# Supporting Information

## Friedlaender et al. 10.1073/pnas.0905222106

### SI Text

**SI Methods.** ***Sample preparation.*** Planarians from the clonal, asexual CIW4 strain of *Schmidtea mediterranea* were starved for 1 week before all experiments. Planarian total RNA was isolated by using the standard TRIzol protocol (Invitrogen). Planarians for the irradiated samples were exposed to 60 Gy in an Irradiator OB29 (STS) and RNA was extracted 8 days after irradiation.

For FACS sorting, *S. mediterranea* were cut twice to release digestive components, rinsed, chopped finely, and dissociated for ≈1.5 h at room temperature in a calcium- and magnesium-free salt solution (CMF). The resulting cell suspension was enriched for neoblasts by filtration through nylon fabric of uniform pore size (Nytex) to a maximum of 20 microns, then stained for 30 min at room temperature with Hoechst 33342 and Calcein (Molecular Probes). Stained cells were washed in CMF and propidium iodide was added (1). Single reagent controls were prepared for all reagents. Stained samples were then analyzed on a Becton Dickinson FACSAria fluorescence-activated cell sorter and putative neoblasts were sorted to microcentrifuge tubes containing CMF, after the method of Agata (2). Immediately after sorting cells were pelleted by centrifugation, supernatant was removed, and flash-frozen in liquid nitrogen and stored at −80 °C. Eighteen dedicated runs were performed to harvest the neoblasts for generating the small RNA library, with each run of 20 to 30 planaria. A total of 5.6 million cells were accumulated.

***Solexa sequencing.*** Small RNAs from planarian total RNA samples were prepared for Solexa sequencing as follows: ≈5 μg total RNA were size-fractionated on 15% TBE-Urea polyacrylamide gel (Sequagel, National Diagnostics) and RNA fragments of lengths between 18 and 40 bases were isolated. Small RNA libraries were prepared by using a modified protocol that prevents small RNA circularization during adapter ligation as described in ref. 3. The purified small RNAs were ligated to preadenylated 3′-adapters by using the mutant RNA ligase Rnl2(1–249)K227Q (3, 4). To remove unligated adapters, the ligation products (40–60 bases in length) were gel purified on 15% TBE-Urea polyacrylamide gel. Subsequently, the RNA fragments with the adapter at the 3′ end were ligated with 5′-adapters (Illumina). After gel purification on 15% TBE-Urea polyacrylamide gel, RNA fragments with the adapters at both ends (70–90 bases in length), were reverse transcribed and the resulting cDNA was subjected to 15 PCR cycles. The ≈100 base-pair amplification products were gel purified on 15% TBE polyacrylamide gel. The libraries thus obtained were used directly for cluster generation and 36 cycles of sequencing analysis by using the Illumina cluster station and 1G Genome Analyzer following manufacturer protocols. Sequencing reads were extracted from the image files generated by Illumina 1G Genome Analyzer by using the open source Firecrest and Bustard applications (Illumina). The Solexa sequencing of the sample of neoblasts yielded 5,943,990 reads, whereas the sequencing of the untreated and irradiated whole-body neoblast samples yielded 3,772,113 and 4,245,548 reads, respectively.

***Preprocessing and mapping of the 454 and Solexa deep-sequencing data.*** The 454 deep sequencing reads were cleaned of 5′ and 3′ adapters (CGTAGGCACCTGAAA and CTGTAGGCACCATCAA, respectively) by using a custom Perl script searching for near-perfect string matches. Both the sequencing reads and their reverse complements were searched. The Solexa deep-sequencing reads were cleaned of 3′-adapters by using another custom Perl script that searches for the longest instance of the adapter sequence TCGTATGCCGTCTTCTGCTTGT, allowing for 2 edits (mismatches and/or insertions/deletions). The longest instance of adapter sequence searched is 18 nt, meaning that the shortest biological transcripts cleaned of adapters are 16 nt (Solexa read length 36 nt, longest adapter instance searched 18 nt, edit distance 2). Subsequently, 454 and Solexa reads containing >1 uncalled nucleotide or any homo-nucleotides stretches of 7 nt or longer were discarded. The remaining deep sequencing reads were aligned to the planarian genome (*Schmidtea mediterranea*-3.1, from GSC at http://genome.wustl.edu/) by using the oligomap application run with the option -m 100 (5). Reads that mapped 100 or more times by using these criteria were discarded, as were suboptimal mappings (edit distance 1 mappings of reads that map perfectly elsewhere on the genome). The following numbers of 454 reads were successfully mapped: 63,278 from neoblasts; 93,412 from untreated; and 61,391 from irradiated. The following numbers of Solexa reads were successfully mapped: 91,371 from neoblasts; 1,784,859 from untreated; and 2,050,669 from irradiated.

***Annotating miRNA star strands, rRNA, tRNA, and mRNA.*** We obtained the available 12 planarian miRNA star strands at miRBase. We used a custom Perl script to annotate the star strands of the remaining miRNAs, integrating precursor secondary structures predicted by RNAfold (6), mature strand coordinates and knowledge of 2 nt 3′ overhangs. To annotate *S. mediterranea* rRNA genes, we first obtained the 28S rRNA of this organism at GenBank (7). We blasted this against the nonredundant nucleotide collection at the National Center for Biotechnology Information (NCBI), and noted the Linnaeus names of the 10 flatworms whose 28S rRNA produced the best matches (*Dugesia gonocephala*, *Dugesia sicula*, *Dugesia tigrina*, *Dugesia japonica*, *Neppia sp.*, *Spathula alba*, *Dugesia ryukyuensis*, *Dolichoplana sp.*, *Geoplana ladislavii* and *Novibipalium venosum*). We then obtained all available rRNA sequences from these flatworms at GenBank, and blasted these against the *S. mediterranea* genome, by using the NCBI blast application with an e-value cut-off of $1e^{-10}$. The matched genomic regions were annotated as rRNA genes. We annotated tRNA genes by running the tRNAscan-SE-1.23 on the *S. mediterranea* genome with the default eukaryotic parameters. For the mRNA annotation, we used the SmedGD annotation (http://smedgd.neuro.utah.edu). Sometimes reads mapped to more than one type of annotation. In these cases, we resolved annotation by a confidence-based hierarchy: miRNA > mRNA > tRNA > rRNA > piRNA. This strategy is comparable to the one used in ref. 5.

***Comparing sets of piRNAs.*** We obtained the set of piRNAs previously discovered by Palakodeti et al. (8). We then mapped these piRNAs against our pooled deep-sequencing datasets by using a custom mapping tool, allowing for 2 edits (mismatches and/or insertions/deletions). Of 4,485 piRNAs previously reported, we found matches for 1,684 (38%).

***Calculating read mapping and overlap intensities.*** For the purposes of piRNA analysis, all read mappings were assigned intensities that were inverse to the number of times the given read mapped to the genome. For instance, a read mapping 100 times would have each mapping assigned an intensity of 0.01. This is equivalent to weighing the mappings. When 2 reads overlapped, the overlap was assigned an intensity that is the mean of the intensities of the 2 reads in log-space. For instance, the overlap between 2 read mappings with intensity 0.1 would be weighted 0.1, as would the overlap between one read mapping with intensity 1 and one with intensity 0.01.

***piRNA repeat association.*** We downloaded the latest version of RepBase (9) and scanned the planarian genome for repeats by using RepeatMasker with the following options: -s -species "schmidtea mediterranea" -gccalc –xsmall. To quantify piRNA enrichment, we compared the fraction of piRNAs that overlaps with repeat annotations with the fraction of the planarian genome that is covered by the same repeat annotations. If the fraction of piRNAs covered by the repeats was larger than expected from the genome coverage of the repeats, we noted the repeats as enriched in piRNAs. This analysis was done for each class of repeats and for the total set of repeats. The set of piRNAs from the untreated planarian sample was used for this analysis. To test whether the piRNA enrichment or depletion of a given transposon type is significant, we used binomial statistics. We set the number of trials $n$ as the number of piRNA reads and the probability of success $p$ as the probability that a piRNA overlaps with the transposon type. The probability $p$ was set equal to the genome coverage of the transposon type (taking into account edge effects of the overlaps). We also made controls by randomly shuffling the annotated repeats to new positions on the same contig strand and comparing with piRNA mappings, with similar results.

***Control for false positives in the piRNA cluster analysis.*** It was important to check that genomic clusters of mapped reads would not be flagged as piRNA clusters if reads would distribute randomly to the genome. We thus moved each individual read to a random position in the genome and repeated the cluster identification procedure. This control did not yield any clusters.

***Prediction of novel planarian miRNAs by using miRDeep.*** Because it is known that miRNA mature and star strands map few times to metazoan genomes, deep-sequencing reads that mapped >5 times were disregarded for purposes of miRNA prediction. The remaining reads were used as guidelines for excising potential precursor sequences from the genome, and these were filtered and scored by the miRDeep core algorithm as previously described (10). We further improved the prediction by taking 2 extra measures: (*i*) potential precursors were only excised if the putative mature miRNA was represented by 2 or more perfectly mapping reads, and (*ii*) a limited set of known mature miRNAs from miRBase was used for purposes of seed conservation. Only mature sequences belonging to families present in invertebrates or that are present in both mammalian and nonmammalian vertebrates were used for conservation scoring. A cut-off of 1 was used for both the 454 and the Solexa data. Redundancy in the predictions caused by errors in the genome assembly was removed by manual curation. No reads were initially discarded based on annotation, but comparison between the predictions and genome annotations revealed that 1 prediction overlapped with a rRNA gene. This prediction was removed. Permutation controls were performed as previously described (10). Table S6 gives an overview of the novel planarian miRNAs. Fig. S3 gives an overview of the family relations and phylogeny of all planarian miRNAs.

***Northern blot analysis.*** Validation of miRNA or piRNA candidates was performed by Northern blot analysis as described earlier (11). In brief, 30 to 50 $\mu$g total RNA and an RNA ladder (Decade Markers, Ambion) were resolved side by side on a 15% TBE-Urea polyacrylamide gel (Sequagel, National Diagnostics). Hybridization and wash steps were performed at 43–45 °C. The $^{32}$P-radiolabeled oligodeoxynucleotide probes are described in Table S8.

***Real time RT-PCR (qPCR).*** Thirty-eight planarian custom TaqMan miRNA assays designed for mature miRNA quantification (Applied Biosystems) were used in this study.

cDNA was synthesized from 50 ng total RNA from either irradiated or untreated worms in a 15-$\mu$L reaction volume according to TaqMan MicroRNA Assay protocol, by using hairpin primers targeting specifically mature miRNAs. Reverse transcription was performed by using the following program: 30 min at 16 °C, 30 min at 42 °C, 5 min at 85 °C and then held at 4 °C. Samples without reverse transcriptase served as the negative control template. For relative quantification by real time, 1.5 $\mu$L cDNA were used in a total reaction volume of 20 $\mu$L with 1 $\mu$L custom TaqMan assay by using an Applied Biosystems StepOne Real-Time PCR System. Thermal cycling program used for quantification was as follows: 50 °C for 2 min and 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 s and 60 °C for 1 min.

For miRNA quantification, it is utterly important to select the right endogenous control to circumvent normalization on total RNA. Noncoding RNAs are the controls of choice in real time PCR assays, i.e., ribosomal RNAs, snoRNAs, or snRNAs. We first used U6, but its level of expression was too low to get an adequate amplification with our methodology. U6 assays also showed that U6 levels were not constant across samples. Other groups have often used another miRNA as an internal control, one observed to have steady level of expression across samples based on total RNA input in preliminary studies. We decided however that such control could bring strong biases. Thus, the constitutively expressed gene *ura4* (accession number: AY068123) was selected in this study as an endogenous control (1). Sets of specific *ura4* primers and a TaqMan probe (Applied Biosystems) were designed to target an exon–exon junction. cDNA was synthesized from 50 ng total RNA from untreated or from irradiated worms with 0.5 $\mu$M final oligo(dT)$_{20}$ in a 20 $\mu$L reaction by using the following program: 5 min at 25 °C, 30 min at 42 °C, 5 min at 85 °C and then held at 4 °C. Real time was conducted on the endogenous control with the exact same conditions used in the miRNA assays described above and run in parallel.

Each measurement was performed in triplicate and no-template controls were included for each assay. At least 2 biological replicates were used in the study. Data were analyzed by using StepOne v2.0 software (Applied Biosystems). *Ct* (threshold cycle) values of real-time PCR were normalized to the endogenous control which was measured in parallel. The relative expression of each miRNA between irradiated and untreated samples is given as log2 of $2^{-\Delta\Delta Ct}$ values.

As an additional control and to check that miRNA down regulation was due to neoblast depletion and not to the $\gamma$-radiation treatment, we performed in parallel the exact same experiments on a neoblast-enriched cell fraction. Planarians were chopped into small pieces in 500 $\mu$L Accutase (Millipore), placed into 5.5 mL Accutase solution, and left to rock for 2 h at room temperature until complete dissociation. The dissociated cells were placed on ice, collected by centrifugation at 4 °C, and resuspended in 10 mL cold CMF (15 mM Hepes, 400 mg/L $NaH_2PO_4$, 800 mg/L NaCl, 1200 mg/L KCl, 800 mg/L, $NaHCO_3$, 240 mg/L glucose, pH 7.3). To obtain the neoblast-enriched cell fraction, cells were filtrated serially through a 70-$\mu$m nylon filter (BD Falcon), a 40-$\mu$m nylon filter (BD Falcon) and a 20-$\mu$m nylon filter (Millipore). Filtrated cells were collected by centrifugation and RNA was extracted using TriZol (Invitrogen) following the manufacturer protocol.

Smedwi-1 mRNA relative expression was assayed by real time PCR on total RNA extracted from filtrated cells, untreated planarians, and irradiated planarians by using *ura4* as an endogenous control and following the protocol described above for the *ura4* gene. Smedwi-1 expression was greatly reduced in the irradiated sample (400-fold down) and increased in the filtrated cells (1.8-fold up).

A 1-to-1 correlation was found between all miRNAs that went significantly down in the neoblast-depleted sample (irradiated planarians) and significantly up in the neoblast-enriched sample (filtrated cells). These results demonstrate that miRNA down regulation in irradiated planarians is not due to $\gamma$-radiation effects but to the depletion of neoblasts.

***Quantifying total miRNA and piRNA expression.*** The total expression of miRNAs and piRNAs in the 3 samples was performed by normalizing to 3 miRNAs that were constant in the 3 samples according to qPCR (miR-71c, miR-36, and miR-36c). The 454 data, produced by specifically sequencing small RNAs of 18–25 nt in length, was used to estimate miRNA expression, whereas the Solexa data were used to estimate piRNA expression. Total miRNA and piRNA read counts were normalized by dividing by the read count of the constantly expressed miRNA. The miRNA and piRNA expression of the untreated sample was set to 1, and the other expression levels were scaled accordingly (Table S3).

***Quantifying expression of individual miRNAs from deep-sequencing data.*** We intersected the genome coordinates of all mapped reads with the genome coordinates of all planarian miRNA mature and star strands. If a mapped read was fully contained within the genome coordinates of a miRNA strand, including 5 nucleotide flanks upstream and downstream, then it was assumed to be the sequencing product of the strand, and was counted toward the read count of the miRNA. Because suboptimal mappings were discarded (see above), a given read was only mapped to >1 miRNA gene if the genes had identical mature sequences. Table S1 gives an overview of miRNA counts in the 6 datasets. When fold-changes between samples were calculated, the read counts of individual miRNAs in a sample were normalized by dividing by the total miRNA read count in the sample.

**SI Results.** ***miRNA analysis and phylogeny.*** We searched the 454 and the Solexa data separately with miRDeep (*SI Methods*). The default cut-off was found to recover miRNA hairpins with high sensitivity (83% for the 454 data and 84% for the Solexa data). The numbers of false positives were estimated computationally as previously described (10) and were found to be low (6 ± 2 s.d. and 7 ± 3 s.d. false positives for the 454 and Solexa data, respectively). In total, 90 and 118 unique hairpins exceeded the score cut-off. Of these, 52 and 53 represented known miRNA hairpins, whereas 38 and 65 represented novel planarian miRNA hairpins. Because there was significant overlap between the miRNAs detected in the 2 types of deep-sequencing data, we report a total number of 70 novel miRNAs. Curation showed that 1 reported miRNA had short loop untypical of Dicer processing. It was therefore omitted from further analysis. The planarian miR-753 and miR-754 families both had several family members with high sequence similarity. Although these miRNAs were individually plausible, only a subset were supported by uniquely mapping reads. To err on the side of caution, we therefore omitted the remaining 8 miRNAs from the main body of the manuscript, although they are retained in Table S6.

We find that planarians share miRNA families with flies, nematodes, and mammals (Fig. S3). For example, our data show that there are 3 *lin-4*/miR-125 and 4 *let-7* homologs in *S. mediterranea*. The *lin-4* and *let-7* families of miRNAs are often present in multiple copies in a genome and are highly conserved across species in sequence and function, i.e., cell differentiation and proliferation (for review see ref. 12). Moreover, *let-7* is posttranscriptionally regulated by *lin-28* (13), an RNA-binding protein that can be used to help reprogram mammalian somatic-differentiated cells into embryonic stem cells (14). Among other miRNA genes deeply conserved in the animal taxa, we report also 4 miRNAs belonging to the miR-1 family and 5 belonging to the miR-124 family. The miR-124 miRNAs are highly conserved and expressed specifically in the developing and adult nervous systems (15) where they promote neuronal differentiation (16, 17). The function of miR-1 in the differentiation of skeletal muscle is conserved from human to fly (18). Because planarians are among those metazoans in which muscle is neither smooth nor striated (19), it will be interesting to investigate the function of these 4 family members.

