Filopodial retraction force is generated by cortical actin dynamics and controlled by reversible tethering at the tip

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Filopodia are dynamic, finger-like plasma membrane protrusions that sense the mechanical and chemical surroundings of the cell. Here, we show in epithelial cells that the dynamics of filopodial extension and retraction are determined by the difference between the actin polymerization rate at the tip and the retrograde flow at the base of the filopodium. Adhesion of a bead to the filopodial tip locally reduces actin polymerization and leads to retraction via retrograde flow, reminiscent of a process used by pathogens to invade cells. Using optical tweezers, we show that filopodial retraction occurs at a constant speed against counteracting forces up to 50 pN. Our measurements point toward retrograde flow in the cortex together with frictional coupling between the filopodial and cortical actin networks as the main retraction-force generator for filopodia. The force exerted by filopodial retraction, however, is limited by the connection between filopodial actin filaments and the membrane at the tip. Upon mechanical rupture of the tip connection, filopodia exert a passive retraction force of 15 pN via their plasma membrane. Transient reconnection at the tip allows filopodia to continuously probe their surroundings in a load-and-fail manner within a well-defined force range.

Significance

Cells can sense their environment by using hair-like structures called filopodia that often exert pulling forces upon adhesive tip contact. We show, using optical tweezers and confocal microscopy, that the retraction force is generated by the dynamics of the cortical actin cytoskeleton, constantly pulling on the filopodial base. The weakest point of force transduction is at the tip between the actin shaft and the membrane. This allows tip-bound filopodia to apply controlled forces and to use a “load-and-fail” sensing process.


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retraction were not limiting within this range. We argue that actin treadmilling in the cell cortex, that functions far from its stall regime, transduces inward forces to the filopodial actin shaft at the base via high friction. In addition we found that filopodia can exert passive inward forces of 15 pN by using cell membrane-based forces. External counterforces that are only 5 pN higher than the membrane force can lead to rupture of connections between the actin shaft and the membrane at the filopodial tip. These weak contacts at the tip define the maximal pulling force of filopodia and allow cytoskeletal inward forces to operate only for short time intervals (<25 s). We found that the mechanical disconnection between membrane and actin filaments is only transient as actin dynamics at the tip are altered after disconnection. A continuous load-and-fail behavior allows thus tip-bound filopodia to probe the mechanics of their environment.

**Results**

**Filopodial Retraction Abruptly Stalls at a Defined Transition Force.** Retraction of filopodia can be induced by attaching beads to their tips and counteracting mechanical forces can stall or even invert filopodial retraction (16, 25, 30). To better understand the mechanism and the dynamics of filopodial retraction against force we analyzed the dependency of retraction speed on counteracting forces, using the experimental setup shown in Fig. 1A. HeLa cells were fluorescently labeled with the lipophilic dye FM4-64 and observed by confocal microscopy. A filopodium that was not attached to the substrate was selected and its tip was approached to an optically trapped, carboxylated bead (COOH bead). After bead binding, most filopodia retracted (75%, n = 101, Fig. S1A), resulting in displacement of the bead relative to the trap center with velocity \(v_p\) (Fig. 1B). For nonmigrating cells, if the positions of the trap and of the substrate are kept constant (“position-clamp” mode), \(v_p\) represents the filopodial retraction speed against the force \(F\) exerted by the optical trap. The force increases with the distance \(\Delta x\) between the bead and the trap center according to \(F = k_S \Delta x\), where \(k_S\) is the trap stiffness (Fig. 1B). Only short pulling events of at most 400 nm could be observed because retraction stalled abruptly at a transition force \(F_t\). This transition was followed by a force plateau (Fig. 1B), as reported previously (30). To study complete retraction of filopodia, we used a feedback system that adjusted the position of the nonmigrating cell by moving the microscope’s stage at a sub-second timescale, thus allowing the displacement of the bead within the trap to remain constant (“force-clamp” mode). In this mode, filopodia could be observed retracting against a controlled force in a linear fashion (Fig. 1B, Insets at \(t > 8\) min). The stage position (Fig. 1B, Lower) is directly related to the filopodial length and the retraction speed \(v_F\) can be measured during the whole retraction process.

Retraction speeds measured in position-clamp mode \(v_p\) (Fig. 1C) and force-clamp mode \(v_F\) (Fig. 1D) of individual filopodia were plotted as a function of \(F\). Filopodia abruptly stalled at an average force \(<F_p>\) of 21 ± 4 pN when probed in the position-clamp mode (Fig. 1E) and a minority of filopodia (30%, \(n = 29\)) retracted to higher individual transition forces (Fig. 1C). Consistently, most filopodia (74%) were observed to retract against counteracting forces below 21 pN in the force-clamp mode. For higher forces, retraction can be observed for some time (26% of cases, Fig. 1D), but they ultimately led to elongation of the filopodial structure (Fig. S1C and see Fig. 3).

The speed of single filopodia while retracting against forces between 0 pN and 50 pN did not show any explicit dependency on force, either in position- or in force-clamp mode until abrupt stall or forced elongation occurred. When averaged, the speeds recorded in different force intervals showed only a slight increase from \(<v_p> = -7 \pm 1\) nm/s to \(-10 \pm 1\) nm/s at forces of 5 pN and 15 pN, respectively, and no dependency of \(<v_p>\) = \(-14 \pm 1\) nm/s for forces between 0 and 20 pN (Fig. 1C and D). For forces higher than 20 pN, \(<v_F>\) decreased to \(-8 \pm 2\) nm/s, which was similar to \(<v_p> = -10 \pm 2\) nm/s observed in this regime. This weak dependency can be explained by the viscoelastic properties of the filopodium (SI Text).

We tested whether similar results could be observed in two other cell lines. Consistent with results observed for HeLa cells, retraction of filopodia emanating from fibroblastic carcinoma (CAR) cells and human embryonic kidney cells (HEK-293T)
could also be observed following bead attachment to their tip. For the tested cell lines, retraction was abruptly stalled at transition forces $F_t$ in the range of 20–40 pN (Fig. 1F). Additionally, the retraction speed did not significantly vary for different counteracting forces, either in force-clamp or in position-clamp mode (Fig. S2). Fig. 1F summarizes filopodial retraction speeds of all tested cell lines regardless of the counteracting force. The average retraction speed was lower when measured in the position-clamp mode compared with the force-clamp mode for all tested cell lines. Stretching of the viscoelastic filopodia against external elastic load can account for this difference (Discussion).

Together, this suggests that whereas the absolute values of $F_t$, $v_{tp}$, and $v_f$ may be cell-line specific, the mechanics underlying filopodial retraction are conserved.

