Molecular Cell, Volume *47*

Supplemental Information

The Cochaperone Shutdown Defines a Group of Biogenesis Factors Essential for All piRNA Populations in *Drosophila*

Daniel Olivieri, Kirsten-André Senti, Sailakshmi Subramanian, Ravi Sachidanandam and Julius Brennecke

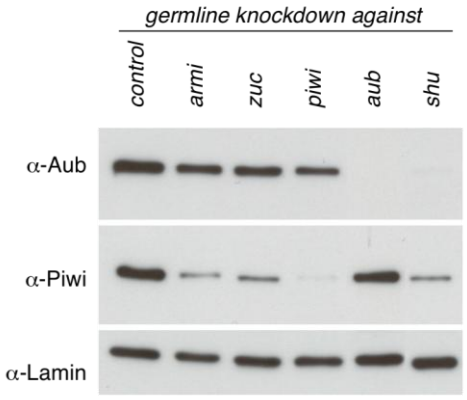

Figure S1.
Western blot analysis indicating protein levels of the PIWI members Piwi and Aub upon knockdown of the indicated genes. Lamin levels are shown for normalization purposes. While Aub levels were essentially undetectable in the *aub*-GLKD ovary lysate, low levels of Piwi were still observed in *piwi*-GLKD ovary lysate. This most likely reflects Piwi from somatic follicle cells that are unaffected by the GLKD.

