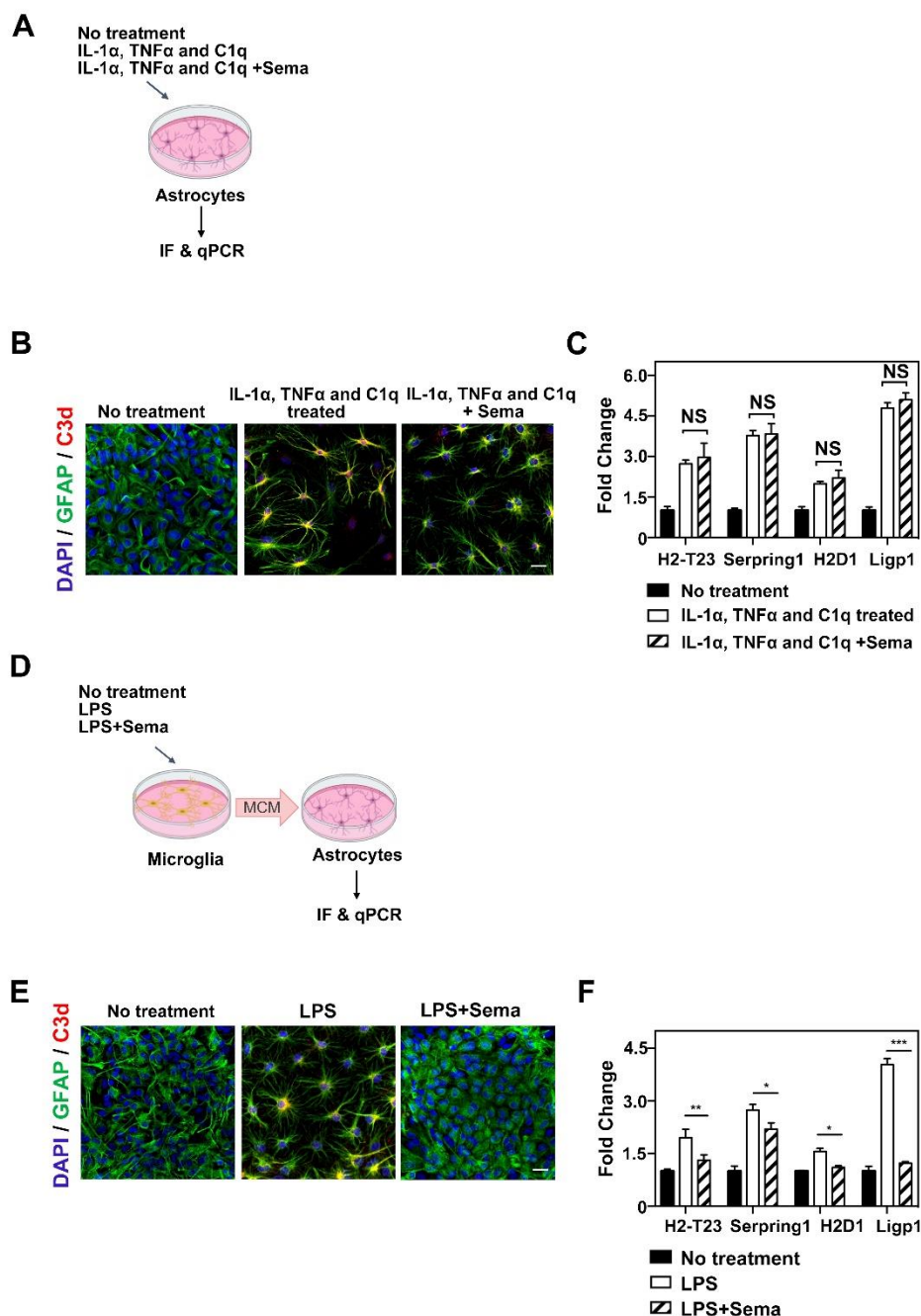


SUPPLEMENTARY DATA

Blocking C3d⁺/GFAP⁺ A1 Astrocyte Conversion with Semaglutide Attenuates Blood-Brain Barrier Disruption in Mice after Ischemic Stroke

