Electronic Supplementary Material (ESI) for Soft Matter.
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Supplementary Information

1. Additional details of the experimental setup

a. Cell Culture

We obtain GFP-labeled MDA-MB-231 human breast carcinoma cells (GenTarget) and maintain them according to the manufacturer's instructions. Briefly, growth media is prepared using Dulbecco's Modified Eagle Medium (Gibco) supplemented with 10% Fetal Bovine Serum (Gibco), 1% 100x Penicillin-Streptomycin (Gibco), and 0.1 mM Non-Essential Amino Acid (ThermoFisher). Cells are kept in culture flasks in a tissue culture incubator at 37°C and 5% CO_2.

b. Preparing Tumor Diskoids with DIGME technique

We prepare collagen gels from high-concentration rat tail collagen 1 in acetic acid (Corning; 9 - 11 mg/mL). We dilute the collagen with DMEM growth medium, 10x PBS, and 0.1M NaOH to a final concentration of 2.0 mg/mL with pH 7.4. We mix and keep all solutions at 4°C prior to the gelation process at 37°C. We employ Diskoid in Geometrically Micropatterned ECM (DIGME) to create 3D cell diskoids in collagen gel [1]. As shown in Fig. S1 We place the neutralized collagen solution in a gridded dish (ibidi μ-dish Grid-500), where a stainless steel cylinder creates a well inside the collagen solution. We allow the collagen solution to gel at 37°C for 45 minutes to fully polymerize before carefully removing the stainless steel cylinder, leaving a molded well inside the gel. We suspend GFP-labeled MDA-MB-231 human breast carcinoma cells at a high density in a 2.0 mg/mL neutralized collagen solution and fill the molded well, creating a diskoid. We allow this diskoid-collagen sample to gel and come to equilibrium for 3 hours in a tissue culture incubator at 37°C and 5 % CO_2.

c. Holographic Optical Tweezers

We use a holographic optical tweezers system with a 1,064 nm trapping laser and a 100x, 1.6 NA, oil-immersion objective for micromechanical measurements [2, 3]. We take video microscopy measurements where the sample is illuminated by a red LED and recorded at 20 fps, while pulsing the displaced trap at 1 Hz by an acousto-optic modulator (AOM). We determine the location of the particle using a tracking algorithm that analyzes the radial symmetry of the particle's image from the video microscopy images [4]. We then calculate the displacements of the particle from the particle trajectories using MATLAB. We use the Equipartition method to determine the stiffness of the trap. We trap a 3 μm particle in water, and due to thermal fluctuations, we observe the Brownian motion of the particle. We record a video of the trapped particle at 200 frames per second for 30 seconds, for a total of 6000 frames. According to the Equipartition theorem, the thermal energy is equal to the average potential energy of the particle for each degree of freedom in motion. We treat the optical

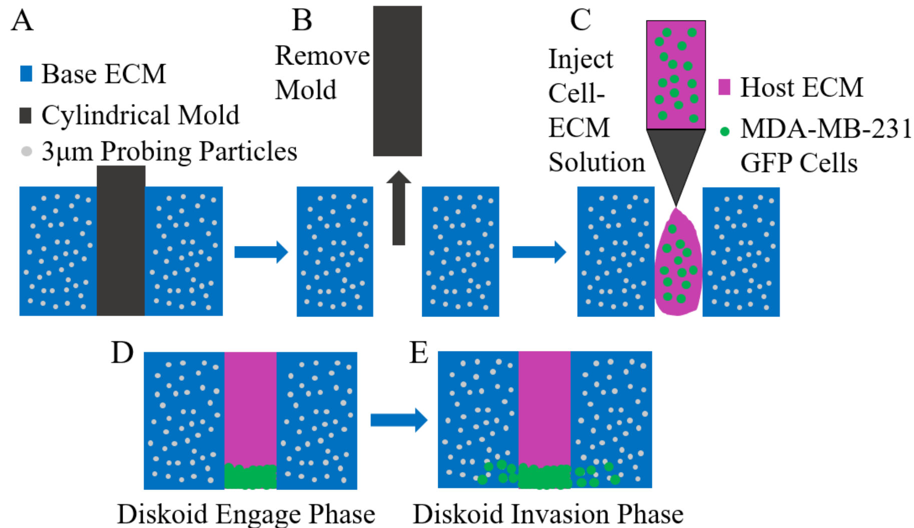

Figure S1: Side-view schematics of DIGME. (A) Base ECM is allowed to gel with a cylindrical mold in the center. (B) The cylindrical mold is removed from the Base ECM after the gelling process, leaving a hole in the shape of the mold in the Base ECM. (C) Cell-ECM solution is injected into the hole created by the mold. (D) The sample is allowed to gel for 3 hours to allow the Cell-ECM solution to gel and engage with the Base ECM. The cells in the Cell-ECM solution sink to the bottom of the hole and form a solid diskoid. (E) The diskoid invades into the Base ECM over the course of 24 hours.

