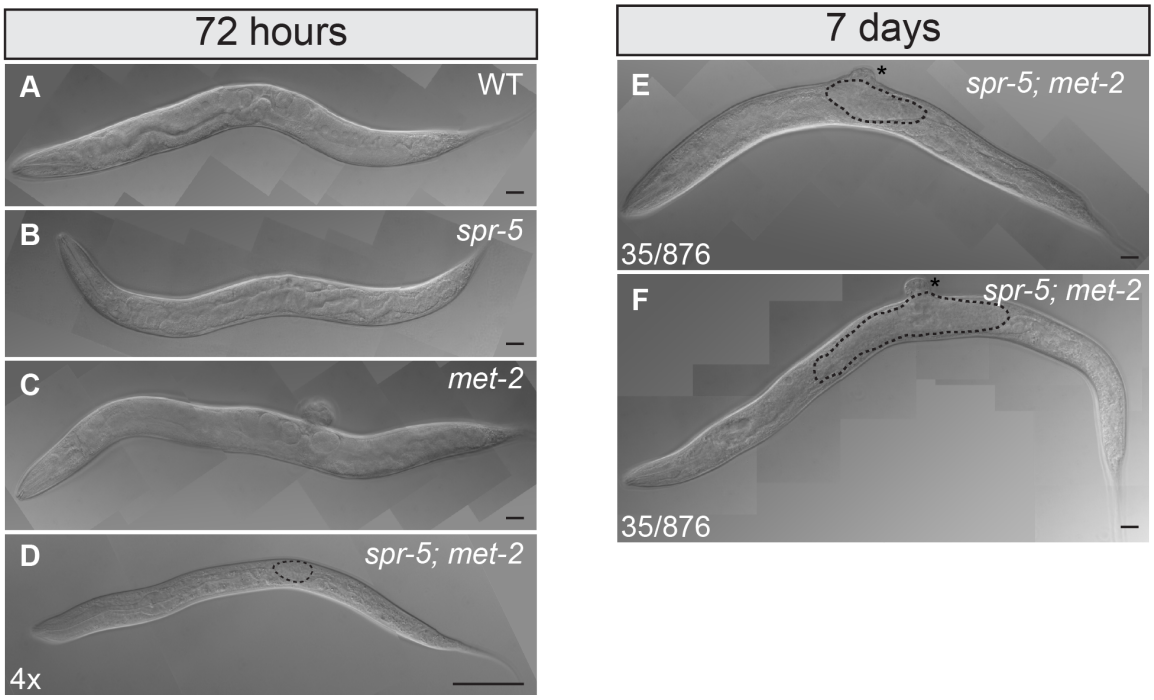
Supplemental Material

Figure S1



G

Total # of progeny scored	Total # of adult progeny	Percent of adult progeny	Total # of adult progeny with Pvl	Percent of adult progeny with Pvl
876	35	3.9%	31	88.5%

Fig. S1. *spr-5; met-2* mutants display severe developmental delay and protruding vulva. 40x DIC images of wild type (A), *spr-5* (B), *met-2* (C), and *spr-5; met-2* (D) progeny at 72 hours post synchronized lay. DIC image in (D) was magnified an additional 4x. 40x DIC images of two examples of *spr-5; met-2* adult progeny (E, F) at seven days post synchronized lay. Dashed-line (D) outlines germline and asterisks (E, F) denote protruding vulva. Scale bar: 100µm. (G) Quantification of *spr-5; met-2* progeny that reached the adult stage by seven days post synchronized lay, along with quantification of protruding vulva (Pvl) in these animals that reached the adult stage. The 876 progeny scored came from a total of 25 hermaphrodites across two independent experiments.

Figure S2

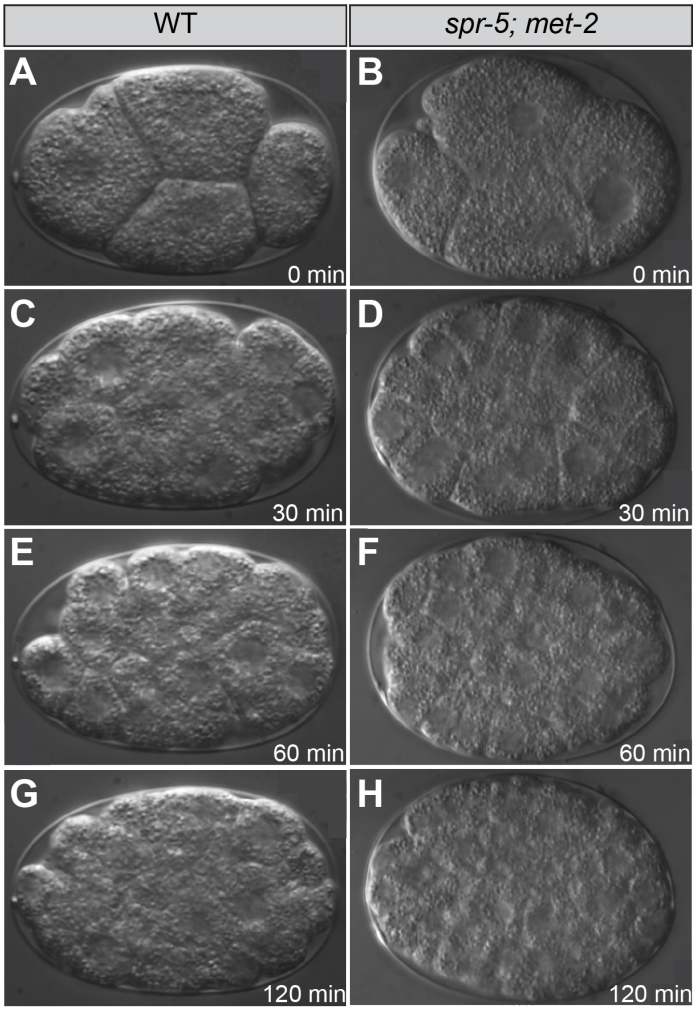


Fig. S2. *spr-5; met-2* mutants display accelerated embryogenesis. 100x DIC images of wild type (WT) (A, C, E and G) and *spr-5; met-2* (B, D, F, and H) embryos. The four-cell stage was established as the starting point, 0 min, for each strain (A, B). Subsequently, time-lapse (minutes) images were obtained at 30 min (C, D), 60 min (E, F) and 120 min (G, H).

