SUPPLEMENTARY MATERIALS AND METHODS

Statistical analysis

For many mRNAs that contain a sequence that is highly complementary to a known miRNA, reads were obtained both at the predicted site of cleavage (at the adjacent site between nt 10 and 11 of the aligned miRNA) and from elsewhere in the mRNA. Assuming no bias in the location of reads across the transcript, the probability (P) of a single read being at a particular nucleotide of interest is equal to 1/L. The probability of X reads at the nucleotide of interest is therefore a binomial process with probability P and with X+Y trials. That is: $p(X=x) = ((x+y)!/x!y!)(1/L^x)(1-1/L)^y$. To calculate $P(X \geq x)$ we used the online calculator at http://strattrek.com/Tables/Bionimial.aspx

SUPP FIG 1 – miRNA-directed target cleavage

Sequence alignments across mRNA : miRNA base pairing sites for all putative examples of RISC-mediated cleavage within PARE libraries. Read numbers at specific locations, across all 6 adult tissues (Supp Fig 1A) and d16.5 whole mouse embryo libraries (Supp Fig 1B) are indicated with arrows. The asterisk denotes the expected cleavage site. Total read numbers mapping to these transcripts and p values for the reads at the putative site of cleavage are shown below each alignment.

SUPP FIG 2 – Specific siRNA mediated cleavage detected by PARE in transfected NMuMg cells

Mouse NMuMg cells were transfected with single siRNAs complementary to SHC1 and cultured for 2 days prior to PARE analysis. The most prominent fragments in PARE map to the exact predicted site of siRNA-directed cleavage.

SUPP FIG 3 – Endogenous miR-145 directs RICS cleavage

Gene specific 5'RACE was used to identify endogenous cleavage sites from rectal mucosa tissue around the site of predicted miR-145 base pairing. From 3 clones, 2 mapped within the region and are indicated by arrows. 1 clone maps precisely to the location of expected miR-145 directed cleavage. The cleavage site is located within a region of high sequence conservation as indicated by 30-way mammalian sequence alignment.

SUPP FIG 4 – Examples of tRNA-associated reads

The majority of tRNA associated reads within PARE libraries map upstream of the mature tRNA 5' terminus (as represented by tRNA Lys, TTT), though there were several examples of reads mapping to the mature 5' end (as for tRNA Glu TTC). 10 tRNA loci yielded RNA species commencing at sites immediately 3' to mature tRNA 3' termini (represented by tRNA Thr CGT), consistent with a recently described RNA class (27).

SUPP FIG 5 – Variability around the 5' end of ncRNAs

Location of 5' ends of PARE reads associated with various ncRNAs, represented as read frequency per million. The 5' ends are indicated by an asterisk.

SUPP FIG 6 – Predicted generation of smaller RNAs from tRNAs and Y-RNAs

Mapping PARE reads to several tRNA and Y-RNA loci indicated reads not just associated with the 5' end but also at specific sites within the ncRNA. Predicted RNA folding with observed cleavage positions in adult PARE libraries are indicated.

