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Figure S1: Analysis of small RNA population with 1-2 mismatches to the genome. Using a mapping protocol that takes into account 1-2 mismatches, we looked at the small RNA distribution at chromosome 10: 80327123-85445335. This is an ~5Mb region with the most prominent oocyte piRNA cluster in the middle, heavy in repetitive elements. The small RNAs that have been mapped with no mismatches are green, while those that have 1-2 mismatches are in red (in both cases, using the 24-30 nt. fraction). As shown in the graph, the number of small RNAs with mismatches in this region is a factor of 10 less than those contributed by perfect matches, and largely conform to the relative peaks of small RNAs in the region. This suggests that including sequences with mismatches contribute to a minor proportion of the total number of small RNAs in this region.

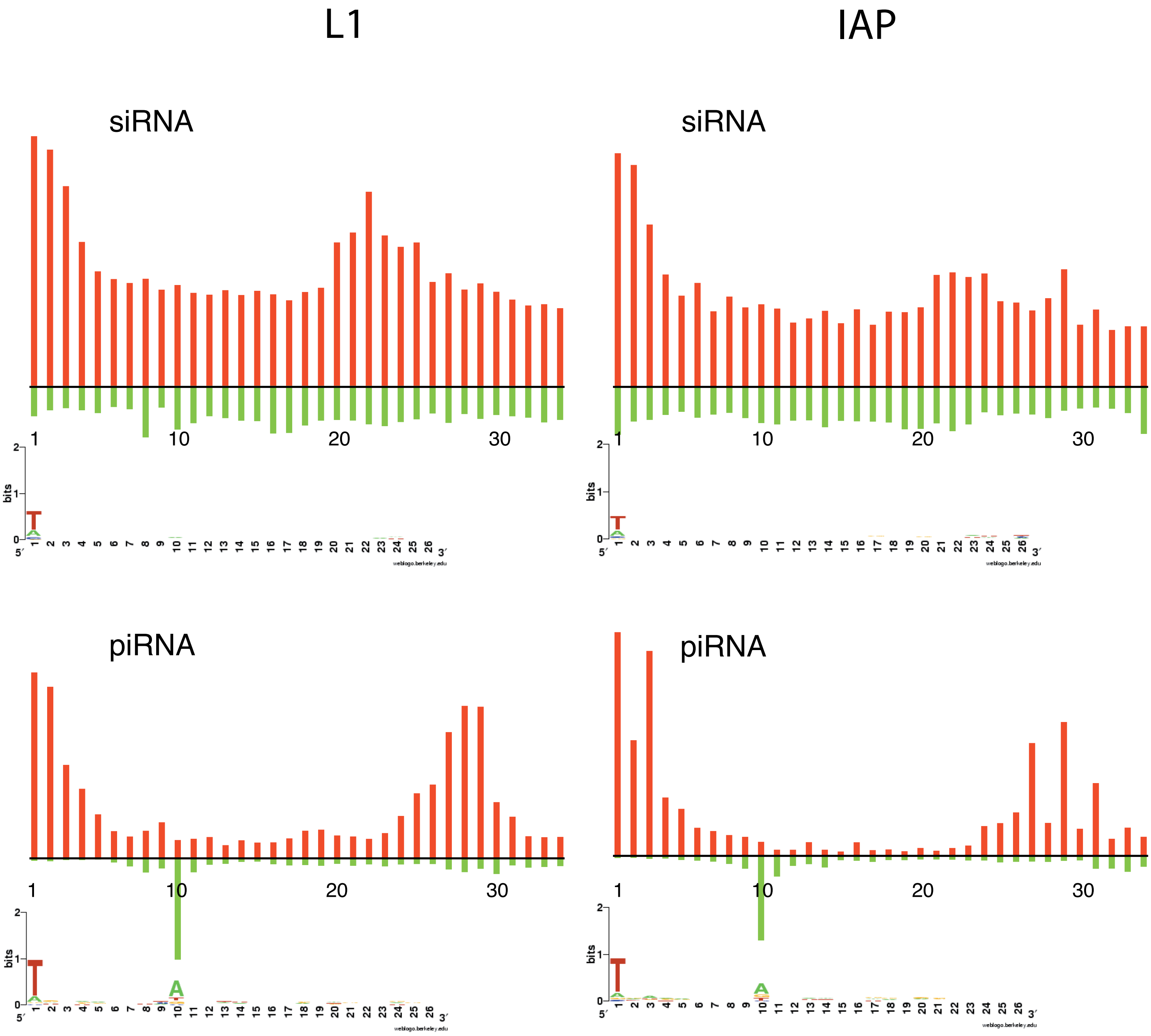

Figure S2. Properties of oocyte siRNAs and piRNAs. Upper plots (bar graphs) show the relative frequencies of small RNAs with a 5′ end at the indicated distance from another siRNA or piRNA (as indicated) on the same strand (red) or the opposite strand (green). Plots were created for sequences matching to L1 or IAP, as indicated. In the piRNA plots, a clear peak at position 10 on the lower strand can be seen for both L1 and IAP. This indicates the 10 nucleotide overlap between two complementary piRNAs that is a signature of the Ping-Pong amplification cycle. Notably, peaks on the same strand for 5′ ends that are ~22 and ~27-29 nucleotides distant can be seen for siRNAs and piRNAs respectively. This indicates some degree of phasing in these populations. The bar for position 1 is truncated to allow visualization of other bars on the graph. Nucleotide bias plots appear below each frequency graph. For piRNAs, a strong U bias at position 1 (T in the reads) can be seen. Moreover, the A at position 10 is also an indication of the Ping-Pong cycle and ongoing transposon defense. A mixture of T and A can be seen at high frequency at the 5′ ends of siRNAs, with no additional nucleotide bias detected.

