Supplemental Note 1.

Kinetochore de-clustering after histone H4 repression

In addition to the differences in kinetochore separation and spindle length between H3 and H4-repressed cells, Nuf2-GFP formed more than two foci following H4 repression. These foci frequently co-localized with spindle pole bodies and along the spindle axis, but were occasionally off the spindle axis as well (Supplemental Figure 1A). We found that kinetochores were de-clustered (>2 Nuf2-GFP foci formed) in 55% of cells following H4 repression, but rarely in wild-type (5% de-clustered) or H3-repressed (10% de-clustered) cells (Supplemental Figure 1B). Kinetochore de-clustering after H4 repression suggests that kinetochore formation or function may be compromised.

Since the centromere is known to contain a specialized nucleosome containing the H3 variant Cse4p, we considered the possibility that H4 depletion might lower the efficiency of kinetochore assembly at the centromere through disruption of the centromere specific nucleosome. To test this idea, chromatin was purified from cells grown in either YPG or YPD, and digested with *Dra*I endonuclease. The budding yeast centromere contains three *Dra*I endonuclease sites, but these sites are protected by centromere-kinetochore binding. As previously reported, *Dra*I accessibility increased following histone H4 repression, but was not increased by H3 repression (Supplemental Figure 1C) [1]. Disruption of proper kinetochore-centromere association may explain the kinetochore de-clustering defect observed following histone H4 repression.

Centromeres replicate in early S phase, and hydroxyurea does not prevent their replication or the assembly of kinetochores at centromeres. When cells were released from HU into YPD, the frequency of Nuf2-GFP declustering was reduced approximately 5-fold to nearly wild-type (Supplemental Figure 1B). These results demonstrate that once centromeres were replicated and centromere-kinetochore attachments were made, lowering histone levels had no direct effect on kinetochore function. The formation of greater than two kinetochore foci in H4-repressed cells is therefore indicative of defects in kinetochore-centromere binding in cells with fewer available nucleosomes.

Spindle structure and function following histone repression

Histone repression could affect spindle length and sister kinetochore separation by indirectly affecting microtubule regulation. To test this possibility we assayed spindle stability in wild-type and H3-repressed cells.

Metaphase spindles maintain a fairly stable spindle length and kinetochore position due to the balance of forces on the structural elements of the spindle. Slight movements of the SPBs or kinetochores are likely due to stochastic on-loading and off-loading of microtubule motor proteins, and the dynamic nature of microtubule plus-ends. To determine whether spindle stability in histone-repressed cells is comparable to wild-type metaphase spindles, we measured SPB separation in cells as they formed their spindles.

We found that the slight changes in spindle length found in wild-type cells are also found after H3 repression (Supplementary Figure 2A). In addition to single-cell analysis, populations of cells were monitored after histone repression. We found spindle length to be consistent among these cells (Supplementary Figure 2B).

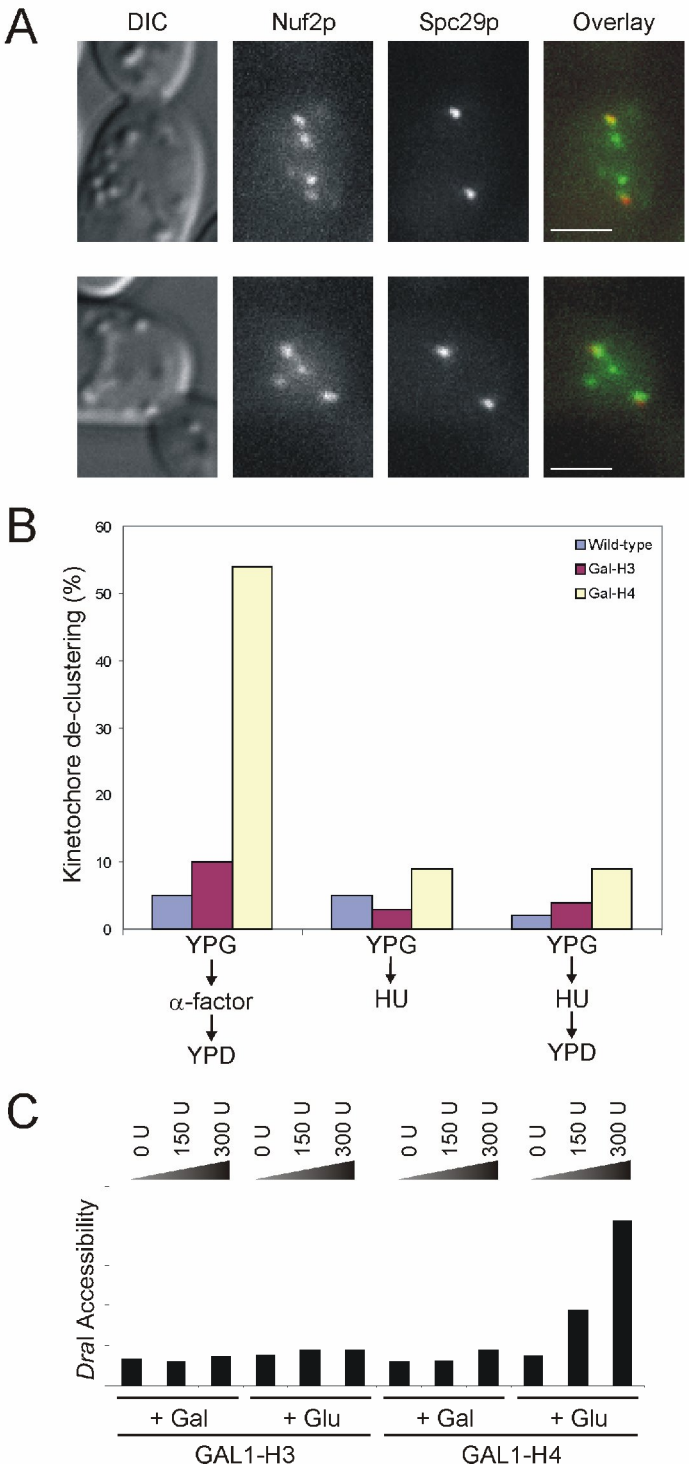
Kinetochore-microtubule attachments are stable once sister chromatid pairs are bi-oriented [2, 3]. To determine whether histone repression affects kinetochore-microtubule stability, the stability of Nuf2-GFP in kinetochore clusters was determined by measuring the fluorescence recovery after photobleaching (FRAP). Following H3 repression, a single Nuf2-GFP cluster was photobleached and images were acquired every 30 s to monitor fluorescence recovery (Supplementary Figure 2C). In nine cells considered, zero cells had fluorescence recovery greater than 10%, demonstrating that Nuf2p-GFP is not exchanging between the two kinetochore clusters or with the unbound, nucleoplasmic pool, consistent with previous reports in wild-type cells [2]. FRAP analysis of Nuf2-GFP in H4-repressed cells yielded variable results, likely reflecting the heterogeneity in proper kinetochore function (data not shown). The lack of recovery in H3-repressed cells shows the stability of kinetochore-microtubule attachments, similar to wild-type cells.

