Supporting Information

Biochemical ligand density regulates YAP translocation in stem cells through cytoskeletal tension and integrins

Alice E. Stanton[1], Xinming Tong[2], Soah Lee[3], and Fan Yang[1,2*]

[1]Department of Bioengineering, [2] Department of Orthopaedic Surgery, [3]Department of Materials Science and Engineering, Stanford University, Stanford, CA 94305, USA

*Address for correspondence:

Fan Yang, Ph.D.
Associate Professor
Departments of Bioengineering and Orthopaedic Surgery
Director of Stem Cells and Biomaterials Engineering Laboratory
Stanford University School of Medicine
300 Pasteur Dr., Edwards R105, Stanford, CA 94305, USA
Phone: 650-725-7128
Fax: 650-723-9370
E-mail: fanyang@stanford.edu

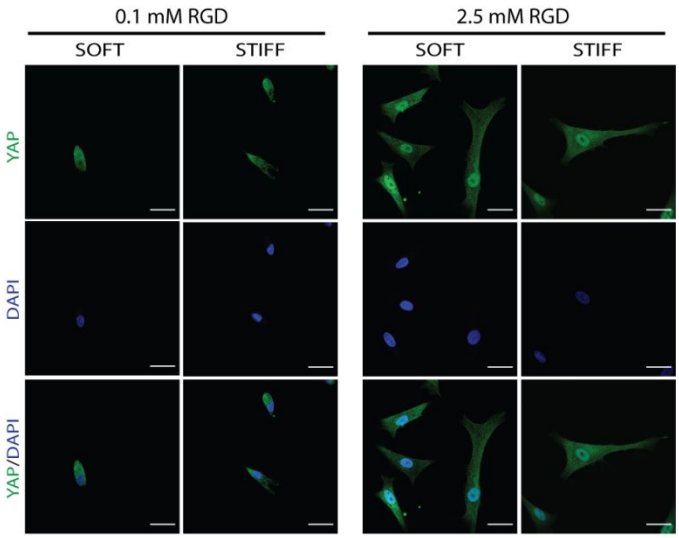

Figure S1: Increasing RGD peptide density led to YAP nuclear translocation. hMSCs were cultured on soft or stiff hydrogels coated with 0.1 mM or 2.5 mM RGD. Green: YAP, Blue: cell nuclei, Scale bar: 30 μm.

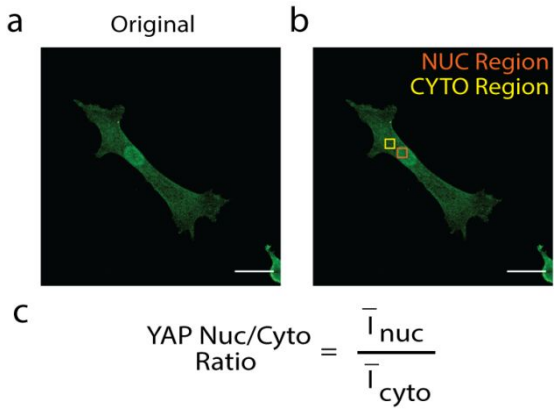

Figure S2: Method of quantifying the ratio of YAP intensity in the nucleus vs. cytoplasm from defined region of interest. (a) Original image of YAP staining in one cell, (b) selecting a region of interest within the nucleus (orange box), and a region of interest of equal area in the adjacent cytoplasm, (c) defining the ratio of nuclear YAP intensity over cytoplasmic YAP intensity.

