Supplementary Materials

Materials and methods

Mouse microglial cell LPS treatment

To stimulate microglia into an M1 phenotype, lipopolysaccharide (LPS, 100 ng/mL) was treated for 24 h. Then the exosomes (200 μg/mL) were added in the medium in different groups for another 24 h.

Immunofluorescence

Immunofluorescence was performed in primary neurons or microglia. Cells grown on coverslips were fixed with 4% PFA and incubated in 5% bovine serum albumin (BSA, Solarbio, Beijing, China) to block nonspecific binding of IgG. The identities of positive cells of the neurons were assessed by antibodies against MAP2 (A01201-1, 1:200, BOSTER, CA, USA) or neuron-specific nuclear protein (NeuN, MAB377, 1:200, Millipore, MA, USA). The microglia was checked by staining with microglial marker ionized calcium binding adapter molecule 1 (Iba1, ab178847, 1:100, Abcam, Cambridge, UK), and astrocyte was measured by glial fibrillary acidic protein (GFAP, BA0056, 1:400, BOSTER). Corresponding secondary antibodies were Alexa Fluor 488 or 594 conjugated antibody respectively (A-11001/A-11005, 1:300, Invitrogen, MA) at room teperature for 1 h in the dark. The nuclei were labeled with DAPI and the photos were acquired under a fluorescence microscope (Leica, Japan).

