

GENETICS

Supporting Information

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**The Conserved miR-51 microRNA Family Is Redundantly Required
for Embryonic Development and Pharynx Attachment
in *Caenorhabditis elegans***

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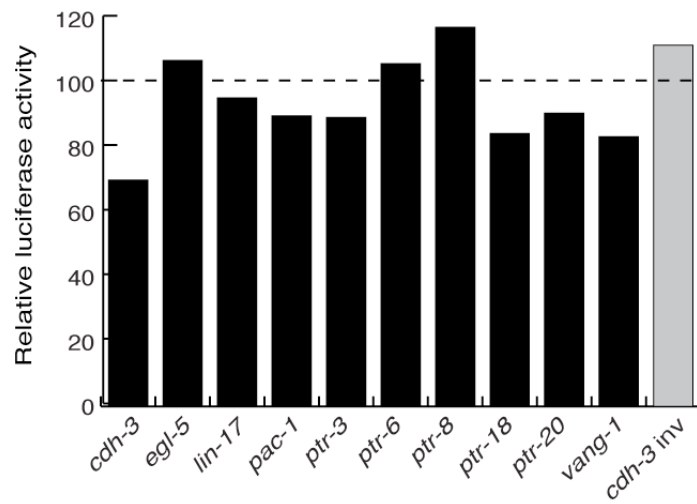


FIGURE S1.—miR-52 can regulate the *cdh-3* 3'UTR in mammalian cells. Luciferase reporter assay. Full length 3'UTRs of predicted miR-51 family target genes were cloned into a modified psiCHECK-2 dual luciferase assay vector (Promega) and transfected into HeLa cells (see Materials and Methods). Luciferase activity shown is relative to transfection of a miR-52 mimic as compared to a control miRNA mimic. Experiments were done in triplicates. *cdh-3 inv*, antisense *cdh-3* 3'UTR.

