Expanded View Figures

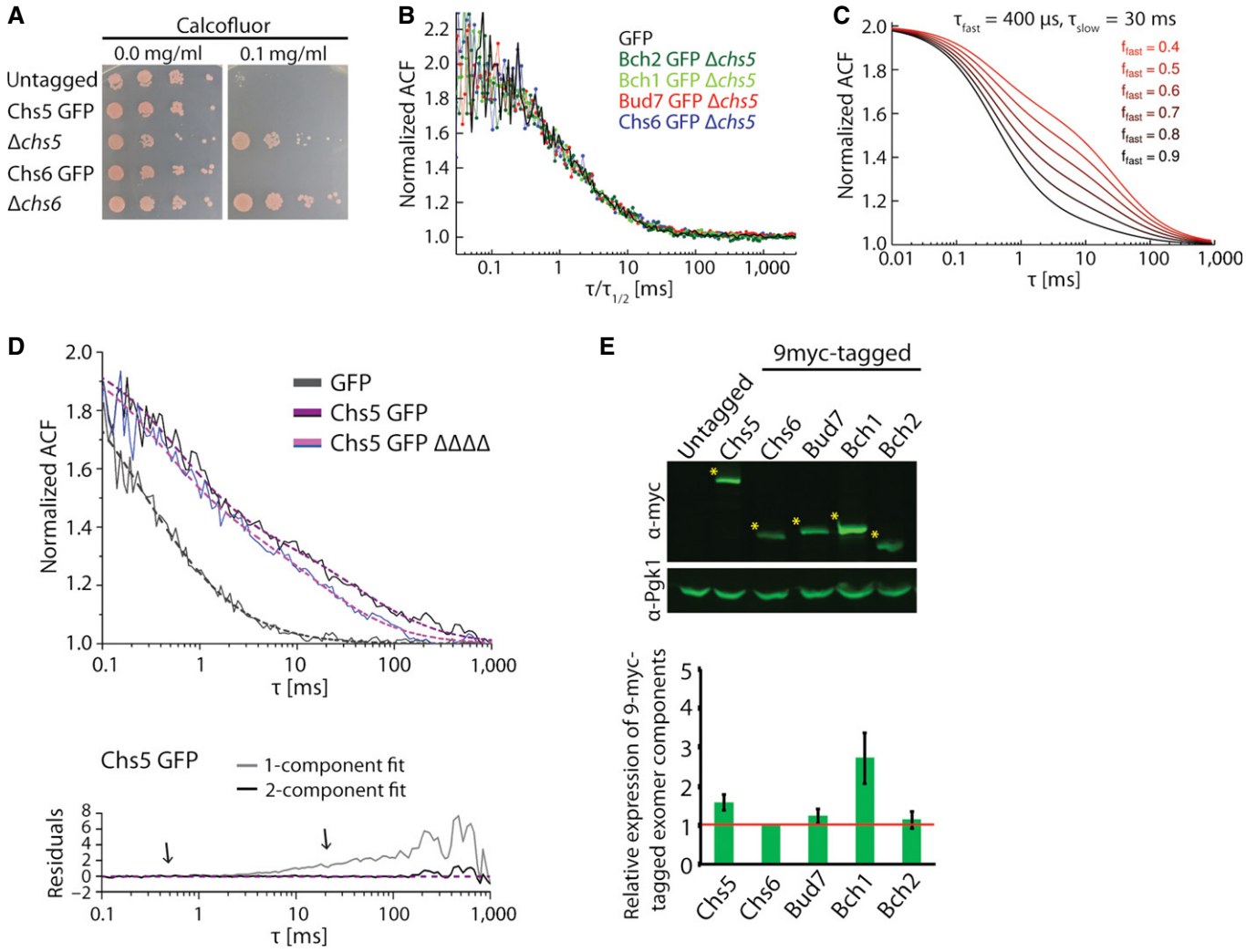


Figure EV1. Characterization of Chs5 and the ChAPs fusion proteins.

A GFP-tag does not affect functionality of the GFP-tagged exomer components. Chs5-GFP- and Chs6-GFP-expressing strains were sensitive to calcofluor similarly as the WT strain. Plates were incubated at 30°C for 2–3 days.

