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

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**Supplementary information 1**

**The details of FC preparation**

FC preparation was according to the previous research[1].FC (Sigma, St. Louis, MO) was dissolved in half of the final volume of distilled water and sonicated until the solution became clear. A slight excess of sodium sulfate was added to the clear solution to precipitate the barium from the solution by forming barium sulfate. This solution was then filtered through a 0.2 μm filter, and distilled water was added to make up the final volume.

**Supplementary information 2**

**Primary enteric glial cells culture**

The isolation, identification, and culture of primary EGCs were performed as previously described[2]. 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**

**E:\博士科研工作\article of master\article revision\实验流程图-1.tifA schematic diagram of the experiments undertaken in this study**

**Supplementary Figure S2**

**Identification of primary enteric glial cells purity E:\博士科研工作\article of master\article revision\figure-V7\原代神经元纯度\原代EGC纯度.tif**

**Supplementary Figure S3**

**Identification of primary enteric neurons purity**

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**Supplementary Figure S4**

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

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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**

**E:\博士科研工作\article of master\article revision\figure-V7\Neuritic length(μm).tifNeuritic 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.

**References**

[1] Paulsen RE, Contestabile A, Villani L, Fonnum F (1987) An in vivo model for studying function of brain tissue temporarily devoid of glial cell metabolism: the use of fluorocitrate. J Neurochem 48(5): 1377–1385. https://doi.org/10.1111/j.1471-4159.1987.tb05674.x

[2] 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