Supplemental Inventory

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- Table S1. Related to Star Methods and Key Resource Table.

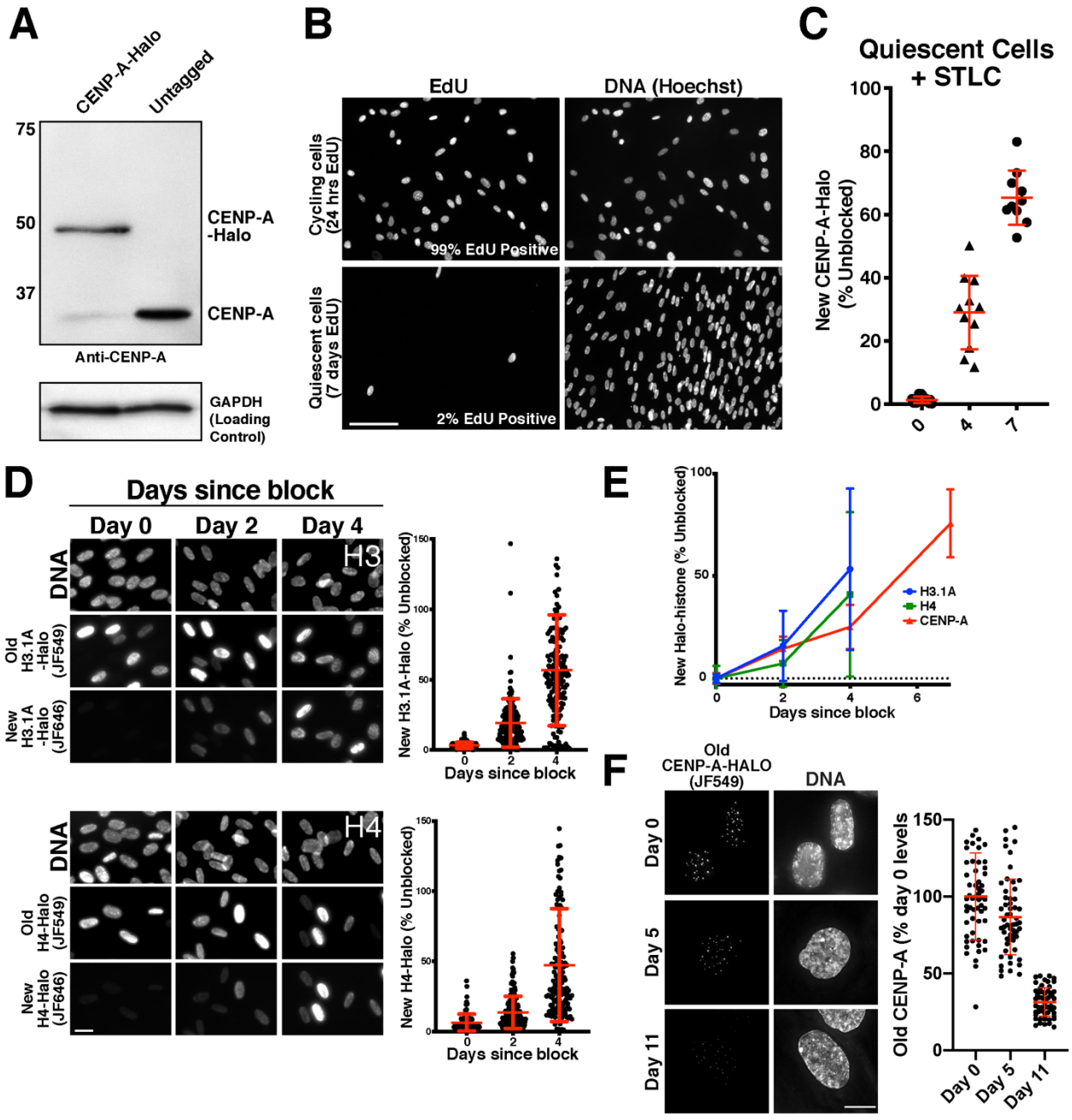

Figure S1. Analysis of CENP-A and other histone incorporation. Related to Figure 1. (A) Western blot showing CENP-A in either parental RPE-1, or RPE-1 cells with a HaloTag introduced at the CENP-A C-terminus at the endogenous locus using CRISPR/Cas9. The presence of a band for both endogenous CENP-A and CENP-A-Halo indicates that the cell line is tagged heterozygously. (B) DNA replication in cycling and quiescent cells, visualized by incorporation of the thymidine analog EdU. 2% of serum-starved cells undergo DNA replication during 1 week in the continuous presence of EdU. Scale bar = 100 μm (C) HaloTag CENP-A incorporation assay in quiescent cells as in Fig. 1E,F, but in cells treated continuously with the Kif11 inhibitor STLC to block any rare mitotic divisions. Graph shows quantification of new CENP-A-Halo fluorescent intensity

