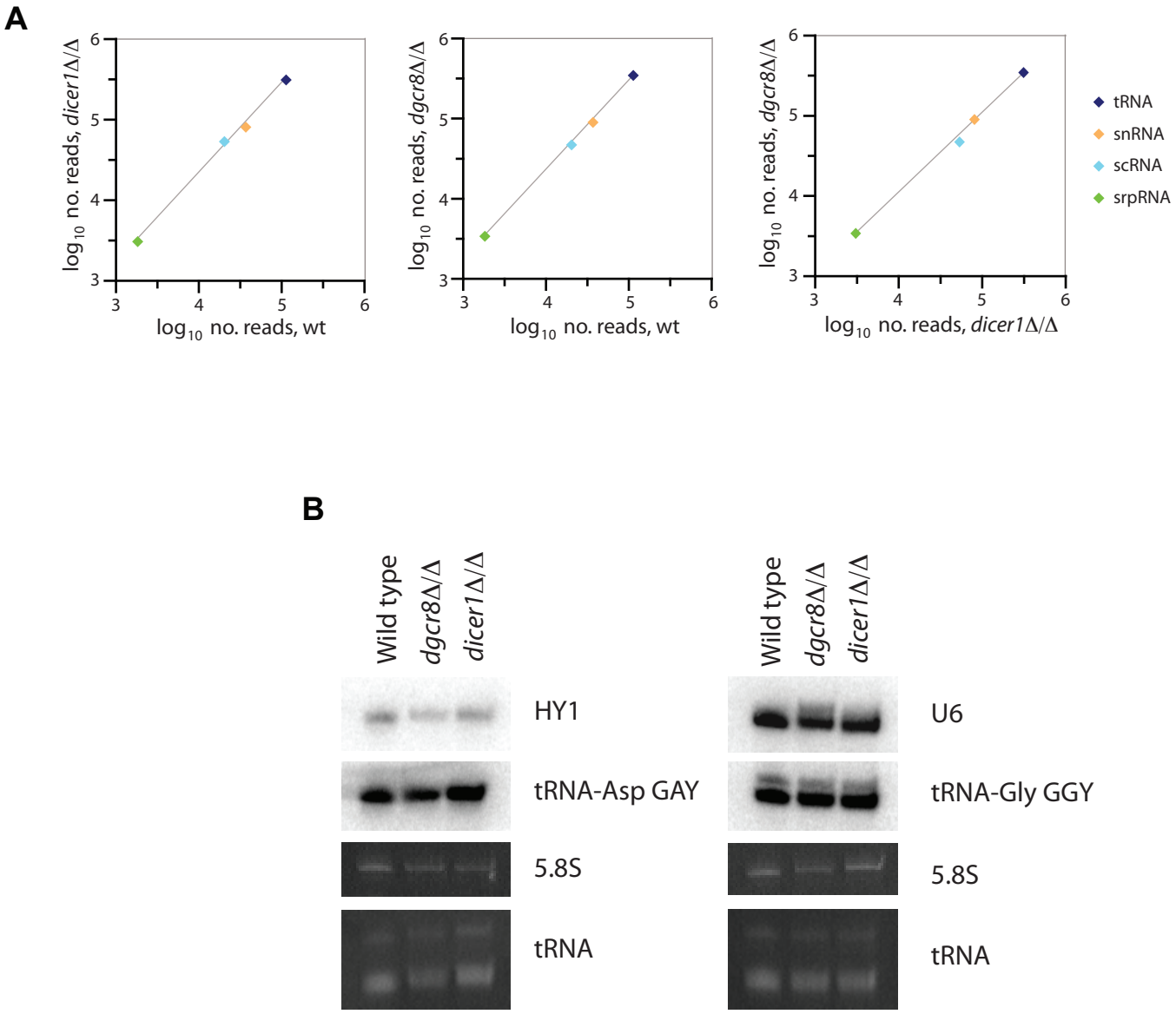


Figure S1. t/sc/sn/srpRNA levels do not change between wt, *dgcr8*Δ/Δ, *dicer1*Δ/Δ. (A) The number of sequences from each catergory in wt, *dgcr8*Δ/Δ, *dicer1*Δ/Δ small RNA libraries. (B) Northern analysis of abundant members of the tRNAs, scRNAs, and snRNAs confirmed no change between the three libraries. Bottom panels show ethidium bromide staining of 5.8S rRNA and tRNAs.

Figure S2A. Wild-type sequence reads mapping to a DGCR8 and Dicer-dependent locus (shown above) that overlaps a DGCR8-dependent, Dicer-independent locus. The DGCR8-dependent, Dicer-independent locus is highlighted in red and extends further to the left, but contains no additional sequences (chr2(+):10386728-10386839). The resulting patterns and enzymatic dependencies of the reads are consistent with them representing a canonical miRNA with 5' remnants of the Microprocessor cleavage. This miRNA has been now annotated as *mir-669l*.

