

Supplemental data

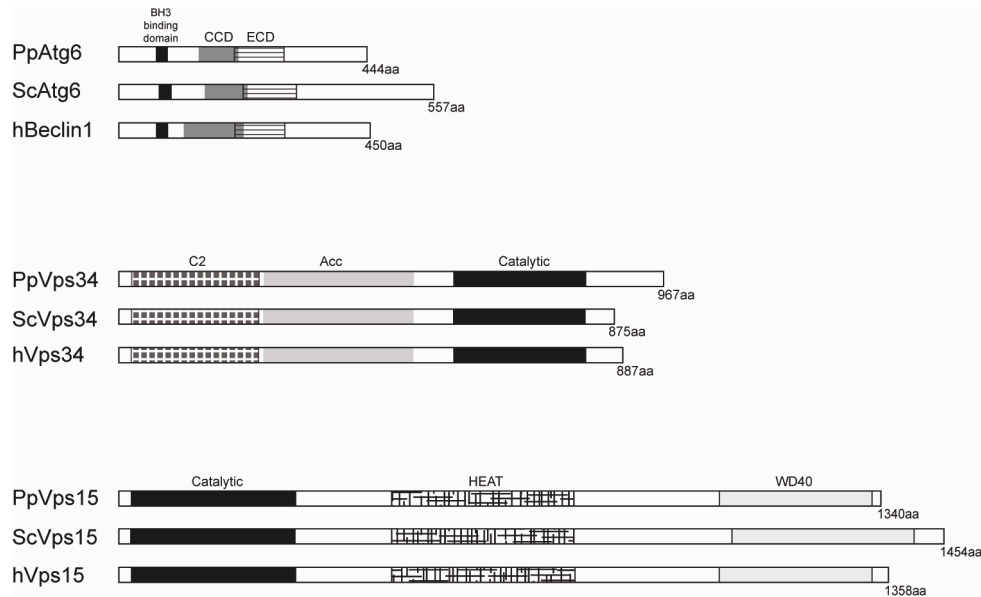


Figure S1. Domain comparisons of Atg6 and Beclin1, Vps34 and Vps15 homologs.

The core proteins of the PI3K complex I and II, Atg6, Vps15 and Vps34 are highly conserved between species, as are most of the domains of these proteins. Like hBeclin 1, PpAtg6 has a central CCD, an evolutionarily conserved domain (ECD), required for interaction with hVps34 in mammals,¹ and a BH3 domain, required in hBeclin 1 for Bcl-2 binding.^{2, 3} We have not identified an ortholog of Bcl-2 in *P. pastoris*. The PpVPS34 gene encodes a 967-amino acid protein (113 kDa) and it shares the Class III PI3K domain structure, with an N-terminal C2 (C2), a helical (Acc), and a C-terminal kinase (catalytic) domain. In mammals, the catalytic domain is responsible for phosphorylation of PtdIns to generate PtdIns3P, and the C2 domain is likely involved in interactions with phospholipids.⁴ The Acc domain has a regulatory function in the class I PI3Ks but its role in Vps34 is unclear. PpVps15 is an extremely large protein (152 kDa) with several distinct domains. These include N-terminal serine/threonine kinase (catalytic) and C-

terminal WD40-repeat (WD40) containing domains, as well as an internal domain (HEAT) that bridges the catalytic and WD40 domains. The precise role of the kinase domain, including its substrates, is unknown. The HEAT and the WD40 domains are important for protein-protein interactions.

