

Fig. S1. Expression pattern of *moody*-Gal4 in third instar. Maximum projection illustrating a dissected *moody*-Gal4>nGFP L3 larva stained for Repo (magenta), Fas2 (blue) and GFP (green) in overview (A) and (boxed area) at higher magnification (B). (B) Individual ePG of the MFA of hs A2 are marked. ePG4, ePG7 and ePG10 are GFP positive. In the MFA the Repo staining is not always clearly recognizable in maximum projections as several PG are hidden behind muscles. Along the NER, *moody*-Gal4-expressing ePG3 is indicated. *moody*-Gal4 is additionally expressed in tracheal cells. Anterior is up.

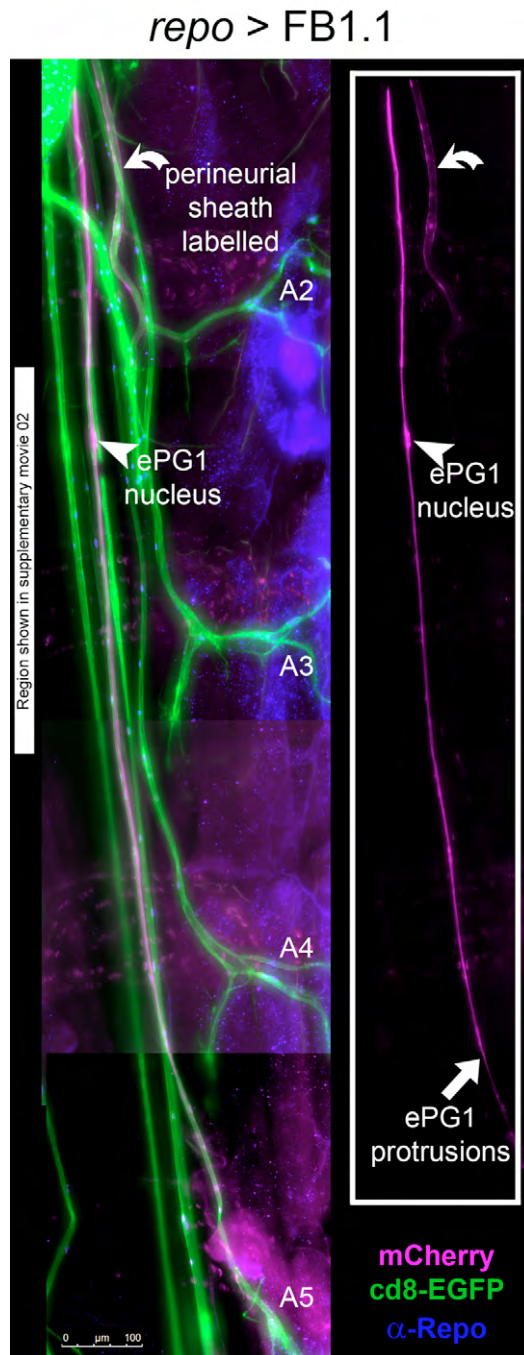


Fig. S2. ePG1 hypertrophy in third instar. Maximum projection showing mCherry-labelled ePG1 in L3 in hs A5. During larval development, ePG1 spreads almost along the entire NER resulting in a total length of more than 1 mm. The ePG1 nucleus is marked by an arrowhead. mCherry signal alone is illustrated in the boxed area. The region presented in Movie 2 is indicated by the white bar on the left side. In addition to ePG1, ePG2 progenies forming the perineurial sheath along the NER in hs A2 are also mCherry labelled (bent arrow). Anterior is up.

