

Supplementary Information

LEGENDS FOR SUPPLEMENTAL FIGURES

Supplemental Figure S1. (P_{GAS1})Wsc1* and (P_{PRC1})Wsc1* are degraded and processed similarly. (A-B) The turnover rate of (A) (P_{PRC1})Wsc1* or (B) (P_{GAS1})Wsc1* in wild type and $\Delta pep4$ cells were pulse-labeled at 30°C with [35 S]methionine/cysteine for 10 min followed by a cold chase for times indicated. Immunoprecipitated proteins using anti-HA monoclonal antibody were resolved by SDS-PAGE and quantified by phosphorimager analysis. The data plotted reflect three independent experiments with the mean \pm SD indicated. The arrowhead denotes the position of a non-specific band that is recognized by anti-HA antibody. (C-E) Western blotting analysis of wild type, $\Delta pep4$, $\Delta vps27$, $rsp5-1$, $\Delta pep4\ rsp5-1$, $\Delta doa4$ and $\Delta pep4\Delta doa4$ cells expressing (P_{GAS1})Wsc1*. (F) Cell lysates from wild type and $\Delta pep4$ strains expressing (P_{GAS1})Wsc1* or (P_{GAS1})Wsc1*-6R were prepared and subject to western blotting.

Supplemental Figure S2. ESCRT mutants disrupt Wsc1*-GFP trafficking to the vacuolar lumen. (A) Pulse-chase analysis was performed in wild type and $\Delta pep4$ cells expressing Wsc1*-GFP as described in supplemental Fig. S1A and B. Anti-GFP monoclonal antibody was used for immunoprecipitation. (B) Wild type (BY4741 background) and various ESCRT mutants (BY4741 background) expressing Wsc1*-GFP were grown to log phase at 30°C and analyzed by confocal and DIC microscopy. Scale bar, 5 μ m.

Supplemental Figure S3. Wsc1*-GFP is localized to PVCs and the vacuolar limiting membrane in $\Delta vps27$ and $\Delta pep4\Delta vps27$ cells. Wild type, $\Delta pep4$, $\Delta vps27$ and $\Delta pep4\Delta vps27$ expressing Wsc1*-GFP were incubated with FM4-64 and analyzed by confocal and DIC microscopy. Scale bar, 5 μ m.

Supplemental Figure S4. Wsc1* is not degraded via the microautophagy pathway. (A) Cycloheximide chase analysis of wild type, $\Delta pep4$ and microautophagy mutants (BY4741 background) expressing Wsc1* was performed as described in Fig. 5A. The arrowhead denotes the position of a non-specific band that is recognized by anti-HA antibody. (B) Confocal and DIC microscopy of wild type, $\Delta pep4$ and microautophagy mutants expressing Wsc1*-GFP. Scale bar, 5 μ m.

Supplemental Figure S5. Entry of Wsc1* into the MVB pathway requires lysine residues in the cytoplasmic domain. Indirect immunofluorescence micrographs of wild type and $\Delta pep4$ expressing Wsc1*-6R. Scale bar, 5 μ m.

Supplemental Figure S6. Toxicity of Wsc1*-6R is dosage dependent. (A) Wild type and $\Delta pep4$ cells containing vector control, (P_{PRC1})Wsc1*, (P_{PRC1})Wsc1*-6R, (P_{GAS1})Wsc1* or (P_{GAS1})Wsc1*-6R were grown in selective synthetic media to log phase and diluted to 0.1 OD/mL. OD₆₀₀ readings were monitored at indicated intervals over 10 hours. The data plotted reflect three independent experiments with the mean \pm SD indicated. * p < 0.01. (B) Wild type and $\Delta pep4$ cells containing vector control, (P_{GAL1})Wsc1* or (P_{GAL1})Wsc1*-6R were grown in synthetic complete media containing 3% raffinose to log phase. Cells were harvested and inoculated in synthetic complete media containing 2% galactose at 0.1 OD/mL. Growth was monitored by OD₆₀₀ for 32 hours. ** p < 0.002. (C-E) Cell lysates prepared from wild type and $\Delta pep4$ cells expressing Wsc1* or Wsc1*-6R driven by the *PRC1*, *GAS1* or *GAL1* promoters at 8 h were prepared and analyzed by western blotting. Membranes were probed with anti-HA and anti-Sec61p antibodies. Protein levels were visualized and quantified using the Odyssey infrared imaging system. Sec61p was used as a control for loading and normalization. The data reflect three independent experiments with mean \pm SD indicated. *** p < 0.001. (F) Wild type and $\Delta pep4$ cells containing

the control vector, (P_{GALI})Wsc1* or (P_{GALI})Wsc1*-6R grown in raffinose media were spotted as 10-fold serial dilutions onto glucose and galactose media plates and incubated at 30°C for 2 days and 3 days, respectively.

Supplemental Figure S7. Electron microscopy reveals disrupted internal membranes in the wild type strain expressing (P_{GAS1})Wsc1*-6R. Log phase cells were processed for TEM analysis as described in materials and methods. Shown are micrographs of (A) wild type cells with the empty vector (pRS315), (B) wild type cells expressing (P_{GAS1})Wsc1*, (C) $\Delta pep4$ cells expressing (P_{GAS1})Wsc1*, (D) wild type cells expressing (P_{GAS1})Wsc1*-6R and (E) $\Delta pep4$ cells expressing (P_{GAS1})Wsc1*-6R. For all panels, a 5,000 magnification micrograph and individual insets at 20,000 magnification are shown. N, nucleus. V, vacuole. Scale bars, 1 μ m.

Supplemental Figure S8. The wild type strain expressing (P_{GAS1})Wsc1*-6R displays an accumulation of lipid droplet. Wild type cells with an empty vector (pRS315), (P_{GAS1})Wsc1* or (P_{GAS1})Wsc1*-6R and $\Delta pep4$ cells expressing (P_{GAS1})Wsc1* or (P_{GAS1})Wsc1*-6R were grown to log phase. They were stained with LD540 and imaged for confocal and DIC microscopy. Scale bar, 5 μ m.

