Microtubules coordinate protrusion-retraction dynamics in migrating dendritic cells

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Abstract

When traversing complex microenvironments, migrating cells extend multiple exploratory protrusions into the interstitial space. In order to preserve cell integrity and maintain polarity as the cell advances, supernumerary protrusions and tethered trailing edges need to be retracted in a coordinated fashion. Here, we demonstrate that spatially distinct microtubule dynamics regulate cell migration by locally specifying the retraction of explorative protrusions. We found that in migrating dendritic cells, local microtubule depolymerization triggers myosin II dependent contractility via the RhoA GEF Lfc. Depletion of Lfc leads to aberrant myosin localization, thereby causing two effects that rate-limit locomotion: i) defective adhesion-resolution and ii) impaired cell edge coordination during path-finding. Such compromised cell shape coordination is particularly hindering when cells navigate through geometrically complex microenvironments, where it leads to entanglement and ultimately fragmentation of the cell body. Our data demonstrate that microtubules regulate dendritic cell shape and coherence by local control of protrusion-retraction dynamics.
Introduction

Cellular trafficking is fundamental for diverse physiological processes such as morphogenesis and immune surveillance (Friedl and Wolf, 2010; Haston et al., 1982; Weijer, 2009). The active locomotion of single cells relies on the dynamic integration of cytoskeletal polymers, including actin, microtubules, and intermediate filaments. Hence their regulators have to be orchestrated in a spatiotemporally coordinated manner (Abercrombie et al., 1970; Horwitz and Parsons, 1999; Petrie and Yamada, 2012; Waterman-Storer and Salmon, 1997). During directed migration, cells adopt a polarized conformation with actin-rich lamellipodia at the leading edge and a contractile trailing edge. The actin cytoskeleton acts as the major force generator: polymerization pushes out the leading edge and actomyosin complexes provide contractility to propel the cell body and retract the trailing edge (Krause and Gautreau, 2014; Mitchison and Cramer, 1996). By contrast, the contribution of the microtubule (MT) cytoskeleton to force-generation is limited and most effects are exerted indirectly via regulation of the actomyosin system (Etienne-Manneville, 2013; Mogilner and Oster, 2003). Typically, MTs nucleate radially from microtubule organizing centers (MTOCs), with slow-growing minus ends anchored at the centrosome. Fast-growing plus ends project to the cell periphery where they undergo stochastic transitions of growth (polymerization) and shrinkage (catastrophe), referred to as dynamic instability (Brouhard, 2015; Desai and Mitchison, 1997; Mitchison and Kirschner, 1984). The MT and actin networks crosstalk at many levels, and in fibroblasts, it was shown that polymerizing MTs impede contractility but promote leading edge extension via activation of Rac (Waterman-Storer et al., 1999). Conversely, MT depolymerization triggers activation of the contractile module by regulating activators of Rho family GTPases, such as RhoA-specific guanine nucleotide exchange factors (RhoGEFs) (Ridley, 2003; Wittmann and Waterman-Storer, 2001). RhoA activates Rho-associated kinase (ROCK), leading to phosphorylation of myosin light chain (MLC), which mediates the formation of contractile actin stress fibers (Amano et al., 1996).

Leukocytes, including dendritic cells (DCs), rely on dynamic cytoskeletal rearrangements to allow for high migration velocities and rapid responses to changes in environmental cues (Sanchez-Madrid and Del Pozo, 1999; Vargas et al., 2017; Vicente-Manzanares and Sánchez-Madrid, 2004). DCs reside in peripheral tissues throughout the mammalian organism and upon pathogen encounter mature from an antigen-capturing to an antigen-presenting state. These cells become highly motile during activation, migrate along chemokine gradients towards afferent lymphatic vessels and finally reach the draining lymph node where priming of naïve T cells is initiated (Banchereau and Steinman, 1998). DCs are one of the best-developed paradigms to study amoeboid polarization, since locomotion and...
the basic patterns of actomyosin and adhesion dynamics are beginning to be understood *in vitro* and *in vivo* (Heuzé et al., 2013; Vargas et al., 2017). However, in comparison to what is known about actin dynamics during leukocyte migration, the role of MT reorganization is poorly understood (Eddy et al., 2002; Niggli, 2003; Yoo et al., 2012).

Here, we investigate MT-mediated processes that govern DC shape and motility. We describe a mechanism by which local modulation of MT dynamics controls both, de-adhesion of cell attachment sites and retraction of entangled protrusions, thereby ensuring that the advancing cell body maintains its morphological coherence.
Results

Microtubules control dendritic cell polarity

To evaluate whether MTs are required for DC migration in physiological environments, we employed mouse ear explants (Supplementary figure 1A). In this system, endogenous DCs undergo maturation, up-regulate the chemokine-receptor 7 (CCR7) and subsequently migrate along an interstitial gradient of the CCR7 ligand CCL21 towards afferent lymphatic vessels (Ohl et al., 2004; Weber et al., 2013). We treated split ears with the MT-destabilizing drug Nocodazole and determined the distance between MHCII expressing mature DCs and lymphatic vessels (Figure 1A). Compared to control conditions (DMSO), mean distances were significantly increased by Nocodazole, and the same was true when in vitro generated mature DCs were allowed to migrate into ear explants (Supplementary figure 1B), suggesting a critical role for MT depolymerization, we in vitro reconstituted DC locomotion along chemotactic gradients in a succession of reductionist environments (Supplementary figure 1A). In three dimensional (3D) collagen matrices (Sixt and Lämmermann, 2011), which largely mimic the geometrically complex dermal interstitium, both migration speed and directionality were significantly reduced upon Nocodazole treatment (Figure 1B, C, and supplementary figure 1C). Under a pad of agarose where cells are physically confined between top and bottom surfaces (“2,5D”) but have free directional choice in the plane of migration (Supplementary figure 1A) (Heit and Kubes, 2003; Lämmermann et al., 2009; Renkawitz et al., 2009), Nocodazole-treatment caused a substantial drop in directional persistence (Figure 1D, E) and cells displayed a rounded morphology and oscillatory protrusion dynamics (Figure 1F). However, velocities were largely unperturbed (Figure 1G) in the absence of filamentous tubulin (Supplementary figure 1D, E). These data suggest that MT depolymerization causes a defect in polar organization rather than actual locomotion, a phenomenon previously observed upon genetic disruption of the GTPase Cdc42, which causes DCs to “entangle” in complex 3D environments but still allows them to migrate in open spaces (Harada et al., 2012; Lämmermann et al., 2009). To challenge this presumption we placed DCs in linear channels, where a polarized shape is enforced by the 1D geometry of the environment, allowing the cells to make binary directional choices only. Here, Nocodazole treatment mildly reduced instantaneous migration velocities but caused a major defect in persistence as cells frequently switched direction (Figure 1H-J and supplementary figure 1F). Together, these data indicate that elimination of MTs preserves the locomotive ability of DCs driven by actomyosin, but causes profound changes in the polar organization that prevent efficient navigation through complex 3D environments.
**Microtubules control polar cellular organization via the contractile module**

The rounded cell morphologies and directional oscillations of Nocodazole-treated cells prompted us to measure parameters of cellular contractility. We found that Nocodazole treatment led to increased activation of RhoA (Figure 2A) and phosphorylation of MLC (Figure 2B). This effect was reversed when Rho-associated protein kinase (ROCK) activity was pharmacologically blocked using Y27632, suggesting that MTs control cell polarity by regulating contractility.

Therefore, we tested whether ROCK inhibition on Nocodazole-treated cells would rescue Nocodazole-induced migratory defects. We again employed the previously used succession of reductionist experimental setups, which allowed us to separate the impact on locomotion versus polarity. In 3D collagen gels, double-treatment with Nocodazole and Y27632 did not rescue the migratory defect and cells slowed down even more than upon Nocodazole-only treatment and often fragmented at their peripheral protrusions (Figure 2C, D and supplementary movie 1). Under agarose, the detrimental effect of Nocodazole on polarized (eccentric) cell shape was rescued, while migration velocities were still reduced (Figure 2E-G, supplementary figure 1G). Interestingly though, in 1D channels inhibition of ROCK largely rescued the oscillatory behavior and also improved locomotion speed of Nocodazole-treated cells (Figure 2H-J and supplementary movie 2). These data suggest that in DCs, global MT depolymerization causes hyperactivity of the contractile module via spatially uncontrolled activation of RhoA and ROCK, leading to oscillations of the contractile trailing edge. Blocking ROCK restores persistent locomotion in 1D channels, in which protrusion coordination is not rate-limiting for migration as the configuration of the environment extrinsically dictates cell shape. By contrast, in complex geometries, where cells need to coordinate cell edge dynamics in order to translocate efficiently (Lämmermann et al., 2009), ROCK inhibition did not rescue locomotion. These findings indicate that global MT depolymerization induces spatially uncontrolled hypercontractility and locally coordinated contractility becomes more rate-limiting for locomotion within increasing geometrical complexity of the environment.

**Polarized microtubule dynamics locally activate the contractile module**

The nocodazole-induced contraction around the entire cell perimeter led us to investigate if local MT depolymerization serves to spatially instruct contractile-events. In order to evaluate if MT dynamics are compatible with the concept of local MT depolymerization causing local retraction, we first mapped MT filaments in migrating DCs. When cells were fixed upon migration under agarose (Supplementary figure 2A, lower panel), MTs polarized along the
axis of migration with highest signal intensities in trailing edge regions (Figure 3A) and a
lower density of MTs protruding towards the leading edge (Figure 3A grey inset). Testing co-
localization between peak intensities of alpha- and gamma-tubulin revealed that the
centrosome acts as the primary MTOC (Supplementary figure 2B) and that the MTOC was
preferentially located behind the nucleus (Figure 3B). To analyze MT dynamics, we co-
transfected DCs with tubulin-GFP and end-binding protein 3 (EB3)-mCherry (Supplementary
movie 3 and supplementary figure 2C). Automated analysis of signal trajectories (Matov et
al., 2010) showed that MT growth occurs across the entire cell area (Supplementary figure
2D) and angular distribution revealed highly polarized growth along the anterior-posterior
axis (Figure 3C and supplementary figure 2E-G). Notably, back-directed MTs frequently
underwent catastrophic events (switch from growth to shrinkage) and rapidly disassembled
towards the MTOC (Figure 3D, supplementary figure 2H and supplementary movie 4). By
contrast, front-directed MTs hardly ever underwent shrinkage but instead continuously grew
into protruding areas (Figure 3D). To substantiate these findings, we stained fixed migratory
DCs for the stabilizing acetylation modification and found that front-oriented were acetylated,
but back oriented MTs had substantially lower levels of acetylation (Figure 3E), irrespective
of MTOC positioning (Supplementary Figure 2I). Together, we find increased dynamism and
high catastrophe-frequency of trailing edge directed MTs.