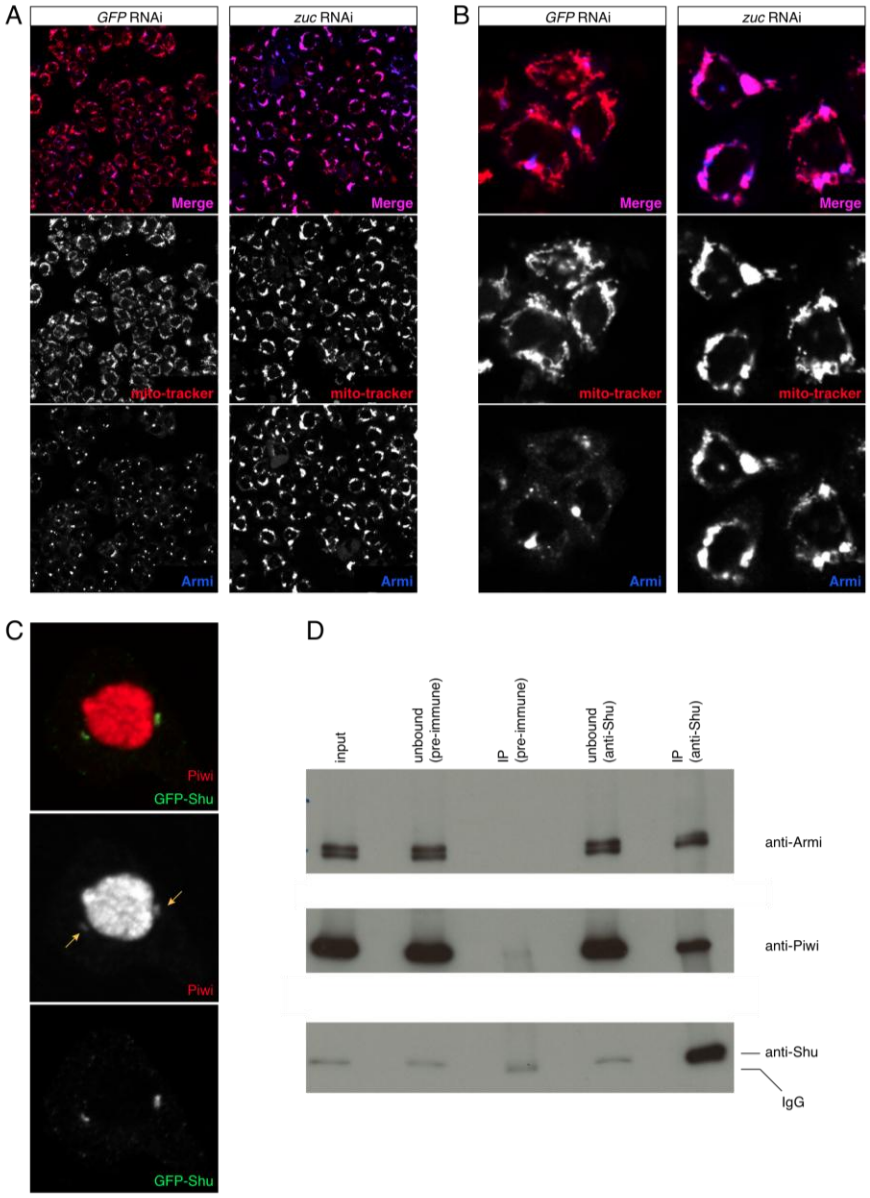

Figure S2.
(A) Shown are immuno fluorescence stainings of OSCs treated with GFP siRNAs (left) or *zuc* siRNAs (right). Mitochondria were visualized with Mito-tracker (red) and Yb-bodies with anti-Armi stainings (blue). In the merge image (top row), overlap of red and blue signal appears magenta. This illustrates the nearly complete co-localization of Armi and mitochondria in *zuc* knockdowns.
(B) Zoom in of an area shown in part (A).
(C) Immuno-fluorescence analysis of OSCs transfected with GFP-Shutdown (green) stained for Piwi (red). Yellow arrows indicate the faint but clearly visible accumulation of Piwi in peri-nuclear bodies that also accumulate Shu and represent therefore Yb-bodies.
(D) Western analysis of an anti-Shu IP experiment in comparison to a control IP experiment with pre-immune serum. Endogenous Armi and Piwi proteins are specifically co-purified with Shu from OSC lysate. The lower running and cross-reacting band in the Shu blot is likely IgG heavy chain, low levels of which are still present in the IP fraction despite the antibody being chemically cross-linked to the beads.

