APPENDIX

Ash1 counteracts Polycomb repression independent of H3K36 methylation.

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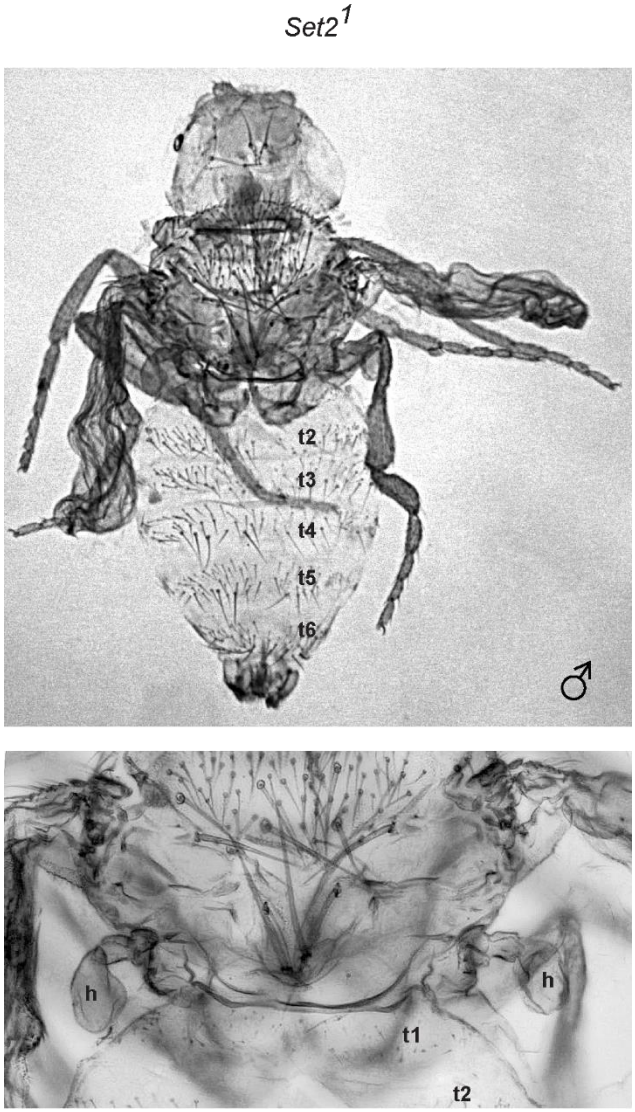
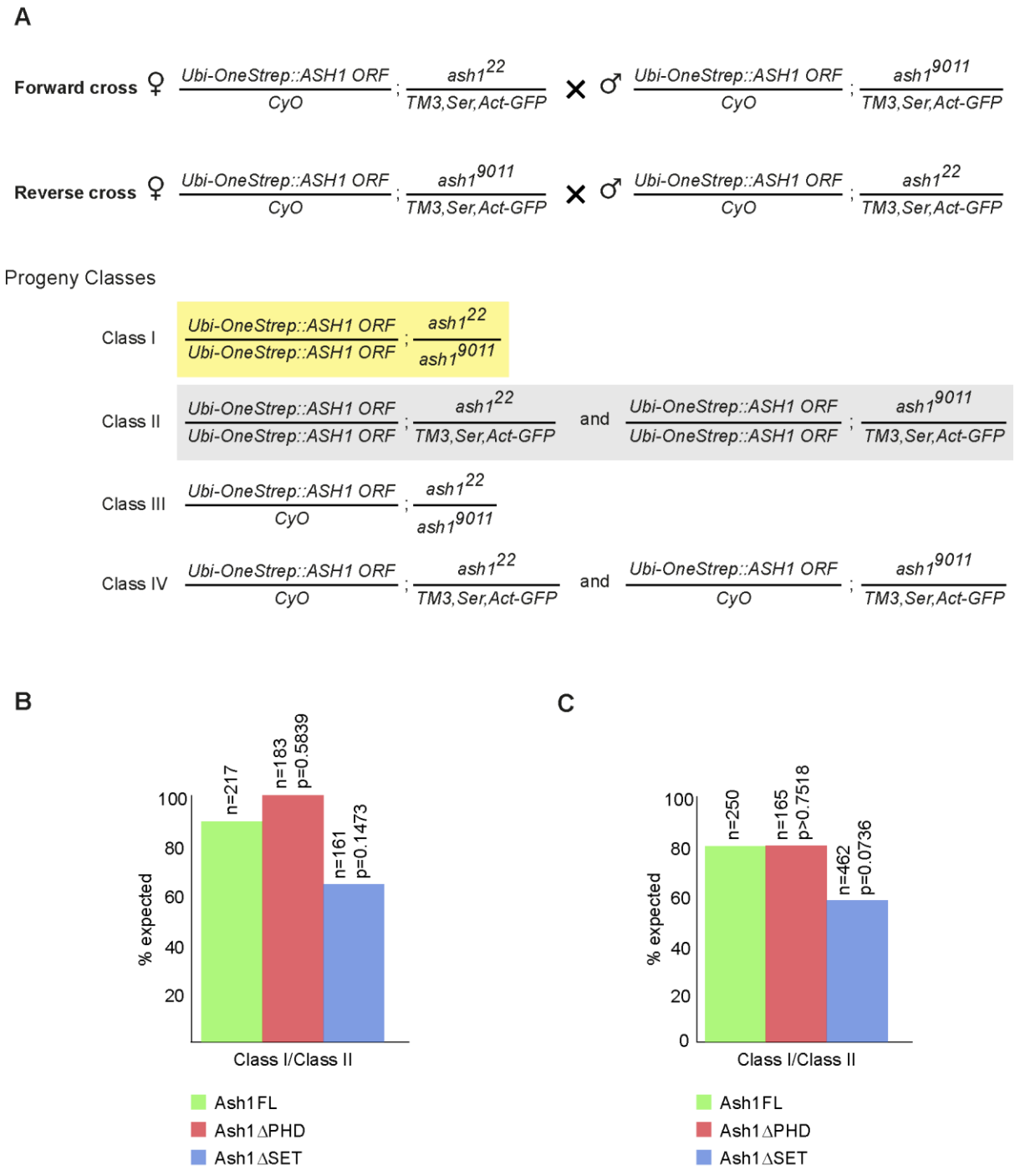
Appendix Figure S1



Appendix Figure S1. Adult phenotype of the *Set2* loss of function mutant. Most flies homozygous for the *Set2*[1] null allele die at the late larval or early pupal stages. Out of 221 homozygous *Set2*[1] pupae, only one male fully developed to pharate adult stage. In that animal, shown here, no homeotic transformations are visible. The halters (h), third legs and all tergites (t1-t6) appear normal and show no signs of posterior to anterior transformations.

