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 $\varepsilon_x$ and $\varepsilon_y$, 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 (69\(\mu\)m 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 GE$\beta$1 and GE$\beta$3 cells preincubated with- and seeded in the absence or presence of integrin blocking antibodies targeting mouse-$\alpha$v, mouse-$\alpha$5, human-$\beta$1 or human-$\beta$3.

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 $\beta$1 and $\beta$3 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.
For 10% stretch we measured \( dx = 1.08 \pm 0.01 \) and \( dy = 0.965 \pm 0.005 \)

\[ r = \sqrt{(\cos(A)dx)^2 + (\sin(A)dy)^2} = r_0 \sqrt{\cos^2(A)dx^2 + \sin^2(A)dy^2} \]

For minimal strain \( r' = 1 \) hence \( \sqrt{\cos^2(A)dx^2 + \sin^2(A)dy^2} = 1 \)

\[ 1.166 \cos^2(A) + 0.931 \sin^2(A) = 1 \]

\[ 0.166 \cos^2(A) - 0.069 \sin^2(A) = 0 \Rightarrow \tan^2(A) = 2.406 \]

\[ A = 57.2^\circ \]

\[ f(A, C) = \left( \frac{1 + \frac{2}{\pi} \frac{C}{L} \cos(A)}{\cos(A)} \right)^{-1} \] for \( A < 45^\circ \) and \[ f(A, C) = \left( \frac{1 + \frac{2}{\pi} \frac{C}{L} \sin(A)}{\sin(A)} \right)^{-1} \] for \( A > 45^\circ \)
A
\[ F(x) = \int_{-\infty}^{x} f(a) \, da \quad f(x) = \frac{1}{\sqrt{2\pi}} e^{-\frac{1}{2} \left( \frac{x-m}{s} \right)^2} \]

Cumulative Gaussian Distribution

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B

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C

Stiffness (kPa)

D

Stiffness (kPa)

E

Stiffness (kPa)

F

Stiffness (kPa)

G

Adhesion size (μm²)

H

Adhesion size (μm²)

I

760 Pa

J

760 Pa

Figure S3