collected in the flow through, concentrated, and purified to homogeneity by gel filtration chromatography (SD200, Amersham, in 50 mM NaCl, 25 mM Tris pH 7.4, 1mM DTT). Yields were typically 25 mg/l culture.

Gel Filtration Chromatography

The gel filtration chromatography experiments shown in Fig. 5, Fig. 6, and Supplemental Fig. 3 were performed on 150 µM Vps4 proteins using a Superdex 200 column in 100 mM NaCl, 25 mM Tris–HCl (pH 7.5), and 1 mM DTT in the presence or absence of ATP. Both columns were calibrated using molecular weight standards (Biorad).

Analytical Ultracentrifugation

Equilibrium sedimentation experiments on full length $Vps4_{E233Q}$, $Vps4_{\Delta MIT,E233Q}$ and $Vps4_{\Delta MIT,E233Q}$ with L151D and Q216A interface mutations, were performed on an XL-A analytical ultracentrifuge (Beckman Coulter). Protein concentrations ranged from 5-30 µm (110-µl) and the corresponding buffer blanks (120 µl; 50 mM NaCl, 25 mM Tris pH 7.5) were run in parallel in sample cells fitted with six-channel equilibrium centerpieces. All data were collected at 4℃ and at two rotor speeds for each protein: Vps4 at 12,000 and 16,000 rpm, for $Vps4_{\Delta MIT}$ at 14,000 and 18,000 rpm, and for $Vps4_{\Delta MIT}$ interface mutants L151D and Q216A at 16,000 and 20,000 rpm. The attainment of equilibrium after 24 h was confirmed by comparing repetitive absorption scans. Theoretical molecular weights, partial specific volumes (v_{bar}), and molar extinction coefficients at 280 nm of the Vps4 variants were calculated using the