File S1

Supporting Materials and Methods

Gene specific PCR:

DNA was isolated from homozygous mutant embryos, and PCR was performed with primers designed based upon the *CG40351* genomic sequence available on Flybase (www.flybase.org). PCR conditions were as follows: 94° for 5 minutes followed by 33 cycles of 94° for 30 s, annealing (varying temperatures listed in Table S1) for 40s and 72° for 1 min. PCR fragments were directly sequenced with the same primers used to amplify each fragment. Resultant sequences were compared to the published *D. melanogaster* genomic sequence available on Flybase (www.flybase.org). Mutations were identified by the presence of mismatches when compared to the reference sequence.

TABLE S1. Sequences and annealing temperatures of *CG40351* PCR primers pairs.

Primer pair used:	Sequences:	Tanneal:
SER1F/	*5'-TTAGCCATAGTCTGAACTAGTTATTATTTACG-3'*	60°
SER2R	*5'-TTGACCAGGTTGATATTCCGAAC-3'*	
SER2F/	*5'-AGGTTTGTTCATAATTGACACAGATGC-3'*	62°
SER3R	*5'-TCATACCTTTCCCATTACAGACTTTTG-3'*	
SER3F/	*5'-ACTTGGGCATTGCACGTATAGTG-3'*	62°
SER4R	*CTACTTATTGTATTCGAAGAACGTCTAACATC*	
SER4F/	*5'-GGATCGCGGAAGTCTTAAGTATTGTAG-3'*	63°
47936M2R	*5'-CCCATAGCCTGAAGCATGGTAATAAAAAGG-3'*	
SER4BF/	*5'-TATCCAGACCAAGGAAGGTGCAG-3'*	64°
SER4CR	*5'-CATTAGAATCCTCCTTATCAGAGTCGGAGC-3'*	
SEQ1F/	*5'-CATCGTTTCGGCGTATAAGAAAACATCC-3'*	65°
SER4ER	*5'-CTCGTTCCATTTGAGCCATGTACTC-3'*	
SER4EF/	*5'-GTGTGATATGTATAAAACTCGTGATC-3'*	56°
SEQ1R	*5'-TACTTATGCTTAGCCTTTTCTCGAACATC-3'*	
SER6F/	*5'-GGTTAACTCGACTAAATCATTCTCTCTTG-3'*	61°
SER6BR	*5'-GTATTAGTTAAGTGTACCCCGACATCC-3'*	
SER6F/	*5'-ATGTAAGTAAGCTGGATGTTGACATTATG-3'*	63°
SER6R	*5'-GAACAGCACCTCTGACCTCGTTG-3'*	
SER4DF/	*5'-TGAATACGATAGAATTTACAGCGACTC-3'*	61°
SEQ1R	*5'-TACTTATGCTTAGCCTTTTCTCGAACATC-3'*	

Cloning of inducible *UAS-dSet1* cDNA transgenes:

Since there was no available cDNA clone containing the full length dSet1 cDNA sequence, we separately amplified and cloned fragments from the DGRC cDNA clones *RE4793*6 and *LD15202*2, which contain incomplete but overlapping regions of the full length *dSet1* cDNA sequence. Regions of the *dSet1* coding sequence contained within *RE47936* and *LD15202* were amplified with *SER2R*/*SER4DR* and *SER4CF*/*SER6BR* respectively (primer sequences listed in Table S1) and each respective PCR product was separately cloned into *pTZ57R/T* using the InsTAclone PCR Cloning Kit (Fermentas). Each fragment was cleaved from the *pTZ57R/T* vector, followed by cleavage of each fragment at an overlapping *Xho*I site, ligated together using T4 DNA ligase (Invitrogen) as per manufacturer's instructions and finally into the *pTZ57R/T* vector. Sequence errors contained within the *dSet1* sequence were corrected with the Quikchange Site Directed Mutagenesis Kit (Stratagene) following manufacturer's instructions so that the full length *dSet1* ORF matched the sequence for *CG40351* (gi: 281366741,68-5018). This corrected *dSet1* coding sequence was cut from *pTZ57R/T* using flanking *Xba*I sites and ligated into *Xba*I digested *pUAST (*BRAND and PERRIMON 1993*).* The completed rescue construct (*pUAST-dSet1*) was fully sequenced with the primers listed in Table S1 prior to microinjection by Bestgene Inc..