Figure S3

A

Genotype	Total # of differentially expressed genes	Up-regulated	Down-regulated
spr-5	343	259	84
met-2	413	262	151
spr-5; met-2	778	482	296

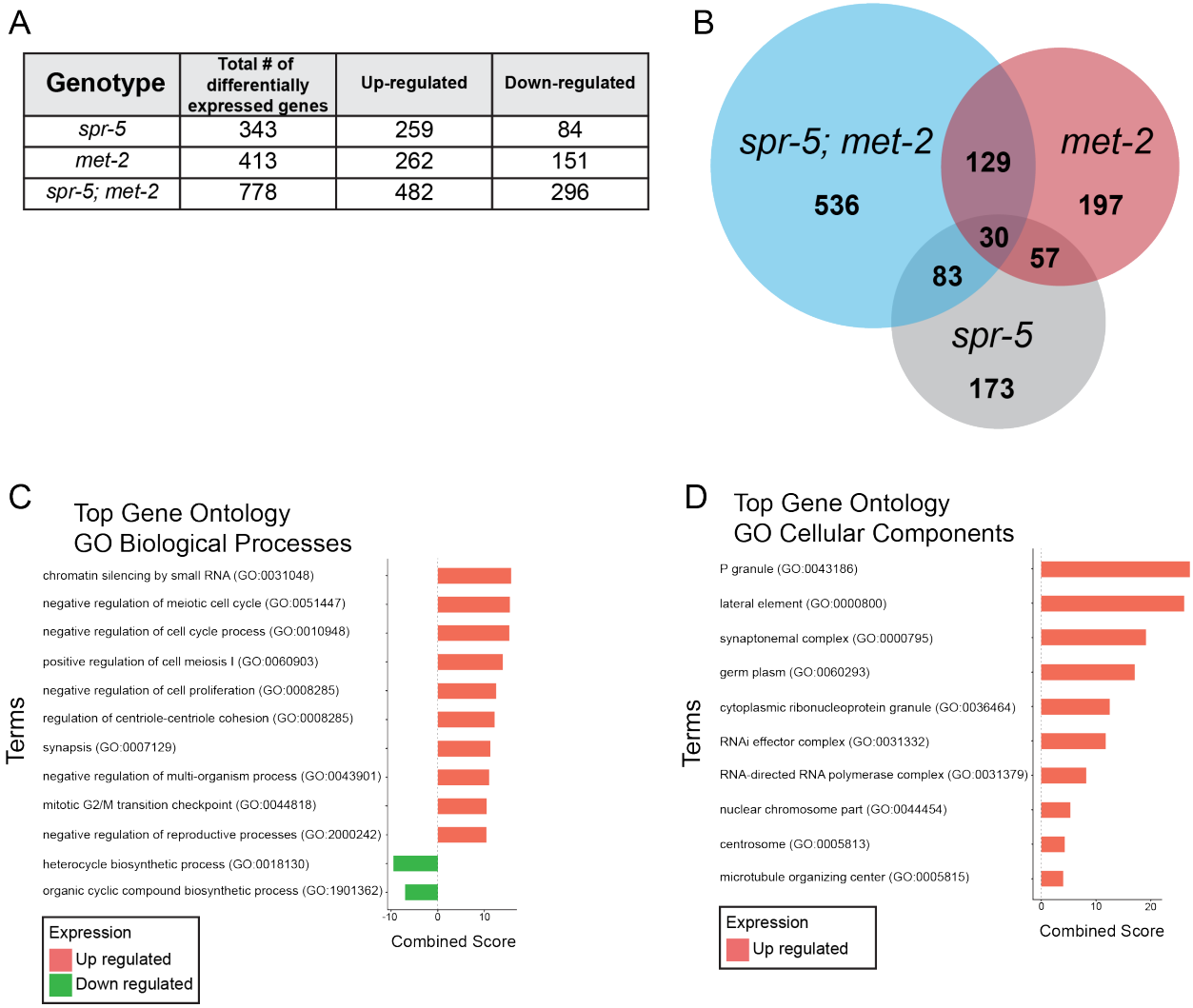


Fig. S3. Differential gene expression in *spr-5, met-2, and spr-5; met-2* progeny *compared* to wild type progeny. (A) Table summary of differentially expressed genes in *spr-5, met-2, and spr-5; met-2* L1 progeny from DESEQ2 analysis (significance cut-off of p-value < 0.05). (B) Overlap of differentially expressed genes between *spr-5, met-2, and spr-5; met-2* L1 progeny. Gene Ontology analysis showing Biological Processes (C) and Cellular Components (D) amongst genes that were up-regulated (red) and down-regulated (green) in *spr-5; met-2* L1 progeny compared to wild type. Combined Score was computed to determine gene set enrichment (Chen et al., 2013) (see GSE143837 for gene ontology R scripts).

