

Aberrant expression of LIMK1 impairs neuronal migration during neocortex development

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Abstract Neuronal migration is essential for the formation of cortical layers, and proper neuronal migration requires the coordination of cytoskeletal regulation. LIMK1 is a serine/threonine protein kinase that mediates actin dynamics by regulating actin depolymerization factor/cofilin. However, the role of LIMK1 in neuronal migration and its potential mechanism remains elusive. Here, we found that using the in utero electroporation to overexpress LIMK1 and its mutants, constitutively active LIMK1 (LIMK1-CA) and dominant-negative LIMK1 (LIMK1-DN), impaired neuronal migration in the embryonic mouse brain. In addition, the aberrant expression of LIMK1-WT and LIMK1-CA induced abnormal branching and increased the length of the leading process, while LIMK1-DN-transfected neurons gave rise to two leading processes. Furthermore, the co-transfection of LIMK1-CA and cofilin-S3A partially rescued the migration deficiency and fully rescued the morphological changes in migrating neurons induced by LIMK1-CA. Our results indicated that LIMK1 negatively regulated neuronal migration by affecting the neuronal cytoskeleton and that its effects were partly mediated by cofilin phosphorylation.

Keywords LIMK1 · Neuronal migration · In utero electroporation · Cofilin phosphorylation · Actin cytoskeleton

Introduction

Mammalian neocortex contains six layers and is formed by the directional migration of post-mitotic neurons from the ventricular zone toward the pial surface (Berry and Rogers 1965; Rakic and Lombroso 1998). When neurons reach their destinations in the cortical plate, apical dendrites extended up to the pial surface, and their axons grow down to the ventricle to establish synaptic connections. Neuronal migration, which is critical for brain function, is a multifaceted process and dependent on a wide variety of cellular functions that modulate cell shape, polarity and motility. Several neurological diseases are caused by defects in neuronal migration, including epilepsy, dysgnosia and mental retardation (Kuzniecky 2006).

Normal central nervous system development relies upon the presence of LIMK1, deletion of which has been implicated in the development of Williams syndrome, a complex human developmental disorder characterized by mental retardation and profound deficits in visuospatial cognition (Frangiskakis et al. 1996; Bellugi et al. 1999). The LIM kinase family is composed of LIMK1 and LIMK2. LIMK1 contains two LIM domains, an S/P-rich domain and a kinase domain, and it plays a central role in numerous cellular processes, including cell proliferation, the establishment of cell morphology, cell motility and structural remodeling.

LIMK1 is restricted to neuronal tissues and accumulates at high levels in mature synapses (Bernard et al. 1994; Wang et al. 2000; Proschel et al. 1995). In the nervous system, it has been shown that LIMK1 is important for neurite outgrowth, spine morphology and synaptic plasticity. LIMK1 knockout in mice or genetic mutation in humans is associated with spine abnormalities and cognitive impairments (Frangiskakis et al. 1996; Tassabehji et al. 1996;

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Meng et al. 2002). Moreover, the overexpression of wild-type LIMK1 in cultured hippocampal pyramidal neurons induces growth cone formation and axon outgrowth at early time points (Rosso et al. 2004). LIMK1 is activated downstream of Rho and Rac1, and evidences indicate that ROCK activates LIMK1 in vitro and in vivo by phosphorylation at Thr-508 (Ohashi et al. 2000b; Scott and Olson 2007; Kapur et al. 2016). In addition, as a serine/threonine kinase, LIMK1 influences the architecture of the actin cytoskeleton by phosphorylating and inactivating its substrate, cofilin family proteins (Arber et al. 1998; Yang et al. 1998; Gorovoy et al. 2005). However, it remains largely unknown whether LIMK1 functions in neuronal migration, and if so, how it contributes to those deficits.

Here, we showed that LIMK1 was essential for neuronal migration in vivo. Overexpression of LIMK1 in cortical neurons by in utero electroporation (IUE) induced neurites and branches. Furthermore, the activation of LIMK1 was significant as well; the two mutants impaired neuronal migration through different morphologies. Finally, we found that cofilin could partially rescue the defects of neuronal migration caused from constitutively active LIMK1 (LIMK1-CA). Thus, our findings indicated that LIMK1 was a negative regulator in proper cortical neuronal migration through the rearrangement of the actin cytoskeleton.

Methods and materials

Animals and tissue preparation

C57BL/6J mice were purchased from the experimental animal center of Xi'an Jiaotong University. The care and handling of animals and the experimental procedures were performed in accordance with the Guide for Care and Experimental of Laboratory Animals of Northwest A&F University.

Plasmid construction

All plasmids used a modified chicken β -actin promoter with the CMV-IE enhancer (CAG promoter), which can drive efficient expression in in utero electroporation experiments. All the fragments were inserted into pCAG-MCS-EGFP, which was constructed by inserting enhanced green fluorescent protein (EGFP) into pCAG-MCS. The full length of *limk1* (GenBank accession No. NM_002314) was cloned from total embryonic neocortex RNA with the specific primers:

LIMK1-WT: forward primer: 5'-gtcgacgccaccatgaggttga cgctactttgttgc-3' and reverse primer: 5'-agatctgtcagggacct cgggg-3'; using gene splicing by overlap extension PCR, the dominant-negative form of LIMK1 was generated by

changing the Asp460 to alanine (Yang et al. 1998; Ishaq et al. 2011), and the constitutively active form of LIMK1 was obtained by transforming Thr508 to valine (Kapur et al. 2016; Scott and Olson 2007).

Cofilin-WT was generated by PCR from a mouse *cofilin* cDNA library (GenBank accession No. NM_007687) using the forward primer: 5'-ccggaattcgccaccatggcctctggt-gtggctgtc-3' and reverse primer: 5'-ggcgggatcccccaagcctt-gccctccagg-3'. Cofilin-S3A was generated to mimic the dephosphorylated (constitutively active) form by changing Ser3 to alanine (Chai et al. 2016; Noguchi et al. 2016).

In utero electroporation

Plasmids for IUE were purified using the Qiagen Plasmid Plus Midi Kit. Pregnant mice with embryos at embryonic day 15.5 (E15.5) were anesthetized with amobarbital sodium (Sigma), followed by inflation of the abdomen and then exposure of the uterine horns. Next, 1–2 μ L of 2–3 μ g/ μ L plasmid solution was injected into the lateral ventricle through a glass micropipette made from a microcapillary tube. Electrical pulses were applied 5 times at intervals of 50 ms using an electroporator. Then, the uterine horns were put back into the peritoneal cavity for continued development (Tabata and Nakajima 2001). At appropriate stages, the electroporated brains were fixed with 4% paraformaldehyde (PFA).

