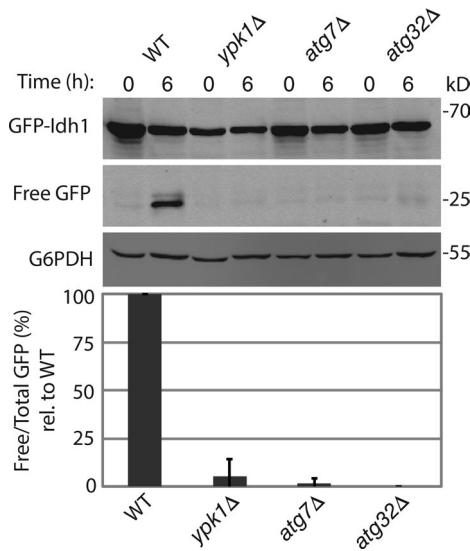
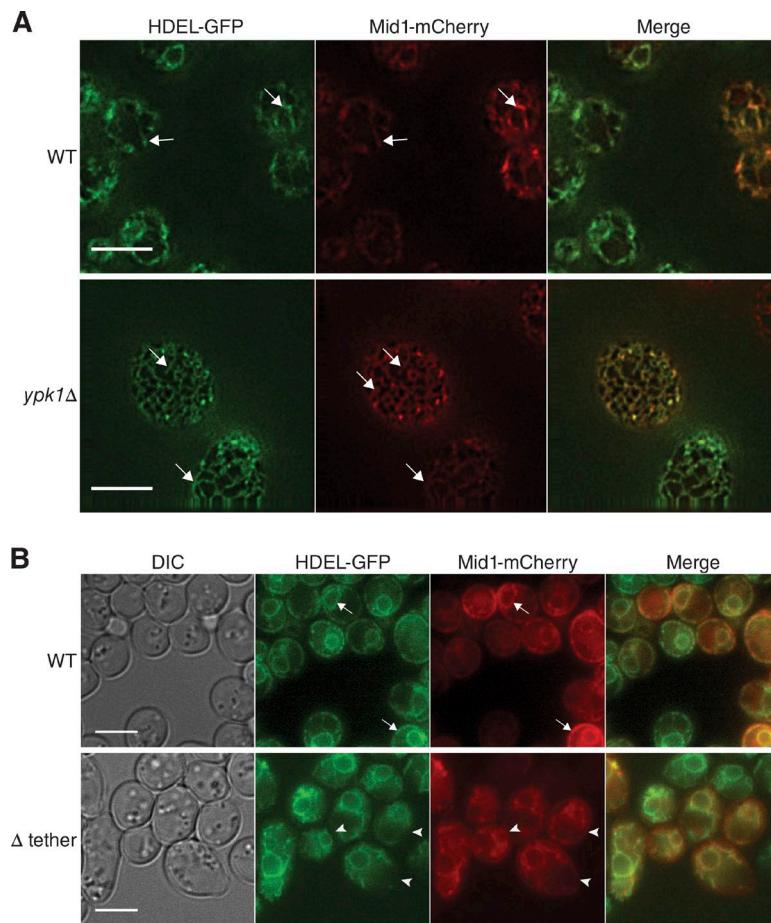


