Supplementary information

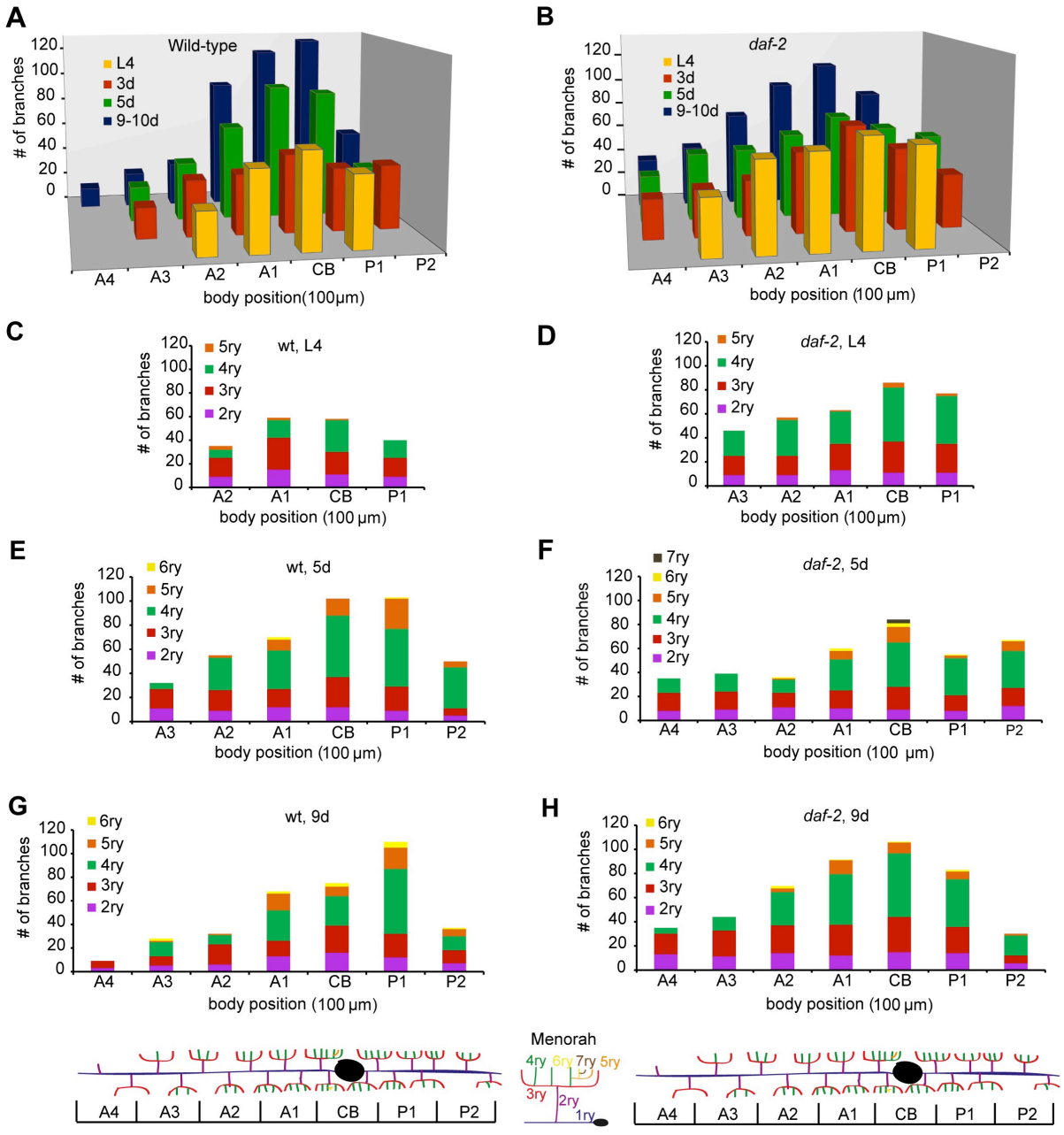


Fig S1. Anterior-posterior branching gradient sharpens as animals age

(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.