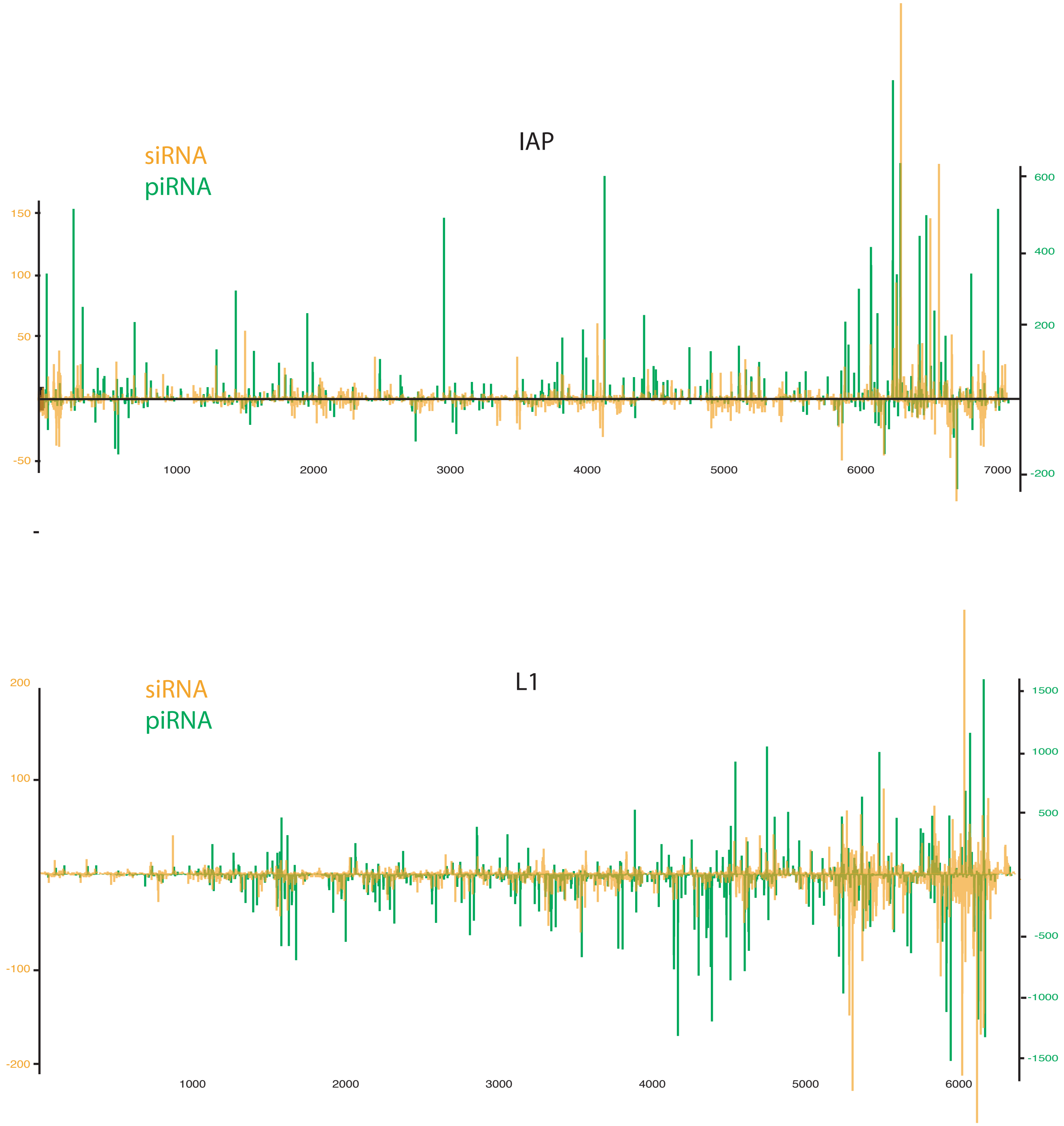

Figure S3. Distribution of piRNAs and siRNAs along L1 and IAP consensus sequences. The number of siRNAs (yellow) or piRNAs (green) whose 5′ end maps with up to 3 mismatches to each