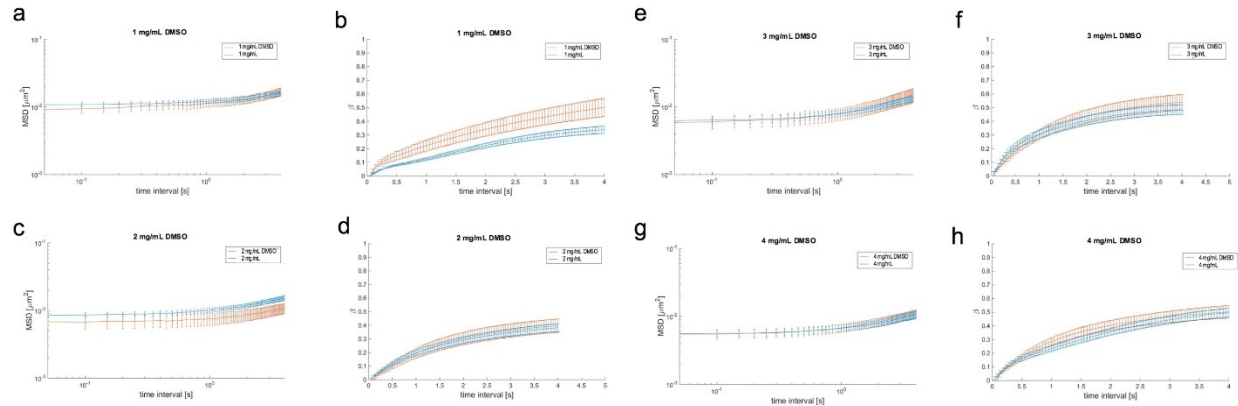
Supplemental Figures



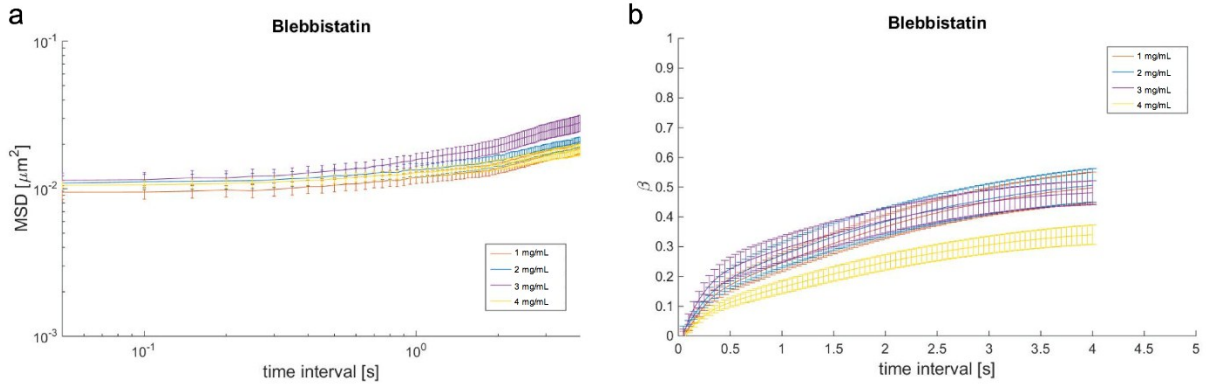
Supplemental Figure S1. Mechanical properties of collagen gels. Collagen gels were measured using a rheometer with a 40 mm aluminum cone (2 degrees) geometry. The angular frequency was 1 Hz and measurements were taken every 1 minute for a duration of 1 hour. The G' and G'' values were taken as an average of 10 data points between 50 minute and 60 minutes.



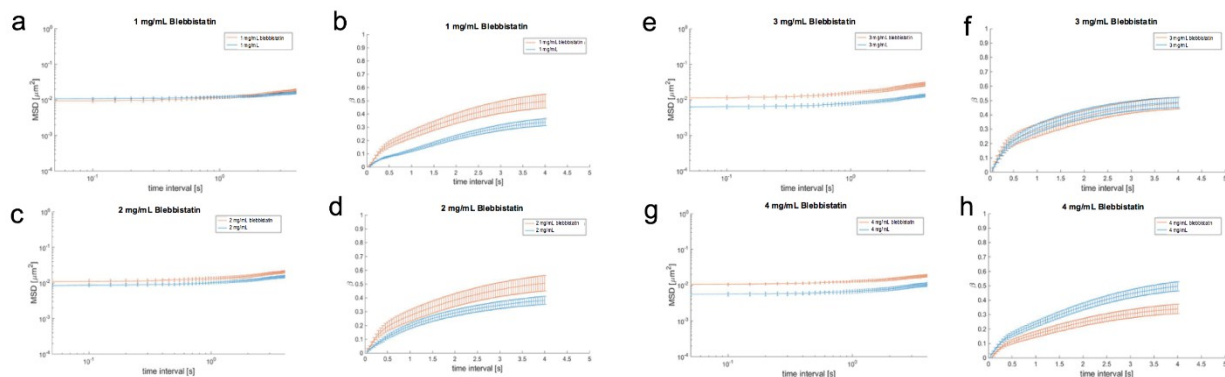
Supplemental Figure S2. Images of untreated cells and corresponding mitochondria in 1-4 mg/mL collagen. a-b) Cell in 1 mg/mL collagen with images of brightfield and mCherry c-d) Cell in 2 mg/mL collagen with images of brightfield and mCherry e-f) Cell in 3 mg/mL collagen with images of brightfield and mCherry g-h) Cell in 4 mg/mL collagen with images of brightfield and mCherry. Scale bar is 10 μ m.



