Supporting Information

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SI Materials and Methods

Generating Deletion Mutations of *hiphop*. The EY09894 P element inserts 5′ to the start codon of *hiphop*. Using P element-induced imprecise excision (1), we generated deletion mutations of *hiphop* based on the loss of the *white*$^{+}$ marker in the P element. PCR followed by sequencing confirmed that four alleles had the *hiphop* locus deleted: *L14*, *L32*, *L35-2*, and *L41*. The extent of the deletions is illustrated in Fig. S1.

N Terminus of K81 Can Interact with HP1 and Orc-Associated Protein and HP1 in Vitro. We previously showed that the N terminus of HipHop interacts with HP1 and Orc-associated protein (HOAP) and HP1 using a GST pull-down assay (2). We purified bacteria-expressed GST fusion proteins carrying the full-length N- or C-terminal fragments of K81 and tested their ability to interact with HOAP and HP1 in *Drosophila* embryonic extracts. As shown in Fig. S3 and similar to results using HipHop fusion proteins, only the N terminus of K81 was able to interact with HOAP and HP1.

Generating a *gfp-hoap* Locus by Gene Targeting. We tagged the endogenous *cav* locus, which encodes HOAP, with *gfp* using SIRT as described previously (3). Briefly, we first targeted an *attP* attachment site for the phiC31 integrase 116-bp 3′ of the stop codon of *cav* using gene targeting by homologous recombination (4). A plasmid that carries *gfp-hoap* and the surrounding genomic region of *cav* (2) along with an *attB* attachment site were injected into flies with *attP* at *cav*. Site-specific integration created a tandem duplication of *cav*, with one copy carrying *gfp*. Site-specific DNA break-induced reduction of this duplication gave rise to a single *gfp-cav* locus. Animals homozygous for this allele are viable and fertile.

Tagging HipHop from *D. virilis*. A 5-kb fragment covering the *hiphop* locus from *virilis* was PCR amplified and cloned into the Gateway donor vector pCR8gwTOPO from Invitrogen. A *gfp* cassette was inserted just downstream of the start codon of *hiphop* using two-step bacteria recombineering as described previously (3). This *gfp-hiphop* fragment was cloned into a Gateway-converted piggyBac transformation vector that carries a miniwhite marker (5). The original piggyBac plasmid was obtained from the Drosophila Genome Resources Center (DGRC) (https://dgrc.cgb.indiana.edu/). The resulting plasmid was transformed into a white-mutant strain of *virilis*. Transformants were identified as flies with pigmented eyes.

1. Witsell A, Kane DP, Rubin S, McVey M (2009) Removal of the bloom syndrome DNA helicase extends the utility of imprecise transposon excision for making null mutations in Drosophila. *Genetics* 183:1187–1193.
2. Gao G, et al. (2010) HipHop interacts with HOAP and HP1 to protect Drosophila telomeres in a sequence-independent manner. *EMBO J* 29:819–829.
3. Gao G, Wesolowska N, Rong YS (2009) SIRT combines homologous recombination, site-specific integration, and bacterial recombineering for targeted mutagenesis in Drosophila. *Cold Spring Harb Protoc* 2009:pdb.prot5236.
4. Rong YS, Golic KG (2000) Gene targeting by homologous recombination in Drosophila. *Science* 288:2013–2018.
5. Holtzman S, et al. (2010) Transgenic tools for members of the genus Drosophila with sequenced genomes. *Fly (Austin)* 4:349–362.

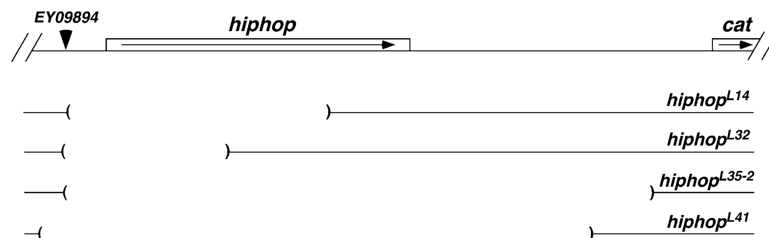

Fig. S1. Deletion mutations of *hiphop*. The coding regions of *hiphop* and *cat* genes are shown as boxes, with the direction of transcription indicated by arrows. The arrowhead marks the insertional position of the original EY element. Underneath the diagram of the coding regions, the extent of the deletion in each of four mutant alleles is depicted as empty space between parentheses.

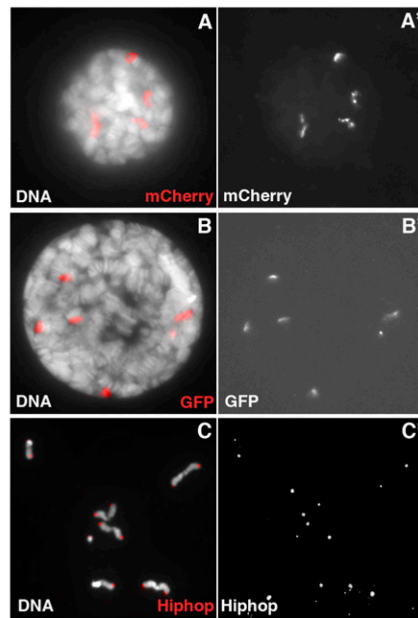

Fig. S2. HipHop and K81 localization at telomeres. (*A*) A merged image of a salivary gland nucleus from a larva expressing *mcherry-hiphop*. In the mCherry channel (*A′*), clusters of fluorescent signals are visible, corresponding the telomeres from major chromosome arms. (*B*) A merged image of a salivary gland nucleus from a larva expressing *gfp-k81* under *hiphop* control. In the GFP channel (*B′*), clusters of fluorescent signals are visible. (*C*) A merged image of a meiosis II nucleus stained with anti-HipHop from k81 mutant animals expressing *hiphop* in the testis under the regulatory control of *k81*. HipHop signals in red decorate telomeres of meiotic chromosomes. (*C′*) Antibody signals only.

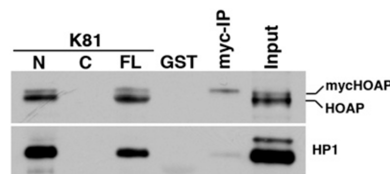

Fig. S3. N terminus of K81 can interact with HOAP and HP1. GST fusion proteins were purified from bacteria and incubated with embryonic extracts made from embryos expressing HOAP and myc-tagged HOAP (4). The materials pulled down were used on Western blots probed with anti-HOAP and anti-HP1. The positions for the mycHOAP, HOAP, and HP1 proteins are indicated to the right. Fusion proteins used are: K81-N (residues 1–94 of K81 fused to GST), K81-C (residues 93–184 fused to GST), K81-FL (full-length K81 fused to GST), and GST (GST alone). Materials from a previous anti-myc immunoprecipitation using the same extract were loaded to indicate the positions of mycHOAP and HP1. Input extracts were also loaded as a control.