

## Supplemental Materials

**Supplemental movie 1.** Time-lapse video of *chc1* $\Delta$  cells expressing Sla2-GFP (Green) and Abp1-RFP (Red) (SL5226). Images were captured every 2 seconds for 360 seconds and movies were sped up 20x for presentation.

**Supplemental movie 2.** Time-lapse video of *chc1* $\Delta$  cells containing p*CHC1* expressing Sla2-GFP (Green) and Abp1-RFP (Red) (SL5386). Images were captured every 2 seconds for 360 seconds and movies were sped up 20x for presentation.

**Supplemental movie 3.** Time-lapse video of *chc1* $\Delta$  cells containing p*CHC-YR* expressing Sla2-GFP (Green) and Abp1-RFP (Red) (SL5729). Images were captured every 2 seconds for 360 seconds and movies were sped up 20x for presentation.

**Table S1. *Saccharomyces cerevisiae* strains and plasmids**

**A. Yeast strains utilized or generated for studies <sup>a</sup>**

<b>Strain</b>	<b>Genotype</b>	<b>Source or Ref.</b>
BJ3556	<i>MAT<math>\alpha</math> sst1-2 ade2-1 his6 met1 cyh2 rme1 ura1 can1</i>	(1)
YRV19	<i>MAT<math>\alpha</math> ade2-101 his3-<math>\Delta</math>200 leu2-<math>\Delta</math>1 trp1 ura3-52 chs6<math>\Delta</math>:HIS3</i>	(2)
SL249	<i>MAT<math>\alpha</math> leu2 ura3-52 trp1 GAL2 chc1<math>\Delta</math>:LEU2</i>	
SL1463	<i>MAT<math>\alpha</math> leu2 ura3-52 trp1 his3<math>\Delta</math>200 GAL2</i>	
SL1915	<i>MAT<math>\alpha</math> leu2 ura3-52 trp1 his3<math>\Delta</math>200 GAL2 clc1<math>\Delta</math>:HIS3 pRS316-CLC1</i>	
SL1916	<i>MAT<math>\alpha</math> leu2 ura3-52 trp1 his3<math>\Delta</math>200 GAL2 clc1<math>\Delta</math>:HIS3 pYCp50</i>	
SL1917	<i>MAT<math>\alpha</math> leu2 ura3-52 trp1 his3<math>\Delta</math>200 GAL2 clc1<math>\Delta</math>:HIS3 pYCp50-CHC1</i>	
SL5226	<i>MAT<math>\alpha</math> leu2 ura3-52 trp1 his3<math>\Delta</math>200 chc1<math>\Delta</math>:LEU2 sla2<math>\Delta</math>:LEU2 Abp1-RFP::KanMx6 p111-SLA2-GFP:TRP1</i>	(3)
SL5386	<i>MAT<math>\alpha</math> leu2 ura3-52 trp1 his3<math>\Delta</math>200 chc1<math>\Delta</math>:LEU2 sla2<math>\Delta</math>:LEU2 Abp1-RFP:KanMx6 YCp50-CHC1 (pCHC1) p111-SLA2-GFP:TRP1</i>	
SL5729	<i>MAT<math>\alpha</math> leu2 ura3-52 trp1 his3<math>\Delta</math>200 chc1<math>\Delta</math>:LEU2 sla2<math>\Delta</math>:LEU2 Abp1-RFP:KanMx6 YCp50-CHC-YR (pCHC-YR) p111-SLA2-GFP:TRP1</i>	

