Supporting Information

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Materials and Methods

Strains. All *Caenorhabditis elegans* strains were grown and maintained at 20 °C under standard conditions, as previously described (1). The *C. elegans spr-5(by101)* strain was provided by R. Baumeister. The *wdr-5(ok1417)* and N2 Bristol (wild-type) strains were provided by the *Caenorhabditis* Genetics Center. The *met-2(n4256)* strain was provided by R. Horvitz. The hT2 [bli-4(e937) let-?(q782) qIs48] balancer strain was used to balance *met-2(n4256)* animals before beginning the germ-line mortality experiment and to maintain *spr-5(by101);met-2(n4256)* double-mutant animals as heterozygotes. Because the hT2[bli-4 (e937) let-?(q782) qIs48] balancer allele does not extend completely to the *spr-5* locus on chromosome I, the F0 animal used to generate F1 *spr-5;met-2* double-mutant progeny was cloned out and genotyped to confirm the presence of the *spr-5(by101)* mutation in all double mutants used in this article. In addition, the balanced allele was always used shortly after thawing to avoid the possibility that the heterozygous balanced stock became homozygous mutant earlier than intended.

Germ-line Mortality Experiment. Germ-line mortality characterization was performed essentially as described in Katz and colleagues (2). Before beginning the experiment, *met-2(n4256)* was balanced with hT2[bli-4(e937) let-?(q782) qIs48] for a minimum of five generations, and then homozygous mutant animals were selected as the F1 generation. Worms were maintained at 20 °C by picking three fertile adults to a fresh 6-cm plate every fourth day to establish the subsequent generation. Brood sizes were counted for wild-type and *met-2(n4256)* animals at F2 and F4 ("early"), F12 and F14 ("middle"), and F24 and F26 ("late") generations. The average brood size of the *met-2(n4256)* strain was calculated from the progeny of 12 worms for early generations, 23 worms for middle generations, and 51 worms for late generations, whereas the brood size for wild-type was calculated from four worms throughout the experiment.

Chromatin Immunoprecipitation. Chromatin immunoprecipitation (ChIP) experiments were performed as described in Katz and colleagues (2). Briefly, mixed-stage worms were collected from three 10-cm plates in PBS, flash frozen in liquid nitrogen, and stored at −80 °C before homogenization. Frozen pellets were disrupted by a glass Dounce homogenizer, fixed with formaldehyde (1% final concentration), and quenched with glycine. ChIP samples were processed with a Chromatin Immunoprecipitation Assay Kit (Millipore), according to manufacturer's instructions. Samples were sonicated using a Diagenode Bioruptor UCD-200 at 4 °C on the "high" setting for a total of 30 min with a cycle of 45 s on and 15 s off. A total of 10 μL H3K4me2 antibody (cma303; Millipore) and 10 μL H3K9me2 (ab1220; Abcam) were used for immunoprecipitation. Immunoprecipitated DNA was quantified by quantitative real-time PCR, using iQ SYBR Green Supermix (Bio-Rad) on the iCycler CFX96 Real Time System (Bio-Rad). The primers used are at the transcription start site and described in Katz and colleagues (2) with the exception of *rme-2*F (ACTCACCATGAGAACCATGC), *rme-2*R (AAAACGGGGAAATTACCTTGA), *ges-1*F (AAACGGAATTGCGAGAAAAA), *ges-1*R)GGCTGGACCAATTGTTGAAA), *unc-98*F (CGGTTTTTCCGTCATCTCTC), and *unc-98*R (ATGAATGCCTGTGATCTTGC). For ChIP on embryos, mixed-stage worms were collected from thirty 10-cm plates and bleached in 20% bleach with 0.5 mM NaOH to degrade worm carcasses. The remaining embryos were washed in 1× PBS, filtered through a 40-μm cell strainer, and flash-frozen in liquid nitrogen before processing. Survival of embryos after bleaching was confirmed by live microscopy.

