Supporting Information

S1.

Animals. Sprague-Dawley rats (SPF, 6-8 weeks of age, half female and half male, weighing 220 ± 10 g, License: SCXK-(military)-2012-0011) were provided by the Experimental Animal Centre of the Third Military Medical University affiliated with the People's Liberation Army (China). Animal feeding, use and surgeries were performed at and subjected to supervision and approval by the Institutional Animal Care and Use Committee of Southwest University

S2.

Transmission electron microscopy. The sciatic nerves, optic nerves and oculomotor nerves were observed under a transmission electron microscope (TEM, JEM 1400 plus, Japan). Samples obtained on the 30th day after surgery included the proximal and distal ends of the receptor sciatic nerve, the graft used for autologous nerve transplantation, and the graft used for nerve tube transplantation.

S3.

Immunofluorescent staining. Tissue samples were frozen (-80℃, Zhongke Duling Commercial Electric Appliance Co, LTD, China), sectioned (6 μm thickness, CM1850, LEICA, Germany), and stained using immunofluorescent markers, while Schwann cell slides were stained directly and observed under a fluorescence microscope (ECLIPSE 80i, NIKON, Japan). Nuclei were stained blue using 4,6-diamidino-2- phenylindole (DAPI, Biosynthesis Biotechnology, China). Primary antibodies against myelin basic protein (MBP101, mouse anti-rat; Abcam, UK) and glial fibrillary acidic protein (GFAP, chicken anti-rat; Thermo Fisher scientific, USA) were used to identify myelinating Schwann cells and nonmyelinating Schwann cells, respectively. Primary antibodies against the nerve growth factor receptor (NGFRP75, mouse anti-rat, Santa Cruz Biotechnology, USA) and neurofilament (NF200, rabbit anti-rat, Sigma, USA) were used to identify Schwann cells and axons, respectively. Goat anti-rabbit (red, Thermo Fisher scientific, USA), goat anti-chicken (green, Thermo Fisher scientific, USA) and goat anti-mouse (red, Thermo Fisher scientific, USA) were used as the secondary antibodies.

S4.

Transcriptome analysis. The sciatic nerves, the sciatic nerves on the 7th,14th and 30th day after surgery, and the primary culture Schwann cells were used for the transcriptome analyses (Biomarker Technologies Co, LTD, China). To obtain their FPKM values (n=3), a follow-up analysis was performed based on their expression levels relative to sciatic nerves. A subsequent analysis was performed using the base 2 logarithm of their relative expression (times), with the values calculated as:

Relative expression (times)= FPKM value of a sample / FPKM value of sciatic nerve

S5.

Statistical analysis. All data were statistically analyzed using the SPSS software (IBM SPSS Statistics, USA) to compare the differences among the different time points in the same group, or among groups at the same time point. Data were illustrated using the Origin 8.0 software and Adobe Photoshop CC 2018.

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S6.

Preparation of a nerve tube. Medical absorbent cotton (Xuwei Health Materials Co., Ltd, China) was used to prepare a membrane of 100 μm thickness. The membrane was immersed for 8 h in a chloroform solution containing 20% (W/V) polylactic acid (PLA, molecular weight 200,000 Daltons, Esun, China), removed from the solution, wound tightly three time around a glass stick which had a diameter of 1.8 mm, and dried using ventilation equipment (CS101–2 A BN, Chongqing Immortalized Experimental Instrument Factory, China) at room temperature. Next, the stick was removed, and a tube with an inner diameter of 1.8 mm and a 300 μm-thick wall was prepared, and it was cut to a 12 mm length before being used as a nerve tube.

S7.

Surgery. One hind limb on each rat was randomly selected for surgery. Preparation of the 10 mm sciatic nerve defect animal model and nerve grafting during surgical implantation were carried out in accordance with a previously described method [1]. In brief, the autologous nerve and nerve tube (n = 90/group) were implanted to bridge the 10 mm sciatic nerve defect. In the end-to-end anastomosis group, the sciatic nerve was transected and end-to-end anastomosis (n = 90) was performed referring to the method described previously [1].

Reference

[1] Mokarram N, Dymanus K, Srinivasan A, Lyon JG, Tipton J, Chu J, English AW, Bellamkonda RV., Immunoengineering nerve repair. Proc Natl Acad Sci U S A 114(26), E5077-E5084 (2017).

S8.

