

1 **MAP1B shapes radial glial cells and promotes postinjury neural recovery**

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15 Running title: MAP1B regulates radial glia growth

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18 ABSTRACT

19 Microtubule-associated protein 1B (MAP1B) is a crucial cytoskeletal regulator essential
20 for microtubule dynamics during neurite elongation and is active in growth cones. While its
21 functions in neuronal development are well-documented, its specific role in glial cells remains
22 largely unexplored. Here, we investigate Map1b's function in radial glia and its potential for
23 facilitating neural repair in zebrafish. We found that knockdown of *map1b* disrupted the
24 morphology of radial glial processes and impaired the development of spinal motor neurons,
25 leading to aberrant swimming behavior. Using transgenic zebrafish models including
26 *Tg(gfap:eGFP)* and *Tg(mnx1:eGFP)*, we found that *map1b* depletion significantly shortened
27 both radial glial processes and motor neuron axons. Specific depletion of *map1b* expression in
28 radial glia caused abnormalities in motor neurons. Mechanistically, we identify that MAP1B
29 interacts with FIGN and is required for normal FIGN-associated microtubule regulation using
30 rat radial glia, RFL-6 cells, and HEK293T. Of notable therapeutic interest, overexpression of
31 mammalian MAP1B accelerated axonal regrowth following traumatic spinal cord injury and
32 restored motor function. Collectively, our findings establish MAP1B as a dual regulator of glial
33 morphogenesis and neural repair, revealing a novel therapeutic strategy for spinal cord injury
34 that targets cytoskeletal plasticity.

35 Keywords: Radial glia; MAP1B; FIGN; Injury; Neuron

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37 INTRODUCTION

38 Microtubule-associated protein 1B (MAP1B) is a versatile cytoskeletal regulator critical
39 for nervous system development. Canonically, MAP1B is enriched in the cytoplasm of growing
40 axons and growth cones (Black et al., 1994; Nozumi et al., 2009), where it stabilizes
41 microtubules and interacts with actin to orchestrate neurite elongation, branching, and neuronal
42 migration (Gonzalez-Billault et al., 2001; Tymanskyj et al., 2012). This well-established
43 cytoplasmic role underpins many of its functions, and its dysfunction is linked to a range of
44 neurodevelopmental disorders, from sensorineural hearing loss to intellectual disability, and
45 structural brain malformations like periventricular heterotopia (PH) (Cui et al., 2020; Heinzen
46 et al., 2018; Walters et al., 2018).

47 However, the complexity of MAP1B function has recently been expanded by a landmark
48 study from Merino et al. (Merino et al., 2026). By profiling the nuclear and cytosolic proteome
49 of neural stem cells (NSCs), they uncovered a substantial nuclear pool of MAP1B. Their work
50 demonstrates that MAP1B dynamically shuttles to the nucleus, where it interacts with the
51 BRG1-containing BAF chromatin remodeling complex. Intriguingly, nuclear MAP1B
52 promotes NSC maintenance, which is functionally opposite to its role in the cytoplasm, where
53 it facilitates neuronal differentiation. This study thereby established MAP1B as a dual-function
54 protein capable of directly linking cytoskeletal dynamics to transcriptional regulation, a finding
55 with profound implications for understanding neurodevelopment and disease.

56 Despite these advancements, a significant gap remains in our understanding of MAP1B's
57 role in glial cells—a cell type where its function is largely unexplored. Radial glial cells (RGCs)

are not only progenitors but also provide essential scaffolds for neuronal migration and axon guidance (Jurisch-Yaksi et al., 2020). Given that the nuclear-cytoplasmic shuttling of MAP1B is crucial for NSC fate decisions in the developing cortex (Merino et al., 2026), we hypothesized that MAP1B may have equally important, yet distinct, functions in radial glia, potentially via both its canonical cytoplasmic roles and its newfound nuclear capacities.

In addition to its role in neural development, MAP1B has been studied for its function in neural regeneration. MAP1B regulates growth cone guidance and suppresses branching of regenerating axons (Bouquet et al., 2004). In the peripheral nervous system, MAP1B restricts axon branching of regenerating dorsal root ganglion (DRG) axons in vitro (Barnat et al., 2016), suggesting its involvement in axonal regeneration through branch modulation. Notably, prior studies implicated MAP1B in axon growth, growth cone behavior, and regeneration-associated neurite remodeling, but direct *in vivo* evidence testing whether MAP1B modulation can enhance spinal cord repair remains limited.

In this study, using *in vivo* zebrafish models, we address this hypothesis by investigating the specific function of MAP1B in spinal cord radial glia. We demonstrate that MAP1B is essential for the morphogenesis of radial glial processes and thereby regulates the development of spinal motor neurons. Mechanistically, we identify that MAP1B interacts with FIGN and is required for normal FIGN-associated microtubule regulation. Finally, leveraging a spinal cord injury (SCI) model, we reveal the therapeutic potential of MAP1B by showing that its overexpression accelerates axonal regrowth and functional recovery. These findings define a

78 novel MAP1B-FIGN signaling axis, extend its functional repertoire from NSCs to glia, and
79 position MAP1B as a promising target for promoting neural repair.

uncorrected proof

80 MATERIALS AND METHODS

81 Animals

82 All the studies reported here were submitted to and approved (S20220311-001) by the
83 Animal Care and Use Committee of Nantong University. All zebrafish were obtained from the
84 Zebrafish Laboratory of Nantong University. Zebrafish were maintained at 28.0°C under a
85 14/10-hour light/dark cycle. The zebrafish strains used in this study were AB, *Tg(mnx1:eGFP)*
86 *ml2Tg*, *Tg(gfap:eGFP)* *mi2001Tg*, and *Tg(corola:eGFP)* *hkz04Tg*. Sprague-Dawley rat pups
87 (postnatal day 1) were purchased from the Laboratory Animal Center of Nantong University.

88 Microinjection

89 Morpholino oligonucleotides (MO) were purchased from Gene Tools (USA). *map1b*
90 gRNAs were designed manually after obtaining sequence information from NCBI. The
91 sequences of zebrafish *map1b* splicing MO and three *map1b* gRNAs used in this study are
92 listed in Supplementary Table S1. A plasmid encoding mouse *Map1b* (Gene ID: 29456,
93 XM_039101889.2) was purchased from Shenzhen Yanming Biotechnology Co., Ltd. (China),
94 and the coding sequences were cloned into the pEGFP-C1 vector. For rescue experiments,
95 capped mouse *Map1b* mRNA was synthesized using the mMessage mMachine T7 Kit to
96 transcribe the template prepared by PCR using primers T7-EGFP-F and SV40pA-R
97 (Supplementary Table S1) and mouse *Map1b* cDNA clone pEGFP-*Map1b*.

98 Trimmed glass capillaries were used to inject 1 nL of MO (0.5, 0.7, or 1.0 mM, as specified
99 in the Results, purchased from GeneTools, USA) into zebrafish embryos at the one-cell stage

at concentrations of 0.5 mM, 0.7 mM, and 1.0 mM. *map1b* gRNA (100 ng/μL, synthesized by Genscript, China) and Cas9 mRNA (300 ng/μL, prepared from pGH-T7-zCas9 (Liu et al., 2014) using mMessage mMachine T7 kit (AM1344, ThermoFisher, USA)) were mixed and injected into 1-cell stage embryos. *map1b* gDNAs (100 ng/μL) were mixed with NgAgo mRNA (340 ng/μL) and injected into 1-cell stage embryos.

For chimeric labeling of *gfap*⁺ cells, microinjections of 50 ng/μL pEGFP-*gfap* (Intron1/5'/Exon1) (Bernardos & Raymond, 2006) with 0.7 mM *map1b* MO were performed.

To achieve *gfap*⁺ cell-specific downregulation of *map1b*, pTol2-mnx1:EGFP-*gfap*:mCherry-*map1b*_shRNA (40 ng/μL, controlled by a plasmid containing control shRNA), constructed based on the test results of three siRNAs targeting zebrafish *map1b* (Supplementary Table S1), was injected with 50 ng/μL SHT transposase mRNA (Wang et al., 2024). Post-injection, embryos were maintained at 28.0°C and treated with 0.2 mM PTU in 1× E3 Buffer from 12 hpf to inhibit pigmentation and facilitate visualization. Embryos were also cultured in 1× E3 Buffer for behavioral tests. Similarly, rescue of *map1b* shRNA-mediated knockdown was performed using pTol2-mnx1:EGFP-*gfap*:mCherry-T2A-*Map1b*-*map1b*_shRNA, which contains an additional murine *Map1b* coding region following the mCherry open reading frame, interspaced with T2A peptide.

Three siRNAs were designed by GenCefe Biotech Co, Ltd., China, for downregulation of *map1b* in zebrafish (Supplementary Table S1), and injected at a concentration of 50 μM. Based on the knockdown efficiency of these siRNAs, *map1b*-zebrafish-3642 was selected for shRNA plasmid (pTol2-mnx1:EGFP-*gfap*:mCherry-*map1b*_shRNA) construction, in which the *map1b*

shRNA was placed in the 3' UTR following mCherry driven by the *gfap* promoter from pEGFP-*gfap* (Intron1/5'/Exon1). For simultaneous radial glia and CaP neuron labeling and *map1b* depletion, pTol2-mnx1:EGFP-*gfap*:mCherry-*map1b*_shRNA was injected into 1-cell stage zebrafish embryos, using pTol2-mnx1:EGFP-*gfap*:mCherry-control_shRNA as a control.

At least five clutches of fertilized eggs were mixed, randomly separated into groups and injected prior to the following experiments.

Zebrafish behavior analysis

Individual zebrafish were placed in a 24-well plate and tracked continuously for 1 hour with light following a 30-minute acclimation period in a DanioVision system (Noldus, Netherlands). Data analysis and video recording were conducted using the Noldus Ethovision XT 13 software.

Live imaging of zebrafish embryos

Depending on the type of transgenic zebrafish, embryos were embedded in 0.8% low-melting-point agarose for confocal imaging. To obtain clear images, embryos were anesthetized with E3 buffer containing 0.16 mg/mL tricaine. Confocal imaging was performed using a Zeiss LSM 900 microscope. Image analysis was carried out with ZEN (Zeiss, Germany) and ImageJ (<https://imagej.net/software/fiji/>).

Spinal cord injury

Spinal cord injury in zebrafish was performed as previously described (Dong et al., 2025). Briefly, zebrafish at 30 hours-fertilization (hpf) were anesthetized by placing them in E3 buffer containing 0.168 mg/mL tricaine (A5040, Sigma, USA), and a 1 mL insulin pen needle (4 mm x 32 G) was used to create a wound on the dorsal side opposite to the cloacal opening.

Cell Culture

GFAP⁺ cells: Sprague-Dawley rat pups (postnatal day 1) were anesthetized with isoflurane. The spinal cords were dissected and digested, and cell suspensions were filtered through a sterile 75 µm filter. After filtration, the collected cells were allowed to adhere to the plate for 30 minutes. Heterogeneous cells were removed as much as possible (GFAP-positive cells adhere to the plate slowly). Cells were seeded into culture flasks. Once the cells reached confluence, they were cultured in DMEM/F12 medium supplemented with 10% fetal bovine serum, 0.5 mM glutamine and 1% penicillin-streptomycin. Cultures were passaged and purified. GFAP-positive cell cultures with a glial fibrillary acidic protein (GFAP) positivity rate of 95% were considered suitable for subsequent experiments.

