Amelioration of experimental autoimmune encephalomyelitis through transplantation of placental derived mesenchymal stem cells

Hong Jiang[1], Yuanyuan Zhang[2#], Kewei Tian[2#], Beibei Wang[3], Shu Han[2*]

[1] Department of Electrophysiology, Sir Run Run Shaw Hospital, Medical College, Zhejiang University, Hangzhou, China

[2] Institute of Anatomy and Cell Biology, Medical College, Zhejiang University, Hangzhou, China

[3] Core Facilities, Zhejiang University School of Medicine, Hangzhou, China

[#]These authors contributed equally to this work.

***Correspondence to**: Dr. Shu Han

Institute of Anatomy and Cell Biology, Medical College, Zhejiang University, 866 Yuhangtang Road, Hangzhou 310058, China. **Tel.:** +86-571-88208160; **Fax:** +86-571-88208094; **Email:** han00shu@zju.edu.cn.

Supplemental figure legends

Figure S1. Differentiation of PMSCs cultured in vitro. PMSCs from passage 3 exhibits morphological characterization of different neural cell lines. (A-D) Astrocytes-like cells differentiate from PMECs. (E-H) Oligodendrocytes-like cells differentiate from PMECs. (I-L) Microcyte-like cells differentiate from PMECs. (M-P) Neuron-like cells differentiate from PMECs.

Figure S2. Both EMSCs and PMECs treatment reverse electrophysiological dysfunction (A-B): EMSCs and PMSCs treatments reduce the clinical severity of EAE in rats at 3 (A) and 8 (B) weeks post-injection, as measured by determining somatosensory-evoked potential (c-SEP) latencies and amplitudes (measured from peak to peak between negative deflection (N) and positive deflection (P). The amplitude of c-SEP is notably lower in vehicle-treated EAE rats and the latency is also significantly prolonged, while treatments with EMSCs and PMSCs effectively reversed these phenomena. (C-D): Motor-evoked potential (MEP) amplitude was also significantly lower in vehicle-treated EAE rats and latency is significantly prolonged at 3 (C) and 8 (D) weeks post-injection. Similarly, these phenomena are reversed following EMSCs and PMSCs treatments.

Figure S3. Compared with the vehicle-treated group, EMSCs and PMSCs treatments effectively reduce the expression of pro-inflammatory factors NF-kB (A-C), TNF-α(**D-F**), COX-2(G-I), but maintained the expression of oligodendrocyte marker MBP (**J-L**) as detected by Western blotting. Data are represented as mean ± SD. n=5, degrees of

freedom=n-1. **NFkB: ***P<0.05*Vs* Normal group (3w Vehicle group: F=0.07, P<0.0001; EMSCs group: F=0.04, P=0.009; 8w PI, Vehicle group: F=0.33,P<0.0001; EMSCs group: F=0.26, P=0.0002) **&** P<0.01 *Vs* Vehicle group (3w PI, EMSCs group: F=0.57,P=0.007; PMECs group: F=4.19,P<0.0001. 8w PI, EMSCs group: F=0.78,P<0.0001; PMECs group: F=0.35, P<0.0001). # P<0.05 *Vs* EMSCs group (3w PI, PMSCs group: F=0.13, P=0.01; 8w PI, PMSCs group: F=0.44, P=0.048). **TNF-α: ***P<0.05 *Vs* Normal group (3w Vehicle group: F=1.76, P<0.0001; EMSCs group: F=0.69, P<0.0001. PMECs group: F=0.65, P=0.0002; 8w PI, Vehicle group: F=0.03, P<0.0001; EMSCs group: F=0.1, P=0.0007. PMECs group: F=0.82, P=0.004) **&** P<0.01 *Vs* Vehicle group (3w PI, EMSCs group: F=0.39, P<0.0001; PMECs group: F=0.37, P<0.0001. 8w PI, EMSCs group: F=3.05, P<0.0001; PMECs group: F=26.23, P<0.0001). # P<0.01 *Vs* EMSCs group (8w PI, PMSCs group: F=0.11, P=0.0003). **COX-2**: *****P<0.05 *Vs* Normal group (3w Vehicle group: F=0.3, P<0.0001; EMSCs group: F=0.15, P=0.0001. PMECs group: F=0.27, P=0.0007; 8w PI, Vehicle group: F=0.05, P<0.0001; PMECs group: F=0.54, P<0.0001) **&** P<0.01 *Vs* Vehicle group (3w PI, EMSCs group: F=0.51, P<0.0001; PMECs group: F=0.89, P<0.0001. 8w PI, EMSCs group: F=1.56, P<0.0001; PMECs group: F=10.62, P<0.0001). # P<0.01 *Vs* EMSCs group (3w PI, PMSCs group: F=0.58, P<0.0001; 8w PI, PMSCs group: F=6.8, P=0.0009), **MBP:***P<0.05*Vs* Normal group (3w Vehicle group: F=0.4, P<0.0001; 8w PI, Vehicle group: F=1.25, P<0.0001; PMECs group: F=0.54, P<0.0001) **&** P<0.01 *Vs* Vehicle group (3w PI, EMSCs group: F=0.91, P<0.0001; PMECs group: F=0.53, P<0.0001. 8w PI, EMSCs group: F=0.07, P=0.0002; PMECs group: F=0.16, P<0.0001).

Figure S4. The expression of pro-inflammatory factors in response to EMSCs and PMSCs treatments. At 3 weeks post-injection, the expression of pro-inflammatory factors NF-kB (A–F) ***P<0.01 *Vs* Normal group (3w PI, in SP, Vehicle group: F=1.07, P<0.0001; EMSCs group: F=0.14, P<0.0001; PMECs group: F=0.23, P<0.0001. In BC, Vehicle group: F=157.26, P<0.0001; EMSCs group: F=286.87, P<0.0001; PMECs group: F=136.57, P<0.0001. 8w PI, in SP, Vehicle group: F=0.92, P<0.0001; EMSCs group: F=0.69, P<0.0001; PMECs group: F=0.34, P<0.0001. In BC, Vehicle group: F=0.08, P<0.0001; EMSCs group: F=0.12, P<0.0001; PMECs group: F=0.49, P<0.0001) **& P<0.01 *Vs* Vehicle group (3w PI, In SP, EMSCs group: F=0.13, P<0.0001; PMECs group: F=0.21, P<0.0001. In BC, EMSCs group: F=0.55, P<0.0001; PMECs group: F=1.15, P<0.0001. 8w PI, In SP, EMSCs group: F=0.37, P<0.0001; PMECs group: F=0.75, P<0.0001. In BC, EMSCs group: F=1.48, P<0.0001; PMECs group: F=6.16, P<0.0001), **COX-2 (G-L) ***P<0.01 *Vs* Normal group (3w PI, in SP, Vehicle group: F=0.05, P<0.0001; EMSCs group: F=0.28, P<0.0001; PMECs group: F=0.24, P<0.0001. In BC, Vehicle group: F=4.36, P<0.0001; EMSCs group: F=0.54, P<0.0001; PMECs group: F=0.7, P<0.0001. 8w PI, in SP, Vehicle group: F=0.22, P<0.0001; EMSCs group: F=0.15, P<0.0001; PMECs group: F=0.54, P<0.0001. In BC, Vehicle group: F=0.03, P<0.0001; EMSCs group: F=0.1, P<0.0001; PMECs group: F=0.06, P<0.0001) **&** P<0.01 *Vs* Vehicle group (3w PI, In SP, EMSCs group: F=0.17, P<0.0001; PMECs group: F=0.2, P<0.0001. In BC, EMSCs group: F=2.36, P<0.0001; PMECs group: F=3.07, P<0.0001. 8w PI, In SP, EMSCs group: F=1.45, P<0.0001; PMECs group: F=2.43, P<0.0001. In BC, EMSCs group: F=0.34, P<0.0001; PMECs group: F=0.55, P<0.0001) **and TNF-α(M–R) ***P<0.01 *Vs* Normal group (3w PI, in SP, Vehicle group: F=7.41, P<0.0001;

