Supplemental Materials

Molecular Biology of the Cell

Anton et al.

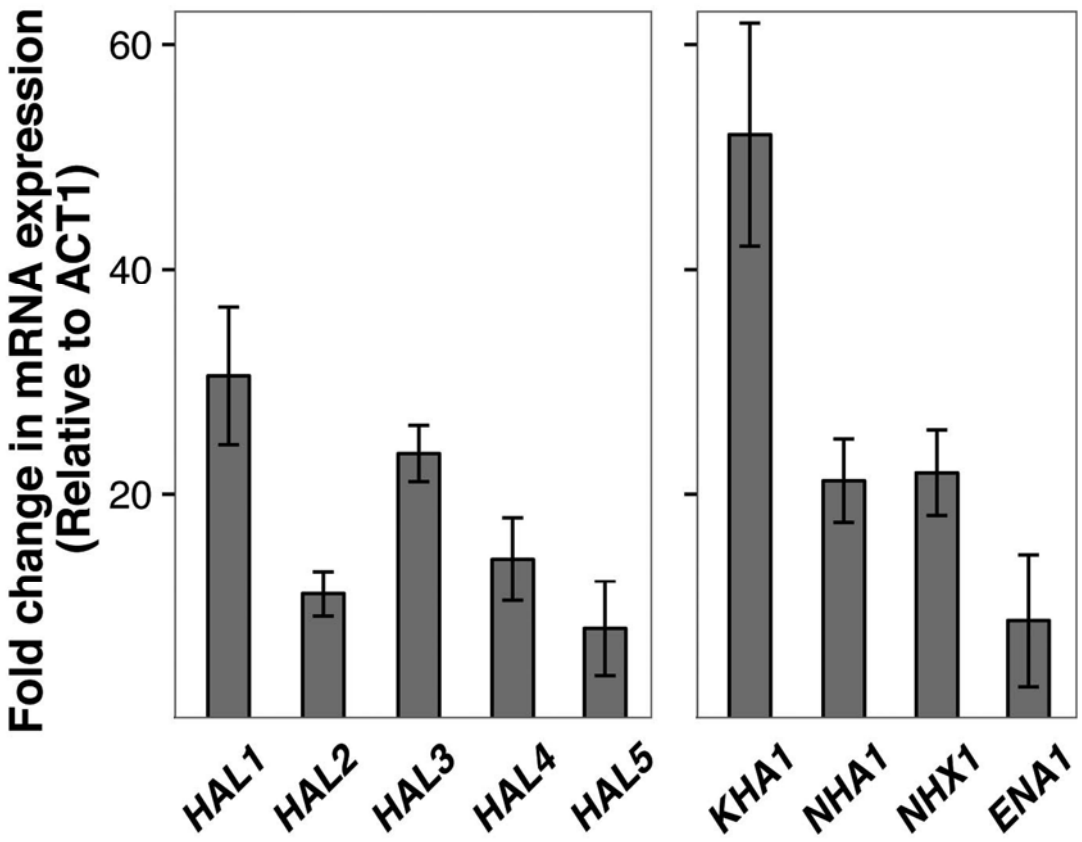

Fig S1: **Quantification of the overexpression levels.** mRNA levels derived for each plasmid were determined in cells growing in SD media using qPCR with dedicated primers for each gene. Values are represented relative to those of the *ACT1* gene measured in the same sample. The values are the average of 4 independent analyses.

