Drosophila Kdm4 demethylases in histone H3 lysine 9 demethylation and ecdysteroid signaling

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Supplementary Table and Figures

Supplementary Table

Table S1. Viability of *Kdm4A, B* double homozygous Larvae

Time (AEL)	Genotype		
	Kdm4A^{KG04636}, *Kdm4B*^{EY10737}/*CyO GFP*	*Kdm4A*^{KG04636}, *Kdm4B*^{EY10737}/ *Kdm4A*^{KG04636}, *Kdm4B*^{EY10737}	
	Observed (n)	Observed (n)	Viability (%)
47 – 48 h	131	64	97.7
49 – 50 h	98	22	44.9
51 – 52 h	258	26	20.2
53 – 54 h	243	4	3.3

Kdm4A^{KG04636}, *Kdm4B*^{EY10737}/*CyO GFP* flies were allowed to lay eggs at 25^{o}C for one hour, and then adult flies were removed and eggs were allowed to hatch. Crawling larvae were counted at indicated time intervals and their genotypes were deduced by the presence or absence of GFP fluorescence, which was detected using an epifluorescent microscope. n denotes the number of live larvae. AEL: after egg laying. Percent viability = observed/expected x100. If viable, the expected number of *Kdm4A,B* double homozygous larvae should be ½ of their GFP+ siblings (homozygotes for the CyO chromosome die as embryos), based on the Mendelian ratio.

Supplementary Figures

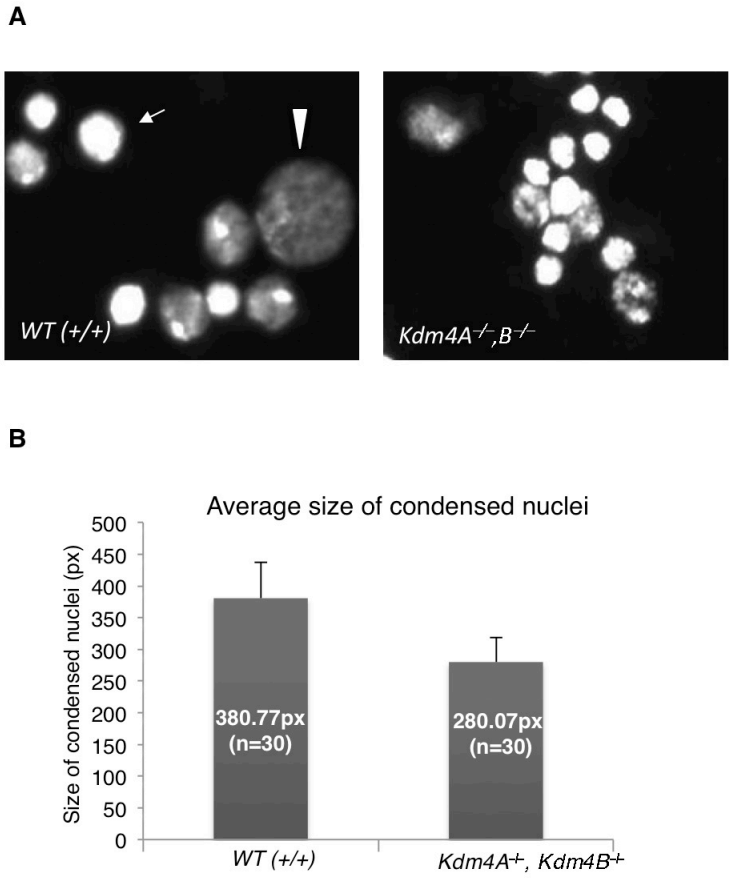

Figure S1. Loss of Kdm4 leads to higher nuclear compaction

(A) Wild-type control and *Kdm4A*$^{-/-}$*, Kdm4B*$^{-/-}$ homozygous double mutant second instar larval brains were dissected and placed on a glass slide in mounting media containing the DNA dye DAPI. Wild-type control and mutant brains were squashed under the same cover slip to allow comparison of nuclear compaction. Nuclei from the brain squash exhibit different size and compaction, as revealed by DAPI stain. The arrow points to a condensed nucleus. The arrowhead points to a less condensed nucleus, with a larger size and diffuse DAPI stain.

(B) Pixel size was used to quantify differences in condensed nuclear size. Note that *Kdm4A*^{KG}*, B*^{EY} homozygous double mutant brains exhibit smaller nuclear size and thus are more condensed.

