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Supplemental Information

The 3′-to-5′ Exoribonuclease Nibbler Shapes the 3′ Ends of MicroRNAs Bound to *Drosophila* Argonaute1

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Supplemental Inventory

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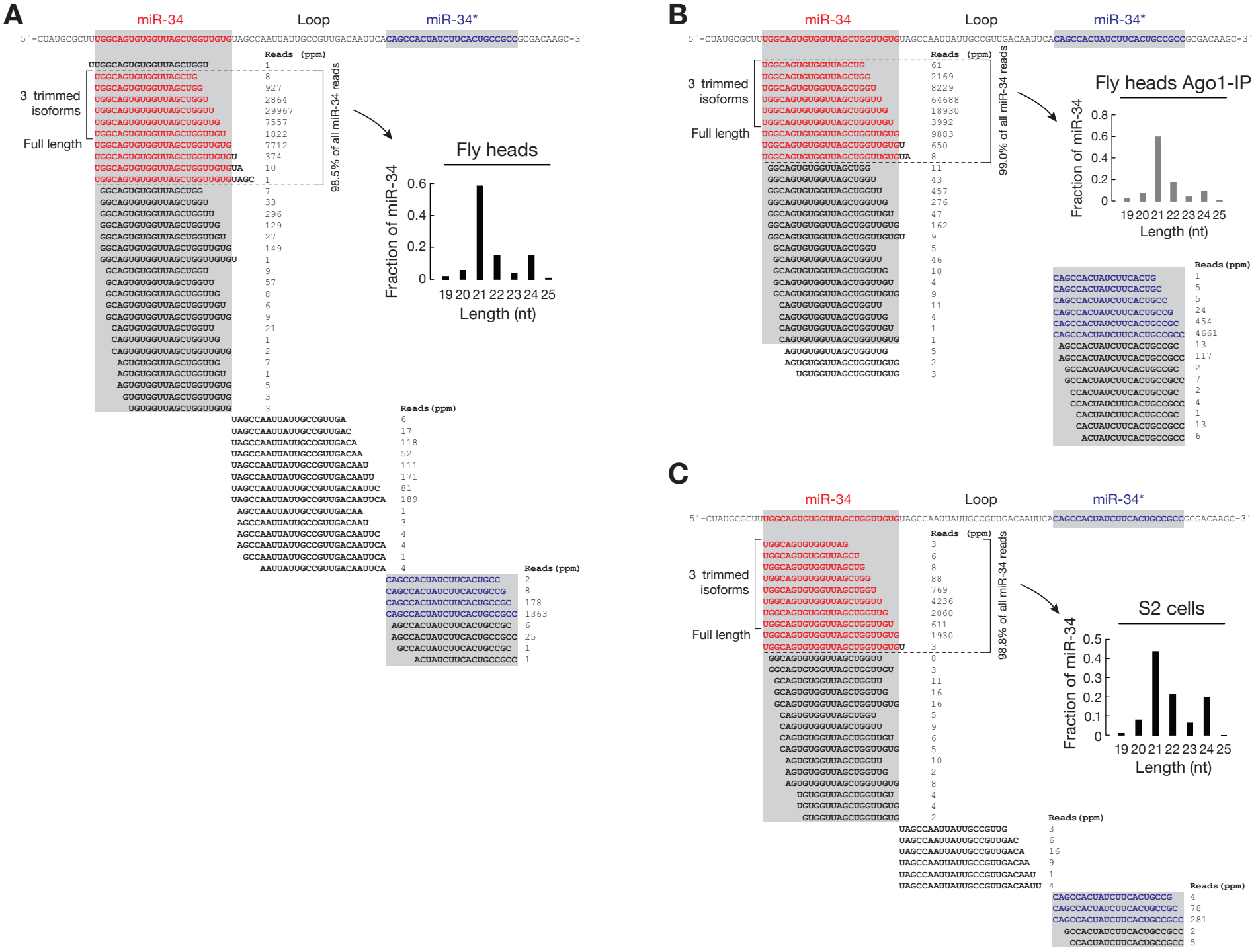

A
miR-34
Loop
miR-34*
5´-CUAUGCGCUUUGGCAGUGUGGUUAGCUGGUUGUGUAGCCAAUUAUUGCCGUUGACAAUUCACAGCCACUAUCUUCACUGCCGCCGCGACAAGC-3´
Reads (ppm)
3 trimmed isoforms
Full length
98.5% of all miR-34 reads
Fly heads
Fraction of miR-34
Length (nt)
B
99.0% of all miR-34 reads
Fly heads Ago1-IP
C
98.8% of all miR-34 reads
S2 cells

Figure S1. 5′ and 3′ miR-34 Isoforms from Fly Heads, Related to Figure 1

Reads mapping to the pre-miR-34 hairpin in high throughput sequencing datasets of total small RNAs from fly heads (A), Ago1-bound small RNAs in fly heads (B), and total small RNAs from S2 cells (C). Red, miR-34 reads that share the most abundant 5′ end; blue, miR-34*. Read abundance is reported as parts per million (ppm). The length distribution of miR-34 reads sharing the most abundant 5′ end is shown.

