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Supplementary Materials for

Curvature and Rho activation differentially control the alignment of cells and stress fibers

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This PDF file includes:

Supplementary Material

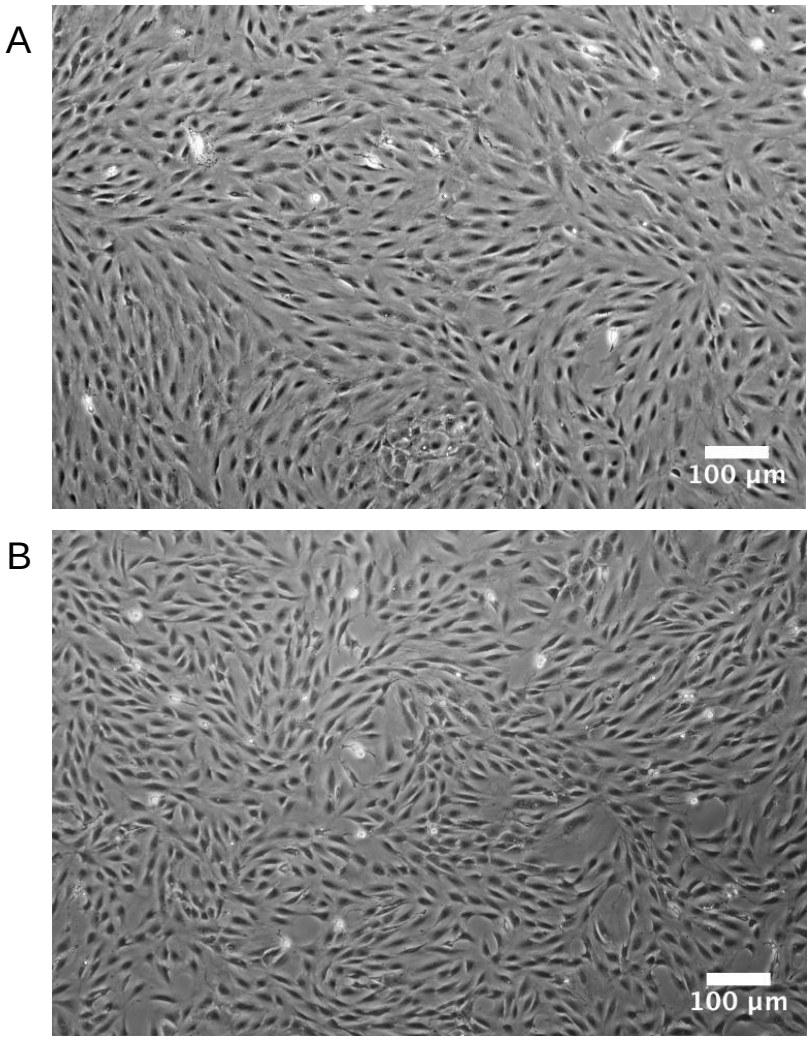

fig. S1. MEFs and hVSMCs coalign on planar surfaces. Phase contrast image of MEFs (**A**) and hVSMCs (**B**) exhibiting nematic order on a flat tissue culture plastic surface.

