Supplementary Online Materials

Microfluidic artificial “vessels” for dynamic mechanical stimulation of mesenchymal stem cells

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Supplementary Materials include Supplementary Figures 1-8 and Supplementary Method.

Supplementary Figure S1. Process flow for the fabrication of microfluidic artificial “vessel” chips.

Supplementary Figure S2. Phase contrast images showing human mesenchymal stem cells cultured on the microchip statically for 1 day and under continuously cyclic mechanical stimulation for 2 and 6 more additional days (total 3 and 7days), respectively.

Supplementary Figure S3. Schematic depiction of cell signaling pathways including TGF-β/SMAD and canonical Wnt/β-catenin.

Supplementary Figure S4. Fluorescence images of all 4 signaling proteins, i.e., β-catenin, GSK-3β, SMAD2, and SMAD1, in response to dynamic mechanical stimulation after 7-day culture.

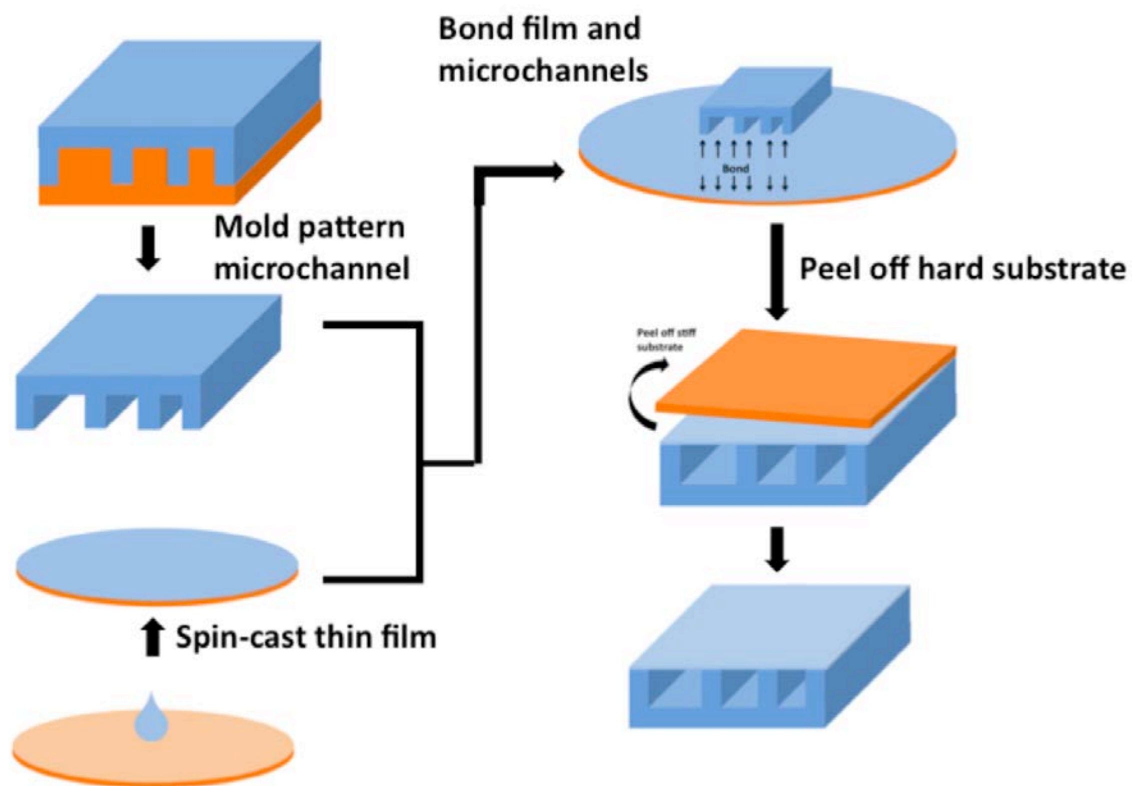
Supplementary Figure S5. Fluorescence images showing SMAD2 signaling in response to cyclic mechanical stimulation.

Supplementary Figure S6. Quantitative analysis of SMAD2 signaling in response to cyclic mechanical stimulation on two devices.

