

Table S1. Male and Female Fertility, Related to Figure 1

	<b>Males</b>	<b>Females</b>				
<b>Genotype</b>	<b>Number of viable offspring</b>	<b>Eggs per female per day</b>	<b>Percent hatching</b>	<b>Dorsal appendage</b>		
				<b>WT</b>	<b>Fused</b>	<b>Absent</b>
<i>w<sup>1118</sup></i>	236 ± 41	45	54	91	6.9	2.2
<i>loqs<sup>KO</sup></i> ; <u>Loqs-PA</u> TM3	177 ± 51	4.3	0.0	5.9	51	43
<i>loqs<sup>KO</sup></i> ; <u>Loqs-PB</u> TM3	186 ± 28	38	53	91	6.5	2.9
<i>loqs<sup>KO</sup></i> ; <u>Loqs-PA,PB</u> TM3	231 ± 46	38	53	88	11	1.6
<i>loqs<sup>KO</sup></i> ; <u>Loqs-PA,PD</u> TM3	186 ± 23	6.0	2.9	14	56	30
<i>loqs<sup>KO</sup></i> ; <u>Loqs-PB,PD</u> TM3	184 ± 57	36	49	89	7.6	3.1
<i>loqs<sup>KO</sup></i> ; <u>Loqs-PA,PB,PD</u> TM3	242 ± 56	40	53	88	10	1.8
<i>loqs<sup>KO</sup></i> CyO	178 ± 46	37	48	88	9.2	2.8

Male fertility (mean ± SD) was assessed by measuring the number of adult progeny for five individual males. For female fertility, virgin females of each genotype were mated to Oregon R virgin males.

Table S2A. Sequencing Statistics for Head Small RNAs: Reads, Related to Figure 2

Genotype	Total reads	Genome-mapping reads	ncRNA reads	Reads excluding ncRNAs	miRNA reads	siRNA reads	Transposon-mapping siRNA reads	Sense siRNA reads	Anti-sense siRNA reads	<i>white</i> -mapping siRNA reads
wIR	5,143,662	4,120,314	114,789	4,005,525	3,956,636	30,055	6,073	2,894	3,381	28,115
wIR; <i>loqs</i> <sup>KO</sup> ; <u>Loqs-PA</u> TM3	5,332,109	3,884,775	250,434	3,634,341	3,584,589	20,753	7,171	3,476	3,765	15,973
wIR; <i>loqs</i> <sup>KO</sup> ; <u>Loqs-PB</u> TM3	4,782,575	3,678,923	90,062	3,588,861	3,560,971	10,415	5,096	2,520	2,611	3,941
wIR; <i>loqs</i> <sup>KO</sup> ; <u>Loqs-PA,PB</u> TM3	6,130,720	4,739,003	98,230	4,640,773	4,584,068	30,115	12,212	5,896	6,510	18,189
wIR; <i>loqs</i> <sup>KO</sup> ; <u>Loqs-PA,PD</u> TM3	10,668,796	8,495,365	392,910	8,102,455	7,364,992	530,353	63,232	30,126	35,150	591,633
wIR; <i>loqs</i> <sup>KO</sup> ; <u>Loqs-PB,PD</u> TM3	12,082,280	9,601,029	513,625	9,087,404	8,656,513	266,425	42,271	19,330	24,354	298,875
wIR; <i>loqs</i> <sup>KO</sup> ; <u>Loqs-PA,PB,PD</u> TM3	3,699,368	3,053,173	153,124	2,900,049	2,801,168	59,263	8,136	3,820	4,555	70,006
wIR; <u><i>loqs</i><sup>KO</sup></u> CyO	6,626,175	5,211,422	163,428	5,047,994	4,959,148	54,788	12,745	6,360	6,594	47,052
wIR; <i>loqs</i> <sup>f00791</sup>	5,574,653	3,791,509	267,899	3,523,610	3,472,433	16,301	5,093	2,641	2,535	9,486
wIR; <u><i>loqs</i><sup>f00791</sup></u> CyO	3,389,994	2,526,164	109,455	2,416,709	2,376,900	21,489	3,772	1,763	2,090	21,244

Table S2B. Sequencing Statistics for Head Small RNAs: Species, Related to Figure 2

