



Figure S1. Characterization of BiFC signals generated from septin–chaperone interactions

(A) Log-phase cultures of cells of the indicated genotypes were imaged for transmitted light and YFP fluorescence. The transmitted light images were used to generate cell outlines (dashed white lines). Yellow arrowheads indicate discrete BiFC signals. “*CDC3-V_C CCT7-V_N*” indicates the haploid strain H00526. Other strains were diploids made by mating the haploid strains H00383, H00384, H00387, H00377, YO802 and YO685 in appropriate combinations. (B) Diploid cells co-expressing Cdc10-V_C and Cct7-V_N made by mating strains H00383 and YO1057 and carrying a plasmid encoding Act1(E364K)-mCherry under control of the *GAL1/10* promoter (pDK304) were cultured in galactose-containing medium to drive Act1(E364K)-mCherry overexpression and shifted to 37° for 2 hours, to drive Act1(E364K)-mCherry aggregation, prior to imaging. Shown is a representative overlay image of the transmitted light, YFP, and mCherry signals. (C) Representative images showing cell outlines overlaid on transmitted images (inverted and false-colored blue) used for measuring intracellular fluorescence. (D) Cellular BiFC signal away from bud necks was quantified for diploid cells co-expressing Cdc10-V_C or Cdc10(D182N)-V_C and the indicated V_N-tagged chaperones. The number of cells analyzed for each genotype ranged from 46 to 460. Strains were diploids made by mating YO1057 or H06530 with H00381, H00383, H00399, H00397, H00393, H00389, H00388, H00403, H00404, or H00395. (E) As in (C) but for the indicated chaperones and the values plotted represent the ratio of signal to area for each cell. Strains were diploids made by mating YO1057 or H06530 with H00382, H00392, H00390, H00398, H00384, H00394, H00385, or H00396. (F) For the data in Figure 1C, the median difference for comparisons between Cdc10(D182N)-V_C and Cdc10-V_C are shown in Cumming estimation plots as bootstrap sampling distributions. Each median difference is depicted as a dot. Each 95% confidence interval is indicated by the ends of the vertical error bars.