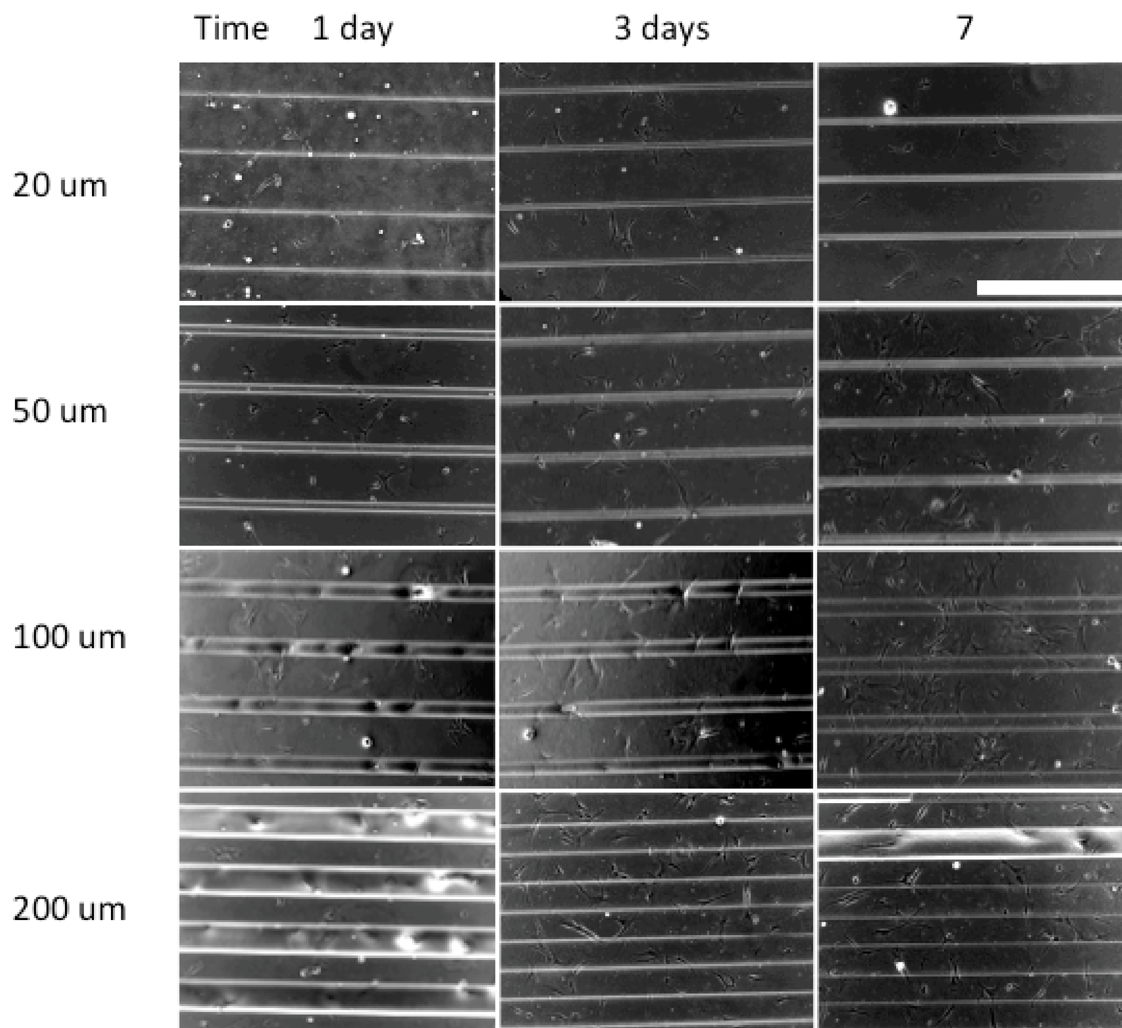
Supplementary Figure S7. β-catenin signaling in response to cyclic mechanical stimulation.

Supplementary Figure S8. Co-staining of SMAD2 and Phospho-SMAD2 signaling in response to cyclic mechanical stimulation at day 7.

