

Supplement Figures

Figure.S1

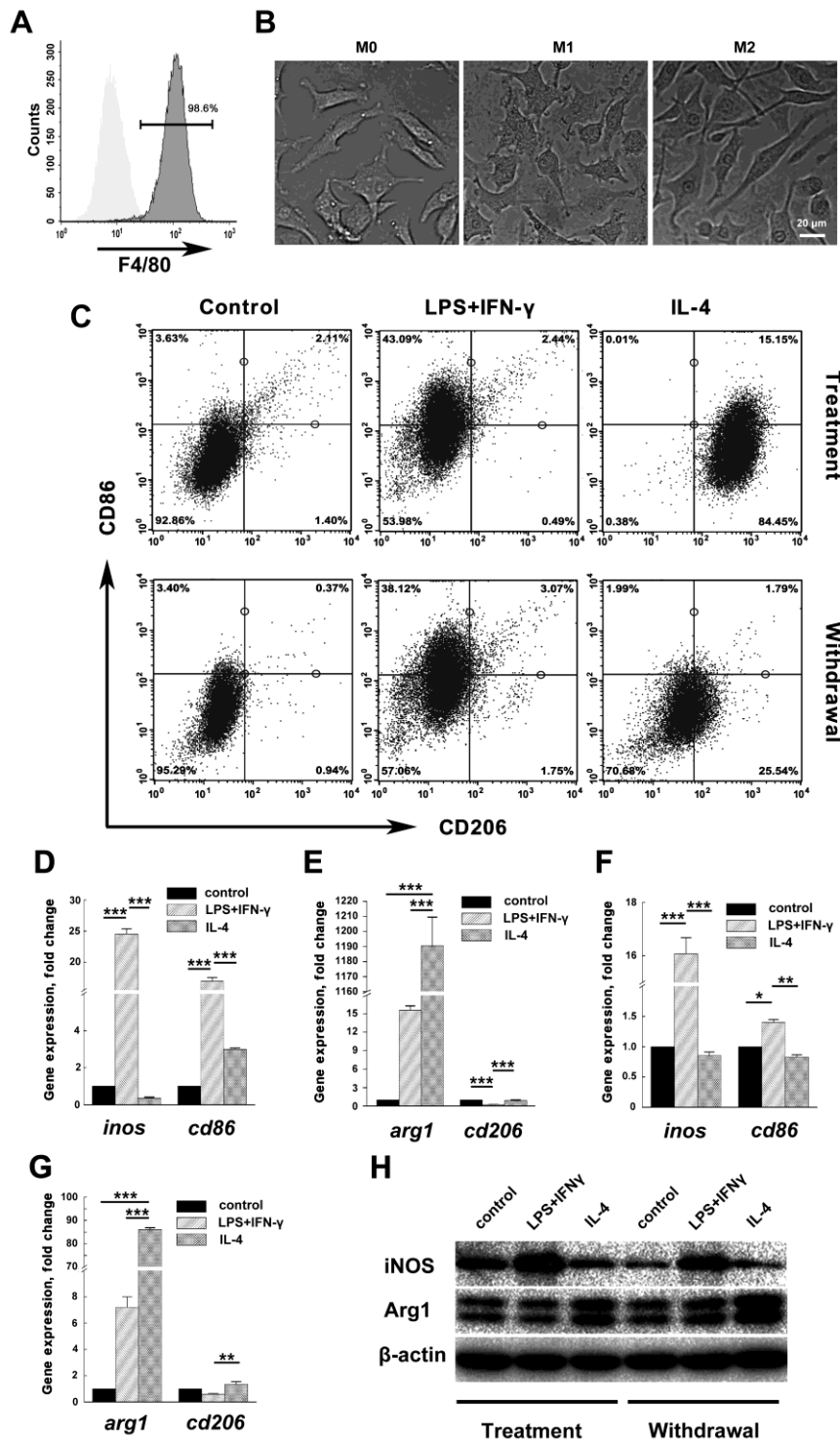


Fig. S1. Polarization states of BMDM-derived macrophages *in vitro*. (A)

