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The insulator protein Suppressor of Hairy-wing is an essential transcriptional repressor in the *Drosophila* ovary

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SUMMARY

Suppressor of Hairy-wing [Su(Hw)] is a DNA-binding factor required for *gypsy* insulator function and female germline development in *Drosophila*. The insulator function of the *gypsy* retrotransposon depends on Su(Hw) binding to clustered Su(Hw) binding sites (SBSs) and recruitment of the insulator proteins Centrosomal Protein 190 kD (CP190) and Modifier of *mdg4* 67.2 kD (Mod67.2). By contrast, the Su(Hw) germline function involves binding to non-clustered SBSs and does not require CP190 or Mod67.2. Here, we identify Su(Hw) target genes, using genome-wide analyses in the ovary to uncover genes with an ovary-bound SBS that are misregulated upon Su(Hw) loss. Most Su(Hw) target genes demonstrate enriched expression in the wild-type CNS. Loss of Su(Hw) leads to increased expression of these CNS-enriched target genes in the ovary and other tissues, suggesting that Su(Hw) is a repressor of neural genes in non-neural tissues. Among the Su(Hw) target genes is *RNA-binding protein 9* (*Rbp9*), a member of the ELAV/Hu gene family. Su(Hw) regulation of *Rbp9* appears to be insulator independent, as *Rbp9* expression is unchanged in a genetic background that compromises the functions of the CP190 and Mod67.2 insulator proteins, even though both localize to *Rbp9* SBSs. *Rbp9* misregulation is central to *su(Hw)*^{-/-} sterility, as *Rbp9*^{+/+}, *su(Hw)*^{-/-} females are fertile. Eggs produced by *Rbp9*^{+/+}, *su(Hw)*^{-/-} females show patterning defects, revealing a somatic requirement for Su(Hw) in the ovary. Our studies demonstrate that Su(Hw) is a versatile transcriptional regulatory protein with an essential developmental function involving transcriptional repression.

KEY WORDS: Chromatin insulator, *Drosophila* oogenesis, Neural gene expression, *Rbp9*, Su(Hw), Transcriptional regulation

INTRODUCTION

Eukaryotic gene expression depends upon the integration of regulatory inputs from multiple classes of elements. Enhancers and silencers represent two classes of regulatory elements, both having the capacity to modulate the activity of target promoters separated by large linear distances (Blackwood and Kadonaga, 1998; Bulger and Groudine, 2010). Enhancers and silencers display limited promoter specificity (Kermekchiev et al., 1991; Schoenherr et al., 1996; Dellino et al., 2004), requiring the presence of a third class of regulatory elements to achieve transcriptional fidelity. This class, called insulators, limits enhancer and silencer action to prevent inappropriate interactions with non-target promoters (Kuhn and Geyer, 2003; Raab and Kamakaka, 2010; Yang and Corces, 2011; Ghirlando et al., 2012). Insulators block enhancer and promoter interactions when positioned between these elements (enhancer-blocking activity) and define boundaries of repressive and active chromatin (barrier activity). Insulator-binding proteins have been identified in most eukaryotes, emphasizing the importance of this regulatory class in establishing transcriptional integrity.

One of the best-characterized insulator-binding proteins is the *Drosophila* Suppressor of Hairy wing [Su(Hw)] zinc-finger (ZF) protein. This DNA-binding protein establishes the chromatin insulator of the *gypsy* retrotransposon (Geyer et al., 1986; Parkhurst et al., 1988; Spana et al., 1988; Geyer and Corces, 1992; Dorsett, 1993). The function of the *gypsy* insulator requires Su(Hw)

recruitment of Centrosomal Protein 190 kD (CP190) (Pai et al., 2004), Modifier of *mdg4* 67.2 kD isoform (Mod67.2) (Georgiev and Kozycina, 1996) and Enhancer of *yellow* 2 [E(y)2; also known as ENY2] (Kurshakova et al., 2007a). Mod67.2 and CP190 are required for enhancer blocking by the *gypsy* insulator (Georgiev and Gerasimova, 1989; Pai et al., 2004). Both proteins carry a Broad-complex, Tramtrack and Bric-a-brac/Poxvirus and Zinc Finger (BTB/POZ) domain (Stogios et al., 2005), which may promote homologous and heterologous associations with other distantly separated BTB/POZ domain proteins to establish chromatin domains that limit enhancer action. E(y)2 is a subunit of the SAGA histone acetylation complex and the TREX RNA export complex (Kurshakova et al., 2007b). E(y)2 is required for barrier function of the *gypsy* insulator (Kurshakova et al., 2007a), possibly through its chromatin-opening activity (Kurshakova et al., 2007b). The *gypsy* insulator displays a versatile capacity to define transcriptional domains, protecting transgenes from enhancer and silencer action when inserted randomly throughout the genome (Roseman et al., 1993; Roseman et al., 1995a; Roseman et al., 1995b; Markstein et al., 2008).

Su(Hw) is globally expressed throughout *Drosophila* development. Even so, loss of Su(Hw) causes a tissue-restricted phenotype, wherein oogenesis is defective owing to oocyte apoptosis during mid-oogenesis (Klug et al., 1968; Parkhurst et al., 1988; Harrison et al., 1993; Baxley et al., 2011). Two observations suggest that the fertility and insulator functions of Su(Hw) are independent. First, a loss of Su(Hw) occupancy at ~60% of genomic SBSs has no effect on fertility (Soshnev et al., 2012), an unexpected finding for an insulator-dependent function that has been linked to the formation of physical chromatin domains important for transcriptional regulation (Gurudatta and Corces, 2009; Yang and Corces, 2011; Hou et al., 2012). Second, fertility is unaffected by loss of CP190 and Mod67.2 (Chodagam et al., 2005; Baxley et al., 2011), two partners that are required for Su(Hw) insulator function.

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Together, these findings suggest that Su(Hw) functions in oogenesis extend beyond the formation of chromatin insulators.

Here, we define transcriptional changes caused by Su(Hw) loss in the ovary and identify direct targets of Su(Hw) regulation during oogenesis. Studies of these target genes show that most have increased expression upon Su(Hw) loss, revealing a major role for Su(Hw) in transcriptional repression. Su(Hw) regulation of target genes is not restricted to the ovary, as loss of Su(Hw) causes gene expression changes in other tissues. The majority of repressed target genes display enriched expression in the CNS and depleted expression in the ovary. These observations suggest that Su(Hw) represses neural genes in non-neural tissues, indicating that Su(Hw) might be a functional homolog of the mammalian repressor for element-1 silencing factor (REST; also known as neuron-restrictive silencing factor, NRSF), a transcription factor that contributes to neural phenotypes via repression of neural genes in non-neural tissues (Lakowski et al., 2006; Ooi and Wood, 2007). Finally, we demonstrate that sterility of *su(Hw)^{-/-}* females depends largely on the repression of one gene within developing germ cells. This gene is *RNA-binding protein 9* (*Rbp9*), which encodes an ELAV/Hu family RNA-binding protein. Strikingly, decreasing the *Rbp9* gene dosage restores oocyte production in *su(Hw)* null females. Eggs produced by the rescued *Rbp9^{+/-}*, *su(Hw)^{-/-}* females show morphological defects, revealing a previously unknown requirement for Su(Hw) in somatic cells of the ovary. Taken together, our studies demonstrate that Su(Hw) is a versatile transcriptional regulatory protein, with an essential developmental function as a transcriptional repressor.

MATERIALS AND METHODS

Fly stocks and culture conditions

Flies were raised at 25°C, 70% humidity on standard cornmeal/agar medium supplemented with yeast. A detailed description of strain genotypes and alleles used in these studies is provided in supplementary material Table S1.

Microarray analyses of gene expression in *Drosophila* ovary

Ovaries were dissected from 4- to 6-hour-old virgin females and stored at -80°C until needed. The *su(Hw)^{+/-}* strains include Canton S and Bloomington Strain 15598. The *su(Hw)^{-/-}* strains include *su(Hw)^{2/2}*, *su(Hw)^{A2663/v}* and *su(Hw)^{Pb/2}*. Total RNA was isolated from ~150 pairs of ovaries per biological replicate using TRIzol (Invitrogen), DNaseI treatment (Qiagen DNasel) and purification on RNeasy columns (Qiagen). Microarray hybridization was performed by the University of Iowa DNA facility using the Affymetrix *Drosophila* 2.0 arrays (Cat. #900532). Data were processed using Partek Genomics Suite 6.5 Gene Expression pipeline, using GeneChip Robust Multiarray Average (GCRMA) normalization (Irizarry et al., 2003), twofold change and 1% false discovery rate (FDR) cutoffs. Three to six independent biological replicates of each sample were studied. Microarray data were deposited to Gene Expression Omnibus (accession numbers GSE36528 and GSE45286).

Quantitative PCR (qPCR) analyses of gene expression

Quantitative (q)PCR analyses used RNA isolated from ~50 ovary pairs, 50 third instar larval wing discs or 25 third instar larval brains per biological sample using TRIzol (Invitrogen). All steps were performed as described previously (Soshnev et al., 2008). Expression levels were determined using housekeeping gene *RPL32* as an internal control. Primers amplified fragments between 100 and 200 bp. Primer sequences are listed in supplementary material Table S2.

Chromatin immunoprecipitation (ChIP)

Chromosome association of Su(Hw), CP190 and Mod67.2 was tested using ChIP from 50–200 ovary pairs per biological sample, as described previously (Baxley et al., 2011). The antibodies used were guinea pig polyclonal anti-Su(Hw) (Baxley et al., 2011; Soshnev et al., 2012), sheep polyclonal anti-CP190 (Baxley et al., 2011) and rabbit polyclonal anti-

Mod67.2 (modEncode D1; kindly provided by K. White, University of Chicago). At least two independent biological samples were analyzed. Primer-amplified fragments between 100 and 200 bp were centered on the Su(Hw) binding consensus when possible. Primer sequences are listed in supplementary material Table S2.

Immunohistochemical analyses

Samples were fixed in 3% electron microscopy grade paraformaldehyde in PBT (PBS with 0.3% v/v Triton X-100) for 30 minutes, washed in PBT and blocked overnight in 5% w/v BSA in PBT at 4°C. Samples were then incubated with primary antibody for at least 8 hours at 4°C, washed in PBT, incubated with the corresponding Alexa Fluor-conjugated secondary antibodies (Molecular Probes), washed in PBT, DAPI stained (0.1 µg/ml, 10 minutes) and mounted in Vectashield (Vector Laboratories). Slides were imaged using a Zeiss 710 confocal microscope and processed using ImageJ. The antibodies used were polyclonal guinea pig anti-Su(Hw) (1:500) (Baxley et al., 2011), polyclonal rabbit anti-Su(Hw) (1:1000) (Parnell et al., 2003), rat anti-ELAV [1:10; 7E8A10; Developmental Studies Hybridoma Bank (DSHB), University of Iowa], mouse anti-Broad Z1 (1:200; Z1.3C11.OA1; DSHB), mouse anti-Repo (1:5; 8D12; DSHB), mouse anti-Gurken (1:200; 1D12; DSHB), polyclonal rabbit anti-Vasa (1:500; Santa Cruz), polyclonal guinea pig anti-Dpn (1:500; a kind gift from J. Skeath, Washington University, St Louis) and polyclonal rabbit anti-Rbp9 (1:3000; a kind gift from M. Buszczak, UT Southwestern) (Kim and Baker, 1993).

Scanning electron microscopy (SEM)

Embryos were collected on orange juice agar plates, washed with distilled water and fixed overnight in 2.5% glutaraldehyde, 2% paraformaldehyde in 0.1 M cacodylate buffer, pH 7.4. Following rinsing, embryos were stained with 1% OsO₄ in 0.1 M cacodylate for 2 hours. Next, embryos were rinsed and dehydrated in a series of ethanol washes (20%, 50%, 75%, 94% and 100%) followed by critical point drying. Embryos were mounted on stubs and coated with gold-palladium using Polaron E5100 sputter coater and imaged using Hitachi S-4800 scanning electron microscope.

Analyses of fertility phenotypes

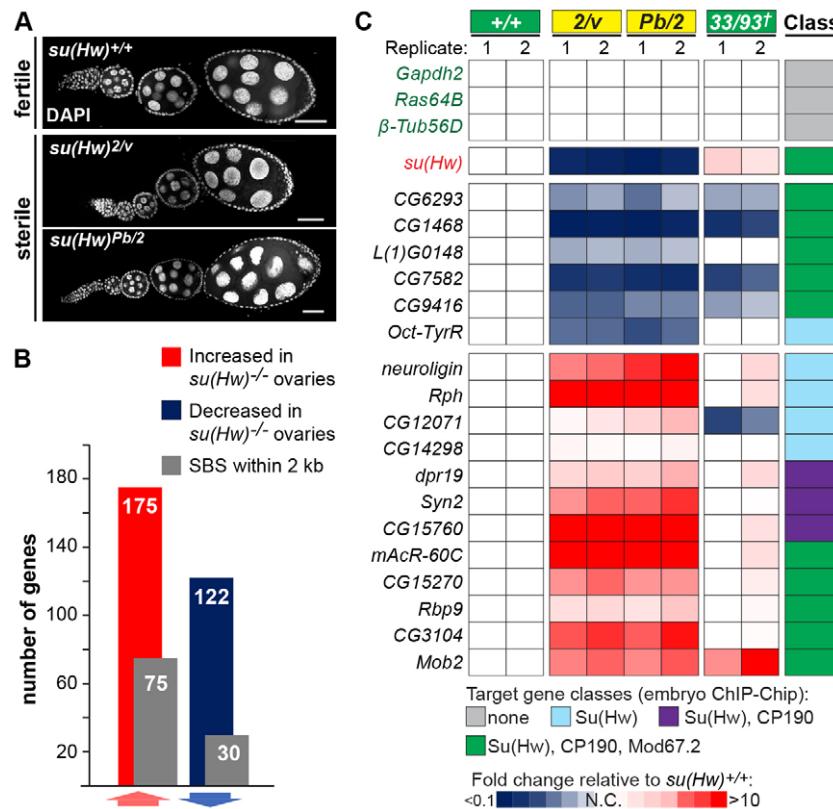
The fertility of 2- to 4-day-old mated females was determined by counting the number of eggs laid on orange juice plates carrying wet yeast paste at 25°C. Egg viability was determined by counting the number of eggs that hatched following egg laying on orange juice plates carrying wet yeast paste.

RESULTS

Identification of Su(Hw)-regulated genes

We investigated the regulatory role of Su(Hw) in the ovary using whole-genome gene expression analyses. We measured gene expression changes in ovaries obtained from females of three null and two *su(Hw)* wild-type genotypes (supplementary material Table S1), to minimize the identification of bystander genes for which expression changes were unrelated to Su(Hw) loss. RNAs were obtained from newly eclosed ovaries, to ensure that our gene expression analyses included equivalent stages of oogenesis. Newly eclosed ovaries lack late-stage egg chambers, establishing a natural method to avoid the stages of oogenesis that are absent in *su(Hw)^{-/-}* ovaries (Fig. 1A) (Klug et al., 1968; Baxley et al., 2011).