To directly test for a potential causal link between MT depolymerization and local activation
of the contractile module, we devised a photo-pharmacological approach to depolymerize
MTs in migratory cells with spatiotemporal control. We used Photostatin-1 (PST-1), a
reversibly photo-switchable analog of combretastatin A-4, one of the most prominent MT
inhibitors. This compound can be functionally toggled between the active and inactive state
by blue and red light, respectively (Borowiak et al., 2015). To validate the approach, we
locally activated the drug and simultaneously visualized MT dynamics using EB3-mCherry.
We found that local photo-activation triggered an almost instantaneous disappearance of the
EB3 signal in the presence but not in the absence of Photostatin (Figure 3F), indicating
immediate stalling of MT polymerization. Local photoactivation in protruding areas of the cell
consistently led to the collapse of the respective protrusion and subsequent re-polarization
(Figure 3G, H and supplementary movie 5). This response was only observed in the
presence of Photostatin, while cells were refractory to illumination in the absence of the
drug. These data demonstrate a causal relationship between MT depolymerization and
protrusion-retraction cycles. This effect can act locally within a cell, raising the possibility that
MTs coordinate subcellular retractions when cells navigate through complex environments
such as collagen gels or a physiological interstitium.
Accumulation of Lfc precedes cellular retraction

One potential link between MT depolymerization and cell contractility is the RhoA specific GEF Lfc (the murine homolog of GEF-H1). Lfc is inactive when bound to MTs, whereas MT depolymerization triggers its release and subsequent activation of the contractile module via RhoA and its effectors ROCK and MLC kinase in other cell types (Chang et al., 2008; Graessl et al., 2017; Krendel et al., 2002; Ren, 1998).

We first mapped Lfc distribution by visualizing a Lfc-GFP fusion protein (Supplementary movie 6). Immunofluorescence of alpha-tubulin in Lfc-GFP-expressing cells confirmed localization of Lfc to MTs (Krendel et al., 2002) (Supplementary figure 3A), with the highest signal in trailing edge areas (Figure 4A, purple arrowhead and B). Besides its clear filamentous appearance across the cell, Lfc-GFP accumulated as a diffuse patch in trailing edges and in retracting protrusions (Figure 4B, supplementary figure 3B and supplementary movie 6). Treatment with Nocodazole globally changed Lfc distribution from filamentous to diffuse (Supplementary figure 3C). To test whether Lfc accumulates in actively retracting areas, we determined the spatiotemporal co-localization of Lfc and MLC by imaging double-transfected cells migrating under agarose. Time course analysis revealed that both proteins are strongly polarized in trailing edge regions and at the cell center in close proximity to the nucleus during phases of cell body translocation. Correlation coefficients of Lfc and MLC in retracting areas were positive over time, indicating that locally increased Lfc levels are paralleled by increased MLC signal intensities in these regions (Figure 4C). Accordingly, quantitative analysis of signal distribution dynamics within sub-cellular compartments revealed a strong increase in Lfc signal before the onset of membrane retraction (Figure 4D). This pattern was particularly prominent when we placed DCs in “pillar forests”, in which cells - confined between two surfaces - navigate through an obstacle course of pillars along a chemokine gradient (Renkawitz et al., 2018) (Supplementary figure 1A). Here, the MTOC moved in a remarkably straight path towards the gradient, while Lfc-GFP transiently accumulated in peripheral explorative protrusions and at the trailing edge (Figure 4E and supplementary movie 7). Together, these data show that Lfc associates with MTs and locally accumulates, together with MLC, at sites of retraction.

Lfc specifies myosin localization at the trailing edge

To gain insight into the localized impact of Lfc on contractile activity, we mapped subcellular MLC dynamics over time. While MLC was largely excluded from the leading lamellipodium, two distinct pools were detectable in the cell body of wild-type cells: one at the trailing edge and one in the cell center, at the base of the lamellipodium and around the nucleus (Figure
5A). Intensities of trailing edge MLC strongly increased during fast directional migration, whereas the central pool was maintained independently of migratory speed (Figure 5B, C, supplementary figure 3D-F, and supplementary movie 8). Upon Nocodazole treatment the trailing edge MLC signal was weakened relative to the perinuclear pool, which displayed dynamic back-to-front oscillations (Figure 5D and supplementary figure 3G and supplementary movie 8).

To test for the contribution of Lfc on MLC positioning in DCs, we generated lfc-deficient mice (Supplementary figure 4A-D) and found that DCs derived from lfc^{-}\text{c}} bone marrow precursor cells differentiated and matured normally as revealed by flow cytometry of surface markers (Kamon et al., 2006) (Supplementary figure 4E, F). Lfc-deficient cells displayed a stable lamellipodium. The MTOC localization and MT distribution were indistinguishable from wild-type cells (Supplementary figure 4G-I). Strikingly, lfc^{-}\text{c}} DCs completely lacked MLC accumulation at the trailing edge but maintained the pool in the cell center (Figure 5E, F, supplementary figure 3H, I and supplementary movie 8). We measured the same localization pattern of the active form of endogenous MLC (phospho-MLC) in fixed samples (Figure 5G and supplementary figure 3J, K). Biochemical analysis revealed reduced but not eliminated RhoA and pMLC levels in lfc^{-}\text{c}} DCs (supplementary figure 4J, K). We next performed quantitative morphometry of immunostainings for moesin, the major ERM (ezrin-radixin-moesin) protein expressed in DCs and a typical component of the leukocyte trailing edge (often termed uropod). Moesin was partially lost from the trailing edge of lfc-deficient DCs (Figure 5H) and biochemical analysis confirmed reduced phospho-ERM levels (Figure 5I). Together, these data indicate that MTs control the positioning of the contractile module via Lfc and that the functional organization of the trailing edge is selectively perturbed in the absence of lfc.

**Lfc controls DC migration by regulating MT-mediated adhesion resolution**

We next measured the migratory capacity of lfc^{-}\text{c}} DCs. *In situ* migration in explanted ear sheets showed that lfc^{-}\text{c}} cells reached the lymphatic vessels later than control cells (Figure 6A) and chemotaxis of lfc^{-}\text{c}} DCs in collagen gels was substantially impaired (Figure 6B). Inhibition of ROCK led to a further drop in migratory speed in both control and lfc^{-}\text{c}} cells, arguing for additional - Lfc-independent - modes of ROCK activation. When we measured cell length in 3D collagen gels (Figure 6C, D) and under agarose (Figure 6E), migrating lfc^{-}\text{c}} DCs were significantly elongated compared to control cells, indicating retraction defects.

In cells that employ an amoeboid mode of migration, trailing edge retraction is essential in two non-exclusive ways: i) under conditions where cells adhere to surfaces, retraction forces...
are required to disassemble integrin adhesion sites and ii) in complex 3D environments the cell has to eventually retract all but one of its exploratory protrusions in order to not get stalled by entanglement (Lämmermann et al., 2009, Renkawitz et al., under revision).

We first tested the role of adhesion-resolution in under agarose assays, where, depending on the surface conditions, DCs can flexibly shift between adhesion-dependent and adhesion-independent locomotion (Renkawitz et al., 2009). Under adhesive conditions lfc\(^{-}\) DCs were elongated compared to wild-type cells (Figure 6E) and this elongation was lost when the migratory substrate at the bottom was passivated with polyethylene glycol (PEG) (Figure 6F). When cells on adhesive surfaces were treated with Nocodazole, wild-type cells shortened, as expected due to hypercontractility. Notably, lfc\(^{-}\) DCs elongated even more upon treatment with Nocodazole (Supplementary movie 9), indicating that elimination of Lfc-mediated hypercontractility unmasked additional modes of MT-mediated length control. Elongation of lfc\(^{-}\) cells by Nocodazole was also largely absent on PEG-coated surfaces. Importantly, not only morphological, but also migratory parameters were restored on passivated surfaces (Figure 6 G, H). Together, these data demonstrate that whenever DCs migrate in an adhesion-mediated manner, MTs control de-adhesion and this is partially mediated via Lfc and myosin.

**Microtubules mediate retraction of supernumerary protrusions via Lfc**

In 3D collagen gels adhesion plays a minor role (Friedl et al., 2012; Lämmermann et al., 2008; van Helvert et al., 2018). Therefore, elongation of lfc\(^{-}\) DCs suggested that cells might entangle rather than fail to de-adhere. To directly address this option, we used a microfluidic setup, in which DCs migrate in a straight channel towards a junction where the channel splits into four paths. In this setup DCs initially insert protrusions into all four channels before they retract all but one protrusion and thereby choose one path (Supplementary figure 4L, M). We could show previously that passage of the MTOC through the junction marks the time-point of retraction and that both MT depolymerization and myosin II inhibition substantially increase passage times due to retraction failure (Renkawitz et al., under revision). In line with the finding that Lfc mediates between MTs and myosin II, lfc\(^{-}\) DCs also showed increased passage times and defective retraction of supernumerary protrusions (Figure 7A, B). Notably, lfc\(^{-}\) DCs often advanced through more than one channel, ultimately resulting in auto-fragmentation into migratory cytoplasts (Figure 7C, D and supplementary movie 10). When DCs migrated through channels with single constrictions, lfc\(^{-}\) cells passed with the same speed and efficiency as wild-type cells (Figure 7E and supplementary figure 4N), demonstrating that not the actual passage through one constriction was perturbed but
rather the coordination of competing protrusions. These data indicate that in complex 3D geometries, where the cell has to choose between different paths, MTs - via Lfc and myosin II - specify entangled protrusions for retraction.

