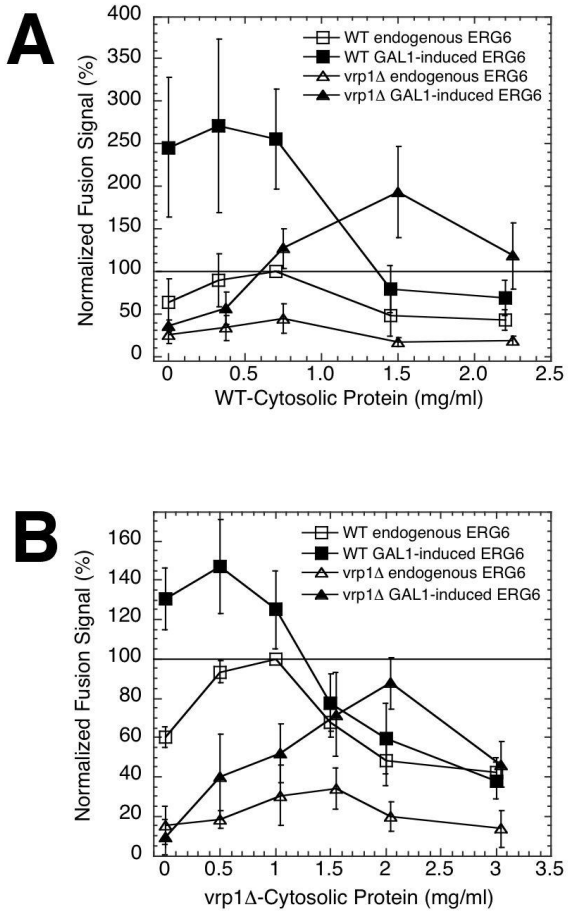
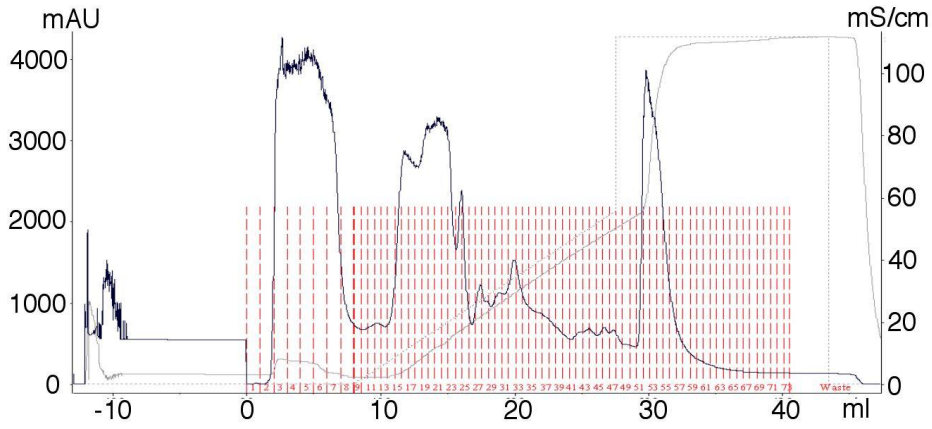
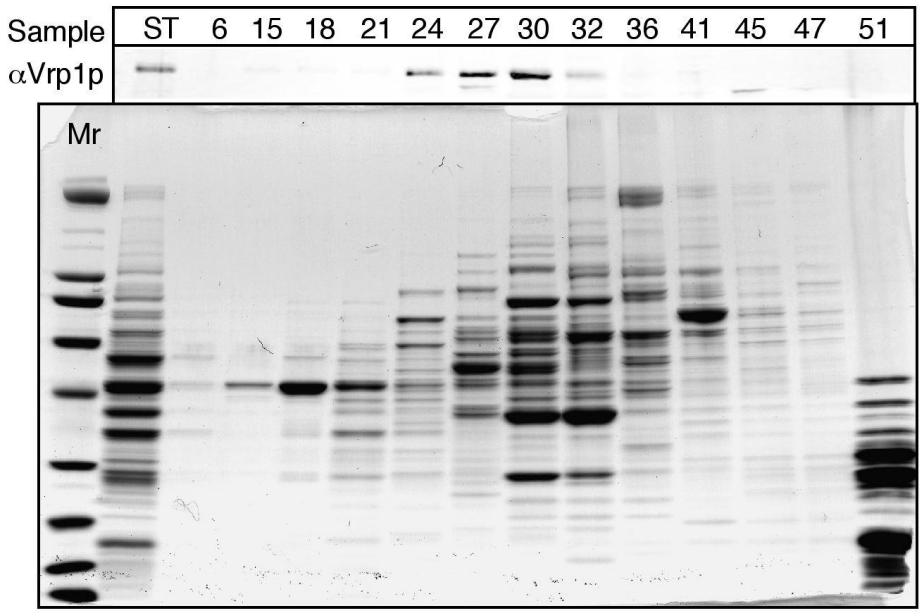