EMSCs group: F=7.89, P<0.0001; PMECs group: F=13.42, P=0.0007. In BC, Vehicle group: F=0.001, P<0.0001; EMSCs group: F=0.001, P<0.0001; PMECs group: F=0.0009, P<0.0001. 8w PI, in SP, Vehicle group: F=0.33, P<0.0001; EMSCs group: F=0.13, P<0.0001; PMECs group: F=0.48, P<0.0001. In BC, Vehicle group: F=7.08, P<0.0001; EMSCs group: F=10.51, P=0.0002; PMECs group: F=9.86, P=0.0002) **&** P<0.01 *Vs* Vehicle group (3w PI, In SP, EMSCs group: F=0.94, P<0.0001; PMECs group: F=1.81, P<0.0001. In BC, EMSCs group: F=1.02, P<0.0001; PMECs group: F=1.23, P<0.0001. 8w PI, In SP, EMSCs group: F=2.53, P<0.0001; PMECs group: F=0.68, P<0.0001. In BC, EMSCs group: F=1.49, P<0.0001; PMECs group: F=1.39, P<0.0001) **are increased in vehicle-treated rats relative to normal control rats, while expressions is alleviated in both EMSCs and PMSCs treated rats. TRIFC-conjugated immunofluorescent staining. Bar = 100μm. Data are represented as mean ± SD. n=5, degrees of freedom=4.**

Figure S5. EAE-induction slightly increases the expression of growth-associated protein GAP-43 in the early stages of disease, but expression decreased as EAE progressed. However, EMSCs and PMSCs treatments markedly up-regulated the expression of GAP-43. (A-N): TRIFC-conjugated red immunofluorescence indicate GAP-43 staining, Scale bar = 100 μm. (BC) Coronal sections of the brain cortex. Data are represented as mean ± SD. n=5, degrees of freedom=4. *****P<0.01 *Vs* Normal group (3w PI, in SP, Vehicle group: F=0.02, P<0.0001; EMSCs group: F=0.003, P<0.0001; PMECs group: F=0.004, P<0.0001. In BC, Vehicle group: F=0.15, P=0.0001; EMSCs group: F=0.03, P<0.0001; PMECs group: F=0.02, P<0.0001. 8w PI, in SP, Vehicle group: F=0.09, P<0.0001; EMSCs group: F=0.03,

P<0.0001; PMECs group: F=0.06, P<0.0001. In BC, Vehicle group: F=0.07, P<0.0001; EMSCs group: F=0.05, P<0.0001; PMECs group: F=0.03, P=0.0006.) **&** P<0.01 *Vs* Vehicle group (3w PI, In SP, EMSCs group: F=0.16, P<0.0001; PMECs group: F=0.19, P<0.0001. In BC, EMSCs group: F=0.11, P<0.0001; PMECs group: F=0.2, P<0.0001. 8w PI, In SP, EMSCs group: F=2.96, P<0.0001; PMECs group: F=0.63, P<0.0001. In BC, EMSCs group: F=0.8, P=0.0003; PMECs group: F=0.39, P=0.008).

Figure S6. EMSCs and PMECs treatments reduce neuronal apoptosis. In vehicle-treated EAE rats, the expression of active caspase-3 is markedly increased. This phenomenon could be reversed by EMSCs and PMSCs treatments, as shown by calculated TRIFC-conjugated (red color) immunofluorescence stained caspase-3 positive cells (scale bar = 100μm). (SC) Transverse sections through the anterior horn of the lumbar spinal. (BC) Coronal sections of the brain cortex. Data are represented as mean ± SD. n=5, degrees of freedom=4. *****P<0.01 *Vs* Normal group (3w PI, in SP, Vehicle group: F=0.03, P<0.0001; EMSCs group: F=0.04, P<0.0001; PMECs group: F=0.17, P<0.0001. In BC, Vehicle group: F=0.04, P<0.0001; EMSCs group: F=0.06, P=0.0002; PMECs group: F=0.2, P<0.0001. 8w PI, in SP, Vehicle group: F=0.2, P<0.0001; EMSCs group: F=0.43, P<0.0001; PMECs group: F=0.39, P<0.0001. In BC, Vehicle group: F=0.08, P<0.0001; EMSCs group: F=0.1, P<0.0001; PMECs group: F=0.3, P<0.0001) **&** P<0.01 *Vs* Vehicle group (3w PI, In SP, EMSCs group: F=1.4, P<0.0001; PMECs group: F=6.03, P<0.0001. In BC, EMSCs group: F=1.39, P<0.0001; PMECs group: F=4.8, P<0.0001. 8w PI, In SP, EMSCs group: F=2.15, P<0.0001; PMECs group: F=1.96, P<0.0001. In BC, EMSCs group: F=1.16, P<0.0001; PMECs group: F=3.49, P<0.0001.) #

P<0.01 *Vs* EMSCs group (8w PI, In SP, PMSCs group: F=1.1, P<0.0001; In BC, PMSCs group: F=3, P=0.0005).