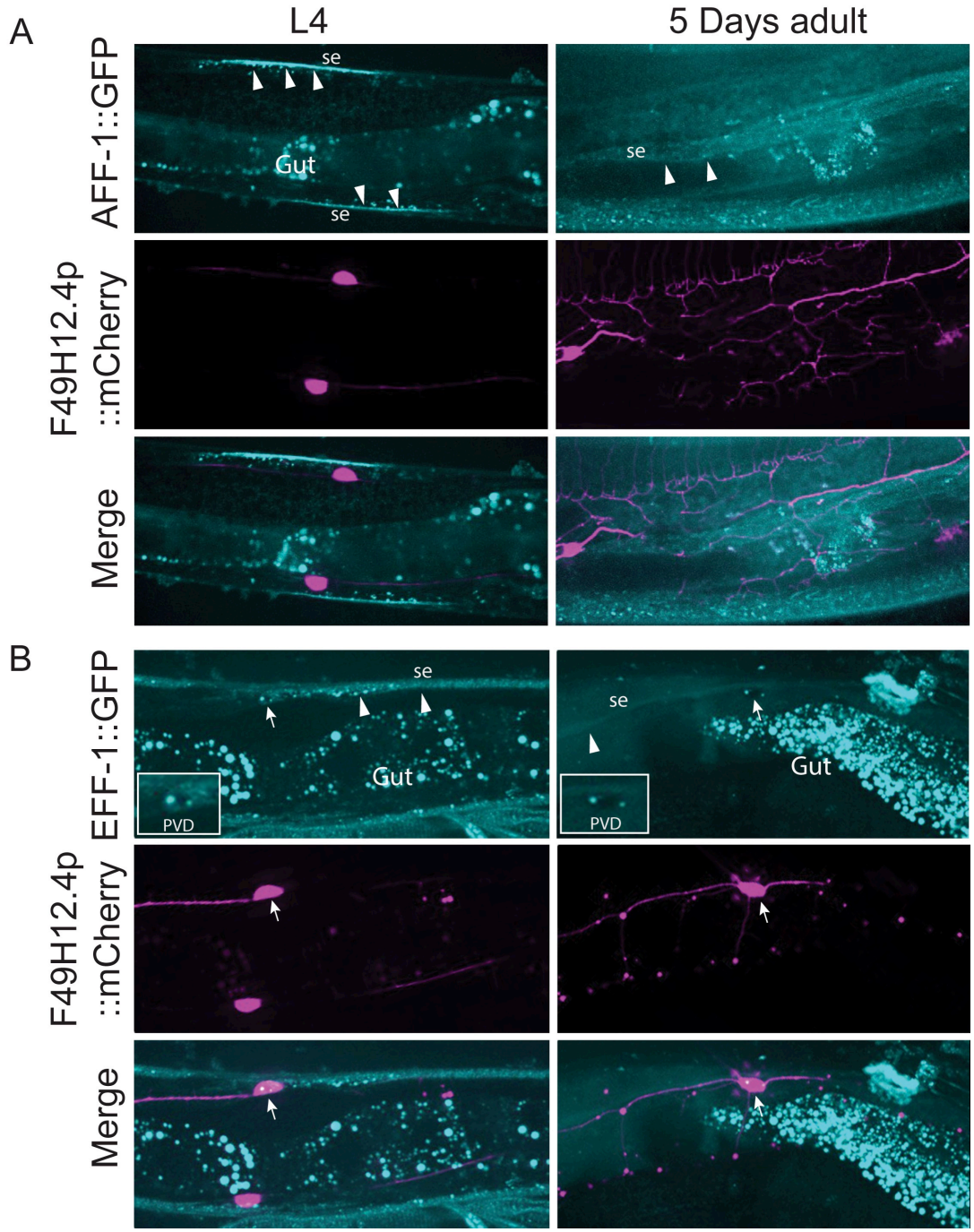


Fig S2. AFF-1 and EFF-1 expression pattern in L4 and aging worms

(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 (see Fig S3 for quantification). AFF-1 is not expressed in the PVD at any stage and cannot rescue *eff-1* PVD morphological defects (Fig S4). 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 (Mohler et al., 2002). EFF-1 is expressed in the seam-cells (arrowheads) and the PVD cell body (arrows and inset) in vesicles. Expression persists but is reduced after 5 days (see Fig S3).

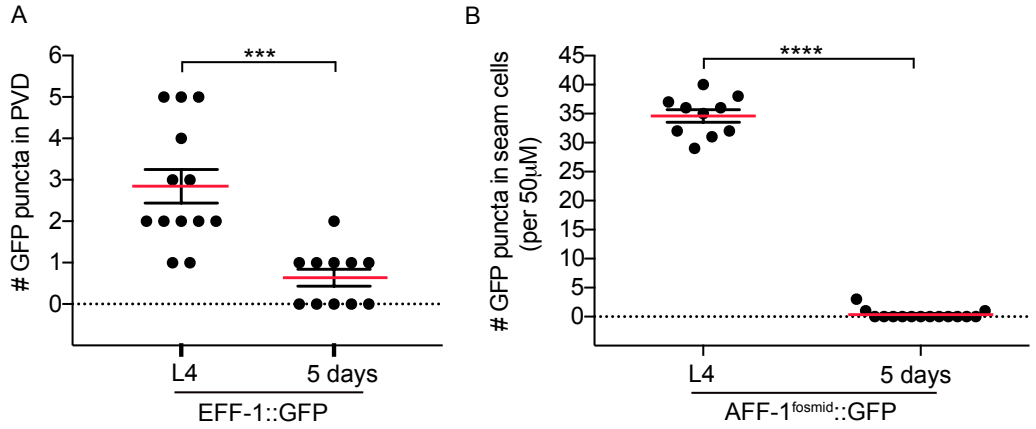


Fig S3. Quantification of EFF-1 PVD localization and AFF-1 seam-cell expression during aging

(A)EFF-1::GFP translational chimera (described above) was used to show a decease in the number of EFF-1 puncta observed in the PVD. Decreased expression in the hypodermis was also observed **(Fig S2)**.

(B) AFF-1 fosmid is expressed strongly as vesicles at the L4 stage in the epithelial seam-cells, and expression is diminished in older animals.

Red horizontal line represents the average. Black lines are SEM. Statistics were calculated using Mann-Whitney test, ***P<0.001, ****P<0.0001.