position along the L1 or IAP consensus (as indicated) is shown. Each RNA class is plotted against a different Y-axis (color coded).

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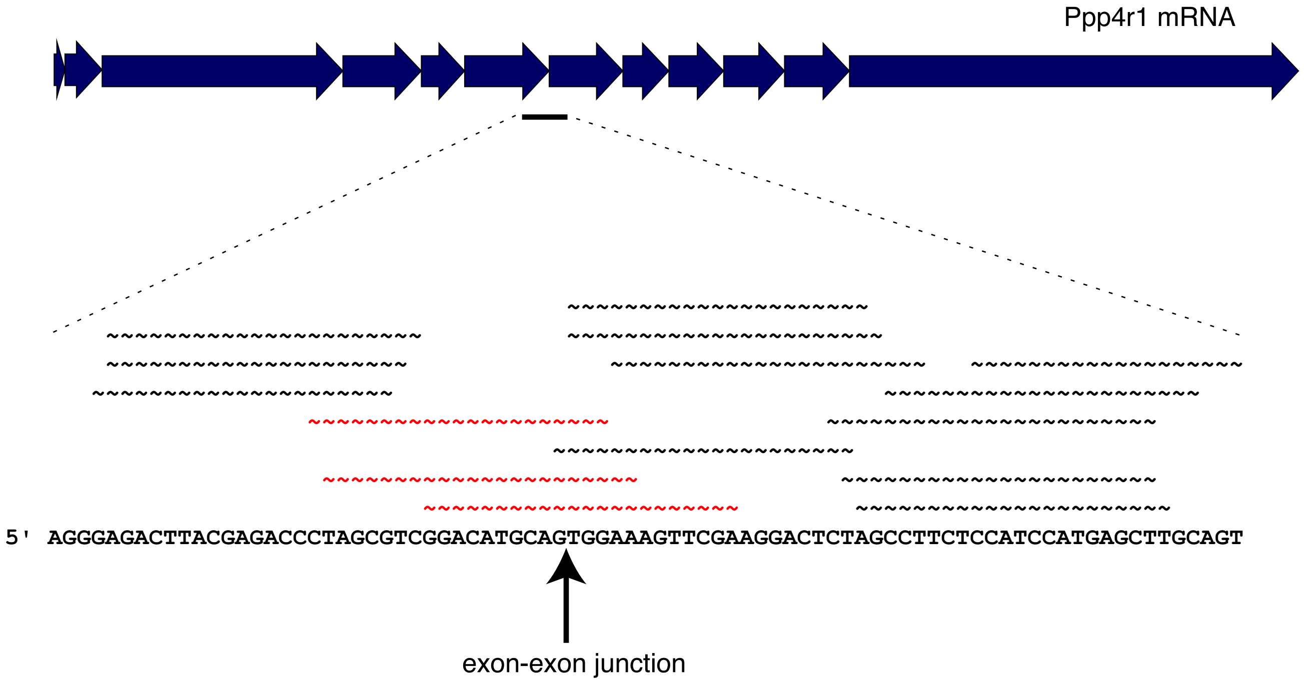

