

### **Supplemental Figures:**

**Figure S1: K11 and K17 are the major acetylated positions of the N-tail of *Drosophila's* H2B.** MS/MS spectra of the monoacetylated (upper panel) and diacetylated (lower panel) N-terminal peptide (1-22) of H2B extracted from asynchronously growing Kc cells. The major acetylation sites are K11 (mono acetylated species) and K11 and K17 (diacetylated species).

**Figure S2: dART8 asymmetrically methylates H3R2. (A)** After incubation of different recombinant core histones (H3, H2B, H2A and H4) with recombinant dNTMT in the presence of  $^3\text{H}$ -SAM, only H2B became methylated. Upper panel, Coomassie stained protein gel; lower panel, corresponding autoradiography. **(B)** Tandem MS spectra of the peptide 1-8 of H3 demonstrate that the observed mono- (upper panel) and dimethylation (lower panel) take place at R2. The mass of the parent ions and the fragment ions were measured with a resolution of 30,000 and 15,000 respectively. The inserts display the signals of the corresponding parent ions and the errors respect to the expected (calculated) values. Note the specific neutral loss of a dimethylamine group (DMA; 45 amu), which is characteristic for an asymmetrically methylated arginine but not of a monomethylamine (MMA; 31 amu) or a dimethylcarbodiimide (DMC; 70 amu) that would be indicative of a symmetric methylation.

**Figure S3: dART8 modulation down does not significantly increase dNTMT expression. (A)** *Drosophila* Art8 mRNA levels were quantified by real time PCR analysis in S2 cells treated with control (complementary to GST), dNTMT-specific or dArt8 specific dsRNAs. Two different amplicons were chosen for the validation of the knockdowns. The given values are given in percent and normalized to the GST control knockdowns and to mRNA levels of GAPDH and Tubulin mRNA levels. Error bars represent standard deviations from three replicates. **(B)** dART8 overexpression does not influence intracellular dNTMT levels. Extracts from cells not expressing additional dART8 (SL2), a low level of dART8 (dART8 –ind.) or high levels of dART8 (dART8 +ind.) were harvested and analysed for dNTMT levels using a monoclonal dNTMT antibody. An anti-Tubulin antibody (Dm1a, Sigma) was used to ensure equal loading.