A sequence encoding a 2X FLAG™ tag was PCR amplified without template DNA using the overlapping primers *2XFLAGpUASTF: 5'-TTTGCGGCCGCGACTACAAGGATGACGATGACAAAGACTACAAGGATGACGATGACAAA- 3'* and *2XFLAGpUASTR: TTTCTCGAGTTATTTGTCATCGTCCATCCTTGTAGTCTTTGTCATCGTCATCCTTGTAGTC-3'*. PCR conditions were as follows: 94° for 5 minutes followed by 20 cycles of 94° for 30s, 60° for 40 s and 72° for 30 s. The 2X FLAG fragment was digested with *Not*I and *Xba*I and ligated into *pUAST* (*pUAST-2X FLAG*). The *dSet1* cDNA sequence was amplified from 20 ng of *pUAST-dSet1* template using a standard PCR recipe with the primers *SETNotIF: 5'-TTTGCGGCCGCTCATGCAGGACGTTCGG-3'*and *SET2XFLAGNotIR: 5'-TTTGCGGCCGCGTTAAGTGTACCCCGACATCC-3'* containing engineered *Not*I restriction sites. PCR conditions were: 95° for 5 min., followed by 33 cycles of 95° for 30 s, 55°C for 40 s and 72° for 5 min. This amplicon and *pUAST-2X FLAG* were digested with *Not*I and ligated together with T4 DNA ligase (Invitrogen). The completed *pUAST-dSet1-2X FLAG* was fully sequenced with primers listed in Table S1 prior to microinjection by Bestgene Inc..

Cloning of GST fusion proteins:

The full length *wds* and *ash2* cDNA sequences were amplified from the Drosophila Gold Collection clones *RE31658* and *LD31689* respectively, with the primers *wdsGSTF: 5'-TTGAATTCGCCAAGGAGCATAAGCAGAAT-3'* and *wdsGSTR: 5'-TTGTCGACAGCTGGATACCAGCCACTCTAT-3'*, and *ash2GSTF: 5'- TTGAATTCATCACTGCAATGGAGGACAG-3'* and *ash2GSTR: 5'-TTCTCGAGCCAAAACTCATGTGTATGACGA-3'*. PCR conditions were 94° for 5 minutes followed by 33 cycles of 94° 30 seconds, 58° for 40 seconds, followed by 72° for 2 minutes. *wds* and *ash2* cDNA amplicons were digested with *Eco*RI/*Sal*I and *Eco*RI/*Xho*I

respectively (sites engineered in each primer set), and were cloned into *pGEX-4T1* in frame with a 5' sequence coding for GST. The *CG17293* cDNA sequence was subcloned from *pEG202* into *pGEX-4T-1* using the restriction sites previously described.

Quantitative PCR:

RNA was extracted from *OreR* animals and animals expressing RNAi targeting Drosophila COMPASS members using TRIzol reagent (Invitrogen) followed by chloroform extraction and isopropanol precipitation using standard procedures. 300 ng of RNA from each stage tested was treated with 1 U of DNAse I for 1 hour, heated at 65° for 15 minutes and converted into cDNA using the iScript Select cDNA synthesis kit (Biorad). Primers were designed for *rp49* and genes coding for COMPASS orthologues and are listed in Table S1. Reaction mixtures were prepared in triplicate as follows: 2 µL cDNA, 1 µL primer/probe mix, 7 µL of ddH2O and 10 µL of KAPA Sybr Fast Mastermix (KAPA). PCR conditions were 40 cycles of 94° for 3 seconds, followed by 60° for 30 seconds, followed by 72° for 1 minute. Primer pairs used are listed in Table S2. Primer efficiencies were estimated from the slope of standard curves generated using dilutions of adult male cDNA. Transcript levels in RNAi knockdown experiments were calculated relative to wild-type using the ΔΔCt method and using *rp49* as a reference gene to normalize differences in input cDNA levels (PFFAFL 2001).