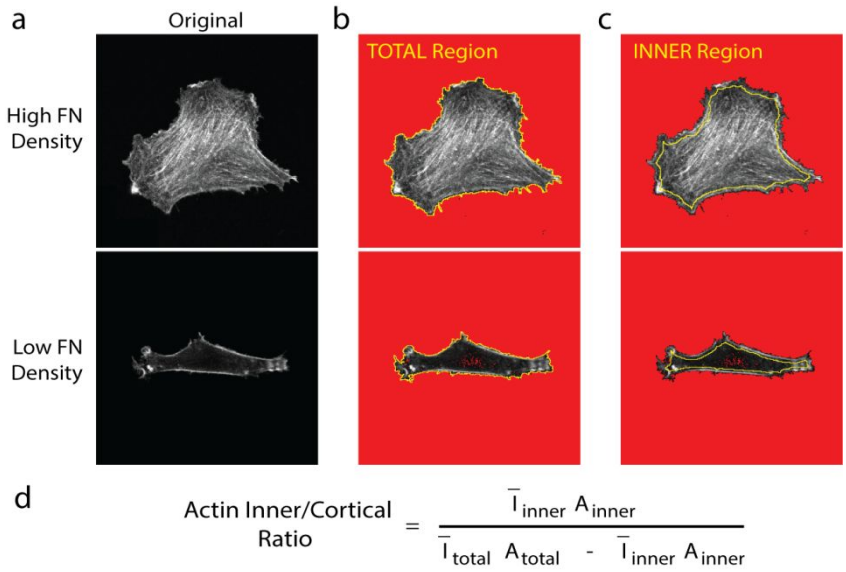

Figure S3: Quantification of F-actin localization. (a) Representative cell images of hMSCs cultured on hydrogels coated with high fibronectin density (top) or low fibronectin density (bottom); (b) Setting a threshold to identify the outer perimeter of the cell, which was used to calculate the total intensity of the actin staining within a cell; and (c) Defining the inner perimeter of a cell by shrinking the cell boundaries inward by 2 μm. The region between the inner and outer perimeters was defined to be the F-actin cortical region. (d) The formula used to quantify the ratio of integrated actin intensity from inner region vs. integrated actin intensity from cortical region.

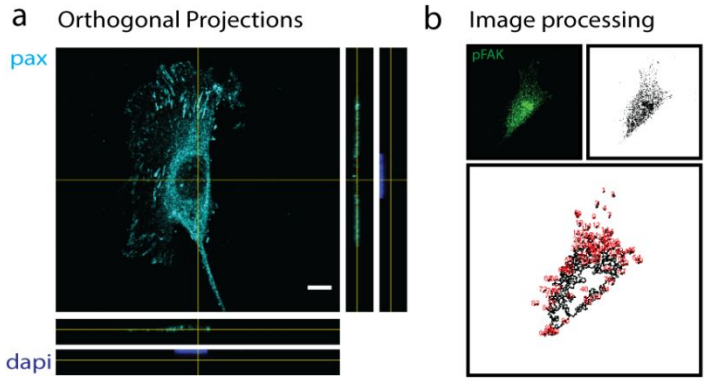

Figure S4: Method for imaging focal adhesion proteins and image analyses. (a) A slice of confocal image of paxillin (cyan) staining in one cell, which shows the locations of focal adhesions and functions to anchor the cells to the underlying matrix. Cell nuclei were counter-stained using DAPI staining (blue). (b) An example of automated image processing used to quantify adhesions showing (top left) pFAK (green) staining, (top right) image processed using macro, and (bottom) output image highlighting segmented focal adhesions as red dots.

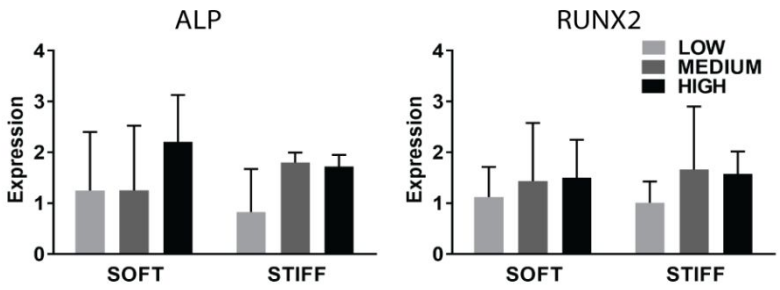

Figure S5: Effects of varying ligand density on gene expressions of early osteogenic markers by hMSCs cultured on soft or stiff substrates. Cells were cultured in osteogenic medium for three days before analyses.