Title: Reactive enteric glial cells participate in paralytic ileus by damaging nitrergic neurons during endotoxemia

Na Li, Jing Xu, Hui Gao, Yuxin Zhang, Yansong Li, Haiqing Chang, Shuwen Tan, Shuang Li, Qiang Wang*

Department of Anesthesiology & Center for Brain Science, The First Affiliated Hospital of Xi'an Jiaotong University, Xi'an, 710061, China.

Correspondence: Qiang Wang, Department of Anesthesiology & Center for Brain Science, The First Affiliated Hospital of Xi'an Jiaotong University, Xi'an 710061, Shaanxi, China, MD.

Tel. & Fax: 029-85323646

Mobile: +86 13991236768

E-mail: Qiang Wang, dr.wangqiang@139.com;

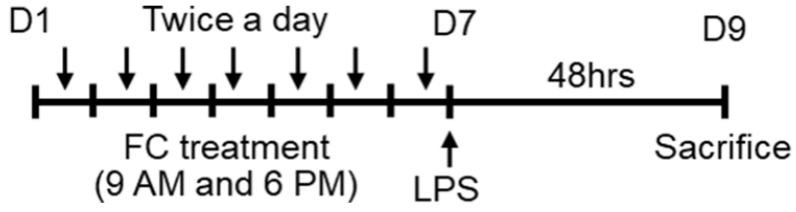
Supplementary information

Primary enteric glial cells culture

The isolation, identification, and culture of primary EGCs were performed as previously described[1]. In brief, newborn mice (1~2 days old, C57BL/6 mice) were deeply anesthetized by isoflurane and decapitated. After dissection of the murine colons, the luminal content was flushed with cold Hank's balanced salt solution (Hank's balanced salt solution, HBSS, Gibco Life Technologies, USA). The external muscularis was peeled off from the underlying circular muscle using a cotton swap and digested for 15 min at 37 °C in trypsin (0.1mg/ml; Gibco Life Technologies, USA). DMEM-F-12 (Gibco Life Technologies, USA) with 10% FBS was used to stop the digestion reaction and then centrifuged at 900 rpm. Cells suspended into DMEM-F-12 supplemented with 10% FBS, 1mM glutamine and 100IU/ml penicillin/streptomycin, 20 μg/ml Gentamicin and 2 mM L-Glutamine were plated on Poly-D-Lysine-coated (0.01%; Sigma Aldrich, USA) plates. Cells were cultured for 12~14 days and passaged to new plates for purity assay and the following experiments.

