Fig. S1. Two FlyLight-GAL4 lines and SPARC-GAL4 label antennal lobe ensheathing glia. Confocal sections of adult antennal lobe are shown. (A1, B1, C1, and D1) Ensheathing glia processes are labeled by GMR56F03-GAL4– (at 25 °C) (A1 and C1), GMR10E12-GAL4– (at 18 °C) (B1), or SPARC-GAL4– (D1) driven (>UAS-mCD8GFP). Ensheathing glia nuclei are marked by UAS-nuclear-LacZ (A2 and B2). Glia nuclei stained by the Repo antibody are shown in green. (A3 and B3) The channel for Repo staining is overlaid on the nuclear-LacZ channel. (C and D) Colabeling of ensheathing glia (mCD8GFP and nuclear-lacZ) and the astrocyte marker GAT, visualized by antibody staining (white). GAT staining is overlaid on the nuclear-LacZ channel (C2 and D2) to show that, with a few exceptions (arrows), GAT does not label GAL4+ cells. (E) Colabeling of ensheathing glia [SPARC-QF > QUAS-mtdT and QUAS-nuclear-lacZ (green and magenta, respectively)] and astrocytes (Alrm-GAL4 > mCD8GFP, white). The GFP channel is overlaid on the nuclear-LacZ channel in E2 to show that the glia marked by SPARC-QF are not astrocytes. (Scale bar: 10 μm.) In this figure, subpanels represent the same sample in different imaging channels, unless otherwise specified.
Fig. S2. P35 suppresses the reduction in ensheathing glia numbers caused by htl knockdown. (A and B) Projections of confocal sections along the z axis for the wild type (A) and UAS-htl RNAi (VDRC 27180) together with UAS-P35 driven by SPARC-GAL4 (B). Ensheathing glia cell bodies marked by SPARC-GAL4 > UAS-nuclear-LacZ are shown in red; Ncad antibody staining for antennal lobe neuropil is shown in blue. (C) Quantification of the number of ensheathing glia cells in each antennal lobe. (D and E) Confocal sections of the antennal lobe at 96 hAPF of wild type (D) and UAS-htl RNAi (VDRC 27180) together with UAS-P35 driven by SPARC-GAL4 (E). Ensheathing glia processes are labeled by SPARC-GAL4 > UAS-mCD8GFP. Neuropil compartments are stained with Ncad antibody. (F–H) Quantification of the intensity of ensheathing process within the antennal lobe (F), relative SD of Ncad staining (G), and the correlation coefficient for the intensities of ensheathing glia processes and neuropil staining (H). (Scale bars: 10 μm.) Error bars represent SD. **P < 0.01; ****P < 0.0001; ns (not significant), P > 0.05. In this figure, subpanels represent the same sample in different imaging channels, unless otherwise specified.

Fig. S3. Additional examples of MARCM clones and analysis. (A–C) Wild-type ensheathing glia processes labeled by GMR10E12-GAL4 > UAS-mCD8GFP are shown in green. Ensheathing glia cell bodies marked by UAS-nuclear-LacZ are shown in blue. Neuropil staining by the Ncad antibody is shown in magenta. (Scale bar: 10 μm.) D, dorsal; L, lateral. An ensheathing glia located on the dorsal surface of the antennal lobe (A3) extends processes ventrally and half-encircles the DA3 glomerulus (A2). An ensheathing glia located on the dorsal surface of the antennal lobe (A3) extends processes laterally to access glomeruli in the center of the antennal lobe (B2 and B3). An ensheathing glia located on the most anterior surface of the antennal lobe (C2) extends processes into posterior antennal lobe (C3). (D) Quantification of the total intensity of processes from each ensheathing glia (wild-type and Htl<sup>Ab42/Ab42</sup>) labeled by MARCM. Error bars represent SD. **P < 0.01. In this figure, subpanels represent the same sample in different imaging channels, unless otherwise specified.
**Fig. S4.** *Orb*^{0449-GAL4} labels LNs in the antennal lobe. (A) *Orb*^{0449-GAL4} > UAS-mCD8GFP are shown in green. (A1) A projection of confocal sections along the z axis at 24 hAPF. Approximately 23 LNs are labeled. (A2) A confocal section of antennal lobe at 96 hAPF. A total of ∼55 LNs are labeled over all confocal sections of the antennal lobe. (B) ey-FLP intersects with *orb*^{0449-GAL4} together with UAS-FRT-stop-FRT-mCD8GFP to show that *orb*^{0449-GAL4} is inactive in the majority of ORNs, except for two classes that project to the medial side of the antennal lobe (arrow). (C) The GH146-FLP intersection shows a rare case in which an interneuron is labeled (one cell out of 10 antennal lobes); no cells are labeled around the other antennal lobes. Neuropil staining by the Ncad antibody is shown in magenta. (Scale bar: 10 μm.)

**Fig. S5.** Loss of Ths from ORNs and PNs does not cause defects in ensheathing glia or glomeruli. (A) GH146-GAL4 drives RNAi against ths. Ensheathing glia processes are labeled by SPARC-QF > QUAS-mtdT. (B) ey-FLP MARCM combined with a cell-lethal strategy creates a ths mutant in nearly all ORNs. Ensheathing glia processes are labeled by GMR56F03-GAL4 > UAS-mCD8GFP. Neuropil staining by the Ncad antibody is shown in magenta. (Scale bar: 10 μm.)

(C–F) Quantification of ensheathing glia process intensities (C and E) and relative SD of Ncad staining intensities (D and F). Error bars represent SD. ns (not significant), *P* > 0.05. In this figure, subpanels represent the same sample in different imaging channels, unless otherwise specified.

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