Expression Level Analysis. Total RNA was isolated and cDNA generated essentially as described in Katz and colleagues (2). Briefly, mixed-stage worms from one 10-cm plate of were collected in PBS, flash frozen in liquid nitrogen, and stored at −80 °C. Pellets were resuspended in TRIzol reagent (Invitrogen), and the pellet was thawed and reflash-frozen four times. The sample was then extracted with bromo-choloropropane and further processed according to the manufacturer's protocol. Isolated total RNA was treated with DNase I (Invitrogen), and RNA was reverse-transcribed using the SuperScript III First-Strand Synthesis System (Invitrogen), using Oligo$(dT)_{20}$ priming. Primers used are previously described in Katz and colleagues (2) except for *rme-2*F (ATACGGAAACCCCATGTACG), *rme-2*R (GAATGACTCCAGCGAATGGT), *ges-1*F (CACTCCTCACTATCGTTTAGTC), *ges-1*R (CGTGAATCCAGAACAGAACTG), *unc-98*F (TTTGGATGGCAGTGATCAACAGG), and *unc-98*R (GAGTGTTCATGAAGTTGAATGTGAG). For expression analysis in L1 larval animals, 100–150 gravid adults were placed on unseeded plates and allowed to lay embryos overnight. Adults and arrested L1s were collected the following day in 1× PBS, and L1 animals were isolated away from adults by filtration through a 40-μm cell strainer and flash frozen in liquid nitrogen before processing.

Differential Interference Contrast Microscopy. Worms were immobilized in 0.1% levamisole and placed on a 2% agarose pad for imaging.

1. Greer EL, et al. (2011) Transgenerational epigenetic inheritance of longevity in Caenorhabditis elegans. *Nature* 479(7373):365–371.

2. Katz DJ, Edwards TM, Reinke V, Kelly WG (2009) A C. elegans LSD1 demethylase contributes to germline immortality by reprogramming epigenetic memory. *Cell* 137(2):308–320.

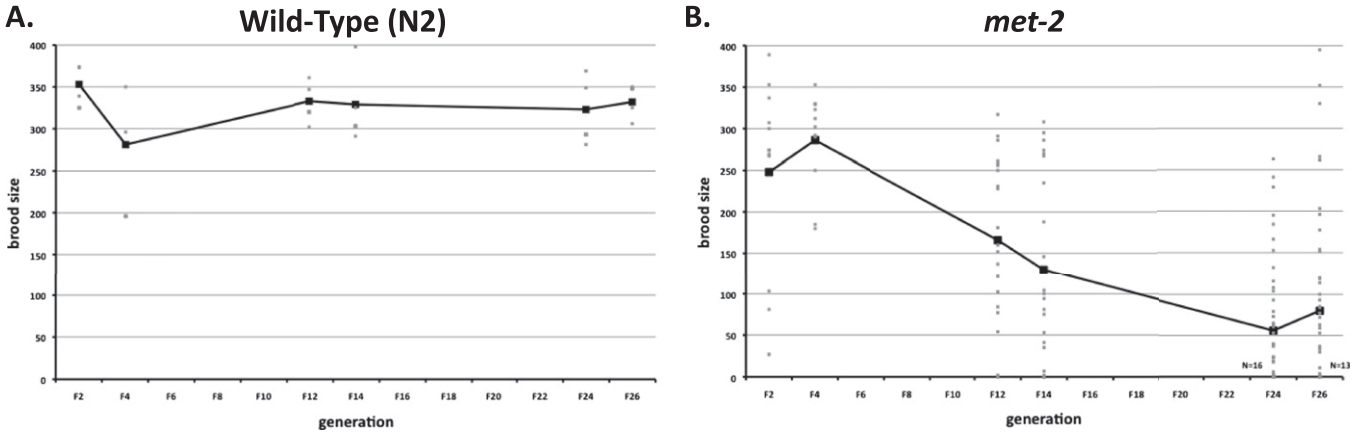

Fig. S1. The *met-2* germ-line mortality phenotype. (*A*) The average brood size of wild-type and (*B*) *met-2* strains in progressive generations are shown. *met-2* mutants display progressive sterility. Offspring from individual wild-type and *met-2* animals, counted at generations 2, 4, 12, 14, 24, and 26, are indicated by the individual boxes. In *met*-2 mutants, $n = 16$ and $n = 13$ refer to the number of animals with zero progeny at generations 24 and 26.

