Supplemental Methods

Western Blots

Cells were plated in tissue culture dishes such that they were confluent after overnight culture. The cells were then lysed in a 20 mM Tris pH 7.5, 150 mM CaCl, 1 M EDTA, and 1% TritonX100 solution that was then supplemented with cOmplete Mini and PhosSTOP (Roche, Indianapolis, IN). After centrifugation (14,000 rpm, 15 min, 4°C), the protein concentration of the supernatant was determined by Bradford assay (BioRad, Hercules, CA). Proteins were separated using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and then were transferred to PVDF membranes. The membranes were blocked with 3% BSA (MP Biomedicals, Santa Ana, CA) in TBST and then incubated in primary antibody (overnight in anti-E-cadherin 1:8000 (Invitrogen) or three hours in anti-actin 1:60,000 (Chemicon)) in 0.3% TBST followed by incubation with a horseradish peroxidase (HRP) conjugated antibody (anti-mouse 1:10,000 (Pierce, Rockford, IL)) for one hour. The antigens were visualized using SuperSignal West Pico Chemiluminescent Substrate (ThermoFisher Scientific, Waltham, MA)

Immunofluorescence

Cells were cultured on 12 well plates as described in the main text. Two hours after the culture medium was lowered to 1% horse serum, cells were fixed with 4% paraformaldehyde in PBS for 10 min. The cells were washed with 0.1 M glycine and then permeabilized with 0.2% Triton. This was followed by blocking with 1% BSA (MP Biomedicals, Santa Ana, CA) in PBS. Cells were incubated with primary antibody (anti-E-Cadherin, 1:800 (Invitrogen)) in 0.1% BSA in PBS at 4°C overnight followed by incubation with anti-mouse antibodies conjugated to Alexa 568 or Alexa 647 (Invitrogen). Nuclei were labled with DAPI and the cells were imaged at 40x using a Zeiss Observer.Z1 inverse microscope.

Supplemental Movies

All movies show 10 hours of time lapse imaging replayed at 15 frames per second. The time stamp on the images is formatted as hours:minutes of real time. The scale bar indicates $100\mu m$.

MovieS1.avi M1 (left) and M4 (right) cell lines

MovieS2.avi M1 nonsense (NS) shRNA control cells (left) and two E-cadherin shRNA cell lines (right two images)

MovieS3.avi M4 nonsense (NS) shRNA control cells (left) and two E-cadherin shRNA cell lines (right two images)
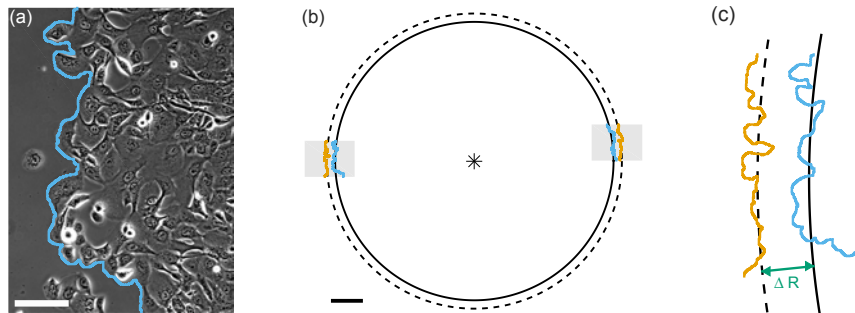
Supplemental Figures

