Supplementary Material

Transplantation of miR-145a-5p modified M2 type microglia promotes the tissue repair of spinal cord injury in mice

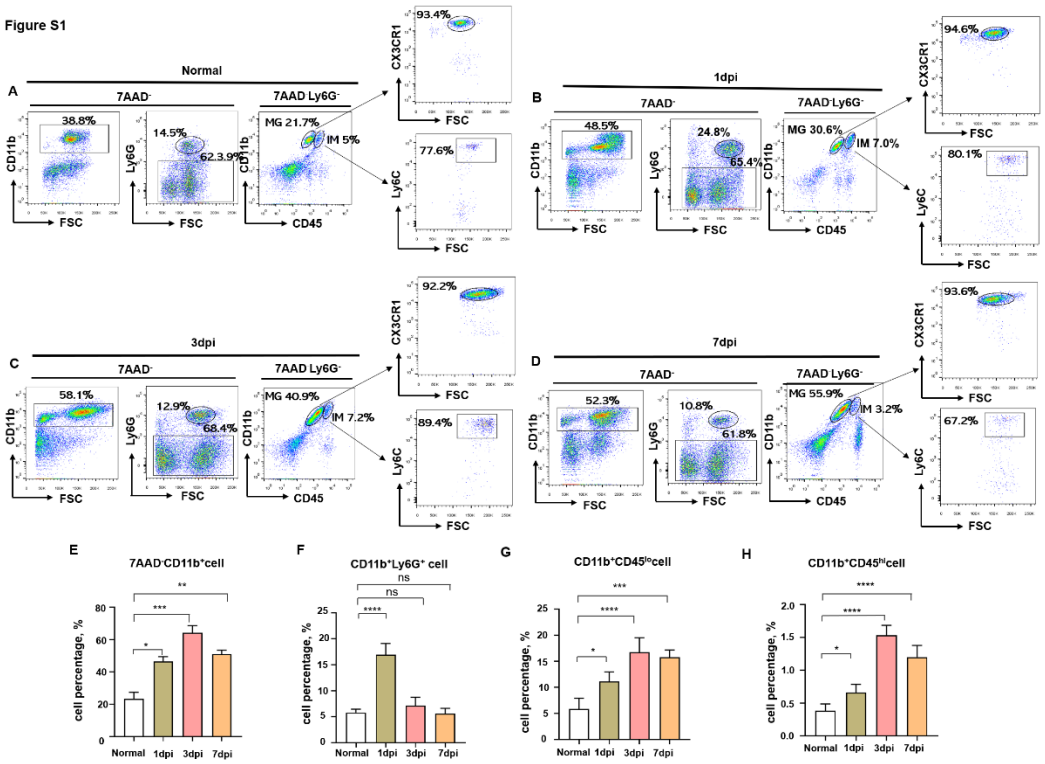

Figure S1. Analysis of immune cell phenotype of spinal cord at different time points during murine SCI progression

(A-D) Analysis of immune cell subsets of spinal cord at different time points after SCI using FACS. Normal group(A); 1 day(B), 3 days(C) and 7 days(D) after SCI. (E-H) The percentage of monocytes (E), granulocytes (F), microglia (G) and inflammatory macrophages (H) was calculated and quantitatively compared (n=6). Data were shown as mean±SEM. *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$; ****, $p<0.0001$ VS normal groups by One-Way ANOVA followed by Tukey's post hoc test.

