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Supporting Online Material for

LIN-12/Notch Activation Leads to MicroRNA-Mediated Down-Regulation of Vav in C. elegans

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Supporting Online Material

I. Lateral signaling defect in *alg-1(RNAi)* hermaphrodites

Mutations that compromise the expression of the lateral signal without affecting the number of anchor cells cause P5.p, P6.p and P7.p to adopt a 3^o-1^o-3^o pattern (*1-4*). *alg-1* is a gene required for microRNA maturation and post-transcriptional gene silencing (*5*). We noticed that *alg-1(RNAi)* hermaphrodites display a lateral signaling defect: P5.px and P7.px express the 3^o marker *arIs101*[*K09H11.1*p*::yfp*] (Fig. S1). This observation suggested to us that a microRNA might be involved in lateral signaling.

'Feeding' RNAi was performed as in (*6*), using the *alg-1* cDNA clone X-6D15 inserted into the L4400 vector (*7*).

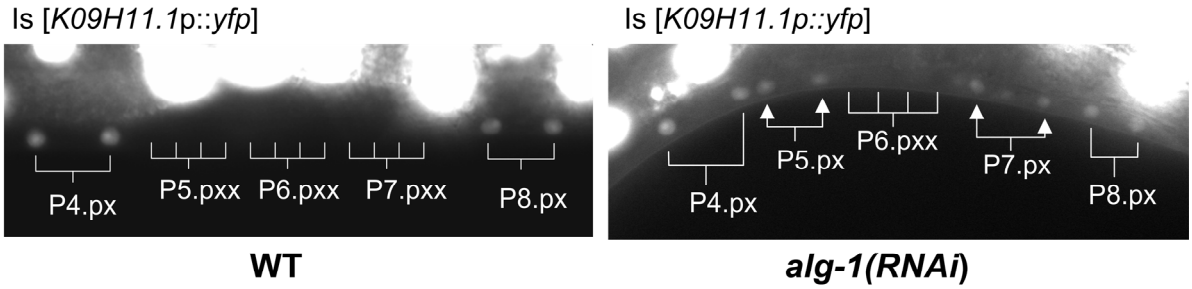

Fig. S1. Lateral signaling defect in *alg-1(RNAi)* hermaphrodites.

II. Identification of *mir* genes that are potential targets of lateral signal

We used the program CATS (*8*) to search a database of predicted *C. elegans* microRNA genes (*9*) for genes that contain two YRTGRGAA sequences (*10, 11*) within 500 bp. The criteria were chosen based on the arrangement of the LBS motifs in *lst-1*, a well-characterized

LIN-12 target gene (*11*). This analysis yielded only *mir-61*. Conserved LBSs are found in the C. briggsae homolog of *mir-61* (Fig. S2, S3).

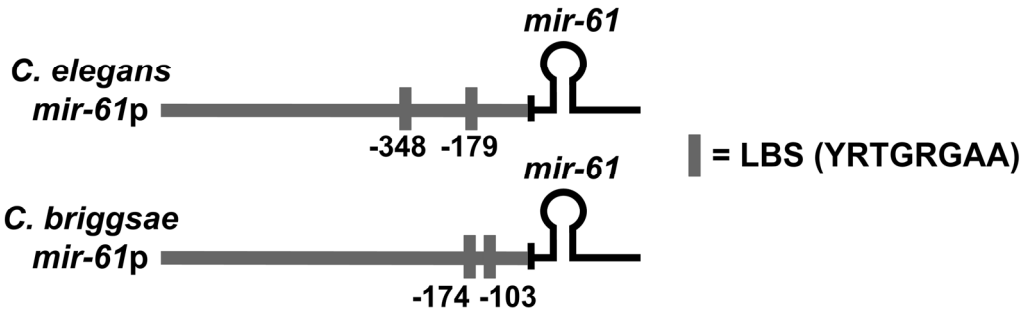

Fig. S2. 5' flanking regions of *C. elegans* and *C. briggsae mir-61*. The 5' flanking regions of *mir-61* in both species are predicted to be about 1 kb; the 5' flanking region is defined as the expanse from the next predicted gene to the start of the *mir-61*precursor. The positions of conserved LBSs are shown with respect to the start of the coding region for the *mir-61* precursor, symbolized by a hairpin.

```
C. elegans mir-61  5' UGACUAGAACCGUUACUCAUC  3'
                        |||||||||||  |  ||||   |
C. briggsae mir-61 5' UGACUAGAACCUUGACUCUGC  3'
```

Fig. S3. Alignment of *C. elegans* and *C. briggsae mir-61*.

