

Identification of piRNAs in the central nervous system

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Supplemental Data

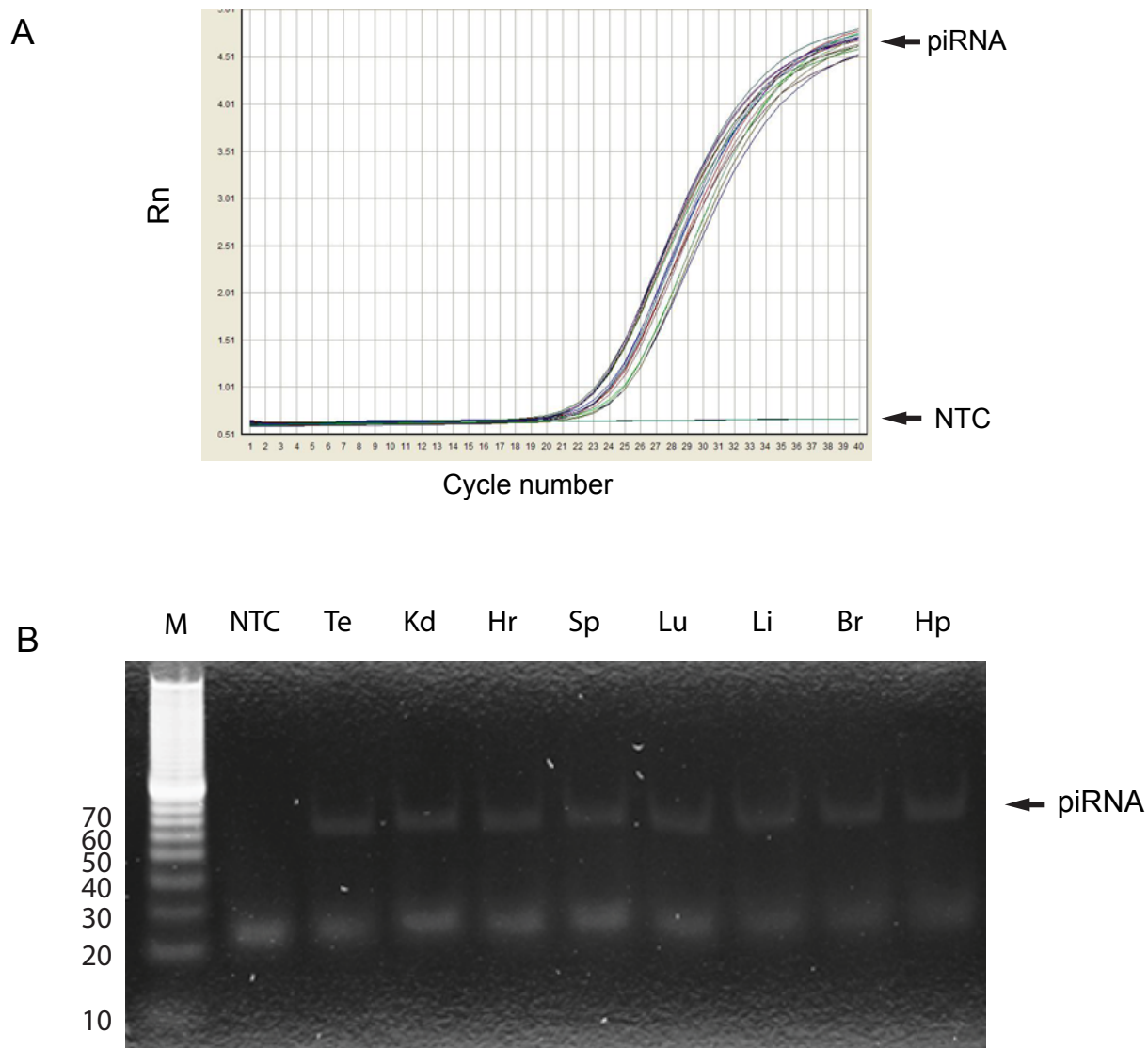
