# Supplementary Figures.

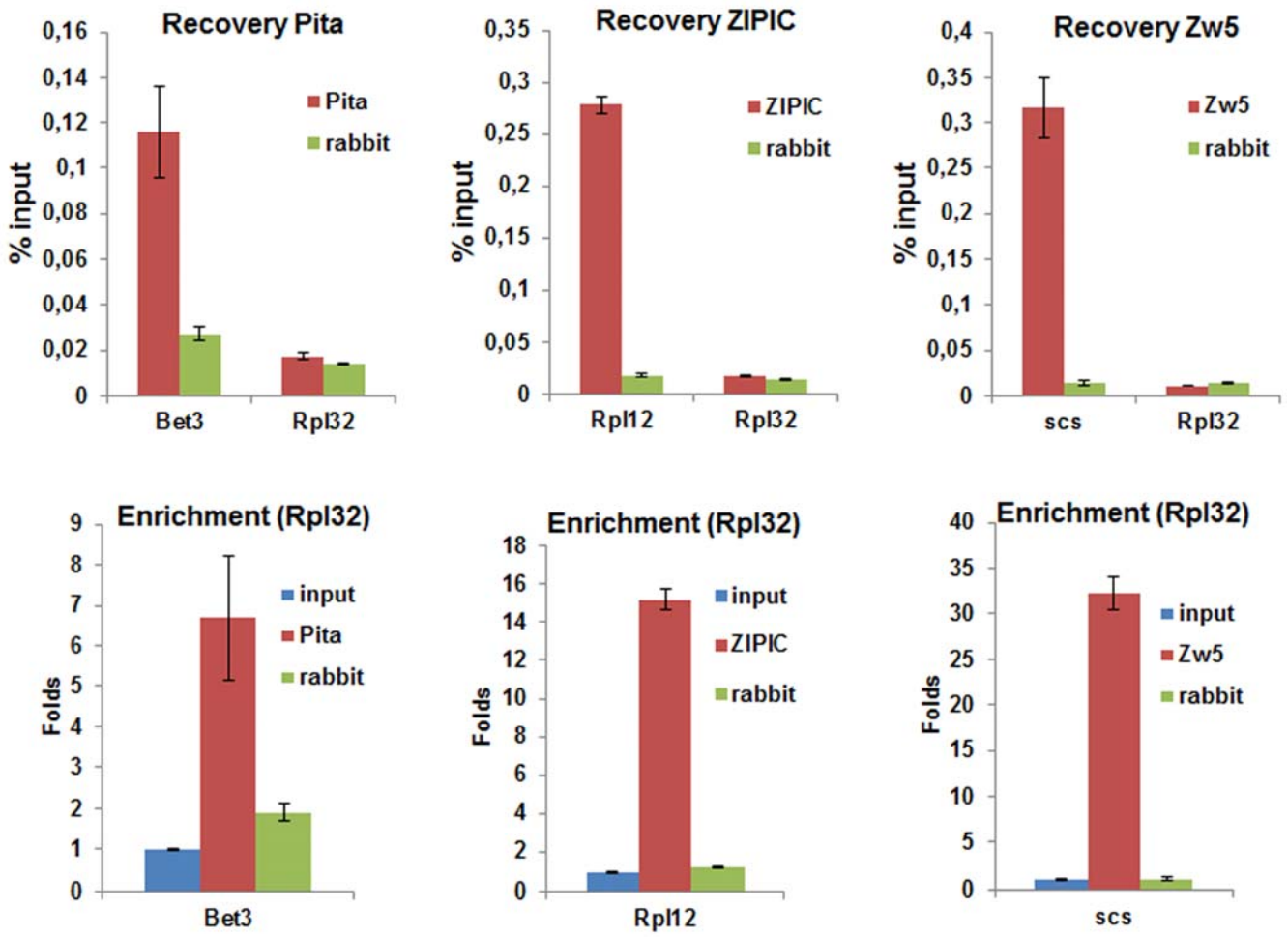


**Figure S1. Localization of Pita, ZIPIC and Zw5 on selected chromatin regions in embryos.** Histograms show ChIP recovery and enrichments (relative to negative rpl32 region) for Pita, ZIPIC and Zw5 in corresponding positive regions on chromatin isolated from embryos and incubated with antibodies against Pita, ZIPIC or Zw5. The results are presented as a percentage of input genomic DNA for recovery and change folds for enrichments. Error bars show standard deviations of triplicate PCR measurements for three independent experiments.

**A**

| | peaks | CapH2 | Nipped-B | Rad21 |
|---|---|---|---|---|
| peaks | | 6051 | 7742 | 7430 |