**Bead Adhesion Reduces Actin Polymerization at the Filopodial Tip, Leading to Retraction Driven by the Cortical Retrograde Flow.** To analyze the underlying actin dynamics, we performed photo-bleaching experiments on cells expressing actin-GFP. First, experiments were done in the absence of bead manipulation and by bleaching areas in the fluorescent filopodium and in the adjacent cortex (Fig. 2 A and B). The displacement of bleached areas (movement of positions b and a, Fig. 2A) as a function of time was analyzed using kymographs (Fig. 2B, Lower).

All analyzed filopodia showed retrograde flow in the actin shaft $v_a$ and in the adjacent cortex $v_c$ with identical mean velocities of $-26 \pm 1$ nm/s and $-27 \pm 2$ nm/s, respectively. In individual filopodia, the retrograde flow speed in the shaft correlated with that in the cortex (Fig. 2C). This points toward high friction between filopodial and cortical actin networks.

The speed of filopodial growth and retraction $v_{filp}$ is determined by the balance between the retrograde flow in the filopodium and the actin polymerization rate of newly incorporated actin at the filopodial tip $v_{poly}$ (19). The rate of actin polymerization corresponding to the assembly of monomers at the filopodial tip was determined from the kymographs as

$$v_{poly} = \frac{\Delta (c - b)}{\Delta t} = v_{filp} - v_f$$

for stationary, growing, or retracting filopodia (Fig. 2A, B, and D). We additionally verified that actin was newly incorporated at the tip, using two independent fluorescence markers (Fig. S3 A and B).

Based on relation [1], in filopodia with a stationary length, the tip-polymerization rate $v_{poly}$ has to exactly balance the speed of filopodial retrograde flow $v_f$ (19), which is similar to the retrograde flow in the cortex $v_c$ (Fig. 2D). When analyzing elongating and retracting filopodia, we found that the mean velocities of retrograde flow ($v_f$ and $v_c$) remained constant (Fig. 2D). Consistently, the mean polymerization rate of actin at the tip $v_{poly}$ was higher for growing filopodia and lower for retracting ones compared with filopodia with stationary length. Of note, we did not observe depolymerization at the tip ($v_{poly} \geq 0$). This suggests that the cortical retrograde flow constantly pulls on the filopodial actin shaft and that filopodia grow or retract, depending on the actin polymerization rate at their tip.

Quantification of the dynamics of individual filopodia confirmed that the cortical retrograde flow did not correlate with filopodial dynamics, whereas the actin polymerization rate at the tip did (Fig. S3 C–E). Thus, the balance between the tip polymerization rate and the cortical retrograde flow accounts for filopodial dynamics (Fig. S3 F and G), because of high friction between the cortical and filopodial actin networks.

We next tested the effect of bead adhesion on filopodial actin dynamics. Beads were approached to the tip of a filopodium emanating from actin-GFP transfected cells, using optical tweezers. We observed filopodial retraction in the force-clamp mode, while simultaneously bleaching an area in the filopodial shaft. Kymograph analysis (Fig. S4A) showed that the actin polymerization rate at the tip was lower than the retrograde flow, with values similar to those for bead-free retracting filopodia (Fig. 2E). We obtained consistent results by analyzing occasionally occurring fluorescence speckles moving backward in the filopodial shaft (Fig. S4 C and D).

Based on these data, we argue that the cortical retrograde flow constantly drives retrograde flow in the filopodial actin shaft through high frictional coupling. Filopodia elongate or retract by controlling the actin polymerization rate at the tip that is reduced upon tip adhesion to a bead.

**Actin Linkage to the Tip Membrane Limits Force Exertion.** To determine components involved in the mechanical stall and forced filopodial elongation that occurs at relatively small transition forces ($<F_t> \sim 20$ pN, Fig. 1E), we applied forces higher than $<F_t>$ while analyzing actin and membrane dynamics, using confocal fluorescence microscopy.
Fig. 3A shows a force and substrate-position trace for a bead-bound filopodium pulling against a constant force $F_0 = 7$ pN in the force-clamp mode (“before”). The filopodium was retracting with a constant velocity $v_t$ (Fig. 3A, Lower). After 160 s, the feedback force was abruptly increased to $F_t = 45$ pN, a value larger than the average transition force $<F_t>$. The filopodium instantaneously stretched over a length $\Delta x$ due to its viscoelastic properties, sustained the force during a time $\tau$, and suddenly elongated (Fig. 3A, “release”). After an elongation of 2–5 $\mu$m, the force-clamp control was turned off (Fig. 3A, $t = 4.1$ min), leading to a force relaxation toward a plateau force $F_p$. A complete detachment of the filopodium from the bead was only rarely observed (8%, $n = 163$). This sudden elongation was reminiscent of the extension dynamics of pure membrane tubes (27), indicating disconnection between the cell’s actin cytoskeleton and the membrane (Fig. S1C). To localize where this disconnection occurred, filopodia were imaged immediately before and after filopodial elongation, using different fluorescent markers (Fig. 3B). Staining with the lipophilic dye FM4-64 confirmed that the filopodial membrane remained attached to the bead and elongated after rupture. In contrast, F-actin labeling with LifeAct-RFP and Fascin-GFP showed a discontinuous distribution after the force release. A quantitative analysis of the fluorescence intensity ratios at the filopodial tip and at its base before (A/B) or following rupture (C/D) revealed a depletion of F-actin at the ruptured tip region (Fig. 3B). These observations are consistent with rupture of the linkage between the membrane and actin filaments at the filopodial tip, suggesting this linkage as the limiting factor for filopodial force application.

To quantify the strength of these links, we determined the time $\tau$ until rupture and rapid elongation occurred for multiple filopodia and for different applied forces $F_t$. Fig. 3C shows the probability distribution $P\tau(\tau)$ that rupture occurred when a force $F_t$ was exerted for a time $\tau$. When applying forces $F_t$ of 20 pN, 35 pN, and 45 pN, most filopodia (~60%) immediately elongated within less than 2 s. In the remaining cases, filopodia continued pulling and showed rupture only after several seconds, as shown in the example in Fig. 3A. For these latter filopodia, the probability of having observed rupture increased with time (Fig. 3C). In addition, the probability for inducing rupture after a defined time increases with increasing forces $F_t$. A Bell–Evans model for the rupture of multiple links explains the observed dependency of tip link stability on force and time (SI Text).

We have shown that filopodia can withstand and pull against high forces up to 50 pN for short times. Our measurements point toward the strength of the connection between actin filaments and the membrane at the tip as the limiting factor for force production.

