# Supporting Information

## Kusch et al. 10.1073/pnas.1320337111

### SI Materials and Methods

**Recombinant Proteins.** The plant homeodomain (PHD) of *Drosophila* Inhibitor of Growth 3 (dIng3) (amino acids 608–84) was fused to GST as described (1, 2). The generation of histone H2Av and canonical histones and the generation of recombinant nucleosomal arrays were published (1). H3K4Cme3 analogs were generated according to Simon et al. (3).

**Tissue Culture and Complex Purification.** For the purification of dTip60 complexes, full-length ORF of the *dTip60* cDNA was amplified from 1 μg of total RNA, and error-free clones were cloned into the FLAGHA-expression vector (1). The generation of stable transgenic cell lines and the tandem-affinity purifications were conducted as published (1, 4). The presence of all dTip60 complex subunits was confirmed by mass-spectrometric analyses. A detailed description has been published (4).

**Immunological Methods and Antibodies.** For immunoblots, the antibodies used were as follows: anti-HSF (rabbit: 1:2,000; a gift of C. Wu, Janelia Farm, Ashburn, VA); anti-dIng3 (rabbit; 1:2,000; ref. 1); anti-dTip60 (rabbit; 1:2,000; ref. 1); anti-dSet1 (guinea pig; 1:3,000; ref. 4); anti-H2Av (rabbit; 1:2,000; ref. 5); anti-H3K4me3 (rabbit; 1:6,000, Active Motif); anti-H2A.ZK4/7/11ac (sheep; 1:1,000; Abcam; catalog no. 18262); anti-H3 (rabbit; 1:25,000; Active Motif); anti-H4K5ac (rabbit; 1:1,000; Millipore; catalog no. 07–327); anti-H4K8ac (rabbit; 1:1,000; Cell Signaling Technology; catalog no. 2594); anti-H4K12ac (rabbit; 1:1,000; Millipore; 06–1352); anti-tubulin (mouse; 1:2,000; Sigma); anti-Flag M2 (rabbit; 1:1,000; Agilent); and anti-GST (rabbit; 1:1,000; Rockland). The preparation of histone extracts from wild-type and *Mrg15* mutant embryos has been published (1).

For immunofluorescence staining experiments, the antibody concentrations used were as follows: anti-dTip60 (rabbit, 1:60), anti-phosphorylated Pol II (1:40; anti-Ser5P CTD, a gift of D. Eick, Hemholtz Center, Munich; ref. 6), anti-H4K5ac (1:500); anti-H4K8ac (1:500); anti-H4K12ac (1:500), and anti-H4K16ac (1:400; Millipore; catalog no. 07–329). The methods for the collection, fixation, and staining of embryos was published (1).

For ChIP/quantitative PCR (qPCR), the following antibodies used were as follows: anti-dSet1 (guinea pig, 2 μL; ref. 4); anti-dIng3 (5 μL); anti-dTip60 (rabbit, 7 μL; ref. 1); anti-Rpb3 (1.5 μL; a gift of J. T. Lis, Cornell University, Ithaca, NY; ref. 4); anti-H3K4me3 (3 μL, 5fmol of competitor peptides were added; for details on competitive ChIP, see ref. 4); anti-H4K5ac (3 μL); anti-H2A.ZK4/7/11ac (5 μL); and anti-H2Av (1.5 μL).

