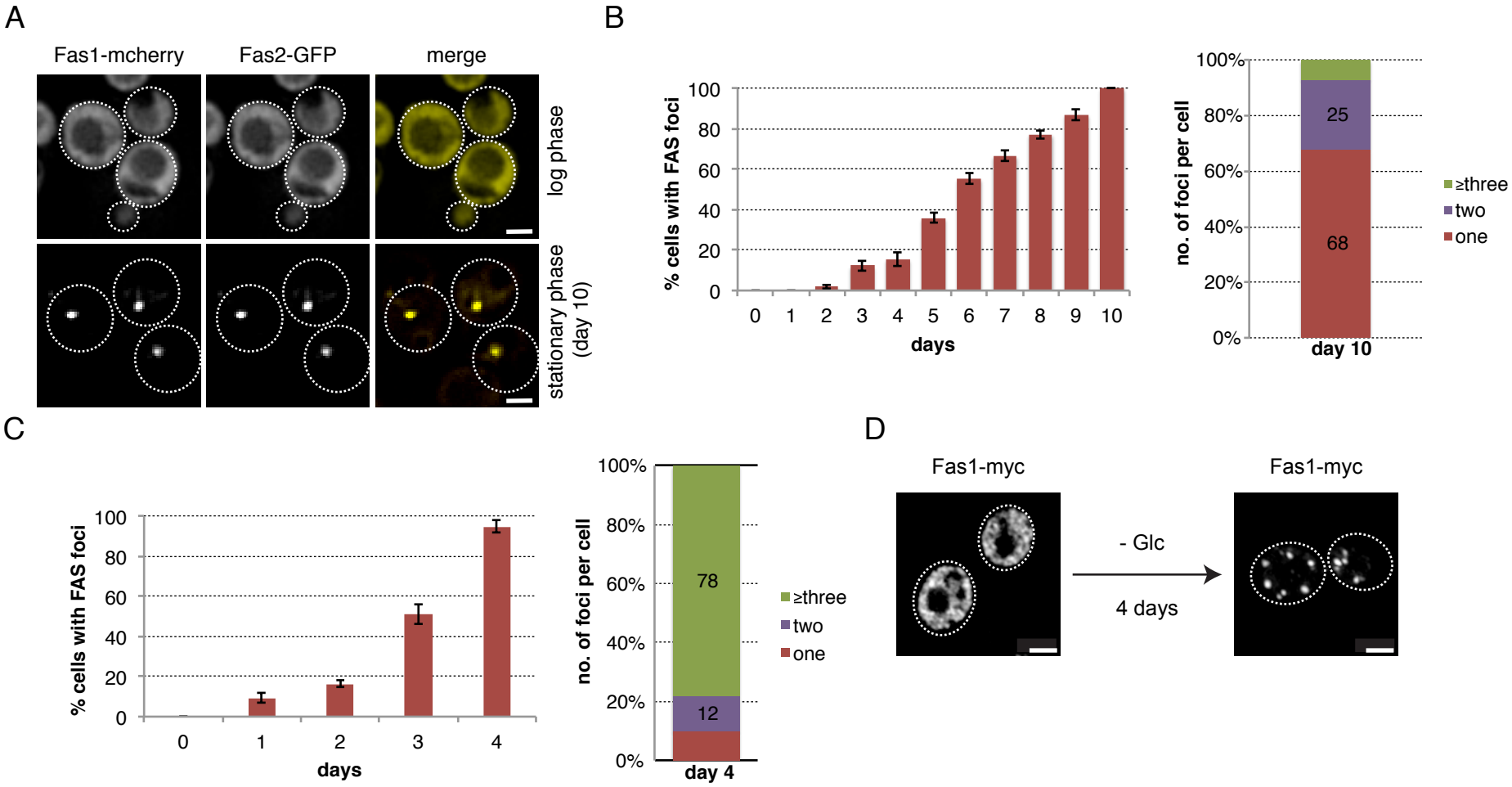
Supplemental Materials

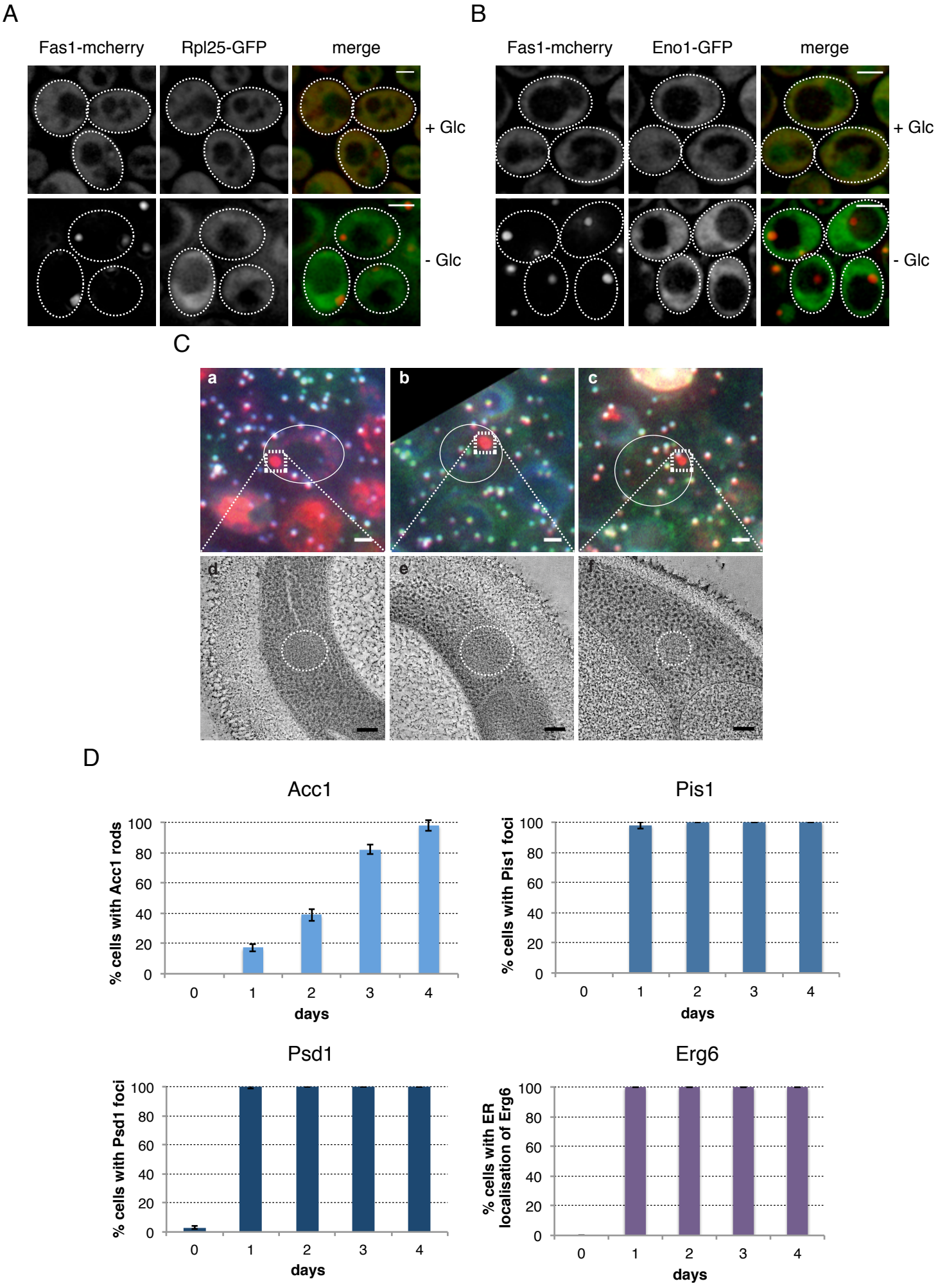
Molecular Biology of the Cell

