Supplementary Materials

Supplementary Methods

Construction of SAH model

The study design and the number of mice used are shown in Supplementary Table 2. Mice were anesthetized by isoflurane inhalation (4% for induction and 1% for maintenance). The rectal temperature was maintained at 37.0 ± 0.5 ℃. A 5-0 monofilament nylon suture was inserted into the left internal carotid artery and used to perforate the bifurcation of the middle cerebral artery. Similar procedures (without perforation) were performed in a sham group.

Behavioral Tests

To evaluate neurological deficits after SAH, the Garcia Score, grip test, rotarod test, and pole test assessments were performed.[1-3] For Garcia Score, mice spontaneous activity, limb movement symmetry, forelimb outstretching, climbing, body proprioception and vibrissae reflex were assessed. The higher score indicates a better neurological function. For grip test, mice were placed on a horizontal wooden pole with two vertical supports about 40 cm in height. Mice were scored according to number of limbs gripping the pole. For rotarod test, mice were placed on an accelerating rotating rod, whose initial speed was 5 rpm and acceleration was 0.2rpm/s. When the mice fell, the time was recorded. For pole test, mice were placed head up on the top of a 50 cm-vertical pole. The time taken for the mice to turn 180° was recorded as T_{Turn}, and the time to reach the bottom was recorded as T_{Total}. If the T_{Turn} >60s, or the mice fell or slipped, then T_{Turn} and T_{Total} were respectively recorded as 60s and 75s.

Administration of recombinant Mouse TIMP1, Transduction of Adeno-Associated Virus (AAV) Vector

Recombinant mouse TIMP1 (rm-TIMP1) was intraperitoneally administered at a dose of 250 μg/kg daily.[4] The first dose was administered at SAH onset, and treatment was continued for 3 days.

The coding sequence region of mouse TIMP1 was cloned into AAV for overexpression (AAV-TIMP1). An siRNA oligomer targeting mouse β1-Integrin (5'-3' sense:

GAACCACAGAAGUUUACAUTT; antisense: AUGUAAACUUCUGUGGUUCTT; designed by GenePharma, Shanghai) was cloned into an miR30-based shRNA expression vector, then packed into AAV for silencing (AAV-siβ1). Five injection locations were selected,[5, 6] and 1×10^{13} units of AAV were injected at each position. The coordinates (relative to bregma) were as follows: 2 locations in the frontoparietal cortex (1 mm posterior, 0.5 mm ventral, 0.5/2.5 mm left lateral), 2 locations in the temporal cortex (1 mm posterior, 5 mm ventral, 0.5/2.5 mm left lateral), and 1 location in the hippocampus (2 mm posterior, 1 mm ventral, 1.5 mm left lateral). For mice that received both AAV-TIMP1 and AAV-siβ1, injections of the two AAV vectors were conducted 1 week apart. At 4 weeks after the last injection, mice were subjected to sham or SAH surgery. The transduction efficacies were determined by quantitative reverse transcription polymerase chain reaction (qRT-PCR) analyses of astrocytes that had been magnetically isolated from brain tissues surrounding the injection locations.

Preparation of Single-Cell Suspensions, Flow Cytometry, and Cell Sorting

Left brain hemispheres were digested with 3 mg/mL collagenase/dispase (Sigma-Aldrich, #11097113001), homogenized, and centrifuged in 30% Percoll (Cytiva, #17089109) to remove the myelin sheath; the resultant single-cell suspensions were used for flow cytometry analysis or cell sorting.[7]

For flow cytometry (FCM) analysis and fluorescence-activated cell sorting (FACS), single-cell suspensions were incubated with antibodies to cell-surface markers (Supplementary Table 3) for 30 min at 4 ℃, then analyzed with a flow cytometer (CytoFLEX LX, Beckman) or sorted with a cell sorter (Aria SORP, BD). CytExpert and FlowJo softwares were used for data analysis. For magnetic-activated cell sorting (MACS) of astrocytes, single-cell suspensions were incubated with ACSA-2 microbeads (Miltenyi Biotec, #130-097-679) at 4 ℃ for 15 min, then filtered through Miltenyi μMACS™ Separator columns (Miltenyi Biotec, #130-042-201) for astrocyte collection.

