Biophysical Journal, Volume *114*

Supplemental Information

Traction Force Screening Enabled by Compliant PDMS Elastomers

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Traction force screening enabled by compliant PDMS elastomers

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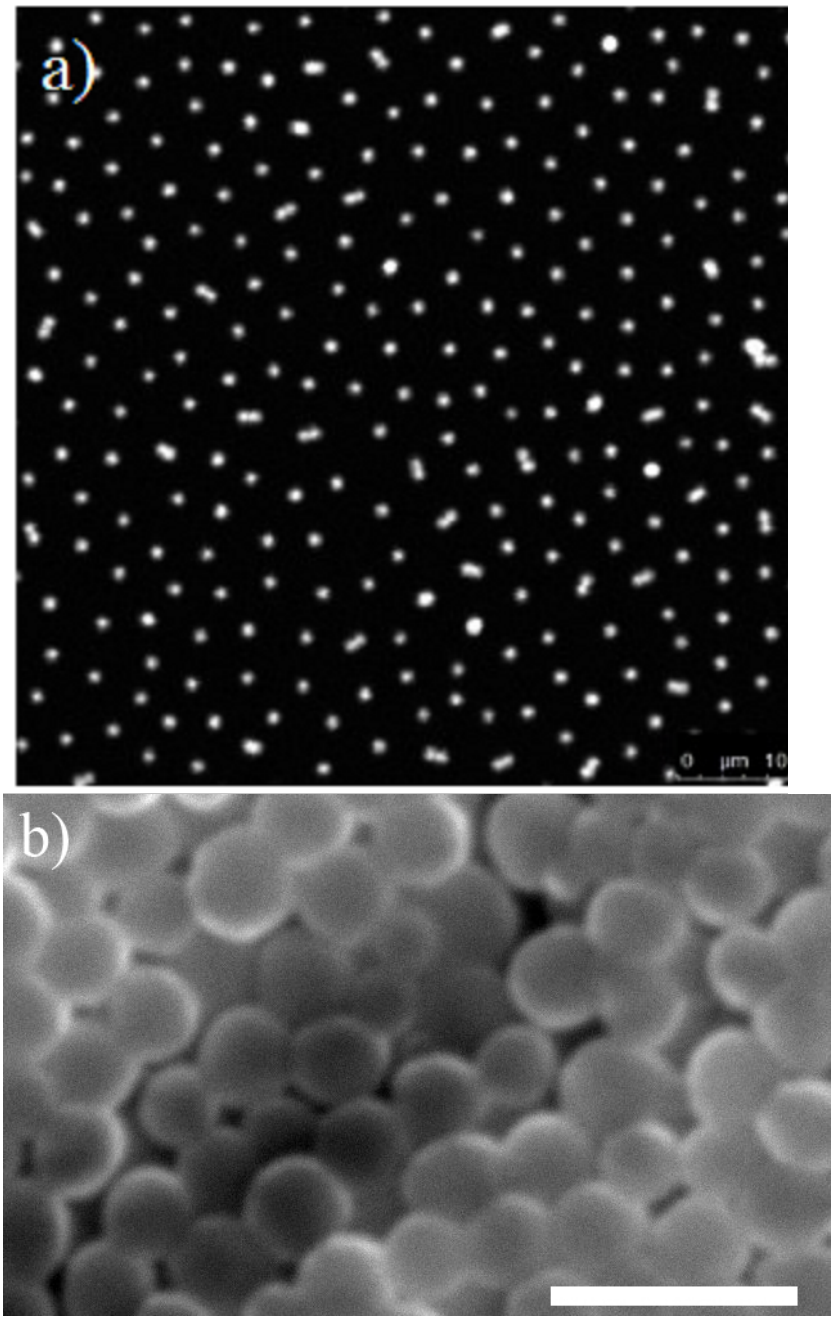
Supplemental Materials:

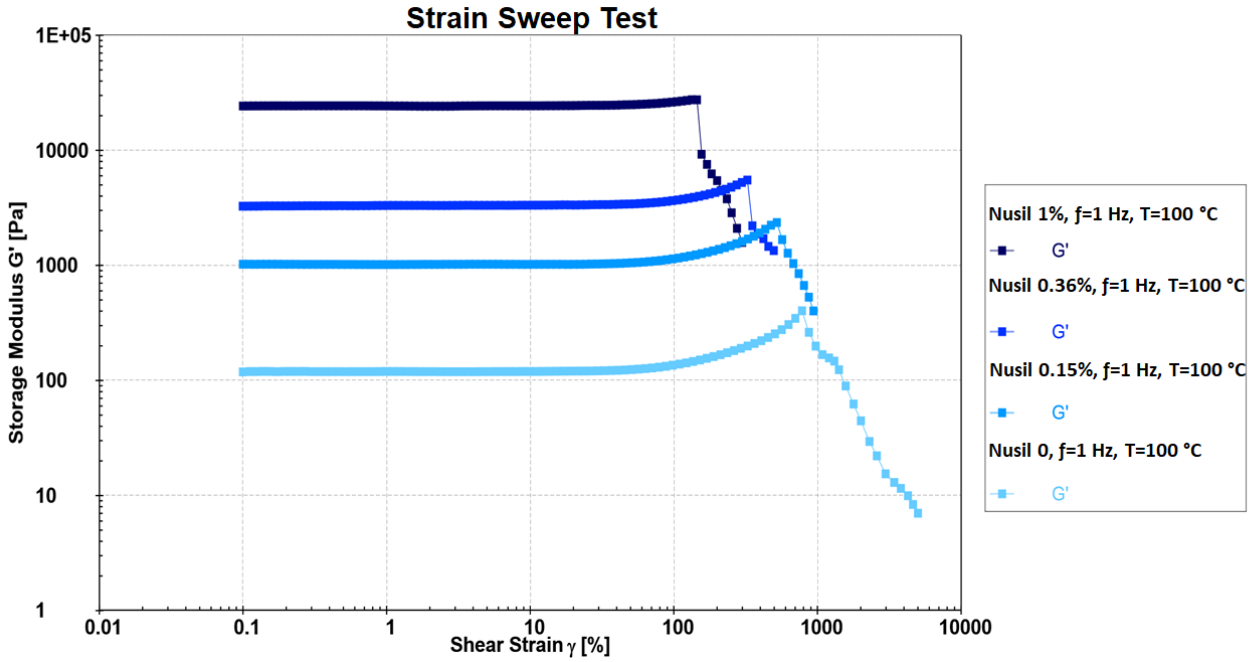
Supplementary Table1:

Condition	Potency(M), 15min	Potency(M), 30min
Formoterol	$4.8*10^{-10}$	$3*10^{-10}$
Salmeterol	$4.3*10^{-10}$	$1.6*10^{-10}$
Isoproterenol	$4.8*10^{-8}$	$3.9*10^{-8}$
Salbutamol	$2.6*10^{-8}$	$2.5*10^{-8}$

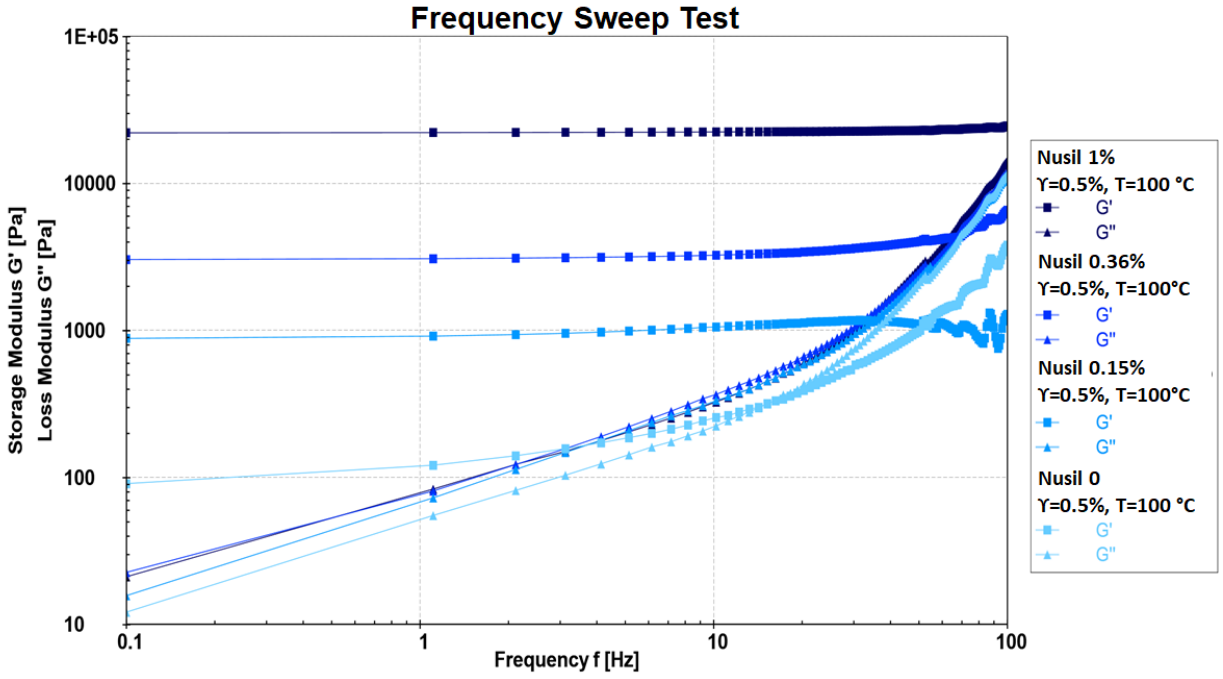
Potency of a panel of beta-agonist compounds measured by Traction Force Screening. The potency value is the concentration of the drug where the force response is reduced by half. This is also called the relative IC50 value. It was calculated from a least squares curve fit of a log(inhibitor) versus response using variable slope (four parameters). The calculation of potency was performed using Prism 6.03 software.



