Supplementary Data

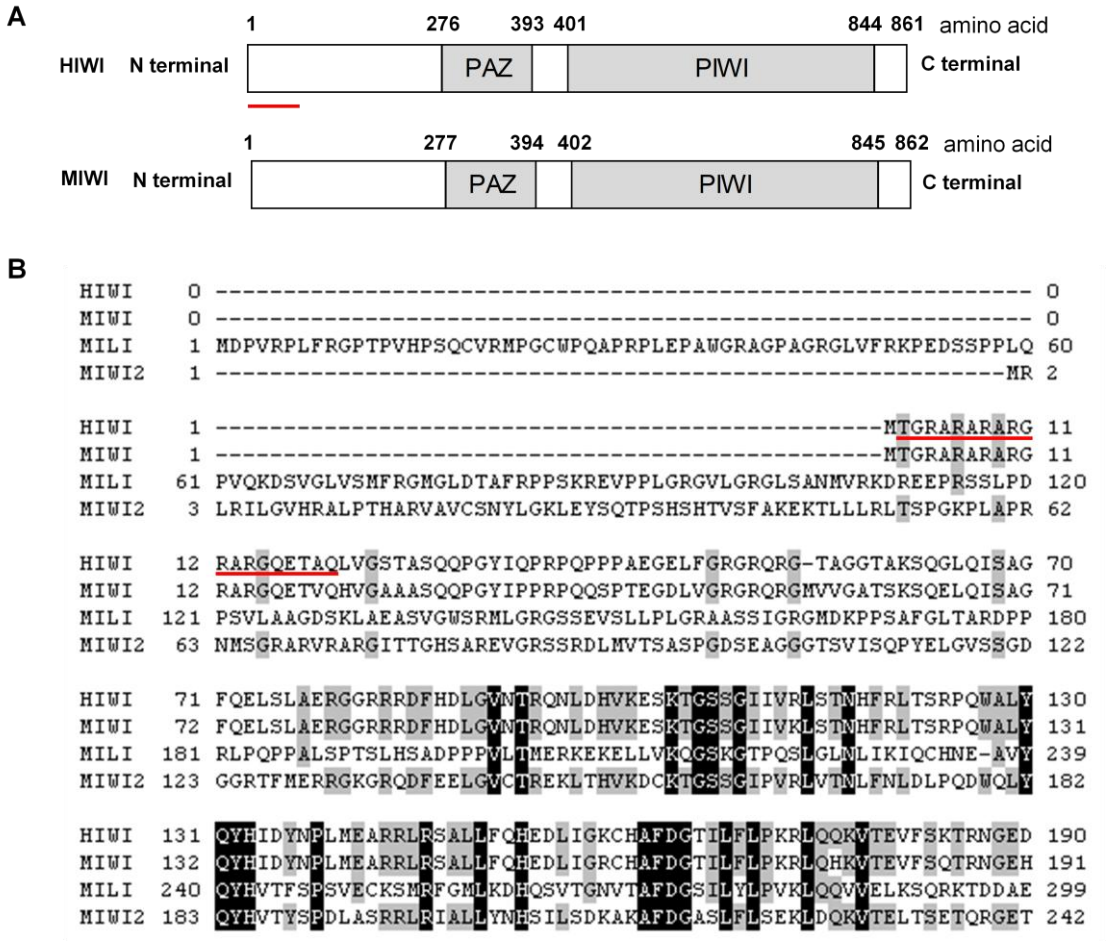

Figure S1. The region and amino acid sequence of antigen for producing anti-HIWI/MIWI monoclonal antibody. (A) Domain maps of HIWI and MIWI. The region of antigen for producing anti-HIWI/MIWI monoclonal antibody is shown by a red line. PAZ, PAZ domain. PIWI, PIWI domain. **(B)** Comparison of amino acid sequences of HIWI and mouse PIWI subfamily proteins. The sequence of peptide used as antigen for producing anti-HIWI/MIWI monoclonal antibody is underlined with a red line.

