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651 Figure S1. (A) Pairwise alignment of SET-21 and SET-32 proteins. Motifs I-IV of the SET domain, pre-

- 652 SET zinc cluster, and post-SET zinc center were highlighted. The SET domain was marked by vertical
- 653 lines. (B) Genome browser shots of *set-21* and *set-32* genes. (C-D) Tissue-specific and developmental

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654 mRNA expression profiles for *set-21, set-32, set-25*, and *met-2* using data generated by <sup>41, 42</sup>. Plots were 655 generated by https://ahringerlab.com/RegAtlas/.

656

Figure S2. SET-21 and SET-32 are expressed in embryo. Anti-FLAG immunofluorescent microscopy
was performed for different stages of N2, *set-21*(native)::3xFLAG, *set-32*(native)::3xFLAG embryos.
Representative IF images were showed for each strain, together with DAPI and DIC images of the same
embryo. Scale bar: 10µm.

661

662 Figure S3. set-32;set-21 mutant animals show germline defects at 25°C. (A-B) Multigenerational 663 brood size analysis. Worms were maintained at 20°C before shifting to 25°C for F1 and the subsequent 664 generations. Strains: WT (N2), set-32(red11), set-21(ok2320), and set-32(red11); set-21(ok2320) mutant 665 animals in (A) and WT (N2), set-21(red109), and set-32(red11);set-21(red109) in (B). We note that the smaller brood size of set-21(ok2320) compared to set-21(red119) or set-32;set-21(ok2320) is likely due 666 667 to some unknown background mutations. (C) Oocytes and sperm of set-32(red11);set-21(red109) young 668 adults (F7 at 25°C) were examined by DAPI staining. Percentages of adult animals with both oocytes 669 and sperm, only either oocyte or sperm, and neither gamete were indicated with representative DAPI-670 staining images.

671

Figure S4. Whole-genome coverage plots of H3K23me1, me2, and me3 comparing WT versus set-*21, set-32, or set-32; set-21 mutant.* The coverage, averaged from two replicates, was normalized to the
ChIP input signal and was calculated for each 10kb window.

675

Figure S5. (A) A scatter plot of whole-genome comparison of H3K9me3 and H3K23me3 levels (1 kb
windows) in the WT animals. (B) A Venn diagram of HRDE-1-dependent H3K23me3 and MET-2 SET25-dependent H3K23me3. (C) A Venn diagram of HRDE-1-dependent H3K9me3 and MET-2 SET-25dependent H3K9me3.

680

Figure S6. RNA-seq (A-C) and sRNA-seq (D-E) comparison of WT and set-32 or set-21 single
mutant.

683

Figure S7. MA-plots comparing *hrde-1* and WT animals for (A) mRNA and (B) siRNA expressions of
all protein-coding genes.

686

**Figure S8.** Venn diagram of genes with decreased or increased siRNA expression (minimal 3-fold

688 change, FDR  $\leq 0.02$ ) comparing *set-21* or *set-32* single mutant with *set-32;set-21* double mutant.

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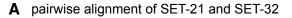
690 Figure S9. *set-32;set-21* mutations cause more wide spread changes in siRNA expression than

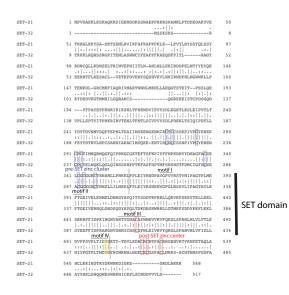
- 691 changes in mRNA expressions. (A) sRNA MA-plot comparing *set-32;set-21* and WT with *set-32/21-*
- 692 sensitive genes (based on mRNA-seq) highlighted. (B-C) mRNA MA-plots comparing set-32;set-21 and

