Supplemental Materials

Molecular Biology of the Cell

Sokolova et al.

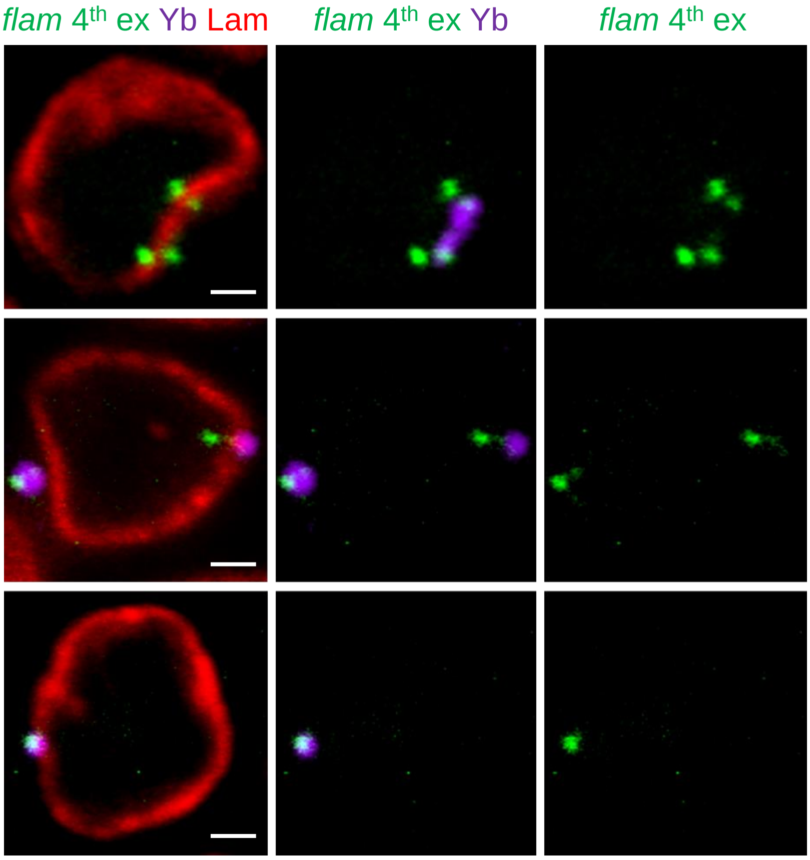

Figure S1: *flam foci* are present both in the nucleus and in the cytoplasm of OSCs. RNA FISH with *flam* 4th exon probe (green) coupled with the immunostaining of nuclear envelope by anti-lamin Dm0 antibody (red) and of Yb bodies by anti-Yb antibody (violet) on OSCs. FISH signals reside at both sides of the nuclear envelope with the cytoplasmic signals colocalizing with the Yb bodies (upper panel), or passing through nuclear envelope to Yb body (middle panel), or completely colocalizing with Yb body (lower panel). Scale bar 1 μm.

