Supporting Online Material for

Endogenous siRNAs Derived from Transposons and mRNAs in *Drosophila* Somatic Cells

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Table S1

Supporting Online Material

Materials and Methods

General Methods

RNA was isolated as described (*S1*) from heads of Oregon R flies or *white*-IR flies (*S2*) or from Schneider 2 (S2) cells, a phagocytic, cultured cell line derived from late-stage *Drosophila* embryos (*S3*). S2 cells were a clonal cell line containing a stably integrated GFP transgene, pKF63, transiently transfected with dsRNA targeting GFP (*S4*). dsRNA was prepared (*S5*) and transfected into S2 cells as described (*S6*).

High-throughput sequencing

High-throughput pyrosequencing was as described (*S1*). Libraries were constructed using a method that selects for RNAs bearing 5´ monophosphates (*S7*). For pyrosequencing, the total small RNA libraries yielded 63,315 (S2 cells) and 71,268 (heads) reads corresponding to 4,971 (S2 cells) and 1,884 (heads) unique sequences. High-throughput sequencing-by-synthesis (Genome Analyzer, Illumina, San Diego, CA, USA) was as for pyrosequencing except that RNA Ligase 2 [Rnl2(1-249)K227Q] (Addgene, Cambridge, MA, USA) was used for 3´ ligation. Linkers and primers for sequencing-by-synthesis were: 5´ adaptor, 5´-rGrUrU rCrArG rArGrU rUrCrU rArCrA rGrUrC rCrGrA rCrGrA rUrC-3´ (Dharmacon, Lafayette, CO, USA); 3´ preadenylated linkers, 5´-rAppdCdT dGdTdA dGdGdC dAdCdC dAdTdC dAdAdT ddC-3´.

After linker addition, the cDNA was synthesized using a reverse-transcriptase primer corresponding to the 3´ adapter and amplified by PCR using forward (5´-dAdAdT dGdDdAdT dAdCdG dGdCdG dAdCdC dAdCdC dGdAdC

dAdGdG dTdTdC dAdGdA dGdTdT dCdTdA dCdAdG dTdCdC dGdA -3´) and reverse (5´-dCdAdA dGdCdA dGdAdA dGdAdC dGdGdC dAdTdA dCdGdA dAdTdT dGdAdT dGdGdT dGdCdC dTdAdC dAdG-3´) primers. The PCR pool was gel purified (4% Metaphor Agarose, Cambrex, East Rutherford, NJ, USA) with Qiaex II (Qiagen, Valencia, CA, USA) then sequenced (Genome Analyzer, Illumina) according to the manufacturer's protocol.

Quantitative RT-PCR analysis

Two micrograms of total RNA was treated with RQ1 DNase (Promega, Madison, WI, USA) or Turbo DNase (Ambion, Austin, TX, USA) according to manufacturer's instructions and then reverse transcribed using oligo(dT) primer and Superscript III and Superscript II reverse transcriptases (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's directions. The resulting cDNA was analyzed by quantitative RT-PCR performed in a DNA Engine OPTICON 2 (MJ Research, Bio-Rad, Hercules, CA, USA) or an iQ5 (Bio-Rad, Hercules, CA, USA) instrument using a SYBR Green PCR kit (Qiagen or Bio-Rad) according to manufacturer's instructions. Relative steady-state mRNA levels were determined from the threshold cycle for amplification using the $\Delta\Delta C_T$ method (*S8*) or DART-PCR (*S9*). Table S6 lists the PCR primer sequences.

Computational Analyses

For each transposon, reads mapping to at least one genomic copy of that transposon were aligned on the transposon consensus sequence using WU-BLAST (http://blast.wustl.edu/) at low stringency (word size, 1; expectancy threshold, 100). For each aligned read, the top-scoring segment pair was selected; if N segment pairs were equally high-scoring, they were all selected, and were

weighted by 1/N (especially true for LTR-matching reads). When the segment pair alignment did not reach the extremities of the read, the alignment was extended in order to cover the complete read. Where reads are reported normalized to sequencing depth, the number of genome-matching reads was used for normalization. Total small RNA data sets correspond to all reads matching the *Drosophila* genome after excluding annotated non-coding RNAs such as ribosomal RNA, snRNAs, snoRNAs, etc. Other computational methods were as described (*S10*). Programs are freely available upon request. Sequencing statistics are in Table S7.

Enrichment of endo-siRNAs in regions of overlapping transcripts

The annotated transcriptome (defined as the genomic regions of all annotated mRNAs, including exons and introns) was first divided into the regions that produced overlapping, complementary transcripts and regions that produce transcripts only from one strand. Then all allowable positions that can be the starting position of a non-transposon-overlapping 21-mer were separately determined for the plus and minus strands. The scope of double-stranded regions was defined as the union of the allowable positions for which the anti-sense positions are also allowable. The scope of single-stranded regions is defined as the union of the remaining allowable positions.

We then mapped the endo-siRNAs from wild-type fly heads or S2 cells onto the transcriptome and computed an enrichment score: [(total number of mapped endo-siRNAs whose 5´-end position falls in the scope of double-stranded regions)*(size of the scope of double-stranded regions + size of the scope of single-

stranded regions)]/[(total number of endo-siRNAs)*(size of the scope of double-stranded regions)].

To determine the statistical significance of the resulting enrichment scores, we randomly selected the same number of allowable positions as the number of endo-siRNAs in the sample and recomputed the enrichment score, 100 times per sample. The random distribution had a mean ~1, as expected. The *p*-values of the actual enrichment scores of the two libraries were determined with reference to the normal distribution. Results are summarized in Table 1.

Supplemental Discussion

The retrotransposon *297* (80 copies per haploid genome) is the second most abundant retroelement in flies. *297* entered *Drosophila* recently through the ancestor of the *melanogaster* species group 44 million years ago (*S11*). Compared to flies, *297* has expanded dramatically in S2 cells (*S12*). *297* matching siRNAs represent 29.2% of all endo-siRNAs in S2 cells, but only 3.3% of endo-siRNAs in heads (Table S2). Remarkably, many of the siRNAs that correspond to *297* in heads map to its LTRs (Fig. 2C). It is difficult to imagine that antisense transcription arising in an adjacent protein-coding gene or an adjacent transposon could produce a precursor dsRNA that would lead to the production of siRNAs so tightly constrained to the LTR sequences. The LTRs of retrotransposons are direct repeats, so intramolecular pairing between LTRs within an RNA transcript—as has been proposed for the terminal inverted repeats (TIRs) of the DNA transposon *TC1* in *C. elegans* (*S13*)—also cannot explain the peculiar pattern of siRNA production from *297*. Perhaps endo-siRNAs arise from an orphaned *297* LTR sequence in flies, but from one or more complete *297* elements in S2 cells. Moreover, somatic

siRNAs are not generally confined to specific regions of the other transposons examined (Fig. 2C). (Notably, the endo-siRNAs derived from the DNA transposon, *S-element*, do not appear to arise from intramolecular base-pairing between the complementary 5′ and 3′ TIRs, as occurs for *TC1* in *C. elegans* (*S13*).)

The *1731* element has also expanded in S2 cells, from a single active copy in the fly to many highly active copies in the cultured cell line (*S14*). Our endo-siRNA data reflects this expansion: *1731* matching siRNAs represent 39% of all endo-siRNAs in S2 cells, but only 0.02% in fly heads, where we found only a single *1731*-matching siRNA (*p*-value $< 2.2 \times 10^{-16}$, chi-square test).

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A

e.g. chr. X (minus strand)
bp 4,815,932

S2 cells:
441 reads 5´-CAGCGUCUGUCGAGCGGAAGG-3´   (21 nt)
 15 reads 5´-CCAGCGUCUGUCGAGCGGAAGG-3´ (22 nt)
  6 reads 5´- AGCGUCUGUCGAGCGGAAGG-3´   (20 nt)
  2 reads 5´- CAGCGUCUGUCGAGCGGAAGGA-3´ (22 nt)
  1 read   5´- CAGCGUCUGUCGAGCGGAAGA-3´ (21 nt)
  1 read   5´- AGCGUCUGUCGAGCGGAAGGA-3´ (21 nt)

adult heads:
41 reads 5´-CAGCGUCUGUCGAGCGGAAGG (21 nt)
 2 reads 5´-CAGCGUCUGUCGAGCGGAAG (20 nt)
 2 reads 5´-AGCGUCUGUCGAGCGGAAGGA (21 nt)
 2 reads 5´-AGCGUCUGUCGAGCGGAAGG (20 nt)
 1 read 5´-CAGCGUCUGUCGAGCGGAA (19 nt)

e.g. chr. X (minus strand) bp 4,815,912
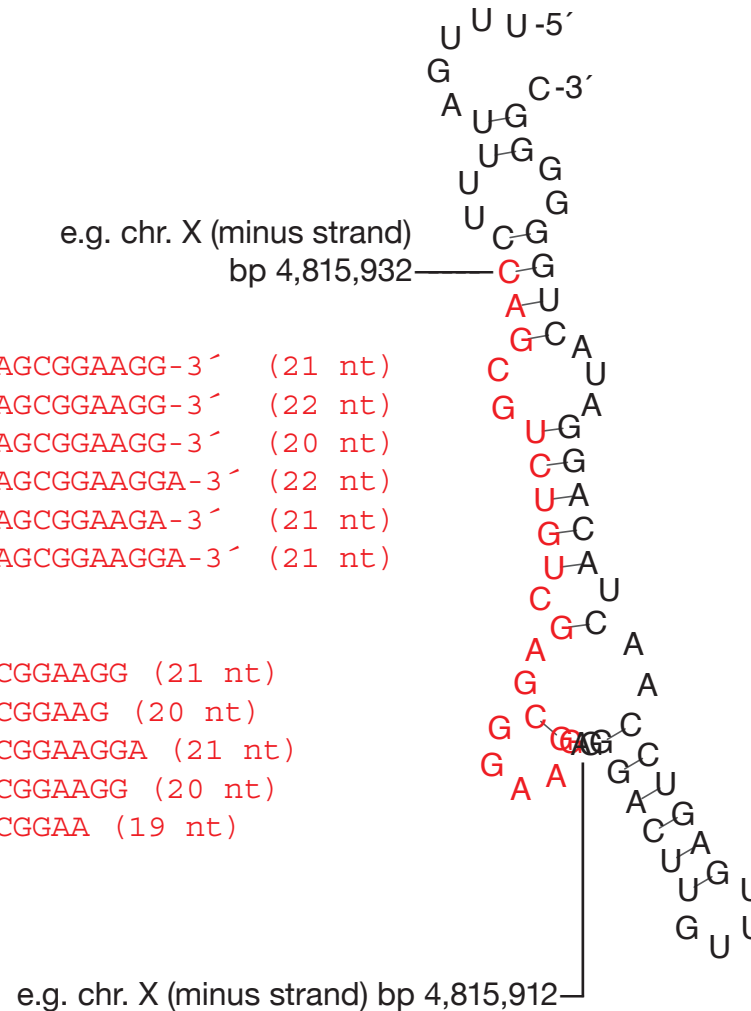
B

perfect matches (bp):

