Supplementary Figure Legends

Figure S1. Density of siRNAs over genes targeted by dsRNAs. Shown are 21-nt siRNAs mapping to the indicated genes (top: piwi; middle: shu; bottom: white) in small RNA libraries from total RNA of knockdown ovaries. Reads mapping to the genomic plus strand are shown in red, those derived from the minus strand in blue-grey. Blue arrows indicate orientation of transcription. Exon (thick bars) and intron (thin lines) structures are displayed. Note that siRNAs are restricted to the regions targeted by the VDRC dsRNA construct used for the indicated knockdowns.

Figure S2. Sub-cellular localization of piRNA pathway components in OSS cells. Transient transfection of OSS cells with GFP-tagged constructs expressing indicated piRNA pathway factors under the ubiquitous *Actin5c or ubiquitin* promoters are shown. MitoTracker CMXRos and DAPI were used as co-stains for mitochondria and nuclei, respectively. GFP-Armi localizes to cytoplasmic, perinuclear structures consistent with the expected Yb body localization. GFP-Piwi is predominantly nuclear, while Zuc-GFP shows significant overlap with mitochondria. Both N- and C-terminal tagged Shu variants localize to the cytoplasm without significant overlap with mitochondria. Minor enrichments of Shu can be seen in perinuclear foci, but the majority is diffusely cytoplasmic.

Figure S3. MicroRNA levels in knockdown libraries are very similar. Shown are size profiles (upper part) and heat maps (lower part) displaying the top 15 abundant miRNAs in the indicated knockdown libraries (driven either by *nos*-GAL4 or *tj*-GAL4). Size profiles show normalized miRNA reads over their length (18- to 26-nt). Heat maps show total number of reads of the 15 most abundant miRNAs in *white* control knockdowns compared to *shu* and *piwi* RNAi. Abundance in reads per million (rpm) is shown in grey scale.

Supplementary Tables

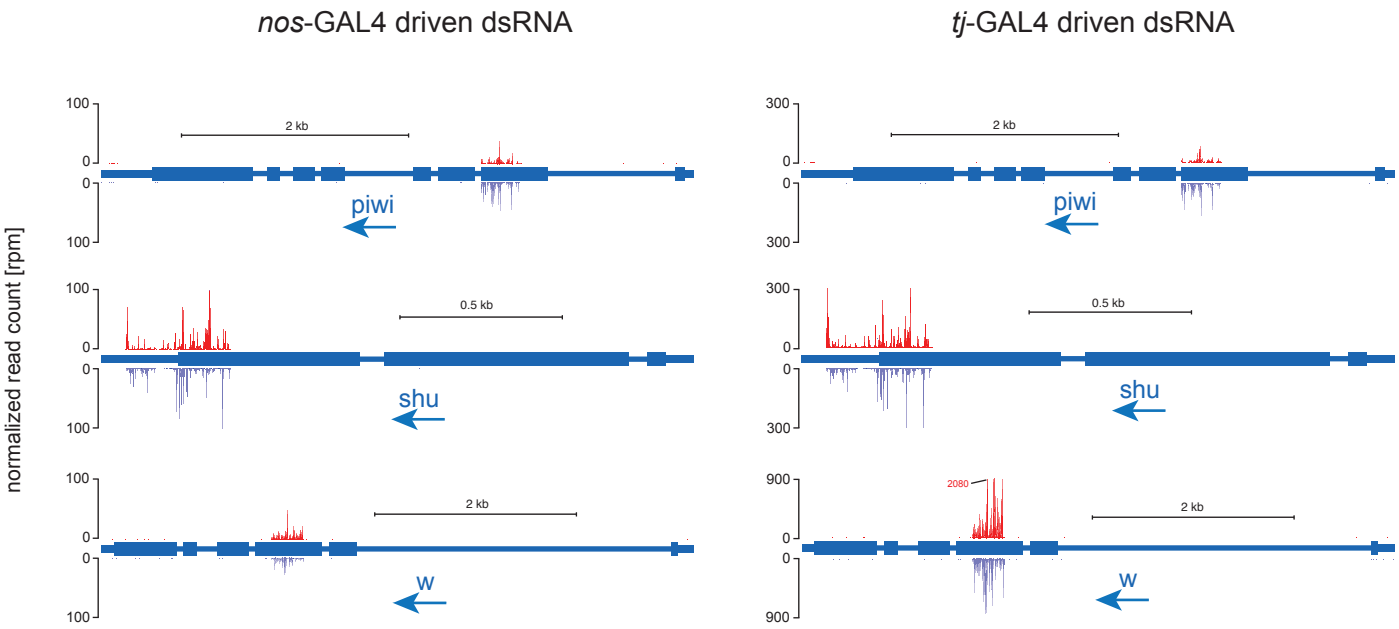
Supplementary Table S1: Fly lines used in this study

Stock Name	Genotype	Source	Stock Number
nos-GAL4	P{w[+mC]=UAS-Dcr-2.D}1, w[1118]; P{w[+mC]=GAL4-nos.NGT}40	Bloomington	25751
tj-GAL4	y* w*; P{GawB}NP1624 / CyO, P{UAS-lacZ.UW14}UW14	Kyoto	104055
dsRNA(*white*)	w^{1118}; ; P{GD14981}v30033	VDRC	v30033
dsRNA(*piwi*)	w^{1118}; P{KK105350}VIE-260B		v101658
dsRNA(*armi*)	w^{1118}; P{KK101517}VIE-260B		v101517
dsRNA(*shu*)	w^{1118}; P{KK102092}VIE-260B		v102092