Figure S4

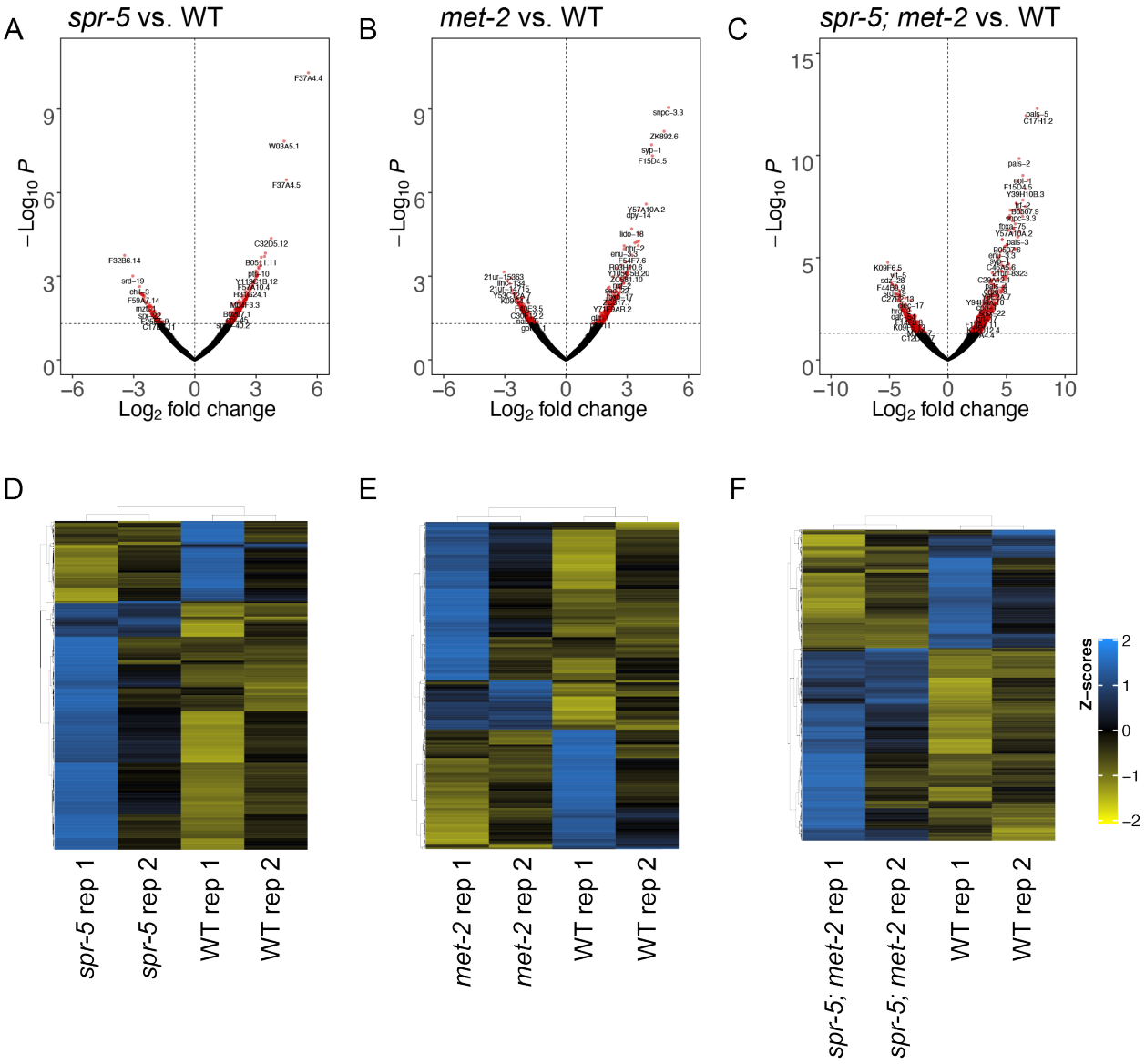


Fig. S4. Differential expression and replicate comparison of RNAseq experiments performed on wild type, *spr-5*; *met-2*, and *spr-5; met-2* L1 progeny. Volcano plot of log2 fold changes in gene expression (x-axis) by statistical significance (-Log_{10} P-value; y-axis) in *spr-5* (A), *met-2* (B), and *spr-5; met-2* (C) L1 Progeny compared to wild type (see GSE143837 for volcano plot R scripts). Heatmap of differentially expressed RNA-seq transcripts between wild type and *spr-5* (D), *met-2* (E), and *spr-5; met-2* (F). Data was scaled and hierarchical clustering was performed using the complete linkage algorithm. Distance was measured by calculating pairwise distance. Higher (Blue) and lower (Yellow) expression is reported as a z-score. (see GSE143837 for heatmap R scripts).

Figure S5

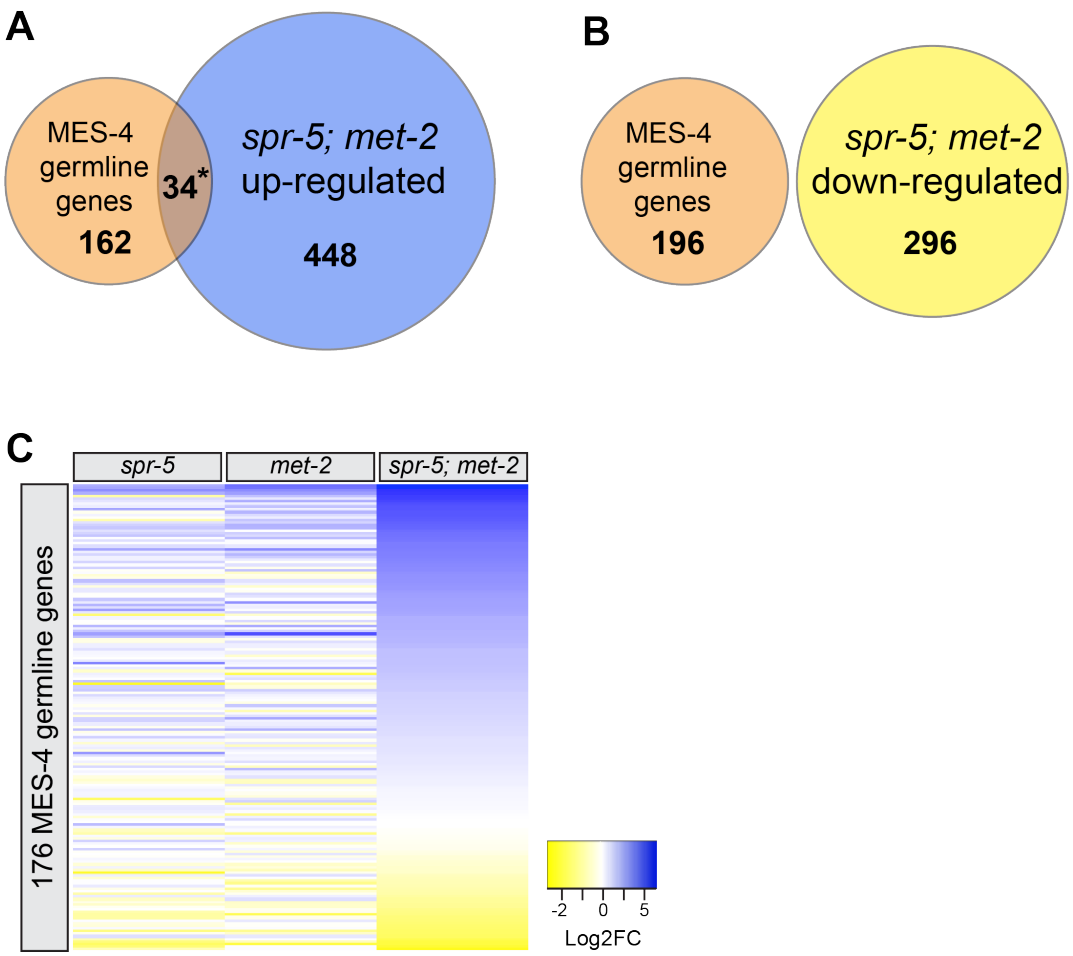


Fig. S5. MES-4 germline genes are ectopically expressed in *spr-5; met-2* mutant soma. Overlap between MES-4 germline genes and genes up-regulated (A) and down-regulated (B) in *spr-5; met-2* L1 progeny. Significant over-enrichment in A was determined by the hypergeometric test (*p-value < 6.44e-20). (C) Heatmap of log2 fold change (FC) of 176 MES-4 germline genes in *spr-5, met-2* and *spr-5; met-2* mutants compared to wild type (see Table S6 for Log2FC values). Log2FC values are represented in a yellow to blue gradient and range from -2 to 5 and were sorted by the log2FC in *spr-5; met-2* mutants. Log2FC values are represented in a yellow to blue gradient and range from -2 to 5. Yellow represents genes with negative log2FC values and blue represents genes with positive log2FC values compared to wild type. The remaining 21 MES-4 germline genes were not included because they do not have an expression value in one or more of the data sets (*spr-5*, *met-2*, or *spr-5; met-2*).