Figure S1. Edge Detection and Analysis Phase contrast images of the cell monolayer were segmented to find the leading edge of migration (see Methods in main text), as shown by the edge overlay in (a). This image corresponds to the first frame in Supplemental Movie 1 and the scale bar shows $100\mu m$. Cells or small clumps of cells that are not attached to the main monolayer are not contained within the edge. For each monolayer, images were taken of both the left and right edge of the monolayer. The imaging field of view is represented by gray rectangles in (b). The blue lines represent the segmented monolayer edge at $t = 0h$ and the orange lines represent the segmented monolayer edges at $t = 10h$. These edges are fit to a circle, shown by a solid line ($t = 0h$) or dashed line ($t = 10h$). The scale bar in (b) shows $500\mu m$. A close up view of the left edge (c) shows the change in edge position over time, denoted by the radial displacement (ΔR). The monolayers have a radius of approximately 2-3 mm and a ΔR of a few hundred microns over $10h$ (see Figure 1, main text).
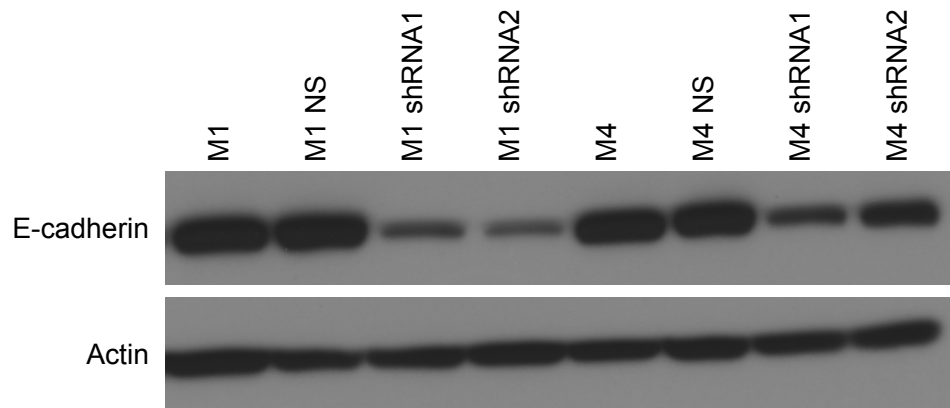

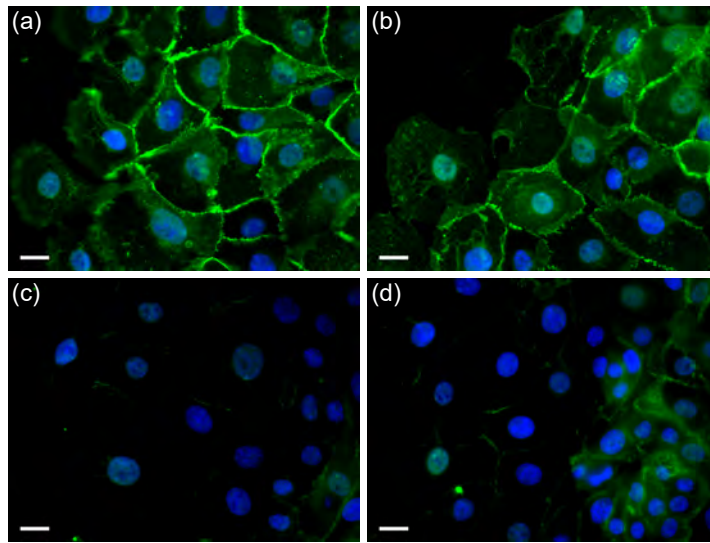**Figure S2.** Western blot showing E-cadherin knockdown cell lines.

Figure S3. E-cadherin in M1 cells Immunofluorescence images of E-cadherin (green) and the nucleus (blue) for the unperturbed M1 (a), M1 nonsense (NS) shRNA (b) and E-cadherin shRNA ($c - d$) cell lines. In all images, the scale bar indicates $25\mu m$.
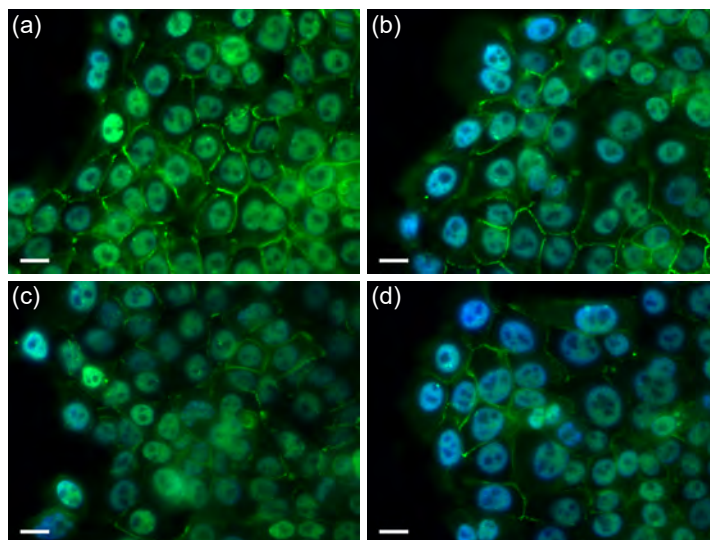

