Table S1: GelMA/Collagen Formulations

E' (kPa)	Final Collagen (mg/mL)	GelMA (20% w/v) (μL)	Collagen (10 mg/mL) (μL)	10X PBS (μL)	LAP (1%) (μL)	DMEM (μL)
2	**1.5**	*360 0.25M*	*216*	*24*	*50*	*350*
12	**1.5**	*250 7M*	*150*	*17*	*50*	*533*

Table S1: Example prepolymer formulations for 1 mL volume of the 2 and 12 kPa GelMA/Coll hydrogels

Table S2: *Mean and standard deviation for number of invading cells per spheroid*

Figure	Condition	Invading Cells (Mean ± SD)
Fig 1B	Low 24 hr	33.6 ± 3.4
	Low 48 hr	62.8 ± 18.4
	Low 72 hr	122.6 ± 34.4
	High 24 hr	0.8 ± 0.8
	High 48 hr	4.9 ± 2.2
	High 72 hr	30.5 ± 7.3
Fig 2C	Low Control	162.4 ± 37.1
	Low +GM6001	86.9 ± 22.1
	High Control	34.5 ± 10.3
	High +GM6001	0
Fig 3D	Low siScr	114.0 ± 15.5
	Low siMena	113.1 ± 33.8
	High siScr	41.8 ± 12.0
	High siMena	14.5 ± 5.7
Fig 4D	Low Control	120.5 ± 18.8
	Low 0 hr PLCg inhibition	114.6 ± 23.1
	Low 24 hr PLCg inhibition	127.2 ± 28.0
	High Control	46.2 ± 10.2
	High 0 hr PLCg inhibition	19.8 ± 7.1
	High 24 hr PLCg inhibition	46.0 ± 9.9
Fig 6D	Low Control for cFN	113.2 ± 16.6
	Low + cFN	100.1 ± 15.7
	High Control for cFN	26.4 ± 9.8
	High + cFN	49.4 ± 12.2
	Low siScr	98.0 ± 26.1
	Low siFN	111.1 ± 26.3
	High siScr	50.3 ± 13.2
	High siFN	21.3 ± 9.8
Fig 7C	Low Control	96.6 ± 24.0
	Low + irigenin	104.7 ± 17.6
	High Control	50.1 ± 10.9
	High + irigenin	17.2 ± 6.3

Table S2: Mean and standard deviations for the number of invading cells per spheroid used to determine the fold change differences shown in Figures 1-7.

