SUPPLEMENTAL INFORMATION

METHODS

Whole genome expression analysis.

Homozygous *Gcn5*^{E333st} and *Ada2a*^{A189} animals follow a slowed-down course of development (Carre et al., 2005; Pankotai et al., 2005). They show an extended third larval instar, then incompletely evert spiracles and form abnormal puparium which fails to shorten. RNA samples were isolated in triplicate from homozygous and heterozygous *Gcn5*^{E333st} and *Ada2a*^{A189} at the end of the third larval instar, as defined by spiracle evertion, and were labeled and hybridized to Affymetrix Drosophila genome arrays. A detailed analysis of these experiments will be published elsewhere (T. Pankotai, et al., manuscript in preparation). Extraction, normalization and computation of the expression indexes were performed using the RMA function of Bioconductor's affy package (Gautier et al., 2004; Gentleman et al., 2004). The statistical significance of transcriptional variations was assessed using SAM software with a fold change (FC) threshold of 3 identical to the threshold used in (Badenhorst et al., 2005) and a false detection rate (FDR) lower than 1% (Tusher et al., 2001).

To compare our data with microarray data from (Badenhorst et al., 2005), we used the correspondence table between probe sets on each array provided by Affymetrix. On this basis, among the probesets downregulated in *Nurf301* mutants, we obtained the number of probesets downregulated in *dGcn5* and *dAda2a* mutants and compared them to the numbers expected from the distribution of the *dGcn5* positive probesets on the complete array. We computed the *p-value* for the null hypothesis of no association between the two distributions, with a binomial distribution.

Quantitative RT-PCR.

Random-primed reverse transcription was performed at 42°C using the IScript cDNA synthesis kit (Bio-Rad) and 1μg of total DNase-treated RNA from third instar larvae (RNeasy mini Kit, Qiagen). Control reactions lacking the RT enzyme were systematically checked for the absence of product. Quantitative real-time PCR measurements were performed in triplicate in an Opticon II thermocycler (Bio-Rad), using the Fast start SYBR Green Master Mix (Roche). Primer sets for genes of interest were as follows: *Hsp70A*: ATCGCCAGCGAATAACCTC and CCTGCTTCACATTGAAGACGTA, *Ada2a*: CGTGAACAAAACGCGTCA and AGGACGGTGGTCTGCTGATA, *Ada2b*: GCGAAAACTTCGATTTGTGTC and CCCCTTTCCACGAAATACAC, *Gcn5*: AGTTCATAGCTGTAATTCGCAGTCA and CAAGTCAAGCCGGGTCTTACTT, *Ubx*: ACTGGCTAGGTACAAATGG and GTAGCGGGTGTATGTCTGTC, *engrailed*: TAAGCGGGAGTTCAACGAG and CGTCGACTTCTTGATCTTGG, *Rp49*: CGGATCGATATGCTAAGCTGT and GCGCTTGTTCGATCCGTA. *Rp49* transcripts levels were used to normalize between samples using the standard curve method.

Immunohistochemistry and western bloating.

Immunostainings of imaginal discs dissected from third instar larvae were performed as described (Dequier et al., 2001). Mousse anti-engrailed 4D9 from Developmental Studies Hybridoma Bank at the University of Iowa (http://www.uiowa.edu/~dshbwww/index.html) was used at 1:500 dilution. Secondary antibody was Cy3-conjugated anti-mousse (1:200) from Jackson Laboratories. Slides were mounted in Vectashield (VECTOR Laboratories) and imaging was carried out using a confocal microscope Zeiss AXIOVERT 200M, Zeiss LSM510 + FCS.

Late larval instar salivary gland extracts were prepared from 20 hand-dissected pairs of salivary glands lysed in 30 μl of cracking buffer (0.125 M Tris ph 6.8, 5% β-mercaptoethanol,

2% SDS, 4M Urea). For western blot analysis, 5 µl of extract were separated of 18% acrylamide PAGE and probed as described (Carre et al., 2005) using anti-H4-AcK12 (Upstate) or anti-H4 antibodies (Abcam) at 1:1000 dilution. Secondary HRP-conjugated anti-rabbit antibody (Vector Laboratory) was used at 1:5000 dilution and detected using the SuperSignal® west Pico Chemiluminescent Substrate (PIERCE).