Preparation of peripheral nerve samples. Before collecting peripheral nerve samples, the procedure to expose the sciatic nerve, optic nerve and oculomotor nerve were carried out referring to previously reported methods [8,27,57]. The part of the sciatic nerve between the lower edge of the piriformis and muscular branch, the part of the optic nerve 1-5 mm distal to the optic chiasm and the part of oculomotor nerve going through the sinus cavernous were taken as normal nerve samples, respectively. Thirty days after surgery, the proximal and distal ends of the receptor sciatic nerve, the graft used for autologous nerve transplantation, and the graft used for nerve tube transplantation were collected for observation via TEM observation and immunofluorescent staining. At 7, 14 and 30 days after surgery, the part between the lower edge of the piriformis and muscular branch of the sciatic nerves, including the proximal and distal ends, and grafts were collected as samples for transcriptome analysis.

S9.

Primary Schwann cell culture. A part of the sciatic nerve between the lower edge of the piriformis and the muscular branch of a male rat was resected and washed three times in PBS (phosphate-buffered saline, Beijing Solarbio Science & Technology Co., Ltd., China) containing antibiotics (250IU penicillin and 250IU streptomycin per 1 mL PBS), cut into pieces in trypsin (0.25%, Hyclone, USA), incubated (37℃, ESCO, Singapore) for 30 min, centrifuged at 800 rpm for 3 min (TDZ4A-WS, Xiangyi Centrifuge Instrument Co., Ltd., China), then the supernatant was removed, and cells were incubated with DMEM (Dulbecco's Modified Eagle Medium, ThermoFisher Scientific Co., Ltd., China) containing 10% fetal bovine serum (Tianhang Biotechnology Co., Ltd., China) at 37℃ with 5% carbon dioxide.

