An *in vivo* RNAi assay identifies major genetic and cellular requirements for primary piRNA biogenesis in *Drosophila*

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Table S1. Phenotypic Summary of *tj*-GAL4 driven RNAi against a collection of house-keeping genes

CG #	Gene Name	VDRC ID	Library	Phenotype
CG13410	mitochondrial ribosomal protein L35	13443	GD	viable; very small ovaries
CG13410	mitochondrial ribosomal protein L35	103388	KK	viable; very small ovaries
CG1913	α-Tubulin at 84B	33427	GD	viable; no ovaries
CG1913	α-Tubulin at 84B	107109	KK	pharate lethal
CG5353	Threonyl-tRNA synthetase	7752	GD	viable; no ovaries
CG5353	Threonyl-tRNA synthetase	107265	KK	viable; very small ovaries
CG5363	cdc2	41838	GD	viable; no ovaries
CG5363	cdc2	106130	KK	viable; no ovaries
CG6253	Ribosomal protein L14	44629	GD	viable; no ovaries
CG6253	Ribosomal protein L14	102011	KK	viable; no ovaries
CG6349	DNA polymerase α 180kD	11227	GD	viable; no ovaries
CG6349	DNA polymerase α 180kD	103699	KK	viable; no ovaries
CG9075	Eukaryotic initiation factor 4a	42202	GD	viable; no ovaries
CG9075	Eukaryotic initiation factor 4a	100310	KK	viable; no ovaries

Table S2. Content Analysis of small RNA libraries obtained from Piwi-IPs.

A. Statistics for reads

	tj > *aub* RNAi	**tj > *piwi* RNAi**	**tj > *armi* RNAi**	**tj > *zuc* RNAi**	**tj > *Yb* RNAi**
Total reads	9.547.451	7.785.649	5.409.376	9.000.057	13.466.053
% of reads matching the genome 100%	7.471.255	5.499.917	3.998.721	5.005.875	10.933.088
# of reads mapping to rRNA, tRNA, snoRNA	522.429	779.521	210.185	645.291	899.389
# of analyzed reads	6.948.826	4.720.396	3.788.536	4.360.584	10.033.699
repeats	83.0 %	84.4 %	85.1 %	84.2 %	83.3 %
no annotation	9.6 %	9.0 %	9.2 %	9.1 %	9.0 %
exons	5.0 %	4.4 %	3.7 %	4.0 %	5.8 %
introns	1.7 %	1.5 %	1.6 %	1.3 %	1.4 %
Pre-miRNAs	0.6 %	0.6 %	0.3 %	1.2 %	0.4 %

B. Statistics for species

	tj > *aub* RNAi	**tj > *piwi* RNAi**	**tj > *armi* RNAi**	**tj > *zuc* RNAi**	**tj > *Yb* RNAi**
Total species	2.433.473	1.988.666	1.796.123	1.566.130	3.193.174
% of species matching the genome 100%	1.336.025	1.001.251	961.431	647.626	1.907.157
# of species mapping to rRNA, tRNA, snoRNA	28.943	28.163	23.412	18.752	44.430
# of analyzed species	1.307.082	973.088	938.019	628.874	1.862.727
repeats	72.8 %	75.8 %	76.9 %	77.7 %	70.1 %
no annotation	11.2 %	10.5 %	10.9 %	10.0 %	11.2 %
exons	11.4 %	10.4 %	8.6 %	9.4 %	15.2 %
introns	4.6 %	3.3 %	3.6 %	2.8 %	3.5 %
Pre-miRNAs	0.0 %	0.1 %	0.0 %	0.1 %	0.0 %

