ONLINE **METHODS**

Hydrogel synthesis, cell encapsulation

Polyethylene glycol diacrylate (PEGDA) was synthesized from polyethylene glycol (PEG, MW 3400, Sigma) and then modified with the collagenase-sensitive peptide CGPQGIWGQGCR to make degradable photoactive hydrogel precursors as described previously[10]. Polyethylene glycol diacrylamide (PEGDAAm, MW 3400) was synthesized from PEG by forming the dimesylate, then the diamine, and finally the diacrylamide as described previously[17]. PEGDAAm was then reacted with the collagenase-sensitive peptide in sodium borate (100 mM, pH 9.0) until the product polydispersity matched that for PEGDA-peptide precursors. For encapsulation, NIH 3T3 cells were resuspended to a final concentration of 60,000 cells/ml in a 10 or 11% w/v solution of degradable PEGDA-peptide macromer in PBS containing 1 μmol/ml acrylate-PEG-CGRGDS, 0.5 mg/ml Irgacure 2959 (I2959, Ciba) and two types of fluorescent beads (0.2 μm diameter, non-functionalized, yellow green dyed, Polysciences, and 0.2 μm diameter, non-functionalized, suncoast yellow dyed, Bangs Labs) at approximately $3.75x10^{10}$ beads/ml each. Note that the pore size of the PEG gels is an order of magnitude smaller than the diameter of the beads used in this study[18]. Therefore, the beads are physically encapsulated within the hydrogel and do not diffuse. Bovine pulmonary artery smooth muscle cells, human mesenchymal stem cells and Lewis lung carcinoma cells were encapsulated in a 7% w/w solution of PEGDAAm-peptide macromer in PBS containing 5 μmol/ml acrylate-PEG-CGRGDS, 5 μmol/ml acrylate-PEG-CGRGES, 0.5 mg/ml Irgacure 2959 (I2959, Ciba) and two types of fluorescent beads. 20 μl of cell-laden prepolymer solution was pipetted onto coverslips (#0 thickness, Fisher Scientific) that were functionalized with 3-(trimethoxysilyl)propyl methacrylate (Sigma) as per the manufacturer's instructions. The solution was contained in annular molds made from poly(dimethyl siloxane) (PDMS, Dow corning) and exposed to 200 mW/cm^2 (measured for 365 nm) UV light from an Omnicure S2000 (320-500 nm, EXFO) for a total of 3,000 mJ. After removing the PDMS mold,

polymerized hydrogels, which now formed a cylindrical disc approximately 4 mm diameter and 500 μm tall that was covalently linked to the coverslip along the bottom surface, were immersed in cell culture media and incubated under standard growth conditions (37 ºC, 5% CO_2) for 72 hrs.

Microscopy, image segmentation, finite element mesh generation, and computational resources

Encapsulated cells were imaged with a 40x, 1.1 NA, water immersion objective (LD C-Apochromat, Carl Zeiss Microimaging Inc.) attached to an Olympus IX71 inverted microscope equipped with a CSU10 spinning disc confocal scan head (Yokogawa Electric Corporation), live cell incubator (Pathology Devices) and an ImagEM 16-bit EMCCD camera (Hamamatsu Photonics). A 147 x 147 x 200 μm volume was imaged around each cell, which corresponded to voxel dimensions of 0.2841 x 0.2841 x 0.8 μm in the horizontal and axial planes, respectively. After the stressed image was acquired, the cells were treated with 0.5% Sodium dodecyl sulfate (SDS) detergent (JT Baker), re-equilibrated for 45 min, and then re-imaged to acquire a reference image of the non-stressed hydrogel. This detergent was chosen so as to completely denature all cellular proteins, although in practice, more mild detergents or specific inhibitors of cytoskeletal contractility could be used as well. Timelapse datasets were acquired at 30 minute intervals and 1 μm spacing in the axial plane. This temporal and spatial resolution was chosen so as to increase the image acquisition speed, (approximately 3 minutes of exposure per volume per cell) and to reduce phototoxicity. Images were saved in multipage TIFF format, imported into Amira (Visage Imaging), and manually segmented to identify the cell and the surrounding hydrogel. A 2D surface mesh of the cell was generated from the segmented image, simplified to the desired number of elements, and smoothed using built in functions. This mesh was then imported into Hypermesh (Altair) as a stereolithography file. To approximate an infinite medium, we generated a 400 μm cube centered on the cell, seeded the edges with 9 nodes (element size of 50 μm), and

