Toulmay and Prinz, http://www.jcb.org/cgi/content/full/jcb.201301039/DC1

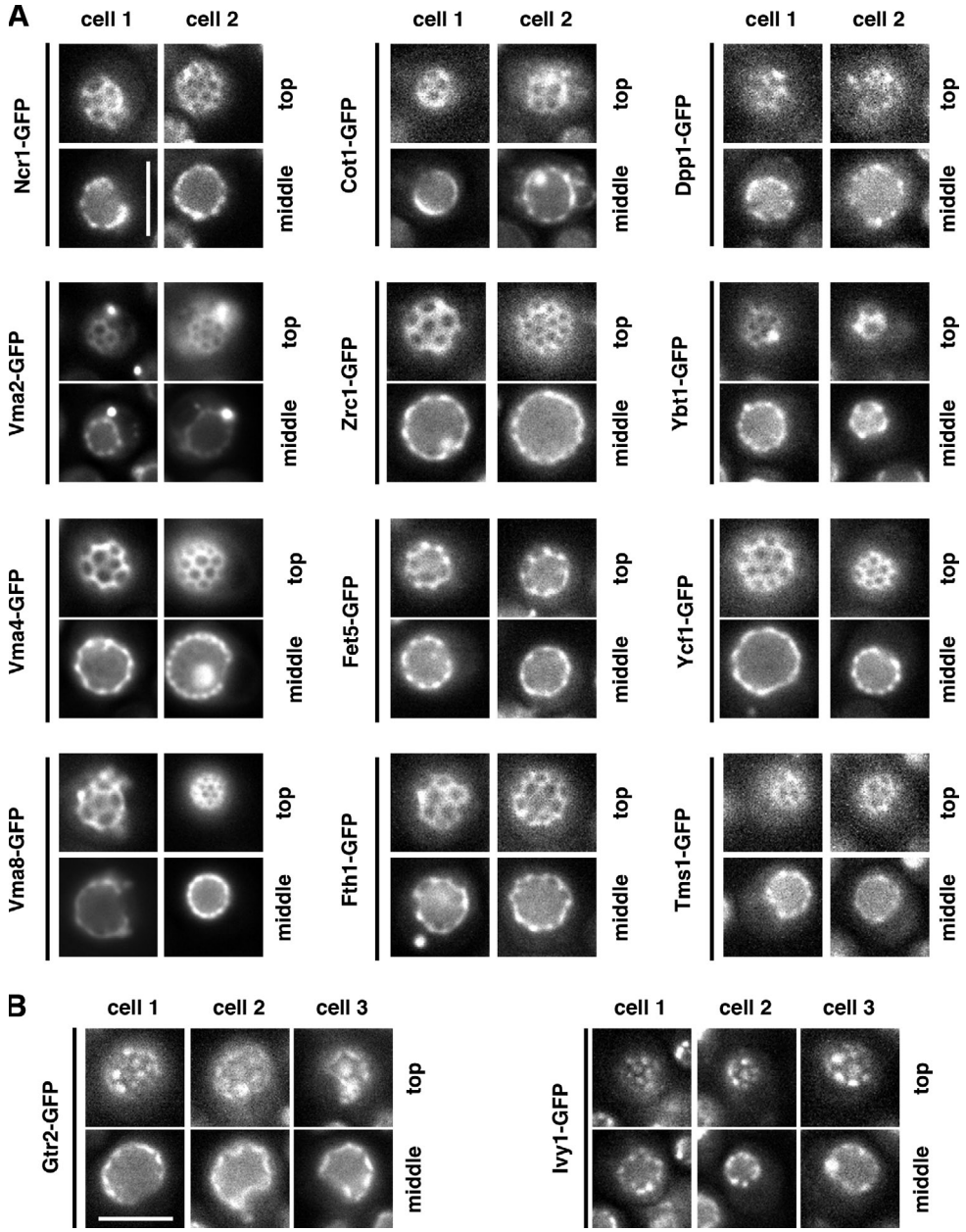


Figure S1. **Localization of 14 vacuolar membrane proteins during Stat-phase.** Cells expressing C-terminal GFP fusions to the indicated proteins were grown to Stat-phase and visualized live by fluorescent microscopy. The fusions were expressed from the chromosome under endogenous promoters. The middle section and top section of the vacuoles are shown. (A) Strains expressing fusions with domain patterns similar to that of Vph1-GFP. (B) Strains expressing fusions with domain patterns opposite to that of Vph1-GFP. Bar, 5 µm.

