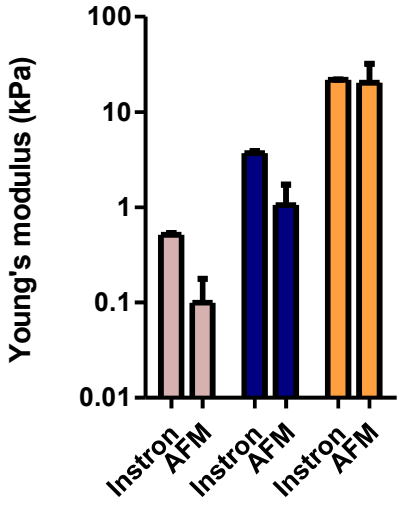
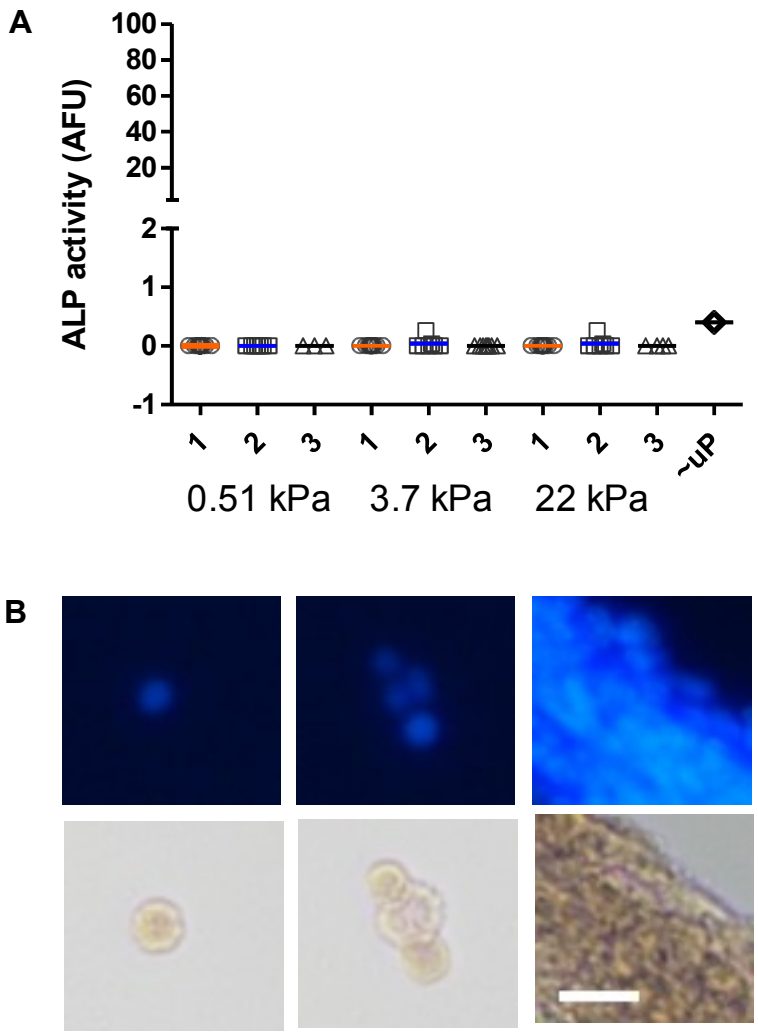
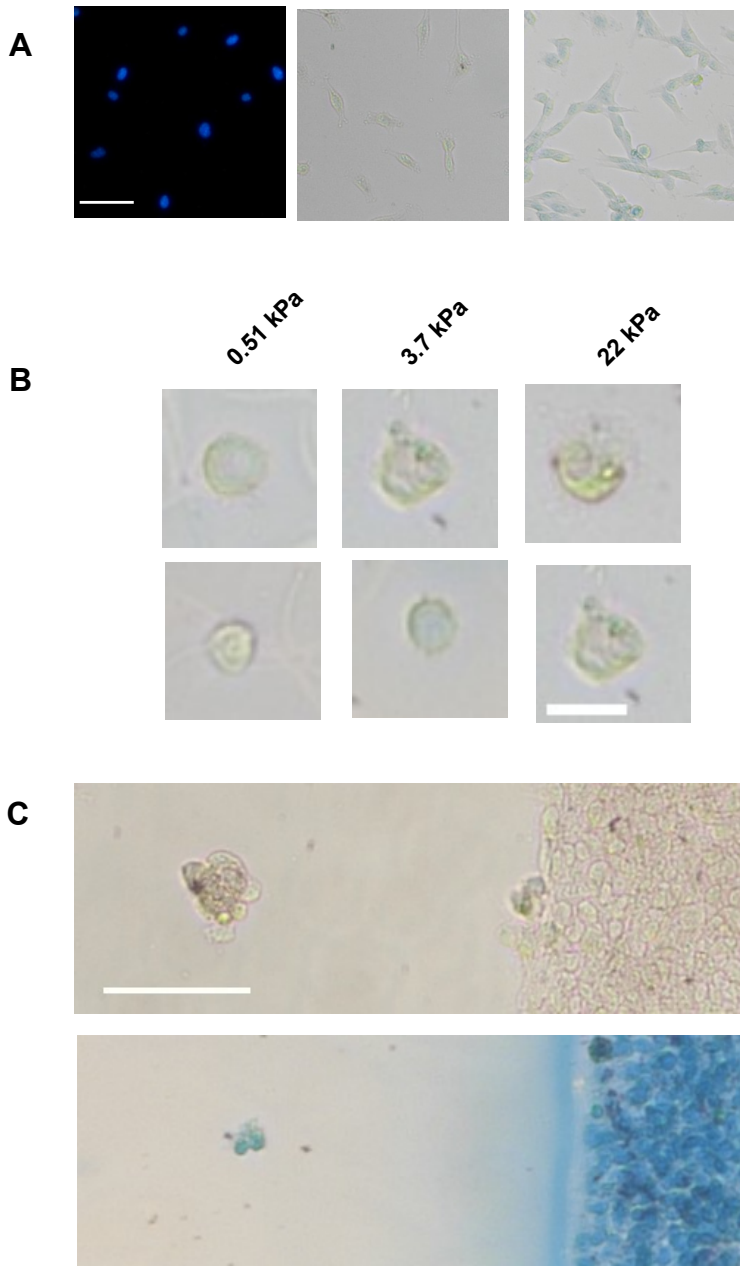


