Supplemental Materials

Molecular Biology of the Cell

Apel et al.

Figure S1. (A) Immunoblot of recombinant binding assay in which WT and mutant Mid2$^{273-376}$ (WY>AAA) fragments were tested for binding to an immobilized GST negative control or GST-Syp1 *μ*HD; top: long exposure anti-His$_6$ immunoblot, middle: short exposure anti-His$_6$ immunoblot, bottom: GelCode Blue (Coomassie) protein stain. (L: loading control, S: supernatant, P: pelleted fraction). (B) Immunoblot of binding assay in which GST and GST-Syp1 *μ*HD were treated with either WT His$_6$-Snc1$^{1-93}$ or a mutant in which the DPY motif was mutated to KPA (L: loading control, S: supernatant, P: pelleted fraction). (C) Immunoblot of binding assay in which GST and GST-Syp1 *μ*HD were treated with either WT His$_6$-Snc1$^{63-93}$ or a mutant in which the WY motif was mutated to alanines (L: loading control, S: supernatant, P: pelleted fraction). (D) Cells expressing full-length Mid2-GFP, Mid2$^{\Delta 286}$-GFP, or Mid2$^{\Delta 289}$-GFP were grown on minimal medium and imaged via live-cell fluorescence microscopy. Scale bar, 2 *μ*m.

Figure S2. (A) Reconstructed WT and *syp1*Δ cells expressing candidate cargos tagged with GFP in strain background W303 were grown on rich medium and imaged via live-cell fluorescence microscopy. Scale bar, 2 μm. (B) Full-length candidate cargo sequences were analyzed using the SPOCTOPUS membrane protein topology prediction algorithm. DxY motifs in regions of proteins that are predicted to be cytoplasmic are indicated in black; in the absence of a DxY motif in these regions, Dx*Φ* and ExY sequences are indicated. One DxY motif residing in a region predicted to be extracellular is indicated in red (Inside: cytoplasmic, Outside: extracellular, TM-Helix: transmembrane).

Figure S3. (A) Cells expressing Ptr2-GFP or Mep3-GFP were treated with vehicle (DMSO) or Latrunculin A (LatA) prior to imaging by live-cell fluorescence microscopy. Scale bar, 2 *μ*m. (B) Intensity of Ptr2-pHluorin or Mep3-pHluorin was quantified for each condition; intensity values were corrected for cell size and expressed in arbitrary units (a.u.) (error bars indicate mean ± SEM; ****, P < 0.0001 compared to WT).

Figure S4. (A) WT cells were grown in nutrient-rich (YPD) medium and resuspended in either fresh YPD or ammonium-rich, minimal (YNB) medium. Cells were imaged every two hours via live-cell fluorescence microscopy. Scale bar, 2 *μ*m. (B) TCA precipitations of total protein from log phase cells expressing Syp1-GFP grown on rich or minimal medium were resolved by SDS-PAGE. Immunoblotting using anti-GFP antibody, or anti-glucose-6-phosphate dehydrogenase (G6PDH) antibody as a loading control, was then performed.

Figure S5. (A) WT and *end3*Δ cells expressing Mid2-GFP were transformed with either an empty or *SYP1*-containing high-copy vector and imaged via live-cell fluorescence microscopy. Scale bar, 2 *μ*m. (B) WT and *end3*Δ cells expressing GFP-Snc1 were transformed with either an empty or *SYP1*-containing high-copy vector and imaged via live-cell fluorescence microscopy. Scale bar, 2 *μ*m. (C) Cells expressing Ptr2-pHluorin were grown on rich medium (YPD) with or without 1M sorbitol. Scale bar, 2 *μ*m. (D) WT and *end3*Δ cells expressing Ptr2-GFP were transformed with either an empty or *ROM1*-

containing high-copy vector and imaged via live-cell fluorescence microscopy. Scale bar, 2 *μ*m. (E) Intensity of Ptr2-pHluorin was quantified for each condition; intensity values were corrected for cell size and expressed in arbitrary units (a.u.) (error bars indicate mean ± SEM; ****, P < 0.0001 compared to WT). (F) WT and *end3*Δ cells expressing Ptr2-GFP were transformed with either an empty, *ROM1*-, or *SYP1*-containing high-copy vector, grown in selective media containing protease inhibitors, and imaged via live-cell fluorescence microscopy. Scale bar, 2 *μ*m.