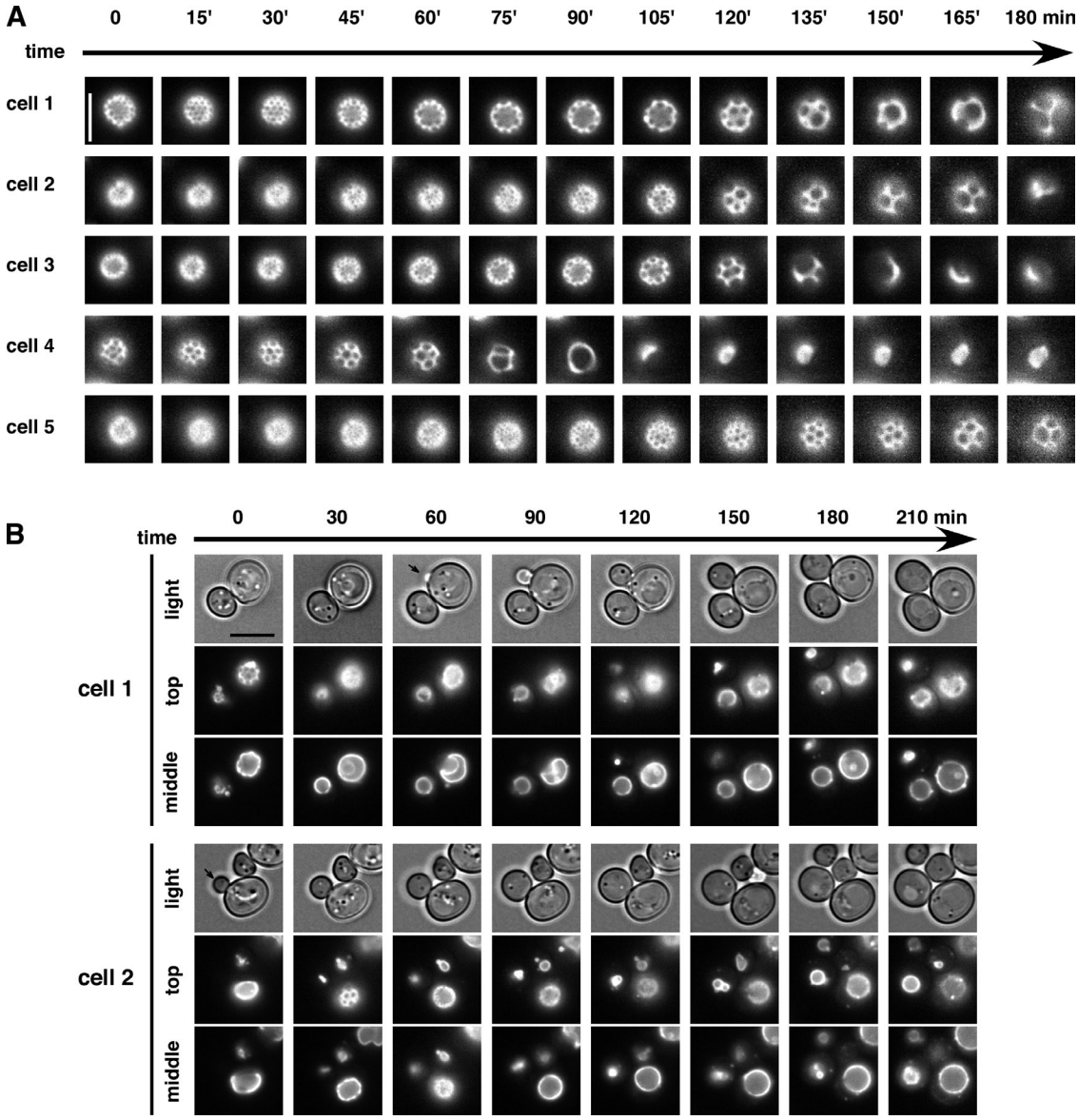


Figure S2. **Time-lapse imaging of vacuole domains and demonstration that cells with vacuolar microdomains are alive and grow.** (A) Cells expressing Vph1-GFP were grown to Stat-phase and imaged every 15 min in SC medium. Five examples are shown. The top sections of the vacuole were imaged. (B) Cells expressing Vph1-GFP were grown to Stat-phase, placed on an agar pad containing fresh SC media (time 0), and imaged every 30 min. Two examples are shown. The middle and top sections of the vacuole were imaged. Arrows show growing buds. Bars, 5 µm.

