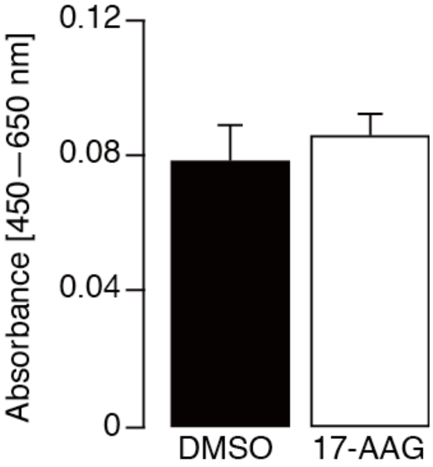
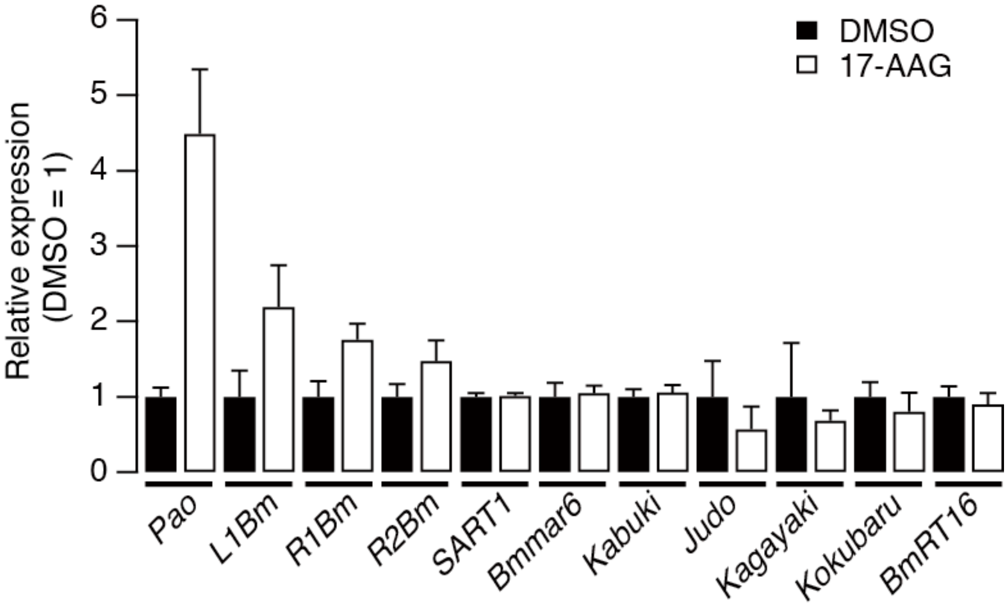
Supplementary Information



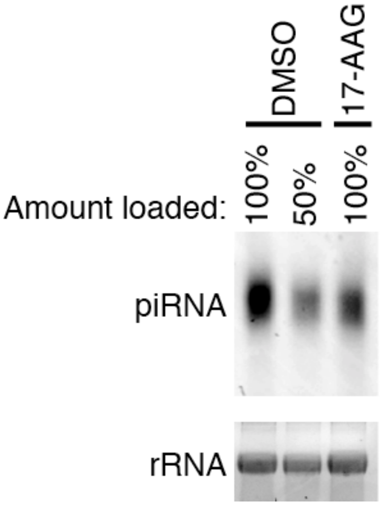
Supplementary Figure S1. Cell viability is unaffected by 17-AAG

Cell viability of BmN4 cells treated with DMSO or 10 μM 17-AAG for 48 hours was assessed by WST-1 assay.



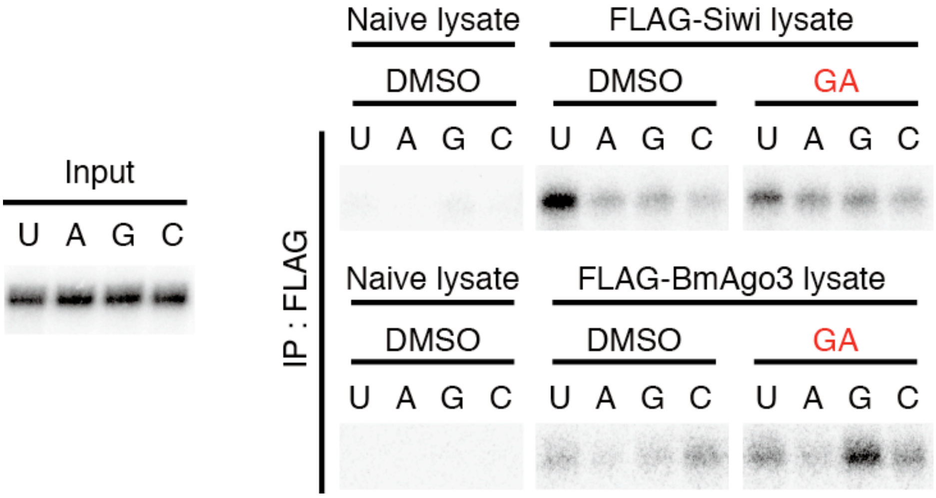
Supplementary Figure S2. Inhibition of Hsp90 causes transposon de-silencing