**Primers.** ***RT/qPCR primer.***

rp49 fwd: CCCAAGGGTATCGACAACAGA

rp49 rev: CGATGTTGGGCATCAGATACTG

hsp70-5′ fwd: GCAAATAAACAAGCGCAGCTG

hsp70-5′ rev: GCAGGCATTGTGTGTGAGTTCTTC

hsp70-3′ fwd: ACGTAAAGCAGTCCGTGGAG

hsp70-3′ rev: TGCTGATGCATCTTGGTCAT

***ChIP primers.***

hsp70 prom fwd: GCAAATAAACAAGCGCAGCTG

hsp70 prom rev: GCAGGCATTGTGTGTGAGTTCTTC

hsp70-148 fwd: GGTCATTTGTTTGGCAGAAAG

hsp70 -148 rev: CCAACGAGAGCAGTATGTCG

hsp70+48 fwd: GGCGCTTCGTCTACGGAGCG

hsp70+48 rev: CTTATAATTGATTCACTTTAA

hsp70+307 fwd: CACAATGCCTGCTATTGGAA

hsp70+307rev: TCCTACGTGGCTTTCACAGA

hsp70+571 fwd: CGCAGAGGACATGAAGCACTGGCC

hsp70+571 rev: CTGCATCCGTGATGCTCTCGCCC

hsp70 +1000 fwd: AGGACTTTGACAACCGGCTA

hsp70 +1000 rev: TCAAACAATGCGTCGATCTC

hsp70 end fwd: ACGTAAAGCAGTCCGTGGAG

hsp70 end rev: TGCTGATGCATCTTGGTCAT

***RNAi** (**T7 promoter sequence not included**).*

dSet1T7 fwd: CGTCTCTGGCAAACTCCAAAATGCC

dSet1T7 rev: GCAAAACACATCTAAGATCTTTCCC

dTip60T7 fwd: CCTGCTGGCATTCCAAACTCTGTGG-CTCC

dTip60T7 rev: CTTGCGGATAGGGTGAGAAATACCAGG

dIng3T7 fwd: TGCCACTCAGATACACGAGC

dIng3T7 rev: GACCACGGGAAGTGAGTGAT

DomT7 fwd: CAACACCATGGAACAGATGC

DomT7 rev: AATGTGCGTTTCGTACCTCC

LacZT7 fwd: GATATCCTGCTGATGAAGC

LacZT7 rev: GCAGGAGCTCGTTATCGC

1. Kusch T, et al. (2004) Acetylation by Tip60 is required for selective histone variant exchange at DNA lesions. *Science* 306(5704):2084–2087.
2. Kusch T, Guelman S, Abmayr SM, Workman JL (2003) Two Drosophila Ada2 homologues function in different multiprotein complexes. *Mol Cell Biol* 23(9):3305–3319.
3. Simon MD, et al. (2007) The site-specific installation of methyl-lysine analogs into recombinant histones. *Cell* 128(5):1003–1012.
4. Ardehali MB, et al. (2011) Drosophila Set1 is the major histone H3 lysine 4 trimethyltransferase with role in transcription. *EMBO J* 30(14):2817–2828.
5. Madigan JP, Chotkowski HL, Glaser RL (2002) DNA double-strand break-induced phosphorylation of Drosophila histone variant H2Av helps prevent radiation-induced apoptosis. *Nucleic Acids Res* 30(17):3698–3705.
6. Chapman RD, et al. (2007) Transcribing RNA polymerase II is phosphorylated at CTD residue serine-7. *Science* 318(5857):1780–1782.

dTip60 Pol IIo

**Fig. S1.** dTip60 colocalizes with Pol II at the activated *hsp70* loci on polytene chromosomes. Polytene chromosomes from heat-shocked third instar larvae stained with antibodies against dTip60 (green) and Pol IIo (red). Boxed area is shown in magnification to the right. Arrowheads indicate the puffed chromosomal regions 87A and 87C, where the major *hsp70* gene clusters are located. Yellow-orange signal in the merged channels indicates colocalization. Microscopy was performed on a Deltavision II Deconvolution system (Applied Precision). The data were processed by using ImageJ and Adobe Photoshop.