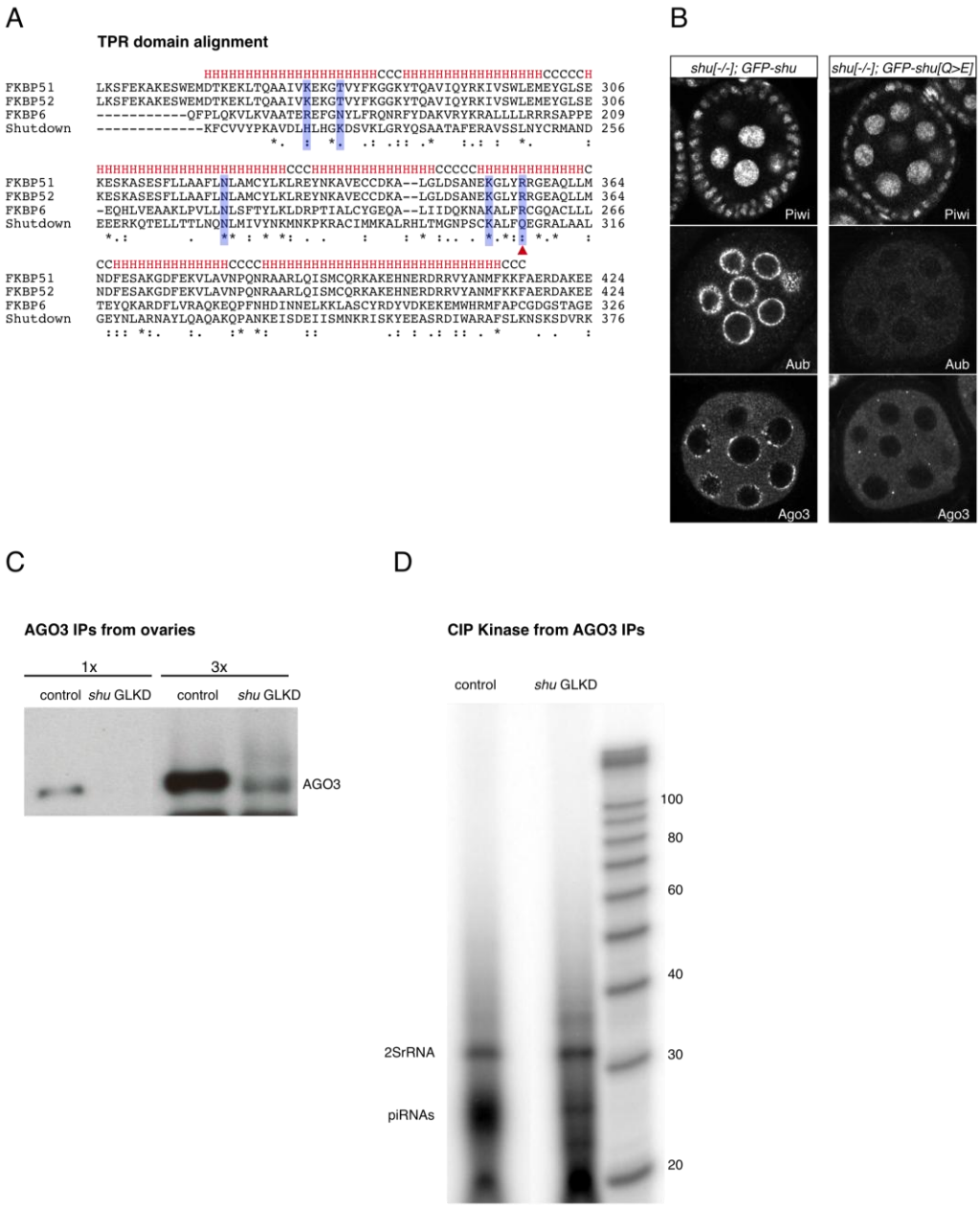

Figure S3.

(A) CLUSTAL alignment of the TPR domains from indicated proteins. α-helical segments indicated with 'H'; residues critical for an Hsp90 interaction highlighted in blue; the red arrowhead indicates the mutated Gln 308 (changed to Glu);

(B) Confocal sections of *shu[1]/shu[Def]* mutant egg chambers expressing wildtype (left) or Q308E mutant (right) GFP-Shu and stained for Piwi, Aub or AGO3.

(C) Western blot against endogenous AGO3 from ovary immuno-precipitations (anti-AGO3). Indicated are the relative loaded amounts. Based on this, the control IP contains roughly 3x more AGO3 compared to the *shu*-GLKD IP.

(D) Denaturing PAA gel (RNA size marker to the right) showing the radioactively marked (CIP Kinase) small RNA populations residing in AGO3 immuno-precipitated from control or *shu*-GLKD ovaries. For the control lane, only 1/3 of the isolated RNAs were loaded to account for the different AGO3 levels in the respective IPs (see C).

