Thomas et al., https://doi.org/10.1083/jcb.201705214

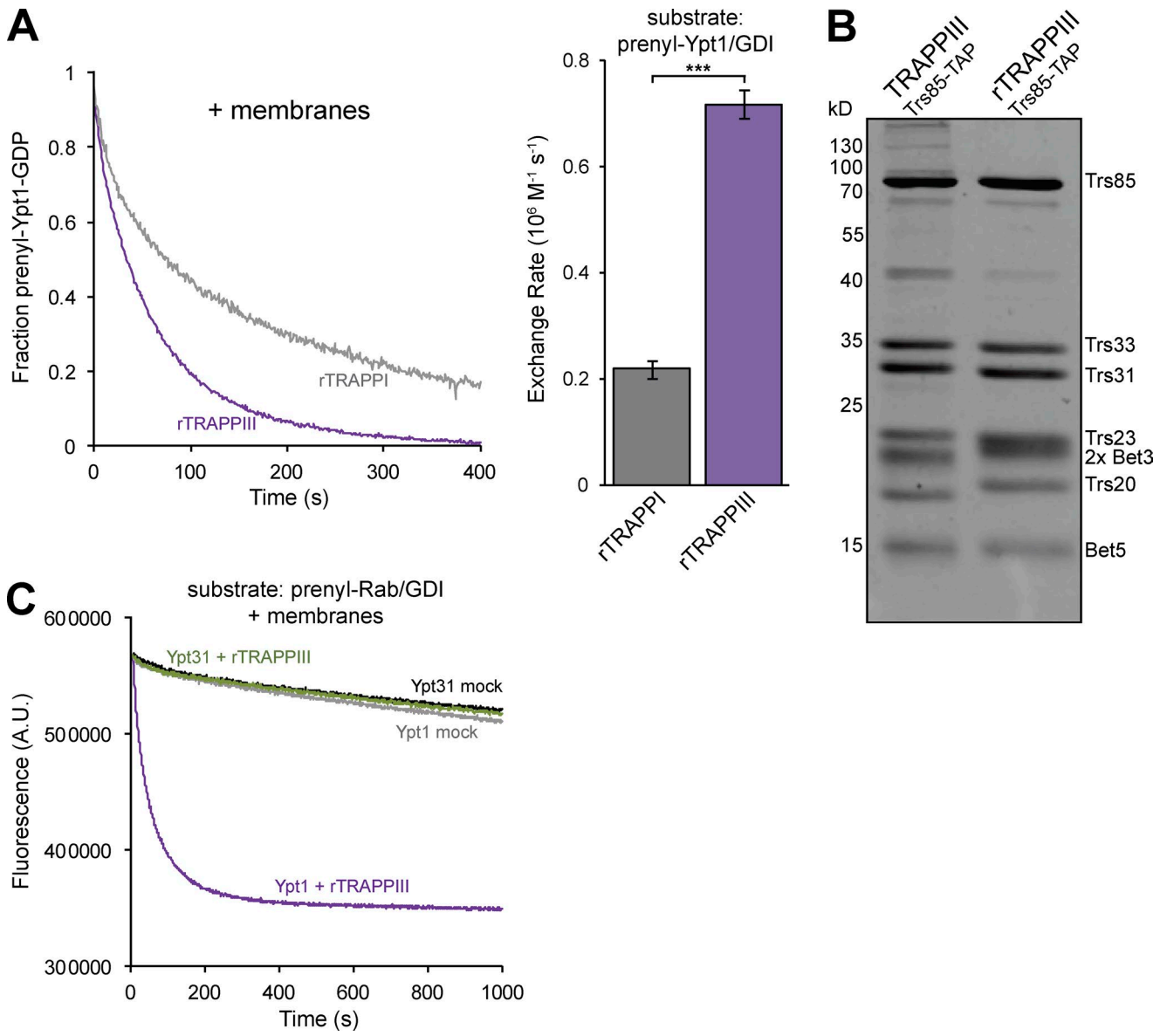

Figure S1. **TRAPPIII is a specific GEF for Ypt1.** (A, left) Normalized representative traces showing activation of prenylated Ypt1 by rTRAPPI and rTRAPPIII in the presence of phosphatidylcholine liposomes. (A, right) Rates of rTRAPP-mediated Ypt1 activation quantified from the traces at left. Error bars represent 95% CIs. $n \geq 3$ reactions. (B) Endogenous and rTRAPPIII was purified from yeast or *E. coli*, respectively, using TAP-tagged Trs85. (C) Representative raw traces showing activation of prenylated Rab substrates by rTRAPPIII in the presence of TGN liposomes. "Mock" traces are buffer-only control reactions. ***, $P < 0.001$ for unpaired two-tailed *t* test with Welch's correction.

