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}H-SAM, only H2B became methylated. Upper panel, Comassie 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: dARTS8 does not significantly influence the activity of dNTMT *in vitro*. Recombinant flag-dNTMT was incubated at 30°C for 60min with ^{3}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 ^{3}H-SAM and H2B (lane 5), with ^{3}H-SAM and flag-dNTMT (lane 3) or with ^{3}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

b ions PP[21]K[20]T[19]S[18]GKAAK$_{10}$(K$_{ac}$)[12]AG[10]K$_{14}$[9]AQKNI$_{19}$T$_{20}$K$_{21}$T y ions

574.8464
574.5964
575.0969
575.3476

Rel. Abund.

m/z

m/z_{exp} = 574.5964 (M+4H)$^{4+}$
m/z_{cal} = 574.5937
Δm= 4.7 ppm

Relative Abundance

y_{10}^{2+}/ b_{21}^{4+} 545.0773
b_{20}^{3+}-H_2O 677.7339
y_{21}^{4+}-H_2O 545.8255
y_{12}^{2+} 658.3860
b_{21}^{4+}-H_2O 540.3251
y_{21}^{4+} 550.3284
b_{20}^{3+} 683.4018
y_{20}^{4+} 526.0646
y_{19}^{3+} 658.3860
b_{21}^{3+} 726.1006
b_{10}^{2+} 483.7883
y_{9}^{2+} 516.3114
b_{19}^{3+} 649.7198
y_{20}^{3+} 701.0854
y_{21}^{3+} 733.4358
b_{14}^{3+} 464.9465
y_{18}^{3+}-H_2O 618.6996

MS/MS K11ac K17ac

b ions PP[21]KTSGK$_7$A[15]AK$_{10}$(K$_{ac}$)$_{11}$AGKAQ$_{16}$(K$_{ac}$)N$_{18}$I$_{19}$T$_{20}$K$_{21}$T y ions

585.3503
585.0995
585.600
585.8521

Rel. Abund.

m/z

m/z_{exp} = 585.0995 (M+4H)$^{4+}$
m/z_{cal} = 585.0964
Δm= 5.3 ppm

Relative Abundance

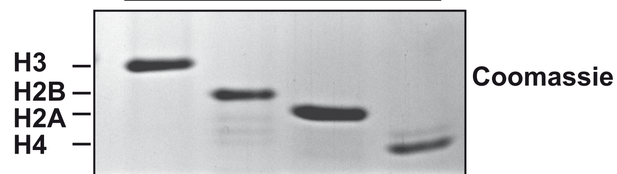
b_{20}^{3+} 697.4050
b_{7}^{+} 696.4002
y_{21}^{4+} 560.8302
b_{20}^{3+}-H_2O 691.4013
y_{15}^{3+} 547.9915
b_{21}^{4+}-H_2O 550.8274
b_{11}^{2+}-H_2O 555.8342
b_{16}^{3+} 531.3123
b_{21}^{4+} 555.3300
b_{19}^{3+} 663.7229
y_{21}^{3+} 747.4393
b_{21}^{3+} 740.1035
b_{7}^{2+} 348.7058
b_{10}^{2+} 483.7889
b_{18}^{3+} 626.0282
y_{15}^{2+} 821.4866