Histology

Histological analysis consisted of immunofluorescence and transmission electron microscopy (TEM). For immunofluorescence, 10-μm-thick frozen coronal brain sections were labeled with

primary and fluorescent secondary antibodies (Supplementary Table 4). Images were captured with a fluorescence microscope (DM550, Leica) or a laser confocal microscope (STELLARIS5, Leica). For TEM analysis, tissue masses separated from the left temporal cortex were cut into 100-nm-thick sections, then stained with 2% uranyl acetate and lead citrate. Images were captured using a 120-kV transmission electron microscope (Tecnai 10, Thermo Fisher).

Assessment of BBB Integrity

The integrity of the BBB was assessed by employing IgG leakage detection and brain water content measurement. For IgG leakage detection, brain frozen sections with a similar anatomical panel were immersed with Alexa Fluor™ 594-labeled anti-mouse IgG (1:500; Thermo Fisher, #A-21203). Samples were photographed by a laser confocal microscope (STELLARIS5, Leica), and the percentage of the stained areas were calculated. For brain water content measurement, the brain tissues were baked dry and the weight was compared before and after. The water content was then calculated using the formula (Wet Weight – Dry Weight) / Wet Weight × 100%.

Cell Culture

For primary astrocyte culture,[8] cerebral cortex tissues from 1-day-old mice were digested with 0.25% trypsin to yield mixed-cell suspensions, and the suspensions were seeded in dishes and cultured in dulbecco's modified eagle medium (DMEM) containing 10% fetal bovine serum (FBS). After incubation for 2 weeks, the mixed cells were shaken at 200 rpm for 12 h to remove microglia; the remaining adherent cells were regarded as astrocytes.

For primary neuron culture,[3] cortexes were dissected from E17 fetal mice. Tissues were digested with 0.25% trypsin, and the suspensions were seeded onto dishes coated with poly-L-lysine and cultured in neurobasal medium containing 1% B27 and 0.5 mmol/L glutamine. At 10 days after seeding, the neurons were obtained for further analysis.

For primary microglia culture,[9] cortexes from 1-day-old mice were digested with 0.25% trypsin, and the suspensions were seeded in dishes and cultured in DMEM containing 10% FBS. After incubation for 2 weeks, the mixed cells were shaken at 200 rpm for 1 h to obtain microglia.

For primary endothelial cell culture,[10] cortexes from 6-8 week old mice (no matter the sex) were homogenized and centrifuged in 15% Dextran-70 to obtain microvascular segments. The

segments were digested with DMEM containing 1% I-type collagenase, 0.25% trypsin and 1% DNase I. The obtained endothelial cells were cultured in DMEM containing 10% FBS.

Red Blood Cell (RBC) Stimulation, Transfection, and Drug Administration

To mimic SAH *in vitro*, mouse RBCs were isolated from peripheral blood via centrifugation and incubated with the adherent astrocytes at a 10:1 ratio (RBCs : astrocytes) for 24 h.[11] Transfections of TIMP1 overexpression plasmid (GenePharma, Shanghai) and Trim21 siRNA (5'-3' sense: GUCUCCUUCUACAACAUAATT; antisense: UUAUGUUGUAGAAGGAGACTT; designed by GenePharma, Shanghai) were conducted 72 h before RBC stimulation using Lipofectamine™ 3000 (Invitrogen, #L3000001). The proteasome inhibitor MG132 (10 μM; Sigma-Aldrich, #M7449) and the autophagy inhibitor chloroquine (CQ, 10 μM; Sigma-Aldrich, #C6628) were added 8 h and 12 h, respectively, before sample collection.[12, 13] The protein synthesis inhibitor cycloheximide (CHX, 100 μg/mL; Sigma-Aldrich, #239763) was added 24 h after RBC stimulation. The samples were collected within 9 h after CHX administration.[13]

RNA Extraction, qRT-PCR, and RNA Sequencing

Total RNA was extracted from brain tissues (left temporal cortex), sorted cells, and *in vitro* cultured cells. cDNA was obtained using a Reverse Transcription Kit (Takara, #RR036Q), and target genes were amplified using a SYBR Green qPCR Kit (Takara, #RR420Q). The primers used are shown in Supplementary Table 5.