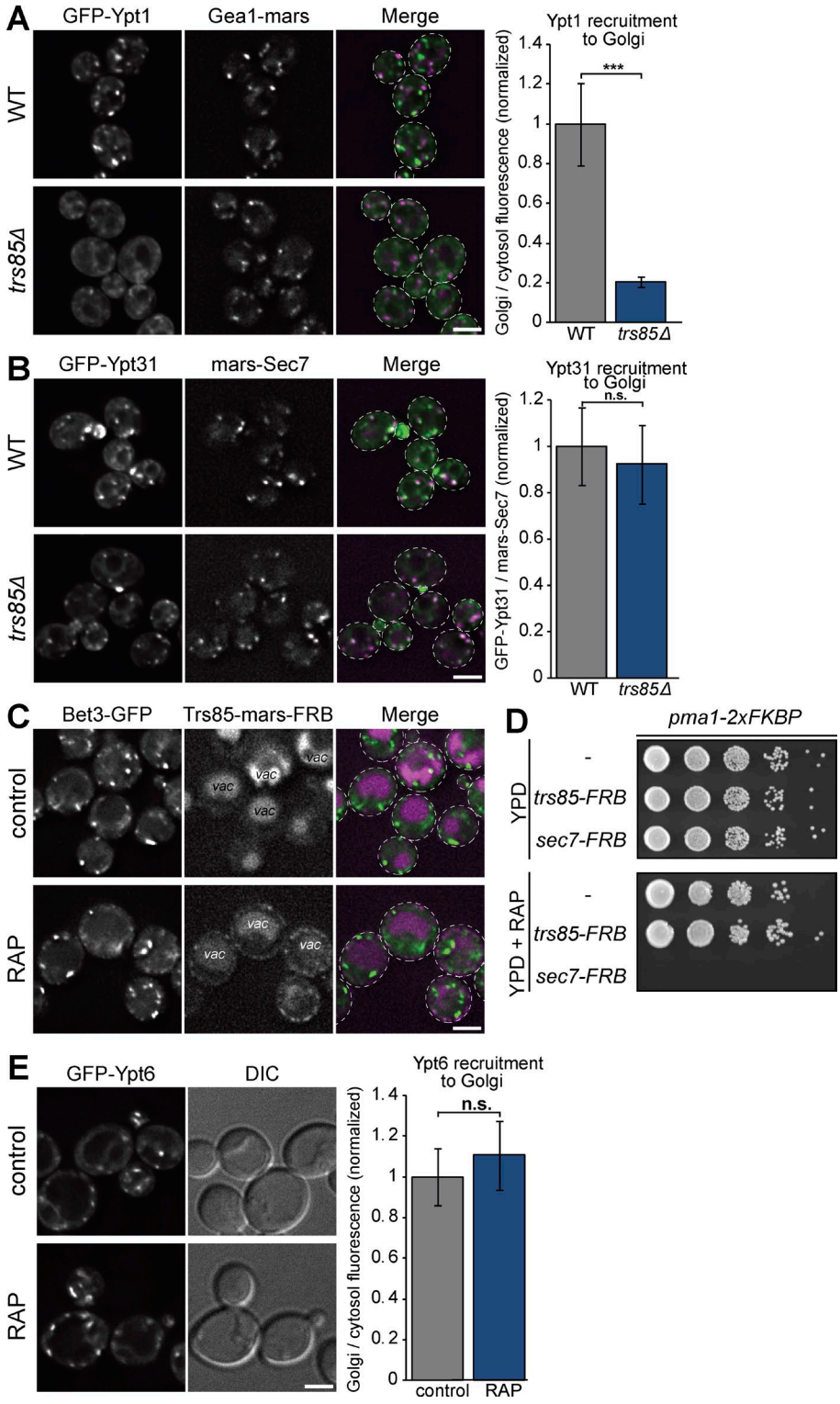

Figure S2. **Loss of TRAPPIII at the Golgi specifically affects Ypt1 activation.** (A, left) Localization of plasmid-borne GFP-Ypt1 relative to the early Golgi Arf-GEF Gea1-3×mRFPmars in WT versus *trs85Δ* mutant cells grown at 30°C. (A, right) Quantification of Golgi and cytosolic Ypt1 in WT versus *trs85Δ* cells. Error bars represent 95% CIs. n = 16 cells. (B, left) Localization of an extra copy of GFP-Ypt31 relative to the late Golgi/TGN marker mRFPmars-Sec7 in WT versus *trs85Δ* cells. (B, right) Recruitment of Ypt31 to Golgi compartments was measured by quantifying the ratio of GFP-Ypt31 to mRFPmars-Sec7 in mRFPmars-Sec7 puncta. Error bars represent 95% CIs. $n \geq 60$ Golgi compartments. (C) Localization of Trs85-mRFPmars-FRB and Bet3-GFP in cells expressing the PM hook Pma1-2×FKBP treated or not treated with rapamycin (RAP). "*Vac*" designates the autofluorescent vacuole. (D) Growth of the Pma1-2×FKBP anchor-away strain in the presence of rapamycin indicates that anchor-away of Trs85-mRFPmars-FRB is not toxic to cells (*TRS85* is not an essential gene). Anchor-away of the essential Sec7 protein causes cell death. (E) Localization of GFP-Ypt6 in untreated cells versus cells treated with rapamycin to relocalize Trs85 to the PM. (E, right) Quantification of Golgi and cytosolic Ypt6 in untreated versus rapamycin-treated cells. Error bars represent 95% CIs. $n \geq 12$ cells. ***, $P < 0.001$ for unpaired two-tailed t test with Welch's correction. Dashed lines represent cell boundaries. Bars, 2 µm. DIC, differential interference contrast.

