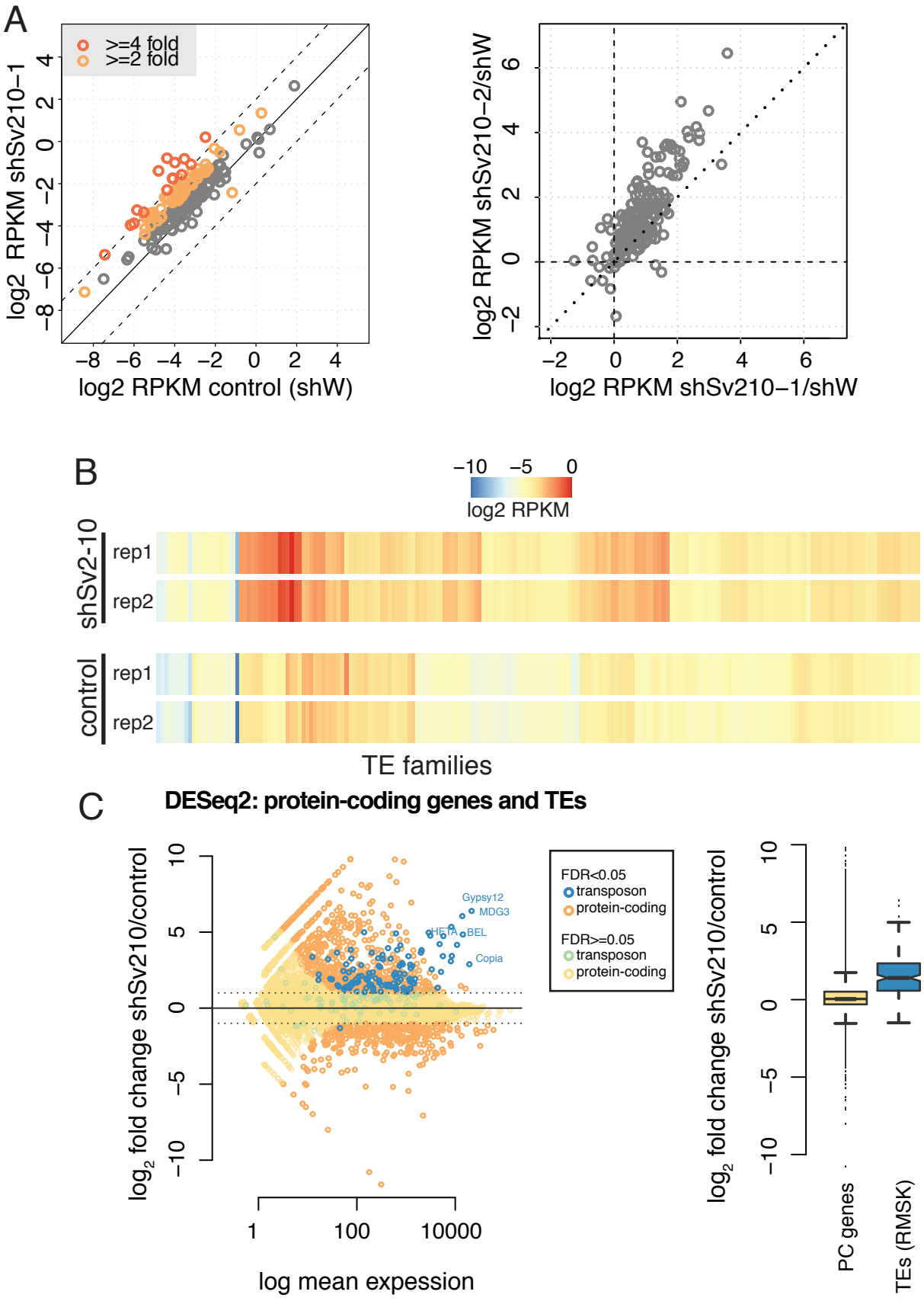
A
>=4 fold
>=2 fold
log2 RPKM shSv210−1
log2 RPKM control (shW)
log2 RPKM shSv210−2/shW
log2 RPKM shSv210−1/shW
B
log2 RPKM
shSv2-10
rep1
rep2
control
rep1
rep2
TE families
C
DESeq2: protein-coding genes and TEs
log2 fold change shSv210/control
log mean expession
Gypsy12
MDG3
BEL
Copia
FDR<0.05
transposon
protein-coding
FDR>=0.05
transposon
protein-coding
PC genes
TEs (RMSK)

