Supplementary Information for

Sex-specific phenotypes of histone H4 point mutants establish dosage compensation as the critical function of H4K16 acetylation in *Drosophila*

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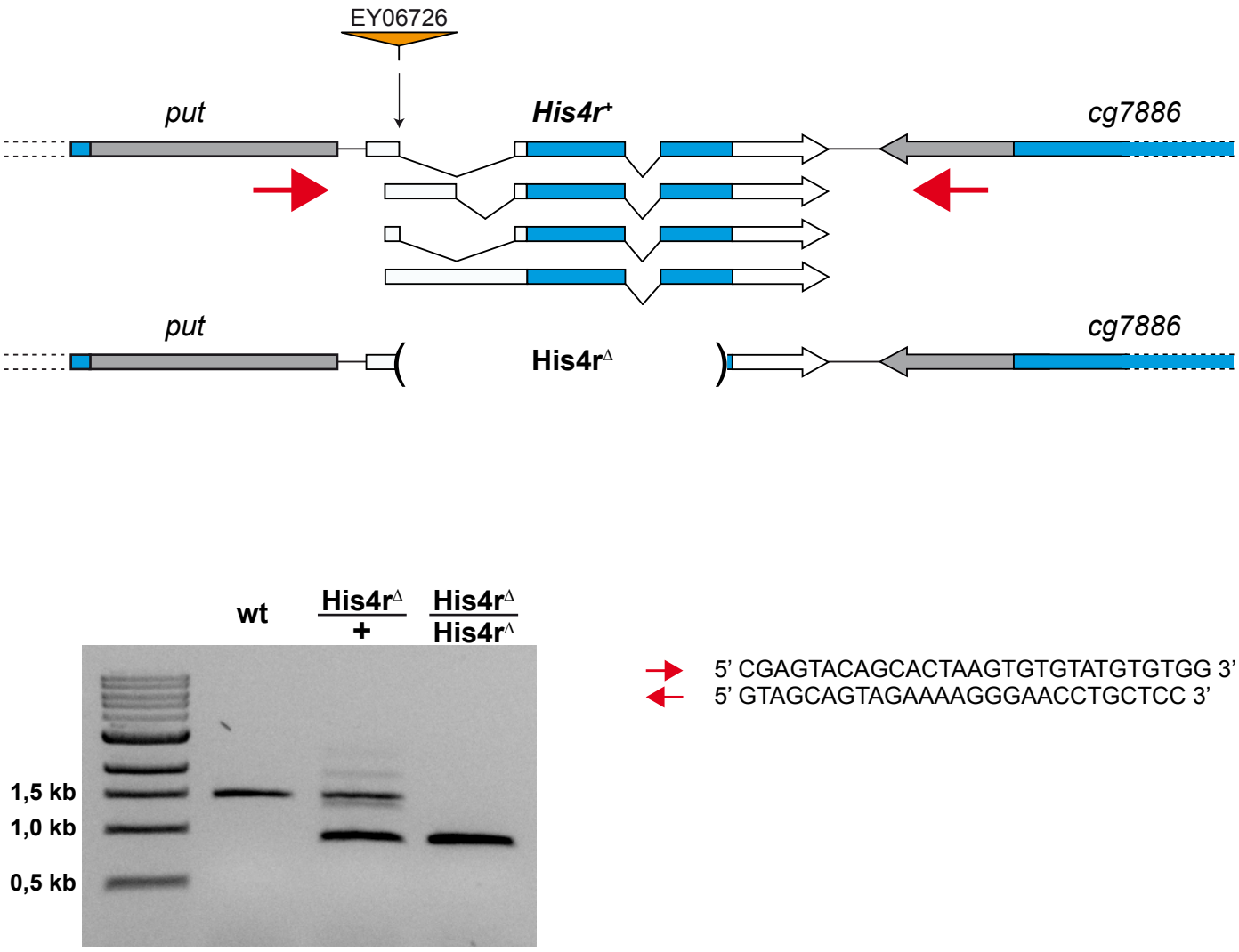

Figure S1
Generation of the His4rΔ deletion allele
(Top) Schematic representation of the *His4r* genomic region and the molecular lesion in *His4r$^{\Delta}$*. *His4r$^{\Delta}$* was generated by imprecise excision of a P-element insertion in the line EY06726 (location of insertion marked by arrow); the excision resulted in the deletion of 594 bp of DNA, including the entire *His4r^{+}* coding region. Location of PCR primers used for analysis is indicated (red arrows). (Below) Diagnostic Polymerase Chain Reactions (PCR) with the indicated primers (red arrows, see also TOP) were performed on genomic DNA from wild type, *His4r$^{\Delta}$*/+ heterozygous and *His4r$^{\Delta}$*/*His4r$^{\Delta}$* homozygous adult flies.