## Supplemental material

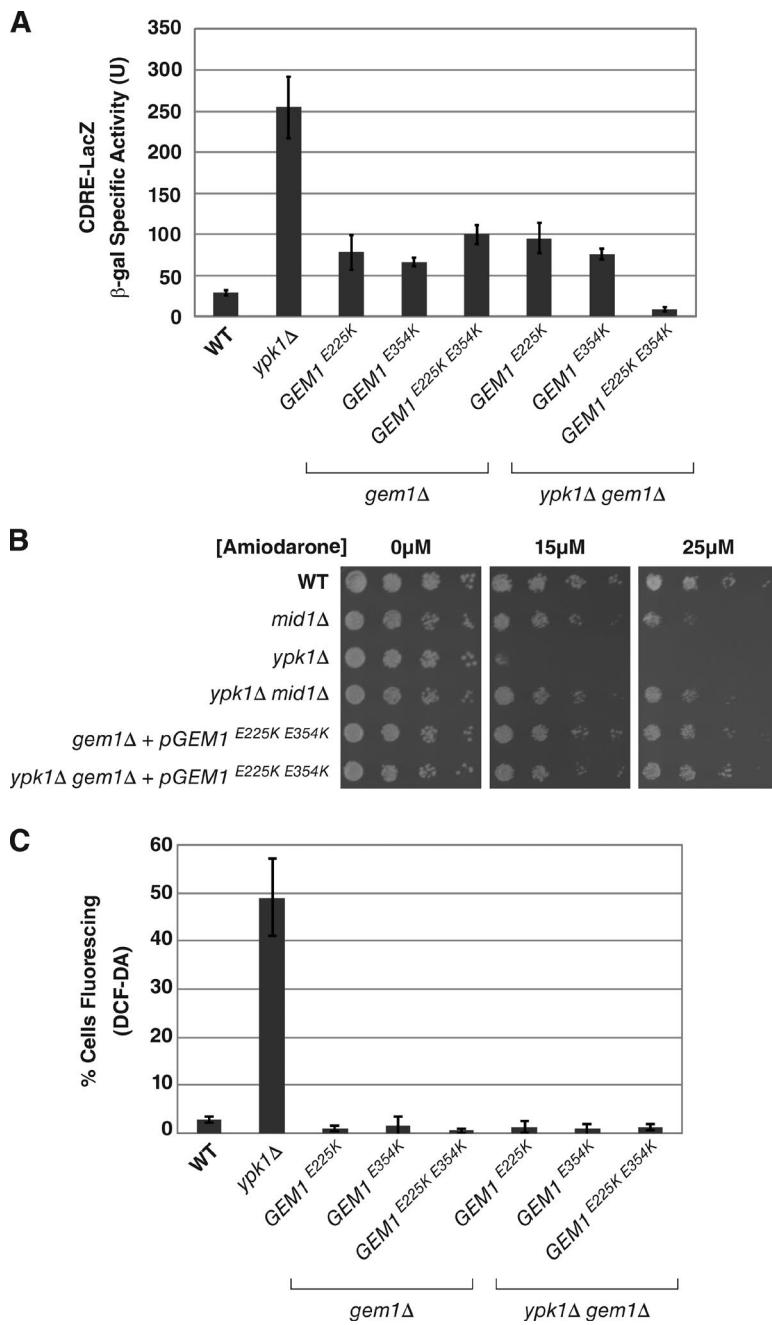
JCB

Vlahakis et al., <https://doi.org/10.1083/jcb.201605030>

**Figure S1. TORC2-Ypk1 signaling is required for mitophagy.** To test whether *ypk1* $\Delta$  cells exhibited defects in mitophagy, we used the mitochondrial matrix protein Idh1, fused to GFP, to monitor degradation of Idh1-GFP and concomitant accumulation of free GFP, under conditions of amino acid starvation-induced mitophagy (see Materials and methods). As controls, we examined both *atg7* $\Delta$  cells, defective for general autophagosome formation, as well as *atg32* $\Delta$  cells, defective for the specific recruitment of Atg proteins to mitochondria. Cells expressing IDH1-GFP at the endogenous locus were grown overnight in YPL medium and subjected to amino acid starvation with 2% lactate for 24 h. Western blot analysis was performed with  $\alpha$ -GFP and  $\alpha$ -G6PDH antibodies, and quantification of Idh1-GFP turnover at 24 h of starvation is represented as a percentage of the ratio of free GFP to total GFP (GFP + GFP-Idh1) signal relative to WT. Data are presented as means  $\pm$  SD of three independent experiments. We observed that Idh1-GFP was efficiently processed in WT cells but not in *ypk1* $\Delta$ , *atg7* $\Delta$ , or *atg32* $\Delta$  cells.



**Figure S2. Mid1 localizes to both the ER and PM.** (A) Cells expressing pRS416 MID1-mCherry and endogenous ER marker HDEL-GFP were grown to log phase and visualized using deconvolution fluorescence microscopy with the Applied Precision Delta Vision microscope as detailed in Materials and methods. Arrows denote examples of coincident signal for Mid1-mCherry and HDEL-GFP. (B) Cells expressing pRS416 MID1-mCherry and endogenous ER marker HDEL-GFP were grown to log phase and visualized using deconvolution fluorescence microscopy using a fluorescent microscope (E600; Nikon) as detailed in Materials and methods. Cells were grown in SCD medium without uracil and with methionine added to reduce expression of MID1-mCherry. Arrows, examples of coincident signal for Mid1-mCherry and HDEL-GFP in WT cells; arrowheads, examples of Mid1-mCherry signal at the PM that does not contain signal from HDEL-GFP in  $\Delta$ -tether cells. Bars, 5  $\mu$ m.