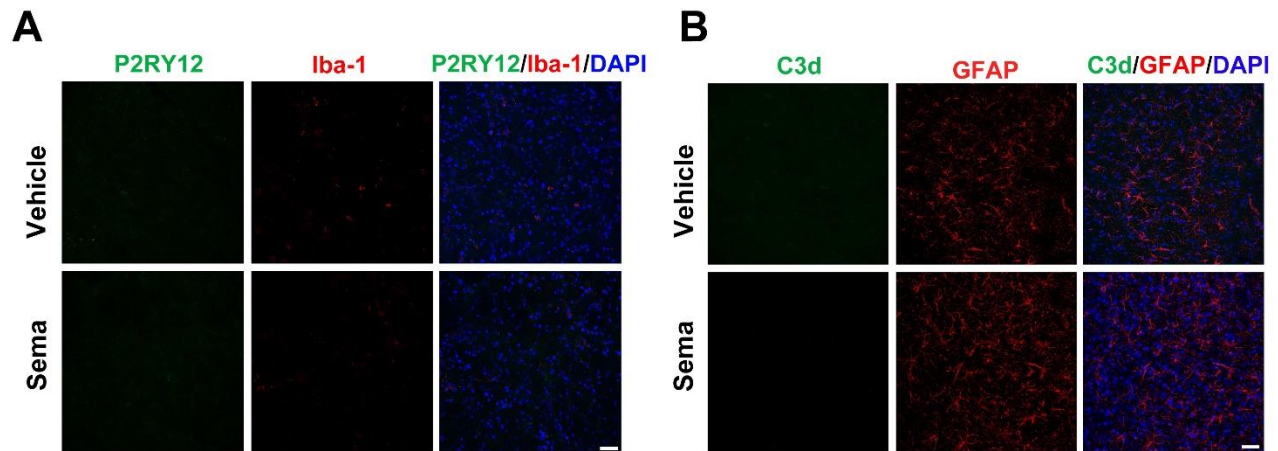
Qi Zhang^{1,#}, Chang Liu^{1,#}, Rubing Shi¹, Shiyi Zhou¹, Huimin Shan¹, Lidong Deng¹, Tingting Chen¹, Yiyao Guo¹, Zhijun Zhang¹, Guo-Yuan Yang^{1,2}, Yongting Wang^{1*}, Yaohui Tang^{1*}