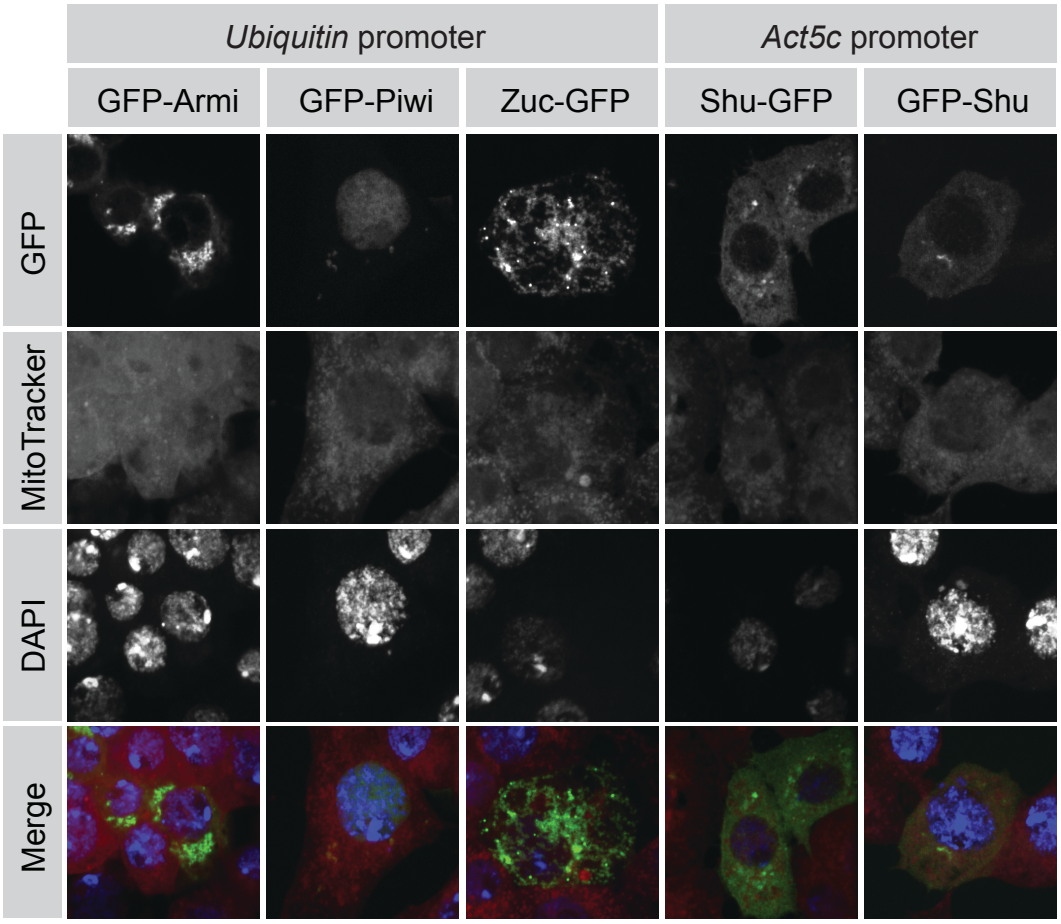
Supplementary Table S2: Primer sequences used for SYBR Green qPCR

Name	forward	reverse
rp49	ATCTCGCCGCAGTAAACGC	CCGCTTCAAGGGACAGTATCTG
Burdock	AGGGAAATATTTGGCCATCC	TTTTGGCCCTGTAAACCTTG
TAHRE	CTGTTGCACAAAGCCAAGAA	GTTGGTAATGTTCGCGTCCT
HetA	CGCGCGGAACCCATCTTCAGA	CGCCGCAGTCGTTTGGTGAGT
Transpac	GGAACGCACCTTCAACATTT	GCAAACTCGCATTTGTCTGA
TART	ACCAGGGAAAAGTGTGAACG	GGTGCAGTGGTATGGCTTTT
McClintock	CCCTAATCCGTTTTCCCAAT	CTGGTCGGTTCTGGTCAAAT
blood	TGCCACAGTACCTGATTTCG	GATTCGCCTTTTACGTTTGC
micropia	CGAATGTTACGCGGTGTATG	CTGGTCAGGTCCAAGGTTGT
Max	ATCTAGCCAGTCGAGGCGTA	TGGAAGAGTGTCGCTTTGTG
Jockey	TCTGCGGTCTCCAGCTTAAT	GTTGGGCAAATGCTAGTGGT
I-element	TGAAATACGGCATACTGCCCCCA	GCTGATAGGGAGTCGGAGCAGATA
Copia	AGCAAACAACCCCTCATGTC	GCAAACCCAATTTGTCTCGT
R2	ATGCTCCCGAAACAACAAAC	GCACTGCAGACTTGGTTCAA
stalker	TTATCAGGCTAGCCACATCTCTG	TTGGCAGATATCACTTCTACCGATTC
ZAM	ACTTGACCTGGATACACTCACAAC	GAGTATTACGGCGACTAGGGATAC
Springer	TGAAGAGCAAGAACCGGAGT	TCCTCCAGCAAAGCTTGTTT
roo	TCCTTTAAGCATCTTACAGCTAAAGG	TTTAGCTGTAAGATGCTTAAAGGAGCT

Preall, Czech et al., Fig. S1



Preall, Czech et al., Fig. S2



Preall, Czech et al., Fig. S3