INTRODUCTION

A large library of actin-associated proteins steer nucleation, cross-linking, capping, and elongation of actin filaments. The precise spatial and temporal co-ordination of these functions is fundamental for the movement of cells that is required for many biological events ranging from organ development to tissue repair. The importance of actin cytoskeleton dynamics

Abbreviations: Arp2/3, actin-related protein 2/3; DC-SIGN, dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin; EB1, end-binding protein 1; FRAP, fluorescence recovery after photobleaching; FHOD1, formin homology domain-containing 1; fMLP, formyl-methionyl-leucyl-phenylalanine; gp120, envelope glycoprotein 120; KD, knockdown; LSP1, leukocyte-specific protein 1; MK2, mitogen-activated protein (MAP) kinase-activated protein kinase 2; PFA, paraformaldehyde; PKC, protein kinase C; SBS, SH3-binding site; SH3, Src-homology 3; TIRF, total internal reflection fluorescence; WASP, Wiskott-Aldrich Syndrome protein.

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LSP1-myosin1e bimolecular complex regulates focal adhesion dynamics and cell migration

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Abstract
Several cytoskeleton-associated proteins and signaling pathways work in concert to regulate actin cytoskeleton remodeling, cell adhesion, and migration. Although the leukocyte-specific protein 1 (LSP1) has been shown to interact with the actin cytoskeleton, its function in the regulation of actin cytoskeleton dynamics is, as yet, not fully understood. We have recently demonstrated that the bimolecular complex between LSP1 and myosin1e controls actin cytoskeleton remodeling during phagocytosis. In this study, we show that LSP1 downregulation severely impairs cell migration, lamellipodia formation, and focal adhesion dynamics in macrophages. Inhibition of the interaction between LSP1 and myosin1e also impairs these processes resulting in poorly motile cells, which are characterized by few and small lamellipodia. Furthermore, cells in which LSP1-myosin1e interaction is inhibited are typically associated with inefficient focal adhesion turnover. Collectively, our findings show that the LSP1-myosin1e bimolecular complex plays a pivotal role in the regulation of actin cytoskeleton remodeling and focal adhesion dynamics required for cell migration.

KEYWORDS
Actin cytoskeleton remodeling, focal adhesions, cell motility, macrophages
is emphasized by the onset and progress of diseases due to cells lacking or expressing mutated variants of actin-associated proteins.\(^1,2\) In spite of several studies, the functions of some actin-associated proteins have not been well defined. One of such proteins is the leukocyte-specific protein 1 (LSP1). LSP1 is expressed in several cell types of the immune system such as T-cells, B-cells, macrophages, and neutrophils. It is also expressed in myeloid and lymphoid cell lines and, despite its name, in endothelial cells.\(^3,9\)

The amino-terminal half of LSP1 incorporates \(Ca^{2+}\)-binding sites and a coiled-coil region.\(^5,10\) suggesting that \(Ca^{2+}\) signaling and dimerization could regulate LSP1 function. The carboxy-terminal half incorporates a caldesmon-like region having a weaker F-actin-binding activity \(^11,12\) and two villin headpiece-like sequences, which primarily mediate the interaction of LSP1 with F-actin.\(^12,14\) We have demonstrated that the carboxy-terminal half of LSP1 directly interacts with the Src-homology 3 (SH3) domain of the molecular motor myosin 1e through the non-canonical SH3-binding site AGDMSKKS.\(^7\) These studies suggest that LSP1 may be involved in the regulation of actin cytoskeleton architecture and dynamics. Indeed, the actin-binding activity of LSP1 is required for the formation of the long, actin-rich cell projections that develop in a wide-ranging variety of cells, which overexpress LSP1.\(^12,15,16\) We have provided a direct evidence that LSP1 regulates actin cytoskeleton dynamics. We found that LSP1 localization and dynamics at the internalization sites during Fc\(\gamma\) receptor-mediated phagocytosis, a process that depends on actin dynamics, spatially and temporally overlap with that of the actin cytoskeleton.\(^7\) Moreover, in LSP1-deficient macrophages and in macrophages in which LSP1-myosin 1e or LSP1-actin interactions are inhibited, Fc\(\gamma\) receptor-mediated phagocytosis is severely reduced.\(^7\) Given the modulation of actin dynamics by LSP1, it is not surprising that LSP1 has been implicated in the regulation of migration of several cell types including neutrophils, dendritic cells, and T-cells.\(^3,15,17,22\)

Although these studies clearly show that LSP1 is involved in the regulation of actin cytoskeleton structural organization and dynamics, the molecular mechanisms underlying the function of this actin-associated protein are still poorly characterized. Current evidence shows that LSP1 is phosphorylated at serine and threonine sites.\(^23-28\) In lymphocytes, LSP1 is phosphorylated by protein kinase C (PKC).\(^25,26,29\) whereas in neutrophils stimulated with the chemotactant formyl-methionyl-leucyl-phenylalanine (fMLP), LSP1 is phosphorylated by the mitogen-activated protein (MAP) kinase-activated protein kinase 2 (MK2).\(^27,28\) Notably, PKC-dependent phosphorylation of LSP1 decreases its localization with the plasma membrane and the actin cytoskeleton.\(^16,29\) In contrast, LSP1 phosphorylated by MK2 results in the accumulation of phosphorylated LSP1 at the leading edge of neutrophils.\(^28\) The importance of the interaction between kinases and LSP1 is further supported by the observation that LSP1 targets proteins of the extracellular signal-regulated kinase/microtubule-associated protein kinase pathway to the actin cytoskeleton.\(^30\) Thus, it is plausible that the localization of LSP1 to actin-rich structures depends on its phosphorylation status and can be regulated by diverse kinases and signaling pathways.

Regardless the positive or negative regulation of cell migration, it is unquestionable that LSP1 controls this important biological process. In contrast, very little is known about the molecular mechanisms underlying this LSP1 function. For instance, it has been shown that LSP1 participates in a complex with Wiskott-Aldrich Syndrome protein (WASP) and the actin-related protein 2/3 (Arp2/3) complex,\(^31\) two important regulators of actin filament nucleation. Furthermore, LSP1 can also be found in a complex together myosin IIA and one of its regulators, the myosin light chain kinase.\(^32\) Since LSP1 does not directly interact with WASP, the Arp2/3 complex and myosin IIA, it is likely that LSP1 is recruited to these complexes via its interaction with F-actin. Notably, we have demonstrated that LSP1 binds to the SH3 domain of myosin 1e and that this bimolecular complex is essential for efficient actin cytoskeleton dynamics during Fc\(\gamma\) receptor-mediated phagocytosis.\(^7\)

In this study, we have added another piece to the puzzle describing the modus operandi of LSP1. We have demonstrated that the interaction of LSP1 with myosin 1e is essential for efficient focal adhesion dynamics and zyxin kinetics at these locations. The LSP1-myo1e binary complex also regulates lamellipodia formation and dynamics. Consequently, interfering with LSP1-myo1e interaction impaired cell migration.