To test for possible effects of Lfc beyond adhesion resolution and protrusion coordination, we placed DCs in straight 1D channels, where neither trailing edge adhesion nor entanglement are rate-limiting. Here, the hypercontractility-caused oscillations triggered by Nocodazole (Figure 1H and 7F) were substantially rescued by knockout of Lfc (Figure 7F-H). Together, our data indicate that the major role of MT dynamics in migrating DCs is to specify sites of protrusion-retraction and that this is partially regulated by the RhoA GEF Lfc and actomyosin contraction.
Discussion

Here we report that MT depolymerization in peripheral regions of migrating DCs locally triggers actomyosin-mediated retraction via the RhoA GEF Lfc. Thereby MTs coordinate protrusion-retraction dynamics and prevent that the cell gets too long or arborized.

How different cell types maintain their typical shape and how cell types with a dynamic shape prevent losing physical coherence is poorly understood. This issue becomes particularly critical in migrating cells, where protrusion of the leading edge has to be balanced by retraction of the tail (Hind et al., 2016) and where multiple protrusions of one cell often compete for dominance, as exemplified in the split pseudopod model of chemotactic migration (Insall, 2010). The two prevalent models of how remote edges of mammalian cells communicate with each other are based on the sensing of endogenous mechanical parameters that in turn control the actomyosin system. In cell types that tightly adhere to substrates via focal adhesion complexes it has been proposed that actomyosin itself is the sensing structure and that adhesion sites communicate mechanically via actin stress fibers: when contractile stress fibers were pharmacologically, physically or genetically perturbed in mesenchymal cells, the cells lost their coherent shape and spread in an uncontrolled manner (Cai et al., 2010; Cai and Sheetz, 2009). A second model suggests that lateral plasma membrane tension, which is thought to rapidly equilibrate across the cell surface, mediates communication between competing protrusions and serves as an input system to control actomyosin dynamics (Diz-Muñoz et al., 2016; Houk et al., 2012; Keren et al., 2008; Murrell et al., 2015).

Based on our findings we propose a third model of shape control, in which MTs take the role of the shape-sensor that signals to actin dynamics. This pathway might be particularly relevant for leukocytes, as they do not develop stress fibers due to low adhesive forces and are often too large and ramified (such as DCs in 3D matrices) to allow equilibration of membrane tension across the cell body (Shi et al., 2018).

Despite being therapeutically targeted, the role of MTs in leukocytes is poorly studied. In neutrophil granulocytes and T cells, it was shown that pharmacological MT depolymerization leads to enhanced cellular polarization, owing to a hypercontractility-induced symmetry break that triggers locomotion but at the same time impairs directional persistence and chemotactic prowess (Redd et al., 2006; Takesono et al., 2010; Xu et al., 2005; Yoo et al., 2012). Although this pharmacological effect might explain the efficacy of MT depolymerizing drugs like Colchicine in the treatment of neutrophilic hyperinflammation, excessive hypercontractility overwrites any morphodynamic subtleties and leaves the question of if
MTs contribute to leukocyte navigation under physiological conditions. Our findings demonstrate that in DCs this is indeed the case and that the MT-sequestered RhoA GEF Lfc is an important mediator between MT dynamics and actomyosin driven retraction. Importantly, we show that DCs lacking both Lfc and MTs had even more severe cell shape defects than the ones lacking Lfc only. This demonstrates that Lfc and myosin II are not the only pathways and that MT depolymerization induces cell retraction via additional modes that remain to be identified.

Although it is likely that multiple feedbacks signal between actin and MTs, we show that there is a strong causal link between local MT catastrophes and cellular retraction, with MTs acting upstream. This raises the key question how MT stability is locally regulated in DCs. Among many possible inputs (adhesion, chemotactic signals etc.) one simple option might be related to the fact that in leukocytes the MTOC is the only site where substantial nucleation of MTs occurs. In complex environments (like the pillar maze we devised) the MTOC of a DC moves a remarkably straight path, while lateral protrusions constantly explore the environment (Figure 4F). In line with this, passage of the MTOC beyond an obstacle is the decisive event determining the further trajectory of the cell (Renkawitz et al, under revision). Upon passage of the MTOC, sheer geometry might dictate that lateral protrusions are cut off MT supply because the filaments are too inflexible to find their way into narrow and ramified spaces. Hence, it is possible that MTs serve as an internal explorative system of the cell that informs actomyosin whenever a peripheral protrusion locates too distant from the centroid and initiates its retraction.
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Conflict of interest

The authors declare no competing financial interests.
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Figure legends

Figure 1. Microtubules control dendritic cell polarity.

(A) *In situ* migration of endogenous DCs on a mouse ear sheet. Z-projections of separated ear sheets upon control conditions (DMSO) or pharmacological treatment (Nocodazole). Lymphatic vessels were stained for Lyve-1, DCs for MHC-II. Mean distance from lymphatic vessels of endogenous DCs was determined 48h after ear separation (lower panel). Per condition, four mouse ears with two fields of view were analyzed. Boxes extend from 25th to 75th percentile. Whiskers span minimum to maximum values. ** P ≤ 0.01. Scale bar, 100µm.

(B) Automated analysis of γ-directed migration speed within three-dimensional collagen network along soluble CCL19 gradient. Plot shows mean migration velocities over time ± S.D. from 4 experiments. Directionality during (C) three-dimensional (n = 50 cells per condition, N = 3 experiments), (D) two-dimensional (n = 50 cells per condition, N = 4 experiments) and (J) one-dimensional (n = minimum of 64 cells per condition, N = 3 experiments) migration was assessed by comparing accumulated- with euclidean-distance of manually tracked cell trajectories. Boxes extend from 25th to 75th percentile. Whiskers span minimum to maximum values **** P ≤ 0.0001 (E) Individual cell migration trajectories within two-dimensional confinement upon control (DMSO) and pharmacological (Nocodazole) treatment of n = 58 cells (DMSO) and n = 52 cells (Noco.) from N = 4 experiments. (F) Individual cell outlines over time upon control or Nocodazole-treated conditions. (G) Migration speed within two-dimensional confinement along soluble CCL19 gradient upon control (DMSO) and pharmacological (Nocodazole) treatment of n = 50 cells per condition from N = 4 experiments. Boxes extend from 25th to 75th percentile. Whiskers span minimum to maximum values. (H) Time-lapse sequence of control or Nocodazole-treated DCs migrating within a one-dimensional microchannel showing oscillatory migration behavior upon Nocodazole-treatment. (I) Migration velocities of control or Nocodazole-treated DCs migrating within a one-dimensional microchannel of n = minimum of 64 cells per condition from N = 3 experiments. Boxes extend from 25th to 75th percentile. Whiskers span minimum to maximum values. ** P ≤ 0.01. See also Figure S1.

Figure 2. Microtubules control polar cellular organization via the contractile module.

(A) Levels of active RhoA upon MT disruption with Nocodazole determined by luminometry. RhoA activity levels were normalized to Nocodazole treated samples. Plotted is mean ± S.D. from N = 3 experiments. **** P ≤ 0.0001. (B) Levels of MLC phosphorylation by Western Blot analysis. Cells were pretreated with the indicated compounds (DMSO, CCL19, CCL21,
Nocodazole, Y27632 together with Nocodazole (Y/N)). Mean fluorescence intensity of pMLC was normalized to GAPDH signal and shown as fold increase relative to DMSO control ± S.D. Blots are representative of N = 3 experiments. ** P ≤ 0.01. (C) DCs migrating within a collagen gel either non-treated (NT) or double-treated with Y27632 and Nocodazole (Y/N). Note the different time intervals per condition. (D) Automated analysis of y-directed speed of non-treated, Nocodazole-treated (Noc.) or double-treated cells using Y27632 and Nocodazole (Y/N). Plot shows mean migration velocities over time ± S.D. from N = 4 experiments. (E) Morphologies of non-treated (NT), Nocodazole- (Noc.) treated and double-treated (Y/N) cells using Y27632 and Nocodazole migrating under agarose. (F) Directionalities of non-treated (NT), Nocodazole- (Noc.) treated and double-treated cells using Y27632 and Nocodazole (Y/N) of n = 25 cells per condition from N = 3 experiments. Boxes extend from 25th to 75th percentile. Whiskers span minimum to maximum values. **** P ≤ 0.0001. (G) Migration velocities within two-dimensional confinement along soluble CCL19 gradient upon control (DMSO), Nocodazole- (Noc) and double-treatment (Y/N) with Y27632 and Nocodazole (n = 25 cells per condition from N = 3 experiments). Boxes extend from 25th to 75th percentile. Whiskers span minimum to maximum values. **** P ≤ 0.0001. (H) Time-lapse sequence of non-treated (NT) or double-treated cells using Y27632 and Nocodazole (Y/N) DCs migrating within microchannels. (I) Directionalities of non-treated (NT), Nocodazole- (Noc.) treated or double-treated (Y/N) cells using Y27632 and Nocodazole within microchannels (n = minimum of 74 cells per condition from N = 4 experiments). Boxes extend from 25th to 75th percentile. Whiskers span minimum to maximum values. *** P ≤ 0.001, **** P ≤ 0.0001. (J) Migration velocities of non-treated (NT), Nocodazole- (Noc.) treated or double-treated (Y/N) cells using Y27632 and Nocodazole within microchannels (n = minimum of 74 cells per condition from N = 4 experiments). Boxes extend from 25th to 75th percentile. Whiskers span minimum to maximum values. **** P ≤ 0.0001.