Supplemental Table S1 Strains used in this study

Strain	Genotype	Source
W303	<i>Mata, leu2-3, 112, his3-11, trp1-1, ura3-1, can1-100, ade2-1</i>	P. Walter (UCSF)
BY4741	<i>Mata, leu2Δ0, his3Δ1, met15Δ0, ura3Δ0</i>	Research Genetics
SWY300	<i>Mata</i> , pRS315, W303 background	This study
SWY342	<i>Mata</i> , pSW104, W303 background	This study
SWY345	<i>Mata, pep4::HIS3</i> , pSW104, W303 background	This study

Strain	Genotype	Source
SWY791	<i>Mata</i> , <i>vps27::KANMX</i> , pSW104, W303 background	This study
SWY792	<i>Mata</i> , <i>pep4::HIS3</i> , <i>vps27::KANMX</i> , pSW104, W303 background	This study
SWY236	<i>Mata</i> , <i>rsp5-1</i> , pSW104, W303 background	This study
SWY1058	<i>Mata</i> , <i>pep4::HIS3</i> , <i>rsp5-1</i> , pSW104, W303 background	This study
SWY1016	<i>Mata</i> , <i>doa4::KANMX</i> , pSW104, W303 background	This study
SWY1018	<i>Mata</i> , <i>pep4::HIS3</i> , <i>doa4::KANMX</i> , pSW104, W303 background	This study
SWY450	<i>Mata</i> , pSW104, BY4741 background	This study
SWY1098	<i>Mata</i> , <i>pep4::KANMX</i> , pSW104, BY4741 background	This study
SWY1099	<i>Mata</i> , <i>ego1::KANMX</i> , pSW104, BY4741 background	This study
SWY1100	<i>Mata</i> , <i>gtr2::KANMX</i> , pSW104, BY4741 background	This study
SWY1101	<i>Mata</i> , <i>ego3::KANMX</i> , pSW104, BY4741 background	This study
SWY552	<i>Mata</i> , pSW148, W303 background	This study
SWY553	<i>Mata</i> , <i>pep4::HIS3</i> , pSW148, W303 background	This study
SWY806	<i>Mata</i> , <i>vps27::KANMX</i> , pSW148, W303 background	This study
SWY807	<i>Mata</i> , <i>pep4::HIS3</i> , <i>vps27::KANMX</i> , pSW148, W303 background	This study
SWY280	<i>Mata</i> , <i>rsp5-1</i> , pSW148, W303 background	This study
SWY1062	<i>Mata</i> , <i>pep4::HIS3</i> , <i>rsp5-1</i> , pSW148, W303 background	This study
SWY292	<i>Mata</i> , <i>rsp5-1</i> , pSW148, W303 background	This study
SWY1063	<i>Mata</i> , <i>pep4::HIS3</i> , <i>rsp5-1</i> , pSW148, W303 background	This study
SWY753	<i>Mata</i> , pSW177, W303 background	This study
SWY754	<i>Mata</i> , <i>pep4::HIS3</i> , pSW177, W303 background	This study
SWY1036	<i>Mata</i> , <i>rsp5-1</i> , pSW177, W303 background	This study
SWY1061	<i>Mata</i> , <i>pep4::HIS3</i> , <i>rsp5-1</i> , pSW177, W303 background	This study
SWY747	<i>Mata</i> , pSW177, BY4741 background	This study

Strain	Genotype	Source
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SWY702	<i>Mata</i> , <i>hse1::KANMX</i> , pSW177, BY4741 background	This study
SWY704	<i>Mata</i> , <i>vps23::KANMX</i> , pSW177, BY4741 background	This study
SWY706	<i>Mata</i> , <i>vps28::KANMX</i> , pSW177, BY4741 background	This study
SWY708	<i>Mata</i> , <i>vps37::KANMX</i> , pSW177, BY4741 background	This study
SWY738	<i>Mata</i> , <i>mvb12::KANMX</i> , pSW177, BY4741 background	This study
SWY710	<i>Mata</i> , <i>vps36::KANMX</i> , pSW177, BY4741 background	This study
SWY712	<i>Mata</i> , <i>vps22::KANMX</i> , pSW177, BY4741 background	This study
SWY714	<i>Mata</i> , <i>vps25::KANMX</i> , pSW177, BY4741 background	This study
SWY716	<i>Mata</i> , <i>snf7::KANMX</i> , pSW177, BY4741 background	This study
SWY718	<i>Mata</i> , <i>vps20::KANMX</i> , pSW177, BY4741 background	This study
SWY720	<i>Mata</i> , <i>vps2::KANMX</i> , pSW177, BY4741 background	This study
SWY722	<i>Mata</i> , <i>vps24::KANMX</i> , pSW177, BY4741 background	This study
SWY672	<i>Mata</i> , <i>vps4::KANMX</i> , pSW177, BY4741 background	This study
SWY724	<i>Mata</i> , <i>bro1::KANMX</i> , pSW177, BY4741 background	This study
SWY728	<i>Mata</i> , <i>did2::KANMX</i> , pSW177, BY4741 background	This study
SWY1073	<i>Mata</i> , <i>ist1::KANMX</i> , pSW177, BY4741 background	This study
SWY730	<i>Mata</i> , <i>vps60::KANMX</i> , pSW177, BY4741 background	This study
SWY732	<i>Mata</i> , <i>vta1::KANMX</i> , pSW177, BY4741 background	This study
SWY1093	<i>Mata</i> , <i>tull::KANMX</i> , pSW177, BY4741 background	This study
SWY233	<i>Mata</i> , pSW252, W303 background	This study
SWY234	<i>Mata</i> , <i>pep4::HIS3</i> , pSW252, W303 background	This study
SWY1066	<i>Mata</i> , pSW257, W303 background	This study