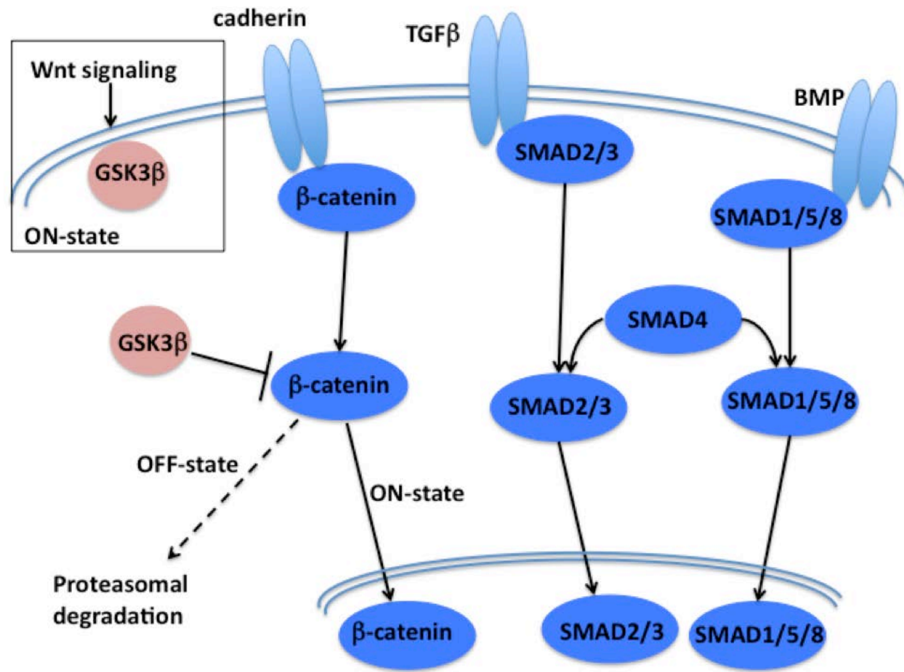
Supplementary Method: How to estimate the average circumferential strain generated on the suspended membranes using a hydrostatic pressure?



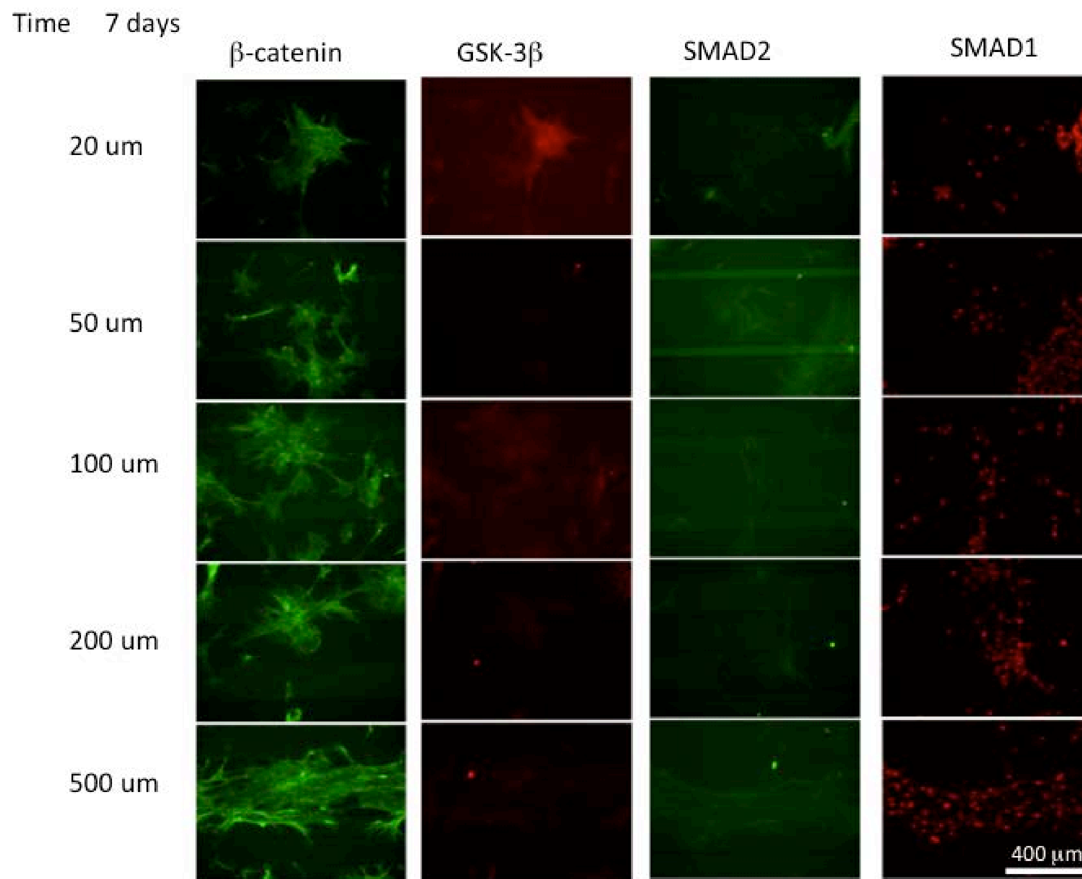
Supplementary Figure S1. Process flow for the fabrication of microfluidic artificial "vessel" chips.



