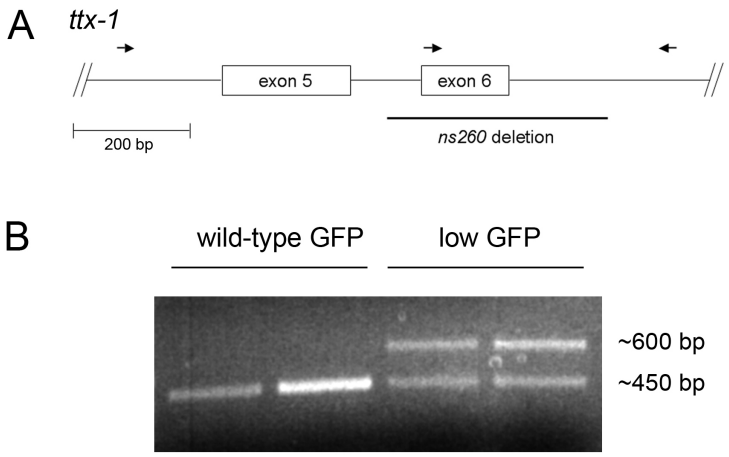

Figure S1 PCR assay for genotyping *ttx-1*(*ns260*) animals. (A) Schematic of the *ttx-1* gene, showing only exons 5 and 6 (boxes). The *ns260* deletion is marked. The location of the three oligonucleotide primers used to genotype individual animals (B) are indicated by arrows (5' to 3'). (B) Genotyping of four individual progeny from an *ns260*/+ heterozygous parent grown at 25° and carrying a *ver-1* promoter::*gfp* transgene (*nsIs22*). Two of these animals had wild-type levels of *gfp* expression, and had a wild-type genotype (left), while the other two had low levels of *gfp* and were *ns260*/+ heterozygous (right). Primers used in the PCR assay are shown in A. Wild-type animals exhibit a single amplified DNA fragment of ~450 bp. *ns260* homozygous animals are predicted to produce only a single DNA band of size ~600 bp.

