Logsdon et al., http://www.jcb.org/cgi/content/full/jcb.201412011/DC1

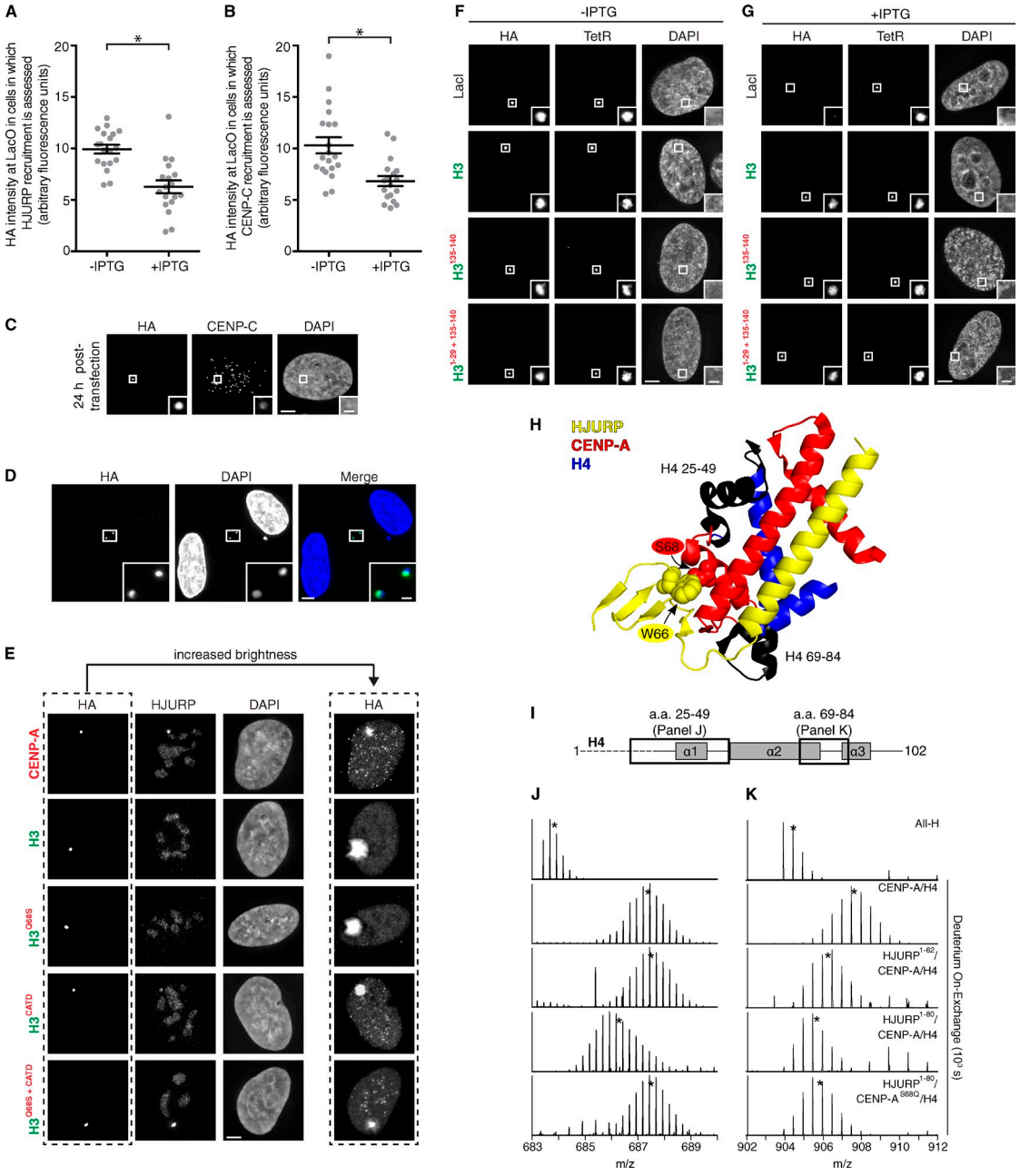

Figure S1. **A significant subset of LacI-tagged chimeras remain at the LacO array upon IPTG treatment while LacI alone is removed, low levels of CENP-C are recruited to the LacO array by LacI–CENP-A at 24 h, missegregation of the LacO-containing chromosome begins to occur at 48 h, LacI-fused H3/CENP-A chimeras containing the CATD localize to endogenous centromeres, and Ser68 of CENP-A transmits stability to distant helices within histone H4.** (A and B) Quantitation of HA-LacI–CENP-A intensity at the LacO array, ±IPTG treatment, in cells in which HJURP (A; Fig. 1, B and C) and CENP-C (B; Fig. 1, D and E) levels are assessed. Error bars show SEM. (C) Rare example of a cell in which low levels of CENP-C are recruited to the LacO array 24 h after transfection of LacI–CENP-A in U2OS-LacO cells. (D) Example of a cell in which missegregation of the LacO array has occurred at 48 h after transfection of LacI–CENP-A in U2OS-LacO cells. (E) Increased brightness of the HA channel from Fig. 1 J, showing that CATD-containing chimeras localize to endogenous centromeres in addition to the dominant localization at the LacO array. (F and G) Representative images of HA-LacI, HA-LacI-H3, or HA-LacI-H3/CENP-A chimeras at the LacO array in U2OS-LacO cells, ±IPTG treatment. TetR, tetracycline receptor. (H) Structure of the HJURP–CENP-A–H4 complex (PDB accession no. 3R45). H4 positions corresponding to the peptides shown in I–K (aa 25–49 and aa 69–84) are in black. (I) Diagram of H4 secondary structure with black boxes corresponding to aa 25–49 and aa 69–84. Peptides of these regions are shown in J and K. (J and K) Raw HX–mass spectrometry data for the H4 peptide corresponding to aa 25–49 (J) and the H4 peptide corresponding to aa 69–84 (K) for the indicated complexes. Data for the CENP-A–H4 complex and HJURP$^{1–62}$–CENP-A–H4 are reproduced from Bassett et al. (2012). Data for the 10^3 s time point are shown because the aa 25–49 peptide is fully exchanged at later time points. The S68Q mutation in CENP-A completely abrogates the protection from HX of aa 25–49 of H4, as seen with HJURP$^{1–80}$ bound to wild-type CENP-A (J). However, the S68Q mutation does not alter binding of HJURP to CENP-A–H4 because of the identical protection observed at other locations (see K for an example peptide of aa 69–84 of H4). m/z, mass per charge. *, $P < 0.05$. Insets show magnification of the boxed regions. Bars: (main images) 5 µm; (insets) 1 µm.