The relative stability of spindle length, organization of kinetochore clusters along the spindle axis, and stability of kinetochore-microtubule attachments collectively indicate that the spindles and kinetochores formed after histone H3 depletion are functionally comparable to wild-type metaphase cells. By lowering chromatin packaging, we have been able to isolate its contribution to the metaphase spindle, allowing us to combine this approach with other mutants to further probe the forces within the spindle.

1. Saunders MJ, Yeh E, Grunstein M, Bloom K: **Nucleosome depletion alters the chromatin structure of Saccharomyces cerevisiae centromeres**. *Mol Cell Biol* 1990, **10**:5721-5727.
2. Joglekar AP, Bouck DC, Molk JN, Bloom KS, Salmon ED: **Molecular architecture of a kinetochore-microtubule attachment site**. *Nat Cell Biol* 2006, **8**:581-585.
3. Pearson CG, Yeh E, Gardner M, Odde D, Salmon ED, Bloom K: **Stable kinetochore-microtubule attachment constrains centromere positioning in metaphase**. *Curr Biol* 2004, **14**:1962-1967.

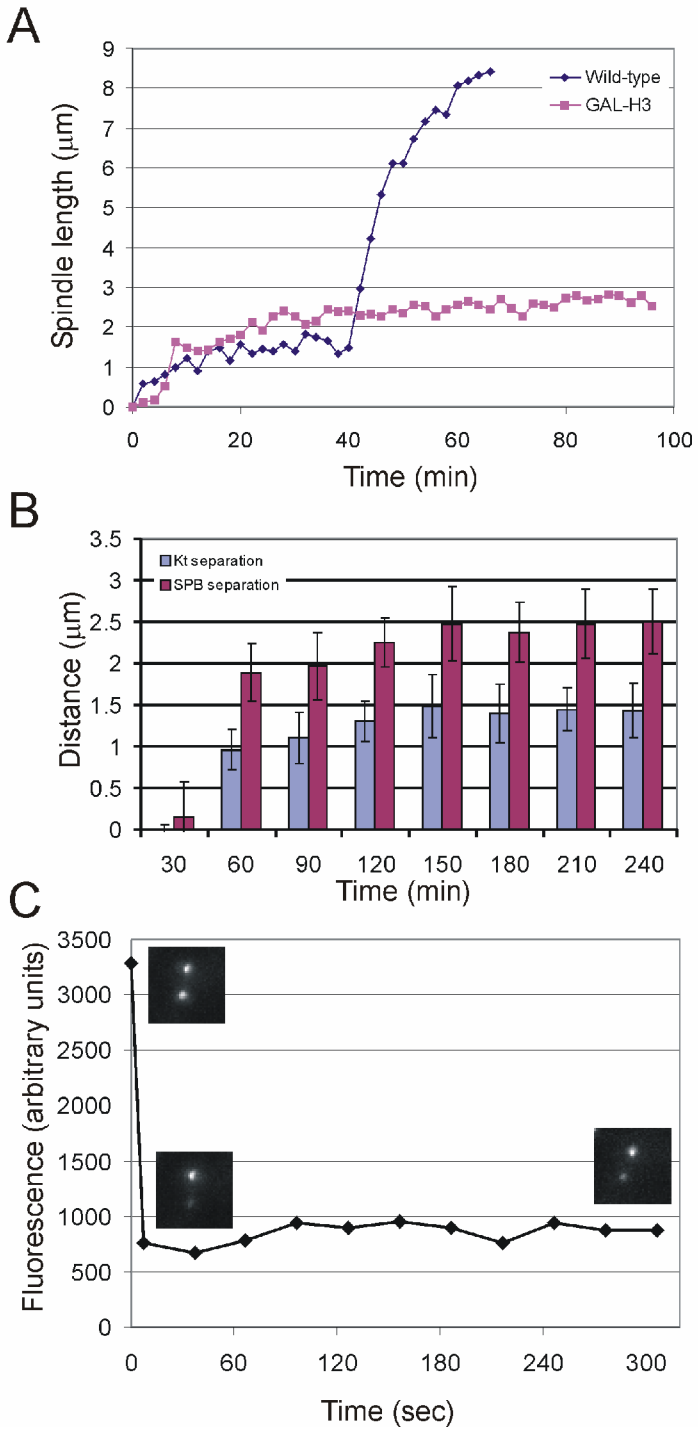
Supplemental Figures

Supplemental Figure 1



Supplementary Figure 1. Kinetochore declustering in H4-repressed cells. (A) Histone H4 repression leads to the formation of multiple (>2) Nuf2p-GFP foci. (B) Histone repression after release from hydroxyurea (HU) suppresses declustering frequency. (C) *Dra*I accessibility at the centromere is elevated following H4 repression, but not H3 repression. (Scale bar, 2 μm).

Supplemental Figure 2