Supplementary Data

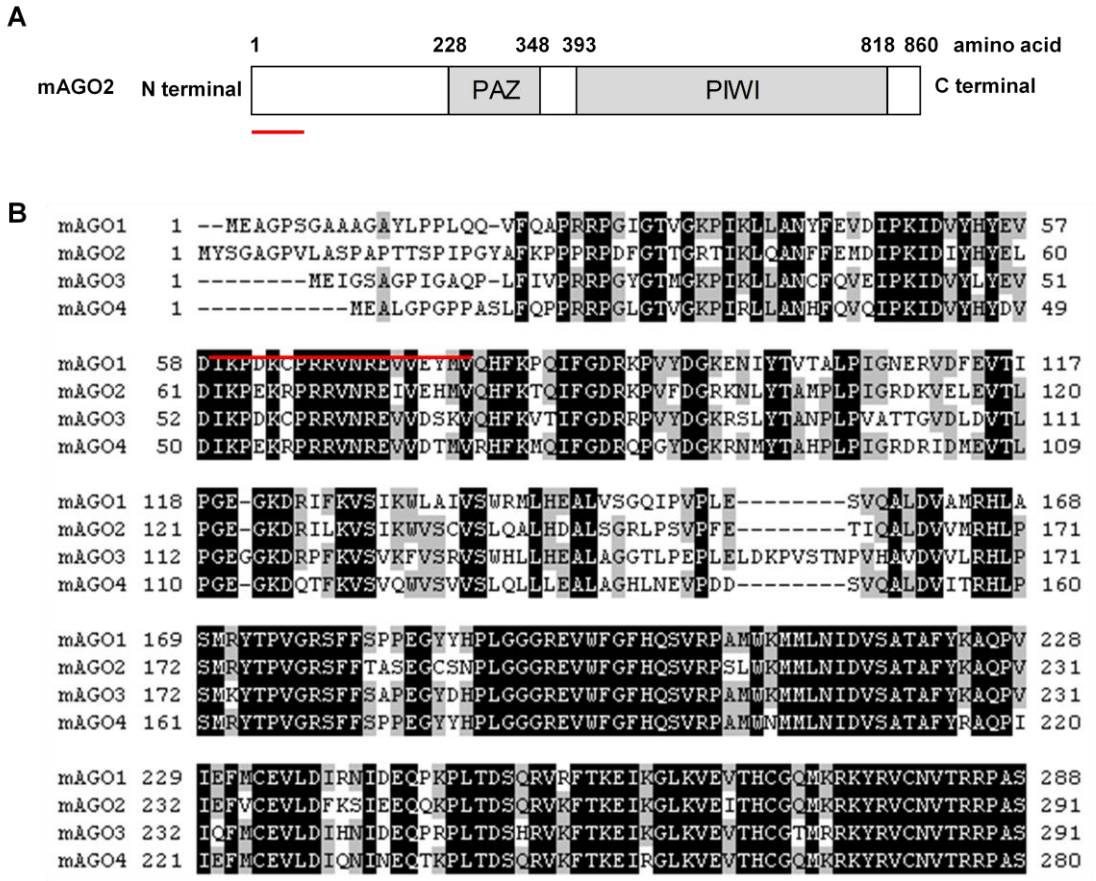

Figure S2. The region and amino acid sequence of antigen for producing anti-AGO2 monoclonal antibody. (A) Domain map of mouse AGO2. The region of antigen for producing anti-AGO2 monoclonal antibody is shown by a red line. PAZ, PAZ domain. PIWI, PIWI domain. **(B)** Comparison of amino acid sequences of mouse AGO subfamily proteins. The sequence of peptide used as antigen for producing anti-AGO2 monoclonal antibody is underlined with a red line.

