Supplementary Data for

msl2 mRNA is bound by free nuclear MSL-complex in *Drosophila melanogaster*

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This PDF includes:

Supplementary Figures S1 to S9

SUPPLEMENTARY FIGURES

Supplementary Figure S1. RIP enrichment levels for all chromosome arms in the MSL2-RIP (N6), The plots show the mean enrichment ratios obtained using a bandwidth of 90 bp. Numbers on the x-axis denote chromosomal position along the chromosome arms in kb. The y-axis shows the RIP enrichment as the log2-ratio. In the resulting profile, all enrichments >0 are shown. The average enrichment levels of the X chromosome and the autosome arms are comparable and range from 0.026 (chromosome 2L) to 0.052 (chromosome 2R).

Supplementary Figure S2. RIP enrichment profiles from the different nuclear extracts at the *roX1* and *roX2* loci. The tiling array results are shown as the ratios between the RIP value and the value of the corresponding nuclear input RNA preparation: MSL2-RIPs (blue), MOF-RIPs (green). Numbers on the *x*-axis denote chromosomal position along the X chromosome in kb. The *y*-axis shows the RIP enrichment as the log2-ratio. Genes expressed from left to right are shown above the horizontal line and genes expressed in the opposite direction are shown below the line. Exons are indicated in black and introns in grey. The described DNAse hypersensitive regions and *roX*-boxes suggested to be of functional importance (36, 51-53) are indicated as deep-purple and yellow boxes, respectively. The *roX1* gene (A) is transcribed from right to left and *roX2* (B) from left to right.

Supplementary Figure S3. MSL-complex associates with *roX1*, *roX2* and *msl2* RNAs. Average calculated enrichment levels in the MSL2-RIP (N6) of genes with at least 10 probes within exons plotted with respect to the genome position (A). *roX1*, *roX2*, *msl2* and *msl1* are indicated by yellow boxes. (B) Enrichment levels for the top 20 genes. The average amounts of nuclear transcript for the individual genes are indicated by the red line. The left *y*-axis shows the average gene MSL2-RIP enrichment as the log2-ratio and the right *y*-axis shows the average amount of nuclear transcript (log2 scale).

Supplementary Figure S4. Enrichment profiles at the *msl2* and *msl1* loci obtained from RIP analyses of the different nuclear extracts. The tiling array results are shown as the ratios between the RIP value and the value of the corresponding nuclear input RNA preparation: MSL2-RIPs (blue), MOF-RIPs (green). Numbers on the *x*-axis denote chromosomal position along chromosome arm 2L in kb. The *y*-axis shows the RIP enrichment as the log2-ratio. Genes expressed from left to right are shown above the horizontal line and genes expressed in the opposite direction are shown below the line. Exons are indicated in black and introns in grey. Both the *msl2* gene (A) and the *msl1* gene (B) are transcribed from right to left.

Supplementary Figure S5. The MSL-complex is targeted to the *roX1* and *roX2* loci but not to the *msl2* locus. ChIP-chip enrichment profiles of the MSL-complex members MSL1, MSL3 and MOF. The genomic region from chromosome arm 2L including *msl2* (A) is compared to regions from the X chromosome including *roX1* (B) and *roX2* (C). The plots show the mean enrichment ratios obtained using a bandwidth of 300 bp. Numbers on the *x*-axis denote chromosomal position along chromosome 2L (A) or the X chromosome (B-C) in kb. The *y*-axis shows the ChIP enrichments, as the log2-ratio. Genes expressed from left to right are shown above the horizontal line and genes expressed in the opposite direction are shown below the line. The *msl2*, *roX1* and *roX2* loci are indicated by yellow boxes. The MSL1, MSL3 and MOF ChIP data is from (32).

Supplementary Figure S6. In *roX1 roX2* mutant males, MSL-complex targets a subset of sites on the X chromosome, the chromocenter and three specific sites on the 4th chromosome. (A-C) Localization of MSL3 on salivary gland polytene chromosomes; (A) DAPI, (B) anti-MSL3 and (C) merged DAPI and MSL3. (D-F) Localization of MSL2 on salivary gland polytene chromosomes; (D) DAPI, (E) anti-MSL2 and (F) merged DAPI and MSL2. Enlargements of the chromocenter and chromosome 4 region are shown for MSL3 (G) and MSL2 (H). The chromocenter is indicated by an arrow and the specific sites on the 4th chromosomes with arrowheads.

Supplementary Figure S7. MLE targets specific sites on the 4th chromosome in wildtype males and females. The specific sites on the 4th chromosome targeted by the MSL-complex in *roX1 roX2* mutant males are targeted by MLE also in wildtype, in both females (left panels) and males (right panels).

Supplementary Figure S8. *msl2* RNA is not associated with chromatin in salivary gland nuclei. *In situ* hybridization shows no hybridization of *msl2* to chromatin with *msl2* overexpression (top row), in a *rox1 roX2* mutant background (middle row) or in a *rox1 roX2* mutant background with *msl2* overexpression (bottom row).