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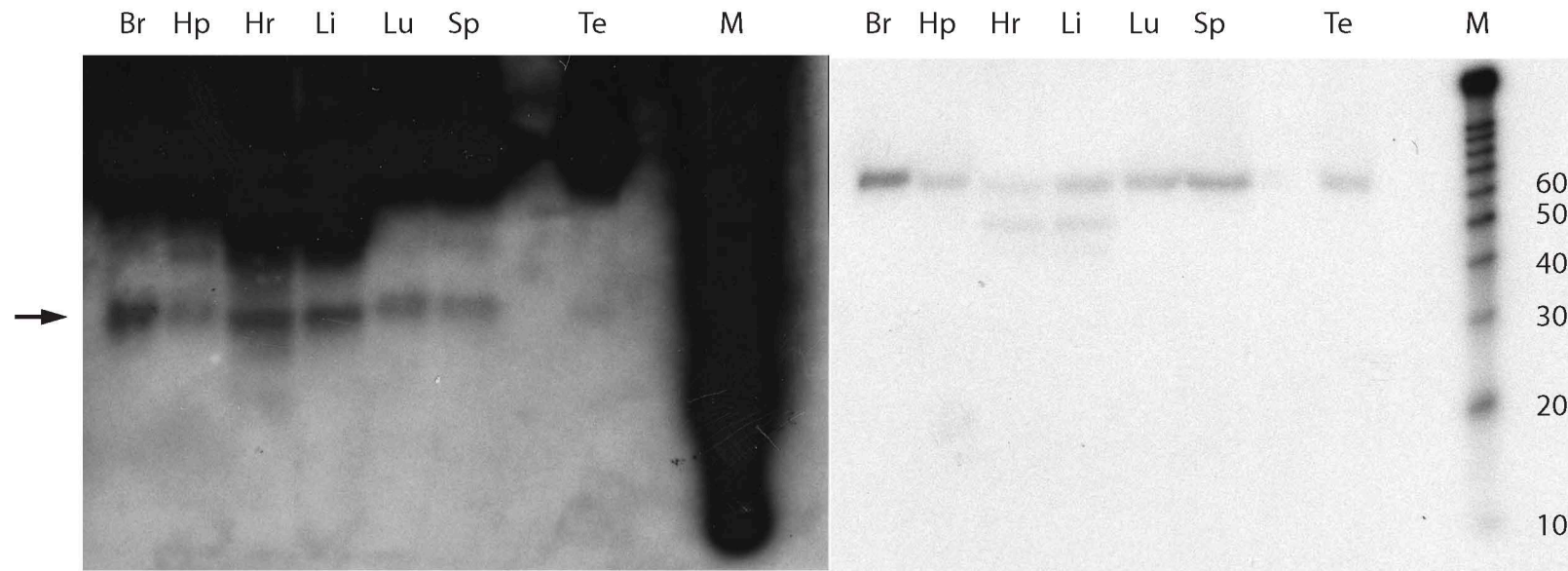
Supplemental Fig. 1. Base distribution for the first 10 bases.