Immunofluorescence

Mouse brains at E18.5 and postnatal day 1 (P1) were sliced into coronal sections using a vibratome (VT 1000S; Leica Microsystems, Wetzlar, Germany). The slices were incubated overnight with rabbit-anti-GFP and goat-anti-myc (1:1000). After rinsing three times with phosphate buffer, they were exposed to Alexa Fluor 488-conjugated donkey anti-rabbit IgG for 3 h at room temperature. After another three washes, they were incubated in Alexa Fluor 568-conjugated donkey anti-goat IgG and DAPI (1:500, Invitrogen, Carlsbad, CA, USA) for another 3 h at room temperature. Then, the slices were mounted in DAKO fluorescent mounting medium (North America, Inc., CA, USA) and photographed using a fluorescent microscope (Axio Observer Z1; ZEISS, Germany).

Statistical analysis

To study the role of LIMK1 in neuronal migration, five slices at the somatosensory cortex level were selected from five transfected mouse brains randomly in each group under a fluorescent microscope (Tabata and Nakajima 2001, 2002). We use rectangular frame to select quantification areas in somatosensory cortex slices, and we counted all

transfected (GFP positive) cells in each layer of the frame, respectively, and then obtained the percentage of cells in each layer to analyze whether migration was affected. The percentage of cells in the each layer equals GFP-positive cells in each layer/GFP-positive cells in the frame. To study the percentage of cells with branches in the LIMK1-WT and control groups, more than 100 transfected cells in the cortical plate (CP) were analyzed at high magnification. To evaluate the percentages of cells with two leading processes in the LIMK1-DN and control groups, we counted at least 100 transfected cells in the CP at high-power magnification. To ensure that the quantitative analyses were objective, we only counted the GFP-positive cells with obvious nuclei stained with DAPI. Thus, the subcellular localizations of GFP in different LIMK1-construct groups did not influence cell counting.

All data were obtained from five independent transfected brains. SPSS 20 software was used for statistical analysis. Student's *t* test was used to determine the statistical significance of differences between two groups; comparisons among multiple groups were made using one-way analysis of variance (ANOVA), and the one-way ANOVA was followed by Dunnett's multiple comparison test ($*P < 0.05$, $**P < 0.01$, $***P < 0.001$).

Results

Overexpression of LIMK1 blocked neuronal migration

To investigate the effect of LIMK1 on neuronal migration, we constructed the LIMK1-WT plasmid and employed in utero electroporation (Tabata and Nakajima 2001) to label new born neurons in embryonic brains at E15.5. The distribution of those transfected neurons was strikingly different between the LIMK1-WT group and the GFP control group. At E18.5, in the control group, numerous GFP-positive cells migrated into both the intermediate zone (IZ) and the cortical plate (CP), and more than 20% transfected cells settled in the CP (Fig. 1a, b). However, neurons in brains transfected with LIMK1-WT accumulated in the sub-ventricular zone (VZ/SVZ) and the IZ, and almost no neurons reached the CP (Fig. 1a, b). Consistently, we also observed a migratory deficiency at P1 (Fig. 1c). More than 60% of the transfected cells in the control group reached the upper region of the CP (UCP), and another 30% were in the IZ and the deeper region of the CP (DCP) (Fig. 1c, d). In contrast, in the LIMK1-WT brain sections, only 15% of the transfected neurons migrated into the UCP, while more than 50% of transfected cells remained in the IZ and VZ/SVZ (Fig. 1c, d). These results indicated that overexpression of LIMK1 impaired neuronal migration.

To explore migratory defects caused by LIMK1 overexpression, high-magnification microscopy was used to observe morphological differences between the transfected cells in the control group and the LIMK1-WT group. LIMK1-WT-transfected cells in the IZ displayed more neurites (Fig. 2a, bottom) compared with the control group (Fig. 2a, top). In addition, we found branches on the leading processes of migrating neurons at P1 (Fig. 2c, right). Nevertheless, in the control group, migrating neurons showed the typical bipolar morphology, and most processes were smooth and straight, oriented toward the marginal zone (MZ) (Fig. 2c, left). Specifically, more than 90% of control cells were bipolar, whereas almost 40% of LIMK1-WT cells gave rise to irregular branches (Fig. 2d). Taken together, the aberrant expression of LIMK1 impaired neuronal migration by affecting neurite branching.

LIMK1 activity was important for its function in neuronal migration

LIMK1 is a serine/threonine kinase, and its activity is regulated by phosphorylation. To further examine how LIMK1 activity affects neuronal migration, we transfected neurons with a constitutively active form, LIMK1-CA, or a dominant-negative form, LIMK1-DN. As expected, we found a marked migration deficiency in the P1 mouse brain transfected at E15.5. In the LIMK1-DN and LIMK1-CA groups, more than 45% of transfected cells were located in the VZ/SVZ and IZ, compared with only 20% of cells in the control group (Figs. 3b, 4c). In addition, fewer than 15% of LIMK1-CA-labeled cells had migrated into the UCP, while in the LIMK1-WT group, almost 20% of transfected neurons reached the UCP. However, more than 25% of cells expressing LIMK1-DN were located in the UCP. These data clearly showed that LIMK1 activity is essential for normal neuronal migration.

