Supporting Information

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SI Materials and Methods

Chromatin Immunoprecipitation. We focused our studies on L3s because genome-wide profiling is available for this stage. ChIP was performed as described in ref. 1 with the following modifications: L3-staged worms or embryos were flash-frozen in liquid nitrogen and ground to a fine powder in liquid nitrogen using a mortar and pestle. L3 worm powder was incubated in PBS containing 1% formaldehyde for 10 min at room temperature. After quenching with glycine (125 mM final), the worm pellet was washed three times in PBS. The worm pellet was resuspended in lysis buffer [50 mM Hepes/KOH (pH 7.6), 150 mM KCl, 1 mM EDTA, 0.5% sarkosyl/protease inhibitors mixture (Roche Biochemicals), and 1 mM PMSF] and sonicated in a Bioruptor water bath (Diagenod) at maximum power for 30 s on and 30 s off for a total of eight pulses on. The homogenate was then centrifuged at 12,000 rpm for 15 min at 4 °C and the supernatant containing the sheared chromatin (chromatin extract) was saved, frozen in liquid nitrogen, and kept at −80 °C for immunoprecipitation. Chromatin sheared profile was checked on an agarose gel, showing fragments between 300 bp and 1,000 bp. For H3K4me3 and H3K4me2 ChIP experiments on N2 or *bn129* strains, chromatin extract (1.2 mg of total protein in 1 mL of lysis buffer) was thawed, diluted with 500 μL dilution buffer [50 mM Hepes/KOH (pH 7.6), 150 mM KCl, 1 mM EDTA, 3% Triton X-100, 0.3% sodium deoxycholate, protease inhibitors mixture, and 1 mM PMSF] and precleared for 1 h at 4 °C with 120 μL of a 1:1 slurry of protein A agarose beads (Millipore; 16157). The cleared extract was split in three Eppendorf tubes in which the following antibodies were added: 5 μL of polyclonal anti-H3K4me2 antibody (Abcam; ab7766), 1 μL of polyclonal anti-H3K4me3 antibody (Diagenode; CS-003-100), or 1 μL of control rabbit IgG. After incubation for 2 h 30 min at 4 °C, 40 μL of a 1:1 slurry of protein A agarose beads was added to each tube and incubation was extended to 1 h. Beads were washed with 1 mL of the following buffer for 5 min at room temperature: two times with FA buffer [50 mM Hepes/KOH (pH 7.5), 1 mM EDTA, 1% Triton X-100, 0.1% sodium deoxycholate, 150 mM KCl, protease inhibitors mixture, 1 mM PMSF], one time with FA-500 [50 mM Hepes/KOH (pH 7.5), 1 mM EDTA, 1% Triton X-100, 0.1% sodium deoxycholate, 500 mM NaCl], one time with FA-1M [50 mM Hepes/KOH (pH 7.5), 1 mM EDTA, 1% Triton X-100, 0.1% sodium deoxycholate, 1 M NaCl], one time with TEL (0.25 M LiCl, 1% Nonidet P-40, 1% sodium deoxycholate, 1 mM EDTA, 10 mM Tris·HCl, pH 8.0), and two times with TE (10 mM Tris·HCl, 1 mM EDTA). Elution was performed by adding two times 75 μL elution buffer (10 mM Tris·HCl, 1 mM EDTA, 250 mM NaCl, 1% SDS) and incubating at 65 °C for 10 min. Supernatants were pooled, incubated for 30 min with 1 μL of RNase A (10 mg/mL), and then incubated overnight at 65 °C with 1 μL of proteinase K (15–20 mg/mL). DNA was recovered using the Qiaquick pure purification kit in 50 μL of 10 mM Tris for qPCR analysis. ChIPs were analyzed by qPCR using the Light Cycler detection system. We focused our studies on genes at least 2 kb away from the nearest 5′ gene, which avoids the possible complication of upstream gene signal contributing to test gene signal. Furthermore, for *pcaf-1* and *lin-13* the transcribed region is long enough to clearly distinguish promoter signal from gene body signal (1).

