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Supplemental Data

A piRNA Pathway Primed by Individual Transposons

Is Linked to De Novo DNA Methylation in Mice

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Supplemental Experimental Procedures

Transgenic Animals

To produce transgenic animals expressing tagged MILI and MIWI2 proteins, BAC clones that contain each gene and its flanking regions (BAC RP23-366F18 and RP23-414K6 for *Mili* and *Miwi2*, respectively) were modified by recombineering using the counter selection BAC modification kit (Gene Bridges, K002). A 3xMyc, 3xFLAG-HA, or EGFP sequence was seamlessly inserted after the start codon of each gene. For *Miwi2* gene, the start codon at position chr9: 14,544,986 - 14,544,988 (according to the July 2007 genome assembly, UCSC genome browser) was used for tag insertion. Correct insertions were verified by sequencing and restriction endonuclease digests of BAC clones. Positive BACs were purified using the Nucleobond BAC 100 kit (Clontech), linearized using I-SceI digestion and submitted for pro-nuclear injection into oocytes of B6xSJL F1 hybrids. Founder animals were genotyped by PCR and positive animals crossed to C57BL/6J mice to establish independent transgenic lines.

Antibody validation

The specificity of antibodies was tested using testis extracts from transgenic animals expressing 3xFLAG-HA-*Miwi2*. MIWI2 protein was immunoprecipitated using specific antibodies and protein bound to beads was probed by Western blotting with anti-HA antibodies. To test specificity for Western blotting, 3xFLAG-HA-MIWI2 fusion protein was first purified using anti-FLAG resin and then probed by Western with specific anti-MIWI2 antibodies.

Northern hybridization

To detect IAP piRNAs, 30 µg of total 10dpp testis RNA isolated from wild-type and *Dnmt3L* KO animals were loaded onto a 17% polyacrylamide, 7M urea gel. Hybridization was performed according to (Varallyay et al., 2007) using an LNA probe detecting IAP sense piRNAs (5'-tcCatcTgAcggcagAAcTgygraa-3', where

LNA substitutions are shown with capital letters). A DNA oligonucleotides probe was used to detect let7 miRNA (5'-aactatacaacctactacctca-3').

Immunofluorescence

For immunofluoresent detection, testes were fixed in either Bouin's solution (Sigma) or 4% paraformaldehyde, sequentially washed in 50% and 70% ethanol and embedded in paraffin. Slides were treated first in xylene (twice, 5 min) and then in an electric pressure cooker for 15 min in Trilogy solution (Cell Marque). After blocking in blocking solution (10 mM Tris pH 7.4, 100 mM $MgCl_2$, 0.5% Tween20, 1% BSA, 5% FBS) for one hour at room temperature, slides were incubated with antibody diluted in blocking solution at 4°C overnight. After washing with PBS (10 min, twice), slides were incubated with secondary antibodies (Alexa Fluor 488 anti-rabbit or Alexa Fluor 568 anti-mouse, Invitrogen, diluted 1:250) for one hour at room temperature. After washing, slides were incubated 5 min in 1:250 solution of DRAQ5 (Biostatus Limited) to visualize DNA and after washing mounted in ProLong Gold antifade reagent (Molecular Probes). Images were taken using a Zeiss confocal microscope.