Figure S2
***H4*^{K16A} mutants arrest development at the end of embryogenesis but show no detectable morphological defects**
Embryonic cuticles of *wildtype* (*wt*) and *H4*^{K16A} mutant embryos. Cuticles from *H4*^{K16A} mutant embryos are indistinguishable from *wt*.

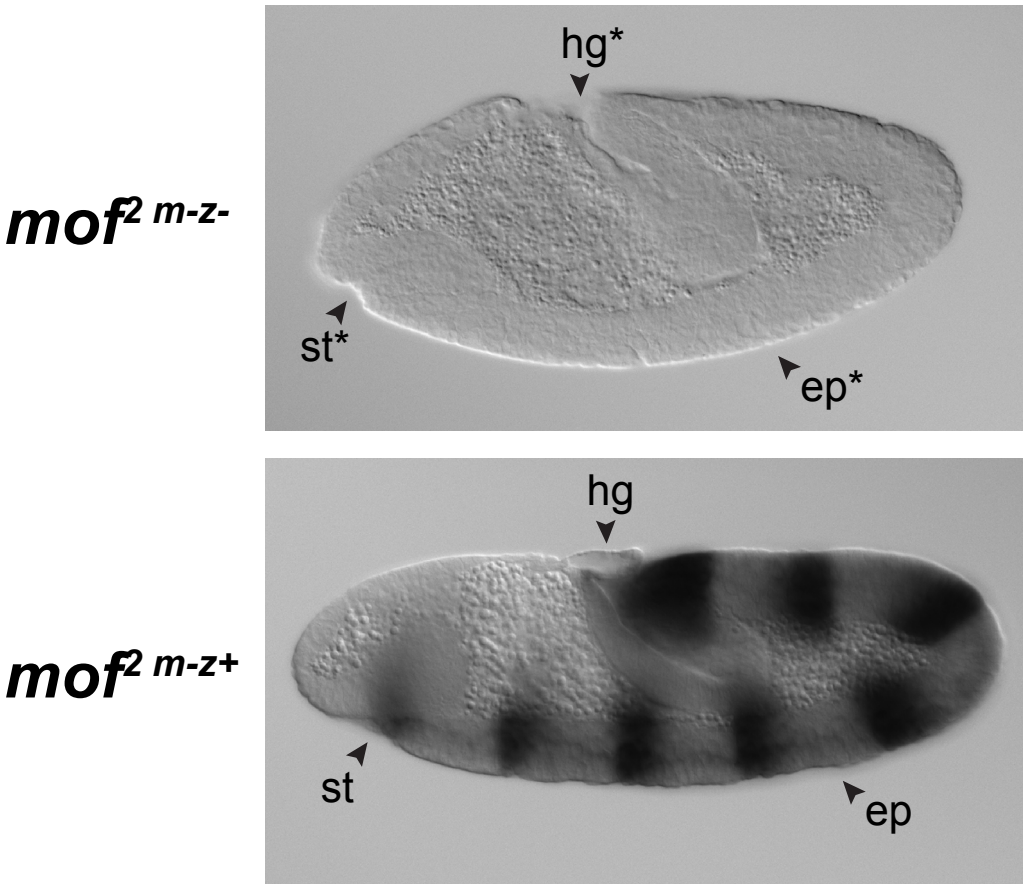

Figure S3
Lack of maternally-deposited and zygotically-expressed Mof protein in males causes developmental arrest shortly after gastrulation.
mof$^{2\ m\text{-}z\text{-}}$ male embryo (top) and *mof*$^{2\ m\text{-}z+}$ female embryo (bottom) at stage 8/9 of development. Embryos were obtained by crossing *mof*^{2} *FRT19A/ ovoD1 hsFLP FRT19A* females producing *mof*^{2} mutant germ cells with *mof*$^{+}$ *FM7c ftz-LacZ/ Y* males, and the embryos were stained with antibody against ß-galactosidase expressed from the *ftz-LacZ* marker gene to distinguish *mof*^{2} *FRT19A/ Y* males and *mof*^{2} *FRT19A/ mof*+ *FM7c ftz-LacZ* females. *mof*$^{2\ m\text{-}z+}$ female embryos showed normal embryonic morphology and developed into adults with apparent normal morphology (Table 1). *mof*$^{2\ m\text{-}z\text{-}}$ male embryos all arrested development during or shortly after gastrulation with highly abnormal morphology such as irregular organization of epidermal tissues (ep*), aberrant invagination of the stomodaeum (st*) and the hindgut (hg*), and failure to form a proper cephalic furrow (not visible); in *mof*$^{2\ m\text{-}z+}$ female embryos these structures, indicated as ep, st and hg, respectively, all appear indistinguishable from wild-type embryos. We note that *mof*$^{2\ m\text{-}z\text{-}}$ male embryos do not secrete any or only very rudimentary fragments of embryonic cuticle. We also note that these phenotypes of *mof*$^{2\ m\text{-}z\text{-}}$ male embryos are all attributed to the lack of Mof protein because a transgene containing a genomic *mof*$^{+}$ DNA fragment (i.e. *mof*$^{+6.8}$) permits to maintain *mof*^{2} *FRT19A* homo- and hemizygotes as a healthy strain.