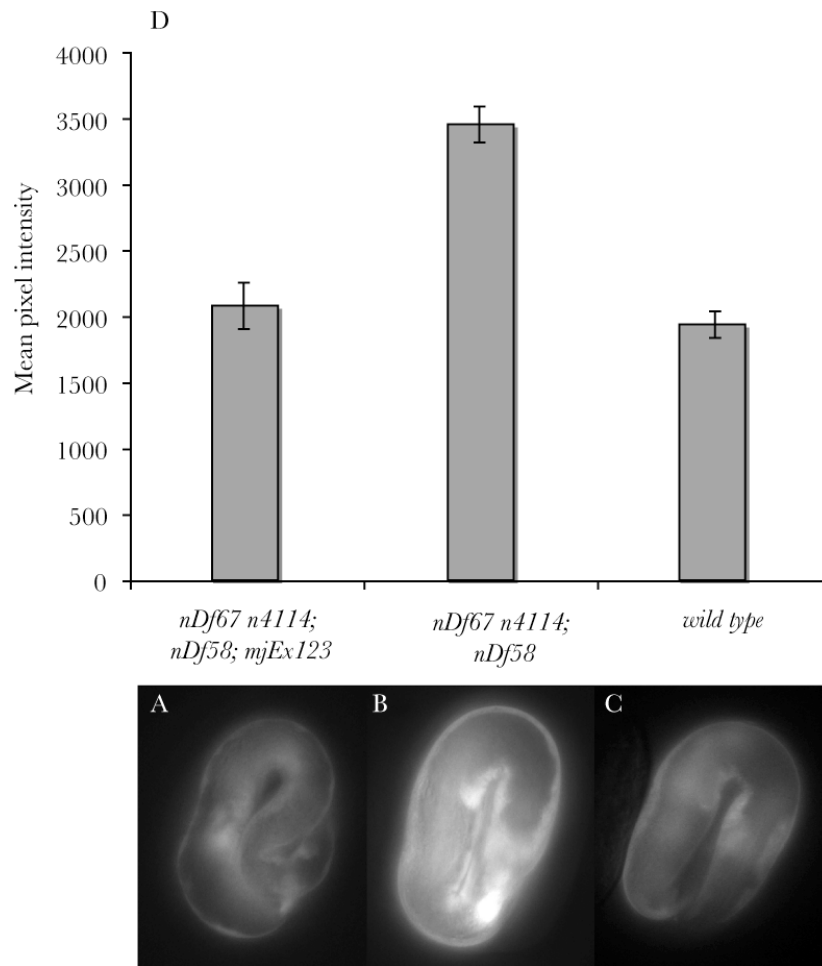


FIGURE S2.—Endogenous levels of miR-51 family miRNAs are able to regulate a sensor transgene containing *cdh-3* 3'UTR sequences. (A-C) GFP was expressed under the *col-10* promoter and the *cdh-3* wild-type 3'UTR. The transgene was expressed at a lower level in the presence of a miR-52-expressing rescue array (A) or in the wild-type background (C) than in the absence of any miR-51 family miRNAs (B). D) Quantification of mean pixel intensity in >30 animals. Bars represent the standard error of the mean.

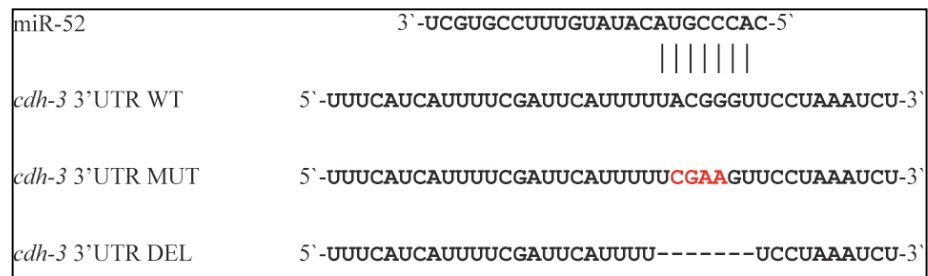


FIGURE S3.—Mutations introduced into the full-length 3'UTR of *cdh-3*. 40 base pairs of the 3'UTR of *cdh-3* encompassing the predicted miR-52 target site is shown. Mutations introduced into the 3'UTR should abolish either some (MUT) or all (DEL) base pairing between the miRNA seed and the 3'UTR.

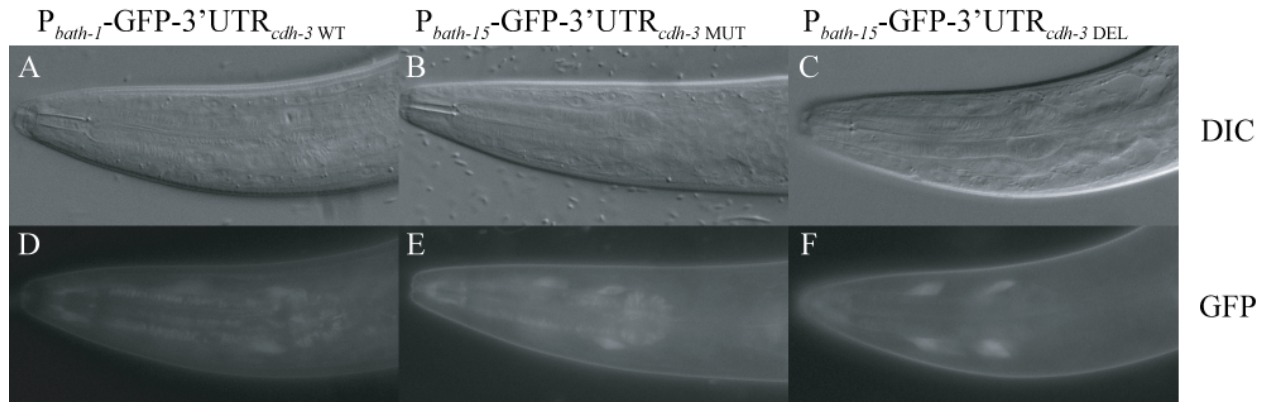


FIGURE S4.—Regulation of GFP::CDH-3 levels in the arcade cells requires the miR-51 family binding site. GFP was expressed under the *bath-15* promoter in the arcade cells (D-F) and compared between constructs with a *cdh-3* 3'UTR that was either wild-type (WT) (A,D), MUT (B,E) or DEL (C-F). For molecular identities of *cdh-3* 3'UTR mutations refer to Fig. S3. Larvae were imaged at the L4 stage.

