

Supplemental Information

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Expanding the MicroRNA Targeting Code: Functional Sites with Centered Pairing

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SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Analysis of miRNA Conservation

Out of 223 miRNA genomic loci producing 197 mouse miRNAs conserved in other mammals (Friedman et al., 2009), 203 miRNA loci producing miRNAs with 5' ends validated from a large scale profiling of mouse miRNAs (Chiang et al., 2010) were used in the analysis of Figure 1A.

Microarray Analyses

To investigate the efficacy of ectopic interactions involving centered sites and other potential sites with related pairing schemes, microarray data reporting the effects of miRNA or siRNA transfections of HeLa cells were collected, considering arrays only for 100 nM transfections and 24 hr time points (Lim et al., 2005; Birmingham et al., 2006; Jackson et al., 2006a; Jackson et al., 2006b; Schwarz et al., 2006; Grimson et al., 2007; Anderson et al., 2008). To identify experiments in which the mi/siRNAs were loaded and active within the silencing complex, the data sets were screened for the efficacy of canonical seed-matched 3' UTR sites using previously described methods (Grimson et al., 2007), requiring that the canonical 8-mer site for the transfected mi/siRNA be associated with downregulated mRNAs with high statistical significance ($P < 0.0001$, K-S test, Table S1). For each of the 78 data sets that passed the screen, cumulative distributions of mRNA changes were generated for each site type, as described (Grimson et al., 2007), considering messages with only one 3' UTR site compared to those with no 3' UTR site (and considering only 7-mer sites that were not part of 8-mer sites, and only 6-mer sites that were not part of 7- or 8-mer sites), and then these 78 distributions were then combined into one composite distribution. To further evaluate the efficacy of centered sites, the centered-site analysis was repeated with 10 cohorts of chimeric control miRNAs, generated using the top 200 miRNAs sequenced from HeLa cells. Analyses of microarray data comparing wild-type and maternal-zygotic *dicer* embryos (Giraldez et al., 2006) were the same, except performed on sites for 21 endogenous miRNAs. These miRNAs were chosen from the top 100 miRNAs sequenced from zebrafish in a screen for detectable miRNA impact, which required that the miRNA canonical 8-mer site be associated with mRNAs derepressed in *dicer* embryos with statistical significance ($P < 0.01$, Table S4). Cohorts of chimeric control miRNAs were generated starting from the top 200 miRNAs expressed in zebrafish.

Reporter Assays

Luciferase reporter constructs were prepared as described (Grimson et al., 2007). Reporter assays were performed as described (Farh et al., 2005). Sequences of reporter inserts and mutants are provided (Supplemental Information).

In Vitro Cleavage Assays

In vitro cleavage reactions were performed essentially as described (Haley and Zamore, 2004; Shin, 2008). mRNA fragments transcribed in vitro and 5'-cap-radiolabeled (Epicentre). HeLa cytoplasmic S100 extract was prepared (Dignam et al., 1983), extensively dialyzed against Buffer D (20 mM Hepes-KOH, pH 7.9, 10% glycerol, 100 mM KCl, 0.2 mM EDTA, 0.5 mM PMSF and 0.5 mM DTT), quick-frozen and stored at -80°C . ATP was further depleted from the extract using hexokinase and glucose (Shin, 2008). NEM and DTT treatment was performed as described (Haley and Zamore, 2004). The amount of endogenous miR-21 in the reaction was 0.06–0.08 nM, as measured by quantitative northern blotting, and the target RNA was present at 0.8 nM.

Gene-Specific 5'-RACE

Uncapped 5' ends of *GSTM3* mRNA degradation products were identified using the 5'-RACE kit (Invitrogen), as described (Jones-Rhoades and Bartel, 2004). *XRNI* mRNA was knocked-down more than 90%, as confirmed by RT-PCR, as described (Aleman et al., 2007). Gene-specific primers for nested amplifications were 5'-ATGGACATGGAGAGTGAGTAGGGCAGA and 5'-CATCCTAGCTTAAGGGTAACTTCTTCCGTTT.

Construction of Degradome Libraries

Degradome libraries were constructed as described (Addo-Quaye et al., 2008) with modifications. Briefly, for the HeLa cell library, cells were treated with two sets of siRNAs targeting *XRNI* for 2 days (Aleman et al., 2007), and total RNA was isolated with TriReagent (Ambion). Poly(A)+ RNA was selected with the Oligotex mRNA kit (QIAGEN), and 5 μg of polyA+ RNA was ligated to a 5' DNA/RNA hybrid adapter containing an MmeI restriction site using T4 RNA ligase. Residual 5' adapter was removed by an additional polyA+ selection, and the ligated products were reverse transcribed with random primers (Cheng et al., 2005). RNA was removed by treatment with NaOH. After second-strand synthesis, 20–21 nt tags, which were obtained by digestion with MmeI, were ligated to a 3' adapter containing a Solexa sequencing primer. An amplified cleavage library was sequenced with an Illumina genome analyzer. For the human brain library, poly(A)+ mRNA was purchased from Ambion. Small RNAs were purified from total RNA (brain sample purchases from Ambion) and libraries were prepared as described (Grimson et al., 2008).

Processing of Degradome Tags and Small RNAs

Linker sequences were removed, requiring a match to at least five of the six most proximal linker nucleotides. After filtering out tags shorter than 20 nt, degradome tags were mapped to RNAs annotated in the ENSEMBL (<http://www.ensembl.org/>), requiring a perfect match. To find “multiple loci tags,” and tags that did not map to annotated RNAs, filtered tags were mapped to the human genome (hg18, <http://genome.ucsc.edu/>). When determining TPRs, filtered tags were mapped to a curated set of mRNAs (Baek et al., 2008). Small RNA sequences from same total RNA samples were mapped to the human genome (hg18), requiring a perfect match, and reads co-localizing to annotated miRNA loci (miRBase, version 11.0) were counted. If ≥ 20 reads had 5' termini matching a 5' terminus of a miRNA, that miRNA was categorized as expressed. If no reads mapped to the miRNA, it was used as a nonexpressed, control miRNA. Expressed mRNAs were those represented by at least one degradome tag.

miRNA:site Duplexes

When searching for miRNA:site duplexes, distinct mRNAs and miRNAs were selected to avoid over-counting duplexes involving miRNA families or mRNA isoforms. miRNA-like control sequences were also generated from the distinct miRNA set. For distinct miRNAs, all human miRNAs and miRNA* sequences (miRBase 11.0) were aligned and classified into groups whose members differed from each other at ≤ 5 positions. The miRNA with the lowest miRBase annotation number was selected as the representative from each group. For distinct mRNAs, the mRNA isoform with the longest 3' UTR (or, if all 3' UTRs were of the same length, a randomly chosen isoform) was selected from a previously filtered set of RefFlat and H-INV annotations (Baek et al., 2008). The total number of distinct human mRNAs was 19,109. For 624 distinct miRNAs (out of 847 human miRNA and miRNA* annotations), we found 37,840 distinct miRNA:site duplexes with at least seven consecutive base pairs, 13 base pairs in total, and alignment penalty score ≤ 15 . For mouse, a search using 418 distinct miRNAs and 17,162 distinct mRNAs found 26,495 miRNA:site duplexes.

CA Score

To search for orthologous sites, we used 165 distinct miRNAs conserved among mammals and a 6-way genome alignment (human, mouse, rat, dog, horse, and pig) from the UCSC genome browser (hg18, <http://genome.ucsc.edu/>). Alignment penalty scores were determined and the second worst score rather than the worst score was selected as the CA score to accommodate some genome-alignment errors, incomplete genome sequences, and species-specific losses.

Generation of miRNA-like Control Sequences

To generate controls with the same seed composition and same trinucleotide composition as authentic miRNAs, chimeric miRNA sequences were created by reciprocally recombining, using the link between nucleotides 10 and 11 as the crossover breakpoint, two miRNAs randomly chosen (without replacement) from miRNA pairs with the same dinucleotide at positions 10 and 11 considering only our set of distinct miRNAs, i.e, miRNA that differ at ≥ 5 positions, including at ≥ 1 seed position. Ten chimeric miRNA cohorts were generated to estimate the signal-to-background ratios.

Analysis of Array Data for Knockdown of Dicer1 and Drosha in S2 Cell

The microarray data comparing wild-type to *dicer* and *drosha* knockdown cells (Kadener et al., 2009) were retrieved from NCBI GEO data set (accession number: GSE14215) and preprocessed using tiling analysis software (TAS). We collected probe sets that mapped to gene annotations (exonic regions, version dm2) and calculated mean values of probes for each gene. To find the miRNAs for which knocking down the biogenesis components had a detectable impact, the top 100 miRNA (or miRNA*) species sequenced from S2 cells (Czech et al., 2008) were screened, requiring that the miRNA canonical 8-mer site be associated with mRNAs derepressed in knockdown cells with statistical significance ($P < 0.01$, Tables S3 and S4). This screen yielded 28 and 48 miRNAs with detectable impact for *dicer* and *drosha* knockdowns, respectively. Cohorts of chimeric control miRNAs were generated starting from top 100 miRNAs expressed in S2 cells.

Sequences of 3' UTR Fragments in Reporter Constructs in Figure 1G and in Figure S11

Reporter plasmids encoded Renilla luciferase were in pIS2 (Grimson et al., 2007). Sequences of 3' UTR fragments are listed with their GenBank accession number and inserted into EcoRV restriction enzyme site in pIS2. The centered matches are indicated (bold type), as are mutated residues (bold italics).

PLXNA1; NM_032242

TCCAGCACCCATGCCCCTGCACCGCTGCCATCCTCAGATT**TCACCGCGTGCT**TCTGCGCGGCCGAGGCCGGAGCACCACATCC
ACCTC

PLXNA1mut

TCCAGCACCCATGCCCCTGCACCGCTGCCATCCTCAGATT**TCACCGCG*****ACG***TCTGCGCGGCCGAGGCCGGAGCACCACATCC
ACCTC

RAPTOR; NM_020761

GCTCCACCCTGGAGCCCACCCCATGGGG**CACCGCGTGCCG**CCTGCACGTGGGCTGTCTTCACAGGTCTGATGTGAAAATTCA
ATCACGACG

RAPTORmut

GCTCCACCCTGGAGCCCACCCCATGGGG**CACCGCG*****ACG***CGCCTGCACGTGGGCTGTCTTCACAGGTCTGATGTGAAAATTCA
ATCACGACG

VAMP1; NM_014231

GCAGGACTTGTTCATCCCTAGAAAGCAGTCCCATGTGCTTCTAGCTGGAAGGAGCTTTCT**CTTCTTTACAT**TTTCTACAGCA
TTCTGTATGAGGGCACC

VAMP1mut

GCAGGACTTGTTCATCCCTAGAAAGCAGTCCCATGTGCTTCTAGCTGGAAGGAGCTTTCTCTTCTTT**AGTA**TTTCTACAGCA
TTCTGTATGAGGGCACC

ZNF586; NM_017652

CCAGATCAAGGTGTTATGAGTGTGACAAATGGGGAATATTCTTTAGCTAGAAATGCTAGCT**TTCTTTACAT**AAAAAGAGTGCTCC
CACTGAAGAAGTGCCTTTTGAGTGC

ZNF586mut

CCAGATCAAGGTGTTATGAGTGTGACAAATGGGGAATATTCTTTAGCTAGAAATGCTAGCTTCTTT**AGTA**AAAAAGAGTGCTCC
CACTGAAGAAGTGCCTTTTGAGTGC

Reporter Assay with S2 Cells

Luciferase reporter constructs were prepared as described (Ruby et al., 2007). Reporter inserts and mutants, provided above, were cloned into Sac I and Not I site of pAS01. Reporter assays were performed as described with modifications (Ruby et al., 2007; Farh et al., 2005). S2 cells were grown in SFM supplemented with 1.6 mM L-glutamine at 25°C in a humid incubator. For transfection, 0.3×10^6 S2 cells were plated in a well of a 24-well plate. After 24 hr, cells were cotransfected with 1.5625 ng of firefly reporter construct, 500 ng of pUC19, 0.78125 ng of *Renilla* reporter construct, and 25 nM of miRNA duplex per well using Dharmafect Duo. Expression of *Renilla* luciferase was induced 24 h post-transfection with 500 µM of CuSO₄. Luciferase assays were performed 24 h post-induction with the Dual-Glo Luciferase system (Promega). Sequences of miRNAs are shown in below.

dme-miR-124: 5' UAAGGCACGCGGUGAAUGCCA

dme-miR-124*: 5' GGUAUCCACUGUAGGCCUAUAUG

dme-miR-1: 5' UGGAAUGUAAAGAAGUAUGGAG

dme-miR-1*: 5' CCAUGCUUCCCUGCAUUCAAUA

Sequences of mRNA Fragments Used in Figure 2

5'-terminal guanosines added to improve transcription yield are shown in lower case. Names of the genes are in italics.

K89 (*KIAA1189*)

5' ggCUCUUUUUCACUGUAGAAUAAUGUGGAAAUAAACCCUAGAUAAAAAU**CAGUCUGAUAA**CCUCAAUCAAAGCUUUU
A

GSTM3

5' ggCAUGGGGGAGCCUUUGGCCAGAAGAGGUAAUAAAGCUCAGAAGUUUUU**CAGUCUGAUAA**CUAUUGAUAAAUUCCAUA

FL9 (*FLJ40919*)

5' ggCUAUCUGACUUUUCUUUGGGAGGAAUGGGAAGUGUGGUUGGCCACU**AGUCUGAUAA**GAGUCUUGGUGAAGCUAAUA

NFI (*NFIA*)

5' ggGCAAGGACAAUGAAUCCACUCACAUUGCAGAACAAUCCGAAAAUGG**CAAACUACUAC**UACUACUGUUCAGUUUUUUA

SUPPLEMENTAL REFERENCES

- Addo-Quaye, C., Eshoo, T.W., Bartel, D.P., and Axtell, M.J. (2008). Endogenous siRNA and miRNA targets identified by sequencing of the Arabidopsis degradome. *Curr Biol* 18, 758-762.
- Aleman, L.M., Doench, J., and Sharp, P.A. (2007). Comparison of siRNA-induced off-target RNA and protein effects. *Rna* 13, 385-395.
- Anderson, E.M., Birmingham, A., Baskerville, S., Reynolds, A., Maksimova, E., Leake, D., Fedorov, Y., Karpilow, J., and Khvorova, A. (2008). Experimental validation of the importance of seed complement frequency to siRNA specificity. *Rna* 14, 853-861.
- Baek, D., Villen, J., Shin, C., Camargo, F.D., Gygi, S.P., and Bartel, D.P. (2008). The impact of microRNAs on protein output. *Nature* 455, 64-71.
- Bartel, D.P. (2009). MicroRNAs: target recognition and regulatory functions. *Cell* 136, 215-233.
- Birmingham, A., Anderson, E.M., Reynolds, A., Ilsley-Tyree, D., Leake, D., Fedorov, Y., Baskerville, S., Maksimova, E., Robinson, K., Karpilow, J., *et al.* (2006). 3' UTR seed matches, but not overall identity, are associated with RNAi off-targets. *Nat Methods* 3, 199-204.
- Cheng, J., Kapranov, P., Drenkow, J., Dike, S., Brubaker, S., Patel, S., Long, J., Stern, D., Tammana, H., Helt, G., *et al.* (2005). Transcriptional maps of 10 human chromosomes at 5-nucleotide resolution. *Science* 308, 1149-1154.
- Chiang, H.R., Schoenfeld, L.W., Ruby, J.G., Auyeung, V.C., Spies, N., Baek, D., Johnston, W.K., Russ, C., Luo, S., Babiarz, J.E., *et al.* (2010). Mammalian microRNAs: experimental evaluation of novel and previously annotated genes. *Genes Dev.*
- Czech, B., Malone, C.D., Zhou, R., Stark, A., Schlingeheyde, C., Dus, M., Perrimon, N., Kellis, M., Wohlschlegel, J.A., Sachidanandam, R., *et al.* (2008). An endogenous small interfering RNA pathway in *Drosophila*. *Nature* 453, 798-802.
- Dignam, J.D., Lebovitz, R.M., and Roeder, R.G. (1983). Accurate transcription initiation by RNA polymerase II in a soluble extract from isolated mammalian nuclei. *Nucleic Acids Res* 11, 1475-1489.
- Farh, K.K., Grimson, A., Jan, C., Lewis, B.P., Johnston, W.K., Lim, L.P., Burge, C.B., and Bartel, D.P. (2005). The widespread impact of mammalian MicroRNAs on mRNA repression and evolution. *Science* 310, 1817-1821.
- Friedman, R.C., Farh, K.K., Burge, C.B., and Bartel, D.P. (2009). Most mammalian mRNAs are conserved targets of microRNAs. *Genome Res* 19, 92-105.
- Giraldez, A.J., Mishima, Y., Rihel, J., Grocock, R.J., Van Dongen, S., Inoue, K., Enright, A.J., and Schier, A.F. (2006). Zebrafish MiR-430 promotes deadenylation and clearance of maternal mRNAs. *Science* 312, 75-79.
- Grimson, A., Farh, K.K., Johnston, W.K., Garrett-Engle, P., Lim, L.P., and Bartel, D.P. (2007). MicroRNA targeting specificity in mammals: determinants beyond seed pairing. *Mol Cell* 27, 91-105.
- Grimson, A., Srivastava, M., Fahey, B., Woodcroft, B.J., Chiang, H.R., King, N., Degen, B.M., Rokhsar, D.S., and Bartel, D.P. (2008). Early origins and evolution of microRNAs and Piwi-interacting RNAs in animals. *Nature* 455, 1193-1197.
- Haley, B., and Zamore, P.D. (2004). Kinetic analysis of the RNAi enzyme complex. *Nat Struct Mol Biol* 11, 599-606.
- Jackson, A.L., Burchard, J., Leake, D., Reynolds, A., Schelter, J., Guo, J., Johnson, J.M., Lim, L., Karpilow, J., Nichols, K., *et al.* (2006a). Position-specific chemical modification of siRNAs reduces "off-target" transcript silencing. *Rna* 12, 1197-1205.
- Jackson, A.L., Burchard, J., Schelter, J., Chau, B.N., Cleary, M., Lim, L., and Linsley, P.S. (2006b). Widespread siRNA "off-target" transcript silencing mediated by seed region sequence complementarity. *Rna* 12, 1179-1187.
- Jones-Rhoades, M.W., and Bartel, D.P. (2004). Computational identification of plant microRNAs and their targets, including a stress-induced miRNA. *Mol Cell* 14, 787-799.

- Kadener, S., Rodriguez, J., Abruzzi, K.C., Khodor, Y.L., Sugino, K., Marr, M.T., 2nd, Nelson, S., and Rosbash, M. (2009). Genome-wide identification of targets of the drosha-pasha/DGCR8 complex. *Rna* 15, 537-545.
- Lim, L.P., Lau, N.C., Garrett-Engele, P., Grimson, A., Schelter, J.M., Castle, J., Bartel, D.P., Linsley, P.S., and Johnson, J.M. (2005). Microarray analysis shows that some microRNAs downregulate large numbers of target mRNAs. *Nature* 433, 769-773.
- Schwarz, D.S., Ding, H., Kennington, L., Moore, J.T., Schelter, J., Burchard, J., Linsley, P.S., Aronin, N., Xu, Z., and Zamore, P.D. (2006). Designing siRNA that distinguish between genes that differ by a single nucleotide. *PLoS Genet* 2, e140.
- Shin, C. (2008). Cleavage of the star strand facilitates assembly of some MicroRNAs into Ago2-containing silencing complexes in mammals. *Mol Cells* 26, 308-313.

Figure S1 (Related to Figure 1)

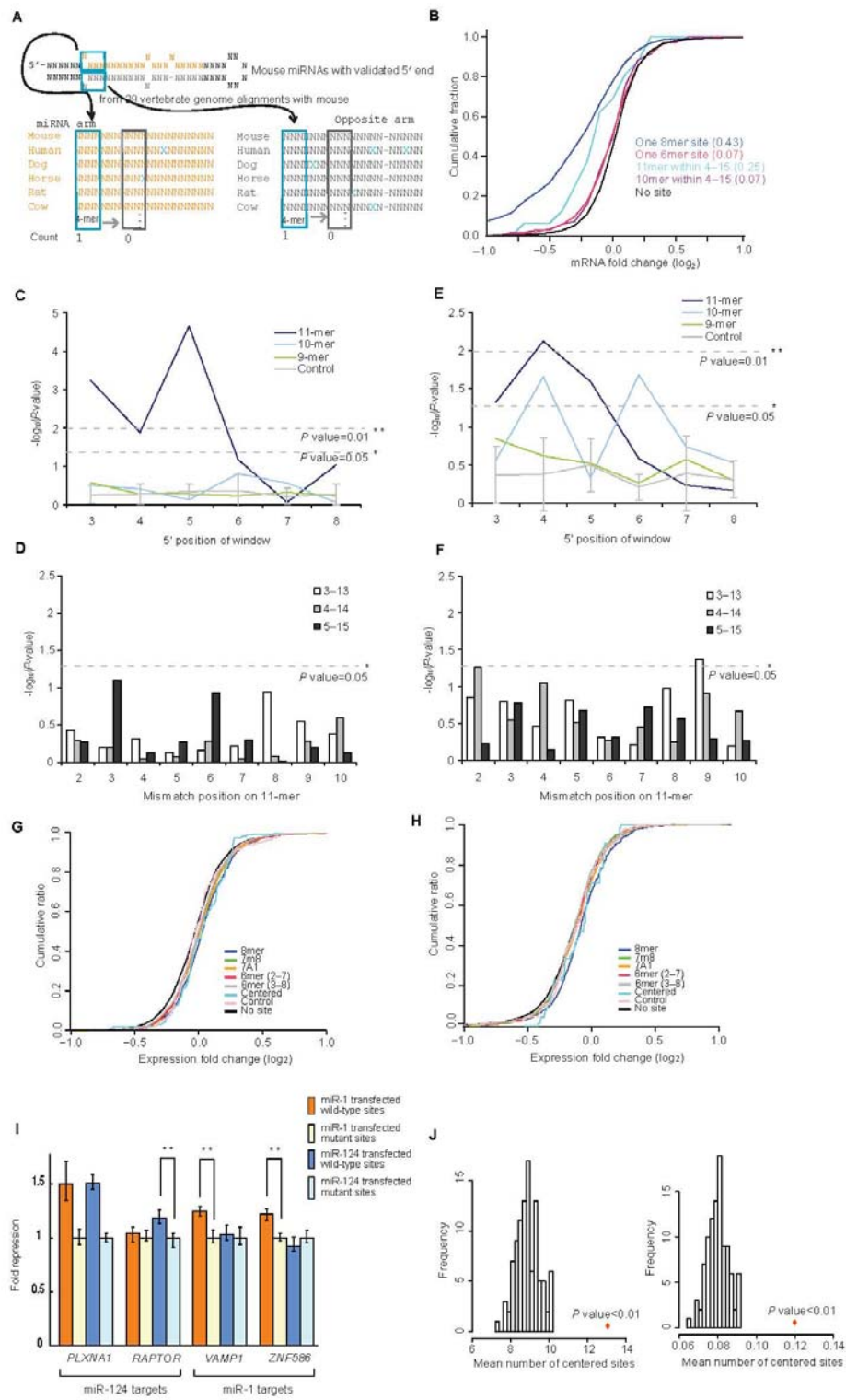


Figure S1. Systemic Analysis of the Centered Sites (Related to Figure 1)

(A) The approach for evaluating the conservation of 4-nt segments of mammalian miRNAs (Figure 1A). The schematic illustrates the approach for a miRNA deriving from the 5' arm of a hairpin.

