Figures and figure supplements

SET-9 and SET-26 are H3K4me3 readers and play critical roles in germline development and longevity

Wenke Wang et al
Figure 1. set-26 but not set-9 is important for longevity. (A) Schematic of the set-9(rw5) and set-26(tm2467) mutants. Red star indicates the position of the sgRNA (single guide RNA) targeting the set-9 gene. Premature stop codons caused by deletions of 38 nucleotides in the set-9 gene and 1090 nucleotides in the set-26 gene are depicted as red hexagons. Loss of set-26 gene but not set-9 gene extended lifespan (B), and increased resistance to heat stress (C). Survival curves for N2, set-26(tm2467), set-9(rw5), and set-9(rw5) set-26(tm2467) strains from representative experiments are shown. Quantitative data for all replicates are shown in Supplementary file 1 Table S1.
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Figure 2. set-9 and set-26 act redundantly to maintain fertility. (A) The set-9(rw5) set-26(tm2467) double mutant worms derived from heterozygous parents (F1) displayed a mild fertility defect. The double mutant worms displayed a much more severe fertility defect at later generations (F2–F6). Average brood size of N2, set-26(tm2467), set-9(rw5), and set-9(rw5) set-26(tm2467) strains at the indicated generation were shown (*p<0.05, ***p<0.001). The error bars represent standard errors. n = 9–10 for N2, set-9 mutant, set-26 mutant, and F1 set-9 set-26 double mutant worms; n = 50

Figure 2 continued on next page
for F2-F6 set-9 set-26 double mutants. (B) The set-9(rw5) set-26(tm2467) double mutant exhibited a mortal germline phenotype. At each generation, 6 L1s for N2, set-26(tm2467), set-9(rw5) and set-9(rw5) set-26(tm2467) strains were transferred to a new plate. Plates were scored as not fertile when no progeny were found. % of fertile lines indicated percentage of plates that were fertile. n = 6 for N2, set-9 and set-26 mutants; n = 25 for set-9 set-26 double mutants. (C) Maternal contribution of set-9 and set-26 appeared important for alleviating the fertility defect in the double mutant. Average brood size of the set-9(rw5) set-26(tm2467) double mutants derived from four different crosses were shown (***p<0.001, n.s. no significant). n = 11 – 12 for assessing the brood size of the set-9(rw5) set-26(tm2467) homozygous progeny from heterozygous male(P0) X heterozygous hermaphrodite(P0) and homozygous male(F1) X heterozygous hermaphrodite(P0); n = 30 – 34 for progeny from homozygous male (F1) X heterozygous hermaphrodite(P0) and heterozygous male(P0) X heterozygous hermaphrodite(F1). (D) The set-9(rw5) set-26(tm2467) double mutant worms that remained fertile nevertheless exhibited reduced number of mitotic germ cells. Whole worms or dissected gonads of fertile set-9(rw5) set-26(tm2467) mutants were stained by DAPI and the mitotic cells were counted. D2 adults were scored. n = 18 – 27, ***p<0.001. Analyses of sterile set-9(rw5) set-26(tm2467) double mutant worms are shown in Figure 2—figure supplement 1. Quantitative data are shown in Supplementary file 1 Table S2.

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Figure 2—figure supplement 1. Fertility defects of the set-9 set-26 double mutant. (A) The set-9(rw5) set-26(tm2467) double mutant exhibited variable percentage of sterility at the different generations. N = 155–517. (B) Representative DAPI staining images showing the germline of three different sterile
Figure 2—figure supplement 1 continued

F3-F4 set-9(rw5) set-26(tm2467) double mutant worms. The germline of these sterile worms showed variable phenotypes, including no differentiated germ cells, no oocytes, or no sperms. D1-D2 adults were scored.

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Figure 3. SET-26 is broadly expressed and SET-9 is only detectable in the germline. (A, B) Fluorescent micrographs of worms carrying gfp knock-in at the C-terminus of set-9 or set-26 gene (set-9::gfp and set-26::gfp). GFP-fused SET-26 was detected in all cells and GFP-fused SET-9 was only detected in the germline. Star indicates head, arrow indicates germline in the images. The signal outside of the germline detected in the set-9::gfp strain represented autofluorescence (marked by hashtag), which appeared yellow under the microscope. (C, D) Germline-specific knockdown of set-9 and set-26 was not sufficient to extend lifespan. RNAi knockdown of set-9 and set-26 or wdr-5.1 extended lifespan in N2 worms (C). RNAi knockdown of wdr-5.1, but not set-9 and set-26, extended lifespan in the rrf-1(pk1417) mutant worms. Quantitative data are shown in Supplementary file 1 Table S3.