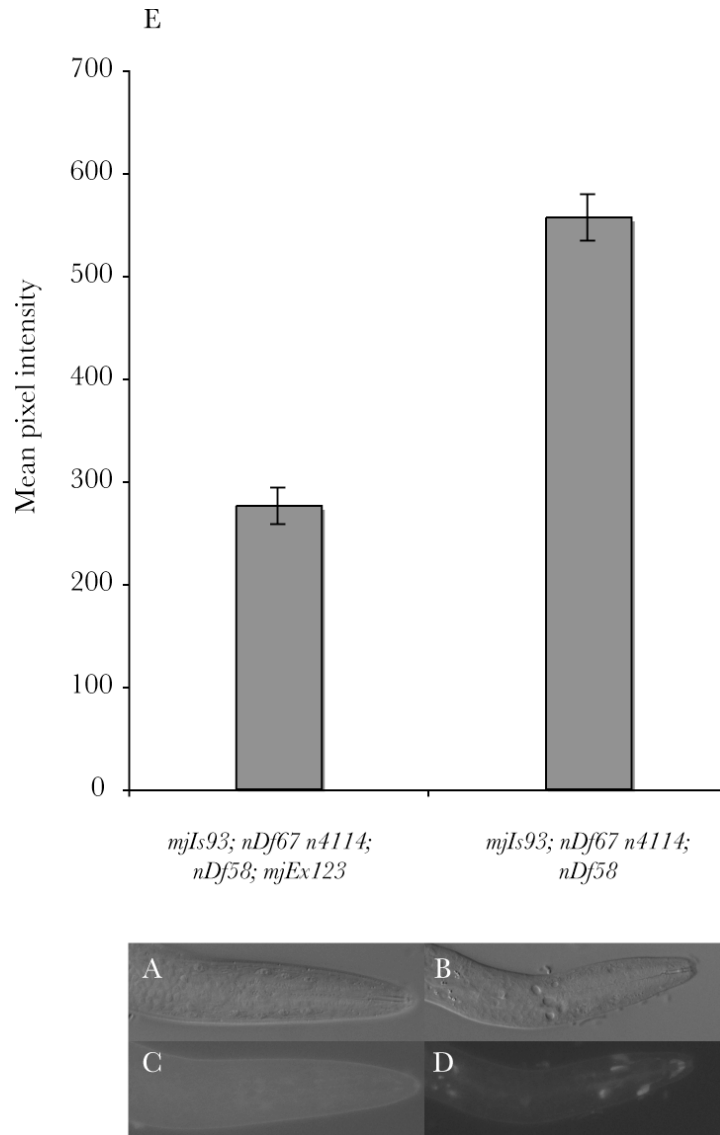


FIGURE S5.—An arcade driven reporter is upregulated in *mir-51* family mutants. (A-D) In a single copy transgene *mjIs93* GFP is expressed in the arcade cells under the *bath-15* promoter from the L1 stage. The majority of *mir-51* family mutant embryos are Pun and do not express this marker prior to their death (Fig. 2). A minority of *mir-51* family mutant embryos are not Pun (B) and do express this arcade marker. GFP under the control of the wild-type *cdh-3* 3'UTR is overexpressed in non-Pun *mir-51* family mutant embryos (D) as compared to non-Pun rescued embryos (C). (E) Mean pixel intensities of the visible arcade cell bodies were determined in 10 animals. Where arcade cell bodies were not visible, pixel intensity was measured between the mouth and the anterior pharyngeal bulb in a position consistent with the arcade cell body position (i.e. outside the pharyngeal basement membrane) at least 3 times per animal. Background fluorescence of the slide was subtracted. Error bars represent standard error of the mean.

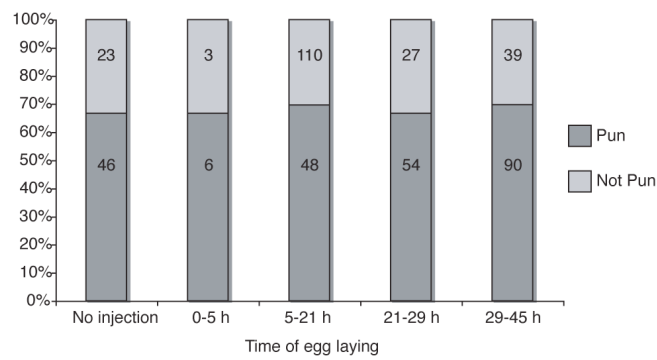


FIGURE S6.—Microinjection of dsRNA against *cdh-3* does not suppress the unattached pharynx (Pun) phenotype. Eight microinjected LA *nDf67 n4114; nDf58; mjEx123* animals were allowed to lay progeny for the indicated time periods. Progeny lacking the *mjEx123* rescuing transgene were identified through the lack of DLG-1::mCHERRY expression and were scored for a Pun phenotype 24 hrs later. No difference was observed in the frequency of Pun phenotype.