Interestingly, we find that the majority of planarian miRNA families do not show sequence similarity to any known miRNAs (Fig. S3). These families might be common to lophotrochozoans or might be specific to planarians. In either case, they underline the need for further molecular and genetic exploration of the Lophotrochozoa, a superphylum representing the largest collection of metazoan body plans.

***Genomic organization of planarian miRNA genes.*** In mammals, flies, and nematodes, the transcriptional regulation of miRNAs is complicated, with many miRNA genes being part of larger transcription units. In contrast, the genomic positions of planarian miRNAs suggest that most are independently transcribed. For instance, only 3 of 124 (2%) planarian miRNA genes are intronic, whereas this percentage is much higher for humans (45%), *D. melanogaster* (43%), and *C. elegans* (36%). Planarians have 28 (23%) miRNAs that locate to miRNA gene clusters, whereas relatively more miRNAs are clustered in human (38%), fly (46%), and nematode (31%). However, given the fragmented nature of the planarian genome draft assembly, we cannot rule out an underestimation of clustered or intronic miRNAs.

***Planarian piRNAs and their associated Piwi proteins.*** The predominant length of planarian piRNAs is 32 nt, longer than observed in other species, and this may be the result of the "footprint" of planarian PIWI proteins. The *S. mediterranea* genome contains 3 piwi-like genes, *smedwi-1* to *-3*. SMEDWI-1 and -2 have been shown previously to be specifically expressed in neoblasts (1) and SMEDWI-2 and -3 are required for regeneration and stem cell differentiation (1, 8). SMEDWI-1 and SMEDWI-2 proteins have high sequence similarity to each other and to the *D. japonica* PIWI-like protein DjPIWI-1 (20), whereas SMEDWI-3 is more closely related to *D. melanogaster* AGO-3 than SMEDWI-1 and -2. In mammals and flies, the sequence biases of piRNA subpopulations reflect association with distinct PIWI proteins, and the same may hold true in planarians. Therefore, we recognize that PIWI protein pull-downs and possibly more cell sorting will be necessary to deconvolute all subpopulations.

***Long and short piRNAs.*** The set of reads annotated as long (>25 nt) piRNAs seem to constitute a homogenous population of small RNAs because the peaked length profile in Fig. 2*C* is smooth, indicating small RNAs generated by the same mechanism. The short piRNAs have similar features as the long piRNAs, but less pronounced (10 nt overlap, transposon association biases). We attempted to estimate the dilution of genuine piRNAs in the set of short piRNA set by simulation. First we randomly sampled the long piRNA mappings to make them comparable in number to the short piRNA mappings. Then we substituted, at random, individual long piRNA mappings with mappings to random positions in the genome, simulating dilution with transcripts not associated with piRNA biology. We found that the long piRNA mappings, when 20–30% of dilution was simulated, had a 10-nt overlap peak of the same height as that of the (untouched) short piRNA mappings. Similarly, we found that the long piRNA mappings with simulated 20–30% dilution had similar biases in transposon association as do the short piRNA mappings. Therefore, we estimate that the sets of reads annotated as short piRNAs contain 20–30% nonpiRNA sequences. This holds for both untreated and irradiated planarians.

***Analysis of genic piRNAs.*** We discovered that substantial numbers of deep-sequencing reads map to annotated exons of protein-coding genes. For instance, in Solexa sequencing data of untreated planarians, we found ≈80,000 reads that map to exons, about one-third of which map antisense. These reads display features of planarian piRNAs, such as a length profile peaking at 32 nt, a tendency to overlap by 10 nt, and sequence biases resembling those of primary piRNAs (antisense) or secondary piRNAs (sense). Similar observations have previously been made in planarians (8) and in vertebrates such as zebrafish (21), and are consistent with a model in which gene transcripts being regulated by antisense piRNAs. To rule out that these genic

piRNAs are artifacts due to genome misannotations or ambiguous read mappings, we performed a rigorous analysis of deep sequencing reads mapping to exons and introns. We counted up the numbers of long (>25 nt) reads that could be traced unambiguously to the following types of annotation: intergenic (the entire genome excluding genes and piRNA clusters), exons of protein coding genes, exons of conserved protein coding genes (true homologs of human genes, analysis as in ref. 22), introns of protein coding genes, introns of conserved protein coding genes, and our annotated piRNA clusters (Table S2).

This more rigorous analysis yielded unforeseen results. First, we found that both exons and introns have fewer reads mapping antisense per kb than do genome strands of the intergenic background. Exons are depleted in antisense reads by a factor of 7, whereas introns are depleted by a factor of 3, suggesting that there is in fact a general selection pressure to avoid short RNAs with perfect complementarity to gene transcripts. Second, when we manually investigated exons that have multiple reads mapping antisense, we found that the exons in the gene models were possible misannotations, i.e., nonconserved exons in otherwise conserved genes. Third, the genes that have reads mapping antisense were not enriched in any gene ontology. In sum, we find little evidence that planarian gene transcripts are targeted by antisense piRNAs.


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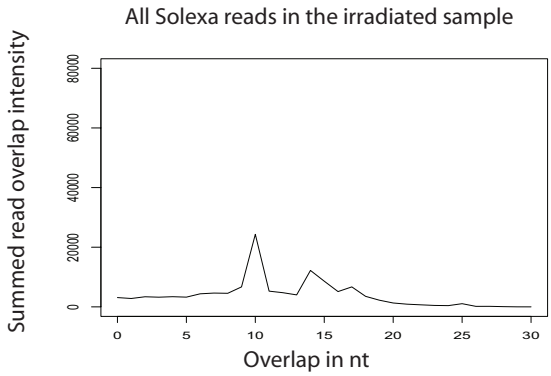



**Fig. S1.** Summed overlap intensities for all Solexa reads in the irradiated sample. The horizontal axis is the length of overlap in nucleotides between the 5′ ends of reads mapping to the same genomic locus on opposite strands. The vertical axis shows the intensity of the overlap, summed over the entire dataset.

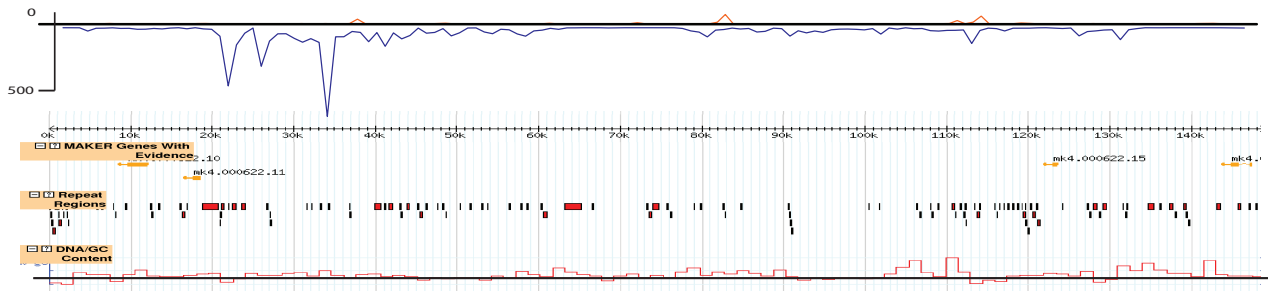
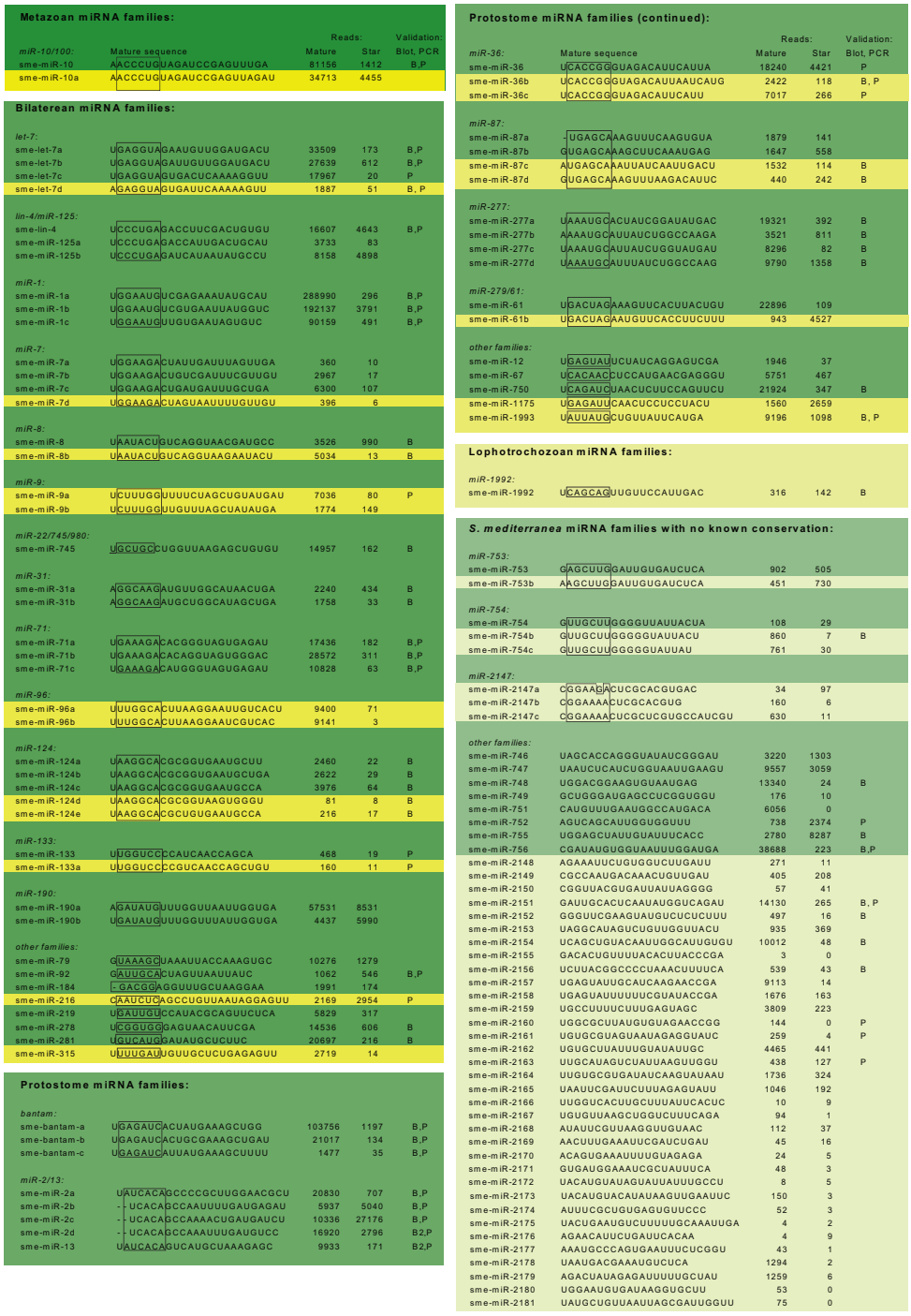



**Fig. S2.** Example of an intergenic planarian piRNA cluster candidate. The top line indicates the piRNA mapping intensities on the genomic plus strand (red) and minus strand (blue). Below, the orange arrows indicate genes annotated by the Maker algorithm, the red squares indicate repeats annotated by Repeatmasker by using custom planarian libraries, and the red line shows the G/C content (from 0% to 100%). The annotation tracks are part of the *S. mediterranea* genome annotation.



**Metazoan miRNA families:**

| miR-10/100: | Mature sequence | Reads: Mature | Star | Validation: Blot, PCR |
|---|---|---|---|---|
| sme-miR-10 | AACCCUGUAGAUCCGAGUUUGA | 81156 | 1412 | B,P |
| sme-miR-10a | AACCCUGUAGAUCCGAGUUAGAU | 34713 | 4455 | |

**Bilaterean miRNA families:**

| | | | | |
|---|---|---|---|---|
| *let-7:* | | | | |
| sme-let-7a | UGAGGUAGAAUGUUGGAUGACU | 33509 | 173 | B,P |
| sme-let-7b | UGAGGUAGAUUGUUGGAUGACU | 27639 | 612 | B,P |
| sme-let-7c | UGAGGUAGUGACUCAAAAGGUU | 17967 | 20 | P |
| sme-let-7d | AGAGGUAGUGAUUCAAAAAGUU | 1887 | 51 | B, P |
| *lin-4/miR-125:* | | | | |
| sme-lin-4 | UCCCUGAGACCUUCGACUGUGU | 16607 | 4643 | B,P |
| sme-miR-125a | UCCCUGAGACCAUUGACUGCAU | 3733 | 83 | |
| sme-miR-125b | UCCCUGAGAUCAUAAUAUGCCU | 8158 | 4898 | |
| *miR-1:* | | | | |
| sme-miR-1a | UGGAAUGUCGAGAAAUAUGCAU | 288990 | 296 | B,P |
| sme-miR-1b | UGGAAUGUCGUGAAUUAUGGUC | 192137 | 3791 | B,P |
| sme-miR-1c | UGGAAUGUUGUGAAUAGUGUC | 90159 | 491 | B,P |
| *miR-7:* | | | | |
| sme-miR-7a | UGGAAGACUAUUGAUUUAGUUGA | 360 | 10 | |
| sme-miR-7b | UGGAAGACUGUCGAUUUCGUUGU | 2967 | 17 | |
| sme-miR-7c | UGGAAGACUGAUGAUUUGCUGA | 6300 | 107 | |
| sme-miR-7d | UGGAAGACUAGUAAUUUUGUUGU | 396 | 6 | |
| *miR-8:* | | | | |
| sme-miR-8 | UAAUACUGUCAGGUAACGAUGCC | 3526 | 990 | B |
| sme-miR-8b | UAAUACUGUCAGGUAAGAAUACU | 5034 | 13 | B |
| *miR-9:* | | | | |
| sme-miR-9a | UCUUUGGUUUUCUAGCUGUAUGAU | 7036 | 80 | P |
| sme-miR-9b | UCUUUGGUUGUUUAGCUAUAUGA | 1774 | 149 | |
| *miR-22/745/980:* | | | | |
| sme-miR-745 | UGCUGCCUGGUUAAGAGCUGUGU | 14957 | 162 | B |
| *miR-31:* | | | | |
| sme-miR-31a | AGGCAAGAUGUUGGCAUAACUGA | 2240 | 434 | B |
| sme-miR-31b | AGGCAAGAUGCUGGCAUAGCUGA | 1758 | 33 | B |
| *miR-71:* | | | | |
| sme-miR-71a | UGAAAGACACGGGUAGUGAGAU | 17436 | 182 | B,P |
| sme-miR-71b | UGAAAGACACAGGUAGUGGGAC | 28572 | 311 | B,P |
| sme-miR-71c | UGAAAGACAUGGGUAGUGAGAU | 10828 | 63 | B,P |
| *miR-96:* | | | | |
| sme-miR-96a | UUUGGCACUUAAGGAAUUGUCACU | 9400 | 71 | |
| sme-miR-96b | UUUGGCACUUAAGGAAUCGUCAC | 9141 | 3 | |
| *miR-124:* | | | | |
| sme-miR-124a | UAAGGCACGCGGUGAAUGCUU | 2460 | 22 | B |
| sme-miR-124b | UAAGGCACGCGGUGAAUGCUGA | 2622 | 29 | B |
| sme-miR-124c | UAAGGCACGCGGUGAAUGCCA | 3976 | 64 | B |
| sme-miR-124d | UAAGGCACGCGGUAAGUGGGU | 81 | 8 | B |
| sme-miR-124e | UAAGGCACGCUGUGAAUGCCA | 216 | 17 | B |
| *miR-133:* | | | | |
| sme-miR-133 | UUGGUCCCCAUCAACCAGCA | 468 | 19 | P |
| sme-miR-133a | UUGGUCCCCGUCAACCAGCUGU | 160 | 11 | P |
| *miR-190:* | | | | |
| sme-miR-190a | AGAUAUGUUUGGUUAAUUGGUGA | 57531 | 8531 | |
| sme-miR-190b | UGAUAUGUUUGGUUUAUUGGUGA | 4437 | 5990 | |
| *other families:* | | | | |
| sme-miR-79 | GUAAAGCUAAAUUACCAAAGUGC | 10276 | 1279 | |
| sme-miR-92 | GAUUGCACUAGUUAAUUAUC | 1062 | 546 | B,P |
| sme-miR-184 | -GACGGAGGUUUGCUAAGGAA | 1991 | 174 | |
| sme-miR-216 | CAAUCUCAGCCUGUUAAUAGGAGUU | 2169 | 2954 | P |
| sme-miR-219 | UGAUUGUCCAUACGCAGUUCUCA | 5829 | 317 | |
| sme-miR-278 | UCGGUGGGAGUAACAUUCGA | 14536 | 606 | B |
| sme-miR-281 | UGUCAUGGAUAUGCUCUUC | 20697 | 216 | B |
| sme-miR-315 | UUUUGAUUGUUGCUCUGAGAGUU | 2719 | 14 | |