Figure S7. EMSCs and PMSCs treatments alleviate neuronal loss in the spinal cord and cerebral cortex when compared with the vehicle-treated group. NF-200 immunofluorescence staining (red), scale bar = 100 μm. (SC) Transverse sections through the anterior horn of the lumbar spinal. (BC) Coronal sections of the brain cortex. Data are represented as mean ± SD. n=5, degrees of freedom=4. *****P<0.01 *Vs* Normal group (3w PI, in SP, Vehicle group: F=0.23, P<0.0001; EMSCs group: F=0.12, P<0.0001; PMECs group: F=0.12, P<0.0001. In BC, Vehicle group: F=0.04, P<0.0001; EMSCs group: F=0.02, P=0.00064; PMECs group: F=0.02, P=0.0001. 8w PI, in SP, Vehicle group: F=0.07, P<0.0001; EMSCs group: F=0.05, P<0.0001; PMECs group: F=0.48, P<0.0001. In BC, Vehicle group: F=0.04, P<0.0001; EMSCs group: F=0.02, P=0.0088; PMECs group: F=0.02, P=0.002) **&** P<0.01 *Vs* Vehicle group (3w PI, In SP, EMSCs group: F=0.51, P<0.0001; PMECs group: F=0.52, P<0.0001. In BC, EMSCs group: F=0.57, P<0.0001; PMECs group: F=0.56, P=0.0004. 8w PI, In SP, EMSCs group: F=0.75, P=0.00011; PMECs group: F=6.57, P<0.0001. In BC, EMSCs group: F=0.41, P<0.0001; PMECs group: F=0.48, P<0.0001.) # P<0.05 *Vs* EMSCs group (3w PI, In SP, PMSCs group: F=0.98, P=0.03; In BC, PMSCs group: F=1, P=0.004. 8w PI, In SP, PMSCs group: F=0.11, P=0.02).

Figure S8. EMSCs and PMECs treatments reverse the decrease of BDNF and CNTF in CNS, increase the expression of growth-associated protein GAP-43, and reduce

apoptosis related enzyme caspase-3 in EAE. (A-C): EAE-induction slightly increases the expression of growth-associated protein GAP-43 compared with healthy controls, but EMSCs and PMSCs treatments markedly increased expression. *****P<0.01 *Vs* Normal group (3w Vehicle group: F=1.85, P=0.0009; EMSCs group: F=0.33, P<0.0001; PMECs group: F=0.65, P<0.0001. 8w PI, Vehicle group: F=0.15, P=0.0002; EMSCs group: F=0.16, P<0.0001; PMECs group: F=0.09, P<0.0001.) **&** P<0.01 *Vs* Vehicle group (3w PI, EMSCs group: F=0.18, P<0.0001; PMECs group: F=0.35, P<0.0001. 8w PI, EMSCs group: F=1.06, P=0.0005; PMECs group: F=0.62, P=0.0002) (D-I): EAE induction remarkably decreased the expression of BDNF and CNTF in CNS as compared with healthy controls, and EMSCs and PMSCs treatments maintained expression of these markers. BDNF: *****P<0.05*Vs* Normal group (3w Vehicle group: F=4.62, P<0.0001;EMSCs group: F=0.56, P=0.003; PMECs group: F=0.13, P=0.02. 8w PI, Vehicle group: F=2.97, P=0.004; EMSCs group: F=8.87, P<0.0001; PMECs group: F=4.12, P<0.0001) **&** P<0.01 *Vs* Vehicle group (3w PI, EMSCs group: F=1.78, P<0.0001; PMECs group: F=0.22, P<0.0001. 8w PI, EMSCs group: F=2.99, P<0.0001; PMECs group: F=1.39, P<0.0001). CNTF: *****P<0.05*Vs* Normal group (3w Vehicle group: F=4.32, P<0.0001; EMSCs group: F=0.24, P=0.02; 8w PI, Vehicle group: F=1.01, P<0.0001; EMSCs group: F=0.47, P=0.0003) **&** P<0.01 *Vs* Vehicle group (3w PI, EMSCs group: F=0.15, P=0.002; PMECs group: F=0.04, P=0.0009. 8w PI, EMSCs group: F=0.46, P<0.0001; PMECs group: F=0.48, P<0.0001). # P<0.05 *Vs* EMSCs group (3w PI, PMSCs group: F=0.64, P=0.04; 8w PI, PMSCs group: F=0.97, P=0.005). (J-O): In vehicle-treated EAE rats, the expression of active caspase-3 is markedly increased and the expression of NF-200 was evidently down regulated. This phenomenon could be reversed by EMSCs and

PMSCs transplantation. Caspase-3: *****P<0.05*Vs* Normal group (3w Vehicle group: F=2.38, P<0.0001; EMSCs group: F=0.29, P<0.0001. PMECs group: F=0.31, P<0.0001; 8w PI, Vehicle group: F=0.01, P=0.0004; EMSCs group: F=0.02, P=0.009. PMECs group: F=0.01, P=0.001) **&** P<0.01 *Vs* Vehicle group (3w PI, EMSCs group: F=0.12, P<0.0001; PMECs group: F=0.13, P<0.0001. 8w PI, EMSCs group: F=1.49, P=0.001; PMECs group: F=0.53, P=0.0006) NF=200: *****P<0.01*Vs* Normal group (3w Vehicle group: F=0.44, P<0.0001; EMSCs group: F=0.11,P=0.0009; PMECs group: F=0.08,P=0.001. 8w PI, Vehicle group: F=0.48,P<0.0001) **&** P<0.01 *Vs* Vehicle group (3w PI, EMSCs group: F=0.25,P<0.0001; PMECs group: F=0.18, P<0.0001. 8w PI, EMSCs group: F=3.85,P<0.0001; PMECs group: F=1.26, P<0.0001). (P-R): GFAP expression visibly increased at both week 3 and week 8 following EAE induction, but decreased following EMSCs and PMSCs treatments as shown by Western blotting. *****P<0.01*Vs* Normal group (3w Vehicle group: F=0.3, P<0.0001; 8w PI, Vehicle group: F=3.6, P<0.0001) **&** P<0.01 *Vs* Vehicle group (3w PI, EMSCs group: F=1.93, P<0.0001; PMECs group: F=2.56, P<0.0001. 8w PI, EMSCs group: F=0.07, P<0.0001; PMECs group: F=0.2, P<0.0001). Data are represented as mean ± SD. n=5, degrees of freedom=n-1.

Figure S9. EMSCs and PMSCs treatments reduce neuronal loss. Visible neuronal loss is observed in the brain cortex (B) and spinal cord (F) in CNS of the vehicle-treated EAE rats at 8 weeks post-injection, when compared with healthy animals (A, E). Nevertheless, in EMSCs and PMSCs-treated EAE rats, more neurons are present in the anterior horn of the spinal cord (G-H) and in the motor cortices (C-D). Nissl staining, scale bar = 100 mm. (I-K): The

transplanted EMSCs and PMECs obviously infiltrated into spinal cord parenchymal (I) and formed MSCs masses inside the brain parenchymal tissue (J, K). (L): Number of surviving neural cells calculated in the different groups at 8 weeks post-injection following Nissl staining (each group is presented as a percentage of the healthy control). Data are represented as mean ± SD. N=5, degrees of freedom=4. *****P<0.01 *Vs* Normal group (In SP, Vehicle group: F=0.15, P<0.0001; EMSCs group: F=0.14, P=0.003; PMECs group: F=0.06, P=0.0018. In BC, Vehicle group: F=1.18, P<0.0001; EMSCs group: F=1.02, P<0.0001; PMECs group: F=0.6, P<0.0001) **&** P<0.01 *Vs* Vehicle group (In SP, EMSCs group: F=0.94, P<0.0001; PMECs group: F=0.37, P<0.0001. In BC, EMSCs group: F=0.86, P<0.0001; PMECs group: F=0.51, P=0.0001.) **#** P<0.01 *Vs* EMSCs group (In SP, PMSCs group: F=0.37, P=0.013).

