

Supplementary figures:

Fig. S1. The NCBI Reference Sequence number and the protein-protein BLAST (BLASTp) search results for the putative conserved domains for each KDM2 proteins that were used in generating the phylogenetic tree presented in Fig. 1A.

Fig. S2. The Coomassie blue staining (left) of the GST-dKDM2 (AA1-220) fusion protein expressed in *E. coli*, note the expected band of ~52.2 kDa. This band can be strongly recognized by our polyclonal dKDM2 antibody, as shown by Western blot (right). This GST-dKDM2 (AA1-220) fusion protein was used to purify the polyclonal dKDM2 antiserum, which was used for the Western blots presented in this work.

Fig. S3 Validation of the five *dKdm2* alleles generated by insertion of transposable elements. (A) Schematic representation showing the insertion sites of the five insertion lines (*d00170*, *DG12810*, *EP3093*, *EY01336*, and *KG04325*) within the *dKdm2* locus. The *EP3093* line was validated using the two-sided PCR approach (B, showing the primers P3 and P4) and the results is shown in (C). The other four insertion lines were verified by the genomic PCR approach (D, showing the primers P5 and P6) and the results are shown in (E). Since the transposons are larger than 10kb, only the wild-type gDNA (has no insertions) allow the PCR

amplification of products that are less than 2kb. Lower panels in **C** and **E** are positive controls using the *dKDM2-34F* and *dKdm2-56R* primers (2682bp apart, as shown in Fig. 2A and 5A) and the same gDNA samples that were used in the upper panels. A 2682bp PCR product was expected from these reactions.

Fig. S4. Analyses of *dKdm2* mRNA and dKDM2 protein levels in several class III *dKdm2* alleles. (**A ~ D**) shows the results of qRT-PCR analysis of *dKdm2* and its neighboring genes in *dKdm2*^{d06730} (**A**), *dKdm2*^{EY01336} (**B**), *dKdm2*^{F11.1} (**C**), and *dKdm2*^{KG04325} (**D**) homozygous mutants during the wandering stage. (**E ~ H**) Results of Western blot analysis showing the levels of dKDM2 protein in the *dKdm2*^{d06730} (**E**), *dKdm2*^{EY01336} (**F**; *Df(3R)J16* mutants were used as the negative control), *dKdm2*^{F11.1} (**G**; *Df(3R)J16*, *Df(3R)J18*, and *dKdm2*^{DG12810} mutants were used as the control), and *dKdm2*^{KG04325} (**H**) homozygous third-instar wandering larvae.

Fig. S5. qRT-PCR analyses of *dKdm2* mRNA levels in several transheterozygous combinations of the *dKdm2* alleles. The genotypes include *w*¹¹¹⁸; +; *Df(3R)J15/dKdm2*^{d00170} (**A**), *w*¹¹¹⁸; +; *Df(3R)J16/dKdm2*^{d00170} (**B**), *w*¹¹¹⁸; +; *dKdm2*^{d00170}/*dKdm2*^{f02828} (**C**), *w*¹¹¹⁸; +; *Df(3R)J16/dKdm2*^{f02828} (**D**), and *w*¹¹¹⁸; +; *dKdm2*^{EP3093}/*dKdm2*^{f02828} (**E**).

Fig. S6. Verification of several transposon insertion lines after four generations of outcrossing with *w*¹¹¹⁸ flies. (**A**) The *dKdm2*^{f02828}, *dKdm2*^{d00170} and *dKdm2*^{EP3093} alleles were verified by genotype PCR using the same scheme illustrated in Fig. S3D, and the *dKdm2*^{EP3093} allele was also validated using hybrid PCR similar to Fig. 3C. To distinguish with their corresponding parental alleles, each allele is marked with “#”. (**B ~ D**) qRT-PCR analyses of the cleaned *dKdm2* alleles to examine the levels of *dKdm2* and its neighboring genes, and the genotypes include *w*¹¹¹⁸; +; *dKdm2*^{f02828#} (**B**), *w*¹¹¹⁸; +; *dKdm2*^{d00170#} (**C**), and *w*¹¹¹⁸; +; *dKdm2*^{EP3093#} (**D**). (**E**) The levels of dKDM2 protein in these mutants detected by Western blot.

All of the homozygous animals are selected at the L3 wandering stage.

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