Figure S4. E-cadherin in M4 cells Immunofluorescence images of E-cadherin (green) and the nucleus (blue) for the unperturbed M4 (a), M4 nonsense (NS) shRNA (b) and E-cadherin shRNA ($c - d$) cell lines. In all images, the scale bar indicates $25\mu m$.
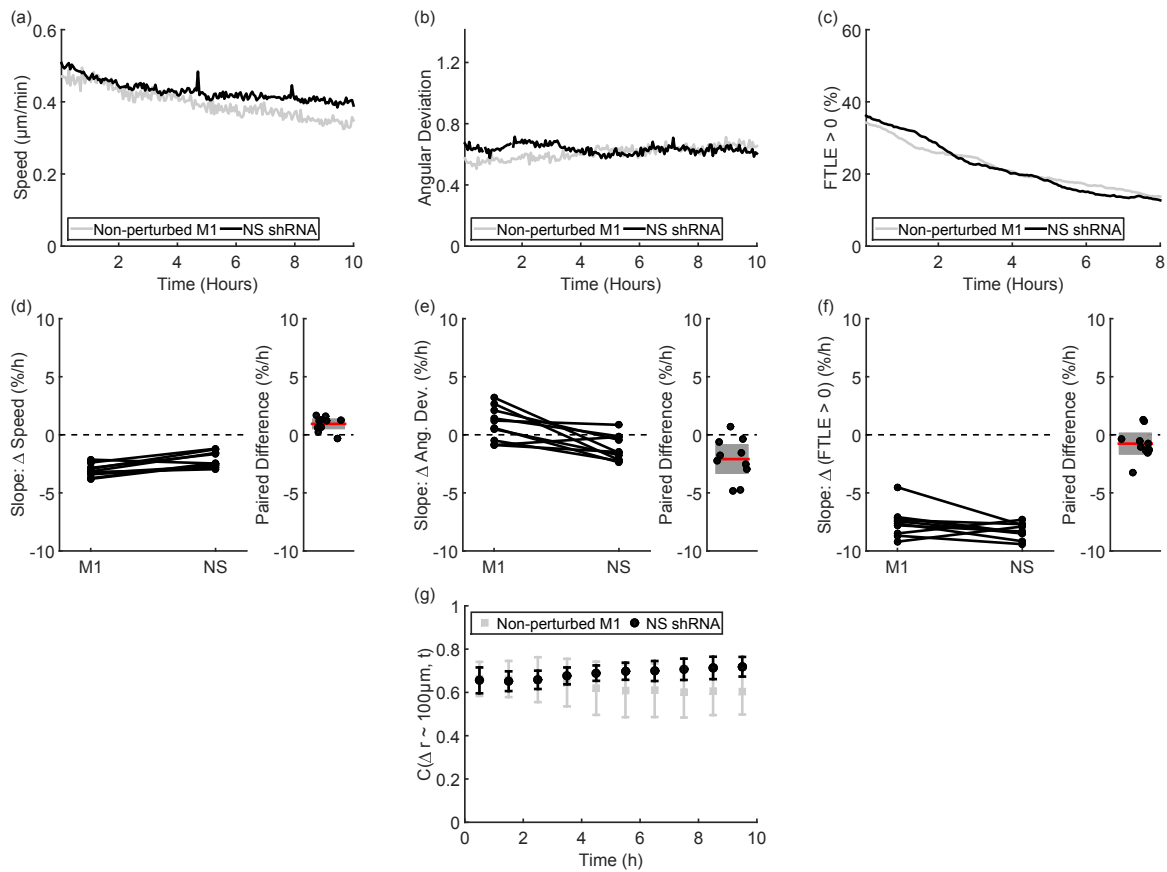
Figure S5. Comparison between M1 cells and shRNA Control cells Representative time traces of speed (a), angular deviation (b), and positive FTLE values (c) show no difference between the M1 cells and the nonsense (NS) shRNA control cells. As shown by the experimentally paired differences between non-perturbed and NS control in the slopes of speed (d), angular deviation (e), and FTLE values (f), there is no change in long time trends. The correlations remain high over time for both cell types, as shown by the correlations in the region $90\mu m < \Delta r < 105\mu m$ (g). Error bars in $(d-g)$ indicate 95% confidence intervals.