Supplementary Data

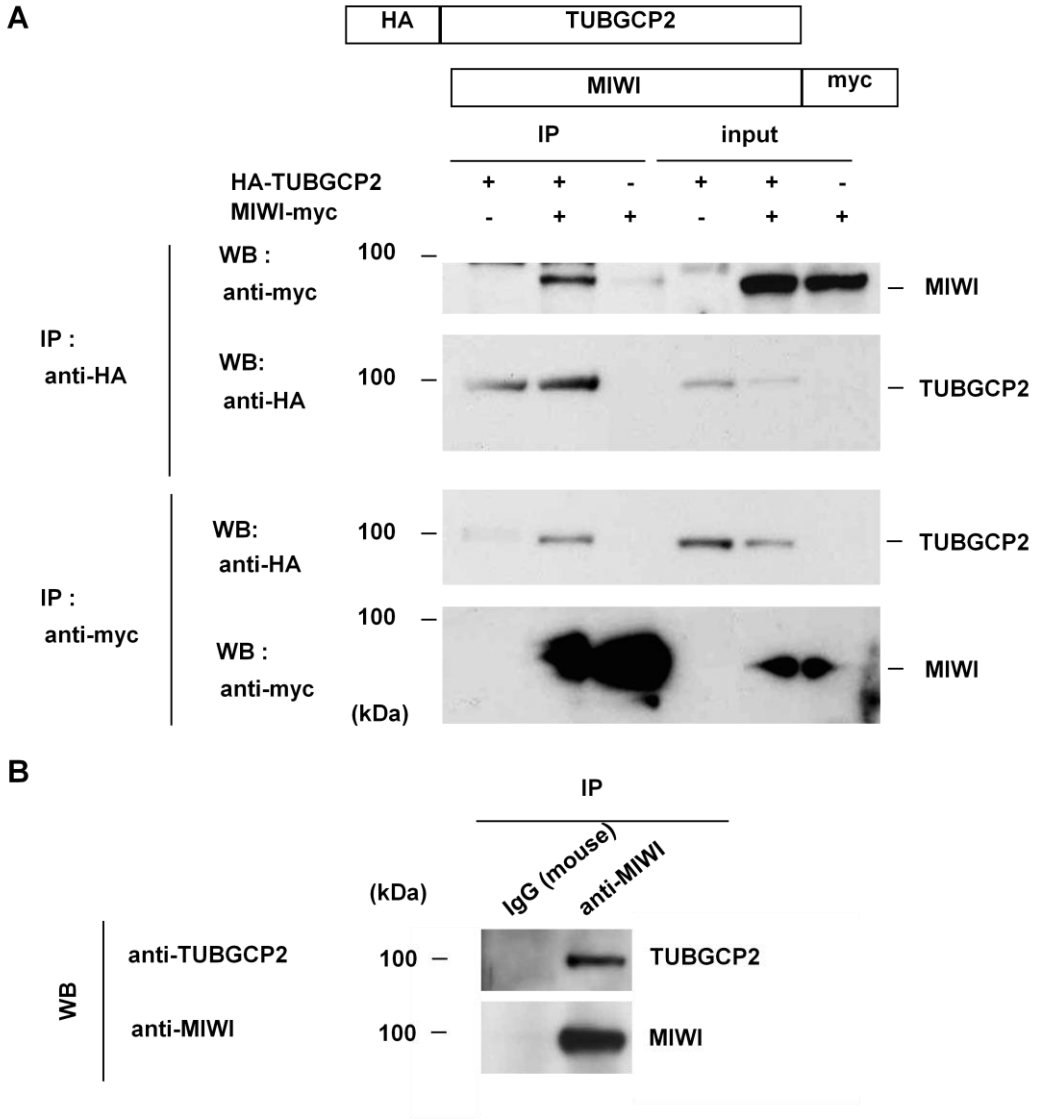

Figure S3. Interaction of MIWI and TUBGCP2. (A) Binding of MIWI and TUBGCP2 in HEK293T cells. Binding of myc tagged MIWI and HA tagged TUBGCP2 was estimated by the pull down assay. Myc tagged MIWI and/or HA tagged TUBGCP2 were transiently expressed in HEK293T cells. Cells were lysed with cell lysis buffer (20 mM Tris-HCl, pH 7.4, 2.5 mM $MgCl_2$, 200 mM NaCl, 0.05% NP40), and the cell lysate was cleared by centrifugation at 15000rpm for 10min at 4℃. Protein G Sepharose 4 Fast Flow (GE Healthcare) bound with anti-HA tag mouse monoclonal antibody 3F10 (Roche) or anti-myc tag mouse monoclonal antibody 9E10 (Santa Cruz Biotechnology) was added to the cell lysate and mixed by rotation for 3h at 4℃. The beads were washed three times with cell lysis buffer, and the immunoprecipitated protein was eluted with SDS sample buffer. Each immunoprecipitated protein and input cell lysate were applied to SDS-PAGE, and Western blotting was performed using anti-HA tag mouse monoclonal antibody 3F10 or anti-myc tag mouse monoclonal antibody 9E10 as the primary antibody and

horseradish peroxidase (HRP)-anti-mouse IgG (GE Healthcare) as the secondary antibody. **(B)** Interaction of MIWI and TUBGCP2 in adult mouse testes. Adult mouse testes were homogenized with cell lysis buffer (20mM HEPES, pH 7.5, 2.5 mM $MgCl_2$, 150 mM NaCl, 0.1% NP40), and the cell lysate was cleared by centrifugation at 15,000rpm for 10min at 4℃. Protein G Sepharose 4 Fast Flow (GE Healthcare) bound with anti-MIWI mouse monoclonal antibody 2C12 or non-specific mouse IgG was used for immunoprecitpitation. Western blotting was performed using anti-TUBGCP2 rabbit polyclonal antibody (sc-67251; Santa Cruz Biotechnology) and anti-MIWI rabbit polyclonal antibody MIWI-C as the primary antibody and HRP-anti-rabbit IgG (Zymed Laboratories) as the secondary antibody.

