Figure S1. *Csi1 is required for centromere clustering*. (A) Loss of Csi1 results in an increase in the number of Cnp20-GFP foci. (B) ChIP analysis of Cnp20-Flag levels at cnt. Error bars represent standard deviations of three experiments. (C) Live-cell imaging of cells containing a LacO array inserted near the centromere of chromosome two that also is expressing LacI-GFP and Sid4-mRFP. (D) Live-cell imaging of cells expressing both Sad1-GFP and Sid4-mRFP. DIC, differential interference contrast; WT, wild type. Bars, 1 μm.
Figure S2. Csi1 associates with the kinetochore and SPB. (A) ChIP analyses of Csi1-Flag protein levels at cnt were performed at a restrictive temperature (37°C for 4 h). (B) ChIP analyses of Csi1-Flag protein levels at cnt were performed at a restrictive temperature (37°C for 6 h). (C) ChIP analyses of Csi1-Flag protein levels at cnt were performed in the presence of napthaleneacetic acid, which induces the degradation of Nuf2 through a fused degron and Skp1-TIR1. (D) Time-lapse microscopy of Csi1-mCherry with Mis6-GFP or Csi1-GFP with Sad1-DsRed during mitosis. Pictures were taken at 2-min intervals. (E) Live-cell imaging of cells expressing Csi1-GFP and Sid4-mRFP in a mis6-302 mutant at a restrictive temperature (37°C for 4 h). Error bars represent standard deviations of three experiments. DIC, differential interference contrast; WT, wild type. Bars, 1 µm.
Csi1 mediates centromere clustering

**Figure S3.** Csi1 is a nuclear protein. (A) Diagram of Csi1 showing the location of a putative NLS. (B) Live-cell imaging of Csi1-mCherry. Csi1-ΔNLS-mCherry removes residues 47–65 of Csi1 and Csi1-ΔNLS-mCherry-NLS is created by the addition of an NLS from the SV40 T antigen to the C terminus of Csi1. (C) Serial dilution analysis of the indicated strains to measure their sensitivity to thiabendazole. (D) Csi1 mutant cells containing Ch16 were grown into single colonies to measure chromosome loss rate, indicated below each picture. n indicates total number of colonies counted. DIC, differential interference contrast; TBZ, thiabendazole. Bar, 1 µm.