

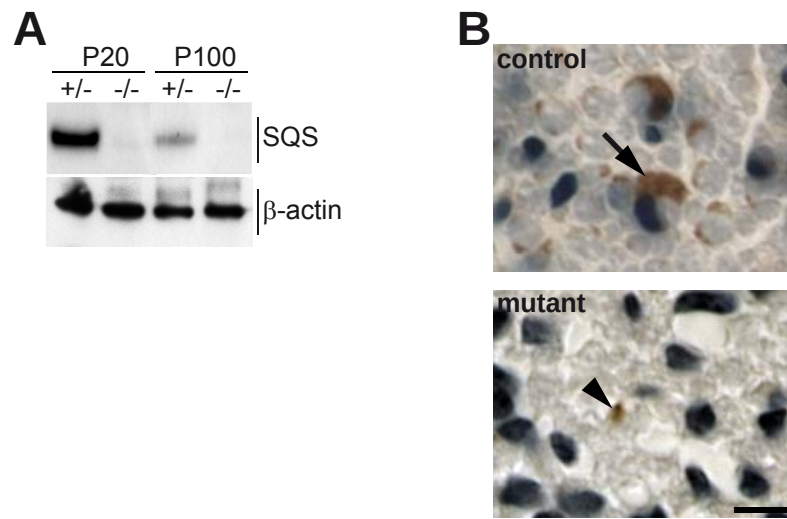


Figure S1 - Squalene Synthase (SQS) conditional mutant mice

A) Western blot of SQS on total sciatic nerve extracts of mutant (-/-) and control (+/-) animals at indicated ages. Detection of β -actin served as loading control.

B) Immunohistochemistry detecting SQS (brown) on P20 sciatic nerves. While Schwann cells in control animals show heavy staining of SQS in the cell soma (arrow), rare and weak SQS specific staining was found in mutants (arrowhead) (scale 25 μ m).

