

SUPPLEMENTAL FIGURE LEGEND

Fig. S1 GAF depletion is as efficient as reported and reduced the focal contact between promoter of *hid* and an intergenic region, related to Fig. 1. sfGFP signal of *sfGFP-GAF(N)* (A) and *GAF^{deGradFP}* (B); (C) Quantification of the signal for *sfGFP-GAF(N)* (n=12) and *GAF^{deGradFP}* (n=13); (D) The focal contact between promoter of *hid* and a distant 5' intergenic region is lost comparing GAF depletion embryos to the control embryos. The scale bar in B is 50 μ m. Boxplot in C are represented as mean \pm SD.

Fig. S2 Insulation is changed at altered toperon anchors and the protein profile at different anchors are different, related to Fig. 2 and Fig. 3. Insulation score around anchor center of changed or unchanged toperon anchors (A) and toperon or non-toperon anchors (B); Protein binding profile around contact anchors different types of contacts (C) and changed or unchanged anchors (D).

Fig. S3. Cooperative binding at the tethering elements is lost in Δ POZ at WT CUT&Tag peaks of Halo-GAF in 3rd instar larval wing discs, related to Fig. 3. (A) Histogram of number of GAGAG elements in CUT&Tag peaks. (B) Violin plots of normalized read counts at WT peaks, plotted by the number of non-overlapping GAGAG elements between the start and end coordinates of each peak identified with HOMER (left panel of A). Red dashed lines mark the median read counts of peaks containing 1 or \geq 7 GAGAG elements. (C) log₁₀ values of median read counts in B show correlation of GAF enrichment and number of GAGAG elements, with Δ POZ showing slower increase of enrichment over number of GAGAG elements indicated by a smaller slope of linear regression. (D) Mean GAF CUT&Tag enrichment for all genes \pm 1kb centered around transcription start sites (TSS). Example Micro-C maps of decreased contacts with GAF CUT&Tag tracks at *smal* and *Ddr* (E) and *tsh* and *tio* (F).

Fig. S4 The loss of Δ POZ proteins at the loop anchors, the changes of loops and the expression level in Δ POZ mutants, related to Fig. 3 and Fig.4. Linear regression of fold change of GAF protein binding and fold change of loop strength in Δ POZ (A) and Δ Q (B). (C) Volcano plot for the differentially expressed genes (DEGs) in Δ POZ mutants. (D) Profile of the WT or Δ POZ GAF near the DEGs transcription start sites. (E) Fraction of changed or unchanged non-toperon contacts in Δ POZ mutant. (F) The numbers of non-overlapping GAGAG elements in changed and unchanged anchors have no significant difference ($p = 0.13$, two sided, unpaired t test). Note: the sizes of the anchors are designated to be 3201bp, which are most of the time wilder than GAF enriched regions (G) Average distances of neighboring non-overlapping GAGAG elements are significantly smaller in changed than in unchanged anchors ($p = 0.017$, two sided, unpaired t test).

Fig. S1 GAF depletion is as efficient as reported and reduced the focal contact between promoter of *hid* and an intergenic region, related to Fig. 1.