Figure 3. Polarized microtubule dynamics locally activate the contractile module.

Cells migrating under agarose along a soluble CCL19 gradient were fixed and stained for alpha-Tubulin and the nucleus (DAPI). Boxed regions indicate trailing edge (purple) or pioneering (gray) MTs towards the leading edge. Scale bar, 10µm. Right panel: Line scan of alpha-tubulin distribution along the anterior-posterior polarization axis, derived from purple line in left panel. Scale bar, 10µm. (B) Determination of MTOC position by alpha- and gamma-tubulin staining with respect to the nucleus. Mean ± S.D. of n = 256 cells from N = 3 experiments. (C) Angular distribution of automatically detected MT growth events along
anterior-posterior polarization axis. (D) MT dynamics during directed migration. Growth and shrinkage frequencies of individual MT filaments (EMTB) were assessed in protrusive (front, grey box) vs. contractile (back, purple box) areas of the same migratory cell. Growth events and catastrophes ≥1μm were tracked for n = 10 filaments in the respective region of N = 8 cells. Mean ± S.D. **** P ≤ 0.0001. Scale bar, 5μm. (E) Acetylated MTs in DCs fixed while migrating under agarose. Levels of acetylation were assessed by measuring mean fluorescence intensity of acetylated Tubulin along individual alpha-Tubulin filaments of (n = 87 filaments per condition of N = 3 experiments) directed towards the front (gray) or back (purple). Boxes extend from 25th to 75th percentile. Whiskers span minimum to maximum values. **** P ≤ 0.0001. Scale bar, 10μm. (F) EB3-mCherry localization of control or PST-1 treated cells migrating under agarose. The red box indicates photo-activated area magnified below. Magnified regions show EB3-mCherry intensities after local photo-activation. Lower panel indicates fluorescence intensity evolution upon photo-activation of control or PST-1 treated cells. The red line highlights the time point of initial photo-activation. (G) Time-lapse sequence of control or PST-1 treated cells, which were locally photo-activated (red box) during migration under agarose. (H) Kymograph analysis of the photo-activated area of (G). The time point of local photo-activation is shown in red. Right panel: Frequency of local retractions upon photo-activation of control or PST-1 treated DCs during migration (n = 26 cells per condition ± S.D. from N = 3 experiments). * P ≤ 0.05, **** P ≤ 0.0001. See also Figure S2.

Figure 4. Accumulation of Lfc precedes cellular retraction

(A) Polarized distribution of Lfc-GFP during DC migration. Shown is a maximum intensity time projection of a double-fluorescent reporter cell expressing Lfc-GFP and EB3-mCherry over 8.5min. Diffuse Lfc-GFP accumulation is highlighted in the trailing edge (purple arrowhead) and in retracting protrusions (orange arrowhead). Scale bar, 10μm. (B) Enrichment of non-filamentous Lfc-GFP or EB3-mCherry signal in the rear versus the front of migrating cells. Shown is a maximum intensity time projection over 100sec. Scale bar, 5μm. Lower panel: Relative enrichment of non-filamentous fluorescence signal intensities of Lfc-GFP and EB3-mCherry in rear versus the front of n = 16 cells per condition from N = 3 experiments. Boxes extend from 25th to 75th percentile. Whiskers span minimum to maximum values. **** P ≤ 0.0001. (C) Localization of Lfc-GFP and MLC-RFP in protrusive (front, gray box) or contractile (back, purple box) areas. Correlation of co-localization between Lfc-GFP and MLC-RFP. Hot colors in right image indicate strong co-localization of both signals, cold colors specify exclusion. Co-localization was determined separately in
actively protruding (grey box) and retracting (purple box) areas in migrating cells. Boxed regions indicate exemplary regions used for analysis of $n = 8$ cells ± S.D. Scale bar, 10µm. (D) Distribution of Lfc-GFP and MLC-RFP during a protrusion-retraction cycle. Time course of protrusion-formation and protrusion-retraction of a migrating double fluorescent reporter cell expressing Lfc-GFP and MLC-RFP. Lower panel represents fluorescence intensity evolution during the entire protrusion-retraction cycle. Scale bar 5 µm. (E) Accumulation of Lfc-GFP precedes retraction of explorative protrusions (orange arrowheads). Time-lapse sequence of an explorative protrusion-retraction cycle of a cell migrating within a complex three-dimensional pillar forest. Scale bar 10 µm. Right panel represents time projection over 16 minutes, showing Lfc accumulation in explorative protrusion (orange arrowhead) while maintaining a straight migration path according to EB3-mCherry signal distribution (lower panel).

**Figure 5. Lfc specifies myosin localization at the trailing edge.**

(A) Myosin light chain-GFP expressing DC migrating under agarose along a soluble CCL19 gradient. Central (orange box) and uropodal (purple box) MLC accumulation is outlined. Scale bar, 10µm. (B) Left panel: Scheme of quantifying relative position of MLC accumulation during directed cell migration. Right panel: Position of MLC accumulation in relation to migration speed. MLC accumulation under (C) untreated, (D) Nocodazole-treated or (E) lfc-deficient conditions. Scale bar 10µm. Middle panels indicate cell shapes over time. Right panels indicate mean MLC fluorescence distribution along the anterior-posterior polarization axis (dashed line) in 80sec intervals. (F) Quantification according to (B) showing MLC accumulation during directed migration of $lfc^{+/+}$ (red) and $lfc^{-/-}$ (blue) DCs. To account for differences in cell length the distance between cell center and MLC accumulation was normalized to cell length. Graph shows distance of $n = 7$ migratory cells per condition ± S.D. (G) Localization of endogenous phospho-MLC(S19) in fixed migratory DCs. Right panel: Quantification of fluorescence intensity in leading versus trailing edge regions of $lfc^{+/+}$ (red) and $lfc^{-/-}$ (blue) DCs of $n = 55$ cells per condition from $N = 3$ experiments. Boxes extend from $25^{th}$ to $75^{th}$ percentile. Whiskers span minimum to maximum values. **** $P \leq 0.0001$. Scale bar 10µm. (H) Localization of Moesin in fixed migratory $lfc^{+/+}$ (red) and $lfc^{-/-}$ (blue) DCs. Right panel: Quantification of fluorescence intensity in leading versus trailing edge regions of $lfc^{+/+}$ (red) and $lfc^{-/-}$ (blue) DCs of $n = 55$ cells per condition from $N = 3$ experiments. Boxes extend from $25^{th}$ to $75^{th}$ percentile. Whiskers span minimum to maximum values. *** $P \leq 0.001$, **** $P \leq 0.0001$. Scale bar 10µm. (I) Western Blot analysis of $lfc^{+/+}$ and $lfc^{-/-}$ DCs for phospho-ERM protein levels. Right panel: Quantification of pERM levels upon treatment with DMSO, CCL19, Nocodazole or Y27632.
Right panel: Increase in signal intensity relative to lfc<sup>+/−</sup> control (DMSO) conditions. Mean fluorescence intensity of pERM signal was normalized to total ERM signal and shown as fold increase relative to lfc<sup>+/−</sup> DMSO control ± S.D. of N = 3 experiments. See also Figure S3.

Figure 6. Lfc controls DC migration by regulating MT mediated adhesion resolution.

(A) In situ migration of exogenous DCs on a mouse ear sheet. Lymphatic vessels were stained for Lyve-1, DCs with TAMRA respectively. Right panel indicates the mean distance of cells from lymphatic vessels. Per experiment two mouse ears with two fields of view were analyzed of N = 4 experiments. Boxes extend from 25<sup>th</sup> to 75<sup>th</sup> percentile. Whiskers span minimum to maximum values. * P ≤ 0.05. Scale bar, 100µm. (B) Automated analysis of y-directed migration speed within a collagen network along soluble CCL19 gradient. Cells were either non-treated or treated with Y27632. Plot shows mean migration velocities over time ± S.D. from N = 7 experiments. (C) Cell outlines of lfc<sup>+/−</sup> (red) and lfc<sup>/−</sup> (blue) DCs migrating within a collagen network along a soluble CCL19 gradient. Scale bar, 10µm. (D) Lengths of cells migrating within a collagen network of n = 85 individual cells per condition from N = 4 experiments. Boxes extend from 25<sup>th</sup> to 75<sup>th</sup> percentile. Whiskers span minimum to maximum values. *** P ≤ 0.001. (E) Cell outlines of lfc<sup>+/−</sup> and lfc<sup>/−</sup> DCs migrating under agarose under adhesive conditions (FCS). Cells were either non-treated (NT) or treated with Nocodazole (Noc.) Lower panel: Cell lengths of n = minimum of 80 cells per condition from N = 5 experiments. Boxes extend from 25<sup>th</sup> to 75<sup>th</sup> percentile. Whiskers span minimum to maximum values. **** P ≤ 0.0001. Scale bar, 10µm. (F) Cell outlines of lfc<sup>+/−</sup> and lfc<sup>/−</sup> DCs migrating under agarose under non-adhesive conditions (PEG). Cells were either non-treated (NT) or treated with Nocodazole (Noc.) Lower panel: Cell lengths of n = minimum of 80 cells per condition from N = 5 experiments. Boxes extend from 25<sup>th</sup> to 75<sup>th</sup> percentile. Whiskers span minimum to maximum values. * P ≤ 0.05, **** P ≤ 0.0001. (G) Migration distance of lfc<sup>+/−</sup> and lfc<sup>/−</sup> DCs migrating under agarose under non-adhesive conditions (PEG) of n = minimum of 80 cells per condition from N = 5 experiments. Cells were either non-treated (NT) or treated with Nocodazole (Noc.). Boxes extend from 25<sup>th</sup> to 75<sup>th</sup> percentile. Whiskers span minimum to maximum values. * P ≤ 0.05, **** P ≤ 0.0001. (H) Directionality of lfc<sup>+/−</sup> and lfc<sup>/−</sup> DCs migrating under agarose under non-adhesive conditions (PEG). Cells were either non-treated (NT) or treated Nocodazole (Noc.) of n = minimum of 80 cells per condition from N = 5 experiments. Boxes extend from 25<sup>th</sup> to 75<sup>th</sup> percentile. Whiskers span minimum to maximum values. **** P ≤ 0.0001. See also Figure S4.
Figure 7. Microtubules mediate retraction of supernumerary protrusions via Lfc.