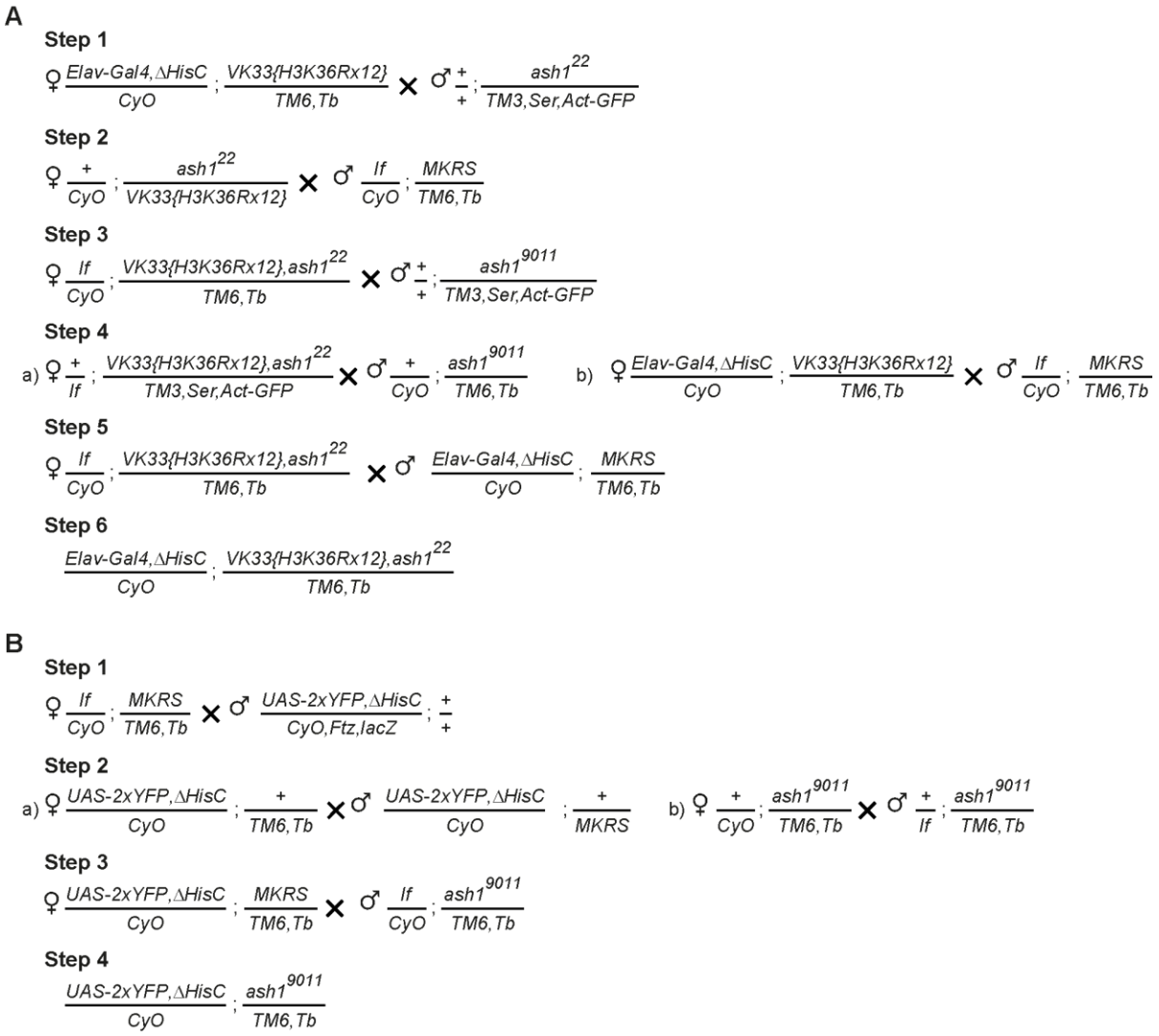
Appendix Figure S2



Appendix Figure S2. Complementation of *ash1* mutations by transgenic Ash1 variants. **(A)** Schematics of crosses and the progeny classes. To test whether different variants of the transgenic Ash1 can compensate for the *ash1* loss of function, the transgenes were introduced into the flies trans-heterozygous for $ash1^{22}$ and $ash1^{9011}$ alleles. To this effect, females of a

strain containing one copy of the Ash1 transgene, balanced over *CyO* on the second chromosome, and the *ash1*^{22} allele, balanced over *TM3,Ser,Act-GFP* on the third chromosome, were crossed to males from a strain containing the same second chromosome combination but *ash1*^{9011} instead of *ash1*^{22} on the third chromosome (Forward cross). The reciprocal cross was also set up, where the genotypes of males and females were switched (Reverse cross). Three different Ash1 transgenes were used in this experiment. One (*Ash1FL*) contained the full-length *ash1* Open Reading Frame (ORF), and the two truncated variants contained ORFs that lacked either the SET domain (*Ash1ΔSET*) or the PHD domain (*Ash1ΔPHD*). The expression of all transgenes was driven by the *Ubiquitin* (*Ubi-p63E*) promoter. In the progeny, Class I (yellow rectangle) and Class II (grey rectangle) contained two copies of the Ash1 transgenes. **(B-C)** The viability of *ash1*^{22}*/ash1*^{9011} flies complemented by different transgenic Ash1 variants. The ratio between Class I/Class II progeny was calculated for each transgenic Ash1 variant to gauge the viability (rescue). In the case of complete rescue, the Class I/Class II ratio is expected to be ½, which we designated as 100%. The χ^2 test shows that the two copies of *Ash1ΔSET*, *Ash1ΔPHD* and *Ash1FL* transgenes restore the viability of *ash1*^{22}*/ash1*^{9011} flies to comparable extent. Graph (**B**) corresponds to the Forward cross in (**A**) and graph (**C**) corresponds to the Reverse cross. n indicates the number of Class I + Class II progeny counted.

Appendix Figure S3

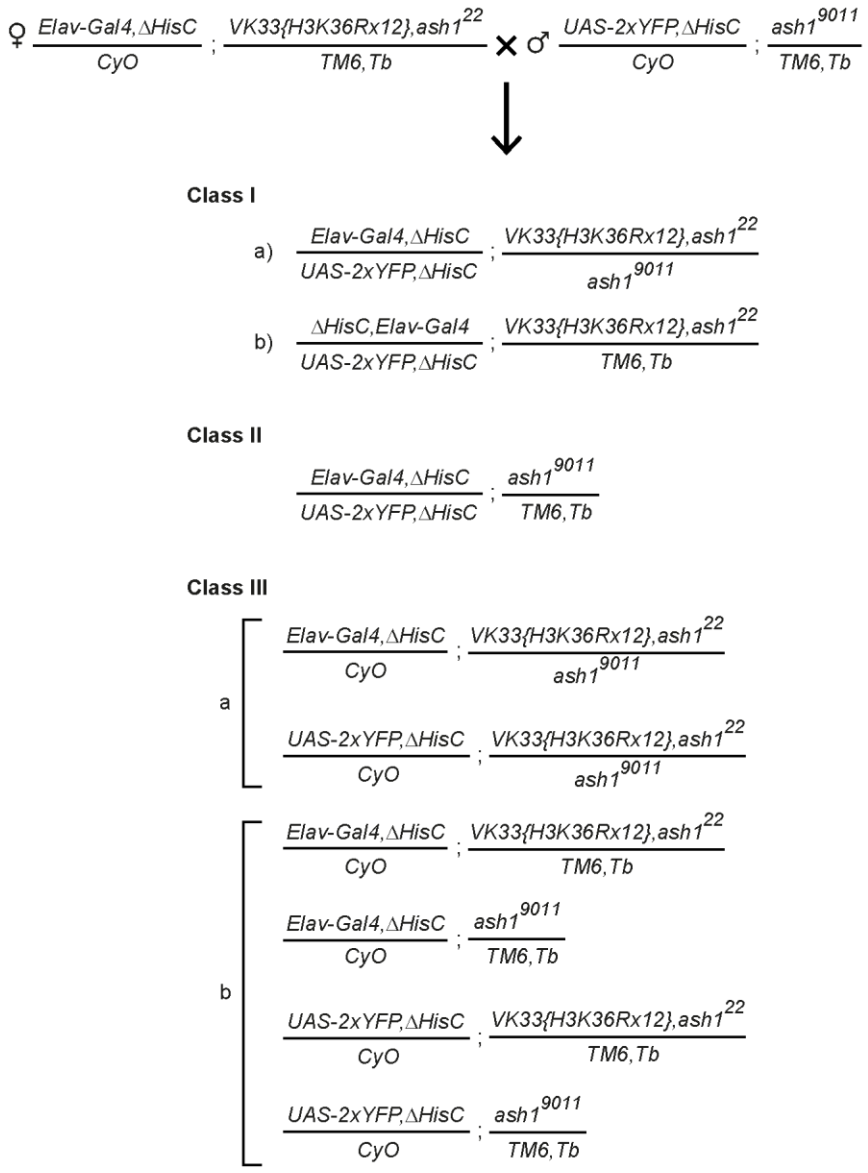


Appendix Figure S3. Generation of fly strains that combine the *Elav-Gal4,ΔHisC or UAS-2xYFP,ΔHisC,* chromosomes with *ash1* mutations and the *VK33{H3K36Rx12}* transgene. (**A**) To combine the *Elav-Gal4,ΔHisC* chromosome with the *ash1*^{22} allele and the *VK33{H3K36Rx12}* transgene, the female flies with the *Elav-Gal4,ΔHisC/CyO* 2nd chromosomes, and the *VK33{H3K36Rx12}* transgene on the third chromosome, were crossed with the *ash1*^{22}*/TM3,Ser,Act-GFP* males (Step 1). From the progeny of Step 1, females lacking the *TM3,Ser,Act-GFP* balancer but containing the *CyO* balancer were crossed to males of the double-balancer strain (*If/CyO; MKRS/TM6,Tb*) (Step 2). The recombination between *ash1*^{22}

and *VK33{H3K36Rx12}* occurred in females in this step. Individual *If/CyO* females with the *TM6,Tb* balancer chromosome, from the progeny of Step 2, were crossed to the *ash1*^{9011}*/TM3,Ser,Act-GFP* males (Step 3). Allowing the females to lay eggs, the genomic DNA of these females was extracted and screened by PCR for the presence of the *ash1*^{22} mutation and the *VK33{H3K36Rx12}* transgene (see Appendix Figure S5). From the progeny of the Step 3 crosses, the females that contained the recombinant *VK33{H3K36Rx12},ash1*^{22} chromosome, the *If* marker and the *TM3,Ser,Act-GFP* balancer were crossed to males that carried the *CyO* and *TM6,Tb* balancers (Step 4a). In parallel (Step 4b), the females from the *Elav-Gal4,ΔHisC/CyO* ; *VK33{H3K36Rx12}/TM6b,Tb* strain were crossed to males of the double-balancer strain. From the progeny of Step 4a, the *If/CyO* females with the *TM6,Tb* balancer were crossed with the *CyO* males carrying both the *MKRS* marker and the *TM6,Tb* balancer but lacking the *If* marker (Step 5). In Step 6, the females and males carrying the *CyO* and *TM6,Tb* balancer chromosomes but lacking the *If* and *MKRS* markers were selected to establish the *Elav-Gal4,ΔHisC/CyO ; VK33{H3K36Rx12},ash1*^{22}*/TM6,Tb* fly stock. **(B)** To combine the *UAS-2xYFP,ΔHisC* chromosome with the *ash1*^{9011} allele, females of the double-balancer line were crossed with the *UAS-2xYFP,ΔHisC/CyO* males (Step 1). In Step 2, the females carrying *CyO* and *TM6,Tb* balancers and lacking the *If* and *MKRS* markers were crossed with the *CyO* males lacking the *If* marker and carrying the *MKRS* chromosome (Step 2a). In parallel (Step 2b), the *CyO* females and the *If* males, both carrying *TM6,Tb* balancer, and both from the progeny of Step 3 in (**A**), were crossed to each other. In Step 3, females with the *MKRS* chromosome and the *TM6,Tb* balancer from the progeny of Step 2a were crossed to the *If/CyO* males carrying *TM6,Tb* balancer, from the progeny of Step 2b. In the next step the flies with the *CyO* and *TM6,Tb* balancers but lacking *If* and *MKRS* were selected from the progeny of Step 3 to establish the strain with the genotype shown in Step 4.

