

- Supplementary Materials -

Substrate area confinement is a key determinant of cell velocity in collective migration

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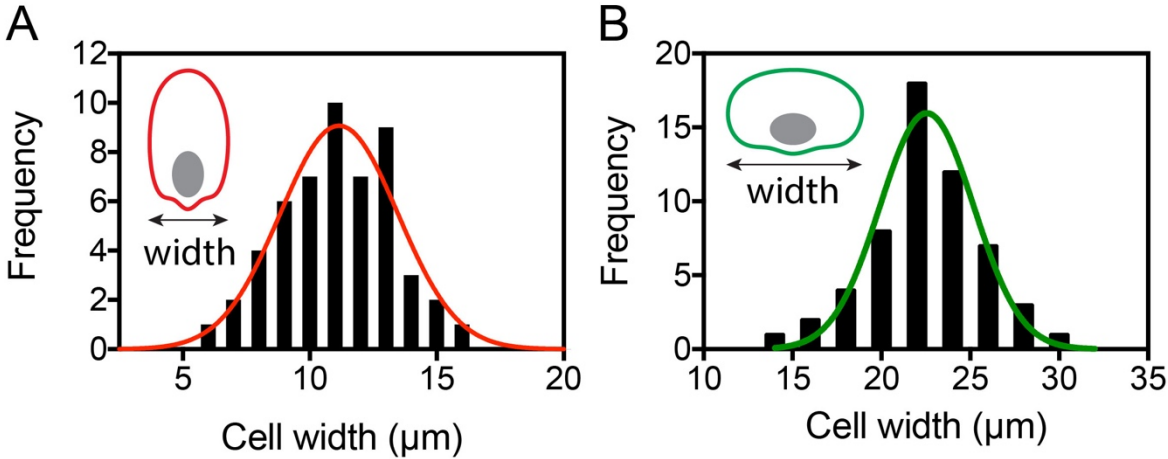
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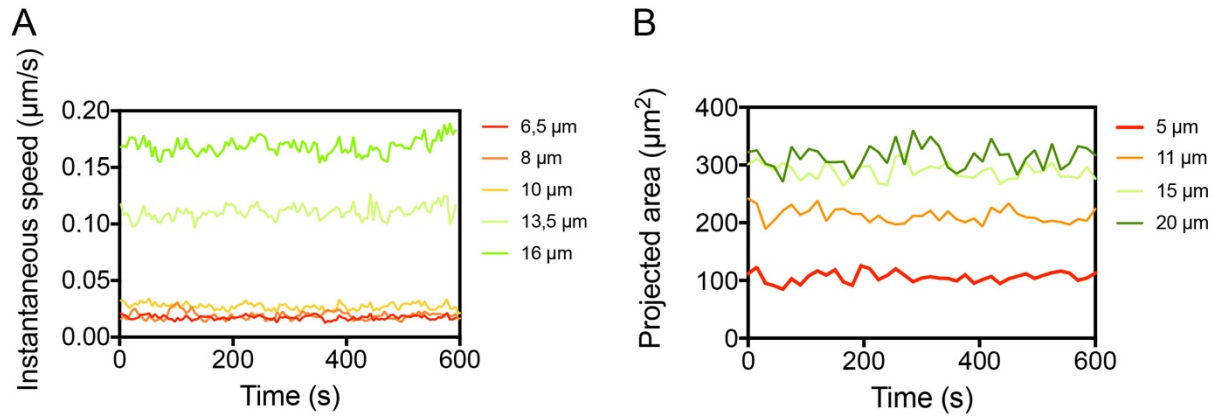
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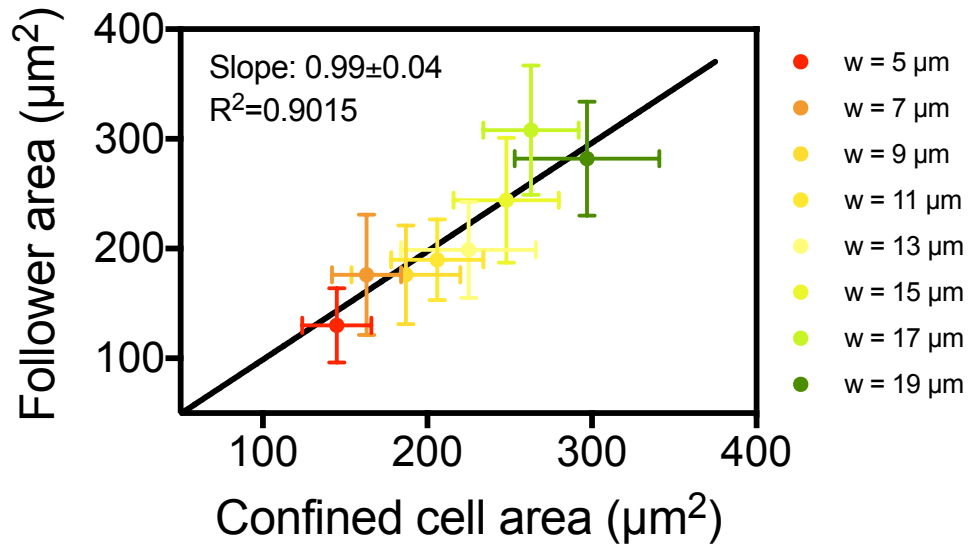
Supplementary Figures



