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Supplemental Experimental Procedures

Detection of Deletion Alleles using Poison Primers

Poison primers were designed to be complementary to the sequences that correspond to the ~22 nt mature microRNA. In the first round of PCR, two products are produced from the wild-type allele: the external forward and external reverse primers generate a full-length product and the external forward and the poison primers generate shorter “poison” product. The shorter “poison” product is amplified more efficiently and thereby effectively competes with the amplification of full-length product, resulting in a reduced level of full-length product. A deletion allele that removes the microRNA sequence and therefore removes the poison primer binding site generates only a single “deletion” product from the external primers. In the second round of PCR, two internal primers designed just inside of the external primers amplify the full-length product, but not the shorter “poison” product, from the wild-type allele and the single “deletion” product from the deletion allele. Because the production of the full-length wild-type product was attenuated by competition from the “poison” primer in the first round of PCR, its production is correspondingly attenuated in the second round of PCR. This gives the “deletion” product an advantage in the second round of PCR and thus enhances its detection.

Supplemental Figure Legends

Supplemental Figure S1. *mir-48*, *mir-84* and *mir-241* and *lin-28* function in parallel pathways.
(A-F) Fluorescent micrographs of (A,D) wild-type, (B,E) *mir-48 mir-241; mir-84* mutant animals and (C,F) *lin-4* animals carrying the *lin-28::gfp::lin-28*-containing

transgene *maIs108.* Larval stage and genotype are indicated for eah image. All fluorescent images were taken with identical exposure times.

(A-C) *lin-28::gfp::lin-28* expression is observed at the L1 stage in multiple cell types, including seam cells and neurons (n).

(B, D) *lin-28::gfp::lin-28* expression is undetectable at the L3-stage in a (B) wild-type and in a (D) *mir-48 mir-241; mir-84* mutant animal.

(F) *lin-28::gfp::lin-28* expression is elevated in an L3-stage *lin-4* mutant animal.

(A'-F') Corresponding Nomarski DIC images for images shown in A-F. Arrowheads indicate seam cell nuclei.

