

Supplemental Data

AFF-1, a FOS-1-regulated Fusogen

Mediates Fusion of the Anchor Cell in *C. elegans*

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Supplemental Experimental Procedures

Worm handling and strains

All *C. elegans* strains were cultured and visualized at 20°C as in (Brenner, 1974). Strains used in this study were:

LGII: *eff-1(ok1021)* (Podbilewicz et al., 2006)

aff-1(tm2214) II; eff-1(ok1021)/mln1[mIs14 dpy-10(e128)] II

aff-1(ty4) II; tyEx14[aff-1 PCR.P + pRabGFP_{Prim3}]

aff-1(tm2214) II; jcls1[ajm-1::GFP + pRF4(rol-6(su1006))] IV

aff-1(tm2214) II; kuls29[unc-119(+)] + egl-13p::GFP(pWH17)] V

aff-1(tm2214) II; syls50 [cdh-3p::GFP] X

LGIV: *eri-1(mg366) IV*

LGV: *kuls29[egl-13p::GFP]* (Hanna-Rose and Han, 1999), *fos-1(ar105)* (Sherwood et al., 2005)

fos-1(ar105) V; hyEx167[aff-1p::GFP + pRF4(rol-6(su1006))]

LGX: *syls50[cdh-3p::GFP]* (Sherwood and Sternberg, 2003)

syls50[cdh-3p::GFP] X; eri-1(mg366) IV

Mapping the *ty4* mutation

To identify the gene mutated in *ty4* background, we performed Tc1 mapping analysis. We assigned the *ty4* mutation to chromosome II and positioned it between the *stP101*(-4.5) and *stP* (1.8) genetic markers. Next, we executed a three-factor mapping and deficiency mapping to further refine the region to which *ty4* maps. The data suggest that *ty4* is positioned between *dpy-2* (0.05) and *vab-13* (0.29), a genetic interval of 0.24 map unit (about 300 kb) to the right of the center of chromosome II. We performed complementation analyses on several candidate genes within or near the mapping region that were known to be involved in cell fusion or whose absence was known to result in an Egl defect. The mutations used were as follows: *eff-1(hy21)*, *egl-27(n170)*, *pvl-5(de4)*, and *snt-1(ad596, md290, n2665)*. All of the above mutations complemented the *ty4* mutation, suggesting that *ty4* was not an allele of the corresponding genes. The *ty4* mutation did not complement *tm2214*, a deletion in *C44B7.3* gene that is localized to this region. *ty4/tm2214* transheterozygous worms exhibited strong Egl phenotype similar to the phenotype of the individual alleles as homozygotes (n=20). This suggests that *ty4* and *tm2214* are alleles of the same gene- *C44B7.3*. The base

substitution in *C44B7.3* gene that resulted in *ty4* mutation was identified by sequencing (See Experimental Procedures).

***aff-1* molecular analysis and constructs**

In all constructs, the DNA was amplified using DNA Expand Taq (Roche) and was sequenced before use. Injection, handling, and characterization of transgenic strains were done according to standard procedures.

The borders of *aff-1* promoter were predicted by promoter comparison between *aff-1* and its putative *C. briggsae* homologue *CBG11169* using Blast two sequences algorithm in NCBI

(<http://www.ncbi.nlm.nih.gov/blast/bl2seq/wblast2.cgi>). Nine short conserved regions were identified in a 4.5Kb region upstream to *aff-1* start codon suggesting that the promoter lays in this interval. To identify candidate transcription factor binding sites we used the online software tool TESS (<http://www.cbil.upenn.edu/cgi-bin/tess/tess>).

aff-1p::gfp was constructed by PCR amplification of this 4.5Kb fragment from C44B7 cosmid and fusion with GFP following (Hobert, 2002). 10ng/μl of *aff-1p::gfp* was co-injected with 26ng/μl genomic DNA and with 80ng/μl of the transformation marker pRF4 [rol-6(su1006)] (hyEx167).

To drive *aff-1* expression ectopically, full length *aff-1* was amplified from C44B7 cosmid and sub cloned into pPD49.78 plasmid (kindly provided by A. Fire) downstream of the heat-shock promoter. 10ng/μl from this construct was co-injected with 10ng/μl of the apical junction marker AJM-1::GFP and with 80ng/μl pRF4 [rol-6(su1006)] (hyEx173). Expression of the reporter *aff-1p::GFP* was also observed in embryonic hyp5, sheath cells, head interneuron 5, chemosensory neurons from the head and tail, pharyngeal cells, and L4/adult posterior male tail.