<b>Strain</b>	<b>Genotype</b>	<b>Source or Ref.</b>
SL5936	<i>MAT<math>\alpha</math> leu2 ura3-52 trp1 his3<math>\Delta</math>200 GAL2 clc1<math>\Delta</math>:HIS3 YCp50-CHC-YR (pCHC-YR)</i>	
SL6049	<i>MAT<math>\alpha</math> leu2 ura3-52 trp1 his3-<math>\Delta</math>200 vrp1<math>\Delta</math>:LEU2 clc1<math>\Delta</math>19-76:NatMX6</i>	
SL6052	<i>MAT<math>\alpha</math> leu2 ura3-52 trp1 his3/4? rvs167<math>\Delta</math>:TRP1 clc1<math>\Delta</math>19-76:NatMX6</i>	
SL6971	<i>MAT<math>\alpha</math> leu2 ura3-52 trp1 chc1<math>\Delta</math>:LEU2 YCp50</i>	
SL6972	<i>MAT<math>\alpha</math> leu2 ura3-52 trp1 chc1<math>\Delta</math>:LEU2 YCp50-CHC1 (pCHC1)</i>	
SL6973	<i>MAT<math>\alpha</math> leu2 ura3-52 trp1 chc1<math>\Delta</math>:LEU2 YCp50-CHC-YR (pCHC-YR)</i>	
SL6974	<i>MAT<math>\alpha</math> leu2 ura3 trp1 his3? chc1<math>\Delta</math>:LEU2 clc1<math>\Delta</math>:HIS3 YCp50</i>	
SL6975	<i>MAT<math>\alpha</math> leu2 ura3 trp1 his3? chc1<math>\Delta</math>:LEU2 clc1<math>\Delta</math>:HIS3 YCp50-CHC1 (pCHC1)</i>	
SL6976	<i>MAT<math>\alpha</math> leu2 ura3 trp1 his3? chc1<math>\Delta</math>:LEU2 clc1<math>\Delta</math>:HIS3 YCp50-CHC-YR (pCHC-YR)</i>	
SL6999	<i>MAT<math>\alpha</math> leu2 ura3-52 trp1 his3<math>\Delta</math>200 GAL2 chc1<math>\Delta</math>:LEU2 Abp1-RFP:KanMx6 GFP-CLC1 YCp50</i>	(4)
SL7000	<i>MAT<math>\alpha</math> leu2 ura3-52 trp1 his3<math>\Delta</math>200 GAL2 chc1<math>\Delta</math>:LEU2 Abp1-RFP:KanMx6 GFP-CLC1 YCp50-CHC1 (pCHC1)</i>	
SL7001	<i>MAT<math>\alpha</math> leu2 ura3-52 trp1 his3<math>\Delta</math>200 GAL2 chc1<math>\Delta</math>:LEU2 Abp1-RFP:KanMx6 GFP-CLC1 YCp50-CHC-YR (pCHC-YR)</i>	
SL7100	<i>MAT<math>\alpha</math> leu2 ura3-52 trp1 GAL2 chc1<math>\Delta</math>:LEU2 YCp50</i>	
SL7101	<i>MAT<math>\alpha</math> leu2 ura3-52 trp1 GAL2 chc1<math>\Delta</math>:LEU2 YCp50-CHC1 (pCHC1)</i>	
SL7102	<i>MAT<math>\alpha</math> leu2 ura3-52 trp1 GAL2 chc1<math>\Delta</math>:LEU2 YCp50-CHC-YR (pCHC-YR)</i>	
SL7103	<i>MAT<math>\alpha</math> leu2 ura3-52 trp1 his3-<math>\Delta</math>200 chs6<math>\Delta</math>:HIS3 chc1<math>\Delta</math>:LEU2 YCp50</i>	
SL7104	<i>MAT<math>\alpha</math> leu2 ura3-52 trp1 his3-<math>\Delta</math>200 chs6<math>\Delta</math>:HIS3 chc1<math>\Delta</math>:LEU2 YCp50-CHC1 (pCHC1)</i>	
SL7105	<i>MAT<math>\alpha</math> leu2 ura3-52 trp1 his3<math>\Delta</math>200 chs6<math>\Delta</math>:HIS3 chc1<math>\Delta</math>:LEU2 YCp50-CHC-YR (pCHC-YR)</i>	
SL7107	<i>MAT<math>\alpha</math> leu2 ura3-52 trp1 his3-<math>\Delta</math>200 GAL2 chc1<math>\Delta</math>:LEU2 clc1<math>\Delta</math>:HIS3 YCp50</i>	
SL7108	<i>MAT<math>\alpha</math> leu2 ura3-52 trp1 his3-<math>\Delta</math>200 GAL2 chc1<math>\Delta</math>:LEU2 clc1<math>\Delta</math>:HIS3 YCp50-CHC1 (pCHC1)</i>	