Figure S1.

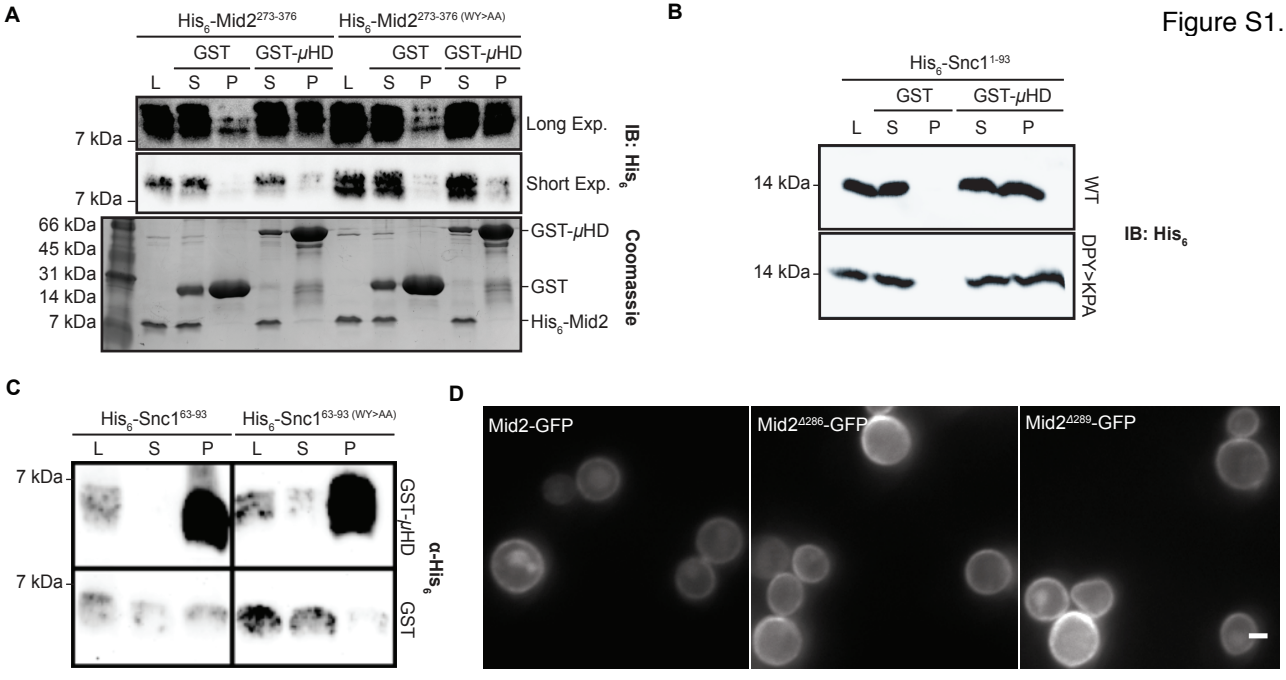
A
His6-Mid2273-376
His6-Mid2273-376 (WY>AA)
GST
GST-μHD
L S P S P L S P S P
7 kDa
Long Exp.
7 kDa
Short Exp.
IB: His6
66 kDa
45 kDa
31 kDa
14 kDa
7 kDa
GST-μHD
GST
His6-Mid2
Coomassie
B
His6-Snc11-93
GST
GST-μHD
L S P S P
14 kDa
WT
14 kDa
DPY>KPA
IB: His6
C
His6-Snc163-93
His6-Snc163-93 (WY>AA)
L S P L S P
7 kDa
GST-μHD
7 kDa
GST
α-His6
D
Mid2-GFP
Mid2Δ286-GFP
Mid2Δ289-GFP

Figure S2.