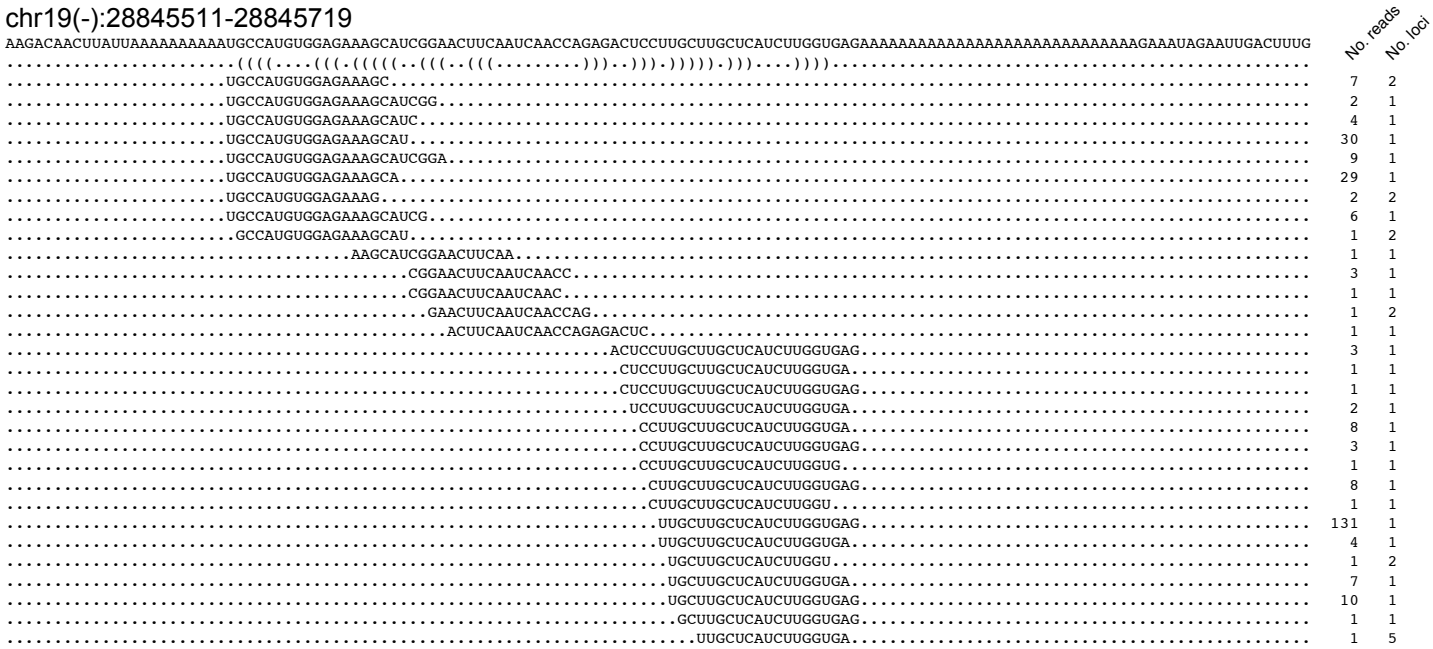


Figure S2B. Wild-type sequence reads mapping to a DGCR8-dependent, Dicer-independent locus that did not flank a pre-miRNA hairpin. No antisense-strand reads were derived from this locus. The coordinates of the dependency-defined locus are given (see methods).