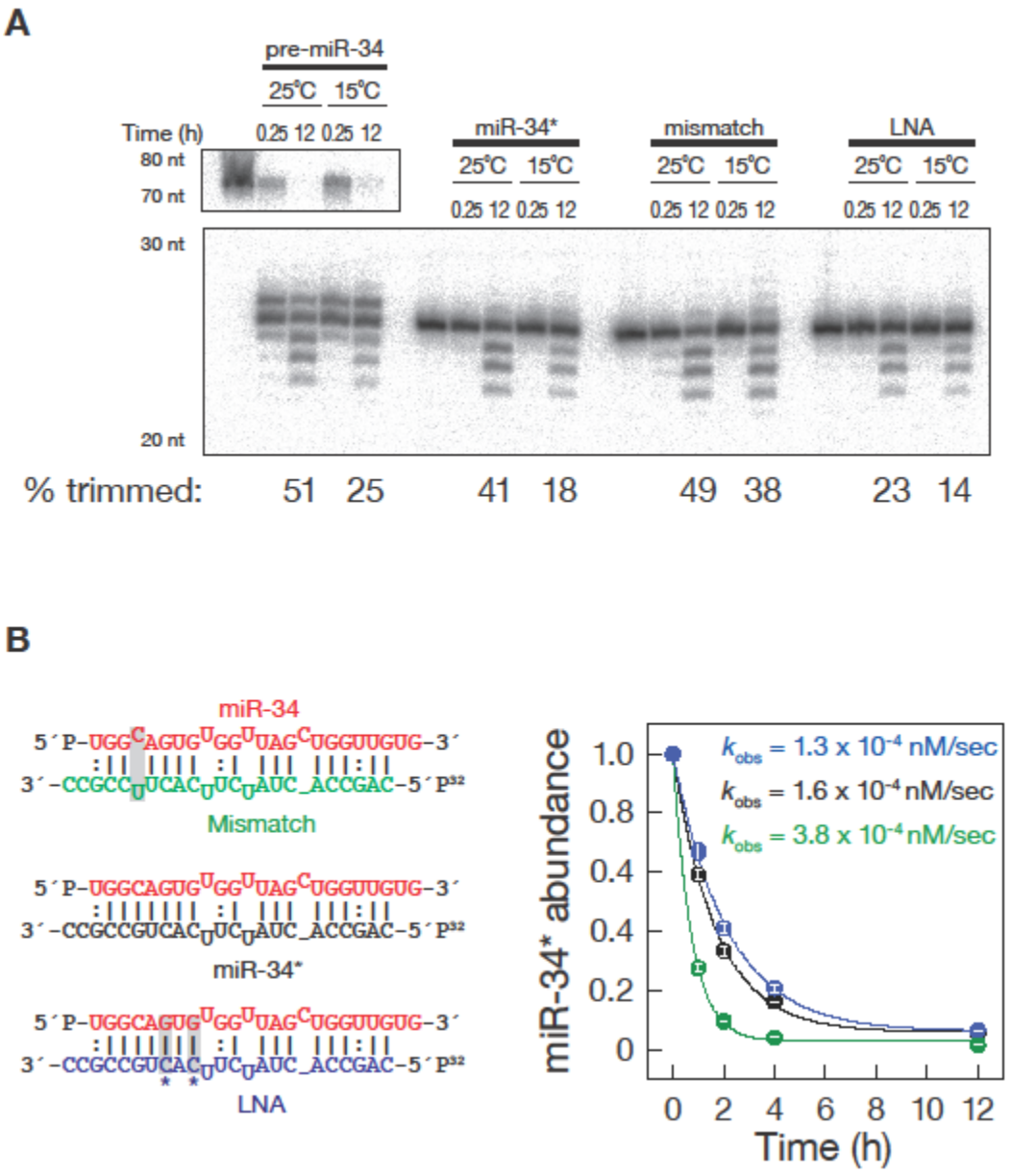


Figure S2. miRNA* Strand Removal Limits miR-34 Trimming, Related to Figure 2

(A) 5′ ^{32}P-radiolabeled pre-miR-34, mature miR-34/miR-34* duplex, miR-34 mismatched duplex or miR-34 LNA duplex was incubated in 0–2 h embryo lysate at 25°C or 15°C, then analyzed by denaturing polyacrylamide gel electrophoresis.

(B) Synthetic duplexes of 5′ ^{32}P-radiolabeled miR-34* variants (green, black, and blue) paired to non-radioactive, phosphorylated miR-34 (red) were incubated in 0–2 h embryo lysate, and then the decrease in abundance was analyzed by denaturing polyacrylamide gel electrophoresis. Mean ± standard deviation for three independent replicates. Decay rates (k_{obs}) were calculated by fitting the data to a single exponential.

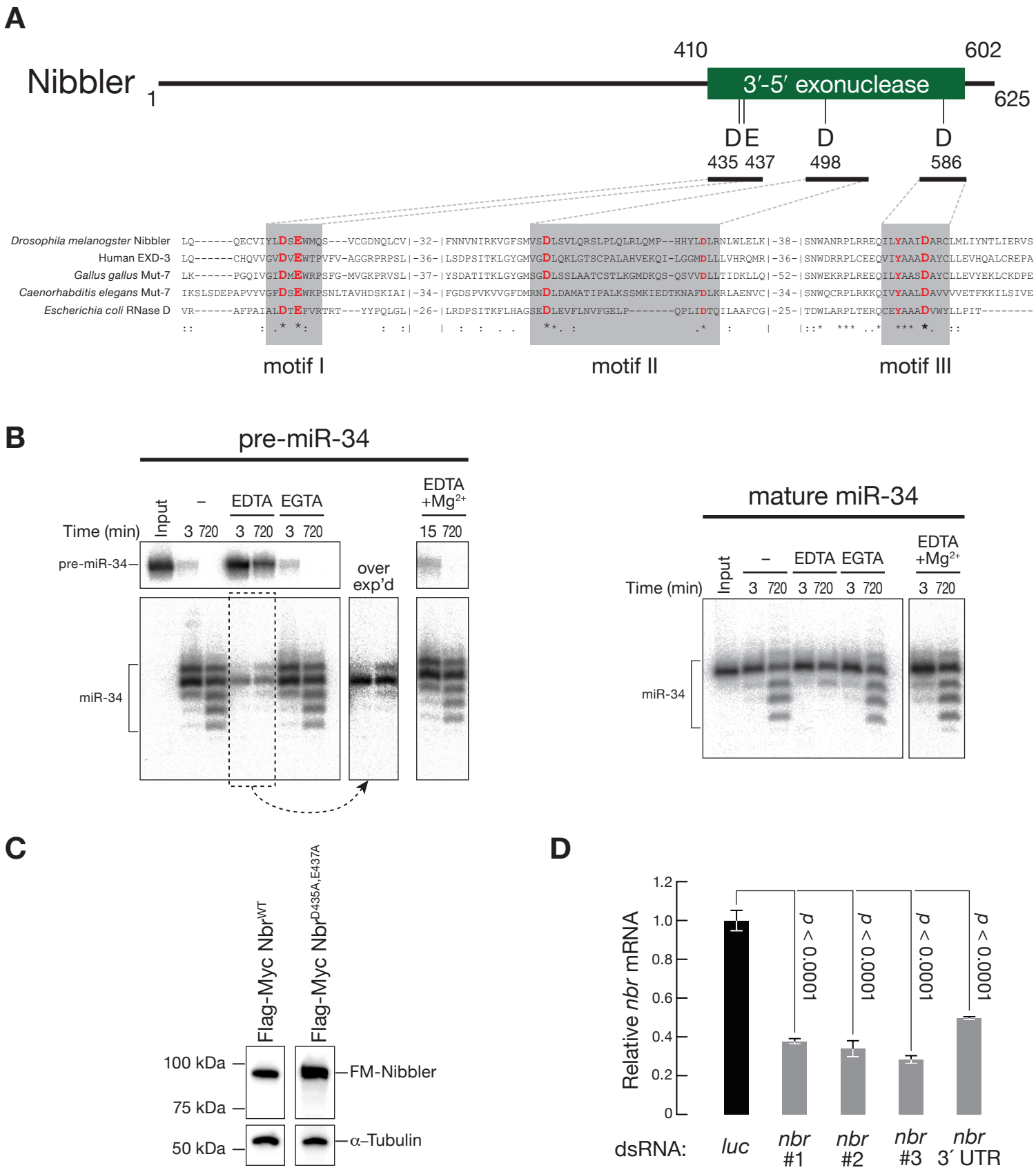

A
Nibbler
1
410
602
625
3′-5′ exonuclease
D E
435 437
D
498
D
586
Drosophila melanogster Nibbler
Human EXD-3
Gallus gallus Mut-7
Caenorhabditis elegans Mut-7
Escherichia coli RNase D
motif I
motif II
motif III
B
pre-miR-34
Input
–
EDTA
EGTA
EDTA +Mg2+
Time (min)
3 720 3 720 3 720 15 720
pre-miR-34
over exp'd
miR-34
mature miR-34
Input
–
EDTA
EGTA
EDTA +Mg2+
Time (min)
3 720 3 720 3 720 3 720
miR-34
C
Flag-Myc Nbr^WT
Flag-Myc Nbr^D435A,E437A
100 kDa
75 kDa
50 kDa
FM-Nibbler
α–Tubulin
D
Relative nbr mRNA
1.2
1.0
0.8
0.6
0.4
0.2
0
p < 0.0001
p < 0.0001
p < 0.0001
p < 0.0001
dsRNA:
luc
nbr #1
nbr #2
nbr #3
nbr 3′ UTR

Figure S3. The 3′-to-5′ Exoribonuclease Nibbler (CG9247) Trims miRNAs, Related to Figure 3

(A) Nibbler belongs to the DEDD superfamily of exonucleases. The four invariant exonuclease domain amino acids (DEDD) required for catalysis are indicated. Multiple sequence alignment of three conserved regions of five DEDD family members is shown below. Red, conserved amino acids required for catalysis.