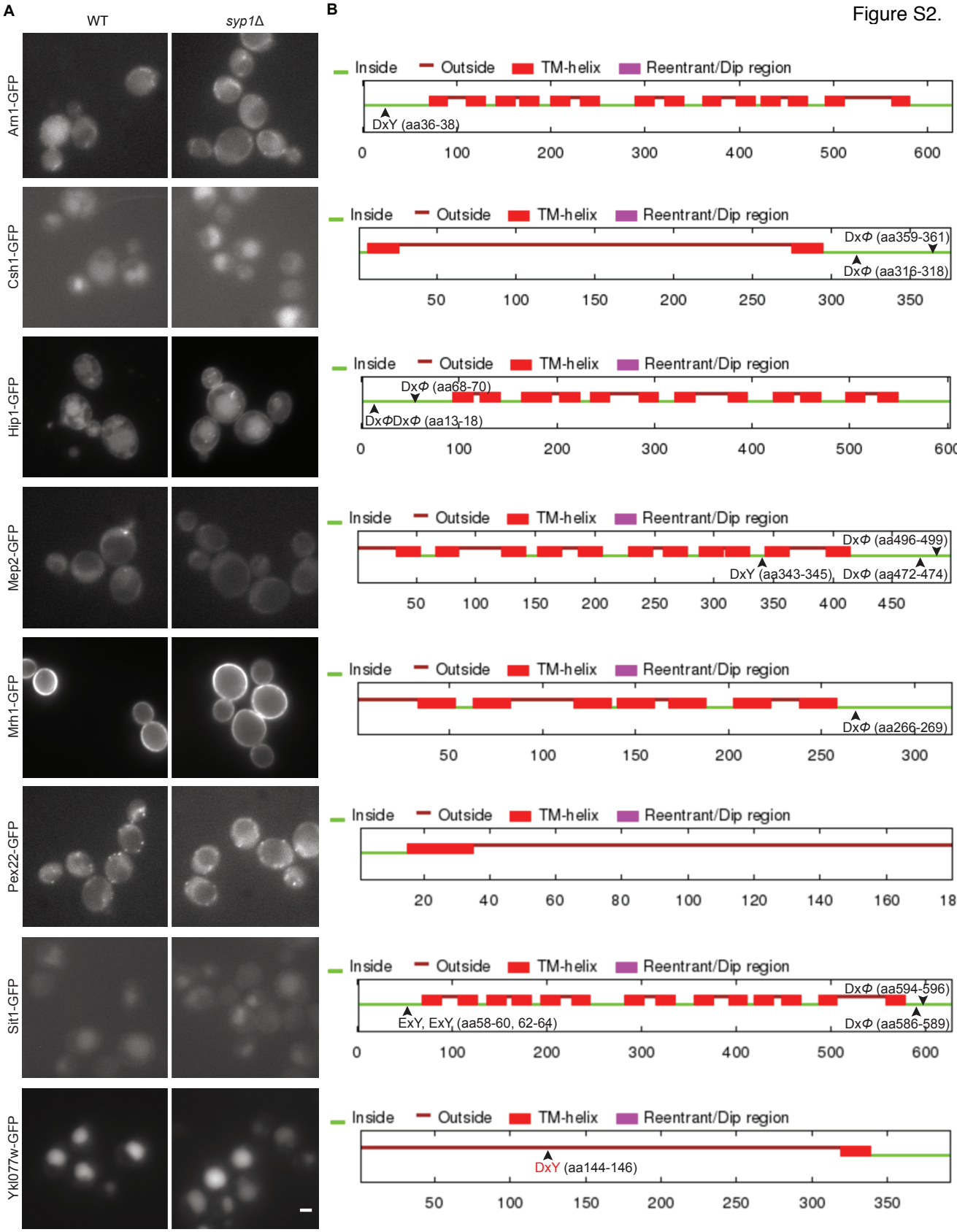
A
WT
syp1Δ
Am1-GFP
Csh1-GFP
Hip1-GFP
Mep2-GFP
Mrh1-GFP
Pex22-GFP
Sit1-GFP
Ykl077w-GFP
B
Inside
Outside
TM-helix
Reentrant/Dip region
DxY (aa36-38)
DxΦ (aa359-361)
DxΦ (aa316-318)
DxΦ (aa68-70)
DxΦDxΦ (aa13-18)
DxΦ (aa496-499)
DxY (aa343-345)
DxΦ (aa472-474)
DxΦ (aa266-269)
DxΦ (aa594-596)
ExY, ExY (aa58-60, 62-64)
DxΦ (aa586-589)
DxY (aa144-146)

