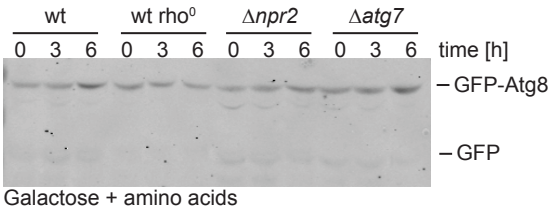

wt
wt rho0
Δnpr2
Δatg7
0 3 6 0 3 6 0 3 6 0 3 6
time [h]
GFP-Atg8
GFP
Galactose + amino acids

Figure S1

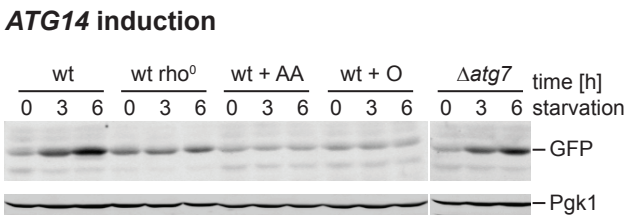

ATG14 induction
wt
wt rho^0
wt + AA
wt + O
Δ*atg7*
time [h]
0 3 6 0 3 6 0 3 6 0 3 6 0 3 6
starvation
GFP
Pgk1