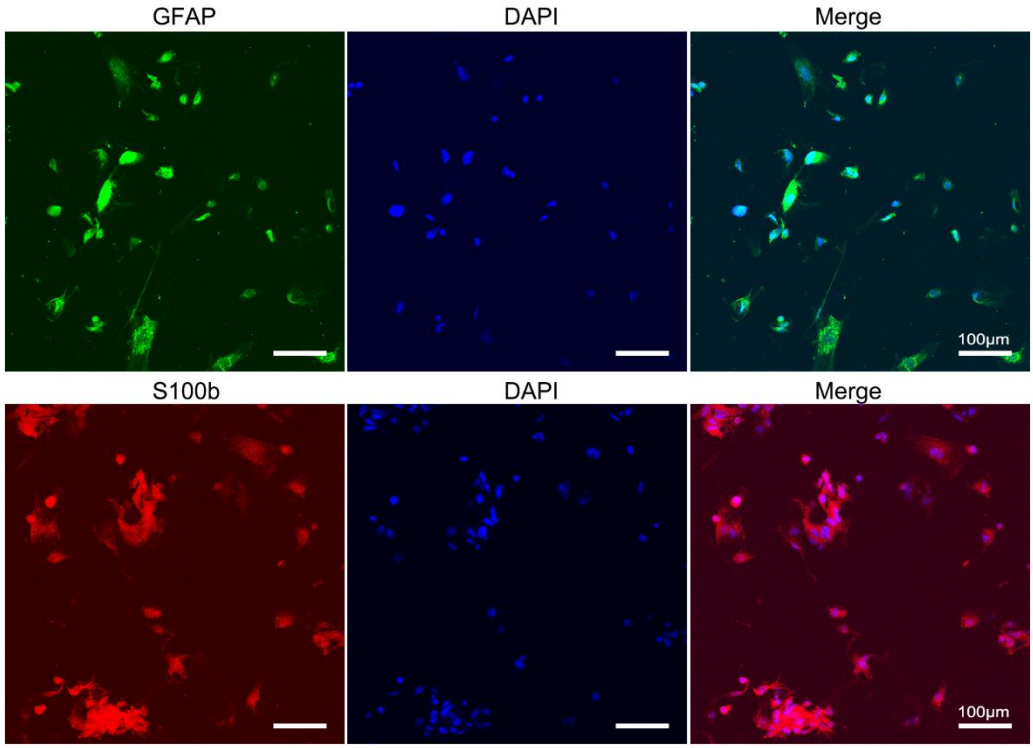
Supplementary Figure S1

A schematic diagram of the experiments undertaken in this study



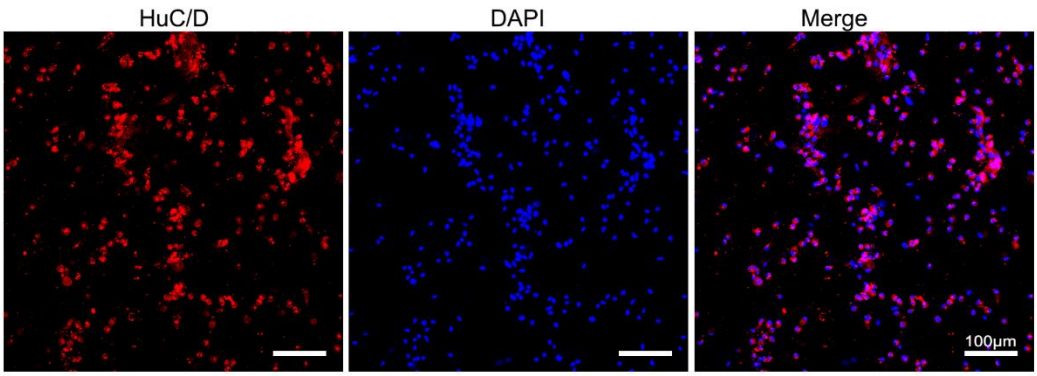
Supplementary Figure S2

Identification of primary enteric glial cells purity



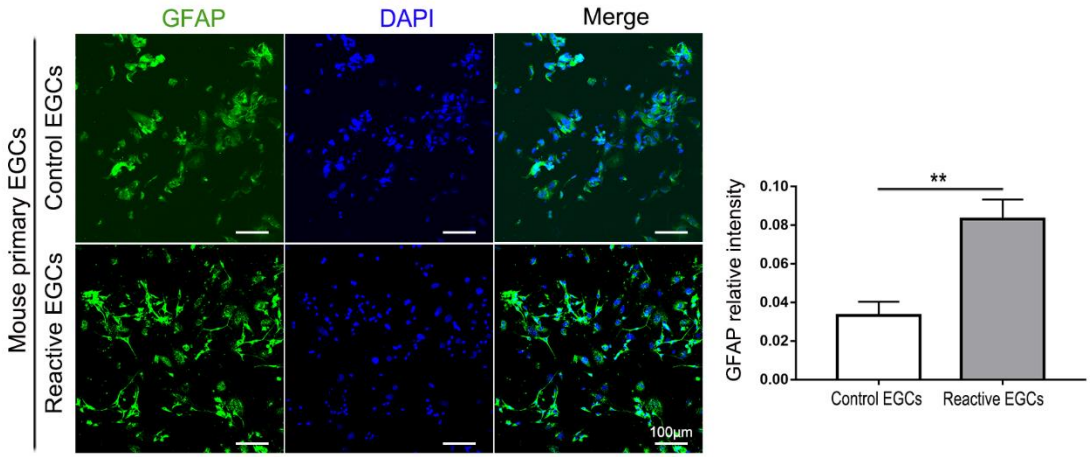
Supplementary Figure S3

Identification of primary enteric neurons purity



Supplementary Figure S4

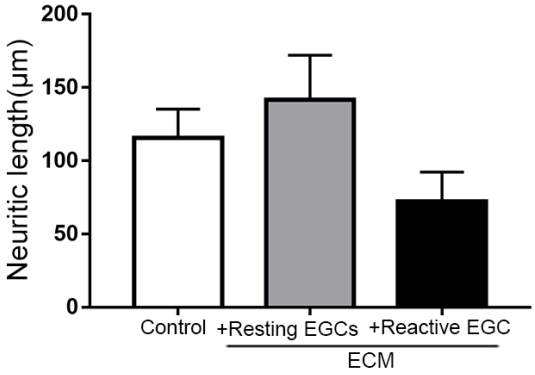
Reactive EGCs were induced by IL-1β and TNF-α in vitro.



