Supplemental material for:

Integrin expression profile modulates orientation and dynamics of force transmission at cell matrix adhesions

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SUPPLEMENTARY FIGURE LEGENDS

Figure S1. Integrin and mCherry-LifeAct expression measurements using FACS. **(A,B)** Human integrin β1 and integrin β3 expression levels for GDβ1 (A) and GDβ3 cells (B). **(C-F)** Human integrin β1, human integrin β3 and mCherry-LifeAct expression levels for either wild type(C,D) or mCherry-LifeAct expressing (E,F) GEβ1 (C,E) and GEβ3 (D,F) cells. **(G,H)** Human integrin β1 (left) and human integrin β3 (right) expression levels in GEβ1 (blue), GEβ3 (red) (G) or GDβ1 (blue) and GDβ3 (red) (H) compared to expression of these integrins in the human breast cancer cell line MDA-MB-435s (green). **(I,J)** Quantification of percentage of cells that are integrin positive, i.e. falls in P2 gate indicated at A-F (I), or positive for mCherry-LifeAct expression, i.e. falls in P3 gate indicated at C-F (J). Mean and standard deviation are shown of three independent experiments for I and J.

Figure S2. Strain field of cyclic stretcher, characterization of PAA gels with bulk rheology and integrin-mediated cell adhesion to PAA and PDMS substrates. **(A)** Magnified homogeneous displacement field under static strain over the entire substrate of 8x10 mm (height x width). Global strain is applied over the x-axis and the net strain from differentiation over this field is homogeneous. **(B)** Positions of fluorescent beads at the minimal and maximal strain during 10% (top) or 20% (bottom) cyclic stretch measured manually and calculated strain (calculations of only point 0 reference is shown, mean and deviation are obtained by taking all points as reference one by one). **(C)** Representation of how the length “r” and orientation angle “*A*” of a filament would change under a horizontal and vertical strain of ε_x and ε_y, respectively to a length of r' and an angle of *A*'. **(D)** Analytical calculation of minimal strain direction, finding *A* where r'(*A*')=r(*A*), for measured strain values (B). **(E)** Correction factor for square imaging window where A is the angle, C is the cell size (obtained from Fig. 1D) and L is the imaging window length (69µm for this experiment). The cosine/sine term in the denominator is due to the variation in maximum measurable fiber length in a given angle and the nominator is the portion of a cell of measured size falling outside of the imaging window if the imaging window was a circle with diameter L. **(F)** Shear storage modulus of a PAA gel of 7.5% acrylamide and 0.2% bis-acrylamide during polymerization and its temperature dependence. **(G)** The final shear elastic modulus measured at 37°C for PAA gels with varying bis-acrylamide concentration. Each bar represents a separate experiment performed on different days and using two different rheometers. **(H,I)** Adhesion to 1:10 (crosslinker:prepolymer) ratio PDMS (H) and 12.2kPa PAA (I) of GEβ1 and GEβ3 cells preincubated with- and seeded in the absence or presence of integrin blocking antibodies targeting mouse-αv, mouse-α5, human-β1 or human-β3.

Figure S3. Average cell-matrix adhesion area remains constant with increasing stiffness. **(A)** Cumulative Gaussian distribution and Gaussian distribution functions used to obtain the fit parameters for cell spreading and cell matrix adhesion formation. **(B)** The fit parameters obtained by fitting cumulative Gaussian distribution model and the p values obtained by comparing the indicated fit parameters between β1 and β3 expressing cells using the F-test. **(C-F)** Slopes of the fits shown in figures 2A,B and 3A,B describing stiffness-dependent induction of cell spreading (C,D) and peripheral cell matrix adhesion formation (E,F) as a function of substrate rigidity at the stiffness range tested. **(G,H)** Quantification of average size of peripheral cell-matrix adhesions of GEβ1 and GEβ3 cells (G) or GDβ1 and GDβ3 (H) for cells with at least 10 adhesions. In all graphs, mean ±95% clearance level is shown and at least 20 cells were measured over 3 different experiments (except for 760 Pa for GEβ1 and GEβ3 cells where results of one experimental replica is shown). P values were calculated by comparing the slope of the linear fits with F-test. **(I,J)** Representative images of Paxillin staining for GDβ1 (I) and GDβ3 cells (J). Upper row shows raw immunofluorescence staining, middle row shows zoomed in region of the boxed area, and bottom row shows adhesions detected by the automated analysis algorithm. Scale bar is 20μm (5μm for zooms).