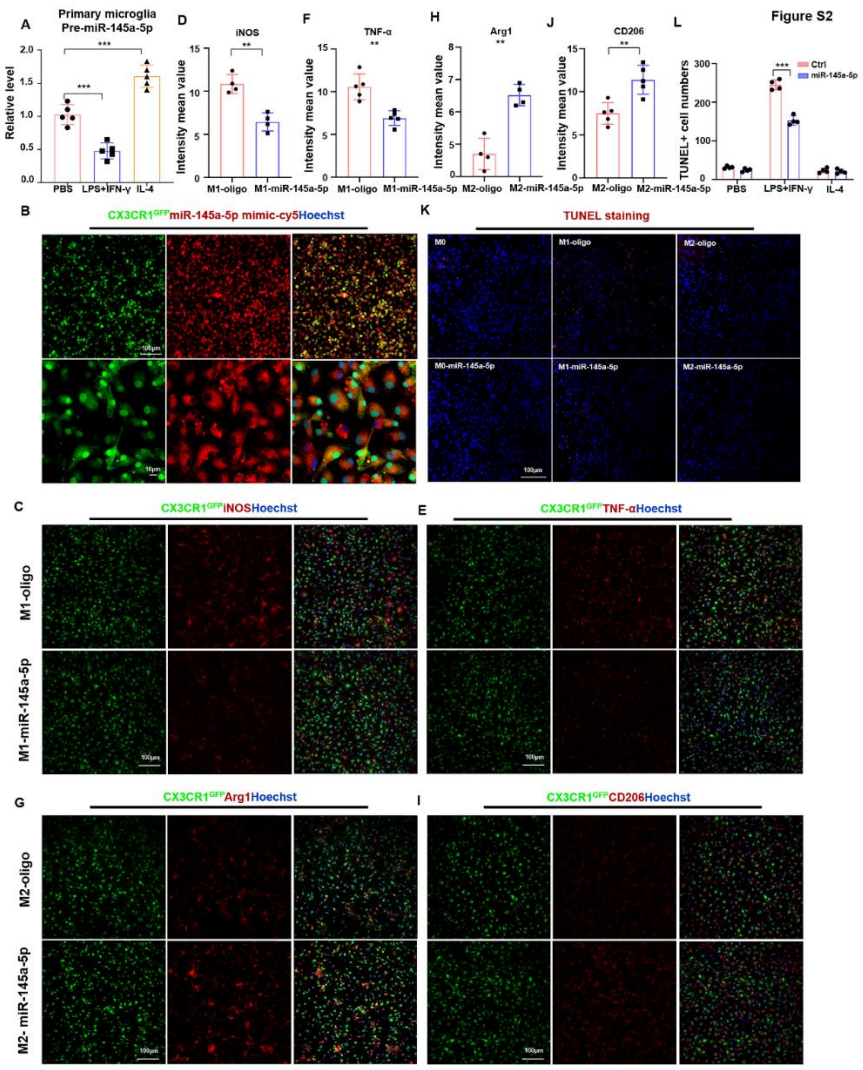

Figure S2. miR-145a-5p promoted M2 while inhibited M1 microglia polarization

(A) The pre-miR-145a-5p level was detected by qRT-PCR in different polarized primary microglia (n=4). (B) Representative images of CX3CR1$^{GFP/+}$ primary microglia that was transfected with miR-145a-5p mimic-Cy5 for 24 hours. Nucleic was co-stained with Hoechst (n=4). (C-J) CX3CR1$^{GFP/+}$ microglia were transfected with miR-145a-5p mimics or Ctrl and stimulated with PBS, LPS+IFN-γ or IL-4 for 24 hours, respectively. The expression of iNOS (C, D) (n=4), TNF-α (E, F) (n=5), Arg1 (G, H) (n=4) and CD206 (I, J) (n=5) were determined by immunofluorescent staining, and then were quantitatively compared. (K, L) TUNEL^{+} microglia with different treatment as shown in Figure 1C was counted and quantitatively compared (n=4). Scale bars=100 μm. Data are shown as the mean±SEM. **, $p<0.01$, ***, $p<0.001$ by unpaired student's t-test.