Figure S6

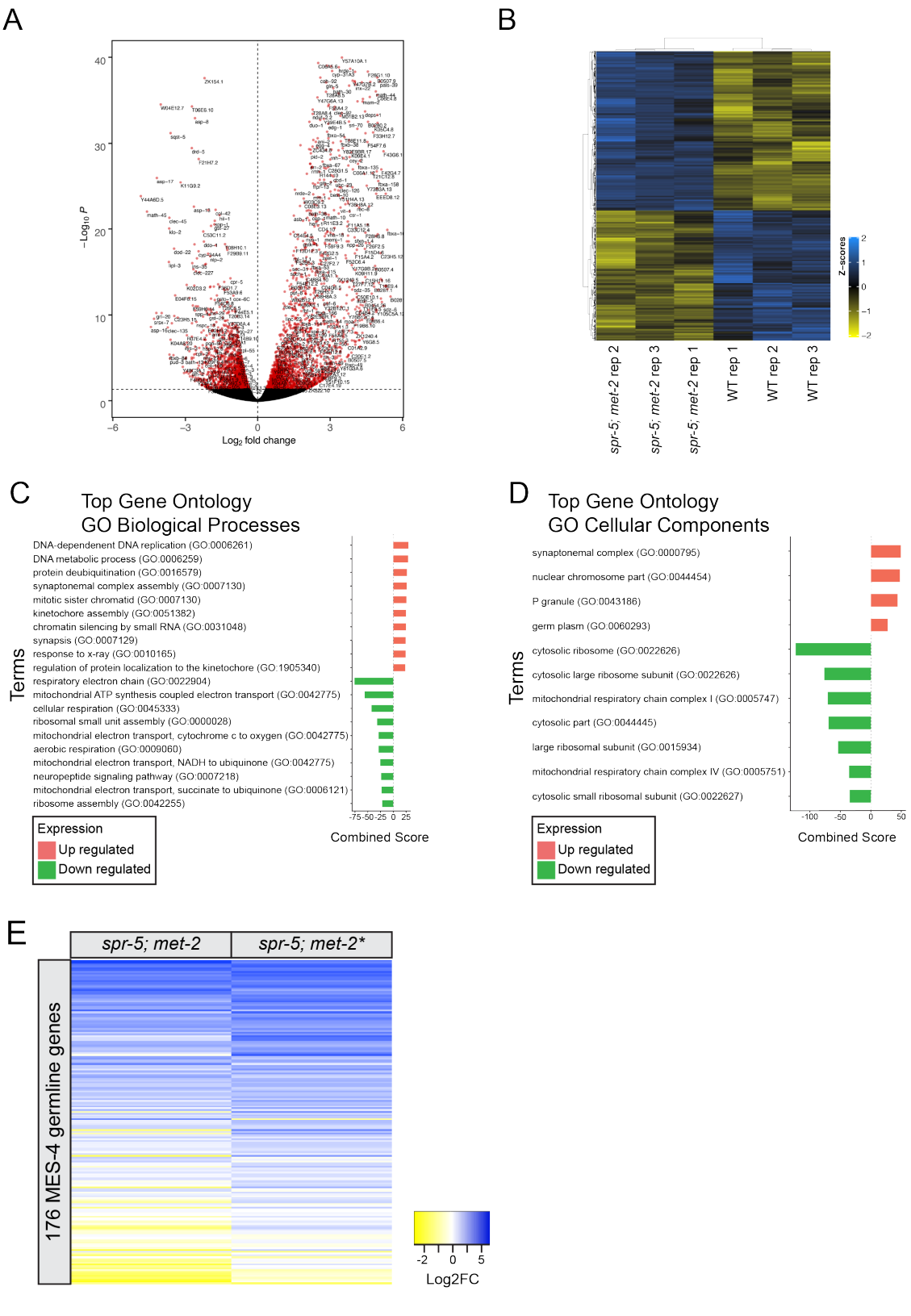


Fig. S6. Differential expression and replicate comparison from repeat experiment two RNAseq experiment performed on *spr-5; met-2* and wild type L1 progeny. Volcano plot (see GSE143837 for volcano plot R scripts) of log2 fold changes (FC) in gene expression (x-axis) by statistical significance ($-Log_{10}$ P-value; y-axis) in *spr-5*; met-2 L1 progeny (A) compared to wild type from repeat experiment two (repeat experiment one shown in Fig. S3-S5) Heatmap of differentially expressed RNA-seq transcripts between wild type and *spr-5; met-2* (B). Data was scaled and hierarchical clustering was performed using the complete linkage algorithm. Distance was measured by calculating pairwise distance. Higher (blue) and lower (yellow) expression is reported as a z-score. (see GSE143837 for heatmap R scripts). (E) Heatmap of log2FC of 176 MES-4 germline genes in *spr-5; met-2* mutants compared to wild type from repeat experiment one (2 replicates, low-input, see methods) vs. repeat experiment two of *spr-5; met-2** mutants compared to wild type (*3 replicates, standard Poly-A selection, see methods and Table S6 for Log2FC values). Log2FC values are represented in a yellow to blue gradient and range from -2 to 5 and were sorted by the average log2FC in *spr-5; met-2* and *spr-5; met-2** progeny. Log2FC values are represented in a yellow to blue gradient and range from -2 to 5. Yellow represents genes with negative log2FC values and blue represents genes with positive log2FC values compared to wild type. The remaining 21 MES-4 germline genes were not included because they do not have an expression value in one or more of the data sets (*spr- 5*, *met-2,* or *spr-5; met-2*).

