The Interactions of Vigilin and the miRISC within

Caenorhabditis elegans

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ABSTRACT

Developmental robustness and redundancy are properties of biological systems that facilitate adaptation and survival in stressful environments. Through use of novel screens for synthetic phenotypes, we have elucidated a fascinating interaction between miRNAs and Vigilin, an RNA binding protein predicted to have a variety of different cellular functions within development. Synthetic screening indicates that mir-52, mir-59, mir-83, mir-254, and mir-265 all interact redundantly with Vigilin to modulate larval progression. Computational analysis and further RNAi testing yielded a list of potential downstream targets that these miRNAs might regulate to control development in a complex and robust manner. RT-qPCR and genetics will be used to study these potential targets to provide further insight into the interactions between miRNAs and Vigilin with the overarching goal of gaining a better understanding of development within *C. elegans.*

INTRODUCTION

Developmental Robustness and Redundancy

Within animal and plant cells, there are numerous regulatory pathways that modulate when, where, and how much of a certain protein is produced and to what extent a gene is expressed. In recent years many of these pathways have been discovered to work together in a redundant fashion in order to dictate when and where a cell will reach a certain fate. In any organism there are numerous different pathways, cell types, signaling molecules, organ systems, and the like that all work together to craft the organism as a whole. Central to the study of molecular and developmental biology is ascertaining when, why, and how these various systems work together on the most basal levels to shape functions on the macro level.

The early developmental biologist C. H. Waddington described development as a bunch of marbles rolling down a hill towards a wall (Van Speybroeck, 2002). As each marble rolls down, it has the potential to fall into any sort of canal, crevice, canyon, or, as Waddington coined, "creode" that lines the path down the hillside. The marbles will compete for the grooves in the path and will all attempt to settle at the lowest point of the hill (the wall). The time and way they get to the bottom, however, is vastly different between the marbles depending on their timing (e.g. if they were released sooner or later than other marbles) and path. Waddington called this crevice-riddled landscape the "epigenetic landscape," referring to the factors and mechanisms outside genetic material that regulate why an organism takes on a certain fate over other fates.

While all cells take a different "path" down the hill, all of the cells must reach the bottom at some point—and more often that not a lot of these "paths" meld together, or level out. In this idea, Waddington presented the idea of robustness—the idea that a number of systems and conditions (a number of different grooves down the hill) work together redundantly to ensure the final fitness of an organism. While superficially rolling marbles down a hill seems like a random process, in actuality it is modulated and regulated by a number of different physical factors like gravity—that is to say each marble has a set trajectory. In biology the same is true. Each cell's "roll down that hill" while seemingly unique and independent is precisely regulated at each and every turn down that hill. The idea of redundancy causing robustness, is that even if one of these marbles misses a certain canal, there are other redundant canals available for it to take, all leading to the same final position, ensuring robustness. Similarly an organism trying to maintain evolutionary fitness also has a number of systems to ensure robust development and survival.

MicroRNA and the miRISC

One regulatory pathway that contributes to developmental robustness involves micro-ribonucleic acid, or MicroRNA. The Han lab has studied the functions of these small RNA molecules for nearly two decades.

MicroRNA (abbreviated miRNA) are small, 22 to 23 nucleotide long RNA molecules that act to protect cells by managing when and where certain proteins are produced. To achieve this goal, the miRNA binds to the end of a target messenger RNA in the 3' untranslated region and either prevents the translation of that messenger RNA into a protein or degrades the messenger RNA (Lim *et al.*, 2005; Bagga *et al.*, 2005, Bartel *et al.,* 2009). These miRNAs accomplish this substantive feat through interactions with a set of RNA binding proteins that bind to the miRNA. Collectively, this combination of miRNA and RNA binding proteins is known as the microRNA Induced Silencing Complex, or the miRISC. Selective gene silencing, a mechanism used by the cell to stop a gene being transcribed or translated, is a vital function in all cells that enables cells with the exact same set of DNA to do strikingly different things.

miRNAs are transcribed in similar fashion to normal mRNAs, however after transcription they form a double stranded hairpin-loop structure with themselves and are processed by a number of proteins (known as the microprocessor complex in humans) within the nucleus to produce a pre-miRNA. This pre-miRNA is then exported out of the nucleus by another set of proteins. After it's exodus to the cytoplasm a pre-miRNA is then processed into its final form by an endoribonuclease, Dicer, and a number of other proteins that lead to a mature single-stranded miRNA (Lund and Dahlberg 2006). This single-stranded miRNA then localizes with the miRISC to promote gene silencing of a target by mRNA destabilization or translational blockage.

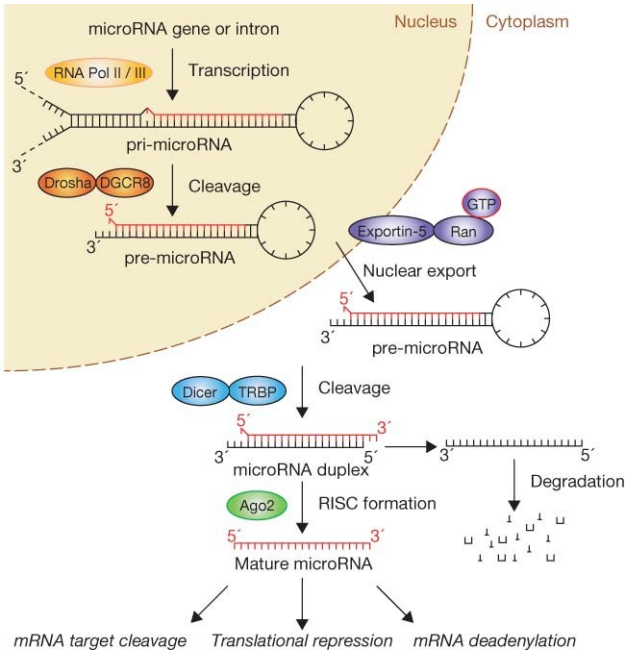


Figure 1: Processing of nascent miRNA (Winter *et al.*, 2009).

The microRNA Induced Silencing Complex (miRISC) is a complex of ribonucleotide binding proteins that function together to facilitate miRNA silencing of a target gene. The complex consists of a microRNA bound to argonaute proteins (ALG-1 and ALG-2 in *C. elegans*), bound to GW182 which is in turn bound to a number of other proteins that assist in silencing (Hafner *et al.*, 2011, Tritschler *et al.*, 2010, Zhang *et al.*, 2005). Within *C. elegans*, AIN-1 and AIN-2 are homologous to GW182. Ain-1 has been shown to interact with worm argonaute homologs in a similar fashion to the human proteins (Ding and Han, 2005). MiRNA function is reduced when ain-1 or ain-2 are knocked out; when both are knocked out in tandem it results in a lethal phenotype.

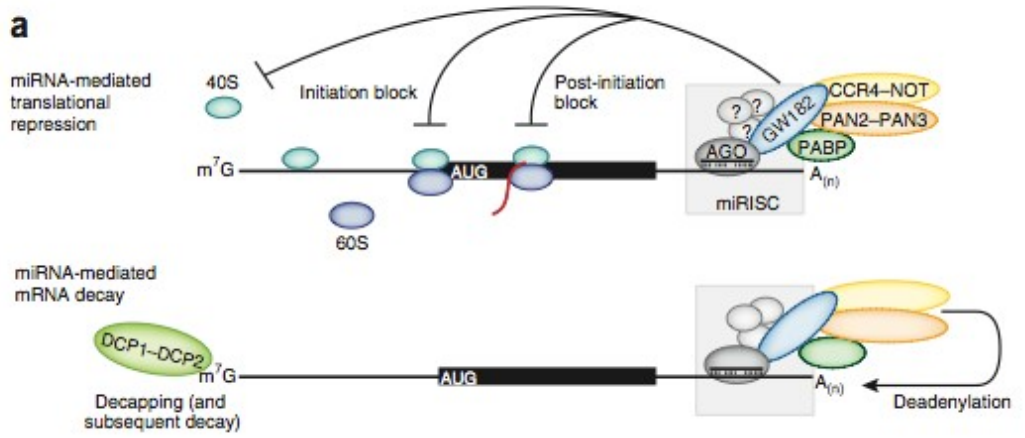


Figure 2: miRISC Function and Group Members (Modified from Fabian *et al.*, 2012)

MicroRNAs are conserved in higher organisms, and mutated miRNAs have been indicated as possible contributing factors in a variety of different human diseases including numerous forms of cancers (Malumbres *et al.*, 2013, Pereira *et al.*, 2013). MicroRNAs function in "families," such that the loss of one miRNA is often not deleterious as other family members make up for its loss of function with a similar activity, binding site, and purpose (Alvarez-Saavedra *et al.*, 2010). This redundancy within the miRISC provides a fascinating glimpse into the system of robustness that has developed among higher organisms. However, it has additionally made the study of miRNA difficult (as single miRNA knockouts often result in no phenotype) so the specific role of many miRNAs remains unknown or clouded. Deleting entire families of miRNAs further revealed that many families are not essential for development or viability (Alvarez-Saavedra *et al.*, 2010). This led the lab to hypothesize that miRNAs function redundantly with other gene regulation mechanisms.

Genome-wide Screen for Developmentally Vital Genes

Within the Han lab, a genome-wide screen was completed that was searching for genes that become essential for development when miRISC function was reduced (Weaver, Zabinsky, *et al.*, 2014). To complete this screen, Post-docs Ben and Yi Weaver and Ph. D. student Rebecca Zabinsky plated *ain-1(lf)* and *ain-2(lf)* worms on the RNAi of genes from the ORF RNAi library. The loss of ain-1 and ain-2 together (that is *ain-1(-); ain-2(-)*) results in a lethal phenotype within worms (due to non functional miRNAs); however, single mutants of either ain-1 or ain-2 results in only a minor developmental timing defect. The *ain-1(-); ain-2(-)* phenotype is an example of a synthetic phenotype: a phenotype that is observed when two genes are knocked out in tandem but not observed when knocked out individually. Within this synthetic screen, worms were scored for any abnormal phenotypes that were seen in the ain-1/2 worms on RNAi, but not observed on ain-1/2 worms on empty vector RNAi. Any phenotypes observed by this method are known as synthetic, since deletions in the genes by themselves result in little observable phenotype but deletion of both results in a severe phenotype.

Vigilin

One of the many interesting genes identified in this screen was *vgl-1* (vigilin). When ain-2 worms were plated on vgl-1 RNAi, a 90% larval (L1) arrest phenotype was observed. Vgl-1 is a member of a family of RNA binding proteins proposed to have roles in a number of cellular processes including telomeric silencing, tRNA splicing, heterochromatin formation, and cholesterol export (Kügler *et al.*, 1996, McKnight *et al.*, 1992, Vollbrandt *et al.*, 2004,Woo *et al.*, 2011, Yang *et al.*, 2014). In humans loss of vigilin function has been observed to contribute to proliferation in several cancers (Woo *et al.*,2011, Molyneux *et al.*, 2014). However, the

exact role and function of vigilin remains difficult to pin down and identifying an interaction with the miRISC is a novel result.