Suresh et al.

Supplemental Figure 1:

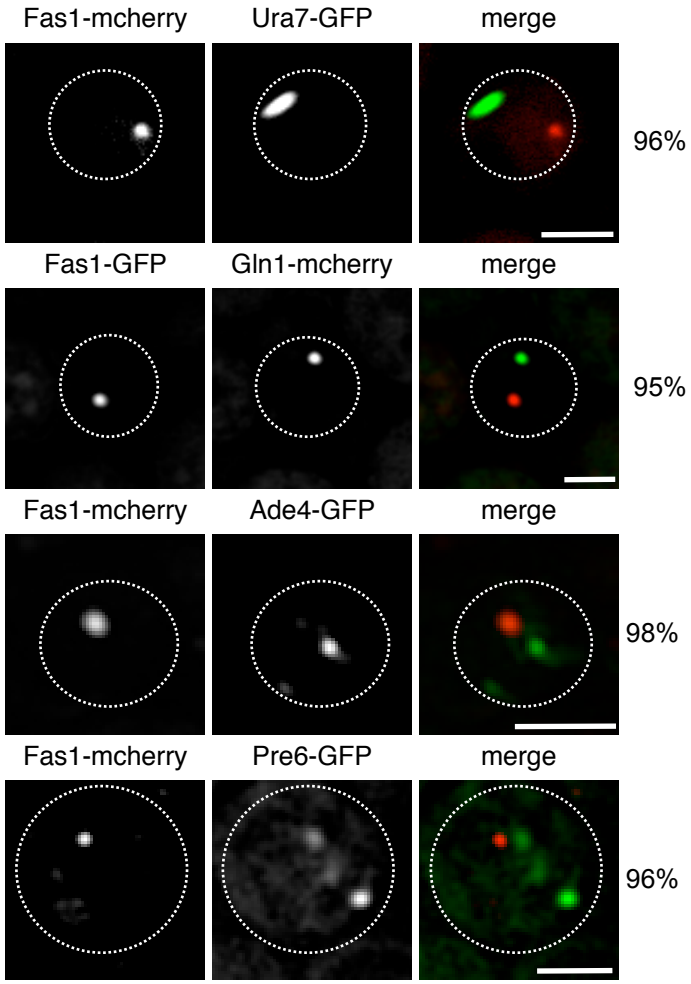


Supplementary Figure 2:



Supplementary Figure 3:

A



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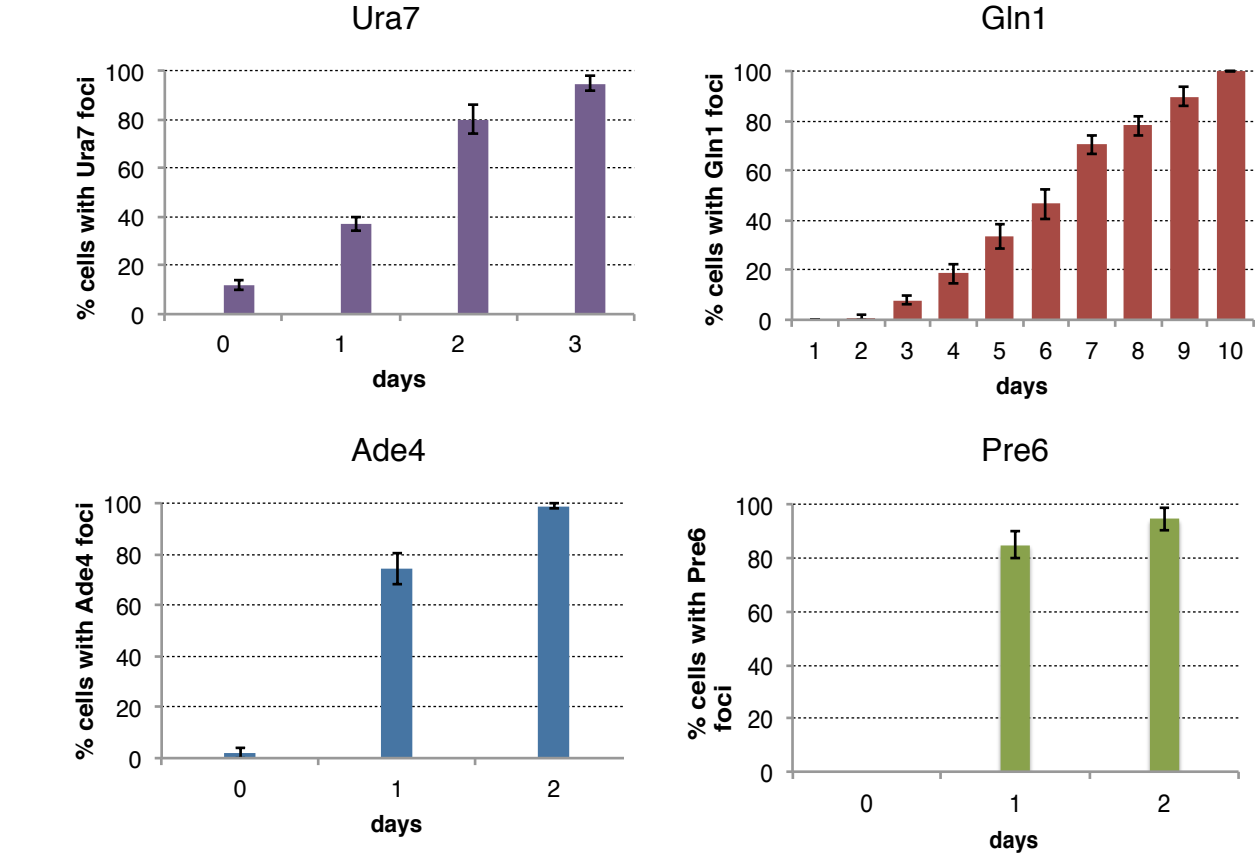
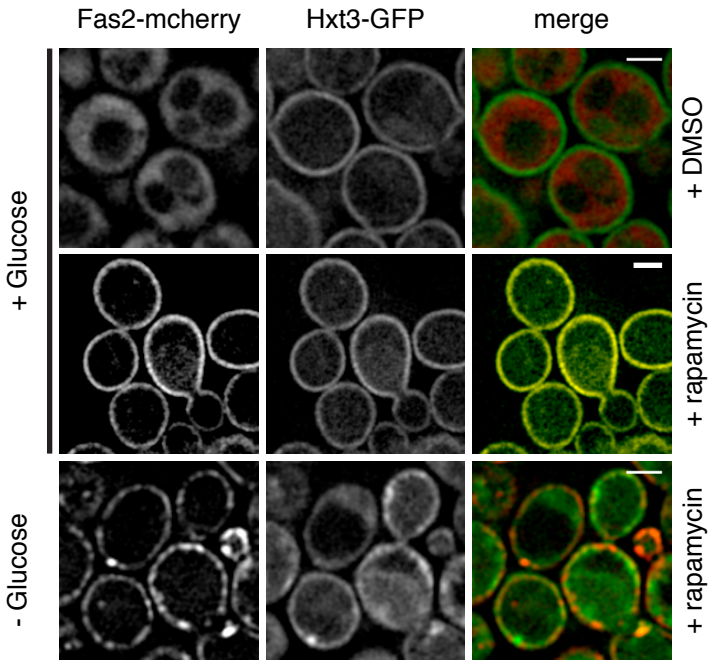
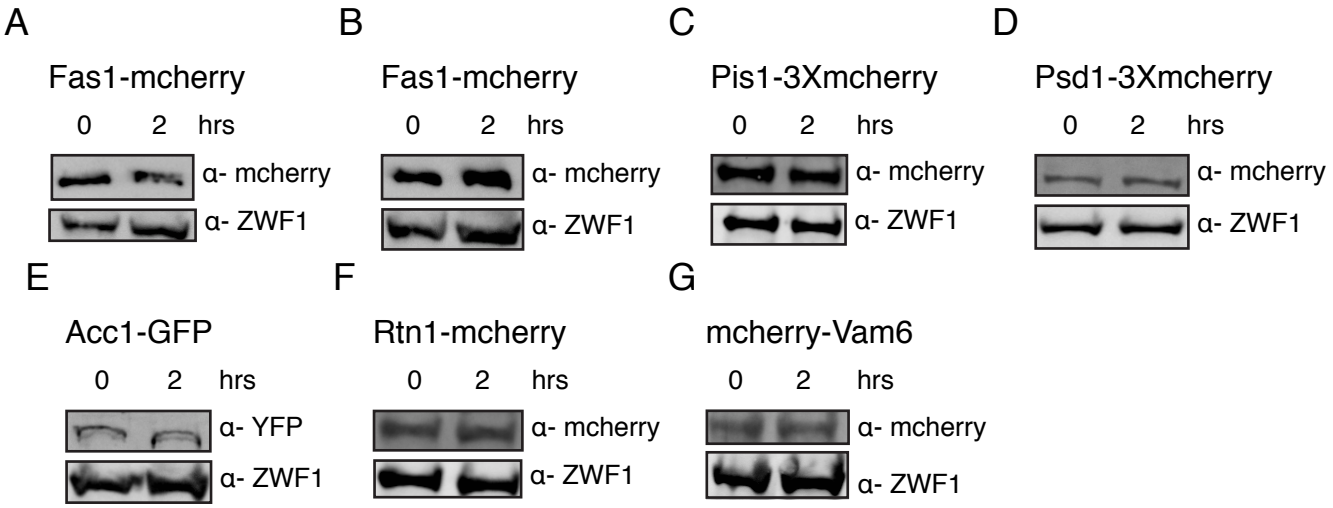