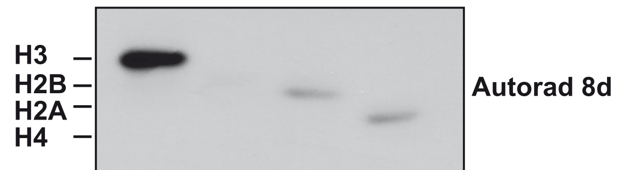
m/z

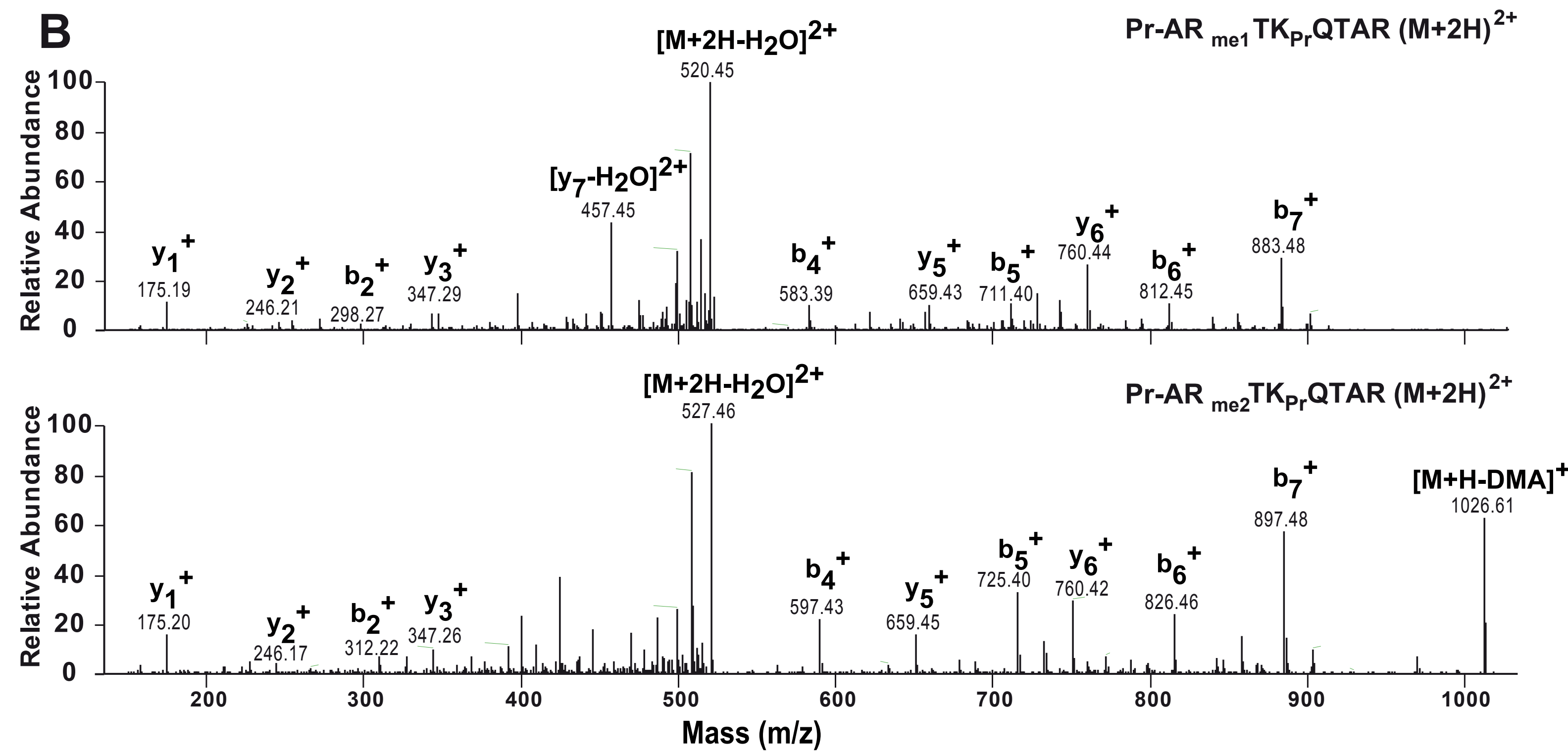
Villar-Garea Figure S2

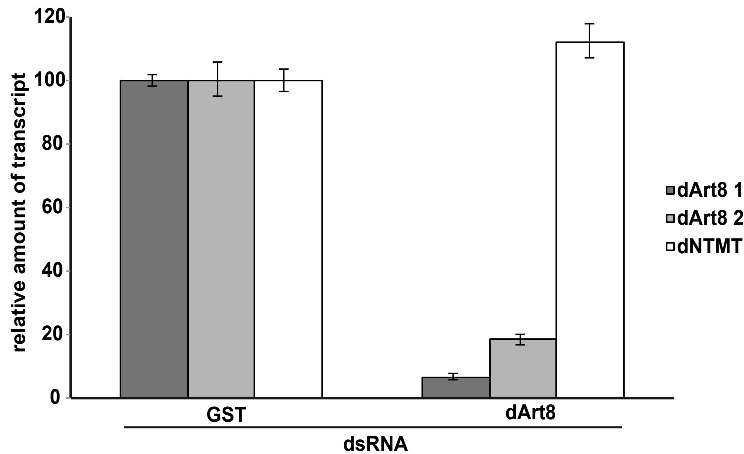
A

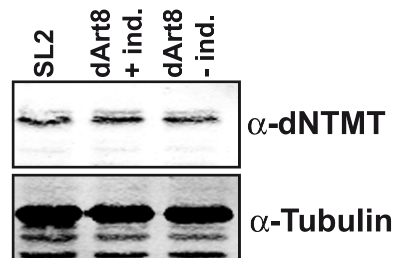
dART8

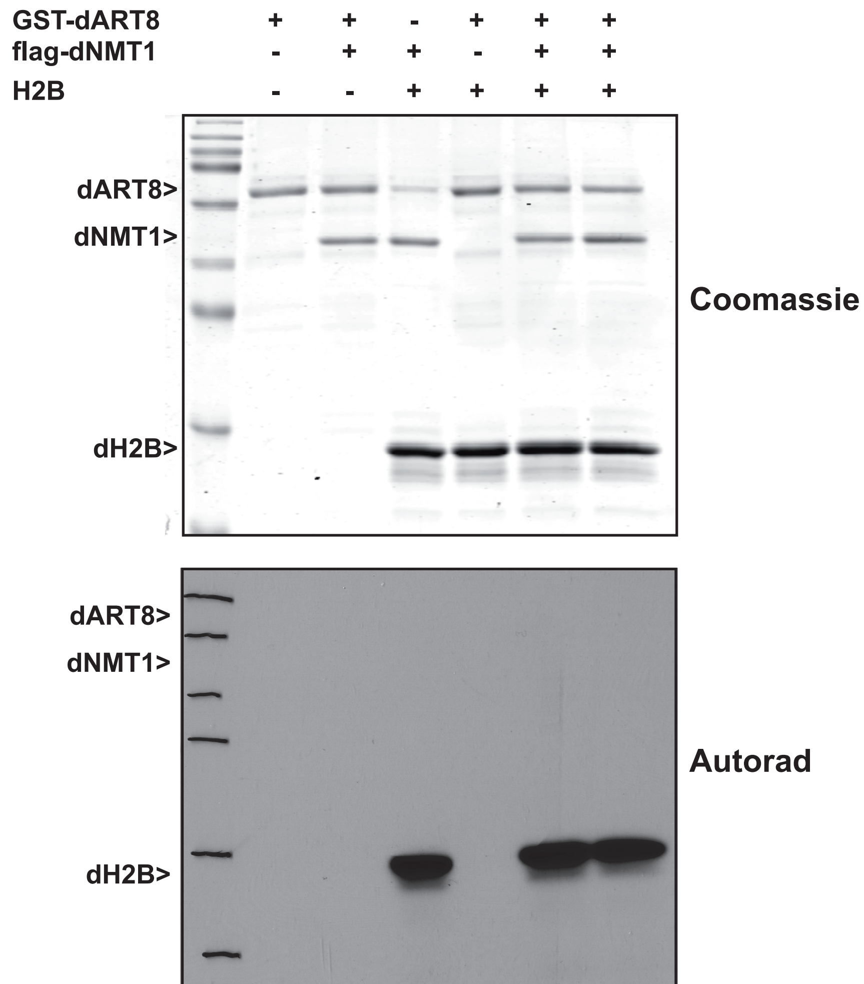


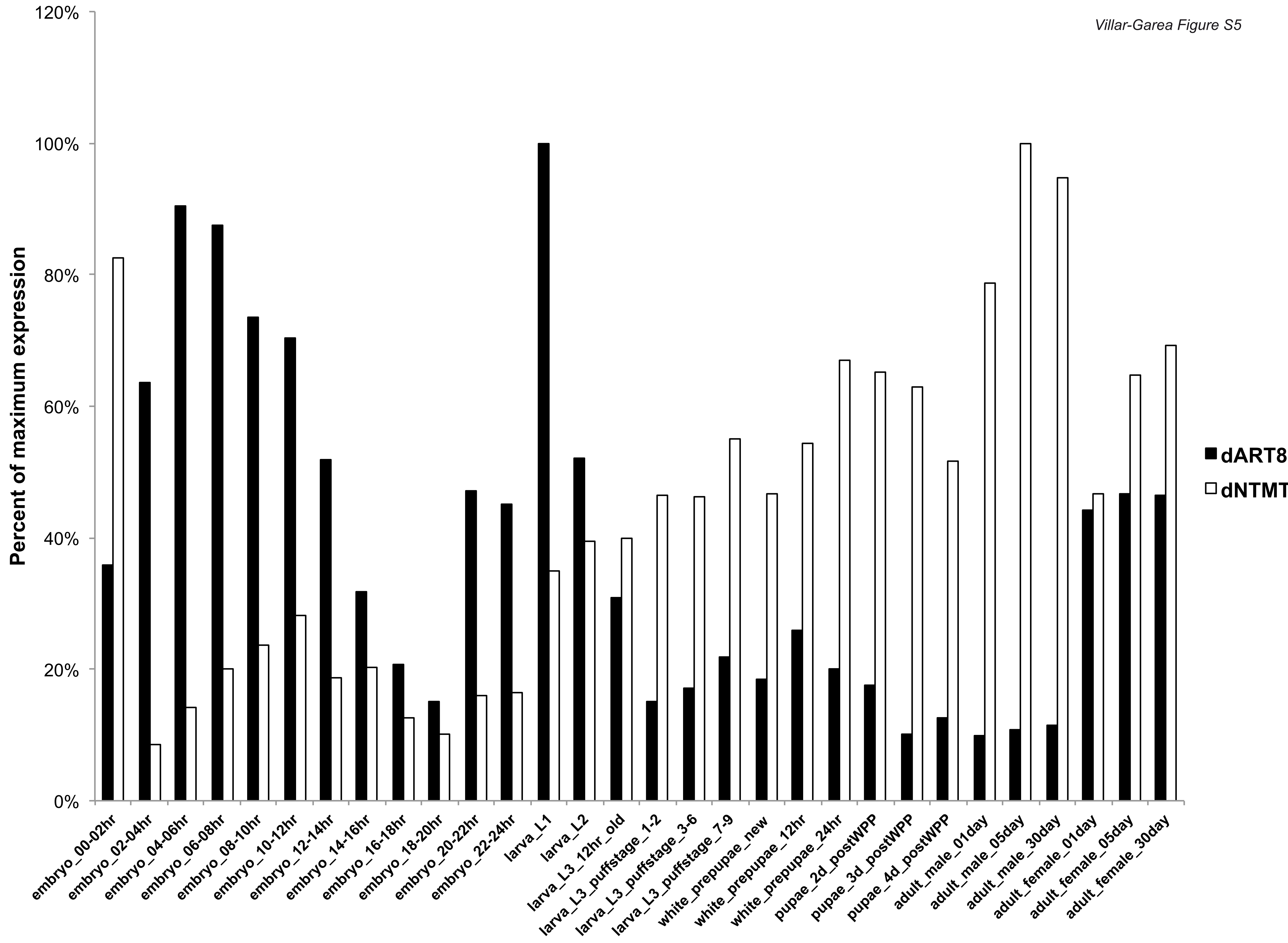





A
120
100
80
60
40
20
0
relative amount of transcript
GST
dArt8
dsRNA
dArt8 1
dArt8 2
dNTMT


B
SL2
dArt8
+ ind.
dArt8
- ind.
α-dNTMT
α-Tubulin


GST-dART8 + + - + + +
flag-dNMT1 - + + - + +
H2B - - + + + +
dART8>
dNMT1>
dH2B>
Coomassie
dART8>
dNMT1>
dH2B>
Autorad



Villar-Garea Figure S5
Percent of maximum expression
120%
100%
80%
60%
40%
20%
0%
dART8
dNTMT
embryo_00-02hr
embryo_02-04hr
embryo_04-06hr
embryo_06-08hr
embryo_08-10hr
embryo_10-12hr
embryo_12-14hr
embryo_14-16hr
embryo_16-18hr
embryo_18-20hr
embryo_20-22hr
embryo_22-24hr
larva_L1
larva_L2
larva_L3_12hr_old
larva_L3_puffstage_1-2
larva_L3_puffstage_3-6
larva_L3_puffstage_7-9
white_prepupae_new
white_prepupae_12hr
white_prepupae_24hr
pupae_2d_postWPP
pupae_3d_postWPP
pupae_4d_postWPP
adult_male_01day
adult_male_05day
adult_male_30day
adult_female_01day
adult_female_05day
adult_female_30day