III. Identification of potential targets of *mir-61*

To identify potential target genes of *mir-61*, we searched for regions containing at least 7 bases of perfect complementarity to the 5' end (position 1-7 or 2-8) of *mir-61* in a database of *C. elegans* 3' UTRs collected from Ensmart: http://www.ensembl.org/Multi/martview, CEL version 140. For the genes without 3' UTR annotations, we took 1 kb of downstream flanking sequences. We then concentrated on the 3' UTRs of genes that have *C. briggsae* orthologs, and used CATS (*8*) to filter out those that did not match *mir-61* 5' region. This analysis resulted in a

set of 201 genes with seed matches present in the 3' UTRs of both *C. elegans* and *C. briggsae*. We used RNAhybrid (*12*) to identify which of these target sites are favorable based on the minimum free energy of the duplex, using -23 kcal/mol as our cutoff; when this threshold was lowered, we obtained candidates that did not have conservation beyond the first 7 nucleotides. Then, we considered the conservation of favorable target sites in both species. Using these criteria, three candidate genes were obtained, *vav-1*, *inx-1* and *egl-46* (Fig. S4).

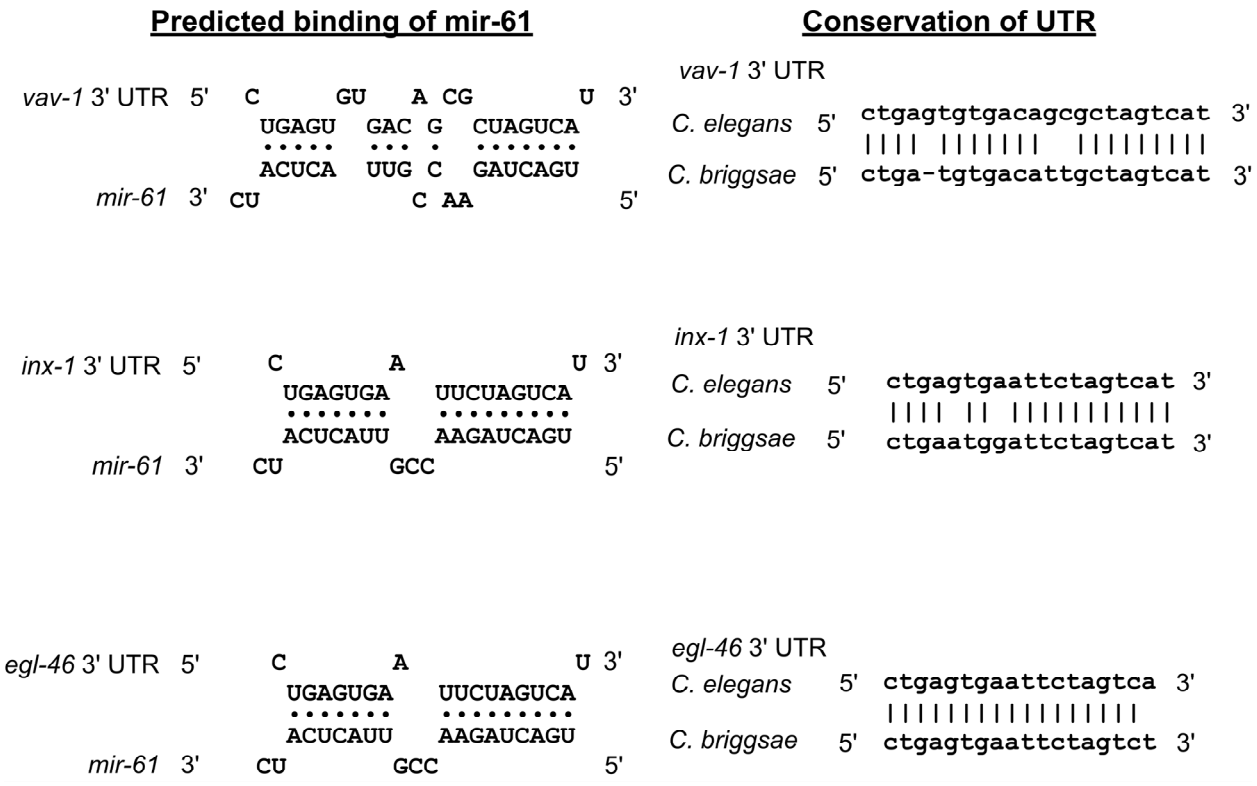

Fig. S4. *mir-61* targets. All were verified using the assay described in Fig. 3; however, only *vav-1* appears to be expressed in VPCs.

IV. Additional negative controls for the coelomocyte assay

In addition to the *unc-54* 3'UTR as a negative control, we tested two additional genes with 3' UTRs that have some resemblance to the *mir-61* target sequence, but did not appear likely to be bona fide targets based on lack of perfect complementarity or energetic considerations (Fig. S5). For *unc-68*, there is a wobble base pair (shaded