Supplementary Figure S1. Related to Figure 2. Expression of transposons and protein-coding genes upon Su(var)2-10 knockdown

(A) (Left) TE upregulation upon germline knockdown of Su(var)2-10 using the Su(var)2-10-1 hairpin. Scatter plot shows log2-tansformed RPKM values for TEs (RepeatMasker) in RNA-seq data from ovaries expressing shSv210-1 versus shW control hairpin under the control of the MT-Gal4 driver. Dashed lines indicate 4-fold change. (Right) Su(var)2-10 GLDK by shSv210-1 and shSv210-2 cause similar up-regulation of TEs. Scatter plot shows fold changes in TE expression in shSv210-1 and shSv210-2 relative to shW control.

(B) TE upregulation is reproducible upon Su(var)2-10 knockdown in the ovary. Heatmaps reflect the expression levels (RPKM normalized) of TE families in two biological replicates of control (shW) and Su(var)2-10 knockdown, using the stronger Su(var)2-10 hairpin (shSv210-2, see main text). Colored bars correspond to RepeatMasker annotated transposon families. Data was sorted by Euclidean distance.

(C) (Left) Scatter plot showing the average expression (X-axis) versus fold change in Su(var)2-10-2 KD (shSv210-2) compared to control (shW) ovaries (Y-axis) for protein-coding genes and transposons in RNA-seq data. Significantly changed protein-coding genes and TEs (5% FDR cutoff) are marked. Analysis was performed with the DESeq2 R-package (Love et al., 2014) with 2 biological replicates for each condition. (Right) Boxplot showing the distribution of RNA-seq fold changes between Su(var)2-10 KD and control (shW) ovaries for protein-coding (PC) genes, and RepeatMasker transposons (TEs RMSK).