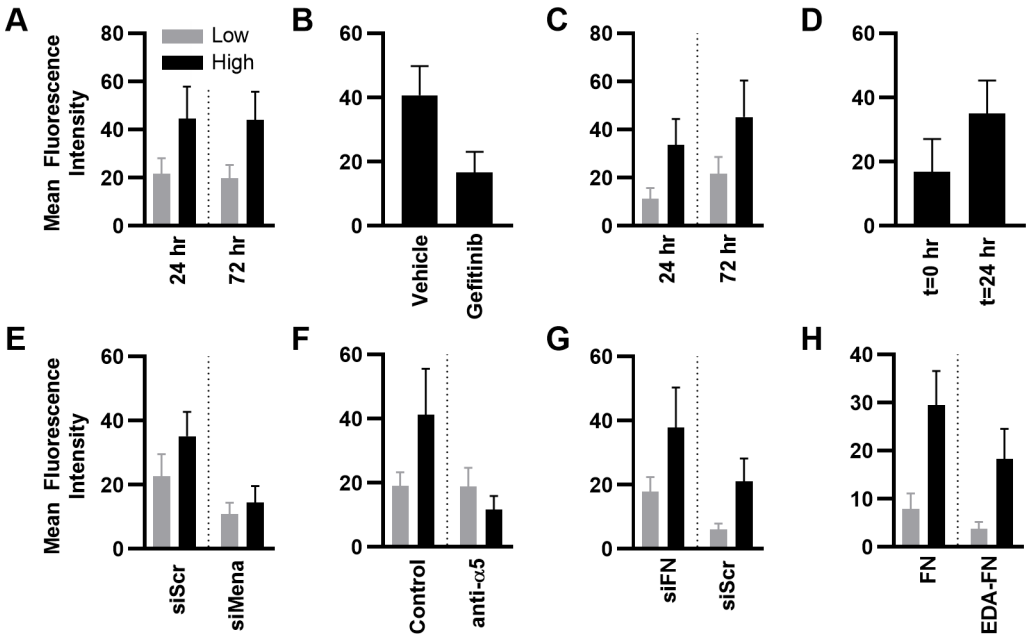

Figure S1: Mean fluorescence intensities of immunofluorescent stainings shown in the main figures. For each image set, n=3 spheroids were imaged and data show is mean and SD. A.) Mena was increased in high stiffness gels; however, staining intensity was unaffected by length of culture time. Corresponds to Figure 3A. B.) Treatment of high stiffness gels with the EGFR inhibitor gefitinib drastically reduced Mena at 72 hours post embedding. Corresponds to Figure 4A. C.) pPLCγ1 was increased in high stiffness gels and this difference persisted throughout the culture. Corresponds to Figure 4B. D.) Inhibiting PLCγ1 directly after embedding (t=0 hr) reduced Mena levels at 72 hours of culture in high stiffness gels compared to inhibition at t=24 hr. Corresponds to Figure 4C. E.) siRNA knockdown of Mena reversed the increase in fibronectin signal associated with stiffness. Corresponds to Figure 5B. F.) Fibronectin signal was decreased in the high stiffness gels through inhibition of α5 integrin. Corresponds to Figure 5C. G.) Knocking down fibronectin with siRNA reduced the intensity of fibronectin staining in both low and high stiffness gels. Corresponds to Figure 6B. H.) Total fibronectin and EDA-fibronectin signal were greatly increased in high stiffness gels. Corresponds to Figure 7A.

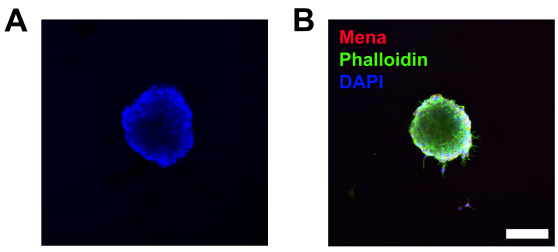

Figure S2: GelMA-only 12 kPa hydrogels failed to replicate results observed in GelMA/Coll gels. A.) MDA-MB-231 spheroids were stained with DAPI at 72 hours post embedding and showed no cell invasion occurred over the duration of the experiment. B.) Staining for Mena demonstrated an almost complete lack of the protein in the 12 kPa GelMA-only gels. Scale bar=150 μm.

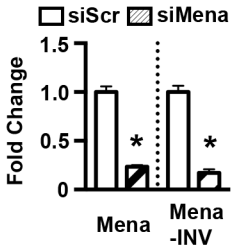

Figure S3: siMena treatment reduced MENA expression in spheroids 24 hours after generation. Treatment with the siRNA decreased expression of MENA and MENA-INV to a similar extent. * denotes $p<0.05$ relative to siScr, n=3 pools of >50 spheroids.

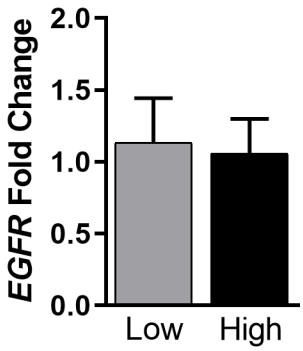

Figure S4: Scaffold stiffness did not affect gene expression of EGFR at 24 hr. n=3 pools of >50 embedded spheroids.