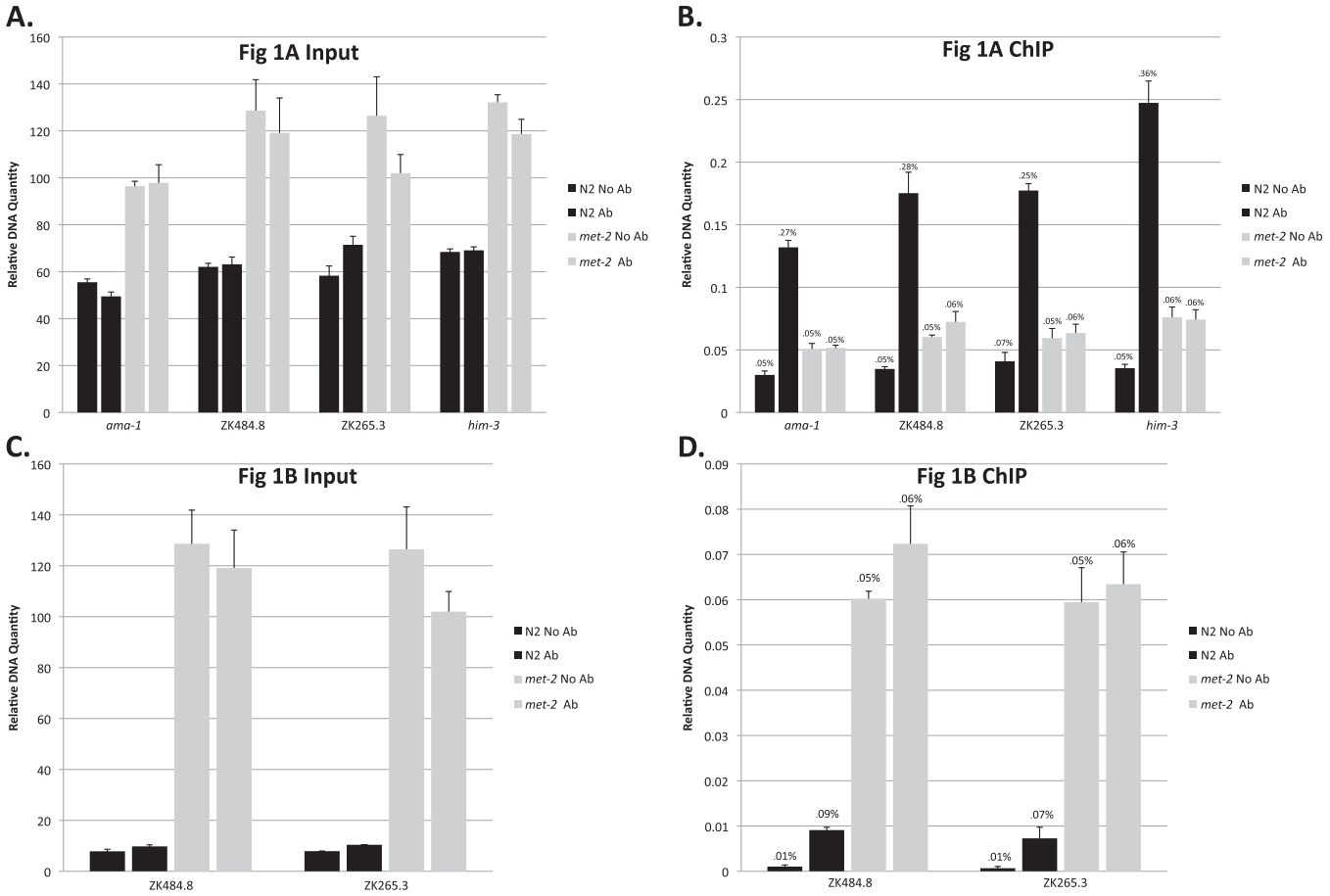

Fig. S2. SPR-5 spermatogenesis targets acquire MET-2-dependent H3K9me2 in the embryo. *A* and *B* correspond to Fig. 1*A* in the main text. *C* and *D* correspond to Fig. 1*B* in the main text. ChIP-qPCR showing the relative DNA quantity of the input (*A* and *C*) and precipitated (*B* and *D*) sample chromatin immunoprecipitated with either no antibody (first bar) or H3K9me2 antibody (second bar) at four germ-line enriched genes from mixed-stage (*A* and *B*) or embryo samples from wild-type or *met-2* mutants (as indicated). In addition, the percentage of input precipitated is indicated above each ChIP bar (*B* and *D*). These individual measurements were used to calculate the fold enrichments in Fig. 1 *A* and *B*. The error bars represent SD.