4815932-4815912
4816215-4816195
4816498-4816478
4816781-4816761
4817051-4817031
4817321-4817301
4817604-4817584
4817887-4817867
4818170-4818150
4818453-4818433
4818736-4818716
4819019-4818999
4819572-4819552

near-perfect matches:

4819302-4819282
4819842-4819822
4820382-4820362
4820652-4820632

Figure S1. An unusual small RNA that maps to 17 (13 exact matches and 4 with one mismatch) stable hairpins on the X chromosome ($\Delta\Delta G$ = -22.90 kcal/mol). The small RNA sequence was enriched in the oxidized, β-eliminated library, suggesting it is 2´-O-methylated in cultured S2 cells. (A) The sequence of the 13 identical hairpins containing the unusual small RNA. Their extraordinary conservation may indicate a recent series of gene duplication events. (B) The genomic locations of the sequence on the minus strand of the X chromosome.
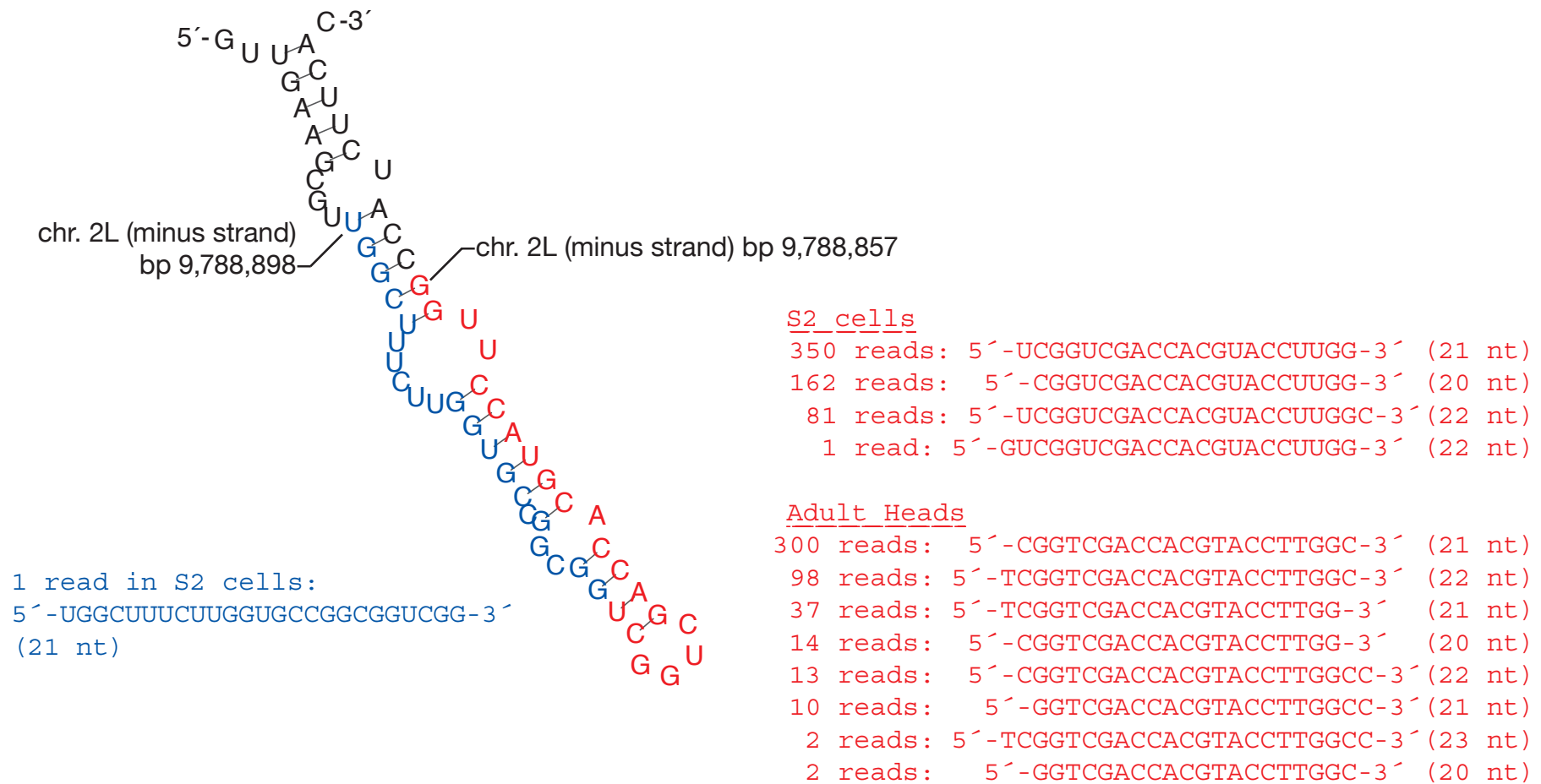
Figure S2. An unusual small RNA derived from a stable hairpin ($\Delta\Delta G$ = -24.20 kcal/mol) on chromosome 2L. The small RNA sequence was enriched in the oxidized, β-eliminated libraries, suggesting it is 2′-O-methylated in cultured S2 cells and adult fly heads.
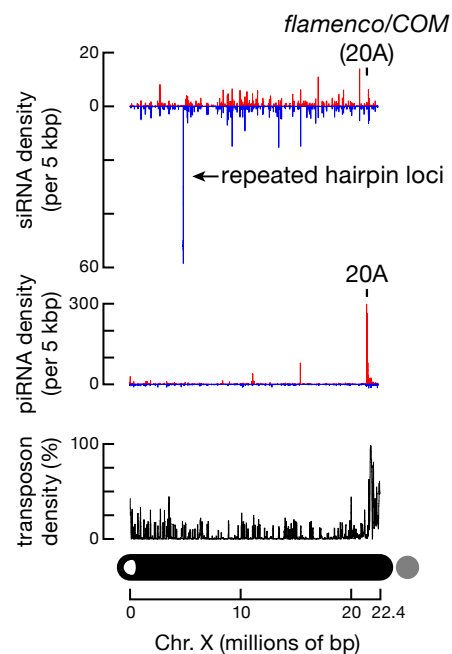
Figure S3. Endogenous siRNAs from adult fly heads. Small RNAs mapping to more than one genomic location were attributed to each site to which they were complementary, but normalized for the number of sites. piRNA data are from Brennecke et al. (*Cell* 2007). The figure was drawn using pooled wild-type head data as indicated in Table S7.
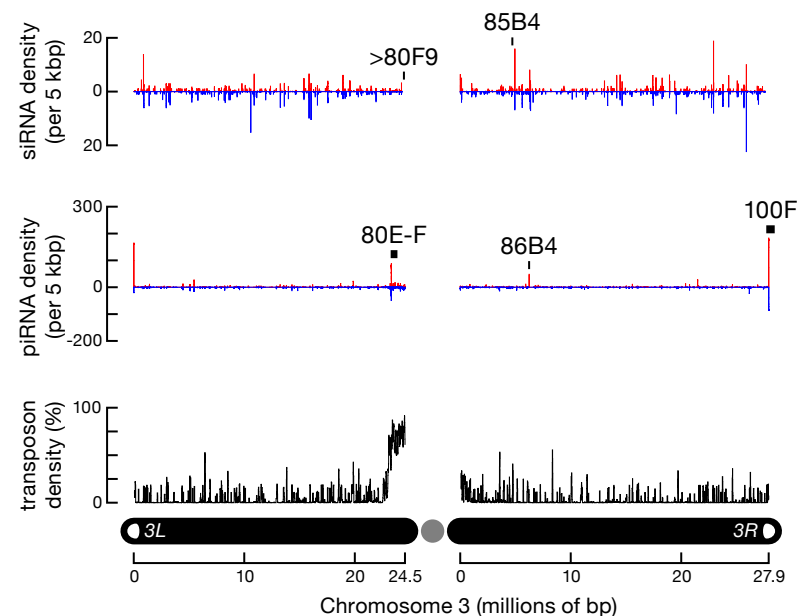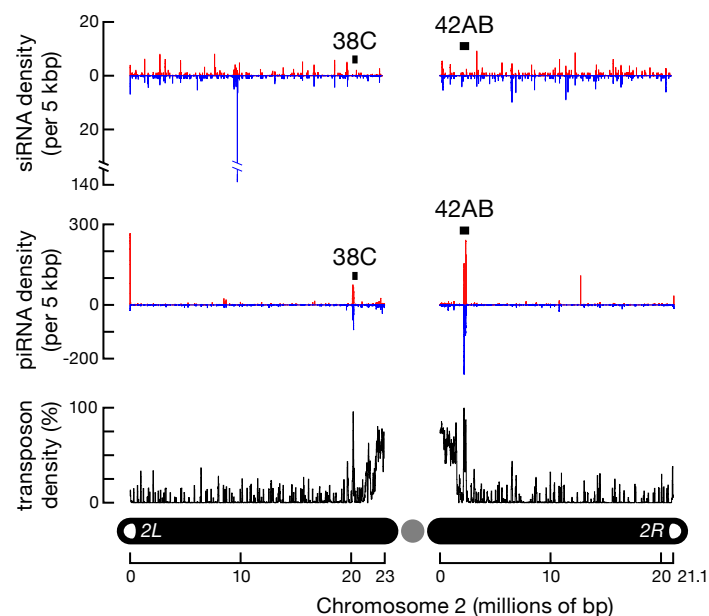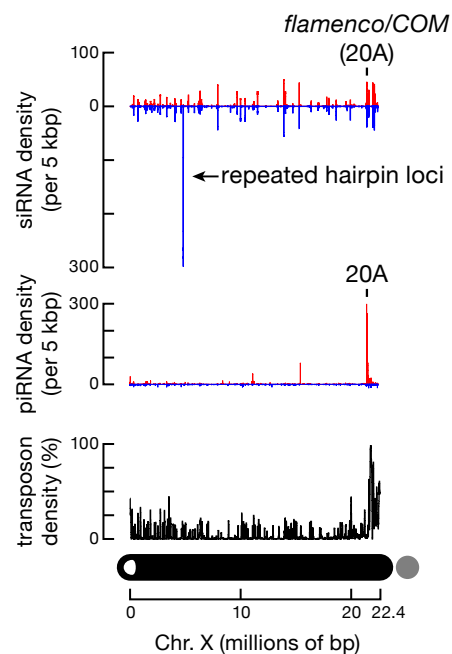
Figure S4. Endogenous siRNAs from cultured S2 cells. Small RNAs mapping to more than one genomic location were attributed to each site to which they were complementary, but normalized for the number of sites. piRNA data are from Brennecke et al. (*Cell* 2007).
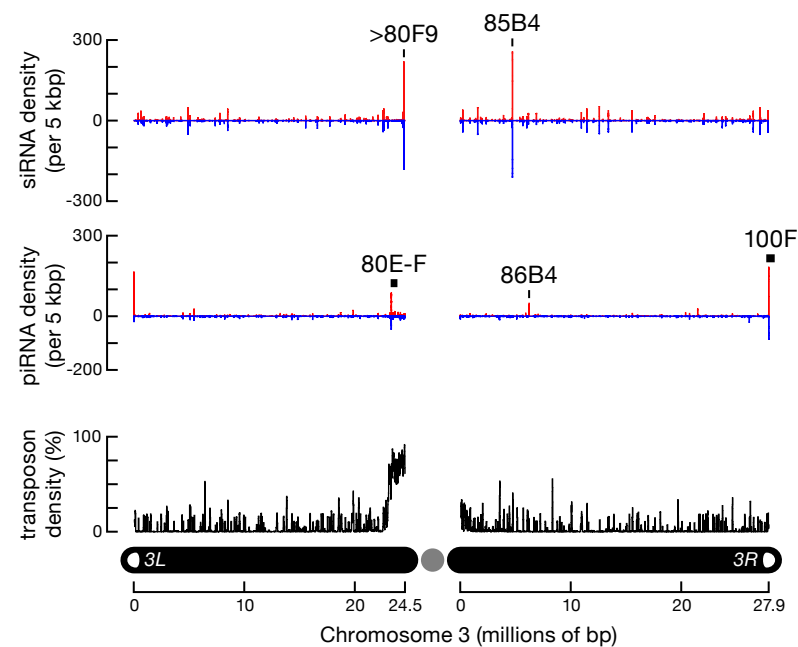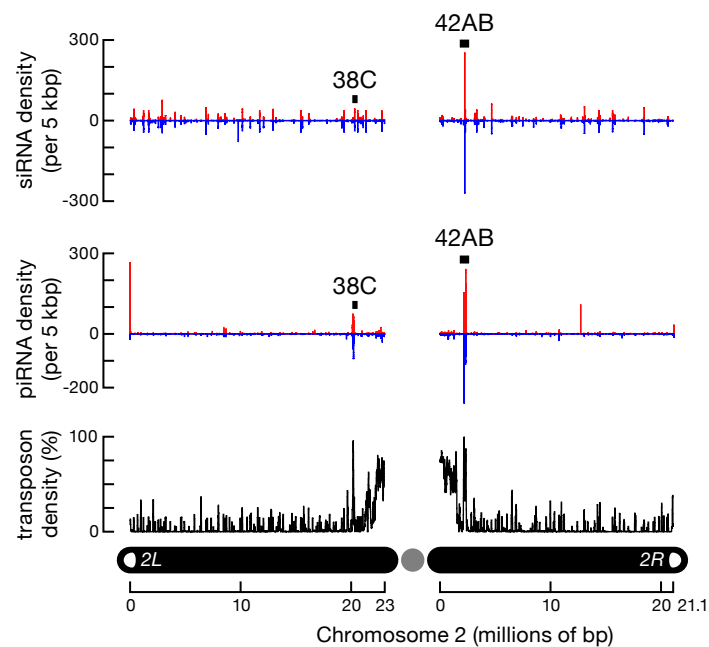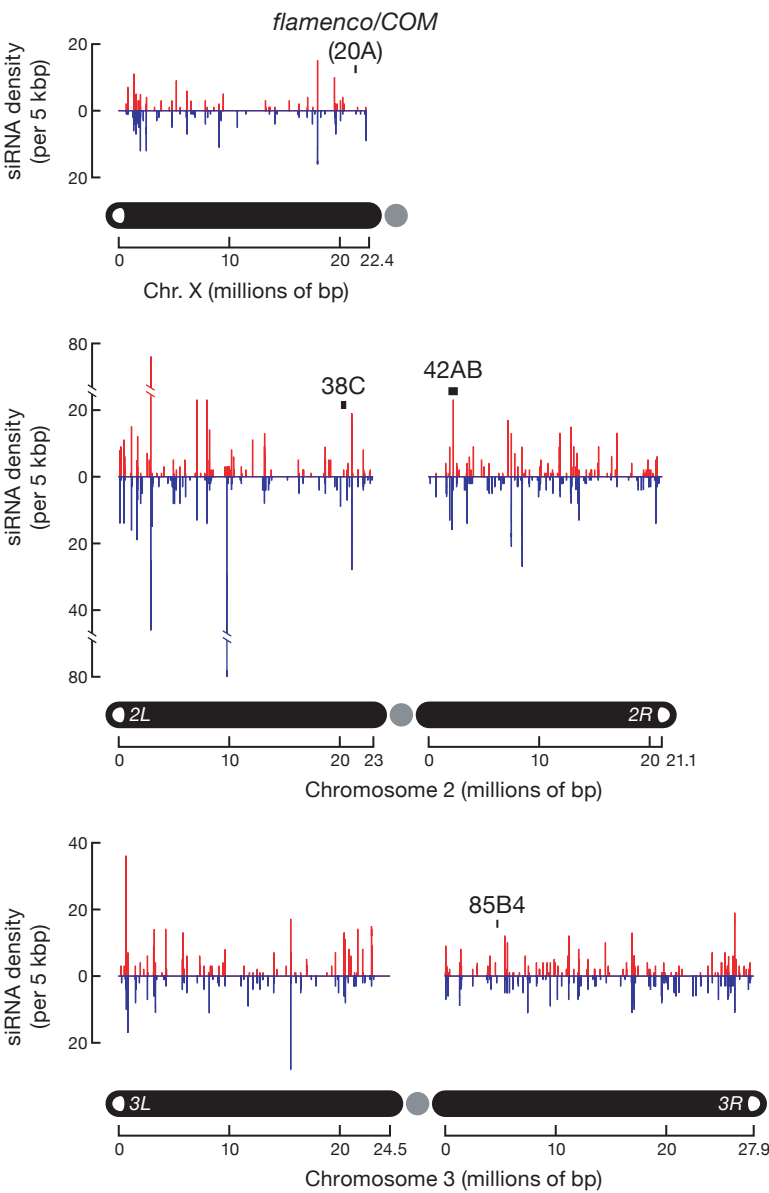
Figure S5. Uniquely mapping endogenous siRNAs from cultured S2 cells.