HEK-293T and RFL-6: Human renal epithelial cell line 293 T derived from human embryonic renal cells and rat lung-derived fibroblast cell line RFL-6 were cultured in DMEM supplemented with 10% fetal bovine serum, 0.5 mM glutamine, and 1% penicillin-streptomycin. All cells were incubated in a humidified atmosphere of 95% air and 5% CO₂ at 37°C.

Neurons: On the 18th day of pregnancy, Sprague-Dawley rats were anesthetized with ether. Subsequently, they were sacrificed by cervical dislocation and immersed in 75% ethanol for 30 seconds. The fetal rats were then carefully retrieved. Their heads were severed and placed in precooled Hanks' dissection buffer for washing. Next, the cerebral cortex of the fetal rats was dissected out, and 0.25% trypsin digestion solution was added. The digestion process was carried out at 37°C for 12 minutes, during which the mixture was gently shaken for 15 seconds every 4 minutes. After digestion, the trypsin solution was discarded, and serum was added to halt the digestion reaction. The tissue was then thoroughly pipetted to ensure uniform mixing. The cell suspension was passed through a 38- μ m mesh (400 mesh) and centrifuged at 1000 $\times$ g for 5 minutes. The supernatant was discarded, and the cell pellet was resuspended in DMEM/F12 complete medium. The cells were seeded at a density of 5×10^5 cells per square centimeter in poly-L-lysine-coated dishes. After 4 – 6 hours of incubation, the medium was replaced with Neurobasal complete medium.

For *Map1b* knockdown, cells were electroporated with *Map1b* siRNA (Supplementary Table S1) at 275V, 1.0 ms, and were cultured in a 35-mm dish for 24 hours prior to further observation of examination.

Reverse transcription–(quantitative) polymerase chain reaction (RT–(q)PCR)

For zebrafish samples, total RNA was extracted from homogenized tissue using a Quick RNA Extraction Kit (Yishan, China), followed by reverse transcription into cDNA with a Quick Reverse Transcription Kit (Vazyme, China) according to the manufacturer's instructions. Primers used for RT–PCR including *map1b* MO F/R for detection of the knockdown efficiency

of *map1b* MO are listed in Supplementary Table S1. PCR products were analyzed by agarose gel electrophoresis.

For cell samples, total RNA was extracted using the Quick RNA Extraction Kit, reverse-transcribed to cDNA, quantified to 100 ng/ μ L, and used for RT – qPCR to assess the knockdown efficiency of *Map1b*.

Cell proliferation assays

Cells were transfected and, after 24 hours, seeded into 96-well plates at a density of 2×10^3 cells per well. After 24 hours, cells were fixed with 4% PFA and cell proliferation was assessed using the EdU Cell Proliferation Kit (Beyotime, China).

Immunohistochemistry and immunocytochemistry

Zebrafish embryos were harvested and fixed in 4% paraformaldehyde for 30 min, blocked with GCB containing 10% v/v goat serum (SL038, Solarbio, China), and incubated overnight with primary antibody Anti-MAP1B (Rabbit, 1:400, 21633-1-AP, Proteintech, China) for 4 days, followed by incubation with Alexa Flour 647-conjugated AffiniPure Goat Anti-Rabbit IgG (H+L) (1:1000, Jackson ImmunoResearch laboratories, USA) for 48 hrs, for visualization of Map1b expression. Images were taken on a Zeiss Axio Imager M2 with Apotome 2 or Zeiss LSM 900 with Airyscan 2.

For observation of microtubules and F-Actin, cells were fixed with a buffer containing 4% paraformaldehyde, 0.2% glutaraldehyde, $1 \times$ PHEM buffer, and 0.1% Triton X-100 for 20 min,

blocked with GCB containing 10% v/v goat serum (SL038, Solarbio, China), 3% w/v BSA (V900933, Merck), and 3% v/v Triton X-100 (C03-03001, Bioss, China) in 1× PBS, and incubated overnight with primary antibody Anti-MAP1B (Rabbit, 1:400, 21633-1-AP, Proteintech, China). The next day, cells were washed with 1× PBS (3× 10 minutes), incubated with Anti-β-tubulin-Cy3 (Mouse, 1:1000, C4585, Sigma, USA)、Phalloidin-TRITC (for F-Actin staining, 200 nM, HY-P2270, MedChemExpress, USA)、Alexa Flour 647-conjugated AffiniPure Goat Anti-Rabbit IgG (H+L) (1:1000, 111-165-003, Jackson ImmunoResearch laboratories, USA) for 1 hour and 45 minutes, followed by DAPI (5 μg/mL in 1x PBS, D9542, Sigma) for 15 minutes, and washed with PBS (3× 10 minutes). Slides were mounted with Fluoromount-G mounting medium (0100-01, Southern Biotech, USA) and images were captured using a Zeiss LSM 900 with Airyscan 2 confocal microscope.

Rat GFAP⁺ cells were transfected with siRNA (400 nM, *Map1b*-specific or Control) in combination with 50 ng/μL pmCherry-C1 or pmCherry-NLSΔ-FIGN plasmid using NEPA Electroporator (275V, 2.0 ms). Cells were cultured for 48 hrs before fixation in a buffer containing 4% paraformaldehyde, 0.2% glutaraldehyde, 1 × PHEM buffer, and 0.1% Triton X-100 for 20 min. The cells were then stained using mouse anti-Ace-tubulin (Abcam ab24610, 1:500) and Rat anti-Tyr-tubulin (Abcam ab6160, 1:1000) and then secondary antibodies: Alexa Fluor 488 AffiniPure Goat Anti-Rat IgG (H+L) (Jackson ImmunoResearch Laboratories, USA, 111-545-003, 1:800) and Alexa Fluor 647 AffiniPure Goat Anti-Mouse IgG (H+L) (Jackson ImmunoResearch Laboratories, USA, 115-605-003, 1:800). Nuclei were stained using DAPI (MeilunBio MA0128, 1:2000). Cells were washed in PBS for 10 min three times and mounted using Fluoromount-G (Southern Biotech, USA) and imaged using a Zeiss LSM 900 with

220 Airyscan 2 confocal microscope.

221 For analysis of Tyr-tubulin and FIGN in *Map1b* knockdown cells, primary antibodies:
222 anti-tyrosinated Tubulin(Rat, 1:800, ab6160, Abcam, UK) and anti-FIGN (Mouse, 1:100, sc-
223 514956, Santa Cruz, USA), and secondary antibodies Alexa Fluor 488 AffiniPure Goat Anti-
224 Rat IgG (H+L), Alexa Flour cy3-conjugated AffiniPure Goat Anti-Mouse IgG (H+L), Alexa
225 Flour 488-conjugated AffiniPure Goat Anti-Mouse IgG (H+L), and Alexa Fluor cy3-
226 conjugated AffiniPure Goat Anti-Rat IgG (H+L) purchased from Jackson ImmunoResearch
227 Laboratories were used.

228 Western blot

229 Cells were electroporated with using NEPA Electroporator (275V, 1.0 ms) to knock down
230 *Map1b*, *Map1b*- or control- siRNA was used at a concentration of 40 μ M (siRNA sequence
231 listed in Supplementary Table S1), then seeded into culture dishes. After 48 hours, cells were
232 lysed with buffer containing protease and phosphatase inhibitors. Total protein was extracted,
233 quantified, and separated by SDS-PAGE. Proteins were transferred to a PVDF membrane
234 (Millipore, Boston, MA, USA), blocked with 5% milk, and incubated overnight at 4°C with
235 primary antibodies anti-MAP1B (Rabbit, 1:800, 21633-1-AP, Proteintech, China), anti-
236 GAPDH (Mouse, 1:1000, Proteintech, China), anti-FIGN (Mouse, 1:100, sc-514956, Santa
237 Cruz), anti-tyrosinated Tubulin(Rat, 1:800, ab6160, Abcam, USA), and anti- α / β -Tubulin
238 (Rabbit, 1:4000, 2148S, Cell Signaling Technology, USA). The membrane was washed with
239 0.1% Tween-20 in 1 $\times$ TBST (13.9 g Tris-base, 60.6 g Tris-HCl, 90 g NaCl, adjusted to pH 7.6
240 with double-distilled water) and incubated with secondary antibodies (HRP-Goat anti mouse

IgG (H+L) A0216, HRP-Goat anti rabbit IgG (H+L) A0208, and HRP-Goat anti rat IgG (H+L) A0192, 1:2000, purchased from Beyotime, China) at room temperature for 2 hours. Bands were visualized and analyzed using ImageJ.

Wound healing Assay

Cells were transfected and, after 24 hours, seeded into dishes at a density of 5×10^5 cells. Wounds were created at 12 hours post-seeding, and migration was observed under a microscope at 0, 12, and 24 hours. Migration areas were analyzed using ImageJ.

Transwell assay

Cells were transfected and, after 24 hours, seeded into Transwell chambers (3422, Corning, USA) at a density of 2×10^3 cells per well. After 24 hours, cells were fixed with 4% PFA and stained with crystal violet for 20 minutes at room temperature. Non-migrated cells were removed with cotton swabs, and migrated cells were imaged and quantified using ImageJ.

Live-cell imaging

Cells transfected with plasmid EB3-EGFP or LifeAct-EGFP (constructed in-house) were seeded into glass-bottom confocal dishes (Biosharp, China) at a density of 1×10^4 cells per well. After 24 hours, live-cell imaging was performed using a Zeiss LSM 900 confocal microscope.

The pmCherry- Δ NLS-FIGN (pmCherry-FIGN) plasmid was constructed in-house based on the methodology described in this article (Li et al., 2020). The pEGFP-MAP1B plasmid was purchased from Shenzhen Yanming Biotechnology Co., Ltd. (China). Cells transfected with

pmCherry-FIGN and pEGFP-MAP1B were seeded into glass-bottom confocal dishes (Biosharp, China) at a density of 1×10^4 cells per well. After 24 hours, live-cell imaging was performed using a Zeiss LSM 900 confocal microscope.

Co-immunoprecipitation of MAP1B and FIGN

The co-immunoprecipitation (co-IP) methods used in this study have been published earlier (Sun et al., 2025). GFAP-positive cells were isolated as described above. The cultured cells were collected, washed twice with PBS, and total proteins were extracted using IP cell lysis buffer (containing protease inhibitors and phosphatase inhibitors). The concentration of total proteins was determined using the BCA protein quantification kit (P0009, Beyotime, China).