Supplementary Figures

Fig. S1

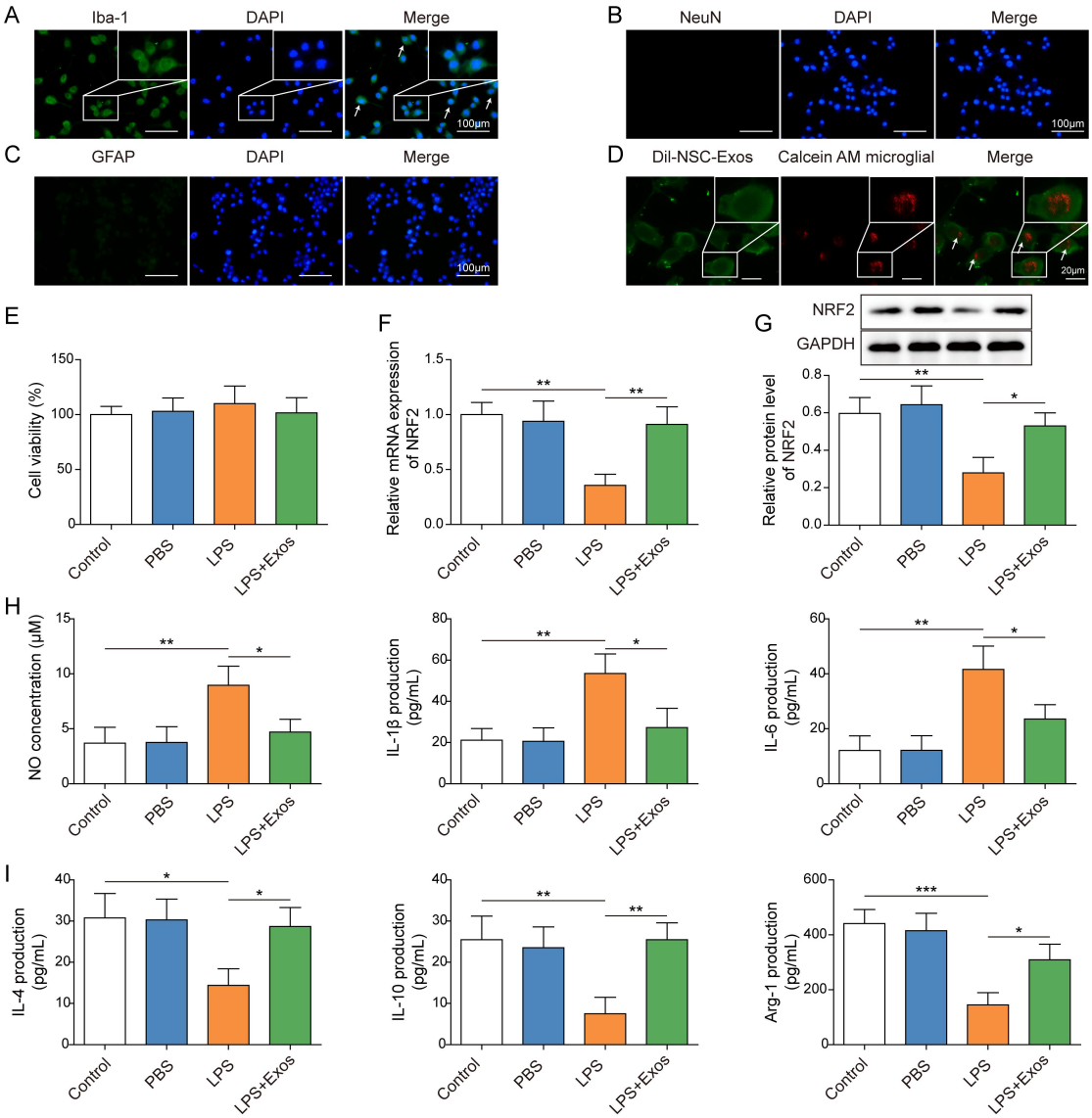

Fig. S2

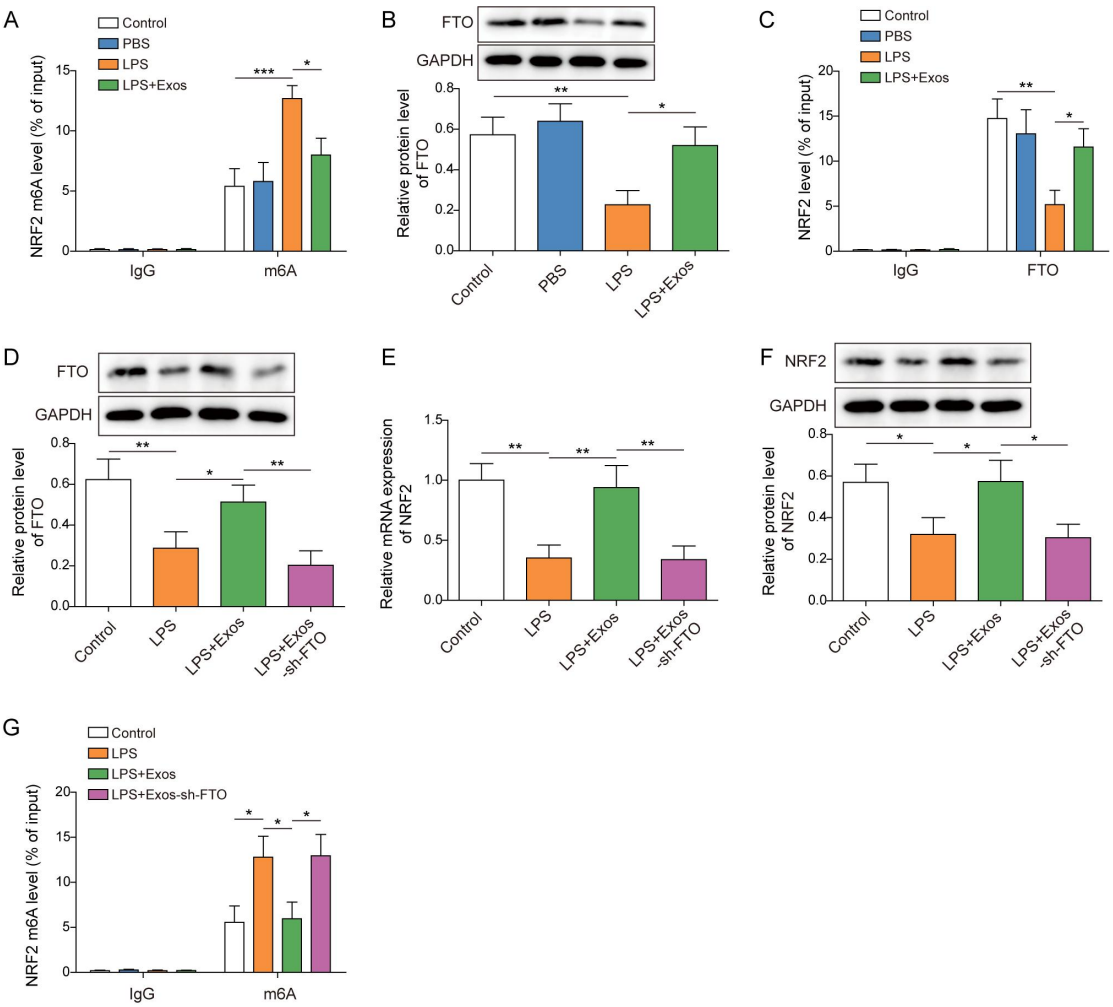
A
Control
PBS
LPS
LPS+Exos
NRF2 m6A level (% of input)
IgG
m6A
B
FTO
GAPDH
Relative protein level of FTO
Control
PBS
LPS
LPS+Exos
C
Control
PBS
LPS
LPS+Exos
NRF2 level (% of input)
IgG
FTO
D
FTO
GAPDH
Relative protein level of FTO
Control
LPS
LPS+Exos
LPS+Exos -sh-FTO
E
Relative mRNA expression of NRF2
Control
LPS
LPS+Exos
LPS+Exos -sh-FTO
F
NRF2
GAPDH
Relative protein level of NRF2
Control
LPS
LPS+Exos
LPS+Exos -sh-FTO
G
Control
LPS
LPS+Exos
LPS+Exos-sh-FTO
NRF2 m6A level (% of input)
IgG
m6A

