Supporting Information

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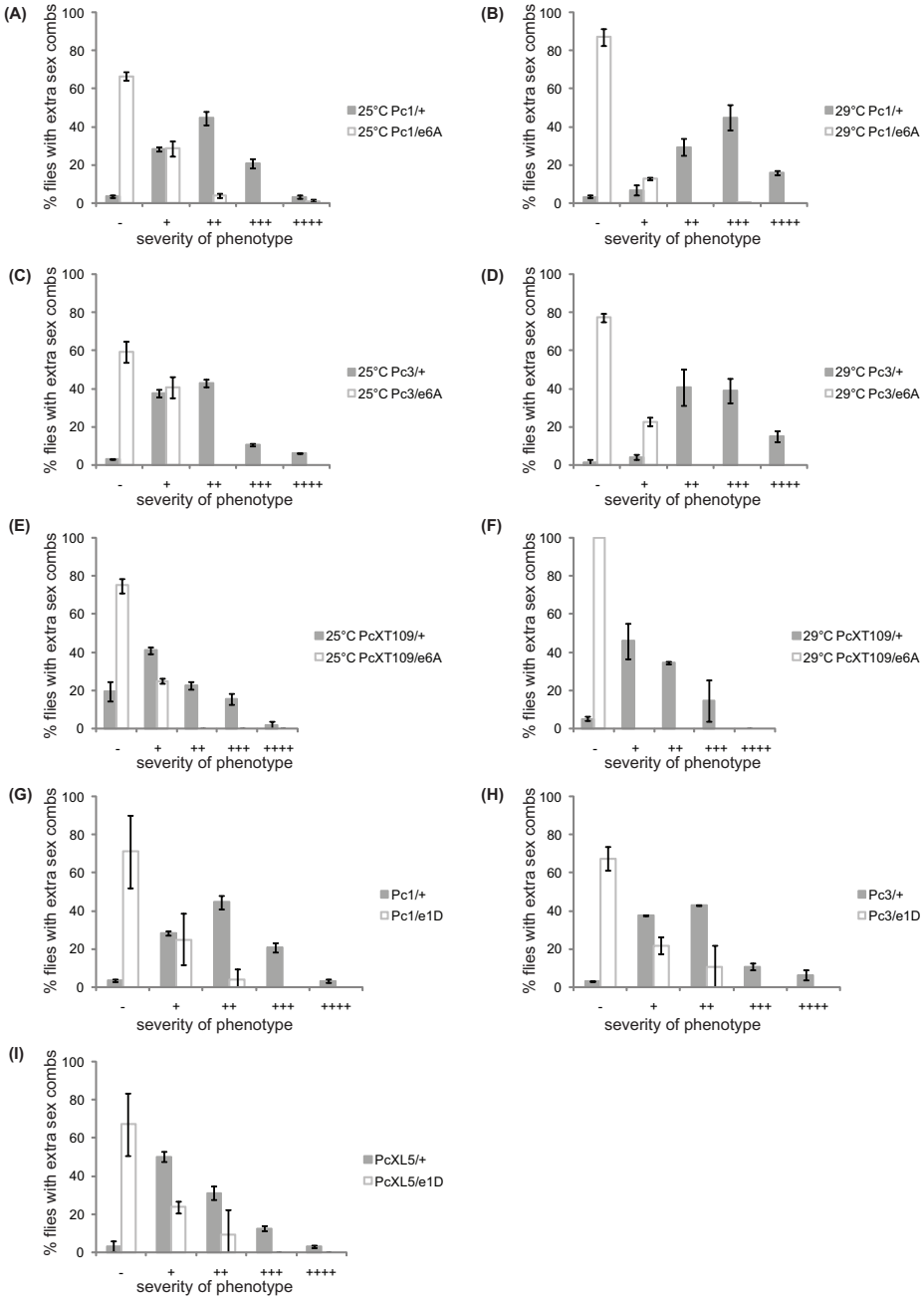


