

PTRH2 promotes the destruction of neurovascular units and glial scar hyperplasia after cerebral ischemia/reperfusion

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Abstract

Peptidyl-tRNA hydrolase 2 (PTRH2) may exert a role in the occurrence of ischemic stroke (IS) and regulate cell death and immunity after IS. Unfortunately, no biological experiments are conducted to explore the function of PTRH2 after cerebral ischemia/reperfusion (I/R). In this study, the roles of PTRH2 in cerebral I/R injury were explored. We found that the protein level of PTRH2 continued to increase after cerebral I/R. Immunofluorescence colocalization indicated that PTRH2 was primarily located in neurons in the early stage after cerebral I/R, but mainly in astrocytes in the later stage. Then, adeno-associated viruses specifically infecting neurons and astrocytes were used to investigate the effects of PTRH2 on early injury and late glial scar formation after cerebral I/R, respectively. When PTRH2 was over-expressed in neurons, it indicated more serious neurological damage, neuronal death, microvascular and blood-brain barrier (BBB) damage, and glial cell activation. In addition, the overexpression of PTRH2 in astrocytes showed that glial scar hyperplasia became more pronounced and neural remodeling was inhibited. When the PTRH2 was knocked down, the above effects were inhibited. Furthermore, the AKT signaling pathway was regulated with the change in the PTRH2 protein level. It was indicated that PTRH2 damaged neurovascular unit (NVU) and promoted glial scar hyperplasia probably through AKT signaling pathway after cerebral I/R. This study preliminarily explored the mechanism of PTRH2 after cerebral I/R and provided a novel therapeutic target for cerebral I/R injury.

1. Introduction

Ischemic stroke (IS), a frequently observed neurological disorder, is a main reason for permanent disability in patients.^[1] Till the present, intravenous thrombolysis and mechanical thrombectomy are the main measures for the clinical treatment of IS to restore blood perfusion. However, with the rapid recovery of blood flow, the large amount of reactive oxygen species produced causes oxidative stress response, damaging the mitochondria and DNA of nuclei and releasing inflammatory factors to promote cell death.^[2] This type of brain injury can exacerbate neuronal damage and even result in brain edema, cerebral hemorrhage, and permanent neurological dysfunction. The phenomenon of severe damage due to cerebral ischemia after blood flow recovery is called cerebral ischemia/reperfusion (IR) injury.^[3–5] Until now, there is a lack of effective treatment methods.

Neurovascular unit (NVU) is not only a dynamic structural complex composed of cerebral microvessels and neurons, but also a structural and functional unit of the brain.^[6] Neurons, glial cells, endothelial cells and extracellular matrix are coupled to form NVU, and maintain brain homeostasis.^[7] After cerebral ischemia, the components of NVUs interact with each other, causing the degeneration of nerve cells and glial cells, as well as microvascular dilation. As time goes on, nerve cells disappear, phagocytes appear, astrocytes proliferate, necrotic tissue is engulfed, and glial cells and microvasculature proliferate.^[8, 9] Evidently, cerebral ischemia can damage NVUs. Therefore, in the treatment of cerebral ischemia, it is essential to protect neurons and strengthen the overall protection of structures including glial cells, endothelial cells, and blood-brain barrier (BBB).

Astrocytes have a strong tolerance to ischemia, which induces the reactivation of astrocytes. Reactive astrocytes have a dual role in damaging or protecting neurons in the pathological process of cerebral ischemia, such as secreting neurotoxins to damage neurons and sealing the damaged area to maintain regional stability.^[10–13] Nevertheless, in the late stage of cerebral ischemia, the excessive proliferation of astrocytes can form glial scars and secrete a large amount of chondroitin sulfate (CS) and chondroitin sulfate proteoglycans (CSPGs).^[14] They not only secrete a series of vital exogenous growth inhibitory factors such as neurocan and phosphacan, but also form a physical barrier that hinders nerve growth.^[15, 16] Therefore, reducing the proliferation of astrocytes in the later stage of cerebral ischemia remains one of the treatment methods to promote neurological rehabilitation after stroke.

Peptidyl-tRNA hydrolase 2 (PTRH2) is a mitochondrial protein that has been conserved throughout evolution.^[17] Furthermore, it is demonstrated to be a proapoptotic protein in cell lines.^[18] Based on sequencing and bioinformatics results, PTRH2 may exert a role in the occurrence of IS and regulate cell death and immunity after IS.^[19] Unfortunately, no biological experiments have been carried out to investigate the actual function of PTRH2 in IS.

In this study, PTRH2 protein expression increased within neurons and astrocytes after cerebral I/R. Afterwards, PTRH2 protein expression was regulated by using adeno-associated virus (AAV) infection, and the impact on NVU and glial scar was explored.

2. Materials and methods

2.1 Animal experiments

The specific pathogen-free grade male Sprague–Dawley rats weighing 240 ± 20 g were provided by Liaoning Changsheng Biotechnology and raised under 25°C and 12-h/12-h light/dark cycle conditions. Animals could take food or water freely. Each experiment was performed following U.K. Animals (Scientific Procedures) Act guidelines, 1986.^[41] In addition, our experimental protocols gained approval from Ethics Committee of the First Affiliated Hospital of Harbin Medical University (Approval number: 2023027).

2.2 AAV infection

Each rat was intracerebroventricularly injected with 10 μ L AAV for infection to achieve PTRH2 overexpression (PTRH2 OE) or PTRH2 knockdown (PTRH2 KD) in the brain tissue. Moreover, AAVs with different promoters were applied to regulate PTRH2 expression in neurons and astrocytes, respectively. AAV9-hSyn-PTRH2 and AAV9-hSyn-shRNAPTRH2 were used to specifically infect neurons.^[20] AAV9-gfaABC1D-PTRH2 and AAV9-gfaABC1D-shRNAPTRH2 were utilized to specifically infect astrocytes.^[21] The shRNA sequence was 5'-GCCTACAAGCAGACTCAAAGG'. Meanwhile, empty AAV9-hSyn and empty AAV9-gfaABC1D were used for vector control (VC). At 2 weeks after infection, surgical treatment was performed in rats.

2.3 Middle cerebral artery occlusion (MCAO) model

At 12 h preoperatively, rats received food fasting. Afterwards, a MCAO model was constructed according to our previous method.^[22] Briefly, following neck skin preparation, rats were given 3% pentobarbital sodium for anesthesia. Then, an incision was made on neck skin to separate cervical arteries, including internal carotid artery (ICA), external carotid artery (ECA), and common carotid artery (CCA). Next, we advanced one suture (Doccol, USA) in the ICA towards the MCA from the ECA. After feeling resistance, the suture was secured, fixed and removed at 2 h later to restore blood flow. Throughout this process, anal temperature was kept at 37°C. To facilitate recovery, animals were put into the warm incubator for 24 h. Animals in Sham group received the same treatment except for suture use. Subsequently, animals were randomized as different groups according to complete random grouping. Totally 10 rats died because of cerebral I/R injury or subarachnoid hemorrhage prior to euthanasia. The MCAO model was successfully constructed in over 90% of animals. A total of 112 rats were enrolled into this study. At 1, 3, 7, 14 and 30 days following MCAO, excessive pentobarbital sodium was given for euthanasia, followed by the acquisition of an ischemic cortical boundary zone in later analysis. (Fig. S1)

2.4 Neurological scores

An inspector assigned scores to rats undergoing neurological deficits in a blinded manner. Modified neurological severity scores (mNSS) and the Grip Strength Test were carried out to evaluate early nerve injury and later neurological function recovery, respectively. mNSS involves motor, reflex, sensory, and balance function assessment, yielding totally 18 points. The functional impairment level is positively related to the score.^[23] A rat grip strength meter was used to evaluate the forelimb grip strength. Gently, the individual rat was lifted from the tail until they grasped the pull-bar with both forepaws. In this study, peak force was deemed as the maximal grip strength (strength in grams). In addition, ten trials were conducted for each animal with the averaged data being considered the final results.^[24]

2.5 Measurement of infarct volume and atrophy volume

Rat euthanasia was performed after anesthesia to collect brain tissues. To prepare brain sections, brain tissues underwent 5 min of rapid freezing at -20°C with stainless steel brain molds. Then, 2-mm brain sections were acquired in the frontal pole. To measure the volume of cerebral infarction, sections were incubated with 2% of 2,3,5-Triphenyl-tetrazoliumchloride (TTC) solution under room temperature in dark. ImageJ software was employed to calculate infarct volumes and atrophy volumes. The weight brain tissue volume/hole brain tissue volume × 100% was used to reflect the condition of infarct volume. In addition, the right brain tissue volume/left brain tissue volume × 100% was used to reflect the condition of brain tissue atrophy.