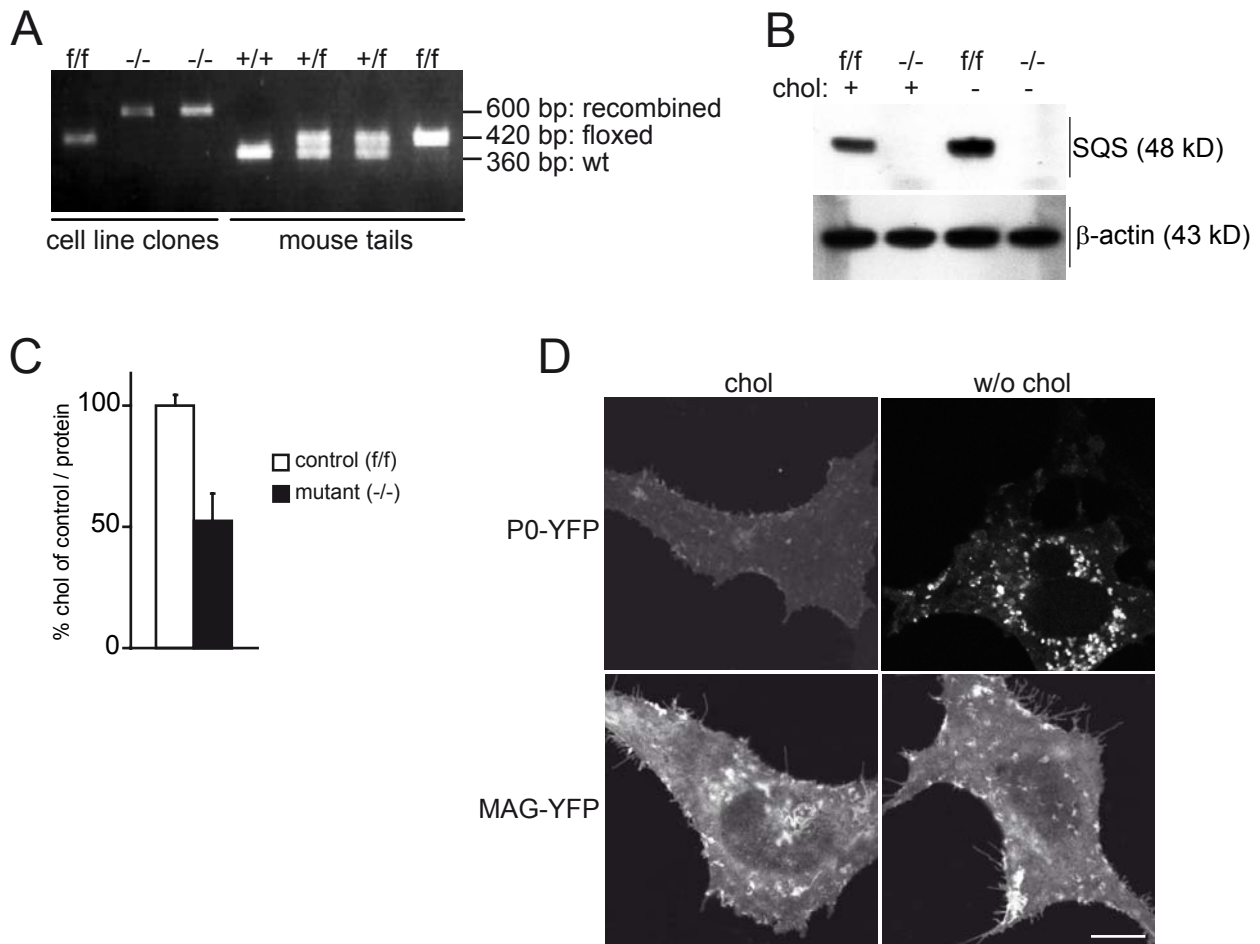


Figure S2 - SQS null fibroblasts

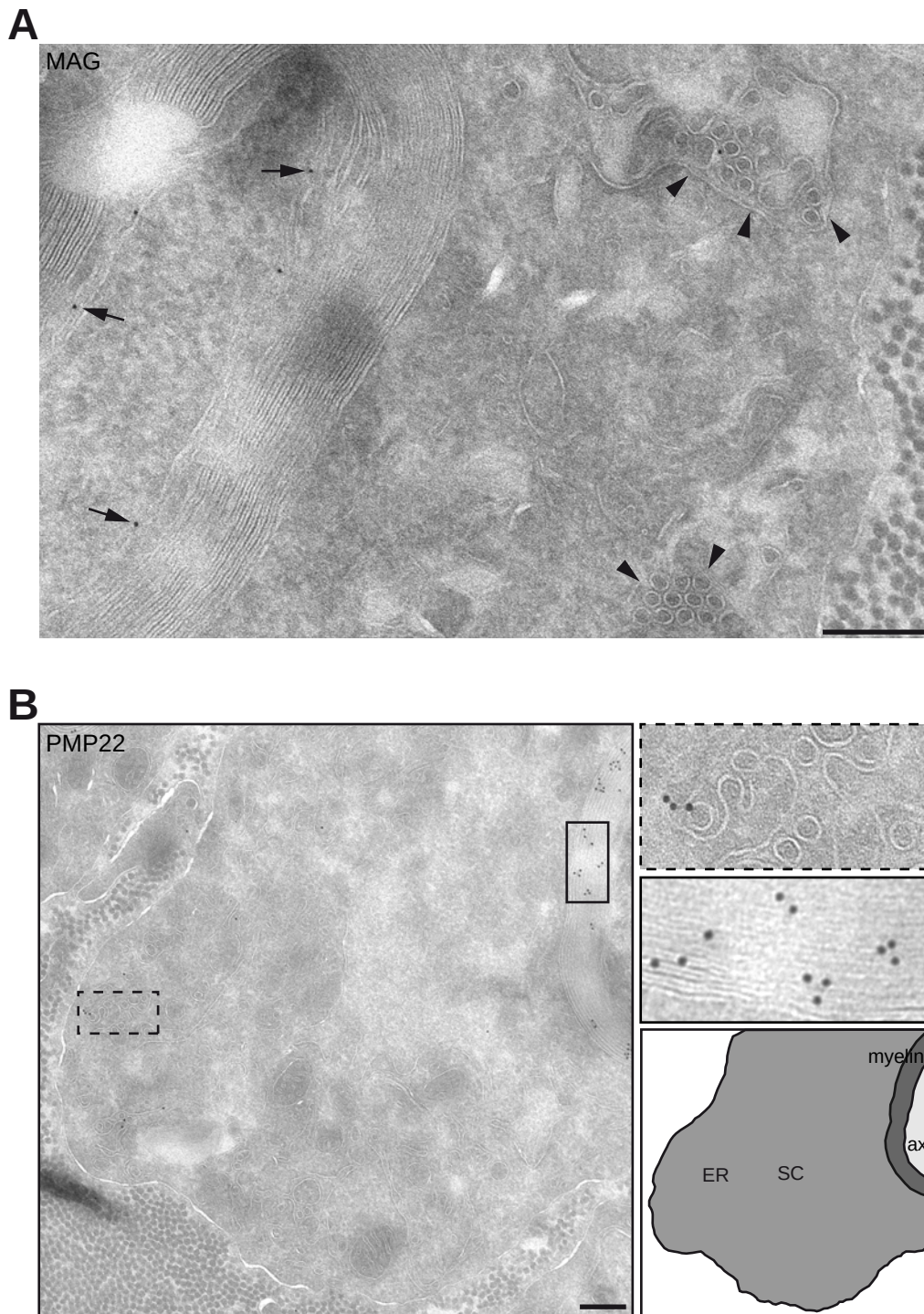
A) Genotyping PCR of stable fibroblastoid clones revealed the floxed SQS allele in the parent clone (left lane) and the recombined allele in clones that were transfected with a cre expression plasmid (-/-). For comparison genotyping of mice showed wildtype (+/+), heterozygous (+/-) and homozygous (f/f) floxed alleles.