| CP190 | 7960 | 0,372 | 0,477 | 0,437 |
| Pita | 1845 | 0,422 | 0,596 | 0,571 |
| ZIPIC | 575 | 0,687 | 0,687 | 0,657 |
| Zw5 | 695 | 0,685 | 0,768 | 0,731 |

**B**

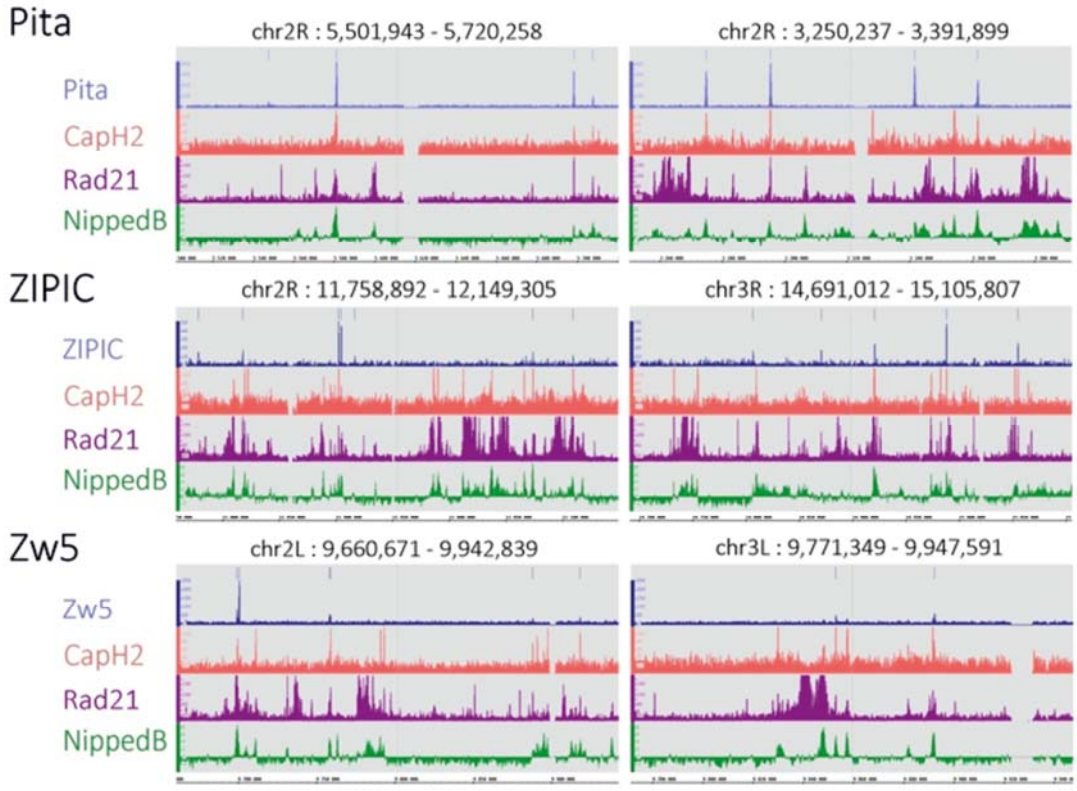


**Figure S2. Co-localization of Pita, ZIPIC, and Zw5 binding sites with subunits of condensin (CapH2) and cohesin (Rad21 and Nipped-B) complexes. (A)** Pairwise comparison of ChIP-Seq data sets. In each matrix, cells with red lettering show the total number of peak calls, and other cells show the proportion of peaks of each particular data set (rows) that overlap peaks of another data set (columns). **(B)** Examples of co-localization of Pita/ZIPIC/Zw5 with CapH2, Rad21, Nipped-B in the Genome Browser window.

