Supplementary Information

Subcellular Control Over Focal Adhesion Anisotropy, Independent of Cell Morphology, Dictates Stem Cell Fate

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Methods

Centroid Fitting

The centroid of circular cells was found using a custom script in MATLAB R2017A. Exported images of actin cytoskeleton were imported into the program. Cell interiors were filled and then fit to disk using the *Regionprops* function. The centroid of the fitted disk was used to evaluate the relative fiber orientation.

Color Deconvolution

Cells on patterned surfaces were imaged with color phase contrast microscopy with a 10x objective (LSM 800, Carl Zeiss). Single cells were identified by counting the number of nuclei in fluorescence images using a DAPI filter set. 200 x 200 pixel or 150 x 150 pixel color images of each cell were then exported, color deconvoluted, and analyzed using the FIJI package of ImageJ (https://imagej.net/Fiji/Downloads). The background intensity of each image was corrected using a pseudo-flatfield correction with a blurring radius of 40 pixels (BioVoxxel Toolbox). Color vectors were assigned using the color deconvolution plugin within ImageJ. The images were then binarized using a set threshold based on visual examination and the intensity of the binarized images was measured for each channel.

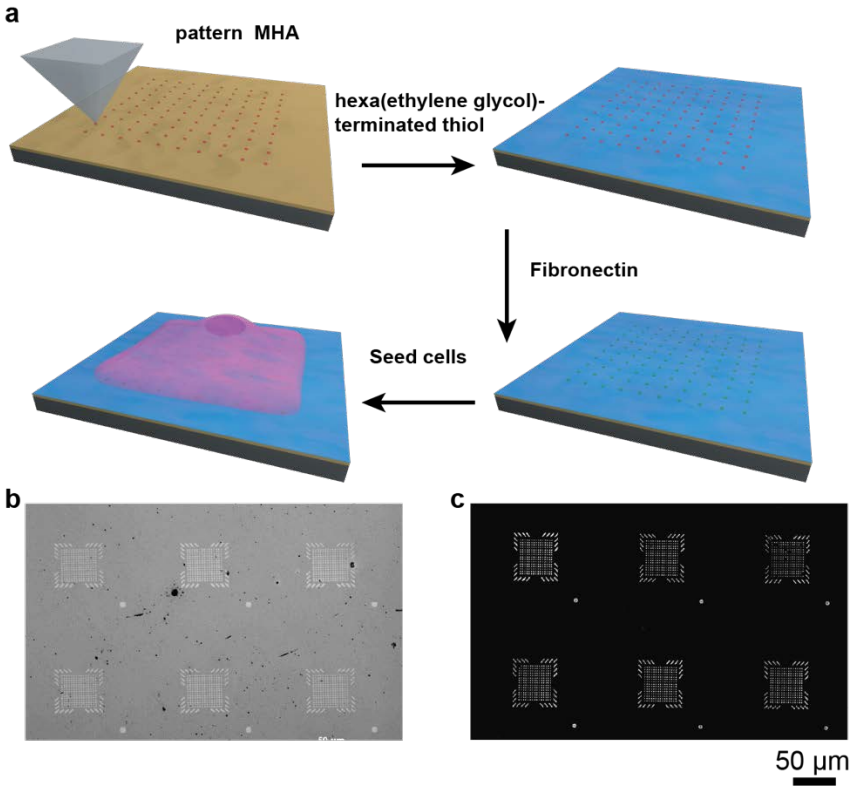

Figure S1. PPL Patterning of Cell Attachment Features. a, Workflow for patterning fibronectin features on a gold substrate for mediating cell attachment. **b,** Micrograph of an etched gold substrate visualizing the mercaptohexadecanoic acid (MHA) features. **c,** Fluorescence micrograph of antibody stained fibronectin that selectively adsorbed to the MHA patterns.