Supplementary Figure S9. Spliced and poly-adenylated *msl2* RNA is associated to a non-chromatin bound nucleoplasmic MSL-complex. The histograms illustrate enrichment of *msl2* RNA in the MSL-complex in the nucleoplasmic fractions (grey) and the corresponding cytoplasmic fractions (light grey). Displayed are the fold enrichments relative to actin RNA. Gel-electrophoretic analyses of amplified genomic DNA (gDNA), total RNA from the cytoplasm (CP) and immunoprecipitated (CP RIP) fractions together with minus reverse transcriptase controls (-RT) following qPCR are shown. The PCR product sizes of the spliced *msl2* amplicon and the unspliced amplicon are indicated by arrowheads and stars, respectively.

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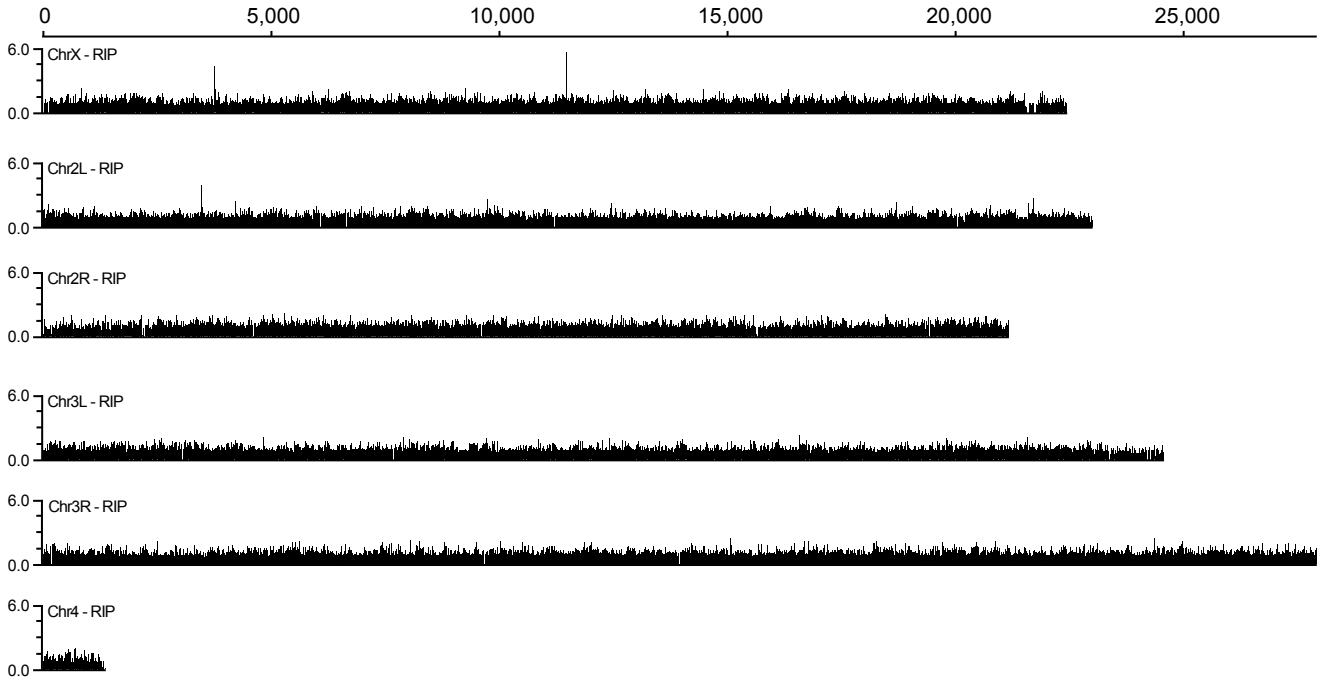
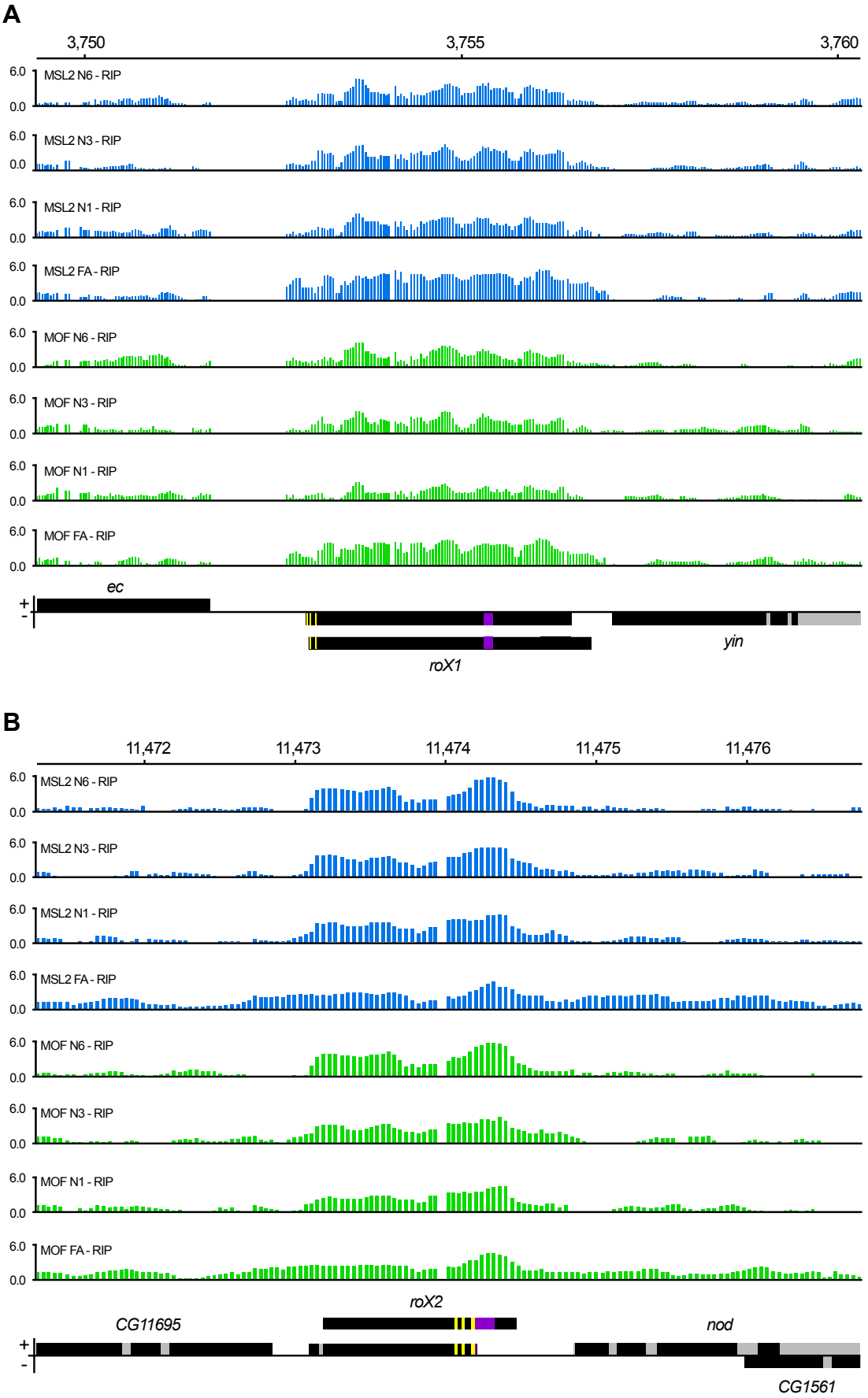
Supplementary Figure S1