**Figure S3. The ERMES component Gem1 is involved in TORC2-Ypk1 signaling to Mid1 and calcineurin.** Data depict an analysis of mutant alleles of Gem1 that disrupt two predicted calcium-binding EF-hand domains (E225K and E354K) without causing mitochondrial morphology defects observable in other ERMES mutants (Kornmann et al., 2011). (A) Cells carrying pAMS363 that expressed 2xCDRE:*lacZ* were grown, and  $\beta$ -galactosidase activity was determined as described in the legend to Fig. 2. Data are presented as means  $\pm$  SD of three independent experiments. (B) Cells were grown to log phase, and serial dilutions were plated on agar plates containing SCD medium and the indicated concentrations of amiodarone. (C) Strains were grown to log phase and incubated with 10  $\mu$ M DCF for 30 min before fluorescence microscopy imaging. Quantification represents the percentage of DCF-positive fluorescing cells where  $n \geq 200$  total cells per strain. Data are presented as means  $\pm$  SD of three independent replicates.

Table S1. *Saccharomyces cerevisiae* strains used in this study

Strain	Genotype	Source
W303α	MAT $\alpha$ <i>leu2-3, -112; his3-11,-15; trp1-1; ura3-1; ade2-1; can1-100</i>	Nasmyth et al., 1990
PLY521	W303α, except <i>ypk1::TRP1</i>	Niles et al., 2014
PLY1569	W303α, except <i>cnb1::kanMX6</i>	Vlahakis et al., 2014
PLY1570	W303α, except <i>ypk1::TRP1 cnb1::kanMX6</i>	Vlahakis et al., 2014
PLY1644	W303α, except <i>rho<sup>0</sup></i>	Graef and Nunnari, 2011
PLY1645	W303α, except <i>rho<sup>0</sup> ypk1::TRP1</i>	This study
PLY1646	W303α, except <i>cbs1::natMX</i>	Graef and Nunnari, 2011
PLY1647	W303α, except <i>mss51::natMX</i>	Graef and Nunnari, 2011
PLY1648	W303α, except <i>atp10::natMX</i>	Graef and Nunnari, 2011
PLY1649	W303α, except <i>ypk1::TRP1 cbs1::natMX</i>	This study
PLY1650	W303α, except <i>ypk1::TRP1 mss51::natMX</i>	This study
PLY1651	W303α, except <i>ypk1::TRP1 atp10::natMX</i>	This study
PLY1652	W303α + pRS416 prATG8-GFP-ATG8	Vlahakis et al., 2014
PLY1653	W303α, except <i>ypk1::TRP1 + pRS416 prATG8-GFP-ATG8</i>	Vlahakis et al., 2014
PLY1654	W303α, except <i>rho<sup>0</sup> + pRS416 prATG8-GFP-ATG8</i>	Graef and Nunnari, 2011
PLY1655	W303α, except <i>rho<sup>0</sup> ypk1::TRP1 + pRS416 prATG8-GFP-ATG8</i>	This study
PLY1656	W303α, except <i>cbs1::natMX + pRS416 prATG8-GFP-ATG8</i>	Graef and Nunnari, 2011
PLY1657	W303α, except <i>mss51::natMX + pRS416 prATG8-GFP-ATG8</i>	Graef and Nunnari, 2011
PLY1658	W303α, except <i>atp10::natMX + pRS416 prATG8-GFP-ATG8</i>	Graef and Nunnari, 2011
PLY1659	W303α, except <i>ypk1::TRP1 cbs1::natMX + pRS416 prATG8-GFP-ATG8</i>	This study
PLY1660	W303α, except <i>ypk1::TRP1 mss51::natMX + pRS416 prATG8-GFP-ATG8</i>	This study
PLY1661	W303α, except <i>ypk1::TRP1 atp10::natMX + pRS416 prATG8-GFP-ATG8</i>	This study
PLY1662	W303α, except <i>tpk3::HIS3MX6 ypk1::TRP1 + pRS416 prATG8-GFP-ATG8</i>	This study
PLY1663	W303α, except <i>tpk3::HIS3MX6 ypk1::TRP1 + pRS416 prATG8-GFP-ATG8</i>	Vlahakis et al., 2014
PLY1664	W303α + pAMS363 2xCDRE: <i>lacZ</i>	Vlahakis et al., 2014