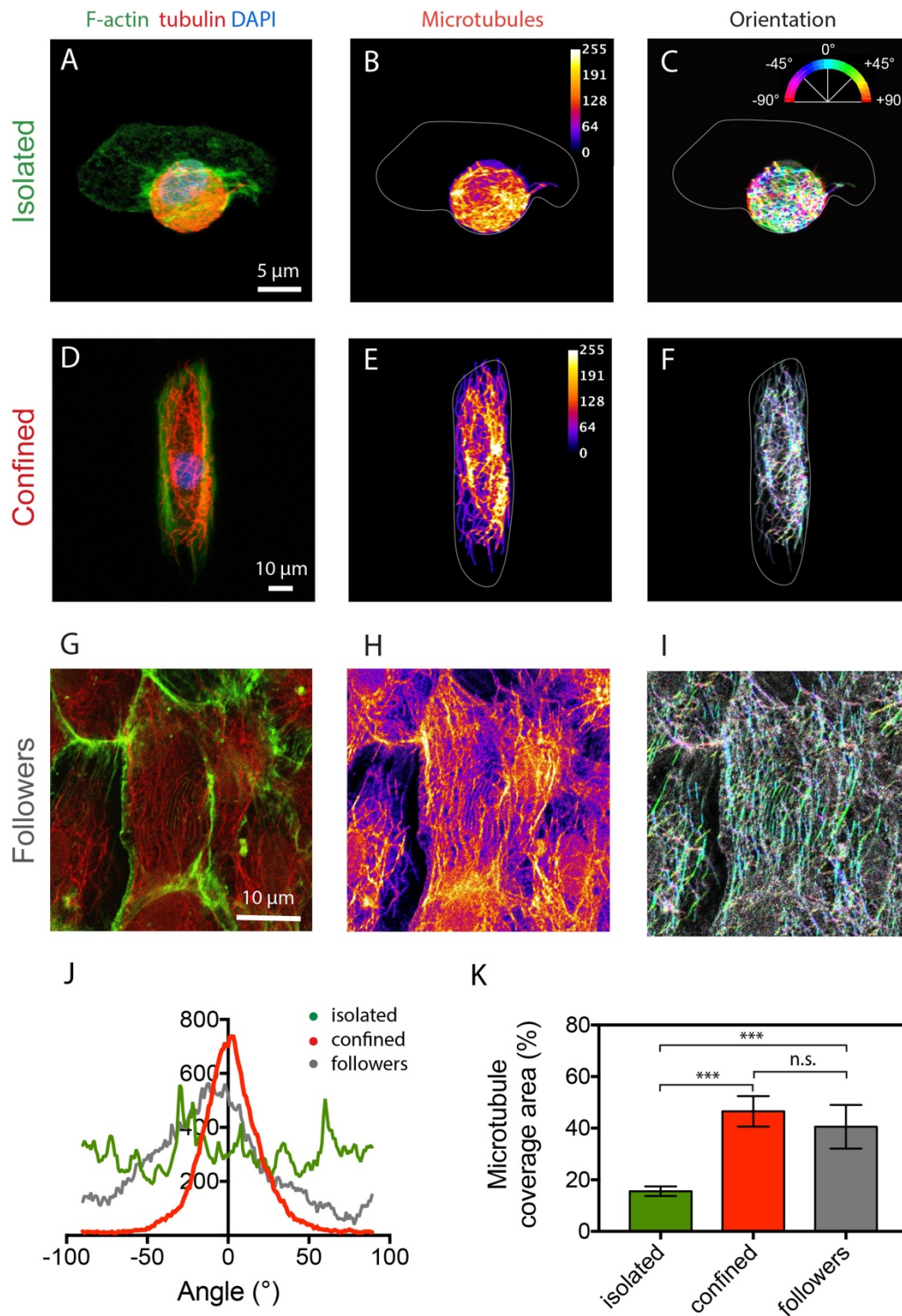
Supplementary Figure 1 – Frequency distribution of the width of (A) the followers (in red) and (B) the leaders (in green) within a cohesive epithelial sheet. Red and green lines correspond to a gaussian fit for followers and leaders, respectively. Mean \pm S.D. is $12.1 \pm 2.2 \mu\text{m}$ ($n=52$) for followers and 22.5 ± 2.6 ($n=49$) for leaders, pooled from three different experiments.



