Supplementary information

AFF-1 fusogen can rejuvenate the regenerative potential of adult dendritic trees via self-fusion

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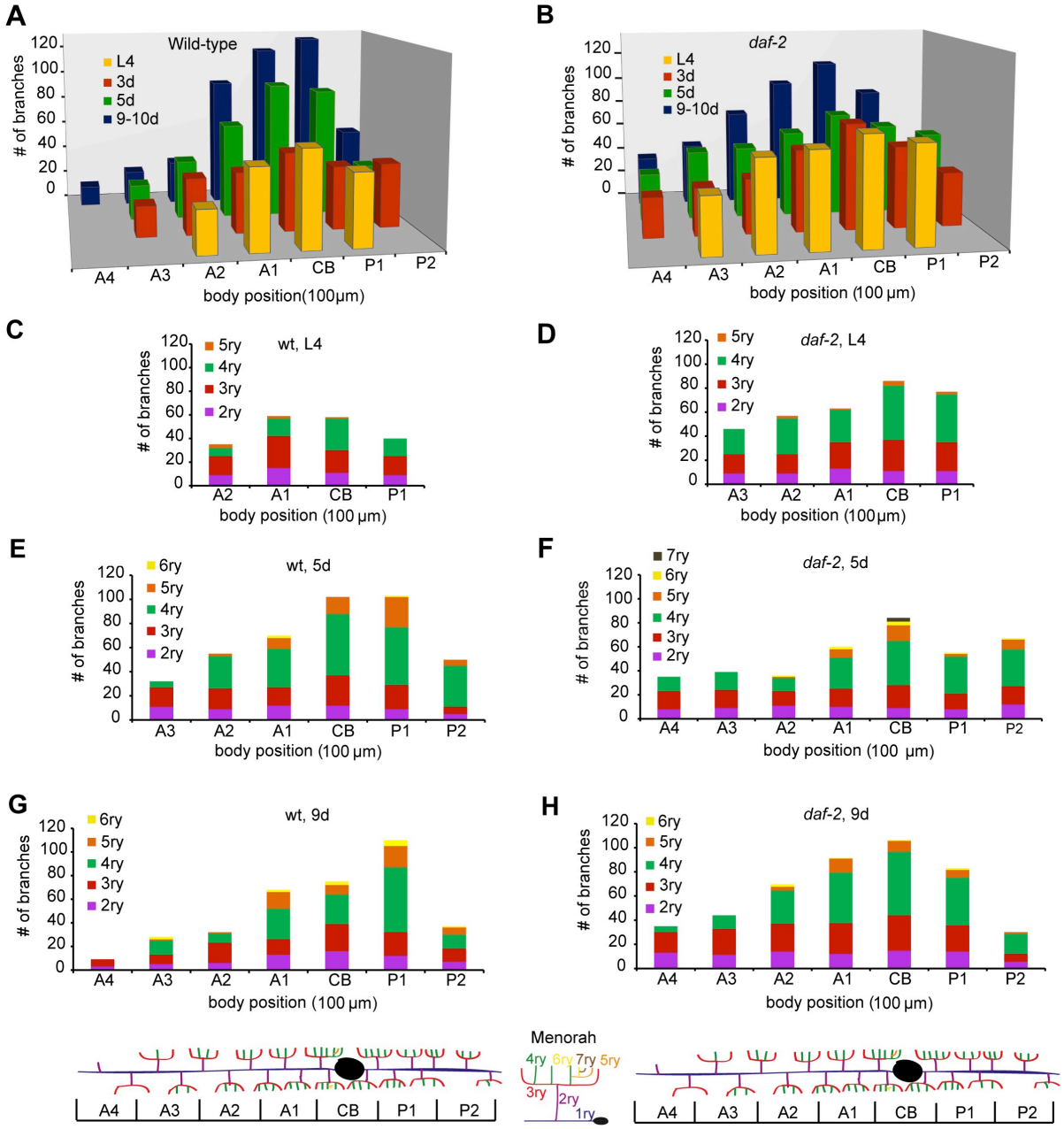

