

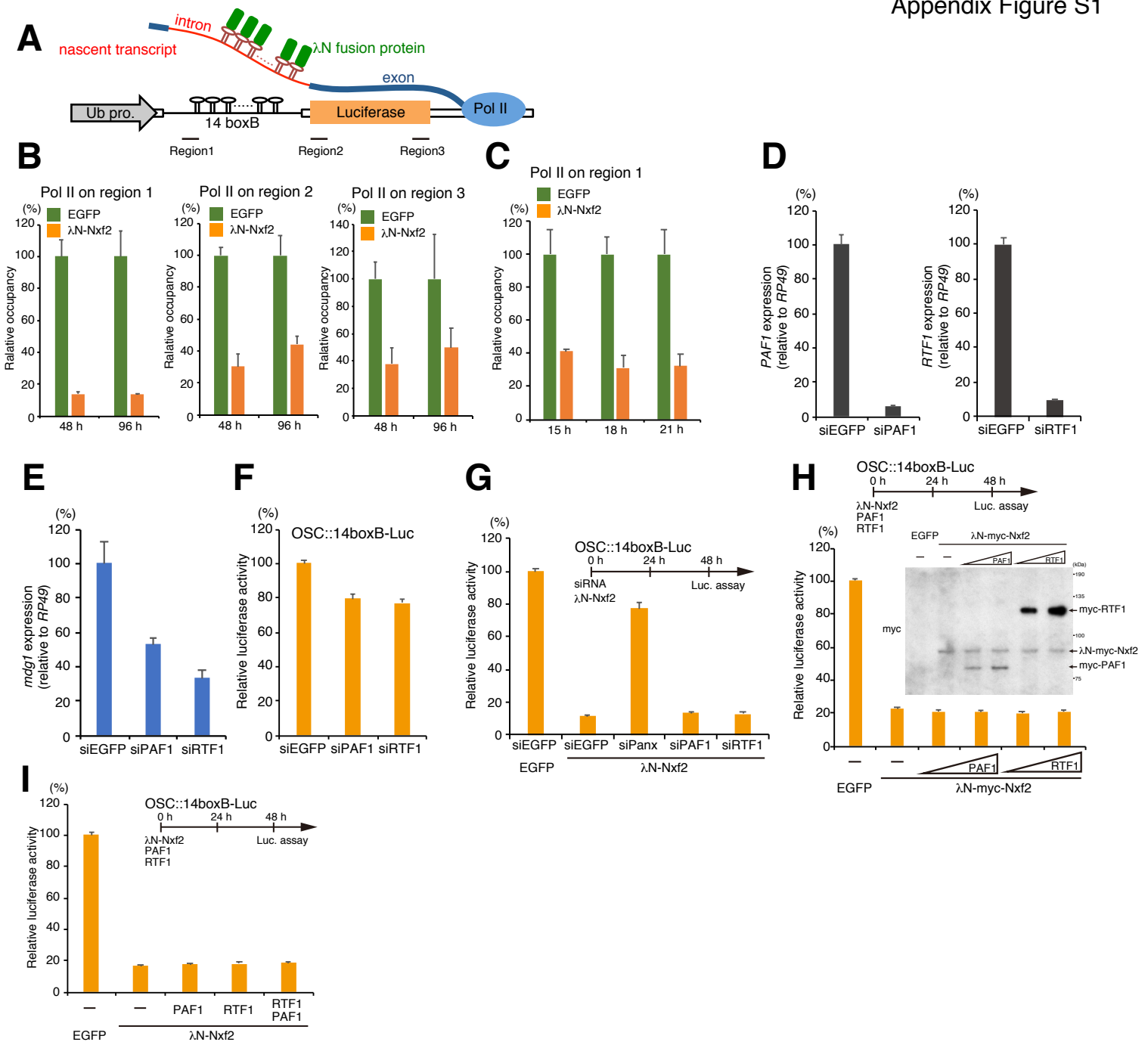
# Appendix

**Nuclear RNA export factor variant initiates piRNA-guided co-transcriptional silencing**

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### Appendix Figure S1. Related to Discussion.

(A) The scheme shows regions targeted by qPCR, following ChIP experiments.