TABLE S1
Strains used in this study

Strain	Genotype
N2	+
SX511	<i>mir-51(n4473) IV</i>
SX512	<i>mir-52(n4114) IV</i>
SX510	<i>mir-52(n4100) IV</i>
SX513	<i>mir-53(n4113) IV</i>
SX514	<i>mir-54 mir-55 mir-56(nDf58) X</i>
SX515	<i>mir-51 mir-53(nDf67) IV</i>
SX138	<i>mir-51(n4473) mir-52(n4114) IV</i>
SX16	<i>mir-53(n4113) mir-52(n4100) IV</i>
SX516	<i>mir-53(n4113) mir-52(n4114) IV</i>
SX517	<i>mir-51 mir-53(nDf67) mir-52 (n4114) IV</i>
SX48	<i>mir-51(n4473) IV; mir-54 mir-55 mir-56(nDf58) X</i>
SX96	<i>mir-52(n4114) IV; mir-54 mir-55 mir-56(nDf58) X</i>
SX27	<i>mir-53(n4113) IV; mir-54 mir-55 mir-56(nDf58) X</i>
SX625	<i>mir-51 mir-53(nDf67) IV; mir-54 mir-55 mir-56(nDf58) X</i>
SX111	<i>mir-53(n4113) mir-52(n4100) IV; mir-54 mir-55 mir-56(nDf58) X</i>
SX173	<i>mir-51(n4473) mir-52(n4114) / mir-51 (n4473) <i>unc-26(e205) dpy-4(e1166) IV; mir-54 mir-55 mir-56 (nDf58) X</i></i>
SX947	<i>mir-51(n4473) mir-52(n4114) IV; mir-54 mir-55 mir-56(nDf58) X; mjEx123 (miR-52 rescue)</i> [miR-52 rescue contains <i>P_{alg-1}::DLG-1-MCHERRY</i>]
SX123	<i>mir-51 mir-53(nDf67) mir-52(n4114) / mir-51 mir-53(nDf67) <i>unc-26(e205) dpy-4(e1166) IV; mir-54 mir-55 mir-56 (nDf58) X</i></i>
SX356	<i>mir-51 mir-53(nDf67) mir-52(n4114) IV; mir-54 mir-55 mir-56(nDf58) X; mjEx123 (miR-52 rescue)</i>
MT1642	<i>lin-15AB(n765ts) X</i>
SX860	<i>lin-15AB(n765ts) X; mjIs60 (P_{mir-51}-GFP, <i>lin-15B(+)</i>)</i>
SX615	<i>mjIs39 (P_{mir-52}-GFP, <i>lin-15B(+)</i>)</i>
SX509	<i>mjIs37 (P_{mir-53}-GFP, <i>lin-15B(+)</i>)</i>
SX59	<i>lin-15AB(n765ts) X; mjEx12 (P_{mir-54-56}-GFP, <i>lin-15B(+)</i>)</i>
EG4322	<i>ttT5605 II; unc-119(ed3)</i>
SX1003	<i>mjIs93 II (P_{bath-15}-GFP-3'UTR_{cdh-3} WT, <i>cbr-unc-119(+)</i>); <i>unc-119(ed3) III</i></i>
SX1005	<i>mjIs95 II (P_{bath-15}-GFP-3'UTR_{cdh-3} WT, <i>cbr-unc-119(+)</i>); <i>unc-119(ed3) III</i></i>
SX1007	<i>mjIs97 II (P_{bath-15}-GFP-3'UTR_{cdh-3} MUT, <i>cbr-unc-119(+)</i>); <i>unc-119(ed3) III</i></i>
SX1008	<i>mjIs98 II (P_{bath-15}-GFP-3'UTR_{cdh-3} DEL, <i>cbr-unc-119(+)</i>); <i>unc-119(ed3) III</i></i>
SX1009	<i>mjIs99 II (P_{bath-15}-GFP-3'UTR_{cdh-3} DEL, <i>cbr-unc-119(+)</i>); <i>unc-119(ed3) III</i></i>
SX669	<i>mir-51 mir-53(nDf67) mir-52(n4114); mir-54 mir-55 mir-56(nDf58) X; mjEx123 (miR-52 rescue); pxIs10 (P_{pha-4}-GFP-PM, <i>rol-6(su1006)</i>)</i>

