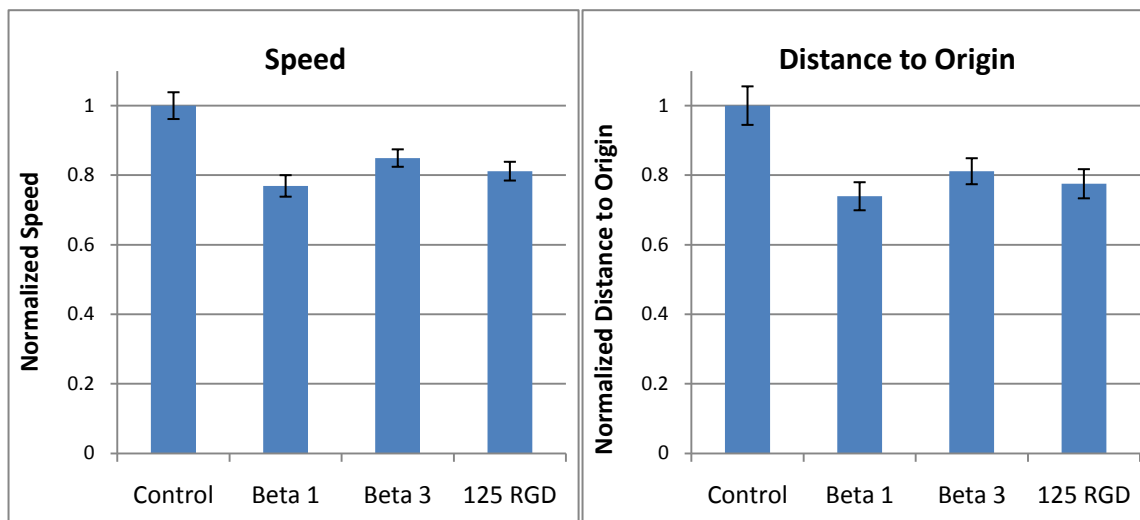Supplemental Data for:

A synthetic strategy for mimicking the extracellular matrix provides new insight about tumor cell migration

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Supplement al Figure 1. **Effects of integrin blocking on migration of HT-1080s in thiol-ene hydrogels**. Integrin blocking experiments were performed for HT-1080s seeded in 3 wt% thiol-ene hydrogels with 1000 μM RGDS. Data is normalized to migration of HT-1080s in 3 wt% thiol-ene control hydrogels prepared from the same monomer/cell solutions as used for blocking experiments. 1 mL of hydrogel monomer solution was prepared and 100 μL was added to each of 3 vials. Either 7 μL of PBS (control) or antibody solutions (see below) was then added to the appropriate vial and mixed by pipetting. 200,000 HT-1080s/mL were suspended in the remaining monomer solution and 150 μL of the suspension was mixed in each of the vials containing monomer/antibody (or PBS) solutions. For β1-integrin blocking, 7 μL of 1 μg/μL mouse monoclonal anti-human β1 antibody ("Beta 1", CD29, 6603113, Beckman Coulter) was added to the hydrogel monomer solution (28 μg/mL final concentration). Cell/hydrogel constructs were then suspended in 1.5 mL media with 37.5 μL of 1 μg/μL β1-antibody solution (25 μg/mL final concentration) and incubated overnight to allow hydrogel/cell constructs to swell. Swollen hydrogel/cell constructs were resuspended in fresh 25 μg/mL anti-β1 antibody solution immediately prior to monitoring migration. For β3-integrin blocking, 2 μL of mouse anti-human β3 antibody ("Beta 3") reagent as received (CD-61, IM3605, Beckman Coulter)was added to 5 μL PBS and then added to monomer solution. 30 μL of anti-β3 antibody solution as received was added to 1.5 mL media for overnight swelling and fresh antibody/media was added prior to monitoring migration.