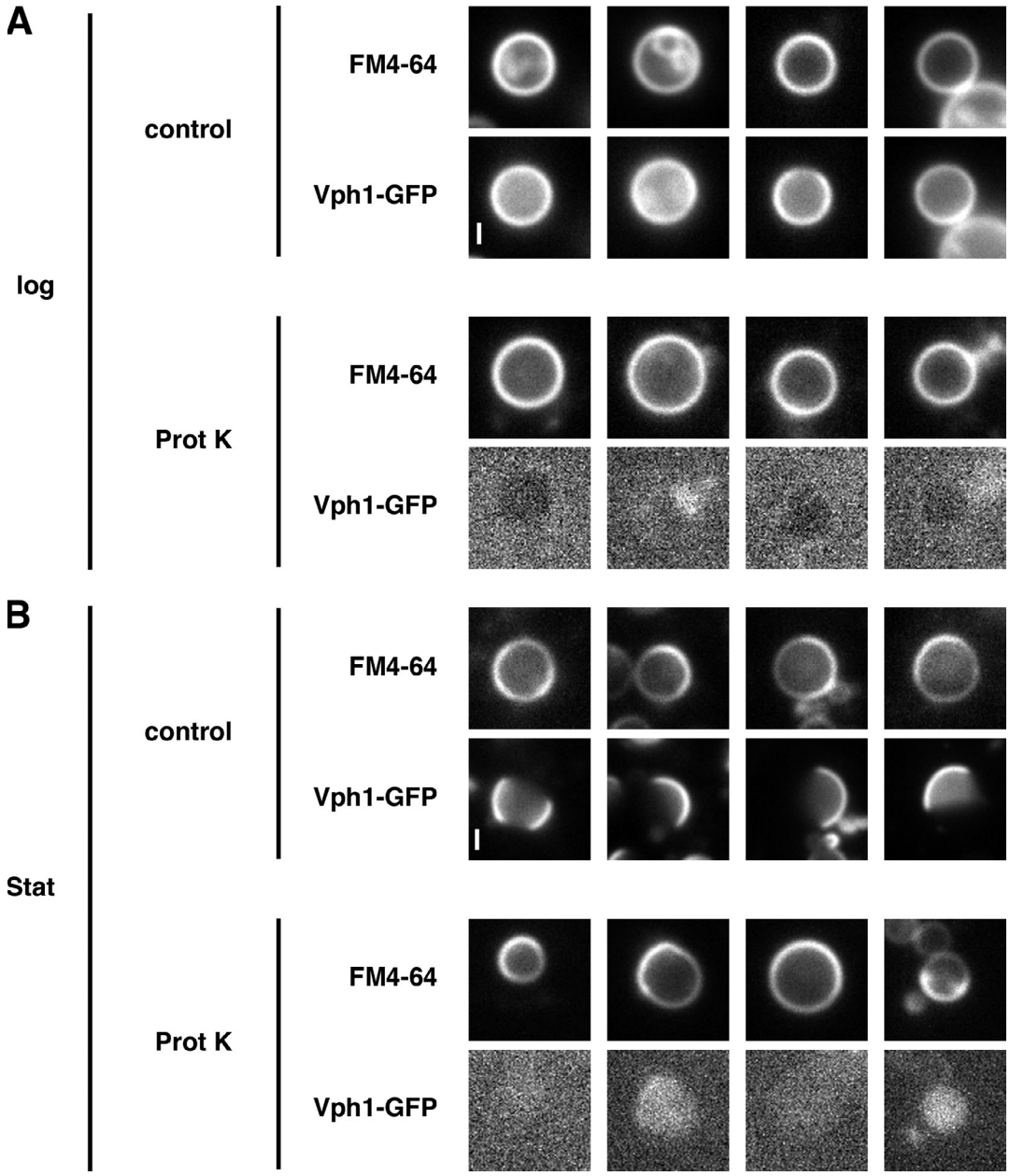


Figure S3. **Cytosolic proteins are not required to maintain vacuolar domains.** Intact vacuoles were isolated from cells expressing Vph1-GFP and grown in medium containing FM4-64. (A and B) Cells were harvested in logarithmic-phase (log) cells (A) or Stat-phase (stat) cells (B). 0.1 mg/ml Proteinase K (Prot K) or breaking buffer (control) was added to the vacuoles, and they were incubated for 1 h at 30°C. The vacuoles were imaged by fluorescence microscopy. The loss of proteins was indicated by the disappearance of the Vph1-GFP signal from the vacuoles. Bars, 1 μm.

Table S1. **Results of screen for mutants with decreased percentage of cells with quasi-symmetrical domains during starvation**

Genes	Functions	Categories	Results[a]	Comments
WT	None	Control	+	
atg8	Required for autophagy/Cvt	Autophagy	+	
atg15	Vacuole lipase	Autophagy	+	
cho2	Phosphatidylcholine synthesis	Lipid	±	
cpt1	PC synthesis	Lipid	+	
erg5	Ergosterol synthesis	Lipid	+	Weak fluorescence, many vacuoles
erg6	Ergosterol synthesis	Lipid	+	Weak fluorescence, many vacuoles
fab1	Phosphoinositide 3.5 kinase	Lipid	−	Big unique vacuole
ipt1	Sphingolipid synthesis	Lipid	+	
isc1	Sphingolipid hydrolase	Lipid	+	
lac1	Ceramide synthase	Lipid	+	
lag1	Ceramide synthase	Lipid	+	Weak fluorescence
lcb3	Long chain base phosphatase	Lipid	+	Dying
ncr1	Niemann Pick C1 protein	Lipid	+	
nem1	Phospholipid homeostasis	Lipid	−	Enriched at vacuole-vacuole contacts sites
opi3	PC synthesis	Lipid	±	Weak fluorescence, unusual domains
osh1	Oxysterol-binding protein	Lipid/NVJ	+	