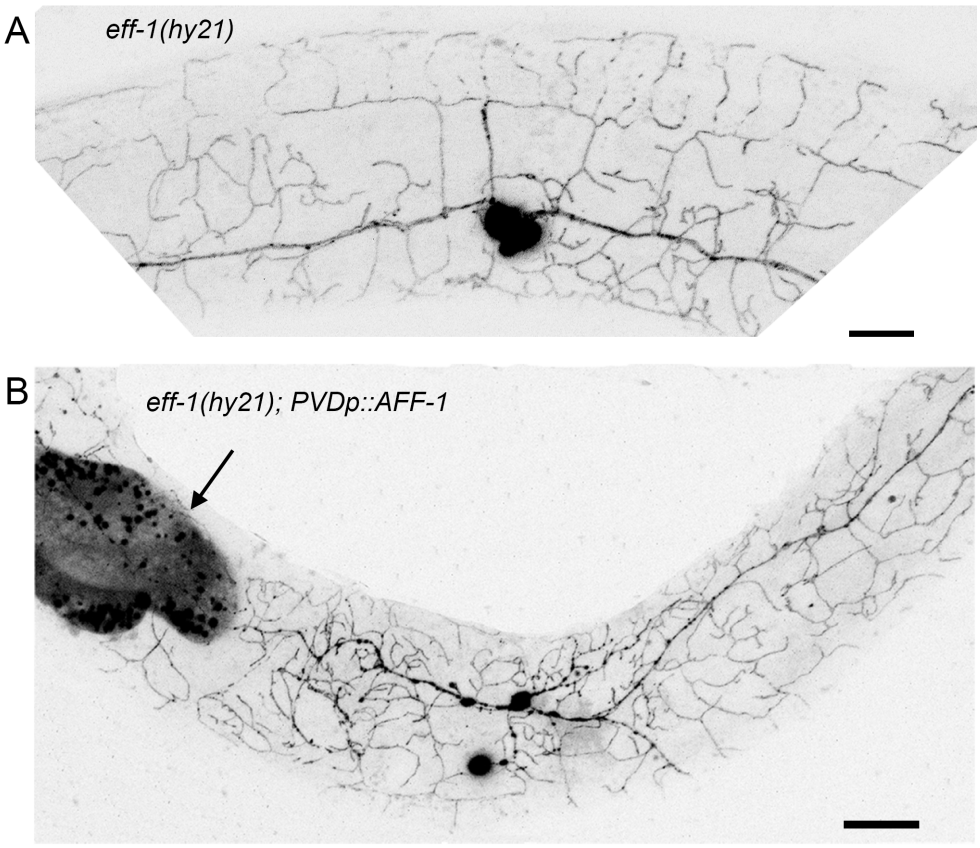


Fig S4. AFF-1 does not retract PVD's dendrites.

eff-1(hy21) animals expressing AFF-1 in the PVD (PVDp::AFF-1) show excess branching **(B)**, similarly to *eff-1* mutants (shown in **(A)**). 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.