(B) Reduced expression of messages with perfect pairing to the center of transfected miRNAs. Shown is analysis of microarray data (Grimson et al., 2007), plotting cumulative changes of mRNAs with the indicated target sites. 8-mer and 6-mer sites were standard sites with either 8 or 6 nt matches centered on the miRNA seed (Bartel, 2009); 11-mer and 10-mer sites had either 11 or 10 contiguous Watson-Crick pairs to segments within miRNA positions 4–15. Shown in parenthesis is the minimal fraction of mRNAs repressed by the miRNA (Grimson et al., 2007).

(C) The statistical significance of repression of messages with perfect pairing to 11 (dark blue), 10 (light blue), and 9 (green) contiguous positions across 78 mi/siRNAs transfected to HeLa cells (Table S1). The analysis was repeated for 11-mer sites matching chimeric control sequences (grey; error bars, standard deviation of 10 control cohorts). All sites and controls were compared to no sites to estimate P values, which were calculated by K-S test. X-axis represents the 5' position of each window. The dotted lines present thresholds for the indicated statistical significance.

(D) Response of HeLa mRNAs with near-perfect pairing to 11-mer sites corresponding the indicated positions of 78 transfected mi/siRNAs. Plotted are mean fold changes for mRNAs with 3' UTR sites for which 10 of 11 positions perfectly paired to the miRNA, and the remaining position was a mismatch or wobble. Sites with the mismatch or wobble at the first or last position were excluded because these were analyzed as perfect 10-mer sites (C). P values were calculated by the K-S test, comparing to mRNAs with no sites.

(E) The statistical significance of derepression of messages with perfect pairing to 11 (dark blue), 10 (light blue), and 9 (green) contiguous positions across 21 miRNAs depleted in MZ Dicer mutant of zebrafish embryo (Table S4). The analysis was repeated for 11-mer sites matching 200 chimeric control sequences (grey; error bars, standard deviation of 10 control cohorts). Otherwise, as in (C).

(F) Response of mRNAs with near-perfect pairing to 11-mer sites corresponding the indicated positions of 21 miRNAs depleted in MZ Dicer mutant of zebrafish embryos. Otherwise, as in (D)

(G) Function of endogenous interactions mediated by centered sites in *Drosophila* S2 cells. Shown is analysis of microarray data (Kadener et al., 2009), as in Figure 1F, plotting cumulative changes of mRNAs with single 3' UTR sites to 48 miRNAs depleted in cells after knocking down Drosha (Table S3).

(H) Function of endogenous interactions mediated by centered sites in *Drosophila* S2 cells. As in (H), but plotting cumulative changes of mRNAs with single 3' UTR sites to 26 miRNAs depleted in cells after knocking down Dicer1 (Table S4).

(I) miRNA-mediated repression at centered sites in S2 cells. Shown is the fold repression of luciferase reporter genes fused to 3' UTR fragments of the indicated genes with the indicated sites or mutant sites. Otherwise as in Figure 1G.

(J) The number of centered sites in our set of non-redundant 3' UTRs. (Left) The mean number of centered sites per miRNA (red diamond), compared to the mean numbers for 100 cohorts of chimeric controls (bars). The analysis was performed with 154 distinct, conserved, non-repeat-derived human miRNAs and our set of 19,109 distinct mRNAs. Statistical significance was determined using the permutation test. (Right) The mean number of centered sites matching miRNAs conserved among mammals per 3' UTR (red diamond), compared to the mean numbers for 100 cohorts of chimeric controls (bars). Statistical significance was determined using the permutation test.

Figure S2 (Related to Figure 2)

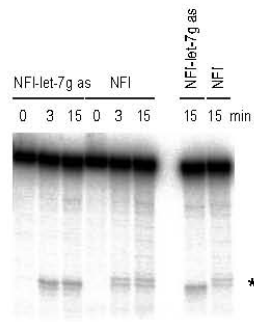


Figure S2. Higher-Resolution Mapping of Cleavage at the *let-7g* Centered Site within NFI (Related to Figure 2)

A longer run of a gel analyzing NFI cleavage products (*) indicated cleavage at positions 9–10 as well as 10–11.

Figure S3 (Related to Figure 4)

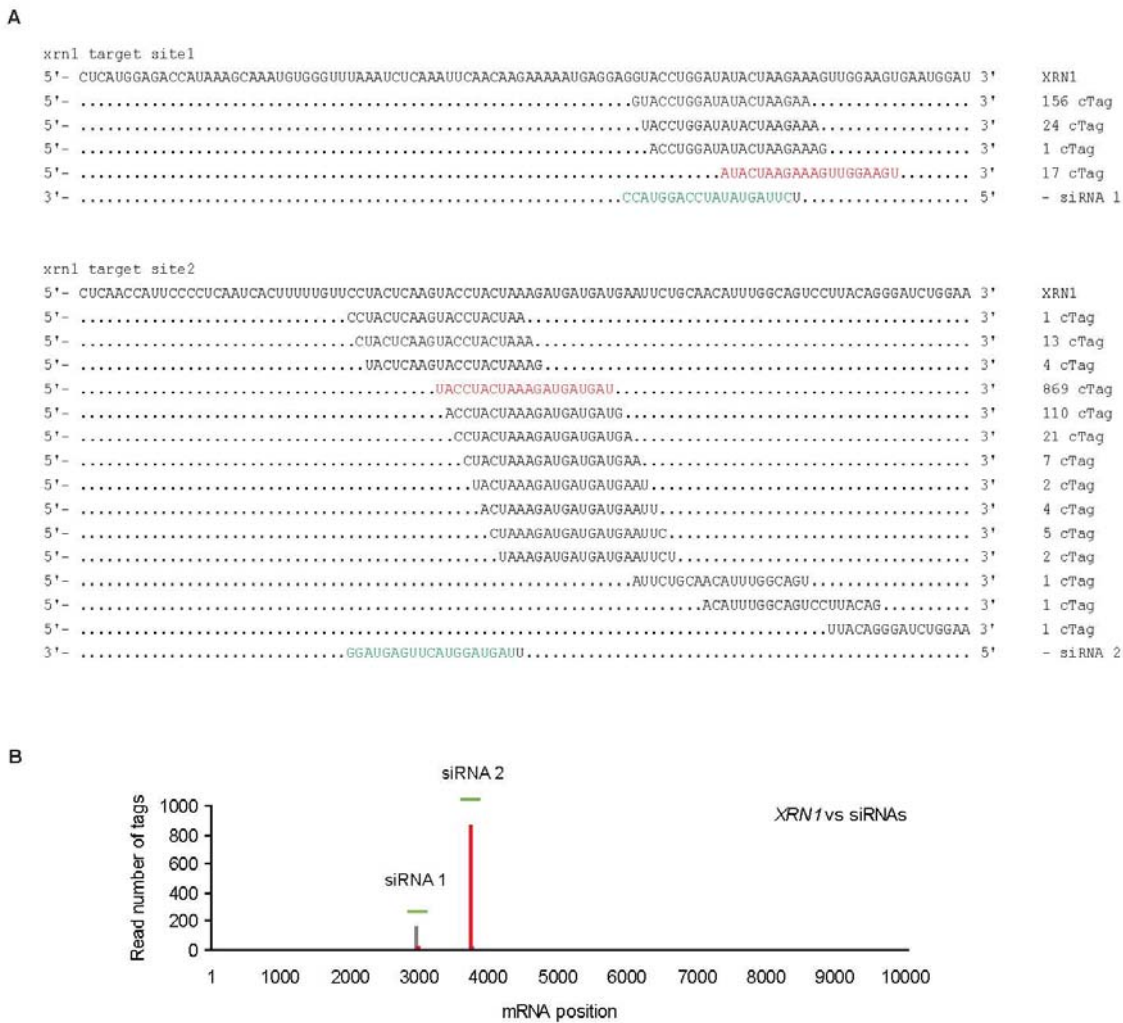


Figure S3. The Cleavage Tags from *XRN1* RNAi and the Distribution of Degradome Tags (Related to Figure 4)

(A) Degradome tags in the vicinity of the sites of siRNAs used to knock down *XRN1* mRNA. Each unique tag is shown below the *XRN1* mRNA fragment, with the expected cleavage tags indicated (red) and the number of tags (cTag) listed on the right. Also shown are the siRNA guide strands (green).

(B) Distribution of degradome tags across the length of the mRNA. The numbers of tags with 5' termini mapping at the expected cleavage sites are plotted in red.

Figure S4 (Related to Figure 5)

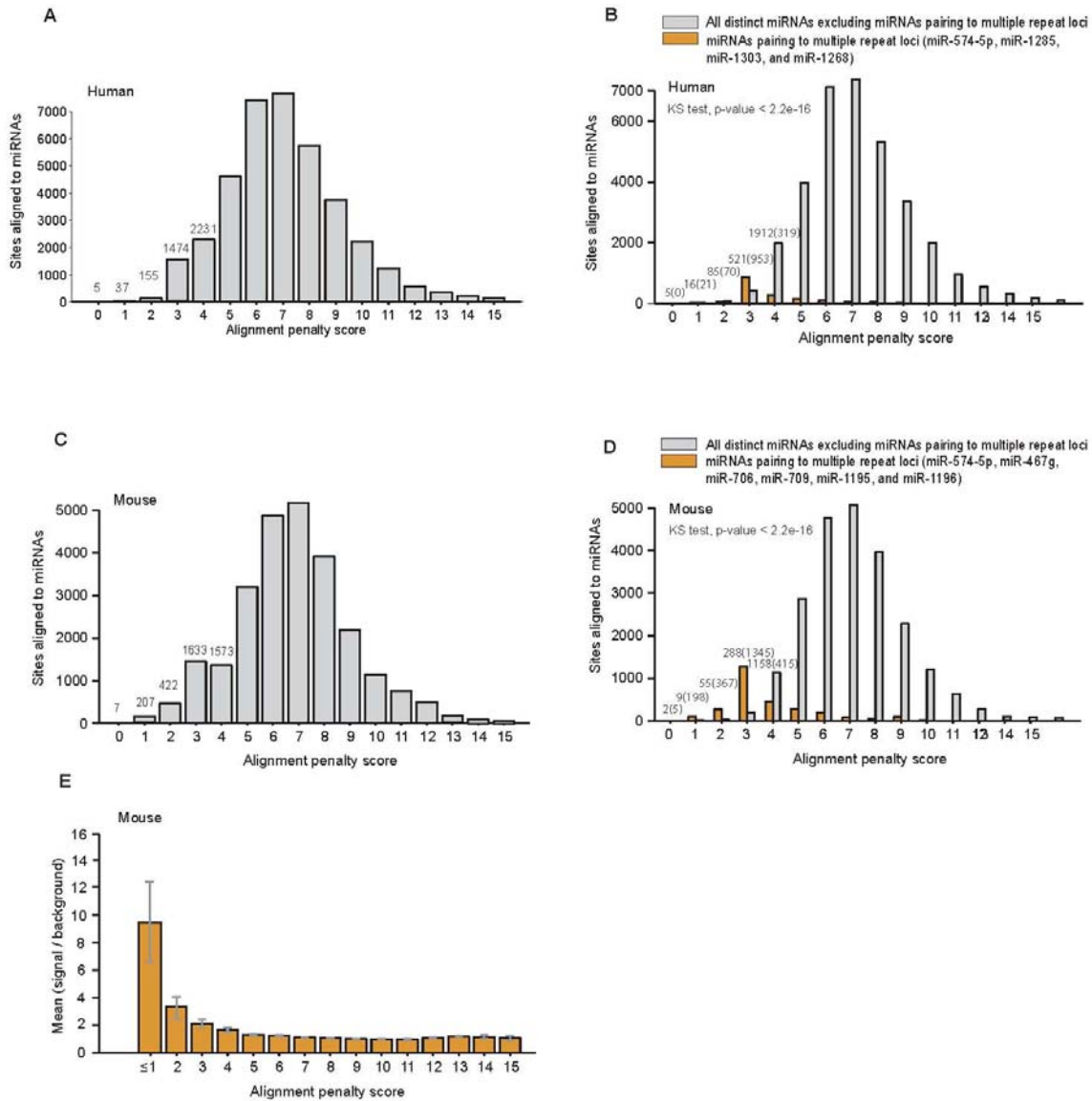


Figure S4. Enrichment of miRNA:site Duplexes with Extensive Complementarity (Related to Figure 5)

(A) Distribution of scores for human miRNA:site duplexes with at least seven consecutive base pairs and 13 base pairs in total.

(B) The distribution of (A), plotting separately the duplexes for four miRNAs that pair to numerous repeat loci. When considering the miRNAs that remain after removing the four pairing to repeat loci, the histogram resembles a Gaussian distribution. To obtain more conservative site-enrichment values (Figure 5C), we used miRNA:site duplexes and chimeric miRNA:site duplexes after excluding miRNAs pairing to multiple repeat loci.

(C) Distribution of scores for murine miRNA:site duplexes with at least seven consecutive base pairs and 13 base pairs in total.

(D) The distribution of (C), plotting separately the duplexes for the six miRNAs that pair to numerous repeat loci. 418 distinct miRNAs out of 627 mouse miRNAs annotated in miRBase (version 11.0) were considered. These paired to 26,495 distinct target sites with score ≤ 15 .

(E) Analysis of site enrichment in mouse, performed as in human (Figure 5C).

Figure S5A (Related to Figure 6)

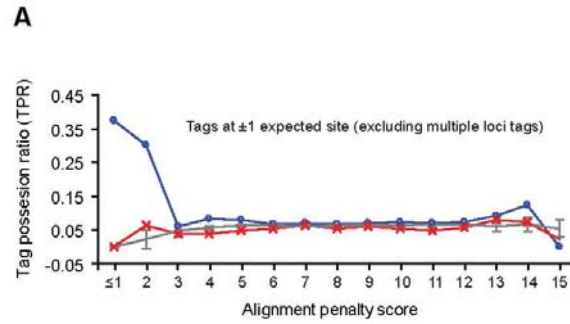
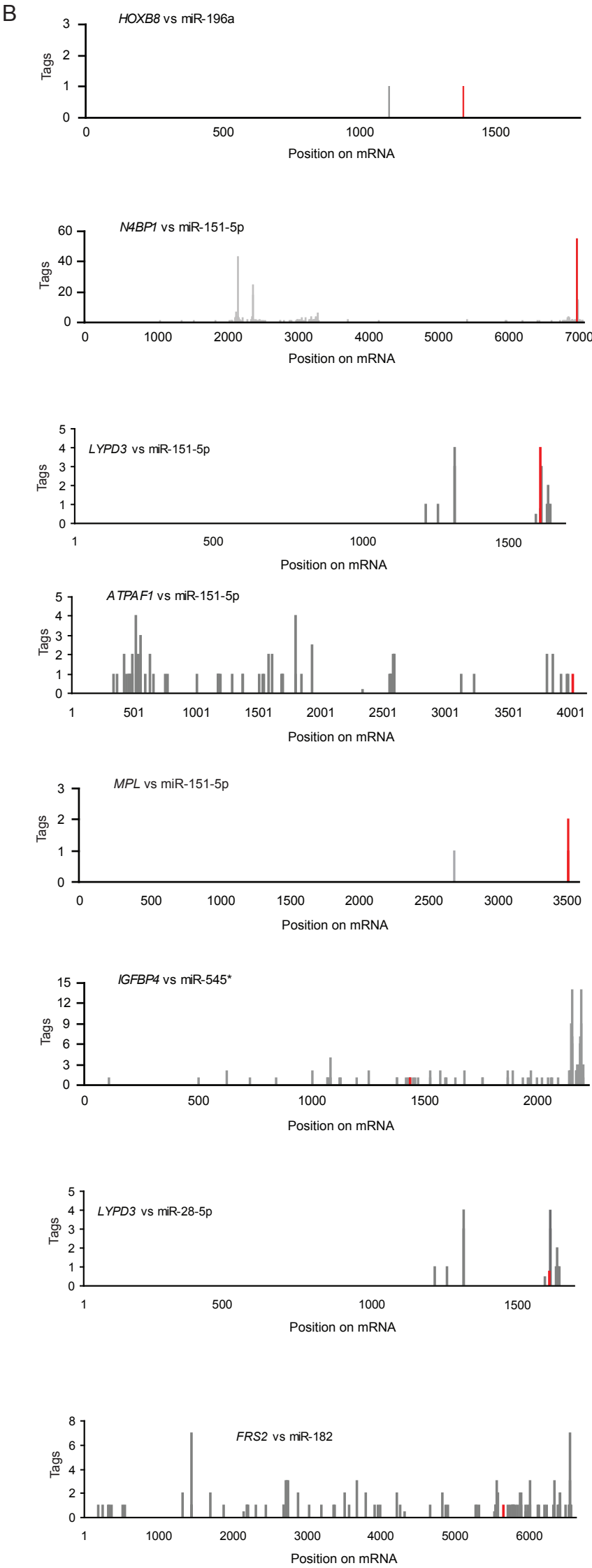


Figure S5A. TPR of miRNA:site Duplexes when Relaxing Classification of Cleavage Tags to Include Tags with 5' Termini Mapping within One Nucleotide of the Expected Cleavage Site (Related to Figure 6)

Figure S5B (continued, Related to Figure 6)



chr17:44,045,131-44,045,152									
5'	CCGATTTCTGCTAAGTTCTCCCAACAACATGAAACTGCCTATTACAGCCGTAATTCTTT	#read	#loci	HoxB8					
5'AACTGCCTATTACAGCCGT.....	3'	1	1	cTag				
3'GGTTGTGTACTTTGATGGAT.....			5'	miR-196a				
chr16:47,130,232-47,130,252									
5'	TCTTGCTGCCCTTCTAGACTGTGAGCTCCTCGTGGGCAGGGACCGCCTGTGTTCTCTGTATTTCACCAGGCGCCTAGCACAGTGCCTTGCACTTGATAGGTGCTTA	#read	#loci	N4BP1					
5'	TCTTGCTGC.....	3'	2	1	cTag				
5'GCCCTTCTAGACTGTGAGC.....	3'	1	1	cTag				
5'TTTCTAGACTGTGAGCTCCT.....	3'	1	4	cTag				
5'AGCTCCTCGTGGGCAGGGAC.....	3'	55	1	cTag				
5'GCTCCTCGTGGGCAGGGAC.....	3'	15	1	cTag				
5'CTCCTCGTGGGCAGGGACCG.....	3'	13	1	cTag				
5'TCCTCGTGGGCAGGGACCGC.....	3'	15	1	cTag				
5'GTGTTCTCTGTATTTCACC.....	3'	1	1	cTag				
5'CTCTGTATTTCACCAGGCGC.....	3'	2	1	cTag				
5'ATTTCACCAGGCGCCTAGCA.....	3'	1	1	cTag				
5'CAGTGCCTTGCACTTGATAG.....	3'	1	1	cTag				
5'GTGCCTTGCACTTGATAGGT.....	3'	1	1	cTag				
5'TTGATAGGTGCTTA.....	3'	1	2	cTag				
5'GATAGGTGCTTA.....	3'	1	2	cTag				
5'TAGGTGCTTA.....	3'	1	6	cTag				
3'TGATCTGACACTCGAGGAGCT.....	5'			miR-151-5p				
chr19:48,656,864-48,656,884									
5'	TCTTCCTTACTAGACTGTGAGCTCCTCGAGGGCAGGGACCGTGCCTTATGTCTGTGTGATCAGTTTCTGGCACATAAATGCCTCAATAAAGATTTAATTACTTTG	#read	#loci	LYPD3					
5'TCCTTACTAGACTGTGAGCT.....	3'	1	2	cTag				
5'TGAGCTCCTCGAGGGCAGGG.....	3'	3	4	cTag				
5'AGCTCCTCGAGGGCAGGGAC.....	3'	12	3	cTag				
5'GCTCCTCGAGGGCAGGGACC.....	3'	6	2	cTag				
5'GCCTTATGTCTGTGTGTGAT.....	3'	1	1	cTag				
5'CTTATGTCTGTGTGTGATCA.....	3'	2	1	cTag				
5'TCTGTGTGTGATCAGTTTCT.....	3'	1	1	cTag				
5'TGTGTGTGTGATCAGTTTCTGG.....	3'	1	1	cTag				
3'TGATCTGACACTCGAGGAGCT.....	5'			miR-151-5p				
chr1:46,871,100-46,871,120									
5'-	TACATTATATTTATTATTTCTTGTGTCCTTTTTTCCCCACTAGACTGTGAGCTCCTTGAGGGCAGGACTTATCTCTGTTCG	3'	#read	#loci	ATPAF1				
5'-	TACATTATATTTATTATTC.....	3'	1	1	cTag				
5'-ATATTTATTTATTTCTTGTGT.....	3'	1	1	cTag				
5'-AGCTCCTTGAGGGCCAGGAC.....	3'	4	4	cTag				
3'-TGATCTGACACTCGAGGAGCT.....	5'			miR-151-5p				
chr1:43,592,603-43,592,623									
5'-	TACTAGATTGTGAGCTCCTTGAGGGAAAGGAACATGATTTATTTGTCCCTTTTCCCCCAGCACCTAGAGTAGTGCTT	3'	#read	#loci	MPL				
5'-AGCTCCTTGAGGGAAAGGAA.....	3'	1	2	cTag				
5'-GCTCCTTGAGGGAAAGGAAC.....	3'	2	1	cTag				
3'-TGATCTGACACTCGAGGAGCT.....	5'			miR-151-5p				
chr17:35,866,700-35,866,720									
5'-	ATCCAGCCACCTAA	AAACATT	TACTGACCATGTACTACGTGCCAGCTCTAGTTTTTCAGCCTTGAGGAGTTTTATTCTGA	3'	#read	#loci	IGFBP4		
5'-	3'	1	1	cTag		
5'-	3'	1	1	cTag		
5'-TCCAGCCACCTAA	AAACATT.....	3'	1	1	cTag		
5'-A	AAACATT	TACTGACCATGT.....	3'	1	1	cTag		
5'-CATT	TACTGACCATGTACTA.....	3'	1	1	cTag		
5'-ATT	TACTGACCATGTACTAC.....	3'	1	1	cTag		
5'-TTT	TACTGACCATGTACTACG.....	3'	1	1	cTag		
5'-CTAC	GTGCCAGCTCTAGTTT.....	3'	1	1	cTag		
5'-CTAC	GTGCCAGCTCTAGTTT.....	3'	1	1	cTag		
5'-CCAG	CTCTAGTTTTCAGCCT.....	3'	1	1	cTag		
5'-AGC	CTTGGGAGTTTTTATTC.....	3'	1	1	cTag		
3'-AGTAGATTATTTGTAAATGACT.....	5'			miR-545*				
chr19:48,656,866-48,656,887									
5'	TCTTCCTTACTAGACTGTGAGCTCCTCGAGGGCAGGGACCGTGCCTTATGTCTGTGTGTGATCAGTTTCTGGCACATAAATGCCTCAATAAAGATTTAATTACTTTG	#read	#loci	LYPD3					
5'TCCTTACTAGACTGTGAGCT.....	3'	1	2	cTag				
5'TGAGCTCCTCGAGGGCAGGG.....	3'	3	4	cTag				
5'AGCTCCTCGAGGGCAGGGAC.....	3'	12	3	cTag				
5'GCTCCTCGAGGGCAGGGACC.....	3'	6	2	cTag				
5'GCCTTATGTCTGTGTGTGAT.....	3'	1	1	cTag				
5'CTTATGTCTGTGTGTGATCA.....	3'	2	1	cTag				
5'TCTGTGTGTGATCAGTTTCT.....	3'	1	1	cTag				
5'TGTGTGTGATCAGTTTCTGG.....	3'	1	1	cTag				
3'GAGTTATCTGACACTCGAGGAA.....	5'			miR-28-5p				
chr12:68,258,886-68,258,908									
5'-	CAACAGTGGG	ACTACATTGCCAATTGATATGA	AATATGAATTTTACCCCCATGGTTAATTTCTTTTATAAACATTCATATTTCTCTAATAAAAAAGACATAAGTGATACTGTAC	3'	#read	#loci	FRS2		
5'-CATTGCCAATTGATATGA.....	3'	1	1	cTag		
5'-TTT	TACCCCCATGGTTAATT.....	3'	1	1	cTag		
5'-TATTT	CTCTAATAAAAAAGAC.....	3'	1	1	cTag		
5'-ATTT	CTCTAATAAAAAAGACA.....	3'	1	1	cTag		
5'-TTT	CTCTAATAAAAAAGACAT.....	3'	1	1	cTag		
5'-CTAC	GTGCCAGCTCTAGTTT.....	3'	1	1	cTag		
5'-TCT	TAATAAAAAAGACATAAGT.....	3'	1	1	cTag		
5'-AATA	AAAAAGACATAAGTGAT.....	3'	1	1	cTag		
3'-TCACACTCAAGATGGTAACGGTTT.....	5'			miR-182				

