

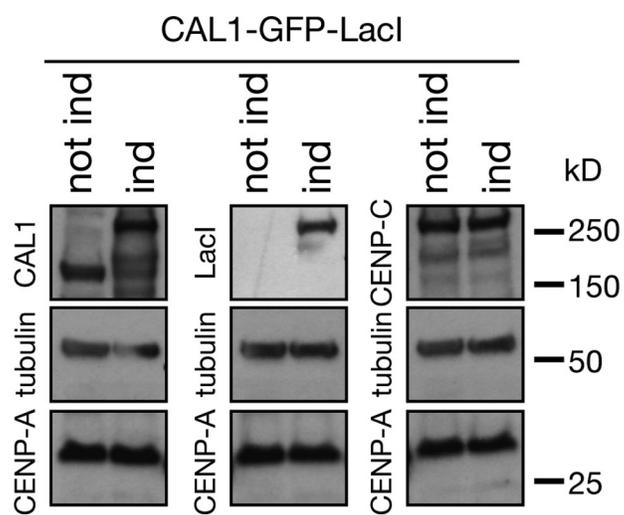
Chen et al., <http://www.jcb.org/cgi/content/full/jcb.201305036/DC1>

Figure S1. **Western blot to detect CAL1-GFP-LacI expression (detected with anti-CAL1 and anti-LacI antibodies) in induced (ind) and uninduced (not ind) cells.** While CENP-A and CENP-C total protein remained unchanged, CAL1-GFP-LacI (top band) levels increase upon induction and endogenous CAL1 (bottom band) levels decreased. Western blot with anti-tubulin is shown as a loading control.

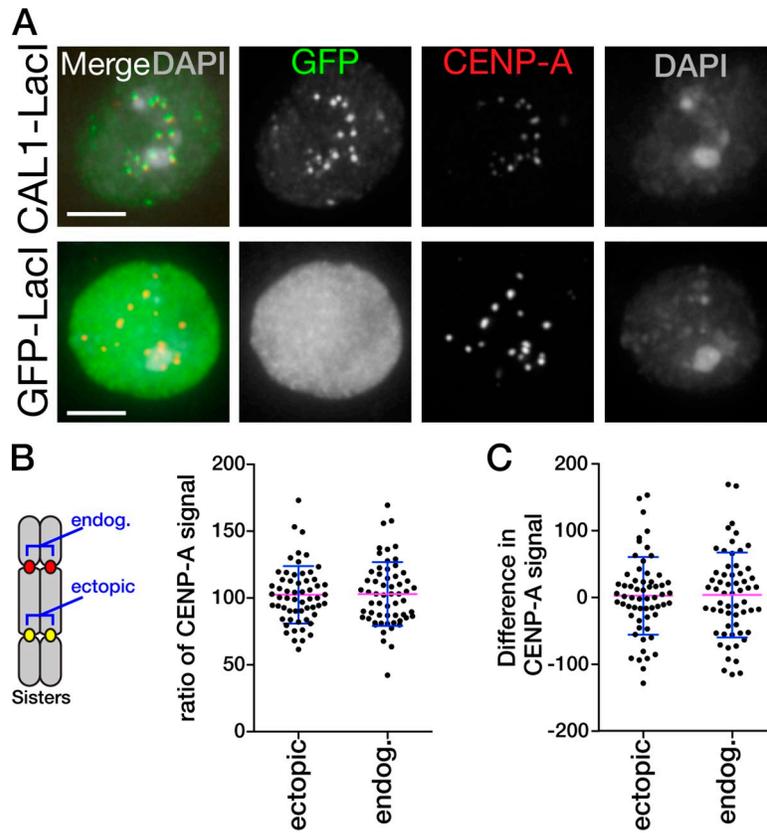


Figure S2. **Localization of CAL1-GFP-LacI and GFP-LacI in interphase and measurements of CENP-A levels at endogenous and ectopic centromeres.** (A) IF of interphase S2 cells showing that although CAL1-GFP-LacI colocalized with CENP-A, GFP-LacI did not (both visualized with anti-GFP antibodies in green). CENP-A is shown in red and DAPI in gray. Bar, 5 μ m. Scatter dot plots showing (B) CENP-A intensity ratios and (C) CENP-A intensity difference between pairs of endogenous (red circles in diagram) and ectopic (yellow circles in diagram) centromeres from sister chromatids, as shown in the cartoon. Each dot in the scatter plot represents a sister pair. The mean value is shown in magenta; error bars (blue) are the SD ($n = 60$ sister chromatids).