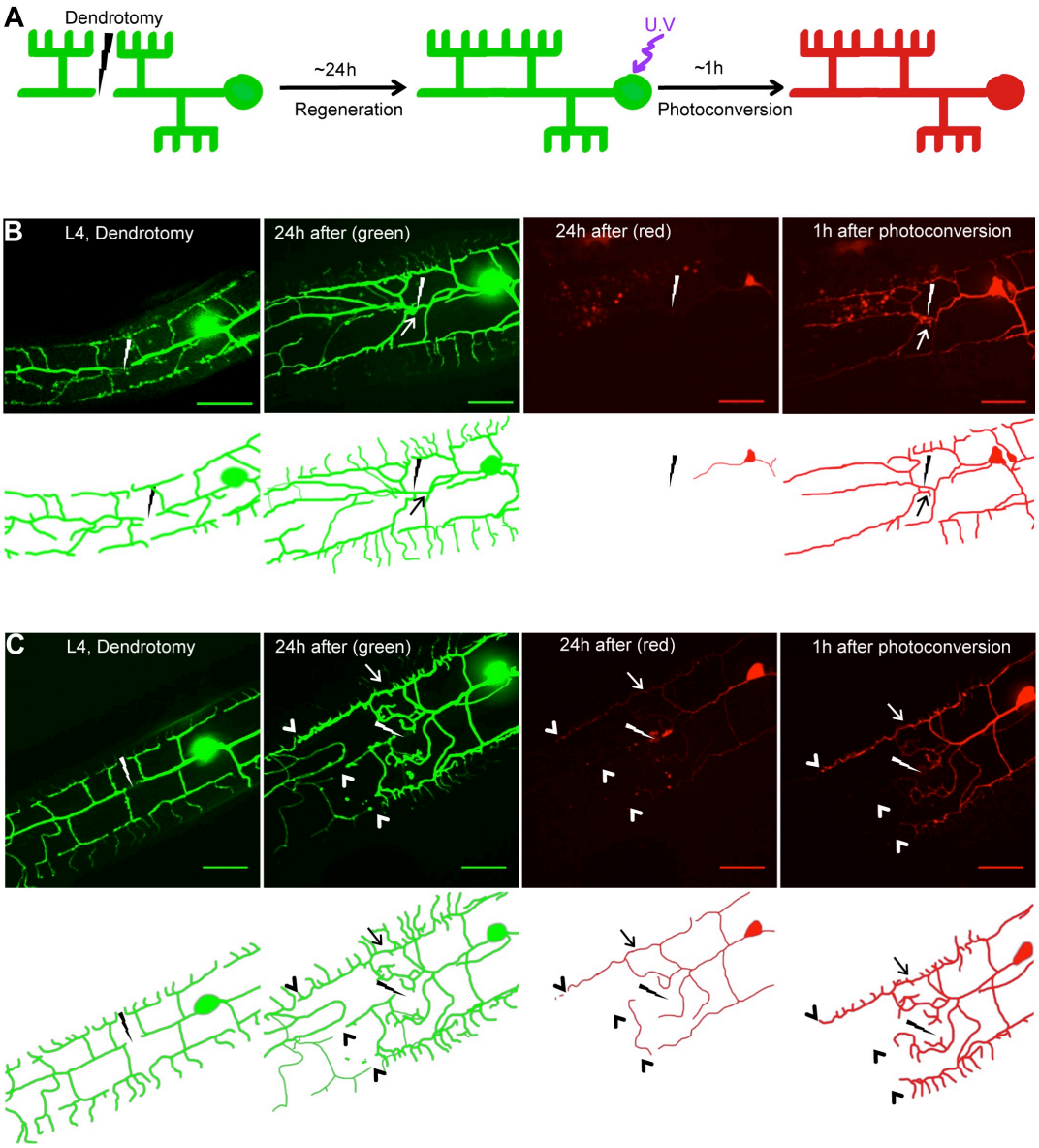


Fig S5. Fusion is a crucial step in regeneration of injured dendrites

(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 (Oren-Suissa et al., 2017; Yip and Heiman, 2016). See Movie 3.

(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.

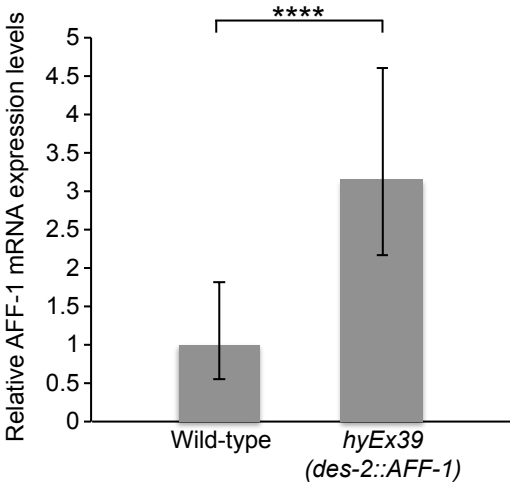


Fig S6. Real-time RT-PCR analysis of AFF-1 mRNA expression in wild-type and transgenic worms over expressing AFF-1 in the PVD

Bar graph representing the fold changes of mRNA levels quantified by normalization to the *act-1* (actin isoform) gene as an internal control. *p* value from *t* test ****$P<0.0001$.

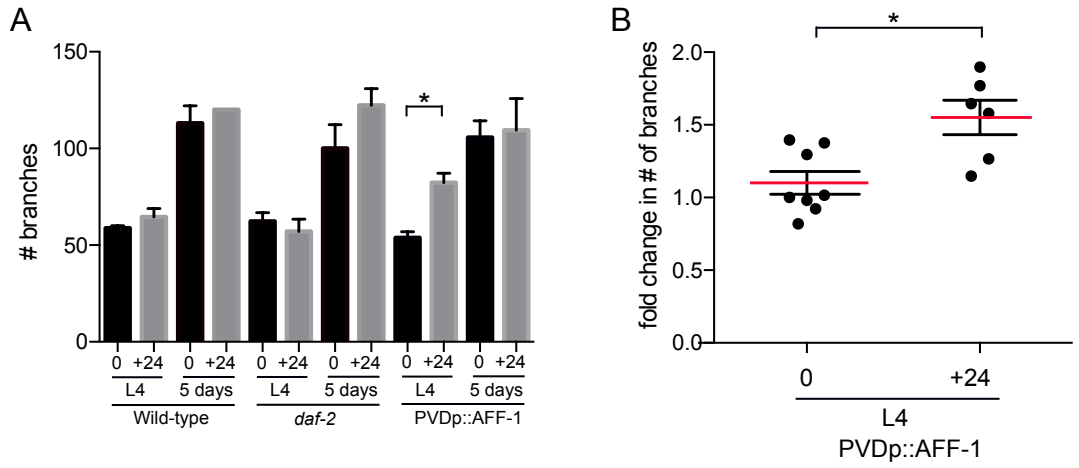


Figure S7. The number of PVD branches increases during development but is kept stable following an injury

(A) Quantification of the number of branches in wild-type, *daf-2* mutants and transgenic worms expressing PVDp::AFF-1, during development (L4 versus 5d) and pre (0) and post (+24) injury (black and grey bars, respectively, in hours). There is a significant increase in the number of branches in L4 animals expressing AFF-1 in the PVD.

(B) The fold difference was calculated from the data shown in (**A**) for PVDp::AFF-1. Red horizontal line represents the average. Black lines are SEM. Statistics were calculated using Mann-Whitney test, *P=0.02.

