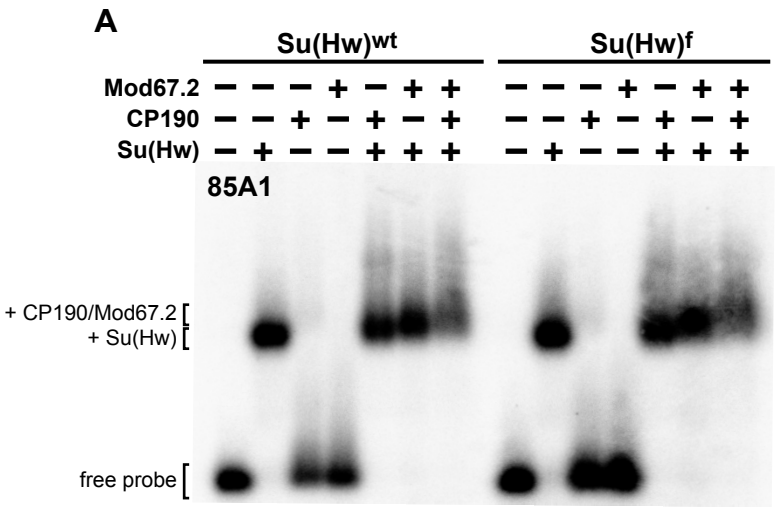
Supplemental Figure 1. Loss of ZnF10 does not affect interaction between Su(Hw) and the *gypsy* insulator proteins Mod67.2 or CP190. A. Electrophoretic mobility shift analyses (EMSA) of a representative SBS with bacterially expressed $Su(Hw)^{+}$ or $Su(Hw)^{f}$, CP190 and Mod67.2. Both $Su(Hw)^{+}$ and $Su(Hw)^{f}$ retard migration of the DNA probe and produce an equivalent supershift upon addition of CP190 or CP190 and Mod67.2 **B.** Bacterially expressed and purified carboxy-terminal HIS-tagged $Su(Hw)^{+}$, $Su(Hw)^{f}$, CP190 and Mod67.2 were resolved on a 7.5% SDS-PAGE and detected by silver staining. Of note, His-tagged CP190 is unstable when produced in bacteria, such that only reduced levels of full length protein (arrowhead) can be obtained. Based on our SDS-PAGE analyses, we estimated that the amount of full length CP190 used in the EMSA studies corresponded to that used for $Su(Hw)^{+}$ and $Su(Hw)^{f}$. Molecular weight markers are shown to the left.

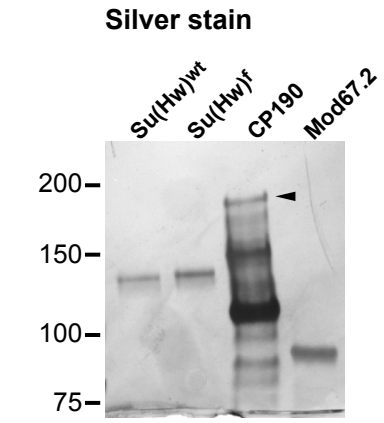
Supplemental Figure 2. Loss of ZnF10 decreases the DNA-binding capacity of $Su(Hw)^{f}$ *in vitro*. A. EMSA of six f-retained and seven f-lost sites. ^{32}P-labeled fragments were incubated with no (-) or increasing amounts of bacterially purified $Su(Hw)^{+}$ (top row) or $Su(Hw)^{f}$ (bottom row) protein. Each lane contained a three-fold increase in protein, beginning at 0.003 μg. The *hsp26* fragment contains no Su(Hw) binding motif and serves as a negative control. **B.** Analysis of recombinant Su(Hw) protein. Bacterially expressed $Su(Hw)^{+}$ (wt) and $Su(Hw)^{f}$ (f) were resolved on an 8% SDS-PAGE and detected by silver staining (left) or western blot with Su(Hw) antibodies (right). Molecular weight markers are shown to the left.

Supplemental Figure 3. Mapping $Su(Hw)^{f}$-lost and $Su(Hw)^{f}$-retained SBSs to chromatin domains defined in Drosophila cell lines. Shown are bar graphs mapping SBSs to chromatin

