Supplementary Material:

Methods

G-ratio

For determination of G-ratio (i.e., ratio between inner axonal diameter to the total outer axonal diameter) of the sciatic nerve, three images per nerve (n=3 nerves) and of the sural nerve one to two images per nerve (n=3 nerves) were chosen and analyzed using a custom-written program (Hunter *et al.*, 2007).

CD68 immunocytochemistry

The left sural nerves of vehicle treated wild-type and SARM1 KO mice as well as vincristine-treated WT and SARM1 KO mice were dissected out and immersed for 1.5 hours in freshly prepared 4% paraformaldehyde in 1x PBS, pH 7.4. After thorough rinsing in PBS, the nerves were immersed in 30% sucrose overnight at 4ºC. Subsequently, they were frozen in OCT (Tissue Tec) in liquid 2-methylbutane cooled by dry ice. Ten micrometer thick cross sections were cut at the cryostat and sections mounted onto slides. Sections were rinsed in 1xPBS, immersed in 10 % normal goat serum (NGS) and 0.1 % Triton X-100 in PBS (PBS-T) for 1 hour and transferred into anti-CD68 (1:500, AbD SeroTec) overnight at 4˚C. The next day, sections were thoroughly rinsed in PBS-T and placed in Alexa Fluor 594 conjugated chicken anti rat secondary antibody (Life Technologies) at a dilution of 1:500 . After further rinsing, nerves were subsequently incubated in 488-Alexa Flour conjugated TUJ1 (1:200; BioLegend) for two hours. After several rinses in PBS, sections were coverslipped using Vectashield with DAPI (Vector Laboratories).