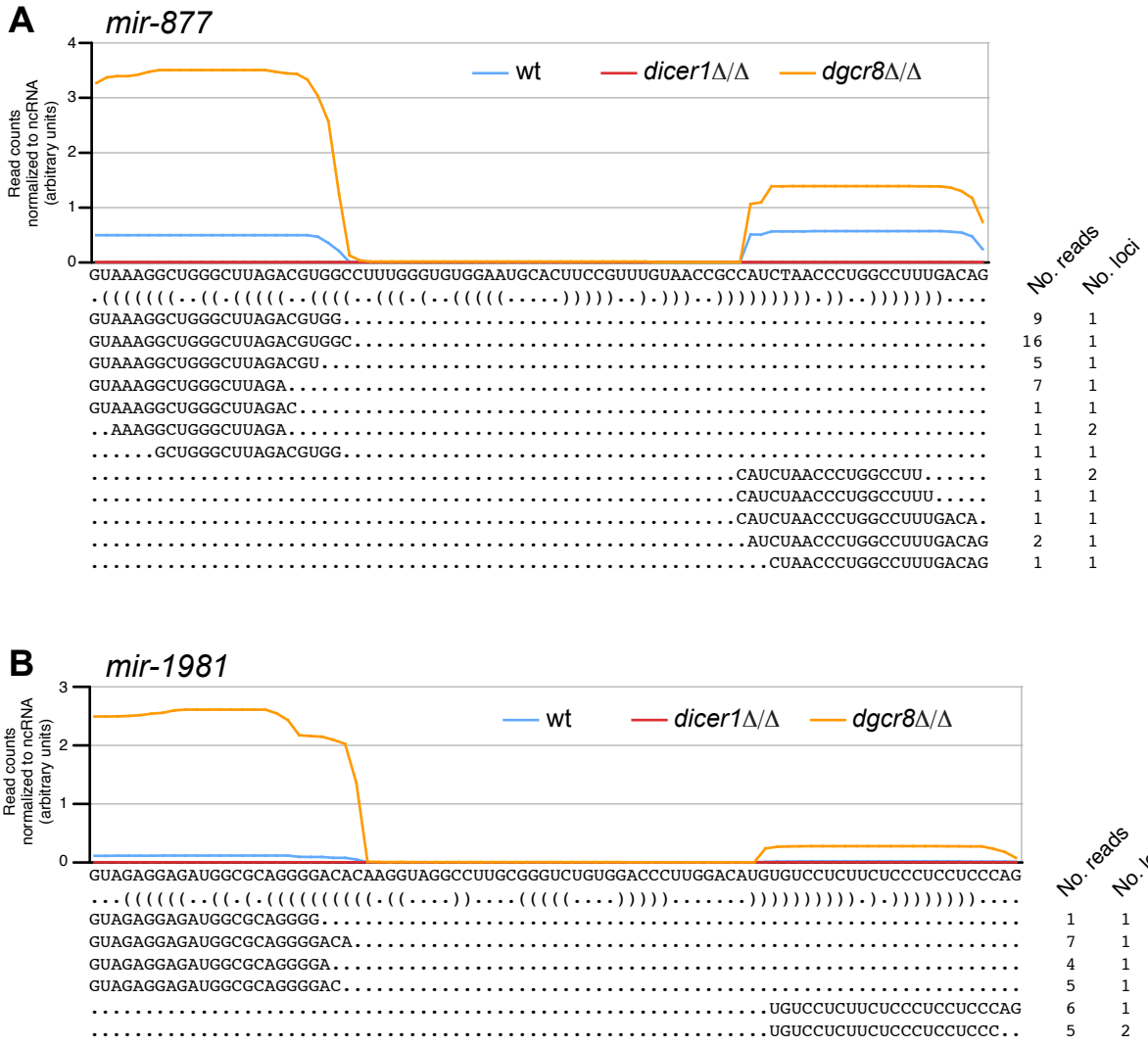


Figure S3. The *mir-877* (A) and *mir-1981* (B) mirtrons, with reads from the wt ES cell libraries. Normalized small-RNA expression across each mirtron for wt, *dicer1*Δ/Δ, and *dgcr8*Δ/Δ samples is shown as in Figure 2A. The dramatic increase in *mir-1981* observed in the *dgcr8*Δ/Δ background was likely due to the increase in *Mosc2* expression in *dgcr8*Δ/Δ relative to wt (Fig. 2I).

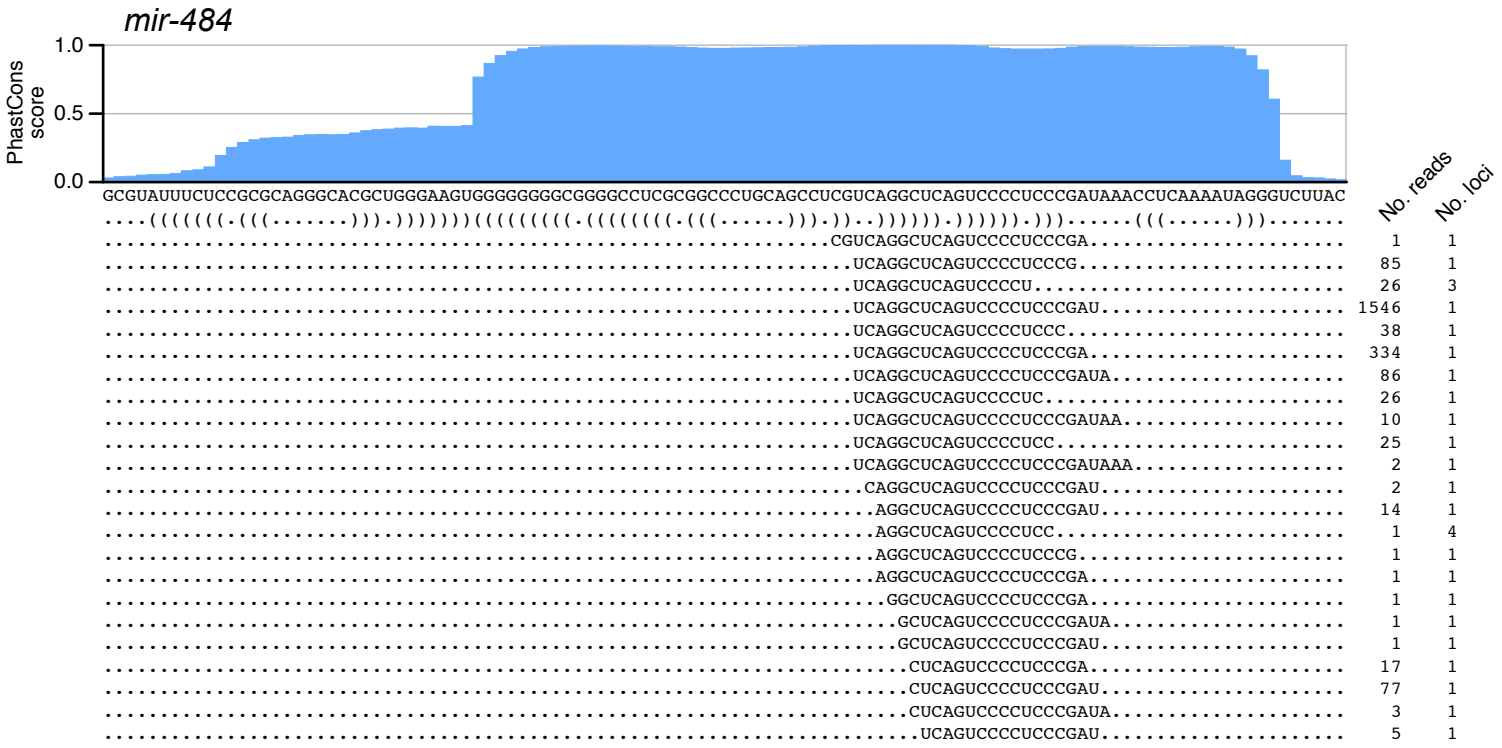


Figure S4. The *mir-484* locus. The locus shown is shifted from the prior hairpin annotation, which had less base pairing across the observed miRNA. PhastCons scores across the locus and reads from the wt dataset are shown aligned to the genomic sequence at this lous.

