Supporting Information

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SI Materials and Methods

Preparation of Small Unilamellar Vesicles. DOPC and DPPC were used as the fluid phase and gel phase, respectively (Avanti Polar Lipids). The desired amount of lipid, in chloroform, was mixed at a 98:2 mol % ratio with b-cap-PE [1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine-*N*-(cap biotinyl); Avanti Polar Lipids] and evaporated under N_2 gas. Excess chloroform was removed by drying under vacuum for ≥1 h.

Lipids were rehydrated in rehydration buffer (150 mM NaCl and 10 mM Tris, pH 7.4) to a concentration of 2 mg/mL and allowed to swell above their transition temperature (DOPC $T_m = -19$ °C, DPPC $T_m = 41$ °C) for 1 h and extruded through a 50-nm polycarbonate membrane (above their T_m) and stored for a maximum of 1 wk.

Preparation of Glass Surfaces. Glass coverslips were cleaned by immersion in 5:1:1 solution of H_2O:NH_4:H_2O_2 at 65 °C for 20 min and were dried under a stream of N_2 gas. Polydimethylsiloxane (PDMS) was prepared in a ratio of 9:1 ratio of elastomer to cross-linker and degassed for 20 min before curing for 2 h. PDMS wells were cut out and the PDMS bonded to the cleaned glass using a handheld plasma device on the surfaces for 20 s. The resulting wells were sterilized by exposure to UV light for 20 min and stored in a sealed, sterile container until use.

Production and Functionalization of SLBs and Glass Control. To produce SLBs, vesicles were diluted in to "fusion buffer" (300 mM NaCl, 10 mM Tris, 10 mM $MgCl_2$) to 0.1 mg/mL under sterile conditions immediately before use and kept at either RT (DOPC) or at 70 °C (DPPC). The glass-bottomed PDMS wells were activated by cleaning with oxygen plasma (Zepto; Diener Electronic) at 75 W for 10 min and exposed to UV light for 5 min to ensure sterility. DOPC and DPPC lipid vesicles were incubated under sterile conditions for 20 min at RT and 70 °C, respectively. Samples were washed with sterile fusion buffer and ultrapure water of the same temperature.

An immobile control was also prepared first by silanizing previously produced glass-bottomed PDMS with 2% (3-aminopropyl) trimethoxysilane in propan-2-ol (Sigma-Aldrich) for 1 h. Samples were then washed and cured at 100 °C overnight and sterilized under UV light for 20 min. Surfaces were incubated with 1 mg/mL of an NHS-biotin cross-linker (biotinamidocaproate *N*-hydroxysuccinimide ester; Sigma-Aldrich) for 1 h and washed thoroughly with ultrapure water.

Both surfaces were functionalized with 0.1 mg/mL neutravidin (Fisher Scientific) and subsequently biotinylated cyclic-RGD peptide (Peptides International) for 15 min each and washed with ultrapure water after each step.

Quantification of SLB Properties. The presence of SLBs in both cases was confirmed via AFM in contact and force mapping mode in ultrapure water (Nanowizard 3 Bioscience AFM; JPK), with cantilevers of nominal spring constant of 0.32 N/m and resonant frequency of 67 kHz (PNP-TR-Au; Nanoworld). For force mapping the relative set point was set to 20 nN with an approach velocity of 1 μm/s. Measurements were taken in an 8 × 8, 5- × 5-μm^2 grid.

