

Filopodia rotate and coil by actively generating twist in their actin shaft

Natascha Leijnse^{*,†}, Younes Farhangi Barooji^{*,†}, Bram Verhagen[†], Lena Wullkopf[‡],
Janine Terra Erler[‡], Szabolcs Semsey[†], Jesper Nylandsted[¶], Amin
Doostmohammadi[†], Lene Broeng Oddershede[†] and Poul Martin Bendix^{**,†}

[†]*Niels Bohr Institute, University of Copenhagen, 2100 Copenhagen, Denmark*

[‡]*Biotech Research and Innovation Centre (BRIC), University of Copenhagen, Ole Maaløes
Vej 5, 2200 Copenhagen, Denmark*

[¶]*Danish Cancer Society Research Center, Strandboulevarden 49, University of Copenhagen,
2100 Copenhagen, Denmark*

E-mail: bendix@nbi.ku.dk

1 Materials and Methods

1.1 Cell culture

All cells were grown in vented T25 flasks (BD Falcon) in a sterile environment at 37°C in a humidified 5 % CO₂ incubator. The cells were passaged at around 80 % confluence.

HEK293, HEK293T, and MCF7. Cells were grown in DMEM growth medium ([+] 4.5 g/L D-Glucose, [+] L-Glutamine, [+] 110 mg/L Sodium Pyruvate, Gibco, supplemented with 10 % FBS, 1 % PenStrep) and passaged by washing with 2 ml DPBS (1X, [-] CaCl₂, [-] MgCl₂, Gibco or PBS pH 7.4, Gibco), detached using 1 ml TrypLE Express ([-] Phenol red, Gibco) and resuspended in growth medium.

MCF7-p95ErbB2. MCF7-p95ErbB2 cells described previously¹ were grown in DMEM growth medium ([+] 4.5 g/L D-Glucose, [+] L-Glutamine, [+] 110 mg/L Sodium Pyruvate, Gibco, supplemented with 10 % FBS, 1 % PenStrep, further supplemented with 200 µg/ml G418, 1 µg/ml Puromycin, 0.5 µg/ml tetracycline). To induce p95ErbB2 expression, the cells were trypsinized and washed 4-5 times in 20 ml of PBS (+CaCl₂) to remove all tetracycline and then plated in a new tissue culture flask. When cells were induced, they were kept in DMEM growth medium ([+] 4.5 g/L D-Glucose, [+] L-Glutamine, [+] 110 mg/L Sodium Pyruvate, Gibco, supplemented with 10 % FBS, 1 % PenStrep). The "spiderlike" phenotype of the MCF7-p95ErbB2 cells appears after 3-5 days.

Uninduced MCF7-p95ErbB2 cells, meaning cells grown in growth medium containing tetracycline, will express some ErbB2 as the promoter is leaky, but will resemble more normal MCF7 cells in their activity levels.

KPC, KP^{fl}C. Cells were cultured in DMEM containing GlutaMAX, 10 % FBS, and 1 % PenStrep.

Stem Cell Lines. Mouse embryonic stem cells (HV.5.1 mESCs) were obtained from the Brickman Group (DanStem, University of Copenhagen, Denmark). HV.5.1 cells are equipped with a Venus reporter which represents the expression state of Hex, line. HV.5.1 mESCs had been passaged 23 times prior to our experiments. The cultures were sustained for no longer than 25 subsequent passages.

Stem cells were either cultured in Serum/LIF (S/L) or 2i culture medium.

Serum/LIF (S/L) medium. The addition of the cytokine Leukemia Inhibitory Factor (LIF) maintains a pluripotent state of the stem cells.²⁻⁴ Cells were cultured in Glasgow modified Eagle's medium (GMEM, Sigma Aldrich, Germany) supplied with 5.5 ml non-essential amino acids (Gibco Science, Paisley, UK), 5.5 ml glutamine, 5.5 ml sodium pyruvate (Gibco Science, Paisley, UK), 560 μ l 0.1 mM 2-mercaptoethanol (Sigma-Aldrich, St. Louis, MO, USA), 50 ml fetal bovine serum (FBS) (obtained from DanStem, University of Copenhagen, Denmark), 560 μ l 1000 U/ml LIF (obtained from DanStem, Copenhagen University, Denmark) per 500 ml of medium. The entire pile of FBS was bought from the manufacturer by DanStem to ensure consistency in the culture medium. For S/L culture, the medium was aspirated, and rinsed once with DPBS and detached with 0.025 % trypsin in DPBS, incubated for 4 min at 37° C and suspended in 5 ml of S/L medium. The cells were then centrifuged for 3 min at 1890 rpm and re-suspended in 5 ml of S/L.

2i medium. In 2i medium, cells remain in a more primitive state. 2i composition: 1:1 mixture of DMEM (Gibco Science, Paisly, UK) and F12 medium, modified N2 medium (25 μ g/ml insulin, 100 μ g/ml apo-transferrin, 6 ng/ml progesterone, 16 μ g/ml putrescine, 30 nM sodium selenite, obtained from DanStem, University of Copenhagen, Demark), 50 μ g/ml bovine serum albumin fraction V, combined with a 1:1 mixture of Neurobasal medium supplemented with B27 (Gibco, Paisley, UK). The culture medium contained two cytokines, a GSK3 inhibitor with a final concentration of 1 μ M and ERK inhibitor with a final concentration of

3 μ M. The cells were cultured in 50 ml Falcon flasks (Falcon Science, Paisley, UK) coated with EmbryoMax ultrapure water with 0.1 % gelatin (Merck Millipore, Billerica, MA, USA) for 10 minutes. At a confluence of approximately 90 % the cells were harvested. Cells detach from the culture flask with time. To retain a large number of cells, the culture medium was collected in a 15 ml Falcon tube and centrifuged for 3 minutes at a speed of 1890 rpm prior to trypsinization. The supernatant was aspirated and the pellet was re-suspended in 1 mL of 0.025 % trypsin by pipetting. The suspension was added to the flask in which the cells had been cultured to detach the clusters of cells that had remained bound to the substrate and incubated at 37°C for 4 min. Cells were transferred to a 15 ml Falcon tube and 5 ml of N2B27 was added to reduce the usage of the cytokines and LIF. The suspension was centrifuged with the same settings and re-suspended in 5 ml of N2B27 medium, by pipetting carefully up and down. Finally, a volume of the cell suspension was added to a new culture flask together with 5 ml of fresh 2i medium.

1.2 F-actin labelling

Transient transfection of HEK293, HEK293T and MCF7 cells. The day prior to transfection, the cells were seeded on a 24 well cell culture plate (Orange Scientific). On day of transfection, the medium in each well was removed and exchanged with 500 μ l fresh growth medium per well. Then 1 μ l of EGFP Lifeact-7 plasmid (mEGFP-Lifeact-7 was a gift from Michael Davidson; Addgene plasmid # 54610), 100 μ l Opti-MEM ([+] HEPES, [+] 2.4 g/L Sodium Bicarbonate, [+] L-Glutamine, Gibco), and 1 μ l Lipofectamine LTX Reagent (Invitrogen) were carefully mixed in an Eppendorf tube and incubated at RT for at least 30 min. This mix was sufficient for a single well. Usually, the cells in at least 3 wells were transfected at a time. The next day, the cells were detached from the wells as described above, the contents of the 3 wells were mixed together. 100 μ l of those cells and ca 1 ml of growth medium was added to a glass bottom dish (35 mm, No. 1.5 coverslip, 20 mm glass diameter, uncoated, MatTek) and left to adhere and express for another day in the

incubator. The samples for experiments were then used 1 or 2 days after transfection.