Appendix Figure S4

A



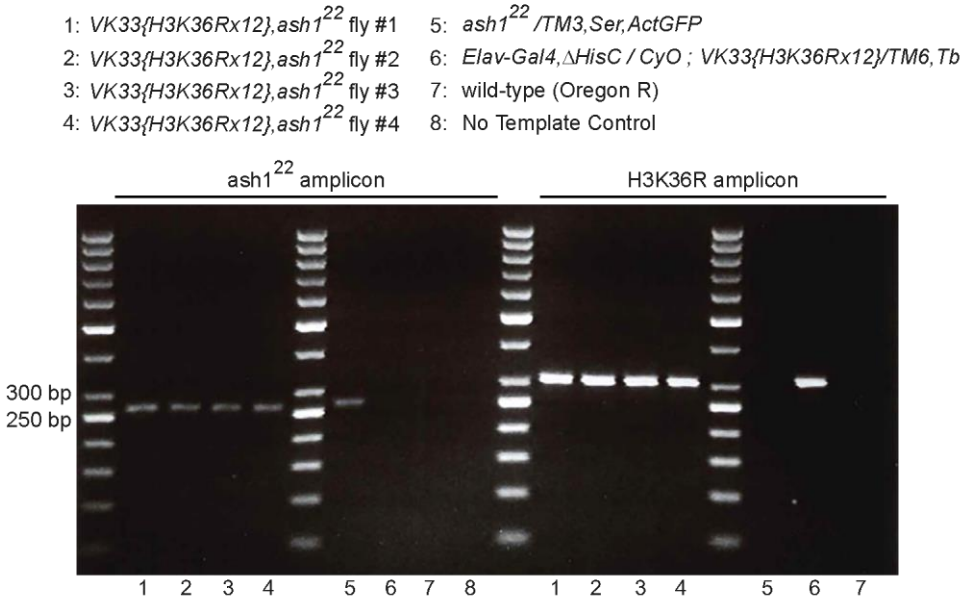
B

	YFP positive (Class I)		YFP negative (Class III)	
Starting number of flies	500		650	
Number of dead pupae	Class Ia (non-Tb)	2	0	
	Class Ib (Tm6,Tb)	186 (37%)	0	
Number of flies hatched	0		Class IIIa (non-Tb)	0
	0		Class IIIb (Tm6,Tb)	403 (62%)

Appendix Figure S4. Genetic interaction between the *ash1* and *H3K36R* mutations. (**A**) To generate the *ΔHisC;ash1⁻,H3K36R* animals, the *Elav-Gal4,ΔHisC /CyO ; VK33{H3K36Rx12}, ash1^{22} /TM6,Tb* flies from Appendix Figure S3A were crossed with the

UAS-2xYFP,ΔHisC/CyO; ash1^{9011}*/TM6,Tb* flies from Appendix Figure S3B. In the progeny, three classes are expected. Classes I and II both show the YFP signal in the fluorescent stereomicroscope and therefore are distinguishable from Class III which lacks the YFP signal. Although both Classes I and II show the YFP signal, the Class II progeny die at early embryonic stage due to the lack of zygotic histone supply. The *Tb* marker on the *TM6,Tb* chromosome is used to distinguish the Class Ib flies from Class Ia and Class IIIb flies from Class IIIa. **(B)** Analysis of Class I and Class III (control) viability. While no adult flies emerge from the Class I larvae, 62% (close to expected) of the Class III (shown as class IIIb) larvae develop to the adult fly stage. Note that Class IIIa larvae are expected to die since they carry both the *ash1*^{22} and *ash1*^{9011} alleles.

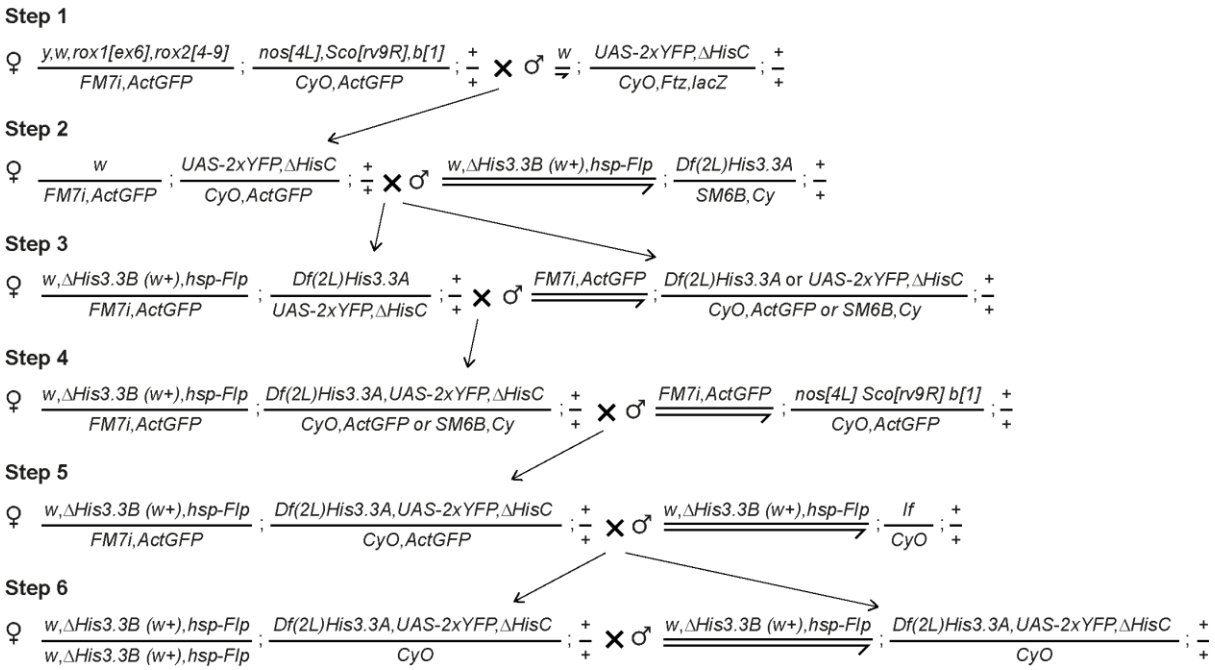
Appendix Figure S5



Amplicons	Forward primer	Reverse primer	Product in mutant/transgenic line	Product in wild-type
ash1^{22}	ash1-22.2	ash1-21.rev	264 bp	No product
H3K36R	H3K36R	His3-rev	300 bp	No product

Appendix Figure S5. The screen for the recombination between *ash1^{22}* and the *H3K36R* transgene. The single flies, described in Appendix Figure S3A, were genotyped by PCR, using the primer specific for the *ash1^{22}* mutation and the *VK33{H3K36Rx12}* transgene. The forward primer in the ash1^{22} amplicon is designed to match the single nucleotide substitution in the *ash1^{22}* mutation and therefore anneals only to the mutant but not to the wild-type genomic DNA. In the H3K36R amplicon, one of the primers (H3K36R) uses the three nucleotide-difference between the wild-type and the transgenic copies of the *His3.2* gene and therefore is specific for the *VK33{H3K36Rx12}* transgene as the template for PCR reaction. Four single flies positive for both the *ash1^{22}* mutation and the *VK33{H3K36Rx12}* transgene are shown here. PCR products were analyzed by the electrophoresis in 2% agarose gel. M = 50bp DNA Ladder.