For RNA sequencing, a next-generation sequencing library was constructed by BGI Genomics, Shenzhen. Data were cleaned with Cutadapt-1.9 software, and reads were mapped with HISAT2 2.0.4 software. The mapped reads were assembled using StringTie-1.3.4d software. Comprehensive transcriptomes were constructed using Gffcompare0.9.8 software. StringTie and ballgown (http://www.bioconductor.org/packages/release/bioc/html/ballgown.html) softwares were used to estimate the expression levels of all transcripts. Differentially expressed genes with fold change values > 2 or < 0.5 and P values < 0.05 were selected for further analysis.

Immunoblotting, Immunoprecipitation, Shotgun Proteomics, and Molecular Docking

Proteins were extracted from brain tissues (left temporal cortex) and *in vitro* cultured cells. For immunoblotting, proteins were separated by electrophoresis and transferred to polyvinylidene fluoride membranes. The membranes were subsequently labeled with primary and horseradish peroxidase-conjugated secondary antibodies. For immunoprecipitation, proteins were precipitated with antibodies to bait proteins and Pierce Protein A/G Magnetic Beads (Invitrogen, #88803); the precipitates were detected by immunoblotting. The antibodies used are shown in Supplementary Table 6.

β1-Integrin antibody-precipitated samples from control or RBC-treated astrocytes were also used for shotgun proteomics (Genechem, Shanghai). Proteins present in both groups (i.e., shared proteins) were analyzed using the JVENN tool (https://jvenn.toulouse.inra.fr/app/example.html). Proteins related to ubiquitination pathways in the shared proteins were filtered by Gene Ontology (GO) enrichment analysis via Metascape software (https://metascape.org/gp/index.html). Potential interaction sites and modes between β1-Integrin (PDB ID: 5XP0) and the E3 ubiquitin ligase Trim21 (PDB ID: 2VOK) were investigated via molecular docking analysis using the online tool HDOCK (http://hdock.phys.hust.edu.cn/).

Enzyme-Linked Immunosorbent Assays

TIMP1 levels in human plasma (Boster Bio, #EK0520), mouse brain tissues (left temporal cortex), and plasma (Boster Bio, #EK0521) were detected by enzyme-linked immunosorbent assays. All procedures were performed in accordance with the manufacturer's instructions.

Supplementary References

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Supplementary Tables

Supplementary Table 1. Basic Characteristics of the Enrolled Patients

	Control (n = 8)	SAH (n = 19)	*p* value
Age, yrs	58.1 ± 10.3	56.4 ± 11.2	0.4098
Male	25.0 (2/8)	31.6 (6/19)	＞0.9999
Smoking	25.0 (2/8)	15.8 (3/19)	0.6159
Hypertension	12.5 (1/8)	31.6 (6/19)	0.6334
Hb, g/L	137.0 ± 8.8	139.5 ± 13.4	0.6532
WBC, ×10^9 /L	5.6 ± 1.6	14.6 ± 6.5	0.0016
PLT, ×10^9 /L	220.9 ± 62.5	206.2 ± 41.6	0.4996
APTT, sec	32.4 ± 9.0	31.5 ± 4.9	0.0817
Aneurysm Location			
ICA	37.5 (3/8)	5.3 (1/19)	0.0646
MCA	0.0 (0/8)	21.1 (4/19)	0.2855
AComA	12.5 (1/8)	36.8 (7/19)	0.3645
PComA	12.5 (1/8)	15.8 (3/19)	＞0.9999
VA	12.5 (1/8)	0.0 (0/19)	0.2963
PCA	12.5 (1/8)	0.0 (0/19)	0.2963
Other	12.5 (1/8)	21.1 (4/19)	＞0.9999
Hunt-Hess Grade	-	2.0 ± 1.0	-
GCS Score	-	13.1 ± 3.3	-