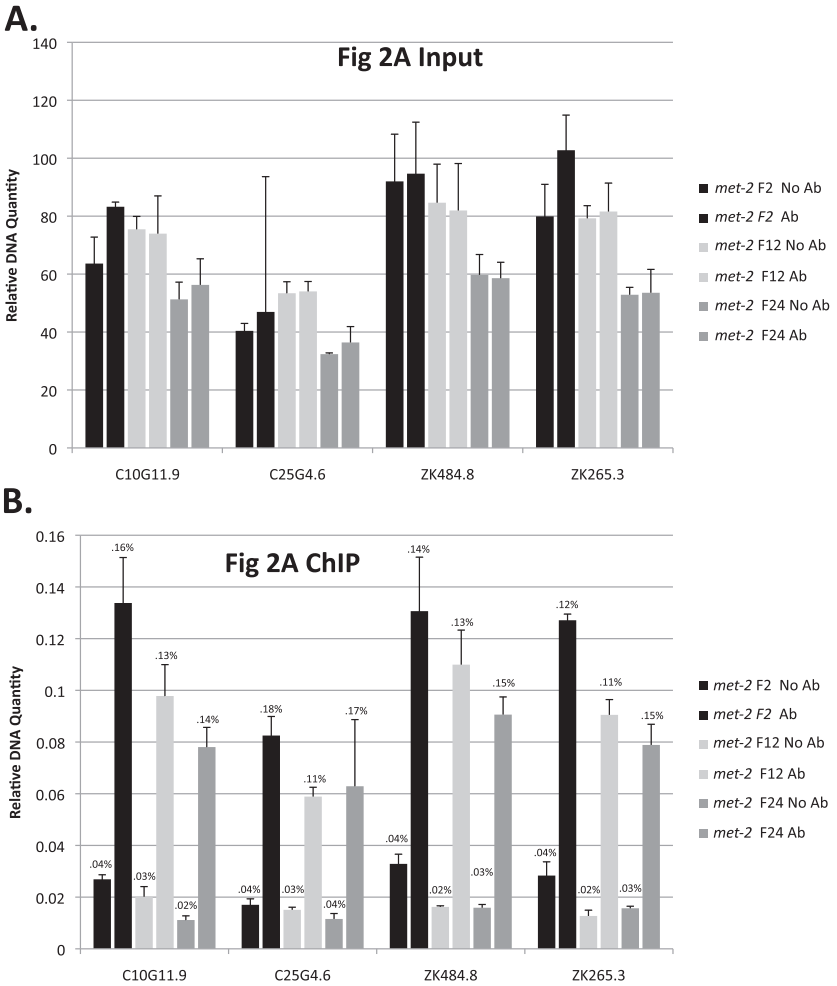

Fig. S3. Heritable accumulation of H3K4me2 across generations in *met-2* mutants. *A* and *B* correspond to Fig. 2*A* in the main text. ChIP-qPCR showing the relative DNA quantity of the input (*A*) and precipitated (*B*) sample chromatin immunoprecipitated with either no antibody (first bar) or H3K4me2 antibody (second bar) at four spermatogenesis genes from mixed-stage *met-2* mutants at generations 2, 12, and 24. In addition, the percentage of input precipitated is indicated above each ChIP bar (*B*). These individual measurements were used to calculate the fold enrichments in Fig. 2*A*. The error bars represent SD.