Supplementary Figure S2



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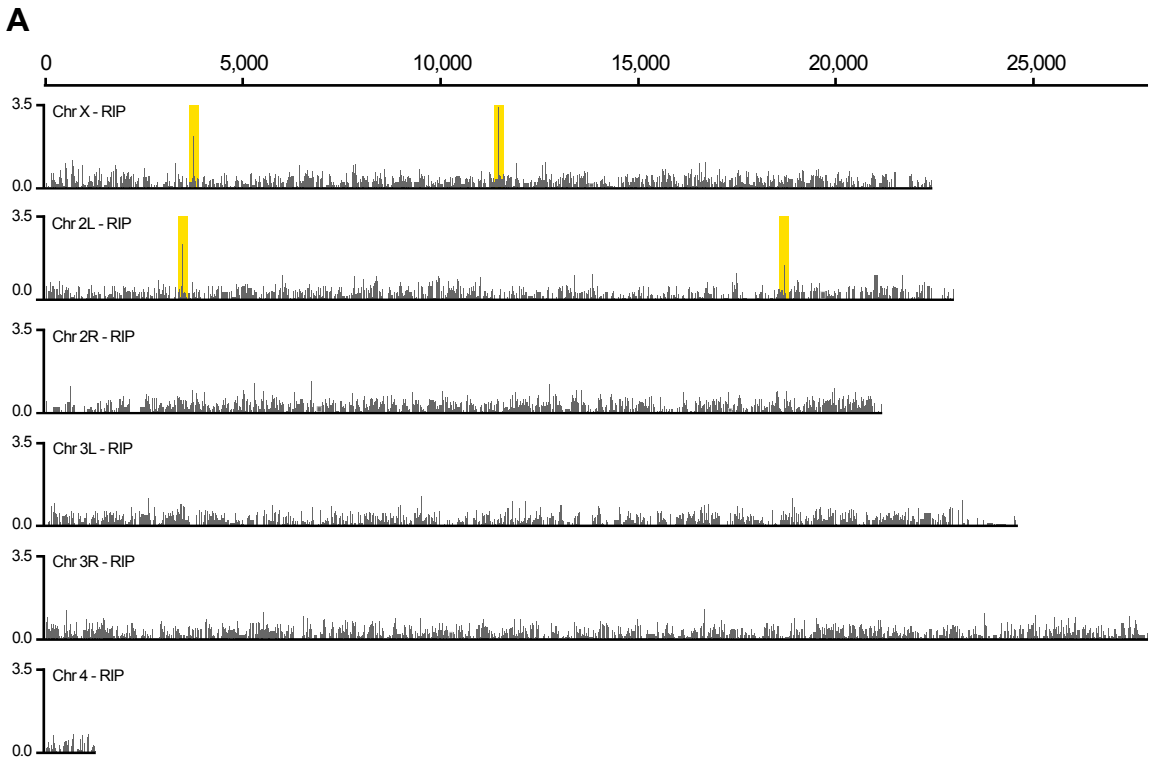

