

## **Supplementary Digital Content 2: Methods**

### **Western blotting**

Rats were deeply anesthetized with pentobarbital (100 mg/kg, intraperitoneally), perfused transcardially with phosphate buffered saline (PBS), and the L5 spinal cord was removed immediately and separated to half at middle of the spinal cord, so that a sample contains dorsal and ventral horn of ipsilateral or contralateral side to peripheral nerve injury. Tissues were immediately homogenized in sample buffer (62.5 mM Tris-HCl (pH 6.8), 2% sodium dodecyl sulfate, 10% Glycerol, 50 mM dithiothreitol, 0.01% bromophenol blue), insoluble fraction was removed by two rounds centrifugation steps and resolved in 4-15% gradient sodium dodecyl sulfate-polyacrylamide gel electrophoresis gels and transferred to polyvinylidene difluoride membranes. After the blocking, the membranes were incubated with primary antibodies [anti-CCR5 (1:1000; Abcam, Cambridge, MA) or anti- $\beta$  actin (1:1000; Sigma, St. Louis, MO)], and then incubated with horseradish peroxidase -conjugated secondary antibody (1:1,000, GE Healthcare, Milwaukee, WI). The blots were visualized using a chemiluminescence method and a LAS-3000 imaging system (Fujifilm, Tokyo, Japan).

### **Chromogenic *in situ* hybridization (ISH)**

Digoxigenin-labeled RNA probes were designed having complementary sequence of rat chemokine (C-C motif) receptor 1 and chemokine (C-C motif) receptor 5 messenger RNA (GenBank accession

number NM\_020542.2 and 053960.3) positioned at 12-396 and 1501-1968 bases. Animals were anesthetized and perfused transcardially with 4% paraformaldehyde in PBS, pH 7.4, 7 days after nerve injury. The L5 spinal cord was removed and again fixed with Tissue Fixative (Genostaff, Tokyo, Japan). Paraffin embedded blocks and sections of rat tissue for ISH were obtained from Genostaff Co., Ltd. The rat tissue was dissected, fixed with Tissue Fixative, and then embedded in paraffin by their proprietary procedures, and sectioned at 6- $\mu$ m. For ISH, tissue sections were de-waxed with xylene, and rehydrate through an ethanol series and PBS. The sections were fixed 4% paraformaldehyde in PBS for 15 min and then washed with PBS. The sections were treated with 6  $\mu$ g/ml Proteinase K in PBS for 30 min at 37°C, washed with PBS, refixed with 4% paraformaldehyde in PBS, again washed with PBS, and placed in 0.2 N HCl for 10 min. After washing with PBS, the sections were acetylated by incubation in 0.1 M tri-ethanolamine-HCl, pH 8.0, 0.25% acetic anhydride for 10 min. After washing with PBS, the sections were dehydrated through a series of ethanol. Hybridization was performed with probes at concentrations of 300 ng/ml in the Probe Diluent-1 (Genostaff) at 60°C for 16 h. After hybridization, the sections were washed in 5xHybriWash (Genostaff), equal to 5xSSC, at 60°C for 20 min and then in 50% formamide, 2xHybriWash at 60°C for 20 min, followed by RNase treatment in 50  $\mu$ g/ml RNaseA in 10 mM Tris-HCl, pH 8.0, 1 M NaCl and 1 mM ethylenediaminetetraacetic acid for 30 min at 37°C. Then the sections were washed twice with 2xHybriWash at 60°C for 20 min, twice 0.2xHybriWash at 60°C for 20 min, and once with TBST (0.1% Tween20 in TBS). After treatment with 1xG-Block (Genostaff) for 15 min at room

temperature, the sections were incubated with antidigoxigenin alkaline phosphatase conjugate (Genostaff) diluted 1:2,000 with 50xG-Block (Genostaff) in TBST for 1 h at room temperature. The sections were washed twice with TBST and then incubated in 100 mM NaCl, 50 mM MgCl<sub>2</sub>, 0.1% Tween20, 100 mM Tris-HCl, pH 9.5. Coloring reactions were performed with nitro-blue tetrazolium chloride/5-Bromo-4-Chloro-3'-Indolylphosphatase p-Toluidine salt solution (Genostaff) overnight and then washed with PBS. The sections were counterstained with Kernechtrot stain solution (Genostaff), and mounted with CC/Mount (Genostaff).