The figure shows the nucleotide distribution for the first 10 bases. About 60% reads show a “U” at position “1”, suggesting bias toward “U” at the 5’ end.



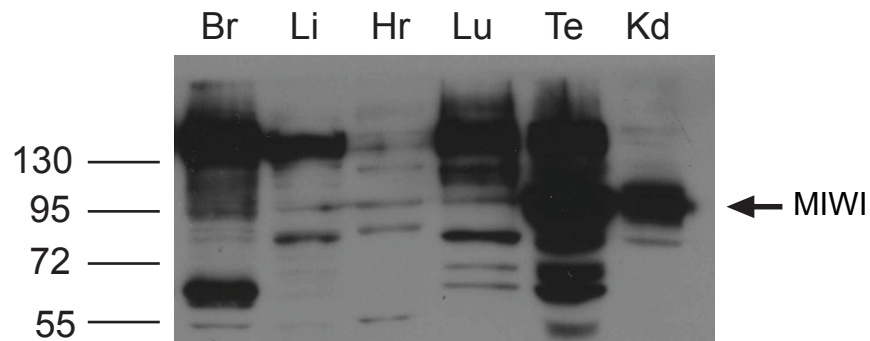
Supplemental Fig. 2. Real-time PCR of piRNA detection.

(A) Real-time PCR for piRNA was performed with Taqman probe. (B) Following the real-time PCR, 10 μ l of each reaction was run on a 6% polyacrylamide gel to demonstrate the reaction specificity. Hippocampus (Hp), Brain (Br), Liver (Li), Lung (Lu), Spleen (Sp), Heart (Hr), Kidney (Kd), Testes (Te), No template control (NTC), M (10 bp DNA ladder).



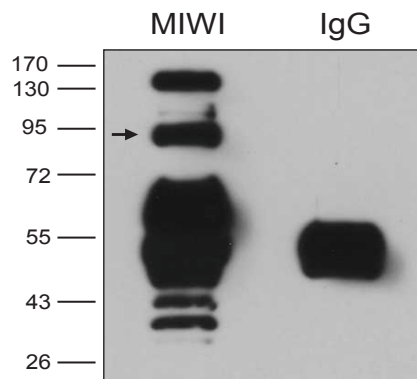
Supplemental Fig. 3. Northern blot analysis of piRNA expression in mouse tissues.

60 µg of total RNA was loaded in a 15% PAGE gel and hybridized with $\gamma^{32}\text{P}$ labeled LNA based antisense probe to DQ705026. The autoradiogram is showing 30 nt long piRNA (DQ705026) band (marked with an arrow) from Brain (Br), Hippocampus (Hp), Heart (Hr), Liver (Li), Lung (Lu), Spleen (Sp), Testes (Te). Decade marker RNA (M) is showing corresponding standard nucleotide length in the autoradiogram.



Supplemental Fig. 4. MIWI expression in mouse tissues.

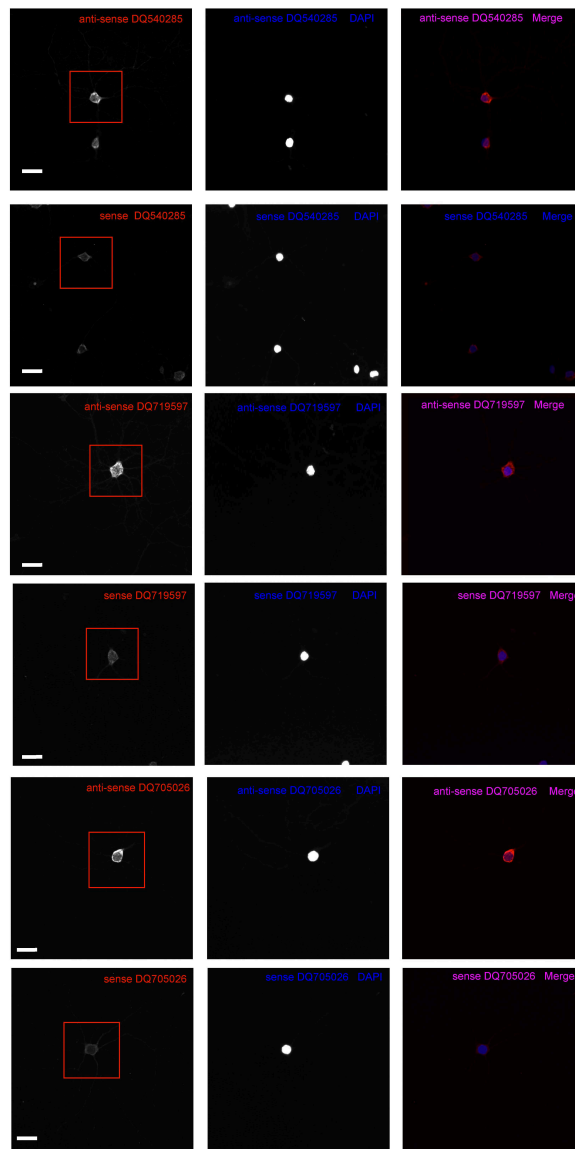
40 μ g of total protein from tissue lysates were blotted with antibody against MIWI. Low level of MIWI expression was observed in the mouse brain as well as hippocampal lysates as compared to lysate from testis. The MIWI immunoreactive band from Brain (Br), Hippocampus (Hp), Heart (Hr), Liver (Li), Lung (Lu), Kidney (Kd), Testes (Te) is marked with an arrow.