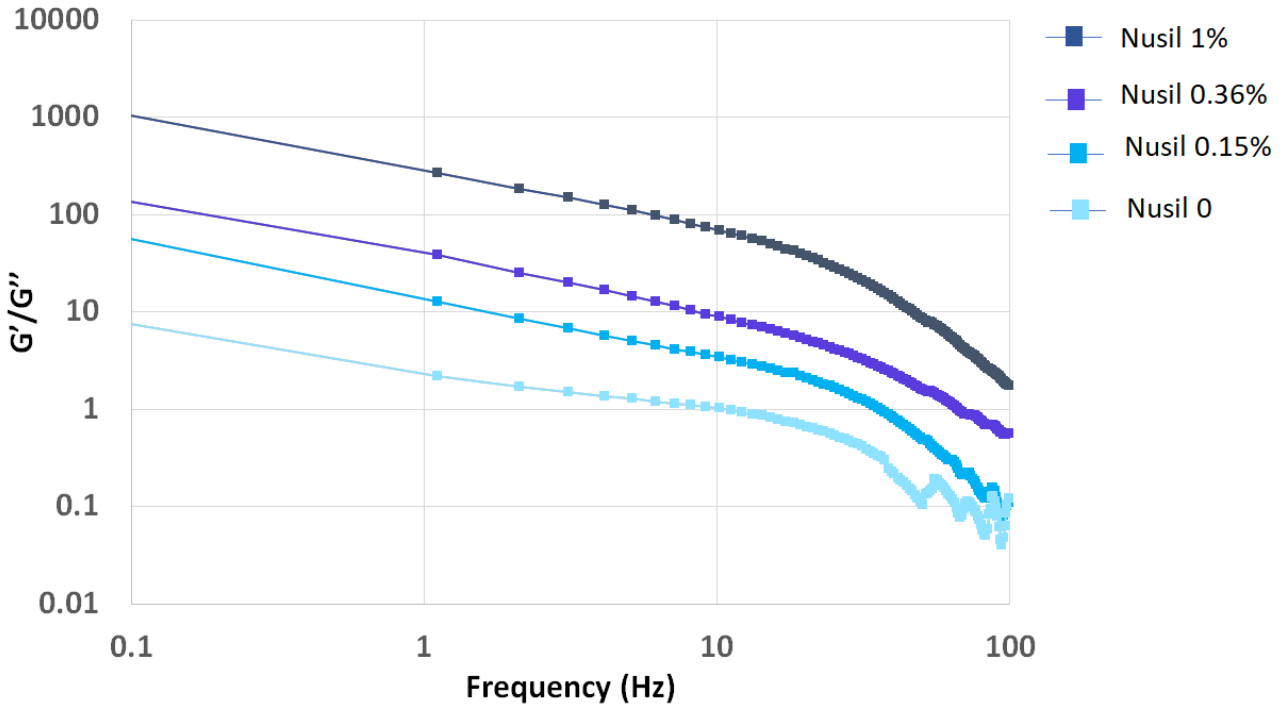
Supplementary Fig 1 – Bead synthesis & characterization.
a) The fluorescent image confirms that the particles were fluorescent and well dispersed in PDMS media (scale bar 10 μm). b) Additionally, particles were sputter-coated with gold and imaged with SEM (Hitachi Scanning Electron Microscope SU3500). The SEM image demonstrates the particle size and shape consistency (scale bar 1 μm).