Affymetrix *Drosophila* 2.0 microarrays were used to measure transcriptional changes resulting from Su(Hw) loss. These arrays contain over 18,500 probe sets corresponding to ~13,000 genes. We identified 297 genes that changed expression at least twofold in *su(Hw)^{-/-}* relative to *su(Hw)^{+/-}* genetic backgrounds (1% FDR; $P < 0.01$; Fig. 1B). We reasoned that if a gene were a direct target of Su(Hw) regulation, then the gene would carry at least one SBS. To this end, we used an ovary-bound SBS dataset to identify mis-regulated genes that carried an SBS within the transcribed region or within 2 kilobases (kb) upstream or downstream (Soshnev et al., 2012). This regulatory distance was chosen for two reasons. First,

**Fig. 1. Identification of Su(Hw) target genes.**

(A) Shown are 4- to 6-hour-old DAPI-stained ovarioles isolated from fertile [*su(Hw)*^{+/+}] and sterile [*su(Hw)*^{2/v} and *su(Hw)*^{Pb/2}] females. Scale bars: 25 μ m. (B) Microarray analyses identified 175 increased (red bar) and 122 decreased (blue bar) genes, of which 75 and 30 correspond to Su(Hw) target genes, respectively (gray bars). (C) Quantitative PCR validation of target genes. Expression is normalized to the housekeeping gene *Rpl32* and shown as heat map of fold change values relative to *su(Hw)*^{+/+}, with blue and red indicating lower and higher expression, respectively. 33/93[†], heteroallelic combination of two independently generated recombinant chromosomes containing double *Cp190*^{H4-1} and *mod(mdg4)*^{U1} mutations. The *gypsy* insulator proteins associated with the SBSs are shown. Three non-target housekeeping genes and *su(Hw)* were included as controls. Two independent biological samples (1, 2) were analyzed.

2 kb matches the distance used in other genome-wide studies in *Drosophila* (Schwartz et al., 2012; Sexton et al., 2012). Second, most target genes identified using the 2-kb window of flanking regulatory DNA are retained at a shorter regulatory distance of 0.5 kb (87% for repressed and 76% of activated target genes; supplementary material Fig. S1A). Using the 2-kb distance, we find that 105 (35%) of the mis-regulated genes carry SBSs compared with 1709 (13.5%) of total genes in the *Drosophila* genome that carry an SBS, representing a highly significant enrichment ($P=6.96E-27$). Of the 105 target genes, 75 display increased expression in *su(Hw)* mutants and correspond to Su(Hw)-repressed genes, whereas 30 display decreased expression and correspond to Su(Hw)-activated genes. Using qPCR, we compared expression of randomly selected target genes in *su(Hw)*^{+/+} and *su(Hw)*^{-/-} RNAs. All but one of the tested genes had altered expression in *su(Hw)* mutants (Fig. 1C), validating our microarray findings. Most target genes increase expression upon Su(Hw) loss (71%, 75/105; Fig. 1B,C), indicating that Su(Hw) has a major role as a transcriptional repressor.

SBSs in repressed and activated target genes show distinct properties

Su(Hw) target genes are dispersed throughout euchromatic regions of the genome. In total, target genes contain 195 SBSs, with an average of 1.9 SBSs per repressed and 1.0 SBS per activated gene (supplementary material Fig. S1B). Previous studies showed that SBSs are distributed in the genome with no apparent enrichment for gene features (Bushey et al., 2009; Nègre et al., 2010; Soshnev et al., 2012). We used the ovary SBS dataset (Soshnev et al., 2012) to determine whether regulatory SBSs in Su(Hw) target genes display different properties from bulk SBSs and whether SBSs associated with activated or repressed target genes had distinct

features. First, we assessed Su(Hw) occupancy. We found that ChIP enrichment at target gene SBSs did not statistically differ from non-target SBSs (Fig. 2A), indicating that Su(Hw) occupancy does not correlate with its effect on transcriptional regulation. Second, we defined the Su(Hw) consensus sequence of SBSs, using the MEME program (Bailey and Elkan, 1994). We found that regulatory and total SBSs possess a similar DNA-binding motif (Adryan et al., 2007; Soshnev et al., 2012) (Fig. 2B), implying that the sequence of an SBS does not predict transcriptional output. Interestingly, these studies revealed that the Su(Hw) binding motif in the *gypsy* insulator differs from total SBSs, as the insulator motif lacks the GC-rich 3' extension, but possesses an AT-rich 5' extension. These observations suggest that Su(Hw) binding at the *gypsy* insulator might be distinct from that at endogenous SBSs. Third, we examined the distribution of SBSs relative to the gene features. As expected, the fraction of intergenic SBSs decreased (Fig. 2C), because target genes were identified based on SBS proximity. Even so, we found that target gene SBSs show enriched localization to exons ($P=3.5E-8$) and transcription start sites (TSSs) ($P=1.5E-11$; Fig. 2C). This latter observation is particularly striking, considering that these regions included only 200 bp flanking each side of the annotated TSS. Localization of many SBSs to target gene promoters is consistent with Su(Hw) having a direct role in target gene transcription.

We investigated whether regulatory SBSs were associated with the *gypsy* insulator proteins. To date, genome-wide mapping of CP190 and Mod67.2 has only been completed in non-ovary tissues, including a dataset obtained from embryos (Nègre et al., 2010). To assess whether the embryonic dataset predicted CP190 and Mod67.2 occupancy in the ovary, we used ChIP-qPCR to analyze the presence of CP190 and Mod67.2 at SBSs within ovary chromatin. These experiments demonstrated that 80% (28/35) and 89% (31/35) of the

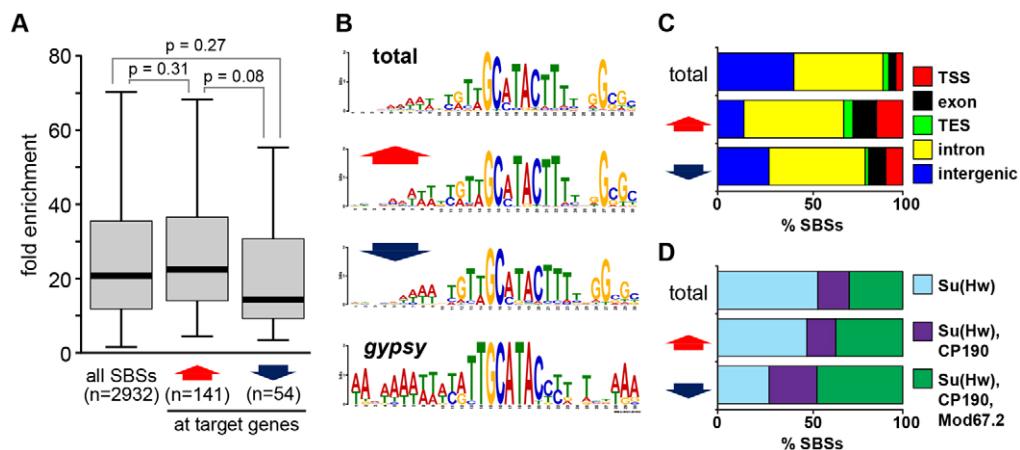


Fig. 2. Characterization of Su(Hw) binding sites (SBSs) at target genes. (A) Shown are box plots of fold enrichment of all SBSs (total) and SBSs in activated (red arrow) and repressed (blue arrow) target genes. Within each box, the black line indicates median enrichment, boxes and whiskers represent 25–75 percentile interval and non-outlier range, respectively. *P*-values of Student's *t*-test are indicated. (B) Weblogo of MEME-derived consensus motifs of all SBSs, SBSs at target genes, and the *gypsy* insulator SBSs. (C) Shown are distributions of SBSs relative to gene features. (D) Shown are enrichments of *gypsy* insulator proteins at SBSs.

embryonic SBSs tested showed the expected occupancy of CP190 and Mod67.2, respectively (supplementary material Fig. S2). As these data endorse the embryonic dataset for predicting ovary binding of the *gypsy* insulator proteins, we examined recruitment of these proteins at target genes. We found that target genes had increased association of CP190 and Mod67.2 relative to total SBSs (Fig. 2D). To determine whether the *gypsy* insulator proteins were required for Su(Hw) regulation of target genes, we tested target gene expression in females carrying one of two independently generated, double mutant recombinant chromosomes (*R33* and *R93*). Each chromosome carried the *Cp190^{H4-1}*, *mod(mdg4)^{ul}* (*mod^{ul}*) alleles (supplementary material Table S1). Homozygous *Cp190^{H4-1/H4-1}*, *mod^{ul/ul}* females are viable, which allowed measurements of RNA levels in newly eclosed ovaries. As predicted, effects of Mod67.2 and CP190 loss occurred mostly at target genes that carry *gypsy*-like SBSs (5/6; Fig. 1C). We found that activated target genes had a stronger dependence on these *gypsy* insulator proteins than did repressed target genes, as expression of 80% (4/5) of activated target genes decreased in *Cp190^{H4-1/H4-1}*, *mod^{ul/ul}* ovaries (Fig. 1C). One target gene, *CG12071*, showed altered expression in *Cp190^{H4-1/H4-1}*, *mod^{ul/ul}* mutants, even though this gene lacks a *gypsy*-like SBS. However, loss of CP190 and Mod67.2 had an opposite effect to that resulting from loss of Su(Hw), implying that altered expression might reflect an indirect effect in the double mutant background. Our data demonstrate that a subset of Su(Hw) target genes require CP190 and Mod67.2 for expression.

Su(Hw) is a repressor of CNS-enriched genes in the ovary

We examined the collection of target genes to identify common features of Su(Hw)-regulated genes. First, we performed Gene Ontology analysis and found no significantly over-represented developmental or signaling pathway (data not shown). Second, we determined whether target genes displayed common tissue expression patterns, using the FlyAtlas anatomical expression dataset that includes larval and adult tissues (Chintapalli et al., 2007). In our analyses, we considered that a gene showed tissue-enriched expression if RNA expression levels were twofold or higher in that tissue relative to the level in the whole fly carcass. These analyses revealed that Su(Hw) target genes showed significantly enriched and depleted gene expression in several

tissues (Fig. 3A; supplementary material Tables S3, S4). We found that Su(Hw)-repressed target genes are significantly enriched for CNS expression [75% (56/75) relative to 28% of total *Drosophila* genes (3654/12856), *P*=5.3E-19], but depleted in the ovary [5% (4/75) relative to 16% (2070/12856) of total *Drosophila* genes, *P*=0.011] and in the testes [8% (6/75) relative to 22% (2778/12856) of total *Drosophila* genes, *P*=0.004]. For Su(Hw)-activated target genes, we found that expression was significantly enriched in the hindgut [40% (12/30) relative to 15% (1890/12856), *P*=4.80E-05]. Based on these findings, we conclude that the major role of Su(Hw) in the ovary is to repress neural genes, as more than half of all target genes show CNS-enriched, but ovary-depleted expression.

One implication of a role for Su(Hw) in the regulation of neural genes in the ovary is that Su(Hw) may not be globally expressed in the CNS. Such tissue-restricted expression was unexpected, as previous studies indicated that Su(Hw) accumulation was ubiquitous throughout development (Harrison et al., 1993). To investigate this, we examined Su(Hw) accumulation in third instar larval CNS and uncovered cell-type specific Su(Hw) expression (Fig. 3B). Immunohistochemical analyses showed that Su(Hw) is present in neuroblasts [Deadpan (Dpn)-positive cells (Doe and Skeath, 1996)] and glia [Reverse Polarity (Repo)-positive cells (Xiong et al., 1994)], but is absent in terminally differentiated post-mitotic neurons [Embryonic Lethal Abnormal Vision (ELAV) (Robinow and White, 1991)]. This cell type-specific expression pattern is established early in development, as Su(Hw) is absent in ELAV-positive cells in embryos (data not shown). These observations indicate that Su(Hw) accumulation is dynamic in neural lineages and is consistent with Su(Hw) acting as a repressor of neural genes in non-neuronal tissues.

Su(Hw) occupancy at SBSs shows little tissue specificity (Adryan et al., 2007; Soshnev et al., 2012). Based on these observations, we predicted that Su(Hw) target genes might be mis-regulated in non-ovary tissues. Previous studies using whole larvae demonstrated that loss of Su(Hw) altered expression of some of the target genes that we identified in the ovary (Adryan et al., 2007). To gain greater insight into the tissue-specificity of Su(Hw) regulation, we examined gene expression in individual tissues, performing qPCR analyses of RNAs isolated from third instar larval brains and wing discs (Fig. 3C). These studies revealed that the majority of

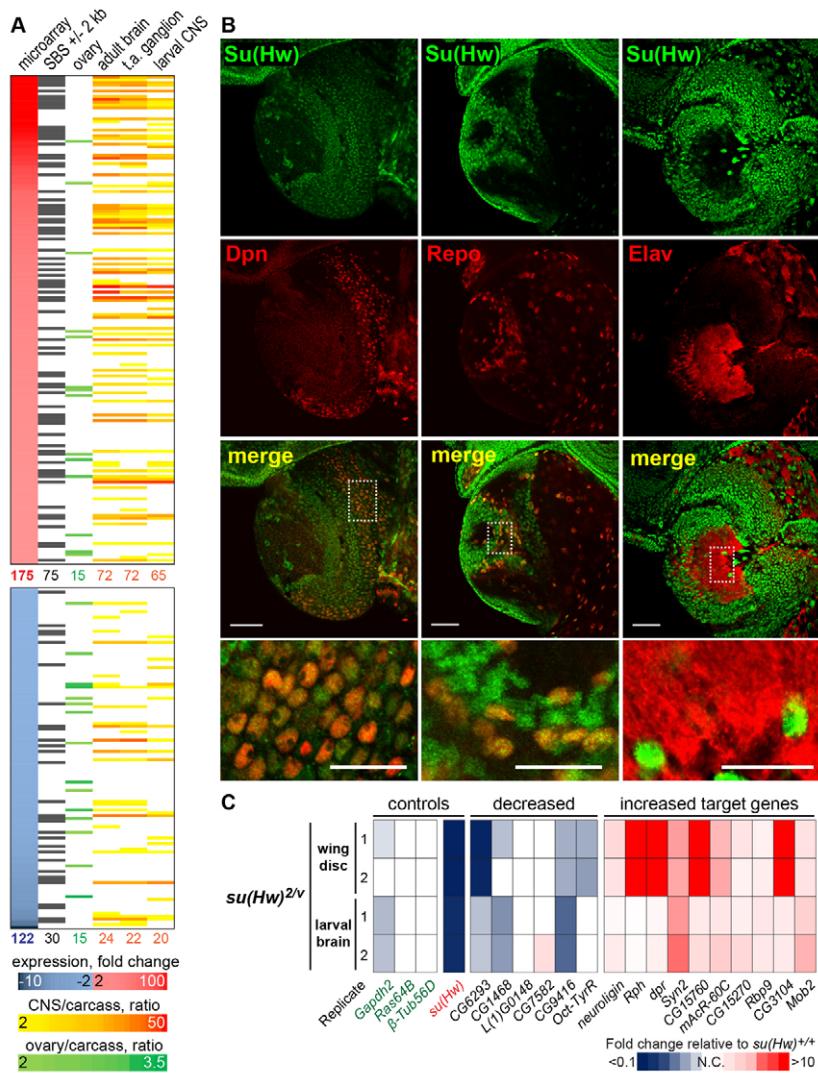


Fig. 3. Su(Hw) is a repressor of CNS-enriched genes. (A) Left to right: target genes ranked by fold changes obtained in microarray analyses, with red corresponding to activated and blue corresponding to repressed genes; genes with SBSs; genes with ovary-enriched expression indicated by green color scale; genes with CNS-enriched expression in three CNS structures [adult brain, thoracoabdominal (t.a.) ganglion and larval CNS] indicated by the orange color scale (Chintapalli et al., 2007). (B) Confocal images of *su(Hw)*^{+/+} third instar larval brains stained for Su(Hw) (green, top) and neural markers (Dpn, Repo, ELAV; red, middle), and merged image (bottom). Scale bars: 50 µm. Magnified areas (shown below) are indicated by dotted rectangles. Scale bars: 25 µm. (C) qPCR analyses of activated and repressed target genes in RNA isolated from *su(Hw)*^{2v} larval brain and wing disc. Expression was normalized to the housekeeping gene *RpL32* and is shown as heat map of fold change values relative to *su(Hw)*^{+/+}, with blue and red indicating low and high expression, respectively. Three non-target housekeeping genes and *su(Hw)* were included as controls. Two biological samples (1, 2) were analyzed.

tested target genes had altered expression in wing or brain tissue (14/16, 88%; Fig. 4B). The largest transcriptional changes were seen in the wing disc, which might reflect the more uniform expression of Su(Hw) in wing discs, but not brain tissue (Fig. 3B; data not shown). These findings demonstrate that Su(Hw) is required for the regulation of target gene expression in multiple tissues.