Fig.1

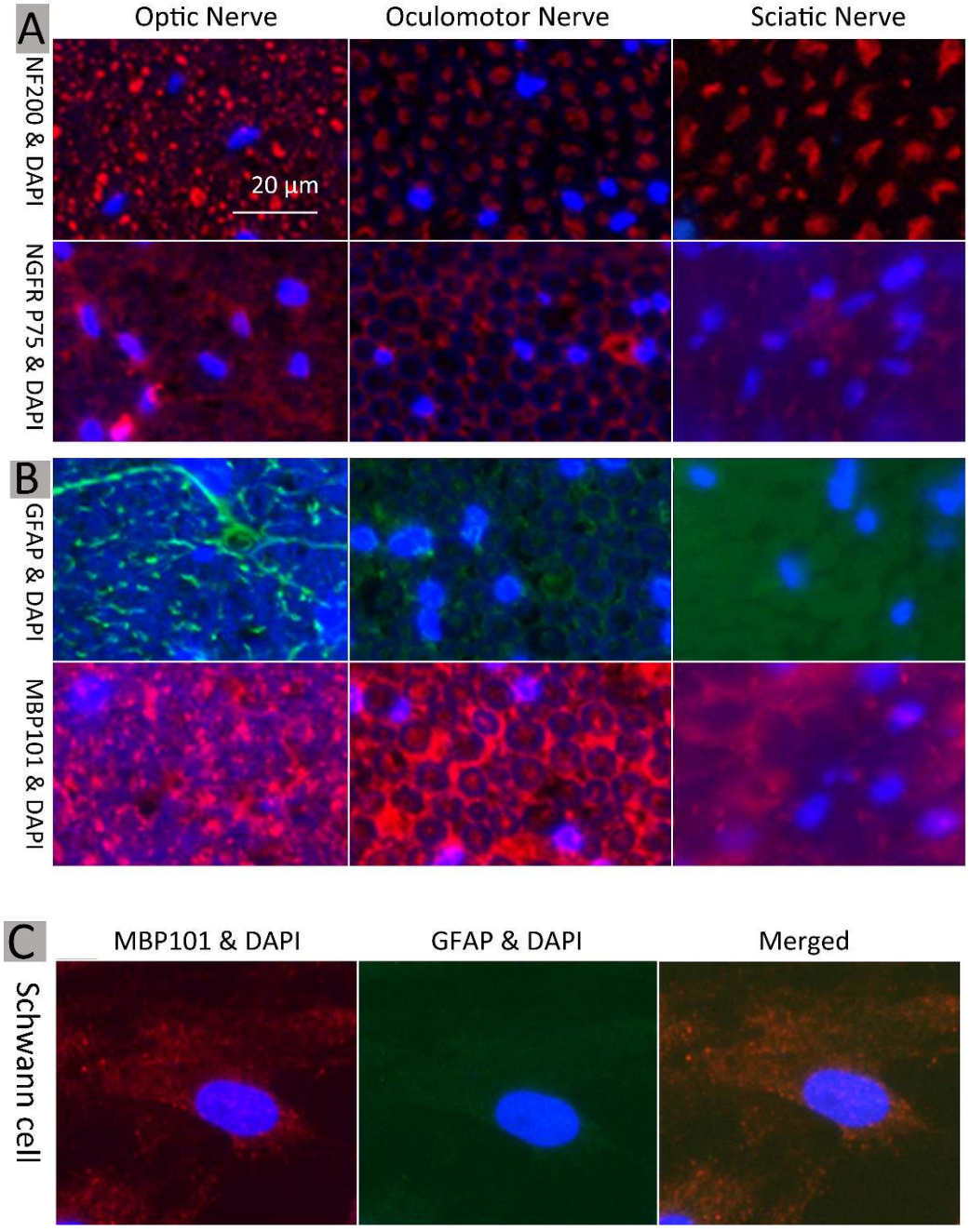

Fig. 1. Immunofluorescent staining of cross-sections of optic nerve, oculomotor nerve, sciatic nerve, and Schwann cells. (A) DAPI (blue), NF200 (red) and NGFR P75 (red) staining of the optic nerve, oculomotor nerve and sciatic nerve. (B) DAPI (blue), GFAP (green) and MBP 101 (red) staining of the optic nerve, oculomotor nerve and sciatic nerve. (C) DAPI (blue), GFAP (green) and MBP 101 (red) staining of primary culture Schwann cells.

