## Supplementary Materials and Methods

### Synthesis of ECM substrates

Solutions of acrylamide and N-N'-methylene-bis-acrylamide (Bio-Rad, Hercules, CA) were combined in various percentages (acrylamide: 3-15%, bis-acrylamide: 0.02-1.2%) with ultra pure water (Gibco BRL, Grand Island, NY), perfused with nitrogen gas for 30 minutes, placed under vacuum for at least an hour, and then transferred to a sterile environment. Free radical initiators ammonium persulfate (10% solution, 1/200 v/v, Bio-Rad) and TEMED (1/2000, Sigma-Aldrich, St. Louis, MO) were added to solution immediately prior to syringe filtering (0.22 μm filter). Droplets of sterile solution were sandwiched between a glutaraldehyde-activated glass coverslip and a hydrophobic glass coverslip (Superhydrophobic Solution, OMS Optochemicals, Pointe Claire, Quebec, Canada). Following polymerization, the substrates were immersed in 50 mM HEPES buffer (Gibco) and the top coverslip was detached with a pair of sterile forceps. All substrates were activated with the heterobifunctional crosslinker Sulfo-SANPAH (0.5 mg/ml, Pierce Chemical Co., Rockford, IL) in two 8 min. UV exposures, functionalized with 10-16 μg/ml human plasma fibronectin (Millipore Corp., Temecula, CA) in HEPES at 4°C overnight in order to achieve a nominal surface density of 2.6 $\mu g/cm^2$, washed with PBS, and soaked in complete culture medium at 37°C and 5% $CO_2$ for at least 30 minutes prior to cell seeding.

### Fluorescence staining of F-actin and vinculin-positive focal adhesions

Cells were washed once with 1x PBS, pH 7.4 (Fisher Scientific, Pittsburgh, PA), prior to fixation with 4% paraformaldehyde (Fisher Scientific) in PBS for 10-12 minutes. A staining buffer of 1% goat serum (Gibco) and 0.05% sodium azide (Sigma-Aldrich) in PBS was used for all subsequent washing steps and dilutions. Cells were permeabilized with 0.1% Triton-X 100 (EMD Biosciences, San Diego, CA) for 10 minutes at room temperature prior to blocking with

5% goat serum in PBS. Cells were subsequently stained for focal adhesions with mouse monoclonal anti-vinculin IgG (Sigma-Aldrich; diluted 1:200) and Alexa Fluor 546-labeled goat anti-mouse IgG (H+L) antibody (Invitrogen, Carlsbad, CA; diluted 1:250). F-actin was stained with Alexa Fluor 488 phalloidin (Invitrogen; diluted 1:200), and the nucleus was stained with 4′,6-diamino-2-phenylindole (DAPI) (Invitrogen; diluted 1:200).

**Measurement of cell proliferation**

Cells were plated at a subconfluent density of 7000 cells/cm$^2$. 50 μM 5-bromodeoxyuridine (BrdU, Sigma-Aldrich) was added to the cell culture medium 48 hours after plating and 90 minutes prior to fixation with 4% paraformaldehyde (Fisher Scientific). Cells were permeabilized with 0.1% TritonX-100 (EMD Biosciences), treated with 4 N HCl for 30 minutes, and blocked with 5% goat serum (Gibco) prior to staining with mouse monoclonal anti-BrdU antibody (Sigma-Aldrich), Alexa Fluor 546-labeled goat anti-mouse IgG (H+L) antibody (Invitrogen), and DAPI (Invitrogen).

## Supplementary Figure Legends

**Supplementary Figure 1: Generality of ECM rigidity-dependent changes in glioma cell morphology across multiple cell lines.** U251-MG, SNB19, C6, U87-MG, and U373-MG glioma cells were cultured on 0.08 kPa and 119 kPa fibronectin-coated ECMs as described in Methods and Fig. 1. In all cases, cells spread on 119 kPa ECMs and adopt a mutually indistinguishable rounded morphology on 0.08 kPa ECMs. Bar is 100 μm.

**Supplementary Figure 2: ECM rigidity modulates cytoskeletal and adhesive architecture of U373-MG cells.** (A) U373-MG cells were cultured on either fibronectin-coated glass (column 1) or polyacrylamide (columns 2-4) ECMs and stained for F-actin (green), vinculin (red) or DAPI (blue). As with U87-MG cells (Fig. 1), cells on either glass or 119 kPa ECMs form extensive stress fiber networks and focal adhesions, with progressive loss of these structures on 0.8 kPa or 0.08 kPa ECMs. Bar is 25 μm. (B) Isolated view of vinculin signal only, showing structure and distributions of cell-ECM adhesions.

**Supplementary Figure 3**: **Pharmacologic inhibition of cytoskeletal contractility reduces stiffness-dependent differences in U373-MG glioma cell morphology.** U373-MG cells cultured on fibronectin-conjugated glass and polyacrylamide substrates in either the absence of drug (control) or 12-24 hours after addition of 25 μM Blebbistatin, 10 μM Y-27632, or 1 μM cytochalasin D. In all cases, cells were cultured for at least 10 hours prior to drug addition. Cells began extending actin-rich processes within an hour after addition of Y-27632 or Blebbistatin. Cytochalasin D induced rounding of cells on all substrates. Bar is 100 μm.

**Supplementary Movie 1:** Phase contrast imaging of U373-MG cells migrating on fibronectin-coated glass. The timer in the upper left corner for this and all subsequent movies represents hours:minutes:seconds.

**Supplementary Movie 2:** Phase contrast imaging of U373-MG cells migrating on a 119 kPa fibronectin-conjugated polyacrylamide ECM.

**Supplementary Movie 3:** Phase contrast imaging of U373-MG cell migration on a 0.8 kPa fibronectin-conjugated polyacrylamide ECM.

**Supplementary Movie 4:** Phase contrast imaging of U373-MG cell migration on a 0.08 kPa fibronectin-conjugated polyacrylamide ECM.

**Supplementary Movie 5:** High-magnification phase contrast imaging of U373-MG cells migrating on fibronectin-coated glass.

**Supplementary Movie 6:** High-magnification phase contrast imaging of U373-MG cells migrating on a 119 kPa fibronectin-conjugated polyacrylamide ECM.

**Supplementary Movie 7:** High-magnification phase contrast imaging of U373-MG cells migrating on a 0.8 kPa fibronectin-conjugated polyacrylamide ECM.

**Supplementary Movie 8:** High-magnification phase contrast imaging of U373-MG cells migrating on a 0.08 kPa fibronectin-conjugated polyacrylamide ECM.

**Supplementary Movie 9:** Phase contrast imaging of U373-MG cell migration on a 119 kPa fibronectin-conjugated polyacrylamide ECM. 10 μM Y-27632 was added to the culture medium after capture of the first frame at time 0.

**Supplementary Movie 10:** Phase contrast imaging of U373-MG cell migration on a 0.08 kPa fibronectin-conjugated polyacrylamide ECM. 10 μM Y-27632 was added to the culture medium after capture of the first frame at time 0.

**Supplementary Movie 11:** Phase contrast imaging of U373-MG cell migration on a 119 kPa fibronectin-conjugated polyacrylamide ECM. 25 μM blebbistatin was added to the culture medium after capture of the first frame at time 0.

**Supplementary Movie 12:** Phase contrast imaging of U373-MG cell migration on a 0.08 kPa fibronectin-conjugated polyacrylamide ECM. 25 μM blebbistatin was added to the culture medium after capture of the first frame at time 0.