Following induction of BMDMs from the bone marrow, approximately 99% of the

cells were F4/80-positive. **(B)** The morphologies of M0, M1 and M2 macrophages are shown under phase contrast microscopy. Space bar = 20 μ m. **(C)** Flow cytometry analysis of CD86/CD206 expression in macrophage cells with or withdrawal the LPS plus IFN- γ or IL-4. **(D–E)** The mRNA expression levels of **(D)** *inos* and *cd86* and **(E)** *arg1* and *cd206* are shown in the polarized macrophages after 24 h in culture with LPS plus IFN- γ or IL-4. **(F–G)** The mRNA expression levels of **(F)** *inos* and *cd86* and **(G)** *arg1* and *cd206* are shown in the polarized macrophages at 24 h in culture after the withdrawal of LPS plus IFN- γ or IL-4. **(H)** The protein expression levels of iNOS and Arg1 are shown under polarizing conditions (LPS plus IFN- γ or IL-4 stimulation) during the first 24 h of culture, followed by withdrawal of the polarizing stimuli for another 24 h in culture. Data in (A), (B),(C) and (H) are representative of three independent experiments; data in (D–G) were pooled from three independent experiments (* p < 0.05, ** p < 0.01, *** p < 0.001).

Figure.S2

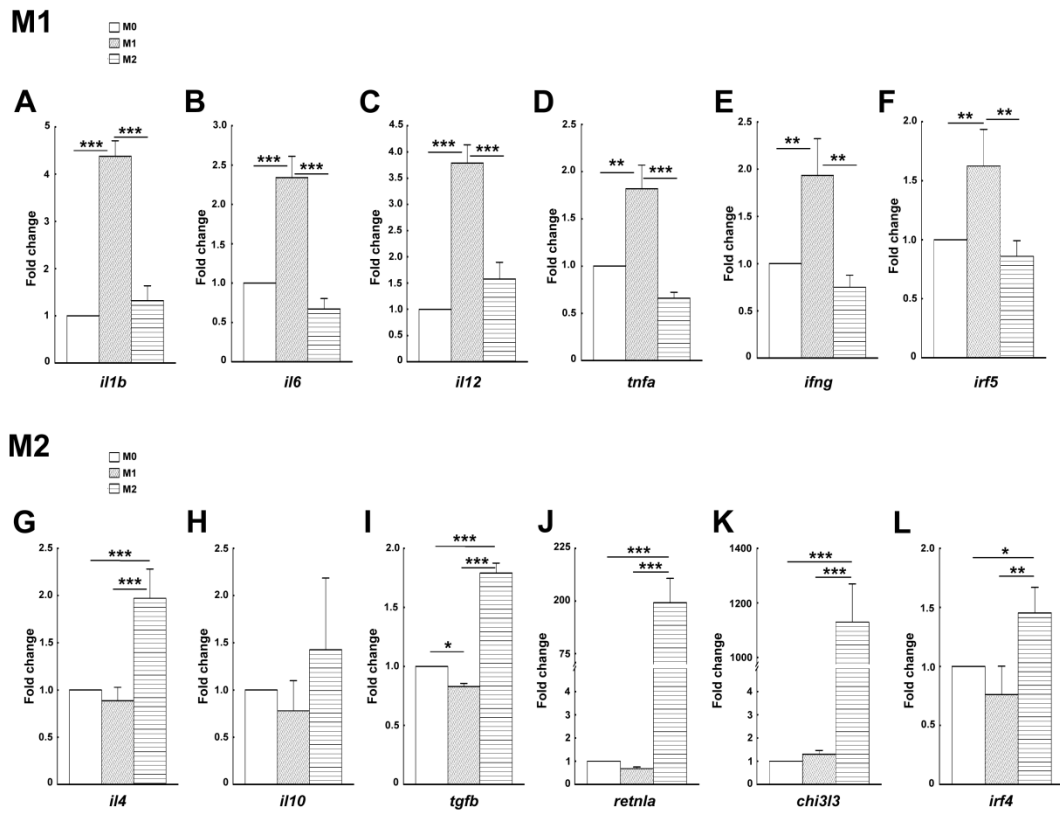


Fig. S2. Gene-specific expression patterns in M1 and M2 macrophages. (A–F) show the mRNA expression levels of M1-specific markers, while (G–L) show the mRNA expression levels of M2-specific markers (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