relative to pre-existing CENP-A. Points represent the sum of all centromeres of individual cells. Error bars represent the mean and standard deviation of at least 10 cells per timepoint. (D) HaloTag incorporation assay for histone H3.1A and H4. Scale bar = 20 μm. For H3.1A, >155 cells were quantified per time point. For H3, >109 cells were quantified per time point. (E) Quantification of new incorporation by HaloTag assay for H3.1A, and H4 as in (D), with CENP-A data reproduced from Fig. 1F and S1D added for comparison. Error bars represent the mean and standard deviation. (F) Live imaging of “old” JF646-labeled CENP-A-Halo loss over time. Quantifications are of raw JF646 signal relative to Day 0. Error bars represent mean and standard deviation of 55 cells per condition. Scale bar = 10 μm.

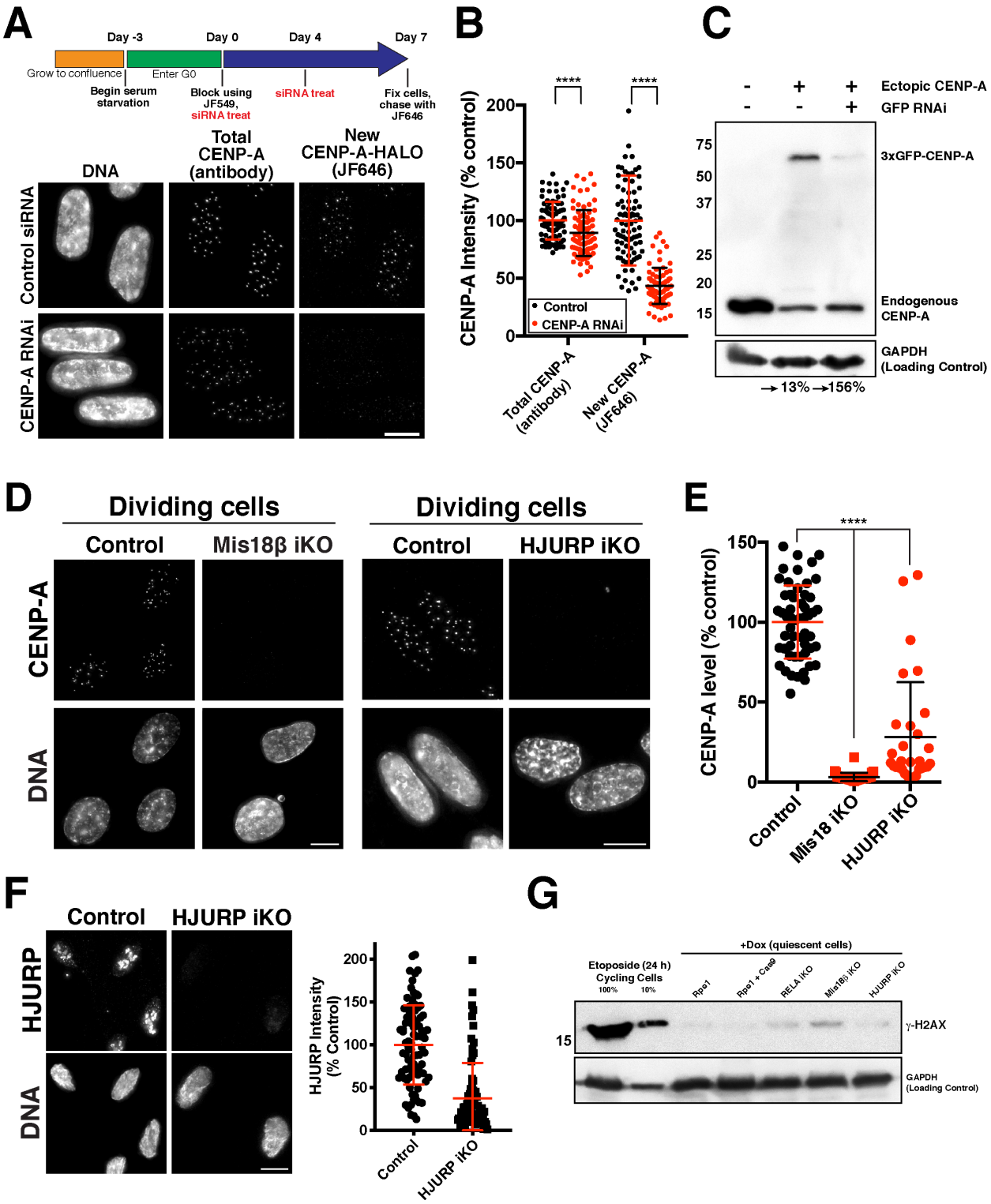

Figure S2. Characterization of HaloTag and inducible knockout cell lines. Related to Figure 2. (A) CENP-A-Halo incorporation requires new CENP-A synthesis. Quiescent CENP-A-Halo RPE-1 cells were treated with siRNAs targeting CENP-A as described in the schematic. Immunofluorescence for total CENP-A by antibody stain and new CENP-A are provided. Scale bar = 10 μm. (B) Quantification of total CENP-A and new CENP-A

