Supplementary Figure 1

A  

| Vehicle  | NPC-Astros | Olig2PC-Astros |
|----------|------------|----------------|
| ![Image](image1.png) | ![Image](image2.png) | ![Image](image3.png) |

B  

![Graph](graph1.png)

C  

![Graph](graph2.png)

D  

![Graph](graph3.png)

E  

![Graph](graph4.png)

F  

![Graph](graph5.png)
Figure S1: Microglia at rostral or caudal 1 mm showed no difference in the number and morphology.

(A) Confocal images of microglia in spinal cord sections taken both rostral (R) and caudal (C) 1 mm to the lesion epicenter. Scale bars: 100 μm. (B) Quantification of microglia branch in spinal cord sections taken both rostral (R) and caudal (C) 1 mm to the lesion epicenter 4 weeks after astroglia transplantation (n = 5 per group). One-way ANOVA followed by Tukey’s test. (C) Quantification of total number of Iba1+ microglia per mm² of tissue in spinal cord sections taken both rostral (R) and caudal (C) to the lesion epicenter at 4 weeks after astroglia transplantation (n = 5 per group). One-way ANOVA followed by Tukey’s multiple comparisons test. (D) Quantification of the total number of Iba1+ microglia per mm² of tissue in spinal cord sections taken both rostral (R) and caudal (C) to the lesion epicenter at 3-56 days after injury (n = 5 per group) (E) Quantification of the total number of Iba1+ microglia per mm² of tissue in spinal cord sections taken both rostral (R) and caudal (C) to the lesion epicenter at 3-56 days after NPC-Astro transplantation (n = 5 per group). (F) Quantification of the total number of Iba1+ microglia per mm² of tissue in spinal cord sections taken both rostral (R) and caudal (C) to the lesion epicenter at 3-56 days after Olig2PC-Astro transplantation (n = 5 per group). Two-way ANOVA followed by Tukey’s multiple comparisons test. Data are mean ± SD; n.s., not significant.
Figure S2: Grafted astroglia did not promote the increase of microglia. (A-C) Representative images showing GFAP (red) and Iba1 (green) labeling in spinal cord sagittal sections at 4 weeks post-transplantation. A1, B1, C1 are higher magnification images of boxed areas in A-C. Scale bars: 200 μm (A-C), 20 μm (A1-C1). (D) Quantification of total Iba1⁺ microglia (n = 5 per group). (E) Quantification of Iba1⁺ microglia in the lesion area (n = 5 per group). (F) Western blotting for Iba1 expression in the lesion area at 3 to 56 days after transplantation. (G) Quantification of Iba1 expression at 3 to 56 days (n = 3 per group). D, E, G: Two-way ANOVA followed by Tukey’s multiple comparisons test. Data are mean ± SD; n.s., not significant.
Supplementary Figure 3

A, A', A'' - ki67/iba1
B, B', B'' - Vehicle
C, C', C'' - NPC-Astro
D, D', D'' - Olg/PC-Astro
E - 28 days post injury
F - 28 days post injury
G - 3 days post injury
H - 3 days post injury
I - 7 days post injury
J - 7 days post injury
K - 14 days post injury
L - 14 days post injury
M - 56 days post injury
N - 56 days post injury
Figure S3: Grafted astroglia did not promote microglia proliferation.

(A-D) Representative images showing ki67 (red) and Iba1 (green) staining in the lesion center at 4 weeks post-transplantation. Scale bars: 20 μm. (E) Quantification of the number of actively proliferating microglia (Iba1⁺ Ki67⁺ cells) at 28 days after astroglia transplantation (n = 5 per group). (F) Percentage of Iba1⁺ microglia undergoing proliferation 28 days after astroglia transplantation (n = 5 per group). (G, I, K, M) Quantification of the number of actively proliferating microglia (Iba1⁺ Ki67⁺ cells) at 3, 7, 14, 56 days after astroglia transplantation (n = 5 per group). (H, J, L, N) Percentage of Iba1⁺ microglia undergoing proliferation 3, 7, 14, 56 days after astroglia transplantation (n = 5 per group). Two-way ANOVA followed by Tukey’s multiple comparisons test. Data are mean ± SD; n.s., not significant.
Supplementary Figure 4

A1

D

Vehicle

NPC-Astro

Olig2PC-Astro

E

F1

G1

H1

I

J

Vehicle

NPC-Astro

Olig2PC-Astro

**n.s.**

* n.s.

** * n.s.

*** n.s.

** *** n.s.

* * n.s.

*** ** n.s.

* *** n.s.
Figure S4: Grafted astroglia promote anti-inflammatory polarization of microglia.

(A-C) Representative images showing Iba1 (green) and iNOS (red) labeling in spinal cord sagittal sections at 4 weeks post-transplantation. Scale bars: 200 μm (A-C), 20 μm (A1-C1). (D) Quantification of total iNOS+ microglia (n = 5 per group). (E) Quantification of iNOS+ microglia in the lesion area (n = 5 per group). (F-H) Representative images showing Iba1 (green) and Arg1 (red) labeling in spinal cord sagittal sections at 4 weeks post-transplantation. Scale bars: 200 μm (F-H), 20 μm (F1-H1). (I) Quantification of total iNOS+ microglia (n = 5 per group). (J) Quantification of iNOS+ microglia in the lesion area (n = 5 per group). D, E, I, J: Two-way ANOVA followed by Tukey’s multiple comparisons test. Data are mean ± SD; *p < 0.05, **p < 0.01, ***p < 0.001, n.s., not significant.