2.6 Brain water content

Brain water content was assessed with TTC-stained brain sections. At first, the section wet weights were determined. To measure dry weight, sections were dried for 24 h in the 90°C drying oven. Brain water content (%) was calculated by (wet weight – dry weight)/wet weight × 100%.

2.7 HE staining

Hematoxylin was added to stain sections for 5 min, followed by another 2 min of HE staining.

2.8 Western blotting (WB)

The brain tissues collected were treated with RIPA Lysis Buffer (Boster, China) to acquire total protein. Then, the BCA kit (P0012, Beyotime, China) was used to measure protein content. Proteins (20–30 µg) were separated through SDS-PAGE, prior to transfer onto polyvinylidene fluoride (PVDF) membrane. Next, 10% defatted milk contained within TBST was added to block PVDF membrane for 60 min. After washing three times by TBST solution, membranes were exposed to overnight incubation at 4°C using primary antibodies, including PTRH2 (1:1000, 51006-2-AP, Proteintech, USA), P-AKT (1:1000, ab192623, Abcam, UK), AKT (1:10000, ab179463, Abcam, UK), TNF-α (1:1000, 60291-1-Ig, Proteintech, USA), IL-6 (1:1000, DF6087, Affinity, USA), Bcl2 (1:5000, 68103-1-Ig, Proteintech, USA), BAX (1:10000, 60267-1-Ig, Proteintech, USA), MMP9 (1:1000, ab228402, Abcam, UK), ZO1 (1:10000, 21773-1-AP, Proteintech, USA), Occludin (1:1000, ab216327, Abcam, UK), IL-17A (1:2000, PA5-106856, Thermo, USA), GFAP (1:1000, DF6040, Affinity, USA), Neurocan (1:1000, ab277525, Abcam, UK), Phosphacan (1:1000, ab302790, Abcam, UK), Neurofilament H (1:1000, ab207176, Abcam, UK), and β-actin (1:2000, TA-09, ZSGB, China). On next day, membrane was further probed using secondary antibody (1:5000, M21003, Abmart, China) for another 60 min. Finally, ECL was utilized for band detection, whereas ImageJ was adopted for protein band quantification.

2.9 Immunofluorescence (IF) and 5-Bromodeoxyuridine (BrdU) staining

GFAP IF and BrdU co-staining was conducted to reflect astrocytes proliferation.^[15] At first, 50 mg/kg BrdU was injected intraperitoneally for 7 consecutive days. After collecting brain tissue, normal saline was added for cardiac perfusion till the liver became white. Afterwards, brain tissue was soaked within 4% paraformaldehyde for 8 h. Then, the sucrose solution was adopted for removing brain tissue water. Brain tissue was prepared in 8-µm slices with the freezing microtome. Then, the slices were blocked and underwent overnight incubation at 4°C using primary antibodies: PTRH2 (1:50, PS04551, Abmart, China), NeuN (1:100, 66836-1-Ig, Proteintech, USA), NeuN (1:200, ab177487, Abcam, UK), GFAP (1:100, BF0345, Affinity, USA), IBA1 (1:200, GTX638147, Gene Tex, USA), ZO1 (1:500, 21773-1-AP, Proteintech, USA), Occludin (1:50, ab216327, Abcam, UK), CD31 (1:50, sc-53411, Santa Cruz, USA), IL-17A (1:100, PA5-106856, Thermo, USA), GFAP (1:100, DF6040, Affinity, USA), Neurocan (1:100, ab277525, Abcam, UK), MAP2 (1:100, ab183830, Abcam, UK) and BrdU (1:200, 66241-1-Ig, Proteintech, USA). Anti-fluorescein was completed for 3 h under room temperature in dark, followed by DAPI staining (Abcam, ab104139, UK). The fluorescence microscope was used for image acquisition.

2.10 TUNEL staining

TUNEL staining was conducted to detect apoptosis using an In Situ Cell Death Detection Kit (Bioscience). Briefly, frozen sections were treated with UNEL solution, followed by 1 h of incubation

under 37°C in dark. Afterwards, the fluorescence microscope was utilized for capturing images and observing green fluorescence channel.

2.11 Fluoro-Jade C (FJC) staining

FJC staining was conducted following the specific protocols (TR-100-FJT, Biosensis, USA). The slide was put into the solution which contained 80% ethanol and 1% NaOH for 5 min, washed by 70% ethanol for 2 min, and immersed with the 0.06% potassium permanganate solution for 10 min. Later, slides were rinsed by distilled water for 2 min, and immersed within the 0.0001% FJC solution for a 10-min period, followed by the addition of DAPI into FJC solution. Finally, the DPX mounting medium was added to fix slides.

2.12 Statistical analysis

Data were obtained and analyzed in a blinded manner. Researchers not participating in all experiments were responsible for data statistical analysis. Test results were indicated by mean $\pm$ standard deviation. Data homogeneity and normality were evaluated by Shapiro–Wilk test, verifying normal data distribution. One-way ANOVA was utilized to compare different groups, whereas subsequent analysis was conducted using Tukey's post hoc test. Statistical analysis was carried out with GraphPad 8.3.0 software. $P < 0.05$ represented statistical significance (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$).

3. Results

3.1 PTRH2 is mainly expressed in neurons and astrocytes in the early and late cerebral I/R injury stages, respectively

Firstly, the WB was employed to detect the PTRH2 protein expression within the ischemic cortical boundary zone at different time points following cerebral I/R. It was found that the PTRH2 protein level increased since the first day ($P < 0.01$) following cerebral I/R and peaked at the seventh day ($P < 0.001$). Until 30 days after brain I/R, the PTRH2 protein remained highly expressed ($P < 0.001$). **(Fig. 1A)** PTRH2 may be expressed in neurons, but neurons will die in a large amount after cerebral I/R.^[25] We speculated that PTRH2 might be expressed in different types of cells at different time points. Subsequently, IF colocalization staining of PTRH2 was performed with neurons, astrocytes, and microglia using different cell markers.^[26] The IF staining results indicated that PTRH2 and NeuN exhibited significant colocalization within 7 days after brain I/R. **(Fig. 1B)** Starting from the seventh day after cerebral I/R, there was significant colocalization between PTRH2 and GFAP.**(Fig. 2A)** However, no overlap was found between PTRH2 and IBA1.**(Fig. 2B)** Therefore, PTRH2 was mainly expressed within neurons and astrocytes during early and late stages of cerebral I/R, respectively. AAVs infecting neurons and astrocytes were utilized to explore the function of PTRH2 after cerebral I/R injury.

