SUPPLEMENTAL DATA

Supplemental methods:

Cell culture. HEK (human embryonic kidney)-293T (ATCC, CRL-3215) and immortal mouse Schwann cells MSC80 (ExPASy, CVCL_S187) were grown in DMEM (Dulbecco's modified Eagle's medium) (Gibco Life Technologies) supplemented with 2 mM L-glutamine, 100 U/ml^{-1} penicillin/streptomycin and 5% (v/v) heat-inactivated fetal bovine serum (all supplements were from Invitrogen). Cells were maintained at 37°C in an atmosphere of 5% CO_2, and were passaged when they were 80–90% confluent, twice a week.

Validation of VDAC1 shRNA in vitro. Five 15 cm dishes of 70 – 80 % confluent mouse Schwann cells were separately transfected with 30 μg of pLKO.1-puro vector containing five commercial VDAC1 shRNA (Sigma-Aldrich, sh1#TRCN0000012388, sh2#TRCN0000012389, sh3#TRCN0000012390, sh4#TRCN0000012391 and sh5#TRCN0000012392) using 80 μl of Lipofectamine 2000 (Invitrogen) and 1,5 ml of Opti-Mem (Gibco Life Technologies). After 7 h, the medium was changed to a fresh complete culture medium enriched with 2mM of glutamine. 48h after transfection cells were treated with 0.5 μg/ml of Puromycin (Gibco Life Technologies) for selection. Antibiotic was maintained in cell medium during one week. Then, cells were collected for western blot.

*Protein extraction and Western blotting.*Transfected cells were washed in PBS, lysed in lysis buffer (10 mM Tris, pH 7.4, 150 mM NaCl, 1% Triton X-100, 0.1% SDS, 0.5% sodium-

deoxycholate, 1mM EDTA, 50mM NaF, 1mM NaVO4, protease inhibitor cocktail (Sigma-Aldrich)) for 15 min on ice, and centrifuged at 14,000 rpm at 4°C to pellet cell debris. Sciatic nerves were dissected from mice, washed in PBS and directly fixed with 4% of PFA for 10 minutes. After removal of the epineurium and perineurium, the nerves were homogenized by sonication in lysis buffer. Cellular debris was removed by centrifugation at 13,000 g for 5 min at 4ºC and protein was quantified by the bicinchoninic acid method using bovine serum albumin as a standard. Then, samples were denatured at 98 °C, loaded on 10% SDS-PAGE, and transferred on PVDF membranes for immunoblotting. Antibody against the phospho-S63-c-jun (1/100, BD Biosciences, Ref. 558036) and the total non-phosphorylated c-jun (1/1000, BD Biosciences, Ref. 610326) were from mouse and antibodies against VDAC (1/1000, Cell Signaling, Ref. 4866), phospho-Thr183/Tyr185-SAPK/JNK (1/1000, Cell Signaling, Ref. 9251), phospho-Thr202/Tyr204-p44/42 MAPK (Erk 1/2) (1/1000, Cell Signaling, Ref. 9101), phospho-Thr180/Tyr182-p38 (1/1000, Cell Signaling, Ref. 9211), Cleaved Caspase-3 (1/1000, Cell Signaling, Ref. 9661), total non-phosphorylated JNK (1/1000, Cell Signaling, Ref. 9252) were from rabbit. Antibody against phospho-S87-bcl2 was from goat (1/500, Santa Cruz Biotechnology, Ref. sc-16323).

Validation of fluorescent probes. Mouse sciatic nerves expressing mito-GCaMP2 or mito-SypHer were isolated three weeks after AAV particles infection. Nerves were washed in PBS and incubated in Leibovitz's L15 medium (Gibco Life Technologies) for 3 hours at 37°C in an atmosphere of 5% CO_2. Sciatic nerve infected with mito-SypHer probe was treated separately with sodium azide 3mM solution at pH 3.2 (Sigma-Aldrich, Ref. S2002) or with ammonium chloride 30 mM solution at pH 8 (Sigma-Aldrich, Ref. A4514) for 5 minutes. Sciatic nerve infected with mito-GCaMP2 was treated separately with calcium chelator EDTA 1mM solution (Sigma-Aldrich, Ref. ED255) or calcium chloride 100 µM solution

(Sigma-Aldrich, Ref. S3014) and saponine 20 µg/µl solution (Sigma-Aldrich, Ref. S4521) for 5 minutes. Mito-SypHer and mito-GCaMP2 probe intensities were quantified at 985 nm using multiphoton microscope.

Ketamine/Xylazine anesthesia. 1 ml of ketamine-HCl (100 mg/ml, IMALGEN, Merial Lab) and 0.1ml of xylazine - HCl (100 mg/ml, ROMPUN, Bayer Sante) were mixed in a sterile tube with 8.9 ml of sterile PBS. The solution was keep away from light and stored to 4 °C. Mice were anesthetized using 0.1 ml of ketamine/xylazine solution per 10 g of body weight by intraperitoneal injection.

Sciatic nerve set up under multiphoton microscope. Firstly, animals were anesthetized with 5% of isoflurane in oxygen (1.5 l/min) into the anesthesia system box (World Precision Instruments, Ref. EZ-B800) for 5 minutes. Then, anesthesia was maintained using an anesthesia mask in 2.5% isoflurane/oxygen (0.4 l/min). The surgery area was shaved and cleaned with betadine and ethanol 70% solution and an incision was realized on the dorsal part of the tight using a scalpel; the skin was retracted using forceps in order to expose the *gluteus superficialis* and *bíceps femoris* muscles. Next, the connective tissue that joins both muscles was cut and the sciatic nerve was gently lifted out using a spatula. A flexible bridge was slide below sciatic nerve, the nerve was embedded in a drop of 3% low melting agarose (Promega, Ref. V2111) in Leibovitz's L15 medium (Gibco Life Technologies) and placed into a first plastic pool fixed to the bridge with a glue. The mouse was then placed under the multiphoton microscope (LSM700 MP-OPO, Zeiss, France) and the bridge was fixed using magnetic brackets to avoid physiological movement. Mouse legs were also fixed using clippers. A dark box fixed on the microscope allowed the control of the temperature to 37 °C during all time-lapse imaging. Finally, a second pool was fixed to the first pool using the drop

of agarose and this second pool was filled with deionized water to immerse the objectives 20x or 63x (Carl Zeiss Microscopy).

Supplemental Figures:

Figure S1

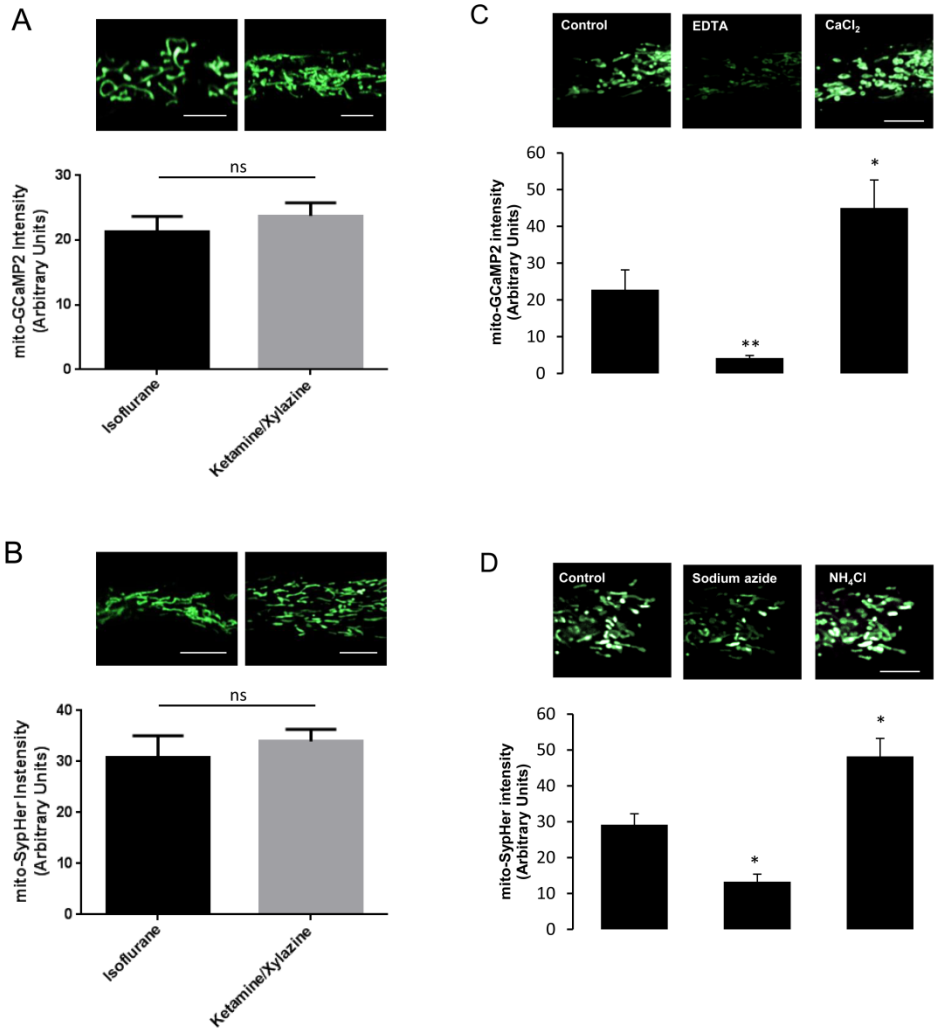


Figure S1. Anesthetic control and *ex vivo* validation of fluorescent mito-GCaMP2 and mito-SypHer probes.