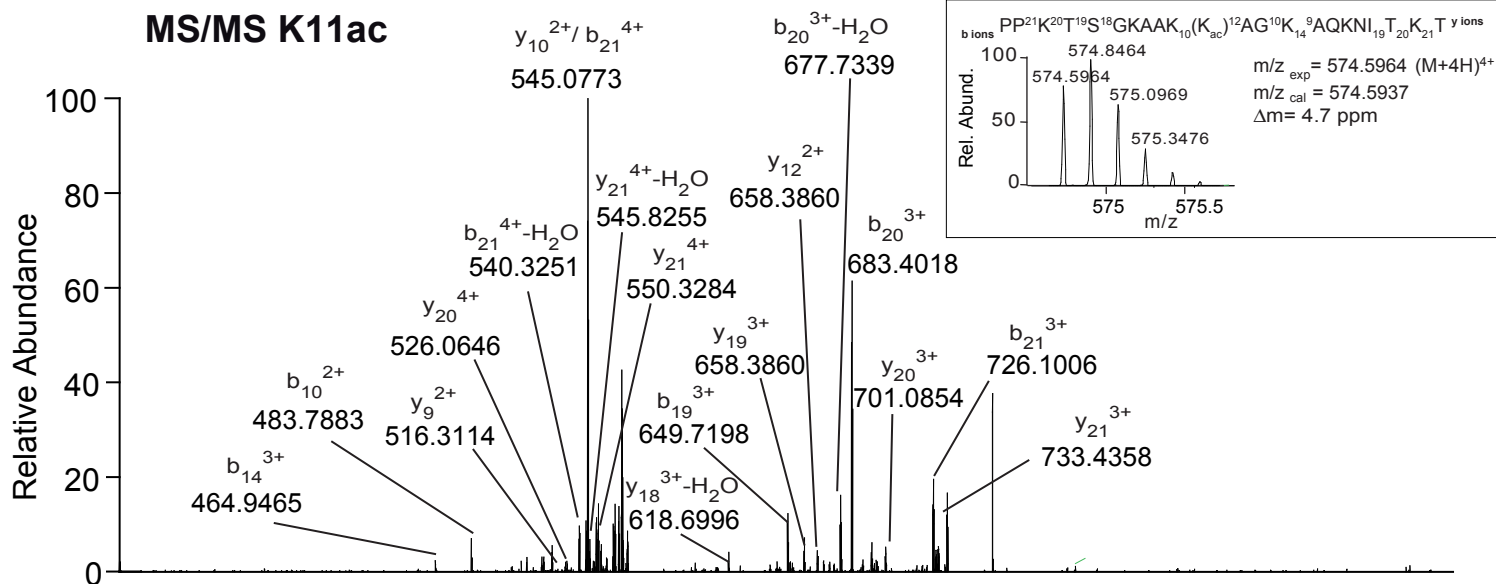
**Figure S4: dART8 does not significantly influence the activity of dNTMT *in vitro*.** Recombinant flag-dNTMT was incubated at 30°C for 60min with <sup>3</sup>H-SAM and recombinant H2B (lanes 4, 6 and 7) in presence (lanes 6 and 7) or absence (lane 4) of recombinant GST-dART8, as indicated. As control, reactions of GST-dART8 with <sup>3</sup>H-SAM and H2B (lane 5), with <sup>3</sup>H-SAM and flag-dNTMT (lane 3) or with <sup>3</sup>H-SAM alone (lane 2) were carried out in parallel. Lane 1 shows the molecular weight marker. Reactions were quenched and proteins were separated through SDS-PAGE to control the protein load (Coomassie staining, upper panel) and to detect the achieved methylation of H2B (autoradiography, lower panel).

**Figure S5: Relative expression levels of dART8 and dNTMT mRNAs throughout the different stages of *Drosophila's* development.** Relative expression data obtained from the modENCODE project (1) are plotted for different developmental stages of the fruit fly. WPP stands for white prepupae.

