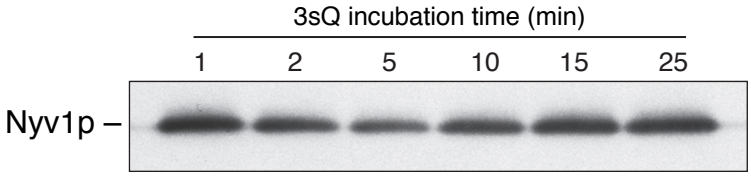
Supplementary Figure S1



Supplementary Figure S1. Kinetics of 3sQ binding to Nyv1p on the vacuole.

Standard fusion reactions were initiated by incubating at 27°C with 3sQ (2 μM; GST-sVti1p, his_6-sVam3p, Vam7p). A portion of reactions was removed at the indicated times (1, 2, 5, 10, 15, or 25 min) and immediately subjected to centrifugation (13,800g, 3 min, 4°C) to remove any unbound 3sQ. Sedimented vacuoles were resuspended in ice-cold solubilization buffer (20 mM HEPES/KOH, pH 7.4, 100 mM NaCl, 2 mM EDTA, 0.5% Triton X-100, 20% glycerol, 1x protease inhibitor cocktail (0.46 μg/ml leupeptin, 3.5 μg/ml pepstatin, 2.4 μg/ml pefabloc-SC, 1 mM PMSF) and incubated at 4°C with rocking for 20 min. Detergent insoluble material was removed by centrifugation (TLA120.2, 52,000 r.p.m., 20 min, 4°C). Clarified detergent extracts were incubated on a nutator for 3 hrs at 4°C with Glutathione Sepharose beads (Amersham). Beads were collected by brief centrifugation (3,000g, 2 min, 4°C) and washed 5 times with icecold solubilization buffer. Under this condition, almost all of the Nyv1p was cleared from the detergent extracts (data not shown). Bound proteins were eluted in reducing SDS sample buffer (95°C, 3 min) for SDS-PAGE followed by immunoblotting.