## 2 MATERIALS AND METHODS

### 2.1 Cell culture

Wild-type and genetically modified J774 macrophage cell lines were grown in DMEM supplemented with 10% fetal calf serum (FCS), 4 mM of L-glutamine, 100 \(\mu\)g/mL of streptomycin, and 100 U/mL of penicillin. The packaging cell line 293T (CRL 11268; ATCC) was grown in DMEM high glucose supplemented with 10% FCS, 2 mM of L-glutamine, 1 mM of sodium pyruvate, 100 \(\mu\)g/mL of streptomycin, and 100 U/mL of penicillin. All cell lines were grown at 37°C and 5% \(CO_2\).

### 2.2 Cloning and generation of genetically modified J774 cells

To generate pWPXL-RFP-zyxin, the coding sequence of zyxin was amplified with the following primer pair:
forward 5'-GCTTCCAATTCCATGGCGGCGCCCCCGCC
CGTCT-3' (containing a EcoRI site) and reverse 5'-CTC
GAGGATCTCCAGGTCTGGGCTCTAGCAGTGT
GCGA-3' (containing a BamHI site) using pMSCV-RFP-Zyxin as the template. The amplified product was then cloned into the EcoRI and BamHI sites of pWPXL-RFP.7 Turquoise-zyxin was cloned as described. The coding sequence of turquoise was amplified from pLL3.7-mTurquoise2-SLBP (18-126)-IRES-H1-mMaroon1 (Addgene vector no. 83842) using the following primer pair: forward 5'-CGTTTTAACCAGGAATGAGCAACGGGCAG-3'
(containing a Pmel site) and reverse 5'-GCAGCGAATT
CCCTCCCAGGGAACGCAACATTGAGTA-3' (containing an EcoRI site). The amplified product was then cloned into pWPXL-RFP-Zyxin after excision of RFP using Pmel and EcoRI to generate pWPXL-Turquoise-zyxin. Both RFP-zyxin and turquoise-zyxin were sequenced to verify the accuracy of the cloning procedure. To generate cells expressing RFP-zyxin or turquoise-zyxin, wild-type, and LSP1-KD J774 cells were transduced with lentiviruses carrying the RFP-zyxin gene, whereas J774 cells expressing the deletion mutant LSP1-ΔSH3-binding site (SBS) or full-length LSP1 were transduced with lentiviruses carrying the turquoise-zyxin gene. Generation of lentiviruses and transduction of cells were done as already described.7

2.3 | Immunofluorescence and live cell imaging

Immunofluorescence labeling was done as previously described.7,33,34 For vinculin labeling, cells were fixed with 1% paraformaldehyde (PFA)/0.5% Triton X-100 in cytoskeleton buffer for 15 minutes at RT and then, post-fixed with 4% PFA in cytoskeleton buffer for 10 minutes at RT. For end-binding protein 1 (EB1) and tubulin labeling, cells were fixed with ice-cold (−20°C) methanol for 4 minutes, rehydrated with 0.1% Triton X-100 in Tris-buffered saline (TBS; 3x, 5 min), and finally washed with TBS. Vinculin, EB1, and tubulin were detected with the monoclonal antibody hVin1 (Sigma-Aldrich), clone 5 (BD Transduction Laboratories, Heidelberg, Germany), and the rat hybridoma supernatant YL1/2,35 respectively. Secondary antibodies included Alexa Fluor 647-conjugated goat anti-rat IgG (for YL1/2) and Alexa Fluor 594- or 647-conjugated goat anti-mouse IgG (for hVin1 and clone 5). The actin cytoskeleton was visualized with Alexa Fluor 647-conjugated phalloidin (Life Technologies). For live cell imaging, phase-contrast and epifluorescence images were acquired with an Axio Observer Z1 inverted microscope (Carl Zeiss, Jena, Germany) equipped with an EMCCD camera (Evolve Delta, Photometrics, Tucson, AZ) driven by ZEN 2.3 software (Carl Zeiss, Jena, Germany).

2.4 | Analysis of cell migration and focal adhesion dynamics

J774 cells were plated onto self-made glass-bottomed dishes (Ø 6 cm) and their migration was recorded continuously for 24 hours (images were acquired every 5 min). The migration of all J774 cell lines was analyzed using the Fiji (https://imagej.net/Fiji) plugin MTrack36 to quantify the parameters such as average speed and directionality. Cells that touched neighboring cells, diving cells, and cells that displayed an oscillating movement were excluded from the analysis. Focal adhesion dynamics was analyzed as previously described.37,38

2.5 | Analysis of lamellipodia dynamics

Lamellipodia dynamics was visualized by phase-contrast microscopy after plating J774 cells at low density onto self-made glass-bottomed dishes (Ø 6 cm). Phase-contrast images were acquired every 5 seconds using an EMCCD camera (Cascade 512B, Photometrics, Tucson, AZ, USA) driven by IPLab Spectrum software (Scanalytics, Fairfax, VA, USA). The following parameters were measured: number of cells associated with lamellipodia (% of total cell number), velocity of lamellipodia spreading, and lamellipodia width (measured from the beginning of lamellipodia spreading until the first signs of lamellipodia retraction).

2.6 | Total internal reflection fluorescence microscopy and confocal microscopy

Total internal reflection fluorescence (TIRF) microscopy was performed on an Axio Observer Z1 inverted microscope equipped with a motorized TIRF slider (Zeiss). Excitation of GFP, RFP, and Turquoise was done using 488, 561, and 458 nm laser lines (at 10% of their nominal output power for 488 and 561, 30% for 458), respectively. The depth of the evanescent field for all wavelengths was ~70 nm. Images were acquired every 10 seconds using an Evolve Delta EMCCD camera driven by ZEN software (Zeiss). For all experiments, exposure time, depth of the evanescent field, and electronic gain were kept constant. Confocal microscopy was done using a Nikon Ti2-E with Crest X-Light V2 Spinning Disk, equipped with a LED light engine Spectra X (Lumencor) illumination source, and a CCD camera (Zyla 4.2, Andor). All images were acquired using a 60x/1.4 NA oil immersion lens (Nikon).