A ctrl RNAi
100 25 10 100 %
dTip60
dSet1
tubulin

B 1.2 1 0.8 0.6 0.4 0.2 0
Dom dTip60 dSet1
lacZi Domi

C dSet1i dTip60i dIng3i
dIng3
tubulin

**Fig. S2.** Knock down (KD) efficiency for dSet1, dTip60, Dom, and dIng3. (*A*) KD efficiency for dSet1 or dTip60. Immunoblots of nuclear extracts from mock- (ctrl: *Escherichia coli lacZ*-specific dsRNA) versus *dSet1*- or *dTip60*-dsRNA–treated cells after 72 h probed for factors indicated at the right. Tubulin served as loading control. Loaded are various amounts of nuclear extract, which are given in percent. (*B*) RT/qPCR values for *lacZi* (= 1) versus *Dom*-RNAi (*Domi*). The relative values for Dom, dTip60, and dSet1 were determined after normalization against *rp49* mRNA levels. Error bars represent the SEM of three independent replicates. (*C*) Immunoblots of nuclear extracts from S2 cells treated for 72 h with dsRNA for *dSet1* (*dSet1i*), *dTip60* (*dTip60i*), or *dIng3* (*dIng3i*). The membranes were probed with antibodies against the proteins indicated to the right. Tubulin served as a loading control.