Fig S1. Anterior-posterior branching gradient sharpens as animals age

(A-B) Average number of branches per 100 µm of body length (A4-P2; A, anterior; P, posterior; CB, cell body; d, days of adulthood) in wild-type (wt) and *daf-2* mutants. Gradient sharpening is delayed in *daf-2*. We performed three way analysis of variance (ANOVA) to compare between wt (**A**) and *daf-2* (**B**). The contribution of body position and age to branching were significant (p=0.0001 and p=1.4X10^{-6}, respectively). Age*genotype, body position*genotype, body position*age and genotype alone were significant (p=0.007, p=0.001, p=0.043 and p=0.023, respectively). Number of animals: wt n ≥ 2; *daf-2* n ≥ 3.

(C-H) Each bar is a 100 µm long PVD region. Bars and schematic menorahs on the bottom: 2ry, magenta; 3ry, red; 4ry, green; 5ry, orange; 6ry, yellow; 7ry, brown.

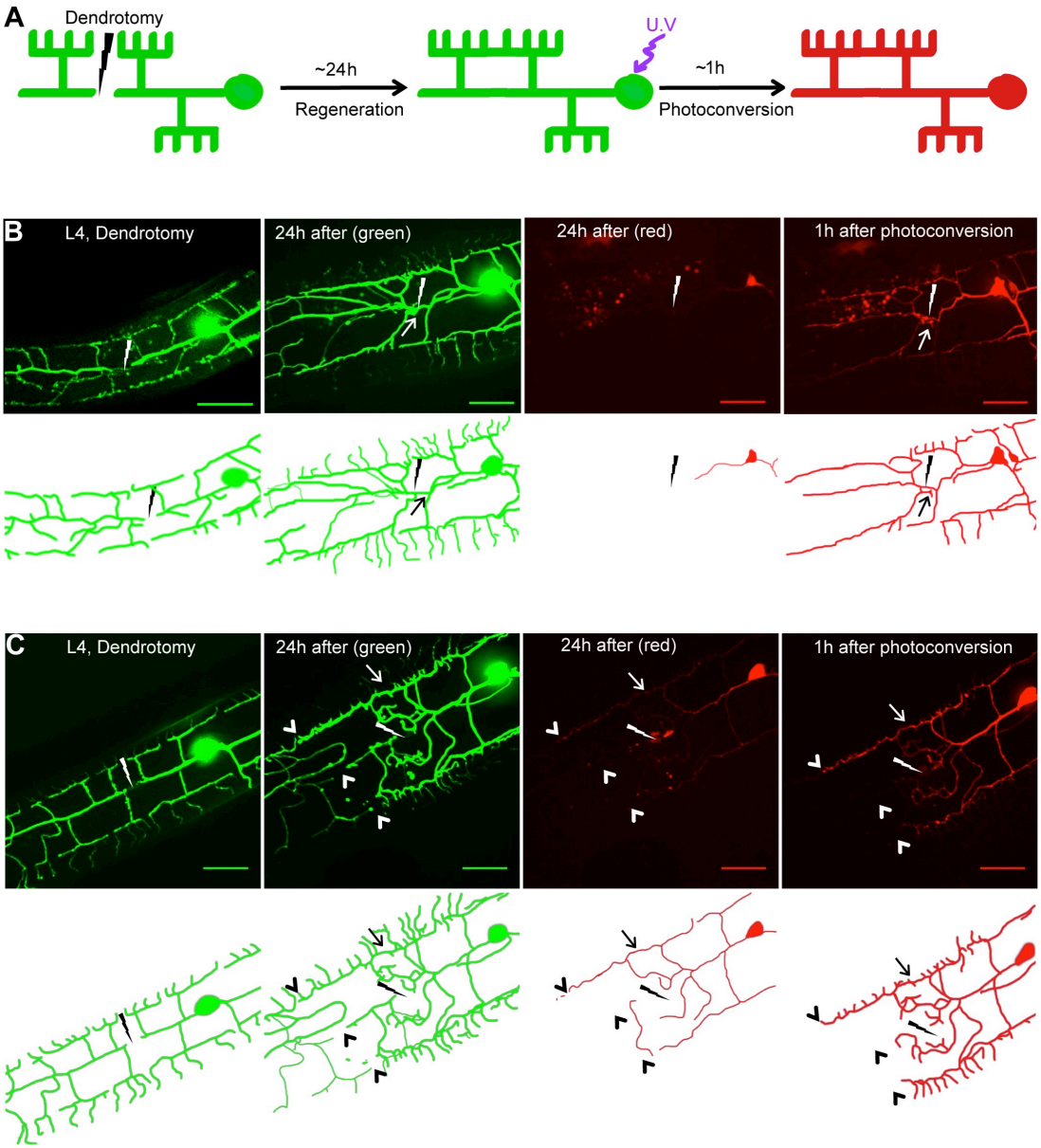

Fig S2. Fusion is a crucial step in regeneration of injured dendrites

(A) Scheme describing the fusion assay using a photoconvertible fluorescent marker Kaede. Primary dendrite is injured using a laser, then the animal is recovered and imaged again after ~24 hours in green and red channels. Green Kaede is photoconverted using a U.V. laser focused to the cell body. After ~1 hour the spread of red Kaede is observed again.

(B) Upper panel: confocal reconstructions of wild-type L4 animal immediately after dendrotomy, green fluorescence 24 hours after injury and before photoconversion, red fluorescence before photoconversion and red fluorescence an hour after photoconversion of the cell-body. In the lower panel are illustrations. Red kaede passed into the distal area, meaning that the broken dendrite fused to the proximal part.

(C) A negative control showing L4 wild-type animal in which the primary branch did not regenerate within 24 hours. Proximal candelabra are fused (arrows), but it did not bridge the gap between the distal and proximal stumps (arrowheads). Indeed red kaede did not spread into the detached distal stump. The order of images is the same as described in **(B)**. Lightings point at injury sites, arrows point at fusion sites. Scale bars, 20 µm.