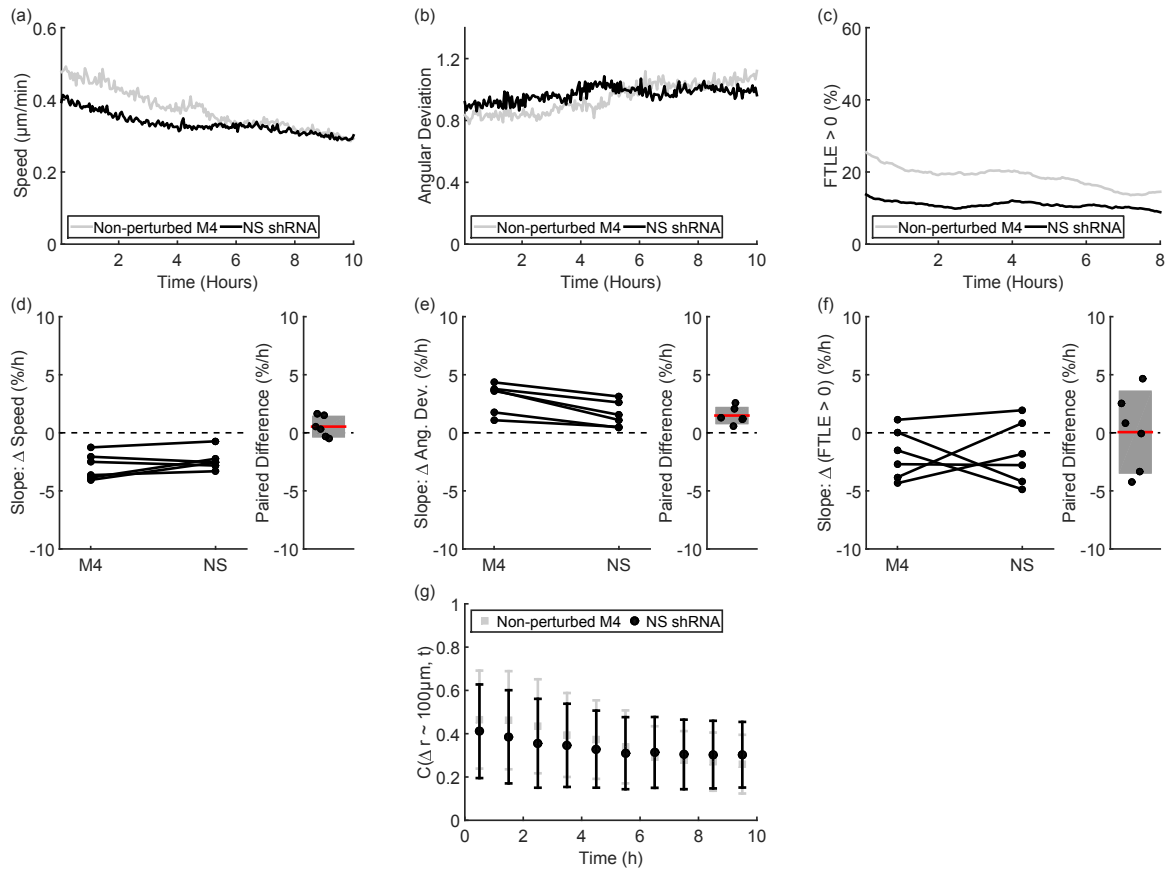
Figure S6. Comparison between M4 cells and shRNA Control cells
Representative time traces of speed (*a*) and angular deviation (*b*) show no difference
between the M4 cells and the nonsense (NS) shRNA control cells. The absolute value
of positive FTLE values (*c*) from paired experiments show some changes between the
non-perturbed M4 cells and the NS control but no changes in long time behavior. As
shown by the experimentally paired differences between non-perturbed and NS control
in the slopes of speed (*d*), angular deviation (*e*), and FTLE values (*f*), there is no
change in long time trends. The correlations decrease over time for both cell types,
as shown by the correlations in the region $90\mu m < \Delta r < 105\mu m$ (*g*). Error bars in
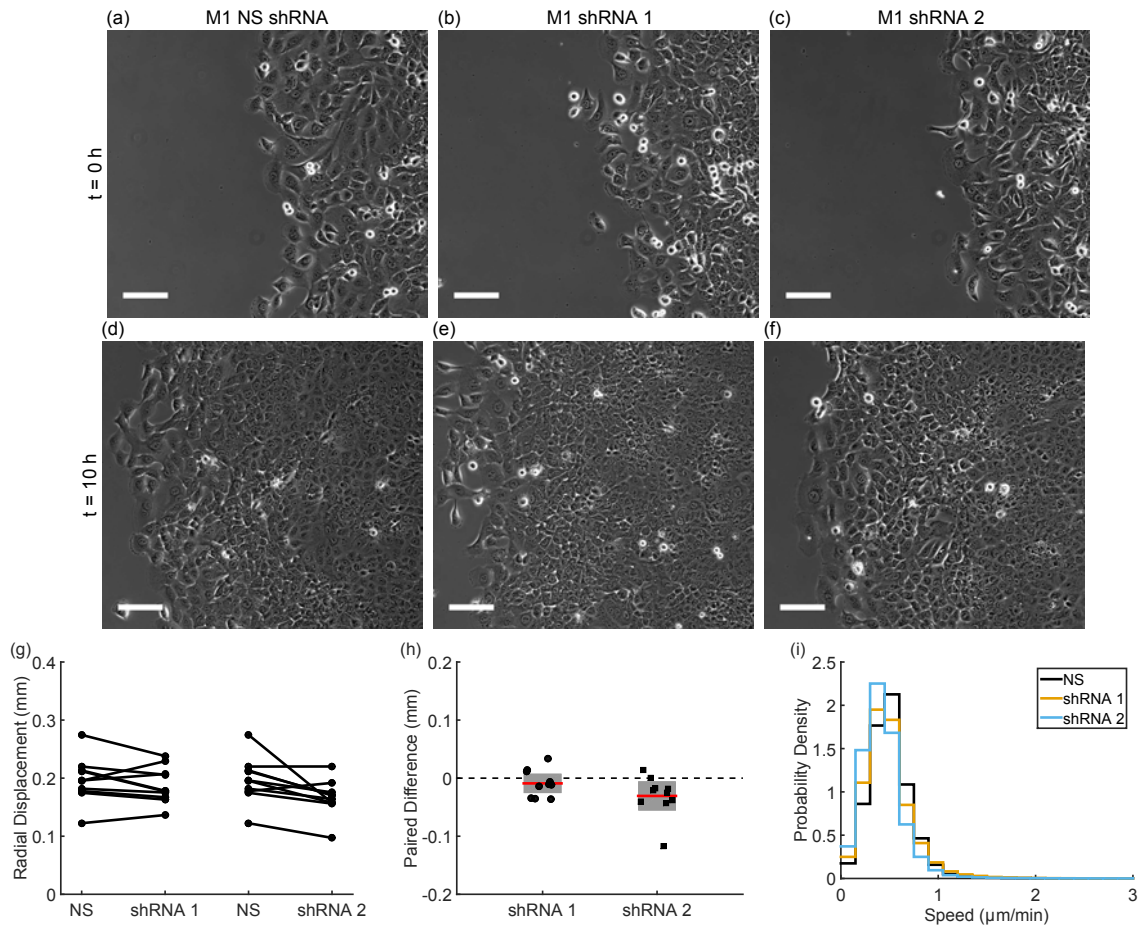(*d* − *g*) indicate 95% confidence intervals.

