Supplemental material

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Provided online is one table in Excel. Table S1 provides information about the sequences of probes and primers used in the experiments.

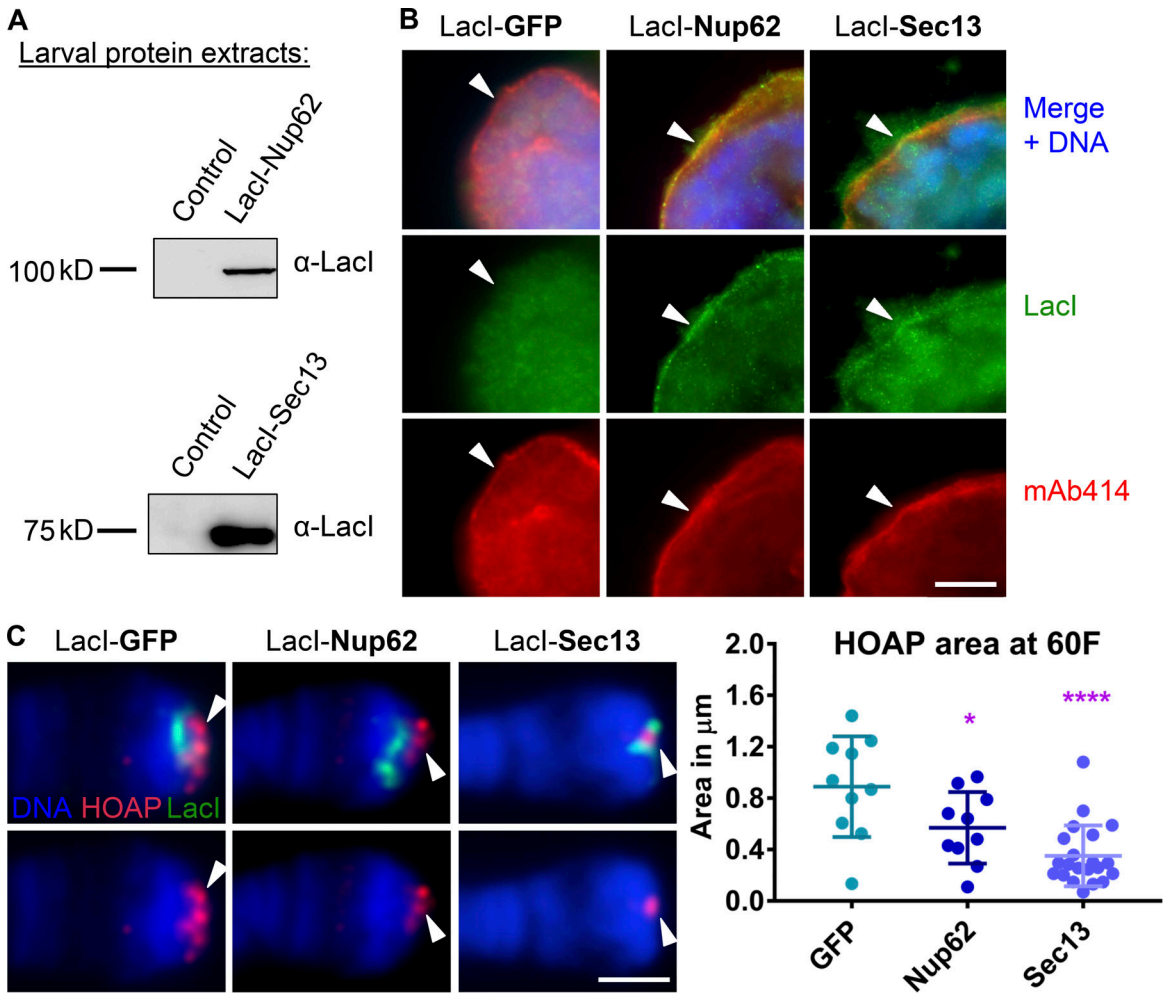

Figure S1. **Generated LacI-Nup fusion proteins localize properly in vivo and affect heterochromatin at the subtelomeric locus. (A)** Western blots of whole larval extracts of indicated control and transgenic animals (five each) stained with α-LacI antibody. **(B)** IF staining of semi-squashed salivary gland nuclei, staining LacI-fusion proteins with α-LacI and NPCs with mAb414, using widefield microscopy. The scale bar is 10 µm. Arrowheads correspond to locations of nuclear membrane, as marked by mAb414 staining, with or without corresponding signal from LacI-fusion proteins. **(C)** IF images of squashed polytene chromosomes displaying the subtelomeric *lacO* integration site at cytological location 60F bound by indicated LacI-fusion proteins and stained with Hoechst, α-LacI, and antibody against telomere capping protein HOAP, using widefield microscopy. Arrowheads indicate locations of existing or reduced HOAP adjacent to/at the LacI signal. The scale bar is 2 µm.The plot shows quantification measuring the area of the HOAP signal. Data are from one to two independent biological replicates from one experiment. GFP, n = 10; Nup62, n = 10; Sec13, n = 21. ****, $P < 0.0001$; *, $P < 0.05$. Error bars represent SDs.