Su(Hw) represses Rbp9 expression

One Su(Hw)-repressed target gene was *RNA-binding protein 9* (*Rbp9*), a gene first identified owing to its CNS-enriched expression (Kim and Baker, 1993). *Rbp9* belongs to the ELAV/Hu gene family that encodes RNA-binding proteins (Kim and Baker, 1993; Pascale et al., 2008). Although loss of *Rbp9* causes female sterility owing to an early arrest in germline development (Kim-Ha et al., 1999), ectopic *Rbp9* expression causes oocyte apoptosis in mid-to-late oogenesis (Jeong and Kim-Ha, 2003). The overlap of this latter phenotype with the *su(Hw)* mutant phenotype prompted us to investigate *Rbp9* regulation.

We studied Su(Hw) and Rbp9 protein localization in the ovary. Oogenesis begins in the germarium by asymmetric division of a germline stem cell (GSC). The resulting daughter cell, termed cystoblast, undergoes four incomplete mitotic divisions to generate a 16-cell cyst, which becomes enveloped by somatic follicle cells to

form an egg chamber. Co-staining ovaries with Su(Hw) and Rbp9 antibodies revealed differences in protein accumulation (Fig. 4A). Su(Hw) is present in somatic and germ cells, whereas Rbp9 is present only in germ cells. In germ cells, Su(Hw) is found at low levels in the GSCs and daughter cystoblasts and is absent in regions of the germarium where the 16-cell cyst is formed and meiosis is initiated (regions 1 and 2a). Su(Hw) reappears in region 2b and increases during egg chamber formation (Baxley et al., 2011). By contrast, Rbp9 is found in region 2a of the germarium, remains high in region 2b, diminishes in region 3 as egg chambers form, and becomes undetectable beyond stage 3 egg chambers (Tastan et al., 2010). We reasoned that if Su(Hw) were required for transcriptional repression of *Rbp9*, then loss of Su(Hw) would prolong Rbp9 protein accumulation. This prediction was met. Loss of Su(Hw) is accompanied by extended presence of Rbp9 protein into late-stage egg chambers (Fig. 4B), supporting a role for Su(Hw) in *Rbp9* regulation.

The *Rbp9* gene contains three alternative TSSs, of which TSS3 is the most active in the ovary (Graveley et al., 2011). Interestingly, Su(Hw) binds near these TSSs, with SBS2 located 357 bp downstream of TSS2 (Fig. 4C). All *Rbp9* SBSs are associated with the *gypsy* insulator proteins CP190 and Mod67.2 (Fig. 4D). We used transcript-specific qPCR primers to investigate

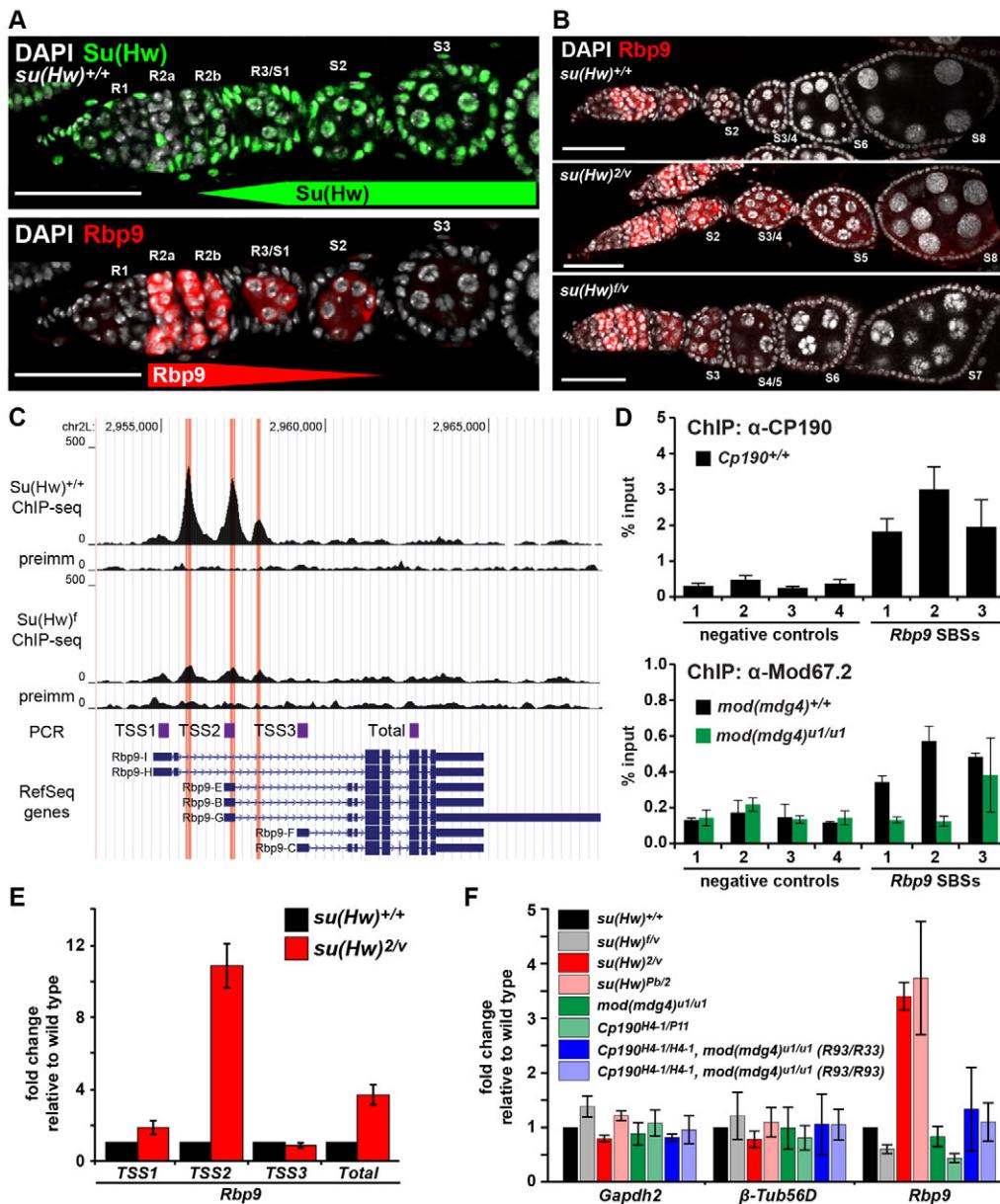


Fig. 4. *Rbp9* is repressed by *Su(Hw)*. (A) Images of *su(Hw)^{+/+}* germarium stained for *Su(Hw)* (top, green), *Rbp9* (bottom, red) and DAPI (white). Developmental regions (R1 to R3) of the germarium and egg chamber stages (S1 to S3) are indicated. Scale bars: 25 μ m. (B) *su(Hw)^{+/+}* (top), sterile *su(Hw)^{2/2}* (middle) and fertile *su(Hw)^{0/0}* (bottom) ovarioles stained for *Rbp9* (red) and DAPI (white). Scale bars: 25 μ m. (C) UCSC genome browser view of the *Rbp9* gene locus, including tracks. Top to bottom: chromosome coordinates, *su(Hw)^{WT}* ChIP-Seq reads, preimmune serum IP control reads, *su(Hw)^{0/0}* ChIP-Seq reads, preimmune serum IP control reads, fragments amplified in qPCR analyses (E), RefSeq gene annotation. (D) ChIP-qPCR analyses of ovary-bound CP190 (top) and Mod67.2 (bottom) at *Rbp9* SBSs. Negative controls (1-4) were genomic regions with no SBS (Soshnev et al., 2012). ChIP from a *mod(mdg4)^{u1}* mutant background was a negative control. (E) qPCR analyses of promoter-specific *Rbp9* transcripts in *su(Hw)^{+/+}* (black bars) and *su(Hw)^{2/2}* (red bars) mutant background. Expression is normalized to housekeeping gene *Rpl32* and is shown as fold change relative to *su(Hw)^{+/+}*. Error bars indicate s.d. of three biological samples. (F) qPCR analyses of gene expression changes in *su(Hw)*, *Cp190* and *mod(mdg4)* mutant ovaries. Expression is normalized to the housekeeping gene *Rpl32* and shown as a fold change relative to *su(Hw)^{+/+}*. *Gapdh2* and β -tubulin are negative controls. R33 and R93 indicate two independently generated recombinant chromosomes containing *Cp190H4-1* and *mod(mdg4)^{u1}* mutations. Error bars indicate s.d. of two independent biological samples.

how *Su(Hw)* loss affects *Rbp9* transcription in *su(Hw)^{2/2}* newly eclosed ovaries. These studies showed that *Rbp9* transcription from TSS1 and TSS3 was largely unaffected, whereas transcription from TSS2 increased ~11-fold (Fig. 4E). These data indicate that *Su(Hw)* repression is specific to TSS2. Such a promoter-specific de-repression was unexpected. Although TSS1

might lack binding sites responsive to ovary transcription factors, this limitation does not apply to TSS3. Based on these data, we suggest that *Su(Hw)* repression of *Rbp9* depends on a localized action targeted to TSS2. To determine whether *Rbp9* repression involves insulator formation, we determined the effects of loss of CP190 or Mod67.2 on *Rbp9* transcription. Gene expression was

measured using qPCR of RNAs isolated from ovaries from (1) *Cp190^{H4-1/PII}* females, (2) *mod(mdg4)^{u1/u1}* (*mod^{u1/u1}*) females and (3) *Cp190^{H4-1/H4-1}*, *mod^{u1/u1}* double mutant females. These studies showed that *Rbp9* transcription is maintained in all *Cp190* and *mod^{u1/u1}* mutant backgrounds (Fig. 4F), implying that Su(Hw) regulation does not involve insulator formation. Taken together, these observations suggest that Su(Hw) is a direct repressor of *Rbp9* transcription, through local effects on TSS2.

Suppression of sterility in *su(Hw)* null females

The shared mutant phenotypes between ectopic *Rbp9* expression and Su(Hw) loss suggested that *Rbp9* de-repression might cause sterility in *su(Hw)^{-/-}* females. We reasoned that if increased transcription of *Rbp9* were responsible for *su(Hw)^{-/-}* sterility, then *su(Hw)* mutants that retain fertility should demonstrate wild-type *Rbp9* regulation. To this end, we studied *Rbp9* transcription and protein accumulation in ovaries obtained from *su(Hw)^{2/v}* females. Importantly, *su(Hw)^f* encodes a full-length Su(Hw) protein with a defective ZF10. Previous studies have shown that *su(Hw)^{2/v}* females display wild-type fertility, even though Su(Hw)^f binds only ~40% of genomic SBSs (Baxley et al., 2011; Soshnev et al., 2012). Strikingly, in *su(Hw)^{2/v}* ovaries, *Rbp9* shows a near-normal accumulation during egg chamber development (Fig. 4B), corresponding to Su(Hw)^f retention at *Rbp9* SBSs *in vivo* (Fig. 4C) (Soshnev et al., 2012) and transcriptional repression of *Rbp9* (Fig. 1C; Fig. 4D). Taken together, these data imply that fertility and *Rbp9* regulation are linked.

Loss of Su(Hw) increases levels of *Rbp9* RNA threefold in the ovaries of newly eclosed females. We postulated that if *Rbp9* de-repression caused *su(Hw)^{-/-}* sterility, then loss of one gene copy of *Rbp9* might reduce *Rbp9* RNA to a level compatible with female fertility. To this end, we generated *Rbp9^{+/+}*, *su(Hw)^{2/v}* double mutants, wherein mutants carried one of four independently generated *Rbp9* null alleles. These *Rbp9* alleles included two *P*-element insertions in the *Rbp9* gene (Kim-Ha, 2000) and two genomic deficiencies that removed *Rbp9* (supplementary material Table S1), as well as other genes. Strikingly, *Rbp9^{+/+}*, *su(Hw)^{2/v}* females derived from any of the four *Rbp9* null alleles produced eggs at ~8–20% of the wild-type level (Table 1). Females carrying alleles of the large genomic deletions produced fewer eggs, a difference that might result from the larger number of genes deleted. Ovaries obtained from *Rbp9^{+/+}*, *su(Hw)^{2/v}* females contained late-stage egg chambers, although evidence of egg chamber apoptosis remained (Fig. 5A). RNA analyses demonstrated that *Rbp9* RNA was not increased above twofold in *Rbp9^{+/+}*, *su(Hw)^{2/v}* ovaries relative to wild type, whereas *su(Hw)* RNA was undetectable (Fig. 5B). We tested whether reduced *Rbp9* expression restored expression of other mis-regulated Su(Hw) target genes, as the *Rbp9* protein belongs to the ELAV/Hu gene family of RNA-binding proteins and might alter mRNA stability (Pascale et al., 2008). These analyses showed that Su(Hw) target genes remained mis-regulated in the *Rbp9^{+/+}*, *su(Hw)^{2/v}* rescued ovaries (Fig. 5C). Strikingly, these data demonstrate that reducing the dosage of a single Su(Hw) target gene restores fertility to *su(Hw)* null females.

As a measure of the specificity of the *Rbp9* rescue, we tested whether hemizygous loss of other genes restored oogenesis in *su(Hw)* null females. To this end, we tested a deficiency that removed *mspo*. This gene was chosen for two reasons. First, Su(Hw)^f retains binding to *mspo*, potentially linking *mspo* de-repression to *su(Hw)^{-/-}* sterility. Second, loss of Su(Hw) increases *mspo* transcription approximately threefold, implying that loss of one gene copy might lower their RNA level to that found in *su(Hw)^{+/+}* ovaries and might lead to rescued

Table 1. Egg lay analyses of *su(Hw)^{2/v}* mutants carrying hemizygous mutations in target and non-target genes

Target gene	Allele	Genes affected (n)	Eggs/female/day (% of wild type*)
<i>Rbp9</i>	<i>Rbp9^{P2690}</i>	1	20
<i>Rbp9</i>	<i>Rbp9^{P2775}</i>	1	20
<i>Rbp9</i>	<i>Df(2L)ED206</i>	57	13
<i>Rbp9</i>	<i>Df(2L)ED4651</i>	122	8
<i>mspo</i>	<i>Df(2R)Exel6284</i>	46	0
<i>mspo</i>	<i>Df(2R)BSC858</i>	58	0
n/a	<i>Df(2R)ED3683</i>	203	0
n/a	<i>Df(2L)ED270</i>	24	0
n/a	<i>Df(2R)50C-38</i>	50	0
n/a	<i>Df(2R)ED1735</i>	144	0

*Wild-type females from the Canton S strain.

n/a, not applicable.

oogenesis. As an additional control, we tested four chromosome deficiencies that collectively remove >400 non-Su(Hw) target genes, to determine whether general decreases in gene dosage reverse the *su(Hw)^{-/-}* phenotype. We found that *mspo^{+/+}*, *su(Hw)^{2/v}* and the *Df(2L)2⁺*, *su(Hw)^{2/v}* females remained sterile (Table 1). Based on these data, we conclude that de-repression of *Rbp9* is the central cause of female sterility in *su(Hw)* null mutants.

