Expanded View Figures

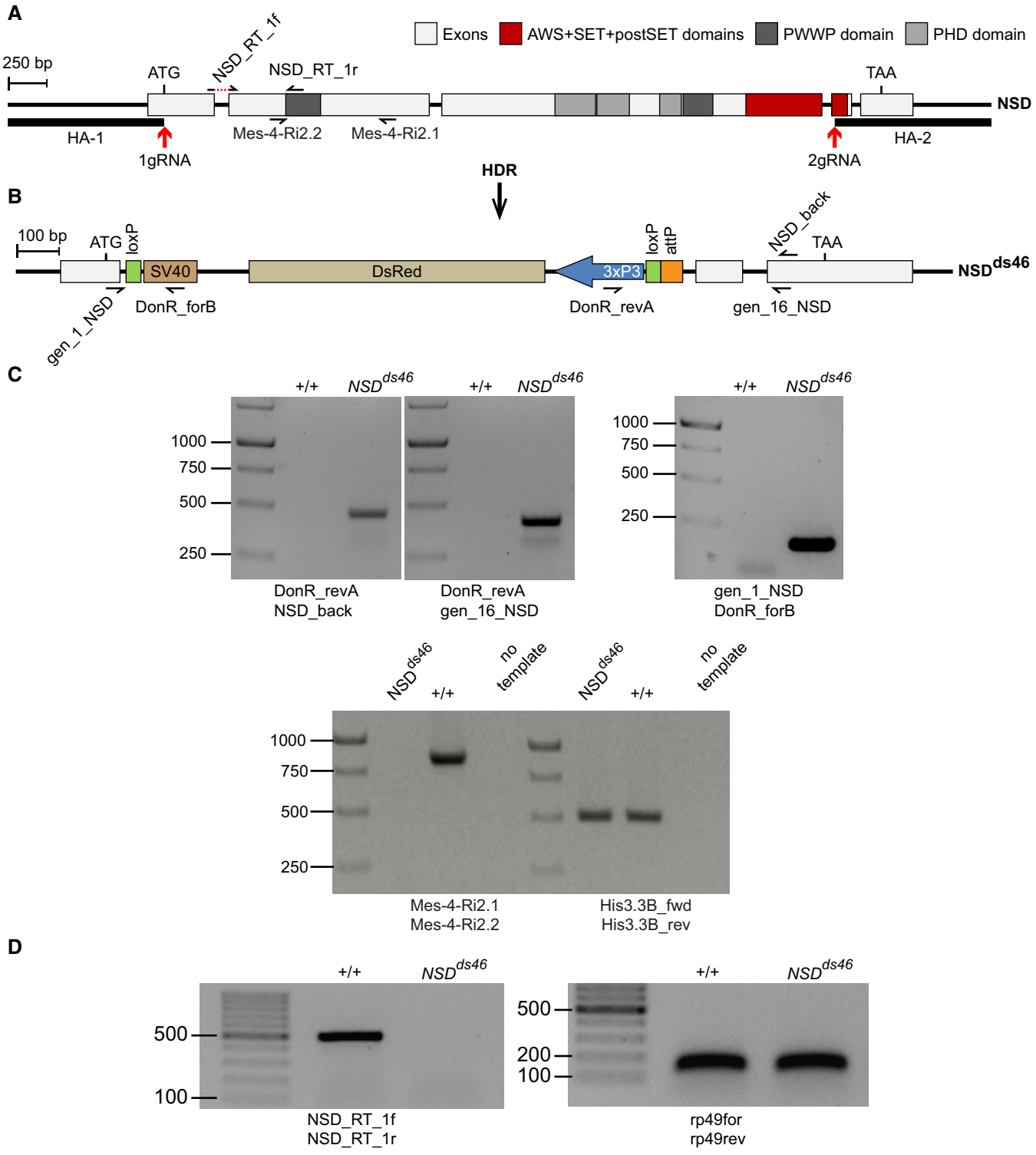

Figure EV1.

Figure EV1. Generation of the *NSD* loss-of-function allele.

A The structure of the *Drosophila NSD* locus. Red arrows indicate locations of the gRNA sites used for CRISPR/Cas9-mediated replacement of the *NSD* Open Reading Frame (ORF) with *DsRed*. The homology regions HA-1 and HA-2, used for the replacement, are shown with bold lines. The half-arrows represent the primers used for genotyping of the mutant allele. The dashed red line in NSD_RT_1f primer, used for RT–qPCR, indicates the intronic region that is excluded from the primer.

