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Supplementary Material

LncRNA xist regulates sepsis associated neuroinflammation in the periventricular white matter of CLP rats by miR-122-5p/PKCη Axis

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Supplementary Figures

Supplementary Figure 1 Experimental group diagram of animals in this study.

To investigate the levels of lncRNA xist, miR-122-5p, PKCη, IL-1β and TNF-α in the PWM in CLP rats. The rats were randomly divided into sham and CLP group. To investigate whether overexpression of miR-122-5p can reduce neuroinflammation in rats with sepsis, rats were divided into 4 groups: Sham, CLP, AAV miR-NC + CLP, and AAV miR-122-5p +CLP. To investigate whether knockdown of lncRNA xist can reduce neuroinflammation in rats with sepsis, rats were divided into 4 groups: Sham, CLP, ASO lncRNA-NC + CLP, and lncRNA-xist ASO +CLP.

Supplementary Figure 2 Experimental group diagram of astrocytes and BV2 in this study.

To investigate whether overexpression of miR-122-5p can reduce neuroinflammation in microglia/astrocyte, cells were treated with mimic-miR-122-5p or mimic-nc, then divided into four groups: control group, LPS group, mimic-nc + LPS group and mimic miR-122-5p+LPS group. To investigate whether knockdown of lncRNA xist can reduce neuroinflammation in microglia/astrocyte, cells were cultured and treated with si-lncRNA xist, then divided into four groups: control group, LPS group, si-lncRNA nc + LPS group and si-lncRNA xist + LPS group. To investigate whether knockdown PKCη can reduce neuroinflammation in microglia/astrocyte, cells were administrated with si-RNA, then divided into four groups: control group, LPS group, si-nc+LPS group and si-PKCη+LPS group.

Supplementary Figure 3 Transfection efficiency of recombinant adeno-associated virus 9 (AAV9) in the PWM of septic CLP rats.

The AAV9 of miR-122-5p were injected into the left lateral ventricle. Double immunofluorescence staining showed the colocalized expression of GFP (green) and Iba1/GFAP (red) after AAV9 injection at the magnification of ×20. The results showed that the AAV9 may be transfected into the microglia and astrocytes with high transfection efficiency.

Supplementary Figure 4 Mimic miR-122-5p can alleviate expression of IL-1β in BV2 cells administrated with LPS *in vitro*.

Double immunofluorescence staining shows the distribution of Iba1 (green) and IL-1β (red) immunoreactive microglial cells after treatment with LPS or LPS + mimic miR-122-5p or LPS +

mimic nc and their matching controls. Co-localized expression of Iba1 and IL-1β could be seen in **Panel A**. The bar graph in **Panel B** shows a significant increase in the number of IL-1β^{+}/Iba1^{+} cells after LPS or LPS + mimic nc in comparison with their corresponding controls or LPS + mimic miR-122-5p. Note mimic miR-122-5p could decrease the expression of IL-1β in microglia induced by LPS.

Supplementary Figure 5 Mimic miR-122-5p alleviate expression of IL-1β in astrocytes administrated with LPS *in vitro*.

Double immunofluorescence staining shows the distribution of GFAP (green) and IL-1β (red) immunoreactive astrocytes after LPS, mimc nc + LPS, mimic miR-122-5p + LPS treatment and their matching controls. Co-localized expression of GFAP and IL-1β could be seen in **Panel A**. The bar graph in **Panel B** shows a significant increase in the number of IL-1β^{+}/GFAP^{+} cells after LPS or mimc nc + LPS in comparison with mimic miR-122-5p + LPS or their corresponding controls. Note mimic miR-122-5p could downregulate the expression of IL-1β in astrocytes induced by LPS.

Supplementary Figure 6 Knockdown lncRNA xist can downregulate expression of IL-1β in BV2 cells treated with LPS.

Double immunofluorescence staining shows the distribution of Iba1 (green) and IL-1β (red) immunoreactive BV2 cells after LPS or si-nc + LPS or si-lncRNA xist + LPS treatment and their matching controls. Co-localized expression of Iba1 and IL-1β could be seen in **Panel A**. The bar graph in **Panel B** shows a significant increase in the number of IL-1β^{+}/ Iba1^{+} cells after LPS or si-nc + LPS in comparison with si-lncRNA xist + LPS or their corresponding controls. Note si-lncRNA xist could downregulate the expression of IL-1β in BV2 cells induced by LPS.

Supplementary Figure 7 Knockdown lncRNA xist can decrease expression of IL-1β in astrocytes administrated with LPS.

Double immunofluorescence staining shows the distribution of GFAP (green) and IL-1β (red) immunoreactive astrocytes after LPS or si-nc + LPS or si-lncRNA xist + LPS treatment and their matching controls. Co-localized expression of GFAP and IL-1β could be seen in **Panel A**. The bar graph in **B** shows a significant increase in the number of IL-1β^{+}/GFAP^{+} cells after LPS or si-nc + LPS in comparison with si-lncRNA xist + LPS or their corresponding controls. Note si-lncRNA xist could downregulate the expression of IL-1β in astrocytes induced by LPS.

Supplementary Figure 8 Knockdown PKCη can reduce the level of p-ikbα and p-p65 in astrocyte and BV2 cells.

A-B. Panel **A and B** shows the immunoreactive bands of PKCη (78kDa), p65 (65kDa), p-p65(65kDa) and p-iKbα (39kDa) and GAPDH (37kDa) after treatment with LPS, si-nc+LPS, si-PKCη+LPS, and the corresponding control (n=3 for each group) in BV2 cells and astrocytes. Bar graphs (**C-J**) show the optical density of protein expression shown in Panel **A and B** (n=3 for each group). For statistical analysis, one-way ANOVA followed by Holm-Sidak tests was used and presented as the mean ± standard error of measurement (SEM). *p<0.05, ** p <0.01, *** p <0.001

Supplementary Table 1. Primary antibodies used in experiments

The antibody name, species, manufacturers and concentrations were included.