Figure S4. Increased cellular traction force in response to substrate stiffening is maintained in post-fixation samples and antibody blocking confirms role for αv integrins in force exertion by GEβ3 but not by GEβ1 cells. **(A,B)** Bar plots of cellular spread area (A) and force per pillar (B) measured in fixed GEβ1, GEβ3, GDβ1, GDβ3 and NIH-3T3 cells on 6.9 and 4.1 μm pillars. In A,B mean ±95% clearance level is shown and at least 15 cells were measured from three independent experiments. **(C)** Representative images from A,B. **(D,E)** Bar plots of cellular spread area (D) and force per pillar (E) analyzed by live cell imaging of mCherry-LifeAct-expressing GEβ1 and GEβ3 cells seeded on 4.1 μm pillars for 5 hours in the presence or absence of blocking antibody against mouse integrin αv. In D,E mean ±95% clearance level is shown and at least 50 cells were measured from a single experiment. NS, $p>0.05$; **, $p<0.005$; ***, $p<0.0005$ compared to control according to Mann-Whitney test. **(F)** Representative images of D,E. White arrows indicate magnitude and direction of forces measured. Scale bar, 20 nN / 10 μm.

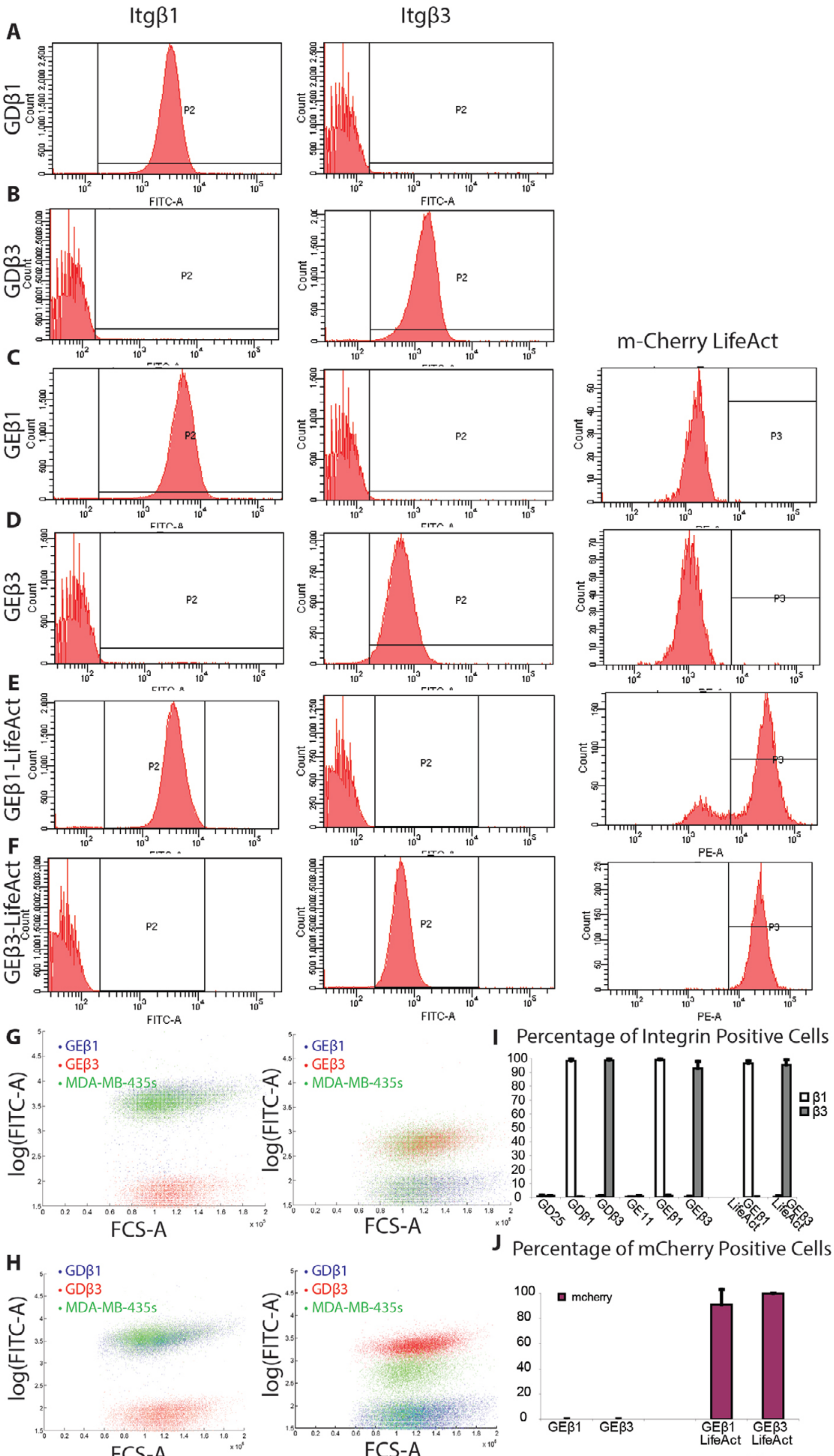
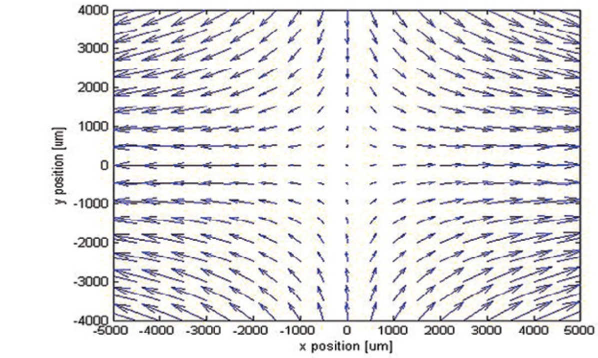