Strain	Genotype	Source
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ESY342	<i>Mata</i> , pES67, W303 background	(1)
ESY349	<i>Mata</i> , <i>pep4::HIS3</i> , pES67, W303 background	(1)
SWY837	<i>Mata</i> , <i>vps27::KANMX</i> , pES67, W303 background	This study
SWY819	<i>Mata</i> , pSW182, W303 background	This study
SWY820	<i>Mata</i> , <i>pep4::HIS3</i> , pSW182, W303 background	This study
SWY821	<i>Mata</i> , <i>vps27::KANMX</i> , pSW182, W303 background	This study
SWY1115	<i>Mata</i> , pSW263, W303 background	This study
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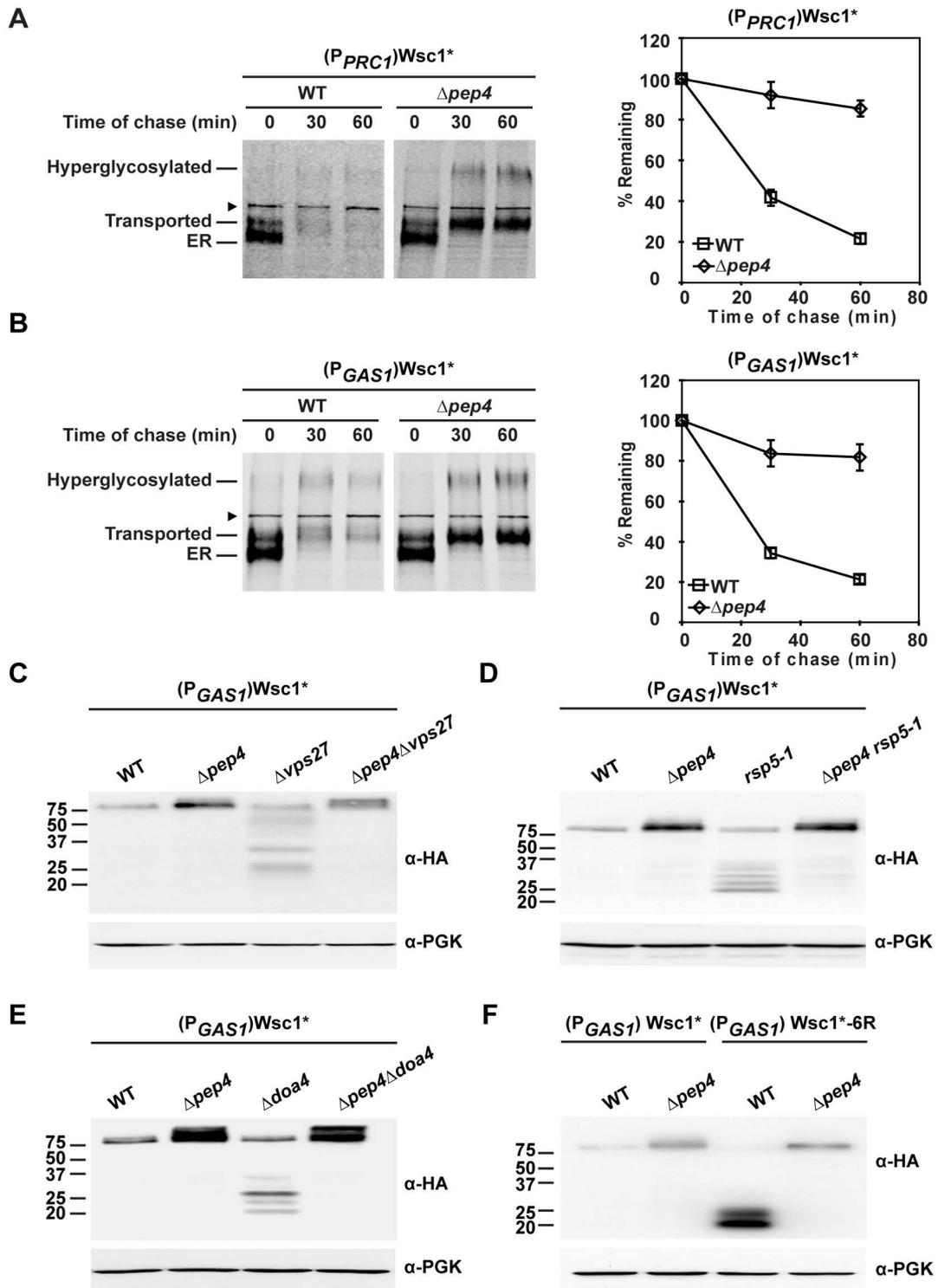
Supplemental Table S2 Oligonucleotide primers used in this study