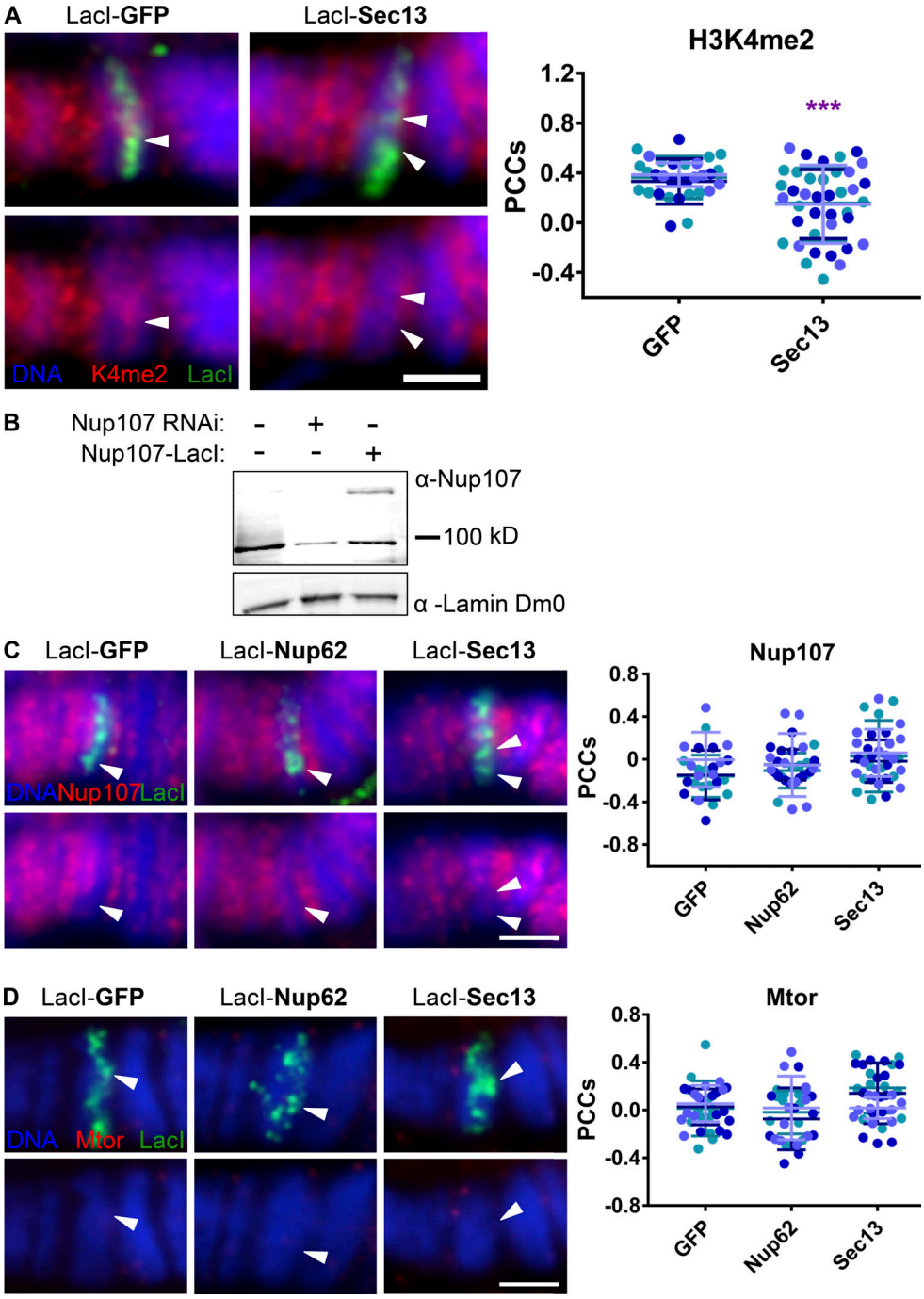

Figure S2. **Chromatin targeting of Sec13 results in lower histone density but not in recruitment of Nup107 or Mtor. (A)** Widefield IF images of LacI-fusion proteins targeted to the *lacO* integration site on squashed polytene chromosomes at cytological location 96C on chromosome 3. Staining was with Hoechst (blue) and antibodies against H3K4me2 (red) and GFP for control/myc (green). LacI-fusion protein expression was driven with second chromosome Nubbin-Gal4. The top row shows the overlay of all 3 channels, whereas the bottom row shows the overlay of blue and red only (here and in C and D). Arrowheads indicate locations of existing or depleted H3K4me2 under LacI signal. The scale bar is 2 µm. Quantification displays PCCs between red and green signal under LacI. Data are from three independent biological replicates (colored) from two independent experiments. GFP, $n = 34$; Sec13, $n = 39$. ***, $P < 0.001$. Error bars represent SDs. **(B)** Validation of the generated Nup107 antibody by Western blot of extracts from S2 cells, either depleted for Nup107 by RNAi or transfected with Nup107-LacI (carried in a cell expression vector), stained with Nup107 antibody or LaminDmO antibody as a loading control. **(C)** Widefield IF images of LacI-fusion proteins targeted to the *lacO* integration site on squashed polytene chromosomes at cytological location 96C on chromosome 3. Staining was with Hoechst (blue) and antibodies against Nup107 (red) and LacI (green). The scale bar is 2 µm. Quantification displays PCCs between red and green signal under LacI. Data are from three independent biological replicates (colored) from two independent experiments. GFP, $n = 26$; Nup62, $n = 31$; Sec13, $n = 34$. Error bars represent SDs. **(D)** Widefield IF images of LacI-fusion proteins targeted to the *lacO* integration site on squashed polytene chromosomes at cytological location 96C on chromosome 3. Staining was with Hoechst (blue) and antibodies against Mtor (red) and LacI (green). The scale bar is 2 µm. Quantification displays PCCs between red and green signal under LacI. Arrowheads indicate LacI signal. Data are from three independent biological replicates (colored) from two independent experiments. GFP, $n = 35$; Nup62, $n = 32$; Sec13, $n = 36$. Error bars represent SDs.