Figure S7. M1 E-cadherin shRNA cells Phase contrast images of the M1 nonsense (NS) shRNA cells (a, d) and two E-cadherin shRNA cell lines $(b, e$ and $c, f)$ show the radial migration assay at $t = 0$ h $(a - c)$ and $t = 10$ h $(d - f)$. These images are taken from Supplemental Movie 2. In all images, the scale bar indicates $100 \mu m$. The edge of the monolayer moves at similar speeds for all cell types. The absolute value of radial displacement varies (g), but paired experiments show no trend in the difference between the cells (h). Lines in (g) pair experiments performed on the same day and error bars in (h) indicate 95% confidence intervals. The speed distributions (cumulative over time and space) within the monolayer show similar mean speeds $(i$, cumulative distributions from $N = 10$ experiments).
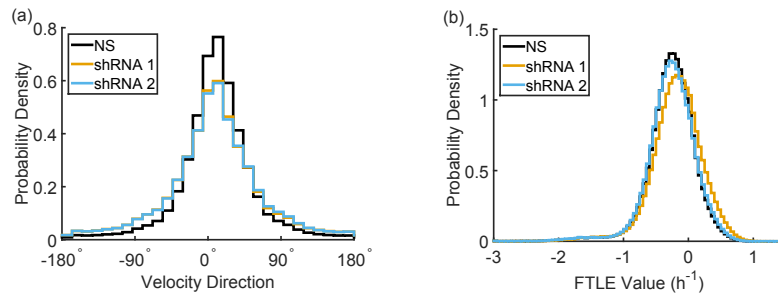
Figure S8. M1 E-cadherin shRNA cells As shown by the angle distributions in (*a*), the M1 E-cadherin shRNA cells are less directional than the nonsense (NS) shRNA cells. However, the long time behavior represented by FTLE values (*b*) only shows a slight difference. Each distribution represents the cumulative data from N = 10 experiments and is cumulative over time and spatial location.
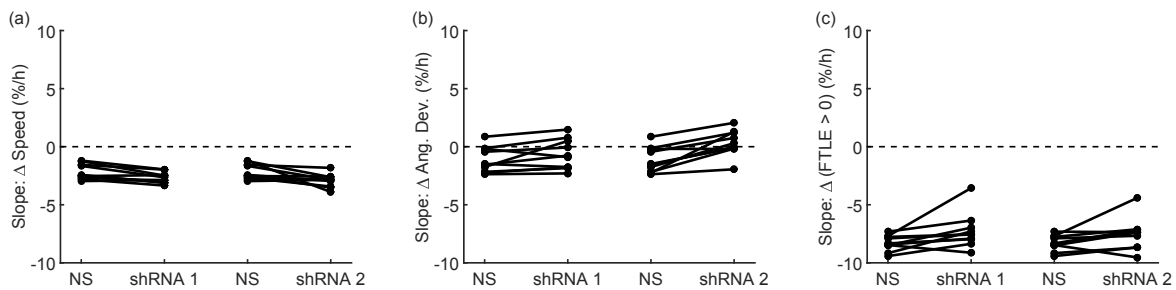