B) Stable fibroblast cell lines were kept in media containing 2% lipoprotein deficient serum (LPDS) with or without 10µg/ml cholesterol for 72h. Western blot revealed increased amounts of SQS protein in the control parent line (f/f) when cholesterol was lacking from the media. No SQS protein could be detected in the mutant cell line (-/-). Detection of β-actin served as loading control.

C) Cells were cultivated in 2% LPDS for 72h, and non-saponifiable lipids were extracted and analysed by HPLC as described (Keller et al., 2004). Briefly, lipids were extracted with chloroform/methanol (1:1, v/v). Lipids were saponified with 20% (w/v) potassium hydroxide in 66% aqueous methanol solution for 1 h at 100°C in the presence of an internal standard (dehydrocholesterol). Hydrolyzed samples were extracted twice with equal volumes of petroleum ether, and the pooled extracts were backwashed with 5% (v/v) aqueous acetic acid and then taken to dryness. Nonsaponifiable lipids were dissolved in mobile phase solvent (methanol-isopropanol, 7:1, v/v), and aliquots were subjected to reverse-phase HPLC (Shandon Hypersil ODS column, 2.1 x 200mm, flow rate 0.2 ml/min) with online ultraviolet detection at either 214 or 282 nm (Smart system, Amersham Pharmacia). A standard curve using cholesterol was used to quantify sterol mass in cell extracts after normalization to the internal standard. Mutant cells contained only 52.5 ± 11.2 % cholesterol of control values (100 ± 4.4%) as mean of three independent experiments.

D) P0-CFP or YFP-MAG fluorescence of SQS null fibroblasts that were starved of cholesterol for 24h in LPDS followed by transfection with P0-CFP or MAG-YFP and cultivation for 24h in LPDS with 10µg/ml cholesterol (chol) or without cholesterol (w/o chol). P0 accumulates intracellularly only when cholesterol is lacking in the media. In contrast, MAG distribution is similar at both conditions. Scale, 10µm.

Keller, R.K., M.Small, and S.J.Fliesler. 2004. Enzyme blockade: a nonradioactive method to determine the absolute rate of cholesterol synthesis in the brain. *J. Lipid Res.* 45: 1952-1957.