SUPPLEMENTARY DATA



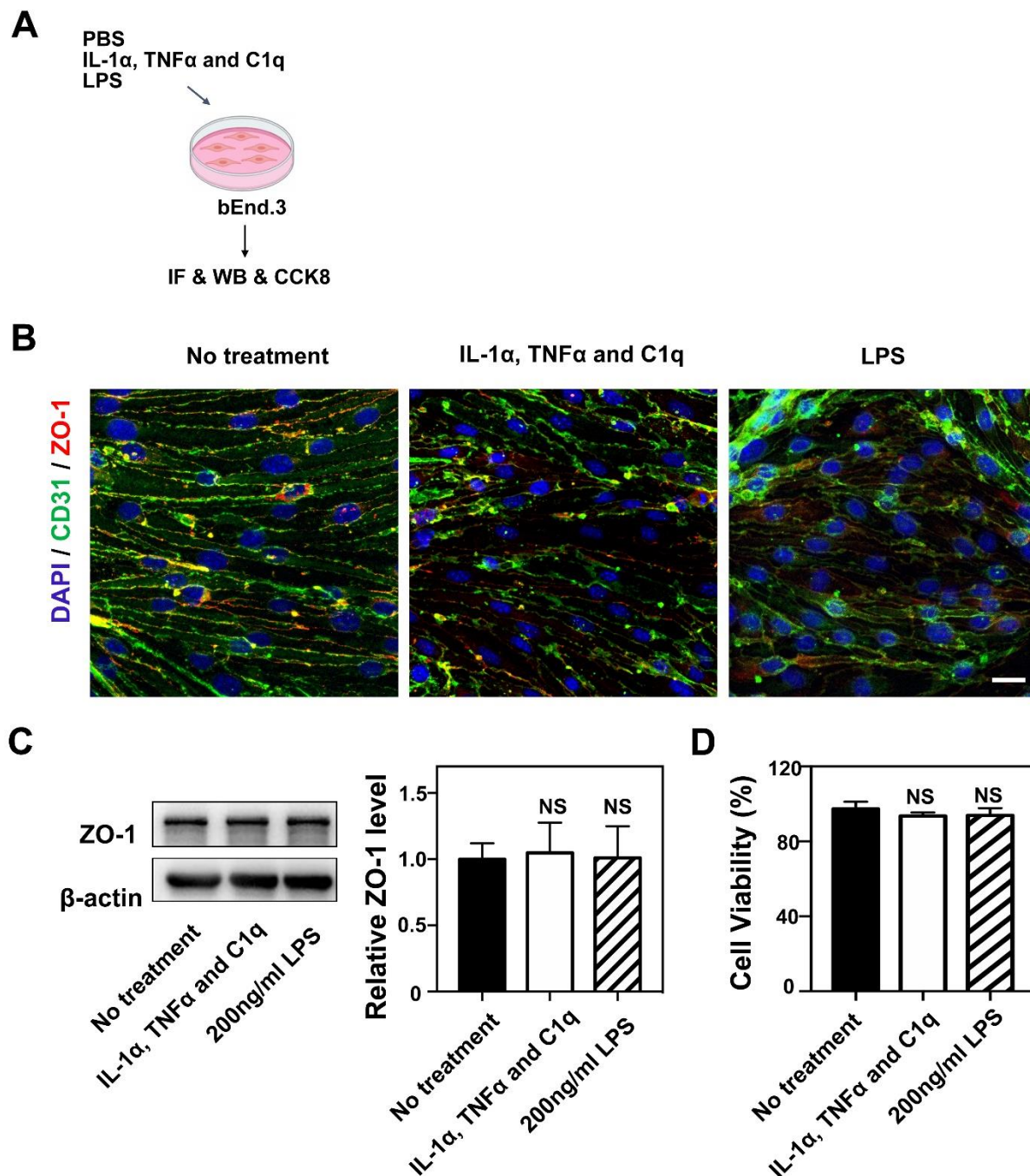
Supplementary Figure 1. Semaglutide treatment did not directly prevent IL-1 α , TNF α and C1q induced phenotype change of astrocyte. **A.** Astrocytes were treated with IL-1 α +TNF α +C1q or IL-1 α +TNF α +C1q+semaglutide for 24 hours and immunocytochemistry and real-time PCR were performed. **B.** Immunostaining of C3d⁺/GFAP⁺ cells (C3d in red color; GFAP in green color; DAPI in blue color). Scale bar=25 μ m. **C.** Bar graph showed the mRNA levels of C3d⁺/GFAP⁺ cells related genes H2-T23, Serping1, H2D1 and Ligp1 expression after IL-1 α +TNF α +C1q or IL-1 α +TNF α +C1q+semaglutide treatment. Data are mean \pm SEM. n=3 per group. NS, $p > 0.05$. **D.** Astrocytes were treated with the medium derived from LPS stimulated microglia or LPS+semaglutide stimulated microglia for 24 hours and the immunocytochemistry and real-time PCR were performed. **E.** Immunostaining of C3d⁺/GFAP⁺ cells (C3d in red color; GFAP in green color; DAPI in blue color). Scale bar=25 μ m. **F.** Bar graph showed the mRNA levels of C3d⁺/GFAP⁺ cells related genes H2-T23, Serping1, H2D1 and Ligp1 expression after treatment with the medium derived from LPS stimulated microglia or LPS+semaglutide stimulated microglia. Data are mean \pm SEM. n=3 per group. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