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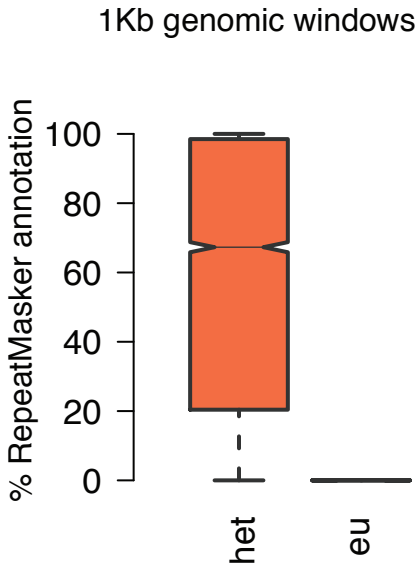
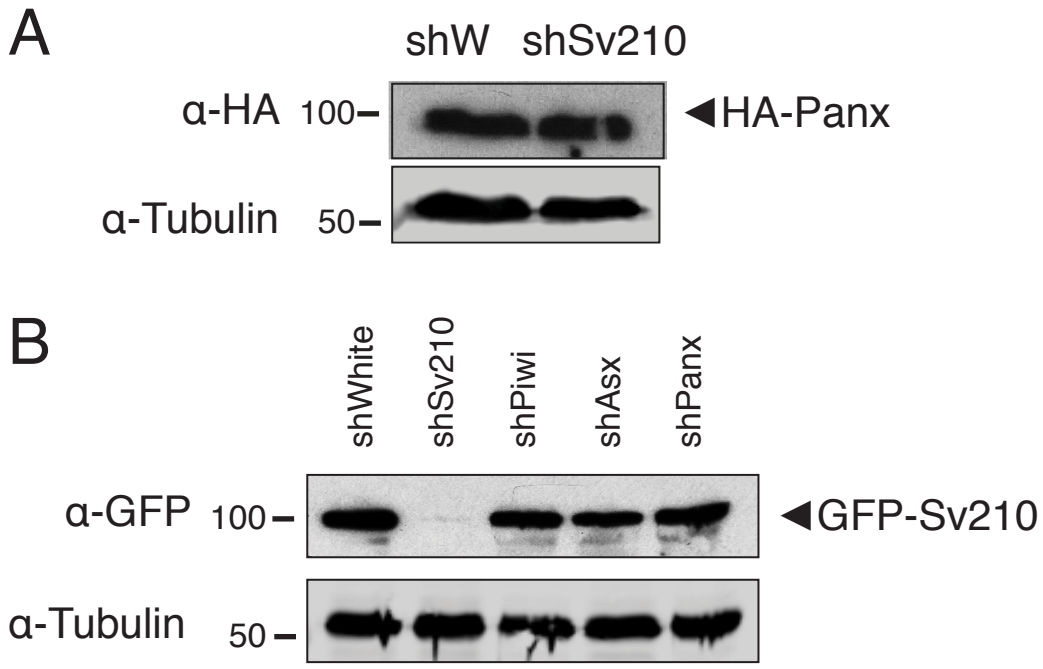
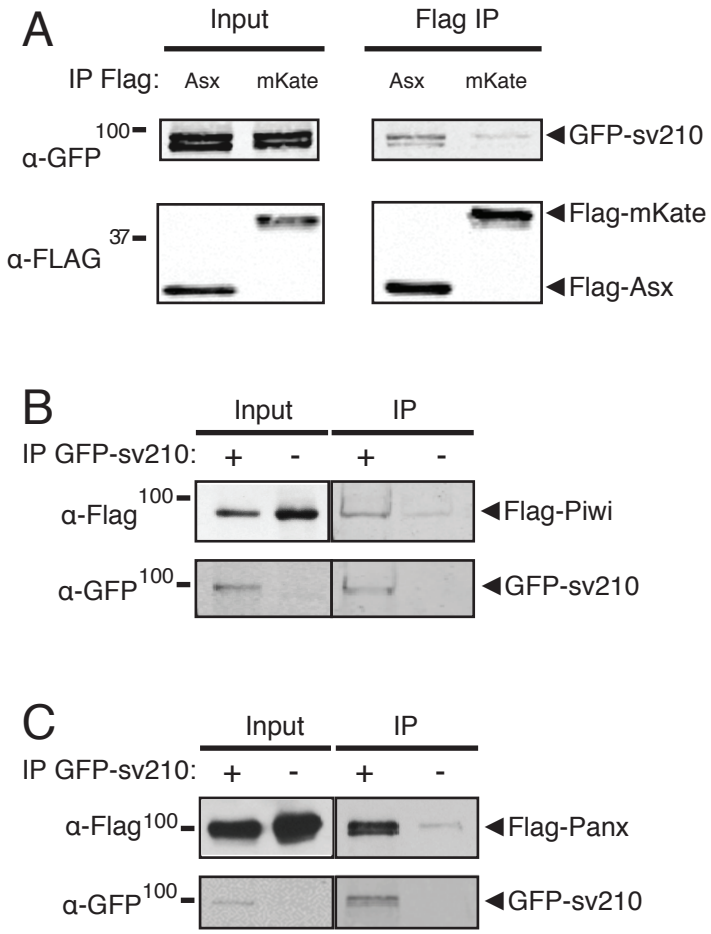


Supplementary Figure S2. Related to Figure 3. Distribution of repetitive sequences on H3K9me3-enriched regions. Boxplots show the proportion of RepeatMasker annotations in the sequence of 1kb genomic intervals which have >2-fold enrichment of H3K9me3 signal (“het”) and H3K9me3-poor intervals (“eu”).