Diffusion coefficients of lipid bilayers were performed via FCS using a Becker and Hickl DCS-120 confocal scanning system, with an excitation laser of 480 nm and emission filter of 535 nm. For this purpose TopFluor-PE lipid [23-(dipyrromethenboron difluoride)-24-norcholesterol and 1-palmitoyl-2-(dipyrromethenboron difluoride) undecanoyl-*sn*-glycero-3-phosphoethanolamine; Avanti Polar Lipids] was used at 0.01 mol % for DOPC and 0.5 mol % for DPPC. The resulting data were fitted to Eq. **S1**:

$$G(\tau) = \frac{1}{N}\frac{1}{\left(1+\tau/\tau_D\right)}, \quad \textbf{[S1]}$$

where $G(\tau)$ is the correlation factor, N the average number of diffusing molecules, τ the correlation time, and τ_D the diffusion time. The diffusion time and previously calibrated beam width was then applied to Eq. **S2** and used to determine the diffusion coefficient of the SLBs:

$$\tau_D = \omega^2/4D, \quad \textbf{[S2]}$$

where ω is the beam radius and D is the diffusion coefficient.

The amount of neutravidin on the surface was quantified using a previously described quantitative fluorescent microscopy technique. Briefly, bulk DOPC vesicle and neutravidin, both containing a Texas Red fluorescent label (TR-DHPE lipid; Molecular Probes and Texas Red Neutravidin; ThermoFisher) were used to create a scaling factor between fluorescent signals. This was then applied to a DOPC SLB containing different concentrations of TR-DHPE per square micrometer to determine the assumed amount of neutravidin per square micrometer, based on a given fluorescence.

Culturing and Staining of C2C12 Mouse Myoblasts. For adhesion experiments C2C12 mouse myoblast cells were seeded at 5,000 cells per square centimeter in DMEM (high glucose, [−]pyruvate, 1% penicillin/streptomycin, and1% fungizone) for 3 h. For differentiation experiments, samples were seeded with 20,000 cells per square centimeter and incubated in said media with 1% insulin-transferrin-selenium for 4 d, with the media changed at day 2.

Samples were then washed in PBS, fixed with 4% paraformaldehyde, and permeabilized with 0.1% Triton X-100. Before staining samples were blocked with 1% BSA for 1 h. Primary antibodies used were antivinculin (1:400, mouse monoclonal), anti-pFAK (1:200), anti-YAP (1:100, mouse monoclonal; Santa Cruz Biotechnologies), and antisarcomeric myosin (1:250, MF-20b; DSHB). Primary antibodies were incubated for 1 h and washed extensively with PBS. The samples were then blocked with 1% BSA for 1 h and incubated with secondary antibodies for 1 h and washed extensively. Secondary antibodies used were Cy3 rabbit anti-mouse (1:100; Jackson Immunoresearch), Alexa Fluor 488 goat anti-mouse (1:100; Molecular Probes), rhodamine phallodin (1:100; Molecular Probes), and Alexa Fluor 488 phalloidin (1:100; Molecular Probes). DAPI containing mounting media was used to stain the nuclei.

Images were taken with a Zeiss Observer.Z1 using Mirco-Manager software. Image processing and analysis was performed using Fiji imaging software. Differentiation levels were calculated using the CellC Counter MATLAB extension to determine the percentage of nuclei staining positive for sarcomeric myosin.

Determining Actin Flow. C2C12 cells were transfected using the Neon transfection system (ThermoFisher Scientific) and performed as described by their cell specific protocol. Plasmids used were LifeAct-RFP, LifeAct-GFP (Ibidi) to label actin and the pEGFPC1/GgVcl 1-258 (aka VD1) plasmid was a gift from Susan Craig (Addgene plasmid no. 46270) (44). Transformed cells were cultured for 24 h and used.

Mattek glass-bottom dishes were used to image live samples, to more effectively maintain sterility. Samples were cleaned by sonicating in propan-2-ol for 30 min and cleaned with oxygen plasma for 20 min. Wells were not used on these samples, but all methods for surface preparation were the same from this point.

Cells were seeded at 5,000 cells per square centimeter in DMEM for 1 h and imaged using a Nikon Eclipse Ti confocal spinning disk microscope with a 60× oil-immersion objective (N.A. 1.40) and Andor Q3 software. Blebbistatin was also used to inhibit cytoskeleton formation at a concentration of 50 μM. Images were taken for 2 min at 1 frame per second at either 488 or 561 nm, depending upon the plasmid. Actin flow was determined kymographs in the Fiji software.