CA score:34 424									
NM_024016 HOXB8 3'TTR	5'	CCCAACAACATGAAACTGCCTA	3'						
		0							
hsa-miR-196a BC	3'	GGGTGTTGTACTTTGATGGAT	5'	1.0					
mouse	5'	CCCAACAACATGAAACTGCCTA	3'						
		0							
hsa-miR-196a BC	3'	GGGTGTTGTACTTTGATGGAT	5'	1.0					
rat	5'	CCCAACAACATGAAACTGCCTA	3'						
		0							
hsa-miR-196a BC	3'	GGGTGTTGTACTTTGATGGAT	5'	1.0					
dog	5'	CCCAACAACATGAAACTGCCTA	3'						
		0							
hsa-miR-196a BC	3'	GGGTGTTGTACTTTGATGGAT	5'	1.0					
CA score:2.5 3898									
NM_001042555 FRS2 3'TTR	5'	ACAGTGGG-ACTACCATTGCCAAA	3'						
		-- 0							
hsa-miR-182 BC	3'	TCACACTCAAGATGGTAACGGTTT	5'	2.5					
mouse	5'	ACAGTGGG-ACTACCATTGCCAAA	3'						
		-- 0							
hsa-miR-182 BC	3'	TCACACTCAAGATGGTAACGGTTT	5'	2.5					
rat	5'	ACAGTGGG-ACTACCATTGCCAAA	3'						
		-- 0							
hsa-miR-182 BC	3'	TCACACTCAAGATGGTAACGGTTT	5'	2.5					
dog	5'	ACAGTGGG-ACTACCATTGCCAAA	3'						
		-- 0							
hsa-miR-182 BC	3'	TCACACTCAAGATGGTAACGGTTT	5'	2.5					
horse	5'	ACAGTGGG-ACTACCATTGCCAAA	3'						
		-- 0							
hsa-miR-182 BC	3'	TCACACTCAAGATGGTAACGGTTT	5'	2.5					
pig	5'	ACAGTGGG-ACTACCATTGCCAAA	3'						
		-- 0							
hsa-miR-182 BC	3'	TCACACTCAAGATGGTAACGGTTT	5'	2.5					

CA score:2.0 2927									
NM_001042546 ATPAF1 3'TTR	5'	ACTAGACTGTGAGCTCCTTGA	3'						
		0							
hsa-miR-151-5p TC	3'	TGATCTGACACTCGAGGAGCT	5'	1.0					
mouse	5'	ACTAGACTGTGAGCTCCTTGA	3'						
		0							
hsa-miR-151-5p TC	3'	TGATCTGACACTCGAGGAGCT	5'	1.0					
rat	5'	ACTAGACTGTGAGCTCCTTGC	3'						
		0							
hsa-miR-151-5p TC	3'	TGATCTGACACTCGAGGAGCT	5'	2.0					
dog	5'	ACTAGACTGTGAGCTCCTTGA	3'						
		0							
hsa-miR-151-5p TC	3'	TGATCTGACACTCGAGGAGCT	5'	1.0					
horse	5'	ACTAGACTGTGAGCTCCTTGA	3'						
		0							
hsa-miR-151-5p TC	3'	TGATCTGACACTCGAGGAGCT	5'	1.0					
pig	5'	GTGAGACTGTGAGCTCCTGTGG	3'						
		00- 0							
hsa-miR-151-5p TC	3'	TGATCTGACACTCGAGGA-GCT	5'	6.0					

CA score:2.0									
NM_153029 N4BP1 3'TTR	5'	TCTAGACTGTGAGCTCCTCGT	3'						
		-							
hsa-miR-151-5p TC	3'	TGATCTGACACTCGAGGAGCT	5'	2					
mouse	5'	TCTAGACTGTGAGCTCCTCGC	3'						
		-							
hsa-miR-151-5p TC	3'	TGATCTGACACTCGAGGAGCT	5'	2					
dog	5'	TCTAGACTGTGAGCTCCTCGC	3'						
		-							
hsa-miR-151-5p TC	3'	TGATCTGACACTCGAGGAGCT	5'	2					
horse	5'	TCTAGACTGTGAGCTCCTCGC	3'						
		-							
hsa-miR-151-5p TC	3'	TGATCTGACACTCGAGGAGCT	5'	2					
pig	5'	TCTAGACTGTGAGCTCCTCGC	3'						
		-							
hsa-miR-151-5p TC	3'	TGATCTGACACTCGAGGAGCT	5'	2					

Figure S5B. The Distribution of HeLa Degradome Tags across the Length of the mRNAs and Conservation of miRNA:site Pairing (Related to Figure 6)

(Upper panel) Distributions are shown for the eight mRNAs with miRNA:site penalty scores ≤ 2.5 and cleavage tags at the expected site (Table 1). The graphs on the left plot tags across the entire mRNAs, indicating the contribution of tags with 5' termini mapping to the expected cleavage site (red). The images to the right zoom in on the tags mapping near the expected cleavage site, highlighting tags with 5' termini mapping to the expected cleavage site (red). Tags mapping to > 10 genomic loci are not considered. The genomic coordinates are those of the microRNA complementary sites.

(Lower panel) Conserved sites with evidence for miRNA-directed cleavage. Shown is the pairing to orthologous sites in the indicated species, which was used to determine the CA scores.

C

5'	TGATTGTGTAAGAGCAGGAGAGCTCTCTCGATGTCAGATCATACAGAGTAGGGAGTGAATCTGACTTCGAGGAATCCTCTCTCCATCCAGATGAGAGTGGAG	3'	chr8:141811844-141811944
5'((((((((((((((.....))	3'	#read #loci
5'TCGAGGAGCTCAGAGCTAG.....	3'	9 6 cTag
5'CGAGGAGCTCAGAGCTAGT.....	3'	7 5 cTag
5'ACAGGGATGGTCATATCTCAC.....	3'	3 1 cTag
5'CTAGACTGGAAGCTCCTTAG.....	3'	19 3
5'TAGACTGGAAGCTCCTTAGG.....	3'	11 3 cTag
5'	CTAAACAGCTCTCTCGCTG.....	3'	2 1 smallRNA
5'	TAAACACTTTCTCGGCC.....	3'	2 1 smallRNA
5'TCGAGGAGCTCAGAGCT.....	3'	11 9 smallRNA
5'TCGAGGAGCTCAGAGCTCA.....	3'	3 8 smallRNA
5'TCGAGGAGCTCAGAGCTAG.....	3'	27 6 smallRNA
5'TCGAGGAGCTCAGAGCTAGT.....	3'	1607 5 smallRNA
5'TCGAGGAGCTCAGAGCTAGTA.....	3'	809 3 smallRNA
5'TCGAGGAGCTCAGAGCTAGT.....	3'	113 3 smallRNA
5'TCGAGGAGCTCAGAGCTAGTAG.....	3'	2 1 smallRNA
5'TACTAGACTGGAAGCTCCTTAG.....	3'	37 1 smallRNA
5'TACTAGACTGGAAGCTCCTTAGG.....	3'	99 1 smallRNA
5'CTAGACTGGAAGCTCCTTG.....	3'	3 3 smallRNA
5'CTAGACTGGAAGCTCCTTGA.....	3'	38 3 smallRNA
5'CTAGACTGGAAGCTCCTTAG.....	3'	126 3 smallRNA
5'CTAGACTGGAAGCTCCTTAGG.....	3'	796 1 smallRNA
5'CTAGACTGGAAGCTCCTTAGGA.....	3'	555 1 smallRNA
5'CTAGACTGGAAGCTCCTTAGGAC.....	3'	9 1 smallRNA
5'CTAGACTGGAAGCTCCTTAGGAC.....	3'	1 1 smallRNA

The secondary structure of the hairpin predicted by RNAfold is shown in bracket notation on the second line. Degradome tags (cTag) are aligned below. These map to sites of presumed Drosha and Dicer cleavage, as expected for a canonical miRNA. Small-RNA sequencing reads (small RNA) are also aligned. These include miR-151-5p (blue) and miR-151-3p (green), which can pair to each other with 2-nt 3' overhangs. These also include some fragments of the pri-miRNA 5' leader ending precisely at the presumed site of Drosha cleavage, as is often observed for canonical miRNAs. Columns at the right list the number of tags/reads for each sequence and the number of genomic loci to which each sequence mapped.

Figure S5D (continued, Related to Figure 6)

D

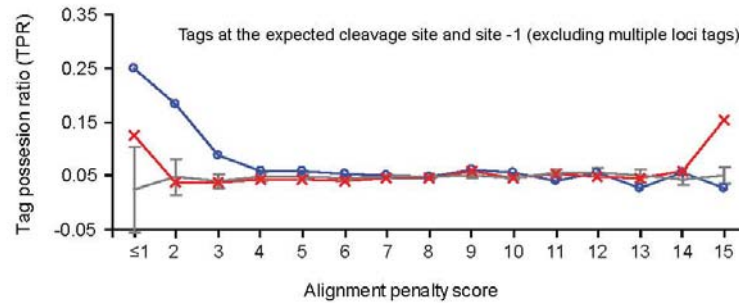
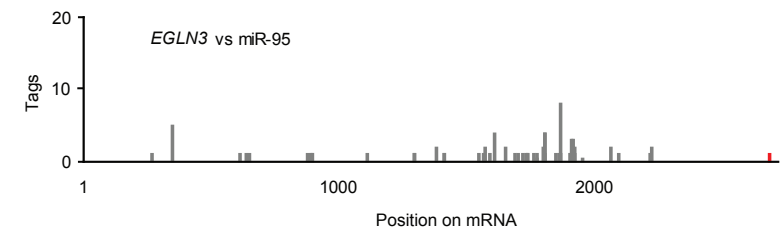
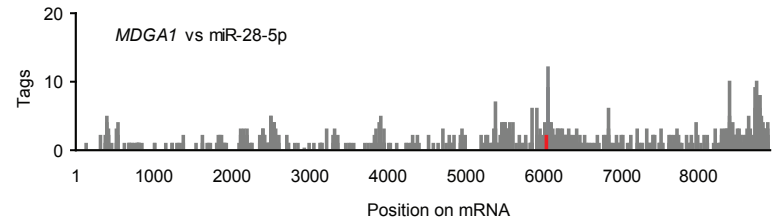
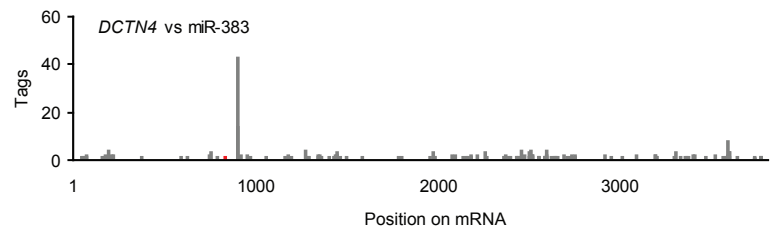
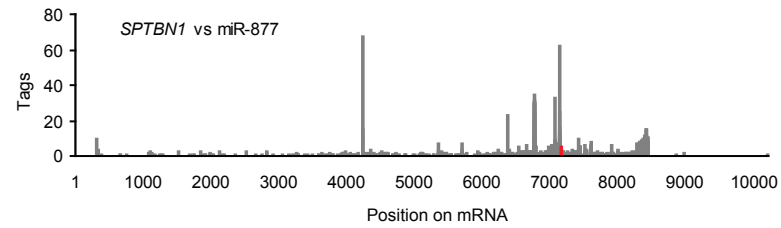
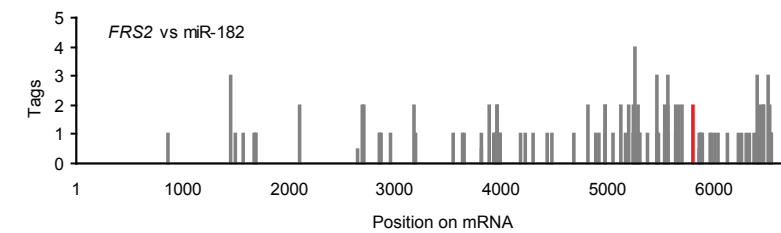
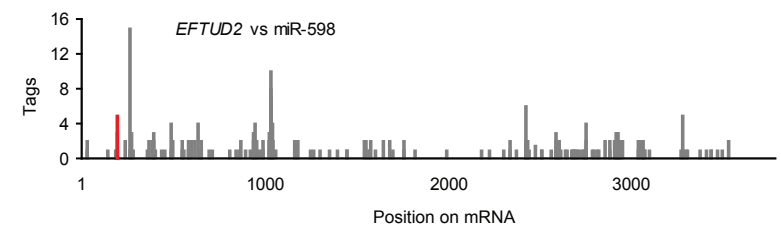
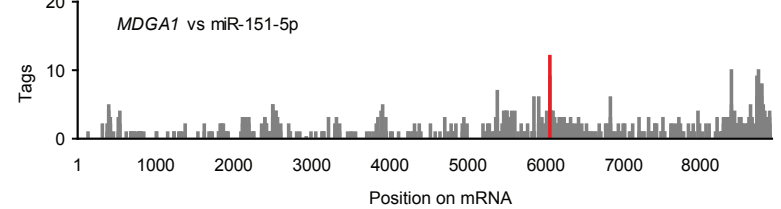


Figure S5D. TPR of miRNA:site Duplexes in Human Brain, including Tags with 5' Termini that Map One Nucleotide Downstream of the Expected Cleavage Site (Related to Figure 6)

TPR values for miRNAs expressed in human brain (blue; Table S12) were compared to values for miRNAs not expressed in our brain sample (red) and values for 10 cohorts of chimeric control miRNAs (gray; error bars indicate standard deviation).

< Cleavage site 0>



```
chr16:47,130,232-47,130,252 3'UTR
5'- CCACGTGATTGTGTAAGTCTTCTGCGCCCTTCTAGACTGTGAGCTCTCTGCGGCGAGGAGCGCCTGTGTTCTCTGATTATCCACGGCGCTAGAC 3' #read #loci N4P4P1
5'- CCACCT..... 3' 1 1 cTag
5'- CCACGTGA..... 3' 1 2 cTag
5'- CCACGTGATT..... 3' 1 1 cTag
5'- CCACGTGATTGTGAC..... 3' 1 1 cTag
5'- CCACGTGATTGTGACT..... 3' 1 1 cTag
5'- .....ATTGTGTAAGTCTTTCGTG..... 3' 2 1 cTag
5'- .....GTGTAAGTCTTTCGTG..... 3' 1 1 cTag
5'- .....GTGTAAGTCTTTCGTG..... 3' 2 1 cTag
5'- .....CTGTGCTTCTGCGCCCTTCTA..... 3' 1 1 cTag
5'- .....CTGTGCGCCCTTCTAGACT..... 3' 5 1 cTag
5'- .....TGCTGCCCTTCTAGACTGT..... 3' 1 2 cTag
5'- .....AGCTCTCTGCTGGCGAGGAC..... 3' 6 1 cTag
5'- .....TCGCTGTGGGACAGCG..... 3' 1 1 cTag
5'- .....CGCCTGTGTTCTCTGATT..... 3' 1 1 cTag
5'- .....TTTCCACGGCGCTAGAC..... 3' 1 1 cTag
3'- .....TGATCTGACACTCGAGAGCT..... 5' miR-151-5p
```

```

chr6:37,711,105-37,711,125 3UTR
5'-GCCATTGCCCACTAGACTGTGAGCTCTCTCTGAGCGTTCCAAAGTCCCCCTCCACCTTGCCACACTCCCGCTCGTGCCACAG 3' #read #loci MDGA1
GTCCATTGCCCACTAGAG... 3' 1 cTag
...CATTGCCCACTAGACTCT... 3' 2 cTag
...CCCCACTAGACTGTGAGCT... 3' 1 3 cTag
...AGACTGTGAGCTCTCTCTCT... 3' 1 cTag
...ACTGTGAGCTCTCTCTGA... 3' 2 cTag
...TGAGCTCTCTCTGAGCT... 3' 1 cTag
...GAGCTCTCTCTGAGCTCT... 3' 1 cTag
...AGCTCTCTCTGAGCTCT... 3' 9 cTag
...GCTCTCTCTGAGCTCT... 3' 12 cTag
...CTCTCTGAGCTTCCAAGT... 3' 2 cTag
...CTTGAGCTTCCAACTCCC... 3' 2 cTag
...CTGAGCTTCCAACTCCCC... 3' 2 cTag
...GAGCTTCCAACTCCCCCT... 3' 2 cTag
...CTTGAGCTCCCCCTCT... 3' 1 cTag
...CTTCAAGTCCCCCTCAGC... 3' 1 cTag
...TCCAACTCCCCCTCACT... 3' 4 cTag
...CAAGTCCCCCTCACTCT... 3' 2 cTag
...AGTCCCCCTCACTTCGG... 3' 4 cTag
...GTCCCCCTCACTTCGCT... 3' 1 cTag
...CCCTCACTCTGGCAGC... 3' 3 cTag
...CCCTCACTCTGGCAGCT... 3' 1 cTag
...CTCTCACTCTGGCAGCTCT... 3' 3 cTag
TGAATCGACACTGAGAGAGCT... 5' miR-151-5p

```

chr17:40,319,615-40,319,636 ORF

5'	GAGAGCAAGCAATCTTGTAGTGGATGATGATGACGACGACGATGAGCTAGAGATCATGACGATGACCAACCT	3'	#read	flori	EFTUD2
5'	GAGAGCAACAAAGATCTTGTAT.....	3'	1	1	cTag
5'CGATGACGTAGGAGATCATG.....	3'	1	1	cTag
5'CATGACGTAGGAGATCATGA.....	3'	5	1	cTag
5'ATGACGTAGGAGATCATGAC.....	3'	1	1	cTag
5'TCTCCAGGACGATGACATGAC.....	3'	1	1	cTag
5'GACGTAGGAGATCATGAGCA.....	3'	3	1	cTag
3'ACTGCTACTGTGGCTACTGAT.....	5'	miR-598		