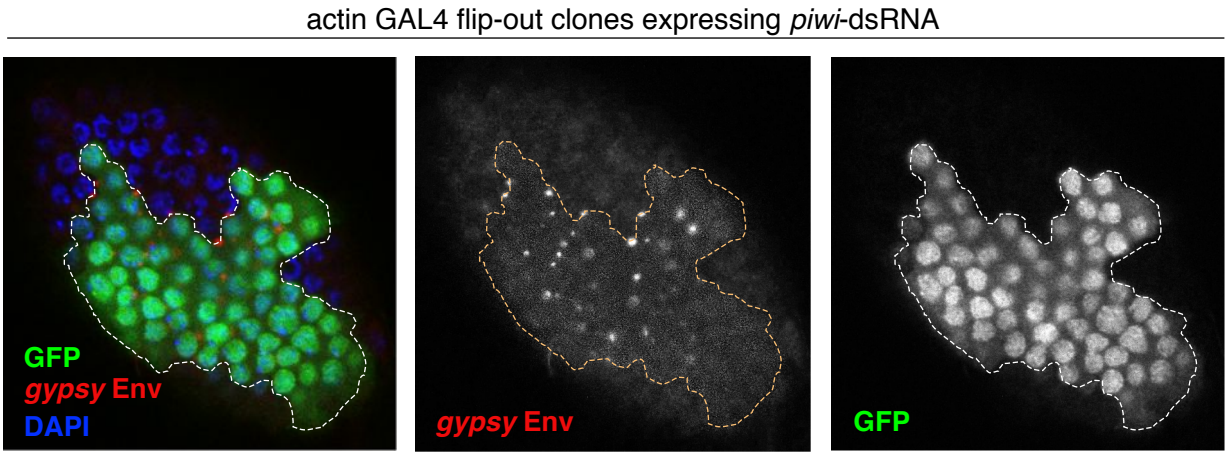

Figure S1. Specific de-silencing of *gypsy* in clones expressing *piwi*-dsRNA.
Shown is a surface view on the follicular epithelium of an egg chamber stained with *gypsy*-Env (red), GFP (green) and DAPI (blue). Cells expressing the dsRNA hairpin against *piwi* are marked with GFP.