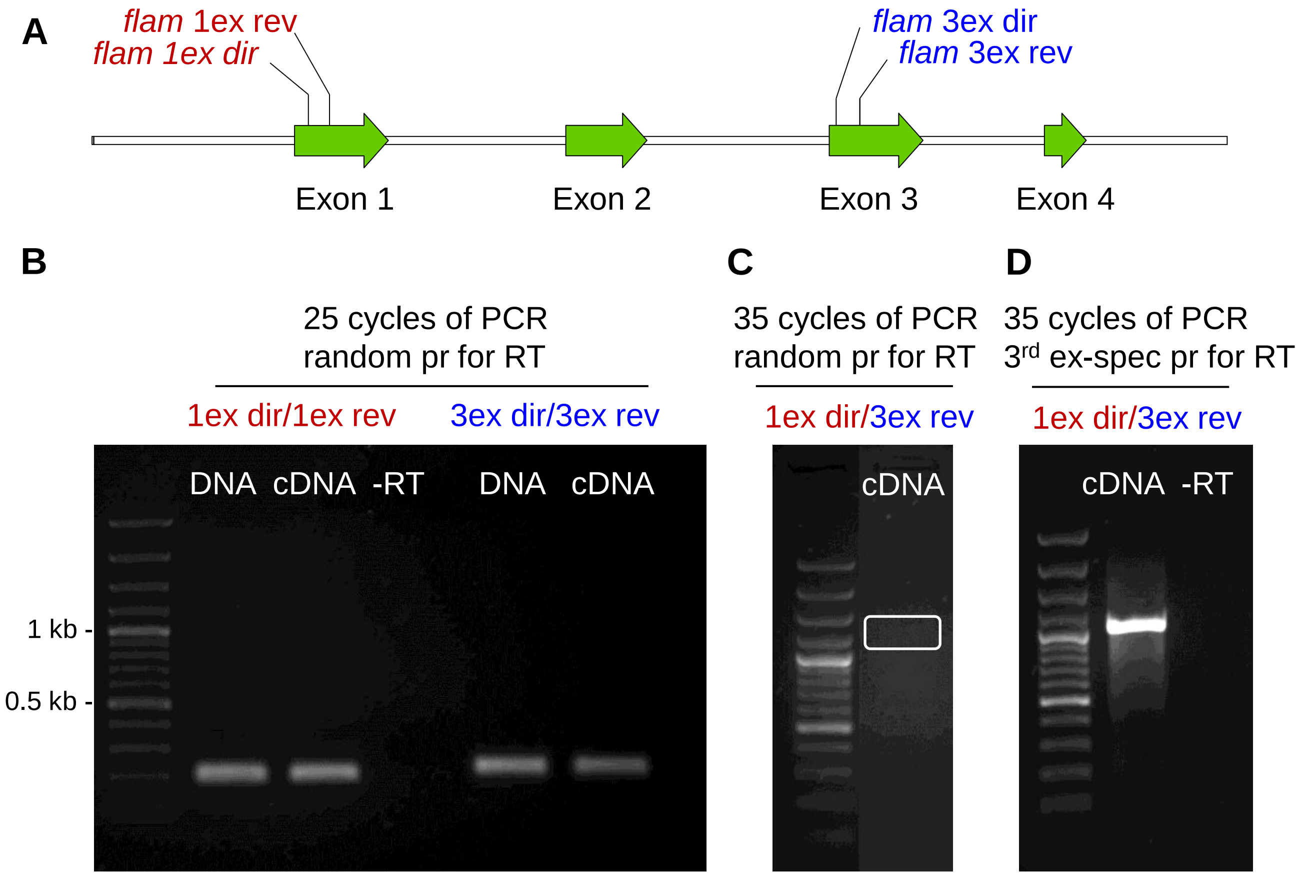

Figure S2: *flam* 1st and 3rd exons are mostly not linked together in the continuous transcripts. (A) Scheme of the 5'-part of *flam* piRNA cluster with the indication of primers used for RT-PCR. (B) After 25 cycles of PCR, *flam* 1st and 3rd exons are readily detected by the corresponding primer pairs in the ovarian cDNA and genomic DNA. (C, D) After 35 cycles of PCR with the 1st exon direct primer and 3rd exon reverse primer, *flam* transcript region corresponding to the 1st and 3rd joint exons is not detected in the ovarian cDNA obtained after RT reaction with the random primer (C), but is detected after RT reaction with the exon-specific primer for the 3rd exon (D).

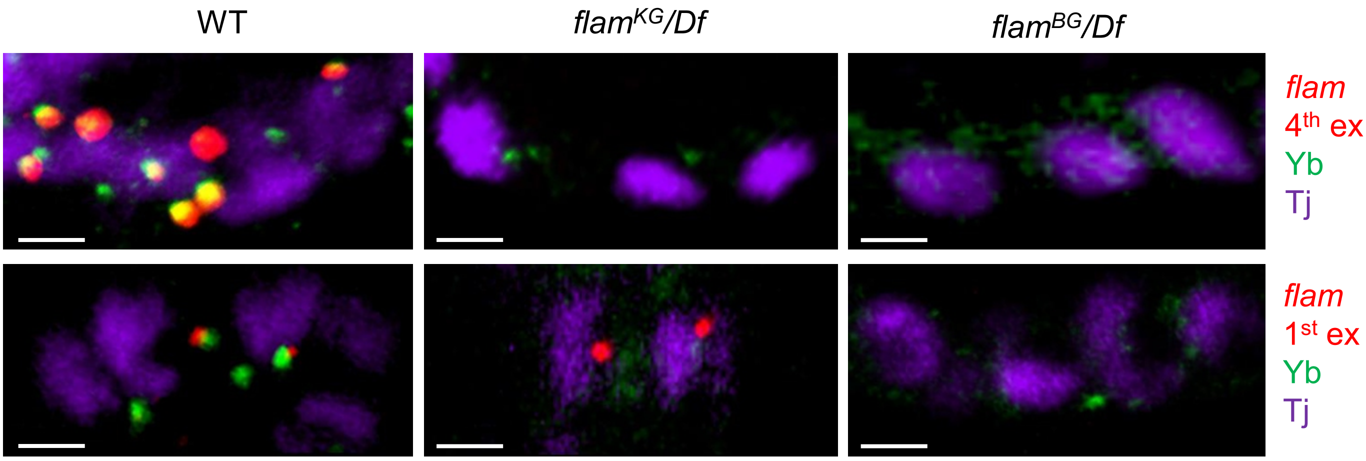

Figure S3: *flam* 1st exon transcripts are gathered in the foci in *flam*^{KG} but not in *flam*^{BG} mutants, but Yb bodies are disintegrated in both mutants. Enlarged view of follicle cells in the early egg chambers marked by immunostaining with anti-Tj antibody (the nuclear marker of ovarian somatic cells; violet) after RNA FISH with 4th (upper panel) or 1st (lower panel) exon probes (red). Yb bodies are marked by anti-Yb antibody (green). Scale bars 3 μm.