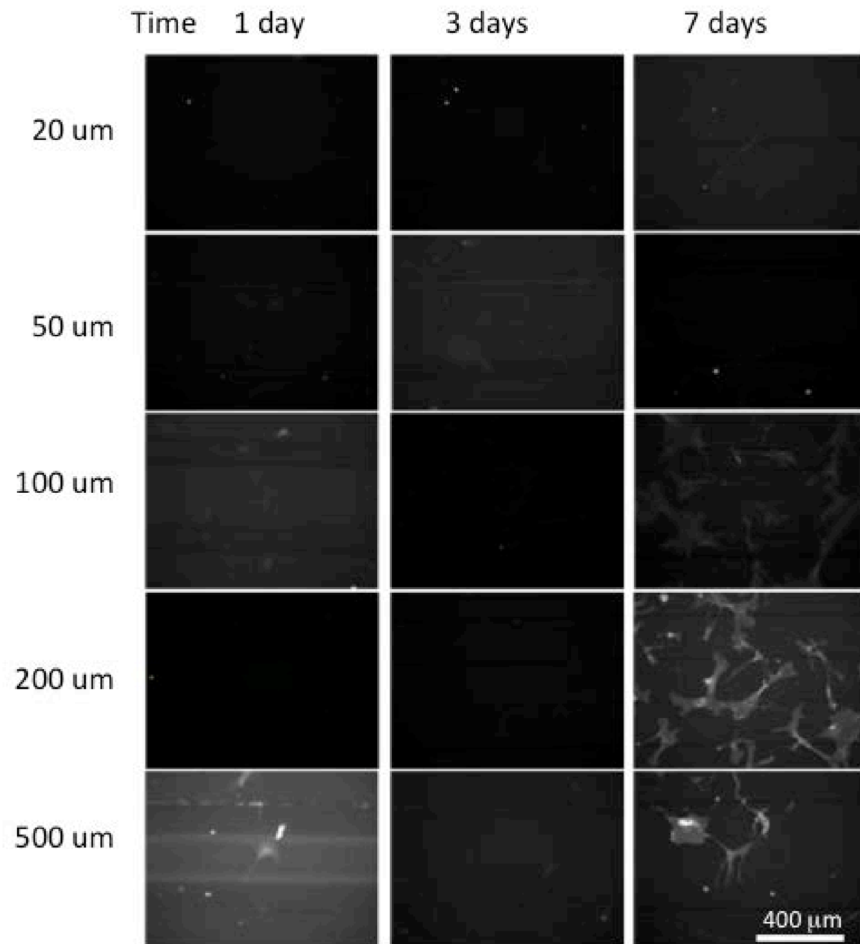
Supplementary Figure S2. Phase contrast images showing human mesenchymal stem cells cultured on the microchip statically for 1 day and under continuously cyclic mechanical stimulation for 2 and 6 more additional days (total 3 and 7days), respectively. Cells on different artificial "vessels" (20, 50, 100 and 200 μm in width) were imaged. Scale bar = 1000 μm.