(B) Trimming of miR-34 requires Mg^{2+}. 5′ ^{32}P-radiolabeled pre-miR-34 or a miR-34/miR-34* duplex was incubated in 0–2 h embryo lysate containing additional water (control), 5 mM (f.c.) EDTA, 5 mM EGTA, or 5 mM EDTA plus 5 mM Mg^{2+}. Samples were analyzed by denaturing polyacrylamide gel electrophoresis.

(C) Western blot analysis of stable S2 cell lines expressing an amino terminally 3xFLAG-6xMyc tagged version of wild-type or catalytic mutant Nibbler. Transgenic Nibbler was detected using mouse anti-FLAG monoclonal antibody (Sigma; F-1804; 1:3,000 dilution). α-Tubulin served as a loading control and was detected using a mouse monoclonal antibody (Sigma; T-9026; 1:10,000 dilution).

(D) *nibbler* mRNA abundance in S2 cells transfected with double-stranded RNA targeting luciferase or different regions of *nibbler* mRNA (see Figure 3B). *nibbler* mRNA levels were measured by quantitative RT-PCR. Data were normalized to mRNA levels of ribosomal protein L32 (alternatively called *rp49* or *RpL32*). Mean ± standard deviation for three biological replicates is shown. Student's t-test was used to determine *p*-values.

Han et al., Figure S4

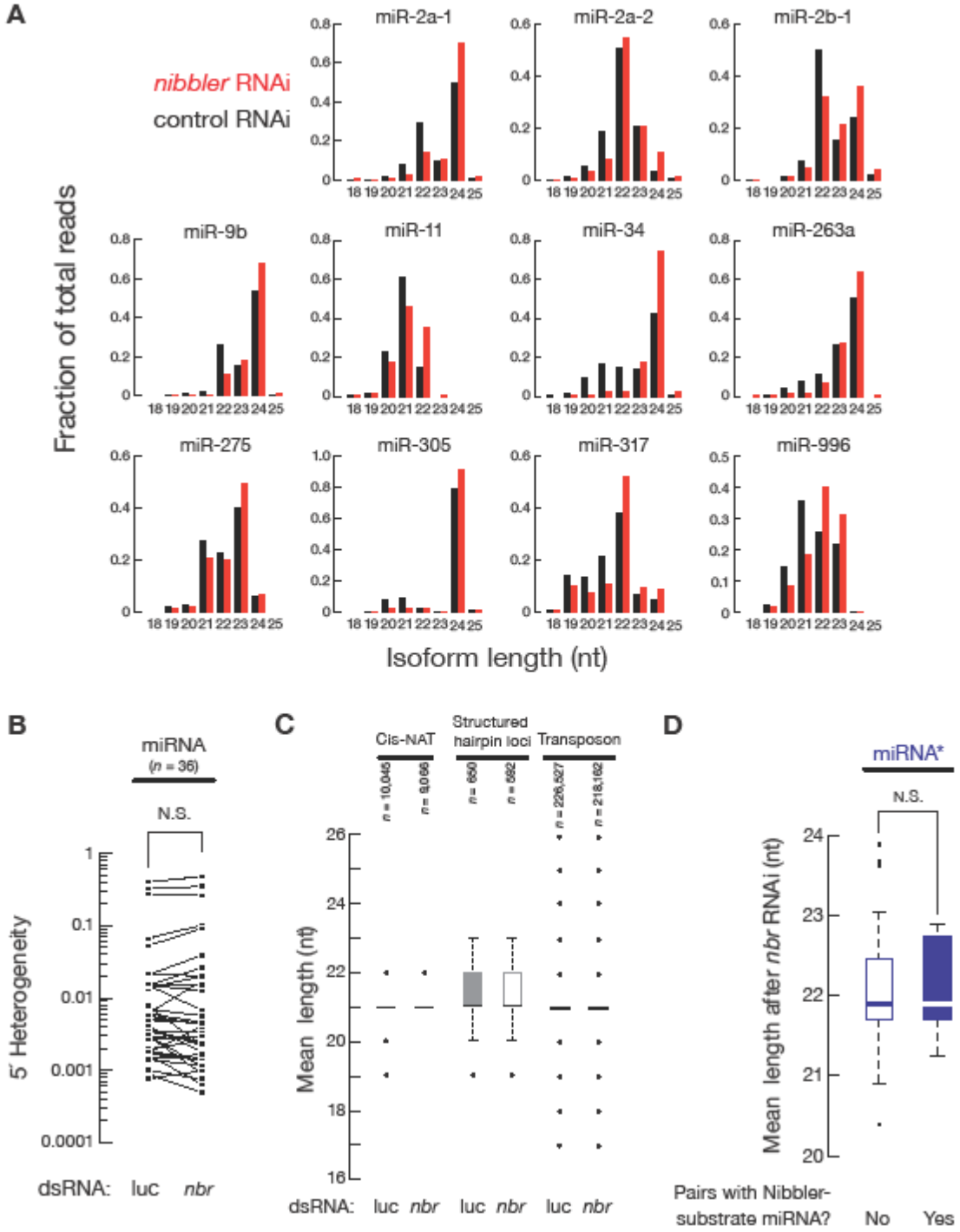


Figure S4. Characterization of miRNA-Trimming by Nibbler in S2 Cells, Related to Figure 4