IL-1β/TNF-α induced primary enteric glia into a reactive EGC phenotype *in vitro*. GFAP expression of primary enteric glia increased under IL-1β/TNF-α treatment. Student's *t*-test, n = 3 biological replications, ** $P < 0.01$.

Supplementary Figure S5

Neuritic length

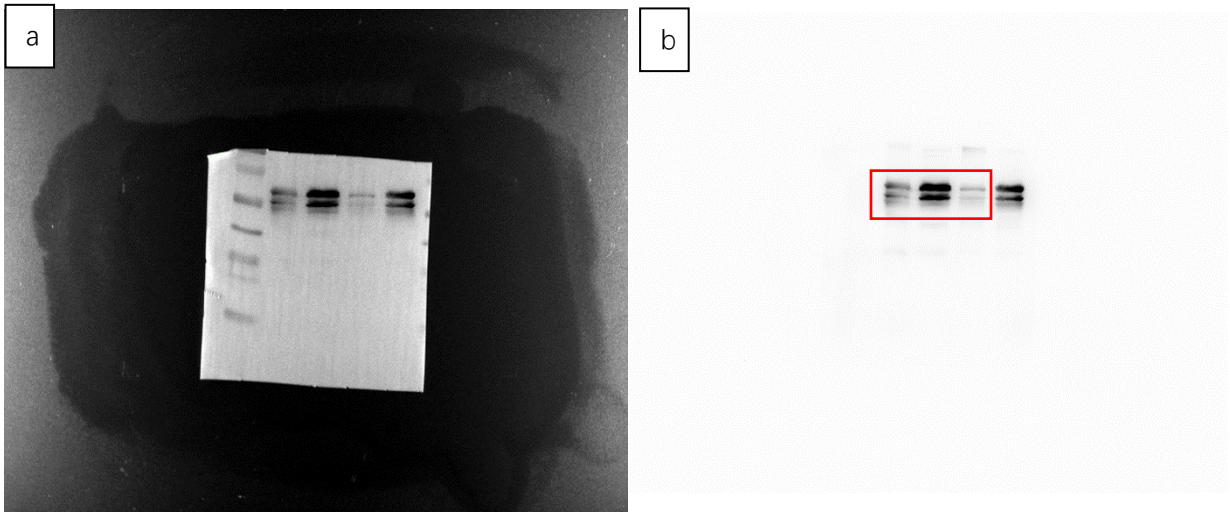


Neurons cultured in reactive EGCs conditioned medium tended to show shorter neurites compared with those grown in the resting EGCs conditioned medium, However, there was no significant difference between them. One-way ANOVA, n = 3 biological replications, ns, no significant difference.

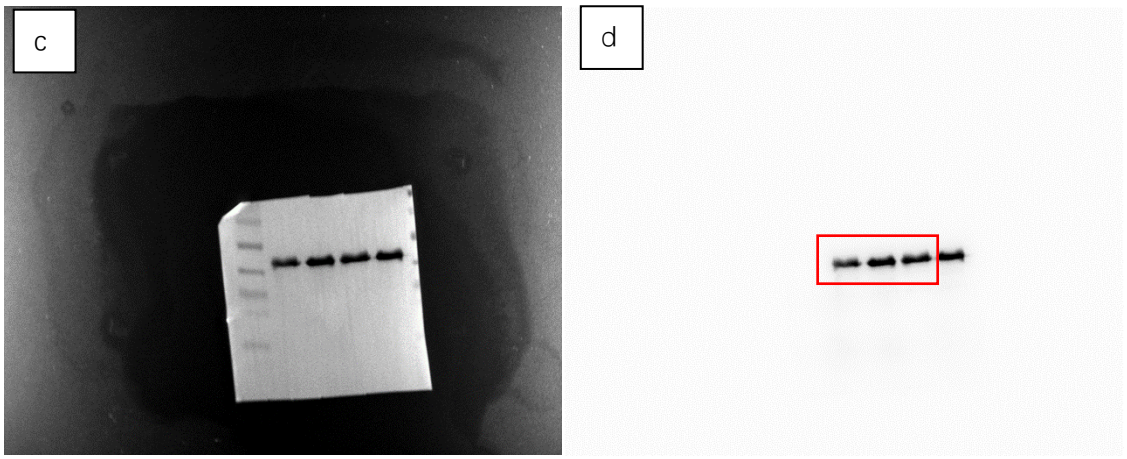
Supplementary Figure S6

Full unedited gel for Fig. 1 (a)