scs7	Sphingolipid-hydroxylase	Lipid	+	
sur2	Phytosphingosine synthase	Lipid	±	Dying, unusual domains
ard1	G0 entry	Quiescence	+	
bck1	PKC signaling	Quiescence	+	Dying
gcs1	ARF GAP, Stat induced	Quiescence	+	
hsp26	Small heat shock protein, Stat induced	Quiescence	+	
mpk1	MAPK in PKC signaling	Quiescence	−	Few unusual domains, dying
snf1	AMP kinase in glc signaling	Quiescence	+	Weak fluorescence
snz1	Stat induced	Quiescence	+	
ego3	EGO complex	Vac localized	+	Dying
gtr1	GTPase EGO complex	Vac localized	+	Dying
gtr2	GTPase EGO complex	Vac localized	+	Dying
ivy1	Unknown	Vac localized	+	
tco89	Torc1	Vac localized	+	Weak fluorescence
tor1	Torc1	Vac localized	+	Dying
nvj1	NVJ	NVJ	+	
nvj2	Unknown	NVJ localized	+	
ccz1	Vac assembly + autophagy	VPS/autophagy	ND	Many tiny vacuoles
end3	Endocytosis	VPS	+	
pep12	Late endosome t-SNARE	VPS	+	Weak fluorescence, many vacuoles
vac8	CVT + NVJ	VPS/NVJ	+	Dying
vam3	Vac t-SNARE	VPS	ND	Many tiny vacuoles
vam7	Vac SNARE	VPS	ND	Many tiny vacuoles
vps33	HOPS	VPS	+	Many tiny vacuoles, but few big ones have domains
vps4	MVB AAA-ATPase	VPS	−	Weak fluorescence, dying
vps41	HOPS	VPS	+	
ypt7	HOPS	VPS	ND	Many tiny vacuoles
				Other growth conditions
are1 are2	Steryl ester synthase	Lipid	+	
ole1	Δ9 fatty acid desaturase	Lipid	+	
psd1 psd2	Phosphatidylserine decarboxylase	Lipid	+	
arv1	Transport glycosylphosphatidylinositol	Lipid	+	
elo3	Very long chain fatty acid elongase	Lipid	+	
vph1	V0-a v-ATPase	Vac localized	+	Strain expressing Ivy1-mCherry
vma3	V0-c v-ATPase	Vac localized	+	
vma4	V1-e v-ATPase	Vac localized	+	
pep4	Main vacuolar portease	Vac localized	+	

