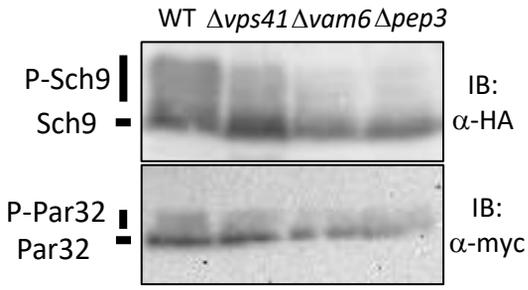


# Supplemental Materials

*Molecular Biology of the Cell*

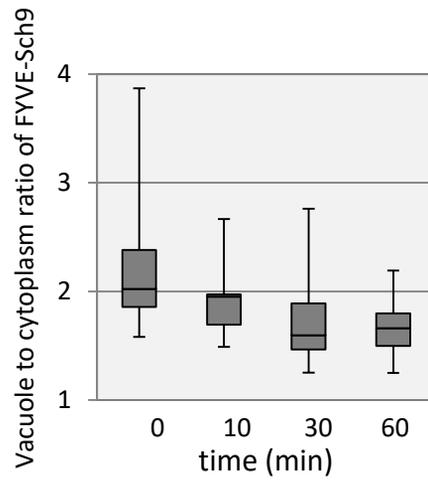
Takeda et al.

1



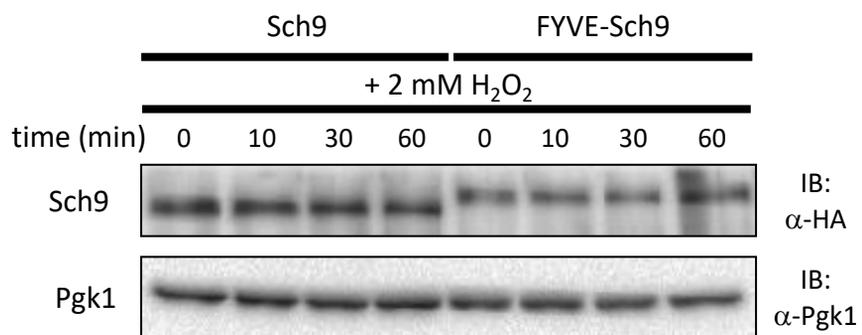
**Supplemental Figure S1:** Western blot analysis of the C-terminal fragment of Sch9-5HA and Par32-13myc. Lysates of WT (yet515), *Δvps41* (yet582), *Δvam6* (yet670), and *Δpep3* (yet723) cells grown in SDC medium were subjected to western blotting.

2



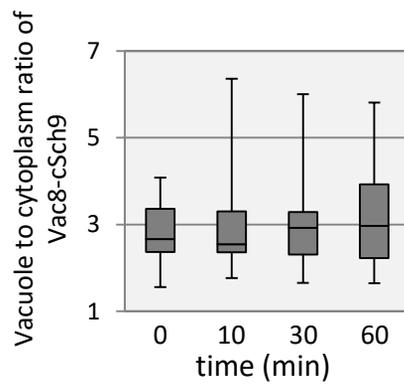
**Supplemental Figure S2:** Quantification of the ratio of FYVE-Sch9 fluorescence intensity of vacuolar membranes to that of the cytoplasm. Related to Figure 6A.

3



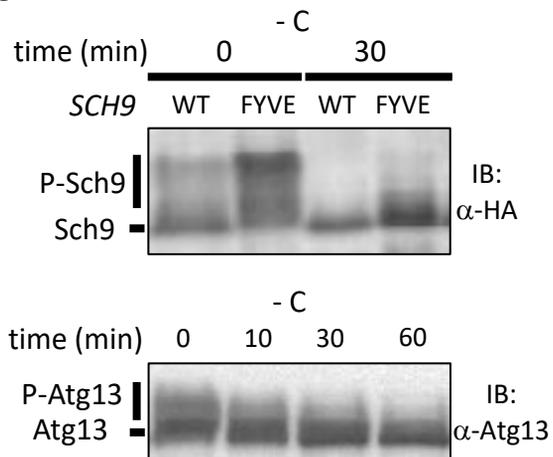
**Supplemental Figure S3:** Western blot analysis of Sch9-5HA (yet628) or FYVE-Sch9-5HA (yet629) with Pgk1 as a loading control. Related to Figure 6B.

