Force transmission during adhesion-independent migration

Martin Bergert1,2,7,8, Anna Erzberger3,7, Ravi A. Desai2,4,5, Irene M. Aspalter1,2, Andrew C. Oates2,4,5, Guillaume Charras2,6, Guillaume Salbreux3,9 and Ewa K. Paluch1,2,9

When cells move using integrin-based focal adhesions, they pull in the direction of motion with large, ~100 Pa, stresses that contract the substrate1. Integrin-mediated adhesions, however, are not required for in vivo confined migration2. During focal adhesion-free migration, the transmission of propelling forces, and their magnitude and orientation, are not understood. Here, we combine theory and experiments to investigate the forces involved in adhesion-free migration. Using a non-adherent blebbing cell line as a model, we show that actin cortex flows drive cell movement through nonspecific substrate friction. Strikingly, the forces propelling the cell forward are several orders of magnitude lower than during focal-adhesion-based motility. Moreover, the force distribution in adhesion-free migration is inverted: it acts to expand, rather than contract, the substrate in the direction of motion. This fundamentally different mode of force transmission may have implications for cell–cell and cell–substrate interactions during migration in vivo.

Cell migration is essential to many physiological and pathological processes, including embryonic development, wound healing, immune response and cancer metastasis. To migrate, cells must exert forces on their substrate to propel the cell body forward. Classical models of cell migration imply that specific adhesion points transmit intracellular pulling forces from the cytoskeleton to the substrate3. Yet, recent studies indicate that in three-dimensional (3D) confinement, migration can be achieved without specific adhesions2,4,5. Here we investigated force generation during such adhesion-independent cell migration.

Traction force microscopy measurements have established that in adherent cells single focal adhesions can transmit forces of tens of nanonewtons (ref. 6), resulting in force densities on the cell surface of several kilopascals (ref. 7). In contrast, force transmission during focal-adhesion-independent migration has not been investigated experimentally and the origin and magnitude of the forces involved remain unclear. Several theoretical models have been proposed. For example, in smooth 3D confinement, such as during migration through dense tissues in cancer invasion or in development8–10, nonspecific substrate friction could account for force transmission11. However, a friction-based mechanism has not been demonstrated experimentally, and it is unknown whether friction alone can generate sufficient force to mediate cell body translocation.

To investigate forces exerted during focal-adhesion-independent migration, we used a non-adherent subline of Walker 256 carcinosarcoma (henceforth Walker) cells as a model. Walker cells polarize spontaneously in suspension and form blebs at their leading edge. They are unable to migrate on 2D substrates, but move effectively when confined in three dimensions12. We verified that Walker cells migrate in a variety of confined environments, including in confinement between an agarose pad and serum-coated glass, within 3D collagen gels and in polydimethylsiloxane (PDMS) microfluidic channels passivated with bovine serum albumin (BSA) or beta-lactoglobulin to prevent cell adhesion13,14 (Fig. 1a and Supplementary Fig. 1a,b). Walker cells remained motile when confined between agarose, which is non-adhesive15, and other non-adhesive surfaces (Fig. 1b and Supplementary Fig. 1d). We therefore reasoned that Walker cells migrate in confinement without using specific substrate adhesions. To test this directly, we first attempted to localize focal adhesion components in these cells. We found that neither GFP-tagged focal adhesion kinase (FAK) nor speckle-GFP–vinculin, a low-expression construct of vinculin allowing for detection of faint focal adhesion points16, formed foci at the basal surface of Walker cells migrating under agarose (Fig. 1c and Supplementary Fig. 1c). Furthermore, Walker cells with reduced
Figure 1 Specific-adhesion-independent migration of blebbing Walker cells in confined environments. (a) Time lapses of migrating blebbing Walker cells under agarose, within a 3D collagen-I gel or in a BSA-coated microfluidic channel. Arrowheads: blebs. (b) Instantaneous velocities of blebbing Walker cells migrating under agarose on glass, PEG-coated glass and commercial low-attachment surfaces (Corning). Cells were manually tracked for 52 min using Fiji. P value: Welch’s two-sided t-test; n: number of cells analysed in 2 independent experiments. Boxes in box plots extend from the 25th to 75th percentiles, with a line at the median. Whiskers extend to 1.5 × IQR (interquartile range) or the max/min data points. (c) Representative images (inverted contrast) of Walker cells expressing the focal adhesion component speckle-GFP–vinculin. Top: blebbing Walker cell migrating under agarose; no focal adhesions are detected. Bottom: control; clear focal adhesions are formed by an adherent Walker cell migrating on glass. Arrows indicate the direction of migration. (d) Representative images of in-plane substrate deformations during migration of adherent and non-adherent cells. Non-adherent blebbing Walker cells migrating under agarose on soft (3 kPa), elastic PDMS substrates with embedded beads do not elicit substantial bead displacements, whereas adherent Walker cells and HeLa cells do. Displacement fields caused by the cells were calculated from images of fluorescent beads using a traction force Fiji plugin. Cell outlines are drawn in white. All scale bars, 10 μm.

We then attempted to measure forces exerted during adhesion-independent migration of Walker cells using traction force microscopy. As positive controls, we plated HeLa cells and an adherent subline of Walker cells22 on soft PDMS substrates with embedded fluorescent beads. We observed clearly detectable in-plane bead displacements, indicative of strong pulling forces (Fig. 1d). In contrast, we did not observe substantial in-plane bead displacements during blebbing Walker cell migration under agarose (Fig. 1d). This indicates that forces exerted during adhesion-free migration are considerably smaller than those exerted during classical, focal-adhesion-based migration.

To understand the forces underlying focal-adhesion-independent migration, we characterized the actomyosin cytoskeleton, which drives migration in most cell types17. We focused on Walker cells migrating in microchannels, as the simple channel geometry imposes a well-defined, static cell morphology easily amenable to quantitative analysis (Fig. 2a and Supplementary Video 2). The medium was supplemented with the Arp2/3 inhibitor CK-666, which does not affect blebbing Walker cell behaviour but prevents the cells from occasionally switching to an adhesion-based, lamellipodia-driven migration mode12. Both F-actin and myosin were enriched at the cell cortex and exhibited a strong gradient towards the cell rear (Fig. 2a and Supplementary Fig. 1g–i), suggesting a rearward gradient in contractility. To test whether the rearward actomyosin gradient was essential for migration, we performed laser ablations to relax cortical contractility locally18. Cortex ablation at the cell rear strongly decreased cell velocity, whereas ablation at the leading edge had no effect (Supplementary Fig. 1f and Supplementary Videos 3 and 4). This suggests that cortical contractility at the rear is necessary for confined migration of Walker cells. We next imaged dynamics of the actomyosin cortex. We found that in BSA-coated channels, both actin and myosin exhibited a strong retrograde flow in the reference frame of the cell (Supplementary Videos 5 and 6). These flows seemed tightly coupled to the surface of the microchannel, as myosin foci in the cortex remained almost stationary in the channel reference frame (Fig. 2b,c and Supplementary Video 6). Taken together, our observations suggest a mechanism in which cortex flows resulting from a rearward actomyosin gradient drive Walker cell migration in the absence of specific adhesions.

Retrograde actin flows in the leading edge have been shown to drive cell migration when coupled to the substrate through integrin-
A minimum friction is required for cortical flow-driven migration of non-adherent Walker cells. (a) F-actin (Lifeact) and myosin (MRLC) gradients in Walker cells, imaged in the middle cross-section of the cell (left) and within the plane of the actomyosin cortex close to the channel wall (right). Scale bars, 10 μm. (b) Time-lapse images of migrating Walker cells in microchannels with different frictions. The cell–substrate friction coefficient \( \alpha \) was measured for the 3 different channel coatings (BSA, F127 and BSA/F127-mix) by applying a pressure to the channel entry and measuring the velocity of non-polarized cells (mean ± s.e.m., see Supplementary Fig. 2 and Methods for details). Dynamics of the actomyosin cortex in a confocal section at the cell surface were visualized with MRLC–GFP. Coloured circles highlight the dynamics of individual myosin foci. Scale bars, 10 μm. (c) Kymographs showing the dynamics of the cortex along the long axis of the cell. Scale bars, horizontal: 10 μm, vertical: 50 s. (d) Summary of the observed cell behaviours and actomyosin cortex dynamics depending on the friction between the cell and the channel walls.

In the absence of adhesions, nonspecific transient interactions between transmembrane proteins and the substrate might generate friction, transmitting flows into cell movement, as suggested by theoretical studies. To test this hypothesis, we modified the coating of the channel walls to decrease friction-based coupling. We passivated the channel walls with Pluronic F127, an analogue of polyethylene glycol (PEG), which has been suggested to provide low-friction substrates. We directly measured friction coefficients on single, unpolarized cells using a microfluidic chip and confirmed that this coating resulted in a very low-friction environment compared with BSA coating (Supplementary Fig. 2 and Supplementary Video 7). In the low-friction F127-coated channels, Walker cells adopted the same polarized morphology as in the high-friction BSA-coated channels but were unable to migrate (Supplementary Video 8).
I. Small friction

\[ \text{Cortex velocity} \ (V_{\text{Cortex}} (\mu m \ min^{-1})) \]

\[ V_{\text{Cortex}} = -\alpha \ V_{\text{Cell}} \]

\[ \text{Fluid drag} = -\alpha_{C} \ V_{\text{Cell}} \]

\[ \text{Viscosity } \eta \]

\[ \text{Large contractility} \]

\[ \text{Small contractility} \]

II. Intermediate friction \( \alpha < \alpha_{C} \)

III. Large friction \( \alpha > \alpha_{C} \)

\[ \text{Contraction} \]

\[ \text{Expansion} \]

\[ \text{Max. stress } f_{\text{Max}}/f_{\text{Norm}} \]

\[ \text{Position } x/L_{\text{Cell}} \]

\[ 0 \quad 0.5 \quad 1 \]

\[ 0 \quad 10 \mu m \ min^{-1} \]

\[ \text{Cortex velocity} \ (V_{\text{Cortex}} (\mu m \ min^{-1})) \]

\[ \text{BSA} \]

\[ \text{BSA/F127} \]

\[ \text{F127} \]

Strikingly, retrograde actomyosin flows in the cell reference frame persisted in these channels but, in contrast to BSA-coated channels, were completely uncoupled from the substrate (Fig. 2b,c and Supplementary Video 9). We then coated the channels with a mixture of BSA and F127 to achieve intermediate friction (Supplementary Fig. 2), and observed that blebbing Walker cells could migrate in such channels, but at significantly slower velocities than in high-friction BSA channels \( (V_{\text{BSA}} = 7.6 \pm 0.5 \mu m \ min^{-1}, n = 18 \) cells from 5 independent experiments, versus \( V_{\text{BSA/F127}} = 5.2 \pm 0.4 \mu m \ min^{-1}, n = 25 \) cells from 3 independent experiments; error: s.e.m.; \( P < 0.01 \), Supplementary Video 10). Furthermore, in contrast to cells in BSA-coated channels, the retrograde cortical flows did not remain stationary in the channel reference frame (Fig. 2b,c), indicating only partial coupling of the cortex to the channel wall. Taken together, these experiments suggest that rearward cortical flows coupled to the substrate through friction drive adhesion-free migration in Walker cells, and that a minimum friction is required to achieve effective force transmission (Fig. 2d).

We then used the measured friction coefficients to obtain an estimate of the forces driving friction-based migration. Assuming that the friction coefficient we measured in unpolarized cells (Supplementary Fig. 2) does not change on cell polarization, the product of the measured friction coefficient and the cortical flow velocity yields an estimate of the force density (stress) exerted by the cells. We thus estimated average cortical flow velocities in the different friction conditions (Fig. 2c), and found that cells in BSA, BSA/F127 and F127 channels exerted stresses lower than \( \sim 1 \) Pa, 5 mPa and 0.5 mPa, respectively, on the channel walls. These stresses are several orders of magnitude smaller than the hundreds of pascal stresses typically exerted by adhesive cells\(^{6,7}\), consistent with our traction force microscopy observations (Fig. 1d).