Given that LIMK1-WT affected neuronal migration through changes in neuronal morphology, we compared the cellular morphology induced by the overexpression of LIMK1-DN and LIMK1-CA. LIMK1-CA-transfected cells not only showed branching but also displayed longer leading processes during migration (Fig. 4d). Compared with the average length of 60 μm for leading processes in the control group, that in the LIMK1-CA group was over 90 μm (Fig. 4e). However, LIMK1-DN brain slices showed no significant difference from the control group in the length of the leading process. Interestingly, LIMK1-DN-transfected cells in the CP possessed two leading processes, unlike cells in the control group, which typically possessed only one leading process (Fig. 3d). The above observation was confirmed using quantitative analysis. More than 60% of neurons transfected with LIMK1-DN gave rise to

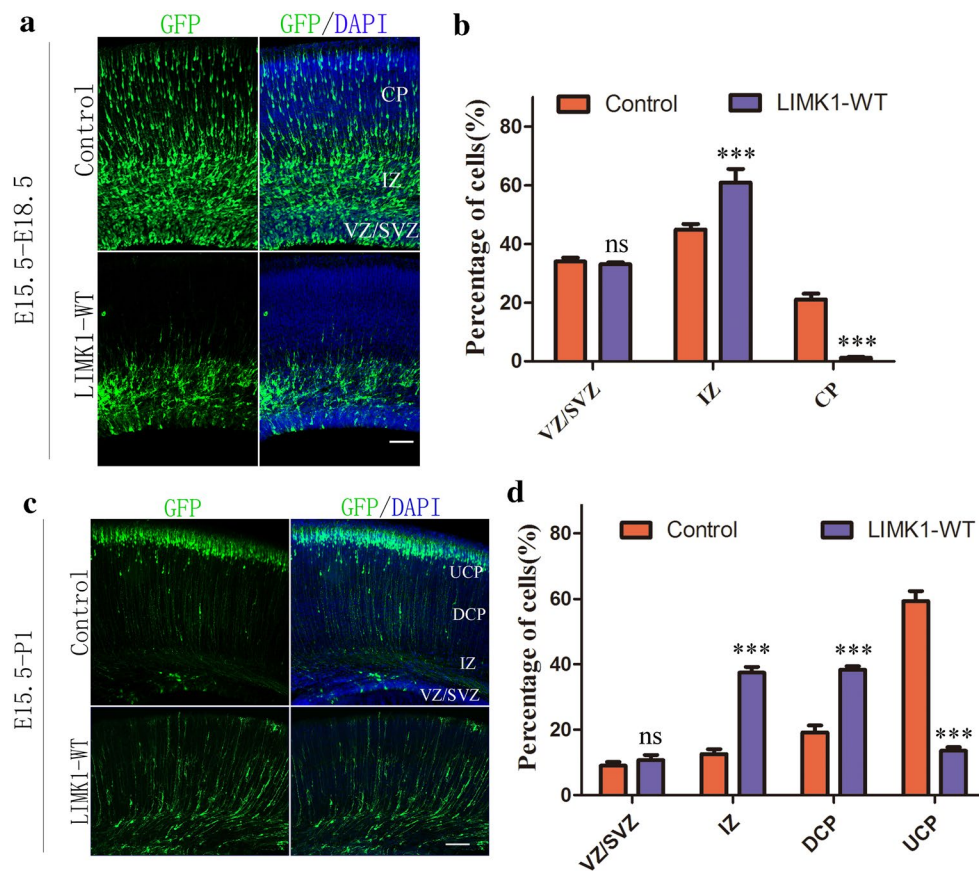


Fig. 1 Overexpression of LIMK1 impairs the radial neuronal migration of cortical neurons in vivo. **a** Coronal sections of the E18.5 brains were electroporated at E15.5 with EGFP (control, *top*) or LIMK1-WT (*bottom*). In the control group, many transfected neurons were located in the CP, whereas the majority of neurons transfected with LIMK1-WT accumulated in the IZ. **b** Quantification of transfected neurons in each layer at E18.5 showed that compared with the control group, the number of LIMK1-transfected neurons in the CP decreased significantly. Statistical significance was assessed using *t* test (DF = 8, $T = 0.6828$, $P = 0.5039$ for VZ/SVZ; DF = 8,

$T = 13.20$, $P < 0.0001$ for IZ; and DF = 8, $T = 15.87$, $P < 0.0001$ for CP). **c** EGFP-positive cells (control, *top*) and LIMK1-WT-positive cells (*bottom*) in the brains fixed at P1. Many control-transfected cells invaded the UCP; however, many neurons expressing LIMK1-WT failed to reach the UCP. **d** Quantification showed the percentage of neurons in each layer at P1, and statistical significance was assessed using *t* test (DF = 8, $T = 0.4459$, $P = 0.6593$ for VZ/SVZ; DF = 8, $T = 6.411$, $P < 0.0001$ for IZ; DF = 8, $T = 6.610$, $P < 0.001$ for DCP; and DF = 8, $T = 15.67$, $P < 0.0001$ for UCP). Scale bar 100 μm . *** $P < 0.001$; ns not significant, E embryo, P postnatal

two leading processes, whereas more than 90% of neurons in control brain slices showed only one leading process (Fig. 3c). These results suggested that both hyperactivation and inactivation of LIMK1 affect neuronal migration but that the underlying mechanisms are different.

LIMK1 regulated neuronal migration by modulating cofilin

In signaling pathways related to actin dynamics, cofilin is an effector of LIMK1, which regulates the cytoskeleton via phosphorylating cofilin (Ohashi et al. 2000a; Salvarezza et al. 2009). To verify whether LIMK1 affects neuronal migration through cofilin, we co-transfected LIMK1-CA and cofilin-S3A into embryonic mouse brains. We found that co-expression of cofilin-S3A partially rescued the migration

defects induced by LIMK1-CA (Fig. 4b). This partial rescue effect was demonstrated by quantitative analysis of the distribution patterns and the lengths of leading processes in the CP—almost 30% of co-transfected cells were located in the UCP, a much higher percentage than in LIMK1-CA brain slices (Fig. 4c). Additionally, the average length of the leading process in co-transfected cells was 60 μm , while it was 90 μm in the LIMK1-CA group (Fig. 4e). These results showed that cofilin is a downstream target of LIMK1 in mediating neuronal migration into the neocortex.

