Supplementary materials and methods

Sciatic nerve transection and analysis

The left sciatic nerve of anaesthetised 8 week-old female rats was exposed at the sciatic notch and transected. At one and three days after injury, the distal nerve stumps were removed and the most proximal segment, approximately 1 cm long, was excised. Nerves were frozen immediately in liquid nitrogen and stored at -80°C. The right uncut sciatic nerve from each animal was used as control. Samples were analysed by Western blotting or immunohistochemistry.

Western blotting- Frozen nerve samples were ground in liquid nitrogen using an Eppendorf tube micropestle. Samples were lysed in RIPA buffer. Levels of ERK activity were determined by Western blot analysis using anti-phospho-ERK antibody (Sigma).

Immunohistochemistry- 5μm sections from OCT embedded frozen tissue were thawed onto gelatin coated slides and immediately fixed in 4% paraformaldehyde/PBS. Phosphatase inhibitors (1mM sodium orthovanadate and 1mM sodium fluoride) were included in all subsequent solutions. Tissue sections were permeablised with 1% NP40, then with 0.2% Triton-X-100 in TBS with 0.1mM levamisol. Slices were quenched with 0.27% NH_4Cl and 0.37% glycin. Endogenous peroxidase activity was inactivated by incubating with 1% H_2O_2 in PBS and the tissue was blocked with 3% BSA in TBS with 0.2% Triton. Slices were incubated at 4^oC overnight with the primary antibodies: phospho-ERK (New England Biolabs) at 1:100 dilution in 3% BSA in TBS with 0.2% Triton-X-100 and P0 (P07 a gift from J.Archelos). Phospho-ERK was detected by using the Tyramine Signal Amplification kit (Molecular probes).