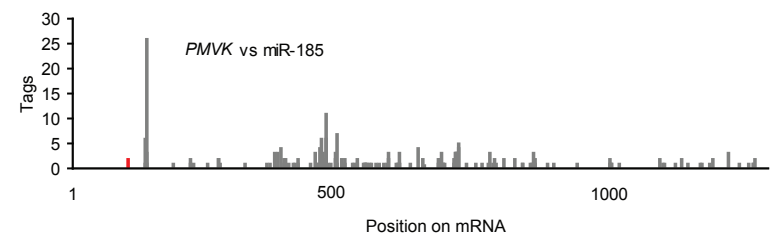
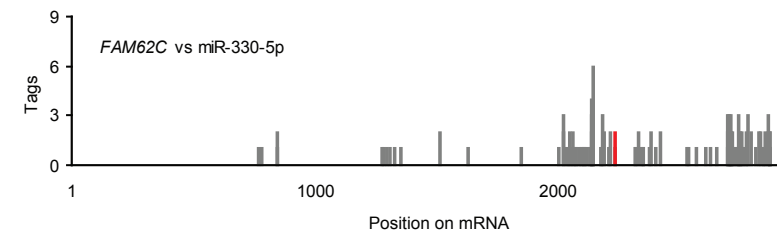
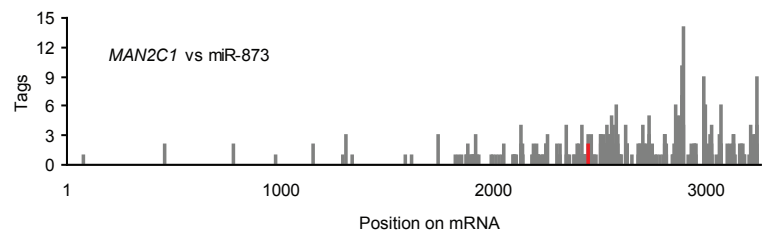
chr12:68,258,886-68,258,908 3'UTR

5'	CAACAGTGGG	ACTACCATTGCCAAATGTATATGAAATATGAATTTTACCCCATCGTTTAATTTCTTTTATAAACAT	3'	#read	#loci	FRS2
5' CATTGCCAAATGTATATGA	3'	2	1
3'TCACACTCAAGATGGTAACGGTTT.....	5'	miR-182	cTag

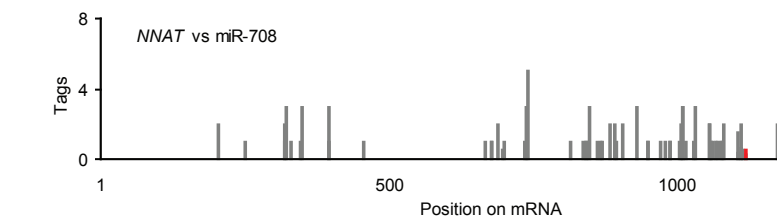
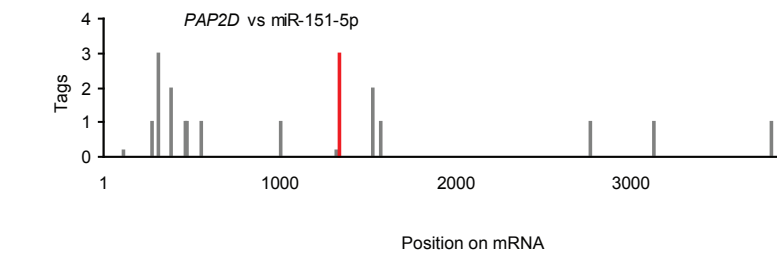
```
chr2:54,749,022-54,749,041 ORF
5'-  AFGGATCCAGGCTATCTTCGGCAATCTTCCTTGATAAAACAGAGGGTCTGCGACACC 3' #read #loci SPBTN1
5'-      ..GCTATCTCTTCGGCAATC.. 3' 2 1 cTag
5'-      ..TATCTCTTCGGCAATCTTCCT.. 3' 2 1 cTag
5'-      ..ATCTCTTCGGCAATCTTCCT.. 3' 3 1 cTag
5'-      ..CTTCGGCAATCTTCCTTGAT.. 3' 1 1 cTag
5'-      ..CTTCGGCAATCTTCCTTGAT.. 3' 1 1 cTag
5'-      ..CTTCGGCAATCTTCCTTGAT.. 3' 1 1 cTag
5'-      ..TCGCGCAATCTTCCTTGATA.. 3' 1 1 cTag
5'-      ..CGCGCAATCTTCCTTGATA.. 3' 4 1 cTag
5'-      ..CCATCTCTTGATAAAACAG.. 3' 4 1 cTag
5'-      ..ATCTCTCTTGATAAAACAGA.. 3' 5 1 cTag
5'-      ..TCTCTCTTGATAAAACAGAG.. 3' 2 1 cTag
5'-      ..TCTCTCTTGATAAAACAGGGT.. 3' 3 1 cTag
5'-      ..CTTCTCTTGATAAAACAGG.. 3' 1 1 cTag
3'-      ..GGAGCGCGGTAGAGGAGATG.. 5' mLR-877
```

chr5:150,090,406-150,090,427 ORF

5'-	GCTTCACAGCTCTATCTCTGGCCACAAACATCTTCTGATCAACGGTCCCTGCGCTGCCG	3'	#read	#loci	DCTC
5'-	GCTTCAC	3'	1	1	cTag
5'-CTCTGATCAACGGTCCCT	3'	1	1	cTag
3'-TCGGTGTAGTGAAGACTAGA	5'	5		miR-383

[illegible][illegible]

< Cleavage site -1>



chr15:73,437,708-73,728 ORF		#read	loci	MAN2C1
5'-	..CGAGTACATCGTCATGAGGACCAATCTCGTAGAGTGGAGTCTCTGCTCCGCTCGGAGTTCCTCCAGGCCACCTATGAGAT	3	1	MAN2C1
5'-TACACTGGCATGAGGCCAC..	3	1	1 ctag
5'-ACTGGCATGAGGCCAAG..	3	1	1 ctag
5'-CTGGCATGAGGCCACAAGT..	3	1	1 ctag
5'-TGAGTATGAGGCCACAAGT..	3	1	1 ctag
5'-GCAATGAGGCCACAAGTCT..	3	1	1 ctag
5'-GCATGAGGCCACAAGTCT..	3	1	1 ctag
5'-CCACAAGTCTCGAAGGTG..	3	1	1 ctag
5'-CCACAAGTCTCGAAGGTG..	3	3	1 ctag
5'-AGTCTCTGAAGGTGAGTT..	3	1	1 ctag
5'-AGTCTCTGAAGGTGAGTCT..	3	1	1 ctag
5'-TGAGTATGAGGCCACCAAGT..	3	1	1 ctag
5'-AGTCCCTGCTCCGCTCGG..	3	1	1 ctag
3'-TCTCTGAGGTTCAGGAGC..	5	1	mIR-873

```
chr3:139,674,129-139,674,150 ORF
5'- TCCCAAGACAGACAGCGCCAGAGCTTAAGCGAAGCAGTGCCAAAGGTTCTGTGAGCCCATCGGGGAGAGAAGATCCAGCCACATCTTCTGACTG 3' #read #loci F
5'- .....GACAGCGCGCCAGCGCTTAAG..... 3' 1
5'- .....GGCCACAGCGCTTAAGGCAA..... 3' 2
5'- .....AAGCAGCTGCCAAAGGTT..... 3' 2
5'- .....GGCAGCTGCCAAAGGTTCT..... 3' 1
3'- CGGATTCTGTGTCGGGCTCTT..... 5' miR-330-5p
```

chr1:153,175,996-153,176,017 5'UTR			
5'	CTGTGAAGGACACCGCTCTTCTCCGGCTGTCCACGCTTTTCCCACATCTGTTGGACATGAGCTGAGGGGACGGTGGCGGGCGGTGAGCCT	3'	#read loci PWM
5'	TTTCTCTCCGGCTGTCCAC	3'	2 1 cTc
5'	..CCATATCTGTTGGACATGA	3'	1 1 cTc
5'	..ATATCTGTTGGACATGAGC	3'	6 1 cTc
5'	TATCTGTTGGACATGAGCT	3'	26 1 cTc
5'	..ATCTCTGTTGGACATGAGCTG	3'	3 1 cTc
5'	..TCTGTTGGACATGAGCTGA	3'	3 1 cTc
3'	AGTCTCTGACGGAAGAGAGGT	5'	mir-185

chr1:99,141,030-99,131,061 3'UTR

```
5'..TTCGCCCTACAGCTGTGAGCTCTCGAGGGCAGGATTGCTTTTTTTTTCTGTGT 3' #read #loci PAF
5'..CCCCCATAGCTAGCTGTGAGCT.. 3' 1 6 cTag
5'.....GCTCTCGAGGGCAGGATTG.. 3' 3 1 cTag
3'.....TGATCTGACACTCGAGGAGCT.. 5' miR-151-5p
```

[illegible]

CA score:2	4076		
NM_153029 NABP1 3'UTR	5'	TCTAGACTGTGAGCTCTCGT	3'
		- ----- ----- -----	
hsa-miR-151-5p TC	3'	TGATTCTGACACTCGAGAGACT	5'
mouse	3'	TCTAGACTGTGAGCTCTCGT	3'
		- ----- ----- -----	
hsa-miR-151-5p TC	3'	TGATTCTGACACTCGAGAGACT	5'
dog	5'	TCTAGACTGTGAGCTCTCGG	3'
		- ----- ----- -----	
hsa-miR-151-5p TC	3'	TGATTCTGACACTCGAGAGACT	5'
horse	3'	TCTAGACTGTGAGCTCTCGG	3'
		- ----- ----- -----	
hsa-miR-151-5p TC	3'	TGATTCTGACACTCGAGAGACT	5'
pig	3'	TCTAGACTGTGAGCTCTCGG	3'
		- ----- ----- -----	
hsa-miR-151-5p TC	3'	TGATTCTGACACTCGAGAGACT	5'

CA score:2.55	3898				
NM_001042555	FRS3	3'	UTR	5'	ACAGTGGG-ATCACCATGTCGAAA 3'
					- 0 -
hsa-miR-182 BC					3' TCACATCAGAAGTTGTAACGGTTT 5'
mouse		5'	ACAGTGGG-ACATACGTCGCGAA 3'		
					- 0 -
hsa-miR-182 BC					3' TCACATCAGAAGTTGTAACGGTTT 5'
rat		5'	ACAGTGGG-ACATCAATGTCGAAA 3'		
					- 0 -
hsa-miR-182 BC					3' TCACATCAGAAGTTGTAACGGTTT 5'
dog		5'	ACAGTGGG-ACATCAATGTCGAAA 3'		
					- 0 -
hsa-miR-182 BC					3' TCACATCAGAAGTTGTAACGGTTT 5'
horse		5'	ACAGTGGG-ATCACCATGTCGAAA 3'		
					- 0 -
hsa-miR-182 BC					3' TCACATCAGAAGTTGTAACGGTTT 5'
pig		5'	ACAGTGGG-ACATCAATGTCGAAA 3'		
					- 0 -
hsa-miR-182 BC					3' TCACATCAGAAGTTGTAACGGTTT 5'

NA score:3 167					
NC_001010861 PAP2D 3'UTR		5'	ACTAGACTGTGAGCTCTCGA	3'	
hsa-miR-151-5p(TC mouse		3'	TGATCTGACACTGGAGGACT	5'	
	5'	ACGAGCACTGTGAGCTCTCTAA	3'		
hsa-miR-151-5p(TC dog			TGATCTGACACTGGAGGACT	5'	3
	5'	ACTAGACTGTGAGCTCTCTAA	3'		
hsa-miR-151-5p(TC horse			TGATCTGACACTGGAGGACT	5'	2
	5'	ACTAGACTGTGAGCTCTCTAA	3'		
hsa-miR-151-5p(TC pig			TGATCTGACACTGGAGGACT	5'	2
	5'	ACTAGACTGTGAGCTCTCTAA	3'		
hsa-miR-151-5p(TC			TGATCTGACACTGGAGGACT	5'	0
	3'	TGATCTGACACTGGAGGACT	5'		

CA score:2.5	806			
nm_016221 DCTN4 ORF		5'	CGCCACAAACACTCTTCTGATCA	3'
			- ----- ----- -----	
hsa-miR-383 BC mouse		3'	TGGTGTTAGTGGGAAGACTAGA	5'
	5'	CGACACAAGCACTTCTGTGATCA		
		- ----- ----- -----		
hsa-miR-383 BC rat		3'	TCGGTGTAGTGGGAAGACTAGA	5' 2
	5'	CGACACAAGCACTTCTGTGATCA		
		- ----- ----- -----		
hsa-miR-383 BC dog		3'	TGGTGTTAGTGGGAAGACTAGA	5' 2
	5'	CGTCACAAACACTTCTGTGATCA		
		- ----- ----- -----		
hsa-miR-383 BC horse		3'	TCGGTGTAGTGGGAAGACTAGA	5' 2.5
	5'	CGTCACAAACACTTCTGTGATCA		
		- ----- ----- -----		
hsa-miR-383 BC pig		3'	TCGGTGTTAGTGGGAAGACTAGA	5' 2.5
	5'	CGCCATAGGCACTTCTGTGATCA		
		- ----- ----- -----		
hsa-miR-383 BC		3'	TGGTGTTAGTGGGAAGACTAGA	5' 1.5

CA score:2.5	2426	5'	ATGAGGCCCAACAGTTCCTGA	3'	
NC_006715	MANC1	ORF			
hsa-miR-873	IC				
mouse	5'	ACGAGGCCCAACAGTTCCTGA	3'	2.5	
hsa-miR-873	IC				
rat	5'	ACGAGGCCCAACAGTTCCTGA	3'	2.5	
hsa-miR-873	IC				
dog	5'	ACGAGGCCCAACAGTTCCTGA	3'	2	
hsa-miR-873	IC				
horse	5'	ATGAGGCCCAACAGTTCCTGA	3'	2.5	
hsa-miR-873	IC				
pig	5'	ACGAGGCCCAACAGTTCCTGA	3'	2.5	
hsa-miR-873	IC				
	5'	ACGAGGCCCAACAGTTCCTGA	3'	3.5	

Accession	Species	Gene	Chromosome	Start (kb)	End (kb)	Strand	Orientation	Sequence
NC_005386	INPAT	3'UTR	5'	CCCGACGTAGATTGAAGCTCTG	3'			
hsa-miR-708	MS		3'	GGGTGCATACAACTCGAGAA	5'			
mouse	5'	CCCGACGTAGATTGAAGCTCTG	3'					
hsa-miR-708	MS		3'	GGGTGCATACAACTCGAGAA	5'	1		
rat	5'	CCCGCGTAGATTGTAAGCTCTG	3'					
hsa-miR-708	MS		3'	GGGTGCATACAACTCGAGAA	5'	1.5		
dog	5'	CCCGACGTAGATTGAAGCTCTG	3'					
hsa-miR-708	MS		3'	GGGTGCATACAACTCGAGAA	5'	1		
horse	5'	CCCGACGTAGATTGAAGCTCTG	3'					
hsa-miR-708	MS		3'	GGGTGCATACAACTCGAGAA	5'	1		
pig	5'	CCCGACGTAGATTGAAGCTCTG	3'					
hsa-miR-708	MS		3'	GGGTGCATACAACTCGAGAA	5'	1		

Figure S5E. The Distribution of Brain Degradome Tags across the Length of the mRNAs and Conservation of miRNA:site Pairing (Related to Figure 6)

(Upper panel) Distributions are shown for the 11 mRNAs with miRNA:site penalty scores ≤ 3.0 and cleavage tags at the expected site (Table 1), and two others with tags one nucleotide downstream of the expected site. For each mRNA, the graph on the left plots tags across the entire mRNA, indicating the contribution of tags with 5' termini mapping to the expected cleavage site (red), and the image to the right zooms in on the tags mapping near the expected cleavage site, highlighting tags with 5' termini mapping to the expected cleavage site (red). Tags mapping to > 10 genomic loci are not considered. The genomic loci are those of the microRNA complementary sites.

(Lower panel) Conserved sites with evidence for miRNA-directed cleavage. Pairing to orthologous sites in the indicated species, which was used to determine the CA scores.

**Table S1. 78 miRNA/siRNA Transfection Data Sets Used to Evaluate the Efficacy of Centered Sites
(Related to Figure 1)**

miRNA/siRNA sequences	Reference	Accession number	<i>P</i> value (8-mer)
TTTGTAGCCAAATCCTTTCTT	(Birmingham et al., 2006)	E-MEXP-668	1.32E-06
TTTGTAGCCAAATCCTTTCTT	(Birmingham et al., 2006)	E-MEXP-668	1.86E-06
AGAACCTCCATCCATGTGCTT	(Birmingham et al., 2006)	E-MEXP-668	3.15E-05
TCAAATCTGCTCTCTGCTT	(Birmingham et al., 2006)	E-MEXP-668	1.15E-08
ACTTGATCCAGAGAACCTCTT	(Birmingham et al., 2006)	E-MEXP-668	1.82E-07
ACTTGATCCAGAGAACCTCTT	(Birmingham et al., 2006)	E-MEXP-668	1.03E-07
AGTTGCTTCAAATCTGCTCTT	(Birmingham et al., 2006)	E-MEXP-668	6.98E-08
AGTTGCTTCAAATCTGCTCTT	(Birmingham et al., 2006)	E-MEXP-668	9.86E-11
TTCATCTCCAATTCGTAGGTT	(Anderson et al., 2008)	E-MEXP-1402	3.63E-16
ATGCTCTTTCCTCTGTGCTT	(Anderson et al., 2008)	E-MEXP-1402	4.14E-11
TTTTGGAACAGTCTTCCGTT	(Anderson et al., 2008)	E-MEXP-1402	2.93E-05
TTTGGAACAGTCTTCCGATT	(Anderson et al., 2008)	E-MEXP-1402	2.43E-08
CCAAACACCACATGCTTGCTT	(Anderson et al., 2008)	E-MEXP-1402	1.15E-08
AAATACACCTTGACGGTGATT	(Anderson et al., 2008)	E-MEXP-1402	3.72E-06
CTTTCCTCTGTGCCATCTTT	(Anderson et al., 2008)	E-MEXP-1402	1.60E-22
CAGGCTGTCTTGACTGTCGTT	(Anderson et al., 2008)	E-MEXP-1402	5.23E-06
TCGTTGAGGGCAATGCCAGTT	(Anderson et al., 2008)	E-MEXP-1402	2.18E-05
AAGCTTCCCCTTCTCAGCCTT	(Anderson et al., 2008)	E-MEXP-1402	2.69E-07
AAGTCAGAGGAGACCACCTTT	(Anderson et al., 2008)	E-MEXP-1402	4.42E-09
ATGAGCCCCAGCCTTCTCCTT	(Anderson et al., 2008)	E-MEXP-1402	1.11E-06
CAAGCTTCCCGTTCTCAGCTT	(Anderson et al., 2008)	E-MEXP-1402	1.81E-09
TGGCAGTGATGGCATGGACTT	(Anderson et al., 2008)	E-MEXP-1402	1.29E-23
ATTTCCATTGATGACAAGCTT	(Anderson et al., 2008)	E-MEXP-1402	2.55E-21
AAAAGCAGCCCTGGTGACCTT	(Anderson et al., 2008)	E-MEXP-1402	9.82E-18
CATATTTGGCAGGTTTTTCTT	(Anderson et al., 2008)	E-MEXP-1402	5.98E-07
GAGGCAGGGATGATGTTCTTT	(Anderson et al., 2008)	E-MEXP-1402	5.51E-10
TTAGACCACATGTAAACCATT	(Anderson et al., 2008)	E-MEXP-1402	3.18E-16
TGGTACCACATGTAAACCATT	(Anderson et al., 2008)	E-MEXP-1402	6.40E-09
TGATATCACATGTAAACCATT	(Anderson et al., 2008)	E-MEXP-1402	2.30E-14
TACACACATGTAAACCATT	(Anderson et al., 2008)	E-MEXP-1402	3.97E-34
TAGGCAGACATGTAAACCATT	(Anderson et al., 2008)	E-MEXP-1402	9.37E-12
AACCGTAGTTCTCTGTAGGTT	(Jackson et al., 2006b)	GSE5814	4.27E-05
AACTGCAGTTCTCTGTAGGTT	(Jackson et al., 2006b)	GSE5814	2.28E-10
AATCGCAGTTCTCTGTAGGTT	(Jackson et al., 2006b)	GSE5814	5.06E-08
TTGTAGAGGATGAGGCGTGTT	(Jackson et al., 2006b)	GSE5814	2.92E-10
AACCGTAGTTCTCTGTAGGTT	(Jackson et al., 2006b)	GSE5814	9.66E-06
AACCTCAGTTCTCTGTAGGTT	(Jackson et al., 2006b)	GSE5814	5.05E-08
AACTGCAGTTCTCTGTAGGTT	(Jackson et al., 2006b)	GSE5814	1.26E-23
AATCGCAGTTCTCTGTAGGTT	(Jackson et al., 2006b)	GSE5814	8.94E-06
ATCCGAGTTCTCTGTAGGTT	(Jackson et al., 2006b)	GSE5814	1.54E-08
ATCCGAGTTCTCTGTAGGTT	(Jackson et al., 2006b)	GSE5814	2.12E-10
AACCGTAGTTCTCTGTAGGTT	(Jackson et al., 2006b)	GSE5814	4.80E-05
AACCTCAGTTCTCTGTAGGTT	(Jackson et al., 2006b)	GSE5814	2.45E-05
AACTGCAGTTCTCTGTAGGTT	(Jackson et al., 2006b)	GSE5814	6.00E-21