Vigilin was first identified and studied within chickens in the 1990s. Early studies suggested that vigilin (known as High Density Lipoprotein Binding Protein, or HDLBP, in humans) functioned to modulate cholesterol export by binding High Density Lipoprotein within cells. Additionally, vigilin weighs 150 kD, has 15 KH-binding domains that enable vigilin to bind to RNA (Kügler *et al.*, 1996), has a nuclear localization signal (Kügler *et al.*, 1996), is found in nearly all cells both cytoplasmically and in the nucleus, and is heavily conserved in a variety of different organisms. More recently vigilin has been shown to bind ribosomal proteins (Vollbrandt *et al.*, 2004) and interact with signal peptide peptidase (Lu *et al.*, 2012). Despite the current research on vigilin identifying these numerous functions and characteristics of the protein, the functional identity of vigilin still remains open to debate and elucidating the overall purpose of vigilin is an interesting mystery within development.

The purpose of this study was to examine this observed novel interaction between vigilin, the miRISC, and predicted downstream targets within *C. elegans* to identify how these interactions are modulating gene expression and perhaps provide clarity on vigilin's function within development. To study this interaction, a combination of methods was used. A synthetic screen for miRNA(-) single mutants on vigilin RNAi was performed to identify which specific miRNAs are contributing to the synthetic larval arrest phenotype. From here, crosses were carried out to confirm any observed phenotypes. Next, through the use of a variety of methods and databases a list (and then a second list) of predicted downstream targets was compiled and tested for any efficacy in suppressing the larval arrest phenotype. To identify specific targets contributing to the larval arrest phenotype, qRT-PCR was used to quantify expression

differences of the predicted miRNA target gene T24B8.5 in miRNA mutant worms and vigilin mutant worms. Together these methods provide novel insights into the role of vigilin and specific miRNAs in development.

METHODS

General C. elegans *Strain Maintenance*

C. elegans strains used were either obtained from the CGC or from the Han Lab's frozen stocks. Strains were maintained and picked weekly to remove contamination and prevent starvation. Strains were stored at 20^{o}C unless otherwise noted. A list of strains used is attached (supplemental table 1).

miRNA(-) worms on Vigilin RNAi

To test for specific miRNA mutants contributing to the larval arrest phenotype observed in *ain-2(-); vgl-1(-)* worms, a list of the miRNAs identified within *C. elegans* was compiled, single mutant strains were unfrozen or ordered from the CGC from this list, and these single mutants were allowed to lay eggs on RNAi plates. The protocol for screening each single mutant is as follows:

Day 1: Inoculate cultures of vgl-1 RNAi and empty vector (control) RNAi.

Day 2: Inoculate cultures of vgl-1 RNAi and empty vector (control) RNAi, using cultures from previous day. Incubate for 4 hours and then spot onto to IPTG+AMP plates.

Day 6: 3 larval-4 stage miRNA-"X"(-) worms were picked to a single vgl-1 RNAi plate. 3 larval-4 stage miRNA-X(-) worms were picked to a mock RNAi plate.

Day 7: After 24 hours adult worms were singled to new RNAi plates.

Day 8: After 24 hours, gravid adults were flamed.

Day 10: F1 generation of worms were scored for larval arrest. The number of larval worms (errant phenotype) and adult worms (normal phenotype) were then compared.

This screen was completed for each of the 88 miRNA strains obtained.

miRNA(-) and vgl-1 Crosses

miRNA(-) single mutants with a larval arrest phenotype (observed in the screen above) on vgl-1 RNAi were crossed to *vgl-1*(-) worms to confirm the phenotype was not just an artifact of the RNAi feeding. NT1, a well-published balancer, was used to balance the strains throughout the crosses. Genotyping by PCR confirmed the genotype of the worms. These crosses are still in progress at time of writing.

miRNA Predicted Target Analysis—Pilot Analysis

The predicted targets of miRNAs identified in the miRNA on vgl-1 RNAi synthetic screen were compiled from the databases of mirbase (mirbase.org), target scan (targetscan.org), wormbase (wormbase.org), and pubmed (ncbi.nlm.nih.gov/pubmed). The compiled database contained data for every miRNA family in *C. elegans* and contained roughly 1,000,000 rows of data. From this set, predicted targets of the miRNAs identified in the synthetic screen with a 6mer, 7mer, or 8mer binding predicted binding site were selected, screened for duplicates (per each miRNA), and then compared to miRNA targets enriched in a neuronal IP, a L1 IP, and a egg IP (all studies performed earlier within the Han lab, Zhang *et al.* 2009, Kudlow *et al.* 2011, Than *et al.* 2012). This narrowed the list down to roughly 20,000. Worm mine (wormbase.org/tools/wormmine), a data mining tool, was then used to convert the data to an easily usable format and create a final predicted target list for each miRNA identified as contributing to the larval arrest phenotype in the synthetic screen. The final list of predicted targets was roughly 500 genes. From here, Venny (bioinfogp.cnb.csic.es/tools/venny/) was used

to compare the list of targets for each miRNA, looking for common predicted targets shared between miRNAs. This list had roughly 50 genes.

ain-2(-); vgl-1(-) Worms on RNAi of Predicted Targets

The RNAi of each of the 50 gene targets found by the protocol described above were obtained through the Ahringer and ORF RNAi libraries. The same protocol that was used for the miRNA single mutants on vigilin RNAi was conducted with these RNAi strains. Heterozygous HT2(with a GFP marker)/ain-2(lf); *vgl-1*(lf) worms were picked to the RNAi of these predicted targets and the F2 generation was scored for rescue. Progeny with homozygous ain-2(lf) are expected to have a 90% larval arrest phenotype when paired with a homozygous loss of function of *vgl-1*. In comparison, worms that keep the HT2 marker will show little to no arrest at the larval stage. Accordingly without rescue, a ratio of 1:25 Non-GFP:GFP worms is expected on the RNAi. The presence of a ratio different from this indicates the possibility that a suppression of the larval arrest has occurred.

miRNA Predicted Target Analysis—Second Round

A second round of miRNA target analysis was completed using data from TarBase and miRWip rather than the sources listed earlier. A list of 350 predicted miRNA targets was compiled and compared to enriched targets in a Dicer microarray (Welker *et al.*, 2007) and enriched hits from an IP of a intestine promoter, ges-3 (Kudlow *et al.* 2011). Results were visualized with Venny like before, and similar target analysis is planned.

GoTerm Analysis

Targets from this second list were then compared to a list of every gene in the *C. elegans* genome (list retrieved from wormbase) using Babelomics (Al-Shahrour *et al.*, 2004),

according to the Fatigo algorithm. GoTerms are a standardized list of descriptors ascribed to genes for specific functions.

RESULTS

Ain-2(lf) worms on Vigilin RNAi

Ain-2(-) worms on vigilin RNAi produced a L1 arrest phenotype of 90% (Fig. 1), similar to results observed previously in the Han Lab

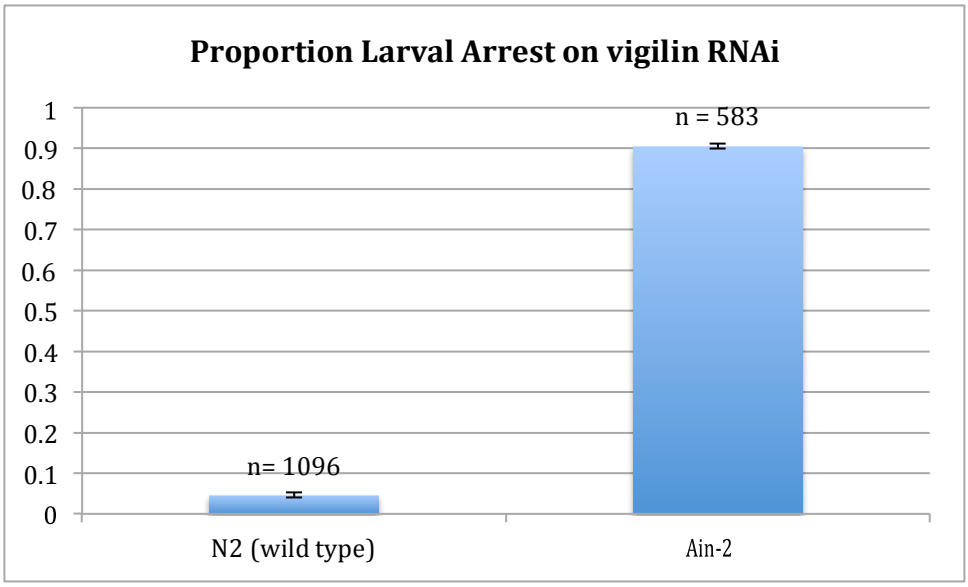


Figure 3: proportion larval arrest of wild type and ain-2 worms on vigilin RNAi. Error Bars represent standard error of proportion. P<<0.05 according to a Fischer's Exact P Test.

miRNA Single Mutants on Vigilin RNAi

Systematic analysis of 88 miRNA single mutant worms on vigilin RNAi in *C. elegans* found that over half of the strains tested contribute statistically significantly to the larval arrest phenotype as is expected according to the redundancy of miRNAs (Fig. 4). Values above 8% are significant according to a Fischer's Exact P Test.