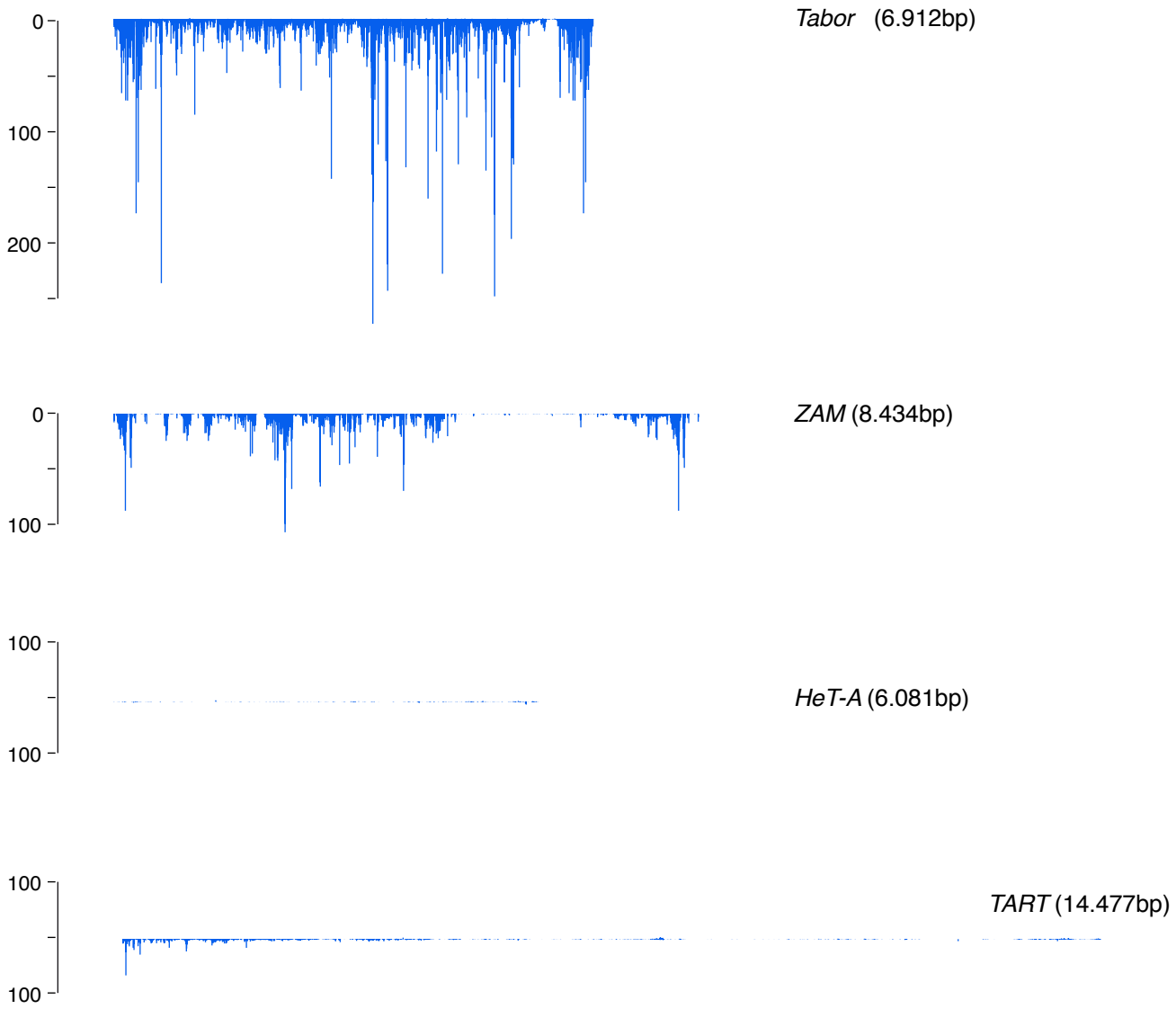

Figure S2. piRNA profiles mapping to *Tabor, ZAM, HeT-A* and *TART* elements in OSC cells.
Small RNA data from Robine et al. 2009 was used to map OSC derived 23-32nt small RNAs to the indicated retro-elements. Peaks pointing down indicate antisense piRNAs, those pointing up correspond to sense piRNAs. All four plots are shown at the same scale. The profiles have a single nt resolution along the x-axis and the y-axis indicates reads per 1 million sequenced RNAs (excluding miRNA, rRNA, tRNA and snoRNA species).

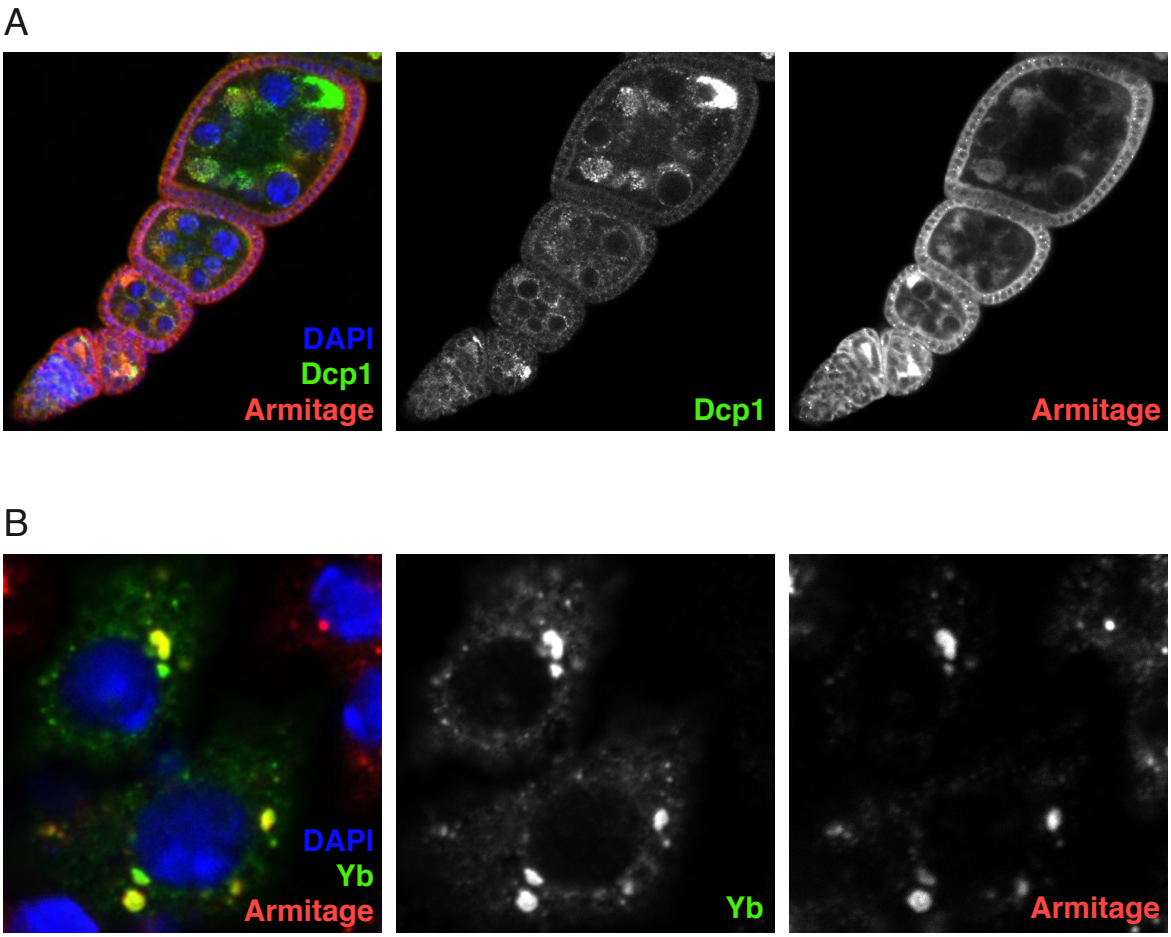

Figure S3. Armitage, Yb and DCP1 localization in OSC cells and ovaries.
(A) Shown is an ovariole expressing YFP-DCP1 (green) stained for Armitage (red) and DNA (blue). **(B)** Shown are OSC cells transiently transfected with GFP tagged Yb (green) and stained for Armitage (red) and DNA (blue).