Mitochondrial calcium **(A)** or mitochondrial pH **(B)** in mice anesthetized during five hours with isoflurane (black bars) or 30 minutes with ketamine/xylazine (grey bars). Mito-GCaMP2 and mito-SypHer specifically measure mitochondrial calcium and pH respectively: **(C)** Quantification of mito-GCaMP2 intensity in mouse sciatic nerve incubated with EDTA 1mM or $CaCl_2$ 100 µM and saponine 20 µg/µl solution. **(D)** Quantification of mito-SypHer intensity in sciatic nerve incubated separately with sodium azide 3mM or with ammonium chloride 30 mM solution. Top panels: image example of mSC mitochondria mito-GCaMP2 **(A and C)** or mito-SypHer **(B and D)** intensity. mito-GCaMP2 and mito-SypHer intensities are represented in Arbitrary Units. Scale bar: 5 µm. Asterisks mark statistical differences over control conditions. Error bars indicate SD. n= 3. Two-tailed Student's *t* test: *$p < 0.05$, **$p < 0.01$. ns: non-significant.

Figure S2

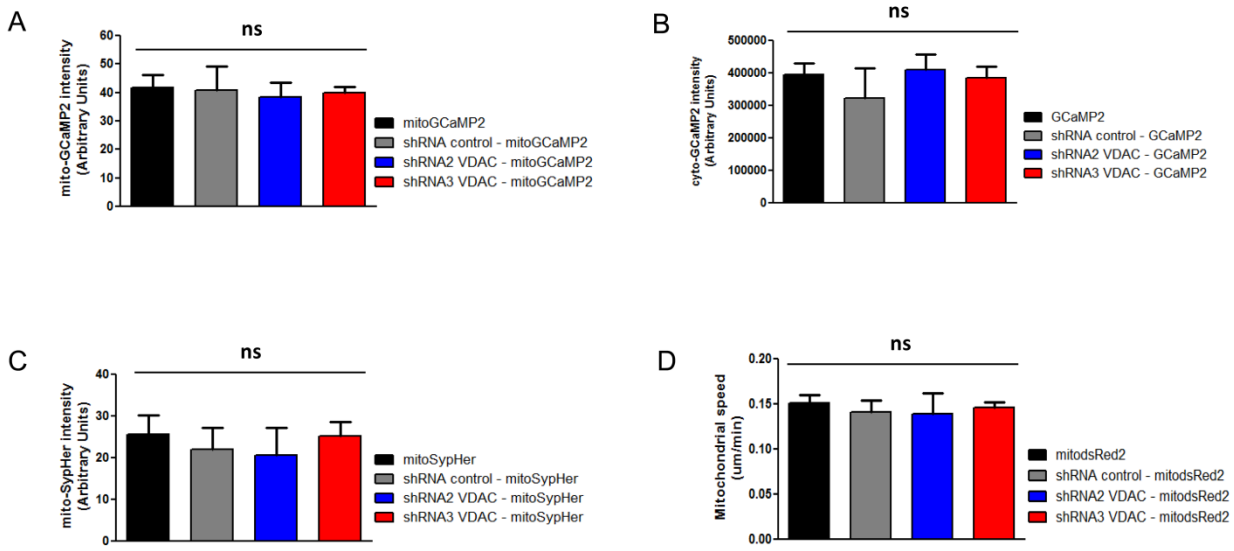
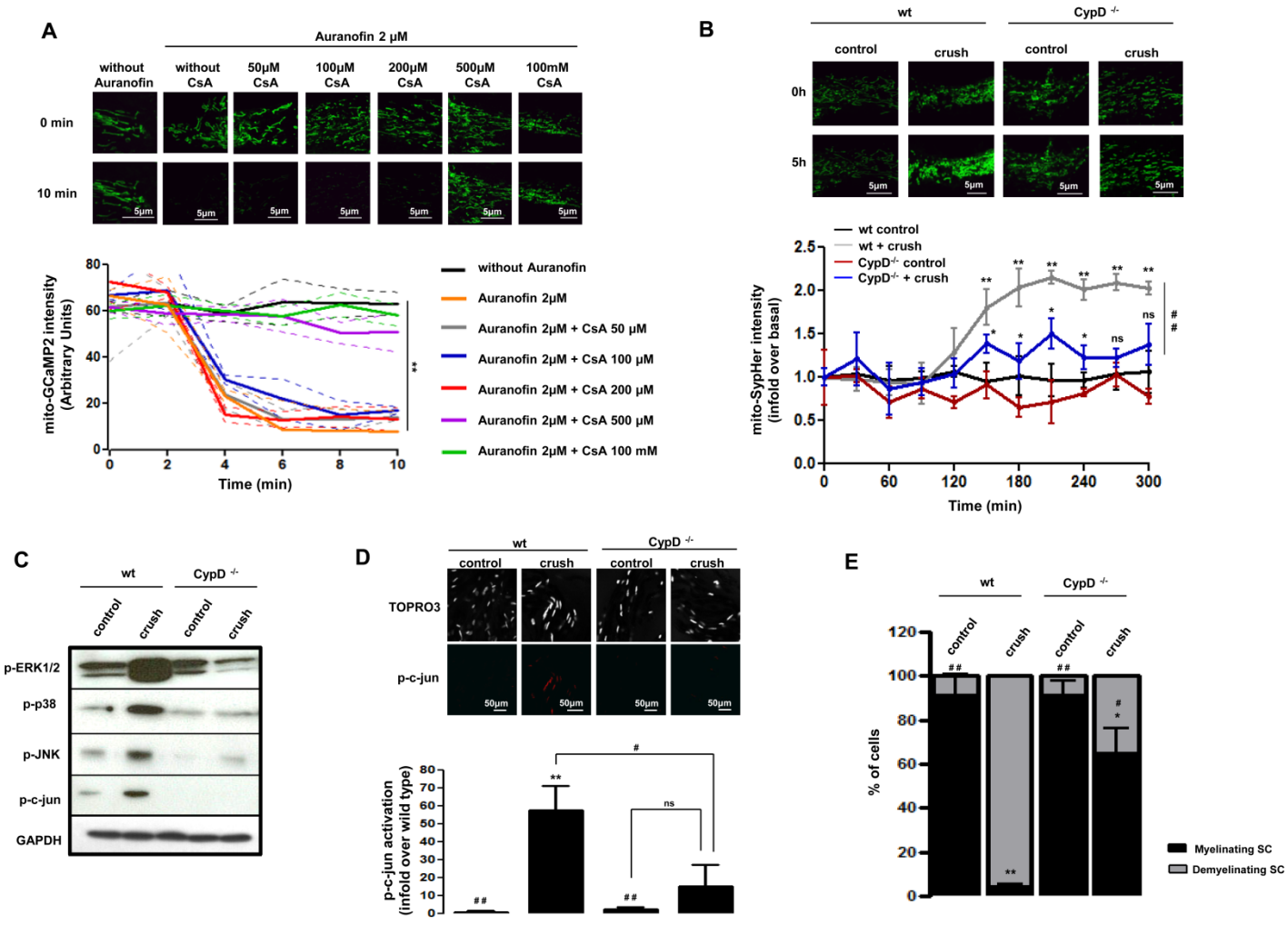


Figure S2. Fluorescent probes intensity during VDAC1 silencing in basal condition.
VDAC1 silencing using shRNA does not change myelinating Schwann cell mitochondrial calcium concentration **(A)**, cytoplasmic calcium **(B)**, mitochondrial pH **(C)** and mitochondrial motility **(D)** in basal condition. Measures were done as described in Multiphoton image acquisition.