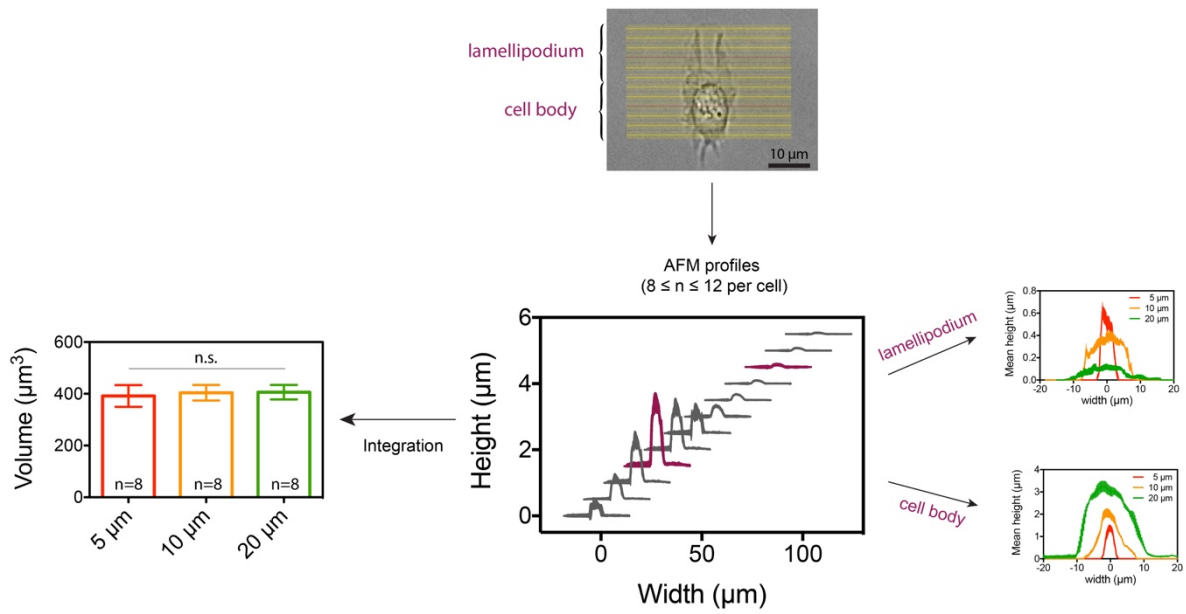
Supplementary Figure 2 – Temporal evolution of (A) the instantaneous speed and (B) the projected area of single keratocytes migrating on microstripes of different widths (from 5 to 20 μm).



