## Supplementary Methods

**Behavioural studies.** A unilateral L5 spinal nerve of male Wistar rats was tightly ligated and cut just distal to the ligature. To assess the mechanical allodynia, the calibrated von Frey filaments (0.4-15.1 g) were applied to the plantar surface of the hindpaw from below the mesh floor. The 50% paw withdrawal threshold was determined using the up-down method (Chaplan, S. R., Bach, F. W., Pogrel, J. W., Chung, J. M. & Yaksh, T. L. Quantitative assessment of tactile allodynia in the rat paw. *J Neurosci Methods* **53**, 55-63, 1994). Rats were implanted with a PE-10 polyethylene tube to the lumber enlargement (Yaksh, T. L., Jessell, T. M., Gamse, R., Mudge, A. W. & Leeman, S. E. Intrathecal morphine inhibits substance P release from mammalian spinal cord in vivo. *Nature* **286**, 155-157, 1980) for intrathecal injection of P2XR antagonists [2',3'-O-(2,4,6-trinitrophenyl)adenosine 5'-triphosphate (TNP-ATP) (Sigma-RBI) and pyridoxalphosphate-6-azophenyl-2',4'-disulphonic acid (PPADS) (Sigma-RBI)], $P2X_4R$ antisense or mismatch ODN. We confirmed that PPADS and TNP-ATP used in the present study show biological activity to inhibit effects of $\alpha,\beta$-methylene ATP *in vitro* and *in vivo*. The 16-base antisense ODN endcapped with phosphorothioate linkages were designed according to the primary sequence of the rat $P2X_4R$ cDNA (X87763). The sequence of the $P2X_4R$ antisense and mismatch ODN was 5'-CAGCCCGCCATGGCTC-3' and 5'-ACCGCCGCCAGTGCCT-3', respectively. The mismatch ODN served as a control. Rats were injected intrathecally with antisense ODN (5 nmol 10 $\mu l^{-1}$) and mismatch ODN (5 nmol 10 $\mu l^{-1}$) using a 25-μl Hamilton syringe with 28-gauge needle once a day from day 0 (immediately after nerve injury) to day 6. On day 7, paw withdrawal threshold of antisense ODN- and mismatch ODN-treated rats were tested. After the test, to quantify the levels of $P2X_4R$ protein in the spinal cord in both groups using Western blot analysis, the membrane fraction from the ipsilateral spinal cord segments L4-L6

was prepared. For the experiments in which cultured microglia were administered intrathecally, the cultured microglia were pre-incubated with ATP (50 μM) or PBS for 1 hr at 37 °C immediately prior to administration. Microglia with their supernatant were injected intrathecally in normal rats and paw withdrawal threshold was tested 1, 3 and 5 hr later. TNP-ATP (10 μM) was preincubated with microglia starting 10 min prior to ATP application.

**Immunohistochemistry.** Transverse L5 spinal cord sections (30 μm) were incubated for 2 hr at room temperature in a blocking solution (3% normal goat serum) and then incubated for 48 hr at 4 °C in the primary antibody for $P2X_4R$ (anti-$P2X_4$ receptor, 1:500, Alomone). Markers of microglia, OX42 (anti-OX42, 1:100, Chemicon) and iba1 (anti-iba1, 1:2000, gifted from S. Kohsaka); astrocytes, glial fibrillary acidic protein (GFAP, anti-GFAP, 1:500, Boehringer Mannheim); spinal cord neurones, neuronal marker (NeuN, anti-NeuN, 1:200, Chemicon) and microtubule-associated protein-2 (MAP2, anti-MAP2, 1:500, Chemicon); perivascular macrophages, ED2 (anti-ED2, 1:800, Serotec) were used to identify the type of $P2X_4R$-positive cells. Following incubation, tissue sections were washed and incubated for 3 hr at room temperature in the secondary antibody solution (anti-rabbit IgG conjugated Alexa Fluor$^{TM}$ 488 or anti-mouse IgG conjugated Alexa Fluor$^{TM}$ 546, 1:1000, Molecular Probes). The spinal cord sections were analysed using a MicroRadiance Confocal Imaging System (Bio-Rad) and an Olympus IX70 microscope equipped for epifluorescence. To assess immunofluorescence staining of cells quantitatively, we randomly selected dorsal horn fields displayed at high magnification. Microglia, as identified by OX42 immunofluorescence were outlined, and the immunofluorescence intensity (using a 9-bit scale) of the $P2X_4R$ or OX42 was determined as the average pixel intensity within each cell. Background fluorescence intensity was determined and was subtracted from the value obtained for microglia.

**Western blotting.** The membrane fraction from spinal cord segments L4-L6 ipsilateral or contralateral to the nerve injury was used. Twenty μg aliquots were subjected to 12.5 % SDS-PAGE, and proteins were transferred electrophoretically to nitrocellulose membranes. After blocking, the membranes were incubated with anti-rat $P2X_4R$ polyclonal antibody (1:200; Oncogene) and then were incubated with HRP-conjugated secondary antibody. The blots were detected using a chemiluminescence method (ECL system; Amersham) and exposed to autoradiography films (Hyperfilm-ECL; Amersham).