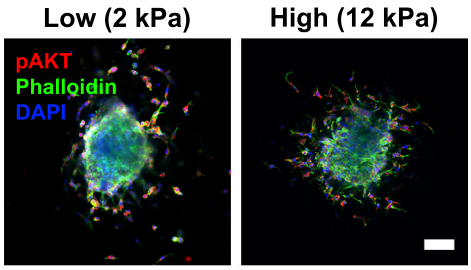

Figure S5: Scaffold stiffness did not substantially affect phosphorylation of Akt at Ser473. At both low and high stiffnesses, pAkt levels and distribution were similar at 72 hr post embedding (rabbit primary monoclonal antibody, Cell Signaling Technologies 193H12, 1:100 dilution, Danvers, MA). Scale bar=100 μm.

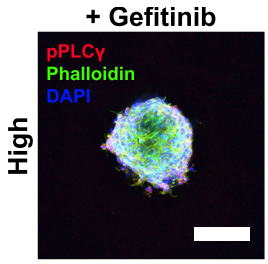

Figure S6: Inhibition of EGFR activity significantly decreased phosphorylation of PLCγ. Treatment with gefitinib, an irreversible EGFRI inhibitor, eliminated most of the signal from pPLCγ immunofluorescent staining. Scale bar=150 μm.

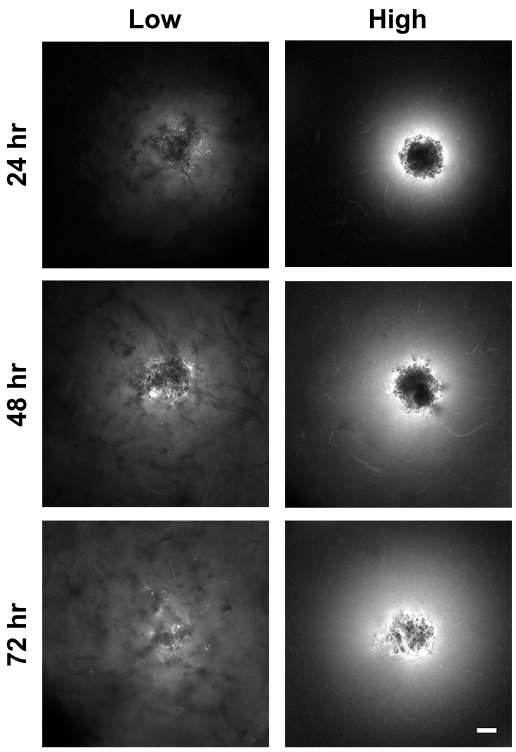

Figure S7: Unprocessed fluorescence images of fibronectin halos. Samples were fixed and imaged for fibronectin content via immunofluorescent staining. The raw fluorescent images for low and high gel stiffnesses at 24, 48, and 72 hours are shown and correspond to the heatmap images presented in Figure 5A. Scale bar = 100 μm

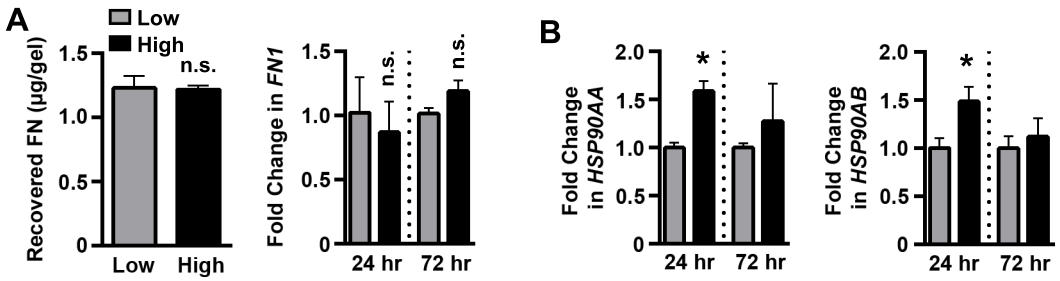

Figure S8: Fibronectin amount and secretion is similar between low and high stiffness conditions. A.) Fibronectin recovered from digested gels (left) and FN1 expression (right) were similar between the 2 and 12 kPa gels. n=3 pools of >50 embedded sheroids. B.) Expression of HSP90A (left) and HSP90B (right), molecular chaperones involved in the secretion of fibronectin, were modestly upregulated in 12 kPa gels at early timepoints (24 hr), but this difference disappeared at the later timepoints (72 hr). * denotes $p<0.05$ relative to low stiffness condition, n=3 pools of >50 embedded spheroids.

