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Supplemental Information

Durotaxis by Human Cancer Cells

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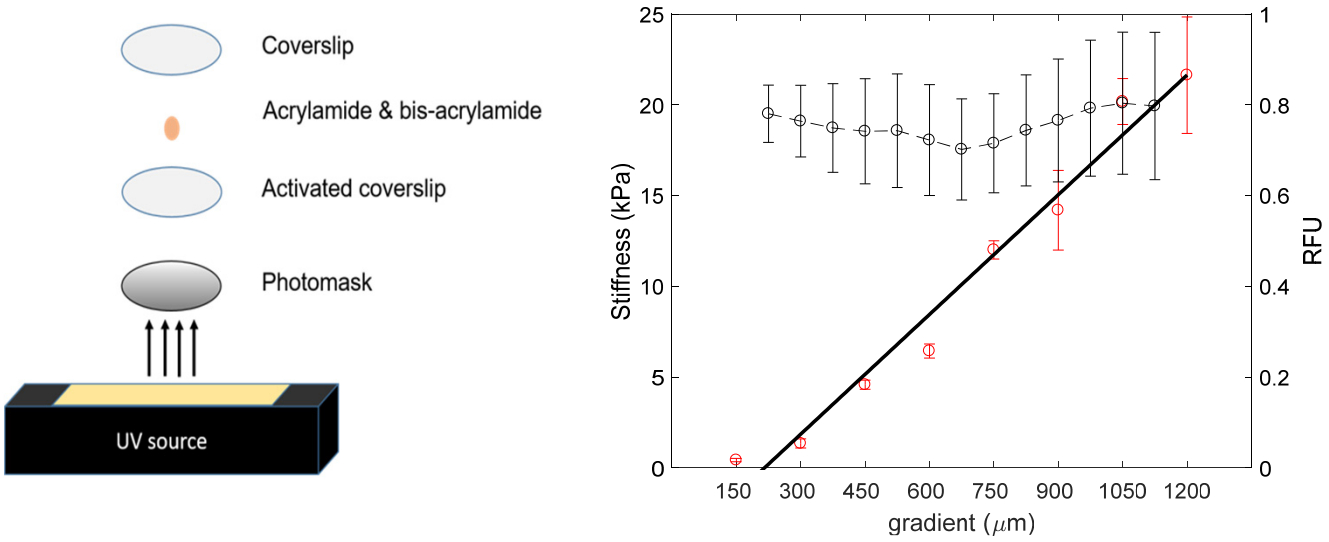

Figure S1. Uniformity of conjugated fibronectin across the stiffness gradient. A gradient substrate was generated and conjugated with human plasma FN protein. The red points and error bars represent the averaged stiffness (elastic modulus) values ± SD along the gradient with the solid black line indicating the line of best fit. The equation for this line is used to infer the stiffness of the gel at any given point along the gradient. The gradient slope measures 22 Pa/μm. The black points and error bars represent the relative fluorescence of the fibronectin conjugated to the gradient as determined by confocal microscopy after immunostaining.

