Supplemental Materials--Sun and Birchler

Supplemental Figure 1. Sequestration of MOF to the X chromosomes in ectopically expressing MSL2 females and metafemales.

Immunofluoresence labeling of polytene chromosomes with anti-MOF (green) from six genotypes of males, females and metafemales with and without the transgene (*w+)H83M2-6I* is shown. DNA is counterstained in blue. The MOF protein is sequestered to the Xs in ectopically expressing MSL2 females (H83M2-□) and metafemales (H83M2-m□), males (□) and overexpressing MSL2 males (H83M2-□). Normal males, females (□) and metafemales (m□) were used as comparisons. Arrows indicate localization on the X chromosome(s). Scale bar =15μm.

Supplemental Figure 2. MOF association with normal male, female and metafemale chromosomes.

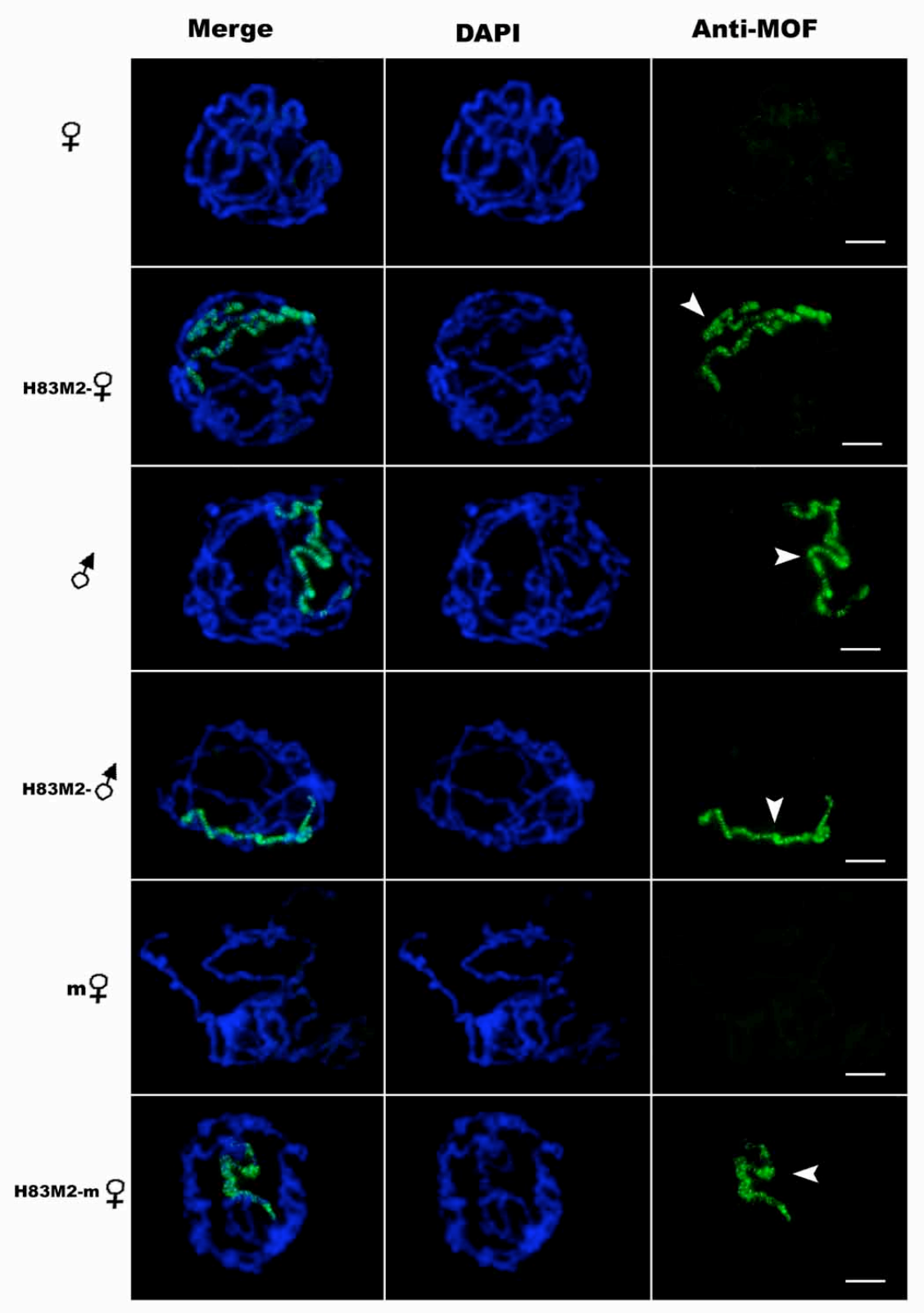
(A) The top panel, at 25X, (a) shows a merged image from a mixture of normal male and female nuclei in the same microscopic field stained with DAPI (b), anti-SXL (c) and anti-MOF (d). Lower panels are magnified images of males (□) or females (□) selected from the top panel. (B) The top panel at 25X (a) shows a merged image from a mixture of normal male and metafemale larval glands stained with DAPI (b), anti-SXL (c) and anti-MOF (d). Lower panels, enlarged images of normal male and metafemale (m□) nuclei selected from the top panel. Only the X chromosome in males is labeled with MOF, which is indicated by the arrowheads in A and B. The scale bar represents 40 μm in both A and B.