Figure S1 Tedrick et al. 2004

A



B



C

Sample	Protein (mg/ml)	Band Intensity	Volume Analyzed	Relative Purity
STart cytosol	14.9	4.48	0.5 µl	0.60
Fraction 24	0.19	3.47	5 µl	3.65
Fraction **27**	0.26	6.20	5 µl	4.77
Fraction 30	0.71	11.08	5 µl	3.12
Fraction 32	1.08	2.12	5 µl	0.39

Figure S2 Tedrick et al. 2004

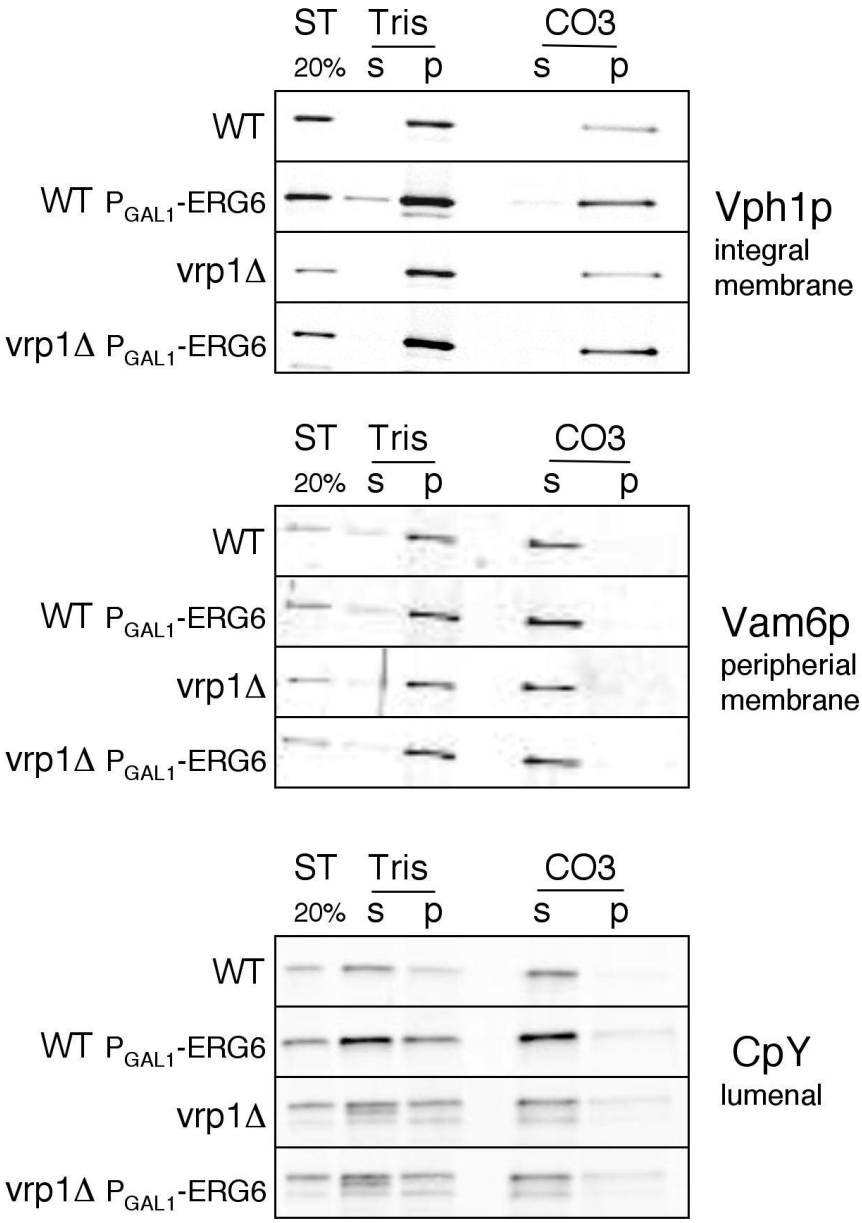


Figure S3. Tedrick et al. 2004

Supplemental Figure Legends

Figure S1. Titration of cytosol stimulation in vacuole fusion reactions. (A) Wild-type cytosol was titrated into fusion reactions using vacuole pairs isolated from strains KTY1 and 2 (*WT endogenous ERG6*) or KTY5 and 6 (*vrp1Δ endogenous ERG6*), and with P_{GAL1}-*ERG6* induction from strains KTY3 and 4 (*WT GAL1-induced ERG6*) or KTY7 and 8 (*vrp1Δ GAL1-induced ERG6*). (B) *vrp1Δ* cytosol was titrated into fusion reactions using vacuole pairs as in part (A). Note that significantly higher concentrations of both wild-type and *vrp1Δ* cytosol is consistently needed to maximally stimulate fusion of vacuoles from *vrp1Δ GAL1-induced ERG6*.

Figure S2. Partial purification of Vrp1p. 25 mg of wild-type cytosol from strain BY4742 was applied to a 1 ml MonoQ column in 5 ml of Q-bind buffer (25 mM Tris-Cl pH 8.5, 5 mM $MgCl_2$, 10 mM KCl, 1 mM DTT). The column was washed with 2 ml Q-bind, eluted with a 20 ml 10 – 500 mM KCl linear salt gradient, and step eluted with 10 ml 1 M KCl. The column was run at 0.5 ml/min, 4°C and 0.5 ml fractions