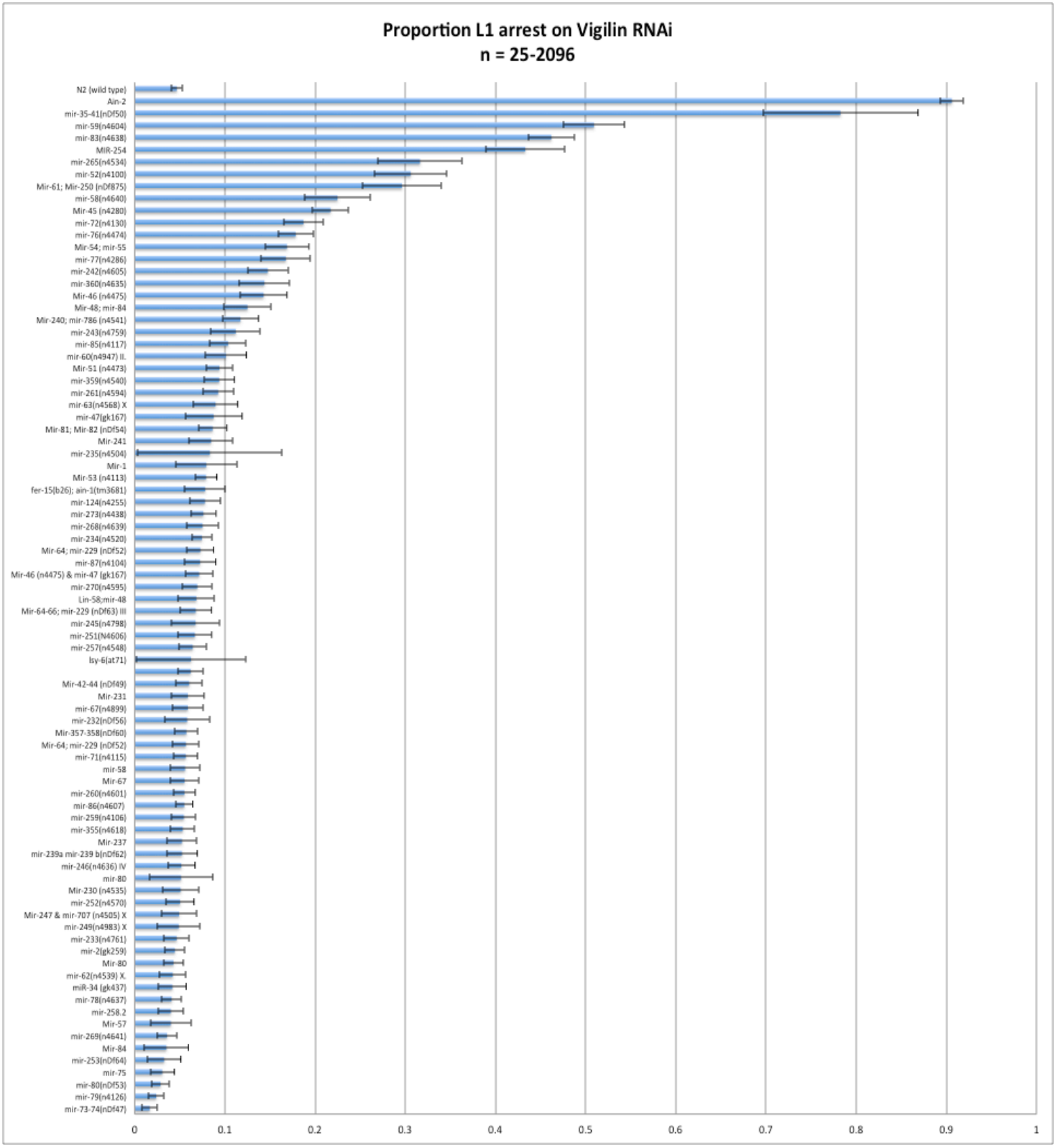


Figure 4: miRNA(-) single mutants on vigilin RNAi. Error bars represent standard error of proportion. Values above 8% have a P value of <0.05 according to a Fischer's Exact P Test.

Strains that had a larval arrest phenotype of >20% were considered "extremely significant" and useful for further procedures. Of the miRNA mutants tested, mir-35-41(-), mir-52(-), mir-58(-), mir-59(-), mir-83(-), mir-254(-), mir-265(-), and mir-61(-); mir-250(-) worms all produced an extremely significant L1 arrest phenotype on vigilin RNAi with $p < 0.00001$ using a Fischer's Exact P-Test (Fig. 5).

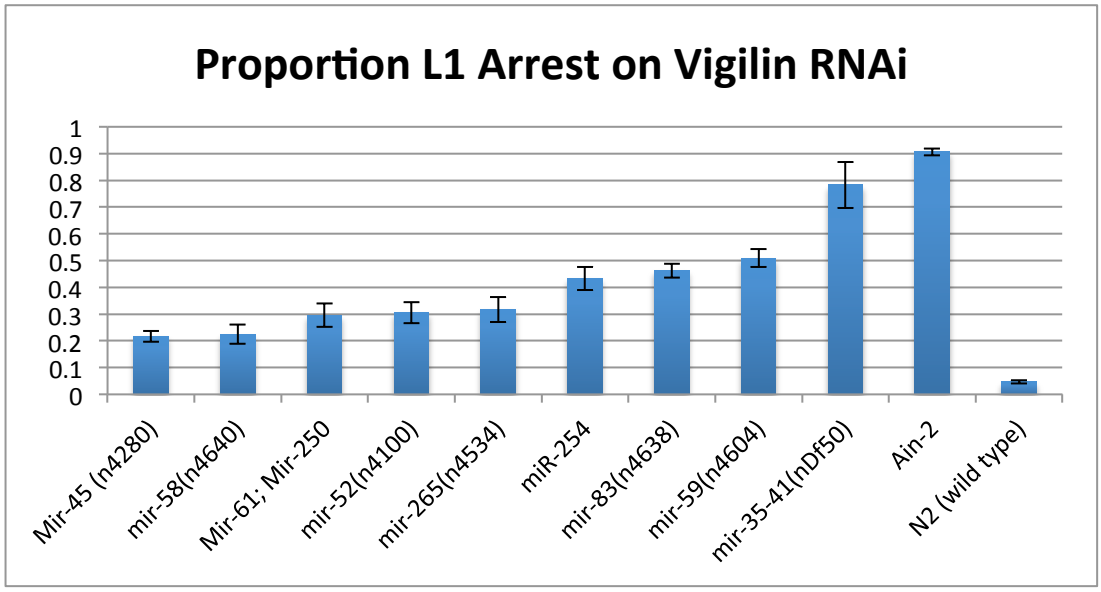
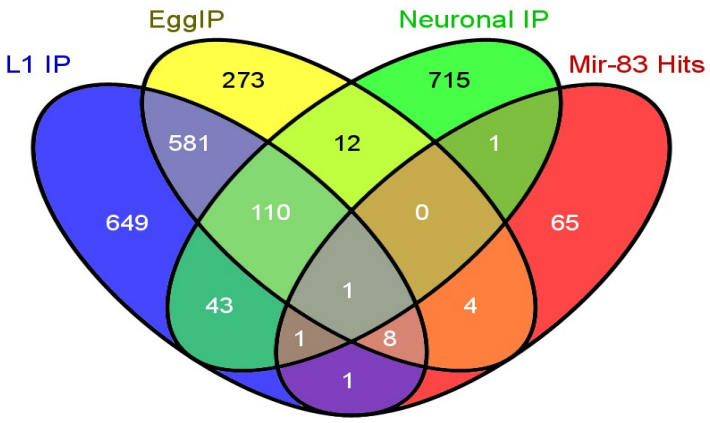


Figure 5: miRNA(-) mutants with elevated L1 arrest on vigilin RNAi. Error bars represent standard error of proportion.

miRNA Predicted Targets and Results (Pilot Analysis)

miRNA(-) single mutant strains with an elevated larval arrest phenotype of more than 25% on vigilin RNAi were analyzed using the methods listed above for potential targets. Accordingly, miRNA-35-41(-), miRNA-59(-), miRNA-83(-), miRNA-254(-), mir-265(-), and miRNA-52 were examined for potential targets using miRbase and targetscan for a base list of hits. This list for each miRNA was then compared to enriched targets in an L1 IP, neuronal IP, and egg IP done previously and common targets between each subset were selected for further examination (example 1).



Example 1: Comparison of mir-83 predicted targets to targets enriched in various IPs. This was performed for each miRNA.

A final list of filtered targets (those predicted to be targeted by a miRNA mutant and enriched in one or more IPs) was assembled in this way, creating a final list of 346 genes (sup. table 2) from the initial list of over 5000. The predicted function of these genes (from the Ahringer RNAi library "suspected function" column) was compiled and compared (Fig. 6).

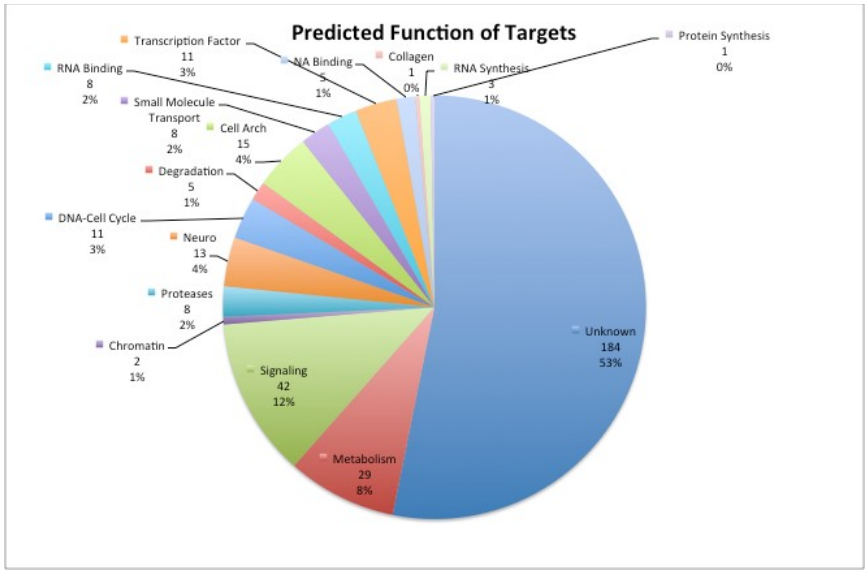
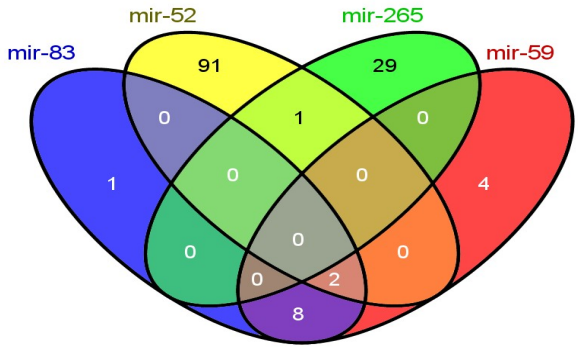


Figure 6: Numbers and percentages of miRNA targets' predicted functions.

Additionally, the predicted targets of each miRNA on this final list were then compared to each other (Example 2) for common hits.



Example 2: Comparison of predicted miRNA targets between multiple miRNAs. This was performed for all miRNAs identified in the synthetic screen as extremely significant to the larval arrest phenotype.

This analysis resulted in a list of 53 predicted targets regulated by 2 or more miRNA (Table 1).