To drive AFF-1::GFP expression specifically in the AC we cloned upstream to *aff-1* genomic sequence a 1.5 kb cis-regulatory region of the *cdh-3* gene (pAC) which drives expression specifically in the AC from L2 to L4 larval stages (Kirouac and Sternberg, 2003). Next we added GFP in frame after the 3' end of the *aff-1* gene by fusion PCR to create the pAC::AFF-1::GFP construct (Hobert, 2002).

To construct *aff-1* promoter::AFF-1::GFP we amplified *aff-1* promoter and coding region from the C44B7 cosmid by PCR. GFP was fused in frame to the 3' end of the last exon of *aff-1* by PCR, to create *aff-1* promoter::AFF-1::GFP.

For Sf9 experiments *aff-1* cDNA was constructed in two steps. In the first step two base substitutions in the EST clone yk747 (G in 1217 instead of A) and (G in 1705 instead of A) were corrected by a standard site directed mutagenesis method. The 3' of this corrected EST was then ligated with 5' of yk1083 to give the correct *aff-1* ORF that was sub cloned into pIZT plasmid to form pIZT::AFF-1-V5-6Xhis (Podbilewicz et al., 2006).

To construct AFF-1::EFF-1cyto, the extracellular portion of AFF-1 ending in isoleucine 540 (see Figure S3) was fused by PCR to EFF-1 transmembrane and intracellular domains starting from serine 550 and cloned into the pZT vector.

Supplemental References

Brenner, S. (1974). The genetics of *Caenorhabditis elegans*. *Genetics* 77, 71-94.

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Hanna-Rose, W., and Han, M. (1999). COG-2, a Sox domain protein necessary for establishing a functional vulval-uterine connection in *Caenorhabditis elegans*. *Development* 126, 169-179.

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Table S1 Characterization of *aff-1* and *eff-1* loss-of-function phenotypes

Strain/treatment	% AC Fusion Failure (Aff) (n)	% Egl (n)	% Sterility (n)	Number of Progeny \pm SD (n)
Wild-type (N2)	0 (135)	0 (59)	0 (59)	294 \pm 43 (59)
<i>eff-1 (ok1021)</i>	1.5* (135)	ND	3.8 (52)	131 \pm 57 (52)
Empty vector, <i>eri-1</i>	0 (66)	4 (117)	ND	ND
<i>aff-1(RNAi), eri-1</i>	38 (77)	79 (130)	ND	ND
<i>aff-1 (tm2214)</i>	98** (135)	100 (48)	3.4 (117)	16 \pm 14 (117)
<i>aff-1 (ty4)</i>	97*** (135)	100 (91)	0 (46)	7 \pm 5 (46)
<i>aff-1 (ty4); aff-1 rescue</i>	5.4 (135)	6 (52)	3 (52)	45 \pm 35 (52)

* In these two *ok1021* worms where the uterus and vulva structure was highly malformed, the AC failed to fuse.

** Of the 132 non-fused anchor cells 124 exhibited bloated like shape while the other eight had the shape of a degenerated cell.

*** 132 non-fused anchor cells, 120 exhibited bloated like shape and 12 had degenerated cell shape.

Table S1 Characterization of *aff-1* and *eff-1* loss-of-function phenotypes

All strains were grown at 20°C and visualized by Nomarski microscopy. In all wild type worms examined the AC fuses with the uste syncytium resulting in formation of a hymen that permits the laying of ~300 progeny. Normal AC fusion was observed in *eff-1 (ok1021)* mutant worms where a moderate reduction in progeny number is resulting from different abnormalities. Attenuating *aff-1* activity by RNAi or mutations resulted in AC fusion failure concomitant with strong EGg Laying defective (Egl) phenotype and gross reduction in the number of progeny.