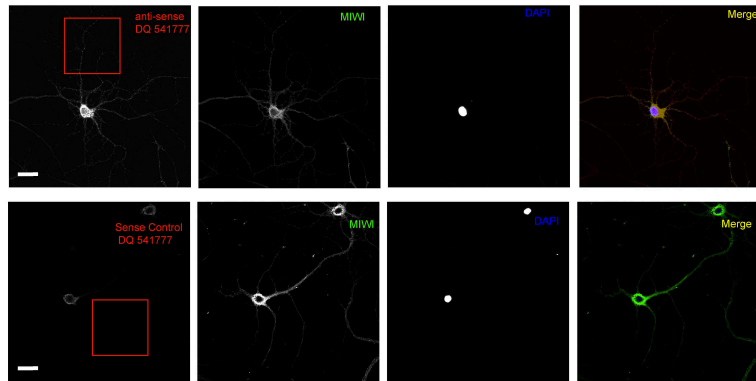
Supplemental Fig. 5. Immunoprecipitation of MIWI.

To visualize piRNA association with MIWI, equal amount of hippocampal lysate was immunoprecipitated with MIWI and IgG antibodies. Immunoprecipitated band of MIWI is marked with an arrow.

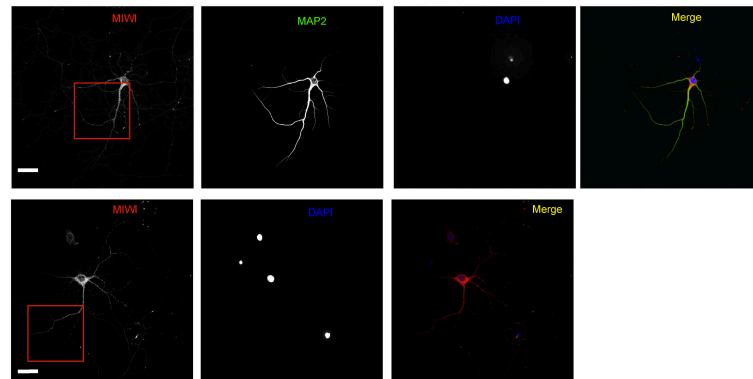
A



B



C

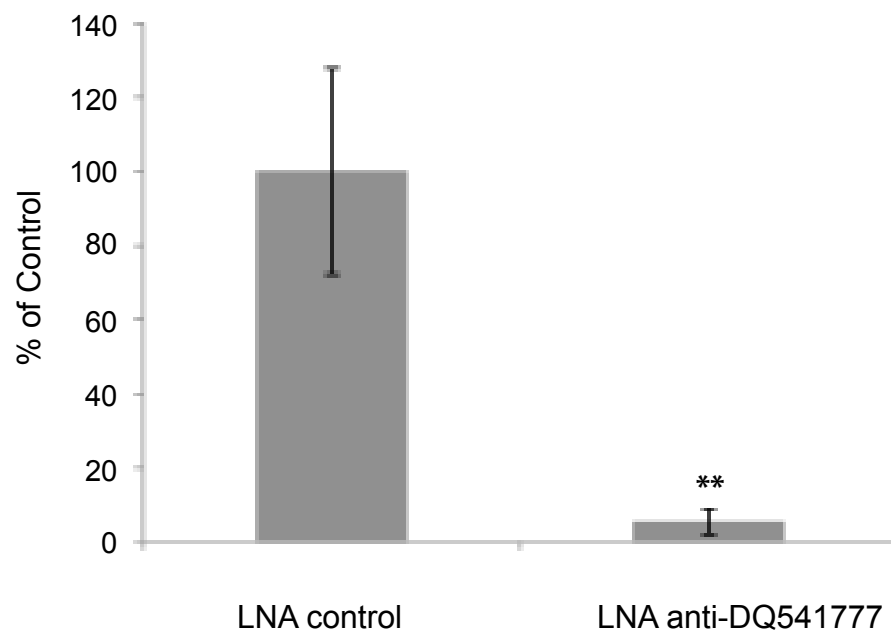


Supplemental Fig. 6. Subcellular localization of piRNA and MIWI

A) *In situ* hybridization of cultured mouse hippocampal neurons showing cytoplasmic localization of specific piRNAs (DQ540285, DQ719597, DQ705026 and DQ541777). The hybridization was performed using LNA based anti-sense as well as sense probes as indicated. The box area in red showing a portion of neuron selected for high resolution images shown in Figure 3. Scale bars 50 μ m.

B) *In situ* hybridization showing piRNA – DQ541777 complexed with MIWI. *In situ* hybridization was performed using LNA based anti sense and sense probe for the specific piRNA and then these neurons (DIV21) were immunostained with antibody against MIWI. High resolution image shown in Figure 3 is marked with red box. Scale bars 50 μ m.

C) Localization of MIWI in neuronal dendrite was detected by double immunostaining of cultured hippocampal neuron (DIV21) using antibodies against MIWI and a dendritic marker, MAP2. High resolution image in Figure 3 is marked by red box. Scale bars 50 μ m.



Supplemental Fig. 7. piRNA levels after LNA transfection.