ARF, ADP ribosylation factor; GAP, GTPase-activating protein; Cvt, cytoplasm to vacuole targeting; HOPS, homotypic fusion and vacuole protein sorting; MVB, multivesicular body; Vac, vacuole; VPS, vacuolar protein sorting.
[a]Percentage of cells with vacuoles with quasi-symmetrical domains, similar to WT (+), moderately less than WT (±), or substantially less than WT (−).

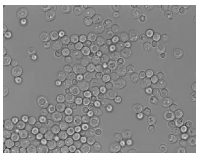
Table S2. **Strains used in this study**

Strains	Relevant genotype	Reference
WPY333	BY4741 mat a *leu2Δ his3Δ ura3Δ met15Δ*	
ATY349	BY4741 mat a *leu2Δ his3Δ ura3Δ met15Δ VPH1-GFP::HIS3*	Huh et al., 2003
–	BY4741 mat a *leu2Δ his3Δ ura3Δ met15Δ XXX-GFP::HIS3*	Huh et al., 2003
ATY385	BY4741 mat a *leu2Δ his3Δ ura3Δ met15Δ VPH1-GFP::HIS3 p416-IVY1-mCherry-URA3*	This study
ATY363	BY4741 mat a *leu2Δ his3Δ ura3Δ met15Δ atg8::KanMX6 VPH1-GFP::HIS3*	This study
ATY387	BY4741 mat a *leu2Δ his3Δ ura3Δ met15Δ atg15::KanMX6 VPH1-GFP::HIS3*	This study
ATY407	BY4741 mat a *leu2Δ his3Δ ura3Δ met15Δ cho2::KanMX6 VPH1-GFP::HIS3*	This study
ATY408	BY4741 mat a *leu2Δ his3Δ ura3Δ met15Δ cpt1::KanMX6 VPH1-GFP::HIS3*	This study
ATY371	BY4741 mat a *leu2Δ his3Δ ura3Δ met15Δ erg5::KanMX6 VPH1-GFP::HIS3*	This study
ATY377	BY4741 mat a *leu2Δ his3Δ ura3Δ met15Δ erg6::KanMX6 VPH1-GFP::HIS3*	This study
ATY364	BY4741 mat a *leu2Δ his3Δ ura3Δ met15Δ fab1::KanMX6 VPH1-GFP::HIS3*	This study
ATY378	BY4741 mat a *leu2Δ his3Δ ura3Δ met15Δ ipt1::KanMX6 VPH1-GFP::HIS3*	This study
ATY396	BY4741 mat a *leu2Δ his3Δ ura3Δ met15Δ isc1::KanMX6 VPH1-GFP::HIS3*	This study
ATY379	BY4741 mat a *leu2Δ his3Δ ura3Δ met15Δ lac1::KanMX6 VPH1-GFP::HIS3*	This study
ATY380	BY4741 mat a *leu2Δ his3Δ ura3Δ met15Δ lag1::KanMX6 VPH1-GFP::HIS3*	This study
ATY365	BY4741 mat a *leu2Δ his3Δ ura3Δ met15Δ lcb3::KanMX6 VPH1-GFP::HIS3*	This study
ATY381	BY4741 mat a *leu2Δ his3Δ ura3Δ met15Δ ncr1::KanMX6 VPH1-GFP::HIS3*	This study
ATY367	BY4741 mat a *leu2Δ his3Δ ura3Δ met15Δ nem1::KanMX6 VPH1-GFP::HIS3*	This study
ATY409	BY4741 mat a *leu2Δ his3Δ ura3Δ met15Δ opi3::KanMX6 VPH1-GFP::HIS3*	This study
ATY382	BY4741 mat a *leu2Δ his3Δ ura3Δ met15Δ osh1::KanMX6 VPH1-GFP::HIS3*	This study
ATY383	BY4741 mat a *leu2Δ his3Δ ura3Δ met15Δ scs7::KanMX6 VPH1-GFP::HIS3*	This study
ATY397	BY4741 mat a *leu2Δ his3Δ ura3Δ met15Δ sur2::KanMX6 VPH1-GFP::HIS3*	This study
ATY406	BY4741 mat a *leu2Δ his3Δ ura3Δ met15Δ ard1::KanMX6 VPH1-GFP::HIS3*	This study
ATY393	BY4741 mat a *leu2Δ his3Δ ura3Δ met15Δ bck1::KanMX6 VPH1-GFP::HIS3*	This study
ATY394	BY4741 mat a *leu2Δ his3Δ ura3Δ met15Δ gcs1::KanMX6 VPH1-GFP::HIS3*	This study
ATY395	BY4741 mat a *leu2Δ his3Δ ura3Δ met15Δ hsp26::KanMX6 VPH1-GFP::HIS3*	This study
ATY399	BY4741 mat a *leu2Δ his3Δ ura3Δ met15Δ mpk1::KanMX6 VPH1-GFP::HIS3*	This study