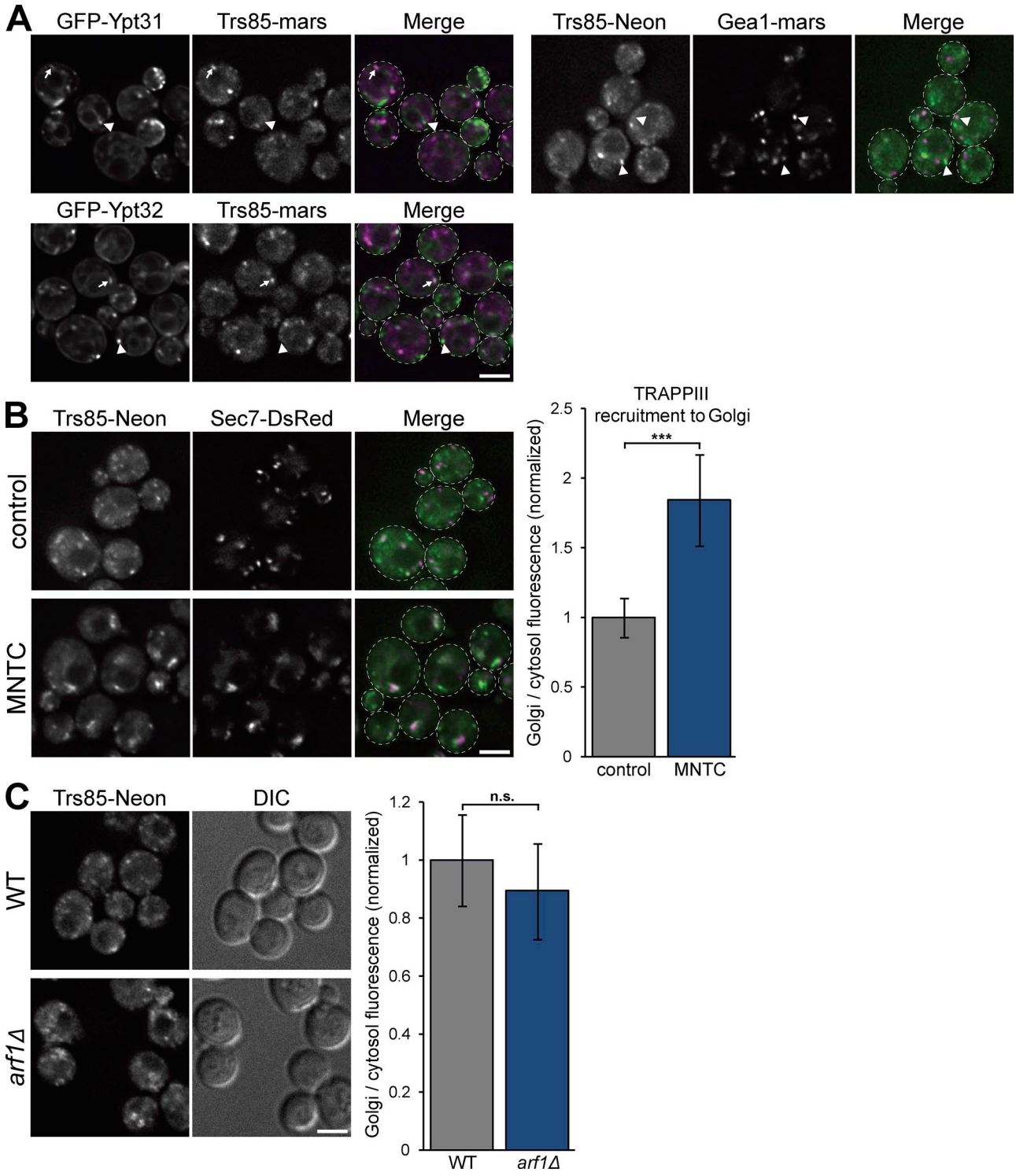

Figure S3. **TRAPPIII localizes to medial/late Golgi compartments.** (A) Localization of Trs85-3×mRFPmars or Trs85-mNeonGreen relative to Gea1-3×mRFPmars (early Golgi) or GFP-Ypt31/32 (TGN) in log-phase cells grown at 30°C. (B, left) Localization of Trs85-mNeonGreen relative to Sec7-6×DsRed in cells treated with the Sec7 inhibitor MNTC. (B, right) Line-trace quantification of Golgi and cytosolic TRAPPIII in untreated versus MNTC-treated cells. Error bars represent 95% CIs. $n = 16$ cells. (C, left) Localization of Trs85-mNeonGreen in WT versus *arf1Δ* mutant cells grown at 30°C. (C, right) Quantification of Golgi and cytosolic TRAPPIII in WT versus *arf1Δ* cells. Error bars represent 95% CIs. $n \geq 10$ cells. ***, $P < 0.001$ for unpaired two-tailed *t* test with Welch's correction. White arrows and arrowheads denote colocalization or lack thereof, respectively. Dashed lines represent cell boundaries. Bars, 2 µm. DIC, differential interference contrast.

