# ADAM17 Aggravates the Inflammatory Response by Modulating Microglia Polarization Through the TGF-β1/Smad Pathway Following Experimental Traumatic Brain Injury

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**Research**

**Keywords:** Traumatic brain injury, ADAM17, Microglia, Neuroinflammation, TGF-β1/Smad pathway

**Posted Date:** February 4th, 2021

**DOI:** https://doi.org/10.21203/rs.3.rs-171893/v1

# Abstract

Background

Microglia-mediated neuroinflammatory responses play important roles in secondary neurological injury after traumatic brain injury (TBI). The TGF-β pathway participates in the regulation of M1/M2 phenotype transformation of microglia. TGF-β can activate the Smad pathway by binding to TGF-βRs, which is regulated by the cleavage function of A disintegrin and metalloproteinase 17 (ADAM17). ADAM17, as an important regulatory factor of the TGF-β pathway, is considered an important factor in regulating the inflammatory response. However, the role of ADAM17 and the associated signaling pathways in the pathological process after TBI remain unclear.

Methods

A rat model of TBI was established by the Feeney weight-drop method. Neurological severity, brain water content, and Nissl staining were used to evaluate the neuroprotective effect of inhibiting ADAM17 expression. In vitro and in vivo experiments were conducted to detect the transformation of microglia M1/M2 phenotype polarization and the neuroinflammatory response after specific inhibition of ADAM17. The formation of TGF-βRs and TGF-β1/TGF-βRII complexes on microglia were detected by immunofluorescence, western blot analysis, and co-immunoprecipitation in order to evaluate the effect of ADAM17 inhibition on the TGF-β1/Smad pathway. Meanwhile, Smad nuclear translocation, secretion, and TGF-β1/ Smad-mediated activation of the inflammatory reaction were referenced to evaluate the effects of ADAM17 inhibition and gain further insight into the mechanisms underlying development of the neuroendocrine response after TBI.

Results

ADAM17 was highly expressed after TBI and mainly located in the microglia. Specific inhibition of ADAM17 reduced permeability of the blood-brain barrier, the degree of brain edema, and apoptosis of nerve cells, and improved neurological function after TBI. Further studies indicated that the neuroprotective effect of ADAM17 inhibition was related to a shift from the M1 microglial phenotype to the M2 microglial phenotype, thus reducing TBI-induced neuroinflammation. ADAM17 inhibition increased expression of TGF-βRs on the microglia membrane, promoted formation of TGF-β1/TGF-βRII complexes, and induced intranuclear translocation of Smads, which activated the TGF-β/Smad pathway. ADAM17 inhibition regulated microglia M1/M2 phenotype polarization through the TGF-β1/Smad pathway and influenced the neuroinflammatory response after TBI.

Conclusions

Microglial activation, the neuroinflammatory response, and the TGF-β1/Smad signaling pathway play essential roles in secondary injury after TBI. Specific inhibition of ADAM17 increased the expression of TGF-βRs on the microglia membrane and promoted formation of TGF-β1/TGF-βRII complexes. The TGF-β1/TGF-βRII complexes then induced intranuclear translocation of Smads, activated the TGF-β 1/Smad

pathway, and regulated M1/M2 polarization of microglia, which influence the neuroinflammatory response as well as play a neuroprotective role after TBI

## Significance Statement

Microglia-mediated neuroinflammatory responses play important roles in secondary neurological injury after TBI. Regulation of the phenotype of microglia and inflammatory factors is a potential strategy for the treatment of TBI. Here, we investigated the potential molecular mechanisms focusing on the phenotypic transition of microglia and TGF-β1/Smad pathway mediated by ADAM17 after TBI. we show that ADAM17 was highly expressed after TBI and mainly located in the microglia. Specific inhibition of ADAM17 increased the expression of TGF-βRs on the microglia membrane and promoted formation of TGF-β1/TGF-βRII complexes. The TGF-β1/TGF-βRII complexes then induced intranuclear translocation of Smads, activated the TGF-β 1/Smad pathway, and regulated M1/M2 polarization of microglia, which influence the neuroinflammatory response as well as play a neuroprotective role after TBI.

## Introduction

Primary brain injury triggers a series of harmful inflammatory processes that aggravate the initial tissue damage and impair neural function, which are important pathological features after traumatic brain injury (TBI).[1] Microglia, the resident immune cells of the central nervous system, play significant roles in neuroinflammation.[2–5] Microglia activated by traumatic stress exhibit two polarized phenotypes: the classic activated M1 (pro-inflammatory) phenotype and the alternative activated M2 (anti-inflammatory) phenotype.[6] Previous our studies have confirmed that the polarization phenotype of microglia is closely related to the outcome of neuroinflammation after TBI.[7]

Post-traumatic activation of M1 phenotype microglia and the subsequent release of inflammatory mediators, such as tumor necrosis factor (TNF), interleukins (ILs), and interferons (IFN), can directly damage nerve cells or induce the expression of cell adhesion molecules and chemokines, leading to increased capillary and blood-brain barrier (BBB) permeability, brain edema.[8, 9] On the contrary, M2 microglia secrete anti-inflammatory factors, such as transforming growth factor-β (TGF-β), IL-10, and IL-8. The recovery of neurological function after TBI can be improved by promoting the activation of M2 microglia and regulating the M1/M2 ratio and neuroinflammation.[10–12]

At the same time, changes to microinflammatory reactions will also cause transformation to the polarization phenotype of microglia.[3, 13, 14] TGF-β secreted by M2 microglia, can promote the transformation of the M1 phenotype to the M2 phenotype, which is characterized by up-regulation of the anti-inflammatory cytokine IL-10 and inhibition of the synthesis of the proinflammatory cytokines TNF-α and IL-6.[15–17] After TGF-β binds to type I and II serine/threonine kinase receptors (TGF-βRI and TGF-βRII, respectively), activated TGF-βRII can phosphorylate TGF-βRI, thus activating the classic Smads pathway and non-Smad pathways, such as the PI3K/Akt and MPAK pathways, which convey a variety of biological effects and play important regulatory roles in inflammatory reactions.[18–20]

The shedding of the extracellular domain of membrane-anchored receptors mediated by the ectodomain shedding enzyme A disintegrin and metalloproteinase 17 (ADAM17) can cleave various substrates, including cytokine precursors (e.g., pro-TNF-α), cytokine receptors (e.g., IL-6R, TNF-R, and TGF-βRs), ErbB ligands (e.g., TGF-α and TGF-β), and adhesion proteins (e.g., L-selectin), etc..[21, 22] ADAM17 cleaves the extracellular domain of TGF-βRs, thereby down-regulating the downstream TGF-β/Smad pathways, and participates in the pathological processes of tumors and autoimmune diseases.[20, 23]

Relatively few studies have explored the role of ADAM17 in diseases of the central nervous system. ADAM17 can promote the survival of microglia through the EGFR pathway after spinal cord injury.[24, 25] Inhibition of ADAM17 after cortical injury can reduce gliosis at the site of injury by promoting neuron formation as well as nerve repair.[24, 26] The results of these studies suggest that ADAM17 plays an important role in nerve repair. However, the role and underlying mechanism of ADAM17 in the regulation of inflammatory responses after TBI remain unclear. Considering that ADAM17 is an important regulatory factor of the TGF-β pathway[27, 28], it may be closely related to microglia polarization and the neuroinflammatory response. Therefore, the aim of the present study was to determine whether ADAM17 can influence the M1/M2 polarization of microglia and the neuroinflammatory response mediated by the TGF-β pathway in the pathological process after TBI.

# Materials And Methods

### Animals

Adult male Sprague-D

awley rats (weighing 230–260 g) were purchased from the Experimental Animal Center of Fujian Medical University (Fuzhou, Fujian, China) and housed in a clean, temperature-controlled environment (23°C ± 2°C) under a 12-h light/dark cycle with free access to food and water. The experimental protocols of the present study, including all surgical procedures and animal usages, were approved by the Experimental Animal Ethics Committee of Fujian Medical University and conducted in accordance with the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health (Bethesda, MD, USA).

### Cell culture and treatment

Murine microglial BV-2 cells were obtained from the Institute of Basic Medical Sciences of the Chinese Academy of Medical Sciences (Beijing, China) and maintained in Dulbecco's modified Eagle's medium (Nanjing KeyGen Biotech. Co., Ltd.) supplemented with 10% fetal bovine serum (Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA) and 1% penicillin/streptomycin (Nanjing KeyGen Biotech. Co. Ltd.) at 37°C under an atmosphere of 5% $CO_2$/95% air. In vitro, BV2 cells were stimulated with 10 μg/ml of lipopolysaccharide (LPS; Dalian Meilun Biotech Co., Ltd., Dalian, China) for 6 h and 20 ng/ml of exogenous IL-4 (Novoprotein Scientific, Shanghai, China) overnight, respectively, to mimic the M1 and M2 polarization environments of the brain. Then, 50 nM LY2157299 (Selleck Chemicals, Houston, TX,USA), a

TGF-βRI kinase inhibitor, was applied to the BV2 cells for further study of the signaling pathways involved in polarization.

### Cell transfection

To evaluate the effect of ADAM17 on microglia polarization, BV2 cells were transfected with small interfering RNA (siRNA) against ADAM17 and the plasmid pcDNA3.1-Flag-ADAM17, respectively. Mouse ADAM17 siRNA and a negative control were chemically synthesized by Guangzhou RiboBio Co., Ltd. (Guangzhou, China). Mouse pcDNA3.1-Flag-ADAM17 (open reading frame sequence MR210844) was purchased from PPL (Nanjing KeyGen Biotech. Co. Ltd.). The BV2 cells were transiently transfected with ADAM17 siRNA and the plasmid with a corresponding negative control using Lipofectamine 2000 reagent (Thermo Fisher Scientific Waltham, MA, USA) for 24 h at 37°C in accordance with the manufacturer's instructions. Afterward, transfected BV2 cells were treated with 50 nM LY2157299 for 24 h at 37°C for signaling pathway assessment. The target sequences of ADAM17 siRNA and RealTime-PCR primers of ADAM17 are showed in table S1, S2.

### Animal model and drug delivery

Rats were randomly assigned to one of four groups: sham, TBI, TBI+Vehicle, or TBI+ TNF-alpha protease inhibitor I (TAPI-1, an ADAM17 inhibitor) (n = 48 each), each of which were further divided into four time-based subgroups (1, 3, 7, and 14 days). The rat TBI model was established as previously described under sodium pentobarbital anesthesia (50 mg/kg by intraperitoneal injection). An incision was made to the scalp and a 5 mm-diameter hole was made to the right side of the coronal suture (anterior-posterior, -2 mm; lateral-anterior, 2 mm). A 40-g hammer was dropped on the brain from a height of 20 cm to simulate TBI. The hole in the posterior bone was then sealed closed with wax. In sham rats, the surgery was performed but the impact was omitted. Rats in the TBI+TAPI-1 group were administered TAPI-1 by intraventricular injection (300 mg/kg/day; Sigma–Aldrich Corporation, St. Louis, MO, USA) at 0.5 h after surgery, while those in the TBI+Vehicle group were administered an equal volume of the vehicle dimethyl sulfoxide as a negative control.

### Neurological impairment score

Rats were subjected to exercise (muscular state and abnormal action), sensation (visual, tactile, and balance), and reflex examinations and assigned a modified neurological severity score (mNSS) that was recorded when the rat failed to complete the task or there was no corresponding reflex. The mNSS ranged from 0 to 18 points, where a total score of 18 points indicated severe neurological deficits and a score of 0 indicated normal performance (13–18 points indicated severe injury, 7–12 indicated moderate injury, and 1–6 indicated mild injury). Neurological function was measured at different time points by investigators who were blinded to group information.