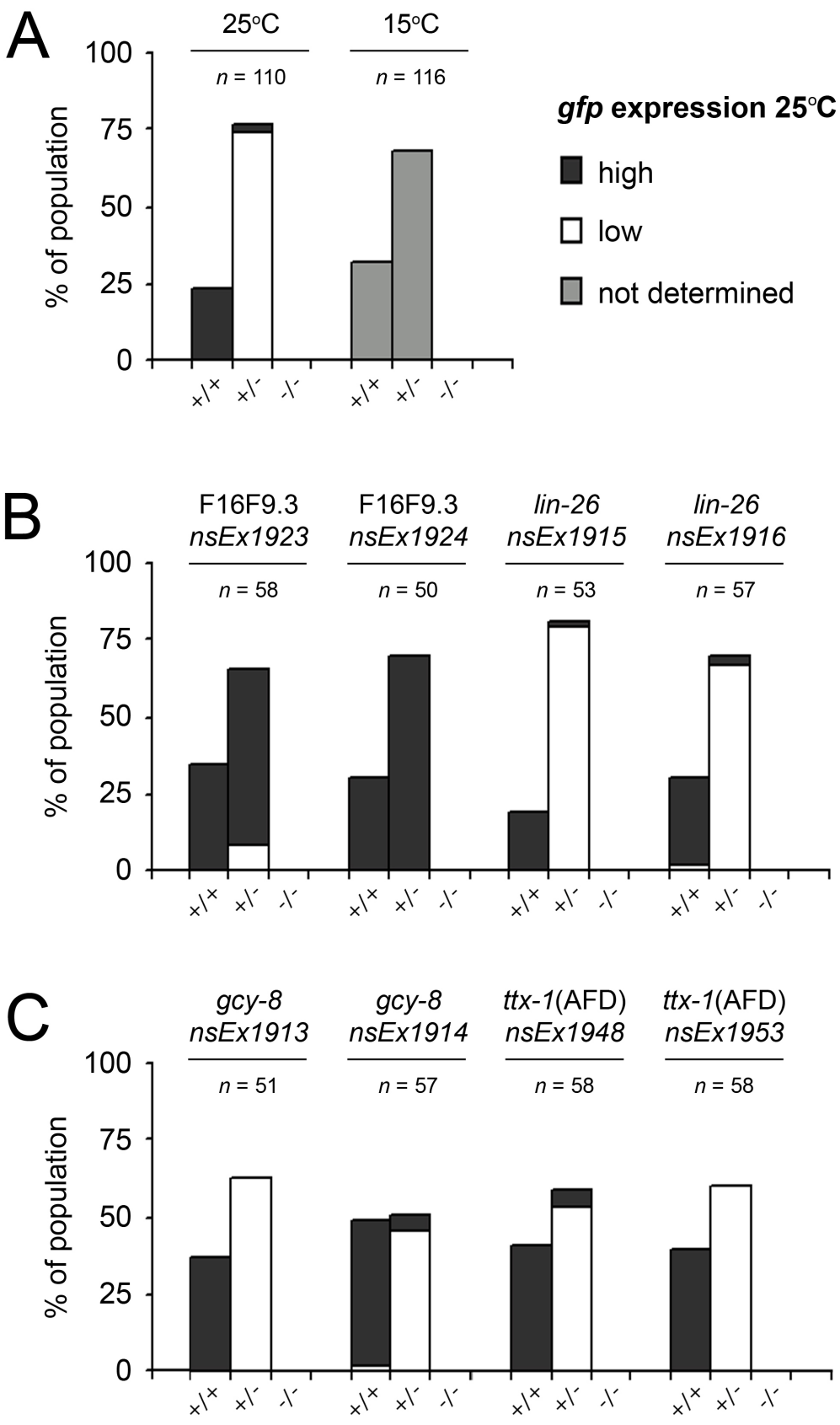
A
25°C
15°C
n = 110
n = 116
% of population
100
75
50
25
0
+/+
+/-
-/-
gfp expression 25°C
high
low
not determined
B
F16F9.3
nsEx1923
F16F9.3
nsEx1924
lin-26
nsEx1915
lin-26
nsEx1916
n = 58
n = 50
n = 53
n = 57
C
gcy-8
nsEx1913
gcy-8
nsEx1914
ttx-1(AFD)
nsEx1948
ttx-1(AFD)
nsEx1953
n = 51
n = 57
n = 58
n = 58

Figure S2 Glial or AFD-specific expression of *ttx-1* cDNA fails to rescue *ns260* lethality. (A) Genotyping of viable progeny from *ns260*/+ heterozygous parents grown at either 15° or 25°. Wild type, +/+; *ns260* heterozygous, +/–; and *ns260* homozygous, –/–. All animals also carry a *ver-1* promoter::*gfp* transgene (*nsIs22*), and levels of *gfp* expression in the sheath glia in each individual animal were scored as either high, low, or not determined. The number of progeny examined (*n*) is indicated. (B) Same as A, except the progeny also carry extrachromosomal arrays restoring *ttx-1* expression in the AMsh glia. Cell-specific promoters driving *ttx-1* cDNA include the *F16F9.3* promoter (late embryo to adult expression (Bacaj *et al.* 2008)) and the *lin-26* promoter (embryonic expression only (Landmann *et al.* 2004)). Two different rescuing arrays using each promoter were scored, and are indicated. The qualitative expression level of *ver-1* promoter::*gfp* at 25° is shown (see legend part A). (C) Same as B, except using AFD-specific *gcy-8* and *ttx-1* promoters. In B and C, the *ttx-1a* splice form was used. In all lines shown in A, B, and C, viable *ns260* homozygous animals (–/–) were never observed.