Supplemental Figures

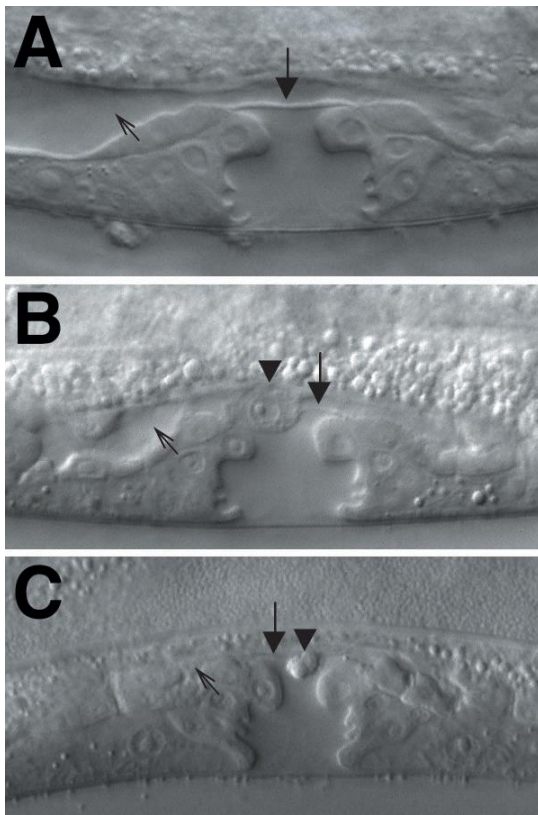


Figure S1 Formation of utse syncytium in *aff-1* mutant worms

(A) Wild-type worm at the L4 stage. Thin laminar process of the utse (large arrow) separates the uterine and vulval lumens. The AC has fused with the utse and is not visible as a distinct cell. Uterus lumen is marked by small arrow.

(B) *aff-1(ty4)* mutant at the L4 stage. The arrowhead indicates unfused AC. The thin laminar process characteristic of utse syncytium formation is evident (large arrow) suggesting that utse syncytium formation is *aff-1*-independent. Uterus lumen is marked by small arrow.

(C) In 12% of *aff-1* mutant worms the AC has a shape of a degenerative cell (arrowhead) while utse syncytium formation occurs (large arrow). It is not clear what mechanism mediates AC degeneration. Uterus is marked by small arrow.

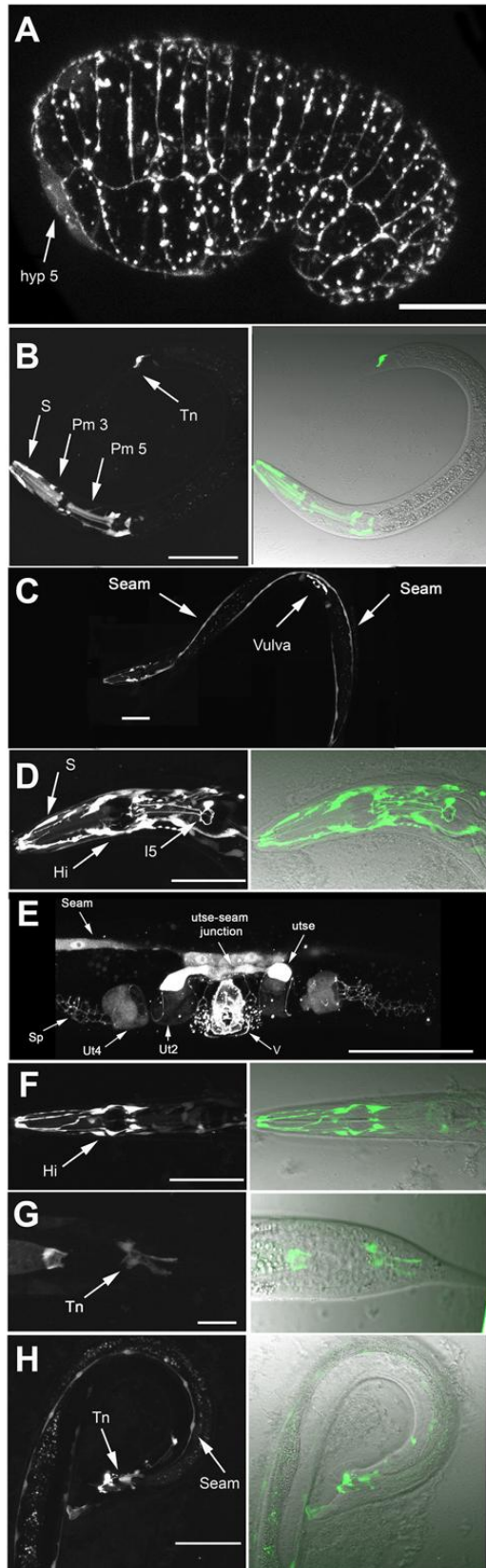


Figure S2 Expression patterns of *aff-1* at different developmental stages

(A) In comma stage embryo *aff-1* is expressed specifically in the two hyp5 cells that undergo fusion to form the hyp5 syncytium. *aff-1* expression is marked by cytoplasmic GFP in hyp5 (arrow). Embryonic cell junctions are marked by AJM-1::GFP.