**Protostome miRNA families:**

| | | | | |
|---|---|---|---|---|
| *bantam:* | | | | |
| sme-bantam-a | UGAGAUCACUAUGAAAGCUGG | 103756 | 1197 | B,P |
| sme-bantam-b | UGAGAUCACUGCGAAAGCUGAU | 21017 | 134 | B,P |
| sme-bantam-c | UGAGAUCAUUAUGAAAGCUUUU | 1477 | 35 | B,P |
| *miR-2/13:* | | | | |
| sme-miR-2a | UAUCACAGCCCCGCUUGGAACGCU | 20830 | 707 | B,P |
| sme-miR-2b | - - UCACAGCCAAUUUUGAUGAGAU | 5937 | 5040 | B,P |
| sme-miR-2c | - - UCACAGCCAAAACUGAUGAUCU | 10336 | 27176 | B,P |
| sme-miR-2d | - - UCACAGCCAAAUUUGAUGUCC | 16920 | 2796 | B2,P |
| sme-miR-13 | UAUCACAGUCAUGCUAAAGAGC | 9933 | 171 | B2,P |

**Protostome miRNA families (continued):**

| miR-36: | Mature sequence | Reads: Mature | Star | Validation: Blot, PCR |
|---|---|---|---|---|
| sme-miR-36 | UCACCGGGUAGACAUUCAUUA | 18240 | 4421 | P |
| sme-miR-36b | UCACCGGGUAGACAUUAAUCAUG | 2422 | 118 | B, P |
| sme-miR-36c | UCACCGGGUAGACAUUCAUU | 7017 | 266 | P |
| *miR-87:* | | | | |
| sme-miR-87a | -UGAGCAAAGUUUCAAGUGUA | 1879 | 141 | |
| sme-miR-87b | GUGAGCAAAGCUUCAAAUGAG | 1647 | 558 | |
| sme-miR-87c | AUGAGCAAAUUAUCAAUUGACU | 1532 | 114 | B |
| sme-miR-87d | GUGAGCAAAGUUUAAGACAUUC | 440 | 242 | B |
| *miR-277:* | | | | |
| sme-miR-277a | UAAAUGCACUAUCGGAUAUGAC | 19321 | 392 | B |
| sme-miR-277b | AAAAUGCAUUAUCUGGCCAAGA | 3521 | 811 | B |
| sme-miR-277c | UAAAUGCAUUAUCUGGUAUGAU | 8296 | 82 | B |
| sme-miR-277d | UAAAUGCAUUUAUCUGGCCAAG | 9790 | 1358 | B |
| *miR-279/61:* | | | | |
| sme-miR-61 | UGACUAGAAAGUUCACUUACUGU | 22896 | 109 | |
| sme-miR-61b | UGACUAGAAUGUUCACCUUCUUU | 943 | 4527 | |
| *other families:* | | | | |
| sme-miR-12 | UGAGUAUUCUAUCAGGAGUCGA | 1946 | 37 | |
| sme-miR-67 | UCACAACCUCCAUGAACGAGGGU | 5751 | 467 | |
| sme-miR-750 | UCAGAUCUAACUCUUCCAGUUCU | 21924 | 347 | B |
| sme-miR-1175 | UGAGAUUCAACUCCUCCUACU | 1560 | 2659 | |
| sme-miR-1993 | UAUUAUGCUGUUAUUCAUGA | 9196 | 1098 | B, P |

**Lophotrochozoan miRNA families:**

| | | | | |
|---|---|---|---|---|
| *miR-1992:* | | | | |
| sme-miR-1992 | UCAGCAGUUGUUCCAUUGAC | 316 | 142 | B |

***S. mediterranea* miRNA families with no known conservation:**

| | | | | |
|---|---|---|---|---|
| *miR-753:* | | | | |
| sme-miR-753 | GAGCUUGGAUUGUGAUCUCA | 902 | 505 | |
| sme-miR-753b | AAGCUUGGAUUGUGAUCUCA | 451 | 730 | |
| *miR-754:* | | | | |
| sme-miR-754 | GUUGCUUGGGGUUAUUACUA | 108 | 29 | |
| sme-miR-754b | GUUGCUUGGGGGUAUUACU | 860 | 7 | B |
| sme-miR-754c | GUUGCUUGGGGGUAUUAU | 761 | 30 | |
| *miR-2147:* | | | | |
| sme-miR-2147a | CGGAAGACUCGCACGUGAC | 34 | 97 | |
| sme-miR-2147b | CGGAAAACUCGCACGUG | 160 | 6 | |
| sme-miR-2147c | CGGAAAACUCGCUCGUGCCAUCGU | 630 | 11 | |
| *other families:* | | | | |
| sme-miR-746 | UAGCACCAGGGUAUAUCGGGAU | 3220 | 1303 | |
| sme-miR-747 | UAAUCUCAUCUGGUAAUUGAAGU | 9557 | 3059 | |
| sme-miR-748 | UGGACGGAAGUGUAAUGAG | 13340 | 24 | B |
| sme-miR-749 | GCUGGGAUGAGCCUCGGUGGU | 176 | 10 | |
| sme-miR-751 | CAUGUUUGAAUGGCCAUGACA | 6056 | 0 | |
| sme-miR-752 | AGUCAGCAUUGGUGGUUU | 738 | 2374 | P |
| sme-miR-755 | UGGAGCUAUUGUAUUUCACC | 2780 | 8287 | B |
| sme-miR-756 | CGAUAUGUGGUAAUUUGGAUGA | 38688 | 223 | B,P |
| sme-miR-2148 | AGAAAUUCUGUGGUCUUGAUU | 271 | 11 | |
| sme-miR-2149 | CGCCAAUGACAAACUGUUUGAU | 405 | 208 | |
| sme-miR-2150 | CGGUUACGUGAUUAUUAGGGG | 57 | 41 | |
| sme-miR-2151 | GAUUGCACUCAAUAUGGUCAGAU | 14130 | 265 | B, P |
| sme-miR-2152 | GGGUUCGAAGUAUGUCUCUCUUU | 497 | 16 | B |
| sme-miR-2153 | UAGGCAUAGUCUGUUGGUUACU | 935 | 369 | |
| sme-miR-2154 | UCAGCUGUACAAUUGGCAUUGUGU | 10012 | 48 | B |
| sme-miR-2155 | GACACUGUUUUACACUUACCCGA | 3 | 0 | |
| sme-miR-2156 | UCUUACGGCCCCUAAACUUUUCA | 539 | 43 | B |
| sme-miR-2157 | UGAGUAUUGCAUCAAGAACCGA | 9113 | 14 | |
| sme-miR-2158 | UGAGUAUUUUUUCGUAUACCGA | 1676 | 163 | |
| sme-miR-2159 | UGCCUUUUCUUUGAGUAGC | 3809 | 223 | |
| sme-miR-2160 | UGGCGCUUAUGUGUAGAACCGG | 144 | 0 | P |
| sme-miR-2161 | UGUGCGUAGUAAUAGAGGUAUC | 259 | 4 | P |
| sme-miR-2162 | UGUGCUUAUUUGUAUAUUGC | 4465 | 441 | |
| sme-miR-2163 | UUGCAUAGUCUAUUAAGUUGGU | 438 | 127 | P |
| sme-miR-2164 | UUGUGCGUGAUAUCAAGUAUAAU | 1736 | 324 | |
| sme-miR-2165 | UAAUUCGAUUCUUUAGAGUAUU | 1046 | 192 | |
| sme-miR-2166 | UUGGUCACUUGCUUUAUUCACUC | 10 | 9 | |
| sme-miR-2167 | UGUGUUAAGCUGGUCUUUCAGA | 94 | 1 | |
| sme-miR-2168 | AUAUUCGUUAAGGUUGUAAC | 112 | 37 | |
| sme-miR-2169 | AACUUUGAAAUUCGAUCUGAU | 45 | 16 | |
| sme-miR-2170 | ACAGUGAAAUUUUGUAGAGA | 24 | 5 | |
| sme-miR-2171 | GUGAUGGAAAUCGCUAUUUCA | 48 | 3 | |
| sme-miR-2172 | UACAUGUAUAGUAUUAUUUGCCU | 8 | 5 | |
| sme-miR-2173 | UACAUGUACAUAUAAGUUGAAUUC | 150 | 3 | |
| sme-miR-2174 | AUUUCGCUGUGAGUGUUCCC | 52 | 3 | |
| sme-miR-2175 | UACUGAAUGUCUUUUUGCAAAUUGA | 4 | 2 | |
| sme-miR-2176 | AGAACAUUCUGAUUCACAA | 4 | 9 | |
| sme-miR-2177 | AAAUGCCCAGUGAAUUUUCUCGGU | 43 | 1 | |
| sme-miR-2178 | UAAUGACGAAAUGUCUCA | 1294 | 2 | |
| sme-miR-2179 | AGACUAUAGAGAUUUUUGCUAU | 1259 | 6 | |
| sme-miR-2180 | UGGAAUGUGAUAAGGUGCUU | 53 | 0 | |
| sme-miR-2181 | UAUGCUGUUAAUUAGCGAUUGGUU | 75 | 0 | |

**Fig. S3.** Phylogeny and family relations of planarian miRNAs. miRNAs in yellow are novel genes reported in this study, whereas miRNAs in dark green were reported by Palakodeti et al. (23). The relative depth of conservation of the miRNA families is indicated by the color saturation, such that more deeply conserved families have a deeper color. A number of the validations by Northern blot analysis shown in this figure were performed in this previous study. ''B'' indicates that the mature miRNA was validated by Northern blot analysis either by Palakodeti et al. or in this study, whereas ''B2'' indicates that validation was performed independently in both studies. ''P'' indicates that the mature miRNA was validated by Taqman qPCR assay using custom primers (current study). Read numbers refer to the entire sequencing data, pooled from all samples that were obtained in this study. All miRNA names and family relations were assigned by miRBase curators, based on analysis of seed sequences and overall mature miRNA sequence similarity. Consensus family seed sequences are boxed in. Phylogeny of the planarian miRNA families was based on the study by Wheeler et al. (24) and the study by Peterson et al. (25). The one ''metazoan'' miRNA family (miR-10/100) is inferred in this study to have originated in the eumetazoan ancestor. The 21 ''bilaterean'' families are inferred to have originated in the tribloblastic or in the nephrozoan ancestor. The 11 ''protostome'' families are inferred to have originated in the protostome ancestor. The ''lophotrochozoan'' family (miR-1992) is inferred to have originated in the eutrochozoan ancestor. Forty-five miRNA planarian families appeared unrelated to miRNAs in other species.

**Table S1. Expression of all annotated planarian miRNA genes in the 6 deep-sequencing datasets as measured by read counts**

| | 454 | | | Solexa | | |
|---|---|---|---|---|---|---|
| miRNA genes | Neoblast | Untreated whole-body | Irradiated whole-body | Neoblast | Untreated whole-body | Irradiated whole-body |
| sme-bantam-a | 4 | 25 | 11 | 27 | 45,940 | 57,749 |
| sme-bantam-a* | 0 | 60 | 35 | 0 | 449 | 653 |
| sme-bantam-b | 453 | 1,546 | 848 | 7 | 81,10 | 10,053 |
| sme-bantam-b* | 0 | 0 | 0 | 2 | 59 | 73 |
| sme-bantam-c | 5 | 2 | 6 | 7 | 670 | 787 |
| sme-bantam-c* | 1 | 1 | 0 | 1 | 13 | 19 |
| sme-let-7a | 78 | 88 | 20 | 337 | 24,510 | 8,476 |
| sme-let-7a* | 1 | 10 | 2 | 2 | 68 | 90 |
| sme-let-7b | 147 | 189 | 61 | 162 | 14545 | 12,535 |
| sme-let-7b* | 1 | 2 | 0 | 1 | 222 | 386 |
| sme-let-7c | 905 | 1,797 | 949 | 2 | 6,187 | 8,127 |
| sme-let-7c* | 0 | 0 | 0 | 0 | 11 | 9 |
| sme-let-7d | 35 | 68 | 24 | 333 | 795 | 632 |
| sme-let-7d* | 0 | 0 | 0 | 0 | 31 | 20 |
| sme-lin-4 | 622 | 834 | 453 | 9 | 6,073 | 8,616 |
| sme-lin-4* | 12 | 209 | 108 | 10 | 1,551 | 2,753 |
| sme-miR-10 | 5 | 52 | 21 | 218 | 24,300 | 56,560 |
| sme-miR-10* | 1 | 57 | 20 | 0 | 420 | 914 |
| sme-miR-10a | 3 | 48 | 20 | 28 | 9,717 | 24,897 |
| sme-miR-10a* | 0 | 0 | 0 | 4 | 1,499 | 2,952 |
| sme-miR-1175 | 8 | 143 | 94 | 0 | 259 | 1,056 |
| sme-miR-1175* | 0 | 13 | 3 | 0 | 721 | 1,922 |
| sme-miR-12 | 30 | 1,136 | 732 | 0 | 162 | 400 |
| sme-miR-12* | 0 | 3 | 0 | 0 | 10 | 9 |
| sme-miR-124a | 53 | 300 | 202 | 0 | 789 | 1,278 |
| sme-miR-124a* | 0 | 1 | 1 | 1 | 8 | 18 |
| sme-miR-124b | 106 | 424 | 256 | 2 | 1,250 | 1,938 |
| sme-miR-124b* | 0 | 23 | 16 | 0 | 8 | 17 |
| sme-miR-124c | 263 | 1,364 | 793 | 0 | 478 | 835 |
| sme-miR-124c* | 0 | 4 | 1 | 0 | 32 | 46 |
| sme-miR-124d | 6 | 9 | 12 | 0 | 26 | 28 |
| sme-miR-124d* | 0 | 4 | 1 | 0 | 0 | 3 |
| sme-miR-124e | 5 | 35 | 11 | 0 | 59 | 106 |
| sme-miR-124e* | 1 | 2 | 1 | 0 | 3 | 10 |
| sme-miR-125a | 6 | 91 | 30 | 27 | 3,267 | 4,737 |
| sme-miR-125a* | 13 | 543 | 262 | 0 | 1,719 | 2,361 |
| sme-miR-125b | 52 | 168 | 85 | 0 | 684 | 957 |
| sme-miR-125b* | 0 | 0 | 2 | 0 | 14 | 21 |
| sme-miR-13 | 339 | 189 | 114 | 19 | 7,002 | 2,270 |
| sme-miR-13* | 0 | 0 | 0 | 0 | 11 | 8 |
| sme-miR-133 | 2 | 73 | 24 | 0 | 70 | 299 |
| sme-miR-133* | 4 | 89 | 42 | 0 | 10 | 26 |
| sme-miR-133a | 2 | 11 | 3 | 0 | 43 | 101 |
| sme-miR-133a* | 0 | 0 | 0 | 0 | 5 | 6 |
| sme-miR-184 | 1 | 14 | 7 | 1 | 566 | 1,402 |
| sme-miR-184* | 2 | 71 | 10 | 0 | 25 | 66 |
| sme-miR-190a | 0 | 4 | 2 | 103 | 22,786 | 34,636 |
| sme-miR-190a* | 39 | 872 | 462 | 2 | 1,995 | 5,161 |
| sme-miR-190b | 0 | 6 | 1 | 0 | 1656 | 2774 |
| sme-miR-190b* | 53 | 1,275 | 622 | 1 | 1,207 | 2,832 |
| sme-miR-1992 | 39 | 136 | 82 | 0 | 33 | 26 |
| sme-miR-1992* | 2 | 19 | 3 | 0 | 63 | 55 |
| sme-miR-1993 | 1 | 24 | 8 | 0 | 2,485 | 6,678 |
| sme-miR-1993* | 0 | 11 | 2 | 0 | 291 | 794 |
| sme-miR-1a | 5 | 156 | 81 | 27 | 92,297 | 196,424 |
| sme-miR-1a* | 1 | 186 | 80 | 0 | 11 | 18 |
| sme-miR-1b | 149 | 1,455 | 799 | 8 | 59,781 | 129,945 |
| sme-miR-1b* | 36 | 1,420 | 838 | 0 | 481 | 1,016 |
| sme-miR-1c | 4 | 247 | 152 | 0 | 26,275 | 63,481 |
| sme-miR-1c* | 0 | 9 | 2 | 0 | 138 | 342 |
| sme-miR-2147a | 0 | 8 | 10 | 0 | 7 | 9 |
| sme-miR-2147a* | 0 | 0 | 0 | 0 | 38 | 59 |
| sme-miR-2147b | 5 | 22 | 7 | 0 | 47 | 79 |