Supplementary Data

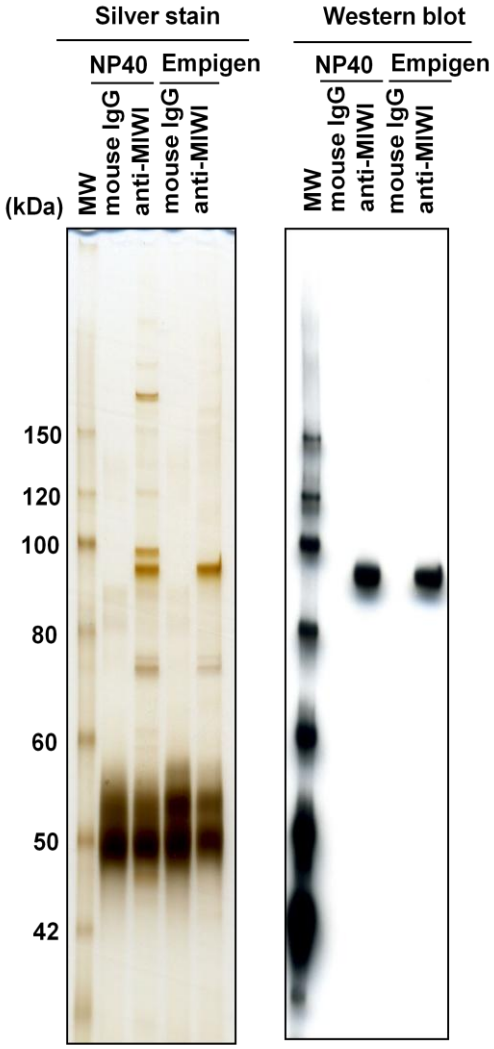

Figure S4. Immunoprecipitation of lysate of adult mouse testes prepared with Empigen BB, a strong zwitterionic detergent. Adult mouse testes were lysed with cell lysis buffer (20 mM Tris-HCl, pH 7.4, 2.5 mM $MgCl_2$, 200 mM NaCl) containing 0.05% NP-40 (a non-ionic detergent) or 1% Empigen BB (a strong zwitterionic detergent). The lysate was immunoprecipitated using an anti-MIWI monoclonal antibody (2C12) or non-specific mouse IgG as a negative control. The immunoprecipitated proteins were confirmed by SDS-polyacrylamide gel electrophoresis followed by silver staining (left panel), or Western blotting using an anti-MIWI polyclonal antibody (MIWI-C) (right panel).

Supplementary Data

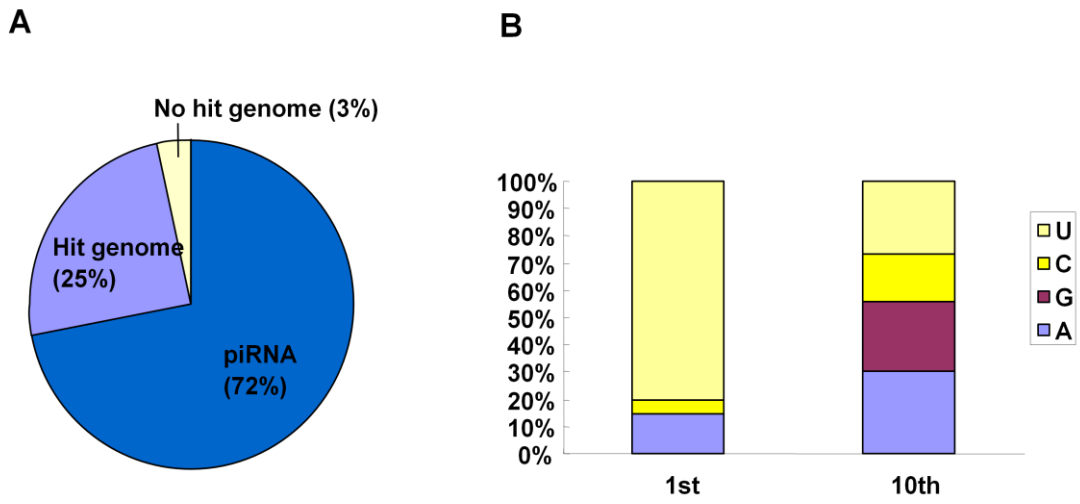

Figure S5. Composition of cDNA clones derived from MIWI-IP small RNAs and the content of the first and tenth nucleotides of the MIWI-IP small RNAs. The small RNAs purified from MIWI-IP products derived from approximately 50 mg of adult mouse testes were used as a template for cDNA synthesis. cDNA synthesis and PCR amplification were performed using a microRNA Cloning Kit (Wako) according to the manufacturer's instructions. PCR-amplified cDNA fragments were subcloned into the pGEM-T easy vector (Promega), and sequenced using a BigDye Terminator Cycle Sequencing Kit (Life Technologies). The sequences of these cDNA clones were analyzed by BLAST searches of DDBJ (http://blast.ddbj.ac.jp/) and homology searches of the piRNABank (http://pirnabank.ibab.ac.in). **(A)** Composition of 93 cDNA clones derived from MIWI-IP small RNAs. All cDNA clones were categorized as *i*) "piRNA" clones that showed significant homology to known piRNAs; *ii*) "hit genome" clones that showed no homology to known piRNAs, but matched perfectly to the genomic sequence; and *iii*) "no hit genome" clones that showed no homology to the genomic sequence. **(B)** Content of the first and tenth nucleotides of MIWI-IP small RNAs.