(B) ChIP-qPCR analysis of RNA polymerase II (Pol II) occupancy on the reporter gene upon λN-Nxf2 tethering. Bar graph shows the occupancy relative to that of EGFP. Error bars indicate SD (n=3). OSCs were harvested at 48 or 96 h.

(C) Time course of Pol II occupancy on region 1, immediate upstream of 14 boxB sites, upon λN-Nxf2 tethering. OSCs were harvested at the indicated time points. Error bars indicate SD (n=3). Contrary to our expectations, Pol II accumulation by the enforced tethering of Nxf2 at 14 boxB sites was not observed.

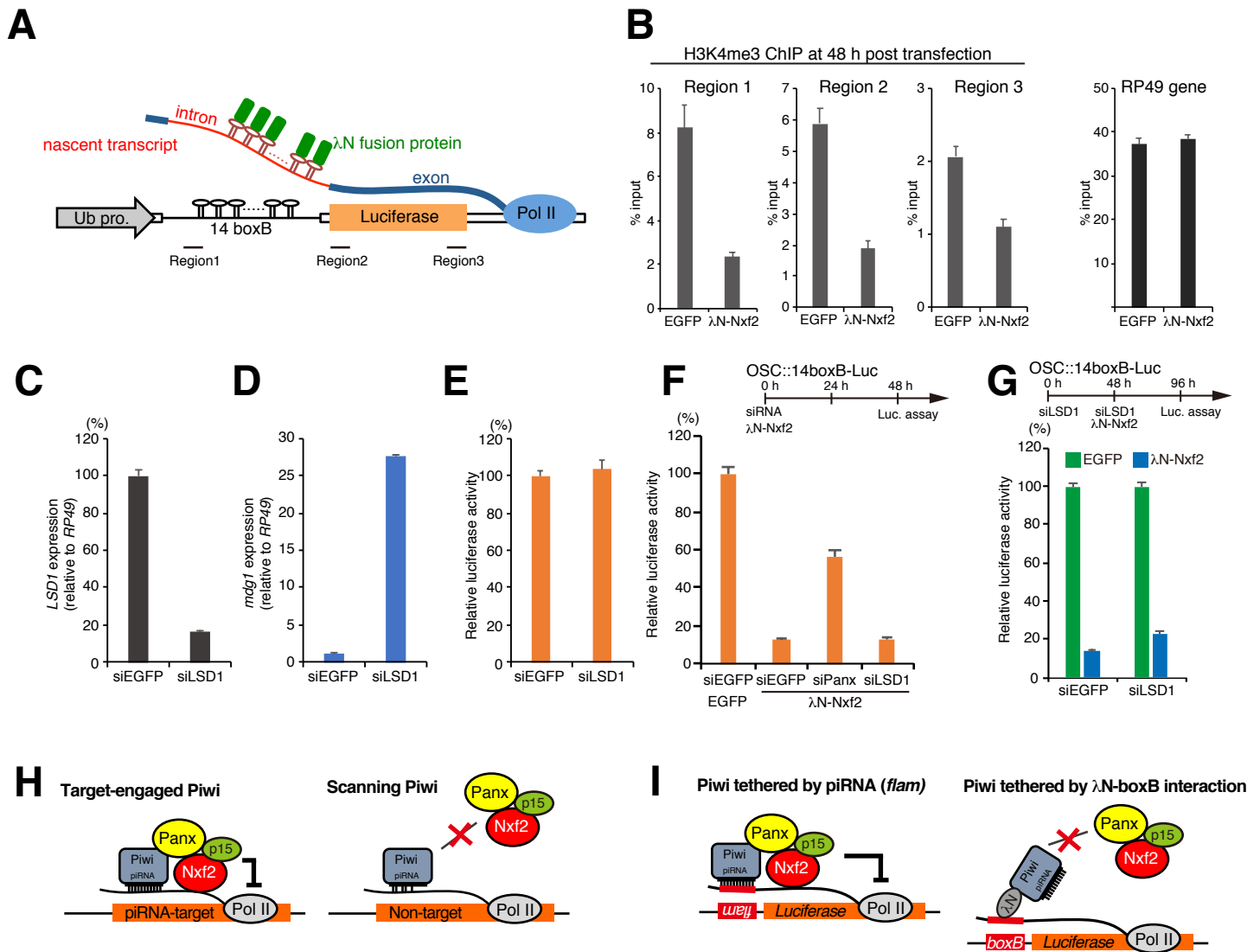
(D) RNA levels of *PAF1* and *RTF1* were quantified by qRT-PCR upon treatment of siEGFP (control), siPAF1, or siRTF1. Error bars represent SD (n=3).

