Supplemental Data

Nuclear Pore Components Are Involved in the Transcriptional Regulation of Dosage Compensation in *Drosophila*

Sascha Mendjan, Mikko Taipale, Jop Kind, Herbert Holz, Philipp Gebhardt, Malgorzata Schelder, Michiel Vermeulen, Alessia Buscaino, Kent Duncan, Juerg Mueller, Matthias Wilm, Henk Stunnenberg, Harald Saumweber, and Asifa Akhtar

Figure S1.

(A) Co-localization of MOF and MSL-3FLAG on the male X chromosome in transgenic larvae, visualized by immunostaining of polytene chromosomes with α–MSL-3 and α–MOF antibodies.

(B) Localization of TAP-MOF protein to the male X chromosome. Squashes of polytene chromosomes were stained with α-MSL-3 and α-protein A (PAP) antibody to detect specifically TAP-MOF.

(C) Western blot analysis of MSL proteins eluted in the MSL-3FLAG purification. I: Input, E: Eluate.

(D) RT-PCR of roX1 and roX2 RNAs in eluates of the MSL-3FLAG purification. Lanes 1-4 (reactions with reverse transcriptase), lanes 5-8 (reactions without reverse transcriptase) U6 RNA is shown as a control.

(E) RT-PCR analysis of roX1 and roX2 RNAs in eluates from TAP-MOF purification. Lanes 1-4 (reactions with reverse transcriptase), lanes 5-8 (reactions without reverse transcriptase). U6 RNA serves as a control.

(F) Control immunoprecipitation (IP) experiment with α-Mi2 (lane 1) or α-Pc (lane 2) antibodies. Following binding to beads, samples were separated by SDS-PAGE. Western blot analysis was performed with antibodies against the indicated proteins. Input represents the 5%, 1% or 0.5% (lanes 1-3) of starting extract used for IP.

(G) HAT activity of HA-2xFLAG-hMOF eluates. (Top panel) Reconstituted mononucleosomes with wild-type H4 (wt) or tailless H4 (H4Δtail) were used as a substrate. (Bottom panel) western blot with specific antibodies against acetylated lysines. Ponceau staining verified equal loading.

Supplementary Figure S1

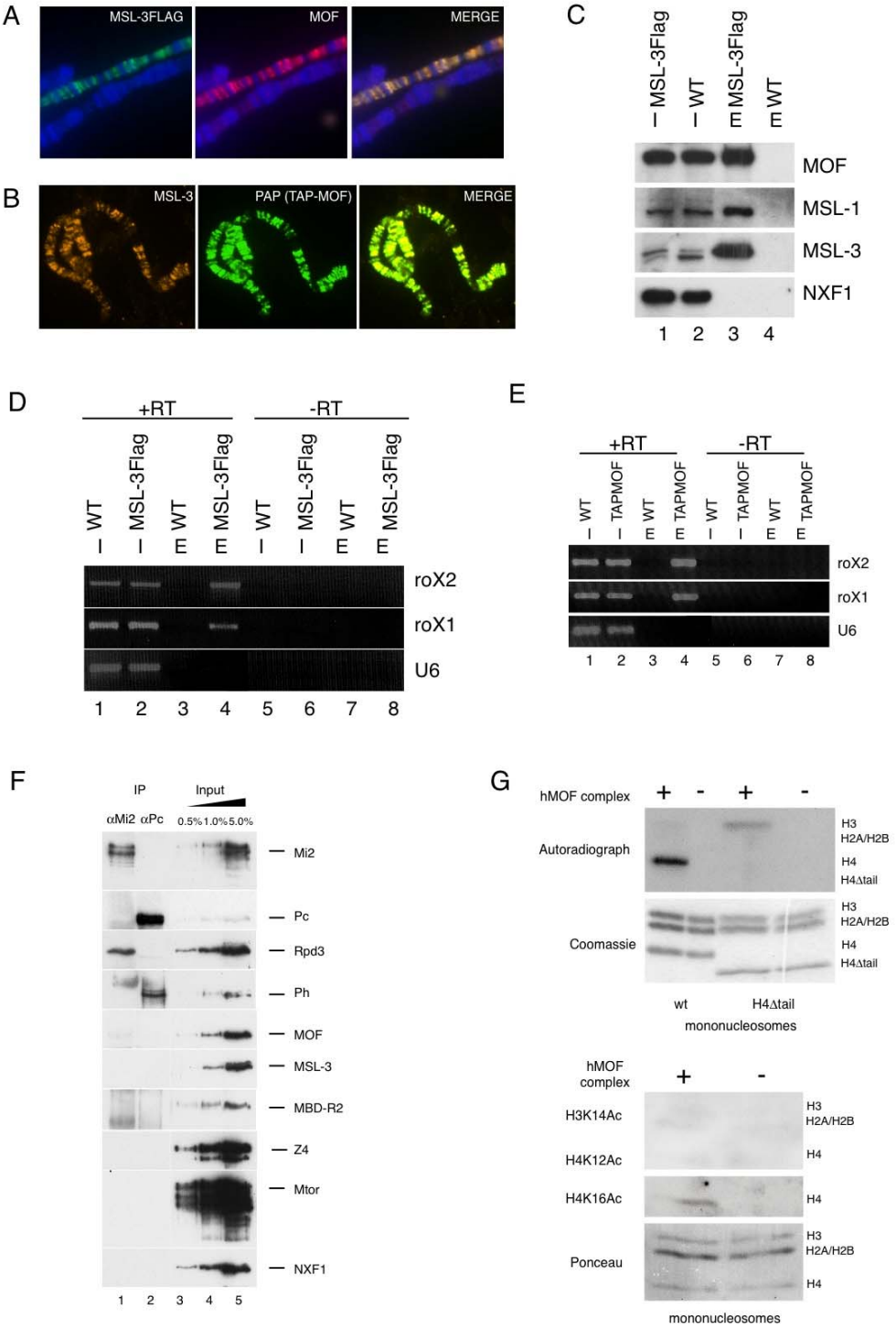


Figure S2.