in the alignment); in the coelomocyte assay, 0/44 individuals showed downregulation of the YFP signal. For *cdh-3*, there are two wobble base pairs and the minimal free energy (-18.7 kcal/mole) is not favorable; in the coelomocyte assay, 0/26 individuals showed downregulation of the YFP signal. These results support the view that expression of a microRNA in coelomocytes will cause downregulation only of bona fide target genes, and that perfect complementarity and favorable energetics are important for target gene selection.

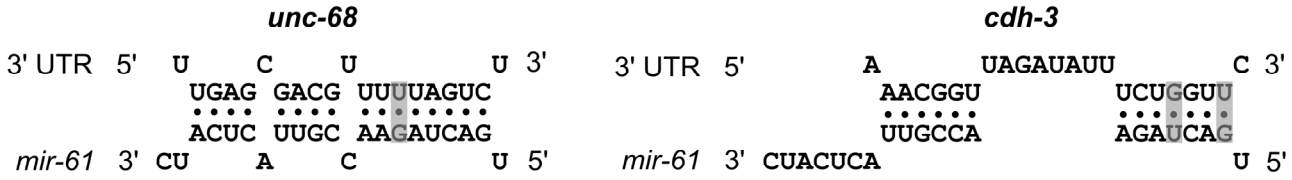

Fig. S5. 3' UTRs that do not permit downregulation by *mir-61* in the coelomocyte assay. The *unc-54* 3'UTR also does not permit downregulation (see text and Fig. 3A).

IV. List of transgenic strains used in this study.

For generating extrachromosomal arrays, the following cotransformation markers were used as described below: *pha-1(+)* (50 ng/µl) (*13*), *dpy-20 (+)* (50 ng/µl) (*14*), *ttx-3*::*gfp* (30 ng/µl) (*15*) or *ceh-22*::*gfp* (30 ng/µl) (*16*). YFP = yellow fluorescent protein. 2XNLS = two

copies of a nuclear localization sequence are present. Additional information about constructs is available upon request.

3^o fate marker (50 ng/µl):

pha-1(e2123); *arIs101* [*K09H11.1*p::2x*nls-yfp, pha-1*(+)]

*mir-61*p integrated reporter (50 ng/µl):

pha-1(e2123); arIs107 [*mir-61*p::2x*nls-yfp, pha-1*(+), *ttx-3*::*gfp*]

Mutated LBS (50 ng/µl):

pha-1(e2123); arEx711 [*mir-61*p LBS mutated::2x*nls-yfp, pha-1*(+), *ttx-3*::*gfp*]

pha-1(e2123); arEx712 [*mir-61p* LBS mutated::2x*nls-yfp, pha-1*(+), *ttx-3*::*gfp*]

egl-17p::*mir-61* (20 ng/µl) (mir-61 genomic region, F55A11 6807-6530)

pha-1(e2123); nIs106; arEx713 [*egl-17*p::*mir-61, pha-1*(+), *ceh-22*::*gfp*]