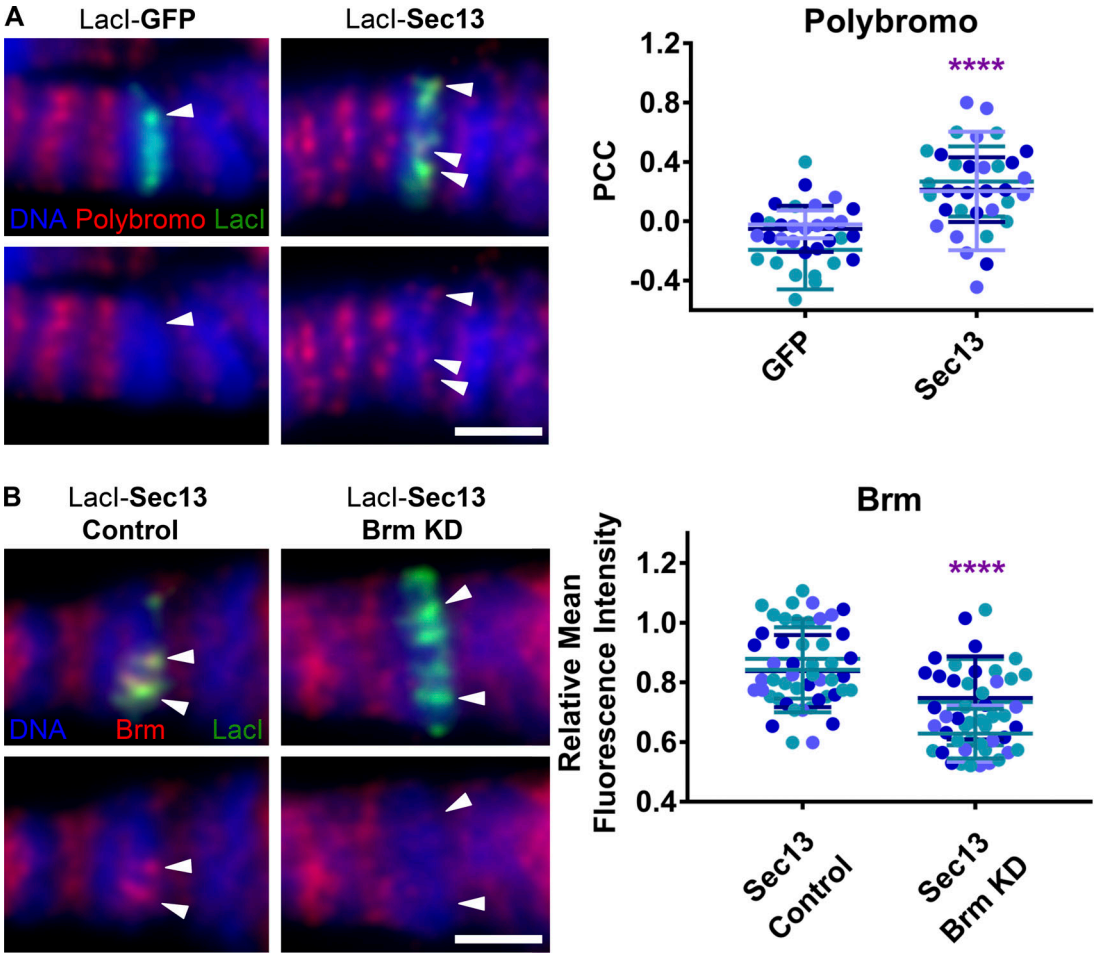

Figure S3. **Recruitment of PBAP components by Sec13-LacI. (A)** Widefield IF images of LacI-fusion proteins targeted to the *lacO* integration site on squashed polytene chromosomes at cytological location 96C on chromosome 3. Staining was with Hoechst (blue) and antibodies against polybromo (red) and GFP for control/myc (green). LacI-fusion protein expression was driven with second chromosome Nubbin-Gal4. The top row shows the overlay of all three channels, whereas the bottom row shows the overlay of blue and red only (here and in B). Arrowheads indicate locations of observed polybromo recruitment or lack thereof under LacI signal. The scale bar is 2 µm. Quantification displays PCCs between red and green signal under LacI. Data are from three independent biological replicates (colored) from two independent experiments. GFP, n = 44; Sec13, n = 45. ****, P < 0.0001. Error bars represent SDs. **(B)** Widefield IF images of LacI-Sec13 targeted to the *lacO* integration site on squashed polytene chromosomes at cytological location 96C on chromosome 3 under control conditions (flies crossed to w1118 WT stock) or Brm KD conditions (flies crossed to Brm RNAi stock BL35211). Staining was with Hoechst (blue) and antibodies against Brm (red) and myc (green) for LacI-Sec13 myc-tagged fusion protein. Arrowheads indicate locations of observed Brm recruitment or lack thereof under LacI signal. Quantification displays mean Brm fluorescence signal intensity at *lacO* relative to the nearby control band. Data are from three independent biological replicates (colored) from two independent experiments. GFP, n = 39; Sec13, n = 39. ****, P < 0.0001. Error bars represent SDs.