The above total protein sample was mixed with 6X SDS loading buffer and boiled for 5 minutes (set at $1 \mu\text{g}/\mu\text{L}$ as the Input control), while the remaining proteins were diluted to $5 \mu\text{g}/\mu\text{L}$ with IP cell lysate for further use. For every 3 mg of total protein, 3 μg of anti-MAP1B (Rabbit, 1:800, 21633-1-AP, Proteintech, China) was added and incubated overnight at 4°C with constant rotation. Protein A/G agarose beads (PR40025, Proteintech, China) were washed three times with prechilled PBS, mixed with the above sample and incubated at 4°C for 4 hrs. The mixture was then centrifuged, and the supernatant was kept as post-binding. The beads were washed using wash buffer for 5 times, and the supernatant was kept as Wash 5. The sample was mixed with SDS loading buffer and boiled for 10 minutes. Finally, the samples were centrifuged at 1000 g for 1 minute to completely remove the beads, and the supernatant was kept. Anti-FIGN (Mouse, 1:100, sc-514956, Santa Cruz, USA) antibody was used to detect

any interaction between MAP1B and FIGN. For the reverse co-immunoprecipitation, the same procedure was carried out using anti-FIGN (Mouse, 1:100, sc-514956, Santa Cruz, USA) antibody, and anti-MAP1B (Rabbit, 1:800, 21633-1-AP, Proteintech, China) was used to detect any interaction between FIGN and MAP1B.

Detection of FIGN-interacting proteins using mass spectrometry

Primary rat neurons were cultured for two days and then were first washed three times with 1xPBS. Subsequently, an appropriate volume of immunoprecipitation lysis buffer (87787, Thermo, USA) was added to extract total nuclear and cytoplasmic proteins (P0028, Beyotime, China). The protein concentration was determined using the BCA assay method. The total proteins were then diluted to a concentration of 1 µg/µL, and a fraction of the sample was set aside as an input control. Next, 40 µL of Pierce Protein A Plus Agarose (Cat No. PR40025, Proteintech, China) was carefully aspirated and added to 800 µL of precooled PBS. This mixture was centrifuged at 5000×g for 1 minute and then washed three times. Using 500 µL of the protein sample as the experimental system, 40 µL of the washed Pierce Protein A Plus Agarose was added. The mixture was incubated at 4°C with gentle rotation for 30 minutes. Following this incubation, the supernatant was centrifuged at 12000 × rpm for 10 minutes at 4°C and then transferred to a new centrifuge tube. According to the proportion specified in the instructions, 2-5 µg of FIGN antibody (Anti FIGN, sc 514956, Santa Cruz, USA) was added. As a control group, an equivalent amount of immunoglobulin G (mouse IgG, 3420S, Cell Signaling Technology, USA) was added. The antigen-antibody mixture was incubated

overnight at 4°C in a rotator. Finally, the enriched protein complex was identified by mass spectrometry.

Bioinformatics

The sequence information for zebrafish *map1b* was obtained from NCBI (<https://www.ncbi.nlm.nih.gov>). Transcripts of *map1b* were analyzed using Snapgene (<https://www.snapgene.com>), and PCR primers were designed using Primer Premier 5 (<http://www.premierbiosoft.com/>). Zebrafish development single cell RNAseq dataset GSE112294 (Wagner et al., 2018) was analyzed for *map1b* expression. Mouse spinal cord injury single cell RNAseq dataset GSE172167 (Matson et al., 2022) was analyzed for *Map1b* expression.

Measurement and statistical analysis

With a setting of $\alpha=0.05$, power $(1-\beta)=80\%$, Cohen's $d=2.5$, the required sample size was estimated to be roughly five.

Axon and process lengths were measured using ImageJ software. All data analysis, statistical comparisons, and graphing were conducted using GraphPad Prism, adhering to methods described in our previous studies (Dong et al., 2019).

Cell migration was quantified by measuring the area using ImageJ. Live cell data were tracked manually using the trackmate plugin for ImageJ for EB3 comets and analyzed for filopodia activity with the Multi Kymograph plugin for Life-Act-eGFP. Microtubule

cytoskeleton orientation data were obtained with the OrientationJ plugin in ImageJ. All figures were prepared using Adobe Illustrator 2021.

One-way analysis of variance (ANOVA) with Dunnett's multiple comparisons tests and Student's *t*-test was used to assess significance of differences. Data are presented as Mean $\pm$ S.D..

RESULTS

Depletion of *map1b* causes deficit in zebrafish motor function

To explore the potential role of Map1b in nervous system development, we first analyzed published single-cell RNA sequencing data (Wagner et al., 2018) of early zebrafish embryogenesis. The data (Figure 1A) revealed broad expression of *map1b* across multiple neural lineages, suggesting its involvement in diverse neurodevelopmental processes. In addition to its high expression in neurons, *map1b* is notably expressed in radial glia (*gfap*⁺) during early development (Figure 1B). This initial broad expression pattern prompted us to investigate its general impact on neural function.

To verify *map1b* distribution during early zebrafish development, we conducted immunofluorescence analysis at 24 and 54 hours post-fertilization (hpf) in transgenic zebrafish line *Tg(gfap:eGFP)*. Using structured illumination microscopy for optical sectioning, Map1b was found widely expressed in various tissues including the spinal cord (Supplementary Figure S1A).

339 To functionally interrogate *map1b*, we designed splice-blocking morpholino
340 oligonucleotides (MOs) targeting the exon 1–intron 1 junction (Supplementary Figure S1B).
341 RT–PCR and quantitative PCR analyses confirmed a significant and sustained reduction in
342 *map1b* mRNA levels from 30 hours post-fertilization (hpf) through 72 hpf following MO
343 injection, indicating effective and persistent knockdown (Supplementary Figure S1C, D). The
344 reduced intensity of the bands may be due to the decay of mis-spliced transcripts.

345 We next performed behavioral analyses on zebrafish larvae subjected to *map1b*
346 knockdown at 7 dpf to identify any deficits in motor function. Compared to controls, *map1b*
347 knockdown larvae exhibited a significant decrease in swimming velocity (Figure 1C, D).
348 Critically, co-injection of *map1b* MO with *Map1b* mRNA to restore Map1b function rescued
349 these behavioral deficits to the control level (Figure 1C, D). Furthermore, CRISPR/Cas9-
350 generated *map1b* crispants recapitulated the same behavioral phenotypes observed with MO
351 knockdown (Supplementary Figure S1E, F). Together, these results suggest that decreased
352 *map1b* expression directly causes locomotor abnormalities in zebrafish.

353 To assess the morphological consequences of *map1b* loss, we examined spinal motor
354 neurons in *Tg(mnx1:eGFP)* transgenic zebrafish, which express EGFP. At 30 and 54 hpf,
355 knockdown of *map1b* resulted in a notable shortening of axons in caudal primary neurons
356 which are labeled by the *mnx1:eGFP* transgene and project their axons ventrally (Figure 1E-
357 H). In addition, *map1b* knockdown did not affect the migration of *coro1a*⁺ cells into the central
358 nervous system at 72 hpf (Supplementary Figure S1G, H).

Together, these results suggest that decreased *map1b* expression directly causes locomotor abnormalities in zebrafish. However, due to the global distribution of MO and CRISPR/Cas9, the downregulation of *map1b* is not cell type specific. As a result, the observed deficits of motor function and motor neuron axon growth might not be solely due to loss of Map1b in neurons. This raised a crucial question: what specific cell types mediate these observed developmental defects, particularly given the prominent expression of *map1b* in radial glia?

Depletion of *map1b* in radial glia disturbs axon growth in CaP motor neurons

Building upon the general developmental defects observed in *map1b* knockdown zebrafish, we next focused on the specific role of Map1b in radial glial cells (RGCs), a cell type where its function is largely unexplored despite their crucial role in nervous system development and as scaffolds for neuronal migration and axon guidance (Johnson et al., 2016; Jurisch-Yaksi et al., 2020; Miranda-Negron & Garcia-Arraras, 2022). We hypothesized that Map1b in radial glia might non-cell-autonomously influence neuronal development.

While Map1b's functions in neurons are well-documented (Dajas-Bailador et al., 2012; Tint et al., 2005; Tymanskyj et al., 2012; Yang et al., 2012; Ziak et al., 2024), its role in glial cells remains poorly understood. Radial glia are crucial supportive cells during nervous system development. Therefore, we investigated the impact of depletion of *map1b* on these cells using *Tg(gfap:eGFP)* transgenic zebrafish, which specifically label radial glia during early neural development.

We found that knockdown of *map1b* significantly shortened the radial processes of spinal cord radial glia at both 30 and 54 hpf (Figure 2A–D). This phenotype was fully rescued by co-injecting *Map1b* mRNA, while overexpression of *Map1b* further promoted process elongation (Figure 2A–D). These results indicate that *Map1b* expression levels is related to the extent of radial glial process outgrowth. To rule out off-target effects, we employed two independent gene perturbation methods—the NgAgo-gDNA system (Dong et al., 2023) and CRISPR/Cas9 mutagenesis (crispants)—both of which reproduced similar phenotypes, reinforcing the specificity of our findings (Supplementary Figures S2, 3). In addition to shorter processes, loss of *map1b* led to mispositioning of radial glial cell somata (Figure 2A, B; Supplementary Figure S2C, D; Supplementary Figure S3C, D).

However, in *Tg(gfap:eGFP)* transgenic animals, radial glia are tightly positioned, and we were unable to identify single radial glia for morphological observation. We then performed sparse labeling of individual *gfap*⁺ cells, by injecting pEGFP-gfap (Intron1/5'/Exon1), to get a clearer view of the morphological alterations in radial processes, and observed shorter radial processes (Figure 2E, F).

Having established that *map1b* is essential for radial glial morphogenesis, we then investigated the critical question of whether *map1b* deficiency specifically in radial glia could affect neurons, thereby mediating the motor neuron defects observed in global *map1b* knockdown animals. A pivotal question was whether *map1b* deficiency specifically in radial glia could affect neurons. We addressed this by constructing an shRNA expression vector for glia-specific *map1b* knockdown under the control of *gfap* promoter (Supplementary Figure S4).

When *map1b* was downregulated only in radial glia, the axons of spinal motor neurons, whose own *map1b* expression remained intact, were markedly shorter (Figure 2G, H). Moreover, these larvae exhibited reduced motor performance at 7 dpf (Figure 2I, J). This suggests that normal Map1b function within radial glia is essential for the proper development and function of neighboring motor neurons, highlighting a critical non-cell-autonomous role for glial Map1b in shaping neural circuits.

Depletion of MAP1B inhibits GFAP⁺ cell migration and affects microtubule and actin cytoskeleton in the cell cortex

To elucidate the cellular and molecular mechanisms underlying how Map1b in radial glia influences both glial morphogenesis and, consequently, motor neuron development, we investigated its function in vitro using primary cultured spinal cord GFAP⁺ cells isolated from P1 rats. Given Map1b's known role as a cytoskeleton-associated protein, we focused on its impact on cell migration and cytoskeletal dynamics. We started by validating the stemness of these cells by immunostaining of SOX2 and Nestin, and confirmed these cells are positive of both (Supplementary Figure S5). EdU labeling showed that MAP1B knockdown did not affect cell proliferation (Figure 3A-D). However, scratch wound healing assays (Figure 3 E, F) and Transwell migration assays (Figure 3G-I) consistently revealed that MAP1B knockdown significantly impaired cell migration.