Transient transfection of KPC and KP^{fl}C. KPC and KP^{fl}C cells were transfected with mEmerald LifeAct-7 (a kind gift from the Ivaska lab, Turku Centre for Biotechnology) using Lipofectamine 2000 as described above. After an incubation period of 12 h they were embedded in collagen I matrices.

Transient transfection of HV.5.1 mESCs. Cells were transfected with EGFP LifeAct-7 or RFP LifeAct-7 using Lipofectamine P3000 in 24 well plates. The day before transfection, the cells were plated at high density (70 – 90% confluency). On the day of transfection, a transfection mixture containing Opti-MEM, plasmid, Lipofectamine P3000, P3000 reagent, and medium was mixed and incubated for 12 minutes at room temperature. The medium was aspirated from the cells and the mix was subsequently added and was equally distributed over the well. Thereafter the well was filled with fresh medium. Although the manufacturer recommends to use Opti-MEM medium for better transfection efficiency, the cells were cultured in S/L or 2i medium, respectively, to maintain an optimal pluri- or multipotent state. The cells were used for experiments between 24 and 48 hours after transfection.

SiR actin labeling Hepa 1-6 cells were incubated in SiR actin (SiR actin kit, Spirochrome, 1 : 2000) and Verapamil (1 : 1000) for 5 h.

1.3 Collagen I gels.

Collagen mixtures of 4 mg/ml were prepared by mixing high concentration acid-extracted and cross-linked rat tail collagen I, sterile phosphate-buffered saline (PBS), and 5X collagen buffer containing 0.1 M HEPES, 2 % NaHCO₃, and α -MEM. Cells were then suspended in the collagen mixtures. After polymerization, the gels were washed once and incubated with normal culture medium for 24 h.

1.4 Vitronectin coated beads

We prepared two stock solutions: ‘tracer beads’ with $d = 0.989 \mu\text{m}$ streptavidin coated Flash Red fluorescent beads (Bangs Laboratories) and ‘tether beads’ with $4.95 \mu\text{m}$ streptavidin coated polymer particles (Bangs Laboratories) coated with biotin labeled human multimeric vitronectin (VN) (Innovative Research) in PBS (pH 7.4, Gibco) which were stored at 4°C . For coating, $5 \mu\text{l}$ of beads were washed twice in 1 ml of PBS. After the second washing step, the beads were re-suspended in $50 \mu\text{l}$ of PBS containing $2 \mu\text{l}$ of VN, enough to exceed the maximal binding affinity of the streptavidin coated beads. The beads were incubated for 20 min at a shaking platform (Eppendorf MixMate, Hamburg, Germany) with a speed of 450 rpm. To remove unbound VN, the beads were centrifuged at 12000 rpm for 5 min and re-suspended in 1 ml of PBS.

1.5 Imaging sample preparation.

On day of experiments, $400 \mu\text{l}$ of ‘imaging DMEM’ ([+] 4.5 g/L D-Glucose, [+] L-Glutamine, [+] 25 mM HEPES, [-] Sodium Pyruvate, Gibco) was added to an Eppendorf tube together with $3 \mu\text{l}$ and $7 \mu\text{l}$ of $1 \mu\text{m}$ and $4.95 \mu\text{m}$ VN coated beads, respectively. The medium in a MatTek dish with transfected cells (see section ‘Transient transfection’) was carefully removed and replaced with the described bead mix in imaging DMEM.

2 Experimental setup and procedures

2.1 Confocal microscopes with integrated optical trap

Experiments were conducted on two different setups. One setup consists of an inverted Leica TCS SP5 II confocal microscope with a 63X water objective (1.2 NA, Leica) with an integrated optical trap based on a 1064 nm laser (Nd:YVO₄, 5 W Spectra Physics BL106C, $\lambda = 1064 \text{ nm}$, TEM_∞) and a photodiode detection system. The laser beam was tightly

focused by the water-immersion objective and the trapping laser light was collected by a condenser (Leica, P1 1.40 oil S1) located in the back-focal plane and focused onto a quadrant photodiode (S5981; Hamamatsu). A three-dimensional LabVIEW controlled piezoelectric stage (Nano-Drive, MCL) allowed positioning of the sample relative to the laser focus with nanometer precision. For some experiments, the sample stage was heated to 39° C using a stage heater which, due to thermal losses, results in a sample temperature of about 37° C inside the sample. Data were acquired by an acquisition card (NI PCI- 6040E) at a sampling frequency of 1 kHz (for force measurements) and 22 kHz (for force calibration) and processed by custom-written LabVIEW programs (LabVIEW 2010; National Instruments).

The second setup, mostly used for force measurements and stem cell experiments, is described in.⁵

2.2 Free filopodia and filopodia in collagen rotations

Cells in MatTek dishes containing imaging medium or grown in collagen I gels were placed on the confocal microscope and filopodia movement was imaged via z -stacks over time. The mEmerald Lifeact-7 or EGFP Lifeact-7 expressing cells were excited at 488 nm and the collagen I gel was imaged in reflection mode using the 633 nm laser without enhanced dynamics.

2.3 Bead rotations on tether

For experiments where the movement of a VN coated bead along a tether was followed, a MatTek dish with transfected cells and VN coated beads was placed on the sample stage. In the following, we distinguish two types of VN coated beads: 'tether beads' with $d = 4.95 \mu\text{m}$, and 'tracer beads', with $d = 0.989 \mu\text{m}$, see section 1.4 for VN coating details. During tether extraction we imaged the sample in bright field mode to avoid confocal laser radiation before the actual data acquisition. A tether bead was optically trapped and brought close to a (usually isolated and spread out) cell. The bead was carefully pressed against the cell

membrane to allow attachment and then slowly moved a distance of about 10 μm (using the LabVIEW controlled piezo stage) such that a membrane tether was extracted between cell and bead. Once a tether was extracted, we switched to confocal mode. The EGFP Lifeact-7 expressing cells were excited at 488 nm, the flash red tracer beads at 633 nm. The tether bead was carefully pushed onto the sample glass surface such that it got stuck and the trapping laser could be turned off. A control z -stack was acquired (the tether beads were imaged in reflection mode using the 633 nm laser without enhanced dynamics) to ensure the tether was held and did not stick on the glass surface. Then a tracer bead was caught using the optical trap and carefully attached onto the tether close to the tether bead. Confocal z -stacks over time were acquired to follow the tracer bead's rotation around the tether towards the cell body.

3 Data analysis

Image processing and analysis were done using ImageJ and custom written MATLAB scripts.

3.1 Tracking the movement of a bead attached to a filopodium

To localize the position of an attached bead on a filopodium, the xy position of the center of the bead and the filopodium were extracted from the z -projection of the image stack, see Figure S6. Afterwards, the z -positions in each time point were extracted from the orthogonal view of the stack, shown in Figure S6B, which corresponds to the red dashed line in Figure S6A.

3.2 Tracking the rotation of filopodial tips

To quantify the tip rotation of filopodia, we increased the resolution of the images using a Gabor filter.^{6,7} Then the center of a filopodium was extracted by fitting Gaussian function to the orthogonal view along the filopodium, see Figure S7.

3.3 Tracking helical filopodial coils

We used a 3D segmentation plugin software (Simple Neurite Tracer) in ImageJ⁸ to track filopodia after removing the background intensity in images using Gabor and Gaussian blur filters (Figure S8).

