

**Figure S1.** Related to Figure 1A. All-vs-all correlation plots between RNA-seq libraries. Data points represent log2-transformed read counts to exonic regions of genes (uniquely mapping reads) or genomic regions corresponding to RepeatMasker-annotated TE families (including repetitive reads corrected for mappability).



**Figure S2.** Related to Figure 3C. Average profiles of H3K9me3, H3K4me2/3 and RNA pol II over gene bodies of heterochromatic genes upregulated upon Su(var)2-10 GLKD in indicated conditions.



**Figure S3. Loss of H3K9me3 islands at euchromatic genes leads to ectopic gene expression.** Related to Figure 5. Regulation of testis-biased endo-siRNA locus *CG44774/CG4068/esi-2* through a Su(var)2-10-dependent H3K9me3 mark. UCSC browser tracks show the RNA-seq and H3K9me3 ChIP-seq signals in the indicated conditions. The *CG44774* gene is marked by a Su(var)2-10- and SUMO-dependent, but Piwi-independent, H3K9me3 peak and is expressed at low levels in the ovary. Su(var)2-10 GLKD leads to H3K9me3 loss and transcript up-regulation. Areas shaded in grey represent repetitive regions where reads did not align uniquely.



**Figure S4. Sov regulates genome-wide H3K9me3 profiles.** Related to Figure 6. (A) Circle plot of the H3K9me3 genome-wide distribution and fold changes upon Sov GLKD. Outer circle shows the enrichment of H3K9me3 (IP/Input) for 5-kb genomic windows in control ovaries. Black tiles indicate 5-kb windows enriched in H3K9me3 (>2 IP/Input signal ratio). Inner circle shows the fold change of H3K9me3 signal between Sov KD and control ovaries for H3K9me3-enriched regions. (B) Boxplot of the log2-transformed fold change of H3K9me3 signal upon Sov GLKD relative to control ovaries for 5-kb genomic windows enriched or depleted of H3K9me3 signal (>2 fold enrichment cutoff). (C) CG30403 interacts with HP1. Flag-CG30403 and GFP-HP1a were co-expressed in S2 cells. Western blot shows results from co-immunoprecipitation using GFP-HP1 as a bait.



**Figure S5. H3K9me3 mark on ATF7IP.** Related to Figure 6. UCSC ENCODE tracks of H3K9me3 enrichment over ATF7IP (Wde homolog) in various human cell lines (hg19). RNA-seq tracks show that ATF7IP is expressed in lines with high H3K9me3 enrichment.