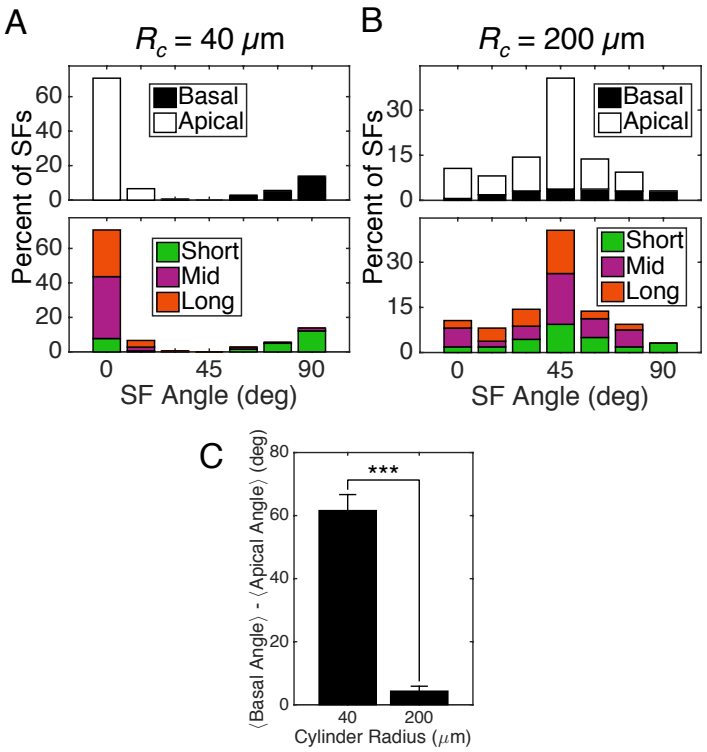

fig. S2. Apical and basal SFs align in distinct patterns in MEFs in response to curvature. Angle distribution plots showing the directions in which basal and apical SFs are oriented (top) on small (**A**) and large (**B**) cylinders. The bottom plots show the longest 30% (Long; 22.4 μm ≤ $L_{40\mu m}$ ≤ 64.0 μm; 19.4 μm ≤ $L_{200\mu m}$ ≤ 41.5 μm), shortest 30% (Short; $L_{40\mu m}$ ≤ 12.3 μm; $L_{200\mu m}$ ≤ 10.0 μm), and the remaining 40% (Mid) SFs, where L_{Rc} denotes the length of stress fibers on cylinders with radius R_c. Axial and circumferential orientations are given by orientation angles of 0˚ and 90˚, respectively. (**C**) Difference between the mean orientation angle of basal and apical SFs within individual cells. At least 165 SFs in 5 cells analyzed for each condition. Results are mean and SE, *** $p \leq 0.001$ (Student's t-test).

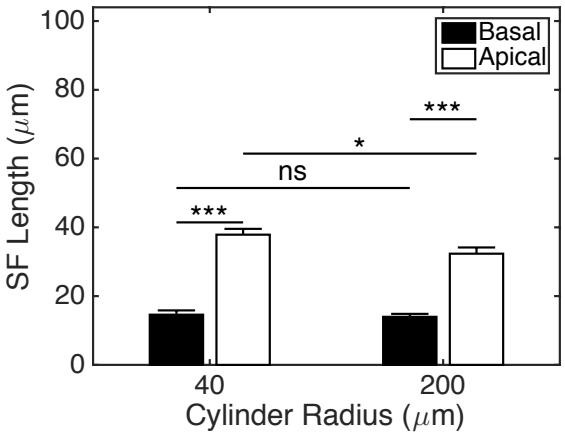

fig. S3. Lengths of apical and basal SFs in hVSMCs on small and large cylinders. At least 272 SFs in at least 10 cells analyzed in each condition. Results are mean and SE, * $p \leq 0.05$, *** $p \leq 0.001$ (Student's t-test).

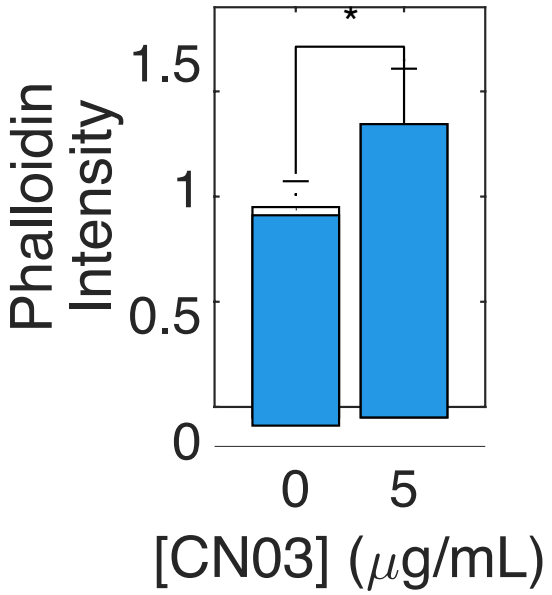

fig. S4. Phalloidin intensity of isolated hVSMCs on coverslips treated with CN03. At least 22 cells were analyzed in each condition in each of four independent experiments. Results show mean and SE, * $p \leq 0.05$ (Student's t-test).