Simulation Methods

We use the 2D equations of active nematohydrodynamics based on the theory of liquid crystals, which have proven successful in describing spatio-temporal dynamics of the cell cytoskeleton, including acto-myosin complexes^{9–12} and microtubule bundles powered by kinesin motor proteins.^{13–15} The orientational order of microscopic actin filaments is represented by the nematic tensor $Q = \frac{3q}{2}(\vec{n}\vec{n} - I/3)$, with q the magnitude of the orientational order, $\vec{n}$ the director, and I the identity tensor, which evolves as

$$(\partial_t + \vec{u} \cdot \nabla) Q - S = \Gamma H, \quad (1)$$

where Γ is a rotational diffusivity and the co-rotation term

$$\begin{aligned} S = & (\lambda E + \Omega) \cdot \left(Q + \frac{I}{3} \right) + \left(Q + \frac{I}{3} \right) \cdot (\lambda E - \Omega) \\ & - 2\lambda \left(Q + \frac{I}{3} \right) (Q : \vec{u}), \end{aligned} \quad (2)$$

accounts for the response of the orientation field to the extensional and rotational components of the velocity gradients characterised by the strain rate $E = (\vec{u}^T + \vec{u})/2$ and vorticity $\Omega = (\vec{u}^T - \vec{u})/2$ tensors. The relative strength of extensional and rotational flows is determined by the alignment parameter λ . Therefore, the alignment parameter accounts for the different responses of particles of different shapes to the symmetric and asymmetric parts of the velocity gradient tensor.¹⁶ Mapping the alignment parameter to the Leslie–Ericksen equation

for liquid crystal dynamics gives $\lambda = \frac{3q+4}{9q} \frac{\beta^2-1}{\beta^2+1}$,¹⁷ where $\beta = a/b$ is the ratio of the length of the cell along its axis of symmetry, a , to its length perpendicular to this axis, b . Therefore, for prolate ellipsoids $\beta > 1$, while for oblate ellipsoids $\beta < 1$ and for spherical particles $\beta = 1$, which correspond to $\lambda > 0$, $\lambda < 0$, and $\lambda = 0$, respectively. Actin filaments are rod-shaped elongated particles and therefore are characterized by $\lambda > 0$. The relaxation of the orientational order is determined by the molecular field,

$$H = -\frac{\delta\mathcal{F}}{\delta Q} + \frac{I}{3}\text{Tr}\left(\frac{\delta\mathcal{F}}{\delta Q}\right), \quad (3)$$

where $\mathcal{F} = \mathcal{F}_b + \mathcal{F}_{el}$ denotes the free energy. We use the Landau-de Gennes bulk free energy for $\mathcal{F}_b$,¹⁶

$$\mathcal{F}_b = \frac{A}{2}\mathbf{Q}^2 + \frac{B}{3}\mathbf{Q}^3 + \frac{C}{4}\mathbf{Q}^4, \quad (4)$$

and $\mathcal{F}_{el} = \frac{K}{2}(\partial_k Q_{ij})^2$ describes the cost of spatial inhomogeneities in the order parameter, assuming a single elastic constant K . There are in general three elastic constants, corresponding to bend K_b , splay K_s , and twist K_t in 3D systems. However, setting different values of K_b , K_s , and K_t does not change the mechanism of the hydrodynamic instability.

The velocity field is evolved according to the incompressible Navier–Stokes equation:

$$\vec{\nabla} \cdot \vec{u} = 0, \quad (5)$$

$$\partial_t \vec{u} + \vec{u} \cdot \vec{\nabla} u = \vec{\nabla} \cdot \Pi, \quad (6)$$

which reduces to the force balance equation $\vec{\nabla} \cdot \Pi = \vec{0}$ in the low Reynolds number limit relevant to actin filaments mechanics, with a stress tensor Π that must account for contributions

from the viscous stress $\Pi^{\text{visc}} = 2\eta E$ and the elastic stress

$$\begin{aligned}\Pi^{\text{elastic}} = & -PI + 2\lambda(Q + I/3)(Q : H) \\ & - \lambda H \cdot (Q + \frac{I}{3}) - \lambda(Q + \frac{I}{3}) \cdot H \\ & - \nabla Q \frac{\delta \mathcal{F}}{\delta \nabla Q} + Q \cdot H - H \cdot Q,\end{aligned}\tag{7}$$

which includes the pressure P .¹⁸ The active contribution to the stress is accounted for by $\Pi^{\text{act}} = -\zeta Q$,¹⁹ such that any gradient in $\mathbf{Q}$ generates a flow field, with strength determined by the activity coefficient, ζ . This active stress term accounts for the local stresses generated by active processes in the cells, including actomyosin polymerization and contractility.^{9,10}

The equations of active nematohydrodynamic are solved using a hybrid lattice Boltzmann and finite difference method²⁰ that does not include thermal fluctuations. The momentum equation is solved using the lattice Boltzmann method to resolve the hydrodynamics, and the method of lines is implemented to determine the order parameter in Eq. 1. A finite-difference approach is used for spatial discretisation of Eq. 1 and the temporal evolution is obtained through an Euler integration scheme.

A three dimensional rectangular domain with a square cross-section is used for simulations. The length (L) of the channel was fixed as 128 while the height (h) was varied between 4 to 32. In our study, simulations are initialized with a stagnant fluid and randomly oriented director field. Zero velocity and no flux of $\mathbf{Q}$ at the walls are used as the boundary conditions, therefore no anchoring boundary condition is imposed for the orientational order parameter on the channel walls.

The parameters used in the simulations are $A = 1.0$, $\Gamma = 0.34$, $\eta = 2/3$, and $\lambda = 0.7$, in lattice units. As usual in lattice Boltzmann schemes, discrete space and time steps are chosen as unity and all quantities can be converted to physical units in a material dependent manner.^{20–22} In order to map the simulation parameters to physical units we consider the typical length of the filopodia as the characteristic length $L_0 \sim 10 \mu\text{m}$, and

the experimentally measured viscosity and elasticity of the actin/myosin mixtures^{11,12} as the characteristic viscosity $\eta_0 = 0.1\text{Pa}\cdot\text{s}$ and force units $F_0 = 10\text{ pN}$, respectively. This maps the confinement sizes studies here to the range $\sim 0.2 - 2\text{ }\mu\text{m}$, elasticities to the range $\sim 0.1 - 1.0\text{ pN}$, and the activities to $\sim 0.01 - 0.1\text{Pa}$, consistent with the values reported for *in vitro* two-dimensional extract of actin/myosin mixtures.¹²

Furthermore, the magnitude of the torque dipole can be estimated based on analytical derivation of,²³ which suggests:

$$Ma = \frac{2\pi f a r^2}{(4\pi^2 r^2 + P^2)^{1/2}}, \quad (8)$$

where M is the torque monopole, $a \sim 100\text{ nm}$ distance between filaments, $f \sim 10\text{ pN}$ is the force generated by myosin motors, $r \sim 5\text{ nm}$ the radius of actin filament, and $P \sim 72\text{ nm}$ is the helical pitch of the actin filaments. Using these values the ratio of the active stress associated with the torque dipole ζ' to the active stress due to force dipole ζ can be estimated as $\zeta'/\zeta \sim M/fa \sim 0.1$, which is the value mentioned in the main text. Therefore, the mechanism of symmetry-breaking and twist generation is governed by hydrodynamic instabilities that are induced by the active stress associated with force dipoles. The role of torque dipole is to set the direction of the twist: in the absence of a torque dipole $\zeta' = 0$ (results in the main text) clockwise and counter-clockwise rotations are equally likely. Including a torque dipole is expected to bias this twist towards clockwise and counter-clockwise rotation depending on the sign of ζ' . To test this, we performed simulations with $\zeta' = (0, 0.1 \times \zeta, \zeta)$ and compared the distribution of clockwise and counter-clockwise rotations for 20 simulations - for each case - with random initial conditions. As evident from Figure S9 increasing the value of ζ' clearly increases the biased rotation and when it becomes comparable to the value of active stress associated with force dipoles it can deterministically set the direction of rotation.