SX1016	<i>mir-51 mir-53(nDf67) mir-52(n4114); mir-54 mir-55 mir-56(nDf58) X; mjEx123</i> (miR-52 rescue); <i>mjEx288</i> (CDH-3(++))
SX1017	<i>mir-51 mir-53(nDf67) mir-52(n4114); mir-54 mir-55 mir-56(nDf58) X; mjEx123</i> (miR-52 rescue); <i>mjEx289</i> (CDH-3(++))
SX1048	<i>mjIs93 [P_{bath-15}-GFP-3'UTR_{cdh-3} WT, <i>unc-119(+)</i>] II ; mir-51 mir-53(nDf67) mir-52(n4114) IV; mir-54 mir-55 mir-56(nDf58) X ; mjEx123</i> (miR-52 rescue)
SX596	<i>mir-51 mir-53(nDf67) mir-52(n4114); mir-54 mir-55 mir-56(nDf58) X; mjEx123</i> (miR-52 rescue); <i>mjEx177</i> (P _{col-10} -GFP-3'UTR _{cdh-3} WT, P _{myo-2} -MCHERRY-3'UTR _{unc-54})
SX597	<i>mir-51 mir-53(nDf67) mir-52(n4114); mir-54 mir-55 mir-56(nDf58) X; mjEx123</i> (miR-52 rescue); <i>mjEx178</i> (P _{col-10} -GFP-3'UTR _{cdh-3} WT, P _{myo-2} -MCHERRY-3'UTR _{unc-54})
SX598	<i>mir-51 mir-53(nDf67) mir-52(n4114); mir-54 mir-55 mir-56(nDf58) X; mjEx123</i> (miR-52 rescue); <i>mjEx179</i> (P _{col-10} -GFP-3'UTR _{cdh-3} WT, P _{myo-2} -MCHERRY-3'UTR _{unc-54})
SX689	<i>mir-51 mir-53(nDf67) mir-52(n4114); mir-54 mir-55 mir-56(nDf58) X; mjEx123</i> (miR-52 rescue); <i>mjEx187</i> (P _{col-10} -GFP-3'UTR _{cdh-3} MUT, P _{myo-2} -MCHERRY-3'UTR _{unc-54})
SX690	<i>mir-51 mir-53(nDf67) mir-52(n4114); mir-54 mir-55 mir-56(nDf58) X; mjEx123</i> (miR-52 rescue); <i>mjEx188</i> (P _{col-10} -GFP-3'UTR _{cdh-3} MUT, P _{myo-2} -MCHERRY-3'UTR _{unc-54})
SX691	<i>mir-51 mir-53(nDf67) mir-52(n4114); mir-54 mir-55 mir-56(nDf58) X; mjEx123</i> (miR-52 rescue); <i>mjEx189</i> (P _{col-10} -GFP-3'UTR _{cdh-3} MUT, P _{myo-2} -MCHERRY-3'UTR _{unc-54})
SX692	<i>mir-51 mir-53(nDf67) mir-52(n4114); mir-54 mir-55 mir-56(nDf58) X; mjEx123</i> (miR-52 rescue); <i>mjEx190</i> (P _{col-10} -GFP-3'UTR _{cdh-3} MUT, P _{myo-2} -MCHERRY-3'UTR _{unc-54})
SX693	<i>mir-51 mir-53(nDf67) mir-52(n4114); mir-54 mir-55 mir-56(nDf58) X; mjEx123</i> (miR-52 rescue); <i>mjEx191</i> (P _{col-10} -GFP-3'UTR _{cdh-3} MUT, P _{myo-2} -MCHERRY-3'UTR _{unc-54})
SX1117	<i>mir-51 mir-53(nDf67) mir-52(n4114); mir-54 mir-55 mir-56(nDf58) X; mjEx123</i> (miR-52 rescue); <i>mjEx298</i> (P _{pha-4} - <i>mir-52-3'UTR_{unc-54}</i> , P _{pha-4} -HIS-58-GFP-3'UTR _{unc-54})
SX1118	<i>mir-51 mir-53(nDf67) mir-52(n4114); mir-54 mir-55 mir-56(nDf58) X; mjEx123</i> (miR-52 rescue); <i>mjEx299</i> (P _{pha-4} - <i>mir-52-3'UTR_{unc-54}</i> , P _{pha-4} -HIS-58-GFP-3'UTR _{unc-54})
SX1119	<i>mir-51 mir-53(nDf67) mir-52(n4114); mir-54 mir-55 mir-56(nDf58) X; mjEx123</i> (miR-52 rescue); <i>mjEx300</i> (P _{pha-4} - <i>mir-52-3'UTR_{unc-54}</i> , P _{pha-4} -HIS-58-GFP-3'UTR _{unc-54})
SX1120	<i>mir-51 mir-53(nDf67) mir-52(n4114); mir-54 mir-55 mir-56(nDf58) X; mjEx123</i> (miR-52 rescue); <i>mjEx301</i> (P _{pha-4} - <i>mir-52-3'UTR_{unc-54}</i> , P _{pha-4} -HIS-58-GFP-3'UTR _{unc-54})
SX1121	<i>mir-51 mir-53(nDf67) mir-52(n4114); mir-54 mir-55 mir-56(nDf58) X; mjEx123</i> (miR-52 rescue); <i>mjEx302</i> (P _{pha-4} - <i>mir-52-3'UTR_{unc-54}</i> , P _{pha-4} -HIS-58-GFP-3'UTR _{unc-54})
SX704	<i>mir-51 mir-53(nDf67) mir-52(n4114); mir-54 mir-55 mir-56(nDf58) X; mjEx123</i> (miR-52 rescue); <i>mjEx301</i> (P _{pha-4} -HIS-58-GFP-3'UTR _{unc-54})
SX1284	<i>mjEx178</i> (P _{col-10} -GFP-3'UTR _{cdh-3} WT, P _{myo-2} -MCHERRY-3'UTR _{unc-54})
SX1285	<i>mjEx179</i> (P _{col-10} -GFP-3'UTR _{cdh-3} WT, P _{myo-2} -MCHERRY-3'UTR _{unc-54})