Supplementary Figure 2

Figure S2. *Kdm4A*$^{-/-}$, *Kdm4B*$^{-/-}$ homozygous larvae show brown patches

A second instar *Kdm4A*$^{-/-}$, *Kdm4B*$^{-/-}$ homozygous larva with brown patches is shown. 28% of *Kdm4A*$^{-/-}$, *Kdm4B*$^{-/-}$ second instar larvae show this phenotype (n=27/95).

Supplementary Figure 3

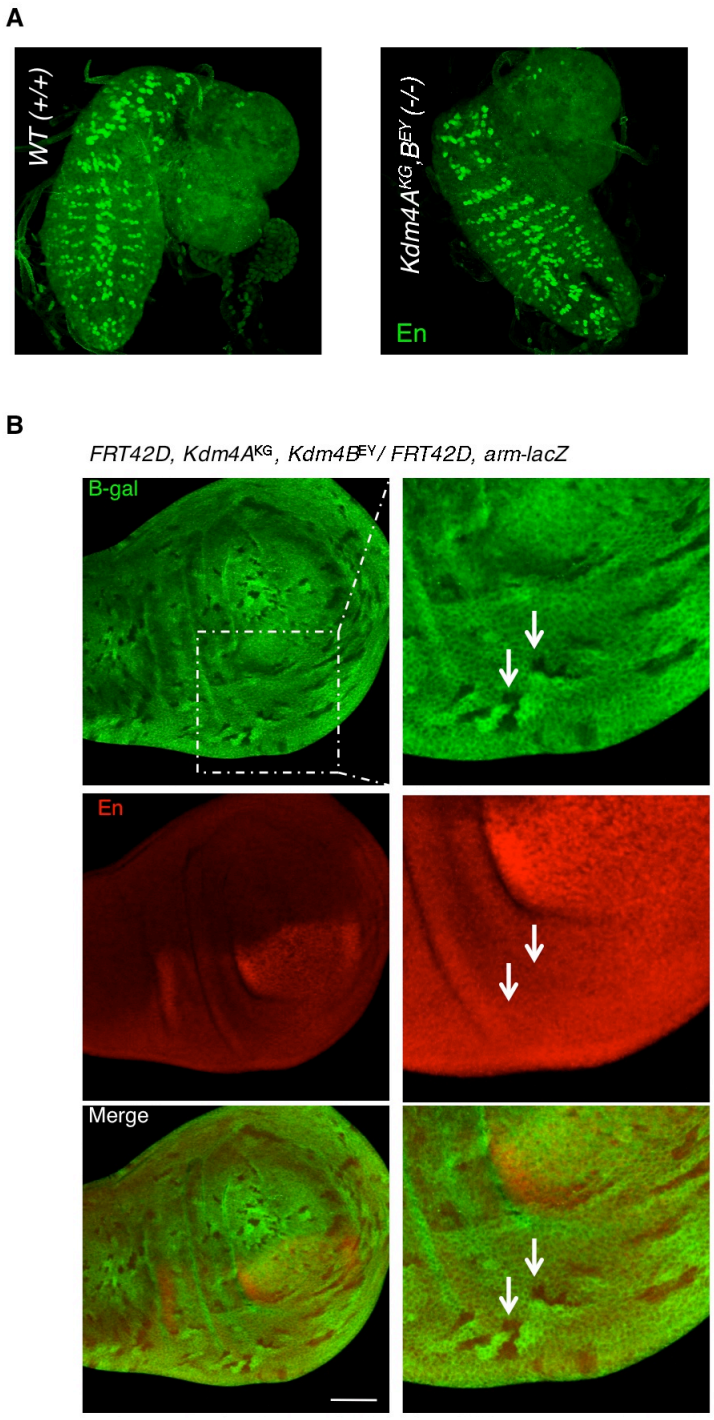

Figure S3. Loss of Kdm4 does not alter En expression

(A) Second instar larval brains from *WT* or $Kdm4A^{KG}$, B^{EY} homozygous double mutant animals were isolated and immunostained with anti-En antibody. **(B)** $Kdm4A^{KG}$, B^{EY} homozygous double mutant clones were induced in the second instar, and larval wing discs were dissected in the third instar. Anti-En and anti-ß-GAL immunostaining were performed. ß-GAL negative regions mark mutant clones, while cells with both signals are wildtype. Higher magnification images of the boxed region in the left panels are shown to the right. White arrows point to two $Kdm4A^{-/-}$, $Kdm4B^{-/-}$ mutant clones (marked by lack of green). Note that En signals (red) in the clones were not altered. The scale bars represent 150μ.