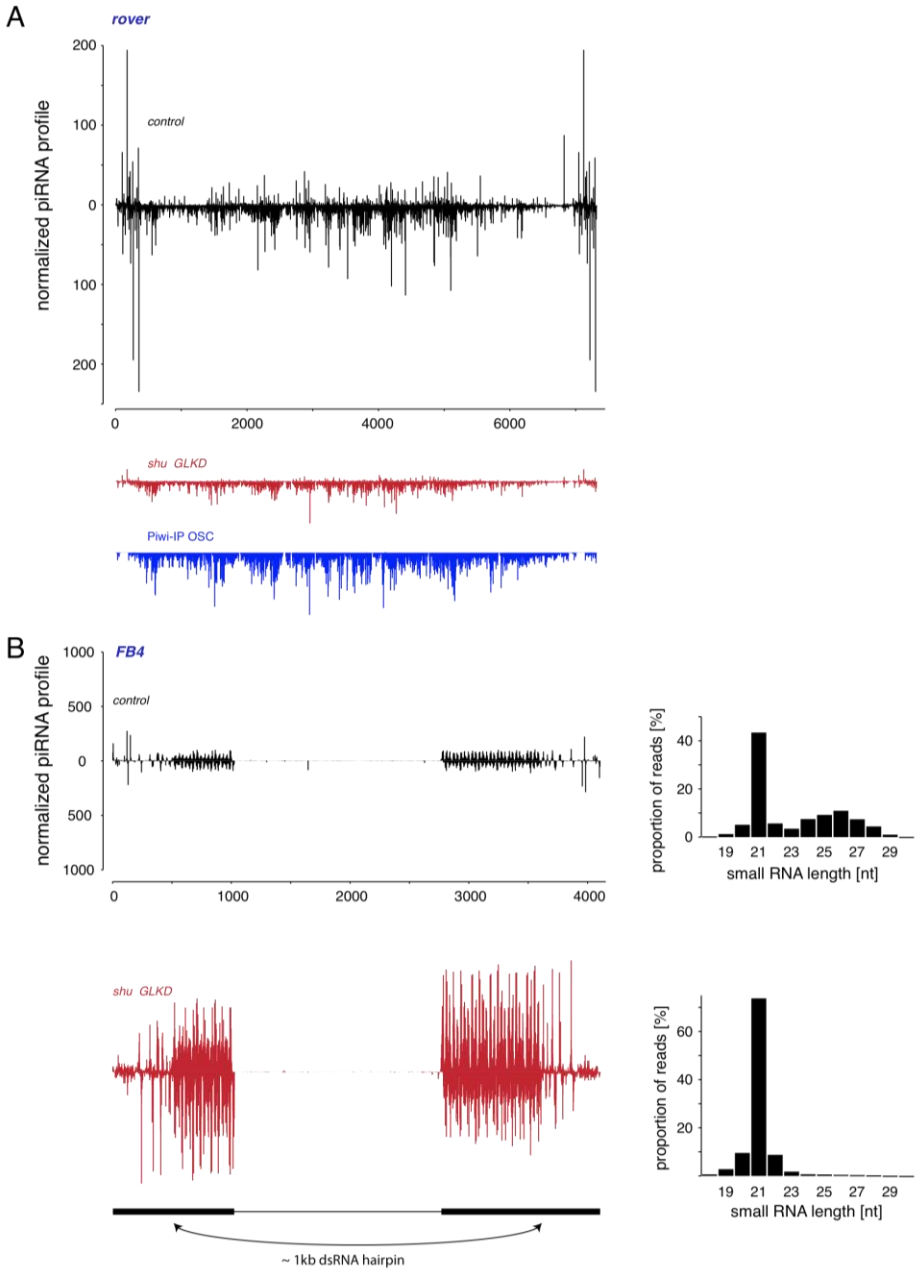

Figure S4.
(A) Shown are normalized piRNA profiles (23-30nt) mapping to the annotated "germline dominant" element *rover* in control GLKD (black) and *shu*-GLKD (red) libraries. Below, the piRNA profile from an OSC Piwi-IP library is shown, indicating that *rover* is a prototypical intermediate element.
(B) Shown are small RNA profiles (18-30nt) mapping to the DNA element *FB4* in control GLKD (black) and *shu*-GLKD (red) libraries. To the right, the length profiles of the corresponding small RNA populations are shown, indicating a complete shift to siRNAs in the *shu*-GLKD library. The cartoon below indicates the sequence stretches that are capable of folding into an extended dsRNA hairpin. For *shu*-GLKD we used the NGT > Dicer2 system and expressed the *shu* VDRC line (expression of an shRNA line resulted in distorted ovarian morphology); it is possible that the over-expression of Dicer2 contributes to the strong increase in siRNAs.