Supplementary Figure 4:

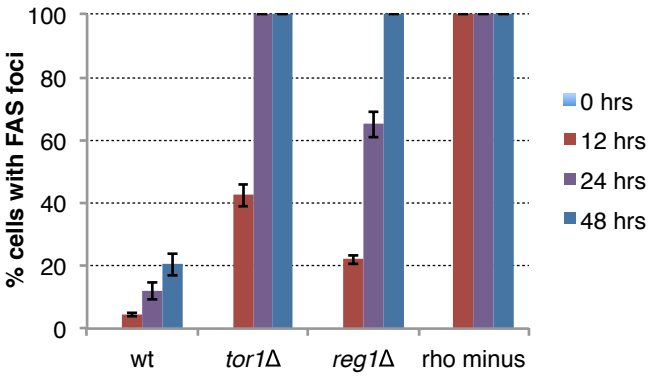


Supplementary Figure 5:



Supplementary Figure 6:

A



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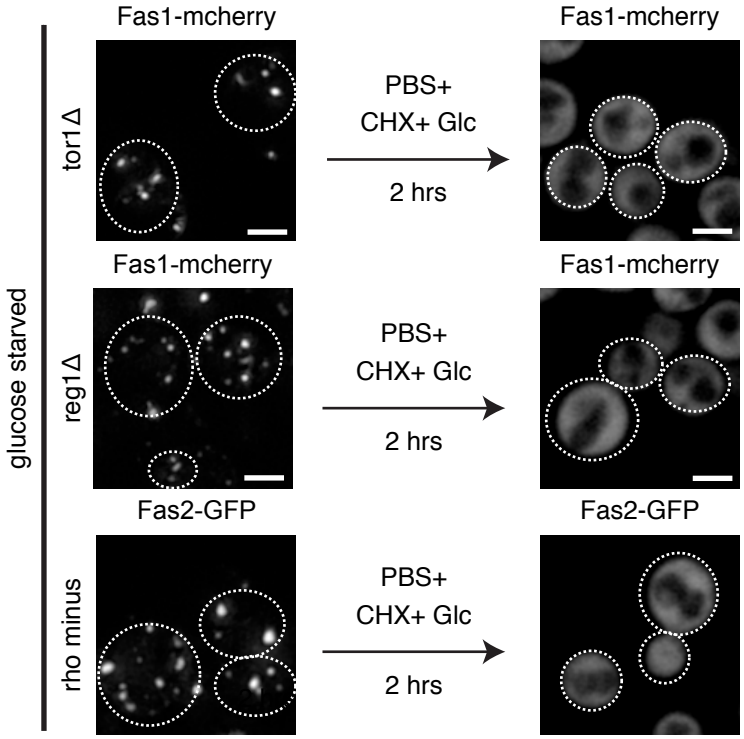
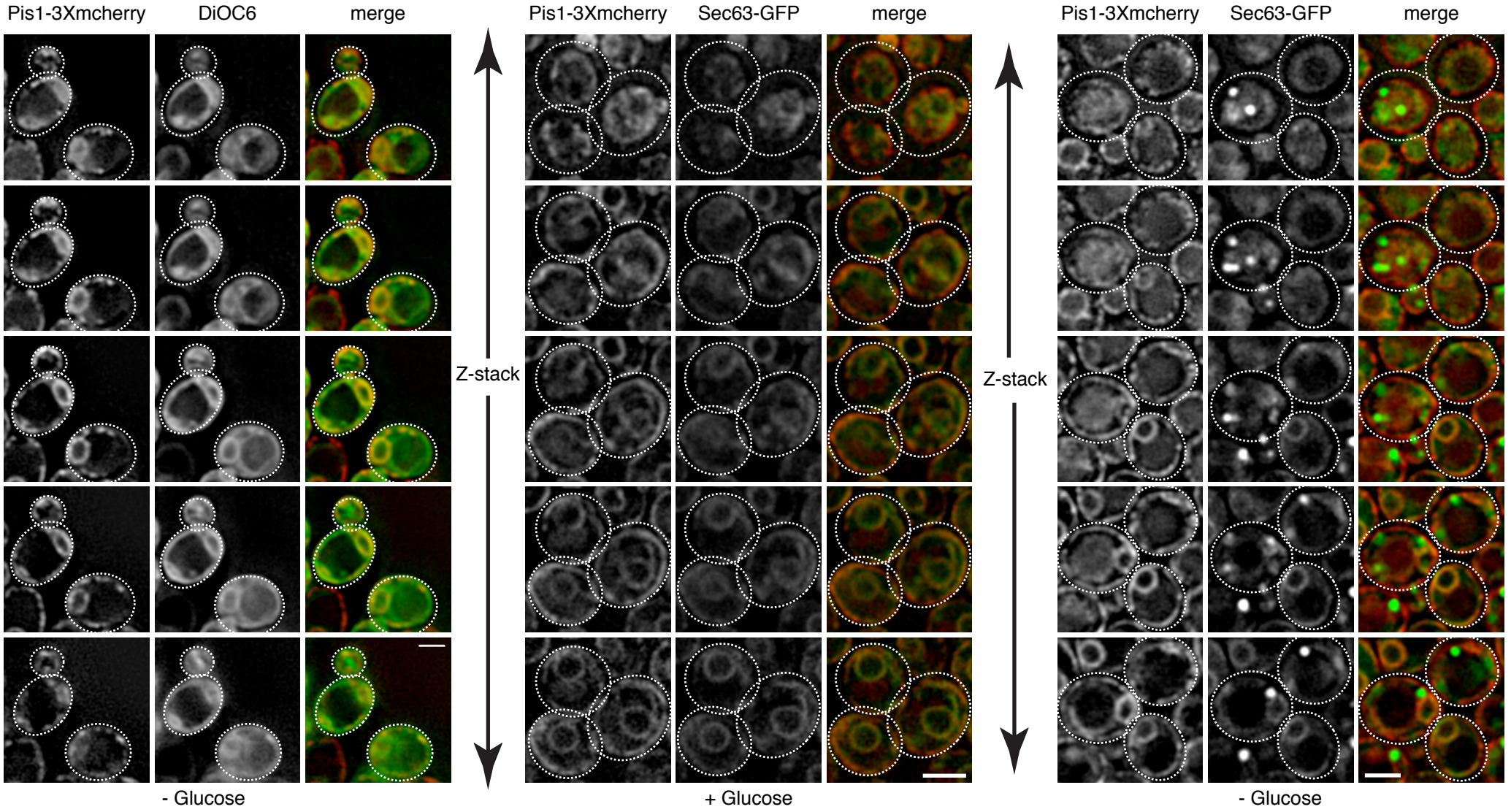
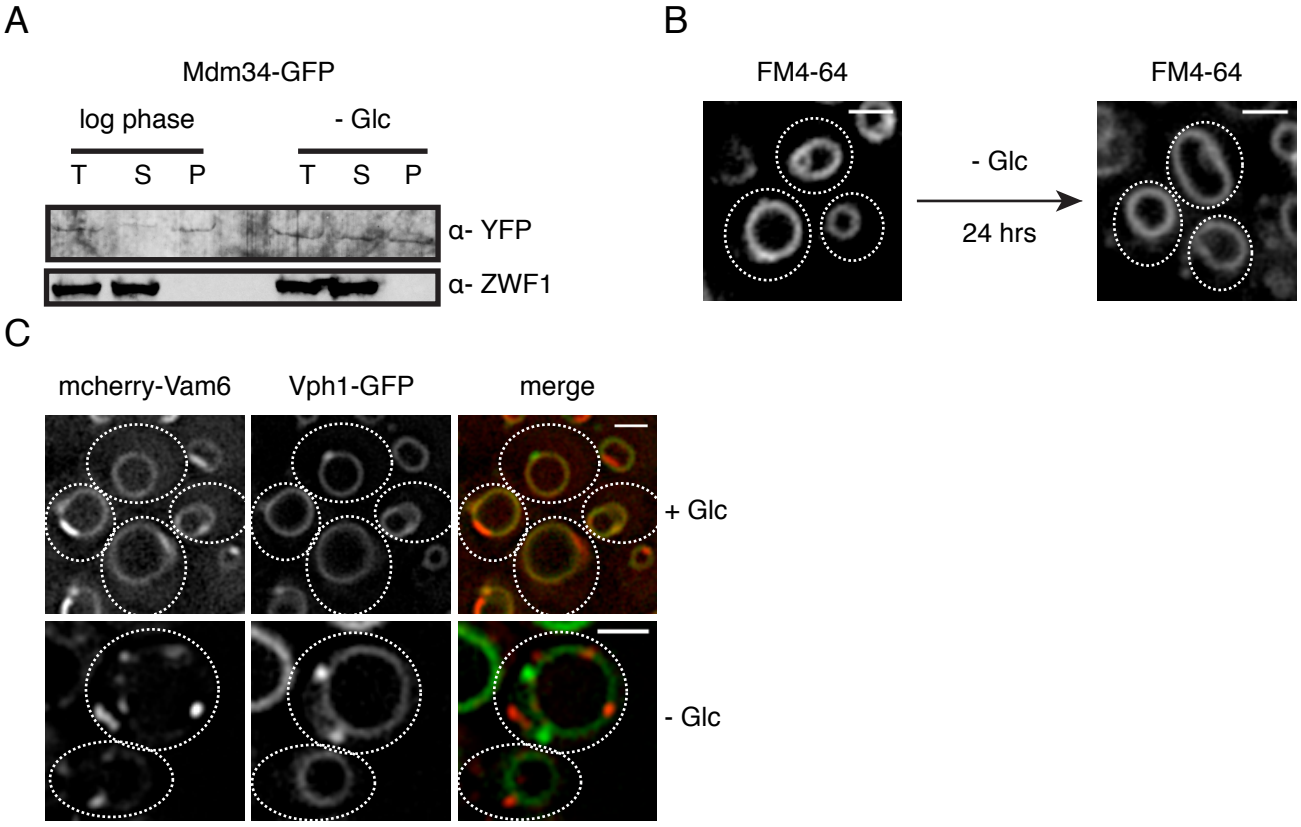


Supplementary Figure 7:



Supplementary Figure 8:



Supplementary information: (Suresh *et.al*, 2015)

Supplementary Figure 1: Kinetics of sequestration of lipid biosynthetic enzymes upon starvation (corresponding to Figure 1). (A) Both subunits of FAS co-sequester into foci that co-localize in stationary phase cells. *S. cerevisiae* cells expressing Fas1-mcherry and Fas2-GFP were grown to log phase or stationary phase (10 days) at 30°C. Changes in protein localizations were recorded. Dashed lines indicate cell boundaries. Single Z-plane images are shown. Bars: 2 μm. (B/C) Time dependent changes in the percentage number of cells harboring FAS foci in cells grown to late stationary phase or upon glucose depletion at 30°C. Percentage of cells having FAS foci (left panel) and the distribution of number of Fas1-mcherry foci per cell (right panel) in stationary phase cells (B) or glucose depleted cells (C) in SD medium at were determined two times (n=100 each). (D) Fas1 accumulates into foci upon glucose depletion. Fas1-7xmyc tagged cells were either grown to log phase or glucose starved for 4 d and localization of Fas1 was probed by immunofluorescence using anti-myc antibody. Maximum intensity projections of Z-stack images are shown. Scale bar: 2 μm.

