Supplementary material

Loss of monocarboxylate transporter 1 aggravates white matter injury after experimental subarachnoid hemorrhage in rats

Running Title: MCT1 in white matter injury after SAH

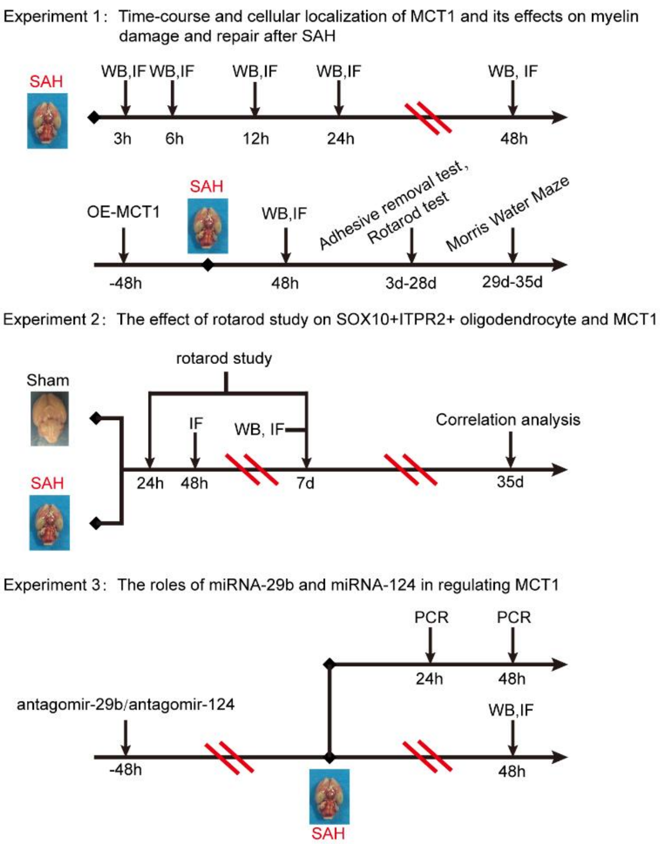

Figure S1. Experimental design. Experimental designs for the three different experiments: Experiment 1, time course and cellular localization of MCT1 and its effects on myelin damage and repair after SAH. Experiment 2, effect of rotarod training on SOX10+ ITPR2+ oligodendrocytes and MCT1. Experiment 3, roles of miRNA-29b and miRNA-124 in regulating MCT1.