Figure.S3

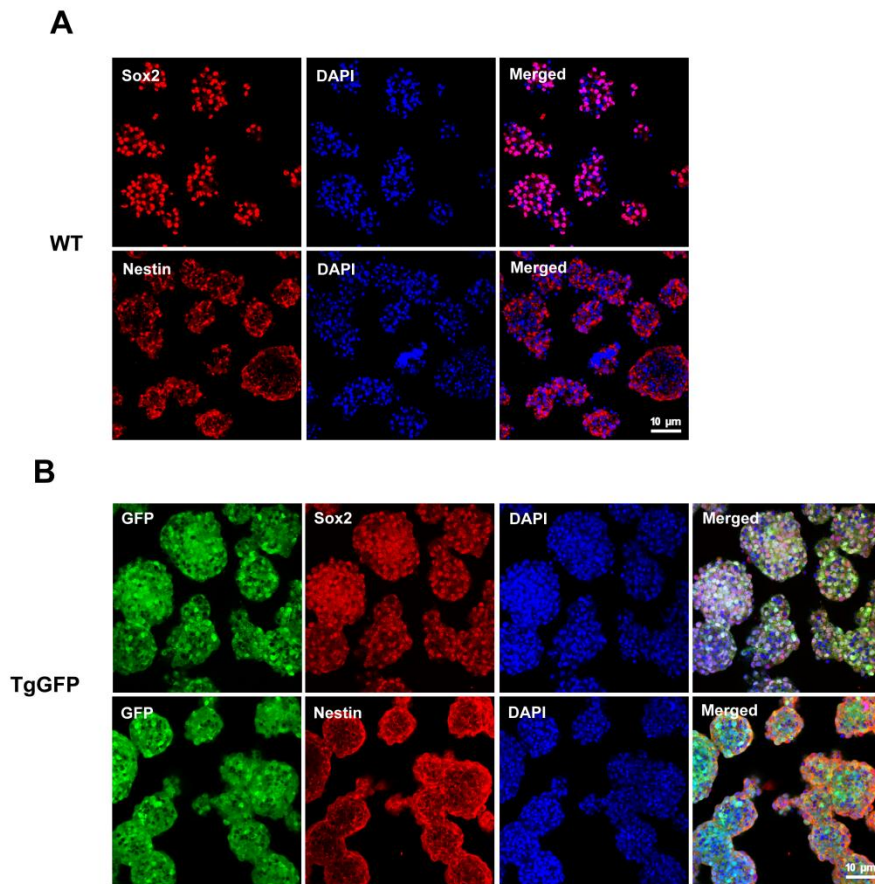


Fig. S3. Confirmation of NS/PC identity. (**A and B**) NS/PCs isolated at E14 from wild-type (WT) B6 or TgGFP/B6 mice expressed the NS/PC-specific markers, Sox2 and nestin.