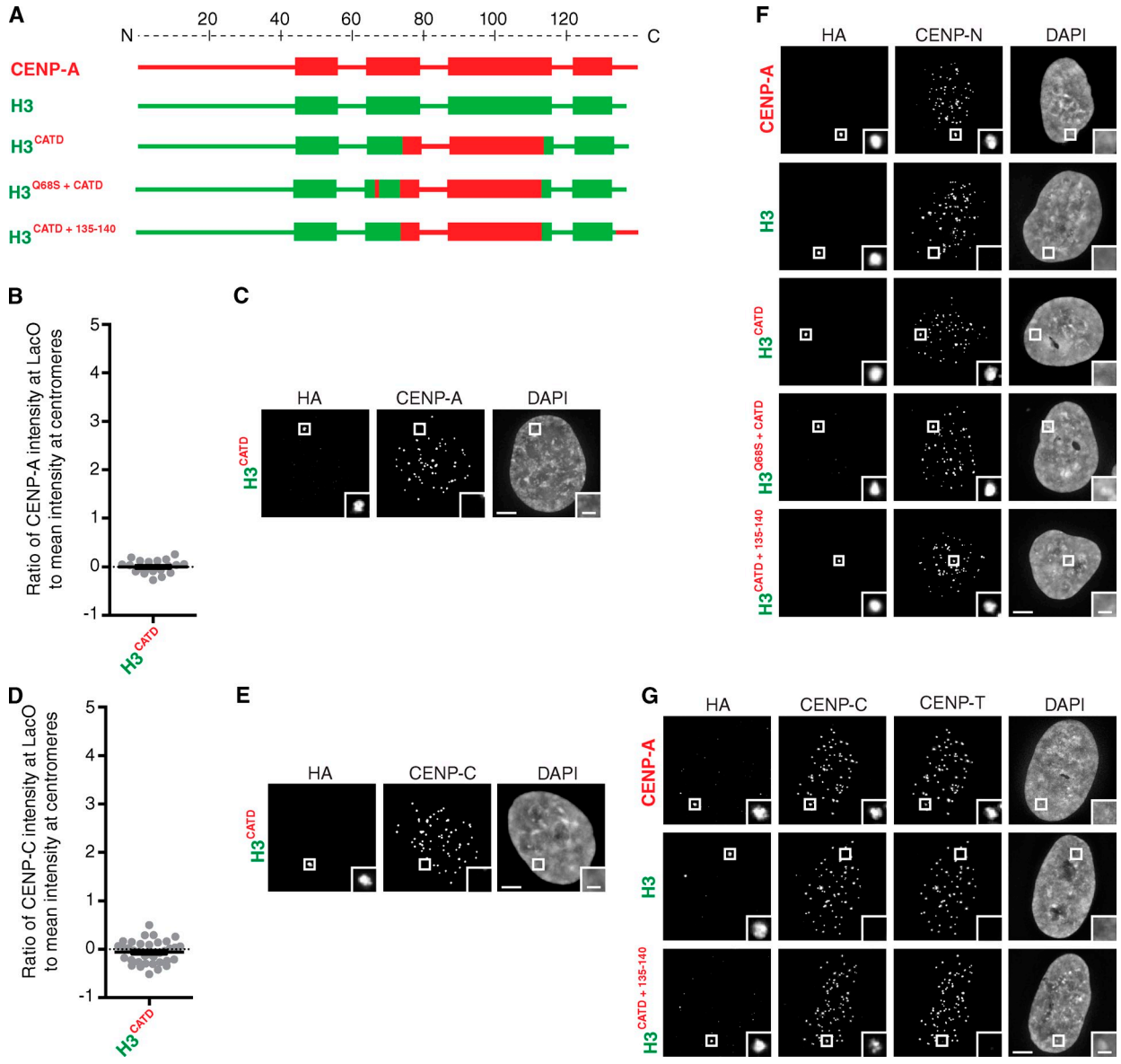

Figure S2. **LacI-H3^{CATD} does not recruit detectable levels of CENP-A or CENP-C to the LacO array, CATD-containing constructs recruit CENP-N to the LacO array, and LacI-H3$^{CATD+135–140}$ recruits CENP-C but not CENP-T to the LacO array.** (A) Diagram of the H3/CENP-A chimeras targeted to the LacO array in U2OS-LacO cells. (B and D) Quantitation of CENP-A (B) and CENP-C (D) intensity at the LacO array normalized to the mean intensity at endogenous centromeres for HA-LacI-H3^{CATD}. Error bars show SEM. (C and E) Representative images of CENP-A (C) and CENP-C (E) recruitment to the LacO array by HA-LacI-H3^{CATD}. (F) Representative images of CENP-N recruitment to the LacO array by the indicated chimeric histones fused to LacI and an HA tag. (G) Representative images of CENP-C and CENP-T recruitment to the same LacO array by the indicated chimeric histones fused to LacI and an HA tag. Insets show magnification of the boxed regions. Bars: (main images) 5 µm; (insets) 1 µm.