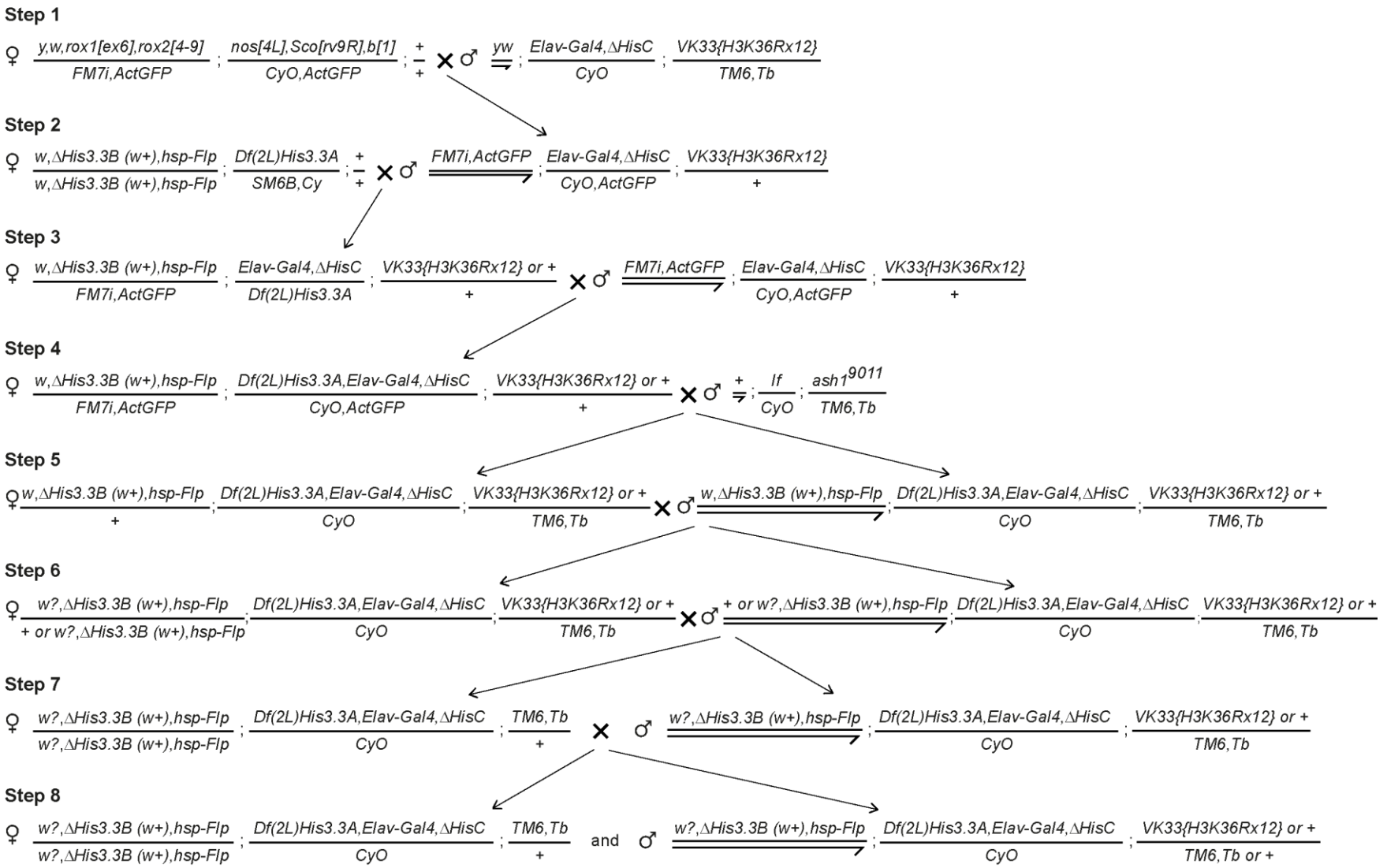
Appendix Figure S6



Appendix Figure S6. Generation of the *Drosophila* strain that combines the *His3.3* deletions and the *UAS-2xYFP,ΔHisC* chromosome. To generate the *w,ΔH3.3B(w^+),hsp-Flp ; Df(2L)His3.3A,ElavGal4,ΔHisC/CyO ; +/+* fly strain, the *FM7i,ActGFP* chromosome was first introduced into the *UAS-2xYFP,ΔHisC/CyO ; +/+* fly strain (Step 1). In the progeny of Step 1, the females with *FM7i,ActGFP* balancer and without the second chromosome marked by *Sco* (therefore carrying the *UAS-2xYFP,ΔHisC* chromosome) were collected and crossed to males of the *w,ΔHis3.3B (w^+),hsp-Flp ; Df(2L)His3.3A/SM6B,Cy* genotype (Step 2). In Step 3, the females with *FM7i,ActGFP* balancer (carrying the *His3.3B* deletion) and without second chromosome balancer were selected. This provides a chance for recombination between *Df(2L)His3.3A* and *UAS-2xYFP,ΔHisC*. These females were crossed with the males hemizygous for *FM7i,ActGFP* and carrying either the *SM6B,Cy* or *CyO,ActGFP* second chromosome balancer. In Step 4, the female progeny of Step 3, carrying either *SM6B,Cy* or *CyO,ActGFP* were selected and crossed individually with the *FM7i,ActGFP; nos[4L],sco[rv9R],b[1]/CyO,ActGFP* males. Note that all females contained the *His3.3B*

deletion balanced over *FM7i,ActGFP*. After several days of egg deposition, genomic DNA was extracted from the single females of each cross. This DNA was used to screen for recombination on the second chromosome (simultaneous presence of the *ΔHisC* deletion, the *UAS-2xYFP* transgene, and the *His3.3A* deletion). Note that the *CyO,ActGFP* balancer will interfere with the detection of the YFP signal used to select for embryos homozygous for *ΔHisC* (for details see Figure EV5). To replace *CyO,ActGFP* with the *CyO* balancer, the female progeny of the crosses with the recombinant second chromosome (from Step 4), were crossed to the males hemizygous for the *His3.3B* deletion, carrying the *If* marker and the *CyO* balancer (Step 5). Finally, in Step 6, the female and male progeny of Step 5, lacking both the *FM7i,ActGFP* balancer and the *If* marker were selected to establish the strain to be used for the cross described in Figure EV5. The founders of this stock were PCR genotyped as described in Appendix Figure S9.

