Brill et al., http://www.jcb.org/cgi/content/full/jcb.201108005/DC1

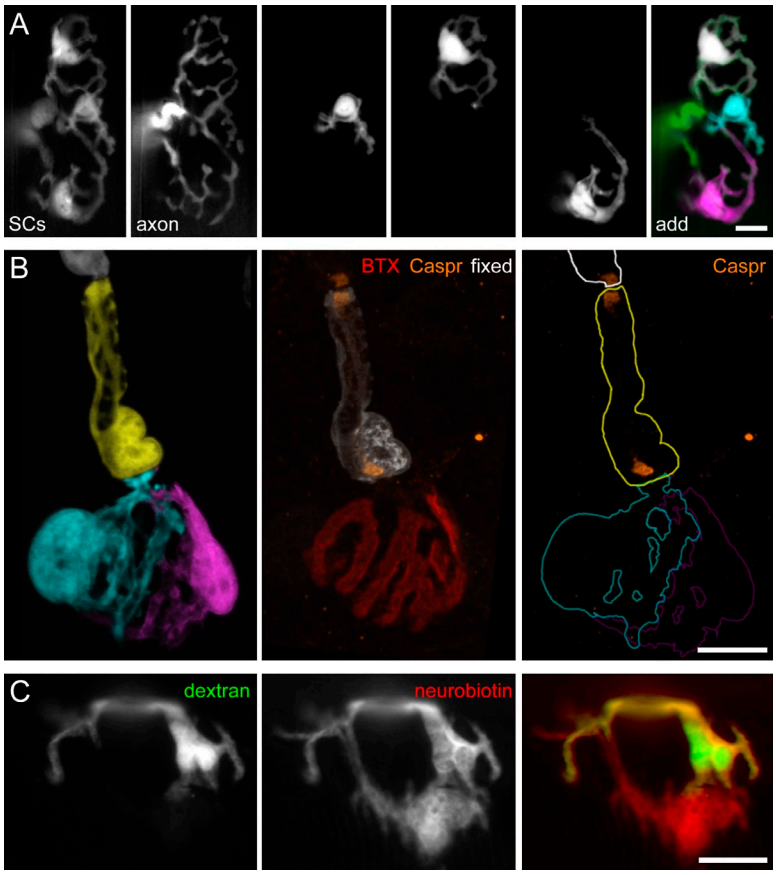


Figure S1. **Terminal SCs form a distinct compartment from axonal SCs.** (A) Terminal SCs (left panels) were sequentially filled with rhodamine dextran, pseudocolored, and merged (right panels). The axon was labeled by *thy1*-CFP transgene (middle panels). SC debris of the filled and subsequently destroyed SCs were removed for clarity. (B) Nonoverlapping territories of terminal SCs and axonal SCs revealed by sequential photobleaching (left; same NMJ as shown in Fig. 1 B). The sample was fixed after photobleaching (middle; one axonal SC remained unbleached) and then stained with BTX and an antibody against the paranodal marker Caspr. Outlines of individual SCs (right) combined with Caspr immunohistochemistry reveal absence of obvious nodelike specializations at contact sites of terminal SCs. (C) Terminal SCs are coupled through gap junctions, as revealed by simultaneously injecting one terminal SC with rhodamine dextran and neurobiotin. Rhodamine dextran is restricted to the injected cell, whereas neurobiotin (developed with Cy5-streptavidin) diffuses into a coupled terminal SC (but not into the axonal SC). Bars, 10 µm.

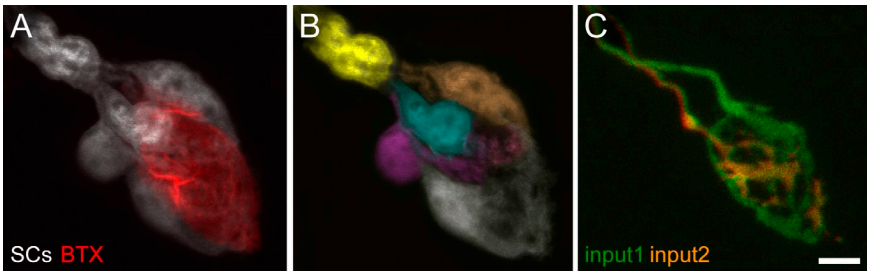


Figure S2. **Terminal SCs are not assigned to single axonal inputs.** (A) Postnatal NMJ (BTX) with four SCs before photobleaching. (B) Single-cell labeling allows individual SC territories to be revealed (pseudocolored), which do not match the synaptic territories of single axonal inputs. (C) Axonal inputs of this NMJ labeled by *thy1*-OFP3 and distinguished by photobleaching, image subtraction, and pseudocoloration. In 9 out of 10 cases that we studied, two axonal inputs shared the same terminal SC. Bar, 5 µm.