Figure S7

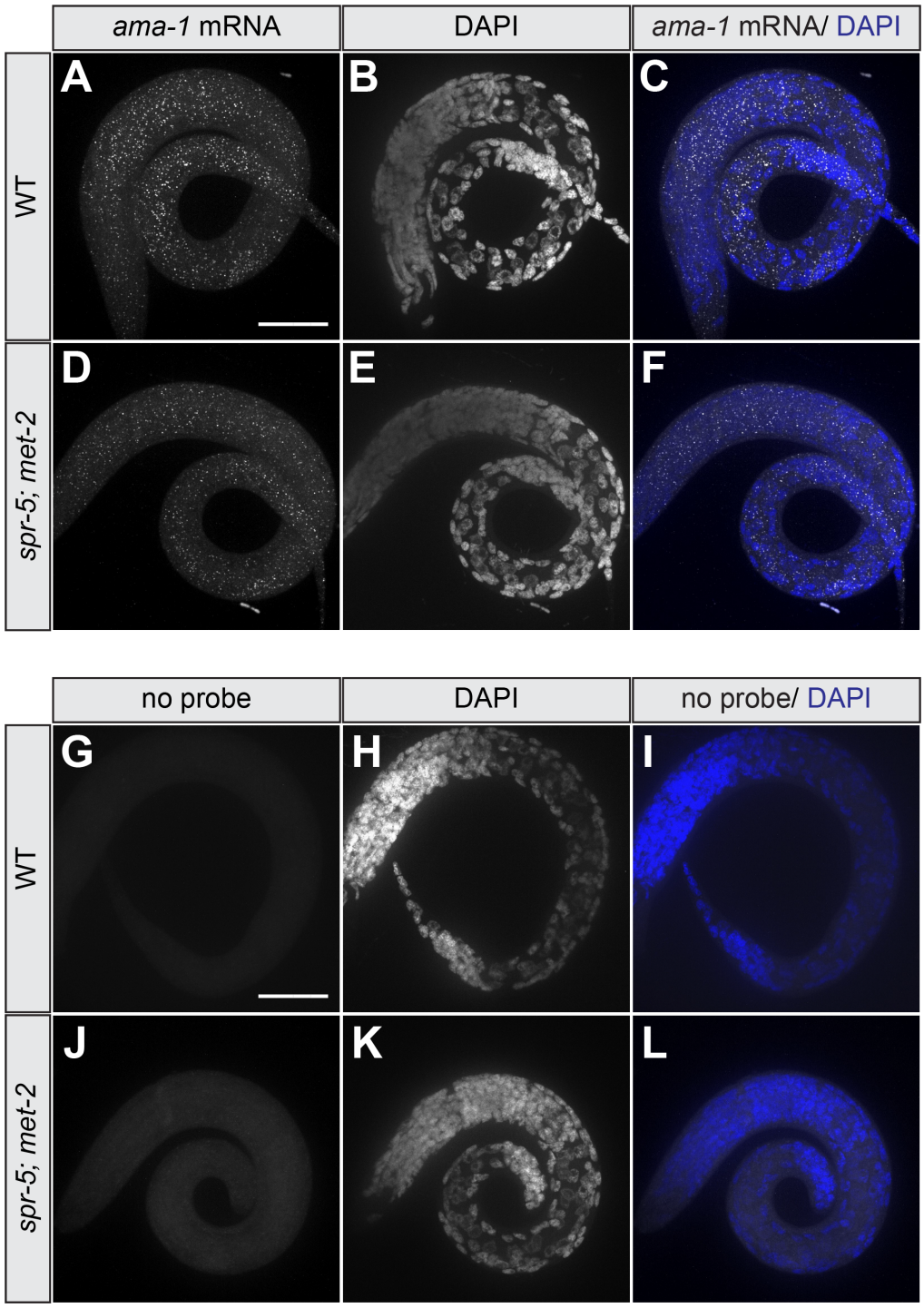


Fig. S7. smFISH control experiments. smFISH images of *ama-1* mRNA (A, C, D, F) and a no probe control (G, I, J, L) in wild type (A-C, G-I) and *spr-5; met-2* (D-F, J-L) L1 progeny. DAPI was used as a nuclear marker (B, C, E, F, H, I, K, L). Scale bar 40µm.

Figure S8

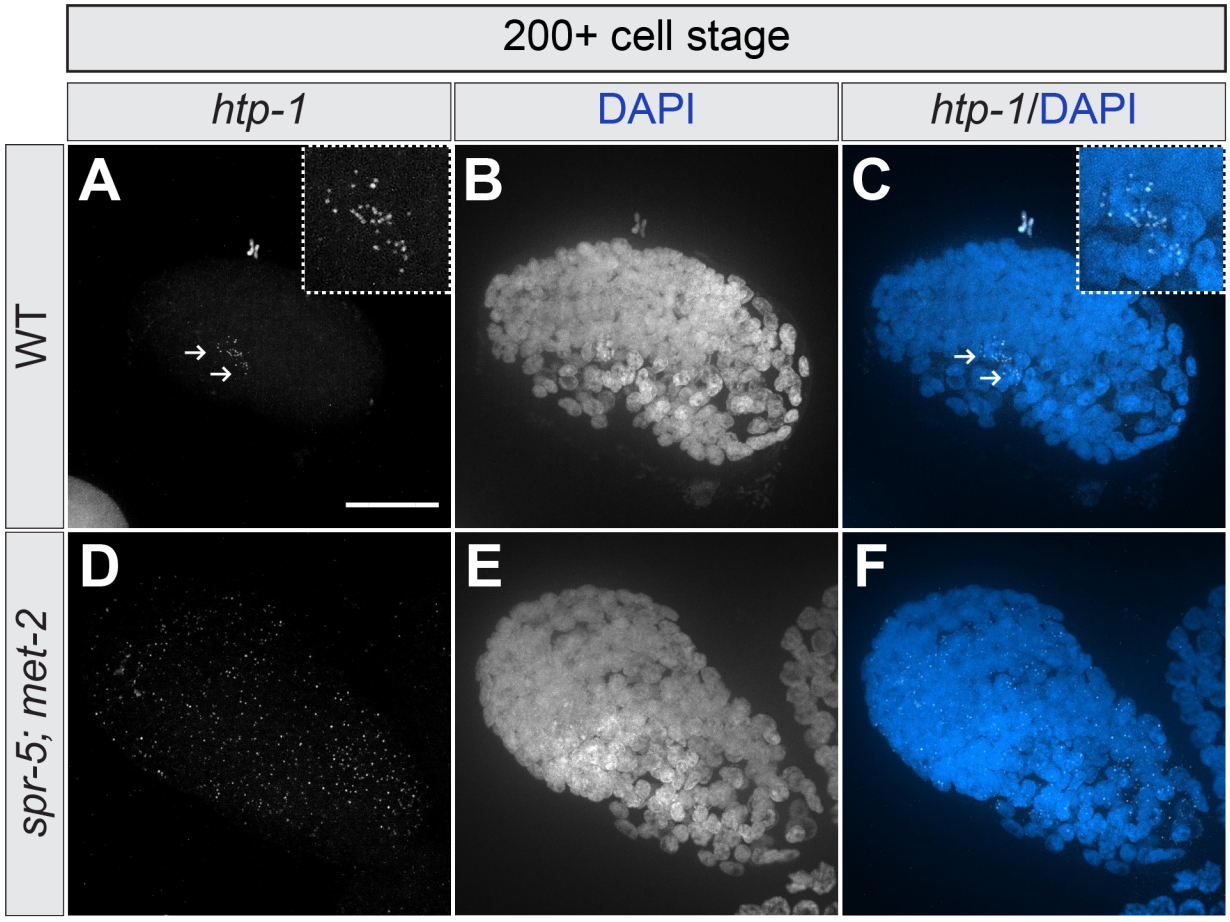


Fig. S8. *htp-1* is ectopically expressed in embryos of *spr-5; met-2* progeny. smFISH images of *htp-1* mRNA (A, C, D, F) in wild type (A-C) and *spr-5; met-2* (D-F) in 200+ cell stage embryos. DAPI was used as a nuclear marker (B, C, E, F). Scale bar 40µm

