

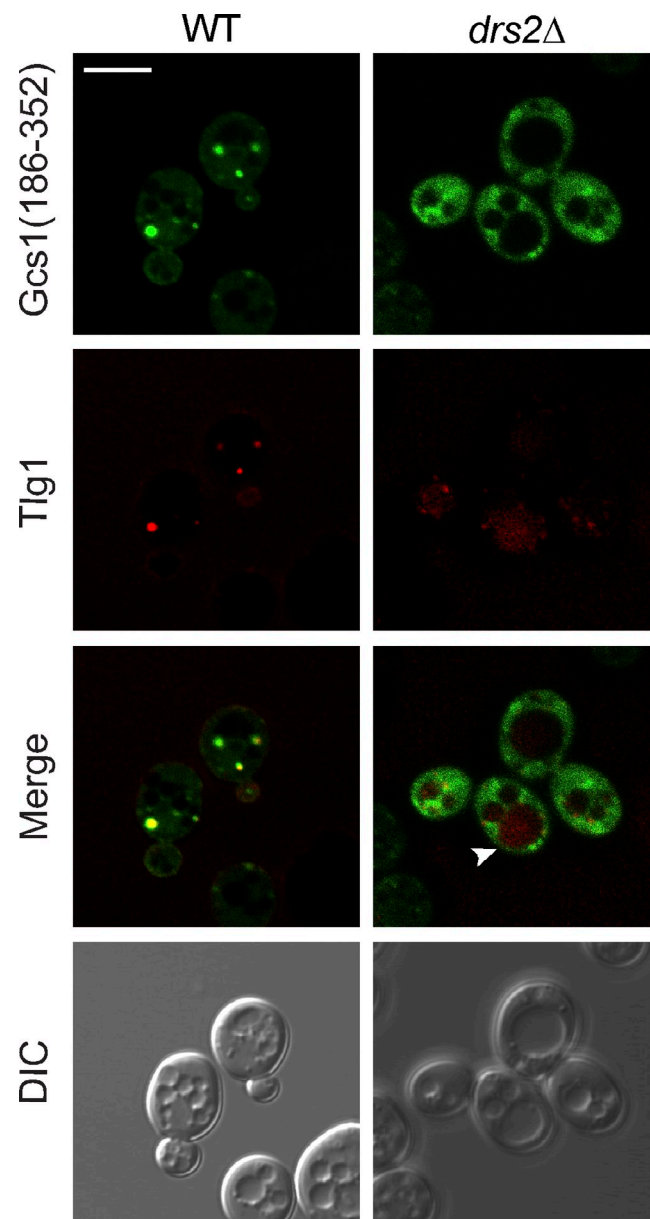
Xu et al., <http://www.jcb.org/cgi/content/full/jcb.201305094/DC1>

Figure S1. **mCherry-Tlg1 is partially mislocalized to the vacuole in *drs2Δ*.** The arrowhead indicates a mother cell where the mCherry-Tlg1 is mislocalized to the vacuole. The two *drs2Δ* buds in this field show localization of mCherry to an enlarged organelle outside of the vacuole. Bar, 5 μ m.

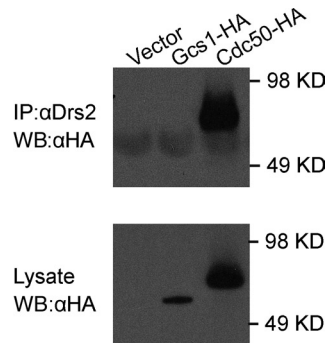


Figure S2. **Drs2 does not physically interact with Gcs1.** The lysates from cells expressing HA-tagged Gcs1 and Cdc50 were immunoprecipitated (IP) with anti-Drs2 antibodies, and the Western blot was probed with anti-HA antibody.