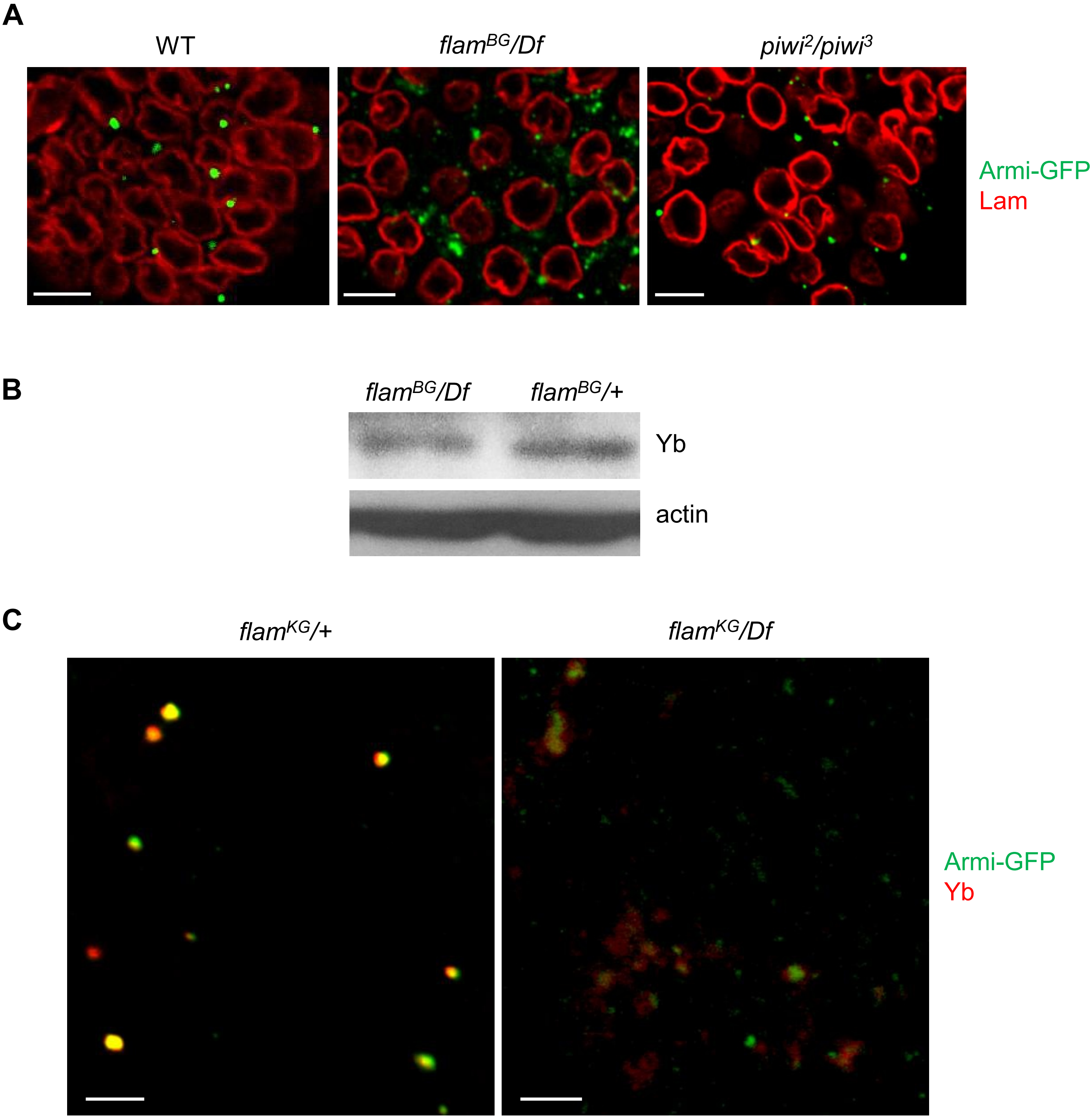

Figure S4: Armi and Yb mark the disintegration of Yb bodies in *flam*, but not in *piwi* mutants. (A) Immunostaining of early egg chamber follicle cells with anti-Armi (green) and anti-lamin Dm0 (red) antibodies showing the disintegration of Yb bodies in *flam*^{BG} mutant, whereas in *piwi*^{2}/*piwi*^{3} mutants Yb bodies remain mostly intact. Scale bar 5 μm. (B) Western blot probed with anti-Yb and anti-actin (for loading control) antibodies showing that the overall amount of Yb protein remains unchanged in *flam*^{BG} mutant ovaries. (C) Immunostaining of control and *flam*^{KG} mutant ovaries, expressing Armi-GFP, with anti-Yb antibody showing the colocalization of Armi (green) and Yb (red) in control (left panel) or in a fraction of dispersed aggregates in the *flam* mutant (right panel). Scale bars 3 μm.