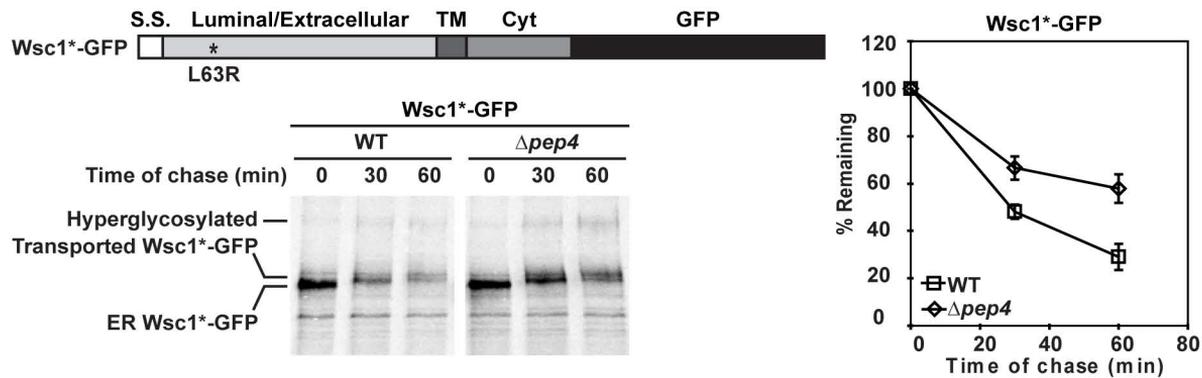
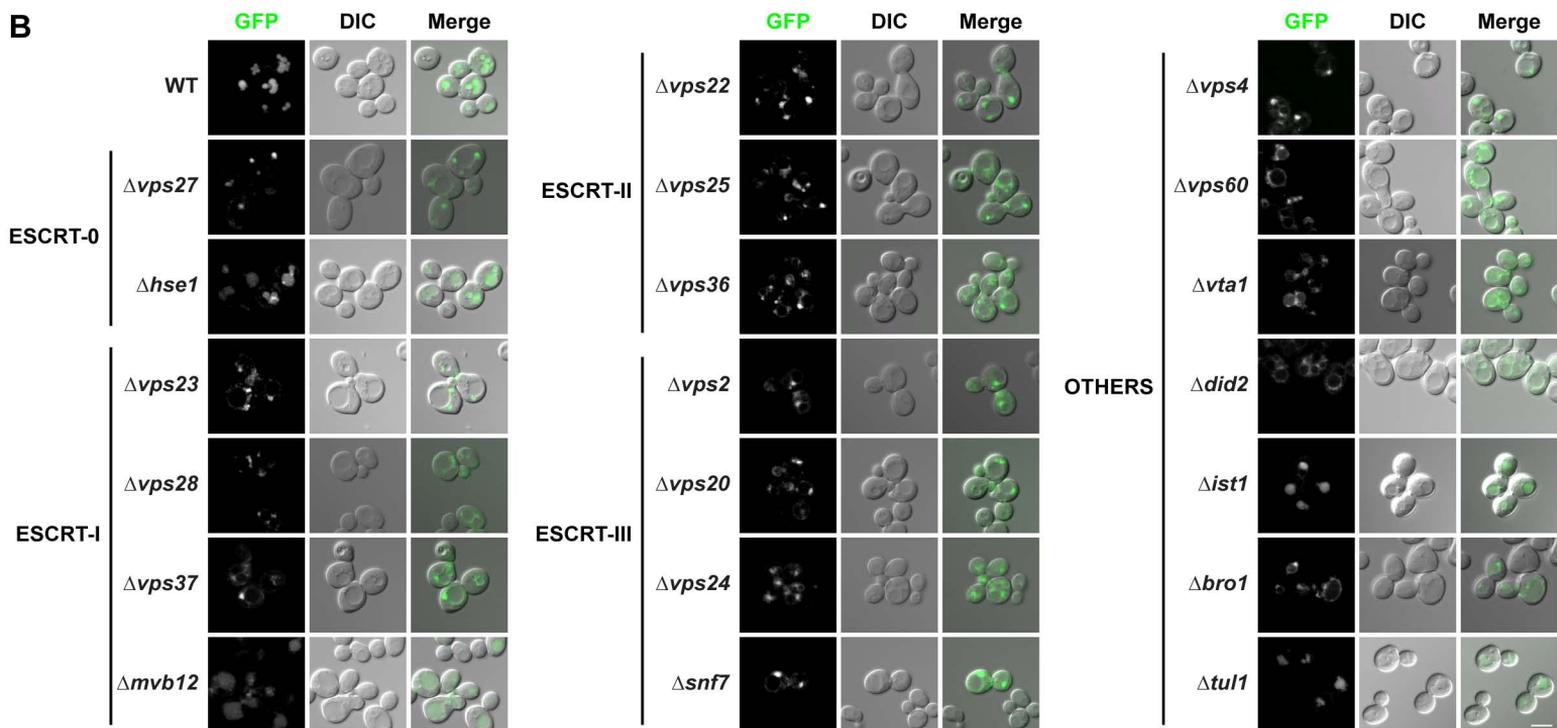
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SWN38	pSW177	CAGAATTCTTTCCACTCCTCC
SWN39	pSW177	CTAGTCTAGATTATTTGTATAGTTCATCCATGCCA
SWN87	pSW257	ATAAGAATGCGGCCGCGGAAGGCACCCTTTTCGAAGG
SWN88	pSW257	ACATGGATCCTGTTGAGATTTAGCTGTGTTTGTG
SWN99	pSW177	ATAAGAATGCGGCCGCGTATATGATGATACATATGTTAGG
SWN100	pSW182	ATAAGAATGCGGCCGCACGGATTAGAAGCCGCGAGCGGGTG
SWN101	pSW182	CGCGGATCCGGTTTTTCTCCTTGACGTAAAGTATAGAGG
SWN103	pSW252	GTTGATTGTCAGACACATTAATATGAGACGGGAACAAGACAGG ATGGAAAAGG
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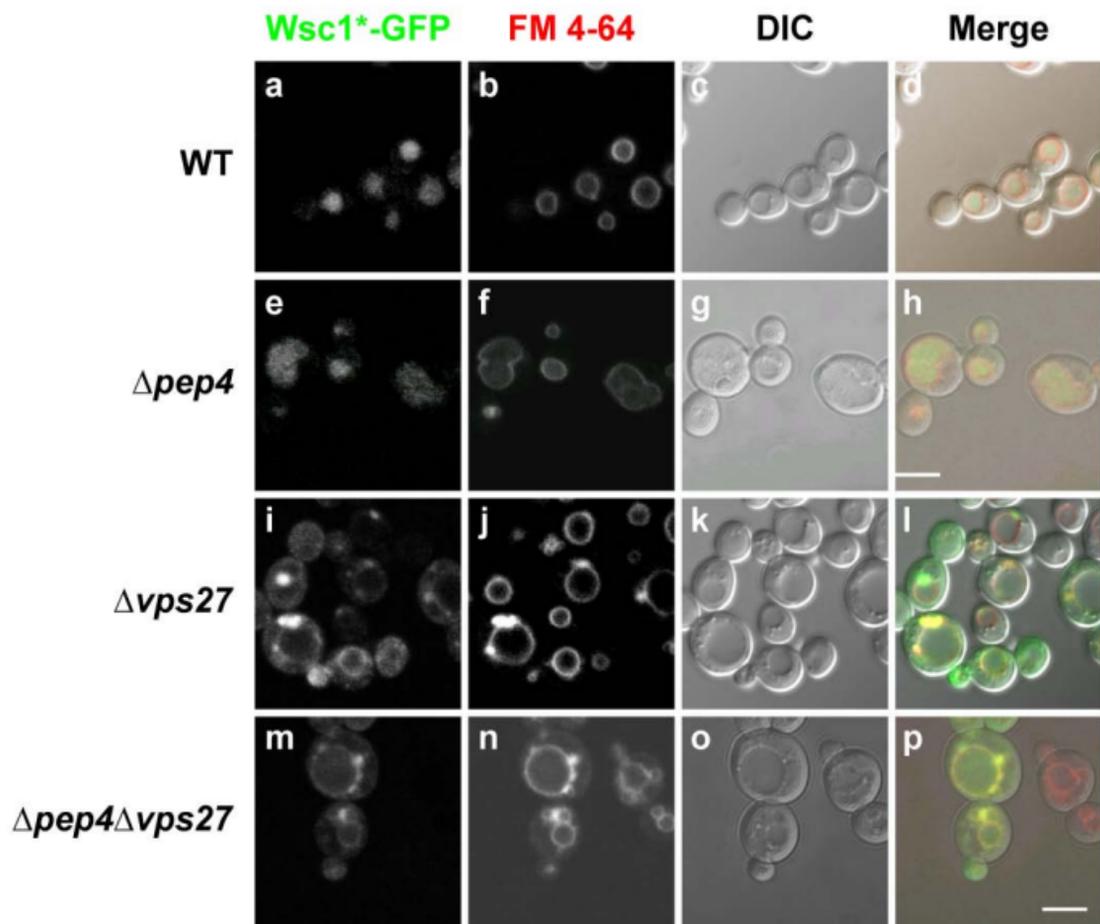
Primer	Construct	Sequence (5'→3')
		GATAAAACCAG
SWN105	pSW252	GGAAAAGGAATACCAAGAGGCGATAAGACCAGTTGAGTACCCT GATAAACTAT
SWN106	pSW252	GATAAAACCAGTTGAGTACCCTGATAGACTATACGCCTCTTCAT TTTCATCTA
SWN107	pSW252	GGTAGCTTCGAGGAGGAGCACACCAGAGGGCAAACCTGATATTA ACCCTTTC
SWN108	pSW252	CATTCATAAATGGCGGACCAGGAGGGAGAAACAACGTTTTAAC AGTGGTCAATCC