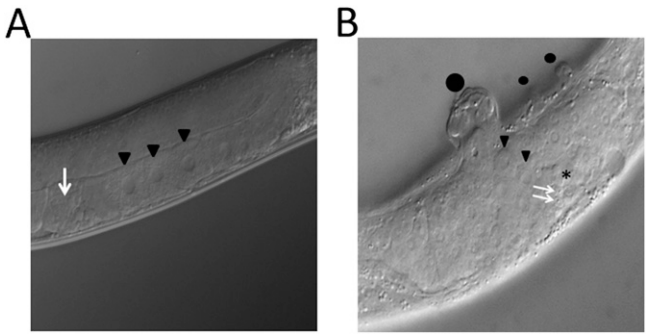

Fig. S4. *spr-5;met-2* synthetic sterile phenotype. Differential interference contrast microscopic imaging of the proximal gonad from (*A*) wild-type and (*B*) sterile *spr-5;met-2* adult worms. Black arrowheads point to (*A*) normal oocytes and (*B*) defective-looking oocytes. White arrows point to (*A*) mature sperm in the spermatheca compared with (*B*) mature sperm and residual bodies (*) in the proximal gonad. Black circles indicate the three protruding vulvas.

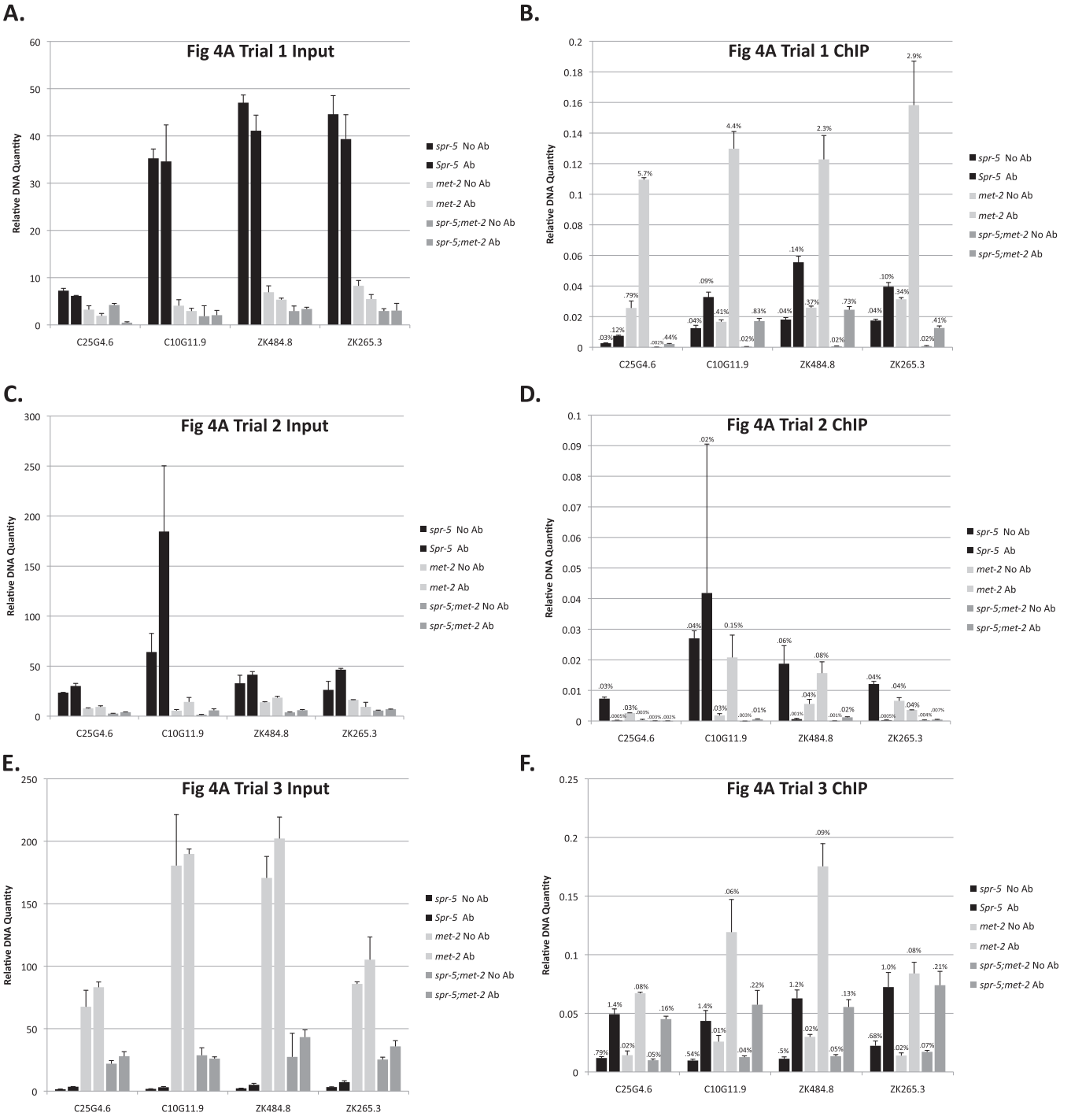

Fig. S5. Synergistic accumulation of H3K4me2 in *spr-5;met-2* mutants. *A* and *B* correspond to replicate 1 from Fig. 4*A* in the main text. *C* and *D* correspond to replicate 2 from Fig. 4*A* in the main text. *E* and *F* correspond to replicate 3 from Fig. 4*A* in the main text. ChIP-qPCR showing the relative DNA quantity of the input (*A*, *C*, and *E*) and precipitated (*B*, *D*, and *F*) sample chromatin immunoprecipitated with either no antibody (first bar) or H3K4me2 antibody (second bar) at four spermatogenesis genes from mixed-stage *spr-5* mutants, *met-2* mutants, or *spr-5;met-2* mutants (as indicated). In addition, the percentage of input precipitated is indicated above each ChIP bar (*B*, *D*, and *F*). These individual measurements were used to calculate the fold enrichments in Fig. 4*A*. The error bars represent SD.