fluorescence following control or CENP-A RNAi treatment, indicating a small but significant decrease in total CENP-A, and a greater than 2-fold reduction in new CENP-A, relative to control. Points represent the mean of all centromeres in a single cell. Error bars represent the mean and standard deviation (total CENP-A, control n = 89, RNAi n = 83; new CENP-A, control = 86, RNAi n = 78). ****p<0.0001 by Mann Whitney test. (C) Western blot for endogenous CENP-A in parental RPE-1 cells, RPE-1 cells stably expressing a 3xGFP-CENP-A transgene, and 3xGFP-CENP-A-expressing cells following GFP RNAi treatment. CENP-A levels were quantified in Adobe Photoshop and normalized to GAPDH for loading. (D) Immunofluorescence for total CENP-A in control, Mis18β inducible knockout, and HJURP inducible knockout following 7 days in proliferation. Scale bar = 10 μm. (E) Quantification of CENP-A fluorescence intensity in control and knockout cells. Points represent the mean of all centromeres in a single cell. Error bars represent the mean and standard deviation (control n = 54, Mis18β iKO n = 34, HJURP iKO n = 30). ****p<0.0001 by Mann Whitney test. (F) Immunofluorescence for HJURP in uninduced and induced HJURP knockout cells. Scale bar = 10 μm. Quantification of HJURP fluorescence intensity (control n = 85, iKO n = 76). ****p<0.0001 by Mann Whitney test. (G) Western blot for the DNA damage marker γH2AX in etoposide-treated cells (100% and 10% of total amount loaded), and indicated inducible knockout cell lines in quiescence.