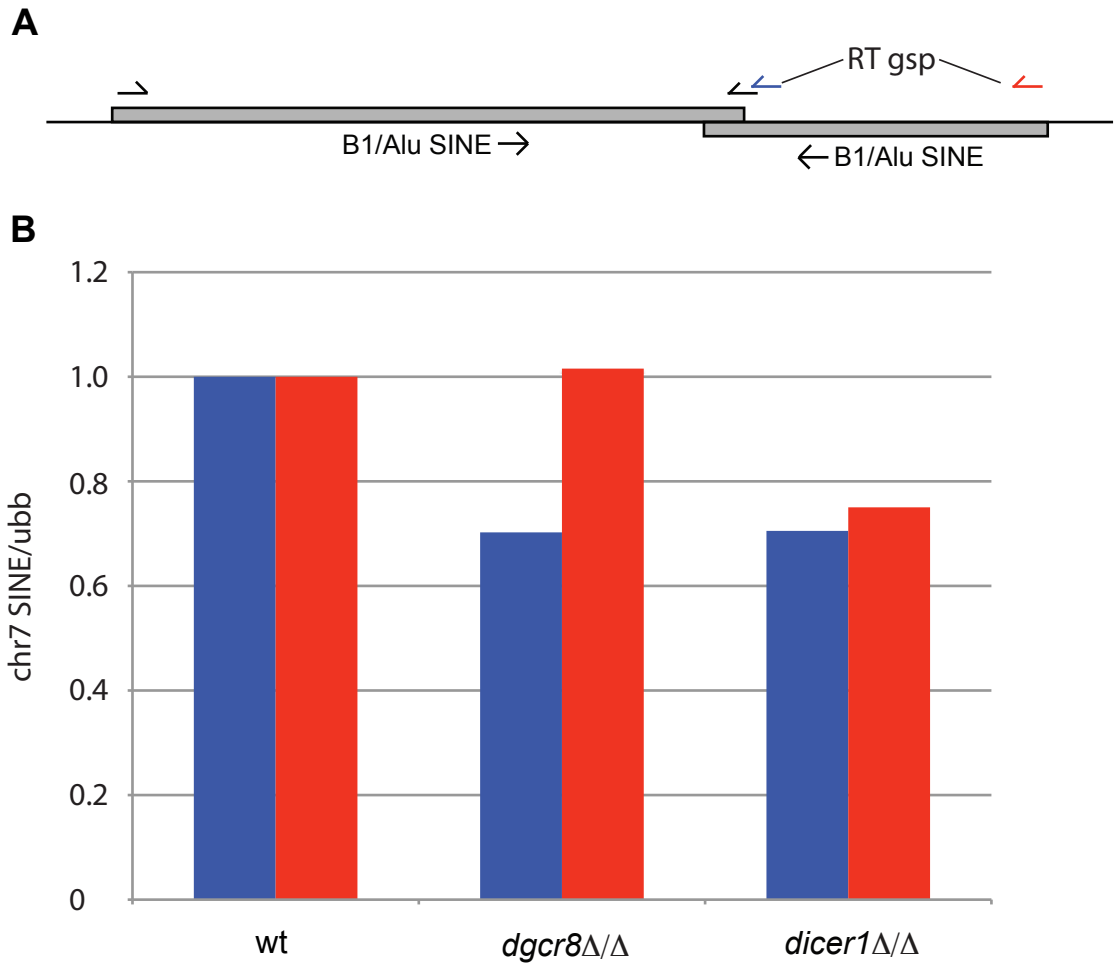
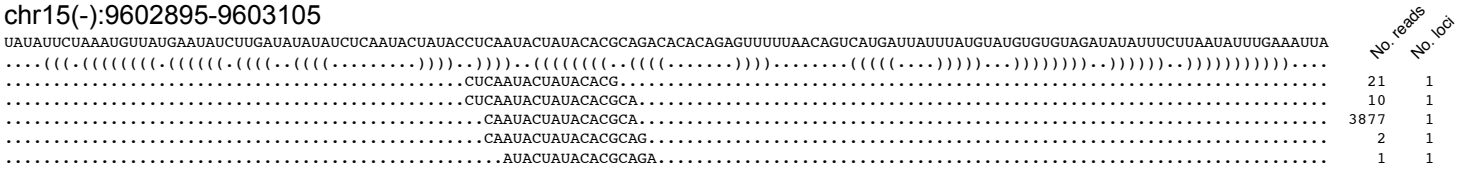
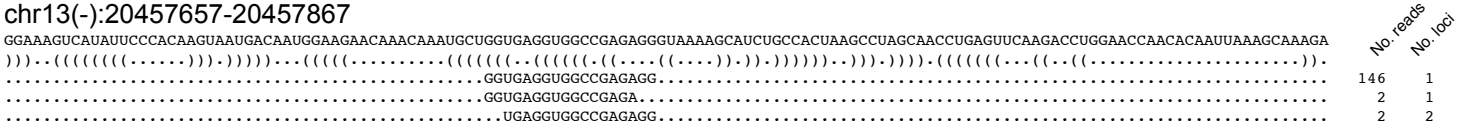