TABLE S2**DNA constructs used in this study**

Construct	Vector	Contents	Cloning	Size (bp)	Start	End	LG	Strand	Template
miRNA reporters									
miR-51::GFP	pPD95.79	miR-51 promoter	Sall/BamH1	1354	11026083	11027436	IV	-	N2 genomic DNA
miR-52::GFP	pPD95.79	miR-52 promoter	Sall/BamH1	2916	14031059	14033974	IV	+	N2 genomic DNA
miR-53::GFP	pPD95.79	miR-53 promoter	Sall/BamH1	767	11027662	11028428	IV	-	N2 genomic DNA
miR-54-56::GFP	pPD95.79	miR-54-56 promoter	Sall/HindIII	2889	13145016	13147904	X	-	N2 genomic DNA
miRNA rescues									
miR-51 rescue	TOPO pCR2.1	miR-51 locus	TA cloning	2011	11025613	11027623	IV	-	N2 genomic DNA
miR-52 rescue	TOPO pCR2.1	miR-52 locus	TA cloning	3337	14031848	14035184	IV	+	N2 genomic DNA
miR-53 rescue	TOPO pCR2.1	miR-53 locus	TA cloning	2211	11026342	11028552	IV	-	N2 genomic DNA
miR-54-56 rescue	TOPO pCR2.1	miR-54-56 locus	TA cloning	3697	13143492	13147188	X	-	N2 genomic DNA
miR-52 (promoterless)		miR-52 locus fragment	MluI	172	14033952	14034123	IV	+	N2 genomic DNA
Gateway® promoters									
Pbath-15	pDONR P4P1R	bath-15 promoter	Multisite Gateway®	1126	7775944	7777069	III	-	N2 genomic DNA
Pcol-10	pDONR P4P1R	col-10 promoter	Multisite Gateway®	1353	9165265	9166617	V	-	N2 genomic DNA
Gateway® gene									
gfp	pDONR 221	gfp	Multisite Gateway®	1002	2	1003			pPD95.75
mir-52 (promoterless)	pDONR 221	miR-52 locus	Multisite Gateway®	172	14033952	14034123	IV	+	N2 genomic DNA

