Supplemental material

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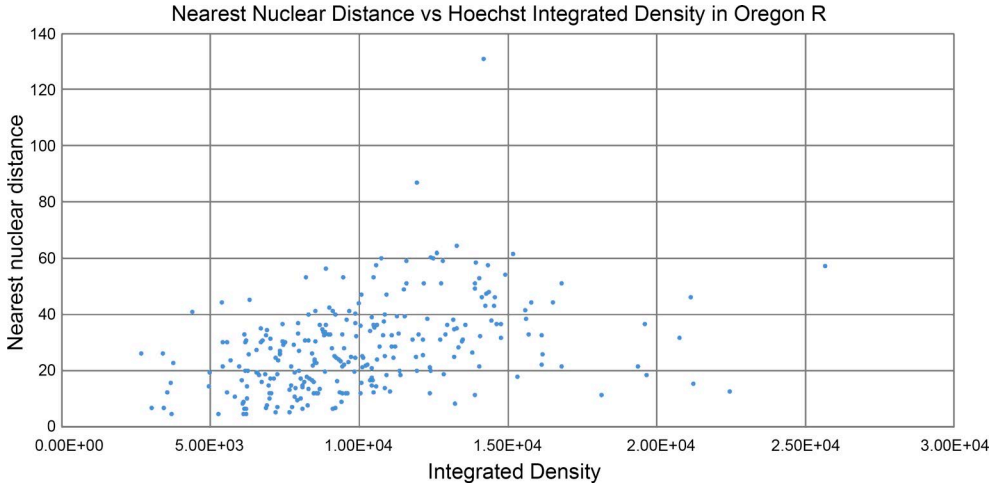

Figure S1. **Distance between nuclei does not correlate with DNA content in Oregon R myonuclei.** Quantification of Hoechst integrated density of WT strain Oregon R in which myonuclei are not evenly positioned. The y axis represents distances between the nearest myonuclei of muscle 7. The x axis represents Hoechst integrated density for each nucleus. The correlation between nuclear position and the amount of myonuclear DNA is not significant (Pearson correlation = 0.4, $P = 1.3 \times 10^{-12}$).

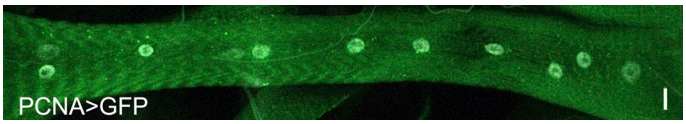

Figure S2. **PCNA-GFP, a direct target of E2F1, is expressed in larval myofibers.** Larval muscle 7 from flies expressing a construct of the promotor of PCNA fused to GFP, as a readout for E2F1 activity, labeled with anti-GFP (green) and Hoechst (white), indicating E2F1 activity in third-instar larval muscle 7. Bar, 10 µm.