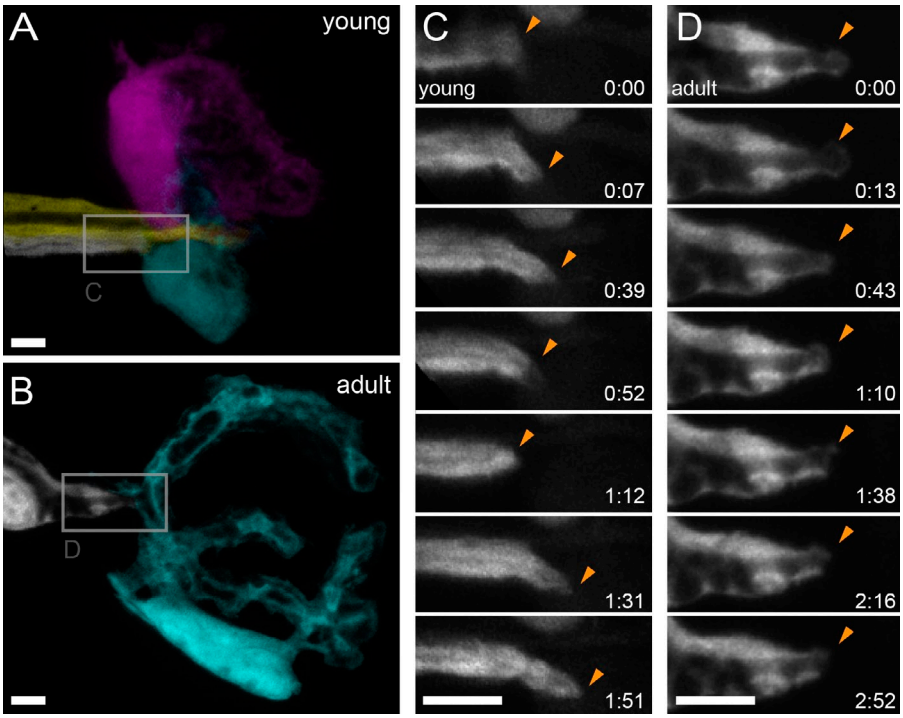


Figure S3. **Immature axonal SCs explore synaptic territory, whereas adult axonal SCs do not.** (A and B) Pseudocolored terminal and axonal SCs at a young (A) and adult (B) NMJ, with an axonal SC remaining unbleached. (C and D) Time-lapse recordings (depicted in boxed areas in A and B) reveal that immature axonal SCs invade NMJs (orange arrowheads; C), whereas mature axonal SCs do not (D). Only minor processes that cross the heminode (orange arrowheads) can be seen in the adult. The timers shown represent hours/minutes. Bars, 5 µm.