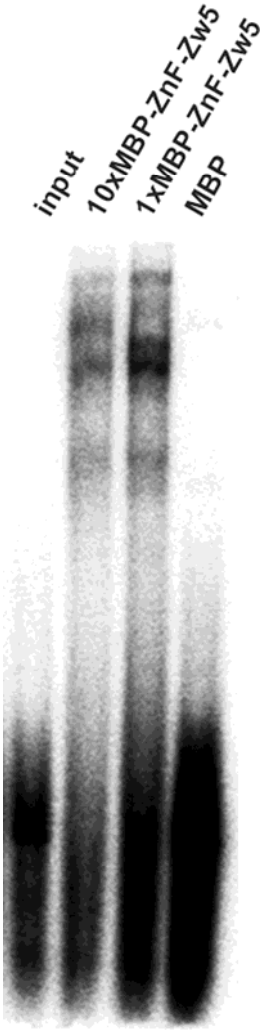


**Figure S3.** Electrophoretic mobility shift assay for the interaction of recombinant Zw5 with a DNA fragment containing four Zw5 binding sites.

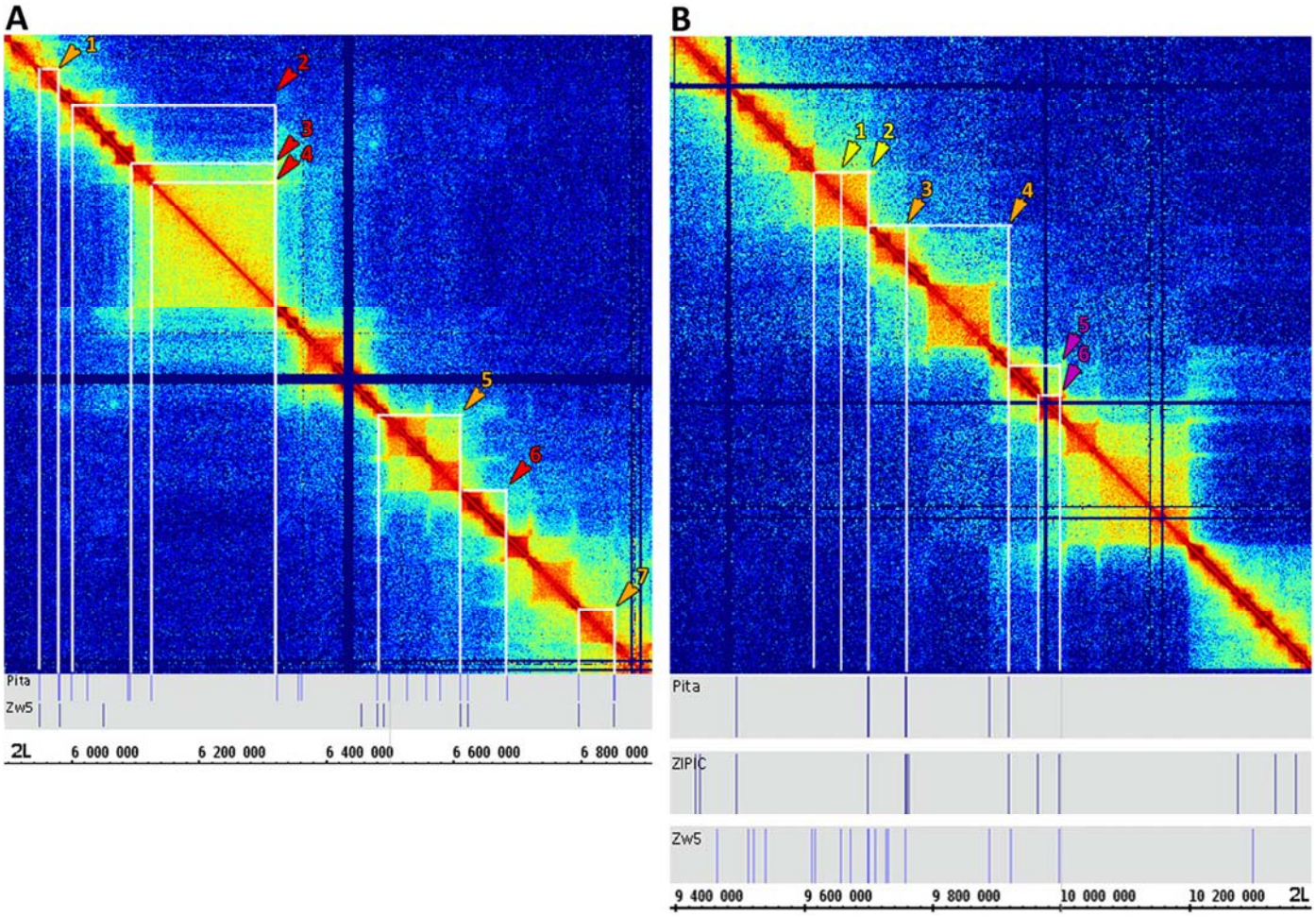


**Figure S4. Co-localization of Pita, ZIPIC and Zw5 ChIP-seq peaks with Hi-C maps** (2 kb resolution, (1)). Panels (A) and (B) show different random locations on chromosome 2L. Arrows indicate examples of contacts surrounded by peaks: red arrowheads, Pita peaks; yellow, Zw5 peaks; purple, ZIPIC peaks; orange, peaks of two or three different proteins. In (A): (1, 3, 5, 6, 7) contacts of TAD boundaries, (4) contact of sub-TAD boundaries; (2) contact of sites from different TADs; in (B): (2, 4, 5) contacts of TAD boundaries, (1, 3, 6) contacts of sub-TAD boundaries.