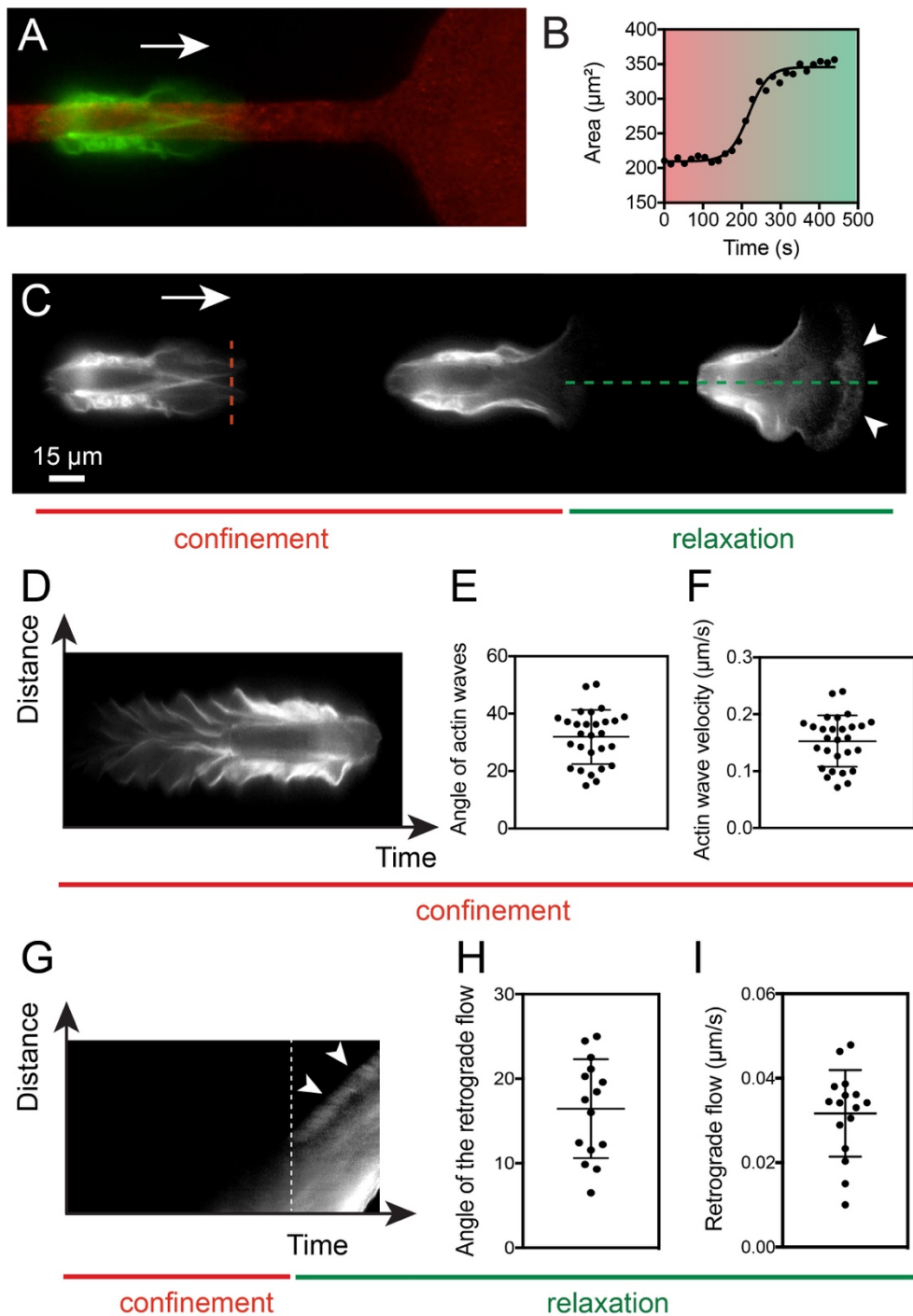
Supplementary Figure 3 – Linear correlation between the projected area of followers binned into 2 μm wide bins and the projected area of cells confined on microstripes of the same width as the bin (from 5 μm in red to 19 μm in green). The slope is 0.99 ± 0.04 and the regression coefficient $R^2 = 0.9015$ for a total number of 93 cells.