Figure S4. siRNAs that have perfect match to a segment of the Ppp4r1 mRNA near one splice junction (indicated) in the sense orientation are shown. Three siRNAs, shown in red span the exon-exon junction. These do not map directly to the genome (i.e. to pseudogenes) and thus must arise from spliced transcripts.

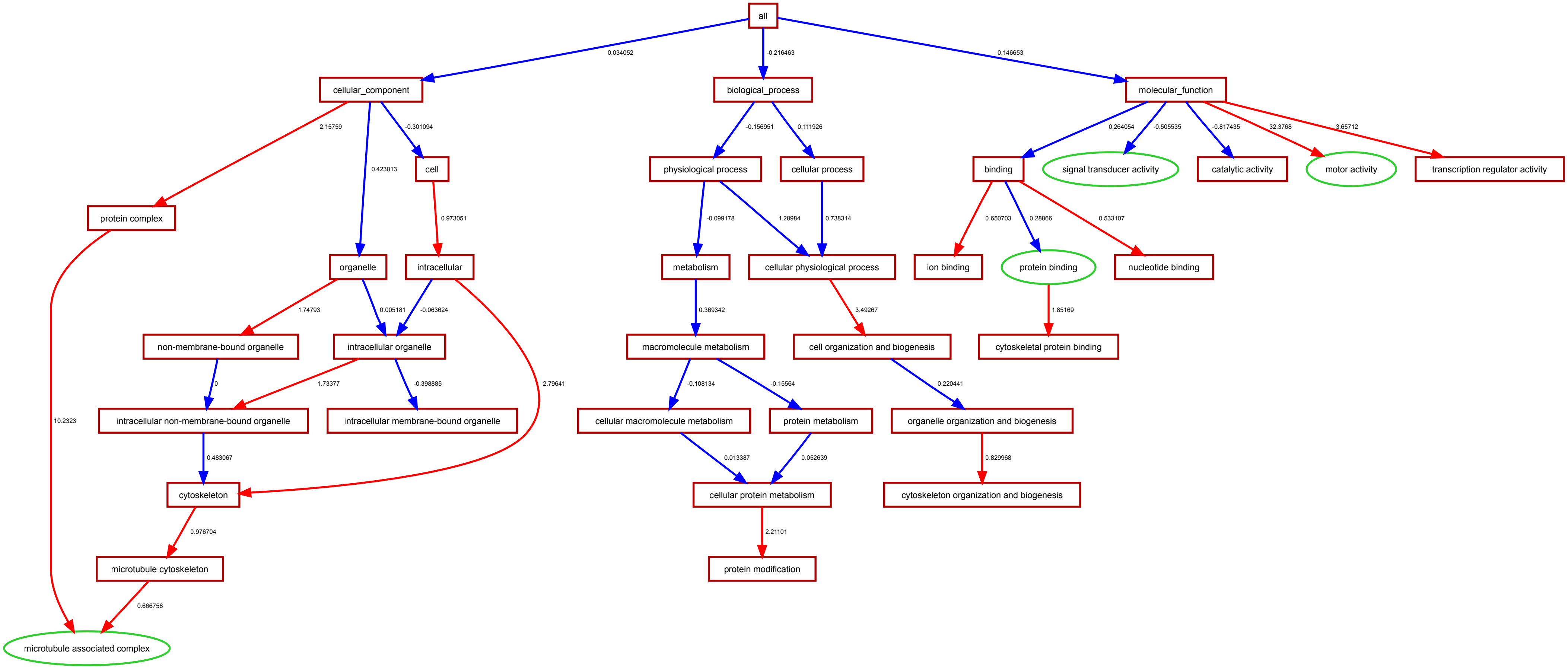

Figure S5. Enrichment in GO categories for endo-siRNA regulated genes. The 20 genes from Table 1 with the greatest fold change in the Dicer KO were analyzed for biological function using GOBAR1. Enriched groups, along with corresponding P-values, in parentheses, were GO:0007017 microtubule-based process (0), GO:0005871 kinesin complex (0), GO:0003774 motor activity (1.198218e-254), GO:0005819 spindle (8.122371e-239), GO:0005875 microtubule associated complex (3.199745e-60), GO:0005102 receptor binding (2.976938e-87), GO:0016564 transcriptional repressor activity (2.067180e-45). Categories of potential interest are highlighted in green ovals.