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Figure 3—figure supplement 1. GFP-tagged SET-9 and SET-26 are largely functional. (A–B) Worms carrying a gfp knock-in at the C-terminus of set-26 exhibited mild lifespan extension and heat resistance phenotypes, but the phenotypes were much weaker than those of the loss-of-function set-26.

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Figure 3—figure supplement 1 continued

(C) Worms carrying gfp knock-ins at the C-termini of set-9 and set-26 genes exhibited a mild fertility defect, which was significantly different from the severe fertility defect observed in the set-9(rw5) set-26(tm2467) double mutant (*p<0.05, ***p<0.001). n = 8-10 for set-9::gfp set-26::gfp and N2; n = 28 for F3 set-9(rw5) set-26(tm2467). Quantitative data are shown in Supplementary file 1 Table S4. (D) SET-26::GFP expression upon RNAi knockdown of set-9 and set-26 and L4440. set-26::gfp; rrf-1(pk1417) and set-26::gfp strains are shown. Arrow heads indicate germline, arrows indicate intestine nuclei in the images.

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**Figure 3—figure supplement 2.** GFP-tagged SET-9 is only detectable in the germline. Fluorescent micrographs of worms carrying gfp knock-in at the C-terminus of set-9 gene (set-9::gfp) is shown. ‘GFP’ indicates signals under GFP channel and ‘Autofluorescence’ indicates signals under YFP channel. Top panel shows L4 stage worm whereas bottom panel shows L2/L3 stage.

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Figure 4. DAF-16-dependent somatic SET-26 regulated genes are enriched for lifespan determinant genes. (A) Venn diagrams show the overlap between up-regulated genes in glp-1(e2141); set-26(tm2467) (comparing with glp-1(e2141)) and down-regulated genes in daf-16(mgDf47); glp-1(e2141); set-26.
Figure 4 continued

set-26(tm2467) (comparing with glp-1(e2141); set-26(tm2467)); and the overlap between down-regulated genes in glp-1(e2141); set-26(tm2467) (comparing with glp-1(e2141)) and up-regulated genes in daf-16(mgDf47); glp-1(e2141); set-26(tm2467) (comparing with glp-1(e2141); set-26(tm2467)).

(B) GO term analysis of DAF-16-dependent somatic SET-26 regulated genes. Gene lists can be found in Supplementary file 2.

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Figure 4—figure supplement 1. Transcriptional profile of glp-1(e2141); set-26(tm2467) mutant. (A) GO term analysis of genes with expression change in glp-1(e2141); set-26(tm2467) (comparing with glp-1(e2141)). (B) Venn diagram shows the overlap between set-26 regulated genes in glp-1(e2141); set-26(tm2467) (comparing with glp-1(e2141)) and set-26 regulated genes in set-26(tm2467) (comparing with N2). Gene lists can be found in Supplementary file 2.

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Figure 5. Transcriptional profiles of set-9(rw5), set-26(tm2467), and F1 set-9(rw5) set-26(tm2467) mutants. (A) Venn diagrams show the overlap among set-9(rw5), set-26(tm2467), and set-9(rw5) set-26(tm2467) down-regulated (left) and up-regulated (right) gene sets. Hashtag indicates genes that only
show expression change in the F1 set-9(rw5) set-26(tm2467) double mutant. GO term analysis of up-regulated (B) and down-regulated (C) genes that only show expression change in the F1 set-9(rw5) set-26(tm2467) double mutant. (D) Venn diagram shows the overlaps between genes that only show expression change in the F1 set-9(rw5) set-26(tm2467) double mutant identified in our RNA-seq data with the previously reported germline-specific gene lists (Reinke et al., 2004). Gene lists can be found in Supplementary file 2.

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**Figure 5—figure supplement 1.** Transcriptional profile comparison between F1 and F3 set-9(rw5) set-26(tm2467) double mutants. (A) GO term analysis of down-regulated and up-regulated genes in F1 set-9(rw5) set-26(tm2467) double mutant. (B) GO term analysis of down-regulated and up-regulated genes in F3 set-9(rw5) set-26(tm2467) double mutant. **Figure 5—figure supplement 1 continued on next page**
genes in F3 set-9(rw5) set-26(tm2467) double mutant. (C) Venn diagrams show the overlaps between down-regulated and up-regulated genes in the F1 and F3 set-9(rw5) set-26(tm2467) double mutants. (D) Scatter plot of log2 fold change of all mRNAs in F3 and F1 set-9(rw5) set-26(tm2467) double mutant. (E) Scatter plot of log2 fold change of genes in F3 and F1 set-9(rw5) set-26(tm2467) double mutants. Only genes that show significant expression change in set-9(rw5) set-26(tm2467) double mutants are shown. Gene lists can be found in Supplementary file 2.