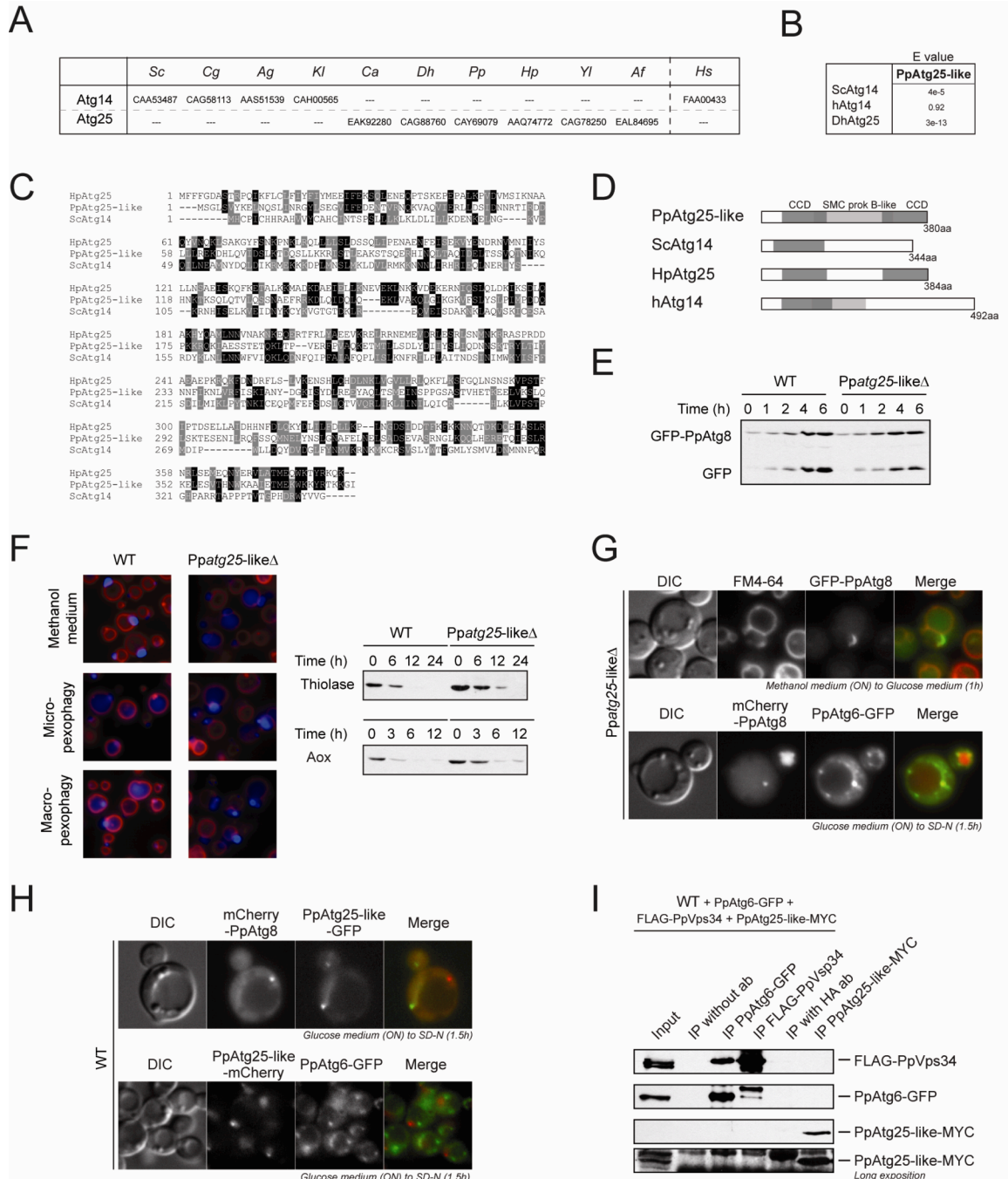



Figure S2: PpAtg25-like protein study. (A) Orthologs of Atg14 and Atg25 in different species adapted from a previous publication.⁵ (B) Homology data expressed as E value and obtained using bl2seq from NCBI. (C) Amino acid alignment of *H. polymorpha* Atg25, *P. pastoris* Atg25-like and *S. cerevisiae* Atg14 proteins. The alignment was generated using CLUSTAL W. Identical residues are in black boxes and similar ones are in grey boxes. (D) Domain comparison of Atg25 and Atg14 proteins. (E) Detection of GFP-PpAtg8 cleavage as an indicator of autophagy. (F) Pexophagy is partially affected in Ppatg25-like Δ cells. Pexophagy was followed by detection of degradation of BFP-SKL by fluorescence microscopy, and thiolase and alcohol oxidase (AOX) degradation by immunoblot. (G)  PPA was normally formed during micropexophagy conditions in Ppatg25-like Δ cells (upper panel), and PpAtg6-GFP and mCherry-PpAtg8 localization was not affected by the absence of the PpAtg25-like protein (lower panel). (H) PpAtg25-like-GFP did not colocalize with mCherry-PpAtg8 or PpAtg6-GFP. (I) PpAtg25 did not interact with PpAtg6 or PpVps34.

Video 1: GFP-PpAtg8 during pexophagy of methanol-grown peroxisomes induced by nitrogen starvation. Cells were grown in methanol medium for 15 h and pexophagy was induced by shift to nitrogen starvation medium for 1 h before the initiation of the time-lapse microscopy experiment. Images were acquired at 2 min intervals and the playback rate is 3 frames per second.

Video 2 and 3: Triple imaging of mCherry-PpAtg8 (red), PpAtg6-GFP (green), and BFP-SKL (blue) during pexophagosome formation. Cells were grown in methanol medium for 15 h and pexophagy was induced by shift to nitrogen starvation medium for 1 h before the initiation of the time-lapse microscopy experiment. Images were acquired at 2 min intervals and the playback rate is 3 frames per second. Also see Fig. 7B.

References

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