AATCGCAGTTCTCTGTAGGTT	(Jackson et al., 2006b)	GSE5814	6.51E-06
ATCCGCAGTTCTCTGTAGGTT	(Jackson et al., 2006b)	GSE5814	3.95E-08
TTGACCATGATGTTGTCACTT	(Jackson et al., 2006b)	GSE5814	1.03E-11
ATAGGATTCATATTAGGAGTT	(Jackson et al., 2006b)	GSE5814	2.04E-32
CTCAAATAGGTCGTCCTCATT	(Jackson et al., 2006a)	GSE5769	3.82E-13
CTTACCCTTTTGGGTTCTGTT	(Jackson et al., 2006a)	GSE5769	1.36E-12
CACACCCTGCCTATTTCCCTT	(Jackson et al., 2006a)	GSE5769	2.38E-13
CTCAAATAGGTCGTCCTCATT	(Jackson et al., 2006a)	GSE5769	3.09E-11
CTTACCCTTTTGGGTTCTGTT	(Jackson et al., 2006a)	GSE5769	2.05E-06
CACACCCTGCCTATTTCCCTT	(Jackson et al., 2006a)	GSE5769	2.30E-15
AACCGCAGTTCTCTGTAGGTT	(Jackson et al., 2006a)	GSE5769	1.14E-05
AACCGCAGTTCTCTGTAGGTT	(Jackson et al., 2006a)	GSE5769	3.80E-05
CTAGTGTCATTGCGCATGCTT	(Jackson et al., 2006a)	GSE5769	1.52E-16
CGCAAGTCTCCAACATGCCTT	(Schwarz et al., 2006)	GSE5291	6.71E-18
CACATTGCGCAAGTCTCCATT	(Schwarz et al., 2006)	GSE5291	4.08E-19
TCACATTGCGCAAGTCTCCTT	(Schwarz et al., 2006)	GSE5291	1.48E-13
GTCACATTGCGCAAGTCTCTT	(Schwarz et al., 2006)	GSE5291	1.55E-21
AGTCACATTGCGCAAGTCTTT	(Schwarz et al., 2006)	GSE5291	1.22E-09
CAGTCACATTGCGCAAGTCTT	(Schwarz et al., 2006)	GSE5291	1.03E-15
AGCAGTCACATTGCGCAAGTT	(Schwarz et al., 2006)	GSE5291	1.00E-24
CAGCAGTCACATTGCGCAATT	(Schwarz et al., 2006)	GSE5291	3.00E-06
GTCAGCAGTCACATTGCGCTT	(Schwarz et al., 2006)	GSE5291	4.82E-44
TGTCAGCAGTCACATTGCGTT	(Schwarz et al., 2006)	GSE5291	9.28E-31
TGGAATGTAAAGAAGTATGTA	(Lim et al., 2005)	GSE2075	1.91E-23
TAAGGCACGCGGTGAATGCCA	(Lim et al., 2005)	GSE2075	9.74E-43
TGGAAGACTAGTGATTTTGTT	(Grimson et al., 2007)	GSE8501	1.71E-32
TCTTTGGTTATCTAGCTGTATGA	(Grimson et al., 2007)	GSE8501	5.28E-22
TGGAGTGTGACAATGGTGTGTT	(Grimson et al., 2007)	GSE8501	6.55E-34
TCACAGTGAACCGTCTCTTTT	(Grimson et al., 2007)	GSE8501	1.39E-33
TAACAGTCTACAGCCATGGTCG	(Grimson et al., 2007)	GSE8501	3.83E-38
TTGGTCCCCTTCAACCAGCTGT	(Grimson et al., 2007)	GSE8501	2.59E-23
TGTAGTGTTTCTACTTTATGGA	(Grimson et al., 2007)	GSE8501	3.04E-45
TCAGTGATCACAGAACTTTGT	(Grimson et al., 2007)	GSE8501	1.18E-47
AACATTCAACGCTGTCGGTGAGT	(Grimson et al., 2007)	GSE8501	1.05E-44

Table S2. Top 200 miRNAs Sequenced from Zebrafish Embryos (Related to Figure 1)

Among the top 100, the 21 miRNAs with 8-mer 3' UTR sites that associate with derepressed mRNAs in the *dicer* mutant are indicated (bold).

miRNAs			miRNAs		
	Read number	<i>P</i> value (8-mer)		Read number	
1	dre-miR-430b	83423	101	dre-miR-7a	135
2	dre-miR-430a	32893	102	dre-miR-152	135
3	dre-miR-430c	30511	103	dre-miR-137	129
4	dre-miR-206	14856	104	dre-miR-204	127
5	dre-miR-17a	12905	105	dre-miR-210	125
6	dre-miR-19b	4273	106	dre-miR-451	125
7	dre-miR-20a	4217	107	dre-miR-135c	124
8	dre-miR-20b	3727	108	dre-miR-199*	113
9	dre-miR-130c	3668	109	dre-miR-9*	111
10	dre-miR-1	3419	110	dre-miR-144	103
11	dre-miR-130b	2727	111	dre-miR-101a	91
12	dre-miR-457a	2565	112	dre-let-7g	90
13	dre-miR-21	2222	113	dre-miR-456	86
14	dre-miR-19a	2126	114	dre-miR-135b	79
15	dre-miR-200a	2068	115	dre-miR-143	73
16	dre-miR-203b	2002	116	dre-miR-194a	73
17	dre-miR-92a	1974	117	dre-miR-153a	69
18	dre-miR-18a	1710	118	dre-miR-27a	67
19	dre-miR-454b	1644	119	dre-miR-22b	67
20	dre-miR-429	1614	120	dre-let-7e	67
21	dre-miR-18c	1471	121	dre-miR-153c	66
22	dre-miR-22a	1413	122	dre-miR-338	65
23	dre-miR-16b	1351	123	dre-miR-19b*	57
24	dre-miR-124	1259	124	dre-miR-30a	57
25	dre-miR-30e	1206	125	dre-miR-192	55
26	dre-miR-26a	1195	126	dre-miR-375	54
27	dre-miR-222	1175	127	dre-miR-10d	54
28	dre-miR-26b	1153	128	dre-miR-34	53
29	dre-miR-15c	1121	129	dre-miR-101b	52
30	dre-miR-301c	936	130	dre-miR-23b	50
31	dre-miR-25	924	131	dre-miR-181c	48
32	dre-miR-9	885	132	dre-miR-139	41
33	dre-miR-200b	799	133	dre-miR-210*	37
34	dre-miR-10c	752	134	dre-miR-146a	36
35	dre-miR-24	750	135	dre-miR-138	34
36	dre-miR-15b	740	136	dre-miR-142a-3p	33
37	dre-miR-19d	730	137	dre-miR-193b	30
38	dre-miR-203b*	701	138	dre-miR-23a	30
39	dre-miR-184	666	139	dre-miR-145	30
40	dre-miR-93	666	140	dre-miR-730	27
41	dre-miR-203a	623	141	dre-miR-107	26
42	dre-miR-18b	619	142	dre-miR-458	24
43	dre-miR-126	608	143	dre-miR-34c	23
44	dre-miR-30c	606	144	dre-miR-150	22
45	dre-miR-96	604	145	dre-miR-10a*	20
46	dre-miR-363	579	146	dre-miR-29a	16
47	dre-miR-196b	578	147	dre-miR-190	16
48	dre-miR-140	577	148	dre-miR-27d	15
49	dre-miR-141	548	149	dre-miR-29b	14
50	dre-miR-301b	546	150	dre-miR-34b	14
51	dre-miR-221	537	151	dre-miR-146b	13
52	dre-miR-218a	506	152	dre-let-7f	11
53	dre-miR-10b	490	153	dre-miR-129	10

54	dre-miR-205	487	0.158166138	154	dre-miR-129*	10
55	dre-miR-301a	485	0.023798257	155	dre-miR-727*	10
56	dre-miR-200c	471	0.214082525	156	dre-miR-430j	10
57	dre-miR-183	456	0.003283033	157	dre-miR-30e*	9
58	dre-miR-15a	456	0.002344751	158	dre-miR-125c	9
59	dre-miR-196a	444	0.181578515	159	dre-miR-122	9
60	dre-miR-16a	441	0.024759062	160	dre-let-7i	8
61	dre-miR-10a	432	0.344155016	161	dre-miR-460-5p	8
62	dre-miR-219	408	0.644480947	162	dre-miR-10d*	8
63	dre-miR-133b	387	0.027168426	163	dre-miR-737	8
64	dre-miR-148	377	0.00441369	164	dre-miR-216a	8
65	dre-miR-133b*	371	0.289806481	165	dre-miR-724	8
66	dre-miR-457b	357	0.023853159	166	dre-miR-430i	8
67	dre-miR-499	344	0.104589707	167	dre-miR-223	7
68	dre-miR-92b	332	0.08525947	168	dre-miR-18b*	7
69	dre-miR-133a*	325	0.427467622	169	dre-miR-732	6
70	dre-miR-7b	299	0.22189028	170	dre-miR-142a-5p	6
71	dre-miR-30d	277	0.221097422	171	dre-miR-462	6
72	dre-miR-182	257	0.057520514	172	dre-miR-135a	6
73	dre-miR-103	252	0.817738452	173	dre-miR-27e	5
74	dre-miR-128	246	0.962580323	174	dre-miR-125a	5
75	dre-miR-454a	242	0.002361776	175	dre-let-7d	5
76	dre-miR-181b	234	0.016651723	176	dre-miR-728	5
77	dre-miR-133a	233	0.027168426	177	dre-miR-155	5
78	dre-miR-217	231	0.03564193	178	dre-miR-19a*	5
79	dre-miR-181a	230	0.017836462	179	dre-miR-15a*	4
80	dre-miR-130a	227	0.002376865	180	dre-miR-731	4
81	dre-miR-133c	223	0.027290714	181	dre-miR-460-3p	4
82	dre-miR-725	222	0.483281714	182	dre-miR-190b	3
83	dre-miR-140*	218	0.193099745	183	dre-miR-459	3
84	dre-miR-199	216	0.306964785	184	dre-let-7j	3
85	dre-miR-27b	212	0.554105668	185	dre-miR-722	3
86	dre-miR-30b	201	0.22409913	186	dre-miR-194b	3
87	dre-let-7c	201	0.105621503	187	dre-miR-733	2
88	dre-miR-181a*	191	0.436580904	188	dre-miR-727	2
89	dre-let-7a	190	0.105621503	189	dre-miR-455	2
90	dre-miR-27c	186	0.555053604	190	dre-miR-193a	1
91	dre-miR-20a*	186	0.019484944	191	dre-let-7h	1
92	dre-miR-19c	175	0.000173428	192	dre-miR-214	1
93	dre-miR-100	173	0.22690878	193	dre-miR-365	1
94	dre-miR-125b	163	0.810184463	194	dre-miR-132	1
95	dre-miR-216b	160	0.156299204	195	dre-miR-202*	1
96	dre-miR-126*	150	0.000170833	196	dre-let-7b	1
97	dre-miR-218b	145	0.476164262	197	dre-miR-729	1
98	dre-miR-99	143	0.22690878	198	dre-miR-723	1
99	dre-miR-153b	140	0.060775028	199	dre-miR-17a*	1
100	dre-miR-736	137	0.198787714	200	dre-miR-187	1

Table S3. 48 miRNAs Used to Evaluate the Efficacy of Centered Sites in *Drosophila* Knockdown S2 Cells**(Related to Figure 1)**

miRNA	<i>P</i> values (8-mer)
dme-mir-8_3p	4.25E-03
dme-mir-980_3p	5.23E-06
dme-mir-11_3p	8.08E-06
dme-mir-2b-2_3p	8.04E-06
dme-mir-2b-1_3p	8.04E-06
dme-mir-13b-2_3p	8.08E-06
dme-mir-13b-1_3p	8.08E-06
dme-mir-33_5p	7.37E-03
dme-mir-275_3p	5.85E-03
dme-mir-9b_5p	8.49E-03
dme-bantam_5p	3.80E-05
dme-mir-970_3p	5.96E-04
dme-mir-9c_5p	8.33E-03
dme-mir-14_3p	9.21E-04
dme-mir-987_5p	2.03E-03
dme-mir-999_3p	7.90E-03
dme-mir-306_5p	5.83E-03
dme-mir-9b_3p	1.88E-05
dme-mir-277_3p	3.67E-07
dme-mir-252_5p	1.57E-04
dme-mir-277_5p	7.00E-04
dme-mir-2b-2_5p	3.53E-05
dme-mir-308_3p	8.14E-06
dme-mir-33_3p	6.26E-11
dme-mir-281-2_5p	2.67E-04
dme-mir-2a-2_5p	5.18E-09
dme-mir-7_5p	2.11E-04
dme-mir-281-1_3p	3.51E-07
dme-mir-281-2_3p	3.51E-07
dme-mir-13a_3p	8.08E-06
dme-mir-2a-1_5p	1.04E-03
dme-mir-995_5p	2.31E-03
dme-mir-9c_3p	6.62E-04
dme-mir-283_5p	8.47E-06
dme-mir-304_3p	5.89E-04
dme-mir-1013_3p	3.14E-09
dme-mir-13b-2_5p	6.25E-03
dme-mir-984_5p	7.76E-03
dme-mir-954_5p	2.54E-07
dme-mir-1006_3p	1.07E-10
dme-mir-87_3p	3.07E-04
dme-mir-994_5p	2.68E-05
dme-mir-190_3p	4.59E-03
dme-mir-989_3p	5.29E-03
dme-mir-92b_3p	6.72E-04
dme-mir-1000_5p	9.30E-04
dme-mir-1005_3p	4.78E-04
dme-mir-274_5p	8.79E-05

Table S4. 26 miRNAs Used to Evaluate the Efficacy of Centered Sites in *Dicer* Knockdown S2 Cells

(Related to Figure 1)

miRNA	<i>P</i> value (8mer)
dme-bantam_3p	7.13E-03
dme-mir-980_3p	9.40E-04
dme-mir-275_3p	5.66E-05
dme-mir-9b_5p	8.67E-09
dme-mir-34_5p	1.51E-04
dme-mir-970_3p	1.68E-03
dme-mir-9c_5p	9.08E-09
dme-mir-305_5p	6.32E-03
dme-mir-987_5p	5.05E-03
dme-mir-999_3p	6.61E-03
dme-mir-306_5p	5.68E-05
dme-mir-2b-2_5p	2.86E-04
dme-mir-988_3p	3.51E-03
dme-mir-33_3p	9.11E-03
dme-mir-996_5p	1.06E-06
dme-mir-304_5p	8.82E-04
dme-mir-9c_3p	5.29E-07
dme-mir-281-1_5p	4.58E-05
dme-mir-304_3p	6.15E-03
dme-mir-984_5p	9.02E-04
dme-mir-994_5p	1.56E-03
dme-mir-1008_3p	5.17E-03
dme-mir-984_3p	1.16E-03
dme-mir-11_5p	8.14E-10
dme-mir-14_5p	3.44E-04
dme-mir-79_5p	6.96E-03

Table S5. Top 20 Cleavage-Tag Clusters from Unclassified Loci (Related to Figure 4)

Clusters were defined as the collection of tags mapping to unannotated loci within a 100 bp window.

Cluster	Annotation	Genomic coordination	Unique tags	Tags
*C1	Noncoding region / Genscan intron	chr9:125882507-125882514	8	532,457.3
*C2	TMEM56 intron	chr1:95393936-95393944	7	531,853.9
*C3	Noncoding region / Genscan intron	chr5:36122996-36123000	5	530,610.4
C4	28s rRNA-like sequence - 3' flanking fragment	chr17:30502259-30502436	43	466,872.0
*C5	CD74 intron	chr5:149767818-149767824	7	356,656.4
*C6	CCDC104 intron	chr2:55623717-55623719	3	176,309.0
°C7	MT tRNA derived - 3' flanking fragment	chr1:558638-558787	21	57,790.8
°C8	MT tRNA derived - 3' flanking fragment	chr2:87905556-87905590	16	57,776.3
§C9	mir-21 Drosha 3' fragment	chr17:55273471-55274456	139	27,967.5
C10	AK023662 intron: sharing same tags as C9	chr9:93952632-93953523	115	27,910.8
§C11	Let-7a-3 Drosha 3' fragment	chr22:44887364-44887372	8	15,950.0
§C12	mir-31 Drosha 3' fragment	chr9:21501769-21502100	18	15,619.5
C13	C20orf94 intron	chr20:10370743-10370745	4	15,278.5
C14	18s rRNA-like sequence - 3' flanking fragment	chr16:33870633-33871075	140	12,439.3
C15	EIF4A2 intron (Cassette exon)	chr3:187988217-187989583	276	10,686.2
C16	MT tRNA derived - 3' flanking fragment	chr1:555957- 555995	7	8,406.8
§C17	Let-7d Drosha 3' fragment	chr9:95981017-95981192	12	8,008.0
§C18	Let-7i Drosha 3' fragment	chr12:61283726-61283816	5	7,308.0
C19	snRNA hostgene8	chr4:119419312-119419791	173	6,396.5
C20	snRNA hostgene6	chr8:67997402-67997496	89	6,169.5
Sum				2,814,690 (~65%)

§ 3' fragment of pri-miRNA following Drosha processing

*, ° shared the tags with other loci.

Table S6. Examples of Clusters Coming from 3' Fragments of Pri-miRNAs (Related to Figure 4)

Dominant 3' fragments of pri-miRNAs are indicated in red, and dominant miRNA reads are indicated in blue. Loci# indicates the number of genomic loci to which the read maps. cTag indicates cleavage tags from degradome sequencing. Asterisk lines indicate the loci of indicated clusters.

Examples				
C9				
<div> <div>C9</div> <div>*****</div> <div> 5'- ACCACCTTGTGGGTAGCTTATCAGACTGATGTTGACTGTTGAATCTCATGGCAACACCACTCGATGGGCTGTCTGACATTTTGGTATCTTTTCATCTGACCAATCCATATCCAAATGTTCTC 3' chr17:55273401-55273521 5'-TGTCGGGTAGCTTATCAGACTGATGTTGACTGTTGAATCTCATGGCAACACCACTCGATGGGCTGTCTGACA..... 3' read# loci# hsa-mir-21 5'-GTAGCTTATCAGACTGATGT..... 3' 1 1 cTag 5'-TAGCTTATCAGACTGATGTT..... 3' 99 1 cTag 5'-AGCTTATCAGACTGATGTTG..... 3' 23 1 cTag 5'-ATGGCAACACCACTCGATGG..... 3' 2 1 cTag 5'-TGCTGACATTTTGGTATCT..... 3' 1 1 cTag 5'-CTGACATTTTGGTATCTTTTC..... 3' 48 2 cTag 5'-TGACATTTTGGTATCTTTCA..... 3' 23284 2 cTag 5'-GACATTTTGGTATCTTTTCAT..... 3' 32263 2 cTag 5'-ACATTTTGGTATCTTTTCATC..... 3' 11 2 cTag 5'-CATTTTGGTATCTTTTCATCT..... 3' 14 2 cTag 5'-ATTTTGGTATCTTTTCATCTG..... 3' 1 2 cTag 5'-TTTTGGTATCTTTTCATCTGA..... 3' 6 2 cTag 5'-TTTGGTATCTTTTCATCTGAC..... 3' 3 2 cTag 5'-TGSTATCTTTTCATCTGACCA..... 3' 3 2 cTag 5'-ATCTTTTCATCTTGACCATCCA..... 3' 2 2 cTag 5'-CATATCCAAATGTTCTC..... 3' 1 2 cTag 5'-GGGTAGCTTATCAGACTGATGTT..... 3' 2 1 smallRNA 5'-GTAGCTTATCAGACTGATGT..... 3' 4 1 smallRNA 5'-GTAGCTTATCAGACTGATGTT..... 3' 1 1 smallRNA 5'-GTAGCTTATCAGACTGATGTTG..... 3' 52 1 smallRNA 5'-GTAGCTTATCAGACTGATGTTGA..... 3' 246 1 smallRNA 5'-GTAGCTTATCAGACTGATGTTGAC..... 3' 86 1 smallRNA 5'-TAGCTTATCAGACTGATGTT..... 3' 858 1 smallRNA 5'-TAGCTTATCAGACTGATGTTG..... 3' 7263 1 smallRNA 5'-TAGCTTATCAGACTGATGTTGA..... 3' 118838 1 smallRNA 5'-TAGCTTATCAGACTGATGTTGAC..... 3' 140843 1 smallRNA 5'-TAGCTTATCAGACTGATGTTGACT..... 3' 6849 1 smallRNA 5'-AGCTTATCAGACTGATGTTG..... 3' 63 1 smallRNA 5'-AGCTTATCAGACTGATGTTGA..... 3' 1099 1 smallRNA 5'-AGCTTATCAGACTGATGTTGAC..... 3' 1395 1 smallRNA 5'-AGCTTATCAGACTGATGTTGACT..... 3' 87 1 smallRNA 5'-GCTTATCAGACTGATGTTGA..... 3' 137 1 smallRNA 5'-GCTTATCAGACTGATGTTGAC..... 3' 143 1 smallRNA 5'-GCTTATCAGACTGATGTTGACT..... 3' 4 1 smallRNA 5'-CTTATCAGACTGATGTTGAC..... 3' 433 1 smallRNA 5'-CTTATCAGACTGATGTTGACT..... 3' 12 1 smallRNA 5'-TTATCAGACTGATGTTGACT..... 3' 20 1 smallRNA 5'-ACTGATGTTGACTGTTGAATCTC..... 3' 1 1 smallRNA 5'-TGTTGAATCTCATGGCAACAC..... 3' 1 1 smallRNA 5'-TCATGGCAACACCACTCGATGGGC..... 3' 1 1 smallRNA 5'-GGCAACACCACTCGATGGGCTGTCT..... 3' 2 1 smallRNA 5'-CAACACCACTCGATGGGCTGT..... 3' 14 1 smallRNA 5'-CAACACCACTCGATGGGCTGT..... 3' 640 1 smallRNA 5'-CAACACCACTCGATGGGCTGTCT..... 3' 670 1 smallRNA 5'-CAACACCACTCGATGGGCTGTCT..... 3' 25 1 smallRNA 5'-AACACCACTCGATGGGCTGT..... 3' 19 1 smallRNA 5'-AACACCACTCGATGGGCTGTCT..... 3' 7 1 smallRNA 5'-AACACCACTCGATGGGCTGTCT..... 3' 15 1 smallRNA 5'-ACACCACTCGATGGGCTGTCT..... 3' 3 1 smallRNA 5'-ACACCACTCGATGGGCTGTCT..... 3' 2 1 smallRNA 5'-TGACATTTTGGTATCTTTTCATCTG..... 3' 1 2 smallRNA </div> </div>				
C17				
<div> <div>C17</div> <div>*****</div> <div> 5'- AAAAAAATGGGTTCTAGGAAGAGGTAGTAGGTTGCATAGTTTTAGGGCAGGGATTTTGCCCAAGAGGAGTAACATACGACCTGCTGCCCTTCTTAGGGCCTTATTATTACCGATAACCTGTTTCCTTCT 3' chr9:95980921:95981061 5'-CCTAGGAAGAGGTAGTAGGTTGCATAGTTTTAGGGCAGGGATTTTGCCCAAGAGGAGTAACATACGACCTGCTGCCCTTCTTAGG..... 3' hsa-let-7d 5'-AGAGGTAGTAGGTTGCATAG..... 3' 29 1 cRNA 5'-GAGGTAGTAGGTTGCATAGT..... 3' 52 1 cRNA 5'-AGGGCAGGGATTTTGCCCAAC..... 3' 2 1 cRNA 5'-TATACGACCTGCTGCCCTTCT..... 3' 2 1 cRNA 5'-CTTAGGGCCTTATTATTTCAC..... 3' 492 1 cRNA 5'-TTAGGGCCTTATTATTTCACC..... 3' 5686 1 cRNA 5'-TAGGGCCTTATTATTTCACCG..... 3' 1815 1 cRNA 5'-AGGGCCTTATTATTTCACCGA..... 3' 5 1 cRNA 5'-GGGCCTTATTATTTCACCGAT..... 3' 1 1 cRNA 5'-CTTATTATTTCACCGATAACC..... 3' 1 1 cRNA 5'-TCACCGATAACCTGTTTCCT..... 3' 1 1 cRNA 5'-CACCGATAACCTGTTTCCTT..... 3' 1 1 cRNA 5'-AAAAAATGGGTTCTAGGA..... 3' 1 1 smallRNA 5'-AAAAAATGGGTTCTAGGA..... 3' 4 2 smallRNA 5'-AAAAAATGGGTTCTAGGA..... 3' 1 1 smallRNA 5'-AGAGGTAGTAGGTTGCATAGTT..... 3' 3 1 smallRNA 5'-AGAGGTAGTAGGTTGCATAG..... 3' 42 1 smallRNA 5'-AGAGGTAGTAGGTTGCATAGT..... 3' 545 1 smallRNA 5'-AGAGGTAGTAGGTTGCATAGTT..... 3' 2840 1 smallRNA 5'-AGAGGTAGTAGGTTGCATAGTTT..... 3' 136 1 smallRNA 5'-AGAGGTAGTAGGTTGCATAGTTT..... 3' 1 1 smallRNA 5'-GAGGTAGTAGGTTGCATAGT..... 3' 2 1 smallRNA 5'-GAGGTAGTAGGTTGCATAGTT..... 3' 23 1 smallRNA 5'-GAGGTAGTAGGTTGCATAGTTT..... 3' 1 1 smallRNA 5'-AGGTAGTAGGTTGCATAGTT..... 3' 7 1 smallRNA 5'-CTATACGACCTGCTGCCCTT..... 3' 10 1 smallRNA 5'-CTATACGACCTGCTGCCCTTCT..... 3' 4 1 smallRNA 5'-CTATACGACCTGCTGCCCTTCT..... 3' 31 1 smallRNA 5'-TATACGACCTGCTGCCCTTCT..... 3' 4 1 smallRNA </div> </div>				

Table S7. Human miRNA:site Duplexes with Alignment Penalty Score ≤ 2.0 (Related to Figure 5)

The 106 miRNA:site duplexes listed excludes several repeat-derived miRNAs (miR-574-5p, miR-1285, miR-1268, and miR-1303), which had a high number of perfectly matched sites compared with other miRNAs (Figure S4B). Out of the 106 sites, 47 were in annotated ORFs, 16 were in 5' UTRs, and 43 were in 3' UTRs.