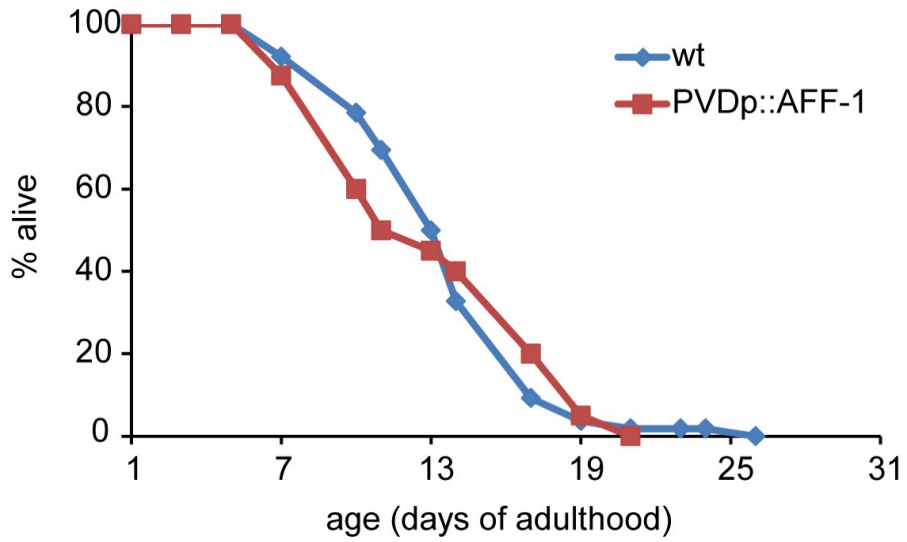
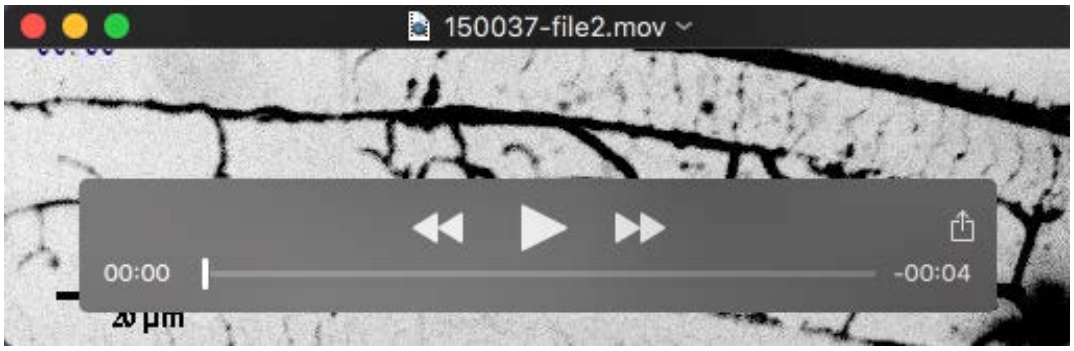


Fig S8. AFF-1 overexpressing animals exhibit wild-type life-span

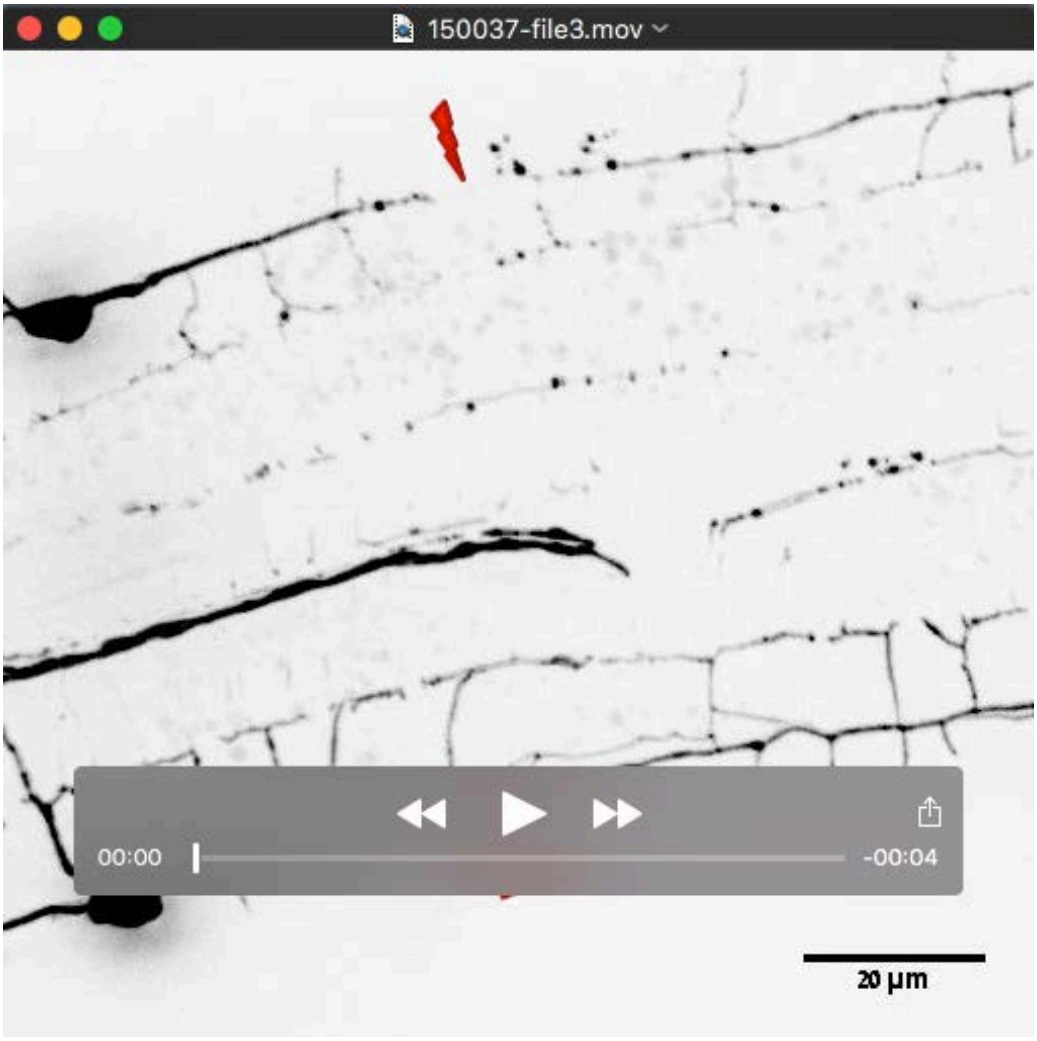
Life-span curve of wild-type (blue) and AFF-1 overexpression in the PVD (PVDp::AFF-1, red) . Median life-span is 14 days of adulthood for wild-types and 13 for PVDp::AFF-1 transgenic animals.