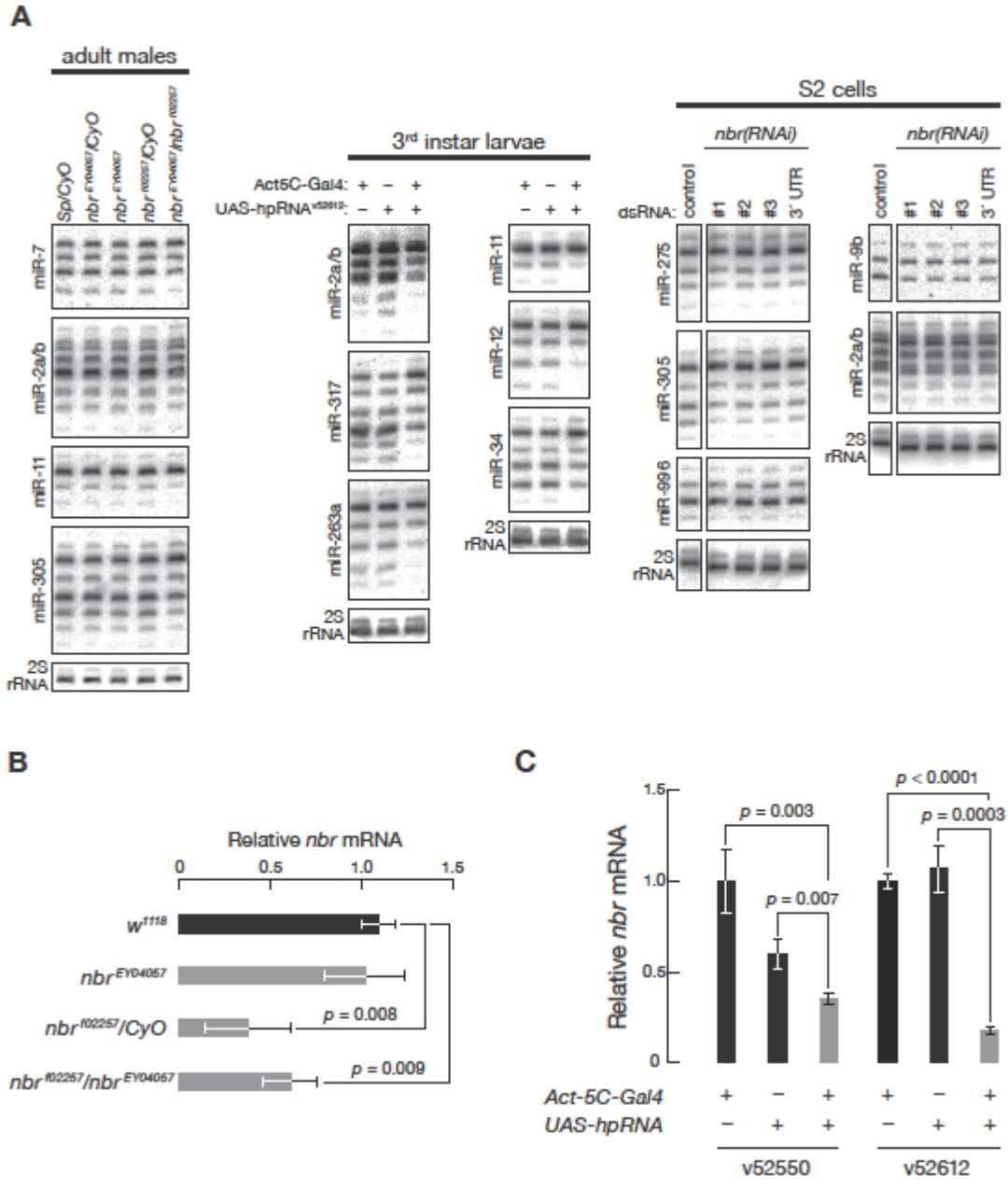
(A) The size distribution of Nibbler substrates in control and *nibbler* dsRNA-treated S2 cell libraries. The size distribution of the 11 miRNAs identified as Nibbler substrates was analyzed by dividing the number of reads for each isoform by the total number reads for that miRNA, using only reads with the canonical seed sequence for each miRNA.

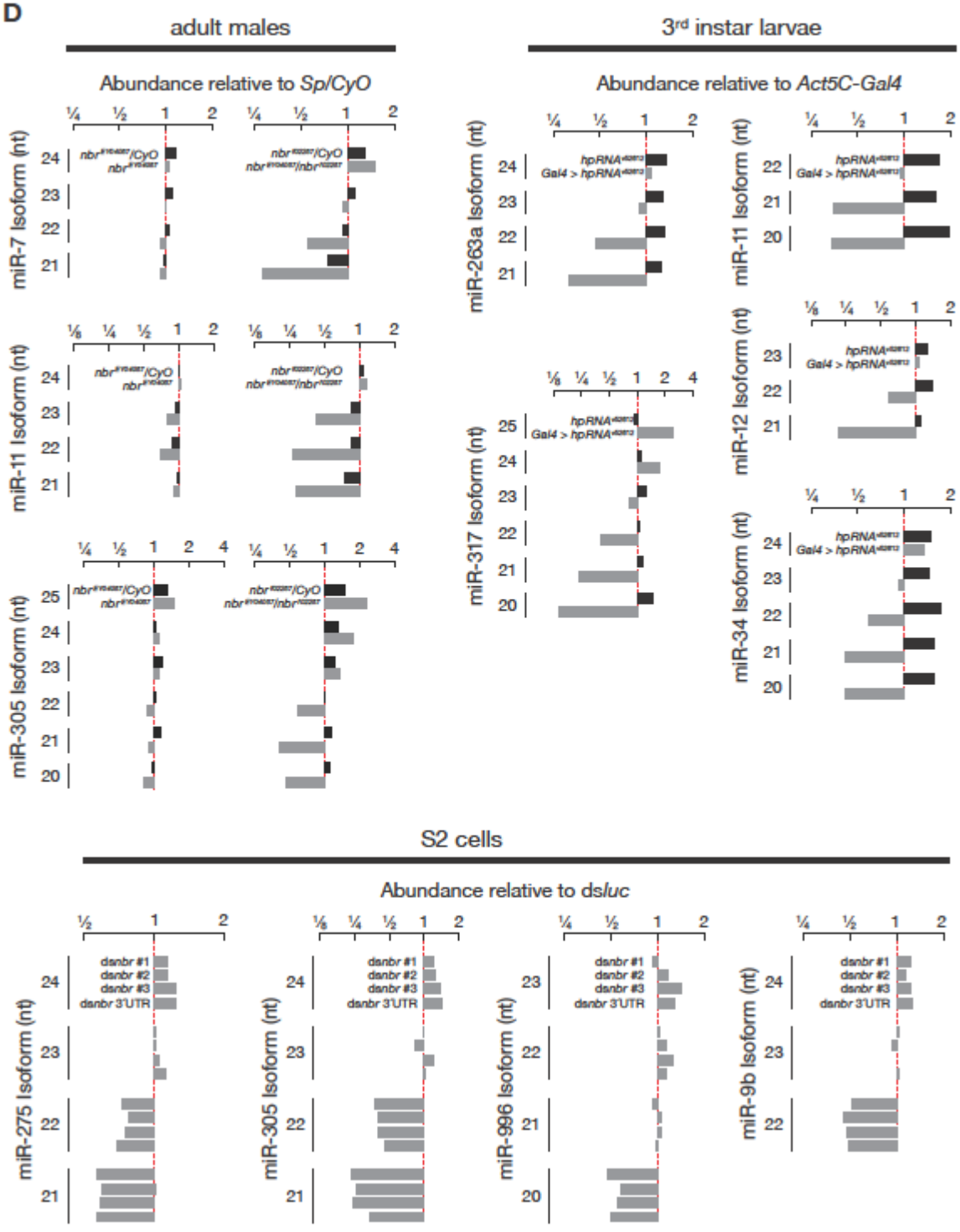
(B) Depletion of Nibbler by RNAi in S2 cells does not influence miRNA 5′ heterogeneity. 5′ heterogeneity of abundantly expressed S2 cell miRNAs (>200 ppm) treated with control *luciferase* (luc) or *nibbler* (*nbr*) dsRNA was analyzed. Wilcoxon signed-rank test was used to calculate *p*-values.

(C) Depletion of Nibbler in S2 cells by RNAi does not alter the length of endo-siRNAs. Box-and-whisker plots display the length of each sequence mapped to the loci previously shown to produce endo-siRNAs (cis-natural antisense transcripts, structured hairpin loci, and transposons). Small RNAs were oxidized before generation of deep sequencing libraries, a procedure previously shown to enrich for Ago2-bound small RNAs [S1]. Unpaired Wilcoxon signed-rank test was used to calculate *p*-values.

(D) Depletion of Nibbler by RNAi in S2 cells does not change the length of the miRNA* strands that pair with Nibbler substrate miRNAs. The mean length of the 11 miRNA* strands that pair with the 11 Nibbler substrate miRNAs identified in this study is shown. Mann-Whitney U test was used to determine *p*-values.