**Ruptured Filopodia Pull with Passive Membrane Forces and an Active Load-and-Fail Mechanism.** The relative weakness of membrane–actin links at the filopodial tip highlights the importance of the plasma membrane for filopodial force exertion during retraction. In intact filopodia, where the actin cytoskeleton is connected to the bead-bound tip, discrimination between membrane- and actin-based force production is difficult because they are closely related (31). We thus analyzed ruptured filopodia to determine their relative force contributions.

After rupture and fast elongation (Fig. 3A, “after”), the remaining structures showed different force dynamics; two distinct examples are shown in Fig. 4A. In all cases, we observed a force relaxation toward an average plateau force $F_p = 13.2 \pm 0.5$ pN (Fig. 4B). In most cases (75%, $n = 131$) the force remained constant, with force fluctuations lower than 5 pN within 3 min after rupture, reminiscent of actin-free membrane tethers pulled from cells (Fig. 4A, Upper) (27). In the remaining cases, distinct load-and-fail events were observed superimposed on the force plateau (Fig. 4A, Lower).

Empty cell-membrane tubes behave as viscous fluids at low frequencies, exhibiting low elasticity as long as the cell membrane reservoir is not depleted (27, 32, 33). To determine the contribution of inward forces that are only due to the plasma membrane, we probed the elasticity of filopodial structures after forced elongation and selected those with low elastic moduli. The viscoelastic properties of ruptured filopodia were measured by imposing a series of step movements to the substrate during 2–3 s and by averaging between 5 and 50 corresponding response traces (Fig. S5 A–C). Fig. 4C shows the averaged response traces (blue) from four filopodia. Fitting the response functions gives the elasticity of the probed structure (dashed red traces in Fig. 4C and Fig. S5C). When they were measured during a force plateau (Fig. 4A, “const-f”), we observed elasticity values widely spread between zero and more than 200 pN/μm (Fig. 4D). These values varied among filopodia and variation could also be observed for a single filopodium over time (Fig. S5D). We postulate that filopodia with a low stiffness (<7 pN/μm) corresponded to ruptured filopodia with actin filaments completely disconnected from the membrane at the tip. The corresponding plateau forces $F_p(k \approx 0)$ for those filopodia are shown in Fig. 4B. The mean force $<F_p(k \approx 0)> = 15.3 \pm 0.7$ pN matches the mean force of...
control tethers pulled directly from the plasma membrane, $<F_{\text{mem}}>$ = 13.6 ± 1.4 pN. This demonstrates that the filopodial plasma membrane itself exerts forces of 15 pN after detachment from the actin shaft.

During the plateau phase, the time-averaged stiffness of these structures showed a low median value of 19 pN/μm (Fig. 4D, const-f), and thus membrane mechanics dominated. In contrast, when measuring the stiffness during active force rise events, high median values around 100 pN/μm were observed (Fig. 4D, “rise”). The pulling speed during these rise phases, $v_p = 10 \pm 2$ nm/s ($n = 28$), was similar to the pulling speed observed for nonruptured retraction filopodia (Fig. 1C), suggesting that active pulling via the retrograde flow resumed, and thus reconnection between membrane and filopodial actin filaments allowed cytoskeletal force transduction.

To elucidate how actin reconnection at the filopodial tip occurred after rupture, LifeAct-RFP fluorescence was quantified at different positions within the filopodium after extended time periods (>3 min) (Fig. 4 E and F). More than 3 min after rupture, the relative fluorescence intensity at the former tip position (“F/G”) was equivalent to that immediately after rupture (“N/D”), showing that the filopodial actin shaft did not further retract. In contrast, the relative fluorescence intensity next to the bead increased in some cases (6 of 16, “E/G”). This indicates that following rupture, increased actin polymerization rates at the tip may allow the reestablishment of the actin–membrane connection for some filopodia.

Taken together, we show that an actin-based inward force can act concomitantly with a passive and constant inward force due to the plasma membrane. The transient aspect of the active rise phases suggests that connection of the actin filaments is reestablished for short periods, until rupture occurs again, leading to a load-and-fail behavior.

**Discussion–Conclusion**

We propose a mechanical model that explains how a single filopodium exerts a pulling force via its tip (Fig. 5). We have shown that two inward forces are exerted by filopodia: a passive force via the membrane and an active cytoskeletal force produced by the retrograde flow in the cortex that is transduced via high frictional coupling to the filopodial actin shaft at its base. Such frictional coupling might also drive retrograde flow in filopodia of other cell types (19, 34), where it could account for high pulling forces up to 1 nN (4, 5). It also allows the filopodial actin filaments to exert pulling and pushing forces against the membrane at the tip, depending on the polymerization rate $v_{poly}$. The filopodial actin shaft can be seen as a viscoelastic Kelvin–Voigt material (25) with a stiffness $k_{mem}$. If the filopodium pulls against an elastic substrate, such as the bead held in the optical trap in the position-clamp mode, stretching of the actin shaft will lead to a reduced apparent pulling speed $v_{load} = \frac{1}{1 + k_{mem}/k_{eff}}$ (SI Text, Eqs. S1 and S2). In this case, $k_{eff}$ is given by the trap stiffness $k_{trap}$ that was between 60 pN/μm and 100 pN/μm in our experiments (SI Text). In the force-clamp mode the effective trap stiffness $k_{eff}$ is zero and the pulling speed reflects the balance between the negative speed of retrograde flow and the polymerization speed at the tip. Consistently we observe smaller pulling speeds $v_p$ in position-clamp mode compared with the force-clamp mode $v_{load}$ for all tested cell lines (Fig. 1F). We also determined the stiffness $k_{trap}$ of intact filopodia from HeLa cells by applying strain fluctuations (Fig. SSE). The measured value $k_{trap} = 73 \pm 22$ pN/μm ($n = 13$, Fig. SSF) accounts well for the dependency of the retraction speed on trap stiffness (SI Text).

We have shown that most filopodia cannot withstand forces higher than 20 pN because of weak connections between the actin shaft and the membrane at the tip (corresponding to $b_{\text{int}}$, Fig. 5). After disruption of these links the membrane alone can exert forces of 15 pN. This value could be an underestimation for membrane forces in intact filopodia, if the actin shaft alters the radius of the filopodium compared with the equilibrium radius of an empty membrane tube (31, 35). Relative tube radii can be deduced by analyzing the fluorescence signal of the membrane.
dye (36). Intact filopodia show a quasi-cylindrical shape (Fig. 3B, Left, “A/B”) in agreement with EM images (30). Actin-depleted tubes pulled from filopodia kept the cylindrical shape after rupture (Fig. 3B, Left, “C/D”), with similar radii to those of the actin-filled filopodia (Fig. 3B, Left, “D/B”), suggesting also membrane forces of 15 pN in intact filopodia. This implies that pulling forces higher than 15 pN are solely due to the retrograde flow of actin filaments.

