

Additional file 1 Detailed procedures.

Sciatic nerve crush

Male, age-matched (3–4 months) wildtype C57BL/6J mice (Janvier Labs, France) were anesthetized for surgery via intraperitoneal injection of a mixture of xylazine (Ceva) (10 mg/kg) and ketamine (Serumwerk Bernburg) (100 mg/kg) and placed on a heating plate (37°C) to maintain constant body temperature. The fur of the lower back was removed with an electric razor, and the skin was disinfected using 70 % ethanol. All instruments were sterilized. A small incision (1 cm) was made in the skin above the right hindlimb between the mm. gluteus maximus and biceps femoris. Opening the fascial plane between both muscles revealed the sciatic nerve which was carefully lifted using bent forceps and crushed right before its distal branches using a non-serrated clamp at maximum intensity for 30 seconds. The nerve was replaced under the muscle, and the incision was closed using non-absorbable suture material. The contralateral nerve was left intact to serve as control.

Treatment with high-dose biotin

Mice received biotin (BioReagent grade, Sigma-Aldrich) at a concentration of 60 mg/kg body weight once daily via intraperitoneal injection. Biotin was dissolved in phosphate buffered saline (sterile, Gibco) containing 10% dimethyl sulfoxide (BioReagent grade, Sigma-Aldrich) and adjusted to physiological pH. Before injection, the solution was sterile-filtered. Control animals received an equal volume of vehicle.

Assessment of nerve functionality by grip strength analysis

Nerve functionality was evaluated via grip strength analysis of the right (crushed) and left (non-crushed) hindlimbs using a modified force gauge (Erichsen Physimeter 906 MC-B) at 2 days before crush injury and 7, 14 and 21 days post-injury. The mouse was handled by the experimenter with a tight grip behind its head, still allowing the hind limbs to move freely. Subsequently, the hind limbs were gently pulled over a metal bar and the maximum force applied before the mouse lost its grip was recorded. Mice were tested three times in succession and data were averaged for each mouse and time point.

Sciatic nerve histology

Nerves were fixed in 0.1 M cacodylate buffer containing 2.5% glutaraldehyde and kept at 4°C overnight. The fixative was discarded and replaced by washing buffer (0.1 M cacodylate + 3% sucrose). Nerves were washed for four days at 4°C. Washing was followed by incubation in an osmium tetroxide reagent for 3 hours. Osmium tetroxide reagent was composed of one part 5% potassium dichromate solution (pH 7.4), one part 3.4% NaCl solution and two parts 2 % osmium tetroxide solution (Sigma-Aldrich). Afterwards, samples were briefly washed in 0.1 M cacodylate buffer. Samples were dehydrated in an ascending ethanol series (70%; 96%; ≥ 99.8% undenatured ethanol) for one hour each. Following dehydration, samples were incubated in propylene oxide (Sigma-Aldrich) in tightly closed containers for one hour at room temperature, then one hour in a 1:1 mixture of propylene oxide/epon (Epoxy embedding medium kit;

Sigma-Aldrich) and finally kept at 4°C in epon only overnight. Samples were placed in silicone molds and covered with epon embedding mixture. Embedded samples were incubated at 37°C for 6 hours, at 47°C for 15 hours and finally at 60°C for 28 hours until epon was completely hardened. Sectioning was performed approximately 3 mm distal from the crush site. Transverse sections were prepared at a thickness of 1 µm at an Ultracut Microtome and immediately stained with toluidine blue (1% toluidine blue (w/v) dissolved in a 1% disodium tetraborate (w/v) solution), washed in distilled H₂O (approximately 10 mL) containing 1-2 drops of acid ethanol (0.01% HCl in absolute ethanol), placed on a microscope slide, dried on a heating plate and mounted. Sections were photographed on a Leica DMI8 microscope. One representative slide per nerve was evaluated through detailed morphometric assessment.

Assessment of morphometric data

Morphometric analysis was performed by a blinded investigator using ImageJ (National Institutes of Health, Bethesda, MA, USA). Axon numbers of whole fascicles were measured manually by marking each individual axon. Axon numbers were normalized to fascicle area. For the evaluation of g-ratios and axonal diameters, the circumference of axons and their respective myelin sheaths was measured within randomly selected fields. A minimum of 200 axons per nerve were evaluated. For the calculation of G-ratios, axonal circumference was divided by the circumference of the respective myelin sheath. Axonal diameters were calculated from the axonal circumference.

Determination of malonyl-coenzyme A levels

Sciatic nerve malonyl-coenzyme A (CoA) levels were determined using the commercially available Mouse Malonyl Coenzyme A ELISA Kit (MyBioSource, San Diego, CA, USA). Sciatic nerve samples were homogenized in assay buffer. Detection of malonyl-CoA was performed as recommended by the manufacturer. Sciatic nerve malonyl-CoA content was normalized to the respective protein content of the sample.

Hemogram profiles

At 21 days post-injury, immediately following animal sacrifice, blood was drawn via cardiac puncture using heparinized cannulae. Blood samples were collected in tubes containing EDTA and were carefully mixed to avoid clotting. The ProCyte Dx hematology analyzer (IDEXX, Manchester, MO, USA) was used for the generation of a complete hemogram.

Image and data analysis

Analysis of images was performed using ImageJ. Data analysis and compilation of graphs was performed using Microsoft (Redmond, WA, USA) Excel and GraphPad Prism 7 (GraphPad Software, San Diego, CA, USA, www.graphpad.com). Statistical analysis was done by Student's *t*-test, for multiple comparisons, multiple *t*-test were corrected by Holm-Sidak method.