Han et al., Figure S5




D
adult males
3rd instar larvae
Abundance relative to Sp/CyO
Abundance relative to Act5C-Gal4
miR-7 Isoform (nt)
miR-11 Isoform (nt)
miR-305 Isoform (nt)
miR-263a Isoform (nt)
miR-317 Isoform (nt)
miR-12 Isoform (nt)
miR-34 Isoform (nt)
S2 cells
Abundance relative to dsluc
miR-275 Isoform (nt)
miR-996 Isoform (nt)
miR-9b Isoform (nt)
dsnbr #1
dsnbr #2
dsnbr #3
dsnbr 3'UTR

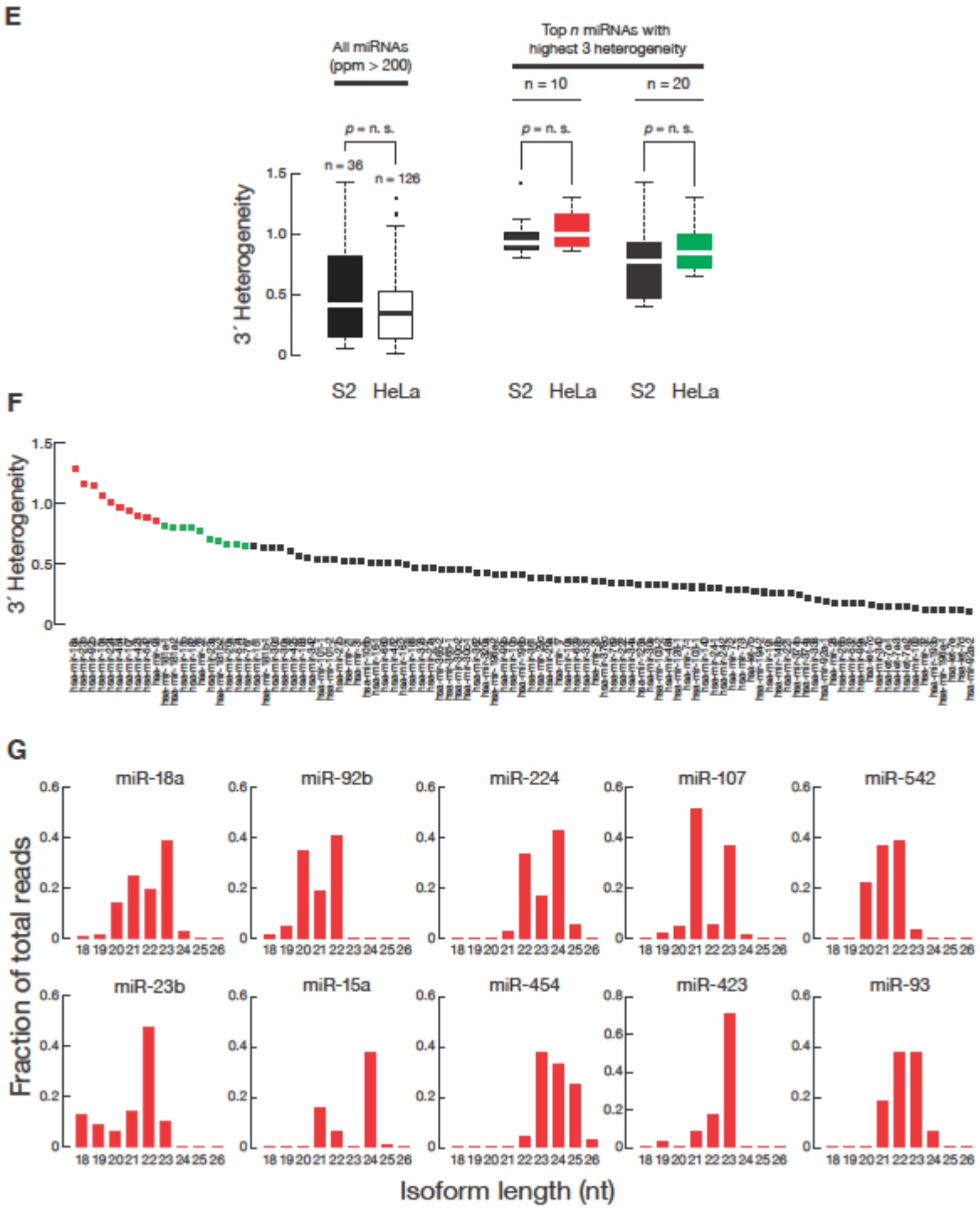

E
All miRNAs (ppm > 200)
Top n miRNAs with highest 3 heterogeneity
n = 10
n = 20
p = n. s.
p = n. s.
p = n. s.
n = 36
n = 126
3′ Heterogeneity
1.5
1.0
0.5
0
S2
HeLa
S2
HeLa
S2
HeLa
F
3′ Heterogeneity
1.5
1.0
0.5
0
G
miR-18a
miR-92b
miR-224
miR-107
miR-542
miR-23b
miR-15a
miR-454
miR-423
miR-93
Fraction of total reads
0.6
0.4
0.2
0
18 19 20 21 22 23 24 25 26
Isoform length (nt)

Figure S5. Nibbler-Mediated miRNA Trimming in Flies and Its Possible Conservation in Mammals, Related to Figure 5

(A) *nibbler* mRNA abundance in wild-type and mutant flies. *nibbler* mRNA levels in 3–5-day-old whole male flies were measured by quantitative RT-PCR. Data were normalized to mRNA levels of ribosomal protein L32 (alternatively called *rp49* or *RpL32*). Mean ± standard deviation for three biological replicates is shown. Student's t-test was used to determine *p*-values.

(B) *nibbler* mRNA abundance in flies depleted of *nibbler* mRNA by RNAi. *nibbler* mRNA levels in 3–5-day-old whole male flies were measured by quantitative RT-PCR. Data were normalized to mRNA levels of ribosomal protein L32. Mean ± standard deviation for three biological replicates is shown. Student's t-test was used to determine *p*-values.

(C) Validation of Nibbler substrate miRNAs. Northern hybridization was used to examine Nibbler substrate miRNAs previously identified by high throughput sequencing experiments. Total RNA from 3–5-day-old whole adult males, third instar larvae, or S2 cells was examined. miR-2a and miR-2b cannot be distinguished by Northern hybridization because they differ by a single nucleotide.

(D) Longer isoforms of Nibbler substrate miRNAs accumulate in the absence of Nibbler-mediated trimming. Quantification of the Northern hybridization shown in (B). For each miRNA, the signal intensities of all isoforms were determined and normalized to the signal for 2S rRNA. The signal intensity of each isoform was then normalized to the intensity of the same isoform in the indicated control to determine the relative abundance.

(E–G) Analysis of miRNA 3′ heterogeneity and length distribution in small RNA deep sequencing libraries of HeLa cells reveals a possible conservation of miRNA trimming in mammals.

(E) 3′ heterogeneity did not significantly differ between miRNAs in S2 cells (small RNA deep sequencing dataset generated in this study) and HeLa cells (NCBI Short Read Archive accession number SRA010045). Left panel compares 3′ heterogeneity for all abundantly expressed miRNAs (> 200 ppm). Right panel compares the 10 or 20 miRNAs with the greatest 3′ heterogeneity. Mann-Whitney U test was used to determine *p*-values.