4



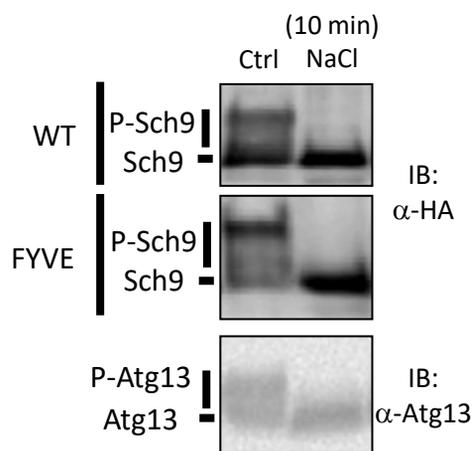
**Supplemental Figure S4:** Quantification of the ratio of Vac8-GFP-cSch9 fluorescence intensity of vacuolar membranes to that of the cytoplasm. Related to Figure 6D.

5

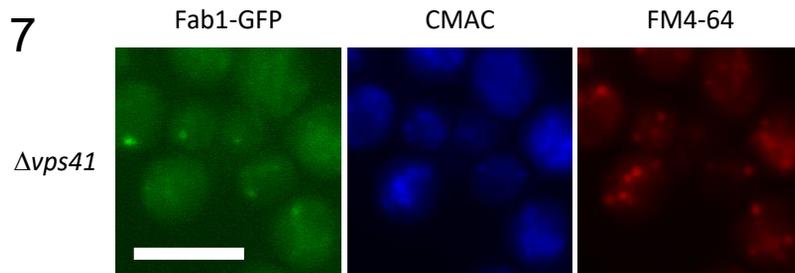


**Supplemental Figure S5:** Western blot analysis of the C-terminal fragment of Sch9-5HA and Atg13. Cells expressing Sch9-5HA (yet628) or FYVE-Sch9-5HA (yet629) were treated with SDC-C medium (carbon starvation) for 30 min during the mid-log phase. Cells overexpressing Atg13 (yet562) on a multi-copy plasmid were treated with SDC-C medium during the mid-log phase in SDC medium. The lysates were subjected to western blotting at each time point.

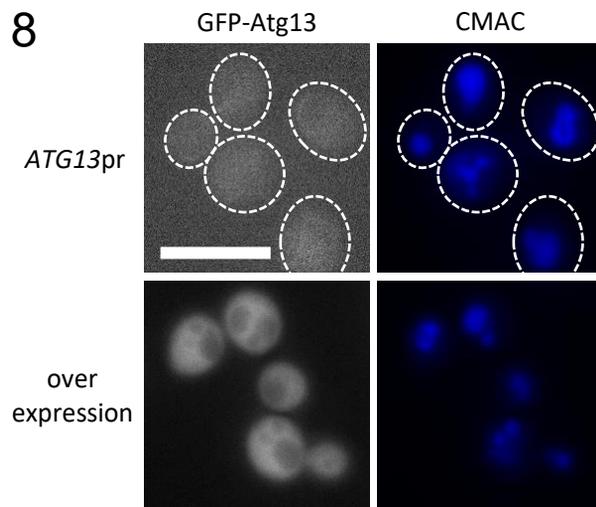
6



**Supplemental Figure S6:** Western blot analysis of the C-terminal fragment of Sch9-5HA and Atg13. Cells expressing Sch9-5HA (yet628) and FYVE-Sch9-5HA (yet629) were treated with SDC medium + 1 M NaCl (hyperosmotic stress) for 10 min during the mid-log phase. Cells overexpressing Atg13 (yet562) on a multi-copy plasmid were treated with SDC + 1 M NaCl medium for 10 min during the mid-log phase. The lysates were subjected to western blotting at each time point.