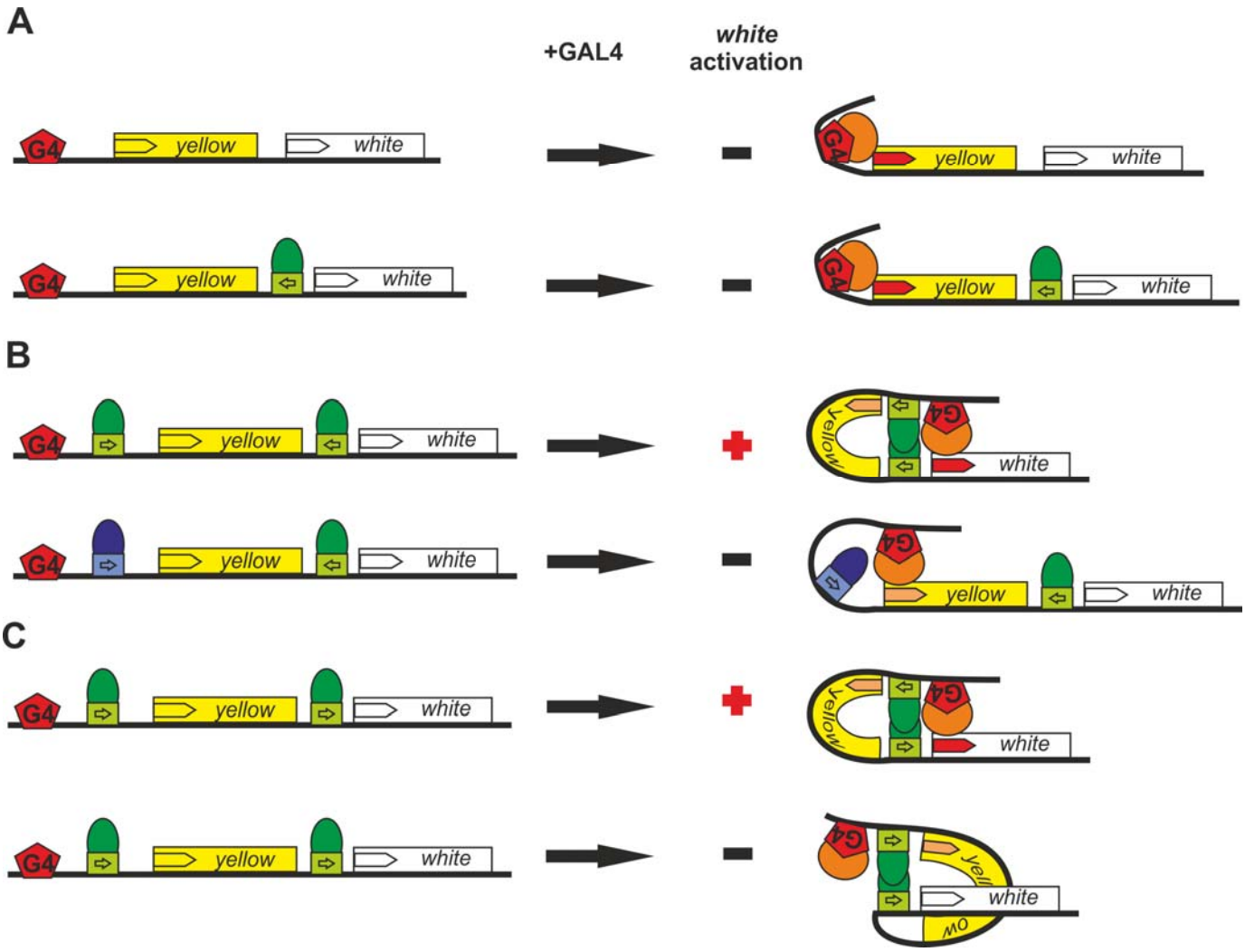


**Figure S5. Scheme of GAL4/*white* assay in *Drosophila* transgenic lines**. The assay is based on the finding that the yeast GAL4 activator (red pentagon) bound to sites located upstream of the *yellow* gene (large yellow rectangle) fails to stimulate the *white* gene (large white rectangle) placed downstream of the *yellow* 3' end. The red, orange and white pointed bars within these rectangles indicate high, moderate, and low transcription levels of the *yellow* and *white* genes. The GAL4 binding sites are inserted at –893 relative to the *yellow* transcription start site. As a result, the distance between the *mini-white* gene and the GAL4 binding sites is almost 5 kb. To express the GAL4 protein, the line carrying the tested transgene is crossed with the transgenic line carrying the GAL4 gene under control of the ubiquitous *tubulin* promoter. Only the interaction between transcription factors (green and purple ovals) expressed in the eyes can be tested by this assay. The insertion of binding sites (small green rectangle with arrow) for the test protein near the *white* promoter cannot stimulate or repress *white* expression. (A) GAL4 can strongly activate the *yellow* gene but fails to stimulate the *white* gene located at a 5-kb distance. The binding sites near the *white* promoter do not facilitate *white* activation by GAL4. (B) Strong

stimulation of the *white* promoter by GAL4 indicates interaction between the same protein bound to the sites (green rectangles with arrows) near the *white* promoter and GAL4 regulatory element. Lack of *white* stimulation by GAL4 indicates the absence of interaction between the proteins bound to different binding sites (green and blue rectangles). (C) According to previous data, pairs of identical insulators interact in an orientation-dependent manner. This is tested by inserting binding sites for the same protein in the same orientation. In this case, lack of *white* stimulation by GAL4 indicates that these sites interact with each other in orientation dependent manner. If GAL4 stimulates *white* expression, we conclude that the binding sites for this protein do not display orientation-dependent interaction.