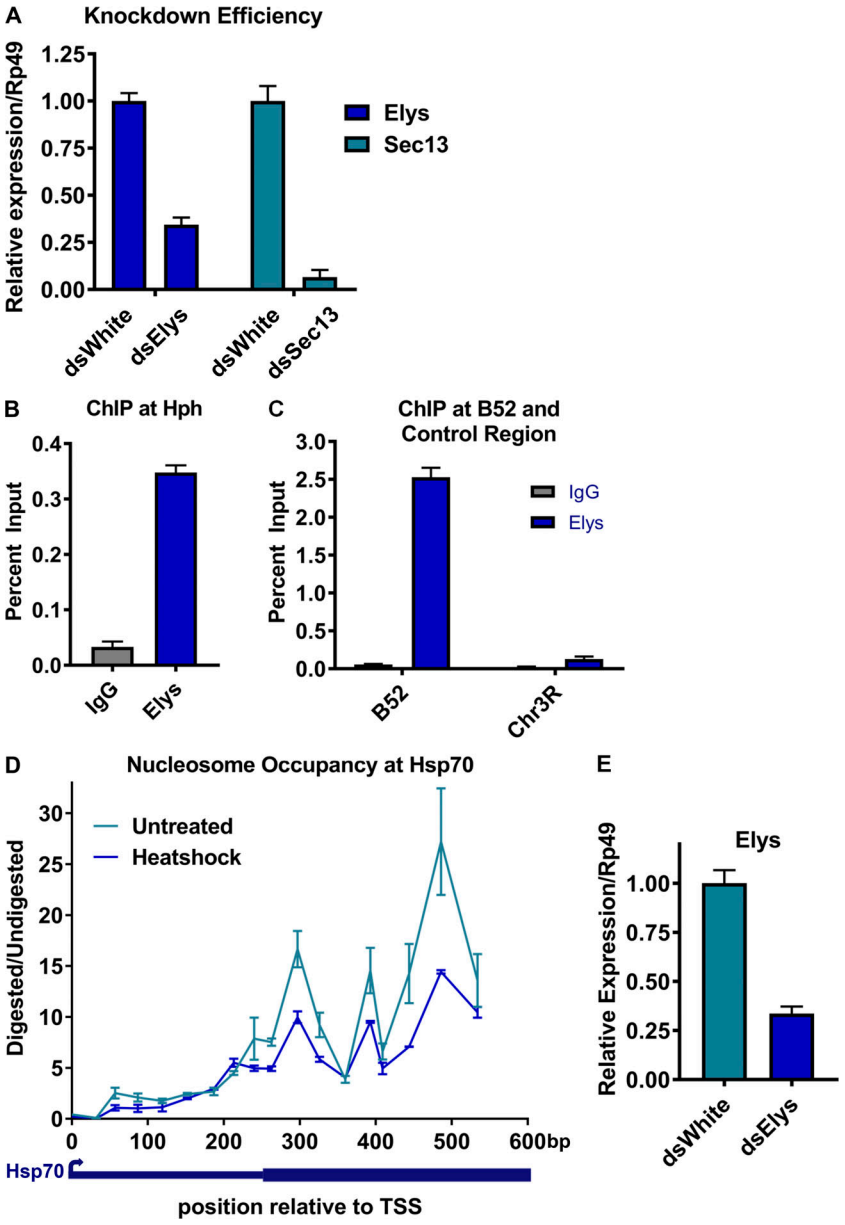

Figure S4. **Control experiments for testing the role of Elys in endogenous chromatin decondensation. (A)** qPCR validation of RNAi-mediated depletion of Elys and Sec13, relative to dsWhite control, in S2 cells for experiments performed in Fig. 6, B and C. Error bars represent SEMs from four biological replicates. **(B)** Graph showing percent input recovery of chromatin in ChIP-qPCR using Elys or control IgG antibodies to detect the level of binding of Elys at gene *Hph* TSS in *Drosophila* S2 cell culture. Error bars represent SEMs from two biological replicates. **(C)** Graph showing percent input recovery of chromatin in ChIP-qPCR using Elys or control IgG antibodies to detect the level of binding of Elys at gene *B52* TSS in *Drosophila* S2 cell culture or negative control region on Chr3R. Error bars represent SEMs from two biological replicates. **(D)** Graph displaying nucleosome occupancy levels along a region spanning the first ∼600 bp of *Hsp70Ab* with the TSS marked as bp "0." Nucleosome occupancy was measured by the ratio of digested to undigested chromatin (quantified by qPCR), retrieved following MNase digestion of genomic DNA from *Drosophila* S2 cultured cells under untreated or heat shock conditions. A schematic of corresponding regions of *Hsp70Ab* transcript is shown below the graph. Error bars represent SEMs from two biological replicates. **(E)** qPCR validation of RNAi-mediated depletion of Elys in S2 cells used for MNase-qPCR experiments in Fig. 7, A–D. Error bars represent SEMs from three biological replicates.