A
shW
shSv210
α-HA
100
◀HA-Panx
α-Tubulin
50
B
shWhite
shSv210
shPiwi
shAsx
shPanx
α-GFP
100
◀GFP-Sv210
α-Tubulin
50

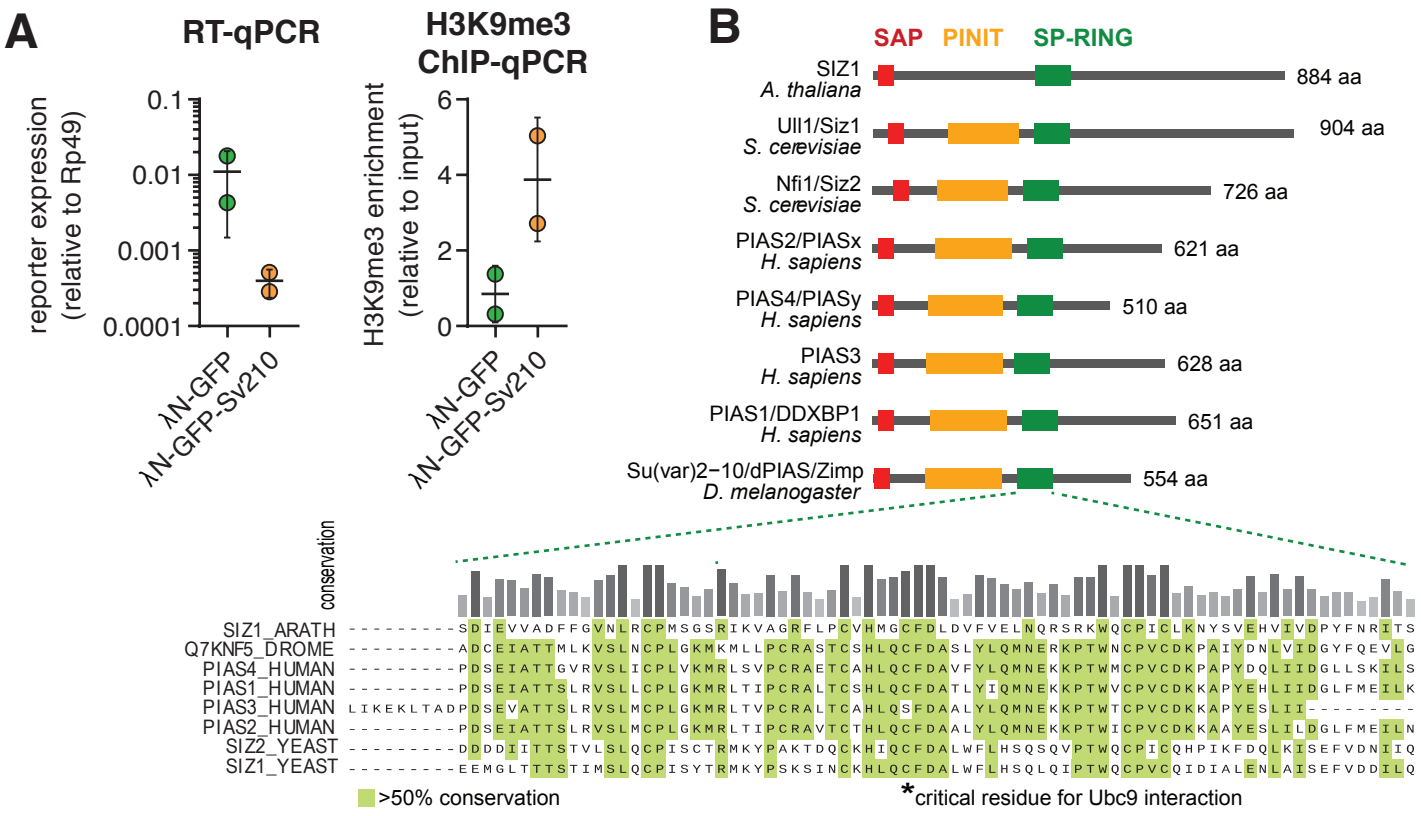
Supplementary Figure S3. Related to Figure 4. Su(var)2-10 and Panx protein stability do not depend on each other. (A) Western-blot analysis of HA-Panx protein level in control (shW) and upon Su(var)2-10 GLKD. (B) Western-blot analysis of GFP-Su(var)2-10 protein level upon GLKD of White (control), Su(var)2-10, Piwi, Arx and Panx.