(B) In L1 hermaphrodite larva *aff-1* is expressed in pharyngeal muscle 3, and 5 (Pm3, Pm5), in sheath cells of chemosensory neurons (S), and in tail neurons (Tn). *aff-1* reporter signal decays in Pm3 and Pm5 until complete disappearance at L4, while the expression in the sheath cells and in head inter neurons is retained until adulthood.

(C) In young adult hermaphrodite *aff-1* is expressed in the two seam cell syncytia that run on each side of the animal body along with expression in the utse syncytium and in vulD vulval ring.

(D) Pharyngeal *aff-1* expression in young adult. *aff-1* is expressed in the sheath cells of chemosensory neurons (S) and in head inter neurons (I5 and Hi).

(E) *aff-1* is expressed in the uterus ut2 (Ut2) and ut4 toroids (Ut4), in the vulva VulD (V), utse, and seam cells.

(F-H) Expression patterns of *aff-1* in the male.

(F) Head interneurons (Hi) and chemosensory neurons expression of *aff-1* in adult pharynx.

(G) *aff-1* is expressed in neurons in the L4 male tail.

(H) In adult male *aff-1* is expressed in the seam cells and in tail neurons (Tn). Autofluorescence was observed in the

spicule and male fan (Af).


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Ce_AFF-1/1-589 1MR-LWQWLS-VAICLVMT-ARLRHRRKRRFVSSNFDEFY-----CGESAHAQSOFEERESNSSKVS SVHS --TQF 70
Cb_AFF-1/1-589 1MR-LWQWLL-TFATFVLLIT-FAIRLRHRRKRRFVSSHDFELY-----CGESAHAQSOFEERESNSSKVS SVHS --TOR 70
Cr_AFF-1/1-588 1MR-LWQWLI-TVALFLLVSA-ETLRHRRKRRRFVTSHFDELY-----CGESANAQSOFEERESNSSKVS SVHS --TOR 70
Pp_AFF-1/1-549 1MGRIR- - - - -YLVLFLPLIS-SSIH-----DLSLPOC-----SDLMIYI-----GQEVSS IHR--PSRF 45
Cb_EFF-1/1-658 1MEPPFEWSP-OFILLLLAVTT-YGFP-----LEEFKDLGRAEPPHC-----SKTPIVRAQTSQNAMSS IARGMQMDF 64
Cr_EFF-1/1-652 1MRPRKMRSPNILLVYVWVATSSGFP-----LEEFKDLGRAEPPHC-----SKTPIVRAQTSQNAMSS IARGMQMDF 68
Pp_EFF-1/1-626 1M-IFSSL-LLYTIIILPHLI-YGFP-----RSFTPOKVIIPPHC-----IKTPSIIAHPSKBEGS--LKSLEMHF 67
C2dD10.7/1-596 1MKPPFEWPP-QFILLLLAVSTI-YGFP-----HEEKPNELLRADPPHC-----SKTPIVNSQTSKSVMS IASGMQMDF 66

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Cb_AFF-1/1-589 71NWGLDNTCIKIL-----QNVVHVLKYEERLEQRYP-ESSYTFVSFL-IDTNGKCHGYGFGSNDVQNV 130
Cr_AFF-1/1-588 71NWGLDNTCIKIL-----QNVVHVLKYEERLEQRYP-ESSYTFVSFL-IDTNGKCHGYGFGSNDVQNV 130
Pp_AFF-1/1-549 46RMQLHQLACLEI-----PTGLNGSLLHSLIFERVEQLHPVLSFYQAYPLVIAKICDCCPGGGSH--CNG 109
Cb_EFF-1/1-658 69SIGLHTAVCFRLYEDTQLASQFIN-DENAGNQTSLLHTIRLEKLEHHHPITQRYTFGPEVHASCICECDATSS-T 144
Cr_EFF-1/1-652 69SIGLHTAVCFRLYEDTQLASQFIN-DENAGNQTSLLHTIRLEKLEHHHPITQRYTFGPEVHASCICECDATSS-T 148
Pp_EFF-1/1-626 68SIGLHTAVCFRLYEDTRLASSINGV-DDDAGNQTSLLHTIRLEKLEHHHPITQRYTFGPEVHASCICECDATSS-T 146
C2dD10.7/1-596 69SIGLHTVYCFRLYEDTQLASQFIN-DENAGNQTSLLHTIRLEKLEHHHPITQRYTFGPEIYASQICEGDSSTK 127