### Measurement of brain water content and BBB permeability

Brain water content was calculated using the wet weight-dry weight method. Animals were sacrificed after neurological assessment and the brain cortex was removed at the edge of the bone window (200 ± 20 mg). Filter paper was used to remove excess blood and cerebrospinal fluid. The wet weight was measured and the brains were dried in an oven at 100°C for 24 h until a constant weight was achieved, at which point the dry weight was measured. The % brain water content was calculated as: (wet weight − dry weight) / wet weight × 100%.

BBB permeability was investigated by measuring the extravasation of Evans blue dye (2% in saline; 4 mL/kg; Sigma-Aldrich Corporation), which was intravenously injected 2 h prior to sacrifice on post-injury day 3. Following sacrifice, the mice were transcardially perfused with PBS followed by PBS containing 4% paraformaldehyde. Each tissue sample was immediately weighed, homogenized in 1 mL of 50% trichloroacetic acid, and centrifuged. Then, the absorption of the supernatant was measured with a spectrophotometer (UV-1800 ENG 240V; Shimadzu Corporation, Kyoto, Japan) at a wavelength of 620 nm. The quantity of Evans blue dye was calculated using a standard curve and expressed as μg / g of brain tissue.

### Nissl staining

Formaldehyde-fixed specimens were embedded in paraffin and cut into 4 μm-thick sections that were deparaffinized with xylene and rehydrated in a graded series of alcohol. Samples were treated with Nissl staining solution for 5 min. Damaged neurons were shrunken or contained vacuoles, whereas normal neurons had a relatively large and full soma and round, large nuclei. Five randomly selected areas were examined by microscopy by investigators who were blinded to the experimental group.

### Immunohistochemical analysis

Formaldehyde-fixed specimens were embedded in paraffin and cut into 4 μm-thick sections that were deparaffinized with xylene and rehydrated in a graded series of alcohol. Antigen retrieval was carried out by microwaving in citric acid buffer. Sections were incubated with an antibody (Ab) against ADAM17 at a dilution of 1:200 (Abcam plc, Cambridge, UK), washed, and then incubated with a secondary Ab for 1 h at room temperature. The negative control was prepared without adding the anti-ADAM17 Ab. Five randomly selected visual fields were analyzed. Signal intensity was evaluated as follows: 0, no positive cells; 1, very few positive cells; 2, moderate number of positive cells; 3, many positive cells; and 4, the highest number of positive cells.

### Immunofluorescence analysis

Formaldehyde-fixed specimens were embedded in paraffin and cut into 4 μm-thick sections that were deparaffinized with xylene and rehydrated in a graded series of alcohol, followed by antigen retrieval. Sections were incubated overnight at 4°C with Abs against neuronal nuclei (1:100; Wuhan Boster Biological Technology, Ltd., Wuhan, China), ionized calcium-binding adapter molecule (Iba)-1 (1:200; Santa Cruz Biotechnology, Inc., Dallas, TX, USA), glial fibrillary acidic protein (GFAP) (1:200; Santa Cruz

Biotechnology, Inc.), cluster of differentiation (CD)16, CD206, TGF-βRII, and p-Smads (1:100; Wuhan Boster Biological Technology, Ltd.). After washing, the sections were incubated with secondary Abs for 1 h at room temperature. The cell nuclei were stained with 4',6-diamidino-2-phenylindole. Immunopositive cells in five randomly selected fields were counted under a microscope (Leica, Wetzlar, Germany) at 400× magnification by investigators who were blinded to the experimental group.

### Terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL) assay

Apoptotic cells were detected using a TUNEL kit (Roche Diagnostics, Indianapolis, IN, USA) in accordance with the manufacturer's instructions. Indicators of apoptosis included a shrunken cell body, irregular shape, nuclear condensation, and brown diaminobenzidine staining, as observed by microscopy at 400× magnification. Positive cells in five random fields per section were counted.

### Enzyme-linked immunosorbent assay (ELISA)

Inflammatory factors (i.e., TNF-α, IL-1 β, IL-6, and IFN-γ) of brain tissues and BV2 cell culture supernatants were detected using mouse ELISA kits (Nanjing KeyGen Biotech. Co., Ltd.) at an optical density of 450 nm using a microplate reader (SpectraMax M3; Molecular Devices, Inc., San Jose, CA, USA).

### Western blot analysis

The samples, including brain tissues and BV2 cells, were prepared using nuclear and cytoplasmic protein purification assays (Nanjing KeyGen Biotech. Co., Ltd.) with modified radioimmunoprecipitation lysis buffer (50 mM Tris-HCl pH 7.4, 150 mM NaCl, 1% Triton X-100, 1% sodium deoxycholate, 0.1% sodium dodecyl sulfate). The protein concentrations of the cell nuclear and cytosolic lysates were determined separately with a bicinchoninic acid assay (Beyotime Institute of Biotechnology, Shanghai, China). Approximately 25 μg of protein were loaded to each well of a polyacrylamide gel, separated by electrophoresis, and then transferred to polyvinylidene difluoride (PVDF) membranes, which were incubated with primary Abs against B cell lymphoma (Bcl)-2 (1:2000; Abcam plc), Bcl-2-associated X factor (Bax) (1:5000; Abcam plc), cleaved caspase-3 (1:3000), Iba-1 (1:1000), CD16, CD206, IL-1β, Arg-1 (1:2000), TGF-β1, TGF-βRI, TGF-βRII, TGF-βRIII, p-Smads, and Smads (1:1000) (all purchased from Abcam, Shanghai, China), followed by incubation with appropriate secondary Abs. Immunoreactivity was visualized with the ECL Western Blotting Detection System (EMD Millipore Corporation, Billerica, MA, USA). Grey value analysis was conducted with UN-Scan-It 6.1 software (Silk Scientific Inc., Orem, UT, USA). Expression levels were normalized against β-actin (1:5000; Wuhan Boster Biological Technology, Ltd.)

### Co-immunoprecipitation (Co-IP) analysis

BV2 cells were homogenized in IP lysis Buffer (Nanjing KeyGen Biotech. Co., Ltd.) and then incubated with 1 μg of TGF-β1 (Santa Cruz Biotechnology, Inc.) or TGF-βR II (Abcam plc) Abs or immunoglobulin G (Abcam plc) for 1 h at 4°C. A 10-μl volume of protein A agarose beads (Roche Diagnostics Deutschland GmbH, Mannheim, Germany) was added to the sample lysate mixture and incubated overnight with

primary Abs at 4°C. After immunoprecipitation and centrifugation, the agarose beads were washed three times with lysis buffer and used for immunoblotting to detect expression of the TGF-βRII and TGF-β1 proteins in order to estimate the expression level of the TGF-β1/TGF-βRII complex.

### Statistical analysis

Data were analyzed using PASW Statistics for Windows, version 18.0. (SPSS Inc., Chicago, IL, USA). All experiments were performed in triplicate unless otherwise noted and the results are expressed as the mean ± standard deviation. Comparisons between groups were made with the unpaired Student's *t*-test. Multiple-group comparisons were assessed by one-way analysis of variance and post hoc multiple comparisons were performed using the Student–Newman–Keuls test. A probability ($p$) value of $< 0.05$ was considered statistically significant.

## Results

### ADAM17 expression increased after TBI and was specific to the microglia of the injured cerebral cortex

In the rat TBI model, a molecular biology study was performed on post-injury days 1, 3, 7, and 14 (Fig. 1a). The western blot results showed that ADAM17 levels were significantly increased on post-injury days 1, 3, and 7, with the most significant increase on post-injury day 3 ($p < 0.05$), which then gradually decreased to almost normal at post-injury day 14 (Fig. 1b). Immunohistochemistry also indicated that ADAM17 was highly expressed in the cortical injury area of the TBI group on post-injury day 3 (Fig. 1c).

In order to clarify the location of ADAM17 in cortical cells after brain injury, immunofluorescence double staining was performed to detect ADAM17 as well as markers specific to neurons (NeuN), microglia (Iba-1), and astrocytes (GAFP). The results showed that ADAM17 was mainly expressed in neurons and microglia, with no obvious expression in astrocytes (Fig. 1d).

### Specific inhibition of ADAM17 can improve neurological function after TBI

TAPI-1 was used to specifically inhibit the activity of ADAM17 and the mNSS was used to evaluate post-TBI neurological function after specific inhibition of ADAM17. The results showed that as compared to the TBI group, the mNSS of the TBI+TAPI-1 group was remarkably improved at post-injury day 3 (10.51 ± 0.38 vs. 12.21 ± 0.52, respectively, $p < 0.05$), suggesting that inhibition of ADAM17 contributes to improved neurological function after TBI (Fig. 2a).

The contents of Evans blue dye and brain water can be used to quantitatively evaluate the degree of BBB destruction and brain edema. The results showed that on post-injury day 3, the brain water content of the TBI+TAPI-1 group was appreciably reduced as compared to the TBI group (Fig. 2b). Concurrently, the corresponding penetration of Evans blue dye was significantly reduced in the damaged cortex (Fig. 2c).

### Specific inhibition of ADAM17 can reduce neuronal apoptosis after TBI

Nissl staining, TUNEL staining, and western blot analysis were used to detect apoptosis-related factors as well as neuronal apoptosis. The results of Nissl staining of the cerebral cortex showed that the apoptosis rate of neurons in the Sham group was low, at about 4 to 6%. In contrast to the Sham group, the apoptosis rate of neurons in the TBI group was significantly increased after injury at each time period ($p$ = 0.00). After TAPI-1-induced inhibition of ADAM17, as compared to the TBI group, the neuronal apoptosis rate in the TBI+TAPI-1 group on post-injury day 3 was significantly reduced (53.00 ± 6.22% vs. 89.05 ± 8.04%, respectively, $p < 0.05$) (Fig. 3a, b).

The western blot results showed that as compared to the TBI group, the expression levels of apoptotic factors (i.e., cleaved capase-3 and Bax) in the cerebral cortex of the TBI+ TAPI-1 group were significantly reduced on post-injury day 3, while expression of the anti-apoptotic factors Bcl-2 was increased ($p$ <0.05) (Fig. 3c).

TUNEL staining was used to further detect neuronal apoptosis, where TUNEL positivity indicated apoptosis. The results showed that the number of TUNEL-positive neurons in the cerebral cortex of the TBI+TAPI-1 group was remarkably lower than that of the TBI group on post-injury day 3 ($p < 0.05$) (Fig. 3d). These results suggest that specific inhibition of ADAM17 can reduce neuronal apoptosis after TBI.

## Specific inhibition of ADAM17 promoted M1/M2 phenotype transformation and inhibited inflammatory reactions

Immunofluorescence double staining and western blot analysis were performed to detect changes in the expression levels of markers of the microglia (positive Iba-1 staining, red), M1 phenotype (positive CD16 staining, green), and M2 phenotype (positive CD206 staining, green) in brain contusion tissues. The results showed that on post-injury day 3, as compared to the sham group, positive staining of Iba-1 and M1 microglia were notably increased ($p < 0.05$) in the TBI group. As compared to the TBI group, CD16 expression was decreased and CD206 expression was increased in the TBI+TAPI-1 group ($p < 0.05$) (Fig. 4a, b).

Western blot analysis also showed that the expression levels of Iba-1 and CD16 were significantly increased in the cortex after TBI ($p < 0.05$). As compared to the TBI group, in the TBI+TAPI-1 group, expression of Iba-1 and CD16 was inhibited, while CD206 expression was increased, and the M1 phenotype was switched to the M2 phenotype ($p < 0.05$) (Fig. 4c).