Hb, Hemoglobin; **WBC**, White Blood Cell; **PLT**, Platelet; **APTT**, Activated Partial Thromboplastin Time; **ICA**, Internal Carotid Artery; **MCA**, Middle Cerebral Artery; **AComA**, Anterior Communicating Artery; **PComA**, Posterior Communicating Artery; **VA**, Vertebral Artery; **PCA**, Posterior Cerebral Artery; **GCS**, Glasgow Coma Scale.

Supplementary Table 2. Study Design and Number of Mice Utilized

Group	Survived	Dead	Excluded	Total	Usage
Experiment 1					1. RNA-seq (n = 5 for sham and n = 4 for SAH group) 2. qPCR in Figure 1C (n = 5 for each time point) 3. ELISA in Figure 1D (n = 6 for each group) 4. FACS in Figure S2A (n = 4 for SAH group) 5. MACS in Figure S2B (n = 5 for each group)
sham	21	0	1	22	
SAH	34	10	6	50	
Experiment 2					1. 18 mice in each group were subjected to behavioral tests. 2. 6 mice in each group were used for qPCR. 3. 6 mice in each group were used for WB. 4. 6 mice in each group were used for immunofluorescence.
sham (Vehicel, ♂)	18	0	0	18	
SAH (Vehicel, ♂)	18	4	3	25	
SAH (rm-TIMP1, ♂)	18	3	2	23	
sham (Vehicel, ♀)	18	0	0	18	
SAH (Vehicel, ♀)	18	2	0	20	
SAH (rm-TIMP1, ♀)	18	0	2	20	
Experiment 3					To verify the transduction efficacies of the AAVs, astrocytes were sorted via MACS for qPCR analysis.
AAV-Control	3	0	0	3	
AAV-TIMP1	3	0	0	3	
AAV-siβ1	3	0	0	3	
Experiment 4					1. FCM in Figure 5A (n = 3 for sham and n = 5 for SAH ± AAV-TIMP1 group) 2. 6 mice in each group were used for WB. 3. 6 mice in each group were used for immunofluorescence. 4. 6 mice in each group were used for detection of brain water content. 5. 3 mice in each group were used for TEM.
sham+AAV-Control	24	0	0	24	
SAH+AAV-Control	26	6	3	35	
SAH+AAV-TIMP1	26	2	0	28	
Experiment 5					1. 6 mice in each group were used for WB. 2. 6 mice in each group were used for immunofluorescence. 3. 3 mice in each group were used for TEM. 4. All mice used for above experiments were subjected to behavioral tests. 5. Samples were shared with Experiment 4.
sham+AAV-Control	-	-	-	-	
SAH+AAV-Control	-	-	-	-	
SAH+AAV-TIMP1	-	-	-	-	
AAV-TIMP1+AAV-siβ1	15	8	0	23	

Total	263	35	17	315	-

Supplementary Table 3. Antibodies for FCM and FACS

Antibodies	Concentration	Manufacturer	Catalog No.
CD45-AF700	1:100	Biolegend	147716
CD11b-PE-Cy7	1:100	Biolegend	260002
ACSA-2-PE	1:50	Miltenyi	130-123-284
CD31-APC	1:100	Biolegend	160209
β1-Integrin-FITC	1:100	Biolegend	102206

Supplementary Table 4. Antibodies for Immunofluorescence

Antibodies	Concentration	Manufacturer	Catalog No.
NeuN	1:500	Abcam	ab104224
CD31	1:100	R & D	AF3628
GFAP	1:500	Abcam	ab279290
Iba1	1:250	Abcam	ab283346
β1-Integrin	1:100	Proteintech	12594-1-AP
ZO-1	1:250	Proteintech	21773-1-AP
Occludin	1:100	Abcam	ab216327
AQP4	1:250	Proteintech	16473-1-AP