(A) Migration within a microchannel path choice assay. Graphs shows junction point passing times of $\text{lfc}^{+/+}$ (n = 79 cells of N = 3 experiments) and $\text{lfc}^{-/-}$ (n = 49 cells of N = 2 experiments) DCs. Boxes extend from 25th to 75th percentile. Whiskers span minimum to maximum values. *** P ≤ 0.001. (B) Junction point passing times depending on presence of non-competing or multiple competing protrusions per cell of $\text{lfc}^{+/+}$ (n = 37 cells of N = 3 experiments) and $\text{lfc}^{-/-}$ (n = 46 cells of N = 2 experiments) DCs. Boxes extend from 25th to 75th percentile. Whiskers span minimum to maximum values. ** P ≤ 0.01. (C) Time-lapse sequence of $\text{lfc}^{+/+}$ and $\text{lfc}^{-/-}$ DCs migrating within a path choice assay. White arrowheads in lower panel highlight cell rupturing events. (D) Frequency of cell rupturing events during path-choice decision of $\text{lfc}^{+/+}$ (n = 79 cells ± S.D. of N = 3 experiments) and $\text{lfc}^{-/-}$ (n = 52 cells ± S.D. of N = 2 experiments) DCs. (E) Migration of DCs within straight constriction-containing microchannels. Graphs shows constriction point passing times of $\text{lfc}^{+/+}$ (n = 114 cells of N = 3 experiments) and $\text{lfc}^{-/-}$ (n = 195 cells of N = 3 experiments) DCs. Boxes extend from 25th to 75th percentile. Whiskers span minimum to maximum values. (F) Time-lapse sequence of $\text{lfc}^{+/+}$ and $\text{lfc}^{-/-}$ DCs migrating within straight microchannels. (G) Directionality of cell trajectories within straight microchannels of n = minimum of 80 cells per condition from N = 5 experiments. Boxes extend from 25th to 75th percentile. Whiskers span minimum to maximum values. * P ≤ 0.05, ** P ≤ 0.01, *** P ≤ 0.001, **** P ≤ 0.0001. (H) Migration speed of $\text{lfc}^{+/+}$ and $\text{lfc}^{-/-}$ DCs within straight microchannels of n = minimum of 80 cells per condition from N = 5 experiments. Boxes extend from 25th to 75th percentile. Whiskers span minimum to maximum values. *** P ≤ 0.001.
Materials and Methods

Generation of Lfc-deficient mice

A cosmid containing the full genomic sequence of the gene that encodes Lfc (Arhgef2) was isolated from a 129 mouse genomic library with Lfc cDNA probes (106-630, 631-1057 and 1060-1478 bp) amplified by RT-PCR. The genomic DNA region between base pairs 1193-1477, coding for amino acids 351-445 in the DH domain and DH/PH domain interface was exchanged for a neomycin cassette flanked by LoxP sites. The targeting construct was linearized with NotI and electroporated into R1 ES cells. Homologous recombinants were selected in the presence of G418 (150 µg/ml) and gancyclovir (2 µM) and analyzed by Southern blotting. Positive embryonic stem cell clones were aggregated with eight cell-stage mouse embryos to generate chimeras. The resulting mice were genotyped by Southern blot and PCR. Primers (5′– CCGGGATCCATTCCGTTGTA –3′) and (5′– AAGCGGCATGGAGTTCAGGA –3′) amplified a 365-bp fragment specific for the wild type allele, whereas primers (5′– AGAGTTCTGCAGCCGCCACACCA–3′) and 5′– GGTGGGGGTGGGGTGGGATTAGATA –3′) amplified a 500-bp fragment specific for the targeted allele. We refer to these mice as lfc−/− mice throughout the entire manuscript.

Western blot analysis using a Lfc-specific antibody was performed to confirm that lfc−/− mice had no expression of Lfc protein. Mice were backcrossed to C57Bl/6 background for more than 12 generations. Dendritic cells were generated from bone marrow isolated from littermates or age-matched wildtype and lfc-deficient 8-12 week-old mice. Mice were bred and housed in accordance with institutional guidelines.

Generation of immortalized hematopoietic progenitor reporter cell lines

Hematopoietic progenitor cell lines were generated by retroviral delivery of an estrogen-regulated form of HoxB8 as described recently (Redecke et al., 2013). Briefly, bone marrow of 6-12 week of lfc+/+ and lfc−/− mice was isolated and retrovirally transduced with an estrogen-regulated form of the HoxB8 transcription factor. After expansion of immortalized cells, lentiviral spin infection (1500g, 1h) was carried out in the presence of 8µg/ml Polybrene and the lentivirus coding for fluorescent expression construct of interest. Following transduction, cells were selected for stable virus insertion using 10µg/ml Blasticidin for at least one week. Cells expressing fluorescent reporter constructs were sorted using fluorescence-activated cell sorting (FACS Aria III, BD Biosciences) prior to migration experiments.
**Dendritic cell culture**

Culture was started either from freshly isolated bone marrow of 6-12 week old mice as described earlier (Lutz et al., 1999) or from stable hematopoietic progenitor cell lines after washing out estrogen. DC differentiation was induced by plating $2 \times 10^6$ cells (bone marrow) or $2 \times 10^5$ cells (progenitor cells) in complete media (RPMI 1640 supplemented with 10% Fetal Calf Serum, 2mM L-Glutamine, 100U/ml Penicillin, 100µg/ml Streptomycin, 50µM β-Mercaptoethanol) (all purchased from Invitrogen) containing 10% Granulocyte-Monocyte colony stimulating factor (GM-CSF, supernatant from hybridoma culture). To induce maturation, cells were stimulated overnight with 200ng/ml Lipopolysaccharide from *E.coli* 0127:B8 (Sigma) and used for experiments on days 9-10.

**In situ migration assay**

Six to eight weeks old female C57BL/6J mice were sacrificed and individual ear sheets separated into dorsal and ventral halves. *Endogenous cell migration*: Cartilage free ventral halves were incubated for 48h at 37°C, 5% CO$_2$ with ventral side facing down in a well plate filled with complete medium. The medium was changed once 24h post-incubation-start. If indicated, pharmacological inhibitors were added to the medium. Ear sheets were fixed with 1% PFA followed by immersion in 0.2% Triton X-100 in PBS for 15min and three washing steps á 10min with PBS. Unspecific binding was prevented by 60min incubation in 1%BSA in PBS at room temperature. Incubation with primary rat-polyclonal antibody against LYVE-1 in combination with rat-polyclonal biotinylated anti-MHC-II antibody (both R&D Systems) was done for 2h at room temperature. After three times 10min washing with 1% BSA in PBS consecutive incubation using Alexa Fluor 488-AffiniPure F(ab')$_2$ fragment donkey anti-rat IgG (H+L) secondary antibody and streptavidin-Cy3 secondary antibody (both Jackson Immuno) was done. Samples were incubated 45min in first secondary antibody in the dark followed by 10min washing in 1% BSA in PBS and subsequent incubation with second secondary antibody. Samples mounted with ventral side up on a microscopy slide, protected with a coverslip and stored at 4°C in the dark. *Exogenous cell migration*: Cartilage free ventral ear halves were mounted on a plastic ring, followed by application of $1 \times 10^5$ exogenously differentiated DCs stained with 7µM TAMRA (Invitrogen). If indicated, cells were pretreated with pharmacological inhibitors. Cells were allowed to migrate into the tissue for 20min followed by three washing steps using complete media. Subsequently, ears were incubated in complete media for 45min at 37°C, 5% CO$_2$ before continuing with fixation and immunostaining procedure (see above).
In order to determine the distance between the lymphatic vessels and DCs a mask was created by manually outlining lymphatic vessels depending on Lyve-1 staining and segmenting cells according to their fluorescence intensity. The distance between cells and lymphatic vessels was quantified using a custom-made Matlab script, which determines the closest distance from the segmented cells to the border of the lymphatic vessel binary image. Image borders were excluded from analysis.

**In vitro collagen gel migration assay**

Custom made migration chambers were assembled by using a plastic dish containing a 17mm hole in the middle, which was covered by coverslips on each side of the hole. Three-dimensional scaffolds consisting of 1.73mg/ml bovine Collagen I were reconstituted *in vitro* by mixing 3x10^5 cells in suspension with Collagen I suspension buffered to physiological pH with Minimum Essential Medium and Sodium Bicarbonate in a 1:2 ratio. To allow polymerization of Collagen fibers, gels were incubated 1h at 37˚C, 5% CO₂. Directional cell migration was induced by overlaying the polymerized gels with 0.63µg/ml CCL19 diluted in complete media (R&D Systems). To prevent drying-out of the gels, migration chambers were sealed with Paraplast X-tra (Sigma-Aldrich). The acquisition was performed in 60sec intervals for five hours at 37˚C, 5% CO₂. Detailed description of experimental procedure can be found elsewhere (Sixt and Lämmermann, 2011).

**Analysis of y-displacement**

Quantification of y-directed migration analysis of cell population was performed as described earlier (Leithner et al., 2016). Briefly, raw data image sequences were background corrected and particles smaller and bigger than an average cell were excluded. For each time point the lateral displacement in y-direction was determined with the previous frame to generate the best overlap, which yields the y-directed migration velocity of a cell population.