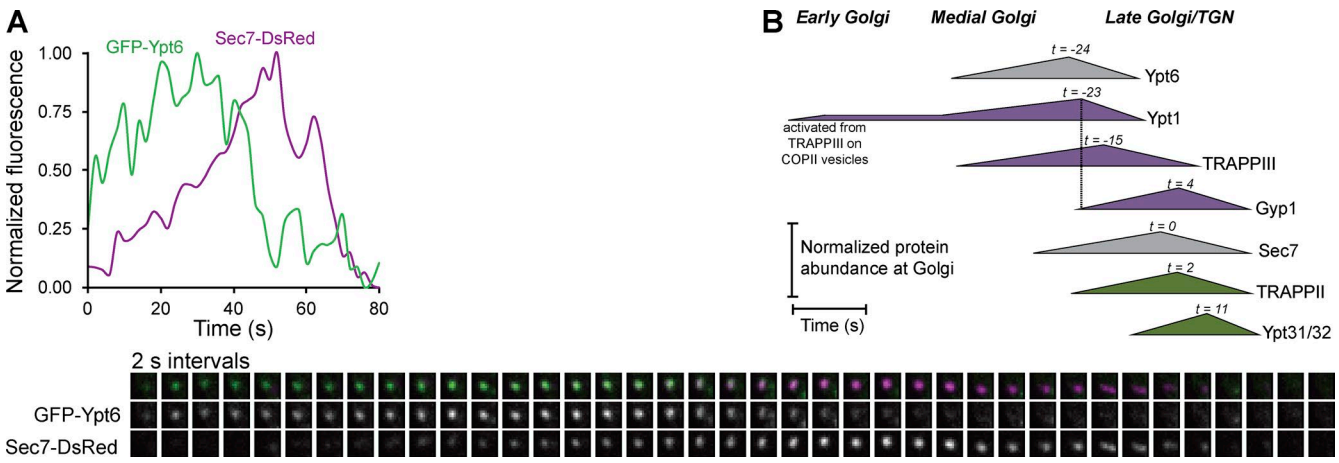

Figure S4. **The Rab GTPase Ypt6 is recruited to the Golgi directly upstream of TRAPPIII.** (A) Time-lapse imaging series (2-s intervals; bottom) and normalized quantification (top) for GFP-Ypt6 and Sec7-6×DsRed at a single Golgi compartment. (B) Summary of the timing of Rab, GEF, and GAP localization to the medial/late Golgi. *t* = 0 is set to peak Sec7 recruitment. Regions of interest for time-lapse imaging are 0.7 × 0.7 µm.