| | 454 | | | Solexa | | |
|---|---|---|---|---|---|---|
| miRNA genes | Neoblast | Untreated whole-body | Irradiated whole-body | Neoblast | Untreated whole-body | Irradiated whole-body |
| sme-miR-2147b* | 0 | 0 | 0 | 0 | 11 | 5 |
| sme-miR-2147c | 10 | 73 | 49 | 0 | 204 | 294 |
| sme-miR-2147c* | 0 | 3 | 1 | 0 | 17 | 6 |
| sme-miR-2148 | 0 | 22 | 2 | 0 | 105 | 142 |
| sme-miR-2148* | 0 | 3 | 0 | 0 | 4 | 4 |
| sme-miR-2149 | 17 | 132 | 49 | 0 | 113 | 94 |
| sme-miR-2149* | 15 | 106 | 58 | 0 | 13 | 16 |
| sme-miR-2150 | 0 | 14 | 7 | 1 | 16 | 19 |
| sme-miR-2150* | 0 | 5 | 0 | 0 | 9 | 27 |
| sme-miR-2151 | 0 | 66 | 16 | 0 | 5,695 | 8,353 |
| sme-miR-2151* | 0 | 10 | 5 | 0 | 82 | 168 |
| sme-miR-2152 | 120 | 4 | 21 | 6 | 36 | 310 |
| sme-miR-2152* | 0 | 0 | 0 | 0 | 0 | 16 |
| sme-miR-2153 | 14 | 143 | 70 | 4 | 272 | 432 |
| sme-miR-2153* | 24 | 18 | 19 | 18 | 86 | 204 |
| sme-miR-2154 | 1 | 99 | 33 | 0 | 3,002 | 6,877 |
| sme-miR-2154* | 0 | 2 | 0 | 0 | 12 | 34 |
| sme-miR-2155 | 0 | 0 | 0 | 0 | 3 | 0 |
| sme-miR-2155* | 0 | 0 | 0 | 0 | 12 | 14 |
| sme-miR-2156 | 10 | 129 | 51 | 1 | 105 | 243 |
| sme-miR-2156* | 0 | 3 | 3 | 0 | 10 | 27 |
| sme-miR-2157 | 0 | 37 | 21 | 0 | 2,511 | 6,544 |
| sme-miR-2157* | 0 | 4 | 0 | 0 | 2 | 8 |
| sme-miR-2158 | 1 | 318 | 195 | 0 | 331 | 831 |
| sme-miR-2158* | 0 | 7 | 1 | 0 | 33 | 122 |
| sme-miR-2159 | 32 | 2,117 | 1,477 | 0 | 49 | 134 |
| sme-miR-2159* | 1 | 68 | 28 | 0 | 38 | 88 |
| sme-miR-216 | 1 | 42 | 15 | 0 | 577 | 1,534 |
| sme-miR-216* | 0 | 68 | 26 | 0 | 1,095 | 1,765 |
| sme-miR-2160 | 45 | 24 | 8 | 0 | 35 | 32 |
| sme-miR-2160* | 0 | 0 | 0 | 0 | 0 | 0 |
| sme-miR-2161 | 18 | 84 | 39 | 0 | 33 | 85 |
| sme-miR-2161* | 0 | 0 | 0 | 0 | 2 | 2 |
| sme-miR-2162 | 73 | 2,786 | 1,525 | 0 | 27 | 54 |
| sme-miR-2162* | 2 | 5 | 2 | 0 | 169 | 263 |
| sme-miR-2163 | 3 | 31 | 17 | 0 | 141 | 246 |
| sme-miR-2163* | 0 | 1 | 0 | 0 | 54 | 72 |
| sme-miR-2164 | 1 | 28 | 14 | 0 | 402 | 1,291 |
| sme-miR-2164* | 0 | 0 | 0 | 0 | 66 | 258 |
| sme-miR-2165 | 0 | 2 | 0 | 0 | 359 | 685 |
| sme-miR-2165* | 0 | 1 | 3 | 0 | 54 | 134 |
| sme-miR-2166 | 0 | 0 | 0 | 0 | 3 | 7 |
| sme-miR-2166* | 1 | 0 | 0 | 0 | 5 | 3 |
| sme-miR-2167 | 0 | 8 | 4 | 0 | 25 | 57 |
| sme-miR-2167* | 0 | 0 | 0 | 0 | 0 | 1 |
| sme-miR-2168 | 0 | 0 | 0 | 0 | 31 | 81 |
| sme-miR-2168* | 0 | 1 | 0 | 0 | 11 | 25 |
| sme-miR-2169 | 0 | 0 | 0 | 0 | 17 | 28 |
| sme-miR-2169* | 0 | 0 | 0 | 0 | 4 | 12 |
| sme-miR-2170 | 0 | 0 | 0 | 0 | 10 | 14 |
| sme-miR-2170* | 0 | 0 | 0 | 0 | 0 | 5 |
| sme-miR-2171 | 0 | 1 | 3 | 0 | 19 | 25 |
| sme-miR-2171* | 0 | 0 | 0 | 0 | 0 | 3 |
| sme-miR-2172 | 0 | 1 | 0 | 0 | 4 | 3 |
| sme-miR-2172* | 0 | 0 | 0 | 0 | 4 | 1 |
| sme-miR-2173 | 1 | 1 | 0 | 0 | 58 | 90 |
| sme-miR-2173* | 0 | 1 | 0 | 0 | 1 | 1 |
| sme-miR-2174 | 0 | 0 | 0 | 0 | 20 | 32 |
| sme-miR-2174* | 0 | 0 | 0 | 0 | 0 | 3 |
| sme-miR-2175 | 0 | 0 | 0 | 0 | 1 | 3 |
| sme-miR-2175* | 0 | 0 | 0 | 0 | 1 | 1 |
| sme-miR-2176 | 0 | 0 | 0 | 0 | 1 | 3 |
| sme-miR-2176* | 0 | 0 | 0 | 0 | 3 | 6 |
| sme-miR-2177 | 0 | 0 | 0 | 0 | 19 | 24 |

| | 454 | | | Solexa | | |
|---|---|---|---|---|---|---|
| miRNA genes | Neoblast | Untreated whole-body | Irradiated whole-body | Neoblast | Untreated whole-body | Irradiated whole-body |
| sme-miR-2177* | 0 | 1 | 0 | 0 | 0 | 0 |
| sme-miR-2178 | 0 | 1 | 0 | 0 | 587 | 706 |
| sme-miR-2178* | 0 | 0 | 0 | 0 | 0 | 2 |
| sme-miR-2179 | 0 | 10 | 3 | 6 | 567 | 673 |
| sme-miR-2179* | 0 | 0 | 0 | 0 | 3 | 3 |
| sme-miR-2180 | 3 | 1 | 2 | 0 | 17 | 30 |
| sme-miR-2180* | 0 | 0 | 0 | 0 | 0 | 0 |
| sme-miR-2181 | 0 | 1 | 0 | 0 | 16 | 58 |
| sme-miR-2181* | 0 | 0 | 0 | 0 | 0 | 0 |
| sme-miR-219 | 13 | 295 | 137 | 1 | 1,622 | 3,761 |
| sme-miR-219* | 0 | 3 | 5 | 0 | 105 | 204 |
| sme-miR-277a | 0 | 10 | 3 | 0 | 6,284 | 13,024 |
| sme-miR-277a* | 0 | 1 | 0 | 2 | 100 | 289 |
| sme-miR-277b | 0 | 0 | 0 | 0 | 1,210 | 2,311 |
| sme-miR-277b* | 10 | 379 | 181 | 0 | 60 | 181 |
| sme-miR-277c | 3 | 74 | 34 | 0 | 2,820 | 5,365 |
| sme-miR-277c* | 0 | 12 | 12 | 0 | 20 | 38 |
| sme-miR-277d | 3 | 137 | 75 | 0 | 3,238 | 6,337 |
| sme-miR-277d* | 1 | 305 | 181 | 0 | 260 | 611 |
| sme-miR-278 | 32 | 3,036 | 1,572 | 0 | 2,770 | 7,126 |
| sme-miR-278* | 17 | 200 | 84 | 0 | 92 | 213 |
| sme-miR-281 | 136 | 1,271 | 646 | 1 | 5,932 | 12,711 |
| sme-miR-281* | 0 | 22 | 6 | 0 | 62 | 126 |
| sme-miR-2a-1 | 987 | 721 | 357 | 0 | 6,898 | 11,867 |
| sme-miR-2a-1* | 15 | 237 | 142 | 0 | 96 | 217 |
| sme-miR-2a-2 | 987 | 721 | 357 | 0 | 6,898 | 11,866 |
| sme-miR-2a-2* | 5 | 176 | 89 | 1 | 916 | 2,294 |
| sme-miR-2b | 1,242 | 1,732 | 1,281 | 0 | 716 | 966 |
| sme-miR-2b* | 0 | 6 | 0 | 0 | 1526 | 3508 |
| sme-miR-2c | 57 | 1,586 | 1,187 | 1 | 2,235 | 5,270 |
| sme-miR-2c* | 0 | 5 | 1 | 15 | 9,002 | 18,153 |
| sme-miR-2d | 7,222 | 5,776 | 3,146 | 1 | 402 | 373 |
| sme-miR-2d* | 0 | 0 | 0 | 3 | 1,276 | 1,517 |
| sme-miR-315 | 0 | 0 | 0 | 0 | 875 | 1,844 |
| sme-miR-315* | 0 | 0 | 0 | 0 | 2 | 12 |
| sme-miR-31a | 2 | 28 | 11 | 20 | 832 | 1,347 |
| sme-miR-31a* | 0 | 0 | 0 | 0 | 126 | 308 |
| sme-miR-31b | 8 | 157 | 57 | 3 | 654 | 879 |
| sme-miR-31b* | 0 | 4 | 1 | 0 | 9 | 19 |
| sme-miR-36 | 2,198 | 4,242 | 2,928 | 25 | 2,529 | 6,318 |
| sme-miR-36* | 164 | 2,564 | 1,610 | 0 | 27 | 56 |
| sme-miR-36b | 320 | 227 | 131 | 12 | 584 | 1,148 |
| sme-miR-36b* | 0 | 0 | 0 | 0 | 33 | 85 |
| sme-miR-36c | 111 | 338 | 240 | 11 | 1,679 | 4,638 |
| sme-miR-36c* | 0 | 0 | 0 | 0 | 72 | 194 |
| sme-miR-61 | 13 | 210 | 98 | 0 | 8,765 | 13,810 |
| sme-miR-61* | 0 | 1 | 2 | 1 | 25 | 87 |
| sme-miR-61b | 0 | 96 | 32 | 0 | 284 | 531 |
| sme-miR-61b* | 3 | 45 | 28 | 0 | 2,111 | 2340 |
| sme-miR-67 | 0 | 46 | 20 | 0 | 1,781 | 3,904 |
| sme-miR-67* | 0 | 5 | 0 | 1 | 137 | 324 |
| sme-miR-71a-1 | 290 | 407 | 254 | 1 | 6,880 | 9,604 |
| sme-miR-71a-1* | 1 | 76 | 61 | 0 | 11 | 33 |
| sme-miR-71a-2 | 289 | 406 | 254 | 3 | 6,847 | 9,581 |
| sme-miR-71a-2* | 3 | 264 | 138 | 1 | 218 | 462 |
| sme-miR-71b | 948 | 857 | 370 | 96 | 14,751 | 11,550 |
| sme-miR-71b* | 10 | 38 | 27 | 1 | 129 | 106 |
| sme-miR-71c | 279 | 688 | 433 | 5 | 3,376 | 6,047 |
| sme-miR-71c* | 0 | 9 | 6 | 0 | 12 | 36 |
| sme-miR-745 | 46 | 392 | 167 | 0 | 4,700 | 9,652 |
| sme-miR-745* | 0 | 0 | 0 | 0 | 50 | 112 |
| sme-miR-746 | 68 | 604 | 351 | 0 | 770 | 1427 |
| sme-miR-746* | 5 | 156 | 65 | 0 | 343 | 734 |
| sme-miR-747 | 0 | 37 | 6 | 0 | 2,925 | 6,589 |

| | 454 | | | Solexa | | |
|---|---|---|---|---|---|---|
| miRNA genes | Neoblast | Untreated whole-body | Irradiated whole-body | Neoblast | Untreated whole-body | Irradiated whole-body |
| sme-miR-747* | 1 | 183 | 102 | 0 | 904 | 1,869 |
| sme-miR-748 | 3 | 0 | 3 | 0 | 3,967 | 9,367 |
| sme-miR-748* | 0 | 0 | 0 | 0 | 5 | 19 |
| sme-miR-749 | 11 | 34 | 21 | 0 | 29 | 81 |
| sme-miR-749* | 0 | 0 | 1 | 0 | 1 | 8 |
| sme-miR-750 | 79 | 998 | 445 | 0 | 6,127 | 14,275 |
| sme-miR-750* | 0 | 6 | 2 | 0 | 113 | 226 |
| sme-miR-751 | 0 | 12 | 1 | 6 | 2,121 | 3,916 |
| sme-miR-751* | 0 | 0 | 0 | 0 | 0 | 0 |
| sme-miR-752 | 157 | 61 | 24 | 52 | 346 | 98 |
| sme-miR-752* | 2 | 4 | 6 | 7 | 1,058 | 1,297 |
| sme-miR-753 | 0 | 3 | 0 | 3 | 429 | 467 |
| sme-miR-753* | 3 | 11 | 8 | 11 | 288 | 407 |
| sme-miR-753b | 0 | 8 | 1 | 2 | 217 | 223 |
| sme-miR-753b* | 52 | 119 | 52 | 11 | 295 | 433 |
| sme-miR-754 | 0 | 23 | 7 | 0 | 14 | 64 |
| sme-miR-754* | 0 | 0 | 0 | 0 | 8 | 21 |
| sme-miR-754b-1 | 6 | 134 | 44 | 2 | 205 | 469 |
| sme-miR-754b-1* | 0 | 0 | 0 | 0 | 4 | 3 |
| sme-miR-754c | 6 | 108 | 33 | 1 | 180 | 433 |
| sme-miR-754c* | 0 | 0 | 0 | 0 | 8 | 22 |
| sme-miR-755 | 4 | 388 | 169 | 0 | 665 | 1,554 |
| sme-miR-755* | 3 | 356 | 164 | 0 | 2,351 | 5,413 |
| sme-miR-756 | 582 | 787 | 398 | 1,802 | 16,520 | 18,599 |
| sme-miR-756* | 0 | 2 | 3 | 0 | 64 | 154 |
| sme-miR-79 | 1,671 | 4,093 | 2,615 | 1 | 781 | 1115 |
| sme-miR-79* | 0 | 4 | 0 | 0 | 582 | 693 |
| sme-miR-7a | 1 | 5 | 1 | 0 | 149 | 204 |
| sme-miR-7a* | 0 | 0 | 0 | 0 | 1 | 9 |
| sme-miR-7b | 49 | 170 | 64 | 0 | 1,163 | 1,521 |
| sme-miR-7b* | 0 | 0 | 0 | 0 | 8 | 9 |
| sme-miR-7c | 89 | 263 | 107 | 1 | 2,119 | 3,721 |
| sme-miR-7c* | 15 | 28 | 11 | 0 | 23 | 30 |
| sme-miR-7d | 18 | 58 | 41 | 0 | 147 | 132 |
| sme-miR-7d* | 0 | 1 | 1 | 0 | 1 | 3 |
| sme-miR-8 | 132 | 264 | 130 | 0 | 453 | 900 |
| sme-miR-8* | 2 | 7 | 3 | 0 | 42 | 87 |
| sme-miR-87a | 83 | 860 | 550 | 0 | 58 | 96 |
| sme-miR-87a* | 3 | 46 | 34 | 0 | 135 | 340 |
| sme-miR-87b | 6 | 91 | 27 | 0 | 1,000 | 2,402 |
| sme-miR-87b* | 7 | 5 | 4 | 1 | 344 | 629 |
| sme-miR-87c | 5 | 158 | 111 | 0 | 432 | 826 |
| sme-miR-87c* | 3 | 19 | 13 | 0 | 24 | 55 |
| sme-miR-87d | 7 | 24 | 4 | 3 | 124 | 278 |
| sme-miR-87d* | 1 | 6 | 1 | 0 | 84 | 150 |
| sme-miR-8b | 37 | 283 | 116 | 0 | 1,459 | 3,139 |
| sme-miR-8b* | 0 | 0 | 0 | 0 | 5 | 8 |
| sme-miR-92 | 1 | 15 | 2 | 0 | 327 | 717 |
| sme-miR-92* | 1 | 307 | 197 | 0 | 11 | 30 |
| sme-miR-96a | 0 | 19 | 11 | 4 | 3,191 | 6,175 |
| sme-miR-96a* | 0 | 0 | 0 | 0 | 21 | 50 |
| sme-miR-96b | 0 | 16 | 10 | 2 | 3,143 | 5,970 |
| sme-miR-96b* | 0 | 0 | 2 | 0 | 0 | 1 |
| sme-miR-9a | 0 | 15 | 6 | 2 | 2,015 | 4,998 |
| sme-miR-9a* | 1 | 1 | 2 | 0 | 24 | 52 |
| sme-miR-9b | 0 | 20 | 5 | 0 | 606 | 1,143 |
| sme-miR-9b* | 0 | 2 | 1 | 0 | 45 | 101 |
| sme-putative-753.1 | 1 | 8 | 6 | 1 | 15 | 40 |
| sme-putative-753.1* | 1 | 6 | 10 | 5 | 366 | 604 |
| sme-putative-753.2 | 5 | 17 | 21 | 5 | 373 | 600 |
| sme-putative-753.2* | 52 | 119 | 52 | 11 | 297 | 434 |
| sme-putative-753.3 | 2 | 99 | 55 | 0 | 1,007 | 2,010 |
| sme-putative-753.3* | 0 | 0 | 0 | 2 | 6 | 16 |
| sme-putative-753.4 | 2 | 99 | 55 | 0 | 1,007 | 2,010 |

| | 454 | | | Solexa | | |
|---|---|---|---|---|---|---|
| miRNA genes | Neoblast | Untreated whole-body | Irradiated whole-body | Neoblast | Untreated whole-body | Irradiated whole-body |
| sme-putative-753.4* | 52 | 119 | 51 | 11 | 280 | 421 |
| sme-putative-753.5 | 0 | 12 | 10 | 2 | 1,072 | 1,860 |
| sme-putative-753.5* | 52 | 119 | 51 | 11 | 290 | 423 |
| sme-putative-754.1 | 0 | 51 | 17 | 0 | 4 | 14 |
| sme-putative-754.1* | 0 | 0 | 0 | 0 | 6 | 18 |
| sme-putative-754.2 | 6 | 134 | 44 | 2 | 205 | 469 |
| sme-putative-754.2* | 0 | 0 | 0 | 0 | 9 | 22 |
| sme-putative-754.3 | 6 | 112 | 33 | 1 | 88 | 161 |
| sme-putative-754.3* | 0 | 0 | 1 | 0 | 8 | 22 |

**Table S2. Analysis of long (>25 nt) reads mapping to intergenic regions, exons of protein coding genes, exons of conserved protein coding genes, introns of protein coding genes, introns of conserved protein coding genes, and our annotated piRNA clusters**

| Region | Long, uniquely mapping reads per mega base |
|---|---|
| Intergenic | 200 ± 10 |
| Exons, sense | 350 ± 10 |
| Exons, antisense | 90 ± 10 |
| Exons from homolog genes, sense | 390 ± 20 |
| Exons from homolog genes, antisense | 30 ± 10 |
| Introns, sense | 210 ± 10 |
| Introns, antisense | 130 ± 10 |
| Introns from homolog genes, sense | 190 ± 40 |
| Introns from homolog genes, antisense | 70 ± 10 |
| piRNA clusters | 16300 ± 700 |

All counts were normalized to the length of the type of annotation summed over the genome. Standard errors were calculated by randomly splitting the mappings into 5 sets and repeating the analysis for each set.