**Migration within micro-fabricated polydimethylsiloxane (PDMS) based devices.**

Generation of PDMS-based devices and detailed experimental protocols can be found elsewhere (Leithner et al., 2016). Briefly, photomasks were designed using Coreldraw X18, printed on a photomask (5” square quartz, 1µm resolution, JD Photo data), followed by a spin coating step using SU-8 2005 (3000 RPM, 30sec, Microchem, USA) and a prebake of 2 min at 95˚C. The wafer was then exposed to ultra-violet light (90 – 105 mJ/cm² on an EVG mask aligner). After a post-exposure bake of 3 min 95˚C, the wafer was developed in PGMEA. A one-hour silanization with Trichloro(1H,1H,2H,2H-perfluoroctyl)siilane was
applied to the wafer. The devices were made with a 1:10 mixture of Sylgard 184 (Dow Corning). Air bubbles were removed with a desiccator. The PDMS was cured overnight at 85°C. Micro-devices were attached to ethanol cleaned coverslips after plasma cleaning for 1h at 85°C.

**In vitro under-agarose migration assay**

To obtain humid migration chambers a 17mm plastic ring was attached to a glass bottom dish using Paraplast X-tra (Sigma-Aldrich) to seal attachment site. For under-agarose migration assay, 4% Ultra Pure Agarose (Invitrogen) was mixed with phenol-free RPMI-1640 (Gibco) supplemented with 20% FCS, 1x Hanks buffered salt solution pH 7.3 in a 1:3 ratio. Ascorbic acid was added to final concentration of 50µM and a total volume of 500µl agarose-mix was cast into each migration chamber. After polymerization, a 2mm hole was punched into the agarose pad and 2.5µg/ml CCL19 (R&D Systems) was placed into the hole to generate a soluble chemokine gradient. Outer parts of the dish were filled with water followed by 30-minute equilibration at 37°C, 5% CO₂. The cell suspension was injected under agarose opposite of the chemokine hole to confine DCs between coverslip and agarose. Prior to acquisition, dishes were incubated at least two hours at 37°C, 5% CO₂ to allow recovery and persistent migration of cells. During acquisition, dishes were held under physiological conditions at 37°C and 5% CO₂.

**Immunofluorescence**

For fixation experiments a round shaped coverslip was placed in glass bottom dish before casting of agarose and injection of cells. Migrating cells were fixed by adding prewarmed 4% Para-Formaldehyde (PFA) diluted in cytoskeleton Buffer pH6.1 (10mM MES, 150mM NaCl, 5mM EGTA, 5mM Glucose, 5mM MgCl₂) directly on top of the agarose. After fixation, agarose pad was carefully removed using a coverslip-tweezer followed by 20min incubation of the coverslip in 0.5% Triton X-100 in PBS and three subsequent washing steps á 10min with Tris-buffered saline (TBS) containing 0.1% Tween-20 (Sigma). Samples were blocked to prevent unspecific binding by incubating 60min in blocking solution (5% BSA, 0.1% Tween-20 in TBS). Immunostainings were carried out consecutively by 2h incubation with rat monoclonal anti-alpha-tubulin (AbD serotec), mouse anti-phospho-Myosin light chain 2 (S19) (Cell signaling), mouse anti-gamma tubulin (Sigma) or rabbit anti-acetylated alpha-tubulin (Sigma). Followed by 3x10min washing with blocking solution and 30min incubation using Alexa Fluor® 488-AffiniPure F(ab’)₂ or Alexa Fluor® 647-AffiniPure F(ab’)₂ Fragment IgG (H+L) (both Jackson Immuno) secondary antibodies. After incubation washing was done at
least three times à 5min. Samples were conserved in non-hardening mounting medium with DAPI (VectorLaboratories) and stored at 4°C in the dark.

Immunodetection of whole cell lysates

3×10⁵ cells were serum starved for 1h followed by drug treatment. After harvesting, cell pellet was snap frozen and lysed using RIPA buffer (Cell Signaling) to which 1mM Phenylmethylsulfonylfluoride was added prior to usage. Samples were supplemented with LDS Sample Buffer and Reducing agent (both Invitrogen) and incubated for 5min at 90°C before loading on pre-cast 4-12% Bis-Tris acrylamide gel (Invitrogen). Subsequently, samples were transferred to Nitrocellulose membrane using iBlot system (Invitrogen) and blocked for 1h in 5% bovine serum albumin in TBS containing 0.01% Tween-20. For whole cell lysate protein detection following antibodies were used: rabbit anti phospho-Myosin Light Chain 2 (S19) (1:500), rabbit anti Myosin Light Chain 2 (1:500), rabbit anti GEF-H1 (the mammalian homologue of Lfc) (1:500), rabbit anti phospho-ERM (1:500), rabbit anti ERM (1:500, all Cell Signaling), mouse anti glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (1:1000, BioRad). As secondary antibodies Horseradish Peroxidase (HRP) Conjugated Anti-rabbit and anti-mouse IgG (H + L) antibodies were used in 1:5000 dilutions and enzymatic reaction was started by addition of chemoluminescent substrate for HRP (Super Signal West Femto). Chemoluminescence was acquired using a VersaDoc imaging system (BioRad). Western blot signals were quantified manually by normalization to input values and subsequent comparison of each treatment to signal intensity of steady-state level (i.e. control sample).

Flow cytometry

Before staining, 1-2×10⁶ cells were incubated for 15 min at +4°C with blocking buffer (1xPBS, 1% BSA, 2mM EDTA) containing 5mg/ml α-CD16/CD32 (2.4G2, BD Biosciences). For surface staining, cells were incubated for 30 min at 37°C with conjugated monoclonal antibodies (mAbs; mouse α-CCR7-PE (4B12), rat α-mouse I-A/I-E-eFluor450 (M5/114.15.2), hamster α-mouse CD11c-APC (N418)) diluted at the recommended concentration in blocking buffer. Flow cytometry analysis was performed on a FACS CANTO II flow cytometer (BD Biosciences).

Pharmacological inhibitors

For perturbation of cytoskeletal and myosin dynamics we used final concentrations of 300nM Nocodazole and 10µM Y27632 (all purchased from Sigma Aldrich). Nocodazole was
dissolved in dimethylsulfoxide (DMSO) and Y27632 in poly-buffered saline. Control samples were usually treated with 1:1000 DMSO if not indicated differentially.

**Fluorescent reporter constructs**

Generation of a C-terminal eGFP fusion construct of Lfc was carried out by amplifying Lfc from DC cDNA using a NotI restriction site containing forward (5’ ATATGCGGCCGCAATCTCGGATCGAATCCCTCACTCGCG 3’) and reverse (5’ ATATGCGGCCGCTTAGCTCTCTGAAGCTGTGGGCTCC 3’) primer pair. After NotI digestion, Lfc was cloned into a pcDNA3.1 backbone containing eGFP (Express Link™ T4 DNA-Ligase). Correct sequence and orientation of clones was verified by sequencing (Eurofins). The fluorescent plasmid DNA reporter construct coding for EB3-GFP was a kind gift of V. Small (IMBA, Austria). M. Olson (Beatson Institute) generously provided MLC constructs (either fused to eGFP or RFP) (Croft et al., 2005) and EMTB-3xmCherry constructs were a kind gift of (W. M. Bement, University of Wisconsin) (Miller and Bement, 2009). Gateway cloning technology™ was employed to generate lentivirus from plasmid DNA constructs. Briefly, corresponding DNA segments were amplified using primers containing overhangs with attB1 and attB2 recombination sites on the 3’- and the 5’-end respectively. In order to obtain an EMTB fusion construct carrying a single mCherry tag, the PCR product was size separated via gel electrophoresis and only the fragment of corresponding size (EMTB: 816bp, mCherry: 705bp) was further processed. Gel purified PCR fragments were inserted into pcDNA221 entry vectors (Invitrogen) via BP recombination reaction, generating the entry clone. Expression clones were obtained by carrying out the LR recombination reaction between entry clone and pLenti6.3 destination vector (Invitrogen). Lentivirus production was carried out by co-transfecting LX-293 cells (Chemicon) with the expression clone of interest in conjunction with pdelta8.9 (packaging plasmid) and pCMV-VSV-G (envelope plasmid) (plasmids were a gift from Bob Weinberg) (Stewart et al., 2003). The supernatant of virus-producing cells was harvested 72h after transfection, snap frozen and stored at -80°C after sterile filtration.

**Transgene delivery**

To induce expression of fluorescently labeled proteins DCs were transfected according to manufacturer guidelines using nucleofector kit for primary T cells (Amaxa, Lonza Group). Briefly, 5x10^6 were resuspended in 100µl reconstituted nucleofector solution, transferred to an electroporation cuvette and a total amount of 4µg plasmid DNA was added. Cells were transfected by using a protocol specifically designed for electroporating immature mouse
DCs (program X-001). After transfection, cells were cultured in 60mm cell culture dishes in complete media and taken for experiments 24h post-transfection. Due to low transfection efficiency of primary cells, transfected cells were FACS sorted prior to experiment using FACS Aria III (BD Biosciences).

**Luminometric RhoA activity assay**

RhoA activities were determined using G-LISA™ RhoA Activation Assay Biochem Kit™ (Cytoskeleton) according to the manufacturer's instructions. Briefly, 4x10^5 mature BMDCs were lysed in 70μl RIPA buffer (Cell Signaling) and protein concentration determined using the Precision Red™ Advanced Protein Assay Reagent (Cytoskeleton). Respective samples were treated with 300nM Nocodazole for 15 min before lysis. All samples were adjusted to a final protein concentration of 0.5mg/ml. Luminescence signals were measured using a microplate photometer at 600nm. Wells containing lysis buffer only were used as reference blanks in all experiments.