B The NSD^{ds46} allele. After Homology-directed Repair (HDR), the insertion of the *DsRed* cassette generates the loss-of-function allele NSD^{ds46}, where *DsRed* substitutes most of the *NSD* ORF. The *DsRed* expression is controlled by the 3xP3 promoter. In addition to *DsRed*, the replacement cassette contains two *loxP* sites to remove *DsRed* via Cre-mediated recombination and an *attP* docking site to insert variants of the *NSD* ORF. The half-arrows represent the primers used to genotype the mutant allele.

C Genotyping of the NSD^{ds46} allele by PCR. The replacement of the *NSD* ORF by the DsRed cassette was confirmed by PCR with four different primers pairs. Three primer pairs (top row of images) yield the product only if the replacement has happened. The expected sizes of the PCR products are 469, 427 and 186 bp for DonR_revA and NSD_back, DonR_revA and gen_16_NSD, and gene_1_NSD and DonR_forB primer pairs, respectively. The PCR with Mes-4-RI-2.1 and Mes-4-RI-2.2 primer pair amplifies the 830 bp product only from the wild-type allele. PCR with His3.3B_fwd and His3.3B_rev primer pair, amplifying the 495 bp DNA fragment from the *His3.3B* gene, was used as a positive control.

D The NSD^{ds46} allele produces no messenger RNA. This was confirmed by RT–PCR with NSD_Rt_1f and NSD_Rt_1r primer pair, which yields the 513 bp product only when the intact *NSD* mRNA is produced. RT–PCR with rp49for and rp49rev primer pair that amplifies 132 bp fragment from the cDNA of constitutively expressed *RpL32* (a.k.a *rp49*) gene was used as positive control.

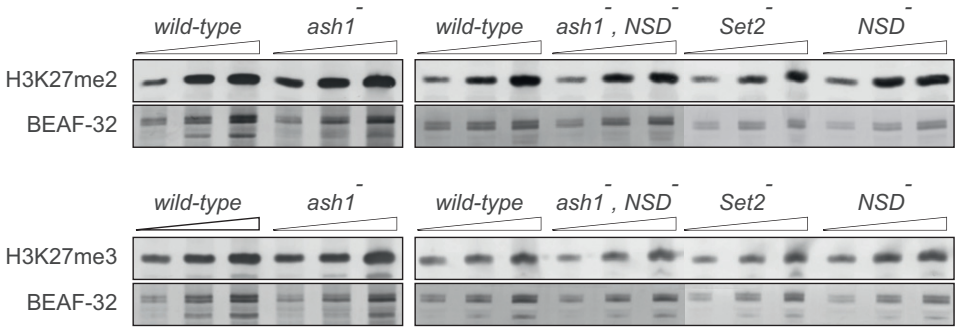

Figure EV2. H3K27 methylation in the *Drosophila* larvae lacking different H3K36-specific histone methyltransferases.

Western blot analysis of the $ash1^{-}$ ($ash1^{22}/ash1^{9011}$), $ash1^{-}$,NSD^{-} ($ash1^{22}$,$NSD^{ds46}/ash1^{9011}$,NSD^{ds46}), $Set2^{-}$ ($Set2^{1}/ Set2^{1}$) and NSD^{-} (NSD^{ds46}/NSD^{ds46}) mutants shows no change in global levels of di- and tri-methyl H3K27 compared to wild-type (*Oregon R*). Twofold serial dilutions of total protein extracts from larval brains, imaginal discs and salivary glands were analysed by Western blot with corresponding antibodies. The protein extracts from the wild-type, double $ash1^{-}$, NSD^{-} and single NSD^{-} and $Set2^{-}$ mutants (right panels) were analysed together on the same membrane; however, the images were modified to splice out the marker lane between the $ash1^{-}$, NSD^{-} and the $Set2^{-}$ extracts. Western blots with constitutively expressed BEAF-32 protein were used as loading controls.

Source data are available online for this figure.