SUPP TABLE 1 – Genomic mapping localities from PARE

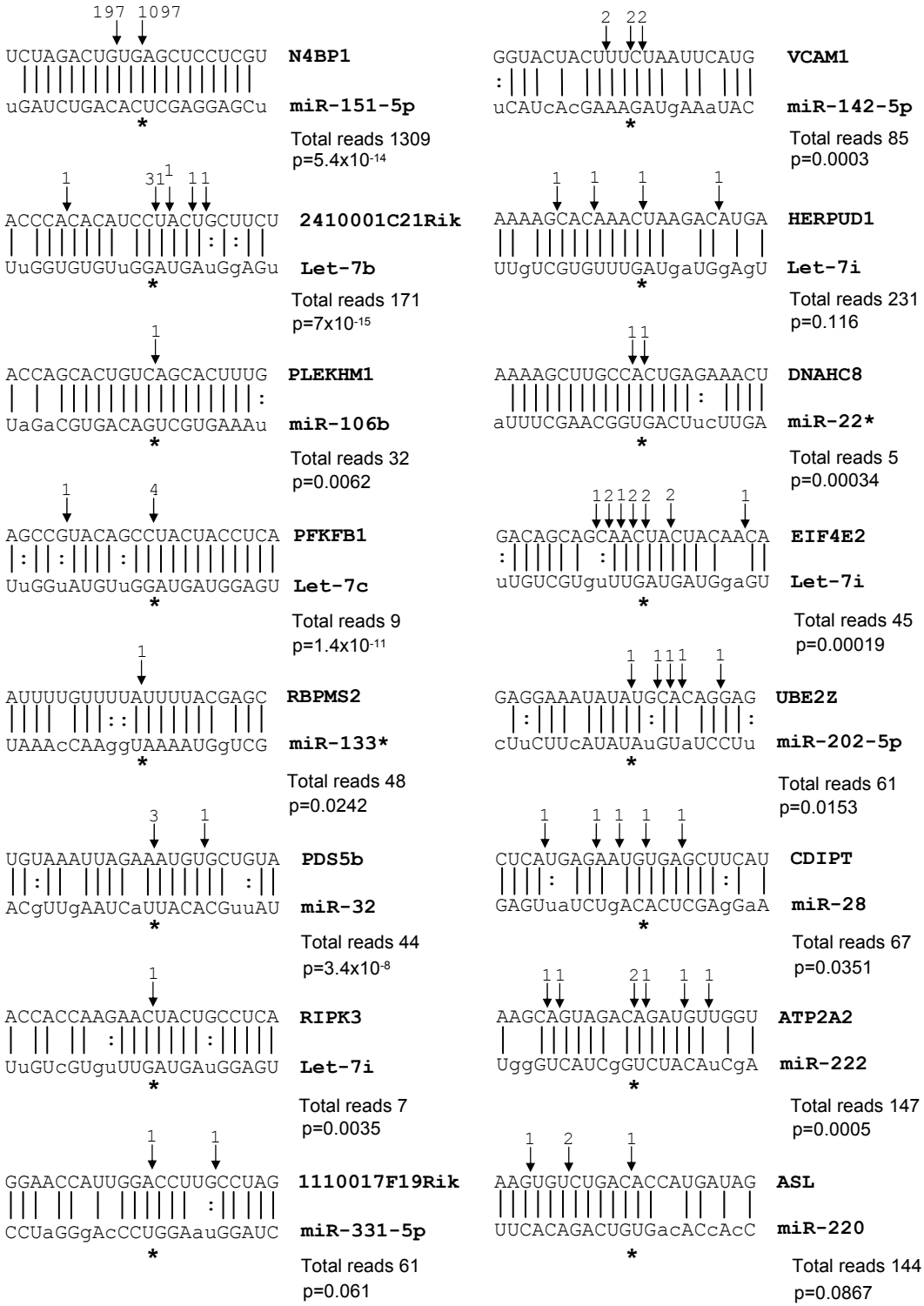
Reads from PARE libraries successfully mapped to the genome (permitting 1 mismatch) are categorised based upon mapping location. MiRNA and tRNA-associated reads refer specifically to reads mapping to these RNA molecules and do not include neighbouring RNAs or post-processing fragments.

SUPP TABLE 2 – Specific non-miRNA associated cleavages detected by PARE
Local accumulations of reads (>10) within adult PARE libraries mapping to specific nucleotides were identified and listed. The total read numbers summed from adult PARE libraries is shown, with most of these reads being identified in multiple libraries. Reads associated with miRNA-directed cleavage or the miRNAs themselves are removed. Reads mapping to mRNAs which are represented by very high read numbers along their length are also removed as they do not represent a locally significant increased frequency.