4 Additional Figures

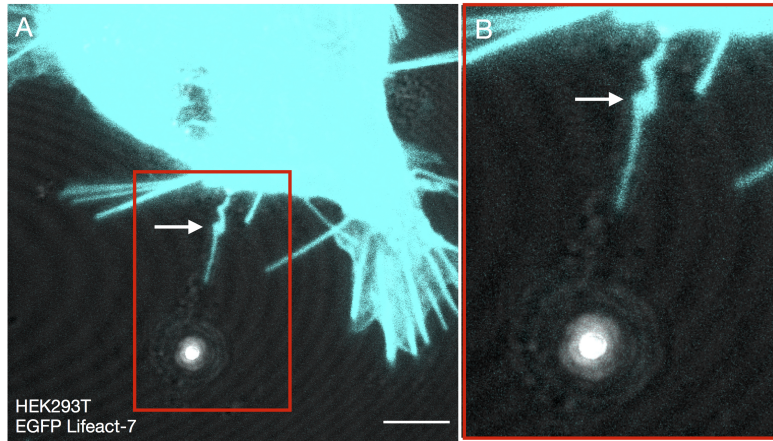


Fig. S1. Filopodia rotation is not an artifact of confocal laser illumination. (A) Confocal z -projection of tether extracted from a HEK293T cell (cyan, EGFP Lifeact-7) using a $4.96\ \mu\text{m}$ bead shows a buckle (white arrow) stemming from rotation of the filopodium. The tether was extracted in bright field mode without exposing the cell to laser illumination prior to confocal imaging. Scale bar is $5\ \mu\text{m}$. (B) Zoom in to the red marked region in (A).

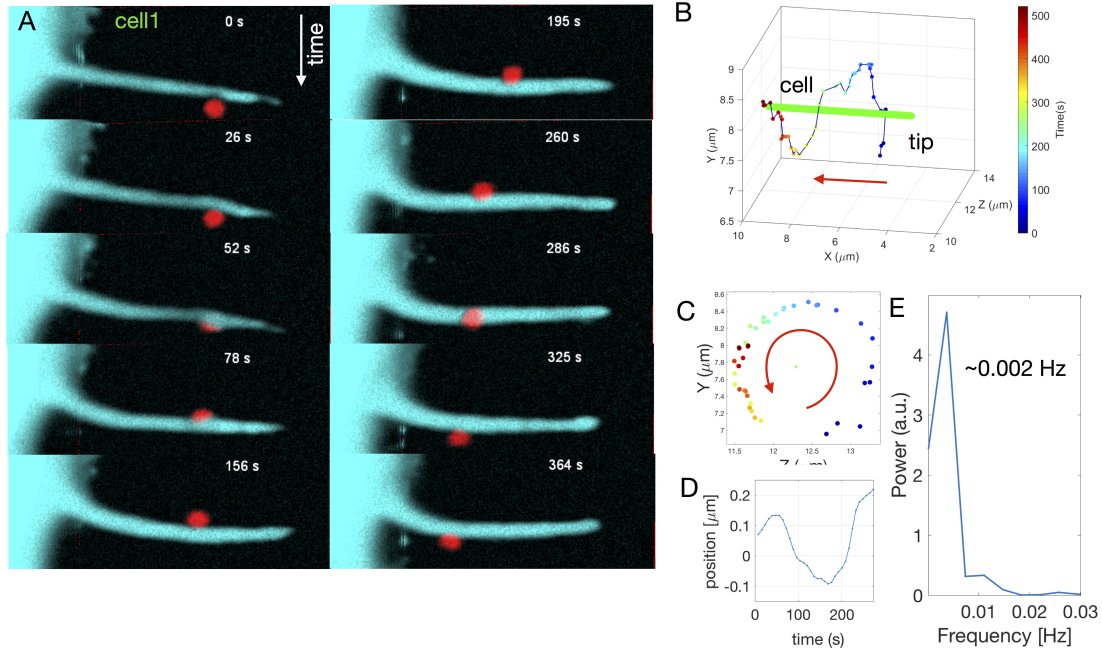


Fig. S2A. Bead rotation assay reveals that the F-actin shaft inside filopodia rotates around its own axis (additional data for cell 1 from Fig. 2B). (A) 3D reconstructed images of the rotation of a VN coated tracer bead ($d = 0.989 \mu\text{m}$, flash red) around an extended filopodium of a HEK293T cell (cyan, EGFP Lifeact-7, cell 1 from Fig. 2B) at consecutive time points. (B) 3D trajectory of the bead in (A) (from blue to red) rotating in a counterclockwise orientation around the filopodium (green) from tip towards the cell body. (C) xz view of the bead movement from (A) over time, time bar is the same as in (B) shows the counterclockwise rotation (seen from tip towards the cell body). (D,E) The bead position (data from A) as a function of time (D) can be Fourier transformed to give the rotation frequency of the tracer bead 0.002 Hz .

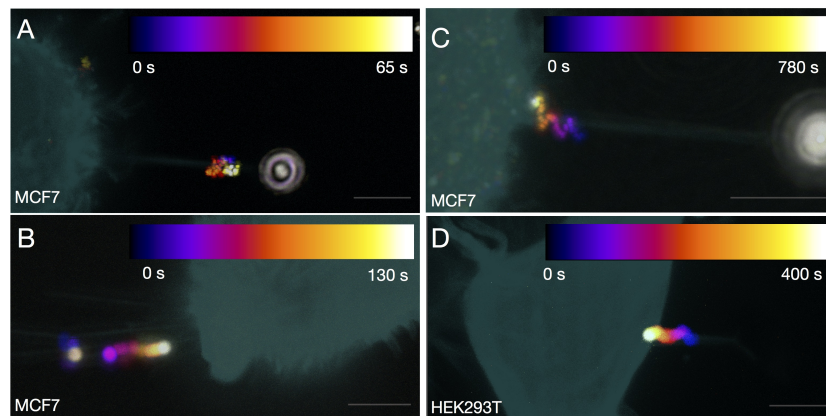


Fig. S2B. Further examples of a bead rotating around or moving along a filopodium. (A-D) Color coded z -projection over time of single beads rotating around (A-C) MCF7 cells (cyan, EGFP Lifeact-7) and (D) a HEK293T cell (cyan, EGFP Lifeact-7). Scale bars are 5 μm .

cell type	bead rotation frequency [Hz]	bead velocity [$\mu\text{m/s}$]
HEK293T	0.002	0.0184
HEK293T	0.005	0.1222
MCF7	0.08	0.3676
MCF7	0.05	0.2309
MCF7	0.005	0.0312
uninduced MCF7-p95ErbB2	0.02	0.1297

Table S2C. Rotation frequencies and velocities for single tracer beads on filopodia of different cell types.