SUPPLEMENTARY DATA



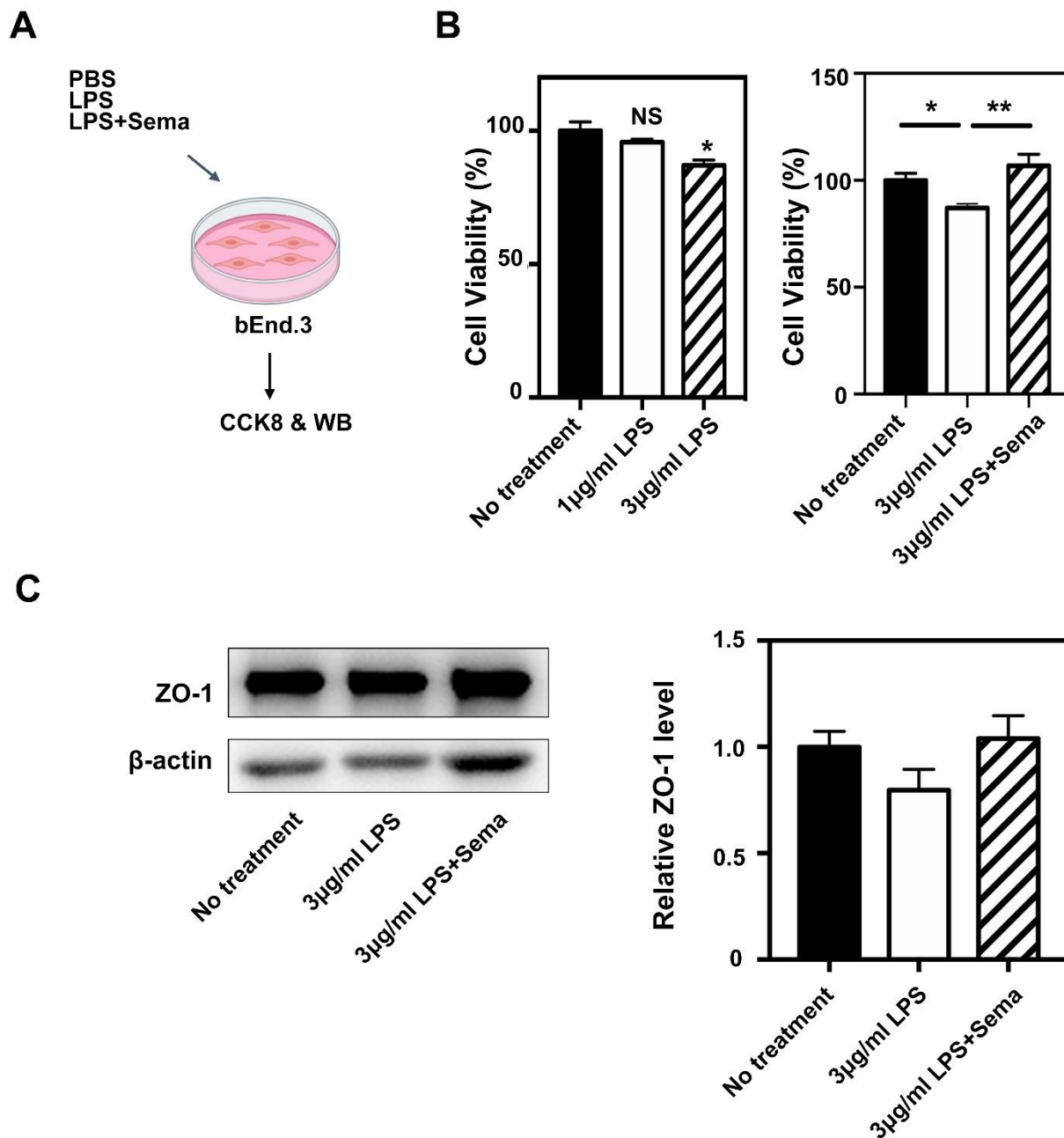
Supplementary Figure 2. The formation of C3d⁺/GFAP⁺ reactive astrocytes are induced by activated microglia. A. Photomicrographs showed that P2RY12⁺/Iba-1⁺ cells (P2RY12 in green color; Iba-1 In red color; DAPI in blue color) in the ipsilateral hemisphere of the perifocal area in tMCAO mice and semaglutide treated tMCAO mice. Scale bar=75 μ m. **B.** Immunofluorescence images showed C3d⁺/GFAP⁺ cells (C3d in red color; GFAP in green color; DAPI in blue color) after microglial depletion. Scale bar=75 μ m.