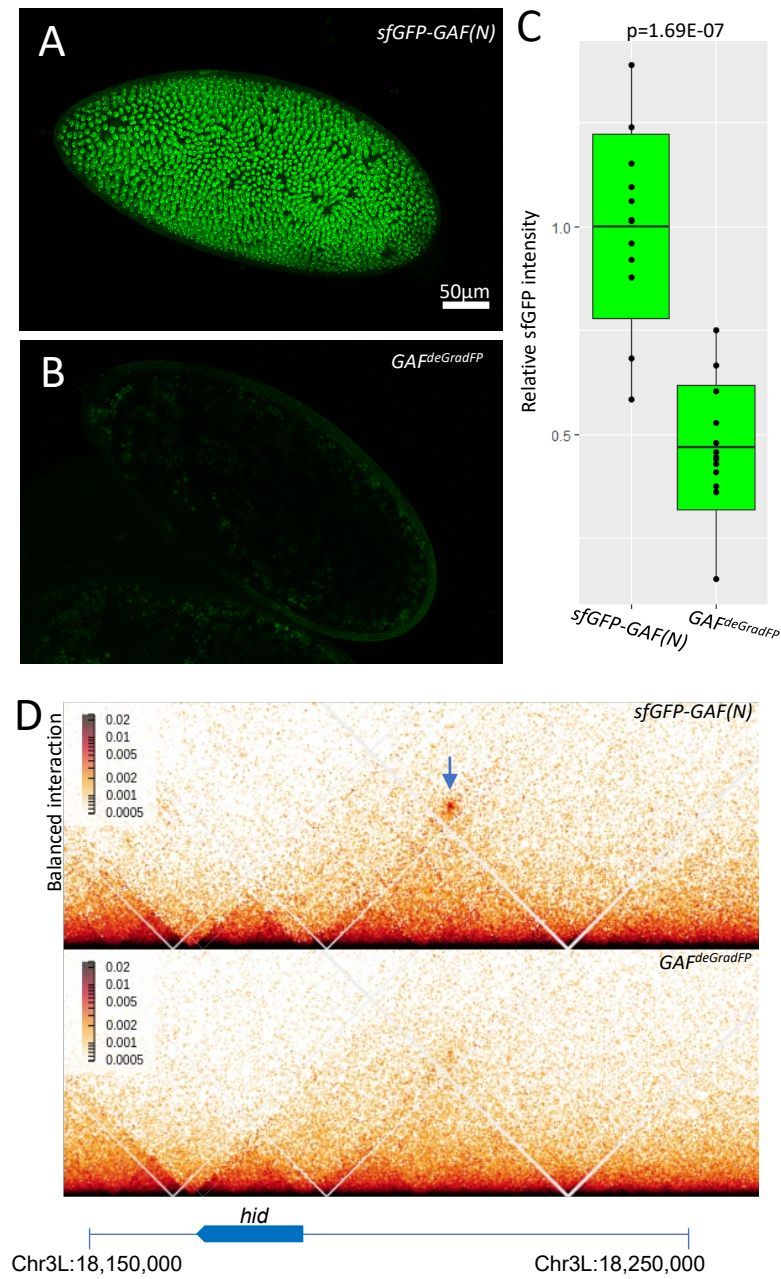


Fig. S2 Insulation is changed at altered toperon anchors and the protein profile at different anchors are different, related to Fig. 2 and Fig. 3.