Figure.S4

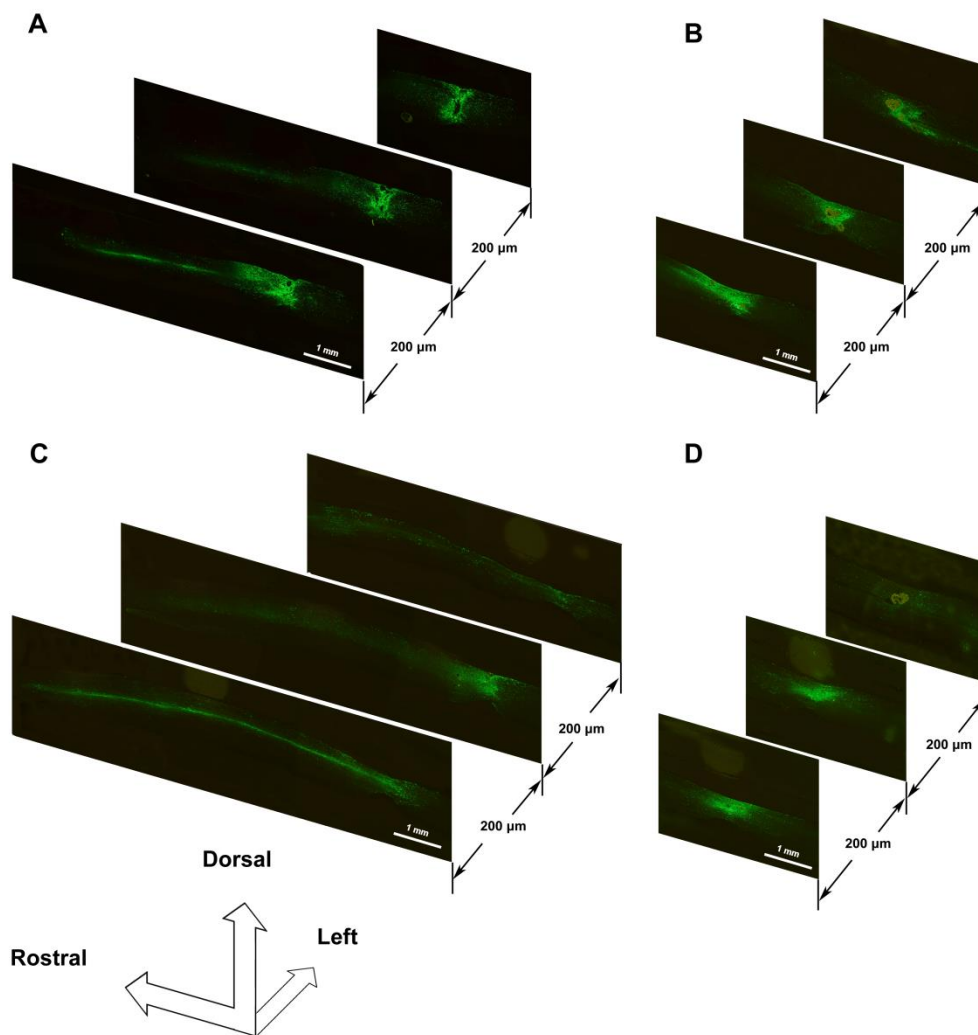


Fig. S4. Migratory patterns of engrafted NS/PC-derived cells after injection of NS/PCs with and without co-transplanted macrophages into the injured spinal cord. Serial sections display the migration stream of NS/PC-derived GFP-positive cells transplanted alone (A) or co-transplanted with M0 (B), M1 (C) or M2 (D) cells. Adjacent sections were taken at 200-µm intervals.

Figure.S5

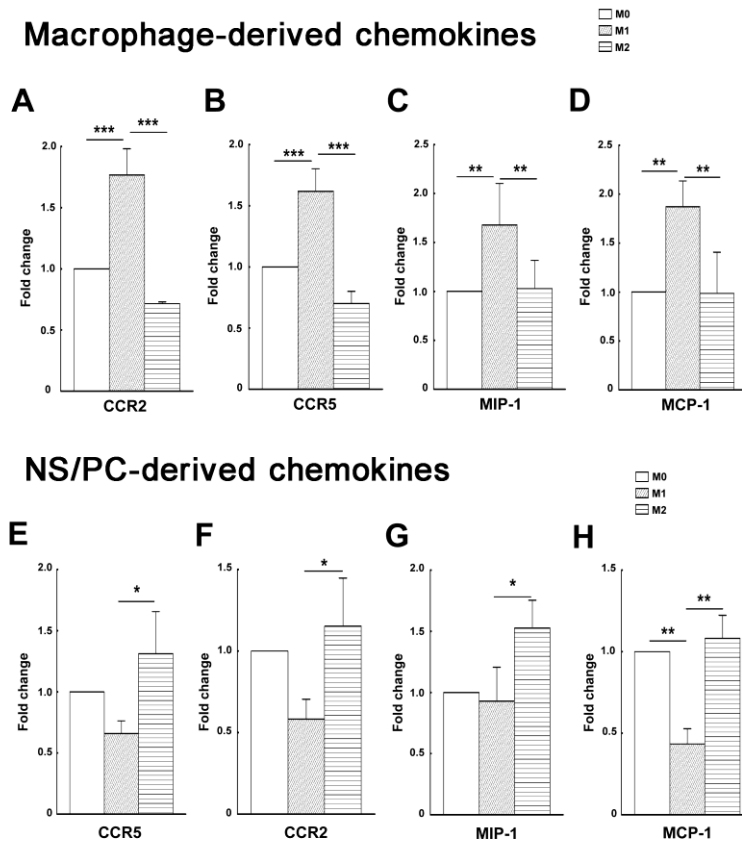


Fig. S5. Chemokine expression patterns in macrophages and NS/PCs. The expression patterns are shown for macrophages (A–D) and NS/PCs simulated with CM derived from M0, M1, or M2 macrophages (E–H) (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