Fig. S3

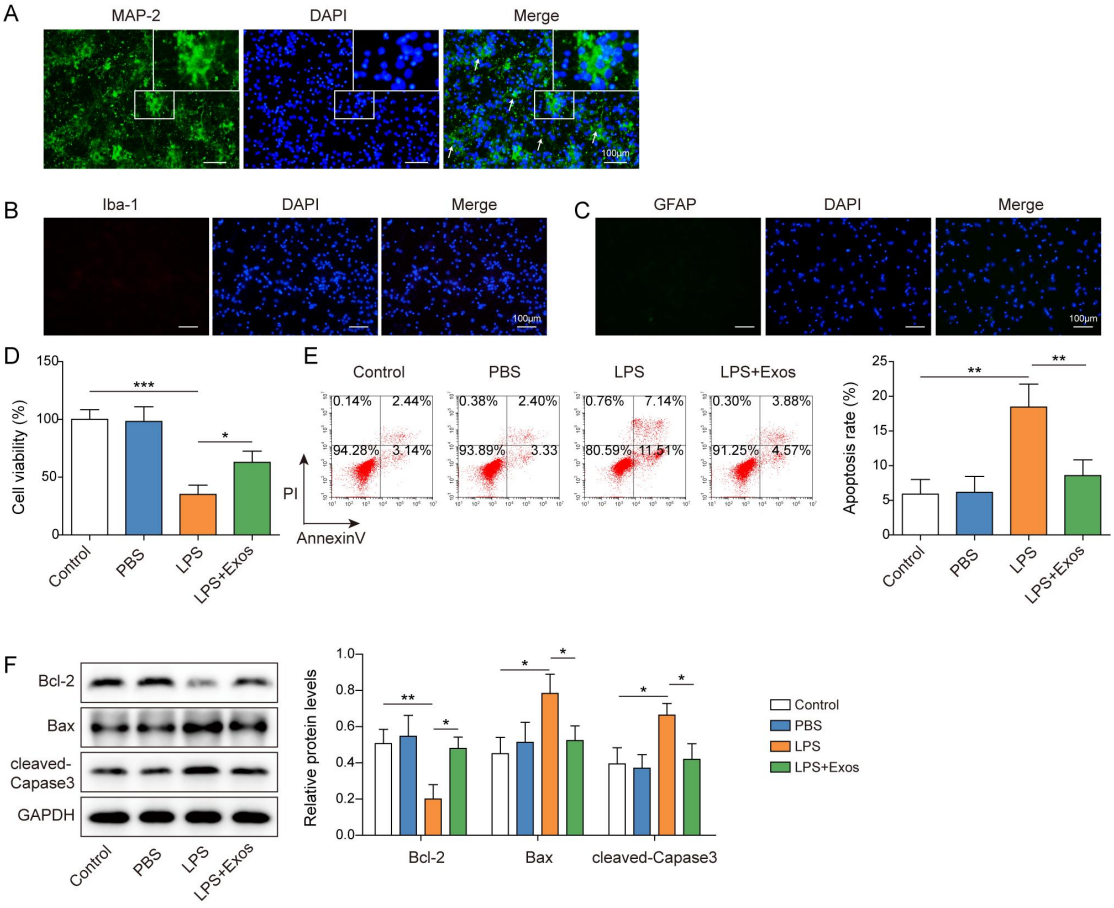
A
MAP-2
DAPI
Merge
100μm
B
Iba-1
DAPI
Merge
100μm
C
GFAP
DAPI
Merge
100μm
D
Cell viability (%)
Control
PBS
LPS
LPS+Exos
E
Control
PBS
LPS
LPS+Exos
0.14%
2.44%
94.28%
3.14%
0.38%
2.40%
93.89%
3.33
0.76%
7.14%
80.59%
11.51%
0.30%
3.88%
91.25%
4.57%
PI
AnnexinV
Apoptosis rate (%)
F
Bcl-2
Bax
cleaved-Capase3
GAPDH
Relative protein levels
Control
PBS
LPS
LPS+Exos