GFAP



β-actin

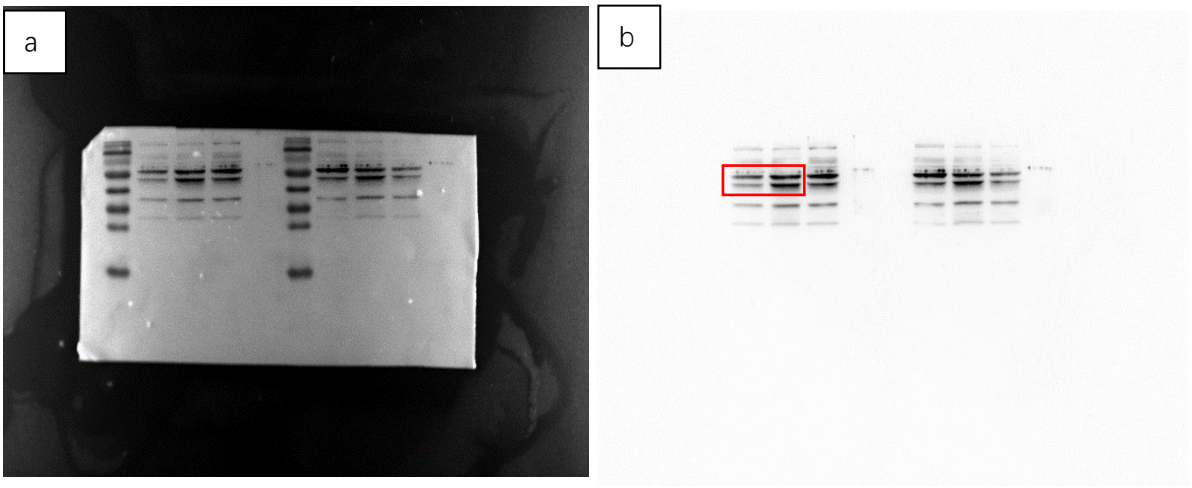


Lanes in the red frame of the unedited gel correspond to those shown in the cropped images within the manuscript. The fourth lane is irrelevant to the experimental results.

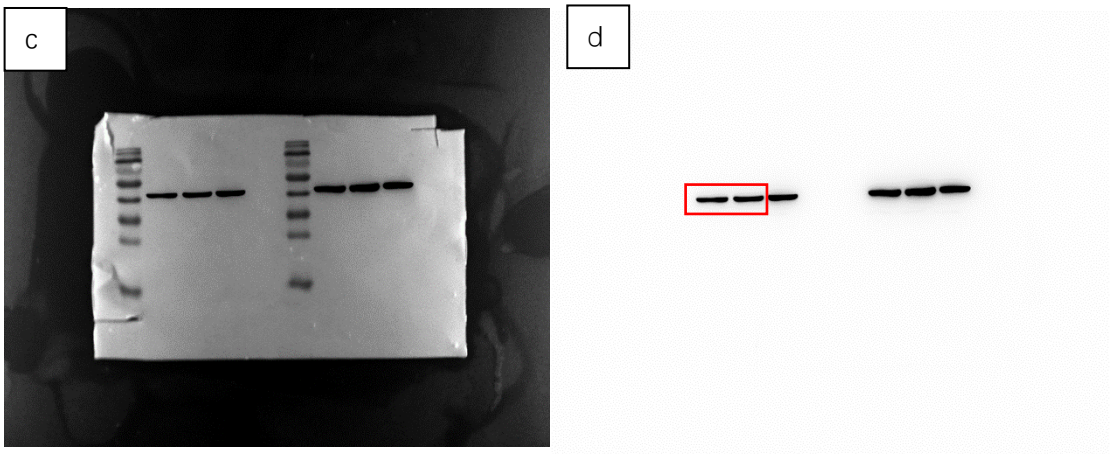
Supplementary Figure S7

Full unedited gel for Fig. 4 (a)

GFAP



β-actin



Lanes in the red frame of the unedited gel correspond to those shown in the cropped images within the manuscript. Other lanes are irrelevant to the experimental results.

References

[1] von Boyen GB, Steinkamp M, Reinshagen M, Schafer KH, Adler G and Kirsch J (2004) Proinflammatory cytokines increase glial fibrillary acidic protein expression in enteric glia. Gut 53:222-8. https://doi.org/10.1136/gut.2003.012625