Figure S5. The primary transcript of the Dicer-dependent SINE element on chr7 does not change dramatically in dicer1Δ/Δ. (A) Design of the qPCR strategy to amplify the chr7 SINE. Two different gene specific primers (RT gsp) were used for reverse transcription (red and blue). qPCR was performed with the denoted primers (black), with the 5´ primer matching the genome uniquely. Primers were chosen such that RT followed by qPCR would amplify only this locus. (B) qPCR of the chr7 relative to ubiquitin b (ubb). The two bars represent two independent RT reactions with the primers indicated in part A.

chr15(-):9602895-9603105



chr13(-):20457657-20457867



chr10(-):38383545-38383753

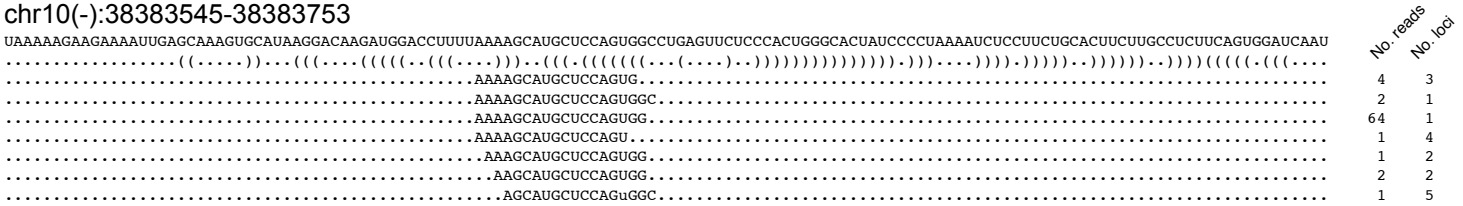


Figure S6. Three Dicer-dependent, Dgcr8-independent loci that give rise to short reads that exhibit both 5′ and 3′ heterogeneity. No antisense-strand reads were derived from any of these loci. The coordinates of the dependency-defined loci are given (see methods), but flanking portions of the locus without reads are omitted from the figure. Reads are shown from the wt datasets.

mir-320 chr8(-):22158429-22158485

```
CCUUCUCUUCCCGGUUCUUCCCGGAGUCGGGAAAAGCUGGGUUGAGAGGGCGAAAAA
(((((((..(((((((.((((((....)))))).)))))))..)))))))........
...........................GAAAAGCUGGGUUGAGAGGGCGA....
............................GAAAAGCUGGGUUGAGAGGG.......
...........................GAAAAGCUGGGUUGAGAGGGCG.....
...........................GAAAAGCUGGGUUGAGAGGGCGAAA..
.............................AAAAGCUGGGUUGAGA..........
.............................AAAAGCUGGGUUGAGAGGGCG.....
...........................AAAAGCUGGGUUGAGAGGGCGAAAAA
...........................AAAAGCUGGGUUGAGAGGGCGA....
...........................AAAAGCUGGGUUGAGAGGGCGAA...
.............................AAAAGCUGGGUUGAG..........
.............................AAAAGCUGGGUUGAGAGG........
.............................AAAAGCUGGGUUGAGAG........
...........................AAAAGCUGGGUUGAGAGGGCGAAAA.
...........................AAAAGCUGGGUUGAGAGGGCGAAA..
.............................AAAAGCUGGGUUGAGAGGGC......
.............................AAAAGCUGGGUUGAGAGGG.......
............................AAAGCUGGGUUGAGAGGGCGAAA..
............................AAAGCUGGGUUGAGAGGGC......
............................AAAGCUGGGUUGAGAGGGCGA....
............................AAAGCUGGGUUGAGAGGG.......
............................AAAGCUGGGUUGAGAGGGCGAA...
............................AAAGCUGGGUUGAGAGGGCGAAAA.
...........................AAAGCUGGGUUGAGAGGGCG.....
..........................AAGCUGGGUUGAGAGGGCGAA...
..........................AAGCUGGGUUGAGAGGGCGA....
..........................AAGCUGGGUUGAGAGGGCGAAA..
..........................AAGCUGGGUUGAGAGGGCGAAAA.
.........................AGCUGGGUUGAGAGGGCGA....
.........................GCUGGGUUGAGAGGGCGAAA..
.........................GCUGGGUUGAGAGGGCGAA...
........................CUGGGUUGAGAGGGCGA....
........................CUGGGUUGAGAGGGCGAAA..
........................CUGGGUUGAGAGGGCGAA...
........................UGGGUUGAGAGGGCGAA...
........................UGGGUUGAGAGGGCGAAA..
```