SUPPLEMENTARY DATA



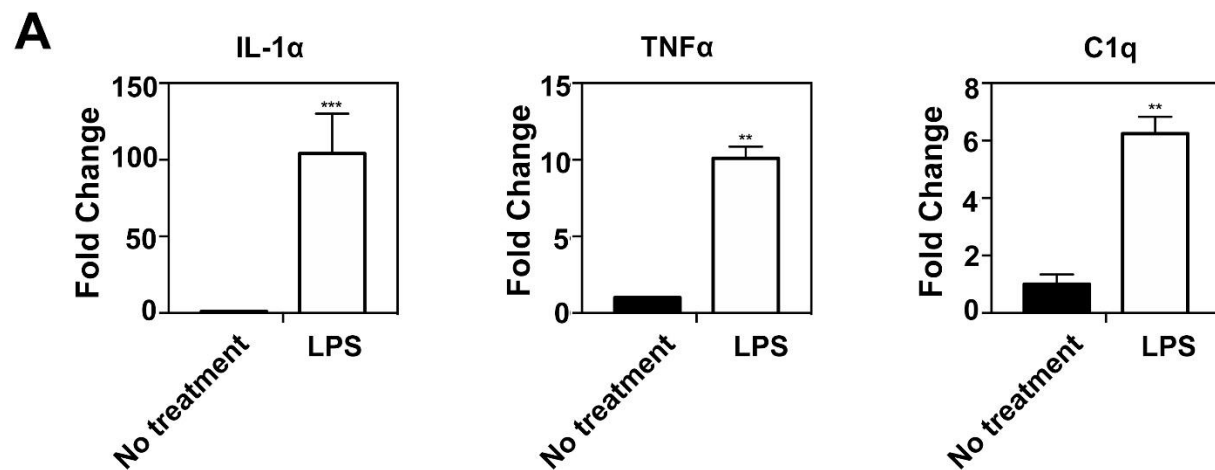
Supplementary Figure 3. IL-1 α (3 ng/ml), TNF α (30 ng/ml) and C1q (400 ng/ml) or LPS (200 ng/ml) treatment did not affect the tight junction integrity and viability of bEnd.3 cells. **A.** bEnd.3 cells were treated with IL-1 α +TNF α +C1q or LPS (200 ng/ml) for 24 hours, then immunocytochemistry and western blot were performed. **B.** Immunostaining of ZO-1/CD31 cells (ZO-1 in red color; CD31 in green color; DAPI in blue color). Scale bar=25 μ m. **C.** Western blot of ZO-1 in bEnd.3 cells. Bar graph showed ZO-1 level in bEnd.3 cells. Data are mean \pm SEM. n=3 per group. NS, $p>0.05$. **D.** CCK8 assay in bEnd.3 cells in no treatment group or treated with IL-1 α +TNF α +C1q or 200 ng/ml LPS.

SUPPLEMENTARY DATA



Supplementary Figure 4. Semaglutide treatment increased TJ integrity of bEnd.3 cells exposed to 3µg/ml LPS. **A.** bEnd.3 cells were treated with LPS or LPS+semaglutide for 24 hours, then CCK-8 and western blot were performed. **B.** CCK-8 assay showed the viability of bEnd.3 cells exposed to different concentration of LPS (1µg/ml and 3µg/ml). Data are mean ± SEM. n=8 per group. *, $p<0.05$; **, $p<0.01$. **C.** Western blot data showed the expression of ZO-1 in bEnd.3 cells treated with 3µg/ml LPS or 3µg/ml LPS+semaglutide. Data are mean ± SEM. n=3 per group.

SUPPLEMENTARY DATA



Supplementary Figure 5. LPS treatment increased the expression of IL-1 α , TNF α and C1q in primary murine microglia. A. Bar graph showed the expression of IL-1 α , TNF α and C1q in microglia after LPS treatment. Data are mean \pm SEM. $n=3$ per group. **, $p<0.01$; ***, $p<0.001$.