🟥	83% antisense
🟧	
🟧	75% antisense
🟨	
🟨	50% each
🟩	
🟩	75% sense
🟩	
🟩	83% sense

|	
🟩	*1360*
🟨	1731
🟧	17.6
🟨	297
🟨	*3S18*
🟨	*blood*
🟨	*Burdock*
🟩	*copia*
🟩	Cr1a
🟨	*diver*
🟩	*diver2*
🟨	*Dm88*
🟨	Doc
🟧	F
🟨	flea
🟩	*gypsy12*
🟩	*gypsy2*
🟩	*gypsy8*
🟨	HB
🟨	*INE−1*
🟨	*invader1*
🟩	Ivk
🟨	*jockey*
🟨	*Juan*
🟥	*McClintock*
🟨	*mdg1*
🟧	*mdg3*
🟨	*micropia*
🟧	NOF
🟩	*Quasimodo*
🟨	roo
🟧	rooA
🟨	Rt1b
🟨	S
🟧	*Stalker*
🟧	*Stalker2*
🟥	*Stalker4*
🟨	*Tirant*
🟨	*transib1*
🟧	*transib3*
🟨	*Transpac*

Figure S6. In cultured S2 cells, transposon-derived siRNAs generally mapped about equally to sense and antisense orientations. Only transposons with more than fifty 21-nt reads were analyzed.

Figure S7. Transposon-matching siRNAs, but not miRNAs, are significantly changed in heads from *dcr-2*[L811fsX] homozygous flies, compared to their heterozygous siblings (*dcr-2*[L811fsX]/CyO). (A) Box plots for the ratio of reads for all miRNAs and transposon-matching siRNAs, normalized to sequencing depth, for the two genotypes. Only miRNAs whose sequence was read ≥ 100 times in at least one of the two genotypes were evaluated. Because miRBase does not always report the most abundant isoform of each miRNA, up to 9 nts were tolerated between the termini of each observed miRNA read and the miRBase entry, provided the miRNA matched the pre-miRNA perfectly. *p*-value calculated using Wilcoxon test. (B) The fold decrease for transposon-derived siRNAs for which ≥ 20 reads were detected in *dcr-2*[L811fsX]/CyO. The changes of all transposons were statistically significant (*p*-value < 0.029, Fisher's exact test); the *p*-values for the change in individual miRNA and siRNA abundance are listed in Tables S4 and S5.

Figure S8. The change (mean ± SD, *N* = 3) for transposon RNA expression in S2 cells was measured for cells treated twice with dsRNA to deplete Ago2 by RNAi, relative to a control dsRNA. Collectively, the transposons in this panel comprise > 88% of the transposon-derived siRNAs in S2 cells. Red, LTR retrotransposons; green, non-LTR retrotransposons, blue, DNA transposons.