Ce_AFF-1/1-589 131EKVADDRNQTTS- - -SEFPTCYTKYHVAPEFLDQ-PVTSIPAKACODIKLKRDRGRMFRAVKLQDPINDMIITHSIFANNSS 208
Cb_AFF-1/1-589 131EKVADDRNQTTS- - -SEFPTCYTKYHVAPEFLDQ-PVTSIPAKACODIKLKRDRGRMFRAVKLQDPINDMIITHSIFANNSS 208
Cr_AFF-1/1-588 131EKVADDRNQTTS- - -SEFPTCYTKYHVAPEFLDQ-PVTSIPAKACODIKLKRDRGRMFRAVKLQDPINDMIITHSIFANNSS 208
Pp_AFF-1/1-549 110DQVYRNGTDG- - - - -STCYRTHYHASSSEGG-FVGGQA-EVCCDIRVEYFVNEIYSAYKIQDPTNIMRROPEKEDG 182
Cb_EFF-1/1-658 145ESH-QFTACPECDKSDSTSSCYRTFFPNQTPIGG-SEDDIP-KLCCDVRFKPKYKMMFLAVLLEQPTTYATFVYAAVDFVNG 223
Cr_EFF-1/1-652 145ESH-QFTACPECDKSDSTSSCYRTFFPNQTPIGG-SEDDIP-KLCCDVRFKPKYKMMFLAVLLEQPTTYATFVYAAVDFVNG 225
Pp_EFF-1/1-626 128SYV-EFKCPEK- -DOTHCYRTFFPNQSNKSESSPMGSLCGLKFNPFENKTYTARLQDPTFAIFTVSVYDVSNG 206
C2dD10.7/1-596 145ESH-QFTACPECDKSDSTSSCYRTFFPNQTPIGG-SEDDIP-KLCCDVRFKPKYKMMFLAVLLEQPTTYATFVYAAVDFVNG 223

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Cb_AFF-1/1-589 209KMMKVLGDFEFRINLLKGGKEQFELYEYRHSVQLVASSPQQQLREGMYVPEENHNDLREGK- - -INEIIESDLDKLGWY 286
Cr_AFF-1/1-588 209KMMKVLGDFEFRINLLKGGKEQFELYEYRHSVQLVASSPQQQLREGMYVPEENHNDLREGK- - -INEIIESDLDKLGWY 286
Pp_AFF-1/1-549 183GKILIHKEPIITVPLNKGPSRMEIGGTSRMIISVTSQSGSKVLPNTNDLYFAREGETTELGGVY- - -LNEVGGSSIEIKGLW 281
Cb_EFF-1/1-658 214YVWEK-DKTKIRSQLDGGTDRHLDSKRRISLAVTAGGRASHQLEIGMYFRSTSNGGETEELRMQPLNEITDNNFDRLGWY 304
Cr_EFF-1/1-652 214YVWEK-DKTKIRSQLDGGTDRHLDSKRRISLAVTAGGRASHQLEIGMYFRSTSNGGETEELRMQPLNEITDNNFDRLGWY 306
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C2dD10.7/1-596 214YVWEK-DKTKIRSQLDGGTDRHLDSKRRISLAVTAGGRASHQLEIGMYFRSTSNGGETEELRMQPLNEITDNNFDRLGWY 304