Figure S3.

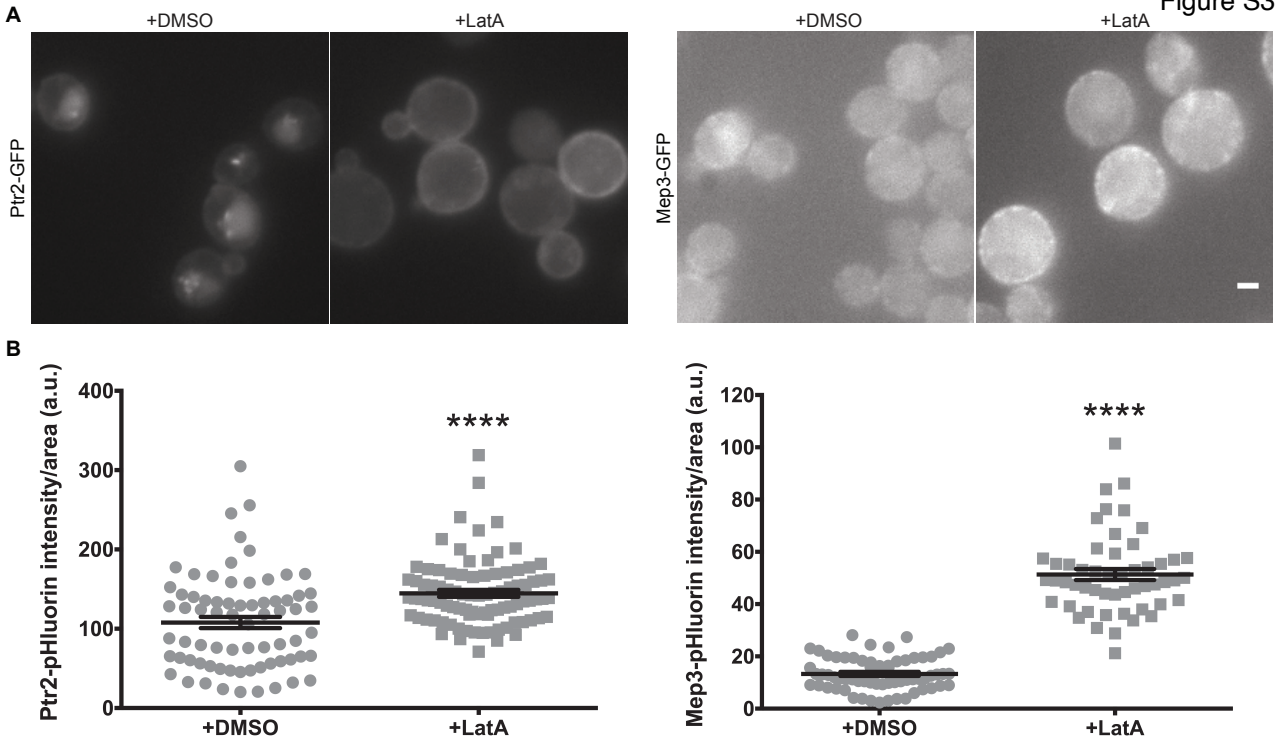
A
+DMSO
+LatA
Ptr2-GFP
+DMSO
+LatA
Mep3-GFP
B
Ptr2-pHluorin intensity/area (a.u.)
400
300
200
100
0
****
+DMSO
+LatA
Mep3-pHluorin intensity/area (a.u.)
120
100
80
60
40
20
0
****
+DMSO
+LatA

Figure S4.