Figure legends

Figure S1. NSC-Exos promoted NRF2 expression and M2 polarization in LPS induced microglia. (A) Primary mouse microglia were immunofluorescent stained with Iba-1 (green), and cell nuclei were stained with DAPI (blue). Scale bar = 100 μm. (B) NeuN expression in primary isolated microglia were identified by immunofluorescent staining. Scale bar = 100 μm. (C) GFAP expression in primary isolated microglia were identified by immunofluorescent staining. Scale bar = 100 μm. (D) Fluorescence microscopy was used to detect the uptake of DiI-labeled NSC-Exos (red) by Calcein AM dye-labeled primary microglia (green). Scale bar = 20 μm. Microglia were treated with control, PBS, LPS, and LPS+NSC-Exos. (E) Cell viability in each group detected via CCK-8 assay. (F) Relative mRNA expression level of NRF2 was determined via RT-qPCR. (G) NRF2 protein level determined by Western blot and quantified by Image J. (H-I) NO production released into the medium measured via the Griess reaction. The M1 cytokine levels (IL-1β, IL-6) and M2 cytokine levels (IL-4, IL-10 and Arg-1) were measured using ELISA. n = 3 per group. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

Figure S2. NSC-Exos transferred FTO to regulate NRF2 mRNA m6A modification in LPS-induced microglia. Microglia were treated with LPS or NSC-Exos. (A) The m6A enrichment of NRF2 mRNA in microglia was measured by MeRIP-qPCR. (B) FTO protein expression level was detected via Western blot. (C) The association between NRF2 mRNA and FTO in microglia was detected by RIP assay. (D) Western blot analysis of FTO in microglia. (E-F) RT-qPCR and Western

blot analyzed NRF2 level in microglia. (G) The m6A enrichment of NRF2 mRNA in microglia was measured by MeRIP-qPCR. n = 3 per group. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

Figure S3. NSC-Exos attenuated damage to neurons caused by microglia's inflammatory response. (A) Primary mouse neuronal cells were immunofluorescent stained with neural marker MAP2 (green) with DAPI nuclei (blue). Scale bar = 100 μm. (B) Iba-1 expression in primary isolated neurons were identified by immunofluorescent staining. Scale bar = 100 μm. (C) GFAP expression in primary isolated neurons were identified by immunofluorescent staining. Scale bar = 100 μm. Microglia were treated with PBS, LPS, and LPS+NSC-Exos. (D) The relative cell viability of neurons detected via CCK-8 assay upon coculture. (E) The percentage of apoptosis rate neurons was detected by flow cytometry. (F) Relative amounts of Bcl-2, Bax, cleaved Caspase-3 in neurons detected by Western blot and quantified by Image J. n = 3 per group. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.