Rescue of sterility phenotype reveals somatic function of Su(Hw) in oogenesis

We noted that most eggs produced by *Rbp9^{+/+}*, *su(Hw)^{2/v}* females had fused and deformed dorsal appendages (Fig. 6A). These observations imply a second, previously unrecognized requirement for Su(Hw) during late oogenesis. Dorsal appendage formation is linked to specification of the dorsal-ventral axis of the oocyte through three classes of genes that regulate signaling pathways (Berg, 2005). The presence of a single, broad dorsal appendage in *Rbp9^{+/+}*, *su(Hw)^{2/v}* eggs suggested that loss of Su(Hw) might compromise the function of the midline-minus class of genes. A hallmark of disruptions in this gene class is the aberrant expression of *broad*, which encodes four BTB/POZ domain ZF transcription factors. Among these, the Z1 isoform is required for dorsal appendage formation, being expressed in two lateral-dorsal fields of dorsal appendage primordia (Deng and Bownes, 1997; Tzolovsky et al., 1999). A failure to define two fields is associated with expansion and fusion of the dorsal appendages (Ward and Berg, 2005). To investigate whether *broad* expression was altered in *Rbp9^{+/+}*, *su(Hw)^{2/v}* egg chambers, ovaries were stained with antibodies against Z1. Whereas wild-type stage 12 egg chambers displayed two fields of Z1 expression, *Rbp9^{+/+}*, *su(Hw)^{2/v}* egg chambers showed a single field (Fig. 6B). The data suggest that loss of Su(Hw) alters the regulation of *broad* expression in late oogenesis.

The dorsal-ventral signaling cascade contributes to the repression of *broad* expression in midline cells (Berg, 2005). Activation of this cascade depends upon Gurken, a TGF α ligand that signals from the oocyte to the overlying follicle cells using the homolog of the epidermal growth factor receptor (Deng and Bownes, 1997). To test the involvement of Gurken, *Rbp9^{+/+}*, *su(Hw)^{-/-}* ovaries were stained with Gurken antibodies, revealing that Gurken production and localization were unaffected in the rescued ovaries (Fig. 6C). These data imply that Su(Hw) loss alters signaling events downstream of Gurken. Based on these findings, we predict that loss of Su(Hw) alters expression of one or more genes within the somatic follicle cells, leading to altered regulation of *broad*. We conclude that Su(Hw) has regulatory functions in somatic and germ cells during oogenesis.

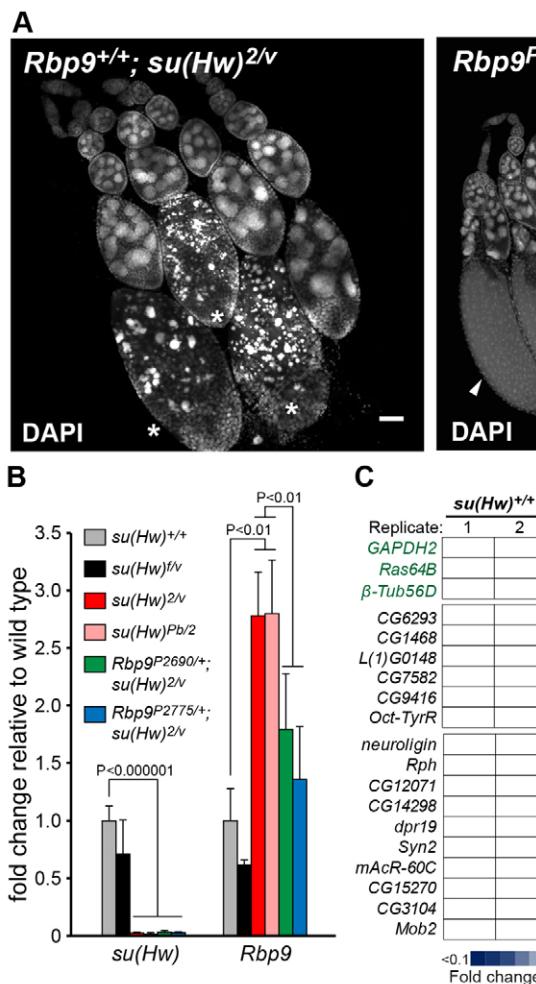


Fig. 5. Decreased Rbp9 expression rescues female sterility of su(Hw) null mutants. (A) DAPI-stained ovarioles isolated from su(Hw)^{2/v} and Rbp9^{P2775/+}; su(Hw)^{2/v} females. Asterisks indicate egg chamber apoptosis. Arrowheads indicate late-stage egg chambers. Scale bars: 50 μ m. (B) qPCR analyses of su(Hw) and Rbp9 RNA levels in the fertile su(Hw)^{+/+} (gray), su(Hw)^{2/v} (black), sterile su(Hw)^{2/v} (red) and su(Hw)^{Pb/2} (pink) and fertile Rbp9^{P2690/+}; su(Hw)^{2/v} (green) and Rbp9^{P2775/+}; su(Hw)^{2/v} (blue) backgrounds. Expression is normalized to housekeeping gene RpL32 and is shown as fold change relative to su(Hw)^{+/+}. Error bars indicate s.d. of three biological samples. (C) qPCR analyses of activated and repressed target genes in ovaries dissected from su(Hw)^{+/+}, su(Hw)^{2/v}, Rbp9^{P2690/+}; su(Hw)^{2/v} and Rbp9^{P2775/+}; su(Hw)^{2/v} females. Expression was normalized to the housekeeping gene RpL32 and is shown as heat map of fold change values relative to su(Hw)^{+/+}, blue and red indicating low and high expression, respectively. Three non-target housekeeping genes were included as controls. Two biological samples (1, 2) were analyzed.

DISCUSSION

Su(Hw) constitutively binds ~3000 SBSs distributed throughout the euchromatic regions of the genome (Soshnev et al., 2012). Endogenous SBSs largely contain a single Su(Hw) binding motif and show context-specific recruitment of CP190 and Mod67.2. Here, we address how Su(Hw) contributes to gene expression in the ovary, in which Su(Hw) function is essential. These studies advance our understanding of the transcriptional role of Su(Hw), revealing an essential function as a transcriptional repressor.

Su(Hw) regulation extends beyond insulator formation

Transcriptional requirements for Su(Hw) were defined using gene expression microarrays, controlling for genetic background and the developmental differences between su(Hw)^{+/+} and su(Hw)^{-/-} ovaries (Fig. 1; supplementary material Table S3). These analyses identified 297 mis-regulated genes, with over a third (105, 35%) corresponding to SBS-containing genes. Most Su(Hw)-target genes are de-repressed upon Su(Hw) loss (71%, 75/105; Fig. 1B,C), suggesting that most Su(Hw) regulation involves transcriptional repression. These data are consistent with previous findings that Su(Hw) localizes to repressive chromatin (Filion et al., 2010) and binds near genes that display low levels of transcription (Bushey et al., 2009; Roy et al., 2010).

Su(Hw) establishes an insulator when bound to the *gypsy* retrotransposon. This function depends upon recruitment of two

non-DNA-binding proteins, CP190 and Mod67.2 (Raab and Kamakaka, 2010). Building from this well-established requirement, we investigated whether Su(Hw) regulation of target genes involved insulator formation. We find that 20% of Su(Hw) target genes lack association of the *gypsy* insulator proteins (supplementary material Table S3), indicating that regulation of these genes might not involve canonical insulator formation. Additionally, even though 80% of target genes bind CP190 or Mod67.2, we found that protein localization does not always predict a regulatory involvement. Of the ten target genes tested that carry SBSs associated with CP190 and Mod67.2, five displayed altered gene expression in *Cp190*, *mod67.2* mutants (Fig. 1C). Interestingly, most of the affected target genes require the *gypsy* insulator proteins for transcriptional activation (Fig. 1C), suggesting that Su(Hw) might establish an insulator at some genes to prevent the spread of repressive chromatin. Alternatively, Su(Hw) might have a direct role in activation of gene expression, as CP190 shows strong association with active promoters (Bartkuhn et al., 2009). Taken together, our findings suggest that Su(Hw) regulation of transcription of most target genes is independent of insulator formation.

SBSs present in Su(Hw) target genes show a distinct distribution relative to gene features than do total SBSs. Interestingly, we observe a bias for location of regulatory SBSs with TSSs (Fig. 2C), indicating that Su(Hw) might have a direct transcriptional repression role. This prediction is supported by studies of Rbp9 regulation. Rbp9 expression involves transcription from three TSSs, with loss

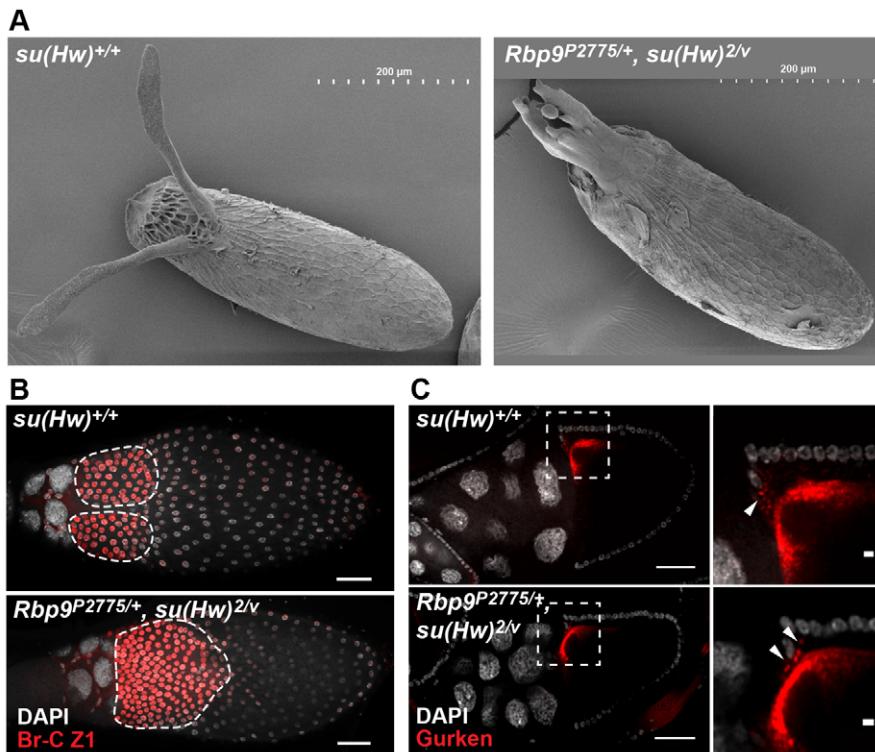


Fig. 6. Rescue of female sterility reveals a somatic function for Su(Hw). (A) SEM image of a *su(Hw)*^{+/+} egg and a *Rbp9*^{P2775/+}, *su(Hw)*^{2/v} egg. (B) Stage 12 egg chambers from *su(Hw)*^{+/+} and *Rbp9*^{P2775/+}, *su(Hw)*^{2/v} mutant females, stained with Broad-Z1 (red) and DAPI (white). Scale bars: 50 µm. (C) Left: whole-mount egg chambers stained for Gurken (red) and DAPI (white) dissected from wild-type (top) and *Rbp9*^{P2775/+}, *su(Hw)*^{2/v} (bottom) females. Dashed rectangles indicate magnified areas (shown to the right). Arrowheads indicate Gurken-positive vesicles. Scale bars: 50 µm (left) and 5 µm (right).

of Su(Hw) causing de-repression of only TSS2 (Fig. 4E). Such a limited transcriptional response is unexpected if *Rbp9* misregulation resulted from loss of a Su(Hw)-dependent insulator, because TSS3 is an active promoter in the ovary (Graveley et al., 2011) and should also respond to an unblocked enhancer. Consistent with an insulator-independent mechanism, transcription from TSS2 is unchanged in *Cp190*, *mod67.2* females, a genetic background that compromises *gypsy* insulator function (Gerasimova et al., 1995; Pai et al., 2004; Baxley et al., 2011). Notably, one *Rbp9* SBS is located ~400 bp downstream of TSS2, suggesting that Su(Hw)-dependent repression may result from a block of RNAP II recruitment or elongation from this promoter. Alternatively, *Rbp9* repression might depend upon interactions between SBSs to form a chromatin loop that constrains TSS2 activity. This latter mechanism shares features of the insulator function, as insulators display an ability for long-range interactions (Yang and Corces, 2012), unifying the mechanism of gene repression and insulator function.

Su(Hw) functions as a global repressor of neural genes in non-neuronal tissues

At the beginning of our studies, we predicted that the tissue-restricted defects caused by loss of the globally expressed and constitutively bound Su(Hw) protein were due to mis-regulation of genes that are expressed primarily in the ovary. However, our data do not support this prediction. Whereas 7% (7/105) of Su(Hw) target genes display ovary-enriched expression (Fig. 3A), 65% show CNS-enriched expression (68/105). Comparisons with global gene expression in these tissues indicate that Su(Hw) target genes show ovary-depleted, CNS-enriched expression (supplementary material Table S3). Interestingly, loss of Su(Hw) causes de-repression of most target genes in multiple tissues (Fig. 1B; Fig. 3C). Based on these data, we conclude that Su(Hw) is a transcriptional repressor of neural genes in non-neuronal tissues. A recent publication studying *Drosophila* chromatin proteins using Bayesian network analysis

support this conclusion, showing that Su(Hw) is associated with gene repression and neurological system processes (van Bemmel et al., 2013).

Properties of Su(Hw) are reminiscent of the REST, a mammalian transcription factor that establishes neural phenotypes owing to repression of neural genes in non-neuronal tissues (Chong et al., 1995). REST is an eight ZF protein that interacts with two separate co-repressor complexes, the transcriptional co-repressor CoREST and a Sin3-histone deacetylase complex (Lakowski et al., 2006; Ooi and Wood, 2007). Although REST is not conserved in drosophilids, a homolog of CoREST has been identified (Dallman et al., 2004; Yamasaki et al., 2011), implying that non-REST transcription factors direct dCoREST to chromosomes. *Drosophila* CoREST is a component of a newly identified transcriptional repressor complex LINT, which contains three subunits, CoREST, *Drosophila* lethal (3) malignant brain tumor [L(3)mbt] and *Drosophila* L(3)mbt interacting protein 1 (dLint-1) (Meier et al., 2012). Interestingly, L(3)mbt is a transcription factor associated with insulator elements (Richter et al., 2011). Based on this connection, we examined whether dLint-1 and L(3)mbt colocalized with Su(Hw) at SBSs in target genes. Strikingly, over a third (18/56) of CNS-enriched repressed target genes contain SBSs that colocalize with L(3)mbt (Richter et al., 2011) and >60% (35/56) contain SBSs that colocalize with dLint-1 (Meier et al., 2012). These data indicate that Su(Hw)-dependent repression might depend upon CoREST recruitment within the LINT complex. Taken together, our observations suggest that Su(Hw) might represent a third functional homolog of REST in *Drosophila*, with Charlatan and Tramtrack representing the other identified homologs (Dallman et al., 2004; Tsuda et al., 2006; Yamasaki et al., 2011).

Mis-regulation of a single Su(Hw) target gene is largely responsible for *su(Hw)*^{2/v} sterility

Repression of one target gene, *Rbp9*, is central to sterility in *su(Hw)*^{2/v} females. This conclusion stems from our striking observation that

oogenesis is rescued in *Rbp9^{+/−}*, *su(Hw)^{2v}* females, with these female producing ~20% of the wild-type number of eggs (Fig. 6; Table 1). *Rbp9* encodes a protein that belongs to the ELAV/Hu gene family of RNA-binding proteins (Kim and Baker, 1993). The ELAV family regulates multiple post-transcriptional steps in gene expression, ranging from alternative splicing to translation (Soller et al., 2010; Hilgers et al., 2012). *Rbp9* is transiently expressed in germ cells of developing cysts, wherein the encoded RNA-binding protein has an essential function to repress translation of the germ cell differentiation factor, Bag of marbles (Kim-Ha et al., 1999). Su(Hw) directs repression of *Rbp9* transcription after the formation of developing cysts to permit egg chamber development (Fig. 4A,B). Rescued *Rbp9^{+/−}*, *su(Hw)^{2v}* females produced fewer eggs than do *su(Hw)^{+/+}* females (Table 1). We consider two possible explanations to account for this partial suppression. First, Su(Hw) target genes besides *Rbp9* might contribute to *su(Hw)* sterility, because these genes remained mis-regulated in *Rbp9^{+/−}*, *su(Hw)^{2v}* ovaries (Fig. 5). However, not all Su(Hw) target genes might contribute to defects in sterility, as oogenesis was not restored in *su(Hw)^{+/−}* females carrying deletions encompassing another upregulated target gene (Table 1). Second, low levels of *Rbp9* protein aberrantly accumulate in *Rbp9^{+/−}*, *su(Hw)^{2v}* late-stage egg chambers. We note that *Rbp9* binds U-rich RNAs and regulates translation and stability of its target RNAs (Park et al., 1998; Kim-Ha et al., 1999). As such, even low levels of ectopically produced *Rbp9* might affect the function of RNAs crucial for late oogenesis, thereby triggering apoptosis. Identification of *Rbp9* target RNAs may provide insight into processes involved in programmed cell death that occurs in mid-oogenesis.