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Table S2. Endogenous siRNAs map to transposons. Percentages total more than 100, because some siRNAs map to more than one transposon. Red, LTR retrotransposons; green, non-LTR retrotransposons, blue, DNA transposons.

| Transposon	S2 cells (36,958 reads excluding pre-miRNA matching)				Total number of siRNAs
	Sense siRNAs		Antisense siRNAs		
	Number of siRNAs	% of total siRNAs	Number of siRNAs	% of total siRNAs	
297	10,918	29.54	10,833	29.31	21,751
1731	7,887	21.34	6,490	17.56	14,377
mdg1	4,565	12.35	5,156	13.95	7,968
roo	3,101	8.39	4,023	10.89	6,745
Doc	1,794	4.85	1,999	5.41	3,793
blood	1,810	4.90	1,952	5.28	3,762
INE-1	1,194	3.23	1,306	3.53	2,476
diver	1,037	2.81	1,126	3.05	2,163
mdg3	569	1.54	914	2.47	1,483
Cr1a	804	2.18	402	1.09	1,183
jockey	593	1.60	565	1.53	1,158
S	490	1.33	518	1.40	999
Juan	508	1.37	480	1.30	988
copia	615	1.66	246	0.67	861
Tirant	308	0.83	380	1.03	688
17.6	237	0.64	400	1.08	637
Quasimodo	383	1.04	236	0.64	597
3S18	264	0.71	245	0.66	509
transib1	242	0.65	256	0.69	498
F	202	0.55	283	0.77	403
Stalker2	293	0.79	313	0.85	332
gypsy12	200	0.54	125	0.34	325
micropia	161	0.44	163	0.44	324
HB	144	0.39	167	0.45	311
Dm88	142	0.38	142	0.38	284
Stalker4	29	0.08	151	0.41	180
Rt1b	90	0.24	84	0.23	171
flea	63	0.17	59	0.16	122
Transpac	59	0.16	48	0.13	107
Ivk	72	0.19	34	0.09	102
transib3	26	0.07	66	0.18	92
diver2	70	0.19	14	0.04	84
Burdock	45	0.12	35	0.09	80
rooA	24	0.06	50	0.14	74
gypsy2	48	0.13	25	0.07	73
invader1	73	0.20	73	0.20	73
Stalker	17	0.05	52	0.14	69
McClintock	1	0.00	62	0.17	63
NOF	14	0.04	48	0.13	62
gypsy8	44	0.12	13	0.04	57
1360	42	0.11	34	0.09	46
412	8	0.02	32	0.09	40
ninja-Dsim-like	19	0.05	21	0.06	40
jockey2	25	0.07	9	0.02	32
HMS-Beagle	24	0.06	5	0.01	29
Fw2	24	0.06	3	0.01	27
gypsy10	23	0.06	4	0.01	27
gypsy4	11	0.03	16	0.04	27
gypsy6	14	0.04	13	0.04	27

HeT-A	16	0.04	25	0.07	27
FB	23	0.06	10	0.03	23
gypsy	11	0.03	10	0.03	21
opus	21	0.06	17	0.05	21
G	18	0.05	2	0.01	20
G3	7	0.02	9	0.02	16
Rt1c	7	0.02	9	0.02	16
R1-element	2	0.01	12	0.03	14
Tabor	4	0.01	9	0.02	13
gypsy11	0	0.00	12	0.03	12
Fw3	9	0.02	7	0.02	11
Idefix	7	0.02	4	0.01	11
G4	4	0.01	6	0.02	10
Max	6	0.02	8	0.02	10
GATE	2	0.01	7	0.02	9
TART	7	0.02	1	0.00	8
baggins	7	0.02	0	0.00	7
G5A	2	0.01	4	0.01	6
S2	5	0.01	0	0.00	5
looper1	4	0.01	2	0.01	4
gypsy3	2	0.01	1	0.00	3
invader3	2	0.01	1	0.00	3
invader4	2	0.01	1	0.00	3
rover	0	0.00	3	0.01	3
springer	2	0.01	1	0.00	3
frogger	0	0.00	2	0.01	2
accord	1	0.00	0	0.00	1
I	0	0.00	1	0.00	1
invader2	0	0.00	1	0.00	1
pogo	1	0.00	0	0.00	1

intergenic	1,606	4.35	1,406	3.80	2,817
unannotated	N/A	N/A	N/A	N/A	1,715
mRNA not transposon	N/A	N/A	N/A	N/A	1,261
mRNA & transposon	3,247	8.79	3,021	8.17	4,597

Fly Heads (5,600 reads excluding pre-miRNA matching)

Transposon	Sense siRNAs		Antisense siRNAs		Total number of siRNAs
	Number of siRNAs	% of total siRNAs	Number of siRNAs	% of total siRNAs	
mdg1	533	10.13	540	10.26	720
roo	350	6.65	338	6.42	571
297	185	3.52	189	3.59	374
jockey	72	1.37	112	2.13	184
F	92	1.75	89	1.69	137
Cr1a	64	1.22	58	1.10	119
INE-1	73	1.39	40	0.76	110
Stalker2	65	1.24	61	1.16	100
gypsy12	47	0.89	53	1.01	99
Doc	49	0.93	37	0.70	86
HB	41	0.78	44	0.84	85
Ivk	39	0.74	61	1.16	81
Rt1b	34	0.65	45	0.86	78
Stalker4	40	0.76	31	0.59	71
opus	59	1.12	61	1.16	65
diver2	25	0.48	35	0.67	60
transib3	37	0.70	16	0.30	53
gypsy2	26	0.49	20	0.38	45
blood	17	0.32	26	0.49	43
invader1	41	0.78	41	0.78	43
gypsy6	21	0.40	16	0.30	37
gypsy	19	0.36	16	0.30	35
rooA	9	0.17	23	0.44	32
FB	30	0.57	29	0.55	30
accord2	17	0.32	12	0.23	29
jockey2	20	0.38	10	0.19	26
Stalker	5	0.10	19	0.36	24
NOF	5	0.10	18	0.34	23
gypsy8	14	0.27	4	0.08	18
1360 (hoppel)	7	0.13	13	0.25	16
Max	7	0.13	10	0.19	16
412	3	0.06	11	0.21	14
GATE	7	0.13	7	0.13	14
gypsy3	6	0.11	8	0.15	14
springer	6	0.11	8	0.15	14
Burdock	5	0.10	7	0.13	12
invader3	6	0.11	6	0.11	12
gypsy4	3	0.06	8	0.15	11
Quasimodo	6	0.11	5	0.10	10
R1	5	0.10	5	0.10	10
17.6	6	0.11	3	0.06	9
gypsy10	2	0.04	6	0.11	8
R1-element	3	0.06	5	0.10	8
HMS-Beagle	3	0.06	4	0.08	7
X	7	0.13	5	0.10	7
HeT-A	5	0.10	2	0.04	5
mdg3	2	0.04	3	0.06	5
S	1	0.02	5	0.10	5
copia	3	0.06	1	0.02	4
Tabor	3	0.06	1	0.02	4
Dm88	1	0.02	2	0.04	3
HMS-Beagle2	1	0.02	2	0.04	3

I	2	0.04	1	0.02	3
rover	1	0.02	2	0.04	3
3S18	2	0.04	0	0.00	2
flea	1	0.02	1	0.02	2
G	1	0.02	1	0.02	2
BS	1	0.02	0	0.00	1
Circe	1	0.02	0	0.00	1
Doc2	1	0.02	0	0.00	1
Doc4	1	0.02	0	0.00	1
G2	1	0.02	0	0.00	1
G3	1	0.02	0	0.00	1
G6	1	0.02	0	0.00	1
hopper2	1	0.02	0	0.00	1
invader2	1	0.02	0	0.00	1
invader6	1	0.02	0	0.00	1
looper1	1	0.02	0	0.00	1
McClintock	1	0.02	0	0.00	1
micropia	1	0.02	0	0.00	1
Rt1a	1	0.02	0	0.00	1
Rt1c	1	0.02	0	0.00	1
transib4	1	0.02	0	0.00	1

intergenic	1,727	32.81	1,487	28.25	2,144
unannotated	N/A	N/A	N/A	N/A	1,779
mRNA not transposon	N/A	N/A	N/A	N/A	2,443
mRNA & transposon	2,006	38.12	1,768	33.59	2,441

Table S3A. Enodogenous siRNAs from S2 cells were clustered as described by Brennecke et al. (2007), using Drosophila *melanogaster* genome release R5.5 (http://flybase.bio.indiana.edu/).

Cluster ID	Chromosome	Start	End	Cluster length (kb)	Number of reads mapping uniquely to cluster	Number of reads mapping to cluster	piRNA cluster I.D. (Brennecke et al., 2007)	Cytogenetic location
S2 cells								
1	2L	9,782,623	9,795,136	13	517	517		30C9-30D1
2	3RHet	782,889	796,491	14	159	897		
3	2L	2,898,870	2,913,985	15	128	128		23C2
4	2L	22,752,903	22,780,367	27	65	619		
5	2L	1,655,404	1,717,432	62	50	4845		22A6-22B1
6	2L	21,079,751	21,094,168	14	50	50		39A1
7	3L	15,547,096	15,559,889	13	45	53		71E1
8	U	5,762,659	5,775,688	13	42	106	cluster #10	
9	2L	13,178,621	13,215,680	37	41	41		34A8-34A10
10	3L	645,955	657,148	11	40	40		61C8
11	2L	7,967,622	7,988,787	21	39	4930		28D3
12	2R	8,459,176	8,469,174	10	37	37		49B5-49B6
13	2L	7,073,818	7,084,359	11	36	36		27E1
14	2L	103,176	123,592	20	35	35		21B2
16	3L	3,192,342	3,242,225	50	35	35		63B11-63C1
15	2R	7,478,837	7,491,292	12	35	35		48A3
17	X	17,983,251	17,995,197	12	32	32		16F6
18	2L	447,944	482,135	34	31	31		21C2-21D1
19	2L	1,153,981	1,164,214	10	31	31		21F1
20	3R	26,182,009	26,195,088	13	30	30		99F1-99F2
21	U	9,199,049	9,230,523	31	29	2917		
22	3L	824,291	870,587	46	28	958		61D2
23	2R	2,229,785	2,243,731	14	27	15206	cluster #1	42A15-42A16
24	3R	19,551,888	19,606,927	55	26	26		95B1-95B5
25	3R	16,891,416	16,901,947	11	24	24		93B9-93B10
26	2L	8,195,702	8,225,201	30	23	23		28F1-28F4
27	2R	12,892,015	12,902,518	11	23	23		53E4
28	2R	1,897,692	1,907,690	10	22	22		42A7-42A8
29	3R	5,591,241	5,634,025	43	22	22		85E8-85E10
31	X	1,346,902	1,378,364	31	21	21		2A3-2B1