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Cr_AFF-1/1-588 287RI- - -GNDWQVATSGLLLRNAHKVVIKKNCKGVMHMDQSGTKNFVLRG- - - - -TQYNDTYNEKKVTENNFRVRSVK-VDESSRE 361
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Ce_AFF-1/1-589 362TVIHEHGAAQVSLKTDTRPN- - - - -LTKSQSLANFTGSI TLHDGGRNMLNVFFGVKGTWHIKMYVNDR-KLIATFAC 437
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Pp_AFF-1/1-549 334VRVHAEGTIEIRMGSEKRPY- - - - -VRRDLSHLGWFDGEITVDTLSQHLNITFHEKGTLLGNVFSSEA-RERTDYSFV 411
Cb_EFF-1/1-658 377VVTHAEGNLQISIHLDDEVESQNLVFFHNAARIDFSGSIIVDSKSNRFLNLTVEYASGKIDGSKVMTGFGSDTIHTFTA 458
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C2dD10.7/1-596 377VVTHAEGNLQISIHLDDEVESQNLVFFHNAARIDFSGSIIVDSKSNRFLNLTVEYASGKIDGSKVMTGFGSDTIHTFTA 458

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Pp_AFF-1/1-549 412TIGDRLNSY-YIAASIDSTVNS-RRVYCFYPTGESNKKNERKWFPEKSTPISTPPVAGLLPOKGECC-ATCFEG- - - - - 484
Cb_EFF-1/1-658 439YVSDLHASNRSMIIPPAIVGGGARAI CLRADSMAD-IDKICHVEYFESPLEDLVEGKWHEMIGTC-PTCNQIN--FN 534
Cr_EFF-1/1-652 443YVSDLHASNRSMIIPPAIVGGGARAI CLRADSMAD-IDKICHVEYFESPLEDLVEGKWHEMIGTC-PTCNQIN--FN 536
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C2dD10.7/1-596 458YVSDLHASNRSMIIPPAIVGGGARAI CLRADSEITDIESICHVTEFFESPLEDLVEGKWHEMIGTC-PTCNQIN--FN 534

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Cb_AFF-1/1-589 517LFPWIMNFDFYMAHGDFTEWLKMGIHIVIAVGLLFLLIILFTKCLVPLACC-----SLSIPFKNRNK 579
Cr_AFF-1/1-588 517LFPWIMNFDFYMAHGDFTEWLKMGIHIVIAVGLLFLLIILFTKCLVPLACC-----SLSIPFKNRNK 579
Pp_AFF-1/1-549 485- - -PQVLPNSWLVEDGVMKMIIVVILFELIFLIIIVVIMIVVIVKLPILIRG-----SLVVKPF- - - - - 542
Cb_EFF-1/1-658 535GMMKFLNPAHWIKGISSIGDGVMIATDIVVYLVGLCIIYLLTKIILVLRQWCPM3IFCNGQ93-9GKNNKDKRRKEREE 415
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C2dD10.7/1-596 535GMMKFLNPAHWIKGISSIGDGVMIATDIVVYLVGLCIIYLLTKIILVLRQWCPM3ICSGGSG-SSKNNSEKRRKEREE 418

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Cr_EFF-1/1-652 620KRRRNSSSASPNSPRRTHASPRDAHTLARHGGSDRSYSSSQYI 657
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Figure S4 Protein sequence alignment of the FF family in nematodes

Alignment color code was according to the Clustal X color scheme with 10% conservation color increment in Jalview software (Clamp et al., 2004). Abbreviations: *Ce*, *C. elegans*; *Pp*, *P. pacificus*; *Cr*, *C. remanei*; *Cb*, *C. briggsae*. Accession numbers: *Ce* AFF-1: EF205023; *Cb* AFF-1: CBG11169 ([BP:CBP17138](#)); *Cr* AFF-1: Supercontig3 ([cr01.sctg3.wum.206.1](#)); *Pp* AFF-1: contig1480; *Ce* EFF-1: C26D10.5 (WP:CE03028); *Cb* EFF-1: [BP:CBP05786](#) (CBG00700); *Cr* EFF-1: Supercontig2 ([cr01.sctg2.wum.648.1](#)); *Pp* EFF-1: Contig2476.