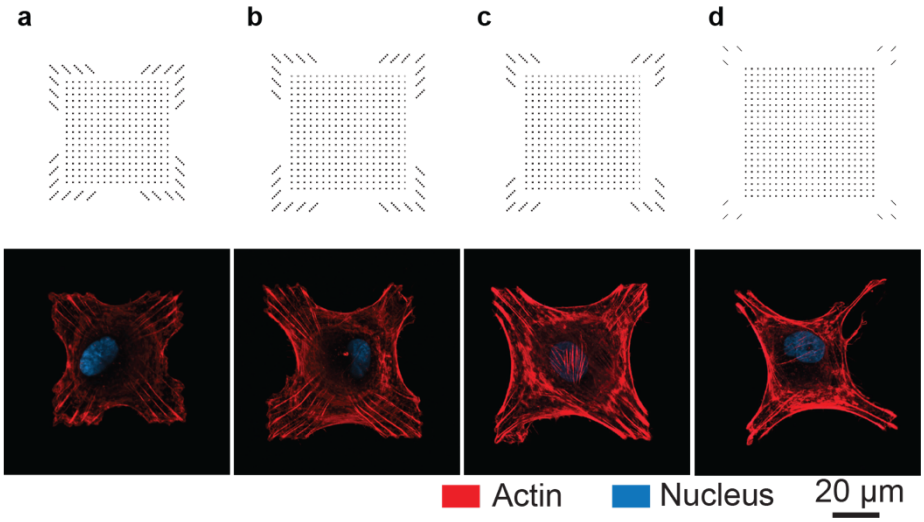

Figure S2. Set of patterns used to identify cytoskeletal controlling features in a square geometry. a-d, Computer generated images of the programmed fibronectin features patterned on gold substrates and representative fluorescence images of the actin cytoskeleton (red) for cells on these patterns are shown along with the nucleus (blue).