S2 cell siRNAs

30	3R	11,159,014	11,191,374	32	21	21		88F1
33	X	1,956,119	1,972,769	17	20	20		2C8-2C10
32	3L	22,860,546	22,871,373	11	20	20		80B1-80B2
34	2L	3,014,084	3,028,983	15	19	19		23C4
39	3R	12,063,559	12,099,214	36	19	19		89B9
35	2L	18,675,544	18,705,832	30	19	19		36F10-37A1
37	3L	20,378,235	20,389,706	11	19	19		77B9-77C1
38	3L	20,473,055	20,511,399	38	19	20		77C4-77C6
36	2R	20,554,111	20,563,855	10	19	19		60D13
40	3L	14,008,142	14,049,713	42	18	18		70C12-70D1
44	U	4,011,232	4,031,670	20	17	96	cluster #7	
43	3R	5,378,423	5,391,934	14	17	17		85D24-85D25
41	2R	7,162,904	7,177,380	14	17	17		47E5-47F1
42	2R	11,876,244	11,887,693	11	17	17		52D9-52D11
46	3R	27,620	57,331	30	16	621		81F6-82A1
48	X	2,491,408	2,503,866	12	16	16		3A6-3A7
45	2R	17,026,606	17,037,555	11	16	16		57C3-57C4
47	3R	17,091,519	17,102,609	11	16	16		93D2
49	3L	3,317,306	3,327,304	10	15	15		63D1
54	U	1,130,212	1,149,062	19	14	11543		
50	3L	4,246,406	4,256,404	10	14	14		64A11-64A12
51	3L	5,798,359	5,808,357	10	14	14		64E11-64E13
53	3R	17,042,277	17,052,592	10	14	14		93C6-93C7
52	3L	21,615,234	21,625,232	10	14	14		78E1
56	3L	2,592,932	2,603,319	10	13	13		62E7
55	2L	4,914,510	4,954,666	40	13	671		25B3-25B4
59	X	6,179,215	6,191,365	12	13	13		5E5-5E6
60	X	9,084,020	9,094,018	10	13	13		8C17-8D1
57	3R	9,500,740	9,510,738	10	13	13		87F7-87F10
58	3R	25,568,106	25,586,472	18	13	14		99B9-99B10
63	3L	1,502,401	1,521,931	20	12	690		62A3
67	X	1,558,009	1,575,576	18	12	12		2B5-2B6
64	3L	9,619,817	9,631,371	12	12	12		67C2-67C3
66	U	9,763,582	9,778,881	15	12	4825		
61	2L	10,200,298	10,210,296	10	12	12		31B1
62	2L	22,127,508	22,137,506	10	12	16		40E4-40E5
65	3R	25,621,570	25,634,567	13	12	12		99C1-99C2
72	3L Het	780,345	791,575	11	11	3535		
68	2L	5,520,595	5,530,593	10	11	11		25E5
70	3L	6,164,197	6,176,924	13	11	11		65A7

S2 cell siRNAs

73	3R	7,458,172	7,468,170	10	11	11		86E13
71	3L	8,184,373	8,194,371	10	11	11		66B11-66B12
69	2L	12,103,912	12,113,910	10	11	11		33C4
74	3R	12,908,322	12,924,213	16	11	11		89E12-89E13
75	3L	686,750	696,889	10	10	10		61C8
78	3R	1,293,230	1,303,419	10	10	10		83A4-83A5
79	3R	14,481,371	14,491,369	10	10	10		91B8
81	X	19,512,463	19,522,461	10	10	10		18D7-18D8
76	3L	19,596,487	19,606,485	10	10	10		76B9
77	3L	22,056,871	22,066,869	10	10	10		79B2
82	X	22,345,871	22,392,860	47	10	1258		20F2-20F3
80	3R	25,303,931	25,313,929	10	10	10		99B1
83	2L	192,335	202,333	10	9	9		21B4
96	3L Het	248,455	258,453	10	9	249	cluster #15	
84	2L	1,975,628	1,985,626	10	9	9		22B8
91	3L	3,147,736	3,157,734	10	9	9		63B6-63B7
87	2R	4,046,783	4,056,781	10	9	9		44B5-44B8
98	X	5,207,694	5,217,692	10	9	12		4F4-4F5
92	3L	5,748,568	5,758,566	10	9	9		64E5-64E6
93	3L	11,698,935	11,708,933	10	9	9		68D2-68D3
88	2R	11,817,707	11,827,705	10	9	9		52D2-52D3
89	2R	13,120,411	13,131,234	11	9	1004		54B1
90	2R	15,369,295	15,379,293	10	9	9		56D11-56D13
94	3L	15,598,077	15,608,075	10	9	9		71E2-71E3
85	2L	20,059,341	20,069,339	10	9	9		38B1-38B2
86	2L	20,652,091	20,662,089	10	9	9		38D2-38D3
95	3L	22,933,272	22,943,270	10	9	9		80C1
97	3R	26,028,924	26,038,922	10	9	9		99E2
109	X	831,709	841,707	10	8	8		1D2-1D3
108	4	1,218,726	1,228,724	10	8	8		102F8
103	3R	1,403,421	1,413,419	10	8	8		83B2-83B3
110	X	1,809,962	1,819,960	10	8	8		2B15-2B16
100	2R	3,685,613	3,693,468	8	8	8		43E17-43E18
111	X	4,810,933	4,826,291	15	8	7978		4D5-4D7
99	2L	5,041,556	5,051,554	10	8	8		25C1-25C3
104	3R	5,508,105	5,524,570	16	8	22		85E4
101	2R	7,780,030	7,790,028	10	8	8		48C5
105	3R	12,008,921	12,018,919	10	8	8		89B7
106	3R	16,927,742	16,937,740	10	8	8		93B12-93B13
102	3L	20,821,682	20,831,680	10	8	8		77F1

107	3R	21,149,905	21,159,903	10	8	98		96D1
112	2L	146,778	156,776	10	7	7		21B3
113	2L	2,560,743	2,586,937	26	7	4649		22F4-23A1
116	3R	4,058,120	4,068,025	10	7	7		84F4-84F5
115	3L	7,708,825	7,718,481	10	7	7		66A10
120	X	7,838,431	7,844,562	6	7	7		7C9-7D1
117	3R	10,142,716	10,152,714	10	7	13		88B3-88B4
114	2R	13,424,091	13,434,089	10	7	7		54C3
121	X	19,632,553	19,642,551	10	7	10		18E5-18F1
118	3R	24,141,329	24,151,327	10	7	7		98C3
119	3R	24,710,511	24,720,509	10	7	7		98F1-98F2
135	3R	229,824	261,348	32	6	983		82A6-82B1
122	2L	542,059	552,057	10	6	6		21E2
134	3L Het	563,098	600,561	37	6	879		
127	2R	666,812	675,630	9	6	6		41C2
136	3R	1,459,740	1,467,999	8	6	6		83B7
123	2L	4,986,739	4,996,737	10	6	6		25B9-25B10
137	3R	5,805,160	5,815,158	10	6	6		85F4
124	2L	6,043,300	6,053,298	10	6	6		26B3
138	3R	7,231,157	7,241,155	10	6	6		86E4
130	3L	7,316,278	7,325,381	9	6	6		65F4
131	3L	9,078,428	9,086,564	8	6	6		66F5
125	2L	10,389,264	10,399,262	10	6	6		31D11-31E1
139	3R	11,092,595	11,099,452	7	6	6		88E9-88E10
126	2L	16,307,231	16,317,229	10	6	6		35F1
132	3L	16,450,201	16,460,199	10	6	6		73A1
128	2R	16,549,517	16,559,515	10	6	6		57A9-57A10
140	3R	19,016,516	19,022,739	6	6	6		94E5-94E6
145	X	20,061,920	20,069,591	8	6	6		19C1
129	2R	20,663,857	20,673,855	10	6	6		60E1
141	3R	20,869,191	20,879,189	10	6	6		96B17-96B19
133	3L	20,986,085	21,020,979	35	6	969		78A2
142	3R	22,405,317	22,415,315	10	6	6		97C1
143	3R	22,687,952	22,697,947	10	6	6		97D3
144	3R	27,568,150	27,577,970	10	6	6		100D2
173	3R Het	31,087	74,992	44	5	10922		
174	4	551,915	561,913	10	5	5		102C4
146	2L	2,132,930	2,142,928	10	5	5		22D1
161	3R	2,479,803	2,486,260	6	5	5		84A1
147	2L	2,764,639	2,783,911	19	5	4784		23A5-23A6

S2 cell siRNAs

154	2R	4,779,425	4,789,423	10	5	5		44F3
148	2L	5,986,382	5,996,380	10	5	5		26B2
162	3R	7,039,531	7,049,529	10	5	5		86D8
163	3R	8,839,679	8,847,105	7	5	5		87D8-87D9
175	X	9,452,424	9,462,422	10	5	5		8E7-8E10
164	3R	9,855,890	9,865,888	10	5	5		88A4
155	2R	10,145,197	10,155,195	10	5	5		5.00E+07
176	X	10,731,645	10,741,643	10	5	5		9F4-9F5
149	2L	11,092,188	11,100,393	8	5	5		32D2-32D3
157	3L	12,131,399	12,140,545	9	5	5		68F5-68F6
158	3L	12,759,827	12,768,208	8	5	5		69E2
165	3R	13,512,031	13,521,424	9	5	9		90C1
150	2L	16,249,303	16,259,301	10	5	5		35F1
159	3L	16,984,985	16,994,983	10	5	5		73E1-73E3
166	3R	18,407,620	18,413,515	6	5	5		94B5
167	3R	18,559,695	18,569,011	9	5	5		94C4
151	2L	19,000,410	19,010,408	10	5	5		37B9
152	2L	19,142,575	19,174,358	32	5	4890		37C1-37C6
156	2R	19,833,985	19,843,983	10	5	5		60A13
160	3L	19,874,250	19,884,248	10	5	5		76D3
177	X	20,257,850	20,267,821	10	5	5		19C5-19C6
168	3R	20,704,520	20,714,518	10	5	5		96B2-96B3
153	2L	21,660,567	21,669,076	9	5	5		39E3-39E6
169	3R	23,765,906	23,775,904	10	5	5		98B6
170	3R	25,507,490	25,515,528	8	5	5		99B7
171	3R	25,816,869	25,826,867	10	5	5		99D1
172	3R	26,303,191	26,319,848	17	5	128		99F6

Table S3B. siRNAs from fly heads were clustered as described by Brennecke et al. (2007), using *Drosophila melanogaster* genome release R5.5.