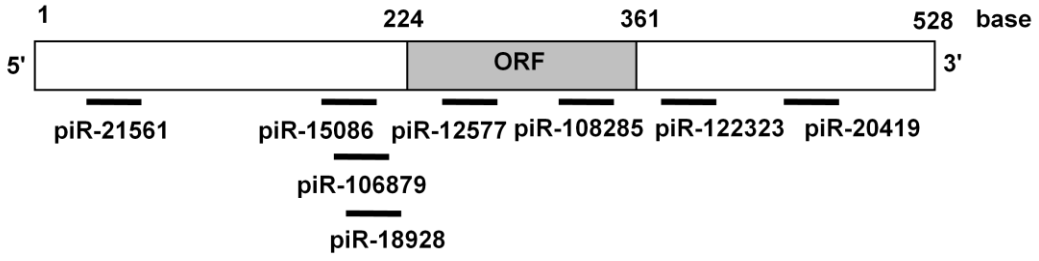

Figure S6. piRNA-encoding portions of *Aym1* mRNA. ORF, open reading frame.

Supplementary Data

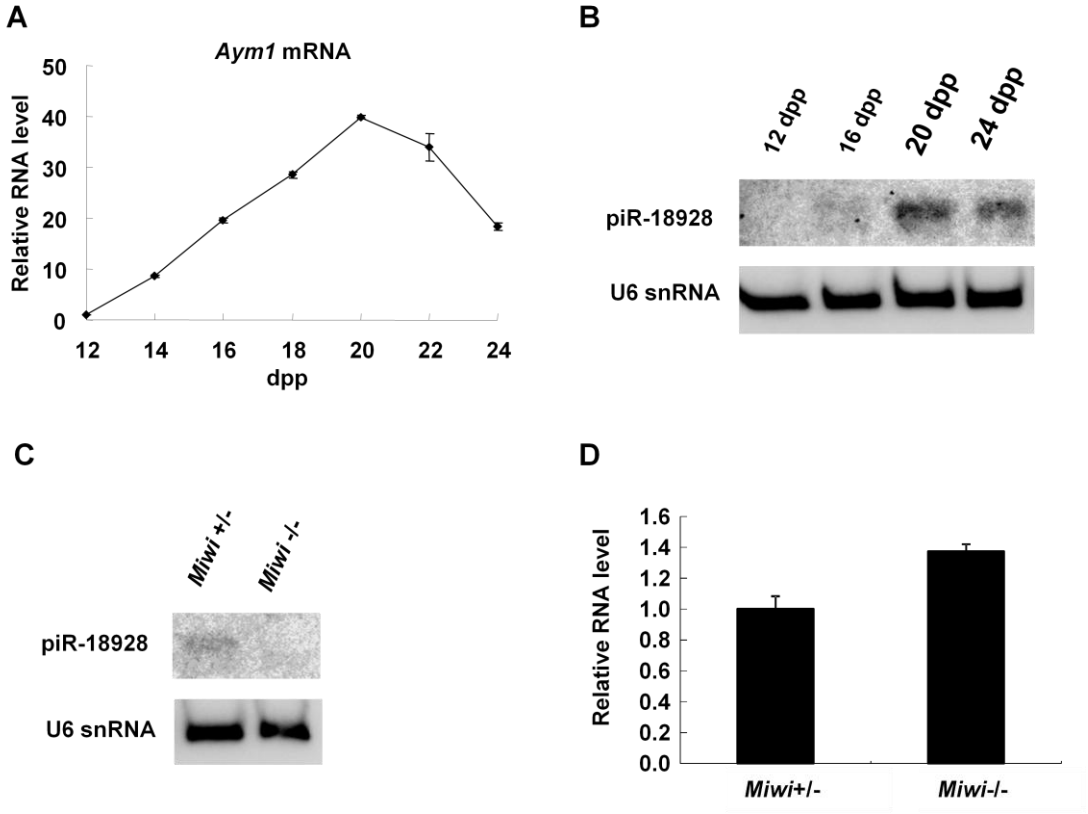

Figure S7. Expression analysis of *Aym1* mRNA and piRNA specifically encoded in *Aym1* mRNA. (A) Expression level of *Aym1* mRNA during spermatogenesis. The expression level of *Aym1* mRNA in mouse testes at 12 to 24 days postpartum (dpp) was analyzed by quantitative PCR and normalized to those of *Gapdh*. The normalized levels were compared with that at 12 dpp. **(B)** Northern blotting analysis of piR-18928, which was specifically encoded in *Aym1* mRNA during spermatogenesis. The expression levels of piR-18928 and U6 small nuclear RNA (snRNA) in mouse testes at 12 to 24 dpp were analyzed by Northern blotting using a LNA-modified DNA probe labeled with digoxigenin at the 5′ end. The nucleotide sequences of the LNA probes were for piR-18928, 5′- TGCTAACCTTGTGAGTCTACACACCCCTCC-3′ and for U6 snRNA, 5′-ATCGTTCCAATTTTAGTATATGTGCTGCCG-3′. The concentrations of the probe for piR-18928 and U6 snRNA were 0.1 nM and 0.05 nM, respectively. **(C)** Northern blotting analysis of piR-18928 in *Miwi*$^{+/-}$ and *Miwi*$^{-/-}$ testes at 24 dpp. The expression levels of piR-18928 and U6 snRNA in *Miwi*$^{+/-}$ and *Miwi*$^{-/-}$ testes at 24 dpp were analyzed by Northern blotting as described in B. **(D)** Comparison of the RNA levels of the *Aym-1* mRNA in *Miwi*$^{+/-}$ and *Miwi*$^{-/-}$ testes at 24 dpp. The expression level of the piRNA-encoding RNA clone no. 5 was analyzed by quantitative PCR and normalized to that of *Gapdh*. The normalized level in *Miwi*$^{-/-}$ testes was compared to that in *Miwi*$^{+/-}$ testes.