Statistical Analysis. In all figures values are given as the mean ± SD. Before statistical comparisons D'Agostino–Pearson normality tests were carried out on the data. Upon ensuring a normal distribution of points one-way or two-way ANOVA tests were carried out as appropriate, with Tukey multiple comparison tests to determine the significance of the results. Significance was taken as the P values, which are given as follows: ns, <0.05, *≤ 0.05, **≤ 0.01, ***≤ 0.001, and ****≤ 0.0001.

Computational Model Implementation. To implement the computational clutch model we took our previously described model as a reference (16) and we carried out a simple modification to consider a viscous rather than elastic substrate, exchanging a spring for a dashpot. To this end, we modified the equation calculating the total force exerted on the substrate F_{sub} as follows:

$$F_{sub} = \frac{v_u}{\frac{1}{n_{eng}\mu} + \frac{v_u}{n_m F_m}},$$

where v_u is the contraction speed of myosin motors in the absence of force, μ is the viscosity of each ligand, n_{eng} is the number of engaged (bound) ligands, n_m is the total number of myosin motors, and F_m is the stall force of a single motor. This equation comes from combining our previously used equation to model a linear reduction in myosin contraction speed v with force:

$$v = v_u\left(1 - \frac{F_{sub}}{n_m F_m}\right)$$

with the equation relating force to speed in a viscous system where each engaged ligand contributes with a given viscosity:

$$F_{sub} = n_{eng} v \mu.$$

Total force F_{sub} was considered to be distributed evenly among all engaged clutches. For simplicity, we neglected the elasticity of the clutches. This is consistent with our previous work (16, 17, 52), where clutch elasticity was successfully modeled with very high values and played a negligible role compared with the mechanical properties of the substrate.