(F) 3′ heterogeneity of abundantly expressed miRNAs in HeLa cells. The 10 (red) or 20 (green) miRNAs with the greatest 3′ heterogeneity are indicated.

(G) Among the 10 HeLa miRNAs with greatest 3´ heterogeneity, several (e.g. miR-18a, miR-92b, miR-224, miR-107, miR-15a, and miR-423) exhibit a length distribution with two abundant isoforms separated in length by at least one nucleotides, reminiscent of the profile of most *Drosophila* Nibbler substrate miRNAs (see Figure S4A).

Table S1A, Related to Figure 4

Abundance (parts per million; ppm) and mean length of abundant miRNAs (>200 ppm; top) or miRNA* strands (>10 ppm; bottom) in S2 cells transfected with double-stranded RNA targeting a luciferase mRNA (control RNAi) or *nibbler*. The *p*-value for the change in mean length was determined by a chi-square test.

miRNA	Seed Sequence	*nibbler* RNAi		Control RNAi		Change in length (*nbr* – control)	*p*-value
		Reads (ppm)	Mean Length (nt)	Reads (ppm)	Mean Length (nt)		
bantam	GAGATCA	433411	22.90	459298	22.87	0.02	1.55×10^{-250}
miR-2a-1	ATCACAG	2733	23.48	3814	23.00	0.48	5.24×10^{-73}
miR-2a-2	CACAGCC	835	22.30	950	21.95	0.35	7.41×10^{-15}
miR-2b-1/2	ATCACAG	6230	22.98	11435	22.60	0.39	1.83×10^{-147}
miR-7	GGAAGAC	550	23.73	523	23.60	0.13	0.08
miR-8	AATACTG	54164	22.71	61941	22.64	0.07	5.94×10^{-111}
miR-9b	CTTTGGT	2395	23.55	1850	23.18	0.37	3.87×10^{-40}
miR-9c	CTTTGGT	778	21.52	862	21.46	0.06	0.53
miR-11	ATCACAG	3132	21.13	6095	20.89	0.24	2.74×10^{-106}
miR-13b-1/2	ATCACAG	8971	22.84	14230	22.83	0.01	0.0179796
miR-14	CAGTCTT	14914	21.77	24596	21.78	-0.02	2.52×10^{-07}
miR-33	TGCATTG	4377	20.90	6153	20.87	0.03	6.13×10^{-07}
miR-34	GGCAGTG	9743	23.68	5641	22.58	1.09	0.00
miR-79	AAAGCTA	1354	21.96	17611	21.96	0.00	0.98
miR-184	GGACGGA	123174	21.67	98481	21.64	0.03	3.45×10^{-28}
miR-252	TAAGTAC	1699	22.02	5807	22.00	0.02	0.53
miR-263a	ATGGCAC	555	23.50	478	23.10	0.39	2.15×10^{-07}
miR-275	CAGGTAC	2076	22.34	3102	22.14	0.21	2.35×10^{-10}
miR-276a	AGGAACT	30366	21.92	34208	21.91	0.01	0.00
miR-277	AAATGCA	3493	22.82	3658	22.74	0.08	3.69×10^{-09}
miR-279	GACTAGA	7057	21.06	7999	21.07	-0.01	0.00
miR-282	AGCCTCT	5421	21.95	4027	21.95	0.00	0.86
miR-283	AATATCA	1111	22.52	1173	22.45	0.07	0.24
miR-304	AATCTCA	268	22.32	310	22.39	-0.07	0.90
miR-305	TTGTACT	9224	23.80	9461	23.34	0.46	3.31×10^{-175}
miR-306	CAGGTAC	332	21.15	396	21.09	0.06	0.98
miR-308	ATCACAG	805	21.11	446	21.12	-0.01	1.00
miR-317	GAACACA	2554	21.68	15748	21.22	0.46	1.09×10^{-84}
miR-965	AAGCGTA	392	21.90	391	21.91	0.00	1.00
miR-970	CATAAGA	1044	20.94	1769	20.94	0.00	1.00
miR-980	AGCTGCC	1150	21.25	1284	21.18	0.07	0.06
miR-988	CCCTTGT	971	21.46	812	21.36	0.10	0.01
miR-995	AGCACCA	3746	20.88	2775	20.89	-0.01	0.61
miR-996	GACTAGA	1758	21.90	2574	21.49	0.41	8.19×10^{-49}
miR-998	AGCACCA	1068	20.89	1317	20.92	-0.03	0.73
miR-1003	CTCACAT	353	21.49	411	21.46	0.03	1.00

miRNA*	Seed	*nibbler* RNAi		Control RNAi		Change in length (*nbr* – control)	*p*-value
	Sequence	Reads (ppm)	Mean Length (nt)	Reads (ppm)	Mean Length (nt)		
*bantam**	CGGTTTT	5574	22.75	16716	22.86	0.11	2.19×10^{-156}
miR-2a-1*	TCTCAAA	17	21.16	19	21.25	0.09	0.98
miR-2a-2*	CCTCATC	46	21.67	35	21.65	-0.02	1.00
miR-2b-2*	TCTTCAA	361	22.21	396	22.29	0.09	0.94
miR-7*	AATAAAT	12	21.89	12	21.89	0.00	1.00
miR-8*	ATCTTAC	4200	21.75	4616	21.80	0.05	0.00
miR-9b*	AGAGCTT	216	21.66	110	21.72	0.06	0.99
miR-9c*	AAAGCTT	33	21.07	44	21.17	0.10	0.99
miR-11*	AAGAACT	34	21.86	32	21.85	-0.02	1.00
miR-13b-2*	CGTCAAA	166	23.64	124	23.63	-0.01	1.00
miR-14*	GGAGCGA	310	21.81	188	21.89	0.08	0.89
miR-33*	AATACAA	97	21.41	82	21.30	-0.11	0.98
miR-34*	AGCCACT	659	22.87	642	22.88	0.02	0.93
miR-79*	CTTTGGC	31	22.23	16	22.26	0.04	1.00
miR-184*	CTTATCA	59	21.70	40	21.68	-0.01	1.00
miR-275*	GCGCTAA	88	22.60	72	22.74	0.13	0.99
miR-277*	GTGTCAG	102	20.39	150	20.39	0.00	1.00
miR-281-1*	AAGAGAG	27	21.23	15	21.20	-0.03	0.99
miR-281-2*	AGAGAGC	278	22.29	314	22.47	0.18	0.59
miR-282*	CATAGCC	1296	21.94	2261	21.95	0.00	1.00
miR-283*	GGAATTT	15	21.89	21	21.87	-0.02	1.00
miR-304*	ACTTTGC	82	21.97	64	21.98	0.01	1.00
miR-305*	GGCACAT	252	21.77	333	21.84	0.07	0.96
miR-317*	GGGATAC	133	21.90	96	21.96	0.06	0.97
miR-954*	ATTCACA	14	21.67	12	21.72	0.04	1.00
miR-965*	GGGTAAA	72	22.31	61	22.32	0.01	1.00
miR-980*	GTTTTTC	550	23.84	550	23.88	0.04	0.95
miR-988*	TGTGATT	39	21.85	33	21.81	-0.04	1.00
miR-995*	CCCGAAT	23	22.89	35	22.91	0.02	1.00
miR-996*	GCGAACA	402	22.71	580	22.74	0.03	0.90
miR-998*	CTGAATT	12	21.72	21	21.52	-0.20	1.00
miR-1012*	TGGGTAG	39	21.31	42	21.28	-0.03	1.00

Table S1B, Related to Figure 4

Sequencing statistics: reads (top) and species (bottom). “Small RNA reads (excluding ncRNAs)” correspond to genome-matching reads after excluding annotated non-coding RNAs (ncRNAs), such as rRNA, snRNA, snoRNA, or tRNA. “Transposon-matching reads” correspond to small RNAs mapped to *Drosophila melanogaster* transposons. “cis-NAT-matching reads” correspond to reads matching to mRNAs [S1-S3]. “Structured loci-matching reads” correspond to reads that map to two distinct loci in the *Drosophila melanogaster* genome (CG18854 and a locus overlapping with CG4068), the transcripts of which fold into long hairpin structures and produce the majority of small RNAs of this class [S2, S4]. Where reads were normalized to genome-matching reads (excluding ncRNAs), they are reported in parts per million (ppm).

