

Figure S1

A,B Dissected filet preparations of third instar larvae during prefixation (**A**) and after plastic embedding (**B**). The needles are required to keep the filet in a stereotyped and straight pattern. **C** Wrapping index (number of wrapped axon clusters / total number of axons in %) for cross sections taken at 2 μm, 50 μm, 200 μm or 400 μm distance from the CNS along the same peripheral nerve from the same animal. The corresponding images are shown (**D-G**). The scale bar size is 2 μm. The arrows indicate one tracheal cell that forms two tubes.

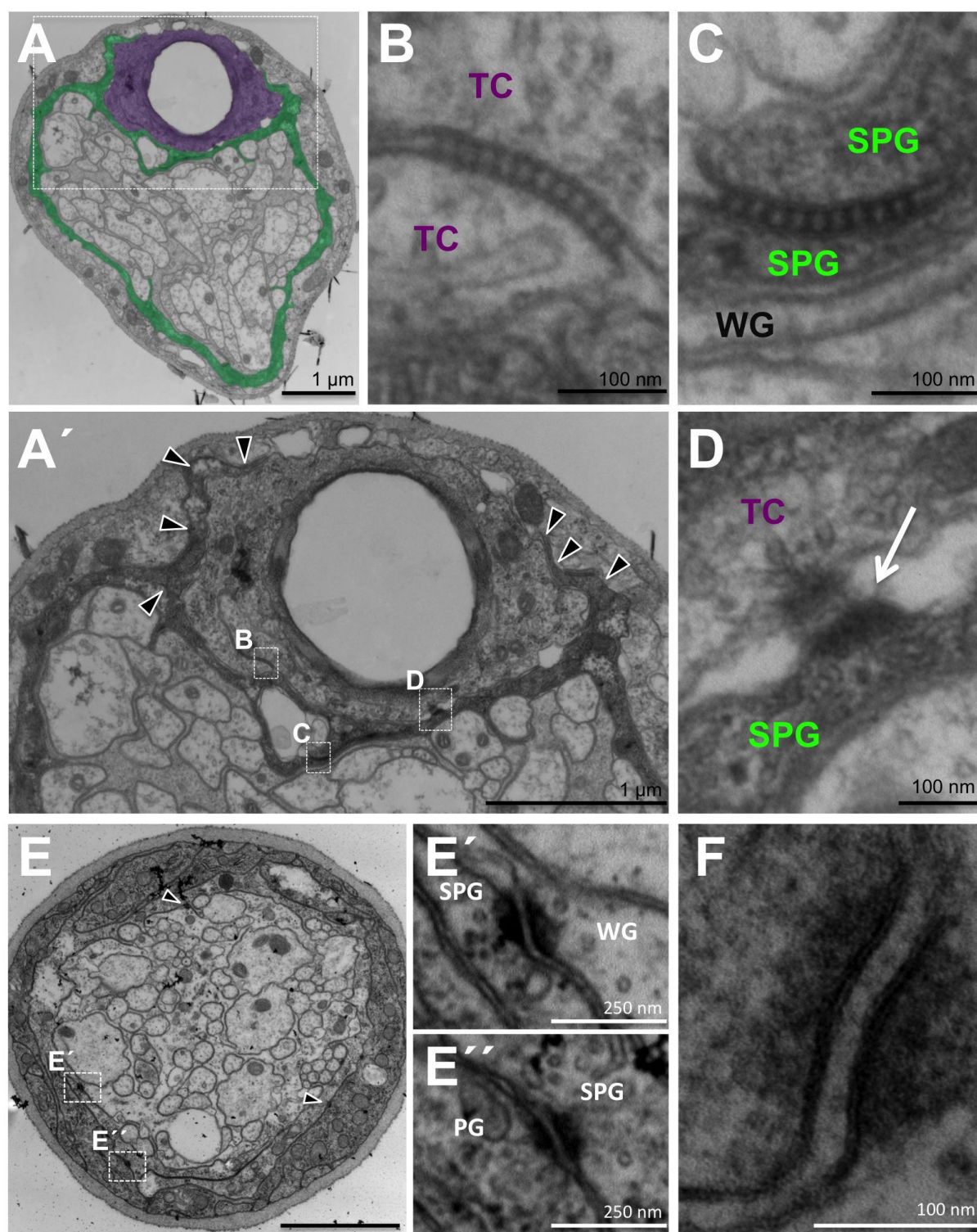


Figure S2

A Tracheal cells are located within the nerve close to the CNS but are not found in the nerve 200 μ m or more distant from the CNS. Tracheal cells are always found in

the perineurial glial layer, often covered by processes of the subperineurial glia (green shading). The boxed area is shown in higher magnification in (A'). **A'** Arrowheads indicate the processes of the subperineurial glia that cover the trachea. The areas boxed in A' are shown in higher magnification in (B-D). **B** Tracheal cells (TC) establish extensive autocellular pleated septate junctions (pSJ). **C** Subperineurial glial cells (SPG) form autocellular pSJ, pSJ are not detected between trachea and subperineurial glia. **D** The subperineurial glia forms spot adherens junctions with the tracheal cells. **E, E', E''** The subperineurial glia forms spot adherens junctions with other glial cells. Boxed areas are shown in higher magnification. **F** High resolution image of a glial-glial spot adherens junction. Scale bars are 2 μm , or as indicated.