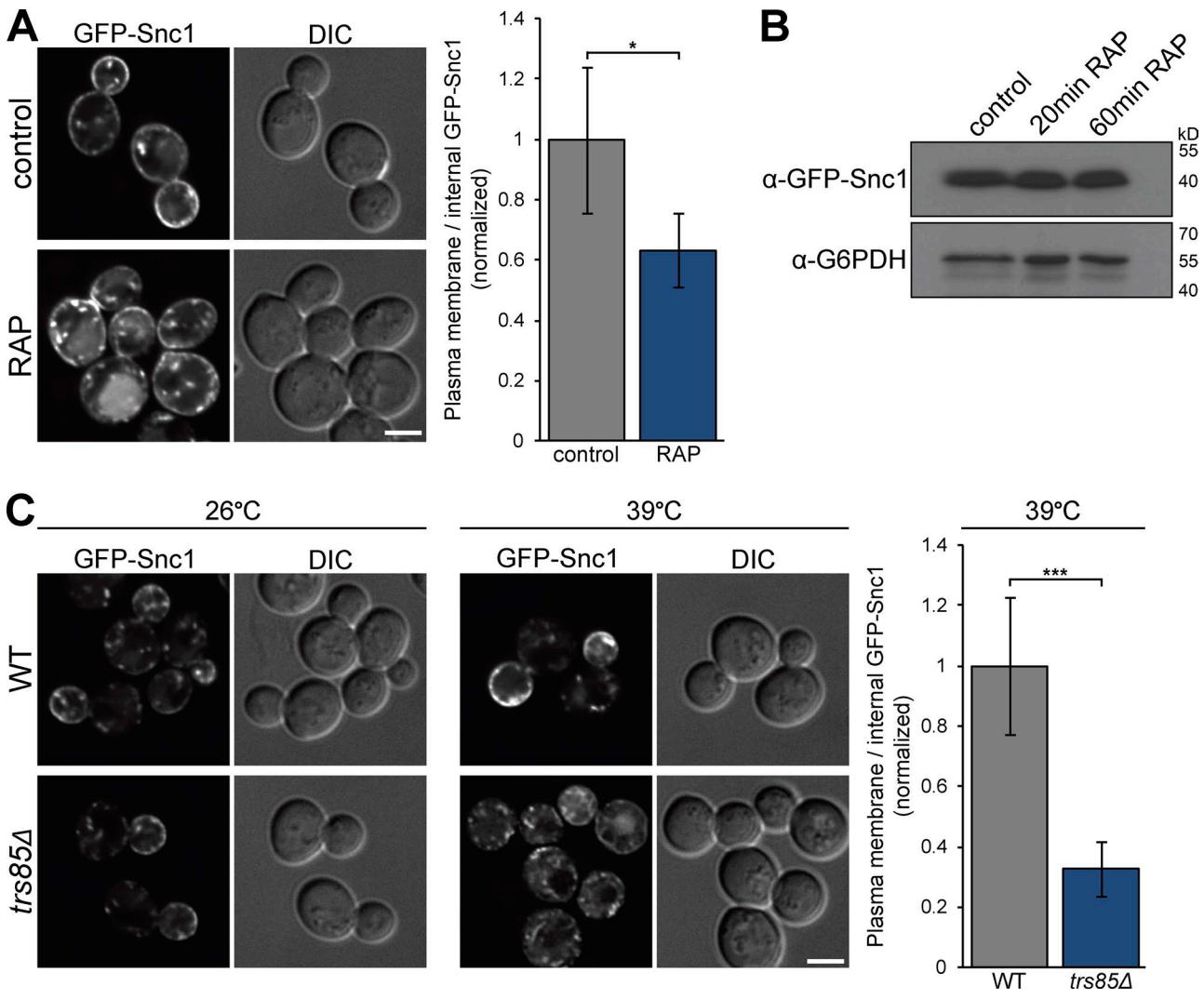

Figure S5. **Disruption of Trs85 function by anchor-away perturbs GFP-Snc1 trafficking.** (A, left) Localization of GFP-Snc1 in untreated cells versus cells treated with rapamycin (RAP) for 60 min to relocalize Trs85 to the PM. (A, right) Line-trace quantification of PM-localized GFP-Snc1 in untreated versus rapamycin-treated cells. Error bars represent 95% CIs. $n \geq 10$ cells. (B) Western blot demonstrating that the total cellular levels of GFP-Snc1 are unchanged by Trs85 anchor-away. G6PDH served as a loading control. (C, left) Localization of GFP-Snc1 in WT versus *trs85Δ* mutant cells grown at 26°C and after shifting to 39°C for 90 min. (C, right) Line-trace quantification of PM-localized GFP-Snc1 in WT versus *trs85Δ* cells. Error bars represent 95% CIs. $n \geq 10$ cells. *, $P < 0.05$; ***, $P < 0.001$ for unpaired two-tailed *t* test with Welch's correction. Bars, 2 µm. DIC, differential interference contrast.

Table S1. **Plasmids used in this study**

Name	Description	Backbone	Source
pLT14	rTRAPPI with Trs33, His_6(TEV)Trs31, Trs23, Bet3, Trs20, and Bet5	pCOLA-Duet-1	(Thomas and Fromme, 2016)
