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Supplementary Materials for

A Histone Mutant Reproduces the Phenotype Caused by Loss of Histone-Modifying Factor Polycomb

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SUPPORTING ONLINE MATERIAL

Materials and Methods

Fly Strains

The following fly strains were used in this study:
w; E(z)^{731} FRT2A/TM6C
w; Df(2L)HisC FRT40A/SM6b
w; Df(2L)HisC FRT40A; 6xHisGU/SM5^TM6B
w; Df(2L)HisC FRT40A; 6xHisGU^{H3K27R}/SM5^TM6B
yw hs-flp122; y+ hs-nGFP FRT2A
yw hs-flp122; hs-nGFP FRT40A
yw hs-flp122; hs-nGFP FRT40A; 6xHisGU
yw hs-flp122; hs-nGFP FRT40A; 6xHisGU$^{H3-K27R}$
yw hs-flp122; y+ FRT40A; 6xHisGU
yw hs-flp122; y+ FRT40A; 6xHisGU$^{H3-K27R}$

The *Df(2L)HisC* deletion allele and the construction of *6xHisGU* chromosomes were described before (*7*). The *E(z)^{731}* null allele was previously described (*8*). A *Df(2L)HisC FRT40A* recombinant was generated for this study. Flies of the appropriate stocks were crossed to generate the genotypes shown in Fig. S1.

Immunostaining of clones in imaginal wing discs

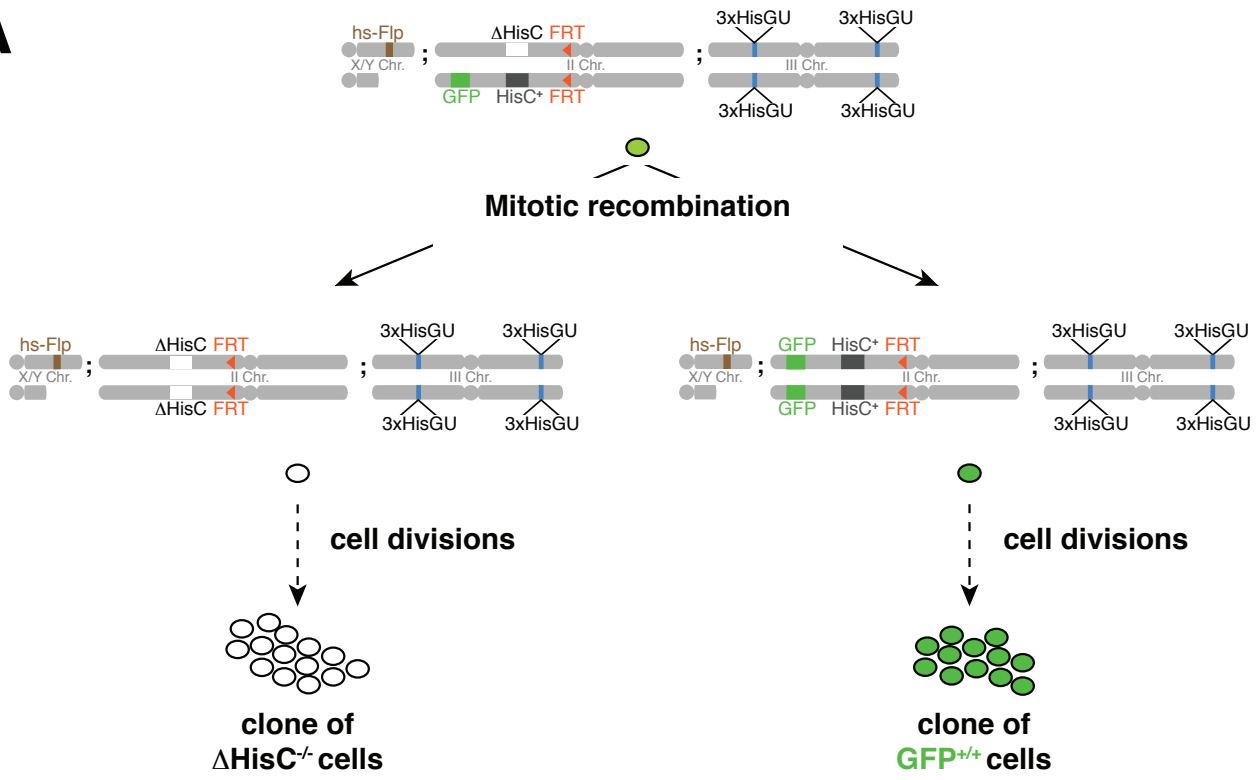
Marked clones in imaginal discs were induced and stained as described (*9*). The following primary antibodies were used: Ubx (DSHB#FP3.38), Abd-B (DSHB#1A2E9), Scr (DSHB#6H4.1), En (DSHB#4D9) and H3K27me3 (Upstate UBI#07-449). Fluorescently-labeled secondary antibodies were labeled with Cy3 (Jackson ImmunoResearch) and DNA was stained with Hoechst 33342. Imaginal discs were mounted in Flouromount G (Southern Biotech) and pictures were taken using a Leica TCS SP2 AOBS confocal microscope.