Figure S4

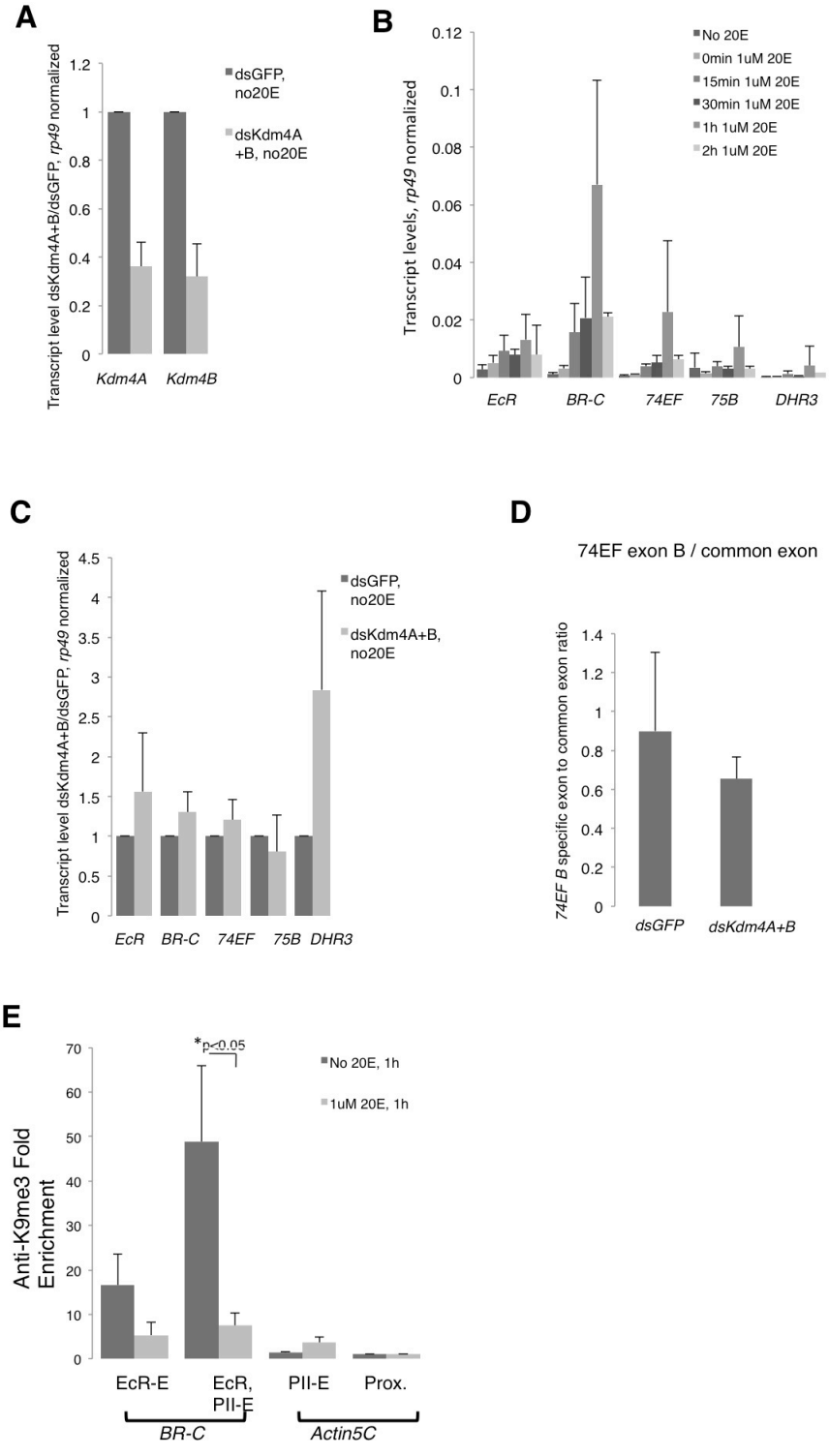
A
Transcript level dsKdm4A+B/dsGFP, rp49 normalized
1.2
1
0.8
0.6
0.4
0.2
0
dsGFP, no20E
dsKdm4A +B, no20E
Kdm4A
Kdm4B
B
Transcript levels, rp49 normalized
0.12
0.1
0.08
0.06
0.04
0.02
0
No 20E
0min 1uM 20E
15min 1uM 20E
30min 1uM 20E
1h 1uM 20E
2h 1uM 20E
EcR
BR-C
74EF
75B
DHR3
C
Transcript level dsKdm4A+B/dsGFP, rp49 normalized
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
dsGFP, no20E
dsKdm4A+B, no20E
EcR
BR-C
74EF
75B
DHR3
D
74EF exon B / common exon
74EF B specific exon to common exon ratio
1.4
1.2
1
0.8
0.6
0.4
0.2
0
dsGFP
dsKdm4A+B
E
Anti-K9me3 Fold Enrichment
70
60
50
40
30
20
10
0
*p<0.05
No 20E, 1h
1uM 20E, 1h
EcR-E
EcR, PII-E
PII-E
Prox.
BR-C
Actin5C