2.7 | Fluorescence recovery after photobleaching

For the analysis of the kinetics of LSP1 variants, GFP-LSP1, RFP-LSP1 (full-length, rescue), or RFP- LSP1-ΔSBS were
expressed in J774 cells. To analyze focal adhesion kinetics, J774 cells expressing RFP- or Turquoise-tagged zyxin were seeded onto self-made glass-bottomed dishes (ø 6 cm). For fluorescence recovery after photobleaching (FRAP), cells were imaged on an Axio Observer Z1 inverted microscope equipped with heating stage and CO2 controller (Zeiss) maintained at a constant temperature of 37°C. A portion of single focal adhesions (approximately ø 3.84 µm) was photobleached using a 405 nm laser driven by the UGA-40 control unit (Rapp Opto Electronic GmbH, Wedel, Germany). The recovery of the fluorescent signal was monitored by imaging cells every second for 15 minutes. Imaging was done using an Evolve Delta EMCCD camera driven by ZEN software (Zeiss). For all experiments the size of the bleached area, the duration, and intensity of the laser impulse were kept constant. The extent of recovery of the fluorescent signal was determined using Fiji to measure the average pixel intensity values within three distinct regions of interest (ROIs): ROI1: bleached area, ROI2: unbleached area within the cell, and ROI3: background. Normalized FRAP recovery curves and the mobile fractions were calculated using the program easyFRAP.\textsuperscript{30}

2.8 Scanning and transmission electron microscopy

For scanning electron microscopy, cells were fixed and processed as already described.\textsuperscript{7,34} Samples were examined with a digital scanning electron microscope (ESEM XL30 FEG; FEI, Hillsboro, OR) using a working distance of 8 mm and an acceleration voltage of 10 kV.

2.9 Statistical analysis

Graphs and statistical tests were done using Prism 8 (GraphPad Software, La Jolla, CA). Differences between sample pairs were analyzed using the two-tailed Mann-Whitney nonparametric \textit{U} test. The null hypotheses (the two samples have the same median values, that is, they are not different) were rejected when \( p > 0.5 \). For the box-and-whiskers plots, the line in the middle of the box indicates the median, the top of the box indicates the 75th quartile, and the bottom of the box indicates the 25th quartile. Whiskers represent the 10th (lower) and 90th (upper) percentiles.

3 RESULTS

3.1 LSP1 is essential for efficient migration of J774 mouse macrophages

Since LSP1 directly interacts with F-actin\textsuperscript{14} and regulates the dynamics of the actin cytoskeleton,\textsuperscript{7} we hypothesized that LSP1 could control cell migration. To this end, we focused on macrophages because LSP1 is essential for another actin-dependent process in this cell type, namely Fcy receptor-mediated phagocytosis.\textsuperscript{7} After seeing control cells or cells in which LSP1 was downregulated by shRNA,\textsuperscript{7} we imaged cell migration by phase-contrast microscopy over a period of 24 hours. Typically, control cells were characterized by a polarized morphology and the formation of large lamellipodia, which developed in the direction of movement (Figure 1A and Figure S1). Furthermore, control J774 cells usually traveled large distances (Figure 1A,C). Conversely, LSP1-deficient J774 cells rarely formed lamellipodia and moved over short distances (Figure 1B,D and Figure S1). Consistent with these observations, the average speed of LSP1-deficient J774 cells was significantly smaller than that of the control cells (0.01083 µm/sec for LSP1-deficient cells (n = 138) vs 0.02889 µm/sec for control cells (Figure 1E; n = 149). These findings clearly show that LSP1 is essential for efficient migration of J774 macrophages.

3.2 LSP1 is essential for the normal development of microfilaments, microtubules, and focal adhesions

The morphological features and largely decreased migration of LSP1-deficient cells suggest that LSP1 is involved in the organization of actin and microtubule cytoskeletons as well as cell-substrate adhesion (ie, focal adhesions). We verified this hypothesis by labeling control and LSP1-deficient J774 cells with anti-tubulin and anti-EB1 antibodies (for assessing microtubule organization) or fluorescent phalloidin and anti-vinculin antibodies (for assessing microfilaments and focal adhesions, respectively). Using TIRF and confocal microscopy, we found that control cells developed a prominent microtubule network characterized by long microtubules emanating from a perinuclear area and projecting toward the cell periphery (Figure 2A, arrows in inset; Figure S2 and corresponding videos). As expected, peripheral microtubule ends were labeled with EB1, a plus-end protein that regulates microtubule dynamics (Figure 2A, arrows in inset). In contrast, the microtubule network in LSP1-deficient cells, which were round and smaller than the control cells, was formed by short microtubules (Figure 2B, arrows in inset; Figure S2 and corresponding videos). In these cells, we could not find any gross alteration of EB1 distribution (Figure 2B, arrows in inset). Next, we analyzed control and LSP1-deficient J774 cells labeled with Alexa Fluor 594-phalloidin and anti-vinculin antibody to visualize actin cytoskeleton and focal adhesion by TIRF microscopy, respectively. Control cells were characterized by a spread and elongated morphology with one or multiple large actin-rich lamellipodia at their periphery (Figure 2C, green
These cells interacted with the substratum via several elongated focal adhesions (Figure 2C, arrows). At variance with these morphological features, LSP1-deficient cells were smaller and round with no or a single small actin-rich lamellipodium (Figure 2D, red arrowhead). Focal adhesions in these cells were strongly reduced in size and number and showed a rounded shape (Figure 2D, arrows). Overall, these findings demonstrate that LSP1 is essential for the normal development of microfilaments, microtubules, and focal adhesions. They also suggest that LSP1-dependent regulation of cell migration is exerted via the control of these cytoskeletal structures.