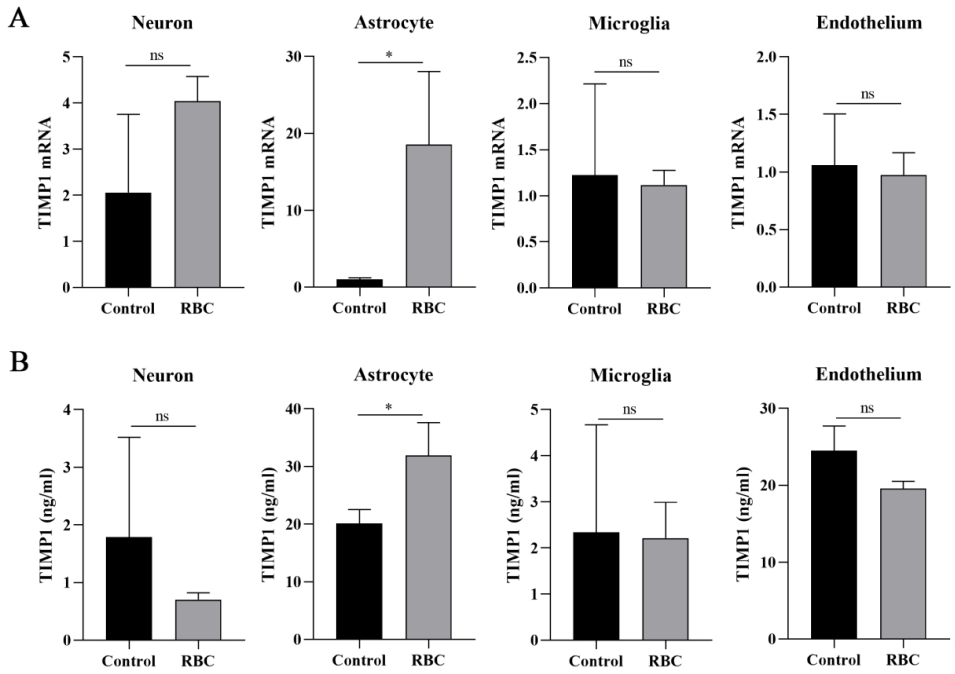
Supplementary Table 5. Primer Sequences

Gene	Sequence (5'-3')
TIMP1	F: GCAACTCGGACCTGGTCATAA
	R: CGGCCCGTGATGAGAAACT
β1-Integrin	F: ATGCCAAATCTTGCGGAGAAT
	R: TTTGCTGCGATTGGTGACATT
IL-1α	F: CGAAGACTACAGTTCTGCCATT
	R: GACGTTTCAGAGGTTCTCAGAG
IL-1β	F: GCAACTGTTCCTGAACTCAACT
	R: ATCTTTTGGGGTCCGTCAACT
TNF-α	F: CCCTCACACTCAGATCATCTTCT
	R: GCTACGACGTGGGCTACAG
IL-6	F: TAGTCCTTCCTACCCCAATTTCC
	R: TTGGTCCTTAGCCACTCCTTC
CD16	F: CAGAATGCACACTCTGGAAGC
	R: GGGTCCCTTCGCACATCAG
CD68	F: TGTCTGATCTTGCTAGGACCG
	R: GAGAGTAACGGCCTTTTTGTGA
iNOS	F: GTTCTCAGCCCAACAATACAAGA
	R: GTGGACGGGTCGATGTCAC
β-Actin	F: GGCTGTATTCCCCTCCATCG
	R: CCAGTTGGTAACAATGCCATGT

