

## Supplemental Figure Legends

### **Figure S1. EGFP-AID-tagged CENP-A is degraded within 1 hr of IAA treatment; Related to Figure 2.**

**(A)** Immunoblot of whole cell lysates from parental DLD-1 TIR1 and CENP-A<sup>WT</sup> cell lines. The CENP-A<sup>WT</sup> cell line was treated with 500  $\mu$ M IAA for 1, 6, or 24 hr to degrade EGFP-AID-tagged CENP-A. The blot was probed with ACA (anti-centromere antibody). CENP-B is shown in all samples as a loading control.

**(B)** Representative image of TMR-*Star*-labeled SNAP-tagged CENP-A<sup>WT</sup> cells immediately after quench. TMR-*Star* is not detected, indicating that the quench is complete. Scale bar: 10  $\mu$ m.

### **Figure S2. LacI-fused versions of CENP-A containing mutations at Ser68 or Lys124 recruit CENP-C and CENP-T to the LacO array to the same extent as LacI-fused wild type CENP-A and are expressed in cells to similar levels; Related to Figure 3.**

**(A,C)** Quantitation of the CENP-C intensity (A) and CENP-T intensity (C) at the LacO array normalized to the mean intensity at endogenous centromeres for the indicated HA-LacI-fused histone.

**(B,D)** Representative immunofluorescence images of CENP-C (B) and CENP-T (D) recruitment to the LacO array by the indicated HA-LacI-fused histone.

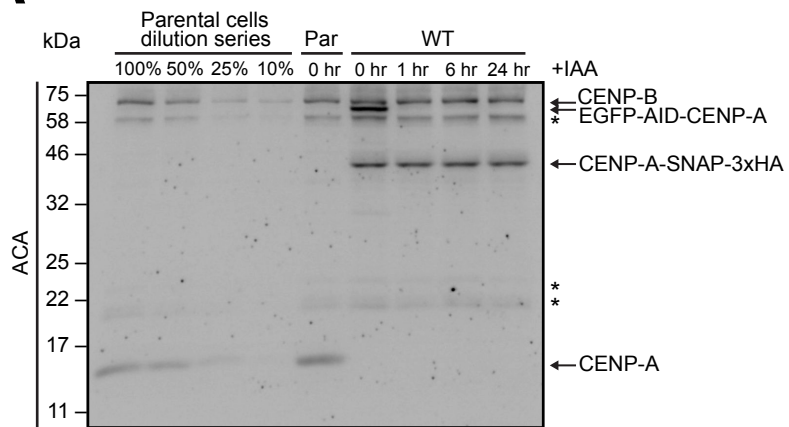
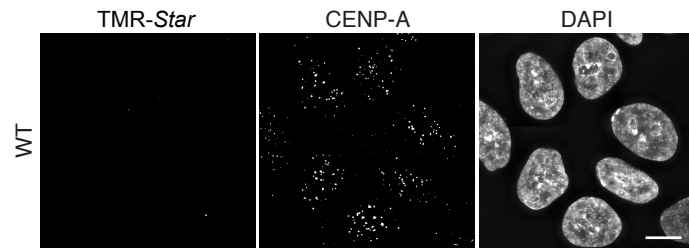
**(E)** Quantitation of total nuclear HA intensity in cells in which CENP-C and CENP-T recruitment to the LacO array was assessed (Panels A-D), with the mean intensity of HA-LacI-CENP-A-transfected cells set to 1. Because transient transfections often result in variation in expression levels from cell-to-cell, we wanted to ensure that the cells that we analyzed in our LacO/LacI tethering assays had similar expression levels between the HA-LacI-fused histones. Therefore, we quantified the total

nuclear HA intensity within all cells and found that all HA-LacI-fused constructs had similar expression levels.

**(F)** Quantification of total nuclear CENP-A intensity in cells that had been transfected with HA-LacI-CENP-A compared to untransfected cells, with the mean intensity measured in untransfected cells set to 1. Transfected cells have ~0.35x more CENP-A than untransfected cells, indicating a moderate increase in total CENP-A levels.

**(G)** Quantification of total nuclear HA intensity in cells that had been transfected with HA-LacI-CENP-A compared to untransfected cells. Using the same cells in which total CENP-A intensity was measured (Panel F), total HA intensity was also assessed, and the mean intensity measured in untransfected cells was set to 0.