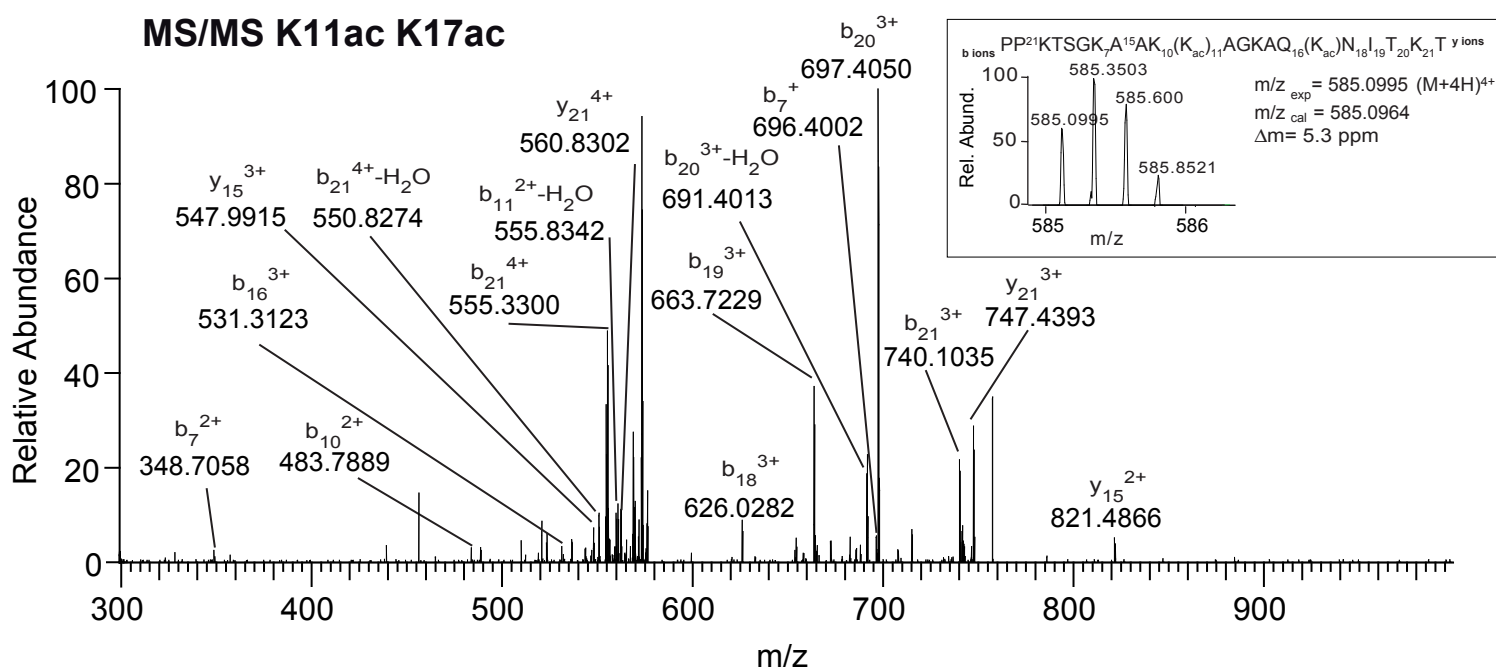
## References

1. Celniker, S.E., Dillon, L.A., Gerstein, M.B., Gunsalus, K.C., Henikoff, S., Karpen, G.H., Kellis, M., Lai, E.C., Lieb, J.D., MacAlpine, D.M. *et al.* (2009) Unlocking the secrets of the genome. *Nature*, **459**, 927-930.