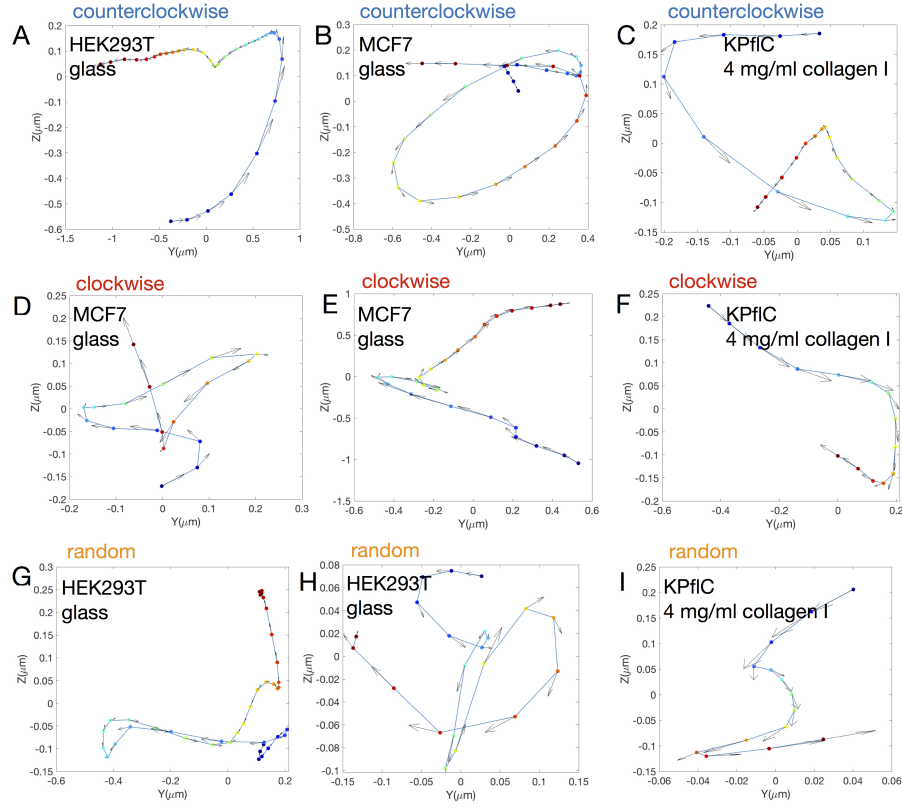


Fig. S3A. Further examples of filopodial tip rotation tracks. 46 % of tracked rotations were counterclockwise, 7 % clockwise, and 47 % random ($N = 74$). (A-C) Traces of the filopodial tip movement over time showing a counterclockwise rotation in yz view for the tip of a HEK293T cell on glass (A), a MCF7 cell on glass (B), and a KP^{fl}C cell in 4 mg/ml collagen I (C). (D-F) Traces of the filopodial tip over time showing a clockwise rotation in yz view for the tip of MCF7 cells on glass (D,E), and a KP^{fl}C cell in 4 mg/ml collagen I (F). (G,H) Traces of the filopodial tip over time showing random rotations in yz view for the tip of HEK293T cells on glass (G,H), and a KP^{fl}C cell in 4 mg/ml collagen I (I).

cell type	N	(mean tip rotation frequency $\pm$ std.) [Hz]
HEK293T on glass	22	0.0055 ± 0.0040
MCF7 on glass	14	0.0136 ± 0.0119
MCF7-p95ErbB2 on glass	4	0.0061 ± 0.0030
KP ^{fl} C in collagen	23	0.0024 ± 0.0011
KPC in collagen	5	0.0023 ± 0.0012
cells on glass	41	0.0084 ± 0.0084
cells in collagen	28	0.0024 ± 0.0011

Table S3B. Mean filopodial tip rotation frequencies for cells on glass and embedded in collagen I gels for the data in Figure 3J.

cell types	<i>p</i> -value
HEK293T (glass), MCF7 (glass)	0.0058 *
HEK293T (glass), KP ^{fl} C (collagen)	$8.2969 \cdot 10^{-4}$ ***
MCF7 (glass), KP ^{fl} C (collagen)	$6.701 \cdot 10^{-5}$ ***
MCF7-p95ErbB2 (glass), KP ^{fl} C (collagen)	$1.0608 \cdot 10^{-4}$ ***
MCF7-p95ErbB2 (glass), KPC (collagen)	0.0357 *
cells on glass, cells in collagen	$3.7910 \cdot 10^{-4}$ ***

Table S3C. *p*-values for the tip rotation frequency data from Figure 3J. * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$. The *p*-values for the not mentioned couples show no significant difference between the compared populations.

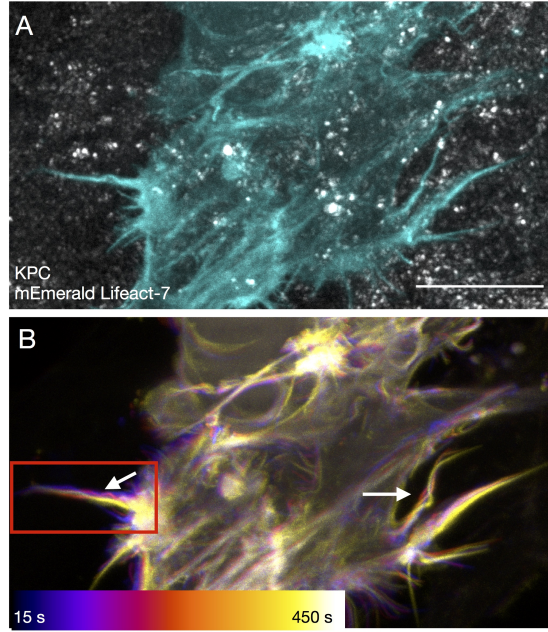


Fig. S4A. Further example of buckling filopodia from the KPC cell embedded in a 4 mg/ml collagen I gel from Fig. 1C,D. (A) Confocal z -projection of a KPC cell (cyan, mEmerald Lifeact-7) in 4 mg/ml collagen I gel (gray, reflection). Scale bar is 10 μm . (B) Color coded temporal development of the cell from (A and Fig. 1C,D). Overlay of 6 z -projections acquired at different time points over an interval of 450 s. Time resolution of each z -stack is 15 s. The arrows highlight buckles. (B) Zoom in to the red region of (A). The right arrow marks the region shown in Fig. 1C,D.