Table S1 Characteristics of the oocyte small RNA populations. The small RNA population of the two fractions are characterized based on total reads (both mapped and unmapped to genome), non-redundant sequences and clonal multiplicity. Relative percentages are also provided for most categories.

	Lower fraction		**Upper fraction**	
	Number	% (% of mapped)	Number	% (% of mapped)
total reads	1568637	100.0	1216443	100.0
mapped to genome	753981	48.1	283374	23.3
- unique	396007	25.2 (52.5)	57086	4.7 (20.1)
- multiple	357974	22.8 (47.5)	226288	18.6 (79.9)
non-mapped	814656	51.9	933069	76.7
Non-redundant sequences	623587	100.0	652490	100.0
mapped	126515	20.3	97807	15.0
- unique	46397	7.4 (36.7)	30150	4.6 (30.8)
- multiple	80118	12.8 (63.3)	67657	10.4 (69.2)
non-mapped	497072	79.7	554683	85.0
cloned once (mapped/unmapped)	101186/461001	16.2/73.9	73715/512099	11.3/78.5
cloned >1 (mapped/unmapped)	25329/36071	4.1/5.8	24092/42584	3.7/6.5

Table S2. Small RNAs mapping to transposons. The repeat-associated small RNAs are sorted into various categories of repeat-elements (LINE, SINE, LTR and others). Percentages of repeat-associated RNAs contributing to each class are also provided.

	Lower fraction		**Upper fraction**	
	# reads	**% total**	**# reads**	**% total**
Total	135359	100	180380	100
LINE	37036	27.4	114023	63.2
SINE	9303	6.8	2396	1.3
LTR	84827	62.6	59449	33.0
MT	44980	33.2	19848	11.0
IAP	12637	9.3	23763	13.2
other LTR	27210	20.1	15838	8.8
other repeats	4193	3.1	4512	2.5

Table S3. Genomic positions of oocyte piRNA clusters. A comprehensive list of piRNA-generating clusters in the genome that are expressed in oocytes is given.

position	size (kb)	unique piRNA (s/as)	total piRNA (s/as)
chr12:68748000-68772000	24	0/2886	6596/13571
chr10:82824000-82945000	121	17/2685	17616/23795
chr16:23105000-23112000	7	0/1580	6/1585
chr1:146067000-146072000	5	1288/0	1425/1
chr16:57387000-57392000	5	1071/0	3570/16
chr9:118580000-118585000	5	969/0	2498/20
chr17:56227000-56232000	5	932/0	1713/51
chr3:5856000-5861000	5	0/875	479/2519
chr10:43936000-43948000	12	0/715	6253/16794
chr6:47727000-47739000	12	327/366	4656/939
chr1:162961000-162969000	8	0/625	16/627
chrX:43749000-43757000	8	589/0	5288/572
chr19:8794000-8801000	7	0/556	33/586
chr10:23346000-23379000	33	490/0	27240/20908
chr6:3147000-3152000	5	1/482	929/3931
chr11:108868000-108874000	6	473/0	1207/21
chr7:118215000-118220000	5	336/0	337/0
chr13:97956000-97961000	5	314/0	962/10
chrX:50402000-50408000	6	313/1	313/1
chr4:130301000-130306000	5	0/281	2/288
chr14:31823000-31830000	7	229/0	246/50
chr16:13433000-13438000	5	0/222	18/231
chr11:68882000-68887000	5	0/213	0/215
chr4:43501000-43506000	5	207/0	1829/1393
chrX:94116000-94127000	11	0/200	2938/12824
chr8:53831000-53841000	10	170/0	10843/1983
chr5:147068000-147073000	5	0/153	7/400
chr11:106358000-106363000	5	0/151	20/183
chr10:117681000-117689000	8	18/124	152/597
chr15:82976000-82981000	5	1/138	11/175
chr4:116858000-116863000	5	0/129	12/196
chr15:79905000-79914000	9	114/0	131/12
chr10:23501000-23507000	6	110/0	116/1
chr12:110914000-110920000	6	0/108	49/108
chr14:115438000-115444000	6	0/108	8/113
chr7:19882000-19887000	5	0/106	25/109
chr10:26458000-26467000	9	0/103	488/392
chr11:48611000-48617000	6	0/103	8/107
chr6:12059000-12065000	6	0/102	1434/8664
chr2:130098000-130104000	6	0/100	2/126
chr11:77882000-77887000	5	0/99	7/121
chr10:90640000-90645000	5	97/0	115/33