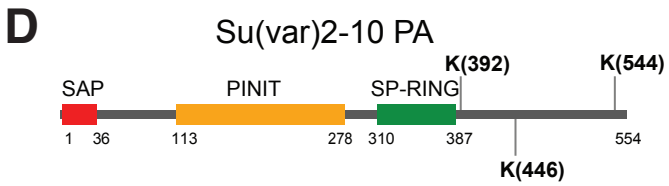

A
Input
Flag IP
IP Flag:
Asx
mKate
Asx
mKate
100
α-GFP
◀GFP-sv210
37
α-FLAG
◀Flag-mKate
◀Flag-Asx
B
Input
IP
IP GFP-sv210:
+
-
+
-
100
α-Flag
◀Flag-Piwi
100
α-GFP
◀GFP-sv210
C
Input
IP
IP GFP-sv210:
+
-
+
-
100
α-Flag
◀Flag-Panx
100
α-GFP
◀GFP-sv210

Supplementary Figure S4. Related to Figure 4. Biochemical interaction of Su(var)2-10 with Panx, Arx and Piwi in S2 cells

(A) Su(var)2-10 interacts with Arx. Lysates from S2 ectopically expressing GFP-Su(var)2-10 and FLAG-tagged Arx or FLAG-mKate (negative control) were analyzed by immunoprecipitation with anti-FLAG antibody. Western blot was probed with anti-FLAG antibody to detect Arx, and anti-GFP antibody to detect Su(var)2-10.

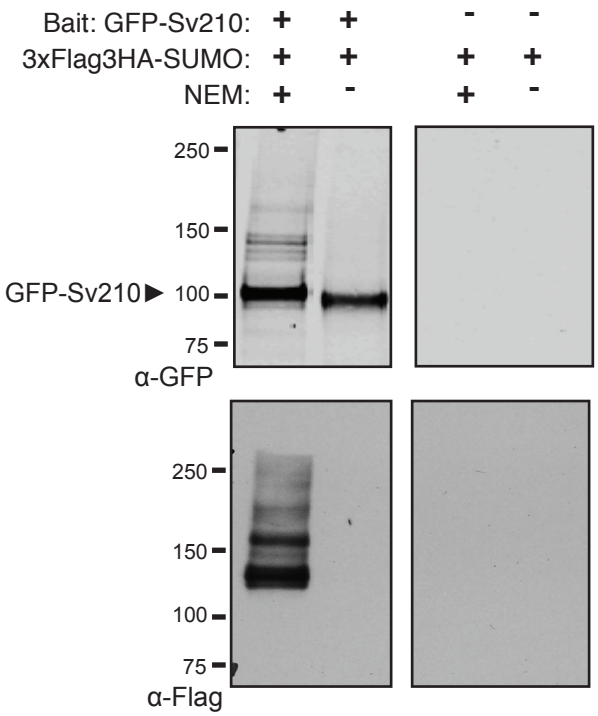
(B, C) Su(var)2-10 interacts with Piwi. Lysate from S2 ectopically expressing FLAG-tagged Piwi(B) or Panx(C) and GFP-Su(var)2-10 were analyzed by immunoprecipitation with anti-GFP nanobody. Cells that do not express the bait protein, GFP-Su(var)2-10, were used as negative control. Western blot was probed with anti-FLAG antibody to detect Piwi/Panx, and with anti-GFP antibody to detect Su(var)2-10.

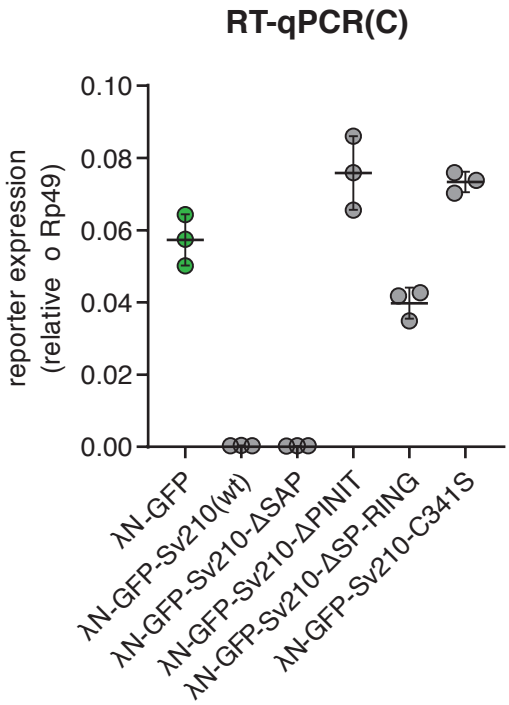




GPS-SUMO output

Position	Sequence	Score	P-Value
392	VLGSSLL**K**SDDTEIQ	4.212	0.034
446	SDDAKPV**K**RDLSPAQ	3.685	0.032
544	WLRPRKP**K**PESSRRP	12.527	0.016





Supplementary Figure S5. Related to Figure 5.