**Table S3. Total expression of miRNAs and piRNAs normalized to 3 miRNAs that were constant in the 3 samples according to qPCR (See *SI Methods* of *SI Text*)**

| | miRNAs | | | piRNAs | | |
|---|---|---|---|---|---|---|
| miRNA | Neoblast | Untreated | Irradiated | Neoblast | Untreated | Irradiated |
| miR-71c | 0.9 | 1 | 0.9 | 46 | 1 | 0.25 |
| miR-36 | 0.7 | 1 | 0.8 | 7.1 | 1 | 0.19 |
| miR-36c | 1.1 | 1 | 0.8 | 10 | 1 | 0.17 |

The miRNA and piRNA expression of the untreated sample was set to 1, and the other expression levels were scaled accordingly. The large variance of piRNA expression in the neoblast sample is likely an effect of the low read counts for the miRNAs used for normalization in this sample.

**Table S4. piRNA transposon association**

| Repeat class | Total genome coverage of repeat, Mb | Total genome coverage of repeat, fraction | Fraction of piRNAs in the untreated sample mapping to repeat type | Fold enrichment, fraction of piRNA coverage/fraction of genome coverage |
|---|---|---|---|---|
| DNA_TcMar | 12.16643208 | 0.014065239 | 0.026605987 | 1.892 |
| DNA_hAT | 9.772936599 | 0.011298193 | 0.018968193 | 1.679 |
| LINE_Penelope | 16.63934215 | 0.019236234 | 0.026799082 | 1.393 |
| LINE_R2 | 23.6842331 | 0.027380616 | 0.02754767 | 1.006 |
| Low_complexity | 21.26549164 | 0.024584383 | 0.019532837 | 0.795 |
| LTR_Gypsy | 116.9148185 | 0.13516164 | 0.106542 | 0.788 |
| DNA_Maverick | 47.64749046 | 0.055083804 | 0.034961253 | 0.635 |
| Simple_repeat | 14.28672666 | 0.016516447 | 0.009141375 | 0.553 |
| DNA_PiggyBac | 40.20635564 | 0.046481336 | 0.023200735 | 0.499 |
| DNA | 2.020804264 | 0.00233619 | 0.001091767 | 0.468 |
| Unknown | 4.755393898 | 0.005497565 | 0.002202752 | 0.401 |

The piRNA enrichment is calculated as the transposon piRNA coverage divided the transposon genome coverage. Only major transposon classes are shown.

**Table S5. Location and intensities of the 119 planarian piRNA clusters**

| Genome contig | Begin position of cluster | End position of cluster | 10 nt overlap intensity of long, uniquely mapping piRNAs | No. of long piRNAs mapping uniquely to the genome plus strand of the cluster | No. of long piRNAs mapping uniquely to the genome minus strand of the cluster | 10 nt overlap intensity of mapped piRNAs | No. of piRNAs mapping to the genome plus strand of the cluster | No. of piRNAs mapping to the genome minus strand of the cluster |
|---|---|---|---|---|---|---|---|---|
| v31.000000 | 290000 | 300000 | 4.41 | 1 | 579 | 4.41 | 4.44 | 737.33 |
| v31.000001 | 100000 | 110000 | 2 | 100 | 5 | 9.69 | 151 | 17.57 |
| v31.000016 | 70000 | 80000 | 6.45 | 14 | 170 | 8.32 | 22.9 | 245.48 |
| v31.000056 | 100000 | 110000 | 1 | 116 | 1 | 1 | 176 | 2.87 |
| v31.000077 | 230000 | 240000 | 2 | 3 | 187 | 2 | 5.99 | 259.67 |
| v31.000111 | 60000 | 70000 | 1 | 1 | 1003 | 1 | 1.8 | 1144.28 |
| v31.000132 | 80000 | 90000 | 2.41 | 110 | 4 | 9.44 | 172 | 13.96 |
| v31.000132 | 100000 | 110000 | 1.41 | 99 | 3 | 21.6 | 215 | 6.51 |
| v31.000144 | 170000 | 180000 | 8.59 | 482 | 3 | 40.9 | 602 | 4.11 |
| v31.000169 | 130000 | 140000 | 136.14 | 1435 | 50 | 204 | 1782 | 66.76 |
| v31.000169 | 170000 | 180000 | 10.14 | 431 | 2 | 15.1 | 556 | 6.99 |
| v31.000179 | 110000 | 120000 | 1 | 3 | 231 | 1.57 | 6.02 | 317.02 |
| v31.000182 | 150000 | 160000 | 79.54 | 47 | 104 | 168 | 107 | 167.01 |
| v31.000200 | 70000 | 80000 | 4 | 206 | 2 | 6.28 | 238 | 6.93 |
| v31.000200 | 190000 | 200000 | 7 | 277 | 2 | 7 | 346 | 2.27 |
| v31.000205 | 120000 | 130000 | 7.65 | 2 | 179 | 9.49 | 3.64 | 230.62 |
| v31.000221 | 30000 | 40000 | 4.41 | 1 | 274 | 4.55 | 1.72 | 346.5 |
| v31.000221 | 40000 | 50000 | 3.73 | 3 | 704 | 3.94 | 4.97 | 815.2 |
| v31.000254 | 30000 | 40000 | 21.48 | 186 | 1 | 21.5 | 235 | 2.14 |
| v31.000265 | 70000 | 80000 | 1 | 98 | 3 | 1 | 115 | 3.8 |
| v31.000265 | 170000 | 180000 | 1 | 5 | 729 | 3.62 | 8.37 | 928.36 |
| v31.000265 | 180000 | 190000 | 5.82 | 2 | 314 | 6.39 | 2.02 | 389.92 |
| v31.000307 | 10000 | 20000 | 3 | 4 | 96 | 3.45 | 11.5 | 110.99 |
| v31.000333 | 30000 | 40000 | 3.41 | 224 | 1 | 4.34 | 327 | 1.7 |
| v31.000338 | 60000 | 70000 | 1 | 2 | 154 | 2 | 2.3 | 191.61 |
| v31.000338 | 100000 | 110000 | 3 | 233 | 6 | 13.3 | 312 | 7.58 |
| v31.000342 | 100000 | 110000 | 21.11 | 8 | 588 | 27.6 | 23.3 | 728.18 |
| v31.000342 | 110000 | 120000 | 2.41 | 4 | 229 | 5.82 | 13.5 | 427.51 |
| v31.000355 | 130000 | 140000 | 4.82 | 4 | 106 | 11.2 | 8.71 | 147.4 |
| v31.000359 | 0 | 10000 | 2.41 | 1 | 121 | 2.41 | 3.04 | 146.73 |
| v31.000361 | 100000 | 110000 | 1 | 3 | 139 | 2.5 | 5.41 | 176.2 |
| v31.000465 | 70000 | 80000 | 2 | 272 | 5 | 6.6 | 442 | 12.37 |
| v31.000466 | 40000 | 50000 | 9.07 | 165 | 3 | 12.7 | 199 | 3.57 |
| v31.000466 | 50000 | 60000 | 12.64 | 122 | 55 | 65.2 | 190 | 176.48 |
| v31.000511 | 90000 | 100000 | 2 | 446 | 1 | 2 | 539 | 1.77 |
| v31.000521 | 70000 | 80000 | 1 | 2 | 141 | 1.98 | 2.27 | 220.07 |
| v31.000521 | 80000 | 90000 | 2 | 4 | 182 | 2 | 5.04 | 233.27 |
| v31.000532 | 20000 | 30000 | 5 | 1 | 117 | 5.32 | 3.94 | 160.16 |
| v31.000554 | 60000 | 70000 | 28.17 | 2 | 134 | 32.4 | 3.65 | 169.11 |
| v31.000558 | 110000 | 120000 | 65.36 | 13 | 235 | 91.1 | 26.4 | 287.6 |
| v31.000558 | 140000 | 147967 | 4.41 | 6 | 199 | 5.04 | 11.1 | 263.91 |
| v31.000564 | 90000 | 100000 | 1 | 3 | 413 | 8.87 | 10 | 548.17 |
| v31.000590 | 110000 | 120000 | 6.41 | 181 | 4 | 9.4 | 341 | 11.01 |
| v31.000617 | 20000 | 30000 | 1 | 125 | 3 | 4.15 | 170 | 3.77 |
| v31.000652 | 120000 | 130000 | 2.41 | 106 | 2 | 24.5 | 168 | 8.84 |
| v31.000672 | 80000 | 90000 | 12 | 4 | 202 | 12.9 | 5.22 | 318.52 |
| v31.000741 | 50000 | 60000 | 1 | 2 | 229 | 5.99 | 2.71 | 352.49 |
| v31.000751 | 50000 | 60000 | 283.67 | 159 | 48 | 405 | 207 | 76.16 |
| v31.000753 | 130000 | 139189 | 1 | 2 | 112 | 2.7 | 5.04 | 138.84 |
| v31.000866 | 20000 | 30000 | 1.41 | 221 | 3 | 1.97 | 277 | 5.45 |
| v31.000886 | 60000 | 70000 | 14.38 | 1 | 234 | 25.8 | 2.53 | 305.75 |
| v31.000900 | 100000 | 110000 | 1 | 1 | 376 | 85.1 | 15.1 | 493.21 |
| v31.000911 | 0 | 10000 | 1 | 98 | 2 | 1 | 162 | 3.74 |
| v31.000918 | 20000 | 30000 | 5.82 | 224 | 1 | 7.97 | 352 | 2.51 |
| v31.000995 | 70000 | 80000 | 2 | 1 | 348 | 2.14 | 2.76 | 424.22 |
| v31.001066 | 90000 | 97060 | 9.23 | 3 | 227 | 14.1 | 3.06 | 300.34 |

| Genome contig | Begin position of cluster | End position of cluster | 10 nt overlap intensity of long, uniquely mapping piRNAs | No. of long piRNAs mapping uniquely to the genome plus strand of the cluster | No. of long piRNAs mapping uniquely to the genome minus strand of the cluster | 10 nt overlap intensity of mapped piRNAs | No. of piRNAs mapping to the genome plus strand of the cluster | No. of piRNAs mapping to the genome minus strand of the cluster |
|---|---|---|---|---|---|---|---|---|
| v31.001076 | 40000 | 49260 | 2 | 2 | 320 | 222 | 7.67 | 2934.65 |
| v31.001093 | 30000 | 40000 | 1 | 108 | 4 | 2.49 | 145 | 6.54 |
| v31.001191 | 30000 | 40000 | 24.86 | 4 | 156 | 33.8 | 7.88 | 203.88 |
| v31.001204 | 30000 | 40000 | 34.21 | 221 | 2 | 41.2 | 284 | 3.67 |
| v31.001206 | 50000 | 60000 | 7.18 | 3 | 443 | 16.1 | 17 | 518.77 |
| v31.001207 | 30000 | 40000 | 1 | 5 | 147 | 3.35 | 7.09 | 184.07 |
| v31.001207 | 40000 | 50000 | 24.33 | 1 | 298 | 26.9 | 1.45 | 373.46 |
| v31.001225 | 40000 | 50000 | 1 | 311 | 2 | 1 | 422 | 3.89 |
| v31.001232 | 0 | 10000 | 1 | 246 | 2 | 2.67 | 324 | 7.11 |
| v31.001345 | 20000 | 30000 | 1.41 | 276 | 4 | 3.54 | 371 | 5.09 |
| v31.001374 | 60000 | 70000 | 1 | 3 | 633 | 1.37 | 4.89 | 862.47 |
| v31.001417 | 40000 | 50000 | 3 | 135 | 2 | 3.02 | 183 | 2.17 |
| v31.001417 | 50000 | 60000 | 2.41 | 142 | 2 | 3.41 | 183 | 4.84 |
| v31.001551 | 20000 | 30000 | 4 | 148 | 1 | 8 | 201 | 1.04 |
| v31.001568 | 70000 | 80000 | 2.41 | 1 | 130 | 7.69 | 2.63 | 186.21 |
| v31.001575 | 40000 | 50000 | 18.37 | 141 | 103 | 44.1 | 211 | 154.43 |
| v31.001576 | 30000 | 40000 | 13.59 | 346 | 2 | 22.2 | 438 | 6.15 |
| v31.001621 | 40000 | 50000 | 1 | 219 | 3 | 1 | 298 | 6.32 |
| v31.001801 | 70000 | 80000 | 2.41 | 2 | 2347 | 9.53 | 3.56 | 2603.73 |
| v31.001826 | 40000 | 50000 | 22.87 | 4 | 175 | 45.5 | 6.33 | 222.99 |
| v31.001892 | 40000 | 50000 | 12.42 | 542 | 22 | 42.3 | 698 | 33 |
| v31.001892 | 50000 | 60000 | 5 | 992 | 2 | 19.5 | 1253 | 6.07 |
| v31.001892 | 60000 | 70000 | 9.38 | 303 | 4 | 14.6 | 399 | 8.37 |
| v31.001892 | 70000 | 78621 | 1 | 186 | 1 | 5.22 | 241 | 3.46 |
| v31.001973 | 20000 | 30000 | 4.41 | 3 | 103 | 17.8 | 6.74 | 134.18 |
| v31.002051 | 0 | 10000 | 58.25 | 2 | 319 | 103 | 3.73 | 357.35 |
| v31.002093 | 10000 | 20000 | 2 | 3 | 418 | 7.74 | 4.35 | 476.96 |
| v31.002339 | 20000 | 30000 | 1 | 2 | 246 | 4.32 | 6.91 | 374.26 |
| v31.002365 | 30000 | 40000 | 1.41 | 221 | 3 | 5.33 | 307 | 4.93 |
| v31.002415 | 30000 | 40000 | 2.41 | 4 | 236 | 13.6 | 5.11 | 322.24 |
| v31.002528 | 10000 | 20000 | 36.95 | 143 | 12 | 59.8 | 181 | 14.87 |
| v31.002537 | 20000 | 30000 | 25.28 | 2 | 668 | 71.7 | 5.42 | 806.57 |
| v31.002537 | 30000 | 40000 | 2 | 3 | 101 | 2 | 4.06 | 139.37 |
| v31.002549 | 50000 | 60000 | 4 | 458 | 3 | 4.33 | 574 | 3.98 |
| v31.002684 | 10000 | 20000 | 2 | 125 | 1 | 7.04 | 252 | 2.04 |
| v31.002688 | 20000 | 30000 | 3.41 | 223 | 1 | 5.27 | 285 | 4.68 |
| v31.002788 | 20000 | 30000 | 2 | 222 | 1 | 2.28 | 301 | 3.06 |
| v31.002788 | 30000 | 40000 | 1 | 234 | 1 | 1.71 | 324 | 3.5 |
| v31.002965 | 40000 | 50000 | 118.07 | 2 | 596 | 137 | 2.27 | 677.09 |
| v31.003034 | 10000 | 20000 | 63.02 | 5 | 199 | 107 | 5.31 | 270.16 |
| v31.003118 | 20000 | 30000 | 2 | 1 | 374 | 3.61 | 3.77 | 487.43 |
| v31.003208 | 60000 | 70000 | 1 | 104 | 3 | 2.2 | 198 | 7.46 |
| v31.003212 | 20000 | 30000 | 12.29 | 246 | 2 | 13.6 | 323 | 5.97 |
| v31.003763 | 20000 | 30000 | 7.41 | 2 | 209 | 13 | 4.17 | 314.47 |
| v31.004482 | 10000 | 20000 | 2.73 | 1 | 331 | 2.73 | 1.86 | 436.82 |
| v31.005110 | 0 | 10000 | 1 | 4 | 133 | 4.97 | 9.89 | 207.91 |
| v31.005212 | 30000 | 40000 | 69.35 | 38 | 98 | 139 | 59.2 | 128.93 |
| v31.005332 | 30000 | 40000 | 69.94 | 103 | 118 | 105 | 166 | 165.17 |
| v31.005594 | 30000 | 40000 | 2 | 4 | 149 | 16.5 | 5.35 | 371.11 |
| v31.005657 | 10000 | 20000 | 6.82 | 3 | 172 | 7.82 | 8.32 | 222.23 |
| v31.005856 | 0 | 10000 | 2 | 93 | 19 | 4.42 | 125 | 28.2 |
| v31.005979 | 10000 | 20000 | 9.73 | 2 | 125 | 23.7 | 3.04 | 165.67 |
| v31.006652 | 20000 | 30000 | 35.48 | 273 | 2 | 80.4 | 652 | 7.87 |
| v31.006721 | 10000 | 20000 | 4 | 33 | 73 | 21.7 | 65.6 | 158.35 |
| v31.006827 | 0 | 10000 | 4 | 6 | 101 | 4 | 7.13 | 135.39 |
| v31.006841 | 10000 | 20000 | 5.14 | 321 | 1 | 5.14 | 407 | 2.21 |
| v31.007165 | 10000 | 20000 | 1 | 160 | 4 | 4.65 | 201 | 5.9 |

| Genome contig | Begin position of cluster | End position of cluster | 10 nt overlap intensity of long, uniquely mapping piRNAs | No. of long piRNAs mapping uniquely to the genome plus strand of the cluster | No. of long piRNAs mapping uniquely to the genome minus strand of the cluster | 10 nt overlap intensity of mapped piRNAs | No. of piRNAs mapping to the genome plus strand of the cluster | No. of piRNAs mapping to the genome minus strand of the cluster |
|---|---|---|---|---|---|---|---|---|
| v31.007275 | 10000 | 20000 | 4.82 | 110 | 3 | 9.32 | 159 | 3.84 |
| v31.008899 | 0 | 10000 | 1 | 1 | 153 | 1.96 | 2.38 | 884.35 |
| v31.008899 | 10000 | 20000 | 1 | 2 | 236 | 1 | 2.08 | 360.35 |
| v31.009965 | 0 | 10000 | 1 | 1 | 203 | 1 | 5.3 | 259.66 |
| v31.014194 | 0 | 10000 | 39.47 | 50 | 93 | 66.2 | 87.7 | 278.34 |
| v31.020292 | 10000 | 17329 | 1 | 1 | 140 | 2.26 | 2.14 | 198.36 |

The positions of the clusters were inferred from the Solexa sequencing of untreated planarians. All numbers in this table derive from this dataset. The first column is the id of the genome contig. The second column is the genomic start position of the cluster bin on the contig. The third column is the genomic end position of the cluster bin on the contig. The fourth to ninth columns indicate the intensity of read mappings and ten nucleotide overlapping events (*Methods*).