WT Heads								
Cluster ID	Chromosome	Start	End	Cluster length (kb)	Number of reads map uniquely to cluster	Number of reads mapping to cluster	piRNA cluster I.D. (Brennecke et al., 2007)	Cytogenetic location
1	2L	9783876	9795136	11.3	478	478		30C9-30D1
173	3L	886,261	896,260	10.0	14	14		61D3-61D4
174	2L	6,855	17,067	10.2	11	11		21A5
175	X	9,940,973	9,953,050	12.1	10	11		9A5-9B1
176	3R	113,708	123,706	10.0	9	9		82A1
49	3L	3,317,197	3,327,189	10.0	8	8		63D1
111	X	4,811,216	4,826,291	15.1	8	1,771		4D5-4D7
177	2L	7,706,540	7,716,536	10.0	8	8		28C1
7	3L	15,549,041	15,558,952	9.9	8	13		71E1
178	2L	16,784,804	16,794,788	10.0	8	8		36B1
179	3R	6,665	15,118	8.5	7	8		81F6
180	2R	14,267,508	14,277,167	9.7	6	6		55C4
181	X	21,604,591	21,614,589	10.0	6	72	cluster #8	20B1
80	3R	25,305,992	25,315,848	9.9	6	6		99B1
182	3R	1,048,181	1,058,035	9.9	5	5		82F6
183	2L	9,817,453	9,827,451	10.0	5	156		30D1
184	3L	10,687,581	10,697,585	10.0	5	926		67E7

Table S3C. piRNA data from Brennecke et al. (2007) were clustered according using *Drosophila melanogaster* genome

				piRNAs (from Brennecke et al., 2007)		
Chromosome	**Start**	**End**	**Cluster length (kb)**	**Number of reads mapping uniquely to cluster**	**Number of reads mapping to cluster**	**Cytogenetic location**
2R	2,140,512	2,389,335	249	1,460	19,441	42A14-42B1
X	21,388,081	21,432,231	44	994	7,351	20A1-20A3
2L	20,143,634	20,232,517	89	445	2,540	38C2-38C3
3L	23,269,813	23,313,601	44	224	1,169	80E3-80F1
4	1,255,371	1,351,506	96	202	5,079	102F8
U	4,010,984	4,077,966	67	162	822	
X	21,501,319	21,548,357	47	122	2,827	20A5-20B1
U	5,743,150	5,797,646	54	115	3,694	
2R	12,713,990	12,723,988	10	109	109	53D11-53D12
X	15,398,513	15,408,511	10	80	80	13C5-13C7
3LHet	2,008,276	2,212,278	204	70	15,385	
3RHet	2,070,375	2,106,781	36	67	1,066	
3LHet	237,482	330,926	93	61	3,703	
U	7,497,140	7,584,470	87	61	8,578	
3R	6,228,871	6,238,915	10	46	46	86B4
X	21,756,108	21,841,785	86	43	3,377	20B3-20C1
4	807,233	867,379	60	41	464	102E1-102E3
2L	20,100,366	20,123,183	23	40	261	38C1-38C2
2L	22,342,790	22,421,219	78	35	3,508	40F7
2L	1	11,667	12	33	20,448	21A5
3LHet	148,660	204,731	56	33	1,701	
3L	24,088,523	24,134,591	46	33	2,113	
3RHet	2,309,480	2,373,211	64	32	2,425	
X	2,061	26,029	24	31	387	1A1

X	11,076,431	11,099,456	23	31	215	10A10-10B1
X	21,580,417	21,687,831	107	31	2,671	20B1
3L	23,449,678	23,478,214	29	30	601	80F6-80F7
3LHet	493,948	685,925	192	29	10,795	
3R	21,467,283	21,482,178	15	29	29	96E6-96E7
2LHet	121,252	266,568	145	27	6,692	
3LHet	285	32,970	33	26	1,164	
2R	742,942	782,203	39	26	2,423	41C4-41C5
U	2,433,298	2,478,920	46	26	1,155	
2L	22,945,885	22,989,803	44	26	1,179	
3L	23,940,894	24,045,838	105	26	6,737	
3R	1,279	23,416	22	25	58	81F6
3RHet	1,607,736	1,674,464	67	25	2,263	
2RHet	1,857,936	1,913,095	55	24	966	
3L	19,845,140	19,864,685	20	23	1,530	76D1-76D3
3RHet	104,786	191,198	86	21	2,505	
3LHet	1,402,112	1,458,965	57	21	1,332	
2L	22,486,772	22,547,558	61	21	3,167	40F7
3L	24,465,528	24,543,475	78	21	1,399	
3RHet	617,618	656,530	39	20	1,197	
2RHet	1,412,742	1,489,780	77	20	824	
3RHet	1,746,563	1,797,611	51	20	3,429	
3RHet	532,053	575,335	43	19	1,169	
3RHet	849,568	921,355	72	19	1,662	
U	2,056,878	2,098,213	41	19	2,277	
U	889,267	1,061,441	172	18	6,217	
3RHet	1,111,034	1,223,916	113	18	3,403	
2R	1,253,143	1,284,240	31	18	1,040	41E5-41E6
2L	19,564,519	19,574,923	10	17	32	37F1-37F2
2L	22,254,319	22,281,479	27	17	701	40F7
3L	23,612,866	23,636,896	24	17	752	80F9

X	8,368,544	8,381,781	13	16	16	7F1
3LHet	770,628	819,852	49	15	2,636	
2R	16,466,415	16,476,583	10	15	15	57A6
3LHet	840,924	895,679	55	14	2,243	
3RHet	1,383,668	1,470,543	87	14	2,624	
3LHet	1,479,139	1,528,684	50	14	4,505	
2R	7,777,083	7,787,544	10	14	14	48C5
2L	8,450,213	8,490,832	41	14	2,178	29C5-29D1
2L	16,693,456	16,703,757	10	14	14	36A10-36A11
X	22,369,187	22,403,875	35	14	1,688	20F3
2R	109,239	149,540	40	13	884	
U	141,712	210,336	69	13	2,023	
3LHet	362,237	394,074	32	13	326	
2R	1,216,294	1,227,635	11	13	39	41E5
3LHet	1,844,970	1,901,261	56	13	2,843	
3L	24,350,206	24,375,909	26	13	819	
3R	27,892,332	27,909,797	17	13	11,215	10E4
U	40,427	117,442	77	12	4,287	
4	1,015,921	1,026,279	10	12	55	102F5
2RHet	2,204,696	2,287,166	82	12	1,695	
2RHet	2,788,079	2,857,172	69	12	2,226	
U	5,625,604	5,649,537	24	12	476	
2L	5,954,935	5,984,574	30	12	12	26A3-26B2
2R	3,316,801	3,331,740	15	11	96	43C1
U	3,519,704	3,551,702	32	11	768	
3R	5,921,675	5,931,673	10	11	11	85F10-85F11
U	9,170,572	9,298,799	128	11	7,794	
2RHet	1,679,952	1,715,467	36	10	730	
2R	9,211,947	9,221,945	10	10	10	50A1-50A3
X	10,164,447	10,174,445	10	10	1,391	9B5-9B6
2R	185,439	225,778	40	9	1,909	

2R	845,724	885,372	40	9	1,552	41C6
2RHet	867,578	909,826	42	9	3,623	
U	5,446,117	5,477,034	31	9	2,751	
2L	7,420,980	7,430,978	10	9	9	27F3-27F4
2R	21,136,534	21,151,342	15	9	1,898	60F5
2L	21,891,204	21,901,202	10	9	9	40B3
3L	24,309,487	24,328,647	19	9	415	
3RHet	9,020	19,018	10	8	71	
3LHet	2,376,347	2,446,273	70	8	2,166	
2RHet	2,878,674	2,939,749	61	8	2,021	
2RHet	2,988,025	3,049,062	61	8	1,036	
U	3,876,652	3,943,760	67	8	1,823	
2L	20,631,611	20,640,251	9	8	18	38D1
XHet	169,257	192,176	23	7	919	
2LHet	302,772	369,442	67	7	3,621	
U	339,589	384,771	45	7	1,130	
3LHet	714,299	741,348	27	7	702	
X	1,371,374	1,381,268	10	7	7	2B1
X	4,017,313	4,027,311	10	7	12	4B1
X	5,201,679	5,211,408	10	7	7	4F4-4F5
U	6,643,127	6,660,684	18	7	1,684	
3R	7,044,221	7,053,379	9	7	7	86D8
3L	8,716,961	8,726,803	10	7	7	66D12
2L	9,891,561	9,901,336	10	7	7	30E1
X	12,660,975	12,670,600	10	7	116	11B16-11C1
X	22,096,745	22,116,991	20	7	129	20D2
3R	27,415,958	27,425,954	10	7	7	100C7
2R	410,424	423,625	13	6	536	41A2
X	652,829	662,184	9	6	6	1C4
4	985,305	995,476	10	6	37	102F4
3LHet	989,120	1,049,237	60	6	4,049	

3RHet	1,252,983	1,338,766	86	6	2,064	
U	1,379,079	1,450,515	71	6	3,660	
2RHet	1,597,675	1,641,429	44	6	2,648	
U	1,962,353	1,972,720	10	6	90	
U	3,109,090	3,139,633	31	6	1,015	
U	5,852,441	6,059,636	207	6	14,423	
U	7,836,590	7,882,892	46	6	6,453	
2L	17,968,726	17,978,719	10	6	6	36E3
3L	23,187,909	23,220,679	33	6	562	80D5-80E1
2RHet	483,216	517,466	34	5	258	
2R	514,257	524,320	10	5	202	41B2
4	609,263	619,456	10	5	1,194	102C6-102D1
3L	825,510	833,058	8	5	5	61D2
2RHet	1,087,648	1,133,687	46	5	1,120	
2RHet	1,339,078	1,389,974	51	5	3,038	
3RHet	2,482,651	2,492,649	10	5	62	
3R	2,909,142	2,918,466	9	5	6	84B2-84B6
X	3,435,144	3,445,036	10	5	7	3D5
U	6,191,840	6,261,703	70	5	6,969	
U	7,020,670	7,055,172	35	5	4,662	
2L	7,825,754	7,830,915	5	5	5	28D1-28D2
3L	10,353,382	10,363,380	10	5	5	67E1-67E2
X	11,787,892	11,794,120	6	5	5	10F4
2L	13,405,034	13,416,223	11	5	646	34B10-34B11
X	19,487,663	19,497,511	10	5	5	18D3
X	21,183,210	21,188,753	6	5	12	19F3-19F4
3L	24,169,238	24,179,236	10	5	95	
3L	24,220,571	24,229,777	9	5	17	

Table S4. Endogenous siRNAs matching transposons are depleted in *dcr-2* null mutant fly heads. Percentages total more than 100, because some siRNAs map to more than one transposon. Red, LTR retrotransposons; green, non-LTR retrotransposons, blue, DNA transposons. "Fold decrease" was calculated by normalizing the total number of siRNAs matching the transposon in each genotype to the total number of 18–29 nt RNA reads, excluding pre-miRNA-matching reads, a measure of the small RNA sequencing depth. "Fold decrease*" was calculated by normalizing to the total number of small RNA reads, including pre-miRNA-matching reads. Some siRNAs match more than one transposon, so the sum of the total number of siRNAs for each transposon is greater than the actual number so 21 nt small RNA reads: 2,524 for *dcr-2*/CyO and 263 for *dcr-2* homozygotes. *p*-value was calculated using Fisher's exact test.