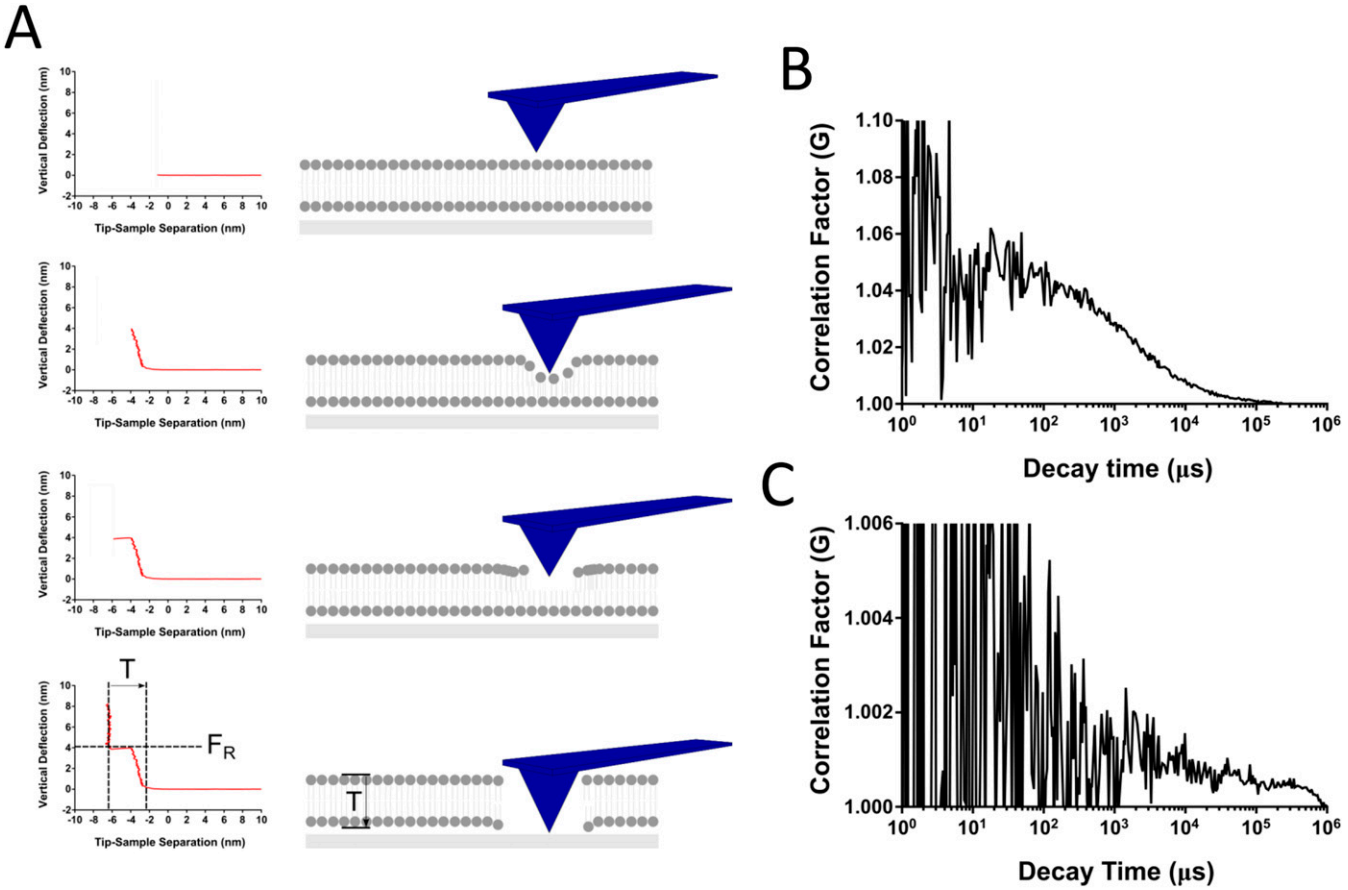

Fig. S1. (*A*) A schematic representation of how the lipid bilayer produces a characteristic force curve, by using a representative curve attained from a SLB. (*B*) The correlation decay of fluorescence TopFluor-PE present in DOPC and (*C*) DPPC SLBs.

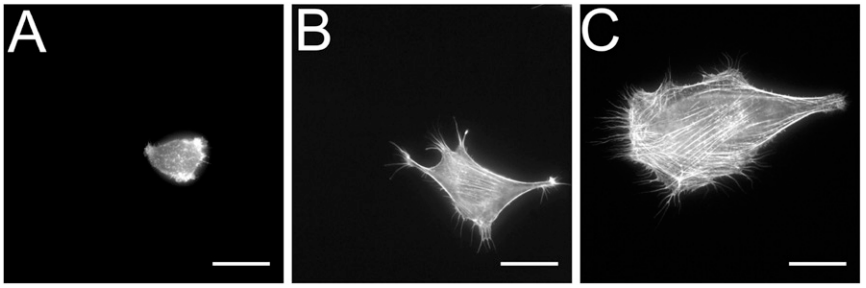

Fig. S2. Representative images of the cellular morphology and actin cytoskeleton of C2C12 mouse myoblast cells on (*A*) DOPC, (*B*) DPPC, and (*C*) RGD-glass. (Scale bars: 25 μm.)

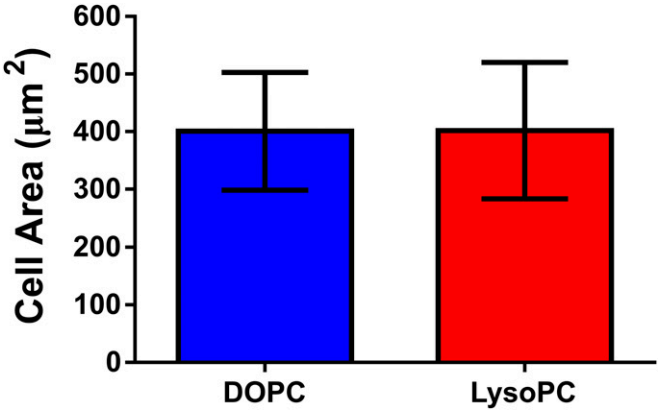

Fig. S3. The average cell area on DOPC and LysoPC lipid, showing no statistical change in cell area.

