Supplementary Information

Rewiring mesenchymal stem cell lineage specification by switching the biophysical microenvironment

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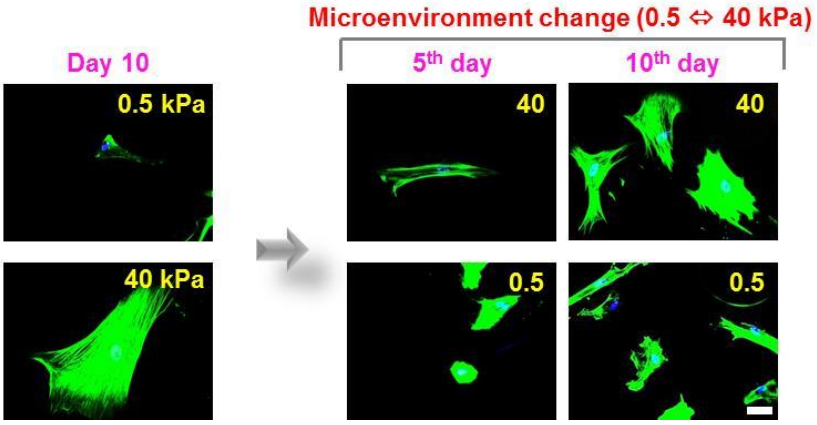
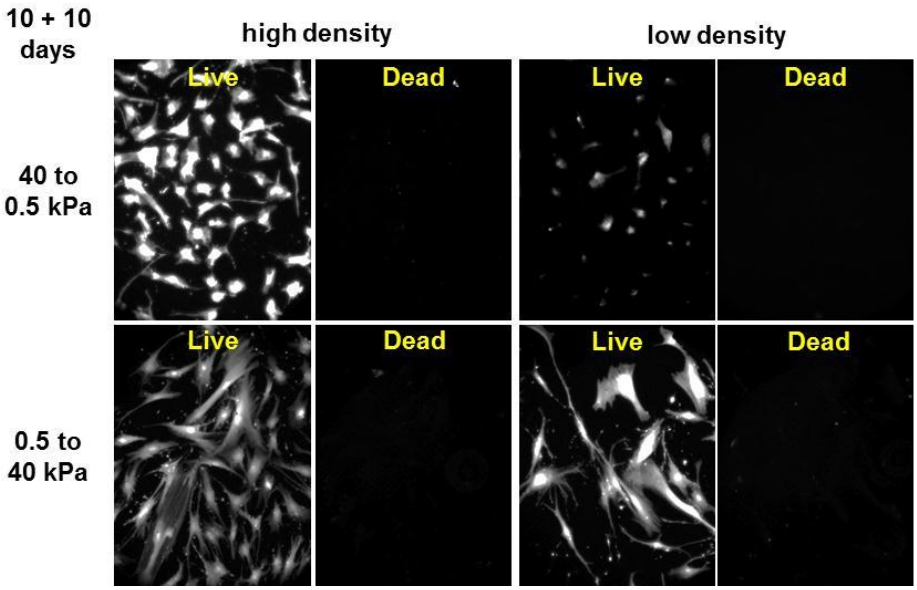
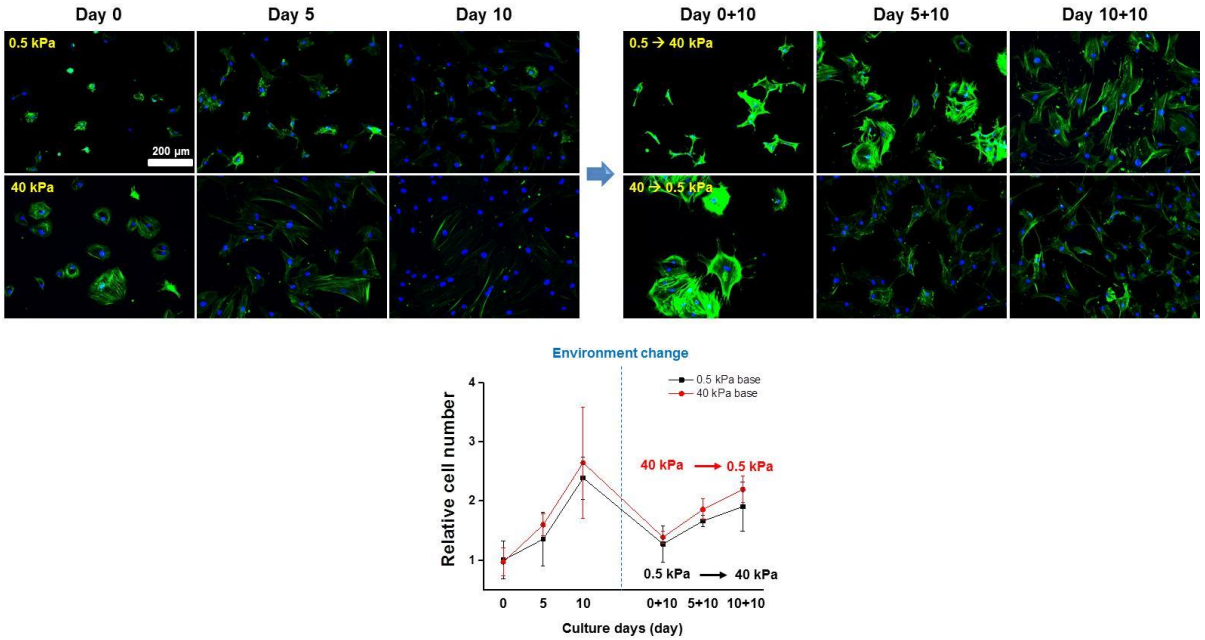