In summary, we demonstrate that Su(Hw) is required for activation and repression of individual target genes, extending the known regulatory properties of this classic insulator protein. A growing body of data suggests that many insulator proteins have transcriptional functions that extend beyond insulator formation. For example, early studies documented roles for CTCF as a direct transcriptional activator and repressor (Lobanenkov et al., 1990; Burcin et al., 1997), findings supported by recent genetic analyses in transgenic mice (Heath et al., 2008; Wan et al., 2008; Ribeiro de Almeida et al., 2009; Soshnikova et al., 2010). Further studies are needed to establish the general principles that govern the interplay between genomic context and transcriptional functions of insulator proteins.

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Competing interests statement

The authors declare no competing financial interests.

Author contributions

A.A.S. and R.M.B. performed the experiments. J.R.M. and K.T. assisted with the analyses of microarray data. A.A.S. and P.K.G. designed the experiments and wrote the paper.

Supplementary material

Supplementary material available online at <http://dev.biologists.org/lookup/suppl/doi:10.1242/dev.094953/-DC1>

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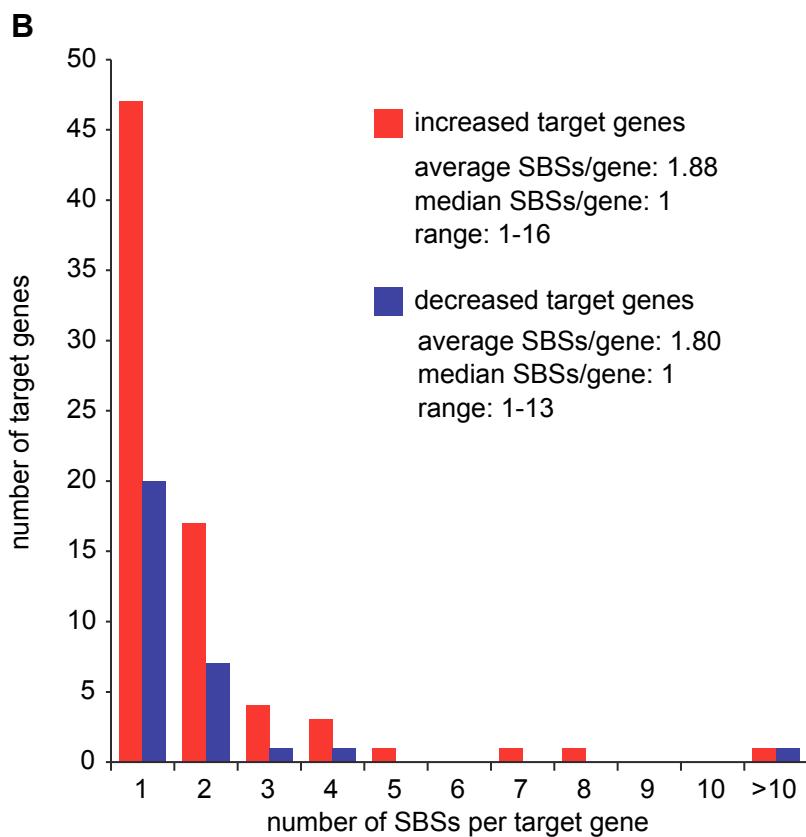
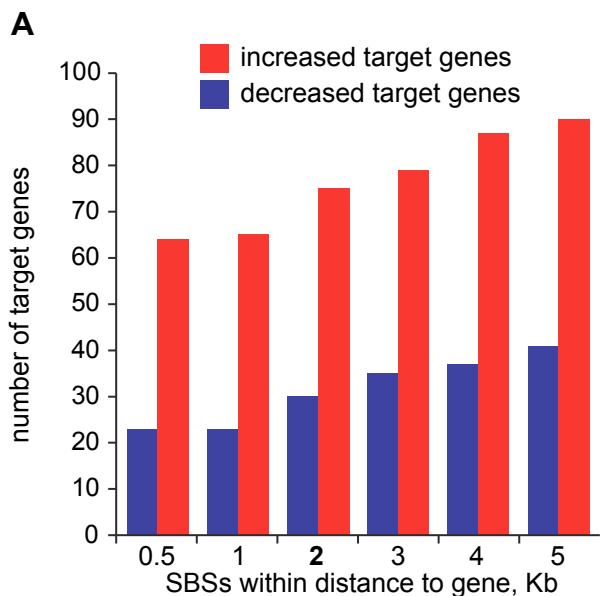


Fig. S1. Distribution of SBSs relative to the genes identified in microarray analyses. (A) Numbers of genes classified as direct targets, considering the presence of an SBS at different distance cutoffs of regulatory DNA flanking the mis-regulated gene (0.5 to 5 Kb). Activated and repressed genes are shown in red and blue, respectively. (B) Numbers of SBSs per target gene, with average, median and range indicated for activated (red) and repressed (blue) genes.

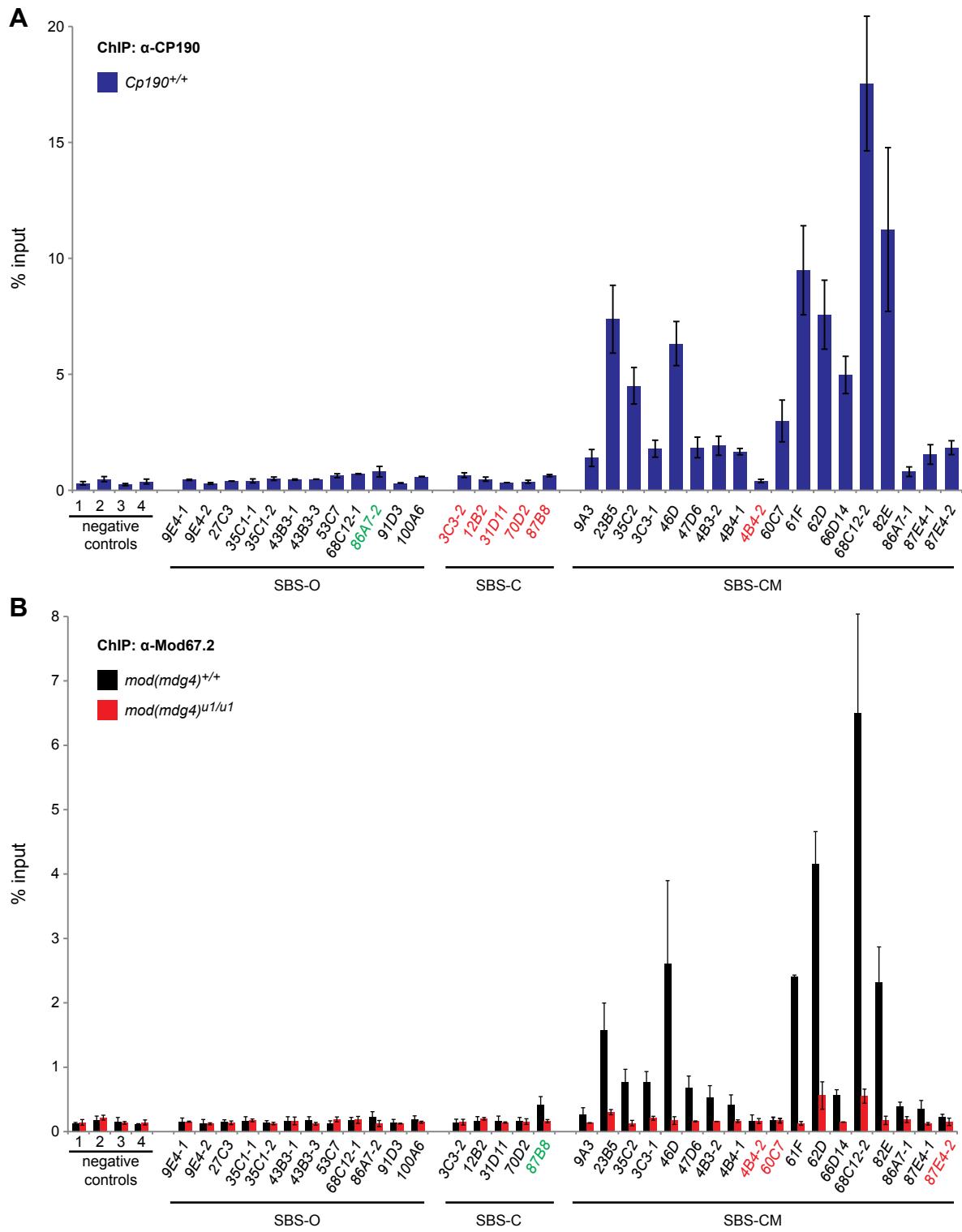


Fig. S2. CP190 and Mod67.2 association at SBSs in the ovary. (A,B) ChIP-qPCR analyses of ovary CP190 (A) and Mod67.2 (B) distribution. Negative controls: genomic regions with no SBS and no reported CP190 and Mod67.2 association. Sites are arranged into classes based on ModEncode results [SBS-O, SBS-C and SBS-CM (Negre et al., 2010, Soshnev et al., 2012)] and are labeled based on cytological location. Red indicates a loss of protein occupancy in the ovary relative to embryos; green indicates gain of occupancy in the ovary relative to embryos. In B, $mod(mdg4)^{u1}$ null mutant background is shown as an additional negative control (black bars). In total, 28/35 (80%) sites demonstrate expected CP190 occupancy and 31/35 (89%) sites demonstrate expected Mod67.2 occupancy. Error bars indicate s.d. of two independent biological replicates.

Table S1. Alleles and genotypes used in the study

su(Hw) mutant alleles				
Allele	Genotype	Stock	Lesion	Reference
<i>su(Hw)²</i>	$y^2 w^{67c23} cf^6 f^l; su(Hw)^2/TM6B Tb$	n/a	jockey insertion in first intron of <i>su(Hw)</i> gene	Harrison et al., 1993
<i>su(Hw)^v</i>	$y^2 cf^6 f^l; su(Hw)^v/TM6B Tb$	n/a	deletion encompassing <i>su(Hw)</i> TSS and <i>RpII 15</i> gene	Harrison et al., 1992
<i>su(Hw)^{Pb}</i>	$y^2 w^{67c23} cf^6 f^l; su(Hw)^{e04061}/TM6B Tb$	n/a	<i>piggyBac</i> insertion in first intron of <i>su(Hw)</i> gene	Baxley et al., 2011
<i>su(Hw)^f</i>	$y^2 w^{67c23} cf^6 f^l; su(Hw)^f Ubx^+/su(Hw)^v$	n/a	C525Y mutation disrupting Su(Hw) ZF10 backbone	Harrison et al., 1993
<i>su(Hw)^{A2663}</i>	$y^2 w^{67c23} cf^6 f^l; su(Hw)^{A2663}/TM6B Tb$	n/a	EMS-generated deletion encompassing +3534 to +4633 bp relative to the TSS	n/a
Reference wild-type alleles				
Allele	Genotype	Stock	Lesion	Reference
<i>Canton S</i>	<i>Canton-S</i>	BL 1	n/a	
<i>BL15598</i>	$y^l w^{67c23}; Pfw^{+mC} y^{+mDint2}=EPgy2; CG6499^{EY02782}$	BL 15598	n/a	
gypsy insulator partners				
Allele	Genotype	Stock	Lesion	Reference
<i>Cp190^{H4-1}</i>	$y^2 w cf^6 omb^{P1-D11}; Cp190^{H4-1}/TM6B$		truncation of the 331 amino acids at the C-terminus of CP190	Pai et al., 2004
<i>Cp190^{P11}</i>	$y^2 w cf^6; Cp190^{P11}/TM6B$		deficiency encompassing <i>Set</i> , <i>Oscp</i> , <i>Cp190</i> and <i>Mrg15</i> genes	Pai et al., 2004
<i>mod(mdg4)^{u1}</i>	$y^2 cf^6; mod(mdg4)u1/mod(mdg4)u1$		<i>Stalker</i> insertion causing Mod67.2 truncation by 145 amino acids at the C-terminus	Gause et al., 2001
Target gene alleles and deficiency lines				
Allele	Genotype	Stock	Lesion	Reference
<i>Rbp9^{P2690}</i>	$w^*; Pfw^{+mC} lacW/Rbp9^{P2690}/CyO$	BL 25778	P-element insertion within the coding sequence of <i>Rbp9</i> gene	Kim-Ha et al., 1999
<i>Rbp9^{P2775}</i>	$w^*; Pfw^{+mC} lacW/Rbp9^{P2775}/CyO$	BL 25777	P-element insertion within the coding sequence of <i>Rbp9</i> gene	Kim-Ha et al., 1999
<i>Df(2L)ED206</i>	$w^{118}; Df(2L)ED206, Pfw^{+mW} Scer/FRT.h3^3'RS5+3.3'ED206/SM6a$	BL 8038	deficiency	
<i>Df(2L)ED270</i>	$w^{118}; Df(2L)ED270, Pfw^{+mW} Scer/FRT.h3^3'RS5+3.3'ED270/SM6a$	BL 8039	deficiency	
<i>Df(2L)ED4651</i>	$w^{118}; Df(2L)ED4651, Pfw^{+mW} Scer/FRT.h3^3'RS5+3.3'ED4651/SM6a$	BL 8904	deficiency	
<i>Df(2R)Exel6284</i>	$w^{118}; Df(2R)Exel6284, Pfw^{+mC} XP-U/Exel6284/CyO$	BL 7749	deficiency	
<i>Df(2R)BSC858</i>	$w^{118}; Df(2R)BSC858, P+PBacfw^{+mC} XP3.WH3/BSC858/SM6a$	BL 27928	deficiency	
<i>Df(2R)ED1735</i>	$w^{118}; Df(2R)ED1735, Pfw^{+mW} Scer/FRT.h3^3'RS5+3.3'ED1735/SM6a$	BL 9275	deficiency	
<i>Df(2R)ED3683</i>	$w^{118}; Df(2R)ED3683, Pfw^{+mW} Scer/FRT.h3^3'RS5+3.3'ED3683/SM6a$	BL 8918	deficiency	
<i>Df(2R)50C-38</i>	$Df(2R)50C-38, al^l b^l cn^l Pfw^{+mC} CaSpeR/CpI^{50C-38}/CyO, amos^{Roi-1} bw^l$	BL 8114	deficiency	