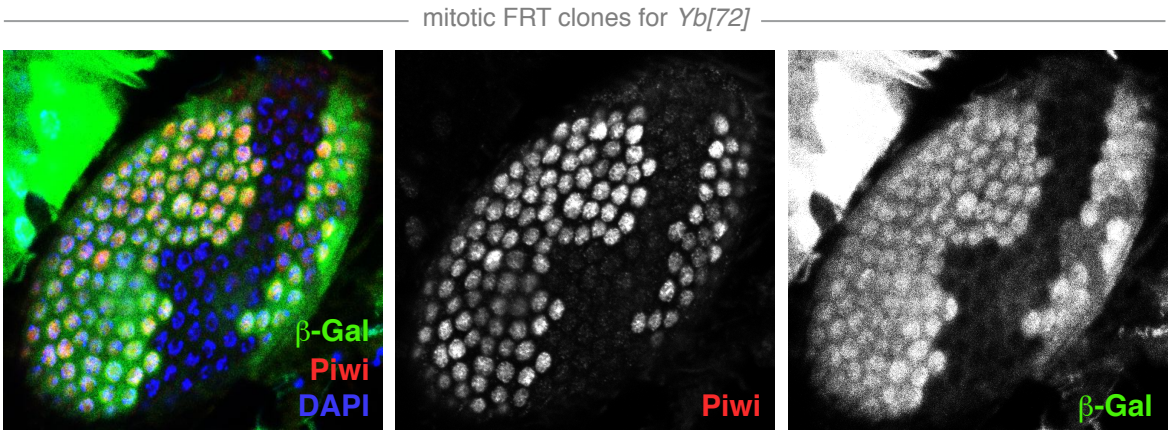

Figure S4. Follicle cells lacking Yb lose nuclear Piwi localization.
Shown is a surface view onto an egg chamber containing a mitotic clone for the *fs(1)Yb[72]* null allele stained for Piwi (red), β-Gal (green) and DNA (blue). The clone is marked by the absence of β-Gal (green).