Supplemental figures

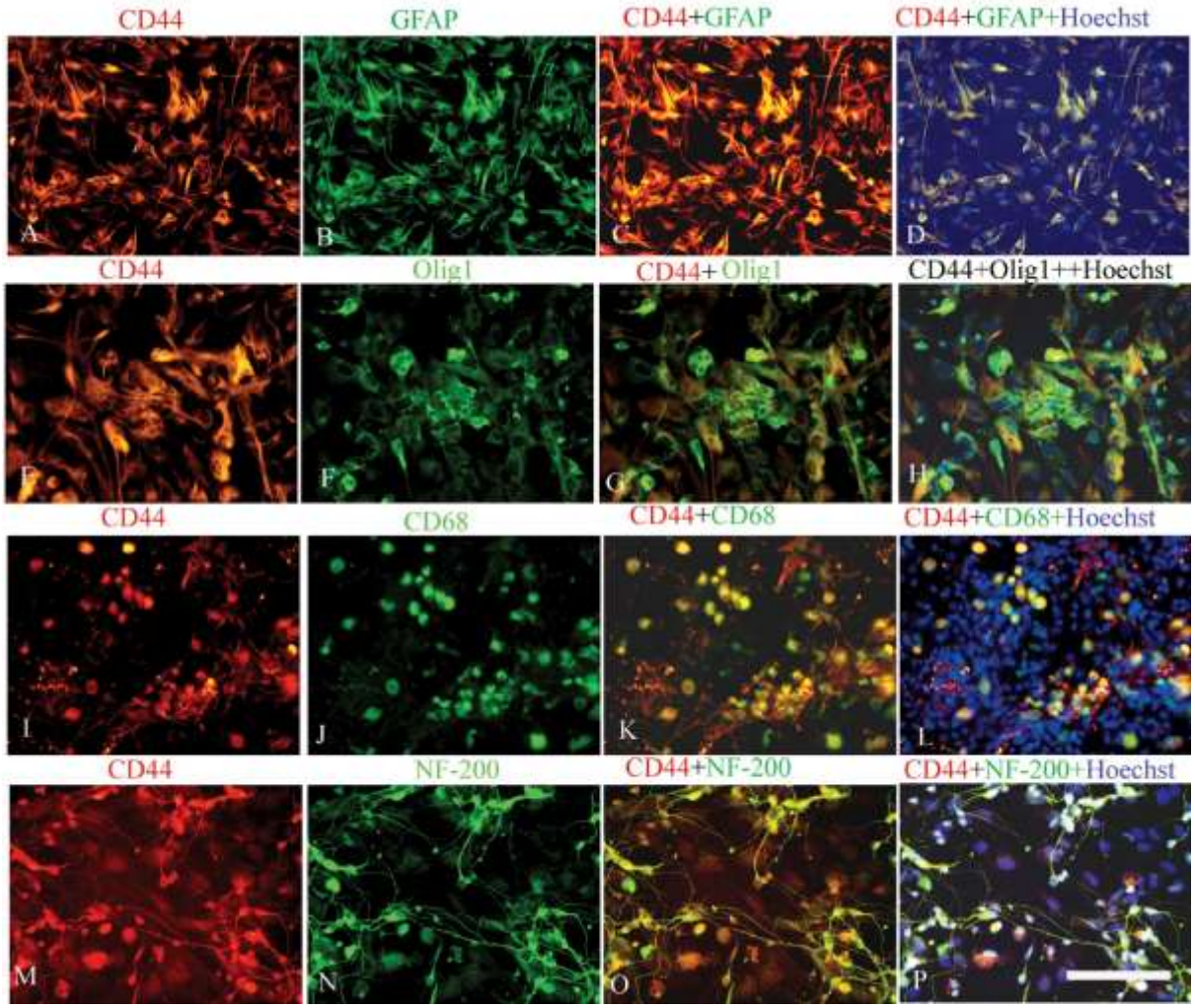

Figure S1

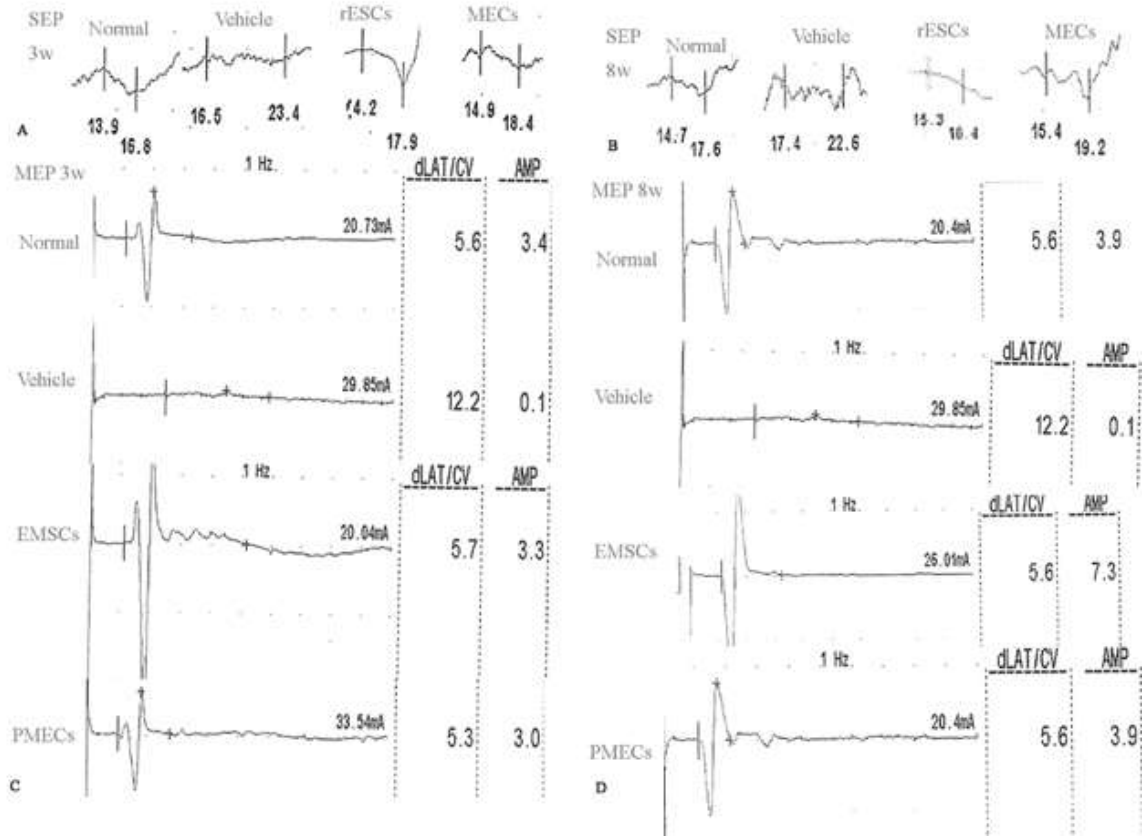
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Figure S2

