File S1

Supplemental methods

Yeast strains and plasmids

Supplemental Table S1 and S2 present the genotype of yeast strains and plasmids used for this study, respectively.

Protein stability assay

Cells were grown in a 2% raffinose synthetic complete medium overnight. Cells were then diluted to an optical density at 600 nm of 0.3 and grown for an additional 3 h at 25°C. A sample was removed as a negative control before the addition of galactose (Raffinose). Galactose was added to the media to a final concentration of 2% to induce expression of Cse4 from the *GAL1* promoter for 2 h at 25°C. Glucose was then added to a final concentration of 5% to stop gene expression, and samples were taken at the indicated time points.

Antibodies

Anti-Cse4 antibody was generated as previously described (OHKUNI *et al.* 2008). Anti-Cdc28 was a gift from Dr. Deshaies's laboratory. Anti-myc (Roche, Indianapolis, IN), anti-HA (Roche, Indianapolis, IN), and anti-tubulin (Serotech, Oxford, UK) antibodies were purchased.

Western blotting and quantitation

Western blotting and quantitation were performed as described previously (ESCAMILLA-POWERS and SEARS 2007). In brief, protein from equal cell numbers were separated by SDS-PAGE gel and transferred to Immobilon-FL membrane (Millipore, Billerica, MA). Membrane was blocked with Odyssey Blocking buffer (LI-COR Biosciences, Lincoln, NE) after shaking the membrane in PBS for several minutes. Primary and secondary antibodies were diluted in Odyssey Blocking buffer with 0.1% Tween 20. Anti-Cse4 was used at a dilution of 1:1000, and anti-tubulin and anti-Cdc28 were used at 1:5000. Secondary antibodies were used at a dilution of 1:15000. Blots were scanned with a LI-COR Odyssey CLx Imager (Lincoln, NE). Protein levels at each time point were quantitated using Image Studio software version 2.0.