Our measurements show that upon binding to a bead, filopodia retract in association with a reduction of the actin polymerization speed at the tip. Surprisingly, although mechanically stalled filopodia still show retrograde flow (Fig. S4C), we did not observe further retraction of the actin shaft after forced rupture (Fig. 4F, F/G ≈ N/D). Following mechanical rupture, the rate of actin polymerization at the tip could increase to compensate or even overcome the retrograde flow. Such behavior would point toward a control mechanism at the tip that senses rupture and adjusts the actin polymerization rate, ensuring contact between the actin shaft and the membrane.

Our paper provides evidence that actin–membrane links at the tip control the extent of cytoskeletal forces transduced to the substrate by filopodia. The nature and strength of these links may depend on the specific type of external tip adhesion to the substrate. Perturbation of surface tension in the tip and density of adhesive links to differently coated beads, but stayed below 15 pN (30). In addition, when probing filopodial dynamics and mechanics using fibronectin-coated beads that specifically bind integrins (Fig. S6A–C), we observed no drastic changes compared with COOH beads. In sharp contrast, filopodia from professional phagocytes such as macrophages can resist mechanical rupture against high forces up to 600 pN (25), arguing for strong tip connections. These filopodia can be mechanically forced to stall (16) and to slowly elongate (25), which may be due to enhanced actin polymerization at the tip (25) or due to weak frictional coupling at the filopodial base mediated, e.g., via molecular motors (16).

Our experiments point toward a sensing mechanism at the filopodial tip that allows the cell to quickly react to external mechnano-chemical signals by controlling the actin polymerization rate. A future challenge will be to identify implicated molecular players and to reveal how they help in translating chemical and mechanical signals to coordinate filopodial actin dynamics.

Materials and Methods

Cell Culture. Human cervical adenocarcinoma cells (HeLa), human embryonic kidney cells (HEK-293T), and goldfish fin fibroblast cells (CAR) were cultured as described in SI Text. Cells were plated on glass coverslips and, if mentioned, transfected using the Fugene HD (Roche) or jetPEI (Polyplus) reagents (SI Text). Before experiments, cells were rinsed three times in EM buffer (120 mM NaCl, 7 mM KCl, 1.8 mM CaCl$_2$, 0.8 mM MgCl$_2$, 5 mM glucose, and 25 mM Hepes, pH 7.3) and mounted on a microscope chamber.

Experimental Setup. Fluorescence recovery after photobleaching (FRAP) experiments without bead manipulation were performed on an Eclipse Ti confocal microscope (Nikon) equipped with a 100× objective (NA 1.4) in a 37 °C controlled environment (SI Text). Experiments with bead manipulation were performed on a Nikon TE2000 confocal microscope with a temperature-controlled objective (100×, NA 1.3) and a custom-build optical trap. The force detection and the calibration of the optical trap via the bead’s power spectrum are detailed in SI Text.

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Supporting Information

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SI Text

Mechanical Properties of the Filopodium

We model the actin shaft of a filopodium as a viscoelastic rod embedded in a highly viscous fluid that accounts for the cytoskeletal network in the lamellipodium (Fig. 5). A similar mechanistic model was already described in ref. 1 and another model was proposed to explain length regulation of structurally related stereocilia (2). The distal end of the actin shaft is connected to the membrane via cell internal mechanic links (btrap) and may also be connected directly to the microbead held in the optical trap via transmembrane receptors. The retrograde flow of the cytoskeletal network pulls the actin bundle backward, whereas actin polymerization at the tip of the filopodium elongates the actin shaft with speed vpoly and pushes the tip forward. We denote by xtrape the position of the piezo surface, by xtrap the displacement of the bead from its equilibrium position in the optical trap, and by xfilo the extension of the viscoelastic filopodium past its equilibrium length, and their velocities are denoted by $\dot{x}$.

The retrograde movement of the cytoskeletal network at a velocity $v_c$ in the cortex produces a drag force $c_{cyt}\cdot v_c$ on the filopodial actin shaft, where $c_{cyt}$ is the effective drag coefficient. Our experiments show that the retrograde flow speeds in filopodia and in lamellipodia are the same (Fig. 2 C and D). Moreover, the filopodial retraction speed below the mean transition force $F_t$ in force clamp measurements is independent of the applied force. This indicates that $c_{cyt}$ is effectively infinite at the timescales and forces of our experiments and thus that the proximal end of the actin shaft reacts with speed $v_c$ (Fig. 5). The actin shaft can be modeled as a Voigt material, and the linkers connecting it to the membrane and the microbead are assumed to be much stiffer than the actin shaft. The filopodial membrane exerts an inward force $F_{mem}$ on the distal end of the actin shaft and the optical tweezers exert an outward force $F_{trap} = k_{trap}x_{trap}$. The relaxation rate of beads in the optical tweezers is high ($\lambda_{bead} \sim 1$ kHz) compared with the relaxation rate of the filopodium ($\lambda_{filo} \sim 1$ Hz). So we can ignore the drag coefficient of the bead and assume that it is instantly displaced in the trap.

Force Clamp Measurement. When the force feedback is turned on, the system is in a quasi-steady state. The forces are constant and thus $\dot{x}_{trap} = x_{filo} = 0$. Then

$$\dot{x}_{piezo} = v_l = v_{poly} + v_{trap}; \quad [S1]$$

e.g., the filopodium retracts with constant speed if the negative and constant retrograde flow $v_{trap}$ is faster than the positive and constant tip polymerization rate $v_{poly}$. A time trace of such retraction is shown in Fig. 1B.

Position Clamp Measurement. In the position-clamp mode the piezo position is held fixed, while the filopodium pulls on the bead. Thus, the bead is displaced in the trap with a speed of

$$v_p(t) = \frac{k_{filo} + k_{trap} e^{-\lambda_{trap} t}}{k_{filo} + k_{trap}} v_l, \quad [S2]$$

where $\dot{x}_{filo} = (k_{filo} + k_{trap})/\lambda_{filo}$ is of the order of 1 Hz or higher (Fig. 4C). Because the retraction of a filopodium in the position-clamp mode typically lasts tens of seconds, the constant mean retraction speed measured is $v_p = v_l/(1 + k_{trap}/k_{filo})$. This explains the apparent difference of the retraction speeds of the filopodia when they are probed in the force- and position-clamp modes (Fig. 1F and Fig. S5).

