Biophysical Journal, Volume *114*

Supplemental Information

Gaussian Curvature Directs Stress Fiber Orientation and Cell Migration

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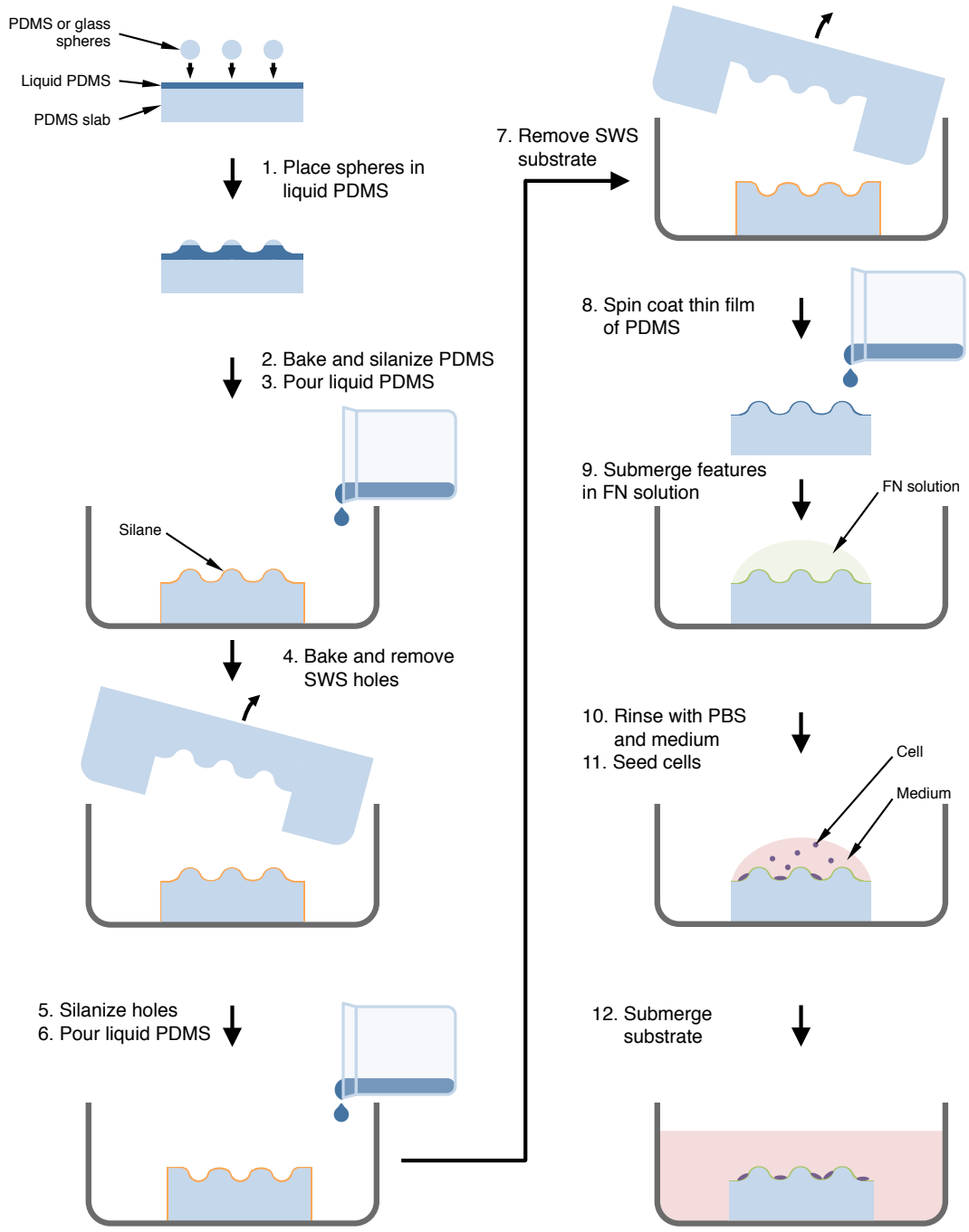