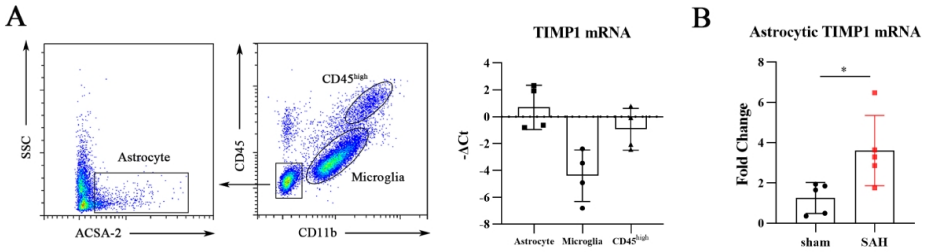
Supplementary Table 6. Antibodies for Immunoblotting and Immunoprecipitation

Antibodies	Concentration	Manufacturer	Catalog No.
β1-Integrin	1:1000 (WB) 1:50 (IP)	Proteintech	12594-1-AP
ZO-1	1:1000 (WB)	Proteintech	21773-1-AP
Occludin	1:1000 (WB)	Abcam	ab216327
Trim21	1:1000 (WB) 1:50 (IP)	Proteintech	67136-1-Ig
PARP	1:1000 (WB)	CST	9532
cleaved-PARP	1:1000 (WB)	CST	9548
cleaved-Caspase 9	1:1000 (WB)	CST	9509
α-Tubulin	1:10000 (WB)	Proteintech	66031-1-Ig

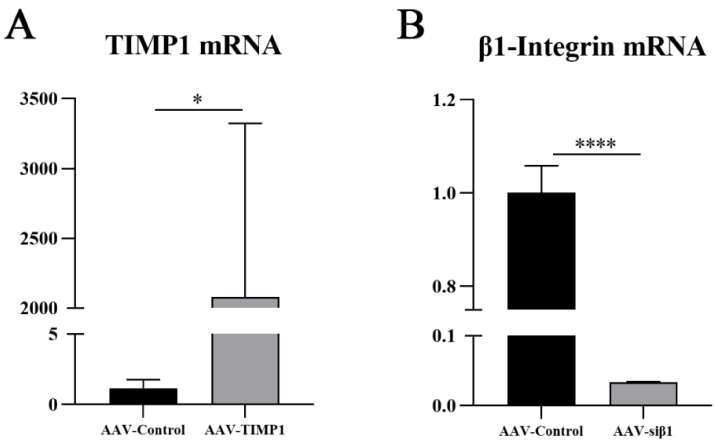
Supplementary Figures



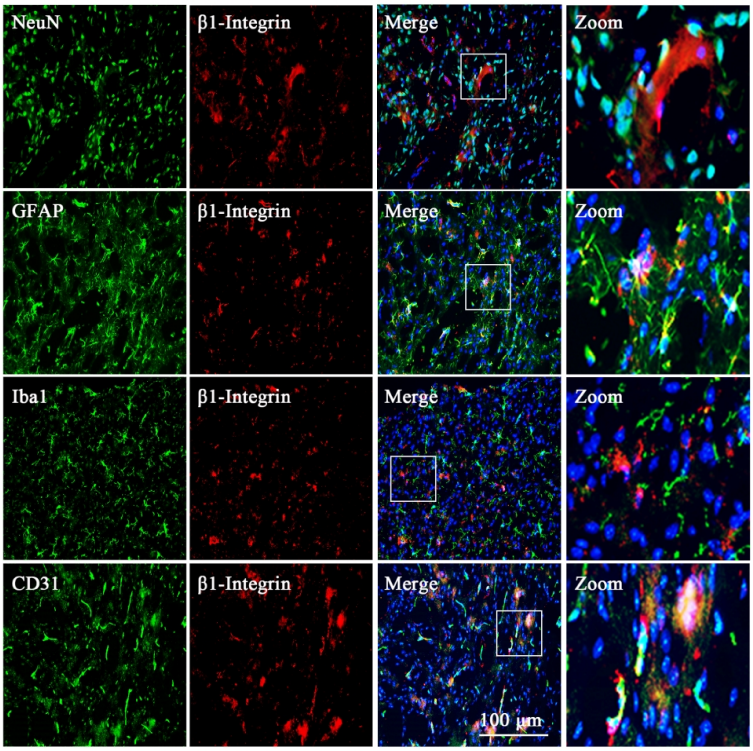
Supplementary Figure 1. RBC stimulation increased TIMP1 levels only in astrocytes. Transcriptional (A) and supernatant (B) TIMP1 levels in primary cultured cells stimulated with control or RBC. n = 3 dependent experiments. * $p < 0.05$.