To understand how such small forces can drive cell motion and to obtain the spatial force distribution, we developed a theoretical description of friction-based cell migration. We modelled the actomyosin cortex as an axisymmetric, viscous surface subjected to the following forces: myosin-generated internal contractile stress \( \tau \) (see Supplementary Note), a gradient in active tension along the cell axis induces deformations of the cell poles and rearward cortical flow of velocity \( V_{\text{Cortex}} \) in the cell reference frame, resulting in cell movement at velocity \( V_{\text{cell}} \) if the generated friction force is sufficient to counteract the fluid drag force. \( (V_{\text{cell}} = V_{\text{cell}} + V_{\text{Cortex}}) \)

(b) Top: cell translocation is achieved by frictional forces resisting a retrograde cortical flow coupled to contraction of the cell rear and expansion of the leading edge. The relative contribution of the two mechanisms depends on the friction coefficient. Bottom: cell velocity as a function of friction (dots: experimental data; error: s.e.m., solid line; fitted theoretical curve). Fluid drag \( \alpha_{C} \) leads to cell stalling below a threshold value of substrate friction \( \alpha/\alpha_{C} \approx 0.1 \). The maximum stress exerted on the channel wall (inset) increases for increasing friction, whereas the cell velocity reaches a plateau. (Normalization: \( V_{\text{norm}} = (\xi - \xi_{0})L/\eta, f_{\text{norm}} = \alpha_{C} V_{\text{norm}} \))

(c) Cortical flow profiles in different friction conditions quantified using particle image velocimetry (PIV). Dots: experimental data; lines: fit theoretical curves calculated for measured myosin gradients (Supplementary Fig. 1i and Supplementary Note for details). Scale bars, 10 \( \mu m \) (left panel) and 0.5 \( \mu m \) (right panel). For b,c, \( \theta_{BSA} = 33, \ \theta_{BSA/F127} = 25 \) and \( n_{BSA} = 18 \) cells were analysed in 5 (BSA), 3 (BSA/F127) and 5 (F127) independent experiments. Data were systematically filtered on the basis of the PIV sample size (error bar: s.e.m. see Methods for details).
friction, cell velocity is roughly constant whereas friction-generated stresses increase (Fig. 3b inset), highlighting that cell velocity does not correlate with the amplitude of the stresses exerted in this migration mode but is dictated by the velocity of the actomyosin flow.

We then tested whether our theoretical description could quantitatively recapitulate observed cell and cortex dynamics in the different friction regimes, and quantified the spatial profile of cortical flows in the different friction conditions (Fig. 3c). We performed a simultaneous fit of the model equations to the cortical flow profiles and the cell velocities measured for different frictions. Flow profiles and cell velocities could be fitted accurately with a single set of three fit parameters (Fig. 3b,c), yielding estimates for the mechanical parameters of the model. We found a cortical tension gradient $\Delta \xi = 68 \pm 7 \text{ Pa m}^{-1}$, consistent with reported cortical tension values$^{12,18}$ and a 2D cortex viscosity $\eta = 27 \pm 3 \times 10^{-6} \text{ Pa s m}^{-1}$, consistent with reported values of 3D cortex viscosities$^{20}$. The drag coefficient was found to be $\alpha_D = 208 \pm 29 \text{ kPa s m}^{-1}$, corresponding to about 50% of the extracellular medium being pushed forward as the cell migrates, in agreement with direct estimates of medium flows (Supplementary Fig. 4e–h and Supplementary Note). In summary, we could accurately fit both cell and cortex dynamics for all three friction conditions using a single set of three fit parameters. The strong agreement between data and theory suggests that rearward cortical flows coupled to the substrate through nonspecific friction are sufficient to drive focal-adhesion-independent migration.

Finally, we used the model and the extracted parameters to compute the spatial distribution of forces exerted on the substrate during adhesion-free migration (Supplementary Note). We found that the total force exerted by migrating Walker cells on the channel walls was a few piconewtons, balancing the drag experienced by cells in microchannels. The spatial force distribution of a migrating cell can be described by a force dipole, a measure of the separation of pushing and pulling forces exerted by the cell (see Supplementary Note for details). A positive force dipole characterizes a cell that pushes itself from the rear, whereas a negative dipole describes a cell that pulls at the front. The force dipole determines the pattern of substrate deformation and can influence cell orientation and cell–cell interactions (reviewed in ref. 1). Cells migrating using focal adhesions exert a contractile, negative force dipole, where strong pulling forces on adhesions at the cell leading edge are counterbalanced by contractile forces at the rear$^{1,21}$. In striking contrast with adherent cells, we found that in migrating Walker cells, the dipolar moment of the force distribution was positive (Fig. 4a). This indicates that the cells tend to expand rather than contract their substrate, with the propulsive thrust being generated in the cell rear (Fig. 4b). Thus, even though force generation relies on actomyosin-driven flows for both migration modes, the force dipoles during adhesion-free and focal-adhesion-based migration have opposite orientations.

In summary, our study reveals fundamental differences in force transmission mechanics between focal-adhesion-dependent and -independent migration modes (Fig. 4). We find that stresses generated during adhesion-free migration are orders of magnitude smaller than stresses typically exerted during specific-adhesion-based motility (Fig. 4b and Supplementary Fig. 4i). Indeed, even cells relying on weak or transient adhesions, such as fast-moving keratocytes or adherent cells with reduced myosin activity, exert forces in the nanonewton per square micrometre range on their substrate$^{22,23}$. We report here that Walker cells exert stresses of $\sim$1 Pa or lower, corresponding to forces on the channel walls in the piconewton per square micrometre range. This range of stresses is under the limit of detection using sensitive traction force measurement methods$^{23-25}$. Instead, our approach, which relies on friction measurements coupled to fitting of imaging data to a mechanical model of migration, provides a technique to extract forces and cell physical properties from cell shape and cortex dynamics. Such fitting-based measurements are increasingly being used in studies of cell mechanics$^{26,27}$. It will be a challenge for future studies to design substrates allowing for a more direct measurement of stresses under 1 Pa during migration in confinement.

Our study suggests that the large forces transmitted by focal adhesions, rather than being essential for motion itself, could function mostly to guide persistent directional migration$^{28}$ or to probe substrate stiffness$^{29,30}$. Furthermore, in integrin-based migration, large forces are required to detach focal adhesions and adhesion that is too strong prevents migration$^{21}$; in friction-based migration, the detachment forces are minimal and above a threshold friction, cell velocity remains...
constant, even for very high frictions (Fig. 3b). Mechanisms giving rise to friction, including possible contributions of substrate irregularities in vivo, remain to be investigated. Finally, we show that during friction-driven migration, the force distribution corresponds to a positive force dipole in striking contrast with adhesive migration, where the force dipole is negative (Fig. 4b). A similar distinction exists for microwimmer organisms, which can be divided into two categories, 'pushers', such as Escherichia coli, and 'pullers', such as Chlamydomonas reinhardtii, according to the sign of their force dipoles (2) (Fig. 4c).

This distinction has important consequences for coupling between swimming cells: side-by-side moving pushing repel each other whereas pullers attract each other. It will be interesting to investigate how cell-cell interactions differ between crawling 'pushing' and 'pulling' cells migrating in vivo.

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METHODS

Methods and any associated references are available in the online version of the paper.

Note: Supplementary Information is available in the online version of the paper.

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AUTHOR CONTRIBUTIONS

M.B., A.E., G.S. and E.K.P. designed the research and wrote the paper, M.B. performed the experiments, M.B. and A.E. analysed the data and A.E. and G.S. developed the theoretical model. M.B. and R.A.D. designed the microfluidic assays and performed microfocib assays. I.M.A. helped to perform revision experiments. G.C. and A.C.O. gave technical support and conceptual advice. All authors discussed the results and implications and commented on the manuscript at all stages.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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1. Schwarz, U. S. & Safran, S. A. Physics of adherent cells. Rev. Mod. Phys. 85, 1327–1381 (2013).

2. Lämmermann, T. et al. Rapid leukocyte migration by integrin-independent flowing and squeezing. Nature 453, 51–55 (2008).

3. Parsons, J. T., Horwitz, A. R. & Schwartz, M. A. Cell adhesion: integrating cytoskeletal dynamics and cellular tension. Nat. Rev. Mol. Cell Biol. 11, 633–643 (2010).

4. Friedl, P. To adhere or not to adhere? Nat. Rev. Mol. Cell Biol. 11, 3 (2010).

5. Renkawitz, J. & Sixt, M. Mechanisms of force generation and force transmission during interstitial leukocyte migration. EMBO Rep. 11, 744–750 (2010).

6. Balaban, N. Q. et al. Force and focal adhesion assembly: a close relationship studied using elastic micropatterned substrates. Nat. Cell Biol. 3, 466–472 (2001).

7. Legant, W. R. et al. Measurement of mechanical tractions exerted by cells in three-dimensional matrices. Nat. Methods 7, 969–971 (2010).

8. Alexander, S. et al. Dynamic imaging of cancer growth and invasion: a modified skin-fold chamber model. Histochem. Cell Biol. 130, 1147–1154 (2008).

9. Beadle, C. et al. The role of myosin II in glioma invasion of the brain. Mol. Biol. Cell 19, 3357–3368 (2008).

10. Stroka, K. M. et al. Water permeation drives tumor cell migration in confined microenvironments. Cell 157, 611–623 (2014).

11. Hawkins, R. J. et al. Spontaneous contractility-mediated cortical flow generates cell migration in three-dimensional environments. Biophys. J. 101, 1041–1045 (2011).

12. Berget, M. et al. Cell mechanics control rapid transitions between blebs and lamellipodia during migration. Proc. Natl Acad. Sci. USA 109, 14434–14439 (2012).

13. Kam, L. & Boxer, S. G. Cell adhesion to protein-micropatterned-supported lipid bilayer membranes. J. Biomed. Mater. Res. 55, 487–495 (2001).

14. Kawamura, R. et al. Controlled cell adhesion using a biocompatible anchor for membrane-conjugated bovine serum albumin/bovine serum albumin mixed layer. Langmuir 29, 6429–6433 (2013).

15. Kaga, H. et al. Tailor-made cell patterning using a near-infrared-responsive composite gel composed of agarose and carbon nanotubes. Biofabrication 5, 015010 (2013).

16. Kubow, K. E. & Horwitz, A. R. Reducing background fluorescence reveals adhesions in 3D matrices. Nat. Cell Biol. 13, 5–9 (2011).

17. Ponti, A., Machacek, M., Gupton, S. L., Waterman-Storer, C. M. & Danuser, G. Two distinct actin networks drive the protrusion of migrating cells. Science 305, 1782–1786 (2004).

18. Tinevez, J. Y. et al. Role of cortical tension in bleb growth. Proc. Natl Acad. Sci. USA 106, 18581–18586 (2009).

19. Byun, S. et al. Characterizing deformability and surface friction of cancer cells. Proc. Natl Acad. Sci. USA 110, 7580–7585 (2013).

20. Norstrom, M. & Gardel, M. L. Shear thickening of F-actin networks crosslinked with non-muscle myosin IIB. Soft Matter 7, 3228–3233 (2011).

21. Tanimoto, H. & Sano, M. A simple force-motion relation for migrating cells revealed by multipole analysis of traction stress. Biophys. J. 106, 16–25 (2014).

22. Lee, J. et al. Traction forces generated by locomoting keratocytes. J. Cell Biol. 127, 1957–1964 (1994).

23. Ricart, B. G. et al. Measuring traction forces of motile dendritic cells on micropost arrays. Biophys. J. 101, 2620–2628 (2011).

24. Fu, J. et al. Mechanical regulation of cell function with geometrically modulated elastomeric substrates. Nat. Methods 7, 733–736 (2010).

25. Morin, T. R. Jr, Ghassem-Zadeh, S. A. & Lee, J. Traction force microscopy in rapidly moving cells reveals separate roles for RCKK and MLCK in the mechanics of retraction. Exp. Cell Res. 326, 280–294 (2014).

26. Mayer, M. et al. Anisotropies in cortical tension reveal the physical basis of polarizing cortical flows. Nature 467, 617–621 (2010).

27. Sedzinski, J. et al. Polar actomyosin contractility destabilizes the position of the cytokinetic furrow. Nature 476, 462–466 (2011).

28. Petrie, R. J., Doyle, A. D. & Yamada, K. M. Random versus directionally persistent cell migration. Nat. Rev. Mol. Cell Biol. 10, 538–549 (2009).

29. Geiger, B. & Bershadsky, A. Exploring the neighborhood: adhesion-coupled cell mechanosensors. Cell 110, 139–142 (2002).

30. D’Cruze, D. E., Janmey, P. & Wang, Y. L. Tissue cells feel and respond to the stiffness of their microenvironments. Science 310, 1139–1143 (2005).

31. Palecek, S. P. et al. Integrin-ligand binding properties govern cell migration speed through cell-substratum adhesiveness. Nature 385, 537–540 (1997).