(A) Su(var)2-10 tethering to pTubulin-GFP-5xBoxB reporter (Sienski et al., 2015) induces transcriptional repression and H3K9me3 deposition. Plots show the expression level (RT-qPCR) and enrichment of H3K9me3 (ChIP-qPCR) at the reporter locus upon tethering of λN-Su(var)2-10 or λN-GFP. Dots correspond to independent biological replicates; bars indicate the mean and SD.

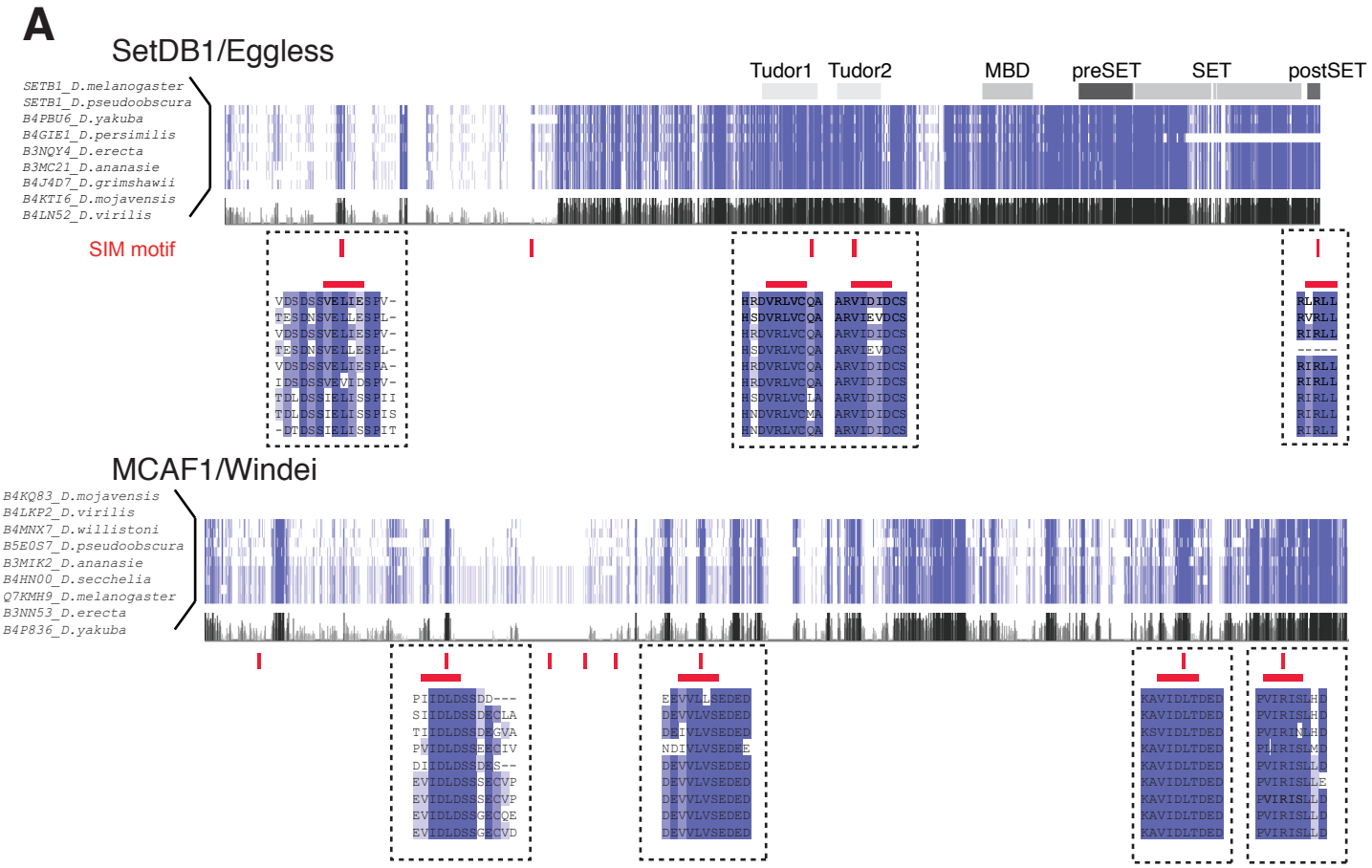
(B) (Top) Siz/PIAS proteins domain structure from various organisms. (Bottom) Sequence alignment of the SP-RING domains.

