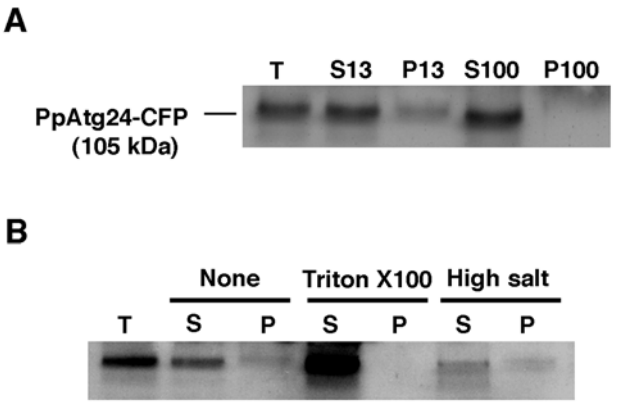

Suppl. Figure 1. Ano et al.

Supplemental Figure 1. Association of PpAtg24 with intracellular membrane.

Methanol-grown *Ppatg24*Δ cells expressing CFP-tagged PpAtg24 fusion protein (strain YAP2404) were spheroplasted, and fractionated as described in the text.

(A) Subcellular fractionation. T, total cell lysate; S13, supernatant fraction after centrifugation at 13,000 *g*; P13, pellet fraction after centrifugation at 13,000 *g*; S100, supernatant fraction after high-speed centrifugation at 100,000 *g*; and P100, pellet fraction after high-speed centrifugation at 100,000 *g*. The fractionated samples were subjected to immunoblot analysis using anti-GFP antiserum. (B) Membrane association of PpAtg24-CFP. Total cell lysate was separated as described above and resuspended in Buffer A containing 1% Triton X-100 or a 1.0 M salt wash (0.67 M KOAc, 0.3 M KCl). After incubation of the sample at 25˚C for 10 min, the treated lysates were separated into T, total membrane; P, pellet fraction after centrifugation at 100,000 *g*, and S, supernatant fraction after centrifugation at 100,000 *g*. The samples were subjected to immunoblot analysis using anti-GFP antiserum.