Figure S3



FigS3. mPTP formation is required for Schwann cell demyelination. (A) Mitochondrial calcium changes after Auranofin 2µM injection and increasing concentrations of Cyclosporine A (CsA) **(B)** Mitochondrial calcium and cytoplasmic calcium changes of wild type and CypD$^{-/-}$ mice after crush or control conditions. **(C)** Sciatic nerve western blot analysis of phosphorylated ERK1/2, p38, JNK and c-jun of wild type (wt) and CypD$^{-/-}$ mice 12 hours after crush or in control conditions. GAPDH was used as loading control. **(D) Upper panels:** Immunohistochemistry for phospho-c-jun in mSC of wild type (wt) and CypD$^{-/-}$ mice 12 hours after crush or in control conditions. **Lower panel**: quantification of phospho-c-jun fluorescence intensity. **(E)** Quantification of myelinating and demyelinating SC frequency in wild type and CypD$^{-/-}$ mice 4 days after crush or in control conditions . Data are expressed as the mean ± SEM. Error bars indicate SEM. n = 3 mice for each group. Asterisks and hashes mark statistical differences over non-crushed and crushed nerves respectively. Two-tailed Student's *t* test: * and $^{\#}p < 0.05$, ** and $^{\#\#}p < 0.01$. ns: non-significant.

Figure S4

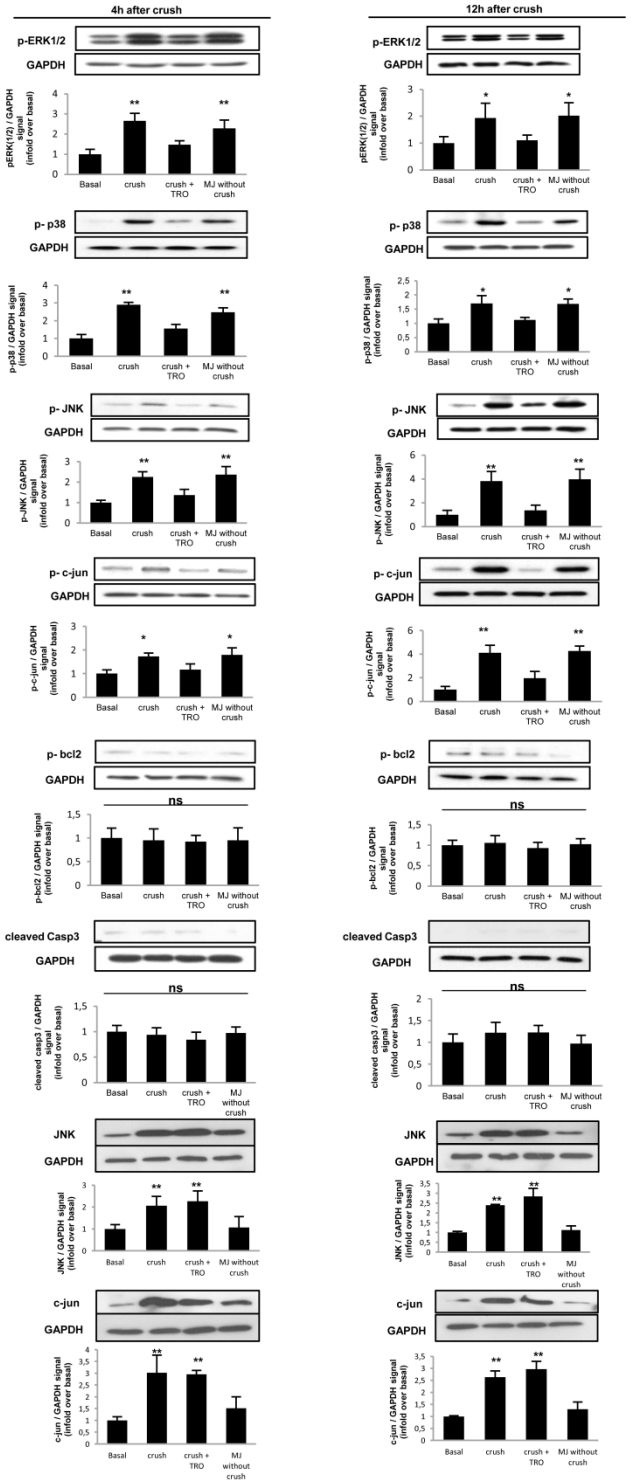


Figure S4. Western blot quantification of cell signaling activation after crush and drug treatments.

Sciatic nerve western blot quantification of activated p-ERK1/2, p-p38, p-JNK, p-c-jun, p-blc2, cleaved caspase3, total c-jun and total JNK, 4 hours (left panels) or 12 hours (right panels) after crush, after crush+TRO19622 and MJ treatments. GAPDH was used as loading control. Ratio protein signaling activation is represented as infold over basal condition (non-crushed nerves). In top panels representative western blot images of each phosphorylated protein. The same data were shown in Figure 3A. Error bars indicate SD. n = 3 – 4 mice for each treatment.. Asterisks mark statistical differences over basal conditions. Two-tailed Student's *t* test: *p< 0.05, **p < 0.01. ns: non-significant.

Figure S5

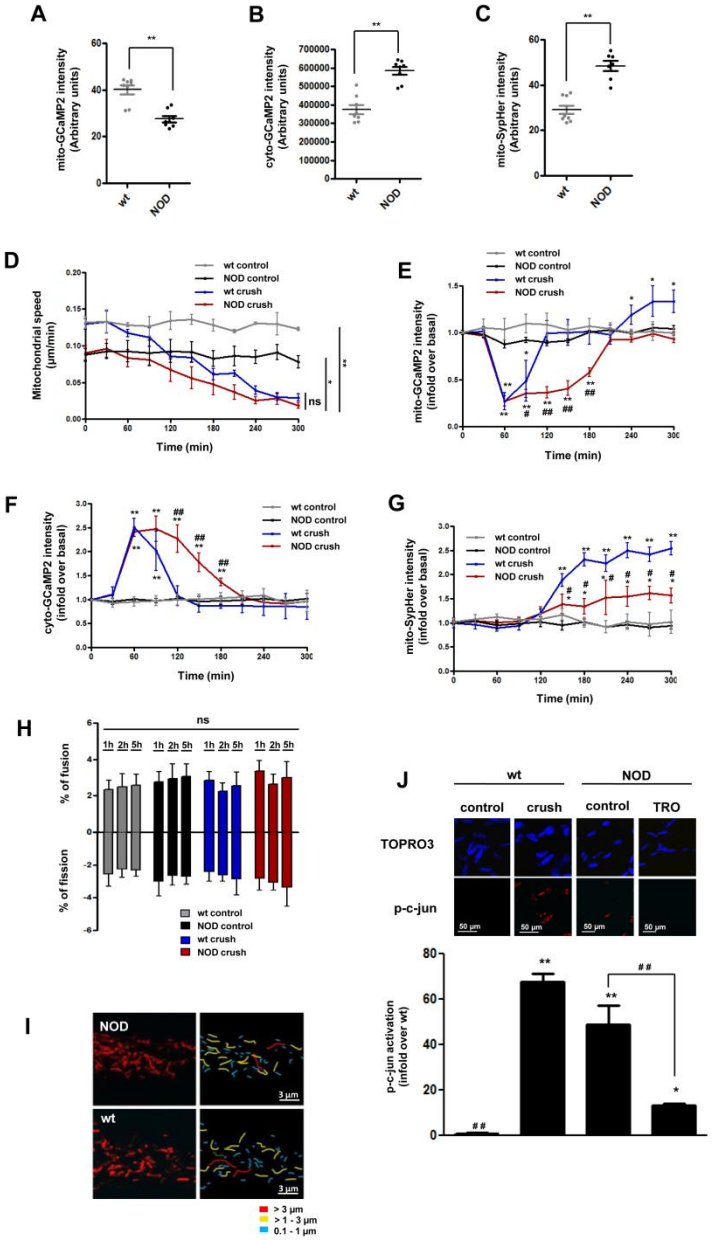


Figure S5. Mitochondrial physiology is altered in Schwann cells of non-obese diabetic (NOD) mice. Mitochondrial calcium **(A)**, cytoplasmic calcium concentration **(B)** and mitochondrial pH **(C)** in mSC of control (wt) and NOD mice in basal conditions. Mitochondrial motility **(D)**, mitochondrial calcium **(E)**, cytoplasmic calcium concentration **(F)** and mitochondrial pH **(G)** changes after crush or in control non-crushed conditions in mSC of control (wt) and NOD mice. **(H)** Ratio of mitochondrial fusion and fission events in mSC of control (wt) and NOD mice in crushed or control non-crushed conditions. **(I)** Representative images of mSC mitochondria of control (wt) and NOD mice after 300 min of time-lapse imaging acquisition. **(J) Upper panels:** Immunohistochemistry for phospho-c-jun in mSC of control (wt) and NOD mice in basal conditions, or treated with TRO19622 (TRO). **Lower panel**: quantification of phospho-c-jun fluorescence intensity. Data are expressed as the mean ± SEM. Error bars indicate SEM. n= 3 mice. Asterisks and hashes mark statistical differences over non-crushed control mice and crushed control mice respectively. Two-tailed Student's *t* test: * and $^{\#}p < 0.05$, ** and $^{\#\#}p < 0.01$. ns: non-significant.