Discussion

Neuronal migration requires a sophisticated interplay of complex molecular machines in its multiple steps (Govek

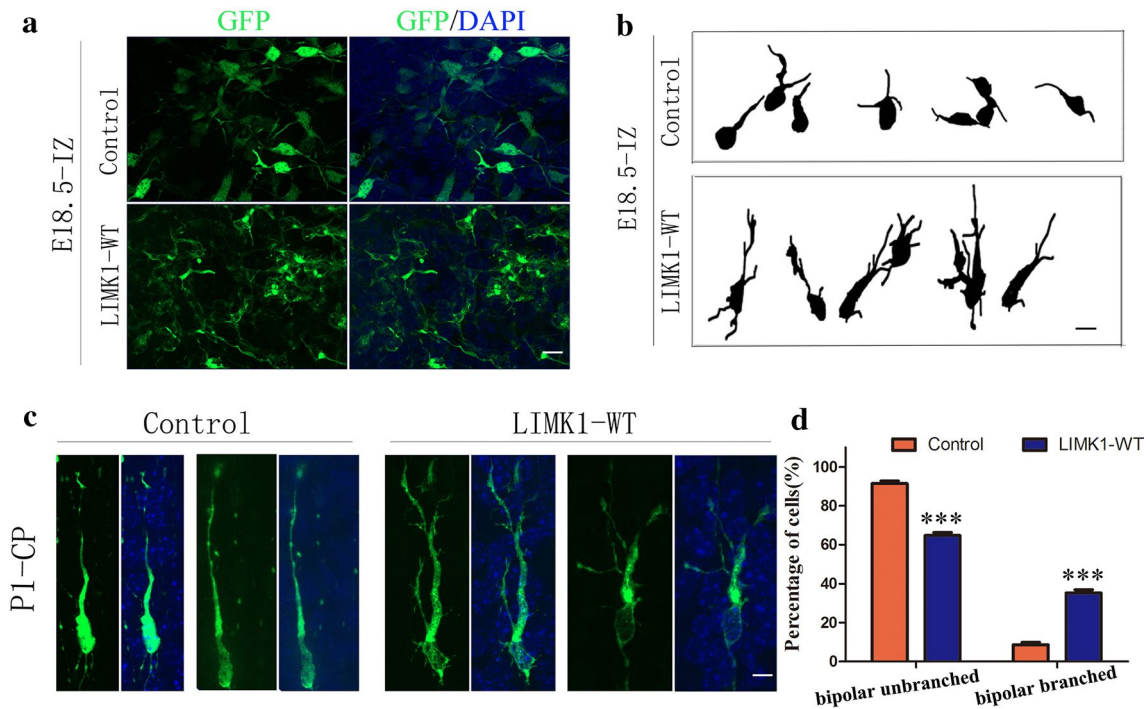


Fig. 2 Overexpression of LIMK1 induces neuronal branching and increases the number of neurites. **a** Increased neurite outgrowth in the IZ at E18.5. **b** Drawings of the cell morphology from high-power magnification. *Scale bar* 10 μ m. **c** High-power magnification of migrating neurons in the CP of P1 brain sections. Sections stained with DAPI (blue). In the control group, the transfected cells showed the typical bipolar morphology of migrating neurons, including the oval soma with a long and thick leading process and a short,

thin trailing process (*left*). In contrast, many LIMK1-WT-transfected neurons gave rise to many branches (*right*). **d** Quantitative analysis of branched neurons in the CP at P1. Data were collected from more than 100 cells in three brains. Statistical significance was assessed using Student's *t* test (DF = 4, $T = 12.853$, $P = 0.0002$ for branched cells; DF = 4, $T = -12.853$, $P = 0.0002$ for unbranched cells; *** $P < 0.001$

et al. 2011; Kawauchi and Hoshino 2008; Ayala et al. 2007). Neurons derived from the ventricular zone (VZ) pass through a multipolar stage to become bipolar and then undergo radial-glia-guided migration to reach their final destination within the cortex (Valiente and Marin 2010; Kriegstein 2005). Recent studies have shown that cofilin plays a critical role in cortical neuronal migration (Chai et al. 2016; Bellenchi et al. 2007). Because LIMK1 is important for the activation of cofilin (Ohashi et al. 2000a; Salvarezza et al. 2009), we investigated whether LIMK1 is also involved in neuronal migration during cortical development. In this study, using in utero electroporation, we found that LIMK1 regulated neuronal migration by changing the morphology of migrating neurons. Increased numbers of neurites and cell branches were found in the LIMK1-WT group. To further examine whether the activation state of LIMK1 was important for its effect on neuronal migration, we analyzed two mutants, LIMK1-CA and LIMK1-DN, and they showed that multiple mechanisms contribute to the migration defects. Moreover, co-transfection of LIMK1-CA and cofilin-S3A partially rescued the neuronal migration defect observed with LIMK1-CA

alone. Taken together, our results suggested that LIMK1 controlled neuronal migration through the regulation of actin dynamics.

LIMK1 is a member of the LIM kinase family, which regulates cell migration, cell morphology and cell proliferation through phosphorylating cofilin (Yoshioka et al. 2003). Because LIMK1 is a critical molecule in Williams syndrome, many studies have focused on neurite outgrowth, spine morphology and synaptic plasticity in the neuronal system (Meng et al. 2002; Rosso et al. 2004). However, the role of LIMK1 in neuronal migration is largely unknown. Using in utero electroporation, we observed that overexpressing LIMK1-WT blocked neuronal migration. At E18.5, neurons had accumulated in the IZ and the VZ/SVZ, and neurons in the IZ showed increased numbers of neurites. Moreover, we obtained tissue at P1, and fewer than 20% of neurons had reached their destination. In addition, more than 30% of neurons remained in the CP and showed abnormal branches. Neurons must transform their morphology from multipolar to bipolar as they migrate from the IZ into the CP, whereas mature neurons possess a similar multipolar morphology (Sakakibara et al. 2014; Sakakibara