Movies



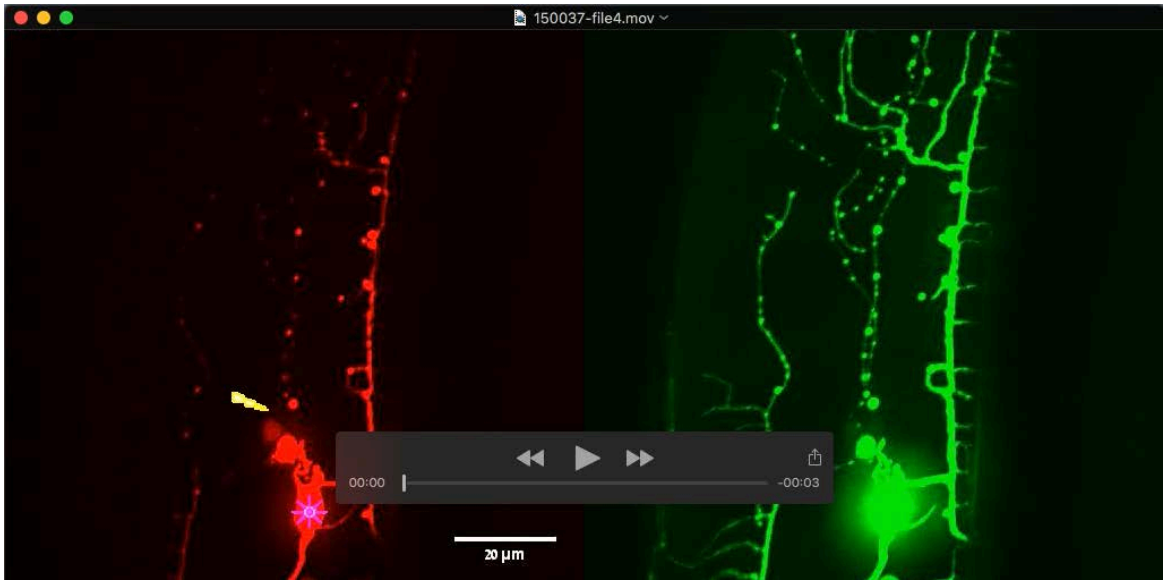
Movie 1. Dendritic plasticity of aged wild-type

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 dendritic disorganization and hyperbranching is dynamic and involves formation of new neurites. The counter in this and other movies are in Hours: minutes. See Fig 3 for details.