Figure S6

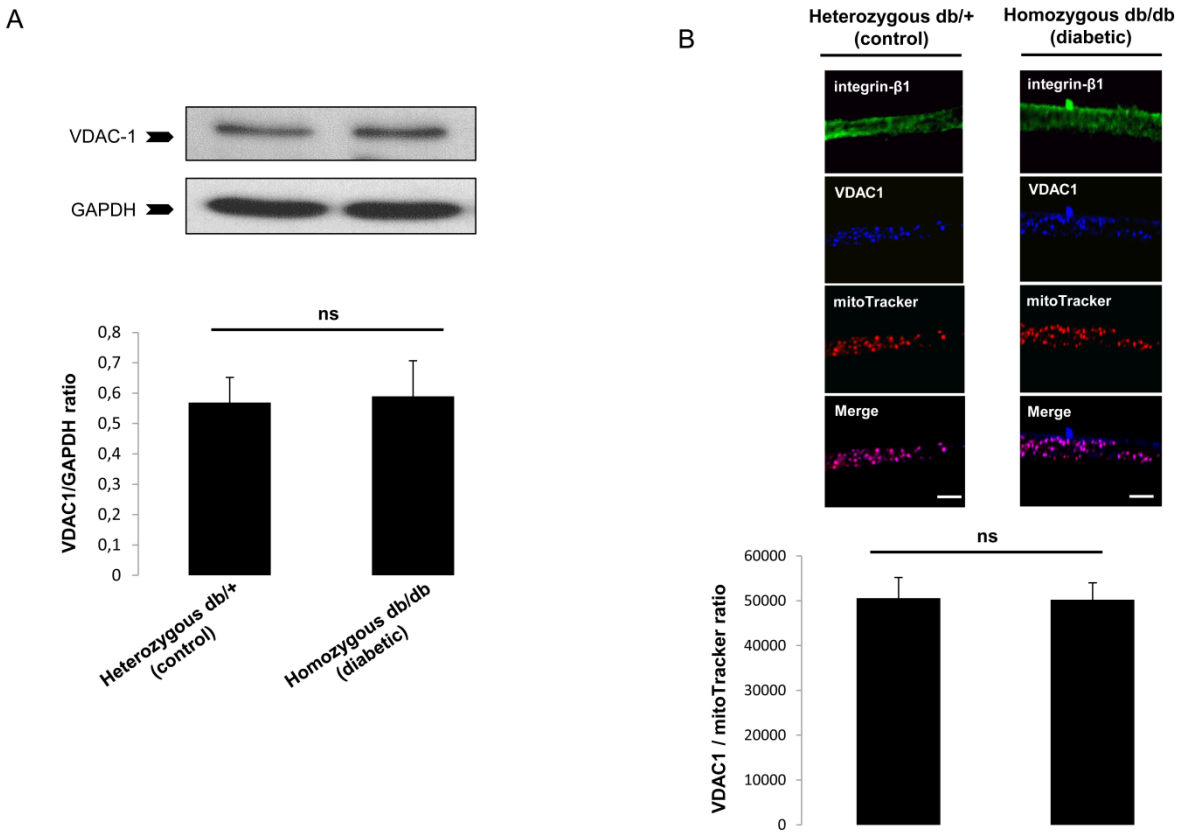


Figure S6. No change in mitochondrial VDAC1 expression in control and diabetic mice
(A) Western blot analysis for VDAC in sciatic nerve of heterozygous db/+ (control) and homozygous db/db (diabetic) mice. GAPDH was used as loading control. n = 3. **(B)** Immunohistochemistry for VDAC1 expression in mSCs of heterozygous db/+ (control) and homozygous db/db (diabetic) mice. In green integrin-β1 labeling myelinating Schwann cell structure, in blue: VDAC1 expression is restricted to mitochondria and in red: mitoTracker labelled mitochondria. Scale bar: 50 µm. Mitochondrial VDAC1 quantification in represented in bottom graph as VDAC1 / mitoTracker ratio intensity. n = 5. Error bars indicate SD. Two-tailed Student's *t* test. ns: non-significant.

Figure S7

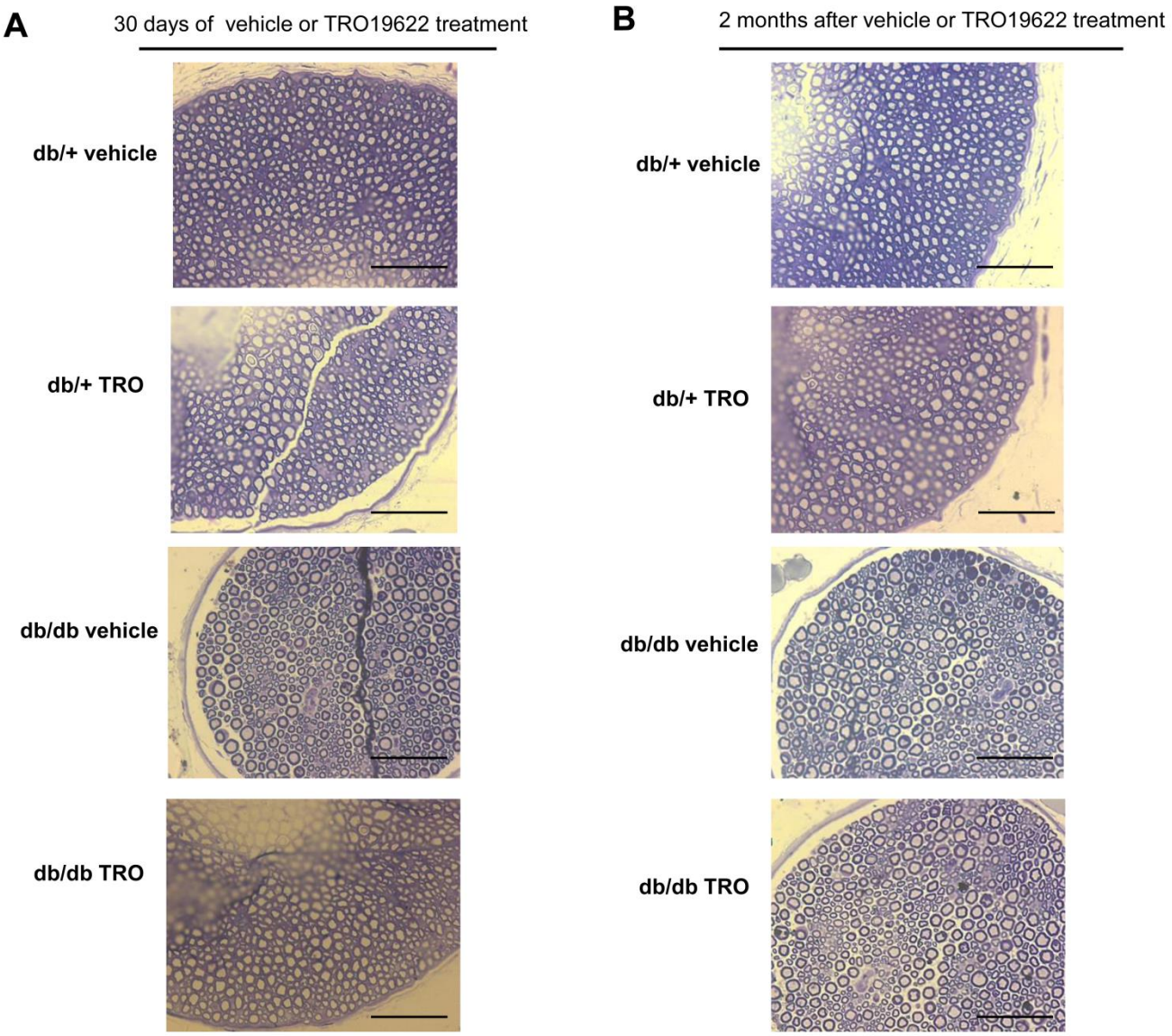


Figure S7. Toluidine blue staining of diabetic mouse sciatic nerve transversal sections

