



**Figure S1, Related to Figure 1.** Pho23 regulates ATG gene transcription in an Rpd3-dependent manner (A) Number of genes that have more than a 1.5-fold change in expression level in *pho23Δ* and *rpd3Δ* [4] strains relative to the wild-type strain are shown. Numbers in parentheses indicate the number of the ATG genes upregulated in the mutant strains. (B) Cells were grown in YPD to mid-log phase and RNA extracted as described in Materials and Methods. The relative mRNA levels of different ATG genes were measured in wild-type and mutant strains. The mRNA level of each gene in wild-type cells was set to 1, and other values were normalized. The error bars represent the SEM of three independent experiments. (C) Protein levels of Atg1, Atg8, and Atg9 were analyzed from protein extracts generated from mid-log phase wild-type (WLY176), *pho23Δ* (JMY048), *rpd3Δ* (JMY093), *pho23Δ rpd3Δ* (JMY094), *ume6Δ* (JMY097), and *pho23Δ ume6Δ* (JMY098) strains. Endogenous Atg proteins were detected by the indicated antisera, and Pgk1 was used as a loading control.

**Table S1, Related to Figure 1.** Strains used in this study

<b>Name</b>	<b>Genotype</b>	<b>Ref</b>
BY4742	MAT $\alpha$ <i>his3<math>\Delta</math>1 leu2<math>\Delta</math>0 ura3<math>\Delta</math>0</i>	Research Genetics/Invitrogen
CWY230	SEY6210 <i>vac8<math>\Delta</math>::KAN</i>	[S1]
FRY143	SEY6210 <i>vps4<math>\Delta</math>::TRP1 pep4<math>\Delta</math>::LEU2</i>	[S2]
HDY001 (A9 $\Delta$ )	WLY176 <i>ATG9<math>\Delta</math>::LEU2 pRS406::URA3</i>	This study
HDY002 (A9WT)	WLY176 <i>ATG9<math>\Delta</math>::LEU2 pRS406-P<sub>ATG9</sub>-ATG9-GFP::URA3</i>	This study
HDY003 (A9Lo)	WLY176 <i>ATG9<math>\Delta</math>::LEU2 pRS406-P<sub>ATG23</sub>-ATG9-GFP::URA3</i>	This study
HDY007 (A9OE)	WLY176 <i>pRS406-P<sub>ATG8</sub>-ATG9-GFP::URA3</i>	This study
JMY015	ZFY202 <i>pho23<math>\Delta</math>::KAN</i>	This study
JMY018	BY4742 <i>pho23<math>\Delta</math>::HIS5</i>	This study
JMY020	TVY1 <i>pho23<math>\Delta</math>::HIS5</i>	This study
JMY047	SEY6210 <i>pho23<math>\Delta</math>::HIS5</i>	This study
JMY048	WLY176 <i>pho23<math>\Delta</math>::HIS5</i>	This study
JMY050	FRY143 <i>pho23<math>\Delta</math>::KAN</i>	This study
JMY093	WLY176 <i>rpd3<math>\Delta</math>::HIS5</i>	This study
JMY094	WLY176 <i>pho23<math>\Delta</math>::KAN rpd3<math>\Delta</math>::HIS5</i>	This study
JMY097	WLY176 <i>ume6<math>\Delta</math>::HIS5</i>	This study
JMY098	WLY176 <i>ume6<math>\Delta</math>::HIS5 pho23<math>\Delta</math>::KAN</i>	This study