Gateway® 3'UTRs

cdh-3 3'UTR WT	pDONR P2RP3	cdh-3 3'UTR WT	Multisite Gateway®	418	7740905	7741322	III	-	WRM066aH 05
cdh-3 3'UTR MUT	pDONR P2RP3	cdh-3 3'UTR MUT	Multisite Gateway®	419	7740905	7741323	III	-	WRM066aH 05
cdh-3 3'UTR DEL	pDONR P2RP3	cdh-3 3'UTR DEL	Multisite Gateway®	419	7740905	7741323	III	-	WRM066aH 05
unc-54 3'UTR	pDONR P2RP3	unc-54 3'UTR	Multisite Gateway®	1159	748	1906			pPD95.75

Assembled
constructs

myo-2:: mcherry::unc-54 pGFP-N	pDEST R4- R3 pJH4.52	pha-4::HIS-58- GFP::unc-54	Multisite Gateway®						
col-10::gfp::cdh-3 WT	pDEST R4- R3		Multisite Gateway®						
col-10::gfp::cdh-3 MUT	pDEST R4- R3		Multisite Gateway®						
bath-15::gfp::cdh-3 WT	pDEST R4- R3		Multisite Gateway®						
bath-15::gfp::cdh-3 MUT	pDEST R4- R3		Multisite Gateway®						
bath-15::gfp::cdh-3 DEL	pDEST R4- R3		Multisite Gateway®						
pha-4:: mir-52::unc-54	pJH4.52		pGFP-N was digested with MluI and a promoterless mir-52 fragment was inserted in the correct orientation						

Luciferase assay

pEM393	Psicheck II (Promega)	Death cassette	Psicheck II						pDEST 14 (Invitrogen)
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pEM394	Psicheck II (Promega)	Death cassette	and PCR product were						pDEST 14 (Invitrogen)
pEM395	Psicheck II (Promega)	Death cassette	digested with XbaI or						pDEST 14 (Invitrogen)
pEM396	Psicheck II (Promega)	Death cassette	XhoI. Death cassette was inserted into the Firefly or Renilla 3'UTR						pDEST 14 (Invitrogen)

Luciferase 3'UTRs

pEM393 cdh-3	pEM393	cdh-3 3'UTR WT Gateway®	419	7740905	7741323	III	-	N2 genomic DNA
pEM393 egl-5	pEM393	egl-5 3'UTR WT Gateway®	780	7816270	7817049	III	+	N2 genomic DNA
pEM393 lin-17	pEM393	lin-17 3'UTR WT Gateway®	230	2717357	2717586	I	+	N2 genomic DNA
pEM393 pac-1	pEM393	pac-1 3'UTR WT Gateway®	478	8518318	8518795	III	+	N2 genomic DNA
pEM393 ptr-3	pEM393	ptr-3 3'UTR WT Gateway®	960	361678	362637	II	-	N2 genomic DNA
pEM393 ptr-6	pEM393	ptr-6 3'UTR WT Gateway®	702	5241842	5242543	II	-	N2 genomic DNA
pEM393 ptr-8	pEM393	ptr-8 3'UTR WT Gateway®	598	10893081	10893678	II	+	N2 genomic DNA
pEM393 ptr-18	pEM393	ptr-18 3'UTR WT Gateway®	548	12977803	12978350	II	+	N2 genomic DNA
pEM393 ptr-20	pEM393	ptr-20 3'UTR WT Gateway®	480	15163375	15163854	II	+	N2 genomic DNA
pEM393 vang-1	pEM393	vang-1 3'UTR WT Gateway®	300	2890296	2890595	X	+	N2 genomic DNA
pEM394 cdh-3 inv	pEM394	cdh-3 3'UTR WT Gateway®	419	7740905	7741323	III	-	N2 genomic DNA