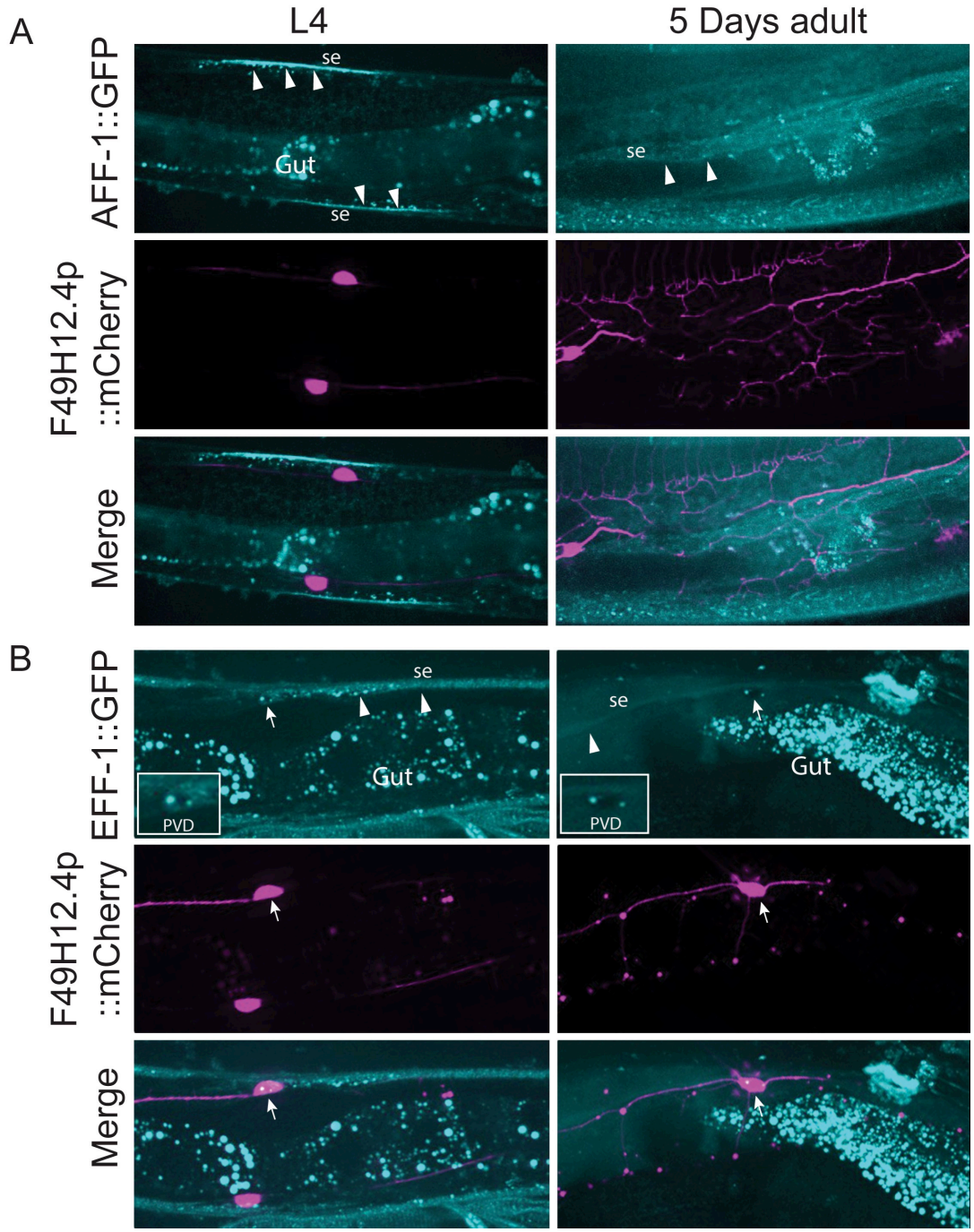

Fig S3. AFF-1 and EFF-1 expression pattern in L4 and aging worms

(A) A 30kb fosmid-based reporter for AFF-1 shows expression in the epithelial seam-cells at the L4 stage (marked with arrowheads), and in 5-day adults. AFF-1 is not expressed at the PVD in any stage. Multiple transgenic lines were analyzed and a similar expression pattern was observed in all.

(B) EFF-1 expression at the L4 stage and 5-days adults was analyzed using an EFF-1::GFP translational chimera: 7.5 kb *eff-1* promoter driving the full-length *eff-1* genomic coding sequence was fused to GFP [1]. EFF-1 is expressed in the seam-cells (arrowheads) and the PVD cell body (arrows and inset) in vesicles. Expression persists after 5 days.