(E) RNA level of *mdg1* was quantified by qRT-PCR upon treatment of siEGFP (control), siPAF1, or siRTF1. Error bars represent SD (n=3). Depletion of PAF1 or RTF1 enhanced the silencing of *mdg1* in OSCs.

(F) Depletion of PAF1 and RTF1 slightly weakened the luciferase activities from the reporter gene with 14 boxB sites integrated in the OSC genome. Error bars represent SD (n=4).

(G) Effects of knockdown of PAF1 and RTF1 on boxB reporter activity upon λN-Nxf2 expression. Bar graph shows luciferase activity relative to that of the sample co-transfected with myc-EGFP and siEGFP (control). Error bars indicate SD (n=4). Transfection schedule of siRNA and protein expression plasmids is shown at the top of the figures. Depletion of PAF1 or RTF1 had no impact on the silencing by the enforced tethering of Nxf2 on a nascent transcript.

(H and I) Effects of exogenous expression of PAF1 and RTF1 on boxB reporter activity upon λN-Nxf2 expression. Bar graph shows luciferase activity relative to that of the sample transfected with myc-EGFP (control). Error bars indicate SD (n=4). Transfection schedule of plasmids is shown at the top of the figures. Exogenous expression of PAF1 or RTF1 did not cancel the silencing by the enforced tethering of Nxf2 on a nascent transcript.



### Appendix Figure S2. Related to Discussion.

(A) The scheme shows regions targeted by qPCR, following ChIP experiments.

(B) ChIP-qPCR analysis of H3K4me3 occupancy on the reporter gene (14 boxB-Luc reporter gene integrated into the genome) upon  $\lambda$ N-Nxf2 tethering. Bar graph shows % input. Error bars indicate SD (n=3). Regions targeted by qPCR are shown in (A). OSCs were harvested at 48 h post-transfection (hpt). H3K4 methylation is a transcriptionally active marker. Although occupancy of H3K4me3 on the *RP49* gene was not affected by  $\lambda$ N-Nxf2 expression, the occupancy of H3K4me3 on the reporter gene decreased even at an earlier time point (48 hpt).

(C) RNA level of *LSD1* was quantified by qRT-PCR upon treatment of siEGFP (control) or siLSD1. Error bars represent SD (n=3).

(D) RNA level of *mdg1* was quantified by qRT-PCR upon treatment of siEGFP (control) or siLSD1. Error bars represent SD (n=3). *mdg1* was de-silenced by LSD1 depletion.

(E) Luciferase activity was not affected by depletion of LSD1. Error bars represent SD (n=4)

(F and G) Effects of knockdown of LSD1 on boxB reporter activity upon  $\lambda$ N-Nxf2 expression. Bar graph shows luciferase activity relative to that of the sample co-transfected with myc-EGFP and siEGFP (control). Error bars indicate SD (n=4). Transfection schedule of siRNA and protein expression plasmids is shown at the top of the figures. Depletion of LSD1 had limited impact on the silencing by the forced tethering of Nxf2 on a nascent transcript.

(H) Schematic model showing the target-engaged Piwi. "The target-engaged Piwi" can recruit the Panx-Nxf2-p15 complex to the target, which elicits co-transcriptional repression. However, "the scanning Piwi" is in the process of searching for targets through random interaction between piRNA and transcripts, many of which likely show partial complementarity. The scanning Piwi cannot recruit Panx-Nxf2-p15 complex to the target.

(I) Schematic model showing Piwi that is targeted by piRNA or is tethered by  $\lambda$ N-boxB interaction in the reporter systems. Piwi targeted by piRNA derived from the *flam* locus can recruit the Panx-Nxf2-p15 complex to the reporter gene, leading to co-transcriptional silencing. Piwi tethered by  $\lambda$ N-boxB interaction cannot recruit the Panx-Nxf2-p15 complex to the reporter gene, and thus fails to induce silencing.