**Supplemental Figure S7:** Representative images of *Δvps41* (yet805) cells expressing Fab1-GFP. Cells at mid-log phase in SDC (+ uracil) medium were stained with CMAC and FM4-64. Scale bar = 5  $\mu$ m.



**Supplemental Figure S8:** Representative images of cells expressing GFP-Atg13 from the endogenous *ATG13* promoter or the *TEF* promoter (overexpression). Cells at mid-log phase in SDC (+ uracil) medium were stained with CMAC as a vacuolar marker. Scale bar = 5  $\mu$ m.

	+ 2mM H <sub>2</sub> O <sub>2</sub>			
	0 min	10 min	30 min	60 min
PI3P	3.97 ± 0.19	4.64 ± 0.21	4.94 ± 0.12	4.66 ± 0.16
PI4P	2.38 ± 0.05	3.38 ± 0.01	4.07 ± 0.28	4.38 ± 0.26
PI(3,5)P <sub>2</sub>	0.27 ± 0.04	0.29 ± 0.02	0.32 ± 0.04	0.32 ± 0.04
PI(4,5)P <sub>2</sub>	1.34 ± 0.05	0.98 ± 0.07	1.01 ± 0.05	0.98 ± 0.03

**Supplemental Table 1:** Changes in the levels of phosphoinositides after oxidative stress.

Values are presented as means ± standard deviation (n = 3).

**Supplemental Table 2: Strains used in this study.**

<b>Strain</b>	<b>Genotype</b>	<b>Figure</b>	<b>Source</b>
BY4741	MATa <i>his3Δ1 leu2Δ0 met15Δ0 ura3Δ0</i>	2A	Laboratory stock
yet38	BY4741 pRS416- <i>SCH9-5HA</i>		This study
yet515	yet38 <i>PAR32-13myc::HIS3MX</i>	1, 2B, 2D, 5AB	This study
yet571	yet562 <i>SCH9-5HA::LEU2</i>		This study
yet610	yet571 <i>NPR1-13myc::HIS3MX</i>	1, 2B, 5B,	This study
yet562	BY4741 YEp352- <i>ATG13</i>	1, 2B, 5AB,	This study
yet567	BY4741 $\Delta$ <i>vps41::kanMX</i>	2A	This study
yet576	BY4741 $\Delta$ <i>ego1::kanMX</i>	2A	This study
yet701	BY4741 $\Delta$ <i>tco89::kanMX</i>	2A	This study
yet569	yet515 $\Delta$ <i>vps41::kanMX</i>	2BD	This study
yet577	yet515 $\Delta$ <i>ego1::kanMX</i>	2BD	This study
yet729	yet515 $\Delta$ <i>tco89::kanMX</i>	2BD	This study
yet618	yet610 $\Delta$ <i>vps41::kanMX</i>	2B	This study
yet619	yet610 $\Delta$ <i>ego1::kanMX</i>	2B	This study
yet780	yet610 $\Delta$ <i>tco89::kanMX</i>	2B	This study
yet574	yet562 $\Delta$ <i>vps41::kanMX</i>	2B	This study
yet580	yet562 $\Delta$ <i>ego1::kanMX</i>	2B	This study
yet726	yet562 $\Delta$ <i>tco89::kanMX</i>	2B	This study
SKY086-A	BY4741 $\Delta$ <i>gtr1::kanMX</i> $\Delta$ <i>gtr2::hphMX</i>		This study
yet593	SKY086-A <i>PAR32-13myc::HIS3MX</i>		This study
yet634	yet593 <i>SCH9-5HA::LEU2</i>		This study
yet639	yet634 pRS316	2E	This study
yet640	yet634 pSK122	2E	This study
yet645	yet634 pSK127	2E	This study
yet647	yet634 pSK129	2E	This study
yet631	BY4741 $\Delta$ <i>gtr1::natMX</i> $\Delta$ <i>gtr2::hphMX</i> <i>SCH9-5HA::LEU2</i>		This study
yet707	yet631 <i>PAR32-myc::HIS3MX</i>		This study
yet754	yet707 $\Delta$ <i>vps41::kanMX</i>		This study
yet755	yet754 pRS316	2E	This study
yet756	yet754 pRS122	2E	This study
yet761	yet754 pRS127	2E	This study
yet763	yet754 pRS129	2E	This study
yet120	BY4741 pSK1	3AB	This study
yet691	yet576 pSK1	3A	This study
yet727	yet701 pSK1	3A	This study
yet732	yet567 pSK1	3B	This study
SKY374-A	BY4741 <i>NUP49-mCherry::hphMX</i> <i>GFP-TOR1</i>	3C	2015 Kira
yet665	SKY374-A $\Delta$ <i>vps41::kanMX</i>	3C	This study