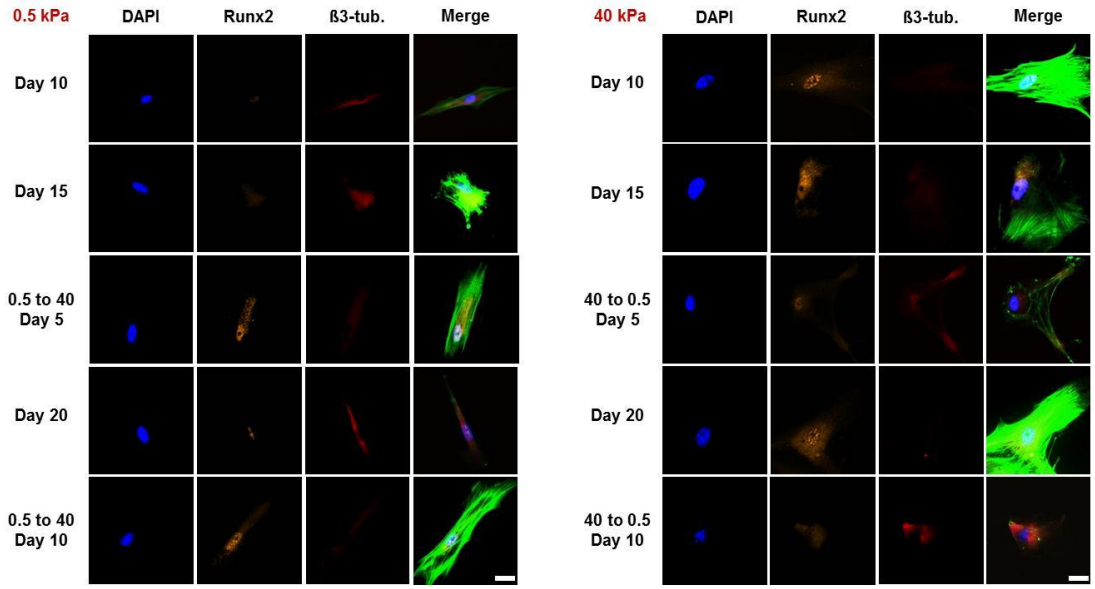
Supplementary Figure S1. Representative immunofluorescence microscopy images of MSCs cultured for 10 days and after the substrate switch (0.5 ⇔ 40 kPa); scale bar: 80 μm.