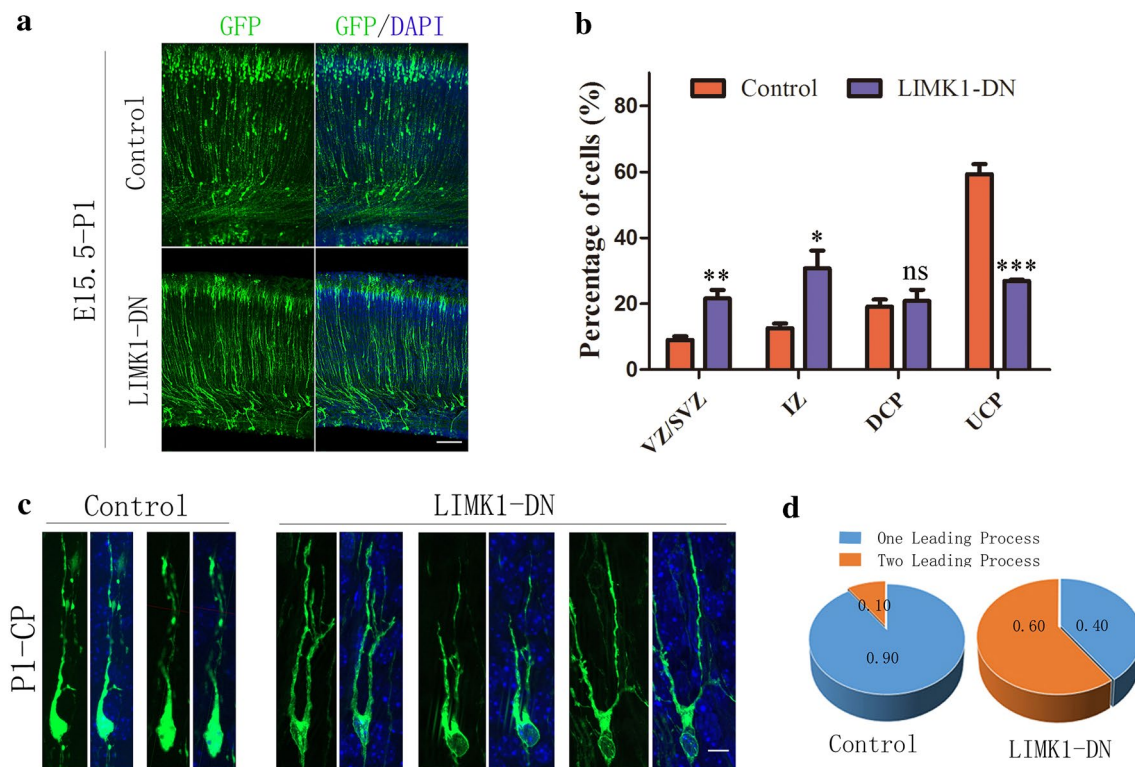


Fig. 3 Overexpression of LIMK1-DN impairs neuronal migration by giving rise to two leading processes. **a** Representative images of EGFP (*top*) and LIMK1-DN (*bottom*) brain sections at P1. Compared to the control group, over 70% of LIMK1-DN-transfected cells failed to migrate to the UCP. Scale bar 100 μ m. **b** Quantitative analysis of the distribution of transfected cells in different layers. More than 5 brain sections were counted. Statistical significance was assessed using Student's *t* test (DF = 8, T = 3.874, P = 0.0047 for VZ/

SVZ; DF = 8, T = 3.119, P = 0.0133 for IZ; DF = 8, T = 0.4233, P = 0.6807 for DCP; and DF = 8, T = 8.928, P < 0.001 for UCP). **c** High-power magnification of neurons transfected with LIMK1-DN in the CP. Some cells gave rise to two leading processes. Scale bar 10 μ m. **d** Quantitative analysis of neurons transfected with LIMK1-DN showing two leading processes. At least 100 cells from three brains were counted. *** P < 0.001; ** P < 0.01; * P < 0.05; *ns* not significant

and Hatanaka 2015). The present study indicated that LIMK1 induced neuronal branching that impaired neuronal migration. Therefore, it is understandable that the transfected neurons accumulated between the IZ and CP but not between the VZ and IZ. We hypothesized that these branches might contribute to the migration defects, with the increase in obstruction inhibiting neuronal migration (Nadarajah et al. 2003; Marin et al. 2006). The morphological changes might result from actin polymerization induced by inactivated cofilin.

Most neurons transfected with LIMK1-CA did not reach their destination, instead remaining in the IZ and DCP. This might be because LIMK1 phosphorylates cofilin and inhibits its ability to bind and depolymerize actin, leading to the accumulation of F-actin aggregates and filaments. Meanwhile, our results showed that the inactivation of LIMK1, in neurons expressing LIMK1-DN, also negatively regulated neuronal migration, possibly because dominant-negative LIMK1 inhibits the accumulation of the F-actin. In migrating cells, the actin cytoskeleton (F-actin) at the leading edge is subjected to an organized process of

assembly/disassembly to move the edge forward (Pollard and Borisy 2003). Therefore, an increase or a decrease in actin cytoskeleton dynamics would impair neuronal migration. Interestingly, overexpressing wild-type LIMK1 in cultured hippocampal pyramidal neurons induced growth cone formation and axon outgrowth at early time points (Rosso et al. 2004). This scenario is similar to our findings that LIMK1-WT gave rise to irregular branches and LIMK1-CA to the formation of longer leading processes in the migratory neurons *in vivo*, even though it is found on the other side of the bipolar neuron. However, LIMK1-DN-transfected cells possessed two leading processes and also impaired neuronal migration. Previous studies have shown that the overexpression of a dominant-negative form of LIMK1 in metastatic cancer cells inhibited motility, invasiveness and metastasis (Davila et al. 2003; Yoshioka et al. 2003). This might be due to the dephosphorylation of cofilin, an actin-depolymerizing protein, which binds to F-actin, thus promoting their disassembly and rearrangement (Jovceva et al. 2007; Kiuchi et al. 2007). Moreover, after the co-transfection of LIMK1-CA and