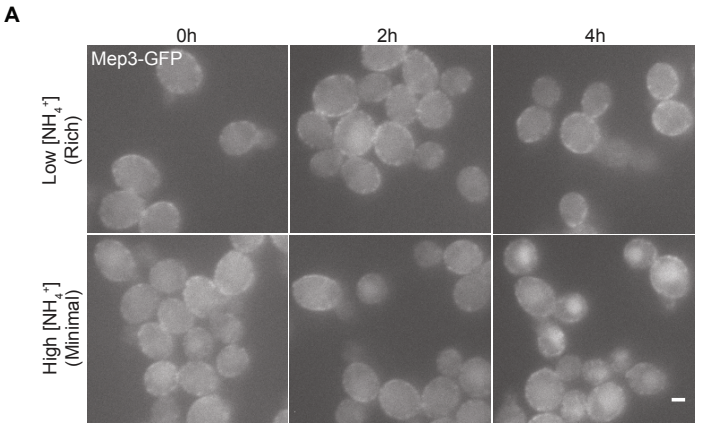
A
0h
2h
4h
Mep3-GFP
Low [NH4+] (Rich)
High [NH4+] (Minimal)
B
Syp1-GFP
Rich
Minimal
116 kDa
67 kDa
IB: GFP
IB: G6PDH

Figure S5.

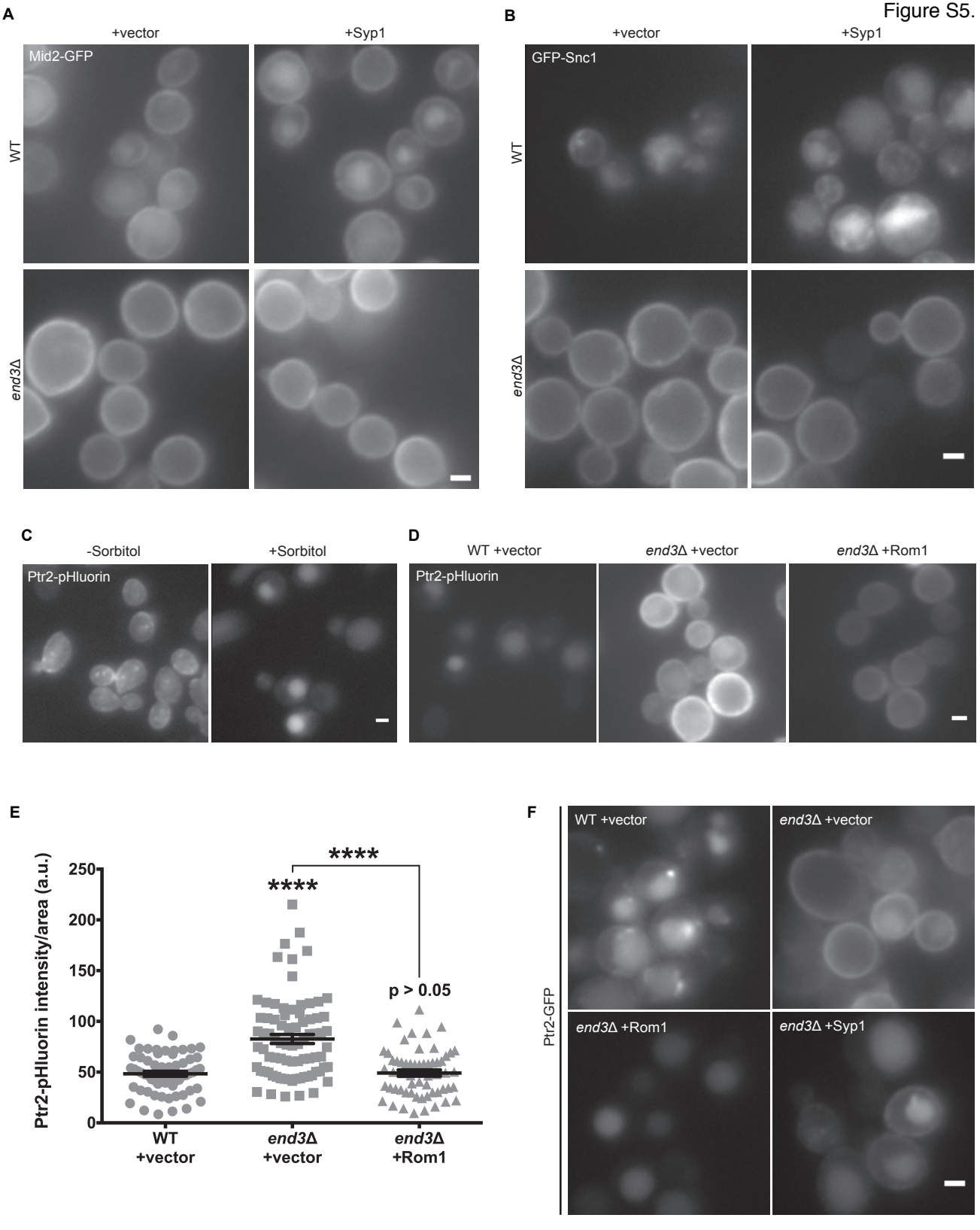
A
+vector
+Syp1
Mid2-GFP
WT
end3Δ
B
+vector
+Syp1
GFP-Snc1
WT
end3Δ
C
-Sorbitol
+Sorbitol
Ptr2-pHluorin
D
WT +vector
end3Δ +vector
end3Δ +Rom1
Ptr2-pHluorin
E
Ptr2-pHluorin intensity/area (a.u.)
250
200
150
100
50
0
****
****
p > 0.05
WT +vector
end3Δ +vector
end3Δ +Rom1
F
Ptr2-GFP
WT +vector
end3Δ +vector
end3Δ +Rom1
end3Δ +Syp1

