Supplemental information (Langemeyer et al.)

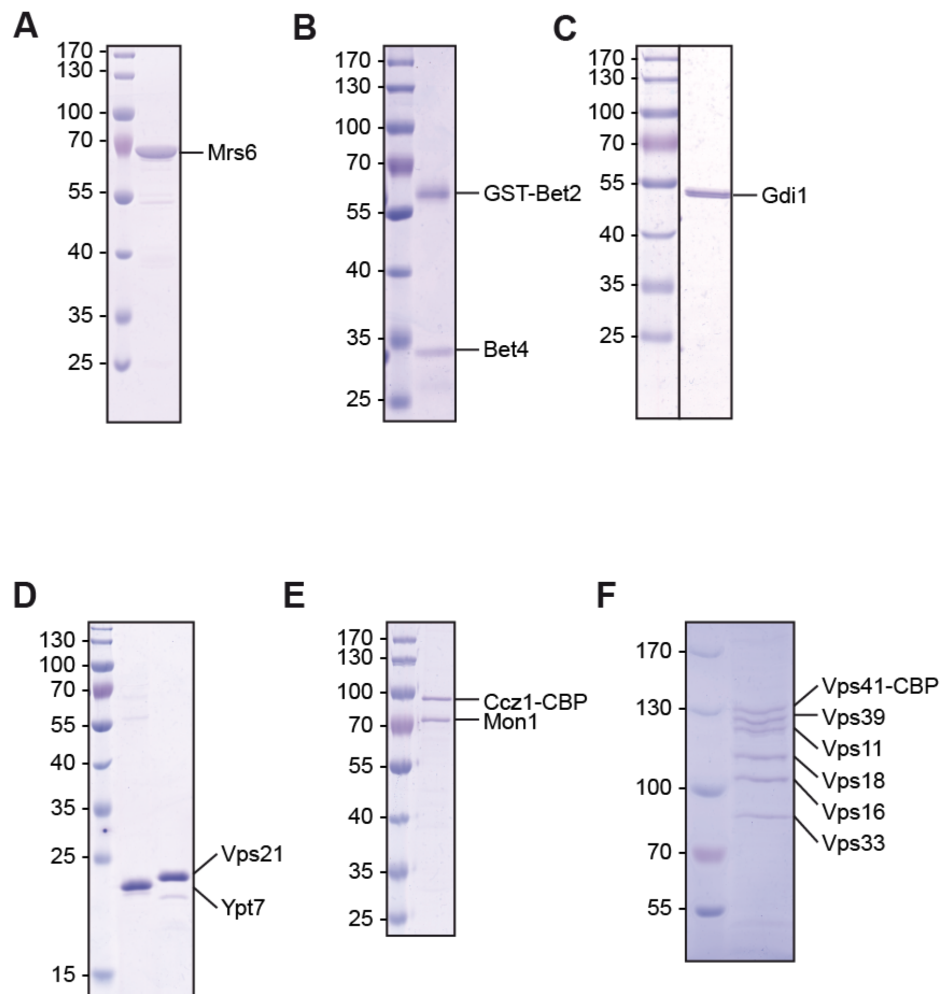

Figure S1. **Representative examples for purified proteins used in this study.** Expressed and purified (A) Rab Escort Protein Mrs6, (B) Geranylgeranyltransferase (GGTase) consisting of subunits Bet2 and Bet4, (C) GDP dissociation inhibitor GDI, (D) Rab-GTPases Ypt7 and Vps21, (E) Guanine Nucleotide Exchange factor (GEF) Mon1-Ccz1, (F) Tethering complex HOPS. For each gel, 1 µg of protein as used for assays was analyzed by SDS-PAGE and Coomassie staining.