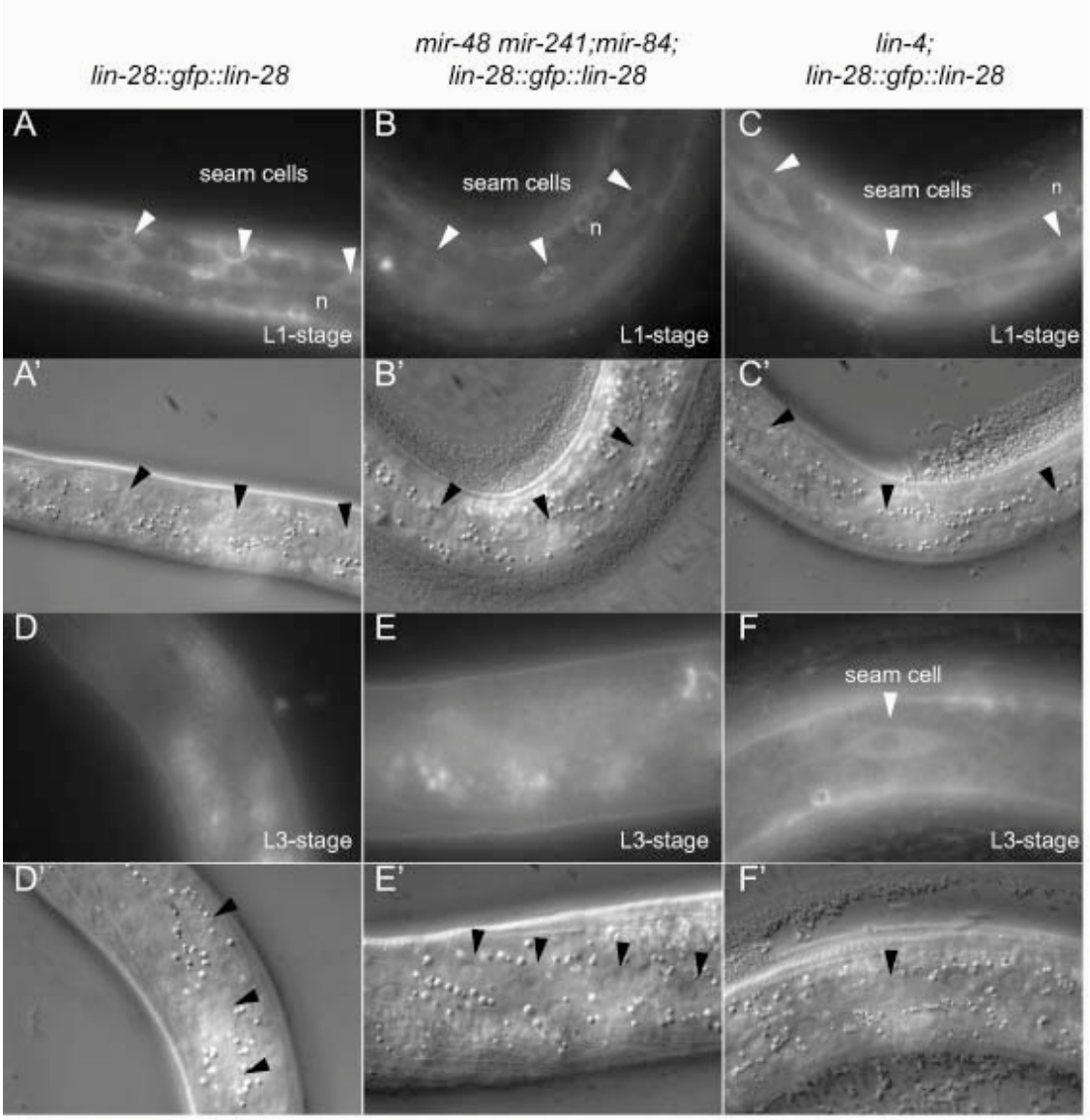

Table S1. List of sequences of primers used in identification of microRNA deletion strains

Primer Name	microRNA	Primer Position[a]	Sequence
SAM 134	*mir-48*	F1	5'-CAGAATTAACTTCCAATTTCAGAGG-3'
SAM 135	*mir-48*	F2	5'-TCGCCTGAATTGATGTTGG-3'
SAM 136	*mir-48*	R1	5'-TCAACACCTGCGTTTTATGC-3'
SAM 137	*mir-48*	R2	5'-TGCGAGAGACCTGGATTAGC-3'
SAM 139	*mir-48*	Poison	5'-CGGTATCTCACATCCACCAGCC-3'
SAM 29	*mir-84*	F1	5'-CGGCTCTTGAAAAAGTTGAC-3'
SAM 30	*mir-84*	F2	5'-GCAACGGGAAGCTCTGTTAC-3'
SAM 32	*mir-84*	R1	5'-AGCTTCACCTTAAATTTCAAAGTATC-3'
SAM 33	*mir-84*	R2	5'-AAGTATCATTCAGCTTCAATTTTGTC-3'
SAM 36	*mir-84*	Poison	5'-ATTGTAGACTGTCTATAATG-3'
SAM 373	*mir-241*	F1	5'-GAGCTGGCAAATAAACTGAAAC-3'
SAM 374	*mir-241*	F2	5'-GAGCAAATTCCTTCCTGTGC-3'
SAM 376	*mir-241*	R1	5'-CGGTGACATTCAATCCCTTC-3'
SAM 377	*mir-241*	R2	5'-AAGTCTGGCGTCCAAAAGTG-3'
SAM 380	*mir-241*	Poison	5'-TCGGCATCCATATAGTAATCGT-3'
SAM 385	*mir-48 mir-241*	F1	5'-TCCCTATTGGGAGCCTTTTC-3'
SAM 386	*mir-48 mir-241*	F2	5'-TTGGGTTTGTTTTGGCTCTC-3'
SAM 388	*mir-48 mir-241*	R1	5'-TCTTCCCTGACCCTCTTGTG-3'
SAM 389	*mir-48 mir-241*	R2	5'-CGTTCGCACTCTCTGTTCTG-3'

[a] For the first PCR reaction, F1 and R1 and Poison were used. For the second PCR reaction, F2 and R2 were included.