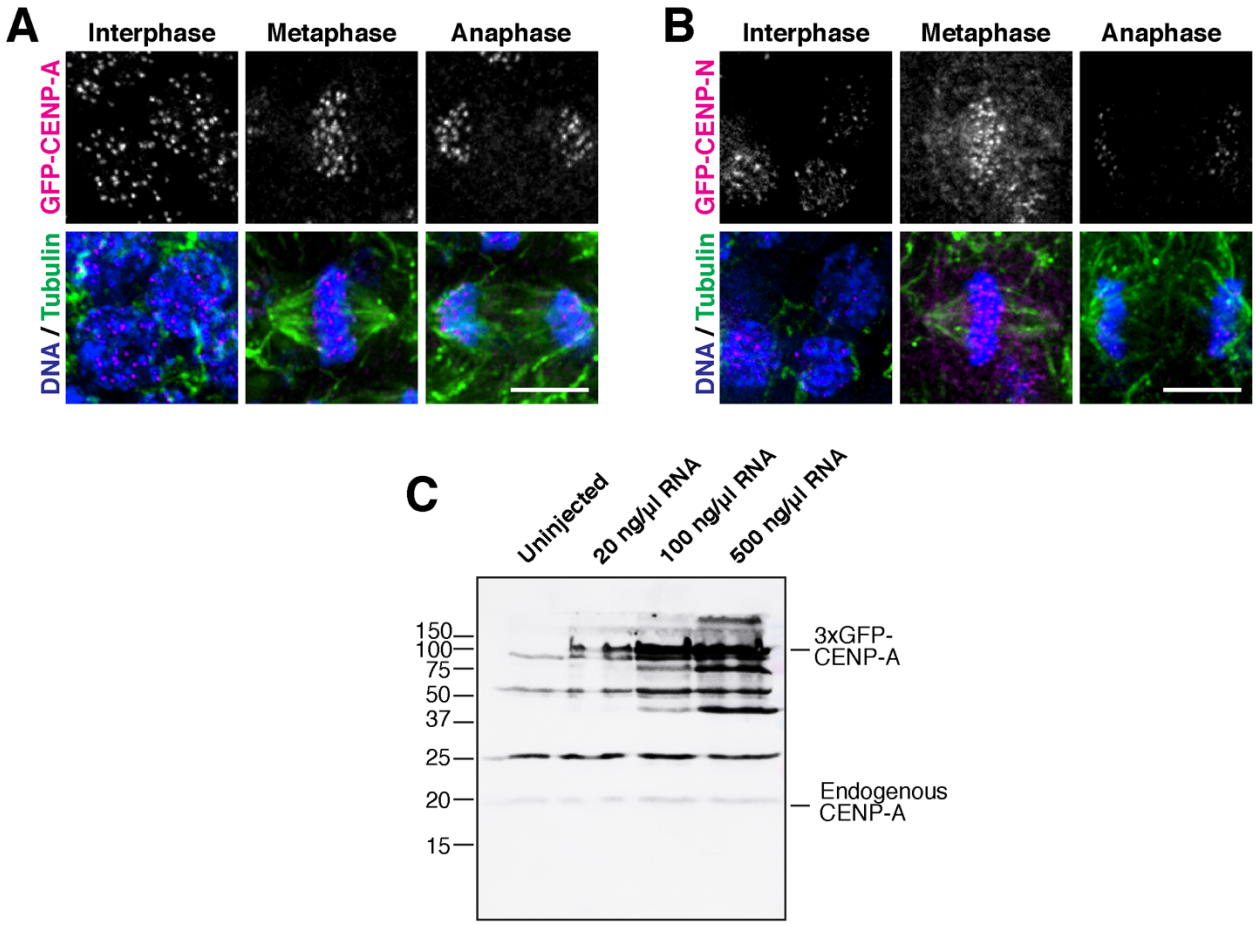

Figure S3. Localization of GFP fusions in oocytes and embryos. Related to Figure 3. (A,B) 3xGFP-CENP-A and CENP-N localize to centromeres in dividing ectodermal cells. Constructs were introduced into prophase I-arrested oocytes by mRNA injection and visualized following fertilization in 18-hour old embryos. Scale bars = 5 µm. (C) Western blot for CENP-A in arrested oocytes 18 hours after injection of the indicated concentrations of mRNA or uninjected controls.