Figure.S6

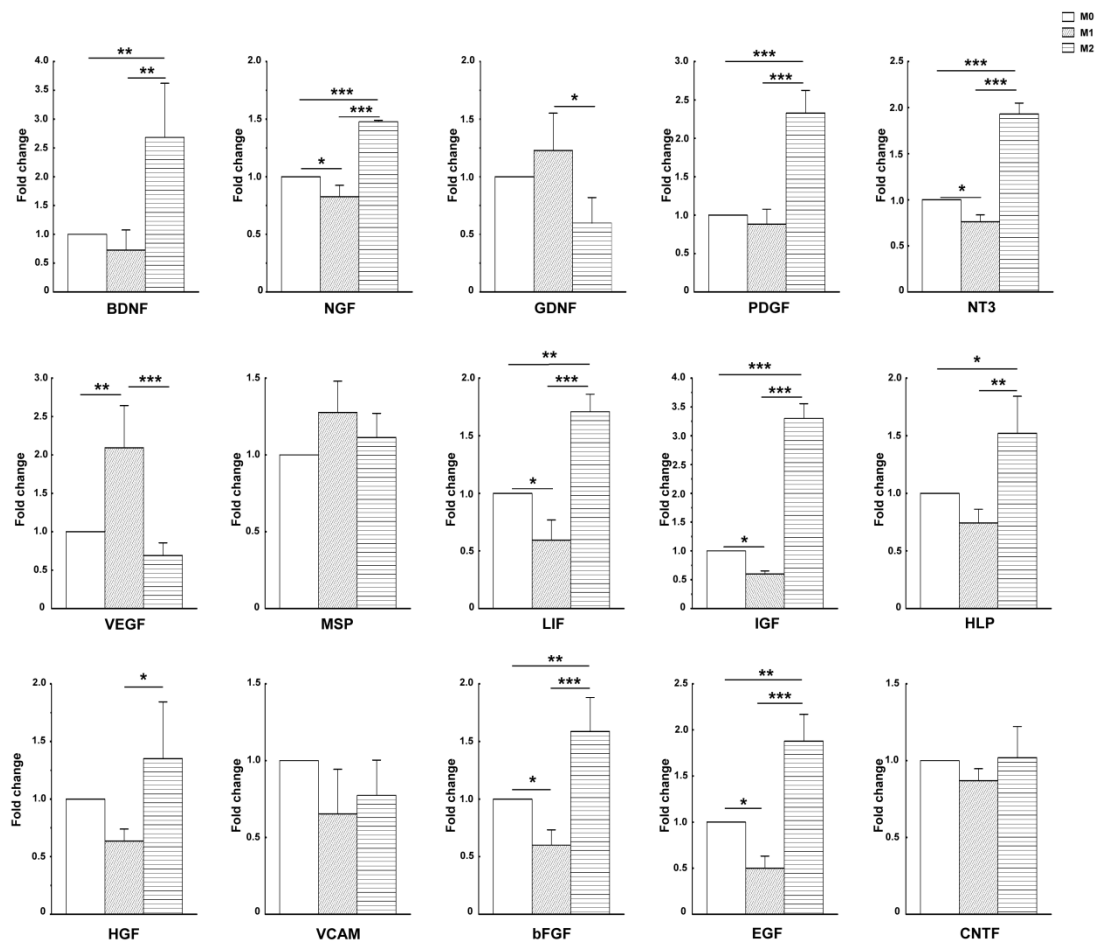


Fig. S6. Expression patterns of growth factors and VCAM in cultured macrophages (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

Figure.S7

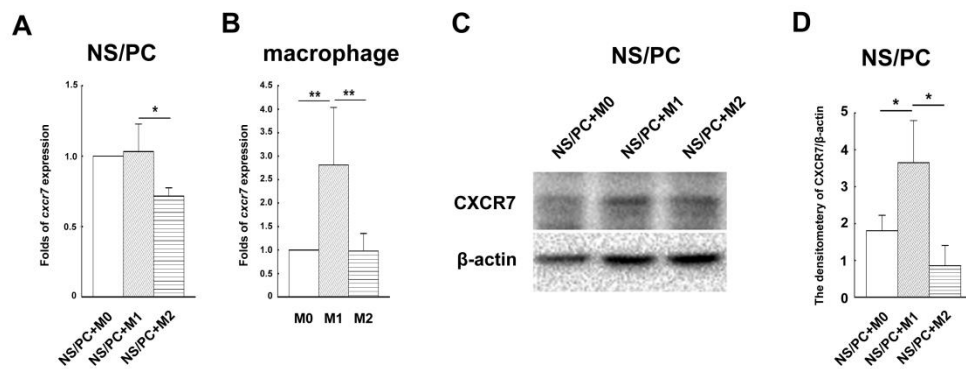


Fig. S7. In vitro expression patterns of CXCR7 in NS/PCs and macrophages. (A and B) The mRNA expression levels of CXCR7 are shown in (A) NS/PCs stimulated with M0-, M1- or M2-CM and (B) M0, M1 and M2 macrophages. **(C and D)** The protein expression levels of CXCR7 are shown in NS/PCs stimulated with M0-, M1- or M2-CM by (C) Western blotting and (D) quantitative densitometric analysis. Data in (A–D) were pooled from three independent experiments (* $p < 0.05$, ** $p < 0.01$).