32. Lauga, E. & Powers, T. R. The hydrodynamics of swimming microorganisms. Rep. Prog. Phys. 72, 096601 (2009).
**METHODS**

Reagents and plasmids. The Arp2/3 complex inhibitor CK-666 (ref. 33) was purchased from Tocris Bioscience and dissolved in dimethylsulfoxide. BSA, β-lactoglobulin and Fluoronic F127 were purchased from Sigma and dissolved in 1× PBS. Poly-L-lysine (pLys)–PEG was from Surface Solutions and diluted in 10 mM HEPES. GFP–FAX was provided by E. Papusheva/C-P. Heisenberg (Institute of Science and Technology Austria, Austria). Speckle-GFP–vinculin was a gift from A. R. Horwitz (University of Virginia, USA). MRLC–GFP was a gift from R.L. Chisholm (Northwestern University, Chicago, USA). siRNAs were Stealth Select RNAi siRNAs from Invitrogen with the following sequences: TLN1 siRNA (RS52 0273): 5′-GGGCAUAUCCAUUGCUCCAGACAAA-3′; TLN2 siRNA (RS5231 0233): 5′-GAGAGGACCUGGAGAUCCCAGGAAAAUUC-3′. Knockdown efficiency was quantified by western blotting with antibodies against TLN1 (monoclonal clone 8d4, Sigma no. T3287, 1:2,000), TLN2 (GeneTex, no. EPR5326(2), 1:1,000) and GAPDH (clone 1D4, Novus Biologicals no. NB300-221, 1:20,000). Representative images of immunoblots are based on at least 2 independent experiments.

Culture and transfection of cells. Walker 256 carcinosarcoma cells were a gift from V. Niggli (University of Bern, Switzerland). Cells were grown in RPMI1640 supplemented with 10% FCS, 1% penicillin–streptomycin and 2 mM glutamine (all Gibco, Invitrogen) at 37 °C and 5% CO2. Transient cell transfection was performed with Amazix Nucleofection systems (Cell Line Nucleofector Kit V or Cell Line 96-well Nucleofector Kit SE, Lonza) using 0.5 to 1 µg plasmid DNA and protocols optimized for HL-60 cells provided by the manufacturer. For MRLC–GFP, a stable cell line was created by transient transfection of WT cells followed by selection with 500 µg ml−1 G418 and clonal separation by FACS (performed at the Wolfson Institute for Biomedical Research).

Cell migration environments. For the under-agarose assay, Walker cells were placed between a glass surface or a commercially available ultralow-attachment surface (Corning Life Sciences) and an agarose layer by using the earlier-described under-agarose assay33, one hour before the experiment. Cell culture-tested agarose with a low gelling temperature was used (Sigma). In under-agarose experiments with PEG-coated glass substrates, small agarose patches were made and placed onto glass-bottom dishes previously coated with 100 µg ml−1 pLys–PEG for one hour at room temperature.

Three-dimensional collagen gels were produced as described earlier35. Microfluidic channels were produced as described earlier18, except that PDMS blocks with channels were not bound to glass but instead onto PDMS-coated glass coverslips. This creates microfluidic channels where all 4 sides are made out of PDMS. Bonding was achieved by partially curing the PDMS on the coverslip as well as the PDMS with the features, and then assembling the device following the final curing step. Microchannels were then coated with 50 µg ml−1 BSA, 2 mg ml−1 F127 or a mix of BSA and F127 (50 µg ml−1 BSA and 50 µg ml−1 F127, all in 1× PBS) for 1 h at room temperature. After a washing step, channels were filled with cell culture medium (supplemented with 50 µM of the Arp2/3 inhibitor CK-666, to prevent cells from switching to an adhesion-based, lamellipodia-driven migration mode33). Cells pretreated with 50 µM CK-666 for 15 min were introduced into the channels by applying a gentle pressure with a syringe containing a concentrated cell suspension. At all steps it was ensured that the tubing and the syringes were free of air bubbles and that the air was introduced into the microfluidic system. After introducing the cells in the channels, the entire chip was covered with medium to ensure an equal hydrostatic pressure through the channel.

**Traction force substrates.** Traction force substrates were prepared as follows: Dow Corning silicone elastomer CY 52-276 components were thoroughly mixed in a 1:1 mass ratio (3KPa substances17,18), degassed and spin-coated on glass coverslips to create a 30-µm-thick layer. After curing for 30 min at 70 °C samples were treated for 30 min with 10% (3-aminopropyl)triethoxysilane (Sigma) in ethanol, followed by several washes with ethanol. Air-dried samples were then incubated at 70 °C for 1 h. Forty-micrometre fluorescent microspheres (Life Technologies) were bound to the surface of the samples by a 1 min incubation with a 1× PBS solution containing microspheres and 50 µg ml−1 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC Sigma). Finally, elastic substrates were coated with a solution containing 50 µg ml−1 fibronectin (Life Technologies) and 50 µg ml−1 EDC for 30 min before cell seeding. In the case of HeLa cells and adherent Walker cells, substrates were relaxed by adding 0.1% SDS to the medium. For suspension, Walker cells, elastic substrates and cells were overlaid with an agarose layer and cells were imaged over time, allowing observation of the bead positions with and without the cell.

**Imaging and laser ablation.** Fluorescence, DIC and IRM imaging were performed on a scanning confocal microscope (Olympus FV1000 or FV 1200, UPlanApo × 60 NA 1.35 oil objective or UPlanApo 60XS NA 1.3 silicone objective) using a 488 nm or a 561 nm laser as the light source. Laser ablation experiments were performed on the same system using a 405 nm picosecond pulsed laser, as described previously33,34. Phase contrast observations were performed on an inverted Zeiss Axiovert 200M wide-field microscope using a Ph2 Plan Neofluar × 40 NA 0.75 or a LD Plan Neofluar × 40 NA 0.6 Ph2 Korr objective. Environmental control (37 °C, 5% CO2) was applied for all imaging set-ups. Representative images of cells shown are based on ≥ 2 independent experiments.

**Cell tracking.** Analysis of migration of cells under agarose was done by manual tracking of the nuclei of cells using the Manual Tracking plugin of Fiji. Quantitation of cell velocities in microchannels from phase contrast videos was done by producing kymographs along the long axis of the cell followed by calculation of the velocity from the angle of the kymograph.

Quantification of cortical flows (PIV). To estimate the velocity field in the cell cortex, we analysed time-lapse videos of migrating Walker cells expressing MRLC–GFP in microchannels (height: 6 µm, width: 10 µm, 2 per frame). In the analysis of cell migration in microchannels with large and intermediate frictions, only actively migrating cells were considered. Cells failing to migrate, or switching direction or dividing during imaging, were excluded. This criterion was pre-established and then applied to all experiments. The videos were taken in the focal plane of the actomyosin cortex close to the cell surface. Videos were first registered using the MultiStackReg Fiji plugin and a Gaussian blur filter of height 3 and width 0.9 was applied to all images to reduce noise before analysis. The PIV algorithm described in ref. 26 was then applied to estimate the frictional cortical flow field by computing the cross-correlation between subsequent images. The same set of PIV parameters was used in all analyses. The ROI for each cell, spanning from the rear to the front excluding the uropod, was selected manually on time-averaged images (see Fig. 3c for example). Assuming axisymmetry and stationarity of the flow field, we averaged the data over the height of the cell and over time. To account for differences in cell length and to average over cells, we binned the data into 33 bins along the cell. In both averaging steps, data points were excluded on the basis of the following criteria: for each cell, we included only data points that came from a sample of n = 300 time and cell-height points; and for the cell average flow profile, we used only points coming from a sample larger than the sample size median for each friction condition. We noticed a systematic underestimation of velocities by the PIV algorithm due to the static noisy background. To correct for this, we generated series of artificial test images, where random patterns of blurred spots were shifted in front of middle-plane images of the analysed cells. The intensity of the spots was adjusted to match real myosin foci. The pattern was advanced at a rate of 3 per frame, in the range of the observed cortical flow. Performing PIV on these artificial cortical flows with known velocity allowed computation of the factor correcting for the bias. We found \( V_{corr} = 1.167 V_{PIV} \). The code for the PIV algorithm is available on request to the corresponding authors.

**Quantification of myosin intensity profiles.** We estimated the relative fluorescence intensity of cortical myosin from the same images the PIV was performed on. Again, assuming axisymmetry and stationarity of the myosin distribution, we binned the data into 50 bins along the cell and averaged each video over time and cell height. Finally, data were averaged between different cells for each friction condition. We subtracted the background fluorescence from the cortex close to the cell surface. Videos were first registered using the MultiStackReg Fiji plugin and a Gaussian blur filter of height 3 and width 0.9 was applied to all images, where random patterns of blurred spots were shifted in front of middle-plane images, using images from the middle section of the cells. All intensities were normalized to the average intensity in a region devoid of myosin spots in the front of the cell.

**Friction measurements.** The friction device was designed on the basis of previous work18,19 and assembled and coated in a way identical to the microchannels (see above). After the device was filled with cell culture medium, the 3 entry ports were connected to reservoirs containing medium with 0.2 µm fluorescent microspheres (Invitrogen), medium with cells and plain medium (see Supplementary Fig. 2). Lowering the output reservoir E3 created a pressure difference, leading to flows of beads and cells into and through the device. The relationship between the mean free flow velocities in the analysis channel of the device and the height of reservoir E3 relative to the other reservoirs E1 and E2 (Δh) was determined by imaging and measuring microsphere velocities using a LD Plan Neuofluar × 20 NA 0.4 Ph2 Korr objective on an inverted Zeiss Axiovert 200M wide-field microscope equipped with a spinning-disc scan head (Andor/Yokogawa; Supplementary Fig. 2 and Supplementary Video 7). Briefly, to extract the mean free flow velocities, kymographs were created along the entire channel width and the mean angle of each kymograph was determined using Fiji’s Directionality plugin. As the imaging set-up allowed imaging of the entire channel height in a single plane, averaging over the channel width gives the mean free flow velocity (see Supplementary Fig. 2). Friction measurements were performed on unpolaredized cells, which did not exhibit any motility. Single cells were introduced into the analysis channel and their velocity was

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recorded for different mean free flow velocities in the device with various coatings (Supplementary Fig. 2).

To estimate the friction, the pressure difference \( P_{\text{friction}} \) was first estimated by measuring the average free flow velocity \( v_{\text{free}} \) in the channel without cells, related to the applied pressure through the following relation:

\[
P_{\text{friction}} = k_c \eta c_{\text{free}}
\]  

(1)

where \( k_c = 8 \eta / R^2 \pi \) is the hydraulic resistance of the channel, with \( \eta \) being the viscosity of water and \( R \) the length of the channel. Once a single cell was introduced into the channel, its velocity \( U \) was measured at the same applied pressure \( P_{\text{friction}} \) as given in equation (1). The cell velocity was then related to the friction coefficient \( \alpha \) and to the applied pressure through the following relation:

\[
P_{\text{applied}} = \left( \frac{k_c}{\xi} + 1 \right) \frac{2 \alpha U}{R} + \xi c S U
\]  

(2)

where \( R \) is the channel radius and \( L \) is the contact length of the cell estimated from the microscopy images. To obtain equation (2), we take into account the hydraulic resistance of the cell \( \xi \) to estimate the fraction of fluid going through the cell. The applied pressure to the channel can be written as \( P_{\text{applied}} = 2 \alpha U / R + \xi c S \), where the first term relates the pressure to the resulting displacement of the cell, which is resisted by friction, and the second term describes the relation to the induced fluid flow in the channel. To obtain the resistance of the cell to displacement in the first term, we integrated the friction force density \( \alpha U \) over the cell surface, assuming the cell behaves as a solid object. The second term depends on the mean fluid velocity in the channel in the presence of a cell, which is given by \( \tau_c = (2 \alpha U / R^2 \pi + 1) U \), leading to the expression given in equation (2). The hydraulic resistance of the cell \( \xi \) was estimated self-consistently together with the fitting procedure for cellular retrograde flows (see Supplementary Theory). Using this estimate, we computed the friction coefficients in different conditions from equation (2).

**Image processing, data analysis and statistics.** Images were processed using Fiji and Adobe Illustrator. They were cropped, rotated, and their contrast and brightness were manually adjusted. Data were analysed, tested for statistical significance, fitted and visualized using R, MATLAB (MathWorks, 2013) and Mathematica (Wolfram Research, 2013) software. In particular, the code used to fit the data to the mechanical model of migration was a custom-made code written in Mathematica. The source code is available on request to the corresponding authors. No statistical method was used to predetermined sample size. The Shapiro–Wilk test or the Kolmogorov–Smirnov test was used to ensure normality of data. Welch’s \( t \)-test was chosen for statistical testing, which is insensitive to the equality of variances. Boxes in all box plots extend from the 25th to 75th percentiles, with a line at the median. Whiskers extend to \( \times 1.5 \) IQR (interquartile range) or the max/min data points if they fall within \( \times 1.5 \) IQR.

