

Supplementary Materials

Celastrol ameliorates hypoxic-ischemic brain injury in neonatal rats by reducing oxidative stress and inflammation

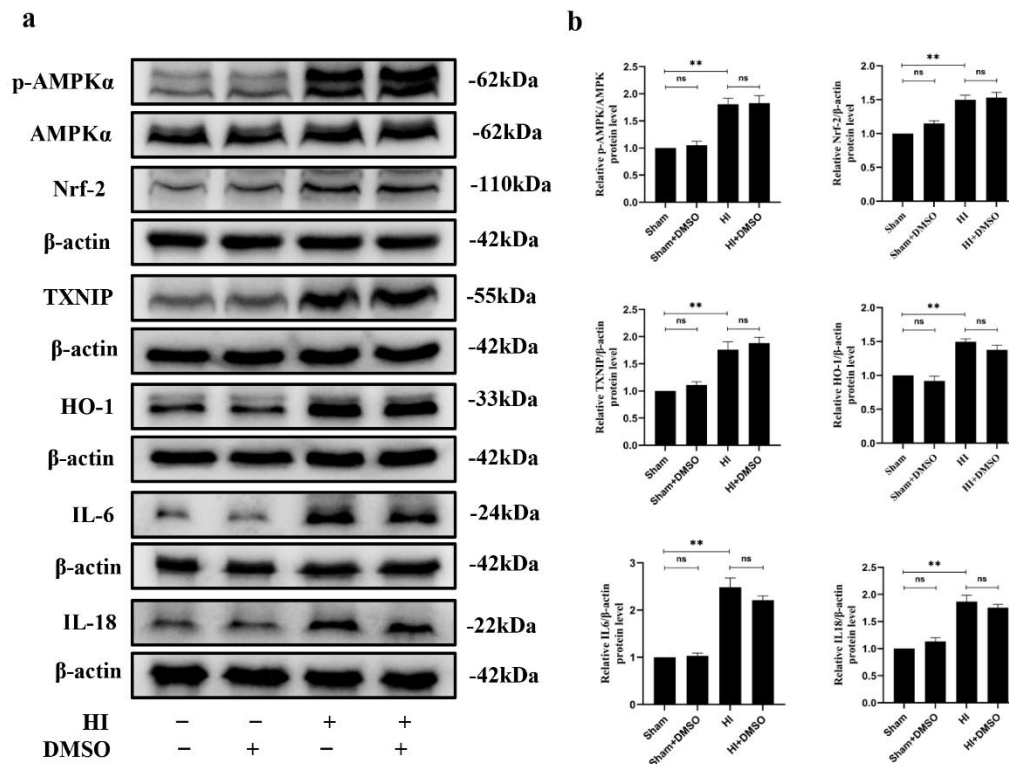


Fig. s1 Effects of 1% DMSO in neonatal rats post HI.

a Protein levels of p-AMPK α , AMPK α , Nrf-2, TXNIP, HO-1, IL-6 and IL18 were evaluated by western blot in neonatal rats after HI damage with or without 1% DMSO in solvent. **b** Quantitative analysis of protein levels. ** $p < 0.01$ vs. Sham (n = 4).

