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Appendix Table S1. Calculated parameters derived from FRAP analysis of ChAPs at the TGN and in the cytoplasm in $\Delta chs5$ strain background. The mean \pm SEM is shown for diffusion coefficient D_f and immobile fraction F_{im} . For the detailed calculation procedures see Materials and Methods section.

Analyzed protein	Strain background	D_f ($\mu\text{m}^2 \cdot \text{s}^{-1}$)		F_{im} (%)	
		TGN	cytoplasm	TGN	cytoplasm
Chs6 GFP	$\Delta chs5$	0.110 \pm 0.008	0.325 \pm 0.030	0.5 \pm 1.2	4.9 \pm 1.4
Bud7 GFP	$\Delta chs5$	0.095 \pm 0.009	0.182 \pm 0.015	1.4 \pm 1.6	4.5 \pm 1.4
Bch1 GFP	$\Delta chs5$	0.145 \pm 0.011	0.269 \pm 0.029	2.2 \pm 1.4	3.2 \pm 0.1
Bch2 GFP	$\Delta chs5$	0.140 \pm 0.020	0.234 \pm 0.020	6.6 \pm 2.0	3.8 \pm 1.5

Appendix Table S2. Colocalization analysis of Chs5 GFP with the TGN marker Sec7 DsRed. The mean \pm SD is shown.

Analyzed protein	Strain background	Manders coefficients	
		M1- Green to Red	M2- Red to Green
Chs5 GFP	WT	0.81 \pm 0.11	0.86 \pm 0.06
	<i>CHS6</i> $\Delta\Delta\Delta$	0.22 \pm 0.07	0.70 \pm 0.02
	<i>BUD7</i> $\Delta\Delta\Delta$	0.38 \pm 0.07	0.85 \pm 0.10
	<i>BCH1</i> $\Delta\Delta\Delta$	0.87 \pm 0.07	0.85 \pm 0.06
	<i>BCH2</i> $\Delta\Delta\Delta$	0.34 \pm 0.07	0.76 \pm 0.11
	$\Delta\Delta\Delta\Delta$	0.40 \pm 0.09	0.82 \pm 0.11

