

Fig. S1. Sequence alignment of Asr1 and Atg35 proteins from Fig. 1C. Sequences were aligned in MultAlin. Residues identical in 3-4 and 5-7 sequences are colored blue and red, respectively. The RING-finger, PHD-finger and C-terminal nuclear localization signal are underlined by red, blue and black lines, respectively.

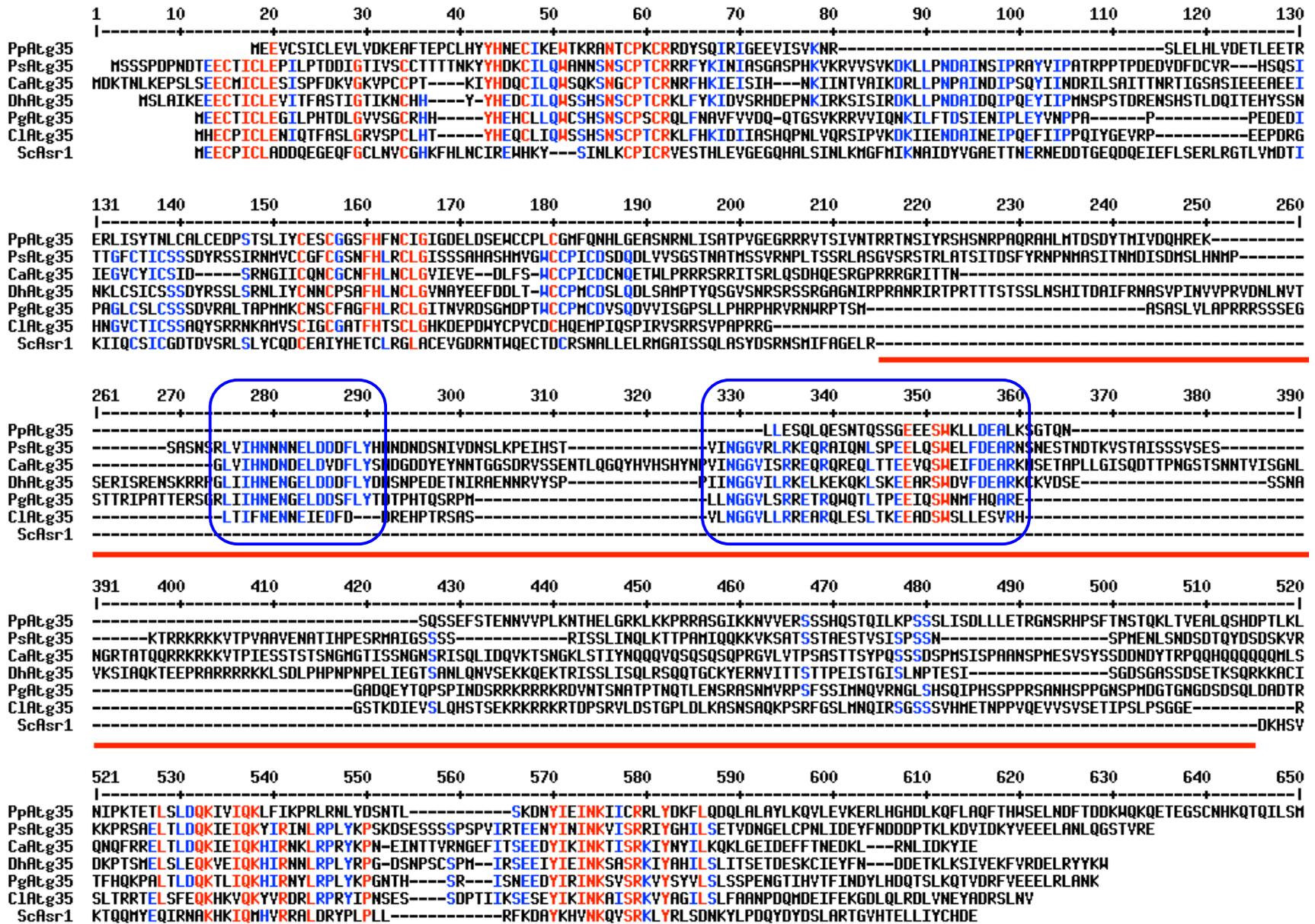


Fig. S2. Sequence alignment of Atg35's from *P. pastoris* (CAY67399), *P. stipitis* (PICST_60919), *C. albicans* (EEQ47214), *D. hansenii* (CAG88739), *P. guilliermondii* (PGUG_03993) and *C. lusitaniae* (CLUG_01611) with *S. cerevisiae* Asr1 (NP_015418). Sequences were aligned in MultAlin. Residues identical in 4-5 and 6-7 sequences are colored blue and red, respectively. The internal fragment of Atg35's absent in ScAsr1 is underlined by red. Two conserved motifs in the internal fragment of Atg35 are boxed in blue.