## Upper band

Rank	Description	Accession	emPAI	Score	Mass	Sequence coverage (%)
1	Piwi [Drosophila melanogaster]	429892668	8.06	1054	97037	70
2	piwi, partial [Drosophila simulans]	418207352	2.65	535	95840	46
3	GH10652p [Drosophila melanogaster]	20976828	2.05	698	88155	51
4	rasputin [Drosophila melanogaster]	7739653	1.63	531	74940	43
5	DNA ligase III [Drosophila melanogaster]	84796152	1.33	579	90791	44
6	Fmr1, isoform G [Drosophila melanogaster]	383292605	1.32	303	80980	29
7	CG10077, partial [Drosophila busckii]	924558740	0.72	294	86515	30
8	combgap, isoform B [Drosophila melanogaster]	21645407	0.67	270	83703	14
9	single-stranded recognition protein [Drosophila melanogaster]	290280	0.6	227	81645	30
10	belle, isoform A [Drosophila melanogaster]	17985987	0.57	162	85029	24

## Middle band

Rank	Description	Accession	emPAI	Score	Mass	Sequence coverage (%)
1	Fmr1, isoform A [Drosophila melanogaster]	19922726	7.94	1237	76030	64
2	hsp 82 [Drosophila melanogaster]	8127	4.9	946	81814	61
3	nuclear RNA export factor 2 (NXF2) [Drosophila melanogaster]	14456090	2.03	703	96573	41
4	Nnp-1 [Drosophila melanogaster]	7298046	1.02	276	78947	34
5	belle, isoform A [Drosophila melanogaster]	7299061	0.83	201	85029	32
6	Bj6 protein [Drosophila melanogaster]	7662	0.74	201	77005	27
7	DNA replication factor MCM7 [Drosophila melanogaster]	4903288	0.69	155	81232	22
8	eIF3-S9, isoform A [Drosophila melanogaster]	7302767	0.61	157	80391	30
9	GH14068 [Drosophila grimshawi]	193891778	0.53	322	80175	19
10	rasputin [Drosophila melanogaster]	7739653	0.41	110	74940	14

## Lower band

Rank	Description	Accession	emPAI	Score	Mass	Sequence coverage (%)
1	CG9754/Panoramix/Silencio [Drosophila melanogaster]	7291281	5.57	617	61110	45
2	no-on-transient A product form I [Drosophila melanogaster]	157978	4	610	76920	48
3	AT19081p [Drosophila melanogaster]	21429758	2.82	499	79722	44
4	RecName: Full=Protein claret segregational	127945	2.75	684	77426	55
5	glucosidase 2 beta subunit, isoform A [Drosophila melanogaster]	7298396	1.46	309	61501	34
6	uncharacterized protein Dmel_CG10565, isoform A [Drosophila melanogaster]	7296390	0.79	150	73575	23
7	Fragile X related [Drosophila melanogaster]	6503206	0.76	208	75773	28
8	belle, isoform A [Drosophila melanogaster]	7299061	0.65	134	85029	32
9	CG8368, isoform A [Drosophila melanogaster]	7295335	0.56	183	76671	35
10	LD25239p [Drosophila melanogaster]	16198023	0.52	196	71051	10

**Appendix Table S1. List of proteins detected in MS analysis (related to Figure 1)**

Proteins detected by MS analysis of indicated bands from Panx-immunoprecipitation followed by silver staining (Fig 1A) are listed with their accession number, emPAI, Score, Mass, and Sequence coverage.