The knockdown effect of anti-piRNA (DQ541777) was measured by real-time PCR after transfection. All data are from 3 independent experiments. Amount represented as mean $\pm$ s.d.

A. DQ541777 16130 GGCTGGTCCGAAGGTAGTGAGTTATCTCAA NM_145515, Mark1

3111 gccttgagctaactcagaagcgccctcgaggaagagttgagtcctttaccaagcttacttggccct

3' aactctattgagtgatggaagcctggtcgg 5'

3261 acccagcacttggtgttgccttcggaagacgcagggtagctgcggaa

3' aactctattgagtgatggaagcctggtcgg 5'

1921 atggggaacagctccgagagcggcgcagcggttacaacggaccacggcctcccat

3' aactctattgagtgatggaagcctggtcgg 5'

3481 tgagcagggcggtgctctccatgcccggttactacggacttatgctgcctgggtggttcaact

3' aactctattgagtgatggaagcctggtcgg 5'

2931 tagaaattattttaaactcacattttaatttaaaccagaagttgacagtggttggtgtcc

3' aactctattgagtgatggaagcctggtcgg 5'

3361 ttggctgagatatgatgccgcctcaggaattgaccgcagttgggagcatctgccccagtg

3' aactctattgagtgatggaagcctggtcgg 5'

3401 tgggagcatctgccccagtgctcctccagcctccaccctccccaccactctcgtatctg

3' aactctattgagtgatggaagcctggtcgg 5'

B. DQ541777 16130 GGCTGGTCCGAAGGTAGTGAGTTATCTCAA NM_007928, Mark2
(transcript variant1)

961 ttaagttgtttgaagtgatcgagactgagaagactctctaccttgtcatggagtatgcc

3' aactctattgagtgatggaagcctggtcgg 5'

2401 ccttccatgctgggcagctccgacaggtgcgggaccagcagaatctaccctacgggtgtga

3' aactctattgagtgatggaagcctggtcgg 5'

601 ccctcccctgagataccggcgccatgtccagcgctcggacccccctaccacgctgaacg

3' aactctattgagtgatggaagcctggtcgg 5'

3' aactctattgagtgatggaagcctggtcgg 5'

41 gtaaacagagccgaacctcgggtcttcggaatctaggaggtgcgcgcgtccccggggatta

3' aactctattgagtgatggaagcctggtcgg 5'