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LEGENDS TO SUPPLEMENTARY FIGURES

Supplementary Figure S1. Gcn5 regulates the expression of *engrailed*, *Ultrabithorax* and *hsp70* genes.

(A) Confocal analysis of Engrailed protein expression in wing imaginal discs from wild type and $Gcn5^{E333st}/Gcn5^{E333st}$ late third instar larvae. **(B)** Quantitative RT-PCR analysis of *Ubx* and *engrailed* transcript abundance in total RNA from $Gcn5^{E333st}$ homozygous late third instar mutant larvae. Transcript levels were normalized to rp49 and are expressed as fold-changes relative to levels in heterozygous control animals. Bars represent the standard deviation of three independent determinations. **(C)** Quantitative RT-PCR analysis of *hsp70Ab* induction in $Gcn5^{f02830}$ and $Gcn5^{E333st}$ late third instar larvae. Bars represent the standard deviation of three independent determinations.

Supplementary Figure S2. (A) *Gcn5* and *Ada2a* mutation do not impair Iswi binding to chromosomes. Polytene chromosomes from wild type (wt), $Gcn5^{E333st}$, $Ada2a^{A189}$ and $Iswi^{2}$ homozygous mutant were co-stained with and anti-Iswi and anti-PolII antibody. **(B) *Iswi* mutation impairs Ada2a binding to chromosomes.** Polytene chromosomes from wild type (wt) and $Iswi^{2}$ (Iswi) homozygous mutant were co-stained with anti Ada2a (red) and anti-PolII (green) antibodies and DAPI (blue) as indicated.

Supplementary Figure S3. *Iswi* mutation does not affect *Gcn5*, *Ada2a* and *Ada2b* expression levels.

Quantitative RT-PCR analysis of Gcn5, Ada2a and Ada2b transcript abundance in total RNA from $Iswi^{2}$ late third instar mutant larvae. Transcript levels were normalized to rp49 and

are expressed as fold-changes relative to levels in wild type control animals. Bars represent the standard deviation of three independent determinations.