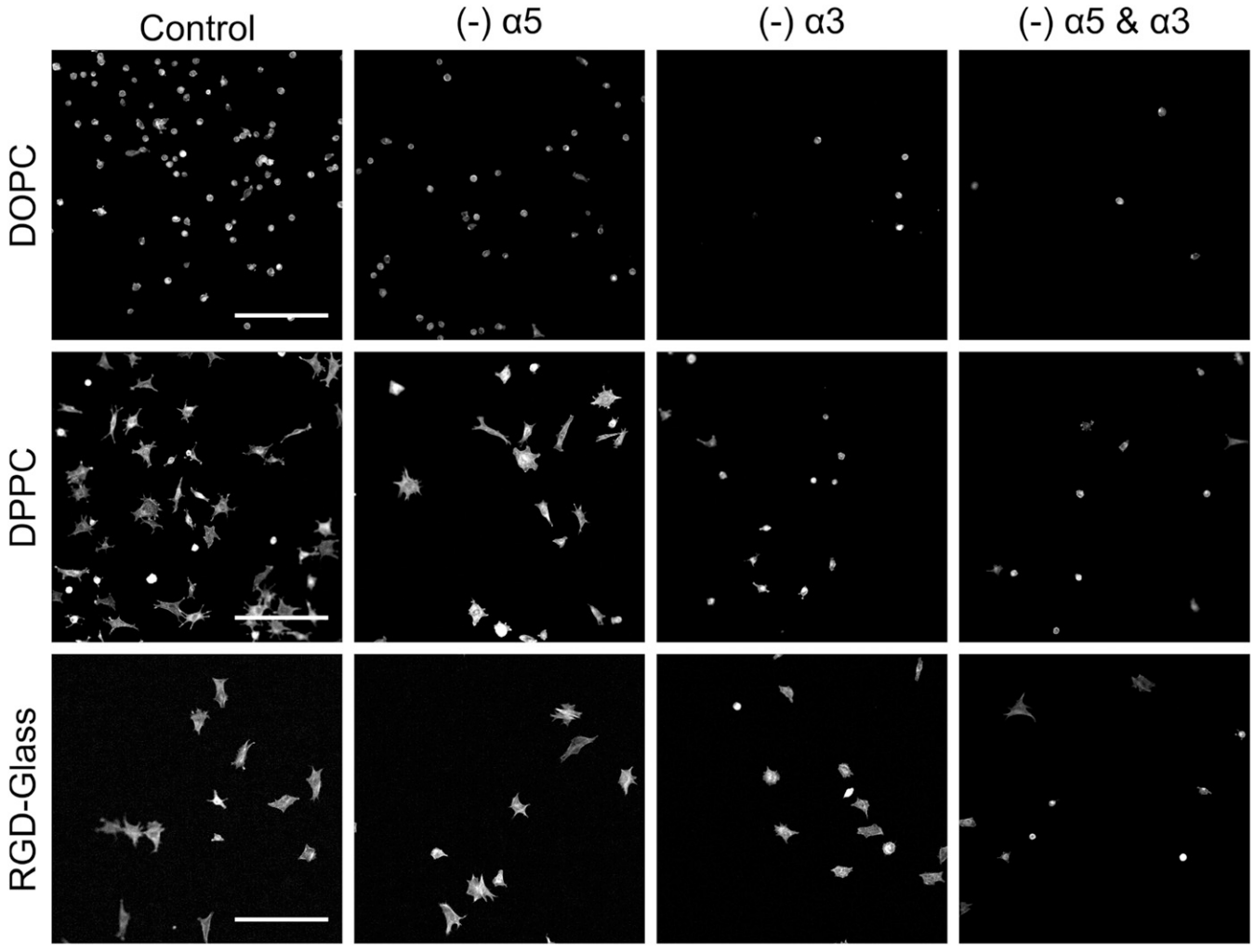

Fig. S4. Representative images of cells on all of the surfaces when incubated with inhibitors for both α5 and α3 integrins, as well as simultaneous inhibition. (Scale bars: 250 μm.)