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Figure 6. SET-9 and SET-26 bind to H3K4me3. (A) PHD domain of SET-9 and SET-26 specifically pulled down H3 in vitro. Coomassie-blue stained gel showing pulldown results using GST–SET-9/26 PHD and GST control. (B) Binding intensity of GST-SET-9/26 PHD to the significant hits from histone peptide arrays. Quantitative data are shown in Supplementary file 1 Table S5. (C) Genome browser view showing ChIP z-scores (standardized log2 ratios of ChIP/Input or ChIP/H3ChIP signals) for SET-9/26, H3K4me3 and H3K9me3 at a representative region. (D) 75% of the SET-9 and SET-26 peaks overlapped with H3K4me3 peaks. (**p<0.001 indicates overlapping more than expected) (E) 49% of the H3K4me3 peaks overlapped with SET-9 and Figure 6 continued on next page
Figure 6 continued

SET-26 peaks. (**p<0.001 indicates overlapping more than expected) (F) 8% of the SET-9/26 peaks overlapped with H3K9me3 peaks. (**p<0.001 indicates overlapping less than expected).

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Figure 6—figure supplement 1. SET-9 and SET-26 are not the major enzymes for H3K9me3 in vivo. (A) Clustal Omega alignment of the SET domains of SET-9 and SET-26 from *C. elegans*, SETD5, MLL5, SET7 and SUV91 from human, UpSET from *Drosophila melanogaster* and SET1 from *Saccharomyces*. Red boxes indicate the proposed key residues. The online tool used is: http://www.ebi.ac.uk/Tools/msa/clustalo/. (B) Western blotting showing H3K9me3 levels in N2 and the set-9(rw5) set-26(tm2467) double mutant (left). Synchronized L4 worms for N2 and the set-9(rw5) set-26(tm2467) double mutant were used. Quantification of three independent Western experiments (right). (C) Representative genome browser views of H3K9me3.
Figure 6—figure supplement 1 continued

Profiles in N2 (black) and the F3 set-9(rw5) set-26(tm2467) double mutant (purple). z-scores for normalized H3K9me3 and SET-9 and SET-26 ChIP signals in N2 and the set-9(rw5) set-26(tm2467) are shown.

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Figure 6—figure supplement 2. SET-9 and SET-26 are not the major enzymes for H3K9me3 in vivo (part 2). (A) Heatmaps showing the H3K9me3 profiles in wild-type and the F3 set-9(rw5) set-26(tm2467) double mutant for all H3K9me3 peaks.
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Figure 6—figure supplement 3. SET-9 and SET-26 share similar genomic binding profiles in vivo. (A) Genome browser view showing ChIP z-scores (standardized log2 ratios of ChIP/Input or ChIP/H3ChIP signals) for SET-9, SET-26 and SET-9/26 at a representative region. (B) A large proportion of SET-9 peaks overlapped with SET-26 peaks. (C) A large proportion of SET-26 peaks overlapped with SET-9 and SET-26 peaks.

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Figure 6—figure supplement 4. SET-9 and SET-26 bind to H3K4me3 in vivo. (A) Metagene plots showing the average H3K4me3 z-score (standardized log2 ratios of H3K4me3 ChIP/H3 ChIP signals) for the H3K4me3 peaks bound (shown in blue) and not bound by SET-9 and SET-26 (shown in grey) in N2.
Figure 6—figure supplement 4 continued

worms. Regions 3000 bp upstream and downstream of the peak summits are shown. The light blue and light grey indicate 95% confidence intervals. (B) Metagene plots showed the average SET-9 and SET-26 z-score (standardized log2 ratios of SET-9 and SET-26 ChIP/input signals) for the H3K4me3 peaks bound (shown in blue) and not bound by SET-9 and SET-26 peaks (shown in grey). The light blue and light grey indicate 95% confidence intervals. (C) Scatter plot of H3K4me3 z-score and SET-9 and SET-26 z-score for H3K4me3 peak regions.