B Comparison of the ACFs for GFP and ChAPs in the Δ*chs5* strain rescaled by their respective half times.

C Simulated FCS traces of the fast and slow component with fixed diffusion correlation times (τ_{fast}, τ_{slow}) and variable fast component fractions (f_{fast}).

D FCS measures the *in vivo* mobility of Chs5 complexes in the cytoplasm. FCS analysis was performed on the GFP and Chs5-GFP in the presence or absence (ΔΔΔΔ) of the ChAPs. 10–20 FCS measurements were performed for each condition using different cells and representative curves are shown. The ACF of free GFP is fitted with a one-component anomalous diffusion model. The ACF of Chs5-GFP in both WT and ΔΔΔΔ background was fitted with a two-component diffusion model. The residuals assess the quality of the fit. Note the ChAP deletion affects the diffusion of the slow component of Chs5 only mildly. Estimated diffusion correlation times, diffusion coefficients, and fractions are shown in Table 1.

E Quantification of the relative expression levels of the exomer components in 9-myc-tagged strains. Representative immunoblot of yeast lysates is shown; Pgk1 serves as a loading control. The asterisks depict the analyzed bands. The plot shows average and SD of three independent biological experiments. The relative expression levels are normalized to Chs6 (depicted by the red line).

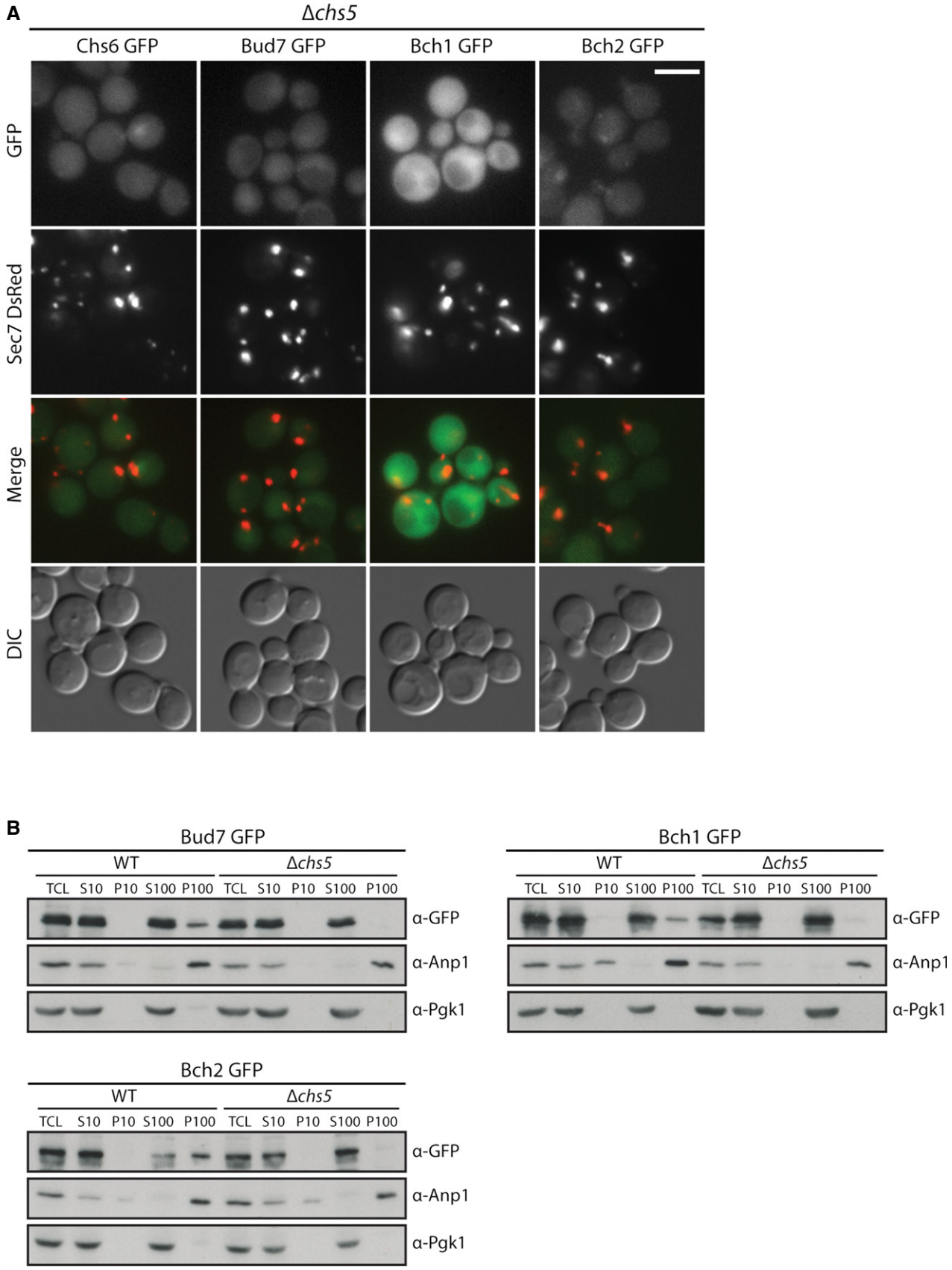


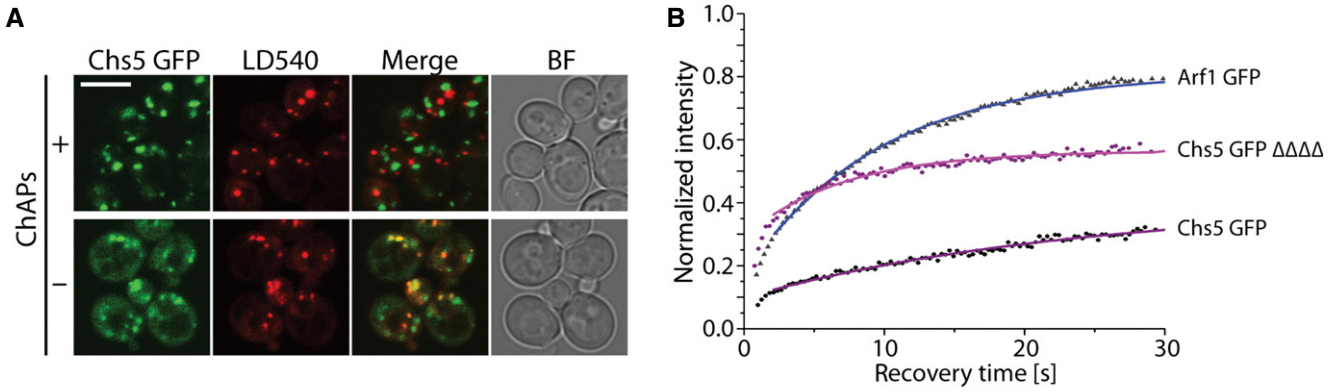
Figure EV2.

Figure EV2. ChAPs distribution is dependent on Chs5.

A Chs5 is necessary for recruitment and stable binding of ChAPs at the TGN. Fluorescence images of cells expressing GFP-tagged ChAPs and TGN marker Sec7-DsRed in Δ*chs5* background. Scale bar, 5 μm.

B Chs5 is necessary for stable binding of ChAPs at the Golgi membranes *in vitro*. Differential centrifugation of cell lysates obtained from Bud7-GFP, Bch1-GFP and Bch2-GFP WT and Δ*chs5* strains. TCL, total cell lysate; S10, 10,000 *g* supernatant; P10, 10,000 *g* pellet; S100, 100,000 *g* supernatant; P100, 100,000 *g* pellet. Anp1 serves as the Golgi marker and Pgk1 as the cytoplasm marker. A representative immunoblot of three independent biological experiments is shown.

Source data are available online for this figure.