Supplemental Figure 3. Colocalization of ectopically expressed MSL2 and MOF in females. Polytene chromosomes from third instar female larvae with transgene (*w+)H83M2-6I* were doubled labeled with anti-MSL2 and anti-MOF antibodies. (a) Merged image. (b) Chromosomes were counterstained with DAPI (blue), (c) labeled with anti-MSL2 (red) and (d) labeled with anti-MOF (green). Part of the image was magnified at the lower right showing individual labels and the colocalized bands of MSL2 and MOF. The scale bar represents 15 μm.

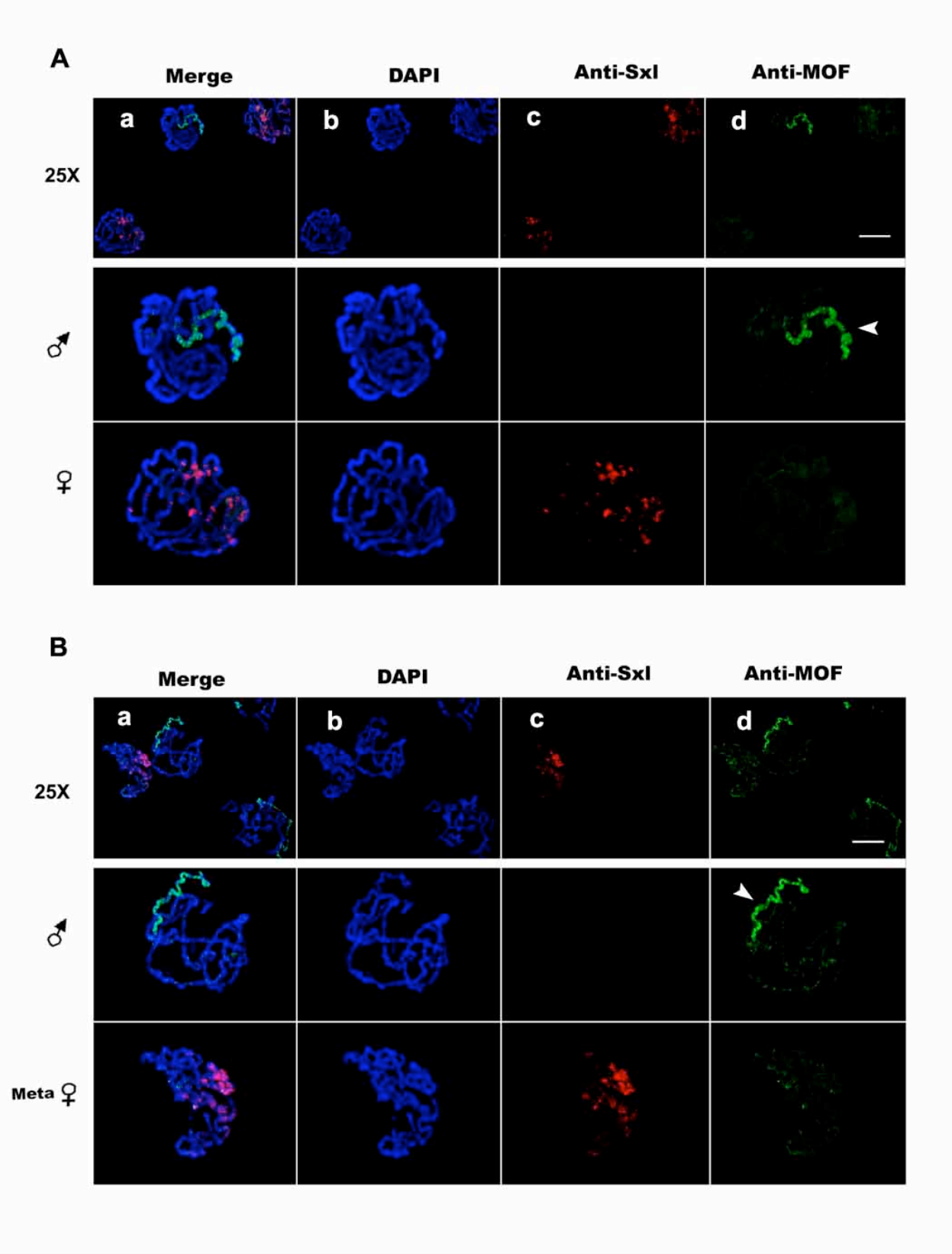
Supplemental Figure 4. H4Ac16 distribution in normal male, female and metafemales.
(A) Top panel at 25X shows (a) merged image from a mixture of male and female nuclei viewed in the same microscopic field. (b) mixture of chromosomes stained with DAPI (blue). (c) The same chromosomes probed with anti-SXL (red) and (d) anti-H4Ac16 (green). Lower panels are the enlarged views of a male (□) and a female (□) nucleus shown in the top panel. (B) Top panel at 25X shows (a) a mixture of polytene chromosomes from normal male and metafemale larvae viewed in the same microscopic field. (b) Chromosomes stained with DAPI in blue, (c) with anti-SXL in red and (d) anti-H4Ac16 in green. Lower panels are the enlargement of images from male and metafemale (m□) chromosomes represented in the top panel. Only the X chromosome in males is labeled with H4LysAc16, which indicated by the arrowheads in A and B. The scale bar represents 40 μm in either A or B.