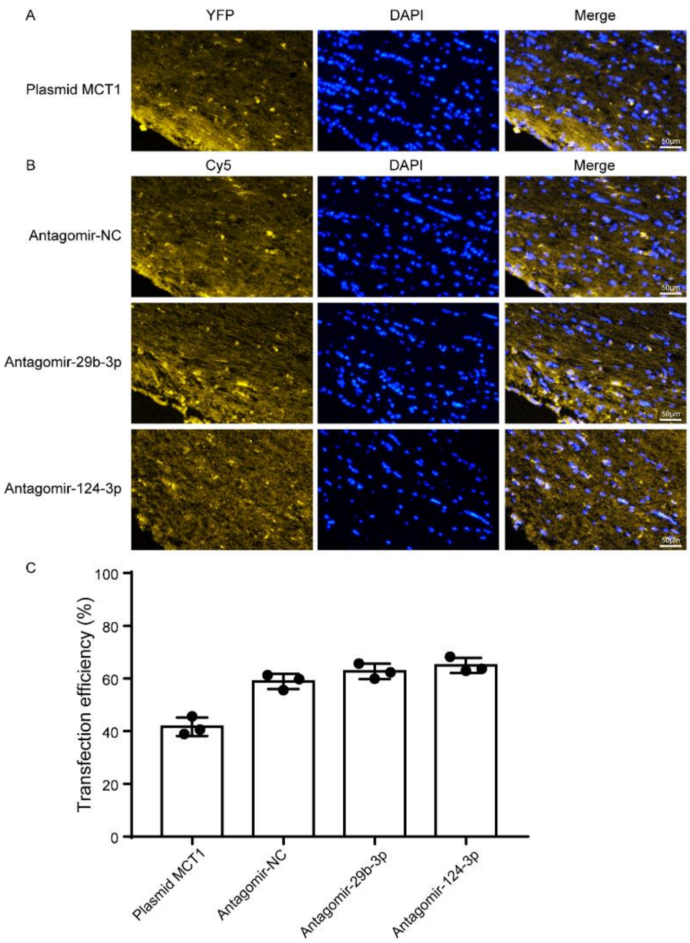

Figure S2. The transfection efficiency of plasmid and antagomir in the corpus callosum region. **A.** The transfection efficiency of plasmids in the corpus callosum region. Plasmid MCT1 have YFP label (yellow) and nuclei were fluorescently labeled with DAPI (blue). The yellow fluorescence signal was observed in corpus callosum region. Scale bar = 50 μm. **B.** The transfection efficiency of antagomir in the corpus callosum region. Antagomir have Cy5 labels (yellow) and nuclei were fluorescently labeled with DAPI (blue). The yellow fluorescence signal was observed in corpus callosum region both in the antagomir-NC group, the antagomir-29b-39 group and the antagomir-124-3p group. Scale bar = 50 μm. **C.** The quantitative graph of the transfection efficiency. Data are presented as mean ± SD. n = 3 per group.

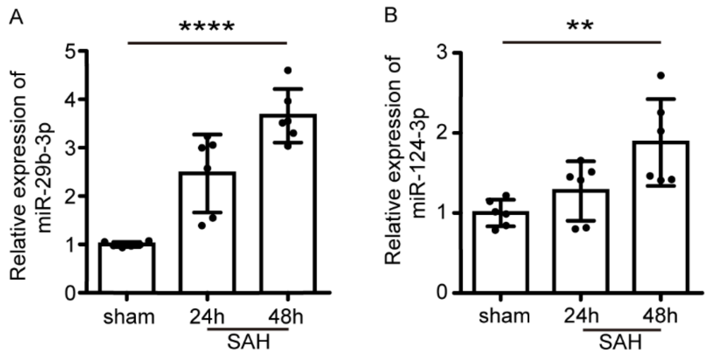

Figure S3. A. Relative expression level of miR-29b-3p in the corpus callosum of SD rats in the sham and SAH groups. **B.** Relative expression level of miR-124-3p in the corpus callosum of SD rats in the sham and SAH groups. Data are presented as mean ± *SD*. *n* = 6 per group. **$P \leqslant 0.01$, ****$P \leqslant 0.0001$.

Table S1 The total mortality and exclusion rates of experimental rats

Group	Mortality Rate	Excluded
Experiment 1		
Part 1		
sham	0% (0/10)	0
SAH(3h,6h,12h,24h,48h)	21.88% (14/64)	6
Part 2		
sham	0% (0/18)	0
SAH	18.18% (4/22)	2
SAH+Vector	10% (2/20)	1
SAH+Over-MCT1	14.29% (3/21)	0
Experiment 2		
sham	0% (0/20)	0
sham+training	0% (0/20)	0
SAH	20.83% (5/24)	2
SAH+training	13.64% (3/22)	2
Experiment 3		
sham	0% (0/12)	0
SAH	14.29% (3/21)	1
SAH+antagomir-NC	25% (2/8)	0
SAH+antagomir-29b-3p	14.29% (1/7)	0
SAH+antagomir-124-3p	14.29% (1/7)	1
Total	12.84% (38/296)	15
sham	0% (0/80)	0
SAH	17.59% (38/216)	15

Table S2 MCT1 overexpression plasmid

Final sequence:

ATGCCACCTGCGATTGGCGGGCCAGTGGGGTACACCCCCCCAGATGGAGGCTGGGGCTGG
GCGGTGGTAGTTGGAGCCTTCATTTCTATTGGCTTCTCCTATGCATTTCCCAAATCCATCAC
TGTCTTCTTTAAAGAGATTGAAATTATATTCAGTGCAACGACCAGTGAAGTGTCATGGATA
TCGTCCATCATGCTGGCTGTCATGTATGCCGGAGGTCCTATCAGCAGTATCTTGGTGAATA
AATATGGCAGCCGTCCAGTAATGATTGCTGGTGGCTGCCTGTCTGGCTGTGGCTTGATTGC
AGCTTCTTTCTGTAACACGGTGCAGGAACTTTACTTCTGCATTGGTGTCATTGGAGGTCTT
GGGCTTGCTTTCAACTTGAACCCAGCTCTGACTATGATTGGCAAGTATTTCTACAAGAAGC
GACCATTGGCCAATGGCCTGGCTATGGCAGGCAGCCCAGTGTTCCTCTCTACCCTGGCTCC
ACTTAATCAGGCTTTCTTTGGTATTTTTGGCTGGAGAGGAAGCTTCCTAATTCTTGGGGGC
CTCCTCCTCAACTGTTGTGTAGCTGGATCCCTGATGCGACCAATAGGGCCTCAGCAAGGC
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TACAGATCTCATTGGAGGAAGTCCCAAAGGAGAAAAGCTGTCAGTCTTCCAAACAGTTAA
TAAATTCCTGGACTTGTCCCTGTTTACCCATAGAGGCTTTTTGCTGTACCTGTCTGGAAAT
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TTTTTGCTGCTTCTGTTGTTGCGAATGGAGTGTGCCATTTGCTGGCACCTTTGTCTACGACC
TATGTTGGGTTCTGCATCTACGCGGGAGTCTTTGGATTTGCCTTTGGTTGGCTCAGCTCCGT
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GTGACCATTGTGGAATGTTGTCCTGTCCTCCTGGGACCACCACTTTTAGGCCGCCTCAATG
ACATGTATGGAGACTA
CAAATACACATACTGGGCTTGTGGCGTGATCCTCATCATCGCAGGCCTCTACCTCTTCATT
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GGACGGTAAAGAGGACGAGACCAGCACTGATGTTGATGAGAAGCCCAAGAAGACAATGA
AAGAAACACAGTCGCCAGCGCCACTGCAGAACAGCTCTGGAGACCCCGCGGAGGAGGAG
AGCCCAGTC

Clone ID: ORa00732D

ORF Clones (Accession No.): NM_012716.2 (ORF Sequence)

Table S3 Resource Identifiers for antibodies

Antibody ID		AB_2756669	AB_570666	AB_306298	AB_94882
Antibody name	MCT1 Antibody	Anti-MCT1 (SLC16A1) Antibody	Olig-2 antibody AF 555 Conjugate	Anti-Neurofilament, Heavy, 200 kD Antibody	Anti-Alzheimer Precursor Protein A4, a.a. 66-81 of APP {N-terminus}
Target antigen	MCT1 human, mouse	MCT1 (SLC16A1) human, mouse, rat	Olig-2 human, mouse, rat	200 kD Neurofilament Heavy-Neuronal Marker human, mouse, rat	Alzheimer Precursor Protein A4 human, mouse, rat
Vendor	Proteintech	Alomone Labs	Millipore	Abcam	Millipore
Cat number	20139-1-AP	AMT-011	ab9610-af555	ab8135	MAB348
Proper Citation	(Proteintech Cat# 20139-1-AP)	(Alomone Labs Cat# AMT-011, RRID:AB_2756669)	(Millipore Cat# ab9610-af555, RRID:AB_570666）	(Abcam Cat# ab8135, RRID:AB_306298)	(Millipore Cat# MAB348, RRID:AB_94882)
Reference	Reference(3)	Reference(2)	Reference(64)	Reference(3)	Reference(20)
Clonality	Polyclonal antibody	Polyclonal antibody	Polyclonal antibody	Polyclonal antibody	Monoclonal antibody
Clone ID					Clone 22C11
Host Organism	Rabbit	Rabbit	Rabbit	Rabbit	Mouse
Comments	WB,IP,IHC,IF	IF, IFC, IHC, WB	IF	IHC,ICC,IF,WB	IHC,IF,WB
Applicate dilution	WB=1:1000	IF=1:200	IF=1:150	IF=1:500	IF=1:250