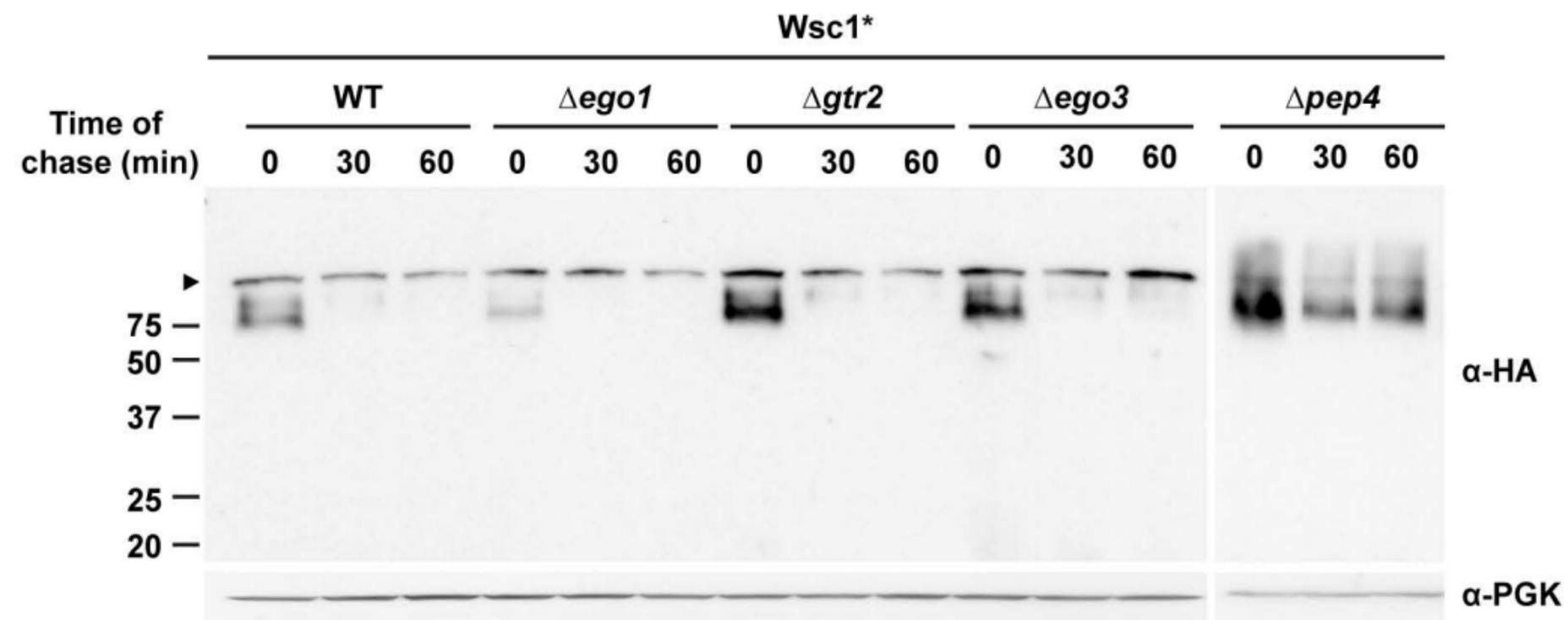
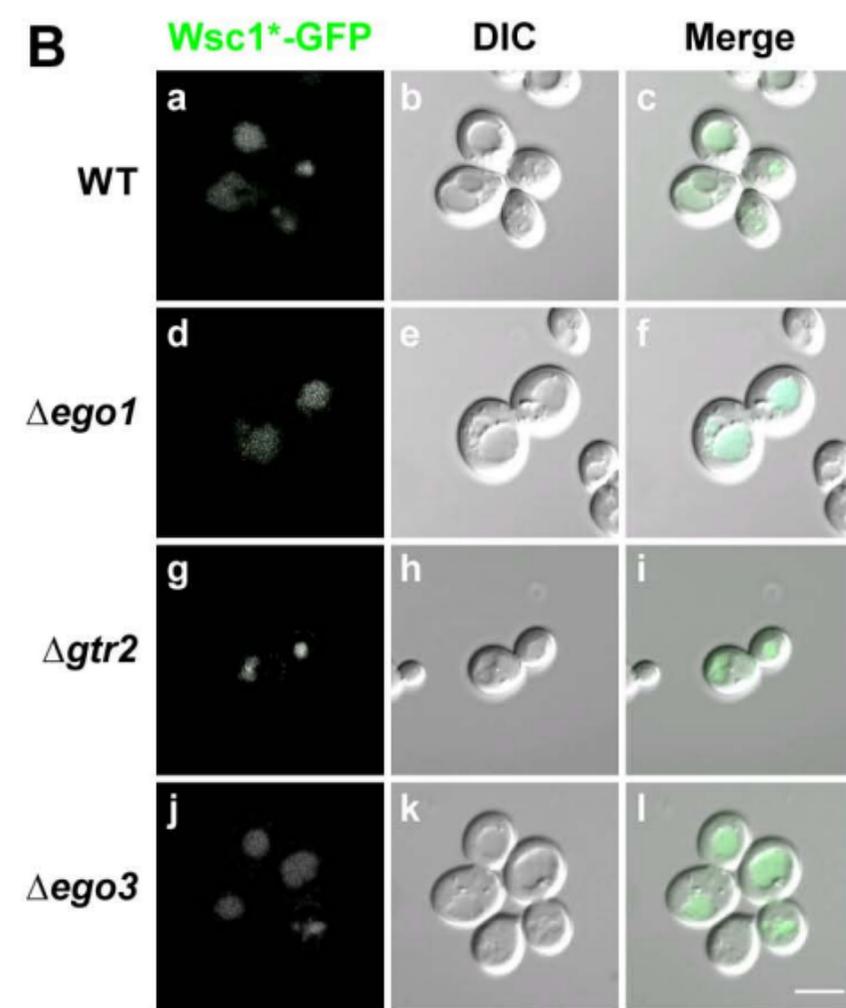
SUPPLEMENTAL REFERENCES