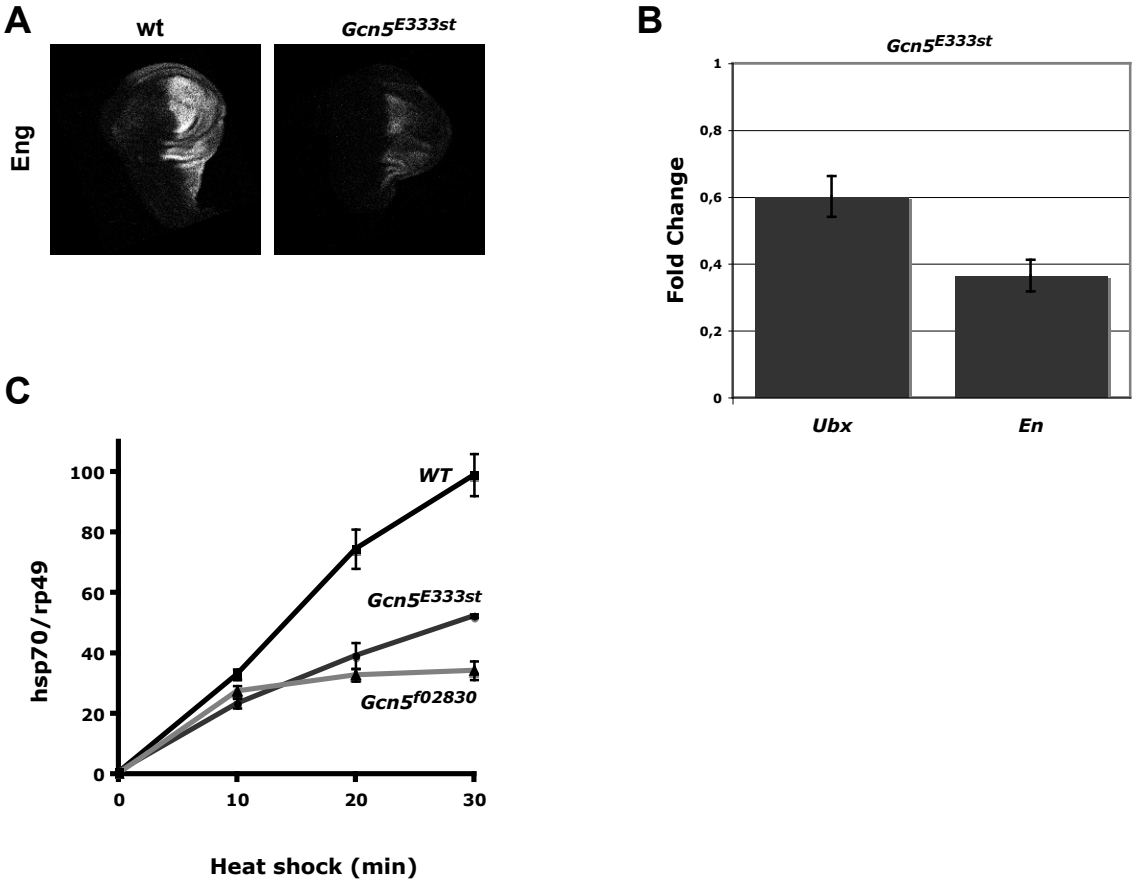
Supplementary Figure S4. Chromosome H4-AcK12 acetylation is impaired by mutations in components of the Iswi-containing NURF remodeling complex.

(A) Polytene chromosomes from wild type (wt) and *Iswi*^{2} homozygous mutant were co-stained with anti H4-AcK12 (red) and anti-PolII (green) antibodies as indicated. **(B)** Magnification of the tip of male X polytene chromosomes from wild type, homozygous Iswi^{2} and Nurf301$^{1/3}$ mutants co-stained with DAPI (blue) and H4-AcK12 (red) antibodies.

Supplementary Figure S5. *Iswi* and *Nurf301* mutations reduce global H4-AcK12 level in salivary glands.

Western blots of salivary gland protein extracts from wild type, *Gcn5*^{E333st}, Iswi^{2}, *Nurf-301*^{3}, *Ada2b*$^{\Delta 842}$ and *Ada2a*$^{\Delta 189}$ late third instar larvae were probed with antibodies against H4-AcK12 and H4 histone (loading control) as indicated.

