

Thompson et al., <http://www.jcb.org/cgi/content/full/jcb.200811119/DC1>

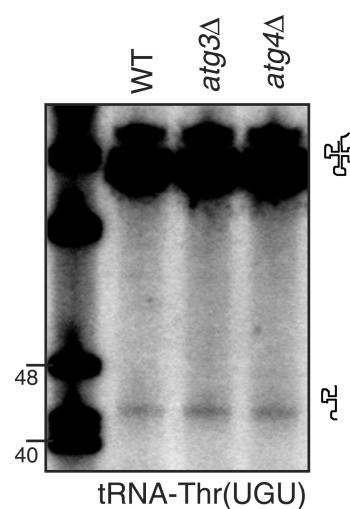


Figure S1. **tRNA cleavage is not affected in autophagy-deficient mutant strains.** 3' Thr (UGU) probe. tRNA cleavage is shown in WT yeast and the autophagy mutant strains *atg3Δ* and *atg4Δ*. Cells were grown to mid-log phase in YEPD. tRNA illustrations indicate full-length and fragment species. L = φX174/*Hinf* I ladder (sizes indicated in nucleotides). The experiment was repeated at least three times; a representative blot is shown.

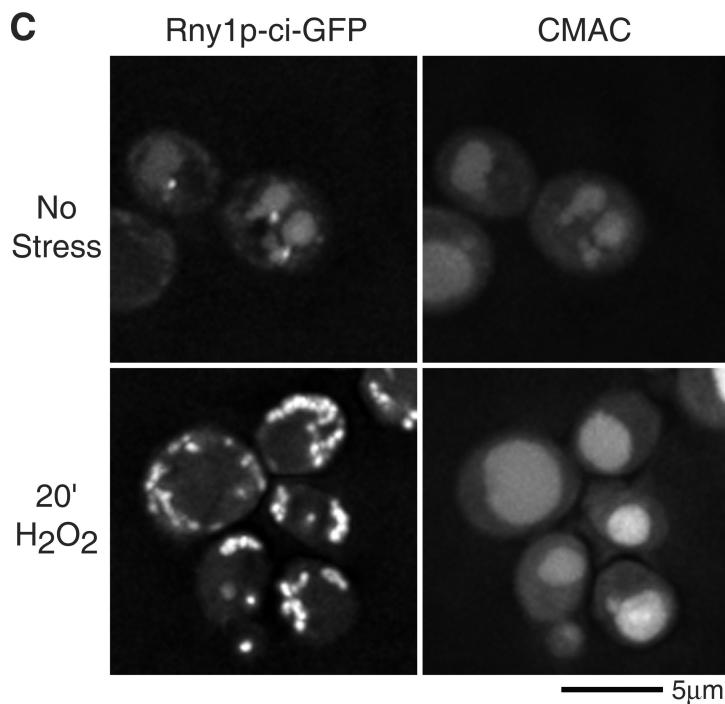
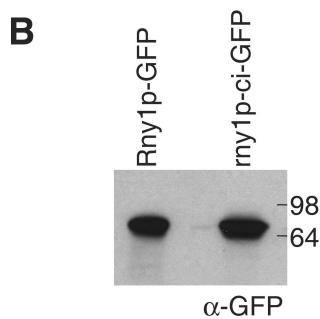
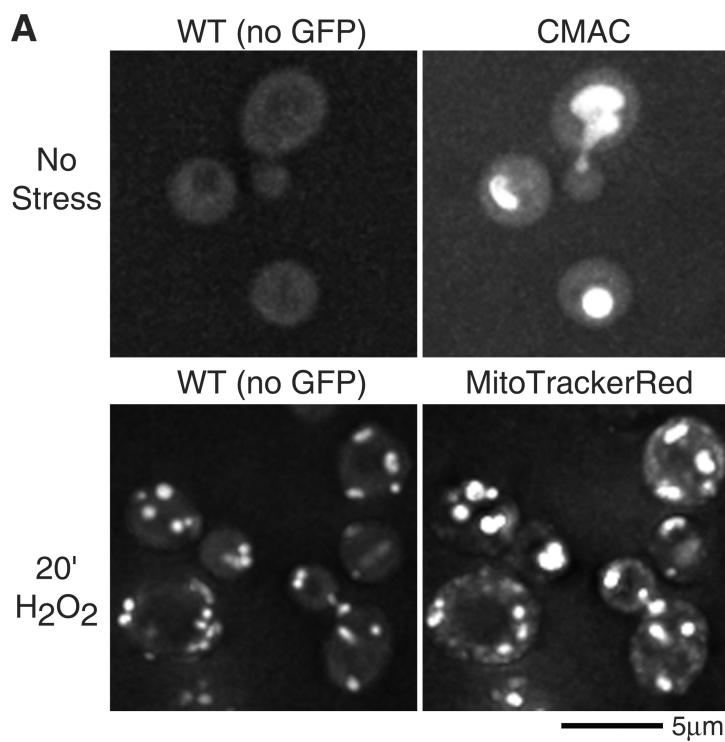