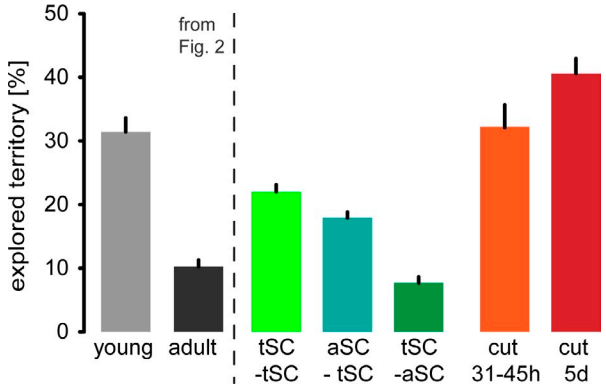


Figure S4. **Territory exploration of terminal SCs after ablation and axotomy.** Quantitative analysis of terminal SC dynamism. Data for young and adult dynamism repeated from Fig. 2 (young: 31.4 ± 2%, *n* = 24 NMJs, eight triangularis sterni explants vs. adult: 10.2 ± 1%, *n* = 14 NMJs, six triangularis sterni explants). After ablation of terminal SCs, the young abutting terminal SCs (tSC) expand and explore significantly more territory than adult SCs (tSC-tSC: 22.0 ± 1%, *n* = 8 NMJs, six triangularis sterni explants; Fig. 5). Terminal SCs expand when axonal SCs (aSC) are ablated (aSC-tSC: 17.9 ± 1%, *n* = 6 NMJs, five triangularis sterni explants). In contrast, axonal SCs do not react to ablation of terminal SCs (tSC-aSC: 7.7 ± 0.9%, *n* = 7 NMJs, four triangularis sterni explants). 31–45 h and 5 d after axotomy, terminal SCs screen significantly more territory (31–45 h: 32.4 ± 4%, *n* = 12 NMJs, five triangularis sterni explants; 5 d: 40.6 ± 2%; *n* = 7 NMJs, four triangularis sterni explants). Data are represented as the mean of NMJs + SEM.

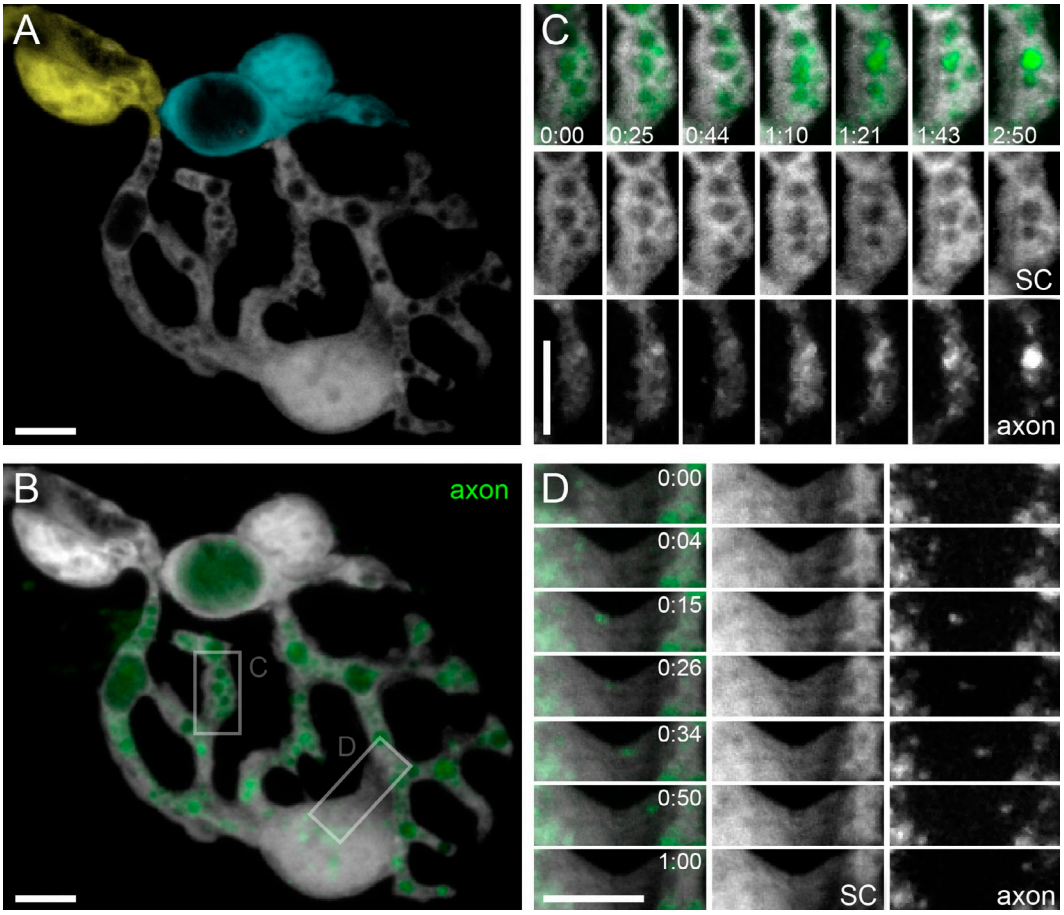
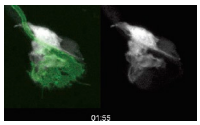
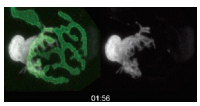