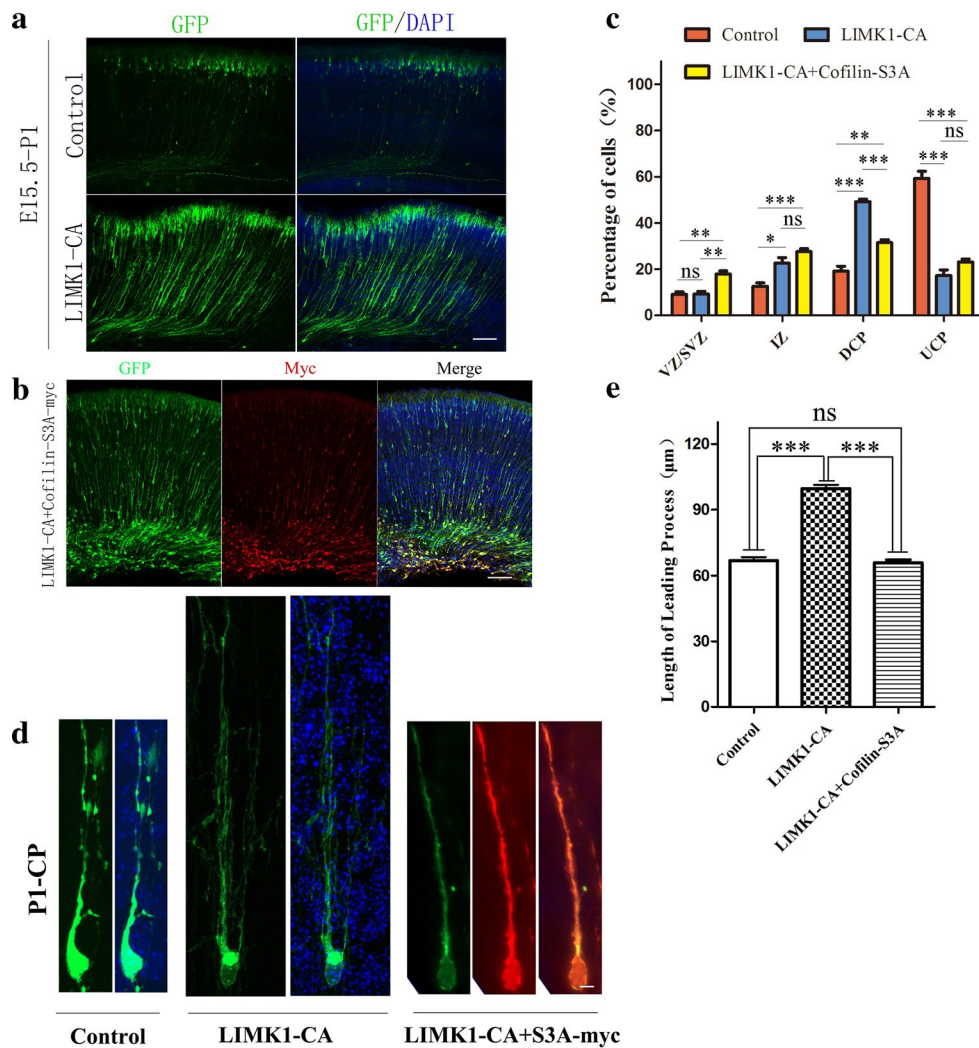


Fig. 4 Cofilin-S3A partially rescued the neuronal migration defect induced by LIMK1-CA. **a** LIMK1-CA was transfected into neurons of the VZ/SVZ, and the brains were fixed and analyzed at P1. Control (top), LIMK1-CA (bottom). Scale bar 100 μm . **b** Co-transfection of LIMK1-CA (green) and cofilin-S3A-myc (red), evaluated at P1. Scale bar 100 μm . **c** Quantitative comparison of the control (EGFP), LIMK1-CA and the co-transfection of LIMK1-CA and cofilin-S3A. Distribution patterns were analyzed at P1. More neurons reached their destinations in LIMK1-CA + cofilin-S3A-co-transfected brains than in LIMK1-CA brains. Statistical significance was assessed using one-way ANOVA (the one-way ANOVA was followed by Dunnett’s multiple comparison test; in VZ/SVZ, $DF = 2$, $F = 17.480$, $P = 0.999$ for control and LIMK1-CA group, $P = 0.002$ for control and LIMK1-CA + cofilin-S3A group, $P = 0.002$ for LIMK1-CA and LIMK1-CA + cofilin-S3A group; in IZ, $DF = 2$, $F = 21.240$, $P = 0.046$ for control and LIMK1-CA group, $P < 0.001$ for control and LIMK1-CA + cofilin-S3A group, $P = 0.307$ for LIMK1-CA and LIMK1-CA + cofilin-S3A group; in DCP, $DF = 2$, $F = 95.046$,

$P < 0.001$ for control and LIMK1-CA group, $P < 0.01$ for control and LIMK1-CA + cofilin-S3A group, $P < 0.001$ for LIMK1-CA and LIMK1-CA + cofilin-S3A group; and in UCP, $DF = 2$, $F = 99.080$, $P < 0.001$ for control and LIMK1-CA group, $P < 0.001$ for control and LIMK1-CA + cofilin-S3A group, $P = 0.192$ for LIMK1-CA and LIMK1-CA + cofilin-S3A group). **d** High-magnification analysis at P1 of neurons in the CP transfected with EGFP, LIMK1-CA or LIMK1-CA + cofilin-S3A. Scale bar 10 μm . **e** Quantitative analysis of the length of the leading process from neurons transfected with EGFP, LIMK1-CA or LIMK1-CA + cofilin-S3A. At least 100 cells from three brains were counted. Statistical significance was assessed using one-way ANOVA (the one-way ANOVA was followed by Dunnett’s multiple comparison test; $DF = 2$, $F = 96.839$, $P < 0.001$ for control and LIMK1-CA group, $P = 0.971$ for control and LIMK1-CA + cofilin-S3A group, $P < 0.001$ for LIMK1-CA and LIMK1-CA + cofilin-S3A group). *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$; ns not significant

of hippocampal pyramidal neurons with cofilin-S3A and LIMK1-WT increased the deployment of Golgi apparatus, while LIMK1-WT alone produced fewer deployments (Quassollo et al. 2015). Surprisingly, we found that

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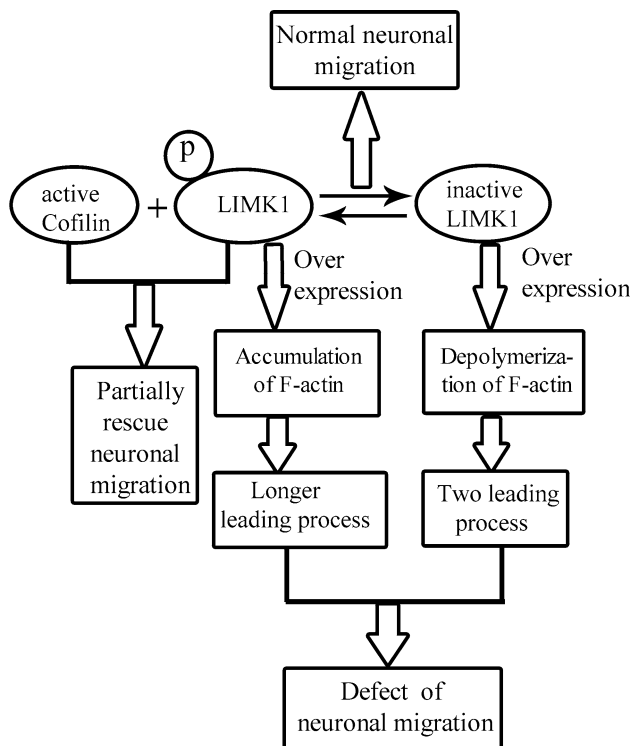


Fig. 5 Schematic drawing of the present study. Neuronal migration was blocked by either the active or the inactive form of LIMK1. After co-transfection of LIMK1-CA and cofilin-S3A, neuronal migration was partially rescued, and the morphological changes in migrating neurons were fully recovered

branches disappeared and that the length of the leading process decreased, which was consistent with previous results regarding the obstruction of neuronal migration. These results showed that cofilin was a downstream target of LIMK1 in mediating neuronal migration into the neocortex. Nevertheless, the cause of this partial rescue requires further study. All in all, we hypothesized that there is a balance between activation and inactivation of LIMK1 in vivo. Once the balance was broken, neuronal migration would be blocked (Fig. 5).