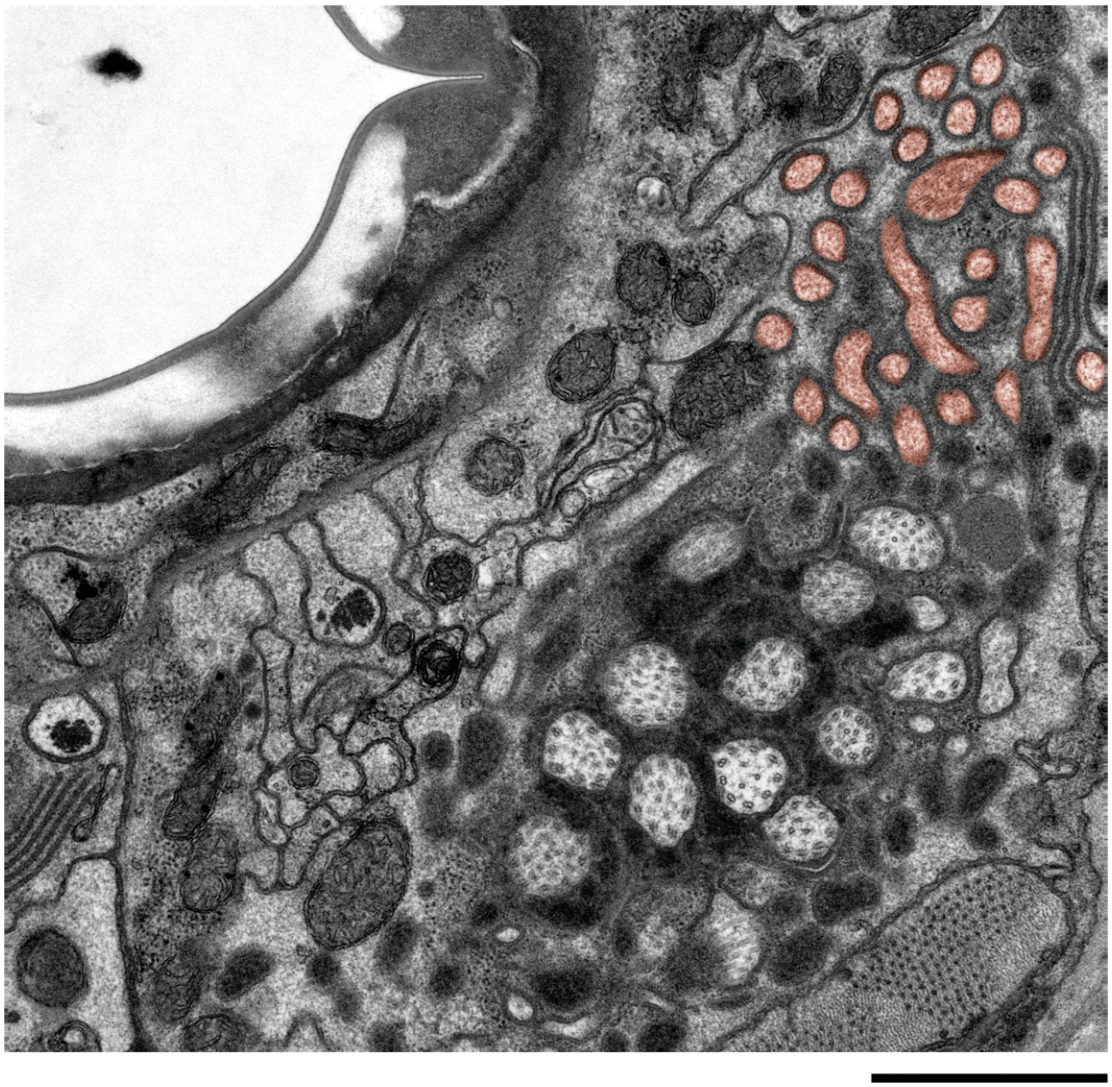

Figure S3 *ns171* mutants have wild-type AFD sensory ending morphology. Electron micrograph (EM) showing a cross-section through an amphid sensory organ of an *ns171* mutant adult animal. AFD microvillar projections, red shading. See also Figure 5, A and B. Scale bar, 1 μm. For comparison, see (Ward *et al.* 1975).