repo > nGFP, FB1.1

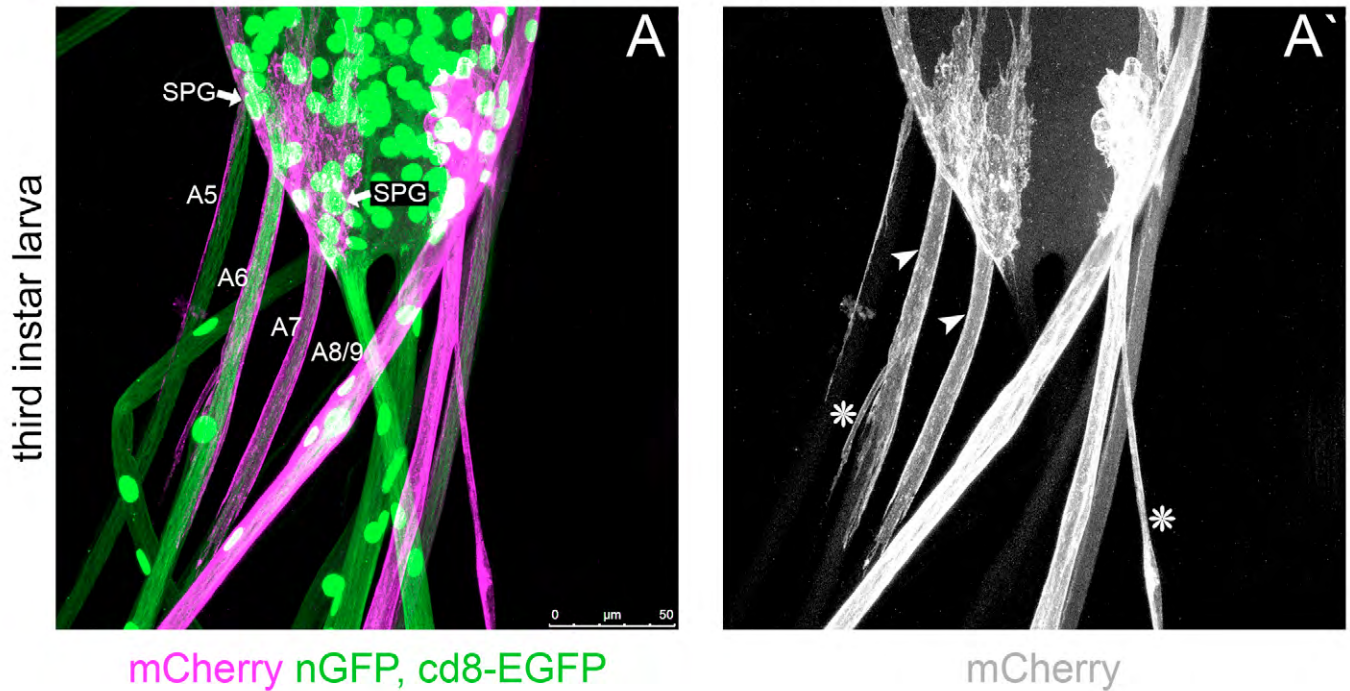


Fig. S3. VNC surface glia cover the proximal part of the NER. (A) Maximum projection showing the posterior tip of the VNC exhibiting several mCherry-labelled glial cells. (A') mCherry alone. Among these are two subperineurial glia of the VNC (nuclei marked by an arrow) that ensheath the proximal part of the peripheral nerve tract in hs A6 and A7 (arrowheads in A'). In addition, glial membranes can be observed covering a structure branching off the peripheral nerve (asterisk). Probably, this carries afferent sensory axons projecting into the VNC. The right-hand side of this animal was not analysed due to multiple labellings within the VNC and the PNS. Anterior is up.

A

Table summarizing the results of ePG2 ablation

specimen	N° of PG nuclei along NER	position of remaining PG along the NER	ablated in hs
larva 01 (B)	5	3 proximal; 2 distal	A5
larva 02 (C)	6	equally distributed but distal part free of nuclei	A5
larva 03	3	proximal located, extending up to middle of the nerve	A5
larva 04 (Fig.4)	2	both proximal	A5
larva 05	4	all distal	A4
larva 06	2	ePG1 proximal; ePG3 distal	A4
larva 07	2-4 precise counting impossible due to close vicinity of A4 and A5 in the proximal section of the NER	all proximal; distal part free of nuclei	A4
larva 08	3	all distal	A4
larva 09 (D)	4	all distal	A4
larva 10	8 - no significant effect	equally distributed	
larva 11	2	both distal	A4
larva 12	2	ePG1 proximal; ePG3 distal	A4
larva 13	15 - no effect	equally distributed	A4
larva 14	17 - no effect	equally distributed	A5