In summary, our results imply LIMK1 is a negative regulator of neuronal migration during brain development. Furthermore, our results might provide a theoretical basis for a cure for Williams syndrome.

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Compliance with ethical standards

Conflict of interest No conflict of interest exists in the submission of this manuscript, and the manuscript was approved by all authors

for publication. The manuscript does not contain clinical studies or patient data.

References

- Arber S, Barbayannis FA, Hanser H, Schneider C, Stanyon CA, Bernard O, Caroni P (1998) Regulation of actin dynamics through phosphorylation of cofilin by LIM-kinase. *Nature* 393(6687):805–809. doi:10.1038/31729
- Ayala R, Shu T, Tsai LH (2007) Trekking across the brain: the journey of neuronal migration. *Cell* 128(1):29–43. doi:10.1016/j.cell.2006.12.021
- Bellenchi GC, Gurniak CB, Perlas E, Middei S, Ammassari-Teule M, Witke W (2007) N-cofilin is associated with neuronal migration disorders and cell cycle control in the cerebral cortex. *Genes Dev* 21(18):2347–2357. doi:10.1101/gad.434307
- Bellugi U, Lichtenberger L, Mills D, Galaburda A, Korenberg JR (1999) Bridging cognition, the brain and molecular genetics: evidence from Williams syndrome. *Trends Neurosci* 22(5):197–207
- Bernard O, Ganiatsas S, Kannourakis G, Dringen R (1994) Kiz-1, a protein with LIM zinc finger and kinase domains, is expressed mainly in neurons. *Cell Growth Differ Mol Biol J Am Assoc Cancer Res* 5(11):1159–1171
- Berry M, Rogers AW (1965) The migration of neuroblasts in the developing cerebral cortex. *J Anat* 99(Pt 4):691–709
- Chai X, Zhao S, Fan L, Zhang W, Lu X, Shao H, Wang S, Song L, Failla AV, Zobiak B, Mannherz HG, Frotscher M (2016) Reelin and cofilin cooperate during the migration of cortical neurons: a quantitative morphological analysis. *Development* 143(6):1029–1040. doi:10.1242/dev.134163
- Davila M, Frost AR, Grizzle WE, Chakrabarti R (2003) LIM kinase 1 is essential for the invasive growth of prostate epithelial cells: implications in prostate cancer. *J Biol Chem* 278(38):36868–36875. doi:10.1074/jbc.M306196200
- Frangiskakis JM, Ewart AK, Morris CA, Mervis CB, Bertrand J, Robinson BF, Klein BP, Ensing GJ, Everett LA, Green ED, Proschel C, Gutowski NJ, Noble M, Atkinson DL, Odelberg SJ, Keating MT (1996) LIM-kinase1 hemizyosity implicated in impaired visuospatial constructive cognition. *Cell* 86(1):59–69
- Gorovoy M, Niu J, Bernard O, Profirovic J, Minshall R, Neamu R, Voyno-Yasenetskaya T (2005) LIM kinase 1 coordinates microtubule stability and actin polymerization in human endothelial cells. *J Biol Chem* 280(28):26533–26542. doi:10.1074/jbc.M502921200
- Govek EE, Hatten ME, Van Aelst L (2011) The role of Rho GTPase proteins in CNS neuronal migration. *Dev Neurobiol* 71(6):528–553. doi:10.1002/dneu.20850
- Ishaq M, Lin BR, Bosche M, Zheng X, Yang J, Huang D, Lempicki RA, Aguilera-Gutierrez A, Natarajan V (2011) LIM kinase 1-dependent cofilin 1 pathway and actin dynamics mediate nuclear retinoid receptor function in T lymphocytes. *BMC Mol Biol* 12:41. doi:10.1186/1471-2199-12-41
- Jovceva E, Larsen MR, Waterfield MD, Baum B, Timms JF (2007) Dynamic cofilin phosphorylation in the control of lamellipodial actin homeostasis. *J Cell Sci* 120(Pt 11):1888–1897. doi:10.1242/jcs.004366
- Kapur R, Shi J, Ghosh J, Munugalavadla V, Sims E, Martin H, Wei L, Mali RS (2016) ROCK1 via LIM kinase regulates growth, maturation and actin based functions in mast cells. *Oncotarget* 7(13):16936–16947. doi:10.18632/oncotarget.7851
- Kawauchi T, Hoshino M (2008) Molecular pathways regulating cytoskeletal organization and morphological changes