**Table S6. Overview of the novel planarian miRNAs**

| Novel miRNA | Mature sequence | Precursor sequence | Genomic location of interest |
|---|---|---|---|
| sme-let-7d | AGAGGUAGUGAUUCAAAAAGUU | CUUCCUAUAUUCAUUUUACUAGAGGUAGUGAUUCAAAAAGUU-AAUUUAACAUUUUGACUUUUUGAAUUAUUAUCUUUGAUAA-AUGAUUGAAAGACAUCA | |
| sme-miR-10a | AACCCUGUAGAUCCGAGUUAGAU | UAAUUUGUUUCCUUGAUAUAAACCCUGUAGAUCCGAGUUAGA-UGGUUUUUCCAAUUCGAAUCUCGGGGAAUUAUAUUAAUGA-GAUUAUCUUCU | |
| sme-miR-1175 | UGAGAUUCAACUCCUCCUACU | UAUUAAAAUUCUAAAAUGAGUGGAGAUGUUGAAAUUCAAAAA-AAUAAUUUCGUUUAUUUUGAGAUUCAACUCCUCCUACUAA-UUUGGGAUUUUCAAACU | |
| sme-miR-124d | UAAGGCACGCGGUAAGUGGGU | UUGGAAUAUGUUUUAAUAAAACAUUUACAAGCGAGCCUUAAU-UAGAAUUCAAUUAAGGCACGCGGUAAGUGGGUUGUUGAAA-UUCCUAUCAUCU | |
| sme-miR-124e | UAAGGCACGCUGUGAAUGCCA | UUCAAUAAAUUAUUCUACUGCCAUUCUCAGUUGGAGUCUUGA-UAUGAUCUAUUAAGGCACGCUGUGAAUGCCAAGGAAUUUU-AUUUCUCUUCU | clusters with sme-mir-8b |
| sme-miR-133a | UUGGUCCCCGUCAACCAGCUGU | AAUCGCAGUAUAUUUAUAGCAGCUGUUGACGAAGUAUCAAAA-UAGUUAAAUGAUGAUAUAAAUUAUUUGGUCCCCGUCAACC-AGCUGUUAUAAUGUACAUUAACAUCC | |
| sme-miR-1992 | UCAGCAGUUGUUCCAUUGAC | UUAUGAAAUAAAGAAUUUGUCUUGAACAACUGCUUAUGAUGA-AAAAAUGUCAUCAGCAGUUGUUCCAUUGACAAGUUAUAUU-UCAUUGAUAU | |
| sme-miR-1993 | UAUUAUGCUGUUAUUCAUGA | UUAACCUUCGAUAUGUAAUUGAUGAAUCAACAGUAUAAAAAC-GGAGUACAACAAAUAUUCGUAUUAUGCUGUUAUUCAUGAG-UUACUAUGGAUUUAUCGUCG | |
| sme-miR-2147a | CGGAAGACUCGCACGUGAC | UCUUCUUUGGAUGCCAACGGCGGAAGACUCGCACGUGACAUU-GCUAAUUUUUUUCCAUUUCACUGCGAGUGUUCCCGCGUUGA-UUCCAAUUCUCAUCA | |
| sme-miR-2147b | CGGAAAACUCGCACGUG | CCUUCUUUGGAUACUAACGGCGGAAAACUCGCACGUGUCCUC-GUCAACUUCACGAUUUCACGGCGAGUGUUCCCACGUUAUU-UCCAAUUCUCGACA | |
| sme-miR-2147c | CGGAAAACUCGCUCGUGCCAUCGU | UUGUUUUGCUGUAAUAACGGCGGAAAACUCGCUCGUGCCAUC-GUCGUCUUCACGAUGUCACGGCAGGUGUUCCCACGUUAAU-CCCAAUUUUCCUCA | close sense to unknown gene |
| sme-miR-2148 | AGAAAUUCUGUGGUCUUGAUU | GUAAGUAACCAGUUUAUCAGAGAAAUUCUGUGGUCUUGAUUG-UAAAUGAUAUUAUACUGGCCCACAAAAUUUCUUUUAUUAA-ACGUUUGAACUUCA | clusters with sme-mir-2177 |
| sme-miR-2149 | CGCCAAUGACAAACUGUUGAU | GCAGAAGAUCAAAUGAGUACCGCCAAUGACAAACUGUUGAUG-AAGACUUUGUCACAAUUAUCAGCAGUUGUCCAUUGACGUU-AUUUACCUUCGUUUUCAUCG | |
| sme-miR-2150 | CGGUUACGUGAUUAUUAGGGG | UCAAAUCGGACUUCUCUAUUCGGUUACGUGAUUAUUAGGGGC-AGCGGCUUCGCUGUCUGGUGAACUGUCACUAGCAAGAAGU-UCCUUAGUAGUCAUGUGACCUCAUGACCGAAUGCAAUUU-CUUC | |
| sme-miR-2151 | GAUUGCACUCAAUAUGGUCAGAU | UGCUUAAGAGAACUCUUUACAGAUCAUGUUAAGUGGAAUUUG-AUAACAGAUUGCACUCAAUAUGGUCAGAUUAGCUUUCUCU-CAACAGCA | |
| sme-miR-2152 | GGGUUCGAAGUAUGUCUCUCUUU | CUUAUUCUCAAACUUUAAAUGGGUUCGAAGUAUGUCUCUCUU-UAUUGGAGAUAUAUAUCGAAUACGAAUGACUAGUGAGCCA-UUUUU | |
| sme-miR-2153 | UAGGCAUAGUCUGUUGGUUACU | UAAAUUACGGAUUUUGAUUAGGUAGGCAUAGUCUGUUGGUUA-CUUCUAUGAAAUUGAAUUUGAUCAAUAAAUCACCCUUGAC-CAUGGACGCGACCCUACCUUUUAUUGAAUGAAAUAUUUCG | |
| sme-miR-2154 | UCAGCUGUACAAUUGGCAUUGUGU | UUCGAGUGAUUAUUAGAAUUUCAGCUGUACAAUUGGCAUUGU-GUAUGAUAAAUGAAUAUAUUAACACAAGUCUUUGUAUAGC-UUAUAUUUUUUAUCACUAAUCCACC | |
| sme-miR-2155 | GACACUGUUUUACACUUACCCGA | UAUUUGAUGUAAAGUGCAUCAGGAUAAGUGUAUAGCAGUGUU-AAUUUGACACUGUUUUACACUUACCCGAUGCUCAGAGAAA-UUUGAAUG | |
| sme-miR-2156 | UCUUACGGCCCCUAAACUUUUCA | UUUUUCAAGUCAUGAAAAUAAAAAGUUUUGGGUCAUAACAUA-AAUAAUUUUAAUAUCUUACGGCCCCUAAACUUUUCAUUUU-UCAAGAUUCUACAGCA | |

| Novel miRNA | Mature sequence | Precursor sequence | Genomic location of interest |
|---|---|---|---|
| sme-miR-2157 | UGAGUAUUGCAUCAAGAACCGA | UUAAAGGUGCAGUCAAAUUUUGAGUAUUGCAUCAAGAACCGA-AUGUUAAUAUCAGUUUUUGAUGAAUUACUUAAAUUAAGCU-CCUCUAAUCUCUU | clusters with sme-mir-216 |
| sme-miR-2158 | UGAGUAUUUUUUCGUAUACCGA | GGAAAGUAAUUUUAAUAUUUUGAGUAUUUUUUCGUAUACCGA-ACAGAUCAUCGUUGACUUUCGGUAACGAAGAAUACUUGGA-AUUGAGAAAUUUAUUCGUCG | clusters with sme-miR-747 |
| sme-miR-2159 | UGCCUUUUCUUUGAGUAGC | AAUGCAAAUGAAUUCAAAACUGCCUUUUCUUUGAGUAGCUGU-CCGCAGACAGGUGGAGCUGCCAAAUAAAGGGUGAUUUGGA-AUUCAUUCAUCGUAA | |
| sme-miR-216 | CAAUCUCAGCCUGUUAAUAGGAGUU | CCUGUCAUGAUUUAGCAAGCAAUCUCAGCCUGUUAAUAGGAG-UUUGGAAAUGUUUAAAUACUCCUAUAACGUCUGAUGAUUA-CUUGUCAAAUCAUUUUACCAA | clusters with sme-mir-2157 |
| sme-miR-2160 | UGGCGCUUAUGUGUAGAACCGG | AAAAUCUUUGUGAUUAAAUAUGGCGCUUAUGUGUAGAACCGG-UUGUACUAUAUUUUUAAACUGGUUUUUCAUGUAAGAGCCC-AAAUAGUAUCACAAAAAUCAUC | close to SMAD gene |
| sme-miR-2161 | UGUGCGUAGUAAUAGAGGUAUC | UUUUCCUUUGUCUGUAAUAGUAUCUUUUUUAUUACGUACAAA-AUGCUUGCUUAGGUUGACAUUUUGUGCGUAGUAAUAGAGG-UAUCAAUAUAGUCAAUUUAUCGUC | |
| sme-miR-2162 | UGUGCUUAUUUGUAUAUUGC | UGGCGUAGUCUAUGGUGUAUUGUGCUUAUUUGUAUAUUGCAC-UGUUAAUCUUUUGUAUUAUGCAAAUAUUCACAAUUUGACC-AAGCUUAAACAUCC | |
| sme-miR-2163 | UUGCAUAGUCUAUUAAGUUGGU | AUUACGAGAUUUUAUUUUCAUAACUUGAUAGAUAUAUGCAUC-AACGAGAUUUGCUAUUAUUUUGUUGCAUAGUCUAUUAAGU-UGGUCGAAAUGAUUUUAAUCGUCA | |
| sme-miR-2164 | UUGUGCGUGAUAUCAAGUAUAAU | CCAUAUUCAGGUAAUAAAAUUAUAUUUGAUUCACGUAGAGAU-UGAGAUCUAUCUUGUGCGUGAUAUCAAGUAUAAUAAUGUU-CUGACUCUUCAUCU | |
| sme-miR-2165 | UAAUUCGAUUCUUUAGAGUAUU | AAACUUUAUAUGAAUCCGAUCAUUCUACAGGAUCGAAUCAGU-UUGUAAAUUUGUAAACUAUCUCUUCAACUAAUUCGAUUCU-UUAGAGUAUUUGAUUUCAUUUUAUUCCUCG | |
| sme-miR-2166 | UUGGUCACUUGCUUUAUUCACUC | AUAUGAAAUCUAUUUUUCUCUAGAAUAAAACAGAUAACCAAU-CAUGUUGCAGUUGAAUCUGAUUGGUCACUUGCUUUAUUCA-CUCAUAAAUCUAAAUUAAAUAGC | intron of unknown gene |
| sme-miR-2167 | UGUGUUAAGCUGGUCUUUCAGA | UGUGAAUGCAAUUCUAUCUUUGAAAGACAAGUCUUAUACAUU-CUUAGUGUCAAACAUCCAAGUGUGUUAAGCUGGUCUUUCA-GAGCAUGAAUUCGUUAAUCGUC | close antisense to unknown gene |
| sme-miR-2168 | AUAUUCGUUAAGGUUGUAAC | AUGCCAGAAAUUCUGAUGCCAUAUUCGUUAAGGUUGUAACCA-GACACUUUGGUCACAACCUUUGACGAAUGAGGAGUCAUUU-UUGGUAAAUCAU | |
| sme-miR-2169 | AACUUUGAAAUUCGAUCUGAU | GAAUUUUUUAUCACUCUAACAACUUUGAAAUUCGAUCUGAUA-UAAAAUAUUAUAAGCUCGAUAUUUAAAGUGGUUUUCGUGA-UAUUUUUCGUCC | close sense to proliferation-associated 2G4 gene |
| sme-miR-2170 | ACAGUGAAAUUUUGUAGAGA | AUAAAUGGCAUUAUAUUUUCUCUACUAAAUUUCACUAUGAAU-UUAAGGUAUUCACAGUGAAAUUUUGUAGAGAAAAUAUAAU-AAAUUGCUUGU | |
| sme-miR-2171 | GUGAUGGAAAUCGCUAUUUCA | GAAAACGCAACAGUACAAAUGUGAUGGAAAUCGCUAUUUCAA-AUGCUCCCUUGAGAUUAUGCAGUGGCCGUUCAUGAUGUAA-GGCAUAAUAGAAA | |
| sme-miR-2172 | UACAUGUAUAGUAUUAUUUGCCU | AAUAAUUUUCCAUUGAUUUGGAAAUAAUGACUAUGCAUGGGA-CAUUAGUAGAGCUUGUACAUGUAUAGUAUUAUUUGCCUGU-CAUUGGAUUUAUCAGCGA | |
| sme-miR-2173 | UACAUGUACAUAUAAGUUGAAUUC | ACAAUUCUAGUAAUCAAUGGAAACAACUUGUAUAUGCAUACG-UAUAGAAAGAAAACAAUAUACAUGUACAUAUAAGUUGAAU-UCAUUGUUAUAAAAUCAUCCGA | |
| sme-miR-2174 | AUUUCGCUGUGAGUGUUCCC | UCUUUGGAACGUUAACAUCGGAAAACCCGCAUUGCGUUAUUG-UCAACCUCACAAUUUCGCUGUGAGUGUUCCCAUGUUAGCU-CCCAUUUUCGA | close sense to thimet oligopeptidase gene |
| sme-miR-2175 | UACUGAAUGUCUUUUUGCAAAUUGA | CUAUUGGUAGCCAUGAUGUUAUUUGCAAUAAAAUAUCAGUAA-AAUGACAUUACUAAGAAUUUUAUUUUUACUGAAUGUCUUU-UUGCAAAUUGACAAUGCUACUUGGCCCAUUA | partly overlapping with gene model, but mature, loop and star represented |
| sme-miR-2176 | AGAACAUUCUGAUUCACAA | UGAUCAAAAGCAAAACCUUUGUGAAUCGGAUUGUUCUUCAGU-UAAGAACAUUCUGAUUCACAAAGGUUUUGCUUCUUAUCACA | |