trap as a harmonic potential well, and we get $\frac{1}{2}k_bT = \frac{1}{2}k_{trap}\langle x^2\rangle$, where k_b is the Boltzmann constant, T is the absolute temperature of the water, and $\langle x^2\rangle$ is the variance, or square of the standard deviation σ_x, of the spatial fluctuations ($\langle x\rangle = 0$). We fit the Brownian motion with a Gaussian distribution, allowing us to find the standard deviation, and therefore calculate the stiffness of our trap to be $k_{trap} = 50\frac{pN}{\mu m}$.

2. Effects of Δd_{dir} on the simulated directional compliance

In the main paper, we reported J_{dir} computed by displacing the micro-bead embedded in the ECM networks by $\Delta d_{dir} = 1\ \mu m$. Here we show the results are not sensitive to the choice of Δd_{dir} by computing J_{dir} with $\Delta d_{dir} = 0.1\ \mu m$ and $0.01\ \mu m$. In particular, we compare J_{dir} computed using the aforementioned three different bead displacements Δd_{dir} in Fig. S5, which are statistically equivalent.

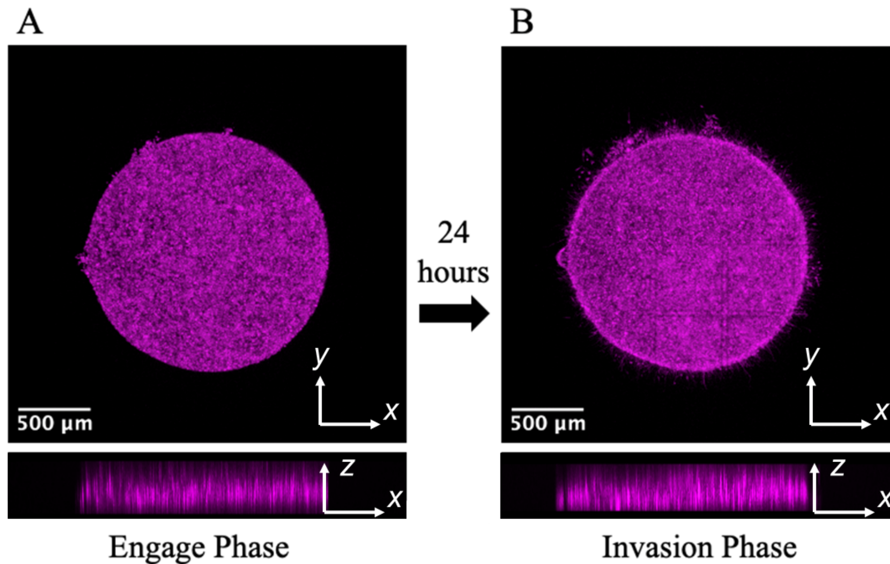

Figure S2: 4x confocal images of a diskoid created using DIGME. The side-view of the diskoid is also imaged to show the height of the cylindrical diskoid on the same scale. (A) The Engage Phase of the diskoid is imaged surrounded by Base ECM. (B) The Invasion Phase of the diskoid is imaged with cells now invading into the surrounding Base ECM. The invasion depth is about 100μm.

3. Additional experimental systems

Our computational model predicts, consistent with previous *in vivo* and *in vitro* observations, that a solid tumor stiffens its surrounding ECM. To further test the predictions, we have measured the micromechanics of cell-free collagen matrix prepared in the same conditions. Fig. S6 shows the average compliance of the cell-free ECM in comparison to the tumor-remodeled ECM reported in the main text (Fig. 7). Consistent with the model prediction, cell-free ECM demonstrate greater micromechanical compliance.

Our computational model also predicts that MMP-associated invasion soften the tumor-associated ECM. To further test this prediction, we have created tumor diskoids with MCF-7 cells. MCF-7 cells generate contractile forces, but are non-invasive in 3D ECM, and do not express MMPs. We therefore expect no change of micromechanics from day 0 to day 1, in contrast to MDA-MB-231 tumor models. This is indeed the case as shown in Fig. S7.