generated a 2D quadrilateral surface mesh. Using these two surface meshes as a template, we then generated a 3D tetrahedral mesh (4 node linear tetrahedron elements "C3D4" in Abaqus) of the enclosed volume. These meshes were then imported into Abaqus (Dassault Systèmes) for finite element analysis with the bottom surface of the cube fixed as a boundary constraint. Validity of the finite element approximation of an infinite medium was verified by fixing the top surface of the cube as an additional boundary constraint and showed no substantial difference in the recovered tractions. Unless otherwise mentioned, for all measurements, the cells were discretized using 2,000 linear elements. The center of mass of the cell was computed using the area weighted centroids of each element on the 2D surface mesh of the cell. Renderings of cellular tractions were computed in Tecplot 360 (Tecplot Inc.), and contour plots were scaled such that approximately 1% of all elements on the cell were saturated. The deviation of the tractions fields from static equilibrium was assessed by summing the projection of the forces (tractions multiplied by facet area) on each facet of the cell along each Cartesian direction. All data presented in the manuscript were calculated using a Dell Precision T7400 workstation equipped with dual quad core Intel Xeon processors and 16 GB of RAM (**Supplementary Note 3**).

Mechanical characterization of hydrogel substrates

The shear modulus of swollen gels was obtained using an AR 2000 oscillating rheometer on a temperature controlled Peltier plate at 37ºC and a 20 mm stainless steel plate with solvent trap geometry (TA Instruments). Cylindrical gel samples were created from 125 μL of identical precursor solution to that used for traction measurements, covalently linked to 3-(trimethoxysilyl)propyl methacrylate (Sigma) treated glass microscope slides and then swollen in growth media at 37 ºC and 5% CO_2 for 72 hours. Immediately prior to testing, the slides were removed from media and carefully blotted dry with laboratory wipes. The heights and diameter of the swollen gels were measured with calipers and were typically ~0.5 mm thick and ~19 mm

diameter. To prevent slipping, 400 grit, wet/dry sandpaper was sectioned to fully cover the geometry and attached with double-stick tape. The head was lowered to a gap corresponding to approximately 0.2 N of normal force. Three consecutive controlled oscillatory strain sweeps were performed from 0.1% to 50% radial strain with 30 linearly spaced measurements at 0.25 Hz (**Supplementary Fig. 1a**). Following the strain sweeps, frequency sweeps were performed from 0.1 to 10 Hz, 10 measurements per decade on a log scale, at 1% controlled strain (**Supplementary Fig. 1b**). These data were acquired for six independent samples from multiple experiments. The data from the strain sweeps were averaged to yield a shear modulus of 196 ± 66, 328 ± 76 and 267 ± 34 Pa (± s.d.) for 10% w/v and 11% w/v PEGDA and 7% w/w PEGDAam hydrogels respectively. These values were used to calculate Young's moduli of 585 ± 196, 978 ± 228 and 796 ± 102 Pa (± s.d., assuming a Poisson's ratio of 0.49) (**Supplementary Fig. 1c**).

To characterize the validity of a homogeneous material assumption, cell-laden degradable matrices were prepared as described above, cultured for 72 hrs, labeled with Cell Tracker Red (Invitrogen) as per the manufacture's instructions and then treated with 0.5% SDS. Non-degradable matrices were prepared in an identical manner using PEGDA (MW 6000, Sigma) in absence of degradable peptides and measured after 48 hrs. These matrices were imaged before and after applying a uniform compression of approximately 5% strain using a microscope mounted micromanipulator pressed against a coverslip laid over the gel (**Supplementary Fig. 5a**) and bead displacements throughout the volume were computed between the unstressed and compressed images. A 3D tetrahedral mesh was constructed in the vicinity of a cell as described above, and nodal displacements of the boundary nodes were interpolated from the experimentally observed bead displacements. The forward finite element solution was then solved for static equilibrium under homogeneous or heterogeneous (i.e., weakening near the cell) material

assumptions, and predicted bead displacements within the simulated volume were compared to experimental observations.