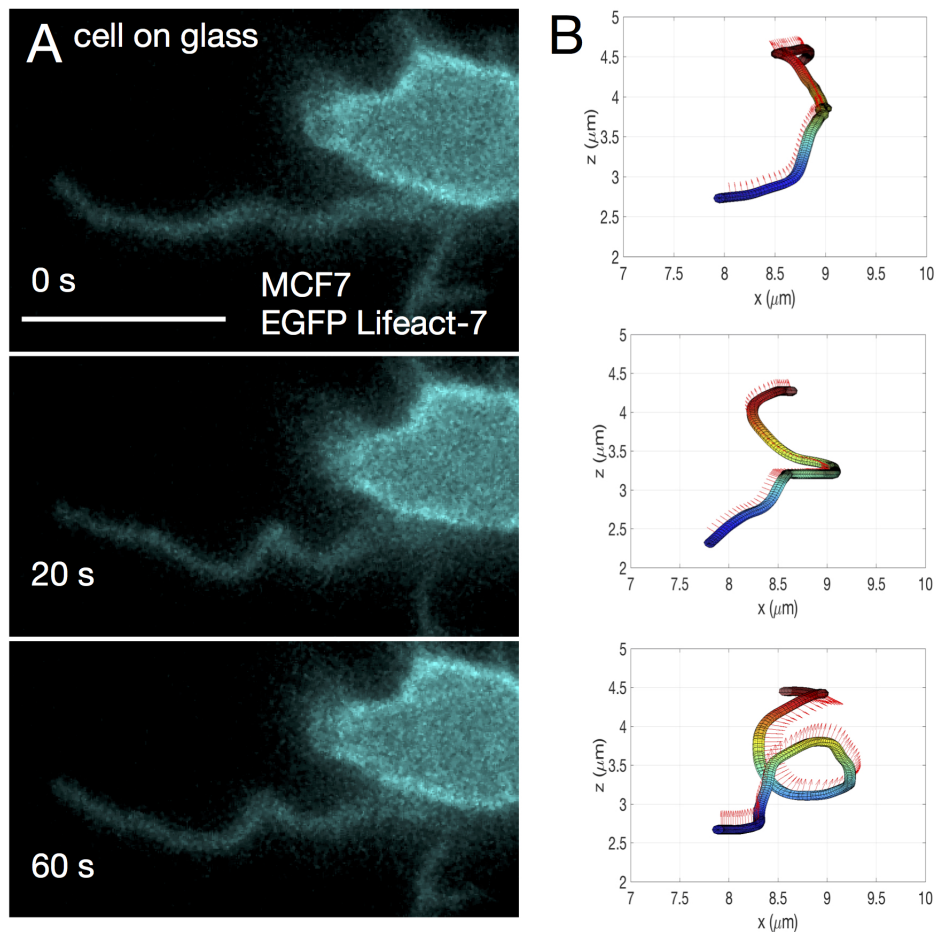


Fig. S4B. Further example of a buckling filopodium of a cell grown on glass. (A) Filopodium from a MCF7 cell (cyan, EGFP Lifeact-7) grown on glass at 3 different time points (at 0 s, 20 s and 60 s). Scale bar is 5 μm . (B) 3D tracks of the filopodium corresponding to the time points from (A) show how it bends and helically coils.

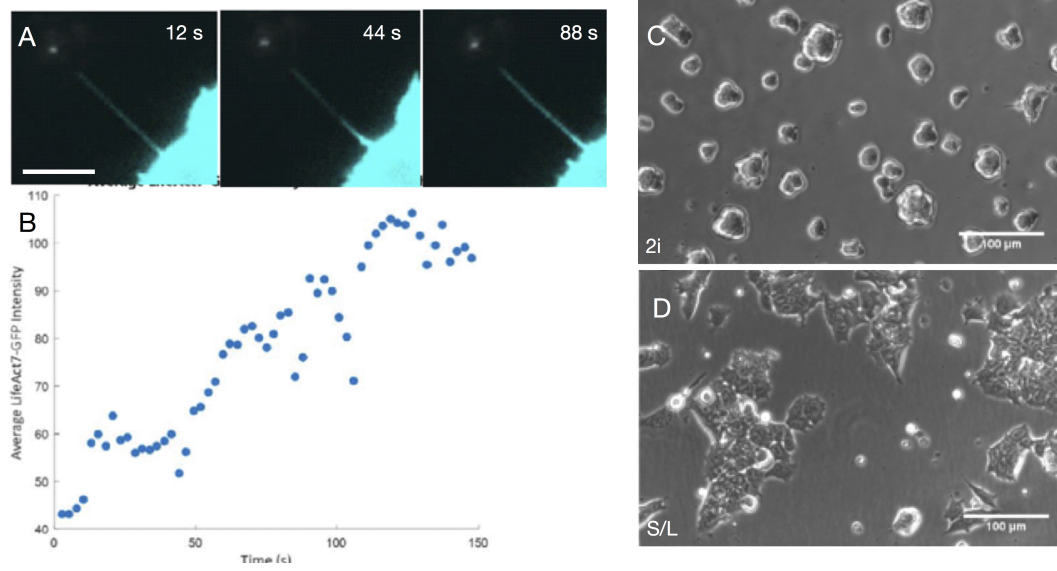


Fig. S5A. Tethers from stem cells show rich actin dynamics. (A) Tether extracted from a mES cell (cyan, EGFP Lifeact-7) grown in S/L medium at 3 different time points (12 s, 44 s, 88 s) after tether extraction. Scale bar is 5 μm . (B) Quantification of the F-actin content of the tether from (A) (via the average intensity of the EGFP Lifeact-7 signal) over a time of 180 s shows how F-actin accumulates inside the tether. (C and D) mES cells cultured in 2i medium (C) are less spread out than cells grown in S/L medium (D). Scale bars are 100 μm .

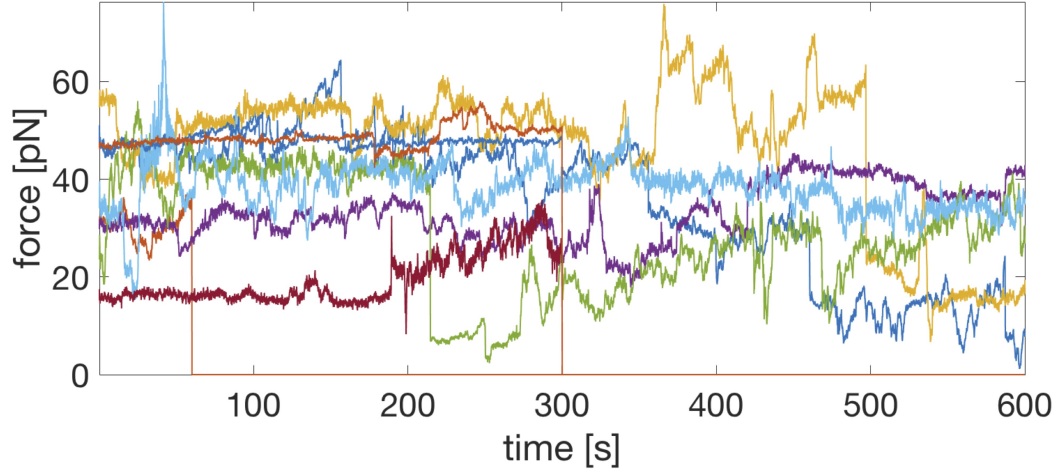


Fig. S5B. Tethers extracted from MCF7 cells show rich dynamics. 9 different force curves of tethers from MCF7 cells extracted with and held by an optically trapped bead ($d = 4.95 \mu\text{m}$). Acquisition of the forces started after tether extraction.

cell type	N	(mean maximum force $\pm$ std.) [pN]
HEK293	60	43.8783 ± 27.1404
MCF7	11	61.0963 ± 17.8468
mESC (2i)	42	31.3262 ± 12.7301
mESC (S/L)	51	25.5621 ± 15.4024

Table S5C. Mean maximum forces for the data in Figure 5F.

cell types	p -value
MCF7, mESC (2i)	$2.68 \cdot 10^{-8}$ ***
MCF7, mESC (S/L)	$5.63 \cdot 10^{-9}$ ***
HEK293, mESC (2i)	0.0071 **
HEK293, mESC (S/L)	$7.87 \cdot 10^{-5}$ ***

Table S5D. p -values for the maximum tether force data from Figure 5F. * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$. The p -values for the not mentioned couples show no significant difference between the compared populations.