TABLE S2. qPCR primers used to validate RNAi experiments.

Gene target:	Sequences:
dWdr82	*5'*- GCA CGG TGA ACT CGA AGA AG *-3'*
	5'- TCG TGC AGG CTG AGA TAG *-3'*
wds	*5'*- AGC TCC TCC GCT GAT AAA CTA ATC *-3'*
	5'- ACC TTG AGC GTC TTG TCA TCA C *-3'*
hcf	*5'*- TTC TTA ACC CAA CCG GAC CG *-3'*
	5'- CGC ATC CGT TCG GTA CAT CA *-3'*
dRbbp5	*5'*- TGA GCG GAT GCG TTT CAT C *-3'*
	5'- GGA ACC GCA TGA CTA TGA GGA *-3'*
trx	*5'- TCG ACT ATG GAT CGG ATC AG-3'*
	5'-GAT GGG CTG CTT GCG ATG TCT-3'
dSet1	*5'*- CGT TCG GAA TAT CAA CCT GGT C *-3'*
	5'- CGT AAC GAT AGA GTC TGG TAC CAC *-3'*
rp49	5'-TGC TAA GCT GTC GCA CAA ATG GCG -3'
	5'-CAT GTG GCG GGT GCG CTT GTT C -3'

Methyl-H3K4 antibody specific tests:

0.5 μg and 0.05 μg of unmodified H3 peptides and H3 peptides containing mono-, di- and trimethylation at lysine 4 (Epigenetek) were spotted onto nitrocellulose membranes and dried for 30 minutes. Immunoblotting was then performed using standard procedures. Antibody dilutions are indicated in the main text.

Quantitative Western blotting:

Two-fold dilutions series of wild-type and *dSet1* mutant (*G12/γ28*) nuclear extracts were run on SDS-PAGE gels and transferred to nitrocellulose membranes using standard procedures. Immunoblotting was then performed using standard procedures with antibody dilutions indicated in the text. Detection was performed using Supersignal Pico or Supersignal Femto (Thermo Scientific) and exposure to film. Exposed films were scanned and integrated density (pixel area multipled by baseline subtracted intensity) of bands were calculated within an appropriate exposure range, and bands from *dSet1* mutant lanes were compared with bands from wild-type lanes exposed to similar levels. These integrated densities were then normalized according to total H3 loaded. Levels of mutant to wild-type methyl-H3K4 were calculated based on the normalized integrated density of the band corresponding to *dSet1* mutant extracts divided by that corresponding to the normalized wild-type extract multiplied by the dilution factor. All blots were performed in duplicate and error is reported as the standard error of the mean (SEM).

SUPPORTING REFERENCES

BRAND, A. H. and N. PERRIMON, 1993 Targeted gene expression as a means of altering cell fates generating dominant phenotypes. *Development* **118**: 401-415.

PFAFFL, M. W., 2001 A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic Acids Res.* **29**(9): e45.