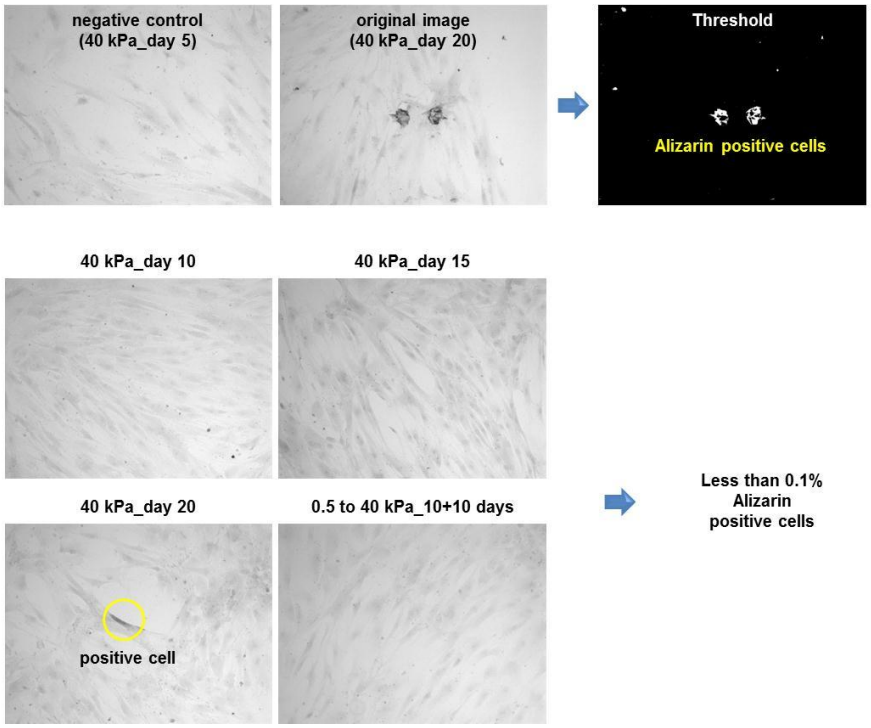
Supplementary Figure S2. Representative live/dead cell images of MSCs with high and low cell density after microenvironment change (0.5 ⇔ 40 kPa).