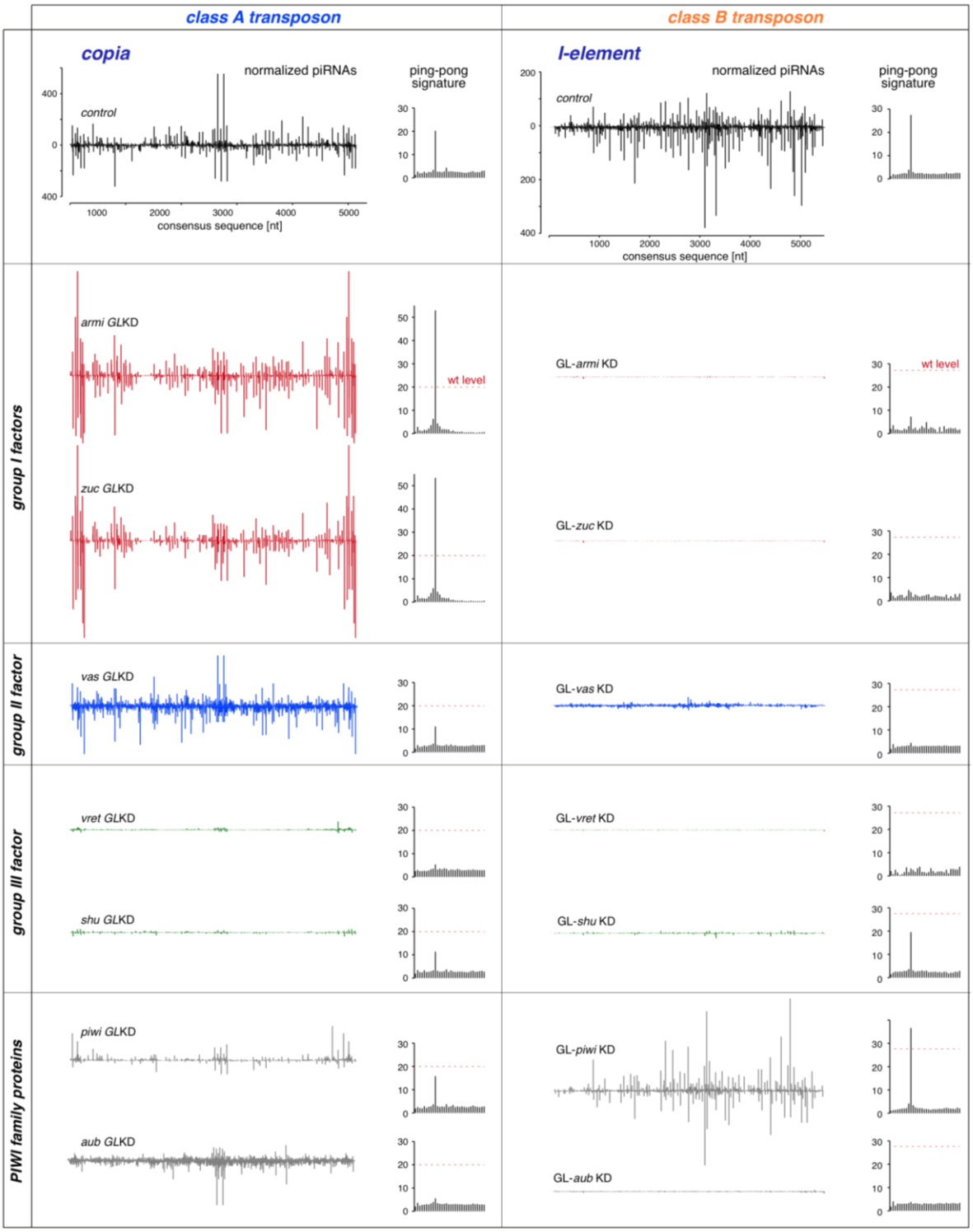

Figure S5.
Shown are normalized piRNA profiles mapping to the class A element *copia* (left column) and the class B element *I-element* (right column) in control-GLKD (top) and all other analyzed GLKD libraries that are classified into the biogenesis groups I-III. Also shown are the ping-pong signatures for each element in each library with the levels obtained from the control-GLKD library indicated with a red dashed line.