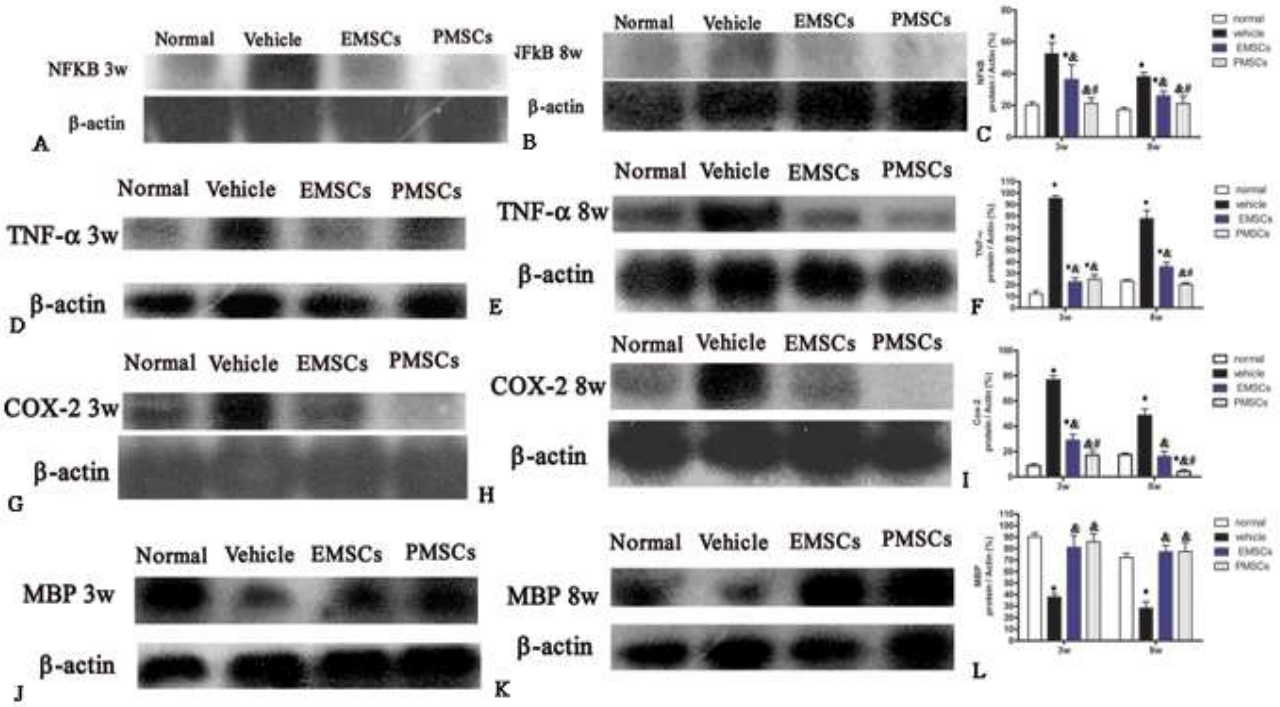
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Figure S3