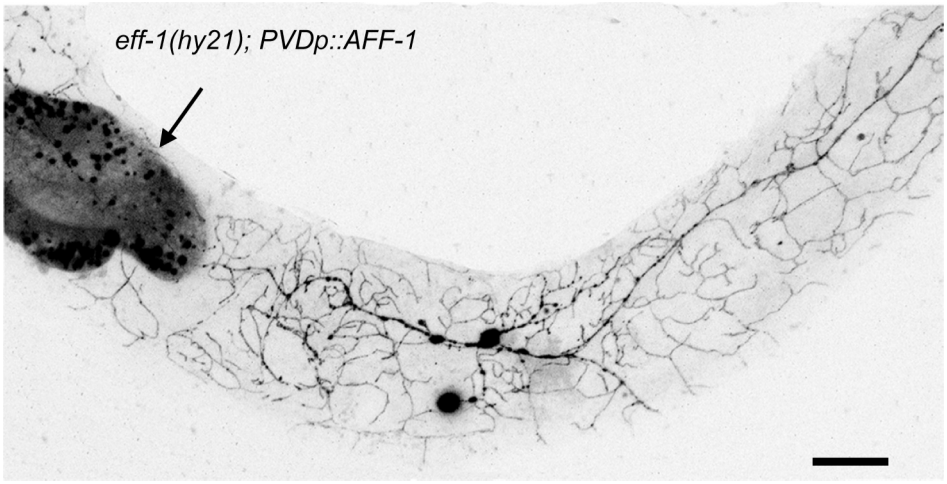

Fig S4. AFF-1 does not retract PVD's dendrites. *eff-1(hy21)* animals expressing AFF-1 in the PVD (PVDp::AFF-1) show excess branching, similarly to *eff-1* mutants

Thus the fusogen AFF-1 cannot rescue *eff-1* loss-of-function phenotype when expressed in the PVD. Arrow points to hypodermal cells expressing GFP after ectopic PVD neurite-epidermal fusion. Scale bar, 10 μm.

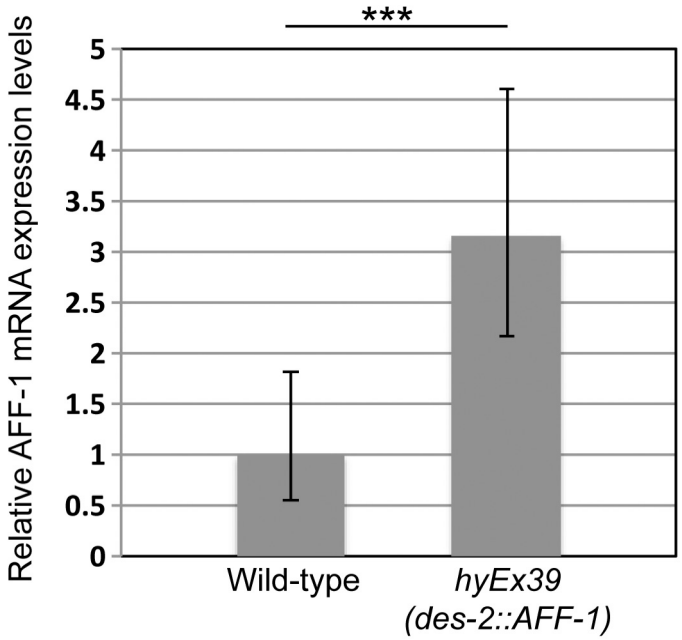

Fig S5. Real-time RT-PCR analysis of AFF-1 mRNA expression in wild-type and transgenic worms over expressing AFF-1 in the PVD. Bar graph representing the fold changes of mRNA levels quantified by normalization to the *act-1* gene as an internal control. *p* value from *t* test ***$p<0.0001$.

