Supplementary information, Fig. S1. Psp2 is a positive regulator of Atg1 synthesis.

a The total protein level in yeast cells is downregulated after nitrogen starvation, and deletion of *PSP2* does not affect global translation. WT (SEY6210) and the *psp2Δ*

(YZY050) cells were grown to mid-log phase and then starved for nitrogen for 1 d. Cells were harvested before and after starvation. Protein extracts were analyzed with Coomassie Brilliant Blue staining. **b** Psp2 is also required for the efficient expression of Atg1 during amino acid starvation. WT (SEY6210) and the *psp2Δ* cells (YZY050) were grown to mid-log phase and then starved for amino acids for the indicated times. Protein extracts were analyzed by western blot and the Atg1 level was quantified as in Fig. 1a. **c** Psp2 is a positive regulator of Atg1 expression after autophagy induction. WT (SEY6210) and *psp2Δ* cells (YZY050) were grown in YPD medium until the mid-log phase and then treated with 100 nM rapamycin for the indicated times. Protein extracts were analyzed by western blot with anti-Atg1 and anti-Pgk1 (loading control) antisera. **d** WT (YZY205) and *ZEO1p-PSP2* (YZY225) cells that lack *PEP4* were grown in YPD medium until the mid-log phase and then starved for nitrogen for the indicated times. Protein extracts were analyzed by western blot with anti-Atg1 and anti-Pgk1 (loading control) antisera. **e** Change in translation (ΔTU) of *ATG1* or *PGK1* mRNAs (measured by bound ribosomes as in Fig. 1e) in *psp2Δ* cells relative to the WT. Data were from two biological replicates. Box plots represent the range; the midline indicates the mean. AU, arbitrary units. **f-g** Autophagy is reduced in *psp2Δ* cells during prolonged nitrogen starvation. WT (YZY286 [for f], YZY283 [for g]), *psp2Δ* (YZY287 [for f], YZY284 [for g]) and *atg1Δ* (YZY288 [for f], YZY285 [for g]) cells in which either Pgk1 or Fba1 was tagged with GFP were grown in YPD medium until mid-log phase and then starved for nitrogen for the indicated times. The ratio of free GFP to total GFP at each time point was quantified and normalized as in Fig. 1f. Mean ± SEM of n = 3 or 4 independent experiments are indicated. Student's t-test; *p<0.05, **p<0.01, ***p<0.001.