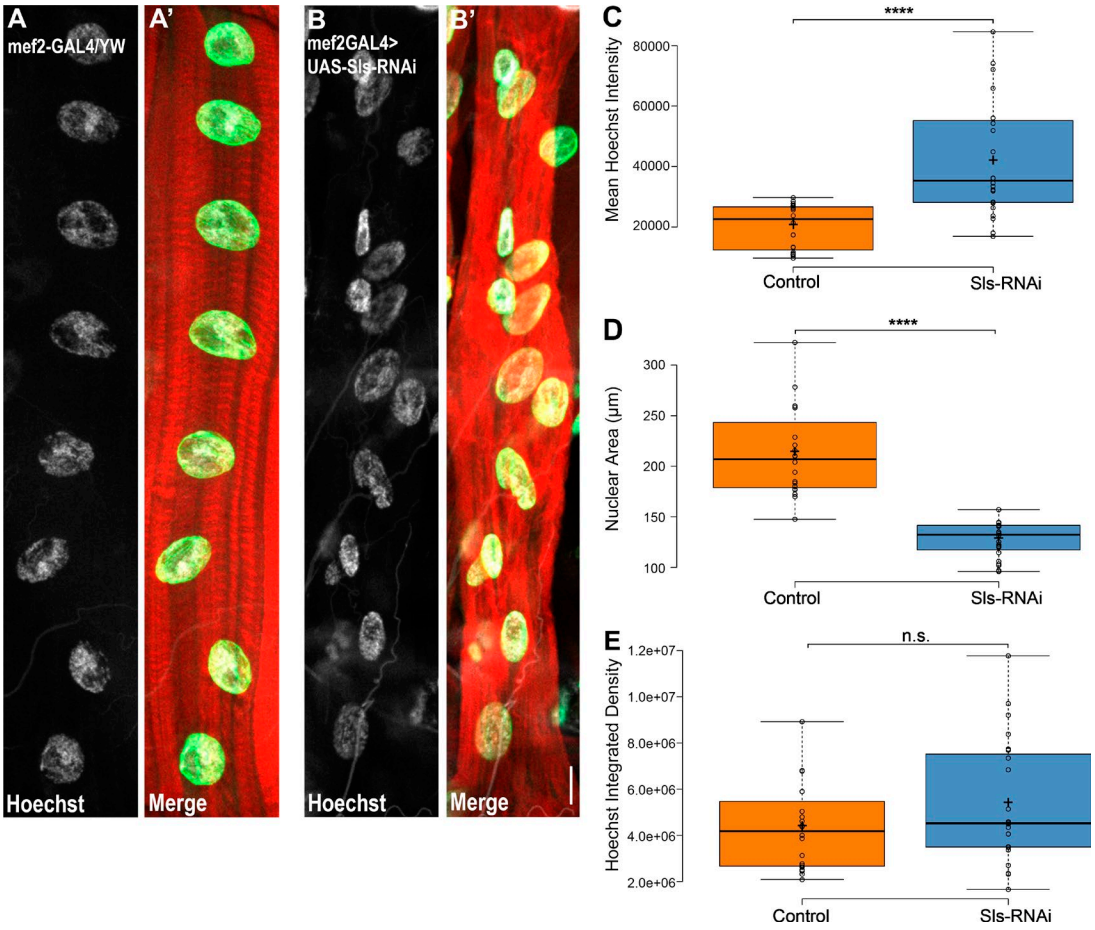

Figure S3. **Muscle-specific knockdown of D-Titin (Sls) reveals smaller nuclei with similar DNA content.** Representative confocal projection image of muscle 7 of control (A and A′) or muscle-specific Sls-knockdown (B and B′) labeled with Hoechst (A and B, white) and with lamin C (green) and phalloidin (red). A′ and B′ show their merged images. All images were taken at the same magnification. Representative scale bar in B′, 10 µm. Quantification of the mean Hoechst intensity per myofibers (C), nuclear area (D), and Hoechst integrated density (E) are shown. A significant difference between the groups is observed in the mean Hoechst intensity (*t* test, ****, $P = 2.14 \times 10^{-5}$). Sls knocked-down myonuclei are smaller than control (*t* test, ****, $P = 4.26 \times 10^{-11}$). The mean value of Hoechst integrated density per myofiber in muscle 7 did not differ significantly from control (P = 0.16). Whiskers extend to data points less than 1.5 interquartile ranges from the first and third quartiles.