3.3 | LSP1 is essential for the regulation of focal adhesion dynamics

Focal adhesions are highly dynamic structures whose spatial and temporal regulation is essential for cell migration. The impaired cell migration and formation of focal adhesions in LSP1-deficient J774 cells suggests that LSP1 plays an important role in the control of focal adhesion dynamics. To test this assumption, we engineered control and LSP1-deficient J774 cells to express RFP-tagged zyxin, a focal adhesion component, to visualize focal adhesion dynamics using TIRF microscopy and analyzed their dynamics using a dedicated algorithm.
The initial examination of time-lapse sequences revealed that focal adhesions in control cells were highly dynamic, assembling or disassembling within short time periods (arrows in Figure 3A and corresponding video). In contrast, focal adhesions in LSP1-deficient cells appeared to be less dynamic requiring longer time periods to assemble and disassemble.
FIGURE 3 LSP1 is essential for the regulation of focal adhesion dynamics. A and B, Representative time-lapse images showing focal adhesion dynamics in control (A) and LSP1-deficient (B; LSP1-KD) J774 cells. Focal adhesions were visualized using RFP-zyxin and images were acquired by TIRF microscopy. Note the faster turnover of focal adhesions in control cells (arrows in A) compared to focal adhesions in LSP1-deficient cells (arrows in B). Numbers indicate the elapsed time in minutes and seconds. Scale bar: 10 µm. C-H, Quantification of focal adhesion parameters. In the box-and-whiskers plots the line in the middle of the box indicates the median, the top of the box indicates the 75th quartile, and the bottom of the box indicates the 25th quartile. Whiskers represent the 10th (lower) and 90th (upper) percentiles. ns, non-significant.
(arrows in Figure 3B and corresponding video). The quantification of several focal adhesion parameters confirmed the impression provided by visually inspecting time-lapse sequences. Precisely, for both growing and shrinking focal adhesions, the change in the area over time, a proxy for assembling and disassembly rates, was faster for focal adhesions in control cells than in LSP1-deficient cells (Figure 3C,D). Accordingly, the assembly and disassembly rates of focal adhesions in LSP1-deficient cells were significantly lower than the corresponding parameters for control focal adhesions (Figure 3E). Furthermore, the average area was significantly reduced in focal adhesions in LSP1-deficient cells (Figure 3F), whereas we could not see any difference in their shape (elongation index, Figure 3G). Finally, the focal adhesion movement relative to the substratum (focal adhesion speed) was also significantly impaired in LSP1-deficient cells (Figure 3H). These findings clearly show that LSP1 regulates cell migration via the modulation of focal adhesion formation and dynamics.

3.4 LSP1-myosin1e binary complex is essential for the regulation of cell migration

We have demonstrated that LSP1 directly interacts with myosin1e through a non-canonical SH3-binding site. Moreover, downregulating LSP1 or blocking its interaction with myosin1e results in severely impaired Fcγ receptor-mediated phagocytosis and the inhibition of actin accumulation and lamellipodia formation around the particles to be internalized. Since LSP1 deficiency impairs cell migration, we reasoned that the LSP1-myosin1e binary complex could play an important role in the regulation of J774 cell migration. To experimentally verify this hypothesis, we scrutinized the migration of J774 cells expressing the deletion mutant LSP1-ΔSBS, which cannot bind to myosin1e, by phase-contrast microscopy for 24 hours. As control, we used J774 cells re-expressing full-length LSP1 (rescue). As expected, cells in which LSP1 was re-expressed did not show any sign of migration defect highly resembling control J774 cells (Figure 4B,C,E). In contrast, it was...
immediately evident that cells expressing LSP1-ΔSBS traveled very short distances (Figure 4A,D) and moved at a significantly reduced speed (Figure 4E). Remarkably, the motile phenotype and the speed of cells expressing LSP1-ΔSBS were undistinguishable from LSP1-deficient cells (compare with Figure 1). These observations clearly demonstrate that the binary complex between LSP1 and myosin1e is essential for efficient J774 migration.

### 3.5 LSP1-myosin1e binary complex is necessary for lamellipodia activity

One of the earliest events of cell migration is the formation and stabilization of one lamellipodium in the direction of movement. Because cell migration is severely impaired in LSP1-deficient cells and in cells in which LSP1 cannot interact with myosin1e, we decided to determine whether lamellipodia activity is compromised in these cell types. The closer inspection of time-lapse sequences at high magnification revealed that motile control cells frequently form one large and persistent lamellipodium in the direction of movement (Figure 5A and corresponding video). LSP1-deficient cells and cells expressing LSP1-ΔSBS greatly differed from this phenotype in that they maintained a round morphology and never formed a large and persistent lamellipodium (Figure 5B,C). These cells were rather characterized by the formation of small lamellipodia that formed around their periphery (Figure 5B.C and corresponding video). Consistent with this visual examination, we found that the frequency of lamellipodia formation and its width were significantly lower in LSP1-deficient cells and cells expressing LSP1-ΔSBS (Figure 5D,F). Interestingly, the speed of lamellipodia spreading was significantly reduced in LSP1-ΔSBS cells, but not in LSP1-deficient cells (Figure 5E) possibly due to a large data variability. Collectively, these findings demonstrate that LSP1 and myosin1e are indispensable for efficient lamellipodia activity.

### 3.6 LSP1-myosin1e interaction regulates the dynamics and kinetics of LSP1

Next, we determined whether the interaction with myosin1e affected the dynamics and kinetics of LSP1. To this end, we expressed GFP-LSP1 in control J774 cells, RFP-LSP1 (full-length) or RFP-tagged LSP1-ΔSBS in LSP1-deficient J774 cells. LSP1 localization and dynamics were visualized by TIRF microscopy over a period of 10-15 minutes. In control cells and cells re-expressing full-length LSP1 (rescue), LSP1 was highly dynamic often localizing to lamellipodia (asterisk in Figure 6A,C; see also corresponding video) and to filamentous-like and focal adhesions-like structures (arrows in Figure 6A,C). In cells expressing LSP1-ΔSBS, LSP1 appeared to be less dynamic and was concentrated at the perinuclear area and at very small lamellipodia (arrows in Figure 6B; see also corresponding video). To corroborate the visual impression that LSP1 was less dynamic when unable to interact with myosin1e, we determined its kinetics using FRAP microscopy. As shown in Figure 6D,E, both the time-course of the fluorescence recovery and the mobile fraction of LSP1 in control cells and cells re-expressing full-length LSP1 (rescue) were indistinguishable. Conversely, the time-course of the fluorescence recovery and the mobile fraction of LSP1-ΔSBS were significantly reduced (Figure 6D,E). Thus, our findings suggest that efficient LSP1 dynamics and kinetics depend on its interaction with myosin1e.

### 3.7 LSP1-myosin1e binary complex is essential for the regulation of focal adhesion dynamics

The above findings provide clear evidence that LSP1 deficiency severely impairs cell migration, focal adhesion dynamics, and lamellipodia activity. Moreover, the interaction of LSP1 with myosin1e is essential for the regulation of cell migration and lamellipodia formation. Based on these observations and given the importance of focal adhesion dynamics for cell migration, we sought to determine whether LSP1-myosin1e binary complex also plays a role in the regulation of focal adhesion dynamics. For this purpose, we genetically modified J774 expressing LSP1-ΔSBS or full-length LSP1 (rescue) with turquoise-zyxin to visualized focal adhesion dynamics using TIRF microscopy.

Similar to LSP1-deficient cells, LSP1-ΔSBS cells were characterized by few and small focal adhesions with a slower turnover (Figure 7B and corresponding video). As expected, focal adhesions in cells re-expressing full-length LSP1 were similar to those in control cells (Figure 7A and corresponding video; compare with Figure 3A). Detailed analysis of focal adhesion dynamics using a dedicated algorithm supported this visual impression showing that assembly and disassembly rates as well as size were significantly reduced in LSP1-ΔSBS cells (Figure 7C-H). Consistent with these observations, we found a reduced amount of vinculin in the actin cytoskeleton fraction of LSP1-deficient and LSP1-ΔSBS cells (Figure 8).

