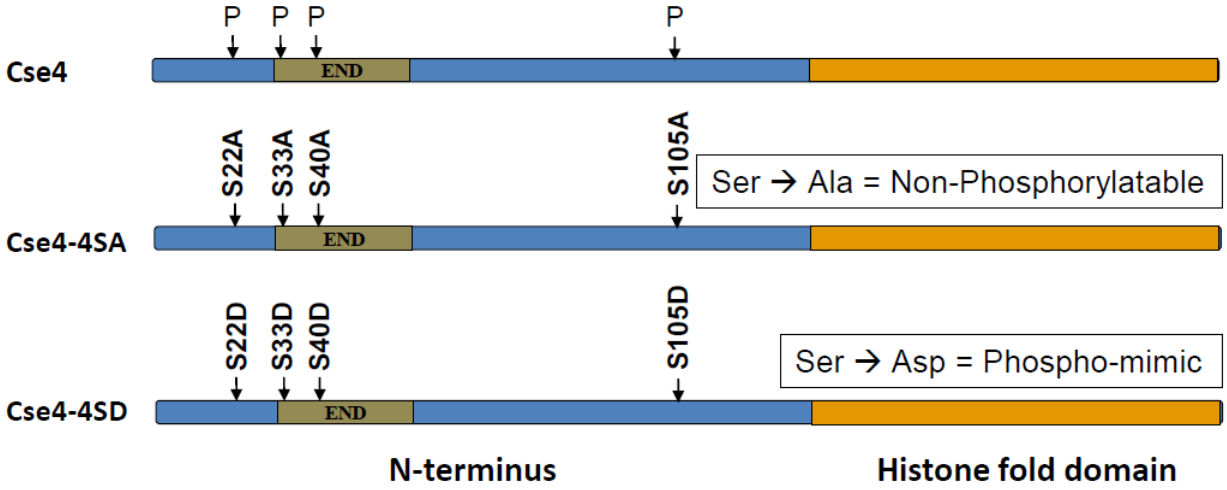
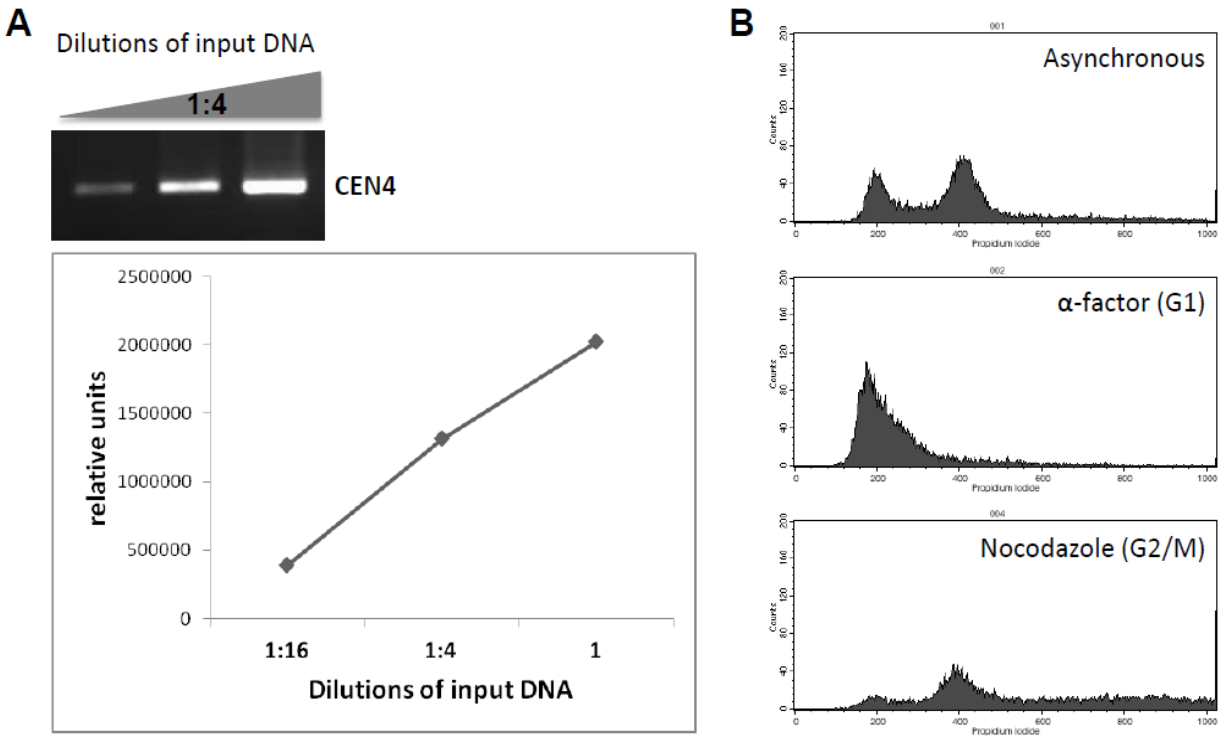
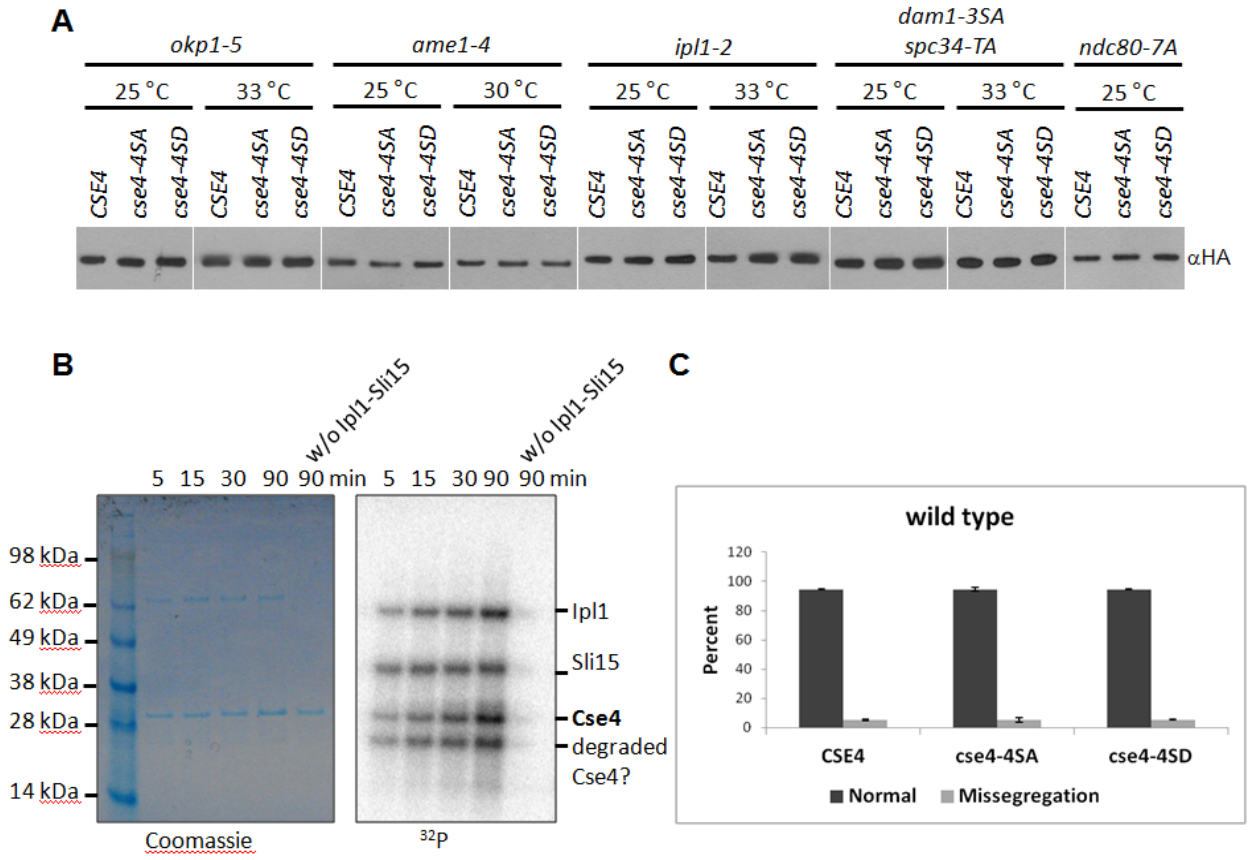
Supplemental Material