Immunofluorescence staining showed co-localization of MAP1B with microtubules and actin filaments in the cell cortex (Figure 3J). Upon MAP1B depletion, the cortical cytoskeleton lost its organized polarity (Figure 3K-M). Live-cell imaging provided further mechanistic

insight: MAP1B knockdown increased the speed and travel distance of EB3 comets (marking growing microtubule plus-ends) but reduced comet density per unit area (Figure 3N, O). Concurrently, the dynamic activity of lamellipodia, including protrusion/retraction switching frequency and velocity, observed using LifeAct (Ryan et al., 2017), was markedly diminished (Figure 3P, Q). These findings indicate that MAP1B regulates migratory capacity of radial glia by modulating the dynamics and organization of the cortical microtubule and actin networks, providing a cellular mechanism for the observed defects in radial glial morphology and their ability to support neuronal development.

MAP1B regulates cell migration by controlling function of FIGN

Having identified that MAP1B regulates radial glial migration and cytoskeletal organization, we next sought to uncover the molecular players mediating these effects. The observed increase in EB3 comet travel speed in MAP1B-depleted cells suggested altered microtubule plus-end stability, potentially due to attenuated microtubule-severing activity. Based on prior co-immunoprecipitation coupled with mass spectrometry (Co-IP/MS) analysis in neurons, we identified a putative interaction between MAP1B and the microtubule-severing protein Fidgetin (FIGN) (Supplementary Table S2), prompting us to investigate this interaction further.

Interestingly, knockdown of FIGN produced phenotypes strikingly similar to those seen after MAP1B depletion: extended microtubule labile domain (reported in our previous study (Hu et al., 2017), and also shown with elongated EB3 comet travel distance in Figure 4A, B) and reduced lamellipodial activity (protrusion/retraction velocity and switching frequency,

Figure 4C, D). This hinted at a functional connection. We then sought to verify if there is a direct physical interaction between MAP1B and FIGN. Plasmids expressing mCherry-FIGN and EGFP-MAP1B, respectively, were transfected into HEK293T cells, and co-immunoprecipitation assays were performed to detect the interaction between mCherry-FIGN and EGFP-MAP1B (Figure 4E, F). Fluorescence co-localization (Figure 4G, H) and Förster resonance energy transfer (FRET) experiments (Supplementary Figure S6) in GFAP⁺ cells further suggested that the two proteins form a complex.

We then tested whether FIGN overexpression could compensate for the loss of MAP1B by expressing *Fign* in *Map1b*-depleted GFAP⁺ cells. However, expressing *Fign* in MAP1B-knockdown cells failed to rescue the disrupted cytoskeletal polarity (Figure 4I). Furthermore, while FIGN remained associated with microtubules after MAP1B depletion (Figure 4J), its protein levels were paradoxically elevated, accompanied by an increase in tyrosinated tubulin (Figure 4K-N), which is a marker of dynamic, labile microtubules, to be severed by FIGN (Hu et al., 2017; Li et al., 2020; Ma et al., 2023). We examined the mRNA level of *Fign* after *Map1b* knockdown. In consistency to its protein level, *Fign* mRNA was upregulated after the depletion of *Map1b* (Figure 4O). These results suggest that the absence of MAP1B weakens the activity of FIGN, resulting elevated level of tyrosinated tubulin, which in turn triggers an increase in the expression of FIGN. These data collectively indicate that FIGN's microtubule-severing activity is compromised in the absence of MAP1B, positioning MAP1B as a necessary factor enabling proper FIGN function. This novel MAP1B-FIGN signaling axis provides a critical mechanism for how MAP1B controls cytoskeletal dynamics in glial cells.

MAP1B facilitates neuronal regeneration and motor function recovery following spinal cord injury in zebrafish

Beyond its crucial roles in neural development and glial morphogenesis, we also investigated Map1b's function in the context of neural regeneration. Analysis of single-cell RNA-seq data from mouse spinal cord after injury (Matson et al., 2022) revealed a dynamic expression pattern for *Map1b*: a transient upregulation followed by recession across multiple cell types, suggesting its involvement in the post-injury repair response (Figure 5A). This dynamic expression pattern prompted us to test whether manipulating Map1b could facilitate functional recovery after spinal cord injury.

We tested this hypothesis in a zebrafish spinal cord injury model (Figure 5B). Following injury in *Tg(mnx1:eGFP)* larvae, knockdown of *map1b* delayed axonal regrowth, as reflected by the fluorescence intensity produced by the transgene in motor neurons at the lesion site (Figure 5C–E), and impaired the recovery of locomotor function at 3 and 7 days post-injury (Figure 5F–H). Conversely, overexpression of *Map1b* significantly enhanced axonal regrowth (Figure 5I–K), and accelerated the restoration of motor function (Figure 5L–N). These results establish that *Map1b* expression facilitates neural repair processes after spinal cord injury, supporting its therapeutic potential beyond its developmental functions.

DISCUSSION

This study establishes a novel and context-dependent role for MAP1B in the nervous system, integrating its functions across different cell types and developmental/pathological stages. We

demonstrate that beyond its well-characterized roles in neurons, MAP1B is indispensable for the morphogenesis and function of radial glial cells. Furthermore, we uncover a novel molecular mechanism—the regulation of the microtubule-severing protein FIGN by MAP1B—which mediates its effects on the cytoskeleton. Finally, we translate these findings to a pathological context, showing that MAP1B overexpression significantly enhances motor recovery after spinal cord injury. Our work, when considered alongside the recent findings of Merino et al. (2026), supports an emerging view of MAP1B as a master integrator of cellular state, whose function is exquisitely dependent on its subcellular localization, cell type, and physiological context.

Motor neuron morphology and function relies on radial glial *map1b* expression

Our data extend the canonical view of Map1b as a neuronal cytoskeletal regulator by revealing a critical non-cell-autonomous role for glial Map1b in shaping neural circuits. The observation that *map1b* knockdown in radial glia, sparing its expression in neurons, is sufficient to cause motor axon shortening and behavioral deficits reveals a non-cell-autonomous mechanism. This suggests that radial glia, acting as crucial neural progenitors and scaffolds, likely influence motor neuron development through multiple communication pathways. These pathways could include contact-mediated guidance, for instance, through direct interactions with neuronal axons via cell adhesion molecules (CAMs) or extracellular matrix components, providing physical support and directional cues for axon growth.

The mispositioning of radial glial somata and morphological deficit in radial processes upon *map1b* loss may further disrupt this supportive architecture, thereby indirectly affecting

neuronal migration and axon pathfinding. Specifically, the altered morphology of radial glial cells, particularly the shortening of their radial processes and their abnormally positioned somata, could directly impact neuronal migration pathways and the physical guidance of axons, leading to impaired motor neuron axon growth (Ferent et al., 2020; Meyerink et al., 2020), as confirmed by our observation that *gfap*-positive cell-specific downregulation of *map1b* lead to changes in the positioning and morphology of neuron cell bodies and axons (Figure 2G). Additionally, as a source of neural stem cells, impaired Map1b function in radial glial cells might affect their proliferation and differentiation capabilities, thereby indirectly influencing neurogenesis and neuronal integration (Ferent et al., 2020; Meyerink et al., 2020). These findings align with emerging evidence that glial cells actively sculpt neural circuits and that their cytoskeletal integrity is fundamental to this role. While this study reveals the non-cell-autonomous effect of Map1b deficiency in radial glial cells on motor neuron development, future research will need to precisely dissect the underlying communication mechanisms through more specific functional experiments, such as co-culture experiments, conditioned medium analysis, or targeted blockade of intercellular signaling pathways.

MAP1B-FIGN interaction in cytoskeletal regulation in migrating glia

To delve into the molecular underpinnings of how MAP1B regulates radial glial function, as demonstrated in Results 3.3, our experiments in cultured cells provide mechanistic depth. We show that MAP1B supports cell migration by modulating cortical microtubule and actin dynamics. The identification of FIGN as a direct MAP1B interactor offers a precise molecular handle. The paradoxical increase in FIGN protein and tyrosinated tubulin upon MAP1B

knockdown, coupled with the loss of FIGN's functional output (evidenced by elongated EB3 comets), indicates that MAP1B does not recruit FIGN but is in support of its proper microtubule-severing activity. We propose a model where the MAP1B-FIGN complex locally fine-tunes microtubule stability and turnover at the cell cortex, which in turn influences lamellipodial dynamics to drive directed migration (Figure 6). This mechanism is likely conserved and operational during radial glial morphogenesis *in vivo*.

In zebrafish with overexpressed murine *Map1b*, in addition to longer radial processes, the cell bodies of radial glia have greater circularity and stronger fluorescence near the cell membrane, suggesting structural changes in the cell bodies, which may be related to MAP1B's function in the nucleus reported by Merino et al. (2026) and requires further studies. Related to this morphological change, we observed a slight behavioral change in zebrafish with overexpressed *Map1b*, however, this subtle change requires further verification in future studies.

MAP1B's nuclear and cytoplasmic functions

The discovery that MAP1B functions in the nucleus to regulate NSC fate (Merino et al., 2026) profoundly reshapes our interpretation of our own results, particularly the morphological changes observed in radial glia. This highlights a dual-compartment model for MAP1B function. The nuclear enrichment of MAP1B promotes stemness, while its cytoplasmic enrichment promotes differentiation. This suggests a fascinating model where MAP1B's subcellular ratio is critical for cell fate and morphology. In NSCs, a higher nuclear/cytosolic ratio sustains progenitor identity. In post-mitotic or non-dividing cells like radial glia, a predominantly cytosolic localization is crucial for executing morphological and supporting

functions, necessary for circuit formation. In our study, the radial glia-specific knockdown of MAP1B likely impairs the cytoplasmic pro-differentiation and motility functions in these supportive cells, resulting in stunted glial processes and a failure to properly guide developing motor neurons. Apart from the morphology of radial glia shaped by Map1b, the morphological and functional changes in neurons may well be related to the nuclear function of Map1b.

This dual-compartment model also offers a powerful lens to reinterpret the etiology of periventricular heterotopia (PH). Merino et al. (2026) showed that disease-linked MAP1B mutations lead to nuclear enrichment and enhanced BRG1 binding, causing neuronal ectopia in organoids. Our data show that MAP1B dysfunction disrupts the cytoskeleton of supporting glial cells, indirectly impairing neuronal migration via a non-cell-autonomous mechanism. Therefore, PH might not be a simple "migration disorder" of neurons alone, but a "developmental niche disorder" where aberrant MAP1B function in both NSCs (within the nucleus) and glial cells (within the cytoplasm) converges to disrupt the complex environment required for proper brain formation. This dual-hit hypothesis, combining intrinsic neuronal fate changes with extrinsic glial support failure, represents a significant conceptual advance.