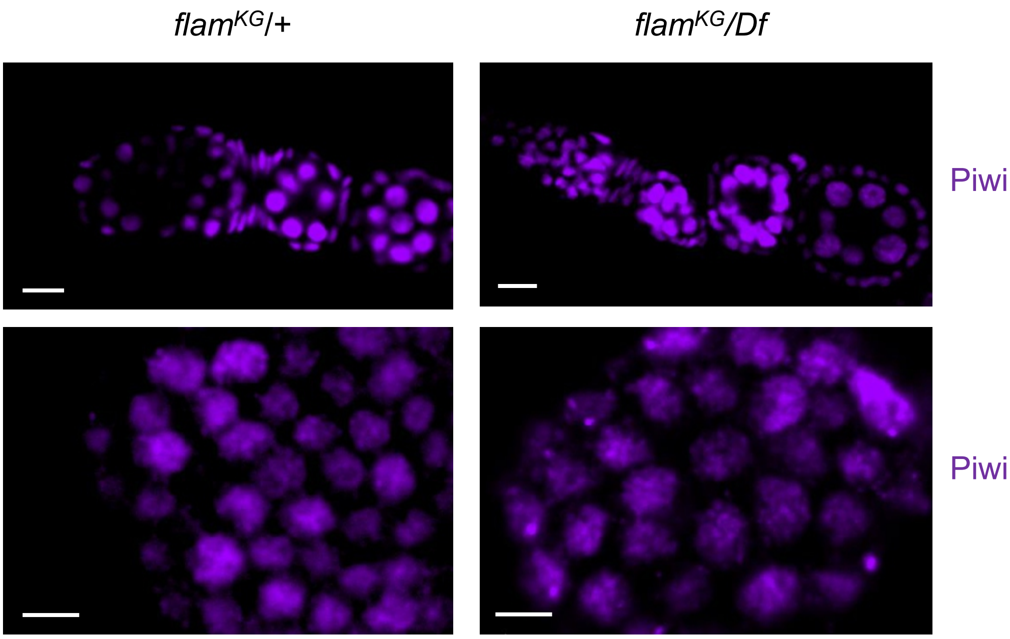

Figure S5: Piwi preserves nuclear localization in *flam*^{KG} mutant. Immunostaining of *flam*^{KG}/+ and *flam*^{KG}/*Df* ovarioles (upper panel, scale bar 10 µm) and of the surface of early egg chambers (lower panel, scale bar 5 µm) with anti-Piwi antibody, showing Piwi localization in the nuclei of follicle cells. More than 20 ovarioles were visually examined.

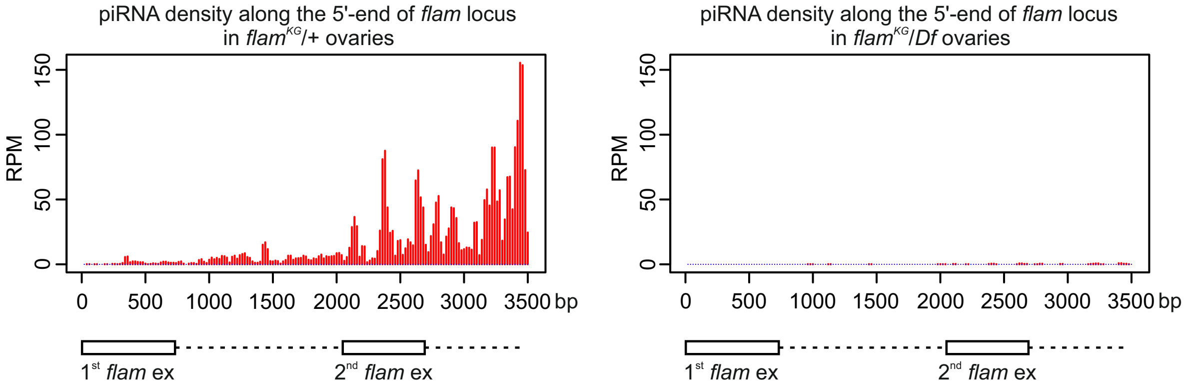

Figure S6: piRNA density (in RPM) for uniquely mapped reads along the 5'-end region of *flam* locus in WT and *flam*^{KG} mutant ovaries. Sense and antisense piRNAs are indicated in red and blue, respectively. Exon/intron *flam* structure (according to Goriaux *et al*. (2014) and FlyBase r6) is represented below.