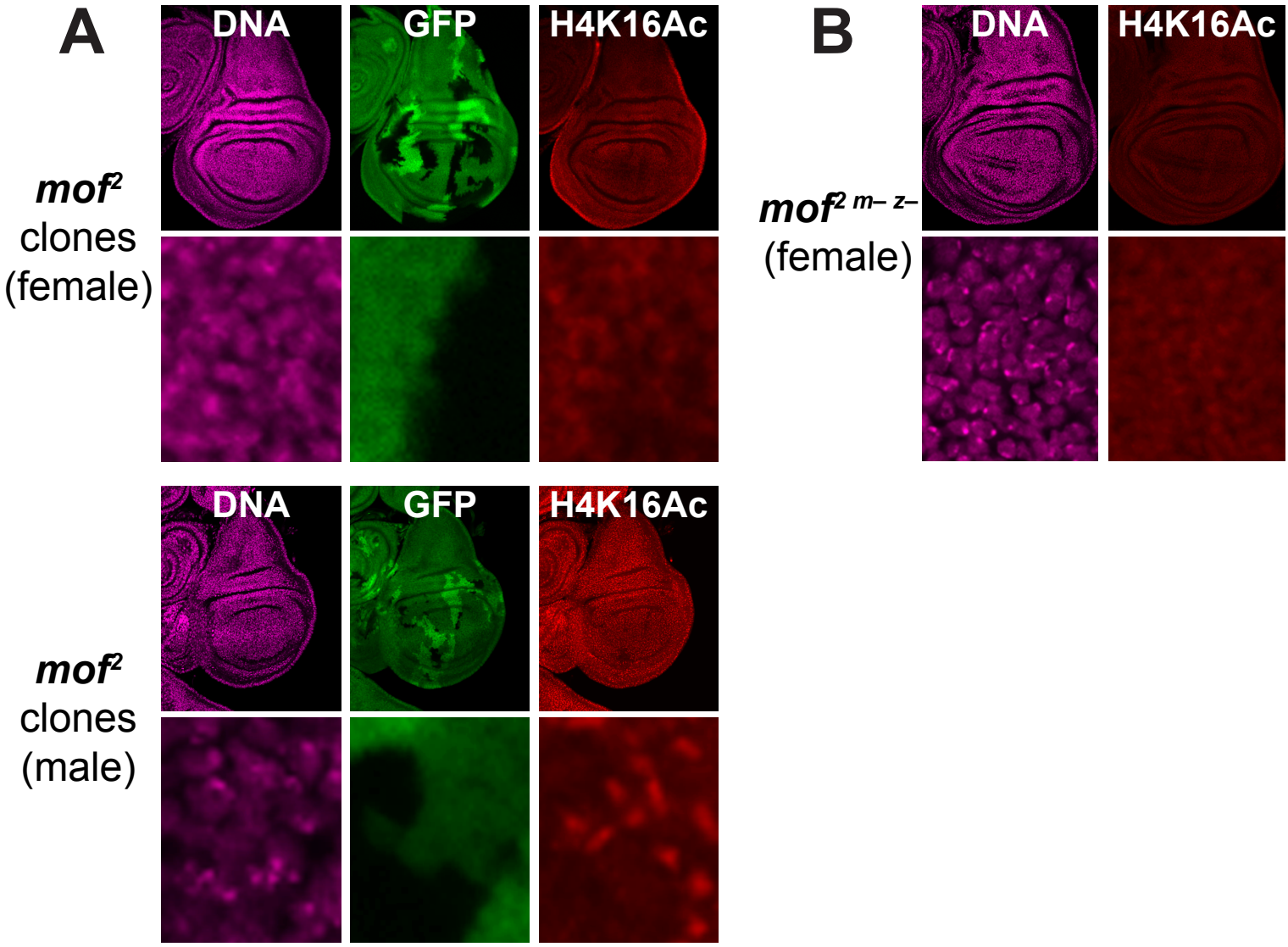

Figure S4

Mof is not the major H4K16 acetyltransferase in females but generates the bulk of H4K16ac in males

(A) Imaginal wing discs from female and male larvae with clones of *mof*^{2} homozygous mutant cells that are marked by the lack of GFP, stained with antibody against H4K16ac and with Hoechst (DNA) to visualize nuclei. Lower rows show representative area of disc with wild-type and *mof*^{2} mutant cells at higher magnification. Wild-type cells in both sexes show a relatively uniform nuclear H4K16Ac signal but males in addition also show a bright spot of H4K16Ac signal that corresponds to the X chromosome territory (1). Note that the nuclear H4K16ac signal in *mof*^{2} mutant cells in females is only mildly reduced compared to neighbouring wild-type cells. In *mof*^{2} mutant cells in males, both the X chromosome-specific and the more uniform nuclear H4K16ac signal are strongly diminished (compare signal in *mof*^{2} mutant cells with lack of H4K16Ac signal in clones of H4K16R mutant cells in Figure 2A). Genotypes and clone induction like in Figure 2.

(B) Imaginal wing disc from a *mof*$^{2\ m-\ z-}$ mutant female, stained in parallel with the discs shown in (A). Note that there is clearly detectable nuclear H4K16ac signal (compare with lack of H4K16Ac signal in clones of H4K16R mutant cells in Figure 2A). Lower row shows representative area of disc at higher magnification.

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Table S1

Description of the genotypes of the strains used in this study and of the animals shown in each figure panel

Drosophila strain genotypes

*w^{1118}/ Dp(1;Y)y^+ P{ry+11} P{w+mC=ActGFP}JMR1 (*designated *Y-GFP$^+$* below*)*