Itgβ1
Itgβ3
m-Cherry LifeAct
A
GDβ1
B
GDβ3
C
GEβ1
D
GEβ3
E
GEβ1-LifeAct
F
GEβ3-LifeAct
Count
FITC-A
PE-A
P2
P3
G
GEβ1
GEβ3
MDA-MB-435s
log(FITC-A)
FCS-A
H
GDβ1
GDβ3
I
Percentage of Integrin Positive Cells
β1
β3
GD25
GDβ1
GDβ3
GE11
GEβ1
GEβ3
GEβ1 LifeAct
GEβ3 LifeAct
J
Percentage of mCherry Positive Cells
mcherry

Figure S1

A

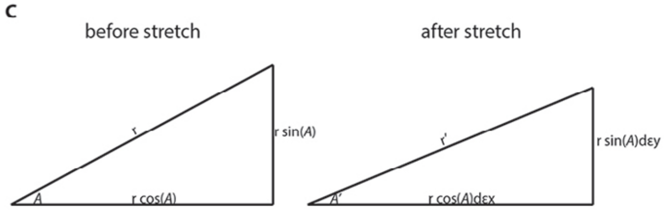


B

10% stretch	absolute positions				reference from point 0				strain when dx(or dy)>100	
	before stretch		after stretch		before stretch		after stretch			
	x	y	x	y	dx	dy	dx	dy	dεx	dεy
point 0	208	372	183	382	0	0	0	0		
point 1	131	439	100	445	-77	67	-83	63		
point 2	392	285	380	298	184	-87	197	-84	1.0707	
point 3	163	144	133	160	-45	-228	-50	-222		0.9737
point 4	431	437	424	445	223	65	241	63	1.0807	
point 5	494	332	491	342	286	-40	308	-40	1.0769	
point 6	185	99	159	119	-23	-273	-24	-263		0.9634
point 7	313	86	299	105	105	-286	116	-277	1.1048	0.9685
point 8	174	31	146	52	-34	-341	-37	-330		0.9677
point 9	400	67	390	87	192	-305	207	-295	1.0781	0.9672
point 10	66	215	31	230	-142	-157	-152	-152	1.0704	0.9682
point 11	415	494	409	499	207	122	226	117	1.0918	0.959
								mean	**1.0806**	**0.9654**
								std	**0.0105**	**0.0053**