Appendix Figure S7

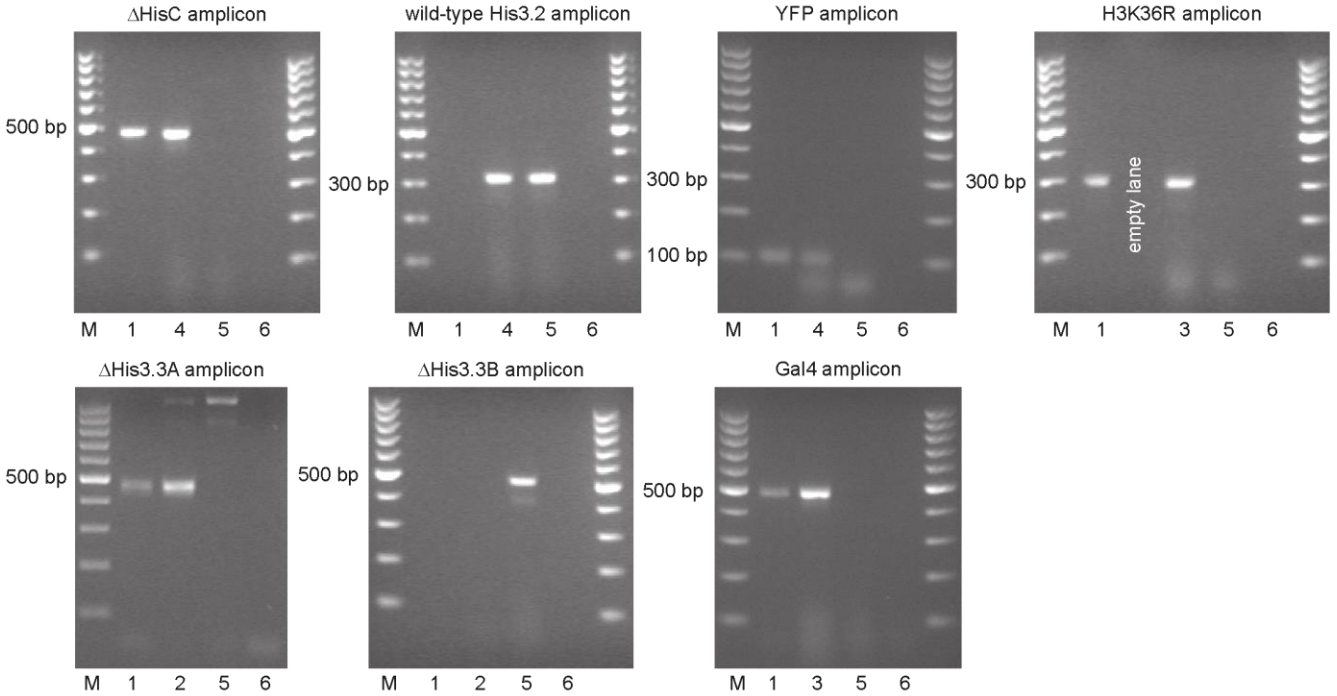


Appendix Figure S7. Generation of *Drosophila* males that combine the *His3.2* and *His3.3* deletions with *Elav-Gal4,ΔHisC* and the *H3K36R* transgene. To generate the *w,ΔH3.3B(w^+),hsp-Flp ; Df(2L)His3.3A,ElavGal4,ΔHisC/CyO ; VK33{H3K36Rx12}/TM6,Tb/+* fly strain, we started by introducing the first chromosome balancer (*FM7i,ActGFP*) into the *Elav-Gal4,ΔHisC/CyO ;VK33{H3K36Rx12}/TM6,Tb* fly strain (Step 1). The *FM7i,ActGFP* balancer is marked with the dominant marker *Bar*. In the progeny of the Step 1 cross, we selected the male flies showing the *Bar* phenotype (hence *FM7i,ActGFP*), lacking the *Sco* marker on the second chromosome (therefore carrying the *Elav-Gal4* transgene) and lacking the *TM6,Tb* third balancer (thus containing *VK33{H3K36Rx12}*). Those males were crossed with the *w,ΔHis3.3B(w^+),hsp-Flp; Df(2L)His3.3A/SM6B,Cy* females (Step 2). This introduces the *His3.3B* deletion (balanced over *FM7i,ActGFP*) and generates conditions to recombine the *Df(2L)His3.3A* deletion and the *Elav-Gal4* transgene. To allow the recombination in Step 3, the female offspring from Step 2 that lacked the second chromosome balancer was backcrossed to the same males as in Step 2. In the next step (Step 4), the female progeny from Step 3 that had been balanced over *CyO,ActGFP* was crossed individually to the *If/CyO ; ash1^{9011}/TM6,Tb* males. Note that the *VK33{H3K36Rx12}* chromosome is homozygous lethal, probably due to additional mutation. After several days of egg deposition, genomic DNA of the single females from each individual cross was extracted and screened by PCR for the presence of the *Elav-Gal4* transgene, the *ΔHisC and ΔHis3.3A* deletions on the second chromosome and the *VK33{H3K36Rx12}* transgene on the third chromosome. For next step, the female and male offspring from the Step 4 individual crosses were selected to have both the *His3.3A,Elav-Gal4,ΔHisC* second chromosome balanced over *CyO,* and the *TM6,Tb* balancer. We also required that the selected flies lacked the *FM7i,ActGFP* balancer, which ensured the presence of the *His3.3B* deletion on the first chromosome. Selected females and males were crossed together (Step 5). In Step 6,

the single females and males with the *TM6,Tb* balancer from the progeny of Step 5 were used to set up individual crosses. Following several days of egg deposition, genomic DNA was of the individual males and females from each cross was extracted and tested by PCR for the presence of the *His3.3B* deletion and the *VK33{H3K36Rx12}* transgene. Note that we can detect the *His3.3B* deletion only if homozygous. In the process, we realized that the females homozygous for the *His3.3B* deletion, heterozygous for the recombinant second chromosome and carrying the *VK33{H3K36Rx12}* transgene on the third chromosome are not viable. Therefore, in step 7, females, homozygous, and males, hemizygous, for the *His3.3B* deletion both carrying the third chromosome balancer were crossed individually. After giving time for egg deposition, DNA was extracted from the single males and females of each cross and used to genotype single flies by PCR. Females were selected for the deletion of *His3.3A*, *His3.3B* and the *HisC* cluster as well as the presence of the *Elav-Gal4* transgene on the second chromosome. Males were additionally selected for the presence of the *VK33{H3K36Rx12}* transgene on the third chromosome. For details of genotyping see Appendix Figure S10. The selected males and females were used in the Step 8 cross. Unfortunately this cross did not result in a stable strain but yielded enough males with the desired genotype (*w,ΔH3.3B(w^+),hsp-Flp ; Df(2L)His3.3A,ElavGal4,ΔHisC/CyO ; VK33{H3K36Rx12}/TM6,Tb or* +) which were used in the cross described in Figure EV5.

Appendix Figure S8

1: *w,ΔHis3.3B (w+),hsp-Flp* ; *Df(2L)His3.3A,Elav-Gal4,ΔHisC* / *Df(2L)His3.3A,UAS-2xYFP,ΔHisC* ; *VK33{H3K36Rx12}* / + Embryo
2: *w,ΔHis3.3B (w+),hsp-Flp* ; *Df(2L)His3.3A* / *SM6B,Cy* ; + / + Control for deletion of *His3.3B* and *His3.3A*
3: *yw* ; *Elav-Gal4,ΔHisC* / *CyO* ; *TM6,Tb* / *VK33{H3K36Rx12}* Positive control for Gal4 and H3K36R amplicons
4: *w* ; *UAS-2xYFP,ΔHisC* / *CyO,Ftz,lacZ* ; + / + Control for deletion of *HisC* and presence of YFP
5: wild-type (Oregon R)
6: no-template control

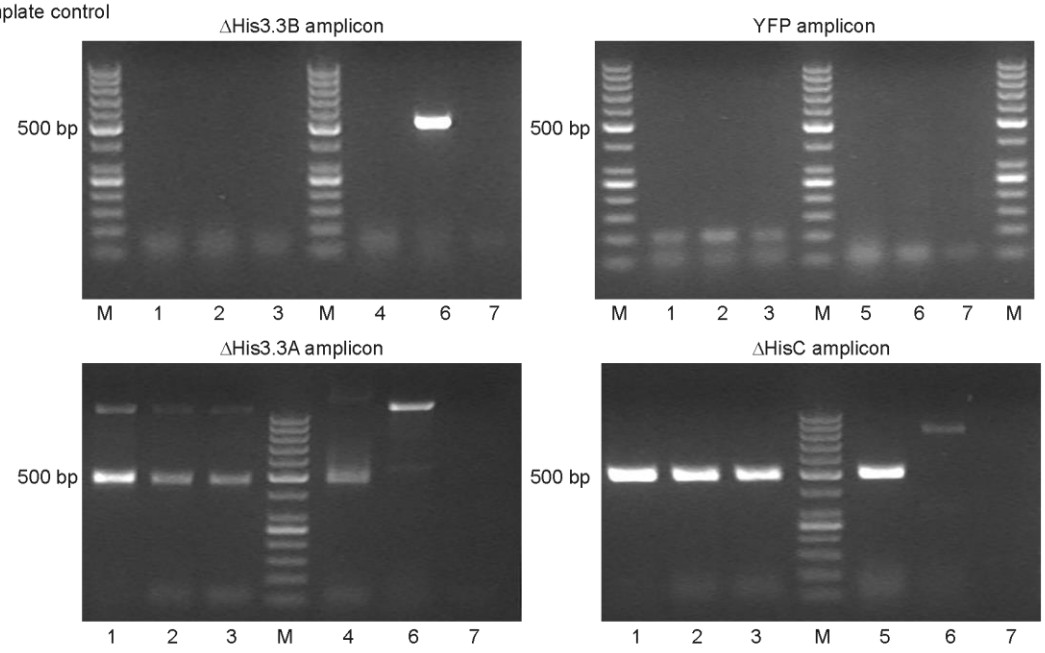


Amplicon	Forward primer	Reverse primer	Product in mutant/transgenic line	Product in wild-type
ΔHis3.3A	His3.3A_fwd	His3.3A_rev	450 bp	1054 bp
ΔHis3.3B	His3.3B_fwd	His3.3B_rev	No product	About 500 bp
ΔHisC	HisC.2	Pry4	About 500 bp	No product
H3K36R	H3K36R	His3-rev	300 bp	No product
Gal4	osGAL4:DBD-fw	CP190-GAL4	About 500 bp	No product
YFP	eGFP_F	eGFP_R	About 100 bp	No product
Wild-type His3.2	H3WT	His3-rev	No product	300 bp

Appendix Figure S8. PCR genotyping of a Class I embryo from the cross on Figure EV5 (YFP positive, wild-type zygotic H3.2 and H3.3 histones replaced with H3R36). Shown are the results of one representative genotyping experiment. To verify the identity of the Class I embryos described in Figure EV5, the genomic DNA from a single YFP+ embryo was extracted and subjected to seven separate PCR reactions using primer pairs specific for different histone H3 deletions, the presence of the *His3.2* gene and the *Elav-Gal4, UAS-2xYFP* and *VK33{H3K36Rx12}* transgenes. The products of each PCR reaction were analyzed by the electrophoresis in 2% agarose gel. M = 100bp DNA Ladder.