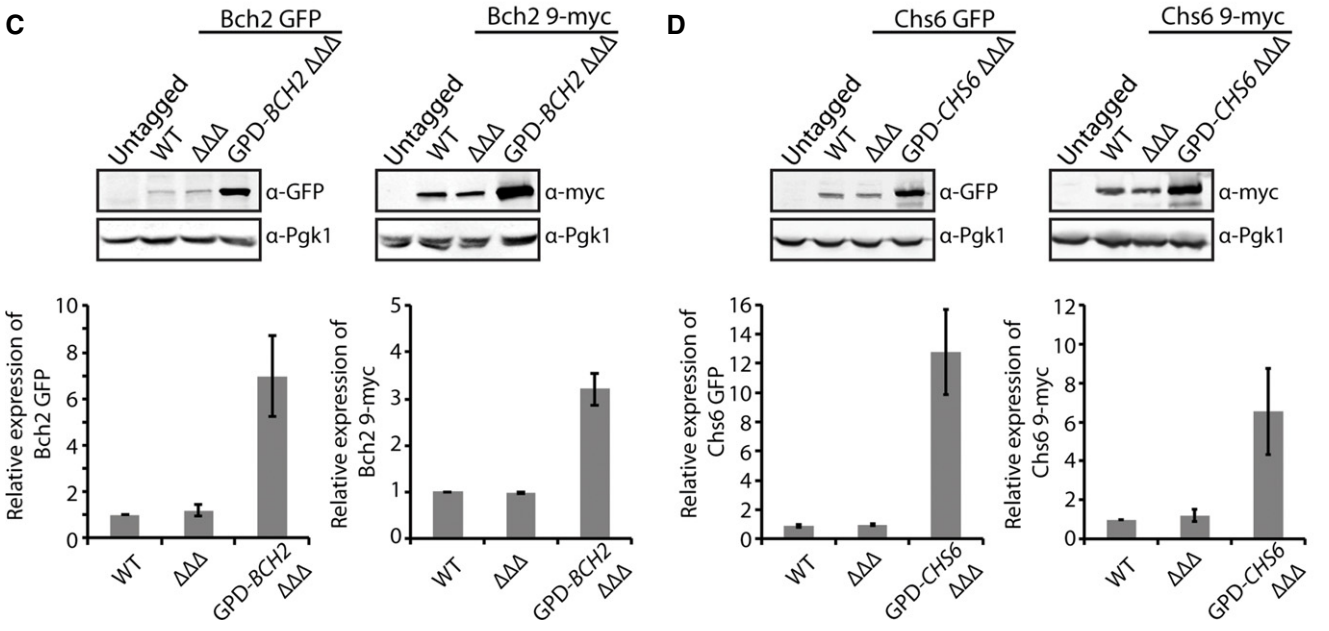


Figure EV3. Analysis of Chs5-GFP and ChAPs expression levels.

A In the absence of ChAPs, Chs5-GFP localizes to the *trans*-Golgi network, cytoplasm, and lipid droplets. Confocal images of strains expressing Chs5-GFP with and without the ChAPs, stained with a lipid droplet staining dye LD540. Scale bar, 5 μm.

B Binding kinetics of Arf1-GFP in the WT and Chs5-GFP in the WT and ΔΔΔΔ strains. The mean of 20–30 FRAP measurements from different cells is shown. Calculated parameters are shown in Table 2.

C Quantification of the relative expression levels of the GFP- and 9-myc-tagged Bch2 in WT, *BCH2* ΔΔΔ, and GPD-*BCH2* ΔΔΔ strains. Representative immunoblots of yeast lysates are shown; Pgk1 serves as a loading control. The plots show average and SD of three independent biological experiments. The relative expression levels are normalized to WT.

D Quantification of the relative expression levels of the GFP- and 9-myc-tagged Chs6 in WT, *CHS6* ΔΔΔ, and GPD-*CHS6* ΔΔΔ strains. Representative immunoblots of yeast lysates are shown; Pgk1 serves as a loading control. The plots show average and SD of three independent biological experiments. The relative expression levels are normalized to WT.