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**Figure 6—figure supplement 5.** SET-9 and SET-26 bind to H3K4me3 in vivo (part 2). (A) 28% of the SET-9 and SET-26 peaks overlapped with H3K9ac peaks. (**p<0.001 indicates overlapping more than expected) (B) Scatter plot of H3K4me3 z-score and H3K9ac z-score for all H3K4me3 peak regions. (C) Scatter plot of H3K4me3 z-score and H3K9ac z-score for H3K4me3 peak regions bound by SET-9 and SET-26. (D) Scatter plot of H3K4me3 z-score and H3K9ac z-score for H3K4me3 peak regions not bound by SET-9 and SET-26.

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Figure 7. Binding of SET-9 and SET-26 regulate the RNA expression of specific target genes. (A) Venn diagram showing comparisons of the genes with increased and decreased expression changes in the F1 set-9 set-26 (RNA-seq) set-9(rw5) set-26(tm2467) double mutant and the SET-9 and SET-26 target genes. (B) GO term analysis of SET-9/26 target genes with increased expression in F1 set-9 set-26. (C) GO term analysis of SET-9/26 target genes with decreased expression in F1 set-9 set-26. (D) Venn diagram showing comparisons of the genes with increased and decreased expression changes in the glp-1; set-26 (RNA-seq) glp-1; set-26 double mutant and the SET-9 and SET-26 target genes. (E) GO term analysis of SET-9/26 target genes with increased expression in glp-1; set-26. (F) GO term analysis of SET-9/26 target genes with decreased expression in glp-1; set-26.
analyses of the up-regulated genes in the F1 set-9(rw5) set-26(tm2467) double mutant that were bound by SET-9 and SET-26. (C) GO term analyses of the down-regulated genes in the F1 set-9(rw5) set-26(tm2467) double mutant that were bound by SET-9 and SET-26. (D) Venn diagram showing comparisons of the up-regulated and down-regulated somatic SET-26 regulated genes and the SET-9 and SET-26 target genes. (E) GO term analyses of the up-regulated somatic SET-26 regulated genes bound by SET-9 and SET-26. (F) GO term analyses of the down-regulated somatic SET-26 regulated genes bound by SET-9 and SET-26. Gene lists can be found in Supplementary file 2.

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Figure 7—figure supplement 1. SET-9 and SET-26 target genes. (A) Venn diagrams showing comparisons of the genes with increased and decreased expression changes in the set-9(rw5) mutant. (B) GO term analyses of the up-regulated genes in set-9(rw5) bound by SET-9 and SET-26. (C) GO term analyses of the down-regulated genes in set-9(rw5) bound by SET-9 and SET-26. (D) Venn diagrams showing comparisons of the genes with increased and decreased expression changes in the set-26(tm2467) mutant. (E) GO term analyses of the up-regulated genes in set-26(tm2467) bound by SET-9 and SET-26. (F) GO term analyses of the down-regulated genes in set-26(tm2467) bound by SET-9 and SET-26. Gene lists can be found in Supplementary file 2.

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Figure 8. SET-9 and SET-26 restrict the spreading of H3K4me3. (A) Western blotting of H3K4me3 levels in three independent replicates of wild type and F3 set-9(rw5) set-26(tm2467) double mutant (left). Synchronized L4 worms for N2 and the F3 set-9(rw5) set-26(tm2467) double mutant were used.
Figure 8 continued

Quantification of normalized H3K4me3 levels from three independent Western experiments (right). (*p<0.05) (B) Elevated H3K4me3 levels were observed in the dissected gonads of the F3 set-9(rw5) set-26(tm2467) double mutant. Synchronized D1-D2 adults for N2 and the F3 set-9(rw5) set-26 (tm2467) double mutant were used. Representative immunostaining images are shown. Right panel shows quantification of images shown on left. (*p<0.05) (C) Metagene plots showing the average H3K4me3 z-score (standardized log2 ratios of ChIP/H3ChIP signals) for the peaks bound by SET-9 and SET-26 in N2 (black) and the F1 (blue) and F3 (purple) set-9(rw5) set-26(tm2467) double mutants. Regions 3000 bp upstream and downstream of the peak summits are shown. The grey areas indicate 95% confidence intervals. (D) Metagene plots showing the average H3K4me3 z-score for the peaks not bound by SET-9 and SET-26 in N2 (black) and the F1 (blue) and F3 (purple) set-9(rw5) set-26(tm2467) double mutant. The grey areas indicate 95% confidence intervals.