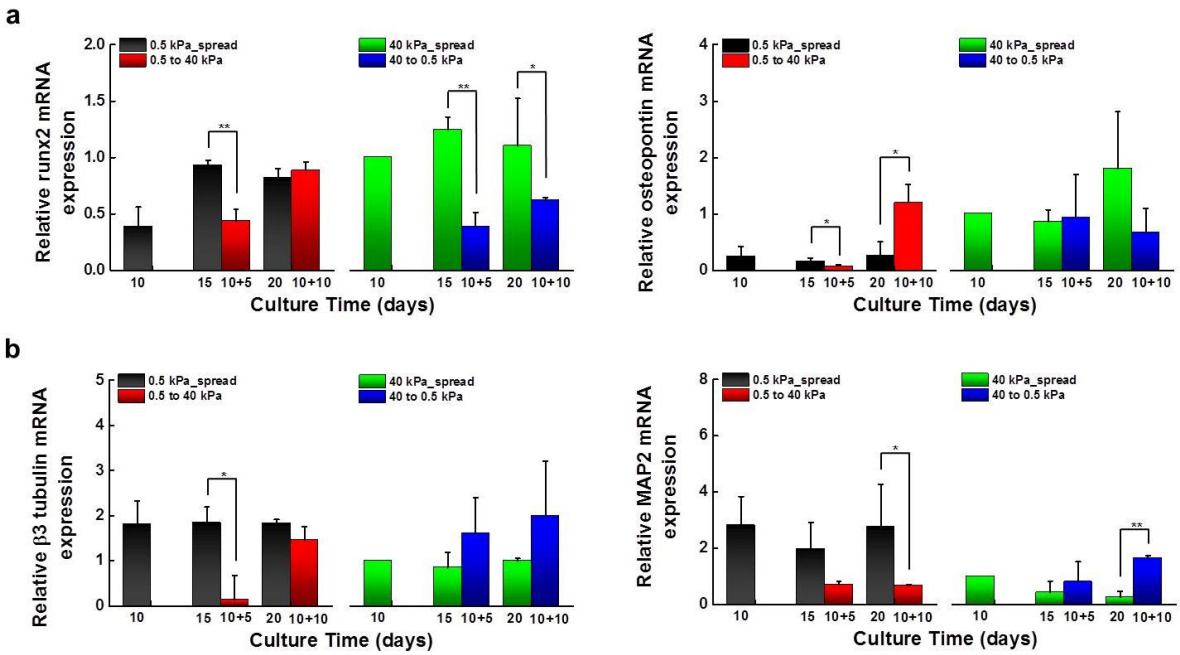
Supplementary Figure S3. Representative fluorescence images show a number of cells on soft (0.5 kPa) or stiff (40 kPa) substrates before and after microenvironment changes. Relative cell numbers increase with cultural days before microenvironment changes, decrease right after the changes, and then increase.



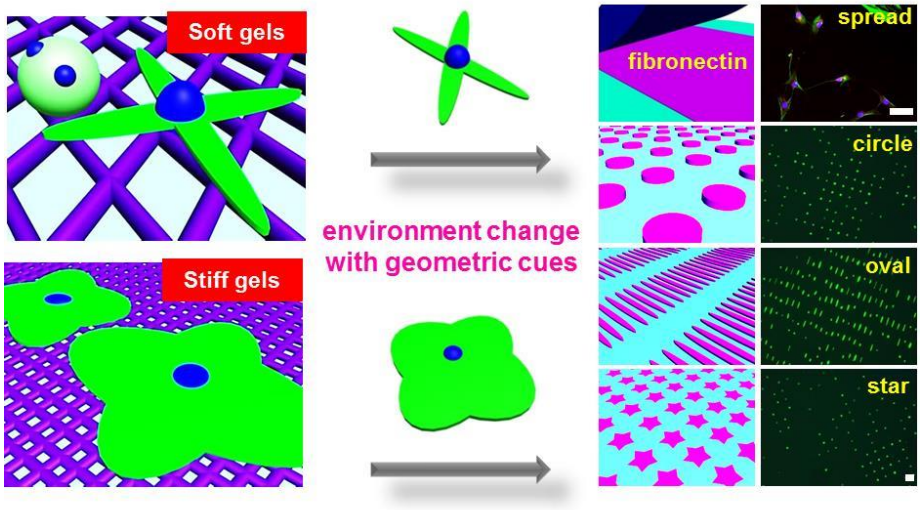
Supplementary Figure S4. Representative immunofluorescence microscope image of MSCs cultured on the unpatterned fibronectin coated substrates after immunostaining for nuclei, runx2, ß3-tubulin and filamentous actin; staining for MSC nuclei (blue), actin (cyan-green), runx2 (orange), ß3-tubulin (red).