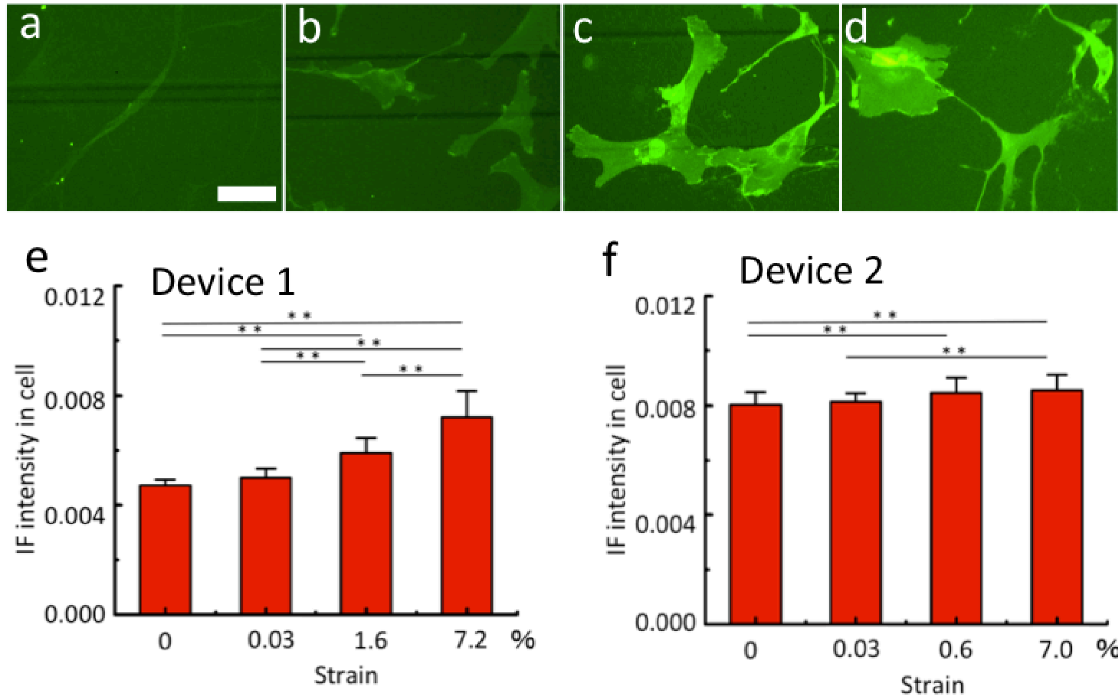
Supplementary Figure S3. Schematic depiction of cell signaling pathways including TGF-β/SMAD and canonical Wnt/β-catenin.