| Novel miRNA | Mature sequence | Precursor sequence | Genomic location of interest |
|---|---|---|---|
| sme-miR-2177 | AAAUGCCCAGUGAAUUUCUCGGU | AAAAAUCAUUGCACAUGAUACAAGAAAUUCAUUGGUUAAUUU-CAUGAUUAUGAAAAUGCCCAGUGAAUUUCUCGGUAGAUGA-ACAAUUAUUCAUUU | clusters with sme-mir-2148, partly overlapping with gene model |
| sme-miR-2178 | UAAUGACGAAAUGUCUCA | UACGAGACAUUUCAUCAUUGCAACAUUUCAUCAUUAUAGCAA-UUCAUCAAUGCACAUUCUAUAAUGACGAAAUGUCUCAAUG-AUGAAAUGUCUCAAUGA | |
| sme-miR-2179 | AGACUAUAGAGAUUUUUGCUAU | CAUACUAAGUCUAUUUGUAUUGCAAAAAUAUUUAUAUUUAAC-UCAUUUCUCUAAAUGAGACUAUAGAGAUUUUUGCUAUGCA-UUAGGCUGGAGCCGUCA | |
| sme-miR-2180 | UGGAAUGUGAUAAGGUGCUU | AUAUAUAUAUAUAUAUACCAACCCUCAUCCCAUACUCAUGGA-AAAAUGGAAUGUGAUAAGGUGCUUGAAUUAUACUUGAAAU-AUUG | |
| sme-miR-2181 | UAUGCUGUUAAUUAGCGAUUGGUU | UCAACUAUUCGAUUUUGUCAUAUGCUGUUAAUUAGCGAUUGG-UUUGAAAGAGUUUCUUUAACCAAUCAUAAUUCACAGCACU-UGUUUAAUAUCGUGAGCCAUCA | |
| sme-miR-315 | UUUUGAUUGUUGCUCUGAGAGUU | GUUGAGCGAGGCUUAUAGAAUUUUGAUUGUUGCUCUGAGAGU-UAAUAUAAUUUCAAUGAUAACUGUCUAACAACCAUCAAAU-UUCAAAGCGAUGCUUGUCAUCG | |
| sme-miR-36b | UCACCGGGUAGACAUUAAUCAUG | UUUUUUAACAGUCUAACUCUGGAUUGAUGUUAUUCGGUAUGA-UCUAGAAUAAAAAUUAUCACCGGGUAGACAUUAAUCAUGC-AGUUAGAGUCUGUAGUCCU | clusters with let-7b |
| sme-miR-36c | UCACCGGGUAGACAUUCAUU | AAUUUCAACAUAAAUUAGCGAUGAUUGUCCAACUGGUUUGGU-GUCGAGUUGAAAAAUGCAUCACCGGGUAGACAUUCAUUCU-CUAAAUUUAUCGUCAAUCUC | clusters with miR-36 |
| sme-miR-61b | UGACUAGAAUGUUCACCUUCUUU | CAAAGCGGCGAUUACACAGAAGAUCGUGUCGUUCUAGAUUAC-AUGGAUAUAUGUGACUAGAAUGUUCACCUUCUUUAGUAGA-AUCCGCGAUCAUCU | |
| sme-miR-753b | AAGCUUGGAUUGUGAUCUCA | UUUUAAUAAAGUAUAUUUAAAAGCUUGGAUUGUGAUCUCAUA-UAUUUAUGAGAUCAUUAUUCAAGCUCUUAAAUAAUACUUU-UCAUCAAC | intron of R3H domain containing protein |
| sme-miR-754b-1 | GUUGCUUGGGGGUAUUACU | GUUUAUCAUCCACCCAGCAUGUUGCUUGGGGGUAUUACUAUA-UUUAUUAAUAUAAUUUUAUCAUCAAGCAAUAUCAGAGGUG-AAAUAUUCAACU | |
| sme-miR-754c | GUUGCUUGGGGGUAUUAU | ACGAUGCAUUCACCCAGCAUGUUGCUUGGGGGUAUUAUUAUA-UUUAUUGAUAUAGUUUUAUCUUCAAGCAAUAUCAGAGGUG-GAAUAUUCAACA | |
| sme-miR-7d | UGGAAGACUAGUAAUUUUGUUGU | AAAAACUAUAAAACAAUCCUUGGAAGACUAGUAAUUUUGUUG-UAUGCGAAAUUUUAUCAGACAUUCAGUUUGAGAGGAUUUA-CAACAUUAUUUCUGUUUUCCAAGAAACUUAUAGUCAAUC-AACG | |
| sme-miR-87c | AUGAGCAAAUUAUCAAUUGACU | GUUCUCGGCUCGUGUCGCAGUCAAUUGAUAAUGGGCCCCAGC-CAUUCCAGGCGAAUGAUGAGCAAAUUAUCAAUUGACUUUC-CAUGGCCAUUGUCAUCU | |
| sme-miR-87d | GUGAGCAAAGUUUAAGACAUUC | UUACAUUGUUUUAGAAUUAUAAUGUCUUAUAUUUUGCUAUUA-UCAUUGUUUCAAUGAAAAUGGUGAGCAAAGUUUAAGAC-AUU-CAUAUUUUGAAAUUUUCUUCCU | |
| sme-miR-8b | UAAUACUGUCAGGUAAGAAUACU | UGCAUUCAUUCUAAUAAUUUGCUUCUUACUUGAUGGUAAGAG-ACAGAUUUUUGUUUAAUACUGUCAGGUAAGAAUACU-GACUAUUAGAAUUAUCUUCG | clusters with sme-mir-124e |
| sme-miR-96a | UUUGGCACUUAAGGAAUUGUCACU | CUUCAUUUGAGUAUAUAUCGUUUGGCACUUAAGGAAUUGUCA-CUAGUGUUCUUUUUAAAGUUCGGUGACAAUACUAAAGAGU-CAAGCGUUUCAACUCGAUUCGCUUCA | intron of unknown gene |
| sme-miR-96b | UUUGGCACUUAAGGAAUCGUCAC | UAUCAAACAGUUGUCUGAAAUUUGGCACUUAAGGAAUCGUCA-CCAGUUGCAUUGAUAUUGUGACAUUUCUAAGUGUCUAUUA-UCAGAGAUUGGUUAUCAUCU | |
| sme-miR-9a | UCUUUGGUUUUCUAGCUGUAUGAU | UUGUUUAAUUGACUAUAUGGUCUUUGGUUUUCUAGCUGUAUG-AUUGUAAAAUUUGUUGAAAUCACACACUAGAUUACCAAUU-UCAUAUAUUAUAUGUGAUCAUCU | |
| sme-miR-9b | UCUUUGGUUGUUUAGCUAUAUGA | ACAAAUGUCAAAUCAAGAUUUCUUUGGUUGUUUAGCUAUAUG-AAUGUCUAGGUCAUAUACUGCAUAAUCAAAGUUGUCUG-AUUUGAGUAAGCUUCC | |

| Novel miRNA | Mature sequence | Precursor sequence | Genomic location of interest |
|---|---|---|---|
| sme-putative-753.1 | AGGCUUGAAUUGUGAUCUCA | UUUUUAUAAAGCAUAUUUUAAGGCUUG-<br>AAUUGUGAUCUCAUAUAUUUAUGAGAUC-<br>AUUGUUCAAGCUCUUAAA-UAAUACUUUUCAUCAUUG | |
| sme-putative-753.2 | AGGCUUGAAUUGUGAUCUCC | UUUUAAUAAAGUAUAUUUUAAGGCUUGAAUUGUGAUCUCCUA-<br>UAUUUAUGAGA-<br>UCAUUAUUCAAGCUCUUAAAUAAUAUUUU-<br>CAUCAACG | |
| sme-putative-753.3 | AGGGUUUGGAUUGUGAUCUC | UUUUUAAUAAAGUAUAUUUUAGGGUUUGGAUUGUGAUCUCAU-<br>AUAUUUAUGAGAUCAUUAUCCAAGCUCUUAAAUAGGAAUU-<br>UAUGCCAAUU | |
| sme-putative-753.4 | AGGGUUUGGAUUGUGAUCUC | UUUUUAAUAAAGUAUAUUUUAGGGUUUGGAUUGUGAUCUCAU-<br>AUAUUUAUGAGAUCAUUAUUCAAGCUCUUAAAAAAUAC-<br>UUC-<br>AUUAACGUGU | |
| sme-putative-753.5 | GAGCUUGGAUUGUGAUCUU | UUUAAUUAAAGUAUAUUUUAGAGCUUGGAUUGUGAUCUUGUA-<br>UAUUUAUGAGAUCAUUAUUCAAGCUCUUAAAUAAUACUUU-<br>CAUCAUCA | |
| sme-putative-754.1 | GUUGCUUGGGGUAUUACUA | AUUUGUCAUCCACCCAGCAUGUUGCUUGGGGUAUUACUAUAU-<br>UUAUUAAUAUAGUUUUAUCUUCAAGCAAUAUCGGAGGUGG-<br>AAUAUUCAACA | |
| sme-putative-754.2 | GUUGCUUGGGGGUAUUACUAU | AUUUAGCAUCCACGCAGCAUGUUGCUUGGGGGUAUUACUAUA-<br>UUUAUUAAUAUAGUUUUAUCUUCAAGCAAUAUCAGAGGUG-<br>GAAUCAUCUUCA | |
| sme-putative-754.3 | GUUGCUAGGGGGUAUUACU | AUUUAACAUCCGCCCAGCAUGUUGCUAGGGGGUAUUACUAUA-<br>CUUAUUAAU-<br>AUAGUUUUAUCUUCAAGCA-<br>AUAUCAGAGGUGGAAUCAUCUUCA | |

The precursor sequences include 20-nt-long flanks.

**Table S7. Fold change of miRNA expression in the untreated, irradiated, and neoblast samples as measured by the 454 and Solexa sequencing**

| miRNA | 454, nb vs. un | 454, nb vs ir | 454, un vs. ir | solexa, un vs. ir |
|---|---|---|---|---|
| sme-bantam-a | 0.52 | 0.64 | 1.23 | 1.41 |
| sme-bantam-a* | 0.04 | 0.04 | 0.96 | 1.22 |
| sme-bantam-b | 0.8 | 0.83 | 1.03 | 1.43 |
| sme-bantam-b* | 2.73 | 1.55 | 0.57 | 1.44 |
| sme-bantam-c | 5.45 | 1.33 | 0.24 | 1.51 |
| sme-bantam-c* | 2.73 | 3.09 | 1.13 | 1.24 |
| sme-let-7a | 2.42 | 5.82 | 2.4 | 5.12 |
| sme-let-7a* | 0.5 | 1.03 | 2.08 | 1.34 |
| sme-let-7b | 2.12 | 3.69 | 1.74 | 2.05 |
| sme-let-7b* | 1.82 | 3.09 | 1.7 | 1.02 |
| sme-let-7c | 1.37 | 1.48 | 1.07 | 1.35 |
| sme-let-7c* | 2.73 | 1.55 | 0.57 | 2.12 |
| sme-let-7d | 1.42 | 2.23 | 1.57 | 2.23 |
| sme-let-7d* | 2.73 | 1.55 | 0.57 | 2.7 |
| sme-lin-4 | 2.03 | 2.12 | 1.04 | 1.25 |
| sme-lin-4* | 0.17 | 0.18 | 1.09 | 1 |
| sme-miR-10 | 0.31 | 0.42 | 1.37 | 0.76 |
| sme-miR-10* | 0.09 | 0.15 | 1.57 | 0.81 |
| sme-miR-10a | 0.22 | 0.29 | 1.32 | 0.69 |
| sme-miR-10a* | 2.73 | 1.55 | 0.57 | 0.9 |
| sme-miR-1175 | 0.17 | 0.15 | 0.86 | 0.44 |
| sme-miR-1175* | 0.19 | 0.39 | 1.99 | 0.66 |
| sme-miR-12 | 0.07 | 0.07 | 0.88 | 0.72 |
| sme-miR-12* | 0.68 | 1.55 | 2.27 | 1.95 |
| sme-miR-124a | 0.49 | 0.41 | 0.84 | 1.09 |
| sme-miR-124a* | 1.36 | 0.77 | 0.57 | 0.84 |
| sme-miR-124b | 0.69 | 0.64 | 0.94 | 1.14 |
| sme-miR-124b* | 0.11 | 0.09 | 0.8 | 0.89 |
| sme-miR-124c | 0.53 | 0.51 | 0.98 | 1.01 |
| sme-miR-124c* | 0.55 | 0.77 | 1.42 | 1.24 |
| sme-miR-124d | 1.91 | 0.83 | 0.44 | 1.65 |
| sme-miR-124d* | 0.55 | 0.77 | 1.42 | 0.44 |
| sme-miR-124e | 0.45 | 0.77 | 1.7 | 0.99 |
| sme-miR-124e* | 1.82 | 1.55 | 0.85 | 0.64 |
| sme-miR-125a | 0.21 | 0.35 | 1.68 | 1.22 |
| sme-miR-125a* | 0.07 | 0.08 | 1.17 | 1.29 |
| sme-miR-125b | 0.86 | 0.95 | 1.11 | 1.27 |
| sme-miR-125b* | 2.73 | 0.52 | 0.19 | 1.21 |
| sme-miR-13 | 4.88 | 4.57 | 0.94 | 5.46 |
| sme-miR-13* | 2.73 | 1.55 | 0.57 | 2.36 |
| sme-miR-133 | 0.11 | 0.19 | 1.68 | 0.42 |
| sme-miR-133* | 0.15 | 0.18 | 1.19 | 0.72 |
| sme-miR-133a | 0.68 | 1.16 | 1.7 | 0.76 |
| sme-miR-133a* | 2.73 | 1.55 | 0.57 | 1.52 |
| sme-miR-184 | 0.36 | 0.39 | 1.06 | 0.72 |
| sme-miR-184* | 0.11 | 0.42 | 3.71 | 0.69 |
| sme-miR-190a | 0.55 | 0.52 | 0.95 | 1.16 |
| sme-miR-190a* | 0.12 | 0.13 | 1.07 | 0.68 |
| sme-miR-190b | 0.39 | 0.77 | 1.99 | 1.06 |
| sme-miR-190b* | 0.12 | 0.13 | 1.16 | 0.75 |
| sme-miR-1992 | 0.8 | 0.75 | 0.94 | 2.23 |
| sme-miR-1992* | 0.41 | 1.16 | 2.84 | 2.02 |
| sme-miR-1993 | 0.22 | 0.34 | 1.58 | 0.66 |
| sme-miR-1993* | 0.23 | 0.52 | 2.27 | 0.65 |
| sme-miR-1a | 0.1 | 0.11 | 1.09 | 0.83 |
| sme-miR-1a* | 0.03 | 0.04 | 1.31 | 1.12 |
| sme-miR-1b | 0.28 | 0.29 | 1.03 | 0.81 |
| sme-miR-1b* | 0.07 | 0.07 | 0.96 | 0.84 |
| sme-miR-1c | 0.05 | 0.05 | 0.92 | 0.73 |
| sme-miR-1c* | 0.27 | 0.52 | 1.89 | 0.72 |
| sme-miR-2147a | 0.3 | 0.14 | 0.46 | 1.42 |
| sme-miR-2147a* | 2.73 | 1.55 | 0.57 | 1.15 |
| sme-miR-2147b | 0.71 | 1.16 | 1.63 | 1.06 |

| miRNA | 454, nb vs. un | 454, nb vs ir | 454, un vs. ir | solexa, un vs. ir |
|---|---|---|---|---|
| sme-miR-2147b* | 2.73 | 1.55 | 0.57 | 3.54 |
| sme-miR-2147c | 0.41 | 0.34 | 0.84 | 1.23 |
| sme-miR-2147c* | 0.68 | 0.77 | 1.13 | 4.55 |
| sme-miR-2148 | 0.12 | 0.52 | 4.35 | 1.31 |
| sme-miR-2148* | 0.68 | 1.55 | 2.27 | 1.77 |
| sme-miR-2149 | 0.37 | 0.56 | 1.51 | 2.12 |
| sme-miR-2149* | 0.41 | 0.42 | 1.03 | 1.46 |
| sme-miR-2150 | 0.18 | 0.19 | 1.06 | 1.5 |
| sme-miR-2150* | 0.45 | 1.55 | 3.4 | 0.63 |
| sme-miR-2151 | 0.04 | 0.09 | 2.24 | 1.21 |
| sme-miR-2151* | 0.25 | 0.26 | 1.04 | 0.87 |
| sme-miR-2152 | 65.99 | 8.51 | 0.13 | 0.21 |
| sme-miR-2152* | 2.73 | 1.55 | 0.57 | 0.1 |
| sme-miR-2153 | 0.28 | 0.33 | 1.15 | 1.12 |
| sme-miR-2153* | 3.59 | 1.93 | 0.54 | 0.75 |
| sme-miR-2154 | 0.05 | 0.09 | 1.67 | 0.77 |
| sme-miR-2154* | 0.91 | 1.55 | 1.7 | 0.66 |
| sme-miR-2155 | 2.73 | 1.55 | 0.57 | 7.08 |
| sme-miR-2155* | 2.73 | 1.55 | 0.57 | 1.53 |
| sme-miR-2156 | 0.23 | 0.33 | 1.42 | 0.77 |
| sme-miR-2156* | 0.68 | 0.39 | 0.57 | 0.7 |
| sme-miR-2157 | 0.07 | 0.07 | 0.98 | 0.68 |
| sme-miR-2157* | 0.55 | 1.55 | 2.84 | 0.59 |
| sme-miR-2158 | 0.02 | 0.02 | 0.92 | 0.71 |
| sme-miR-2158* | 0.34 | 0.77 | 2.27 | 0.49 |
| sme-miR-2159 | 0.04 | 0.03 | 0.81 | 0.66 |
| sme-miR-2159* | 0.08 | 0.11 | 1.35 | 0.78 |
| sme-miR-216 | 0.13 | 0.19 | 1.52 | 0.67 |
| sme-miR-216* | 0.04 | 0.06 | 1.45 | 1.1 |
| sme-miR-2160 | 5.02 | 7.91 | 1.58 | 1.93 |
| sme-miR-2160* | 2.73 | 1.55 | 0.57 | 1.77 |
| sme-miR-2161 | 0.61 | 0.73 | 1.21 | 0.7 |
| sme-miR-2161* | 2.73 | 1.55 | 0.57 | 1.77 |
| sme-miR-2162 | 0.07 | 0.08 | 1.04 | 0.9 |
| sme-miR-2162* | 1.36 | 1.55 | 1.13 | 1.14 |
| sme-miR-2163 | 0.34 | 0.34 | 1.01 | 1.02 |
| sme-miR-2163* | 1.36 | 1.55 | 1.13 | 1.33 |
| sme-miR-2164 | 0.19 | 0.21 | 1.1 | 0.55 |
| sme-miR-2164* | 2.73 | 1.55 | 0.57 | 0.46 |
| sme-miR-2165 | 0.91 | 1.55 | 1.7 | 0.93 |
| sme-miR-2165* | 1.36 | 0.39 | 0.28 | 0.72 |
| sme-miR-2166 | 2.73 | 1.55 | 0.57 | 0.89 |
| sme-miR-2166* | 5.45 | 3.09 | 0.57 | 2.66 |
| sme-miR-2167 | 0.3 | 0.31 | 1.02 | 0.79 |
| sme-miR-2167* | 2.73 | 1.55 | 0.57 | 0.89 |
| sme-miR-2168 | 2.73 | 1.55 | 0.57 | 0.69 |
| sme-miR-2168* | 1.36 | 1.55 | 1.13 | 0.82 |
| sme-miR-2169 | 2.73 | 1.55 | 0.57 | 1.1 |
| sme-miR-2169* | 2.73 | 1.55 | 0.57 | 0.68 |
| sme-miR-2170 | 2.73 | 1.55 | 0.57 | 1.3 |
| sme-miR-2170* | 2.73 | 1.55 | 0.57 | 0.3 |
| sme-miR-2171 | 1.36 | 0.39 | 0.28 | 1.36 |
| sme-miR-2171* | 2.73 | 1.55 | 0.57 | 0.44 |
| sme-miR-2172 | 1.36 | 1.55 | 1.13 | 2.21 |
| sme-miR-2172* | 2.73 | 1.55 | 0.57 | 4.43 |
| sme-miR-2173 | 2.73 | 3.09 | 1.13 | 1.15 |
| sme-miR-2173* | 1.36 | 1.55 | 1.13 | 1.77 |
| sme-miR-2174 | 2.73 | 1.55 | 0.57 | 1.13 |
| sme-miR-2174* | 2.73 | 1.55 | 0.57 | 0.44 |
| sme-miR-2175 | 2.73 | 1.55 | 0.57 | 0.89 |
| sme-miR-2175* | 2.73 | 1.55 | 0.57 | 1.77 |
| sme-miR-2176 | 2.73 | 1.55 | 0.57 | 0.89 |
| sme-miR-2176* | 2.73 | 1.55 | 0.57 | 1.01 |
| sme-miR-2177 | 2.73 | 1.55 | 0.57 | 1.42 |
| sme-miR-2177* | 1.36 | 1.55 | 1.13 | 1.77 |