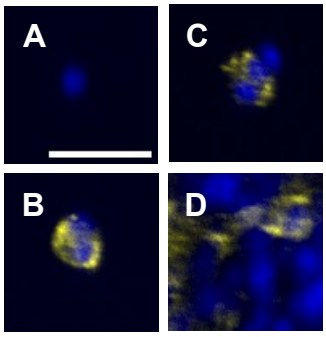
Supp Figure 1.
Comparison of Young's modulus of the three substrate stiffnesses as assessed by uniaxial compression of bulk hydrogel or by atomic force microscopy (AFM).



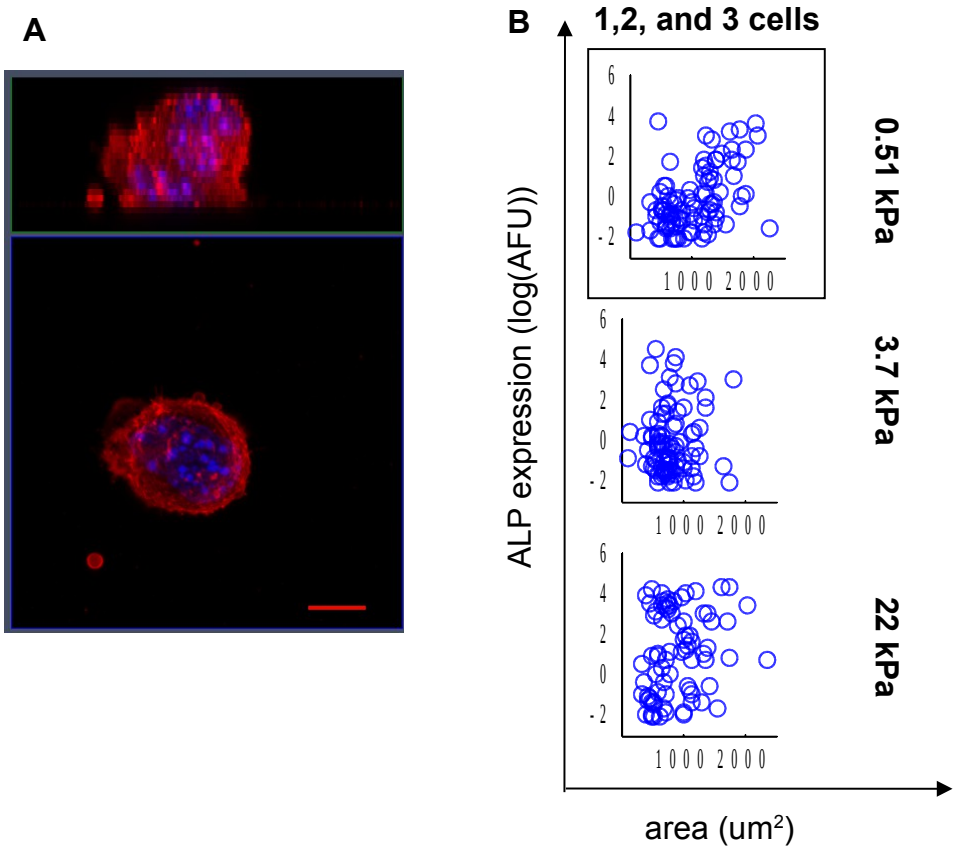
Supp Figure 2. Osteogenesis in basal media. (A) Quantification of ALP production in D1s grown on islands of different sizes as well as on unconstrained 22 kPa substrate. (B) Representative images of negative Alizarin Red staining of D1s after two weeks of culture, showing single island (left), four cells (middle), and multiple cells on an unpatterned area (right).