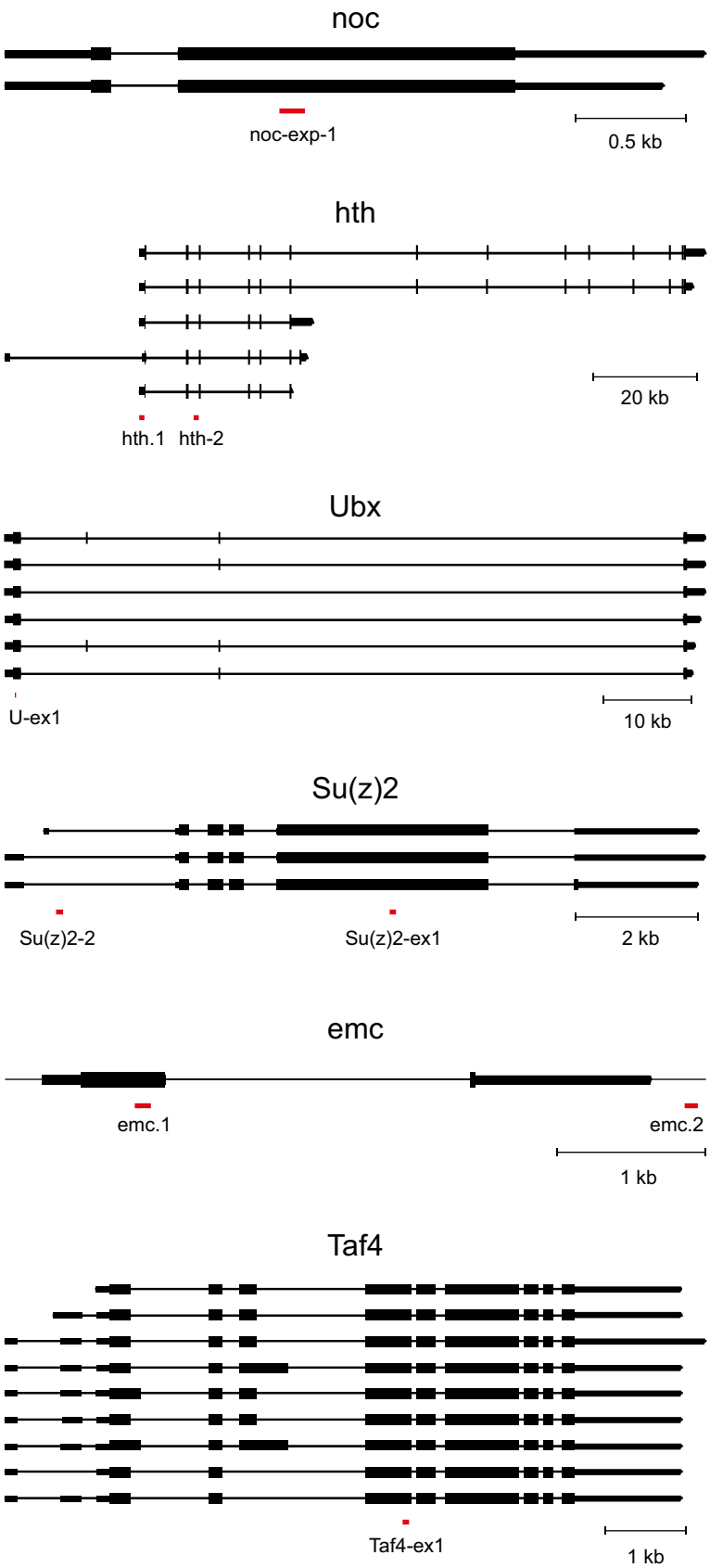

Figure EV3. Schematic of target genes selected for ChIP-qPCR analyses.

Gene exons are shown as thick boxes for coding parts and thin boxes for 5′- and 3′-UTRs, the introns are indicated with strait lines. All isoforms are listed. The schematics are drawn to scales indicated with scale bars below each gene. The positions of PCR amplicons used for the analyses are marked with red boxes.

C

ΔHisC,ElavGal4/ΔHisC,UAS-2xYFP ; VK33{H3K36Rx12}/+							ΔHisC,ElavGal4/ΔHisC,UAS-2xYFP ; VK33{H3WTx12}/+						
Larvae	Light Pupae	(%)	Dark Pupae	(%)	Hatched flies	(%)	Larvae	Light Pupae	(%)	Dark Pupae	(%)	Hatched flies	(%)
750	341	45.5	157	20.9	40	5.3	715	0	0	37	5.2	573	80.1

Figure EV4. Generation of flies with zygotic H3.2K36 replaced by H3.2R36.

A, B To generate the desired flies, females carrying *ΔHisC* recombined with the *UAS-2xYFP* transgene were crossed to males carrying *ΔHisC* recombined with the *Elav-Gal4* transgene and one of the two variants of the transgene with 12 copies of the histone gene cluster. In one of the transgenes, all copies of His3.2 gene contained H3K36R mutation (A) and in the other, all copies of His3.2 were wild-type (B). The crossing scheme and the progeny classes in (A) and (B) were identical. The Class I larvae were separated from the larvae of the other classes, based on the YFP signal. Although the Class II animals also show YFP signal, they die at early embryonic stages after running out of maternally deposited histones. The Class III larvae lack YFP signal and are easily distinguishable from Class I.

C Twelve transgenic copies of the wild-type histone gene cluster complement the loss of endogenous *HisC* locus nearly completely (80.1%). However, the transgene carrying *H3K36R* mutation fails to compensate for the deletion of the endogenous histones cluster. Although a large fraction of these animals reaches the pupal stage (about 66%), only 5% of them reach the adult stage.