No. reads	No. loci
33	1
1	1
8	5
2	1
1	9
221	1
2	1
3595	1
2322	1
2	16
9	8
1	8
63	1
670	1
14	3
17	5
66	1
3	3
74	1
3	5
126	1
2	1
17	1
18	1
9	1
43	1
7	1
1	1
1	1
2	1
4	1
1	1
1	4
1	4

mir-484 chr16(+):15644623-15644683

```
CCGGGGGGGGCGGGGCCUCGCGGCCCUGCAGCCUCGUCAGGCUCAGUCCCCUCCCGAUAAA
.((((((((((.((((((((.(((......))).)).)).)))))).)))))).)).....
.................................UCAGGCUCAGUCCCCUCCCGA....
.................................UCAGGCUCAGUCCCCUCCCGAU...
.................................UCAGGCUCAGUCCCCUCCCGAUAA.
.................................UCAGGCUCAGUCCCCUCCCGAUA..
..................................AGGCUCAGUCCCCUCCCGAU...
```

No. reads	No. loci
3	1
53	1
1	1
5	1
1	1

Figure S7. The human *mir-320* and *mir-484* loci had a paucity of 5' hairpin sequences. Human ES cell small RNA sequences (Morin, et al., 2008) were aligned to the human genome (March 2006, hg18) with ELAND (Pipeline version 0.2.2.6, Illumina). Shown are the number of reads observed and the number of times that read matched the genome.

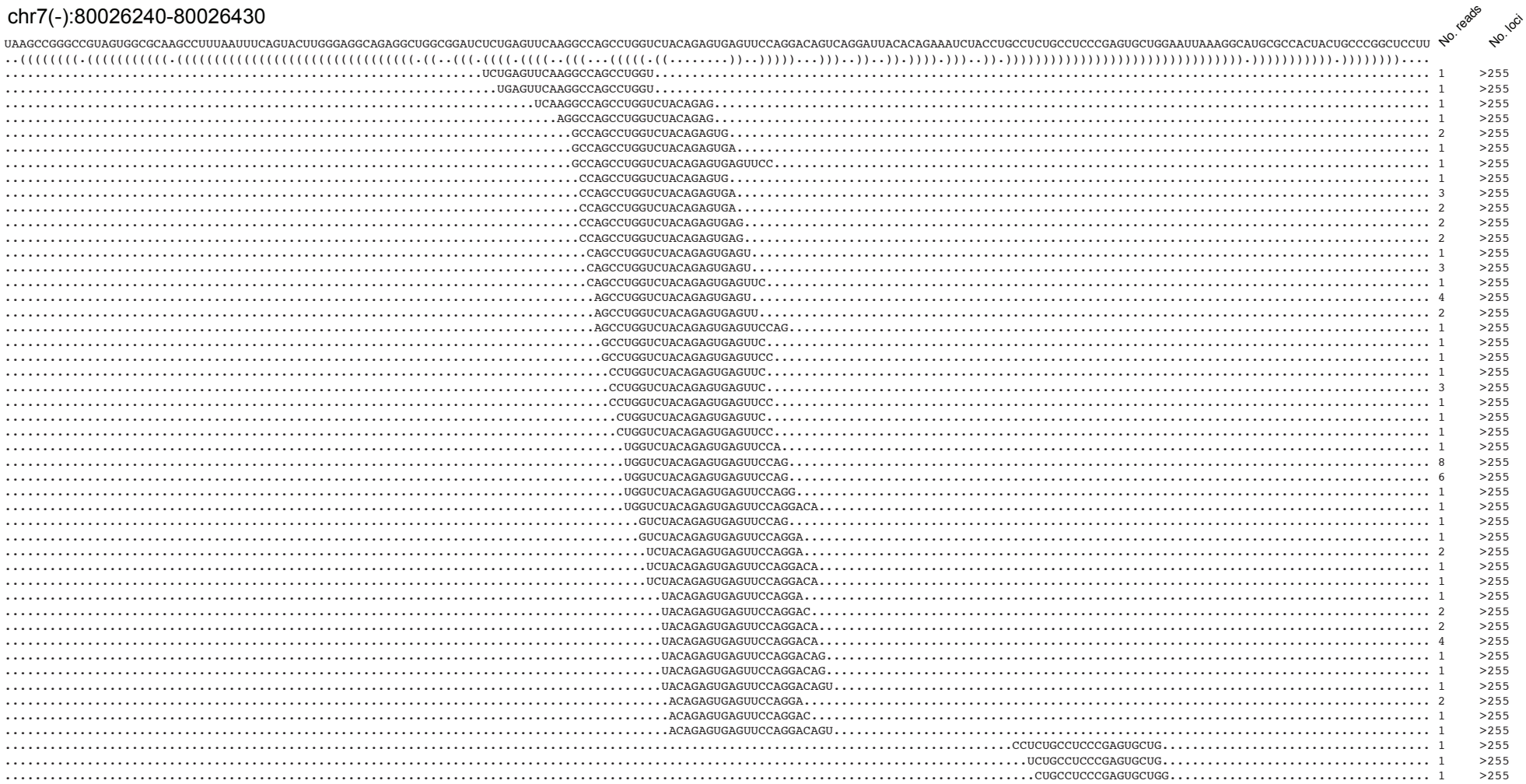


Figure S8. No small RNAs from mouse oocytes aligned to the SINE hp-siRNA locus uniquely. Previously published small RNA sequences from mouse oocytes (Tam et al. 2008) were aligned to the mouse genome (February 2006 mm8) using ELAND (Pipeline version 0.2.2.6, Illumina). The number of times a sequence mapped to the genome was computed for each sequence that aligned to the hp-siRNA locus. Each sequence mapped to the genome more than 255 times, which is the limit for ELAND.