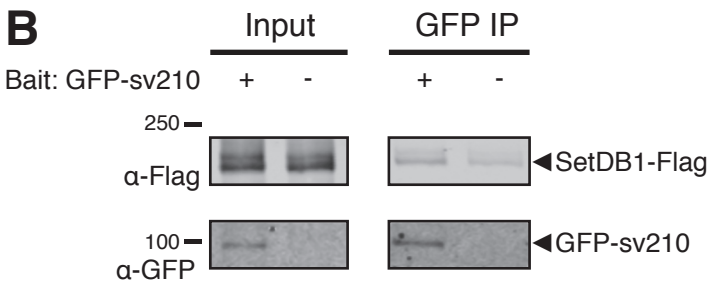
(C) Su(var)2-10 interacts with SUMO *in vitro*. Bacterially purified His-tagged Su(var)2-10 was incubated with GST-SUMO (wild type) or interaction-deficient mutant GST-SUMO (QFI>AAA). GST-SUMO was affinity purified using glutathione sepharose beads and His-Su(var)2-10 was detected by Western blotting using anti-His antibody.

(D) Diagram of SUMOylation sites in Su(var)2-10-PA protein.

(E) Su(var)2-10 is SUMOylated. GFP-Su(var)2-10 and 3XFLAG3XHA-SUMO were co-expressed in S2 cells. GFP-Su(var)2-10 was immunopurified from total cell lysates under stringent wash conditions in the presence or absence of 20 mM NEM and analyzed by Western blot.

(F) The role of Su(var)2-10 domains in reporter silencing. λN-GFP, λN-GFP-Su(var)2-10 (wild type) or λN-GFP-Su(var)2-10 with deletions of the entire SAP, PINIT or SP-RING domain (see main text) were recruited to the mKate-4BoxB reporter. Plots show reporter expression levels in indicated conditions estimated by RT-qPCR. Dots correspond to three independent biological replicates; bars indicate the mean and SD.


A
SetDB1/Eggless
Tudor1
Tudor2
MBD
preSET
SET
postSET
SETB1_D.melanogaster
SETB1_D.pseudoobscura
B4PBU6_D.yakuba
B4GIE1_D.persimilis
B3NQY4_D.erecta
B3MC21_D.ananasie
B4J4D7_D.grimshawii
B4KTI6_D.mojavensis
B4LN52_D.virilis
SIM motif
MCAF1/Windei
B4KQ83_D.mojavensis
B4LKP2_D.virilis
B4MNX7_D.willistoni
B5E0S7_D.pseudoobscura
B3MIK2_D.ananasie
B4HN00_D.secchelia
Q7KMH9_D.melanogaster
B3NN53_D.erecta
B4P836_D.yakuba


B
Input
GFP IP
Bait: GFP-sv210
+
-
+
-
250
α-Flag
SetDB1-Flag
100
α-GFP
GFP-sv210

Supplementary Figure S6. Related to Figure 7

(A) Multiple sequence alignments of homologs of SetDB1 and Wde in *Drosophila* species with annotated genomes. Orthologs were extracted from OrthoDB and aligned using MUSCLE. For full-length alignments, schematic of positions colored by % identity is shown. Histograms reflect conservation. Predicted SIMs (Zhao et al., 2014) in *D. melanogaster* are marked by red bars. Regions of sequence alignments flanking the most highly conserved SIMs are magnified. Images are based on alignments edited in Jalview (Clamp et al., 2004).