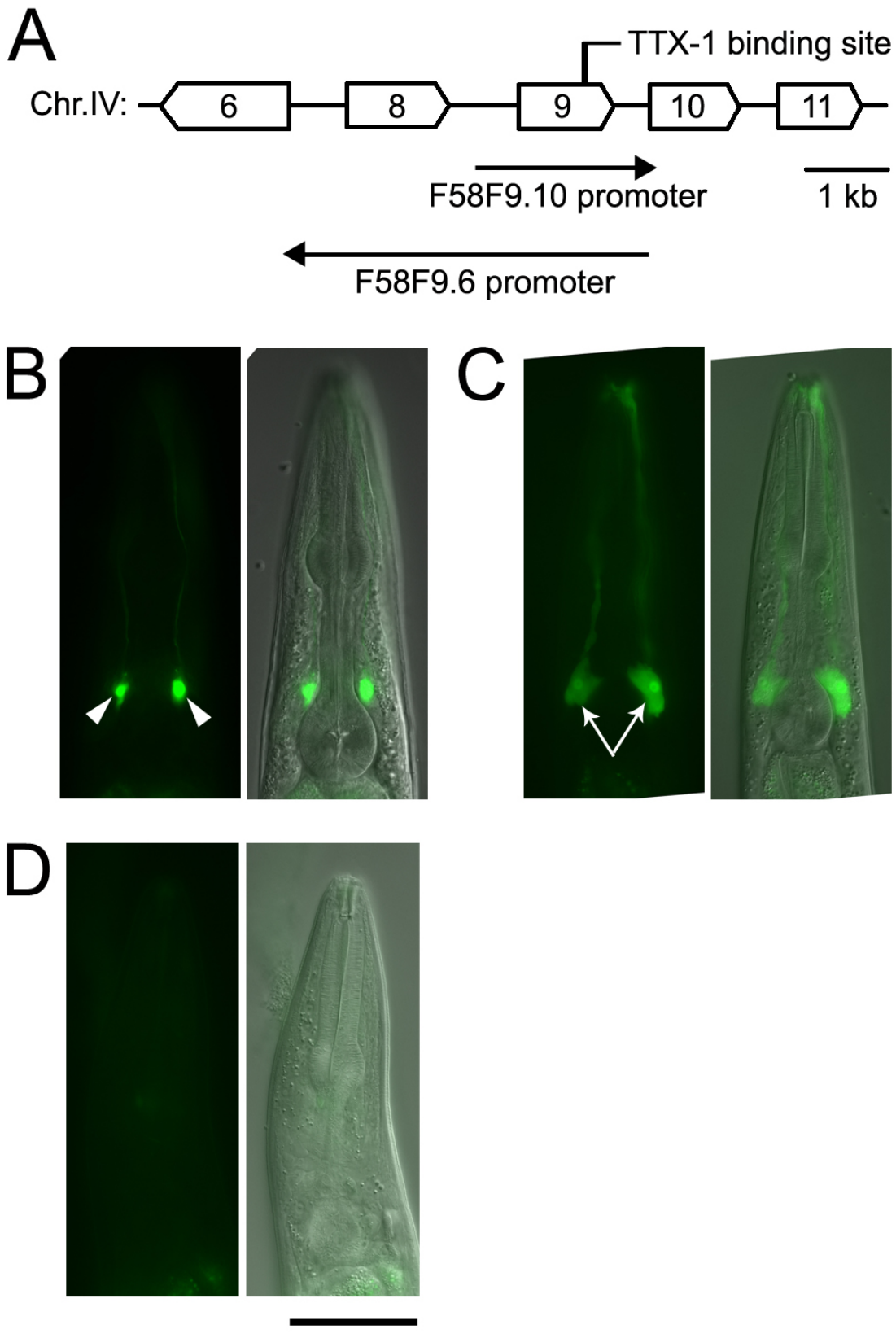
A
TTX-1 binding site
Chr.IV:
6
8
9
10
11
F58F9.10 promoter
1 kb
F58F9.6 promoter
B
C
D

