

Supplemental Figure Legends:

Supplemental Figure 1: GFP-Clc1p accumulation at the cell cortex in *chc1-TD* mutants in the presence of latrunculin A (LAT-A) at 37°C. *GFP-CLC1 ABP1-mRFP* *chc1Δ* (SL5538) was transformed with pAP4 (*CEN, CHC1*), pJRC3 (*CEN, chc1-2*), pJRC5 (*CEN, chc1-4*), or pJRC19 (*CEN, chc1-box*). Cells were shifted to 37°C for 90 minutes and then treated with LAT-A (250μM) for 20 min prior to imaging. Abp1-mRFP was completely cytosolic after 20 min in LAT-A indicating the disassembly of actin (data not shown). In the *chc1-box* mutant there is significant cortical recruitment even at high temperature, while in *chc1-2* and *chc1-4* very few cells had any cortical clathrin.

Supplemental Figure 2: Immunoblot showing GBD-TD fusions are expressed similarly in the two-hybrid analysis. All GBD-TD's were expressed well, although we noted that the GBD-Chc1-4-TD migrated slightly slower on the gel, suggesting some structural alteration. Methods: Cell extracts were prepared from the two-hybrid reporter strain (SL2793) transformed with two hybrid bait plasmids encoding GBD-empty or GBD-TD fusions. Cultures were grown at 30°C to log phase, cells (5×10^7) were harvested and washed once in water, and pellets were resuspended in 400μl of 0.1 M NaOH for 5 minutes. Cells were resedimented for 30 seconds at $13,000 \times g$, resuspended in 200μl SDS-PAGE sample buffer (containing DTT) and then boiled for 5 minutes. Lysates were centrifuged for 5 min at $10,000 \times g$ to remove debris, and 20μl of supernatant were separated by SDS-PAGE and transferred for blotting. Blots were

probed with a 1:200 dilution of anti-GBD rabbit polyclonal antiserum (sc-577, Santa Cruz Biotechnology Inc. Santa Cruz, CA) and detected by IRDye800 conjugated goat anti-rabbit antiserum (LiCor, Lincoln, NE). Pgk1 was detected with 2 µg/ml mouse monoclonal anti-Pgk1 antibody (Molecular Probes, Eugene OR) and detected by IRDye700 conjugated goat anti-mouse antiserum (LiCor, Lincoln, NE). Blots were imaged using an Odyssey Infrared Imaging System (LiCor, Lincoln, NE).