Supplementary Data

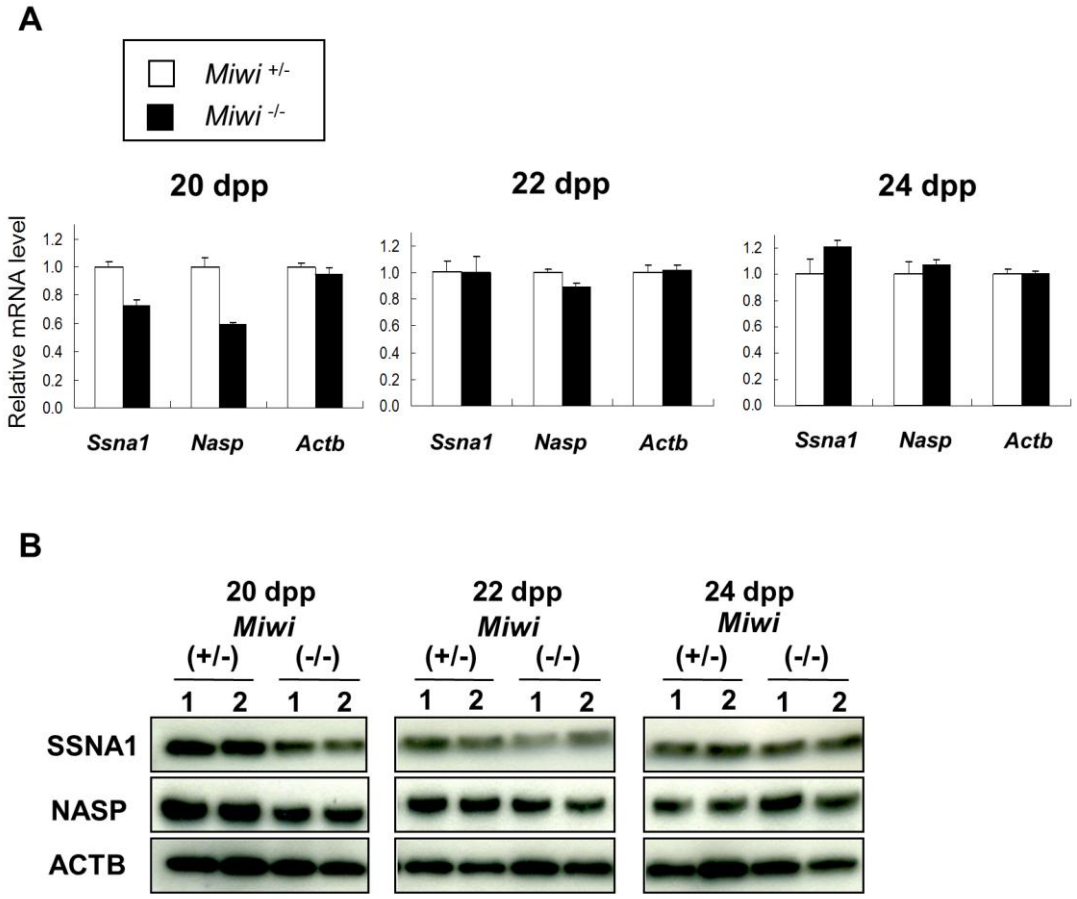

Figure S8. mRNA levels and protein levels of *Ssna1* and *tNasp* in *Miwi*$^{+/-}$ and *Miwi*$^{-/-}$ testes at 20, 22 and 24 dpp. (A) Expression levels of *Ssna1*, *Nasp* and *Actb* mRNA in *Miwi*$^{+/-}$ and *Miwi*$^{-/-}$ testes at 20, 22 and 24 dpp were analyzed by quantitative PCR and normalized to those of *Gapdh*. The normalized levels in *Miwi*$^{-/-}$ testes were compared with those in *Miwi*$^{+/-}$ testes. **(B)** Expression levels of SSNA1, NASP and ACTB proteins in *Miwi*$^{+/-}$ and *Miwi*$^{-/-}$ testes at 20, 22 and 24 dpp analyzed by Western blotting. Each protein lysate (2.5 μg) was loaded onto a 5-20%, for NASP and ACTB, or 15-20%, for SSNA1, gradient SDS-polyacrylamide gel. Western blotting was performed using anti-SSNA1 rabbit polyclonal antibody (1:500; Proteintech Group) or anti-NASP rabbit polyclonal antibody (1:500; Proteintech Group) as the primary antibody and horseradish peroxidase-conjugated anti-rabbit IgG goat polyclonal antibody (1:2,000; Dako) as the secondary antibody, or anti-ACTB mouse monoclonal antibody 3G4-F9 (1:250; Abnova) as the primary antibody and horseradish peroxidase-conjugated anti-mouse IgG rabbit polyclonal antibody (1:5,000; Dako) as the secondary antibody. dpp, days postpartum.

Supplementary Data

Table S1. Primers used for quantitative PCR

Gene symbol	Sequence	
	Forward primer	**Reverse primer**
8430410K20Rik	CCCAACACAAACAGAATAGCA	AACTCCACTGGAGAATCAC
Actb	CTTCTACAATGAGCTGCGT	ACCAGAGGCATACAGGGA
Atpif1	AAAGGCTGAAGAGGATCG	TCTTCTTCTTATGGCGTTCAATTT
Aym1	GGCAACTGAACTGTGGAC	CCTCTCTCTAAGAGATAATGGAACTA
Bcmo1	CCTACCCTCAGGAGGTTT	CTTCGTAGAGGACCTCGG
Btg3	AGGAAGTGGACGTGAAAC	AACAGGAGGAGGGTAGT
Ccna1	TCCTGCTGGATTTCAACAC	GCTTCTCGAAGGTAGCG