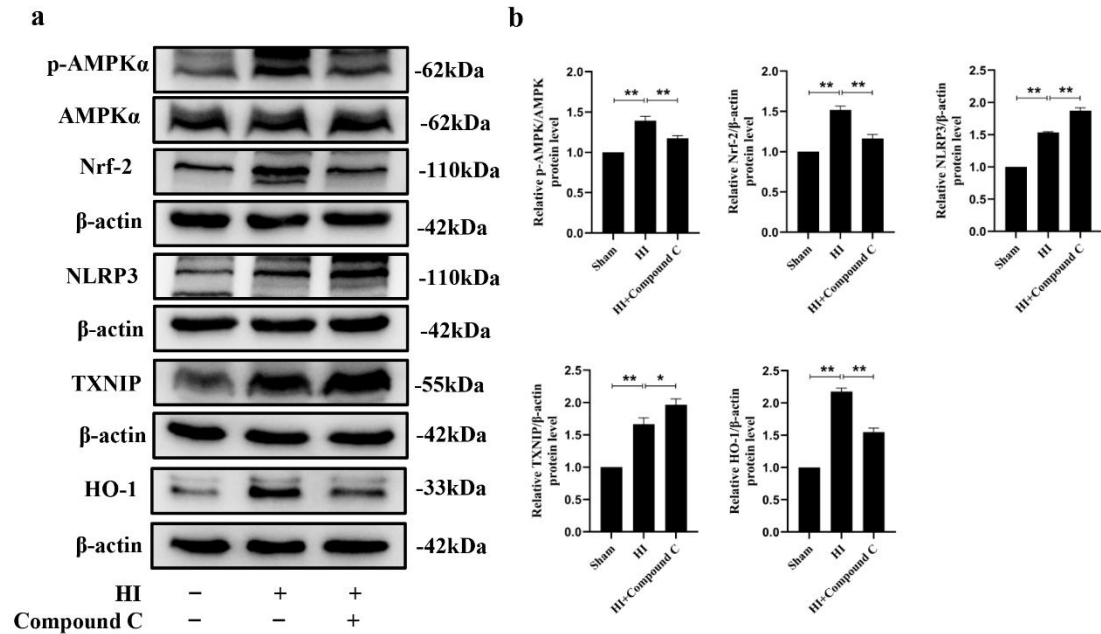


Fig. s2 AMPK inhibition suppressed anti-oxidative ability and upregulated inflammations in neonatal rats after HI.

a Protein levels of p-AMPK α , AMPK α , Nrf-2, NLRP3, TXNIP, and HO-1 were evaluated by western blot in neonatal rats 24 h after HI brain injury with or without the treatment of compound C. **b** Quantitative analysis of protein levels. * $p < 0.05$ and ** $p < 0.01$ vs. Sham or HI (n = 5).

Table s1. Antibodies

Antibody	Vendor (City, State, catalogue)	Dilution	
		WB	IF
AMPK α	Cell Signaling Technology (Danvers, MA, 5831)	1:1000	ND
p-AMPK α	Cell Signaling Technology (Danvers, MA, 2535)	1:1000	ND
NLRP3	Abclonal (Wuhan, China, A5652)	1:1000	ND
Nrf-2	Proteintech (Wuhan, China, 16396-1-AP)	1:1000	1:200
TXNIP	Abcam (Cambridge, UK, ab188865)	1:1000	ND
HO-1	Proteintech (Wuhan, China, 10701-1-AP)	1:1000	ND
MAP-2	Abcam (Cambridge, UK, ab254264)	1:1000	1:2000
MBP	Abcam (Cambridge, UK, ab218011)	1:1000	1:5000
Iba-1	Abcam (Cambridge, UK, ab178846)	1:1000	1:1000
TNF- α	Affinity Biosciences (OH, USA, AF7014)	1:1000	ND
IL-18	Proteintech (Wuhan, China, 10663-1-AP)	1:1000	ND
IL-6	Affinity Biosciences (OH, USA, DF6087)	1:1000	ND
IL-1 β	Affinity Biosciences (OH, USA, AF5103)	1:1000	ND
ACTB	Abclonal (Wuhan, China, AC026)	1:10000	ND
Lamin B1	Proteintech (Wuhan, China, 12987-1-AP)	1:10000	ND

ND = Not detected; WB = Western blot; IF: Immunofluorescence.

Table s2. Primer information

Primer	Gene name	Primer	Sequences (5'to 3')
Symbol		direction	
<i>TNF-α</i>	Tumor necrosis factor	Forward	GAGGTCAACCTGCCCAAGTA
	alpha	Reverse	GCTGGGTAGAGAACGGATGA
<i>IL-6</i>	Interleukin 6	Forward	CCAGTTGCCMDATTGGGACT
		Reverse	TCTGACAGTGCATCATCGCT
<i>IL-18</i>	Interleukin 18	Forward	CGAACAGCCAACGAATCCCAGAC
		Reverse	TCACAGATAGGGTCACAGCCAGTC
<i>IL-1β</i>	Interleukin 1 beta	Forward	AATCTCACAGCAGCATCTCGACAAG
		Reverse	TCCACGGGCAAGACATAGGTAGC
<i>β-actin</i>	Actin, beta (Actb)	Forward	AAGTCCCTCACCTCCCAAAAG
		Reverse	AAGCAATGCTGTCACCTTCCC