Table S4 Resource Identifiers for antibodies

Antibody ID	AB_305869	AB_2564642	AB_11091087	
Antibody name	Myelin Basic Protein Monoclonal Antibody	Neurofilament H (NF-H), Nonphosphorylated antibody	ITPR2 antibody	SOX10 Antibody [SOX10/991]
Target antigen	Myelin Basic Protein human, mouse, rabbit, rat	Neurofilament H NF-H human, mouse, rat,	Rabbit ITPR2 human, mouse, rat	SOX10 Mouse, human
Vendor	Abcam	BioLegend	Bioss	Abcam
Cat number	ab7349	801701	bs-4243R	ab212843
Proper Citation	(Abcam Cat# ab7349, RRID:AB_305869)	(BioLegend Cat# 801701, RRID:AB_2564642)	(Bioss Cat# bs-4243R, RRID:AB_11091087)	(Abcam Cat# ab212843
Reference	Reference(24)	Reference(11)	Reference()	Reference(1)
Clonality	Monoclonal antibody	Monoclonal antibody	Polyclonal antibody	Monoclonal antibody
Clone ID	Clone 12	Clone SMI 32		SOX10/991
Host Organism	Rat	Mouse	Rabbit	Mouse
Comments	WB,ELISA, RIA,IHC,IF	IHC-P,WB,ICC	ELISA,IHC-P, IHC-F,ICC,IF	WB,IHC-P ICC/IF,Flow Cyt
Applicate dilution	WB=1:1000 IF=1:300	WB=1:1000 IF=1:300	IF=1:500	IF=1:500

Table S5 Statistical table

	Description	Hours after SAH or Sham surgery	in vivo or in vitro	Test used	Stat-value	One- or two-tailed P value?
Fig. 1A	Relative protein level of MCT 1	3h-48h	in vivo	One-way ANOVA	F(5,54)=3.154, P=0.0357, 95% CI=0.02105 to 0.8159 (sham vs. SAH 48h) η^2= 0.2261	Two-tailed
Fig. 1B	Mean density of MCT 1	48h	in vivo	t-test	P=0.0004, t = 5.292, df = 10, 95% CI= -0.5995 to -0.2443 (sham vs. SAH 48h)	Two-tailed
Fig. 2A	Relative protein level of MCT 1	48h	in vivo	One-way ANOVA	F(3,20)=37.98, P=0.0002, 95% CI=0.2804 to 0.6883 (sham vs. SAH); P=0.0003,95% CI=-0.7923 to -0.3843 (SAH+ Vector vs. SAH+ Over-MCT 1) η^2= 0.8505	Two-tailed
Fig. 2C	Relative fluorescent intensity of MCT 1	48h	in vivo	One-way ANOVA	F(3,20)=25.52, P=0.0002, 95% CI=0.2786 to 0.6475 (sham vs. SAH); P=0.0002, 95% CI=-0.5267 to -0.1577 (SAH+ Vector vs. SAH+ Over-MCT	Two-tailed