Next, as a complementary approach to demonstrate the role of LSP1-myosin1e binary complex in the regulation of focal adhesions, we analyzed zyxin kinetics by FRAP. To this end, focal adhesions in control, LSP1-deficient, LSP1-ΔSBS and cells re-expressing full-length LSP1 (rescue) were
bleached and the recovery of zyxin fluorescence within the bleached areas was monitored over a period of several minutes. According to the above analysis of focal adhesion dynamics, zyxin kinetics was not significantly different in control cells and cells re-expressing full-length LSP1 (Figure 9A,D-F). Conversely, zyxin recovery was much slower and less complete at focal adhesions in LSP1-deficient and LSP1-ΔSBS cells (Figure 9B,C,E,F). Taken together, these findings demonstrate that the LSP1-myosin1e binary complex is required for the regulation of focal adhesion dynamics.

4 DISCUSSION

Deciphering the mechanisms of cell migration is essential for fully understanding this process in normal and pathological conditions. Understanding how the LSP1-myosin1e complex regulates focal adhesion dynamics can provide insights into cell migration processes and potential therapeutic targets for diseases involving impaired cell migration, such as cancer metastasis.
conditions. In this study, we demonstrate that LSP1 is essential for efficient cell migration in J774 macrophages. LSP1 controls this process through the regulation of the formation and dynamics of lamellipodia and focal adhesions. In this context, the interaction of LSP1 with the molecular motor myosin1e is indispensable for LSP1 function in cell migration since inhibiting the formation of LSP1-myosin1e binary complex severely impairs cell migration, formation and dynamics of lamellipodia, and focal adhesions. These findings provide novel insights into the regulation of cell migration in immune cells and demonstrate a pivotal role for the LSP1-myosin1e binary complex in the regulation of this process.

Previous investigations have addressed the role of LSP1 in cell migration in a variety of cell types. For instance, LSP1 deficiency significantly reduces the migration of dendritic cells induced by envelope glycoprotein 120 (gp120). Likewise, LSP1−/− neutrophils show both impaired migration and chemotactic response. Using a complementary approach, it was shown that the expression of LSP1 to physiological levels in a myeloid cell line, which does not express LSP1, results in a significant enhancement of cell migration. Notably, large lamellipodia are rarely observed in LSP1−/− cells, which rather form small and transient lamellipodia. In this study, we have deepened our knowledge of...
LSP1 function demonstrating that its deficiency severely impairs the formation of lamellipodia, focal adhesion dynamics, and cell migration in macrophages. Hence, LSP1 unequivocally plays a key role in the regulation of cell migration in different cellular systems.

For the sake of clarity, it should be mentioned that other studies show that the loss, or reduced expression, of LSP1 increases rather than reduces cell migration. However, this effect has been described in pathological situations such as in T-cells derived from patients with rheumatoid arthritis and in hepatoma cells.\(^20,21\) Since, in hepatoma cells, a classical scratch assay and not single-cell tracking was used to study cell migration, it is not possible to rule out that the described increase of cell migration was not due to the increase of cell proliferation observed in LSP1\(^{-/-}\) cells. In addition, the marginal (1.36-fold) increase of migration of LSP1\(^{-/-}\) neutrophils could be observed when cells were seeded only on fibrinogen, but not on other substrates.\(^44\) Hence, when interpreting these studies ascribing a negative regulatory effect on cell migration to LSP1, experimental parameters that may impact on this effect must be considered in order to avoid inaccurate interpretations.

Although it is known that LSP1 interacts with actin filaments,\(^12-14\) the mechanisms underlying LSP1 function in the

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**FIGURE 7** LSP1-myosin1e interaction is essential for efficient focal adhesion dynamics in J774 cells. A and B, J774 cells expressing full-length LSP1 (A; rescue) or the LSP1 mutant unable to interact with myosin 1e (B; LSP1-\(\Delta\)SBS) were transfected with RFP-zyxin to visualize focal adhesions and images were acquired by TIRF microscopy. Note the faster turnover of focal adhesions in cells expressing full-length LSP1 (arrows in A) compared to focal adhesions in cells expressing the LSP1 mutant unable to interact with myosin 1e (arrows in B). Numbers indicate the elapsed time in minutes and seconds. Scale bar: 10 µm. C-H, Quantification of focal adhesion parameters. In the box-and-whiskers plots the line in the middle of the box indicates the median, the top of the box indicates the 75th quartile, and the bottom of the box indicates the 25th quartile. Whiskers represent the 10th (lower) and 90th (upper) percentiles. ns, non-significant.
regulation of actin-driven processes are, as yet, poorly defined. Before discussing potential scenarios describing LSP1 function, our previous study\(^7\) and the findings described here clearly indicate that one possible mechanism for LSP1 function requires its interaction with myosin Ie. In this context, we envisage that LSP1 and myosin Ie will synergize to activate the signaling pathways specific for actin cytoskeleton remodeling. How does, then, this binary complex regulate cell migration and focal adhesion dynamics? It has been shown that LSP1 is a component of a complex including WASP and the Arp2/3 complex.\(^{31}\) LSP1 also co-precipitates with regulators of myosin II such as calmodulin and the myosin light chain kinase.\(^{32}\) Since WASP and the Arp2/3 complex are essential for actin filament nucleation in lamellipodia and myosin II activity controls actin cytoskeleton contraction and cell body displacement required for cell migration,\(^{45}\) it is reasonable to envisage a signaling scenario in which LSP1 controls both actin filament nucleation and cell body translocation (Figure 10). Our present study supports this hypothesis since in LSP1-deficient cells both lamellipodia formation and cell migration are severely impaired.

It is interesting to note that targeting myosin Ie to mitochondria induces the accumulation at the surface of these organelles of actin filaments and activators of the Arp2/3 complex WASP-interacting protein and neuronal WASP.\(^{46}\) Furthermore, in yeast, a type I myosin has been involved in actin filament formation induced by WASP and the Arp2/3 complex.\(^{47}\) These studies suggest a role for myosin Ie in the formation of actin filaments. This role for myosin Ie is further supported by two additional studies showing that lamellipodia formation and stability is impaired in myosin Ie-deficient cells.\(^{48,49}\) It is also important to note that cells expressing a variant of LSP1 that cannot interact with myosin Ie. Thus, it is conceivable that LSP1 and myosin Ie work in concert to regulate actin filament assembly through WASP-family proteins and the Arp2/3 complex (Figure 10). In this context, it should also be taken into account that myosin 1e has been found in actin-rich structures containing formin homology domain-containing 1 (FHOD1).\(^{48}\) In mammalian cells, FHOD1 stimulates the formation of stress fibers and cell migration and is recruited to integrin clusters.\(^{50-52}\) Moreover, FHOD1 knockdown impairs cell spreading and focal adhesion maturation.\(^{51}\) In Drosophila, the mutation of the FHOD1 homolog Knittrig results in smaller macrophages, which show reduced migration.\(^{53}\) These observations are, again, in agreement with our findings showing a similar phenotype in LSP1-deficient cells and in cells expressing a variant of LSP1 that cannot interact with myosin Ie. Hence, we propose that the LSP1-myosin Ie binary complex exert its function also via FHOD1 (Figure 10).