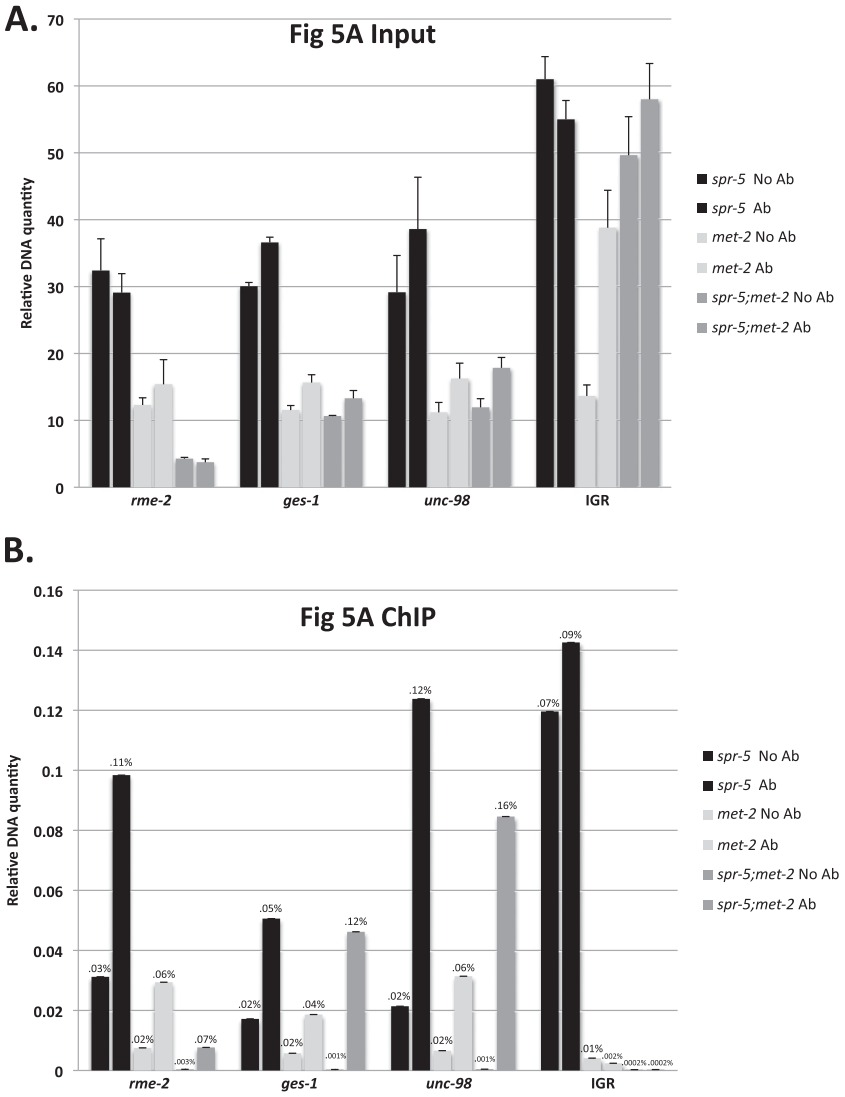

Fig. S6. Synergistic accumulation of H3K4me2 at nonspermatogenesis targets. *A* and *B* correspond to Fig. 5*A* in the main text. ChIP-qPCR showing the relative DNA quantity of the input (*A*) and precipitated (*B*) sample chromatin immunoprecipitated with either no antibody (first bar) or H3K4me2 antibody (second bar) at an oocyte (*rme-2*), gut (*ges-1*), and muscle (*unc-98*) gene in mixed-stage *met-2*, *spr-5*, and *spr-5;met-2* mutants (as indicated). In addition, the percentage of input precipitated is indicated above each ChIP bar (*B*). These individual measurements were used to calculate the fold enrichments in Fig. 5*A*. The error bars represent SD.

