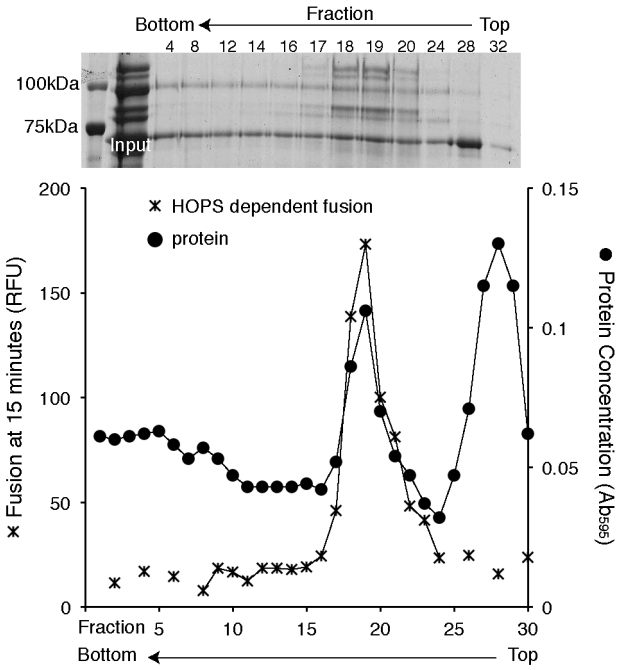
Supplemental Materials

Molecular Biology of the Cell

Orr et al.



SUPPLEMENTAL FIGURE S1: Velocity gradient purification of HOPS. Affinity-isolated HOPS was gradient purified as described in Materials and Methods. Aliquots of select fractions from the sedimentation gradient were boiled in Sample Buffer and analyzed for HOPS subunits by SDS-PAGE. Fractions 2 through 30 were analyzed for protein concentration by Bradford (closed circles) and for fusion activity via lumenal compartment mixing (stars). RPLs of vacuolar mimic lipid composition, bearing Ypt7 and either 1R or 3Q and containing either biotinylated R-phycoerythrin or Cy5 labeled streptavidin in their lumens were prepared as described (Zick *et al.*, 2014) with a 1:2000 protein to lipid ratio of Ypt7, and a 1:2600 protein to lipid ratio of each of the 4 SNAREs. Fusion was assayed in Rb150 as described (Zick *et al.*, 2014), with each fusion reaction containing 1mM lipid, 14.5μM external streptavidin, 28.8μM GTP, 3mM EDTA, 1.5mM ATP, 1.6mM $MgCl_2$, 177nM Sec17, 1.3μM Sec18 and 2μl of each fraction from the HOPS gradient, in a total of 10μl. RFUs (Raw Fluorescence Units) at t=15min are shown here.

SUPPLEMENTAL TABLE S1: Kinetics of the association of SNAREs and HOPS with GST-tagged Qc. Immunobot images from Figure 6 were analyzed using UN-SCAN-IT Software (Silk Scientific, Orem, UT). Data represents the average of 3 independent experiments.

		complete			30 min time points for controls				
		5min	30min	90min	detergent	no HOPS	no sQa	no sQb	w.t. Qc
pMols	Vps16	0.06	0.08	0.10	0.04	0.02	0.07	0.06	0.02
	R	0.53	0.82	0.85	0.08	0.11	0.07	0.07	0.08
	sQa	0.38	0.56	0.52	0.05	0.14	0.03	0.11	0.04
	sQb	0.25	0.48	0.57	0.02	0.07	0.01	0.01	0.05

REFERENCES

Zick M, Wickner W (2014). A distinct tethering step is vital for vacuole membrane fusion. Elife 3, e03251.