Rheology Measurements. The experimental procedure for rheology measurements is shown in Fig. S5A for a filopodium where rupture and fast elongation occurred at $t = 5.5$ min. To probe the viscoelasticity of the elongated filopodium, 20 stepwise oscillations were imposed between $t = 6$ and $t = 8$ min (Fig. SSB). To this end the piezo stage was moved over a distance of $x_{piezo} = 100$ nm during a time $T = 0.1$ s (Fig. SSB, Lower). After a time interval of 2–3 s, the stepwise movement was repeated in the other direction. For the trace shown in Fig. SSB, the overall distance of the bead from the trap center remained roughly constant (Fig. SSB, Upper, const-f) during the imposed steps. A single response to one step displacement is noisy. To determine the average viscoelastic properties, we averaged between 5 and 50 single forward and backward step responses. Fig. SSC shows the average over the applied strain steps in gray and the averaged response function in blue. For a Kelvin–Voigt body that is retracting with speed $v$ the response is

$$x_{trap}(t) = -\frac{\kappa_{filo} + \kappa_{trap} G(T)e^{-\lambda_{trap} t}}{\kappa_{filo} + \kappa_{trap}} x_{piezo} + \frac{\kappa_{filo} + \kappa_{trap} G(t + T)}{\kappa_{filo} + \kappa_{trap}} v \cdot (t + T), \quad [S3]$$

where $g(t) = [1 - \exp(-\lambda_{trap} t)]/\lambda_{trap}$ and $t$ is the time after the piezo is stopped; i.e., the total time is $t + T$. The red traces in Fig. SSC and in Fig. 4C show fits with Eq. S3 for different stalled filopodia with $v = 0$ (const-f). Note that the response can show variations between filopodia and also with time in a single filopodium (e.g., Fig. SSB at $t = 8$ min and Fig. SSD). Fig. SSD shows an example of a filopodium exhibiting a low stiffness directly after forced elongation of 3 μm that suddenly increases to higher stiffness although the overall bead displacement stays constant (const-f). Fig. SSF summarizes the measured stiffness during different filopodial states. The values obtained for $\lambda_{filo}$ were around 4 Hz and their averages did not significantly differ between filopodial states. Together, this shows the high sensitivity of our method to detect stiffness changes. The observed stiffness changes indicate internal reconnection of actin filaments to the membrane.

Filopodia Stiffness Changes Can Account for Retraction Speed Dependency. The pulling speed in the position-clamp mode $v_p$ depends on the filopodial stiffness $\kappa_{filo}$ and the trap stiffness $\kappa_{trap}$ (Eq. S2); thus, with a constant retrograde flow speed $v_{trap}$, softer filopodia show slower pulling speed. Analysis of pulling speeds $v_p$ against counteracting forces showed a slight increase from $-7 \pm 1$ nm/s to $-10 \pm 1$ nm/s for filopodia that start pulling against 5 pN and 15 pN, respectively (Fig. 1C). We can determine the filopodial stiffness during such a first pull, before rupture occurs, by applying stepwise oscillations. An example trace is shown in Fig. S5E and the averaged stiffness over the entire first force rise is shown in Fig. S5F (first pull). The mean stiffness of different filopodia of $\kappa_{filo} = 73 \pm 22$ pN/mm is smaller than the stiffness related to active pulling of filopodia observed later on (e.g., during “rise” phases after forced elongation; Fig. S5F, rise). Thus, filopodia may stiffen with time (e.g., due to stretching out of entropic springs), which could account for the observed slower speeds during initial filopodia pulling. Such stiffening cannot
explain the slower retraction speeds \( \nu \) against high forces \((>21 \text{ pN}\)) when measured in force-clamp mode compared with small counteracting forces. However, because we inevitably filter away filopodia that have a transition force lower than 21 pN, an underlying correlation between high stability at the tip and slow retraction could explain the lower observed retraction speeds in this force region \((>21 \text{ pN})\). Alternatively, an additional serial Maxwell material could account for this. Because the measured speed dependencies are very subtle and resolvable only after extensive averaging, and due to possible statistical screening effects as mentioned above, we have used a simple Kevin–Voigt material as the less complex minimal system to describe filopodia in HeLa cells.

Stochastic Detachment of the Actin Core from the Tip Under External Load. An external load \( F \) applied to the filopodium leads to stochastic detachment of the membrane from the actin core and subsequent elongation of a membrane tube. Assuming that the actin core is attached to the membrane and the microbead through a set of \( N \) parallel molecular bonds, the detachment involves the rupture of these \( N \) bonds.

For an external load \( F \) higher than the transition force, we expect the rate of bond breaking to dominate the rebinding rate, which we thus neglect \((3)\). For \( N \) identical bonds the probability density for complete detachment at time \( t \) after the application of the constant load \( F \) is \((4)\)

\[
p_d(t) = N! \left( \prod_{i=1}^{N} r(F/i) \right)^{N} \sum_{i=1}^{N} \frac{\exp[-r(F/i)\tau]}{\prod_{j=1+1}^{N} [j! (r(F/i) - ir(F/i))]}, \tag{S4}\]

where \( r \) is the force-dependent detachment rate of a single bond under a load \( F \), i.e., the external load experienced by a single bond when \( i \) bonds are attached. Using the Bells–Evans model for bond rupture, \( r \) is given by

\[
r(F/i) = r_0 e^{-\lambda_{0} F/i} \tag{S5}\]

where \( r_0 \) is the detachment rate at zero force.

We see \( p(t) \) is a sum of exponential distributions with weights given by Eq. \( S4 \) and individual rates given by Eq. \( S5 \). In practice, multiple species of molecular linkers may be involved in the attachment and the number of linkers may differ between filopodia. Thus, in general we expect that the probability density for detachment is of the form

\[
p_d(t) = \sum_{i=1}^{N} a_i \lambda e^{-\lambda_i t}, \tag{S6}\]

with \( \sum a_i = 1 \).

Left and right censoring of the data. In practice, we cannot measure the rupture time \( \tau \) for filopodia that elongate faster than \( T_\text{start} = 100 \text{ ms} \), but detect only that they have detached during this time interval. Thus, we have left censoring of the data and for the \( M_\text{fast} \) filopodia experiencing complete detachment before \( \tau = T_\text{start} \) we know only that \( \tau < T_\text{start} \). The probability that a single filopodium detaches before \( T_\text{start} \) is

\[
P_\tau(T) = \int_{0}^{T} p_\tau(t) dt = \sum_{i=1}^{N} a_i (1 - e^{-\lambda_i T_\text{start}}). \tag{S7}\]

So the likelihood for a model given that we have measured \( M_\text{fast} \) rupture events happening before \( T_\text{start} \) and \( M - M_\text{fast} \) rupture events happening at the measured times \( (\tau)_m \) is

\[
L[\theta](\tau)_m, M_\text{fast}] = \left( \prod_{m=1}^{M_\text{fast}} \sum_{i=1}^{N} a_i \lambda e^{-\lambda_i \tau_m} \right) \times \left( \sum_{i=1}^{N} a_i (1 - e^{-\lambda_i T_\text{start}}) \right)^{M_\text{fast}}. \tag{S8}\]