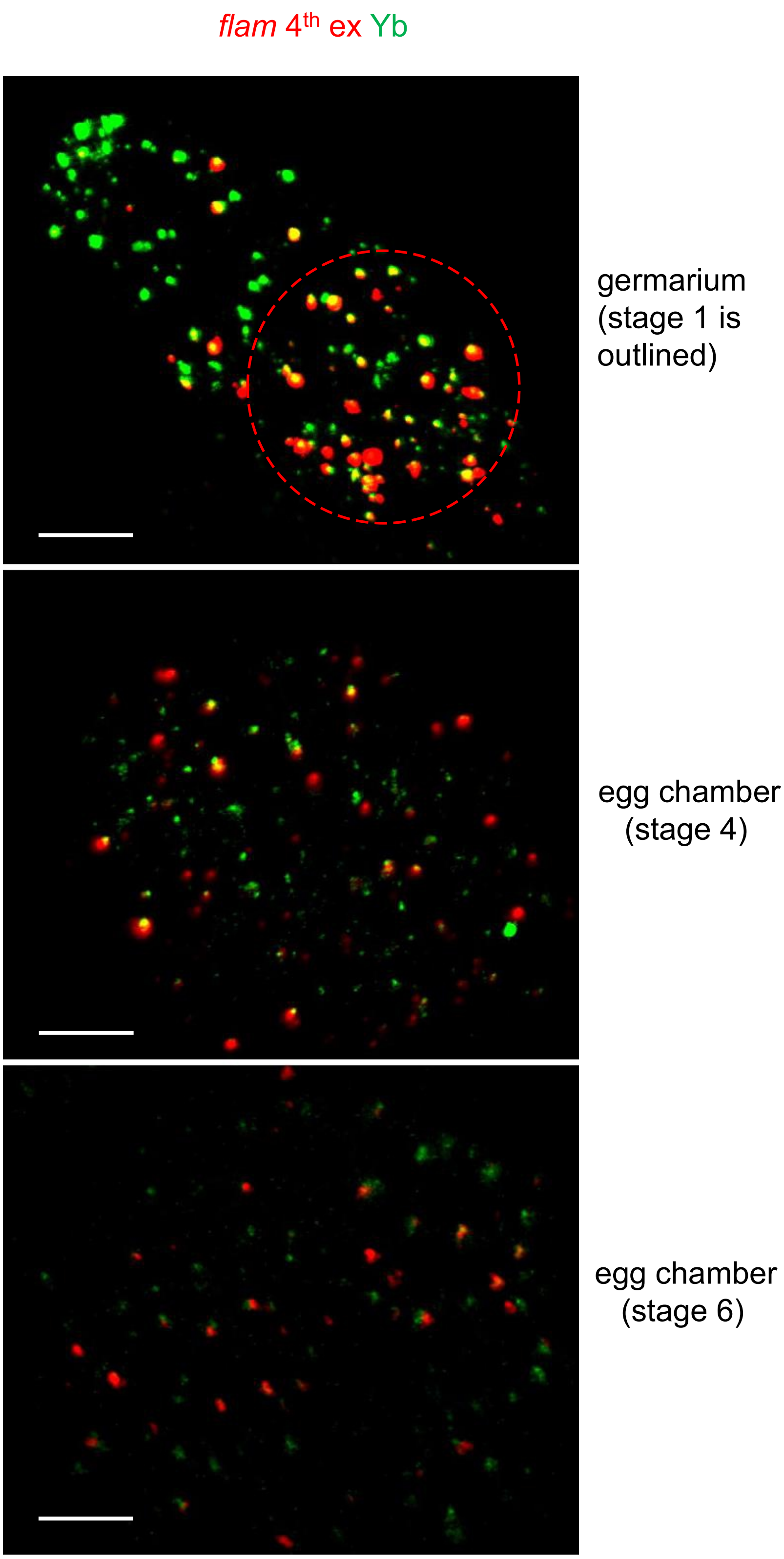

Figure S7: In ovarian follicle cells *flam* foci as well as the Yb bodies become progressively smaller during egg chamber maturation. The enlarged and signal enhanced version of Figure 5A, right panels. RNA FISH with *flam* 4^{th} exon probe (red) coupled with the immunostaining of Yb bodies by anti-Yb antibody (green) in germarium (upper panel, stage 1 egg chamber is outlined by red dotted line), stage 4 egg chamber (middle panel) or stage 6 egg chamber (lower panel) from WT ovariole. Scale bars are 10 μm in the upper panel and 7 μm in the middle and lower panels.

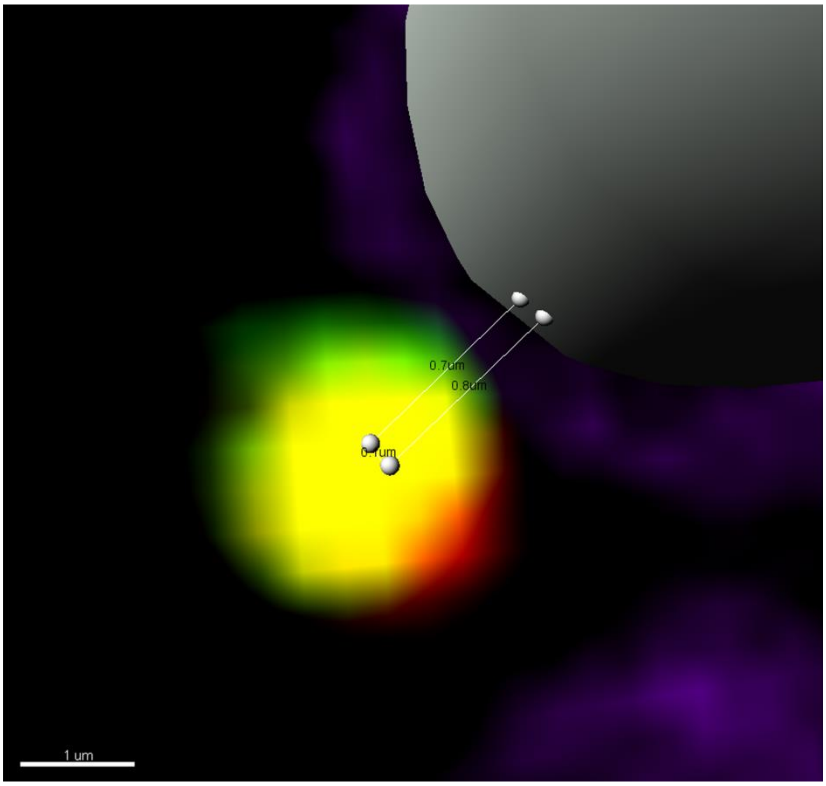

Figure S8: Measuring distances between RNA FISH or Yb body signals and nuclear envelope using IMARIS. Shown is the automatically reconstructed by the middle of nuclear lamina (violet) surface of the nucleus (grey). White spheres are the IMARIS “measurement points” positioned on the centers of signals and at the nuclear envelope. White lines connecting the spheres show the distances from nuclear envelope to the Yb body signal (green) and to the *flam* 4^{th} exon signal (red) (their overlap results in yellow).