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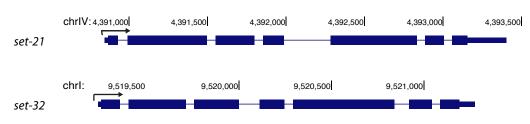
- 693 WT with genes that had increased (B) and decreased (C) siRNA expression in the *set-32;set-21* 694 compared to WT highlighted.
- 695
- Figure S10. For genes of which mRNAs are desilenced in *set-32;set-21*, as well as genes of which
  siRNAs are differentially expressed in *set-32;set-21* (either decreased or increased), their siRNAs
  tend to be bound by HRDE-1, instead of CSR-1. The same CSR-1 vs HRDE-1-coIP sRNA MA-plot
  was shown in all three panels with each highlighting a different set of genes (marked in blue): (A)
  desilenced genes (mRNA-seq) in *set-32;set-21*, (B-C) genes with decreased (B) or increased (C) siRNA
  expression in the *set-32;set-21* mutant. CSR-1 vs HRDE-1-coIP sRNA data were from <sup>50</sup>. Genes with a
- minimal of 3-fold difference in CSR-1-vs-HRDE-1-coIP siRNA (FDR≤0.02) were highlighted in red.
- 703
- Table 1. A list of H3K23me3-enriched regions in WT identified in WT adult animals, with H3K23me3
- 705 ChIP-seq differential analysis outputs (log2 ratio, FDR and mean) for WT vs *hrde-1* and WT vs *set-*
- 706 *32;set-21* comparisons calculated by BaySeq.
- 707
- Table 2. Protein-coding gene differential analysis results of H3K23me3 ChIP-seq, Pol II ChIP-seq,
- 709 RNA-seq, and sRNA-seq for the comparisons between WT and various mutant animals. Set-32/21-
- 710 sensitive genes, based on RNA-seq analysis, were indicated.
- 711
- 712 Table 3. A list of high-throughput sequencing libraries used in this study.

#### Figure S1

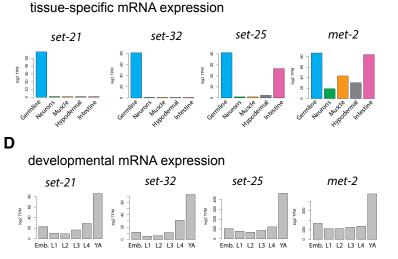




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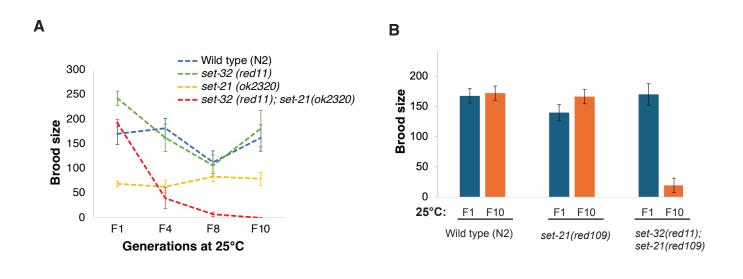
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25°C G7 generation

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### H3K23me1

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coverage (WT and *set-32;set-21*)