1. Spear, E. D., and Ng, D. T. (2003) *Mol Biol Cell* **14**(7), 2756-2767



A**B**



A**B**

Wsc1^{*}-6R

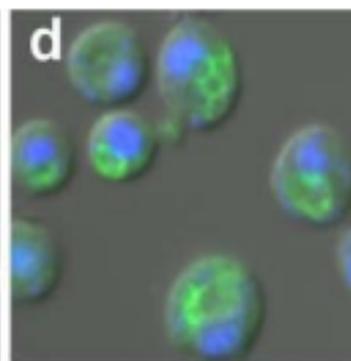
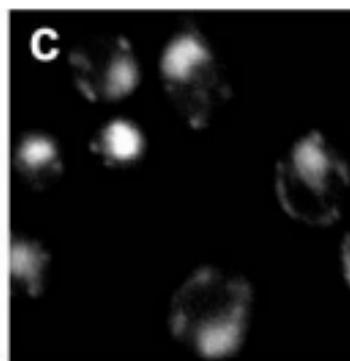
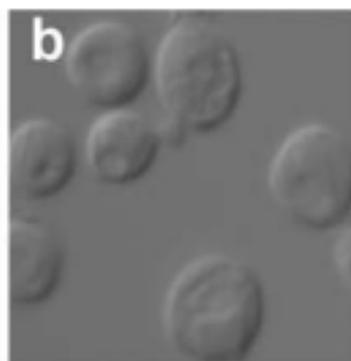
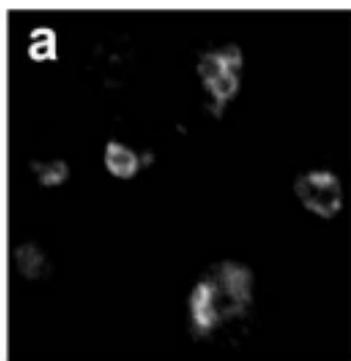
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DIC

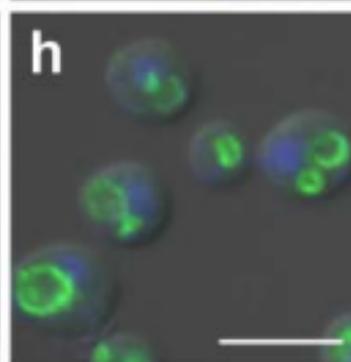
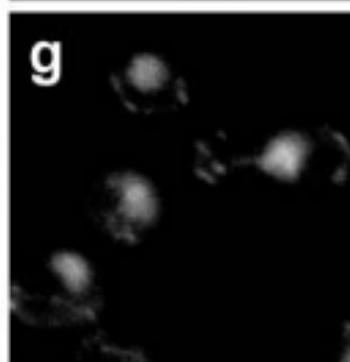
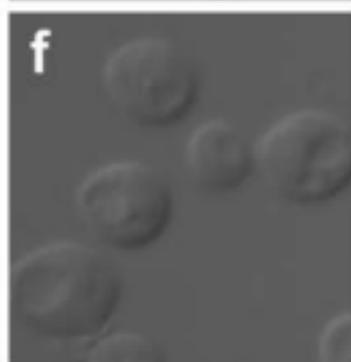
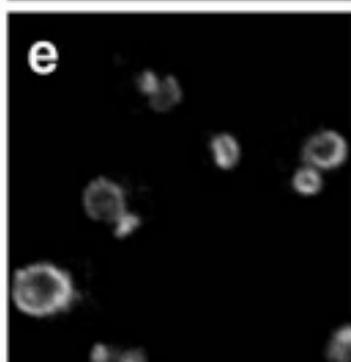
DAPI

