

## Condensin II regulates interphase chromatin organization through the Mrg-binding motif of Cap-H2.

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**Figure S1** Confirmation of guinea pig anti-Mrg15 antibody specificity. Lysates from Kc cells treated with non-targeting control (SK) or Mrg15 dsRNA immunoblotted for anti-Mrg15. Anti-KHC (Kinesin Heavy Chain) was used as a loading control.



**Figure S2** Quantification of percentage of co-localization between Cap-H2 and Mrg15, Pol IIo<sup>pSer5</sup>, or Pol IIo<sup>pSer2</sup> polytene immunostaining signals shown in Figures 1 and 2.



**Figure S3** Cap-H2 ChIP-seq peak regions validated by ChIP-qPCR. (A-D) Integrated Genome Viewer (IGV) screenshot of ChIP-seq peaks for Cap-H2 and Mrg15 in regions near *Esc* (A), *squ* (B), *Orc2* (C), and *RpL27* (D) validated by ChIP-qPCR in Figure 2B. Grey arrows indicate the region that was amplified by qPCR.



**Figure S4 Mrg15 is required for Cap-D3 localization at interband regions on polytene chromosomes.** (A-B) Salivary gland polytene chromosomes from wild type larvae stained for Cap-D3 (red), Mrg15 (green), and DNA (blue) as indicated. Scale bar, 10 μm.



**Figure S5 Mrg-binding motif is required for Cap-H2-mediated axial compaction.** (A-E) Kc cells transiently expressing EGFP (A), Cap-H2-EGFP (B), Cap-H2-MBM-EGFP (C), Cap-H2-ΔC23-EGFP (D), or Cap-H2-ΔC23-MBM-EGFP (E) labeled with FISH probes for two chromosome 2L loci, chromosome 2L probe 1 (2L1, red) and chromosome 2L probe 2 (2L2, green). Scale bar, 2.5 µm. (F) Pairwise distances were measured for 2L1-2L2 FISH probes (n = 60). \**p*<0.05, \*\**p*<0.01, two-tailed student's t test. P-values correspond to statistical significance relative to control, except where indicated by horizontal lines. Asterisks located above horizontal black line indicate significance between cells expressing Cap-H2-EGFP or Cap-H2-ΔC23-EGFP and the corresponding MBM mutant. Error bars, s.e.m.

## Table S1. Primers used for RNAi and qPCR.

Gene	Primer sequences
RpL49	5'-CTTGGGAAATCTGTTAGTTTTTAGCCAAA-3' 5'-TTACTGGAATCCTTTGGTTTATCTGCT-3'
Esc	5'-CCCGAGTTCCATCACAAGAT-3' 5'-CTGTGTATATCCCGCGTTGAA-3'
squ	5'- CCCGAAGTAAAACCACCAGA-3' 5'- ATTCTTCGTTCGGGGAATCT-3'
Orc2	5'-AGTCCATGGATCAGCCAAAG-3' 5'- GGAAATCGCTTTCATCTCCAG-3'
RpL27	5'-GCACGACATCAGCTTTGAGA-3' 5'-CCTCCTTGTAGACGGACTCG-3'

## File S1

## Raw data file

Available for download as an Excel file at http://www.g3journal.org/lookup/suppl/doi:10.1534/g3.115.016634/-/DC1