Supplemental Figure 5. Effects of ectopically expressing MSL2 on the expression of the X chromosome genes in larvae.
The top panel image represents gene transcripts detected by Northern analysis from five genotypes of males, females with and without (*w+)H83M2-6I* and metafemales The bottom

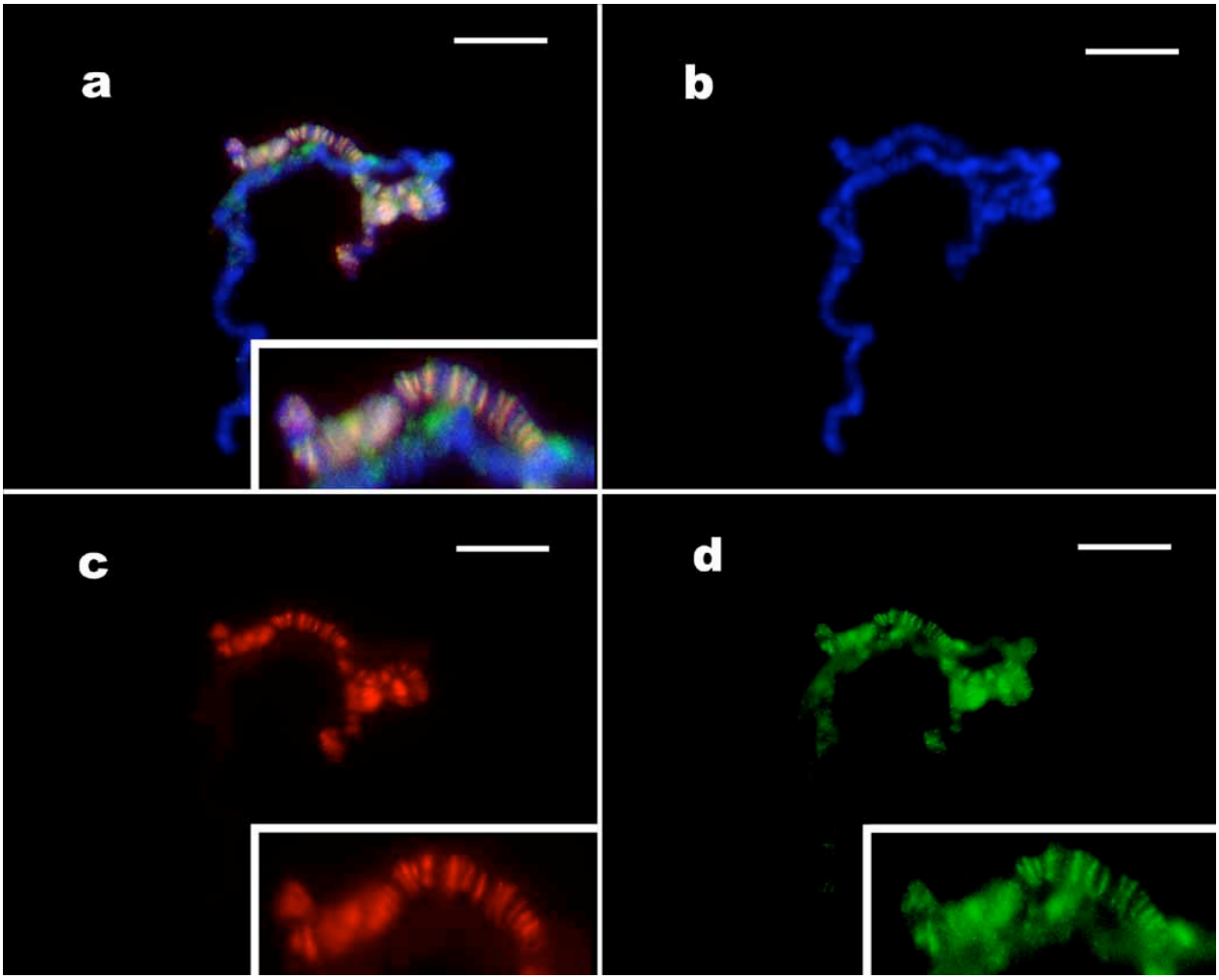
panel is rRNA as a loading control. Each lane was loaded with 10 μg of total RNA. The intensity of bands was measured by a Fujifilm Fluorescent Image