Figure S9

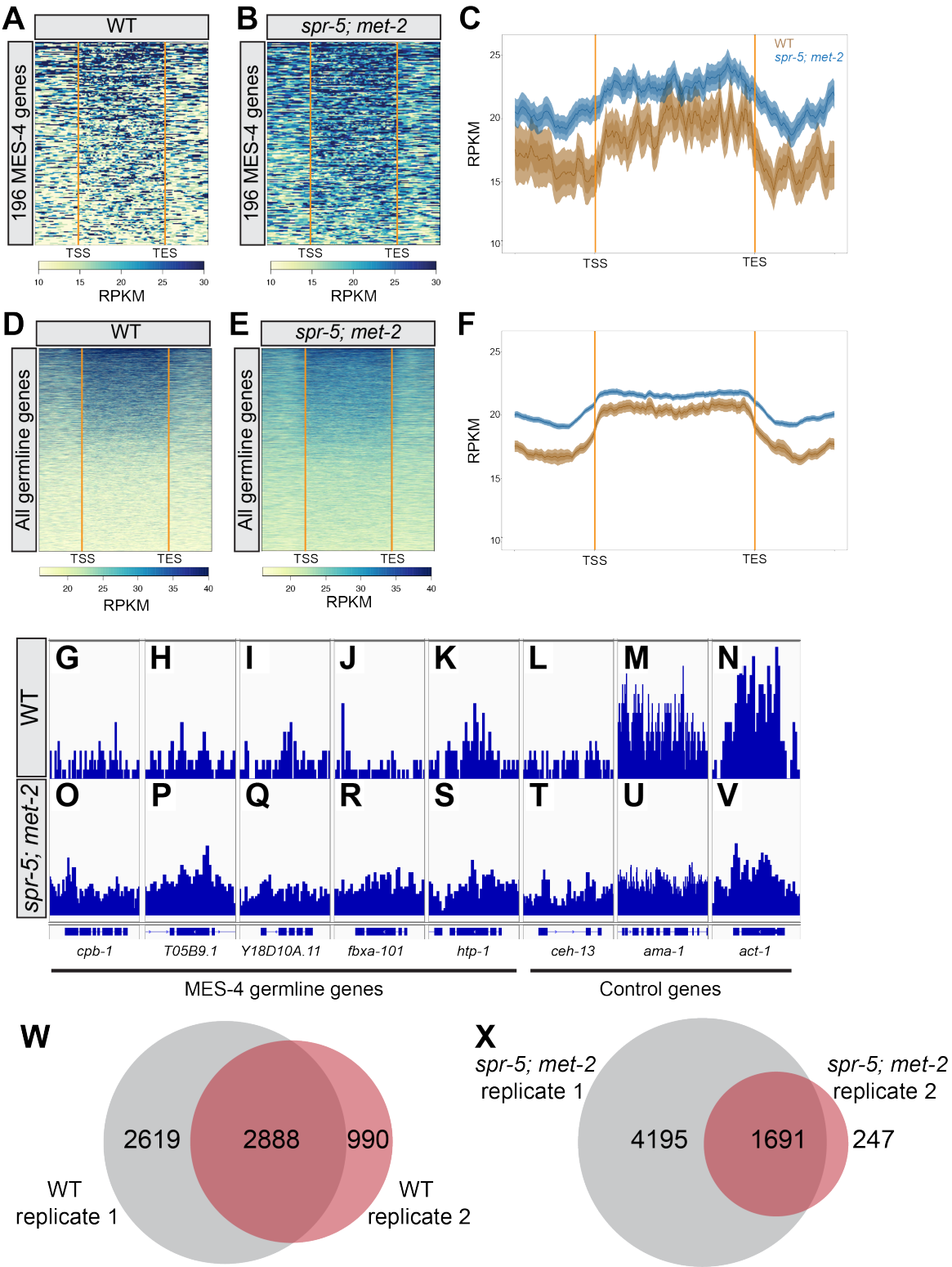


Fig. S9. Replicate comparison of H3K36me3 ChIP-seq analysis in *spr-5; met-2* L1 progeny. Heatmap of H3K36me3 ChIP-seq reads from a second replicate (1st replicate in Figure 4) normalized to reads per kilobase million (RPKM) over the gene bodies of 196 MES-4 germline genes in wild type (A) versus *spr-5; met-2* (B) L1 progeny. (C) Plot profile corresponding to heatmaps in (A) (wild type, brown) and (C) (*spr-5; met-2*, blue). Heatmap of H3K36me3 ChIP-seq reads normalized to reads per kilobase million (RPKM) over the gene bodies of all germline genes in wild type (D) versus *spr-5; met-2* (E) L1 progeny. (F) Plot profile corresponding to heatmaps in (D) (wild type, brown) and (E) (*spr-5; met-2*, blue). Gene bodies were pseudoscaled to 1kb with 500bp borders separated by orange bars that represent the transcriptional start site (TSS) and transcriptional end site (TES). Integrative Genome Viewer (IGV) image of H3K36me3 ChIP-seq reads normalized to RPKM at MES-4 germline genes (G-K) and control genes (O-S) in wild type (G-N) versus *spr-5; met-2* (O-V) L1 progeny. RPKM IGV windows were scaled between 0 and 202 RPKM for all genes. Venn-diagram displaying the overlap of H3K36me3 ChIP-seq called broad peaks for wild type (W) and *spr-5; met-2* (X) replicates.

