Supplementary methods

**Animals.** All animal experiments were approved by the national ethics committee on animal experimentation, and were done in compliance with the European community and national directives for the care and use of laboratory animals. *Hb9::GFP* mice (T.M. Jessell's laboratory, Columbia University, NY, USA) were maintained on a CD1 background (Charles River laboratories, Wilmington, MA, USA). *Light<sup>−/−</sup>* mice (K. Pfeffer's laboratory, Heinrich-Heine University, Düsseldorf, Germany) were maintained on a C57BL/6 background.

**Reagents.** Soluble human recombinant protein LIGHT was purchased from Alexis Biochemicals, San Diego, CA, USA. Agonistic polyclonal anti-LT-βR antibodies and antagonistic anti-HVEM antibodies were purchased from R&D systems, Minneapolis, MN, USA. Domoic acid was purchased from Sigma-Aldrich, St Louis, MO, USA. The caspase inhibitors Ac-LEHD-cmk (LEHD), z-DEVD-fmk (DEVD) and the MAPK/ERK inhibitors PD98059 and U0126 were purchased from Calbiochem, San Diego, CA, USA. AlexaFluor 555-conjugated wheat germ agglutinin and the tetramethylrhodamine conjugated α-bungarotoxin were purchased from Life Technologies, Carlsbad, CA, USA.

**Neuron cultures.** Motoneurons were isolated from embryonic day (E)12.5 spinal cord of *Hb9::GFP, Light<sup>+/+</sup>* or *Light<sup>−/−</sup>* embryos as previously described [1], using iodixanol density gradient centrifugation. Motoneurons were plated on poly-ornithine/laminin-treated wells in the presence of a cocktail of neurotrophic factors (0.1 ng/ml glial-derived neurotrophic factor (GDNF), 1 ng/ml brain-derived neurotrophic factor (BDNF) and 10 ng/ml ciliary neurotrophic factor (CNTF) in supplemented Neurobasal medium (Life Technologies). Supplemented Neurobasal contains 2% (vol/vol) horse serum, 25 mM L-glutamate, 25 mM β-mercaptoethanol, 0.5 mM L-glutamine and 2% (vol/vol) B-27 supplement (Life Technologies). Adult sensory neurons were prepared from lumbar (L)4-L5 dorsal root ganglia (DRG) of mice at 50 days of age as previously described [2]. Sensory
neurons were plated on poly-ornithine/laminin-treated glass coverslips in Neurobasal medium supplemented with 2% (vol/vol) B-27 supplement and 2 mM glutamine.

**Survival assays.** Between 60 and 130 Hb9::GFP motoneurons per cm² were counted by using Leica DM fluorescent microscope (Leica Microsystems GmbH, Wetzlar, Germany). In our conditions, the number of living motoneurons remained constant between 24 and 96 h of culture [1]. To allow for comparison of values from different experiments, survival values were expressed relative to the value in the presence of neurotrophic factors only (taken as 100%). Optimal concentration of sLIGHT, agonistic anti-LT-βR antibodies, antagonistic anti-HVEM antibodies, domoic acid, Ac-LEHD-cmk, z-DEVD-fmk has been determined previously [3-7]. PD98059 and U0126 were used at the maximum concentration motoneurons can tolerate regarding survival as we previously determined (not shown).

**Quantification of axon outgrowth and branching.** Neurons were first fixed on ice for 15 min with 2% formaldehyde in PBS-culture medium (1:1) and then for 15 min with 3.7% formaldehyde in PBS. Cells were washed 3 times with PBS and incubated for 1 h at room temperature in PBS containing 4% BSA, 4% serum 0.1% Triton X-100. Neurons were immunostained using primary antibodies directed against: GFP (TP401; Torrey Pines Biolabs, East Orange, NJ, USA; 1:500), non-phosphorylated neurofilament (SMI32; Sternberger Monoclonals, Covance, Princeton, NJ, USA; 1:500), βIII-tubulin (T8660; Sigma-Aldrich; 1:500), pan-axonal neurofilament (SMI312; Sternberger Monoclonals; 1:1,000) and MAP2 (MAP2A,B; Sternberger Monoclonals; 1:1,000). The secondary antibodies used were AlexaFluor 488 or 555-conjugated anti-mouse or anti-rabbit antibodies (Life Technologies). Pictures were taken with a Leica DM IRB camera and axonal length was determined using the ImageJ software and NeuronJ plugin (National Institutes of Health, USA). Axonal outgrowth in dissociated cell cultures and in microfluidic chamber cultures
was analyzed by measuring the length of the longest neurite for SMI32- or GFP-positive motoneuron or βIII-tubulin-positive DRG neurons. The branching was determined by direct counting of the number of branches connected to the longest neurite.

