Electronic Supplementary Material (ESI) for Soft Matter.

Supplementary Information

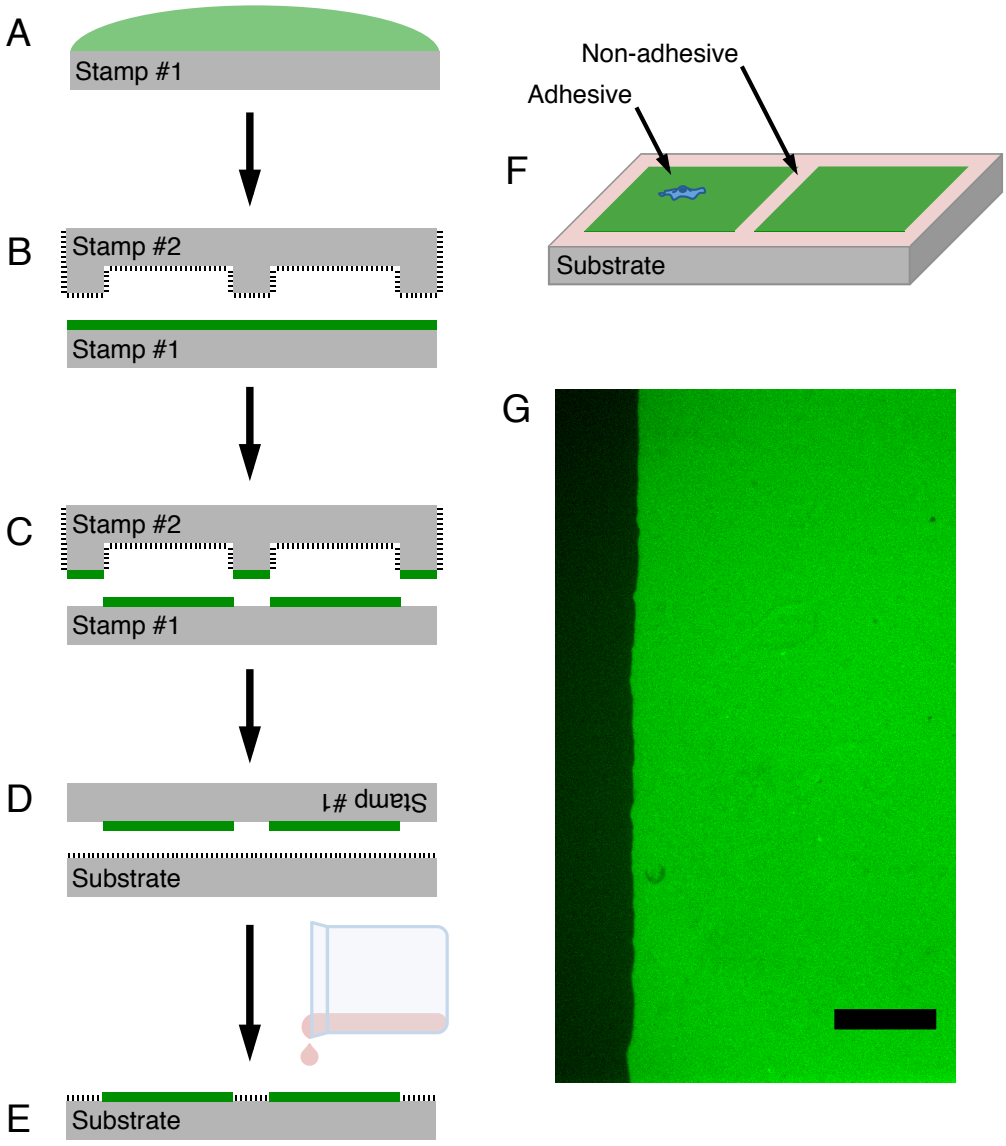

Figure S1: A stamp-off microcontact printing method creates edges free of FN gradients. (A) A flat PDMS stamp (Stamp #1) is inked by placing a sessile drop of labeled FN (green) on its surface. **(B)** A second stamp (Stamp #2) with holes of the shape of interest is treated with UV ozone to make the surface hydrophilic. Short black lines indicate hydrophilic regions of the surface. Stamp #2 is pressed onto Stamp #1. **(C)** Upon separation of the two stamps, FN from Stamp #1 is removed where the stamps were in contact. **(D)** The remaining FN on Stamp #1 is stamped onto a UV ozone-treated PDMS substrate. **(E)** The substrate surface is submerged in a solution of blocking surfactant (pink). **(F)** Cells (blue) are cultured on the printed substrate. The interface between FN and the blocking surfactant is the edge experienced by cells. **(G)** Representative edge generated using stamp-off method. Green: Alexa Fluor 488-labeled FN. Scale bar is 50 µm.