Figure S2

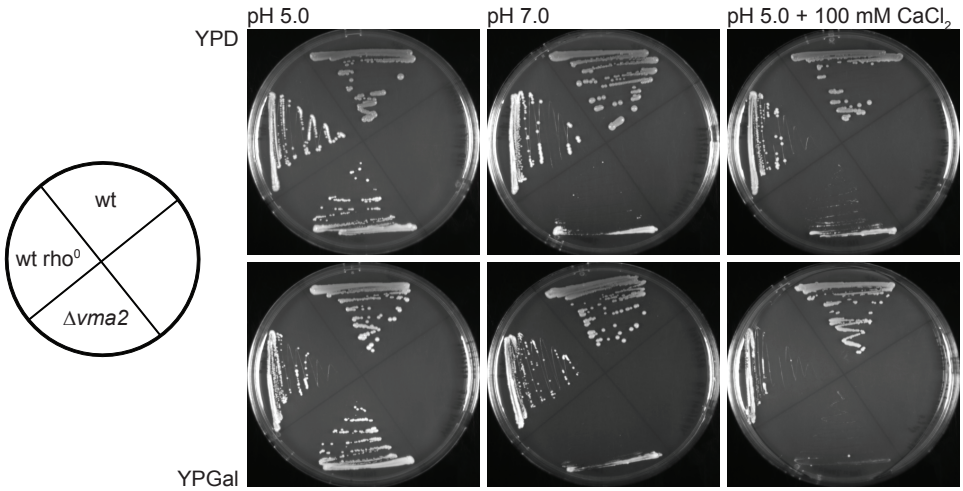


Figure S3

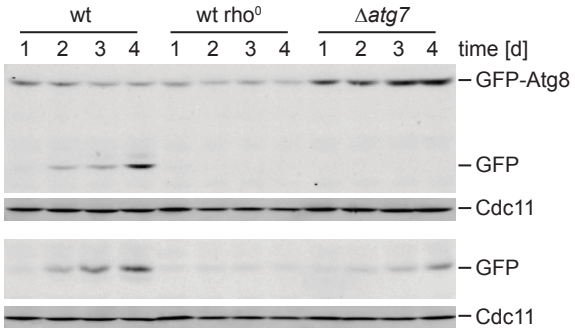

wt
wt rho0
Δatg7
1 2 3 4 1 2 3 4 1 2 3 4 time [d]
GFP-Atg8
GFP
Cdc11
GFP
Cdc11

Figure S4

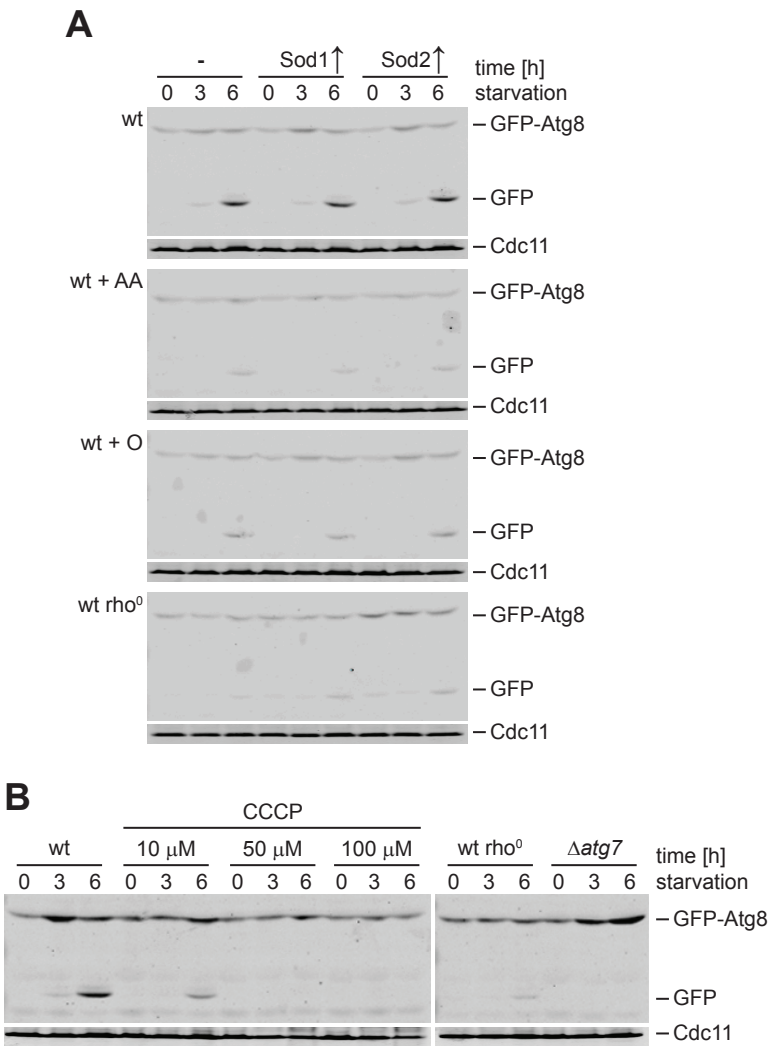

A
-
Sod1↑
Sod2↑
time [h]
0 3 6 0 3 6 0 3 6
starvation
wt
wt + AA
wt + O
wt rho0
GFP-Atg8
GFP
Cdc11
B
CCCP
wt
10 μM
50 μM
100 μM
wt rho0
Δatg7
time [h]
0 3 6 0 3 6 0 3 6 0 3 6 0 3 6 0 3 6
starvation
GFP-Atg8
GFP
Cdc11

Figure S5

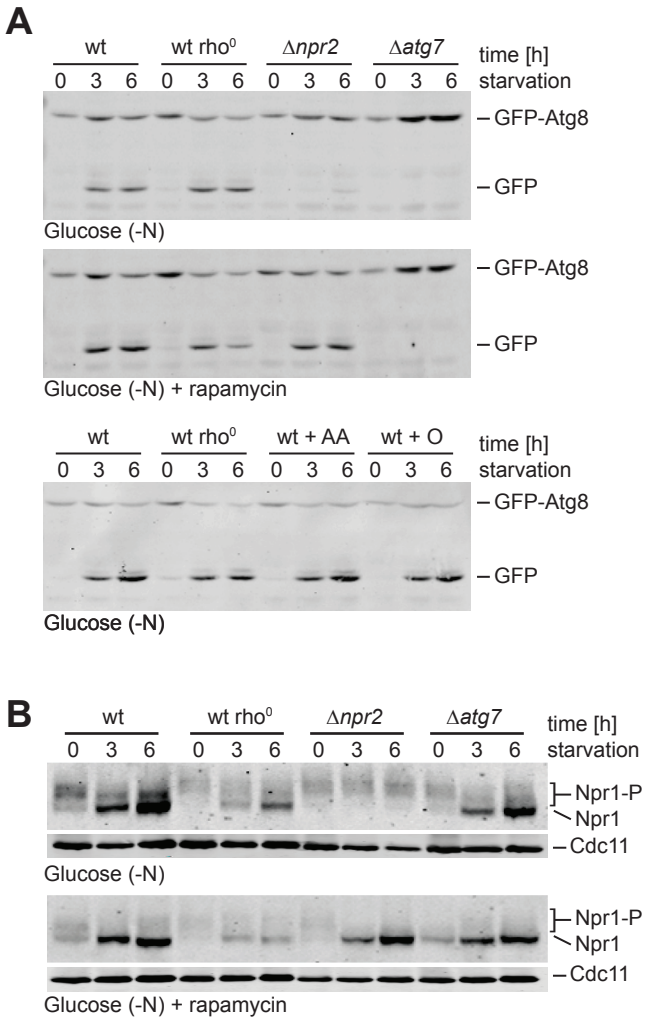
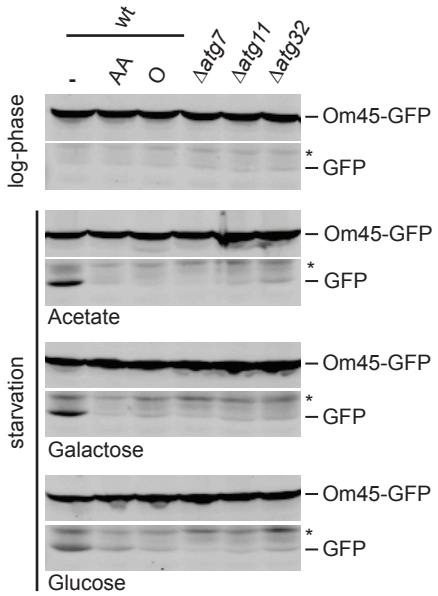

