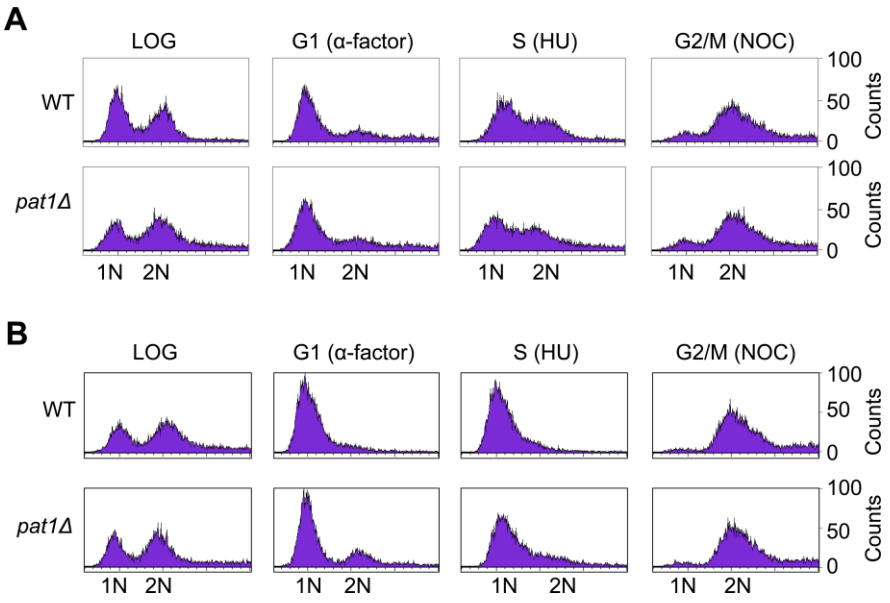
# Supplemental Materials

***Molecular Biology of the Cell***

Mishra et al.