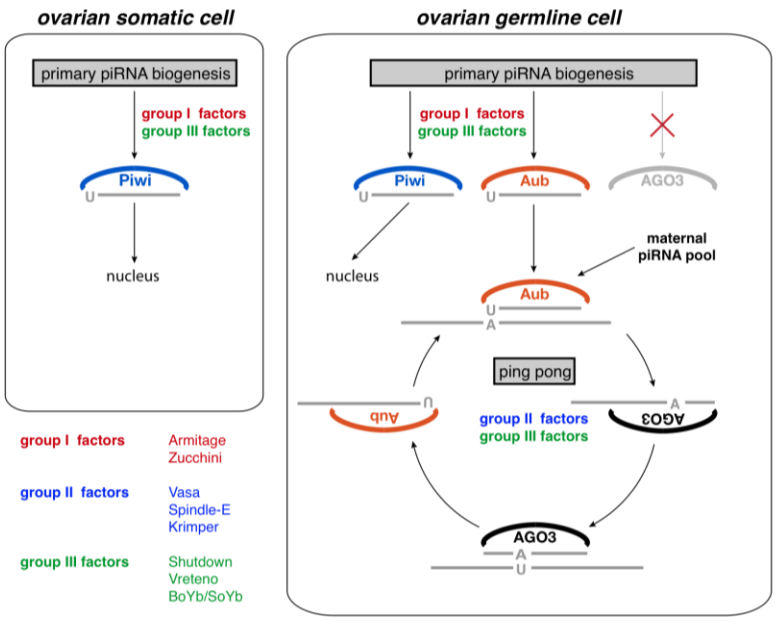

Figure S6.
Model of primary and secondary (ping-pong) piRNA biogenesis in *Drosophila*. Depicted are a somatic and a germline cell from the *Drosophila* ovary. The involvement of group I-III biogenesis factors as well as their identity is indicated.

Table S4 (used fly stocks)

shu^{1}/CyO (WQ41) and *shu*^{2}/CyO (WM40) (Schupbach and Wieschaus, 1991)
Df(2R)BSC665/SM6a (Bloom. # 26517);
tj-GAL4 (DGRC # 104055);
UAS-*Dcr-2*; NGT (Bloom. # 25751);
P{GAL4::VP16-*nos*.UTR}CG6325^{MVD1} (Bloom. # 4937);
MTD-GAL4 (Ni et al., 2011);
w^{1118} (Bloom. # 3605);
armi shRNA, *vret* shRNA, *SoYb* shRNA, *BoYb* shRNA, *spn-E* shRNA (Handler et al., 2011);
piwi shRNA, *aub* shRNA (Ni et al., 2011);
armi RNAi (VDRC # 16205);
zuc shRNA (TRiP # GL00111);
shu RNAi (VDRC # 105832);
shu shRNA (TRiP # GL00379);
vas, *krimp* and *AGO3* shRNAs (sequences in Table S1) were integrated into attP2.
eGFP_Shu, eGFP_Aub, and eGFP_Hsp83 were cloned by inserting N-terminal eGFP via bacterial recombineering into genomic rescue constructs and integrated into the attp2 landing site.
shu^{2} mutant mitotic clones were obtained with *hsflp*^{122}; FRT42D *arm*-lacZ;
control GLKD was obtained from MTD x *w*^{1118};
GFP, armi, *zuc, piwi, vret*, *SoYb/BoYb*, *vas*, *spn-E*, *krimp*, *aub* and *AGO3* GLKDs were obtained from MTD-GAL4 and the respective shRNAs. *shu* GLKD was obtained from UAS-*Dcr-2*; NGT; P{GAL4::VP16-*nos*.UTR}CG6325^{MVD1} and *shu* RNAi.
GLKDs of *shu* and *armi* for Fig. 1 were obtained with UAS-*Dcr-2*; NGT; nosGAL4 and the respective VDRC lines.
All flies were aged 5 days at 25°C before analysis.