Table S1. Cargoes tested in *syp1Δ* visual screen.

Systematic Name	Standard Name
YAL053W	FLC2
YAL055W	PEX22
YBL017C	PEP1
YBL042C	FUI1
YBR008C	FLR1
YBR014C	GRX7
YBR043C	QDR3
YBR054W	YRO2
YBR068C	BAP2
YBR069C	TAT1
YBR086C	IST2
YBR161W	CSH1
YBR187W	GDT1
YBR199W	KTR4
YBR205W	KTR3
YBR255W	MTC4
YBR302C	COS2
YCL001W	RER1
YCL027W	FUS1
YCR024C-A	PMP1
YCR024C-B	
YCR028C	FEN2
YCR061W	
YDL010W	GRX6
YDL046W	NPC2
YDL091C	UBX3
YDL180W	
YDL211C	
YDL222C	FMP45
YDR011W	SNQ2
YDR033W	MRH1
YDR038C	ENA5
YDR039C	ENA2

Systematic Name	Standard Name
YDR040C	ENA1
YDR046C	BAP3
YDR084C	TVP23
YDR090C	
YDR093W	DNF2
YDR233C	RTN1
YDR270W	CCC2
YDR281C	PHM6
YDR343C	HXT6
YDR345C	HXT3
YDR367W	KEI1
YDR384C	ATO3
YDR452W	PPN1
YDR456W	NHX1
YDR479C	PEX29
YDR481C	PHO8
YDR497C	ITR1
YDR508C	GNP1
YDR525W-A	SNA2
YEL017C-A	PMP2
YEL063C	CAN1
YEL065W	SIT1
YER001W	MNN1
YER056C	FCY2
YER118C	SHO1
YER145C	FTR1
YFL026W	STE2
YFL062W	COS4
YGL008C	PMA1
YGL077C	HNM1
YGL139W	FLC3
YGL200C	EMP24
YGL203C	KEX1

