Supporting Information

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Substrates with Patterned Extracellular Matrix and Subcellular Stiffness Gradients Reveal Local Biomechanical Responses

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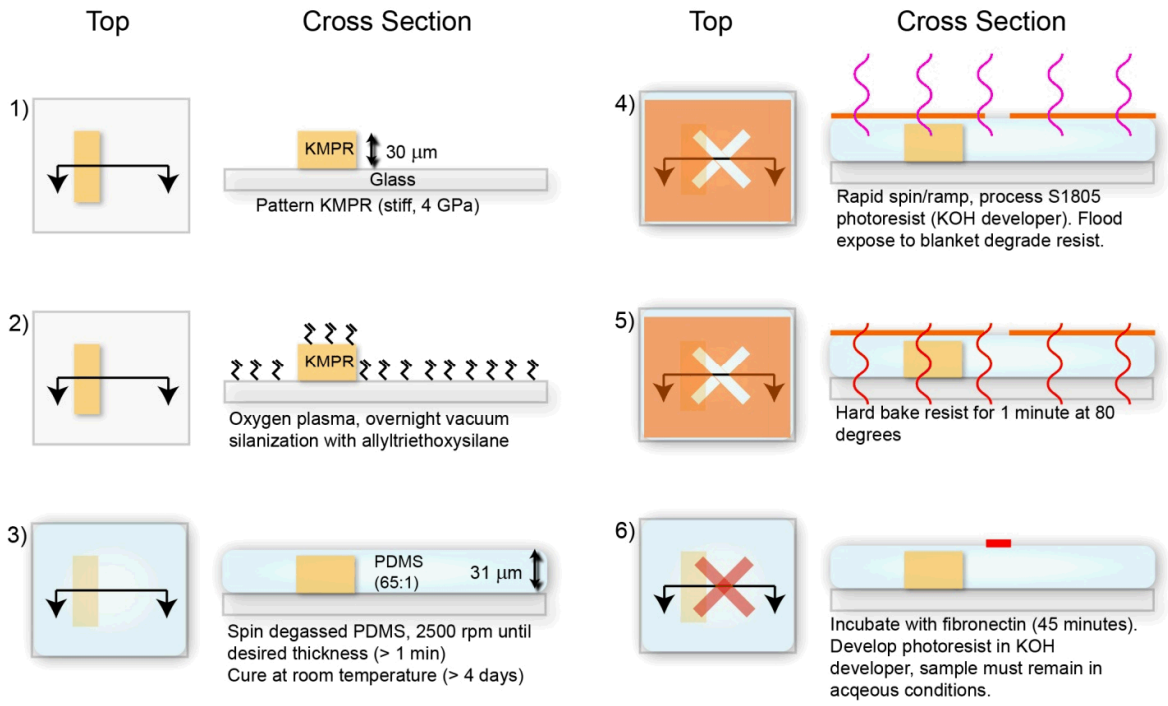
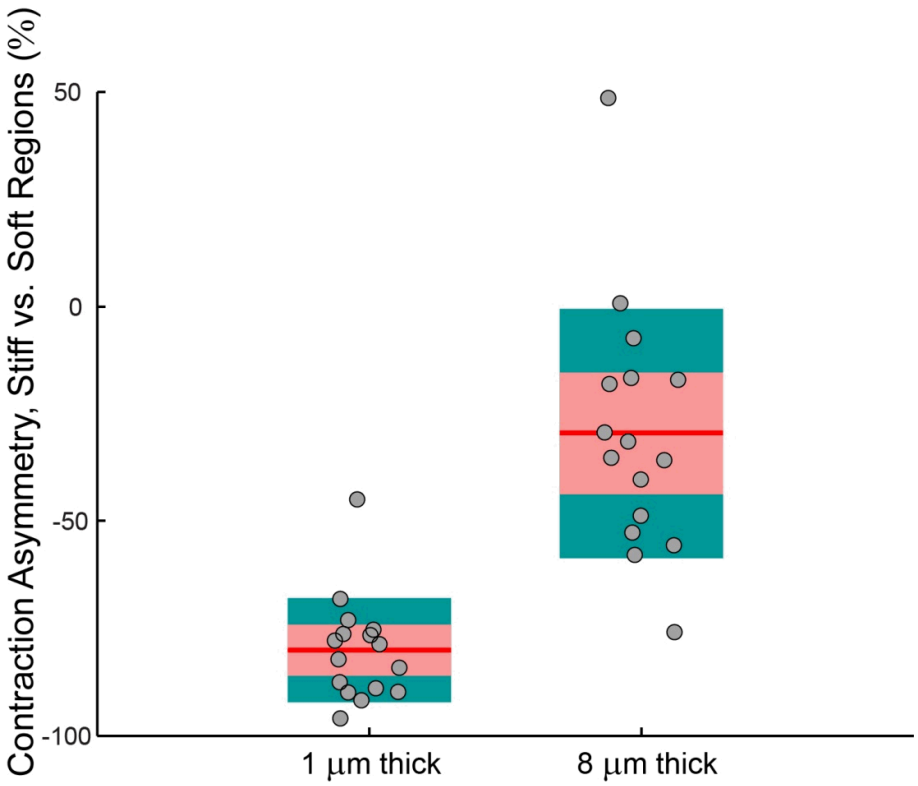
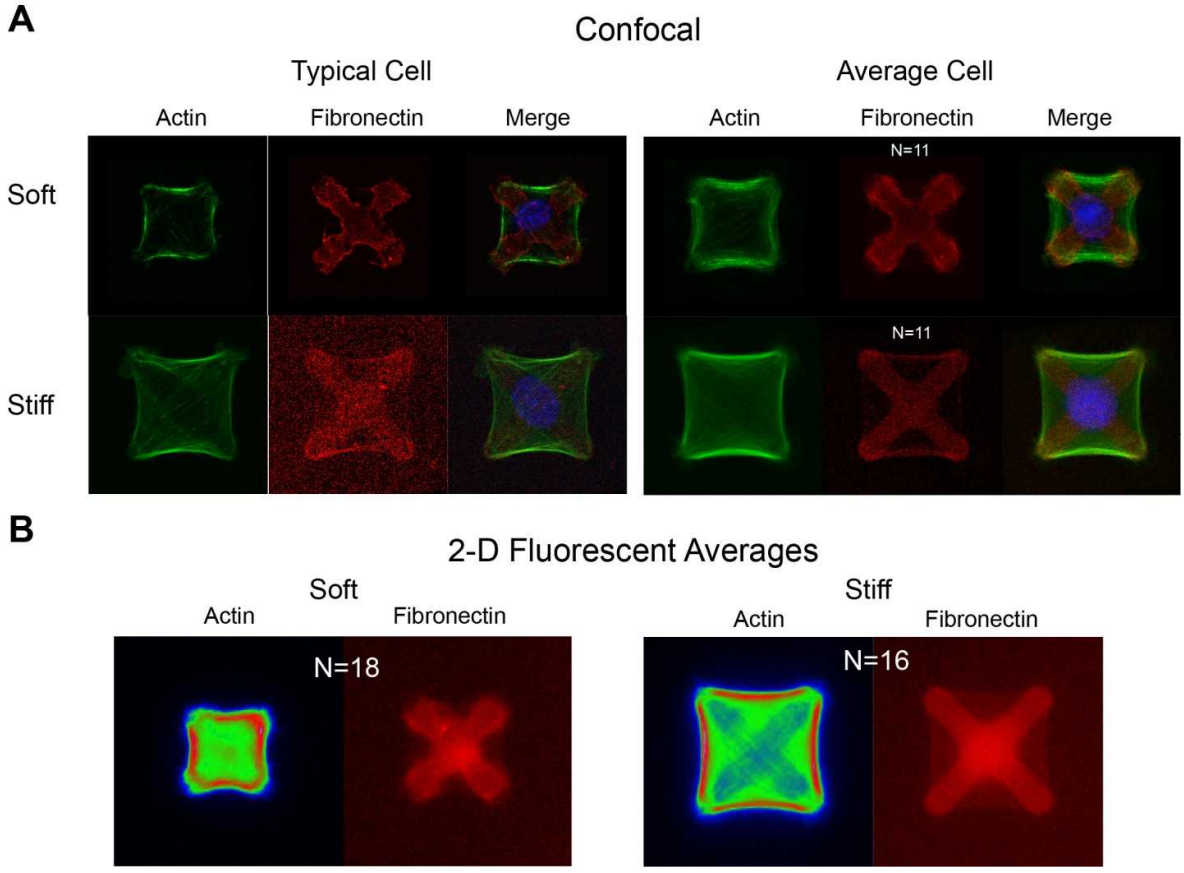