At the lowest force \( F = 20 \text{ pN} \) not all filopodia elongate during the measurements’ duration \( T_\text{end} \). So we also have right censoring of the data. If there are \( M_\text{slow} \) of these filopodia, the likelihood is

\[
L[\theta](\tau)_m, M_\text{fast}, M_\text{slow}] = \left( \prod_{m=1}^{M_\text{fast}} \sum_{i=1}^{N} a_i \lambda e^{-\lambda_i \tau_m} \right) \times \left( \sum_{i=1}^{N} a_i (1 - e^{-\lambda_i T_\text{end}}) \right)^{M_\text{slow}}. \tag{S9}\]

Maximum-likelihood fit. We fit the model parameters \( \theta = (a_1, \lambda_1, a_2, \lambda_2, \ldots, a_n, \lambda_n) \) by maximizing the likelihood with respect to \( \theta \) given the experimentally measured rupture times \( (\tau)_m = (\tau_1, \tau_2, \ldots, \tau_M) \). This is equivalent to minimizing the minus-log-likelihood \(-\ln L\), which is computationally more convenient,

\[
L[\theta](\tau)_m, M_\text{fast}, (T)_m] = - \sum_{m=1}^{M_\text{fast}} \ln \left( \sum_{i=1}^{N} a_i \lambda e^{-\lambda_i \tau_m} \right) - M_\text{fast} \ln \left( \sum_{i=1}^{N} a_i (1 - e^{-\lambda_i T_\text{end}}) \right) - M_\text{slow} \ln \left( \sum_{i=1}^{N} a_i e^{-\lambda_i T_\text{end}} \right). \tag{S10}\]

Determining the best number of parameters in the fit. The number of exponential functions \( n \) in Eqs. \( S6–S10 \) that we need to include to accurately model the probability density for breaking is unknown a priori. We thus need to infer the number \( n \) of exponentials that best fit the data. This is done using a version of Akaike’s information criterion corrected for small sample size,

\[
\text{AICc} = -2 \ln L[\theta](\tau)_m, M_\text{fast}, M_\text{slow}] + \frac{2(n-1)M}{M-2n}. \tag{S11}\]

The model that minimizes AICc with respect to both \( \theta \) and \( n \) is the best fit to the data.

Fit to data. By minimizing Eq. \( S11 \) with \( n \) between 1 and 4 the best fits were obtained with two exponentials \((n = 2)\) for \( F = 20 \text{ pN} \) and 35 pN and with \( n = 3 \) for \( F = 45 \text{ pN} \) for the data shown in Fig. \( 3 C \). The dashed lines in Fig. \( 3 C \) show Eq. \( S7 \) with the fitted values for 20 pN, \( a_1 = 0.64, \lambda_1 = 1.8 \text{ s}^{-1}, a_2 = 0.37, \lambda_2 = 6.5 \text{ s}^{-1}; \) for 35 pN, \( a_1 = 0.57, \lambda_1 = 33.4 \text{ s}^{-1}, a_2 = 0.43, \lambda_2 = 4.1 \text{ s}^{-1}; \) and for 45 pN, \( a_1 = 0.49, \lambda_1 = 27.8 \text{ s}^{-1}, a_2 = 0.23, \lambda_2 = 1.97 \text{ s}^{-1}, a_3 = 0.28, \lambda_3 = 3.3 \text{ s}^{-1}. \) This shows that a Bell–Evans model for bond rupture can explain the measured probability of tip link rupture with the assumption that the number of tip links can vary between filopodia or that they are heterogeneous. This means that we do not need to postulate a strengthening of the internal linkage of filopodia bound to COOH beads when probed with higher forces. Precise control of the number and species of links should in future experiments allow us to investigate whether specific tip links lead to strengthening of the connection if higher forces are applied as one may expect for filopodia that have been shown to be precursors of focal adhesions \((5)\).

Mean time before rupture. The mean time it takes before the tip detaches from the actin shaft after application of the external force \( F \) characterizes the strength of the tip connection. From Eq. \( S6 \) we find that this mean time is equal to

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The variance of the estimate $\hat{\tau}$ is given by

$$\sigma^2(\hat{\tau}) = \sum_{i,j=1}^{n} \frac{\partial^2 \ln L}{\partial \theta_i \partial \theta_j} \bigg|_{\hat{\theta}} \sigma^2(\hat{\theta})$$

where the covariances $\sigma^2(\hat{\theta})$ of the maximum-likelihood estimates of the parameters are estimated by $\frac{\partial^2 \ln L}{\partial \theta_i \partial \theta_j} \bigg|_{\hat{\theta}}$. $J(\theta)$ is the observed Fisher information matrix with entries given by

$$J(\theta)_{ij} = \frac{\partial^2 \ln L}{\partial \theta_i \partial \theta_j} \bigg|_{\hat{\theta}}$$

The mean times before rupture for COOH- and fibronectin-coated beads are compared in the legend of Fig. S6.

**Experimental Protocol, Setup, and Data Analysis**

**Cell Culture and Transfection.** HeLa cells (American Type Culture Collection) were grown in RPMI medium (GIBCO) containing 10% (vol/vol) FCS (GIBCO) at 37 °C in a 5% CO$_2$ incubator. Goldfish fin fibroblasts cells (FishCAR; CCL71, American Type Culture Collection) were grown as described elsewhere (5). Human embryonic kidney cells (HEK-293T) were grown in DMEM (GIBCO) containing 10% FCS at 37 °C in a 10% incubator. Cells were plated 1 d before the experiment at a density of 2 × 10$^5$ cells on 22 × 22-mm glass coverslips (ESCO; Erie Scientific Company). When mentioned, cells were plated ~16 h before transfection, using the Fugene HD (Roche) or jetPEI (Polyplus) reagents following the manufacturer’s instructions, and incubated for another 8–20 h before performing measurements. Plasmids used for transfection were described elsewhere (6–8).

**Experimental Setup and Protocol for FRAP Experiments.** Actin-GFP transfected HeLa cells grown on 25-mm-diameter glass coverslips were rinsed three times in EM buffer (120 mM NaCl, 7 mM KCl, 1.8 mM CaCl$_2$, 0.8 mM MgCl$_2$, 5 mM glucose, and 25 mM Hepes, pH 7.3), mounted on a microscope chamber, put for 10 min and observed with a microscope with a 100× objective (NA 1.4), a CSU-X1 spinning disk confocal head (Yokogawa), an Evolve camera (Roper Scientific Instruments), and a fluorescence recovery after photobleaching (FRAP) module (Roper Scientific Instruments) in a 37 °C controlled environment. Images were acquired every 2 s for 10 min on a 2-μm z-section with a 200-ms exposure time, and photobleached regions in the filopodia were determined during the acquisition.