Expression of transposon mRNAs in BmN4 cells treated with DMSO or 10 μM 17-AAG for 48 hours was measured by qPCR. *rp49* served as an internal control. The average ± standard deviations from three independent trials are shown.



Supplementary Figure S3. The overall level of piRNAs is reduced by 17-AAG

Ten microgram of total RNAs from BmN4 cells treated with DMSO or 10 μM 17-AAG for 48 hours were resolved by 15% denaturing PAGE and stained with SYBRGold. rRNA was used as a loading control.



Supplementary Figure S4. Accurate pre-piRNA loading is impaired by Geldanamycin in vitro

50-nt 5′-radiolabeled RNAs with 5′ U, A, G, or C were incubated in lysates prepared from wild-type BmN4 cells (naive), FLAG-Siwi or FLAG-BmAgo3 expressing cells in the presence or absence of 1 mM Geldanamycin (GA). After immunoprecipitation with anti-FLAG antibody, Siwi or BmAgo3-bound RNAs were detected.

Supplementary Table 1. Summary of piRNA deep sequencing in this study

Library name	**Total reads**	**Total genome mapping**	**Genome mapping (%)**	**Total TE mapping**	**TE mapping (%)**
Total_DMSO	32031092	13330009	41.6	2565560	8.0
Total_17-AAG	27881490	10227258	36.7	1829248	6.6
Siwi_DMSO	31831288	12118225	38.1	2346582	7.4
Siwi_17-AAG	28287765	10954181	38.7	2239138	7.9
BmAgo3_DMSO	29149468	14850765	50.9	3873590	13.3
BmAgo3_17-AAG	28893120	14805694	51.2	4075341	14.1

TE: Transposable element.

Supplementary Table 2. The primer sequences for quantitative PCR analysis used in this study.

Name	**Sequence (5′–3′)**
Siwi forward	CGCCGAAGTCCACATTTAAAATGCG
Siwi reverse	TAGCTCGCATCTCGTCGGAGAGGCC
BmAgo3 forward	TGTGATGTTCAAATGTATAATGA
BmAgo3 reverse	CCTTCGTATTCGTCTACTGC
rp49 forward	AGGTATTGACAACAGAGTCC
rp49 reverse	GGAGCATATGACGGGTCTTC
Pao forward	CGCCATGAAAAGTGGAAGTGCCA
Pao reverse	AACCAAGGGTGGAGTTCTACGGAGA
L1Bm forward	TTACGTCATCATCACGCGCTACGTC
L1Bm reverse	CTTTAAGTTCGAGCGCACGTTCCAA
R1Bm forward	TACCCTCCATTGCACGGGATCT
R1Bm reverse	TGTGTATATCCGCAATAGGGTGCT
R2Bm forward	GAGATTCCAGCACGGTTTTGTGC
R2Bm reverse	GATTTTGAGGCCTCTGGATGCGT
SART1 forward	TCGCAGGCAGTTATTGTCAA
SART1 reverse	GGCTAAAATCGATACGGCAGA
Bmmar6 forward	ATGAAGACTCTAGTGTAGATGACAGG
Bmmar6 reverse	GTGATAAAAGCTGTGAAGGCGCGAA
Kabuki forward	GTCCAACCATGTGCATGGAAACCAAGTTC
Kabuki reverse	TGGACTAAATTTTTTGGTGGACACAGGTGC
Judo forward	CGTACAAAGGAGCCAATGGT
Judo reverse	CAAATGATCGCGCTACTGAA
Kagayaki forward	CACTGGTTCCATACGTAACA
Kagayaki reverse	GGTTTCTGGACGAAGCCAAG
Kokubaru forward	TACAATGCTCACTGAGAAGG
Kokubaru reverse	GACTGGGTCATTATAACTACTGG
BmRT16 forward	GTGGATTAGGCGTTTCACTAC
BmRT16 reverse	GTGTGACCACGTGTGTACGCA