The expression levels of inflammatory factors were detected by ELISA on post-injury day 3. As compared to the sham group, the serum expression levels of the inflammatory factor TNF-α, IL-1 β, IL-6, and IFN-γ were significantly increased in the TBI group on post-injury day 3 (all $p < 0.05$). As compared to the TBI group, the expression levels of the serum inflammatory factors were significantly decreased in the TBI + TAPI-1 group ($p < 0.05$) (Fig. 4d).

At the cellular level, exposure of BV2 microglia to LPS induced a switch to the M1 phenotype and positive expression of CD16 was notably increased. As compared to the siCtrl+LPS group, CD16 expression was

decreased in the siADAM17+LPS group, while CD206 expression was increased, indicating M2 phenotype polarization ($p < 0.05$). After overexpression of ADAM17, the BV2 microglia in the ADAM17 group were switched to the M1 phenotype. As compared to the ADAM17 group, CD16 expression was decreased and CD206 expression was increased in the ADAM17+TAPI-1 group, indicating M2 phenotype polarization ($p < 0.05$) (Fig. 5a, b).

The results of western blot analysis also showed that as compared to the siCrl+LPS group, the expression levels of CD16 and IL-1β were significantly inhibited, while those of CD206 and Arg-1 were increased in the siADAM17+LPS group, indicating a switch from the M1 phenotype to the M2 phenotype ($p < 0.05$) (Fig. 5c). As compared to the ADAM17 group, the expression levels of CD16 and IL-1β were significantly decreased, while those of CD206 and Arg-1 were increased in the ADAM17+TAPI-1 group. In the ADAM17+TAPI-1 group, the expression levels of CD16 and IL-1β were decreased, while those of CD206 and Arg-1 were observably increased, indicating M2 phenotype polarization ($p < 0.05$) (Fig. 5c).

The ELISA results showed that as compared to the siCtrl+LPS group, the expression levels of inducible nitric oxide synthase (iNOS) and IL-1β were significantly decreased, while those of Arg-1 and TGF-β1 were increased in the siADAM17+LPS group ($p < 0.05$). Overexpression of ADAM17 promoted the expression of iNOS and IL-1 β, but decreased that of Arg-1 and TGF-β1. TAPI-1 specifically inhibited the expression of ADAM17, which counteracted the M1 phenotype polarization of microglia induced by ADAM17. Meanwhile, cells in the ADAM17+TAPI-1 group had the M2 phenotype ($p < 0.05$) (Fig. 5d). As compared to the siCtrl+LPS group, the expression levels of TNF-α, IL-6, and IFN-γ were significantly decreased in the supernatant of the siADAM17+LPS group (all $p < 0.05$). Similarly, the expression levels of inflammatory factors were considerably lower in the ADAM17+TAPI-1 group than in the ADAM17 group ($p < 0.05$) (Fig. 5e).

### Specific inhibition of ADAM17 activate genes related to the TGF-β 1 pathway

TGF-β1 and its receptors (TGF-βRI and, TGF-βRII), affected by the cleavage function of ADAM17, are important factors in regulating M1/M2 polarization of microglia.[16] Western blot analysis showed that after specific inhibition of ADAM17, the expression levels of TGF-β1, TGF-βRI, and TGF-βRII were significantly increased in the TBI+TAPI-1 group. Meanwhile, the downstream classic Smads pathway was activated and the expression of p-Smads was significantly increased ($p < 0.05$) (Fig. 6a). Immunofluorescence double staining showed that the expression of TGF-βRII (red) on microglia (positive Iba-1 staining, green) was significantly higher in the TBI+TAPI-1 group than the TBI group (Fig. 6b).

As compared to the siCtrl+LPS group, the expression levels of TGF-β1, TGF-βRI and TGF-βRII were appreciably increased in the siADAM17+LPS group. The downstream classical Smads pathway was activated and the expression of p-Smads was markedly increased. Overexpression of ADAM17 inhibited expression of TGF-β1, TGF-βRI, and TGF-βRII, and the activation of the downstream Smads pathway, while the expression of p-Smads was significantly decreased. As compared to the ADAM17 group, the TGF-β1 pathway was activated after inhibition of ADAM17 expression in the ADAM17+TAPI-1 group ($p < 0.05$) (Fig. 7a).

As compared to the siCtrl+LPS group, TGF-βRII (red) expression by microglia (Iba-1 positive, green) remarkably increased in the siADAM17+LPS group (Fig. 7b), while inhibited in the ADAM17 group. As compared to the ADAM17 group, TGF-βRII expression was significantly increased in the ADAM17+TAPI-1 group (Fig. 7b).

Co-IP analysis showed that as compared to the siCtrl+LPS group, TGF-β1/TGF-βRII complex formation was notably increased in the siADAM17+LPS group. ADAM17 inhibited TGF-β1/TGF-βRII complex formation. As compared to the ADAM17 group, TGF-β1/TGF-βRII complex formation was promoted in the ADAM17+TAPI-1 group (Fig. 7c).

TGF-β1/TGF-β RII complex formation can activate the downstream classic Smads pathway. Western blot analysis and immunofluorescence staining further showed that as compared to the siCtrl+LPS group, siADAM17 significantly increased translocation of p-Smads from the cytosol to the nucleus and increased p-Smads expression, while ADAM17 inhibited intranuclear translocation of p-Smads. On the contrary, the ADAM17 inhibitor TAPI-1 counteracted the inhibitory effect of ADAM17 on translocation of p-Smads (Fig. 8a, b).

### Inhibition of the TGF-β1 pathway hindered ADAM17 regulation of the M1/M2 phenotype transformation of microglia

In order to further clarify whether the TGF-β1 pathway participates in the regulation of microglia polarization by ADAM17, TGF-β receptor inhibitor (LY2157299) was used to specifically inhibit these pathways. As compared to the siADAM17+LPS group, LY2157299 notably inhibited the expression of TGF-βRI, TGF-βRII, and downstream p-Smads (Fig. 9a). As compared to the siADAM17+LPS group, the expression levels of iNOS and IL-1β were increased, while those of Arg-1 and TGF-β were decreased in the siADAM17+LPS+LY group, indicating counteraction of the effect of ADAM17 on M2 phenotype polarization of microglia and a switch from the M1 phenotype in the siADAM17+LPS+LY group ($p < 0.05$) (Fig. 9b). After LY2157299 specifically inhibited TGF-βRs, the expression levels of related inflammatory factors were considerably increased in the siADAM17+LPS+LY group as compared to the siADAM17+LPS group, which offset the anti-inflammatory effect of siADAM17 ($p < 0.05$) (Fig. 9c).

## Discussion

Our previous studies have confirmed that the M1/M2 polarization phenotype of microglia is closely related to the outcome of neuroinflammation. Inducing polarization of the M2 phenotype and adjusting the M1/M2 ratio can improve the prognosis of neuroinflammation after TBI and restore nerve function.[29] Exploring the molecular mechanisms that regulate the M1/M2 polarization of microglia is particularly vital to improve neurological function after TBI.[15, 29] The results of the present study revealed high expression of ADAM17 in the damaged area of the cerebral cortex after TBI injury and was mainly located in microglia, which are closely related to the inflammatory response. In TBI rats, inhibition of ADAM17 lowered permeability of the BBB, reduced the degree of brain edema, and inhibited apoptosis of nerve cells, thereby improving nerve function. Inhibition of ADAM17 suppressed microglia M1

phenotype polarization and induced M2 phenotype polarization, which can alleviate neuroinflammation. Further studies indicated that the neuroprotective function of ADAM17 inhibition is correlated with the mechanism of microglia M1/M2 phenotype polarization. Inhibition of ADAM17 can increase the expression of TGF-βRs on the microglia membrane and promote the formation of TGF-β1/TGF-βRII complexes in addition to inducing nuclear transfer of Smads, then activating the TGF-β1/Smad pathway and finally regulating M1/M2 polarization of microglia.

Shedding of the extracellular domain of the membrane anchored receptor mediated by the shedding enzyme ADAM17 can affect the expression level and biological effects of the substrate, which plays a significant role in the regulation of inflammatory responses.[23, 25, 30] After TBI, the expression levels of metalloproteinases, including ADAM17, are increased. However, the underlying pathological mechanism remains unclear. The results of the present study show that in the TBI model, ADAM17 was highly expressed in the cerebral cortex at the site of injury and specifically localized on microglia, suggesting essential roles in neuromodulation and microglia-mediated neuroinflammation. After specifically inhibiting ADAM17, the detection of markers of the M1 phenotype was remarkably reduced, while the expression of markers of the M2 phenotype was increased. Meanwhile, the expression levels of inflammatory cytokines were decreased, while those of anti-inflammatory factors were increased. Collectively, these results demonstrate that the ADAM17 pathway can accelerate polarization of the M1/M2 phenotypes and repress activation of microglia as well as the neuroinflammatory response, thereby facilitating neural repairment after TBI. ADAM17 is relatively specific to the microglia specificity and, thus, should be considered as a therapeutic target for neuroinflammation after TBI.

The TGF-β pathway is critical to polarization of the M1/M2 phenotypes of microglia.[11, 31] TGF-β participates in neural damage and repair by binding to TGF-βRs and activating related signaling pathways (e.g., Smads, PI3K/AKT, p38/MAPK, etc.) and downstream gene expression.[17, 32] The activity of TGF-β1/Smad pathway is closely associated with the number of TGF-βRs.[18] ADAM17 cleaves the extracellular domain of TGF-βRs, thereby reducing expression, and down-regulates signal transduction of the downstream pathways.[23] The results of this study suggest that the expression and activity of ADAM17 were increased in the cortical microglia after TBI. At the same time, the expression levels of TGF-βRI and TGF-βRII were notably reduced on the cell membrane, which also affects downstream intranuclear translocation and phosphorylation of Smads. High expression of ADAM17 cleaves TGF-βR on the cell membrane leading to the formation of TGF-β1/TGF-βRII complexes, which affects the downstream Smad pathways. Specific inhibition of ADAM17 reduces the cleavage and detachment of TGF-βR on the cell membrane to increase expression levels. Consequently, TGF-β1 and TGF-βRII form a complex that induces intranuclear translocation of Smads and then activates the TGF-β1/Smad pathway. The activated pathway can regulate M1/M2 phenotype polarization of microglia and the neuroinflammatory response, which play significant roles in neuroprotection. Specific inhibition of TGF-βRs can reverse the neuroprotective effect of ADAM17 inhibition mentioned above.[24] These mechanisms demonstrate that the TGF-β1/Smad pathway plays a crucial role in the regulatory role of ADAM17 in the M1/M2 phenotype polarization of microglia as well as the neuroinflammatory response. In short, inhibition of ADAM17 up-regulated the expression levels of TGF-βRs on the microglia membrane

and boosted the formation of TGF-β1/TGF-βR complexes, while inducing intranuclear translocation of Smads, activating the TGF-β1/Smad pathway, and promoting switching to the M2 phenotype of microglia, ultimately inhibiting the neuroinflammatory response. Future studies involving ADAM17 knockout mice are warranted to explore the mechanism underlying the effect of ADAM17 on inflammation after TBI. Meanwhile, primary microglia cells are also needed to confirm the direct effects of ADAM17 on microglial activation.

# Conclusions

In summary, M1 phenotype polarization of microglia along with neuroinflammation plays a significant role in neural damage after TBI injury. ADAM17, which is highly expressed on the membrane of microglia, mediates shedding of the extracellular domain of TGF-βRs on the cell membrane, thereby decreasing the quantity of receptors. In addition, ADAM17 negatively regulates the TGF-β1/Smad pathway to promote polarization of the M1 phenotype and inhibit the M2 phenotype of microglia, resulting in the aggravation of neuroinflammation as well as neuronal damage after TBI. Inhibition of ADAM17 can up-regulate the expression of TGF-βRs, which are located on the membrane of microglia, and then activate the TGF-β1/Smad pathway and promote the polarization of the M2 phenotype in order to suppress neuroinflammation and improve neural function after TBI.