3.2 PTRH2 aggravates cerebral I/R injury

The Syn promoter AAV that specifically infected neurons were applied to explore the function of PTRH2 in the early cerebral I/R injury stage. After three days, animals were used in subsequent experiments. The WB showed that the protein level of PTRH2 was successfully regulated. **(Fig. 3J, K)** Compared with the VC group, the neurological function of PTRH2 OE group deteriorated significantly ($P < 0.05$). On the contrary, the neurological function of PTRH2 KD group significantly improved ($P < 0.05$). **(Fig. 3A)** The TTC staining showed that the infarct volume increased after PTRH2 overexpression ($P < 0.01$), but declined following PTRH2 knockdown ($P < 0.05$). **(Fig. 3B, C)** Moreover, the PTRH2 OE group also displayed more severe cerebral edema ($P < 0.01$), but the PTRH2 KD group exhibited the contrary trend ($P < 0.01$). **(Fig. 3D)** These results indicated that PTRH2 damaged the BBB and increased cerebral I/R injury. After PTRH2 overexpression, tissue structure was significantly disrupted and the morphology of the cell nucleus was significantly altered. The FJC staining showed significant increase in damaged neurons ($P < 0.05$). Additionally, the apoptotic cells increased, as evidenced by TUNEL staining ($P < 0.01$). These situations showed the opposite trend after PTRH2 knockdown. **(Fig. 3E-I)** It indicated that PTRH2 exacerbated neuronal damage after cerebral I/R. We also tested the AKT pathway and cell death related proteins using WB. The AKT pathway, which plays a protecting role in cerebral I/R, was inhibited after PTRH2 overexpression ($P < 0.001$) and activated after PTRH2 knockdown ($P < 0.01$). **(Fig. 3J, L)** Additionally, TNF- α ($P < 0.0001$) and IL-6 ($P < 0.0001$) protein levels elevated, whereas Bcl2/Bax ratio ($P < 0.001$) declined in PTRH2 OE group. The results in PTRH2 KD group were reversed. **(Fig. 3J, M-O)**

3.3 PTRH2 promotes microvascular damage and exacerbates tight junction proteins (TJPs) loss

TJP, like occludin and *zonula occludens protein 1* (ZO1), represent the important BBB structural components, which form tight junctions of vascular endothelial cells with additional central nervous system cell types.^[26] The WB analysis results revealed that MMP9 expression ($p < 0.0001$) increased, while ZO1 ($p < 0.0001$) and occludin ($p < 0.05$) expression decreased after PTRH2 up-regulation. On the contrary, MMP9 ($p < 0.01$) was down-regulated, while ZO1 ($p < 0.001$) and occludin ($p < 0.001$) expression elevated after PTRH2 knockdown. **(Fig. 4A)** To further investigate the effects of PTRH2 on microvessels and BBB, we co-stained TJPs with vascular marker CD31. The IF staining results showed that ZO1 ($p < 0.01$) and occludin ($p < 0.01$) fluorescence intensities reduced after PTRH2 up-regulation. When the PTRH2 was knocked down, the degradation of TJPs was reversed. It was also observed that CD31 showed the same trend. **(Fig. 4B, C)** This further confirms that PTRH2 exacerbates microvascular damage and BBB damage after cerebral I/R.

3.4 PTRH2 enhances cerebral I/R induced glial cell activation and IL-17A expression

IL-17A promotes astrocyte activation following cerebral ischemia-reperfusion injury. It triggers reactive astrogliosis, upregulates GFAP expression, and amplifies neuroinflammatory responses, which further exacerbate neuronal damage in the ischemic penumbra.^[27] In comparison with VC group, IL-17A protein expression of PTRH2 OE group increased ($p < 0.001$), while that of PTRH2 KD group decreased ($p < 0.01$). **(Fig. 5A)** For investigating PTRH2's effect on NVUs, we co-stained the markers of astrocytes and microglia with IL-17A. In IF staining, colocalization of IL-17A with GFAP and IBA1 was observed.

Overexpression of PTRH2 further activated astrocytes ($p < 0.05$) and microglia ($p < 0.01$). When PTRH2 was knocked down, the opposite effect was observed. **(Fig. 5B-E)** This indicates that PTRH2 not only exacerbates the inflammatory response, but also promotes the activation of glial cells after cerebral I/R.

3.5 PTRH2 inhibits neurological function recovery

Because PTRH2 is mainly expressed in astrocytes in the late stage of brain I/R. AAV specifically infecting astrocytes was used to investigate its effects on the neurological function recovery. At 30 days after cerebral I/R, animals in the PTRH2 OE group had lighter body weight whereas lower neurological function scores (both $p < 0.05$). After slicing the brain tissue, it was observed that the PTRH2 OE group exhibited more significant brain tissue atrophy ($p < 0.05$). In the PTRH2 KD group, opposite results were observed. **(Fig. 6)** Consequently, PTRH2 may affect neurological function recovery by exacerbating brain tissue atrophy.

3.6 PTRH2 promotes glial scar formation

The proliferation of astrocytes forms glial scars, hinders nerve remodeling, affects nerve function recovery, and exacerbates brain tissue atrophy.^[28] Therefore, we further investigated how PTRH2 affected glial scar in the ischemic cortical boundary zone. In the PTRH2 OE group, the PTRH2 protein expression increased ($p < 0.0001$), the AKT signaling pathway was activated ($p < 0.001$), and more GFAP proteins were discovered ($p < 0.001$). **(Fig. 7A)** In order to further observe the activation of astrocytes, GFAP and BrdU co-staining was conducted to reflect the activation of astrocytes. In the PTRH2 OE group, we observed the stronger fluorescent intensity of astrocyte markers and more activated astrocytes ($p < 0.01$). **(Fig. 7B)** Similarly, the PTRH2 OE group showed a significant increase in glial scar width. **(Fig. 7C)** The above results were reversed in the PTRH2 KD group. This suggests that PTRH2 may promote glial scar hyperplasia by activating the AKT pathway.

3.7 PTRH2 weakens neural remodeling

Finally, we investigated the effect of PTRH2 on neural remodeling. To this end, two proteins, Neurocan and Phosphacan, which inhibit neural remodeling, were utilized. Neurofilament H and MAP2, the markers of axons and dendrites, were also detected separately. After PTRH2 overexpression, the Neurocan ($p < 0.01$) and Phosphacan ($p < 0.0001$) protein expression elevated. IF staining showed the same trend ($p < 0.01$). **(Fig. 8A, B)** The WB showed the less Neurofilament H expression in the PTRH2 OE group ($p < 0.01$). **(Fig. 8A)** Similarly, IF staining exhibited the poor continuity and fluorescent intensity of MAP2 for PTRH2 OE group ($p < 0.05$). **(Fig. 8C)** In the meanwhile, these above results were reversed in the PTRH2 KD group. This indicates that PTRH2 promotes the formation of a neural growth barrier and inhibits neural remodeling.

4. Discussion

Currently, the IS mechanism needs to be further explored. Our results indicate that PTRH2 is mostly located in neurons in the early stage of IS, but mainly in astrocytes in the late stage after cerebral I/R. Firstly, AAV was used to regulate the PTRH2 expression in neurons, finding that PTRH2 promoted NVU damage after cerebral I/R. Then, AAV was utilized to regulate the PTRH2 expression in astrocytes and discovered that PTRH2 promoted the activation of astrocytes and inhibited neural remodeling. In addition, AKT pathway activity altered after the alteration in PTRH2 expression.

Recently, the research direction has gradually shifted from a single structure to the integrated structure of NVUs. NVU is a functional unit composed of neurons, microglia, brain microvascular endothelial cells, astrocytes, pericytes, and basement membrane in the cerebral vascular system. Among them, the cerebral microvascular endothelial cells, astrocytes and pericytes constitute the BBB, contributing to maintaining the central nervous system homeostasis.^[29] Neurons are vital building blocks of NVUs and are interconnected with other components of NVUs. After cerebral I/R, the increased BBB permeability can cause significant loss of neurons.^[30] Cerebral I/R can also lead to neuronal damage through mechanisms including excitotoxicity, inflammation, oxidative stress, and apoptosis. Cell apoptosis is a series of highly regulated intracellular events that can participate in neuronal death after cerebral ischemia through signaling pathways (such as JANK/STAT3, NF- κ B, and PI3K/AKT), apoptosis-inducing factors and various kinases.^[31] Subsequently, the damaged neurons release a large amount of harmful cytokines to activate glial cells and damage the BBB. This further exacerbates neuronal death and deterioration of neurological function.^[32, 33] Additionally, the Akt signaling pathway exerts a vital effect on the inflammation, cell apoptosis, and oxidative stress.^[34] The activated AKT pathway can protect neurons and reduce cerebral I/R damage after cerebral I/R.^[35] The mutation of PTRH2 is associated with some neurological disorders. Therefore, it can probably be participated in neuron development.^[25] PTRH2 can also regulate BCL2 transcription and influence cell survival by modulating the AKT signaling pathway.^[36] Although the function of PTRH2 in cerebral I/R has not been experimentally investigated, bioinformatics analysis predicts its possible involvement in early injury and immune regulation after cerebral I/R.^[19] In this study, we first explored the function of PTRH2, finding that it was mainly localized in neurons early after cerebral I/R. Subsequent experiments demonstrated that PTRH2 not only reduced AKT signaling pathway activity and promoted neuronal death, but also exacerbated the damage of NVUs. This phenomenon is probably related to the regulation of AKT pathway.