ATY400	BY4741 mat a *leu2Δ his3Δ ura3Δ met15Δ snf1::KanMX6 VPH1-GFP::HIS3*	This study
ATY401	BY4741 mat a *leu2Δ his3Δ ura3Δ met15Δ snz1::KanMX6 VPH1-GFP::HIS3*	This study
ATY411	BY4741 mat a *leu2Δ his3Δ ura3Δ met15Δ ego3::KanMX6 VPH1-GFP::HIS3*	This study
ATY412	BY4741 mat a *leu2Δ his3Δ ura3Δ met15Δ gtr1::KanMX6 VPH1-GFP::HIS3*	This study
ATY413	BY4741 mat a *leu2Δ his3Δ ura3Δ met15Δ gtr2::KanMX6 VPH1-GFP::HIS3*	This study
ATY414	BY4741 mat a *leu2Δ his3Δ ura3Δ met15Δ ivy1::KanMX6 VPH1-GFP::HIS3*	This study
ATY390	BY4741 mat a *leu2Δ his3Δ ura3Δ met15Δ tco89::KanMX6 VPH1-GFP::HIS3*	This study
ATY391	BY4741 mat a *leu2Δ his3Δ ura3Δ met15Δ tor1::KanMX6 VPH1-GFP::HIS3*	This study
ATY366	BY4741 mat a *leu2Δ his3Δ ura3Δ met15Δ nvj1::KanMX6 VPH1-GFP::HIS3*	This study
ATY368	BY4741 mat a *leu2Δ his3Δ ura3Δ met15Δ nvj2::KanMX6 VPH1-GFP::HIS3*	This study
ATY415	BY4741 mat a *leu2Δ his3Δ ura3Δ met15Δ ccz1::KanMX6 VPH1-GFP::HIS3*	This study
ATY388	BY4741 mat a *leu2Δ his3Δ ura3Δ met15Δ end3::KanMX6 VPH1-GFP::HIS3*	This study
ATY389	BY4741 mat a *leu2Δ his3Δ ura3Δ met15Δ pep12::KanMX6 VPH1-GFP::HIS3*	This study
ATY402	BY4741 mat a *leu2Δ his3Δ ura3Δ met15Δ vac8::KanMX6 VPH1-GFP::HIS3*	This study
ATY403	BY4741 mat a *leu2Δ his3Δ ura3Δ met15Δ vam3::KanMX6 VPH1-GFP::HIS3*	This study
ATY416	BY4741 mat a *leu2Δ his3Δ ura3Δ met15Δ vam7::KanMX6 VPH1-GFP::HIS3*	This study
ATY404	BY4741 mat a *leu2Δ his3Δ ura3Δ met15Δ vps33::KanMX6 VPH1-GFP::HIS3*	This study
ATY417	BY4741 mat a *leu2Δ his3Δ ura3Δ met15Δ vps4::KanMX6 VPH1-GFP::HIS3*	This study
ATY405	BY4741 mat a *leu2Δ his3Δ ura3Δ met15Δ vps41::KanMX6 VPH1-GFP::HIS3*	This study
ATY418	BY4741 mat a *leu2Δ his3Δ ura3Δ met15Δ ypt7::KanMX6 VPH1-GFP::HIS3*	This study
ATY462	BY4741 mat a *leu2Δ his3Δ ura3Δ met15Δ are1::KanMX6 are2::Leu2 VPH1-GFP::HIS3*	This study
ATY370	BY4741 mat a *leu2Δ his3Δ ura3Δ met15Δ elo3::KanMX6 VPH1-GFP::HIS3*	This study
ATY463	BY4741 mat a *leu2Δ his3Δ ura3Δ met15Δ ole1::KanMX6 VPH1-GFP::HIS3*	This study
ATY386	BY4741 mat a *leu2Δ his3Δ ura3Δ met15Δ vph1::KanMX6 p416-IVY1-mCherry-URA3*	This study
ATY575	BY4741 mat a *leu2Δ his3Δ ura3Δ met15Δ vma3::KanMX6 VPH1-GFP::HIS3*	This study
ATY574	BY4741 mat a *leu2Δ his3Δ ura3Δ met15Δ vma4::KanMX6 VPH1-GFP::HIS3*	This study
ATY547	BY4741 mat a *leu2Δ his3Δ ura3Δ psd1::KanMX6 psd2::KanMX6 VPH1-GFP::HIS3*	This study
ATY546	BY4741 mat a *leu2Δ his3Δ ura3Δ met15Δ arv1::KanMX6 VPH1-GFP::HIS3*	This study
ATY587	mat α *ade2Δ his3Δ ura3-52Δ erg1::URA3 upc2-1 Ycplac111-VPH1-GFP-KanMX6-LEU2*	This study
ATY567	BY4741 mat a *leu2Δ his3Δ ura3Δ met15Δ SEC18-1::LEU2 VPH1-GFP::HIS3*	This study
ATY572	BY4741 mat a *leu2Δ his3Δ ura3Δ met15Δ rim21::KanMX6 VPH1-GFP::HIS3*	This study