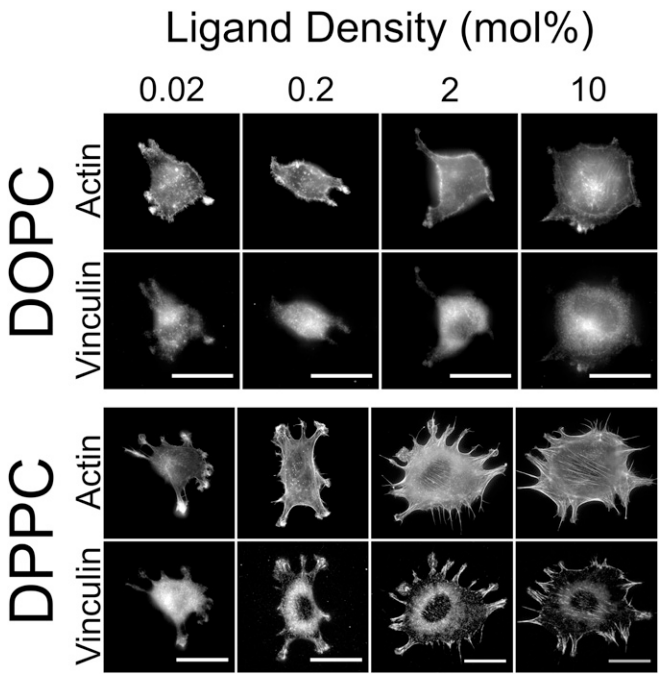

Fig. S5. Representative images of cells on DOPC and DPPC upon the alteration of the ligand density. The actin and vinculin stains used to quantify cell area and FA size in Figs. 3 and 5, respectively, are shown. (Scale bars: 25 μm.)

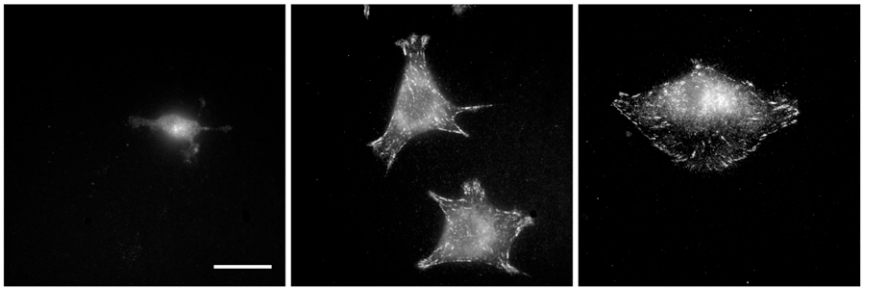

Fig. S6. Staining of representative images of pFAK on DOPC, DPPC, and RGD-glass (from left to right). (Scale bar: 25 μm.)