Sequence	Gene Name	Number of miRNA predicted to be targeted by
R10E12.2		4
Y38F1A.5	ddl-3	4
C01C7.1	madf-5	3
F07C3.4	adt-2	3
F13D11.2	lips-10	3
F13H6.1	acy-1	3
H13N06.2		3
K08H10.4		3
B0410.3		2
B0464.4	bre-3	2
B0464.4	ark-1	2
C01G12.1	swt-3	2
C06G8.1	nas-7	2
C07D10.4		2
C13B4.1	dyci-1	2
C17H12.1	anat-1	2
C17H12.13	cab-1	2
C23H4.1		2
C26B9.1		2
C30F12.2		2
C34B2.11	lon-2	2
C39E6.1		2
C53B4.3	nhr-19	2
E02H1.7	glo-4	2
F07C3.4	hbl-1	2
F08C6.1		2
F14E5.5		2
F17C8.1	egl-1	2
F18C5.10	snb-2	2
F23B12.9	cdh-4	2
F23H12.1	rgl-1	2

F25F2.2		2
F28B4.2	vha-5	2
F29B9.8	wrt-3	2
F35H10.4	dep-1	2
F38E11.7	swan-2	2
F44G4.8		2
F53C11.7		2
F59B2.2		2
H10E21.5	ser-3	2
K02F2.6	uda-1	2
K08E3.4	erv-46	2
K09E9.2	msi-1	2
R10E9.1	tlp-1	2
T23B12.6		2
T23G4.1	snf-1	2
T28D6.4	vab-2	2
W03G9.1	tcl-2	2
Y37E11AR.6	cyd-1	2
Y38C1AA.4	rig-4	2
Y42H9B.2	lron-11	2
Y54G11A.8		2
Y71F9B.8		2

Table 1: list of predicted targets regulated by more than one miRNA identified in the synthetic screen.

To test if any of these 53 genes are contributing to the larval arrest phenotype, ain-2(-);*vgl-1*(-) worms were plated on RNAi of each of the targets from table 2 to observe if knockout of these predicted targets can suppress the larval arrest, however no statistically significant suppression was observed.

miRNA Predicted Target Analysis (Second Round)

Following the analysis of the first data list, a second list of downstream potential miRNA targets was compiled using similar but refined methods to those used above. Predicted targets of miR-52, miR-59, miR-83, miR-254, and miR-265 were compiled using recent data from miRWip (Hammell *et al.*, 2006) and TarBase (Vlachos *et al.*, 2014), creating a list of roughly 550 targets. Predicted targets from the mirWIP list were only considered if identified in an Ain-2 or Alg-1 IP. This list was then compared to a list of mRNAs that are increased in a Dicer mutant (Welker *et al.*, 2007). Additionally, the initial list was compared to enriched targets in a Pges-1:Ain-2 IP, because it was found that intestine specific ain-2 expression can somewhat suppress the larval arrest phenotype within the double mutant. The comparison is shown below (Figure 7). This list was then compared to the list compiled in the pilot analysis (Figure 8).

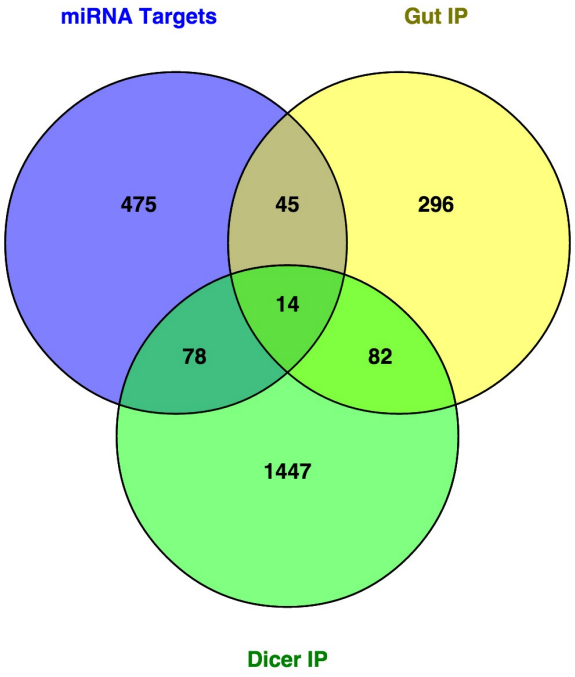


Figure 7: Comparison of Raw list of predicted miRNA targets (blue), Intestine IP enriched targets (yellow), and enriched targets from the Dicer IP (green).

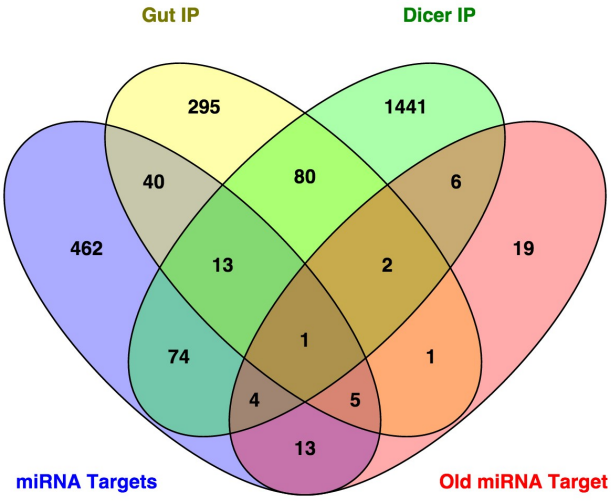
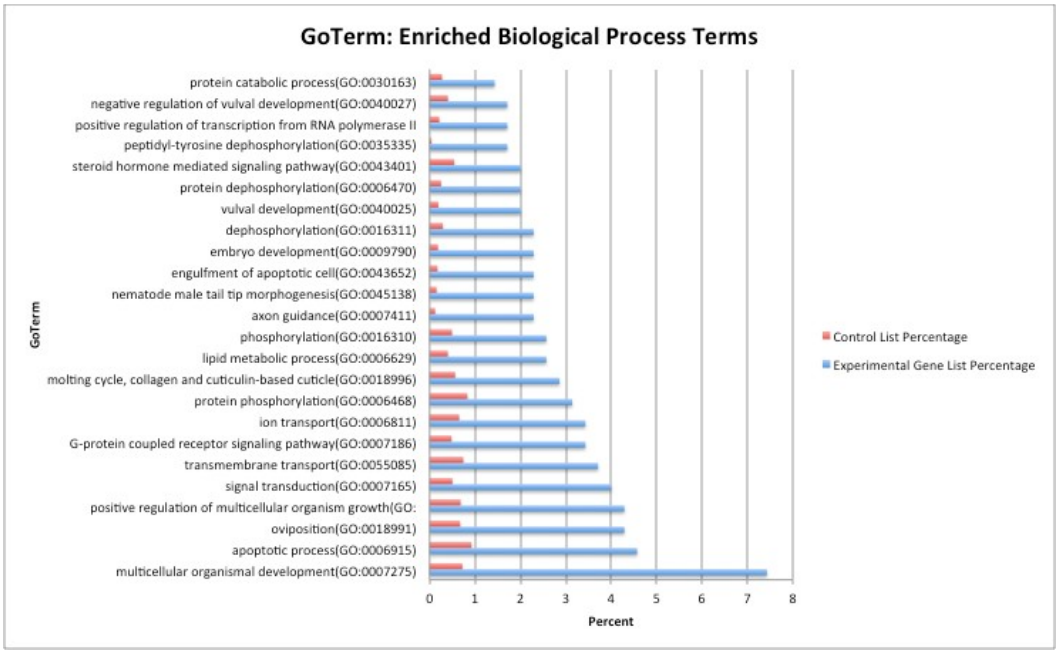


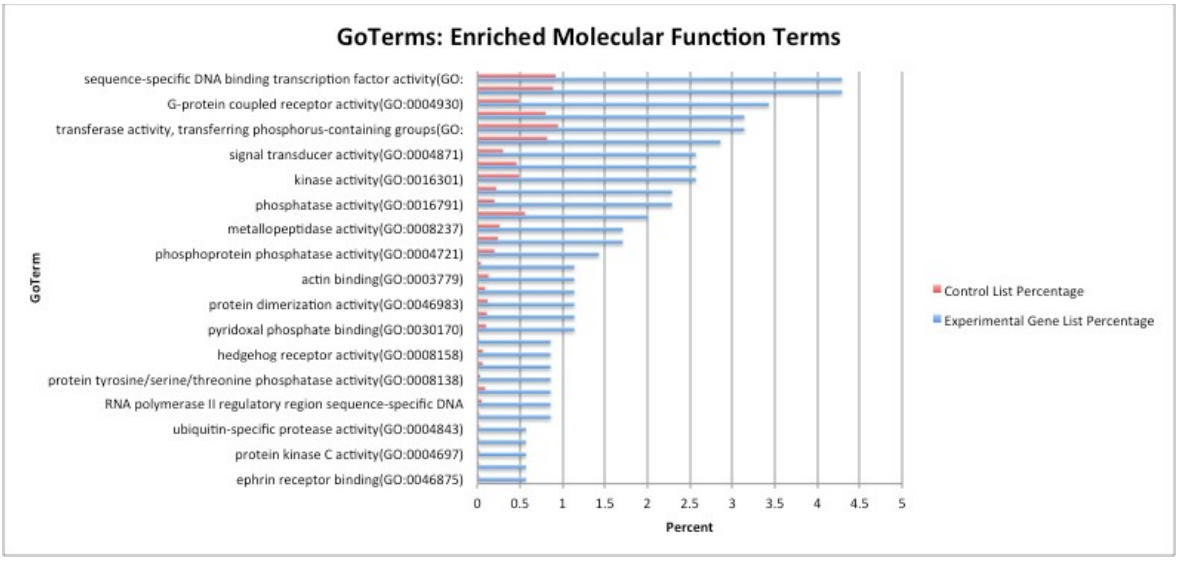
Figure 8: Comparison of Old miRNA predicted targets (from the pilot screen) to the "new" list of potential hits. List of targets within each segment of the diagram are found in supplemental table 3.

This list of final targets and their commonalities with the Intestine IP, Dicer mutant, and old targets is attached (supplemental table 3).

GoTerm Analysis

GoTerm analysis was completed for this new list of targets, computing significant (according to a Fischer's P test) differences between terms identified with the compiled gene list and a control gene list of all the genes in the *C. elegans* genome (list of genes taken from Wormbase). Comparisons of significant GoTerms by percentages are shown below for biological function, molecular function, and cellular components (Figure 9). Each comparison compares the percentage of the potential list of targets ("Experimental Gene List Percentage") to the percentage in overall genome ("Control List Percentage").