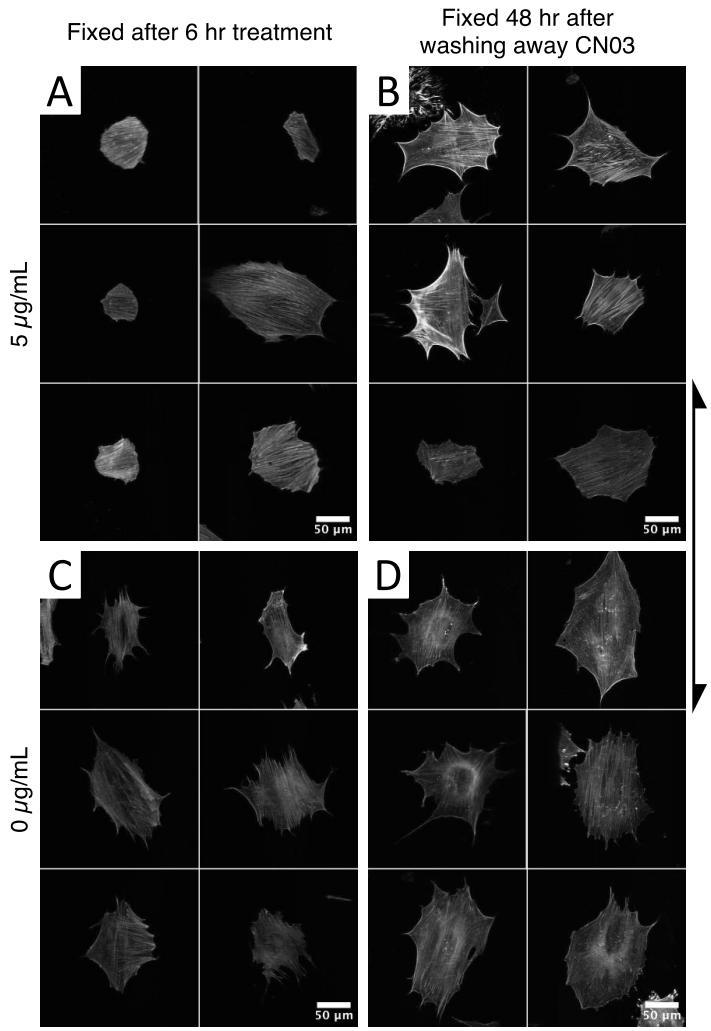

fig. S5. F-actin organization is not recovered after CN03 washout. Six representative mapped images of phalloidin-stained hVSMCs on cylinders with R_c = 125 μm. Cells treated with 5 μg/ml CN03 had predominantly basal SFs oriented in the circumferential direction both after being treated for 6 hours (**A**) and 48 hours after washing away the activator (**B**). The apical, axially-oriented SFs were more prominent in control cells (0 μg/ml) at both time points (**C** and **D**). Arrow indicates cylinder axis orientation.

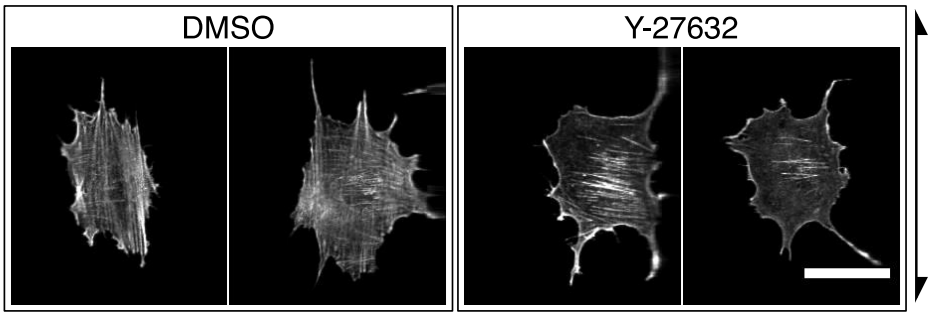

fig. S6. Basal SFs remain after inhibition of ROCK. Serum-starved MEFs were seeded on cylinders with R_c = 40 μm overnight before being treated with 10 μM Y-27632 or an equivalent concentration of DMSO for 6 hours. Two representative mapped images are shown for each condition. Arrow indicates cylinder axis orientation. Scale bar is 50 μm.

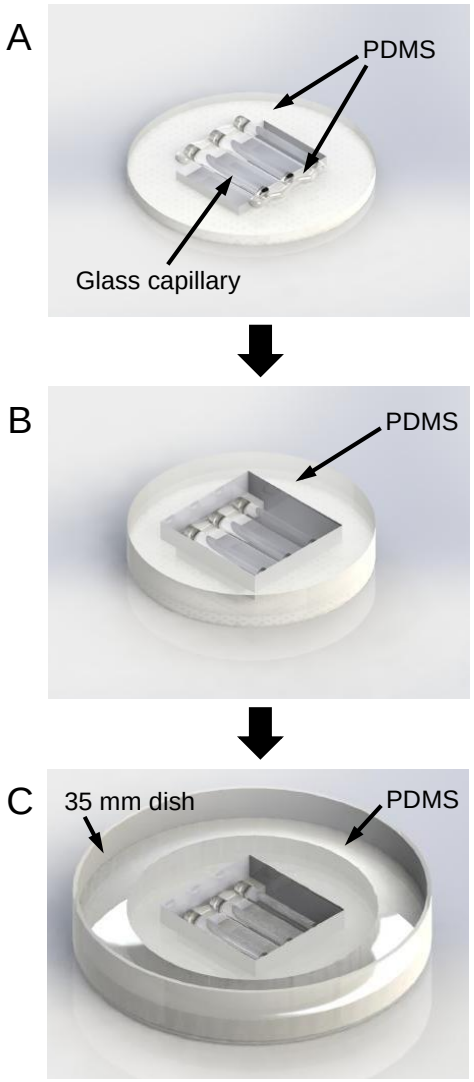

fig. S7. Assembly of cylinder substrates. (**A**) A 5 mm × 10 mm hole is first cut out of the center of a 1 mm thick slab of PDMS that is 25 mm in diameter. Then, 8 mm long sections of capillary tubes are placed over the hole and secured in place using liquid PDMS as the adhesive. (**B**) A 10 mm x 10 mm hole is cut out of the center of a second slab of PDMS; this second slab is also 25 mm in diameter but is 2 mm thick. Once the PDMS securing the cylinders has cured, the second slab is placed on top of the first slab. (**C**) The two-slab structure is placed in a 35 mm dish and secured in place by pouring liquid PDMS around the structure and curing it.