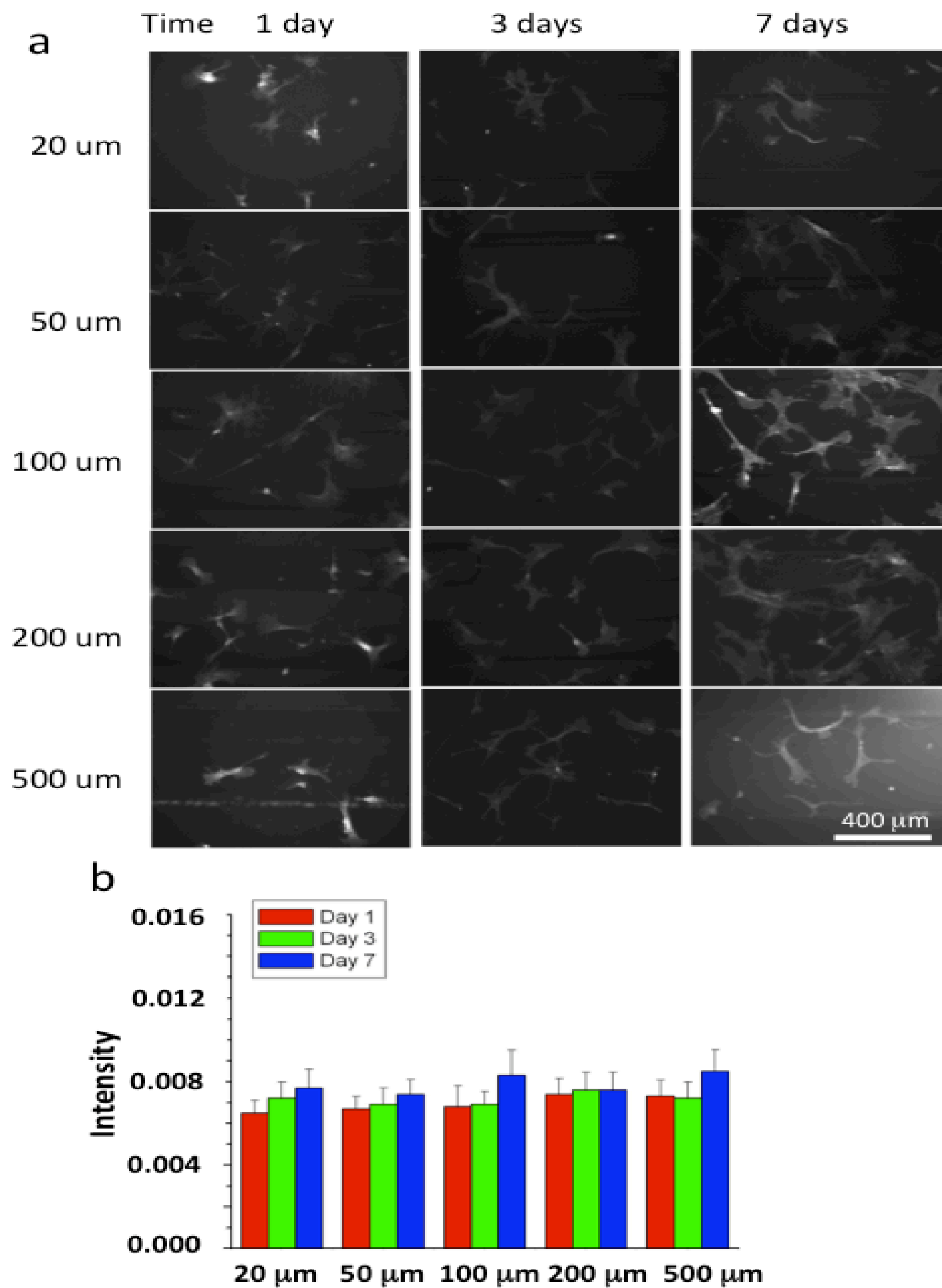
Supplementary Figure S4. Fluorescence images of 4 signaling proteins, i.e., β-catenin, GSK-3β, SMAD2, and SMAD1, in response to dynamic mechanical stimulation after 7-day culture.



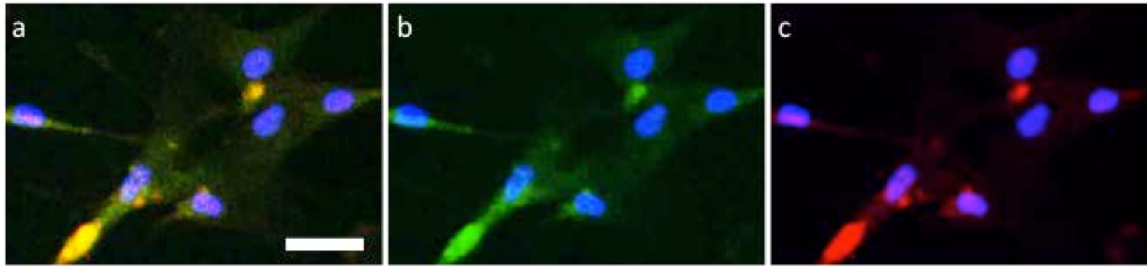
Supplementary Figure S5. SMAD2 signaling in response to cyclic mechanical stimulation. Fluorescence images showing SMAD2 in mesenchymal stem cells cultured on microfluidic "vessels" of different dimensions and stimulated for various periods (1, 3, and 7 days).



