Supplemental Figures.

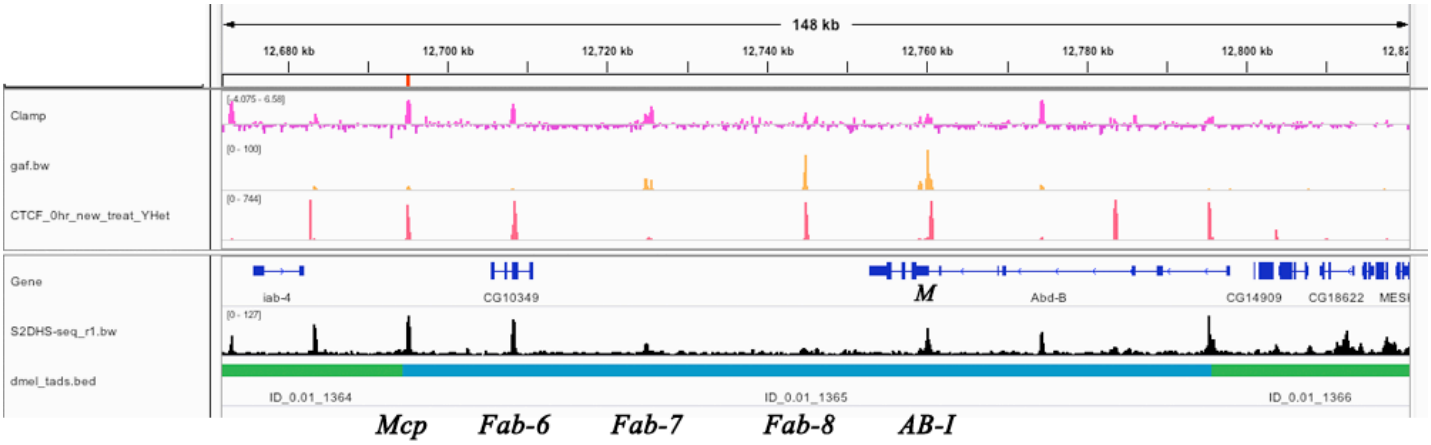

Figure S1: Map of the *Abd-B* TAD: Map of *Abd-B* TAD in KC tissue culture cells. Integrated Genome Viewer (IGV; James et al., 2011) screenshot of available ChIP-seq binding profiles for Clamp, GAF, dCTCF, and DNase Hypersensitive Sites (DHS-seq) across the *Abd-B locus*. The last track represents the regions identified as Topologically Associated Domains (TADs) based on Hi-C data. Green left: *abd-A* TAD; blue middle: *Abd-B* TAD; green right: neighboring TAD. The genomic coordinates are shown at the top (FlyBase R5). The data files were downloaded from Chorogenome Navigator (http://chorogenome.ie-freiburg.mpg.de/) (Ramírez et al., 2018). The ChIP-seq data for Clamp were downloaded from GEO Database (accession number GSE39271) (Soruco et al., 2013).

References

Robinson J.T., H. Thorvaldsdóttir, W. Winckler, M. Guttman, E. S. Lander, *et al.*, 2011 Integrative Genomics Viewer. Nature Biotechnology 29, 24–26.

Ramírez, F., V. Bhardwaj, L. Arrigoni, K.C. Lam, B.A. Grüning, *et al.*, 2018 High-resolution TADs reveal DNA sequences underlying genome organization in flies. Nature Communications, 9(1):189.

Soruco M.M., J. Chery, E.P. Bishop, T. Siggers, M.Y. Tolstorukov *et al.*, 2013 The CLAMP protein links the MSL complex to the X chromosome during Drosophila dosage compensation. Genes Dev 27(14):1551-6.