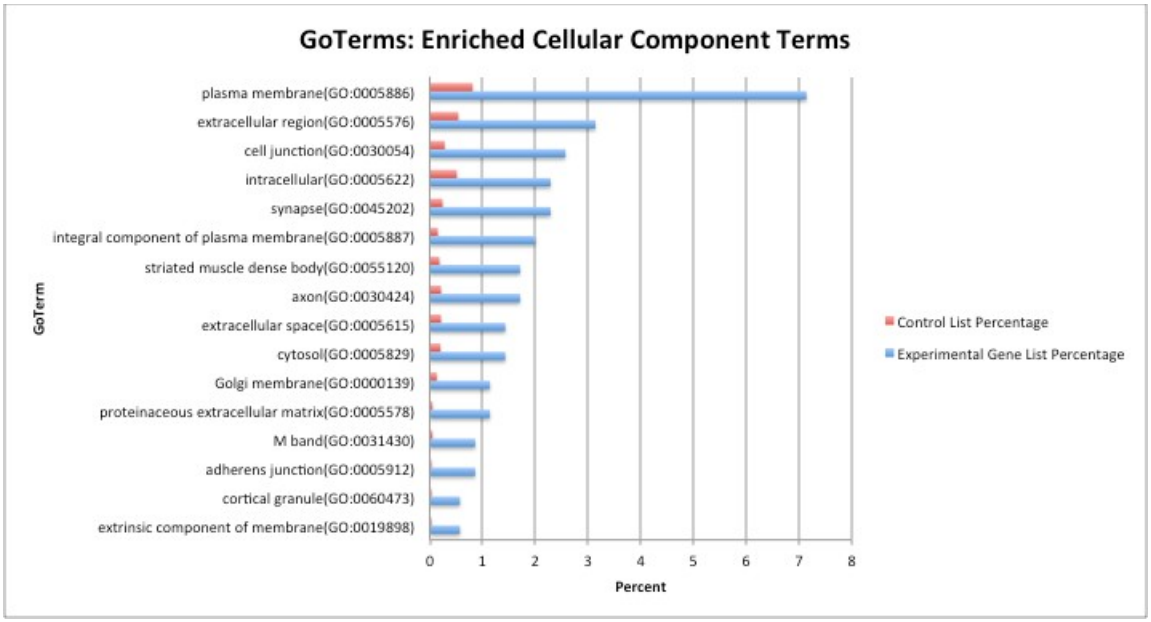


Figure 9: GoTerms with significant ($p<0.05$) enrichment. Chart A represents biological process GoTerm enriched differences, Chart B represents molecular function GoTerm enriched differences, and Chart C represents cellular component enriched term differences.

Assorted Other RNAi Results

In addition to the miRNA(-) on vgl-1 RNAi screen that was completed, a number of other synthetic screens were completed with candidate genes we hypothesized might be synthetic either with vgl-1 or ain-2. Notably, the insulin pathway was screened for potential interactions due to the known role of insulin signaling in dauer and larval arrest formation (Fig. 10).

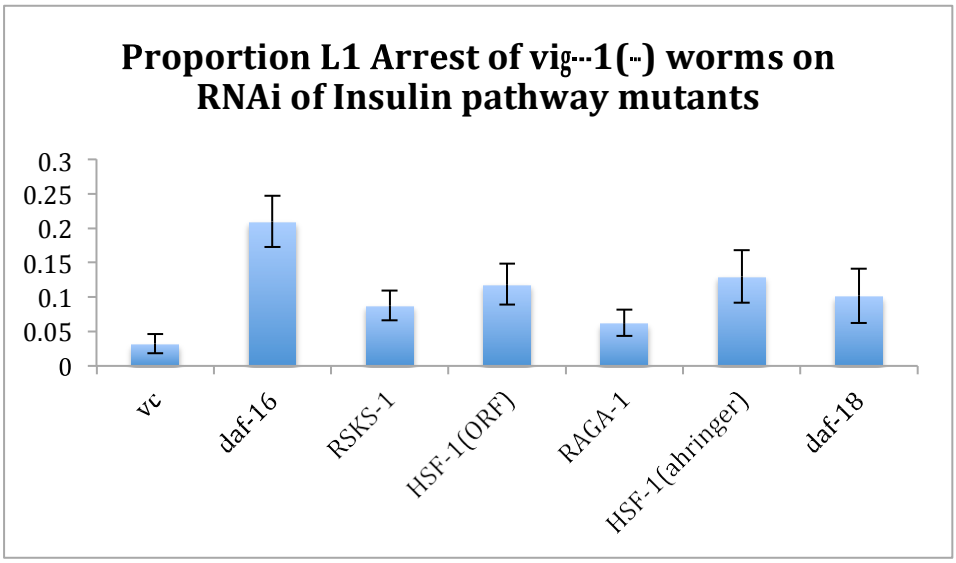


Figure 10: *vgl-1*(-) worms on insulin pathway RNAi. Error bars represent standard error of proportion.

Additionally, a number of other interesting genes known to contribute to development were also screened (Fig. 11).

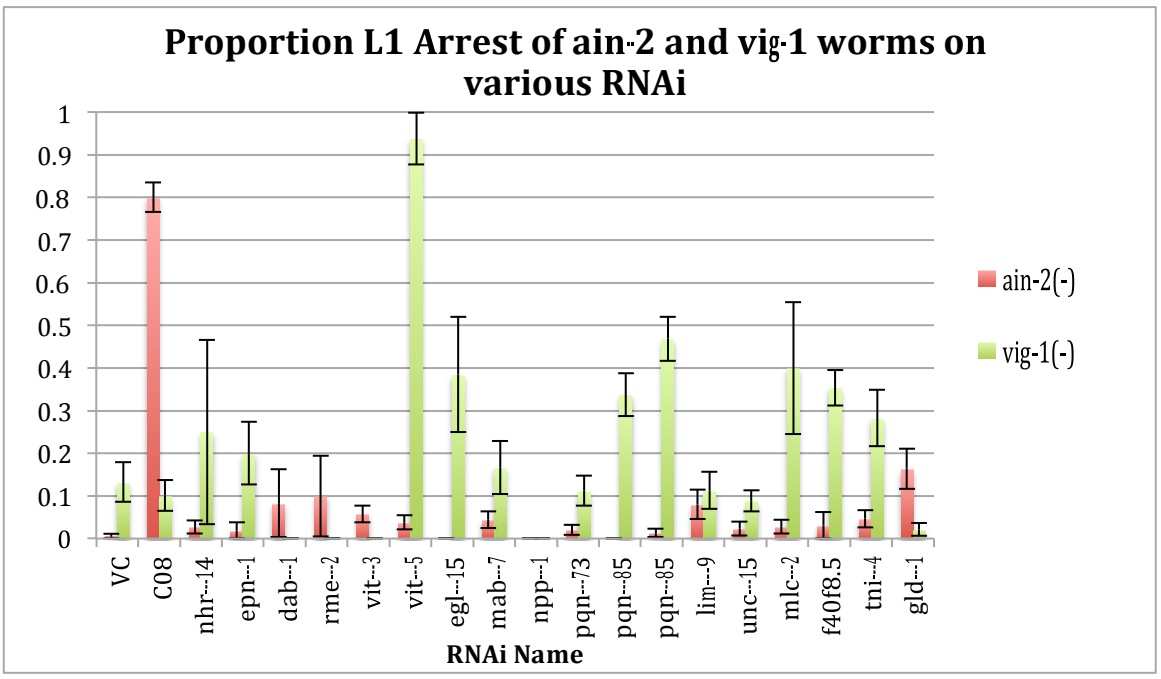


Figure 11: ain-2(-) and *vgl-1*(-) worms on various RNAi. Here "CO8" refers to vigilin RNAi. Error bars represent standard error of proportion.

DISCUSSION

Based upon the data presented in figure 4 in the miRNA and vgl-1 synthetic screen, it is likely that many microRNAs act redundantly in parallel with Vigilin to modulate development within *C. Elegans.* The fact that so many of the screened miRNAs contributed to the phenotype is consistent with the redundancy of microRNAs. Additionally, the high number of significant strains could indicate sensitivity to the RNAi; however the large range of larval arrest between significant strains makes RNAi sensitivity likely not the only causative factor for the larval arrest phenotype. This issue of RNAi sensitivity will be addressed once crosses of the highly significant (>20% larval arrest) miRNAs with *vgl-1*(-) are complete, but this is still in progress.

Of the microRNAs that produced a phenotype of greater than 20% larval arrest, mir-52, mir-59, mir-83, mir-254, and mir-265 seem to be the most reliable for future study. The mir-35 family, while producing an extremely significant larval arrest phenotype, has been already shown to have some deleterious effects on development and has been shown to be sensitive to RNAi. Additionally initial results were misleading, as further testing of both strains used to test the miR-35 family produced varying degrees of embryonic lethality, indicating a possible sensitivity to the RNAi. Of the 5 remaining targets, miR-254 and miR-52 are conserved while miR-59, miR-83, and miR-265 are not. Mir-254 is homologous to human miR-19, a miRNA indicated in a number of cancers. Of the other four targets, little study has been done and their overall function remains unknown, so the results postulated here present an interesting link between these miRNA and early development within *C. elegans*.

The pilot target analysis of each of the miRNAs individually and in comparison to each other and various microarrays and immunoprecipitation data has yielded a list of potential downstream targets whose misregulation might be contributing to this larval arrest phenotype.

Further analysis, in the form of tests to see if the RNAi of these targets can suppress the larval arrest phenotype, remain to be done as presented methods of testing HT2/*ain-2(-); vgl-1(-)/vgl(-)* remain somewhat ineffective as the double mutant is extremely sick, making screens difficult. Testing with the less ill miRNA-"X"; *vgl-1*(-) crosses might yield better and more conclusive results and should be easier to screen. It is expected, according to the general mechanism of miRNA action, that the larval arrest phenotype is induced by a number of misregulated downstream targets acting together so any suppression by an individual gene is expected to not be a complete rescue.

The second round of data analysis done recently has yielded a list of predicted targets perhaps more promising than the last, as each target was identified as enriched within a previous ain-2 IP or alg-1 IP (tarbase, Zisoulis *et al.* 2010) in comparison to the pilot list where this was not the case. Between these two data analyses, targets selected for further analysis include hbl-1, lin-17, F26A3.4, skn-1, and daf-12. These targets were selected because they are downstream within known growth signaling pathways and it is hypothesized that overexpression of these targets (caused by loss of specific miRNAs) is causing or contributing to the larval arrest.

Testing RNAi of these targets with the crosses for suppression, in addition to qRT-PCR of the targets to ascertain if mRNA levels of the targets change between the mutants remains to be completed. Initial RT-qPCR data with T24B8.5 (an initial target from the first analysis), as a previously published test gene (Troemel *et al.*, 2006), remains cursory and in need of further replicates. It is expected that mRNA levels of the predicted targets should rise within the respective miRNA mutants, as these miRNA should be downregulating these targets normally. RT-qPCR should provide another meaningful avenue for approaching the remaining question of whether or not these potential targets are actual targets.

Together, the miRNA synthetic screen, RNAi data, and data analysis yielding potential downstream targets provide interesting clues as to how vigilin and the miRISC are interacting together to influence development. Much work remains to be done to further investigate the potential targets, however the list presented here provides an ample starting position. Further analysis with genetic approaches and RT-qPCR should solidify the interactions of these miRNA with specific previously unexplored downstream targets and further our understanding of the interactions between vigilin and the microRNA induced silencing complex.