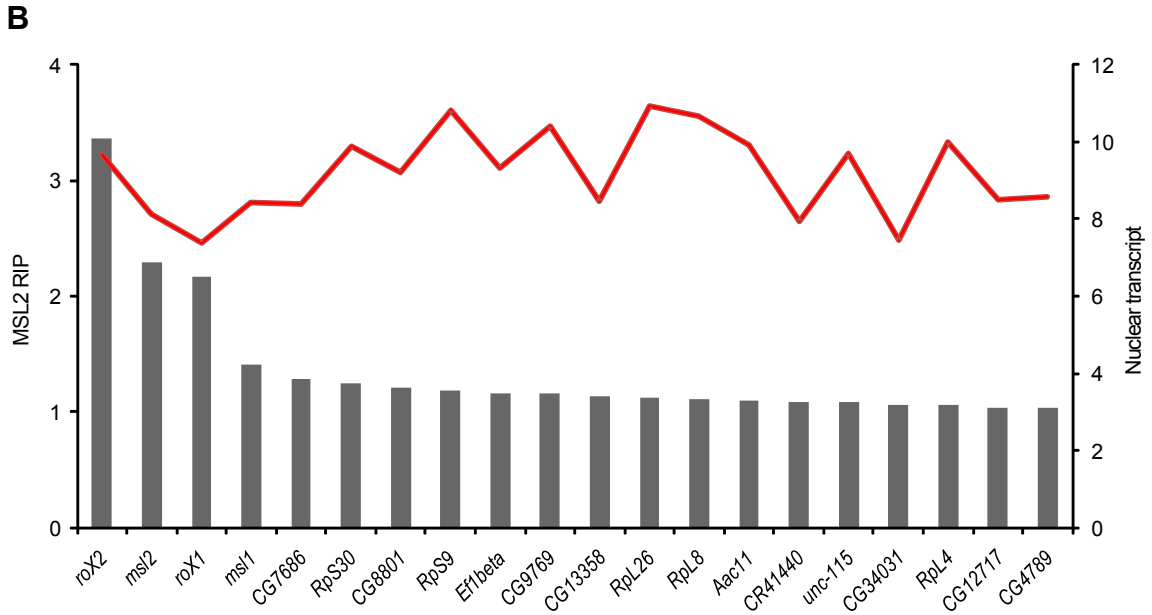

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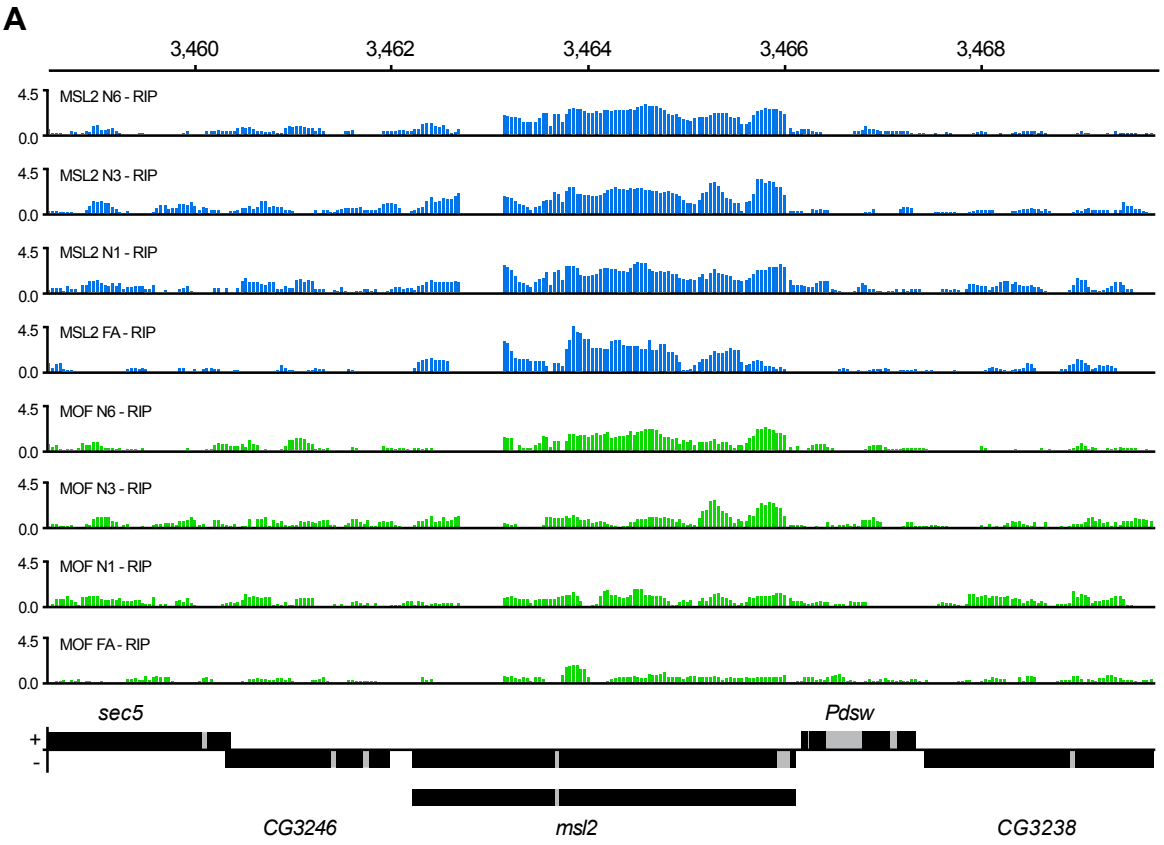