Figure S10

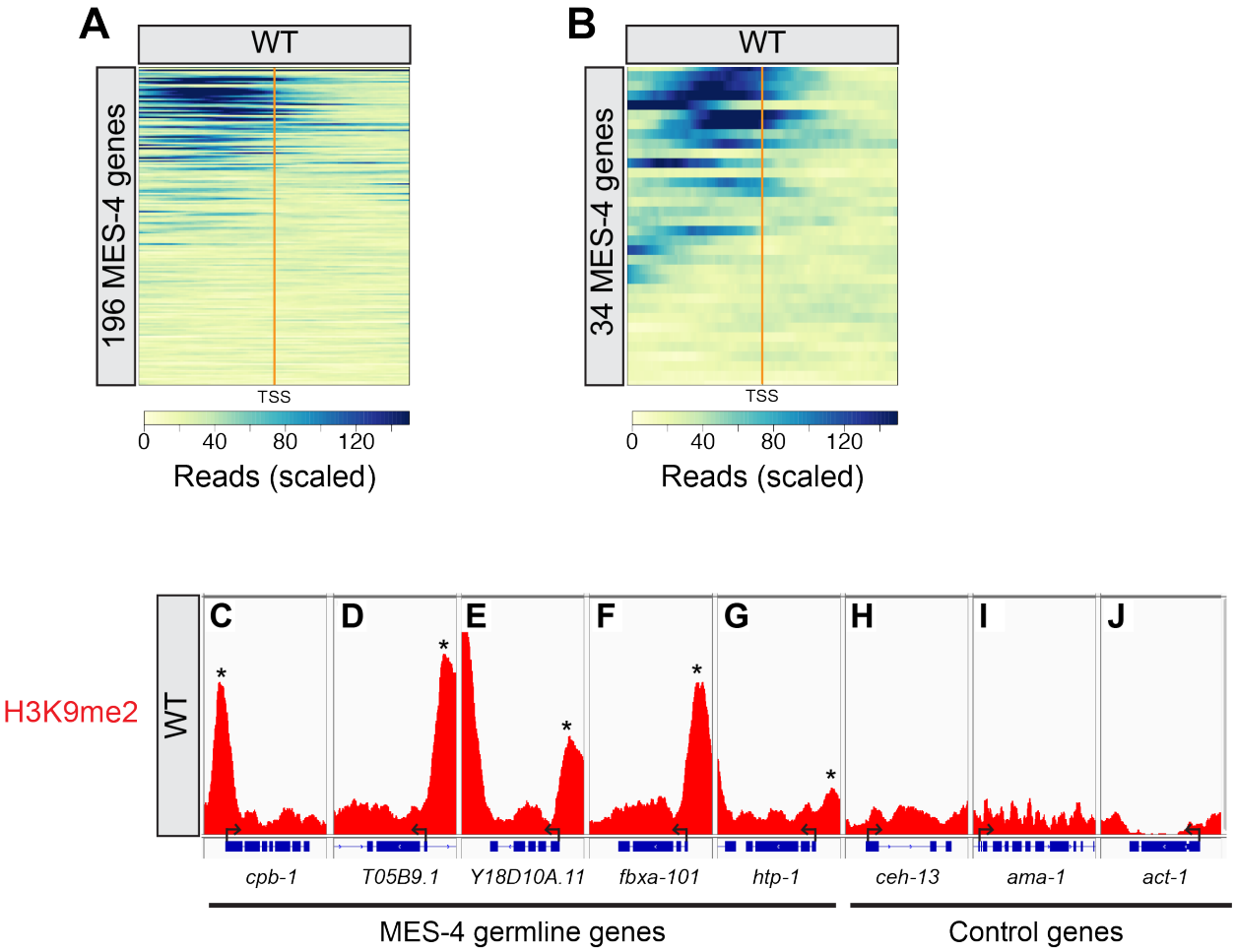


Fig. S10. MES-4 germline genes display H3K9me2 at their promoters. Heatmap of H3K9me2 promoter peaks in wild type L1 progeny at all 196 MES-4 germline genes (A), and at the 34 MES-4 germline genes that were ectopically expressed in the soma of *spr-5; met-2* progeny (B). ChIP-seq reads were scaled to genome wide coverage for H3K9me2 (15 million reads). The transcriptional start site (TSS) is denoted by an orange bar with 500bp flanking regions upstream and downstream of the TSS. Integrative Genome Viewer (IGV) image of ChIP-seq reads from wild type L1 progeny scaled to genome wide coverage for H3K9me2 (15 million reads) over the promoters of MES-4 germline genes that were ectopically expressed in the soma of *spr-5; met-2* progeny (C-G) and control genes (H-J). IGV windows were scaled between 0 and 250 RPKM. Asterisks (*) denotes H3K9me2 promoter peaks at MES-4 germline genes. To enable a comparison of gene expression, H3K36me3 and H3K9me2, we have also included a table summarizing these data across the critical MES-4 germline genes highlighted in this paper (Table S12).

Figure S11

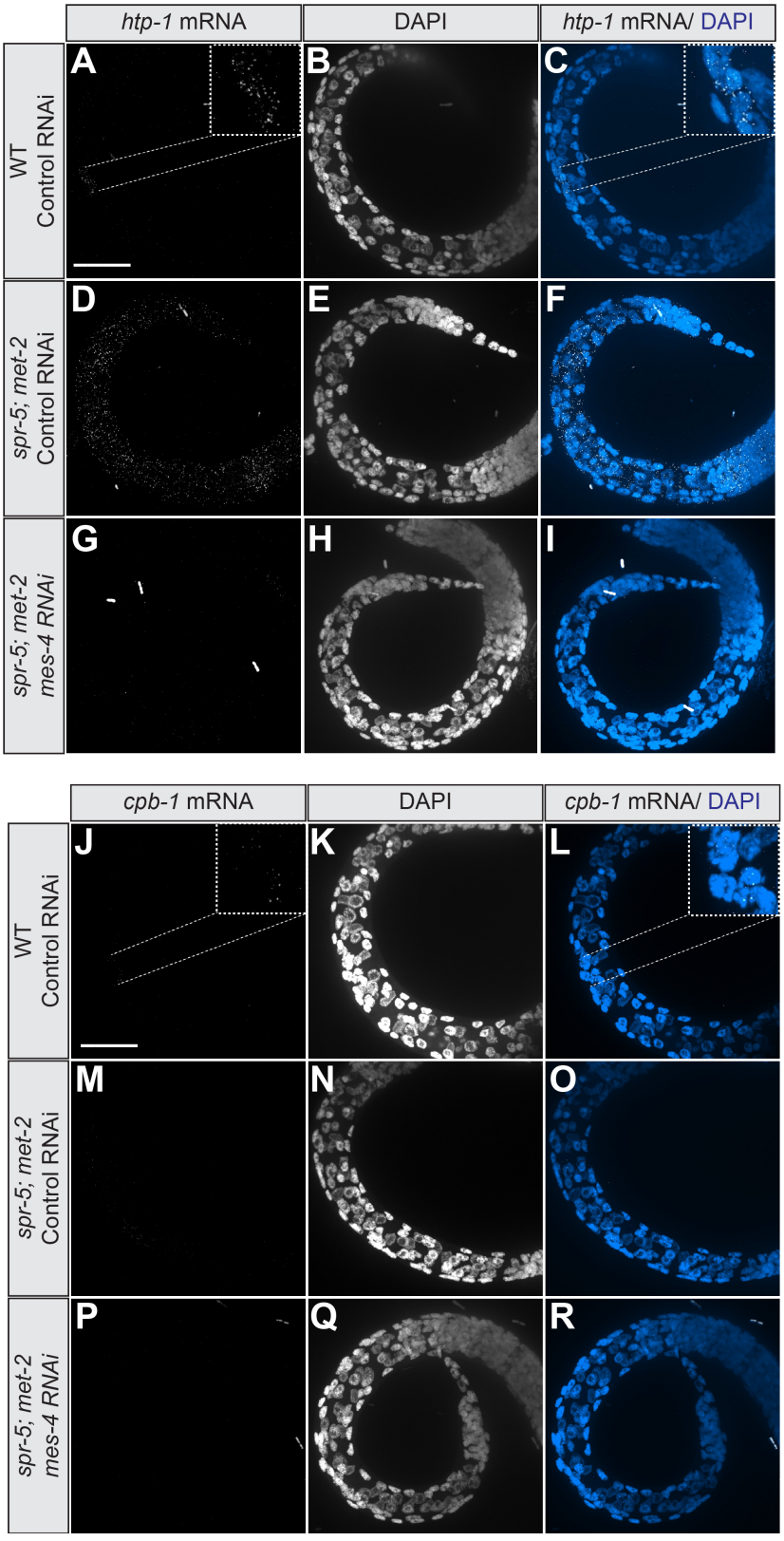


Fig. S11. Ectopic expression of *htp-1* and *cpb-1* in the soma of *spr-5; met-2* progeny requires MES-4. smFISH images of *htp-1* mRNA (A, C, D, F, G, I) in wild type (A, C), *spr-5; met-2* fed control RNAi (D, F), and *spr-5; met-2* fed *mes-4* RNAi (G, I). smFISH images of *cpb-1* mRNA (J, L, M, O, P, R) in wild type (J, L), *spr-5; met-2* fed control RNAi (M, O), and *spr-5; met-2* fed *mes-4* RNAi (P, R). DAPI was used as a nuclear marker (B, C, E, F, H, I, K, L, N, O, Q, R). Scale bar 40μm