Merge

WT



Δpep4



12

Figure S5

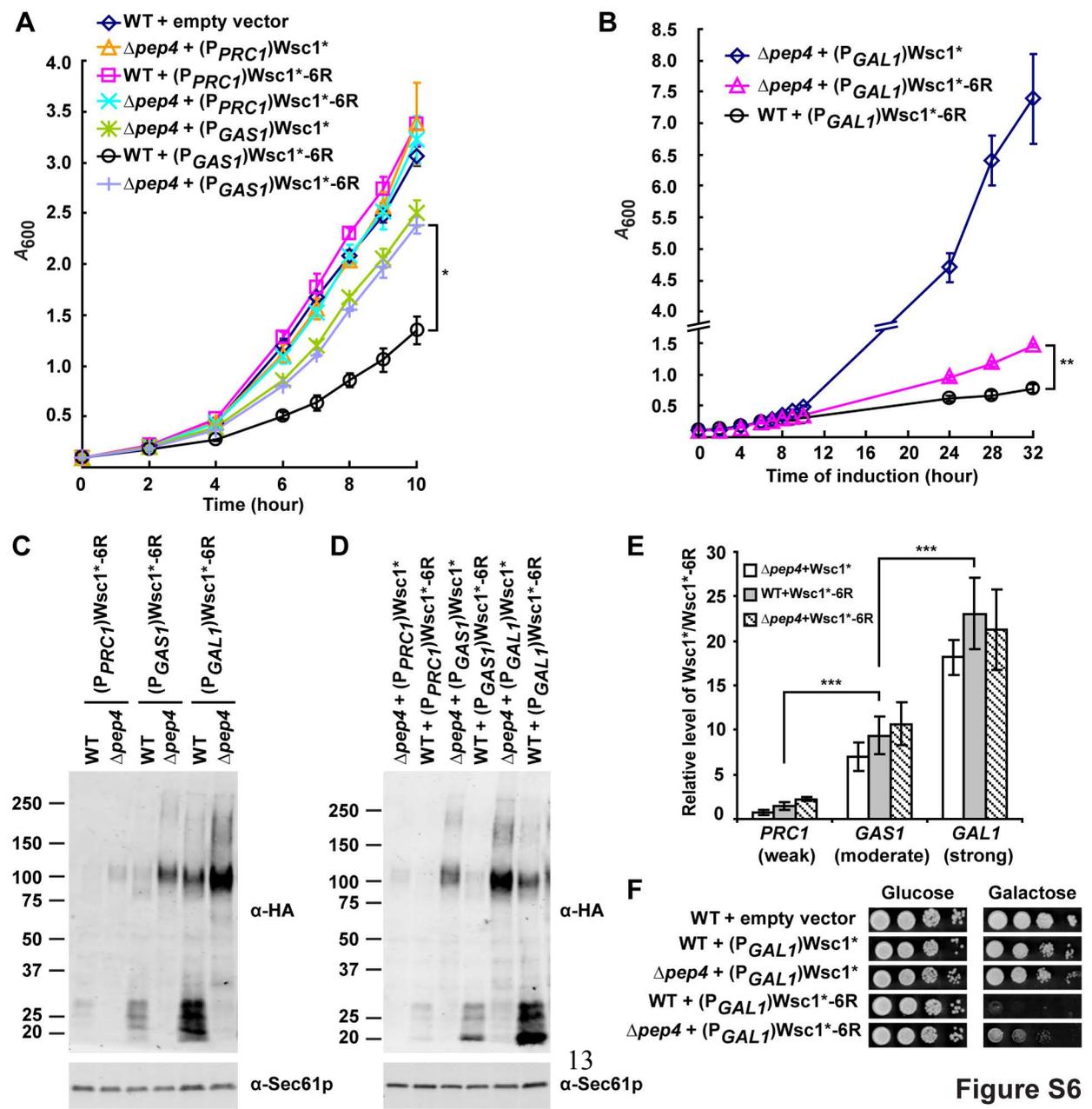
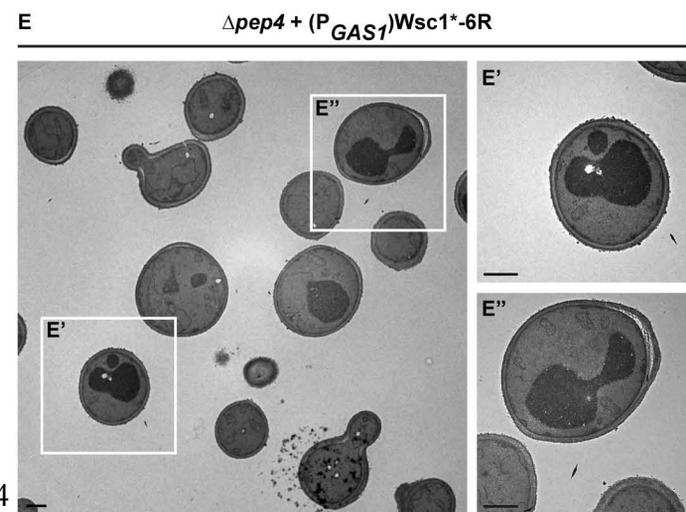
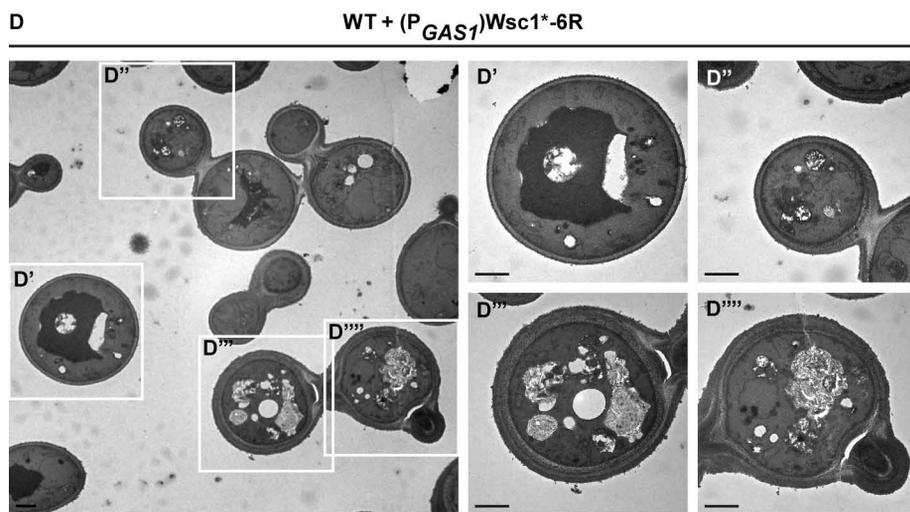
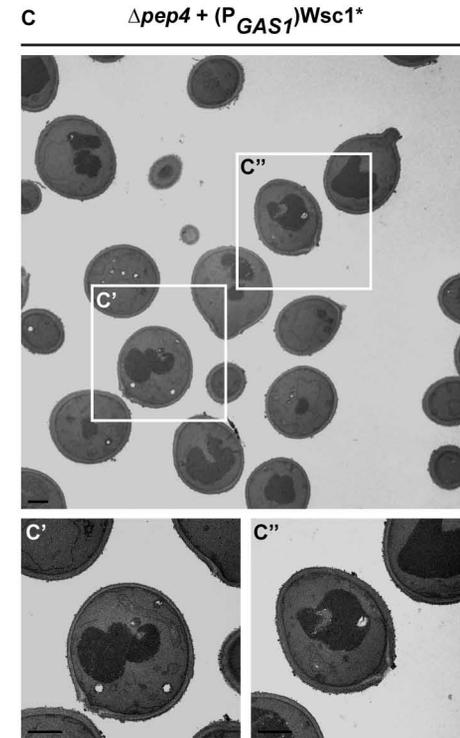
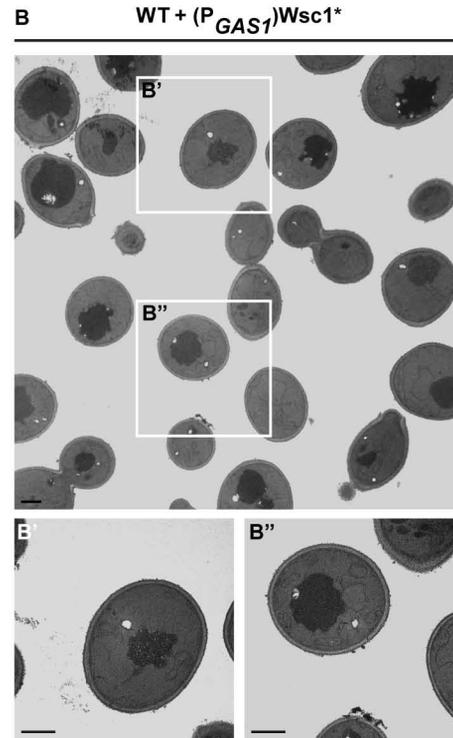
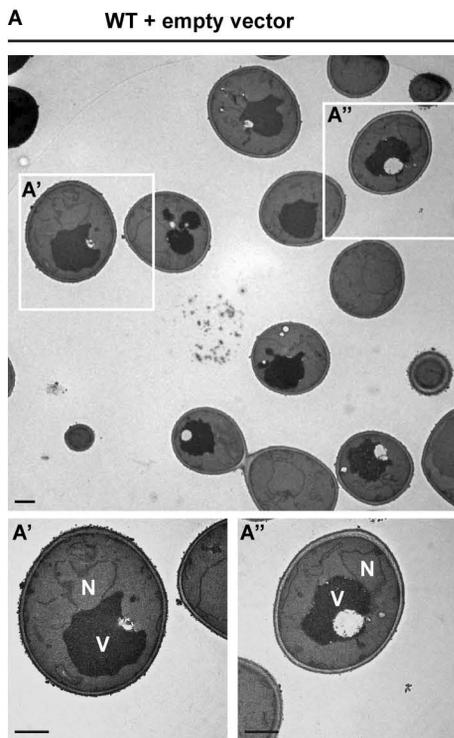