Supplementary Figure 2. Spindle and kinetochore stability following H3 repression. (A) Spindle length is plotted for wild-type and H3-repressed cells during spindle formation and metaphase. Wild-type cells proceed through anaphase (anaphase onset at timepoint ~40 min), while H3-repressed cells maintain a stable spindle length. (B) Kinetochore separation and spindle length in cells following release from alpha factor (T=0) under histone repression. (C) FRAP of Nuf2p-GFP in H3-repressed cells shows that Nuf2p-GFP is stable.

Supplemental Table 1. Relevant plasmids and strains.

Plasmid name	Description	Source
pMA1186	*CIN8::LEU2* plasmid (digested with *Pst*I and *Sal*I)	M.A. Hoyt
pJK67	*NUF2-GFP-URA3* integrating vector (digested with *BstE*II)	P. Silver
pVG270	*MCD1-6HA-URA3* integrating vector (digested with *Age*I)	P. Megee
pDH7	*CFP-HIS3* plasmid	T. Davis

Strain name	Description	Source
YEF473A	*MATa trp1-63 leu2-1 ura3-52 his3-200 lys2-801*	J. Pringle
DCB 200.1	As YEF473A except *HHT1::TRP1 KAN-GAL1p-HHT2*	This study
DCB 220.1	As YEF473A except *HHF1::TRP1 KAN-GAL1p-HHF2*	This study
DCB 206.1	As YEF473A except *HHT1::TRP1 KAN-GAL1p-HHT2 MCD1-6HA-URA3*	This study
DCB 202.1	As YEF473A except *HHT1::TRP1 KAN-GAL1p-HHT2 ura3::NUF2-GFP-URA3*	This study
DCB 204.1	As YEF473A except *HHT1::TRP1 KAN-GAL1p-HHT2 ura3::NUF2-GFP-URA3 SPC29-RFP-HYG*	This study
DCB 222.1	As YEF473A except *HHF1::TRP1 KAN-GAL1p-HHF2 ura3::NUF2-GFP-URA3*	This study
DCB 224.1	As YEF473A except *HHF1::TRP1 KAN-GAL1p-HHF2 ura3::NUF2-GFP-URA3 SPC29-RFP-HYG*	This study
DCB 208.1	As YEF473A except *HHT1::TRP1 KAN-GAL1p-HHT2 ura3::NUF2-GFP-URA3 SPC29-CFP-HIS3 cin8::LEU2*	This study
DCB 209.1	As YEF473A except *HHT1::TRP1 KAN-GAL1p-HHT2 ura3::NUF2-GFP-URA3 SPC29-CFP-HIS3 kip1::LEU2*	This study
CEN15(1.8)-GFP	*MATa ade2 his3 trp1 ura3 leu2 can1 LacINLSGFP:HIS3 lacO::URA3* (1.8kb from CEN15)	M. Yanagida
CEN15(1.8)-GFP GAL-H3	*MATa ade2 his3 trp1 ura3 leu2 can1 LacINLSGFP:HIS3 lacO::URA3* (1.8kb from CEN15) *HHT1::TRP1 KAN-GAL1p-HHT2*.	This study