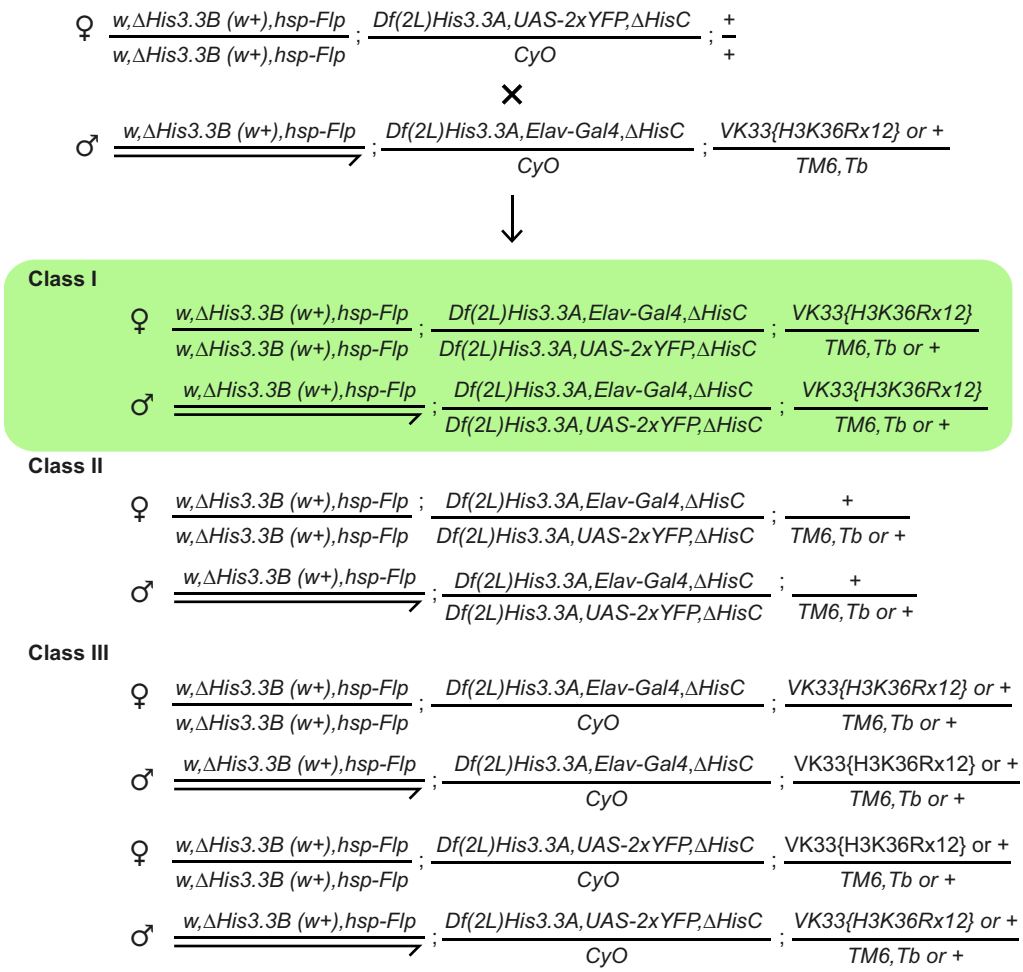

Figure EV5. Zygotic replacement of the wild-type H3.2 and H3.3 histones with H3R36.

To generate the embryos where all zygotic H3 is replaced with H3R36, the *ω,ΔH3.3B($ω^+$),hsp-Flp; Df(2L)His3.3A,UAS-2xYFP,ΔHisC/CyO; +/+* females (from the stock generated as described in Appendix Fig S6) were crossed to the *ω,ΔH3.3B($ω^+$),hsp-Flp; Df(2L)His3.3A,ElavGal4,ΔHisC/CyO; VK33{H3K36Rx12} or +/TM6,Tb* males (generated as described in Appendix Fig S7). In the progeny, all embryos are homozygous for *ΔHis3.3B* deletion. The YFP-positive embryos are also homozygous for both *ΔHis3.3A* and *ΔHisC* (deletion of histone cluster). From those, the Class II embryos die as they enter the S phase of cycle 15 when the pool of maternally loaded histones is depleted. However, the embryos supplemented with 12 copies of the histone repeat unit from the *H3K36Rx12* transgene (Class I, highlighted in green) survive until the first instar larval stage. In these embryos, all zygotic histone H3 has Lys 36 substituted to Arg. Late embryos of this class were collected, genotyped by PCR as described in Appendix Fig S8 to verify their identity and examined for the *Abd-B* expression. The remaining embryos (Class III) show no YFP signal since they do not combine the *Elav-Gal4* driver and *UAS-2xYFP* reporter.