yet19	BY4741 <i>SCH9p::GFPΔC-URA3-NOP1p-GFP-SCH9</i>		This study
yet36	yet36 <i>SCH9p::GFP-SCH9</i>	4C	This study
yet231	BY4741 <i>SCH9p::GFPΔC-URA3-TEF1p-GFP-FYVE-SCH9</i>		This study
yet234	yet231 <i>SCH9p::GFP-FYVE-SCH9</i>	4ACF, 6A	This study
yet606	yet234 <i>Δvps41::kanMX</i>	4A	This study
yet628	yet36 <i>SCH9p::GFP-SCH9-5HA-LEU2</i>	4B, 6B	This study
yet629	yet234 <i>SCH9p::GFP-FYVE-SCH9-5HA-LEU2</i>	4B, 6B	This study
yet661	yet628 <i>Δvps41::kanMX</i>	4B	This study
yet662	yet629 <i>Δvps41::kanMX</i>	4B	This study
yet605	yet36 <i>Δvps41::kanMX</i>	4C	This study
yet606	yet234 <i>Δvps41::kanMX</i>	4C	This study
yet653	BY4741 <i>EGO3-3mCherry::hphMX</i>		This study
yet765	yet653 pSK1	5D	This study
yet857	BY4741 pRS413- <i>VAC8-GFP-csch9-5HA</i>	6D	This study
yet859	BY4741 pRS413 <i>VAC8-csch9-5HA</i>	6D	This study
yet681	BY4741 <i>ATG18p::GFPΔC-URA3-NOP1p-GFP-ATG18</i>	7A	This study
yet709	yet681 <i>Δvps41::kanMX</i>	7A	This study
yet782	yet681 <i>Δvac7::kanMX</i>	7A	This study
yet777	yet681 <i>EGO3-3mCherry::hphMX</i>	7B	This study
yet793	BY4741 <i>FAB1-GFP::kanMX</i>	7D	This study
yet685	BY4741 <i>ATG18p::mCherryΔC-URA3-TEFp-mCherry-ATG18</i>		This study
yet838	yet685 pTN1015	7F	This study

**Supplemental Table 3: Plasmids used in this study.**

Plasmid	Source
pRS416- <i>SCH9-5HA</i>	2007 Urban <i>et al.</i>
YEp352- <i>ATG13</i>	2010 Kamada <i>et al.</i>
pRS316	Laboratory stock
pSK122	pRS316- <i>GTR1 GTR2</i>
pSK127	pRS316- <i>GTR1(S20L) GTR2(Q66L)</i>
pSK129	pRS316- <i>GTR1(Q65L) GTR2(S23L)</i>
pSK1	pRS316- <i>SCH9-2GFP</i>
	pRS413- <i>VAC8-GFP-csch9-5HA</i>
	BY4741 pRS413 <i>VAC8-csch9-5HA</i>
pTN1015	pRS425- <i>GFP-FYVE</i>