**Microscopy**

Low magnification bright field or DIC time-lapse acquisition was carried out using inverted routine microscopes (Leica), equipped with PAL cameras (Prosilica, Brunaby, BC) controlled by SVS-Visitek software (Seefeld, Germany). Acquisition was conducted using 4x, 10x, 20x objectives. For high magnification live cell acquisition, either an Andor spinning disc confocal scanhead installed on an inverted Axio observer microscope (Zeiss), using a C-Apochromat 63x/1.2 W Korr UV-VIS-IR objective, or a total internal reflection (TIRF) setup consisting of an inverted Axio observer microscope (Zeiss), a TIRF 488/561 nm laser system (Visitron systems) and an Evolve™ EMCCD camera (Photometrics) triggered by VisiView software (Visitron) was chosen. Photo-activation experiments were conducted on an inverted Spinning disc microscope (iMic) using a 60x/1.35 Oil objective. TAMRA stained DCs were either untreated or treated with 10µM PST-1 in the dark and recorded using a 561nm laser line in 2-second intervals. Photoactivation was carried out on directionally migrating cells using a 405 nm laser line (pixel dwell time: 10 ms, interval: 40 sec). FRAP calibration was carried out on separate samples before each experiment. During live cell imaging cells were held under physiological conditions at 37°C, 5% CO₂ in a humidified chamber. Acquisition of fixed samples (*in situ* ear crawl in and immunofluorescence samples) was carried out using an upright confocal microscope (LSM700, Zeiss) equipped with a Plan-Apochromat 20x/1.0 W DIC (UV) VIS-IR or a Plan-Apochromat 63x/1.4 Oil objective.
Statistics
All boxes in Box-Whisker plots boxes extend from 25th to 75th percentile and whiskers span minimum to maximum values. Graphs represent pooled data of several cells (n) from independent biological experiments (N) as mentioned in the figure legends. Individual experiments were validated separately and only pooled if showing the same trend. For representation of frequencies, bar charts depict mean values from several independent biological experiments (N) ± S.D. Statistical analysis was conducted out using GraphPad Prism.

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Supplementary figure legends

Supplementary figure 1. (A) Schematic representation of migration assays used in this study. Assays range from highly complex (top) and relatively uncontrollable geometries to very simple and precisely controllable PDMS-based structures (bottom). Complexity of the geometrical confinement correlates with dynamic shape changes of cells. Upward-facing arrows indicate high geometrical complexity and cell shape changes respectively. Downward-facing arrows indicate low complexity. (B) In situ migration of exogenously generated DCs on a mouse ear sheet. Shown are z-projections of separated ear sheets upon control (DMSO) or pharmacological treatment (Nocodazole). Lymphatic vessels were stained for Lyve-1, DCs with 7µM TAMRA before application on ear sheets. Pictures below represent z-stack of magnified region rotated around the Y-axis. Scale bar, 100µm in left images, 10µm in middle and right images. (C) Individual cell migration trajectories of automatically tracked DCs migrating within a 3D collagen network. Scale bar 100µm. (D) Non-treated (NT) or Nocodazole-treated cells migrating under agarose were fixed and stained for endogenous distribution of alpha-tubulin. Scale bar 10µm. (E) Time-lapse montage of a Nocodazole-treated Tubulin-GFP and membrane-targeted tdTomato expressing cell under agarose. Scale bar 10µm. (F) Kymograph of DCs migrating within a straight microchannel. Cells were either untreated (NT) or Nocodazole (Noco.) treated. Scale bar 10µm. (F) Cell lengths of non-treated (NT), Nocodazole (Noco.) treated and double-treated cells using Y27632 and Nocodazole (Y/N) of n = 25 cells per condition from N = 3 experiments. Boxes extend from 25th to 75th percentile. Whiskers span minimum to maximum values. **** P ≤ 0.0001.

Supplementary figure 2. (A) Time-lapse montage of cells migrating along a CCL19 gradient when confined under agarose (top panel) and when fixed with 4% PFA during migration (bottom panel). Small panels below image sequences are montages of black-boxed regions over time to visualize advancement of the leading edge. The red dotted line represents the cell leading edge. Scale bar, 10µm. (B) Determination of MTOC position by alpha- and gamma-tubulin staining (left panels). MT nucleation from centrosomal origin visualized by line scan of signal intensities along purple line in merged image (right panel). Scale bar, 10µm. (C) Still images of a cell expressing EB3-mCherry and tubulin-GFP migrating under agarose. Scale bar 10µm. Right panel highlights localization of EB3 signal at the tip of polymerizing tubulin filaments as the cell advances. Scale bar 5µm. (D) Automatically detected EB3 comets (cyan) overlaid on maximum intensity time projection (120 sec) of an EB3-mCherry expressing cell migrating under agarose. Lower panel:
Quantification of MT growth events of front (gray) vs. back (purple) directed MT tracks over a time period of 120 sec of n = 7 cells. Boxes extend from 25th to 75th percentile. Whiskers span minimum to maximum values. Scale bar, 10 µm. (E) MT growth dynamics in protrusive (gray box) vs. contractile (purple box) areas. Scale bar, 10 µm. Lower panel: Differential EB3-mCherry fluorescence intensities in protrusive (front) vs. contractile (back) areas of n = 16 cells from N = 3 experiments. **** P ≤ 0.0001. (F) Quantification of 2D projected cell area of polarized DCs expressing membrane-targeted tdTomato. Boxes extend from 25th to 75th percentile and whiskers span minimum to maximum values of n = 13 cells. *** P ≤ 0.001. (G) Quantification of EB3 growth events relative to the cell area. Mean ± S.D. of n = 7 cells. (H) Time-course analysis of MT filament dynamics of migrating DCs expressing EMTB-mCherry. Upper panel indicates leading edge area. The white arrow represents membrane protrusion and the green arrowhead represents elongating MT filaments. Lower panel indicates trailing edge area in which purple arrow represents membrane retraction and purple arrowheads MT filament depolymerization. Red line represents cell edges. Scale bar, 10 µm. (I) Acetylated MTs in fixed migratory. Levels of acetylation were assessed by measuring mean fluorescence intensity of acetylated Tubulin along individual alpha-Tubulin filaments of (n = XY filaments per condition of N = 3 experiments) directed towards the front (gray) or back (purple). Boxes extend from 25th to 75th percentile. Whiskers span minimum to maximum values. **** P ≤ 0.0001. Scale bar, 10 µm.

Supplementary figure 3. (A) Co-localization of Lfc-GFP on alpha-tubulin structures. A Lfc-GFP expressing cells was fixed while migrating under agarose and stained for alpha-tubulin distribution. Scale bar, 10 µm. (B) Polarized distribution of Lfc-GFP in trailing edges and retracting protrusions. A double reporter cell expressing Lfc-GFP and EB3-mCherry was followed while migrating under agarose. Purple arrowhead denotes trailing edge, orange arrowhead highlights retracting protrusion followed by cell repolarization. Scale bar 10 µm. (C) Lfc-GFP distribution upon Nocodazole treatment. A Nocodazole-treated double fluorescent reporter cell was followed while migrating under agarose. Note the absence of filamentous structures in both channels and the strong diffuse signal distribution of Lfc-GFP. Scale bar 10 µm. (D) Time-lapse montage of a MLC-GFP expressing DC migrating under agarose towards a soluble CCL19 gradient. A cycle of migration, retraction, and pausing is shown. Scale bar, 10 µm. Dotted lines indicate positions further analyzed by Kymograph in (E). (E) Leading edge kymograph was derived from grey dotted line in leading edge region of (D). Trailing edge kymograph was derived from red dotted line in trailing edge region of (D). Scale bar, 5 µm. (F) Time-lapse sequence showing spatiotemporal MLC accumulation of
Supplementary figure 4. (A) Integration of the lfc targeting vector into genomic locus. Black boxes represent exons. The neo-lox P cassette was cloned in reverse orientation into two, replacing a Smal-Xhol segment. Locations of primers used for PCR are indicated with triangles. Probes A and B were used for Southern blot detection of short and long arms, respectively. S, Smal; Xh, Xhol; X, XbaI: N, Nhel (B) Southern blot analysis. Genomic DNA from lfc+/+, lfc+/ and lfc−/− mice was digested with XbaI and hybridized with probes B (left panel) and genomic DNA from lfc+/+ and lfc−/− embryonic stem cells were hybridized with probe A (right panel). (C) PCR analysis of tail DNA from lfc+/+, lfc+/ and lfc−/− mice. Locations of primers used for PCR are indicated with triangles in (A). (D) Immunoblot analysis of total thymus cell lysates probed for Lfc protein content. (E) Cell morphologies of immature (NT) and mature (+LPS) lfc wildtype (upper-lane) and lfc-deficient (lower-lane) littermate DCs. Note the presence of multiple veils in both LPS-treated samples. (F) DC differentiation markers (MHC-II and CCR7) of lfc wildtype (blue line) and lfc-deficient (red line) littermate DCs compared to unstained cells (grey peak). (G) Endogenous MT organization in lfc−/− DCs. Cells migrating under agarose were fixed and stained for alpha- (left) and gamma-tubulin (middle). Scale bar, 10µm. (H) Line scans across the highest gamma-tubulin signal along the left-right axis (dashed line in (G)). The purple line indicates gamma-tubulin signal intensity. The black line indicates alpha-tubulin signal distribution. (I) Quantification of centrosome localization in lfc-deficient DCs of n = 117 cells from N = 2 experiments. (J) Levels of active RhoA upon MT disruption with Nocodazole in wildtype (lfc+/+) and lfc-deficient cells (lfc−/−) determined by luminometry. Activity levels are normalized to Nocodazole-treated wildtype samples, showing mean intensities ± S.D. from N = 3 experiments. **** P ≤ 0.0001. (K) Levels of MLC phosphorylation in lfc+/+ and lfc−/− DCs assessed by Western Blot analysis. Cells were pretreated with the indicated compounds
(DMSO, CCL19, CCL21, Nocodazole, Y27632 together with Nocodazole). Mean fluorescence intensity of phospho-MLC was normalized to GAPDH signal and shown as fold increase relative to DMSO control ± S.D. Blots are representative of N = 3 experiments. (L) Path choice preference of Ifc<sup>+/−</sup> and Ifc<sup>−/−</sup> DCs migrating within a complex path choice assay. Shown are mean frequencies of Ifc<sup>−/−</sup> n = 49 cells of N = 2 experiments and Ifc<sup>+/−</sup> n = 79 cells of N = 3 experiments. (M) Centrosome position with respect to nucleus of Ifc<sup>−/−</sup> DCs migrating within a path choice assay. Shown are mean frequencies of Ifc<sup>−/−</sup> n = 49 cells of N = 2 experiments. (N) Frequencies of cell rupturing events of Ifc<sup>+/−</sup> (n = 73 cells, N = 3 experiments) and Ifc<sup>−/−</sup> (n = 128 cells, N = 3 experiments) DCs migrating within a simple 1D single constriction channel.
Supplementary movie legends

Supplementary movie 1. Perturbation of MT and myosin dynamics impairs DC migration in complex 3D scaffolds. Mature DCs migrating along a soluble CCL19 gradient within a 3D collagen matrix. Shown are separately acquired bright field movies of control-(DMSO), Nocodazole-treated and double-treated cells using Y27632 and Nocodazole (Y/N) reconstructed in a single file. Images were acquired every 60sec for 5h and are represented as single movie in 4min intervals. Time in [min:sec]. Scale bar, 100µm for representative movie of bulk cell movement, scale bar, 10µm for movie showing single cell dynamics.