Table S2. Primers used in qPCR analyses

Gene expression analyses		
Target	Forward	Reverse
GAPDH2	CACTCGTGGTTCGATGCCAAG	TCGATGACGCCGGTTGGAGTAGC
Ras64B	AGGAAGTGCTGCCATGAGAAG	TTATATGTTGGCTCTGCTTCCGC
bTub56D	AGTTCACCGCTATGTTCA	CGAAAACATTGATCGAG
su(Hw)	ATACCCTGCGACGGCACATACG	TTCACGCACCACAAGGCCATT
CG6293	TGCCATCATTAACATTATCATGCC	ATCGGTGCTACTGGGACTACAAATTCTC
CG1468	ATAGGAGGCCACGGGTTCTTCACT	ATTTGTCGCGAGACGCATATCACC
L(1)G0148	CAACTGGCGCAGATGGATCAAACA	AGACAGTTGAGGCAAAGTGAGGG
CG7582	TCGGTTCCACATCCATCAG	GAACGCAACCACCAAACTTAG
CG9416	GCGAGCACAGTGAGTCATAA	AGCTCCGAGTCACAATCAATAC
Oct-TyrR	CGCTGGGCTCTTCTTATT	GCGATCGTGTAAAGCTTGTG
neuroligin	TCGTGTCGGGAGTATAGCTGCTAC	GCAGTTCACCAAGCAGCTTCTTC
Rph	AACAACTCCGCAACTCACAAACG	AGGCCTCACGATAGCTAATGGCAA
CG12071	TGCCACATTGGAGGAAGTGTCTG	AACAACAAACAACAGCCAGAACATG
CG14298	AATTCAACTGCTATGTGGCCGC	GATTGCGATTGCCGTTGCATCCTT
dpr19	TATGACGCAACAGCATCAATCCGC	AACATGCACTGTAATGCTGGCAGG
Syn2	AATGCCAACGTCCTGCAAAGTCTGCT	TATATCATTGAGTGTGAGTCCATGCG
CG15760	GCAACTCGTCCACGATTCAAAG	GCAACTCGTCCACGATTCAAAG
mAcR-60C	CTGCTGGGTATGACCTGGCGTTG	TTCAGCCTGCCACTCGGACGG
CG15270	TTCACAGCACCAAGCAGCAAATAGTG	ATGAACAGATGAAGAGTTGATAATGCG
Rbp9	GCAGAGCAACAAAGTCAAGGGC	AGCTGACCTGAAGCACTCGGTTGC
CG3104	TGTGGATAGCAGCCCAGATGGGAC	TGTGGATAGCAGCCCAGATGGGAC
Mob2	AAATCCGAAGCATAAATCTGGTTATCTCC	CCAACACAACCAAAATCGCACC
Rbp9-TSS1	CGCAAATTCCAGGGTATTAACAGG	TTGATGGTCATATCCTCTCGCTGG
Rbp9-TSS2	GTGGAATCCGAATCAGTATCGAAATC	CATACAACACCGATTTCCTAACTC
Rbp9-TSS3	CTTCGTTGTCGCCTATGTTTG	ACTGGTTGCTAATCAGTAGATTCCACG
ChIP-QPCR		
Target	Forward	Reverse
chip neg 1	GTATATCCACATCACCAAGACCTCAGG	ACATCCTCGAATCACTATGCAAGTCG
chip neg 2	CACTCGTGGTTCGATGCCAAG	TCGATGACGCCGGTTGGAGTAGC
chip neg 3	CTTCGACTTGTATGTGATACTTCTGCT	AACGGATTGGAGATCGCATCAGC
chip neg 4	ACATCGGAGCCAGTGCCTCG	TATCCGACACCGTGGTAGTACTCTGC
Rbp9 SBS1	ATACTGTAGGCAGCGGGATGGG	AGAGAGAGCAAGGCCAGGAGCTAGG
Rbp9 SBS2	AAGAACATTTCAGTCCAGTCCAG	TTCTCTTCGCTTACATTATTGCCAG
Rbp9 SBS3	TAAGCTCGCTCTGGCTCCCATC	AGATAGAGCGGGAGAGACAGTGAGAGC
9E4-1	TGCGGAGCGTTGCGAATCGGTTG	ATAACCGTAAACGCCAGCCTGTTG
9E4-2	AACCTTCAACTCCCCACCGCACAC	TTCCAGAACCAACGACTATACCCAAAC
27C3	ACAAATGCTTGTGCTTCCGCC	GTTGGCTTCGCGTACACGGAATT
35C1-1	ACACAAACACTGAGAGCGCCGATA	TTCTATGCCAGGATCGTTGGTGA
35C1-2	TGCAAGCCGACAATATCAGACCT	GCGCACTTAAGCGCCATGAAGTAT
4B3-1	CAGTGGAGAAGCGAGACAATC	CGGACTGACTGACAAGTACATAAA
4B3-3	TACGCCAGTGTGACCCATAACTA	AGCGTTCAGTTCAGCTACACCCA
53C7	TCCGCTAGCGAAGTGTATAACG	TTGCCCACTTCAGGCCAAATGTAC
68C12-1	AGGCAGAAGCATGTGGTATAGTGGT	ATGCGCCCTGATGTAGGCTATGAA
86A7-2	TGCCAGCGGCTATTACAACACCTA	TCGTTCGATGGCGTATGGACGAA
91D3	ATTGTTGCTCTGGTGCCTGTTG	GTTTGCCTACTTCTGTGGCAGCA
100A6	CGTTGCATATCTCAAGGGCTCCAAAC	GCCATAACCAACTGTGCGTGACCCAG
3C3-2	AGCATCTAACGAAAGCCACCGA	AATCACACAGGGCGACGTACATCTA
12B2	GCAGCACTCAGTTGCAACAAATTCTCTC	TCCGTTATTGTACCATGCTGTTGTTGC
31D11	CTCATAAACTGAGTTCGTTGCAGGAGG	AGTAGCAAAAAACATGATAAAATGCAAGTG
70D2	GCACAGATAAGACCGCCAACTGT	AATTAGCGGCCACGTTGTTCGC
87B8	TTTGGGCAACACTAGAACATCTGGGAC	AGCAGGGAACCAAATTACTTCAGGAC
9A3	AGCTCGAGGCAATTATGTTGCGG	AAGAATTGGCGAAGAACGCACCG
23B5	AACCGATTGCTTCCAAAGTGGCG	TAGGCGTCGCTCACTCAATGAAC
35C2	ATAGATGCACCCAAACACAAACGGC	ACACCCAACTGACCATCCAGTCAA
3C3-1	ACCGCTACTTCACCACCATAGC	CGTGTGCAACATCCATTCC
46D	AGCAGTTATTGTAGGGACAGGTTGATGG	AAGTGGCAACGCTAAAGGAAAGAGTG
47D6	ATCTGGAGCTCTGCTATTGGCATT	AGCAGCGAAGAGAGTTGCTAAGA

4B3-2	AGTTCCATCTGCAGTATCGCTCCA	AGCTGATTGAATGCGGCTGTGTC
4B4-1	ATGCCACGACTCAATGGGCAAATC	ATGCGTGTGTGCCAGTATCTGT
4B4-2	TGCACTTGTGGATGTCTCTGTGGA	AATCCTCCAGCACTACAGCACCTT
60C7	TGTAACCGGTGACATCGCTGCTGTT	AGAGGCAGCATCTTAGCCCCAA
61F	CAGACCAATGTCTACAGCTCAATAGGCG	AGGTACACATAAGTATGAAAGAATGACCG
62D	GAATCGCGCAAATTGGTCGG	CGGGCAAAACGTATTTAAATTCAGCC
66D14	GAGCGCCGCCAACATACATTACATT	AGCTGCTGATCCTCGCTTCCATA
68C12-2	TCGGTGCGATTGTGGTTGTGTTG	CCAGCCTGAAAGTATGCACCGAAA
82E	TAAGACCAATAATGAATGAGTCAAGCACAG	TTAACGCATGTGAAACAGTAGTGTGAAACTG
86A7-1	AAACTAATTGGGCTAACGACGGG	TTCGATAGAAAGATCACGCCATTGAG
87E4-1	TCCAGGGTGTCTTGAAGTTCCC	GCAGCAGATGGGCATAACCACAAA
87E4-2	TACGACCAGTGCTTGGTTCAAGT	ACATCCTGCTCGGACTTTGCCTAT

Table S3. Results of gene expression microarray analyses and identification of direct target genes

NULL vs WT 1% FDR				FLYATLAS				dm3 coordinates			Su(Hw) targets			
Probeset ID	Gene Symbol	Fold	P-value	Brain	TAG	L CNS	Ovary	chr	start	end	Su(Hw)	Su(Hw)-F	CP190	Mod67.2
1631004_s_at	su(Hw)	-130.4	1.58E-20	0.50	0.40	1.45	2.70	chr3R	10130177	10134309				
1637063_at	CG33099	-9.06	3.41E-04	0.00	0.10	0.20	0.40	chr3R	18354642	18356294				
1636954_at	NetB	-7.72	2.00E-06	20.30	19.00	25.29	0.30	chrX	14580071	14643409				
1624580_at	GstZ1	-7.68	8.64E-14	0.30	0.40	0.28	1.30	chr3R	5281239	5282268				
1640327_at	CG6023	-5.94	2.12E-11	1.00	0.90	0.39	1.70	chrX	18208156	18215198				
1637281_at	trol	-4.50	2.84E-05	1.60	0.80	2.96	0.50	chrX	2364493	2438795				
1625688_at	CG6293	-4.25	6.54E-09	0.30	0.30	0.65	2.00	chr3R	6133528	6137335				
1641327_at	CG9416	-4.00	4.56E-07	0.10	0.30	0.28	0.20	chr2R	15254753	15261207				
1630886_at	SelR	-3.66	3.47E-05	4.10	2.90	0.63	0.80	chr3R	6689077	6694364				
1639411_at	I(1)G0148	-3.00	5.54E-13	0.50	0.30	0.56	1.60	chrX	6548649	6553742				
1636099_s_at	mbl	-2.88	7.82E-06	20.80	15.20	5.18	0.40	chr2R	13104054	13266881				
1629897_a_at	CG6044	-2.74	1.45E-04	26.50	32.70	2.84	0.10	chr2R	18291678	18295880				
1632932_a_at	mp	-2.68	1.29E-06	22.90	15.00	8.26	0.80	chr3L	6991943	7046528				
1625780_a_at	CG9812	-2.15	1.56E-04	0.20	0.10	0.14	0.00	chr2R	19299859	19307207				
1637590_at	CG42329	-7.30	1.70E-04	0.10	0.00	0.09	0.10	chr2L	1219293	1229802				
1627441_at	Oct-TyrR	-3.98	4.94E-05	27.90	26.80	16.30	0.70	chr3L	22028103	22058379				
1629072_at	CG42259 / su(w[al])	-2.11	3.52E-04	0.30	0.30	0.53	1.40	chrX	906812	941042				
1639733_s_at	CG14275	-6.74	4.13E-06	0.10	0.20	0.25	0.20	chr2L	8327456	8332911				
1632021_at	Cyp6a20	-16.78	6.62E-20	1.10	2.20	0.14	0.10	chr2R	10769625	10771551				
1633427_at	CG7582	-12.36	4.29E-12	22.00	21.40	2.69	0.20	chr3R	25505963	25508000				
1638996_at	CG11727	-5.19	3.88E-08	3.50	2.30	2.21	1.50	chrX	11324556	11348371				
1636119_at	CG1468	-4.95	1.13E-08	0.30	0.50	0.05	0.00	chrX	9772455	9773312				
1623949_s_at	CG32816	-2.83	1.70E-05	0.30	0.10	0.37	1.60	chrX	210609	364670				
1631326_at	Zasp66	-2.70	2.74E-04	0.10	0.30	0.19	0.00	chr3L	8617471	8631773				
1635742_s_at	Cad74A	-2.53	1.09E-04	0.00	0.00	0.74	2.10	chr3L	17360853	17372630				
1626028_at	CG4783	-2.33	9.24E-05	0.00	0.00	0.00	0.00	chr3R	15774192	15774629				
1634440_s_at	Eip74EF	-2.14	1.97E-04	3.20	1.90	0.24	0.60	chr3L	17551772	17612425				
1641042_at	CG16820	-8.68	4.12E-07	0.20	0.00	0.07	0.00	chr2L	13246232	13250189				
1637670_s_at	CHES-1-like	-3.99	1.86E-06	1.70	1.40	0.72	2.50	chrX	7576371	7603418				
1628859_at	shi	-2.21	1.74E-05	14.10	14.60	5.74	0.70	chrX	15786149	15800725				
1630026_s_at	dpp	-2.01	8.39E-05	0.70	0.90	1.98	0.40	chr2L	2428372	2459823				
1633645_at	CG42565	-39.31	1.04E-08	0.00	0.10	0.06	1.40	chr2R	18547988	18548894				
1636485_at	CTCF	-17.52	1.45E-04	2.00	3.50	0.66	1.50	chr3L	7346678	7349813				
1637180_at	CG17977	-11.15	9.61E-15	3.70	3.10	3.11	1.60	chr2R	3953779	3955160				
1640681_at	CG4950	-10.35	1.85E-05	2.10	0.80	0.03	0.10	chr3L	16258897	16261256				
1636826_at	CG14072	-10.26	6.43E-09	0.00	0.10	0.04	0.10	chr2L	10936662	10937931				
1637366_at	nimC4	-8.62	9.43E-07	0.20	0.20	0.16	0.10	chr2L	14047582	14048863				
1631803_at	Lsp1alpha	-8.46	9.00E-09	1.60	1.30	5.62	0.50	chrX	12384416	12386933				
1628918_at	CG17478	-8.40	2.73E-04	0.10	0.20	0.10	4.20	chr2R	508621	510016				
1625063_a_at	CG31606	-8.40	3.38E-06	0.50	0.70	0.15	0.40	chr2L	8147626	8148356				
1628896_a_at	CG4210	-8.38	1.04E-04	0.00	0.10	0.01	0.70	chr3R	11039696	11040567				
1630256_at	lectin-24Db	-8.07	1.76E-06	1.90	0.90	0.05	0.10	chr2L	4188123	4189284				
1631555_at	CG10062	-8.01	2.53E-11	0.20	0.20	0.03	0.10	chr2R	15262818	15267679				
1628882_at	CG7378	-6.96	1.25E-07	1.20	1.00	0.23	0.10	chrX	18794443	18803330				
1640386_at	wbl	-6.62	1.00E-04	0.00	0.00	0.01	0.40	chr2R	15137266	15138419				
1629310_at	Trf4-1	-6.52	4.76E-09	0.60	0.40	0.21	2.40	chrX	8454662	8464192				
1628258_at	CG14526	-6.36	1.24E-04	0.20	0.20	0.05	0.10	chr3R	24709415	24711737				
1639502_at	nimC1	-5.91	1.19E-07	1.70	1.10	0.42	0.20	chr2L	13974119	13976753				
1624101_at	Cyp6a23	-5.35	1.23E-07	0.10	0.10	0.03	0.10	chr2R	10763338	10765159				
1629572_a_at	fat-spondin	-5.11	3.30E-05	1.40	2.90	0.21	0.00	chr2R	12941508	12946810				
1641477_at	CG32447	-4.89	1.61E-06	0.70	0.30	3.73	0.40	chr3L	21683712	21699465				