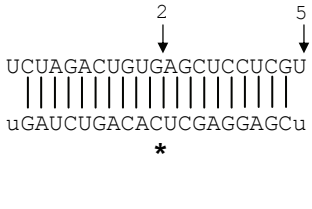
SUPP TABLE 3 – PARE reads mapping to miRNA loci
Reads commencing at the mature 5'-termini of miRNAs, their star forms or the 3' fragment remaining after processing are tallied across the adult PARE libraries. MiRNAs are listed in the order corresponding to their position within polycistronic transcripts.

SUPP TABLE 4 – Polycistronic miRNA and 3' fragment reads within PARE libraries
Reads commencing at the mature 5'-termini of miRNAs, their star forms or the 3' fragment remaining after processing are tallied across the adult PARE libraries and ordered according to their location within polycistronic transcripts. For inclusion within the table, there must be a minimum of 5 reads mapping to 2 or more miRNAs within the transcript. The relative percentages of the mature + star miRNA and 3' fragment reads are shown.

SUPPLEMENTARY FIGURE 1a



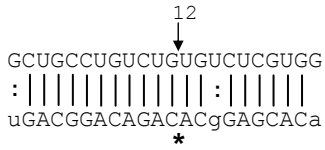
SUPPLEMENTARY FIGURE 1b



N4BP1

miR-151-5p

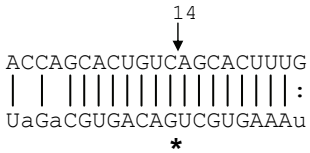
Total Reads : 38
p=1.7x10^{-7}



BC037034

miR-214

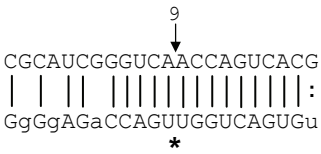
Total Reads : 42
p=10x10^{-16}



PLEKHM1

miR-106b

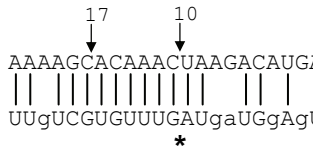
Total Reads : 39
p=10x10^{-16}



2410075B13RIK

miR-134

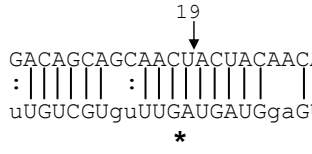
Total Reads : 105
p=5x10^{-15}



HERPUD1

Let-7i

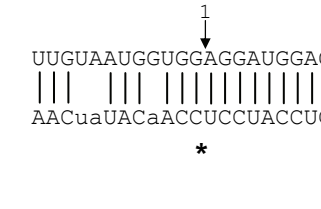
Total Reads : ~500