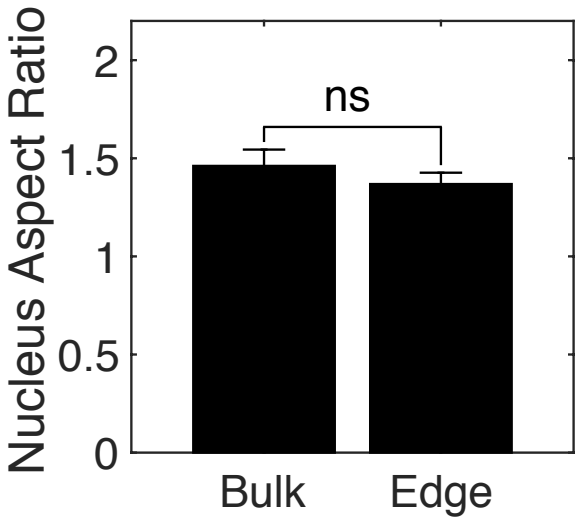

Figure S2: Edges do not affect nuclear elongation. The aspect ratio of NucBlue-stained nuclei in MEFs migrating in the bulk and at edges were measured. 10 cells in each of two experiments per condition. ns = not significant.

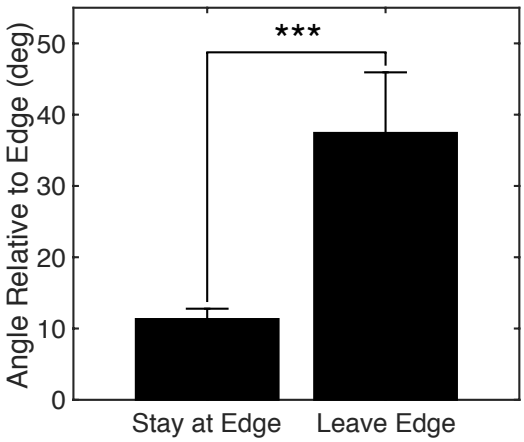

Figure S3: Cells that leave edges orient away from edge. Orientation angles of cells that remain at edges (n = 38) and cells that leave edges (n = 6) after encountering them were measured from ellipses fit to cytoplasmic mCherry signals. Student's t-test *** $p < 0.001$.

