

Figure S1: expected cumulative distribution of calibration images. the cumulative distribution of several patterned gels (n=7) that lack cells provides an understanding of the false positive error in traction measurements resulting from patterning and image processing errors. the 25th, 50th, and 75th percentile of dots are displaced 0.06, 0.106, and 0.16 μ m, respectively.



Figure S2: cell on paam with fn dots and embedded beads. (a) a brightfield image shows a pair of cells on the surface of a 3.6 kpa paa hydrogel that has been micropatterned with fluorescent fn adhesion points. (b) the cell tractions can be calculated from the deflection of the micropatterned dots. the units on the colorbar are nn. (c) furthermore, the presence of fluorescent microbeads and removal of the cells to obtain images of the relaxed bead positions allows calculation of the traction field using fttc of the same cell pair. the units of the colorbar are in pa. (d) and (e) represent an area in the top left corner of the cell that has been enlarged to show the locations of the adhesion points in that region. scale bars represent 5 μ m.



Figure S3: cell tractions of paxillin stained cells. cell traction forces (a, d, g, and j) were measured for cells that had been fixed and then stained with anti-paxillin antibody (b, e, h, and m). the images were then overlaid onto one another (c, f, i, and l) to demonstrate the cells ability to adhere only to the micropatterned fibronectin (red). scale bars are 20µm. color bars represent tractions from 0 to 35 nn (blue to red). the scale arrow in the upper left corner of the traction images represents 35 nn.



Figure S4: traction forces of cells treated with Ipa and y27632. traction forces of cells (n = 4) were measured before (-) and after treatment with either Ipa or y27632 (+). the average of the magnitude of traction forces are shown with standard deviation error bars. the p-value of a 2 tailed student's t-test was determined to be 0.15 between the untreated and the Ipa-treated cells, and 0.0029 between the untreated and the y2763-treated cells.