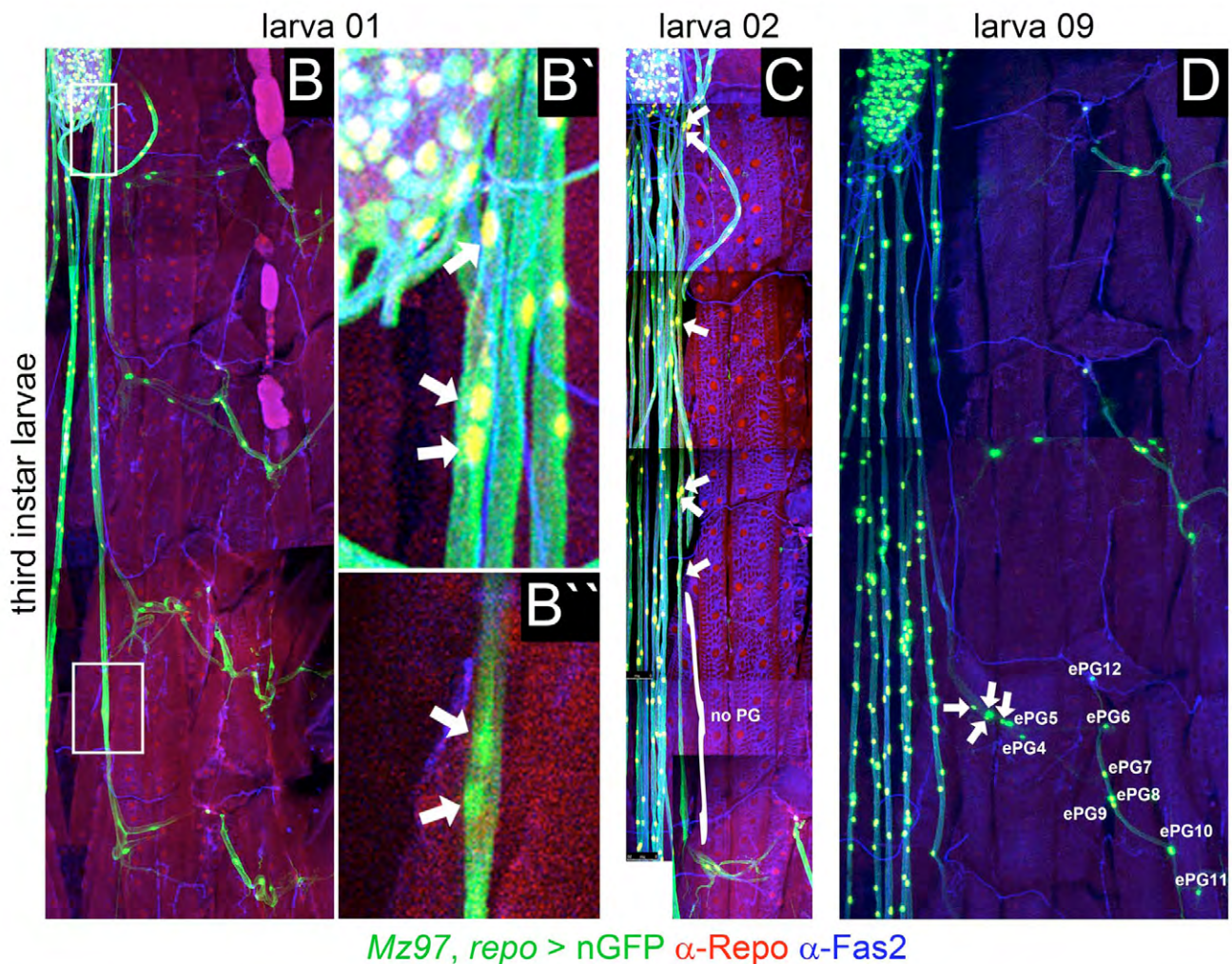


Fig. S4. Phenotypes resulting from embryonic UV irradiation of ePG2. (A) Summary of the number and position of remaining PG along the NER of irradiated hs in L3. Larva 10, 13 and 14 display no significant PG reduction along the NER. (B-D) Position of remaining PG along the NER of three dissected larvae stained for Repo (red) and Fas2 (blue). Anterior is up.