Appendix Figure S9

1: *w,ΔHis3.3B (w+),hsp-Flp* ; *Df(2L)His3.3A,UAS-2xYFP,ΔHisC* / *CyO* ; +/+ Female #1
2: *w,ΔHis3.3B (w+),hsp-Flp* ; *Df(2L)His3.3A,UAS-2xYFP,ΔHisC* / *CyO* ; +/+ Female #2
3: *w,ΔHis3.3B (w+),hsp-Flp* ; *Df(2L)His3.3A,UAS-2xYFP,ΔHisC* / *CyO* ; +/+ Male #1
4: *w,ΔHis3.3B (w+),hsp-Flp* ; *Df(2L)His3.3A* / *SM6B,Cy* ; +/+ Control for deletion of *His3.3B* and *His3.3A*
5: *w* ; *UAS-2xYFP,ΔHisC* / *CyO,Ftz,lacZ* ; +/+ Control for deletion of *HisC* and presence of *YFP*
6: wild-type (Oregon R)
7: no-template control

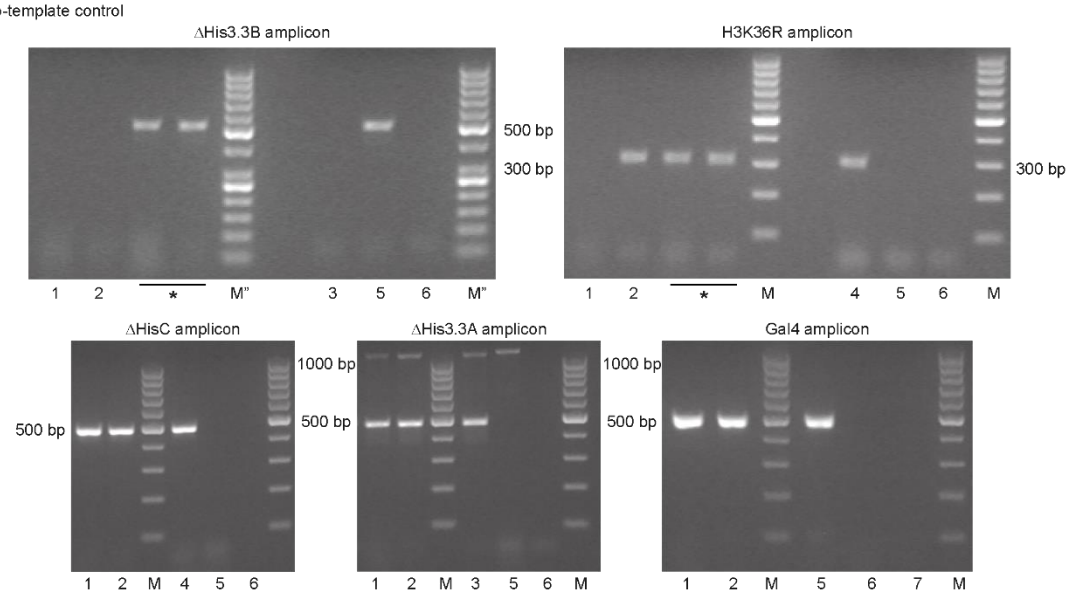


Amplicon	Forward primer	Reverse primer	Product in mutant/transgenic line	Product in wild-type
ΔHis3.3A	His3.3A_fwd	His3.3A_rev	450 bp	1054 bp
ΔHis3.3B	His3.3B_fwd	His3.3B_rev	No product	About 500 bp
ΔHisC	HisC.2	Pry4	About 500 bp	No product
YFP	eGFP_F	eGFP_R	About 100 bp	No product

Appendix Figure S9. PCR-genotyping of the parental flies for the cross of Step 6 on Appendix Figure S6. The selection of the parents (2 females and 1 male) for the correct cross described in Appendix Figure S6 (Step 6) was done by PCR. Genomic DNA was extracted from individual flies and a set of PCR reactions with specific primer pairs (described in the table below) were used detect the deletion of the histone cluster (*ΔHisC*), the deletions of the *His3.3A* and *His3.3B* genes and the presence of the *UAS-2xYFP* transgene. Shown are the pictures of the indicated PCR products separated by the electrophoresis in 2% agarose gel. 100bp DNA Ladder marker is indicated as M.

Appendix Figure S10

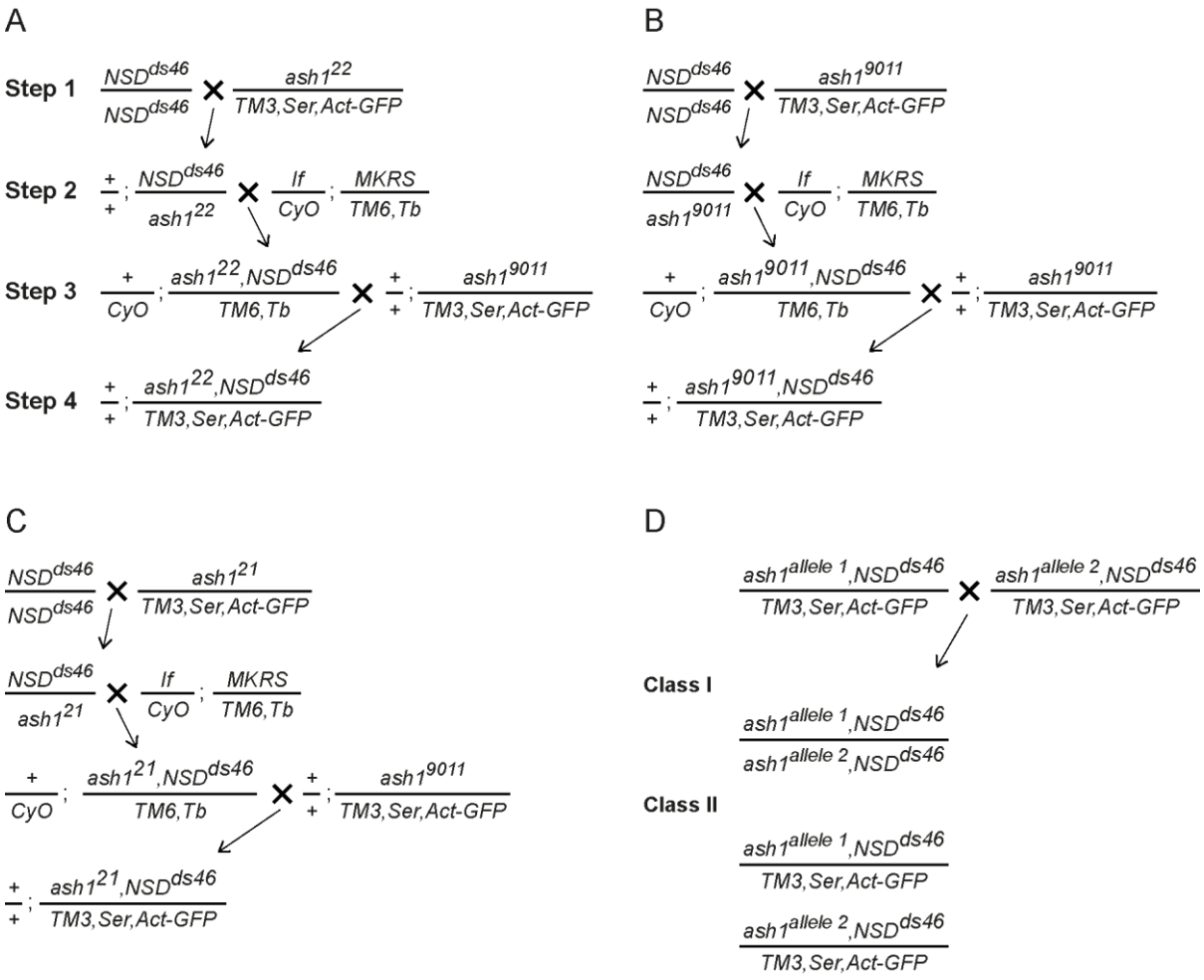
1: *w?,ΔHis3.3B (w+),hsp-Flp* ; *Df(2L)His3.3A,Elav-Gal4,ΔHisC / CyO* ; *TM6,Tb / +* Female
2: *w?,ΔHis3.3B (w+),hsp-Flp* ; *Df(2L)His3.3A,Elav-Gal4,ΔHisC / CyO* ; *TM6,Tb / VK33{H3K36Rx12}* Male
3: *w,ΔHis3.3B (w+),hsp-Flp* ; *Df(2L)His3.3A / SM6B,Cy* ; *+ / +* Control for deletion of *His3.3B* and *His3.3A*
4: *yw* ; *Elav-Gal4,ΔHisC / CyO* ; *TM6,Tb / VK33{H3K36Rx12}* Control for deletion of *HisC* and presence of *Gal4* and *H3K36R*
5: wild-type (Oregon R)
6: no-template control



Amplicon	Forward primer	Reverse primer	Product in mutant/transgenic line	Product in wild-type
ΔHis3.3A	His3.3A_fwd	His3.3A_rev	450 bp	1054 bp
ΔHis3.3B	His3.3B_fwd	His3.3B_rev	No product	About 500 bp
ΔHisC	HisC.2	Pry4	About 500 bp	No product
H3K36R	H3K36R	His3-rev	300 bp	No product
Gal4	osGAL4:DBD-fw	CP190-GAL4	About 500 bp	No product

Appendix Figure S10. PCR-genotyping of the parental flies for the cross of Step 7 on Appendix Figure S7. The selection of the parents (1 female and 1 male) for the correct cross described in Appendix Figure S7 (Step 7) was done by PCR genotyping. Genomic DNA was extracted from individual flies and a set of PCR reactions with specific primer pairs (described in the table below) were used detect the deletion of the histone cluster (*ΔHisC*), the deletions of the *His3.3A* and *His3.3B* genes and the presence of the *Elav-Gal4* and *VK33{H3K36Rx12}* transgenes. Shown are the pictures of the indicated PCR products separated by the electrophoresis in 2% agarose gel. Lanes marked with asterisks correspond to PCR reactions with genomic DNAs of single flies with undesirable genotypes. Crosses with such parents were discarded. 100bp DNA Ladder marker is indicated as M; 50bp DNA Ladder marker is indicated as M".

