Supplemental Digital Content 1

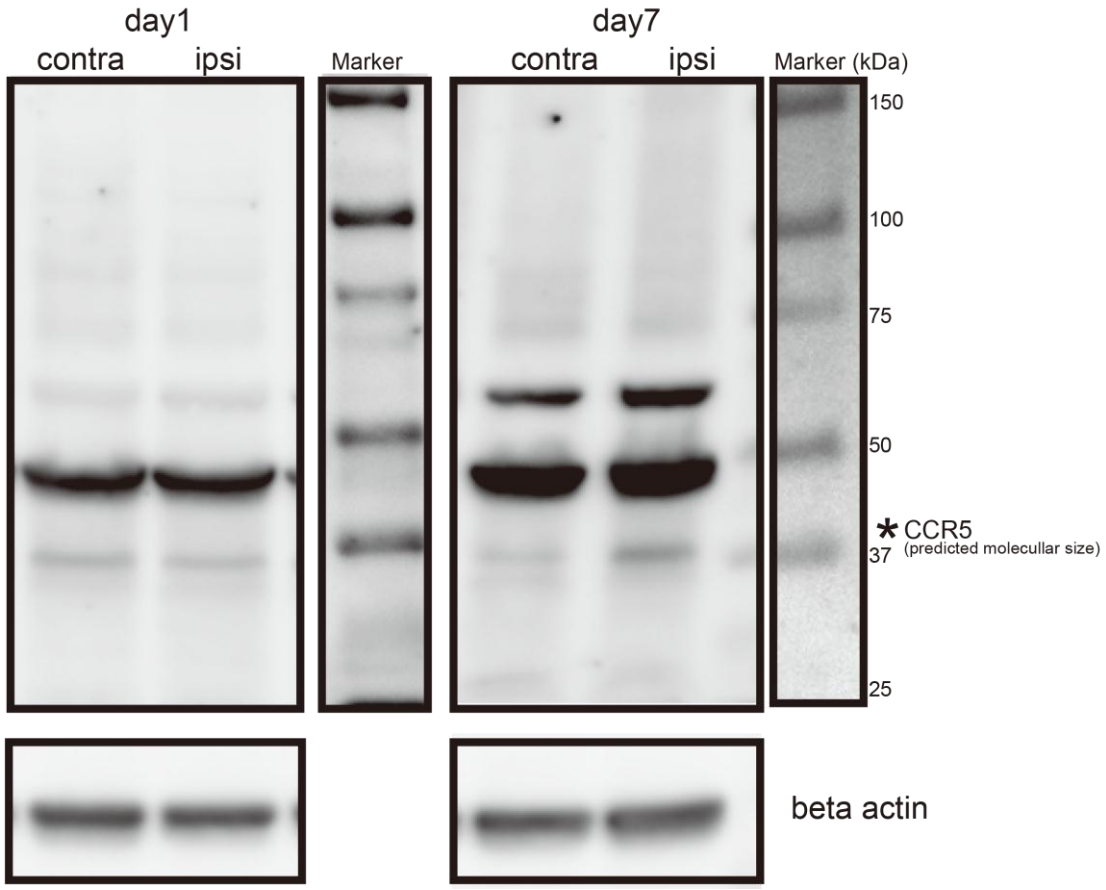

Fig. 1. Western blotting using anti-chemokine (C-C motif) receptor 5 (CCR5) antibody was performed at day 1 and day 7 after peripheral nerve injury. Blots show representatives of data from three animals at each time points. Asterisk indicates predicted molecular size (about 40 kDa) of CCR5 from amino acid sequence. Blot of beta actin for loading control represents under blot of CCR5. When focused on the bands with corresponding molecular weight, intensity of the bands was increased in the day 7 spinal cord ipsilateral side to the peripheral nerve injury but not in the day 1 spinal cord. Detailed methods is described in supplemental digital content 2.