Although this study is centered on the impact of the LSP1-myosin Ie binary complex on actin cytoskeleton remodeling (therefore, on cell migration and focal adhesion dynamics), it is reasonable that the impairment of cell migration and focal adhesion turnover in LSP1-deficient cells and in cells expressing the mutant ΔSBS could, in part, be due to the alteration of the microtubules in these cells. This possibility is consistent with classical studies showing that microtubule dynamics is essential for both polarized cell migration\(^{54-56}\) and focal adhesion turnover.\(^{57-60}\)

At present, it is not possible to rule out that the deletion of the binding site for myosin Ie may alter the 3D folding of LSP1 thus, in part, contributing to impaired cell migration and focal adhesion turnover. However, we think that the LSP1-myosin Ie complex plays a major role for the following reasons. First, we have clearly demonstrated that LSP1-deficient cells (LSP1-KD) and cells expressing the LSP1 mutant ΔSBS exhibit very similar cell migration and focal adhesion
turnover. Thus, the inhibition of the formation of the LSP1-myosin1e complex results in the same phenotype typical of cells lacking LSP1 function emphasizing the role of LSP1-myosin1e interaction. Moreover, if the LSP1 mutant ΔSBS were deficient in other LSP1 functions that are linked to the regulation of cell migration and focal adhesion turnover, then we would have expected to observe a stronger impairment (possibly caused by the loss of yet unknown LSP1 interaction partners) of cell migration and focal adhesion turnover in cells expressing LSP1 ΔSBS. Second, we have previously

**FIGURE 9** LSP1-myosin1e interaction is essential for efficient kinetics of zyxin at focal adhesions. A-D, Control J774 cells (A), LSP1-deficient cells (LSP1-KD; B), and cells expressing the LSP1 mutant unable to interact with myosin 1e (LSP1-ΔSBS; C) or full-length LSP1 (rescue; D) were stably transfected with RFP-zyxin or turquoise-zyxin to visualize focal adhesions. RFP-zyxin and turquoise-zyxin kinetics was determined by fluorescence recovery after photobleaching. Arrows in A-D point to bleached focal adhesions. Note the slower recovery of the fluorescence signal at bleached focal adhesions in LSP1-deficient cells (B) and in cells expressing the LSP1 mutant unable to interact with myosin1e (C). E and F, Quantification of the fluorescence recovery (E) and mobile fraction (F) of zyxin. Error bars show s.e.m. In the box-and-whiskers plots, the line in the middle of the box indicates the median, the top of the box indicates the 75th quartile, and the bottom of the box indicates the 25th quartile. Whiskers represent the 10th (lower) and 90th (upper) percentiles.
shown that the ability of LSP1 ΔSBS to interact with the actin cytoskeleton is not grossly altered. This is very important since LSP1 ΔSBS retains an essential feature, which is required for the regulation of actin cytoskeleton-dependent functions, that is, the ability to interact with the actin cytoskeleton. Hence, our findings support the conclusion that the LSP1-myosin1e complex primarily regulates cell migration and focal adhesion turnover. Future studies should unravel the network of molecular interactions involving the modulation of the actin cytoskeleton dynamics by LSP1.

Finally, it is important to note that the prominence of LSP1 in the regulation of cell migration is further supported by studies on pathogen-cell interactions. It has been clearly demonstrated that several types of pathogens such as *Listeria monocytogenes* and vaccinia viruses have developed elegant strategies to subvert fundamental steps of actin cytoskeleton remodeling for their spreading and survival. In this context, it has been shown that the human immunodeficiency virus can induce migration of dendritic cells. These viruses control dendritic cell migration by the binding of their envelope protein gp120 to dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin (DC-SIGN) on dendritic cells. Remarkably, gp120-DC-SIGN interaction triggers a signaling cascade that involves LSP1 and leads to Rho GTPase (a regulator of focal adhesion dynamics) activation. This study, thus, support our findings highlighting the key role of LSP1 in the regulation of focal adhesion dynamics and cell migration.

Collectively, our findings provide novel evidence about LSP1 function and its ability to regulate two crucial aspects of cell migration in co-operation with myosin1e: a) actin cytoskeleton assembly (required for lamellipodia formation and dynamics) and b) focal adhesion formation and dynamics. Several questions remain to be addressed. For instance, is the function of myosin1e dependent on its interaction with LSP1? Is LSP1 phosphorylation required for its role in cell migration? Has LSP1 other binding partners in addition to myosin1e? Could LSP1 be a target for novel pharmaceutical treatments of HIV infections? The answers to these questions will certainly help to better understand the role of LPS1 and its interaction with myosin1e not only in the regulation of cell migration and adhesion but also in other processes dependent on actin cytoskeleton remodeling.

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**CONFLICT OF INTEREST**

Authors have no conflict of interest to declare.

**AUTHOR CONTRIBUTIONS**

A. Sechi conceived and designed the study; K. Schäringer, S. Maxeiner, C. Schalla, S. Rütten, and M. Zenke performed the research; A. Sechi, K. Schäringer, and S. Maxeiner analyzed the data; A. Sechi and K. Schäringer wrote the manuscript; all authors have read and approved the manuscript.

**REFERENCES**

1. Ramaekers FC, Bosman FT. The cytoskeleton and disease. J Pathol. 2004;204:351-354.
2. Mathieson PW. The podocyte cytoskeleton in health and in disease. Clin Kidney J. 2012;5:498-501.
3. Jongstra-Bilen J, Misener VL, Wang C, et al. LSP1 modulates leukocyte populations in resting and inflamed peritoneum. Blood. 2000;96:1827-1835.

4. Liu L, Cara DC, Kaur J, et al. LSP1 is an endothelial gatekeeper of leukocyte transendothelial migration. J Exp Med. 2005;201:409-418.

5. Jongstra J, Jongstra-Bilen J, Tidmarsh GF, Davis MM. The in vitro translation product of the murine lambda 5 gene contains a functional signal peptide. Mol Immunol. 1988;25:687-693.