# Abbreviations

**TBI** = traumatic brain injury; **TGF-β =** transforming growth factor-β; **ADAM17 =** a disintegrin and metalloproteinase 17; **TNF** = tumor necrosis factor; **IL** = interleukin; **IFN** = interferon; **BBB** = blood-brain barrier; **SCI =** spinal cord injury; **LPS =** lipopolysaccharide**; siRNA** = small interfering RNA; **TAPI-1** = TNF-alpha protease inhibitor I; **mNSS =** modified neurological severity score; **Ab** = antibody; **GFAP** = glial fibrillary acidic protein; **CD** = cluster of differentiation; **TUNEL =** transferase dUTP nick-end labeling; **ELISA =** enzyme-linked immunosorbent assay; **PVDF** = polyvinylidene difluoride; **Bcl** = B cell lymphoma; **Bax** = Bcl-2-associated X factor; **Co-IP =** Co-immunoprecipitation.

# Declarations


### Acknowledgments

Sincere appreciation is also given to the teachers and our colleagues from the Second Affiliated Hospital of Fujian Medical University, who participated in this study with great cooperation. We thank International Science Editing (http://www.internationalscienceediting.com ) for editing this manuscript.

### Funding

This work was supported by grants from the funds for Fujian Province Scientific Foundation (2019J01168), Young and middle-aged backbone talent foundation of Fujian Provincial Commission of

Health Construction (2020GGA058) and Quanzhou High-level talent Innovation Foundation（2019C024R） from Dr. Xiangrong Chen.

### Availability of data and materials

All the datasets and materials supporting the conclusions of this article are presented in the manuscript.

### Competing interests

The authors declare that they have no competing interests.

### Consent for publication

Consent for publication is not applicable for this manuscript.

### Authors' contributions

X.C.;conception and design, writing of the manuscript. Y.Z., F.W., J.Y. and W.L.; supported several experiments, acquisition of data, analysis and interpretation of data. C.D., and X.T.;statistical analysis and revision of the manuscript. All authors read and approved the final manuscript.

### Ethics approval

The experimental protocols in the present study including all the surgical procedures and animal usages conformed to the guidelines for the care and use of laboratory animals by the National Institutes of Health (NIH) and were approved by the Fujian Medical University Experimental Animal Ethics Committee (Fuzhou, China).

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## References

1. Sandsmark DK, Bashir A, Wellington CL, Diaz-Arrastia R: Cerebral Microvascular Injury: A Potentially Treatable Endophenotype of Traumatic Brain Injury-Induced Neurodegeneration. Neuron 2019, 103:367-379.
2. Corps KN, Roth TL, McGavern DB: Inflammation and neuroprotection in traumatic brain injury. Jama Neurol 2015, 72:355-362.

3. Jassam YN, Izzy S, Whalen M, McGavern DB, El KJ: Neuroimmunology of Traumatic Brain Injury: Time for a Paradigm Shift. Neuron 2017, 95:1246-1265.
4. Shandra O, Winemiller AR, Heithoff BP, Munoz-Ballester C, George KK, Benko MJ, Zuidhoek IA, Besser MN, Curley DE, Edwards GR, et al: Repetitive Diffuse Mild Traumatic Brain Injury Causes an Atypical Astrocyte Response and Spontaneous Recurrent Seizures. J Neurosci 2019, 39:1944-1963.
5. Henry RJ, Ritzel RM, Barrett JP, Doran SJ, Jiao Y, Leach JB, Szeto GL, Wu J, Stoica BA, Faden AI, Loane DJ: Microglial Depletion with CSF1R Inhibitor During Chronic Phase of Experimental Traumatic Brain Injury Reduces Neurodegeneration and Neurological Deficits. J Neurosci 2020, 40:2960-2974.
6. Xiong XY, Liu L, Yang QW: Functions and mechanisms of microglia/macrophages in neuroinflammation and neurogenesis after stroke. Prog Neurobiol 2016, 142:23-44.
7. Ma Y, Wang J, Wang Y, Yang GY: The biphasic function of microglia in ischemic stroke. Prog Neurobiol 2017, 157:247-272.
8. Wolf SA, Boddeke HW, Kettenmann H: Microglia in Physiology and Disease. Annu Rev Physiol 2017, 79:619-643.
9. Tang Y, Le W: Differential Roles of M1 and M2 Microglia in Neurodegenerative Diseases. Mol Neurobiol 2016, 53:1181-1194.
10. Heneka MT, Carson MJ, El KJ, Landreth GE, Brosseron F, Feinstein DL, Jacobs AH, Wyss-Coray T, Vitorica J, Ransohoff RM, et al: Neuroinflammation in Alzheimer's disease. Lancet Neurol 2015, 14:388-405.
11. Liu Z, Chen HQ, Huang Y, Qiu YH, Peng YP: Transforming growth factor-beta1 acts via TbetaR-I on microglia to protect against MPP(+)-induced dopaminergic neuronal loss. Brain Behav Immun 2016, 51:131-143.
12. Lan X, Han X, Li Q, Yang QW, Wang J: Modulators of microglial activation and polarization after intracerebral haemorrhage. Nat Rev Neurol 2017, 13:420-433.
13. Sarlus H, Heneka MT: Microglia in Alzheimer's disease. J Clin Invest 2017, 127:3240-3249.
14. Eyolfson E, Khan A, Mychasiuk R, Lohman AW: Microglia dynamics in adolescent traumatic brain injury. J Neuroinflammation 2020, 17:326.
15. Ma Y, Wang J, Wang Y, Yang GY: The biphasic function of microglia in ischemic stroke. Prog Neurobiol 2017, 157:247-272.
16. Qin Y, Garrison BS, Ma W, Wang R, Jiang A, Li J, Mistry M, Bronson RT, Santoro D, Franco C, et al: A Milieu Molecule for TGF-beta Required for Microglia Function in the Nervous System. Cell 2018, 174:156-171.
17. Devanney NA, Stewart AN, Gensel JC: Microglia and macrophage metabolism in CNS injury and disease: The role of immunometabolism in neurodegeneration and neurotrauma. Exp Neurol 2020, 329:113310.

18. Hata A, Chen YG: TGF-beta Signaling from Receptors to Smads. Cold Spring Harb Perspect Biol 2016, 8.
19. Meng XM, Nikolic-Paterson DJ, Lan HY: TGF-beta: the master regulator of fibrosis. Nat Rev Nephrol 2016, 12:325-338.
20. Macias MJ, Martin-Malpartida P, Massague J: Structural determinants of Smad function in TGF-beta signaling. Trends Biochem Sci 2015, 40:296-308.
21. Black RA, Rauch CT, Kozlosky CJ, Peschon JJ, Slack JL, Wolfson MF, Castner BJ, Stocking KL, Reddy P, Srinivasan S, et al: A metalloproteinase disintegrin that releases tumour-necrosis factor-alpha from cells. Nature 1997, 385:729-733.
22. Peschon JJ, Slack JL, Reddy P, Stocking KL, Sunnarborg SW, Lee DC, Russell WE, Castner BJ, Johnson RS, Fitzner JN, et al: An essential role for ectodomain shedding in mammalian development. Science 1998, 282:1281-1284.
23. Malapeira J, Esselens C, Bech-Serra JJ, Canals F, Arribas J: ADAM17 (TACE) regulates TGFbeta signaling through the cleavage of vasorin. Oncogene 2011, 30:1912-1922.
24. Geribaldi-Doldan N, Carrasco M, Murillo-Carretero M, Dominguez-Garcia S, Garcia-Cozar FJ, Munoz-Miranda JP, Del RV, Verastegui C, Castro C: Specific inhibition of ADAM17/TACE promotes neurogenesis in the injured motor cortex. Cell Death Dis 2018, 9:862.
25. Sommer D, Corstjens I, Sanchez S, Dooley D, Lemmens S, Van Broeckhoven J, Bogie J, Vanmierlo T, Vidal PM, Rose-John S, et al: ADAM17-deficiency on microglia but not on macrophages promotes phagocytosis and functional recovery after spinal cord injury. Brain Behav Immun 2019, 80:129-145.
26. Zhang S, Kojic L, Tsang M, Grewal P, Liu J, Namjoshi D, Wellington CL, Tetzlaff W, Cynader MS, Jia W: Distinct roles for metalloproteinases during traumatic brain injury. Neurochem Int 2016, 96:46-55.
27. Kawasaki K, Freimuth J, Meyer DS, Lee MM, Tochimoto-Okamoto A, Benzinou M, Clermont FF, Wu G, Roy R, Letteboer TG, et al: Genetic variants of Adam17 differentially regulate TGFbeta signaling to modify vascular pathology in mice and humans. Proc Natl Acad Sci U S A 2014, 111:7723-7728.
28. Ou SC, Bai KJ, Cheng WH, Chen JY, Lin CH, Wen HC, Chen BC: TGF-beta Induced CTGF Expression in Human Lung Epithelial Cells through ERK, ADAM17, RSK1, and C/EBPbeta Pathways. Int J Mol Sci 2020, 21.
29. Lan X, Han X, Li Q, Yang QW, Wang J: Modulators of microglial activation and polarization after intracerebral haemorrhage. Nat Rev Neurol 2017, 13:420-433.
30. Schumacher N, Rose-John S: ADAM17 Activity and IL-6 Trans-Signaling in Inflammation and Cancer. Cancers (Basel) 2019, 11.
31. Xiang B, Xiao C, Shen T, Li X: Anti-inflammatory effects of anisalcohol on lipopolysaccharide-stimulated BV2 microglia via selective modulation of microglia polarization and down-regulation of NF-kappaB p65 and JNK activation. Mol Immunol 2018, 95:39-46.
32. Divolis G, Stavropoulos A, Manioudaki M, Apostolidou A, Doulou A, Gavriil A, Dafnis I, Chroni A, Mummery C, Xilouri M, Sideras P: Activation of both transforming growth factor-beta and bone morphogenetic protein signalling pathways upon traumatic brain injury restrains pro-inflammatory

and boosts tissue reparatory responses of reactive astrocytes and microglia. Brain Commun 2019, 1:z28.