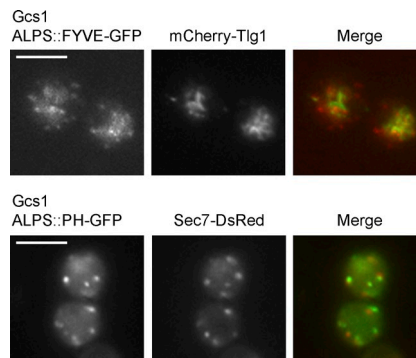


Figure S3. **GFP-tagged GCS1-ALPS::FYVE in wild-type cells were imaged relative to the TGN/EE marker mCherry-Tlg1, and GFP-tagged GCS1-ALPS::PH were imaged relative to the TGN marker Sec7-DsRed.** Bars, 5 μ m.

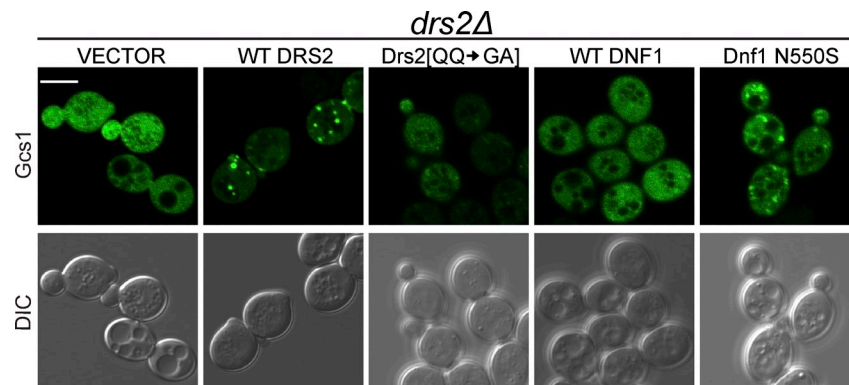
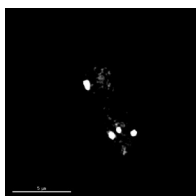
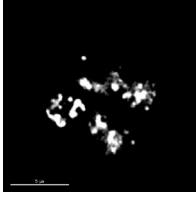


Figure S4. **Gcs1-GFP localization to membranes is restored in *drs2Δ* cells expressing wild-type (WT) Drs2, but not the PS-deficient Drs2 [QQ → GA] mutant.** An extra copy of WT Dnf1 failed to suppress the Gcs1-GFP localization defect, but the Dnf1 N550S mutant that can flip PS restored Gcs1-GFP endosome localization. Bar, 5 μ m.



Video 1. **Membrane dynamics labeled with GFP-Tlg1 in wild-type cells.** Wild-type cells transformed with GFP-Tlg1 were grown to mid-log phase before being imaged using a DeltaVision Elite workstation (Applied Precision). Frames were taken every 170 ms for several seconds (see Materials and methods for more details on image acquisition).



Video 2. **Membrane dynamics labeled with GFP-Tlg1 in *drs2Δ* cells.** *drs2Δ* cells transformed with GFP-Tlg1 were grown to mid-log phase before being imaged using a DeltaVision Elite workstation (Applied Precision). Frames were taken every 170 ms for several seconds (see Materials and methods for more details on image acquisition).

Table S1. **Yeast strains used in this study**

Strain	Genotype	Reference
BY4741	<i>MATa his3 leu2 ura3 met15</i>	Invitrogen
BY4742	<i>MATα his3Δ1 leu2Δ0 ura3Δ0 lys2Δ0</i>	Invitrogen
ZHY615M2D	BY4742 <i>drs2Δ::KanMX6</i>	Hua et al., 2002
BY4742 YJL036W	BY4742 <i>snx4Δ::KanMX6</i>	Invitrogen
BY4742 YDL226C	BY4742 <i>gcs1Δ::KanMX6</i>	Invitrogen
BY4741 YLR240W	BY4741 <i>vps34Δ::KanMX6</i>	Invitrogen
BY4742 YFR019W	BY4742 <i>fab1Δ::KanMX6</i>	Invitrogen
KLY022	BY4741 <i>drs2Δ::KanMX6 chs6Δ::KanMX6</i>	Liu et al., 2008
RBY8700	BY4741 <i>cho1Δ::natNT2</i>	Baldrige et al. 2013