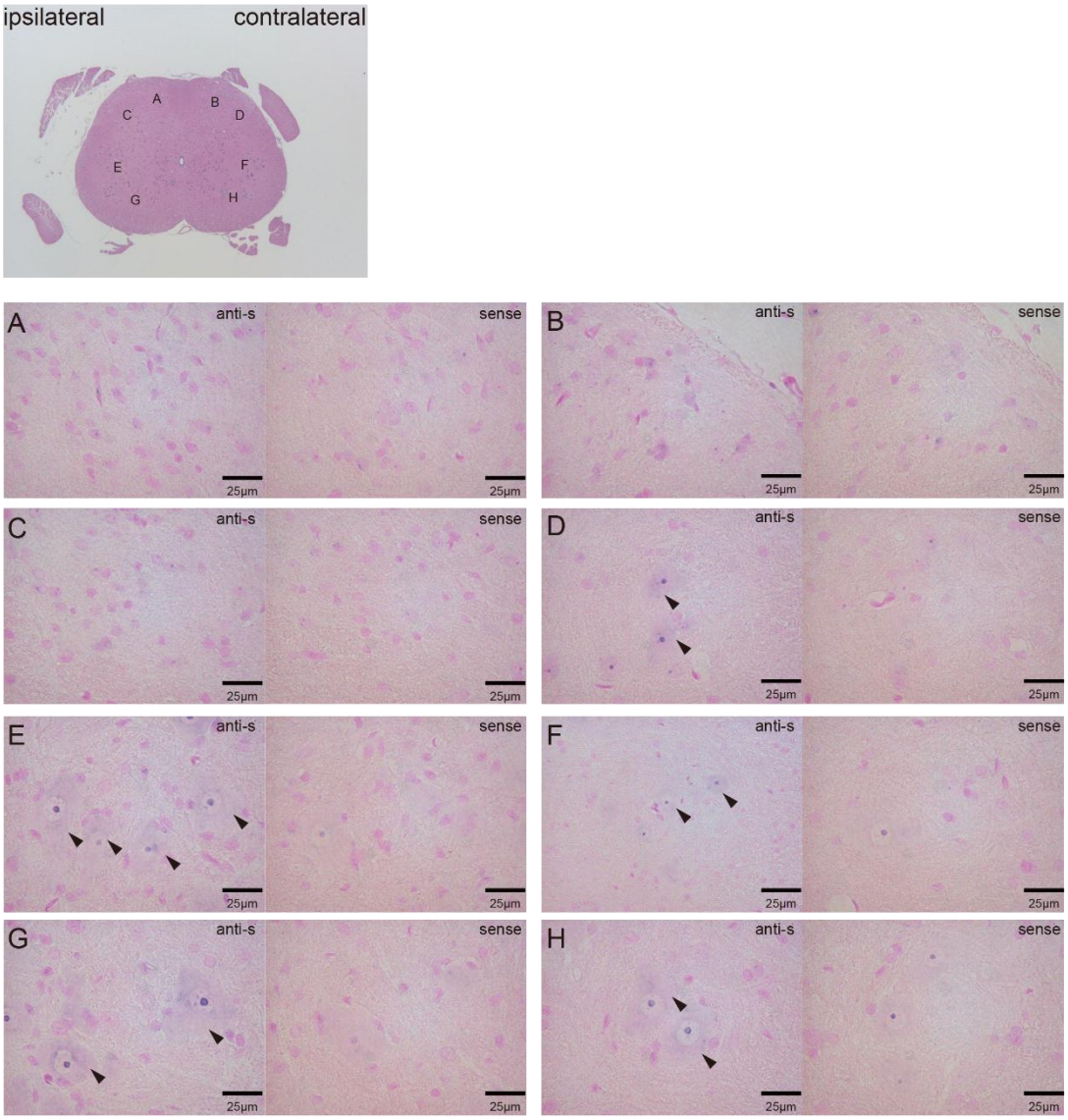

Fig. 2. Histological analyses of chemokine (C-C motif) receptor 1 (CCR1) messenger RNA (mRNA) expression in the spinal cord 7 days after peripheral nerve injury. Detailed method for staining was described in supplemental digital content 2. Each labeled images (A-H) represent corresponding locus in upper left low magnification image. Localization of CCR1 mRNA (anti-s) is shown as deep purple of nitro-blue tetrazolium chloride/5-Bromo-4-Chloro-3'-Indolylphosphatase p-Toluidine salt and counter staining was performed with Kernechtrot nuclear fast red staining. The right of each image (sense) was obtained using probe having complementary sequence of anti-s probe which is used as negative control of staining. CCR1 mRNA positive staining were overall weak but part of neuron-like (having large cell body) cells had positive staining (arrowheads). Apparently, no difference was observed between ipsilateral and contralateral side to nerve injury.