Abdominal adult clone analysis

To generate adult clones in the abdomen, animals of the genotypes shown in Fig. S1B were heat shocked for 1 hour at 37 C, at the white pre-pupal stage.
Adults were dissected in PBS containing 0,1% Tween20, the fat body was removed and abdominal cuticles were mounted in Hoyers medium (*10*) and photographed using a Zeiss Axio Scope A1 microscope.

Figure S1

A



B

Genotypes of animals in figure panels:

Fig. 1A, top row:	yw hs-flp122 ; Df(2L)HisC FRT40A / hs-nGFP FRT40A ; + / +
Fig. 1A, second row:	yw hs-flp122 ; Df(2L)HisC FRT40A / hs-nGFP FRT40A ; 6xHisGU / 6xHisGU
Fig. 1A, third row, Fig. 1B, first row:	yw hs-flp122 ; Df(2L)HisC FRT40A / hs-nGFP FRT40A ; 6xHisGU$^{H3\text{-}K27R}$ / 6xHisGU$^{H3\text{-}K27R}$
Fig. 1B, second row:	yw hs-flp122 ; + / + ; E(z)^{731} FRT2A / y^{+} hs-nGFP FRT2A
Fig. 2, top row:	yw hs-flp122 ; Df(2L)HisC FRT40A / y^{+} FRT40A ; 6xHisGU / 6xHisGU
Fig. 2, second row:	yw hs-flp122 ; Df(2L)HisC FRT40A / y^{+} FRT40A ; 6xHisGU$^{H3\text{-}K27R}$ / 6xHisGU$^{H3\text{-}K27R}$
Fig. 2, third row:	yw hs-flp122 ; + / + ; E(z)^{731} FRT2A / y^{+} hs-nGFP FRT2A
Fig. 2, fourth row:	w ; + / + ; + / +

Clonal analysis in imaginal discs and adults

(**A**) Experimental strategy for generating histone mutant clones.
Top: Genotype of animals in which mutant clones were analyzed. Chromosomes X/Y, II and III are depicted with the following genetic elements. HisC^{+} (black box): wild-type allele of the histone gene cluster; ΔHisC (empty box): deletion allele lacking the entire histone gene cluster; GFP (green box): hsp70-nGFP transgene; hs-Flp(brown box): hsp70-Flp transgene expressing Flp recombinase under control of the hsp70 promoter to induce recombination at FRT elements (orange triangles); 3xHisGU: cassette containing 3 histone gene units, inserted at two specific integration sites on chromosome arm 3L and 3R to generate a total of 12xHisGU, as described (7), the same integration sites were used for 12xHisGU and 12xHisGU$^{H3\text{-}K27R}$. Below: cell of the above genotype (green oval). Flp-mediated recombination between sister chromatids on homologous chromosomes (i.e. after S-phase) results in two genetically different daughter cells: a ΔHisC homozygous cell lacking the GFP marker gene (empty oval) and a HisC^{+} homozygous cell carrying two copies of the GFP marker gene (dark green oval). For generation of ΔHisC homozygous cell clones marked with the yellow mutation (light pigmentation) in adults (Fig. 2), a yellow^{+} (y^{+}) marker gene instead of the GFP marker was placed in *cis* with the HisC^{+} allele and the clones were induced in the background of animals that carried a yellow (y) mutation on the X-chromosome (see B).
(**B**) Genotypes of animals analyzed in Figs. 1 and 2. For simplicity, only the male genotype is shown .

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