Figure S2. Mitochondrial autofluorescence in WT cells during oxidative stress and localization of catalytically inactive Rny1p. (A) Visualization of WT cells using GFP settings during log-phase growth and after 20 min of exposure to 3 mM H₂O₂. (B) Western blot analysis showing protein expression levels of overexpressed *RNY1* and *rny1-ci* (GFP-tagged forms, internal GFP tag). Approximate molecular weights are indicated in kD. (C) Localization of GFP-tagged *rny1-p-ci* during log-phase growth and after 20 min of exposure to 3 mM H₂O₂. The vacuolar luminal dye CMAC was used to mark vacuoles, and MitoTracker red was used to mark mitochondria. Experiments were repeated at least three times; representative examples are shown.

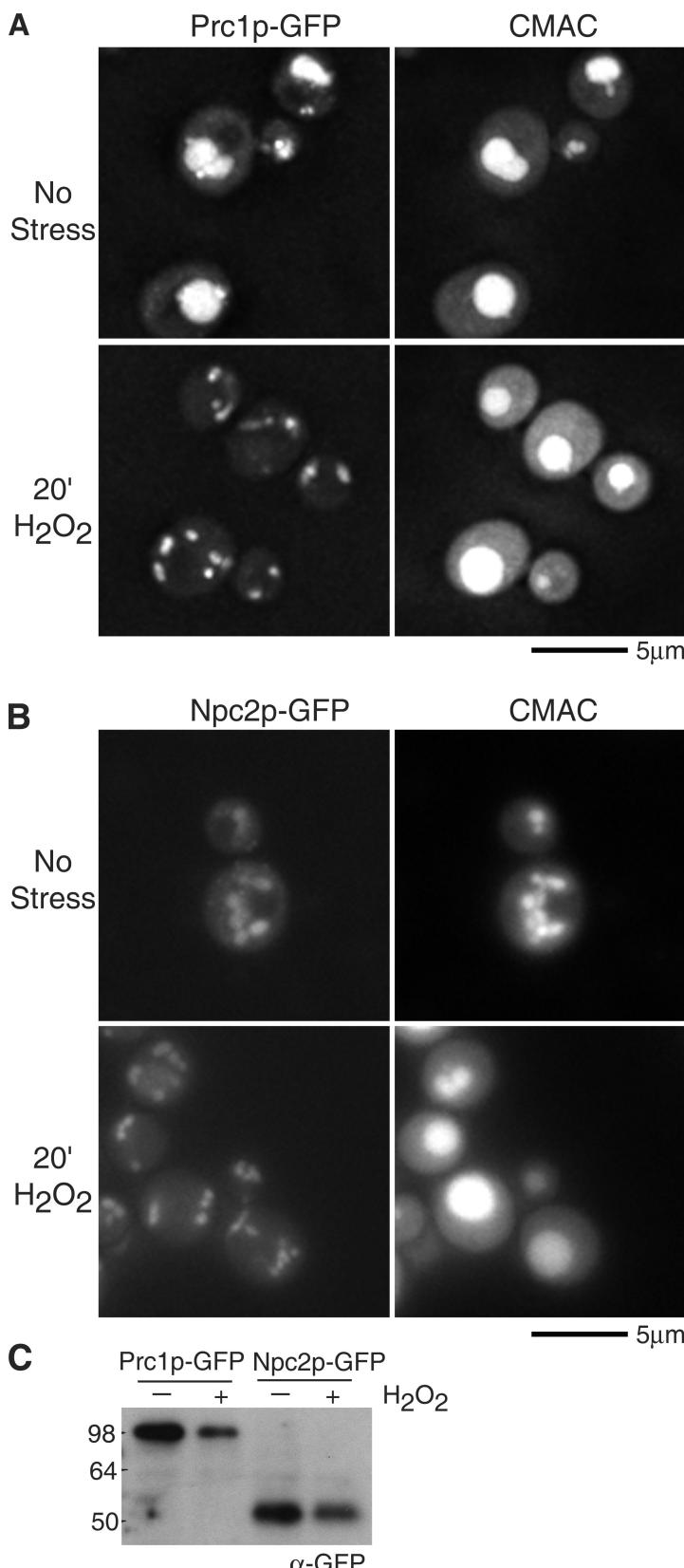


Figure S3. Release of other vacuolar proteins during oxidative stress. (A) Localization of Prc1p-GFP during log phase and after 20 min of exposure to 3 mM H₂O₂. (B) Localization of Npc2p-GFP during log phase and after 20 min of exposure to 3 mM H₂O₂. (C) Western blot analysis of Prc1p-GFP or Npc2p-GFP levels before and after a 1-h exposure to 3 mM H₂O₂. Approximate molecular weights are indicated in kD. The vacuolar luminal dye CMAC was used to mark vacuoles. Experiments were repeated at least three times; representative examples are shown.