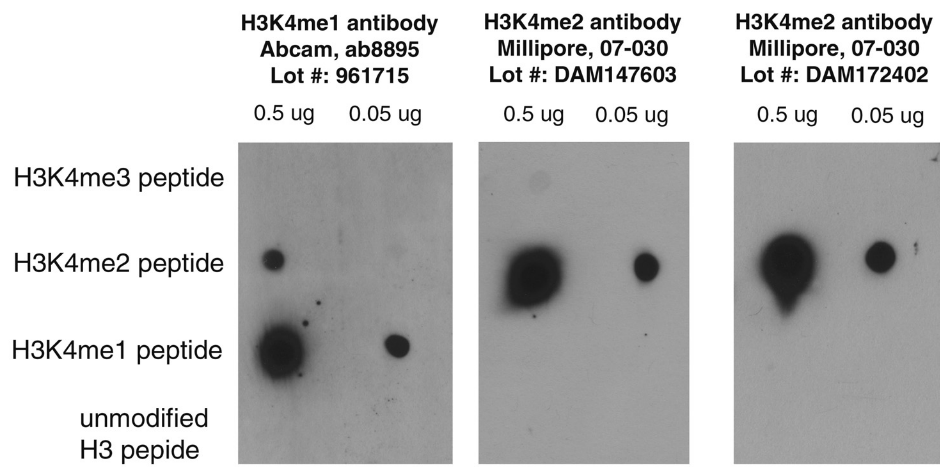

Figure S1 Methyl-H3K4 antibody specificity tests.

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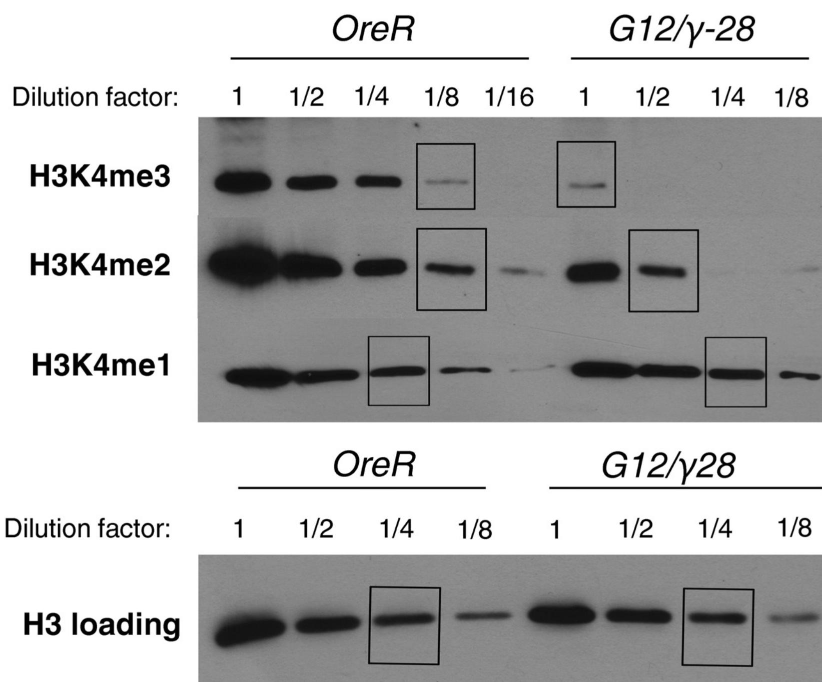

Figure S2 An example of our quantitative approach for calculating methyl H3K4 levels in *dSet1* mutants (*G12*/*γ28*) relative to wild-type (*OreR*) levels. This example contains dilution series of pupal extracts. Boxed bands correspond to bands used for quantification purposes.