MAP1B as a modulator of neural repair

The dynamic regulation of *Map1b* expression after spinal cord injury and the plausible effects of its manipulation on regeneration outcomes position MAP1B as a key intrinsic regulator of repair. Its transient upregulation may represent an early, pro-regenerative response, while its subsequent decline could contribute to the inhibitory environment of the chronic lesion, as suggested by (Barnat et al., 2010; Soares et al., 2007; Wang et al., 2022). Our gain-of-function

experiments, where *Map1b* overexpression enhanced both axonal sprouting and functional recovery, are particularly promising. They suggest that bolstering MAP1B function, potentially in both neurons and glia, may overcome some of the intrinsic and extrinsic barriers to regeneration. However, this study is based on larval zebrafish and early injury response, which is different from the case of adult mammalian SCI. Future work should investigate the efficacy of manipulating the MAP1B-FIGN axis in adult mammalian SCI models.

Strengths and limitations

We used zebrafish as a visualizable *in vivo* model to identify a novel role of Map1b in regulating neural development *via* cell-cell interaction, supporting that Map1b is essential for active cytoskeleton dynamics in radial glia and the proper radial glia scaffold to support neuron development. However, the larval zebrafish *in vivo* model, undergoes development during injury and following observation. Although uninjured baseline data have been included to correct post-injury recovery data, this study does not directly establish mammalian MAP1B as an independent therapeutic target for SCI.

The FRET experiments were performed in RFL-6 and not radial glia because primary radial glia were hard to accept both plasmid in the transfection. However, combined with endogenous co-IP data, the FRET data support the interaction between MAP1B and FIGN. Based on our observation in radial glia, RFL-6, and HEK-293T cells, the interaction between MAP1B and FIGN is conserved in various cell types.

Actin dynamics was affected by the loss of MAP1B in radial glia. Although MAP1B has been studied in neurons for its function on the cross-talk between microtubules and microfilaments (Gonzalez-Billault et al., 2001), future studies are required to learn how actin dynamics is regulated by MAP1B in radial glia.

Conclusions and future directions

In conclusion, we have uncovered a multifaceted role for MAP1B that bridges glial biology, cytoskeletal regulation, and neural repair. The study shifts the paradigm by showing that MAP1B's function in glia is as critical as its role in neurons for circuit formation. The mechanistic link to FIGN provides a new target for modulating cytoskeletal dynamics in development and disease. Our findings collectively highlight MAP1B as a master integrator of cellular state, orchestrating both neuronal and glial functions to maintain nervous system integrity and facilitate repair, thus offering a comprehensive understanding of its pivotal role in neurodevelopment and regeneration.

Several important questions arise from this work, guiding our future research endeavors. First, to precisely delineate the non-cell-autonomous mechanism, we will investigate the specific signals exchanged between MAP1B-deficient radial glia and motor neurons that lead to stalled axon growth. This will involve employing advanced co-culture systems and *in vivo* genetic manipulation in zebrafish, coupled with single-cell transcriptomics and proteomics, to identify novel paracrine factors or cell-surface adhesion molecules. Second, we aim to determine whether the MAP1B-FIGN complex exhibits distinct roles across different neural cell types. This will be achieved through cell-type-specific knockout/knockdown studies *in vitro* and *in*

606 vivo, combined with high-resolution imaging techniques like super-resolution microscopy and
607 FRET, to map the precise spatiotemporal interactions and their functional consequences on
608 microtubule dynamics and cellular processes in neurons, astrocytes, and oligodendrocytes.
609 Finally, a critical future direction is to translate these fundamental insights into therapeutic
610 strategies. We will explore the development of small molecules or gene therapies targeting the
611 MAP1B-FIGN pathway to promote recovery after central nervous system trauma or in
612 neurodegenerative conditions. This will involve high-throughput screening for novel MAP1B
613 agonists or FIGN modulators, and optimizing gene delivery approaches, such as engineered
614 AAV vectors, to overcome the current limitations of MAP1B cDNA size. Furthermore,
615 rigorous validation in adult mammalian SCI models will be essential to bridge the gap between
616 our zebrafish findings and potential clinical applications, paving the way for novel
617 interventions in neurological disorders.

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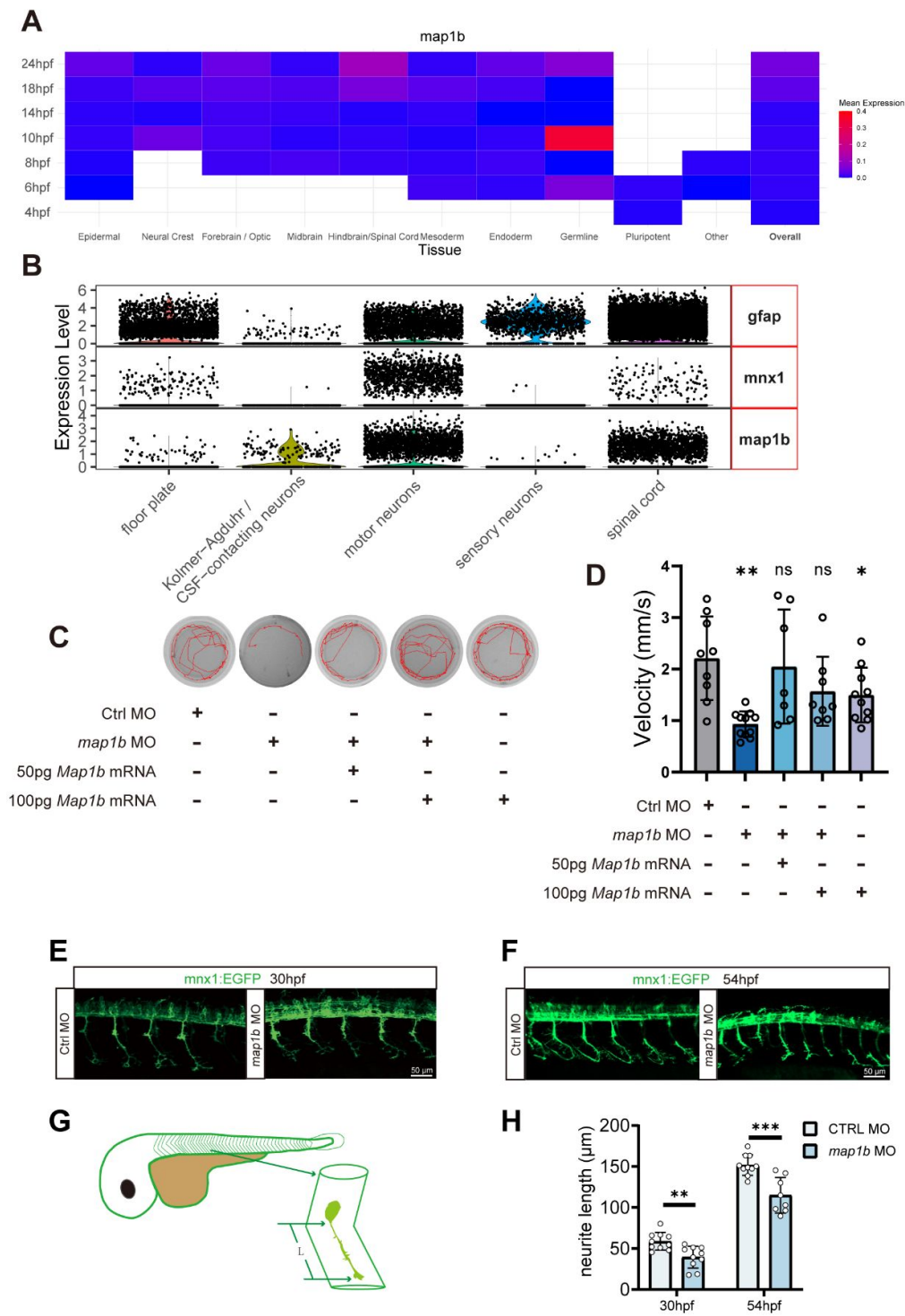
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708 FUNDING INFORMATION

709 This work was supported by grants from the National Natural Science Foundation of China
710 (31701049, 32070725), the Major Program of The Natural Science Foundation of the Jiangsu
711 Higher Education Institutions of China to ZD (23KJA180005), the Science and Technology
712 Project of Nantong City (JC12022011), the Large Instruments Open Foundation of Nantong
713 University (KFJN2515), and the Postgraduate Research & Practice Innovation Program of
714 Jiangsu Province to QW (KYCX23_3384).

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719 **Figure 1. Expression pattern of *map1b* during zebrafish embryonic development, and**

knockdown of *map1b* in zebrafish embryos. A–B: Expression pattern of *map1b* in developing zebrafish. Data sourced from Wagner et al. (2018) and Daniocell (<https://daniocell.nichd.nih.gov>). C: Representative action trajectory plots during two minutes of free swimming following *map1b* knockdown by Mos. D: Statistical results of swimming speed over one hour. One way ANOVA was used with Dunnett's multiple comparisons test. E–F: Representative images of spinal motor neuron axon length at 30 hpf and 54 hpf in *Tg(mnx1:eGFP)* zebrafish in lateral view. G: Schematic diagram of somites used for statistical analysis of spinal motor neuron axon length at 30 hpf and 54 hpf. H: Statistical results of spinal motor neuron axon length following 0.7 mM MOs-mediated knockdown of *map1b*. 30 hpf, Ctrl MO and *map1b* MO, n=10; 54 hpf, Ctrl MO, n=10, *map1b* MO, n=8. Student's *t*-test was used. *: $P<0.05$; **: $P<0.01$; ***: $P<0.001$; ns: not significant.