Supplementary Figure 2: (A/B) Glucose starvation leads to sequestration of specific cytosolic cellular factors. *S.cerevisiae* cells were grown to log phase and starved of glucose for 4 days. Fas1-mcherry/Fas2-mcherry but not other tested cytosolic factors, Rpl25-GFP/Eno1-GFP, sequestered into foci upon glucose starvation. Single plane images are shown. Scale bar: 2 μm. (C) Correlated fluorescence and electron microscopic analysis of Fas1-mcherry foci formed upon glucose depletion. (a-c) An overlay of GFP, mcherry and blue fluorescence channels

to visualize Fas1-mCherry spots as well as fiducial markers, which are visible in all three channels and therefore appear white. Circles mark the boundaries of the cells and the foci are marked by dashed squares. (d-e) are close-up views of virtual slices through the electron tomograms, corresponding approximately to the boxed areas in (a-c), respectively. The predicted centroid positions of the mcherry signals are marked by the center of the white dashed curves. Scale bars: 1 μm in (a-c) and 100 nm in (d-f). (D) Cells expressing Acc1-GFP/Pis1-3Xmcherry/Psd1-3Xmcherry/Erg6-mcherry were grown to log phase in SD medium. Cells were washed twice with water and glucose depleted by transferring them to fresh SC medium. Cells were monitored for foci formation on different days by fluorescence microscopy. Time dependent sequestration of different proteins was quantified from multiple repetitions (n=100 cells). Standard deviations are given.

Supplementary Figure 3: FAS foci unlike misfolded protein aggregates do not co-localize with other known sequestrations during starvation (corresponding to Figure 2). (A) FAS is sequestered at a site distinct from other known sequestrations. *S. cerevisiae* cells coexpressing Fas1-mcherry/Fas1-GFP with Ura7-GFP, Gln1-mcherry, Ade4-GFP or Pre6-GFP were grown to stationary phase in SD medium for 10 days. Localizations of protein fusions were determined by fluorescence microscopy (n=100/each pair). Single Z-plane images are shown. Bars: 2 μm. (B) Proteins sequester at different rates in stationary phase. Cells expressing Ura7-GFP/Gln1-mcherry/Ade4-GFP /Pre6-GFP were grown to stationary phase in SD medium. Time dependent changes in the percentage no. of cells harboring foci for the above-mentioned proteins were determined. The experiments were repeated twice based on 100 cells and standard deviations are given.

Supplementary Figure 4: Glucose starvation leads to alterations in distribution of plasma membrane proteins. *S.cerevisiae* cells expressing Fas1-FRB, Pma1-FKBP12, Fas2-mcherry and Hxt3-GFP were grown to log phase. While an aliquot of the culture was treated with either DMSO (control) or rapamycin (upper panel), another aliquot was treated with rapamycin and starved of glucose for a day (lower panel). In the log phase culture, addition of rapamycin leads to anchoring of FAS (monitored by Fas2-mcherry) uniformly to the plasma membrane, stained by Hxt3-GFP (upper panel). In glucose-starved cells, Fas2-mcherry while anchored to the plasma membrane accumulates into punctate structures that are largely distinct from those formed by Hxt3-GFP (lower panel). Scale bar: 2 μm.

Supplementary Figure 5: Protein levels of sequestered proteins do not change upon addition of glucose and cycloheximide. *S.cerevisiae* cells expressing fluorescent protein tagged marker proteins (Fas1-mcherry/Acc1-GFP/Pis1-3Xmcherry/Psd1-3Xmcherry/Rtn1-mcherry/mcherry-Vam6) were grown to log phase and starved of glucose. Foci formation of the above mentioned markers were confirmed by fluorescence miscroscopy. 2% (v/v) Glucose (A, C-G) or entire medium (B) was added to cycloheximide (100 μg/ml) treated cells and incubated for 2 h at 30°C. Total cell lysates were prepared and protein levels were determined before and after glucose addition using anti-YFP/anti-mcherry antibodies. Anti-ZWF1 antibodies were used for loading controls.

Supplementary Figure 6: FAS sequestration is modulated by nutrient sensing pathways and is independent of its subcellular localization (corresponding to Figure 2). (A) *S.cerevisiae* wt, *tor1*Δ, *reg1*Δ and rho minus cells expressing Fas1-mcherry/Fas2-GFP were grown to log phase and subsequently starved of glucose by transferring them to media lacking glucose. Changes in localization of FAS monitored by fluorescence microscopy at different time points and their kinetics were determined (n=100). Standard deviations are given (n=2). (B) FAS foci formed in nutrient sensing mutants are reversible. Glucose starved *tor1Δ*, *reg1Δ* and rho minus cells expressing Fas1-mcherry/Fas2-GFP were replenished with glucose in the presence of cycloheximide (100 μg/ml) and changes in localization monitored by fluorescence microscopy. Images represent maximum intensity projections of the Z-stack images. Bars: 2 μm.