					1) η^2= 0.7926	
Fig. 2E	Number of β-APP per mm²	48h	in vivo	One-way ANOVA	F(3,20)=26.37, P=0.0001, 95% CI=-291.6 to -141.4 (sham vs. SAH); P=0.0479, 95% CI=0.5554 to 150.8 (SAH+ Vector vs. SAH+ Over-MCT 1) η^2= 0.7982	Two-tailed
Fig. 3A	Relative protein level of MBP	48h	in vivo	One-way ANOVA	F(3,20)=13.82, P=0.0002, 95% CI=0.2036 to 0.6633 (sham vs. SAH); P=0.0169, 95% CI=-0.5019 to -0.04209 (SAH+ Vector vs. SAH+ Over-MCT 1) η^2= 0.6747	Two-tailed
Fig. 3B	Relative protein level of SMI 32	48h	in vivo	One-way ANOVA	F(3,20)=14.18, P=0.0008, 95% CI=-2.096 to -0.7349 (sham vs. SAH); P=0.0399, 95% CI=0.02673 to 1.388 (SAH+ Vector vs. SAH+ Over-MCT 1)	Two-tailed

					η^2= 0.6802	
Fig. 3D	SMI32/MBP ratio	48h	in vivo	One-way ANOVA	F(3,20)=12.69, P=0.0001, 95% CI=-4.058 to -1.308 (sham vs. SAH); P=0.0498, 95% CI=0.001149 to 2.751 (SAH+ Vector vs. SAH+ Over -MCT 1) η^2= 0.6555	Two-tailed
Fig. 4A	Rotarod test	pre-35d	in vivo	Two-way repeated ANOVA	F(3,40)=367.8, P=0.002, 95% CI=-46.53 to -10.74 (Bonferroni's test, day 21, SAH+ Vector vs. SAH+ Over-MCT 1); P=0.0479, 95% CI=-35.9 to -0.1017 (Bonferroni’s test, day 28, SAH+ Vector vs. SAH+ Over-MCT 1); P=0.003, 95% CI=-45.72 to -9.92 (Bonferroni’s test, day 35, SAH+ Vector vs. SAH+ Over-MCT 1) partial η^2= 0.7841	Two-tailed
Fig. 4B	Adhesive removal test	pre-35d	in vivo	Two-way repeated ANOVA	F(3,40)=118.8, P=0.0457, 95% CI=0.03406 to	Two-tailed

					5.966 (Bonferroni's test, day 21, SAH+ Vector vs. SAH+ Over-MCT 1); P=0.005, 95% CI=1.489 to 7.42 (Bonferroni's test, day 28, SAH+ Vector vs. SAH+ Over-MCT 1); P=0.0457, 95% CI=0.03406 to 5.966 (Bonferroni's test, day 35, SAH+ Vector vs. SAH+ Over-MCT 1) partial η^2= 0.6836	
Fig. 4C	Morris water maze learning test	29d-33d	in vivo	Two-way ANOVA	F(3,200)=57.45, P=0.0301, 95% CI=0.2842 to 9.061 (Bonferroni's test, SAH+ Vector vs. SAH+ Over-MCT 1) partial η^2= 0.4629	Two-tailed
Fig. 4E	Morris water maze memory test	34d	in vivo	One-way ANOVA	F(3,40)=7.878, P=0.0046, 95% CI=1.807 to 12.28 (sham vs. SAH); P=0.0464, 95% CI=-10.49 to -0.06147 (SAH+ Vector vs. SAH+ Over-MCT	Two-tailed

					1) η^2= 0.3714	
Fig. 4F	Morris water maze swimming speed	34d	in vivo	One-way ANOVA	F(3,40)=0.3952, P=0.9803, 95% CI=-5.688 to 4.259 (sham vs. SAH); P=0.7914, 95% CI=-3.243 to 6.663 (SAH+ Vector vs. SAH+ Over-MCT 1) η^2= 0.0288	Two-tailed
Fig. 5B	%ITPR2+ out of SOX10+	48h	in vivo	Two-way ANOVA	F(1,12)=19.85, P=0.0008, 95% CI=-12.12 to -0.01835 (Bonferroni's test, sham vs. sham+ training); P=0.0494, 95% CI=-12.12 to -0.01835 (SAH vs. SAH+ training) partial η^2= 0.6234	Two-tailed
Fig. 5D	Relative protein level of MCT 1	7d	in vivo	One-way ANOVA	F(3,20)=12.5, P=0.0436, 95% CI=-1.027 to -0.01199 (sham vs. sham+ training); P=0.0003, 95% CI=-1.425 to -0.4104 (SAH vs. SAH+	Two-tailed