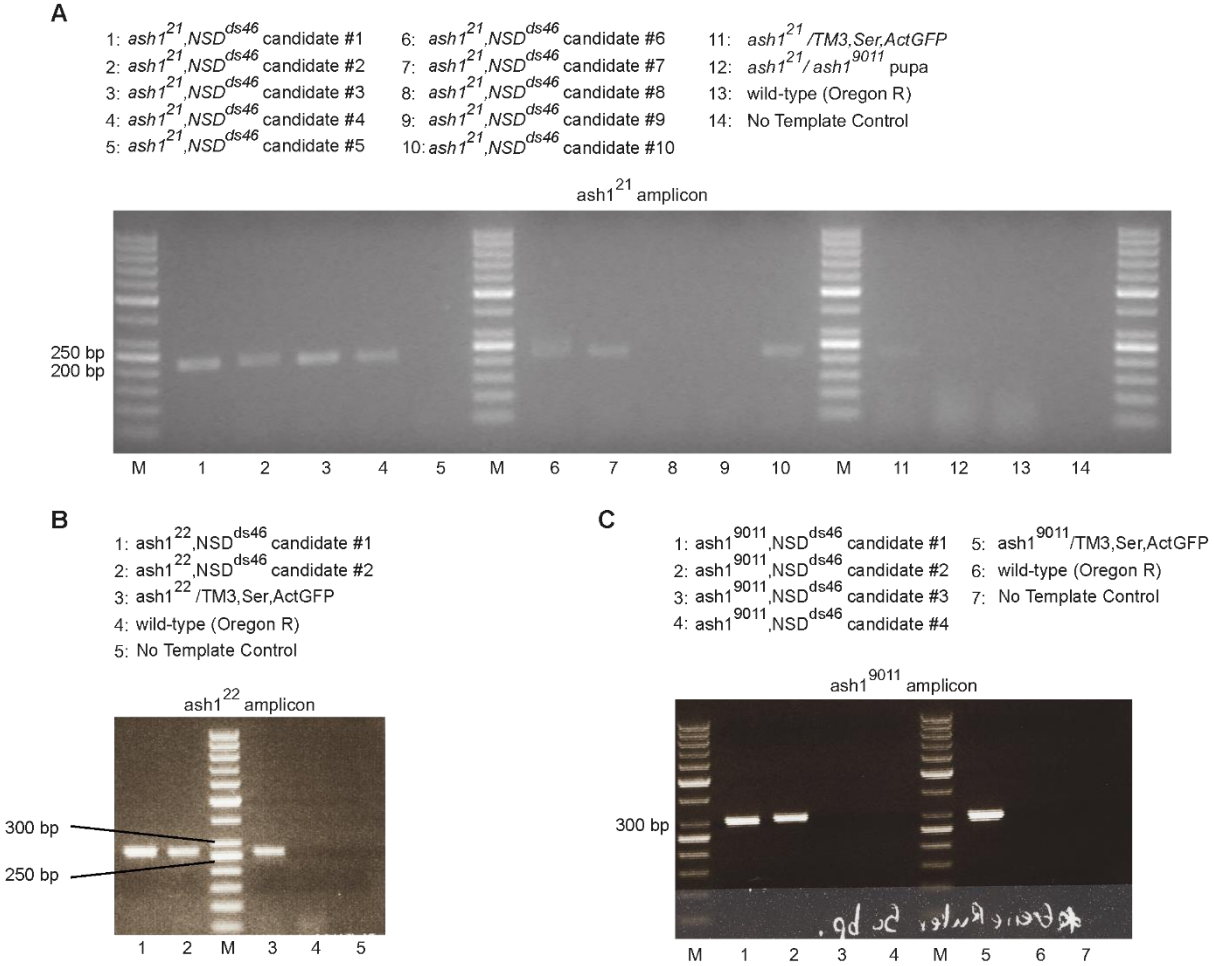
Appendix Figure S11



Appendix Figure S11. Recombination of *ash1* and *NSD* mutations. The *NSD* loss of function allele, NSD^{ds46}, was recombined with three different *ash1* alleles, namely $ash1^{22}$ (**A**), $ash1^{9011}$ (**B**), and $ash1^{21}$ (**C**) using identical set of crosses. First, the homozygous NSD^{ds46} fly strain was crossed with the *ash1* mutant strain in which the *ash1* allele was balanced over the *TM3,Ser,Act-GFP* balancer chromosome (Step 1). In Step 2, the males of the *TM6,Tb* balancer chromosome strain were crossed with the female progeny of Step 1 that lacked the *TM3* balancer. The recombination between the NSD^{ds46} and *ash1* alleles occurred in these females. In the NSD^{ds46} deletion, the coding region of *NSD* is replaced with the *DsRed* ORF. Therefore, the female progeny of Step 2 were selected based on the RFP signal and the presence of the

TM6,Tb balancer and crossed individually to the *ash1*^{9011}*/TM3,Ser,Act-GFP* males (Step 3). After egg deposition, genomic DNA was extracted from the females in each individual cross and screened by PCR for the presence of the relevant *ash1* allele (see Appendix Figure S12). The progeny of the crosses with females carrying the recombinant chromosome, balanced over *TM3,Ser,Act-GFP* balancer was used to establish the strains shown as Step 4. (**D**) Generation of the *ash1,NSD* double mutant animals. The *NSD*^{ds46} allele is homozygous viable, however, the *ash1*^{22}, *ash1*^{9011}, and *ash1*^{21} chromosomes all carry additional unrelated lethal mutations. Therefore, to obtain the *ash1,NSD* double-mutants, different *NSD*^{ds46}*,ash1* fly strains were crossed to each other and the GFP-negative first instar larvae (Class I, double mutants) were separated from the GFP-positive (Class II) larvae and grown separately to avoid competition.

Appendix Figure S12



Amplicons	Forward primer	Reverse primer	Product in mutant/transgenic line	Product in wild-type
ash1^{22}	ash1-22.2	ash1-21.rev	264 bp	No product
ash1^{9011}	Plac1	9011-1.2	300 bp	No product
ash1^{21}	ash1-21.mut	ash1-21.rev	217 bp	No product

Appendix Figure S12. Genotyping *ash1, NSD^{ds46}* recombinants. The single flies described in Appendix Figure S11A-C, were genotyped by PCR, using primers specific for *ash1* mutations. The presence of RFP (thus deletion of *NSD*) was checked prior to DNA extraction and only the RFP-positive flies were selected for further screening for the *ash1* mutations. **(A)** The screen for the *ash1^{21}* allele. Forward primer in the ash1^{21} amplicon is designed to match the single nucleotide substitution in the *ash1^{21}* mutation and therefore anneals only to the mutant genomic DNA but not to the wild-type *ash1*. Seven out of ten single flies shown here contain the *ash1^{21},NSD^{ds46}* recombinant chromosome. **(B)** The screen for the *ash1^{22}* allele. In

this case, one of the primers is specific for the *ash1*^{22} allele, therefore the PCR product is seen only in the mutant but not in the wild-type flies. Only two single flies positive for *ash1*^{22} mutation are shown here. **(C)** The screen for the *ash1*^{9011} allele. One of the primers (9011-1.2) in the ash1^{9011} amplicon is specific to the genomic DNA flanking the deletion and the other primer is specific to the remnant of the transposon used to generate the deletion. Therefore, the ash1^{9011} amplicon yields the product only from the mutant but not the wild-type DNA. Out of four flies shown here, the two contain the *ash1*^{9011} deletion. PCR products were analyzed by the electrophoresis in 2% agarose gel. M = 50bp DNA Ladder.

Appendix Table S1. The list of oligonucleotide sequences.