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Figure 8—figure supplement 1. SET-9 and SET-26 restrict the spreading of H3K4me3. (A) H3K4me3 and H3K9me3 levels in N2, set-9(rw5) and set-26 (tm2467) mutants. Synchronized L4 worms were used. Quantification showed results from three independent experiments. (B) Western blotting showing elevated H3K9ac levels in the set-9(rw5) set-26(tm2467) double mutant (left). Synchronized L4 worms for N2 and the set-9(rw5) set-26(tm2467) double mutant were used. Quantification of normalized H3K9ac levels from three independent Western experiments (right). (*p<0.05).

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Figure 8—figure supplement 2. SET-9 and SET-26 restrict the spreading of H3K4me3 (part 2). (A) Representative genome browser views of H3K4me3 profiles in N2 (black) and the F1 (blue) and F3 (purple) set-9(rw5) set-26(tm2467) double mutant surrounding SET-9 and SET-26 binding sites (red). z-scores for normalized H3K4me3 and SET-9 and SET-26 ChIP signals in N2 and the F1 and F3 set-9(rw5) set-26(tm2467) are shown. (B) Heatmaps showing the H3K4me3 profiles in wild-type and the F1 and F3 set-9(rw5) set-26(tm2467) double mutant for H3K4me3 peaks bound and not bound by SET-9/26.

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Figure 8—figure supplement 3. SET-9 and SET-26 restrict the spreading of H3K4me3 (part 3). (A) Metagene plots showing the average H3K4me3 z-score (standardized log2 ratios of ChIP/H3ChIP signals) in N2 (black) and the F1 (blue) and F3 (purple) set-9(rw5) set-26(tm2467) double mutants.

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Regions 3000 bp upstream and downstream of the peak summits of SET-9 and SET-26 binding are shown. The light grey and light purple areas indicate 95% confidence intervals. (B) Metagene plots showing the average H3K4me3 z-score (standardized log2 ratios of ChIP/H3ChIP signals) in *glp-1(e2141)* (black) and *glp-1(e2141); set-26(tm2467)* (orange) for H3K4me3 peaks bound by SET-9 and SET-26 (left), not bound by SET-9 and SET-26 (middle) and SET-9 and SET-26 peaks overlapping H3K4me3 (right). The light grey and light purple areas indicate 95% confidence intervals. (C, D) Venn diagrams showing genes with altered expression in the F1 and F3 *set-9(rw5) set-26(tm2467)* double mutant identified by RNA-seq and genes with altered H3K4me3 in the F1 and F3 *set-9(rw5) set-26(tm2467)* double mutant identified by ChIP-seq. (**p<0.001 indicate less than expected). Gene lists can be found in Supplementary file 2.

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Figure 8—figure supplement 4. Germline-specific genes show correlated changes in H3K4me3 and RNA expression. (A) Venn diagram shows the overlaps between SET-9/26 target genes with expression change in F1 set-9 set-26 and germline-specific genes. (B) Average H3K4me3 z-score.
score in N2 (black) and the F1 (blue) and F3 (purple) set-9(rw5) set-26(tm2467) double mutants for the SET-9/26 germline target genes that showed elevated expression in F1 set-9(rw5) set-26(tm2467) (compared to N2). (C) Venn diagram shows the overlap between SET-9/26 germline target genes with increased RNA expression in F1 set-9(rw5) set-26(tm2467) double mutant and genes with expanded H3K4me3 marking in F1 set-9(rw5) set-26 (tm2467) double mutant. Gene lists can be found in Supplementary file 2.

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Figure 9. SET-9 and SET-26 and SET-2 act synergistically to regulate fertility. (A) Ratio of eggs laid by F1 set-9(rw5) set-26(tm2467) double mutant worms fed dsRNA of *C. elegans* potential histone modifiers (mainly methyltransferases and demethylases) or empty vector (L4440) for one generation.

(B) Average brood size in N2, set-2(ok952), F1 set-9(rw5) set-26(tm2467) double mutant and F1 set-2(ok952); set-9(rw5) set-26(tm2467) triple mutant.

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Figure 10. SET-9 and SET-26 regulate H3K4me3 and target gene expression. In the germline, loss of set-9 and set-26 results in the broadening of H3K4me3 domains surrounding most SET-9 and SET-26 binding regions and up-regulation of germline genes. In the soma, loss of set-26 modulates lifespan by indirectly regulating DAF-16-dependent genes. We propose that SET-9 and SET-26 are critical for organizing local chromatin environment and regulating the expression of specific target genes, and these activities together contribute to their roles in germline development and longevity.

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