Inflammation, oxidative stress, glutamate excitotoxicity, activation of microglia and oligodendrocytes after cerebral ischemia can lead to the activation of astrocytes and the formation of glial scars.^[37, 38] Research has suggested that at different stages of stroke injury, the role of glial scars shows a dual and dynamic nature, which is a double-edged sword for neuronal survival and functional protection. In the early stage of cerebral ischemia injury, the formation of glial scars is beneficial for sealing the injury site and secreting neurotrophic factors, antagonizing inflammation and oxidative stress, and inhibiting the expansion of secondary damage. In the middle and late stages of stroke injury, glial scars in the core area of the stroke secrete a large amount of CS and CSPGs. Additionally, they inhibit the migration of new neurons and form physical and chemical barriers that hinder axonal regeneration, affecting the

recovery of neural function.^[15, 16] Previous studies have reported that inhibiting the proliferation of glial scars can effectively promote neural remodeling and improve neurological function recovery.^[24] Additionally, AKT, STAT3 and TGF- β signaling pathways also participate in the activation of astrocytes and the formation of glial scars. The results suggest that the activated AKT signaling pathway can promote glial scar hyperplasia.^[39] Our findings indicated that PTRH2 showed main expression in astrocytes in the late stage of cerebral I/R. After regulating PTRH2 protein expression using AAV that specifically infected astrocytes, we found that PTRH2 promoted AKT pathway activation and glial scar hyperplasia. Moreover, PTRH2 exhibited the opposite regulatory effects in astrocytes and neurons. This may be due to the dynamic changes in the state of immune cells after cerebral I/R. Neuronal injury and glial scar are both regulated by immune cells, and the AKT pathway also plays a key role. This will cause different immune microenvironments at different stages.^[37, 40]

However, this study still had the following limitations. As there is a lack of research on the regulation of AKT pathway, we can only assume that PTRH2 may damage NVUs and promote glial scar formation via the AKT pathway. In addition to glial scars, factors inhibiting neural remodeling also include mitochondrial aggregation and cytokines that promote nerve growth. PTRH2 is expressed in multiple cell populations within brain tissue. This study selectively explores its functions only in neurons and astrocytes, leaving substantial uninvestigated biological roles to be characterized in future research. Finally, the detailed mechanism of PTRH2 after cerebral I/R needs to be explored.

In conclusion, PTRH2 promotes the destruction of NVUs and glial scar hyperplasia after cerebral I/R. The above effects are probably obtained by modulating the AKT pathway. Our study preliminarily investigates the mechanism of PTRH2 after cerebral I/R and provides a new target for treating cerebral I/R injury.

Declarations

Conflict of interests

The authors confirm that there are no conflicts of interest.

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Author Contribution

Yingju Wang and Min Xu were in charge of experimental implementation and manuscript writing. Yifan Wang and Zhengqin Gao analyzed the data. Hongxue Sun was in charge of animal experiments. Qiming

Feng and Chen Wang contributed to the study conceptualization and revised the eventual manuscript.

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Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Figures

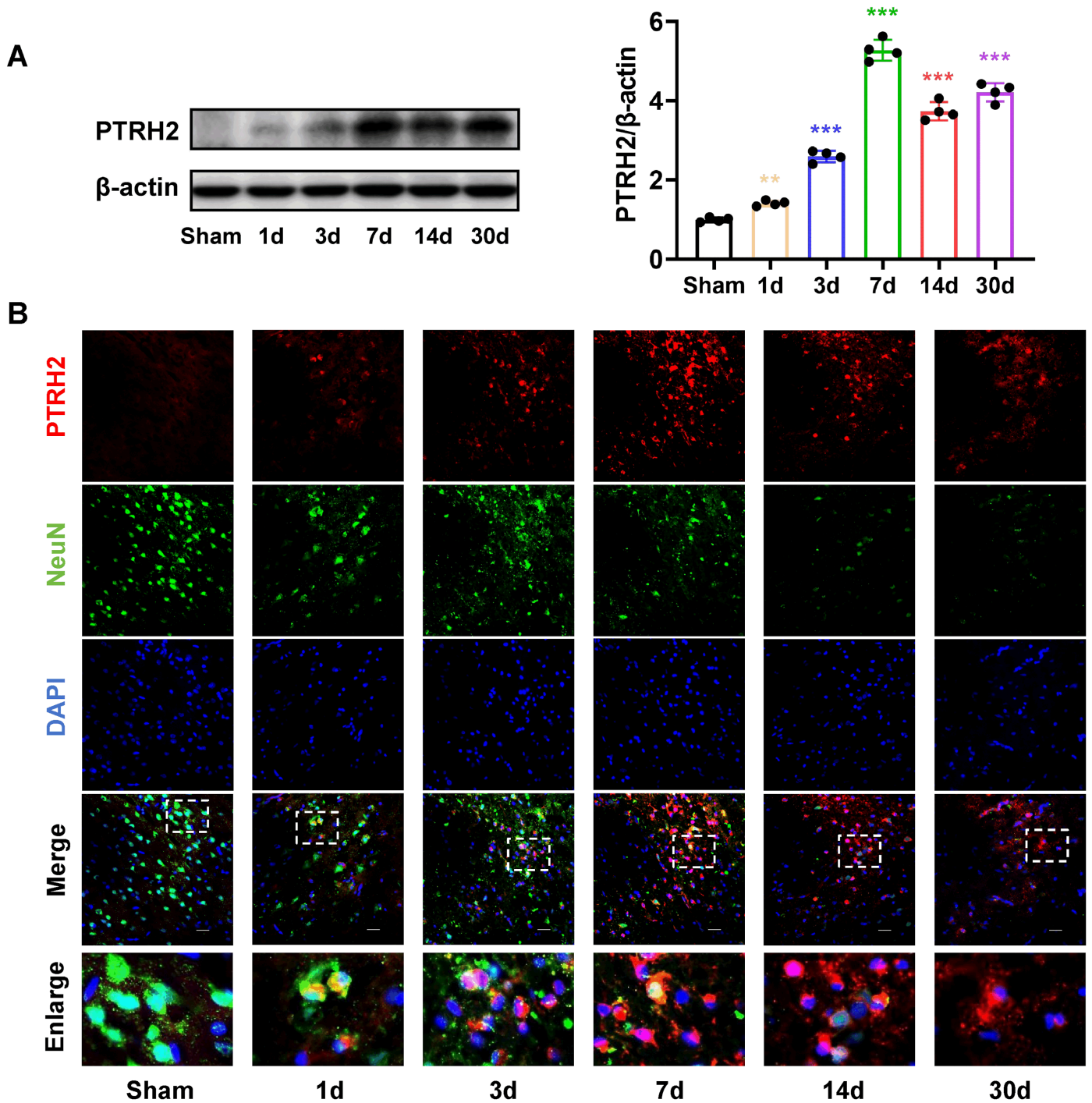


Figure 1

Expression of PTRH2 in the cortical boundary zone of ischemic area at different time points. **(A)** Representative western blotting (WB) bands and quantification of PTRH2 at varying time points after cerebral I/R (n = 4 per group). **(B)** Immunofluorescence colocalization staining of PTRH2 and NeuN (scale bar = 50 μ m). * p < 0.05, ** p < 0.01, *** p < 0.001, **** p < 0.0001 vs. Sham group. Results are indicated as mean $\pm$ SD (one-way analysis followed by Tukey's post hoc test).