Score:0 167 NM_001010861 PAP2D 3'UTR	5' ACTAGACTGTGAGCTCCTCGA 3'				
hsa-miR-151-5p Score:0 471 NM_014400 LYPD3 3'UTR	3' TGATCTGACACTCGAGGAGCT 5' 0				
hsa-miR-151-5p Score:0 3688 NM_007007 CPSF6 3'UTR	5' AGAAGAAGCAATATGA 3'				
hsa-miR-1279 Score:0 150 NM_001018108 SERF2 ORF	3' TCTTTCTTCGTTTACT 5' 0				
hsa-miR-1282 Score:0 2734 NM_001025190 MPFL ORF	5' AAGCAGAAAAGGCAACGA 3'				
hsa-miR-1282 Score:0 2734 NM_001025190 MPFL ORF	3' TTGCTCTTTTTCGTTGCT 5' 0				
hsa-miR-662 Score:1 297 NM_021257 NGB 3'UTR	5' CTGCTGGGCGACACGTGGGA 3'				
hsa-miR-662 Score:1 297 NM_021257 NGB 3'UTR	3' GACGACCCGCTGTTGCAACCT 5' 0				
hsa-miR-1260 Score:1 745 NM_018715 RCC2 3'UTR	5' TGGTGGCAGAGTGGGAT 3'				
hsa-miR-1260 Score:1 600 NM_153202 ADAM33 3'UTR	3' ACCACCGTCTCCACCTA 5' 1				
hsa-miR-1260 Score:1 600 NM_153202 ADAM33 3'UTR	5' TGGTGGCAGAGTGGGAT 3'				
hsa-miR-1275 Score:1 521 NM_017594 DIRAS2 3'UTR	3' ACCACCGTCTCCACCTA 5' 1				
hsa-miR-1275 Score:1 521 NM_017594 DIRAS2 3'UTR	5' GACAGCCTCTCCCCAT 3'				
hsa-miR-1275 Score:1 3075 NM_020923 ZDBF2 ORF	3' CTGTGGAGAGGGGGTG 5' 1				
hsa-miR-1275 Score:1 3075 NM_020923 ZDBF2 ORF	5' CACAGCCTCTCCCCAA 3'				
hsa-miR-1279 Score:1 631 NM_001007559 SS18 ORF	3' CTGTGGAGAGGGGGTG 5' 1				
hsa-miR-1279 Score:1 631 NM_001007559 SS18 ORF	5' AGAAGAAGCAATATGT 3'				
hsa-miR-1300 Score:1 2927 NM_001042546 ATPAF1 3'UTR	3' TCTTTCTTCGTTTACT 5' 1				
hsa-miR-1300 Score:1 2927 NM_001042546 ATPAF1 3'UTR	5' CAGCAGCCTCTCTTCAG 3'				
hsa-miR-151-5p Score:1 695 NM_182646 CPEB2 3'UTR	3' GTCTCGGAGGAGAGT 5' 1				
hsa-miR-151-5p Score:1 695 NM_182646 CPEB2 3'UTR	5' ACTAGACTGTGAGCTCCTTGA 3'				
hsa-miR-15a* Score:1.0 424 NM_024016 HOXB8 3'UTR	3' TGATCTGACACTCGAGGAGCT 5' 1.0				
hsa-miR-15a* Score:1.0 424 NM_024016 HOXB8 3'UTR	5' -GCAGGACGACAAATATGGCTA 3'				
hsa-miR-196a Score:1 838 NM_005386 NNAT 3'UTR	3' AC-TCCGTCTGTATACCGGAC 5' 1				
hsa-miR-196a Score:1 838 NM_005386 NNAT 3'UTR	5' CCCAACACATGAACTGCCTA 3'				
hsa-miR-339-5p Score:1 4820 NM_032012 C9ORF5 3'UTR	3' GGTTGTGTACTTTGATGGAT 5' 1.0				
hsa-miR-339-5p Score:1 4820 NM_032012 C9ORF5 3'UTR	5' TGTAACTCTCTGGAGCAGGGA 3'				
hsa-miR-378 Score:1 1904 NM_001014380 KATNAL1 3'UTR	3' GCACTCGAGAGCTCTGTCCCT 5' 1				
hsa-miR-378 Score:1 1904 NM_001014380 KATNAL1 3'UTR	5' ACTTCTGACTCCAAATTCAGTA 3'				
hsa-miR-548c-5p Score:1 733 NM_198798 ANKRD5 3'UTR	3' GGAAGACTGAGGTTTCAGTCA 5' 1				
hsa-miR-548c-5p Score:1 733 NM_198798 ANKRD5 3'UTR	5' GGCAAAAACCGCAATTAATTTT 3'				
hsa-miR-548c-5p Score:1 557 NM_032444 BTBD12 3'UTR	3' CCGTTTTTGGCGTTAATGAAA 5' 1				
hsa-miR-548c-5p Score:1 557 NM_032444 BTBD12 3'UTR	5' AGCAAAAACCGCAATTAATTTT 3'				
hsa-miR-566 Score:1.0 3704 NM_181832 NF2 3'UTR	3' CCGTTTTTGGCGTTAATGAAA 5' 1				
hsa-miR-566 Score:1.0 3704 NM_181832 NF2 3'UTR	5' GCTGGGATCACAGCGCCCA 3'				
hsa-miR-671-5p Score:1 827 NM_005386 NNAT 3'UTR	3' CAACCTAGTGTCCGCGG 5' 1				
hsa-miR-671-5p Score:1 827 NM_005386 NNAT 3'UTR	5' AGCCAGCCCCCTCAGGGCTTTCA 3'				
hsa-miR-708 Score:1.5 558 NM_005317 GZM ORF	3' GAGTTCGGGAGGTCCTCGCT 5' 1.0				
hsa-miR-708 Score:1.5 558 NM_005317 GZM ORF	5' CCGTTTTTGGCGTTAATGAAA 5' 1				
hsa-miR-1275 Score:1.5 119 NM_000093 COL5A1 5'UTR	3' GCGAGCCTCTCCCCAG 3'				
hsa-miR-1275 Score:1.5 119 NM_000093 COL5A1 5'UTR	5' GAGTTCGGGAGGTCCTCGCT 5' 1.0				
hsa-miR-1281 Score:1.5 39 NM_001310 CREBL2 5'UTR	3' GGTTCGATCTAACATTCGAGGA 5' 1				
hsa-miR-1281 Score:1.5 39 NM_001310 CREBL2 5'UTR	5' GCGAGCCTCTCCCCAG 3'				
hsa-miR-1281 Score:1.5 251 NM_002843 PTPRJ 5'UTR	3' GGTTCGATCTAACATTCGAGGA 5' 1				
hsa-miR-1281 Score:1.5 26 NM_022078 GPATCH3 ORF	5' GCGAGCCTCTCCCCAG 3'				
hsa-miR-1281 Score:1.5 26 NM_022078 GPATCH3 ORF	3' GGTTCGATCTAACATTCGAGGA 5' 1				
Score:2 440 NM_005705 TSPAN32 ORF	5' GGGAGAGGAGGCGGCGA 3'				
hsa-miR-1281 Score:2 440 NM_005705 TSPAN32 ORF	3' CCCTCTCTCTCTCGCT 5' 2				
Score:2 207 NM_000719 CACNA1C 3'UTR	5' GGGAGAGGAGGCGGCGA 3'				
hsa-miR-1281 Score:2 514 AL050388.1 Q5TCM0_HUMAN 3'UTR	3' CCCTCTCTCTCTCGCT 5' 2				
hsa-miR-1281 Score:2 775 NM_002316 LMXB1 3'UTR	5' GGGAGAGGAGGAGGCGA 3'				
hsa-miR-1281 Score:2 731 NM_031229 RBCK1 ORF	3' CCCTCTCTCTCTCGCT 5' 2				
hsa-miR-1281 Score:2 87 NM_000093 COL5A1 5'UTR	5' GGGAGAGGAGGAGGCGA 3'				
hsa-miR-1281 Score:2 286 NM_006885 ZFXH3 5'UTR	3' CCCTCTCTCTCTCGCT 5' 2				
hsa-miR-1281 Score:2 695 NM_016358 IRX4 ORF	5' GGGAGAGGAGGAGGCGG 3'				
hsa-miR-1281 Score:2 119 NM_005392 PHF2 ORF	3' CCCTCTCTCTCTCGCT 5' 2				
hsa-miR-1281 Score:2.0 1109 NM_001297 CNGB1 ORF	5' GGAAGAGGAGGAGGCGC 3'				
hsa-miR-1281 Score:2.0 176 NM_002500 NEUROD1 ORF	3' CCCTCTCTCTCTCGCT 5' 2				
hsa-miR-1281 Score:2 367 NM_016585 THEG ORF	5' GGAAGAGGAGGAGGCGG 3'				
hsa-miR-1281 Score:2 177 NM_004389 CTNNA2 5'UTR	3' CCCTCTCTCTCTCGCT 5' 2				
hsa-miR-1281 Score:2 78 NM_003601 SMARCA5 5'UTR	5' GGAAGAGGAGGAGGCGG 3'				
hsa-miR-1281 Score:2 60 NM_001018112 FANCA 3'UTR	3' CCCTCTCTCTCTCGCT 5' 2				
hsa-miR-1281 Score:2 122 NM_198576 AGRN ORF	5' GGGAGAGGAGGAGGCGA 3'				
hsa-miR-1281 Score:2.0 2408 NM_001005360 DNM2 ORF	3' CCCTCTCTCTCTCGCT 5' 2				
hsa-miR-1300 Score:2.0 608 NM_024321 RBM42 ORF	5' GCGAGGAGGAGGCGG 3'				
hsa-miR-1300 Score:2 808 NM_015409 EP400 ORF	3' CCCTCTCTCTCTCGCT 5' 2.0				
hsa-miR-1322 Score:2 4076 NM_153029 N4BP1 3'UTR	5' GGTGACTTGGTGGGTACG 5' 2.0				
hsa-miR-151-5p Score:2 2166 NM_182580 CYB561D1 3'UTR	3' CAG-ATCAGCAGCATCATC 3'				
hsa-miR-1825 Score:2.0 1362 NM_003247 THBS2 ORF	5' TCTAGACTGTGAGCTCCTCGT 3'				
hsa-miR-192* Score:2.0 80 BC000939.3 Q9BVT6_HUMAN 5'UTR	3' TGATCTGACACTCGAGGAGCT 5' 2				
hsa-miR-214 Score:2.0 265 NM_004573 PLCB2 5'UTR	5' AGAGAGGAGGCGACTGGT 3'				
hsa-miR-214 Score:2 250 NM_016240 SCARA3 3'UTR	3' CCTCTCTCTCTCGCT 5' 2				
hsa-miR-297 Score:2.0 140	5' TCTGCCTGCCTGTGTCTGCTGA 3'				
hsa-miR-297 Score:2.0 140	3' TGACGGACAGACACGGACGACA 5' 2.0				

Table S8. Murine miRNA:site Duplexes with Alignment Penalty Score ≤ 2.0 (Related to Figure 5)

Of the 66 miRNA:site duplexes found, 25 were in annotated ORFs, two were in 5' UTRs, and 39 were in 3' UTRs.

Score:0 1093 (Opposite strand) NM_001039586 Glyctk 3'UTR 5' TCACATAGGAATAAAAGCCATA 3' mmu-miR-135a 3' AGTGATATCCTTATTTTCGGTAT 5' 0 Score:0 1054 (Opposite strand) NM_001039586 Glyctk 3'UTR 5' CGCCACGGCTCCCAATCCCTATA 3' mmu-miR-135a* 3' GCGGTGCCGAGGTTAGGGATAT 5' 0	Score:2 1630 NM_001037916 Ccdc17 ORF 5' TGACC-CCCCACCTCCCCACA 3' - - mmu-miR-705 3' ACGGGTGGGGTGGAGGGTGG 5' 2 Score:2 1912 NM_010800 Bhlhb8 3'UTR 5' CGGGACCTCAGCCTCCTGGC 3' - mmu-miR-665 3' TCCCTGGAGTCGGAGGACCA 5' 2 Score:2 138 NM_001039676 Slc39a2 ORF 5' AGGGTCCTCAGCCTCCTGGG 3' --- mmu-miR-665 3' TCCCTGGAGTCGGAGGACCA 5' 2 Score:2.0 345 NM_198029 5830467p10rik 5'UTR 5' GAGCGGGGAGGGGAGGAGTGT 3' 0 0 mmu-miR-483* 3' TTCTGCCCTCCCTCCTCACT 5' 2.0 Score:2.0 1389 NM_172767 Lohllcr2a 3'UTR 5' GTGTGTATATATTATACAT 3' 0 mmu-miR-568 3' CACACATATGTAAATATGTA 5' 2.0
Score:1 mouse Atpaf1 5' ACTAGACTGTGAGCTCCCTGA 3' 0 mmu-miR-151-5p 3' TGATCTGACACTCGAGGAGCT 5' 1.0 Score:1 187 NM_152825 Usp45 3'UTR 5' CATGCCCTTAAATTCAGAGCTT 3' mmu-miR-1186 3' GTACGGAAATTAAGTTCGTGAG 5' 1 Score:1 225 NM_009746 Bcl7c 5'UTR 5' GCTCTGTCTCCGCGCCAGCCCC 3' mmu-miR-762 3' CGAGACGGGCGCGGGTCGGGG 5' 1 Score:1 785 NM_010923 Nnat 3'UTR 5' CCCAGCTAGATTGTAAAGCTCCTG 3' mmu-miR-708 3' GGGTCGATCTAACATTGAGGAA 5' 1 Score:1 579 F830014H21 Cables1 ORF 5' GGAACCCCGGAGTACGAGCGG 3' mmu-miR-1901 3' CCTGGGGGCCCTCATGCTCGCC 5' 1 Score:1 3406 NM_026283 Samd8 3'UTR 5' TTGACTTGAAGGGAAAAC 3' mmu-miR-684 3' AACTGAACCTCCCTTTTGA 5' 1 Score:1 365 NM_001004367 Cxox4 ORF 5' TCCTCCTCGTCTCCTCGGGGG 3' mmu-miR-1895 3' AGGAGGAGCAGAGGAGCCCC 5' 1 Score:1 737 NM_001013786 Zfp187 ORF 5' CTCCTCACCCACCATCAGAGAG 3' mmu-miR-1896 3' GAGGAGTGGGTGTAGTCTCTC 5' 1 Score:1.0 431 NM_010461 Hoxb8 3'UTR 5' CCCAACACATGTAAAGTCCCTA 3' 0 mmu-miR-196a 3' GGGTGTGTGTACTTGTATGGAT 5' 1.0 Score:1.5 1749 NM_026735 Mobk11a 3'UTR 5' CTAGGAAGTCCCTTTTCTCCA 3' -0 0 0 mmu-miR-185 3' AGTCCTTGACGGAAGAGAGAGT 5' 1.5 Score:1.5 670 NM_008352 Il12b 3'UTR 5' TATGCTTTTAAATTCAGACTC 3' 0 0 mmu-miR-1186 3' GTACGGAAATTAAGTTCGTGAG 5' 1.5 Score:1.5 268 NM_008909 Pp1 ORF 5' GGAGGCGAGATTGGCCATCGCC 3' 0 mmu-miR-1899 3' CCTTCGTCTAGCCGGTAGCGA 5' 1.5	Score:2 189 NM_172567 Mett12 3'UTR 5' CAGTGATACACAGAGAACTCT 3' - mmu-miR-876-5p 3' ATCACTCAAGTGTCTCTTAGGT 5' 2 Score:2.0 3664 NM_177386 Sfmbt2 3'UTR 5' TTGAGTGTGTATTATTGGA 3' mmu-miR-488* 3' AACTCTCAGGATAATAGACCC 5' 2.0 Score:2 912 NM_011768 Zfx ORF 5' ACTCTCAGCAAGAAGAAGAAGA 3' -- mmu-miR-1903 3' ACAGAGTCTCTCTCTCTCTCC 5' 2 Score:2.0 384 NM_053098 Lmod2 ORF 5' AGTCTCAGGAGGAAGAAGAGGA 3' - 0 0 mmu-miR-1903 3' ACAGAGTCTCTCTCTCTCTCC 5' 2.0 Score:2 981 NM_001081308 Taok3 ORF 5' AGTCGCAAGAAGAAGAAGAAGA 3' - - - mmu-miR-1903 3' ACAGAGTCTCTCTCTCTCTCC 5' 2 Score:2 1014 NM_025310 Ftsj3 ORF 5' AG-CTCA-GAAGAAGAAGAAGA 3' - - - mmu-miR-1903 3' ACAGAGTCTCTCTCTCTCTCC 5' 2 Score:2 1400 NM_023585 Ube2v2 3'UTR 5' CAACAAGGGCCCTCACTGCAGA 3' - mmu-miR-17* 3' GATGTTCAGGGGAGTACGCTA 5' 2
Score:1.5 527 NM_001033535 Tnfaip813 3'UTR 5' TTTAGTAATCAGCACTACTGTA 3' 0 - mmu-miR-101a 3' AAGTCAATAGTGCATGACAT 5' 1.5 Score:1.5 1463 NM_028799 Tgm5 ORF 5' AGCTCCGTCGCAACCA 3' -0 mmu-miR-546 3' CTGAGGCACGGTGGTA 5' 1.5 Score:2 mouse N4bp1 BC004022 5' CTAGACTGTGAGCTCCTCGT 3' - mmu-miR-151-5p 3' TGATCTGACACTCGAGGAGCT 5' 2 Score:2.0 293 NM_080463 Pofut1 3'UTR 5' ATATATGTGTGTGTGTATGTA 3' -0- 0 0 mmu-miR-466b-3p 3' AGAATACACACGACATACATAT 5' 2.0 Score:2.0 1483 NM_009260 Spm2 3'UTR 5' ATATATGTGTGTGTGTATGTA 3' -0- 0 0 mmu-miR-466b-3p 3' AGAATACACACGACATACATAT 5' 2.0 Score:2 1193 NM_183116 l110021109rik ORF 5' CTGGGCG-GATTCTGTGTGAGA 3' -0 0 - mmu-miR-342-3p 3' TGCCCAAGCTAAGACACACTCT 5' 2	Score:2 533 NM_023279 Tubb3 ORF 5' AAGTGTGGGACACTGTGGTGG 3' mmu-miR-220 3' TTCACAGACTGTGACACCACC 5' 2 Score:2 1044 ILC0042E05 Q8r4r6 3'UTR 5' TATATGTATGTGTGTATGTA 3' --- mmu-miR-297c* 3' ATACCCATACACATACATAT 5' 2 Score:2 1426 NM_177203 A730037c10rik 3'UTR 5' GATGTATATGTGTGTATGTA 3' - --- mmu-miR-297c* 3' ATACCCATACACATACATAT 5' 2 Score:2.0 421 NM_144552 Stxbp6 3'UTR 5' TGTGTGTGTGTGTGTATGTA 3' 0 - 0 mmu-miR-297c* 3' ATACCCATACACATACATAT 5' 2.0 Score:2.0 1034 C130073N23 4930420ollrik 3'UTR 5' TGTGTGTGTGTGTGTATGTA 3' 0 - 0 mmu-miR-297c* 3' ATACCCATACACATACATAT 5' 2.0 Score:2.0 1483 NM_007691 Chek1 3'UTR 5' TGTGTGTGTGTGTGTATGTA 3' 0 - 0 mmu-miR-297c* 3' ATACCCATACACATACATAT 5' 2.0 Score:2.0 804 NM_028228 2610028a01rik ORF 5' TGGAGGCCCACTGAGGA 3' 0 0 mmu-miR-720 3' ACCTCCGGGTGCTCTTA 5' 2.0 Score:2.0 1422 NM_022882 Lpin2 ORF 5' TGGAGGCCCTCAGTGAGAA 3' 0 0 mmu-miR-720 3' ACCTCCGGGTGCTCTTA 5' 2.0 Score:2.0 708 NM_013500 Hapln1 ORF 5' ACACGGTGCCTGGAGTCAGGAA 3' 0 -0 mmu-miR-378* 3' TGTGTCTGGACCTCAGTCCTC 5' 2.0 Score:2.0 263 NM_011889 Sept3 3'UTR 5' GTATGCATGTGTGTGTATGTA 3' 0 0 mmu-miR-669i 3' CATACGTACACATATACGT 5' 2.0 Score:2.0 2072 NM_178750 Ssl1811 3'UTR 5' GTATGTGTGTGTGTATGTA 3' 0 0 mmu-miR-669i 3' CATACGTACACATATACGT 5' 2.0 Score:2 1255 NM_025903 Ifird2 ORF 5' -AAAAGCACTGTACAATGCTGCA 3' -- - - mmu-miR-103 3' AGTATCG-GGACATGTTACGAGCA 5' 2 Score:2.0 842 A030009H04 Np_065616 3'UTR 5' CAGCTTGCTAGAAATGCAGAAT 3' - 0 mmu-miR-544 3' CTCGACGATTTTACGTCTTA 5' 2.0 Score:2 831 NM_016846 Rgl1 ORF 5' GA-TCCGTGCCACCAT 3' - mmu-miR-546 3' CTGAGGCACGGTGGTA 5' 2 Score:2 1334