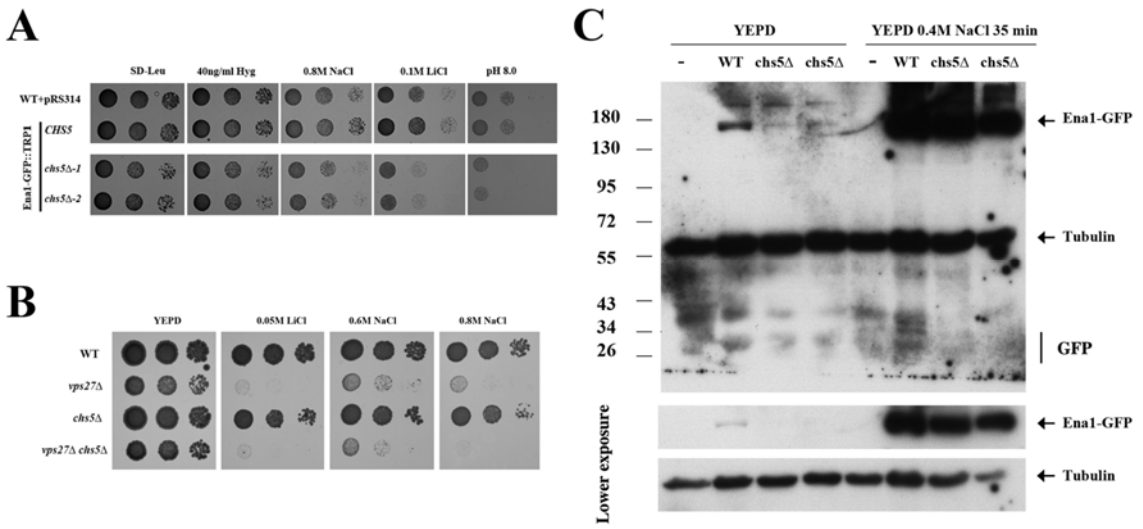

Fig S2: Characterization of Ena1-GFP strains. (A) Sensitivity of the strains containing the *ENA1-GFP::TRP1* construct. Note the normal sensitivity of this strain compared to a wild type and the increased sensitivity to different compounds of the *chs5*Δ mutant in the same genetic background. (B) Sensitivity of the indicated strains to lithium and sodium. Notice the increased sensitivity shown by the double *chs5*Δ *vps27*Δ mutant. (C) Western blot showing Ena1-GFP levels in wild type and *chs5*Δ strain under different growth conditions. Lower images are from the same experiment but with lower exposure time. Note the reduced levels of Ena1-GFP in the *chs5*Δ strains, but also the almost complete absence of degradation in both strains, consistent with the faint vacuolar staining observed (See Figure 5). NaCl treatment clearly increases Ena1 levels in both strains. Note that the experiment was performed in YEPD media in order to detect basal expression levels of Ena1-GFP, undetectable after growth in SD media (see Figure 5B).