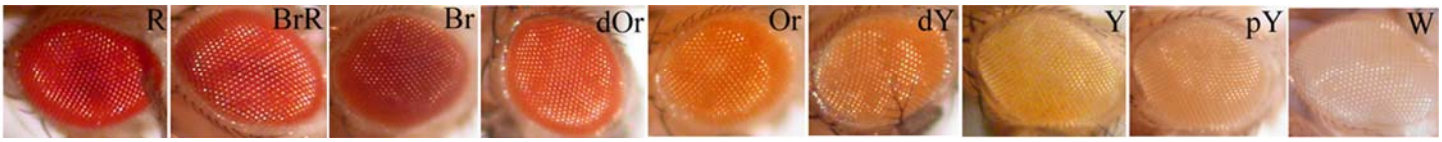


**Figure S6. Nine classes of eye pigmentation.**

The nine-grade scale of the *eye* pigmentation level in 2- to 3-day adult males (reflecting the activity of the *eye* enhancer), with wild-type expression and the absence of expression assigned scores Red (R) and White (W), respectively.

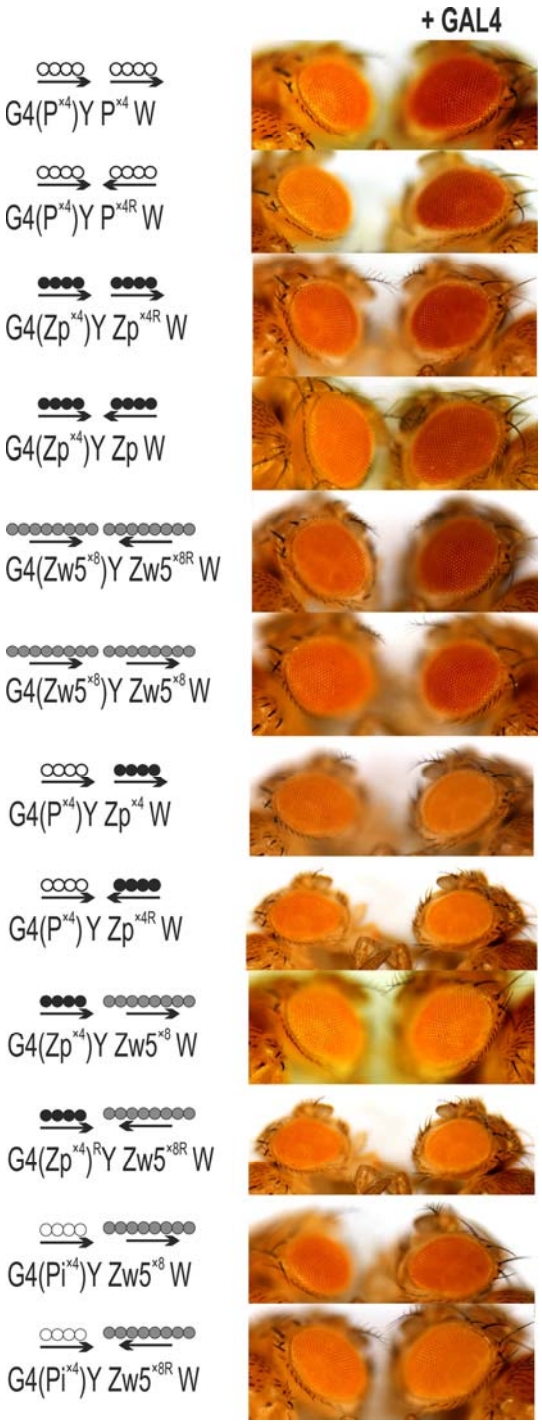


**Figure S7. Testing for the functional interaction between DNA fragments containing binding sites for Pita (white circles), ZIPIC (black circles) and Zw5 (gray circles) at region 86Fb in the GAL4/*white* model system.**

