

Supplementary materials

Supplementary figure 1

Insulator activity assay using the ct^6 mutation show that flies with genotype $su(Hw)^{v/e04061}$ lack insulator activity. The ct^6 mutation results from an insertion of the gypsy retrotransposon between the wing margin enhancer of the cut gene and the cut gene

promoter, showing a jagged shape with cuts in the wing edge (GAUSE et al. 2001). Here, y^2ct^6 ; $su(Hw)/TM6$ flies show jagged wings, yet the y^2ct^6 ; $su(Hw)^{v/e04061}$ does not, indicating lack of functional insulator activity.

Supplementary figure 2

Degenerated egg chambers during mid-oogenesis in $su(Hw)$ mutants. Nuclear lamin (green) is disrupted in nurse cells from stage 9 egg chambers in $su(Hw)$ mutants (D-F) but not in wildtype (A -C). Arrows point at stage 9 egg chambers. Nucleus from oocytes stage 6 (S6) and stage 9 (S9) were cropped for better resolution. Nuclei from wildtype oocytes are shown in B and C and from mutant oocytes in E and F. Scale bars are 50 μm in A and D, and 5 μm in B, C, E and F.

Supplementary figure 3

$Su(Hw)::GFP$ expression in ovaries driven by various GAL4 drivers. Egg chambers were stained using GFP antibody (in green) and Phalloidin conjugated with Texas Red to stain F-actin (in red). $nanosGal4>Su(Hw)::eGFP$; $su(Hw)^{v/e 04061}$ (A); $tjGal4>Su(Hw)::eGFP$; $su(Hw)^{v/e 04061}$ (B); $metaGal4>Su(Hw)::eGFP$; $su(Hw)^{v/e 04061}$ (C); and $nosGal4>Su(Hw)::eGFP$; $su(Hw)^{v/e 04061}$ (D). Scale bar is equivalent to 50 μm .

Supplementary figure 4

Dumping occurs normally in egg chambers from $su(Hw)$ mutant ovaries if $Su(Hw)$ expression is driven by Gal4 drivers. $nosGal4$ rescued ovaries were stained for F-actin

using Phalloidin conjugated with Texas Red at stage 10B (A). F actin fibers connecting the outer cell with the nucleus in a zoom-in image shown in B. Details are shown in C and D. Scale bar is 50 μm in A and 10 μm in B.

Supplementary figure 5

Outer diameters of ring canals are significantly different between wildtype and su(Hw) mutants at stage 8 egg chambers. The outer diameter of rings was measured in egg chambers from both genotypes and the growth curve of rings shows the average of ring outer diameter at each stage. Single asterisks indicate $P < 0.05$ and two asterisks indicate $P < 0.001$.

Supplementary figure 6

Heatmap of genes grouped based on different terms. The array data from different su(Hw) mutants were analyzed using the Gene set enrichment analysis (GSEA) and flies were clustered based on genes grouped in structural constituent of chorion (A), multi-cellular organism development (B) and eggshell chorion assembly (C). Each sample has three replicates.

Supplementary materials and methods

Gene Set Enrichment Analysis (GSEA). GSEA (SUBRAMANIAN et al. 2005) was used to identify sets of related genes which expression levels were significantly influenced by the su(Hw) mutation. Gene ontology (GO) information (http://flybase.org/static_pages/downloads/FB2014_03/ontologies/gene_ontology.obo.gz) and GO term associations (http://flybase.org/static_pages/downloads/FB2013_06/go/gene_association.fb.gz) were downloaded from FlyBase and used to assemble a gene sets file. Affymetrix IDs were ranked according to their absolute mean fold-change between the su(Hw) and wildtype groups. The gene sets file and the ranked probe list were used as inputs for running GSEA with default settings. GSEA determines how significantly each gene set is skewed toward the top of a ranked list, thus avoiding the task of arbitrarily defining a significance threshold before performing GO enrichment analysis. Eleven gene sets were identified as significant, based on the software developers' "rule-of-thumb" of having an enrichment p-value less than 0.05 and an FDR-adjusted q-value less than 0.25 (<http://www.broadinstitute.org/gsea/doc/GSEAUUserGuideFrame.html>). Public microarray data from Geyer's group were downloaded from GEO (<http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE36528>) and processed together with our data as described above. Expression data for each of the eleven gene sets identified from GSEA in our data were used to generate heatmaps with hierarchical clustering based on the same genes in the combined data, using the heatmap.2 function in the R gplots package.