NM_009831 Ccngl 3'UTR	5' CATGCCTTTAATCCCAGCACTA 3'			NM_010950 Numbl ORF	5' -ACCTCCGTGCCACCAA 3'		
					- -		
mmu-miR-1186	3' GTACGGAAATTAAAGGTCGTGAG 5'	2		mmu-miR-546	3' CT-GAGGCACGGTGGTA 5'	2	
Score:2	1669			Score:2.0	1775		
NM_026185 1300007f04rik 3'UTR	5' CATGCCTTTAATCCCAGCACTA 3'			NM_019456 Apbb1ip ORF	5' GACTTCGTGCCACCAC 3'		
					0		
mmu-miR-1186	3' GTACGGAAATTAAAGGTCGTGAG 5'	2		mmu-miR-546	3' CTGAGGCACGGTGGTA 5'	2.0	
Score:2.0	1060			Score:2	1417		
NM_178662 Atcay 3'UTR	5' TATGCCTTTAATTTCCAGACTC 3'			NM_177475 Zfp280b ORF	5' GACTCAGTGCCACCAA 3'		
	0 0				-		
mmu-miR-1186	3' GTACGGAAATTAAAGGTCGTGAG 5'	2.0		mmu-miR-546	3' CTGAGGCACGGTGGTA 5'	2	
Score:2	215			Score:2	882		
NM_029545 653040ln04rik 3'UTR	5' CATACCTTTAATTCAGCACTT 3'			NM_028142 Nsun4 ORF	5' GACTCCTTGCCACCAA 3'		
					-		
mmu-miR-1186	3' GTACGGAAATTAAAGGTCGTGAG 5'	2		mmu-miR-546	3' CTGAGGCACGGTGGTA 5'	2	
Score:2.0	974			Score:2.0	2690		
6820402c04 Arp2 3'UTR	5' CATGCCTTTAATTCAGTACTT 3'			NM_178705 Luzp2 3'UTR	5' CACAGCCCATCGATTGCTGTTG 3'		
	0				- 0		
mmu-miR-1186	3' GTACGGAAATTAAAGGTCGTGAG 5'	2.0		mmu-miR-21*	3' CTGTGGGTAGCTGACGACAAC 5'	2.0	
Score:2	1526			Score:2.0	123		
NM_178623 2010005j08rik 3'UTR	5' CACACCTTTAATTCAGCACTA 3'			NM_153161 Bc037034 3'UTR	5' GCTGCCTGTCTGTCTGTCTGG 3'		
	--				0 0		
mmu-miR-1186	3' GTACGGAAATTAAAGGTCGTGAG 5'	2		mmu-miR-214	3' TGACGGACAGACACGGACGACA 5'	2.0	
Score:2.0	1383			Score:2.0	459		
NM_153058 Mapre2 3'UTR	5' TGCCACCCCCACCTTCCACT 3'			NM_012025 Racgap1 ORF	5' ATCTGGTTCCATTTTATCAGA 3'		
	0				- 0		
mmu-miR-705	3' ACGGGTGGGGTGAGGGTGG 5'	2.0		mmu-miR-133a*	3' TAAACCAAGGTAAAAATGGTCG 5'	2.0	

Table S9. 17 Top CA-Scoring Sites (Related to Figure 5)

Conserved sites with CA score ≤ 3.0 . Because the miR-196a:*HOXB8* duplex is not conserved in horse and pig, it was not on this list. In other analyses, however, it is classified as a conserved cleavage target duplex because it is conserved in human, mouse, rat, and dog, as well as many non-mammalian species, including frog and fish. Of the 17 conserved miRNA:site duplexes found, 6 were in annotated ORFs and 11 were in 3' UTRs.

Nine top CA-scoring sites									
CA score:3 195					CA score:3.0 39				
NM_001489 NR6A1 3'UTR					NM_001100588 RC3H2 3'UTR				
5' ATTTCA-CGACAGAGTTGAATGTA 3'					5' TGACCACACCATGGAAGCATTTA 3'				
-0 - -					- - - 0				
hsa-miR-181a BC	3'	T-GAGTGGCTGTCGCAACTTACAA	5'	3	hsa-miR-302a BC	3'	AGTGGTTTTGTACCTTCGTGAAT	5'	3.0
mouse	5'	ATTTCA-CGACAGAGTTGAATGTA	3'		mouse	5'	TGACCACACCATGGAAGCATTTA	3'	
-0 - -					- - 0				
hsa-miR-181a BC	3'	T-GAGTGGCTGTCGCAACTTACAA	5'	3	hsa-miR-302a BC	3'	AGTGGTTTTGTACCTTCGTGAAT	5'	3.0
rat	5'	ATTTCA-CGACAGAGTTGAATGTA	3'		rat	5'	TGACCACATCATGGAAGCATTTA	3'	
-0 - -					- - 0				
hsa-miR-181a BC	3'	T-GAGTGGCTGTCGCAACTTACAA	5'	3	hsa-miR-302a BC	3'	AGTGGTTTTGTACCTTCGTGAAT	5'	3.0
dog	5'	ATTTCA-CGACAGAGTTGAATGTA	3'		dog	5'	TGACCATACCATGGAAGCATTTA	3'	
-0 - -					- - 0				
hsa-miR-181a BC	3'	T-GAGTGGCTGTCGCAACTTACAA	5'	3	hsa-miR-302a BC	3'	AGTGGTTTTGTACCTTCGTGAAT	5'	3.0
horse	5'	ATTTCA-CGACAGAGTTGAATGTA	3'		horse	5'	TGACCATACCATGGAAGCATTTA	3'	
-0 - -					- - 0				
hsa-miR-181a BC	3'	T-GAGTGGCTGTCGCAACTTACAA	5'	3	hsa-miR-302a BC	3'	AGTGGTTTTGTACCTTCGTGAAT	5'	3.0
pig	5'	ATTTCA-CGACAGAGTTGAATGTA	3'		pig	5'	TGACCACACCATGGAAGCATTTA	3'	
-0 - -					- - 0				
hsa-miR-181a BC	3'	T-GAGTGGCTGTCGCAACTTACAA	5'	3	hsa-miR-302a BC	3'	AGTGGTTTTGTACCTTCGTGAAT	5'	3.0
CA score:3 167					CA score:2.5 2426				
NM_001010861 PAP2D 3'UTR					NM_006715 MAN2C1 ORF				
5' ACTAGACTGTGAGCTCCTCGA 3'					5' ATGAGGCCACCAAGTTCCTGA 3'				
					- 0 -				
hsa-miR-151-5p TC	3'	TGATCTGACACTCGAGGAGCT	5'	0	hsa-miR-873 IC	3'	TCCTCTGAGTGTTCGAAGGACG	5'	2.5
mouse	5'	ACGAGACTGTGAGCTCCTCAA	3'		mouse	5'	ACGAGGCCCCACAAGTTCCTGA	3'	
-					- 0 -				
hsa-miR-151-5p TC	3'	TGATCTGACACTCGAGGAGCT	5'	3	hsa-miR-873 IC	3'	TCCTCTGAGTGTTCGAAGGACG	5'	2.5
dog	5'	ACTAGACTGTGAGCTCCTCAA	3'		rat	5'	ACGAGGCCCCACAAGTTCCTGA	3'	
					-				
hsa-miR-151-5p TC	3'	TGATCTGACACTCGAGGAGCT	5'	2	hsa-miR-873 IC	3'	TCCTCTGAGTGTTCGAAGGACG	5'	2
horse	5'	ACTAGACTGTGAGCTCCTCAA	3'		dog	5'	ACGAGGCCCCACAAGTTCCTGA	3'	
					- 0 -				
hsa-miR-151-5p TC	3'	TGATCTGACACTCGAGGAGCT	5'	2	hsa-miR-873 IC	3'	TCCTCTGAGTGTTCGAAGGACG	5'	2.5
pig	5'	ACTAGACTGTGAGCTCCTCGA	3'		horse	5'	ATGAGGCCCCACAAGTTCCTGA	3'	
					- 0 -				
hsa-miR-151-5p TC	3'	TGATCTGACACTCGAGGAGCT	5'	0	hsa-miR-873 IC	3'	TCCTCTGAGTGTTCGAAGGACG	5'	2.5
CA score:2 4076					CA score:2.5 1698				
NM_153029 N4BP1 3'UTR					NM_173468 MOBK1A 3'UTR				
5' TCTAGACTGTGAGCTCCTCGT 3'					5' CTAGAAACTGCCTTTTCTCCA 3'				
-					-0 - 0				
hsa-miR-151-5p TC	3'	TGATCTGACACTCGAGGAGCT	5'	2	hsa-miR-185 MS	3'	AGTCCTTGACGGAAGAGAGGT	5'	2.5
mouse	5'	TCTAGACTGTGAGCTCCTCGT	3'		mouse	5'	CTAGGAACCTGCCTTTTCTCCA	3'	
-					-0 0				
hsa-miR-151-5p TC	3'	TGATCTGACACTCGAGGAGCT	5'	2	hsa-miR-185 MS	3'	AGTCCTTGACGGAAGAGAGGT	5'	1.5
dog	5'	TCTAGACTGTGAGCTCCTCGC	3'		rat	5'	CTAGGAACCTGCCTTTTCTCCA	3'	
-					-0 0				
hsa-miR-151-5p TC	3'	TGATCTGACACTCGAGGAGCT	5'	2	hsa-miR-185 MS	3'	AGTCCTTGACGGAAGAGAGGT	5'	1.5
horse	5'	TCTAGACTGTGAGCTCCTCGC	3'		dog	5'	TTAGGAACCTGCCTTTTCTCCA	3'	
-					0 0				
hsa-miR-151-5p TC	3'	TGATCTGACACTCGAGGAGCT	5'	2	hsa-miR-185 MS	3'	AGTCCTTGACGGAAGAGAGGT	5'	1.5
pig	5'	TCTAGACTGTGAGCTCCTCGC	3'		horse	5'	CTAGGAGCTCCCTTTTCTCCA	3'	
					-0 0 - 0				
hsa-miR-151-5p TC	3'	TGATCTGACACTCGAGGAGCT	5'	2	hsa-miR-185 MS	3'	AGTCCTTGACGGAAGAGAGGT	5'	4.0
CA score:2.0 2927					CA score:3 984				
NM_001042546 ATPAF1 3'UTR					NM_153816 SNX14 ORF				
5' ACTAGACTGTGAGCTCCTTGA 3'					5' GAACCTAGAAATAAAAAGCCATC 3'				
					-- - -				
hsa-miR-151-5p TC	3'	TGATCTGACACTCGAGGAGCT	5'	1.0	hsa-miR-135a BC	3'	AGTGTATCCTTATTTTTCGGTAT	5'	3
mouse	5'	ACTAGACTGTGAGCTCCTTGA	3'		mouse	5'	GAACCTAGAAATAAAAAGCCATC	3'	
					-- - -				
hsa-miR-151-5p TC	3'	TGATCTGACACTCGAGGAGCT	5'	2.0	hsa-miR-135a BC	3'	AGTGTATCCTTATTTTTCGGTAT	5'	3
rat	5'	ACTAGACTGTGAGCTCCTTGC	3'		rat	5'	GAACCTAGAAATAAAAAGCCATC	3'	
					-- - -				
hsa-miR-151-5p TC	3'	TGATCTGACACTCGAGGAGCT	5'	1.0	hsa-miR-135a BC	3'	AGTGTATCCTTATTTTTCGGTAT	5'	5
dog	5'	ACTAGACTGTGAGCTCCTTGA	3'		dog	5'	GAACCTAGAAATAAAAAGCCATC	3'	
					-- - -				
hsa-miR-151-5p TC	3'	TGATCTGACACTCGAGGAGCT	5'	1.0	hsa-miR-135a BC	3'	AGTGTATCCTTATTTTTCGGTAT	5'	3
horse	5'	ACTAGACTGTGAGCTCCTTGA	3'		horse	5'	GAACCTAGAAATAAAAAGCCATC	3'	
					-- - -				
hsa-miR-151-5p TC	3'	TGATCTGACACTCGAGGAGCT	5'	1.0	hsa-miR-135a BC	3'	AGTGTATCCTTATTTTTCGGTAT	5'	3
pig	5'	GTGAGACTGTGAGCTCCTGTGG	3'		pig	5'	GAACCTAGAAATAAAAAGCCATC	3'	
00- -0					-- - -				
hsa-miR-151-5p TC	3'	TGATCTGACACTCGAGGA-GCT	5'	6.0	hsa-miR-135a BC	3'	AGTGTATCCTTATTTTTCGGTAT	5'	3
CA score:3.0 1087					CA score:3.0 1087				
NM_198256 E2F6 3'UTR					NM_198256 E2F6 3'UTR				
5' ATTAGACTGTGAGCTCCTTGA 3'					5' ATTAGACTGTGAGCTCCTTGA 3'				
0 0					0 0				
hsa-miR-151-5p TC	3'	TGATCTGACACTCGAGGAGCT	5'	1.5	hsa-miR-151-5p TC	3'	TGATCTGACACTCGAGGAGCT	5'	1.5
mouse	5'	ACTAGACTGTGAGCTCCGTGA	3'		mouse	5'	ACTAGACTGTGAGCTCCGTGA	3'	

hsa-miR-151-5p TC rat	3'		5'	3.0	CA score:2.5 3898 NM_001042555 FRS2 3'UTR	5'	ACAGTGGG-ACTACCATTGCCAAA 3'	
hsa-miR-151-5p TC dog	3'		5'	2	hsa-miR-182 BC mouse	3'	TCACACTCAAGATGGTAACGGTTT 5'	2.5
hsa-miR-151-5p TC horse	3'		5'	1.5	hsa-miR-182 BC rat	3'	TCACACTCAAGATGGTAACGGTTT 5'	2.5
hsa-miR-151-5p TC pig	3'		5'	1.5	hsa-miR-182 BC dog	3'	TCACACTCAAGATGGTAACGGTTT 5'	2.5
hsa-miR-151-5p TC	3'		5'	10.0	hsa-miR-182 BC horse	3'	TCACACTCAAGATGGTAACGGTTT 5'	2.5
CA score:3 2925 NM_001042546 ATPAF1 3'UTR	5'		3'		hsa-miR-182 BC pig	3'	TCACACTCAAGATGGTAACGGTTT 5'	2.5
hsa-miR-28-5p MS mouse	3'		5'	3	hsa-miR-182 BC	3'	TCACACTCAAGATGGTAACGGTTT 5'	2.5
hsa-miR-28-5p MS rat	3'		5'	3	CA score:3 838 NM_005386 NNAT 3'UTR	5'	TGTAAGCTCCTGGAGGACAGGGA 3'	
hsa-miR-28-5p MS dog	3'		5'	3	hsa-miR-339-5p MS mouse	3'	GCACCTCGAGGACCTCCTGTCCCT 5'	1
hsa-miR-28-5p MS horse	3'		5'	3	hsa-miR-339-5p MS rat	3'	GCACCTCGAGGACCTCCTGTCCCT 5'	3
hsa-miR-28-5p MS pig	3'		5'	3	hsa-miR-339-5p MS dog	3'	GCACCTCGAGGACCTCCTGTCCCT 5'	3
hsa-miR-28-5p MS	3'		5'	4.5	hsa-miR-339-5p MS horse	3'	GCACCTCGAGGACCTCCTGTCCCT 5'	1
CA score:3 4352 NM_014715 RICS ORF	5'		3'		hsa-miR-339-5p MS pig	3'	GCACCTCGAGGACCTCCTGTCCCT 5'	1
hsa-miR-145 BC mouse	3'		5'	3	hsa-miR-339-5p MS	3'	GCACCTCGAGGACCTCCTGTCCCT 5'	1
hsa-miR-145 BC rat	3'		5'	3	CA score:2.5 806 NM_016221 DCTN4 ORF	5'	CGCCACAAACATCTTCTGATCA 3'	
hsa-miR-145 BC dog	3'		5'	3	hsa-miR-383 BC mouse	3'	TCGGTGTTAGTGGAAAGACTAGA 5'	2.0
hsa-miR-145 BC horse	3'		5'	3	hsa-miR-383 BC rat	3'	TCGGTGTTAGTGGAAAGACTAGA 5'	2
hsa-miR-145 BC pig	3'		5'	3	hsa-miR-383 BC dog	3'	TCGGTGTTAGTGGAAAGACTAGA 5'	2
hsa-miR-145 BC	3'		5'	3	hsa-miR-383 BC horse	3'	TCGGTGTTAGTGGAAAGACTAGA 5'	2.5
CA score:3.0 388 NM_194318 B3GALTL ORF	5'		3'		hsa-miR-383 BC pig	3'	TCGGTGTTAGTGGAAAGACTAGA 5'	2.5
hsa-miR-376b MS mouse	3'		5'	4.0	hsa-miR-383 BC	3'	TCGGTGTTAGTGGAAAGACTAGA 5'	1.5
hsa-miR-376b MS rat	3'		5'	3.0	CA score:2.0 188 NM_012410 SEZ6L2 ORF	5'	CT-GAGGAGGGGCCAGAGA 3'	
hsa-miR-376b MS dog	3'		5'	3.0	hsa-miR-326 MS mouse	3'	GACCTCCTTCCCGGGTCTCC 5'	2.0
hsa-miR-376b MS horse	3'		5'	3.0	hsa-miR-326 MS rat	3'	GACCTCCTTCCCGGGTCTCC 5'	2
hsa-miR-376b MS pig	3'		5'	3.0	hsa-miR-326 MS dog	3'	GACCTCCTTCCCGGGTCTCC 5'	2
hsa-miR-376b MS	3'		5'	3.0	hsa-miR-326 MS horse	3'	GACCTCCTTCCCGGGTCTCC 5'	3.0
CA score:1 827 NM_005386 NNAT 3'UTR	5'		3'		hsa-miR-326 MS pig	3'	GACCTCCTTCCCGGGTCTCC 5'	1
hsa-miR-708 MS mouse	3'		5'	1	hsa-miR-326 MS	3'	GACCTCCTTCCCGGGTCTCC 5'	1
hsa-miR-708 MS rat	3'		5'	1				
hsa-miR-708 MS dog	3'		5'	1.5				
hsa-miR-708 MS horse	3'		5'	1				
hsa-miR-708 MS pig	3'		5'	1				
hsa-miR-708 MS	3'		5'	1				

Table S10. The miRNAs Expressed in HeLa Cells, as Indicated by High-Throughput Sequencing (Related to Figure 6)

Listed are read numbers of 300 miRNAs with at least twenty of reads in an Illumina sequencing run. The small-RNA library was prepared as described previously (Grimson et al., 2008).