pha-1(e2123); nIs106; arEx714 [*egl-17*p::*mir-61, pha-1*(+), *ceh-22*::*gfp*]

pha-1(e2123); nIs106; arEx715 [*egl-17*p::*mir-61, pha-1*(+), *ceh-22*::*gfp*]

pha-1(e2123); nIs106; arEx716 [*egl-17*p::*mir-61, pha-1*(+), *ceh-22*::*gfp*]

egl-17p::*lsy-6* (20 ng/µl)

pha-1(e2123); nIs106; arEx717 [*egl-17*p::*lsy-6, pha-1*(+), *ttx-3*::*gfp*]

pha-1(e2123); nIs106; arEx718 [*egl-17*p::*lsy-6, pha-1*(+), *ttx-3*::*gfp*]

pha-1(e2123); nIs106; arEx719 [*egl-17*p::*lsy-6, pha-1*(+), *ttx-3*::*gfp*]

vav-1 transcriptional reporter (5 ng/µl):

pha-1(e2123); arEx720 [*vav-1*p::2x*nls yfp-unc-54 3' UTR, pha-1*(+), *ttx-3*::*gfp*]

pha-1(e2123); arEx721 [*vav-1*p::2x*nls yfp-unc-54 3' UTR, pha-1*(+), *ttx-3*::*gfp*]

pha-1(e2123); arEx722 [*vav-1*p::2x*nls yfp-unc-54 3' UTR, pha-1*(+), *ttx-3*::*gfp*]

vav-1 sensor (5 ng/µl):

pha-*1(e2123); arEx723* [*vav-1*p::2x*nls yfp-vav-1 3' UTR, pha-1*(+), *ttx-3*::*gfp*]

pha-1(e2123); arEx724 [*vav-1*p::2x*nls yfp-vav-1 3' UTR, pha-1*(+), *ttx-3*::*gfp*]

pha-1(e2123); arEx725 [*vav-1*p::2x*nls yfp-vav-1 3' UTR, pha-1*(+), *ttx-3*::*gfp*]

vav-1(mut) sensor (5 ng/µl):

pha-1(e2123); arEx726 [*vav-1*p::2x*nls yfp-vav-1*(mutated) *3' UTR, pha-1*(+), *ttx-3*::*gfp*]

pha-1(e2123); arEx727 [*vav-1*p::2x*nls yfp-vav-1*(mutated) *3' UTR, pha-1*(+), *ttx-3*::*gfp*]

pha-1(e2123); arEx728 [*vav-1*p::2x*nls yfp-vav-1*(mutated) *3' UTR, pha-1*(+), *ttx-3*::*gfp*]

coelomocyte assay

Ex array 1 (microRNA-expressing) (20 ng/µl) (mir-61 genomic region, F55A11 6807-6415)

dpy-20(e1282); arEx729 [*unc-122*p::*mir-61, dpy-20(+)*, *ceh-22*::*gfp*]

Ex array 2 [neutral (20 ng/µl) + test 3' UTR (20 ng/µl)]:

inx-1 test:

dpy-20(e1282); arEx730 [*unc-122*p::*cfp-unc-54 UTR*, *hlh-8*p::*yfp-inx-1 UTR*, *ttx-3*::*gfp*]

egl-46 test:

dpy-20(e1282); *arEx731* [*unc-122*p::*cfp-unc-54 UTR*, *hlh-8*p::*yfp-egl-46 UTR*, *ttx-3*::*gfp*]

vav-1 test:

dpy-20(e1282); *arEx732* [*unc-122*p::*cfp-unc-54 UTR*, *hlh-8*p::*yfp-vav-1 UTR*, *ttx-3*::*gfp*]

unc-54 test:

dpy-20(e1282); *arEx733* [*unc-122*p::*cfp-unc-54 UTR*, *hlh-8*p::*yfp-unc-54 UTR*, *ttx-3*::*gfp*]

V. *vav-1(RNAi)*

L4 hermaphrodites of genotype *lin-12(n379)* or *lin-12(n676)* were placed on plates containing bacteria with the following plasmid: *vav-1* cDNA amplified from yk1531d03 (*17*) inserted into the L4400 vector (*6*). F1 progeny were scored for the presence of pseudovulvae in the dissecting microscope.

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