Supplemental Table S1: Primers used in this study.

probes for RNA FISH:	
flam 1st ex dir	5'-AGTTGCTTTATGACGCCG
flam 1st ex rev	5'-GAATTAATACGACTCACTATAGGGAGAACAAAATCTTGGGTGACGTAAGT
flam 2nd ex dir	5’-TTGTCAAACCAGCCAAGGTATT
flam 2nd ex rev	5’-GAATTAATACGACTCACTATAGGGAGAGGCAAAACGAGCCCTTACTA
flam 4th ex (#508) dir	5'-ATTCTCCTTTCTCAGGATGC
flam 4th ex (#508) rev	5'-GAATTAATACGACTCACTATAGGGAGAGCATTGCTACCTTACGTTTC
flam distal ex (#681) dir	5'-AGTGCTCAAAGGTTGTCTCAACTCCG
flam distal ex (#681) rev	5'-GAATTAATACGACTCACTATAGGGAGAATGGATTAGGCAGTCTCGTGAAAGC
primers for RT-qPCR:	
flam 1st ex dir	5'-GGTATGGACGCAAGAAAAAGGAAT
flam 1st ex rev	5'-GAAACAAACAAATTAACCGCAGAAC
flam 3rd ex dir	5'-CAGATTACCATTTGGCTATGAGGATCAGAC
flam 3rd ex rev	5'-TGGTGAAATACCAAAGTCTTGGGTCAAC
flam 3rd ex RT	5’-GAATTAATACGACTCACTATAGGGAGATGGTGAAATACCAAAGTCTTGGGTCAAC
adh dir	5'-GCCTGCGTACATAGCCGAGAT
adh rev	5'-GCTCCGTTAGTTGTTGGTTTCC

Supplemental Table S2: Counted in IMARIS distances between *flam* RNA FISH and Yb body signals, or between *flam* or Yb body signals and nuclear lamina.

4th *flam* exon RNA FISH_WT_exp1

N	volume of nucleus, mkm^3	distance between *flam* RNA FISH signal and nuclear lamina, mkm	distance between Yb body and nuclear lamina, mkm	distance between *flam* RNA FISH and Yb signals, mkm	"-" inside nucleus, "+" outside nucleus
1	40	0,213	0,059	0,131	
2	45	-0,058	0,333	0,406	
3	60	0,232	0,792	0,619	
4	48	1,150	0,722	0,428	
5	41	0,051	0,040	0,185	
6	47	0,086	0,390	0,395	
7	40	0,320	0,100	0,361	
8	58	0,076	0,189	0,157	
9	29	0,550	0,688	0,696	
10	63	-0,465	0,199	0,658	
11	52	-0,032	0,450	0,698	
12	51	0,225	0,317	0,277	
13	70	0,372	0,646	0,295	
14	52	-0,039	0,010	0,338	
15	65	-0,037	0,417	0,455	
16	43	0,321	0,212	0,153	
17	44	-0,819	0,026	0,928	
18	44	0,650	0,632	0,208	
19	44	0,563	0,300	0,312	
20	57	-0,084	0,497	0,657	
21	44	0,502	0,314	0,272	
22	64	0,427	0,327	0,326	
23	64	0,029	0,092	0,267	
24	48	0,088	0,202	0,475	
25	48	0,202	0,102	0,281	
26	59	0,451	0,563	0,291	
27	59	0,812	1,168	0,383	
28	50	0,541	0,958	0,466	
29	46	1,133	1,594	0,593	
30	43	-0,014	0,183	0,179	
31	55	-0,043	0,279	0,379	
32	36	0,704	1,000	0,327	
33	39	-0,055	0,167	0,733	
34	43	0,969	1,195	0,424	
35	43	1,165	1,239	0,183	
36	64	0,198	0,143	0,510	
37	64	0,880	0,741	0,246	
38	78	0,316	1,042	0,786	
39	35	0,789	0,717	0,302	
40	62	0,316	0,047	0,297	

4th *flam* exon RNA FISH_WT_exp2

N	volume of nucleus, mkm^3	distance between *flam* RNA FISH signal and nuclear lamina, mkm	distance between Yb body and nuclear lamina, mkm	distance between *flam* RNA FISH and Yb signals, mkm	"-" inside nucleus, "+" outside nucleus
1	45	0,578	0,200	0,517	
2	45	0,984	0,781	0,254	
3	57	0,188	0,333	0,283	
4	89	0,347	0,240	0,364	
5	54	0,156	0,020	0,219	
6	56	0,359	0,429	0,195	
7	85	0,488	0,354	0,312	
8	64	-0,065	0,122	0,215	
9	68	0,092	0,208	0,284	
10	40	0,623	0,713	0,118	
11	87	0,535	0,525	0,210	
12	92	0,791	0,740	0,284	
13	92	-0,291	0,062	0,686	
14	58	0,087	0,086	0,079	
15	60	0,421	0,257	0,290	
16	71	-0,115	0,292	0,430	
17	72	0,259	0,741	0,571	
18	72	1,405	1,101	0,393	
19	85	0,285	0,780	0,481	
20	75	0,793	0,876	0,309	
21	38	0,088	0,438	0,378	
22	35	-0,008	0,255	0,279	
23	34	0,030	0,602	1,533	
24	78	0,297	0,326	0,075	
25	78	0,912	0,523	0,388	
26	40	0,054	0,101	0,159	
27	67	0,523	0,698	0,224	
28	57	0,018	0,025	0,121	
29	57	0,644	0,634	0,201	
30	78	-0,066	0,097	0,170	
31	78	0,291	0,290	0,138	
32	88	0,119	0,080	0,050	
33	88	0,395	0,383	0,032	
34	77	1,124	0,745	0,261	
35	106	0,144	0,260	0,145	
36	106	0,911	0,786	0,898	
37	82	0,330	0,416	0,137	
38	96	0,529	0,318	0,193	
39	96	0,313	0,263	0,133	
40	70	0,637	0,463	0,115	