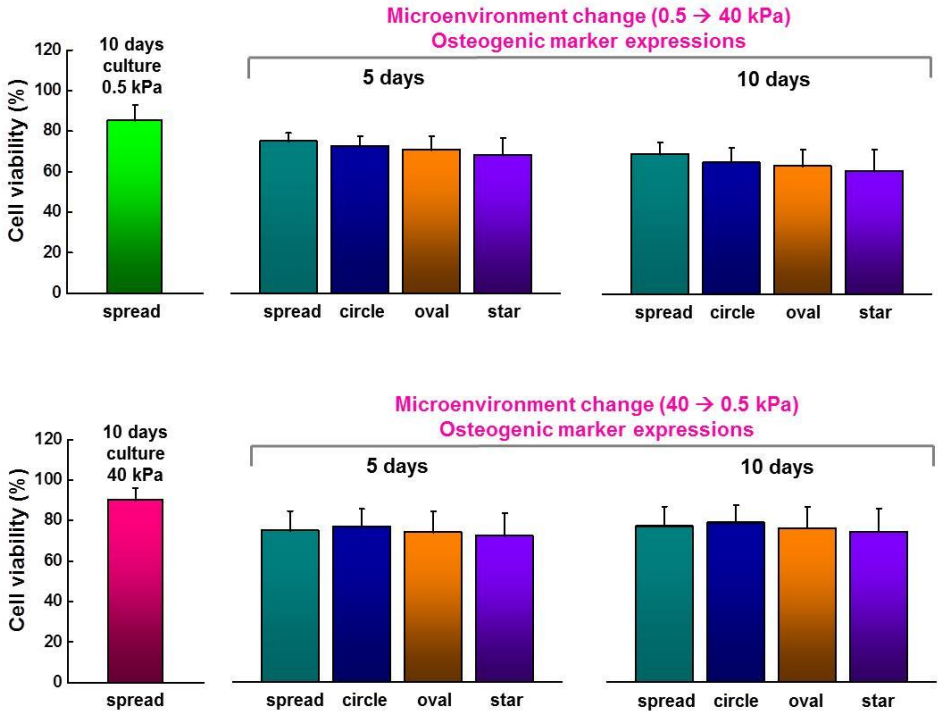
Supplementary Figure S5. Representative optical microscope images show alizarin-stained cells (40 kPa). Although alizarin positive cells exist after day 20 on stiff substrates, the percentage is below than 0.1%. Cells cultured 5 days on stiff substrates were used as minus control and threshold were obtained after image inverter and above intensity of control.



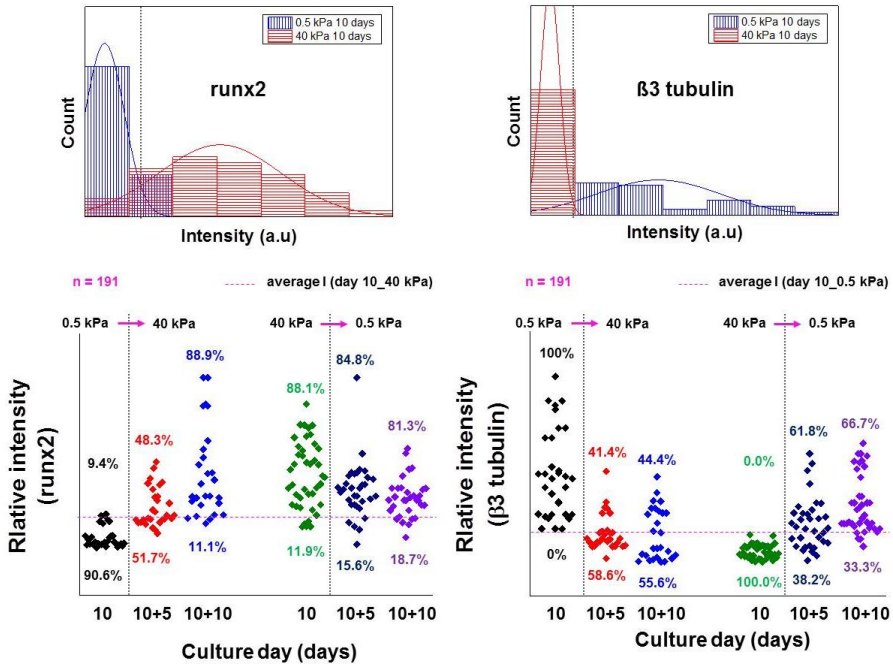
Supplementary Figure S6. Lineage-specific gene expression analysis of MSCs with and without microenvironmental change. (a) Results of real-time PCR to measure the gene expression of runx2 and osteopontin as early and late indicators of osteogenesis of MSCs, respectively (*P<0.05, **P<0.005, Fisher's exact test). (b) Results of real-time PCR for quantitation of β-tubulin and MAP2 as early and late indicators of neurogenesis mRNA expression of MSCs, respectively (*P<0.05, **P<0.005, Fisher's exact test).