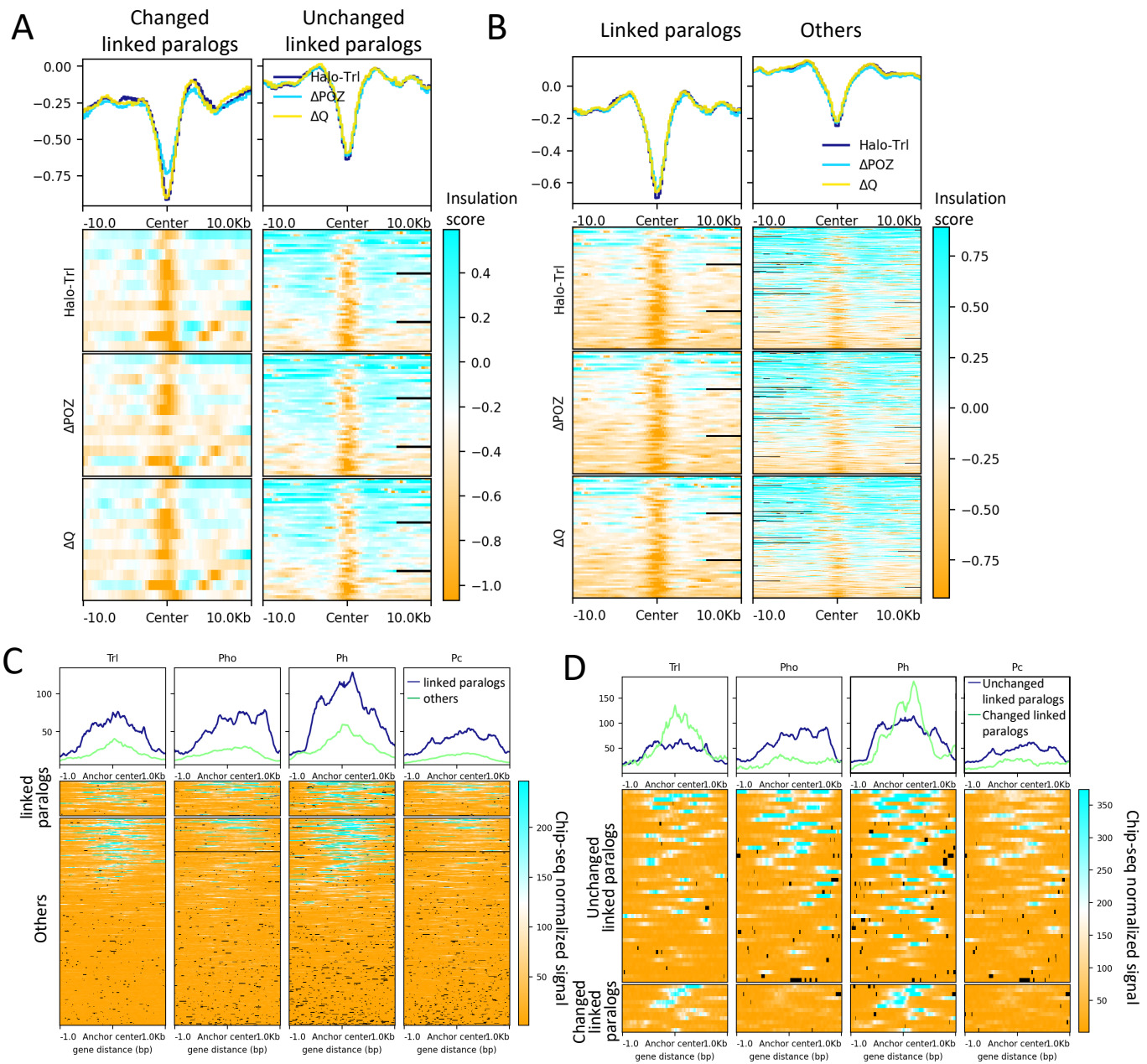


Fig. S3. Cooperative binding of Halo-GAF in 3rd instar larval wing discs, related to Fig. 3.