Genotype	Total species	Genome-mapping species	ncRNA species	Species excluding ncRNAs	miRNA species	siRNA species	Transposon-mapping siRNA species	Sense siRNA species	Anti-sense siRNA species	white-mapping siRNA species
<i>wIR</i>	461,579	43,365	21,087	22,278	1,883	7,991	4,186	2,027	2,240	2,432
<i>wIR; loqs<sup>KO</sup> ; Loqs-PA<sub>TM3</sub></i>	629,736	65,174	34,752	30,422	2,175	9,393	5,739	2,759	3,037	2,650
<i>wIR; loqs<sup>KO</sup> ; Loqs-PB<sub>TM3</sub></i>	503,847	42,865	21,148	21,717	1,668	7,083	4,242	2,062	2,210	1,184
<i>wIR; loqs<sup>KO</sup> ; Loqs-PA,PB<sub>TM3</sub></i>	555,458	56,479	20,730	35,749	2,204	14,788	9,451	4,535	5,053	2,366
<i>wIR; loqs<sup>KO</sup> ; Loqs-PA,PD<sub>TM3</sub></i>	876,748	170,903	41,522	129,381	2,581	58,435	32,868	15,923	17,765	7,430
<i>wIR; loqs<sup>KO</sup> ; Loqs-PB,PD<sub>TM3</sub></i>	957,662	150,797	47,344	103,453	2,704	43,074	21,883	10,257	12,171	6,973
<i>wIR; loqs<sup>KO</sup> ; Loqs-PA,PB,PD<sub>TM3</sub></i>	299,728	61,983	29,178	32,805	1,925	11,884	6,162	2,919	3,380	4,828
<i>wIR; loqs<sup>KO</sup> CyO</i>	605,000	64,530	26,539	37,991	1,866	14,749	8,265	4,100	4,279	3,029
<i>wIR; loqs<sup>100791</sup></i>	702,842	70,142	37,826	32,316	2,261	9,078	4,417	2,318	2,171	1,022
<i>wIR; loqs<sup>100791</sup> CyO</i>	360,373	43,549	22,552	20,997	1,777	6,726	2,774	1,339	1,485	2,933

Table S3A. Sequencing Statistics for Ovary Small RNAs: Reads, Related to Figure 2

Genotype	Total reads	Genome-mapping reads	ncRNA reads	Reads excluding ncRNAs	miRNA reads	siRNA reads	Transposon-mapping siRNA reads	Sense siRNA reads	Anti-sense siRNA reads
$w^{1118}$	20,285,360	11,977,108	1,478,344	10,498,764	7,534,273	145,334	76,945	27,743	50,814
$w^{1118}; loqs^{KO}; \underline{Loqs-PA}$ TM3	24,338,576	15,087,578	5,543,991	9,543,587	5,697,600	202,654	109,441	57,637	53,943
$w^{1118}; loqs^{KO}; \underline{Loqs-PB}$ TM3	16,857,747	12,172,239	3,278,461	8,893,778	7,042,089	87,957	37,154	15,018	22,862
$w^{1118}; loqs^{KO}; \underline{Loqs-PA,PB}$ TM3	20,940,001	10,784,229	2,547,131	8,237,098	6,525,639	88,580	38,065	15,914	23,079
$w^{1118}; loqs^{KO}; \underline{Loqs-PA,PD}$ TM3	38,011,406	19,955,061	5,186,462	14,768,599	9,236,453	303,876	139,811	61,167	82,012
$w^{1118}; loqs^{KO}; \underline{Loqs-PB,PD}$ TM3	22,774,181	14,566,892	2,981,469	11,585,423	9,068,347	194,015	84,673	35,854	50,803
$w^{1118}; loqs^{KO}; \underline{Loqs-PA,PB,PD}$ TM3	40,114,682	30,501,930	5,828,677	24,673,253	18,282,367	389,501	192,941	80,855	116,502
$w^{1118}; \underline{loqs^{KO}}$ CyO	20,334,011	14,144,591	4,443,759	9,700,832	6,872,591	42,181	20,703	7,084	14,063
$w^{1118}; loqs^{f00791}$	23,728,591	16,209,591	11,935,822	4,273,769	2,556,306	121,801	50,290	19,695	31,635
$w^{1118}; \underline{loqs^{f00791}}$ CyO	23,879,843	17,066,428	2,535,062	14,531,366	11,499,746	233,961	127,962	52,903	77,551

Table S3B. Sequencing Statistics for Ovary Small RNAs: Species, Related to Figure 2

