

Fig. S1. Retrograde axonal transport defect is not restricted to a subtype of organelles in $Sp^{-/-}$ neurons. (A-A'') Primary cultures of $Sp^{-/-}$ cortical neurons were incubated at DIV5, with 1 $\mu\text{g}/\text{ml}$ of the neuronal retrograde tracer AlexaFlour488-conjugated CTb (A), washed in CTb-free medium, fixed and processed for immunolabeling with acetylated α -tubulin antibody (A', A''). Many CTb-positive vesicles are accumulated within the proximal part of the swellings (A-A''; arrows). (B-D'') Co-immunolabeling of acetylated α -tubulin (tubulin; B', B'', C', C'', D' and D'') and endosomes (EEA1; B, B''), lysosomes (Lamp1; C, C''), synaptic vesicles (Snap23a; D, D'') in DIV6 primary cultures of $Sp^{-/-}$ cortical neurons. All these organelles are equally accumulated within axonal swellings (B-D''; arrows). (A-D'') Nuclei are stained with DAPI (asterisks). A''-D'' are higher magnifications of A'-D'. Scale bars: 10 μm .

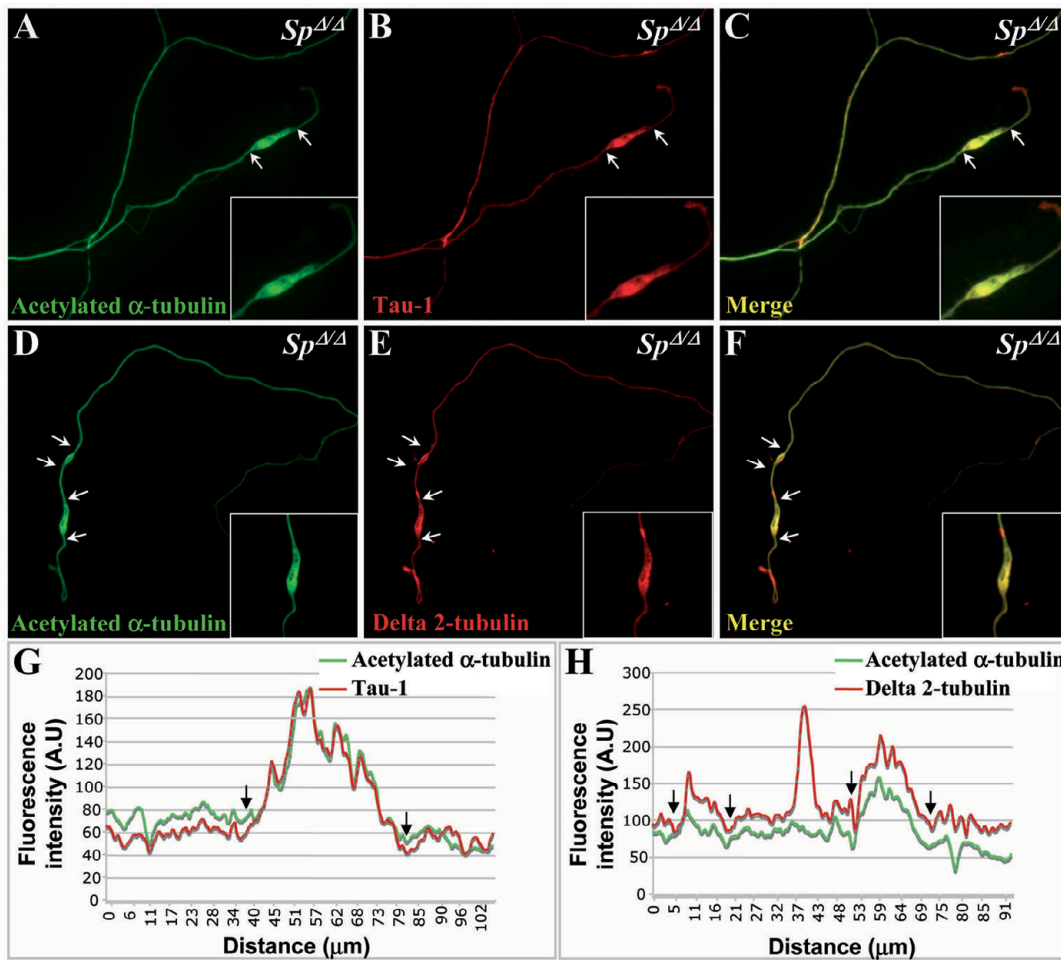


Fig. S2. Excessive stabilization of microtubules in axonal swellings of $Sp^{-/-}$ cortical neurons. Primary cultures of DIV6 $Sp^{-/-}$ cortical neurons were immunolabeled for acetylated α -tubulin (A, C, D, F) and the microtubule-stabilizing protein Tau (Tau-1; B, C) or delta 2-tubulin, a marker of very long-lived microtubules (E, F). Insets show higher magnifications of axonal swellings. The accumulation of Tau and the two markers of long lived microtubules (acetylated α -tubulin and delta 2-tubulin) within the swellings strongly suggest that microtubules are abnormally stabilized in this axonal region. Scale bars: (A-F) 50 μ m; (insets) 10 μ m. (G-H) Quantitative fluorescence intensity profile of acetylated α -tubulin and Tau-1 (G) or acetylated α -tubulin and delta 2-tubulin (H) within and on either side of the swellings. Arrows indicate the proximal and distal parts of the swellings.