33. Nolen, B. J. et al. Characterization of two classes of small molecule inhibitors of Arp2/3 complex. *Nature* **460**, 1031–1034 (2009).
34. Heit, B. & Kubes, P. Measuring chemotaxis and chemokinesis: the under-agarose cell migration assay. *Sci. STKE* **170**, PL5 (2003).
35. Artym, V. V. & Matsumoto, K. *Imaging Cells in Three-Dimensional Collagen Matrix*, Ch. 10, 1–20 (2010).
36. Heuze, M. L. et al. Cell migration in confinement: a micro-channel-based assay. *Methods Mol. Biol.* **769**, 415–434 (2011).
37. Style, R. W. et al. Traction force microscopy in physics and biology. *Soft Matter* **10**, 4047–4055 (2014).
38. Iwadate, Y. & Yumura, S. Molecular dynamics and forces of a motile cells simultaneously visualized by TIRF and force microscopies. *Biotechniques* **44**, 739–750 (2008).
39. Preira, P. et al. Single cell rheometry with a microfluidic constriction: Quantitative control of friction and fluid leaks between cell and channel walls. *Biomicrofluidics* **7**, 24111 (2013).
40. Gabriele, S. et al. A simple microfluidic method to select, isolate, and manipulate single cells in mechanical and biochemical assays. *Lab Chip* **10**, 1459–1467 (2010).
**Supplementary Figure 1** Blebbing Walker cell migration is focal adhesion independent but depends on a rearward myosin contractility gradient. 

**a**. Snapshot and kymograph of a blebbing Walker cell in microchannel (8x8µm) coated with 50µg/ml β-Lactoglobulin. Scale bars: horizontal: 10µm, vertical: 50s. 

**b**. Plot shows instantaneous velocity of Walker cells in microchannels coated with 50µg/ml BSA or β-Lactoglobulin. P-Value: Welch’s two-sided T-Test, n: number of cells analyzed in 2 (BSA) and 3 (β-Lactoglobulin) independent experiments. Boxes in boxplots extend from the 25th to 75th percentiles, with a line at the median. Whiskers extend to 1.5x IQR (interquartile range) or the max/min datapoints. 

**c**. Blebbing Walker cells expressing GFP-FAK do not form detectable focal adhesions during migration under agarose, while adherent Walker cells show focal adhesions during 2D migration on glass. Arrows indicate migration direction. Scale bars: 10 µm. 

**d**. Tracks of migrating blebbing Walker cells under agarose on glass, PEG-coated glass and commercially available low attachment surfaces. Cells were manually tracked for 52min. Scale bars: 50µm. 

**e**. Double laser ablation of the actomyosin cortex at the rear of the cell body (highest Myosin and F-Actin intensities) caused an instantaneous, strong decrease in cell velocity compared to a double ablation at the cell front. Scale bars: horizontal: 10 µm, vertical: 50 s. Plot shows reduction in cell velocity in % relative to the pre-ablation velocity. Horizontal bars: mean values. P-Value: Welch's two-sided T-Test; n: number of cells analyzed in 3 (rear) and 2 (front) independent experiments. 

**f**. Confocal images of Walker cells labelled with MRLC-GFP: upper panel: cortex section, lower panel: middle cross section. Scale bar: 10 µm. The white rectangles highlight the ROI chosen for the analysis of myosin II intensity. 

**g**. To obtain cortical myosin intensity profiles (panel i), we subtracted the normalized fluorescence intensity profile in the middle cell section (cytoplasmic background, grey) from the myosin intensity profile acquired at the cell surface (black). n=33 cells from 5 independent experiments. 

**h**. Relative myosin fluorescence intensity profile at the cortex for different friction conditions. n: number of cells analyzed in 5 (BSA), 3 (BSA/F127) and 5 (F127) independent experiments. All error bars: SEM.
Supplementary Figure 2  Varying and measuring substrate friction. a, Design of the microfluidic chip used for friction measurements. 3 entry ports were connected to reservoirs. Blowup shows region with bypass (h:8µm, w:50µm) and analysis channel (h:8µm, w:8µm). Micrograph shows single cell entering the analysis channel. Scale bar: 10µm. b, Schematic of the measurement setup and principle of flow control. The pressure difference and thus the flow were controlled by adjusting the height of reservoir E3 relative to the other reservoirs E1 and E2. c, Calibration of the free average flow in the microfluidic friction device based on tracking of fluorescent beads. n: number of measurements pooled from several independent experiments as indicated in the figure. d, Maximum intensity projections of Z-stacks taken at the entry regions to microchannels coated with 50µg/ml fluorescent BSA-488 mixed with 0µg/ml, 30µg/ml or 200µg/ml F127. Scale bars: 10 µm. e-g, Cell velocities and linear fits in microfluidic friction devices coated with F127, BSA-F127 mix or BSA. Error bars represent SEMs. For e n=9 cells analyzed from 3 independent experiments; for f n= 10 cells analyzed from 2 independent experiments; for g n=9 cells analyzed from 3 independent experiments. h. Cell substrate friction coefficient measured for channels coated with F127, BSA-F127 mix and BSA. n: number of cells analyzed from 3 (F127), 2 (BSA/F127) and 3 (BSA) independent experiments.
**Supplementary Figure 3** Mechanical description of focal adhesion-independent migration.  

**a**, Contact between a Walker cell and the channel wall visualized using interference reflection microscopy (IRM). Scale bar: 10 μm.  

**b**, Upper panel: Time-average (3min) maximum intensity projection of the surface of a cell expressing MRLC-GFP. On timescales exceeding the bleb life cycle, the cell front has a near-hemispherical average shape. Scale bar: 10 μm. Lower panel: Parametrization and geometry of the axisymmetric cell surface.  

**c**, Coordinates used in different cell parts.  

**d**, Upper panel: Linear profile of myosin-generated active tension $\zeta(x)$ along the cell axis used for analytical calculations. Lower panel: Retrograde cortical flow profiles along the cell (reference frame of the cell). The cortical flow velocity depends on the friction coefficient. $v_{\text{norm}} = (\zeta(r) - \zeta(f))/L$.  

**e**, Cell velocity and external pressure. Upper panel: Schematic of internal and external pressures acting on the cell. Lower panel: Comparison between numerical evaluation of the inflexion point of the cell velocity $U(\alpha)$ (dots) and the proposed approximate relation $\alpha^* = R_0/2L$ (line), see Eq. 49. [$\alpha_{\text{norm}} = \eta L^2/\eta$] Red dot: value obtained from the fitting procedure to experimental data.  

The stalling pressure of −15 Pa is three orders of magnitude lower than reported values for adhesive cells in micropipettes (around −2 kPa).
Supplementary Figure 4 Role of the nucleus and internal friction (a-d), hydraulics of the channel-cell system (e), validation of the estimated fluid drag coefficient (f - h) and forces exerted on the channel walls (i). a, Quantification of nuclear cross-sectional area. Scale bar: 10 μm. b/c, Nuclear cross-sectional area does not correlate with cell velocity in intermediate (b) or high (c) friction channels. NExp= 1(b) or 4(c). d, Schematic of internal friction arising from intracellular material. In this description, the intracellular pressure difference between the rear and front of the migrating cell is balanced by the frictional forces \( f - h \) and forces exerted on the channel walls (i). e, A single cell migrating in a channel experiences drag forces governed by the fluid flow resistances in the channel. A channel segment containing a cell offers a flow resistance \( \xi \) while the fluid-filled channel is characterized by the resistance \( \xi_c \). When multiple cells migrate in a channel, the fluid drag coefficient depends predominantly on the flow resistance of cell segments \( \xi \). For definition of the fluid flow resistances of the rear and front part of the channel \( \xi_{\text{r}} \) and \( \xi_{\text{f}} \) see Equations 51-52. f, Average fluid flow velocity induced by migrating cells was estimated by tracking microspheres in channels with rapidly migrating cells. Scale bar: 10 μm. g, Kymograph of BSA-coated microchannel array with multiple migrating cells labelled with MRLC-GFP. Scale bars: horizontal: 500 μm, vertical: 30 min. h, Histogram of cell velocities in BSA-coated channels obtained from kymographs of the full microchannel array. To allow for comparison with fluid flow measurements in channels with rapidly migrating cells, time-windows without cell velocities above 5.45 μm/min were discarded. The threshold was chosen according to the 0.1 quantile of the velocity distribution of polarised, fast cells used for cortical flow analysis. n=164 cells from 2 independent experiments. i, Distribution of forces exerted by migrating Walker cells on the channel wall in large, intermediate and small friction channels. Cell migration direction is to the right, the force is oriented on average in the direction opposite to cell motion, and the stress magnitudes are in the mPa-Pa range.
Supplementary Figure 5 Uncropped Western Blots and images of molecular weight makers. Dashed lines indicate how the blot was cropped in Supplementary Fig. 1e.
Supplementary Video Legends

**Video S1: Migration of blebbing Walker cell in collagen gel in the presence of EDTA.**
Blebbing Walker cell migrating in a three-dimensional collagen gel in the presence of 2 mM EDTA. Scale bar: 10 μm; 25 s/frame; DIC, confocal microscopy (Lifeact-mCherry, maximum intensity projection) and confocal reflection microscopy (collagen fibers).

**Video S2: Migration of a non-adherent Walker cell in a BSA-coated microchannel.**
Non-adherent Walker cell placed in a microchannel (10 μm x 8 μm) coated with BSA in the presence of 50 μm CK-666. Scale bar: 10 μm; 5 s/frame; phase contrast.

**Video S3: Ablation of the cortex in the rear part of a non-adherent Walker cell in a microchannel.**
Non-adherent Walker cell expressing Lifeact-mCherry in a BSA-coated microchannel (6 μm x 8 μm) in the presence of 50 μm CK-666. The cortex at the rear of the cell body was ablated twice. Scale bar: 10 μm; 1 s/frame; DIC and confocal microscopy (Lifeact-mCherry).

**Video S4: Ablation of the cortex at the leading edge of a non-adherent Walker cell in a microchannel.**
Blebbing Walker cell expressing Lifeact-mCherry in a BSA-coated microchannel (6 μm x 8 μm) in the presence of 50 μm CK-666. The cortex at the leading edge was ablated twice. Scale bar: 10 μm; 1 s/frame; DIC and confocal microscopy (Lifeact-mCherry).

**Video S5: F-Actin dynamics at the cortex of a Walker cell migrating in a BSA-coated channel.**
F-Actin dynamics in a non-adherent Walker cell expressing Lifeact-GFP in a BSA-coated microchannel (8 μm x 6 μm) in the presence of 50 μm CK-666. Scale bar: 10 μm; 2 s/frame; DIC and confocal microscopy (Lifeact-GFP). Left: images in the reference frame of the channel; right: images in the reference frame of the cell.

**Video S6: Myosin dynamics at the cortex of a Walker cell migrating in a BSA-coated channel.**
Myosin dynamics in a non-adherent Walker cell expressing MRLC-GFP in a BSA-coated microchannel (10 μm x 6 μm) in the presence of 50 μm CK-666. Scale bar: 10 μm; 2 s/frame; DIC and confocal microscopy (MRLC-GFP). Left: images in the reference frame of the channel; right: images in the reference frame of the cell.

**Video S7: Microfluidic-based friction measurements.**
Summary of performed friction measurements. The microfluidic device in this example was coated with F127. Scale bar: 10 μm; confocal microscopy (beads) or phase contrast (cells).

**Video S8: Non-adherent Walker cell in an F127-coated microchannel (low friction).**
Non-adherent Walker cell placed in a microchannel (10 μm x 8 μm) coated with F127 in the presence of 50 μm CK-666. The cell is polarized but does not migrate. Scale bar: 10 μm; 5 s/frame; phase contrast.

**Video S9: Myosin dynamics at the cortex of a Walker cell in an F127-coated channel (low friction).**
Myosin dynamics in a non-adherent Walker cell expressing MRLC-GFP in an F127-coated microchannel (10 μm x 6 μm) in the presence of 50 μm CK-666. Scale bar: 10 μm; 2 s/frame; DIC and confocal microscopy (MRLC-GFP). Images in the reference frame of the channel.