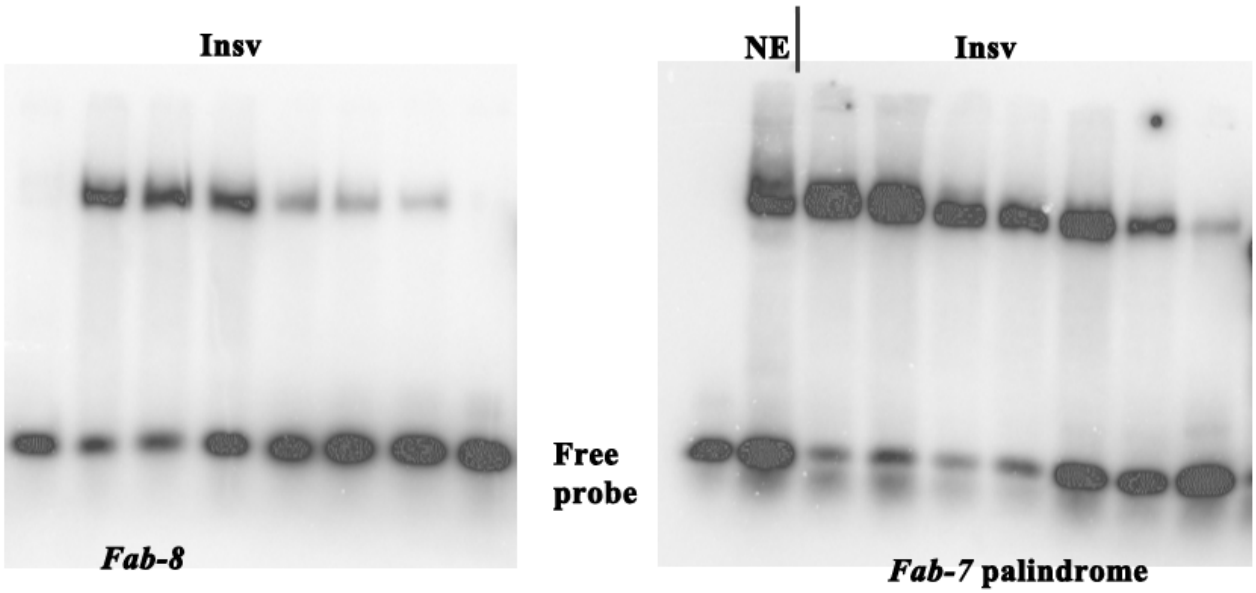

Figure S2: **Recombinant Insv binds to sequences at the proximal end of the *Fab-8* boundary.** Increasing amounts of recombinant Insv protein was added to a reaction mix containing labeled probe from *Fab-8* that contain sequences which bind to the Elba factor. A *Fab-7* probe containing the Elba/Insv palindrome sequences is used as a positive control. Amounts of recombinant Insv protein added (left to right) to the reaction mix were estimated based on the relative intensity of the Coomassie stained protein bands in SDS-PAGE gels: 0.5 μM, 0.25 μM, 0.05 μM, 0.025 μM, 0.005 μM, 0.0025 μM, and 0.0005 μM. NE: nuclear extract.

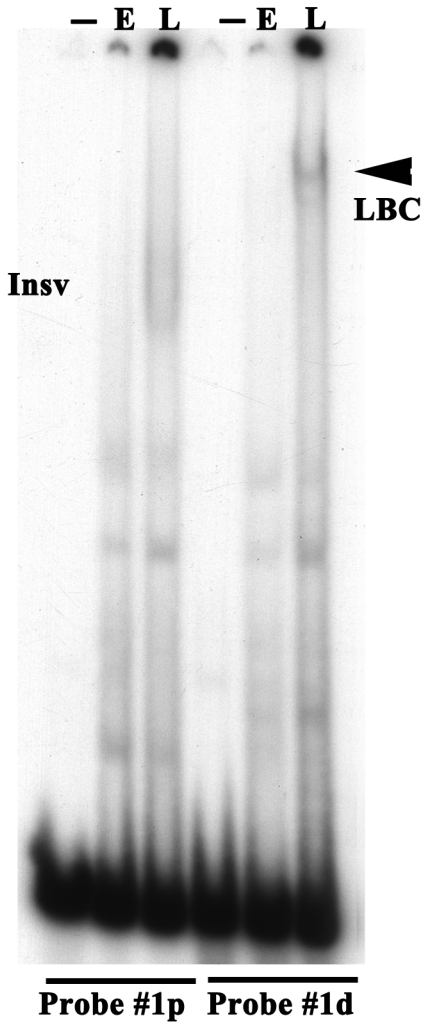

Figure S3: The LBC binds to sequences on the distal side of probe #1. Late nuclear extracts were used for EMSA experiments with two 70 bp overlapping probes, #1p and #1d, derived from the larger 100 bp probe #1 shown in Fig. 1. The LBC binds to probe #1d, but not to probe #1p. Probe #1p has the Elba/Insv recognition sequence shown in diagram in Fig. 1. Based on the mobility of the blurry shift in the late extract with probe #1p, it is likely to correspond to an Insv shift. Probe #1d gives the LBC like shift. -: no extract; E: early extract; L: late extract.