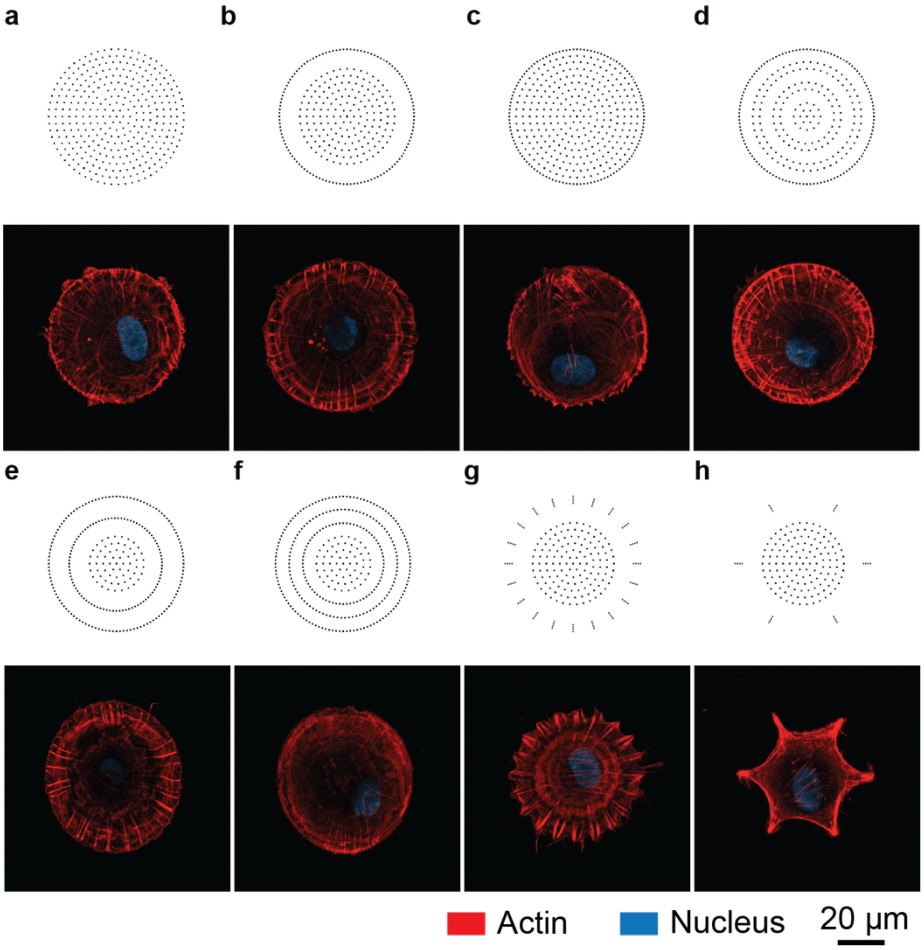

Figure S3. Set of patterns used to identify cytoskeletal controlling features in a circular geometry. a-h, Computer generated images of the programmed fibronectin features patterned on gold substrates and representative fluorescence images of the actin cytoskeleton (red) for cells on these patterns are shown along with the nucleus (blue).

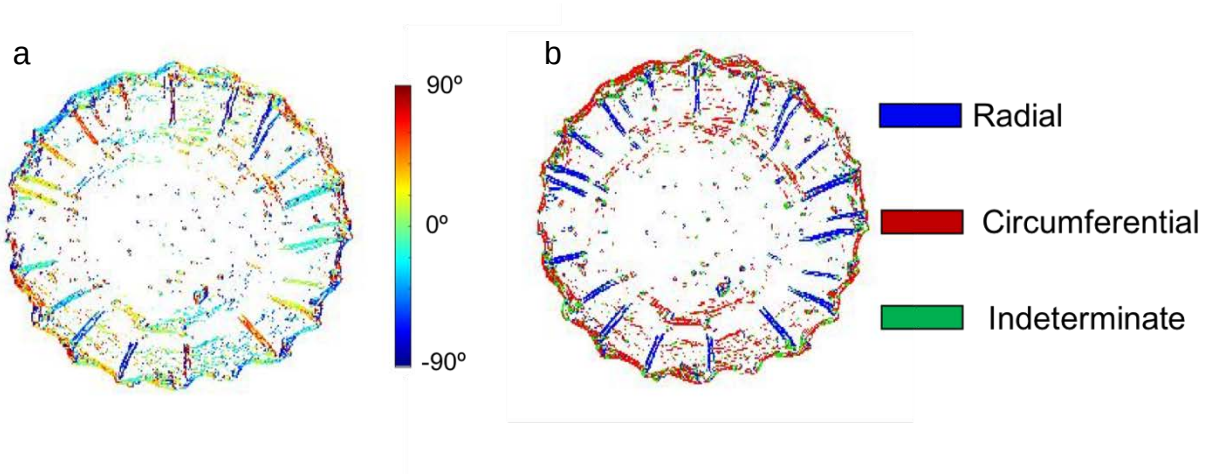

Figure S4. Fiber Classification within cells on circular geometries. a, Local fiber orientation of all fibers within the cell. **b,** Classification of fiber directionality within the cell with respect to the centroid.