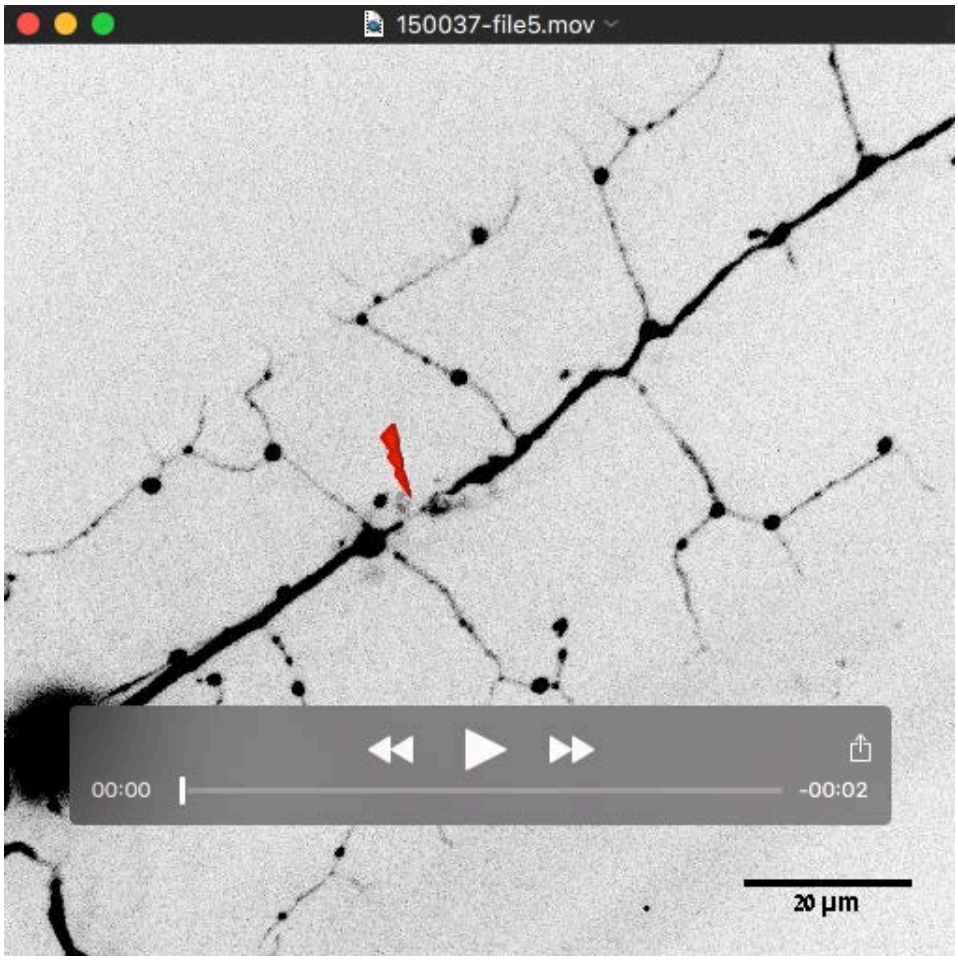


Movie 2. Young wild-types regenerate rapidly after dendrotomy

Inverted fluorescence confocal maximum intensity projections time lapse movie of two L4 wild-type animals. PVDs marked with Kaede. Red lightnings mark the sites of injury. Both animals regenerated bypassing the injury sites within 2 hours after laser-induced dendrotomy via menorah-menorah fusion. Note at time 01:40 how two pairs of 3ry dendrites merge and form giant menorahs (blue arrows). At time 01:45 the branch below the bottom blue arrow also connected to the 1ry dendrite bypassing the injury site.

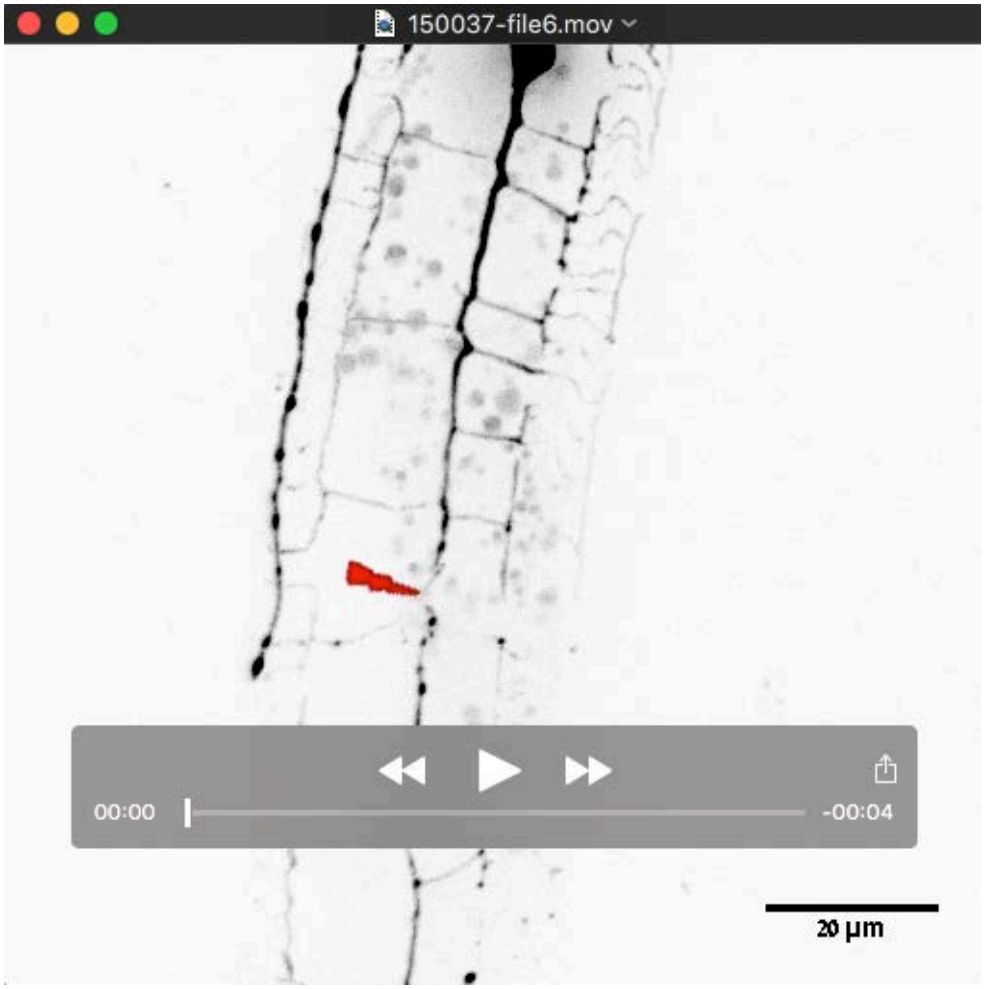


Movie 3. Kaede photoconversion confirms auto-fusion as part of regeneration Confocal time lapse movie of wild-type L4 worm expressing the photoconvertible protein Kaede in the PVD. The 1ry branch of this worm was dendrotomized 24 hours prior to the movie (yellow lightning), then green kaede was photoconverted 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 starting at time 0:35 (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 since the green kaede can be seen in the 1ry branch and there is no detectable increase in photoconverted red kaede going across the injury site (see Fig S5).