Fig. S1. *Hsp90* mutations strongly suppress the extra sex comb phenotype in *Pc* (*Polycomb*) mutants. (*A–F*) *Hsp90* allele *e6A* crossed to *Pc* alleles *Pc1* (*A* and *B*), *Pc3* (*C* and *D*), and *PcXT109* (*E* and *F*) at 25°C (*A*, *C*, and *E*) and 29°C (*B*, *D*, and *F*). The *Pc* alleles crossed to wild-type flies, *Pc*/+, represent the control cross and strong extra sex combs are seen at 29°C (*B*, *D*, and *F*) as compared with 25°C (*A*, *C*, and *E*). The *Hsp90* allele *e6A* strongly suppressed to none or few extra sex combs in all of the *Pc* alleles at 25°C and 29°C (*A–F*). (*G–I*) *Hsp90* allele *e1D* crossed with *Pc1*, *Pc3*, and *PcXL5* at 25°C. As compared with control *Pc*/+, *Hsp90* mutations show strong suppression of sex comb phenotype. More than 200 male flies were analyzed for each cross and mean values of 2 independent experiments are presented. Error bars represent the standard deviation and where error bars are absent shows small standard deviation values. On the basis of strength of phenotype (number of hairs on second and third legs), male flies were categorized as follows: −, no extra sex combs; +, 1–2 hairs on second leg; ++, 3 or more hairs on second leg; +++, 3 or more hairs on second leg and 1–2 hairs on third leg; and ++++, strong sex combs on both the second and the third pairs of legs.

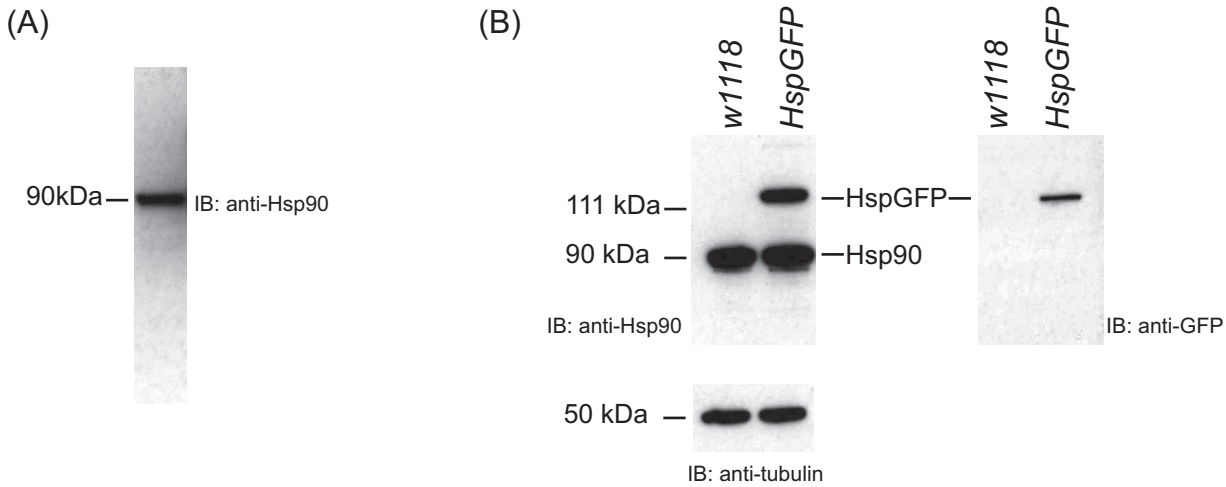


Fig. S2. Western blot analysis of Hsp90GFP-expressing transgenic flies. (*A*) Anti-Hsp90 antibody generated against a peptide detected a single band of the expected size (90 kDa) in wild-type flies. (*B*) Total protein extracts from Hsp90GFP transgenic flies probed with anti-Hsp90 and anti-GFP antibodies show an expected size of fusion protein HspGFP, which is absent in w1118 control flies. Compared with wild-type Hsp90 protein, Hsp90GFP is less abundant. Anti-tubulin was used as a loading control.