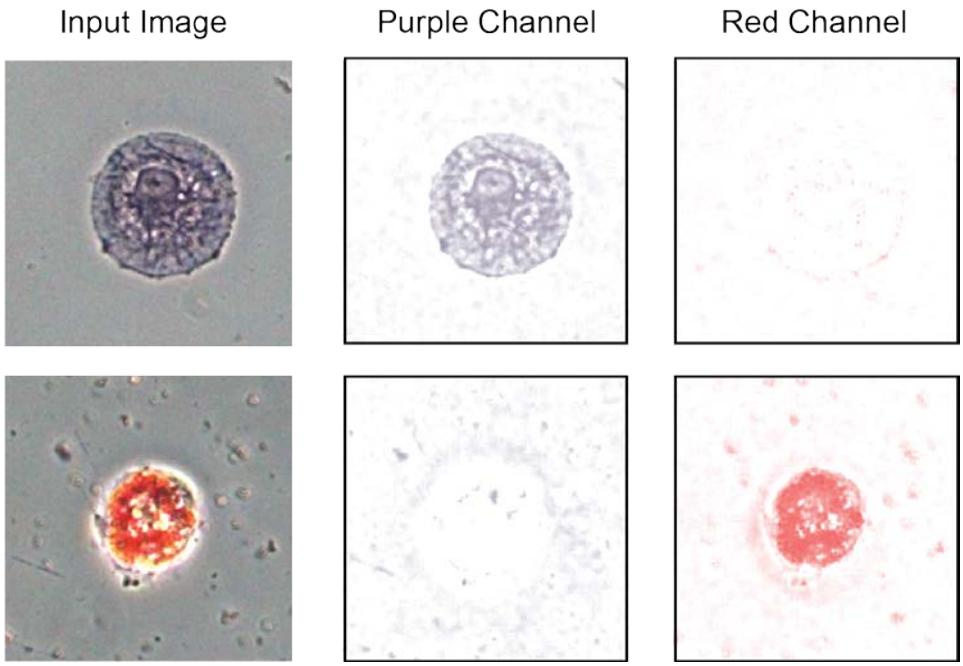

Figure S5. Color Deconvolution of cells stained for alkaline phosphatase (purple channel) and lipid vacuoles (red channel). Top: The purple channel and red channel intensity for a cell staining positive for osteogenesis. Bottom: The purple channel and red channel intensity for a cell staining positive for adipogenesis.

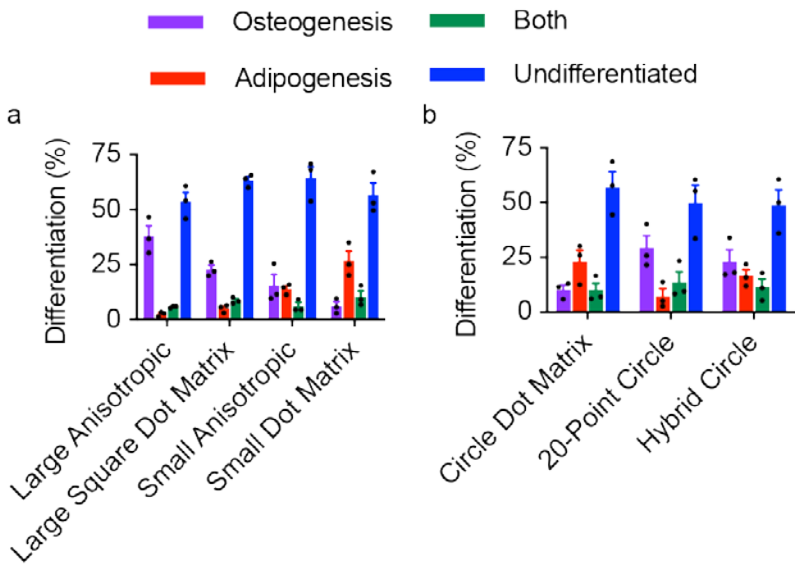

Figure S6. Total differentiation of hMSCs seeded on patterns. Response of cells seeded on the square patterns (**a)** and circular patterns (**b**) when exposed to mixed differentiation media.

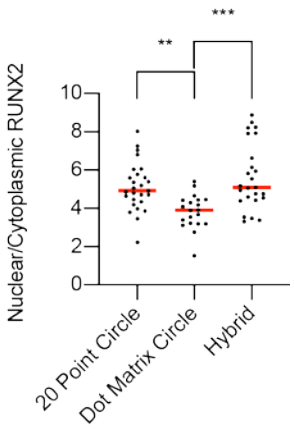

Figure S7. Translocation of RUNX2 into the nucleus in hMSCs on circular patterns. Ratio of the staining intensity of nuclear and cytoplasmic RUNX2 (median; *** $p<0.001$; ** $p=0.0013$; Kruskal-Wallis)