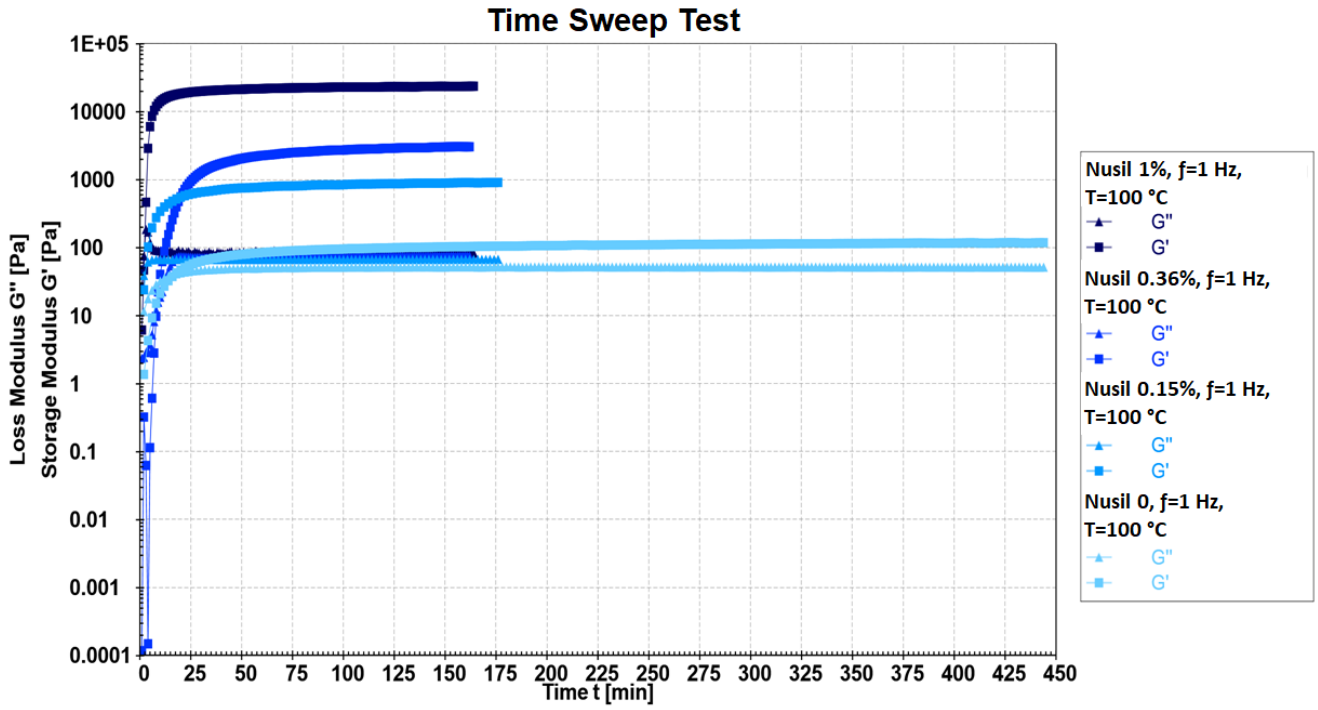
Supplementary Fig 2. PDMS samples exhibit linear elasticity.
Different formulations of PDMS were tested for nonlinear behavior such as strain-stiffening. Only at deformations approaching 100% strain did samples exhibit any change in the measured storage moduli.



Supplementary Fig 3. Frequency dependent response of PDMS samples.
Frequency sweep of PDMS formulations at 0.5% strain shows a relatively flat response in the storage modulus of all samples, with 0% crosslinker increasing above 2Hz, and other formulations remaining flat even at higher frequencies.



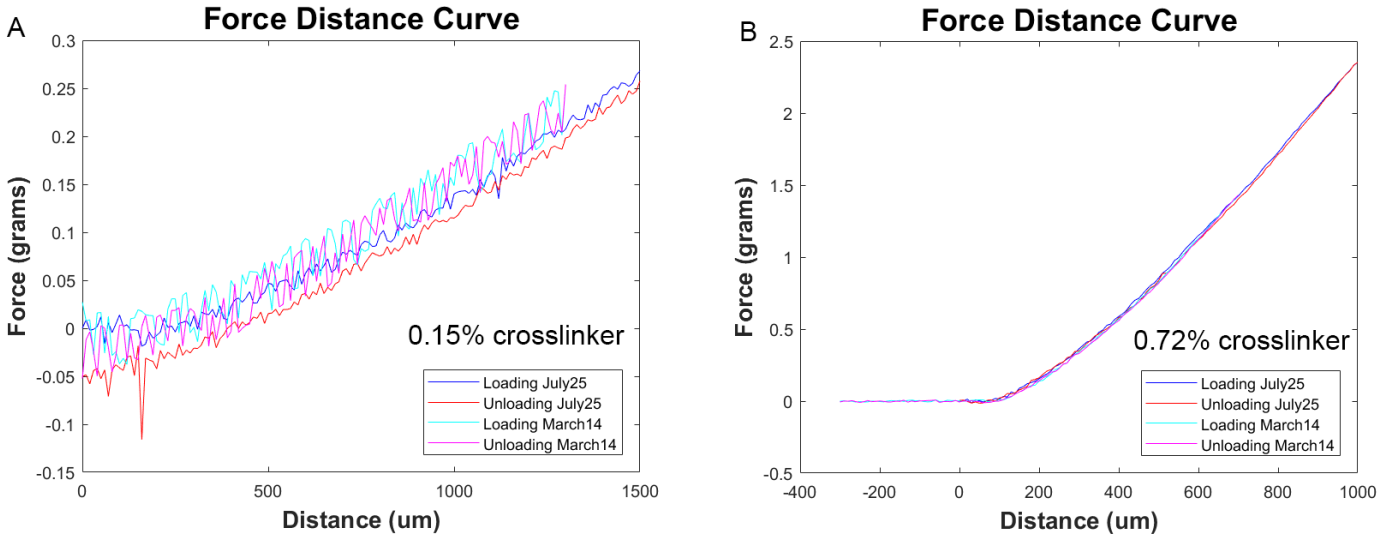
Supplementary Fig 4. Inverse Loss Tangent (ILT) of PDMS samples.
The ILT quantifies G'/G", and is a measure of the relative solid-like to fluid-like response of the material, and a material with an ILT of 1 would be highly viscoelastic. All formulations of PDMS are predominantly elastic, with the lowest being approximately 9 for 0% additional crosslinker, and approximately 1000 for 1% additional crosslinker. These data suggest that the PDMS formulations may be treated as elastic solids for the purposes of TFM calculations.