20% stretch	absolute positions				reference from point 0				strain when dx(or dy)>100	
	before stretch		after stretch		before stretch		after stretch			
	x	y	x	y	dx	dy	dx	dy	dεx	dεy
point 0	401	64	316	190	0	0	0	0		
point 1	214	123	102	246	-187	59	-214	56	1.1444	
point 2	265	314	158	424	-136	250	-158	234	1.1618	0.936
point 3	400	365	314	475	-1	301	-2	285		0.9468
point 4	232	246	127	363	-169	182	-189	173	1.1183	0.9505
point 5	163	259	43	374	-238	195	-273	184	1.1471	0.9436
point 6	465	345	385	453	64	281	69	263		0.9359
point 7	242	18	133	144	-159	-46	-183	-46	1.1509	
point 8	317	314	217	427	-84	250	-99	237		0.948
point 9	315	202	218	321	-86	138	-98	131		0.9493
point 10	495	312	429	428	94	248	113	238		0.9597
point 11	370	156	280	275	-31	92	-36	85		
								mean	**1.1445**	**0.9499**
								std	**0.022**	**0.0129**

C



D

$$r' = \sqrt{(r\cos(A)d\varepsilon x)^2 + (r\sin(A)d\varepsilon y)^2} = r^2\sqrt{\cos^2(A)d\varepsilon x^2 + \sin^2(A)d\varepsilon y^2}$$

For minimal strain $\frac{r'}{r} = 1$ hence $\sqrt{\cos^2(A)d\varepsilon x^2 + \sin^2(A)d\varepsilon y^2} = 1$

For 10% stretch we measured $d\varepsilon x = 1.08 \pm 0.01$ and $d\varepsilon y = 0.965 \pm 0.005$

And for 20% stretch we measured $dx = 1.14 \pm 0.02$ and $dy = 0.95 \pm 0.01$

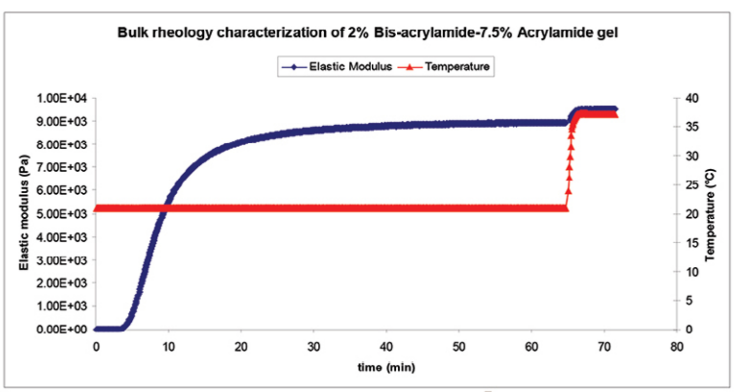
$$\sqrt{\cos^2(A)(1.08)^2 + \sin^2(A)(0.965)^2} = 1$$
$$1.166\cos^2(A) + 0.931\sin^2(A) = 1$$
$$0.166\cos^2(A) - 0.069\sin^2(A) = 0 \quad \Rightarrow \quad \tan^2(A) = 2.406$$
$$A = 57.2^{\circ}$$

$$\sqrt{\cos^2(A)(1.14)^2 + \sin^2(A)(0.95)^2} = 1$$
$$1.300\cos^2(A) + 0.903\sin^2(A) = 1$$
$$0.300\cos^2(A) - 0.097\sin^2(A) = 0 \quad \Rightarrow \quad \tan^2(A) = 3.093$$
$$A = 60.4^{\circ}$$