1st *flam* exon RNA FISH_WT_exp1

N	volume of nucleus, mkm^3	distance between *flam* RNA FISH signal and nuclear lamina, mkm	distance between Yb body and nuclear lamina, mkm	distance between *flam* RNA FISH and Yb signals, mkm	"-" inside nucleus, "+" outside nucleus
1	35	0,133	0,616	0,643	
2	38	0,106	0,572	0,552	
3	35	-0,753	0,032	0,898	
4	37	0,201	0,338	0,142	
5	36	0,019	0,034	1,038	
6	56	-0,055	0,019	0,571	
7	32	0,476	0,688	1,562	
8	33	0,005	0,110	0,433	
9	33	0,183	0,097	0,491	
10	14	-0,375	0,643	1,187	
11	48	0,515	0,757	0,388	
12	16	-0,086	0,660	2,027	
13	18	0,095	0,232	0,240	
14	34	-0,580	0,256	1,065	
15	13	-0,194	0,094	0,391	
16	21	0,383	0,876	0,531	
17	41	-0,281	0,019	0,481	
18	33	0,135	0,709	1,082	
19	20	-0,243	0,090	0,675	
20	35	0,164	0,095	0,184	
21	27	0,144	0,543	0,479	
22	23	-0,035	0,196	0,340	
23	28	0,342	0,482	0,218	
24	41	-0,166	0,423	0,738	
25	45	-0,202	0,039	0,433	
26	23	0,159	1,267	1,106	
27	38	-0,012	0,493	0,482	
28	53	-0,371	0,072	0,457	
29	33	-0,045	0,018	0,528	
30	30	0,364	0,069	0,407	
31	37	-0,133	0,300	0,633	
32	36	0,450	0,758	0,319	
33	48	-0,181	0,285	0,641	
34	42	-0,449	0,001	0,488	
35	28	0,680	0,721	0,255	
36	38	0,587	0,789	0,282	
37	40	0,330	0,670	0,317	
38	36	0,329	0,114	1,697	
39	39	0,090	0,290	0,456	
40	40	0,365	0,255	0,351	

1st *flam* exon RNA FISH_WT_exp2

N	volume of nucleus, mkm^3	distance between *flam* RNA FISH signal and nuclear lamina, mkm	distance between Yb body and nuclear lamina, mkm	distance between *flam* RNA FISH and Yb signals, mkm	"-" inside nucleus, "+" outside nucleus
1	43	-0,236	0,135	0,390	
2	58	0,069	0,003	0,087	
3	47	-0,019	0,080	0,364	
4	41	0,799	0,289	1,042	
5	31	-0,341	0,090	0,488	
6	31	0,405	0,391	0,550	
7	52	0,242	0,189	0,265	
8	52	0,135	0,040	0,130	
9	72	0,275	0,050	0,472	
10	58	-0,300	0,284	1,025	
11	58	1,661	0,822	0,756	
12	48	-0,346	0,856	1,266	
13	71	-0,183	0,043	0,582	
14	71	-0,105	0,019	0,134	
15	70	-0,458	0,009	0,682	
16	34	-0,064	0,040	1,078	
17	47	-0,923	0,015	0,946	
18	47	0,488	1,011	0,554	
19	30	-0,328	0,052	0,550	
20	29	0,565	0,154	0,532	
21	54	-0,107	0,100	0,239	
22	52	0,198	0,868	0,588	
23	33	-0,123	0,119	0,434	
24	34	-0,156	0,669	1,159	
25	45	0,791	0,810	0,815	
26	45	-0,459	0,061	0,592	
27	45	0,058	0,092	0,253	
28	41	0,048	0,270	0,207	
29	50	0,660	0,318	0,321	
30	50	0,096	0,085	0,747	
31	16	0,559	0,372	0,189	
32	39	0,346	0,214	0,416	
33	36	0,288	0,508	0,356	
34	53	0,836	1,596	0,596	
35	53	0,143	0,151	0,056	
36	81	-0,333	0,169	0,494	
37	83	-0,143	0,013	0,235	
38	70	0,092	0,388	0,608	
39	78	-0,146	1,255	1,225	
40	60	0,025	0,449	0,426	