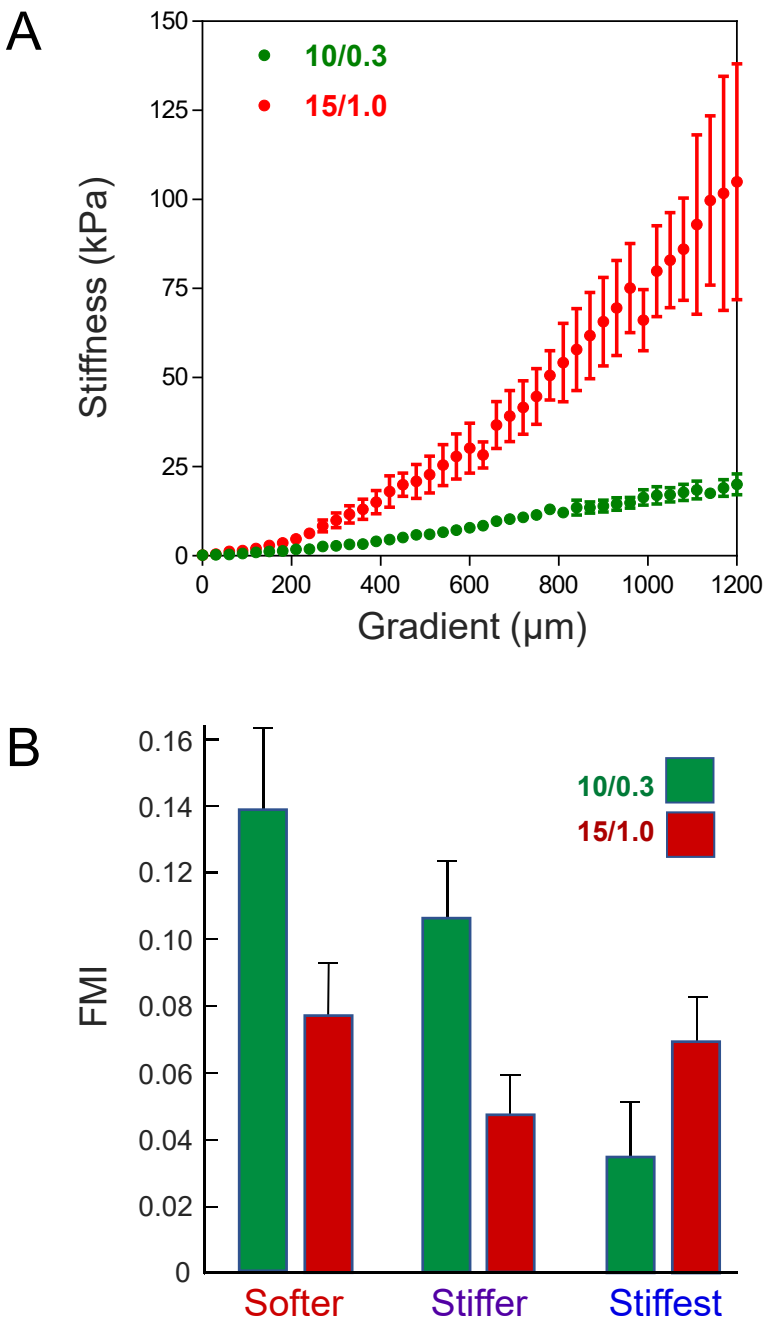

Figure S2. Durotactic efficiency is dependent on the absolute ECM stiffness for U87 glioblastomas cells. A) Polyacrylamide gels were generated using different acrylamide/bis-acrylamide ratios to generate low- and high-stiffness gradient gels (10/0.3 and 15/1, respectively). Data shown indicate the average elastic modulus and SEM for 3 replicate gels for low-stiffness (green) and high-stiffness (red) gradient gels. B) Forward migration index (FMI) for U-87 glioblastoma cells migrating in a durotactic fashion on 10/0.3 (low-stiffness gradient: green) and 15/1.0 (high-stiffness gradient: red) in the three regions used throughout this study, indicated here as softer, stiffer, and stiffest. The results indicate that the local overall substrate stiffness is important for durotactic efficiency, but also that durotaxis can still occur at high stiffness. N=4 replicate gels, n>170 cells for each region.

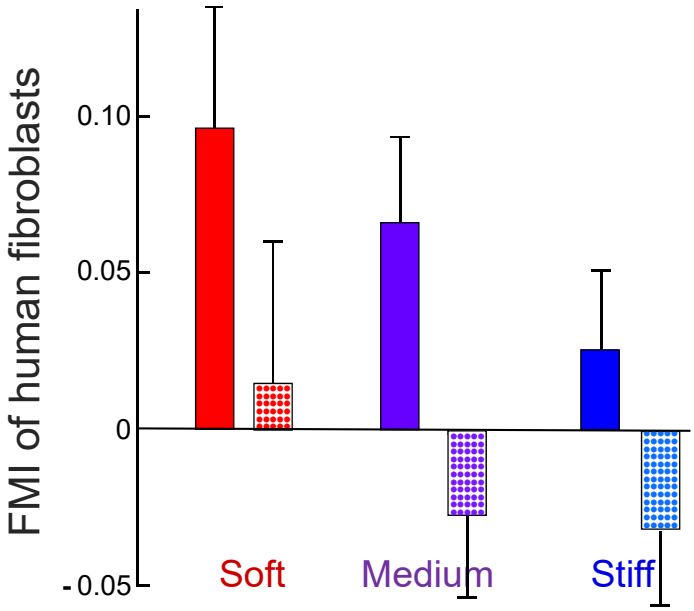

Figure S3. Durotaxis efficiency for normal human fibroblasts. Forward migration index (FMI) values for the BJ-5ta fibroblast cell line are shown on soft (2-7 kPa), medium (7-13 kPa), and stiff (13-18 kPa) regions of stiffness gradients. Solid bars indicate FMI along (up) the stiffness gradient, and stippled bars indicate FMI perpendicular to the gradient. Error bars represent ± SEM.