Table S2. **Plasmids used in this study**

Plasmid	Description	Reference
pRS315	<i>LEU2 CEN</i>	ATCC
pRS313	<i>HIS3</i>	ATCC
pRS416	<i>URA CEN</i>	ATCC
pRS416-mCherry-Tlg1	Tlg1 tagged mCherry	From David Katzman
pRS315-mCherry-Tlg1	Tlg1 tagged mCherry	This study
pRS313-mCherry-Tlg1	Tlg1 tagged mCherry	This study
pRS416-ADH	ADH promoter URA CEN	Mumberg et al., 1995
pRS416-ADH-Gcs1-GFP	Gcs1 tagged with GFP	This study
pRS416-ADH-Gcs1 ^{L246D} -GFP	Gcs1 ALPS mutant	This study
pRS416-ADH-Gcs1(216-352)-GFP	truncated Gcs1	This study
pRS416-ADH-Gcs1(186-352)-GFP	truncated Gcs1	This study
pRS416-ADH-Gcs1(242-352)-GFP	truncated Gcs1	This study
pRS416-ADH-Gcs1(186-285)-GFP	truncated Gcs1	This study
pRS416-ADH-Gcs1(186-285) ^{R199Q} -GFP	R199Q	This study
pRS416-ADH-Gcs1(186-285) ^{K210Q} -GFP	K210Q	This study
pGST-Gcs1(186-352)	Gcs1(186-352) tagged GST	This study
pGST-Gcs1(186-285)	Gcs1(186-285) tagged GST	This study
pGST-Gcs1(186-285) ^{K210Q}	Gcs1(186-285) mutant tagged GST	This study
pRS416-ADH-GCS1-ALPS::FYVE-GFP	GCS1-GFP ALPS replaced FYVE	This study
pRS416-ADH-GCS1-ALPS::PH-GFP	GCS1-GFP ALPS replaced PH	This study
pRS315-GCS1	GCS1 LEU2 CEN	Pak Poon
pRS315-GCS1 GAP(R54K)	GCS1 catalytic dead mutant	This study
pRS315-GCS1 ALPS(L246D)	GCS1 ALPS mutant	This study
pRS315-GCS1-ALPS::FYVE	GCS1 ALPS replaced by FYVE domain	This study
pRS315-GCS1-ALPS::PH	GCS1 ALPS replaced by PH domain	This study
pRS416-GFP-Snc1	Snc1 tagged GFP	Lewis et al., 2000
pRS315-DRS2	DRS2	Gall et al., 2002
pRS315-drs2 ^{ts}	drs2 ^{ts}	Gall et al., 2002
pRS416-GPD-GFP-Lact-C2	PS biosensor	Fairn et al., 2011
pRS425-GPD-mRFP-Lact-C2	PS biosensor	Fairn et al., 2011
pRS313-DRS2	DRS2	Natarajan, et al., 2004
pRS313-Drs2 [QQ->GA]	PS-deficient DRS2 mutant	Baldrige et al., 2013
pRS313-DNF1	DNF1	Liu et al., 2008
pRS313-Dnf1 N550S	DNF1 mutant	Baldrige et al., 2013
pRS313-GPD-3xFLAG-DNF1		Baldrige and Graham, 2012
pRS425-LEM3		Baldrige and Graham, 2012
pRS313-GPD-DRS2		Baldrige and Graham, 2012
pRS425-CDC50		Baldrige and Graham, 2012
pCB866	2 μ . Osh4-YFP::HIS3MX	Alfaro et al., 2011

ATCC, American Type Culture Collection.

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