hsa-miR-21	292271	hsa-miR-590-5p	747	hsa-miR-502-3p	103	hsa-miR-570	30
hsa-let-7i	118276	hsa-miR-185	720	hsa-miR-1	102	hsa-miR-323-3p	29
hsa-let-7a	113422	hsa-miR-204	704	hsa-miR-93*	101	hsa-miR-550*	29
hsa-let-7f	72336	hsa-miR-31*	692	hsa-miR-92a-1*	101	hsa-miR-1185	29
hsa-let-7c*	66781	hsa-miR-886-5p	675	hsa-miR-873	99	hsa-miR-411*	29
hsa-let-7c	66781	hsa-miR-590-3p	667	hsa-miR-181a-2*	98	hsa-miR-548o	28
hsa-let-7b	55167	hsa-miR-425	663	hsa-miR-1259	96	hsa-miR-136*	26
hsa-miR-24	36663	hsa-miR-345	651	hsa-miR-379	96	hsa-miR-380	26
hsa-miR-27a	32951	hsa-miR-182	624	hsa-miR-143*	96	hsa-miR-505*	26
hsa-miR-17	29782	hsa-miR-28-3p	596	hsa-miR-410	93	hsa-miR-199b-3p	26
hsa-miR-16	25653	hsa-miR-744	586	hsa-miR-200c	93	hsa-miR-199a-3p	26
hsa-let-7g	21431	hsa-miR-18b	559	hsa-miR-33b*	92	hsa-miR-369-5p	25
hsa-miR-26a	19753	hsa-miR-340	558	hsa-miR-889	91	hsa-miR-874	25
hsa-miR-20a	19321	hsa-miR-140-5p	541	hsa-miR-30c-2*	91	hsa-miR-548a-3p	24
hsa-miR-30a	17965	hsa-miR-339-5p	538	hsa-miR-1296	91	hsa-miR-148b*	23
hsa-miR-191	17266	hsa-miR-629	519	hsa-miR-125b-1*	89	hsa-miR-1255a	22
hsa-miR-106b	16566	hsa-miR-376c	479	hsa-miR-376b	88	hsa-miR-376a*	22
hsa-miR-27b	14843	hsa-miR-196b	477	hsa-miR-296-3p	85	hsa-miR-191*	22
hsa-miR-22	13685	hsa-miR-342-3p	472	hsa-miR-16-1*	84	hsa-miR-1285	22
hsa-miR-92a	13511	hsa-miR-19b-1*	466	hsa-miR-99a*	83	hsa-miR-101*	21
hsa-miR-19b	12848	hsa-miR-190	445	hsa-miR-378*	77	hsa-miR-551b*	21
hsa-miR-26b	12739	hsa-miR-136	430	hsa-miR-194	76	hsa-miR-548c-5p	20
hsa-miR-130a	10600	hsa-miR-148a	430	hsa-miR-545	75	hsa-miR-29b-1*	20
hsa-let-7e	10452	hsa-miR-27a*	430	hsa-miR-503	74	hsa-miR-376a	20
hsa-miR-29a	10394	hsa-miR-152	429	hsa-miR-497	73		
hsa-miR-125b	10176	hsa-miR-301b	423	hsa-miR-339-3p	73		
hsa-miR-93	9914	hsa-miR-193a-3p	407	hsa-miR-501-5p	72		
hsa-miR-29b	9406	hsa-miR-183	406	hsa-miR-369-3p	70		
hsa-let-7d	9267	hsa-miR-331-3p	401	hsa-miR-542-3p	69		
hsa-miR-103	9221	hsa-miR-210	397	hsa-miR-505	68		
hsa-miR-23a	8918	hsa-miR-15b*	386	hsa-miR-450a	67		
hsa-miR-186	8371	hsa-miR-1308	373	hsa-miR-654-3p	66		
hsa-miR-30c	8286	hsa-miR-7	366	hsa-miR-141	65		
hsa-miR-100	7496	hsa-miR-17*	353	hsa-miR-409-3p	64		
hsa-miR-33a	6457	hsa-miR-100*	350	hsa-miR-134	63		
hsa-miR-320a	6225	hsa-miR-671-5p	348	hsa-miR-1307	63		
hsa-miR-19a	5996	hsa-miR-33b	346	hsa-let-7e*	62		
hsa-miR-99a	5919	hsa-miR-660	329	hsa-miR-7-1*	61		
hsa-miR-30d	5655	hsa-miR-140-3p	326	hsa-miR-548d-5p	60		
hsa-miR-181a	4903	hsa-miR-886-3p	311	hsa-miR-411	60		
hsa-miR-193b	4625	hsa-miR-193a-5p	310	hsa-miR-548e	59		
hsa-miR-15a	4498	hsa-miR-197	290	hsa-miR-409-5p	59		
hsa-miR-143	4393	hsa-miR-574-3p	288	hsa-miR-132	57		
hsa-miR-18a	4002	hsa-miR-128	274	hsa-miR-23a*	57		

hsa-miR-196a	3682	hsa-miR-127-3p	266	hsa-miR-30d*	56
hsa-miR-101	3616	hsa-miR-181c	252	hsa-miR-769-5p	56
hsa-miR-423-3p	3613	hsa-miR-27b*	238	hsa-miR-1271	55
hsa-miR-23b	3585	hsa-miR-335*	230	hsa-miR-19a*	54
hsa-miR-98	3566	hsa-miR-381	230	hsa-miR-127-5p	53
hsa-miR-224	3300	hsa-miR-29a*	226	hsa-miR-99b*	52
hsa-miR-30e	3205	hsa-miR-421	225	hsa-miR-33a*	51
hsa-miR-222	3159	hsa-miR-18a*	224	hsa-miR-32*	51
hsa-miR-148b	3153	hsa-miR-574-5p	222	hsa-miR-24-1*	49
hsa-miR-125a-5p	3137	hsa-miR-582-3p	215	hsa-miR-218	49
hsa-miR-365	2971	hsa-miR-377	212	hsa-miR-24-2*	48
hsa-miR-15b	2741	hsa-miR-95	210	hsa-miR-548b-3p	47
hsa-miR-320b	2647	hsa-miR-362-5p	203	hsa-miR-154*	47
hsa-miR-378	2627	hsa-miR-532-5p	200	hsa-miR-324-3p	46
hsa-miR-25	2603	hsa-miR-130b*	183	hsa-let-7d*	45
hsa-miR-107	2590	hsa-miR-767-5p	179	hsa-miR-548k	45
hsa-miR-301a	2584	hsa-miR-30e*	174	hsa-miR-331-5p	44
hsa-miR-151-5p	2526	hsa-miR-181a*	169	hsa-miR-432	44
hsa-miR-221	2469	hsa-miR-615-3p	169	hsa-miR-424*	44
hsa-miR-320c	2413	hsa-miR-1277	159	hsa-miR-126	43
hsa-miR-34a	2274	hsa-miR-320d	158	hsa-miR-370	43
hsa-miR-454	2229	hsa-miR-335	156	hsa-miR-625	43
hsa-miR-30b	2106	hsa-miR-551b	155	hsa-miR-628-5p	41
hsa-miR-130b	2057	hsa-miR-137	153	hsa-miR-651	41
hsa-miR-424	2020	hsa-miR-92b*	148	hsa-miR-1287	41
hsa-miR-374a	2018	hsa-let-7b*	140	hsa-miR-708	40
hsa-miR-92b	1939	hsa-miR-296-5p	136	hsa-miR-629*	40
hsa-miR-28-5p	1829	hsa-miR-193b*	135	hsa-miR-1301	40
hsa-miR-96	1655	hsa-miR-374a*	130	hsa-miR-203	40
hsa-miR-99b	1590	hsa-miR-361-3p	129	hsa-miR-330-5p	40
hsa-miR-151-3p	1522	hsa-miR-652	126	hsa-miR-876-5p	40
hsa-miR-21*	1378	hsa-miR-181d	124	hsa-miR-493*	39
hsa-miR-145	1347	hsa-miR-494	123	hsa-miR-532-3p	38
hsa-miR-423-5p	1329	hsa-miR-149	122	hsa-miR-501-3p	38
hsa-miR-484	1289	hsa-miR-10b	121	hsa-miR-664	38
hsa-miR-452	1258	hsa-miR-941	120	hsa-miR-1180	37
hsa-miR-30a*	1242	hsa-miR-105	120	hsa-miR-374b*	36
hsa-miR-374b	1176	hsa-miR-22*	119	hsa-miR-425*	36
hsa-miR-324-5p	1132	hsa-miR-500	118	hsa-miR-362-3p	36
hsa-miR-582-5p	1106	hsa-miR-487b	114	hsa-miR-576-5p	35
hsa-let-7i*	1038	hsa-miR-145*	113	hsa-miR-192	35
hsa-miR-181b	1025	hsa-miR-125a-3p	112	hsa-miR-500*	34
hsa-miR-32	1003	hsa-miR-188-5p	112	hsa-miR-186*	34
hsa-miR-361-5p	952	hsa-miR-589	112	hsa-miR-545*	34
hsa-miR-155	943	hsa-miR-195	110	hsa-miR-665	32
hsa-miR-31	874	hsa-miR-491-5p	107	hsa-miR-1826	31
hsa-miR-138	843	hsa-miR-20a*	106	hsa-miR-138-1*	31
hsa-miR-29c	769	hsa-miR-106b*	105	hsa-miR-34a*	30

Table S11. The Significance Tests of Cumulative TPR with Indicated Thresholds and Window Sizes (Related to Figure 6)

Fisher exact tests were performed on results obtained with the indicated thresholds and window sizes, using either filtered or non-filtered tags. The filtered tags were those remaining after subtracting tags mapping to ≥ 10 genomic loci. P value 1 was estimated by comparing the TPR of expressed miRNA:site duplexes to the TPR of nonexpressed miRNA:site duplexes. P value 2 was estimated by comparing the TPR of expressed miRNA:site duplexes to the mean TPR of chimeric miRNA:site duplexes. Bold values represent most significant cumulative TPRs with indicated thresholds and windows.

A. HeLa cells

Window sizes	Non-filtered tags		Filtered tags		Thresholds
	P value 1	P value 2	P value 1	P value 2	
Window ± 0	3.2×10^{-5}	1.8×10^{-4}	7.3×10^{-5}	3.1×10^{-4}	≤ 2
	1.4×10^{-5}	1.1×10^{-4}	5.3×10^{-5}	2.6×10^{-4}	≤ 2.5
	7.6×10^{-3}	5.8×10^{-4}	9.3×10^{-4}	1.6×10^{-3}	≤ 3
Window ± 1	0.35	0.013	0.023	0.025	≤ 3

B. Human brain

Window sizes	Non-filtered tags		Filtered tags		Thresholds
	P value 1	P value 2	P value 1	P value 2	
Window ± 0	0.12	0.18	0.14	0.21	≤ 2
	0.065	0.019	0.028	0.021	≤ 2.5
	0.013	2.4×10^{-3}	8.0×10^{-3}	0.030	≤ 3
Window -1~0	0.39	9.8×10^{-3}	0.011	0.013	≤ 3

Table S12. The miRNAs Expressed in Human Brain, as Indicated by High-Throughput Sequencing (Related to Figure 6)

Listed are read numbers of 382 miRNAs with at least twenty of reads in a Solexa sequencing run.

hsa-let-7a	479248	hsa-miR-432	1733	hsa-miR-421	347	hsa-miR-376a	79
hsa-let-7f	387923	hsa-miR-15a	1684	hsa-miR-18a	347	hsa-miR-193b*	79
hsa-let-7b	273022	hsa-miR-320c	1673	hsa-miR-484	346	hsa-miR-501-3p	76
hsa-miR-125b	229226	hsa-miR-146a	1627	hsa-miR-502-3p	342	hsa-miR-490-3p	71
hsa-miR-9	195679	hsa-miR-329	1624	hsa-miR-887	339	hsa-miR-185*	68
hsa-miR-181a	140272	hsa-miR-485-3p	1618	hsa-miR-190	339	hsa-let-7b*	67
hsa-miR-26a	112220	hsa-miR-15b	1437	hsa-miR-656	314	hsa-miR-590-3p	66
hsa-let-7c*	111228	hsa-miR-345	1210	hsa-miR-671-5p	305	hsa-miR-886-5p	64
hsa-let-7c	111228	hsa-miR-154*	1183	hsa-miR-504	296	hsa-miR-450a	63
hsa-let-7g	105025	hsa-miR-409-3p	1179	hsa-miR-411	287	hsa-miR-106a	63
hsa-miR-22	94798	hsa-miR-146b-5p	1156	hsa-miR-144	285	hsa-miR-34b*	61
hsa-miR-9*	63341	hsa-miR-152	1154	hsa-miR-376c	274	hsa-miR-1247	61
hsa-miR-100	57942	hsa-miR-424	1083	hsa-miR-628-5p	274	hsa-miR-770-5p	61
hsa-miR-7	56403	hsa-miR-92b	1077	hsa-miR-346	274	hsa-miR-299-5p	61
hsa-miR-126	48025	hsa-miR-889	1075	hsa-miR-625*	261	hsa-miR-212	61
hsa-miR-30c	43858	hsa-miR-17	1021	hsa-miR-374b*	256	hsa-miR-376b	60
hsa-miR-103	40567	hsa-miR-185	1014	hsa-miR-30c-2*	251	hsa-miR-301b	59
hsa-miR-340	38259	hsa-miR-331-3p	1012	hsa-miR-362-5p	250	hsa-miR-1252	57
hsa-miR-29a	33619	hsa-miR-148b	995	hsa-miR-369-5p	249	hsa-miR-655	56
hsa-miR-181b	33525	hsa-miR-363	992	hsa-miR-33b	246	hsa-miR-532-3p	53
hsa-miR-151-5p	33007	hsa-miR-1298	991	hsa-miR-144*	239	hsa-miR-206	53
hsa-miR-99a	32472	hsa-miR-127-3p	967	hsa-miR-184	238	hsa-miR-766	53
hsa-let-7i	29869	hsa-miR-423-5p	960	hsa-miR-130b*	236	hsa-miR-671-3p	51
hsa-miR-24	28486	hsa-miR-374a	939	hsa-miR-381	226	hsa-miR-377*	50
hsa-let-7d	27861	hsa-miR-30a*	937	hsa-miR-34a	221	hsa-miR-654-5p	49
hsa-miR-27b	24707	hsa-miR-223	922	hsa-miR-505	220	hsa-miR-340*	49
hsa-miR-191	23608	hsa-miR-140-5p	913	hsa-miR-22*	219	hsa-miR-503	47
hsa-miR-29b	22306	hsa-miR-374b	912	hsa-miR-106b*	219	hsa-miR-1308	46
hsa-miR-107	21564	hsa-miR-181a*	903	hsa-miR-1307	215	hsa-miR-335*	46
hsa-miR-124	20299	hsa-miR-195	877	hsa-miR-499-5p	211	hsa-miR-618	45
hsa-miR-125a-5p	19464	hsa-miR-132*	841	hsa-miR-130b	209	hsa-miR-548e	45
hsa-miR-26b	16651	hsa-miR-323-3p	836	hsa-miR-1301	201	hsa-miR-500*	44
hsa-miR-320a	16420	hsa-miR-126*	829	hsa-miR-199a-5p	197	hsa-miR-30d*	44
hsa-miR-219-2-3p	16209	hsa-miR-153	820	hsa-miR-769-5p	197	hsa-miR-214	43
hsa-miR-30a	16146	hsa-miR-382	818	hsa-miR-885-3p	193	hsa-miR-29a*	42
hsa-miR-221	15963	hsa-miR-324-5p	807	hsa-let-7i*	193	hsa-miR-877	41
hsa-miR-139-5p	15742	hsa-miR-584	796	hsa-miR-454	189	hsa-miR-448	41
hsa-miR-222	15605	hsa-miR-129-5p	783	hsa-miR-138-2*	187	hsa-miR-493*	40
hsa-miR-23b	14817	hsa-miR-378	783	hsa-miR-181a-2*	186	hsa-miR-876-3p	39
hsa-miR-143	14205	hsa-miR-145	766	hsa-miR-590-5p	181	hsa-miR-29b-2*	39
hsa-miR-451	13779	hsa-miR-361-5p	757	hsa-miR-486-5p	169	hsa-miR-362-3p	39
hsa-miR-30d	12536	hsa-miR-1185	746	hsa-miR-148a	167	hsa-miR-886-3p	39
hsa-let-7e	12253	hsa-miR-330-5p	744	hsa-miR-299-3p	166	hsa-miR-552	37
hsa-miR-30e	11168	hsa-miR-20a	734	hsa-miR-708*	160	hsa-miR-455-3p	37
hsa-miR-138	11121	hsa-miR-134	728	hsa-miR-28-3p	160	hsa-miR-10a	37
hsa-miR-335	10896	hsa-miR-192	712	hsa-miR-374a*	160	hsa-miR-582-3p	36

hsa-miR-128	10037	hsa-miR-136*	690	hsa-miR-339-5p	150	hsa-miR-375	36
hsa-miR-99b	9851	hsa-miR-197	690	hsa-miR-339-3p	150	hsa-miR-105	36
hsa-miR-21	9780	hsa-miR-7-1*	685	hsa-miR-99b*	150	hsa-miR-154	34
hsa-miR-30b	9721	hsa-miR-874	678	hsa-miR-1224-5p	147	hsa-miR-758	34
hsa-miR-34c-5p	9144	hsa-miR-129-3p	661	hsa-miR-654-3p	145	hsa-miR-767-5p	34
hsa-miR-598	8131	hsa-miR-136	637	hsa-miR-431*	143	hsa-miR-574-5p	34
hsa-miR-137	8099	hsa-miR-1296	635	hsa-miR-491-5p	143	hsa-miR-18a*	33
hsa-miR-423-3p	8076	hsa-miR-660	632	hsa-miR-769-3p	141	hsa-miR-99a*	33
hsa-miR-338-5p	7588	hsa-miR-17*	629	hsa-miR-1287	141	hsa-miR-1226	33
hsa-miR-93	7297	hsa-miR-125b-1*	627	hsa-miR-488	139	hsa-miR-24-1*	33
hsa-miR-29c	6819	hsa-miR-193b	620	hsa-miR-210	136	hsa-miR-30b*	32
hsa-miR-106b	6670	hsa-miR-1271	619	hsa-miR-29c*	133	hsa-miR-1826	32
hsa-miR-92a	6603	hsa-miR-652	617	hsa-miR-20b	131	hsa-miR-135b	31
hsa-miR-151-3p	5000	hsa-miR-320d	616	hsa-miR-155	129	hsa-miR-183	30
hsa-miR-204	4922	hsa-miR-425*	614	hsa-miR-424*	129	hsa-miR-876-5p	30
hsa-miR-1	4716	hsa-miR-19b	600	hsa-let-7e*	126	hsa-miR-141	29
hsa-miR-218	4554	hsa-miR-383	593	hsa-miR-744*	126	hsa-miR-378*	29
hsa-miR-101	4530	hsa-miR-409-5p	586	hsa-miR-582-5p	126	hsa-miR-629	29
hsa-miR-23a	4426	hsa-miR-127-5p	578	hsa-miR-641	125	hsa-miR-296-3p	28
hsa-miR-98	4382	hsa-miR-132	575	hsa-miR-143*	122	hsa-miR-551a	28
hsa-miR-342-3p	4356	hsa-let-7d*	569	hsa-miR-377	118	hsa-miR-199b-5p	28
hsa-miR-149	4253	hsa-miR-138-1*	569	hsa-miR-182	115	hsa-miR-760	28
hsa-miR-33a	4230	hsa-miR-485-5p	567	hsa-miR-200b	113	hsa-miR-1229	28
hsa-miR-744	4187	hsa-miR-941	565	hsa-miR-21*	112	hsa-miR-431	28
hsa-miR-16	4143	hsa-miR-532-5p	552	hsa-miR-380	110	hsa-miR-496	27
hsa-miR-379	3979	hsa-miR-361-3p	538	hsa-miR-193a-5p	109	hsa-miR-500	27
hsa-miR-487b	3944	hsa-miR-142-3p	534	hsa-miR-133a	108	hsa-miR-34b	26
hsa-miR-27a	3549	hsa-miR-32	529	hsa-miR-93*	108	hsa-miR-664	26
hsa-miR-708	3454	hsa-miR-95	523	hsa-miR-1250	107	hsa-miR-331-5p	26
hsa-miR-328	3407	hsa-miR-425	516	hsa-miR-145*	107	hsa-miR-494	26
hsa-miR-140-3p	3189	hsa-miR-129*	511	hsa-miR-92b*	107	hsa-miR-668	26
hsa-miR-433	3023	hsa-miR-142-5p	509	hsa-miR-224	105	hsa-miR-326	26
hsa-miR-186	2820	hsa-miR-150	506	hsa-miR-19a	102	hsa-miR-548a-3p	25
hsa-miR-25	2805	hsa-miR-369-3p	485	hsa-miR-27b*	102	hsa-miR-1277	25
hsa-miR-370	2599	hsa-miR-365	481	hsa-miR-379*	100	hsa-miR-628-3p	25
hsa-miR-28-5p	2544	hsa-miR-130a	467	hsa-miR-200a	98	hsa-miR-100*	25
hsa-miR-199b-3p	2503	hsa-miR-495	462	hsa-miR-101*	97	hsa-miR-665	25
hsa-miR-199a-3p	2503	hsa-miR-1249	460	hsa-miR-542-3p	97	hsa-miR-7-2*	24
hsa-miR-30c*	2459	hsa-miR-487a	459	hsa-miR-505*	96	hsa-miR-539	23
hsa-miR-194	2333	hsa-miR-885-5p	403	hsa-miR-20a*	94	hsa-miR-432*	22
hsa-miR-181c	2215	hsa-miR-488*	402	hsa-miR-30c-1*	94	hsa-miR-216a	22
hsa-miR-497	2158	hsa-miR-625	394	hsa-miR-592	91	hsa-miR-23b*	22
hsa-miR-574-3p	2122	hsa-miR-31	391	hsa-let-7g*	91	hsa-miR-1251	21
hsa-miR-410	2098	hsa-miR-135a	382	hsa-miR-551b	89	hsa-miR-516a-5p	21
hsa-miR-330-3p	2057	hsa-miR-203	374	hsa-miR-124*	89	hsa-miR-221*	21
hsa-miR-873	2033	hsa-miR-301a	373	hsa-miR-664*	89	hsa-miR-483-5p	21
hsa-miR-338-3p	1966	hsa-miR-342-5p	367	hsa-miR-429	88	hsa-miR-25*	21
hsa-miR-181d	1881	hsa-miR-543	361	hsa-miR-1197	88	hsa-miR-32*	20
hsa-miR-219-5p	1810	hsa-miR-1180	354	hsa-miR-323-5p	85		
hsa-miR-320b	1803	hsa-miR-376a*	352	hsa-miR-411*	80		

Table S13. Extended Evidence for miRNA-Directed Cleavage in Human Brain (Related to Figure 6)

miRNA-directed cleavage targets alignment penalty score ≤ 3.0 . This table presents extended results considering tags mapping to position -1 as well as position 0 relative to the cleavage site. The number of cleavage-tag reads, normalized by genomic loci number is listed (Cleavage tags). Percentage of all degradome tags were calculated using the sum of cleavage tags at positions 0 and -1. Conservation sites (Cons.) are shown in Supplemental Figure S5E.

miRNA	mRNA	Location	Score	Cleavage site	Cleavage tags	miRNA reads	Percentage of all degradome tags (%)	Cons.
hsa-miR-28-5p	<i>MDGA1</i>	3'UTR, LINE L2	3	0 / -1	1 / 1	2544	0.2	
hsa-miR-151-5p	<i>MDGA1</i>	3'UTR, LINE L2	3	0 / -1	9 / 12	33007	2.0	
hsa-miR-151-5p	<i>N4BP1</i>	3'UTR, LINE L2	2	0	6	33007	2.1	Yes
hsa-miR-151-5p	<i>PAP2D</i>	3'UTR, LINE L2	0	-1	3	33007	15.5	Yes
hsa-miR-873	<i>MAN2C1</i>	ORF	2.5	0 / -1	1 / 2	2033	0.6	Yes
hsa-miR-330-5p	<i>FAM62C</i>	ORF	3	0	2	744	1.3	
hsa-miR-95	<i>EGLN3</i>	3'UTR	2.5	0	1	523	1.2	
hsa-miR-182	<i>FRS2</i>	3'UTR	2.5	0	2	115	1.4	Yes
hsa-miR-877	<i>SPTBN1</i>	ORF	3	0	2	41	0.1	
hsa-miR-185	<i>PMVK</i>	5'UTR	3	0	2	1014	0.8	
hsa-miR-383	<i>DCTN4</i>	ORF	2	0	1	593	0.4	Yes
hsa-miR-598	<i>EFTUD2</i>	ORF	2.5	0 / -1	1 / 5	8131	2.0	
hsa-miR-708	<i>NNAT</i>	3'UTR, LINE L2	1	-1	0.25	3454	0.3	Yes