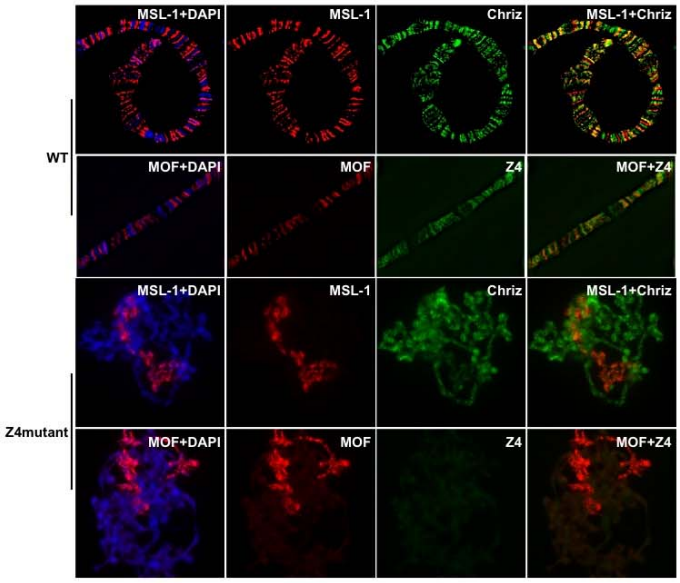
(A) Polytene chromosomes were isolated from 3rd instar larvae from wildtype (WT) or Z4 hypomorphic mutants. Chromosomes were immunostained with the combination of either MSL-1 (red) and Chriz (green) antisera or MOF (red) and Z4 (green) antisera. All polytene preparations were co-stained with DAPI to visualize DNA (blue). Last panel also shows the merge of green and red channels.

(B) SL-2 cells were incubated with EGFP or MBD-R2 dsRNA. Following protein knockdown, cells were harvested and nuclear (N) and cytoplasmic extracts (C) were prepared and separated by SDS-PAGE. Western blot analysis was performed with the antibodies against MBD-R2, MOF, Mtor, MSL-3 and Tubulin as indicated. The top two panels show short and long exposure of the MBD-R2 blot demonstrating efficient knockdown.

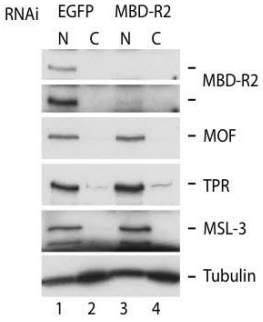
(C) Confocal microscopy was performed on SL-2 cells treated with EGFP or MBD-R2 dsRNA. For this purpose cells were immunostained with MBD-R2, Z4, MSL-1, MOF, Nup153 or WGA as indicated. Merge pictures are indicated in corresponding right panels.

Supplementary Figure S2

A



B



C

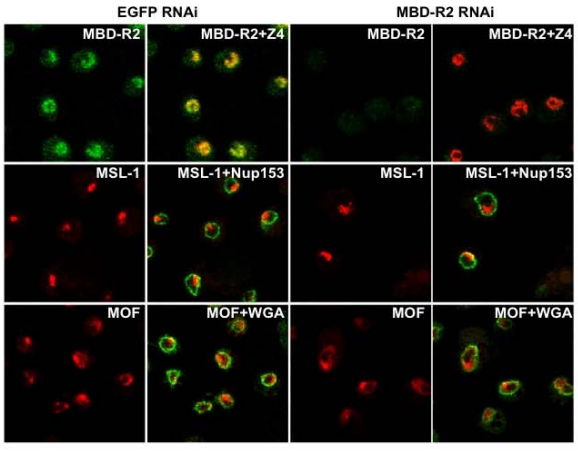


Figure S3.

(A) Western blot analysis of nuclear (N) versus cytoplasmic (C) extracts prepared from cells treated with Mtor, Nup153 or EGFP dsRNA (same experiment as in Figure 3). Blots were probed with antibodies generated against MLE, MSL-2, or tubulin as indicated.

(B) RNA was isolated from nuclei or cytoplasm of corresponding cells and analyzed by quantitative RT-PCR for roX2, GAPDH and PolII RNA. Y-axis corresponds to RNA levels in percentage (%).

(C) Confocal microscopy was performed on Schneider cells treated with MSL-1 dsRNA. For this purpose cells were immunostained for MSL-1, MSL-3, MOF, Nup153, Mtor or Z4. In addition, all cells were incubated with WGA (green) to visualize the nuclear envelope (+WGA).

Supplementary Figure S3