A
wt
wt rho0
Δnpr2
Δatg7
time [h]
0 3 6 0 3 6 0 3 6 0 3 6
starvation
GFP-Atg8
GFP
Glucose (-N)
GFP-Atg8
GFP
Glucose (-N) + rapamycin
wt
wt rho0
wt + AA
wt + O
time [h]
0 3 6 0 3 6 0 3 6 0 3 6
starvation
GFP-Atg8
GFP
Glucose (-N)
B
wt
wt rho0
Δnpr2
Δatg7
time [h]
0 3 6 0 3 6 0 3 6 0 3 6
starvation
Npr1-P
Npr1
Cdc11
Glucose (-N)
Npr1-P
Npr1
Cdc11
Glucose (-N) + rapamycin

Figure S6


wt
-
AA
O
Δatg7
Δatg11
Δatg32
log-phase
Om45-GFP
*
GFP
starvation
Om45-GFP
*
GFP
Acetate
Om45-GFP
*
GFP
Galactose
Om45-GFP
*
GFP
Glucose

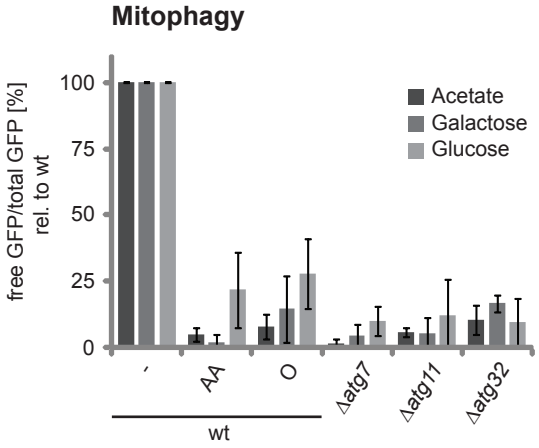

Mitophagy
free GFP/total GFP [%]
rel. to wt
100
75
50
25
0
Acetate
Galactose
Glucose
-
AA
O
Δatg7
Δatg11
Δatg32
wt

Figure S7

Supplementary information

Fig. S1: Wild type, rho^0, Δ*npr2*, and Δ*atg7* cells harboring pr^{ATG8}-*GFP-ATG8* were exposed to amino acid starvation medium supplemented with amino acids and galactose. Samples were analyzed by whole cell extraction and western blot analysis using a α-GFP antibody.

Fig. S2: *ATG14* induction during amino acid starvation. Wild type, rho^0, and Δ*atg7* cells harboring pr^{ATG14}-*GFP* were exposed to amino acid starvation medium supplemented with acetate in the presence or absence of antimycin A (AA) or oligomycin (O). Cells were analyzed at indicated time points by whole cell extraction and western blot analysis using α-GFP and α-Pgk1 antibodies.