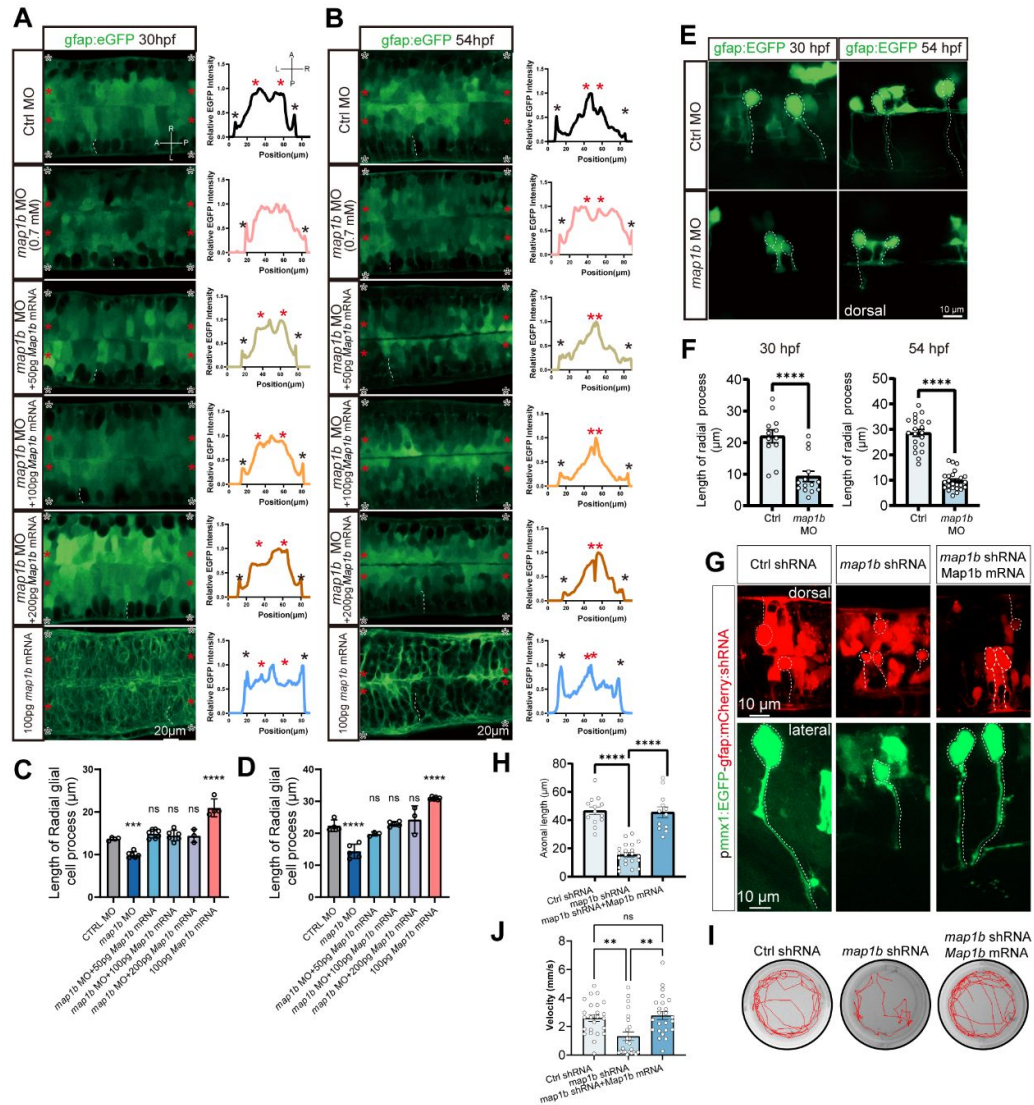


Figure 2. Depletion of *map1b* in radial glia cells disrupts localization of radial glia cells and dampens neurite growth in caudal primary (CaP) neurons. A–B: Representative images and statistical analysis of radial glia process length following *map1b* rescue and overexpression at 30 hpf and 54 hpf in *Tg(gfap:eGFP)* zebrafish in dorsal view. Integrated fluorescence along the anteroposterior axis was calculated to show the positioning of radial glia cell bodies. The peaks (red asterisks) represent the integrated fluorescence of the radial glial cell bodies along the anteroposterior axis in the zebrafish spinal cord. The black asterisks

740 represent the integrated fluorescence along the anteroposterior axis of the boundaries of the
 741 spinal cord. Intensities were calculated and plotted by integrating the intensity values along the
 742 anteroposterior axis, of the entire length of the imaged region of the fish. C: Statistical analysis
 743 of radial glia process length at 30 hpf following six different experimental treatments. One-
 744 way ANOVA test used. Sample sizes: $n = (4, 6, 7, 6, 3, 4)$. D: Statistical analysis of radial glia
 745 process length at 54 hpf following six different experimental treatments. One-way ANOVA
 746 test used. Sample sizes: $n = (4, 5, 3, 5, 3, 6)$. E: Representative images of the radial process
 747 length of sparsely labeled *gfap*-EGFP⁺ cells at 30 and 54 hpf following *map1b* knockdown in
 748 dorsal view. Student's *t*-test used. Sample sizes: $n = (12, 14)$. F: Statistical results of the radial
 749 process length of sparsely labeled *gfap*⁺ cells at 30 and 54 hpf following *map1b* knockdown.
 750 Student's *t*-test used. Sample sizes: $n = (21, 24)$ cells, from 12 and 14 individuals, respectively.
 751 Student's *t*-test was used. G: Fluorescent images of *gfap*:mCherry⁺ radial glia and
 752 *mnx1*:EGFP⁺ CaP neurons after injection of pTol2-*mnx1*:EGFP-*gfap*:mCherry (ctrl shRNA),
 753 pTol2-*mnx1*:EGFP-*gfap*:mCherry-*map1b*_shRNA (*map1b* shRNA), or pTol2-*mnx1*:EGFP-
 754 *gfap*:mCherry-T2A-*Map1b*-*map1b*_shRNA (*map1b* shRNA + *Map1b* mRNA). H: Statistic
 755 diagram of axonal length of CaP neurons after injection of ctrl shRNA or *map1b* shRNA.
 756 Student's *t*-test was used. I–J: Representative swimming trajectories and statistic diagram of
 757 swimming velocity of zebrafish larvae after injection of pTol2-*mnx1*:EGFP-*gfap*:mCherry (ctrl
 758 shRNA), pTol2-*mnx1*:EGFP-*gfap*:mCherry-*map1b*_shRNA (*map1b* shRNA), or pTol2-
 759 *mnx1*:EGFP-*gfap*:mCherry-T2A-*Map1b*-*map1b*_shRNA (*map1b* shRNA + *Map1b* mRNA).
 760 Sample sizes: $n = (24, 24, 24)$ zebrafish. One-way ANOVA was used followed by Tukey's
 761 multiple comparisons. **: $P < 0.01$; ***: $P < 0.001$; ****: $P < 0.0001$; ns: not significant.

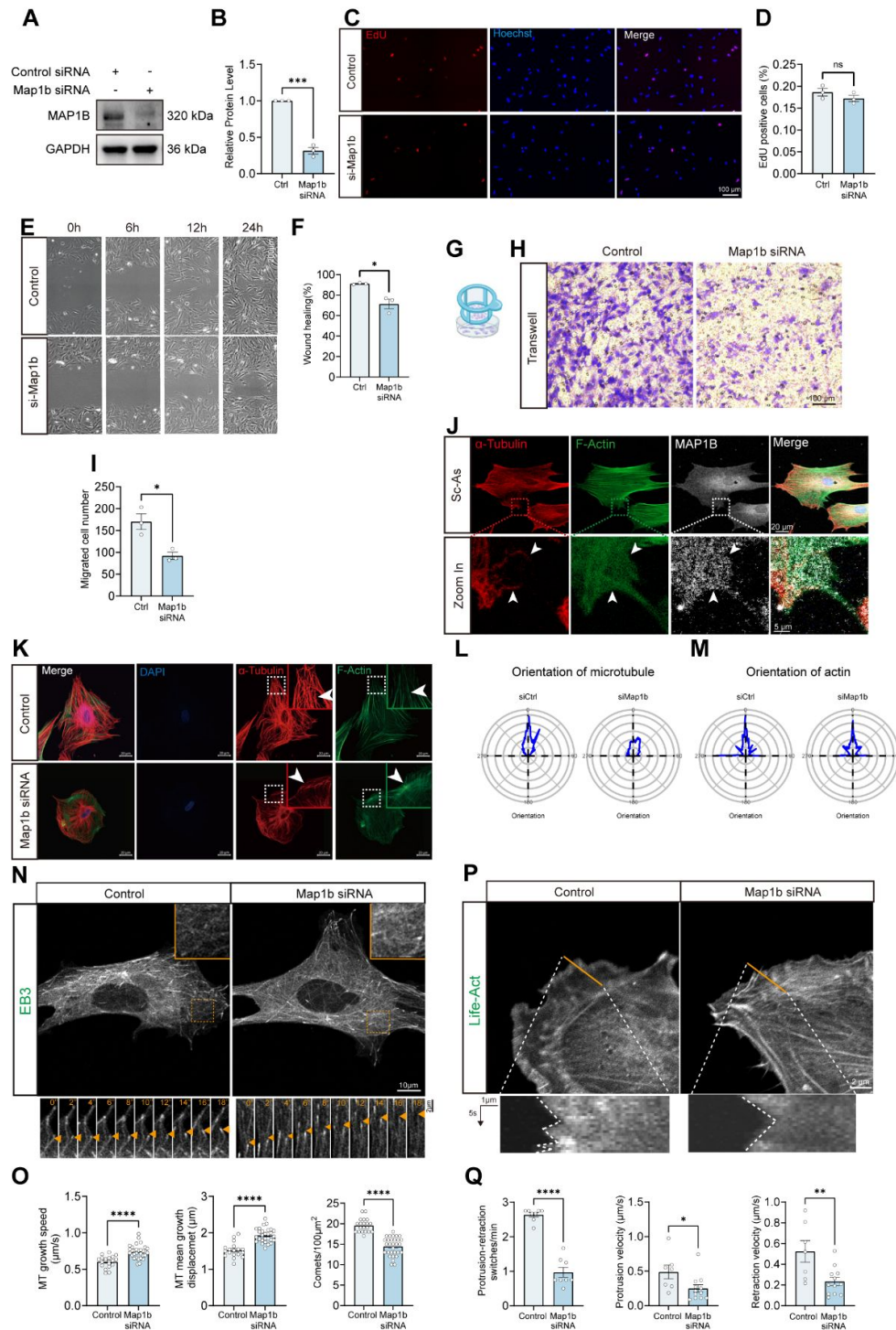
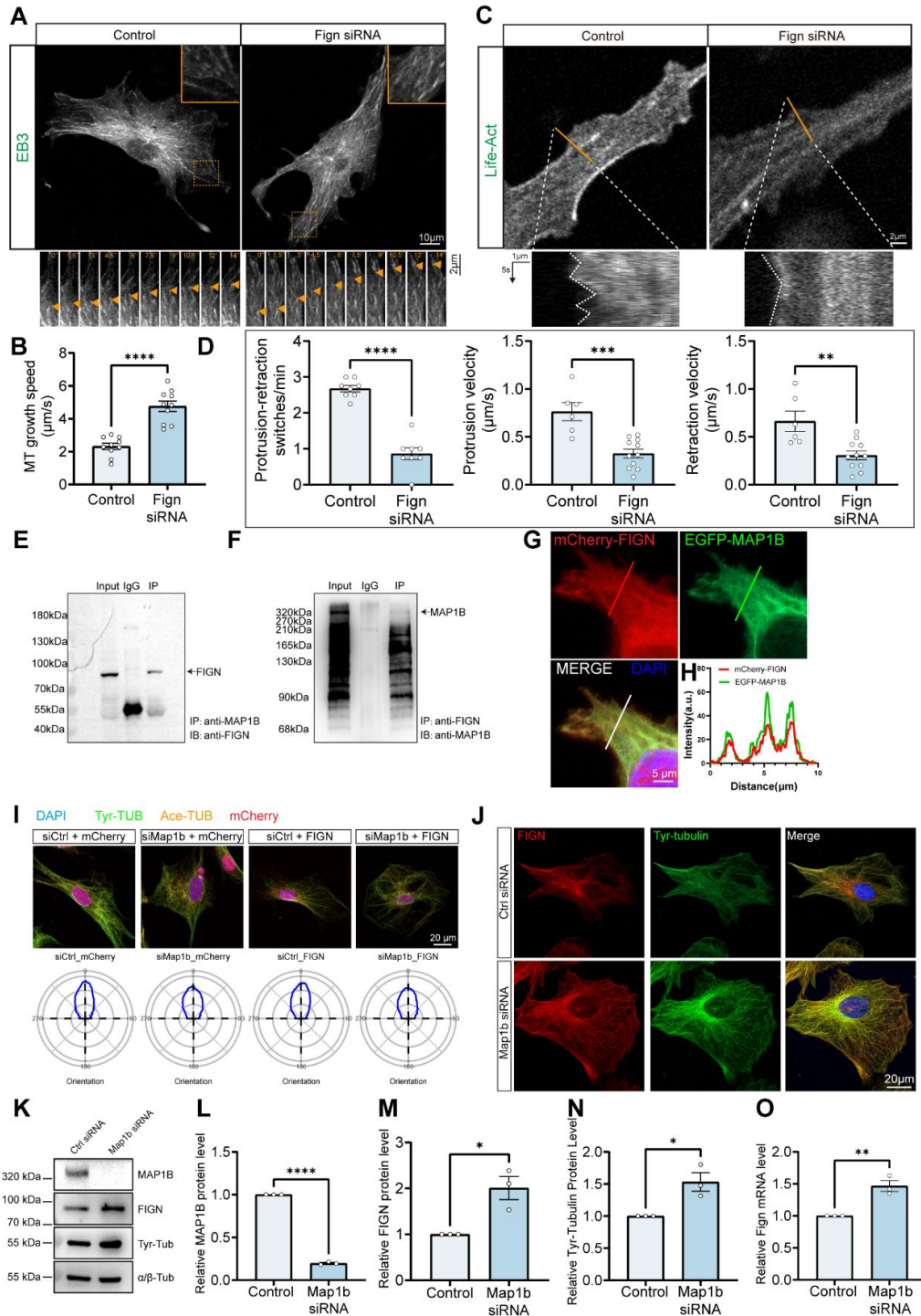