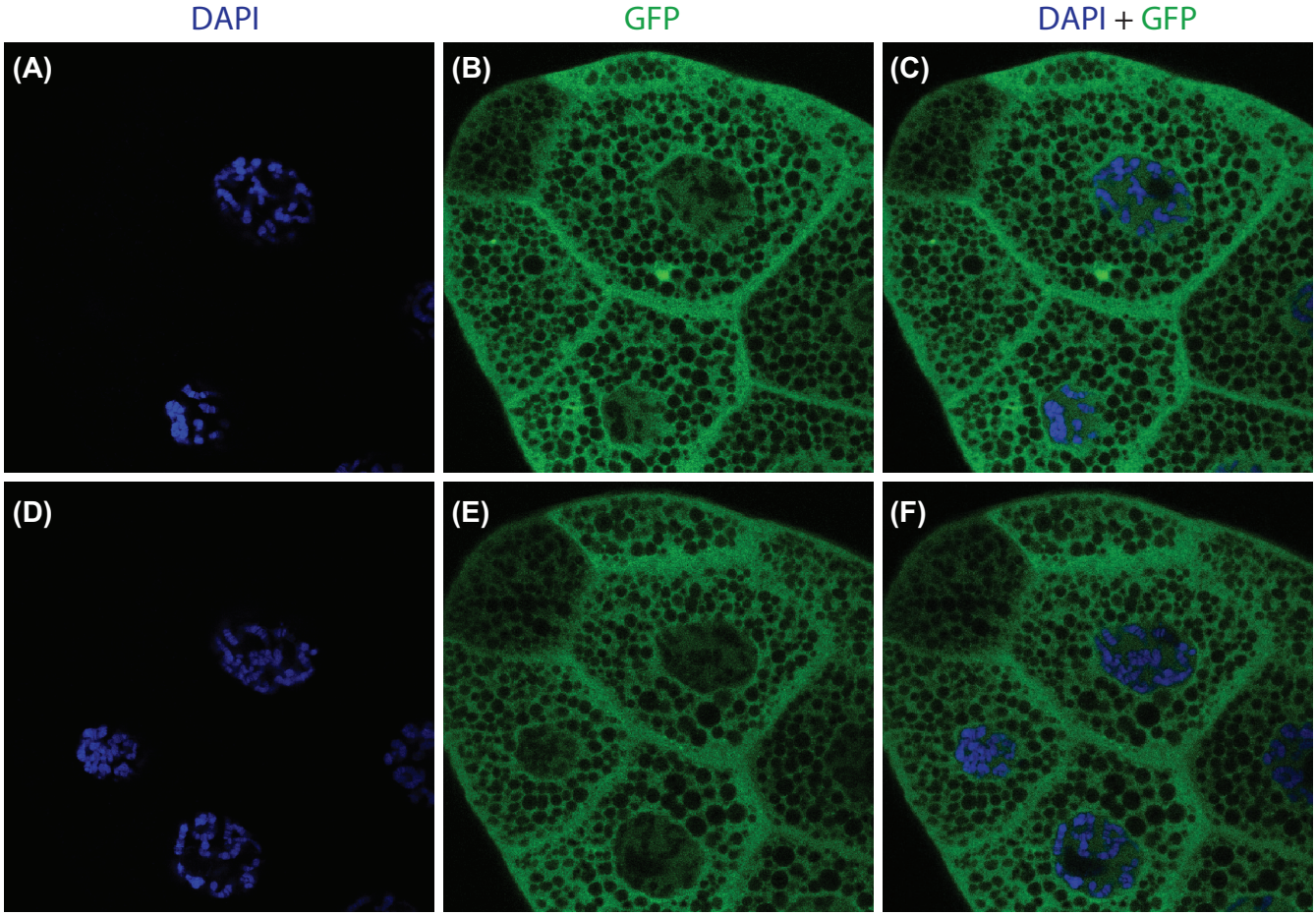


Fig. S3. Subcellular localization of Hsp90GFP, visualized by confocal microscope, in unfixed salivary glands containing polytene chromosomes. (*A*–*F*) Two z-frames of the same salivary gland. The HspGFP is predominantly visible in the cytoplasm around granular structures (*B*, *C*, *E*, *F*) but Hsp90 is also uniformly distributed in the nucleus around chromatin, which is visible as blue DAPI (*A*, *C*, *D*, *F*) regions.