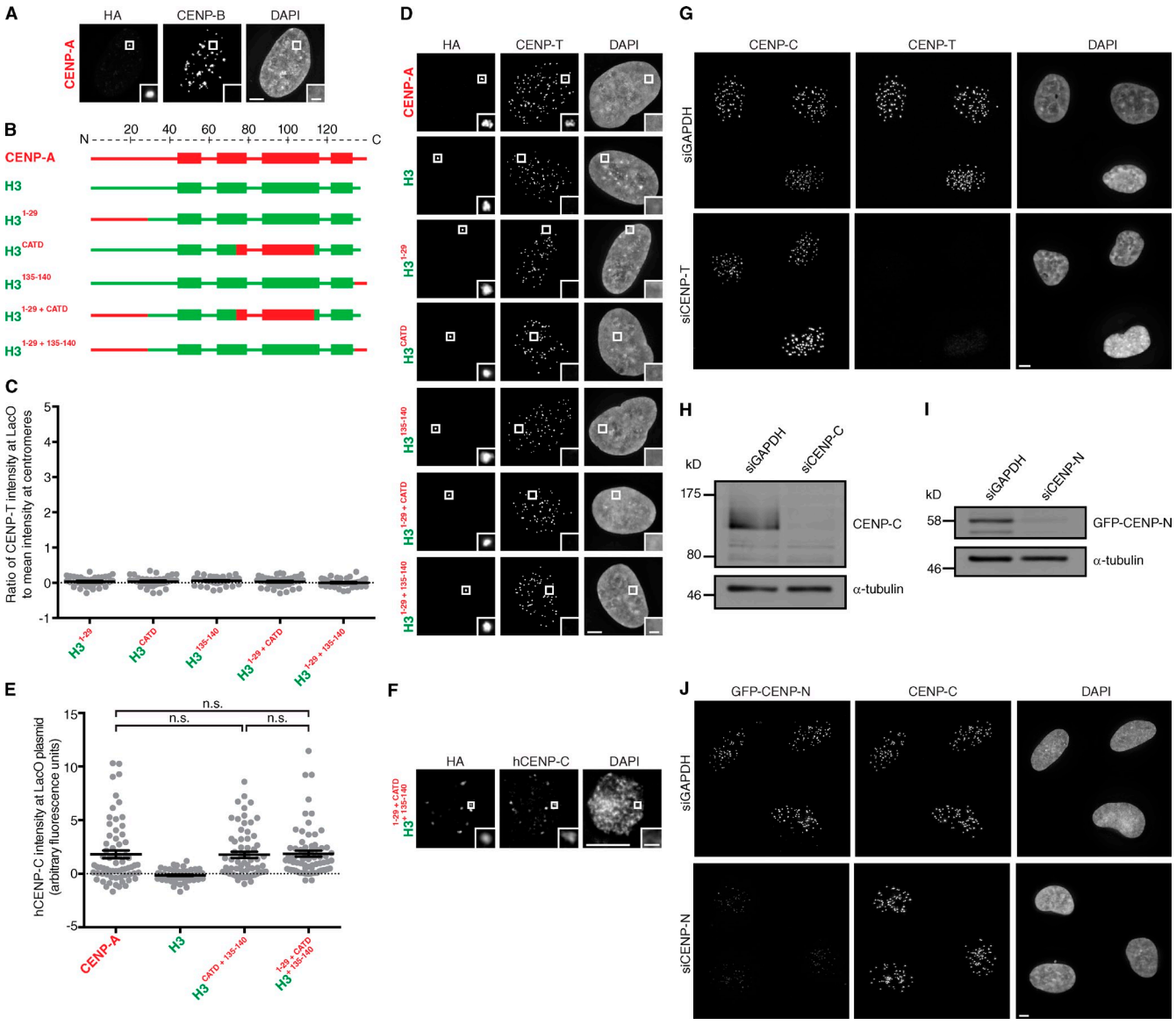

Figure S3. **CENP-B is not recruited to the LacO array by LacI–CENP-A, LacI-tagged chimeric histones containing combinations of the CATD and N- and C-terminal tails of CENP-A do not recruit CENP-T to the LacO array, LacI-H3$^{1–29+CATD+135–140}$ does not enhance CENP-C recruitment to the LacO-containing plasmid in *Drosophila* S2 cells, and CENP-T, CENP-C, and CENP-N are depleted after siRNA treatment.** (A) Representative image of CENP-B recruitment to the LacO array by HA-LacI–CENP-A. (B) Diagram of the H3/CENP-A chimeric histones targeted to the LacO array in U2OS-LacO cells and assessed for recruitment of CENP-T. (C) Quantitation of CENP-T intensity at the LacO array normalized to the mean intensity at endogenous centromeres for the indicated chimeric histones fused to LacI and an HA tag. (D) Representative images of CENP-T recruitment to the LacO array by the indicated chimeric histones fused to LacI and an HA tag. (E) Quantitation of hCENP-C intensity at the LacO-containing plasmids targeted by HA-LacI-H3/CENP-A chimeras in *Drosophila* S2 cells. Data points for HA-LacI–tagged CENP-A, H3, and H3$^{CATD+135–140}$ are reproduced from Fig. 3. Error bars