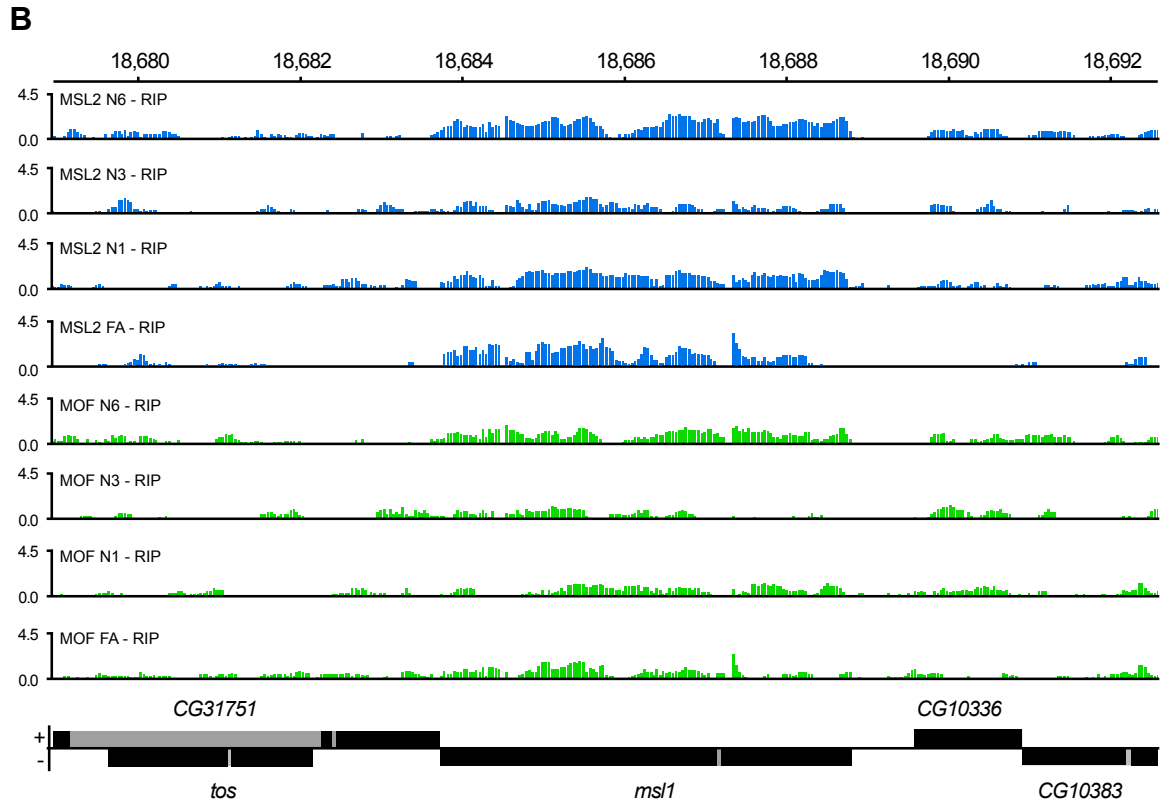

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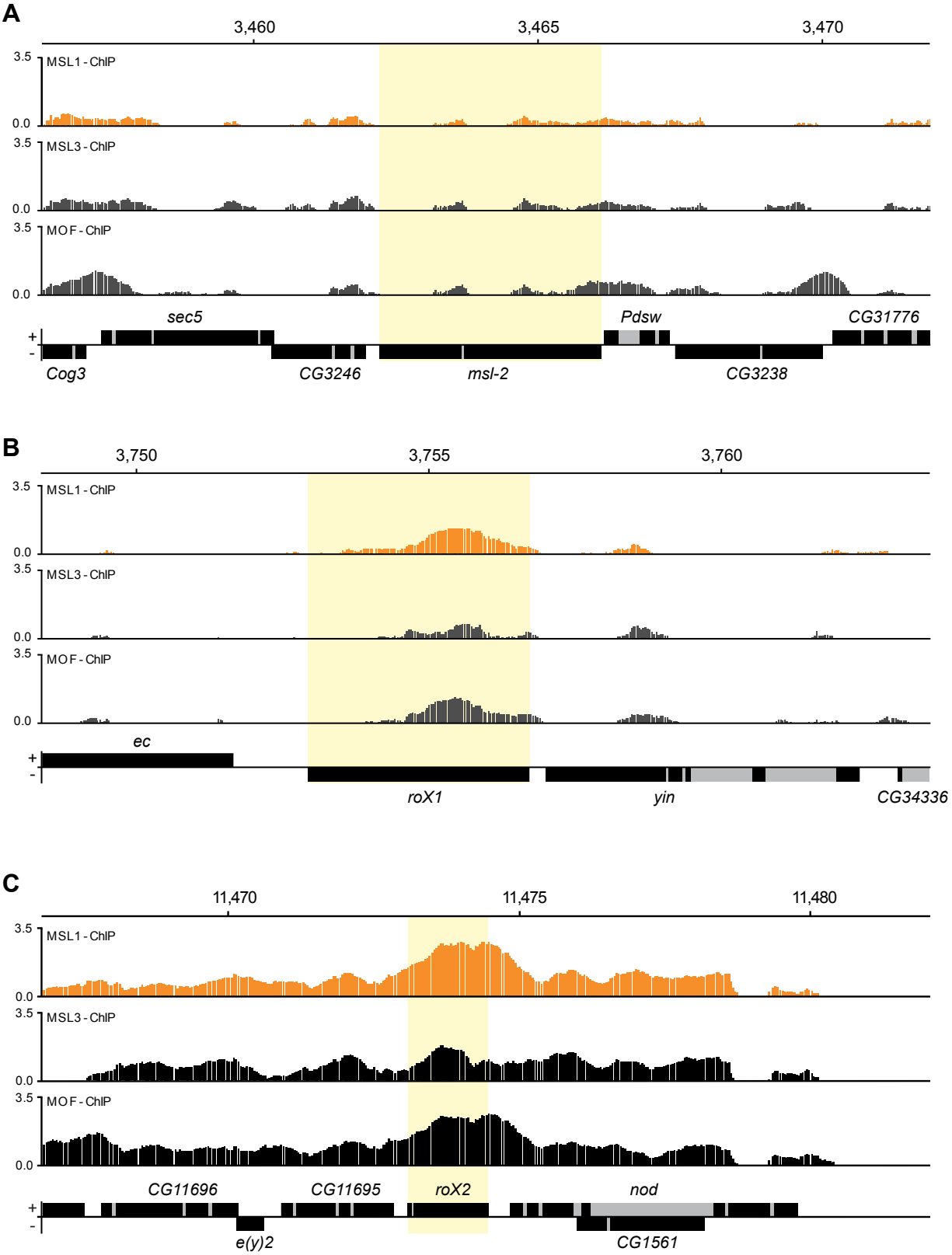

