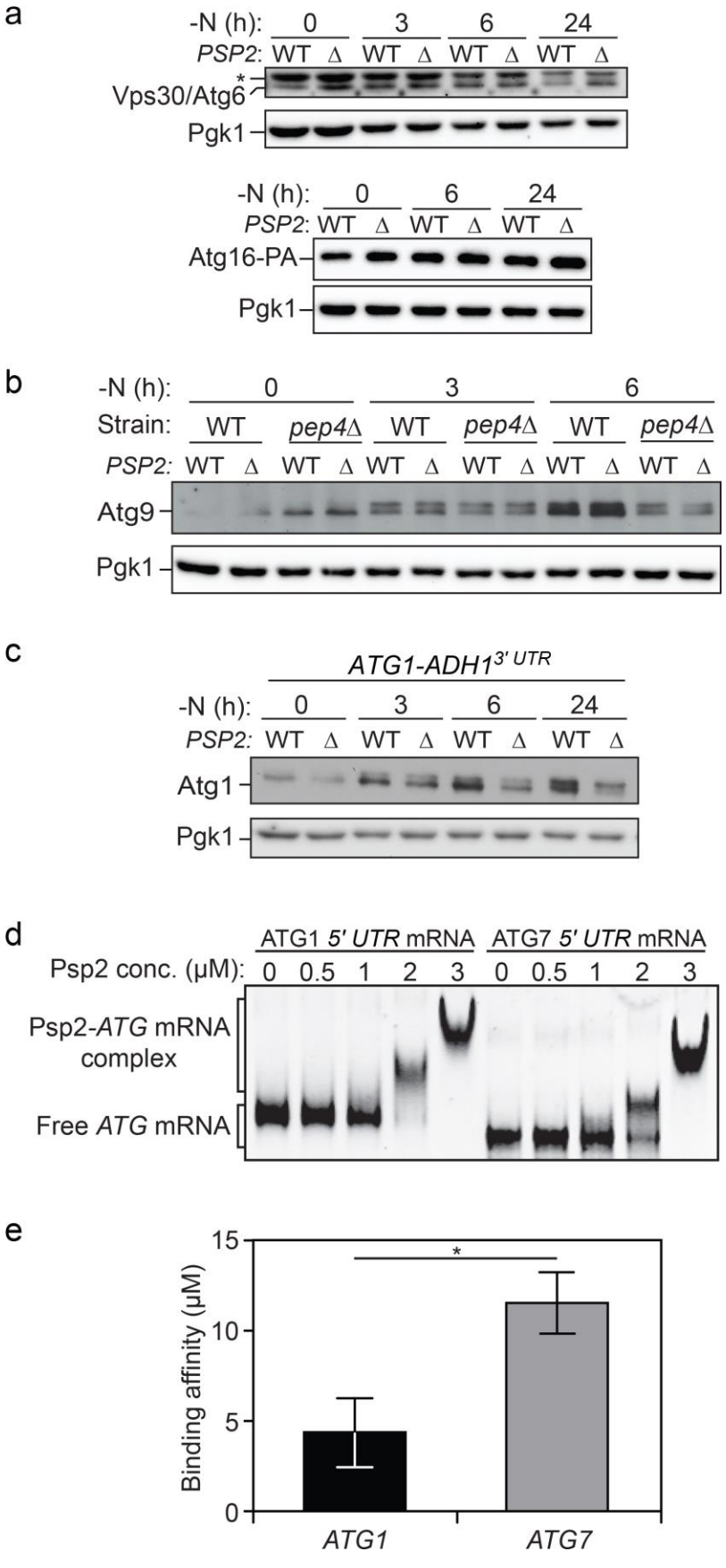
a
-N (h): 0 3 6 24
PSP2: WT Δ WT Δ WT Δ WT Δ
*
Vps30/Atg6
Pgk1
-N (h): 0 6 24
PSP2: WT Δ WT Δ WT Δ
Atg16-PA
Pgk1
b
-N (h): 0 3 6
Strain: WT pep4Δ WT pep4Δ WT pep4Δ
PSP2: WT Δ WT Δ WT Δ WT Δ WT Δ WT Δ
Atg9
Pgk1
c
ATG1-ADH1 3′ UTR
-N (h): 0 3 6 24
PSP2: WT Δ WT Δ WT Δ WT Δ
Atg1
Pgk1
d
ATG1 5′ UTR mRNA
ATG7 5′ UTR mRNA
Psp2 conc. (μM): 0 0.5 1 2 3 0 0.5 1 2 3
Psp2-ATG mRNA complex
Free ATG mRNA
e
Binding affinity (μM)
15
10
5
0
*
ATG1
ATG7

Supplementary information, Fig. S2. Psp2 binds *ATG1* mRNA to regulate its expression.

a Psp2 does not promote Vps30/Atg6 and Atg16 expression. WT (YZY241) and *psp2*Δ (YZY242) cells 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-Vps30/Atg6, anti-PA and anti-Pgk1 (loading control) antisera or antibodies. **b** Psp2 does not regulate Atg9 expression. WT and *psp2*Δ cells in both WT (SEY6210 and YZY050) and *pep4*Δ (TVY1 and YZY092) backgrounds 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-Atg9 and anti-Pgk1 (loading control) antisera. **c** The regulation of Atg1 translation by Psp2 is independent of the 3' UTR of *ATG1* mRNA. Atg1 protein levels were measured in WT (XLY324) and *psp2*Δ (XLY444) cells expressing *ATG1* with the *ADH1* 3' UTR under the indicated conditions by western blot. A representative image is shown. **d-e** Psp2 has stronger binding affinity to *ATG1* mRNA in comparison to *ATG7* mRNA. 500-bp constructs representing the 5' UTR transcripts of the *ATG1* or *ATG7* mRNAs were incubated with increasing concentrations of purified recombinant Psp2. A representative image is shown in (d). Titration gel-shift assay was performed to calculate the K_d of the binding, as shown in (e). Mean ± SEM of n = 3 independent experiments are indicated. Student's t-test; *$p<0.05$.