Cdc42se2	CTTCCTGTCTGAAGACTTGG	TGGCTCTCCGATCATACT
Cdk5rap1	ATGAACTTCTAGGAAGGCAG	CGTGACAAGAAGGATCACAT
Chchd1	AGCAGAATGAATTCCGCGA	TAACAACTTGGTCATCTTGTGG
Cmpk	AGGGTATCAGCTAATTCTTGTT	TAAATGTGAAGCAAGAGGAAGT
Cox5b	ACCCTAATCTAGTCCCGT	ACCAGCTTGTAATGGGTTC
Cox7c	CGACATGTTGGGCCAGA	TCCAAAGTACACGGTCATCA
Ddt	ACAAATCCACAGAGCCT	GGGAAGAAGCGGATAACG
Dmrtc2	TGCTCTCCACCACTGTT	GCTTGTGTCCCTTGAGATG
Dynlrb2	ATTCAGAACCCATGTGAGTAG	AGTGCAGTTCCATTGTCAAA
Gapdh	TGGACCTCATGGCCTAC	GGTCTGGGATGGAAATTGT
Gca	GCAGAATTATGATTGCCATGT	TGTTCTACCGTGCCACT
H2-DMb2	CTTCTGGACTGTGCCTC	GTCACATCCGCTGGATAG
Mex3c	TACCAACATCTGCTCAAACTATC	GGAAATGTAGGAGACAGACGA
Miwi	TCGTGGGCATCGACTGTTA	TTGCAAGCACACCTTGAG
Mrpl13	ATTTAGTGGAAGAGCTTCCTCA	AGTCTCTTGCTGTAATGTTCTATC
Mrpl22	CCTGAAGCGCATCCGTTA	CTGAATATAGTCCTTGGCGTG
Mrpl36	GCGCTCAGTTCTCTGTG	GGGTTGGTCTTACAGAGGAT
Mrps18c	GCTCTGTGCAGTGGTATAG	CTGGCAGGTCCTCATTG
Myadm	CAAGCTGCACTCGTAGTATT	AATCTCAGACCTTGACGAAC
Ndufb9	GTATATCTTCCCAGACTCTCCG	CATCCTCAGCTTCTTCCAC
Ndufs4	AAACGGATGGAGCTATGAT	CAGCTGACACTTCAGTCAC
Ndufs6	GTTCAAGTGTCGCCGAG	TTCACAGGCTGTTGTGC
Ndufv3	CCACAGTCCAGTGAGATG	TGTGTGGGTACTCTGACTT
Ovol1	CAAGAACAGGCTGGGAG	TCGGTTCAAAGGCATTTGG
piRNA-encoding RNA clone no.4	CCTTCATTCTGACCTTCAAGT	AGAGACAAGCACCATTGCATA
piRNA-encoding RNA clone no.5	CAGGAGTCAGGGAGCAG	GTGGATGCACCTGAGTTC
piRNA-encoding RNA clone no.6	ATGGGCCTTAGCAACATT	GGGCTTGATCTCAACACT
Reep1	CTTGTATGTAGAAGTGCGGT	TGCAGAAATGGAGAGTAACTCA
Rgs2	TTCCTCTTACGTTCCAACCT	GTGGTGAGCAAGAGCTAAA
Rnf11	TGACTGGTTGATGAGATCCTT	CTTTGGGCTCAGTGACA
Rpl24	AAAGGAATCCTCGGCAGATAAA	TATATCAGCAAGAGATGCACCA
Rpl35a	TGCTGGGAACAGGACTTCTAA	ATCTCGGGCATAAACGC
Rpp21	GCCAGAGATTCCTCAACG	CCTGAGTCTGAATGTTCTCTTT
Rps19bp1	GAAGTTTATGACCAGCATGAGA	GAACACAGTGCCCTCAG
Rps27l	TGGCTAGAGATCTGTTACACC	AGAACCACAGTCTGTGC
Uqcrb	GGACCTTTATAATGACAGGATGTT	CTTCTCTCTCCTTTCTTTCCC