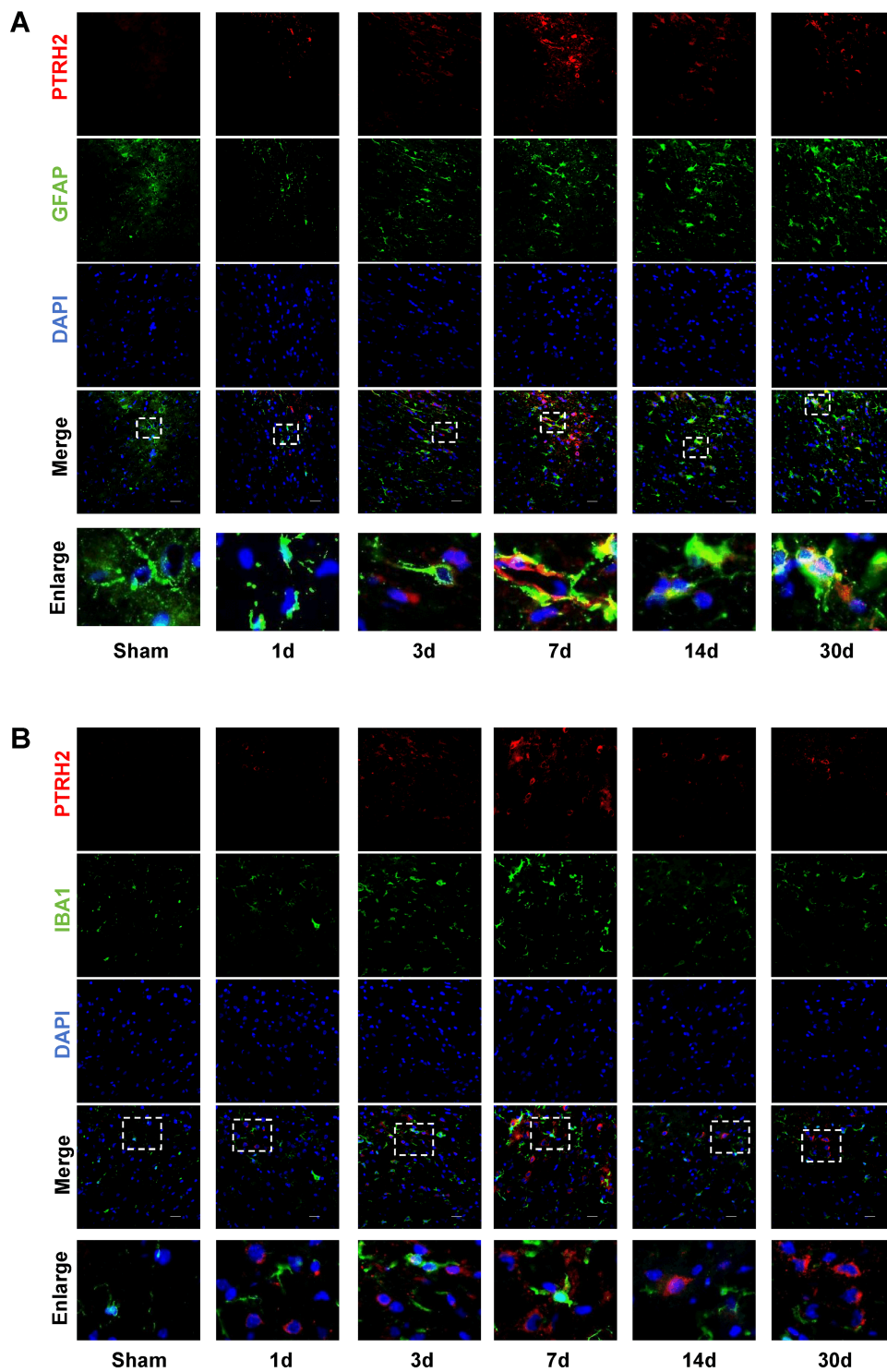


Figure 2

Immunofluorescence colocalization staining of PTRH2 with GFAP and IBA1, respectively. **(A)** Immunofluorescence colocalization staining of PTRH2 and GFAP (scale bar = 50 μ m). **(B)** Immunofluorescence colocalization staining of PTRH2 and IBA1 (scale bar = 50 μ m).

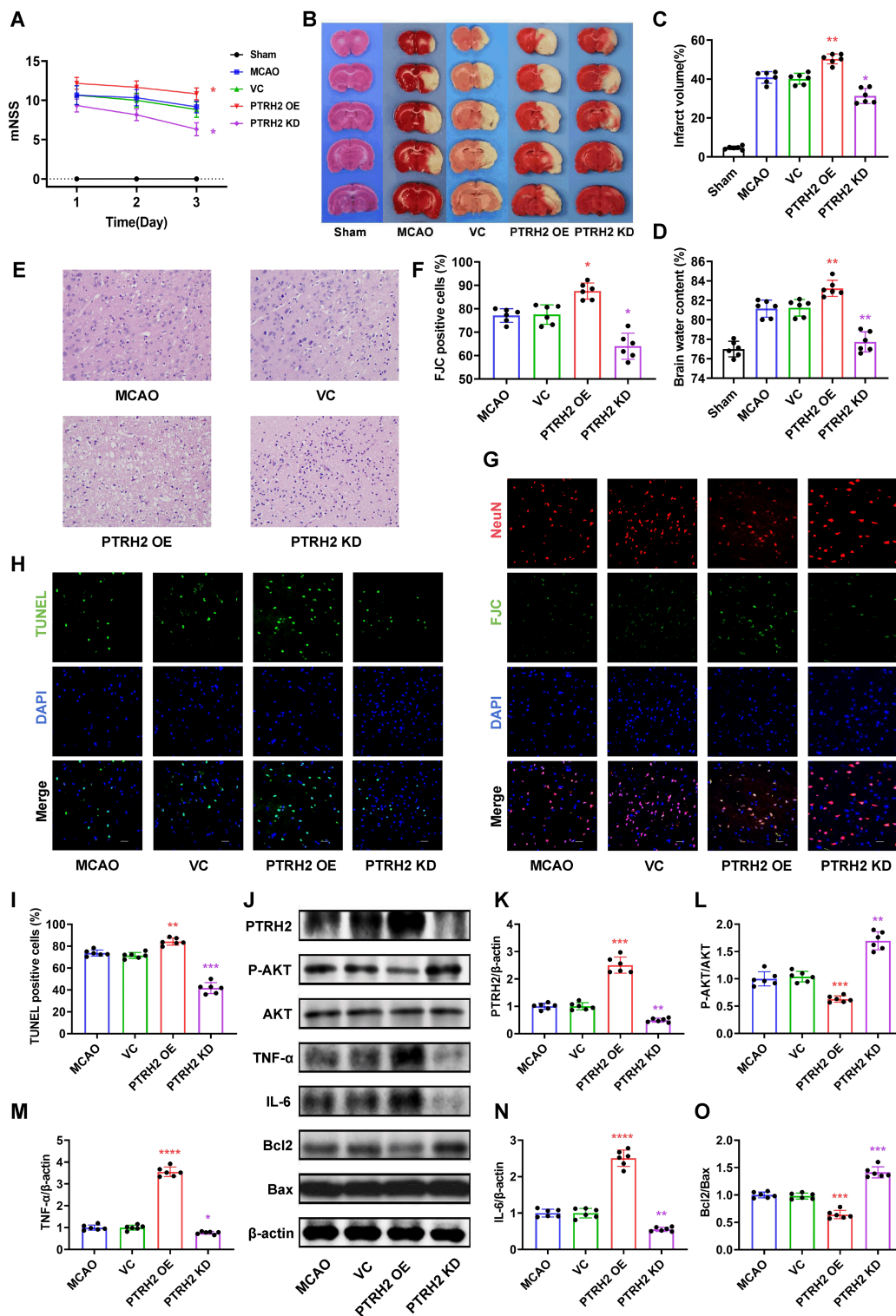


Figure 3

PTRH2 promotes brain injury and neuronal death after cerebral I/R. **(A)** The mNSS (n = 6 per group). **(B, C)** The infarct volume (n = 6 per group). **(D)** The brain water content (n = 6 per group). **(E)** The HE staining (× 400 magnification). **(F, G)** FJC positive cells in the ischemic cortical boundary zone (scale bar = 50 μm) (n = 6 per group). **(H, I)** TUNEL positive cells in the ischemic cortical boundary zone (scale bar = 50 μm) (n = 6 per group). **(J-O)** Representative WB bands and quantification of PTRH2, P-AKT, AKT, TNF-α,