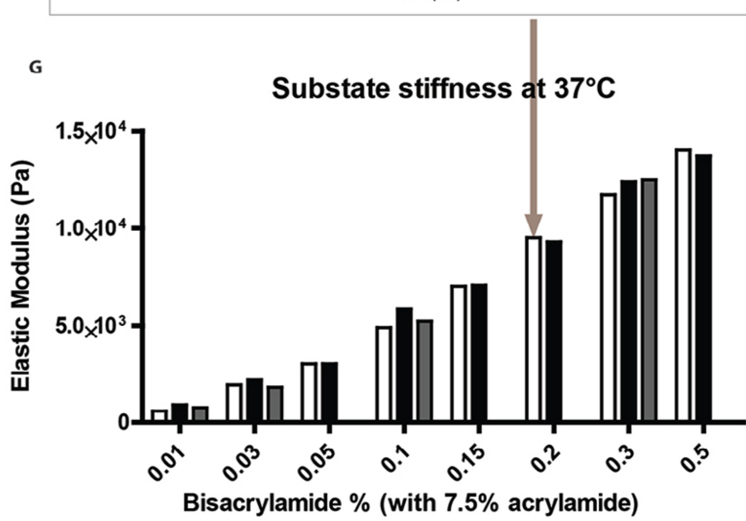
E

$$f(A,C) = \frac{\left(1 + \frac{2}{L}\sqrt{\frac{C}{\pi}}\right)^2 - 1}{\cos(A)} + 1 \quad for\ A < 45^{\circ}\ and \quad f(A,C) = \frac{\left(1 + \frac{2}{L}\sqrt{\frac{C}{\pi}}\right)^2 - 1}{\sin(A)} + 1 \quad for\ A > 45^{\circ}$$

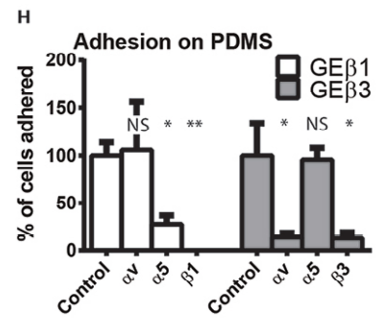
F



G



H



I

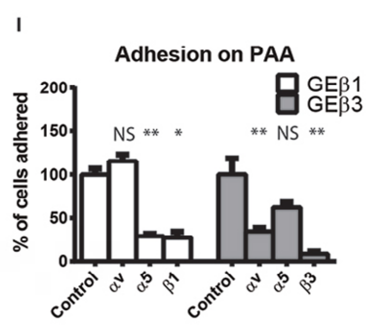


Figure S2

A

$$F(x) = \int_{-\infty}^{x} f(a)da$$

Cumulative Gaussian Distribution

$$f(x) = \frac{N}{\sigma\sqrt{2\pi}} e^{-\frac{1}{2}\left(\frac{x-\mu}{\sigma}\right)^2}$$

Gaussian Distribution

B

	Spreading				Adhesion formation			
cell line	GEβ1	GEβ3	GDβ1	GDβ3	GEβ1	GEβ3	GDβ1	GDβ3
Mean (μ,E½)	2.692	2.820	1.727	2.16	4.556	3.070	2.685	2.005
	p=0.1156		p=0.0668		p=0.0235		p=0.0103	
SD (σ)	3.17	3.253	2.204	2.109	3.138	3.137	1.385	0.7485
	p=0.9105		p=0.816		p=0.9995		p=0.1425	
Max value (N)	1168	1524	499.5	869.2	15.99	20.44	33.33	40.3
	p<0.0001		p< 0.0001		p=0.0041		p=0.1135	

