Combination of Electroacupuncture and Grafted Mesenchymal Stem Cells Overexpressing TrkC Improves Remyelination and Function in Demyelinated Spinal Cord of Rats

Ying Ding^{a#}, Rong-Yi Zhang^{a#}, Bing He^{a#}, Zhou Liu^a, Ke Zhang^a, Jing-Wen Ruan^{e*}, Eng-Ang Ling^f, Jin-Lang Wu^g, Yuan-Shan Zeng^{a,b,c,d,*}

^aDepartment of Histology and Embryology, Zhongshan School of Medicine, Sun Yat-sen University, Guangzhou, Guangdong 510080, China

^bKey Laboratory for Stem Cells and Tissue Engineering Ministry of Education, Sun Yat-sen University, Guangzhou, Guangdong 510080, China

^cInstitute of Spinal Cord Injury, Sun Yat-sen University, Guangzhou, Guangdong 510127, China

^dCo-innovation Center of Neuroregeneration, University, Nantong, Nantong, Jiangsu 226011, China

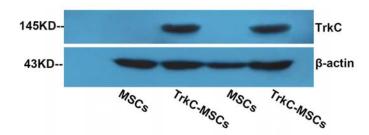
^eDepartment of Acupuncture, the 1st Affiliated Hospital, Sun Yat-sen University, Guangzhou, Guangdong 510080, China

^fDepartment of Anatomy, Yong Loo Lin School of Medicine, National University of Singapore, Singapore 117597, Singapore

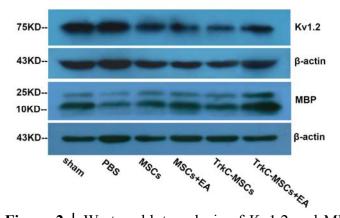
^gDepartment of Electron Microscope, Zhongshan School of Medicine, Sun Yat-sen University, Guangzhou, Guangdong 510080, China

[#] These authors contributed equally to this manuscript

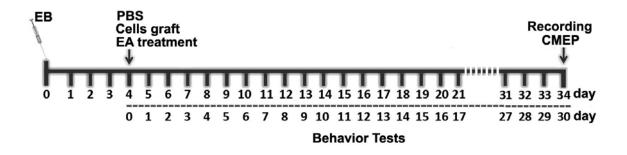
* Corresponding Authors: Yuan-Shan Zeng, M.D., Ph.D. Department of Histology and Embryology Zhongshan School of Medicine Sun Yat-sen University 74# Zhongshan 2nd Road Guangzhou 510080, China Tel: +86-20-87332698 Fax: +86-20-87332698 E-mail address: zengysh@mail.sysu.edu.cn or Jing-Wen Ruan, M.D. Department of Acupuncture The 1st Affiliated Hospital Sun Yat-sen University 58# Zhongshan 2nd Road Guangzhou 510080, China Tel: +86-20-87755766-8390 E-mail address: ruanjw@163.com



Supplementary Figure 1 | Western blot analysis of TrkC expression in cultured MSCs and Ad-TrkC transduced MSCs. Samples were run twice in the independent experiment and the experiments were repeated three times under the same experimental conditions. β -actin was used as a control.



Supplementary Figure 2 | Western blot analysis of Kv1.2 and MBP expression in the sham, PBS, MSCs, MSCs+EA, TrkC-MSCs and TrkC-MSCs+EA groups. Samples were run five times in the 6 groups and the experiments were repeated twice under the same experimental conditions. β -actin was used as a control.



Supplementary Figure 3 | Illustrating the time points of EB injection, PBS injection, cells graft, behavior tests and recording CMEP.

Different experimental	Number of rats in each group					
procedures	sham	PBS	MSCs	MSCs +EA	TrkC-MSCs	TrkC-MSCs +EA
ELISA	5	5	5	5	5	5
Western blot	5	5	5	5	5	5
Behavioural testing; CMEPs recording; Differentiation of MSCs; Myelin counting and electron microscopy	5	5	5	5	5	5
Longitudinal sections were used to NF/Kv1.2 /MBP triple-label immunofluorescence staining.	3	3	3	3	3	3
Immunoelectron microscope	0	0	0	0	0	3
Total	18	18	18	18	18	21

Supplementary Table 1 Application of animals in different experimental procedures