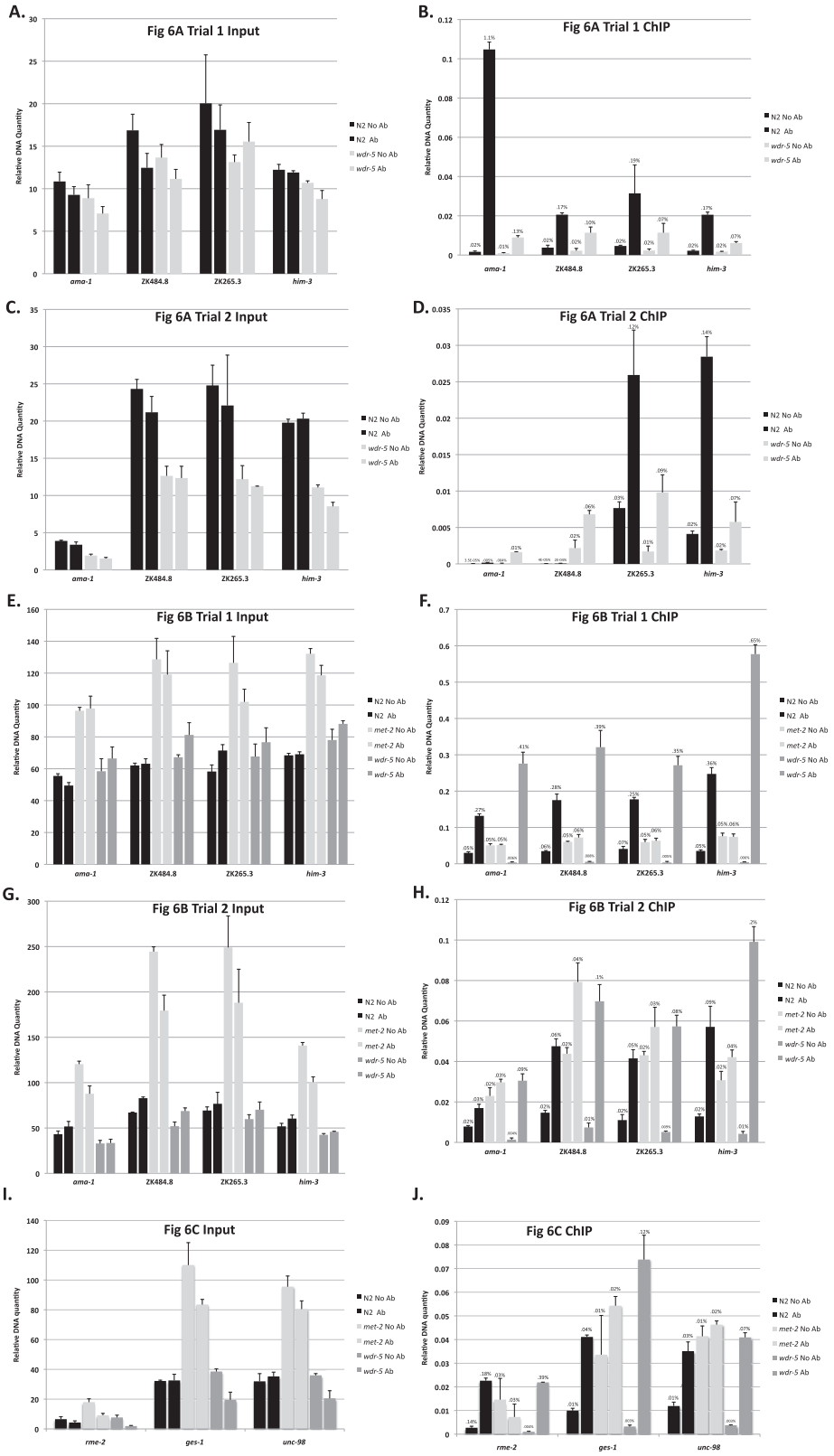

Fig. S7. Decreased H3K4me2 and increased H3K9me2 in *wdr-5* mutants. *A* and *B* correspond to replicate 1 from Fig. 6*A* in the main text. *C* and *D* correspond to replicate 2 from Fig. 6*A* in the main text. *E* and *F* correspond to replicate 1 from Fig. 6*B* in the main text. *G* and *H* correspond to replicate 2 from Fig. 6*B* in the main text. *I* and *J* correspond to Fig. 6*C* in the main text. ChIP-qPCR showing the relative DNA quantity of the input (*A*, *C*, *E*, *G*, *I*) and precipitated (*B*, *D*, *F*, *H*, *J*) sample chromatin immunoprecipitated with either (*A*, *B*, *C*, *D*) no antibody (first bar) or H3K4me2 antibody (second bar), or (*E*, *F*, *G*, *H*, *I*, *J*) no antibody (first bar) or H3K9me2 (second bar) at four germ-line enriched genes (*A*, *B*, *C*, *D*, *E*, *F*, *G*, *H*), or an oocyte (*rme-2*), gut (*ges-1*), and muscle (*unc-98*) gene (*I* and *J*) from mixed-stage wild-type (N2), *met-2*, or *wdr-5* mutants (as indicated). In addition, the percentage of input precipitated is indicated above each ChIP bar (*B*, *D*, *F*, *H*, *J*). These individual measurements were used to calculate the fold enrichments in Fig. 6 *A*–*C*. The error bars represent SD.