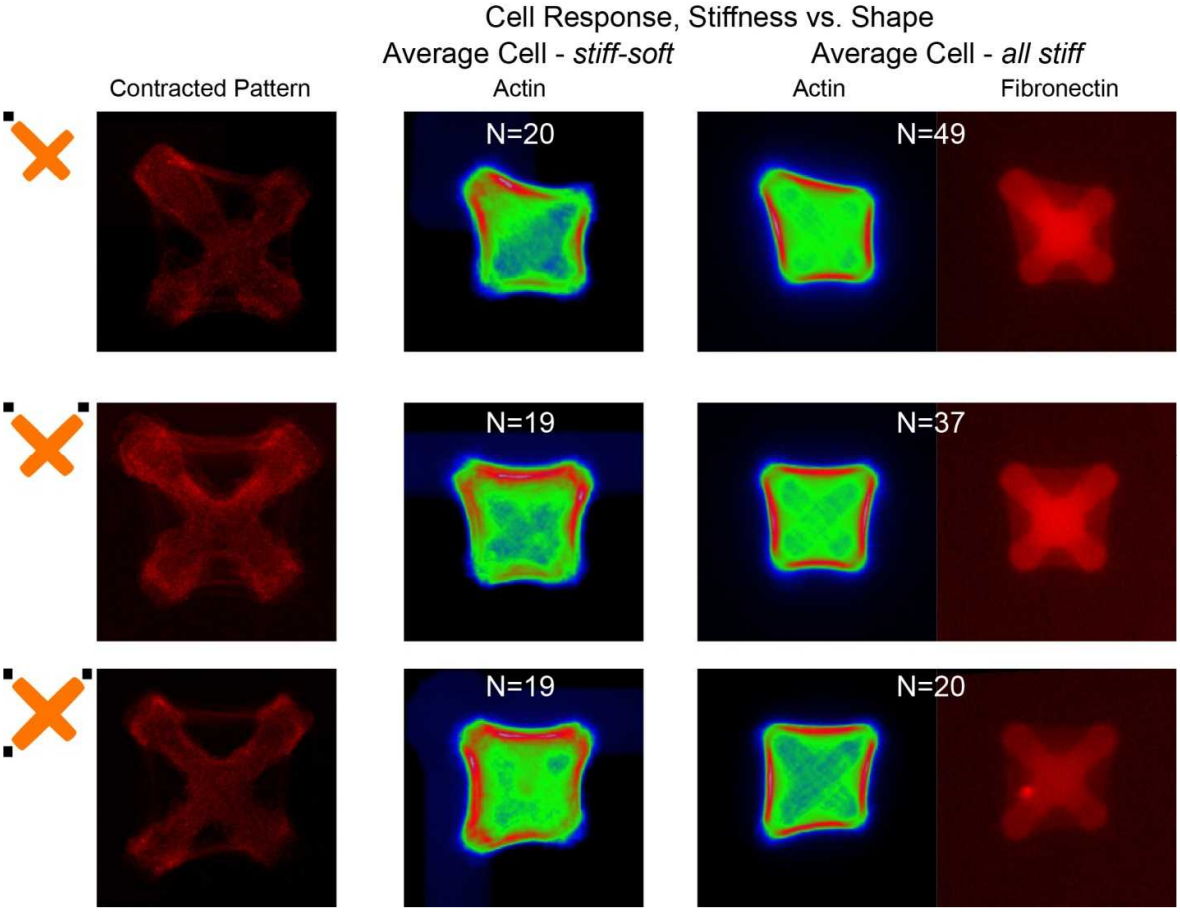
Supplementary Figure 1. Process flow for creating gradients in substrate stiffness across patterned cells. The process is modified from standard lithography techniques to reduce solvent exposure to the photoresist, allow aqueous development, and reduce adhesion and rippling issues in the resist layer.



