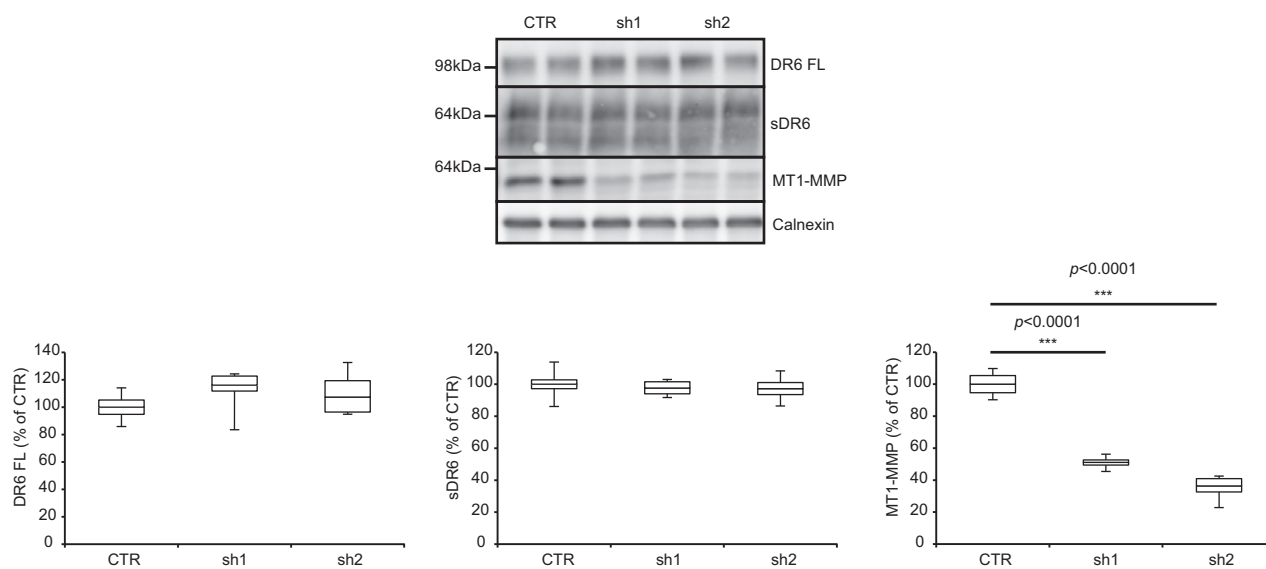


## Expanded View Figures

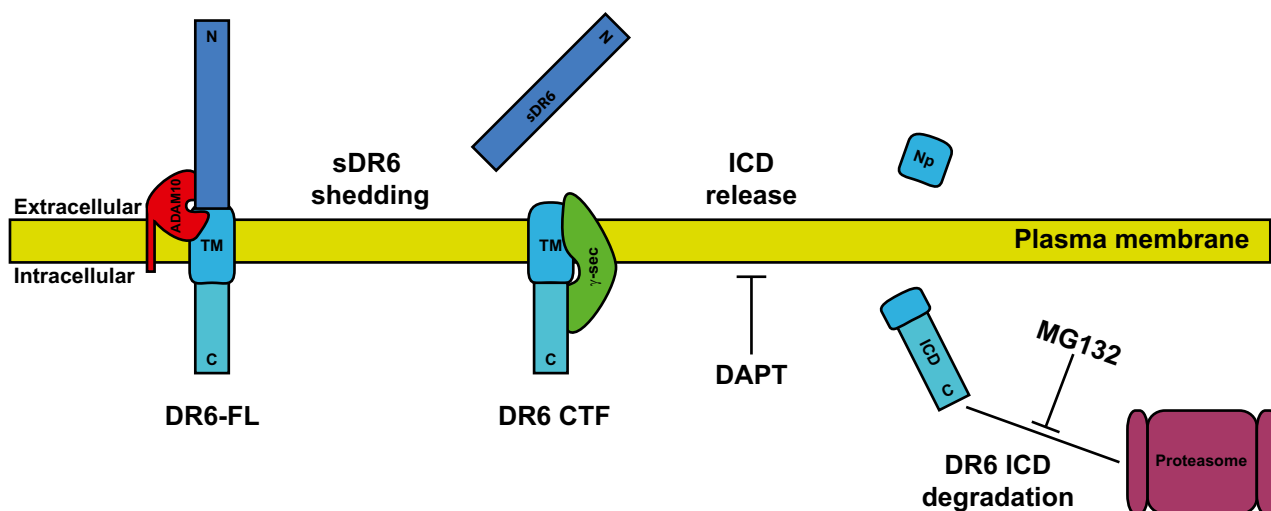


**Figure EV1. Analysis of sDR6 level in WT primary neurons upon MT1-MMP knockdown.**

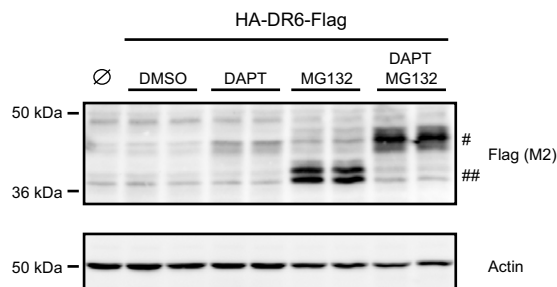
Specific short hairpin RNAs targeting MT1-MMP (sh1 and sh2) were expressed in primary neurons using a lentiviral system. A scrambled shRNA sequence was used as negative control. Levels of full-length DR6 (DR6 FL) and its released ectodomain (sDR6) were analyzed in cell lysates and culture supernatant, respectively ( $n = 6$  for each group), showing that upon MT1-MMP knockdown, full-length protein and its processing were not affected. Western blot quantifications are represented using box plots showing the first and the third quartile together with the median. The whiskers show the maximum and the minimum data point. Statistical test: unpaired Student's  $t$ -test with two-tailed distribution.

Source data are available online for this figure.