**Video S10: Myosin dynamics at the cortex of a Walker cell migrating in a channel with intermediate friction.**
Myosin dynamics in a non-adherent Walker cell expressing MRLC-GFP in a microchannel coated with a mix of BSA and F127 (10 μm x 6 μm) in the presence of 50 μm CK-666. Scale bar: 10 μm; 2 s/frame; DIC and confocal microscopy (MRLC-GFP). Images in the reference frame of the channel.

**Video S11: Simulation of cell motion and cortical flow velocity predicted by the theoretical description of flow-friction based cell migration.**
Simulations are shown for the three different values of friction measured in F127, F127/BSA and BSA coated channels respectively (from top to bottom). Black dots at the cell surface depict cortex flows.
Supplementary Note: Mechanical description of focal adhesion-independent cell migration

Contents

1 Model description and main equations 2
  1.1 Cell shape parametrization 2
  1.2 Material equation for the actomyosin cortex 3
  1.3 Force balance equations 4
  1.4 External fluid pressure and drag coefficient 5

2 Cortical velocity profiles and cell velocity 5
  2.1 Profile of active tension 5
  2.2 Flow in the cylindrical region of the cell 6
  2.3 Flow and deformation in the cell pole regions 6
  2.4 Cortical velocity and tension at the contact points 7
  2.5 Cell velocity 8
  2.6 Threshold friction for cell movement 9

3 Fluid drag coefficient in a channel 9

4 Comparison of theoretical profiles to experimental measurements 10
  4.1 Cortical flow profiles and cell velocities at different frictions 10
  4.2 Role of internal friction 12
  4.3 Fluid flow in the channel 12

5 Force density on the cell surface 13
We propose here a theoretical description of the mechanics of non-adherent blebbing Walker cells migrating in microchannels. Our assumptions on cell mechanics and the main equations are detailed in Section 1. We focus on the mechanics of the actomyosin cortical network, since it is the structure powering cell motion (Supplementary Fig. 1f), and describe the actin cortex as a thin layer of active, viscous fluid. This essential assumption is captured in Eq. 7, which states that the tension in the cortex is a sum of a viscous part, proportional to the gradient of flow, and an active part, reflecting internal contractile tension. The internal contractile tension is generated by myosin II motor proteins and thus depends on their concentration: the polarised myosin distribution observed in cell migration (Supplementary Fig. 1g, Supplementary Fig. 3b) translates into a gradient of internal tension, which we assume to be proportional to the gradient in myosin intensity (Supplementary Fig. 1f). A gradient in active tension can drive actomyosin flows in the cortical layer, and deformations of the cell surface. We assume that the cell shape is confined to a cylinder in the region where it is in contact with the microchannel walls, and that the rear and front pole regions are free to take the shape satisfying force balance (Fig. 3a, Supplementary Fig. 3b).

In addition to the stresses arising in the actomyosin cortex, two external forces act on the cell when it moves in the microchannel:

- (i) Forces between the cortex and the channel walls: we assume that cortical flows in the region in contact with the channel are resisted by a tangential friction force, proportional to the velocity of the flow relative to the channel wall. A friction coefficient $\alpha$ characterizes this proportionality relation (Eq. 11). Possibly, transient binding and unbinding events between proteins at the cell surface and the channel coating are contributing to the friction coefficient.

- (ii) Forces arising from the fluid surrounding the cell: to take into account forces arising from the external medium, we introduce an effective drag coefficient arising from the motion of the external medium, which must flow around or through the cell, or be pushed forward as the cell moves. In Section 3, we evaluate the different contributions to the effective drag acting against cell motion in a channel.

In Section 2, we solve the model equations analytically and obtain expressions for the cell shape, cell velocity and the cortical flow profile as a function of the mechanical parameters. Our central result, the expression for the cell velocity (Eq. 45), indicates that myosin-dependent cortical flows produce sufficient thrust to propel the cell through the surrounding fluid only above a critical threshold of substrate friction (see Fig. 3b and Supplementary Fig. 3f). The threshold friction coefficient depends on the drag coefficient and on the shape of the cell. For friction coefficients above the threshold, the mechanism for cell migration can be understood as follows: the active tension gradient gives rise to rearward cortical flows, which are opposed by friction at the cell-channel contact surface, giving rise to a force propelling the cell forward (Supplementary Fig. 3f). In addition, active tension dependent contraction of the cell rear and expansion of the front allow the cell to move, even in the limiting condition of infinite friction, when the cell body cannot move relative to the wall.

In Section 4, we proceed to compare the predictions of the theoretical description to experimental measurements of the cortical flow profiles and cell velocities in three different conditions of channel friction. A fitting procedure allows us to obtain estimates of the mechanical parameters introduced in the theoretical description. Remarkably, all our quantitative data on cell and cortex dynamics in three different friction conditions can be fitted very accurately with three parameters (Fig. 3b and c), yielding estimates consistent with previous studies and additional validating experiments (see Section 4.3 and Supplementary Fig. 4e-h).

Finally, using parameters extracted from the fitting procedure, we obtain in Section 5 the distribution of forces created by the cells (Supplementary Fig. 4i). We measure the first moments of the force distribution along the axis of the channel and we find that in contrast with adhesion-based motility, the dipolar moment is positive (Fig. 4).

## 1 Model description and main equations

### 1.1 Cell shape parametrization

We start by introducing geometrical quantities characterizing the shape of the cell surface, in the reference frame of the cell. The cell shape is taken to be cylindrical where confined by the channel, and is free in the rear and front regions (see Supplementary Fig. 3b). The cell volume is assumed to be conserved during cell...
movement. For simplicity, we describe a shape, which is averaged over the fluctuations induced by membrane blebbing at the front of the cell. We also do not include the uropod in our description, as laser ablation experiments show that most of the driving force is generated in the rear of the cell, in the region of the cortex adjacent to the uropod (Supplementary Fig. 1f). We consider the channel to be cylindrical and the cell shape to be axisymmetric around the central axis of the channel. The cell surface is parametrized by coordinates $s$ and $\phi$, such that a point on the surface is denoted by

$$X(s, \phi) = x(s)e_x + r(s)\sin \phi e_y + r(s)\cos \phi e_z,$$

where $e_x$, $e_y$, and $e_z$ are unit vectors of the cartesian coordinates (Supplementary Fig. 3b).

To formulate constitutive equations and force balance on the curved cell surface, we first need to obtain the local basis of tangent and normal vectors to the surface, as well as the metric and curvature tensors. In what follows, the Einstein summation convention is used and lower and upper indices denote respectively covariant and contravariant tensors on the curved surface (for an introduction to differential geometry, please refer to e.g. [2]).

The vectors tangent to the surface are given by

$$e_s = \partial_s X = \begin{pmatrix} \partial_s x \\ \partial_s r \sin \phi \\ \partial_s r \cos \phi \end{pmatrix}, \quad e_\phi = \partial_\phi X = \begin{pmatrix} 0 \\ r \cos \phi \\ -r \sin \phi \end{pmatrix}.$$  

(2)

In what follows, $s$ is imposed to be an arc length coordinate, such that $|e_s| = (\partial_s r)^2 + (\partial_s x)^2 = 1$. The normal vector to the surface, $n = \frac{e_s \times e_\phi}{|e_s \times e_\phi|}$, is then given by

$$n = \begin{pmatrix} -\partial_s r \\ \partial_s x \sin \phi \\ \partial_s x \cos \phi \end{pmatrix}.$$  

(3)

For convenience, we introduce $\psi(s)$ the angle formed by $e_s$ with the plane normal to the $x$-axis (Supplementary Fig. 3b), such that the coordinates $r(s)$ and $x(s)$ are related to $\psi$ by $\partial_s r = \cos \psi$ and $\partial_s x = \sin \psi$. With these notations, the first fundamental form, the metric tensor is defined by $g_{ij} = e_i e_j$ and reads

$$g_{ij} = \begin{pmatrix} 1 & 0 \\ 0 & r^2 \end{pmatrix}.$$  

(4)

The area element is thus $dS = \sqrt{\det g} \; dsd\phi = r \; dsd\phi$. The curvature tensor of the surface, the second fundamental form, has the definition $C_{ij} = e_i, \partial_\phi n$. In the coordinates introduced above, the curvature tensor is given by

$$C_i^j = C_{ik} g^{kj} = \begin{pmatrix} \partial_s \psi & 0 \\ 0 & \frac{\sin \psi}{r} \end{pmatrix}.$$  

(5)

Note that as a result of axisymmetry, the curvature tensor is diagonal and the radii of curvature can be immediately identified, $R_s = \frac{1}{\partial_s \psi}$ and $R_\phi = \frac{r}{\sin \psi}$.

Finally, we introduce the velocity field of the cell surface $v$, which can be decomposed into a tangential and a normal part

$$v = v^s e_s + v^\phi e_\phi + v^n n.$$  

(6)

The velocity component $v^\phi$ cancels because of the rotational symmetry. The normal component $v^n$ is related to shape changes of the cell, and the tangential component of the velocity $v^s$ represents cortical flows. Note that for the sake of clarity, the cortical velocity $v^s$ is denoted $V_{\text{Cortex}}$ in the main text.

### 1.2 Material equation for the actomyosin cortex

We describe the actomyosin network at the cell surface as a two-dimensional viscous fluid subjected to active tension [30]. Migrating Walker cells indeed exhibit cortical flows on the timescale of minutes, exceeding the turnover rate of cortical crosslinkers (~ 20 s, Ref [30]), at which elastic stresses in the actin network are released. Furthermore, contractile stresses arising from the ATP driven activity of myosin motor proteins in the cortical actin network [2] give rise to an active contractile tension, which depends on the spatial density...
1.1, the linearized strain rate tensor is

\[ \dot{\varepsilon}^i_j = \eta_k \left[ v^i_j - \frac{1}{2} v^k_k \delta^i_j \right] + \eta \omega^k_k \delta^i_j + \zeta(c) \delta^i_j, \]

(7)

where \( \eta_k \) and \( \eta \) are the 2D shear and bulk viscosity of the actomyosin cortical network, \( v^i_j \) is the strain rate tensor, and \( \zeta(c) \) is the active myosin induced tension, which depends on the myosin concentration \( c \). For simplicity, we assume here that the shear and bulk viscosities are related through \( \eta_k = 2 \eta \), corresponding to a ratio of bulk to shear viscosity \( \eta_k/\eta = 1/2 \). Other choices for this ratio renormalize the viscosities in expressions given in these supplementary materials. In the coordinates introduced in Section 1.1, the linearized strain rate tensor is

\[ v^i_j = \begin{pmatrix} \partial_s v^s + \partial_\phi \psi v^n & 0 \\ 0 & \cos \psi v^s + \sin \psi v^n \end{pmatrix} \]

(8)

with \( v^s \) and \( v^n \) the flow velocity tangential and normal to the surface. Note that in the case of a stationary cell shape in the reference of the cell, the normal surface velocity has to vanish \( (v^n = 0) \), since the shape of the surface is modified by the normal velocity only.

### 1.3 Force balance equations

Newton’s second law at low Reynolds number imposes that the sum of all forces acting on an element of the cell surface must vanish. Therefore, normal and tangential stresses arising from the intrinsic viscous and active tensions must balance pressures and shear stresses exerted by the medium surrounding the cortex. The tangential force balance reads

\[ \nabla_i t^i_j = \Sigma^i_{\text{in}} - \Sigma^i_{\text{out}} \]

(9)

where \( \nabla_i \) denotes the covariant derivative on the curved surface indexed by \( i, j \) and \( \Sigma_{\text{in}} - \Sigma_{\text{out}} \) is the external shear stress acting on the cell surface, respectively inside and outside the cell. The normal force balance is the Young-Laplace equation

\[ C_{ij} t^i_j = P_{\text{in}} - P_{\text{out}} \]

(10)

with \( P_{\text{in}} \) the intracellular pressure, \( P_{\text{out}} \) the extracellular pressure, such that \( P_{\text{in}} - P_{\text{out}} \) is the pressure drop across the surface.