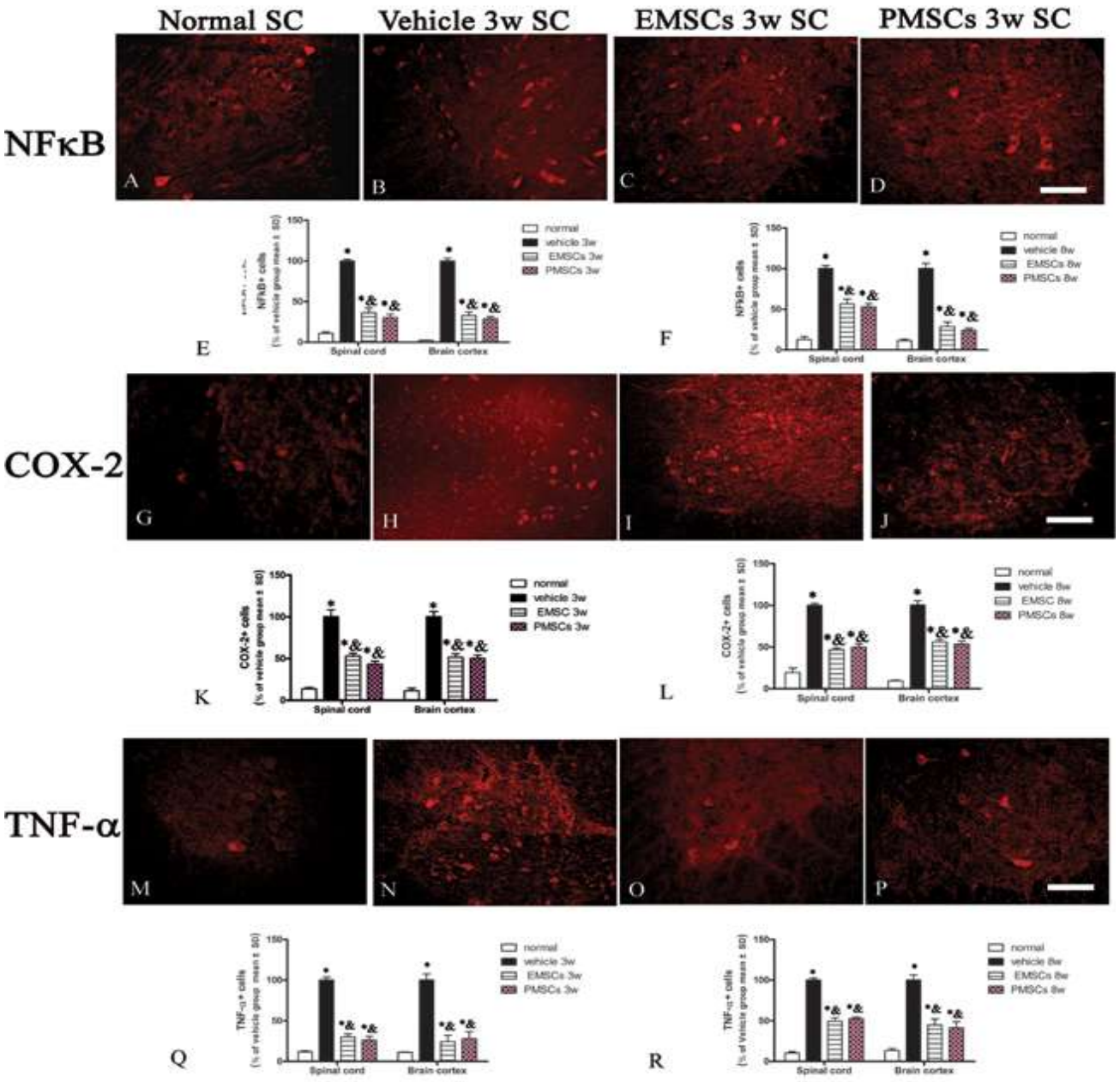
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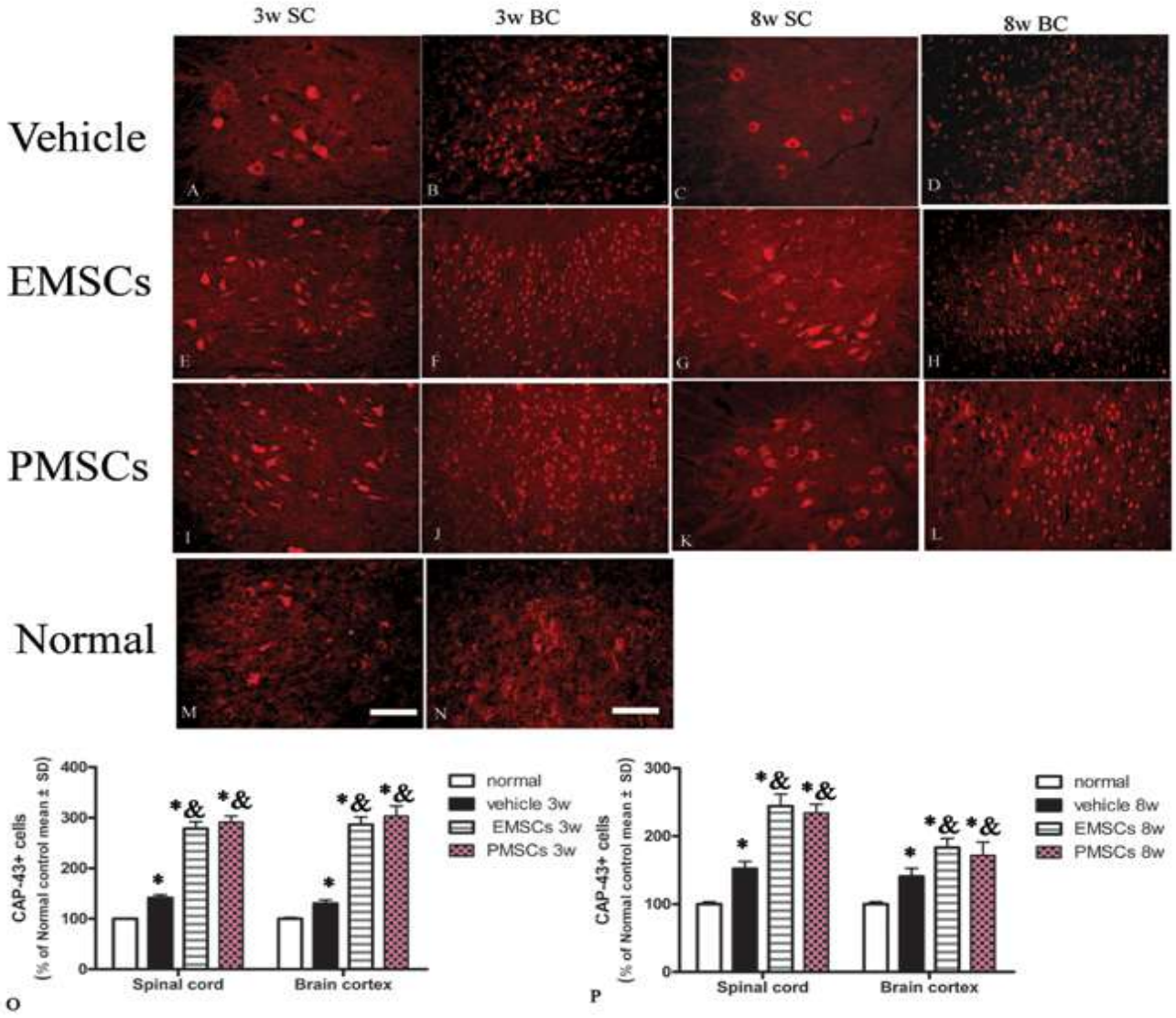
Figure S4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Spinal cord
Brain cortex
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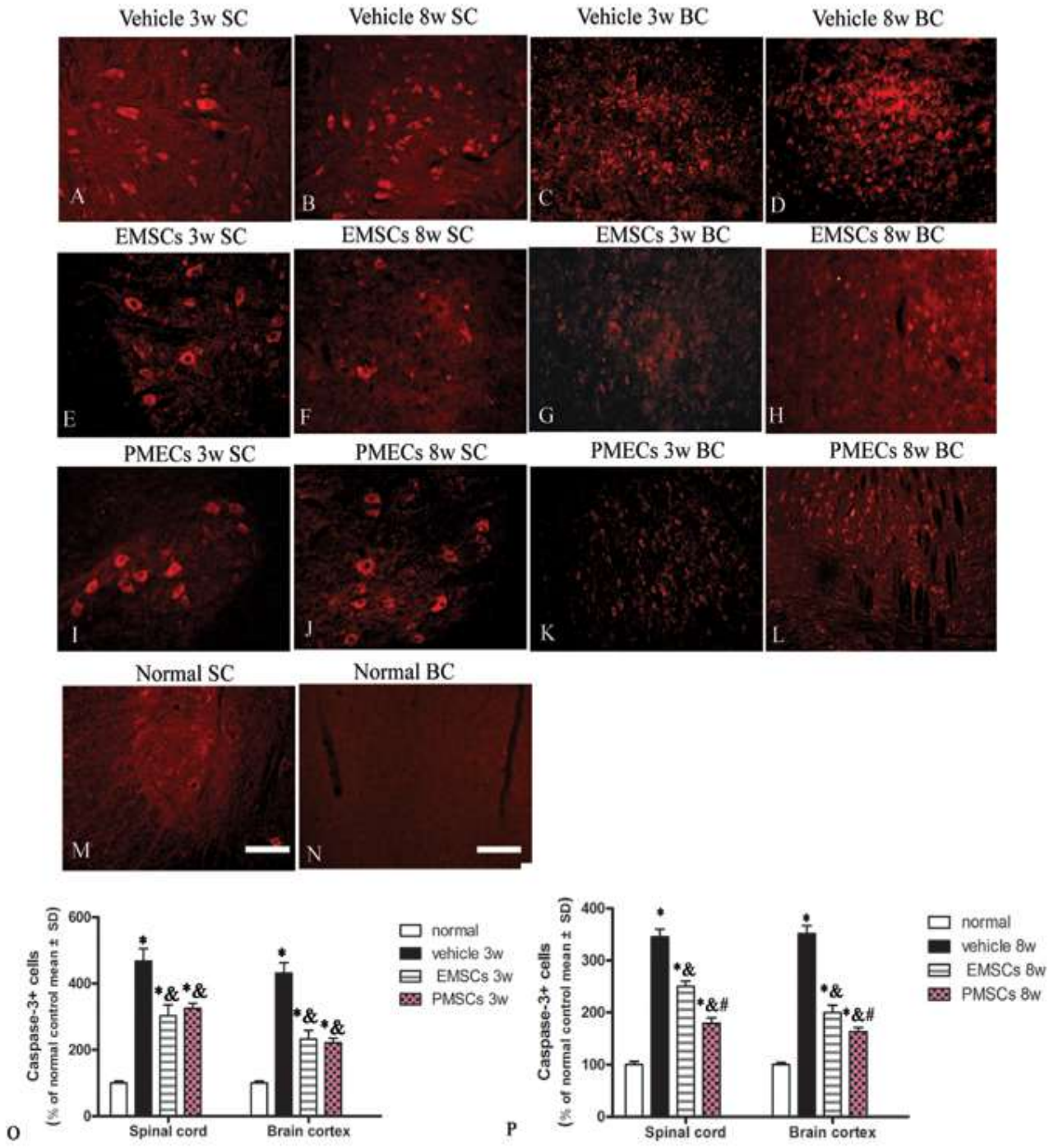
Figure S5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Spinal cord
Brain cortex
normal
vehicle 3w
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Spinal cord
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PMSCs 8w