**Time-lapse imaging of neurons.** *Hb9::GFP* motoneurons or sensory neurons were plated on poly-ornithine/laminin coated 24 wells plate at the density of 1,000 cells/cm². Cells were placed on motorized Zeiss Axiovert 200M (Zeiss Microscopy GmbH, Jena, Germany) inverted microscope with an environmental chamber (*temperature* and *CO₂* control). The microscope is equipped with a CCD camera (Micromax, Princeton Instruments, Trenton, NJ, USA) controlled by Metamorph 7.0 microscopy automation and image analysis software (Molecular devices, Sunnyvale, CA, USA). We determined coordinate of motoneurons using GFP native fluorescence. Phase contrast images of about 20 neurons were acquired 24 h after sLIGHT treatment (100 ng/ml) using a LD A-Plan x20/0.5 objective every 30 min for 24 h and after analyzed using the Metamorph 7.0 software. The axon outgrowth velocity was calculated by averaging axon length measures quantified every 30 min for 24 h. Each condition is done in quadruplicate and experiments are repeated independently three times.

**Microfluidic assays.** The microfluidic devices with 750 μm long, 6 μm high and 10 μm wide microchannels were used for compartmentalized cultures. After incubation in 0.8% BSA in L15 medium (Life Technologies) at 37°C, the devices were coated with poly-ornithine (3 μg/ml, 24h at 37°C) and laminin (7 μg/ml, 24 h at 37°C). About 25,000 motoneurons are seeded in the somatic compartment in 250 μl of supplemented Neurobasal medium. The axonal compartment is then filled with the same culture medium. To fluidically isolate the two compartments a 50 μl volume difference is applied between both. The fluidic isolation was validated using retrograde transport of fluorochrome-conjugated WGA [8]. To calculate the number of motoneurons that reached
the axonal compartment, AlexaFluor 555-conjugated WGA (2 µg/ml) was incubated for 2 h at 37°C in the axonal compartment. The number of GFP-positive and AlexaFluor 555 positive-soma was then determined by direct counting in different conditions. Quantification of axon outgrowth and branching was determined as described above.

**Sciatic nerve lesions.** For sciatic nerve crush experiments, we anaesthetized 50-day-old \( \text{Light}^{+/+} \) and \( \text{Light}^{-/-} \) mice with an intra-peritoneal injection of a ketamine and xylazine mixture. Sciatic nerve was exposed and grasped using a halsted-mosquito hemostats (13008-12, Fine ScienceTools Inc., Heidelberg, Germany) 5 mm distal to the sciatic notch. The nerve was crushed by applying a pressure for 30 s. As a surgical control (sham-operated control), the contralateral nerve was exposed and mobilized, but left intact. Axotomy of the sciatic nerve was done in neonates as described previously [9]. Briefly, postnatal day 3 (P3) mice were anesthetized and immobilized by hypothermia. The skin of the right leg was incised parallel to the femur. The right sciatic nerve was exposed and a 3 mm-long segment of the nerve was removed using microscissors.

For peripheral delivery of sLIGHT following the lesion, PBS, sLIGHT (250 ng/ml in PBS) and inactivated LIGHT (sLIGHT\(^{\text{inact}}\), 250 ng/ml) were injected in the tibialis anterior and gastrocnemius muscles. To inactivate sLIGHT, mouse recombinant sLIGHT was heated at 95°C for 15 min. To confirm inactivation, sLIGHT and sLIGHT\(^{\text{inact}}\) were added to motoneuron cultures after 24 h. Cell survival was determined 48 h later (none, 100±10.6%; sLIGHT, 52.4±8.4; sLIGHT\(^{\text{inact}}\) 94.9±6.5%, means ±SEM, \( n = 3 \)).