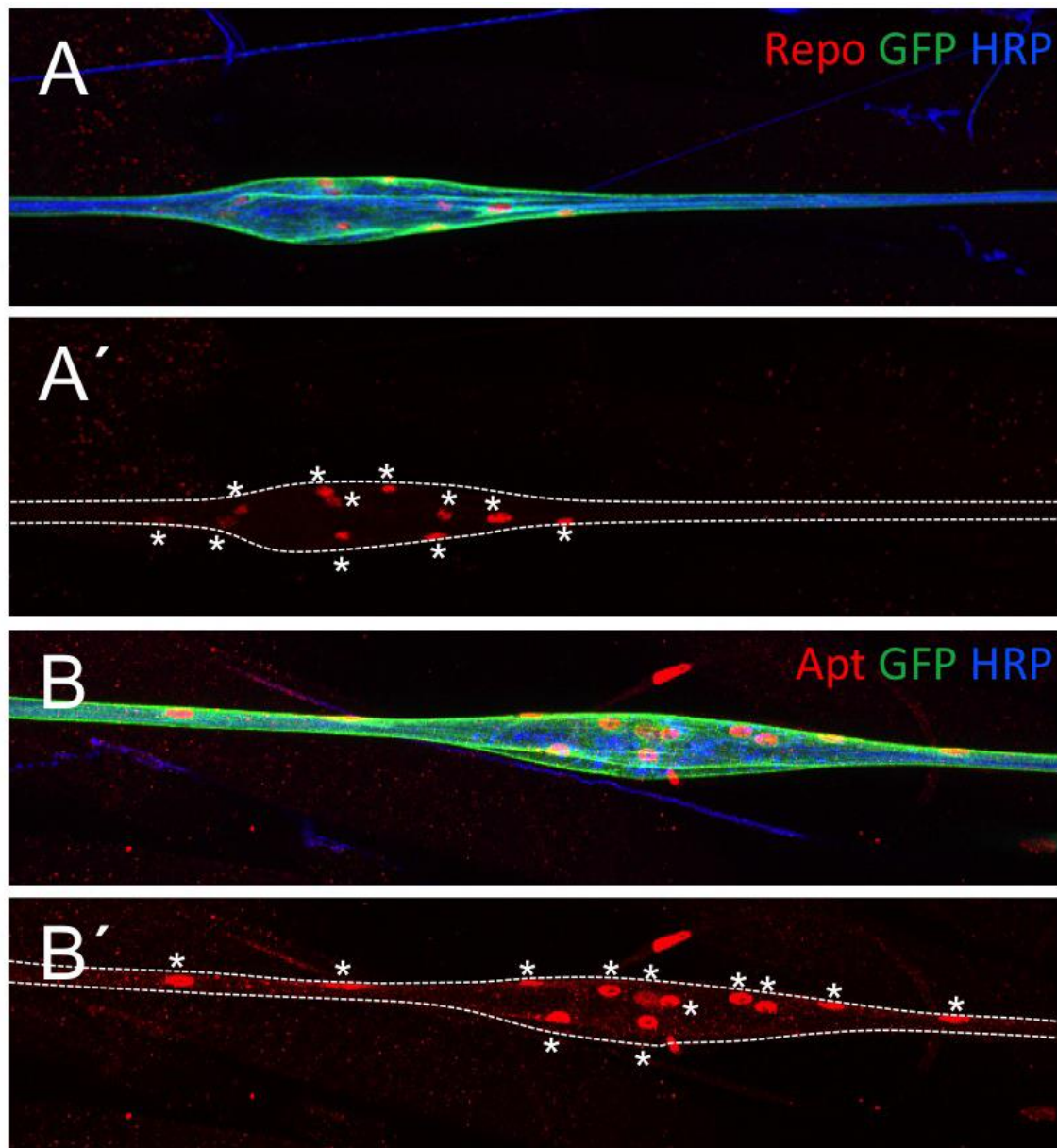


Figure S3

A,A' Nerve bulge induced by expressing *fray*^{dsRNA} in glial cells. Glial nuclei are in red (Repo staining), glial membranes are in green (*repo>>CD8GFP*) and axonal membranes are in blue. **B,B'** Nerve bulge induced by expressing *fray*^{dsRNA} in glial cells. Perineurial glial nuclei are in red (Apontic staining), glial membranes are in green (*repo>>CD8GFP*) and axonal membranes are in blue. Note the increased number of glial nuclei (asterisks) in the bulge area. The dashed line denote the nerve.