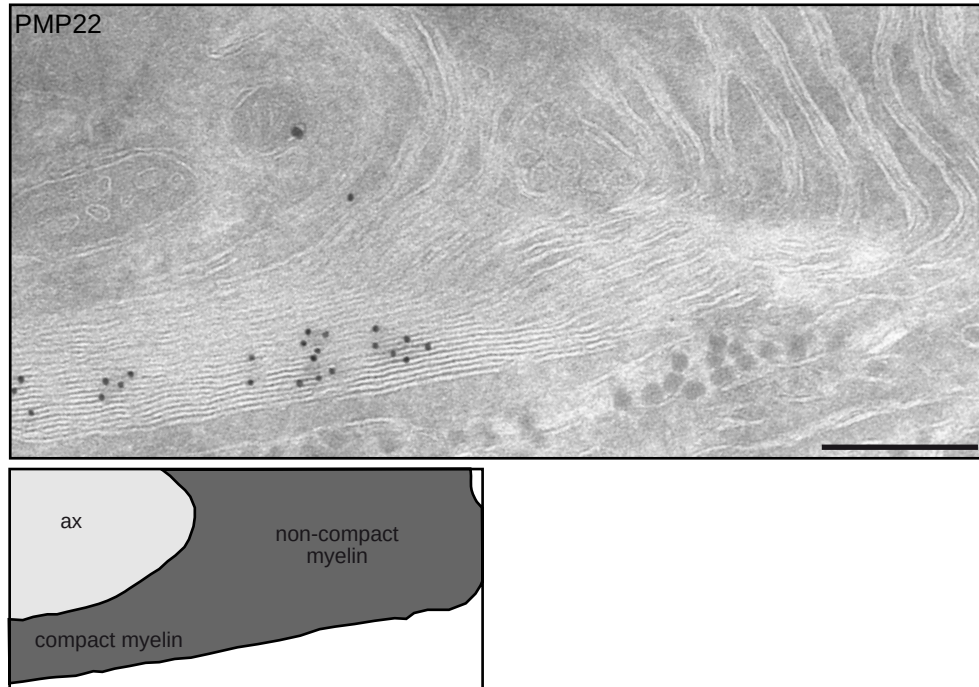


Figure S4 - PMP22 localization

Cryo immunoelectron microscopic images of mutant sciatic nerve (P14) show PMP22 in compact myelin. PMP22 is absent from non-compact myelin. Scale, 250 nm.

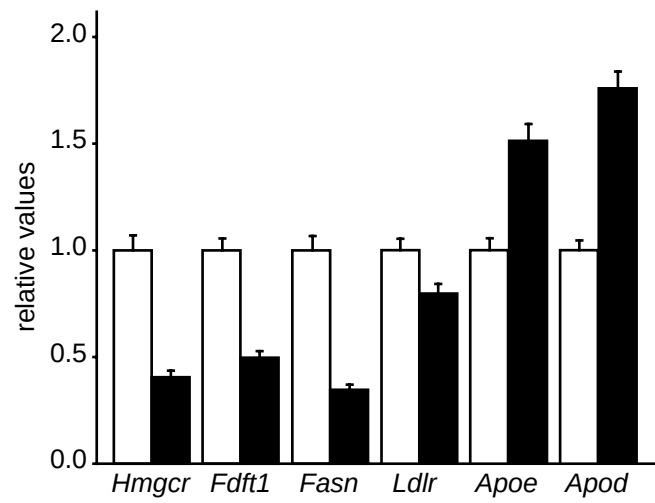


Figure S5 - Expression levels of genes involved in lipid homeostasis

Quantification of mRNA from genes involved in cholesterol homeostasis in control and mutant sciatic nerves at P20 (n=3) by real-time PCR (HMG-CoA reductase, *Hmgcr*; squalene synthase, *Fdft1*; fatty acid synthase, *Fasn*; LDL receptor, *Ldlr*; Apolipoprotein E, *Apoe*; Apolipoprotein D, *Apod*).

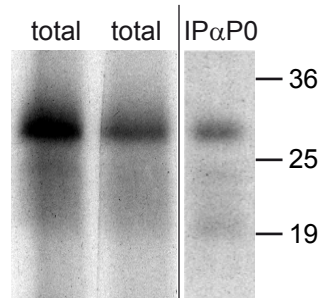


Figure S6 - Photo-[³H]cholesterol labeling of myelin

Myelin membranes purified from wild-type sciatic nerve (50 µg total protein in 100 µl PBS) were incubated for 1 h at 37°C with photoactivatable [³H]cholesterol (5 µCi) complexed to methyl-β-cyclodextrin to supplement myelin membranes with the cholesterol analogue. Covalent modification of cholesterol-interacting proteins was induced by UV-irradiation using a mercury lamp. Analysis of the total fraction by SDS-PAGE and fluorography revealed one major cholesterol-modified protein in the range of 30 kD (total), which was identified as P0 by immunoprecipitation (IPαP0).