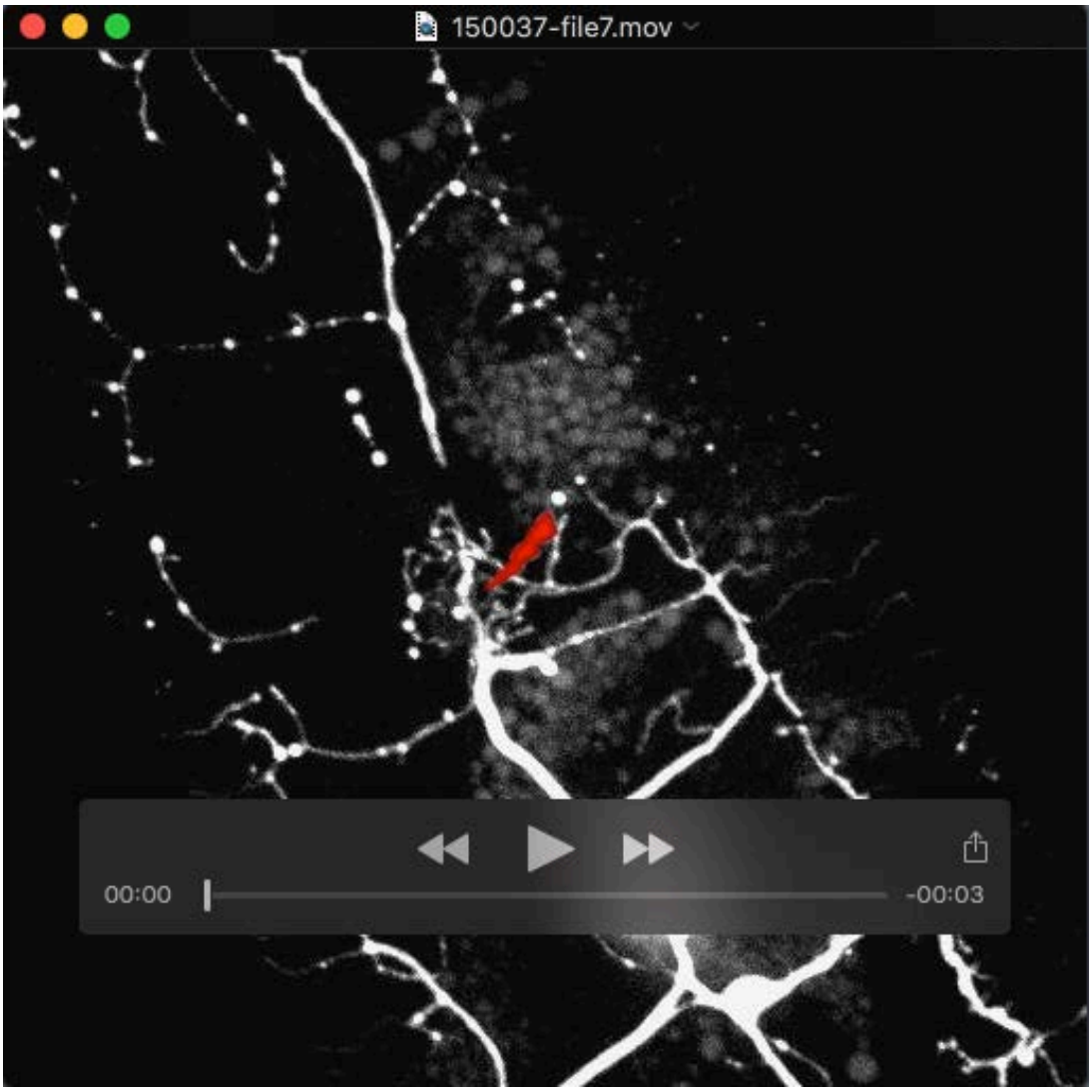
Movie 4. Adult wild-type responds slowly to injury

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. There is dynamic growth and retraction around the injury site but there is no loss of self avoidance between the 3ry branches. Red lightning points at injury site.



Movie 5. 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, the PVD 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 cut primary that bridges the gap (at time 03:00 see connection between 3ry branch pointed by bottom blue arrow and the distal fragment of severed 1ry branch). Red lightning points to injury site (time 0:05). Red arrowhead marks a candelabrum that degenerated during the movie (starting at time 01:45).



Movie 6. 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 (time 5:00). Growth is seen near site of dendrotomy. The primary branch grows toward the distal primary (time 08:40) and reconnects with it 10 hours after injury (blue arrow). 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 (right blue arrow) and menorah-menorah fusion (left blue arrow) Hyperbranching is clearly seen by comparing the last time lapse images at times 10:00 and 25:00. Red lightning points at injury site.

Note: Use arrows on keyboard to watch the movies frame by frame.

References

Mohler, W. A., Shemer, G., del Campo, J., Valansi, C., Opoku-Serebuoh, E., Scranton, V., Assaf, N., White, J. G. and Podbilewicz, B. (2002). The type I membrane protein EFF-1 is essential for developmental cell fusion in *C. elegans*. *Dev Cell* **2**, 355-362.

Oren-Suissa, M., Gattegno, T., Kravtsov, V. and Podbilewicz, B. (2017). Extrinsic Repair of Injured Dendrites as a Paradigm for Regeneration by Fusion in Caenorhabditis elegans. *Genetics* **206**, 215-230.

Yip, Z. C. and Heiman, M. G. (2016). Duplication of a Single Neuron in C. elegans Reveals a Pathway for Dendrite Tiling by Mutual Repulsion. *Cell reports* **15**, 2109-2117.