Supplementary Figure 7: Glucose starvation drives sequestration of ER proteins into distinct foci. Left panel: *S. cerevisiae* cells expressing Pis1-3Xmcherry were grown to log phase and starved of glucose for 1 day. The ER was stained by DiOC6 (5 ng/ml) and imaged by fluorescence microscopy. While the peripheral Pis1-3Xmcherry accumulates into foci, the ER membrane staining remains continuously uniform. Middle and right panel: *S. cerevisiae* cells expressing Pis1-3Xmcherry and Sec63-GFP were grown to log phase and/or starved of glucose for 1 day. Sec63-GFP and Pis1-3Xmcherry accumulate into distinct foci upon glucose starvation (right panel). Scale bar: 2 μm.

Supplementary Figure 8: Glucose starvation causes re-localization of ERMES and vCLAMP markers. (A) *S. cerevisiae* cells expressing Mdm34-GFP were grown at

30°C to either log phase or were depleted of glucose for one day Total cells lysates (T) were separated into soluble/cytosolic (S) and insoluble/organellar (P) fractions by centrifugation. Distribution of Mdm34-GFP was determined by western blot analysis using GFP-specific antibodies. (B) *S.cerevisiae* cells were either grown to log phase or starved of glucose for one day and vacuoles were stained with FM4-64. (C) *S.cerevisiae* cells expressing mcherry-Vam6 and Vph1-GFP were either grown to log phase or starved of glucose for one day. mcherry-Vam6 and Vph1-GFP colocalize to the vacuolar membrane in log phase cultures (upper panel). Glucose starvation leads to sequestration of mcherry-Vam6 into distinct without affecting Vph1-GFP vacuolar membrane localization (lower panel). Scale bar: 2 μm.

Supplementary Table 1: Alterations in lipid profiles upon glucose starvation (corresponding to Figure 8). Relative levels of different phospholipid species measured for 10 biological replicates at different time points of glucose starvation.

		% of total			
	Time(h)	PC	PE	PI	PS
EXP01	0h	39,5347	25,6417	15,8809	18,9427
EXP01	0h	41,893	25,7552	15,0387	17,3131
EXP01	0h	37,2324	25,8011	18,4714	18,4952
EXP03	0h	50,5319	30,5214	6,1925	12,7542
EXP03	0h	49,692	31,2266	6,39497	12,6864
EXP03	0h	46,7757	23,2329	17,0016	12,9898
EXP05	0h	52,252	23,286	5,00446	19,4575
EXP05	0h	51,6425	23,2192	5,40399	19,7343
EXP05	0h	50,1223	23,2778	5,58068	21,0192
EXP05	0h	50,786	23,2553	5,43558	20,5231
EXP01	24h	44,7142	20,2868	18,3102	16,6889
EXP01	24h	42,0035	22,2782	22,7559	12,9624
EXP01	24h	37,1858	18,5369	30,6102	13,6671
EXP03	24h	47,7375	20,6492	19,3377	12,2756
EXP03	24h	44,5014	22,921	19,8501	12,7275
EXP03	24h	43,6936	23,3431	21,2054	11,7579
EXP05	24h	49,0948	12,5944	18,7367	19,574
EXP05	24h	49,0283	12,0925	18,6842	20,195
EXP05	24h	48,1188	12,0094	19,7969	20,075
EXP05	24h	48,6435	12,7889	19,6229	18,9447
EXP01	48h	42,0636	25,5254	19,0981	13,3129
EXP01	48h	38,5178	21,1545	25,1437	15,184
EXP01	48h	41,977	20,1174	19,3543	18,5513
EXP03	48h	42,911	22,0559	20,2374	14,7958
EXP03	48h	40,5843	25,4525	20,1956	13,7676
EXP03	48h	40,0512	24,8678	21,8527	13,2284
EXP05	48h	45,8385	13,4051	20,3142	20,4422
EXP05	48h	45,0753	13,0205	19,6307	22,2735
EXP05	48h	47,5523	12,938	19,5203	19,9895
EXP05	48h	47,2074	13,1913	19,751	19,8503
EXP01	72h	36,7845	19,4802	27,1497	16,5856
EXP01	72h	40,3456	21,6106	22,8464	15,1975
EXP01	72h	38,1057	23,3527	25,1849	13,3567
EXP03	72h	38,5634	26,386	20,0647	14,9858
EXP03	72h	38,7548	25,5098	20,9233	14,8121
EXP03	72h	36,7598	26,9457	20,9953	15,2991

EXP05	72h	43,7002	13,9781	20,1328	22,1889
EXP05	72h	42,6137	13,3654	20,9161	23,1048
EXP05	72h	42,4945	13,9151	21,3113	22,2791
EXP05	72h	44,3804	13,6239	19,3993	22,5963
EXP01	96h	42,4311	15,5031	22,9527	19,1131
EXP01	96h	42,1351	16,584	20,6042	20,6767
EXP01	96h	42,7748	15,5146	22,9525	18,7581
EXP03	96h	39,6699	25,524	19,9299	14,8762
EXP03	96h	37,8194	23,9275	24,8931	13,36
EXP03	96h	38,2666	26,9496	21,3219	13,462
EXP05	96h	41,049	14,3718	19,7552	24,8241
EXP05	96h	41,9615	13,9249	20,876	23,2375
EXP05	96h	42,5337	13,7842	20,8599	22,8221
EXP05	96h	42,0385	14,0423	20,9655	22,9537
	Time(h)	Total_PC	Total_PE	Total_PI	Total_PS
Mean	0h	47,0463	25,5217	10,0405	17,3916
Mean	24h	45,4721	17,75	20,891	15,8868
Mean	48h	43,1778	19,1728	20,5098	17,1395
Mean	72h	40,2503	19,8168	21,8924	18,0406
Mean	96h	41,068	18,0126	21,5111	19,4083
SD	0h	5,47912	3,04412	5,72209	3,32285
SD	24h	3,84908	4,83208	3,65927	3,54714
SD	48h	3,10959	5,48861	1,79949	3,42144
SD	72h	2,85711	5,68734	2,47379	3,95789
SD	96h	1,83044	5,2648	1,60418	4,25332
ttest	**t-Test versus Time 0h**				
ttest	24h	0,46683	0,00043	0,00008	0,34055
ttest	48h	0,06799	0,00497	0,00003	0,86917
ttest	72h	0,00269	0,01192	0,00001	0,69592
ttest	96h	0,00423	0,00104	0,00001	0,25273

PC: Phosphatidyl choline; PE: Phosphatidyl ethanolamine; PS: Phosphatidyl serine; PI: Phosphatidyl inositol. While the legends for the mean values and the respective standard deviations for 10 biological repeats are highlighted in yellow, the significant P values (as determined by t-test) are highlighted in pink.