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Strain name	Gene Mutant name
CU8018	miR-34 (gk437)
DR721	lin-4(e912) II.
MH 4379	Mir-80
MH4360	mir-253(nDf64)
MH4364	fer-15(b26); ain-1(tm3681)
MH4368	mir-58(n4640)
MH4379	mir-80(nDf53)
MT12945	mir-52(n4100)
MT12954	Mir-1
MT12958	mir-87(n4104)
MT12969	mir-259(n4106)
MT12988	
MT12989	Mir-53 (n4113)
MT12993	mir-71(n4115)
MT12999	mir-85(n4117)
MT13015	mir-72(n4130)
MT13016	Mir-64; mir-229 (nDf52)
MT13016	Mir-64; mir-229 (nDf52)
MT13078	mir-73-74(nDf47)
MT13292	mir-124(n4255)
MT13372	Mir-42-44 (nDf49)
MT13433	Mir-45 (n4280)
MT13650	Lin-58;mir-48
MT13651	Mir-84
MT13652	Mir-48; mir-84
MT13653	Mir-237
MT13897	Mir-241
MT13949	mir-80
MT13954	Mir-81; Mir-82 (nDf54)

Strain Name	Gene Mutant Name
MT14091	mir-79(n4126)
MT14118	mir-241; mir-84
MT14119	mir-35-41(nDf50)
MT14347	mir-273(n4438)
MT14449	mir-232(nDf56)
MT14450	Mir-51 (n4473)
MT14451	mir-76(n4474)
MT14452	Mir-46 (n4475)
MT14525	MIR-254
MT14588	mir-234(n4520)
MT14661	mir-265(n4534)
MT14662	Mir-230 (n4535)
MT14673	mir-359(n4540)
MT14682	mir-257(n4548)
MT14767	Mir-54; mir-55
MT14768	Mir-231
MT14875	Mir-61; Mir-250 (nDf875)
MT14876	mir-261(n4594)
MT14878	mir-270(n4595)
MT14919	mir-260(n4601)
MT14935	mir-59(n4604)
MT14936	mir-242(n4605)
MT14993	Mir-46 (n4475) & mir-47 (gk167)
MT15018	mir-360(n4635)
MT15019	Mir-357-358(nDf60)
MT15020	mir-246(n4636) IV
MT15021	mir-78(n4637)
MT15022	mir-83(n4638)
MT15023	mir-268(n4639)
MT15024	mir-58

Strain Name	Gene Mutant Name
MT15312	mir-239a mir-239 b(nDf62)
MT15454	mir-243(n4759)
MT15517	mir-233(n4761)
MT15767	mir-258.2
MT15873	Mir-240; mir-786 (n4541)
MT15982	Mir-67
MT15982	mir-67(n4899)
MT16308	mir-252(n4570)
MT16309	Mir-247 & mir-707 (n4505) X
MT16310	mir-269(n4641)
MT16311	mir-77(n4286)
MT16316	mir-355(n4618)
MT16335	mir-251(N4606)
MT16336	mir-86(n4607)
MT16337	mir-245(n4798)
MT16471	mir-60(n4947) II.
MT16494	Mir-64-66; mir-229 (nDf63) III
MT16696	mir-244(n4367)
MT16848	mir-249(n4983) X
MT17445	mir-62(n4539) X.
MT17997	mir-235(n4504)
MT18037	mir-75
MT7626	Let-7
OH2535	lsy-6(at71)
VC328	mir-47(gk167)
VC347	Mir-57
VC495	mir-2(gk259)
VC514	mir-35(gk262)
VT1289	mir-63(n4568) X
	N2 (wild type)
	Ain-2

Supplemental Table 1: List of Strains used.