S2 cell condition	Total Reads	Reads perfectly matching genome	Reads matching annotated ncRNAs	Small RNA reads (excluding ncRNAs)	Pre-miRNA-matching reads (ppm)	Reads excluding ncRNA and pre-miRNA-matching (ppm)	Transposon-matching reads (ppm)	cis-NAT-matching reads (ppm)	Structured loci-matching reads (ppm)
control (*luc*) RNAi	10,392,044	7,598,980	701,543	6,897,430	838,169	69,105	33,082	266	2,150
nibbler RNAi	11,660,479	8,516,830	931,335	7,585,490	838,718	74,923	35,976	271	2,436
control (*luc*) RNAi, oxidized	12,652,801	4,336,100	118,984	4,217,110	56,410	675,858	554,656	4,022	16,442
nibbler RNAi, oxidized	10,945,880	3,982,380	110,034	3,872,350	66,213	759,628	620,552	4,391	20,977

S2 cell condition	Total number of species	Number of species perfectly matching genome	Number of species matching annotated ncRNAs	Number of small RNA species (excluding ncRNAs)	Number of pre-miRNA-matching species	Number of species excluding ncRNA and pre-miRNA-matching	Number of transposon-matching species	Number of cis-NAT-matching species	Number of structured loci-matching species
control (*luc*) RNAi	443,253	254,492	38,170	216,322	1,590	214,732	83,238	1,639	369
nibbler RNAi	560,481	313,547	45,107	268,440	1,702	266,738	94,072	1,862	432
control (*luc*) RNAi, oxidized	1,307,000	609,746	14,376	595,370	770	594,600	226,527	10,045	650
nibbler RNAi, oxidized	1,161,140	566,296	13,843	552,453	770	551,683	218,162	9,066	592

Supplemental Experimental Procedures

General Methods

Preparation of embryo and S2 cell lysate [S5, S6], recombinant Dicer-1 and Loquacious-PB [S7], clonal S2 cell lines [S8], and small RNA libraries for high throughput sequencing [S1] have been described previously. Northern hybridization was as described [S8], except that *N*-(3-Dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (Sigma-Aldrich, St. Louis, MO, USA) was used to crosslink 5′ phosphorylated small RNAs to Hybond-NX (Amersham, GE Healthcare, Piscataway, NJ) [S9]. Published small RNA libraries used in this study were total S2 cell RNA (Figures 1D, 2C, and S1C) and *ago1* RNAi (Figure 2C) [S10], total fly head RNA (Figures 1D and S1A) [S1], and anti-Ago1 immunoprecipitated small RNAs (Figure S1B) [S11]. Sequence data generated in this study are available from the NIH Gene Expression Omnibus (www.ncbi.nlm.nih.gov/geo) using accession number GSE31689. Fly strain $y^{1}\ w^{67c23}$; P{w^{+mC}, $y^{+mDint2}$, EPgy2}CG9247[EY04057] was from the *Drosophila* Stock Center (Bloomington, IN, USA); PBac{WH}CG9247[f02257] was from the Exelixis Collection at Harvard Medical School (Boston, MA, USA); and flies expressing hpRNAs were from the *Drosophila* RNAi Center (Vienna, Austria).

Quantitative RT-PCR

Total RNA purified from S2 cells or flies was treated with Turbo DNase (Ambion), extracted with phenol:chloroform (1:1), and precipitated with 3 volumes ethanol and 1/10th volume sodium acetate (Ambion). Purified RNA was reverse transcribed with SuperScript III (Invitrogen, Carlsbad, CA, USA), and quantitative PCR was performed using SsoFast EvaGreen Supermix (Bio-Rad, Hercules, CA, USA).

Oligonucleotides

The following table lists oligonucleotide sequences used in this manuscript. Synthetic DNA (Coralville, IA, USA), RNA and modified RNA oligonucleotides (Dharmacon, Lafayette, CO, USA) were deprotected according to the manufacturer's instructions and PAGE purified. Upper case, unmodified nucleotides; lower case, 2′-*O*-methyl ribose; underlined, locked-nucleic acid (LNA) ribose.

To clone pENTR/D-Nibbler

Name	Type	Sequence
MCS-*nibbler* fwd	DNA	CAC CAT GGC ACG CAA GAG CCA CAT
MCS-*nibbler* rev	DNA	TCA CTT AAC ATG GGC ACC C

To generate long double-stranded RNA

Name	Type	Sequence
luciferase fwd	DNA	TAA TAC GAC TCA CTA TAG GGA TAT CGC CCT GAT CAA G
luciferase rev	DNA	TAA TAC GAC TCA CTA TAG CCC TCG ACA ATA GCG TTG G
GFP fwd	DNA	TAA TAC GAC TCA CTA TAG GGC CAC AAG TTC AGC GTG TCC