Figure S9. M1 E-cadherin shRNA cells Paired slope values for speed (*a*), angular deviation (*b*), and FTLE values (*c*) as a supplement to the slopes shown in Figure 6 of the main text. Lines pair experiments performed on the same day.
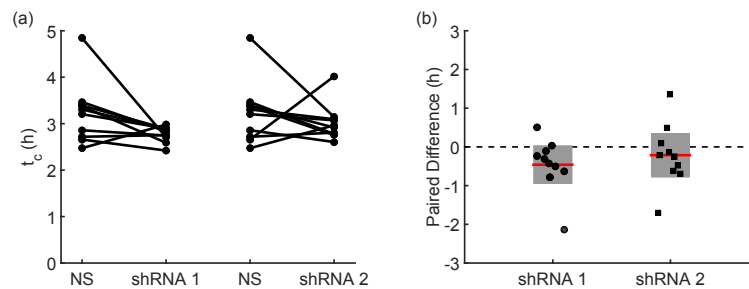

Figure S10. M1 E-cadherin shRNA cells Coarse graining the cells' PIV flow field over increasing time intervals decreases the variance (σ) of the velocity field. By fitting the variance of the velocity orthogonal to the cell migration to an exponential decay (see methods), we calculate a characteristic time scale of fluctuations in the flow. As shown by the paired values (*a*) and paired differences (*b*), the M1 E-cadherin shRNA cells show a similar characteristic time scale to the nonsense shRNA cells (NS). Lines in (*a*) pair experiments performed on the same day and error bars in (*b*) indicate 95% confidence intervals.
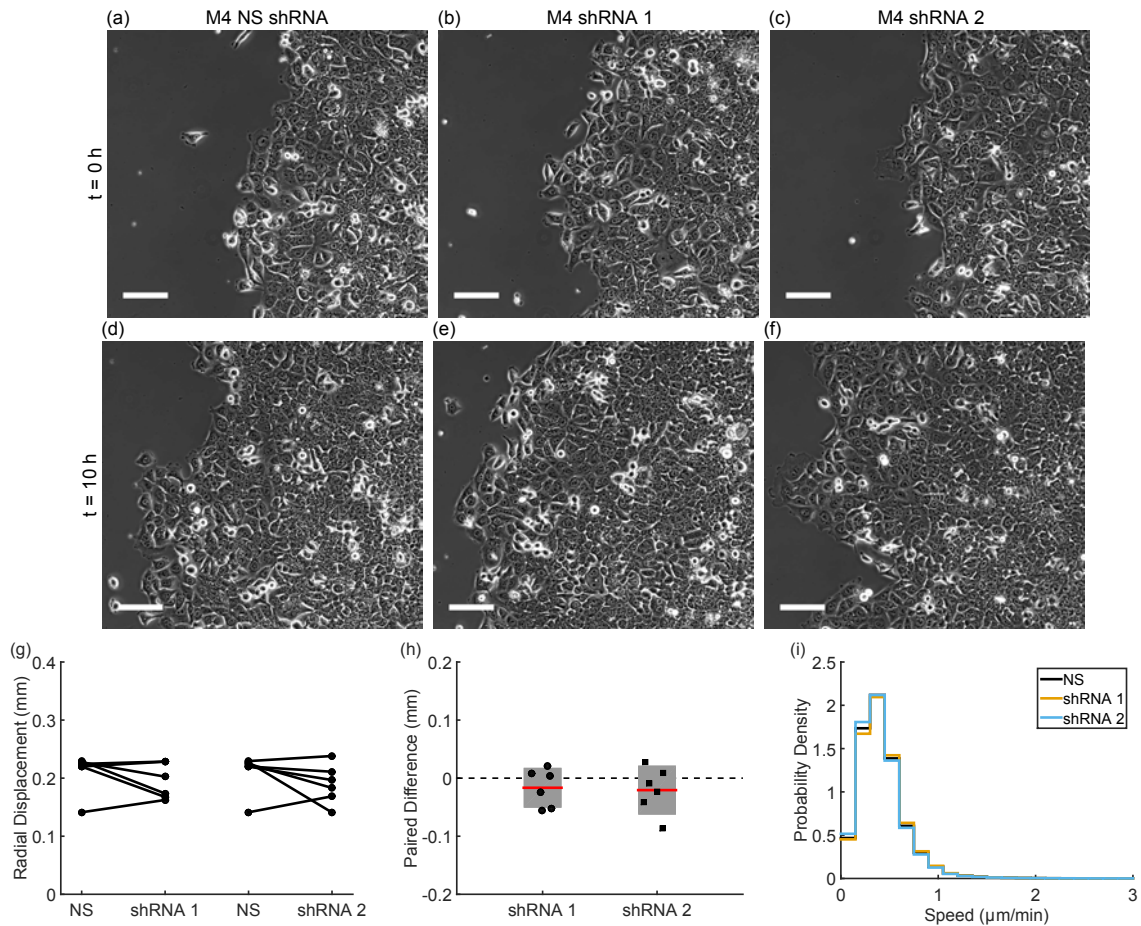