1638376	at	Osi15	-4.51	1.36E-04	0.00	0.10	0.13	0.00	chr3R	2127526	2129375					
1631710	at	KaiRIA	-4.26	1.63E-04	0.40	0.40	0.37	0.30	chr3R	16474317	16478172					
1634427	at	CG15461	-4.14	2.78E-09	0.10	0.10	0.08	0.10	chrX	20357207	20357615					
1641455	at	CG6762	-4.03	4.61E-07	2.20	1.50	0.74	0.60	chrX	17751007	17752643					
1623025	at	CG3457	-4.02	2.73E-04	0.10	0.10	0.08	1.80	chrX	2081853	2082743					
1624816	at	CG30283	-3.83	6.70E-07	1.50	1.60	2.47	1.40	chr2R	17427089	17428078					
1633152	at	mod(mdg4)	-3.82	8.89E-05	3.00	2.50	0.89	3.20	chr3R	17177331	17203121					
1624663	a	at	vis	-3.75	2.38E-10	0.10	0.10	0.10	0.10	chr2R	8395396	8398311				
1640957	at	Hexo2	-3.72	6.55E-05	3.90	4.50	1.64	0.60	chrX	8603685	8606513					
1623327	at	CG43103	-3.58	6.15E-07	0.20	0.20	0.01	0.10	chr2R	13026956	13027430					
1626534	at	tko	-3.55	3.37E-12	0.80	1.00	0.98	1.30	chrX	2336346	2338009					
1629430	s	at	regucalcin	-3.46	3.66E-04	1.60	0.40	0.05	0.00	chrX	11906467	11911091				
1632656	at	CG12112	-3.33	7.77E-13	0.30	0.40	0.64	2.10	chrX	8452912	8454225					
1625901	s	at	CG34172	-3.33	1.35E-04	0.00	0.10	0.00	0.00	chr2L	2192525	2197912				
1639476	at	Vago	-3.30	1.96E-07	0.10	0.10	0.07	0.00	chrX	10983323	10984138					
1628425	at	CG31898	-3.29	1.13E-05	0.50	0.60	0.89	3.40	chr2L	8400457	8401477					
1629106	at	CG2233	-3.28	3.06E-05	0.30	0.30	0.01	0.00	chrX	7929774	7931550					
1634191	at	CG6398	-3.22	2.19E-20	0.10	0.10	0.40	1.90	chrX	17724873	17735628					
1623555	at	CG10131	-3.07	9.14E-05	1.20	0.80	0.12	0.10	chr2R	10517418	10518526					
1637315	at	larp	-3.06	5.65E-05	0.80	1.00	0.75	1.30	chr3R	24143884	24162162					
1633329	at	yellow-h	-3.06	1.02E-05	1.20	1.10	0.44	0.10	chr4	248548	251054					
1635125	a	at	LM408	-3.04	2.64E-04	0.80	1.00	0.31	0.10	chr2L	10457656	10462475				
1627324	at	lola	-2.98	2.15E-04	5.30	3.60	15.11	1.20	chr2R	6369712	6430794					
1624599	at	CG34331	-2.98	4.97E-07	0.30	0.20	0.03	0.00	chrX	20016486	20017094					
1639737	at	CG34330	-2.98	2.17E-04	0.00	0.00	0.01	0.00	chrX	18962306	18962925					
1632650	at	CG5867	-2.83	1.67E-05	0.20	0.10	0.37	0.10	chr2L	13236494	13239297					
1637421	at	Cht2	-2.83	1.48E-05	0.50	0.40	1.50	2.00	chr3L	1752328	1755934					
1624836	at	CG13795	-2.73	2.91E-05	0.20	0.40	0.11	0.00	chr2L	7723310	7726298					
1632200	s	at	Edem1	-2.71	4.83E-05	0.70	0.80	1.32	1.30	chrX	1797735	1802860				
1638127	s	at	Dlic	-2.71	1.45E-10	1.80	2.10	1.27	1.70	chrX	11048434	11057245				
1630170	at	Cyp12b2	-2.63	7.81E-07	3.40	5.10	0.71	0.10	chr2R	14642500	14644588					
1625140	at	NtR	-2.63	9.95E-08	1.60	0.90	0.14	0.10	chr2R	18015314	18017689					
1631165	at	GstE14	-2.61	1.99E-05	0.10	0.00	1.05	0.70	chr2R	9127233	9128523					
1632591	at	Phk-3	-2.56	3.08E-04	0.10	0.10	0.20	0.10	chr2R	20858366	20859365					
1637066	at	CG8239	-2.55	6.61E-06	0.40	0.20	0.93	2.60	chrX	15643210	15645255					
1629395	at	CG9743	-2.55	9.13E-06	0.80	1.60	0.77	0.10	chr3R	26022082	26028715					
1633880	s	at	Ir76a	-2.54	2.89E-04	6.60	4.80	1.62	1.50	chr3L	19792378	19796527				
1625023	a	at	nAcRbeta-21C	-2.51	1.69E-04	1.40	0.60	0.14	0.00	chr2L	545129	547096				
1637144	a	at	Map205	-2.49	5.63E-06	1.00	1.10	2.81	2.20	chr3R	27881001	27894163				
1623486	at	CG7900	-2.48	1.07E-04	0.10	0.10	0.39	0.30	chr3R	3919846	3930805					
1638567	at	CG1092	-2.47	1.09E-04	1.30	0.50	0.13	0.10	chr3R	92492	94038					
1639059	s	at	exu	-2.43	2.84E-04	0.00	0.00	0.00	1.70	chr2R	16554924	16558379				
1637135	at	nej	-2.41	7.23E-05	5.10	3.10	2.69	3.50	chrX	9559475	9581099					
1628344	at	CkIIbeta	-2.38	3.43E-04	2.20	1.30	13.79	3.30	chrX	11686566	11695620					
1638481	at	CG2736	-2.38	1.04E-04	0.20	0.20	0.03	0.00	chr2R	20860954	20862894					
1628345	at	Cyp6a9	-2.38	1.59E-04	0.60	0.60	0.25	0.80	chr2R	10766861	10768877					
1623804	a	at	hep	-2.36	5.62E-05	4.10	1.90	1.46	1.90	chrX	12973240	12984466				
1630034	at	CG12119	-2.36	5.10E-05	0.00	0.10	0.06	0.10	chrX	9100153	9104580					
1636112	s	at	CG33521	-2.35	2.97E-04	0.10	0.10	0.06	0.10	chr4	1206394	1213482				
1629857	at	CG10359	-2.33	3.31E-04	0.80	1.90	7.43	0.10	chr3L	3612192	3615244					
1625705	s	at	E(Pc)	-2.31	6.26E-05	0.90	0.90	0.82	1.80	chr2R	7339548	7352775				
1630707	at	edl	-2.26	1.21E-05	0.30	0.90	3.50	0.40	chr2R	14555026	14561037					
1630640	at	CG4594	-2.26	1.02E-05	0.10	0.20	0.38	1.80	chr2L	9920160	9921459					
1629851	at	CG31998	-2.26	1.58E-05	1.10	0.80	1.33	2.50	chr4	217301	226571					
1633254	at	CG7787	-2.23	2.04E-08	0.70	0.60	0.71	1.80	chr2L	8320328	8321285					
1626694	at	CG1674	-2.21	3.27E-04	0.10	0.10	0.07	0.00	chr4	251356	266529					
1636296	at	CG12945	-2.21	3.09E-04	0.60	0.30	0.37	1.70	chr3R	5614574	5616943					

1632744_a_at	if	-2.19	2.27E-05	0.60	0.90	1.12	0.20	chrX	16646222	16677467				
1640845_at	CG10581	-2.17	1.72E-06	1.80	1.90	2.13	0.40	chr3L	21026654	21027303				
1638687_at	CG40298	-2.11	2.16E-05	0.30	0.20	0.26	0.40	chr3L	23719929	23721089				
1631331_a_at	Pxt	-2.09	4.86E-09	0.00	0.00	0.01	1.70	chr3R	13545176	13548677				
1634685_at	RpL3	-2.07	3.49E-05	1.10	0.80	1.11	1.10	chr3R	7047616	7050895				
1641117_a_at	CG17600	-2.07	3.52E-06	2.10	1.50	1.87	1.90	chrX	21917033	21943860				
1636900_at	CG5390	-2.05	2.68E-05	0.80	0.90	1.11	0.10	chr2L	10304067	10306594				
1626908_at	CG8066	-2.03	6.30E-06	1.30	2.50	1.75	0.70	chr3R	10393333	10394569				
1637873_at	scpr-C	-2.03	2.64E-04	0.00	0.00	0.02	0.10	chr3R	7051242	7052204				
1629181_at	CG33494	-2.03	1.36E-06	0.30	0.20	0.24	0.00	chr3R	21319203	21320883				
1638782_at	CG8195	-2.02	5.40E-06	3.20	3.70	1.66	2.20	chr2R	11450201	11453291				
1630129_at	CG43078	-2.02	1.07E-04	1.00	0.40	0.14	0.00	chr3L	8571842	8592717				
1636835_at	CG16700	-2.02	2.48E-04	1.50	1.90	0.85	1.40	chrX	16985887	16992520				
1624851_at	Tango13	-2.02	4.15E-05	1.50	1.00	0.63	1.30	chrX	13457856	13487257				
1640857_at	CG10208	-2.00	1.77E-06	1.10	1.40	0.79	0.70	chr3R	19598720	19599849				
1638809_at	CG34284	8.06	1.55E-04	0.00	0.00	0.01	0.00	chr3R	14728060	14728655				
1633646_at	inaF-B / inaF-D	2.08	3.37E-04	0.40	0.10	0.02	0.00	chrX	11618701	11626803				
1636409_at	CG11034	2.19	2.50E-07	0.10	0.10	0.06	0.40	chr2L	5805395	5808858				
1638198_at	CG12768	2.36	4.69E-06	13.80	13.50	20.52	0.40	chr3L	22903591	22916632				
1639106_at	Grip	2.41	6.98E-05	0.70	1.40	1.28	0.70	chrX	5860314	5880729				
1628009_at	fas	2.55	5.09E-05	35.90	41.50	41.39	0.10	chr2R	9510593	9636872				
1625265_at	CG9119	2.59	1.39E-04	0.20	0.30	0.09	0.10	chr3L	1203316	1204795				
1623173_at	CG10013	2.93	1.76E-07	0.30	0.50	0.34	0.40	chr3R	8149243	8150932				
1637957_s_at	Rbp9	3.08	1.92E-18	1.00	0.60	0.06	0.10	chr2L	2954762	2968434				
1627961_a_at	CG18507	3.11	2.79E-04	0.10	0.10	0.13	0.20	chr2L	13665525	13672967				
1627436_s_at	Pdp1	3.34	1.58E-04	7.70	8.20	1.01	0.20	chr3L	7805015	7860472				
1628083_at	CG5036	3.43	7.91E-10	8.50	7.50	4.48	1.30	chr2R	13680387	13685633				
1640097_at	Pxn	3.49	2.89E-04	8.10	6.70	0.62	0.40	chr3L	2601455	2630072				
1629205_at	CG32032	4.09	6.41E-06	9.80	10.80	9.82	0.10	chr3L	9086153	9087956				
1637294_at	nwk	4.50	1.81E-06	21.60	19.00	5.17	0.10	chr3L	9130960	9138793				
1629269_at	CG32204	4.70	1.50E-04	42.90	14.90	18.01	0.10	chr3L	18915519	18939138				
1626457_s_at	SKIP	5.27	2.22E-06	15.20	9.80	22.24	1.00	chr3R	17959552	18121848				
1641339_at	CG10137	5.36	1.59E-07	15.20	12.70	5.11	0.40	chr2L	19526686	19532992				
1639110_at	Slc45-1	5.97	3.14E-06	2.90	4.10	2.29	0.30	chr3L	9196165	9201953				
1640914_at	CG8838	6.40	8.65E-09	0.00	0.00	0.01	0.00	chr2L	3357646	3358618				
1638381_s_at	CG1695 / CG32506	7.78	3.04E-08	18.00	7.80	4.14	0.10	chrX	20304977	20315623				
1627297_at	CG15270	7.90	8.39E-06	22.30	21.00	8.98	0.00	chr2L	15074776	15096915				
1626262_s_at	CG3104	8.02	1.19E-14	20.60	11.50	3.91	1.40	chr2L	2848989	2855778				
1624577_at	Ace	8.56	9.14E-07	7.00	15.10	6.13	0.10	chr3R	9048673	9085239				
1640729_s_at	nrv3	20.93	6.37E-10	9.40	11.30	4.77	0.00	chr2L	21380000	21393319				
1625951_at	CG17778	21.59	1.93E-13	18.40	9.60	3.71	0.10	chrX	388933	390402				
1634016_at	CG2781	22.52	9.84E-05	0.00	0.00	0.05	0.30	chr3R	3807539	3821574				
1640774_a_at	Mob2	40.93	2.99E-17	8.70	10.20	3.39	0.00	chr3L	11515857	11555333				
1638016_at	CG2993	41.30	1.44E-20	20.00	11.00	4.54	0.80	chr3R	3630387	3635736				
1624833_a_at	mAcR-60C	47.66	6.00E-19	40.40	24.70	13.46	0.10	chr2R	20266159	20277235				
1634855_s_at	CG9813	52.38	1.66E-18	14.40	13.90	1.52	0.00	chr3R	9198826	9205497				
1633582_at	Ih	2.03	4.56E-06	12.80	14.10	3.56	0.40	chr2R	10163809	10187349				
1640976_at	Ykt6	2.08	3.58E-04	2.50	3.00	3.30	1.30	chrX	7814442	7815999				
1637853_a_at	Hk	6.74	6.45E-13	28.30	34.10	2.55	0.20	chrX	10128791	10157518				
1641390_at	dpr19	6.95	5.70E-14	28.70	26.30	16.21	0.10	chr2L	10376168	10379907				
1625911_at	CG15760	7.55	2.23E-06	10.40	6.50	2.75	0.20	chrX	13563732	13570265				
1635398_at	Scp2	15.21	1.39E-05	7.40	15.80	0.73	0.00	chr3R	12400265	12409385				
1639743_s_at	Syn2	23.07	5.56E-17	14.70	18.10	3.24	0.10	chr2R	12439765	12445991				
1626200_s_at	tipE	79.25	3.20E-19	15.00	22.00	8.54	0.20	chr3L	4188499	4193788				
1624098_s_at	CG18675 / tipE	110.7	1.29E-21	3.40	4.10	0.85	0.00	chr3L	4173884	4192968				
1631038_at	CG32773	3.39	1.48E-06	0.20	0.30	0.38	2.40	chrX	4281829	4505418				
1638280_at	CG43102	2.79	7.52E-05	8.40	2.70	2.22	0.30	chr3R	13594157	13620296				