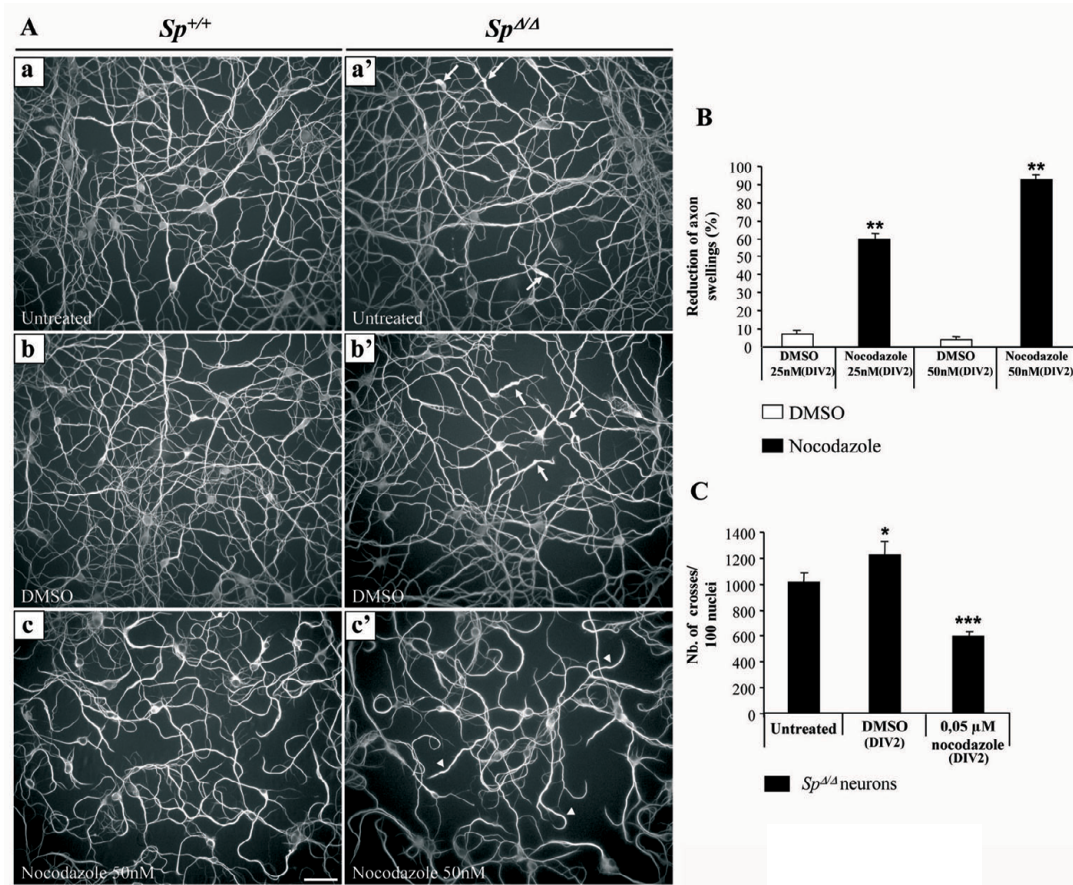


Fig. S3. Nocodazole treatment at DIV2 prevents the formation of axonal swellings but affects *Sp*^{-/-} cortical axon outgrowth. (A) Immunolabeling of acetylated α -tubulin on DIV6 primary cultures of *Sp*^{+/+} (Aa-Ac) and *Sp*^{-/-} (Aa'-Ac') cortical neurons untreated (Aa, Aa') or treated 2 days post-plating with 50 nM nocodazole (Ac, Ac') or with an equivalent volume of DMSO (Ab, Ab'). Note the absence of axonal swellings in the distal region of *Sp*^{-/-} cortical neurons treated with nocodazole (Ac'; arrowheads) compared with untreated or DMSO-treated *Sp*^{-/-} neurons (arrows). Scale bar: 50 μ m. (B). The percentage of axonal swellings in *Sp*^{-/-} cortical neurons was evaluated at DIV6. Note that 50 nM nocodazole significantly decreases the proportion of neurite swellings in primary cultures of *Sp*^{-/-} neurons compared with DMSO-treated cultures. Asterisks indicate statistically different percentages between DMSO-treated neurons and 50 nM nocodazole-treated cells (** $P < 0.001$). Vertical bars indicate s.e.m. (C). Analysis of neurite outgrowth at DIV6 in primary cultures of *Sp*^{-/-} neurons treated with 50 nM nocodazole or with an equivalent volume of DMSO. The treatment of *Sp*^{-/-} neurons with nocodazole at DIV2 dramatically and significantly affects neurite outgrowth compared with DMSO control treatment ($P < 0.0001$). More than 1000 neurons were analyzed in each condition.