distal *flam* exon RNA FISH_WT_exp1

N	volume of nucleus, mkm^3	distance between *flam* RNA FISH signal and nuclear lamina, mkm	distance between Yb body and nuclear lamina, mkm	distance between *flam* RNA FISH and Yb signals, mkm	"-" inside nucleus, "+" outside nucleus
1	116	0,537	0,511	0,253	
2	116	0,847	0,752	0,131	
3	72	0,218	0,294	0,256	
4	81	0,059	0,101	0,483	
5	77	0,845	0,634	0,194	
6	59	0,326	0,228	0,219	
7	95	1,029	0,716	1,023	
8	95	0,483	0,710	0,402	
9	84	1,446	1,262	0,171	
10	84	0,735	0,925	0,499	
11	75	0,087	0,031	0,233	
12	67	0,135	0,517	0,415	
13	89	0,472	0,610	0,252	
14	38	0,334	0,265	0,128	
15	86	0,842	0,498	0,387	
16	86	0,262	0,335	0,154	
17	126	0,087	0,252	0,530	
18	126	1,295	0,308	0,886	
19	65	-0,132	0,308	0,579	
20	76	0,479	0,600	0,243	
21	71	0,220	0,274	0,224	
22	68	0,749	0,690	0,213	
23	44	0,276	0,161	0,197	
24	50	0,521	0,415	0,152	
25	50	0,785	0,525	0,180	
26	55	0,587	0,011	0,604	
27	142	0,383	0,700	0,626	
28	57	-0,568	0,019	0,613	
29	120	-0,048	0,054	0,330	
30	74	0,328	0,896	0,353	
31	84	0,096	0,148	0,286	
32	38	0,145	0,398	0,253	
33	73	0,206	0,139	0,119	
34	56	0,448	0,363	0,161	
35	64	0,590	0,525	0,226	
36	64	0,972	0,313	0,818	
37	64	0,558	0,607	0,525	
38	44	0,262	0,758	0,363	
39	44	0,708	0,929	0,204	
40	55	0,126	0,046	0,106	

distal *flam* exon RNA FISH_WT_exp2

N	volume of nucleus, mkm^3	distance between *flam* RNA FISH signal and nuclear lamina, mkm	distance between Yb body and nuclear lamina, mkm	distance between *flam* RNA FISH and Yb signals, mkm	"-" inside nucleus, "+" outside nucleus
1	69	0,171	0,334	0,177	
2	16	1,226	0,630	0,601	
3	40	0,167	0,196	0,194	
4	40	0,725	0,586	0,143	
5	73	0,161	0,165	0,114	
6	73	0,649	0,365	0,255	
7	68	0,221	0,329	0,124	
8	68	0,581	0,461	0,164	
9	46	1,606	1,362	0,308	
10	46	0,281	0,280	0,194	
11	55	-0,214	0,010	0,409	
12	41	-0,110	0,020	0,148	
13	67	0,503	0,568	0,095	
14	67	0,376	0,429	0,296	
15	49	-0,182	0,042	0,341	
16	55	-0,090	0,027	0,107	
17	89	0,042	0,017	0,099	
18	87	-0,037	0,062	0,168	
19	87	-0,029	0,012	0,086	
20	87	0,256	0,005	0,324	
21	84	0,976	0,726	0,278	
22	91	0,125	0,123	0,101	
23	91	0,319	0,585	0,269	
24	53	0,803	0,314	0,589	
25	67	0,136	0,100	0,136	
26	62	0,181	0,042	0,244	
27	62	0,557	0,937	0,387	
28	32	0,369	0,507	0,144	
29	88	-0,073	0,007	0,222	
30	67	0,263	0,326	0,079	
31	52	0,473	0,422	0,203	
32	64	-0,065	0,301	0,398	
33	50	0,966	0,592	0,361	
34	57	0,504	0,668	0,243	
35	66	0,416	0,512	0,192	
36	66	0,259	0,191	0,203	
37	61	0,752	0,732	0,096	
38	55	0,171	0,394	0,449	
39	55	0,223	0,258	0,099	
40	68	0,531	0,478	0,056	

1st *flam* exon RNA FISH_*flam*^{KG}_exp1

N	volume of nucleus, mkm^3	distance between *flam* RNA FISH signal and nuclear lamina, mkm	"-" inside nucleus, "+" outside nucleus
1	66	-0,195	
2	78	1,060	
3	76	-0,023	
4	76	-0,849	
5	48	0,262	
6	48	-0,381	
7	57	-0,711	
8	57	0,621	
9	43	0,014	
10	43	-0,079	
11	56	0,053	
12	41	-0,157	
13	62	0,359	
14	64	-0,113	
15	64	0,023	
16	64	0,258	
17	62	0,152	
18	52	0,234	
19	63	0,609	
20	16	0,488	
21	16	-0,660	
22	52	0,047	
23	49	-0,447	
24	59	0,113	
25	56	-0,153	
26	56	-0,036	
27	80	-0,046	
28	80	0,216	
29	49	0,093	
30	47	0,063	
31	49	0,041	
32	84	-0,101	
33	84	-0,653	
34	76	0,243	
35	80	-0,238	
36	80	0,034	
37	59	0,068	
38	89	-0,139	
39	89	-0,874	
40	104	0,058	

Supplemental Data S1.

This Excel file includes data for RPM values of small RNAs uniquely mapped to the 3'-UTRs of genic piRNA clusters, to *flamenco*, to other predominantly germline-specific piRNA clusters, and to the top 10 most abundant in OSCs microRNAs based on the deep sequencing of small RNA libraries prepared from $flam^{KG}$/+ and $flam^{KG}$/*Df* ovaries (this work; Sheet #1), or based on the data taken from (Malone *et al*., 2009; Sheet #2).