6. Jongstra J, Ittel ME, Iscove NN, Brady G. The LSP1 gene is expressed in cultured normal and transformed mouse macrophages. Mol Immunol. 1994;31:1125-1131.

7. Maxeiner S, Shi N, Schalla C, et al. Crucial role for the LSP1-myosin1e bimolecular complex in the regulation of Fcgamma receptor-driven phagocytosis. Mol Biol Cell. 2015;26:1652-1664.

8. Kadiyala RK, McIntyre BW, Krensky AM. Molecular cloning of human lymphocyte-specific protein 1, a Ca2+-binding protein. J Immunol. 1990;20:2417-2423.

9. Klein DP, Jongstra-Bilen J, Ogyrizlo K, Chong R, Jongstra J. Lymphocyte-specific Ca2+-binding protein LSP1 is associated with the cytoplasmic face of the plasma membrane. Mol Cell Biol. 1989;9:3043-3048.

10. Zhang Q, Li Y, Howard TH. Human lymphocyte-specific protein 1, the protein overexpressed in neutrophil actin dysfunction with 47-Kd and 89-Kd protein abnormalities (NAD 47/89), has multiple F-actin binding domains. J Immunol. 2000;165:2052-2058.

11. Zhang Q, Li Y, Howard TH. Hair-forming activity of human lymphocyte specific protein 1 requires cooperation between its caldesmon-like domains and the villin headpiece-like domains. Cell Motil Cytoskel. 2001;49:179-188.

12. Klein DP, Galea S, Jongstra J. The lymphocyte-specific protein LSP1 is associated with the cytoskeleton and co-caps with membrane IgM. J Immunol. 1990;145:2967-2973.

13. Yong J, Malapitan IA, Sikorski BA, Jongstra J. A cell-free binding assay maps the LSP1 cytoskeletal binding site to the COOH-terminal 30 amino acids. Biochem Biophys Acta. 2003;1642:17-24.

14. Howard TH, Hartwig J, Cunningham C. Lymphocyte-specific protein 1 expression in eukaryotic cells reproduces the morphologic and motile abnormality of NAD 47/89 neutrophils. Blood. 1998;91:4786-4795.

15. Miyoshi EK, Stewart PL, Kincade PW, Lee MB, Thompson AA, Wall R. Abl expression and localization of the cytoskeleton-binding pp52 (LSP1) protein in hairy cell leukemia. Leuk Res. 2001;25:57-67.

16. Coates TD, Torkildson JC, Torres M, Church JA, Howard TH. An inherited defect of neutrophil motility and microfilamentous cytoskeleton associated with abnormalities in 47-Kd and 89-Kd proteins. Blood. 1991;78:1338-1346.

17. Howard T, Li Y, Torres M, Guerrero A, Coates T. The 47-kD protein increased in neutrophil actin dysfunction with 47- and 89-kD protein abnormalities is lymphocyte-specific protein. Blood. 1994;83:231-241.

18. Li Y, Zhang Q, Aaron R, Hilliard L, Howard TH. LSP1 modulates the locomotion of monocyte-differentiated U937 cells. Blood. 2000;96:1100-1105.

19. Hwang SH, Jung SH, Lee S, et al. Leukocyte-specific protein 1 regulates T-cell migration in rheumatoid arthritis. Proc Natl Acad Sci U S A. 2015;112:E6535-6543.

20. Koral K, Paranjpe S, Bowen WC, Mars W, Luo J, Michalopoulos GK. Leukocyte-specific protein 1: a novel regulator of hepatocellular proliferation and migration deleted in human hepatocellular carcinoma. Hepatology. 2015;61:537-547.

21. Petri B, Kaur J, Long EM, et al. Endothelial LSP1 is involved in endothelial dome formation, minimizing vascular permeability changes during neutrophil transmigration in vivo. Blood. 2011;117:942-951.

22. Jongstra-Bilen J, Young AJ, Chong R, Jongstra J. Human and mouse LSP1 genes code for highly conserved phosphoproteins. J Immunol. 1990;144:1104-1110.

23. Matsumoto N, Kita K, Kojima S, et al. Lymphocyte isoforms of mouse p50 LSP1, which are phosphorylated in mitogen-activated T cells, are formed through alternative splicing and phosphorylation. J Biochem. 1995;118:237-243.

24. Matsumoto N, Toyoshima S, Osawa T. Characterization of the 50 kDa protein phosphorylated in concanavalin A-stimulated mouse T cells. J Immunol. 1993;113:630-636.

25. Carballo E, Colomer D, Vives-Corrons JL, Blackshear PJ, Gil J. Characterization and purification of a protein kinase C substrate in human B cells. Identification as lymphocyte-specific protein 1 (LSP1). J Immunol. 1996;156:1709-1713.

26. Huang CK, Zhan L, Ai Y, Jongstra J. LSP1 is the major substrate for mitogen-activated protein kinase-activated protein kinase 2 in human neutrophils. J Biol Chem. 1997;272:17-19.

27. Wu Y, Zhan L, Ai Y, et al. MAPKAPK2-mediated LSP1 phosphorylation and FMLP-induced neutrophil polarization. Biochem Biophys Res Comm. 2007;358:170-175.

28. Matsumoto N, Kojima S, Osawa T, Toyoshima S. Protein kinase C phosphotyrosines p50 LSP1 and induces translocation of p50 LSP1 in T lymphocytes. J Biochem. 1995;117:222-229.

29. Harrison RE, Sikorski BA, Jongstra J. Leukocyte-specific protein 1 targets the ERK/MAP kinase scaffold protein KSR and MEK1 and ERK2 to the actin cytoskeleton. J Cell Sci. 2004;117:2151-2157.

30. Prasad A, Kuzontkoski PM, Shrivastava A, Zhu W, Li DY, Groopman JE. Slit2N/Robo1 inhibit HIV-gp120-induced migration and podosome formation in immature dendritic cells by sequestering LSP1 and WASp. PLoS One. 2012;7:e48854.

31. Cervero P, Wiesner C, Bouissou A, Poincloux R, Linder S. Lymphocyte-specific protein 1 regulates mechanosensory oscillation of podosomes and actin isoform-based actomyosin symmetry breaking. Nat Commun. 2018;9:515.

32. Gamper I, Fleck D, Barlin M, et al. GAR22beta regulates cell migration, sperm motility, and axoneme structure. Mol Biol Cell. 2016;27:277-294.

33. Sechi A, Freitas JMG, Wunnemann P, et al. Surface-grafted nanogel arrays direct cell adhesion and motility. Adv Mater Interfaces. 2016;3(20):1600455.