(A) Toluidine blue staining of 0.7 µm sciatic nerve cross-sections of control (db/+) and diabetic (db/db) mice after 30 days of vehicle, or TRO 19622 treatment or **(B)** two months after the last vehicle or TRO 19622 treatment. Mice aged 20 weeks **(A)** and 28 weeks **(B)**. Scale bar: 50 µm

Figure S8

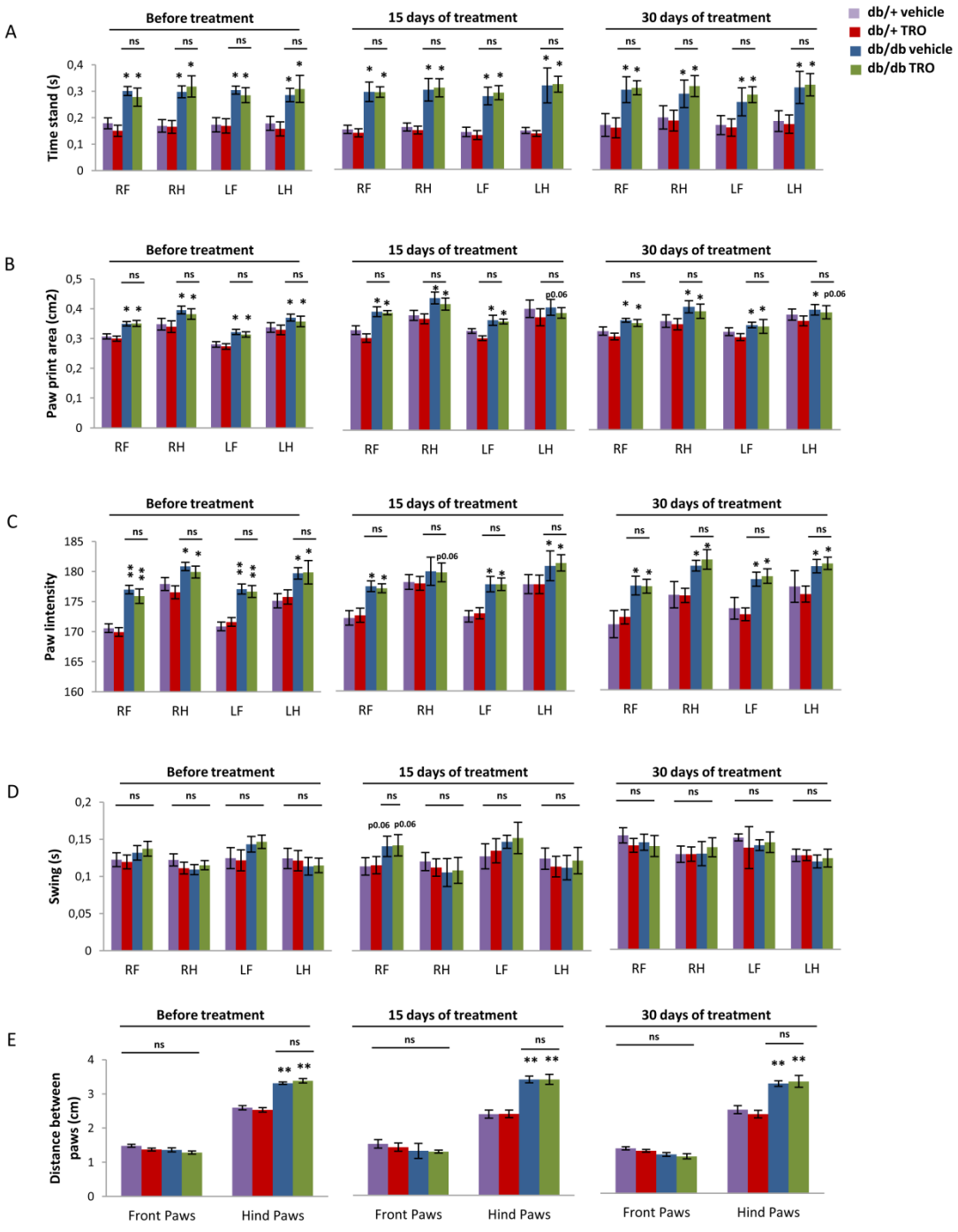


Figure S8. Complete catwalk test parameters during diabetic mice TRO19622 treatment

Evolution of time of paw stand **(A)**, paw print area **(B)**, paw intensity **(C)**, time of fly between paws (swing) **(D)** and paw distance **(E)** of 20 weeks old control (db/+) (purple and red bars) and diabetic (db/db) (blue and green bars) mice, after 15 and 30 days of vehicle (purple and blue bars) or TRO19622 (red and green bars) treatment. Parameters were determined using Catwalk XT 10.5 test system. Error bars indicate SEM. n = 12 mice for each group. Asterisks mark statistical differences over control mice and ns marks non-significant differences over diabetic vehicle treatment and/or control mice. One-way ANOVA followed by a Dunnett's multiple comparison *post hoc* test: *p < 0.05 and **p < 0.01. RF: right front paw, RH: right hind paw, LF: left front paw, LH: left hind paw.

Figure S9

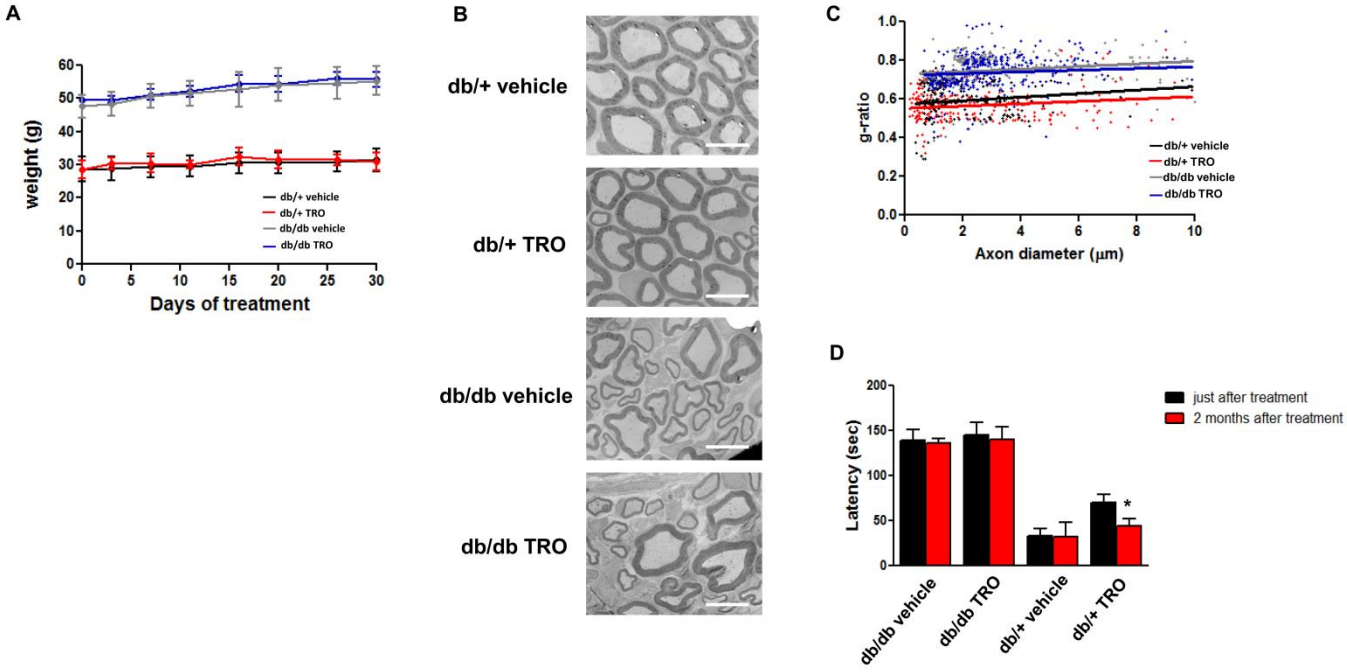


Figure S9. Diabetic and control mice weight during TRO 19622 treatment and TEM, g-ratio and rotarod latency of control and diabetic mice two months after vehicle and TRO 19622 treatments.