					training η^2= 0.6521	
Fig. 5F	%MCT1+ out of SOX10+	7d	in vivo	Two-way ANOVA	F(1,20)=19.57, P=0.0244, 95% CI=-13.4 to -0.711 (Bonferroni's test, sham vs. sham+ training); P=0.0413, 95% CI=-12.89 to -0.2052 (SAH vs. SAH+ training) partial η^2= 0.4947	Two-tailed
Fig. 6A	Correlation in sham group between Rotarod test and %MCT1+ out of SOX10+	35d	in vivo	Pearson Product linear regression analyses	r=0.6475, **P=0.0027; Y = 2.896*X + 129.6; 95% CI=0.2738 to 0.8513	Two-tailed
Fig. 6B	Correlation in SAH group between Rotarod test and %MCT1+ out of SOX10+	35d	in vivo	Pearson Product linear regression analyses	r=0.6668, **P=0.0025; Y = 3.089*X + 78; 95% CI=0.2903 to 0.8645	Two-tailed
Fig. 6C	Correlation in sham group between Adhesive removal test and %MCT1+ out of	35d	in vivo	Pearson Product linear regression analyses	r=-0.5521, *P=0.0142; Y = -0.1736*X + 10.32; 95% CI=-0.8046 to -0.1307	Two-tailed

	SOX10+					
Fig. 6D	Correlation in SAH group between Adhesive removal test and %MCT1+ out of SOX10+	35d	in vivo	Pearson Product linear regression analyses	r=-0.4722, *P=0.0478; Y = -0.2772*X + 16.25; 95% CI=-0.7695 to -0.00689	Two-tailed
Fig. 7A	Relative expression of miR-29b-3p	24h and 48h	in vivo	One-way ANOVA	F(2,15)=26.84, P=0.0002, 95% CI=-7.197 to -3.541 (sham vs. SAH 48h) η^2= 0.7816	Two-tailed
Fig. 7B	Relative expression of miR-124-3p	24h and 48h	in vivo	One-way ANOVA	F(2,15)=9.486, P=0.0053, 95% CI=-2.403 to -0.4524 (sham vs. SAH 48h) η^2= 0.5584	Two-tailed
Fig. 7C	Relative protein level of MCT 1	48h	in vivo	One-way ANOVA	F(4,35)=12.81, P=0.0074, 95% CI=0.09376 to 0.8052 (sham vs. SAH); P=0.0060, 95% CI=-0.815 to -0.1036 (SAH+ antagomir-NC vs. SAH+antagomir-29b-3p); P=0.0003, 95% CI=-0.9395 to -0.2281(SAH+ antagomir-NC vs.	Two-tailed

					SAH+antagomir-124-3p) η^2= 0.5941	
Fig. 7E	Relative fluorescent intensity of MCT 1	48h	in vivo	One-way ANOVA	F(4,25)=6.714, P=0.0410, 95% CI=0.01481 to 0.9742 (sham vs. SAH); P=0.0066, 95% CI=-1.102 to -0.1424 (SAH+ antagomir-NC vs. SAH+antagomir-29b-3p); P=0.0462, 95% CI=-0.9653 to -0.00597(SAH+ antagomir-NC vs. SAH+antagomir-124-3p) η^2= 0.5178	Two-tailed
Fig. S3A	Relative expression of miR-29b-3p	24h and 48h	in vivo	One-way ANOVA	F(2,15)=33.4, P=0.0001, 95% CI=-3.458 to -1.866 (sham vs. SAH 48h) η^2= 0.8166	Two-tailed
Fig. S3B	Relative expression of miR-124-3p	24h and 48h	in vivo	One-way ANOVA	F(2,15)=7.967, P=0.0027, 95% CI=-1.433 to -0.3304 (sham vs. SAH 48h) η^2= 0.5151	Two-tailed