Isolation of *set-2*(*bn129*). The first round of amplification primers used to screen the UV-TMP deletion library were 130F, 5′-CCTTGTTCCGTGTTCAAGGT-3′; 1020R (central poison primer), 5′-GTCGCTTGTCATTGCTCAAA-3′; and 2005R, 5′-TTCTCCAGGGCAATTCATC-3′. Primers used in the second round were 182F, 5′-TCAATTCGGTGAGCGTTATA-3′; and 1953R, 5′-GTGCTGCCCTCAAATACCAT-3′. The last two primers and the poison primer were used for subsequent genotyping by PCR. The prediction of the molecular lesion associated with *bn129*, based on sequencing genomic DNA, was confirmed by sequencing RT-PCR products from mutants.

SET-2 Contribution to H3K4me3 in the Germ Line. To calculate the contribution of *set-2* to germ-line methylation in Fig. 2*E*, we reasoned as follows: In *glp-1* mutants, which have only soma, global methylation levels are reduced to 40% of wild-type levels. Therefore, ∼60% of the wild-type signal is derived from the germ line and ∼40% is derived from the soma. The *set-2* mutation reduces the soma signal by ∼50% (compare *set-2 ;glp-1* to *glp-1*), so SET-2 is responsible for half of H3K4me3 in the soma. In the WT histogram, 40% of the total signal is from the soma (compare WT to *glp-1*). According to the previous estimate, half of this soma signal (20%) is from SET-2, leaving the remaining 20% to another HMT. The *set-2* mutation reduces total H3K4me3 signal by ∼70% (compare *set-2* to WT), leaving 30% of the total H3K4me3 signal for another HMT. Of this 30%, we know that ∼20% corresponds to a somatic signal, leaving the remaining ∼10% for germ-line signal. Because in the WT histogram ∼60% of the total signal is derived from the germ line and 10% of this total signal is due to an HMT different from SET-2, we can deduce that the remaining ∼50% of total signal is due to SET-2 in the germ line. Therefore, ∼50/60 of the H3K4me3 signal (i.e., ∼83%) is due to SET-2 in the germ line.

Plasmid Construction. The *set-2*::GFP construct was previously described (2). *wdr-5*::HA was obtained by replacing the GFP coding sequence from the *wdr-5*::GFP rescuing construct pTS1 with HA coding sequence.

Quantification of Germ-Line Fluorescence. Images were taken at 63× magnification as stacks, using a Zeiss Axioplan 2 microscope coupled to a CoolSNAP CCD camera. The same integration time was used for all of the measurements. Stacks were merged into a single image using the ImageJ software package. A 300 × 300 pixel-wide region was enclosed and the "Measure" tool in the Analyze menu applied to this region to measure the maximal fluorescence intensity value. Measurements were taken from six to eight dissected gonads for each genotype and averaged.

1. Kolasinska-Zwierz P, et al. (2009) Differential chromatin marking of introns and expressed exons by H3K36me3. *Nat Genet* 41:376–381.

2. Simonet T, Dulermo R, Schott S, Palladino F (2007) Antagonistic functions of SET-2/SET1 and HPL/HP1 proteins in *C. elegans* development. *Dev Biol* 312:367–383.

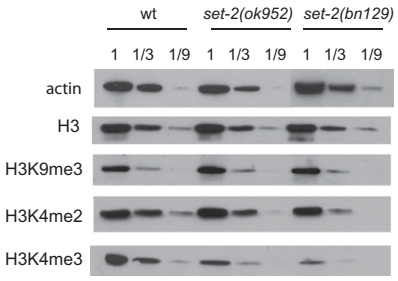

Fig. S1. Quantitative Western blot analysis by serial dilution of loading samples to examine H3K4me2 and -me3 levels. The highest amount of protein loaded per lane was 15–20 μg. Following transfer, the same membrane was cut into two parts (according to the molecular weight differences) and probed with H3K4me3, H3K4me2, H3, or actin antibodies. For detection of H3K4me2/3 and H3, which have similar molecular weights, we either used different membranes or stripped and reprobed the same membrane. H3 and actin loading controls gave similar results.