Oligonucleotide	sequence
1gRNA_NSD_asen	AAACTCGATCTCGGAGTGCGCGTC
1gRNA_NSD_sens	CTTCGACGCGCACTCCGAGATCGA
2gRNA_NSD_asen	AAACTCAACTACTTGTGGGACGAC
2gRNA_NSD_sens	CTTCGTCGTCCCACAAGTAGTTGA
3R-nc-1.1	TTGCGGAGCGTGAGCGGAAG
3R-nc-1.2	AAGCCAGGAGAACGGCACGC
9011-1.2	CAGTGCCTTGGCAGTTGATGTC
Abd-B_F_ex	CCACTGCATATACCCGCCAT
Abd-B_R_ex	AATCCCTCGTCGTTGTAGGC
ASH1_mE_Fwd	TGGATCCCCGGAATTGCAGCTCTTTGTGGAGGCCAAG
ASH1_seq10	AGCCATTGATTACCAAGAGTGCG
ASH1_seq11	ATTCGGGTGTGATAAGCACCAC
ASH1_seq12	TTTCGTCAGAGGAGGAACCTGG
ASH1_seq2	TGCTAAGTGCTATCATCCAGGC
ASH1_seq3	CCAGGAGTAAGCGACGGCAG
ASH1_seq4	TGATGAACCAGCGTAAAGAAACCC
ASH1_seq5	AATGGGAAATTGAACGCAGAGGC
ASH1_seq6	ACAACAGCCGTGATTGATGAGC
ASH1_seq7	ATTCCTACCGAGCACGATCCC
ASH1_seq8	ACTAAGCTACCCATTGCGAAGGG
ASH1_seq9	AAAAGATACGCAGATGCAAGGCC
ash1-21.mut	CAACCGCATGGTTTATACGA
ash1-21.rev	CTGCTTGAATTCCTTTTCCG
ash1-22.2	GTGCAGCGTTCGGACACCGA
ash1-22.R	CCGACTGTGCCGCCTGGATG
ash1-22-rev	TCCGAGGAAAATGCCGAGATCTA
ASH1CN_Cfwd	AGATCTCCAAGAGCTATGCGC
ASH1CN_Crev	GTCTAGATATCTCGATTACTCGAGTGTTGAGTTGGCCGTAGAACTG
ASH1CN_Nfwd	AGTCGACTGGATCCGACCATGGGTACCAGCTGTAGCCAAAATGAGACGG
ASH1CN_Nrev	AGCTCTTGGAGATCTGTGACTTCTTAGACTTAGACAC
CP190-GAL4:DBD-rev	ACTTCACCCAGTGCGGCCGCCGATACAGTCAACTGTCTTTGACCTT
deltaPHD_A	CAGAAGGCCAAAAAGCATGCTC
deltaPHD_B	CCACTTCATCCTCATCAGGAGAGGCCTC
deltaPHD_C	ATGAGGATGAAGTGGATCGAGAAATTCCCC
deltaPHD_D	TGACCAACTCAATGGGTACCAC
deltaSET_A	ATCCGCACCAATGTATACGCAG
deltaSET_B	AGGGATTGACGGCATGTCGCTGGATCTTC
deltaSET_C	ATGCCGTCAATCCCTCAGAGGGTCAGCC

Oligonucleotide	**sequence**
deltaSET_D	CCGCCTGCCGTTGAGC
DonR_forB	CAATAGCATCACAAATTTCACAAATAAAG
DonR_revA	GATCCTAATTGAATTAGCTCTAATTG
eGFP_F	CAAGGACGACGGCAACTACA
eGFP_R	CCTCCTTGAAGTCGATGCC
emc.1.1	GATCCAGATATCCTCGCCCAGC
emc.1.2	CCACAAGAGTGTTGGGCGTTTG
emc-2.1	GAGCGAGTTTTCTCACGCAG
emc-2.2	GCAGGTCGAGTTTTCAACGG
gen_1_NSD	TAAACTGGACCGCAGAATGAAG
gen_16_NSD	GTGTTTCACCTTGGGAGTCTTC
H3K36R	GCCACCGGAGGTGTGCGC
H3WT	CAGCCACCGGAGGTGTGAAG
HA-1-PstI	TACTGCAGTCTTGATAAAGTCGTCCATGCTCCTTG
HA-1-SpeI	TAACTAGTATCTCGGAGTGCGCGTCCGTGCTCAGC
HA-2-EcoRI	TA GAATTC TCCGTGCGCAACTGCCTGAAGCTG
HA-2-NotI	TAGCGGCCGC ACTACTTGTGGGACGACTTAATG
His3.3A_fwd	TAGGGTCACACTGAGCAGACGC
His3.3A_rev	TGAATGCATTTACTACATGG
His3.3B_fwd	TCTGTGTCAATCTGGAACGC
His3.3B_rev	GAGAAATTCATATGAGTTGGATAACC
His3-rev	AATGCGTCGCGCTAACTGGATG
HisC.2	TTACGCCGATAAGACACTAACG
hth.1.1	TGTGTTTTTGCGACGGCAATTTG
hth.1.2	GCCGCGGAGGGAATTTCTTTC
hth-2.1	GACCGCTCCGAAAAGTAGGG
hth-2.2	AACTCCGGACTCGGACTCTT
Mes-4-RI-2.1	CTAATACGACTCACTATAGGGAGTTCAGTCCGGCAAAGGTCAGC
Mes-4-RI-2.2	CTAATACGACTCACTATAGGGAGTACATGAGCCCCATCGAGAAGC
noc-exp-1.1	AGATTTCCGCGCACGATTCCTC
noc-exp-1.2	TTCCGTTGGCATTGTTCAGCACC
NSD_back	TTACTCGTCCTTCGCATCCATTGGCTCA
NSD-Rt-1f	CCATGGTGAACAGGAAACGAG
NSD-Rt-1r	CATCTGTCCGGTCAACTTCCTCATC
OneStrep_fwd	TGGATCCGACCATGGCTAGCTGGAGCCACCCGCAGTTCGAGAAAGGTGG AGGTTCCGGAGGTGGATCGGGAGGTGGATCGTGGAGCCACCCGCAGTTC GAAAAAGGCGC
OneStrep_rev	TTTTGGCTACAGCTGGCGCCTTTTTCGAACTGCGGGTGGCTCCACGATCCA CCTCCCGATCCACCTCCGGAACCTCCACCTTTCTCGAACTGCGGGTGGCTC CAGGTAC
osGAL4:DBD-fw	TTCGAAAAAGCGGCCAAGCTACTGTCTTCTATCGAACAAGCAT
Plac1	CACCCAAGGCTCTGCTCCCACAAT

Oligonucleotide	**sequence**
Pry4	CAATCATATCGCTGTCTCACTCA
rp49for	TGTCCTTCCAGCTTCAAGATGACCATC
rp49rev	CTTGGGCTTGCGCCATTTGTG
RpL32-ex1.1	TGGGCGATCTCGCCGCAGTA
RpL32-ex1.2	CAGAGTGCGTCGCCGCTTCA
Su(z)2-2.1	CGTGCCGGTCGAACTGTTAT
Su(z)2-2.2	TCCTTAGCCCGCTTTCTGTT
Su(z)2-ex1.1	GCCAAGGCCCAGAGCTACGC
Su(z)2-ex1.2	GGTGGTCCCATCAAGCGGGC
Taf4-ex1.1	AGCCCGCAGCCGTGTTTGAT
Taf4-ex1.2	CGTGCTGCGGCGGAGGTTTA
Ubi-1.1	GGCGGCCGTTACTAGAAGGATCCTTTAGGCTTTTGCC
Ubi-1.2	TTGATGATCCACTAGTTTGGATTATTCTGCGGGAAGAAA
U-ex1.1	GTCTTTGTAGCCATTCACCG
U-ex1.2	CCCTATGCCAACCACCATC

Appendix Table S2. The list of antibodies.

Antigen	Host	Reference/company/catalogue #	IF	Western	ChIP
H3K36me1	rabbit monoclonal	Cell Signaling, D9J1D, Cat#14111	-	1:2000	1:250
H3K36me2	mouse monoclonal	Active motif, MABI0332, Cat#61019	-	1:1000	1:100
H3K36me3	rabbit monoclonal	Cell Signaling, D5A7, Cat#4909	1:200	1:2000	-
H3K27me2	rabbit monoclonal	Cell Signaling, Cat#9728S	-	1:2000	-
H3K27me3	rabbit polyclonal	Millipore (Upstate), 07-449	-	1:2000	-
BEAF-32	rabbit polyclonal	#21352 gift from H. Saumweber	-	1:2000	-
H3K18Ac	rabbit polyclonal	Abcam, # ab1191	1:200	-	-
Ash1	rabbit polyclonal	Kahn at al., 2016	-	-	1:100
Ash1	rabbit polyclonal	against aa 2156-2217, gift from V.Pirrotta	-	1:500	-
Abd-B	mouse monoclonal	DHSB, 1A2E9	1:10	-	-
Ubx	mouse monoclonal	DHSB, Ubx FP3.38	1:50	-	-
Anti-rabbit Alexa-488	Goat	Invitrogen, # A-11008	1:500	-	-
anti-mouse Alexa-555	Goat	Abcam, # ab150114	1:500	-	-
Anti-Rabbit AP conjugated	Goat polyclonal	Promega, #S3731	-	1:10000	-
Anti-Mouse IgG AP conjugated	Goat polyclonal	Sigma, A3562	-	1:10000	-
Anti-Elav (68 μg/ml)	mouse monoclonal	DHSB, Elav-9F8A9-s	5 μg/ml	-	-

Appendix Table S3. Amplicons used in ChIP-qPCR.

Amplicon name	Oligo name	ChIP
intergenic	3R-nc-1.1	Ash1, H3K36me1, H3K36me2
	3R-nc-1.2	
noc	noc-exp-1.1	Ash1, H3K36me1, H3K36me2
	noc-exp-1.2	
hth	hth.1.1	Ash1
	hth.1.2	
	hth-2.1	H3K36me1, H3K36me2
	hth-2.2	
Ubx	U-ex1.1	Ash1, H3K36me1, H3K36me2
	U-ex1.2	
Su(z)2	Su(z)2-2.1	Ash1
	Su(z)2-2.2	
	Su(z)2-ex1.1	H3K36me1, H3K36me2
	Su(z)2-ex1.2	
emc	emc.1.1	Ash1, H3K36me1
	emc.1.2	
	emc-2.1	H3K36me2
	emc-2.2	
Taf4	Taf4-ex1.1	Ash1, H3K36me1, H3K36me2
	Taf4-ex1.2	