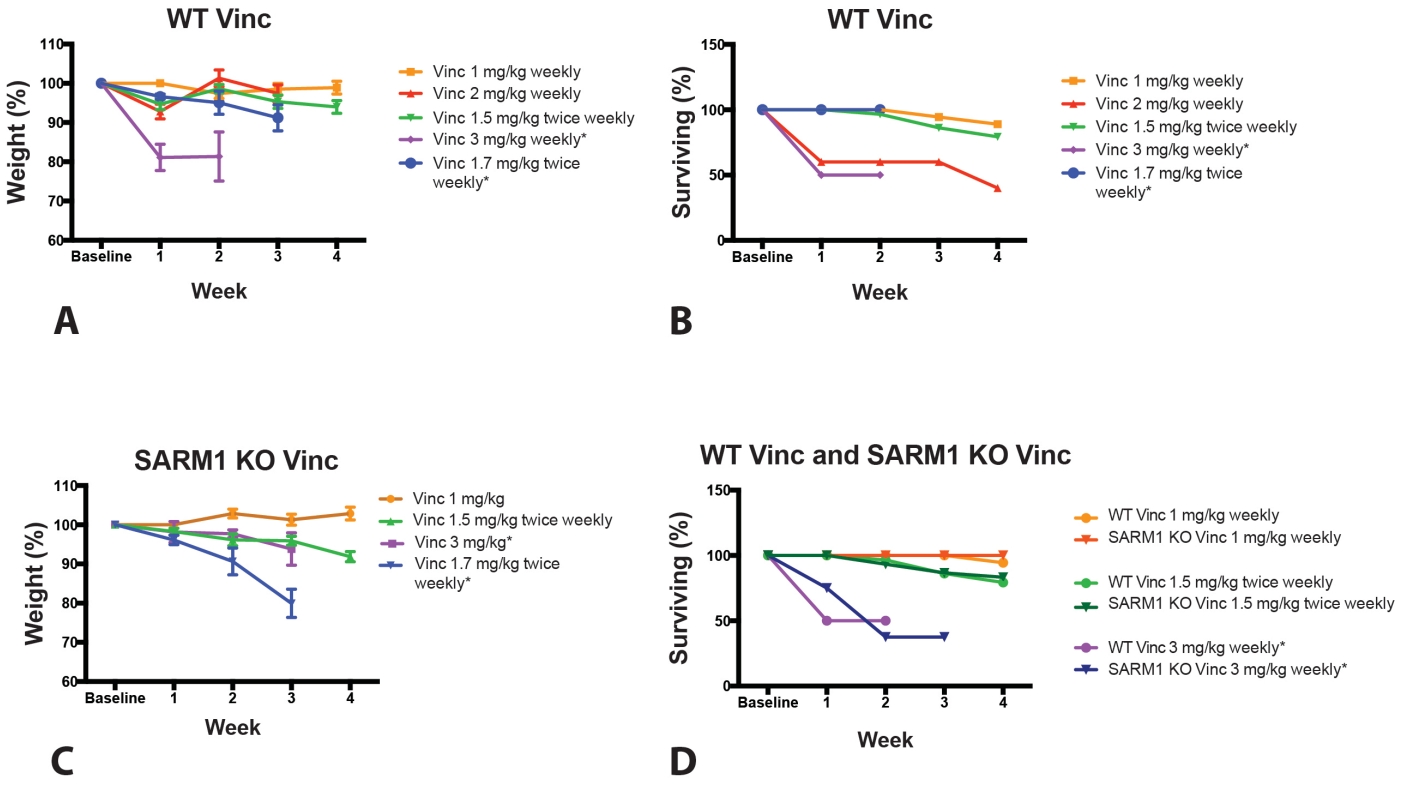
Determination of macrophages

Three Z stack images per sural nerve were obtained with a Leica DMI 4000B using a 40x oil objective and Leica DFC 7000-T Camera. Macrophages were identified by scrolling through the image to ascertain that both the red label for CD68 and the blue label for nuclei are in the same plane of the section and touching. Imaging and analysis was done with the samples blinded to the observer.

References

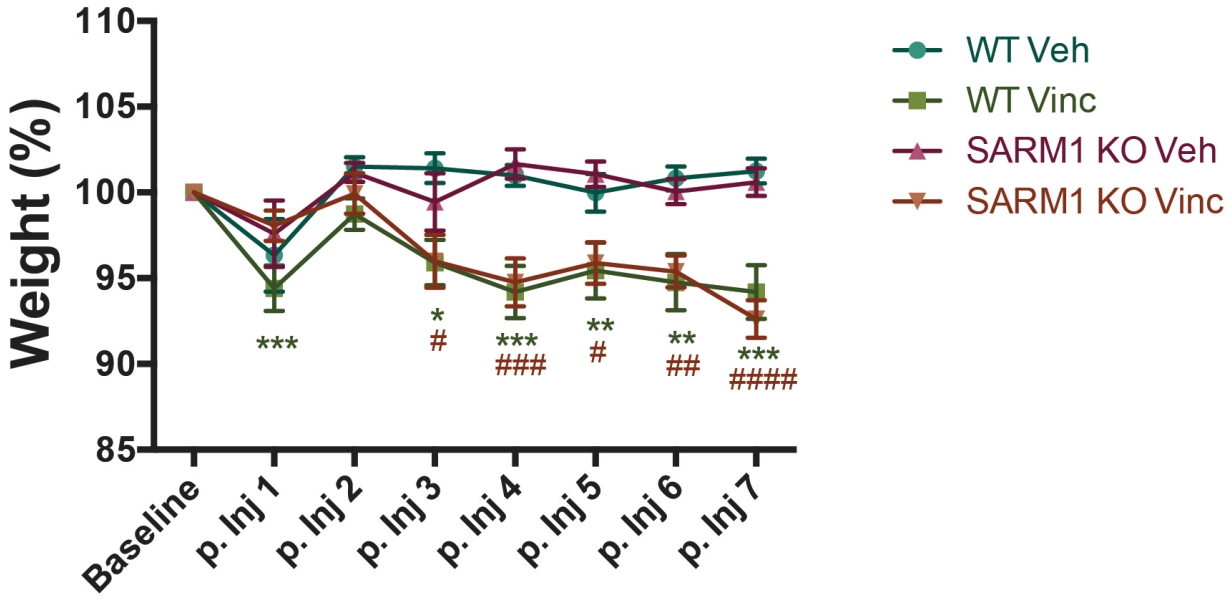
Hunter DA, Moradzadeh A, Whitlock EL, Brenner MJ, Myckatyn TM, Wei CH, et al. Binary imaging analysis for comprehensive quantitative histomorphometry of peripheral nerve. J Neurosci Methods 2007; 166: 116-24.

Supplementary Figures:

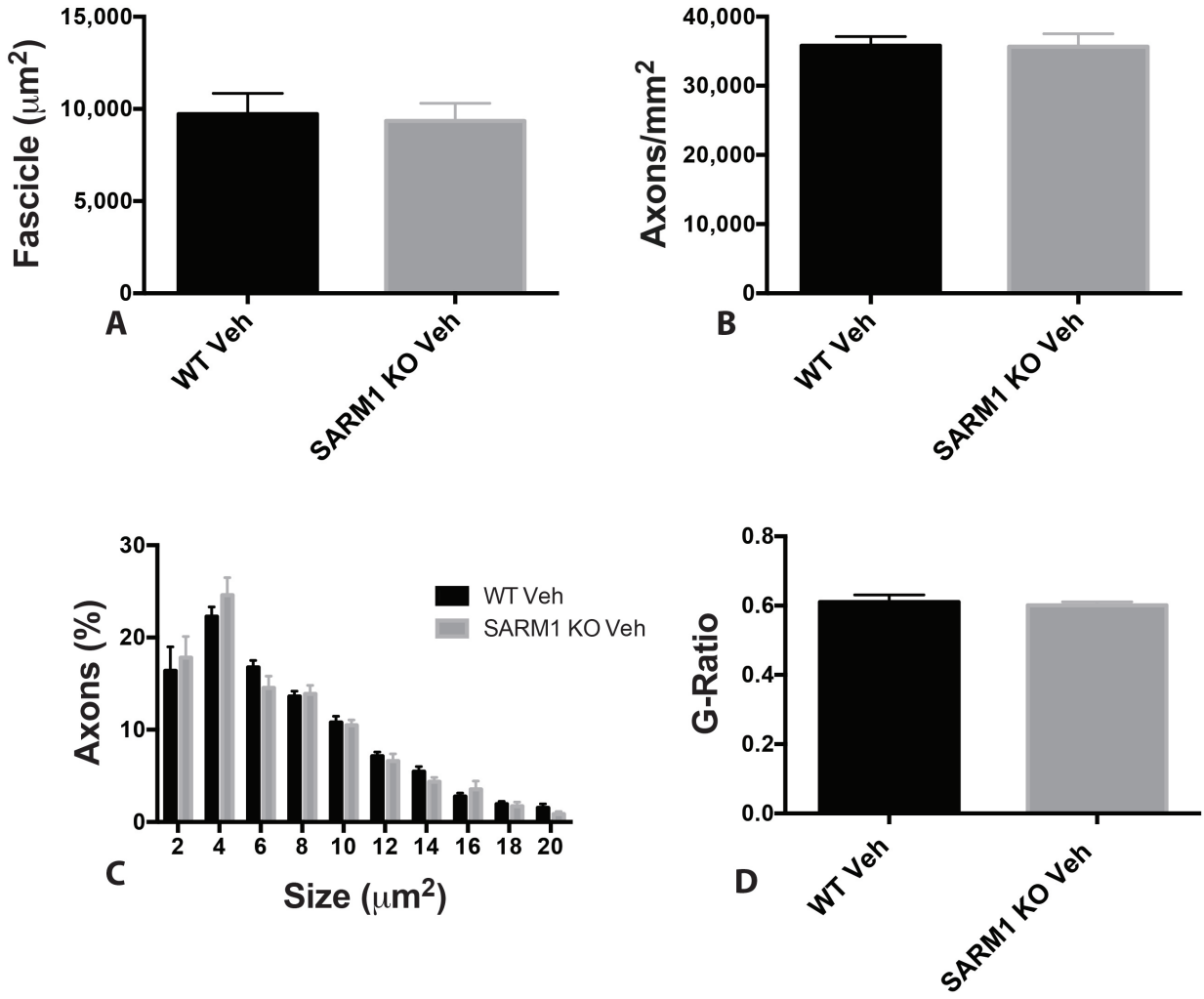


Supplementary figure 1: **Vincristine dose-response exploration in WT and SARM1 KO mice**. A, B: WT mice receiving vincristine 1 mg/kg (n=10) weekly for four weeks did not lose weight (A) and had a low mortality (B). WT mice administered vincristine 1.5 mg/kg twice weekly (n=23) had mild weight loss and a slightly higher cumulative mortality (A, B). WT mice receiving 2 mg/kg (n=5) weekly stabilized their weight after an initial loss (A) but overall had a high mortality (B). The experiment had to be terminated early in WT mice receiving vincristine 3 mg/kg or 1.7 mg/kg twice weekly (n=6) because the mice became sick (B). (C) Similar to WT mice, SARM1 KO showed mild weight loss at 1 mg/kg weekly (n=10) and 1.5 mg/kg twice weekly (n=24) and the experiment had to be terminated early for SARM1 KO mice treated either