Protein	Annotation Symbol	Significance (p-value, $-\log_{10}$ )	Enrichment (peptide count in flag-Nxf2 IP +1/Control IP +1, $\log_2$ )
Nxf2	CG4118	5.32	5.71
Hsc70-4	CG4264	4.05	5.23
betaTub56D	CG9277	3.50	4.52
Panx	CG9754	3.37	5.09
alphaTub84B alphaTub84D alphaTub85E	CG1913	3.36	4.86
HtrA2	CG8464	3.35	4.17
betaTub56D betaTub85D	CG9277	2.92	3.91
alphaTub84B alphaTub84D	CG1913	2.52	4.17
blw	CG3612	2.49	3.46
Hsc70-3 Hsc70-4	CG4147	2.49	3.46
CG7194	CG7194	2.22	4.46
CklIIalpha	CG17520	2.22	3.00
Hrb98DE	CG9983	2.22	3.00
Ant2 sesB	CG1683	1.98	3.58
Droj2	CG8863	1.91	3.91
DnaJ-1	CG10578	1.86	2.81
betaTub56D betaTub60D betaTub85D	CG9277	1.81	2.58
Cctgamma	CG8977	1.81	2.58
p15 (Nxt1)	CG12752	1.79	3.17
UQCR-C2	CG4169	1.74	4.25
Fib	CG9888	1.74	3.32
Ssrp	CG4817	1.72	3.58
ATPsynbeta	CG11154	1.62	3.70
lost	CG14648	1.55	4.46
porin	CG6647	1.52	3.46
Ef1alpha100E Ef1alpha48D	CG1873	1.49	4.25
Hsp68	CG5436	1.45	3.00
sqd	CG16901	1.45	4.00
T-cp1	CG5374	1.45	4.00
Hsp26	CG4183	1.39	3.17
Rpn2	CG11888	1.24	3.32
Grip71	CG10346	1.20	3.46
CG14207	CG14207	1.10	2.58
Rpt6	CG1489	1.10	2.58
AGO2	CG7439	1.08	4.17
Hsp83	CG1242	1.06	4.32
Hsc70-3	CG4147	0.99	2.81
I(2)gl	CG2671	0.90	3.91
CG6439	CG6439	0.88	3.00
Hsp23	CG4463	0.87	3.32
Hsp27	CG4466	0.85	2.58
hyd	CG9484	0.82	4.39
Tcp-1zeta	CG8231	0.80	4.25
CG9281	CG9281	0.74	4.39
dre4	CG1828	0.74	3.17
eIF-4a	CG9075	0.74	3.17
spn-E	CG3158	0.74	2.81
pAbp	CG5119	0.73	4.09
Piwi	CG6122	0.71	2.58
14-3-3zeta	CG17870	0.71	2.58
bel	CG9748	0.71	2.58
Hop	CG2720	0.71	2.58
Nap1	CG5330	0.71	2.58
PyK	CG7070	0.71	2.58
r	CG18572	0.71	2.58
Rpt3	CG16916	0.71	2.58
Mtor	CG8274	0.70	3.81
Dcr-2	CG6493	0.70	3.17
CG8258	CG8258	0.56	2.58
smid	CG8571	0.56	2.58

### Appendix Table S2. List of proteins detected in shotgun proteome analysis of flag-Nxf2 (related to Figure 2)

Proteins detected with shotgun proteome analysis of flag-Nxf2 immunoprecipitants are listed. Enrichment of peptide obtained by flag-Nxf2 IP over Control IP ( $\log_2$  value of peptide count+1), and significance calculated by replicate experiments as the p-value ( $-\log_{10}$ ) are listed along with protein name and annotation symbol. Proteins with significance over 0.5 and enrichment over 2.5 are indicated (ribosomal proteins are excluded from the list).