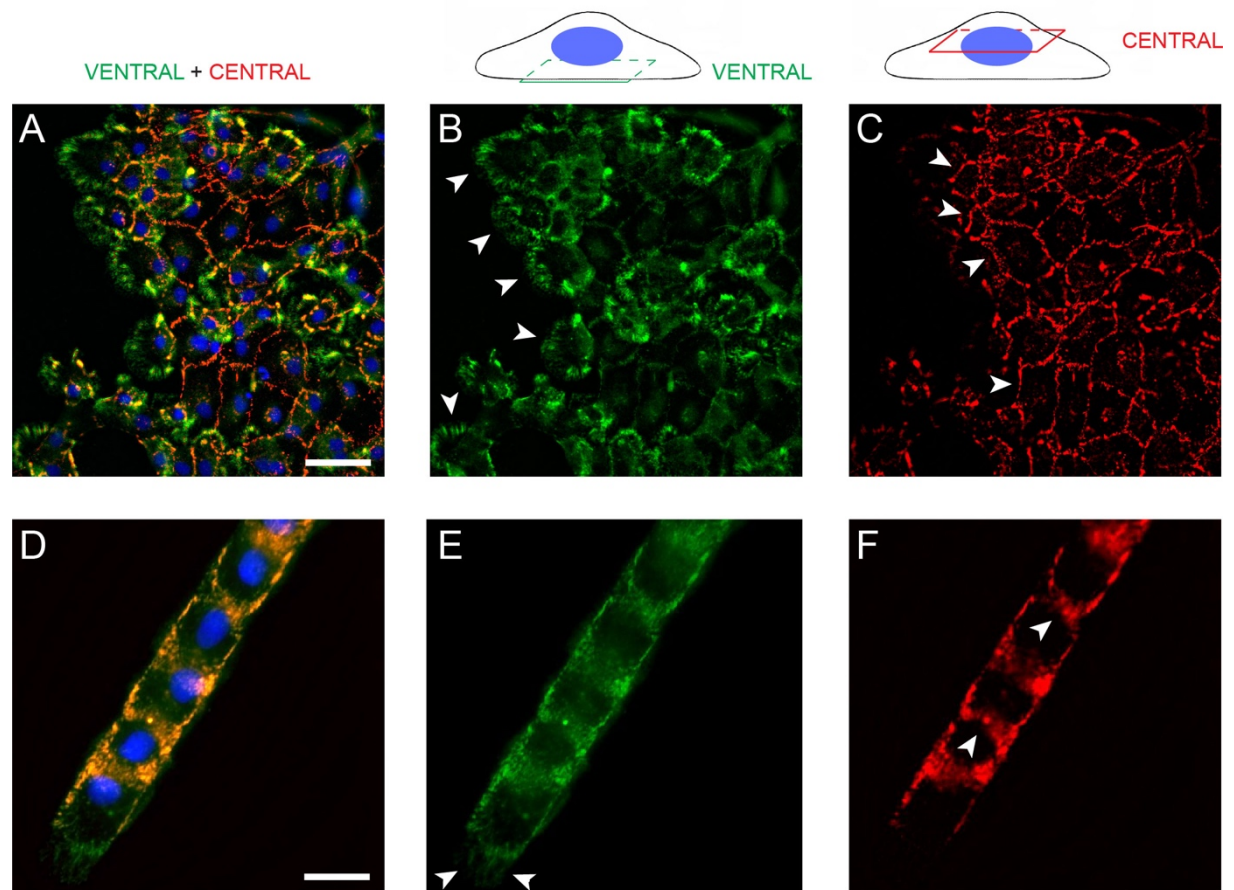
Supplementary Figure 4 – Epifluorescence imaging of isolated cells (A), confined cells (D) and followers (G) stained for actin (in green), microtubules (in red) and DNA (in blue). Isolated cells show a dense accumulation of microtubules in the cell body (B) with a random orientation (C). Microtubules in confined cells (E) and follower cells (H) extend throughout the whole cell body parallel to the cell long axis (F and I for confined cells and follower cells, respectively). (C, E, H) Individual microtubules are color coded to show their orientation. The inset in (C) shows the color code. (J) Distribution of microtubule orientations computed from images such as C, E, H. (K) Percentage of the cell area covered by microtubules in isolated cells (green), confined cells (red) and followers (gray). *** $p < 0.05$ and n.s. not significant.



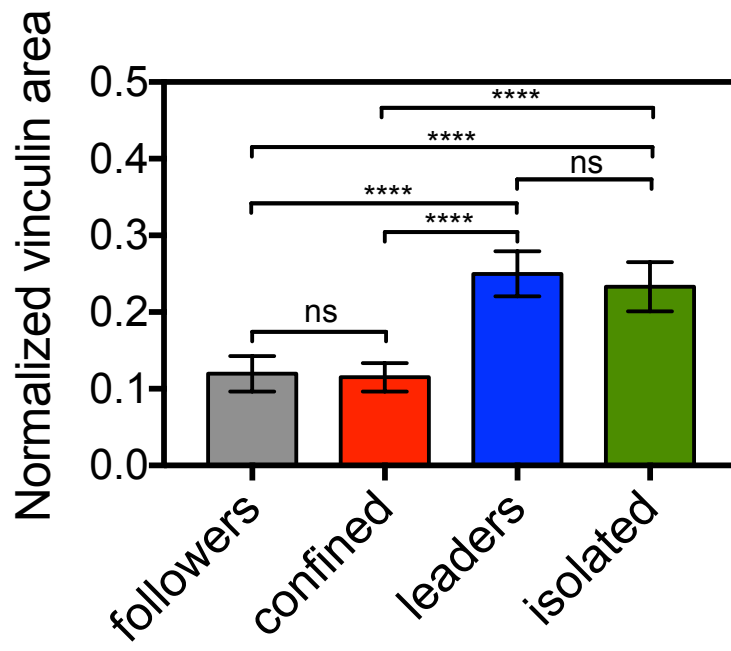
Supplementary Figure 5 – Schematic representation of the determination of the cellular volume of cells confined on 5, 10 and 20 μm stripe from AFM profiles acquired on fixed confined cells.