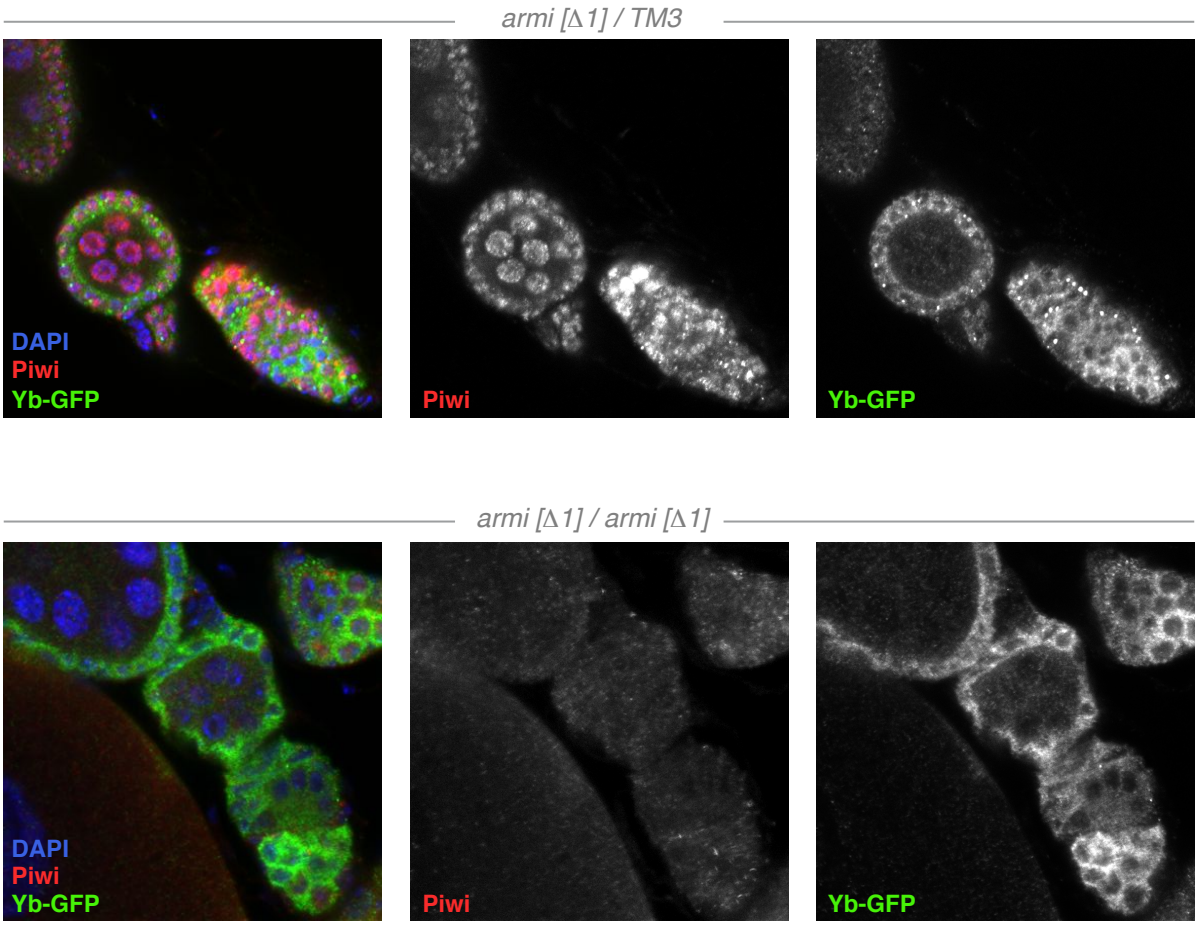

Figure S5. Armitage is required for Yb localization to cytoplasmic foci.
Shown are confocal sections through ovarioles heterozygous (top) or homozygous (bottom) for the *armi[Δ1]* deletion carrying a Yb-GFP (green) transgene and stained for Piwi (red) and DNA (blue).