w/Y-GFP$^+$: Df(2L)His^C FRT40A/CyO-RFP; 3xHisGU^{wt}(68E) 3xHisGU^{wt}(86Fb), His4r$^\Delta$
w/Y-GFP$^+$: Df(2L)His^C FRT40A/CyO-RFP; 3xHisGU^{H4K16R}(VK33) 3xHisGU^{H4K16R}(86Fb), His4r$^\Delta$
w/Y-GFP$^+$: Df(2L)His^C FRT40A/CyO-RFP; 3xHisGU^{H4K16Q}(VK33) 3xHisGU^{H4K16Q}(86Fb), His4r$^\Delta$/TM6B
w/Y-GFP$^+$: Df(2L)His^C FRT40A/CyO-RFP; 3xHisGU^{H4K16A}(68E) 3xHisGU^{H4K16A} (86Fb) His4r$^\Delta$
w; Df(2L)His^C FRT40A; 3xHisGU^{H4K16R}(VK33) 3xHisGU^{H4K16R}(86Fb), His4r$^\Delta$ / SM5⍁TM6
ywhsFLP; hs-nGFP FRT40A; 3xHisGU^{H4K16R}(VK33) 3xHisGU^{H4K16R}(86Fb), His4r$^\Delta$
w; Df(2L)His^C FRT40A; 3xHisGU^{H4K16Q}(VK33) 3xHisGU^{H4K16Q}(86Fb), His4r$^\Delta$/ SM5⍁TM6
ywhsFLP; hs-nGFP FRT40A; 3xHisGU^{H4K16Q}(VK33) 3xHisGU^{H4K16Q}(86Fb), His4r$^\Delta$
w; Df(2L)His^C FRT40A; 3xHisGU^{H4K16A}(68E) 3xHisGU^{H4K16A} (86Fb) His4r$^\Delta$ / SM5⍁TM6
ywhsFLP; hs-nGFP FRT40A; 3xHisGU^{H4K16A}(68E) 3xHisGU^{H4K16A} (86F)b His4r$^\Delta$
yw;Df(2L)His^C FRT40A; 3xHisGU^{H4K16R}(86Fb) His4r$^\Delta$/ SM5⍁TM6
yw hsFLP; ry$^+$ y$^+$ FRT40A; 3xHisGU^{H4K16R}(86Fb) His4r$^\Delta$
yw; Df(2L)His^C FRT40A; 3xHisGU^{H4K16Q}(86Fb), His4r$^\Delta$/ SM5⍁TM6
yw hsFLP; ry$^+$ y$^+$ FRT40A; 3xHisGU^{H4K16Q}(86Fb) His4r$^\Delta$
yw; Df(2L)His^C FRT40A; 3xHisGU^{H4K16A}(86Fb), His4r$^\Delta$/ SM5⍁TM6
yw hsFLP; ry$^+$ y$^+$ FRT40A; 3xHisGU^{H4K16A}(86Fb) His4r$^\Delta$
yw, mof^2/FM7c, P{2xTb[1]-RFP}, sn[+]
yw,mof^2; mof$^{+6.8}$ (refs. 2,3)
w; UbiGFPnls FRT40A
ovo^{D1} yw hsFLP FRT19A/ C(1)DX
yw mof^2 FRT19A/ FM7, Act-GFP
w, ph^{504}/FM7c,ftz-LacZ
yw, mof^2; UbiGFPnls mof$^{+6.8}$ FRT40A
yw, hsFLP, mof^2/FM7c; FRT40A
yw, mof^2; y$^+$ mof$^{+6.8}$ FRT40A