Figure 3. Knockdown of MAP1B inhibits GFAP⁺ cell migration and affects cortical microtubule and actin cytoskeleton. A–B: Representative Western Blot images and statistical analysis showing MAP1B knockdown efficiency. C: Representative images of EdU labeling showing that proliferation was not affected following MAP1B knockdown. D: Statistical results of EdU positive cells in panel B. E–F: Inhibition of rat spinal GFAP⁺ cell migration following MAP1B knockdown and corresponding statistical analysis. G–I: Schematic diagram and representative images and statistical analysis of Transwell migration assay. J: Representative images showing the localization of MAP1B in primary rat spinal GFAP⁺ cells. K–M: Representative images and statistical analysis of changes in cell cortical microtubules and actin cytoskeleton following MAP1B knockdown. Sample sizes for microtubule and actin analysis: n = (8, 10), n = (16, 31) respectively. N: Representative live-cell imaging of actin following MAP1B knockdown. O: Statistical diagram of microtubule growth speed, displacement and count of comet density. Control and *Map1b* knockdown groups, n = (18, 31), (18, 28), (20, 30) cells respectively. P: Representative live-cell image following MAP1B knockdown. Q: Statistic diagram of protrusion/retraction switching frequency and movement velocity of MAP1B knockdown cells with actin cytoskeleton labeled. For each group, cells were randomly selected, and from each cell three to five filopodium regions were selected, measured and averaged for analysis. For protrusion-retraction, n=(8, 8) cells; for protrusion velocity, n=(7, 12) cells; for retraction velocity, n=(7, 12) cells. C, D, F, I, O, and Q: Student's *t*-test was used. *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$; ****: $P < 0.0001$; ns: not significant.



784

785 **Figure 4. MAP1B interacts with FIGN to regulate cytoskeleton dynamics. A:**

786 **Representative images of EB3 comets following *Fign* knockdown. B: Statistic diagram of**

microtubule plus-end displacement and growth speed. Ten cells were selected for analysis in each group, and three to five comets in each cell were analyzed and averaged. C: Representative images lamellipodia dynamics in Life-Act labeled cells following *Fign* knockdown. D: Statistic diagram of lamellipodia dynamics (protrusion/retraction switching frequency and movement velocity) of *Fign* knockdown cells with actin cytoskeleton labeled. For each group, cells were randomly selected, and from each cell three to five filopodium regions were selected, measured and averaged for analysis. For protrusion-retraction, n=(8, 8) cells; for protrusion velocity, n=(6, 11) cells; for retraction velocity, n=(6, 11) cells. E–F: Representative images of co-immunoprecipitation of MAP1B and FIGN. GFAP⁺ cell lysate was immunoprecipitated with anti-MAP1B or anti-FIGN antibody and immunoblotted with anti-FIGN (E) or anti-MAP1B antibody (F). G: Representative image of colocalization of mCherry-FIGN and EGFP-MAP1B in RFL-6 cells. H: Profile of fluorescence intensity of mCherry-FIGN and EGFP-MAP1B in (G). I: The directional distribution of microtubules is disturbed in MAP1B knockdown cells, which cannot be rescued by *Fign* overexpression. n=(10, 8, 11, 12) cells. J: Immunofluorescence images of FIGN and Tyrosinated tubulin after depletion of MAP1B in GFAP⁺ cells. K–N: Western blotting images (K) and statistic diagram of relative protein amount (L–N) of MAP1B, FIGN, Tyrosinated Tubulin and α/β Tubulin after depletion of MAP1B in GFAP⁺ cells. O: Relative *Fign* mRNA expression after depletion of MAP1B. B, D, L–N, and O: Student's *t*-test was used. *: $P < 0.05$; ****: $P < 0.0001$.

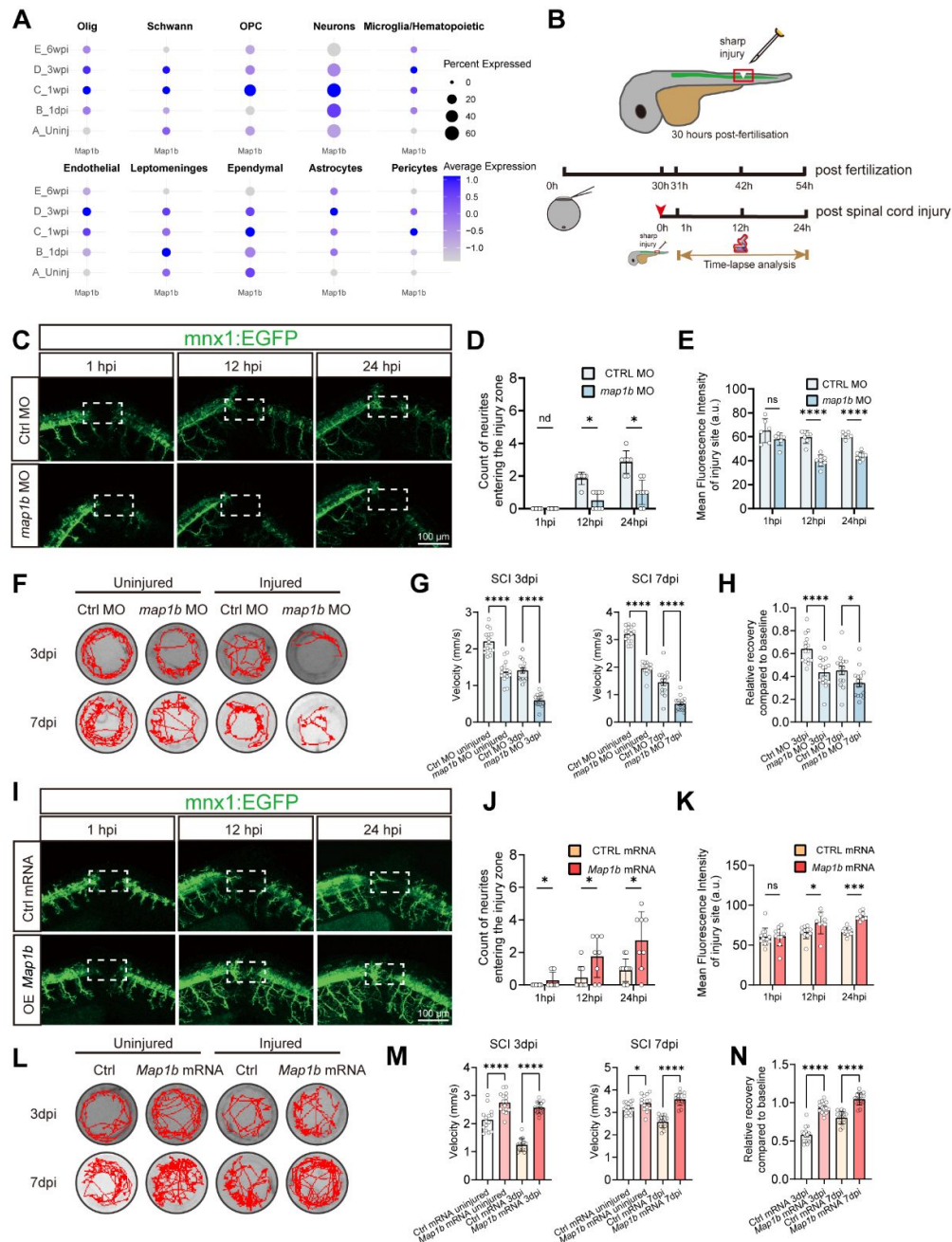


Figure 5. Role of Map1b in neuronal regeneration and functional recovery of zebrafish spinal cord post-injury. A: Schematic diagram showing changes in Map1b expression during the recovery process after spinal cord injury, based on single-cell sequencing data in mice. B: Schematic representation of zebrafish spinal cord injury and the workflow for monitoring morphological changes during recovery post-injury. C: Representative images of inhibited

814 neuronal regeneration following *map1b* knockdown at 1 hpi, 12 hpi, and 24 hpi in
 815 *Tg(mnx1:eGFP)* zebrafish in lateral view. D: Statistical graph of neurites entering the injury
 816 site following *map1b* knockdown and spinal cord injury. Sample sizes: 1 hpi, n = (6, 7); 12 hpi,
 817 n = (7, 8); 24 hpi, n = (7, 8). E: Statistical analysis of average fluorescence intensity at the
 818 injury site at 1hpi, 12hpi, and 24hpi. Student's *t*-test used. Sample sizes: 1 hpi, n = (6, 7); 12
 819 hpi, n = (7, 8); 24 hpi, n = (7, 8). F: Representative action trajectory plots during 10 minutes of
 820 free swimming following *map1b* knockdown at 3dpi. G–H: Statistical analysis of swimming
 821 speed and relative recovery (calculated by normalizing swimming speed to uninjured zebrafish
 822 at the same age) at 3 dpi and 7 dpi following *map1b* knockdown. Sample sizes: n = 15 for each
 823 group. I: Representative images of increased neuronal regeneration following *Map1b*
 824 overexpression at 1hpi, 12hpi, and 24hpi in *Tg(mnx1:eGFP)* zebrafish in lateral view. J:
 825 Statistical graph of neurites entering the injury site following *Map1b* overexpression and spinal
 826 cord injury. Sample sizes: 1 hpi, n = (13, 10); 12 hpi, n = (11, 8); 24 hpi, n = (11, 8). K:
 827 Statistical analysis of average fluorescence intensity at the injury site at 1 hpi, 12 hpi, and 24
 828 hpi following *Map1b* overexpression. Student's *t*-test used. Sample sizes: 1 hpi, n = (13, 10);
 829 12 hpi, n = (11, 8); 24 hpi, n = (11, 8). L: Representative action trajectory plots during 10
 830 minutes of free swimming following *Map1b* overexpression at 3dpi. M–N: Statistical analysis
 831 of swimming speed and relative recovery (calculated by normalizing swimming speed to
 832 uninjured zebrafish at the same age) at 3dpi and 7dpi following *Map1b* overexpression. Sample
 833 sizes: n = 15 for each group. D and J: Mann-Whitney test used, false discovery rate (FDR)
 834 calculated using two-stage step-up method of Benjamini, Krieger, and Yekutieli, and a *Q*-value
 835 less than 0.05 is considered a discovery, while a *Q*-value ≥ 0.05 not a discovery (nd). E, G, H,