Supplemental Figure 1. Schematic diagram of wild-type Cse4 and mutants showing the N-terminus, which contains the **E**ssential **N**-terminal **D**omain (END), and the C-terminal Histone Fold Domain (Chen *et al.*, 2000). Serines mutated to either Alanine (A, non-phosphorylatable) or Aspartic acid (D, phosphomimetic) are indicated.



Supplemental Figure 2. Quantification of ChIP experiments and flow cytometry analysis. (A) Linear range of band intensities used in quantification of PCR products from ChIP experiments. Serial dilutions of input DNA from asynchronously growing wild type cells was PCR-amplified using primers specific to CEN4. (B) DNA content determined by flow cytometry of cells used for the analyses in Figure 2E (α-factor or nocodazole arrest).



Supplemental Figure 3. (A) Expression of wild-type and mutant Cse4 proteins in strain backgrounds used for genetic analysis of Cse4 phosphorylation. Western blot analysis of protein extracts prepared from *okp1-5*, *ame1-4*, *ipl1-2*, *dam1-3SA spc34-TA* and *ndc80-7SA* strains expressing HA-tagged *CSE4*, *cse4-4SA* or *cse4-4SD* from alleles integrated at the endogenous *cse4* locus and driven by the endogenous promoter. Strains were grown in YPD at 25 °C for 5 hours then shifted to 25 °C, 30 °C or 33 °C for 3 hours. Blots were probed with αHA. (B) Ipl1 phosphorylates Cse4 *in vitro*. *In vitro* kinase assays were carried out using purified Cse4, Ipl1-Sli15 and radiolabeled ATP at 30 °C for 5, 15, 30 and 90 minutes and products analyzed by SDS gel electrophoresis followed by Coomassie Blue staining and autoradiography of radiolabeled proteins. (C) Control for Figures 5B and C. *cse4* phosphorylation mutants do not exhibit increased chromosome loss in a wild-type strain background. Wild type strain (DDY1925) containing either *CSE4* (YMB8378), *cse4-4SA* (YMB8382) or *cse4-4SD* (YMB8379) was α-factor arrested and shifted to medium lacking α-factor at 33 °C for 3 hours. Segregation of a GFP-labeled chromosome was monitored in large budded cells undergoing mitosis. In each of two independent experiments, at least 30 cells were counted for each strain. Error bars represent mean deviations.