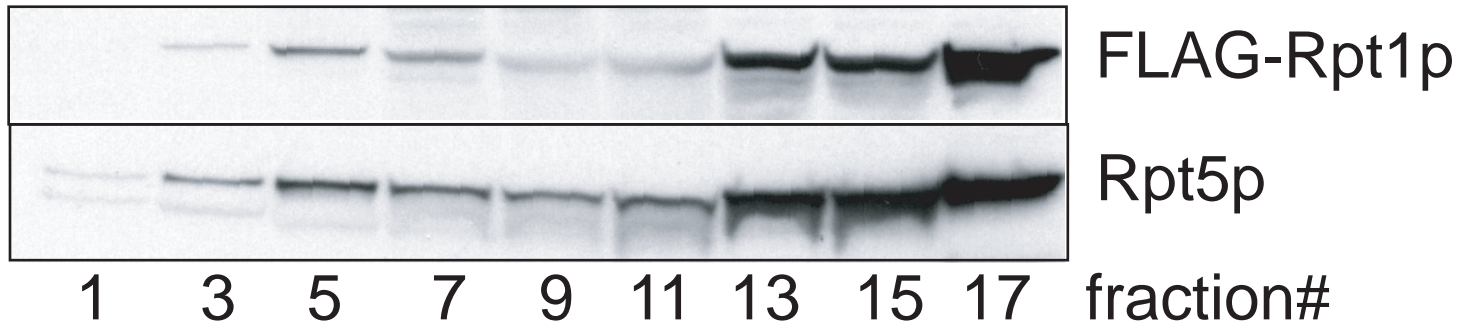
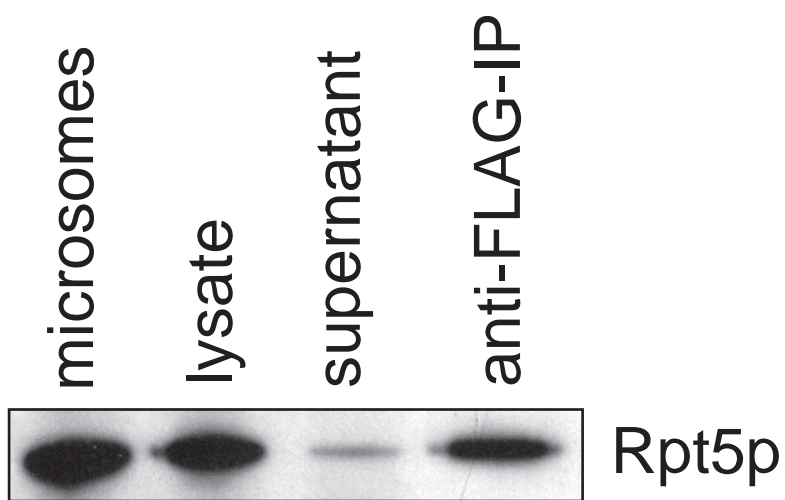


A



B



Supp. Figure 3 Epitope-tagged proteasome subunits are quantitatively assembled into proteasomes

(A) The FLAG-RPT1 strain was grown to OD₆₀₀=2.5, and cells harvested and lysed by liquid nitrogen lysis. The lysate was cleared by centrifugation at 36,000 g for 20 min and applied to a 10-40% glycerol gradient in 50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 5 mM MgCl₂, 1 mM EDTA, 1 mM PMSF, and protease-inhibitors (Roche). Proteasomes were sedimented for 16h at 4°C in a SW40 rotor at 40,000 rpm. Fractions were collected from the top and proteasome subunits detected by immunoblotting.

(B) Microsomes were prepared from the FLAG-RPT1 strain, membranes solubilized as described in Figure 3, and FLAG-Rpt1p and associated proteins precipitated with anti-FLAG-agarose (Sigma). Proteins were eluted from the beads with FLAG-peptide and proteins in each fraction analyzed by immunoblotting.