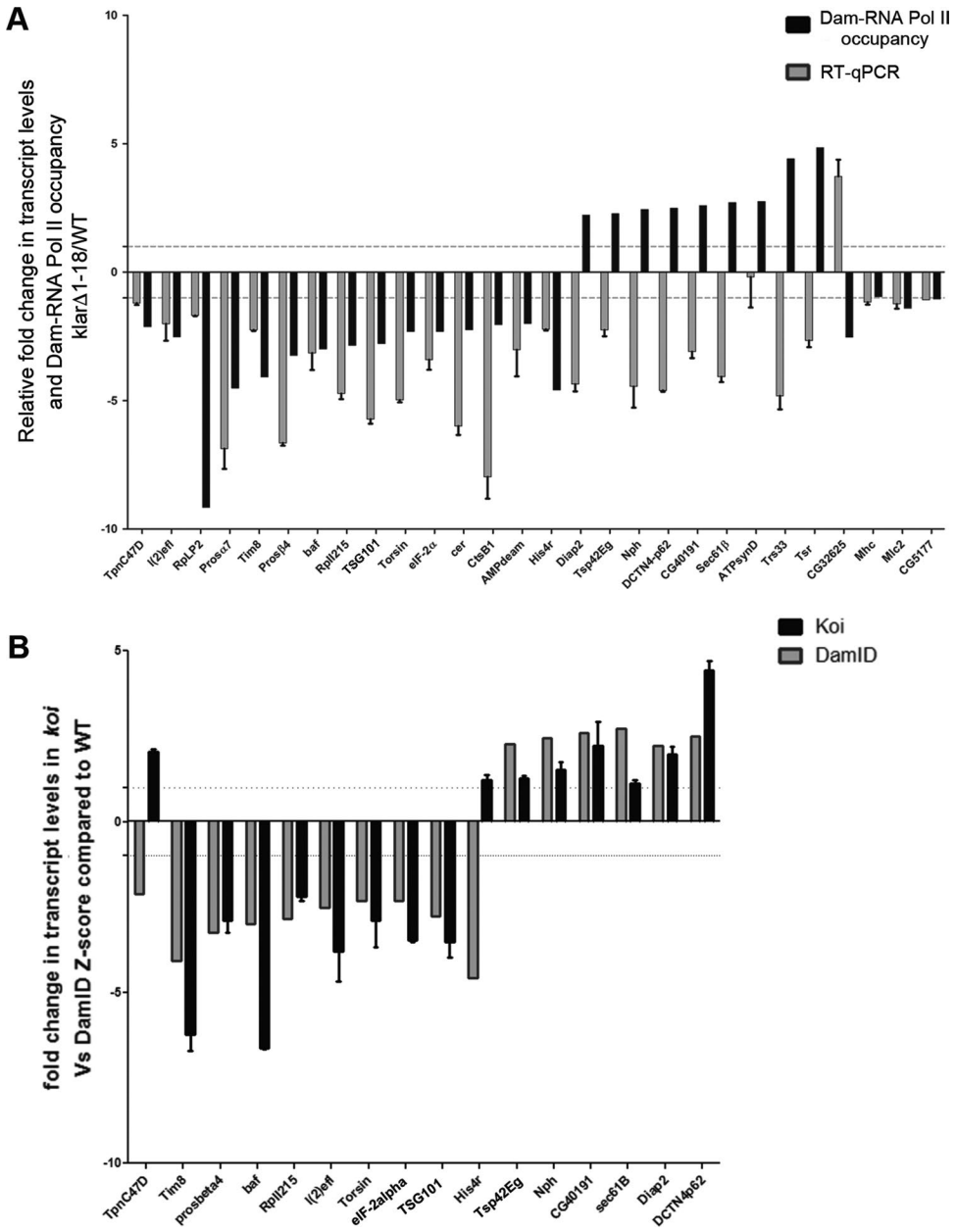

Figure S4. **Comparison of the muscle-specific Dam-Pol II occupancy gene hits and qPCR analysis in klar and in koi mutant dissected larvae. (A)** Real-time qPCR analysis of the mRNA levels extracted from dissected larvae preparations by using primers for 25 genes that exhibited statistically significant Z-score (≥2) in the Dam-Pol II binding in *klar* versus control muscles, as well as three genes that did not change. The qPCR analysis was performed in triplicate and normalized to housekeeping mRNA of GAPDH. The results represent two independent experiments. Values >1.5-fold are highly statistically significant (see Materials and methods). The Dam-Pol II z-score values for each gene are indicated by black bars. A table of the actual values is presented in Table S1. **(B)** Comparison between some of the genes identified in the Dam-Pol II binding profile in *klar* mutant, their mRNA levels measured by qPCR analysis in *klar,* and the SUN domain protein *koi,* compared with WT. Bars represent SEM. Most of the genes exhibit a similar tendency between the Dam-Pol II binding and the qPCR analysis in both LINC complex mutants.

Provided online are three supplemental tables and a supplemental text file. Table S1 shows a summary of the actual values obtained by the Dam-ID pipeline. Table S2 shows a summary of the major hits identified by the DamID pipeline. Table S3 shows the primer sequences used in this study. The supplemental data file shows a macro designed to allow the user to specify a range of conditions in which he or she can select desired nuclei, flatten the images, and obtain a range of different measurements for all nuclei in a given cell; the macro uses IJ1 Macro language.