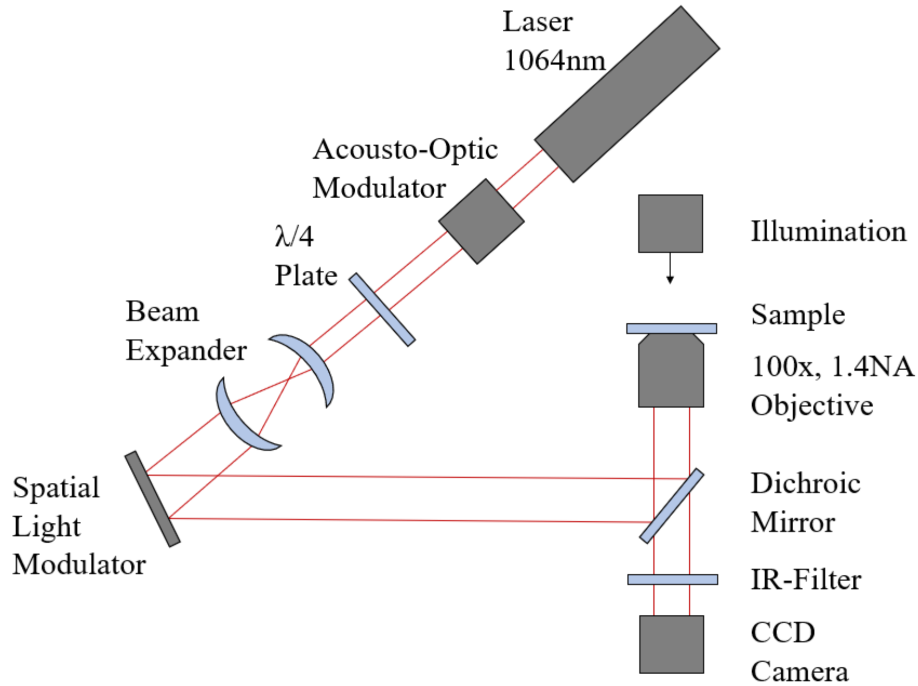

Figure S3: Schematic of the light path of the Holographic Optical Tweezers. The 1064nm infrared laser first passes through an acousto-optic modulator that we use to pulse the laser on and off at a 1Hz frequency. The beam then goes through a quarter-wave plate and beam expander to create a large, circularly polarized beam to optimize the effectiveness of the spatial light modulator. The spatial light modulator creates a hologram that the beam interacts with to create the optical trap in the precise position wanted in the sample. The beam then travels through the dichroic mirror of the microscope to split the beam. Part of the beam travels through an infrared filter into the CCD camera for viewing, while the other part of the beam travels through the 100x, 1.4NA objective lens, creating an optical trap inside the sample. The sample is illuminated from above by a red LED.

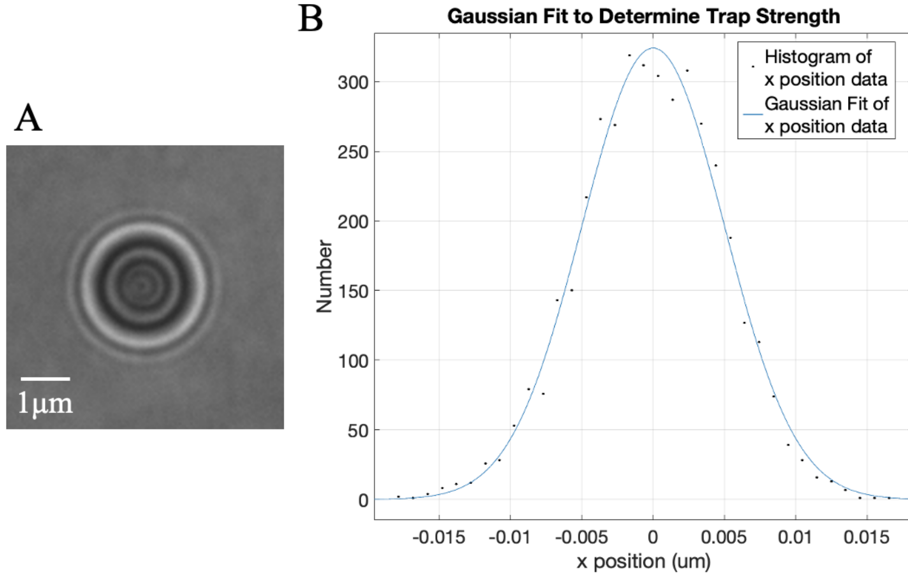

Figure S4: An example of determining the strength of the holographic optical tweezers. (A) Microscope image of a 3μm polystyrene bead is trapped by the holographic optical tweezers. The bead is illuminated by a semi-coherent light source that generates concentric diffraction patterns. (B) The position data for the trapped bead fits a Gaussian distribution and allows us to determine the stiffness of our trap. Position data is made into a histogram with 10nm bins.