PLY1665	W303α, except <i>cbs1::natMX + pAMS363 2xCDRE:<i>lacZ</i></i>	This study
PLY1666	W303α, except <i>mss51::natMX + pAMS363 2xCDRE:<i>lacZ</i></i>	This study
PLY1667	W303α, except <i>atp10::natMX + pAMS363 2xCDRE:<i>lacZ</i></i>	This study
PLY1668	W303α, except <i>ypk1::TRP1 + pAMS363 2xCDRE:<i>lacZ</i></i>	Vlahakis et al., 2014
PLY1669	W303α, except <i>ypk1::TRP1 cbs1::natMX + pAMS363 2xCDRE:<i>lacZ</i></i>	This study
PLY1670	W303α, except <i>ypk1::TRP1 mss51::natMX + pAMS363 2xCDRE:<i>lacZ</i></i>	This study
PLY1671	W303α, except <i>ypk1::TRP1 atp10::natMX + pAMS363 2xCDRE:<i>lacZ</i></i>	This study
PLY1672	W303α, + p180 GCN4 URE -1 to -4: <i>lacZ</i>	Vlahakis et al., 2014
PLY1673	W303α, except <i>cbs1::natMX + p180 GCN4 URE -1 to -4:<i>lacZ</i></i>	This study
PLY1674	W303α, except <i>mss51::natMX + p180 GCN4 URE -1 to -4:<i>lacZ</i></i>	This study
PLY1675	W303α, except <i>atp10::natMX + p180 GCN4 URE -1 to -4:<i>lacZ</i></i>	This study
PLY1676	W303α, except <i>ypk1::TRP1 + p180 GCN4 URE -1 to -4:<i>lacZ</i></i>	Vlahakis et al., 2014
PLY1677	W303α, except <i>ypk1::TRP1 cbs1::natMX + p180 GCN4 URE -1 to -4:<i>lacZ</i></i>	This study
PLY1678	W303α, except <i>ypk1::TRP1 mss51::natMX + p180 GCN4 URE -1 to -4:<i>lacZ</i></i>	This study
PLY1679	W303α, except <i>ypk1::TRP1 atp10::natMX + p180 GCN4 URE -1 to -4:<i>lacZ</i></i>	This study
PLY1680	W303α, except <i>gcn2::kanMX6 + p180 GCN4 URE -1 to -4:<i>lacZ</i></i>	Vlahakis et al., 2014
PLY1691	W303α, except <i>mid1::kanMX6 + pAMS363 2xCDRE:<i>lacZ</i></i>	This study
PLY1692	W303α, except <i>mid1::kanMX6 ypk1::TRP1 + pAMS363 2xCDRE:<i>lacZ</i></i>	This study
PLY1693	W303α, except <i>mid1::kanMX6 + pRS416 prATG8-GFP-ATG8</i>	This study
PLY1694	W303α, except <i>mid1::kanMX6 ypk1::TRP1 + pRS416 prATG8-GFP-ATG8</i>	This study
PLY1695	W303α, except <i>vpx1::kanMX6 + pAMS363 2xCDRE:<i>lacZ</i></i>	This study
PLY1696	W303α, except <i>ypk1::TRP1 vpx1::kanMX6 + pAMS363 2xCDRE:<i>lacZ</i></i>	This study
PLY1697	W303α, except <i>yvc1::kanMX6 + pAMS363 2xCDRE:<i>lacZ</i></i>	This study
PLY1698	W303α, except <i>ypk1::TRP1 yvc1::kanMX6 + pAMS363 2xCDRE:<i>lacZ</i></i>	This study
PLY1699	W303α, except <i>cch1::kanMX6 + pAMS363 2xCDRE:<i>lacZ</i></i>	This study
PLY1700	W303α, except <i>ypk1::TRP1 cch1::kanMX6 + pAMS363 2xCDRE:<i>lacZ</i></i>	This study
PLY1701	W303α, except <i>mid1::kanMX6</i>	This study
PLY1702	W303α, except <i>mid1::kanMX6 ypk1::TRP1</i>	This study
PLY1703	W303α, except <i>HDEL:GFP:LEU2 + pRS416 MET25 MID1-mCherry (pPL610)</i>	This study
PLY1704	W303α, except <i>ypk1::TRP1 HDEL:GFP:LEU2 + pRS416 MET25 MID1-mCherry (pPL610)</i>	This study
PLY1705	SEY6210.1, except <i>HDEL:GFP:LEU2 + p416 MET25 MID1-mCherry (pL610)</i>	This study
PLY1706	SEY6210.1 Δether (ANDY198), except <i>HDEL:GFP:LEU2 + p416 MET25 MID1-mCherry (pPL610)</i>	This study
PLY1707	W303α, + pRS416	This study
PLY1708	W303α, except <i>ypk1::TRP1 + pRS416</i>	This study
PLY1709	W303α, except <i>mid1::KanMX6 + pRS416</i>	This study
PLY1710	W303α, except <i>ypk1::TRP1 mid1::KanMX6 + pRS416</i>	This study

Table S1. *Saccharomyces cerevisiae* strains used in this study (Continued)