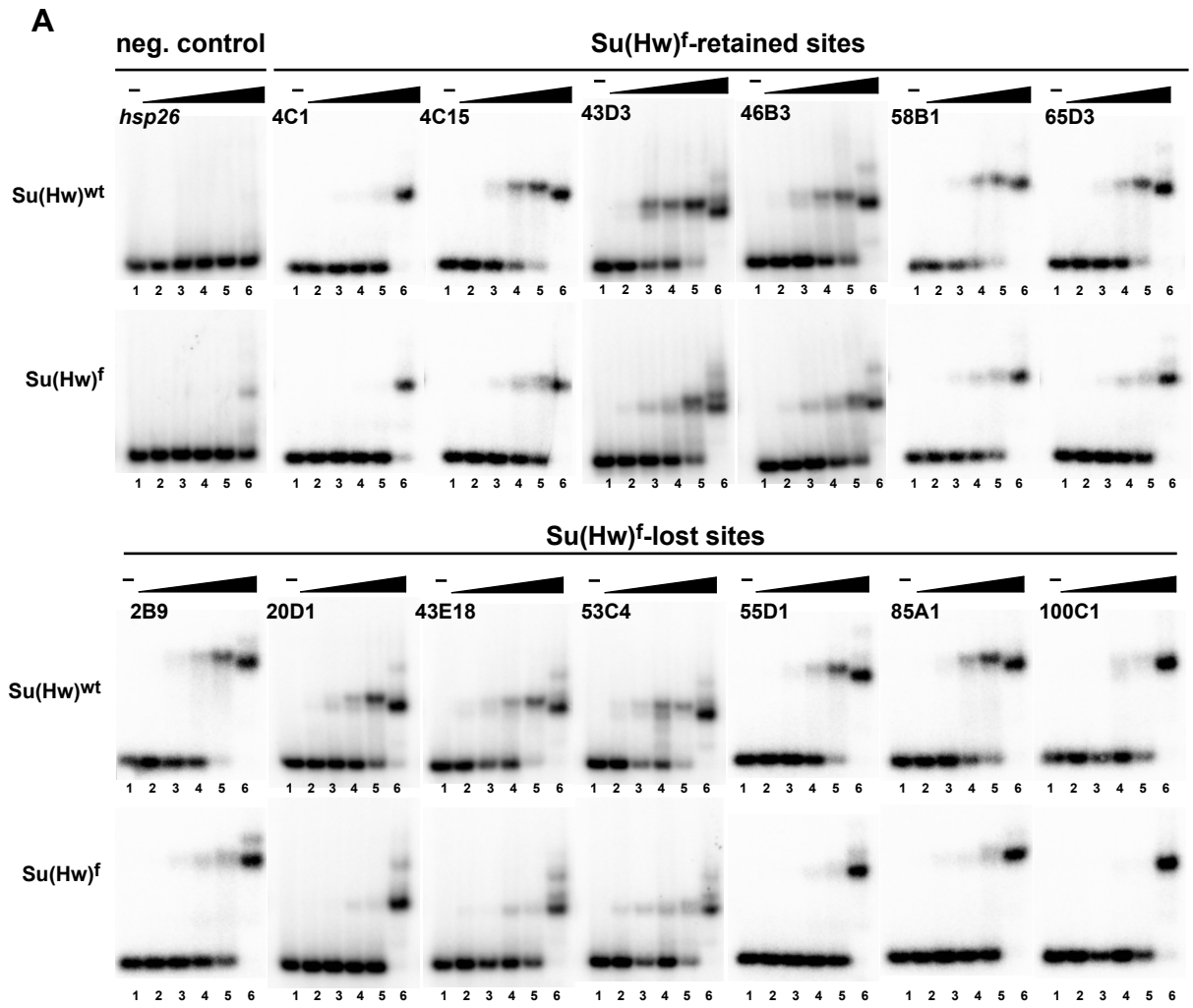
domains defined in the S2 (left) and BG3 (right) cell lines (Kharchenko et al. 2011). These analyses revealed no significant differences between f-retained and f-lost SBSs.