Figure S1: Preparation of an SWS substrate

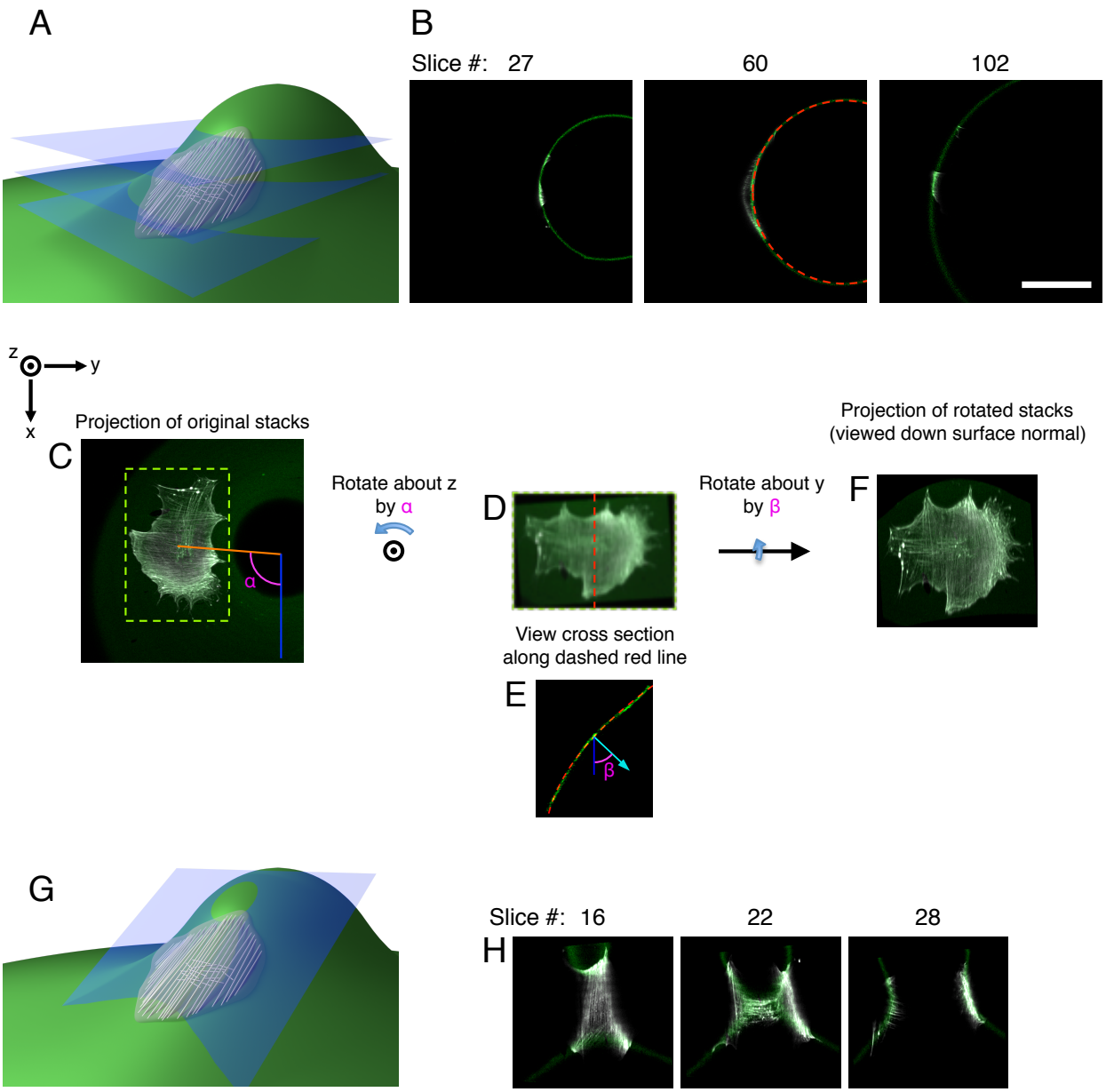

Figure S2: Processing of Confocal Stacks for SF Alignment Analysis. (A) Schematic of the actin cytoskeleton of a cell on an SWS skirt. Green: fluorophore-labeled FN on SWS surface; solid grey: SFs; transparent grey: plasma membrane; blue: planes scanned by the laser scanning confocal microscope. Only three scan planes are shown for clarity. **(B)** Three representative slices from the stack of confocal images. Slices roughly correspond to the three planes shown in A. Green: Alexa Fluor 647-labeled FN; grey: phalloidin:TRITC. Red dashed line is a circle fit to the FN channel in the slice passing through the cell center. Slice step size: 0.6 µm. Scale bar: 50 µm. All images in this figure are to scale with this figure except A and G. **(C)** Maximum intensity projection of the raw FN and F-actin channels. Dashed chartreuse lines represent the bounding rectangle at which the stacks are cropped. The orange line connects the center of this bounding rectangle to the center of the fit circle shown in B (i.e., the center of the radially-symmetric SWS). The angle α defines the angular position of the cell in the original scan. **(D)** Cropped region of the projection rotated about the z-axis by the angle α. The red dashed line is the radius of the SWS through which the FN stack is sliced. **(E)** Cross section of the FN stack along the red dashed line in D. Here, the red dashed line is the parabola fit to the FN cross section. The normal vector to the surface (cyan arrow) is calculated from the fit parabola. The angle between the normal vector and the x-axis, β, is also calculated. **(F)** Maximum intensity projection of the FN and F-actin stacks rotated about the y-axis by the angle β. The plane tangent to the surface at the center of the cell is now parallel to the plane of the page. The rotated stacks are resliced along parallel planes in

Fiji. All SFs are analyzed after undergoing these transformations. **(G)** A blue plane represents the direction along which the cell and SWS in A are resliced after the series of transformations. Transformations and reslicing were performed using Fiji's TransformJ function. **(H)** Three representative slices from the rotated and resliced stacks of FN (green) and phalloidin (grey).