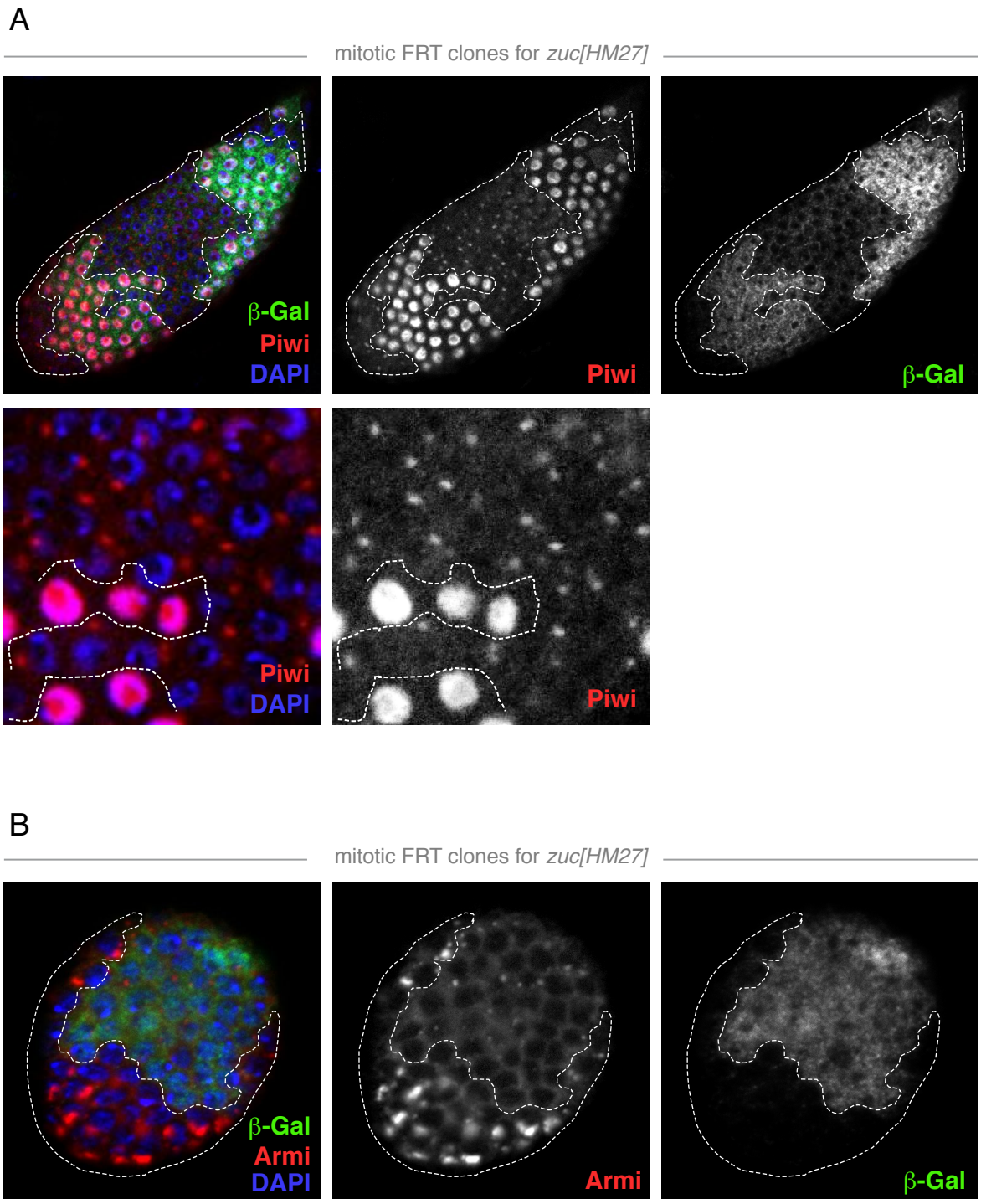

Figure S6. Follicle cells lacking Zuc exhibit Piwi localization to Yb-bodies.
(A) Shown is a surface view onto an egg chamber containing mitotic clones for the *zuc[HM27]* null allele stained for Piwi (red), β-Gal (green) and DNA (blue). The clones are marked by the absence of β-Gal (green) and is encircled by a dashed line. The lower panels show a magnified view. **(B)** Shown is a surface view onto an egg chamber containing mitotic clones for the *zuc[HM27]* null allele stained for Armi (red), β-Gal (green) and DNA (blue). The clone is marked by the absence of β-Gal (green) and encircled by a dashed line.

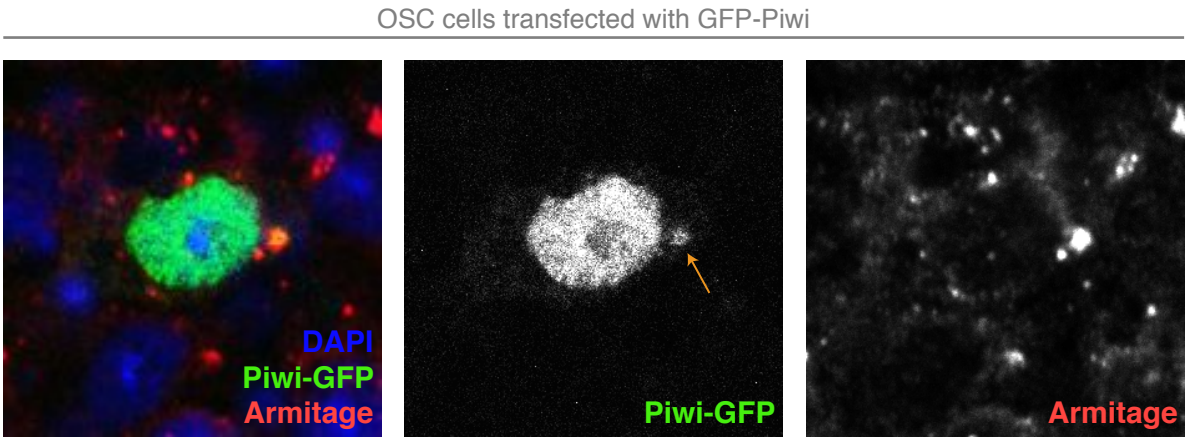

Figure S7. OSC cells transiently expressing GFP-Piwi show Piwi localization to Armi-foci.
Shown are OSC cells chemically transfected with GFP-Piwi (green) stained for Armitage (red) and DNA (DAPI, blue). The arrow points to the Piwi staining co-localizing with Armi.