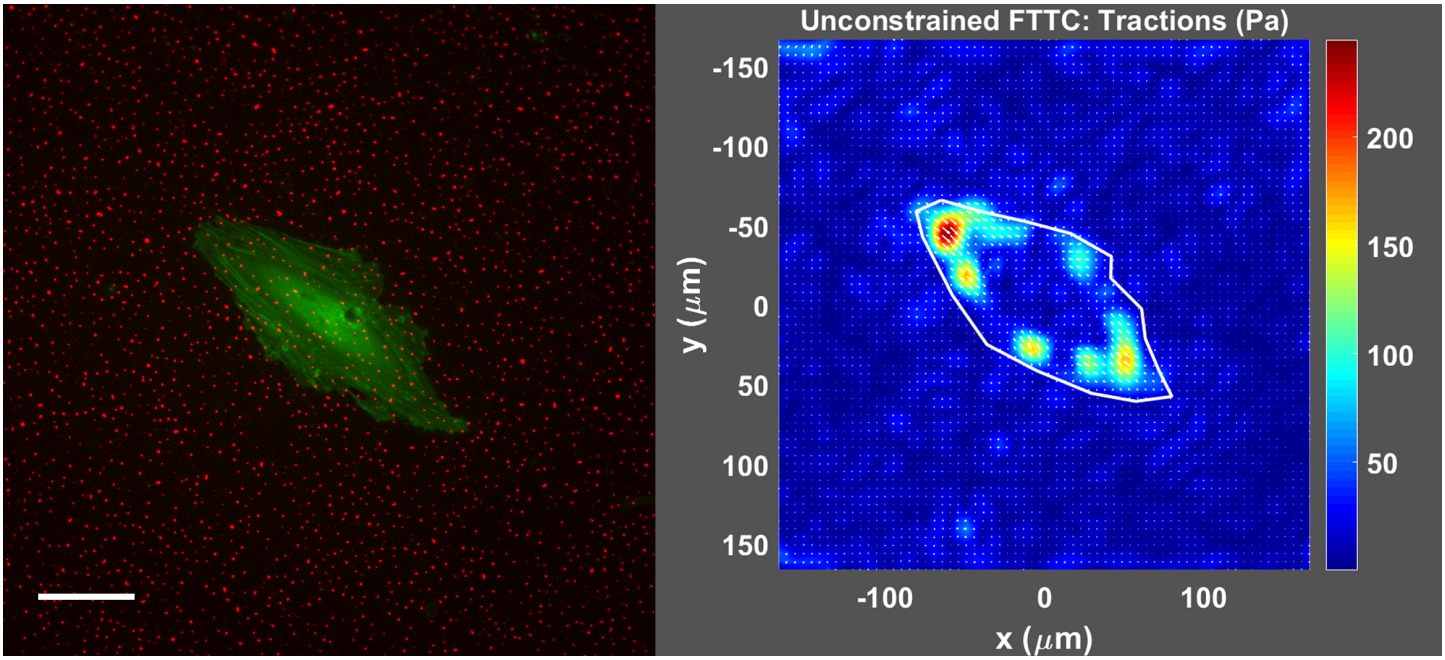
Supplementary Fig 5. Gelation curves for curing PDMS samples.
Uncured PDMS samples were mixed and loaded onto the rheometer, and shear rheology was performed during the curing process at 100C. These data reveal that the samples have largely cured after 25 minutes, and are completely cured within three hours.

additional crosslinker %	Average Young's Modulus (kPa)	Standard Deviation (kPa)	n (number of points)
0%	0.9894	0.0797	10
0.15%	2.106	0.4768	10
0.36%	4.934	0.1133	10
0.72%	19.22	0.6206	10