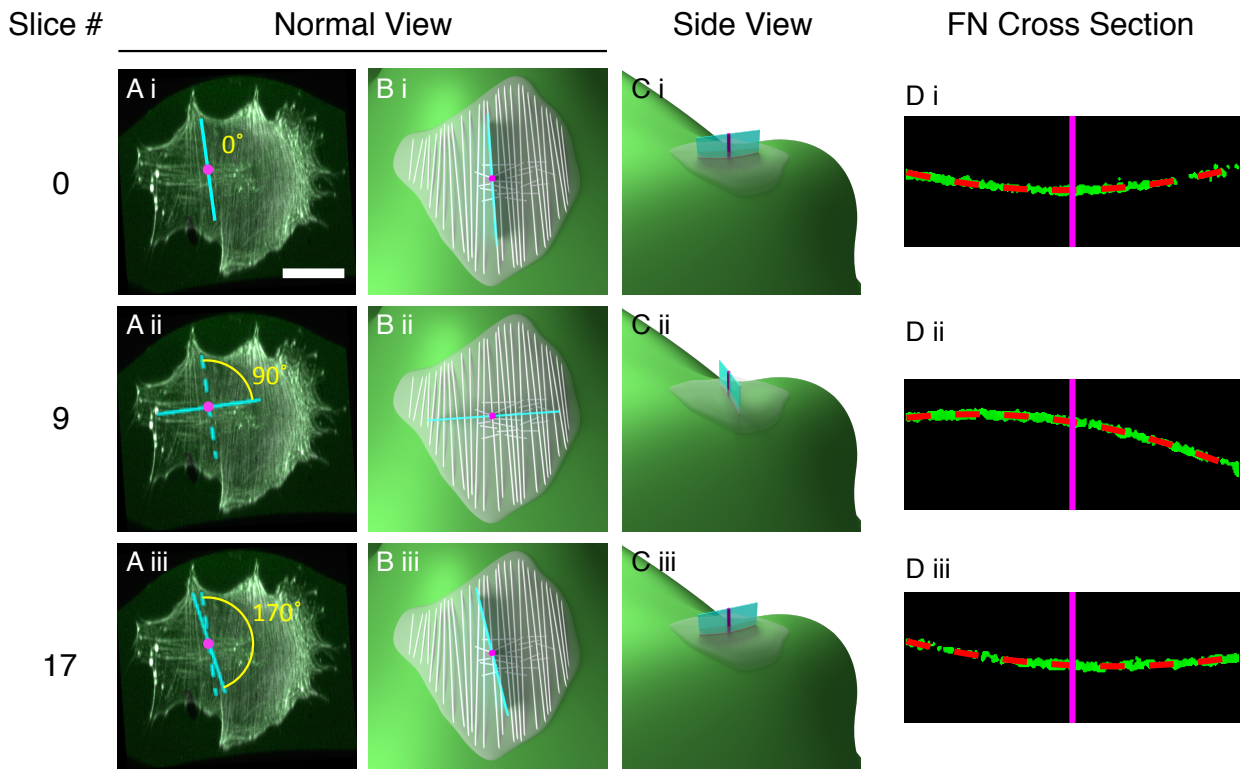

Figure S3: Method of reslicing FN confocal stacks. Three representative slices are shown from different perspectives. **(A)** Maximum intensity projections of the merged Alexa Fluor 647-labeled FN (green) and phalloidin:TRITC (grey) channels after undergoing the transformations described in Fig. S2. The cyan line is a 50 μm-long line along which the FN channel is resliced. In Ai, this line is oriented along and centered on an SF of interest. Three-dimensional models of the system described in A are shown from two different perspectives in **B** and **C**. Solid grey cylinders represent SFs and the transparent grey surface represents the plasma membrane. The FN-coated SWS surface is green. In Bi, the cyan reslicing plane intersects the SWS surface along the contour highlighted by the solid red line in C. The cross section of the FN stack along the cyan plane is shown in Di. The green dots are the processed FN data points and the red dashed line is a parabola fit to the points. Reslice angles are measured in the clockwise direction from the original orientation of the reslice line. Figs. Aii-Dii show the same