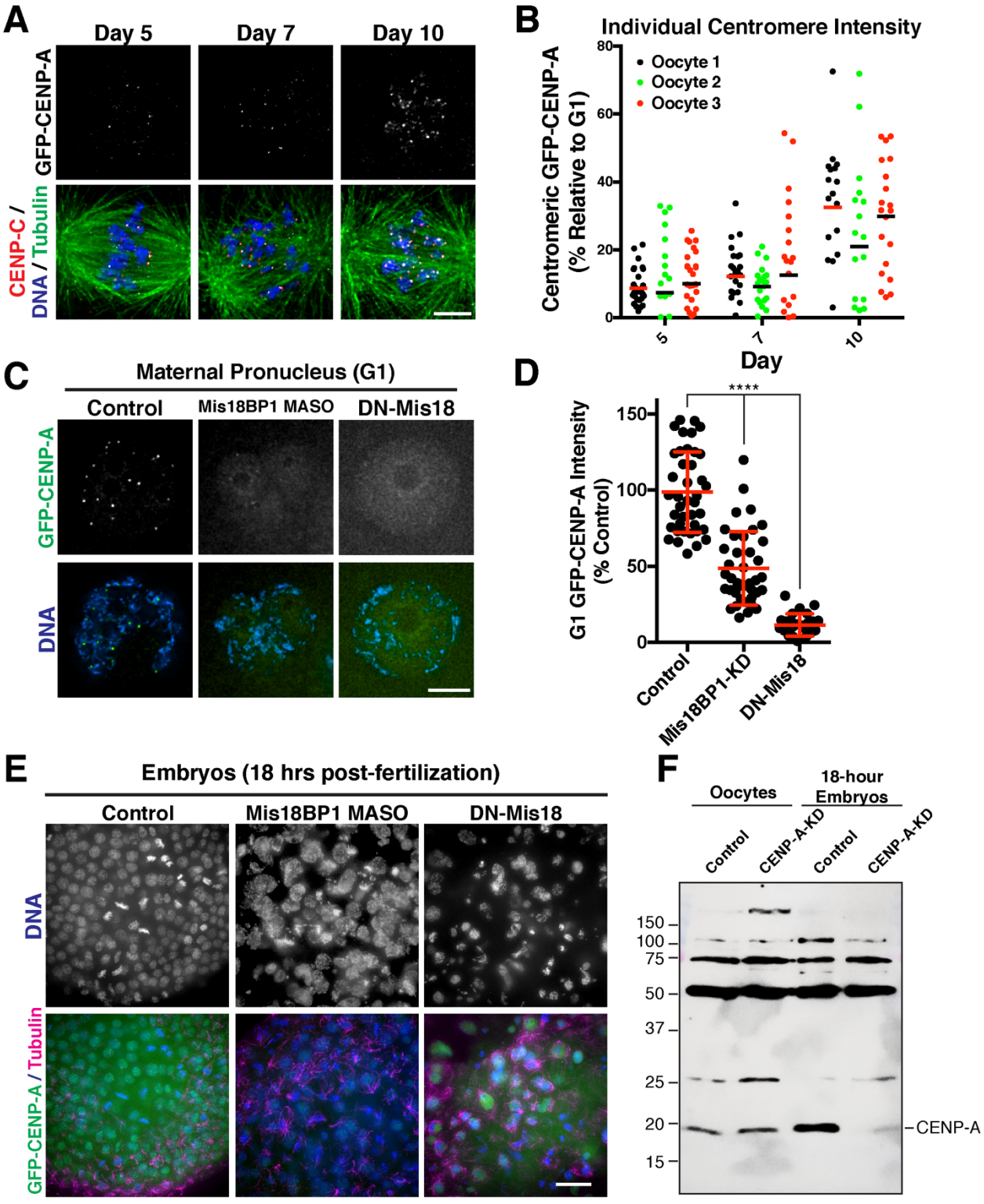

Figure S4. CENP-A protein is stable but gradually deposited in arrested oocytes. Related to Figure 4. (A) CENP-A displays stochastic incorporation at some centromeres over time. Immunofluorescence images showing prophase I deposited CENP-A visualized after meiotic entry for clarity. Images are scaled individually relative to a G1

control from the same day to normalize for injection behavior. Scale bar = 5 µm. (B) 3xGFP-CENP-A levels at individual centromeres (each point represents one centromere) from three representative oocytes from the average centromere data presented in Fig, 2b. (C) G1 replenishment of 3xGFP-CENP-A is reduced following Mis18BP1 knockdown or DN-Mis18 expression in starfish oocytes. Prophase arrested oocytes were injected with mRNA encoding 3xGFP-CENP-A either alone, or together with a Mis18BP1 morpholino or dominant negative Mis18 construct (DN-Mis18). Images are scaled individually to adjust for nuclear CENP-A background. Scale bar = 5 µm. (D) Quantification of 3xGFP-CENP-A levels at centromeres in the G1 female pronucleus (from c). Each point represents the average of all identifiable centromeres in one egg. Error bars represent the mean and standard deviation (control, 40 eggs; Mis18BP1 knockdown, 39 eggs; DN-Mis18, 26 eggs) ****$p \leq 0.0001$ by two-tailed unpaired T-test. (E) 3xGFP-CENP-A incorporation and chromosome segregation are disrupted in 18-hour embryos following Mis18BP1 knockdown or DN-Mis18 expression. Scale bar = 20 µm. (F) Endogenous CENP-A protein is stable in prophase I-arrested oocytes. Anti-CENP-A Western blot for oocytes 10 days after injection of control or CENP-A translation blocking morpholino to test CENP-A levels in prophase I-arrested oocytes, or following 18 hours of embryogenesis.