Figure S6

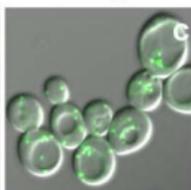
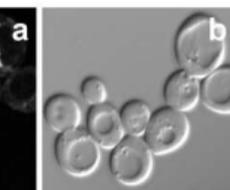
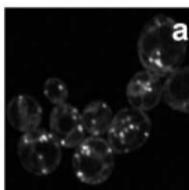
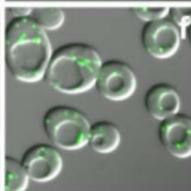
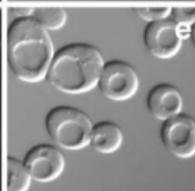
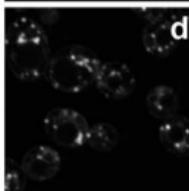
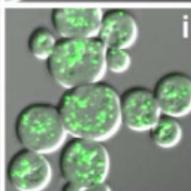
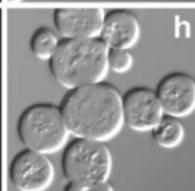
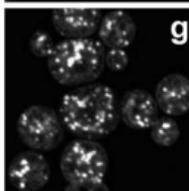
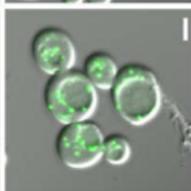
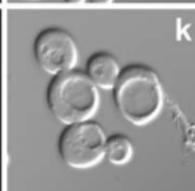
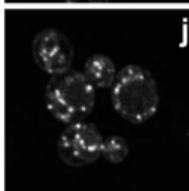
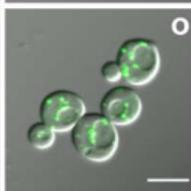
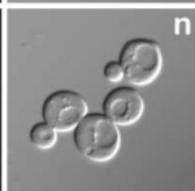
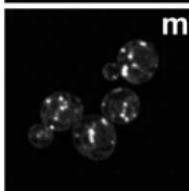


LD540

DIC

Merge

WT + empty vector

WT + (P_{GAS1})Wsc1*WT + (P_{GAS1})Wsc1*-6R $\Delta pep4$ + (P_{GAS1})Wsc1* $\Delta pep4$ + (P_{GAS1})Wsc1*-6R

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Figure S8