(A) Weight evolution of 20 weeks old diabetic (blue and grey lines) and control (black and red lines) mice after the vehicle or TRO19622 treatment. Non-significant differences are found in vehicle and TRO19622 treatment. Error bars indicate SEM. n = 12 mice for each group. **(B)** Representative transmission electronic microscopy images of 70 nm sciatic cross-sections of 32 weeks old control (db/+) and diabetic (db/db) mice, two months after vehicle or TRO 19622 treatment. Scale bar: 5 µm. **(C)** Scatterplots showing the myelin sheath thickness (g-ratio) plotted against the axon diameter of sciatic nerve fibers from control (db/+) and diabetic (db/db) mice, two months after vehicle or TRO16922 treatment. n = 6 mice for each group. Minimum 200 axons per animal. Non-significant differences are found in diabetic vehicle and TRO19622 treatments. **(D)** Rotarod test latency of control and diabetic mice just after or 2 months after vehicle or TRO 19622 treatment. n = 6 mice for each group. Asterisks mark statistical differences over mouse latency just after vehicle or TRO19622 treatment. One-way ANOVA followed by a Dunnett's multiple comparison *post hoc* test: *$p < 0.05$.

Figure S10

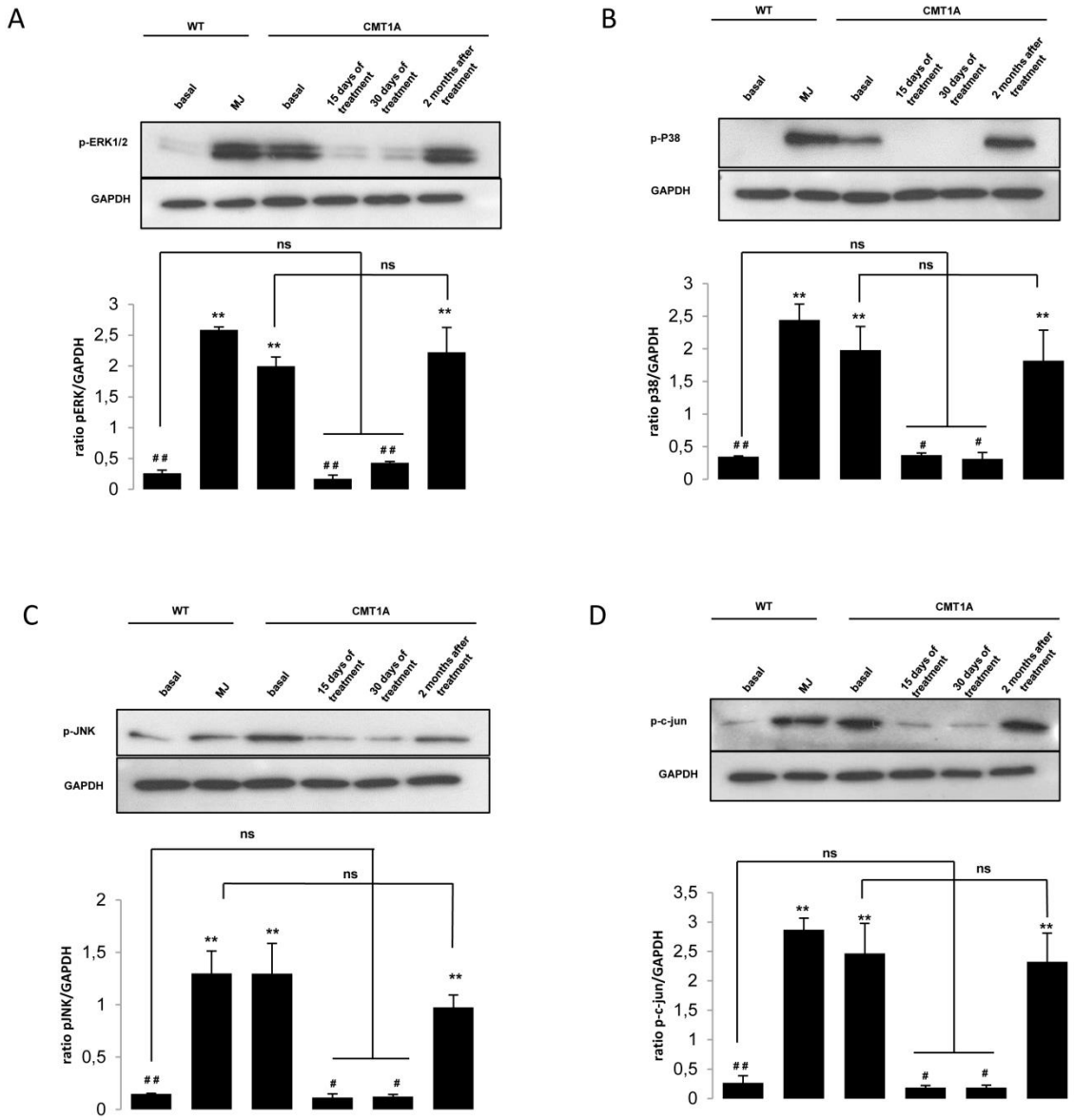


Figure S10. Western blot quantification of cell signaling activation in sciatic nerve from CMT1A rats treated with TRO 19622.

Western blot quantification of activated p-ERK1/2 **(A)**, p-p38 **(B)**, p-JNK **(C)** and p-c-jun **(D)** pathways from sciatic nerve from wt rat in basal conditions or treated with MJ and CMT1A rat treated for 15 days or 30 days with TRO19622 or two months after TRO 19622 treatment. GAPDH was used as loading control. Top panels shown representative Western blot images of each phosphorylated protein. Error bars indicate SD. n = 3 rats for each treatment. Asterisks mark statistical differences over wild type (wt) rats and hashes mark statistical differences over non-treated CMT1A rat (basal). Two-tailed Student's *t* test: * and $^{\#}$ $p < 0.05$, ** and $^{\#\#}$ $p < 0.01$. ns: non-significant.