pLT21	rTRAPPI plasmid with His_6 tag removed from Trs31	pCOLA-Duet-1	(Thomas and Fromme, 2016)
pLT92	Trs85 with C-terminal TAP tag	pETDuet-1	This study
Ypt1-His_7	Ypt1 with C-terminal His_7 tag and cleavable N-terminal GST tag	pGEX-6P	(McDonold and Fromme, 2014)
pLT50	Full-length Ypt1 with cleavable N-terminal GST tag	pGEX-6P	(Thomas and Fromme, 2016)
pLT72	Full-length Ypt31 with cleavable N-terminal GST tag	pGEX-6P	(Thomas and Fromme, 2016)
pLT40	Gdi1 with cleavable N-terminal GST tag	pGEX-6P	(Thomas and Fromme, 2016)
pLT35	Mrs6 with cleavable N-terminal His_6 tag	pET28	(Thomas and Fromme, 2016)
pLT41	Bet2 with cleavable N-terminal His_6 tag and Bet4	pCDF-Duet-1	(Thomas and Fromme, 2016)
pRC2100	GFP-Ypt1 in pRS415	pRS415	(Buvelot Frei et al., 2006)
pRC650	GFP-Ypt6 in pRS415	pRS415	(Buvelot Frei et al., 2006)
pAS602	HA-GFP-Sed5 in pRS315	pRS315	(Weinberger et al., 2005)
pLT45	mRFPmars-Sec7 in pRS415	pRS415	(Thomas and Fromme, 2016)
pRC678	GFP-Ypt31 integration plasmid	pRS306	(Buvelot Frei et al., 2006)
Sec7-6xDsRed	Sec7-6×DsRed integration plasmid	pRS406	(Losev et al., 2006)
iGFP-Vrg4	iGFP-Vrg4 integration plasmid	YIplac211	B. Glick
GFP-Snc1	GFP-Snc1 integration plasmid	pRS306	(Lewis et al., 2000)