In what follows we assume that the shear stress \( \Sigma_{\text{in}} \) acting on the surface from inside the cell can be neglected compared to viscous and active stresses acting in the surface. This choice is consistent with our measurements of cortical flow fields, see section 4.2. The external shear stress \( \Sigma_{\text{out}} \) and the pressure drop \( P_{\text{in}} - P_{\text{out}} \) have different values in the cylindrically confined part of the cell and in the pole regions, as detailed below:

- In the region where the cell is in contact with the channel walls, an external shear stress \( \Sigma_{\text{out}} \) acts on the surface. This shear stress is proportional to the relative velocity between the flowing cortex and the channel, \( \Sigma_{\text{out}} = -\alpha v^s \), with \( \alpha \) the associated friction coefficient (Supplementary Fig. 3f). The tangential force balance then has the simple expression

\[ \partial_s t^s_s = \alpha v^s. \]

(11)

The shape of the cell is constrained by the channel wall and the normal force balance Equation 10 thus yields the pressure exerted by the cell on the channel. Note that forces exerted by the channel wall normal to the cell surface have no direct mechanical effect on cell migration. Indeed, in the region where the cell is confined by the channel, forces normal to the cell surface are perpendicular to the direction of cell displacement, and therefore do not contribute in propelling the cell. In agreement with this, cells do not move in channels with low friction coefficient, despite the fact that normal forces arising from confinement are still present (Fig. 2 and 3).

- In the unconfined pole regions, the cell is in contact with the external medium. Given the low viscosity of the medium (of the order of the viscosity of water, \( \eta_c \approx 10^{-3} \text{Pa.s} \), compared to cytoskeletal viscosities, we neglect viscous shear stresses arising in the fluid surrounding the cell. Therefore, the tangential and normal force balance equations Eqs. 11 and 10 read

\[ \partial_s t^s_s + \frac{\cos \psi}{r} t^s_\phi = 0 \]

(12)

\[ \partial_\psi t^s_s + \frac{\sin \psi}{r} t^s_\phi = P_{\text{in}} - P_{\text{out}} \]

(13)
1.4 External fluid pressure and drag coefficient

The force balance equation involves the external pressures acting on the rear and front of the cell, \( P_{\text{out}}^{(r)} \) and \( P_{\text{out}}^{(f)} \). In a fluid-filled channel subjected to a zero pressure difference at its boundaries, the only contribution to the difference of pressure acting on the cell \( P_{\text{out}}^{(r)} - P_{\text{out}}^{(f)} \) results from the drag of the medium acting against the motion of the cell. We assume here that the external pressure opposing cell movement is proportional to the cell velocity and can be written in terms of an effective drag coefficient \( \alpha_D \):

\[
P_{\text{out}}^{(r)} - P_{\text{out}}^{(f)} = -\alpha_D U.
\]

Here \( U \) denotes the velocity of the cell, which in the main article text is referred to by \( V_{\text{Cell}} \). Note that such a proportionality relation between the pressure difference on the cell and the cell velocity does not necessarily hold. Indeed, the drag depends also on the motion of the fluid in the channel. For instance, an externally applied pressure difference at the channel boundaries, generating a fluid flow, would modify Eq. \([14]\). We show however in Section 3 that under the experimental conditions in this work, the pressure acting on the cell can be expressed in the form of Eq. \([14]\) and we detail how the value of \( \alpha_D \) is related to the flow permeability of the channel and the migrating cells.

Combining the constitutive material equation introduced in Section 1.2, the force balance equations and the fluid drag equation then allows to obtain the velocity and shape equations of the cell cortex.

2 Cortical velocity profiles and cell velocity

We now proceed to solve the model equations separately in the cylindrical and pole cell parts. In the following, the superscripts \( (r), (m) \) and \( (f) \) denote rear, middle and front cell region respectively (see Supplementary Fig. 3c). First, for convenience we introduce the following coordinates used in the different cell parts:

\[
\begin{align*}
\theta^{(r)} &= \frac{s}{R} \quad \text{in the rear pole region,} \\
x^{(m)} &= s - R \frac{\pi}{2} \quad \text{in the contact region,} \\
\theta^{(f)} &= \pi - \frac{s - L}{R} \quad \text{in the front pole region.}
\end{align*}
\]

We denote by \( R \) and \( L \) the radius of the microchannel and the length of the contact area between channel and cell (see Supplementary Fig. 3b).

In the next sections, we derive analytical expressions for i) the profile of cortical velocity \( v^s \) (Eq. \([6]\)) in the reference frame of the cell, and ii) the cell velocity in the reference frame of the channel, \( U \).

2.1 Profile of active tension

Cell motility relies on the polarized distribution of myosin motors within the cell cortex. Experiments indicate that myosin accumulates at the cell rear (Fig. 2a, Supplementary Fig. 3b). The inhomogeneous distribution of myosin gives rise to a spatial profile of active tension \( \zeta(x) \), decreasing from the cell rear to the front. For analytical calculation, we now postulate a simple form for this profile. We assume that the cell is subjected to a piecewise linear profile of active tension:

\[
\zeta = \begin{cases} 
\zeta^{(r)} & \text{in the rear pole region} \\
\zeta^{(r)} - \frac{\zeta^{(r)} - \zeta^{(f)}}{L} x^{(m)} & \text{in the contact region} \\
\zeta^{(f)} & \text{in the front pole region}
\end{cases}
\]

This choice represents a good approximation of the experimentally measured myosin fluorescence profiles (Supplementary Fig. 1). In Section 3, we evaluate more precisely the active tension profile by assuming a proportionality relation between the active tension and the myosin intensity profile \( I(x) \), \( \zeta(x) = \zeta_0 I(x) \), in line with previous studies\([7-9]\).
2.2 Flow in the cylindrical region of the cell

We first calculate the flow field in the cylindrical part of the cell, which is in contact with the channel walls. In the reference frame of the moving cell, Eq. [11] can be written

$$\partial_t t^s_s = \alpha (v^s + U)$$  \hspace{1cm} (19)

where \(v^s\) is now a velocity taken in the cell reference frame. Together with the constitutive equation [7], this yields the following equation for the cortical flow velocity

$$\eta \partial^2_t v^s = \alpha (v^s + U) - \partial_s \zeta$$  \hspace{1cm} (20)

which admits the general solution

$$v^s = C_1 \exp \left( \frac{x^{(m)}}{l} \right) + C_2 \exp \left( - \frac{x^{(m)}}{l} \right) - U - \frac{\zeta^{(r)} - \zeta^{(f)}}{\alpha L} \quad \text{for } s \in [R \frac{\pi}{2}, R \frac{\pi}{2} + L]$$  \hspace{1cm} (21)

$$t^s_s = \frac{\eta}{l} \left( C_1 \exp \left( \frac{x^{(m)}}{l} \right) - C_2 \exp \left( - \frac{x^{(m)}}{l} \right) \right) + \zeta,$$  \hspace{1cm} (22)

where \(l = \sqrt{\frac{\pi}{4}}\) is a hydrodynamic length characterizing the range of cortical flows. The integration constants \(C_1\) and \(C_2\) can be determined from boundary conditions on the velocity and tension at the contact points where the pole regions are connected to the cylindrical part of the cell (see Section 2.4).

2.3 Flow and deformation in the cell pole regions

Next, we work out the flow field and the shape of the free cell pole surfaces, in the reference frame of the cell and for a stationary shape, implying that the normal velocity \(v^s\) vanishes (see Section 1.2). Furthermore, taking the active tension to be uniform in the pole surfaces (18) implies \(\partial_s \zeta = 0\). Without loss of generality, we explicitly solve here the equations in the rear part of the cell; however the solution in the front can be found by a simple symmetry transformation. Combining Equations 7 and 13, we obtain the velocity and shape equations

$$\partial^2_t v^s + \frac{\cos \psi}{r} \left( \partial_r v^s - \frac{\cos \psi}{r} v^s \right) = 0$$  \hspace{1cm} (23)

$$\left( \partial_r \psi + \frac{\sin \psi}{r} \right) \zeta + \eta \left( \partial_r \psi \partial_r v^s + \frac{\sin \psi \cos \psi}{r^2} v^s \right) = P_{in} - P_{out}. \hspace{1cm} (24)$$

To solve these equations, we perform an expansion around an unpolarised cell state where the active tension is uniform and no flow arises in the cortex. In this case, the rear and front surfaces take the shape of hemispherical caps (in the rear, \(v^s = 0, r_0 = R \sin(s/R), \psi_0 = s/R\)). We linearize the shape and flow equations around this solution \((\psi = \psi_0 + \delta \psi, r = r_0 + \delta r, P_{in} - P_{out} = 2 \zeta / R + \delta P)\). This expansion is valid in the limit where the variation of active tension is small compared to the average cellular surface tension \((\zeta^{(r)} - \zeta^{(f)}) / l \ll 1\), where the average cellular surface tension \(l\) includes both the average cortical tension and the cell membrane tension. For the Walker cells studied here, the total surface tension has been measured to be \(279 \pm 50 \ pN/\mu m\) (Ref.13). Our final estimate of \(\zeta^{(r)} - \zeta^{(f)} = 68 \pm 7 \ pN/\mu m\) (see Section 4, Table 2) is thus consistent with the above assumption. Furthermore, the observed time-average cell shape in the pole regions indeed corresponds approximately to a hemisphere (see Supplementary Fig. 3b).

Neglecting higher order terms and using [15] Equations 23-24 become

$$[\partial^2_\theta + \cot \theta \partial_\theta - \cot^2 \theta] v^s = 0$$  \hspace{1cm} (25)

$$[\partial^2_\theta + 1] \delta r = - \frac{\delta PR^2}{\zeta} \sin \theta + \frac{\eta}{\zeta} \partial_\theta (v^s \sin \theta). \hspace{1cm} (26)$$

Here, \(\theta\) stands for \(\theta^{(r)}\). The solution of Equation 25 is a linear combination of the associated Legendre polynomials \(P^1_{\nu} (\cos \theta)\) and \(Q^1_{\nu} (\cos \theta)\), with \(\nu(\nu + 1) = 1\). The solution cannot diverge in \(\theta = 0\), therefore, \(Q^1_{\nu} (\cos \theta)\) is not part of the solution and the velocity and tension profiles are given by

$$v^s = v^{(r)} \frac{P^1_{\nu} (\cos \theta)}{P^1_{\nu} (0)}$$  \hspace{1cm} (27)

$$t^s_s = \zeta + \frac{v^{(r)} \partial_\theta P^1_{\nu} (\cos \theta)}{R} \frac{P^1_{\nu} (0)}{P^1_{\nu} (0)} \hspace{1cm} (28)$$
where we denote by $v^{(r)}$ the cortical velocity at the contact point between the cell surface and the channel wall (see Supplementary Fig. 3d).

Integrating Equation [24] yields the following solution for the shape equation

$$
\delta r = \eta \frac{v^{(r)}}{\zeta} \left( a \left( \cos(\theta) - \frac{\pi}{2} \right) - \left( \int_0^{\frac{\pi}{2}} \cos(\theta') \sin(\theta') \frac{P_1^{(r)}(\cos(\theta'))}{P_1^{(r)}(0)} d\theta' - 1 \right) \cos(\theta) \right)
+ \eta \frac{v^{(r)}}{\zeta} \int_0^\theta \cos(\theta - \theta') \frac{P_1^{(r)}(\theta')}{P_1^{(r)}(0)} \sin(\theta') d\theta',
$$

(29)

where we have introduced the numerical coefficient $a = \int_0^{\frac{\pi}{2}} d\theta' \sin^2(\theta') \frac{P_1^{(r)}(\cos(\theta'))}{P_1^{(r)}(0)} \simeq 0.56$. The following three boundary conditions have to be satisfied by the shape profile:

- The shape has to be smooth at the cell apex ($\partial_{\theta} \delta r = 0$ at $\theta = 0$).
- At the contact point with the cylinder, the shape has to be tangent to the wall ($\partial_{\theta} \delta r = 0$ at $\theta = \frac{\pi}{2}$).
- At the contact point with the cylinder, the cortex cannot penetrate the wall ($\delta r(\frac{\pi}{2}) = 0$ at $\theta = \frac{\pi}{2}$).

These conditions were used to determine the integration constants in Eq. [29] and yield the following expression for the pressure drop across the cell surface:

$$
P_{in} - P_{out} = 2\zeta \frac{v^{(r)}}{R} + 2\eta \frac{v^{(r)}}{R^2} a.
$$

(30)

Thus, we have derived the expressions for the flow velocity, the tension and the shape, as well as for the difference of pressure across the cell surface.