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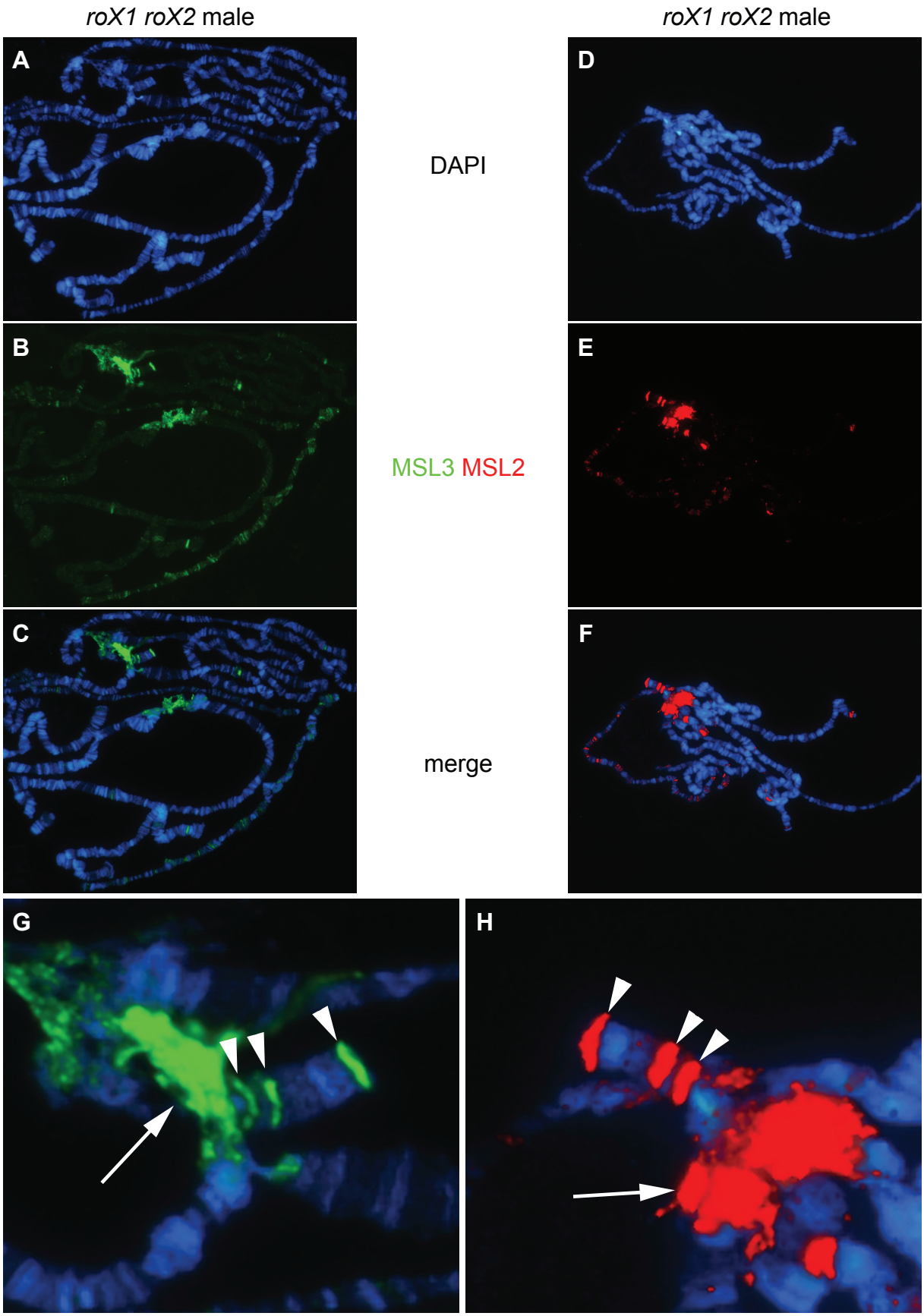