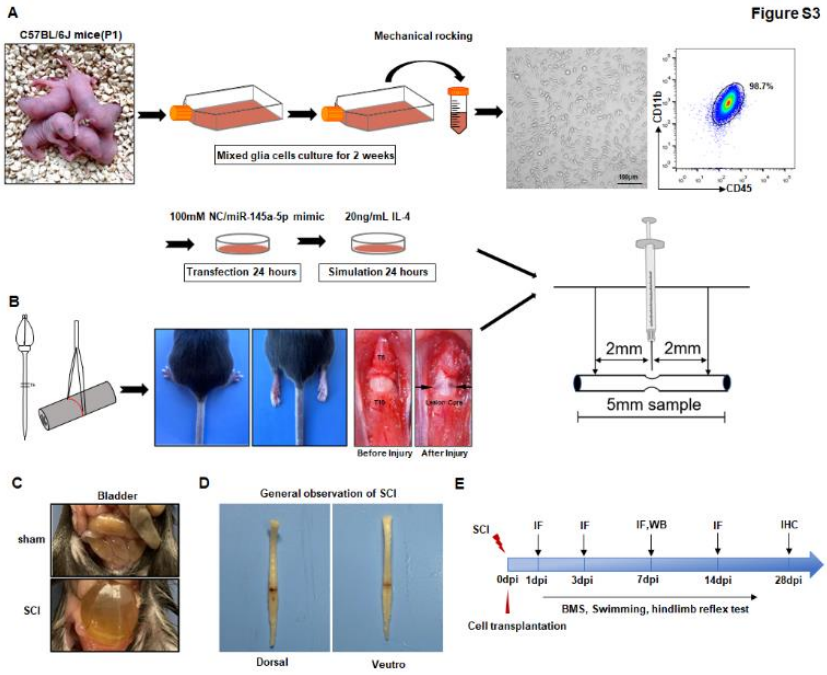

Figure S3. The procedure of murine SCI model establishment and microglia transplantation

(A) The scheme showed the procedure of primary microglia preparation from neonatal mice (postnatal day 1) and the purity detection of acquired microglia with anti-CD45 and anti-CD11b antibody staining by FACS. (B) Schematic illustration of murine SCI model establishment (left panel). Then, the hindlimb phenotype of SCI mice was observed (middle panel). miR-145a-5p overexpressed M2 microglia or M2 microglia was injected into the injured spinal cord site of mice (right panel). (C) Representative images of mice's bladder on the 1st day after surgery. (D) Representative images of dorsal and ventral of injured spinal cord. (E) The schematic experimental strategies after modified microglia transplantation at different time points.

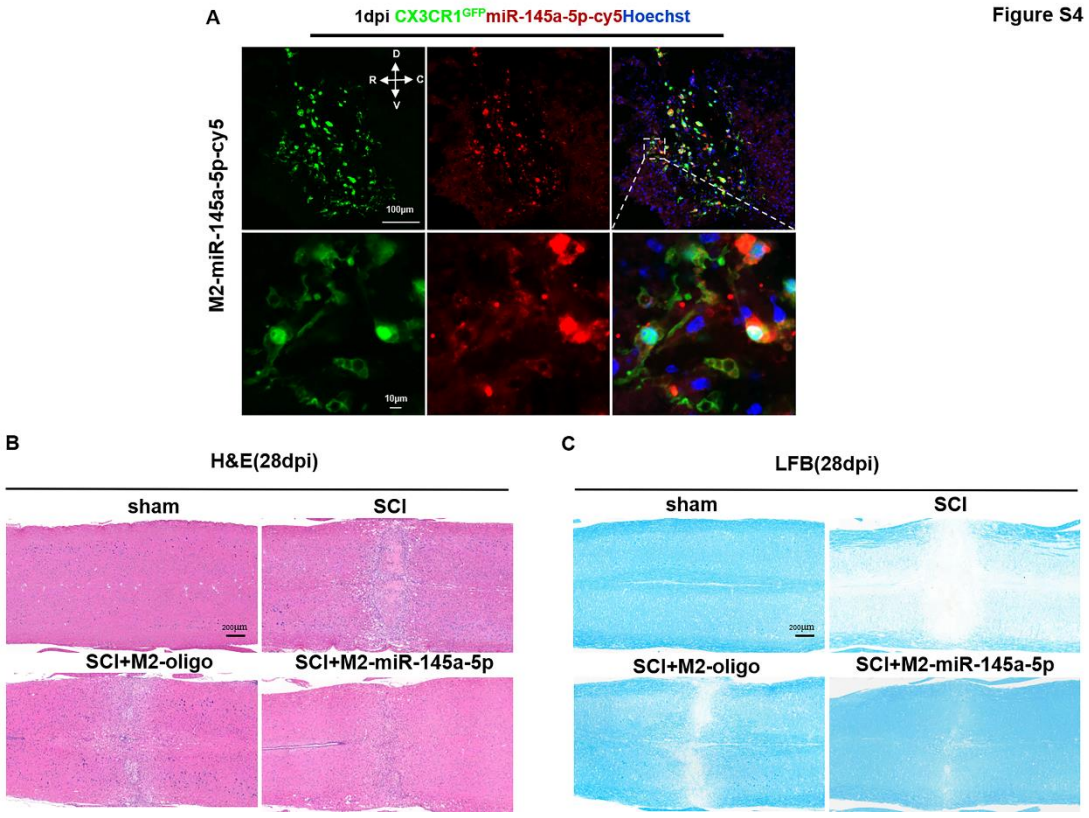

Figure S4. Histopathology evaluation on injured spinal cord after modified microglia transplantation

