

Supplemental Figure 1: Table of Statistical Significance for Figure 4. Statistics were calculated using a two-tailed student's t-test, $n \leq 175$ cells per condition. * = $p < 0.01$, ** = $p < 0.001$, NS = not significant

Supplemental Figure 2: "Reverse Durotaxis" of HT-1080 cells at modulus interface. **A.** A cell migrating from 100 Pa hydrogel is unable to penetrate into 360 Pa region and reverses. **B.** A cell migrating in the 360 Pa region enters the 100 Pa region and continues migration. (15 min time intervals).

Supplemental Figure 3: HT-1080 cells aggregate at interface. No cells are able to enter the 360 Pa pattern but instead aggregate along the interface. Single cells are able to migrate from 360 to 100 Pa regions. (15 min time intervals).