**Figure S1: Characterization of *gin4-AID* and *hsl1-AID* alleles.**

**(A)** Wild type, *2xTIR1* control cells*, gin4-AID hsl1-AID 2xTIR1 cells* and *gin4-AID hsl1-AID swe1∆*

*2xTIR1* cells were grown overnight at room temperature in YPD and cell size distributions were analyzed with a Coulter counter. **(B)** Control cells and *gin4-AID hsl1-AID* cells growing in YPD were released from a G1 phase arrest. After release, the *gin4-AID hsl1-AID* cells were split into two aliquots and 0.5 mM auxin was added to one aliquot and an equivalent amount of the solvent for auxin was added to the other. Auxin was also added to the control strain. Samples were taken at the indicated intervals and the behavior of Gin4 was analyzed by western blot. All strains contain 2 copies of the *TIR1* gene (*2xTIR1*). **(C)** Quantification of Gin4 protein after addition of auxin. The plots show an average of two biological replicates with the error bars representing SD of the mean. Quantification was carried out as described in Materials and Methods. (D) *hsl1- AID 2xTIR1* cells growing in YPD were released from a G1 phase arrest. After release, the cells were split into two aliquots and 0.5 mM auxin was added to one aliquot and an equivalent amount of the solvent for auxin was added to the other. Samples were taken at the indicated intervals

and the behavior of Hsl1-AID was analyzed by western blot. \* marks a background band seen with the V5 antibody that was used to detect the V5-epitope on the AID-tagged Hsl1 protein. Anti- Nap1 was used as a loading control. The Hsl1-AID protein was at the limit of detection so it was not possible to accurately quantify the signal due to the high background signal caused by long exposure times. (E) *gin4-AID hsl1-AID 2xTIR1* cells growing in YPD were released from a G1 phase arrest. After release, the cells were split into two aliquots and 0.5 mM auxin was added to one aliquot and an equivalent amount of the solvent for auxin was added to the other. Samples were taken at the indicated intervals and the behavior of Clb2 was analyzed by western blot.

**Figure S2: Gin4 and Hsl1 control bud growth during mitosis.**

**(A)** A scatter plot showing the growth rate of the daughter buds during mitosis for the indicated genotypes. The growth rate (fL/min) was determined as the increase in bud volume from initiation of metaphase to the completion of anaphase, divided by the total time spent in metaphase and anaphase. **(B**) *gin4∆, hsl1∆, gin4∆ hsl1∆, and gin4∆ hsl1∆ swe1∆* cells were grown to log phase in YPD media at 25˚C and images were obtained using DIC optics. Scale bar represents 5 µm.

**Figure S3: The severity of the *gin4-AID hsl1-AID* phenotype increases with time.**

Control cells and *gin4-AID hsl1-AID* cells were grown to log phase at room temperature in YPD medium and auxin was added to both strains. Both strains included 2 copies of the *TIR1* gene. DIC images of the cells were taken at the indicated times. Scale bar represents 5 µm.

**Figure S4: Swe1 is rapidly dephosphorylated in asynchronous cells upon inactivation of**

**Gin4 and Hsl1.**

*gin4-AID hsl1-AID 2xTIR1* cells were grown to log phase in YPD and were then split into two aliquots. 0.5 mM auxin was added to one aliquot and an equivalent amount of the solvent for auxin was added to the other. Samples were taken at the indicated intervals and the behavior of Swe1 was analyzed by western blot. Anti-Nap1 was used as loading control.

**Figure S5: Gin4 phosphorylation is correlated with the extent of bud growth, rather than the rate of bud growth.**

Wild type and *sec6-4* cells were released from a G1 arrest in YPD at room temperature, incubated in a 25˚C shaking water bath until a shift to the restrictive temperature (34˚C) at 30 min **(A)**, 60 min **(B)** or 90 min **(C)** after release from arrest. Samples were taken at the indicated intervals and the behavior of Gin4 and Clb2 was analyzed by western blot. The timepoint labeled in red indicates the time at which cultures were shifted to 34˚C.

**Figure S6: Quantification of Gin4-GFP localization at the bud neck.**

Cells of the indicated genotypes were analyzed to determine the maximum pixel intensity for GFP fluorescence at the bud neck. The Y-axis on the scatter plot shows the maximum pixel intensity in arbitrary units after subtracting background signal.

**Video 1: Time-lapse imaging of *2xTIR1* and *gin4-AID hsl1-AID* cells.**

The two strains were mixed together prior to imaging as described in Materials and methods. The spindle pole bodies were differentially tagged in the two strains. The cells in top right corner show

*2xTIR1* cells with mRuby2-tagged SPC42 and the cells in the bottom left corner show *gin4-AID hsl1-AID* with GFP-tagged SPC42. The *gin4-AID hsl1-AID* cells undergo a prolonged metaphase delay with polarized bud growth while the *2xTIR1* cells begin the next cell cycle. The cells were imaged using time-lapse confocal microscopy with image acquisition every 3 min. The movie was converted to AVI format using Fiji and shows the time-lapse at a speed of 5 frames per second (fps). Scale bar represents 5 µm.

**Video 2: *gin4-AID hsl1-AID* cells show spindle pole and cytokinesis defects.**

A tile showing *2xTIR1* cells with mRuby2-tagged SPC42 and *gin4-AID hsl1-AID* cells with GFP- tagged SPC42 imaged together. Cells were imaged using time-lapse confocal microscopy with image acquisition every 3 min. The *gin4-AID hsl1-AID* cells exhibit defects in bud separation and results in the formation of cell chains. These defects are also accompanied by spindle defects (magenta arrows) in the second cell cycle. The movie was converted to AVI format using Fiji and shows the time-lapse at a speed of 5 fps.