Analyzer FLA-2000 and analyzed by Fujifilm Image Gauge V 3.3 program (Fuji, Tokyo, Japan). The relative transcript (transcripts/rRNA) levels in the six genotypes are presented in the bar graph. The labels of H83M2-male and H83M2-female indicate the transgenic MSL2 males and females, respectively. No significant differences between the normal and transgenic genotypes are observed in males, females, or metafemales.



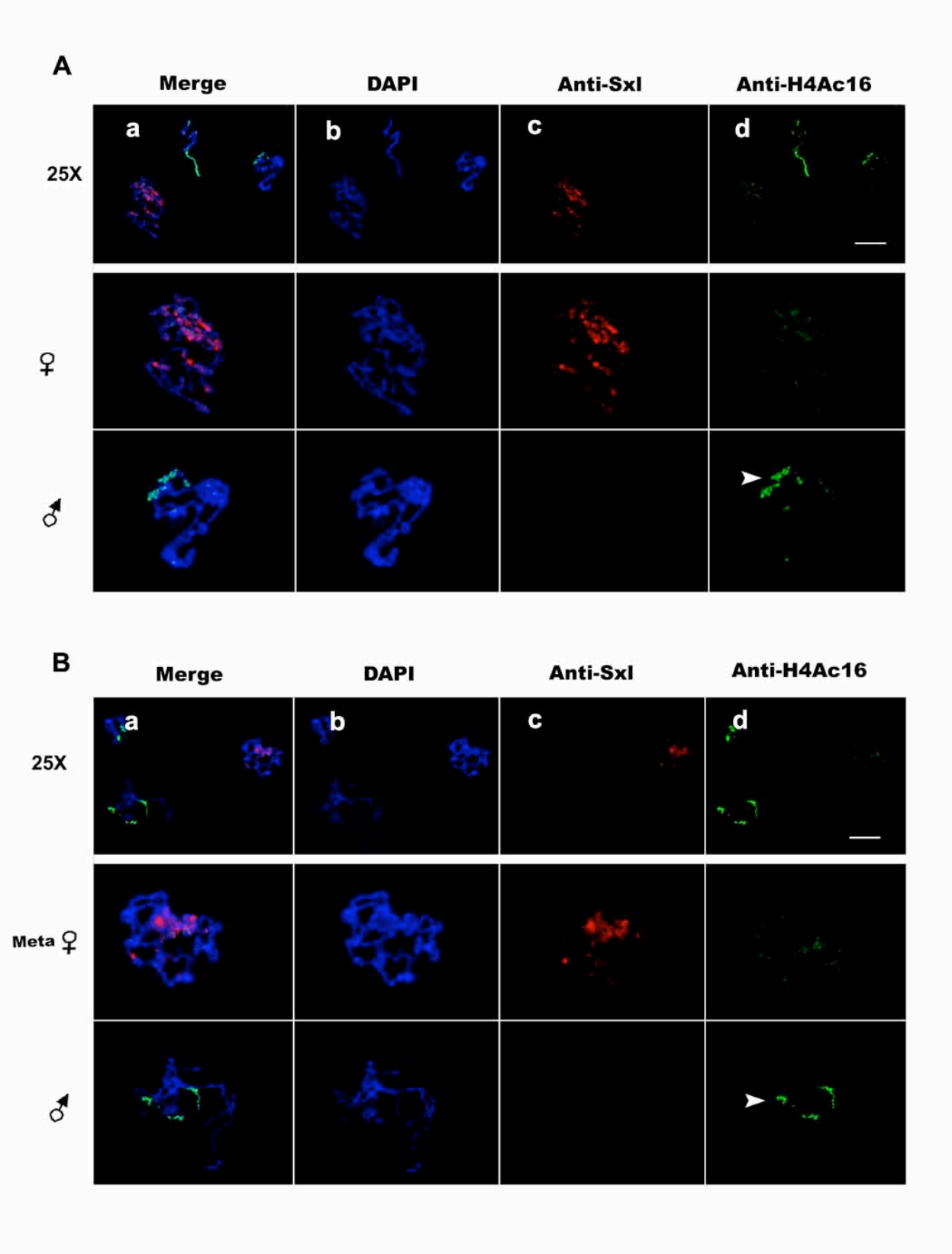
Supplemental Figure 1.