	dcr-2^L811fsX/CyO (25,822 reads, excluding pre-miRNA-matching reads)					dcr-2^L811fsX (16,917 reads, excluding pre-miRNA-matching reads)							
	Sense siRNAs		Antisense siRNAs		Total number of siRNAs		Sense siRNAs		Antisense siRNAs		Total number of siRNAs	fold decrease	*p*-value	fold decrease*
Transposon	Number of siRNAs	% of total siRNAs	Number of siRNAs	% of total siRNAs		Transposon	Number of siRNAs	% of total siRNAs	Number of siRNAs	% of total siRNAs				
roo	802	71%	897	80%	1,126	roo	56	64%	64	74%	87	8.5	0.000	10.4
jockey	351	47%	400	53%	751	jockey	30	63%	18	38%	48	10.3	0.000	12.5
mdg1	297	43%	396	58%	687	mdg1	35	60%	27	47%	58	7.8	0.000	9.5
297	288	45%	354	55%	642	297	43	55%	35	45%	78	5.4	0.000	6.6
Cr1a	295	60%	202	41%	490	Cr1a	26	58%	19	42%	45	7.1	0.000	8.7
blood	202	42%	281	58%	483	blood	20	54%	17	46%	37	8.6	0.000	10.5
HB	180	40%	270	60%	450	HB	11	44%	14	56%	25	11.8	0.000	14.4
springer	189	42%	256	58%	445	springer	16	64%	9	36%	25	11.7	0.000	14.3
gypsy3	162	42%	221	58%	383	gypsy3	14	64%	8	36%	22	11.4	0.000	13.9
invader3	162	42%	221	58%	383	invader3	14	64%	8	36%	22	11.4	0.000	13.9
Stalker4	118	32%	246	68%	364	Stalker4	11	37%	19	63%	30	7.9	0.000	9.7
Stalker2	228	68%	265	79%	337	Stalker2	7	58%	11	92%	12	18.4	0.000	22.5
F	115	43%	181	68%	268	F	7	41%	13	76%	17	10.3	0.000	12.6
Doc	85	33%	170	66%	258	Doc	6	21%	21	75%	28	6.0	0.000	7.4
gypsy12	156	62%	97	38%	253	gypsy12	8	73%	3	27%	11	15.1	0.000	18.4
Ivk	186	74%	118	47%	251	Ivk	14	88%	3	19%	16	10.3	0.000	12.6
INE-1	78	38%	133	65%	204	INE-1	5	25%	16	80%	20	6.7	0.000	8.2
Stalker	61	32%	128	68%	189	Stalker	3	43%	4	57%	7	17.7	0.000	21.6
transib3	57	31%	125	69%	182	transib3	2	13%	13	87%	15	7.9	0.000	9.7
rooA	59	33%	122	67%	181	rooA	2	25%	6	75%	8	14.8	0.000	18.1
NOF	58	33%	116	66%	176	NOF	2	33%	4	67%	6	19.2	0.000	23.5
Rt1b	56	42%	77	58%	133	Rt1b	4	31%	9	69%	13	6.7	0.000	8.2
FB	105	81%	112	87%	129	FB	2	50%	3	75%	4	21.1	0.000	25.8
412	39	37%	66	63%	105	412	0	0%	1	100%	1	68.8	0.000	84.1
jockey2	69	68%	36	35%	102	jockey2	2	67%	0	0%	3	22.3	0.000	27.2
gypsy8	44	67%	22	33%	66	gypsy8	0	0%	1	100%	1	43.2	0.000	52.9
opus	39	89%	39	89%	44	opus	4	57%	7	100%	7	4.1	0.000	5.0
diver2	24	57%	18	43%	42	diver2	3	33%	6	67%	9	3.1	0.001	3.7
invader1	31	82%	32	84%	38	invader1	2	100%	2	100%	2	12.4	0.000	15.2
Burdock	13	37%	22	63%	35	Burdock	5	100%	0	0%	5	4.6	0.000	5.6
1360	25	76%	17	52%	33	1360	7	70%	4	40%	10	2.2	0.029	2.6
Quasimodo	20	61%	17	52%	33	Quasimodo	5	50%	5	50%	10	2.2	0.029	2.6
gypsy	13	42%	18	58%	31	gypsy	2	50%	2	50%	4	5.1	0.000	6.2
HMS-Beagle	7	24%	22	76%	29	HMS-Beagle	3	43%	4	57%	7	2.7	0.016	3.3
gypsy2	16	67%	8	33%	24	gypsy2	1	33%	2	67%	3	5.2	0.002	6.4
gypsy6	11	46%	13	54%	24	gypsy6	0	0%	2	100%	2	7.9	0.000	9.6
Transpac	12	55%	10	45%	22	Transpac	5	100%	0	0%	5	2.9	0.029	3.5
gypsy4	7	33%	14	67%	21									
17.6	4	20%	16	80%	20	17.6	1	25%	3	75%	4	3.3	0.022	4.0
GATE	8	47%	12	71%	17	GATE	2	50%	2	50%	4	2.8	0.072	3.4

Name	n	%	n	%	Total	Name	n	%	n	%	Total			
gypsy10	5	29%	12	71%	17	gypsy10	2	50%	2	50%	4	**2.8**	0.072	**3.4**
mdg3	5	29%	12	71%	17	mdg3	1	8%	12	92%	13	**0.9**	0.711	**1.0**
Dm88	5	31%	11	69%	16	Dm88	1	25%	3	75%	4	**2.6**	0.107	**3.2**
S	7	50%	11	79%	14	S	4	57%	3	43%	7	**1.3**	0.659	**1.6**
copia	9	69%	4	31%	13	copia	10	83%	2	17%	12	**0.7**	0.417	**0.9**
Max	8	62%	8	62%	13									
accord2	3	30%	7	70%	10	accord2	0	0%	1	100%	1	**6.6**	0.059	**8.0**
rover	4	40%	6	60%	10									
flea	6	67%	3	33%	9	flea	3	100%	0	0%	3	**2.0**	0.385	**2.4**
I	5	63%	3	38%	8									
HeT-A	4	67%	5	83%	6	HeT-A	2	40%	3	60%	5	**0.8**	0.762	**1.0**
R1-element	4	67%	2	33%	6	R1-element	3	50%	3	50%	6	**0.7**	0.558	**0.8**
X	5	83%	6	100%	6	X	1	100%	1	100%	1	**3.9**	0.256	**4.8**
baggins	1	20%	4	80%	5	baggins	0	0%	2	100%	2	**1.6**	0.711	**2.0**
G	2	40%	3	60%	5	G	1	17%	5	83%	6	**0.5**	0.362	**0.7**
ninja-Dsim-like	2	40%	3	60%	5									
1731	1	25%	3	75%	4	1731	9	56%	7	44%	16	**0.2**	0.000	**0.2**
Idefix	1	25%	3	75%	4	Idefix	0	0%	1	100%	1	**2.6**	0.654	**3.2**
Rt1a	0	0%	4	100%	4	Rt1a	0	0%	1	100%	1	**2.6**	0.654	**3.2**
Tabor	3	75%	1	25%	4	Tirant	3	75%	1	25%	4	**0.7**	0.720	**0.8**
frogger	1	33%	2	67%	3									
Juan	2	67%	1	33%	3	Juan	0	0%	4	100%	4	**0.5**	0.446	**0.6**
3S18	1	50%	1	50%	2									
Circe	1	50%	1	50%	2									
diver	1	50%	1	50%	2	diver	2	67%	1	33%	3	**0.4**	0.391	**0.5**
Fw2	2	100%	0	0%	2									
Fw3	1	50%	1	50%	2									
G3	1	50%	1	50%	2									
gypsy9	0	0%	2	100%	2									
hopper2	0	0%	2	100%	2	hopper2	0	0%	3	100%	3	**0.4**	0.391	**0.5**
invader4	1	50%	1	50%	2	invader4	0	0%	3	100%	3	**0.4**	0.391	**0.5**
micropia	0	0%	2	100%	2	micropia	0	0%	1	100%	1	**1.3**	1.000	**1.6**
Rt1c	1	50%	1	50%	2									
transib1	0	0%	2	100%	2	transib1	0	0%	2	100%	2	**0.7**	0.651	**0.8**
accord	0	0%	1	100%	1									
BS3	1	100%	0	0%	1									
G2	0	0%	1	100%	1	G2	0	0%	3	100%	3	**0.2**	0.308	**0.3**
G4	1	100%	0	0%	1									
G5	1	100%	0	0%	1									
G6	0	0%	1	100%	1									
HMS-Beagle2	0	0%	1	100%	1									
invader2	0	0%	1	100%	1	invader2	1	50%	1	50%	2	**0.3**	0.567	**0.4**
invader6	0	0%	1	100%	1	invader6	0	0%	2	100%	2	**0.3**	0.567	**0.4**
McClintock	0	0%	1	100%	1									
transib4	0	0%	1	100%	1	transib4	0	0%	2	100%	2	**0.3**	0.567	**0.4**
						pogo	1	100%	0	0%	1			
						S2	0	0%	1	100%	1			

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Table S5. The abundance of miRNA-matching reads was unchanged in *dcr-2*^{L811fsX} heads, compared to their heterozygous siblings. Fold change was calculated by normalizing the total number of miRNAs in each genotype to small RNA sequencing depth, i.e., the total number of 18–29 nt RNA reads (688,323 for *dcr-2* homozygotes; 859,436 for heterozygotes).