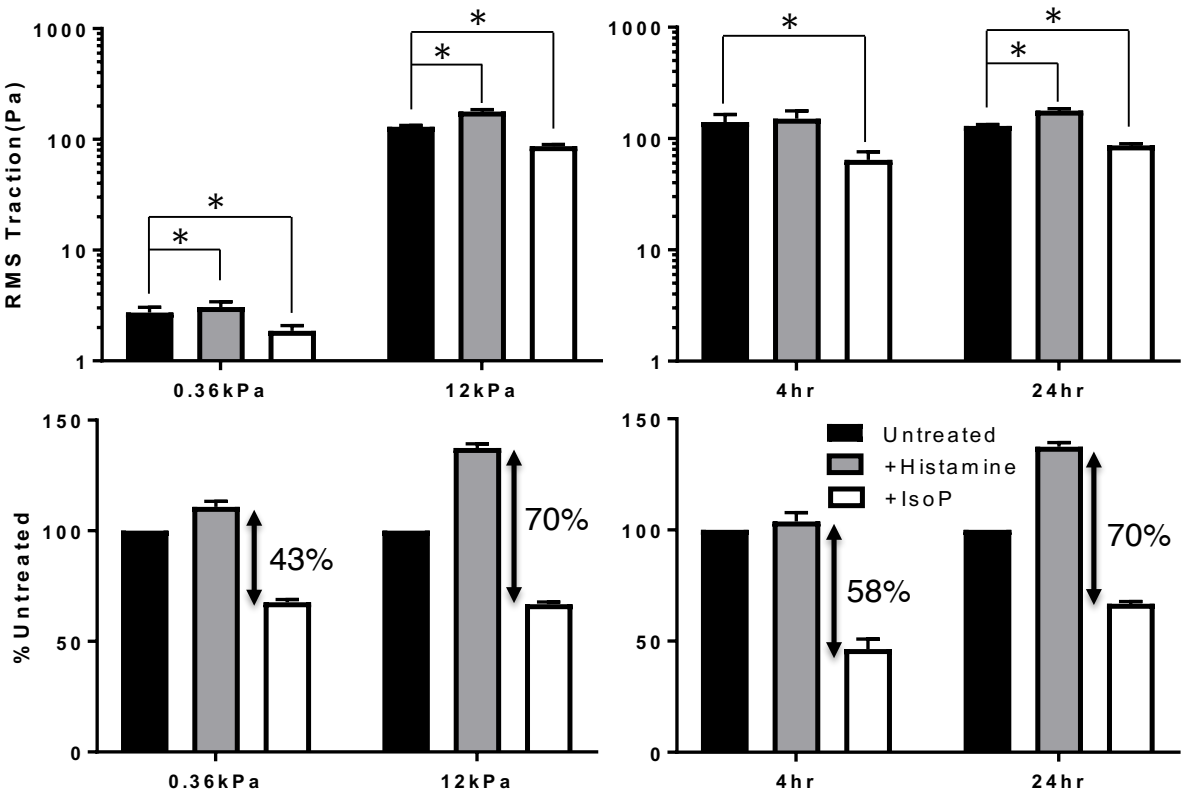
Supplementary Fig 6. Spatial heterogeneity of modulus as measured with AFM.
To examine the uniformity of modulus on PDMS substrates, we created TFM substrates with several different formulations, and then used an AFM (JPK Nanowizard 3, JPK Berlin Germany) to measure 10 independent positions, spaced at least 100 μm from each other. These data demonstrate that the substrates display little variability and are uniform. Deviation in absolute moduli values reported here from those measured in shear rheology are attributed to challenges in contact mechanics modeling (Notbohm et al 2011, Pham et al 2017)



Supplementary Fig 7. Long-term elastic measurements of PDMS substrates.
To assess any long-term changes in the elastic modulus of prepared PDMS samples, we used a custom-built microindenter as described previously (Zhang 2015). In brief, the instrument uses a load cell (S256, Strain Measurement Devices, CT USA) which is moved by a 3-axis micromanipulator (Sutter MP285, Olympus ON Canada). The movement of the micromanipulator and the load-cell data collection are run by custom Matlab software, and the load/strain curve is calibrated using an analytical balance.



Supplementary Fig 8. Single cell TFM PDMS substrates.
To provide an example for comparison with other single-cell traction force studies, we examined HEK293 cells on a 3.6 kPa PDMS substrate. The left panel depicts a HEK293 cell transfected with EGFP-LifeAct (Michael Davidson, Addgene plasmid #54610) on a PDMS substrate with red fiduciary beads. The right panel shows the calculated unconstrained traction stresses generated by the cell. This reveals that single cells display traction stress profiles similar to those previously shown in PAA studies, and the maximum traction stress is observed to be approximately 200 Pa. Scale bar in left panel is 50 μm.



Supplementary Fig 9. The scope of ASM relaxation. Bar graphs reflect the difference in contractility, as reported by RMS traction in Pa (top) and % of untreated (bottom) in untreated, with histamine, or with isoproterenol, with the left panel depicting changes on two different substrate moduli, and the right panel quantifying changes with two different serum deprivation times. In the bottom panels, also shown is the difference between contraction with 10μM histamine (gray bar) and relaxation with additional 1μM isoproterenol (white bar). This difference is greater on 12 kPa than 0.4kPa stiff substrate (n=22-24 separate wells per stiffness group), and, only marginally affected by serum deprivation (n=8 and 24 wells for 4 hr and 24 hr deprivation, respectively). Plotted data are mean±std.error. Data sets were compared using the Wilcoxon matched-pairs signed rank test and differences are reported as * for $p<0.05$.

Detailed methods:

Mechanical testing of PDMS mixtures:

Shear Rheology: Measurements of PDMS moduli as functions of formulation, curing time, frequency, and strain were performed with a stress-controlled shear rheometer using a 25mm parallel plate geometry (Anton Paar, MCR 302, Montreal Canada).

Microindentation measurements: To assess any long-term changes in the elastic modulus of prepared PDMS samples, we used a custom-built microindenter as described previously (Zhang 2015). In brief, the instrument uses a load cell (S256, Strain Measurement Devices, CT USA) which is moved by a 3-axis micromanipulator (Sutter MP285, Olympus ON Canada). The movement of the micromanipulator and the load-cell data collection are run by custom Matlab software, and the load/strain curve is calibrated using an analytical balance.