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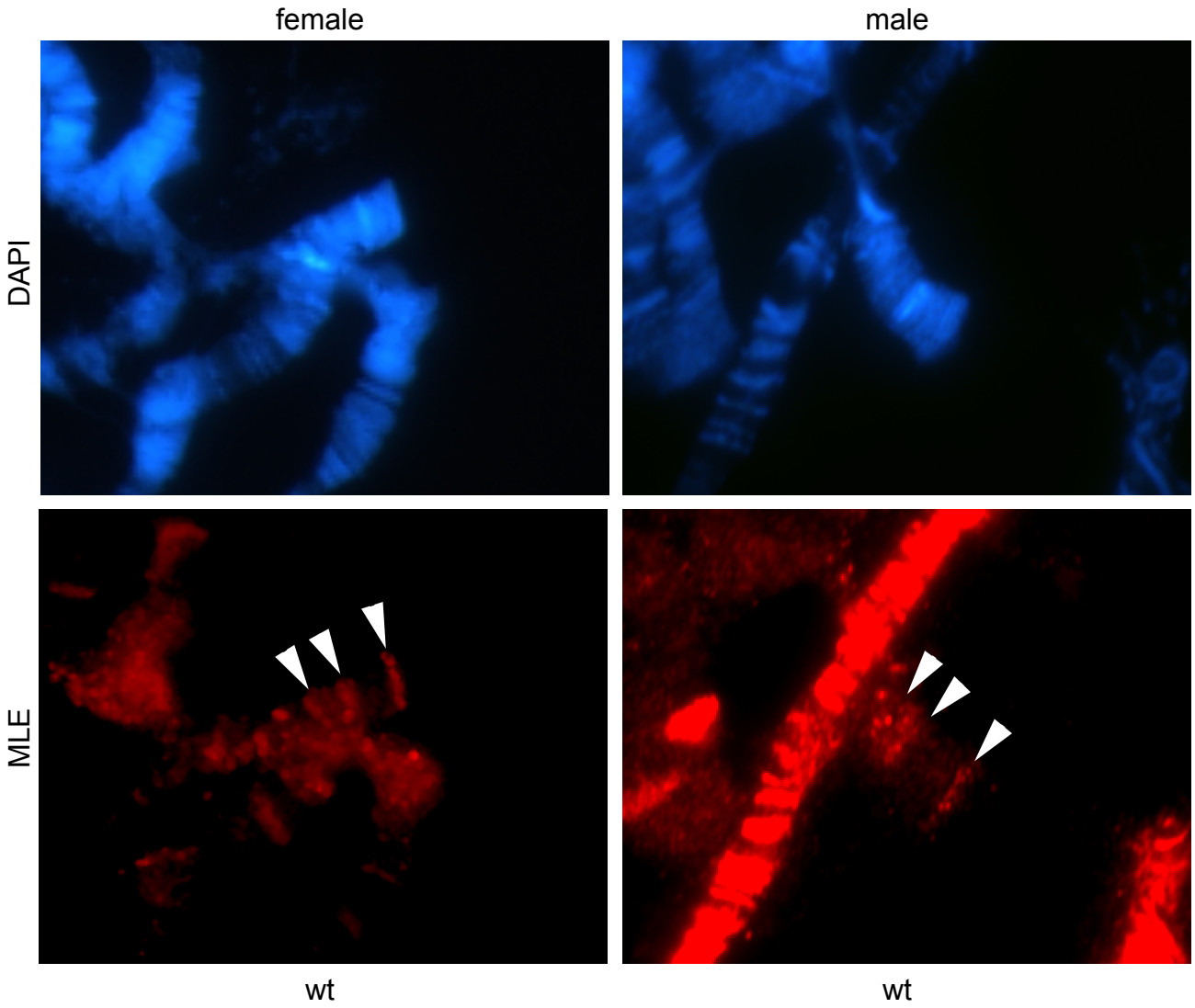