(A) CX3CR1$^{GFP/+}$ microglia were transfected with cy5-labeled miR-145a-5p mimics and induced M2 microglia polarization with IL-4 stimulation for 24 hrs, and then 1.5×10^5 modified M2 microglia were delivered into SCI mice. After 1 day, the location of transplanted cells was observed under microscope. (B) Representative images of HE staining on longitudinal section from sham, SCI, M2 microglia treated SCI and miR-145a-5p overexpressed microglia treated SCI group after 28 days (). Scale bars=200 μm. (C) Representative images of LFB staining on longitudinal section from sham, SCI, M2 microglia treated SCI and miR-145a-5p overexpressed microglia treated SCI group after 28 days. Scale bars=200 μm.

Tables

Table S1. Primers and oligonucleotides used for PCR in this study

Name	Purpose	Sequence
β-actin-F	RT-qPCR	CAT CCG TAA AGA CCT CTA TGC CAA C
β-actin-R	RT-qPCR	ATG GAG CCA CCG ATC CAC A
TNF-α-F	M1 marker	CAG GAG GGA GAA CAG AAA CTC CA
TNF-α-R	M1 marker	CCT GGT TGG CTG CTT GCT T
IL-6-F	M1 marker	CCA CTT CAC AAG TCG GAG GCT TA
IL-6-R	M1 marker	GCA AGT GCA TCA TCG TTG TTC ATA C
IL-1β-F	M1 marker	TCC AGG ATG AGG ACA TGA GCA C
IL-1β-R	M1 marker	GAA CGT CAC ACA CCA GCA GGT TA
iNOS-F	M1 marker	GCA GAG ATT GGA GGC CTT GTG
iNOS-R	M1 marker	GGG TTG TTG CTG AAC TTC CAG TC
Arg1-F	M2 marker	AGA CAG CAG AGG AGG TGA AGA G
Arg1-R	M2 marker	CGA AGC AAG CCA AGG TTA AAG C
Mcr1-F	M2 marker	AAA CAC AGA CTG ACC CTT CCC
Mcr1-R	M2 marker	GTT AGT GTA CCG CAC CCT CC
YM1-F	M2 marker	CAT TCA GTC AGT TAT CAG ATT CC
YM1-R	M2 marker	AGT GAG TAG CAG CCT TGG
IL-10-F	M2 marker	CCC TTT GCT ATG GTG TCC TT
IL-10-R	M2 marker	TGG TTT CTC TTC CCA AGA CC
CX3CR1-Mutant-F	PCR	GTC TTC ACG TTC GGT CTG GT
CX3CR1-Wild type-F	PCR	CTC CCC CTG AAC CTG AAA C
CX3CR1-Common-R	PCR	CCC AGA CAC TCG TTG TCC TT
miR-145a-5p	qPCR	GUC CAG UUU UCC CAG GAA UCC CU
TLR4-mus-1567-sense		CCUCCAUAGACUUCAAUUATT
TLR4-mus-1567-antisense		UAAUUGAAGUCUAUGGAGGTT

*F, forward; R, reverse

Table S2. Antibodies and related reagents used in this study

Name	Supplier	Catalog #	Titration
β-actin	Cell Signaling Technology	4970	1:1000
iNOS	Abcam	Ab49999	1:500
Arg1	Abcam	Ab60176	1:500
TNF-α	Cell Signaling Technology	11948	1:1000
IL-6	Cell Signaling Technology	12912	1:1000
IL-1β	Cell Signaling Technology	31202	1:1000
Iba1	WAKO	019-19741	1:500
CS56	Sigma	C8035	1:200
GFAP	Abcam	Ab53554	1:500
Cleaved-caspase-3	Cell Signaling Technology	9664s	1:1000
TLR4	Abcam	Ab47093	1:400
NF-κB/p65	Cell Signaling Technology	8264	1:1000
P- NF-κB/P-p65	Cell Signaling Technology	3033s	1:1000