A



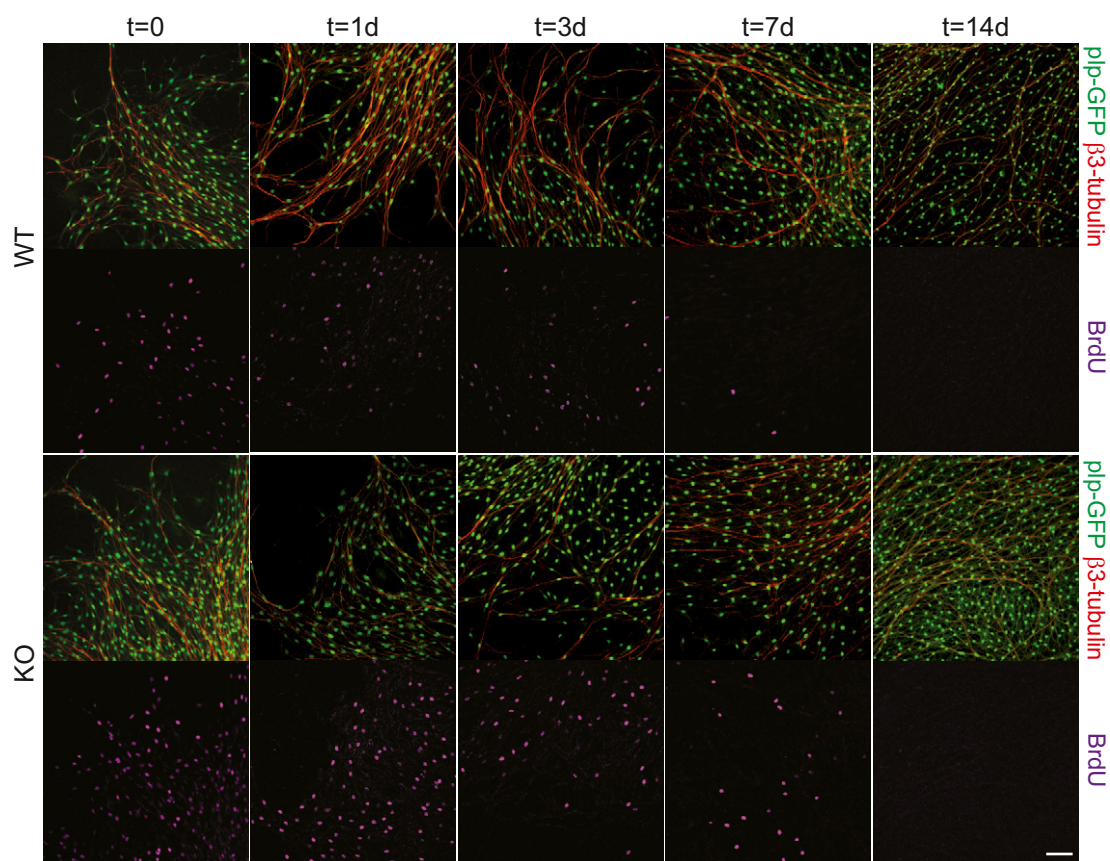
B



**Figure EV2.  $\gamma$ -Secretase cleavage of DR6 CTF.**

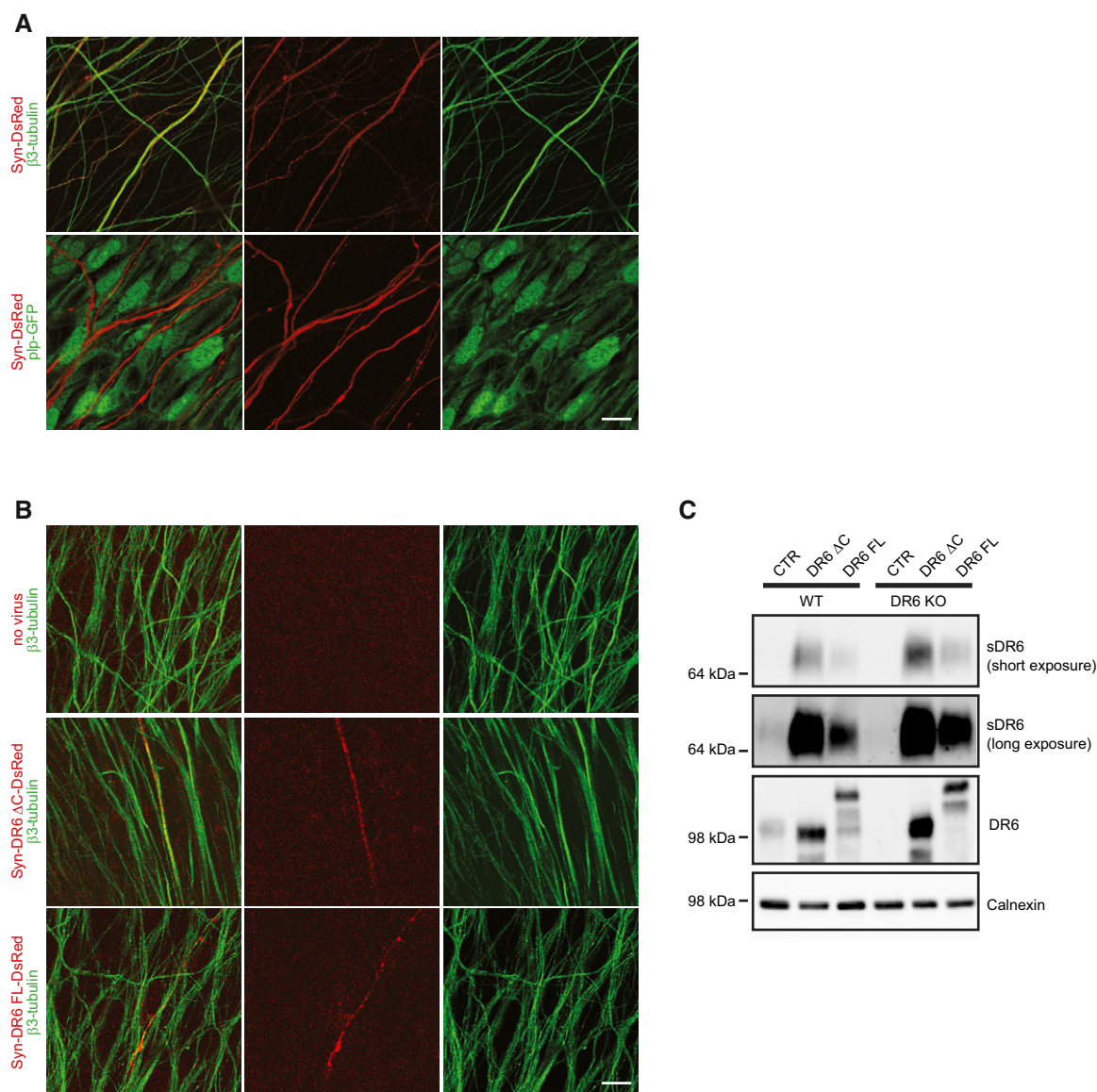
- A Regulated intramembrane proteolysis (RIP) scheme. Type I transmembrane proteins such as DR6 can be cleaved by a protease (e.g., ADAM10), thus releasing their soluble ectodomain (sDR6) into the extracellular environment. The resulting C-terminal fragment (CTF) can be further cleaved by the protease  $\gamma$ -secretase within the transmembrane domain (TM). As a result, a short N-terminal peptide (Np) is typically secreted from cells, while the intracellular domain (ICD) is released into the cytosol. The latter is mostly rapidly degraded, unless proteasomal activity is blocked through specific inhibition with MG132. Typically, the  $\gamma$ -secretase inhibitor DAPT is used to induce accumulation of the CTFs and to validate candidates as  $\gamma$ -secretase substrates.
- B HEK293E cells overexpressing hDR6 with a Flag tag at the C-terminus were treated for 24 h with either the  $\gamma$ -secretase inhibitor DAPT (1  $\mu$ M) or the proteasome inhibitor MG132 (1  $\mu$ M). Cells transfected with an empty vector were used as control (Ø). Western blot analysis of cell lysates showed that DAPT treatment, alone or together with MG132, blocked ICD formation and a 45-kDa fragment (##) corresponding to DR6 CTF was accumulating, while proteasome blockade induces accumulation of a smaller fragment (#) corresponding to DR6 ICD. Together, our data demonstrate that DR6, after initial ectodomain shedding, is further processed by the  $\gamma$ -secretase complex.

Source data are available online for this figure.



**Figure EV3. Time course of SC proliferation in DR6 KO cultures.**

BrdU proliferation assay of SCs in WT and DR6 KO DRGs along the myelination period ( $t$  = days of ascorbic acid (AA) treatment to induce myelination). During the first week of AA treatment, quantification of plp-GFP (green)- and BrdU (magenta)-double-positive cells shows a 1.4- to 2.5-fold increase in proliferating SC in KO cultures in comparison with WT controls. Neurons are labeled with  $\beta$ 3-tubulin (WT,  $n = 5$ ; KO,  $n = 4$ ; scale bar = 100  $\mu$ m, quantification shown in Fig 2B;  $t = 0$ , the same as in Fig 2B).