Table S1. Yeast strains used in this study

Strain	Genotype	Reference
BY4741	MAT α ; his3Δ1; leu2Δ0; met15Δ0; ura3Δ0	Winzeler et al., 1999
Y0 2129	MAT α ; his3Δ1; leu2Δ0; met15Δ0; ura3Δ0; rny1Δ::kanMX4	Winzeler et al., 1999
Y05382	MAT α ; his3Δ1; leu2Δ0; met15Δ0; ura3Δ0; atg3Δ::kanMX4	Winzeler et al., 1999
Y01989	MAT α ; his3Δ1; leu2Δ0; met15Δ0; ura3Δ0; atg4Δ::kanMX4	Winzeler et al., 1999
yRP 840	MAT α ; leu2-3,112; his4-539; trp1; ura3-52; cup1::LEU2/PGK1pG/MFA2pG	Hatfield et al., 1996
yRP 2420	MAT α ; his3Δ1; leu2Δ0; met15Δ0; ura3Δ0; [pRP 1584]	This study
yRP 2427	MAT α ; his3Δ1; leu2Δ0; met15Δ0; ura3Δ0; [pRS 426]	This study
bir1Δ	MAT α ; his3Δ1; leu2Δ0; met15Δ0; ura3Δ0; bir1Δ::kanMX4	Walter et al., 2006
yRP 2446	MAT α ; his3Δ1; leu2Δ0; met15Δ0; ura3Δ0; bir1Δ::kanMX4; rny1Δ::URA3	This study
yRP 2447	MAT α ; his3Δ1; leu2Δ0; met15Δ0; ura3Δ0; bir1Δ::kanMX4; [pRP 1584]	This study
yRP 2448	MAT α ; his3Δ1; leu2Δ0; met15Δ0; ura3Δ0; bir1Δ::kanMX4; [pRS 426]	This study
yRP 2449	MAT α ; leu2-3,112; his4-539; trp1; ura3-52; cup1::LEU2/PGK1pG/MFA2pG; rny1Δ::kanMX4	This study
yRP 2450	MAT α ; leu2-3,112; his4-539; trp1; ura3-52; cup1::LEU2/PGK1pG/MFA2pG; rny1Δ::kanMX4; [pTRNA-GluD]	This study
yRP 2451	MAT α ; leu2-3,112; his4-539; trp1; ura3-52; cup1::LEU2/PGK1pG/MFA2pG; [pTRNA-GluD]	This study
yRP 2463	MAT α ; his3Δ1; leu2Δ0; met15Δ0; ura3Δ0; rny1Δ::kanMX4; [pRP 1584]	This study
yRP 2464	MAT α ; his3Δ1; leu2Δ0; met15Δ0; ura3Δ0; rny1Δ::kanMX4; [pRS 426]	This study
yRP 2467	MAT α ; his3Δ1; leu2Δ0; met15Δ0; ura3Δ0; rny1Δ::kanMX4; [pRP 1587]	This study
yRP 2470	MAT α ; his3Δ1; leu2Δ0; met15Δ0; ura3Δ0; [pRP 1587]	This study
yRP 2496	MAT α ; his3Δ1; leu2Δ0; met15Δ0; ura3Δ0; bir1Δ::kanMX4; rny1Δ (unmarked)	This study
yRP 2497	MAT α ; his3Δ1; leu2Δ0; met15Δ0; ura3Δ0; bir1Δ::kanMX4; rny1Δ (unmarked); [pRP 1618]	This study
yRP 2498	MAT α ; his3Δ1; leu2Δ0; met15Δ0; ura3Δ0; bir1Δ::kanMX4; rny1Δ (unmarked); [pRP 1619]	This study
yRP 2499	MAT α ; his3Δ1; leu2Δ0; met15Δ0; ura3Δ0; bir1Δ::kanMX4; rny1Δ (unmarked); [pRS 416]	This study
yRP 2500	MAT α ; his3Δ1; leu2Δ0; met15Δ0; ura3Δ0; rny1Δ::kanMX4; [pRP 1621]	This study
yRP 2501	MAT α ; his3Δ1; leu2Δ0; met15Δ0; ura3Δ0; rny1Δ::kanMX4; [pRP 1622]	This study
yRP 2502	MAT α ; his3Δ1; leu2Δ0; met15Δ0; ura3Δ0; yap1Δ::kanMX4; [pRP 1584]	This study
yRP 2503	MAT α ; his3Δ1; leu2Δ0; met15Δ0; ura3Δ0; yap1Δ::kanMX4; [pRS 426]	This study
yRP 2504	MAT α ; his3Δ1; leu2Δ0; met15Δ0; ura3Δ0; yap1Δ::kanMX4; [pRP 1587]	This study
yRP 2505	MAT α ; his3Δ1; leu2Δ0; met15Δ0; ura3Δ0; bir1Δ::kanMX4; [pRP 1587]	This study
yRP 2507	MAT α ; his3Δ1; leu2Δ0; met15Δ0; ura3Δ0; rny1Δ::kanMX4; [pRP 1547]	This study
yRP 2599	MAT α ; his3Δ1; leu2Δ0; met15Δ0; ura3Δ0; [pRP 1729]	This study
Y0 3883	MAT α ; his3Δ1; leu2Δ0; met15Δ0; ura3Δ0; vma1Δ::kanMX4	Winzeler et al., 1999
Prc1-GFP	MAT α ; his3Δ1; leu2Δ0; met15Δ0; ura3Δ0; PRC1-GFP	Huh et al., 2003
Npc2-GFP	MAT α ; his3Δ1; leu2Δ0; met15Δ0; ura3Δ0; NPC2-GFP	Huh et al., 2003
Pep4-GFP	MAT α ; his3Δ1; leu2Δ0; met15Δ0; ura3Δ0; PEP4-GFP	Huh et al., 2003