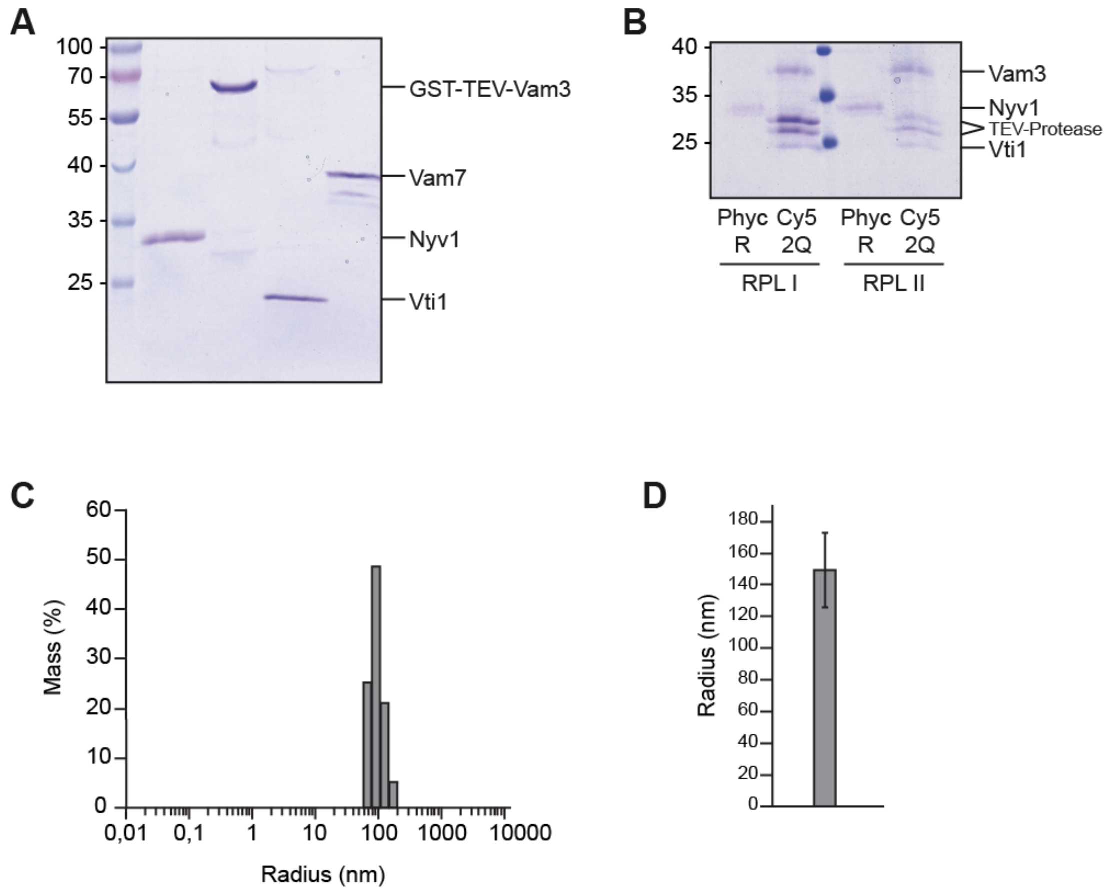

Figure S2. **Characterization of reconstituted proteoliposomes (RPL).** *A.* Purified SNAREs. 0.5 µg of the indicated purified vacuolar SNAREs were analyzed by SDS-PAGE. *B.* Isolated proteoliposomes. Two representative sets, each consisting of liposomes containing either the R-SNARE Nyv1 and the reporter Biotin-R-Phycoerythrin or the two Q-SNAREs Vam3/Vti1 and the reporter Cy5-Streptavidin, of RPLs used for fusion assays were analyzed by SDS-PAGE. The amount loaded on the gel is the equivalent needed for 20 fusion reactions. TEV-protease was added to RPL-preparation containing Vam3 to cleave of the GST-tag during dialysis of RPLs. *C.* Representative Dynamic Light Scattering measurement to estimate the size of RPLs used for fusion assays. A 1:200 dilution of a 2 mM RPL suspension was measured using a DynaPro (Wyatt Technology Corporation) Laser Light Scattering instrument. Settings used: Acquisition time 10 sec, Number of Acquistions 40, Laser Power 10%, Temperature 25°C. *D.* Average radius measured as described in (C) over six independent RPL preparations.