Cell culture media:
Primary human airway smooth muscle cell culture and measurements were performed either in serum containing medium comprising DMEM/F12 supplemented with 10% FBS (35-010-CV, Corning Life Sciences, Tewksbury, MA) and 1% penicillin-streptomycin (P0781; Sigma-Aldrich, St. Louis, MO), 1% L-glutamine (25030149; Thermo-Fisher Scientific, Waltham, MA), 1% amphotericin B (15290018; Thermo-Fisher Scientific, Waltham, MA), 0.17% 1M CaCl2*2H20, and 1.2% 1M NaOH or in serum deprived medium comprising F12 with the above supplements except for replacement of FBS with 1% insulin-transferrin-selenium supplement (25-800-CR; Corning Life Sciences, Tewksbury, MA).

ARPE-19 (retinal pigment epithelium) cells were obtained from American Type Culture Collection and cultured in DMEM/F12 medium supplemented with 1% penicillin-streptomycin, 1% L-glutamine and 10% FBS. For monolayer preparation, ARPE-19 (1.7×10^5 cells/cm^2) were seeded on collagen-coated soft PDMS substrates in low serum media for 2-12 hours before the experiment.

Preparation of NuSil substrates in custom 96-well plates.
As a material for our substrates, we used a very compliant commercial PDMS (NuSil® 8100, NuSil Silicone Technologies, Carpinteria, CA). When prepared as per manufacturer instructions, i.e. 1:1 component mixing, we measured these silicone substrates to have Young's moduli of approximately of 0.36 +/- 0.043 kPa, as determined with shear rheology (Anton Paar MCR302, Montreal Canada). From this baseline, we increased the modulus of the PDMS by including a small amount of additional crosslinker (Sylgard 184 curing agent, Dow Corning, Midland, MI), allowing us to create substrates with higher Young's moduli as desired, and we tested up to 73.32+/-2.96 kPa with 1% additional crosslinker (Fig. 1F&G), spanning two orders of magnitude in compliance.

To prepare the multiwell plates, we selected custom cut glass slides (109.6 mm x 78mm x 1mm, Hausser Scientific, Horsham PA) so that our plates are compatible with existing multiwell tools (Fig. 1A-E). Optionally, the glass surface can be coated with fluorescent bead markers for de-drifting images during an experiment (Fig 1A). To create the deformable layer with a particular modulus, PDMS was mixed to the by combining mixing NuSil 8100 or as per manufacturer instructions, and then adding additional Sylgard 184 crosslinking agent and slowly mixing on a rotator for approximately 30 minutes to achieve the desired elastic modulus (Fig 1F&G).

Next, we spin coat the uncured PDMS mixture, prepared as described above, on the glass slide (Fig 1B). To ease loading and centering the glass slides onto the spin coater, we mark the XY center of the glass slide with a solvent resistant marker, and align that mark with the vacuum chuck on the spin coater (Laurell WS-650Mz-23, Laurell Technologies, USA). We then add 3-4 ml of uncured PDMS to cover the substrate, and use a pipette tip to coarsely spread it from the center to the edges. The slide is then spun with the following protocol: 1) 200 rpm 1min, acceleration 50rpm/s; 2) 300 rpm for 1 min, acceleration 200 rpm/s; 3) deceleration to stop 50 rpm/s. This protocol produces a layer approximately 100 microns thick. The slide with uncured PDMS is then removed from the chuck and placed on a solid surface (i.e. not a wire rack) in a preheated 100^0C oven for 90 minutes. While it may be cured longer, it should not be done hotter than 100^0C as this may cause a degradation and reduction in stiffness. Care should be taken that the oven surface is precisely level to ensure that the PDMS layer has a uniform thickness.

To form the fiduciary bead layer, ~2ml of uncured PDMS mixture from the previous step is mixed with a stock bead solution. Our fluorescent beads are synthesized with a PMMA core and a PDMS shell based on work published previously [14] (Suppl. Fig. 1) and had a final diameter of ~300nm. Their complete synthesis is described in detail below. Beads are stored in hexane, and prior to addition to the uncured PDMS, they are mixed for 30 minutes at approximately 20% volume fraction to the uncured PDMS. The actual bead concentration depends on the desired final bead density, which for our experiments is ~0.05- 0.2 beads 1 μm^2, and the stock bead concentrations, which is approximately $9.2x10^{11}$ beads per ml. To produce a thin layer of bead-embedded PDMS, the uncured mixture is spun on the slide with the following protocol: 1) 500 rpm for 1 minute, acceleration 100 rpm/s; 2) 5000 rpm for 20 sec, acceleration 200 rpm/s; 3) Deceleration to stop at 100 rpm/s. The slide is then placed back in the 100^0C oven for 1 hour to cure the top bead layer. This protocol produces a bead layer approximately 1 micron thick, with an approximate density of 0.05-0.2 beads per 1 μm^2. The elastic substrate and bead layers are now complete (Fig 1C).