GFP rev	DNA	TAA TAC GAC TCA CTA TAG GGT AGT GGT TGT CGG GCA GCA G
Ago1 fwd	DNA	TAA TAC GAC TCA CTA TAG GGC TCG CCC AAC CAG GG
Ago1 rev	DNA	TAA TAC GAC TCA CTA TAG GGA GAG AGC GTC TGT GGC GAT
Ago2 fwd	DNA	TAA TAC GAC TCA CTA TAG GGT CAG GCC CTA AAC CGC AAG GA
Ago2 rev	DNA	TAA TAC GAC TCA CTA TAG GGA TCG CAA TAC AAG ACC GCA CA
CG10210 fwd	DNA	TAA TAC GAC TCA CTA TAG GGG GCT TTC ACT CTG TCC CGC AAT C
CG10210 rev	DNA	TAA TAC GAC TCA CTA TAG GGG GTT TCA GGT TAC GCA TCT CCA GTC C
CG12877 fwd	DNA	TAA TAC GAC TCA CTA TAG GGC TCC CTT CCA CTG CCC CAG A
CG12877 rev	DNA	TAA TAC GAC TCA CTA TAG GGT CCC TTT CCC GCT CCT TGC T
CG42666 fwd	DNA	TAA TAC GAC TCA CTA TAG GGC AGA AAG CCA AGC AGG GGA ATG
CG42666 rev	DNA	TAA TAC GAC TCA CTA TAG GGG CGG AGA GGA GGA ACC AGG AA
CG8368 fwd	DNA	TAA TAC GAC TCA CTA TAG GGG CCA TTC TGG TGG GGC AAT CT
CG8368 rev	DNA	TAA TAC GAC TCA CTA TAG GGC GTG CGC TTA TCC CGC TTT G
CG6744 fwd	DNA	TAA TAC GAC TCA CTA TAG GGT GCG CTT CCT CTG CGT AAT GG
CG6744 rev	DNA	TAA TAC GAC TCA CTA TAG GGC TGC CGG ACG GGT TGA GTG T
CG6833 fwd	DNA	TAA TAC GAC TCA CTA TAG GGA AGA CGG TCA CAC TGG TCG
CG6833 rev	DNA	TAA TAC GAC TCA CTA TAG GGT GTG TTC ACC TGT TGC ACC T
CG7670 fwd	DNA	TAA TAC GAC TCA CTA TAG GGA CGC CAA AGC AAA CGA AAG GAA G
CG7670 rev	DNA	TAA TAC GAC TCA CTA TAG GGC GCA TAT CTG GAT GAC GGC AGA
nibbler-1 fwd	DNA	TAA TAC GAC TCA CTA TAG GGA TGG ACC TGC CCG ACG AGT G
nibbler-1 rev	DNA	TAA TAC GAC TCA CTA TAG GGT TTA ACT CCA ACC AGA GAT TGC GAA GG
nibbler-2 fwd	DNA	TAA TAC GAC TCA CTA TAG GGA GTA TGG TTT TGA CGA CGC C
nibbler-2 rev	DNA	TAA TAC GAC TCA CTA TAG GGC GAT CTC CAA CGG ACA ATC T
nibbler-3 fwd	DNA	TAA TAC GAC TCA CTA TAG GGA TCC GTA AGG TGG GGT TCT C
nibbler-3 rev	DNA	TAA TAC GAC TCA CTA TAG GGG GTC TAT TGG CCC AGT TTG A
nibbler-UTR fwd	DNA	TAA TAC GAC TCA CTA TAG GGT TGA GGA GCT GGC CTC TG
nibbler-UTR rev	DNA	TAA TAC GAC TCA CTA TAG GGT TCA ATC CTA TTC ATT GCT TTT
CG3931 fwd	DNA	TAA TAC GAC TCA CTA TAG GGG GCG AGG GCG AGC ACA AGA T
CG3931 rev	DNA	TAA TAC GAC TCA CTA TAG GGC ACG GCA GGT GGG GAA TGA AG

CG42257 fwd	DNA	TAA TAC GAC TCA CTA TAG GGT GAC GGG CAT CCA GCA GAA GA
CG42257 rev	DNA	TAA TAC GAC TCA CTA TAG GGG CGA CAG GGC ATC CGT GAA G
CG8232 fwd	DNA	TAA TAC GAC TCA CTA TAG GGG GTG AGC ATG CTG CAG CTA
CG8232 rev	DNA	TAA TAC GAC TCA CTA TAG GGG GCG CAG CAT GCG CAG A
CG3291 fwd	DNA	TAA TAC GAC TCA CTA TAG GGG GCG AGG GCG AGC ACA AGA T
CG3291 rev	DNA	TAA TAC GAC TCA CTA TAG GGC ACG GCA GGT GGG GAA TGA AG
CG10214 fwd	DNA	TAA TAC GAC TCA CTA TAG GGT CGG AGC CAC ATC GCA TTC A
CG10214 rev	DNA	TAA TAC GAC TCA CTA TAG GGA GTT CCC ACC AAG CGG ACA GG

Northern probes

Name	Type	Sequence
miR-11 probe	DNA	GCA AGA ACT CAG ACT GTG ATG
miR-12 probe	DNA	ACC AGT ACC TGA TGT AAT ACT CA
bantam probe	DNA	AAT CAG CTT TCA AAA TGA TCT CA
miR-34 probe	DNA	AAC CAG CTA ACC ACA CTG CCA
miR-7 probe	DNA	ACA ACA AAA TCA CTA GTC TTC CA
miR-9b probe	DNA	TAC AGC TAA AAT CAC CAA AGA
miR-996 probe	DNA	GAC GAG CAT GAA ATC TAG TCA
2S rRNA probe	DNA	TAC AAC CCT CAA CCA TAT GTA GTC CAA GCA
miR-2a probe	DNA	TCA TCA AAG CTG GCT GTG ATA
miR-275 probe	DNA	CGC GCG CTA CTT CAG GTA CCT GA
miR-2b-2 probe	DNA	TCC TCA AAG CTG GCT GTG ATA
miR-317 probe	DNA	GGA TAC CAC CAG CTG TGT TCA
miR-305 probe	DNA	GAG CAC CTG ATG AAG TAC AAT

For quantitative PCR

rp49 fwd	DNA	TGT CCT TCC AGC TTC AAG ATG ACC ATC
rp49 rev	DNA	CTT GGG CTT GCG CCA TTT GTG
nibbler fwd	DNA	TCT TCG CCA ACT GCC CAG ACA G
nibbler rev	DNA	GCC AGC AAG CCG GAG GTC TTG

For T7 RNA polymerase transcription of pre-miR-34

Name	Type	Sequence
Forward	DNA	GCG AAT TTA ATA CGA C TCA CTA TAT GGC AGT GTG GTT AGC TGG TTG TGT AGC CAA TTA TTG CCG TTG ACA ATT CAC AGC CAC TAT CTT CAC TGC CGC C
Reverse	DNA	ggC GGC AGT GAA GAT AGT GGC TGT GAA TTG TCA ACG GCA ATA ATT GGC TAC ACA ACC AGC TAA CCA CAC TGC CAT ATA GTG AGT CGT ATT AAA TTC GC

For Nibbler activity assays

Name	Type	Sequence
miR-34-5p (miR-34)	RNA	UGG CAG UGU GGU UAG CUG GUU GUG
miR-34-3p (miR-34*)	RNA	CAG CCA CUA UCU UCA CUG CCG CC
miR-34* MM	RNA	CAG CCA CUA UCU UCA CUU CCG CC
miR-34* LNA	RNA	CAG CCA CUA UCU UCA CUG CCG CC
miR-305-22mer	RNA	AUU GUA CUU CAU CAG GUG CUC U
miR-305*-22mer	RNA	GGC ACA UGU UGA AGU ACA CUC A
miR-305-24mer	RNA	AUU GUA CUU CAU CAG GUG CUC UGG
miR-305*-23mer	RNA	CGG CAC AUG UUG AAG UAC ACU CA
let-7	RNA	UGA GGU AGU AGG UUG UAU AGU
*let-7**	RNA	CUA UAC AAU GUG CUA GCU UUC U

For miRNA reporter assay

Name	Type	Sequence
miR-34 target site S	DNA	TCG AGG TGT TGA TGC TAA GGT CAC TGC CAG TGT TGA TGC TAA GGTCAC TGC CAG TGT TGA TGC TAA GGT CAC TGC CAG C
miR-34 target site AS	DNA	GGC CGC TGG CAG TGA CCT TAG CAT CAA CAC TGG CAG TGA CCT TAG CAT CAA CAC TGG CAG TGA CCT TAG CAT CAA CAC C
miR-34 ASO	2′-*O*-methyl	cac aac cag cta acc aca ctg cca
let-7 ASO	2′-*O*-methyl	cta cta tac aac cta cta cct ca

Supplemental References

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