All primers are given in the 5′-3′ direction.

Supplementary Data

Table S2. Expression analysis of mRNAs encoding electron transport chain proteins or ribosomal proteins during spermatogenesis

Category	Gene symbol	Microarray signal ratio		Relative mRNA level
		AGO2-IP / Total	MIWI-IP / Total	(20 dpp / 12 dpp)
Electron transport chain protein	*Ndufv3*	0.8	9.7	1.0
	Uqcrb	0.8	8.8	3.0
	Cox7c	1.4	8.0	3.0
	Ndufb9	1.2	6.2	2.7
	Ndufs6	1.0	6.2	4.7
	Cox5b	0.7	5.1	2.9
	Ndufs4	0.6	4.6	1.8
	Atpif1	0.5	4.2	3.6
	Mean	0.9	6.6	2.8
Ribosomal protein	*Mrps18c*	1.1	18.8	3.1
	Rps27l	1.2	8.8	1.9
	Rpl24	1.1	7.6	2.6
	Mrpl13	0.7	6.8	3.0
	Mrpl36	0.4	5.1	2.6
	Mrpl22	1.3	5.1	2.4
	Rps19bp1	1.2	4.5	2.3
	Rpl35a	1.0	4.4	1.8
	Mean	1.0	7.6	2.5

The expression levels of eight mRNAs each, encoding electron transport chain and ribosomal proteins that were enriched in the MIWI-IP products, were analyzed by quantitative PCR in mouse testes at 12 and 20 dpp, and normalized to those of *Gapdh*. The normalized levels at 20 dpp were compared to those at 12 dpp. dpp, days postpartum.

Supplementary Data

Table S3. Expression analysis of mRNAs encoding electron transport chain proteins or ribosomal proteins in *Miwi*-deficient testes

		Relative mRNA level (*Miwi*$^{-/-}$/ *Miwi*$^{+/-}$)		
Category	**Gene symbol**	**20 dpp**	**22 dpp**	**24 dpp**
Electron transport chain protein	*Ndufv3*	0.79	1.09	1.06
	Uqcrb	0.84	1.04	1.02
	Cox7c	0.76	1.04	1.02
	Ndufb9	0.77	1.06	1.21
	Ndufs6	0.76	0.98	1.26
	Cox5b	0.83	1.07	1.26
	Ndufs4	0.80	1.02	1.14
	Atpif1	0.65	0.98	1.13
	Mean	0.77	1.03	1.14
Ribosomal protein	*Mrps18c*	0.88	1.04	1.14
	Rps27l	0.87	0.99	1.16
	Rpl24	0.84	0.90	1.17
	Mrpl13	0.81	0.96	1.22
	Mrpl36	0.79	0.97	1.08
	Mrpl22	0.82	0.96	1.16
	Rps19bp1	0.77	0.88	1.17
	Rpl35a	0.72	0.92	1.21
	Mean	0.81	0.95	1.16

The expression levels of eight mRNAs each, encoding electron transport chain and ribosomal proteins that were enriched in the MIWI-IP products, were analyzed by quantitative PCR in *Miwi*$^{+/-}$ and *Miwi*$^{-/-}$ testes at 20, 22 and 24 dpp, and normalized to those of *Gapdh*. The normalized levels in *Miwi*$^{-/-}$ testes were compared to those in *Miwi*$^{+/-}$ testes. dpp, days postpartum.