## MS/MS K11ac

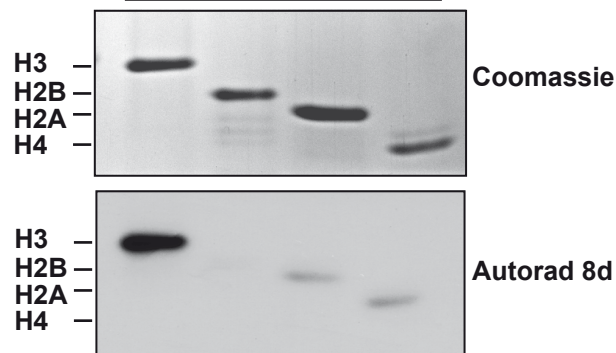
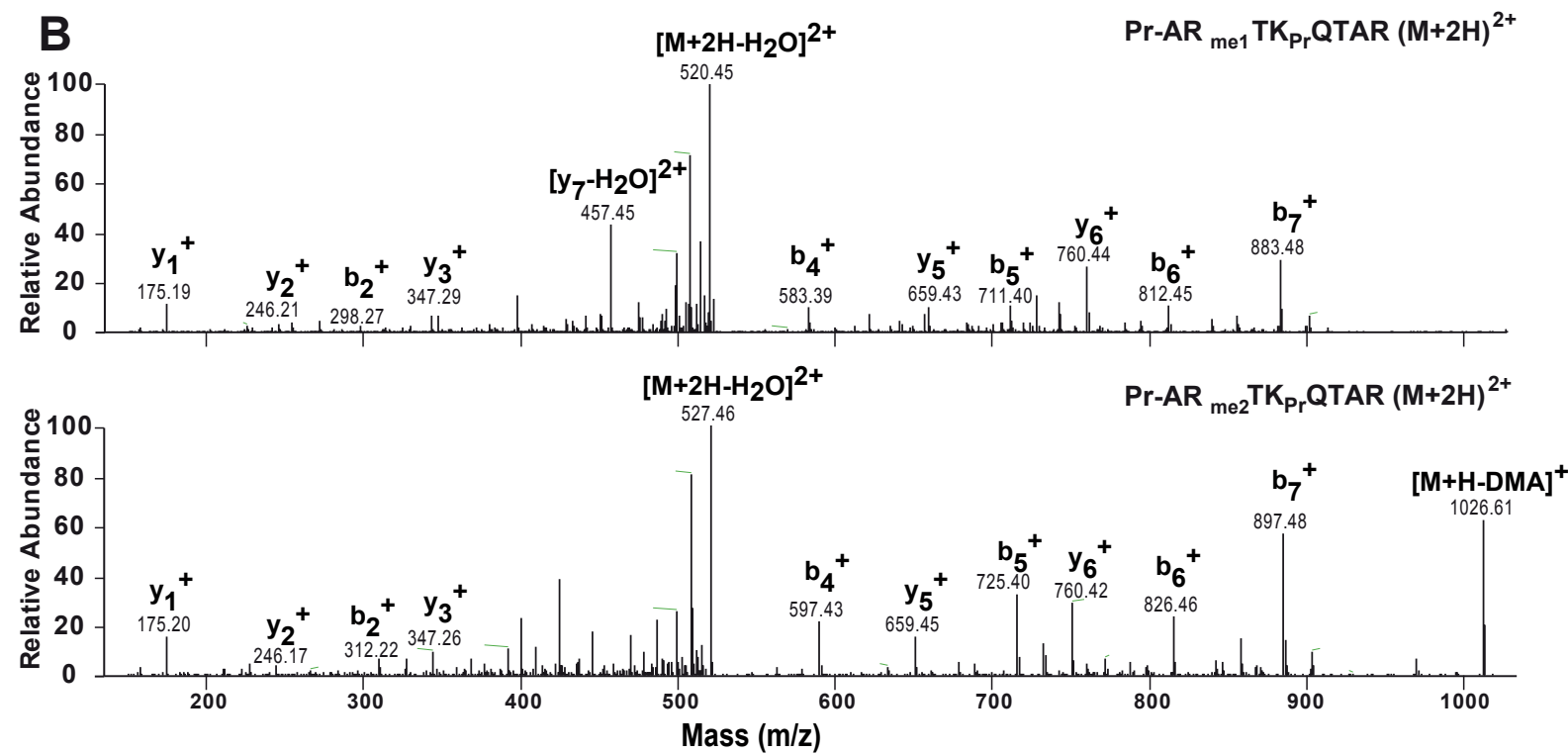


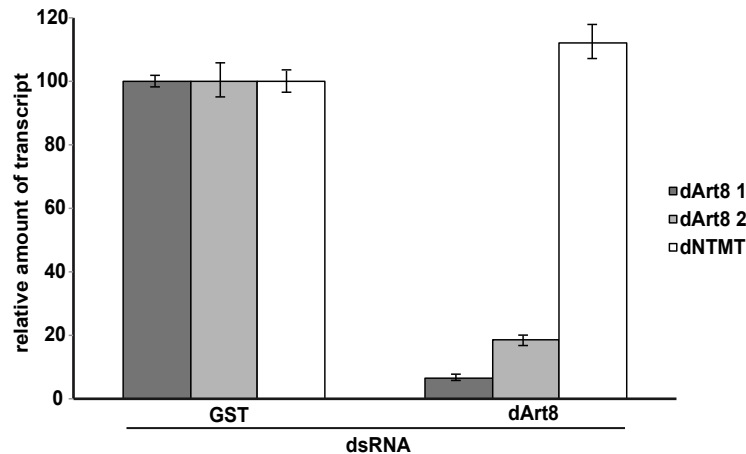
## MS/MS K11ac K17ac



**A**

dART8

**B**

**A****B**