Figure.S8

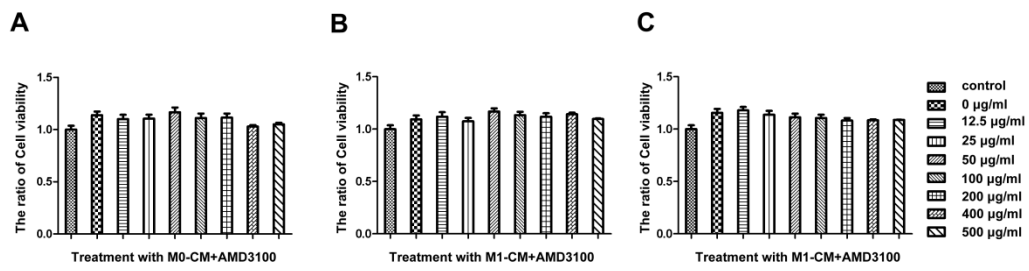


Fig.S8. Cytotoxicity analysis of AMD3100. A dose-dependent assessment was conducted for NS/PCs treated with M0-, M1- or M2-CM and AMD3100. No cytotoxicity was observed.

Figure.S9

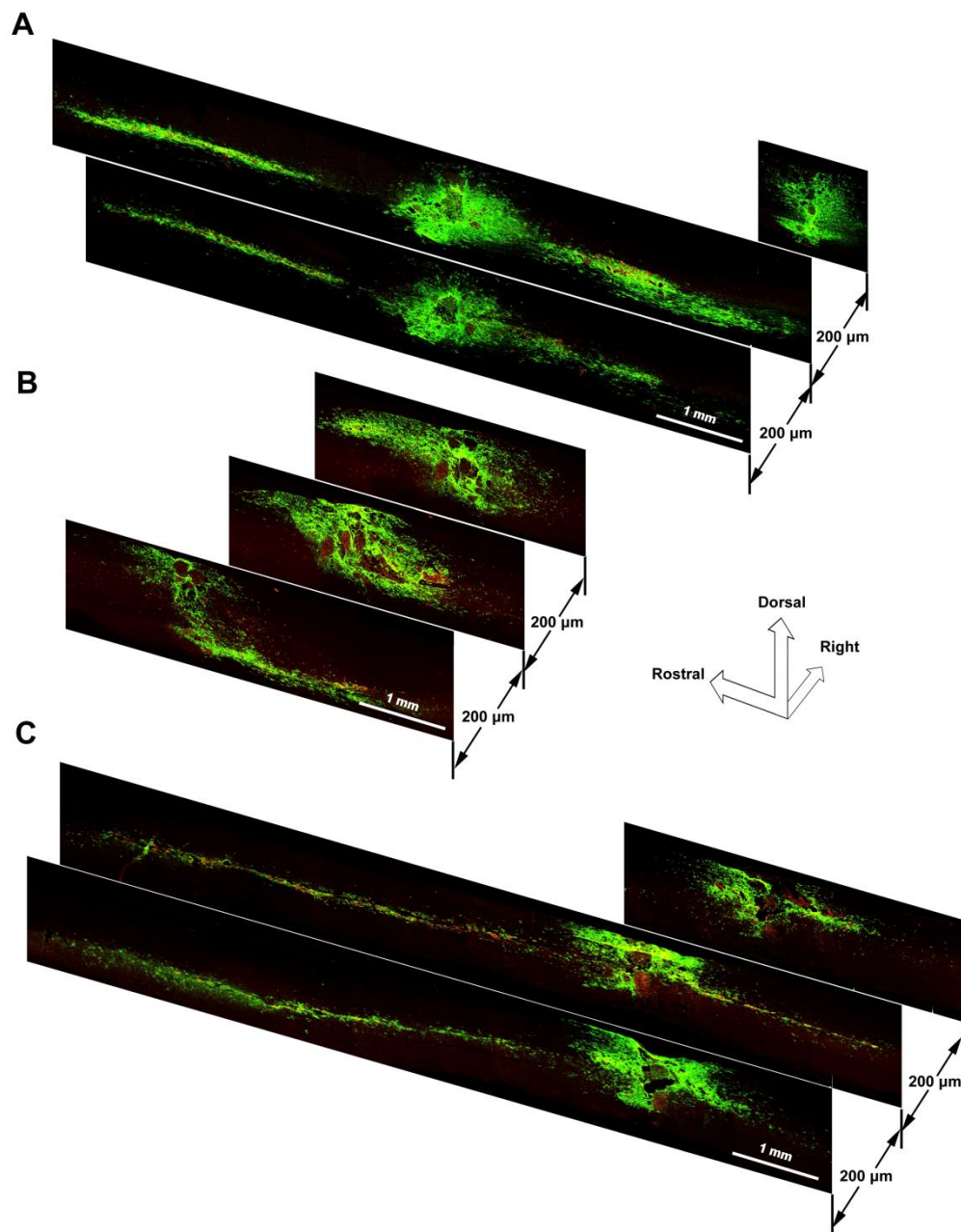


Fig.S9. Migratory patterns of engrafted NS/PC-derived cells after co-transplantation of AMD3100-treated NS/PCs and macrophages. Serial sections display the migratory patterns of NS/PC-derived GFP-positive cells co-transplanted with M0 (A), M1 (B) or M2 (C) macrophages. Adjacent sections were taken at 200-μm intervals.

Table S1. Antibodies for IHC

Antibodies	Host	Vendor	Dilution
iNOS	Rabbit	abcam	1:200
Arginase 1	Goat	Santa Cruz Biotechnology	1:200
GFAP	Rabbit	DakoCytomation	1:500
Oligo2	Rabbit	abcam	1:1000
β -tubulinIII	Mouse	R &D Systems	1:500
NeuN	Mouse	Millipore	1:500
MBP	Rat	Millipore	1:500
Synapsin-1	Rabbit	abcam	1:500
CXCR4	Goat	abcam	1:200
CXCR7	Goat	Santa Cruz Biotechnology	1:200
CXCL12	Rabbit	abcam	1:500
F4/80	Rat	AbD Serotec	1:100
Iba1	Rabbit	Wako	1:1000
Nestin	Rabbit	abcam	1:500
Sox2	Rabbit	Millipore	1:500