<b>Strain</b>	<b>Genotype</b>	<b>Source or Ref.</b>
SL7109	<i>MAT<math>\alpha</math> leu2 ura3-52 trp1 his3-<math>\Delta</math>200 GAL2 chc1<math>\Delta</math>:LEU2 clc1<math>\Delta</math>:HIS3 YCp50-CHC-YR (pCHC-YR)</i>	
SL7111	<i>MAT<math>\alpha</math> leu2 ura3-52 trp1 GAL2 chc1<math>\Delta</math>:LEU2 pCHC-YR-GFP:TRP1</i>	
SL7116	<i>MAT<math>\alpha</math> leu2 ura3-52 trp1 GAL2 chc1<math>\Delta</math>:LEU2 pCHC-YR-GFP:TRP1 pRS316-SEC7-DsRed</i>	
SL7125	<i>MAT<math>\alpha</math> leu2 ura3 trp1 his3-<math>\Delta</math>200 vrp1<math>\Delta</math>:LEU2 clc1<math>\Delta</math>:HIS3 YCp50</i>	
SL7126	<i>MAT<math>\alpha</math> leu2 ura3 trp1 his3<math>\Delta</math>-200 vrp1<math>\Delta</math>:LEU2 clc1<math>\Delta</math>:HIS3 YCp50-CHC1 (pCHC1)</i>	
SL7127	<i>MAT<math>\alpha</math> leu2 ura3 trp1 his3<math>\Delta</math>-200 vrp1<math>\Delta</math>:LEU2 clc1<math>\Delta</math>:HIS3 YCp50-CHC-YR (pCHC-YR)</i>	
SL7131	<i>MAT<math>\alpha</math> leu2 ura3 trp1 his3<math>\Delta</math>-200 rvs167<math>\Delta</math>:TRP1 clc1<math>\Delta</math>:HIS3 YCp50</i>	
SL7132	<i>MAT<math>\alpha</math> leu2 ura3 trp1 his3<math>\Delta</math>-200 rvs167<math>\Delta</math>:TRP1 clc1<math>\Delta</math>:HIS3 YCp50-CHC1 (pCHC1)</i>	
SL7133	<i>MAT<math>\alpha</math> leu2 ura3 trp1 his3<math>\Delta</math>-200 rvs167<math>\Delta</math>:TRP1 clc1<math>\Delta</math>:HIS3 YCp50-CHC-YR (pCHC-YR)</i>	
SL7236	<i>MAT<math>\alpha</math> leu2 ura3-52 trp1 his3-<math>\Delta</math>200 GAL2 chc1<math>\Delta</math>:LEU2 clc1<math>\Delta</math>:HIS3 p111SLA2-GFP:TRP1</i>	
SL7237	<i>MAT<math>\alpha</math> leu2 ura3-52 trp1 his3-<math>\Delta</math>200 GAL2 chc1<math>\Delta</math>:LEU2 clc1<math>\Delta</math>:HIS3 YCp50-CHC1 (pCHC1) p111SLA2-GFP:TRP1</i>	
SL7238	<i>MAT<math>\alpha</math> leu2 ura3-52 trp1 his3-<math>\Delta</math>200 GAL2 chc1<math>\Delta</math>:LEU2 clc1<math>\Delta</math>:HIS3 YCp50-CHC-YR (pCHC-YR) p111SLA2-GFP:TRP1</i>	
SL7239	<i>MAT<math>\alpha</math> leu2 ura3-52 trp1 his3-<math>\Delta</math>200 GAL2 chc1<math>\Delta</math>:LEU2 clc1<math>\Delta</math>:HIS3 YCp50 pKEX2-GFP-TRP1</i>	
SL7240	<i>MAT<math>\alpha</math> leu2 ura3-52 trp1 his3-<math>\Delta</math>200 GAL2 chc1<math>\Delta</math>:LEU2 clc1<math>\Delta</math>:HIS3 YCp50-CHC1 (pCHC1) pKEX2-GFP-TRP1</i>	
SL7241	<i>MAT<math>\alpha</math> leu2 ura3-52 trp1 his3-<math>\Delta</math>200 GAL2 chc1<math>\Delta</math>:LEU2 clc1<math>\Delta</math>:HIS3 YCp50-CHC-YR (pCHC-YR) pKEX2-GFP-TRP1</i>	

## **B. Plasmids utilized or generated for these studies <sup>a</sup>**

Description	Source or Ref.
<i>YCp50-CHC1 (CEN, URA3; pCHC1)</i>	(5)
<i>YCp50-CHC-YR (CEN, URA3; pCHC-YR)</i>	
<i>pRS316-CLC1 (CEN, URA3; pKH4)</i>	(6)
<i>p111SLA2-GFP:TRP1 (CEN, TRP1, LEU2)</i>	(3)
<i>pKEX2-GFP-TRP1 (2 micron, TRP1)</i>	
<i>pRS316-SEC7-DsRed (CEN, URA3; pRC2240)</i>	(7)

*pCHC-YR-GFP:TRP1 (CEN, TRP; URA3 gene in YCp50 deleted)*

<sup>a</sup> Strains and plasmids were generated in our laboratory unless otherwise noted. All yeast strains are *scd1-v*, which confers viability to clathrin-deficient yeast.

#### Supplemental References.

1. Chan RK, Otte CA. Isolation and genetic analysis of *Saccharomyces cerevisiae* mutants supersensitive to G1 arrest by a factor and alpha factor pheromones. *Mol Cell Biol* 1982;2(1):11-20.
2. Valdivia RH, Schekman R. The yeasts Rho1p and Pkc1p regulate the transport of chitin synthase III (Chs3p) from internal stores to the plasma membrane. *Proc Natl Acad Sci U S A* 2003;100(18):10287-10292.
3. Newpher TM, Lemmon SK. Clathrin is important for normal actin dynamics and progression of Sla2p-containing patches during endocytosis in yeast. *Traffic* 2006;7(5):574-588.
4. Collette JR, Chi RJ, Boettner DR, Fernandez-Golbano IM, Plemel R, Merz AJ, Geli MI, Traub LM, Lemmon SK. Clathrin functions in the absence of the terminal domain binding site for adaptor-associated clathrin-box motifs. *Mol Biol Cell* 2009;20(14):3401-3413.
5. Lemmon S, Lemmon VP, Jones EW. Characterization of yeast clathrin and anticlathrin heavy-chain monoclonal antibodies. *J Cell Biochem* 1988;36(4):329-340.
6. Huang KM, Gullberg L, Nelson KK, Stefan CJ, Blumer K, Lemmon SK. Novel functions of clathrin light chains: clathrin heavy chain trimerization is defective in light chain-deficient yeast. *J Cell Sci* 1997;110 ( Pt 7):899-910.
7. Calero M, Chen CZ, Zhu W, Winand N, Havas KA, Gilbert PM, Burd CG, Collins RN. Dual prenylation is required for Rab protein localization and function. *Mol Biol Cell* 2003;14(5):1852-1867.