Table S2. **Yeast strains used in this study**

Name	Description	Source
SEY6210	*MATα ura3-52 his3-Δ200 leu2-3,112 lys2-801 trp1-Δ901 suc2-Δ9*	(Robinson et al., 1988)
SEY6210.1	*MATa ura3-52 his3-Δ200 leu2-3,112 lys2-801 trp1-Δ901 suc2-Δ9*	(Robinson et al., 1988)
BY4741a	*MATa ura3-Δ0 his3-Δ1 leu2-Δ0 met15-Δ0*	(Brachmann et al., 1998)
CUY4655	*MATα ura3 his3-11,15 leu2-3,112 trp1-1 can1-100 tor1-1 fpr1::NatMX Pma1-2xFKBP::TRP1*	(Auffarth et al., 2014)
CFY1066	SEY6210.1 *ypt1-3::KanMX*	This study
CFY1681	SEY6210.1 *Sec7-6xDsRed*	(McDonold and Fromme, 2014)
CFY1784	SEY6210 *GFP-Ypt31::URA3*	(McDonold and Fromme, 2014)
CFY1854	CUY4655 *Sec7-FRB::KanMX*	This study
CFY1905	BY4741a *Bet3-TAP::HIS3*	Open Biosystems
CFY1903	BY4741a *Trs120-TAP::HIS3*	Open Biosystems
CFY2067	SEY6210 *Trs85-TAP::HIS3*	This study
CFY2223	SEY6210.1 *ura3::GFP-Snc1::URA3*	(Thomas and Fromme, 2016)
CFY2398	SEY6210.1 *Gea1-3xmRFPmars::TRP1*	This study
CFY2449	SEY6210.1 *Trs85-mNeonGreen::HIS3*	This study
CFY2544	SEY6210.1 *Trs85-mNeonGreen::HIS3 arf1Δ::KanMX*	This study
CFY2605	SEY6210.1 *GFP-Vrg4 Trs85-3xmRFPmars::TRP1*	This study
CFY2607	SEY6210.1 *Trs85-mNeonGreen::HIS3 Trs130-3xmRFPmars::TRP1*	This study
CFY2628	SEY6210.1 *Trs85-mNeonGreen::HIS3 Gea1-3xmRFPmars*	This study
CFY2640	CUY4655 *Trs85-mRFPmars-FRB::HIS3*	This study
CFY2692	SEY6210 *trs85Δ::KanMX*	This study
CFY2715	SEY6210 *Trs85-mNeonGreen::HIS3 Bet3-3xmRFPmars::TRP1*	This study
CFY2717	SEY6210 *Trs130-mNeonGreen::HIS3 Bet3-3xmRFPmars::TRP1*	This study
CFY2732	SEY6210 *Trs130-mNeonGreen::HIS3 Trs85-mNeonGreen-3xHA::KanMX Bet3-3xmRFPmars::TRP1*	This study
CFY2808	SEY6210 *Trs85-mNeonGreen::HIS3 Sec7-6xDsRed::URA3*	This study
CFY2851	SEY6210.1 *Trs85-3xmRFPmars::TRP1*	This study
CFY2854	SEY6210.1 *GFP-Ypt31::URA3 Trs85-3xmRFPmars::TRP1*	This study
CFY2856	SEY6210.1 *GFP-Ypt32::URA3 Trs85-3xmRFPmars::TRP1*	This study
CFY2963	SEY6210 *Sec7-6xDsRed::URA3 trs85Δ::KanMX*	This study
CFY2976	SEY6210 *GFP-Ypt31::URA3 trs85Δ::KanMX*	This study
CFY2983	SEY6210 *Gea1-3xmRFPmars::TRP1 trs85Δ::KanMX*	This study
CFY2987	SEY6210 *Gyp1-mNeonGreen::HIS3 Sec7-6xDsRed::URA3*	This study
CFY3015	SEY6210 *atg13Δ::KanMX*	This study
CFY3017	SEY6210 *atg1Δ::KanMX*	This study
CFY3043	BY4141a *get1Δ::KanMX*	Open Biosystems
CFY3144	CFY2640 *ura3::GFP-Snc1::URA3*	This study
CFY3146	CFY2640 *Bet3-GFP::KanMX*	This study
CFY3149	CFY2692 *ura3::GFP-Snc1::URA3*	This study
CFY3168	BY4141a *tlg2Δ::KanMX*	Open Biosystems
CFY3169	BY4141a *snx4Δ::KanMX*	Open Biosystems
CFY3170	BY4141a *vps21Δ::KanMX*	Open Biosystems
CFY3171	BY4141a *vps17Δ::KanMX*	Open Biosystems

Table S3. **Liposome formulations used in this study**

Lipid	TGN	PC[a]
DOPC	24%	94%
POPC	6%	
DOPE	7%	
POPE	3%	
DOPS	1%	
POPS	2%	
DOPA	1%	
POPA	2%	
PI	29%	
PI(4)P	1%	
CDP-DAG	2%	
DO-DAG	2%	
PO-DAG	4%	
Ceramide (C18)	5%	
Cholesterol	10%	
Ni^{2+}-DOGS		5%
DiR dye	1%	1%

[a]Phosphatidylcholine

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