Figure S4 TTX-1 directly regulates glial and AFD genes. (A) A schematic showing part of the F58F9 cosmid sequence, which includes a cluster of five thrombospondin (TSP)-domain containing genes (boxes). The gene numbers are designated by WormBase. The putative TTX-1 binding site, based on conservation with the *ver-1* promoter, is indicated (conserved residues between *ver-1* and F58F9 are 5' GATTATCGGATTCAG 3', with core TTX-1 binding residues underlined). Also shown are the *F58F9.10* and *F58F9.6* promoter regions used in expression studies. (B and C) Fluorescence images (left), and DIC and fluorescence merged images (right) showing *gfp* expression in the AFD neurons of an adult wild-type animal carrying an *F58F9.10* promoter::*gfp* transgene (*nsEx2284*) (B), or in the AMsh glia of a wild-type animal carrying an *F58F9.6* promoter::*gfp* transgene (*nsEx2330*) (C). GFP expression in AFD is indicated by arrowheads, and in AMsh glia by arrows. Expression of *F58F9.6* promoter::*gfp* in AMsh glia was rare (1/13 lines). (D) As in (C), except in a *ttx-1(p767)* mutant. Exposure (C and D), 500 ms. Scale bar (B-D), 50 μm. Anterior is up. All animals grown at 25°C.

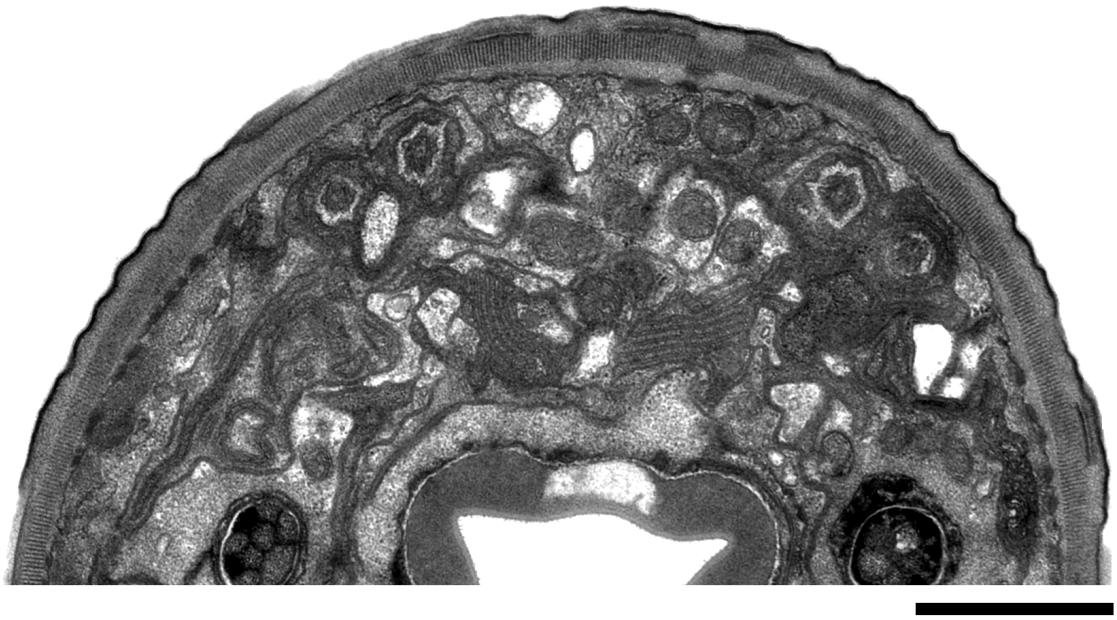

Figure S5 High resolution image of Figure 9C.