Figure S12

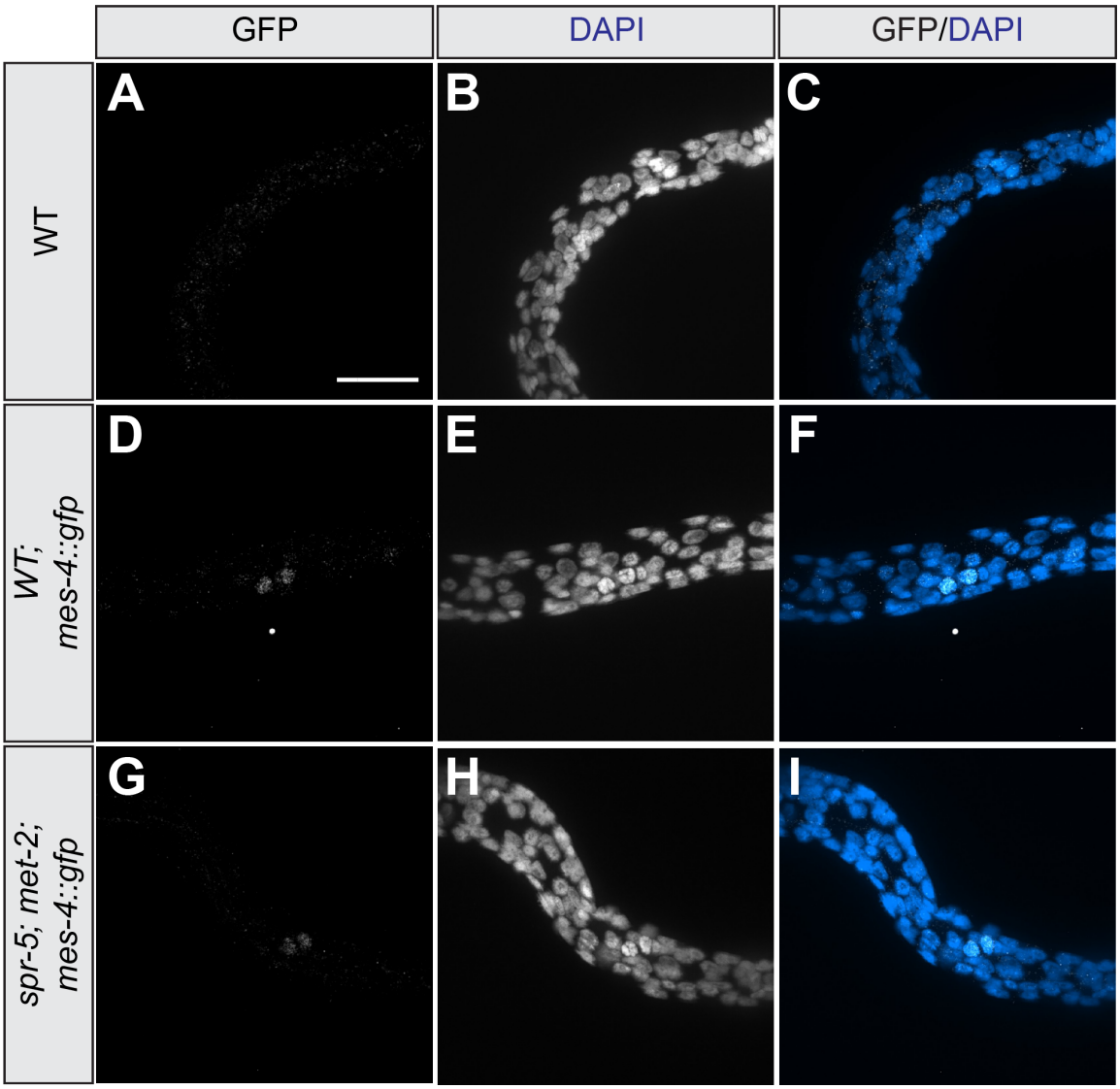


Fig. S12. MES-4 expression is restricted to Z2 and Z3 in *spr-5; met-2* progeny. Immunofluorescent images of wild type L1 larvae (A-C), wild type L1 Larvae containing a *mes-4::gfp* transgene (D-E, see methods), and *spr-5; met-2* L1 Larvae containing a *mes-4::gfp* transgene (G-I). DAPI was used as a nuclear marker (B, C, E, F, H, I). Scale bar 40μm

Table S1. **Raw scores for developmental delay in wild type, *spr-5*, *met-2*, and *spr-5; met-2* progeny.** Progeny were scored 72 hours after a synchronized lay as developing to the L1, L2, L3, L4, or young adult (YA) stage (Fig. 1A-E). N= the total number of progeny from a total of 20-25 hermaphrodites scored over three experiments.

Click here to Download Table S1

Table S2. **Differentially expressed transcripts in *spr-5* progeny**. List of differentially expressed transcripts in *spr-5* progeny (Wald test, P-value<0.05). L1 progeny compared to wild type progeny from DESEQ2 analysis (Fig. S3-S5).

Click here to Download Table S2

Table S3. **Differentially expressed transcripts in *met-2* progeny.** List of differentially expressed transcripts in *met-2* progeny (Wald test, P-value<0.05). L1 progeny compared to wild type progeny from DESEQ2 analysis (Fig. S3-S5).

Click here to Download Table S3

Table S4. **Differentially expressed transcripts in *spr-5; met-2* progeny**. List of differentially expressed transcripts in spr-5; *met-2* progeny (Wald test, P- value<0.05). L1 progeny compared to wild type progeny from DESEQ2 analysis (Fig. S3- S6).

Click here to Download Table S4

Table S5. **Differentially expressed transcripts in *spr-5; met-2* progeny repeat experiment.** List of differentially expressed transcripts in the repeat RNA-seq experiment with three additional *spr-5; met-2* replicates (Wald test, P-adj<0.05). L1 progeny compared to wild type progeny from DESEQ2 analysis (Fig. 2 and Fig. S6).

Click here to Download Table S5

Table S6. **176 MES-4 germline gene log2(FC) values used to generate heatmap.** The log2(FC) values of the 176 MES-4 germline genes from the DESEQ2 analysis performed on *spr-5, met-2, spr-5; met-2*, and the repeat RNA-seq experiment of *spr-5; met-2* (*) L1 progeny compared to wild type progeny. log2(FC) values were sorted from highest to lowest based on the differential expression of genes in *spr-5; met-2* progeny compared to wild type (Fig. S5C and Fig. S6E).

Click here to Download Table S6

Table S7. smFISH htp-1 probe sequences. List of smFISH probe sequences for *htp-1* used in smFISH experiments (Fig. 3 and Fig. S7).

Click here to Download Table S7

Table S8. smFISH cpb-1 probe sequences. List of smFISH probe sequences for *cpb-1* used in smFISH experiments (Fig. 3 and Fig. S7).

Click here to Download Table S8

Table S9. Primer sequences used for genotyping. List of genotyping primers.

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Table S10. Primer sequences used for and RT-PCR. List of RT-PCR primers (Fig. 5).

Click here to Download Table S10

Table S11. Raw values from quantitative RT-PCR analysis. Raw SQ means and standard deviations for RT-PCR experiments (Figure 5).

Click here to Download Table S11

Table S12. Summary of critical genes highlighted in this paper across experiments.

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Table S13. piRNA pathway components misexpressed in *spr-5; met-2* L1 progeny.

Click here to Download Table S13