chr10:26312000-26320000	8	92/0	4139/1605
chr1:89669000-89682000	13	0/89	4/90
chr2:144087000-144092000	5	89/0	101/4
chr8:3750000-3755000	5	87/0	834/5
chr5:90049000-90055000	6	0/85	392/702
chr13:96096000-96107000	11	0/81	0/81
chr15:64120000-64125000	5	81/0	81/0
chr19:5792000-5803000	11	79/0	416/115
chr16:17218000-17223000	5	0/78	73/442
chr11:116531000-116536000	5	0/77	0/89
chr17:35083000-35090000	7	71/0	81/38
chr15:32167000-32172000	5	70/0	90/0
chr15:81396000-81401000	5	0/64	3/147
chr17:23673000-23688000	15	15/49	189/3529
chr5:40963000-40973000	10	64/0	4340/2181
chr13:10722000-10728000	6	63/0	3779/922
chr18:35098000-35103000	5	62/0	64/6
chr7:6559000-6594000	35	62/0	837/812
chr5:108329000-108334000	5	0/61	4/74
chr6:128967000-128975000	8	0/60	1624/4615
chr11:62412000-62417000	5	0/59	5/72
chr16:35977000-35982000	5	57/2	761/45
chr18:13124000-13130000	6	0/58	1143/152
chr2:22596000-22601000	5	58/0	119/16
chr9:72946000-72954000	8	0/58	25/61
chr2:122197000-122204000	7	57/0	421/175
chr2:90231000-90236000	5	57/0	7861/3294
chr1:88248000-88253000	5	56/0	61/6
chr13:48627000-48634000	7	56/0	952/16
chr4:116822000-116829000	7	55/0	67/39
chr4:13343000-13348000	5	0/55	529/909
chr11:77990000-77997000	7	54/0	55/1
chr18:16219000-16232000	13	54/0	2317/659
chr6:47925000-47930000	5	0/54	4/789
chr6:75591000-75600000	9	0/53	2098/4535
chr7:106624000-106632000	8	53/0	65/14
chr16:11140000-11145000	5	0/52	1/35
chr17:5769000-5779000	10	0/52	165/2339
chr1:9928000-9933000	5	51/0	123/89
chr7:82911000-82925000	14	51/0	214/92
chr5:138602000-138607000	5	49/1	49/1

Table S4. microRNAs in oocytes. This table presents a list of miRNAs found in oocytes arranged from the most abundant to the least abundant.

annotation	reads in oocyte_lower
mmu-let-7c-2	67714
mmu-mir-322	45012
mmu-mir-22	29153
mmu-let-7f-1	21598
mmu-let-7a-1	20904
mmu-mir-16-1	19495
mmu-mir-503	19159
mmu-let-7b	17124
mmu-mir-26a-2	15965
mmu-mir-21	14691
mmu-let-7g	13048
mmu-mir-103-1	12016
mmu-mir-20a	10888
mmu-mir-672	9555
mmu-let-7d	8601
mmu-mir-29a	7812
mmu-mir-29b-2	7267
mmu-mir-130a	6780
mmu-let-7i	6748
mmu-mir-93	5868
mmu-mir-451	5676
mmu-mir-202	5100
mmu-mir-143	4897
mmu-mir-30e	4711
mmu-mir-26b	4430
mmu-mir-126	4324
mmu-mir-181a-1	4292
mmu-mir-210	4290
mmu-mir-351	4257
mmu-mir-24-1	4105
mmu-mir-17	3535
mmu-let-7e	3416
mmu-mir-450a-1	3238
mmu-mir-30d	3232
mmu-mir-15b	2950
mmu-mir-92a-1	2946
mmu-mir-10b	2779
mmu-mir-30a	2690
mmu-mir-34c	2529
mmu-mir-99a	2309
mmu-mir-191	2224
mmu-mir-125a	2205
mmu-mir-107	2171
mmu-mir-27b	2097
mmu-mir-181c	2079