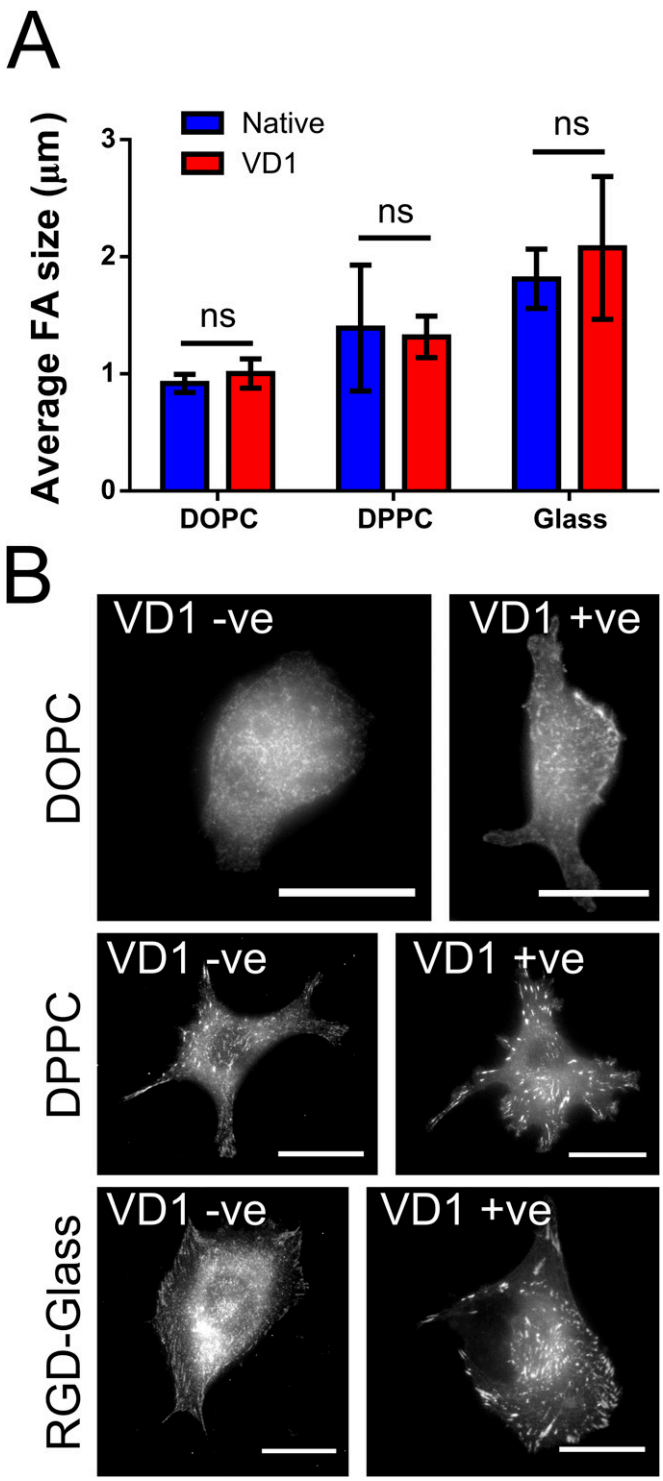

Fig. S7. (*A*) Upon transfection with VD1, encoding for the vinculin head group, there was no change in FA size between transfected (indicated as VD1 +ve) and native (VD1 −ve) cells. In VD1-positive cells this equates to difference between actin flow on the different viscosity surfaces. Representative images are shown in *B*. (Scale bars: 25 μm.)