Name	Sequence	Usage	Vector construction method
BoxB_EcoRIfor	gatGAATTTCGCTAGCTCCCTAAGTCCAACT	Described in the Methods section	Described in the Methods section
BoxB_XhoIrev	gacCTCGAGATAATATCCTCGATAGGGCC		
BoxB_Sallfor	gatGTGACGCTAGCTCCCTAAGTCCAACT		
Fluc_KpnIfor	acgIggIaccacatgagcgaagcgcacaaacataaagaaag	pAc-Fluc-5boxB vector construction	RE digest with KpnI/EcoRI
Fluc_EcoRIrev	acgtggaattcttcaCAATTTGGAACTTTCCGCCCTTCTTGG		
Ubpro_BamHIfor	GA1TgattccGAAGCGACTGGGATTTCGGTG	Described in the Methods section	Described in the Methods section
Ubpro_KpnIrev	ACGTggtaccGGATTATTCTCGGGGAAGAAAATAGAGATGTG		
lambdaN_KpnIfor	GTTCATTGGTACCAACATGGAGCGCA	pUASp-lambdaN-MCS vector construction	RE digest with KpnI/NotI
lambdaN_NotIrev	ctcgagcgccgcgaattggtaccGCTAGCgcGTAATCTGGAAACATC GTATGGGTAAAGC		
lamdaN_NheI_for	acgtGCTAGCaacATGGAOCGACAAACACGACGCGTG	Described in the Methods section	Described in the Methods section
lamdaN_NheI_rev	acgtGCTAGCgAGCGTATCTGGAACATCGTATGGGTAAG		
Nx2_HindIIIfor	ATAAGCTTATGCCAAACAGATGAGAG	pAcM-Nx2 vector construction	RE digest with HindIII/EcoRI
Nx2_EcoRIrev	ATGAATTCCTAGGGCGAATGCTAGATC		
Nx2-sires-INVfor	GAACGAGATAAAGCTGCCATCATAAGGGCCCGCTGGAA	pAcM-Nx2_sires vector construction	inverse PCR from pAcM-Nx2
Nx2-sires-INVrev	CTTATGATGGACAGTTATCTCGTTCCAATGGGTACCC		
Nx2delLR_HindIIIfor	ATAAGCTTAACTGCTGCGACAAGATCTTT	pAcM-Nx2-ΔLR vector construction	RE digest with HindIII/EcoRI
Nx2delLR_EcoRIrev	ATGAATTCCTAGGGCGAATGCTAGATC		
Nx2delUBA_HindIIIfor	ATAAGCTTATGCCAAACAGATGAGAG	pAcM-Nx2-ΔUBA vector construction	RE digest with HindIII/EcoRI
Nx2delUBA_EcoRIrev	ATGAATTCCTAAACCGGAAATTTACACTGAAAG		
Nx2delIC-HindIIIfor	ATAAGCTTATGCCAAACAGATGAGAG	pAcM-Nx2-ΔC vector construction	RE digest with HindIII/EcoRI
Nx2delIC_EcoRIrev	ATGAATTCCTAAACCGGAAATTTACACTGAAAG		
Nx2delINTF2_INVfor	CCAAGTGAACGCTCAGGCGGA	pAcM-Nx2-ΔNTF2 vector construction	inverse PCR from pAcM-Nx2-sires
Nx2delINTF2_INVrev	CAACGACTGGCCAGGATTCCG		
Nx1_HindIIIfor	ATAAGCTTATGCCAAACGCGCGGT	pAcM-Nx1 vector construction	RE digest with HindIII/EcoRI
Nx1_EcoRIrev	ATGAATTCCTACTCATAAAGCGCTCAGGCGG		
Nx3_HindIIIfor	ATAAGCTTATGGGATCCGCTGCGAC	pAcM-Nx3 vector construction	RE digest with HindIII/EcoRI
Nx3_EcoRIrev	ATGAATTCCTAAAGGTCATCGCATCCAACAGG		
Panx_KpnIfor	AATGGTACCAGTGAAGCTCCGATGAAGCT	pAcEGFP-Panx vector construction	RE digest with KpnI/NotI