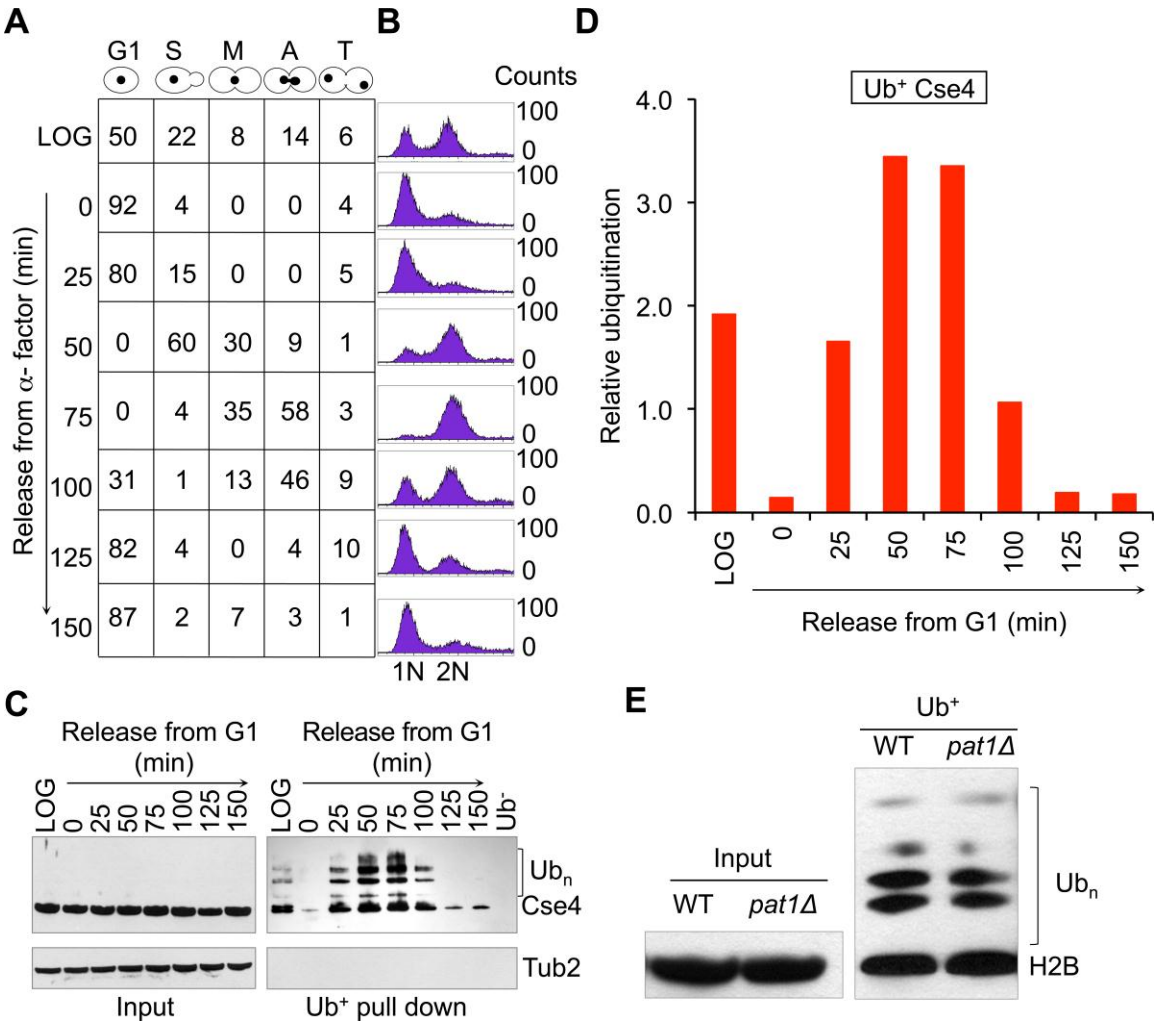



**Supplemental Figure S1:**

FACS profile showing cell cycle arrest in WT and *pat1Δ* strains.

(A) FACS profile showing synchronization of WT (YMB6398) and *pat1Δ* (YMB8422) strains in LOG, G1, S-phase, and G2/M stages of the cell cycle. Related to Figure 1A.

(B) FACS profile showing synchronization of WT (YMB8126) and *pat1Δ* (YMB9096) strains in LOG, G1, S-phase, and G2/M stages of the cell cycle. Related to Figure1C.




**Supplemental Figure S2:**

Ubiquitination of Cse4 is cell cycle regulated. A WT strain (YMB6398) with Myc-tagged Cse4 expressed from its endogenous promoter was grown in YPD, synchronized in G1 with α-factor, washed, and released into pheromone-free YPD medium. α-factor was re-added at 75 min after release to block cells in next G1. Samples were taken at time points (min) after release from G1.

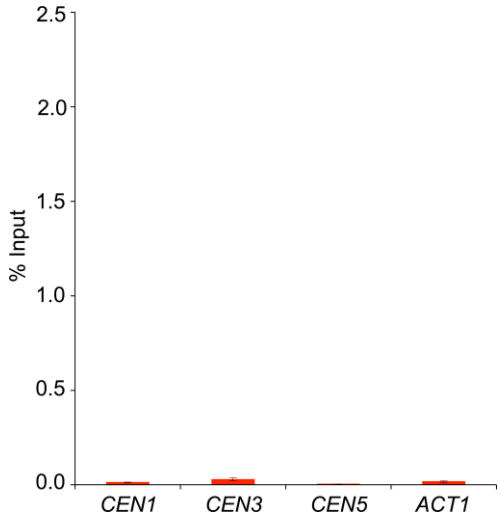
(A) Cell cycle stages were determined based on cell morphology and nuclear position by microscopic examination of 100 cells for each time point as described in Materials and Methods.

(B) DNA content was determined by FACS.

(C) Western blotting showing the levels of Cse4 ubiquitination through the cell cycle stages.

(D) Relative levels of Cse4 ubiquitination through the cell cycle stages. Values were calculated as described in Figure 1B.

(E) Histone H2B ubiquitination is not altered in a *pat1Δ* strain. Western blotting showing the levels of Histone H2B ubiquitination ($Ub_n$) in WT (YMB6398) and *pat1Δ* (YMB8422) strains grown to logarithmic phase of the growth. Ubiquitinated proteins were pulled down as described in Materials and Methods. Eluted proteins were analyzed by Western blotting with $\alpha$-histone H2B antibodies.



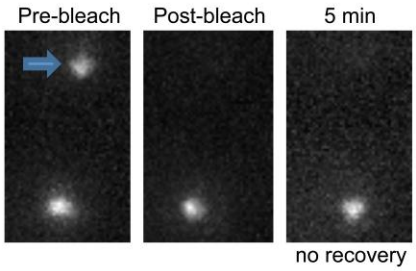

**Supplemental Figure S3:**

Control ChIP-qPCR data using an untagged strain. ChIP experiments were performed with an untagged strain (BY4741) using α-TAP antibodies as described in Figure 1C. Enrichment to the *CEN* (*CEN1, CEN3,* and *CEN5*) andnon-*CEN (ACT1)* regionswas determined by qPCR and is shown as % input. Average from three independent biological replicates ± standard error is shown.