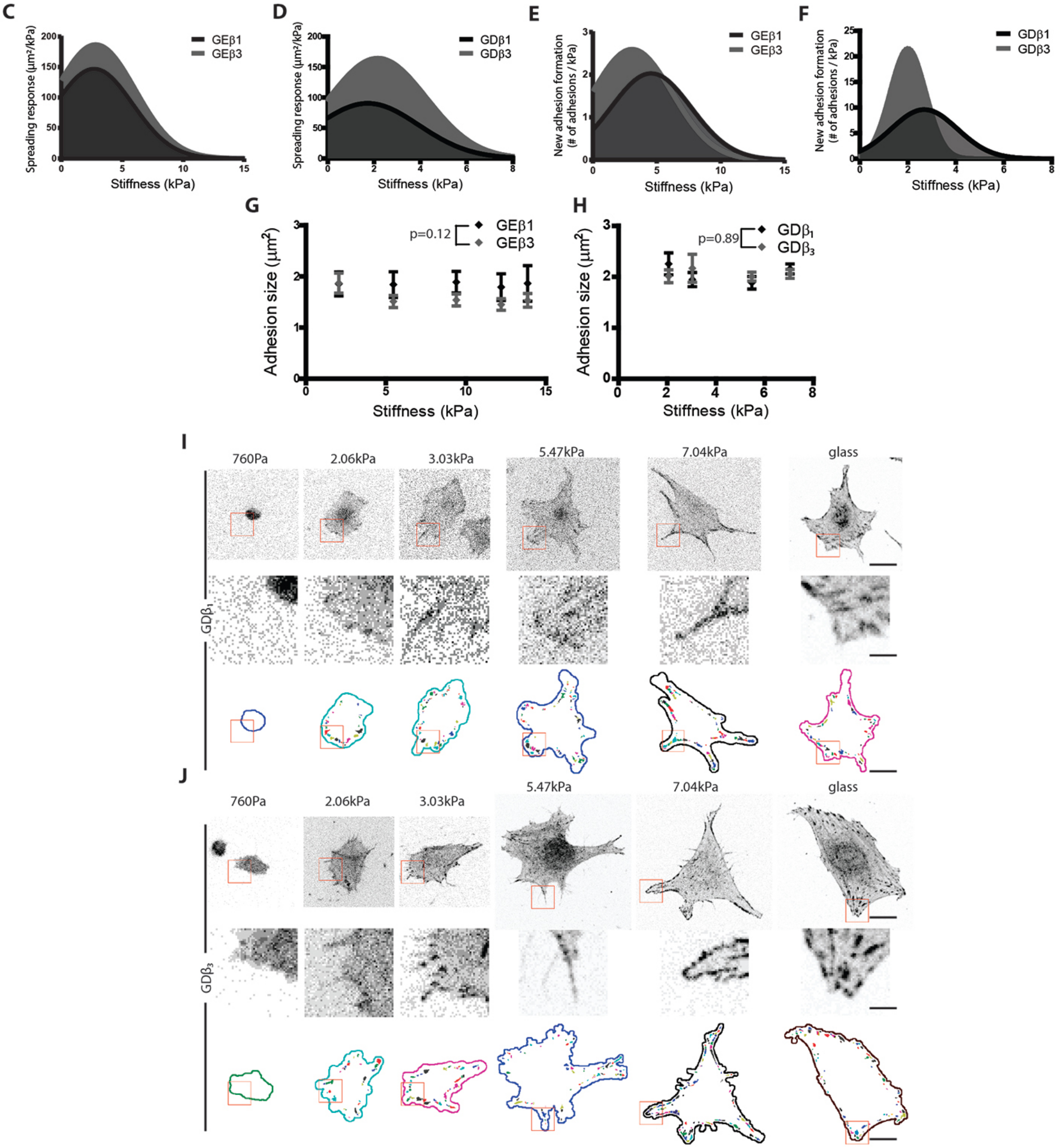


Figure S3

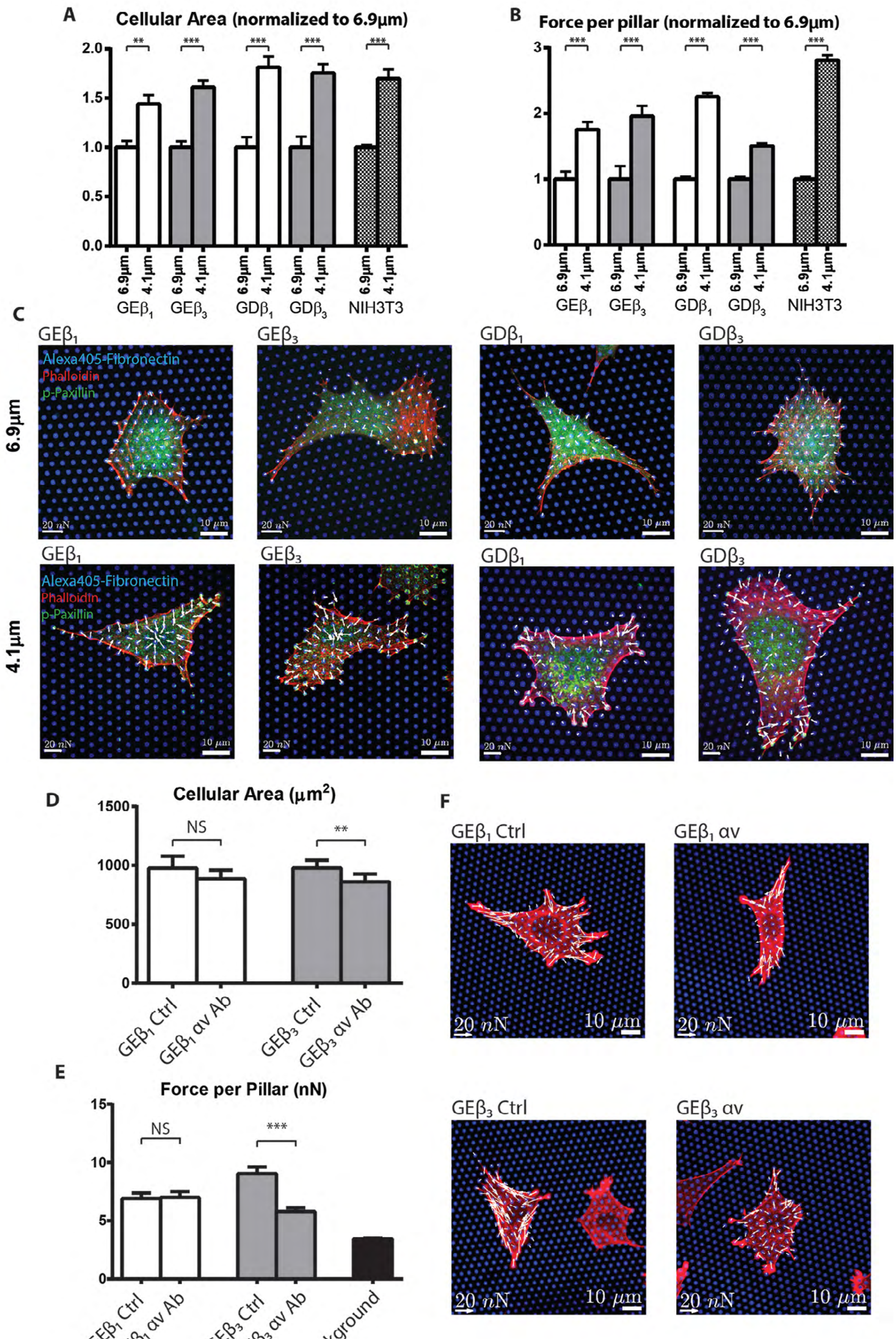

A
Cellular Area (normalized to 6.9μm)
B
Force per pillar (normalized to 6.9μm)
6.9μm
4.1μm
GEβ1
GEβ3
GDβ1
GDβ3
NIH3T3
C
Alexa405-Fibronectin
Phalloidin
p-Paxillin
20 nN
10 μm
D
Cellular Area (μm2)
NS
GEβ1 Ctrl
GEβ1 αv Ab
GEβ3 Ctrl
GEβ3 αv Ab
E
Force per Pillar (nN)
background
F
GEβ1 Ctrl
GEβ1 αv
GEβ3 Ctrl
GEβ3 αv

Figure S4