1625065_s_at	SK	3.16	1.71E-04	20.60	19.90	6.76	0.10	chrX	5234269	5298760				
1628666_at	CG3328	3.68	5.50E-09	5.40	6.80	1.85	0.10	chr2R	20050013	20061139				
1637820_at	X11Lbeta	3.91	2.86E-05	27.70	21.30	16.40	0.20	chrX	10521770	10604900				
1630285_at	RhoGAP100F	4.75	5.13E-05	51.90	36.10	42.99	0.20	chr3R	27638331	27670966				
1631417_s_at	CG6282	5.38	1.06E-07	20.80	17.80	5.02	0.10	chr3L	8597757	8610174				
1631327_at	mspo	6.52	9.62E-10	21.90	14.60	10.31	0.10	chr2R	10559458	10601127				
1624143_a_at	CG12071	7.22	9.56E-07	27.20	17.20	18.65	0.10	chr3R	26702806	26706597				
1626537_at	CG17321	12.40	8.17E-15	25.20	16.40	12.33	0.10	chr2L	18845184	18855118				
1639237_at	CG32814	13.59	1.27E-15	0.60	2.00	0.86	1.90	chrX	913828	920733				
1628147_at	neuroligin	15.87	4.20E-15	37.40	27.00	15.91	0.20	chr2L	6874900	6906658				
1637913_at	CG11638	17.19	3.28E-11	20.90	29.20	11.63	0.30	chrX	908017	920736				
1629228_at	Hs3st-A	18.96	1.47E-14	21.50	15.10	3.93	0.00	chr2R	14605634	14642026				
1637150_at	CG13928	55.98	3.88E-20	21.40	24.40	20.50	0.00	chr3L	1625791	1628377				
1639837_at	Rph	171.4	5.11E-18	28.00	13.90	16.82	0.20	chrX	10643108	10651699				
1635353_at	CG6329	2.36	1.88E-04	15.30	20.80	10.20	0.00	chr2R	9713880	9720419				
1625249_at	fend	2.59	3.25E-04	6.80	7.70	2.78	2.80	chrX	9016006	9029073				
1632313_at	CG1998	2.61	5.43E-07	7.80	9.20	2.70	0.20	chrX	13303402	13307107				
1626675_at	XRCC1	2.78	4.40E-05	0.60	0.50	1.41	2.40	chrX	5223944	5226468				
1638225_a_at	inx7	2.85	3.16E-05	0.20	0.10	0.04	0.20	chrX	6885645	6903902				
1636846_at	lap	3.11	1.08E-08	25.60	26.30	12.59	0.10	chr3R	3012716	3026663				
1634794_at	CG10924	3.22	1.74E-05	0.00	0.00	0.03	0.00	chr2R	14414937	14423263				
1625840_at	CG12001	4.44	1.73E-05	1.50	1.70	0.75	1.10	chr3R	480522	483707				
1627835_a_at	Cda5	4.80	3.05E-05	4.10	4.00	1.92	0.30	chr2L	25402	65404				
1635196_at	CG6836	4.89	1.51E-07	3.00	1.00	0.06	0.30	chr3L	18943729	18948506				
1641146_at	st	5.10	1.71E-13	0.10	0.00	0.07	0.00	chr3L	16490751	16493562				
1623166_at	CG6071	6.64	3.19E-07	0.20	0.30	0.32	0.10	chr3L	11636006	11639133				
1625621_s_at	f	23.72	6.08E-15	0.60	0.70	1.56	0.80	chrX	17126975	17174997				
1641460_at	CanB	100.8	6.77E-22	19.70	20.50	12.30	0.00	chrX	5226532	5228482				
1638334_at	CG13168	3.75	9.08E-07	0.20	0.10	0.06	0.00	chr2R	8141497	8143197				
1637105_at	rgr	2.72	1.45E-04	2.50	1.20	4.31	0.90	chr2R	4487938	4498743				
1629667_at	CG14298	3.52	3.90E-05	0.20	0.00	0.26	0.10	chr3R	14701250	14702926				
1634134_at	sff	4.61	8.28E-09	37.00	40.00	30.06	0.30	chr3L	15893256	15917961				
1634093_at	CG32017	39.26	5.03E-15	20.20	19.80	10.81	0.00	chr4	1158456	1167155				
1638654_at	CG14645	2.00	2.14E-04	0.00	0.00	0.00	0.00	chr3R	160820	161228				
1634742_at	Sodh-2	2.05	5.66E-05	1.80	2.90	0.65	1.10	chr3R	6702106	6703979				
1637610_at	Chrac-16	2.06	5.92E-05	0.30	0.20	2.04	2.30	chrX	11911307	11911989				
1634736_at	hts	2.07	4.10E-13	0.00	0.00	0.01	2.70	chr2R	15284537	15312454				
1634113_at	gk	2.07	2.36E-05	0.10	0.10	0.08	0.20	chr3L	18132502	18138518				
1625652_s_at	CG12428	2.11	4.94E-10	0.40	0.40	0.49	1.00	chr3R	23529101	23532468				
1637517_s_at	KrT95D	2.13	2.18E-05	1.10	1.00	0.48	0.90	chr3R	19824162	19857532				
1625083_at	rhi	2.13	8.24E-06	0.00	0.00	0.05	2.70	chr2R	13515994	13521018				
1634145_s_at	CG14968	2.15	2.30E-04	0.20	0.20	0.52	0.10	chr3L	3299336	3303112				
1623566_at	CG13957	2.19	1.25E-06	1.20	1.20	1.87	1.70	chrX	16207742	16211783				
1624995_at	CG12355	2.23	2.40E-09	5.70	9.80	1.40	0.20	chr3L	15486064	15488649				
1634630_at	CG5707	2.28	1.23E-05	0.20	0.20	0.16	0.90	chr3L	2189160	2192221				
1627302_at	Fmo-2	2.37	1.71E-05	0.10	0.20	0.07	0.20	chr2R	2529923	2532603				
1630038_at	pyd3	2.41	2.35E-12	1.50	2.20	0.97	0.50	chr3R	3573635	3575798				
1625945_a_at	CG1233	2.41	1.81E-04	1.00	0.70	0.96	1.80	chr3L	361472	365681				
1640344_at	CG18480	2.42	3.43E-04	0.00	0.10	0.07	0.10	chr2L	15630084	15632618				
1635807_at	CG17739	2.42	2.35E-05	2.20	4.20	1.18	0.00	chr2R	8194029	8197917				
1638810_at	Faa	2.45	3.88E-04	0.30	0.50	0.35	0.10	chr3L	4072032	4073954				
1633946_at	CG31955	2.48	3.82E-04	0.50	0.50	0.67	0.70	chr2L	3712154	3712866				
1630244_s_at	CG31809	2.51	2.56E-04	0.10	0.60	0.13	0.00	chr2L	16849018	16858714				
1624435_at	Tsp42Ej	2.52	3.47E-04	6.40	8.50	1.36	0.00	chr2R	2927641	2930267				
1639313_at	CG17650	2.53	8.30E-06	0.20	0.20	0.18	0.20	chr2L	1758286	1759093				
1638199_s_at	RhoGAPp190	2.58	6.96E-07	15.70	12.40	14.46	0.50	chrX	17533180	17546595				
1634019_at	CG2064	2.67	7.98E-09	0.60	0.90	0.41	0.10	chr2R	3553333	3554848				

1626384	at	CG12182	2.69	2.86E-06	2.20	1.90	3.51	1.80	chr3L	2596967	2599579				
1624381	at	CG17658	2.74	3.27E-06	0.00	0.10	0.21	3.00	chr2R	19782601	19784520				
1640884	at	CG15784	2.75	7.85E-05	0.10	0.10	0.21	0.10	chrX	5324713	5326494				
1640489	at	CG18522	2.82	8.14E-05	0.30	0.60	0.27	0.10	chr3R	11357859	11363014				
1632087	at	CG9928	2.86	9.65E-05	0.10	0.10	0.00	0.00	chr2L	13142494	13142903				
1623963	at	CG32115	2.91	2.08E-04	0.40	0.30	1.03	0.00	chr3L	12905127	12906746				
1637821	at	CG32023	2.92	1.67E-04	0.00	0.10	0.05	0.10	chr3L	8796702	8797282				
1627854	at	CG9914	2.96	1.71E-08	0.10	0.10	0.27	0.20	chrX	16200945	16202426				
1635020	s at	phr6-4	3.07	9.51E-05	0.20	0.40	0.94	1.20	chr2L	20643351	20645791				
1634351	at	CG7860	3.07	1.86E-06	0.50	0.30	1.43	0.60	chrX	15463026	15464474				
1639712	at	CG17261	3.19	2.95E-07	0.00	0.00	0.03	0.00	chr2L	3042551	3043593				
1623903	at	CG18473	3.20	6.45E-05	0.10	0.10	0.20	0.00	chr3R	5189027	5190560				
1637476	at	CG15579	3.22	1.47E-05	0.40	0.40	0.47	0.10	chrX	4205397	4205832				
1634195	at	CORL	3.24	1.86E-05	5.40	3.20	7.80	1.90	chr4	982967	999048				
1630147	at	Hex-C	3.28	3.07E-04	0.10	0.30	0.01	0.00	chr2R	11106342	11107917				
1635376	at	CG9086	3.33	2.78E-07	1.70	1.00	0.86	2.40	chrX	16850978	16862682				
1623281	s at	His2B	3.38	1.01E-05	0.10	0.10	0.44	2.30	n/a						
1633949	at	CG15152	3.47	5.89E-06	0.00	0.00	0.03	0.00	chr2L	18114547	18115220				
1627188	at	phm	3.49	3.20E-04	0.10	0.20	2.53	0.70	chrX	18579802	18582257				
1629418	s at	sei	3.53	1.67E-07	9.90	10.20	3.14	1.30	chr2R	19936728	19940560				
1640488	a at	CG9510	3.57	2.24E-10	0.10	0.10	0.05	0.00	chr2L	8939780	8943264				
1628701	a at	CG14798	3.58	3.71E-05	1.50	2.00	1.56	1.50	chrX	1586857	1587876				
1635370	at	Tsp42El	3.59	1.44E-08	0.20	0.40	0.90	0.10	chr2R	2933274	2936043				
1623126	at	CG13912	3.60	9.35E-09	0.10	0.20	0.02	0.20	chr3L	1189590	1190472				
1631072	at	CG9512	3.64	3.79E-05	0.80	0.20	0.06	0.00	chrX	14795985	14799574				
1628690	at	CG1503	3.74	1.66E-06	0.20	0.30	0.23	0.80	chrX	20975890	20977394				
1632100	s at	sina	3.82	4.29E-09	3.10	3.60	5.12	1.00	chr3L	16846851	16851812				
1634959	at	TfIIIA-S-2	3.90	2.55E-09	0.20	0.30	0.39	0.20	chrX	905134	905676				
1630696	at	ebd2	3.93	1.02E-07	0.90	0.30	1.08	2.20	chr3L	21337868	21342388				
1624862	at	CG43117	3.98	2.54E-04	2.30	2.90	4.34	0.70	chr3R	22280516	22280834				
1625046	at	Rbm13	3.99	5.79E-07	0.30	0.30	1.08	2.10	chrX	8795724	8797497				
1623609	at	CG15172	4.17	1.17E-07	0.00	0.00	0.03	0.00	chr2L	19022417	19023646				
1632775	at	CG42254	4.20	4.97E-06	1.30	0.90	0.82	1.10	chr2R	10657104	10658804				
1629434	at	CG9380	4.29	1.94E-04	0.00	0.00	0.02	0.00	chr2R	21071151	21142841				
1627096	s at	CG16778	4.35	7.80E-05	22.20	13.80	32.35	0.40	chr2R	20973303	209867111				
1627862	at	dao	4.38	1.09E-07	8.20	11.40	3.14	1.60	chr2L	15248879	15254715				
1628696	at	CG12643	4.47	8.14E-07	0.40	0.50	12.22	0.20	chrX	10158266	10159296				
1628611	at	CG11241	4.48	1.16E-04	1.20	0.70	0.32	1.00	chr3L	22726252	22731520				
1629853	at	CG3699	4.49	5.39E-08	0.00	0.10	0.00	0.00	chrX	840687	841517				
1627613	at	Mtk	4.65	6.02E-05	0.30	0.10	0.01	0.00	chr2R	11296351	11296618				
1637146	at	CG42694	4.70	3.97E-10	0.50	0.60	0.69	1.70	chr2R	18829772	18832670				
1630600	at	Fst / Scm	5.04	5.44E-05	0.00	0.10	0.04	0.00	chr3R	5459081	5471872				
1630829	at	PIP5K5B	5.20	5.23E-11	31.90	27.20	11.49	0.30	chr2R	18762294	18768959				
1633599	a at	Pepck	5.32	1.82E-09	0.00	0.10	0.04	0.00	chr2R	14424272	14426924				
1637275	a at	CG42807	5.37	1.21E-04	0.10	0.00	0.01	0.00	chr2R	9459029	9460045				
1630334	at	CG15406	5.42	4.67E-05	0.00	0.00	0.02	0.00	chr2L	3309222	3311682				
1637282	at	CG6171	5.93	1.36E-04	1.30	1.40	1.04	2.10	chr3R	11188518	11189579				
1635321	at	CG14688	6.18	1.21E-06	1.10	1.80	0.20	0.80	chr3R	6503459	6506647				
1633401	s at	Cyp12d1	6.28	2.71E-08	0.10	0.10	0.01	1.10	chr2R	7011163	7013152				
1632677	a at	GM130	6.28	2.95E-04	1.20	1.50	0.90	1.70	chr2R	18012072	18015002				
1625644	at	CG2641	6.50	4.86E-05	0.60	1.50	1.18	1.40	chr3R	3619931	3622321				
1629473	at	edi	6.93	2.74E-11	4.80	4.40	6.52	0.10	chr3R	14870604	14920076				
1636431	at	CG8768	7.23	3.83E-04	2.40	3.00	0.51	0.30	chr2R	8578813	8580193				
1641722	at	Reg-2	8.03	5.18E-10	0.10	0.10	0.03	0.20	chr3L	605165	606569				
1634064	at	CG13311	8.13	6.33E-05	0.00	0.00	0.03	0.00	chr3L	8794640	8795264				
1634514	at	CG33258	8.42	2.84E-04	0.00	0.00	0.01	0.00	chr3L	16075651	16076738				
1627741	at	CG13086	10.09	5.30E-14	0.10	0.00	0.01	0.00	chr2L	19364375	19365470				

1624377	s at	be	10.15	3.53E-13	3.20	2.80	7.49	0.70	chrX	14918467	14936093			
1639118	a at	Hrb87F	11.58	1.85E-04	0.80	0.90	3.18	2.10	chr3R	9482568	9486253			
1624505	at	Lip4	11.66	4.22E-05	0.20	0.20	1.00	0.30	chr2L	10527572	10531777			
1630085	s at	Peritrophin-A	12.21	3.92E-16	0.20	0.30	0.96	0.70	chrX	20115727	20119277			
1623491	at	CG15155	12.72	2.92E-05	0.00	0.00	0.02	0.00	chr2L	18154864	18155942			
1637224	at	CG13905	13.12	1.01E-06	0.00	0.20	0.32	0.10	chr3L	902898	903810			
1640881	at	CG16762	15.67	7.02E-06	0.00	0.00	0.01	0.00	chr3L	2474667	2475614			
1633959	s at	CG30345	17.43	3.26E-10	0.60	0.30	0.17	0.00	chr2R	5036190	5039397			
1626098	at	CG11453	17.72	1.51E-09	0.10	0.10	0.14	0.20	chr3R	15609244	15611241			
1629125	at	gp210	18.75	2.67E-14	1.30	1.70	19.34	1.10	chr2R	1648367	1661829			
1637691	at	CG4570	19.71	2.42E-04	0.00	0.00	1.17	2.30	chr3R	6682529	6683788			
1635306	at	CG4650	24.84	2.08E-08	1.10	1.00	2.00	0.80	chr2L	14773812	14774981			
1628052	at	Cyp6a17	25.61	1.13E-14	2.10	1.40	0.14	0.10	chr2R	10761459	10763063			
1627736	at	Actbeta	26.91	1.46E-18	13.80	17.50	11.40	0.10	chr4	1097957	1105422			
1627551	s at	AttA / AttB	27.02	3.80E-08	0.20	0.00	0.02	0.50	chr2R	10636728	10637670			
1625436	at	Uro	27.90	3.97E-06	0.00	0.00	0.03	0.00	chr2L	7780085	7781415			
1625124	at	AttA	39.24	4.01E-09	0.50	0.20	0.08	0.60	chr2R	10634876	10635698			
1625246	at	Her	52.93	9.94E-11	0.10	0.10	0.03	1.20	chrX	18098436	18099177			
1623477	at	CG42857	73.28	4.41E-15	0.10	0.10	0.02	0.50	chr3R	5646236	5646913			

Table S4. Tissue-specific expression of Su(Hw) target genes using anatomical expression data (FlyAtlas)

	FlyAtlas		Su(Hw) target genes					
	(n=12856)		Repressed (n=75)			Activated (n=30)		
	#	%	#	%	P	#	%	P
CNS	3654	28	56	75	5.3E-19	12	40	0.159
Ovary	2070	16	4	5	0.011	3	10	0.363
Ovary & CNS	703	5	2	3	0.289	0	0	0.187
Testis	2778	22	6	8	0.004	3	10	0.124
Hindgut	1890	15	14	19	0.332	12	40	4.8E-05
Tubule	1788	14	10	13	0.889	5	17	0.660
Salivary gland	2853	22	11	15	0.119	8	27	0.555
Fat body	1925	15	9	12	0.472	2	7	0.204
Midgut	1946	15	6	8	0.085	5	17	0.810