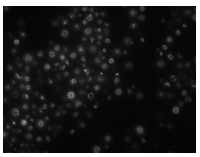
Table S2. **Strains used in this study** *(Continued)*

Strains	Relevant genotype	Reference
ATY568	BY4741 mat a *leu2Δ his3Δ ura3Δ met15Δ dfg16::KanMX6 VPH1-GFP::HIS3*	This study
ATY570	BY4741 mat a *leu2Δ his3Δ ura3Δ met15Δ rim13::KanMX6 VPH1-GFP::HIS3*	This study
ATY571	BY4741 mat a *leu2Δ his3Δ ura3Δ met15Δ rim20::KanMX6 VPH1-GFP::HIS3*	This study
ATY481	BY4741 mat a *leu2Δ his3Δ ura3Δ met15Δ rim101::KanMX6 VPH1-GFP::HIS3*	This study
ATY552	BY4741 mat a *leu2Δ his3Δ ura3Δ met15Δ pep4::KanMX6 VPH1-GFP::HIS3*	This study

The minus sign indicates that there is no strain number for this strain.



Video 1. **Vph1-GFP is segregated into microdomains at the vacuole membrane in Stat-phase.** Cells expressing Vph1-GFP were cultured to Stat-phase, and a stack of six fluorescent images was taken with increments of 0.4 µm between images using a wide-field microscope (BX61; Olympus). Each frame in the video shows one image in the stack. The images are displayed from the bottom of the stack to the top and then back to the bottom.



Video 2. **Microdomains on vacuole membrane are stable over minutes.** A strain expressing Vph1-GFP was cultured to Stat-phase, and fluorescent images were taken every minute for 10 min using a wide-field microscope (BX61; Olympus).

Reference

Huh, W.K., J.V. Falvo, L.C. Gerke, A.S. Carroll, R.W. Howson, J.S. Weissman, and E.K. O'Shea. 2003. Global analysis of protein localization in budding yeast. *Nature*. 425:686–691. http://dx.doi.org/10.1038/nature02026