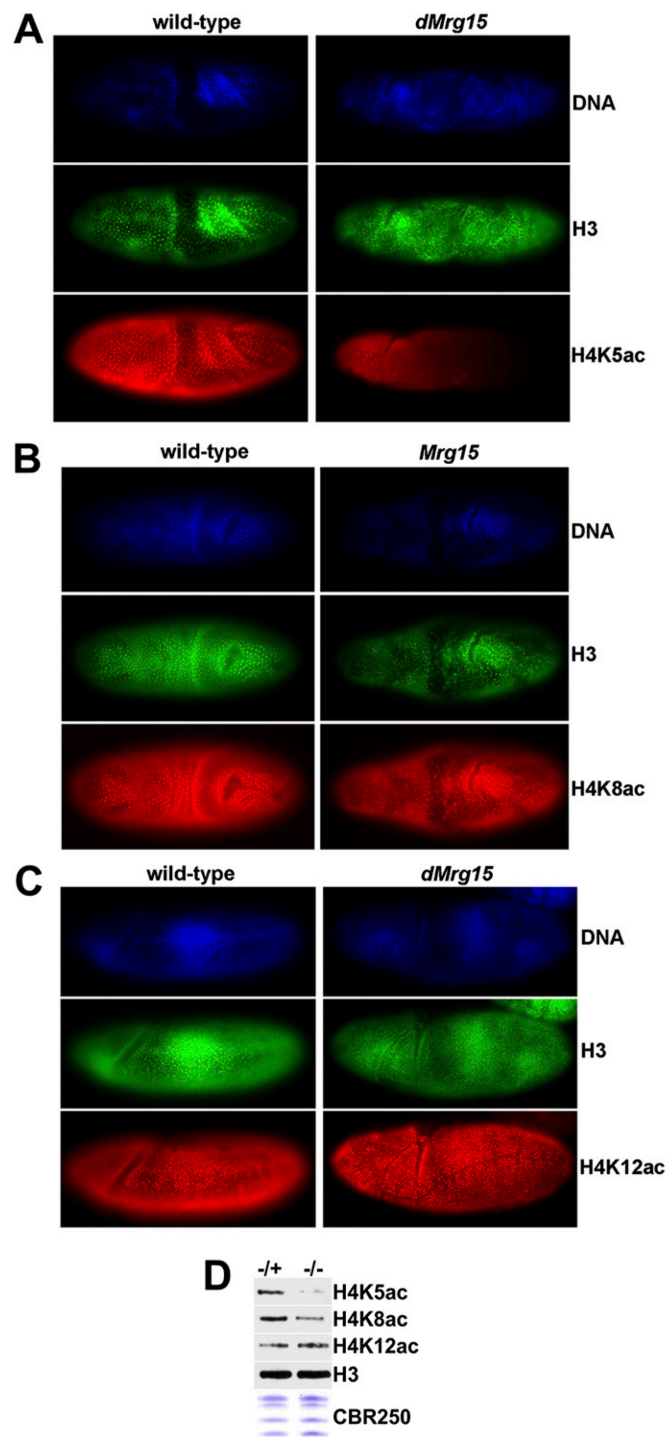


**Fig. S3.** H4K5 acetylation is reduced in *dMrg15* mutants. Shown are embryos colabeled with antibodies against H3 (green) and H4K5ac (*A*), H4K8ac (*B*), or H4K12ac (*C*) (all in red). DNA was counterstained with DAPI (blue). *dMrg15* mutants are recognizable by their gastrulation and germ band retraction defects. The red channel in *dMrg15* mutants stained for H4K5ac has been enhanced to visualize reduced signals in the anterior of the embryo. Embryos stained for H4K5ac and H4K8ac are in stage 6, by which both acetyl marks have reached the highest intensities in all embryonic tissues. Embryos stained for H4K12ac are at stage 10–11, during which this acetylation reached peak levels. (*D*) Immunoblots of separated histones isolated from *Mrg15* heterozygous ($^{-/+}$) and homozygous ($^{-/-}$) mutant embryos. Antibodies used for immunoblotting experiments are indicated to the right. CBR250, Coomassie Blue R-250 stained gel with histones served as loading control. Microscopy was performed on a Deltavision II Deconvolution system (Applied Precision). The images were processed by using ImageJ and Adobe Photoshop.

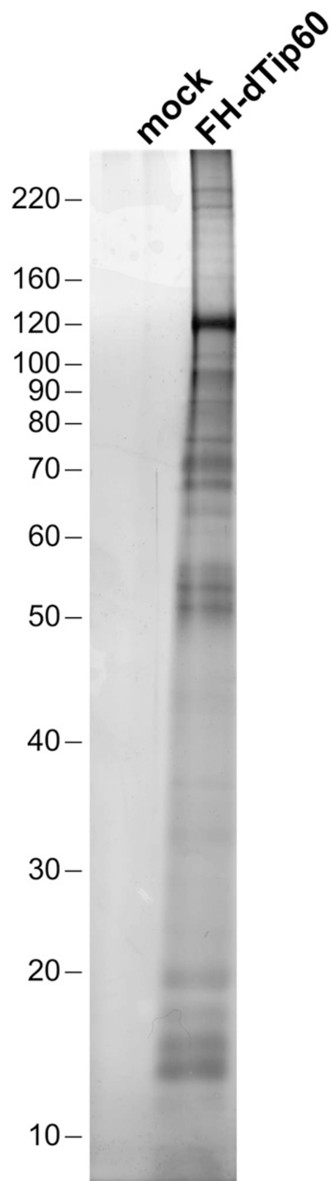


**Fig. S4.** Silverstain of tandem-affinity purified FH-–dTip60 complexes. Silverstain of polypeptides were tandem-affinity purified from nuclear extracts of S2 cells stably transfected with a FLAG/HA-dTip60 expression vector as published (1). Mock, purifications from nuclear extracts of cells carrying empty vector. Apparent molecular masses of marker proteins are indicated to the left in kilodaltons.

1. Kusch T, et al. (2004) Acetylation by Tip60 is required for selective histone variant exchange at DNA lesions. *Science* 306(5704):2084–2087.

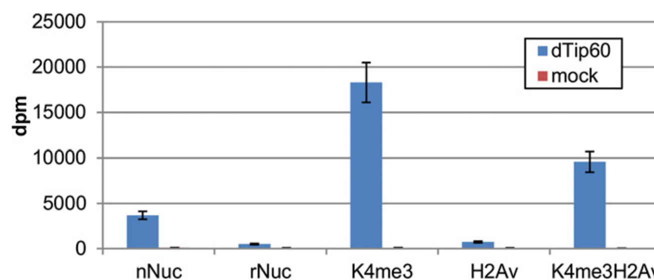


**Fig. S5.** Quantification of [$^3$H]acetate incorporation by purified dTip60 complexes on different nucleosomal substrates. The plotted values correspond to dpm as determined by liquid scintillation counting. One microgram of native (nNuc), canonical recombinant (rNuc), H3K4me3 analog-containing (K4me3), H2Av-containing nucleosomes (H2Av), or a combination (K4me3H2Av) were used as substrates in KAT assays with 15 fmol of Flag/HA tandem-affinity purified Tip60 complexes. Mock, material from cells carrying the empty FH vector. The samples exhibited essentially background signals.