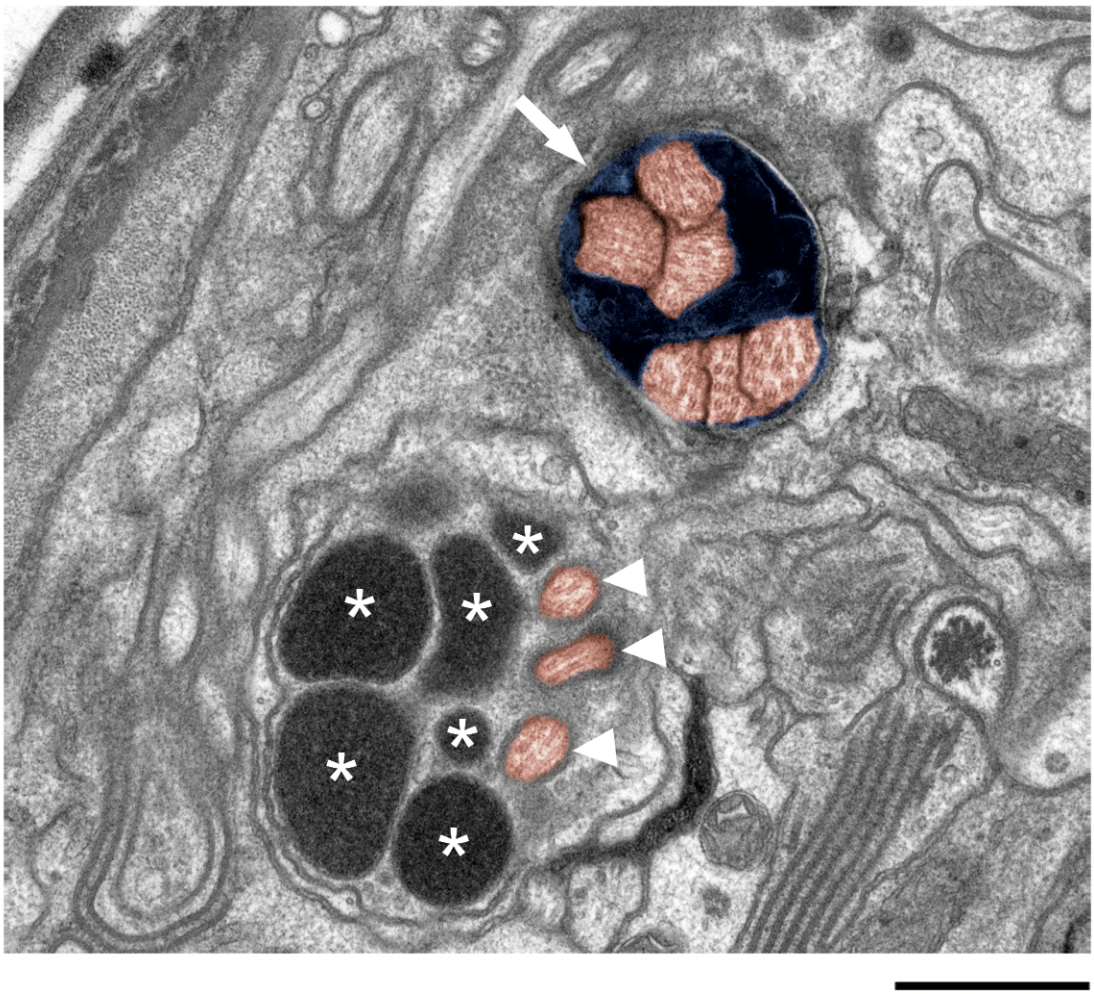

Figure S6 Electron micrograph (EM) showing a cross-section through the amphid sensory channel of a *ztf-16*(*ns171*) mutant adult animal. In 2/3 animals examined, the amphid sensory channel (arrow, blue shading) stained abnormally darkly, as did pockets within the AMsh glia (asterisks). Some sensory neurons (red shading) failed to traverse the channel, and were trapped inside the AMsh glial cell (arrowheads). Scale bar, 600 nm. For comparison, see (Ward *et al.* 1975).