Figure S11

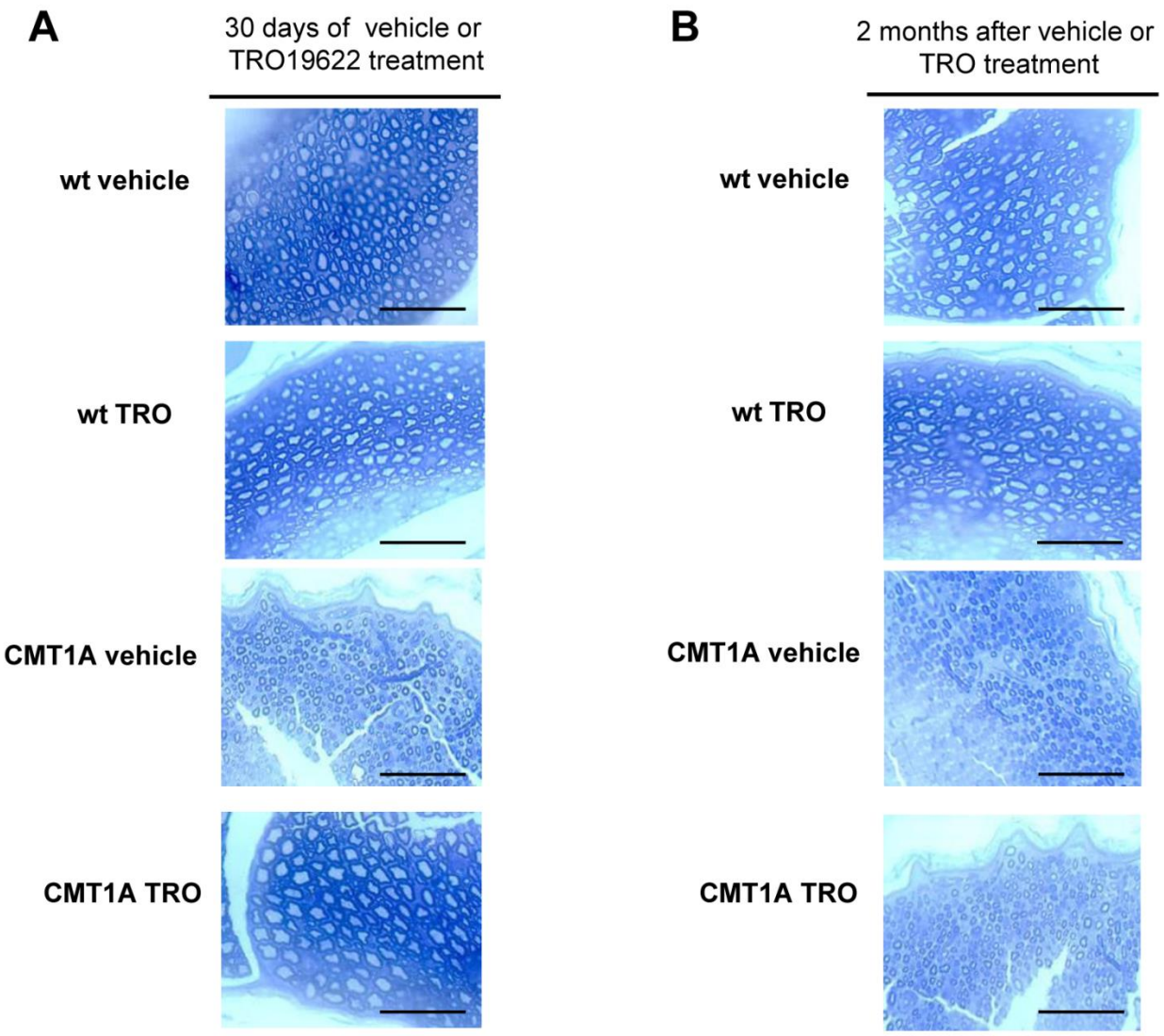


Figure S11. Toluidine blue staining of CMT1A rat sciatic nerve transversal sections
(A) Toluidine blue staining of 0.7 μm sciatic nerve cross-sections of control (wt) and CMT1A rats after 30 days of vehicle or TRO 19622 treatment, or **(B)** two months after the last vehicle or TRO 19622 treatment. Rat aged 16 weeks **(A)** and 24 weeks **(B)**. Scale bar: 50 μm

Figure S12

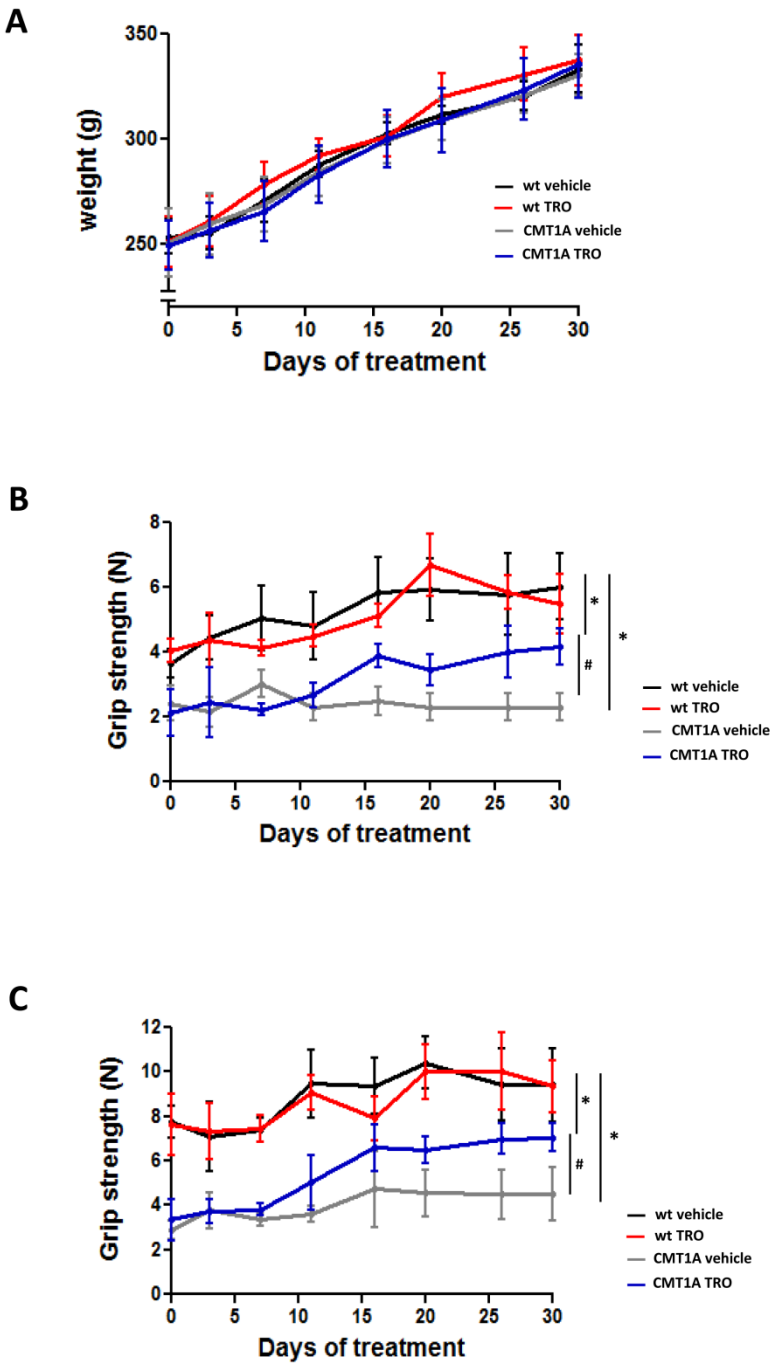


Figure S12. CMT1A and control rat weight and grip strength during TRO 19622 treatment
(A) 12 weeks old CMT1A (blue and grey lines) and control (wt) (black and red lines) rat weight evolution during the vehicle or TRO19622 treatment. Non-significant differences are found in vehicle and TRO19622 treatment. Analysis of front-limb **(B)** and hind-limb **(C)** grip strength of control (wt) and CMT1A rats, during 30 days of vehicle and TRO 19622 treatments. Error bars indicate SD. n = 6 rats for each group. Asterisks mark statistical differences over control rats, and hashes mark statistical differences over CMT1A rats treated with vehicle. One-way ANOVA followed by a Dunnett's multiple comparison *post hoc* test: $^{\#}$ and $^{*}p < 0.05$.