Strain	Genotype	Source
PLY1711	W303 $\alpha$ , except <i>mid1::KanMX6</i> + pRS416 MET25 MID1-mCherry (pPL610)	This study
PLY1712	W303 $\alpha$ , except <i>ypk1::TRP1 mid1::KanMX6</i> + pRS416 MET25 MID1-mCherry (pPL610)	This study
PLY1713	W303 $\alpha$ , except <i>ypk1::TRP1 mid1::KanMX6</i> + pRS416 MET25 MID1 $\Delta$ C-mCherry (pPL611)	This study
PLY1714	W303 $\alpha$ , except <i>IDH1::GFP:HISMX6</i>	This study
PLY1715	W303 $\alpha$ , except <i>ypk1::TRP1 IDH1::GFP:HISMX6</i>	This study
PLY1716	W303 $\alpha$ , except <i>atg7::KanMX6 IDH1::GFP:HISMX6</i>	This study
PLY1717	W303 $\alpha$ , except <i>atg32::KanMX6 IDH1::GFP:HISMX6</i>	This study
PLY1718	W303 $\alpha$ + pRS315	This study
PLY1719	W303 $\alpha$ , except <i>ypk1::TRP1</i> + pRS315	This study
PLY1720	W303 $\alpha$ , except <i>mid1::KanMX6</i> + pRS315	This study
PLY1721	W303 $\alpha$ , except <i>ypk1::TRP1 mid1::KanMX6</i> + pRS315	This study
PLY1722	W303 $\alpha$ , except <i>gem1::KanMX6</i> + pRS315 GEM1 E225K E364K	This study
PLY1723	W303 $\alpha$ , except <i>ypk1::TRP1 gem1::KanMX6</i> + pRS315 GEM1 E225K E364K	This study
PLY1724	W303 $\alpha$ , except <i>gem1::KanMX6</i> + pRS315 GEM1 E225K + pAMS363 2xCDRE: <i>lacZ</i>	This study
PLY1725	W303 $\alpha$ , except <i>gem1::KanMX6</i> + pRS315 GEM1 E364K + pAMS363 2xCDRE: <i>lacZ</i>	This study
PLY1726	W303 $\alpha$ , except <i>gem1::KanMX6</i> + pRS315 GEM1 E225K E364K + pAMS363 2xCDRE: <i>lacZ</i>	This study
PLY1727	W303 $\alpha$ , except <i>ypk1::TRP1 gem1::KanMX6</i> + pRS315 GEM1 E225K + pAMS363 2xCDRE: <i>lacZ</i>	This study
PLY1728	W303 $\alpha$ , except <i>ypk1::TRP1 gem1::KanMX6</i> + pRS315 GEM1 E364K + pAMS363 2xCDRE: <i>lacZ</i>	This study
PLY1729	W303 $\alpha$ , except <i>ypk1::TRP1 gem1::KanMX6</i> + pRS315 GEM1 E225K E364K + pAMS363 2xCDRE: <i>lacZ</i>	This study

## References

- Graef, M., and J. Nunnari. 2011. Mitochondria regulate autophagy by conserved signalling pathways. *EMBO J.* 30:2101–2114. <http://dx.doi.org/10.1038/emboj.2011.104>
- Kornmann, B., C. Osman, and P. Walter. 2011. The conserved GTPase Gem1 regulates endoplasmic reticulum-mitochondria connections. *Proc. Natl. Acad. Sci. USA.* 108:14151–14156. <http://dx.doi.org/10.1073/pnas.1111314108>
- Nasmyth, K., G. Adolf, D. Lydall, and A. Seddon. 1990. The identification of a second cell cycle control on the HO promoter in yeast: Cell cycle regulation of SW15 nuclear entry. *Cell.* 62:631–647. [http://dx.doi.org/10.1016/0092-8674\(90\)90110-Z](http://dx.doi.org/10.1016/0092-8674(90)90110-Z)
- Niles, B.J., A.C. Joslin, T. Fresques, and T. Powers. 2014. TOR complex 2-Ypk1 signaling maintains sphingolipid homeostasis by sensing and regulating ROS accumulation. *Cell Reports.* 6:541–552. <http://dx.doi.org/10.1016/j.celrep.2013.12.040>
- Vlahakis, A., M. Graef, J. Nunnari, and T. Powers. 2014. TOR complex 2-Ypk1 signaling is an essential positive regulator of the general amino acid control response and autophagy. *Proc. Natl. Acad. Sci. USA.* 111:10586–10591. <http://dx.doi.org/10.1073/pnas.1406305111>