IL-6, Bcl2 and Bax (n = 6 per group). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$ vs. VC group. The results are indicated as mean $\pm$ SD (one-way analysis followed by Tukey's post hoc test).

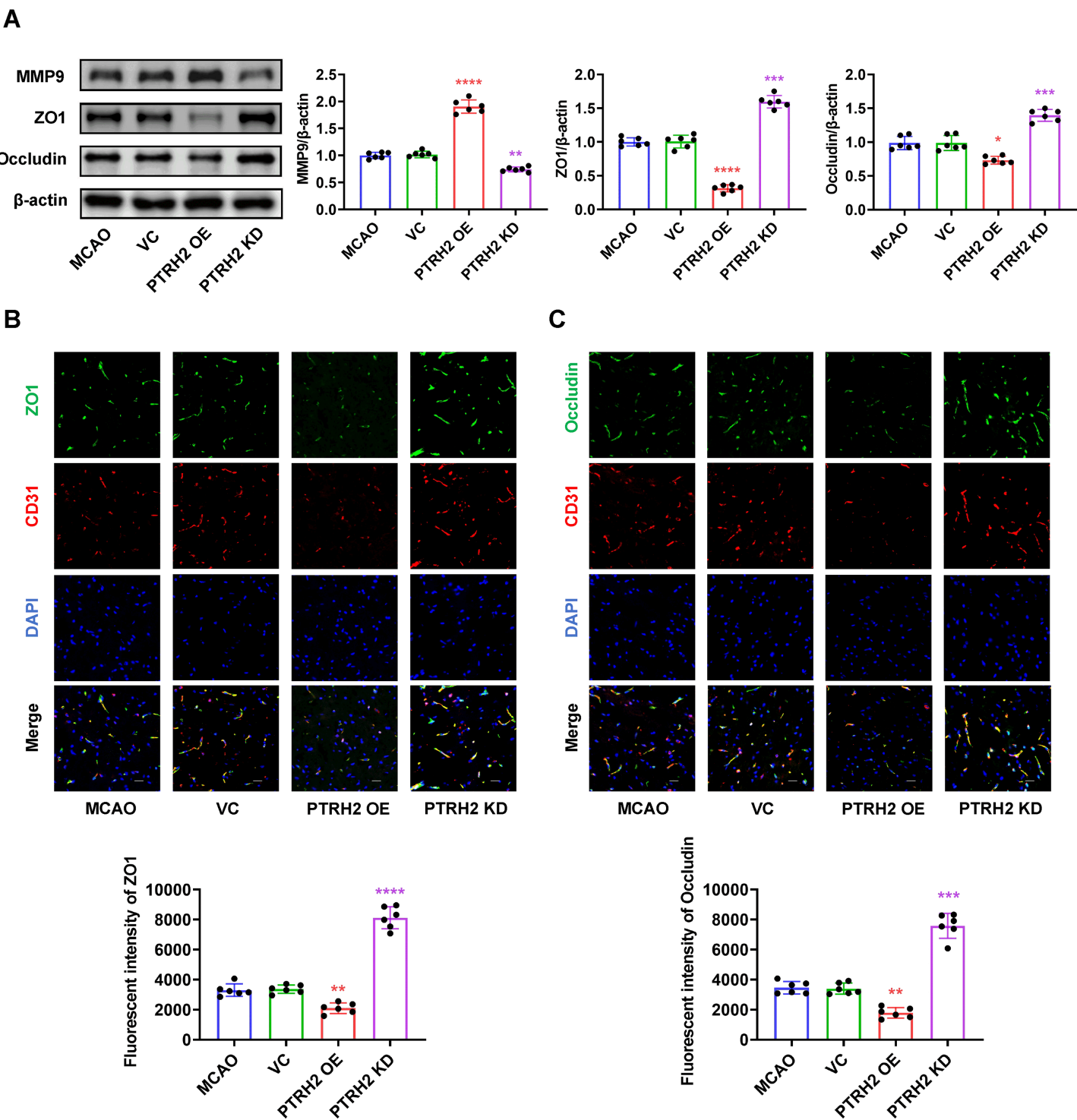


Figure 4

PTRH2 alleviates the BBB damage after cerebral I/R (A) Representative WB bands and quantification of MMP9, ZO1 and Occludin (n = 6 per group). (B) ZO1 IF staining and quantification in the ischemic cortical boundary zone (scale bar = 50 μ m) (n = 6 per group). (C) Occludin IF staining and quantification

in the ischemic cortical boundary zone (scale bar = 50 μ m) (n = 6 per group). * p < 0.05, ** p < 0.01, *** p < 0.001, **** p < 0.0001 vs. VC group. The results are indicated as mean $\pm$ SD (one-way analysis followed by Tukey's post hoc test).