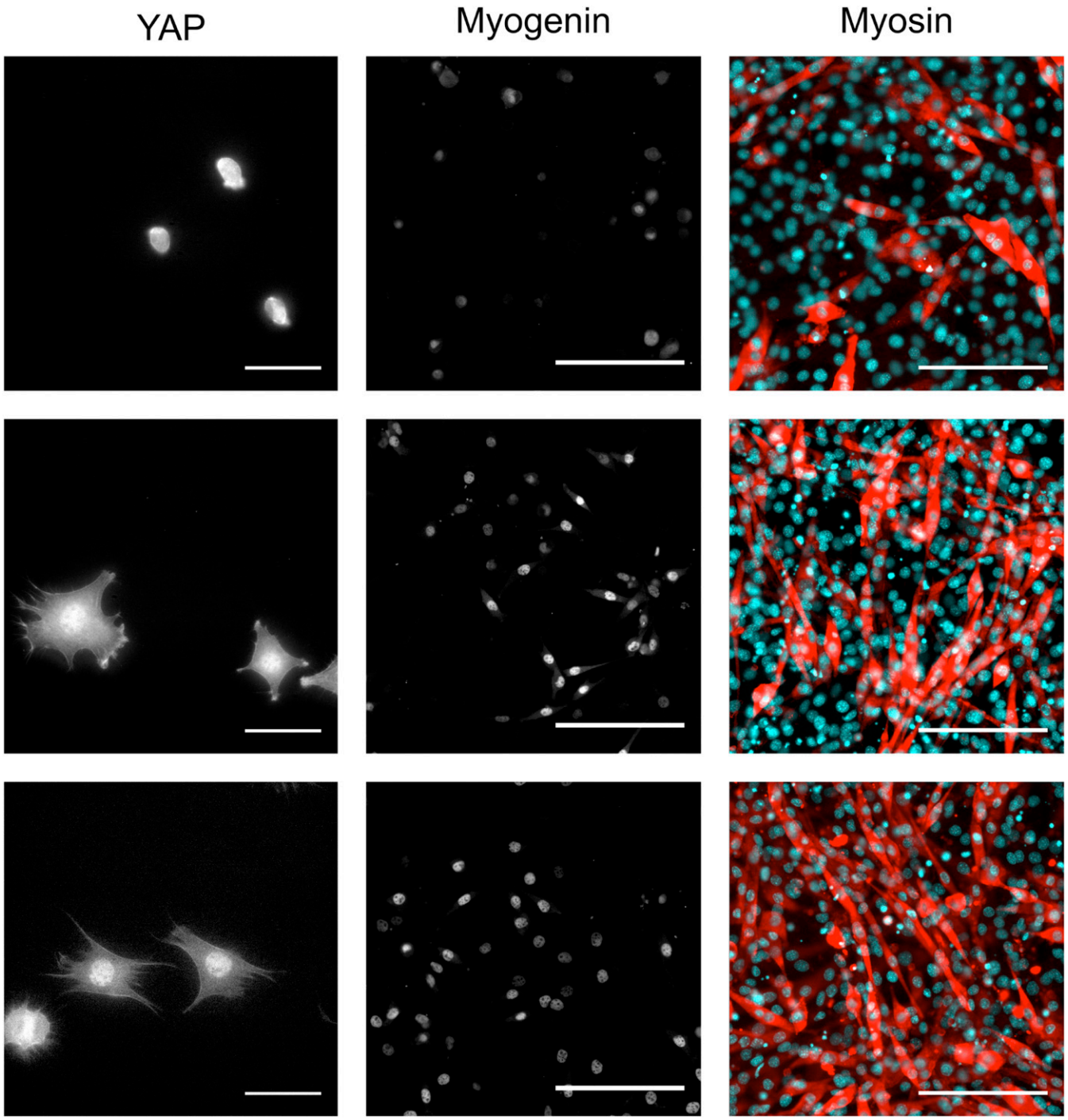

Fig. S8. An array of representative images of the relevant staining on each of the surfaces. Sarcomeric myosin is stained red and the nuclei are blue. (YAP scale bar: 50 μm; myogenin and sarcomeric myosin scale bars: 150 μm.)

Table S1. Model parameters

Parameter	Meaning	Value	Origin
n_m	No. of myosin motors	100	Adjusted
n_l	No. of ligands	75	Adjusted
F_m	Myosin motor stall force	2 pN	(53)
v_u	Unloaded myosin motor velocity	110 nm/s	(16, 17)
d_{int}	Initial integrin density on the membrane	300 per square micrometer	(16)
d_{intmax}	Maximum integrin density on the membrane	1,000 per square micrometer	Adjusted
K_{ont}	True binding rate	1×10^{-4} $\mu m^2/s$	Adjusted, of the order of values reported for $\alpha IIB\beta 3$ (54)
K_{off}	Unbinding rate, scaling factor applied to force curve reported in ref. 1	0.5	Adjusted, catch bond dependency from ref. 54
$F_{threshold}$	Threshold reinforcement force	90 pN	Adjusted, of the order of reported values (55)
d_{add}	Integrins added after each reinforcement event	6 per square micrometer	Does not affect model output

All parameter values are of the same order as those employed in previous simulations considering elasticity rather than viscosity (59, 21, and 51).