34. Wehland J, Willingham MC, Sandoval IV. A rat monoclonal antibody reacting specifically with the tyrosylated form of alpha-tubulin. I. Biochemical characterization, effects on microtubule polymerization in vitro, and microtubule polymerization and organization in vivo. J Cell Biol. 1983;97:1467-1475.

35. Meijering E, Dzyubachyk O, Smaal I. Methods for cell and particle tracking. Methods Enzymol. 2012;504:183-200.
37. Würflinger T, Gamper I, Aach T, Sechi AS. Automated segmentation and tracking for large-scale analysis of focal adhesion dynamics. J Microsc. 2011;241:37-53.

38. Berginski ME, Gomez SM. The focal adhesion analysis server: a web tool for analyzing focal adhesion dynamics. F1000Res. 2013;2:68.

39. Rapsomaniki MA, Kotsantis P, Symeonidou IE, Giakoumakis NN, Taraviras S, Lygerou Z. easyFRAP: an interactive, easy-to-use tool for qualitative and quantitative analysis of FRAP data. Bioinformatics. 2012;28:1800-1801.

40. Sechi AS, Wehland J. ENA/VASP proteins: multifunctional regulators of actin cytoskeleton dynamics. Front Biosci. 2004;9:1294-1310.

41. Zamir E, Geiger B. Molecular complexity and dynamics of cell-matrix adhesions. J Cell Sci. 2001;114:3583-3590.

42. Anand AR, Prasad A, Bradley RR, et al. HIV-1 gp120-induced migration of dendritic cells is regulated by a novel kinase cascade involving Pyk2, p38 MAP kinase, and LSP1. Blood. 2009;114:3588-3600.

43. Hannigan M, Zhan L, Ai Y, Huang CK. Leukocyte-specific gene 1 protein (LSP1) is involved in chemokine KC-activated cytoskeletal reorganization in murine neutrophils in vitro. J Leukoc Biol. 2001;69:497-504.

44. Wang C, Hayashi H, Harrison R, et al. Modulation of Mac-1 (CD11b/CD18)-mediated adhesion by the leukocyte-specific protein 1 is key to its role in neutrophil polarization and chemotaxis. J Immunol. 2002;169:415-423.

45. Blanchin L, Boujemaa-Paterski R, Sykes C, Plastino J. Actin dynamics, architecture, and mechanics in cell motility. Physiol Rev. 2014;94:235-263.

46. Cheng J, Grassart A, Drubin DG. Myosin 1E coordinates actin assembly and cargo trafficking during clathrin-mediated endocytosis. Mol Biol Cell. 2012;23:2891-2904.

47. Sirotnik V, Beltzner CC, Marchand JB, Pollard TD. Interactions of WASp, myosin-I, and verprolin with Arp2/3 complex during actin patch assembly in fission yeast. J Cell Biol. 2005;170:637-648.

48. Gupta P, Gauthier NC, Cheng-Han Y, et al. Myosin 1E localizes to actin polymerization sites in lamellipodia, affecting actin dynamics and adhesion formation. Biol Open. 2013;2:1288-1299.

49. Tanimura S, Hashizume J, Arichika N, et al. ERK signaling promotes cell motility by inducing the localization of myosin 1E to lamellipodial tips. J Cell Biol. 2016;214:475-489.

50. Schulze N, Graessl M, Blancke Soares A, Geyer M, Dehmeit L, Nalbant P. FHOD1 regulates stress fiber organization by controlling the dynamics of transverse arcs and dorsal fibers. J Cell Sci. 2014;127:1379-1393.

51. Iskratsch T, Yu CH, Mathur A, et al. FHOD1 is needed for directed forces and adhesion maturation during cell spreading and migration. Dev Cell. 2013;27:545-559.

52. Koka S, Neudauer CL, Li X, Lewis RE, McCarthy JB, Westendorf JJ. The formin-homology-domain-containing protein FHOD1 enhances cell migration. J Cell Sci. 2003;116:1745-1755.

53. Lamell U, Bechtold M, Risse B, et al. The Drosophila FHOD1-like formin Knittrig acts through Rok to promote stress fiber formation and directed macrophage migration during the cellular immune response. Development. 2014;141:1366-1380.

54. Vasiliev JM, Gelfand IM, Domnina LV, Ivanova OY, Koms SG, Olshevskaja LV. Effect of colcemid on the locomotory behaviour of fibroblasts. J Embryol Exp Morphol. 1970;24:625-640.

55. Finkelstein E, Chang W, Chao PH, et al. Roles of microtubules, cell polarity and adhesion in electric-field-mediated motility of 3T3 fibroblasts. J Cell Sci. 2004;117:1533-1545.

56. Bershadsky AD, Vaisberg EA, Vasiliev JM. Pseudopodal activity at the active edge of migrating fibroblast is decreased after drug-induced microtubule depolymerization. Cell Motil Cytoskeleton. 1991;19:152-158.

57. Kaverina I, Krylyshkina O, Small JV. Microtubule targeting of substrate contacts promotes their relaxation and dissociation. J Cell Biol. 1999;146:1033-1044.

58. Kaverina I, Rottner K, Small JV. Targeting, capture, and stabilization of microtubules at early focal adhesions. J Cell Biol. 1998;142:181-190.

59. Krylyshkina O, Anderson KI, Kaverina I, et al. Nanometer targeting of microtubules to focal adhesions. J Cell Biol. 2003;161:853-859.

60. Krylyshkina O, Kaverina I, Kranevitter W, et al. Modulation of substrate adhesion dynamics via microtubule targeting requires kinesin-1. J Cell Biol. 2002;156:349-359.

61. Geese M, Loureiro JJ, Bear JE, Wehland J, Gertler FB, Sechi AS. Contribution of Ena/VASP proteins to intracellular motility of listeria requires phosphorylation and proline-rich core but not F-actin binding or multimerization. Mol Biol Cell. 2002;13:2383-2396.

62. May RC, Hall ME, Higgs HN, et al. The Arp2/3 complex is essential for the actin-based motility of Listeria monocytogenes. Curr Biol. 1999;9:759-762.

63. Pust S, Morrison H, Wehland J, Sechi AS, Herrlich P. Listeria monocytogenes exploits ERM protein functions to efficiently spread from cell to cell. EMBO J. 2005;24:1287-1300.

64. Way M. Interaction of vaccinia virus with the actin cytoskeleton. Folia Microbiol (Praha). 1998;43:305-310.

65. Prasad A, Kulkarni R, Jiang S, Groopman JE. Cocaine enhances DC to T-cell HIV-1 transmission by activating DC-SIGN/LARG/ LSP1 complex and facilitating infectious synapse formation. Sci Rep. 2017;7:40648.

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