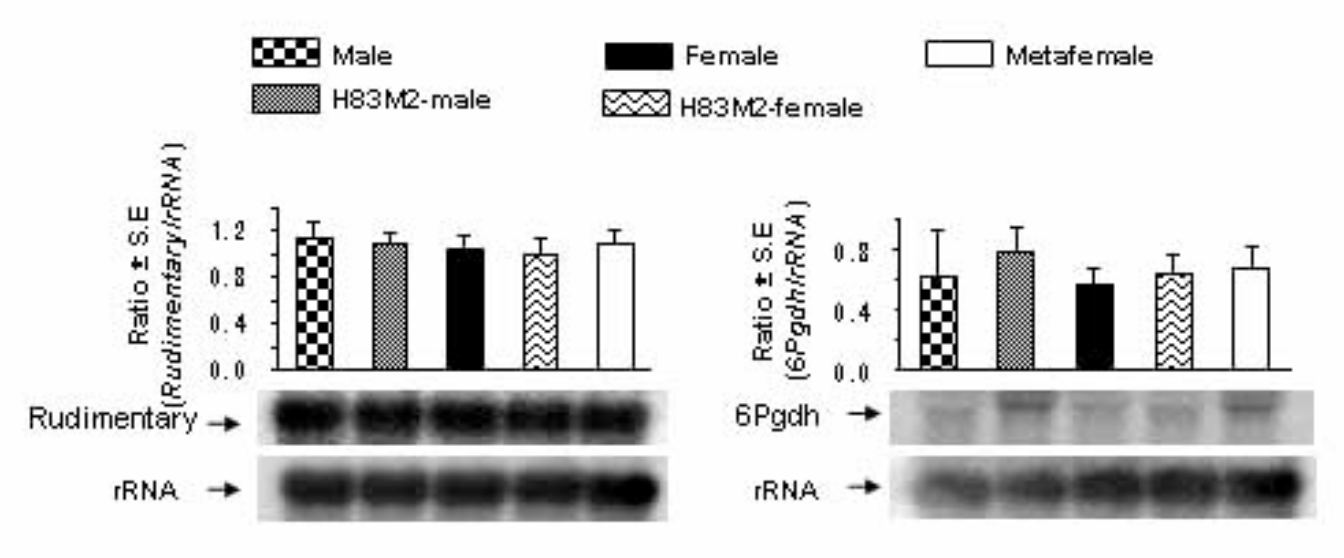
Supplemental Figure 2.



Supplemental Figure 3.



Supplemental Figure 4



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