Fig.2

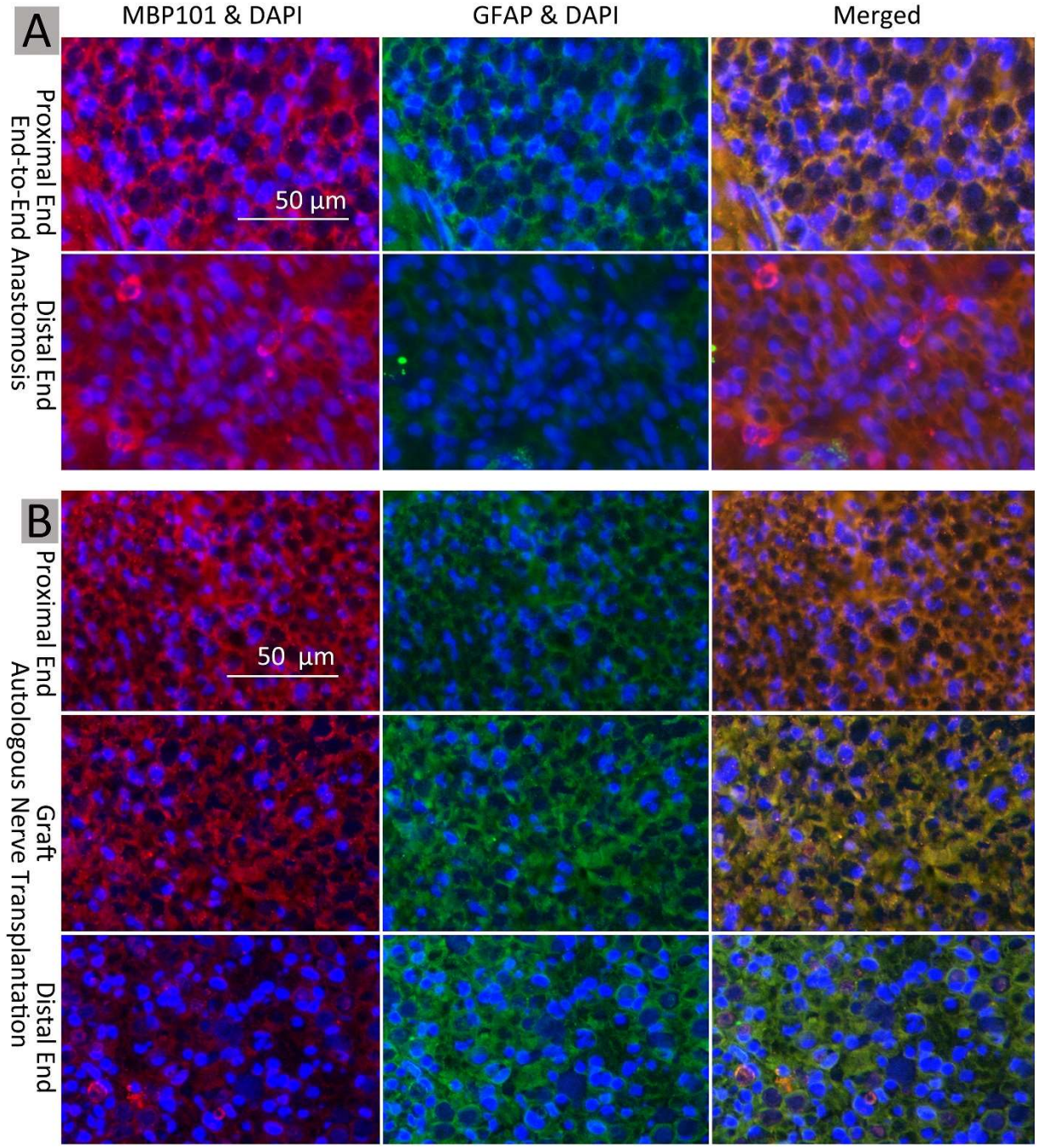

Fig. 2. DAPI, MBP 101, GFAP NGFR P75 and NF200 immunofluorescent staining of the cross-sections from samples obtained on the 30th day after surgery. (A) End-to-end anastomosis group. (B) Autologous nerve transplantation group.

Fig.3

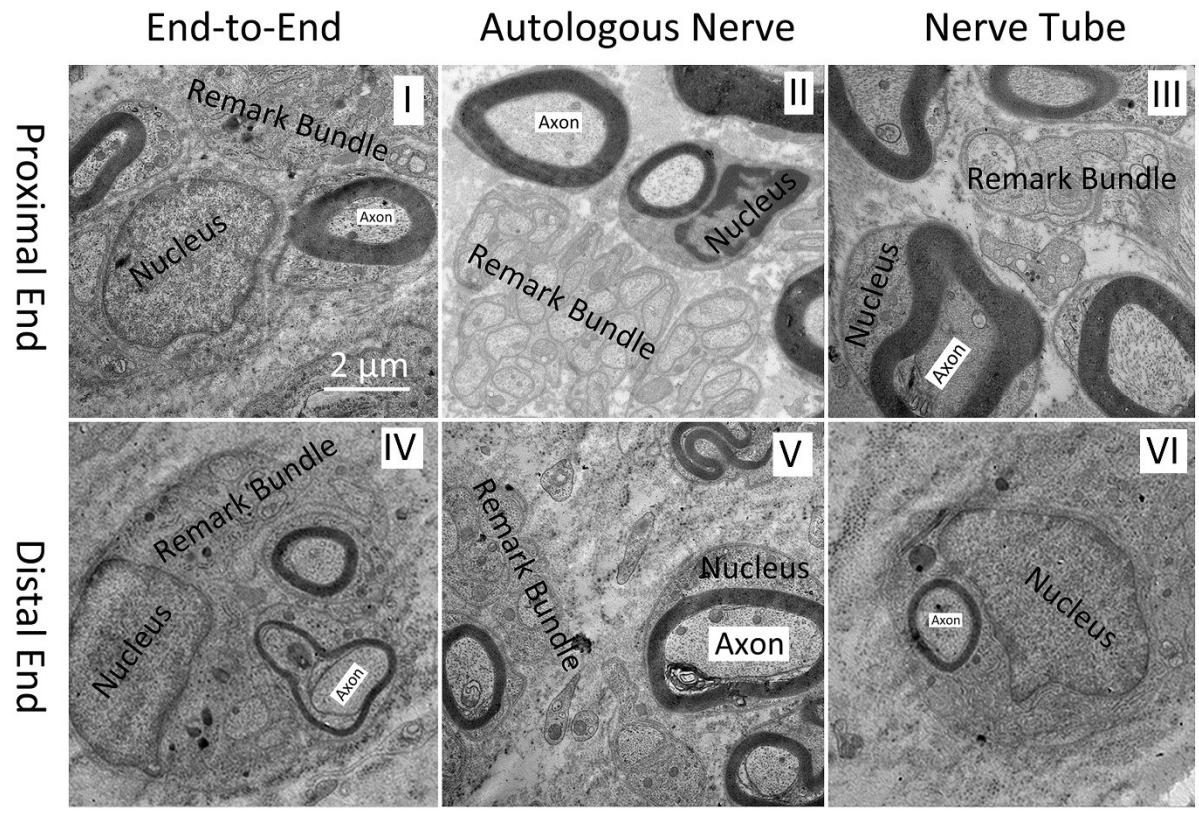

Fig. 3. TEM images of the cross-sections of the proximal and distal ends of samples obtained on the 30th day after surgery.