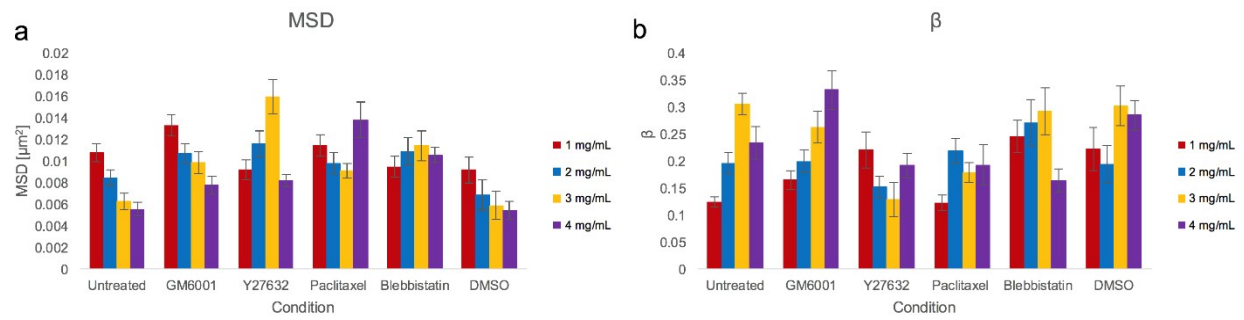
Supplemental Figure S3. DMSO treatment of cells. a) MSD curves of MDA-MB-231 cells treated with DMSO and control cells embedded in 1 mg/mL collagen. b) β curves of of MDA-MB-231 cells treated with DMSO and control cells embedded in 1 mg/mL collagen. c) MSD curves of MDA-MB-231 cells treated with DMSO and control cells embedded in 2 mg/mL collagen. d) β curves of of MDA-MB-231 cells treated with DMSO and control cells embedded in 2 mg/mL collagen e) MSD curves of MDA-MB-231 cells treated with DMSO and control cells embedded in 3 mg/mL collagen. f) β curves of of MDA-MB-231 cells treated with DMSO and control cells embedded in 3 mg/mL collagen g) MSD curves of MDA-MB-231 cells treated with DMSO and control cells embedded in 4 mg/mL collagen. h) β curves of of MDA-MB-231 cells treated with DMSO and control cells embedded in 4 mg/mL collagen



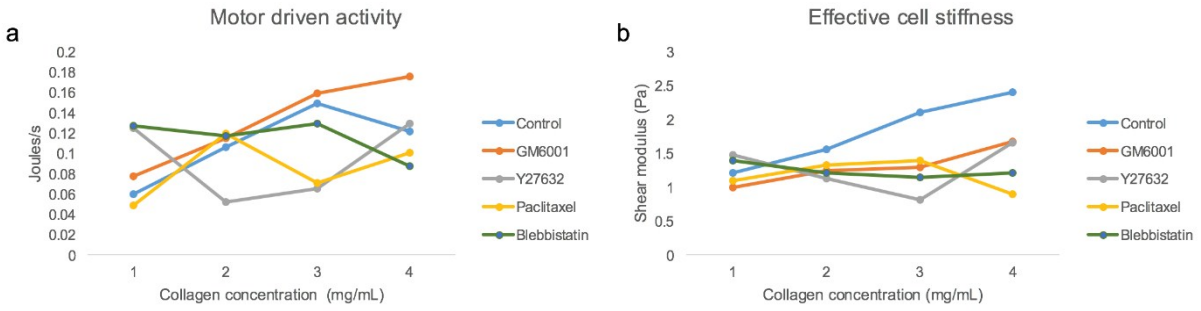
Supplemental Figure S4. Blebbistatin treated cells. Cells were incubated with blebbistatin (50 μM) for 2 hours in 1-4 mg/mL collagen gels. a) MSD curves of MDA-MB-231 cells treated with blebbistatin embedded in 1-4 mg/mL collagen. b) β curves of of MDA-MB-231 cells treated with blebbistatin embedded in 1-4 mg/mL collagen