Appendix Table S3. Strains used in the study

Strain number	Designation	Genotype and mutations	Source
YPH499		<i>MAT a ade2 his3 leu2 lys1 trp1 ura3</i>	Sikorski and Hieter, 1989
YPH500		<i>MAT α ade2 his3 leu2 lys1 trp1 ura3</i>	Sikorski and Hieter, 1989
YAS431	<i>Δchs5</i>	<i>chs5::LEU2</i>	Trautwein et al. 2006
YAS563-2A	<i>Δchs6</i>	<i>chs6::URA3</i>	Trautwein et al. 2006
YAS563-5A	<i>Δbch1</i>	<i>bch1::HIS5</i>	Trautwein et al. 2006
YAS3302	Chs5 GFP	<i>CHS5::CHS5-yEGFP(TRP1)</i>	This study
YAS3303	Chs6 GFP	<i>CHS6::CHS6-yEGFP(TRP1)</i>	This study
YAS3304	Bud7 GFP	<i>BUD7::BUD7-yEGFP(TRP1)</i>	This study
YAS3305	Bch1 GFP	<i>BCH1::BCH1-yEGFP(TRP1)</i>	This study
YAS3306	Bch2 GFP	<i>BCH2::BCH2-yEGFP(TRP1)</i>	This study
YAS3859	Chs6 GFP <i>Δchs5</i>	<i>CHS6::CHS6-yEGFP(TRP1) chs5::URA3</i>	This study
YAS3864	Bud7 GFP <i>Δchs5</i>	<i>BUD7::BUD7-yEGFP(TRP1) chs5::URA3</i>	This study
YAS3865	Bch1 GFP <i>Δchs5</i>	<i>BCH1::BCH1-yEGFP(TRP1) chs5::URA3</i>	This study
YAS3866	Bch2 GFP <i>Δchs5</i>	<i>BCH2::BCH2-yEGFP(TRP1) chs5::URA3</i>	This study
YAS3315	Cop1 GFP	<i>COP1::COP1-yEGFP(TRP1)</i>	This study
YAS3839	Sec28 GFP	<i>SEC28::SEC28-yEGFP(TRP1)</i>	This study
YAS3842	Clc1 GFP	<i>CLC1::CLC1-yEGFP(TRP1)</i>	This study
YAS3843	Apm1 GFP	<i>APM1::APM1-yEGFP(TRP1)</i>	This study
YAS328	Chs6 9myc	<i>CHS6::CHS6-9myc(TRP1)</i>	Trautwein et al. 2006
YAS339	Bch1 9myc	<i>BCH1::BCH1-9myc(TRP1)</i>	Trautwein et al. 2006
YAS589	Bch2 9myc	<i>BCH2::BCH2-9myc(TRP1)</i>	Trautwein et al. 2006
YAS4143	Chs5 GFP <i>ΔΔΔΔ</i>	<i>CHS5::CHS5-yEGFP(TRP1) chs6::URA3 bch2::kanMX bch1::HIS5 bud7::natNT2</i>	This study
YAS4361	Chs5 GFP <i>CHS6 ΔΔΔ</i>	<i>CHS5::CHS5-yEGFP(TRP1) bch1::HIS5 bud7::URA3 bch2::natNT2</i>	This study
YAS4137	Chs5 GFP <i>BUD7 ΔΔΔ</i>	<i>CHS5::CHS5-yEGFP(TRP1) chs6::URA3 bch2::kanMX bch1::HIS5</i>	This study
YAS4544	Chs5 GFP <i>BCH1 ΔΔΔ</i>	<i>CHS5::CHS5-yEGFP(TRP1) bud7::URA3 chs6::HIS5 bch2::natNT2</i>	This study
YAS4134	Chs5 GFP <i>BCH2 ΔΔΔ</i>	<i>CHS5::CHS5-yEGFP(TRP1) bud7::URA3 chs6::HIS5 bch1::natNT2</i>	This study
YAS4368	Chs5 GFP <i>GPD-BCH2 ΔΔΔ</i>	<i>CHS5::CHS5-yEGFP(TRP1) BCH2::GPD(kanMX4)-BCH2 bud7::URA3 chs6::HIS5 bch1::natNT2</i>	This study
YAS4159	Chs5 GFP <i>GPD-CHS6 ΔΔΔ</i>	<i>CHS5::CHS5-yEGFP(TRP1) CHS6::GPD(kanMX4)-CHS6 bud7::URA3 bch2::HIS5 bch1::natNT2</i>	This study
YAS4513	Chs5 GFP <i>CHS6(PSSF) ΔΔΔ</i>	<i>CHS5::CHS5-yEGFP(TRP1) bch2::HIS5 bch1::hphNTI CHS6::CHS6(PSSF) bud7::URA3</i>	This study
YAS4512	Chs5 GFP <i>CHS6(PSSF) BUD7 ΔΔ</i>	<i>CHS5::CHS5-yEGFP(TRP1) bch2::HIS5 bch1::hphNTI CHS6::CHS6(PSSF)</i>	This study
YAS4129	Chs5 GFP <i>CHS6 BUD7 ΔΔ</i>	<i>CHS5::CHS5-yEGFP(TRP1) bch2::HIS5 bch1::natNT2</i>	This study
YAS4356	Chs5 GFP <i>CHS6 BCH2 ΔΔ</i>	<i>CHS5::CHS5-yEGFP(TRP1) bch1::HIS5 bud7::URA3</i>	This study
YAS3871	Chs6 GFP <i>ΔΔΔ</i>	<i>CHS6::CHS6-yEGFP(TRP1) bch2::HIS5 bud7::URA3 bch1::natNT2</i>	This study