Supplementary movie 2. Perturbation of MT and myosin dynamics permits DC migration within simple 1D microenvironments. Mature DCs migrating along a soluble CCL19 gradient within a 1D microchannel. Shown are separately acquired bright field movies of non-treated, Nocodazole-treated and double-treated cells using Y27632 and Nocodazole cells reconstructed in a single file. Images were acquired in 20sec intervals for 5h. Note the frequent directional oscillations of Nocodazole only treated cells. Time in [min:sec]. Scale bar, 10µm.

Supplementary movie 3. MT dynamics in migratory DCs. Mature DC co-expressing EB3-mCherry and tubulin-GFP. Migration of a double fluorescent cell along a soluble CCL19 gradient during 2D confinement under agarose was acquired in 0.5sec intervals using an inverted spinning disc microscope setup. Time in [min:sec]. Scale bar, 10µm.

Supplementary movie 4. MT dynamics are polarized in migratory DCs. DC is expressing EMTB-mCherry. Migration during 2D confinement under agarose was acquired in 2sec intervals using a TIRF setup. For representation, the signal was inverted after the acquisition. The upper panel shows the protruding leading edge, in which grey arrowheads indicate elongating MT filaments. The lower panel shows retracting trailing edge of the same cell in which purple arrowheads highlight MT shrinking events. Time in [min:sec]. Scale bar, 5µm.

Supplementary movie 5. Polarized MT dynamics locally activate the contractile module. TAMRA stained DCs migrating under agarose were recorded every 2sec on an inverted spinning disc microscope and locally photo-activated (red box) every 40sec using a 405nm laser line. Cells were either untreated (upper panel) or treated with PST-1 (lower panel). Time in [min:sec]. Scale bar 10µm.
Supplementary movie 6. Polarized Lfc-GFP distribution from filamentous to diffuse along anterior-posterior polarization axis. A double fluorescent Lfc-GFP and EB3-mCherry reporter cell was acquired while migrating under agarose towards a soluble CCL19 gradient in 2sec intervals on an inverted spinning disc microscope. For better visualization, the signal was inverted after the acquisition. Purple arrowhead highlights persistent diffuse trailing edge Lfc-GFP accumulation during directed migration. Orange arrowhead indicates Lfc-GFP signal distribution upon protrusion-retraction. Black arrowhead indicates filamentous Lfc-GFP signal distribution in protruding areas after repolarization. Time in [min:sec]. Scale bar, 10µm.

Supplementary movie 7. Lfc-GFP signal accumulation defines retraction of explorative protrusions. A double fluorescent Lfc-GFP and EB3-mCherry reporter cell was acquired while migrating within a complex 3D pillar array towards a soluble CCL19 gradient in 2sec intervals on an inverted spinning disc microscope. Diffuse Lfc-GFP signal accumulation is prominent in the trailing edge (purple arrowhead) and the explorative protrusion (orange arrowhead), while the MTOC moves in a straight path towards the top. Time in [min:sec]. Scale bar, 10µm.

Supplementary movie 8. Lfc specifies myosin localization at the trailing edge. Combined movies of MLC-GFP expressing $lfc^{+/+}$ DCs (left panel), Nocodazole-treated $lfc^{+/+}$ DCs (middle panel) and $lfc^{-/-}$ DCs (right panel) migrating under agarose along a soluble CCL19 gradient, acquired in 2sec intervals on an inverted spinning disc microscope. Purple arrowheads indicate trailing edge MLC accumulation, which is absent in $lfc$-deficient cells. Orange arrowheads highlight central MLC accumulation. Note the different time intervals of Nocodazole-treated $lfc^{+/+}$ DCs (middle panel). Fluorescence signal was color coded (LUT: Fire). Time in [min:sec]. Scale bar 10µm.

Supplementary movie 9. Lfc regulates MT-mediated adhesion resolution. Nocodazole-treated $lfc^{+/+}$ and $lfc^{-/-}$ DCs were acquired while migrating under agarose towards a soluble CCL19 gradient in 20 second intervals on an inverted cell culture microscope. Left panels represent Nocodazole effects on adhesive migration. Note the loss of directionality in $lfc^{+/+}$ DCs and the pronounced elongation of $lfc^{-/-}$ DCs. Right panels highlight Nocodazole effects during adhesion-independent migration on PEG coated coverslips. Note the persistent loss
of directionality in \( lfc^{+/+} \) DCs but the restored cell lengths of \( lfc^{-/-} \) DCs. Time in [min:sec].

Scale bar 100\( \mu \)m.

Supplementary movie 10. Microtubules mediate retraction of supernumerary protrusions via Lfc. \( Lfc^{+/+} \) and \( lfc^{-/-} \) DCs were recorded while migrating within a decision assay towards a soluble CCL19 gradient in 30sec intervals. Note that both genotypes insert multiple protrusions into different channels when reaching the junction point (black arrowheads). Red arrowheads highlight rupturing events and loss of cellular coherence only observed in \( lfc \)-deficient cells. Time in [min:sec]. Scale bar, 10\( \mu \)m.
Microtubules coordinate protrusion-retraction dynamics in migrating dendritic cells

Figure 1 - Microtubules control dendritic cells polarity
Microtubules coordinate protrusion-retraction dynamics in migrating dendritic cells

Figure 2 - Microtubules control polar cellular organization via the contractile module
Microtubules coordinate protrusion-retraction dynamics in migrating dendritic cells

Figure 3 - Polarized microtubule dynamics locally activate the contractile module
Microtubules coordinate protrusion-retraction dynamics in migrating dendritic cells

Figure 4 - Accumulation of Lfc precedes cellular retraction
Microtubules coordinate protrusion-retraction dynamics in migrating dendritic cells

Figure 5 - Lfc specifies myosin localization at the trailing edge
Microtubules coordinate protrusion-retraction dynamics in migrating dendritic cells

**Figure 6** - Lfc controls dendritic cell migration by regulating MT-mediated adhesion resolution
Microtubules coordinate protrusion-retraction dynamics in migrating dendritic cells

Figure 7 - Microtubules mediate retraction of supernumerary protrusions via Lfc
Supplementary Figure 1

|        | confinement | complexity | shape change |
|--------|-------------|------------|--------------|
| in situ| ↑           | ↑          | ↑            |
| 3D - Col| ↑           | ↑          | ↑            |
| 3D - Pillar| ↑     | ↑          | ↑            |
| 3D - Path | ↑       | ↑          | ↑            |
| 2D     | ↓           | ↓          | ↓            |
| 1D     | ↓           | ↓          | ↓            |

**B**

DMSO

Nocodazole

**C**

DMSO

Nocodazole

**D**

alpha-Tubulin, Actin, a-Tub., DAPI

**F**

NT

+Nocodazole

**G**

distance vs. cell length

**E**

Tubulin-GFP

membrane

+ Nocodazole
Microtubules coordinate protrusion-retraction dynamics in migrating dendritic cells

Supplementary Figure 2

A 2D

B
gamma-Tub. alpha-Tub. merged

C EB3-mcherry Tubulin-GFP merged

D tracked comets (EB3)

E MT growth (EB3), membrane (tdTomato)

F [number of growth events]

G MT growth (EB3), membrane (tdTomato)

H EB3-MFI [a.u.]

I acetyl tubulin alpha Tubulin

J MTOC in front of nucleus

K MTOC in front of nucleus
Microtubules coordinate protrusion-retraction dynamics in migrating dendritic cells

Supplementary Figure 3

A  Lfc-GFP  aTub-DL549  B  Ufc-GFP  EB3-mcherry

C  +Nocodazole  

D  MLC-GFP

E  leading  trailing

F  MLC-GFP

G  Lfc +/+ +Noc.

H  Lfc -/-

I  MLC-GFP

J  pMLC along polarization axis

K  pMLC in MLC expressing cells

% of Max

| MLC-GFP | % of Max |
|---------|---------|
| Lfc +/+ | red     |
| Lfc -/- | blue    |

Migration, protrusion, retraction, stalled

pMLC in MLC expressing cells

Normalized distance

Lfc +/+, Lfc -/-
Microtubules coordinate protrusion-retraction dynamics in migrating dendritic cells

Supplementary Figure 4

A. Lfc protein domains

- Wild type allele
- Targeting vector
- Targeted allele

B. 

C. 

D. 

E. NT | +LPS

F. MHC Class II

G. 

H. 

I. 

J. RhoA activity

K. 

L. 3D - Path

M. MTOC position

N. 1D - Path