Panx_NotIrev	ATTGCGCGCGCTCACTATGGCTGCGACCCCTTTA		
Panx-sires_INVfor	CCTAAGATGGAAACCGAAGATCAAAGAAGATGCGG	pAcEGFP-Panx_sires vector construction	inverse PCR from pAcEGFP-Panx
Panx-sires_INVrev	CGGCATCTCTTTGATCTCGGTTCCACTCTTAGG		
PanxdelC1_INVfor	TAGGCGGCGCTCGAGTCTA	pAcEGFP-Panx-ΔC1 vector construction	inverse PCR from pAcEGFP-Panx-sires
PanxdelC1_INVrev	ATTGAGAATTTAATAGGTC		
PanxdelC2_INVfor	TAGGCGGCGCTCGAGTCTA	pAcEGFP-Panx-ΔC2 vector construction	inverse PCR from pAcEGFP-Panx-sires
PanxdelC2_INVrev	CAAGCCCATTTAGACAAAT		
PanxdelN_INVfor	CCGCAAAATGATACGCCCAT	pAcEGFP-Panx-ΔN vector construction	inverse PCR from pAcEGFP-Panx-sires
PanxdelN_INVrev	CATGGTACCCTGACTGCAG		
p15_HindIIIfor	AATAAGCTTATGGACAGCGATTTGAAAGC	pAcM-p15 vector construction	RE digest with HindIII/NotI
p15_NotIrev	AATGCGCGCGCTCAGACCTCCGATCCGGT		
p15-sires_INVfor	CAGCAGATAGTTCGCTCTATTGG	pAcM-p15_sires vector construction	inverse PCR from pAcM-p15
p15-sires_INVrev	TCTCCTATTGTCACGAGGCGTAG		
Panx-mid_BamHIfor	AATGGATCCCGGAGCTGATCTCATTGCACA	pGEX-Panx-mid vector construction	RE digest with BamHI/NotI
Panx-mid_NotIrev	AATGCGCGCGCTCAGGACTCAATTAACAGGGAGTT		
Nx2Full-EcoRIfor	atgaaTTCAGCCAAACCGATGAGAG	pPET-Nx2-full vector construction	RE digest with EcoRI/Sall
Nx2Full-Sallrev	ATGTGACCTAGGGCGAATGCTAGATC		
Nx2LR_BamHIfor	ATTGATCCCATGCGCAACACAGATGAGAG	pGEX-Nx2-LRR vector construction	RE digest with BamHI/NotI
Nx2LR_NotIrev	ATGCGCGCGCTGAGAAAGGCTTCCATCCA		
p15_BamHIfor	ATTGATCCCATGCGCAACAGCGATTTGAAAG	pGEX-p15 vector construction	RE digest with BamHI/NotI
p15_NotIrev	AATGCGCGCGCTCAGACCTCCGATCCGGT		
egg_INVfor	TGCGAATTTGCTCCCAAGT	pGEX-Egg-Nter vector construction	inverse PCR from pGEX-Egg-full
egg_INVrev	TAAGCGGCGCATCGT		
PAF1for_KpnI	acgtggtaccATGCCAACCCAGTCAACAAATCCGGC	pAcM-PAF1 vector construction	RE digest with KpnI/NotI
PAF1rev_NotI	ACGTgagcgcgCTCATCTACTGAGCGCTTCCAGAGC		
RTF1for_KpnI	acgtggtaccATGGGAAACCGCGGAGCGCAATC	pAcM-RTF1 vector construction	RE digest with KpnI/NotI
RTF1rev_NotI	ACGTgagcgcgCTCATGATAAGACCGCGCTTTTTTGTAACTCT		
Panx_for	CAAAGAGGAGCCACCGGAAT	Panx qPCR	
Panx_rev	CTGGATGGCTTAACGTGTC		
Nx2_for	CCTCGAACGGTTTCACTCCA	Nx2 qPCR	
Nx2_rev	AGATCCTGCACCCAGTTGTG		
Piwi_for	CGACCAAGAACCCAGTGCCT	Piwi qPCR	
Piwi_rev	CTCCGTGACTGTGCTGAAGT		
mdg1_for	AACAGAAACGCCAGCAACAGC	mdg1 qPCR	
mdg1_rev	CGTCCCATGTCCGTTGTGAT		
roo_for	CGTCTGCAATGACTGGCTCT	roo qPCR	