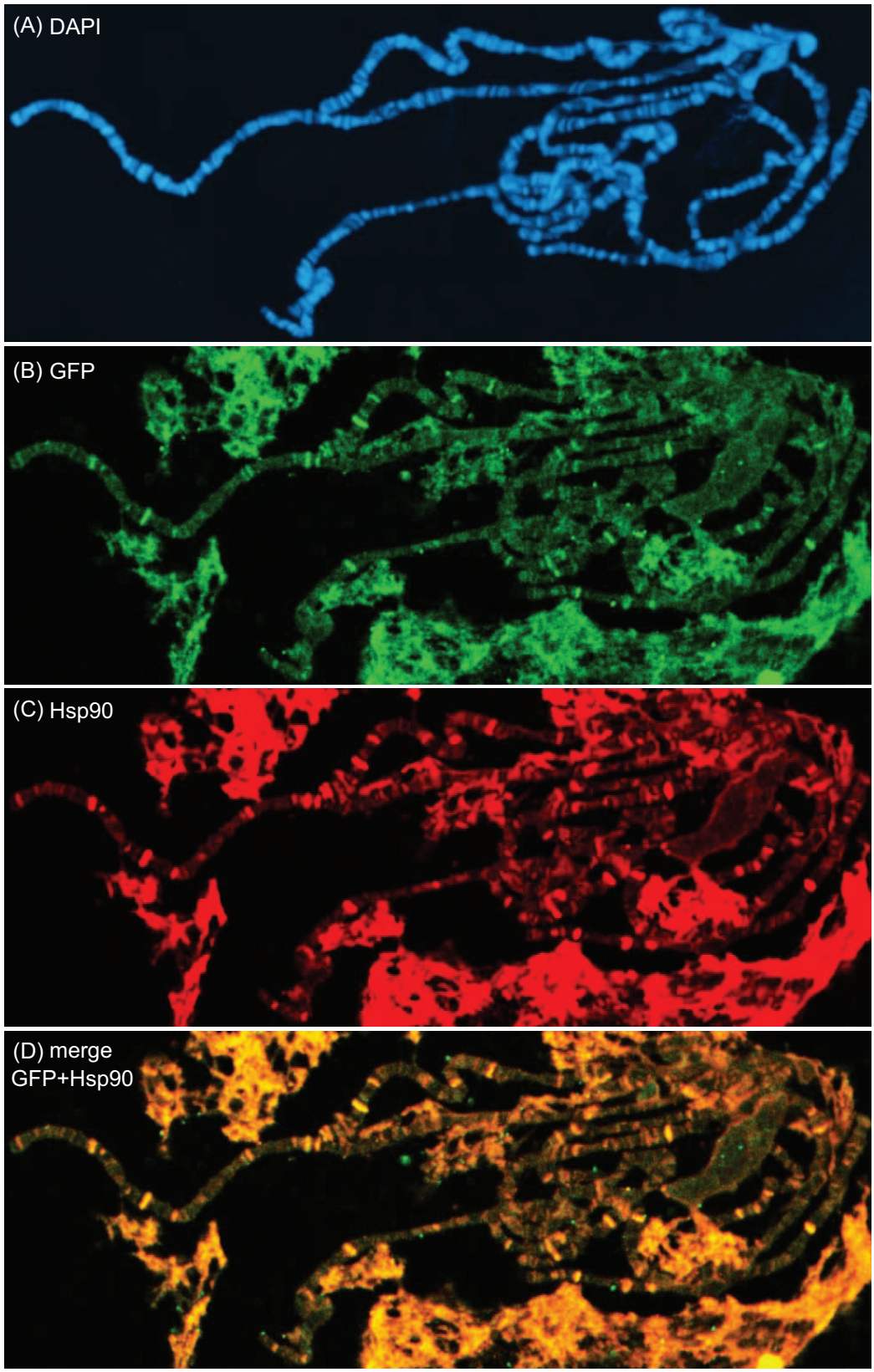
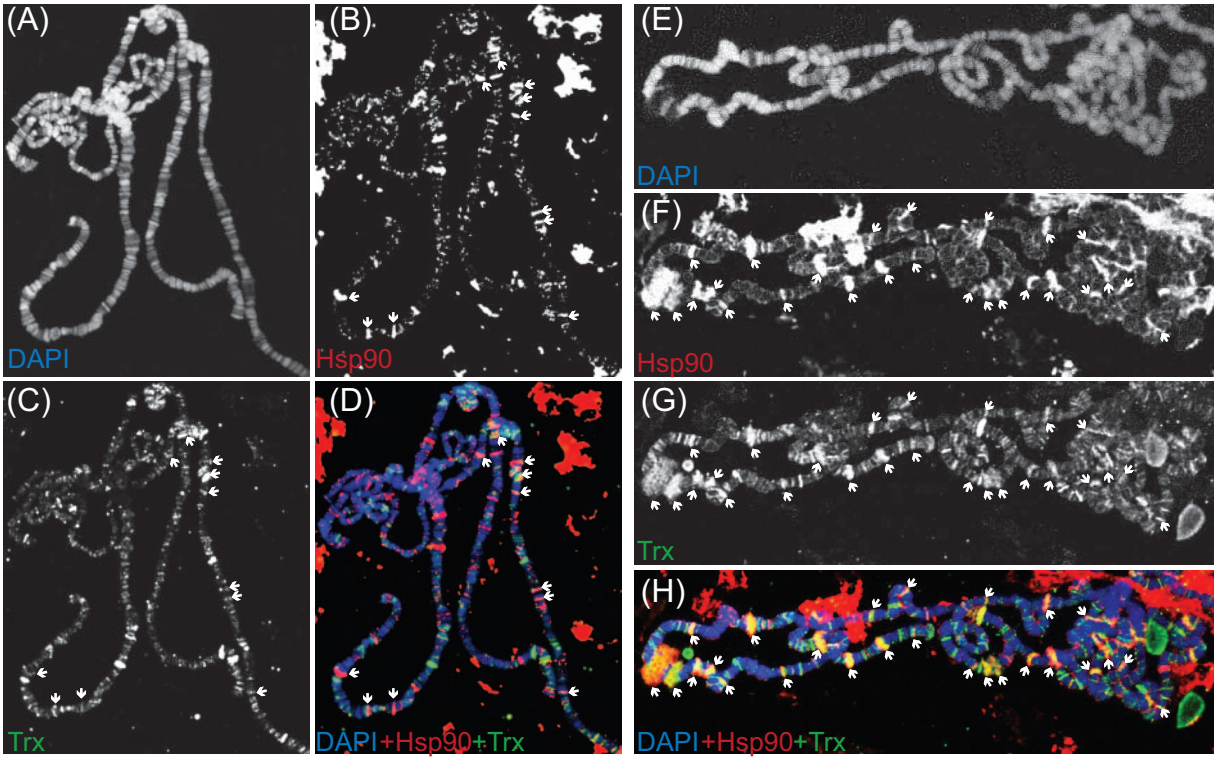


Fig. S4. Hsp90 binds to chromatin in polytene chromosomes. (*A–D*) Immunostaining of polytene chromosomes from HspGFP containing third instar larvae with anti-GFP (*B*) and anti-Hsp90 (*C*) antibodies showed clear overlap (*D*), indicating specific Hsp90 association in polytene chromosomes.