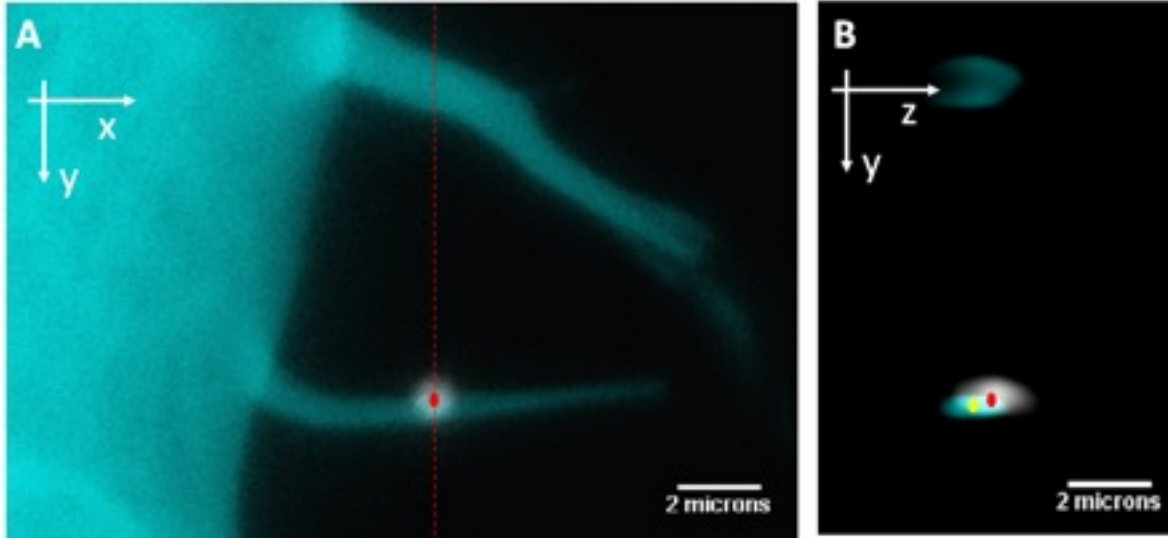


Fig. S6. Tracking the bead movement along a filopodium. (A) The xy -position of a bead on a filopodium was extracted from the z -projection image (red point). (B) The orthogonal view along red dashed line in (A) was used to localize the yz -positions of the bead (red point) and center of the filopodium (yellow point).

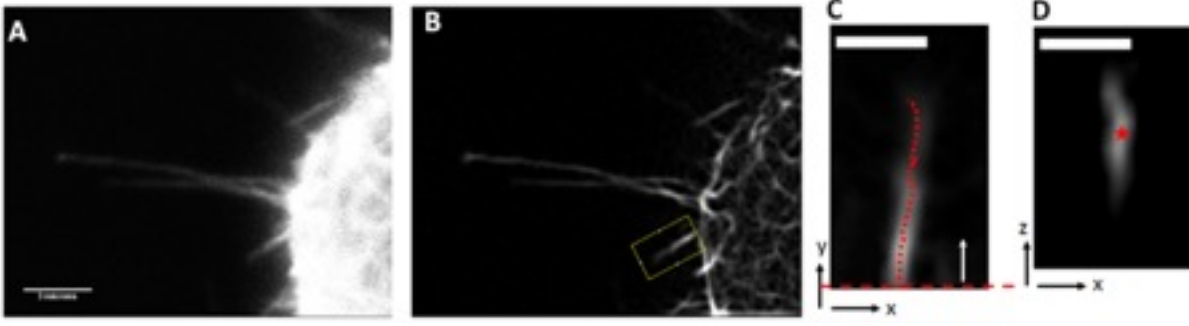


Fig. S7. Filopodial tip tracking. (A) z -projection of 3-dimensional volume of raw confocal images of a cell with filopodia. (B) The same image from (A) after applying linear Gabor filter to enhance the resolution. (C) The xy position of the desired filopodium (yellow box in (B)) was found by scanning the red dashed line along filopodium and applying a Gaussian fit to the intensity profile. (D) The z location of the filopodium in each point (red points in (C)) was extracted from the orthogonal view of the image.

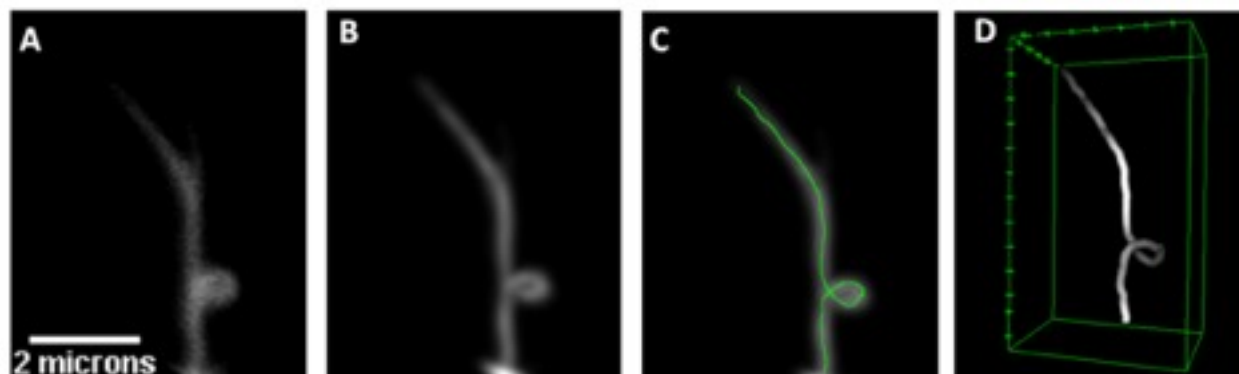


Fig. S8. Tracking helical coils. (A) z -projection of the 3-dimensional volume containing a coiling filopodium. (B) Gabor and Gaussian blur filtered result of the image from (A). (C and D) Segmentation of the filopodium in 2D (C) and in a 3D (D) view; both obtained using the “Simple Neurite Tracer” plugin in ImageJ.

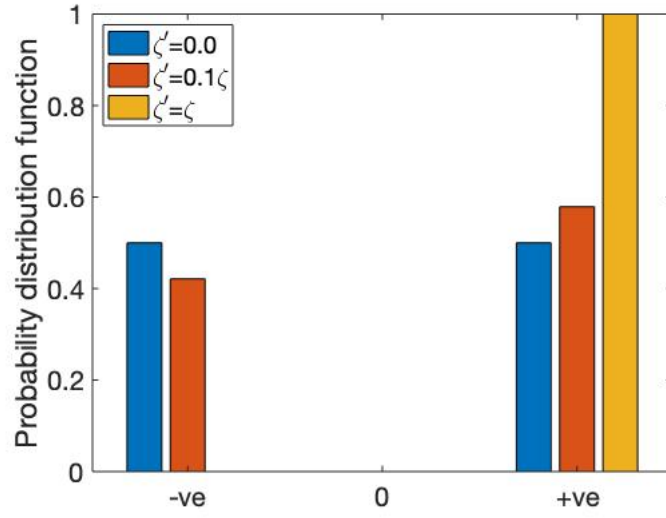


Fig. S9. Probability distribution functions for the direction of rotation. Increasing the magnitude of the active torque dipole ζ' increases the biased rotation. Positive (negative) values are indicated by *+ve* (*-ve*).

5 Supplementary Movies

- **SI movie 1a HEK293T filopodia sweeping.avi** Sweeping filopodia from the HEK293T cell (cyan, EGFP Lifeact-7) from Figure 1A,B over a time span of 400 s. Scale bar is 5 μm .
- **SI movie 1b KPC 4mg/ml collagen I bending arr.avi** Bending filopodia from the KPC cell (cyan, EGFP Lifeact-7) from Figure 1C,D and Figure S4a in 4 mg/ml collagen I (gray, reflection) over a time span of 475 s. Red arrows highlight bending regions. Scale bar is 5 μm .
- **SI movie 1c MCF7 tether force.avi** Bending tether pulled from the MCF7 cell (cyan, EGFP Lifeact-7) from Figure 1E (rotated) using a VN coated 4.95 μm bead (gray, reflection). Movie corresponds to the force curve in Figure 1G. Scale bar is 5 μm .
- **SI movie 2a bead rotation MCF7-p95ErbB2.avi** 3D reconstruction of confocal *xyzt* data for the data from Figures 1C-G. VN coated 0.989 μm bead (cyan, flash red) rotating counterclockwise (as seen from tip towards cell body) around a pulled filopodium from a not activated MCF7-p95ErbB2 cell (red, EGFP Lifeact-7). The filopodium is held by a 4.95 μm VN coated bead (gray, reflection) which is glued to the sample surface. Frame interval is 8 sec, total time is 384 s.
- **SI movie 3a mESC filopodia 2i.avi** Filopodia on a HV.5.1 mESC (cyan, EGFP Lifeact-7) grown in 2i medium.
- **SI movie 3b hepa cell SiR actin filopodia rotation arr.avi** Rotating filopodia on Hepa 1-6 cell (red, SiR-actin) over 71.5 min, highlighted via a white arrow. Scale bar is 10 μm .
- **SI movie 3c hepa cell SiR actin filopodia rotation arr.avi** Rotating filopodia on Hepa 1-6 cell (red, SiR-actin) over 58 min, highlighted via a white arrow. Scale bar is 10 μm .