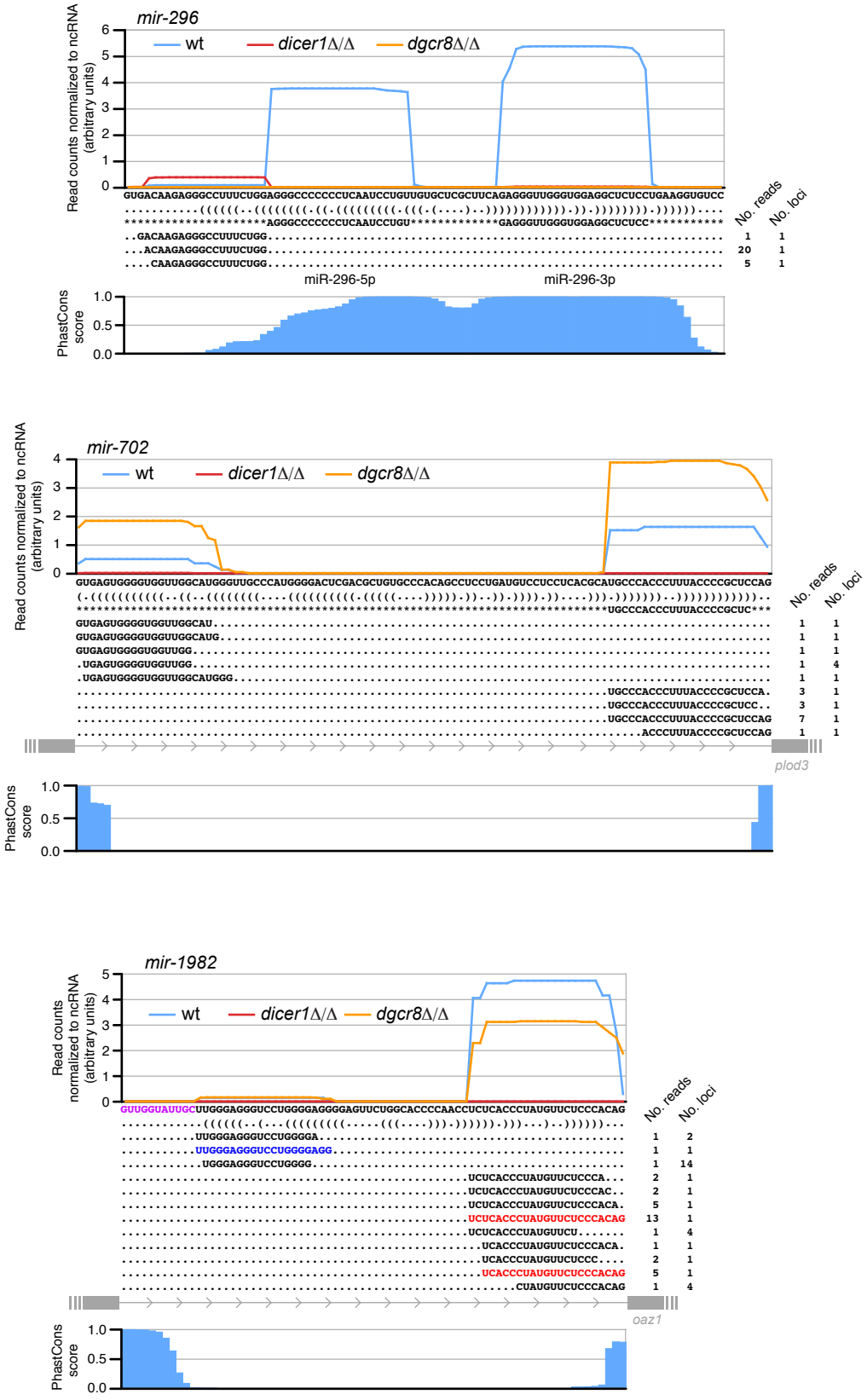


Figure S9. Conservation of miRNA loci in Figure 2. Shown are PhastCons scores (Siepel et al. 2005) of *mir-296*, *mir-702*, and *mir-1982*.

Supplemental Table 1. Values for the stacked bar graph presented in Figure 1A.

	wt	dicer1Δ/Δ	dgcr8Δ/Δ
annotated miRNAs	60.6%	0.4%	2.2%
transposable elements	2.2%	3.3%	3.9%
rRNA	10.5%	27.2%	23.7%
tRNA	2.8%	14.1%	11.8%
other	23.8%	54.9%	58.4%

Supplemental Table 2. Small RNA Loci from Figure 1B, C, and D.
Excel spreadsheet containing the following information:

For each small-RNA-generating genomic locus (see methods), the following information is provided:

Column	Description
chr	chromosome name
start	start coordinate (mm8 assembly)
end	end coordinate (mm8 assembly)
strand	genomic strand
reads(wt)	number of reads in the wt datasets (each normalized to no. genomic loci)
reads(dcr)	number of reads in the dicer(-/-) datasets (each normalized to no. genomic loci)
reads(dgcr8)	number of reads in the dgcr8(-/-) datasets (each normalized to no. genomic loci)
dcrDep	calculated dependency on dicer (see methods)
dgcr8Dep	calculated dependency on dgcr8 (see methods)