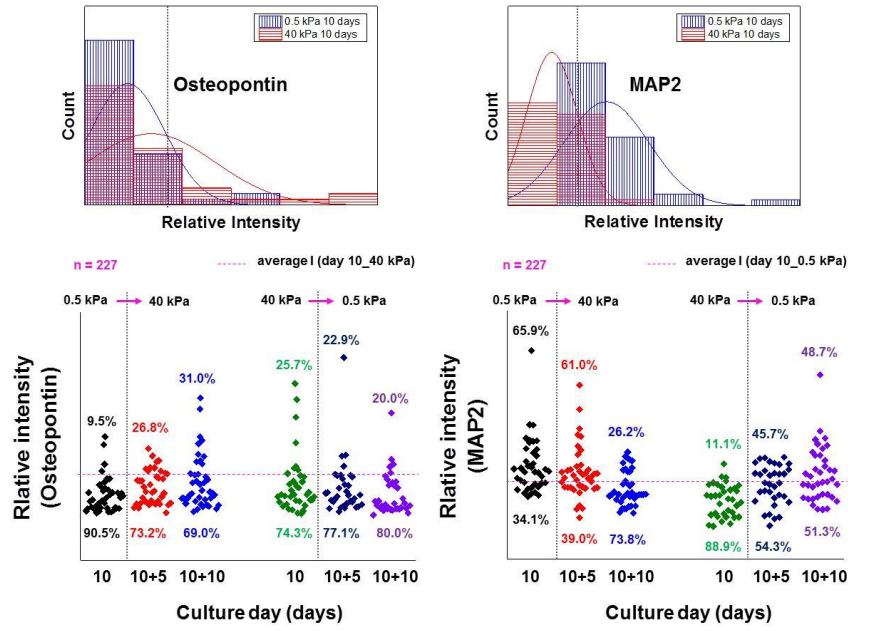
Supplementary Figure S7. Combining matrix stiffness and geometric cues to study stem cell plasticity of lineage specification. Schematic illustration of mechanical microenvironment changes of MSCs between soft (0.5 kPa) and stiff (40 kPa) substrates with geometric cues to control stem cell lineage commitment; scale bar: 120 μm (top), 700 μm (rest).



Supplementary Figure S8. MSC viability when cultured in patterns (circle, oval, star) after changing the microenvironment (0.5 ⇔ 40 kPa).



Supplementary Figure S9. Population analysis of MSCs stained for early osteogenic (runx2) and neurogenic (β-tubulin) markers before and after switching the substrate (0.5 ⇔ 40 kPa). The numbers on the scatter plots indicate the percentage of cells above a threshold intensity used to designate lineage specification. Threshold intensities are obtained by comparing histograms between the marker intensity of MSCs on soft and stiff substrates before switching microenvironments.



Supplementary Figure S10. Population analysis of MSCs stained for late osteogenic (osteopontin) and neurogenic (MAP2) markers before and after switching the substrate (0.5 ⇔ 40 kPa). The numbers on the scatter plots indicate the percentage of cells above a threshold intensity used to designate lineage specification. Threshold intensities are obtained by comparing histograms between the marker intensity of MSCs on soft and stiff substrates before switching microenvironments.