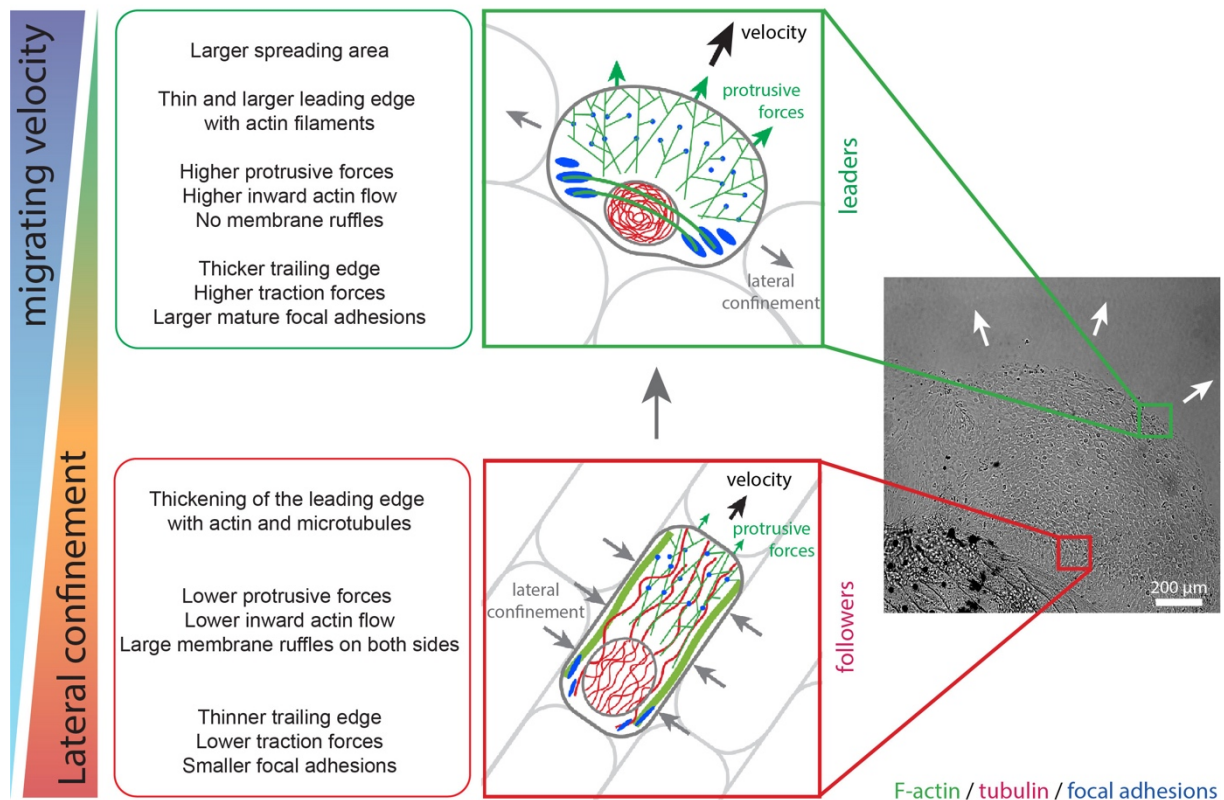
Supplementary Figure 6 – (A) Epifluorescent image of a GFP-UTR cell (in green) migrating on a FN-coated micropattern (in red). The white arrow shows the direction of migration. (B) Evolution of the projected area during the transition from a confined (red zone) to an unconfined (green zone) area. (C) Red and green dashed lines show the axis used to generate kymographs for the confined (in red) and the relaxed (in green) zones of the micropattern. (D) Kymograph and distribution of (E) the angles and (F) the velocities of actin waves for keratocytes confined on a microstripe of 15 μm wide. (G) Kymograph and distribution of (H) the angles and (I) the velocities of the retrograde flow in the leading edge for keratocyte migrating on a relaxed zone.