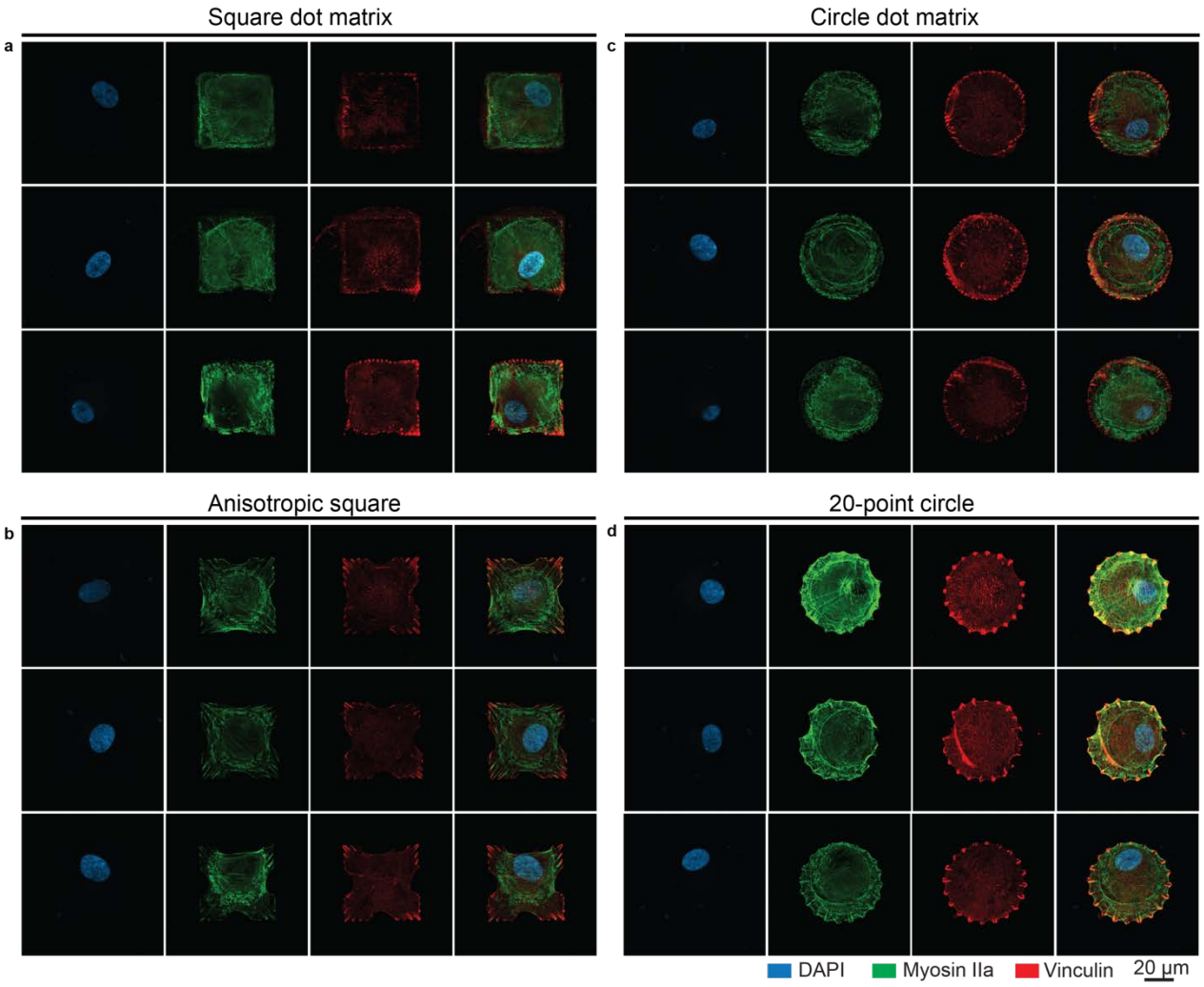

Figure S8. Fluorescence micrographs of myosin IIA within cells on patterns. a-d, Additional confocal images of single cells grown on different patterns: square dot matrix (**a)**, anisotropic square (**b)**, dot matrix circles (**c)**, and 20-point circles (**d)**. The nucleus (**Panel 1**), myosin IIa (**Panel 2**), and vinculin (focal adhesions) (**Panel 3**) are labeled within each cell. **Panel 4** shows the overlay of the different structures.