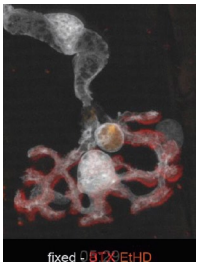
Figure S5. **Engulfment of axonal fragments 12 h after axotomy.** (A and B) Pseudocolored images of SCs at an NMJ (white, cyan, and yellow) in a nerve–muscle explant 12 h after transection of the intercostal nerve. Note that the axon (labeled by *thy1*-Membow; B) has already fragmented. (C and D) Time-lapse recordings over 1–3 h (areas boxed in B). (C) Time-lapse of terminal SC (white) shows engulfment and subsequent aggregation of axonal particles (green). (D) Movement of an axonal fragment within the terminal SC. The timers shown represent hours/minutes. Bars, 5 µm.



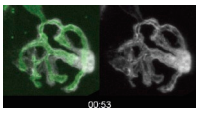
Video 1. **Dynamism of SCs at a young NMJ.** A young (P11) NMJ with labeled SCs (transgenic labeling in SC-GFP mice) and axon (thy1-Membow13). Images were obtained by time-lapse confocal microscopy using a laser-scanning confocal microscope (FV1000). Frames were taken every 5–10 min for 2 h and 21 min. The first frames show sequential photobleaching of SCs. The video shown corresponds to Fig. 2 (A–C).



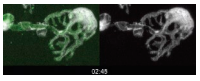
Video 2. **Static tiling of SCs in an adult NMJ.** Adult NMJ showing SCs (SC-GFP) and axon (*thy1*-Membow13). Images were analyzed by time-lapse confocal microscopy using a laser-scanning confocal microscope (FV1000). Frames were taken every 5–10 min for 3 h and 8 min. The first frames show sequential photobleaching of SCs. The video shown corresponds to Fig. 2 (D–F).



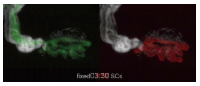
Video 3. **Combined laser ablation and bleaching of terminal SCs in an adult NMJ.** An adult NMJ with three terminal SCs (SC-GFP), one of which was bleached and the other one killed using a femtosecond-pulsed near-infrared laser (EtHD). Images were analyzed by time-lapse confocal microscopy using a laser-scanning confocal microscope (FV1000). Frames were taken every 10–20 min for 5 h and 29 min. The fixed sample is shown at the end of the video. The video shown corresponds to Fig. 4.



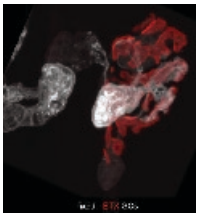
Video 4. **Ablation of a terminal SC leading to expansion of its neighbor in the NMJ.** An adult NMJ showing two terminal SCs (SC-GFP) and the axon (thy1-Membow13). The left SC was laser ablated. Images were analyzed by time-lapse confocal microscopy using a laser-scanning confocal microscope (FV1000). Frames were taken every 5–10 min for 3 h and 25 min. The fixed sample is shown at the end of the video. The video shown corresponds to Fig. 5 (A–C).



Video 5. **Ablation of an axonal SC leading to internodal expansion of a terminal SC.** An adult NMJ with a labeled terminal SC (SC-GFP) and axon (thy1-Membow13). An axonal SC was laser ablated. Images were analyzed by time-lapse confocal microscopy using a laser-scanning confocal microscope (FV1000). Frames were taken every 5–10 min for 3 h and 26 min. The fixed sample is shown at the end of the video. The video shown corresponds to Fig. 5 (D–F).



Video 6. **Ablation of a terminal SC does not result in expansion of the neighboring axonal SC.** An adult NMJ with one terminal SC (SC-GFP; axon labeled by thy1-OFP3). The terminal SC was laser ablated. Images were analyzed by time-lapse confocal microscopy using a laser-scanning confocal microscope (FV1000). Frames were taken every 5–20 min for 3 h and 30 min. The fixed sample is shown at the end of the video. The video shown corresponds to Fig. 5 (G–I).



Video 7. **Terminal SC dynamism 31 h after axotomy.** An adult NMJ with three terminal SCs and two axonal SCs (SC-GFP). Images were analyzed by time-lapse confocal microscopy using a laser-scanning confocal microscope (FV1000). Frames were taken every 5–10 min for 1 h and 43 min. The fixed sample is shown at the end of the video. The video shown corresponds to Fig. 6 (A–D).