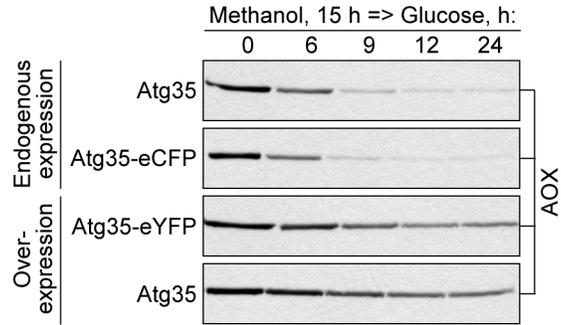


Fig. S3. Overexpression of Atg35, but not its C-terminal tag, inhibits micropexophagy. The WT cells with endogenous expression of Atg35 (PPY12h) and Atg35-eCFP (STN227) or with overexpression of Atg35-eYFP (STN274) and untagged Atg35 (SJCF1472) were grown overnight in SM and transferred to SD. At the indicated time-points, culture samples were collected and processed for immunoblotting for AOX.

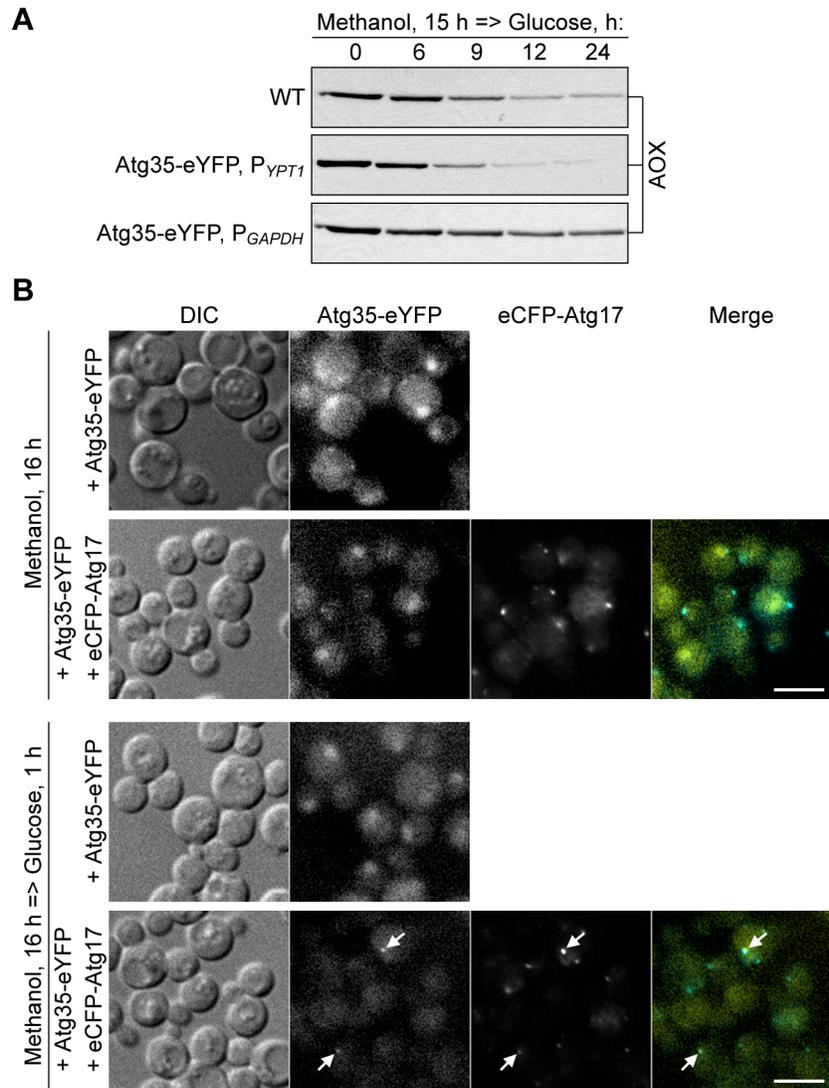


Fig. S4. Atg17 recruits moderately expressed Atg35 to the PNS during micropexophagy. (A) Moderate expression of Atg35-eYFP from the *YPT1* promoter does not inhibit micropexophagy. The WT (PPY12h) cells and WT cells with Atg35-eYFP expressed from moderate *YPT1* (SJCF1474) or strong *GAPDH* (STN274) promoter were grown overnight in SM and transferred to SD. At the indicated time-points, culture samples were collected and processed for immunoblotting for AOX. (B) Atg17 recruits Atg35 to the PNS during micropexophagy. The WT strains with moderate expression of Atg35-eYFP without (SJCF1474) or with eCFP-Atg17 (SJCF1475) overexpression were grown overnight in SM (two top panels) and transferred to SD (two bottom panels) for 1 h. Arrows point to the PNS. Bar, 5 μ m.