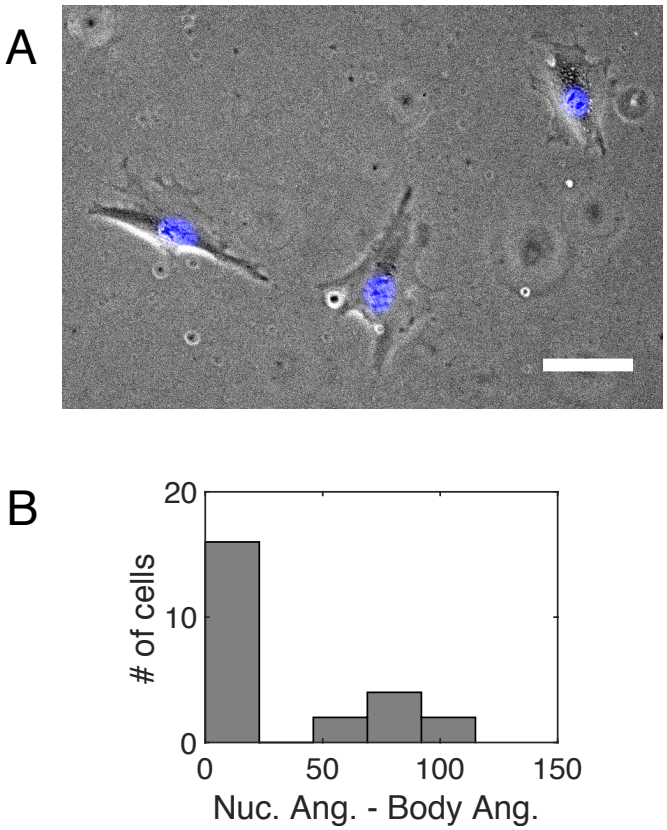

Figure S4: Nuclear orientation is a reasonable indicator of cell body orientation. (A) Representative phase contrast image of mCherry-infected MEFs on microcontact-printed FN island with NucBlue-stained nuclei (blue). (B) Difference between orientation angles of long axes of ellipses fit to nuclei and cytoplasmic mCherry signal averaged over the length of the experiment (≤ 6 hr). 24 cells analyzed in two independent experiments.

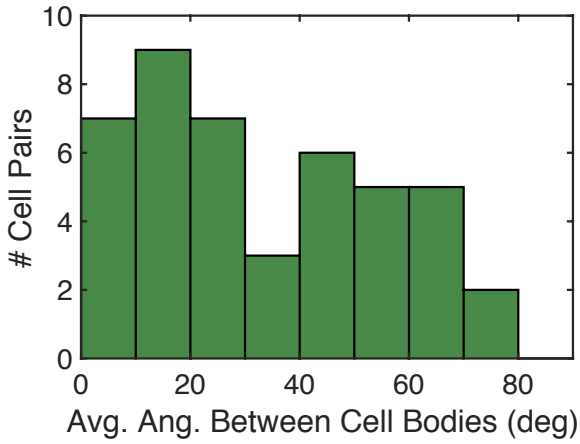

Figure S5: Cell bodies do not co-align over extended periods of time. Mean angle between the orientation of the long axes of cell bodies. 44 cell pairs analyzed in two independent experiments.

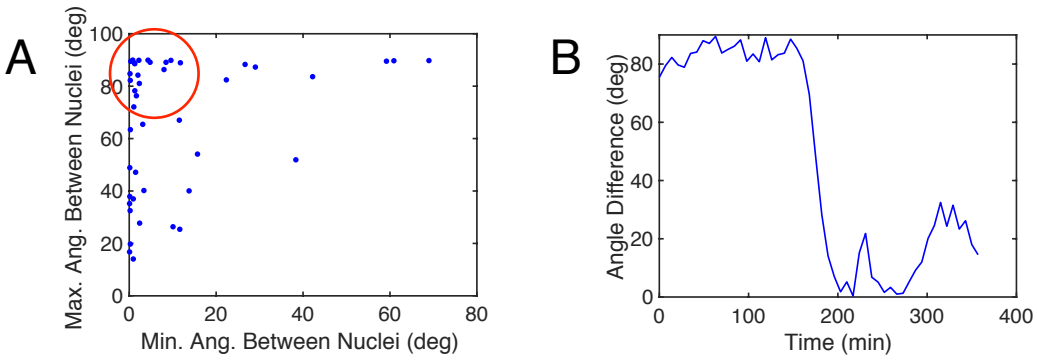

Figure S6: Relative nuclear orientation in cell pairs varies significantly over time. (A) The maximum and minimum angle difference between the two nuclear orientation angles for cells in pars. The red circle indicates the cells that were both nearly parallel and perpendicular to each other for at least a short period of time during the experiment. **(B)** Angle difference between the two nuclei vs. time for an example cell within the red circle in A.

Movie S1: MEF leaving an edge. This cell encounters an edge while oriented at an oblique angle and eventually leaves the edge. Red: mCherry. Green: FN. Duration: 14.7 hr.

Movie S2: MEF elongating at edge but not migrating. This cell remains at the edge over the entire period of observation, but does not migrate along the edge. Red: mCherry. Green: FN. Duration: 16.7 hr.

Movie S3: MEF migrating along edge. This cell persistently migrates along the edge over the entire observation period. Red: mCherry. Green: FN. Duration: 14.7 hr.

Movie S4: 1/2-integer defects emerge in confluent monolayers of MEFs. Three fields of view from three separate monolayers showing 1/2-integer defects. All three monolayers have -1/2 defects. The rightmost monolayer additionally has a +1/2 defect. One frame captured every seven minutes. Scale bar is 100 μm.

Movie S5: Movies of MEFs migrating at the two corners described in Fig. 3.