Table S5 (oligos used for shRNA cloning)

GENE	Primer	Sequence
vas	vas_top	ctagcagtAGCGATGTTCCACAACCTATAtagttatattcaagcataTATAGGTTGTGGAACATCGCTgcg
	vas_bottom	aattcgcAGCGATGTTCCACAACCTATAtatgcttgaatataactaTATAGGTTGTGGAACATCGCTactg
krimp	krimp_top	ctagcagtCAGATTGGGAGACTACGAATAtagttatattcaagcataTATTCGTAGTCTCCCAATCTGgcg
	krimp_bottom	aattcgcCAGATTGGGAGACTACGAATAtatgcttgaatataactaTATTCGTAGTCTCCCAATCTGactg
AGO3	AGO3_top	ctagcagtCTGGTTGATCTTTATCAGCAAtagttatattcaagcataTTGCTGATAAAGATCAACCAGgcg
	AGO3_bottom	aattcgcCTGGTTGATCTTTATCAGCAAtatgcttgaatataactaTTGCTGATAAAGATCAACCAGactg

Table S6 (used siRNAs for RNAi in OSCs)

GENE	siRNA	Sequence
eGFP	eGFP_guide	ACUUCAGGGUCAGCUUGCCTT
	eGFP_passenger	GGCAAGCUGACCCUGAAGUTT
fs(1)Yb	Fs(1)Yb_guide	UUGAAGAUCUUAUCGCAGCTT
	Fs(1)Yb_passenger	GCUGCGAUAAGAUCUUCAATT
Armi	Armi_guide	UAAACUUAGCUUGACAGCGTT
	Armi_passenger	CGCUGUCAAGCUAAGUUUATT
Vret	Vret1_guide	UUGUAGAGCACAAUUUGUCTT
	Vret1_passenger	GACAAAUUGUGCUCUACAATT
	Vret2_guide	UUCCUUGAUAUUAUCCACGTT
	Vret2_passenger	CGUGGAUAAUAUCAAGGAATT
Shu	Shu1_guide	UAGUCGCUGUCGCUGUCGCTT
	Shu1_passenger	GCGACAGCGACAGCGACUATT
	Shu2_guide	UUCGCAUCUCCUAUGAGCGTT
	Shu2_passenger	CGCUCAUAGGAGAUGCGAATT
Piwi	Piwi_passenger	CACCUUCACGCCUGGGAGCTT
	Piwi_guide	GCUCCCAGGCGUGAAGGUGTT
Zuc	Zuc_passenger	UUGUUGUGCAUCAAGUUCGTT
	Zuc_guide	CGAACUUGAUGCACAACAATT
Krimp	Krimp_guide	UAUAUAUUUCCAAUCGUCCTT
	Krimp_passenger	GGACGAUUGGAAAUAUAUATT

Table S7 (primers for QPCR analysis)

GENE	Primer	Sequence
rp49	rp49_fwd	CCGCTTCAAGGGACAGTATCTG
	rp49_rev	ATCTCGCCGCAGTAAACGC
ZAM	ZAM_fwd	ACTTGACCTGGATACACTCACAAC
	ZAM_rev	GAGTATTACGGCGACTAGGGATAC
HeT-A	HeT-A_fwd	CGCGCGGAACCCATCTTCAGA
	HeT-A_rev	CGCCGCAGTCGTTTGGTGAGT
blood	blood_fwd	AACAATAGAAAGAAGCCACCGAAC
	blood_rev	AGTCATGGACTATTGAGGGTGTTG
FLAG_HA_tag	TAG_fwd	GCCGCATCTTTTACCCATACGA
	TAG_rev	GAGCAGCGTAATCTGGAACGTCA