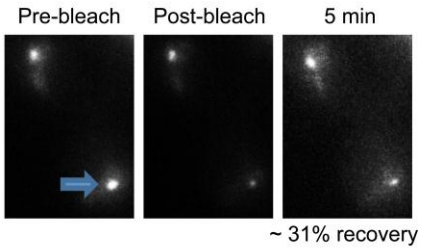
**A**

| Kinetochore Clusters | % recovery of Cse4-GFP | |
|---|---|---|
| | WT | *pat1Δ* |
| 1 | 6 | 10 |
| 2 | 12 | 49 |
| 3 | 11 | 35 |
| 4 | 5 | 31 |
| 5 | 23 | 62 |
| 6 | 22 | 27 |
| 7 | n.d. | 6 |
| 8 | n.d. | 13 |
| 9 | n.d. | 22 |
| 10 | n.d. | 17 |
| 11 | | 1 |
| 12 | | 31 |
| 13 | | 1 |
| 14 | | n.d. |
| 15 | | n.d. |
| 16 | | n.d. |
| 17 | | n.d. |
| Average: | 13.16 | 23.46 |
| Standard error: | 3.15 | 5.09 |

**B**




**C**



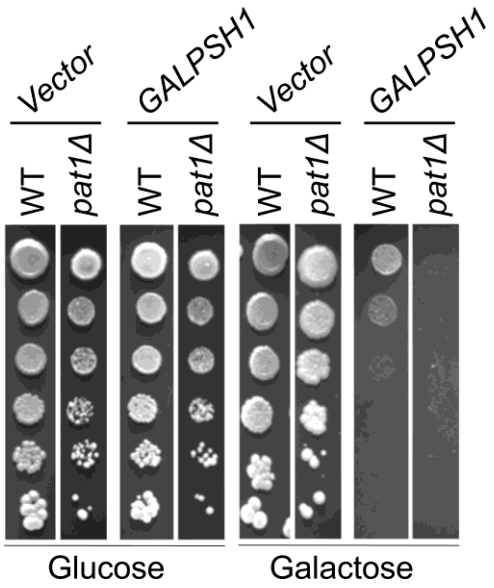

**Supplemental Figure S4:**

FRAP assay revealed a faster turnover of Cse4 at kinetochores in *pat1Δ* strains.

(A) Percent Cse4-GFP fluorescence recovery for WT (KBY2012) and ***pat1Δ*** (KBY8166) strains 5 min post-bleaching. Cse4-GFP-labeled centromeres were photobleached during metaphase. One of the two kinetochore clusters was bleached andfluorescence recovery observed after 5 min of photobleaching is presented. Average from examination of independent kinetochore clusters ± standard error is shown. Letter n.d. represents “no recovery” of fluorescence signal.

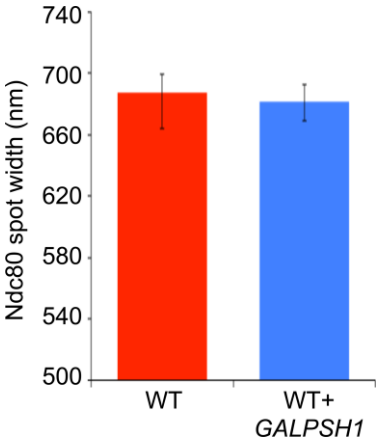
(B) Representative images showing no recovery of Cse4-GFP fluorescence after photobleaching of Cse4-GFP labeled centromeres.

(C) Representative images showing recovery of Cse4-GFP fluorescence (~31%) after photobleaching of Cse4-GFP labeled centromeres in a ***pat1Δ*** strain.




**Supplemental Figure S5:**

Constitutive overexpression of *PSH1* causes loss of viability in budding yeast. Serial dilutions (5-fold) of (YMB6398) and ***pat1Δ*** (YMB8948) strains containing vector (pRS426 *GAL1*) or *GALPSH1HA* (pMB1628) were plated on SC-URA plates with glucose (2%) or galactose + raffinose (2% each) at 30°C.




**Supplemental Figure S6:**

Induction of Psh1by its overexpression does not affect the appearance of Ndc80 at kinetochores. The height of the cluster of Ndc80-GFP at kinetochores was determined from line scans (perpendicular to the spindle axis) through the protein cluster as previously described (Haase *et al.*, 2013). The height was determined from the full-width full-maximum of the Gaussian distribution. The average spot width of Ndc80-GFP foci is shown in WT (KBY8116) overexpressing *PSH1* (*GALPSH1HA,* pMB1628) for 6 hours. Sample sizes range from 11-15 cells for each condition.