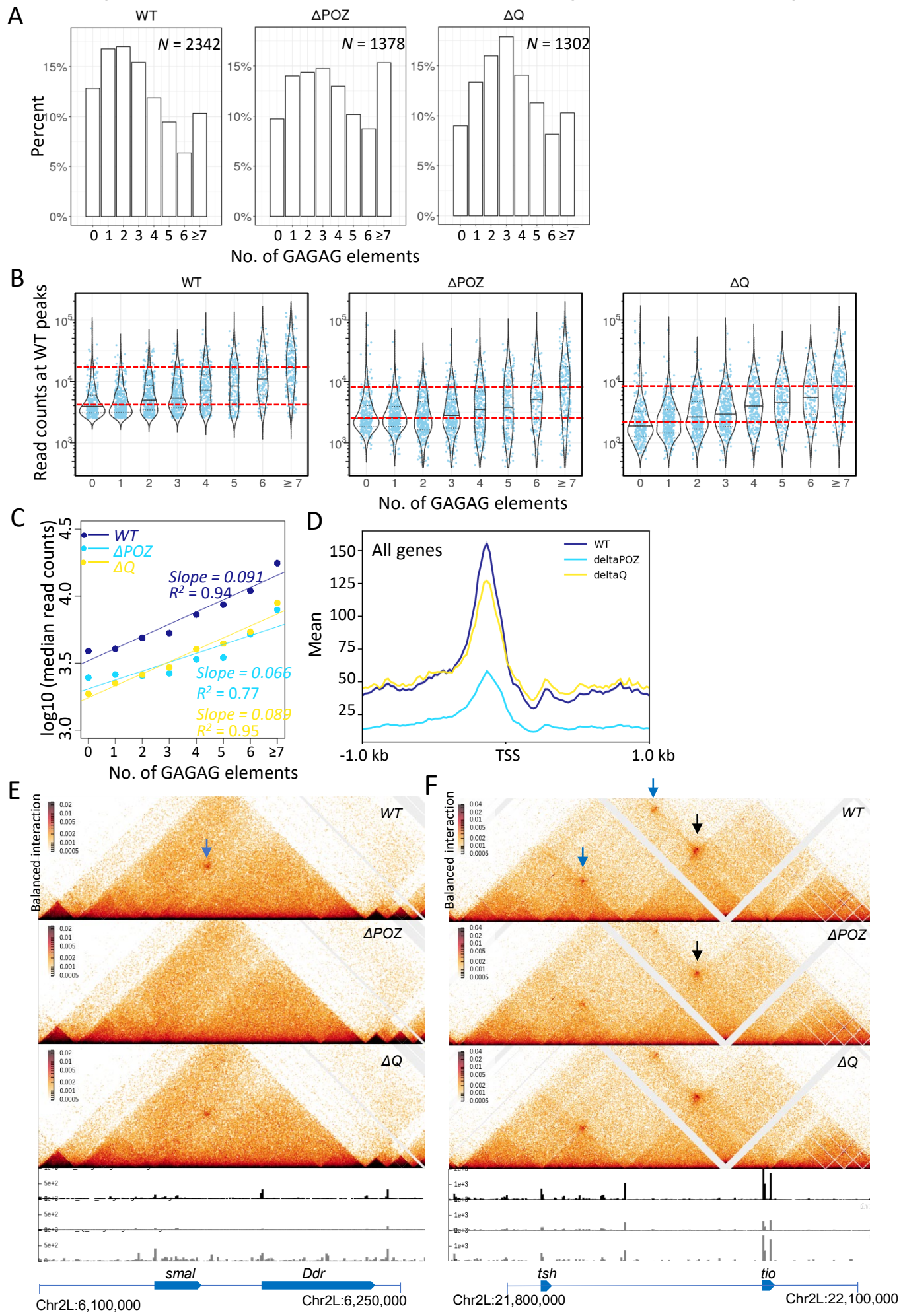
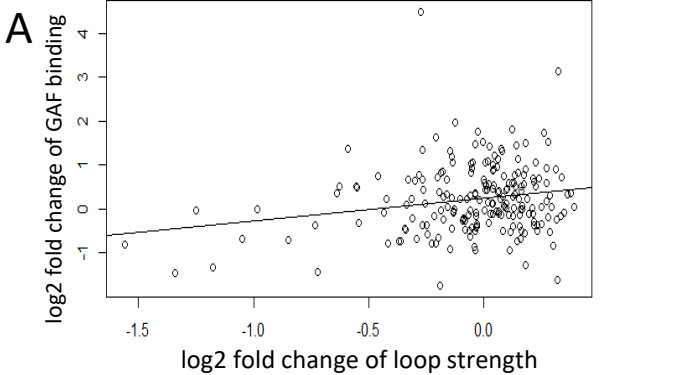


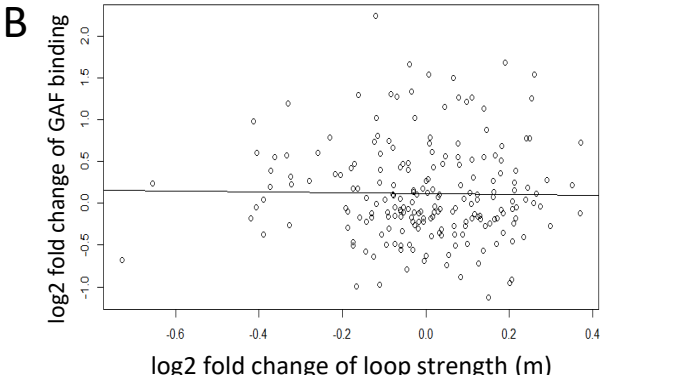
Fig. S4 The loss of Δ POZ proteins at the loop anchors, the changes of loops and the expression level in Δ POZ mutants, related to Fig. 3 and Fig.4.



Coefficients:

	Estimate	Std. Error	t value	Pr(> t)
Intercept	0.24205	0.05371	4.507	1.10E-05
log2 fold change of loop strength	0.52363	0.17291	3.028	0.00277

Multiple R-squared: 0.04262, Adjusted R-squared: 0.03797
 F-statistic: 9.171 on 1 and 206 DF
 p-value: 0.002774



Coefficients:

	Estimate	Std. Error	t value	Pr(> t)
Intercept	0.11736	0.03946	2.974	0.00329
log2 fold change of loop strength	-0.05613	0.22235	-0.252	0.80096

Multiple R-squared: 0.0003092, Adjusted R-squared: -0.04544
 F-statistic: 0.06372 on 1 and 206 DF
 p-value: 0.801