Figure S11. M4 E-cadherin shRNA cells Phase contrast images of the M4 nonsense shRNA cells (a, d) and two E-cadherin shRNA cell lines $(b, e$ and $c, f)$ show the radial migration assay at t = 0 h $(a - c)$ and t = 10 h $(d - f)$. These images are taken from Supplemental Movie 3. In all images, the scale bar indicates $100 \mu m$. The edge of the monolayer moves at similar speeds for all cell types. The absolute value of radial displacement varies (g), but paired experiments show no trend in the difference between the cells (h). Lines in (g) pair experiments performed on the same day and error bars in (h) indicate 95% confidence intervals. The speed distributions (cumulative over time and space) within the monolayer show similar mean speeds $(i,$ cumulative distributions from N = 6 experiments).
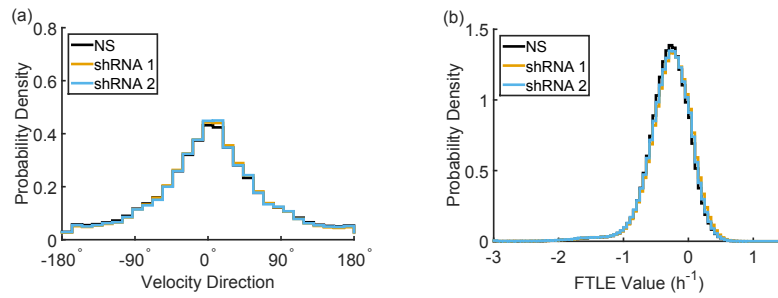
Figure S12. M4 E-cadherin shRNA cells The M4 nonsense (NS) and E-cadherin shRNA cell all show low directionality, as shown by the angle distributions in (*a*). The long time behavior represented by FTLE values also shows a similar distribution (*b*). Each distribution represents the cumulative data from N = 6 experiments and is cumulative over time and spatial location.
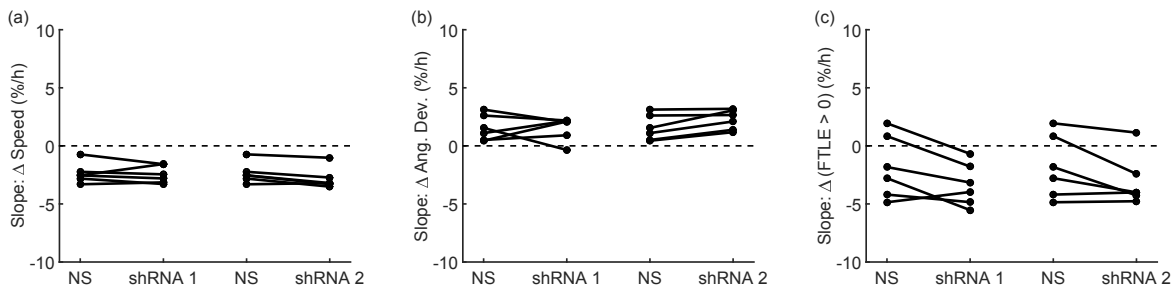