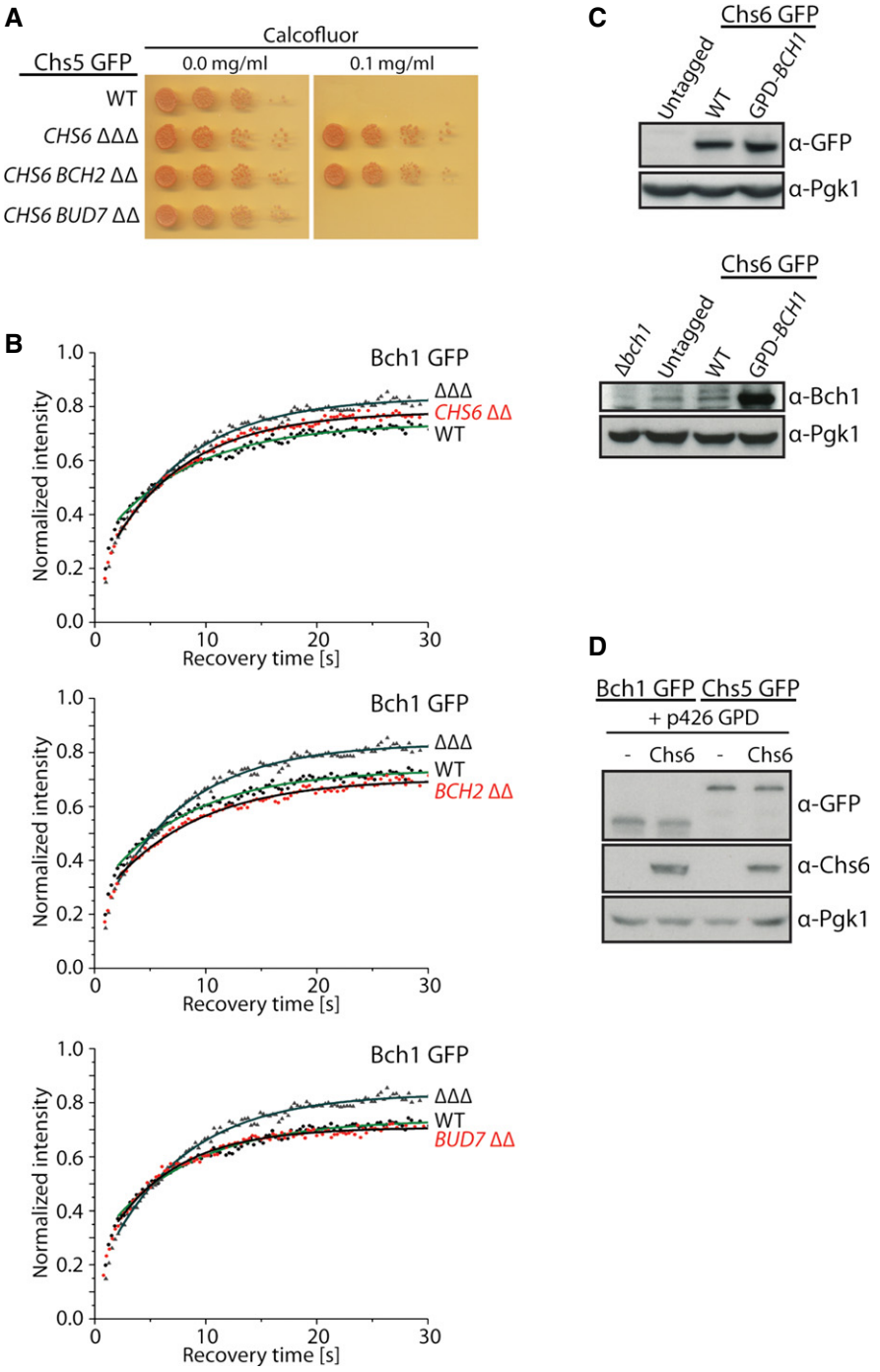


Figure EV4. ChAPs influence each others behavior.

A Chs6 with Bud7 but not Bch2 promotes export of Chs3 to the plasma membrane. Cells bearing Chs6 alone or in combination with Bch2 are resistant to calcofluor, while the combination of Chs6 and Bud7 is sensitive to calcofluor. Plates were incubated at 30°C for 2–3 days.

B Bch1 requires the presence of at least one other ChAP to be stabilized at the *trans*-Golgi network. Binding kinetics of Bch1-GFP in WT, ΔΔΔ, and *ChAP* ΔΔ strains. The mean of 20–30 FRAP measurements from different cells is shown. Calculated parameters are shown in Table 2.

C Comparison of endogenous (WT) and overexpressed (GPD-*BCH1*) Bch1 expression levels in Chs6-GFP cells. Immunoblot of yeast lysates is shown; Pgk1 serves as a loading control.

D Overexpression of Chs6 from GPD-p426 plasmid in Bch1-GFP and Chs5-GFP strains analyzed by immunoblot; Pgk1 serves as a loading control.

Source data are available online for this figure.