Supplementary information, Data S1 Materials and Methods

Animal model and tissue preparation

All efforts were made to minimize animal discomfort after nerve injury. Sprague-Dawley male rats (200-250 g, Shanghai center of experimental animals, CAS, Shanghai, China) were anaesthetized with sodium pentobarbital (60 mg/kg). To analyze the expression of FSTL1 and the effect of FSTL1 on nociceptive responses, we prepared the spared nerve injury (SNI) model as described for rats (Decosterd and Woolf, 2000). The SNI model involves a lesion of two of the three terminal branches of the left sciatic nerve (tibial and common peroneal nerves) leaving the remaining sural nerve intact. To further analyze the effect of nerve injury on FSTL1 expression, we also made the transection of the left sciatic nerve of rats at mid-thigh level and a 5 mm portion of the nerve was removed. All surgical interventions and post-operational care were carried out in accordance with the guidelines of the Committee for Research and Ethical Issues of the International Association for the Study of Pain, and approved by Institute of Neuroscience, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences. The rats were allowed to survive for 2, 7, 14 and 21 days (6 rats for each time point). L4 and L5 DRGs of these rats and of 6 normal rats were then processed for *in situ* hybridization, immunohistochemistry or immunoblotting.

For *in situ* hybridization, operated rats and control rats were anesthetized. L4 and L5 DRGs were dissected and frozen on dry ice. For immunohistochemistry, operated rats and normal rats were anaesthetized and perfused via the ascending aorta with

warm (37 ℃) saline followed by warm solution composed of 4% paraformaldehyde and 0.2% picric acid in 0.1 M phosphate buffer. The perfusion was then immediately followed by 200 ml of the same fixative (4 ℃) for another 5 min. L4 and L5 DRGs and spinal cord segments were dissected out. The tissues were post-fixed in the same fixative for 90 min at 4 ℃, and were then immersed in 10% sucrose in 0.1 M phosphate buffer overnight.

In Situ Hybridization

Antisense oligo DNA probe for FSTL1 (5'TCCCTGGTGCTTCTGGAGTTCCTGGGCATATCTCGTCATCTCCTCCTCTGTG3') was synthesized and labeled with digoxigenin. Sections of L4 and L5 DRGs from adult male rats were hybridized for 18 h at 42°C. The hybridization signal was detected with an alkaline phosphatase-coupled antibody against digoxigenin, as well as nitroblue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate as color reaction substrates.

Immunoblotting

Tissues were sonicated/homogenized and centrifuged. The samples was loaded on SDS-PAGE, transferred, probed with antibodies and visualized with enhanced chemiluminescence (ECL) (Roche). Antibodies against FSTL1 (1:10,000) or actin (1:10,000; Chemicon) were used. Quantification was based on 3 independent experiments. ECL signal intensity of FSTL1 versus GADPH was quantified with NIH

image program.

Immunostaining

Adult male rats were fixed with 4% paraformaldehyde. For all experimental groups, 14 μm-thick sections of the fixed L4-5 DRGs and L4-5 spinal cord segments were cut in series in a cryostat and mounted on same gelatin-coated slides. The sections were processed with indirect immunofluorescent staining. The antisera were diluted in phosphate buffered saline with 1% bovine serum albumin and 0.3% Triton X-100. Briefly, the sections were incubated with a mixture of rabbit anti-FSTL1 antibodies (1:4,000~10,000) and goat anti-GSLI antibody (1:1,000; Vector) to identify IB4 overnight at 4 °C. After several rinses in PBS, the sections were incubated with fluorescence-conjugated donkey anti–rabbit (1:100; Jackson ImmunoResearch) and rhodamine-conjugated donkey anti-goat IgG (1:100) for 30 min at 37 °C. The sections were rinsed and mounted with a mixture of glycerol/PBS (3:1) containing 0.1% paraphenylenediamine and examined under a Leica SP2 confocal microscope (Leica Microsystem).

For quantitative analysis, at least 5 sections from each DRG at each time point were used for quantitative analysis. The sections were randomly selected in every three sections. Three animals were analyzed in each group. The number of immunostained neuron profiles divided the total number of neuron profiles, and the percentage of labeled neuron profiles was determined. To determine the percentage of labeled neuron within a subset of DRG neurons, we counted the neuron profiles with

a clear nucleus.

Behavioral Tests

Adult male rats were housed under a 12:12 h light/dark cycle at 22-26ºC. Mechanical stimuli were applied using ascending graded individual monofilaments. A von Frey filament was applied 5 times (several seconds for each stimulus) to each testing site of the hindpaw. The bending force of the von Frey filament to evoke paw withdrawal with over 50% occurrence frequency was determined as the mechanical threshold.

Statistical analysis

Paired data were evaluated by Student *t*-test. Comparisons between multiple groups were performed using two-way ANOVA with a *post hoc* Bonferroni's test. All data are shown as mean ± s.e.m.. P values < 0.05 were considered significant.