# Figures

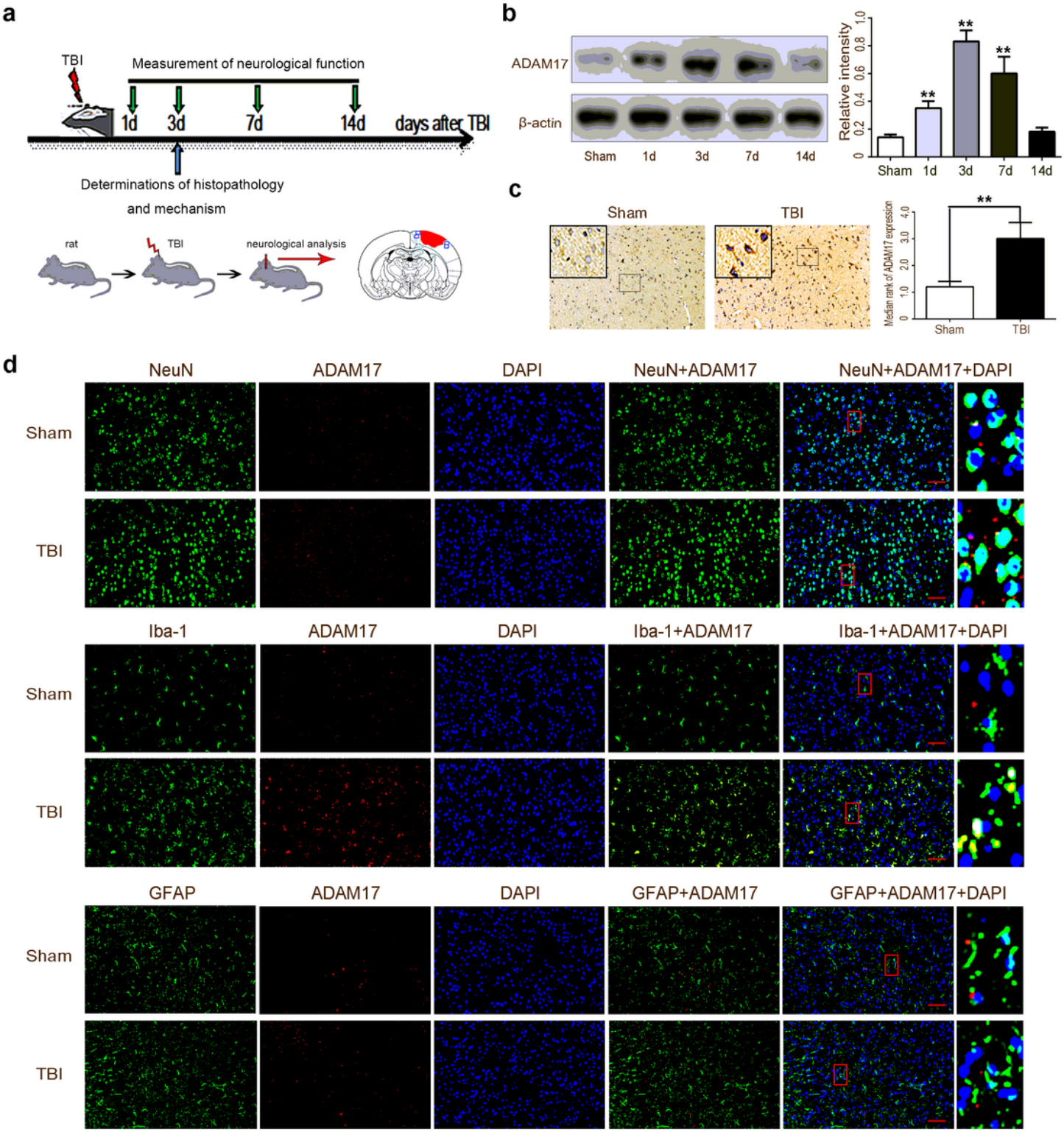
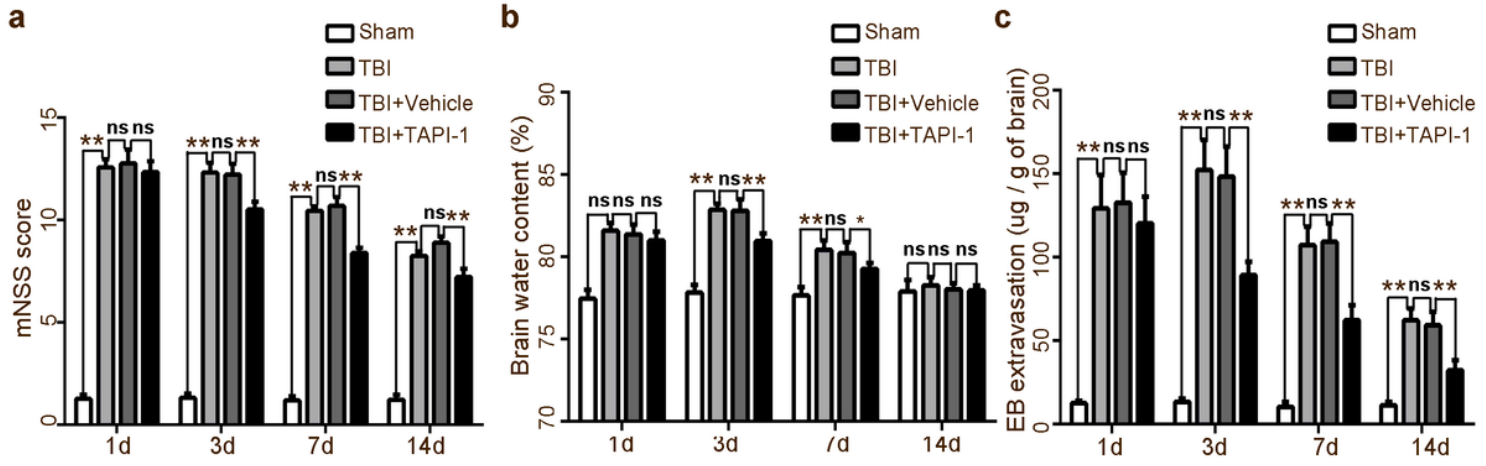


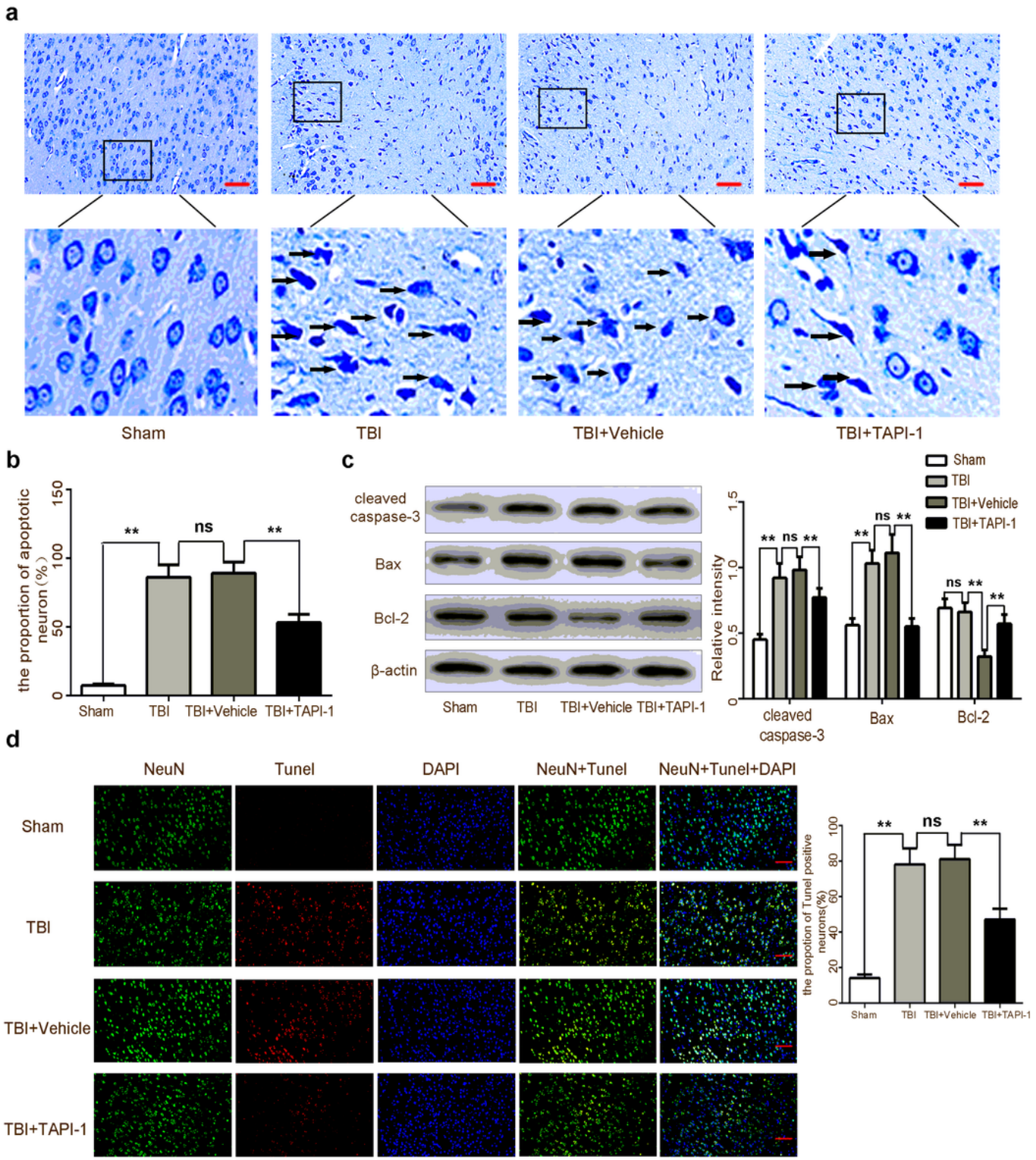

Figure 1

The expression of ADAM17 increased after TBI, and it is specifically located in the microglia at the injured cerebral cortex. (a) Experimental scheme and aschematic of a brain section after TBI. The molecular biology study was performed on post-injury days 1, 3, 7, and 14. Areas in red refer to lesion sites and areas in blue refer to sample points; (b) ADAM17 levels were significantly increased on post-injury days 1, 3, and 7. Among them, the level of ADAM17 increased most significantly on post-injury day 3, and it slowly decreased to approximately normal on post-injury day 14 ($p < 0.05$); (c) Immunohistochemistry indicated that ADAM17 was highly expressed in the cortical injury area of the TBI group on post-injury day 3; (d) Immunofluorescence double staining showed that ADAM17 was mainly expressed in neurons (NeuN+) and microglia (Iba-1+), while the expression in astrocytes (GAFP+) was not obvious. Representative photomicrographs of immunofluorescence double staining are shown. The values are expressed as mean ± standard deviation: *$p < 0.05$, **$p < 0.01$, scale bars = 50 μm.