Supplementary Figure 7 – Confocal images of (A-C) an epithelial tissue and (D-E) a one-dimensional train of cells (n=5) immunostained for vinculin at the ventral focus plane (B and E, in green) and the central focal plane (C and F, in red). White arrows show zones of interest.



Supplementary Figure 8 – Area of vinculin-containing focal adhesions normalized by the cell area for followers (in grey, n=18), confined cells on microstripes of 12 μm in width (in red, n=19), leader cells (in blue, n=17) and isolated cells (in green, n=21). **** $p < 0.0001$ and n.s. not significant.



Supplementary Figure 9 – Schematic representation of the migration behaviour between followers and leaders in response to the spatial confinement imposed by neighbours. The lateral confinement modulates linearly the spreading area of collective (native tissue) and individual (cells on microstripes) states and decreases the adhesiveness of keratocytes. The modulation of the spreading area by a 2D confinement requires a 3D thickening of the leading edge to retain a constant cell volume. Thicker leading edges in confined cells exert less protrusive forces and display a lower adhesiveness than unconfined cells. No retrograde flow is noticeable in the thick lamellipodia of confined cells, whereas inward dynamic actin ruffles generated on both cell sides lead to a less efficient migration. The low cell adhesiveness associated with less efficient actin flows in confined cells lead to a drop of the cell velocity modulated by the level of lateral confinement. This global mechanism is supported by the extension of microtubules in thicker leading edges which was related to a slower actin flow and by the formation of actin ruffles which were associated with low adhesion modes.

Supplementary Movies

Supplementary Movie 1 – Time-lapse sequence in DIC mode of the growth of an epithelial tissue from a fish scale deposited on a glass coverslip coated with fibronectin. Total duration: 304 min. The scale bar is 100 μm .

Supplementary Movie 2 – Time-lapse sequence in DIC mode of the migration of a single keratocyte migrating on a 15 μm -wide microstripe. The fibronectin micropattern is shown in fluorescence mode at $t = 5$ min. Total duration: 09 min 11 sec. The scale bar is 10 μm .

Supplementary Movie 3 – Time-lapse sequence in DIC mode of the migration of a single keratocyte migrating on a FN micropattern composed of five interconnected stripes of different widths: 5, 9, 13, 17 and 21 μm . Each segment has a length of 80 μm and the total length of this micropattern with variable width is 400 μm . The total time is 109 min and the scale bar is 10 μm .

Supplementary Movie 4 – Time-lapse sequence in DIC mode of the cantilever assay to measure protrusive forces. A single keratocyte migrating on a 15 μm -wide microstripe pushed the cantilever. One can observe the cantilever deflection in response to the pushing force exerted by the leading edge of the oncoming cell.

Supplementary Movie 5 – Time-lapse sequence in DIC mode showing a leader cell that escaped from an epithelial tissue. The red arrow at $t = 0$ min shows the initial position of the leader cell. The total duration time is 25 min. The scale bar is 25 μm .

Supplementary Movie 6 – Time-lapse sequence in DIC mode showing a single keratocyte that escaped from a narrow microstripe. The escape process was reproduced by using micropatterns composed of adhesive lines of 12 μm wide connected to a circular disk of 50 μm in diameter. The total duration time is 5 min 50 sec. The scale bar is 10 μm .

Supplementary Movie 7 – Time-lapse sequence in Sir-actin mode showing a single keratocyte that escaped from a narrow microstripe. The escape process was reproduced by using micropatterns composed of adhesive lines of 12 μm wide connected to a circular disk of 50 μm in diameter. The total duration time is 4 min 25 sec. The fluorescence intensity is color-coded on 256 levels. The scale bar is 15 μm .

Supplementary Movie 8 – Time-lapse sequence in epifluorescent mode of a GFP-UTR zebrafish keratocyte (in green) migrating on a fibronectin-coated microstripe of 15 μm wide connected to a circular disk of 50 μm in diameter. The FN micropattern is labeled in red.

Supplementary Movie 9 – Time-lapse sequence in DIC mode of the migration of a train of cells ($n=2$ cells) on a fibronectin-coated microstripe of 15 μm wide.