836 K, M, and N: Student's *t*-test was used. *: $P<0.05$; **: $P<0.01$; ***: $P<0.001$; ns: not significant.

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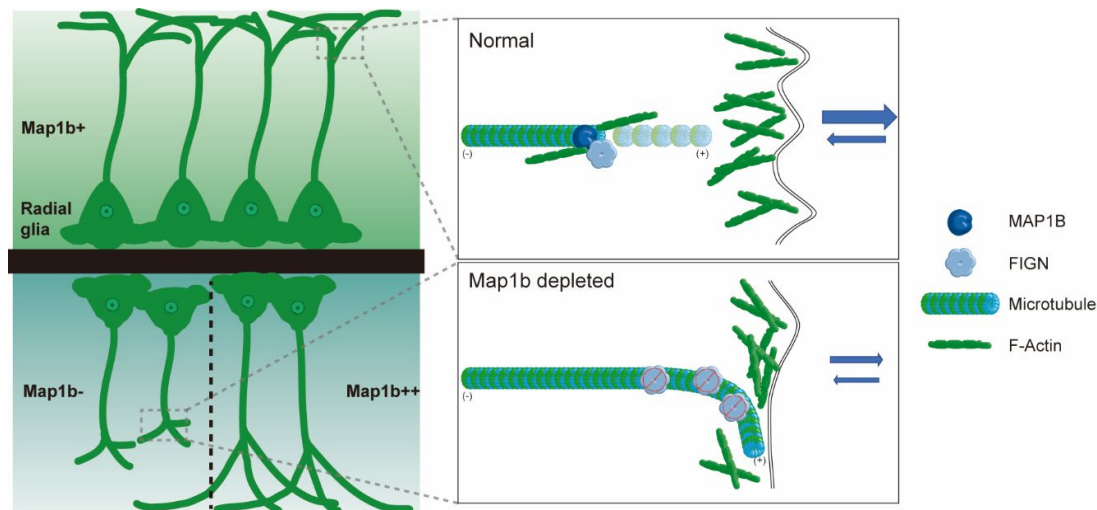


Figure 6. Working model of Map1b regulating cytoskeleton dynamics by regulating Fign.

Map1b interacts with Fign and is required for normal Fign-associated microtubule regulation. Depletion of Map1b is related to failure of Fign-mediated severing of tyrosinated microtubules, compromising migration ability, resulting in misalignment of the somata of radial glia and malformed radial processes in the spinal cord. Accumulated tyrosinated microtubules triggers expression of Fign. However, Fign does not regain its severing activity in the absence of Map1b.

DATA AVAILABILITY

All data generated in this study is available from the corresponding authors upon request.

SUPPLEMENTARY FIGURES

Supplementary Figure S1. Depletion of *map1b* causes deficits in swimming behavior, spinal motor neuron axon development, and *coro1a*⁺ cell migration in zebrafish. A:

Representative fluorescent images showing the expression of Map1b in *gfap*⁺ cells, spinal motor neurons and *coro1a*⁺ cells in lateral view. B: Schematic diagram of *map1b* MO binding site and working principle. C: Knockdown efficiency using *map1b* MO. RT-PCR analysis was

performed at 30, 54, 60, and 72 hpf following *map1b* MO injection. RNA was extracted from 10 zebrafish embryos per time point and analyzed by agarose gel electrophoresis. D:

Measurement of grayscale values in panel C, three biological replicates at each time point were calculated using ImageJ to determine knockdown efficiency. Student's *t*-test used. Data presented as mean $\pm$ SEM. Student's *t*-test was used. *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$; ns:

not significant. E and F. Depletion of *map1b* causes deficits in zebrafish swimming behavior. E: Representative action trajectory plots during two minutes of free swimming following *map1b* knockout by CRISPR-Cas9. F: Statistical results of swimming speed over one hour.

One-way ANOVA with Dunnett's multiple comparisons tests was used. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$, ns, not significant. G: Representative images of *coro1a*⁺ cell migration in the spinal cord at 72 hpf following *map1b* knockdown with three different MO

concentrations in *Tg(coro1a:eGFP)* zebrafish. H: Statistical results of *coro1a*⁺ cell migration in selected spinal cord regions at 72 hpf. Data presented as mean $\pm$ SEM. Student's *t*-test was used for comparison between Ctrl MO and *map1b* MO. Sample sizes: 0.5 mM, 0.7 mM, 1 mM,

869 n = (7, 7), (9, 6), (11, 13), respectively. *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$; ****: $P < 0.0001$;

870 ns: not significant.

871

872 **Supplementary Figure S2. Effects of NgAgo-mediated *map1b* knockdown on radial glia**

873 **process length.** A: Preparation of NgAgo mRNA *via* in vitro transcription and its co-injection

874 with *map1b* guide DNA in zebrafish embryos. B: Efficiency of NgAgo mediated *map1b* gene

875 knockdown. Total RNA was extracted from 10 zebrafish embryos 12 hours after microinjection

876 and *map1b* expression was analyzed by RT-qPCR. Student's *t*-test was used. C, D:

877 Representative images of radial glia process length in the spinal cord and cumulative

878 fluorescence intensity along the anteroposterior axis at 30 hpf and 54 hpf for *map1b*

879 knockdown in Tg(*gfap:eGFP*) zebrafish, rescue, and *map1b* overexpression zebrafish embryos.

880 E: Statistical analysis of radial glia process length at 30 hpf. One-way ANOVA test with

881 Dunnet's multiple comparisons tests was used. Sample sizes: n = (6, 5, 4, 4). F: Statistical

882 analysis of radial glia process length at 54 hpf. One-way ANOVA test with Dunnet's multiple

883 comparisons tests was used. Sample sizes: n = (6, 6, 3, 6).

884

885 **Supplementary Figure S3. Effects of CRISPR-Cas9-mediated *map1b* knockout on radial**

886 **glia process length.** A: Schematic diagram of *map1b* sgRNA target site. B: Representative

887 images of mutations in *map1b* at 24 hpf and 48 hpf. C,.D: Representative images and statistical

888 analysis of radial glia process length at 30 hpf and 54 hpf in *map1b* crispants in Tg(*gfap:eGFP*)

889 zebrafish in dorsal view. E: Statistical analysis of radial glia process length at 30 hpf after

890 CRISPR/Cas9 mutagenesis or rescue. One-way ANOVA test used. Sample sizes: n = (6, 4, 5,

8, 5). F: Statistical analysis of radial glia process length at 54 hpi after CRISPR/Cas9 mutagenesis or rescue. One-way ANOVA test with Dunnett's multiple comparisons tests was used. Sample sizes: n = (7, 8, 5, 6, 6). *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$; ****: $P < 0.0001$; ns: not significant.

Supplementary Figure S4. Expression of Map1b after shRNA-mediated knockdown and rescue in radial glia and Mnx1⁺ neurons. A, B: Immunofluorescence staining of Map1b in zebrafish larvae that had received injection of pTol2-mnx1:EGFP-gfap:mCherry-control_shRNA, pTol2-mnx1:EGFP-gfap:mCherry-map1b_shRNA, or pTol2-mnx1:EGFP-gfap:mCherry-T2A-Map1b-map1b_shRNA. C and D. Statistic diagrams of relative Map1b fluorescence. n=(15,15,15) animals. One-way ANOVA was used, followed by Tukey's multiple comparisons. ****: $P < 0.0001$; ns: not significant.

Supplementary Figure S5. GFAP-positive cells isolated from P1 rat express stemness markers. The isolated cells were stained with anti-GFAP and anti-SOX2 or anti-Nestin antibodies.

Supplementary Figure S6. FRET demonstrating interaction between MAP1B and FIGN.

A. Representative images of FRET between FIGN and MAP1B. mCherry-FIGN and GFP-MAP1B constructs were co-transfected into cultured RFL-6 with GFP as the donor and mCherry as the acceptor. Groups with co transfection of mCherry + GFP, mCherry + GFP-MAP1B, and mCherry-FIGN + GFP served as negative controls. Cytoplasmic regions of

interest were manually selected (red boxes), mCherry was bleached using 561 nm laser, and images were taken before and after photobleaching of mCherry. B, Statistic diagram of FRET efficiencies. n=(10,10,10,30) cells were analyzed for each group. Student's *t*-test was used. ****: $P < 0.0001$.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

Z.J.D. and M.L. conceived the study, designed the experiments, performed data analyses, and wrote the manuscript. Z.J.D, Q.W., H.Q.P., and Y.Q.Y. performed the experiments. All authors read and approved the final version of the manuscript.

926 **MAP1B 塑造放射状胶质细胞形态并促进损伤后神经恢复**

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934 摘要

935 微管相关蛋白 1B (MAP1B) 是一种关键的细胞骨架调节因子, 在神经元突起延伸过程中对微
936 管动力学至关重要, 并在生长锥中发挥功能。尽管其在神经元发育中的功能已有充分研究, 但
937 在胶质细胞中的具体作用仍未被阐明。本研究在斑马鱼中探究 *map1b* 在放射状胶质细胞中的
938 功能及其促进神经修复的潜力。我们发现, 敲降 *map1b* 会破坏放射状胶质细胞突起的形态并
939 损害脊髓运动神经元的发育, 导致异常的游动行为。利用 *Tg(gfap:eGFP)* 和 *Tg(mnx1:eGFP)*
940 等转基因斑马鱼模型, 我们发现 *map1b* 的缺失显著缩短了放射状胶质细胞突起和运动神经元
941 轴突的长度。特异性地在放射状胶质细胞中敲降 *map1b* 表达会导致运动神经元出现异常。在
942 机制层面, 我们利用大鼠放射状胶质细胞、RFL-6 细胞和 HEK293T 细胞鉴定出 MAP1B 与
943 FIGN 存在相互作用, 并且 MAP1B 对于 FIGN 相关的微管调控是必需的。特别具有治疗意
944 义的是, 哺乳动物 MAP1B 的过表达可在创伤性脊髓损伤后加速轴突再生并恢复运动功能。
945 综上所述, 我们的研究确立了 MAP1B 作为胶质形态发生和神经修复的双重调节因子, 揭示
946 了一种通过靶向细胞骨架可塑性来治疗脊髓损伤的新策略。

947 **关键词:** 放射状胶质; MAP1B; FIGN; 损伤; 神经元