81 gcgcgcgtccccggggattaccttcttcggtagtttccggaattcacccatcgtctgggc

3' aactctattgagtgatggaagcctggtcgg 5'

C. DQ541777 16130 GGCTGGTCCGAAGGTAGTGAGTTATCTCAA NM_025876, Cdk5rap1

421 cttacagaagagtggctaccttcggaccagcaacctccaagaggctgatgtgatccttct

3' aactctattgagtgatggaagcctggtcgg 5'

1 agtttccggttttcggacgcggaccttgcagttgtgggagatttggggctg

3' aactctattgagtgatggaagcctggtcgg 5'

D.

DQ555093 775 TGGGGGGCCCAAGTCCTTCTGATCGAGGC NM_001101471, AKAP5

DQ555094 3439 TGGGGGGCCCAAGTCCTTCTGATCGAGGCCCA NM_01101471, AKAP5

2281 aaccgagatcagaagaaagcaaacgaatggagccaattgctattatcattactgacactg

3' cggagctagtcttctgaacccgggggt 5'

5221 cacagaggatacaagcacttgcctcgatctttttgtgtgtgactattcacttaacatttg

3' cggagctagtcttctgaaccgggggt 5'

1 agagggctgcgcgctccgcggtctccggggctctccgttggttgggcccgtgctcctcga

3' cggagctagtcttctgaaccgggggt 5'

E. DQ719597 2459 GGTCGATGATGAGAGCTTTGTTCTGAGC NM_010864, Myo5a

2041 tgagaagccccgcatgtcaaacaagctttcatcatcaaacattttgctgacaaagtga

3' caggtcttgttttcgagagtagtagctgg 5'

3001 caagatactccgtgaatacaaaagcagtcattcagaaacgtgtccgtggctggctggc

3' caggtcttgttttcgagagtagtagctgg 5'

1621 tgcccagagatgcttttagcaaagcacatctatgcaaagctctttaactggattgttgacca

3' caggtcttgttttcgagagtagtagctgg 5'

2121 cttgaaaagaataaagatactgtttttgaagaacaaattaaagtccttaagtcaagcaagt

3' caggtcttgttttcgagagtagtagctgg 5'

5341 ggtgaagccacagggctcagaaaacgaacctccagtatcgagatgagggcacctacac
 3' cgagtccttgtttcgagagtagtagctgg 5'

F. DQ705026 6257 CTGAAATGAAGAGAATACTCTTGCTGATC NM_007495, ASTN1

2051 attgcagcaaagataacggaggctgcagtaagaatcttcgctgcatttcagatcgcaagt
 3' ctagtcgttctcataagagaagtaaagtc 5'

6511 tctttcagtgctccttggtgttttctcttcagatgtgtgtgtgtctatacacacactcac
 3' ctagtcgttctcataagagaagtaaagtc 5'

Supplemental Fig. 8. Candidate target alignments of piRNAs.

Candidate targets of five piRNAs (DQ 541777, DQ 705026, DQ 555093, DQ 555094 and DQ719597) were searched bioinformatically. The base pairing between mRNA and piRNA is colored with yellow (Watson-Crick base pairing) or green (GU wobble base pairing). (A) DQ541777-Mark1, (B) DQ541777-Mark2, (C) DQ541777-Cdk5rap1, (D) DQ555093, DQ555094-AKAP5, (E) DQ719597-Myo5a, (F) DQ705026-ASTN1.

piRNA	Deep sequencing tag number	Real-time PCR expression
DQ541777	16130	4.87
DQ705026	6257	222.86
DQ555094	3439	153.81
DQ719597	2459	55.91
DQ689086	1514	560.28
DQ540285	1433	3.68
DQ715799	0	0

Supplemental Table 1. Comparison of piRNA expression by deep sequencing and real-time PCR methods.

Deep-sequencing analysis of piRNA expression is represented by tag number. Normalized piRNA expression by real-time PCR is represented by $2^{\Delta\Delta CT}$ and multiplied by 10^{10} to simplify the data presentation.