Supplemental Material

Supplemental Figure S1. *bir1-107* cells show chromosome missegregation by flow cytometry. Asynchronous cells were shifted to the restrictive temperature of 37ºC and fixed for flow cytometry at the indicated times. The wild-type strain (CRY2) maintains distinct populations of cells with 1N or 2N DNA content, while an *ipl1-321* strain (SFY233-2D) accumulates cells with varying DNA content. The *bir1-107* strain (PWY167-13D) shows a similar, albeit less severe, missegregation phenotype.

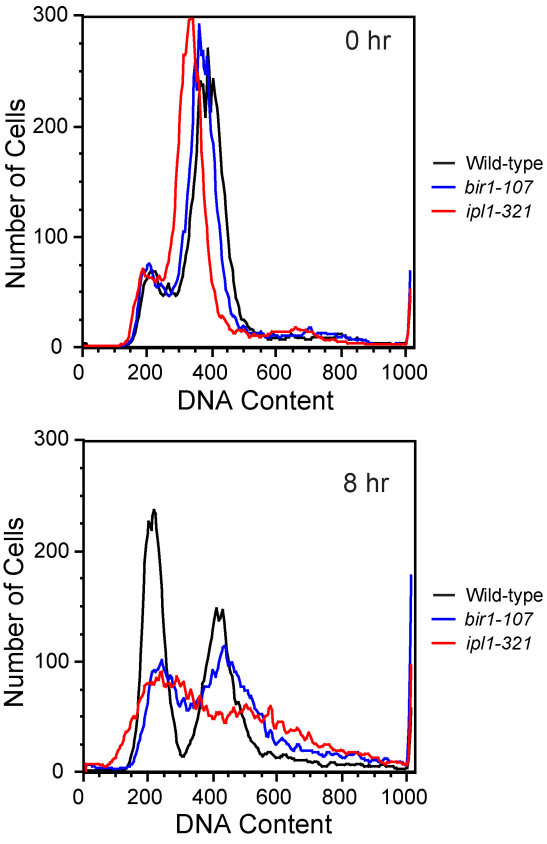
Supplemental Figure S2. Sli15 is required to properly localize Bir1 and Ipl1. p*GAL-SLI15* cells were grown on YPGal plates and streaked onto YPD plates to shut off Sli15 expression. (A) Bir1 (TDY188-2A) and (B) Ipl1 (TDY189-12A) localizations were compared on galactose and after 2 hours on glucose. Bir1 and Ipl1 are green in the merged images and the kinetochore marker Ndc10 is red. Images are scaled equally for each strain. Bar, 1 μm.

Supplemental Figure S3. Metaphase Ndc10 localization is similar between strains. Ndc10-CFP localization at metaphase in strains PWY4-2B (Sli15-Venus), PWY5-5A (Bir1-Venus), PWY303-11A (Ipl1-Venus) and PWY346-1A (Nuf2-Venus). Fluorescence intensity was measured across the same half-spindles analyzed in Figure 6. Background was subtracted, and the average half-spindle distributions were reflected about the spindle midpoint. Fluorescence distribution across the spindle is shown as fraction of total fluorescence. The average Ndc10 separations for each strain were as follows (mean ± standard deviation): PWY4-2B (0.89 μm ± 0.12 μm), PWY5-5A (0.85 μm ± 0.10 μm), PWY303-11A (0.85 μm ± 0.13 μm), PWY346-1A (0.86 μm ± 0.12 μm).

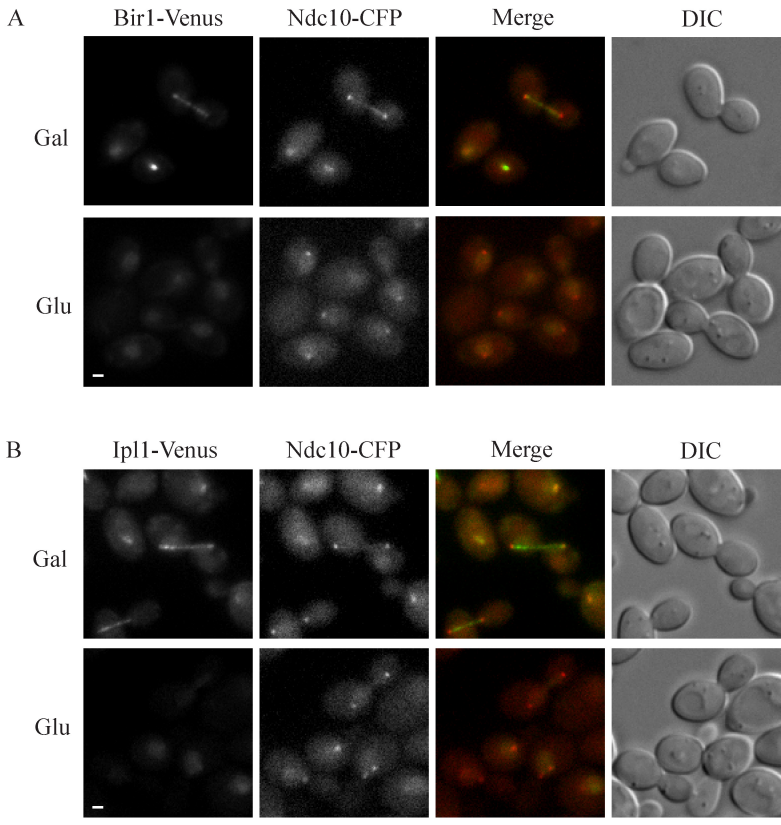
Supplemental Figure S4. Chromosomal passenger localization is distinct from kinetochores at metaphase. Metaphase Bir1-3xGFP (PWY341-12D), Ipl1-3xGFP (PWY334-1B) and Nuf2-

3xGFP (PWY335-1B) localizations were compared in strains carrying the spindle pole body marker Spc110-mCherry at 22ºC. Fluorescence intensity was measured across ≥90 half-spindles for each strain. Background was subtracted, and the average half-spindle distributions were reflected about the spindle midpoint. The error bars represent the standard error of the mean.

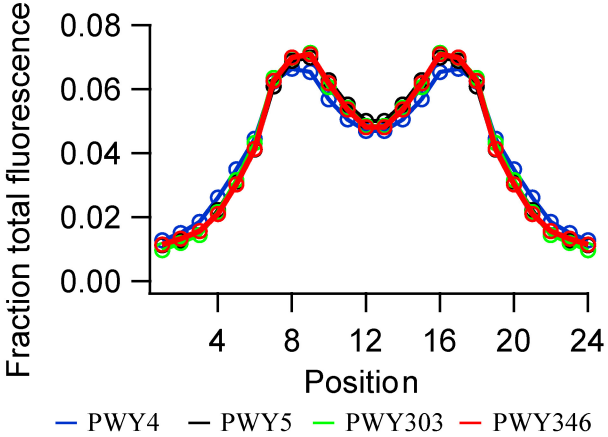
Supplemental Figure S1



Supplemental Figure S2



Supplemental Figure S3



Supplemental Figure S4