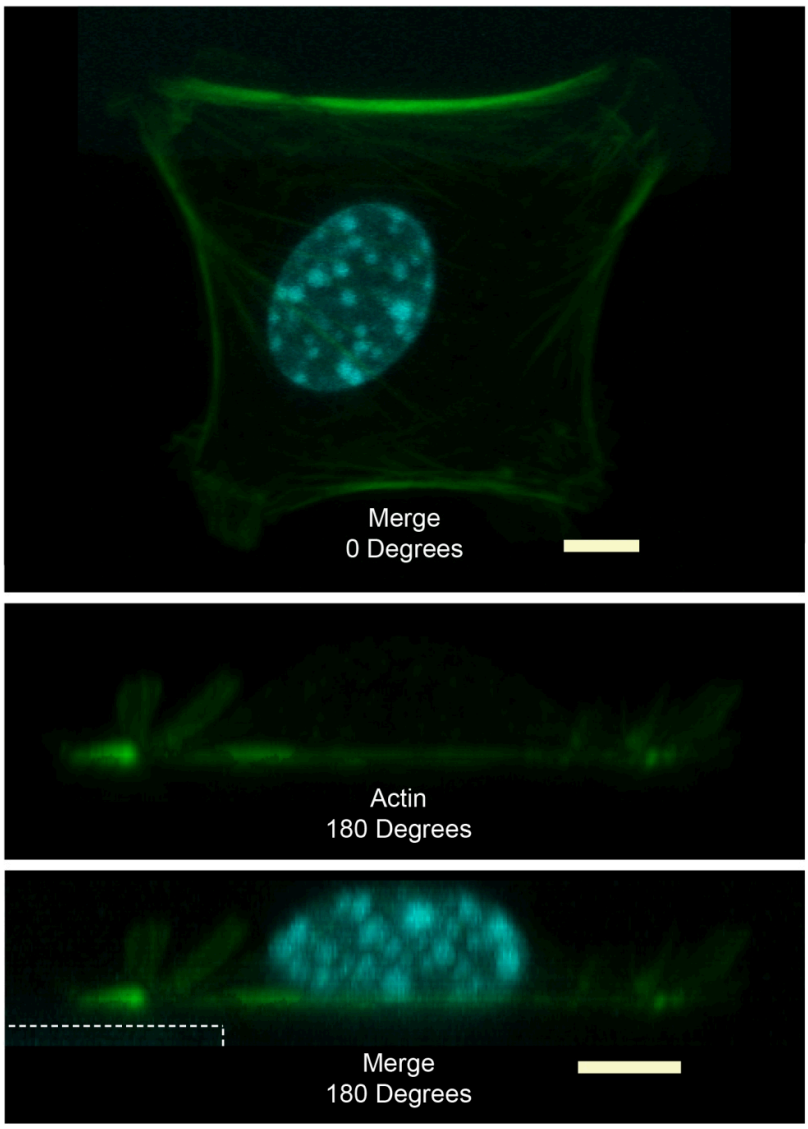
Supplementary Figure 2. Contraction differences between stiff (underlying KMPR layer) and soft regions for different thicknesses of the PDMS layer above the KMPR photopolymer. Contraction asymmetry is defined as the contraction distance of the fibronectin pattern on the stiff region divided by contraction distance of the pattern on the soft region. Zero-point is a presumably infinitely thick layer of PDMS, with no asymmetry between stiff and soft regions. Thicker layers of PDMS yield softer apparent moduli than thinner layers. Red line represents mean, pink region represents the standard deviation, and cyan the 95 % confidence interval of the scatter plot.



Supplementary Figure 3. Typical and average cells for two special cases: all soft and all stiff boundary conditions. Averages from both confocal and inverted fluorescence-based microscopy are shown, confocal for finer detail on the interior stress fibers, and fluorescent images for better quantification of the average actin stress-fiber structure. Cells on all-stiff substrates exhibit a large number of long, well-defined stress fibers in comparison to cells patterned on all-soft substrates.



Supplementary Figure 4. Controlling for cell shape. We patterned cells on stiff substrates to the same end-shape observed for cells patterned on substrates with gradients in stiffness. Direct comparisons of average fluorescence images of cells show differences in the actin distribution that depends on the the soft mechanical properties of the substrate. Cells of the same shape with all stiff boundary conditions have stronger actin stress fibers in comparison over soft regions and overall reduced curvature.



Supplementary Figure 5. 3-D confocal image and reconstruction of a single cell patterned to occupy an X shape in which the upper half of the cell is patterned to adhere to a stiff region. 180 degree cross-sectional view reveals the minimal topology of the PDMS layer (smooth features), and its thickness of 1 to 1.5 μm above the photopolymer backbone (region outlined by a dashed line).