third instar larva

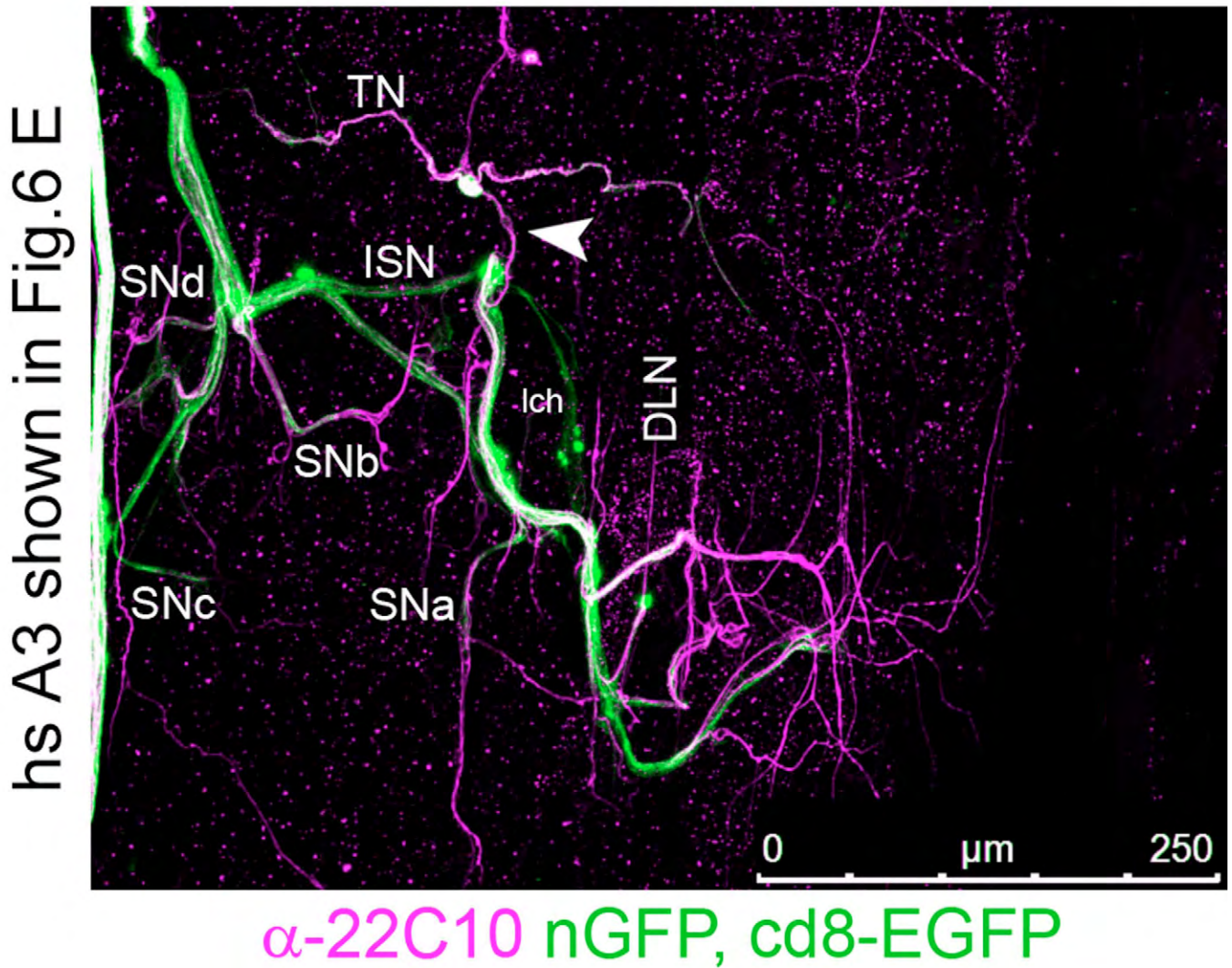


Fig. S5. 22C10-expressing neurons can be detected between ISN and TN. Confocal stacks of the identical hs as presented in Fig. 6E; however, antibody staining against 22C10 (magenta) was performed on this larva subsequently. 22C10-expressing neurons can be seen projecting between ISN and TN (arrowhead). Probably, these are covered by ePG7 protrusions in the corresponding region (arrowhead in Fig. 6E). Anterior is up.