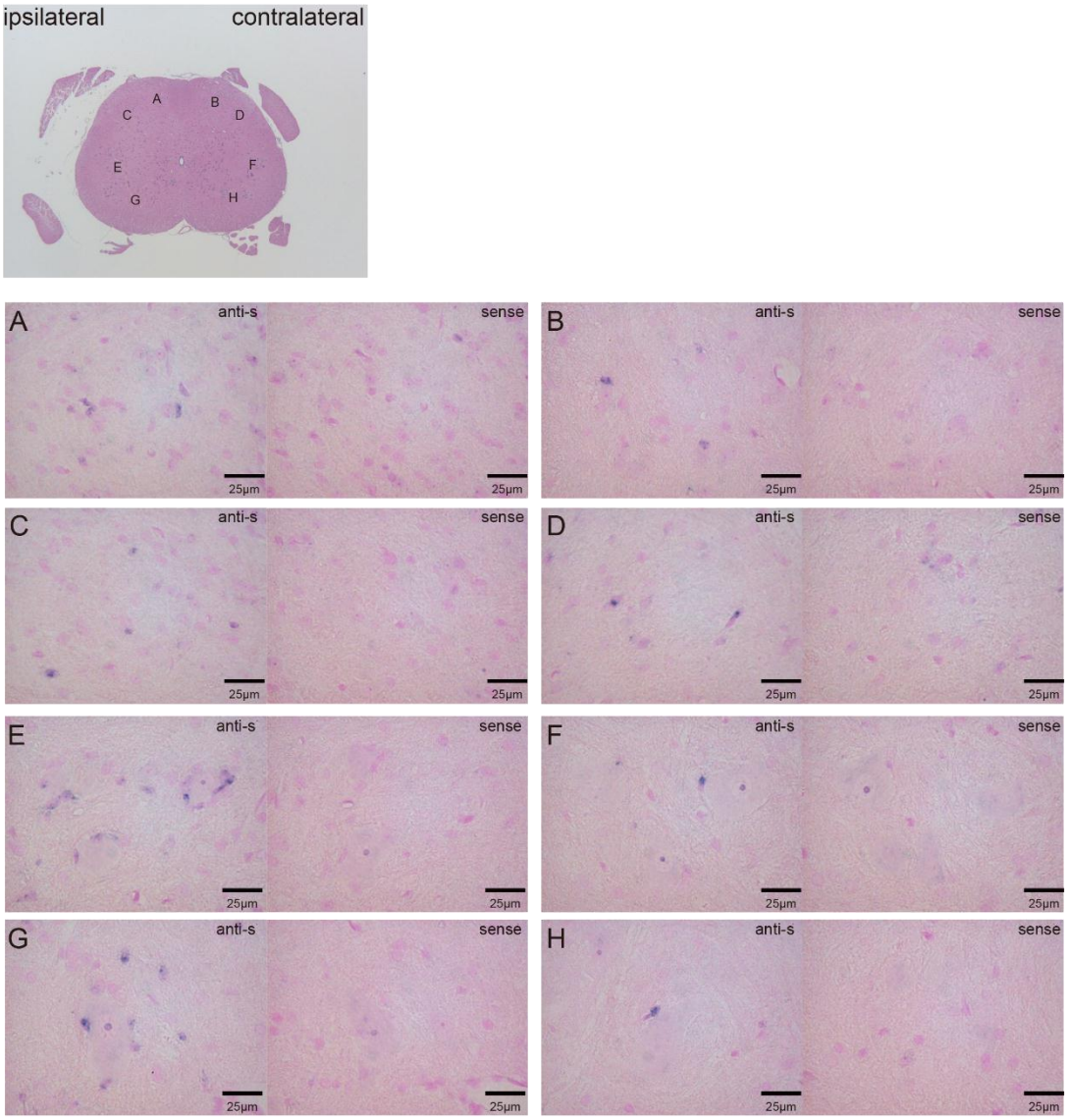

Fig. 3. Histological analyses of chemokine (C-C motif) receptor 5 (CCR5) messenger RNA (mRNA) expression in the spinal cord 7 days after peripheral nerve injury. Each labeled images (A-H) represent corresponding locus in upper left low magnification image. Detailed method for staining was described in supplemental digital content 2. Localization of CCR5 mRNA (anti-s) is shown as deep purple of nitro-blue tetrazolium chloride/5-Bromo-4-Chloro-3'-Indolylphosphatase p-Toluidine salt and counter staining was performed with Kernechtrot nuclear fast red staining. The right of each image (sense) was obtained using probe having complementary sequence of anti-s probe which is used as negative control of staining. CCR5 mRNA positive staining were overall weak but part of cells with small cell body had positive staining. Apparently, nuclear fast red staining indicates increased cell number in ipsilateral side of spinal cord, that is consistent with increased cell number of microglia. In the ventral horn some of motor neuron-like cells were surrounded by CCR5 mRNA-positive cells that is typical of microglia after

spinal nerve injury. There is no difference of staining intensity in individual cells between ipsilateral and contralateral side, however, number of CCR5 mRNA-positive cells were obviously increased in ipsilateral side to the peripheral nerve injury.