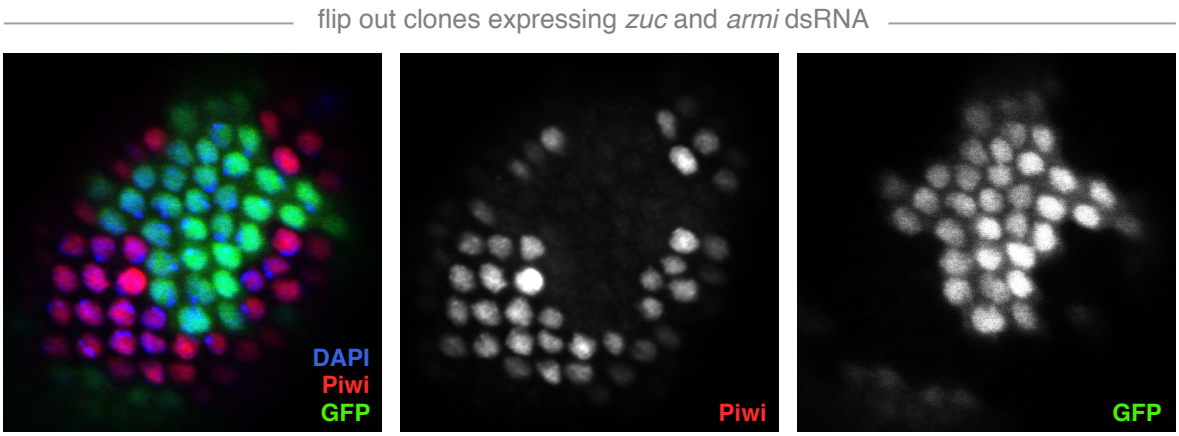

Figure S8. Armitage is required for Piwi localization to Yb-bodies
Shown is a surface view onto the follicular epithelium of an egg chamber containing clones expressing dsRNA hairpins against *zuc* and *armi* stained for Piwi (red) and DNA (DAPI; blue). The clone is marked by the presence of GFP (green).