Fig. S3: Rho^0 cells display growth behavior consistent with intact vacuolar acidification. Wild type, rho^0, and Δ*vma2* cells were grown on solid YP medium in the presence of glucose (YPD) or galactose (YPGal) at indicated pH and $CaCl_2$ concentration for 3-5 days at 30ºC. Note that, in contrast to wild type and rho^0 cells, the growth of cells lacking subunit B of the vacuolar H^+-ATPase (Δ*vma2*) is strongly impaired at pH 7.0 and in the presence of 100 mM $CaCl_2$ consistent with defects in vacuolar acidification described previously (Graham et al., 2000).

Fig. S4: Autophagic response during prolonged stationary phase. Log-phase wild type, rho^0, and Δ*atg7* cells harboring pr^{ATG8}-*GFP-ATG8* (upper panels) or pr^{ATG8}-*GFP* (lower panels) were grown to stationary phase in galactose medium. Cells were analyzed at indicated time points by whole cell extraction and western blot analysis using α-GFP and α-Cdc11 antibodies.

Fig. S5: (A) Overexpression of cytosolic or mitochondrial superoxide dismutase does not affect the autophagic response in the presence of mitochondrial respiratory deficiency. Wild type and rho^0 cells harboring pr^{ATG8}-*GFP-ATG8* were exposed to amino acid starvation medium supplemented with galactose in the presence of antimycin A (AA) or oligomycin (O) when indicated. Wild type and rho^0 cells overexpressed cytosolic (Sod1) or mitochondrial (Sod2) superoxide dismutase when indicated. Samples were analyzed at indicated time points by whole cell extraction and western blot analysis using α-GFP and α-Cdc11 antibodies. **(B)** Effect of lowered membrane potential on the autophagic response during amino acid starvation. Wild type, rho^0, and Δ*atg7* cells harboring pr^{ATG8}-

GFP-ATG8 were exposed to amino acid starvation medium supplemented with galactose. Wild type cells were exposed to CCCP at indicated concentrations during starvation. Samples were analyzed as described in (A).

Fig. S6: Autophagic response of respiratory deficient cells during nitrogen starvation. (A) Wild type, rho^0, Δ*npr2*, and Δ*atg7* cells were exposed to nitrogen starvation medium supplemented with glucose in the absence (upper panel) or presence (middle panel) of rapamycin (400 ng/ml). Wild type cells were exposed to antimycin (AA) or oligomycin (O) during nitrogen starvation as indicated (lower panel). Samples were analyzed by whole cell extraction and western blot analysis using a α-GFP antibody. **(B)** Wild type, rho^0, Δ*npr2*, and Δ*atg7* cells expressing pr^{NPR1}-NPR1-HA were exposed to nitrogen starvation medium supplemented with glucose in the absence (upper panels) or presence (lower panels) of rapamycin (400 ng/ml). The hyperphosphorylated (Npr1-P) and dephosphorylated (Npr1) forms of Npr1 are indicated. Cells were analyzed at indicated time points by whole cell extraction and western blot analysis using α-HA and α-Cdc11 antibodies.

Fig. S7: Mitochondrial respiratory deficiency impairs mitophagy under amino acid starvation. Wild type, Δ*atg7*, Δ*atg11*, and Δ*atg32* cells expressing the outer mitochondrial membrane protein and established mitophagy reporter pr^{OM45}-*OM45-GFP* (Kanki and Klionsky, 2008) were grown in YPlactate medium (log-phase) and exposed to amino acid starvation medium (starvation) supplemented with indicated carbon sources for 24 h. To impair mitochondrial function, wild type cells were exposed to antimycin A (AA) or oligomycin (O) during starvation. Om45-GFP (upper panels) and free GFP levels (lower panels) were monitored by whole cell extraction and western blot analysis using a α-GFP antibody. In wild type cells, a significant amount of free GFP was detected by western blot analysis under all tested amino acid starvation conditions, indicating that mitochondria were degraded. Consistent with published work, Om45-GFP degradation was blocked in Δ*atg7*, Δ*atg11*, and Δ*atg32* cells, demonstrating that mitochondrial degradation depends on both the general autophagy (Atg7) and mitophagy-specific machinery (Atg11 and Atg32) (Kanki and Klionsky, 2008; Kanki et al., 2009; Okamoto et al., 2009). Significantly, mitochondrial turnover was completely blocked in wild type cells in the presence of antimycin A or oligomycin under all carbon source conditions. (*) indicates an unspecific α-GFP signal. Quantification of the ratio of free GFP to total GFP (Om45-GFP and free GFP) relative to untreated wild type (wt=1) after starvation is shown as means and standard deviations of three (n=3) independent experiments.