Each locus corresponds to one point in Figures 1B, 1C, and 1D.
Loci are divided onto worksheets based on their dependency category as defined in Figure 1.

dep = dependent
indep = independent
int = intermediate

Supplemental Table 3. Values for the stacked bar graph presented in Figure 1E.

	wt genome-matching reads
Dicer-independent, DGCR8-independent	789,383 reads
Dicer-dependent, DGCR8-independent	19,716 reads
Dicer-independent, DGCR8-dependent	396 reads
Dicer-dependent, DGCR8-dependent	1,234,945 reads
intermediate	329,943 reads

Supplemental Table 4. Annotated miRNAs from Figure 2B.
Excel spreadsheet containing the following information:

For each miRNA hairpin (mirbase v.10.0) meeting the locus criteria for classification (see Methods), the following information is provided:

Column	Description
miRNA	name of miRNA hairpin
reads(wt)	number of reads in the wt datasets (each normalized to no. genomic loci)
reads(dcr)	number of reads in the dicer(-/-) datasets (each normalized to no. genomic loci)
reads(dgcr8)	number of reads in the dgcr8(-/-) datasets (each normalized to no. genomic loci)
dcrDep	calculated dependency on dicer (see methods)
dgcr8Dep	calculated dependency on dgcr8 (see methods)

Each hairpin corresponds to one point in Figure 2B.
Hairpins are divided onto worksheets based on their dependency category as defined in Figure 1.
In this table, the three intermediate sub-categories have been compressed into one sheet.

Note: there were no dicer-independent, dgcr8-dependent miRNA hairpins, although
portions of many of the hairpins met that description (see text, Figure 2A).

dep = dependent
indep = independent

Supplemental Table 5. Small RNA reads mapping to introns < 500 bp from Figure 2D. Excel spreadsheet containing the following information:

For each refseq intron <500 nt meeting the locus criteria for classification (see Methods), the following information is provided:

Column	Description
chr	chromosome name
start	start coordinate (mm8 assembly)
end	end coordinate (mm8 assembly)
strand	genomic strand
reads(wt)	number of reads in the wt datasets (each normalized to no. genomic loci)
reads(dcr)	number of reads in the dicer(-/-) datasets (each normalized to no. genomic loci)
reads(dgcr8)	number of reads in the dgcr8(-/-) datasets (each normalized to no. genomic loci)
dcrDep	calculated dependency on dicer (see methods)
dgcr8Dep	calculated dependency on dgcr8 (see methods)

Each intron corresponds to one point in Figure 2D.
Introns are divided onto worksheets based on their dependency category as defined in Figure 1.
In this table, the three intermediate sub-categories have been compressed into one sheet.

Note: there were no dicer-independent, dgcr8-dependent introns.

dep = dependent
indep = independent

Supplemental Table 6. Values for the stacked bar graph presented in Figure 4A.

	Dicer-dependent wt reads
other	5304
LTR	58
SINE	7726
miRNA/shRNA	7506

	Dicer-dependent wt SINE reads
B2	18
B4	149
B1/Alu	7559

	Dicer-dependent wt miRNA/shRNA reads
mir-344/668/702	94
shRNA-X(chr7)	248
mir-320	2925
mir-484	2285
shRNA/tRNA-Ile-ATA	1954

Supplemental Methods

SINE qRT-PCR

WT, *dgcr8Δ/Δ* and *dicer1Δ/Δ* ES cells were plated on 60 mm plates and total RNA was purified using Trizol reagent, following the manufacturer's protocol (Invitrogen). Total RNA was digested with amplification grade DNase I (Invitrogen) and cleaned up with phenol/chloroform extraction. 200 ng DNase-treated RNA was reverse transcribed with oligo dT, SINE gsp1 AAGGAGCCGGGCAGTAGTG, or SINE gsp2 GTCCTGGAACTCACTCTGT using the Superscript III First Strand cDNA Synthesis Kit (Invitrogen). Quantitative PCR was performed on an ABI 7300. SINE primers: CGCAAGCCTTTAATTTCAGTA and CACTCTGTAGACCAGGCTG. Ubiquitin B primers: TTCGGCGGTCTTTCTGTGA and TTAACAAATGTGATGAAAGCACAAAC.

RNA blot analysis

RNA blots were performed as previously described (Lau et al. 2001). 4 µg total RNA was loaded onto a 10% denaturing polyacrylamide gel. Following transfer and blocking, the blots were probed with end-labeled oligos to HY1 (TTCAATCTGTAACT GACTGTGAACAATCAATTGAGATAACTCACTACCTTCGGACCAGCC) or U6 (See Wang et al. 2006). The blots were stripped with 1% SDS at 85^{o}C for 1 hour. The blots were then reprobed for either tRNA-Asp GAY (GTCGGGGAATCGAACC CCGGTCTCCCGCGTGACAGGCGGGGATACTCACCACTATACTAA) or tRNA-Gly GGY (TGGCCGGGAATCGAACCCGGGCCTCCCGCGTGGCAGG CGAGAATTCTACCACTGAACCAC), respectively. Blots were exposed to a phosphorimager and scanned on a Storm (GE Healthcare).

References

Lau, N. C., Lim, L. P., Weinstein, E. G. and Bartel, D. P. 2001. An abundant class of tiny RNAs with probable regulatory roles in *Caenorhabditis elegans*. Science. 294: 858-62.