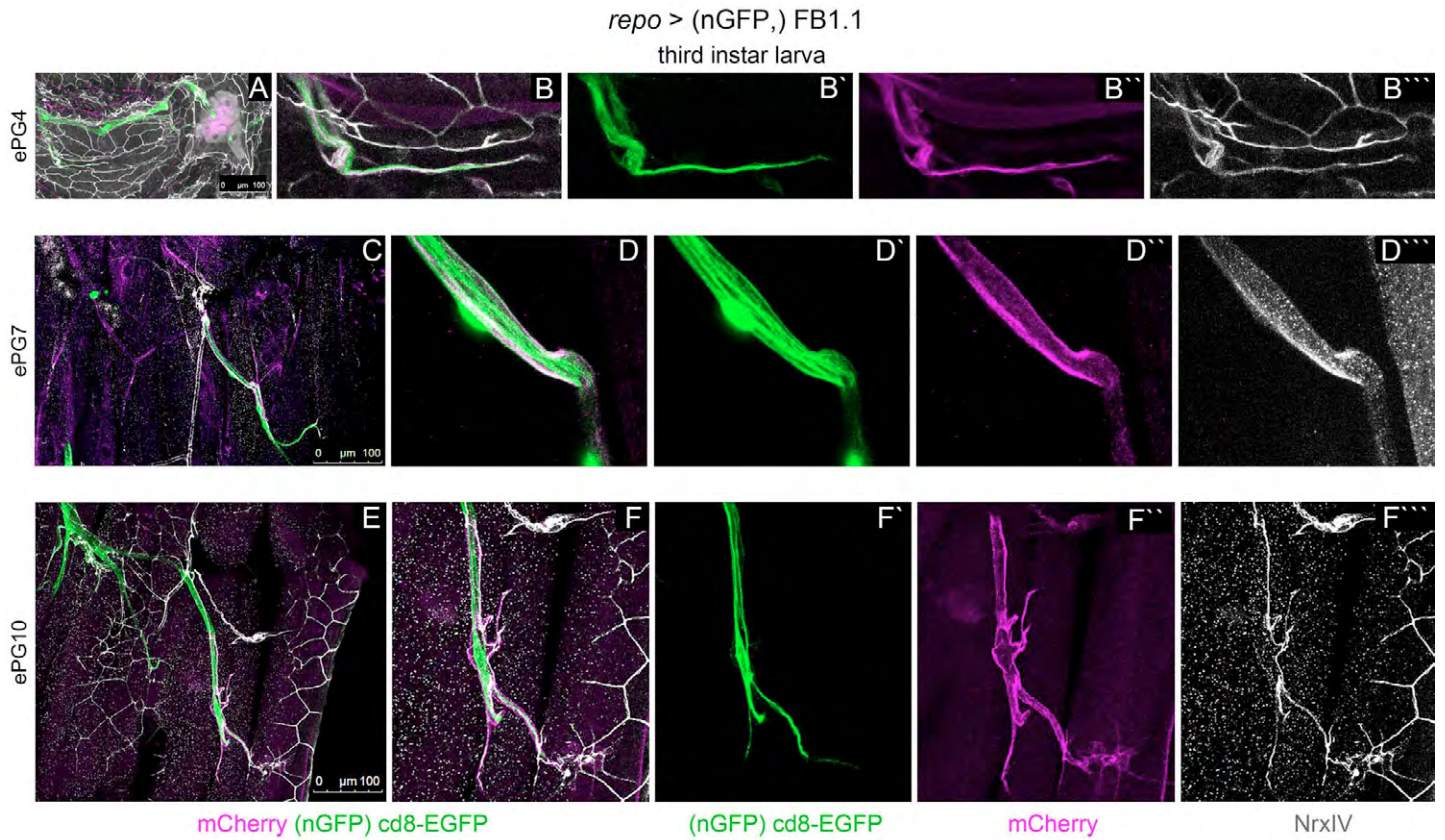


Fig. S6. ePG4, ePG7 and ePG10 express Neurexin IV. (A-F''') Antibody staining against the cell-adhesion molecule Neurexin IV (NrIV) was performed on Flybow-labelled ePG4, ePG7 and ePG10 in L3 to confirm their subperineurial identity. NrIV staining for ePG4 and ePG10 shows the same mCherry-labelled ePG as that presented in Fig. 6B,H. (A,C,E) Maximum projections of triple-labelled subperineurial glia (mCherry, GFP, NrIV). Enlarged views of selected focal planes (B,D,F) are followed by the expression of (nGFP) cd8-EGFP (B',D',F'), mCherry (B'',D'',F'') and NrIV (B''',D''',F''') alone. Anterior is up.