## Figure 2

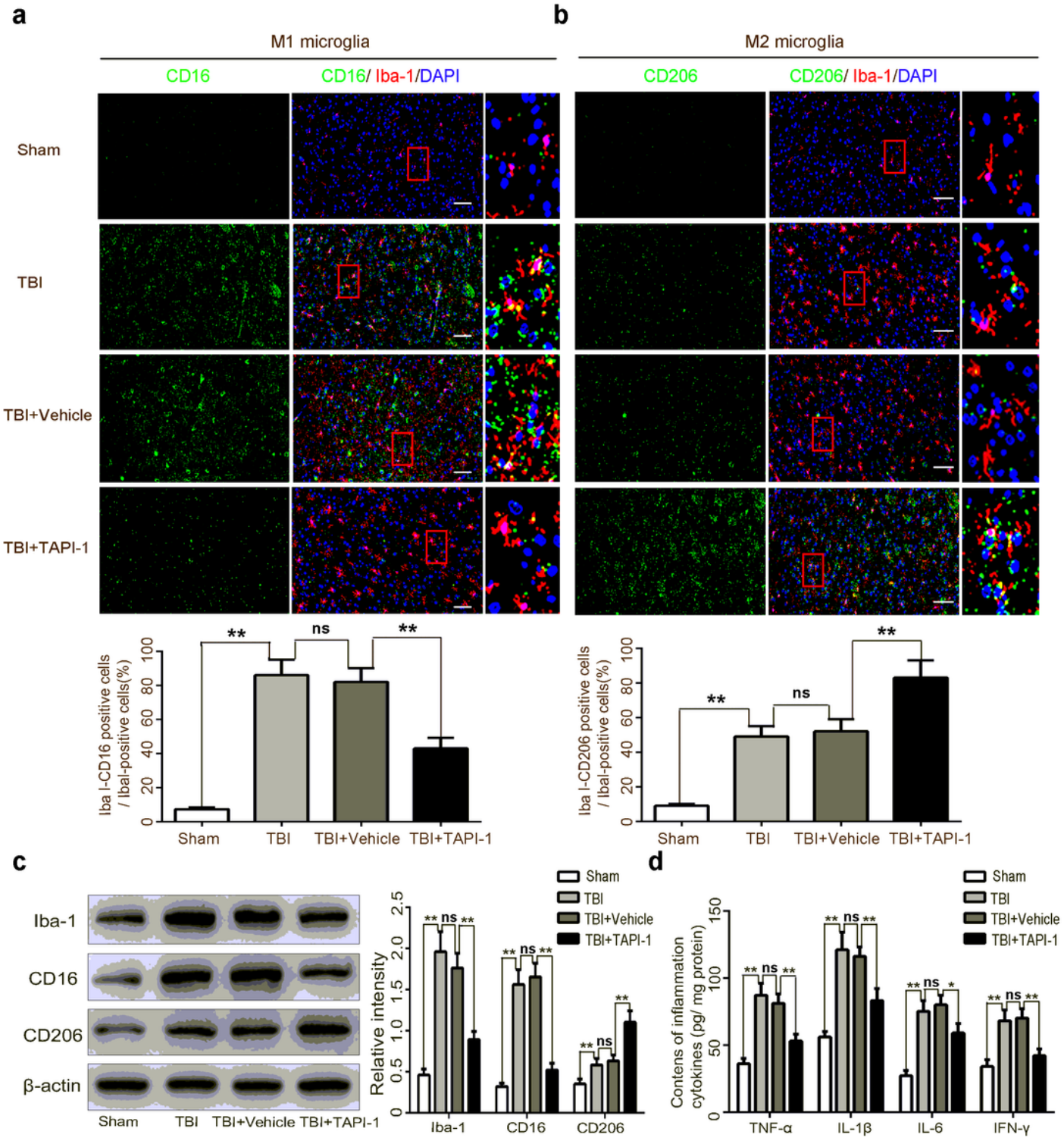
Specific inhibition of ADAM17 can improve neurological function after TBI. (a) Compared with the TBI group, the neurological function score of the TBI+TAPI-1 group was remarkably improved on post-injury day 3 (10.51 ± 0.38 vs. 12.21 ± 0.52, $p < 0.05$); (b) Compared with the TBI group, the brain water content of the TBI+TAPI-1 group was appreciably reduced on post-injury day 3 ($p < 0.05$); (c) The TBI group obtained more Evans Blue dye extravasation 3 days after the TBI than the sham group ($p < 0.05$). The TBI+TAPI-1 group had significantly less extravasation of Evans Blue dye han the TBI group ($p < 0.05$); n = 6 in each group. The values are expressed as mean ± standard deviation: *$p < 0.05$, **$p < 0.01$.




## Figure 3

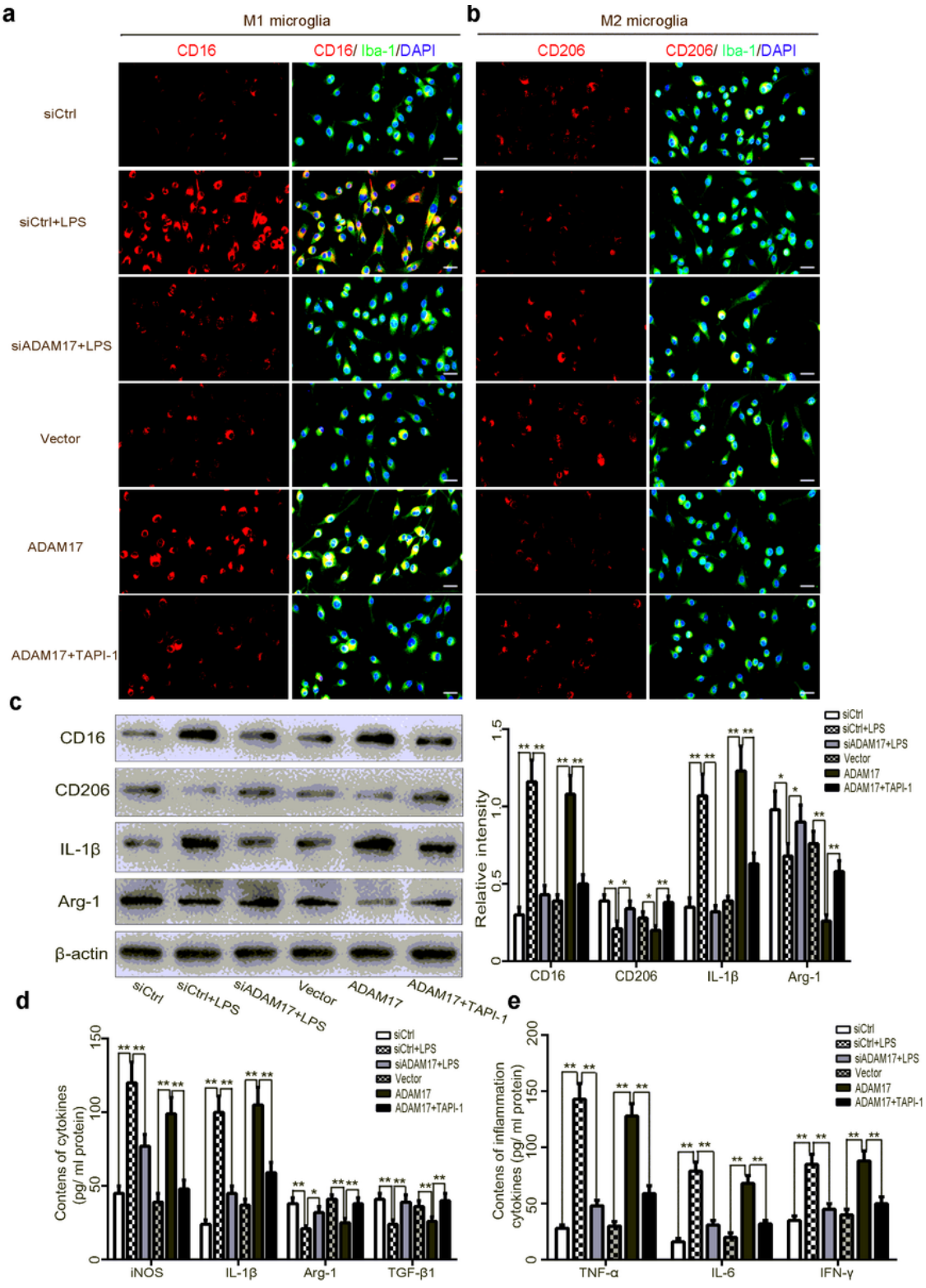
Specific inhibition of ADAM17 can reduce neuronal apoptosis after TBI. (a, b) The sham group obtained low apoptotic fraction of neurons 3 days after TBI. The percentage of apoptotic cells was higher in the TBI than in the sham group ($p < 0.05$). Compared with the TBI group, the neuronal apoptosis rate in the TBI+TAPI-1 group on post-injury day 3 was significantly reduced (53.00±6.22% vs. 89.05±8.04%, $p < 0.05$). Representative photomicrographs of the Nissl-stained neurons are shown. The arrows indicate the

apoptotic neurons; (c) Western blot analyses revealed that the TBI resulted in the upregulation of apoptotic factors in the lesioned cortex 3 days after TBI. The cleaved caspase-3 and Bax levels decreased and the anti-apoptotic factor Bcl-2 increased in the TBI+TAPI-1 group more than the TBI group ($p < 0.05$) ; (d) The TUNEL staining demonstrated that the TUNEL-positive neurons decreased significantly more in the TBI+TAPI-1 group than in the TBI group. Representative photomicrographs of the TUNEL-positive neurons are shown. The arrows indicate the apoptotic neurons. Values are expressed as the mean ± standard deviation (n = 6 per group). N.S., $p > 0.05$, *$p < 0.05$, **$p < 0.01$. Scale bars = 50 μm.



## Figure 4

Specific inhibition of ADAM17 promoted M1/M2 phenotype transformation and inhibited inflammatory reaction in lesion cortex after TBI . (a, b) The double immuohistochemical staining for the microglia (Iba1+) and the M1-associated marker (CD16+) or the M2-associated marker (CD206+) 3 days after the TBI was assessed. Compared with sham group, the expression of microglia (Iba-1+) and M1 microglia (CD16+) in TBI group were notably increased ($p < 0.05$). Compared with TBI group, TBI+TAPI-1 group decreased the expression of CD16 and increased the expression of CD206 ($p < 0.05$). Representative photomicrographs of the CD16 or the CD206-positive microglia are shown; (c) The expression of Iba-1 and CD16 protein increased significantly in lesion cortex after TBI ($p < 0.05$). Compared with TBI group, TBI +TAPI-1 group significantly inhibited the expression of Iba-1 and CD16, increased the expression of CD206 protein; (d) Compared with sham group, the expressions of serum inflammatory factor TNF-α, IL-1 β, IL-6 and IFN-γ were significantly increased in TBI group on post-injury day 3 ($p < 0.05$); Compared with TBI group, the expression of serum inflammatory factors in TBI + TAPI-1 group was significantly decreased ($p < 0.05$). Values are expressed as the mean ± standard deviation ($n = 6$ per group). N.S., $p > 0.05$, *$p < 0.05$, **$p < 0.01$. Scale bars = 50 μm.




## Figure 5

ADAM17 inhibition promoted M1/M2 phenotype transformation and inhibited inflammatory reaction in vitor. (a, b) The positive expression of CD16 was notably increased after BV2 of microglia activated by LPS. Compared with siCtrl+LPS group, the expression of CD16 decreased in siADAM17+LPS group, but increased the expression of CD206 ($p < 0.05$). The positive expression of CD16 was notably increased after overexpression of ADAM17. Compared with ADAM17 group, ADAM17+TAPI-1 group decreased

CD16 expression and increased CD206 expression, showing M2 phenotype polarization ($p < 0.05$). Representative photomicrographs of the CD16 or the CD206-positive microglia are shown; (c) Compared with siCrl+LPS group, siADAM17+LPS group significantly inhibited the expression of CD16 and IL-1β, increased the expression of CD206 and Arg-1 protein ($p < 0.05$). Compared with ADAM17 group, ADAM17+TAPI-1 group significantly inhibited the expression of CD16, IL-1β and protein, and increased the expression of CD206 and Arg-1 ($p < 0.05$); (d) ELISA Kit results showed that compared with siCtrl+LPS group, siADAM17+LPS group significantly inhibited the expression of iNOS, IL-1β and increased the expression of Arg-1 and TGF-β1 ($p < 0.05$). Compared with the ADAM17 group, ADAM17+TAPI-1 group reduced the expression of iNOS, IL-1 β and increased the expression of Arg-1 and TGF-β1 ($p < 0.05$); (e) The expressions of TNF-α, IL-6 and IFN-γ in the supernatant of siADAM17+LPS group were significantly lower than those of siCtrl+LPS group ($p < 0.05$). The expression of inflammatory factors in ADAM17+TAPI-1 group was considerably lower than that in ADAM17 group ($p < 0.05$). Values are expressed as the mean ± standard deviation ($n = 6$ per group). N.S., $p > 0.05$, *$p < 0.05$, **$p < 0.01$. Scale bars = 50 μm.