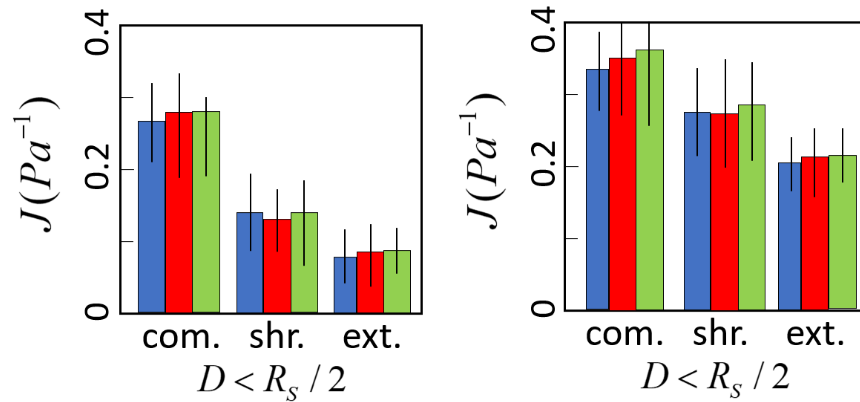

Figure S5: Comparison of average compliance J_{dir} resulted from a contracting tumor diskoid in ECM along different directions using three different values of $\Delta d_{dir} = 1\ \mu m$ (blue), $0.1\ \mu m$ (red) and $0.01\ \mu m$ (green), in the region with $D < R_s/2$ (A) and the region with $D > R_s/2$ (B).

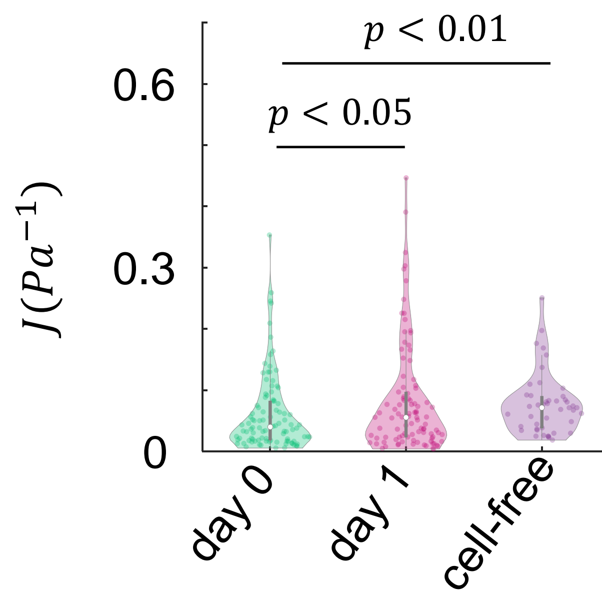

Figure S6: Average micromechanical compliance of a tumor remodeled ECM and a cell-free collagen ECM. The violin plots show the distribution functions. Statistical comparison are done with one way ANOVA. For cell remodeled ECM, approximately 100 probing particles are sampled at each day. For cell-free ECM, 45 particles are sampled.

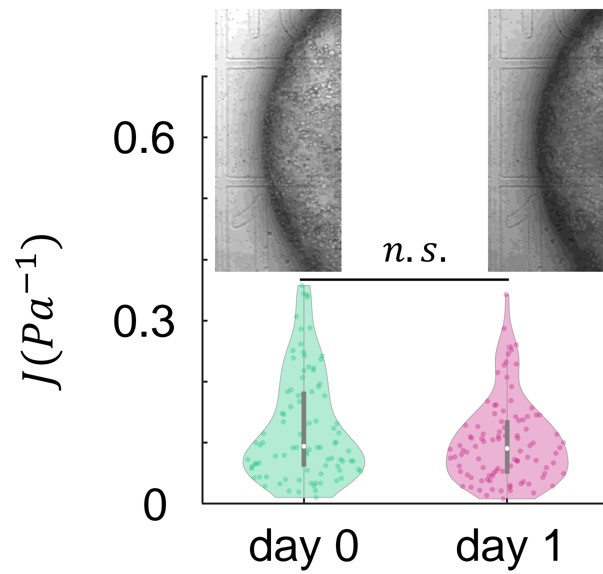

Figure S7: Average micromechanical compliance of ECM remodeled by non-invasive tumor diskoids consists of MCF-7 cells. The violin plots show the distribution functions. Statistical comparison are done with one way ANOVA. Approximately 50 probing particles are sampled at each day. Insets: bright field images of an MCF-7 diskoid at day 0 and day 1.

References

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