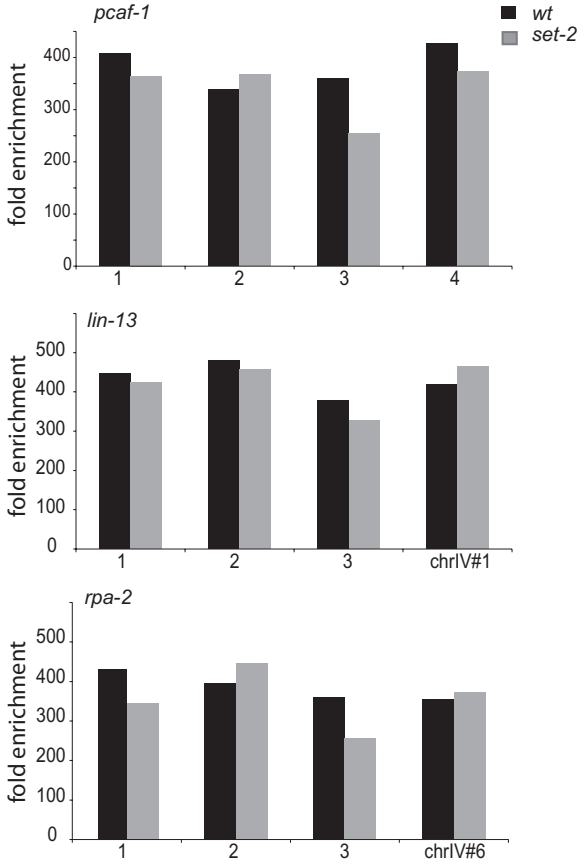

Fig. S2. Histone H3 occupancy is not altered in *set-2*(*bn129*) mutant strains. ChIP analysis was performed on L3 staged wild-type and *set-2(bn129)* mutant animals using histone H3-specific antibodies (Abcam; ab1791) and a preimmune serum as a control. The position of the primers used for qPCR analysis is shown in Fig. 3. Fold enrichment is calculated as the ratio between specific binding by the antibodies and nonspecific binding by preimmune serum.

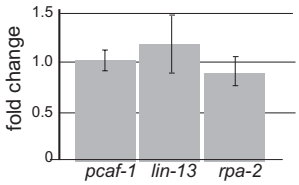

Fig. S3. Expression of *pcaf-1*, *lin-13*, and *rba-2* is not affected by *set-2* loss in L3 larvae. RNA levels were normalized to the mean of internal control genes *pmp-3* and *cdc-42*. Two technical replicates were performed for each of two independent biological samples.

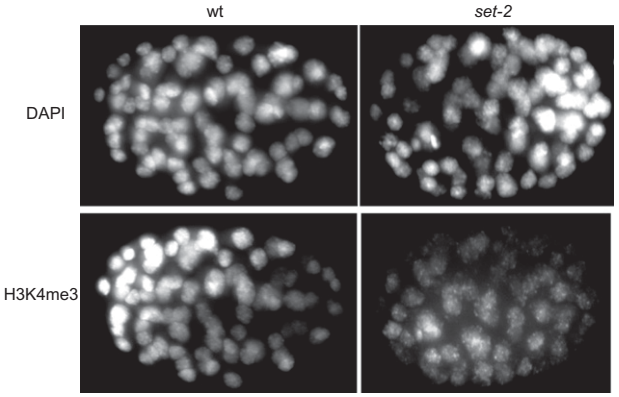

Fig. S4. Residual H3K4me3 signal in *set-2(bn129)* mutant embryos. Fixed embryos were stained with anti-H3K4me3 antibodies from Diagenode (cs-003-100).

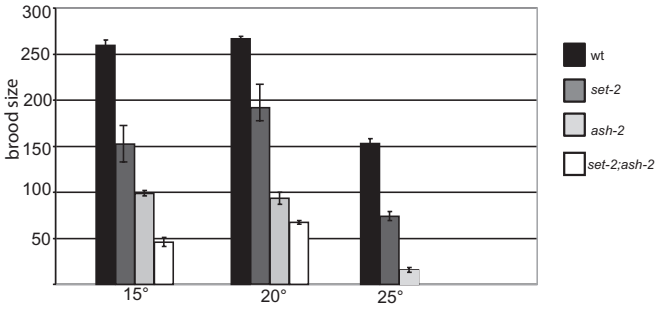

Fig. S5. Reduced fertility in the absence of ASH-2 activity. Five to 10 L4 stage larvae were picked on single plates at the indicated temperature and allowed to lay eggs for 3–4 d, transferring the mother to a fresh plate each day, until no more eggs were laid. Bars represent the average brood size for wild-type and mutant animals. Error bars show SEM.