Supplementary Table 2: Yeast strains used in this study.

Strain	Genotype	Source
BY4741	MATa his3Δ0;leu2Δ0;met15Δ0;ura3Δ0	Lab collection
BY4742	MATα his3Δ1;leu2Δ0;lys2Δ0;ura3Δ0	Lab collection
GSHY210	Fas1-mcherry:: kanMX4;Fas2-GFP::hphNT1	This study
GSHY185	BY4742 Fas1-mcherry::kanMX4;Hsp104-GFP::hphNT1	This study
GSHY191	BY4742 Fas1-mcherry:: kanMX4	This study
GSHY97	BY4741 Fas1-GFP::kanMX4;hsp104Δ::natNT1	This study
GSHY182	BY4741 Acc1-GFP::His3;Fas1-mcherry::hphNT1	This study
GSHY621	BY4742 Fas2-GFP::kanMX4;Pis1-3Xmcherry::hphNT1	This study
GSHY619	BY4742 Fas2-GFP::kanMX4;Psd1-3Xmcherry::hphNT1	This study
GSHY479	BY4742 Erg6-GFP::hphNT1;Fas1-mcherry::kanMX4	This study
GSHY604	BY4742 Sec63-3Xmcherry::hphNT1	This study
GSHY583	BY4742 ss-dsRed-HDEL::natNT1	This study
GSHY514	BY4741 Shm1-GFP::His3	GFP library
GSHY642	BY4741 Mdm34-GFP::His3	GFP library
GSHY639	BY4742 Fas2-GFP::kanMX4;Psd1-3Xmcherry::hphNT1; rho^-	This study
GSHY409	HY110 Fas1-FRB-GFP::His3MX6;Fas2-mcherry::hphNT1	This study
HY110	leu2-3,112;his3-11,15;trp1-1;can1-100;ade2-1;ura3-1;tor1-1;fpr1 Δ:: nat;PMA1-2XFKBP12:: Trp1	Euroscarf
GSHY408	HY110 Fas1-FRB::His3MX6;Fas2-mcherry::hphNT1	This study
GSHY154	BY4742 Ura7-GFP::hphNT1;Fas1-mcherry::kanMX4	This study
GSHY126	BY4741 Fas1-GFP::kanMX4;Gln1-mcherry::hphNT1	This study
GSHY164	BY4742 Ade4-GFP::hphNT1;Fas1-mcherry::kanMX4	This study
GSHY636	BY4742 Pre6-GFP::hphNT1;Fas1-mcherry::natNT2	This study

GSHY637	BY4742 Fas1-7xmyc::natNT2	This study
GSHY634	YND53 TEF1-mcherry-Vam6::Ura	This study
SSY412	W303 Sec63-GFP::His3; Rtn1-mcherry::Trp1	Schuck laboratory
GSHY638	BY4742 Fas2-GFP::kanMX4;Psd1-3Xmcherry::hphNT1; dnm1Δ::natNT2	This study
GSHY208	BY4741 Fas2-GFP::hphNT1; reg1Δ::loxP	This study
GSHY547	BY4742 Fas1-mcherry::hphNT1; tor1Δ::natNT2	This study
GSHY637	YND53 TEF1-mcherry-Vam6:: Ura; Vph1-GFP::hphNT1	This study
GSHY643	HY110 Fas1-FRB::His3MX6, Fas2-mcherry::hphNT1, Eno1-GFP::KanMX4	This study
GSHY644	HY110 Fas1-FRB::His3MX6, Fas2-mcherry::hphNT1, Hxt3-GFP::KanMX4	This study
GSHY645	BY4742 Pis1-3Xmcherry::hphNT1, Sec63-GFP::His3	This study
GSHY294	BY4742 Rpl25-GFP:: leu Rpl25Δ::Nat, Fas1-mcherry::kanMX4	This study

Supplementary Table 3: Plasmids used in this study

Name of plasmid	Description	Source
pKT127	Amp;kan	Euroscarf
pBS34	Amp; kan	Yeast resource centre
pBS35	Amp; hph	Yeast resource centre
pYM25	Amp; hph	Euroscarf (Janke *et.al*, 2004)
pYM21	Amp; nat	Euroscarf (Janke *et.al*, 2004)
pYM27	Amp; kan	Euroscarf (Janke *et.al*, 2004)
pFA6a-natNT2	Amp; nat	Euroscarf (Janke *et.al*, 2004)
pFA6a-FRB-His3MX6	Amp; His3	Euroscarf (Hirohito *et.al*, 2008)
pFA6a-FRB-GFP-His3MX6	Amp; His3	Euroscarf (Hirohito *et.al*, 2008)
pMaM57	Amp; hph	Knop laboratory
pMaM56	Amp; nat	Knop laboratory
YIplac-dsRedHDEL	Amp; nat	Schuck laboratory
pMaM144	Amp; hph	Knop laboratory
pMaM172	Amp; Ura3	Knop laboratory