p=3.6x10^{-13}



EIF4E2

Let-7i

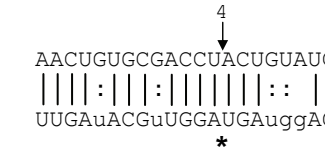
Total Reads : 146



P2RX4

Let-7e

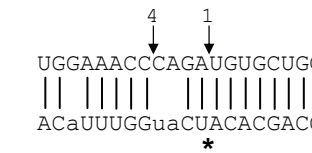
Total Reads : 12
p=0.006



FIGNL1

Let-7d

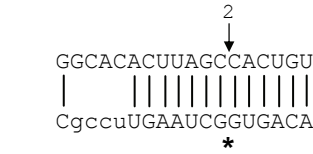
Total Reads : 14



BC030863

miR-15b

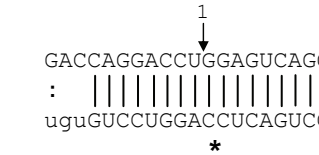
Total Reads : 50
p=0.017



TMEM51

miR-27a

Total Reads : 9
p=2.3x10^{-5}

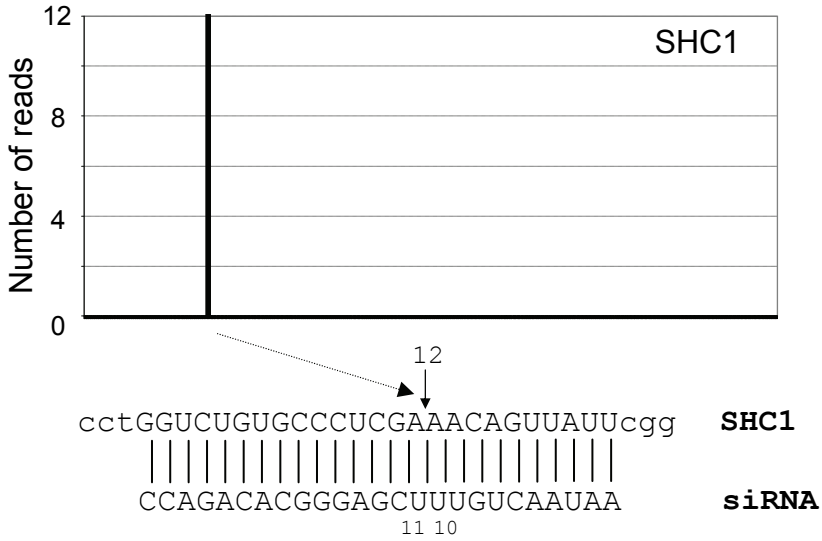


RNF26

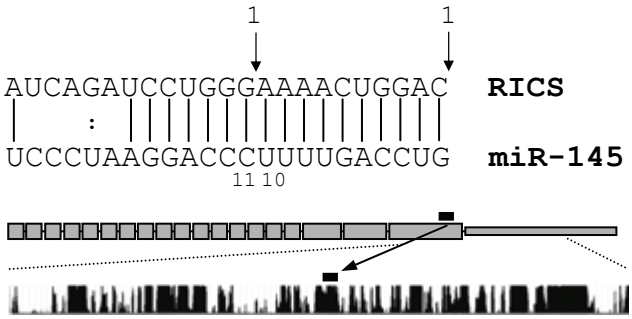
miR-378*

Total Reads : 12

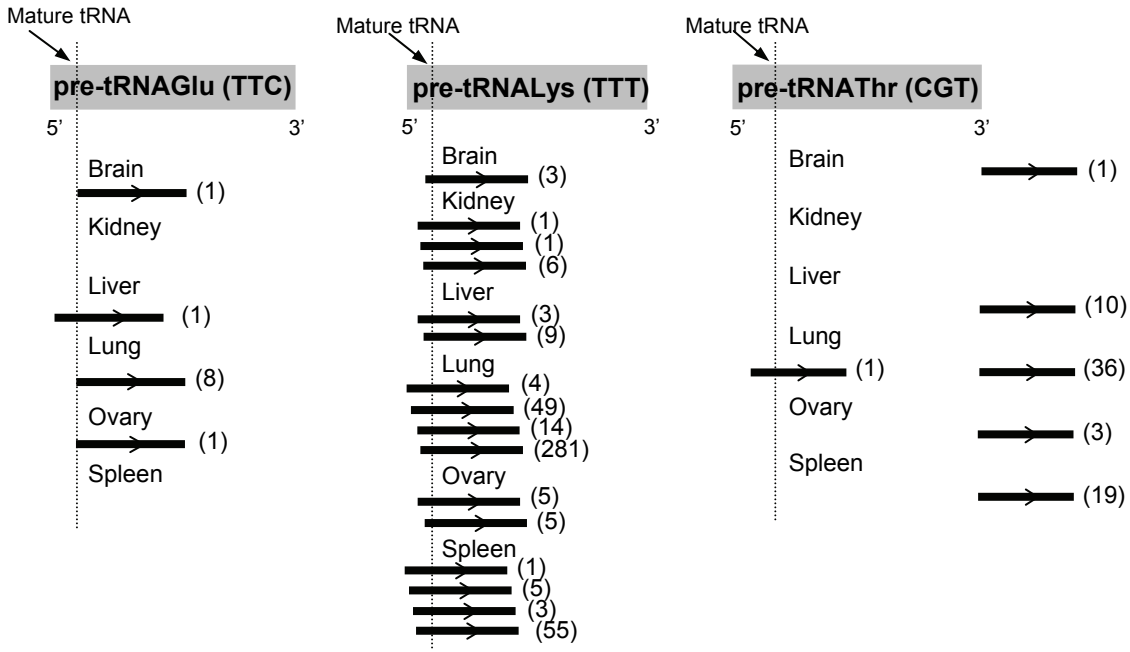
SUPPLEMENTARY FIGURE 2



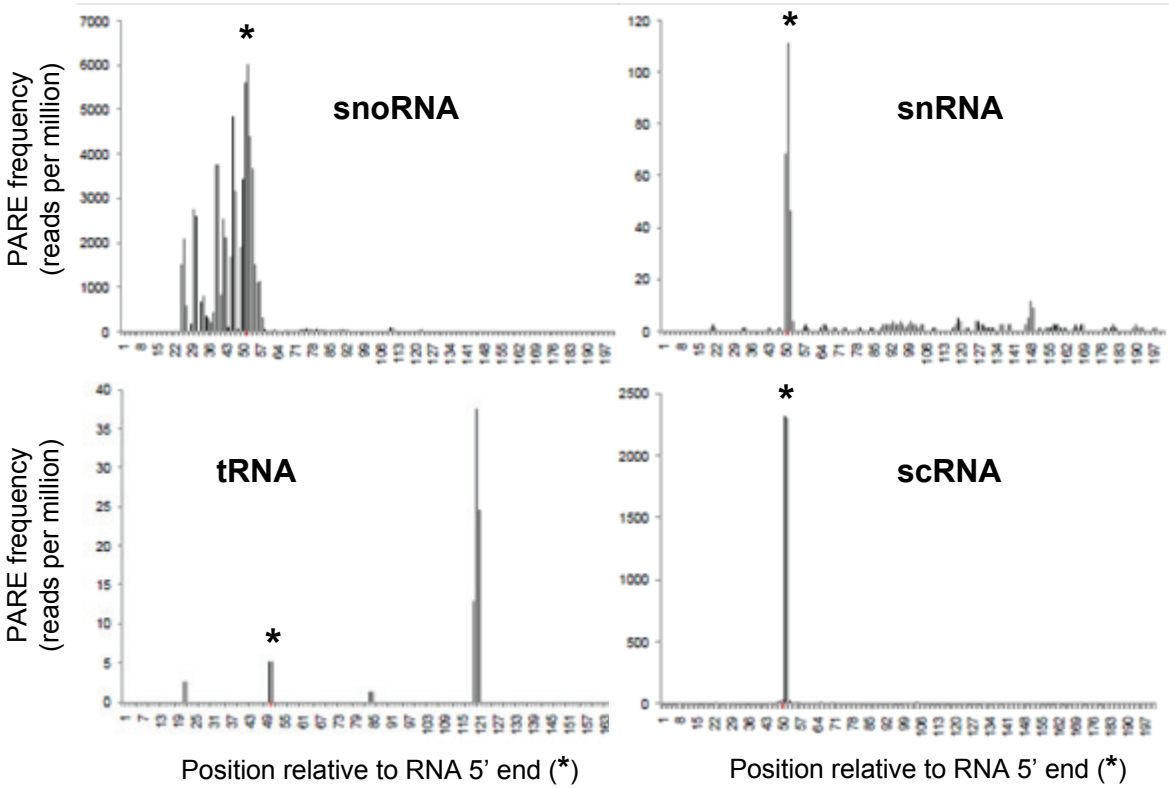
SUPPLEMENTARY FIGURE 3



SUPPLEMENTARY FIGURE 4



SUPPLEMENTARY FIGURE 5



SUPPLEMENTARY FIGURE 6