Supplementary Figure 1

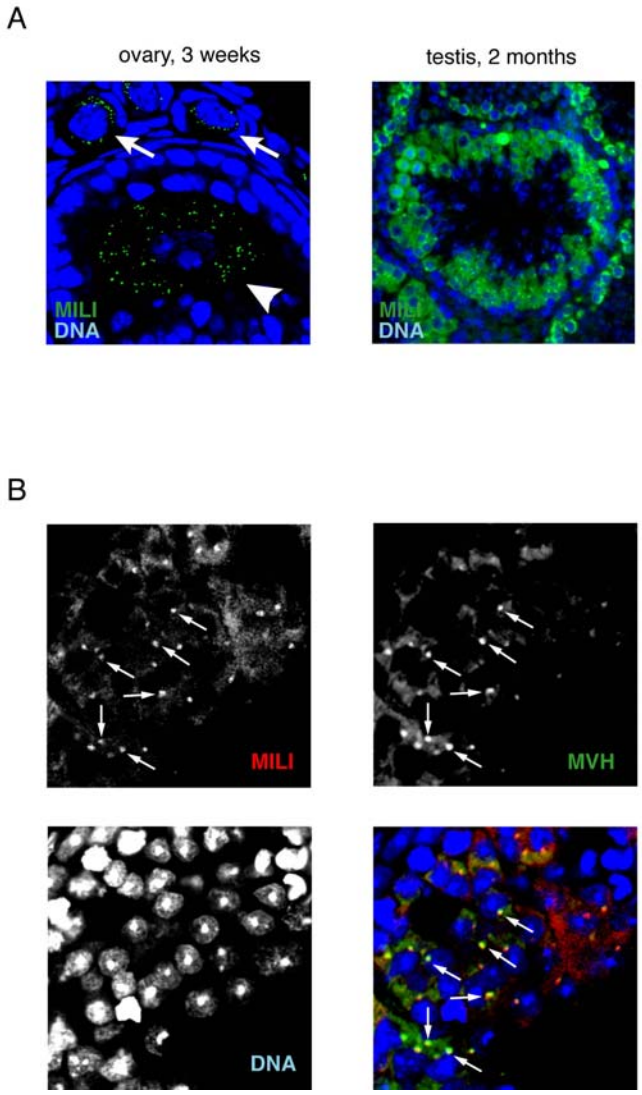

Figure S1. (A) MILI expression in post-natal germ cells. GFP-MILI is present in cytoplasmic granules in arrested (arrows) and growing (arrowhead) oocytes in 3-week old females (left panel). In adult testis MILI is expressed in all stages of germ cell development until the round spermatid stage. **(B**) Co-localization of MILI and MVH in chromatoid bodies. Immunofluorescence detection of MILI and MVH was performed in testes of 6 month old GFP-MILI transgenic animals using chicken anti-EGFP (Abcam ab13970) and rabbit anti-MVH (Abcam ab13840) antibodies.

Supplementary Figure 2

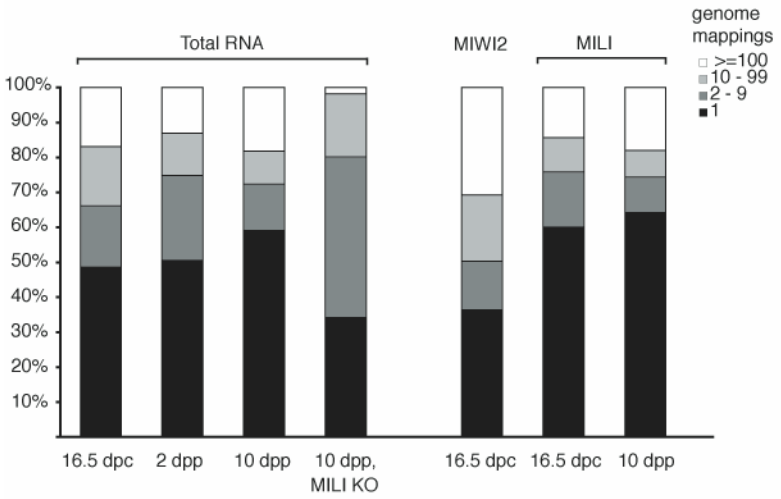

Figure S2. The distribution of small RNAs with the indicated frequency of matching to the mouse genome is presented graphically for libraries described in Fig. 1E.

Table S1

Library (Age, genotype, purification)	16.5 dpc, WT, total RNA 24-33nt	2 dpp, WT, total RNA 24-33nt	10 dpp, WT, total RNA 24-33 nt	10 dpp, MILI KO, total RNA 24-33 nt	16.5 dpc, WT, MIWI2 IP	16.5 dpc, WT, MILI IP	10 dpp, WT, MILI IP	10 dpp, Dnmt3L KO, MILI IP
Number of reads	2,658,307	3,103,747	4,353,823	3,608,352	3,878,709	2,388,544	2,667,543	3,543,193
Number of reads, mapped to genome	1,647,958	1,464,120	2,003,695	487,378	1,940,312	471,657	1,326,644	2,150,954
Annotation categories								
LTR	421,240	286,096	309,300	4,465	901,024	125,982	197,402	674,288
LINE	280,206	178,771	197,479	3,514	573,484	89,486	92,573	220,867
SINE	82,240	113,836	293,321	14,695	113,962	31,140	288,465	276,282
DNA transposons	7,061	8,264	8,437	796	9,158	2,504	5,924	8,164
Exon (sense)	35,497	69,265	362,373	19,284	17,923	19,459	303,113	373,490
Exon (antisense)	6,835	11,930	10,284	439	5,530	3,045	7,002	11,140
non-coding RNAs	487,734	357,994	298,325	405,345	35,527	68,041	35,479	34,234
Unannotated	334,206	446,228	532,613	39,636	292,862	134,504	402,610	560,653
Mapping to the genome								
1 position	802,098	740,569	1,183,851	166,770	706,383	283,326	851,310	1,136,534
2-9 position	288,076	355,749	266,030	223,814	268,953	74,347	134,853	184,070
10-99 position	278,457	176,282	190,250	87,999	368,553	46,108	101,008	195,687
>=100 positions	279,327	191,520	363,564	8,795	596,423	67,876	239,473	634,663
Specific transposable elements								
LTR IAP (sense)	63,519	24,293	97,414	1,464	144,401	21,836	62,097	421,905
LTR IAP (antisense)	57,270	34,868	41,657	221	164,437	19,993	22,812	32,025
LINE L1 (sense)	96,143	50,938	73,767	2,167	173,065	62,920	27,107	92,445
LINE L1 (antisense)	151,029	101,617	101,356	783	343,664	16,070	50,950	102,743
SINE B1 (sense)	11,570	19,589	103,580	4,023	15,302	5,032	103,408	73,665
SINE B1 (antisense)	12,553	15,626	41,026	439	20,967	3,785	64,038	53,795