**Quantitative RT-PCR.** Total RNA was extracted from lumbar spinal cord 10 days after a bilateral sciatic nerve crush using Trizol reagent according to manufacturer's instructions (Qiagen, Germantown, MD, USA). Reverse transcription was done on 1 µg of RNA extract using QuantiTect reverse transcription kit (Qiagen). Real-time PCR was performed in triplicate on 2 µl of reverse transcription product using the Rotor-Gene cycler (Corbett,
RG-300) with rotor-gene SYBR green PCR kit and QuantiTect primer assays from Qiagen, (Light: QT00164080 and Actin: QT00095242). Relative Light mRNA abundance was determined using the \( \Delta \Delta C_t \) method using Actin for normalization [10].

**Immunohistochemistry.** Animals were deeply anesthetized and transcardially perfused with phosphate-buffered saline (PBS, pH 7.4) for collecting fresh tissues or followed by 4% PFA in PBS for immunohistochemistry. The lumbar spinal cord (L4-L5), the sciatic nerve and the tibialis anterior muscle were dissected out. Spinal cord was immersion-fixed in the same fixative over night, sciatic nerve was post-fixed for 6 h and tibialis anterior for 15 min at +4°C. Tissues were immersed in 30% sucrose in PBS and embedded in optimal cutting temperature compound (OCT, CML, Nemours, France).

Eighteen micrometers cryostat sections of the lumbar spinal cord and sciatic nerve (3 mm distal) were washed with PBS and then blocked with 4% bovine serum albumin, 4% heat-inactivated donkey serum in PBS containing 0.1% Triton X-100. Sections were incubated with the following primary antibodies: anti-LIGHT (sc-28880; Santa Cruz Biotechnology, Santa Cruz, CA, USA; 1:50), anti-Iba1 (Wako Chemical Industries, Osaka, Japan; 1:100) or anti-CD3 (C7930; Sigma-Aldrich; 1:200), anti-CD79a (3351; Cell Signaling Technology, Beverly, MA, USA; 1:100), anti-VAcHt (V5387; Sigma-Aldrich; 1:2,500), anti-S100β (Ab868; Abcam, Cambridge, MA, USA; 1:500), CD11b (M1/70.15.11.5.2; DHSB, Iowa, IA, USA; 1:100) or MBP (MAB386; Millipore; 1:300) diluted in blocking solution. Proteins were detected using either fluorochrome-conjugated secondary antibodies (Life Technologies) or Vectastain ABC kit (Vector Laboratories, Burlingame, CA, USA), following manufacturer’s instructions.

For quantification of LIGHT immunoreactivity, spinal cords were collected following PBS perfusion and snap frozen in OCT. Sections (27 sections in total per genotype) were immersed in ice-cold acetone for 10 min and processed for immunohistochemistry as described above using anti-LIGHT antibodies (AF1794; R&D systems; 1:20). All sections
were processed for immunolabeling on the same day and LIGHT-labeling intensities in large ventral horn neurons was determined using the ImageJ software.

**Neuromuscular junctions.** Longitudinal muscle sections were washed with PBS, followed by a 2 h incubation in 5% BSA, 0.5% Triton X-100 in PBS and incubated overnight at 4 °C with rabbit anti-neurofilament-M (AB1987; Millipore, Bedford, MA, USA; 1:1,000) and anti-VACHT (V5387; Sigma-Aldrich; 1:2,500) antibodies diluted in 5% BSA, 0.5% Triton X-100 in PBS. Then, the muscles were incubated in conjugated donkey anti-rabbit AlexaFluor488 secondary antibody combined with α-bungarotoxin rhodamine conjugate (1:1,000) for 2 h at room temperature diluted in PBS containing 5% BSA and 0.5% Triton X-100. Sections were mounted in moviol containing 1,4-diazabicyclo-[2.2.2]-octane (DABCO, Sigma-Aldrich). Images were collected using an Apotome Axio Imager Z2 microscope (Zeiss).