For comparison between blocking integrins and lowering RGDS receptor density, the normalized value for HT-1080s seeded in thiol-ene hydrogels with 125 μM RGD ("125 RGD") are also shown (normalized to 1000 μM RGD experiments performed at the same time, as detailed in experimental section of manuscript). Data for antibody experiments represents three hydrogels per experiment and two experiments (6 spots total). Error bars are standard error relative to individual cells (N>100 for all experiments).   Statistical analysis using a two-tailed Student's t-Test yielded P < 0.005 for all conditions relative to control (1000 μM RGD). These results demonstrate that blocking β1 or β3 integrins has a minimal, although statistically significant, influence on migration. Interestingly, the influence of integrin blocking is similar to the effect of lowering RGD concentration, and in both cases, HT-1080s remain highly invasive.
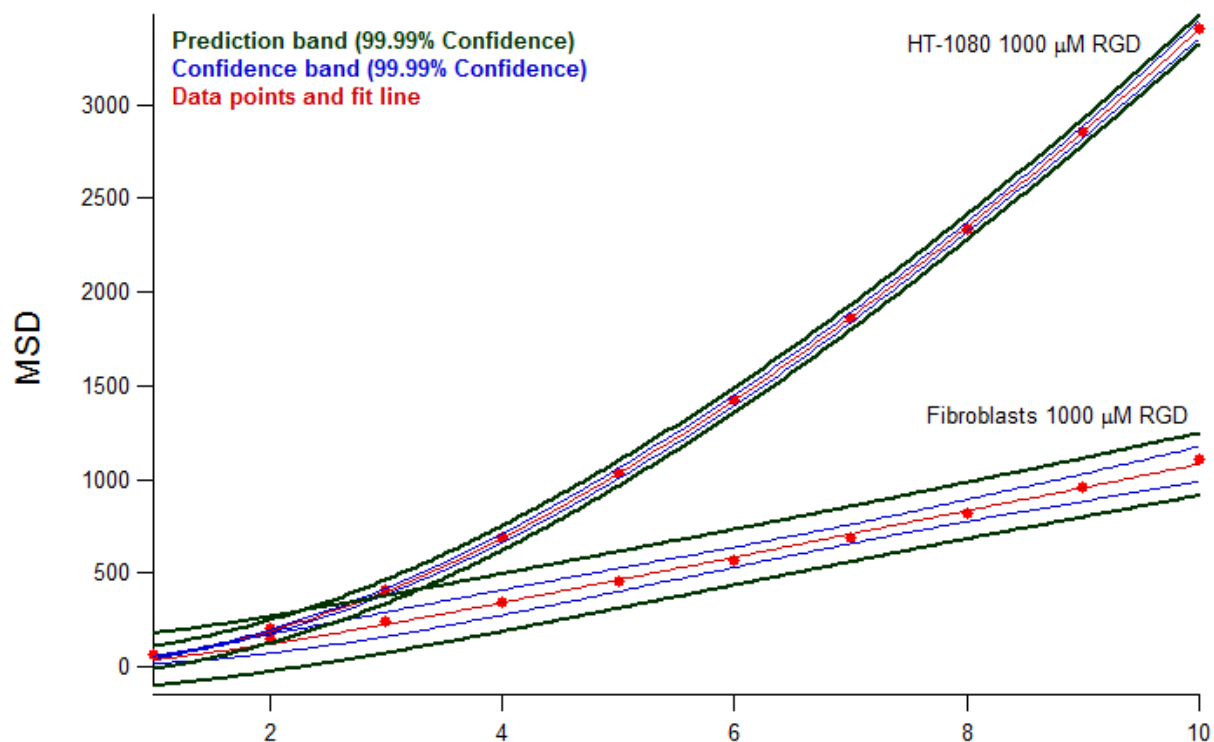
Supplemental Figure 2. Fit data for calulating persistence. See Experimental for details about Mean Square Displacement (MSD) and the equations used for fitting. Red points represent raw data with the red line being the fit to the equation described in the Experimental section. The confidence and prediction bands were determined using IGOR software (Wavemetrics). The confidence band represents the region in which the modeled data is expected to fall (based on the confidence level) while the prediction band represents where random samples plus random error are expected to fall. Bands are calculated at the 99.99% confidence level.

Calculating Mesh Size (See References 31 and 32 from manuscript)

The theory of rubber elasticity for solvent swollen polymer networks allows us to calculate a crosslinking density for the hydrogel environment in which the HT1080s were encapsulated. Equation 1 relates the measured shear elastic modulus, G, and the measured equilibrium volumetric swelling ratio, Q, to the crosslinking density, ρ_x. The gas constant and absolute temperature are represented by R and T respectively.

$$G = \rho_x \, RTQ^{\frac{-1}{3}}$$
Equation 1

Applying the theory of rubber elasticity to the measured shear modulus of our materials as prepared here (G=300 ± 20 Pa) and the equilibrium volumetric swelling ratio (40 ± 4), we calculate a crosslinking density of 0.42 mM which can subsequently be applied to the determination of the mesh size by Equation 2.

$$\xi = Q^{\frac{1}{3}} \, C_n^{\frac{1}{2}} ln^{\frac{1}{2}}$$
Equation 2

Here, ξ is the mesh size, C_n is the characteristic ratio of the polymer (the value is 4 for PEG), l is the average bond length (approximately 0.15 nm) and n is the number of bond lengths between crosslinks, which for PEG is calculated by Equation 3.

$$n = \frac{3}{M_r \bar{v} \rho_x}$$
Equation 3

Here, M_r is the molecular weight of a single polymer repeat unit (44 for PEG) and $\bar{v}$ is the specific gravity of the polymer (approximately 1). Because the contribution of PEG will dominate the mesh size, C_n and M_r of the peptide were omitted from these calculations. From these three equations the mesh size of the hydrogels is estimated at 13 ± 1 nm.