	Sequence Name	Gene Name	Predicted Function		Sequence Name	Gene Name	Predicted Function		Sequence Name	Gene Name	Predicted Function		Sequence Name	Gene Name	Predicted Function		Sequence Name	Gene Name	Predicted Function
1	B0024.13		Unknown	81	Y42H9B.2	rig-4	Unknown	161	Y45F10D.3	gly-10	Metabolism	241	Y38E10A.12	nspe-3	Unknown	321	F20D1.7	iglr-1	Unknown
2	B0379.4	scpl-1	Unknown	82	Y44A6D.3		Unknown	162	B0416.6	gly-13	Metabolism	242	Y43F8C.3		Unknown	322	F32D8.1		Signalling
3	B0454.6		Metabolism	83	Y47H9C.4	ced-1	Signalling	163	C26C6.2	goa-1	Signalling	243	Y48C3A.11	dmsr-3	Unknown	323	F58E10.1	ric-7	Unknown
4	B0564.6		Unknown	84	Y53C12B.5	mab-3	Unknown	164	F13D11.2	hbl-1	Transcription Factor	244	Y106G6H.14		DNA-Cell Cycle	324	K09E9.2	erv-46	Unknown
5	C01C7.1	ark-1	Signalling	85	Y54G11A.8	ddl-3	Unknown	165	R13H4.4	hmp-1	Signalling	245	Y111B2A.15	tpst-1	Unknown	325	R10E12.2		Unknown
6	C03E10.4	gly-20	Unknown	86	Y57G11C.11	coq-3	Unknown	166	K07A1.8	ile-1	Unknown	246	ZK757.4	dhhc-4	Unknown	326	T28D6.4		Unknown
7	C04A2.3	egl-27	Chromatin	87	Y60A3A.21		Unknown	167	F26D11.11	let-413	Signalling	247	ZK795.1		Unknown	327	Y54G11A.8	ddl-3	Unknown
8	C04D8.1	pac-1	Unknown	88	Y67D8C.10	mca-3	Unknown	168	F57B9.2	let-711	Unknown	248	ZK1128.4		RNA Synthesis	328	C06E1.3	doxa-1	Unknown
9	C05D11.4	let-756	Signalling	89	Y67D8C.9		Unknown	169	C39E6.1	lon-2	Unknown	249	B0238.11		Unknown	329	C24B5.1		Neuro
10	C07D10.4	nas-7	Proteases	90	Y71F9B.8	lron-11	Unknown	170	R03E9.1	mdl-1	Transcription Factor	250	B0410.3		Unknown	330	C30F12.2		Unknown
11	C08B11.1	zyg-11	Unknown	91	ZK370.3	hipr-1	Unknown	171	Y57E12AL.5	mdt-6	Unknown	251	C01F4.2	rga-6	Unknown	331	C34B2.11		Unknown
12	C13B4.1		Unknown	92	ZK418.7		Neuro	172	R10E9.1	msi-1	RNA Binding	252	C14C11.3	hex-2	Unknown	332	C34H3.1	tag-275	Unknown
13	C16C8.2		Metabolism	93	ZK682.2		Cell Arch	173	W04H10.3	nhl-3	Unknown	253	C14F5.4	sfxn-2	Metabolism	333	C54D1.5	lam-2	Cell Arch
14	C16D9.6		Unknown	94	ZK867.1	syd-9	Unknown	174	E02H1.7	nhr-19	Transcription Factor	254	C17H12.13	anat-1	Unknown	334	F07C3.4	glo-4	DNA-Cell Cycle
15	C23H4.1	cab-1	Neuro	95	C17H12.1	dyci-1	Unknown	175	F11C1.6	nhr-25	Transcription Factor	255	C26B9.1		Unknown	335	F13H6.1		NA Binding
16	C24A8.4	cst-2	Unknown	96	C52A11.4	mpz-1	Signalling	176	F48F7.4	pqn-39	Unknown	256	C30A5.3		Unknown	336	F20D6.10		Unknown
17	C30F12.2		Unknown	97	C53B4.3		Unknown	177	F45H7.4	prk-2	Signalling	257	C30H7.2		Metabolism	337	F28E10.2	igeg-1	Signalling
18	C33A11.2		Unknown	98	F13D11.2	hbl-1	Transcription Factor	178	R07H5.1	prx-14	Unknown	258	C48E7.1		Unknown	338	F29B9.8		Unknown
19	C34B2.11		Unknown	99	F14E5.5	lips-10	Unknown	179	ZK675.1	ptc-1	Signalling	259	C55C3.5	perm-5	Unknown	339	F42H10.3		Signalling
20	C39E6.1	lon-2	Unknown	100	F47G9.4		Unknown	180	C53C11.3	ptr-5	Signalling	260	EEED8.10		RNA Binding	340	H10E21.5		Unknown
21	C43F9.8	efn-2	Unknown	101	F53C11.7	swan-2	Unknown	181	F46G10.5	ptr-24	Signalling	261	EGAP2.1		Unknown	341	K07C11.8		Unknown
22	C52E4.5	mans-2	Unknown	102	H13N06.2		Unknown	182	K09E2.4	rig-1	Signalling	262	F07C3.4	glo-4	DNA-Cell Cycle	342	M03F8.1		Unknown
23	D1046.5	tpra-1	Unknown	103	K03C7.2	fkh-9	Transcription Factor	183	ZK20.5	rpn-12	Degradation	263	F13H6.1		NA Binding	343	R05H11.2		Unknown
24	F07C3.4	glo-4	DNA-Cell Cycle	104	K08H10.4	uda-1	Metabolism	184	C18F3.2	sax-7	Signalling	264	F18C5.10		Unknown	344	R12E2.2		Unknown
25	F10C5.2		Degradation	105	R10E12.2		Unknown	185	R12H7.3	skr-19	Degradation	265	F22D3.2	flcn-1	Unknown	345	T23B12.6		Unknown
26	F16A11.1		Unknown	106	T23G4.1	tlp-1	NA Binding	186	W03G9.1	snf-1	Neuro	266	F28B4.2	rgl-1	Signalling	346	Y71F9B.8	lron-11	Unknown
27	F16F9.4		Metabolism	107	Y38F1A.5	cyd-1	DNA-Cell Cycle	187	Y110A2AL.14	sqv-2	Unknown	267	F28C10.3		Signalling				
28	F20B10.1	nlr-1	Signalling	108	Y54E10A.16	mab-31	Unknown	188	C52E12.3	sqv-7	Cell Arch	268	F28H1.1		Unknown				
29	F20G4.3	nmy-2	Cell Arch	109	Y38F1A.5	cyd-1	DNA-Cell Cycle	189	ZK1307.5	sqv-8	Metabolism	269	F32B5.7		Unknown				
30	F23B12.9	egl-1	Unknown	110	F13D11.2	hbl-1	Transcription Factor	190	F54C9.2	stc-1	Protein Synthesis	270	F39G3.5		Metabolism				
31	F23H12.1	snb-2	Cell Arch	111	T23G4.1	tlp-1	NA Binding	191	D1014.1	sul-2	Metabolism	271	F40A3.7		Neuro				
32	F25H2.13	rtel-1	DNA-Cell Cycle	112	C53B4.3		Unknown	192	T22B2.4	sup-12	RNA Binding	272	F41A4.1	cutl-28	Unknown				
33	F26A3.4		Signalling	113	F14E5.5	lips-10	Unknown	193	K03A1.5	sur-5	Metabolism	273	F41H10.5		Proteases				
34	F26E4.11	hrdl-1	Degradation	114	F53C11.7	swan-2	Unknown	194	R13A1.2	kcc-1	Small Molecule Transp	274	F42A10.7		Unknown				
35	F28B4.2	rgl-1	Signalling	115	H13N06.2		Unknown	195	Y38C1AA.4	tcl-2	Unknown	275	F54A3.6		Unknown				
36	F28D1.10	gex-3	Signalling	116	K08H10.4	uda-1	Metabolism	196	F39H11.2	tlf-1	RNA Synthesis	276	F55D10.3	glit-1	Metabolism				
37	F29B9.8		Unknown	117	R10E12.2		Unknown	197	T23D8.2	tsp-7	Unknown	277	F56H1.5	ccpp-1	Unknown				
38	F29F11.4	twk-12	Small Molecule Transport	118	C17H12.1	dyci-1	Unknown	198	K07B1.3	ucp-4	Metabolism	278	F59A3.2		Unknown				
39	F32F2.1	uig-1	Signalling	119	H04M03.4	glf-1	Unknown	199	R12H7.1	unc-9	Cell Arch	279	F59A3.4		Unknown				
40	F35H10.4	vha-5	Small Molecule Transport	120	B0238.11		Unknown	200	Y37E11AR.6	vab-2	Unknown	280	K02E10.1		Cell Arch				
41	F37D6.6	tag-68	Unknown	121	B0410.3		Unknown	201	F20B6.2	vha-12	Metabolism	281	K05F1.6		Cell Arch				
42	F38E11.7	wrt-3	Neuro	122	B0464.4	bre-3	Unknown	202	C01G12.1	madf-5	Unknown	282	M01E11.1		Metabolism				
43	F44G4.8	dep-1	Signalling	123	C01C7.1	ark-1	Signalling	203	C03C10.4		Unknown	283	M01H9.1	trx-3	Metabolism				
44	F45H7.2	gei-1	Signalling	124	C01G12.1	madf-5	Unknown	204	C06G8.1	swt-3	Unknown	284	R05G6.10		Signalling				
45	F53B1.8		Small Molecule Transport	125	C05C8.7		Metabolism	205	C08H9.1		Proteases	285	T02C5.3	igcm-3	Signalling				
46	F54G8.3	ina-1	Cell Arch	126	C06E7.3	sams-4	Metabolism	206	C16C10.1		Metabolism	286	T02G5.13	mmaa-1	Unknown				
47	F58H1.5		Unknown	127	C06G8.1	swt-3	Unknown	207	C39E9.10		Signalling	287	T03F1.1		Unknown				
48	F59B2.2		Small Molecule Transport	128	C10B5.3		Unknown	208	C53B4.6	nstp-1	Unknown	288	T05A7.1		Unknown				
49	H03A11.1		Unknown	129	C13G3.1		Unknown	209	E02A10.4		Unknown	289	T19A5.1		Unknown				
50	H10E21.5		Unknown	130	C17H12.13	anat-1	Unknown	210	F11A5.9		Cell Arch	290	T24H7.3		Unknown				
51	H12C20.2	pms-2	DNA-Cell Cycle	131	C18A3.2		Unknown	211	F11A10.6		Unknown	291	W04B5.5		Signalling				
52	K02F2.6	ser-3	Neuro	132	C26B9.1		Unknown	212	F21G4.1		Proteases	292	W06B11.1	atat-2	Unknown				
53	K07G5.1	crml-1	Unknown	133	D1014.4		Unknown	213	F25H9.6		Metabolism	293	Y37E11B.6		Unknown				
54	K08E3.4		Cell Arch	134	F08C6.1	adt-2	Proteases	214	F28D1.9	acs-20	Metabolism	294	Y43H11AL.1		Unknown				
55	K08H10.7	rde-1	RNA Binding	135	F09E10.3	dhs-25	Metabolism	215	F36H2.1	tat-5	Small Molecule Transp	295	Y49G5B.1		Unknown				
56	M01F1.4		Unknown	136	F13H6.1		NA Binding	216	F37A8.5		Unknown	296	Y55B1AL.1		Unknown				
57	M110.5	dab-1	Signalling	137	F16B4.8	cdc-25.2	DNA-Cell Cycle	217	F44G4.8	dep-1	Signalling	297	Y59H11AR.2	catp-7	Unknown				
58	M28.6	lact-3	Unknown	138	F17C11.3	col-153	Collagen	218	F46G10.2		Unknown	298	Y97E10AR.2		Unknown				
59	R08C7.12		Unknown	139	F17C8.1	acy-1	Signalling	219	F53E4.1		Unknown	299	R11B5.1	tbc-12	Cell Arch				
60	R10E12.2		Unknown	140	F17E5.2		Metabolism	220	F55H2.7		Unknown	300	C33H5.19	tag-321	Unknown				
61	R13H8.1	daf-16	Transcription Factor	141	F18C5.10		Unknown	221	F56H11.2		Unknown	301	T14B1.2	aex-2	Neuro				
62	T03F6.4		Unknown	142	F20D1.1		Unknown	222	F59B2.2		Small Molecule Transp	302	C01C7.1	ark-1	Signalling				
63	T07F12.2		Unknown	143	F25B3.1	ehbp-1	Cell Arch	223	H13N06.2		Unknown	303	C23H4.1	cab-1	Neuro				
64	T13H5.6		Unknown	144	F25F2.2	cdh-4	Signalling	224	K08E3.4		Cell Arch	304	Y38F1A.5	cyd-1	DNA-Cell Cycle				
65	T22D1.8		Unknown	145	F26A1.6		Unknown	225	K08H10.4	uda-1	Metabolism	305	F23B12.9	egl-1	Unknown				
66	T23B12.6		Unknown	146	K08F8.2	atf-2	Transcription Factor	226	K09A9.6		Unknown	306	R10E9.1	msi-1	RNA Binding				
67	T24C4.6	zer-1	Unknown	147	T07F10.4	bus-19	Unknown	227	K09E9.2	erv-46	Unknown	307	C07D10.4	nas-7	Proteases				
68	T27F7.2	shc-2	Signalling	148	Y108G3AL.1	cul-3	Unknown	228	R03A10.4	nkat-3	Metabolism	308	E02H1.7	nhr-19	Transcription Factor				
69	T28B11.1		Degradation	149	Y65B4BL.5	acs-13	Unknown	229	R90.1		Signalling	309	ZK270.1	ptr-23	Signalling				
70	T28C12.4		Metabolism	150	F17C8.1	acy-1	Signalling	230	T04F3.2		Unknown	310	Y42H9B.2	rig-4	Unknown				
71	T28D6.4		Unknown	151	F08C6.1	adt-2	Proteases	231	T05H10.7		Unknown	311	K02F2.6	ser-3	Neuro				
72	T28F3.3	hke-4.1	Unknown	152	B0464.4	bre-3	Unknown	232	T13H5.1		Signalling	312	F23H12.1	snb-2	Cell Arch				
73	W01G7.1	daf-5	Unknown	153	F25F2.2	cdh-4	Signalling	233	T21B10.6	cutl-15	Unknown	313	ZC404.8	spn-4	RNA Binding				
74	W03G9.1	snf-1	Neuro	154	C07H6.5	cgh-1	RNA Binding	234	T21C9.4		Unknown	314	Y38C1AA.4	tcl-2	Unknown				
75	W10D5.1	mef-2	Transcription Factor	155	C01C10.1	clc-2	Unknown	235	T23F11.3	cdka-1	Unknown	315	T24A11.3	toh-1	Proteases				
76	Y14H12A.1		Unknown	156	F42G9.2	cyn-6	Metabolism	236	T24B8.4		Unknown	316	Y71A12B.4	trp-4	Unknown				
77	Y22D7AR.13	ser-4	Unknown	157	C01F6.4	fem-3	RNA Synthesis	237	T24B8.5		Unknown	317	F35H10.4	vha-5	Small Molecule Transport				
78	Y37E11AR.6	vab-2	Unknown	158	T07D1.4	fox-1	RNA Binding	238	W02A11.3	toe-4	Unknown	318	B0336.1	wrm-1	Signalling				
79	Y38F1A.5	cyd-1	DNA-Cell Cycle	159	C51F7.1	frm-7	Unknown	239	W09D6.5		Unknown	319	F38E11.7	wrt-3	Neuro				
80	Y40H4A.1	gar-3	Neuro	160	T22H6.6	gei-3	Chromatin	240	W09G3.6		Unknown	320	C13B4.1		Unknown				

mir-52 predicted target

mir-59 predicted target

mir-83 predicted target

mir-265 predicted target

mir-254 predicted target

mir-35-41 predicted target

Supplemental Table 2: 1st List of predicted miRNA(-) targets and predicted function.

Mir-83	Sequence	Gene
1	C23H4.1	cab-1
2	F25F2.2	cdh-4
3	F46C8.5	ceh-14
4	ZC64.3	ceh-18
5	T25F10.2	dbl-1
6	C56C10.13	dnj-8
7	K09G1.4	dop-2
8	C26D10.5	eff-1
9	F35D6.1	fem-1
10	F56A11.1	gex-2
11	C03E10.4	gly-20
12	C16E9.4	inx-1
13	C05D11.4	let-756
14	W06F12.1	lit-1
15	ZK112.2	ncl-1
16	F25G6.6	asns-1
17	T21D12.4	pat-6
18	T27B1.2	pat-9
19	T14F9.4	peb-1
20	W01C9.3	pqn-73
21	C48D5.2	ptp-1
22	F53G12.1	rab-11.1
23	K01G5.6	rib-2
24	F18C12.2	rme-8
25	F57C7.3	sdn-1
26	F15C11.1	sem-4
27	C14F5.5	sem-5
28	T10H9.4	snb-1
29	W03G9.1	snf-1
30	F21F8.10	str-135
31	K02E10.8	syg-1
32	F39C12.3	tsp-14
33	F41C6.1	unc-6
34	F14D12.2	unc-97
35	F09B9.2	unc-115
36	Y54E10A.9	vbh-1
37	Y53C12A.1	wee-1.3
38	B0344.2	wrt-9
39	F28F9.1	zag-1
40	C01G12.1	madf-5
41	C03C10.4	
42	C17E4.10	
43	C33A11.2	
44	F01D5.10	
45	F13E9.1	
46	F21A3.3	
47	F43D9.1	
48	H19N07.2	math-33
49	K09E9.2	erv-46
50	R07B1.9	
51	T04F3.2	
52	T07F10.4	bus-19
53	T23F11.3	cdka-1
54	T24B8.4	
55	W01F3.1	
56	Y11D7A.13	flh-3
57	Y38H6C.14	
58	Y41E3.1	
59	Y45F10D.2	
60	C04E6.7	
61	C06G1.4	ain-1
62	C16A3.2	
63	C34H3.1	tag-275
64	C42C1.4	
65	C45E5.4	
66	C53D5.1	
67	F42H10.3	
68	H18N23.2	
69	K02E10.1	
70	R05C11.3	mca-2
71	T28D9.7	del-10
72	W08A12.1	unc-132
73	Y34D9A.2	npr-23
74	Y73B6BL.6	sqd-1