(B) Su(var)2-10 interacts with SetDB1 in S2 cells. Lysate from S2 cells ectopically expressing FLAG-tagged SetDB1 and GFP-Su(var)2-10 was analyzed by immunoprecipitation with anti-GFP nanobody. Cells that do not express the bait protein, GFP-Su(var)2-10, were used as negative control. Western blot was probed with anti-FLAG antibody to detect SetDB1 and anti-GFP antibody to detect Su(var)2-10.

Supplementary Table 1. Related to Figure 5. Su(var)2-10 interaction with SUMO pathway components from yeast two-hybrid screen. The table shows results from ULTImate Y2H (Hybrigenics) screen using N-terminal DNA-binding GAL4 domain fusion Su(var)2-10-PA (aa 1-554 ; pB35 N-GAL4-bait-C fusion, inducible) as a bait and Drosophila melanogaster ovary cDNA library fused to GAL4 activation domain.

Clone Name	Type Seq	Gene name	FlyBase ID	Start	Stop	Frame	Sense	% Id 5p/3p
DMOV_RP1_hgx5051v1_pB35_B-67	5p 3p	**Uba2**	FBgn0029113	1059	2224	IF	Sense	98.7 / 97.7
DMOV_RP1_hgx5051v1_pB35_B-162	5p	**lwr**	FBgn0010602	-64	No Data	IF	Sense	98.3
DMOV_RP1_hgx5051v1_pB35_B-122	5p 3p	**smt3**	FBgn0264922	-210	349	IF*	Sense	99.3 / 99.7
DMOV_RP1_hgx5051v1_pB35_B-262	5p 3p	**smt3**	FBgn0264922	-201	359	IF*	Sense	98.7 / 99.1
DMOV_RP1_hgx5051v1_pB35_B-199	5p 3p	**smt3**	FBgn0264922	-198	348	IF*	Sense	98.3 / 99.7
DMOV_RP1_hgx5051v1_pB35_B-272	5p 3p	**smt3**	FBgn0264922	-171	356	IF*	Sense	98.2 / 99.0
DMOV_RP1_hgx5051v1_pB35_B-9	5p 3p	**smt3**	FBgn0264922	-141	348	IF*	Sense	98.4 / 99.6
DMOV_RP1_hgx5051v1_pB35_B-3	5p 3p	**smt3**	FBgn0264922	-141	391	IF*	Sense	98.9 / 96.8
DMOV_RP1_hgx5051v1_pB35_B-253	5p 3p	**smt3**	FBgn0264922	-141	361	IF*	Sense	98.2 / 98.4
DMOV_RP1_hgx5051v1_pB35_B-242	5p 3p	**smt3**	FBgn0264922	-141	391	IF*	Sense	98.7 / 99.2
DMOV_RP1_hgx5051v1_pB35_B-306	5p 3p	**smt3**	FBgn0264922	-141	321	IF*	Sense	98.3 / 99.1
DMOV_RP1_hgx5051v1_pB35_B-281	5p 3p	**smt3**	FBgn0264922	-141	360	IF*	Sense	98.2 / 98.4
DMOV_RP1_hgx5051v1_pB35_B-187	5p 3p	**smt3**	FBgn0264922	-138	346	IF*	Sense	98.8 / 99.0
DMOV_RP1_hgx5051v1_pB35_B-112	5p 3p	**smt3**	FBgn0264922	-138	346	IF*	Sense	99.2 / 99.6
DMOV_RP1_hgx5051v1_pB35_B-380	3p	**smt3**	FBgn0264922	No Data	397	??	Sense	99.5
DMOV_RP1_hgx5051v1_pB35_B-57	3p	**smt3**	FBgn0264922	No Data	347	??	Sense	99.5

Start/Stop: Position of the 5p and 3p prey fragment ends, relative to the position of the ATG start codon (A=0)

Frame: With regard to the theoretical frame of each corresponding CDS (GeneBank), fragments are cloned in frame (IF) if they are in the same frame as Gal4AD. *the fragment contains full-length ORF.