**Motoneuron survival in vivo.** We determined the number of surviving motoneurons 10 days after sciatic nerve crush in the adult mice and after axotomy of P10 mice. Sixteen µm lumbar spinal sections were immunostained with the motoneuron marker VACHT as mentioned above. VACHT-positive cells in both sham-operated and lesion sides were counted every five sections for a total of 20 sections per spinal cord.

**Western blotting.** Lumbar spinal cord, sciatic nerve and tibialis anterior were homogenized in 50 mM Tris-HCl pH 7.5, 150 mM NaCl, 2 mM EDTA, 2 mM EGTA, 1% SDS and a cocktail of protease inhibitors (Roche Diagnostics, Indianapolis, IN, USA). Thirty µg of total proteins, as determined by the BCA protein assay reagent (Thermo scientific, Waltham, MA, USA) were separated by a sodium dodecyl sulfate polyacrylamide gel electrophoresis (4-12% polyacrylamide gel) and transferred onto nitrocellulose membrane (Millipore)[1, 3]. For protein extraction from primary motoneuron cultures,
cells from 4 wells (15,000 motoneurons per 2 cm² well) were collected and pooled together in 50 µl of 50 mM Tris-HCl, 150 mM NaCl, 0.1% SDS, 0.5% sodium deoxycholate and 1% Triton X-100. Due to limited amounts of material, protein extracts were not quantified prior to gel electrophoresis but normalized to the actin loading control. Protein extracts were separated by electrophoresis on a 4-15% TGX gel (Bio-Rad, Hercules, CA, USA) and blotted onto a PVDF membrane. Membrane saturating step was done with 5% non-fat dry milk in PBS containing 0.1% Tween-20. Antibody dilution was done in 4% BSA in PBS containing 0.1% Tween-20 and washing steps performed with PBS containing 0.1% Tween-20. The primary antibodies were: anti-phospho-ERK (9101; Cell Signaling Technology; 1:500), anti-ERK (137F5; Cell Signaling Technology; 1:500), anti-LIGHT (sc-28880; Santa Cruz Biotechnology; 1:500), anti-LT-βR (sc-8377, Santa Cruz Biotechnology; 1:500), anti-IFNγ (sc-52557; Santa Cruz Biotechnology; 1:500), anti-actin (AC-40; Sigma-Aldrich; 1:20,000) and anti-GAP-43 (AB5220; Millipore; 1:5,000). The proteins were visualized using ECL detection system (GE Healthcare, Buckinghamshire, UK). For densitometric analysis, immunoblot images were quantified and normalized relative to actin levels using ImageJ software.

**Walking track analysis and hot plate assay.** For walking track analysis, hind paws of unilaterally lesioned Light +/- and Light -/- mice were coated with non-toxic paint and mice were then allowed to walk along a 45 cm-long paper-covered ramp. At indicated times, we determined print length and toe spread for at least four left (sham-operated side) and four right (operated side) footprints per animal. Measures were performed using Axiovision release 4.8. The sciatic functional index was then calculated using the formulas established by Inserra, M and colleagues [11, 12].

For the hot plate test, mice were placed onto a heated surface with a constant temperature of 53 ± 0.1 °C. To restrain mice, the hot plate was surrounded by a plastic barrier of 10 cm in diameter and 20 cm in height. The latency to jump off the plate or lick a hind paw was
determined. The experiment was repeated 3 times with a 15 min interval (cut-off time: 30 sec) between each assay and the mean value was calculated.

**Statistical analysis.** Statistical significance was determined by unpaired two-tailed t test or by a one-way analysis of variance (ANOVA) followed by a Tukey-Kramer’s *post hoc* tests. Statistical analysis of the walking track and the hot-plate response was done using a two-way repeated-measure ANOVA followed by a Newman-Keuls’s *post hoc* test. GraphPad Prism (GraphPad Software, La Jolla, CA, USA) and StatSoft Statistica software (Statsoft, Tulsa, OK, USA) were used for calculations. Significance was accepted at the level of *p* < 0.05.

**SUPPLEMENTARY REFERENCES**