Supplementary Figure S6. Quantitative analysis of SMAD2 signaling in response to cyclic mechanical stimulation on two devices. **a-d.** Fluorescence images showing that the level of SMAD2 is apparently upregulated in response to cyclic mechanical stimulation at strain = 0, 0.03%, 1.6%, and 7.2% (Device 1, 7 days). (scale bar = 100 μm) **b**. and **c.** Quantitative analysis of SMAD2 intensity in MSCs studied on Device 1 and Device 2 over 7 days, respectively.



Supplementary Figure S7. β-catenin signaling in response to cyclic mechanical stimulation. **a.** Fluorescence images showing β-catenin in mesenchymal stem cells cultured on microfluidic "vessels" with different dimensions and stimulated for various periods (1, 3, and 7 days). **b.** Quantitative analysis of all the MSCs cultured for different times showing no significant change of the intensity of β-catenin during the whole culture.

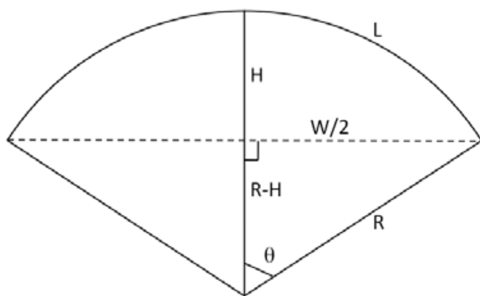


Supplementary Figure S8. Co-staining of SMAD2 (green) and Phospho-SMAD2 (red) signaling in response to cyclic mechanical stimulation at day 7 (a). (b) SMAD2 with nuclei and (c) Phospho-SMAD2 with nuclei indicate that SMAD2 signal overlaps with Phospho-SMAD2 signal, although the latter one is weaker. Nuclei are stained with DAPI (blue). (Scale bar = 50 μm)

Supplementary Method

How to estimate the average circumferential strain generated on the suspended membranes using a hydrostatic pressure?

To estimate the average circumferential strain in the suspended membrane, we assume the deformation of the suspended membrane takes an ideal circular contour, which is approximately suitable for the membranes under small deformation and minimal edge effect (wide channels), e.g., 500 μm channels under a pressure of 3 psi (21 kPa) (the typical experimental condition in our work). The height change of the center of the membrane under a given pressure was measured by a fine micrometer equipped on a light field microscope. Then we can compute the arch width of the deformed membrane and compare to the original membrane to calculate the average circumferential strain by assuming that the strain is relatively uniform over the whole membrane. This assumption is valid for wide membrane with the region of the edges effect is small. The majority of the membrane experiences a constant hydrostatic pressure exerted by the external force applied at the inlets. In order to meet the force balance anywhere on the membrane, the local strain of membrane in the circumferential direction has to be relatively uniform. To count for the condition of large deformation, i.e., > 5% strain, we use true strain ($\varepsilon_{true} = \ln(1+\varepsilon_{engineering})$) to show the effect of circumferential stretch.



Schematic depiction showing the cross-section of a microchannel before (dashed line) and after the application of a hydrostatic pressure (solid line).

W = the width of the channel
H = the change of the height at the center of the membrane under pressure
W = the channel width
R = the radius of the inflated membrane under pressure
L = half of the arch length of the inflated membrane under pressure
θ = half of the angle of the inflated membrane according to its origin

W is defined by the device design, and H is measured by a micrometer equipped on a light field microscope. The other parameters can be deduced from W and H.

Since

$$R^2 = (W/2)^2 + (R-H)^2,$$

then

$$R = \frac{W^2}{8H} + \frac{H}{2},$$

and

$$\theta = a\tan[\frac{W}{2(R-H)}].$$

The half of the length change of the suspended membrane is

$$\Delta L = L - W/2 = \theta \cdot R - W/2$$

The engineering circumferential strain in the membrane is defined as

$$\varepsilon = \Delta L/(W/2).$$

To consider larger deformation, i.e., $> 5\%$ strain, true strain is used to describe the deformation of the membrane, which is defined as

$$\varepsilon_{true} = \ln(1 + \varepsilon_{engineering}).$$

Using the gold nanoparticle tracers and a simple light microscope imaging approach, we measured the height change of the center of membrane in response to the pressure applied. Then we can compute the estimated average circumferential strain using the equations described above.