Systematic Name	Standard Name
YGL225W	VRG4
YGR014W	MSB2
YGR026W	
YGR041W	BUD9
YGR055W	MUP1
YGR065C	VHT1
YGR105W	VMA21
YGR121C	MEP1
YGR125W	
YGR138C	TPO2
YGR191W	HIP1
YGR281W	YOR1
YGR295C	COS6
YHL008C	
YHL028W	WSC4
YHL036W	MUP3
YHL040C	ARN1
YHR004C	NEM1
YHR123W	EPT1
YHR132C	ECM14
YHR140W	
YHR142W	CHS7
YHR149C	SKG6
YIL005W	EPS1
YIL015W	BAR1
YIL037C	PRM2
YIL067C	
YIL117C	PRM5
YIL121W	QDR2
YIL140W	AXL2
YIL147C	SLN1
YIL158W	AIM20
YIL173W	VTH1

Table S1. Cargoes tested in *syp1Δ* visual screen. Continued

Systematic Name	Standard Name
YJL151C	SNA3
YJL178C	ATG27
YJL186W	MNN5
YJR044C	VPS55
YJR054W	KCH1
YJR161C	COS5
YKL051W	SFK1
YKL073W	LHS1
YKL077W	PSG1
YKL165C	MCD4
YKL175W	ZRT3
YKL209C	STE6
YKR039W	GAP1
YKR088C	TVP38
YKR093W	PTR2
YKR100C	SKG1
YLL006W	MMM1
YLL028W	TPO1
YLL043W	FPS1
YLL051C	FRE6
YLR072W	LAM6
YLR080W	EMP46
YLR083C	EMP70
YLR084C	RAX2
YLR093C	NYV1
YLR138W	NHA1
YLR220W	CCC1
YLR237W	THI7
YLR297W	
YLR332W	MID2
YLR342W	FKS1
YLR353W	BUD8
YLR413W	INA1

Systematic Name	Standard Name
YLR414C	PUN1
YML028W	TSA1
YML052W	SUR7
YML072C	TCB3
YML116W	ATR1
YML132W	COS3
YMR010W	ANY1
YMR011W	HXT2
YMR058W	FET3
YMR110C	HFD1
YMR171C	EAR1
YMR183C	SSO2
YMR221C	FMP42
YMR253C	
YMR266W	RSN1
YMR313C	TGL3
YMR319C	FET4
YNL058C	
YNL065W	AQR1
YNL087W	TCB2
YNL115C	
YNL130C	CPT1
YNL142W	MEP2
YNL176C	TDA7
YNL194C	
YNL217W	
YNL238W	KEX2
YNL268W	LYP1
YNL279W	PRM1
YNL283C	WSC2
YNL305C	BXI1
YNL326C	PFA3
YNL336W	COS1

Systematic Name	Standard Name
YNR028W	CPR8
YNR055C	HOL1
YNR056C	BIO5
YNR061C	
YOL007C	CSI2
YOL019W	
YOL020W	TAT2
YOL047C	LDS2
YOL084W	PHM7
YOL088C	MPD2
YOL105C	WSC3
YOR008C	SLG1
YOR086C	TCB1
YOR099W	KTR1
YOR104W	PIN2
YOR153W	PDR5
YOR161C	PNS1
YOR165W	SEY1
YOR273C	TPO4
YOR292C	
YOR320C	GNT1
YOR327C	SNC2
YPL019C	VTC3
YPL053C	KTR6
YPL057C	SUR1
YPL058C	PDR12
YPL087W	YDC1
YPL156C	PRM4
YPL221W	FLC1
YPL265W	DIP5
YPR037C	ERV2
YPR075C	OPY2
YPR079W	MRL1

Table S1. Cargoes tested in *syp1Δ* visual screen. Continued

Systematic Name	Standard Name
YPR124W	CTR1
YPR138C	MEP3
YPR149W	NCE102
YPR156C	TPO3
YPR159W	KRE6