Genotype	Total species	Genome-mapping species	ncRNA species	Species excluding ncRNAs	miRNA species	siRNA species	Transposon mapping siRNA species	Sense siRNA species	Anti-sense siRNA species
$w^{1118}$	4,211,574	1,027,935	61,865	966,070	2,237	67,021	34,611	13,963	21,339
$w^{1118}; loqs^{KO}; \underline{Loqs-PA}_{TM3}$	5,242,781	1,343,890	98,574	1,245,316	2,855	98,998	41,672	18,442	24,058
$w^{1118}; loqs^{KO}; \underline{Loqs-PB}_{TM3}$	3,098,806	847,317	74,999	772,318	2,402	54,237	20,608	8,666	12,328
$w^{1118}; loqs^{KO}; \underline{Loqs-PA,PB}_{TM3}$	5,826,232	767,601	79,939	687,662	2,593	50,455	19,914	8,648	11,704
$w^{1118}; loqs^{KO}; \underline{Loqs-PA,PD}_{TM3}$	8,445,739	1,909,704	105,767	1,803,937	3,970	166,579	68,526	31,481	38,440
$w^{1118}; loqs^{KO}; \underline{Loqs-PB,PD}_{TM3}$	4,604,486	971,713	73,899	897,814	2,487	99,985	40,848	18,164	23,498
$w^{1118}; loqs^{KO}; \underline{Loqs-PA,PB,PD}_{TM3}$	5,516,798	1,705,107	89,316	1,615,791	2,943	164,126	70,037	31,030	40,371
$w^{1118}; \underline{loqs^{KO}}_{CyO}$	2,782,341	1,213,556	73,674	1,139,882	2,271	29,963	13,581	5,068	8,739
$w^{1118}; loqs^{f00791}$	5,217,078	1,021,679	112,106	909,573	2,602	89,627	34,347	14,193	20,814
$w^{1118}; \underline{loqs^{f00791}}_{CyO}$	4,360,684	1,320,887	88,279	1,232,608	3,074	138,076	72,108	31,792	41,492

Table S4. Michaelis-Menten Analysis of Dicing of Pre-esi2.1, Pre-miR-305, and Pre-*let*-7 RNAs,  
Related to Figure 2

Substrate	Enzyme	$K_M$ (nM)	Change in $K_M$	$k_{cat}$ (min <sup>-1</sup> )	Change in $k_{cat}$	$k_{cat} / K_M$ (nM <sup>-1</sup> min <sup>-1</sup> )	Change in $k_{cat} / K_M$
pre- esi-2.1	Dcr-2	135 ± 32	1.0	0.073 ± 0.003	1.0	0.0006 ± 0.0002	1.0
	Dcr-2 + Loqs-PD	31 ± 5	0.23	0.085 ± 0.007	1.2	0.0028 ± 0.0002	5
pre- miR-305	Dcr-1	2.2 ± 0.1	1.0	0.11 ± 0.00	1.0	0.048 ± 0.001	1.0
	Dcr-1 + Loqs-PA	1.5 ± 0.3	0.69	0.11 ± 0.00	1.0	0.072 ± 0.001	1.5
	Dcr-1 + Loqs-PB	1.4 ± 0.1	0.62	0.15 ± 0.00	1.4	0.105 ± 0.002	2.2
pre- <i>let</i> -7	Dcr-1	3.0 ± 0.2	1.0	0.52 ± 0.01	1.0	0.18 ± 0.01	1.0
	Dcr-1 + Loqs-PA	2.7 ± 0.7	0.91	0.51 ± 0.02	0.98	0.20 ± 0.05	1.1
	Dcr-1 + Loqs-PB	1.8 ± 0.2	0.60	0.49 ± 0.02	0.94	0.32 ± 0.04	1.6

Data are mean ± SD for three independent trials.

Table S5A. Sequencing Statistics for Small RNAs from Mouse Embryonic Fibroblasts: Reads, Related to Figure 7

	<b>Total Reads</b>	<b>Genome- mapping reads</b>	<b>ncRNA reads</b>	<b>Reads excluding ncRNAs</b>	<b>miRNA reads</b>	<b>siRNA reads</b>	<b>trans- poson mapping siRNA reads</b>	<b>Sense siRNA reads</b>	<b>Anti- sense siRNA reads</b>
Wild-type	24,312,750	15,393,202	335,492	15,057,710	14,865,314	19,584	1,120	431	727
<i>PACT</i> <sup>-/-</sup>	24,067,347	14,151,935	178,512	13,973,423	13,816,263	13,588	477	160	351
<i>TRBP</i> <sup>+/-</sup>	24,667,276	14,893,408	442,714	14,450,694	14,264,724	20,821	901	377	569
<i>TRBP</i> <sup>-/-</sup>	25,566,992	13,807,806	541,041	13,266,765	12,988,864	31,121	1,920	884	1,087

Table S5B. Sequencing Statistics for Small RNAs from Mouse Embryonic Fibroblasts: Species, Related to Figure 7

	<b>Total Species</b>	<b>Genome- mapping species</b>	<b>ncRNA species</b>	<b>Species excluding ncRNAs</b>	<b>miRNA species</b>	<b>siRNA species</b>	<b>trans- poson mapping siRNA species</b>	<b>Sense siRNA species</b>	<b>Anti- sense siRNA species</b>
Wild-type	1,890,704	87,832	26,428	61,404	6,560	8,229	575	338	268
<i>PACT</i> <sup>-/-</sup>	1,912,168	64,322	18,055	46,267	6,074	5,276	274	139	165
<i>TRBP</i> <sup>+/-</sup>	2,371,800	113,718	33,103	80,615	6,559	10,538	553	304	292
<i>TRBP</i> <sup>-/-</sup>	3,457,985	151,253	35,464	115,789	7,524	15,287	834	517	365