JMY099	WLY176 <i>ATG12-PA::HIS5</i>	This study
JMY100	WLY176 <i>pho23Δ::KAN ATG12-PA::HIS5</i>	This study
JMY102	YZX94 <i>pho23Δ::KAN</i>	This study
JMY103	HCY129 <i>pho23Δ::KAN</i>	This study
JMY104	WLY176 <i>ATG7-PA::HIS5</i>	This study
JMY105	WLY176 <i>ATG7-PA::HIS5 pho23Δ::KAN</i>	This study
JMY146	CWY230 <i>pho23Δ::HIS5</i>	This study
HCY129	SEY6210 <i>ATG29-PA::TRP1</i>	[S3]
MZY089	SEY6210 <i>atg8Δ::HIS5 P<sub>ATG8</sub>-GFP-Atg8::LEU2</i>	[S4]
SEY6210	MAT $\alpha$ <i>leu2-3,112 ura3-52 his3-Δ200 trp1-Δ901 suc2-Δ9 lys2-801 GAL</i>	[S5]
SKB233	SEY6210 <i>atg8Δ::HIS5 P<sub>ATG8</sub>-GFP-Atg8::LEU2 pho23Δ::KAN</i>	This study
TVY1	SEY6210 <i>pep4Δ::LEU2</i>	[S6]
W303-1B	MAT $\alpha$ <i>leu2-3,112 ura3-1 his3-11,15 trp1-1 ade2-1 can1-100</i>	[S7]
WLY176	SEY6210 <i>pho13Δ pho8Δ60</i>	[S8]
XLY054	WLY176; <i>ATG9Δ::LEU2 pRS406::URA3 pep4Δ::KAN</i>	This study
XLY055	WLY176; <i>ATG9Δ::LEU2 pRS406-P<sub>ATG23</sub>-ATG9-GFP::URA3 pep4Δ::KAN</i>	This study
XLY056	WLY176; <i>ATG9Δ::LEU2 pRS406-P<sub>ATG9</sub>-ATG9-GFP::URA3 pep4Δ::KAN</i>	This study
XLY057	WLY176; <i>pRS406-P<sub>ATG8</sub>-ATG9-GFP::URA3 pep4Δ::KAN</i>	This study
YZX94	BY4742; <i>ATG14-PA::HIS5</i>	This study
ZFY202	W303-1B; <i>pho13Δ pho8Δ60</i>	[S9]

**Table S2, Related to Figure 3. WT vs. *pho23Δ* TEM data**

	Autophagic body size					Vacuole size				Autophagic body number		Total flux
	Measured cross-sectional		Estimated original			Measured cross-sectional		Estimated original		Measured cross-sections/cell	Estimated bodies/cell	
	Mean rad	SD rad	Mean rad	SD rad	Volume	Mean rad	SD rad	Mean rad	SD rad			
<b>WT sample set 1</b>	154.134	61.579	164.174	57.207	2.61E+07	996.298	276.647	1132.894	237.068	5.982	32.714	8.55E+08
<b>WT sample set 2</b>	145.456	61.472	150.492	57.964	2.16E+07	989.502	265.114	1132.552	218.821	7.463	43.532	9.41E+08
<b>WT average</b>			157.333	57.586	2.39E+07						38.123	8.98E+08
<b><i>pho23Δ</i> sample set 1</b>	140.472	47.293	153.910	42.607	1.91E+07	1015.804	293.056	1148.214	257.471	13.725	80.583	1.54E+09
<b><i>pho23Δ</i> sample set 2</b>	133.372	49.776	142.444	46.510	1.64E+07	978.013	269.367	1107.574	232.527	15.000	90.619	1.49E+09
<b><i>pho23Δ</i> average</b>			148.177	44.558	1.77E+07						85.601	1.51E+09

**Table S3, Related to Figure 4. De-multiplexing results in 22M-31M reads of RNA-seq samples**

Sample Description	Barcode (5'→3')	Raw reads	% of raw reads		% bases ≥ Q30	Mean quality score	Mean error rate
			0 mismatch barcode	1 mismatch barcode			
WT growing	CGATGT	25,263,020	98.4%	1.6%	96.3%	37.88	1.6/10,000
<i>pho23Δ</i> growing	TGACCA	30,612,154	97.8%	2.3%	96.2%	37.86	1.6/10,000
WT starved	GCCAAT	21,992,351	97.8%	2.2%	96.2%	37.85	1.6/10,000
<i>pho23Δ</i> starved	CAGATC	24,344,369	97.9%	2.1%	96.2%	37.87	1.6/10,000
No barcode	NA	3,124,074	NA	NA	93.8%	37.03	2.0/10,000
	<i>Total:</i>	105,335,968					

**Table S4, Related to Figure 4.** Mapping the reads of RNA-seq samples to the yeast transcriptome using Bowtie2

Sample Description	Raw reads	Mapped reads	% of raw	Mapped, score $\geq 88$	% of raw	Uniquely mapped, score $\geq 88$	% of raw	Multi mapped, score $\geq 88$	% of raw
WT growing	25,263,020	23,690,179	93.8%	22,965,459	90.9%	17,685,706	70.0%	5,279,753	20.9%
<i>pho23Δ</i> growing	30,612,154	28,348,799	92.6%	27,483,468	89.8%	21,475,572	70.2%	6,007,896	19.6%
WT starved	21,992,351	20,296,160	92.3%	19,636,451	89.3%	17,049,729	77.5%	2,586,722	11.8%
<i>pho23Δ</i> starved	24,344,369	22,545,466	92.6%	21,846,077	89.7%	18,481,608	75.9%	3,364,469	13.8%