mmu-mir-379	2066
mmu-mir-34a	2037
mmu-mir-99b	1897
mmu-mir-125b-2	1860
mmu-mir-199a-2	1792
mmu-mir-101b	1785
mmu-mir-320	1702
mmu-mir-541	1411
mmu-mir-25	1386
mmu-mir-411	1299
mmu-mir-151	1282
mmu-let-7f-2	1279
mmu-mir-127	1250
mmu-mir-98	1156
mmu-mir-497	1140
mmu-mir-181b-1	1125
mmu-mir-486	1121
mmu-mir-195	1089
mmu-mir-15a	1056
mmu-mir-744	1012
mmu-mir-181d	958
mmu-mir-423	942
mmu-mir-30c-2	877
mmu-mir-23a	864
mmu-mir-689-1	859
mmu-mir-30b	813
mmu-mir-23b	788
mmu-mir-339	781
mmu-mir-674	768
mmu-mir-205	740
mmu-mir-382	728
mmu-mir-29c	706
mmu-mir-296	700
mmu-mir-369	698
mmu-mir-138-2	681
mmu-mir-324	678
mmu-mir-542	647
mmu-mir-18a	645
mmu-mir-301a	632
mmu-mir-425	631
mmu-mir-342	627
mmu-mir-145	625
mmu-mir-433	609
mmu-mir-652	608
mmu-mir-378	578
mmu-mir-141	575
mmu-mir-376a	575
mmu-mir-19b-1	563
mmu-mir-34b	507

mmu-mir-28	502
mmu-mir-106b	475
mmu-mir-294	474
mmu-mir-340	467
mmu-mir-154	466
mmu-mir-292	453
mmu-mir-328	439
mmu-mir-669a-1	422
mmu-mir-138-1	409
mmu-mir-10a	401
mmu-mir-136	399
mmu-mir-196a-1	394
mmu-mir-200a	392
mmu-mir-467a	391
mmu-mir-361	389
mmu-mir-221	379
mmu-mir-201	365
mmu-mir-299	365
mmu-mir-409	360
mmu-mir-27a	345
mmu-mir-376b	345
mmu-mir-140	343
mmu-mir-450b	333
mmu-mir-298	331
mmu-mir-708	331
mmu-mir-337	325
mmu-mir-144	322
mmu-mir-434	318
mmu-mir-341	313
mmu-mir-370	298
mmu-mir-547	298
mmu-mir-532	296
mmu-mir-494	289
mmu-mir-130b	287
mmu-mir-185	287
mmu-mir-186	280
mmu-mir-381	271
mmu-mir-200c	266
mmu-mir-300	260
mmu-mir-431	254
mmu-mir-223	253
mmu-mir-100	251
mmu-mir-376c	249
mmu-mir-155	247
mmu-mir-805	246
mmu-mir-214	236
mmu-mir-665	235
mmu-mir-218-2	233
mmu-mir-467c	232

mmu-mir-671	231
mmu-let-7c-1	217
mmu-mir-134	215
mmu-mir-128a	211
mmu-mir-31	208
mmu-mir-181b-2	205
mmu-mir-872	200
mmu-mir-455	198
mmu-mir-484	187
mmu-mir-182	186
mmu-mir-669b	186
mmu-mir-374	180
mmu-mir-741	176
mmu-mir-32	175
mmu-mir-295	168
mmu-mir-335	167
mmu-mir-669c	167
mmu-mir-500	150
mmu-mir-181a-2	132
mmu-mir-142	130
mmu-mir-7a-1	130
mmu-mir-871	126
mmu-mir-429	125
mmu-mir-487b	122
mmu-mir-203	115
mmu-mir-362	111
mmu-mir-377	110
mmu-mir-540	110
mmu-mir-101a	105
mmu-mir-16-2	102
mmu-mir-19a	102
mmu-mir-183	87
mmu-mir-149	86
mmu-mir-470	86
mmu-mir-410	85
mmu-mir-465c-1	85
mmu-mir-297a-2	83
mmu-mir-501	83
mmu-mir-743a	83
mmu-mir-615	82
mmu-mir-676	82
mmu-mir-291b	80
mmu-mir-139	77
mmu-mir-1-2	76
mmu-mir-196a-2	76
mmu-mir-421	74
mmu-mir-188	73
mmu-mir-485	69
mmu-mir-146b	67