Figure S4. Kdm4 and expression of the Ecdysone pathway components in S2 cells

(A) S2 cells were treated with 20μg of dsRNA directed against *Kdm4A* and *Kdm4B*, or *GFP*. Quantitative RT-PCR was conducted in triplicate to detect levels of *Kdm4A* and *Kdm4B* transcripts, whwich were normalized to *rp49* transcript levels.

(B) The ecdysteroid pathway was induced in S2 cells by adding 1μM of the 20E hormone. Cells were harvested at the indicated time points and subjected to quantitative RT-PCR in triplicates, with primer pairs specific for *EcR, BR-C, 74EF, 75B* and *DHR3*, respectively. Peak activation was detected at the one-hour time point.

(C) S2 cells were treated with *Kdm4A* and *Kdm4B* dsRNA, or *GFP* control dsRNA. *EcR, BR-C, 74EF, 75B* and *DHR3* transcript levels were measured by quantitative RT-PCR in triplicate, with normalization to *rp49,* prior to 20E induction to determine changes in basal levels upon depletion of both *Kdm4A* and *Kdm4B*. Error bars represent standard deviations.

(D) S2 cells treated with dsRNA directed against *Kdm4A* and *Kdm4B,* or with control dsRNA against *GFP* were harvested one hour after induction with 1μM 20E. The ratio between splice form specific to commonly expressed *74EF* exon was determined by quanitative-PCR analysis. Error bars represent standard deviation.

(E) S2 cells treated with 1μM of 20E for 1 hour, were harvested and subjected to ChIP analysis with anti-H3K9me3 antibody in triplicates. The Fold Enrichment method was used for calculation, normalized to primer pairs amplifying the *actin5C* proximal promoter region. Error bars represent standard error.

Supplementary Figure 5

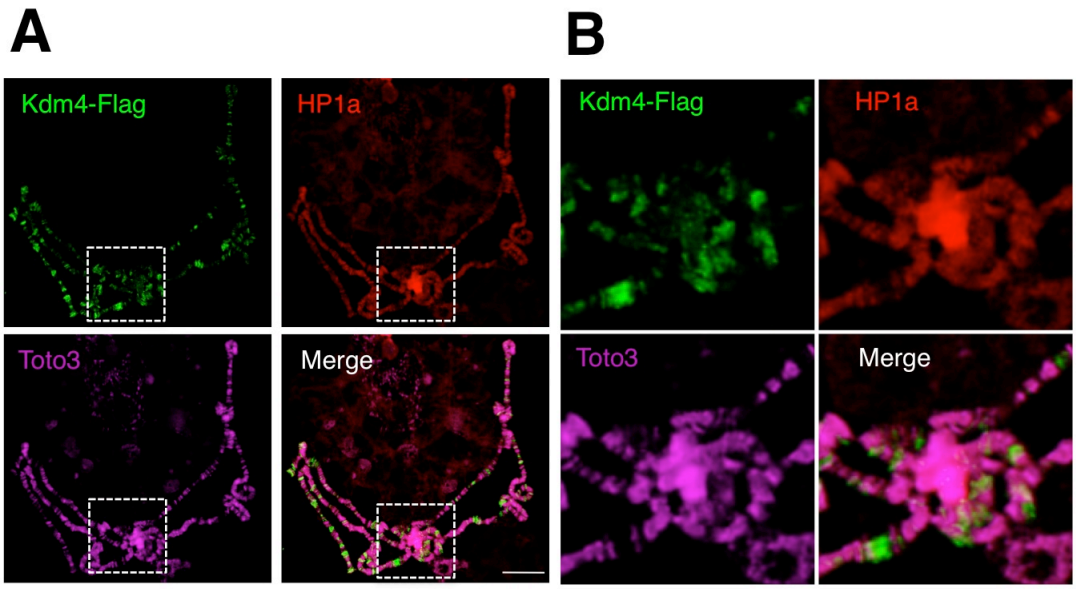

Figure S5. Distribution of Kdm4A and HP1a on polytene chromosomes

The *Sgs-GAL4* driver was used to express Flag-tagged Kdm4A and third instar larval polytene chromosomes were prepared. Immunostaining was performed with anti-Flag and anti-HP1a antibodies, and with Toto-3 to reveal DNA. The scale bar represents 150 μ.