### 2.4 Cortical velocity and tension at the contact points

To summarise, we give below the full solutions for the velocity, tension and pressure drop of the pole regions on each side:

- **Rear pole region:**
  \[
  v^s = v^{(r)} \frac{P_\nu(\cos(\theta^{(r)}))}{P_\nu(0)}
  \]
  \[
  t^s = \zeta^{(r)} + \eta \frac{v^{(r)}}{R} \partial_{\theta^{(r)}} \frac{P_\nu(\cos(\theta^{(r)}))}{P_\nu(0)}
  \]
  \[
  P_{in}^{(r)} - P_{out}^{(r)} = 2\zeta^{(r)} \frac{v^{(r)}}{R} + 2\eta \frac{v^{(r)}}{R^2} a,
  \]

- **Front pole region:**
  \[
  v^s = v^{(f)} \frac{P_\nu(\cos(\theta^{(f)}))}{P_\nu(0)}
  \]
  \[
  t^s = \zeta^{(f)} - \eta \frac{v^{(f)}}{R} \partial_{\theta^{(f)}} \frac{P_\nu(\cos(\theta^{(f)}))}{P_\nu(0)}
  \]
  \[
  P_{in}^{(f)} - P_{out}^{(f)} = 2\zeta^{(f)} \frac{v^{(f)}}{R} - 2\eta \frac{v^{(f)}}{R^2} a.
  \]

Five unknown constants remain to be determined from boundary conditions: $C_1$, $C_2$, $v^{(c)}$, $v^{(f)}$ and $U$. Four of them can be obtained by imposing that the velocity and tension fields have to be continuous at the contact.
given by

\[ C_1 + C_2 - U - \frac{\zeta^{(i)} - \zeta^{(f)}}{\alpha L} = v^{(i)} \]  

\[ \frac{C_1 - C_2}{l} = a \frac{R}{R} v^{(i)} \]  

\[ C_1 \exp \left( \frac{L}{\ell} \right) + C_2 \exp \left( -\frac{L}{\ell} \right) - U - \frac{\zeta^{(i)} - \zeta^{(f)}}{\alpha L} = v^{(i)} \]  

\[ \frac{C_1 \exp \left( \frac{\ell}{\ell} \right) - C_2 \exp \left( -\frac{\ell}{\ell} \right)}{l} = -a \frac{R}{R} v^{(i)}. \]  

A fifth condition is required to close the system of equations and obtain an expression for the cell velocity. This last condition can be obtained by considering the balance of pressure inside the cell, which we discuss in the next section.

### 2.5 Cell velocity

The cytoplasm has been described as a poroelastic material with a rheological timescale below one second (e.g. in[10]). Accordingly, equilibration of intracellular pressure differences can be assumed fast compared to the timescales of cell motion, and the hydrostatic pressure inside the cell can be taken uniform:

\[ P_{in}^{(i)} = P_{in}^{(f)}. \]  

From this relation and Equations 33 and 36, an expression can be obtained for the cell velocity of the following form

\[ U = U_0 + \frac{1}{\chi} (P_{out}^{(i)} - P_{out}^{(f)}). \]  

with \( U_0 \) the intrinsic velocity the cell would achieve in the absence of any external resistance to cell motion, given by

\[ U_0 = \frac{L(\zeta^{(i)} - \zeta^{(f)})}{\eta} \left( \frac{l \exp \left( \frac{L}{L} \right) + 1}{2L \exp \left( \frac{L}{L} \right) - 1} + R \frac{2aL}{2aL - \frac{l^2}{L^2}} \right), \]  

and \( \chi \) an effective cell friction,

\[ \chi = \frac{a4\eta \left( \exp \left( \frac{L}{L} \right) - 1 \right)}{R \left( a - R + \exp \left( \frac{L}{L} \right) \left( aL + R \right) \right)}. \]  

The flow-velocity relationship predicted by the theory (Eq. [12]) is shown in Supplementary Fig. 3e. The cell moves with a spontaneous cell velocity \( U_0 \) in the absence of any external force. Under the presence of an external force proportional to the cell velocity Eq. [13] we can solve Eq. [12] for the cell velocity \( U \), obtaining

\[ U = \frac{2(\zeta^{(i)} - \zeta^{(f)})(aL + L + \exp \left( \frac{L}{L} \right)(L - 2l)) + \left( \exp \left( \frac{L}{L} \right) - 1 \right)LR}{L(aL + L + \exp \left( \frac{L}{L} \right)(aL + R))}. \]  

This expression is the central result of our calculations and describes the cell velocity as a function of the hydrodynamic length \( l \), the cell geometric parameters \( R \) and \( L \), the gradient of active tension \( (\zeta^{(i)} - \zeta^{(f)})/L \) and the external drag \( \alpha \). Fig. 3b and Supplementary Fig. 3f show the predicted cell velocity as a function of the friction coefficient \( \alpha \). The cell velocity \( U \) exhibits two different regimes: for small friction compared to the external drag, the velocity \( U \) vanishes, as the resistance to cortical flow is not sufficient to propel the cell. For infinite friction \( \alpha \to \infty \), the cell velocity is given by the simple expression

\[ U = \frac{(\zeta^{(i)} - \zeta^{(f)})R}{2a\eta + \alphaD R^2/2}. \]  

In this limit, a no-slip boundary condition holds between the cortex and the channel, the cortex cannot flow relative to the wall in the cylindrical cell region. However, cell movement is still possible due to the contraction and expansion of the cell rear and front respectively (Video S11). As a result, in that limit and for small external drag \( \alphaD \), the cell velocity depends on the ratio \( \frac{\Delta \zeta}{R} \), which is a characteristic timescale for contraction of the cortex under the active tension difference \( \Delta \zeta \).
2.6 Threshold friction for cell movement

As shown in Fig. 3b, the cell velocity $U$ exhibits two different regimes for small and large friction $\alpha$. In this section, we perform an approximate calculation of the threshold friction enabling cell movement. To this end, we expand $U$ around the limit of zero friction ($\frac{1}{\alpha} \to \infty$)

$$U = \frac{(\zeta^{(i)} - \zeta^{(f)})L(aL + 6R)}{6aRaDl^2} - \frac{(\zeta^{(i)} - \zeta^{(f)})L^2(30\alpha_DR^3 + 10a(LRa_D + 12\eta) + a^2L(LRa_D + 20\eta))}{60(aRa_D)^2 l^4} + O\left(\frac{1}{\alpha^5}\right).$$

The second term is negative and is responsible for the cell velocity levelling off as the friction coefficient $\alpha$ is increased. The ratio of the first two terms in the expansion therefore defines a critical hydrodynamic length $l^*$, below which friction is not sufficient to drive cell movement

$$\left(\frac{l^*}{L}\right)^2 = \frac{R}{2La} + \frac{1}{60} \left(5 + \frac{aL}{aL + 6R}\right) + \frac{2\eta}{LRa_D}. \tag{48}$$

For the fitted values of $\eta$ and $\alpha_D$ (see Section 4 and Tables 1 - 2), the last term dominates $\left(\frac{U^*}{U}\right)^2 \approx \frac{2\eta}{LRa_D}$. Thus, we find the following expression for the critical friction coefficient enabling cell movement

$$\alpha^* \approx \frac{Ra_D}{2L}. \tag{49}$$

To estimate more precisely the critical friction for cell motion, we numerically evaluated the first inflexion point of $U(\alpha)$ for increased friction, for different values of $\alpha_D$ (Supplementary Fig. 3g). We find that the approximate estimate Eq. 49 actually yields a very good estimate for the inflexion point of $U(\alpha)$ in the regime of small drag coefficients.

Eq. 49 indicates that the cell velocity is critically dependent on the ratio of the friction to drag coefficient, $\alpha^*/\alpha_D$. Cell locomotion can only be achieved provided that the friction coefficient is of the same order or larger than the drag coefficient. This very simple criterion provides a quantitative prediction for conditions enabling cell motion driven by cortical flow and friction.

To summarize, we have calculated the cell velocity $U$ and the rearward cortical flow field $v^*$ resulting from a gradient of active cortical tension, and we find that cortical flows enable cell movement at a finite velocity only if sufficient substrate friction is available to overcome fluid drag. Our predictions on cortical flows and cell velocities at different friction coefficients can be directly compared to experimental measurements (Section 4).

3 Fluid drag coefficient in a channel

In this section, we relate the fluid drag coefficient $\alpha_D$ introduced in Eq. 14 to the hydraulic properties of the channel and the cell. In general, the external medium can flow from the front to the rear of the cell, either due to fluid crossing the cell membrane, or due to fluid flowing in the narrow space between the cell and the channel wall (Supplementary Fig. 4e). Depending on the cell permeability and the effective permeability of the space between the cell surface and the channel wall, this flow gives rise to a difference of pressure between the medium in front of the cell and the medium at the rear of the cell. For a single cell, this pressure difference can be written

$$P^{(c)}_{\text{out}} - P^{(c)}_{\text{out}} = \xi S(\tilde{v}_c - U), \tag{50}$$

where $\xi$ is the effective fluid flow resistance of a channel segment containing a cell, $S$ is the cross-sectional area of the channel, and $\tilde{v}_c$ is the average fluid flow. No pressure difference is created when the velocities of the fluid $\tilde{v}_c$ and the cell $U$ are equal. In addition, the microchannel offers resistance to fluid flow (Supplementary Fig. 4e), such that the relation between a pressure drop across some distance in the channel and the fluid flow is given by Poiseuille’s law. Considering a single cell moving in a channel, the pressure difference between channel inlet and cell rear on one side of the cell and channel outlet and cell front on the other side of the cell are thus given by

$$P^{(c)}_{\text{applied}} - P^{(c)}_{\text{out}} = \frac{8\eta Lc^{(f)}}{R^2} \tilde{v}_c = \xi^* S\tilde{v}_c \tag{51}$$

$$P^{(c)}_{\text{out}} - P^{(c)}_{\text{applied}} = \frac{8\eta Lc^{(f)}}{R^2} \tilde{v}_c = \xi^* S\tilde{v}_c \tag{52}$$
where $L_c^{(r)}$ and $L_c^{(f)}$ are the channel lengths in the rear and in the front of the cell, and $P_{\text{applied}}^{(r)}$ and $P_{\text{applied}}^{(f)}$ are the pressures applied to the two ends of the channel. When these two pressures are equal, the difference of pressure acting on the cell is simply

$$P_{\text{out}}^{(r)} - P_{\text{out}}^{(f)} = -\xi_c S \bar{v}_c,$$

(53)

where $\xi_c = \xi_c^r + \xi_c^f \approx 8 \eta_c L_c / (R^4 \pi)$ is the overall resistance of the channel, with $L_c \approx L_c^{(r)} + L_c^{(f)}$.

Combining Eq. 50 and 53 we obtain a relation between the fluid flow created by the cell and the cell velocity:

$$\bar{v}_c = \frac{\xi}{\xi + \xi_c} U.$$

(54)

The pressure acting on the cell can then be determined self-consistently from Eqs. 50 and 54:

$$P_{\text{out}}^{(r)} - P_{\text{out}}^{(f)} = \left(\frac{\xi}{\xi + \xi_c} - 1\right) \xi S U,$$

(55)

which has the form of Eq. 14 with $\alpha_D = \left(\frac{\xi}{\xi + \xi_c} - 1\right) \xi S$.

We now investigate the case of a cell migrating in a channel containing $N-1$ other motile cells (Supplementary Fig. 4e) and derive the expression for the fluid drag coefficient $\alpha_D$ for that case, since we analyzed experiments where several cells were moving in each channel. We assume here that individual cells differ predominantly in their spontaneous velocities $U_0$, which scale with the active tension differences between cell rear and front (see Eq. 43) and thus depend on cell polarity state. For $N$ simultaneously migrating cells, the induced flow velocity $\bar{v}_c$ reads

$$\bar{v}_c = \frac{\xi}{\xi + N \xi_c} \sum_{i=1}^{N} U_i,$$

(56)

where $U_i$ is the velocity of cell $i$. Using eqs. 42 and 50, $U_i$ can be rewritten as

$$U_i = \frac{\chi}{\chi + \xi S} U_{i,0} + \frac{\xi S}{\chi + \xi S} \bar{v}_c.$$

(57)

The external pressure difference acting on cell $i$ can be written

$$P_{i,\text{out}}^{(r)} - P_{i,\text{out}}^{(f)} = \xi S U_i \left(\frac{\chi + S \xi}{\chi \bar{v}_c + S \xi} - 1\right).$$

(58)

Analysis of the distribution of cell velocities indicates that cells can be grouped into fast and slow moving cells (Supplementary Fig. 4h). For fast moving cells analysed in this work, we find that $U_{i,0}$ is large compared to $\bar{v}_c$. Our analysis also indicates that $S \xi / \chi < 1$ for high and intermediate friction conditions. We therefore simplify Eq. 58 to $P_{i,\text{out}}^{(r)} - P_{i,\text{out}}^{(f)} \simeq -\xi S U_{i,0}$, and we approximate the drag coefficient by

$$\alpha_D \simeq \xi S.$$

(59)

In that case, the drag coefficient depends mainly on the effective fluid flow resistance $\xi$ of the cell.