**Table S5, Related to Figure 5.** A9 strains TEM data

		Autophagic body size					Vacuole size				Autophagic body number		Total flux	% flux
		Measured cross-sectional		Estimated original			Measured cross-sectional		Estimated original		Measured cross-sections/cell	Estimated bodies/cell		
		Mean rad	SD rad	Mean rad	SD rad	Volume	Mean rad	SD rad	Mean rad	SD rad				
Sample 1	A9Lo	151.214	44.113	169.029	36.365	2.32E+07	839.859	216.434	939.549	194.337	2.856	12.686	2.94E+08	50.7%
	A9WT	133.150	35.521	146.124	31.534	1.50E+07	835.052	219.762	929.285	201.215	8.232	41.660	6.24E+08	107.6%
	A9OE	130.633	33.522	143.491	29.268	1.40E+07	893.063	257.700	989.008	241.469	10.000	55.496	7.76E+08	133.8%
Sample 2	A9Lo	150.904	49.666	163.046	46.375	2.29E+07	885.798	251.927	982.226	234.885	3.088	14.314	3.28E+08	56.6%
	A9WT	126.450	33.223	137.063	30.134	1.24E+07	914.461	255.760	1026.609	233.004	7.309	43.105	5.36E+08	92.4%
	A9OE	128.857	35.898	138.916	33.148	1.33E+07	906.265	255.857	1012.629	235.507	9.155	54.567	7.23E+08	124.8%

## Supplemental References

- S1. Umekawa, M., and Klionsky, D.J. (2012). Ksp1 kinase regulates autophagy via the target of rapamycin complex 1 (TORC1) pathway. *J Biol Chem* 287, 16300-16310.
- S2. Cheong, H., Yorimitsu, T., Reggiori, F., Legakis, J.E., Wang, C.-W., and Klionsky, D.J. (2005). Atg17 regulates the magnitude of the autophagic response. *Mol Biol Cell* 16, 3438-3453.
- S3. Mao, K., Chew, L.H., Inoue-Aono, Y., Cheong, H., Nair, U., Popelka, H., Yip, C.K., and Klionsky, D.J. (2013). Atg29 phosphorylation regulates coordination of the Atg17-Atg31-Atg29 complex with the Atg11 scaffold during autophagy initiation. *Proc Natl Acad Sci U S A* 110, E2875-2884.
- S4. Cebollero, E., van der Vaart, A., Zhao, M., Rieter, E., Klionsky, D.J., Helms, J.B., and Reggiori, F. (2012). Phosphatidylinositol-3-phosphate clearance plays a key role in autophagosome completion. *Curr Biol* 22, 1545-1553.
- S5. Robinson, J.S., Klionsky, D.J., Banta, L.M., and Emr, S.D. (1988). Protein sorting in *Saccharomyces cerevisiae*: isolation of mutants defective in the delivery and processing of multiple vacuolar hydrolases. *Mol Cell Biol* 8, 4936-4948.
- S6. Gerhardt, B., Kordas, T.J., Thompson, C.M., Patel, P., and Vida, T. (1998). The vesicle transport protein Vps33p is an ATP-binding protein that localizes to the cytosol in an energy-dependent manner. *J Biol Chem* 273, 15818-15829.
- S7. Thomas, B.J., and Rothstein, R. (1989). Elevated recombination rates in transcriptionally active DNA. *Cell* 56, 619-630.
- S8. Kanki, T., Wang, K., Baba, M., Bartholomew, C.R., Lynch-Day, M.A., Du, Z., Geng, J., Mao, K., Yang, Z., Yen, W.-L., et al. (2009). A genomic screen for yeast mutants defective in selective mitochondria autophagy. *Mol Biol Cell* 20, 4730-4738.
- S9. Yang, Z., Geng, J., Yen, W.-L., Wang, K., and Klionsky, D.J. (2010). Positive or negative roles of different cyclin-dependent kinase Pho85-cyclin complexes orchestrate induction of autophagy in *Saccharomyces cerevisiae*. *Mol Cell* 38, 250-264.