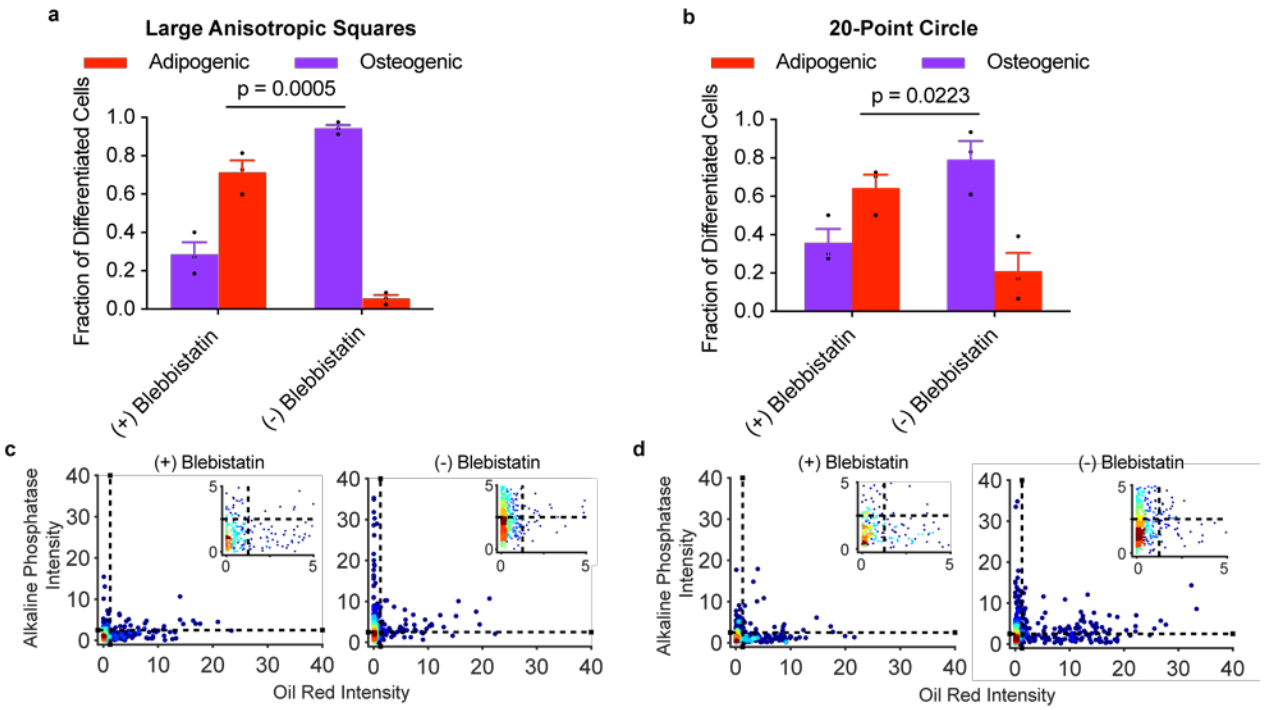

Figure S9. Myosin IIa inhibition with blebbistatin. Differentiation of hMSCs seeded on large anisotropic square (**a**) and 20-point circle (**b**) patterns with and without blebbistatin treatment. Scatter plots of staining intensity of individual cells, with and without treatment with blebbistatin, after color deconvolution of the red and purple channels from cells seeded on anisotropic square (**c**) and 20-point circle (**d**) patterns (n_{cells} = 321-849).