4 Comparison of theoretical profiles to experimental measurements

4.1 Cortical flow profiles and cell velocities at different frictions

To directly compare the model predictions to experimental measurements of Walker cells migrating in microchannels, we measured the distribution of myosin II fluorescence intensity and the cortical flow velocity profiles in moving cells. Measurements were performed for three different surface coatings providing large, small and intermediate friction conditions. The actomyosin flow field was estimated from time-lapse images of cells with fluorescent myosin II using particle image velocimetry (see Materials and Methods for details). We use the density distribution of myosin to provide an estimate of the active tension field driving cortical flow.
Table 1: Model parameters. Quantities obtained from the fitting procedure are highlighted in bold.

| Parameter                  | Units  | Value     |
|----------------------------|--------|-----------|
| Large friction $\alpha$    | Pa s/m | 27 ± 2 10^4 |
| Intermediate friction $\alpha$ | Pa s/m | 18 ± 3 10^3 |
| Small friction $\alpha$    | Pa s/m | 15 ± 6 10^2 |
| Contact length $L$         | $\mu$m | 24.7 ± 0.5 |
| Channel radius $R$         | $\mu$m | 4.4       |
| Viscosity $\eta_{2D}$      | Pa s m | 27 ± 3 10^{-4} |
| Cell flow resistance $\xi$ | Pa s/m$^2$ | 3.5 ± 0.5 10^{15} |
| Myosin scaling factor $\zeta_0$ | pN/ $\mu$m | 54 ± 6 |
4.2 Role of internal friction

While in some systems, the cell nucleus appears to have an important influence on cell migration mechanics in confined environments (see e.g. Refs 14,15), we see no indication of a dissipating effect due to the presence of the nucleus in Walker cells. Indeed, cell migration velocity does not correlate with nuclear size in both large and intermediate friction conditions, suggesting that nuclear properties do not affect cell migration mechanics (see Supplementary Fig. 4a-c). Furthermore, cortical flows are not slowed at the location of the nucleus, which would be expected in the case of mechanical coupling between nucleoskeletal and cortical elements (see Fig. 3c). Accordingly, as stated in section 3.3, we assume that internal shear stresses acting on the cortex from the intracellular material are negligible compared to external shear stresses acting along the contact surface between the cell and the channel walls. To further test this assumption, we introduced in our physical description an internal friction force resisting cortical flows in the reference frame of the cell. Such a friction force can arise from the dissipation in the cytoplasm entrained by the cortical flow, or from possible links between the cortex with internal organelles, such as the nucleus. Accordingly, we modified Eq. 10 to

$$\partial_x t^s_s = (\alpha_{\text{ext}} + \alpha_{\text{int}})v^s + \alpha_{\text{ext}}U, \quad (66)$$

where \(\alpha_{\text{int}}\) is an internal friction coefficient, and the friction coefficient corresponding to dissipation between the cortex and the channel wall has been renamed \(\alpha_{\text{ext}}\) (previously \(\alpha\)). For simplicity, we consider internal friction to act only in the region where the cell is confined by the microchannel (see Supplementary Fig. 4d). By action-reaction principle, the friction force exerted by the intracellular material on the cortex results in a counterforce, acting from the cortex on the intracellular material. In addition, the sum of forces acting on the intracellular material has to vanish for force balance to be satisfied. We therefore find

$$\left( P^{(c)} - P^{(f)} \right) \pi R^2 = -\alpha_{\text{int}} 2\pi R \int_0^L v^s dx, \quad (67)$$

stating that the forces acting on the intracellular material arising i) from the difference of pressure at the front and rear of the cell, and ii) from the friction force with the cortex, sum to zero. Eq. 67 replaces Eq. 11 in section 2.3.

We then proceeded to derive the solutions for the cortical flow velocity and the cell velocity as described in the previous sections. We fitted the resulting equations to the cortical flow data measured in three different friction conditions as in section 4, now with the additional unknown parameter \(\alpha_{\text{int}}\). We find that the error between the experimentally measured velocity points and the model curve is minimised with the following set of parameter values: \(\eta_{\text{BD}} = 27 \pm 1 \times 10^{-14}\ \text{Pa.s.m}, \quad \xi = 3.5 \pm 0.3 \times 10^{15}\ \text{Pa.s/m}^3, \quad \zeta_0 = 53 \pm 1\ \text{pN/\mu m}\) and \(\alpha_{\text{int}} = 5.2 \times 10^{-8} \pm 22\ \text{Pa.s/m}\). The fit values for the viscosity, the cell flow resistance and the myosin scaling factor correspond closely to what we find in the absence of internal friction (see Table 1), while the ratio of internal to external friction is found to be very small \((\alpha_{\text{int}}/\alpha_{\text{ext}} \approx 0\) for the small friction condition). Thus, we conclude that the measured cortical flow fields are consistent with a small effect of internal friction compared to other dissipative processes.

4.3 Fluid flow in the channel

In order to directly assess the value of the cell flow resistance \(\xi\) yielded by the fit of the model to the data, we investigated the fluid flow induced by migrating cells in large friction channels and we compared it to the theoretical expression for the average fluid flow velocity in the channel (Eq. 56). To estimate the fluid velocity from Eq. 56, we quantified the average number of cells per channel \(N = 14 \pm 1\) and the average cell velocity \(\bar{U} = 2.22 \pm 0.36\ \mu m/min\) (see Supplementary Fig. 4f-h). We then predict that the cells induce an average fluid flow of \(\bar{v} = 1.4 \pm 0.2\ \mu m/min\) in the channel. To test this prediction and experimentally measure the average fluid flow, we injected microspheres into channels together with migrating cells and tracked their position over time in a bright-field microscope (Supplementary Fig. 4f). We proceeded to relate the average velocity of microspheres \(\bar{v}_{\text{bead}}\) to the average fluid flow velocity \(\bar{v}\) in the following way:

| Parameter                  | Units   | Value   |
|----------------------------|---------|---------|
| Bulk viscosity \(\eta_{\text{BD}}\) | kPa.s   | 13.4 ± 1.4 |
| Active tension drop \((\zeta^{(c)} - \zeta^{(f)})\) | pN/\mu m | 68 ± 7 |
| Fluid drag coefficient \(\alpha_{\text{D}}\) | kPa.s/m | 208 ± 29 |

Table 2: Mechanical parameters derived from fitted estimates.
- Assuming Hagen-Poiseuille flow in the channel, \( v_c(r) \) is related to the average fluid velocity \( \bar{v}_c \) by

\[
v_c(r) = 2\bar{v}_c \frac{R^2 - r^2}{R^2}.
\] (68)

- The average velocity of the microspheres is related to the fluid flow \( v_c(r) \) by

\[
\bar{v}_{\text{bead}} = \frac{2\pi}{\pi} \int r \, dr \, p(r) v_c(r),
\] (69)

where \( p(r) \) is the radial distribution of bead positions.

- The distribution of bead positions obtained in the bright-field microscope is projected onto the \( y \)-axis. To estimate the radial distribution \( p(r) \) from the projected distribution of bead positions \( p(y) \), we used the inverse Abel transform \(^{14}\).

From Equations 68-69 we then find \( \bar{v}_c = 2.61 \pm 0.31 \, \mu m/min \), close to our prediction \( \bar{v}_c = 1.4 \pm 0.2 \, \mu m/min \).

5 Force density on the cell surface

Finally, we describe the calculation of the forces exerted by the cells on the channel walls during migration (see Supplementary Fig. 4i and Fig. 4a). The 2D force density on the wall is given by the product of the friction coefficient \( \alpha \) and the relative velocity between the cortex and the wall:

\[
f = \alpha (v^* + U).
\] (70)

The resulting spatial force pattern \( f \) is plotted in Supplementary Fig. 4i, and can be characterized by computing the first three terms of a multipole expansion:

\[
\tau = \int \! f dS
\] (71)

\[
\rho = \int \! x f dS
\] (72)

\[
\gamma = \int \! x^2 f dS.
\] (73)

where the integrals are taken over the contact surface between the channel and the cell, \( \tau \) is a force monopole, \( \rho \) is a force dipole and \( \gamma \) a force quadrupole. At low Reynolds number, the total force transmitted by the cell to its environment is zero. Thus, the force transmitted to the substrate by a crawling cell equals the drag force exerted on it by the surrounding fluid. The drag forces in a narrow channel are comparable to forces exerted by the cell on the substrate; therefore, the total force exerted by the cell on the channel does not vanish and gives rise to a non-zero force monopole \( \tau \) (Table. 3). We find that the next term in the multipole expansion, the force dipole \( \rho \), is positive for all friction conditions (Table. 3), indicating that a higher propulsive force is generated at the cell rear. This observation is in sharp contrast with adhesion-based motility, where the force dipole has been measured to be negative\(^{13}\). Finally, we note that the quadrupole \( \gamma \) has a significant contribution to the force distribution for cells moving in large friction conditions. The ratio of quadrupole to dipole moments in this case yields a length much larger than the size of a single cell \( (\gamma/\rho \approx 2 \, \text{mm}) \). Thus, flow and/or deformation fields induced by the cellular forces at large friction conditions are predominantly characterized by the quadrupole moment of the force distribution at distances below a few millimetres.

| Multipole        | Large friction | Intermediate friction | Low friction   |
|------------------|----------------|-----------------------|----------------|
| \( \tau \) (monopole) | \(-1.55 \times 10^{-14} \, \text{N}\) | \(-1.04 \times 10^{-14} \, \text{N}\) | \(-1.37 \times 10^{-13} \, \text{N}\) |
| \( \rho \) (dipole)      | \(7.7 \times 10^{-17} \, \text{N.m}\)     | \(2.5 \times 10^{-15} \, \text{N.m}\)     | \(4.4 \times 10^{-20} \, \text{N.m}\)     |
| \( \gamma \) (quadrupole) | \(1.43 \times 10^{-20} \, \text{N.m}^2\) | \(-3.9 \times 10^{-23} \, \text{N.m}^2\) | \(-5.9 \times 10^{-24} \, \text{N.m}^2\) |

Table 3: Multipole moments of the force density exerted by migrating Walker cells on large, intermediate and small friction channels.
References

[1] Norstrom, M. and Gardel, M.L. Shear thickening of f-actin networks crosslinked with non-muscle myosin iiib. Soft Matter 2011, 3228–3233 (2011)

[2] Mohan, K., Iglesias, P.A., and Robinson, D.N. Separation anxiety: stress, tension and cytokinesis. Exp Cell Res 318, 1428–1434 (2012)

[3] Bergert, M. et al. Cell mechanics control rapid transitions between blebs and lamellipodia during migration. Proceedings of the National Academy of Sciences 109, 14434–14439 (2012)

[4] Deserno, M. Notes on differential geometry. http://www.cmu.edu/biolphys/deserno/pdf/diff_geom.pdf (2004). Accessed: 2014-04-14

[5] Salbreux, G., Prost, J., and Joanny, J.F. Hydrodynamics of cellular cortical flows and the formation of contractile rings. Phys Rev Lett 103, 058102 (2009)

[6] Mukhina, S., Wang, Y.L., and Murata-Hori, M. Alpha-actinin is required for tightly regulated remodeling of the actin cortical network during cytokinesis. Dev Cell 13, 554–65 (2007)

[7] Mayer, M. et al. Anisotropies in cortical tension reveal the physical basis of polarizing cortical flows. Nature 467, 617–21 (2010)

[8] Sedzinski, J. et al. Polar actomyosin contractility destabilizes the position of the cytokinetic furrow. Nature 476, 462–6 (2011)

[9] Behrndt, M. et al. Forces driving epithelial spreading in zebrafish gastrulation. Science 338, 257–60 (2012)

[10] Moeendarbary, E. et al. The cytoplasm of living cells behaves as a poroelastic material. Nature materials 12, 1–9 (2013)

[11] Clark, A.G., Dierkes, K., and Paluch, E.K. Monitoring actin cortex thickness in live cells. Biophys J 105, 570–80 (2013)

[12] Harada, T. et al. Nuclear lamin stiffness is a barrier to 3D migration, but softness can limit survival. The Journal of cell biology 204, 669–82 (2014)

[13] Khatau, S.B. et al. The distinct roles of the nucleus and nucleus-cytoskeleton connections in three-dimensional cell migration. Scientific reports 2, 488 (2012)

[14] Pretzler, G. A new method for numerical abel inversion. Z Naturforsch 46, 639–641 (1991)

[15] Schwarz, U.S. and Safran, S.A. Physics of adherent cells. Reviews of Modern Physics 85, 1327 (2013)