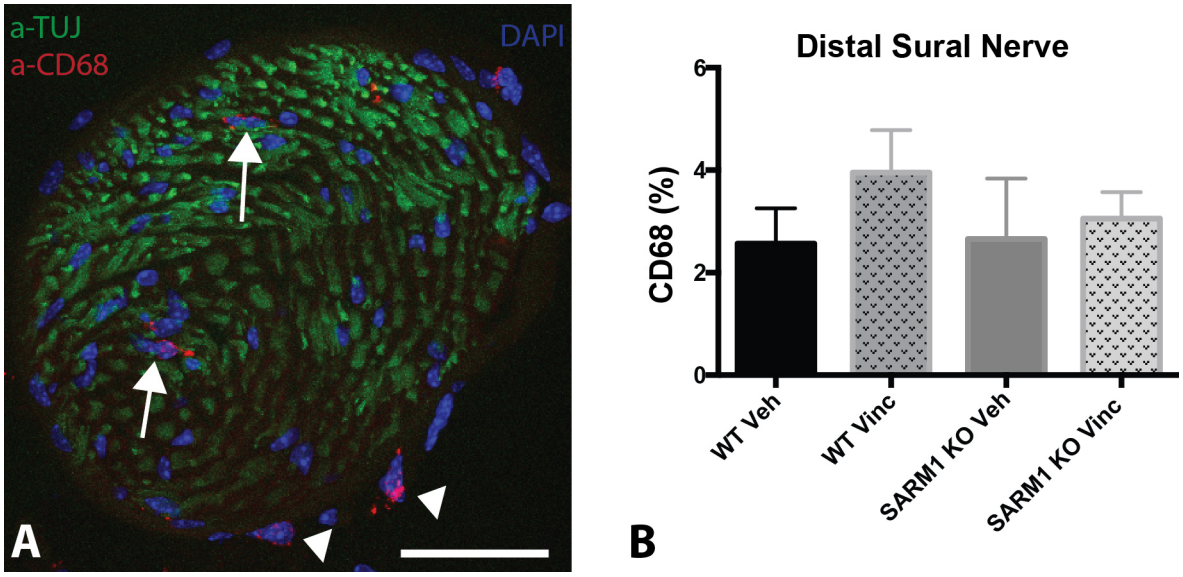
with vincristine 3 mg/kg (n=8) or 1.7 mg/kg twice weekly (n=7), because the mice became sick. (D) The mortality in response to the different vincristine dosages was similar between WT and SARM1 KO mice. For ease of comparison, selected traces (orange, green and purple) from B are also shown in D (WT and SARM1 KO vincristine 1 mg/kg weekly p=0.35; vincristine 1.5 mg/kg twice weekly p=0.97, vincristine 3 mg/kg weekly p=0.86, unpaired t-tests). * stopped early because mice became sick. Vinc – vincristine treated



Supplementary figure 2: WT and SARM1 KO mice treated with vincristine 1.5 mg/kg twice weekly lost weight to a similar extent. After the first injection, both vincristine and vehicle treated WT and SARM1 KO mice lost weight, possibly due to stress associated with the injection. All groups recovered weight before the second injection. At the end of the experiment both vincristine treated WT and SARM1 KO mice showed a similar modest weight loss of 6% ± 2% and 7% ±1 %, respectively ($p<0.01$ for WT and $p<0.001$ for SARM1 KO). There were significant time ($p<0.0001$), group ($p=0.0009$) and time x group interactions ($p<0.0001$; two way repeated measures ANOVA; Tukey's multiple comparison; *-WT Vinc, #-SARM1 KO Vinc; $^{*,\#}$ $p<0.05$; $^{**,\#\#}$ $p<0.01$; $^{***,\#\#\#}p<0.001$; $^{\#\#\#\#}p<0.0001$) Veh – vehicle treated, vinc – vincristine treated p. Inj. – post injection.



Supplementary figure 3: Vehicle treated WT and SARM1 KO distal sural nerves are similar. There is no difference in the mean average fascicle size (A; unpaired t-test, p=0.81; n=9-11), mean axon density (B; unpaired t-test, p=0.96; n=9-11), axon size distribution (C, multiple t-tests) and G-ratios (D, unpaired t-test; p=0.41; n=3) between vehicle treated WT and SARM1 KO mice.



Supplementary figure 4: CD68-positive cells in the distal sural nerves of WT and SARM1 KO mice. (A) Cross-sections of frozen distal sural nerves were stained with the macrophage marker anti-CD68 (red), and DAPI to identify cell nuclei (blue). To distinguish nerve fascicles from surrounding tissue, we immuno-labeled axons with anti-tubulin III (green). CD68-positive cells were identified in z-stacks by red immunostaining surrounding blue nuclei in the same plane of section. Few CD68-positive cells were found in the distal sural nerves (A, arrows) and around it (A, arrowheads). (B) Quantification of CD68-positive cells as percent of all cells observed in the nerve revealed no difference in CD68-positive cells between the vehicle and vincristine treated WT and SARM1 KO mice (One way ANOVA $F(3,28)=0.5678$; $p=0.64$; $n=8$ per group). Scale bar = 50 μm.