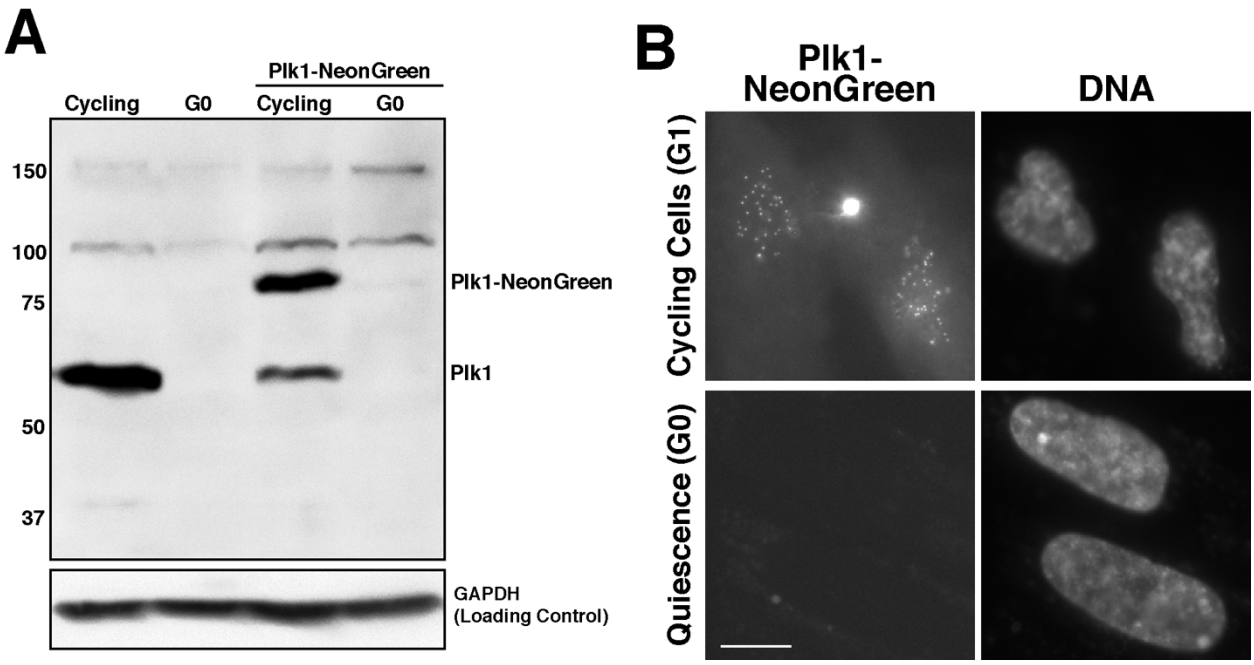

Figure S5. Plk1 levels are strongly reduced in quiescent human cells. Related to Figure 5. (A) Western blot for Plk1 in cycling and G0 quiescent RPE-1 cells, and RPE-1 cells in which Plk1 is endogenously tagged with NeonGreen. Both untagged and tagged Plk1 are strongly reduced in G0. (B) Plk1-NeonGreen (live cells) visualized in cycling and G0 quiescent cells. Scale bar = 10 μm.