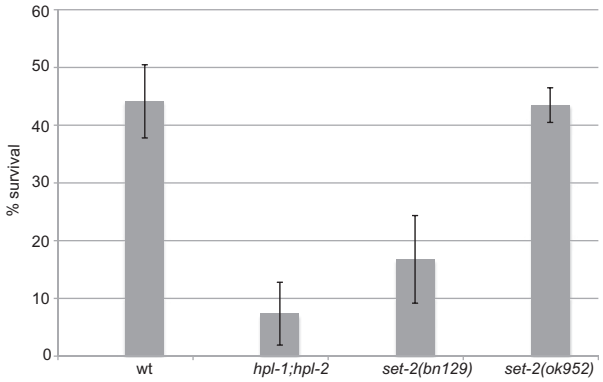

Fig. S6. Increased UV sensitivity of *set-2(bn129)* mutant animals: hatching of wild-type, *set-2(ok952)*, and *set-2(bn129)* mutant eggs after irradiation with UV-B at 80 J/m^2. Eggs were collected from gravid adult animals by ClNaO/NaOH treatment and placed on culture plates seeded with OP50 bacteria at 20 °C (1). Eggs were irradiated using a Bio-Link BLX-E365 device, and the percentage of lethality was scored after 20 h. *hpl-1;hpl-2* double mutants are highly sensitive to UV-induced DNA damage and were used as positive controls (1).

1. Luijsterburg MS, et al. (2009) Heterochromatin protein 1 is recruited to various types of DNA damage. *J Cell Biol* 185:577–586.

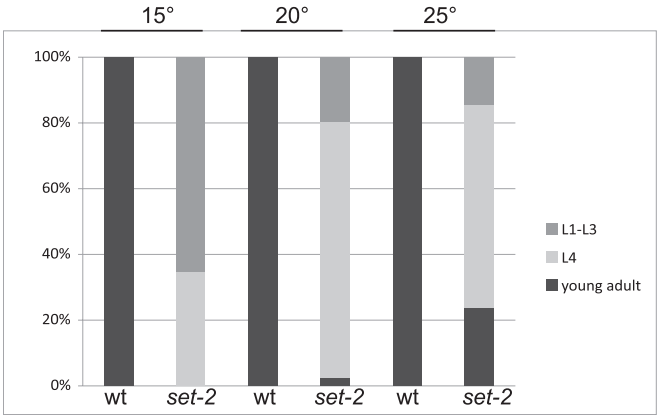

Fig. S7. *set-2(bn129)* mutants display cold-sensitive growth defects. L1 animals from synchronized populations grown at 15 °C were individually picked on plates at the indicated temperature. Once 100% of wild-type animals reached adulthood, the developmental stage of mutant animals at the same temperature was scored as L1–L3, L4, or young adult. Data show averages of three independent experiments.

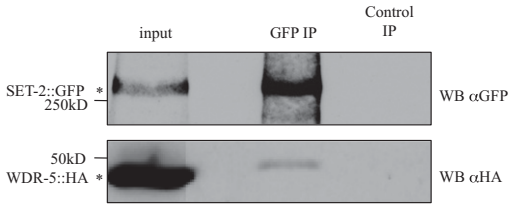

Fig. S8. Interaction between SET-2 and WDR-5 is conserved in *C. elegans*. Populations of mixed-stage embryos were collected from transgenic animals expressing *set-2*::GFP and *wdr-5*::HA. Lysates were subjected to immunoprecipitation using either GFP-Trap (ChromoTek) coupled to beads or control beads. A total of 2.5% of input was loaded in lane 1 and half of the immunoprecipitated material in lanes 2 and 3. Samples were subjected to SDS/PAGE and analyzed by Western blot with the indicated antibodies. Anti-HA antibody (MMS-101P) was from Covance. Anti-GFP (11 814 460 001) was from Roche.