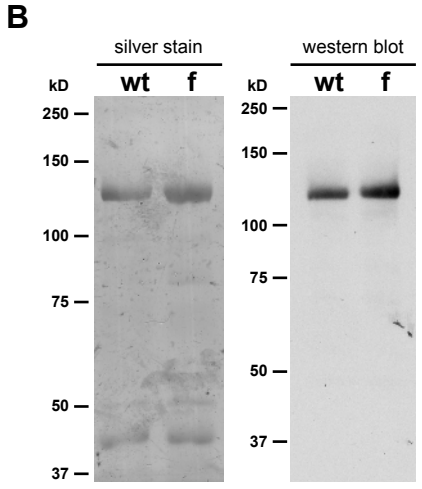
SUPPLEMENTAL FIGURE 1



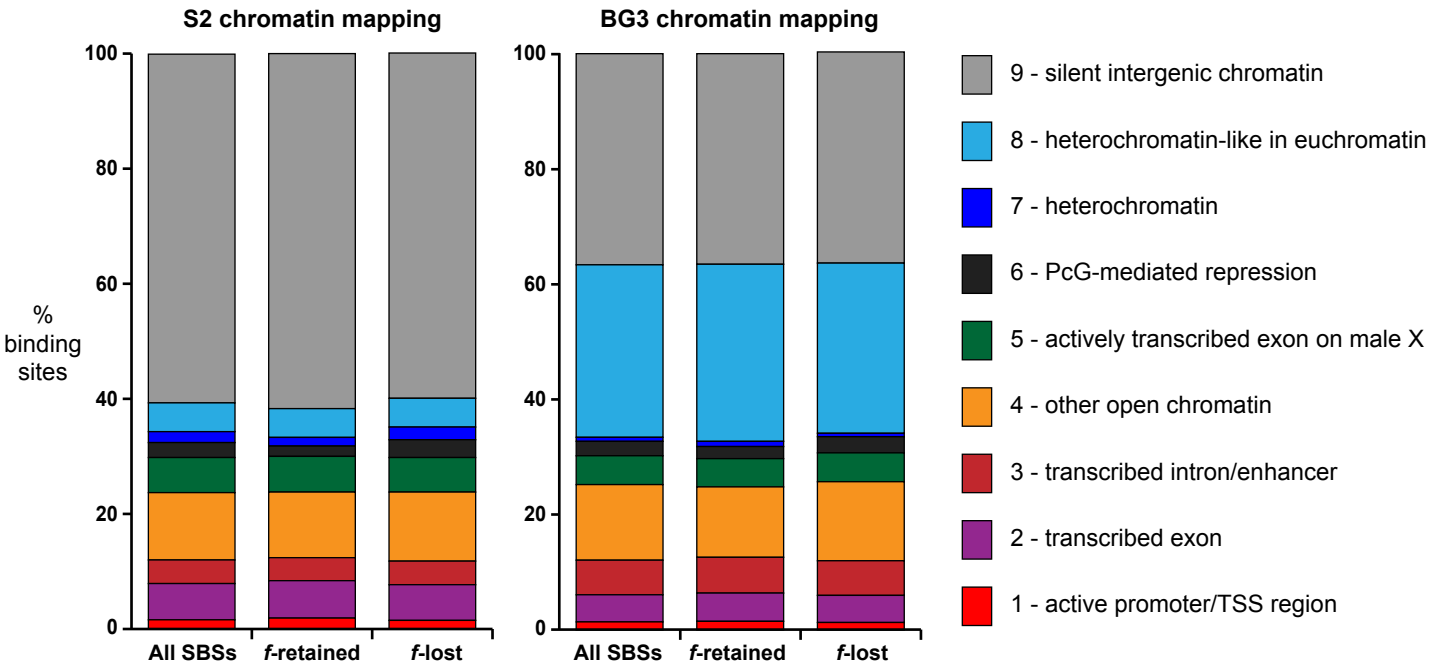


SUPPLEMENTAL FIGURE 2





SUPPLEMENTAL FIGURE 3



Supplemental Table 1

Dataset	Total tags	Unique aligned	Multiple aligned	Unaligned	% mappable tags	% genome coverage
***su(Hw)$^{f/v}$* IP**	21369901	20685020	390223	294658	96.8	7.22
***su(Hw)$^{f/v}$* input**	21247086	20596448	426521	224117	96.9	7.18
***su(Hw)$^{f/v}$* IgG**	20803937	20130453	470581	202903	96.8	7.03
***su(Hw)^{WT}* IP**	21541101	20988838	323940	228323	97.4	7.28
***su(Hw)^{WT}* input**	14573806	13993613	336326	243867	96	4.92
***su(Hw)^{WT}* IgG**	12835674	12173587	415600	246487	94.8	4.34

Supplemental Table 2. Overlap between ovary and non-ovary SBSs

Dataset	Accession number / Reference	# of sites	Median site length (bp)	# of sites (%) overlap
Geyer lab ovary: *su(Hw)^{WT}*	GSE33052	2932	293	
Geyer lab ovary: *su(Hw)^{f}*	GSE33052	1210	249	
Corces lab KC cells	Bushey et al., 2009	3747	340	2503 (78.8)
Corces lab Mbn2 cells	Bushey et al., 2009	3481	340	2100 (66.1)
White lab 0-12 hr embryos	modENCODE_27	4779	903	2700 (85.0)
White lab 0-12 hr embryos PG ab*	modENCODE_901	3947	1620	2957 (93.1)
Pirrotta lab S2 cells	modENCODE_330	4173	2519	2887 (90.9)
Pirrotta lab BG3 cells	modENCODE_951	4459	2490	2900 (91.3)
Pirrotta lab S2 cells VC ab**	modENCODE_331	4094	2501	2874 (90.5)

* Rabbit α-Su(Hw) antibody from the Geyer lab

** α-Su(Hw) antibody from the Corces lab