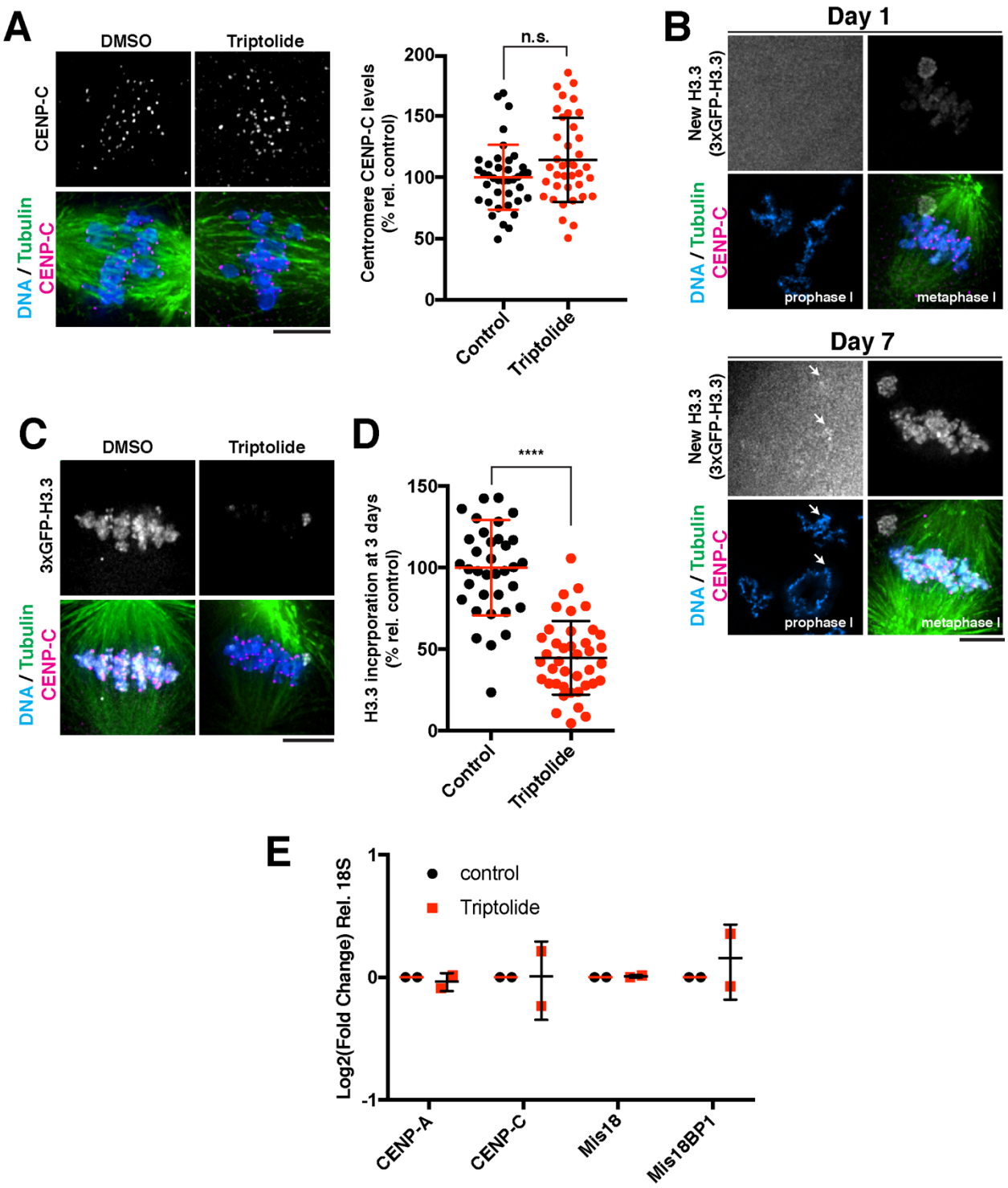

Figure S6. Role of transcription in H3.3 incorporation and centromere identity. Related to Figure 6. (A) Immunofluorescence for endogenous CENP-C following 9 days culture in DMSO or triptolide. Scale bar = 5 µm. Right, fluorescence intensity quantification for CENP-C at centromeres after 9 days of control or triptolide treatment. Error bars represent the mean and standard deviation (control n = 39, triptolide n = 38). Significance determined by Mann-Whitney test. (B) Immunofluorescence for new 3xGFP-

H3.3 incorporation at 1 or 7 days after expression. Metaphase, when the DNA is condensed and the soluble nuclear pool is dispersed, is shown for easier visualization. Metaphase images are scaled equivalently and prophase images are scaled individually. Arrows indicate the position of the chromosomes in prophase images. Scale bar = 5 µm. (C) New 3xGFP-H3.3 incorporation after 3 days of expression in the presence of DMSO or triptolide. Scale bar = 5 µm. (D) Fluorescence intensity quantification of new H3.3 incorporation for control and triptolide-treated oocytes. Each point represents a single oocyte. Error bars represent mean and standard deviation (control n = 36, triptolide n = 40). ****$p<0.0001$ by unpaired two-tailed t-test. (E) qPCR analysis showing expression level of genes involved in CENP-A deposition are unchanged following 9 days of culture with DMSO or triptolide. Each point represents the mean of 3 technical replicates for cDNA extracted from 200 oocytes from a genetically distinct female cultured in separate experiments.