Experimental Setup and Protocol for Force Experiments. Cells grown on coverslips were rinsed three times with EM buffer. Carboxylated beads (Spherotech, polystyrene d = 2.8 or d = 1.9 μm; or Molecular Probes Europe, yellow-green fluorescent, latex, d = 2.0 μm) were added to the cells (0.01% dilution) in the same buffer. When mentioned, the membrane fluorescent dye FM4-64 (Invitrogen) was added at a final concentration of 14 μM. Samples were transferred to a custom-built microscopy observation chamber and placed onto a NikonTE2000 confocal microscope with a custom-built optical trap, using a Ytterbium fiber laser at 1,064 nm (IPG Photonics; ~160 mW at the objective), a feedback-controlled piezo stage (MadCityLabs), and a 37 °C temperature-controlled oil immersion objective (Nikon Plan Fluor 100x, NA = 1.3). The LabView-based integral-feedback loop had a response time of ~100 ms. Confocal and brightfield images, the substrate position, and the 4QD-signal data were recorded with LabView and analyzed using MatLab (MathWorks). N denotes the number of individually measured filopodia protruding from different cells. Per experiment between one and four filopodia were probed.

**Optical Trap Calibration and Force Detection.** The position of the trapped bead was detected by recording the light pattern of the outgoing trapping laser in the back focal plane of the condenser lens with a custom-built four-quadrant photodiode detection system (9). The deflection signal $x_{\text{def}}$ can be translated into bead displacement and force if the conversion values $β$ [nm/V] and $k_{\text{trap}}$ [pN/nm] are known. We determined $β$ and $k_{\text{trap}}$ by measuring the power spectrum of the beams’ thermal motion between 5 Hz and 50 kHz when the piezo stage was driven by a sinusoidal motion with amplitude of 200 nm at 25 Hz (10). We fitted the power spectrum of every bead before the experiment, using a published MatLab routine (11). Typical stiffness values of the beads’ thermal motion between 5 Hz and 50 kHz were measured with nanometer resolution but only at low frequencies. Occasional trap calibrations by detecting bead displacements using the CCD camera upon constant applied viscous drag via linear movement of the piezo stage led to trap stiffnesses consistent with those gained by analyzing the thermal noise spectrum. Undisturbed, optically trapped beads can sometimes show low-frequency (0.1–1 Hz) fluctuations of ~20 nm, limiting spatial resolution. The bead position, when determined via the bright-field image, is absolute, whereas the back focal plane detection detects bead deflections relative to the laser position (12). Comparison of these datasets allowed identifying unwanted experimental drift on very low frequencies (0.001–0.1 Hz). During the experiments the deflection signal from the four-quadrant photodiode was recorded at 1 kHz. Traces were averaged in intervals containing 10 data points, transformed to distance or force values, and displayed using MatLab.
**Fig. S1.** Elongation mechanics of filopodia. Within 1 min after binding of a bead to a filopodial tip most filopodia (75%, n = 101) start pulling and subsequently retract against a force of ~5 pN in the force feedback mode (as shown in Fig. 3A). This indicates that retraction was induced due to bead binding, because free filopodia are mostly found in a stationary state (8). Nevertheless, a different behavior was observed in a minority of cases. (A) Representative trace for the remaining 25% of cases where bead adhesion did not induce retraction. After 1.5 min without force increase during position-clamp mode, the force clamp was turned on, and the filopodium first extended against 6 pN with a slow speed (<150 nm/s) (v_{growth}) and then started retracting. Following the stepwise increase to the set force of 30 pN, the filopodium grew again for ~1 min and then ruptured, leading to fast elongation (v_{rupture}). (B) Slow filopodial growth speed (v_{growth} < 150 nm/s) vs. counteracting force observed during force-clamp measurements. Squares represent mean values of 15-pN intervals ±SD. The growth speed is constant and independent on force within our resolution, in agreement with ref. 1. (C) Fast filopodial elongation speed (v_{rupture} > 150 nm/s, "rupture events"), as a function of the set-force value applied by the feedback control. The fast elongation speed depended linearly on the set value, as expected for a feedback that pulls against a structure with no stiffness. The linear fit gave 17 pN as a force value for stall (red dashed line), in very good agreement with the membrane stall force of 15 pN. This indicates that membrane tension is the only cause for filopodial force exertion during fast elongation events.