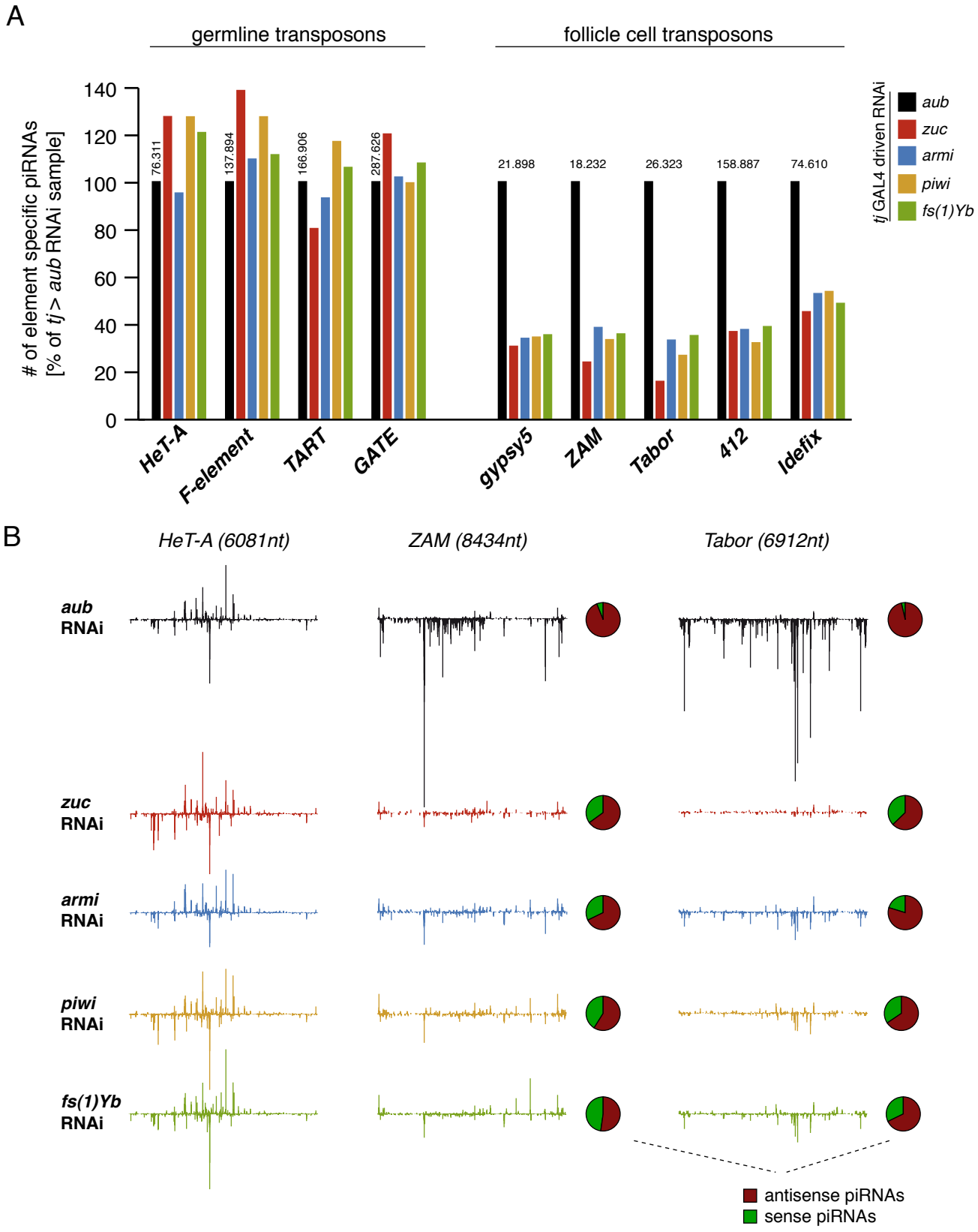

Figure S9. piRNA biogenesis defects in the absence of Zuc, Armi and Yb.
(A) Shown are relative levels of Piwi-piRNAs from ovaries of the indicated genotypes mapping to the indicated transposons. Elements silenced preferentially in the germline are to the left whereas elements with specific silencing in somatic support cells are to the right (based on Malone et al. 2009). Libraries were normalized via the genome-unique mappers to 42AB, a germline specific piRNA cluster (Fig. 5). The *tj*-GAL4>*aub*-RNAi sample acts as control and the raw numbers of this sample are indicated above the black bars. **(B)** Detailed piRNA profiles across the transposons *HetA, ZAM* and *Tabor*. The color code is as in (A). Cake diagrams indicate the levels of sense (green) and antisense (red) piRNAs mapping to the indicated transposons.

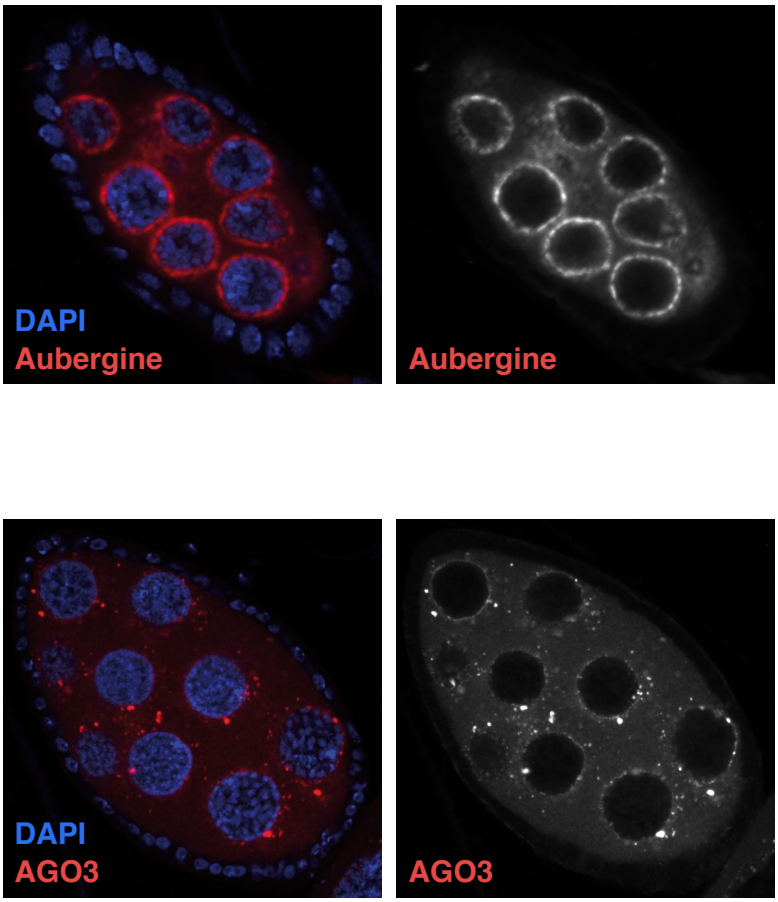

Figure S10. Aubergine and AGO3 localization in *zuc* mutant egg chambers.
Shown are confocal sections through *zuc[HM27]/zuc[Def]* egg chambers stained for Aubergine (top) and AGO3 (bottom). DNA was visualized with DAPI (blue).

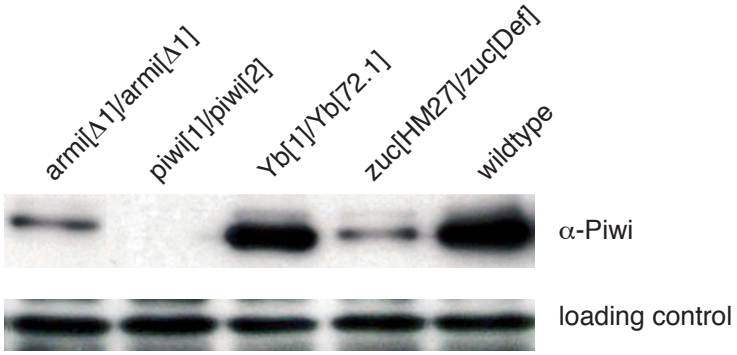

Figure S11. Piwi levels are reduced in primary piRNA biogenesis mutants.
Shown is a western blot against Piwi from ovarian extracts of the indicated mutants. The lower western blot indicates consistent loading.