Appendix:

Supplementary Materials

Materials and methods

Bioinformatics Analysis of Small RNA Libraries

Small RNA sequences were stripped of the 3' adaptor and mapped to the *Drosophila* release 5 genome (Ensembl/BDGP5.25) with up to 2 mismatch using Bowtie (version 1.0.0). Only uniquely mapped sequences were retained (this typically corresponds to roughly 76-85% of all obtained reads) for future analysis. For annotations, we used Flybase for protein coding genes, UCSC for non-coding RNAs, Repbase for transposons and repeats, miRBase for the most recent miRNA. Prior to any analysis, small RNAs annotated as rRNA, tRNA and snoRNA fragments were removed. Libraries were normalized to a subset of miRNAs to allow for cross-analysis. The identity of piRNA clusters is as previously described [1].

Western Blot Analysis

Protein was extracted from ovaries by standard methods using RIPA lysis buffer (150 mM sodium chloride, 1.0% (v/v) Triton X-100, 0.5% sodium deoxycholate, 0.1%

SDS, 50 mM Tris, pH 8.0) containing protease inhibitors (cØmplete Protease Inhibitor Cocktail Tablets, Roche) and PMSF (Sigma). Sixty μg total protein was resolved by standard SDS-PAGE on a 10% polyacrylamide/SDS gel electrophoresis and then transferred to PVDF Western Blotting Membrane (Roche) by standard methods. The membrane was blocked in TBST-milk (25 mM Tris-Cl, pH 7.4, 3.0 mM KCl, 140 mM NaCl, 0.02% [v/v] Tween-20, 5% [w/v] non-fat dry milk) at room temperature for 1 h. After blocking, the membrane was cut according the molecular weight markers and incubated overnight at 4℃ in TBST-milk containing primary antibody anti-Piwi diluted at 1:5000, anti-Vasa (1:3000), anti-Krimp (1:100000), anti-Tubulin (DSHB, 1:2000), anti-Flag (Sigma-Aldrich, 1:5000), anti-Myc (Abmart, 1:2000), anti-GFP (Life Technologies, 1:5000). The membrane was subsequently washed three times with TBST for 15 min each and incubated with either anti-mouse or anti-rabbit IgG-HRP (1:10000, ZSJQ-BIO) at room temperature for 2 hour. Next, the membrane was washed three times with TBST for 15 min each and developed by Immobilon Western Chemiluminescent HRP Substrate kit (Millipore) according to the manufacturer's instructions.

Co-Immunoprecipitation

Female flies of appropriate genotypes were dissected in ice cold PBS. Ovaries were lysed in RIPA containing protease inhibitors. The protein extracts were incubated with anti-Flag resin (Sigma) for 3-4 hour shaking at 4℃ and precipitated by centrifugation. The protein-bound beads were then washed three times in TBS and eluted with 3X FLAG peptide (Sigma), followed by Western blotting.

Yeast Two-Hybrid Experiment

The pelo and Hbs1 cDNA were cloned into pGBKT7 bait vector and pGAD prey vector (Clontech). The pGAD-Nbr1 and pGBKT7-sst6 are kind gifts from Lilin Du. The pGBKT7 and pGAD plasmid carrying interesting genes were co-transformed to AH109 yeast cells by following a previously described protocol [2]. Colonies appearing on media lacking tryptophan and leucine (SC-WL) were picked onto another media lacking tryptophan, leucine, histidine and adenine (SC-WLHA) to determine proteins interaction.

Supplemental References

1. Brennecke, J., Aravin, A.A., Stark, A., Dus, M., Kellis, M., Sachidanandam, R., and Hannon, G.J. (2007). Discrete small RNA-generating loci as master regulators of transposon activity in Drosophila. Cell *128*, 1089-1103.
2. Gietz, R.D., and Woods, R.A. (2002). Transformation of yeast by lithium acetate/single-stranded carrier DNA/polyethylene glycol method. Methods in enzymology *350*, 87-96.