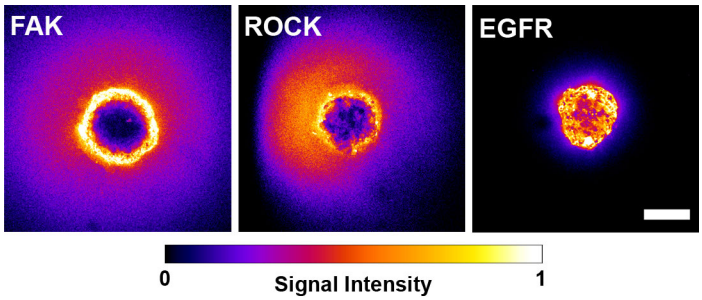

Figure S9: The development of the fibronectin halo is dependent upon EGFR signaling. Inhibition of FAK or ROCK, proteins involved in mechanosensing, did not affect the intensity or spread of the halo in 12 kPa gels at 72 hrs post embeding, while inhibition of EGFR with gefitinib ablated the halo. ROCK was inhibited by addition of 10 μM Y-27632 (Tocris Biosciences, Minneapolis, MN) in media after cell embedding, FAK was inhibited via the addition of 5 μM PF-573228 (Selleck Chem, Houston, TX). Scale bar=150 μm.

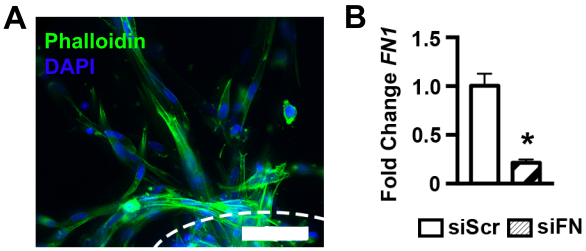

Figure S10: Fibronectin levels were manipulated by the addition of exogenous protein or the elimination of endogenous production. A.) Exogenous cFN did not affect cell morphology in 2 kPa scaffolds. Scale bar=50 μm. B.) siFN1 treatment reduced endogenous FN1 expression in spheroids 24 hours after generation. * denotes $p<0.05$ relative to siScr, n=3 pools of >50 spheroids.

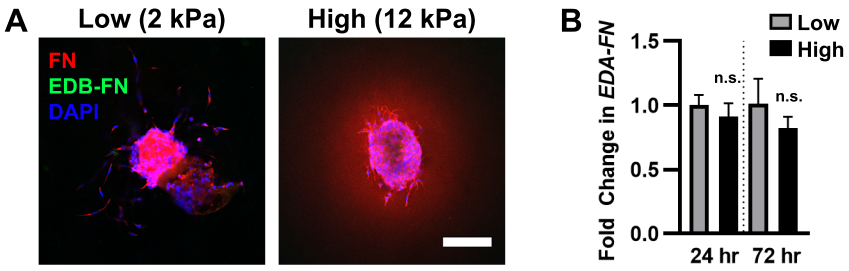

Figure S11: The EDB-fibronectin isoform is not present in the fibronectin halo and EDA-fibronectin gene expression is not upregulated in stiff matrices. A.) At 72 hr post embedding, no staining for EDB-fibronectin was observed in either the low or high stiffnesses. EDB-FN primary mouse monoclonal antibody BC-1 was used (Abcam, cat#: ab154210, 1:100 dilution). Scale bar = 150 μm. B.) Gene expression of EDA-fibronectin was similar in spheroids embedded in low and high stifness gels at both 24 and 72 hours post embedding. n=3 pools of >50 embedded spheroids.