Figure S6

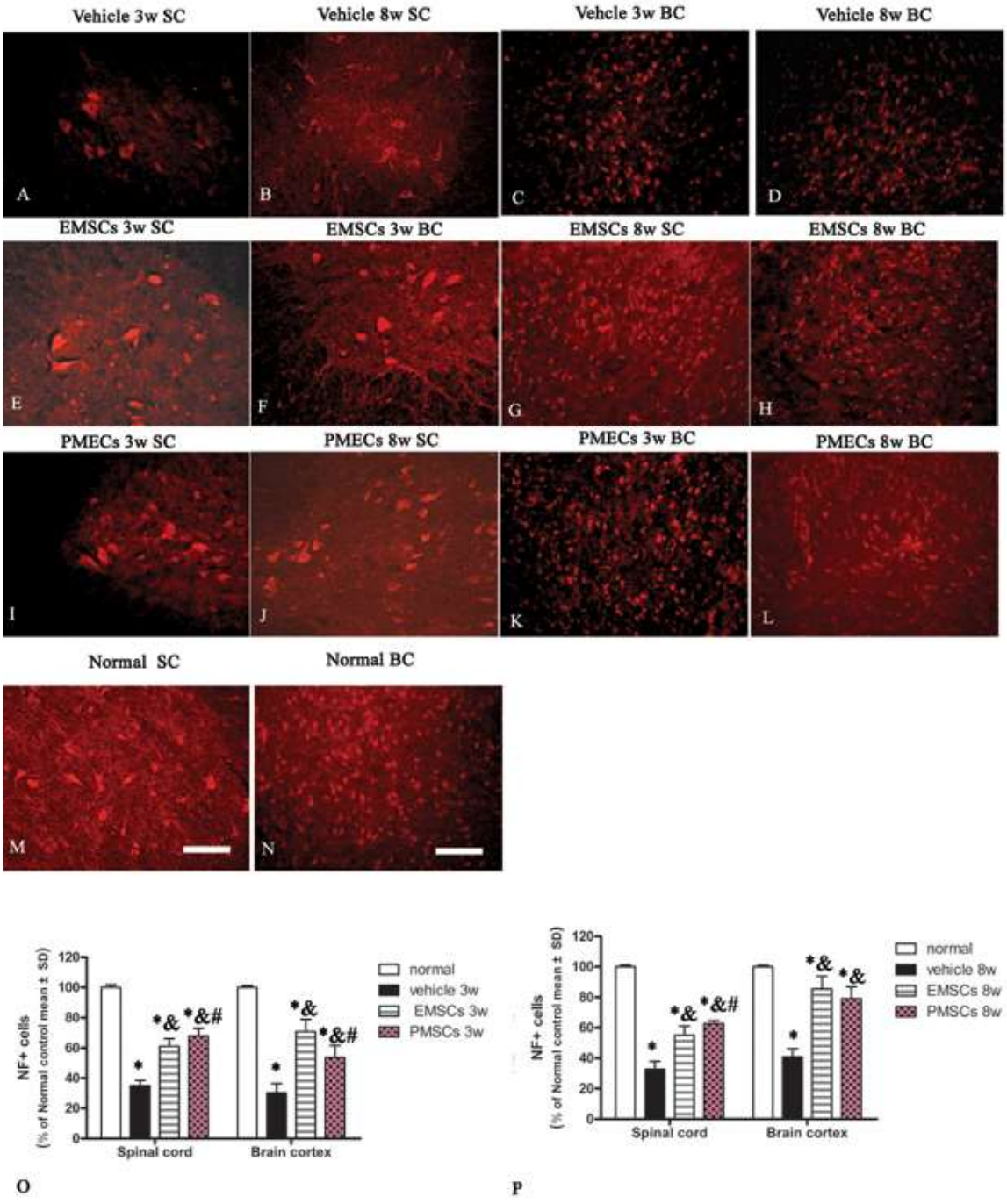
Vehicle 3w SC
Vehicle 8w SC
Vehcle 3w BC
Vehicle 8w BC
A
B
C
D
EMSCs 3w SC
EMSCs 3w BC
EMSCs 8w SC
EMSCs 8w BC
E
F
G
H
PMECs 3w SC
PMECs 8w SC
PMECs 3w BC
PMECs 8w BC
I
J
K
L
Normal SC
Normal BC
M
N
NF+ cells
(% of Normal control mean ± SD)
normal
vehicle 3w
EMSCs 3w
PMSCs 3w
Spinal cord
Brain cortex
O
NF+ cells
(% of Normal control mean ± SD)
normal
vehicle 8w
EMSCs 8w
PMSCs 8w
Spinal cord
Brain cortex
P