miR-59	Sequence	Gene
75	F44E5.1	
76	H19N07.1	erfa-3
77	R107.5	
78	T21C9.3	del-6
79	T24B8.3	
80	W02B12.9	mfn-1
81	Y17G7B.20	
82	B0041.5	
83	C06A8.3	
84	C06E7.1	sams-3
85	C06E7.4	
86	C16A3.10	
87	C32F10.8	
88	D1009.1	acs-22
89	F13H6.1	
90	F21F3.6	
91	F23F12.12	
92	F35H10.10	
93	K07B1.8	
94	K08D12.6	
95	R151.2	
96	Y73B6BL.24	acp-6

miR-254	Sequence	Gene
97	B0464.4	bre-3
98	F11C7.4	crb-1
99	F11A1.3	daf-12
100	C43C3.3	dyf-7
101	C02C6.1	dyn-1
102	T01H8.5	gon-2
103	F13D11.2	hbl-1
104	C49H3.10	xpo-3
105	R07E4.6	kin-2
106	F58H12.1	kin-29
107	F36H1.4	lin-3
108	T12F5.4	lin-59
109	T24A11.1	mtm-3
110	C10G8.5	ncx-2
111	T09A12.4	nhr-66
112	D2005.2	nlp-8
113	F35C8.6	pfn-2
114	E01H11.1	pkc-2
115	F55A12.3	ppk-1
116	C09D8.1	ptp-3
117	F53G12.1	rab-11.1
118	C05B5.7	rgs-1
119	C18F3.2	sax-7
120	H20J18.1	scd-1
121	F57C7.3	sdn-1
122	F35G12.3	sel-5
123	W03G9.1	snf-1
124	T01G5.3	srj-27
125	F35B12.1	srx-84
126	R03E1.1	sym-4
127	F44A2.1	tag-153
128	R13A1.2	kcc-1
129	C07A9.3	tlk-1
130	T23G4.1	tlp-1
131	F39C12.3	tsp-14
132	C47E12.5	uba-1
133	Y54E10A.9	vbh-1
134	C04C11.2	arrd-25
135	C05C12.6	
136	C07A9.9	
137	C13G3.1	
138	C17G1.4	nra-3
139	C34E11.2	
140	DH11.5	
141	F09B12.3	
142	F14B4.3	rpoa-2
143	F17C11.9	eef-1G
144	F31D4.8	
145	F39B2.1	
146	F55H2.2	vha-14
147	F57G12.1	
148	T05D4.1	aldo-1
149	T05H10.7	
150	T12G3.2	
151	T14B1.1	
152	T24B8.4	
153	T25E12.4	dkf-2
154	W08D2.5	catp-6
155	Y11D7A.13	flh-3
156	Y45F10D.2	
157	Y49E10.11	tat-1
158	Y57A10B.1	42068
159	Y76A2B.5	
160	ZK1037.3	srt-22
161	B0336.3	
162	C10G6.1	egal-1
163	C13F10.4	
164	C26F1.6	frpr-3
165	C30B5.7	
166	C35A11.1	dmsr-7
167	C42C1.4	
168	C43G2.1	paqr-1
169	C48A7.2	pitr-1
170	CC8.2	
171	F21A9.2	
172	F26F12.3	
173	F31A3.5	
174	F32B5.6	
175	F52G3.1	
176	F55C12.1	
177	F56C9.8	
178	H34C03.2	
179	K07H8.2	
180	R01B10.5	jamp-1
181	R12E2.2	
182	T07E3.6	pdf-1
183	T07F12.2	
184	T22F3.3	
185	T24H7.3	
186	T26A5.5	jhdm-1
187	W02C12.3	hlh-30
188	Y39G10AR.18	
189	Y51F10.7	
190	Y59E9AL.4	
191	Y71H2AM.5	
192	ZK418.7	

miR-265	Sequence	Gene
193	C01G6.5	
194	C18E9.2	
195	C34B4.2	
196	C37E2.1	idhb-1
197	F26A3.4	
198	F45D3.3	
199	F47G4.4	
200	F58E10.1	ric-7
201	H36L18.2	
202	T21C9.3	del-6
203	T27D12.1	
204	T28F4.5	
205	Y39A1A.9	
206	ZK131.11	
207	B0361.9	
208	C30G4.7	
209	C48A7.2	pitr-1
210	D1009.1	acs-22
211	F35H10.10	
212	F36D4.5	
213	K04F10.3	
214	K09H9.7	
215	R04E5.8	
216	R05D3.2	
217	T27E4.7	
218	ZK370.4	

miR-52	Sequence	Gene
219	F31A3.1	abu-3
220	F17C8.1	acy-1
221	F08C6.1	adt-2
222	F25F2.2	cdh-4
223	F56D1.4	clr-1
224	F11C7.4	crb-1
225	K10B4.6	cwn-1
226	T25F10.2	dbl-1
227	C18D1.1	die-1
228	F54D5.8	dnj-13
229	C26D10.5	eff-1
230	C43F9.8	efn-2
231	C08C3.1	egl-5
232	W08D2.1	egl-20
233	Y40H4A.1	gar-3
234	F28D1.10	gex-3
235	ZC308.1	gld-2
236	B0416.6	gly-13
237	C03E10.4	gly-20
238	C18A3.8	hlh-14
239	C16E9.4	inx-1
240	K11D9.1	klp-7
241	C01G8.9	let-526
242	C05D11.4	let-756
243	F36H1.4	lin-3
244	Y71F9B.5	lin-17
245	M04B2.1	mep-1
246	R07E4.4	mig-23
247	T20B12.6	mml-1
248	F46G10.6	mxl-3
249	K08E5.2	nac-3
250	C07D10.4	nas-7
251	C02B4.2	nhr-17
252	E02H1.7	nhr-19
253	C01H6.5	nhr-23
254	C07A12.3	nhr-35
255	C45E5.6	nhr-46
256	T09A12.4	nhr-66
257	F20B10.1	nlr-1
258	C07G1.3	pct-1
259	T14F9.4	peb-1
260	C09D8.1	ptp-3
261	C54A12.1	ptr-6
262	Y38F1A.3	ptr-18
263	C18F3.2	sax-7
264	Y22D7AR.13	ser-4
265	T19E7.2	skn-1
266	Y51A2D.19	slo-1
267	F35G12.8	smc-4
268	Y22D7AL.8	sms-3
269	AC7.2	soc-2
270	C09H6.1	spr-4
271	F54C9.2	stc-1
272	F12F6.5	srgp-1
273	B0222.4	tag-38
274	F37D6.6	tag-68
275	T23G4.1	tlp-1
276	F29F11.4	twk-12
277	F25H2.8	ubc-25
278	Y37E11AR.6	vab-2
279	F08B1.1	vhp-1
280	C46C2.1	wnk-1
281	F38E11.7	wrt-3
282	C08B11.1	zyg-11
283	B0024.14	crm-1
284	B0334.6	
285	B0491.1	
286	C03C10.4	
287	C06G8.1	swt-3
288	C08B11.3	swsn-7
289	C18E9.7	
290	C29A12.4	nrx-1
291	C35C5.6	
292	C54G10.4	
293	F01D5.10	
294	F11E6.3	
295	F11E6.8	
296	F14E5.2	
297	F16A11.1	
298	F21A3.3	
299	F25D7.2	tag-353
300	F26A3.4	
301	F26E4.11	hrdl-1
302	F31C3.3	
303	F40F12.5	cyld-1
304	F44G4.8	dep-1
305	F45D3.3	
306	F45D3.4	
307	F46C5.1	
308	F46G10.2	
309	F52D10.2	
310	F58H1.5	
311	F59B2.13	
312	H03A11.1	
313	H27A22.1	
314	K01B6.1	fozi-1
315	K01D12.6	
316	K07A12.2	egg-6
317	K08E4.3	
318	K10D6.4	
319	M88.5	zbp-1
320	R08D7.6	pde-2
321	R10E12.2	
322	R13H4.5	
323	R107.5	
324	T02E9.1	npr-25
325	T04F3.2	
326	T05B9.2	
327	T07F10.1	
328	T12G3.2	
329	T13H5.6	
330	T24B8.4	
331	T24C2.5	
332	W01F3.1	
333	W03C9.5	
334	W04G3.2	lpr-5
335	Y48C3A.4	ztf-22
336	Y57G11C.31	
337	Y64G10A.6	
338	ZC84.3	cls-3
339	ZC376.7	atfs-1
340	ZK131.11	
341	ZK673.2	
342	ZK1321.2	shk-1
343	D2023.1	
344	B0034.1	
345	B0410.2	vang-1
346	B0410.3	
347	B0454.6	
348	C01F1.6	
349	C04D8.1	pac-1
350	C05D11.7	
351	C06E1.3	doxa-1
352	C06G1.1	
353	C16D9.6	
354	C23H3.2	
355	C26B9.1	
356	C31H1.8	
357	C49H3.9	
358	C53D5.1	
359	C56C10.11	
360	C56E6.6	lron-15
361	CC8.2	
362	F13H8.11	
363	F18A12.8	nep-11
364	F21A9.2	
365	F21F3.6	
366	F41C3.2	
367	F44A2.3	
368	F49H12.6	acl-4
369	F52F10.2	
370	F53B1.2	
371	F57F10.1	abts-3
372	F59G1.1	cgt-3
373	K03E6.7	
374	M01B12.4	
375	R01B10.5	jamp-1
376	R05G6.10	
377	R12C12.3	frpr-16
378	T01A4.1	gcy-28
379	T06F4.1	
380	T07F12.2	
381	T19A5.1	
382	T27E4.7	
383	W02C12.3	hlh-30
384	Y39A3B.2	lgc-42
385	Y58A7A.6	glb-32
386	Y61A9LA.1	
387	Y65B4BL.5	acs-13
388	Y67D8C.9	
389	Y71D11A.5	lgc-46
390	Y71H2AL.2	
391	Y73E7A.3	
392	Y102A11A.8	igcm-4
393	ZK154.6	
394	ZK355.2	
395	ZK418.7	
396	ZK682.2	

Enriched in Dicer Microarray and Gut IP

Enriched in Gut IP

Enriched in Dicer Microarray

mir-83 from mirWIP

mir-59 from tarbase

mir-254 from mirWIP and tarbase!

mir-265 from tarbase

mir-52 from mirWIP and tarbase!

Supplemental Table 3: 2nd list of predicted miRNA targets and associated Dicer microarray and Gut IP enrichment.