| miRNA | 454, nb vs. un | 454, nb vs ir | 454, un vs. ir | solexa, un vs. ir |
|---|---|---|---|---|
| sme-miR-2178 | 1.36 | 1.55 | 1.13 | 1.47 |
| sme-miR-2178* | 2.73 | 1.55 | 0.57 | 0.59 |
| sme-miR-2179 | 0.25 | 0.39 | 1.56 | 1.49 |
| sme-miR-2179* | 2.73 | 1.55 | 0.57 | 1.77 |
| sme-miR-2180 | 5.45 | 2.06 | 0.38 | 1.03 |
| sme-miR-2180* | 2.73 | 1.55 | 0.57 | 1.77 |
| sme-miR-2181 | 1.36 | 1.55 | 1.13 | 0.51 |
| sme-miR-2181* | 2.73 | 1.55 | 0.57 | 1.77 |
| sme-miR-219 | 0.13 | 0.16 | 1.22 | 0.76 |
| sme-miR-219* | 0.68 | 0.26 | 0.38 | 0.92 |
| sme-miR-277a | 0.25 | 0.39 | 1.56 | 0.85 |
| sme-miR-277a* | 1.36 | 1.55 | 1.13 | 0.62 |
| sme-miR-277b | 2.73 | 1.55 | 0.57 | 0.93 |
| sme-miR-277b* | 0.08 | 0.09 | 1.18 | 0.59 |
| sme-miR-277c | 0.15 | 0.18 | 1.22 | 0.93 |
| sme-miR-277c* | 0.21 | 0.12 | 0.57 | 0.95 |
| sme-miR-277d | 0.08 | 0.08 | 1.03 | 0.9 |
| sme-miR-277d* | 0.02 | 0.02 | 0.95 | 0.76 |
| sme-miR-278 | 0.03 | 0.03 | 1.1 | 0.69 |
| sme-miR-278* | 0.24 | 0.33 | 1.34 | 0.77 |
| sme-miR-281 | 0.29 | 0.33 | 1.12 | 0.83 |
| sme-miR-281* | 0.12 | 0.22 | 1.86 | 0.88 |
| sme-miR-2a-1 | 3.73 | 4.27 | 1.14 | 1.03 |
| sme-miR-2a-1* | 0.18 | 0.17 | 0.94 | 0.79 |
| sme-miR-2a-2 | 3.73 | 4.27 | 1.14 | 1.03 |
| sme-miR-2a-2* | 0.09 | 0.1 | 1.12 | 0.71 |
| sme-miR-2b | 1.96 | 1.5 | 0.77 | 1.31 |
| sme-miR-2b* | 0.39 | 1.55 | 3.97 | 0.77 |
| sme-miR-2c | 0.1 | 0.08 | 0.76 | 0.75 |
| sme-miR-2c* | 0.45 | 0.77 | 1.7 | 0.88 |
| sme-miR-2d | 3.41 | 3.55 | 1.04 | 1.91 |
| sme-miR-2d* | 2.73 | 1.55 | 0.57 | 1.49 |
| sme-miR-315 | 2.73 | 1.55 | 0.57 | 0.84 |
| sme-miR-315* | 2.73 | 1.55 | 0.57 | 0.41 |
| sme-miR-31a | 0.28 | 0.39 | 1.37 | 1.09 |
| sme-miR-31a* | 2.73 | 1.55 | 0.57 | 0.73 |
| sme-miR-31b | 0.16 | 0.24 | 1.55 | 1.32 |
| sme-miR-31b* | 0.55 | 0.77 | 1.42 | 0.89 |
| sme-miR-36 | 1.41 | 1.16 | 0.82 | 0.71 |
| sme-miR-36* | 0.18 | 0.16 | 0.9 | 0.87 |
| sme-miR-36b | 3.84 | 3.76 | 0.98 | 0.9 |
| sme-miR-36b* | 2.73 | 1.55 | 0.57 | 0.7 |
| sme-miR-36c | 0.9 | 0.72 | 0.8 | 0.64 |
| sme-miR-36c* | 2.73 | 1.55 | 0.57 | 0.66 |
| sme-miR-61 | 0.18 | 0.22 | 1.21 | 1.12 |
| sme-miR-61* | 1.36 | 0.52 | 0.38 | 0.52 |
| sme-miR-61b | 0.03 | 0.05 | 1.67 | 0.95 |
| sme-miR-61b* | 0.24 | 0.21 | 0.9 | 1.6 |
| sme-miR-67 | 0.06 | 0.07 | 1.27 | 0.81 |
| sme-miR-67* | 0.45 | 1.55 | 3.4 | 0.75 |
| sme-miR-71a-1 | 1.94 | 1.77 | 0.91 | 1.27 |
| sme-miR-71a-1* | 0.07 | 0.05 | 0.7 | 0.62 |
| sme-miR-71a-2 | 1.94 | 1.76 | 0.91 | 1.27 |
| sme-miR-71a-2* | 0.04 | 0.04 | 1.08 | 0.84 |
| sme-miR-71b | 3.02 | 3.96 | 1.31 | 2.26 |
| sme-miR-71b* | 0.77 | 0.61 | 0.79 | 2.15 |
| sme-miR-71c | 1.11 | 1 | 0.9 | 0.99 |
| sme-miR-71c* | 0.27 | 0.22 | 0.81 | 0.62 |
| sme-miR-745 | 0.33 | 0.43 | 1.33 | 0.86 |
| sme-miR-745* | 2.73 | 1.55 | 0.57 | 0.8 |
| sme-miR-746 | 0.31 | 0.3 | 0.98 | 0.96 |
| sme-miR-746* | 0.1 | 0.14 | 1.35 | 0.83 |
| sme-miR-747 | 0.07 | 0.22 | 3.08 | 0.79 |
| sme-miR-747* | 0.03 | 0.03 | 1.01 | 0.86 |
| sme-miR-748 | 10.91 | 1.55 | 0.14 | 0.75 |

| miRNA | 454, nb vs. un | 454, nb vs ir | 454, un vs. ir | solexa, un vs. ir |
|---|---|---|---|---|
| sme-miR-748* | 2.73 | 1.55 | 0.57 | 0.53 |
| sme-miR-749 | 0.93 | 0.84 | 0.9 | 0.65 |
| sme-miR-749* | 2.73 | 0.77 | 0.28 | 0.39 |
| sme-miR-750 | 0.22 | 0.28 | 1.27 | 0.76 |
| sme-miR-750* | 0.39 | 0.52 | 1.32 | 0.89 |
| sme-miR-751 | 0.21 | 0.77 | 3.69 | 0.96 |
| sme-miR-751* | 2.73 | 1.55 | 0.57 | 1.77 |
| sme-miR-752 | 6.95 | 9.78 | 1.41 | 6.21 |
| sme-miR-752* | 1.64 | 0.66 | 0.41 | 1.44 |
| sme-miR-753 | 0.68 | 1.55 | 2.27 | 1.63 |
| sme-miR-753* | 0.91 | 0.69 | 0.76 | 1.25 |
| sme-miR-753b | 0.3 | 0.77 | 2.55 | 1.72 |
| sme-miR-753b* | 1.2 | 1.55 | 1.28 | 1.21 |
| sme-miR-754 | 0.11 | 0.19 | 1.7 | 0.41 |
| sme-miR-754* | 2.73 | 1.55 | 0.57 | 0.72 |
| sme-miR-754b-1 | 0.14 | 0.24 | 1.7 | 0.78 |
| sme-miR-754b-1* | 2.73 | 1.55 | 0.57 | 2.21 |
| sme-miR-754c | 0.18 | 0.32 | 1.82 | 0.74 |
| sme-miR-754c* | 2.73 | 1.55 | 0.57 | 0.69 |
| sme-miR-755 | 0.04 | 0.05 | 1.3 | 0.76 |
| sme-miR-755* | 0.03 | 0.04 | 1.23 | 0.77 |
| sme-miR-756 | 2.02 | 2.26 | 1.12 | 1.57 |
| sme-miR-756* | 0.91 | 0.39 | 0.43 | 0.74 |
| sme-miR-79 | 1.11 | 0.99 | 0.89 | 1.24 |
| sme-miR-79* | 0.55 | 1.55 | 2.84 | 1.49 |
| sme-miR-7a | 0.91 | 1.55 | 1.7 | 1.3 |
| sme-miR-7a* | 2.73 | 1.55 | 0.57 | 0.35 |
| sme-miR-7b | 0.8 | 1.19 | 1.49 | 1.35 |
| sme-miR-7b* | 2.73 | 1.55 | 0.57 | 1.59 |
| sme-miR-7c | 0.93 | 1.29 | 1.39 | 1.01 |
| sme-miR-7c* | 1.5 | 2.06 | 1.37 | 1.37 |
| sme-miR-7d | 0.88 | 0.7 | 0.8 | 1.97 |
| sme-miR-7d* | 1.36 | 0.77 | 0.57 | 0.89 |
| sme-miR-8 | 1.37 | 1.57 | 1.15 | 0.89 |
| sme-miR-8* | 1.02 | 1.16 | 1.13 | 0.87 |
| sme-miR-87a | 0.27 | 0.24 | 0.89 | 1.08 |
| sme-miR-87a* | 0.23 | 0.18 | 0.76 | 0.71 |
| sme-miR-87b | 0.21 | 0.39 | 1.86 | 0.74 |
| sme-miR-87b* | 3.64 | 2.48 | 0.68 | 0.97 |
| sme-miR-87c | 0.1 | 0.08 | 0.81 | 0.93 |
| sme-miR-87c* | 0.55 | 0.44 | 0.81 | 0.79 |
| sme-miR-87d | 0.87 | 2.48 | 2.84 | 0.79 |
| sme-miR-87d* | 0.78 | 1.55 | 1.99 | 1 |
| sme-miR-8b | 0.36 | 0.5 | 1.38 | 0.82 |
| sme-miR-8b* | 2.73 | 1.55 | 0.57 | 1.18 |
| sme-miR-92 | 0.34 | 1.03 | 3.03 | 0.81 |
| sme-miR-92* | 0.02 | 0.02 | 0.88 | 0.69 |
| sme-miR-96a | 0.14 | 0.13 | 0.95 | 0.92 |
| sme-miR-96a* | 2.73 | 1.55 | 0.57 | 0.76 |
| sme-miR-96b | 0.16 | 0.14 | 0.88 | 0.93 |
| sme-miR-96b* | 2.73 | 0.52 | 0.19 | 0.89 |
| sme-miR-9a | 0.17 | 0.22 | 1.3 | 0.71 |
| sme-miR-9a* | 2.73 | 1.03 | 0.38 | 0.84 |
| sme-miR-9b | 0.13 | 0.26 | 1.99 | 0.94 |
| sme-miR-9b* | 0.91 | 0.77 | 0.85 | 0.8 |
| sme-putative-753.1 | 0.61 | 0.44 | 0.73 | 0.69 |
| sme-putative-753.1* | 0.78 | 0.28 | 0.36 | 1.07 |
| sme-putative-753.2 | 0.91 | 0.42 | 0.46 | 1.1 |
| sme-putative-753.2* | 1.2 | 1.55 | 1.28 | 1.21 |
| sme-putative-753.3 | 0.08 | 0.08 | 1.01 | 0.89 |
| sme-putative-753.3* | 2.73 | 1.55 | 0.57 | 0.73 |
| sme-putative-753.4 | 0.08 | 0.08 | 1.01 | 0.89 |
| sme-putative-753.4* | 1.2 | 1.58 | 1.31 | 1.18 |
| sme-putative-753.5 | 0.21 | 0.14 | 0.67 | 1.02 |
| sme-putative-753.5* | 1.2 | 1.58 | 1.31 | 1.22 |

| miRNA | 454, nb vs. un | 454, nb vs ir | 454, un vs. ir | solexa, un vs. ir |
|---|---|---|---|---|
| sme-putative-754.1 | 0.05 | 0.09 | 1.64 | 0.59 |
| sme-putative-754.1* | 2.73 | 1.55 | 0.57 | 0.65 |
| sme-putative-754.2 | 0.14 | 0.24 | 1.7 | 0.78 |
| sme-putative-754.2* | 2.73 | 1.55 | 0.57 | 0.77 |
| sme-putative-754.3 | 0.17 | 0.32 | 1.89 | 0.97 |
| sme-putative-754.3* | 2.73 | 0.77 | 0.28 | 0.69 |

Normalization as described in the *Methods* of *SI Text*.

**Table S8. Sequences of the $^{32}$P-radiolabeled oligodeoxynucleotides used as Northern blot probes for the validation of miRNA or piRNA candidates**

| | | |
|---|---|---|
| **SMECAND-02** | AACTTTTTGAATCACTACCTCT | >sme-miR-candidate-02 |
| | | AGAGGUAGUGAUUCAAAAAGUU |
| **SMECAND-05** | AGTCAATTGATAATTTGCTCAT | >sme-miR-candidate-05 |
| | | AUGAGCAAAUUAUCAAUUGACU |
| **SMECAND-09** | TCTGACCATATTGAGTGCAATC | >sme-miR-candidate-09 |
| | | GAUUGCACUCAAUAUGGUCAGA |
| **SMECAND-12** | TGAATGTCTTAAACTTTGCTCA | >sme-miR-candidate-12 |
| | | GUGAGCAAAGUUUAAGACAUUCA |
| **SMECAND-13** | AGTAATACCCCCAAGCAAC | >sme-miR-candidate-13 |
| | | GUUGCUUGGGGGUAUUACU |
| **SMECAND-15** | ACCCACTTACCGCGTGCCTTA | >sme-miR-candidate-15 |
| | | UAAGGCACGCGGUAAGUGGGU |
| **SMECAND-16** | TGGCATTCACAGCGTGCCTTA | >sme-miR-candidate-16 |
| | | UAAGGCACGCUGUGAAUGCCA |
| **SMECAND-17** | AGTATTCTTACCTGACAGTATTA | >sme-miR-candidate-17 |
| | | UAAUACUGUCAGGUAAGAAUACU |
| **SMECAND-18** | CTCATGAATAACAGCATAATA | >sme-miR-candidate-18 |
| | | UAUUAUGCUGUUAUUCAUGAG |
| **SMECAND-19** | ATGATTAATGTCTACCCGGTGA | >sme-miR-candidate-19 |
| | | UCACCGGGUAGACAUUAAUCAUG |
| **SMECAND-20** | GTCAATGGAACAACTGCTGA | >sme-miR-candidate-20 |
| | | UCAGCAGUUGUUCCAUUGAC |
| **SMECAND-21** | ACACAATGCCAATTGTACAGCTGA | >sme-miR-candidate-21 |
| | | UCAGCUGUACAAUUGGCAUUGUGU |
| **SMECAND-23** | TGAAAAGTTTAGGGGCCGTAAGA | >sme-miR-candidate-23 |
| | | GGUAGGCAUAGUCUGUUGGUUAC |
| | | >sme-piR-2618212 |
| **piR-2618212** | CATTCACTACTTCATTCTTTTCACATCCA | UUGGAUGUGAAAAGAAUGAAGUAGUGAAUGAG |
| | | >sme-piR-1139655 |
| **piR-1139655** | CTGTTGTTTATTAATTCCACATATTCAACTTA | UAAGUUGAAUAUGUGGAAUUAAUAAACAACAGUU |
| | | >sme-piR-2038493 |
| **piR-2038493** | GCAATCGACCTCCAACGATAATCTTCGCA | UGCGAAGAUUAUCGUUGGAGGUCGAUUGCCC |
| | | >sme- piR-1816361 |
| **piR-1816361** | TGGGTATTTGTGTTCATAGCACTTGCTTCA | UGAAGCAAGUGCUAUGAACACAAAUACCCA |