Figure S1 - Dynamics of intracellular nucleotides and nucleosides under nitrogen starvation in WT, *atg2*Δ, and *phm*8Δ cells.

Time-dependent changes in nucleoside and nucleotide contents under nitrogen starvation. Wild-type, *atg2*Δ, and *phm8*Δ cells were grown in SD and transferred to SD(-N) at time 0. Metabolites were analyzed as described in Figure 2. The results are presented as normalized intensities on the basis of peak height of each metabolite in wild-type cells. All data are means of triplicate samples. The error bars represent the standard deviation.

Figure S2 - Role of Rny1 and Pho8 during autophagy

(A) Time-dependent changes in nucleosides and nucleotides under nitrogen starvation for up to 24 h. Wild-type and *rny1*Δ cells were grown in SD and transferred to SD(-N) at time 0. Metabolites were analyzed as described in Figure 2. The results are presented as normalized intensities on the basis of peak height of each metabolite in wild-type cells. (B) ALP assay (Pho8Δ60). Wild-type, *atg2*Δ, and *rny1*Δ cells expressing Pho8Δ60 were grown in SD to mid-log phase and transferred to SD(-N) at time 0. At the indicated time points, lysates were prepared and subjected to the ALP assay. The bars present the standard deviation of three independent experiments. (C) GFP-Atg8 transport. To monitor starvation-induced PAS (Kawamata *et al.*, 2008), we used *atg11*Δ cells. Cells expressing GFP-Atg8 in *atg11*Δ, *atg11*Δ*rny1*Δ, or *atg11*Δ*pho8*Δ were grown in SD to mid-log phase and transferred to SD(-N). After 2 h of starvation, GFP-Atg8 was analyzed by fluorescence microscopy. In starvation conditions, almost GFP-Atg8 strongly stained vacuoles in these cells, indicating that deletion of Rny1 and Pho8 does not affect the autophagic pathway to the vacuole.

Figure S3 - Processing and activity of vacuolar enzymes in *ubp3*Δ and *bre5*Δ cells

(A) Processing of vacuolar enzymes. Wild-type, *atg2*Δ, *ubp3*Δ, *bre5*Δ, *pep4*Δ, and *pep4*Δ*prb1*Δ cells were grown in SD to mid-log phase and transferred to SD(-N). At the indicated time points, lysates were prepared and analyzed by western blotting using anti-Pep4, anti-Prb1, anti-ALP, and anti-PGK antibodies. m-Pho8 and m-Pho8** are membrane-bound or soluble active form of Pho8, as demonstrated in the literature (Qiao *et al.*, 2009). (B) Enzymatic activity of endogenous Pho8. Cells were cultured as in (A). After incubation in SD(-N) medium for 0 or 1 h, all cell lysates were subjected to the ALP assay. The bars represent the standard deviation of three independent experiments. Asterisk, $P<0.05$; double asterisk, $P<0.005$; triple asterisk, $P<0.0005$ (paired *t*-test, two-tailed).

Figure S4 - Dynamics of intracellular nucleosides and nucleobases under nitrogen starvation in WT, *atg2*Δ, *pnp1*Δ, and *urh1*Δ cells. (A and B) Time-dependent changes in nucleoside and nucleobase contents after nitrogen starvation. (A) Wild-type, *atg2*Δ and *pnp1*Δ cells were grown in SD and transferred to SD(-N) at time 0. At the indicated time points, the cells were collected; their metabolites were extracted and analyzed by LC/MS as described in Materials and Methods and Expanded View, Supplemental Experimental Procedures. All data are means of quadruplicates. The error bars represent the standard deviation. (B) Wild-type, *atg2*Δ, and *urh1*Δ cells were grown in SD and transferred to SD(-N) at time 0. Metabolites were analyzed as in (A). All data are means of quadruplicates. The error bars represent the standard deviation. (C) Expression and localization of Pnp1 and Urh1. Wild-type cells expressing Pnp1-GFP or Urh1-GFP were grown in SD to mid-log phase and transferred to SD(-N). After 2 h of starvation, GFP-tagged proteins were analyzed by fluorescence microscopy.

Figure S5 - Dynamics of nucleotides, nucleosides, and nucleobases under nitrogen starvation

(A and B) Time-dependent changes in nucleotide, nucleoside and nucleobase contents after nitrogen starvation. Intracellular (A) and extracellular (B) nucleotides, nucleosides and nucleobases. Data of nucleosides and nucleobases were the same with in Figure 5B-5E, except for adding *pho8*Δ. Note that 3'-AMP in *pho8*Δ was separately presented.

Figure S6 - Amounts of intracellular RNAs upon nitrogen starvation in wild-type, *atg2*Δ, and *rny1*Δ cells. (A) Northern blot analysis. Wild-type, *atg2*Δ and *rny1*Δ cells were grown in SD, transferred to SD(-N) and cultured for 5 h. The cells were collected and the intracellular RNAs were extracted as described in Expanded View, supplemental experimental procedures. Five micrograms of the extracted RNAs were separated on either denaturing formaldehyde agarose gel (1%) (left) or 10 % polyacrylamide gel containing 8 M Urea (right). Blots were probed with specific oligonucleotides. 18S and 25S rRNA, tRNAs (Leu, Phe, Arg, Val), snRNAs (U4), snoRNAs (SnR13 and SnR50), and mRNAs (HSP26) were indicated. RNAs were stained by methylene blue. *HSP26* mRNAs were mRNAs known to be upregulated by nitrogen starvation (Onodera and Ohsumi, 2005) (B) Quantification of (A). The fold changes were calculated relative to the respective values in SD (wild-type). The RNA measurements were normalized to U4 snRNA or SnR50 snoRNA.(C) qRT-PCR analysis. RNAs were extracted as in (A) and the amount of RNAs was measured by qRT-PCR. The mean fold changes were calculated relative to the respective values of the wild-type in SD (left) or SD(-N) (right). *TAF10* mRNA has recently been shown to be a good reference gene (Teste *et al.*, 2009). The RNA measurements were made on three independent biological replicates and normalized to U4 snRNA or SnR189 snoRNA.