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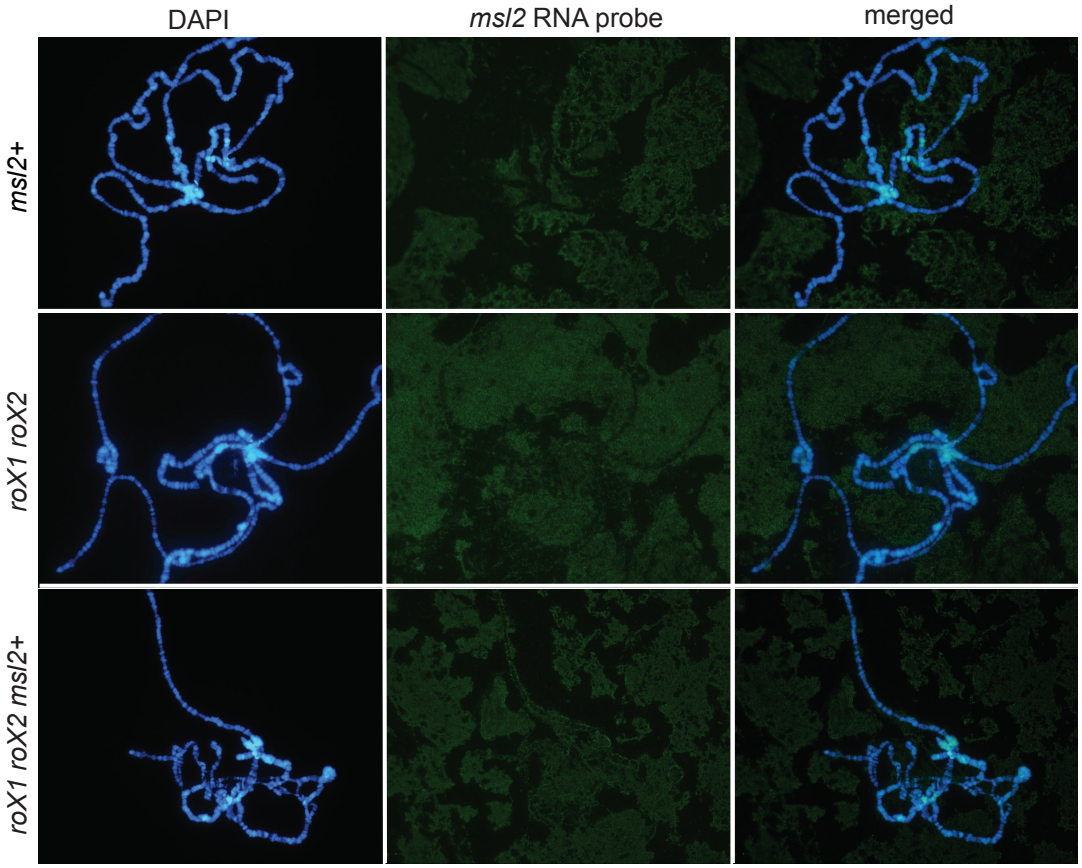

Supplementary Figure S9