**(H)** Representative image showing the increase in CENP-A intensity at centromeres upon transfection with HA-LacI-CENP-A. Error bars show SEM. An asterisk denotes significant differences between the means of two data sets (\*,  $p < 0.05$ ), with others marked as not significant (n.s.). Scale bar: 5  $\mu\text{m}$ .

**A****B**

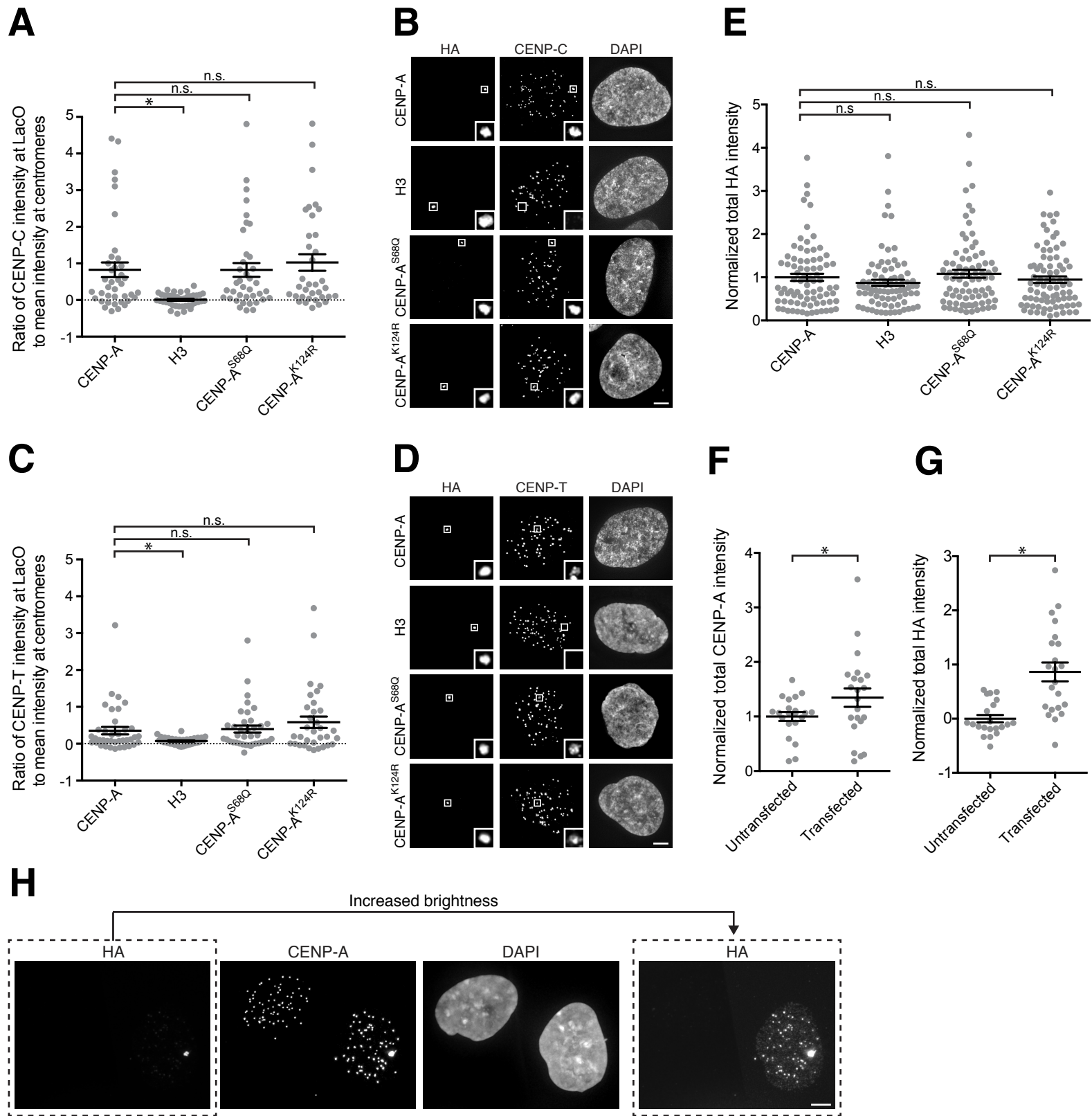


Figure S2