Table S2. The primer sequences for QRT-PCR analysis

Gene	Forward primer(5'- 3')	Reverse primer(5'- 3')
<i>bactin</i>	AGAAGGACTCCTATGTGGGTGA	CATGAGCTGGGTCATCTTTTCA
<i>gfap</i>	TCTCGAATGACTCCTCCACTC	CCAGACCGAACACTGTCCA
<i>btubulinIII</i>	CCCAGCGGCAACTATGTAGG	AAGCTCCGCCTGGTAGACAT
<i>pdgfra</i>	GGAGACTCAAGTAACCTTGCCAC	TCAGTTCTGACGTTGCTTTCAA
<i>il1b</i>	CTTCAGGCAGGCAGTATCAC	CCAGCAGGTTATCATCATCATCC
<i>il6</i>	TAGTCCTTCTACCCCAATTTCC	TTGGTCCTTAGCCACTCCTTC
<i>il4</i>	GGTCTCAACCCCCAGCTAGT	GCCGATGATCTCTCTCAAGTGAT
<i>il10</i>	GCTCTTACTGACTGGCATGAG	CGCAGCTCTAGGAGCATGTG
<i>mip1</i>	ACTGTTACTCTTGGCATCATCA	CCTTCTTCTCACTGGGTCTTC
<i>ccr5</i>	TCCCTGGTATTTCATCTTTGGTT	CAATGTGATAGAGCCCTGTGA
<i>mcp1</i>	CTCTCTTCTCCACCACCAT	GCTCTCCAGCCTACTCATTG
<i>ccr2</i>	GCTGTGTTTGCCTCTCTACC	CTGTGCCTCTTCTTCTCATTCC
<i>cxcr4</i>	CTACAGCAGCGTTCTCATCC	TTTCAGCCAGCAGTTTCTT
<i>cxcl12</i>	CTGTAGCCTGACGGACCAAT	GAGCCCATAGAGCCACTGTT
<i>cxcr7</i>	CCGAGCACAGCATCAAGGA	GCAGCCAACATAACCAGGAAG
<i>bdnf</i>	TTCACAGGAGACATCAGCAAT	ACAAGAGACCACAGCAAGAC
<i>nt3</i>	GCCCCCTCCCTTATACCTAATG	CATAGCGTTTCTCCGTGGT
<i>gdnf</i>	GCCACCATTAAGACTGAAAAGG	GCCTGCCGATTCTCTCTCT
<i>igf1</i>	GATACACATCATGTGCTTTCACA	CAGTACATCTCCAGTCTCCTCAGA
<i>pdgfa</i>	TGTAACACCAGCAGCGTCAAG	CTGGACCTCTTTCAATTTGGC
<i>vcam1</i>	ATTATGCCGTGCGGAGGTT	CAGTCCAAGCAACTCTCTGATT
<i>vegf</i>	CATCTTCAAGCCGTCCTGTGT	CTCCAG-GGCTTCATCGTTACA
<i>bfgf</i>	CCCAC-CAGGCCACTTCAA	GATGGATGCGCAGGAAGAA
<i>lif</i>	TGCTCTTTCATTTCTATTACAC	AACTTGGTCTTCTCTGTCC
<i>hgf</i>	TCACACAGAATCAGGCAAGACT	AAGGGGTGTCAGGGTCAAG
<i>msp</i>	AGTTAAGGAACCTGTTACAC	ACCATGGCTGCTCATGTTGT
<i>ngf</i>	TGGGCTTACAGGACAGAGTC	CAGCTTCTATACTGGCCGAG
<i>cntf</i>	AGGCAGAGCGACTCCAAGA	GGTAGGCGAAGGCAGAAACT
<i>egf</i>	TTGCCTGGTTGTGCCTGT	GCTGTGACGCTGAGTATGC