mmu-mir-152	59
mmu-mir-193b	59
mmu-mir-466a	58
mmu-mir-880	57
mmu-mir-345	55
mmu-mir-133a-1	54
mmu-mir-290	52
mmu-mir-96	51
mmu-mir-148b	49
mmu-mir-222	48
mmu-mir-380	48
mmu-mir-9-2	47
mmu-mir-194-2	46
mmu-mir-375	42
mmu-mir-146a	40
mmu-mir-190	40
mmu-mir-291a	40
mmu-mir-323	40
mmu-mir-350	37
mmu-mir-466f-1	37
mmu-mir-200b	36
mmu-mir-193	33
mmu-mir-574	32
mmu-mir-196b	31
mmu-mir-878	30
mmu-mir-881	30
mmu-mir-297a-3	29
mmu-mir-465a	29
mmu-mir-874	29
mmu-mir-466h	28
mmu-mir-33	27
mmu-mir-326	26
mmu-mir-192	25
mmu-mir-338	25
mmu-mir-449a	25
mmu-mir-125b-1	24
mmu-mir-30c-1	24
mmu-mir-412	24
mmu-mir-450a-2	24
mmu-mir-466d	24
mmu-let-7a-2	23
mmu-mir-743b	23
mmu-mir-132	22
mmu-mir-19b-2	21
mmu-mir-224	21
mmu-mir-543	21
mmu-mir-700	21
mmu-mir-103-2	20
mmu-mir-199b	20

mmu-mir-219-1	20
mmu-mir-194-1	19
mmu-mir-301b	18
mmu-mir-220	17
mmu-mir-293	17
mmu-mir-329	17
mmu-mir-150	16
mmu-mir-92a-2	16
mmu-mir-331	15
mmu-mir-129-2	14
mmu-mir-715	14
mmu-mir-471	13
mmu-mir-505	13
mmu-mir-217	12
mmu-mir-873	12
mmu-mir-877	12
mmu-mir-883a	12
mmu-mir-20b	11
mmu-mir-511	10
mmu-mir-592	10
mmu-mir-742	10

Table S5. Potential regulatory targets of endogenous siRNAs. The 20 genes with the largest number of siRNAs are listed. The number of siRNAs that match the protein coding mRNA corresponding to that gene with no mismatches (perfect) or with from 1 to 4 mismatches (mismatched) in the sense (s) or antisense (as) orientation are listed. Also indicated is whether siRNAs can also be mapped to a homologous pseudogene or whether convergent transcription of two annotated genes can potentially produce dsRNA.

Gene	**Perfect siRNA (s/as)**	**Mismatched siRNA (s/as)**	**siRNA-producing pseudogene**	**Convergent transcription of two genes**
BC003993	1853/1510	665/648	-	-
Sp110	475/88	3606/154	+	-
Rnf168	721/599	1214/658	-	-
Rangap1	433/925	1037/779	+	-
2610016C23Rik	245/45	1857/62	+	-
2410002F23Rik	3/157	50/1679	-	-
Kifc1	523/223	253/248	+	-
prc1	249/156	226/160	+	-
Lcp1	249/231	123/153	+	-
oog4	411/90	94/74	+	-
optn	261/148	121/92	+	-
Trim71	144/146	179/131	-	-
1110067D22Rik	128/62	100/263	+	-
Ppp4r1	208/92	113/90	+	-
Fbxo34	180/132	98/91	+	-
Kif4	189/124	102/49	-	+
ncoa2	56/75	72/250	+	-
kif2c	174/75	97/90	+	-
Ankrd28	39/24	219/147	-	-
traip	199/79	71/77	+	-