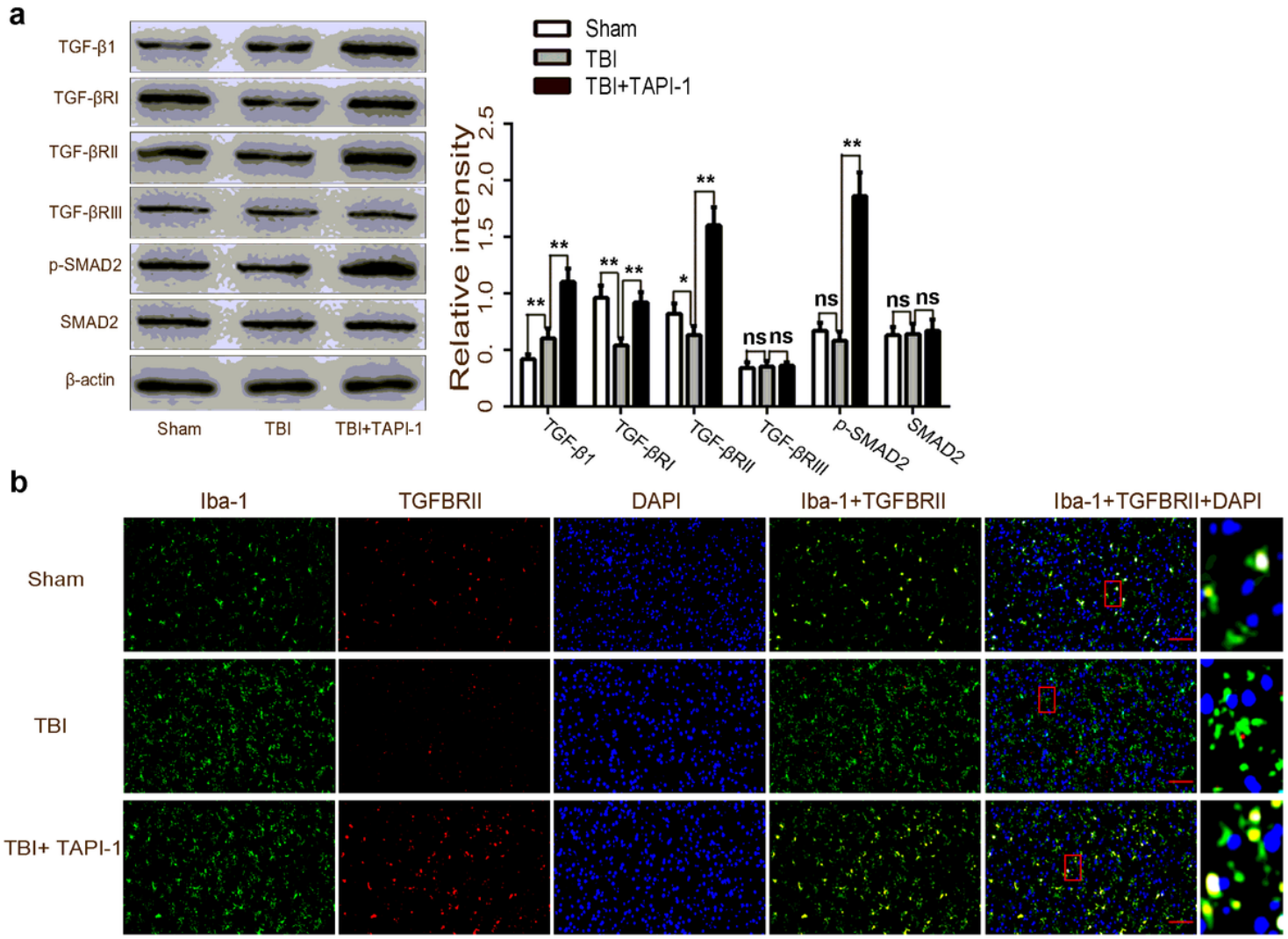
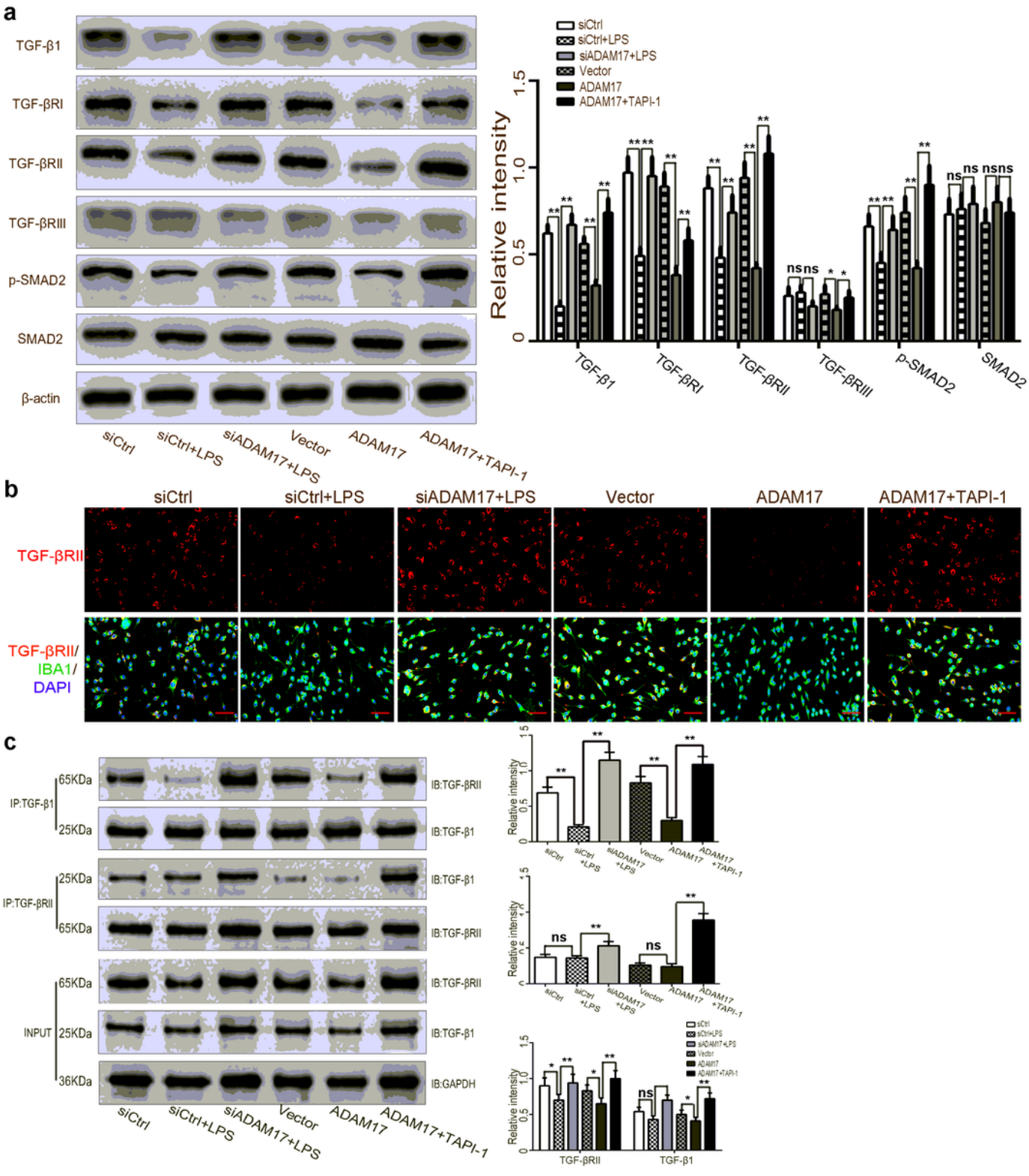


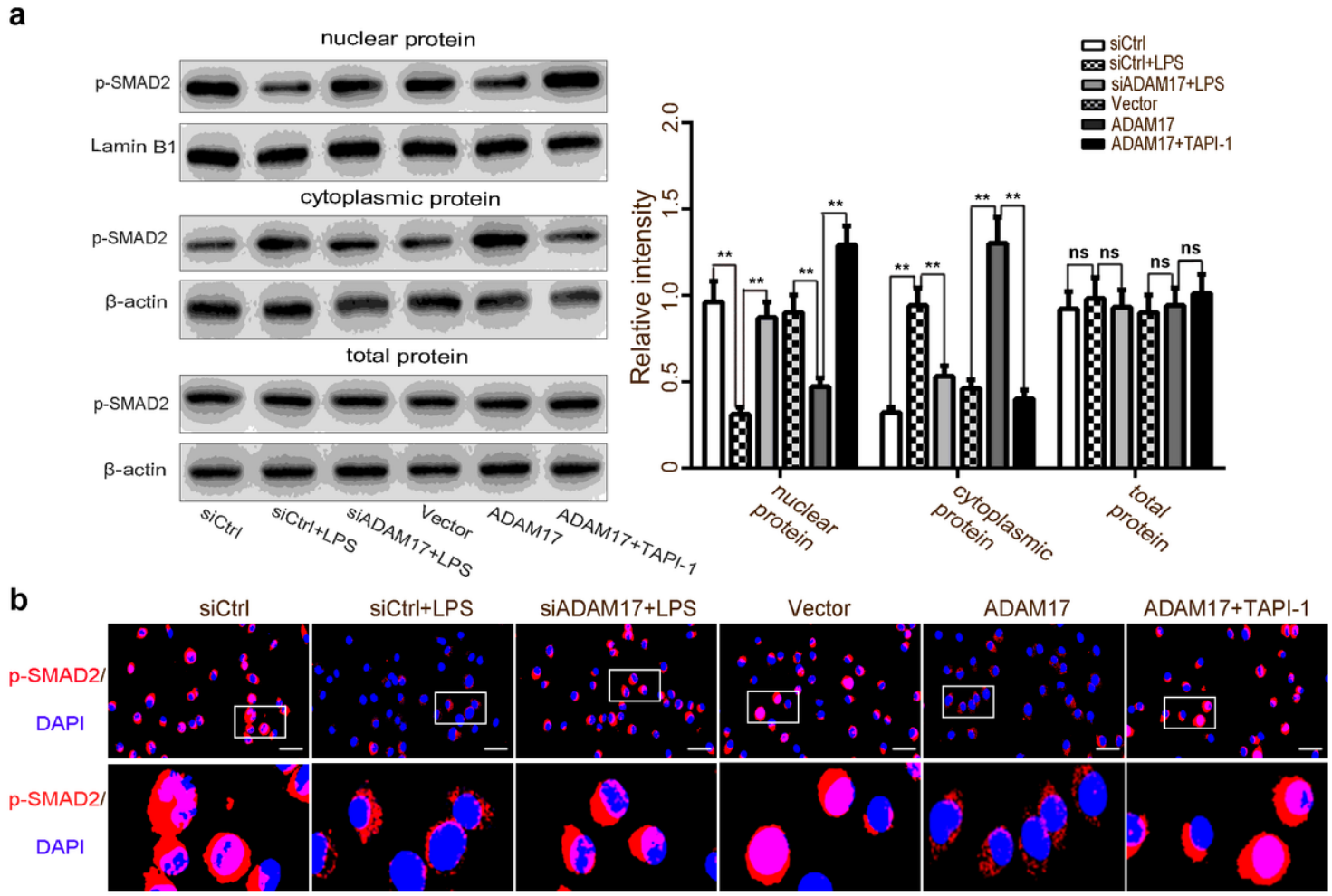

**Figure 6**

Specific inhibition of ADAM17 activated the TGF-β1 pathway. (a) Compared with TBI group, the expressions of TGF-β1, TGF-βRI and TGF-βRII were significantly increased in TBI+TAPI-1 group. The expression of p-Smads was significantly increased in TBI+TAPI-1 group ($p < 0.05$); (b) Immunofluorescence double staining showed that the expression of TGF-βRII on microglia (Iba-1 +) in TBI+TAPI-1 group was significantly higher than that in TBI group ($p < 0.05$). Representative photomicrographs of the TGF-βRII-positive microglia are shown. Values are expressed as the mean ± standard deviation (n = 6 per group). N.S., $p > 0.05$, *$p < 0.05$, **$p < 0.01$. Scale bars = 50 μm.