- **SI movie 4a KPC 4mg/ml collagen I bending arr.avi** Bending filopodia from KPC cell (cyan, EGFP Lifeact-7) in 4 mg/ml collagen I (gray, reflection) over a time span of 399 s. Red arrows highlight bending regions. Scale bar is 10 μm .
- **SI movie 4b MCF7 coil.avi** Coiling filopodium from the MCF7 cell (cyan, EGFP Lifeact-7) from Figures S4b, 7C,D over 160 s. Scale bar is 5 μm .
- **SI movie 5a MCF7 extraction of bending tether.mov** Tether pulling from a MCF7 cell (cyan, EGFP Lifeact-7) using a VN coated 4.95 μm bead (grey, reflection). After extraction, the image zoomed in and the focus is adjusted such that the tether is aligned with the imaging plane. Total time is 450 s. Scale bar is 5 μm .
- **SI movie 5b mESC tether.avi** Bending tether pulled from a HV.5.1 mESC (cyan, EGFP Lifeact-7) using a VN coated 4.95 μm bead (grey, reflection).

References

- (1) Egeblad, M.; Mortensen, O. H.; Jaattela, M. Truncated ErbB2 receptor enhances ErbB1 signaling and induces reversible, ERK-independent loss of epithelial morphology. *Int J Cancer* **2001**, *94*, 185–91.
- (2) Williams, R. L.; Hilton, D. J.; Pease, S.; Willson, T. A.; Stewart, C. L.; Gearing, D. P.; Wagner, E. F.; Metcalf, D.; Nicola, N. A.; Gough, N. M. Myeloid leukaemia inhibitory factor maintains the developmental potential of embryonic stem cells. *Nature* **1988**, *336*, 684–7.
- (3) Niwa, H. How is pluripotency determined and maintained? *Development* **2007**, *134*, 635–46.
- (4) Matsui, Y.; Zsebo, K.; Hogan, B. L. Derivation of pluripotential embryonic stem cells from murine primordial germ cells in culture. *Cell* **1992**, *70*, 841–7.
- (5) Ramesh, P.; Baroji, Y. F.; Reihani, S. N.; Stamou, D.; Oddershede, L. B.; Bendix, P. M. FBAR syndapin 1 recognizes and stabilizes highly curved tubular membranes in a concentration dependent manner. *Sci Rep* **2013**, *3*, 1565.
- (6) Dai, P. S.; Luo, H. Y.; Sheng, H. W.; Zhao, Y. L.; Li, L.; Wu, J.; Zhao, Y. Q.; Suzuki, K. J. A New Approach to Segment Both Main and Peripheral Retinal Vessels Based on Gray-Voting and Gaussian Mixture Model. *Plos One* **2015**, *10*.
- (7) Hendargo, H. C.; Estrada, R.; Chiu, S. J.; Tomasi, C.; Farsiu, S.; Izatt, J. A. Automated non-rigid registration and mosaicing for robust imaging of distinct retinal capillary beds using speckle variance optical coherence tomography. *Biomedical Optics Express* **2013**, *4*, 803–821.
- (8) Longair, M. H.; Baker, D. A.; Armstrong, J. D. Simple Neurite Tracer: open source

- software for reconstruction, visualization and analysis of neuronal processes. *Bioinformatics* **2011**, *27*, 2453–2454.
- (9) Juelicher, F.; Kruse, K.; Prost, J.; Joanny, J.-F. Active behavior of the cytoskeleton. *Physics reports* **2007**, *449*, 3–28.
 - (10) Prost, J.; Jülicher, F.; Joanny, J. F. Active gel physics. *Nat. Phys.* **2015**, *11*, 111–117.
 - (11) Kumar, N.; Zhang, R.; de Pablo, J. J.; Gardel, M. L. Tunable structure and dynamics of active liquid crystals. *Science advances* **2018**, *4*, eaat7779.
 - (12) Zhang, R.; Kumar, N.; Ross, J. L.; Gardel, M. L.; De Pablo, J. J. Interplay of structure, elasticity, and dynamics in actin-based nematic materials. *Proceedings of the National Academy of Sciences* **2018**, *115*, E124–E133.
 - (13) Ellis, P. W.; Pearce, D. J.; Chang, Y.-W.; Goldsztein, G.; Giomi, L.; Fernandez-Nieves, A. Curvature-induced defect unbinding and dynamics in active nematic toroids. *Nature Physics* **2018**, *14*, 85–90.
 - (14) Hardoüin, J.; Hughes, R.; Doostmohammadi, A.; Laurent, J.; Lopez-Leon, T.; Yeomans, J. M.; Ignés-Mullol, J.; Sagués, F. Reconfigurable flows and defect landscape of confined active nematics. *Communications Physics* **2019**, *2*, 1–9.
 - (15) Duclos, G.; Adkins, R.; Banerjee, D.; Peterson, M. S.; Varghese, M.; Kolvin, I.; Baskaran, A.; Pelcovits, R. A.; Powers, T. R.; Baskaran, A. et al. Topological structure and dynamics of three-dimensional active nematics. *Science* **2020**, *367*, 1120–1124.
 - (16) De Gennes, P.-G.; Prost, J. *The physics of liquid crystals*; Oxford university press, 1993; Vol. 83.
 - (17) Doostmohammadi, A.; Ignés-Mullol, J.; Yeomans, J. M.; Sagués, F. Active nematics. *Nature Communications* **2018**, *9*, 1–13.

- (18) Beris, A. N.; Edwards, B. J.; Edwards, B. J. *Thermodynamics of flowing systems: with internal microstructure*; Oxford University Press on Demand, 1994.
- (19) Simha, R. A.; Ramaswamy, S. Hydrodynamic fluctuations and instabilities in ordered suspensions of self-propelled particles. *Physical review letters* **2002**, *89*, 058101.
- (20) Thampi, S. P.; Golestanian, R.; Yeomans, J. M. Vorticity, defects and correlations in active turbulence. *Philosophical Transactions of the Royal Society A: Mathematical, Physical and Engineering Sciences* **2014**, *372*, 20130366.
- (21) Cates, M.; Fielding, S.; Marenduzzo, D.; Orlandini, E.; Yeomans, J. Shearing active gels close to the isotropic-nematic transition. *Physical review letters* **2008**, *101*, 068102.
- (22) Plan, E. L. C. V.; Yeomans, J.; Doostmohammadi, A. Active matter in a viscoelastic environment. *Phys. Rev. Fluids* **2020**, *5*, 023102.
- (23) Fürthauer, S.; Stempel, M.; Grill, S. W.; Jülicher, F. Active chiral fluids. *The European physical journal E* **2012**, *35*, 1–13.