miRNA	Total number of reads		Fold change (homozygotes vs heterozygotes)		*p*-value
	dcr2^{L811fsX}/CyO	*dcr2*^{L811fsX}			
miR-14	172,360	101,066	0.73		0.000
miR-276a	141,107	99,817	0.88		0.000
miR-8	84,901	56,233	0.83		0.000
miR-317	47,027	41,865	1.11		0.000
miR-277	40,372	36,318	1.12		0.000
miR-34	34,350	59,032	2.15		0.000
miR-276b	21,520	13,092	0.76		0.000
bantam	17,977	13,336	0.93		0.000
miR-274	16,754	20,625	1.54		0.169
miR-210	16,142	18,754	1.45		1.2E-11
miR-1	14,885	13,926	1.17		8.9E-135
miR-133	12,532	9,555	0.95		1.6E-296
miR-999	12,065	8,549	0.88		0.000
miR-7	11,707	7,085	0.76		0.000
miR-184	11,679	15,992	1.71		1.8E-14
let-7	11,192	14,617	1.63		3.0E-04
miR-33	10,529	6,842	0.81		0.000
miR-9a	10,101	6,985	0.86		0.000
miR-125	9,397	8,268	1.10		8.1E-121
miR-278	6,942	7,849	1.41		1.5E-09
miR-11	6,562	4,849	0.92		1.5E-172
miR-284	5,486	4,089	0.93		2.2E-140
miR-252	5,188	3,911	0.94		8.9E-128
miR-124	4,181	7,615	2.27		6.2E-89
miR-305	3,398	5,428	1.99		5.4E-30
miR-279	3,395	3,441	1.27		6.1E-18
miR-285	3,198	1,781	0.70		3.5E-173
miR-13a	3,111	1,596	0.64		3.0E-196
miR-996	3,012	1,766	0.73		8.3E-147
miR-987	2,915	2,148	0.92		4.5E-78
miR-981	2,682	2,759	1.28		9.4E-13
miR-932	2,593	1,514	0.73		1.0E-127
miR-307	2,496	2,426	1.21		1.5E-18
miR-12	2,386	1,410	0.74		1.1E-114

miR-927	2,365	1,563	0.83	3.4E-87
miR-306	2,299	2,341	1.27	3.8E-12
miR-282	2,167	2,426	1.40	0.0002
miR-957	1,775	1,998	1.41	1.5E-03
miR-965	1,775	685	0.48	4.2E-170
miR-275	1,647	2,493	1.89	1.1E-09
miR-1000	1,493	1,841	1.54	0.727
miR-79	1,421	1,082	0.95	5.6E-35
miR-304	1,382	770	0.70	1.1E-75
miR-1010	1,300	899	0.86	3.6E-43
miR-263b	1,298	761	0.73	2.9E-64
miR-31a	1,227	1,303	1.33	4.8E-05
miR-970	1,188	1,338	1.41	0.0097
miR-219	980	946	1.21	1.8E-08
miR-1003	917	513	0.70	2.5E-50
miR-315	861	591	0.86	1.2E-29
miR-137	851	729	1.07	8.2E-14
miR-9b	844	473	0.70	2.1E-46
miR-1006	813	533	0.82	1.2E-31
miR-986	714	1,053	1.84	0.0006
miR-316	589	657	1.39	0.049
miR-995	570	690	1.51	0.590
miR-263a	562	872	1.94	5.9E-05
miR-1012	543	377	0.87	1.1E-18
miR-1001	531	392	0.92	2.2E-15
miR-998	526	513	1.22	7.2E-05
miR-1017	497	304	0.76	2.4E-23
miR-9c	478	588	1.54	0.829
miR-993	449	395	1.10	4.0E-07
miR-1009	414	234	0.71	3.2E-23
miR-980	336	276	1.03	2.7E-07
miR-929	335	287	1.07	2.7E-06
miR-190	319	518	2.03	0.0002
miR-2a-2	316	348	1.38	0.109
miR-87	266	139	0.65	1.0E-17
miR-1008	248	200	1.01	4.6E-06
miR-375	243	199	1.02	1.0E-05
miR-100	241	224	1.16	0.0015
miR-988	223	150	0.84	3.1E-09
miR-1004	182	135	0.93	4.2E-06
miR-308	166	281	2.11	0.0019
miR-193	116	126	1.36	0.301
miR-2b-2	87	102	1.46	0.714
miR-2b-1	86	90	1.31	0.255
miR-283	85	92	1.35	0.364
miR-2c	81	117	1.80	0.318
miR-1005	62	40	0.81	0.0013

miR-1007	62	51	1.03	0.029
miR-2a-1	60	63	1.31	0.365
miR-958	58	112	2.41	0.0068
miR-10	53	61	1.44	0.706
miR-971	48	56	1.46	0.768
miR-956	34	78	2.86	0.0030
miR-969	34	36	1.32	0.548
miR-311	30	11	0.46	0.0002
miR-314	21	38	2.26	0.191
miR-3	17	15	1.10	0.375
miR-954	17	27	1.98	0.453
miR-310	16	8	0.62	0.038
miR-312	16	13	1.01	0.266
miR-31b	13	17	1.63	1.000
miR-1016	11	16	1.82	0.847
miR-286	10	1	0.12	0.0035
miR-990	10	24	3.00	0.086
miR-318	7	6	1.07	0.582
miR-92b	7	19	3.39	0.078
miR-960	7	2	0.36	0.088
miR-982	7	5	0.89	0.391
miR-966	6	4	0.83	0.356
miR-991	6	2	0.42	0.151
miR-1013	5	6	1.50	1.000
miR-92a	5	5	1.25	0.759
miR-1011	4	4	1.25	1.000
miR-984	4	5	1.56	1.000
miR-309	3	3	1.25	1.000
miR-313	2	1	0.62	0.589
miR-976	2	4	2.50	0.699
miR-977	2	2	1.25	1.000
miR-303	1	6	7.49	0.141
miR-4	1	2	2.50	1.000
miR-959	1	1	1.25	1.000
miR-961	1	3	3.75	0.634
miR-964	1	2	2.50	1.000
miR-973	1	1	1.25	1.000
miR-989	1	2	2.50	1.000
miR-iab4as	1	4	4.99	0.390

Table S6. Probes and Primers

Primers for quantitative RT-PCR

Detects	Forward primer, reverse primer
Gypsy	CCAGGTCGGGCTGTTATAGG, GAACCGGTGTACTCAAGAGC
297	AAAGGGCGCTCATACAAATG, TGTGCACATAAAATGGTTCG
roo	CGTCTGCAATGTACTGGCTCT, CGGCACTCCACTAACTTCTCC
I-element	TGAAATACGGCATACTGCCCCCA, GCTGATAGGGAGTCGGAGCAGATA
mdg1	CACATGTTCTCATTCCCAACC, TTCGCTTTTTATATTTGCGCTAC
jockey	TGCAGTTGTTTCCCCTAACC, AGTTGGGCAAATGCTAGTGG
INE-1	GGCCATGTCCGTCTGTCC, AGCTAGTGTGAATGCGAACG
blood	TGCCACAGTACCTGATTTCG, GATTCGCCTTTTACGTTTGC
S-element	TGAAAAGCGTCATTCATTCG, TGTTTCTAGCGCACTCAACG
Doc	GGGTGACTATAACGCCAAGC, GCAAAATCGATCAGGTCTGG
1731	AGCAAACGTCTGTTGGAAGG, CGACAGCAAAACAACACTGC
F-element	GCTGGTAGATACCGCTGAGG, GTAGTCGTCCTCCGTTTTCG
412	CACCGGTTTGGTCGAAAG, GGACATGCCTGGTATTTTGG
NOF	AGTTGGACCTGGAATTGTGG, AATGCACACGGAAGAGGAAC
Idefix	AACAAAATCGTGGCAGGAAG, TCCATTTTTCGCGTTTACTG
Het-A	CGCGCGGAACCCATCTTCAGA, CGCCGCAGTCGTTTGGTGAGT
dcr-1	GCTAACGATGGCATCAATCTG, GCTTGGAGCGCAGGTGACTTA
dcr-2	GAGCTGCTCCATCAGTTTCA, TCCCAGTCAAAGCATTTCTGT
ago2	CAAGAAAGGAGGACAGGATAGC, TTGTTGCTGATGCGGTTG

Table S7. Sequencing statistics. "Small RNA reads" correspond to genome matching reads after excluding annotated non-coding RNAs. 454, pyrosequencing; Solexa/Illumina, sequencing-by-synthesis.
An asterisk indicates data that was pooled as described in the legend to Figure 2. Ambiguous: the reads map to the indicated category and another category or in both orientations within a single category.

Genotype	Enriched for modified 3′ ends?	Sequencing method	Genome-matching reads	annotated ncRNAs	Total Small RNA reads	All pre-miRNA-matching reads	Annotated miRNAs only
S2 cells	no	454	81,226	16,921	64,207	56,463	47,599
	yes	454	72,012	5,875	66,056	11,014	7,476
IR-wild-type heads*	no	454	94,772	23,206	71,268	68,596	61,688
	yes	454	30,250	1,526	22,690	11,089	8,740
	no	Illumina	1,245,354	33,429	1,187,572	1,152,293	949,190
	yes	Illumina	33,558	2,219	28,344	10,792	8,849
wild-type male heads*	no	Illumina	387,855	15,671	357,300	347,089	304,740
	yes	Illumina	4,928	422	4,208	3,261	2,856
wild-type female heads*	no	Illumina	916,026	43,081	790,126	754,602	673,105
	yes	Illumina	61,748	2,214	54,495	47,231	41,598
dcr-$2^{L811fsX}$/CyO heads	no	Illumina	908,508	2,683	859,436	833,614	638,085
dcr-$2^{L811fsX}$ heads	no	Illumina	734,343	7,105	688,323	671,408	549,508
untreated ago2 heads	no	Illumina	749,674	27,908	684,388	649,398	1,094,293
oxidized ago2 heads	yes	Illumina	228,112	871	183,572	73,518	17,327

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matching coding genes (unambiguous)		matching coding genes (ambiguous)		matching transposons (unambiguous)		matching transposons (ambiguous)		matching only *white* IR trigger		matching *white IR trigger and others*	
sense	antisense	sense	antisense	sense	antisense	sense	antisense				
1,394	670	21,742	20,290	4	15	2,821	2,950				
6,586	4,995	44,670	42,945	23	61	24,442	24,148	sense	antisense	sense	antisense
1,163	927	23,752	22,362	1	12	171	224	708	834	0	12
4,068	5,503	9,197	8,841	9	8	703	792	3,102	5,283	0	19
14,518	8,326	64,396	34,109	26	59	2,873	3,411	7,149	7,229	0	5
1,556	1,522	11,506	9,860	4	4	306	294	904	1,165	0	0
5,078	215	33,053	12,706	2	2	438	753				
114	13	1,150	686	0	0	18	22				
16,312	851	132,279	59,124	20	85	1,751	2,874				
1,258	279	10,398	5,969	5	17	550	664				
5,066	1,458	56,057	32,819	40	107	3,430	4,164				
3,476	876	49,723	33,036	20	52	1,510	1,686				
7,188	1,146	71,620	39,516	103	136	13,106	13,179				
323	93	5,532	4,228	4	12	336	440				