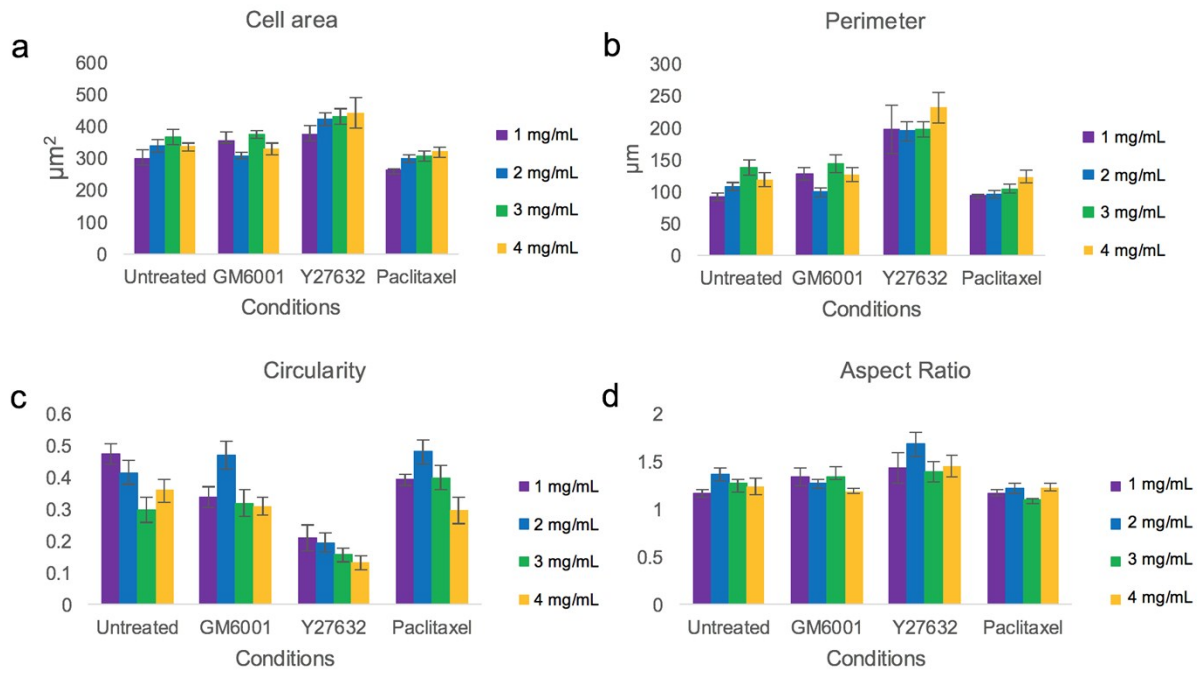
Supplemental Figure S5. Comparison of Blebbistatin treated cells with control cells. a) MSD curves of MDA-MB-231 cells treated with blebbistatin and control cells embedded in 1 mg/mL collagen. b) β curves of of MDA-MB-231 cells treated with blebbistatin and control cells embedded in 1 mg/mL collagen. c) MSD curves of MDA-MB-231 cells treated with blebbistatin and control cells embedded in 2 mg/mL collagen. d) β curves of of MDA-MB-231 cells treated with blebbistatin and control cells embedded in 2 mg/mL collagen e) MSD curves of MDA-MB-231 cells treated with blebbistatin and control cells embedded in 3 mg/mL collagen. f) β curves of of MDA-MB-231 cells treated with blebbistatin and control cells embedded in 3 mg/mL collagen g) MSD curves of MDA-MB-231 cells treated with blebbistatin and control cells embedded in 4 mg/mL collagen. h) β curves of of MDA-MB-231 cells treated with blebbistatin and control cells embedded in 4 mg/mL collagen



Supplemental Figure S6. Comparison of untreated with drug treated cells. a) Comparison of MSDs at $t = 50$ ms of untreated and drug treated cells in 1-4 mg/mL collagen. b) Comparison of β 's at $t = 1$ s of untreated and drug treated cells in 1-4 mg/mL collagen. Blebbistatin and DMSO treated conditions are also shown for comparison. Error bars are s.e.m. * indicates $p < 0.05$.



Supplemental Figure S7. Relationship between collagen concentration and effective cell stiffness. a) Power output generated by molecular motors of cells embedded in 1-4 mg/mL collagen b) Shear modulus of cells embedded in 1-4 mg/mL collagen. Assumed mitochondria with radius $r_{\text{tracer}} = 70 \text{ nm}$.



Supplemental Figure S8. Cell morphology analysis of untreated, GM6001, Y27632, and Paclitaxel treated cells in 1-4mg/mL collagen. a) Cell area b) Cell perimeter c) Aspect ratio, and d) Circularity of phalloidin stained cells. Number of cells for untreated (1 mg/mL n=15, 2 mg/mL n=21, 3 mg/mL=15, 4 mg/mL n=15), GM6001 treated cells (1 mg/mL n=14, 2 mg/mL n=22, 3 mg/mL n=15, 4 mg/mL n=15), Y27632 treated cells (1 mg/mL n=15, 2 mg/mL n=22, 3 mg/mL n=15, 4 mg/mL n=14), and Paclitaxel treated cells (1 mg/mL n=15, 2 mg/mL n=23, 3 mg/mL n=15, 4 mg/mL n=15). Error bars are s.e.m.