- in migrating neurons. *Dev Neurosci* 30(1–3):36–46. doi:[10.1159/000109850](https://doi.org/10.1159/000109850)
- Kiuchi T, Ohashi K, Kurita S, Mizuno K (2007) Cofilin promotes stimulus-induced lamellipodium formation by generating an abundant supply of actin monomers. *J Cell Biol* 177(3):465–476. doi:[10.1083/jcb.200610005](https://doi.org/10.1083/jcb.200610005)
- Kriegstein AR (2005) Constructing circuits: neurogenesis and migration in the developing neocortex. *Epilepsia* 46(Suppl 7):15–21. doi:[10.1111/j.1528-1167.2005.00304.x](https://doi.org/10.1111/j.1528-1167.2005.00304.x)
- Kuzniecky RI (2006) Malformations of cortical development and epilepsy, part 1: diagnosis and classification scheme. *Rev Neurol Dis* 3(4):151–162
- Marin O, Valdeolmillos M, Moya F (2006) Neurons in motion: same principles for different shapes? *Trends Neurosci* 29(12):655–661. doi:[10.1016/j.tins.2006.10.001](https://doi.org/10.1016/j.tins.2006.10.001)
- Meng Y, Zhang Y, Tregoubov V, Janus C, Cruz L, Jackson M, Lu WY, MacDonald JF, Wang JY, Falls DL, Jia Z (2002) Abnormal spine morphology and enhanced LTP in LIMK-1 knockout mice. *Neuron* 35(1):121–133
- Nadarajah B, Alifragis P, Wong RO, Parnavelas JG (2003) Neuronal migration in the developing cerebral cortex: observations based on real-time imaging. *Cereb Cortex* 13(6):607–611
- Noguchi J, Hayama T, Watanabe S, Ucar H, Yagishita S, Takahashi N, Kasai H (2016) State-dependent diffusion of actin-depolymerizing factor/cofilin underlies the enlargement and shrinkage of dendritic spines. *Sci Rep* 6:32897. doi:[10.1038/srep32897](https://doi.org/10.1038/srep32897)
- Ohashi K, Hosoya T, Takahashi K, Hing H, Mizuno K (2000a) A Drosophila homolog of LIM-kinase phosphorylates cofilin and induces actin cytoskeletal reorganization. *Biochem Biophys Res Commun* 276(3):1178–1185. doi:[10.1006/bbrc.2000.3599](https://doi.org/10.1006/bbrc.2000.3599)
- Ohashi K, Nagata K, Maekawa M, Ishizaki T, Narumiya S, Mizuno K (2000b) Rho-associated kinase ROCK activates LIM-kinase 1 by phosphorylation at threonine 508 within the activation loop. *J Biol Chem* 275(5):3577–3582
- Pollard TD, Borisy GG (2003) Cellular motility driven by assembly and disassembly of actin filaments. *Cell* 112(4):453–465
- Proschel C, Blouin MJ, Gutowski NJ, Ludwig R, Noble M (1995) Limk1 is predominantly expressed in neural tissues and phosphorylates serine, threonine and tyrosine residues in vitro. *Oncogene* 11(7):1271–1281
- Quassollo G, Wojnacki J, Salas DA, Gastaldi L, Marzolo MP, Conde C, Bisbal M, Couve A, Caceres A (2015) A RhoA signaling pathway regulates dendritic golgi outpost formation. *Curr Biol* 25(8):971–982. doi:[10.1016/j.cub.2015.01.075](https://doi.org/10.1016/j.cub.2015.01.075)
- Rakic P, Lombroso PJ (1998) Development of the cerebral cortex: I. Forming the cortical structure. *J Am Acad Child Adolesc Psychiatry* 37(1):116–117. doi:[10.1097/00004583-199801000-00026](https://doi.org/10.1097/00004583-199801000-00026)
- Rosso S, Bollati F, Bisbal M, Peretti D, Sumi T, Nakamura T, Quiroga S, Ferreira A, Caceres A (2004) LIMK1 regulates Golgi dynamics, traffic of Golgi-derived vesicles, and process extension in primary cultured neurons. *Mol Biol Cell* 15(7):3433–3449. doi:[10.1091/mbc.E03-05-0328](https://doi.org/10.1091/mbc.E03-05-0328)
- Sakakibara A, Hatanaka Y (2015) Neuronal polarization in the developing cerebral cortex. *Front Neurosci* 9:116. doi:[10.3389/fnins.2015.00116](https://doi.org/10.3389/fnins.2015.00116)
- Sakakibara A, Sato T, Ando R, Noguchi N, Masaoka M, Miyata T (2014) Dynamics of centrosome translocation and microtubule organization in neocortical neurons during distinct modes of polarization. *Cereb Cortex* 24(5):1301–1310. doi:[10.1093/cercor/bhs411](https://doi.org/10.1093/cercor/bhs411)
- Salvarezza SB, Deborde S, Schreiner R, Campagne F, Kessels MM, Qualmann B, Caceres A, Kreitzer G, Rodriguez-Boulan E (2009) LIM kinase 1 and cofilin regulate actin filament population required for dynamin-dependent apical carrier fission from the trans-Golgi network. *Mol Biol Cell* 20(1):438–451. doi:[10.1091/mbc.E08-08-0891](https://doi.org/10.1091/mbc.E08-08-0891)
- Scott RW, Olson MF (2007) LIM kinases: function, regulation and association with human disease. *J Mol Med* 85(6):555–568. doi:[10.1007/s00109-007-0165-6](https://doi.org/10.1007/s00109-007-0165-6)
- Tabata H, Nakajima K (2001) Efficient in utero gene transfer system to the developing mouse brain using electroporation: visualization of neuronal migration in the developing cortex. *Neuroscience* 103(4):865–872
- Tabata H, Nakajima K (2002) Neurons tend to stop migration and differentiate along the cortical internal plexiform zones in the Reelin signal-deficient mice. *J Neurosci Res* 69(6):723–730. doi:[10.1002/jnr.10345](https://doi.org/10.1002/jnr.10345)
- Tassabehji M, Metcalfe K, Fergusson WD, Carette MJ, Dore JK, Donnai D, Read AP, Proschel C, Gutowski NJ, Mao X, Sheer D (1996) LIM-kinase deleted in Williams syndrome. *Nat Genet* 13(3):272–273. doi:[10.1038/ng0796-272](https://doi.org/10.1038/ng0796-272)
- Valiente M, Marin O (2010) Neuronal migration mechanisms in development and disease. *Curr Opin Neurobiol* 20(1):68–78. doi:[10.1016/j.conb.2009.12.003](https://doi.org/10.1016/j.conb.2009.12.003)
- Wang JY, Wigston DJ, Rees HD, Levey AI, Falls DL (2000) LIM kinase 1 accumulates in presynaptic terminals during synapse maturation. *J Comp Neurol* 416(3):319–334
- Yang N, Higuchi O, Ohashi K, Nagata K, Wada A, Kangawa K, Nishida E, Mizuno K (1998) Cofilin phosphorylation by LIM-kinase 1 and its role in Rac-mediated actin reorganization. *Nature* 393(6687):809–812. doi:[10.1038/31735](https://doi.org/10.1038/31735)
- Yoshioka K, Foletta V, Bernard O, Itoh K (2003) A role for LIM kinase in cancer invasion. *Proc Natl Acad Sci USA* 100(12):7247–7252. doi:[10.1073/pnas.1232344100](https://doi.org/10.1073/pnas.1232344100)