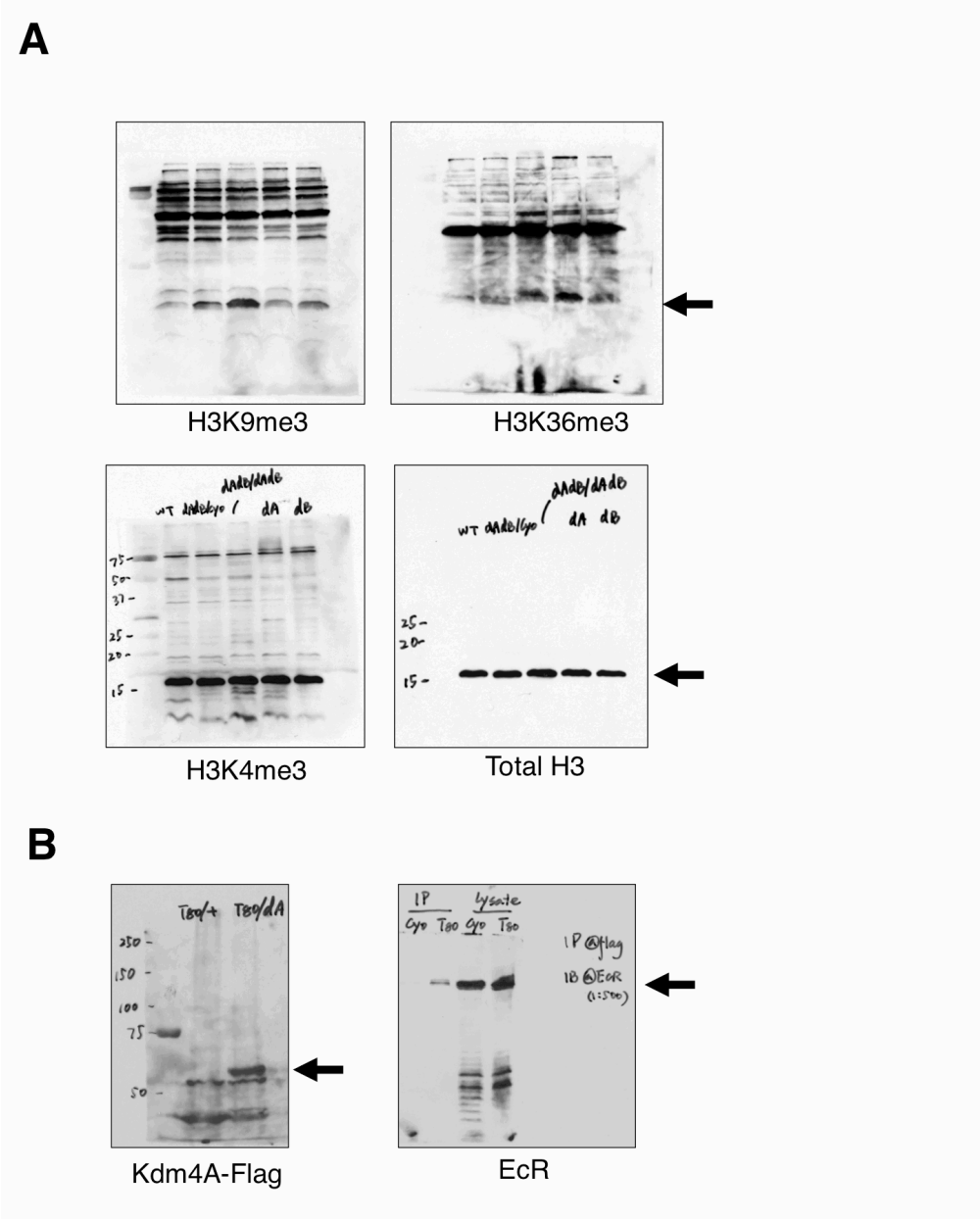

Figure S6. Full-length gel images

Antibodies used for blotting are indicated at the bottom of gel images. Arrows point to specific bands. Other bands are non-specific bands due to cross-reactions of the antibodies.
(A) Full-length Western blot gel images of the cropped bands shown in Figure 1C. **(B)** Full-length images of cropped bands shown in Figure 4B.