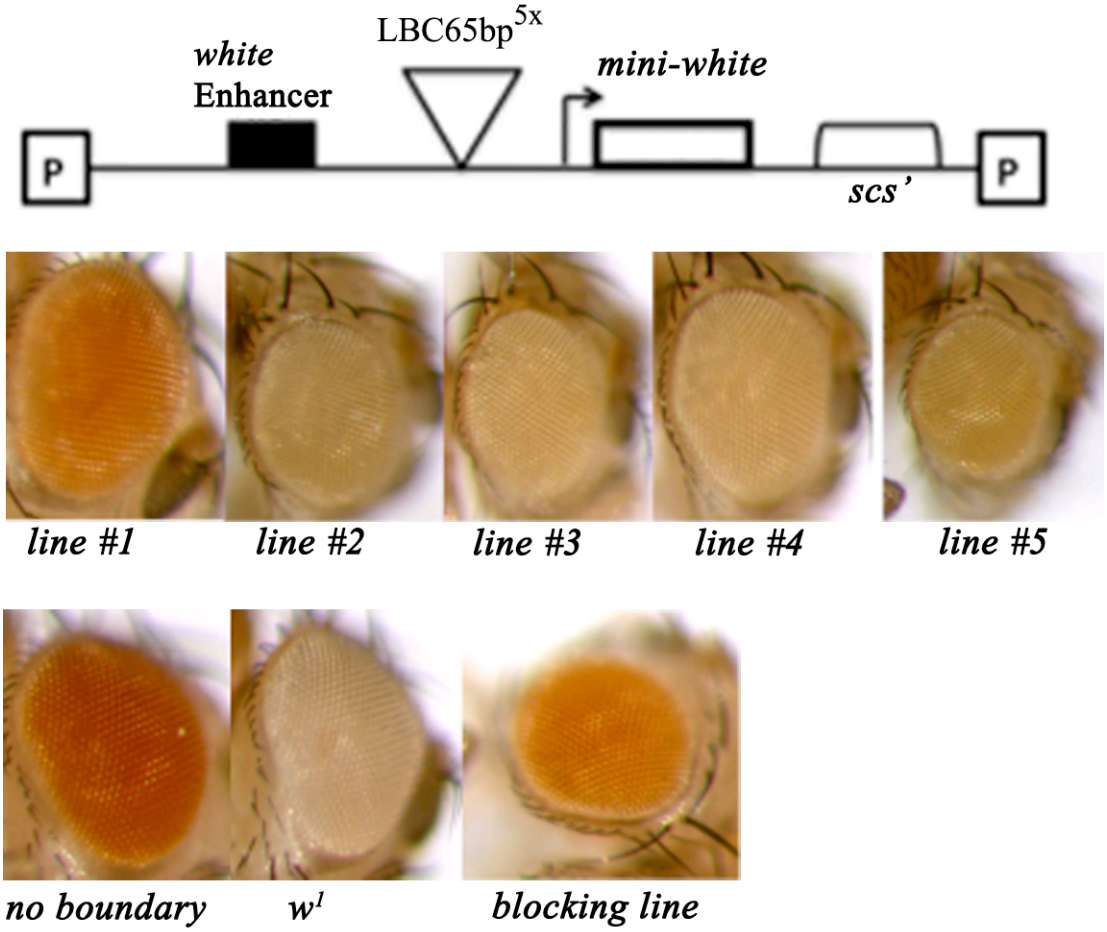

Figure S4: A multimerized *Fab-8* 65 bp LBC recognition sequence has enhancer blocking activity. A *mini-white* gene based enhancer blocking assay (Vazquez and Schedl, 1994; Hagstrom et al., 1996) was used to test a multimerized 65 bp LBC sequence for insulator activity. Five independent lines were recovered. In all five *white* expression (as judged by eye color) was reduced compared to a control line that a lacked a boundary in the blocking position. Lines as indicated in figure.

Vazquez J, and Schedl 1994 Sequences required for enhancer blocking activity of scs are located within two nuclease-hypersensitive regions. EMBO J. 13(24):5984-93.

Hagstrom K, M. Muller, and Schedl. 1996 Fab-7 functions as a chromatin domain boundary to ensure proper segment specification by the Drosophila bithorax complex. Genes Dev.10(24):3202-15. PMID: 8985188