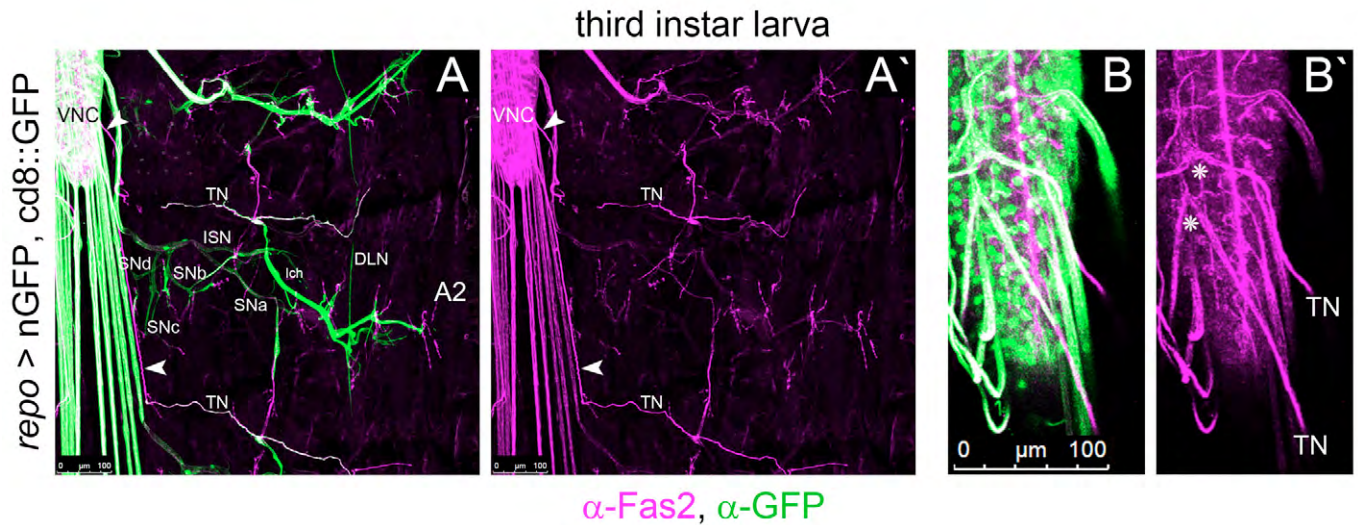


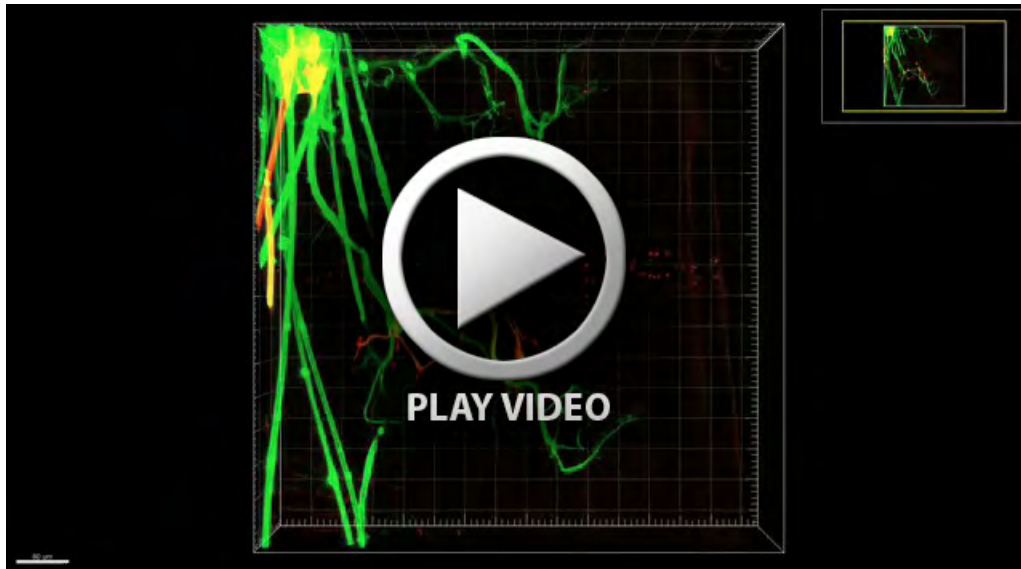
Fig. S7. TN projections enter the VNC separately through the dorsal median cells. Confocal stacks of L3 carrying *repo*-Gal4>UAS-nuclear-GFP (nGFP) and UAS-CD8::GFP transgenes stained for Fas2 (magenta) and GFP (green). (A,A') The overview (A) shows the VNC and a section of the PNS. Main peripheral nerve tracts are labelled in hs A2. Fas2-expressing TN projections can be followed due to their exposed position (arrowheads; A' shows Fas2 staining alone). The TN does not fasciculate with the main peripheral nerve tract along the NER, but enters the VNC at the dorsal midline. (B,B') At higher magnification the Y-shaped entry point of both TNs projecting into the VNC from each side can be seen (asterisks). Anterior is up.