were collected. (A) Elution profile showing protein as OD_{280} (*mAU*, blue), fractions (vertical dashed lines) and conductivity representing salt concentration (*mS/cm*, calculated, dashed line; observed, solid line). (B) Immunoblot showing the position of Vrp1p elution (top panel) and silver stained gel (bottom panel) of selected fractions . ST, 10% starting cytosol equivalent. (C) Purification table of fractions with Vrp1p using immunoblot band intensity divided by amount of protein analyzed to determine relative purity. Vrp1p

elutes from 180 – 240 mM KCl at pH 8.5. Fraction 27 showed the highest relative purity (8-fold overall) and was therefore used for further studies.

Figure S3. Determination of membrane extraction efficiency. 100 μg of total vacuolar protein isolated from the indicated strains was precipitated by centrifugation and resuspended in ice cold 50 mM Tris-Cl pH 8.5 (*Tris*) or 100 mM Na_2CO_3 pH 11.5 (*CO3*). After 30 min incubation on ice samples were centrifuged at 100,000 *g*, 4°C for 30 min to pellet membranes and associated proteins. Soluble and pellet fractions were carefully separated and protein determination was carried out. Equal portion of supernatant (*s*) and pellet (*p*) fractions and a sample representing 20% of the starting material (*ST*) was separated by SDS-PAGE and immunobloted for V-ATPase subunit (*Vph1p*, integral membrane), Vam6p (peripheral membrane) and carboxypeptidase Y (*CpY*, lumenal).