Carré_Sup_Fig. S1



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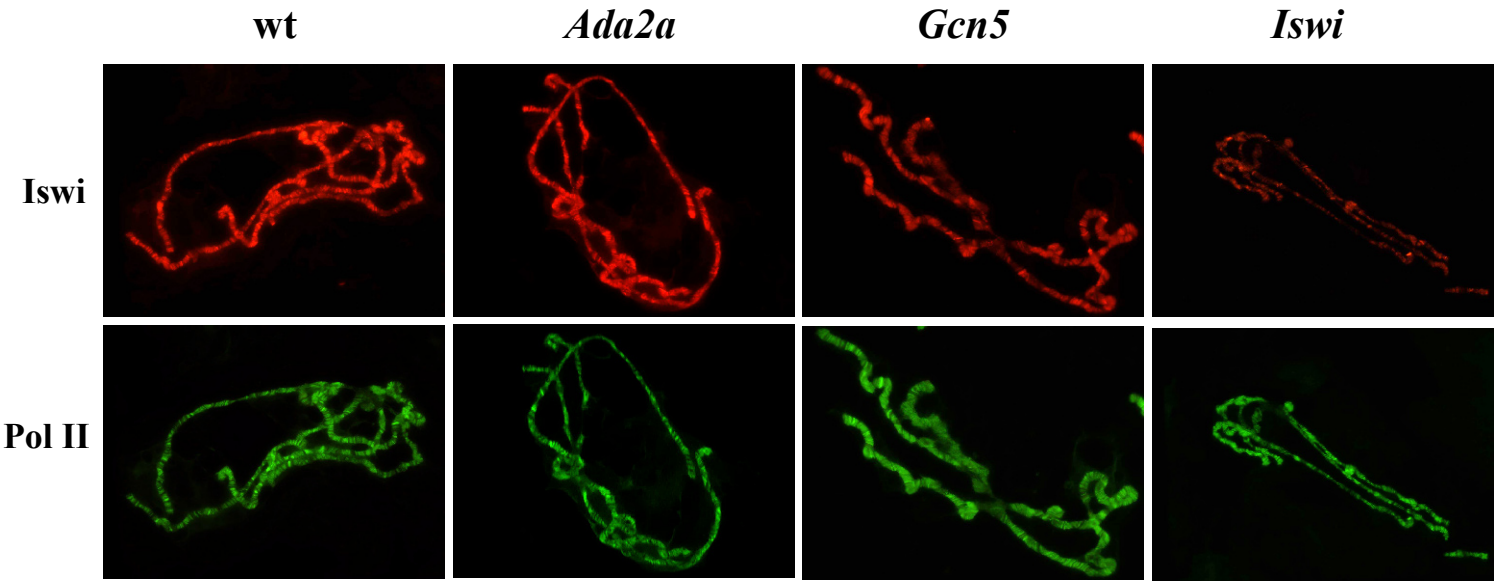

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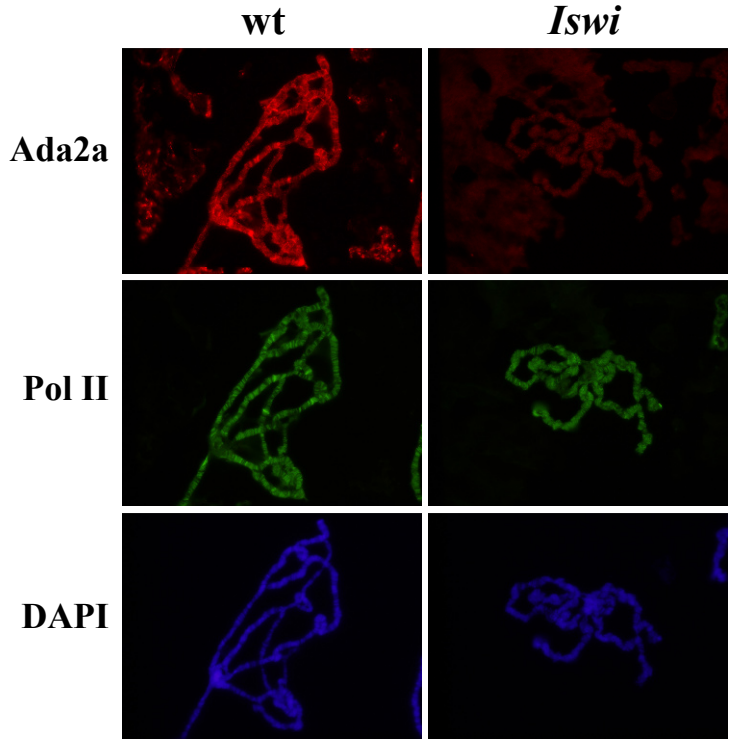