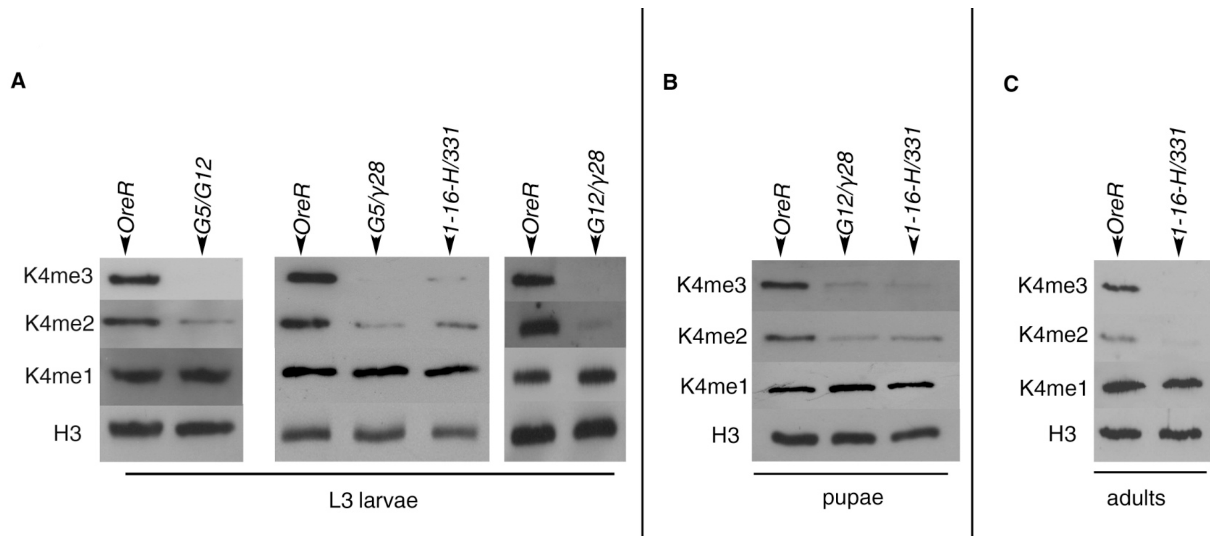

Figure S3 Comparisons of total H3K4 levels between wild-type (*OreR*) and *dSet1* mutant transheterozygous (A) third instar larvae (B) pupae and (C) adults.

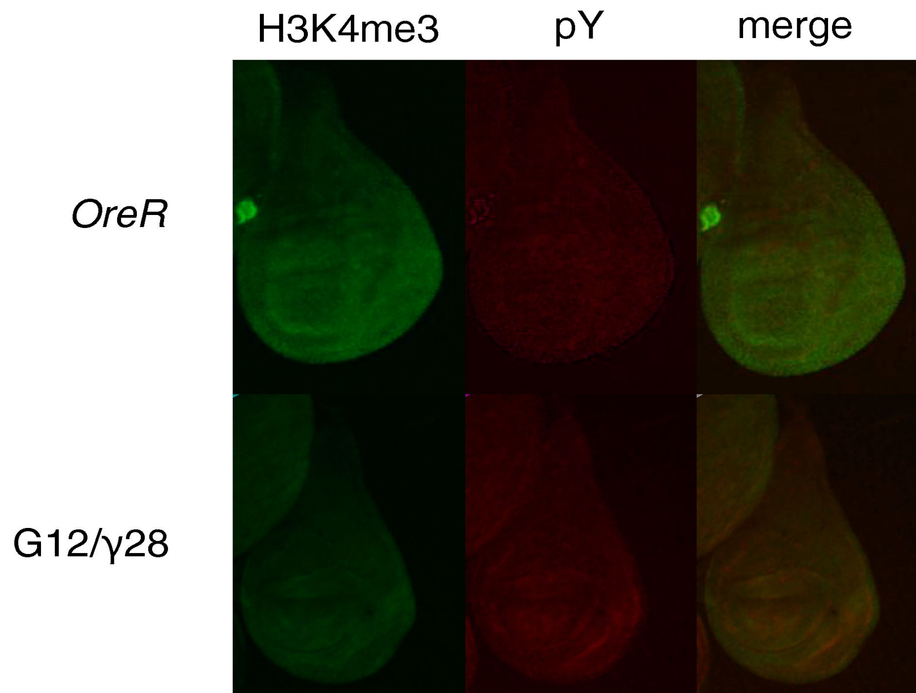

Figure S4 *dSet1* mutations result in reduced H3K4 trimethylation in halterediscs. Left to right: H3K4me3 (green), phosphotyrosine (red), merge. Immunostaining was performed as described in the main text using FITC anti-rabbit and Texas-Red anti-mouse secondaries (Cell Signalling) for detection.