Figure S7

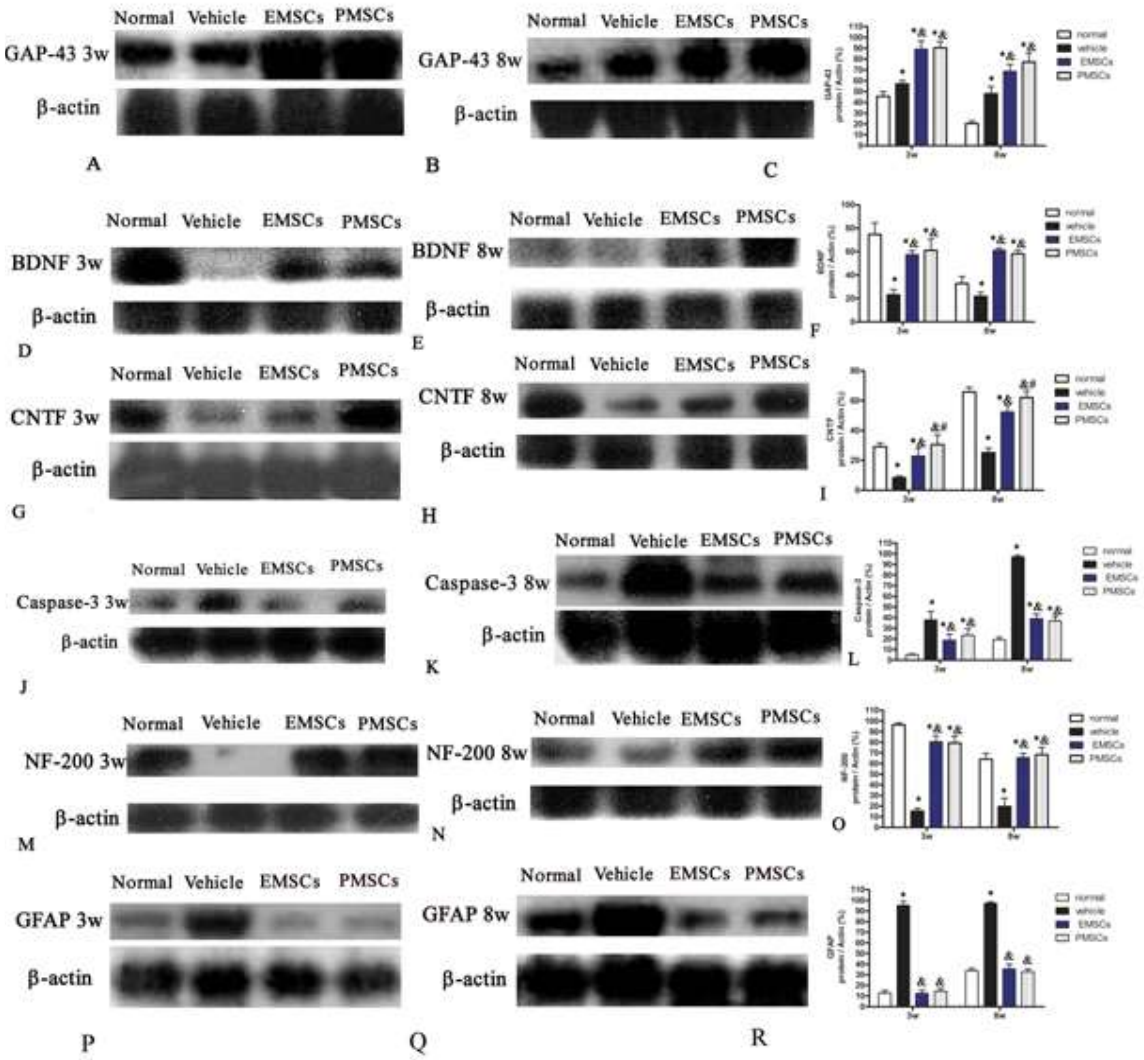
Normal Vehicle EMSCs PMSCs
GAP-43 3w
β-actin
A
GAP-43 8w
B
C
BDNF 3w
D
BDNF 8w
E
F
CNTF 3w
G
CNTF 8w
H
I
Caspase-3 3w
J
Caspase-3 8w
K
L
NF-200 3w
M
NF-200 8w
N
O
GFAP 3w
P
GFAP 8w
Q
R

Figure S8

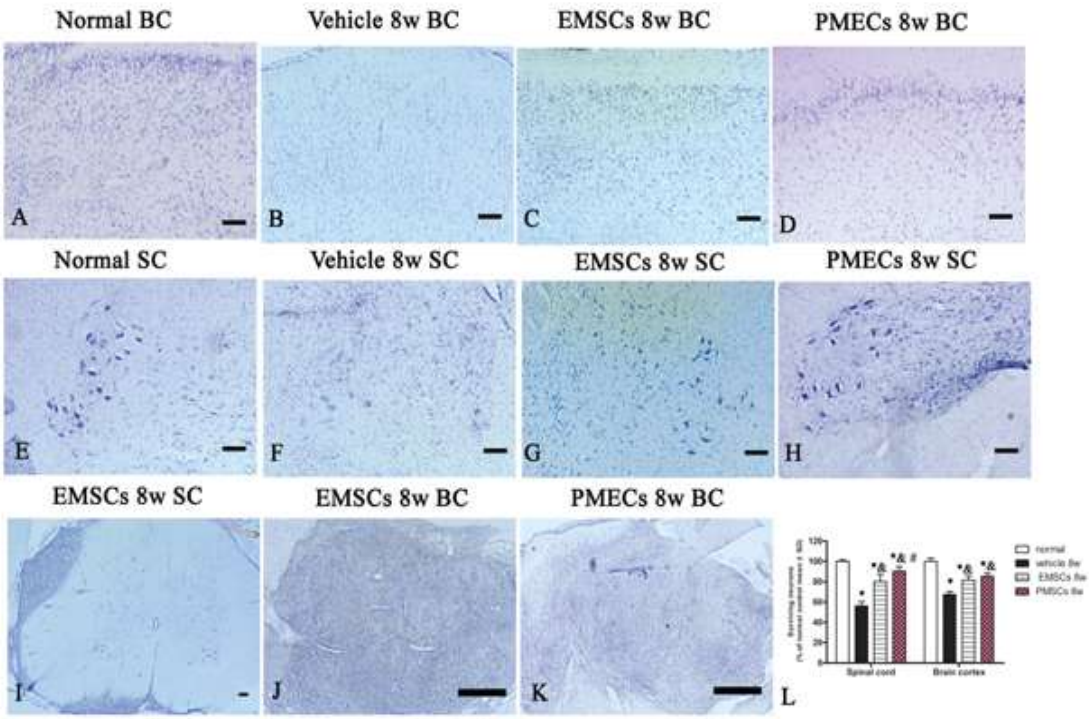
Normal BC
Vehicle 8w BC
EMSCs 8w BC
PMECs 8w BC
A
B
C
D
Normal SC
Vehicle 8w SC
EMSCs 8w SC
PMECs 8w SC
E
F
G
H
EMSCs 8w SC
EMSCs 8w BC
PMECs 8w BC
I
J
K
L

Figure S9