data for a reslice angle that is 90˚ from the original reslice line. In Aii, the dashed cyan line represents the orientation of the SF and the solid cyan line represents the reslice line. The magenta dot in Aii is the center of the reslice line about which the line is rotated. Finally, Aiii-Diii show a reslice angle of 170˚ for the same SF.

The curvature of the surface at any reslice angle is calculated from the fit parabola where the parabola intersects the magenta line. Note the change in sign of the curvature: the curvature is positive in Di and Diii, but negative in Dii; these two changes in sign while reslicing from 0˚ to 170˚ are also shown in Fig 1E. Only three reslice angles are shown here, but the FN channel was resliced 18 times (0˚-170˚ in 10˚ intervals) for each SF in the complete analysis (see Fig. 1D,E).

In C, SFs are excluded for clarity. Scale bar: 30 μm.

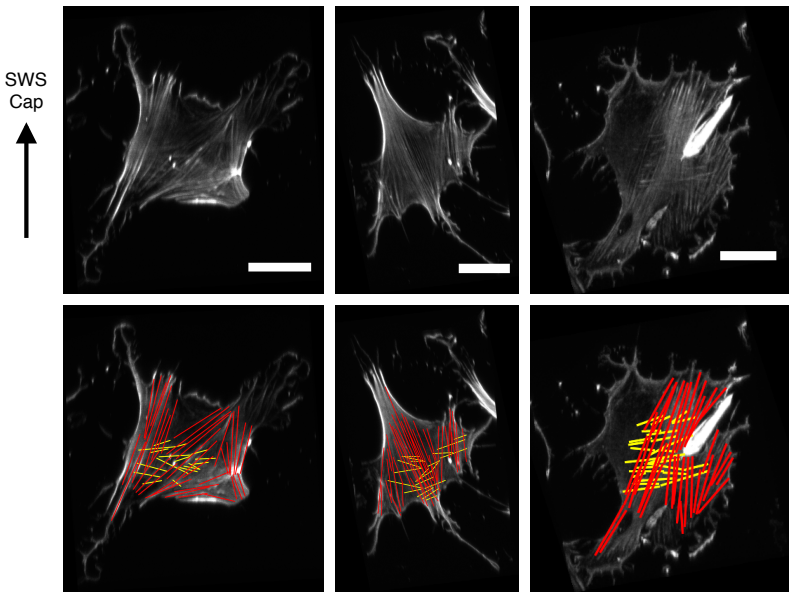

Figure S4: Human vascular smooth muscle cells on SWS skirts. Three representative, rotated projections of human vascular smooth muscles cells on SWS skirts. Grey: phalloidin:TRITC; red: apical SFs; yellow: basal SFs. Scale bars are 30 μm.