<i>hlp</i>	ACACCTGCCACCAGTATT	AGTCTCCATAGCCATGAACCA
<i>retnla</i>	CCAATCCAGCTAACTATCCCTCC	ACCCAGTAGCAGTCATCCCA
<i>chi3l3</i>	CAGGTCTGGCAATTCTTCTGAA	GTCTTGCTCATGTGTGTAAGTGA
<i>irf4</i>	TCCGACAGTGGTTGATCGAC	CCTCACGATTGTAGTCCTGCTT
<i>irf5</i>	GGTCAACGGGGAAAAGAAACT	CATCCACCCCTTCAAGTGTACT
<i>stat1</i>	TCACAGTGGTTTCGAGCTTCAAG	GCAAACGAGACATCATAGGCA
<i>stat6</i>	CTCTGTGGGGCCTAATTTCCA	CATCTGAACCGACCAGGAACT
<i>cd86</i>	TTGTGTGTGTTCTGGAAACGGAG	AACTTAGAGGCTGTGTTGCTGGG
<i>inos</i>	CCCTTCAATGGTTGGTACATGG	ACATTGATCTCCGTGACAGCC
<i>arginase1</i>	GAACACGGCAGTGGCTTTAAC	TGCTTAGCTCTGTCTGCTTTGC
<i>cd206</i>	TCTTTGCCTTTCCAGTCTCC	TGACACCCAGCGGAATTTCC

Materials and Methods

Flow cytometry analysis of surface antigens: BMDMs were incubated in blocking solution (rat serum, 20 min at 4°C) and were subsequently stained with FITC-conjugated anti- F4/80 , APC-conjugated anti- CD86 and PE-conjugated anti- CD206 in the dark for 20 min at 4°C. Acquisitions were performed on a Millipore flow cytometer (Guava 6HT). Subsequent data analyses were completed using FlowJo software version 7.6.2 (Tree Star, Ashland, OR, USA). Results are expressed as % of positive cells.

MTT assay: The cytotoxic effect of AMD3100 in NS/PCs was evaluated by the MTT assay . Briefly, NS/PCs grown in 200 µL M-CM containing different concentrations (0, 12.5, 25, 50, 100, 200, 400 and 500 µg/mL) of AMD3100 (Sigma) were seeded in 96-well plates and incubated for 24 hrs. Then 20 µL of MTT solution (5 mg/mL in PBS; Sigma) was added to each well and incubated for 4 h at 37 °C. The supernatant was then discarded, and 150 µL dimethyl sulfoxide (DMSO; Guanghua Co., Ltd) was added. Absorbance was read at 490 nm using an automated enzyme immunoassay instrument (Bio-Rad, Hercules, CA, USA).