Supp Figure 3. Characterization of D1 cell line. **(A)** Representative images showing negative stains of elf-97 for alkaline phosphatase (left), Oil-Red-O (middle), and Alcian Blue (right) on naïve D1s. Scale bar = 50 um. **(B)** Representative images showing Oil-Red-O (above) and Alcian Blue (bottom) stains of D1s cultured in media with osteogenic supplements for 1 week. Scale bar = 20 um. **(C)** Representative images showing negative stains of Oil-Red-O (above) and Alcian Blue (bottom) of D1s cultured in media with osteogenic supplements for 2 week. Scale bar = 100 um.

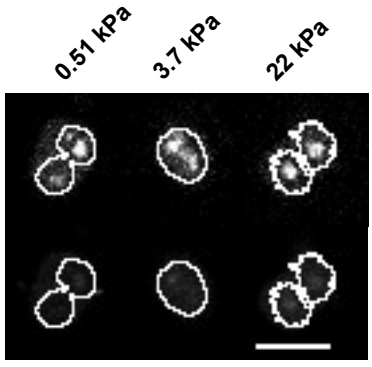


Supp Figure 4. Representative images of single, doublet, and triplet MSCs growing on micropatterned islands (A – C) as well as on an unpatterned area (D).



Supp Figure 5. (A) Confocal image with orthographic views of MSCs grown on a fibronectin island after 6 days of culture, viewed from the side (top) and from above (bottom). Scale bar = 10 um. **(B)** Scatter/density plots of differentiation as a function of projected cell area and modulus. Each point represents a cell or cell cluster grown on a FN island. Boxed plot represents significant correlation ($r = 0.42$, $p < 0.001$).

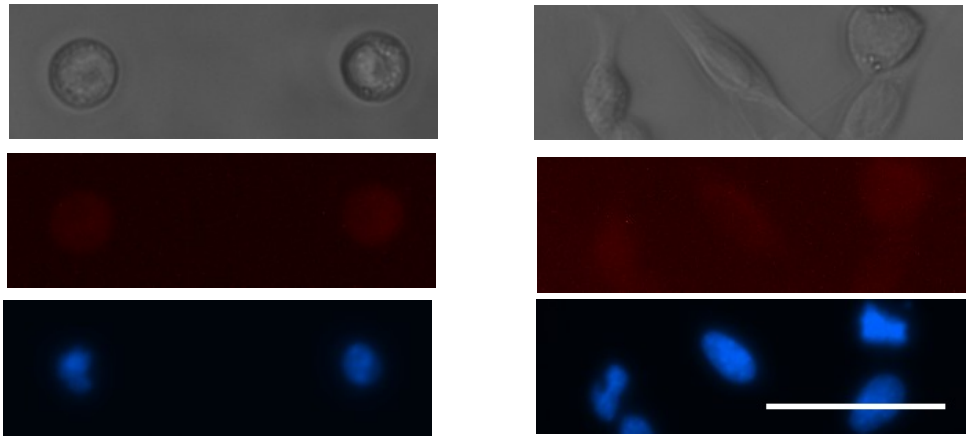
A



B

0.51 kPa	3.7 kPa	22 kPa
0.534	0.924	0.433
0.616	0.723	0.403
0.655	0.913	0.377

C



Supp Figure 6. (A) YAP and Runx2 nuclear localization of micropatterned cells at 24 hr. White lines outline nuclei. Scale bar, 20 um. **(B)** Slopes of best fit lines determined in a least-squares sense of three different cells on each of the three substrate stiffnesses. **(C)** Sclerostin staining (left) and TRITC secondary antibody control (right) of D1s, showing bright field (top) image, the same field under a 555 nm laser (middle), and DAPI stain (bottom). Scale bar = 50 um.