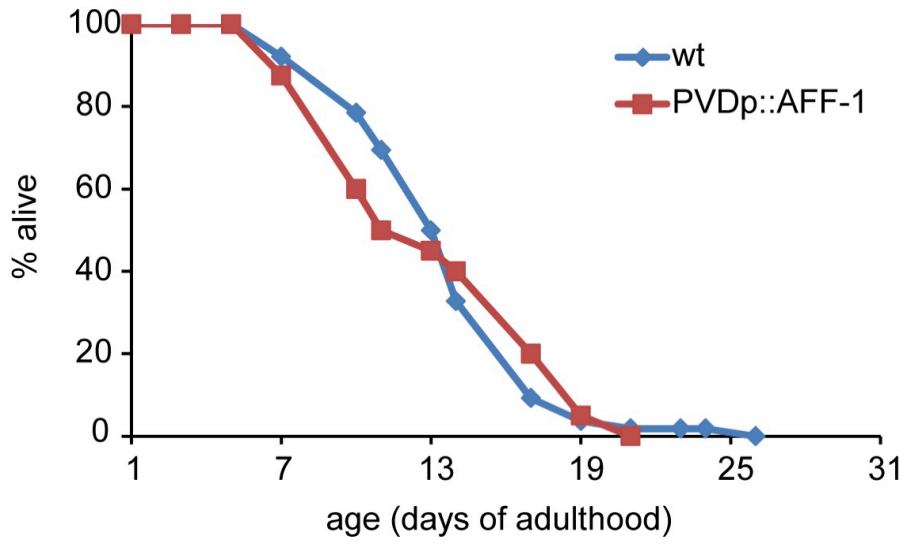

Fig S6. AFF-1 overexpressing animals exhibit wild-type life-span

Life-span curve of wild-type (blue) and AFF-1 overexpression in the PVD (PVDp::AFF-1, red) . Median life-span is 14 days of adulthood for wild-types and 13 for PVDp::AFF-1 containing animals.