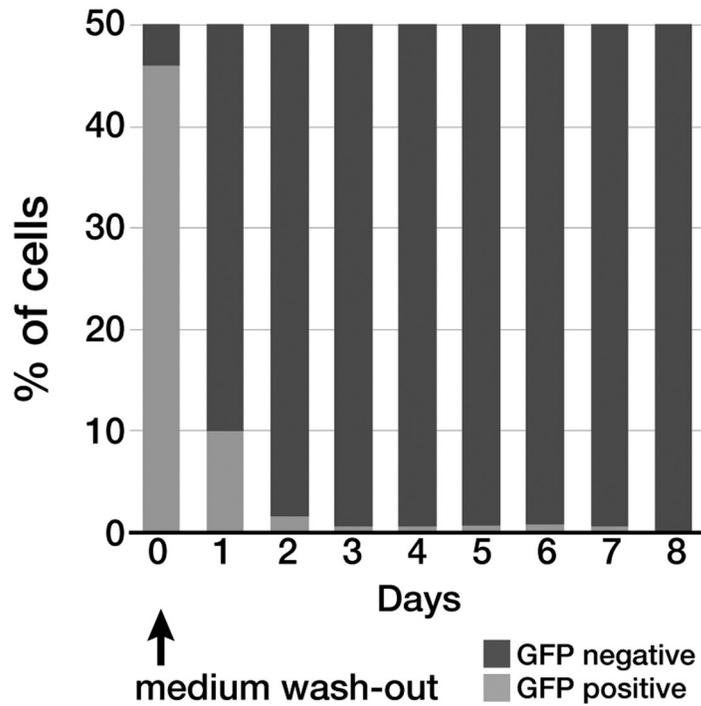


Figure S3. Quantification (from Fig. 2 A) of the percentage of cells displaying CAL1-GFP-LacI expression (GFP positive) or lack thereof (GFP negative) in a time course after medium washout. Two days after CuSO_4 removal, the signal of CAL1-GFP-LacI decreased to an undetectable level in virtually all cells ($n > 100$ cells for each time point). The data shown are from a single experiment.

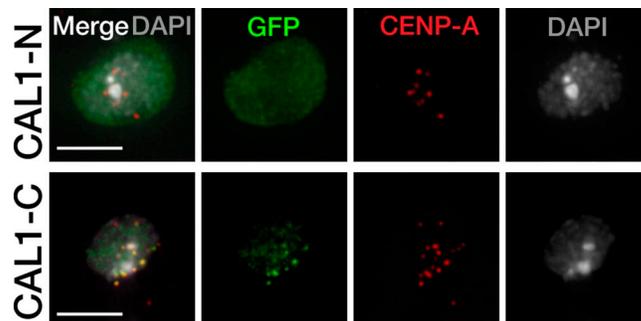


Figure S4. IF of interphase S2 cells (without lacO array) expressing the N terminus (aa 1-407) or the C terminus (aa 700-979) of CAL1-GFP-LacI (green). Although the C terminus can localize to endogenous centromeres (visualized by anti-CENP-A IF in red), the N terminus cannot. DAPI is shown in gray. Bar, 5 μm .

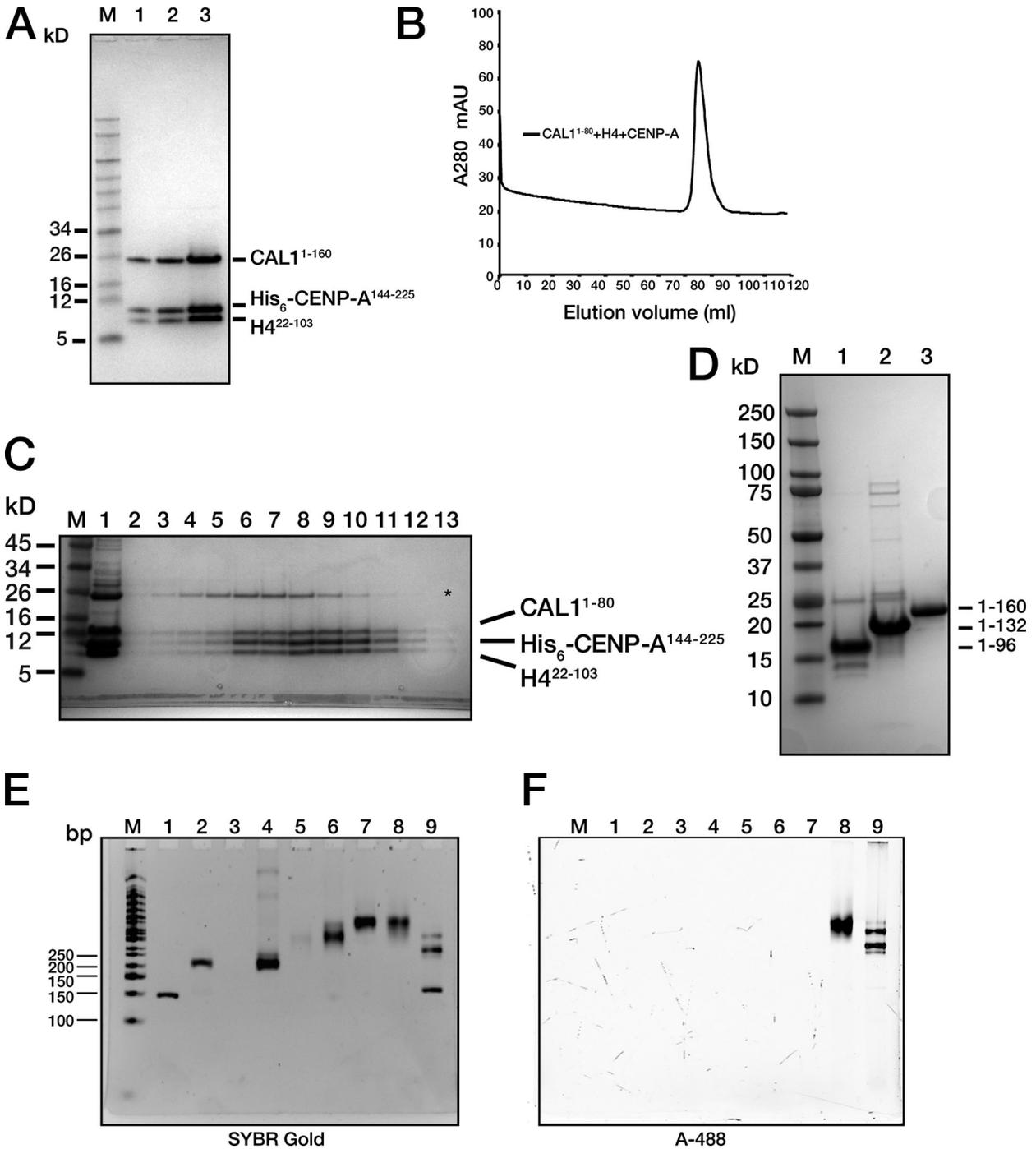
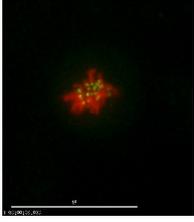
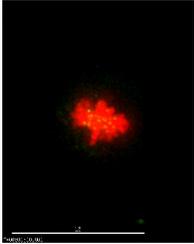