show SEM. (F) Representative image of hCENP-C recruitment to LacO-containing plasmids by HA-LacI-H3$^{1–29+CATD+135–140}$ in *Drosophila* S2 cells. (G) Representative images of U2OS-LacO cells treated with GAPDH or CENP-T siRNA. CENP-T levels are reduced to 8.1 ± 2.7% in cells treated with CENP-T siRNA relative to cells treated with GAPDH siRNA. (H) Immunoblots with anti–CENP-C or anti–α-tubulin of whole cell lysates from U2OS-LacO cells after treatment with GAPDH or CENP-T siRNA. (I) Immunoblots with anti-GFP or anti–α-tubulin of whole cell lysates from U2OS-LacO cells stably expressing GFP-tagged CENP-N after treatment with GAPDH or CENP-N siRNA. (J) Representative images of U2OS-LacO cells stably expressing GFP-tagged CENP-N after treatment with GAPDH or CENP-N siRNA. Insets show magnification of the boxed regions. Bars: (main images) 5 µm; (insets, except F) 1 µm; (F, inset) 0.5 µm.

Reference

Bassett, E.A., J. DeNizio, M.C. Barnhart-Dailey, T. Panchenko, N. Sekulic, D.J. Rogers, D.R. Foltz, and B.E. Black. 2012. HJURP uses distinct CENP-A surfaces to recognize and to stabilize CENP-A/histone H4 for centromere assembly. *Dev. Cell*. 22:749–762. http://dx.doi.org/10.1016/j.devcel.2012.02.001