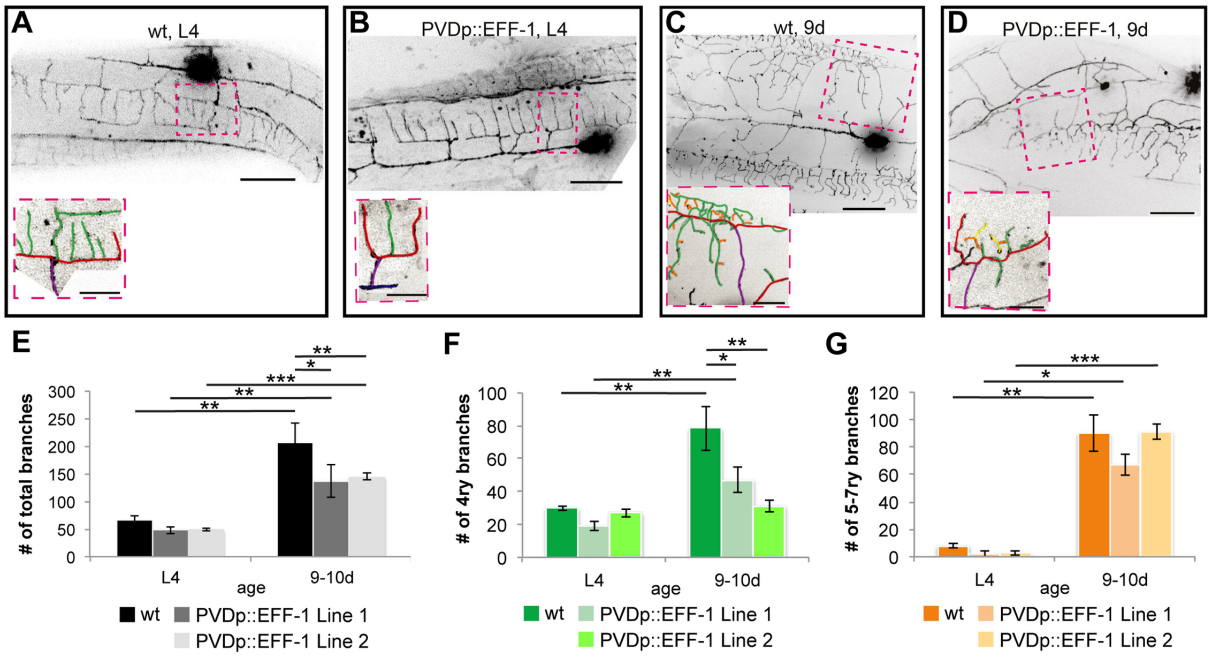

Fig S7. EFF-1 overexpression in the PVD simplifies "aged" dendritic trees

(A-D) Inverted fluorescence images of PVD neurons.
(**A** and **C)** Represent wild-type neurons from L4 and 9 days of adulthood (9d).
(**B** and **D)** Represent EFF-1 overexpression under PVD specific promoter (PVDp) at L4 and 9d. In each panel one candelabrum unit (boxed) is enlarged and colored (see **Figure 1A**). Scale bars, 20 µm and 10 µm in the enlarged images.
(E-G) Graphs showing number of branches in 100 µm of length around cell body. Error bars, ± s.e.m. According to one way ANOVA test, age is a significant factor when comparing number of branches ($p<0.0001$). p values from t tests: * $p<0.05$, ** $p<0.001$, *** $p<0.0001$
Number of animals: n ≥ 4. PVDp::EFF-1 line 1 and 2 correspond to worms carrying the extrachromosomal arrays *hyEx392* and *hyEx23,* respectively.

Captions for movies

Movie S1. Dendritic plasticity of aged wild-type

Inverted fluorescence confocal maximum intensity projections time lapse movie of wild-type PVD marked with GFP at 5 days of adulthood. Some branches show dynamic growth and retraction (arrow). The counter in this and other movies are in Hours: minutes.

Movie S2. Young wild-types regenerate rapidly after dendrotomy

Inverted fluorescence confocal maximum intensity projections time lapse movie of two L4 wild-type animals. PVD marked with Kaede. Both animals regenerated within 2 hours after laser induced dendrotomy via menorah-menorah fusion (blue arrows). Red lightnings mark the sites of injury.

Movie S3. Kaede photoconversion confirms auto-fusion as part of regeneration

Confocal time lapse movie of wild-type L4 worm expressing the photoconvertible protein Kaede in the PVD. The 1ry branch of this worm was dendrotomized 24 hours prior to the movie, then green kaede was photoconverted in the cell body, using U.V. laser (marked with purple asterisk). In the left panel the red channel is seen, with red for