Figure S5. **CAL1 expression in *E. coli* and complex purifications.** (A) CAL1¹⁻¹⁶⁰, CENP-A¹⁴⁴⁻²²⁵, and H4²²⁻¹⁰³ were coexpressed and purified by gradient Ni-NTA and gel filtration. The purified sample was analyzed on 4–12% Bis-Tris SDS-PAGE and was Coomassie blue stained. The different lanes reflect different amounts of the purified proteins loaded onto the gel. (B) Gel filtration elution profile of the complex obtained after coexpression of CAL1¹⁻⁸⁰, CENP-A¹⁴⁴⁻²²⁵ and H4²²⁻¹⁰³ followed by purification by gradient Ni-NTA and gel filtration. (C) SDS-PAGE analysis of fractions from the peak shown in B. Asterisk indicates an unidentified band. (D) N-terminally His₆-tagged CAL1 constructs (1–96, 1–132, and 1–160) were purified under native conditions using Ni-NTA, ion exchange, and gel filtration. The final purified samples were run on 4–12% Bis-Tris SDS-PAGE and stained with Coomassie blue. Lane 1, CAL1¹⁻⁹⁶; lane 2, CAL1¹⁻¹³²; lane 3, CAL1¹⁻¹⁶⁰. (E and F) Mononucleosomes were assembled on the 147-bp Widom DNA at low salt (270 mM NaCl) in the presence of Nap1 or CAL1¹⁻¹⁶⁰. The nucleosomes were analyzed by 6% native PAGE. (E) Gel stained with SYBR gold. (F) The same gel shown in E scanned for Alexa 488 fluorescent signal. Lane 1, 147-bp Widom DNA alone; lane 2, H3 nucleosomes reconstituted by salt dialysis; lane 3, H3 octamer (2.8 μM) without the Nap1 chaperone; lane 4, as in lane 3 but with 20 μM Nap1; lane 5, CENP-A octamer (3.8 μM) without the Nap1 or CAL1 chaperones; lane 6, as in lane 5 but with Nap1 (20 μM); lane 7, CENP-A–H4–CAL1¹⁻¹⁶⁰ complex (3.8 μM); lane 8, as in lane 7 but with and Alexa 488–labeled H2A–H2B (2.5 μM); lane 9, Alexa 488–labeled H2A–H2B (2.5 μM). Note that in lanes 3 and 5 nucleosomes do not form, causing precipitation of histone and DNA complexes.



Video 1. **Representative video showing accurate chromosome segregation in a mitotic *Drosophila* S2 cell (no lacO array) stably expressing H2B-RFP and CAL1-GFP-LacI.** Videos were taken using a DeltaVision microscope (PersonalDV; Applied Precision) with a 60x/1.42 NA objective (Olympus) at 25°C. Frames were taken every 3 min for 60 min. Note that in this video CAL1-GFP-LacI can be seen localized to the endogenous centromeres.



Video 2. **Representative video showing chromosome stretching, due to ectopic kinetochore formation, in a mitotic *Drosophila* S2 cell expressing H2B-RFP and CAL1-GFP-LacI and harboring a lacO array inserted on chromosome 3.** Videos were taken using a DeltaVision microscope (PersonalDV; Applied Precision) with a 60x/1.42 NA objective (Olympus) at 25°C. Frames were taken every 3 min for 60 min. Note that in this video CAL1-GFP-LacI can be seen localized to the endogenous centromeres.