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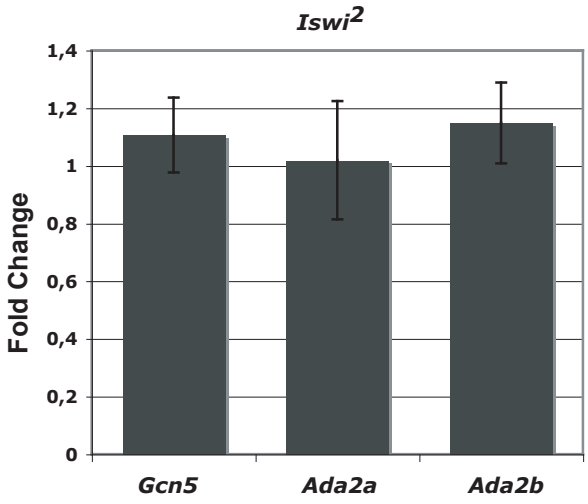

Carré_Sup_Fig. S4

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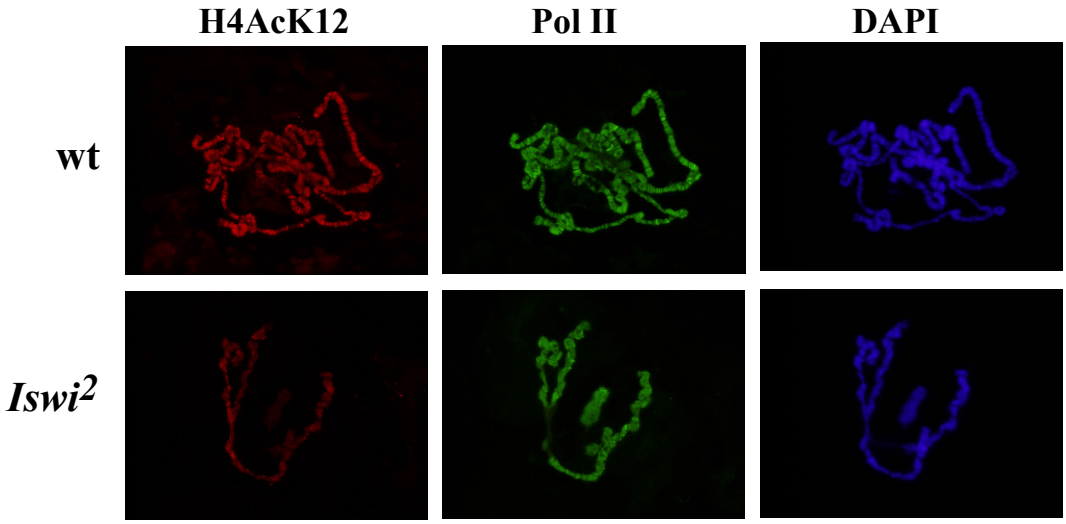

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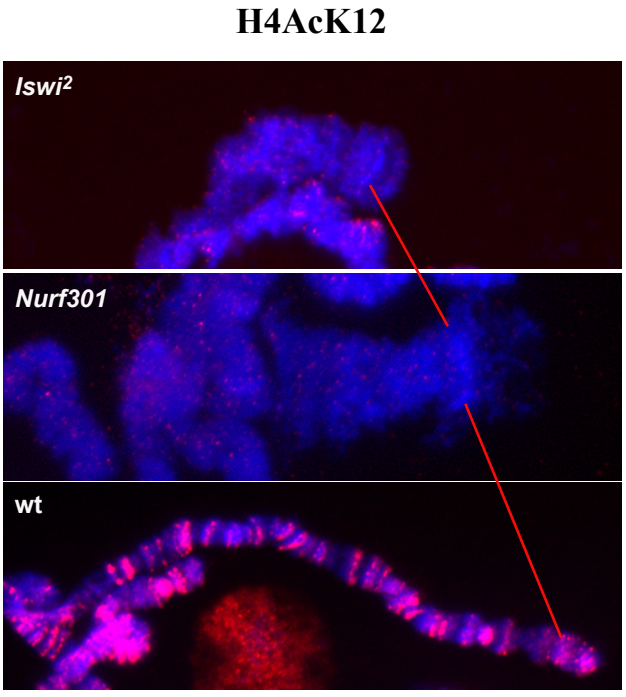

Carré_Sup_Fig. S5