All combinations of binding sites for the Pita, ZIPIC and Zw5 proteins were inserted in the same genomic region 86Fb using a phiC31-based integration system. A reductive scheme of transgenic construct used to examine the functional interaction between the binding sites for tested proteins is presented in the upper part of

Fig.4. The GAL4 binding sites (indicated as G4) are at a distance of 5 kb from the *white* gene. The *yellow* gene is inserted between the GAL4 binding sites and the *white* promoter. '+GAL4' indicates that photos of eye phenotypes in transgenic lines were taken after induction of GAL4 expression.

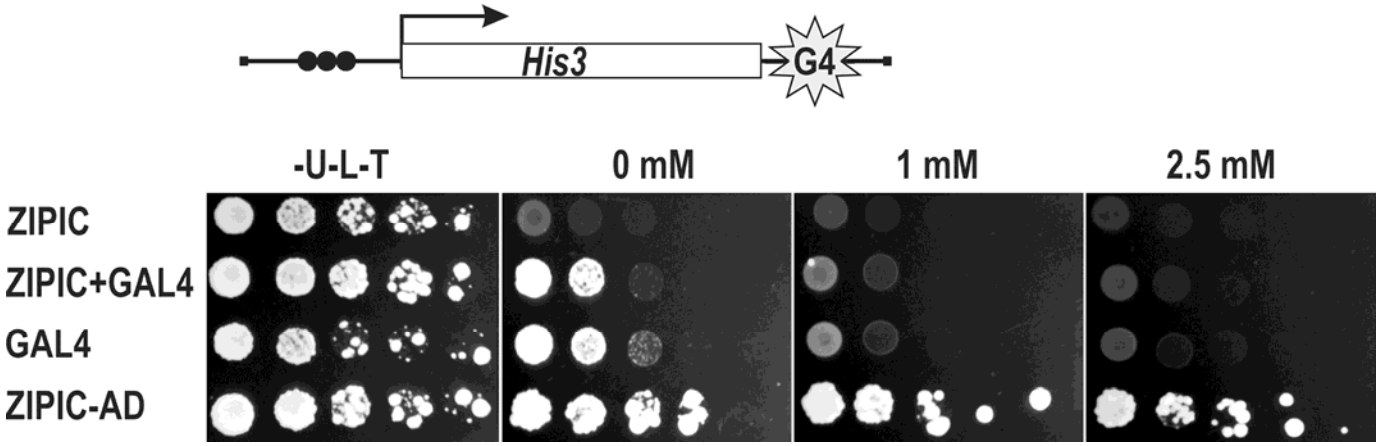


**Figure S8. ZIPIC binding region near the promoter is not able to activate the reporter gene in yeast.** Structure of the *his* gene reporter system to monitor ZIPIC activity in yeast. Ten Gal4 binding sites (G4) were inserted at the 3' end of the *his* gene. The DNA fragments containing three ZIPIC sites were inserted upstream of the *his* reporter. The reporter construct was integrated into the genome. Expression of the *his* reporter was spot-assayed by measuring colony formation on plates containing the histidine analog 3-amino-1,2,4-triazol (3-AT): the higher the concentration of 3-AT, the higher the level of HIS3 protein required for growth.

## REFERENCES

1. Li, L., Lyu, X., Hou, C., Takenaka, N., Nguyen, H.Q., Ong, C.T., Cubenas-Potts, C., Hu, M., Lei, E.P., Bosco, G. *et al.* (2015) Widespread rearrangement of 3D chromatin organization underlies polycomb-mediated stress-induced silencing. *Molecular cell*, **58**, 216-231.