Genotypes of animals shown in each figure panel

Figure 1

wt (M)	*w/ Y-GFP$^+$*
wt (F)	*w/w*
H4^{wt} (M)	*w/Y-GFP$^+$; Df(2L)His^{C}FRT40A; 3xHisGU^{wt}(68E) 3xHisGU^{wt}(86Fb), His4r$^\Delta$*
H4^{wt} (F)	*w/w; Df(2L)His^C FRT40A; 6xHisGU^{wt}, 3xHisGU^{wt}(68E) 3xHisGU^{wt}(86Fb), His4r$^\Delta$*
H4^{H4K16R} (M)	*w/Y-GFP$^+$; Df(2L)His^C FRT40A; 3xHisGU^{H4K16R}(VK33) 3xHisGU^{H4K16R}(86Fb), His4r$^\Delta$*
H4^{H4K16R} (F)	*w/w; Df(2L)His^C FRT40A; 3xHisGU^{H4K16R}(VK33) 3xHisGU^{H4K16R}(86Fb), His4r$^\Delta$*
H4^{H4K16Q} (M)	*w/Y-GFP$^+$; Df(2L)His^C FRT40A; 3xHisGU^{H4K16Q}(VK33) 3xHisGU^{H4K16Q}(86Fb), His4r$^\Delta$*
H4^{H4K16Q} (F)	*w/w; Df(2L)His^C FRT40A; 3xHisGU^{H4K16Q}(VK33) 3xHisGU^{H4K16Q}(86Fb), His4r$^\Delta$*
H4^{H4K16A} (M)	*w/Y-GFP$^+$; Df(2L)His^C FRT40A; 3xHisGU^{H4K16A}(68E) 3xHisGUH4^{K16A}(86Fb) His4r$^\Delta$*
H4^{H4K16A} (F)	*w/w; Df(2L)His^C FRT40A; 3xHisGU^{H4K16A}(68E) 3xHisGU^{H4K16A}(86Fb) His4r$^\Delta$*
mof^{z-} (M)	*yw mof^2/Y*
mof^{z-} (F)	*yw mof^2/ yw mof^2; UbiGFPnls FRT40A/+*
mof^{m-z-} (M)	*yw mof^2 FRT19A/Y*
mof^{m-z-} (F)	*yw mof^2 FRT19A/ yw mof^2; UbiGFPnls FRT40A/+*

mof$^{2\ z-}$ females were obtained by selecting RFP-negative and GFP-positive animals among the progeny from a cross of *yw mof^2/FM7c, P{2xTb[1]-RFP}, sn[+]* females with *yw mof^2; mof$^{+6.8}$ / UbiGFPnls FRT40A* males. *mof$^{2\ m-\ z-}$* females were obtained by selecting GFP-positive progeny produced by a cross of heat shock-treated *ovo^{D1} yw hsFLP FRT19A / yw mof^2 FRT19A* females with *yw mof^2; mof$^{+6.8}$ / UbiGFPnls FRT40A* males.