Figure S13

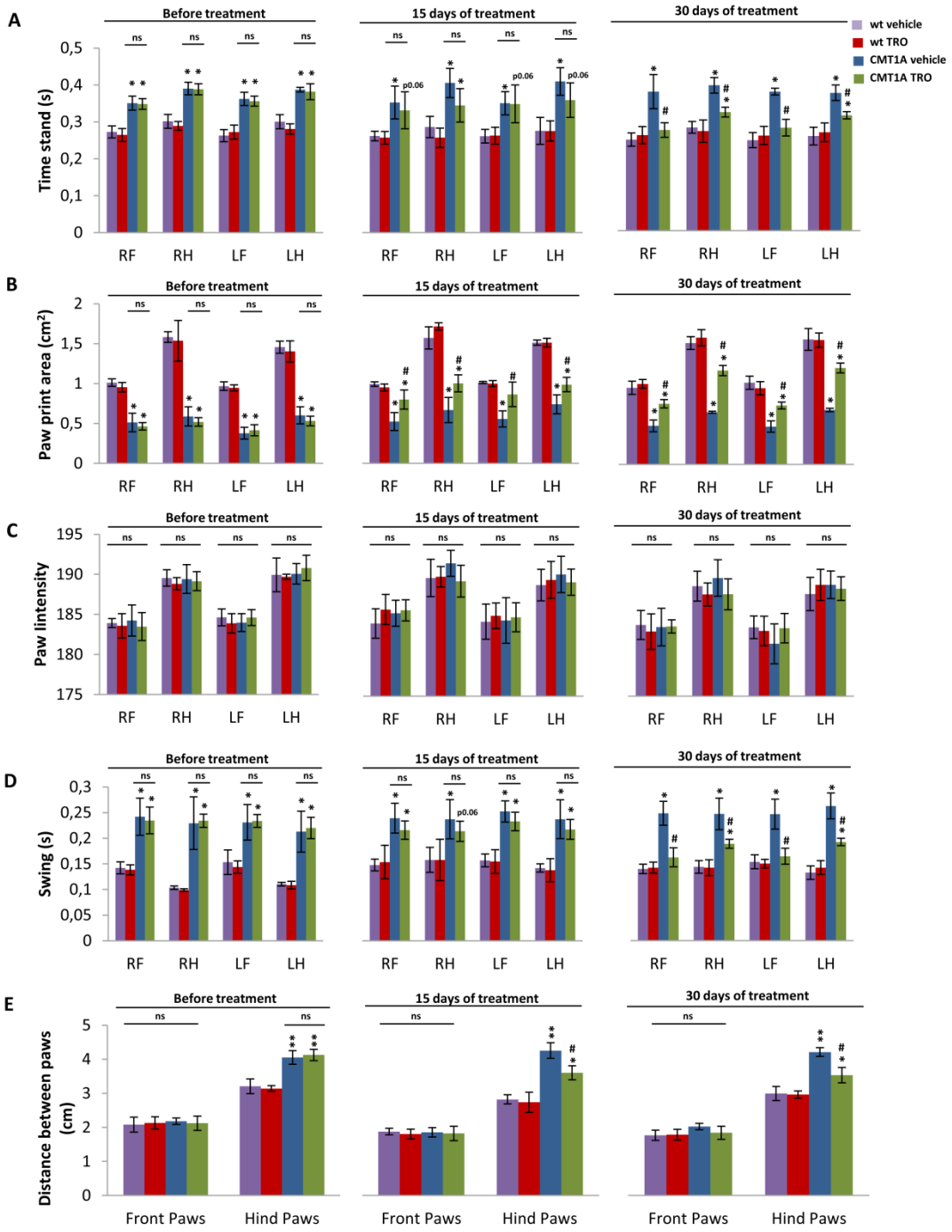


Figure S13. Complete catwalk test parameters during TRO 19622 treatment of CMT1A rats. Evolution of time of paw stand **(A)**, paw print area **(B)**, paw intensity **(C)**, time of fly between paws (swing) **(D)** and paw distance **(E)** of 12 weeks old control (wt) (purple and red bars) and CMT1A (blue and green bars) rats after 30 days of vehicle (purple and blue bars) or TRO19622 (red and green bars) treatment. Parameters were determined using Catwalk XT 10.5 test system. Error bars indicate SD. n = 6 rats for each group. Asterisks mark statistical differences over control rats; hashes mark statistical differences over CMT1A rats treated with vehicle and ns marks non-significant differences over CMT1A rats treated with vehicle. One-way ANOVA followed by a Dunnett's multiple comparison *post hoc* test: *and # $p < 0.05$ and **$p < 0.01$. RF: right front paw, RH: right hind paw, LF: left front paw, LH: left hind paw.

Figure S14

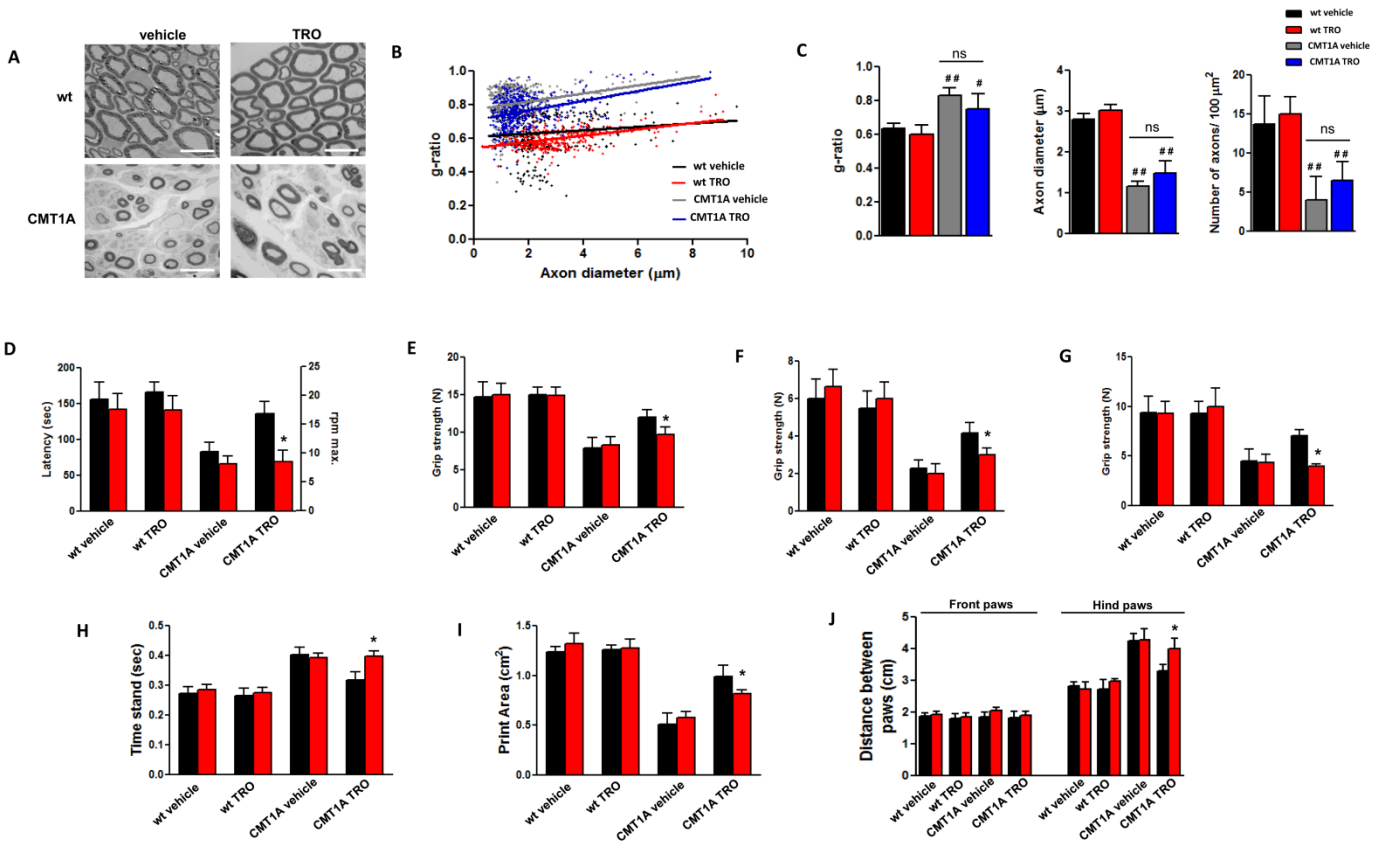


Figure S14. TEM, g-ratio and neuromuscular performance of control and CMT1A rats two months after vehicle and TRO 19622 treatments.

(A) Representative transmission electronic microscopy images of sciatic nerve cross-sections of 24 weeks old control (wt) and CMT1A rats two months after vehicle and TRO 19622 treatments. Scale bar: 5 µm. **(B)** Scatterplots showing the myelin sheath thickness (g-ratio) plotted against the axon diameter of sciatic nerve fibers from control (wt) (black and red lines) and CMT1A (grey and blue lines) rats, two months after vehicle or TRO16922 treatment. n = 3 rats for each group. Minimum 200 axons per animal. Non-significant differences are found in CMT1A vehicle and TRO19622 treatment. **(C)** Average myelin g-ratio (left panel), axonal diameter (middel panel) and number of myelinated axons (right panel) of control and CMT rats described in B. Hashes mark statistical differences over control rats. **(D)** Accelerated rotarod test latency of control and CMT1A rats just after (black bars) or 2 months after (red bars) vehicle or TRO 19622 treatment. All-limb **(E)**, front-limb **(F)** and hind-limb **(G)** grip strength analysis of control (wt) and CMT rats just after (black bars) or 2 months after (red bars) vehicle or TRO 19622 treatment. Paw stand **(H)**, paw print area **(I)** and distance between paws **(J)** of control and CMT1A rats just after (black bars) or 2 months after (red bars) vehicle or TRO 19622 treatment, determined using the Catwalk XT 10.5 test system. Error bars indicate SD. n = 3 rats for each group. Asterisks mark statistical differences over CMT1A rat performance just after TRO19622 treatment. One-way ANOVA followed by a Dunnett's multiple comparison *post hoc* test: *$p < 0.05$.

Supplemental movies:

Movie S1. Schwann cell mitochondrial motility of control mouse. Five hour time-lapse imaging of mitochondria in a control sciatic nerve infected with mito-dsRed2. Mitochondrial motility quantification is shown as µm/min in Figure 1E (grey line). Time is indicated in hours. Scale bar: 4 µm.

Movie S2. Schwann cell mitochondrial motility of crushed mouse. Five hour time-lapse imaging of mitochondria in a crushed sciatic nerve infected with mito-dsRed2. Mitochondrial motility quantification is shown as µm/min in Figure 1E (black line). Time is indicated in hours post crush. Scale bar: 4 µm.

Movie S3. Schwann cell mitochondrial motility of crushed mouse infected with shRNA3 VDAC1 – mito-dsRed2. Five hour time-lapse imaging of mitochondria in a crushed sciatic nerve infected with shRNA3 VDAC1-mito-dsRed2. Mitochondrial motility quantification is shown as µm/min in Figure 2F (red line). Time is indicated in hours post crush. Scale bar: 4 µm.