**Fig. S2.** Retraction speed dependency on counteracting force for different cell types. Retraction speed was measured in position clamp v_p (Left, red circles) and in force clamp v_f (Right, green circles). Squares show mean values within 15-pN intervals ±SD. Different cell lines were probed: (A) HeLa cells stably transfected with LifeAct-RFP (S-LA, n = 16); (B) goldfish fin fibroblast (CAR, n = 9); (C) human embryonic kidney cell line HEK-293T (293T, n = 8). Averaging retraction speeds over all applied forces gives mean values as follows: S-LA, <v_p> = −8 ± 2 nm/s, n = 6; <v_f> = −3 ± 3 nm/s, n = 8; CAR, <v_p> = −13 ± 3 nm/s, n = 9; and HEK-293T, <v_p> = −3 ± 8 nm/s, n = 7; <v_f> = −21 ± 8 nm/s, n = 8. The mean retraction speeds of HeLa cells shown in Fig. 1F are <v_p> = −9 ± 1 nm/s, n = 129; <v_f> = −9 ± 1 nm/s, n = 29.
Fig. S3. Actin dynamics in filopodia. (A) Z-projections of a filopodium protruding from a cell transfected with LifeAct-mcherry (Upper) and GFP-actin (Lower). GFP-actin was bleached in the whole cell, and both fluorescence signals were recorded every 2 s (images correspond to −2, 0, and +20 s). (B) Kymographs taken along the filopodium shown in A. The LifeAct-mcherry signal determines the length changes of the filopodium (Upper, white dashed line, \( v_{filo} \)). Actin-GFP monomers photobleached in the cell body assemble at the tip of the filopodium and lead to a loss of fluorescence, starting from the tip and moving backward, with velocity \( v_{rf} \) (Lower, white dashed line). (Scale bars: horizontal, 20 s; vertical, 1 μm.) (Right) Merged and tilted kymographs. The LifeAct-mcherry signal is shown in red, and actin-GFP is shown in green. (C) Correlation of filopodial retrograde flow \( v_{rf} \) with filopodial dynamics\( v_{filo} \) determined as described in Fig. 2 (blue open circles) or measured with two independent fluorescent actin markers as shown in A (red circles) gives similar results. (D) Correlation of actin polymerization speed at the tip \( v_{poly} = v_{filo} + v_{rf} \) with filopodial growth or retraction speed \( v_{filo} \) determined by FRAP experiments as described in Fig. 2 (blue open circles). Open red circles show the polymerization rates measured with the two independent fluorescent actin markers and give a comparable result. The line fit to both datasets gives \( v_{poly} = 1.07 v_{filo} + 25.4 \) with a Pearson correlation of 0.88. (E) Dependency of retrograde flow in the cortex \( v_{rc} \) on filopodial growth or retraction speed \( v_{filo} \). (F) Correlation of \( v_{poly} \) (open circles) and \( v_{rc} \) (solid circles) with filopodial dynamics. The differences between \( v_{poly} \) and \( v_{rc} \) are represented by blue dashed lines. For growing and retracting filopodia, \( v_{poly} \) is higher or lower than \( v_{rc} \), respectively. (G) Balance between the polymerization rate at the tip and the negative retrograde flow speed in the cortex \( v_{poly} + v_{rc} \) plotted against the filopodial speed \( v_{filo} \) shown for individual filopodia (68 circles, \( n = 26 \)). Linear fit corresponds to \( v_{poly} + v_{rc} = 1.00 v_{filo} − 0.4 \) (red line).
Fig. S4. (A) Three successive confocal microscopy fluorescence images of a filopodium bound to a nonfluorescent bead at the tip (white circle). A spot was bleached in the filopodial shaft containing actin-GFP while the cell pulled the bead toward the cortex in the force-clamp mode. (Scale bar: 1 μm.) (Right) A kymograph taken along the filopodial axis. (Scale bars: vertical, 1 μm; horizontal, 20 s.) The actin polymerization speed at the tip \( v_{\text{poly}} = \Delta(c - b)/\Delta t \) can be directly determined from the kymographs, because the filopodial tip (position c) does not move in the force feedback mode. The cell cortex is moved by the feedback system with a speed \( v_f \). (B) Measured values for \( v_{\text{poly}} \) and \( v_f \) (n = 9). The retrograde flow of actin in the filopodial shaft was calculated via \( v_{\text{rf}} = -v_{\text{poly}} - v_f \) and is comparable to the values observed in filopodia without bound beads (Fig. 2D). (C) Image of a filopodium from a HeLa cell, stably expressing LifeAct-RFP (S-LA) in contact with a bead. (Right) Kymograph along the filopodium. (Scale bars: horizontal, 3 min; vertical, 3 μm.) The force-clamp mode was used twice at the marked intervals and filopodial retraction was observed followed by rupture and forced elongation [r]. Occasionally occurring speckles were observed moving backward with speed \( v_{\text{rf}} \) during position-clamp mode. (D) Retrograde speed of speckles \( <v_{\text{rf}} > = 40 \pm 9 \) nm/s from stably transfected HeLa cells (S-LA, n = 4) and from transiently transfected HeLa cells \( <v_{\text{rf}} > = 16 \) nm/s (HeLa, n = 2), in contact with bead. Retraction speeds \( v_f \) in force-clamp mode are given for S-LA cells \( <v_f > = 56 \pm 8 \) nm/s, n = 16) and HeLa cells \( <v_f > = 11 \pm 8 \) nm/s, n = 10). The average speed of retrograde flow determined by the speckles is faster or similar to the average speed of filopodial retraction and can together with a low tip polymerization speed account for filopodial retraction. Note that when filopodia are probed in position-clamp mode, repetitive pulling on the bead for ~400 nm with speeds slower then the retrograde flow can be observed (Fig. 4A).
**Fig. S5.** Experimental protocol to measure filopodial viscoelasticity. (A) Representative trace of a single filopodium with forced elongation at $t = 5.5$ min. (Upper) Bead displacement from the trap center; (Lower) the position of the substrate. After forced elongation ($t > 6$ min) small, 100-nm step oscillations were applied. (B) A zoom in of the marked region. (C) Average of the responses (blue) on forward and backward steps (shown as gray average). The red line is a fit of Eq. S3 with $v = 0$. (D) Exemplary trace showing a distinct change in filopodial stiffness at $t = 6.6$ min, ca. 40 s after forced elongation. (E) Representative trace where filopodial stiffness was determined directly after first contact with the bead. “First pull” denotes the first force increase due to the induced filopodial retraction, and “plateau” denotes the stall regime that follows. (F) Stiffness values determined during a force plateau phase after forced elongation (const-$f$, $n = 100$), during active pulling events after forced elongation (rise, $n = 11$), during first pulling after initial bead contact (first pull, $n = 13$), and during the stall phase following the first pull (plateau, $n = 18$). The measured stiffness value during the first pulling event of $k_{	ext{filo}} = 73 \pm 22$ pN/μm gives that the theoretically expected ratio of pulling speeds $v_p/v_f$ lies in the range of $0.42 \pm 0.07$–$0.55 \pm 0.07$. This is in agreement with the experimentally measured ratio for HeLa cells of $v_p/v_f = 0.69 \pm 0.09$ (Fig. 1F).

The last column shows stiffness values that are determined via one single stepwise force increase during the force-clamp experiment. Increasing the force by a value of $\Delta F = F_t - F_s$ leads to a stretching of the filopodia by a value $\Delta x$ (Fig. 3A). The stiffness can then be calculated via $k_{\text{filo}} = (\Delta x/\Delta F - k_{\text{OT}})^{-1}$.

**Fig. S6.** Filopodial dynamics and mechanics probed with fibronectin-coated beads. Fibronectin-coated beads were brought in contact with filopodial tips ($n = 20$), inducing retraction in 16 cases. (A) Retraction speeds in the force-feedback mode as a function of the feedback force ($<v_{\text{reret}}>$ = 14 ± 3 nm/s; $n = 16$). (B) Measured slow elongation speeds ($<100$ nm/s) for the remaining cases as well as for initially retracting filopodia that elongated after a feedback force change ($<v_{\text{growth}}>$ = 16 ± 10 nm/s; $n = 8$). (C) Probability that mechanical rupture (fast elongation $>100$ nm/s) occurred when a force of 20 pN was applied for a time $\tau$. The dashed line shows a fit of Eq. S7 with the values: $a_1 = 0.53$, $\lambda_1 = 75$ s$^{-1}$, $a_2 = 0.47$, and $\lambda_2 = 0.016$ s$^{-1}$. The mean time before elongation (Eq. S12) after application of the transition force $F_t = 20$ pN is equal to $\tau = 30 \pm 17$ s for the fibronectin-covered beads whereas it is equal to $\tau = 57 \pm 40$ s for the COOH-covered beads (Fig. 3C).