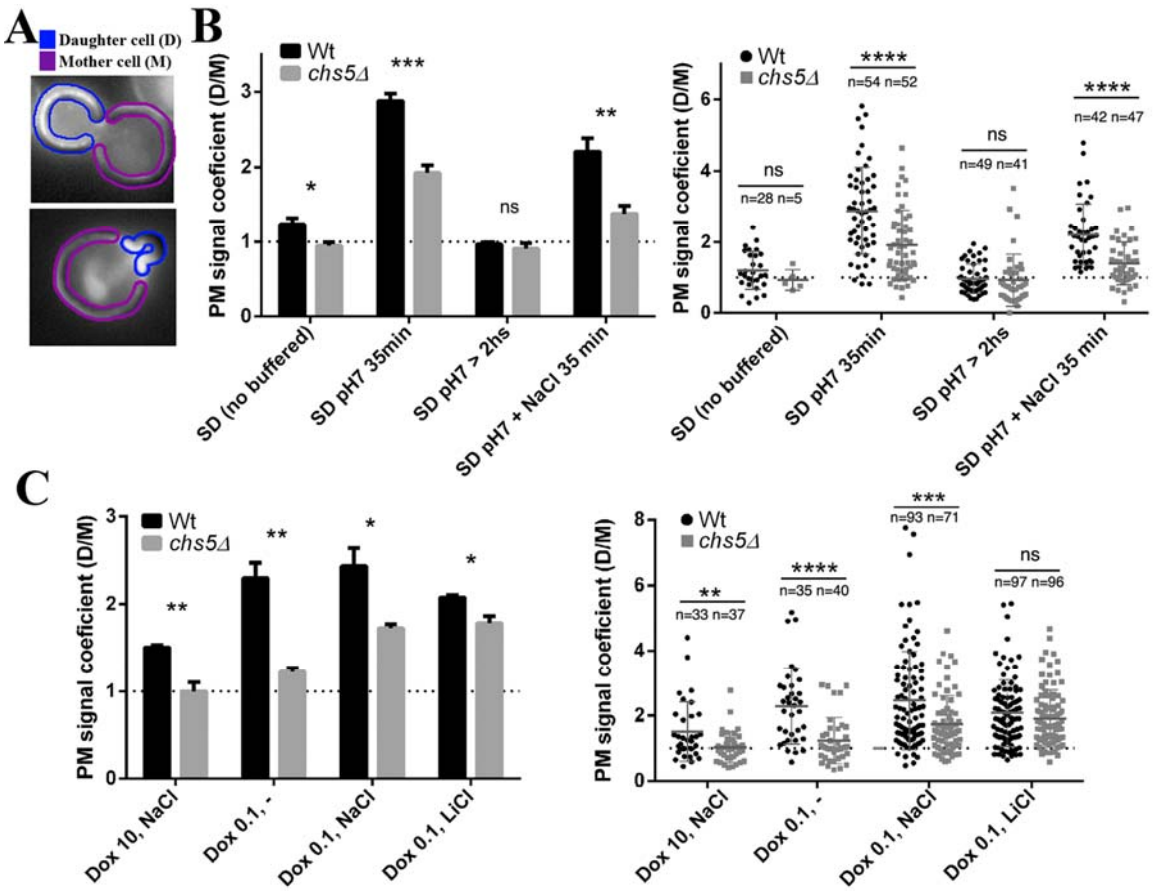

Fig S3:Quantification ofEna1-GFP polarization. (A) Scheme representing the measured area to obtain the Daughter/Mother Polarization Coefficient of Ena1-GFP. (B) Localization of a chromosomally-tagged version of Ena1-GFP in wild type and *chs5*Δ. Cells were grown on selective SD media to logarithmic phase and then incubated under the indicated conditions as described for experiments shown in Figure 5A. Left panel represents of the Ena1-GFP polarization coefficient using the average of the coefficients obtained for any experiment (n=4 experiments). Right panel shows the coefficients for any measured cell (n=number of cells). (C) Ena1-GFP expressed from the tetO promoter was visualized by fluorescence microscopy after growth on the indicated dox concentration for 2 hrs. Cation treatment was performed for additional 30 min. Experimental conditions are identical to those described for Figure 5D. As in (B), left panel represents the average of the coefficient from 3 independent experiments, and right the coefficient for single cells (n=number of cells). The values represented correspond to the mean (top of the bar or central line) and the standard error of the mean (whiskers). The horizontal dashed line indicates the situation of no polarization (daughter and mother

intensities are equal). For each condition, the total number of cells measured (n) is indicated. See materials and methods for details on fluorescence quantification.

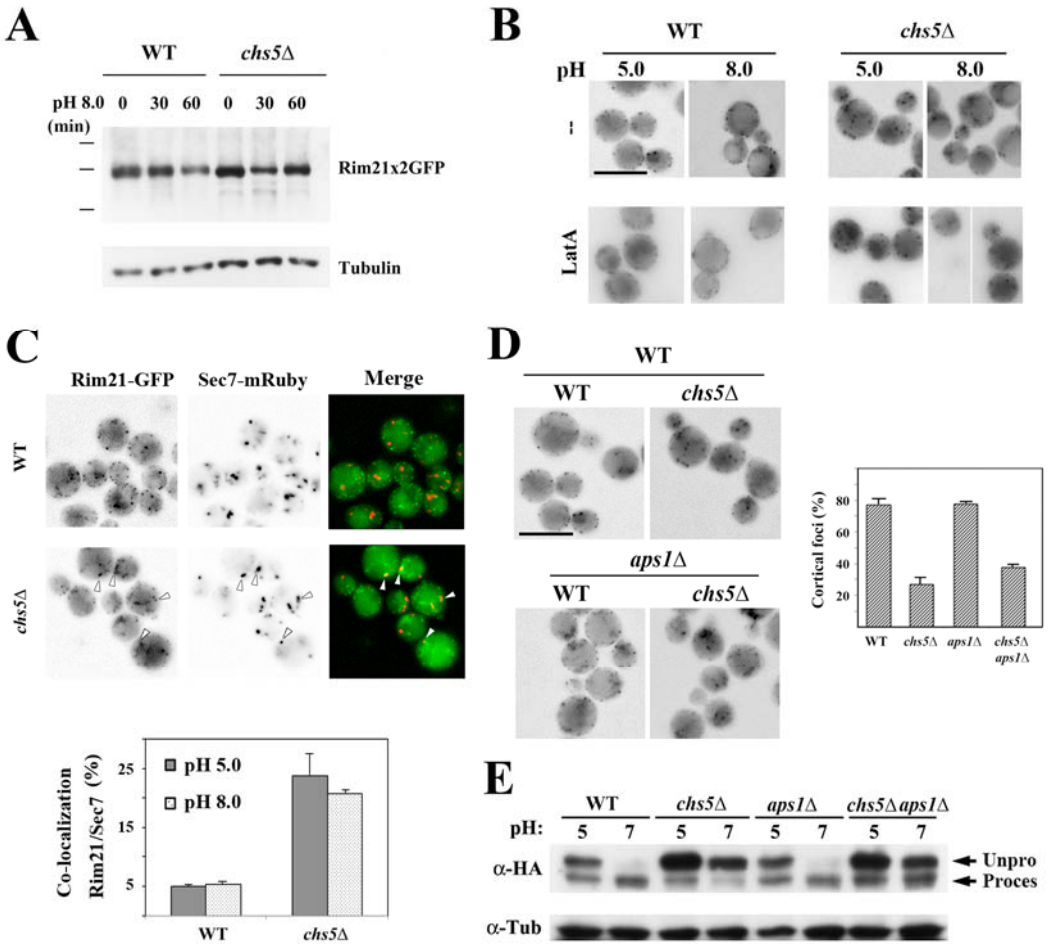

Fig S4: The behavior of Rim21 in the absence of exomer. (A) Rim21-2xGFP levels in the wild-type and *chs5*Δ strains after alkalinization of the media. (B) Localization of Rim21-2xGFP foci in the indicated strains at acidic and alkaline pH, before and after endocytosis block with LatA for 1 hour. (C) Co-localization of Rim21-2xGFP and Sec7-mRuby in wild type and *chs5*Δ strains as indicated. Graph represents the levels of co-localization of the Rim21 foci with Sec7 spots at indicated pH. See the quantification procedure at the Materials and Methods section. (D) Localization of Rim21-2xGFP in the mutant strains at acidic pH. Quantitative results from two independents experiments (n>100) are represented in the graphs, which show percentage of foci associated to the

PM. (E) Induction of the Rim101 processing at pH 7.0 in the indicated mutants. Rim101 processing was determined as described in the materials and methods.