# Figure S5

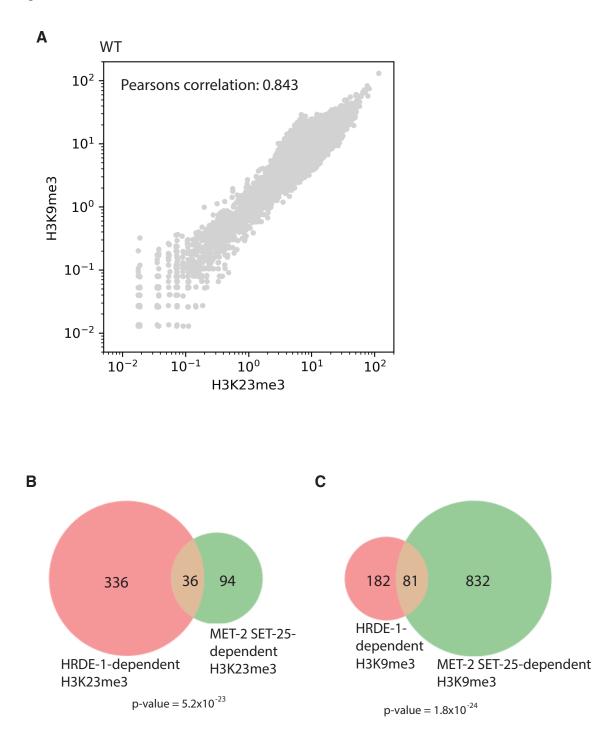


Figure S6

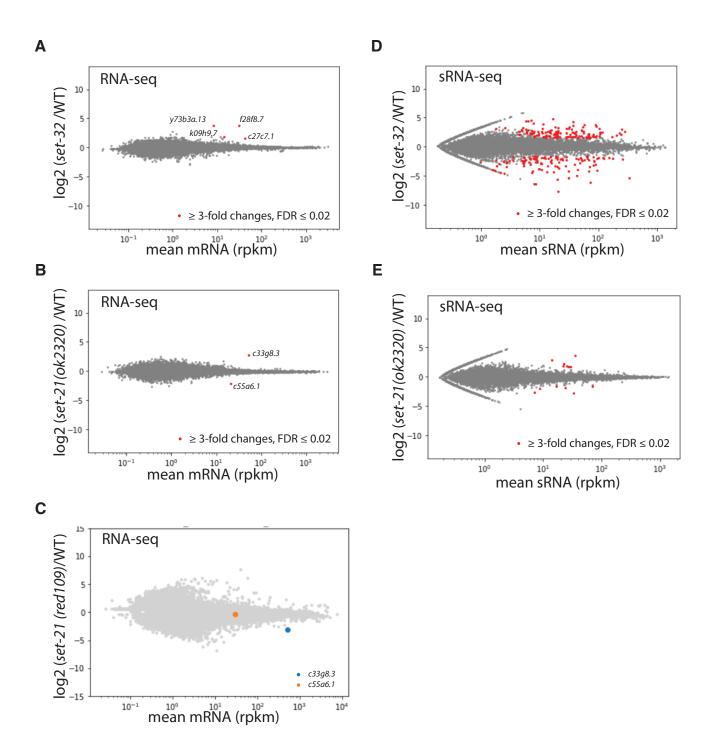
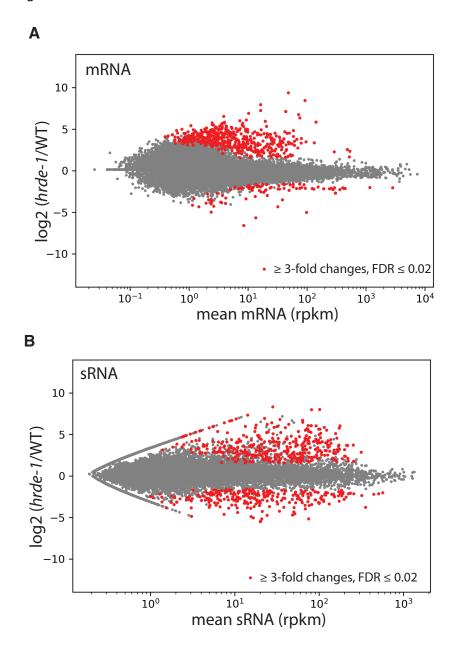


Figure S7



# Figure S8

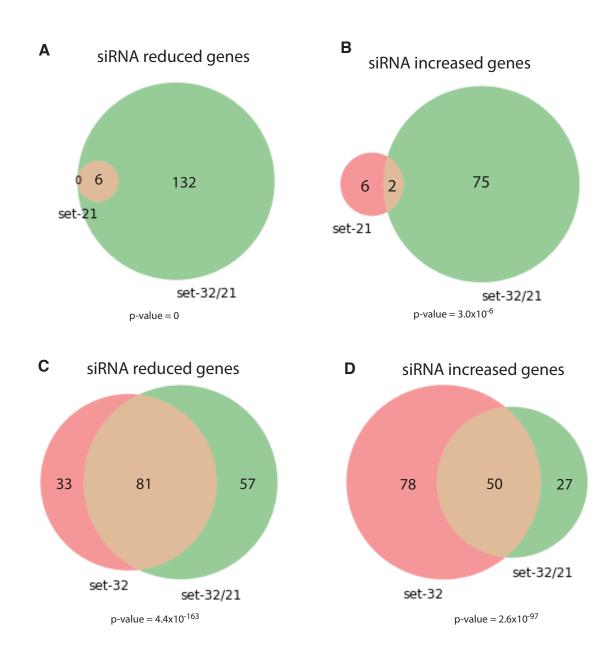


Figure S9