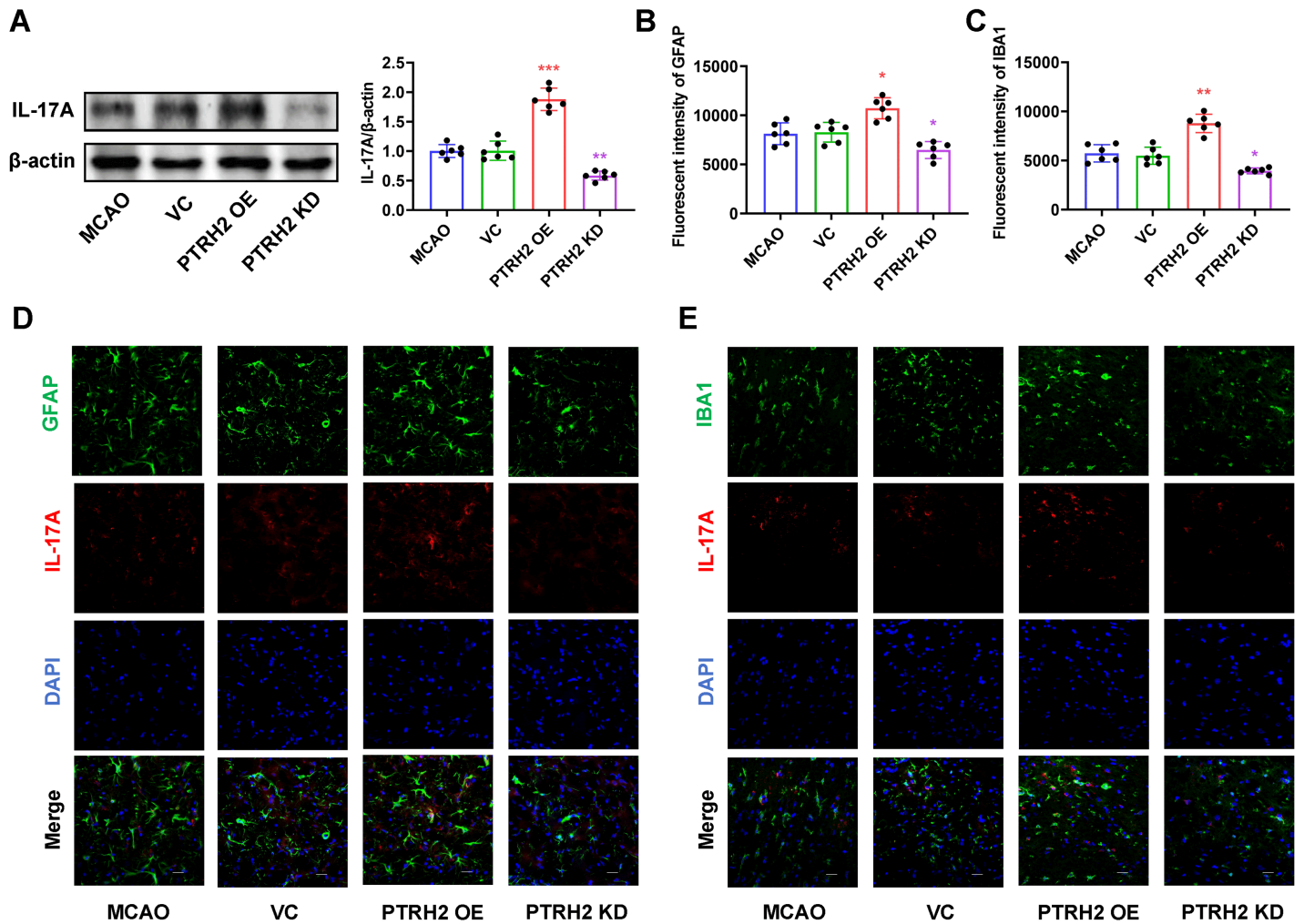


Figure 5

PTRH2 promotes cerebral I/R-induced glial cell activation and IL-17A expression. **(A)** Representative WB bands and quantification of IL-17A (n = 6 per group). **(B, D)** GFAP IF staining and quantification in the ischemic cortical boundary zone (scale bar = 50 μ m) (n = 6 per group). **(C, E)** IBA1 IF staining and quantification in the ischemic cortical boundary zone (scale bar = 50 μ m) (n = 6 per group). * p < 0.05, ** p < 0.01, *** p < 0.001, **** p < 0.0001 vs. VC group. The results are shown to be mean $\pm$ SD (one-way analysis followed by Tukey's post hoc test).

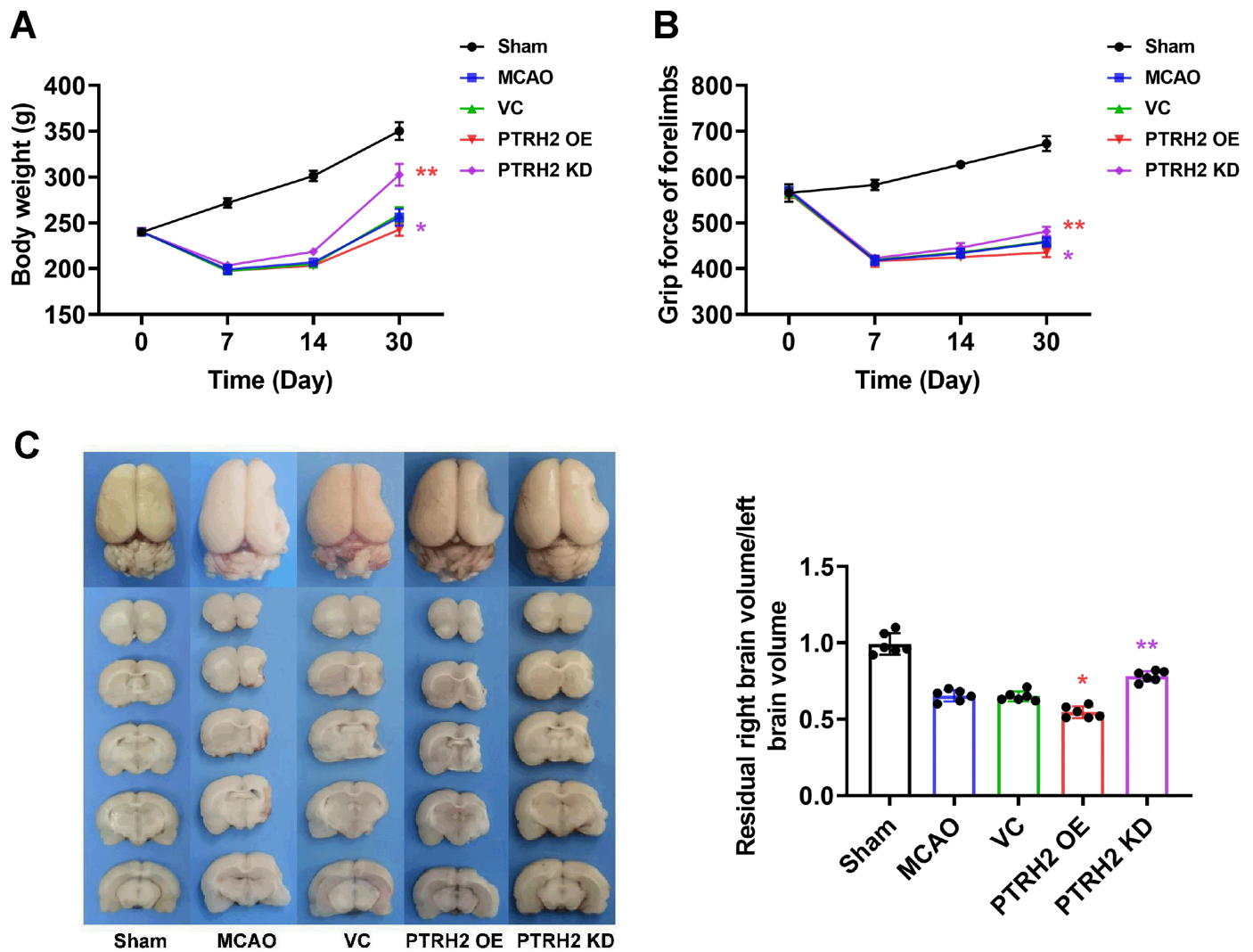


Figure 6

PTRH2 attenuates neurological function recovery after cerebral I/R. **(A)** The body weight at different time points (n = 6 per group). **(B)** The grip force of forelimbs at different time points (n = 6 per group). **(C)** The brain atrophy volume (n = 6 per group). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$ vs. VC group. Results are indicated to be mean $\pm$ SD (one-way analysis followed by Tukey's post hoc test).