YAS4349	GPD-Chs6 GFP $\Delta\Delta\Delta$	<i>CHS6::GPD(kanMX4)-CHS6-yEGFP(TRP1) bch2::HIS5 bud7::URA3 bch1::natNT2</i>	This study
YAS653	Chs6 9myc $\Delta\Delta\Delta$	<i>CHS6::CHS6-9myc(TRP1) bud7::LEU2 bch2::kanMX bch1::HIS5</i>	Trautwein et al. 2006
YAS4161	GPD-Chs6 9myc $\Delta\Delta\Delta$	<i>CHS6::GPD(natNT2)-CHS6-9myc(TRP1) bud7::LEU2 bch2::kanMX bch1::HIS5</i>	This study
YAS3890	Bud7 GFP $\Delta\Delta\Delta$	<i>BUD7::BUD7-yEGFP(TRP1) chs6::URA3 bch2::kanMX bch1::HIS5</i>	This study
YAS3872	Bch1 GFP $\Delta\Delta\Delta$	<i>BCH1::BCH1-yEGFP(TRP1) bud7::URA3 chs6::HIS5 bch2::kanMX</i>	This study
YAS3883	Bch2 GFP $\Delta\Delta\Delta$	<i>BCH2::BCH2-yEGFP(TRP1) bud7::URA3 bch1::HIS5 chs6::natNT2</i>	This study
YAS4500	GPD-Bch2 GFP $\Delta\Delta\Delta$	<i>BCH2::GPD(kanMX4)-BCH2-yEGFP(TRP1) bud7::URA3 bch1::HIS5 chs6::natNT2</i>	This study
YAS654	Bch2 9-myc $\Delta\Delta\Delta$	<i>BCH2::BCH2-9myc (TRP1) chs6::URA3 bud7::LEU2 bch1::HIS5</i>	Trautwein et al. 2006
YAS4499	GPD-Bch2 9-myc $\Delta\Delta\Delta$	<i>BCH2::GPD(natNT2)-BCH2-9myc (TRP1) chs6::URA3 bud7::LEU2 bch1::HIS5</i>	This study
YAS3879	Bch1 GFP <i>CHS6</i> $\Delta\Delta$	<i>BCH1::BCH1-yEGFP(TRP1) bud7::URA3 bch2::kanMX</i>	This study
YAS3904	Bch1 GFP <i>BUD7</i> $\Delta\Delta$	<i>BCH1::BCH1-yEGFP(TRP1) bch2::HIS5 chs6::kanMX</i>	This study
YAS3851	Bch1 GFP <i>BCH2</i> $\Delta\Delta$	<i>BCH1::BCH1-yEGFP(TRP1) bud7::URA3 chs6::HIS5</i>	This study
YAS921	Chs3 GFP	<i>CHS3::CHS3-yEGFP(kanMX4)</i>	Trautwein et al. 2006
YAS4351	Chs3 GFP GPD- <i>BCH1</i>	<i>CHS3::CHS3-yEGFP(kanMX4) BCH1::GPD(natNT2)-BCH1</i>	This study
YAS4491	Chs6 GFP GPD- <i>BCH1</i>	<i>CHS6::CHS6-yEGFP(TRP1) BCH1::GPD(natNT2)-BCH1</i>	This study
YAS4634	Chs3 GFP <i>CHS6</i> $\Delta\Delta\Delta$	<i>CHS3::CHS3-yEGFP(TRP1) bch1::HIS5 bud7::LEU2 bch2::kanMX</i>	This study
YAS4638	Chs3 GFP GPD- <i>CHS6</i> $\Delta\Delta\Delta$	<i>CHS3::CHS3-yEGFP(TRP1) CHS6::GPD(natNT2)-CHS6 bch1::HIS5 bud7::LEU2 bch2::kanMX</i>	This study

MAT α ade2 his3 leu2 lys1 trp1 ura3

YAS216	Arf1 GFP	<i>ARF1::ARF1-yEGFP (kanMX4)</i>	This study
YAS1905	Pin2 GFP	<i>PIN2::PIN2-yEGFP (kanMX4)</i>	Ritz et al. 2014
YAS4488	Pin2 GFP GPD- <i>BCH1</i>	<i>PIN2::PIN2-yEGFP (kanMX4) BCH1::GPD(natNT2)-BCH1</i>	This study
YAS576	Bud7 9myc	<i>BUD7::BUD7-9myc (TRP1)</i>	Trautwein et al. 2006
YAS601	Chs5 9myc	<i>CHS5::CHS5-9myc (TRP1)</i>	This study