Figure 2

A:

H4^{H4K16R} (M): *ywhsFLP; Df(2L)His^{C} FRT40A/ hs-nGFP FRT40A; 3xHisGU^{H4K16R}(VK33) 3xHisGU^{H4K16R}(86Fb), His4r$^{\Delta}$*

H4^{H4K16R} (F): *ywhsFLP/w; Df(2L)His^{C} FRT40A/ hs-nGFP FRT40A; 3xHisGU^{H4K16R}(VK33) 3xHisGU^{H4K16R}(86Fb), His4r$^{\Delta}$*

B,C:

H4^{H4K16R} (M): *ywhsFLP; Df(2L)His^{C} FRT40A/ hs-nGFP FRT40A; 3xHisGU^{H4K16R}(VK33) 3xHisGU^{H4K16R}(86Fb), His4r$^{\Delta}$*

H4^{H4K16R} (F): *ywhsFLP/w; Df(2L)His^{C}FRT40A/ hs-nGFP FRT40A; 3xHisGU^{H4K16R}(VK33) 3xHisGU^{H4K16R}(86Fb), His4r$^{\Delta}$*

H4^{H4K16Q} (M): *ywhsFLP; Df(2L)His^{C} FRT40A/ hs-nGFP FRT40A; 3xHisGU^{H4K16Q}(VK33) 3xHisGU^{H4K16Q}(86Fb), His4r$^{\Delta}$*

H4^{H4K16Q}(F): *ywhsFLP/w; Df(2L)His^{C}FRT40A/ hs-nGFP FRT40A; 3xHisGU^{H4K16Q}(VK33) 3xHisGU^{H4K16Q}(86Fb), His4r$^{\Delta}$*

H4^{H4K16A} (M): *ywhsFLP; Df(2L)His^{C} FRT40A/ hs-nGFP FRT40A; 3xHisGU^{H4K16A}(68E) 3xHisGUH4K16A(86Fb) His4r$^{\Delta}$*

H4^{H4K16A}(F): *ywhsFLP/w; Df(2L)His^{C} FRT40A/ hs-nGFP FRT40A; 3xHisGU^{H4K16A}(68E) 3xHisGUH4K16A(86Fb) His4r$^{\Delta}$*

mof^{2-} (M): *ywhsFLP mof^{2}/Y; UbiGFPnls mof$^{+6.8}$ FRT40A/ FRT40A*

mof^{2-} (F): *ywhsFLP mof^{2}/yw mof^{2}; UbiGFPnls mof$^{+6.8}$ FRT40A/ FRT40A*

Because of the location of *mof^{2}* on the X chromosome, the following strategy was used to generated male and female mutant clones with the same genetic background: *ywhsFLP mof^{2}/FM7c; FRT40A* females were crossed to *yw mof^{2}; UbiGFPnls mof$^{+6.8}$ FRT40A* males.

Figure 3

H4^{H4K16R} (M): *ywhsFLP; Df(2L)His^{C} FRT40A/ ry^{+} y^{+} FRT40A; 3xHisGU^{H4K16R}(86Fb), His4r$^{\Delta}$*

H4^{H4K16Q} (M): *ywhsFLP; Df(2L)His^{C} FRT40A/ ry^{+} y^{+} FRT40A; 3xHisGU^{H4K16Q}(86Fb), His4r$^{\Delta}$*

H4^{H4K16A} (M): *ywhsFLP; Df(2L)His^{C} FRT40A/ ry^{+} y^{+} FRT40A; 3xHisGU^{H4K16A}(86Fb), His4r$^{\Delta}$*

mof^{2-} (M): *ywhsFLP mof^{2}/Y; y^{+} mof$^{+6.8}$ FRT40A/ FRT40A*

For generating *mof^{2}* mutant clones in adult males, *ywhsFLP mof^{2}/Y; y^{+} mof$^{+6.8}$ FRT40A/ FRT40A* were generated by crossing *ywhsFLP mof^{2}/FM7c; FRT40A* females with *yw mof^{2}; y^{+} mof$^{+6.8}$ FRT40A* males.

References

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3. Gu W, Szauter P, Lucchesi JC (1998) Targeting of MOF, a putative histone acetyl transferase, to the X chromosome of Drosophila melanogaster. *Dev Genet* 22(1):56–64.