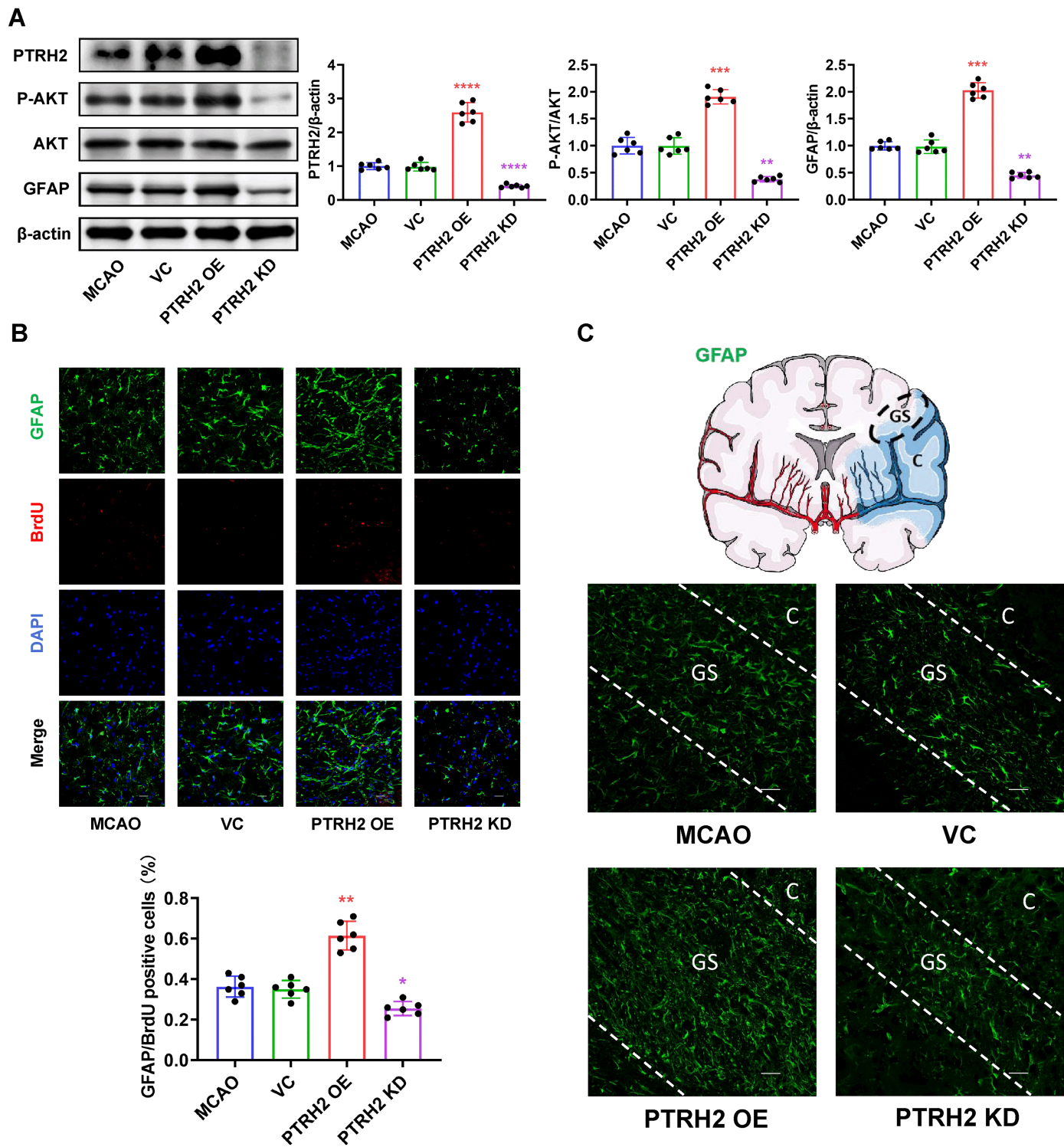


Figure 7

PTRH2 promotes glial scar formation after cerebral I/R. **(A)** Representative WB bands and quantification of PTRH2, P-AKT, AKT and GFAP ($n = 6$ per group). **(B)** GFAP and BrdU IF staining and quantification in the ischemic cortical boundary zone (scale bar = 50 μ m) ($n = 6$ per group). **(C)** Glial scar width IF staining in the ischemic cortical boundary zone (scale bar = 100 μ m) ($n = 6$ per group). * $p < 0.05$, ** $p < 0.01$, *** p

< 0.001, **** p < 0.0001 vs. VC group. Results are indicated as mean $\pm$ SD (one-way analysis followed by Tukey's post hoc test).

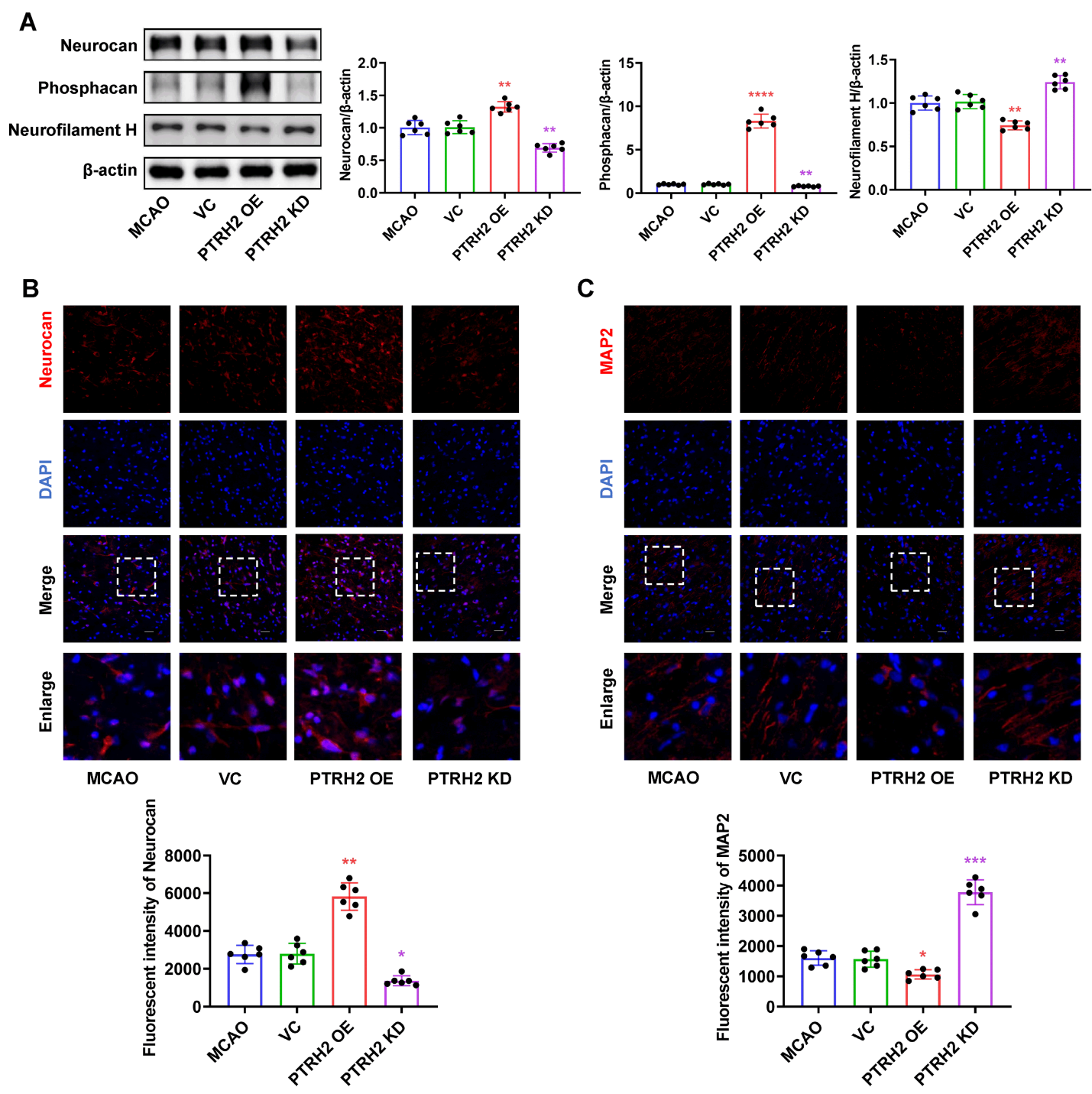


Figure 8

PTRH2 inhibits neural remodeling after cerebral I/R. **(A)** Representative WB bands and quantification of Neurocan, Phosphacan and Neurofilament H ($n = 6$ per group). **(B)** Neurocan IF staining and quantification in the ischemic cortical boundary zone (scale bar = 50 μ m) ($n = 6$ per group). **(C)** MAP2 IF staining and quantification in the ischemic cortical boundary zone (scale bar = 50 μ m) ($n = 6$ per group). * p < 0.05, ** p

< 0.01, *** p < 0.001, **** p < 0.0001 vs. VC group. The results are indicated as mean $\pm$ SD (one-way analysis followed by Tukey's post hoc test).

Supplementary Files

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