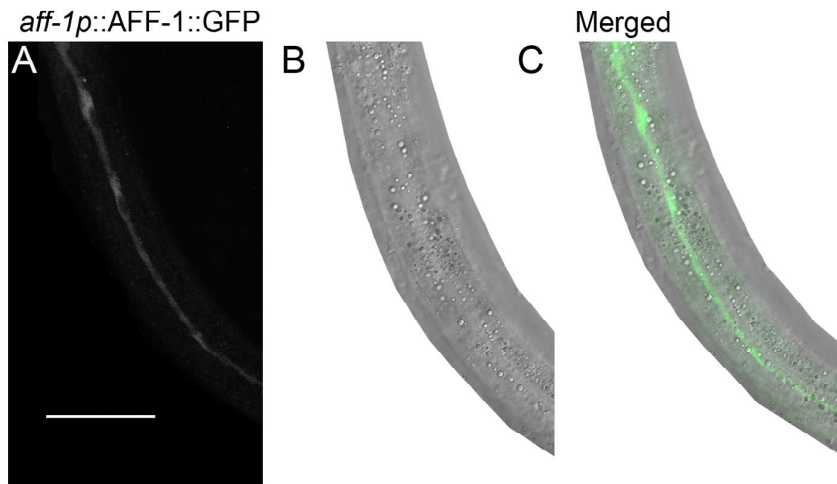


Figure S5 AFF-1 protein localization in L4 seam cells

(A) Dorsal view of L4 larva expressing AFF-1::GFP under the regulation of AFF-1 endogenous promoter. AFF-1 expression in the seam cells correlates with the time of seam cells fusion (L4).

(B) Nomarski image of the same larva.

(C) Merged view demonstrating the distribution of AFF-1 protein in the seam cells (side view).

Scale bar 50 μ m.

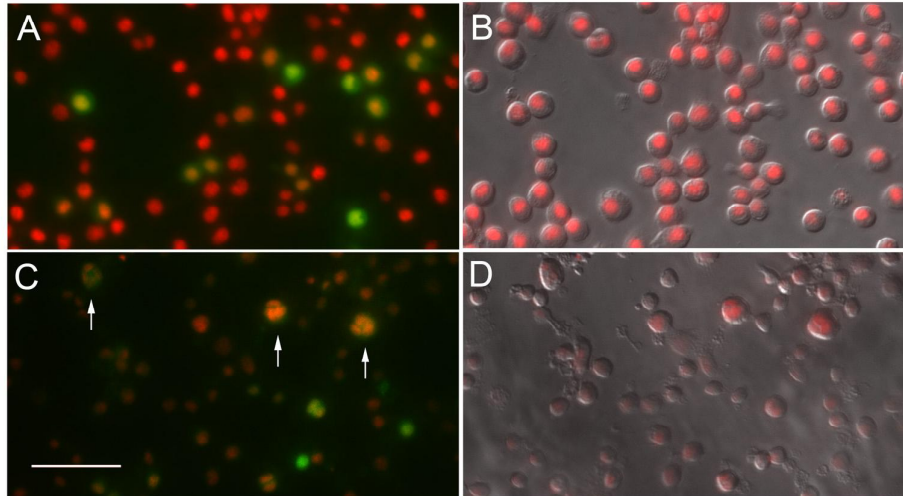


Figure S6 Expression of AFF-1 in Sf9 cells results in multinucleation

(A) Insect Sf9 cells transfected with pIZT/v5-His empty vector shows GFP expression (green) and nuclei (red; Hoechst).

(B) Merged image between nuclei and DIC images of the same field indicates that all cells (transfected and non-transfected) are mononucleated.

(C) Cells transfected with pIZT-AFF-1 (green) became multinucleated (arrows).

(D) Multinucleate AFF-1 expressing cells where 2-4 nuclei are detected per single cell.

All panels are with the same magnifications, scale bar 50 μ m.

Supplemental Movie Legends

Supplemental movie 1 shown in Figure 5E

In non heat shocked *hsp::aff-1* embryo there is no ectopic fusion. Time-
animated volume projections are shown from a three dimensional confocal
recording of a control live embryo 0 to 40 minutes covering comma to 1.5 fold
stage transition. All GFPs are shown as white signal. Cytoplasmic GFP is *eff-
1p::GFP* that labels individual cells cytoplasm. AJM-1::GFP at cell-cell
junctions mark the cell borders.

Supplemental movie 2 shown in Figure 5F

In heat shocked *hsp::aff-1* embryo there is cytoplasmic mixing and ectopic
fusion of most embryonic cells. Time-animated volume projections are shown
from a three dimensional confocal recording of a live embryo 0 to 61 minutes
covering comma to 1.5 fold stage transition.

Movies may be navigated by clicking and dragging (time-animation) by using
corresponding arrows on the keyboard. Requires QuickTime version 4 or later
(<http://www.apple.com/quicktime/>).