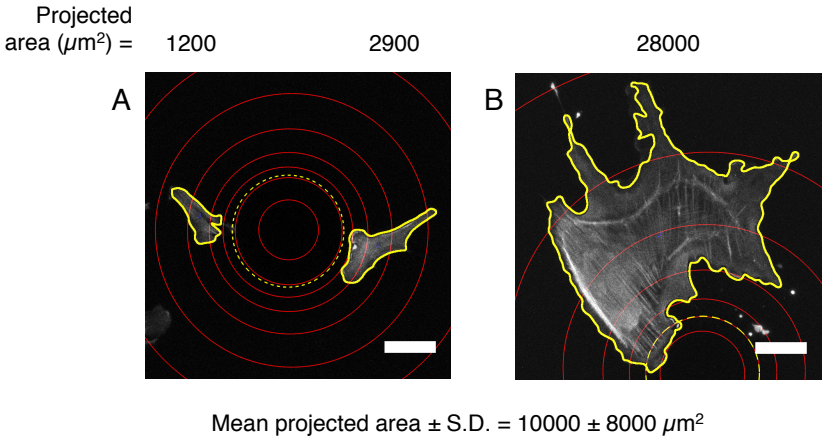

Figure S5: Size heterogeneity of primary Lifeact-GFP MEFs. (A) Two representative small cells on an SWS skirt. The left cell is the smallest cell analyzed. **(B)** Largest cell analyzed on a different SWS skirt. Grey is Lifeact-GFP. Red concentric circles indicate rings of common height separated by 14 µm in height. Yellow dashed ring indicates approximate location of line of inflection. Solid yellow lines are cell outlines generated by ADAPT. Scale bars are 50 µm.

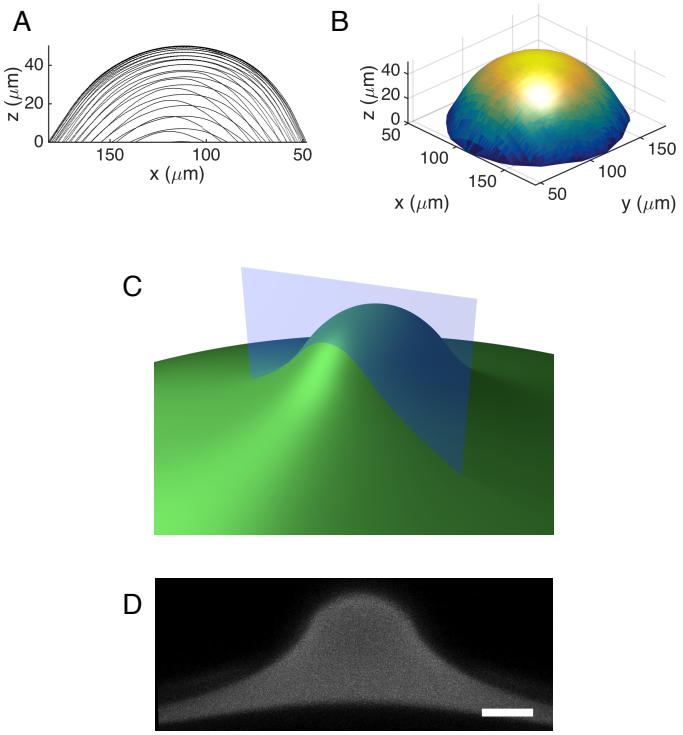

Figure S6: Characterization of SWS surface. (A) Overlaid profilometer scans of a representative SWS cap. **(B)** Surface visualization of the data in A. To examine the FN adsorbed to the surface near the line of inflection, SWS surfaces were scanned in the plane parallel to the surface's symmetry axis (**C**, blue plane). Green represents FN on the surface. **(D)** A projection of FN slices obtained by scanning the surface in the direction shown in C.

Movie S1: MEF on SWS skirt. Grey: phalloidin:TRITC; green: Alexa Fluor 647-labeled FN. Scale bar is 20 µm.

Movie S2: Projection of primary Lifeact-GFP MEFs migrating on SWS. Red concentric circles indicate rings of common height separated by 18 µm in height. Yellow dashed ring indicates approximate location of line of inflection. Grey is Lifeact-GFP. Total duration = 11.3 hr.

Movie S3: Projection of primary Lifeact-GFP MEF migrating on flat region between SWSs. Grey is Lifeact-GFP. Total duration = 11.8 hr.