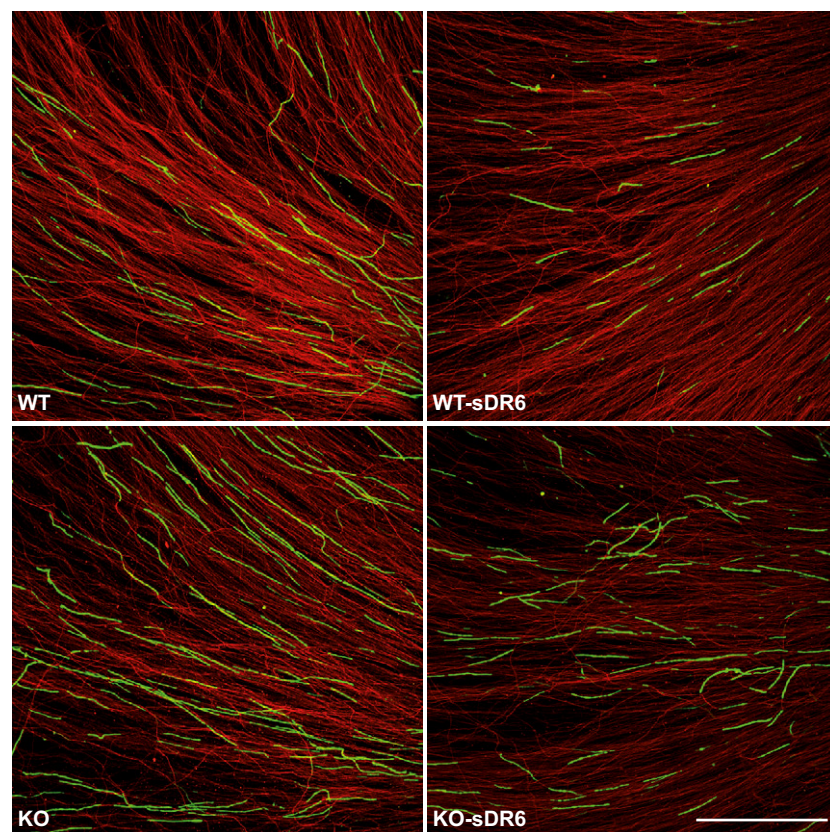


**Figure EV4. Neuronally expressed DR6 rescues myelin phenotype.**

- A DRG cultures transduced with lentiviral vector encoding for DsRed (red) under a neuronal-specific promoter (synapsin), showing that the construct is expressed in neurons (labeled with  $\beta$ 3-tubulin, upper panels, green) but not in SCs (expressing plp-GFP, lower panels, green). Scale bar = 10  $\mu$ m.
- B DRG cultures without viruses (upper panels) or transduced with lentiviral vectors encoding DR6  $\Delta$ C-DsRed (middle panels) and DR6 FL-DsRed (bottom panels) under the synapsin promoter. Note that both DR6  $\Delta$ C-DsRed and DR6 FL-DsRed (red) are expressed in axons (labeled with  $\beta$ 3-tubulin, green). Scale bar = 10  $\mu$ m.
- C Western blot analysis of lentivirally transduced DRG cultures from WT and DR6 KO mice. DR6  $\Delta$ C-DsRed (lysates), DR6 FL-DsRed (lysates), and sDR6 (media) were detectable in both genotypes with the 6B6 antibody. Additionally, WT samples showed bands corresponding to endogenous DR6 FL and its shed ectodomain. DsRed construct alone was used as control.

Source data are available online for this figure.





**Figure EV5. Rescue of knockout phenotype by sDR6.**

In order to test whether sDR6 is able to rescue the myelination phenotype, WT and DR6 KO cultures were incubated with complete medium supplemented with 1  $\mu\text{g/ml}$  murine recombinant sDR6. Quantification of myelin segments (seg., MBP-positive, green) showed that the treatment with sDR6 was sufficient to downregulate myelination. Neurons were labeled with  $\beta 3$ -tubulin (red). Myelin segment quantification is represented using box plots showing the first and the third quartile together with the median. The whiskers show the maximum and the minimum data point.  $n = 6$  for each group; scale bar = 500  $\mu\text{m}$ .