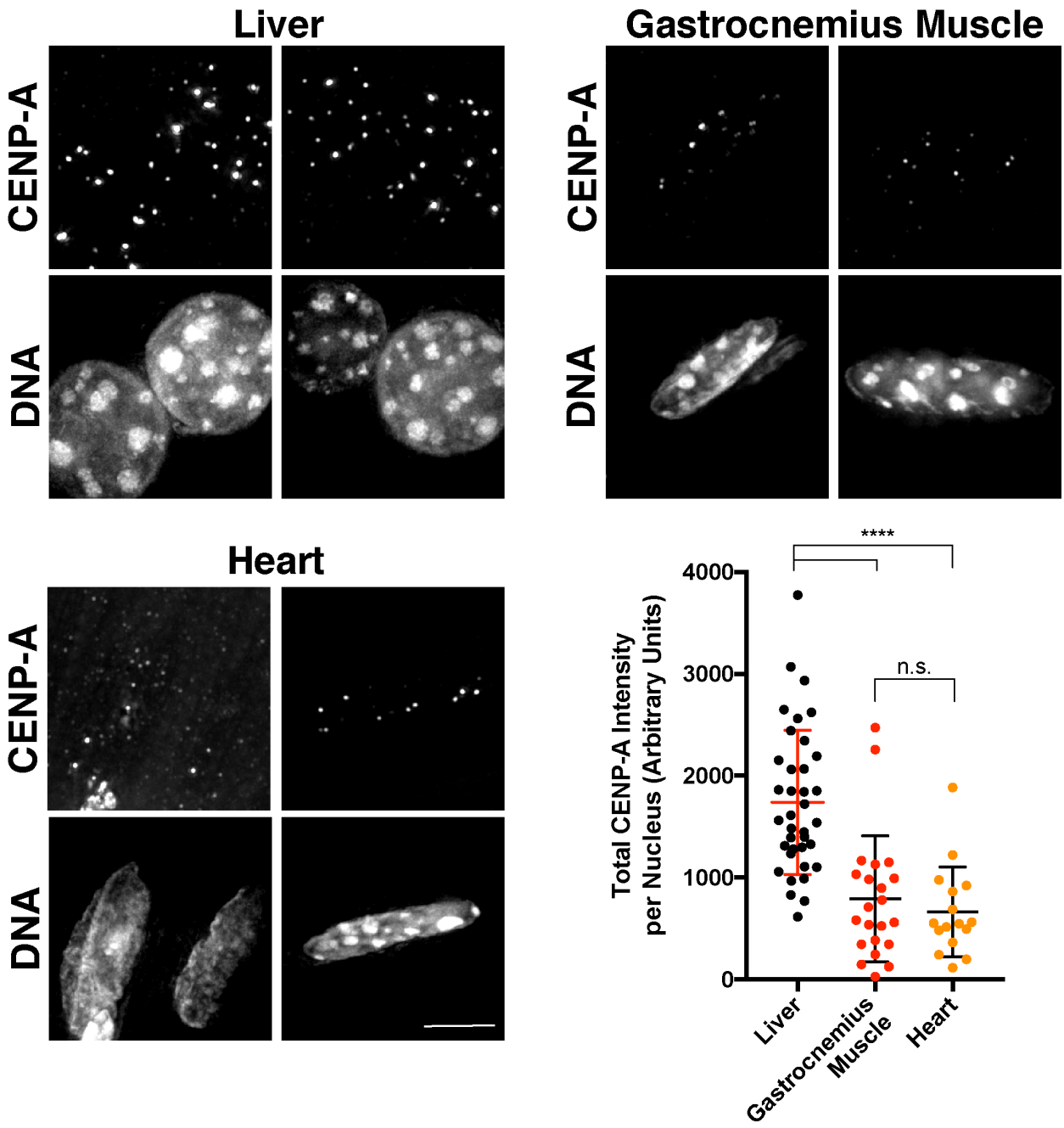

Figure S7. **CENP-A levels decline in post-mitotic tissues.** Related to Figure 7. Immunofluorescence for CENP-A in tissue sections from adult mouse liver, gastrocnemius muscle, and heart. Fluorescence intensity quantification for CENP-A at centromeres per cell. Each data point represents the sum of nuclear fluorescence for each cell. Error bars represent the mean and standard deviation (Liver, n=37 nuclei; Gastrocnemius muscle, n = 22; Heart, n=16). For analysis of CENP-A levels in liver hepatocytes, the ploidy of an individual nucleus was determined based on its diameter (see (Knouse et al., 2014)). The intensity of tetraploid nuclei was divided by 2 to compare to the diploid state in muscle cells. **** $p < 0.0001$.

Oligo Sequence 5'→3'
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Table S1. Stellaris ASAT RNA FISH probe sequences. Related to Star Methods and Key Resources Table.