To create a multiwell dish from the single piece of PDMS-coated glass, we then bonded a 96-well insert (2572; Corning, Tewksbury, MA) on top of the bead layer, allowing each compartment from the insert to function as an individual well on the compliant PDMS substrate, forming the complete multiwell dish (Fig 1D).
To facilitate attachment, we apply a thin coat of uncured PDMS (Sylgard 184, Dow Corning, USA) mixed per manufacturer instructions of 10:1 polymer base to crosslinking agent to the insert bottom, invert, lay the slide and deformable PDMS substrate upside-down onto the insert, and incubate the insert together with the substrate at 65°C for one hour. Inversion is important as it prevents uncured PDMS from flowing down and covering the substrate surface.

Substrate functionalization and ligand binding
The wells are washed and surface-activated using the cross-linker, Sulfo-SANPAH (Proteochem, Hurricane, UT). Briefly, Sulfo-SANPAH is dissolved in 0.1M HEPES buffer at a final concentration of 0.4mM and exposed to UV (Wavelength=254nm, Power=40W, Philips, USA) for 6 minutes. Upon activation, the SANPAH will visibly darken. The SANPAH is then removed from the wells by washing twice with phosphate-buffered saline (PBS). Finally, the wells are ligated with 0.05 mg/ml of collagen

type 1 solution in PBS (5005; Advanced Biomatrix, Carlsbad, CA) overnight at 4°C in preparation for measurements, and sterilized by UV exposure in a Biosafety cabinet in preparation for measurements. While our experiments have used collagen, this process should also be successful with other ligands such as fibronectin.

Fluorescent Bead Synthesis

Commonly available fluorescent polystyrene spheres do not readily disperse in non-polar fluids such as uncured PDMS, requiring in house synthesis of compatible spheres.

The following procedure for fluorescent particle synthesis is based on the method described by Klein et. al.

Materials used for PDMS-coated fluorescent bead synthesis

1,1'-Dioctadecyl-3,3,3',3'-Tetramethylindocarbocyanine Perchlorate (DiI)	1-5 mg	Sigma-Aldrich 468495-100MG
Methyl methacrylate, 99%, contains ≤30 ppm MEHQ as inhibitor	15 mL	Sigma-Aldrich M55909-500ML
Inhibitor Remover		Sigma-Aldrich 306312-1EA
Polydimethylsiloxane stabilizer (25,000g/mol) *Methacryloxypropyl-terminated	0.5 g	Gelest DMS-R31 (25,000g/mol)
2,2'-azobisisobutyronitrile (AIBN 98%) (=2,2'-Azobis(2-methylpropionitrile)	0.15g	Sigma-Aldrich 441090-25G
Hexane Anhydrous (for reaction)	100 mL	Sigma-Aldrich 296989-1L
Hexane, mixture of isomers	~ 200 mL	Sigma-Aldrich 227064-1L

0.5 g of PDMS stabilizer and 5 mg of fluorophore were dissolved in 100 mL of anhydrous hexane in 250 mL two-neck flask. The necks were prepared with a water cooled reflux condenser, a rubber septum with a nitrogen inlet needle and an outlet needle, and a rubber septum for adding monomer solution via a syringe, respectively. The flask was placed in the mineral oil bath at 75 °C and purged with nitrogen gas for 1 hour. To ensure uniform heating, a small magnetic stir bar was placed in the reaction flask. 0.100g of AIBN was dissolved in 6 g of methyl methacrylate and purged with nitrogen for 1 hour. **Methyl methacrylate was flushed through prepacked column to remove inhibitors before use*. After purging both hexanes and the initiator with monomers, the reaction was initiated by adding a monomer and initiator mix solution to the three-neck flask. The initially transparent solution became cloudy as nuclei for the particle growth were formed and tuned milky as they continue to grow. After 3 hours, the reaction flask was placed in an ice water bath after 3 hours to terminate the reaction. The solution was vacuum-filtered through a coarse filter paper. The filtrate was then centrifuged to remove unreacted stabilizer, and re-suspended in fresh hexane. To facilitate the redispersion, the particles in hexane were placed in an ultrasonic bath - the final hexane volume to be added depends on the product yield and desired bead concentration.

Synthesized beads were found to be 300-400 nm in diameter as measured by SEM (Supplemental Figure 1b).

Bead Addition to Uncured PDMS

Prior to mixing, the bead solution was sonicated for 15-30 minutes and vortexed for ~60 seconds to prevent beads from aggregating. Each corresponding PDMS mixtures left from the previous step (NuSil GEL-8100 mixtures) was mixed with fluorescent beads (suspended in hexane) in 10:1 (equal

PDMS mix : bead solution) ratio (the amount of bead solution to be added depends on the concentration of the bead solution) by weight, and the whole mixture was vortexed for 1-2 minutes - **Beads can be filtered through 5 µm pores using syringe filters right before adding to PDMS mixture to further avoid bead clumping – this is especially important when using beads in high density.*

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