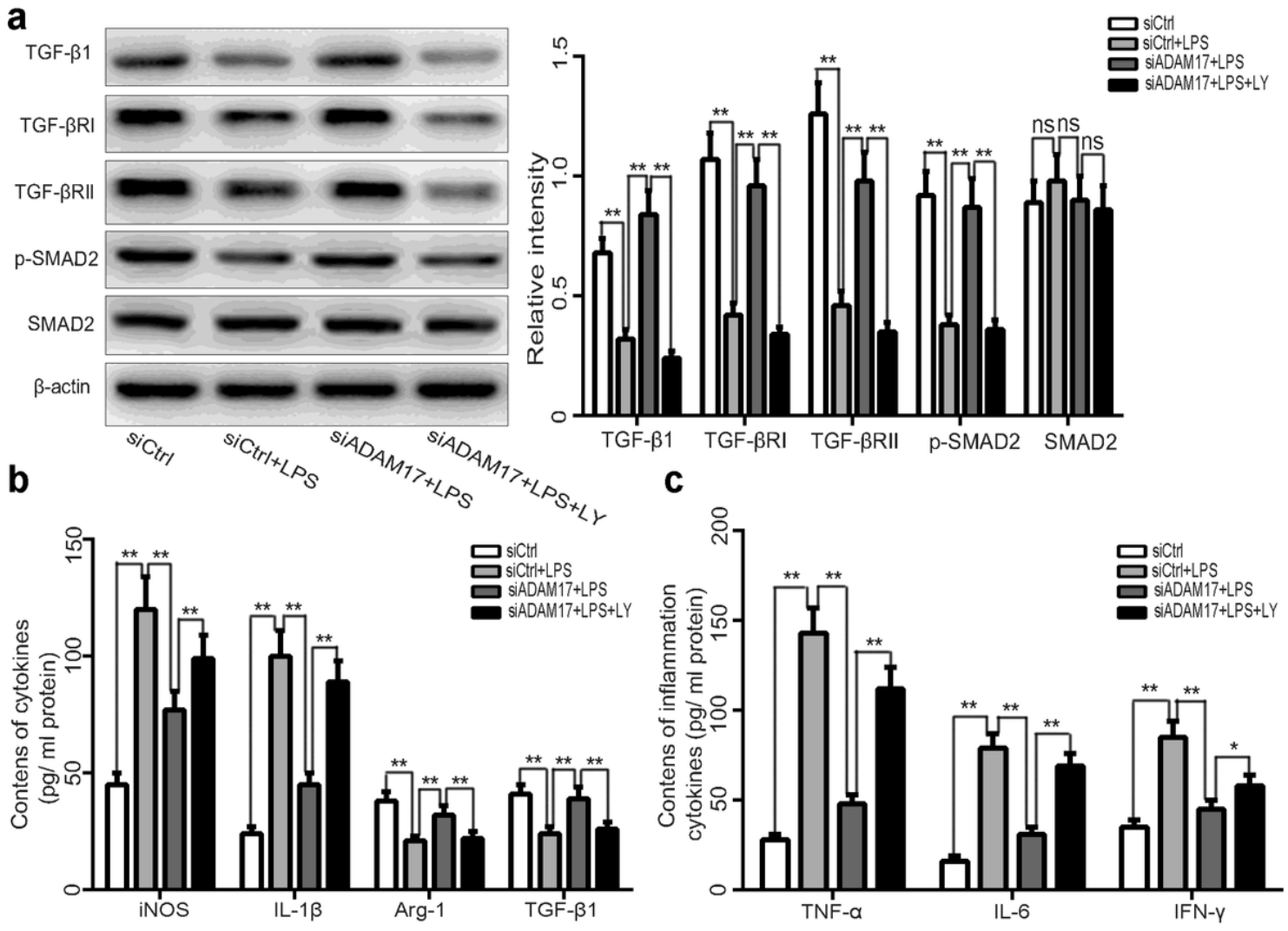
## Figure 7

Specific inhibition of ADAM17 activated the TGF-β1 pathway in vitor. (a) Compared with siCtrl+LPS group, the expressions of TGF-β1, TGF-βRI and TGF-βRII in siADAM17+LPS group were appreciably increased. The expression of p-Smads was markedly increased. ADAM17 inhibited the expressions of TGF-β1, TGF-βRI, TGF-βRII and the activation of downstream Smads pathway, while the expression of p-Smads decreased significantly. Compared with ADAM17 group, ADAM17+TAPI-1 group can activate TGF-β1 pathway after inhibiting ADAM17 expression ($p < 0.05$) ; (b) Compared with siCtrl+LPS group, the expression of TGF-βRII on microglia (Iba-1+) in siADAM17+LPS group remarkably increased. ADAM17 inhibited TGF-βRII expression. Compared with ADAM17 group, ADAM17+TAPI-1 group significantly increased the expression of TGF-βRII. Representative photomicrographs of the TGF-βRII-positive microglia are shown ; (c) Co-IP analysis showed that compared with siCtrl+LPS group, the formation of TGF-β1/TGF-βRII complex in siADAM17+LPS group increased notably ($p < 0.05$). ADAM17 inhibited the formation of TGF-β1/TGF-βRII complex. Compared with ADAM17 group, ADAM17+TAPI-1 group promoted the formation of TGF-β1/TGF-βRII complex ($p < 0.05$). Values are expressed as the mean ± standard deviation ($n = 6$ per group). N.S., $p > 0.05$, *$p < 0.05$, **$p < 0.01$. Scale bars = 50 μm.




## Figure 8

Specific inhibition of ADAM17 promoted activated intranuclear translocation of p-Smads in vitor. (a) Compared with the siCtrl+LPS group, siADAM17 significantly increased the translocation of p-Smads from the cytosol to the nucleus; increased p-Smads expression ($p < 0.05$). ADAM17 inhibited the intranuclear translocation of p-Smads. The ADAM17 inhibitor TAPI-1 counteracts the inhibitory effect of ADAM17 on the metastasis of p-Smads ($p < 0.05$); (b) Representative photomicrographs of p-Smads staining in the experimental groups. Values are expressed as mean ± standard deviation (n = 6 per group). N.S., $p > 0.05$, *$p < 0.05$, **$p < 0.01$. Scale bars = 50 μm.




## Figure 9

Inhibition of TGF-β1 pathway hinders ADAM17's regulation of M1/M2 phenotype transformation of microglia in vitor. (a) Compared with siADAM17+LPS group, the expressions of TGF-βRI, TGF-βRII and downstream p-Smads notably were decreased in siADAM17+LPS+LY group ($p < 0.05$); (b) Compared with siADAM17+LPS group, siADAM17+LPS+LY group promoted the expression of iNOS, IL-1β protein, decreased the expression of Arg-1 and TGF-β ($p < 0.05$); (c) The expression of related inflammatory factors in siADAM17+LPS+LY group increased considerably in comparison to siADAM17+LPS group, which offset the anti-inflammatory effect of siADAM17 ($p < 0.05$). Values are expressed as mean ± standard deviation (n = 6 per group). N.S., $p > 0.05$, *$p < 0.05$, **$p < 0.01$.

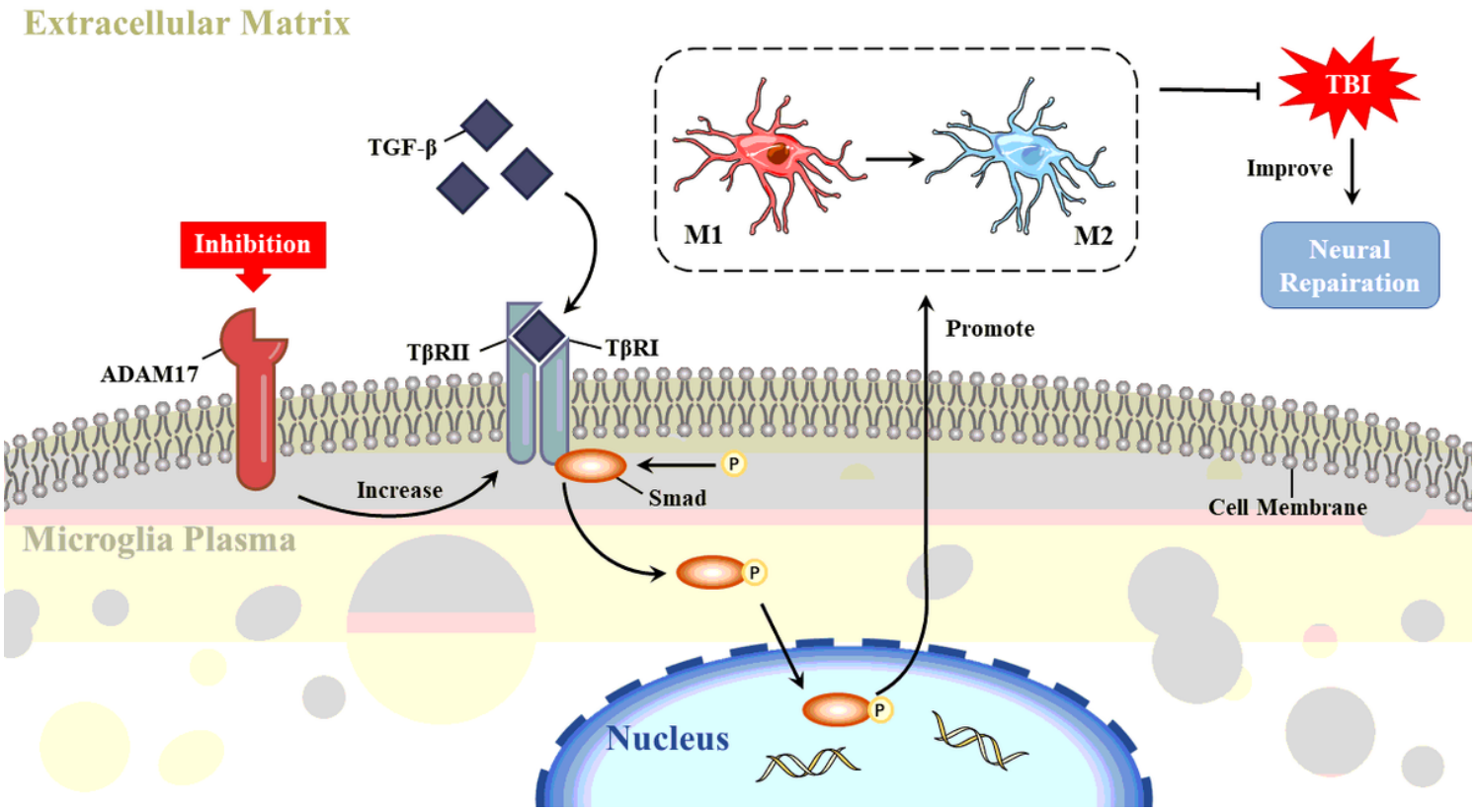



**Figure 10**

Schematic illustrating the possible neuroprotective mechanisms of ADAM17 inhibition after TBI. As illustrated, ADAM17mediates the shedding of the extracellular domain of TGF-βRs on the cell membrane and decrease of its quantity. In addition, the ADAM17 negatively regulates the TGF-β1/Smad pathway, so that it can promote the polarization of M1 phenotype of microglia and inhibit M2 phenotype of microglia. Specific inhibition of ADAM17 can increase the expression of TGF-βRs on microglia membrane, and promote the formation of TGF-β1/TGF-βRII complex. TGF-β1/TGF-βRII complex then induces intranuclear translocation of Smads, activates TGF-β 1/Smad pathway, regulates M1/M2 polarization of microglia, and finally affects the neuroinflammatory response as well as play a neuroprotective role after TBI.

## Supplementary Files

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