the photoconverted kaede protein traveling from the proximal part to the fused distal part (blue arrows). In the right panel the green channel is shown. Injury site is indicated by yellow lightning. The photoconverted red-Kaede was transferred to the distal part (top of the image) by menorah-menorah fusion and there was no 1ry-1ry fusion.

Movie S4. Adult wild-type responds slowly to injury

Inverted fluorescence confocal maximum intensity projections time lapse movie of wild-type animal at the age of 2 days of adulthood. PVD is marked by Kaede. There is neither

regeneration nor degeneration within the first 3 hours after cut. Red lightning points at injury site.

Movie S5. PVDp::AFF-1 L4 regenerates similarly to wild-type

Inverted fluorescence confocal maximum intensity projections time lapse movie of PVDp::AFF-1 (AFF-1 overexpression in the PVD) L4 nematode. The worm was injured and imaged immediately after. In this movie, it rapidly regenerates (within 3 hours from cut) via menorah-menorah fusion, as marked by blue arrows and by novel outgrowth that fuses to the distal primary and by that bridges the gap (yellow arrow). Red lightning points to injury site. Red arrowhead marks a candelabrum that degenerated during the movie.

Movie S6. PVDp::AFF-1 5d old animal reconnects after cut

Confocal time lapse movie of 5 days old PVDp::AFF-1 transgenic animal, beginning 5 hours post injury. Growth is seen near site of dendrotomy. The primary branch grows toward the distal primary and reconnects with it 10 hours after injury (blue arrows). After this time point the worm was recovered and imaged 15 hours later (25 hours after cut), where regeneration via novel outgrowth from 1ry to distal fragment of 1ry branch fusion and menorah-menorah fusion is indicated by blue arrows. Red lightning points at injury site.

References

1 Mohler, W. A. *et al.* The type I membrane protein EFF-1 is essential for developmental cell fusion in *C. elegans*. *Dev Cell* **2**, 355-362 (2002).