Measurement of uncertainties in the displacement field and discretized cell surface and validation using simulated data

The errors of the displacement measurements were measured from bead displacements before and after treatment with 0.5% SDS in six separate regions of the gel that contained no cells from multiple experiments. These six datasets were used to accurately reflect our experimental bead distribution and displacement resolution in all numerical simulations. To determine the uncertainty in our discretization of the cell surface, two separate surfaces were generated (starting with raw confocal data, proceeding through manual image segmentation and finally to surface reconstruction) of seven cells from multiple experiments. The differences between the two surface meshes for each cell were computed by finding the minimal distance between the nodes of one surface and the closest plane of the alternate surface. To model the cell in our numerical analysis, we used a simplified geometry of a 50 μm diameter sphere meshed using 2,000 triangular elements and generated a 3D tetrahedral mesh as described above. We first tested our ability to recover a uniform traction of 183 Pa oriented in the outward normal direction on each facet. The forward solution for this loading was solved and measurements of bead displacements were computed at the centroid locations of each bead for each of the six fields measured above, thus giving six separate datasets of simulated bead displacements. The tractions were recovered, as described in **Supplementary Note 2**, for each of these simulated displacement fields and compared to the applied loading, thus giving a measurement of the mean error and deviation of the recovered tractions. To simulate the effect of bead displacement noise on the recovered tractions, the experimentally measured displacements from each of the six noise fields was superimposed on the displacement due to the simulated loadings, and the tractions recomputed. To simulate the effect of surface discretization error, for each node of our spherical surface mesh,

we randomly sampled measurements of the surface discretization error (computed as described above). As the most accurate discretization can be expected to lie in between the two experimentally generated surfaces, the spatial coordinates of each node from our spherical mesh were shifted either in the inward or outward normal direction (chosen randomly) by one half the magnitude of the experimentally measured noise. The restriction of the noise to the normal directions was necessary to avoid element intersections. This procedure was repeated to generate six independent samples of the surface discretization noise (ie we generated six independent "noisy" spherical surfaces) on which to recover tractions.

To test the spatial resolution of the recovery, we applied oscillatory loadings normal to the cell surface. The magnitudes of these loadings varied sinusoidally with respect to the spherical coordinate θ and ranged from $\pm$ 183, $\pm$ 743 and $\pm$ 1467 Pa. The frequency of these loadings was then increased progressively from two to ten oscillations per 360 degrees, and six separate measurements of the recovered tractions were obtained for each loading. The characteristic length of the simulated loadings was calculated as the average period of oscillation on the surface of the sphere. The relative error between the simulated and recovered loadings was computed by summing over all elements as:

$$\text{Relative Error} = \frac{\left|\mathbf{T}_{\text{recovered}} - \mathbf{T}_{\text{simulated}}\right|^2}{\left|\mathbf{T}_{\text{simulated}}\right|^2} \qquad (1)$$

where $\mathbf{T}_{\text{recovered}}$ and $\mathbf{T}_{\text{simulated}}$ are n x 3 matrices containing the recovered and simulated tractions respectively, n is the number of facets used to discretize the cell, and each row contains the Cartesian components of the traction computed at a given facet. In this manner, a value of 0 indicates perfectly recovered tractions, 1 indicates that the errors are of equal magnitude to the

simulated loadings, and a value of greater than 1 indicates that the errors are larger than the simulated loadings. For cases in which this value lies between 0 and 1, it is possible to express a percent traction recovery as (1-Relative Error) x 100.

Cell culture

NIH 3T3 cells obtained from ATCC were cultured in high glucose DMEM containing 10% bovine serum, 2mM L-glutamine, 100 units/ml penicillin, and 100 mg/ml streptomycin (all from Invitrogen). Cell culture media was changed every 3 days. EGFP-lentiviral infection was carried out as described previously[19]. Human mesenchymal stem cells from Lonza and Lewis lung carcinoma (LLC) cells from ATCC were cultured in growth medium (low glucose DMEM containing 10% fetal bovine serum, 0.3 mg/ml glutamine, 100 units/ml streptomycin and 100 units/ml penicillin). Immediately upon encapsulation of single LLC cells, the medium was supplemented with 50 ng/ml of recombinant human hepatocyte growth factor (R & D systems) to drive proliferation and spheroid formation.