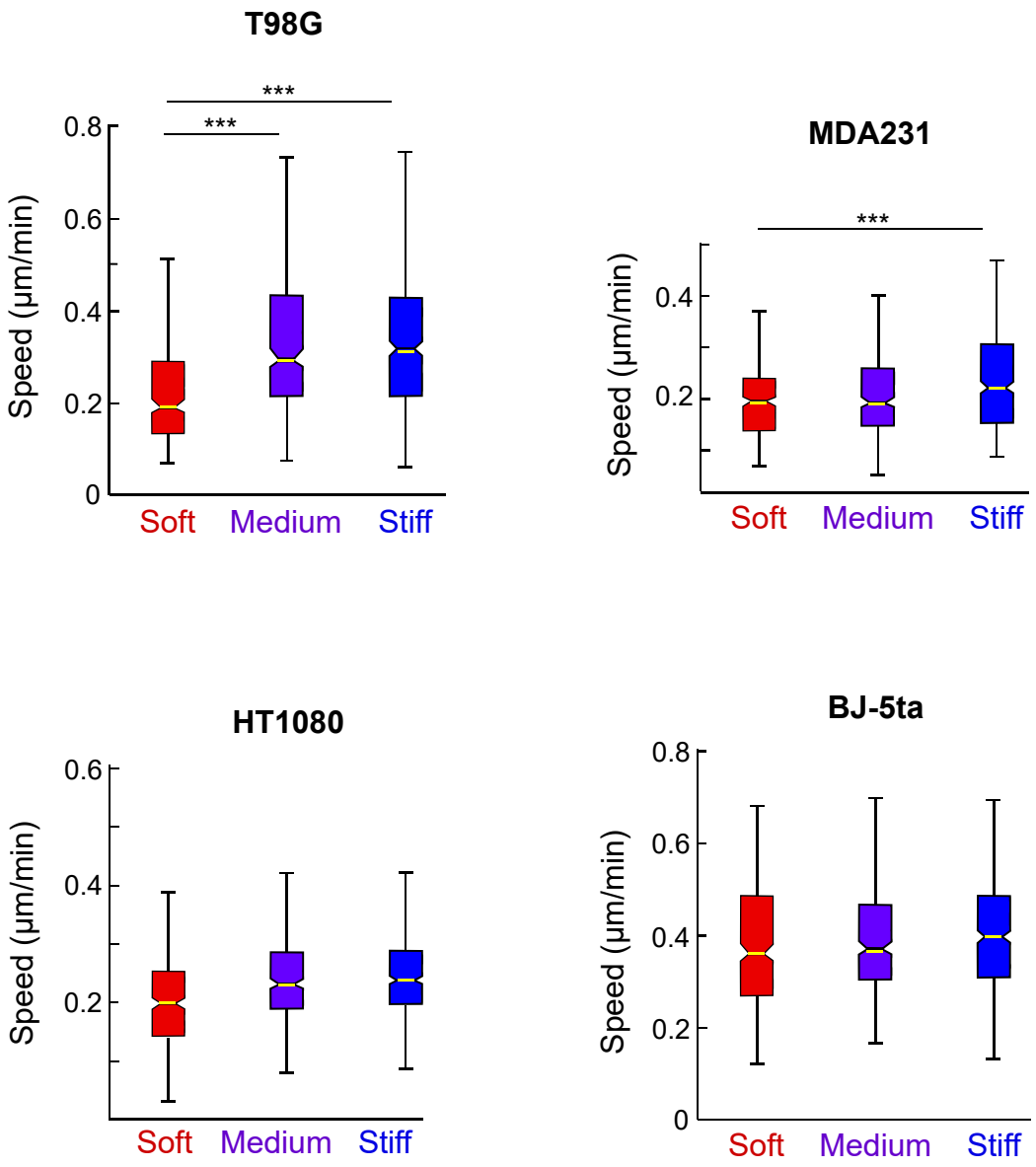

Figure S4. Comparisons of migration speeds on gradient gels. Speed on soft, medium, and stiff regions of gradient gels of different cancer cell lines compared to a normal human fibroblast line (BJ-5ta). Statistical analyses were performed using one-way ANOVA and Tukey's post-hoc test (***, $p \leq 0.001$).