References

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- Walter, D., S. Wissing, F. Madeo, and B. Fahrenkrog. 2006. The inhibitor-of-apoptosis protein Bir1p protects against apoptosis in *S. cerevisiae* and is a substrate for the yeast homologue of Omi/HtrA2. *J. Cell Sci.* 119:1843–1851.
- Winzeler, E.A., D.D. Shoemaker, A. Astromoff, H. Liang, K. Anderson, B. Andre, R. Bangham, R. Benito, J.D. Boeke, H. Bussey, et al. 1999. Functional characterization of the *S. cerevisiae* genome by gene deletion and parallel analysis. *Science*. 285:901–906.

Table S2. Oligonucleotide sequences used in this study

oRP No.	Sequence	Details
oRP 1381	5'-CCAATGGTATCACTGCCGAC-3'	
oRP 1382	5'-GTAATACGCGACTACCTCCTCCAC-3'	
oRP 1383	5'-CAATGGTATCACTGCCGACCAAATCATGCCATCTGGAGCTGTGGGTATTCACACCG-3'	
oRP 1384	5'-GTAATACGCGACTACCTCCTCCACCATTCCAGCTACGGAGATTGACTGAGAGTGCAC-3'	
oRP 1385	5'-CAACTACCAATTTCGAATGGAGCGGACCGGGGTATAGAACCAAATGCCCTATAA-3'	
oRP 1386	5'-TTAGGGCAGTTGGTCTATACCCGGTCCGCTCCATTGAAAAAAATTGGTAGTG-3'	
oRP 1387	5'-CTTACACAGTCTTACAACCAATTTCGAATGGAGACAAGCGCCTGCGTGACAA-3'	
oRP 1388	5'-AGAAGGGTAAAGCTAAATGAAGTTCAATCGAAAATTCAATGCTGGCTTTAG-3'	
oRP 1398	5'-GTACTAACCAACTATACTAAC-3'	5' tRNA-Histidine (GTG)
oRP 1401	5'-GCGCCGCTCGGTTCGATCC-3'	3' tRNA-Methionine (CAT)
oRP 1402	5'-CCCCATTGGGAATCGAAC-3'	<i>D. discoideum</i> 3' tRNA-GluD
oRP 1404	5'-ATGTTACTGAAAAACTTACAC-3'	
oRP 1423	5'-CAAAGGCTTAATCTCAGCAGATCG-3'	3' 25S rRNA
oRP 1428	5'-CTTGTACAAAGTGGTTGATGTGAGTAAAGGAGAAGAAACTTTC-3'	
oRP 1429	5'-GTGAGCGCGCGTAATACGACTCCTATTGTATAGTTCATCC-3'	
oRP 1430	5'-CCTGTCAGAAATGAACAAAC-3'	3' tRNA-Thr (UGU)

For tRNA probes, tRNA name and 5' or 3' specificity are indicated (Details).