roo_rev	CGGCATCCACTAATTCTCC		
HetA_for	CGCGCGAACCCATCTCAGA	HetA qPCR	
HetA_rev	CGCCGAGTCTGTTGGTGAAT		
Ref1_for	GGCTTGGAAAGCAGATATG	Ref1 qPCR	
Ref1_rev	CGTAGTCCAGGTTACCGACG		
RP49_for	CCGCTTCAAGGAGCAGATCTG	RP49 qPCR	
RP49_rev	ATCTGCCGCGATAAACGC		
14BoxBLuc_ChIPfor	CAGCCAGCATCTCGTTTTTC	boxB qPCR	
14BoxBLuc_ChIPrev	AGGGAAGCTAGCGTCGAGTGCCT		
Fluc_4_ChIPfor	GCCGAAGACGCCAAAACAT	Fluc(4-300) qPCR	
Fluc_300_ChIPrev	TCCGATAAATAACGCGCCCA		
Fluc_1277RTfor	ACTGGGACGAAGCGAACAC	Fluc(1277-1512) qPCR	
Fluc_1512RTrev	GGGACGTAATCCACGATCT		
PAF1_for	GCTGGAGGAGGAGACTCTGA	PAF1 qPCR	
PAF1_rev	TCTTGGTTCGCTGAAGGTC		
RTF1_for	CGCTGGCGTCTCTGATTACT	RTF1 qPCR	
RTF1_rev	TCACGGCCGATTTACAACCA		
LSD1_for	TCCAATCTCGCTGCCATT	LSD1 qPCR	
LSD1_rev	ACTCGCTTACCAACTGGG		
Piwi-siRNA_for	GCUCCAGGCGUGAAGUGIT	Piwi RNAi-KD	
Piwi-siRNA_rev	CACUUCACGCGUGGAGCIt		
Panx-siRNA_for	CCGAAGAUJGAGCCCAAGAT	Panx RNAi-KD	
Panx-siRNA_rev	UCUJGGGCUCAUCUJCGGIt		
Nx2-siRNA_for	CGAAUUCACAGUGCACCAUIt	Nx2 RNAi-KD	
Nx2-siRNA_rev	AUGGUGCACUGUAUUJCGIt		
p15-siRNA_for	CCGACGCCAACAAATGGAT	p15 RNAi-KD	
p15-siRNA_rev	UCCAAUUJGUGGCGUGGIt		
HP1a-siRNA_for	GAUCUJGGGUGCCUCGACIt	HP1a RNAi-KD	
HP1a-siRNA_rev	GUCGAGGACCCCAAGAUIt		
H1-siRNA_for	GAAAGAUCCAGAUCCACCCIt	H1 RNAi-KD	
H1-siRNA_rev	GGUGCAUCUGGAUCUUJUCIt		
Mael-siRNA_for	GCACAAGAUCCCAUGAUIt	Mael RNAi-KD	
Mael-siRNA_rev	AUCAUGGACAUUCUJGCGIt		
Gtsf1-siRNA_for	GCTCCAGCAGCACATCTTAt	Gtsf1 RNAi-KD	
Gtsf1-siRNA_rev	UAAGAUJGUGUGGUGGAGCIt		
Egg-siRNA_for	GGTCAAAAGCTATTAGCTIt	Egg RNAi-KD	
Egg-siRNA_rev	AGCUAAUCGUGUJGAGCIt		
Ref1-siRNA_for	CAGCGUJGAGGACGACAUIt	Ref1 RNAi-KD	
Ref1-siRNA_rev	AUCGUGUJGAGGCGUGIt		
EGFP-siRNA_for	GGCAAGCUGACCCUGAAGUIt	EGFP RNAi-KD	
EGFP-siRNA_rev	ACUJGAGGUGUGGUGGCGIt		
Luc-siRNA_for	CGUACGCGGAAUUCUJGAGIt	Luc RNAi-KD	
Luc-siRNA_rev	UCGAAAGUJGUGGUGGUGIt		
PAF1-siRNA_for	CCACCGCUCUGGACGUAIt	PAF1 RNAi-KD	
PAF1-siRNA_rev	UACUGCACGAAAGCGUGGIt		
RTF1-siRNA_for	GGACGAAAGUACAUUGCAIt	RTF1 RNAi-KD	
RTF1-siRNA_rev	UJGCAUGUCAUCUJGUGCIt		
LSD1-siRNA_for	GUCAGGAUCGCAUCAUGIt	LSD1 RNAi-KD	
LSD1-siRNA_rev	CAUGGAUGCCGAUCCUGACIt		
endo-siRNA-sit	GGAGCGAACTTGTGGAGTCAA	esiRNA sit northern blot	
lj-piRNA	GGTAATGGGAAATGCACCTCTCTTGAA	lj-piRNA northern blot	
idexif-piRNA	AAACTACTGGCAATCGTTGGGAA	idexif-piRNA northern blot	

**Appendix Table S3. DNA oligonucleotides and siRNAs used in this study, related to experimental procedures**