Figure S13. M4 E-cadherin shRNA cells Paired slope values for speed (*a*), angular deviation (*b*), and FTLE values (*c*) as a supplement to the slopes shown in Figure 7 of the main text. Lines pair experiments performed on the same day.
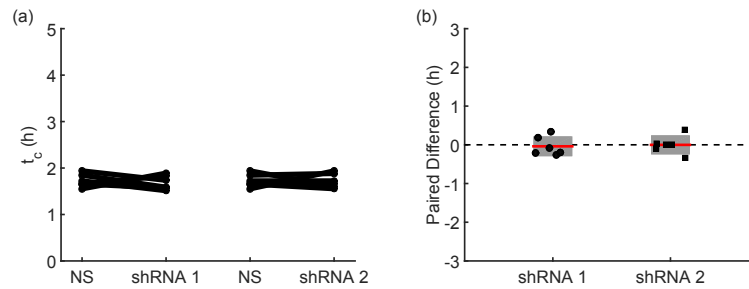

Figure S14. M4 E-cadherin shRNA cells Coarse graining the cells' PIV flow field over increasing time intervals decreases the variance (σ) of the velocity field. By fitting the variance of the velocity orthogonal to the cell migration to an exponential decay (see methods), we calculate a characteristic time scale of fluctuations in the flow. As shown by the paired values (*a*) and paired differences (*b*), the M4 E-cadherin shRNA cells show a similar characteristic time scale to the nonsense shRNA cells (NS). Lines in (*a*) pair experiments performed on the same day and error bars in (*b*) indicate 95% confidence intervals.