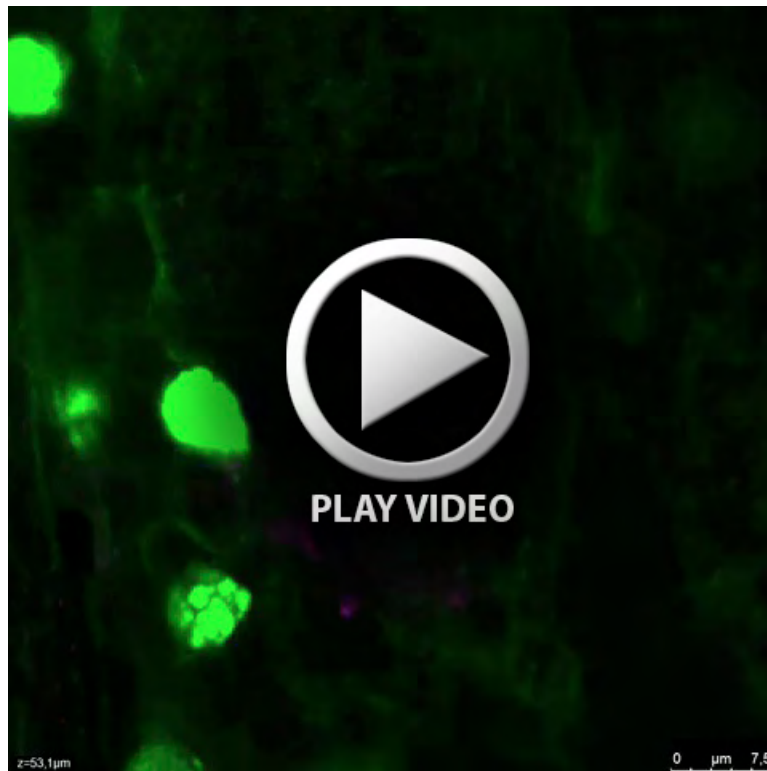
Movie 1. Expression pattern of *moody*-Gal4 in third instar. Confocal z-stacks of the region designated in Fig. S1. Individual PG in the MFA and *moody*-Gal4-expressing ePG3 are labelled. Anterior is up.



Movie 2. ePG1 protrusions form the innermost wrapping sheath along the NER. Confocal z-stacks of the NER region designated in Fig. S2. Due to its morphology, the mCherry-labelled ePG1 can clearly be identified as wrapping glia lying within the fascicle surrounded by cd8-EGFP-positive subperineurial and perineurial glia. Anterior is up.



Movie 3. Three-dimensional reconstruction of ePG7 and PNS morphology. A three-dimensional reconstruction of the mCherry-labelled ePG7 (red) shown in Fig. 6E. It was generated using Imaris software based on confocal stacks (z-step size, 0.8 μm). The reconstruction demonstrates the actual course of the peripheral nerve tracts through the larval tissue. For example, the proximal part of the ISN projects in a more superficial layer (underneath the muscle field in that region) compared with the distal part of the ISN, which lies on top of the muscles. Anterior is up.



Movie 4. Sponge-like morphology of a CBG in the VNC. Confocal z-stacks of an mCherry-labelled CBG (magenta) in the VNC of L3. This glial subtype surrounds neuronal somata in the cortex resulting in a sponge-like morphology. Anterior is up.