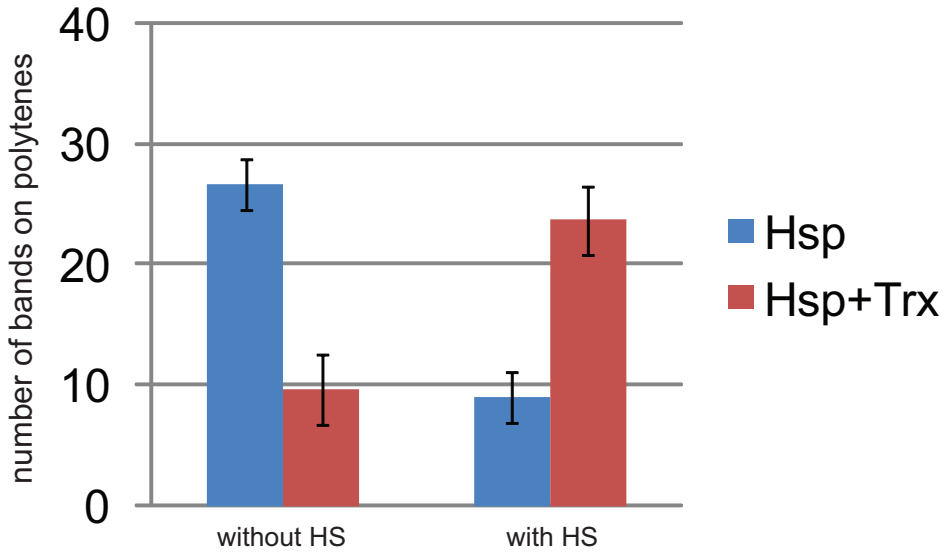


Fig. S5. Hsp90 and Trx colocalize at polytene chromosomes. Polytene chromosomes without heat shock (*A–D*) and with heat shock (*E–H*) were stained with anti-Hsp (*B* and *F*) and anti-Trx (*C* and *G*) antibodies and overlap between Hsp90 and Trx is indicated with arrows (*D* and *H*). Shown below *A–H* is a column chart showing overlap between Hsp90 and Trx (Hsp+Trx) increased after heat shock as compared with non-heat-shocked polytene chromosomes. The data represent the mean of 3 different polytene chromosomes prepared without heat shock as well as with heat shock. Hsp, nonoverlapping Hsp90 bands; Hsp+Trx, Hsp90 bands overlapping with Trx.

Table S1. Summary of Hsp90 and Pc alleles used

Mutant allele	Nature of mutation	Coordinates of mutation	Mutagen	Genotype
$Hsp83^{e1D}$	Point mutation	S38L	EMS	w*;$Hsp83^{e1D}$/TM6B, Tb
$Hsp83^{e6A}$	Point mutation	S592F	EMS	w*;$Hsp83^{e6A}$/TM6B, Tb
$Hsp83^{j5C2}$	P-element activity	—	P element	w*;P{lacW}$Hsp83^{j5C2}$/TM2
Pc^{1}	Deletion + frameshift	Δ2047–2062	X-ray	Pc^{1}/TM3, Ser
Pc^{3}	Null allele	No protein detected	EMS	Pc^{3}/TM3, Ser, Sb
Pc^{XL5}	6-bp deletion	Δ1363–1368	X-ray	Pc^{XL5}/TM3,Ser, Sb
Pc^{XT109}	2-kb deletion	No protein detected	X-ray	w*;Pc^{XT109},b, pr,cu,sbd[2]/TM3, Ser

In *Drosophila*, the gene encoding the Hsp90 protein is named *Hsp83*.