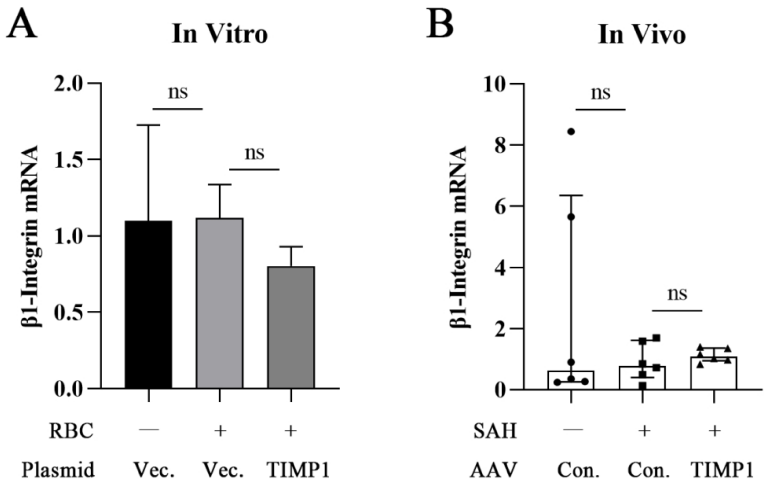
Supplementary Figure 2. TIMP1 is primarily expressed in astrocytes. A. mRNA levels of TIMP1 in astrocytes, microglia, and infiltrated cells sorted from SAH mouse brains via flow cytometry were detected at 1 day post-SAH. Four mice were analyzed. B. TIMP1 mRNA levels in astrocytes sorted from sham and SAH brains by magnetic adsorption were detected at 1 day post-SAH. n = 5 mice in each group.



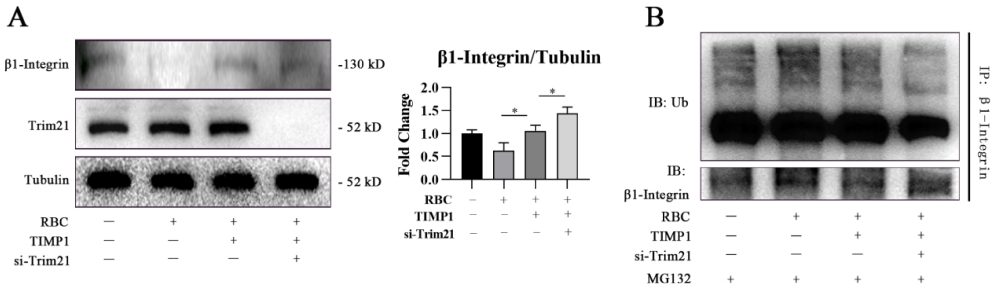
Supplementary Figure 3. Verification of transduction efficacy. TIMP1 (A) and β1-Integrin (B) mRNA levels were detected in astrocytes sorted with magnetic absorption. n = 3 mice in each group. * $p < 0.05$, **** $p < 0.0001$.



Supplementary Figure 4. Cellular co-localization of β1-Integrin in sham-operated brains.



Supplementary Figure 5. RBC stimulation and TIMP1 overexpression could not affect mRNA levels of astrocytic β1-Integrin both in vitro (A; n = 3 dependent experiments) and in vivo (B; n = 6 mice in each group).



Supplementary Figure 6. Silence of Trim21 further ameliorated the levels of β1-Integrin. A. Western blotting analysis for levels of β1-Integrin in astrocytes. n = 3 dependent experiments. B. Detection of ubiquitination of β1-Integrin. * $p < 0.05$.