Supporting Information

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SI Materials and Methods

Yeast Strains. Yeast strains BY4742 (MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0), BJ3505 [MATa ura3-52 trp1-Δ101 his3-Δ200 lys2-801 Gal2 (gal3) can1 prb1-Δ1.6R pep4::HIS3] (1), DKY6281 (MATa ura3-52 leu2-3,112 trp1-Δ901 his3-Δ200 lys2-801 suc2-Δ9 pho8::TRP1) (2), BJ3505-Fos-ω, and BJ3505-α-Jun (3) were used for vacuole production. pRS4xx-Gal1-VOPQ was constructed as described previously (4, 5). For GFP-localization studies in yeast, VopQ was cloned into the BamHI and KpnI sites of the pDGFP vector (6).

Reagent Preparation. Purified recombinant yeast proteins Pbi2p (7), Gyp1-46 (8), Gdi1p (9), and Sec18p (10) were isolated as previously described. Inhibitory antibodies against Vam3p and Ypt7p were purified from serum as previously described (8) and were used routinely in fusion assays at 450 nM and 467 nM, respectively. Antisera against Nyv1p, Vam3p, and Vps33p were generous gifts from William Wickner (Geisel School of Medicine at Dartmouth College, Hanover, NH); antiserum against Vma6p was a gift from Christian Ungermann (University of Osnabrück, Osnabrück, Germany). Nitrocefin was a generous gift from Shahriar Mobashery (University of Notre Dame, South Bend, IN). All other chemicals were purchased from Sigma-Aldrich. Monoclonal antiserum against yeast Vph1p (clone 10D7A7B2) was purchased from Life Technologies.

MBP-VopQ was purified using a standard nickel-affinity purification protocol, as previously described (11), buffer exchanged into storage buffer [10 mM Tris·HCl (pH 8.0), 50 mM NaCl], and stored at −80 °C. MBP-VopQ^{S200P} was purified in the same manner.

Vacuole Isolation and in Vitro ALP Fusion Assay. Vacuoles were freshly prepared from BJ3505 (*pep4*Δ) and DKY6281 (*pho8*Δ) yeast strains on discontinuous Ficoll gradients, as previously reported (12). Standard in vitro vacuole fusion assays (30 μL final volume, 27 °C, 90 min) contained 3 μg each of BJ3505 and DKY6281 vacuoles (6 μg total), fusion reaction buffer [20 mM piperizine-*N*,*N*′-bis(2-ethanesulfonic acid) (Pipes)-KOH (pH 6.8), 200 mM sorbitol, 10 μM CoA, 125 mM KCl, 5 mM $MgCl_2$, 815 nM Pbi2p (I_2^B)], and the ATP regenerating system (1 mM ATP, 1 mg/mL creatine kinase, and 29 mM creatine phosphate). As a measure of vacuole fusion, reactions were assayed for Pho8p ALP activity as described (12), except that $CaCl_2$ was omitted from the development solution. Units of fusion are reported as nmol *p*-nitrophenylate formed·min^{-1}·μg *pep4*Δ vacuole^{-1}.

Yeast Vacuolar Staining. BY4742 yeast strains harboring the galactose-inducible pRS413-Gal1 (wild type), pRS413-Gal1-*VOPQ* (*VopQ*$^+$), or deletions in *VPS33* (*vps33*Δ) were grown overnight in Complete Supplement Mixture (CSM, Sunrise Science Products)-histidine medium supplemented with 2% glucose. Saturated cultures were subcultured to fresh 5-mL CSM-histidine medium supplemented with either 2% glucose or 2% galactose and were grown for 16 h at 30 °C with shaking. Cells in midlogarithmic phase were suspended in yeast extract/peptone/dextrose with 8 μM FM4-64 and were incubated at 30 °C for 30 min (13). After incubation, cells were pelleted, suspended in minimal medium, and shaken for an additional 30 min. Cells then were harvested and visualized using fluorescence microscopy.

β-Lactamase Vacuole Fusion Assay. Assays of homotypic vacuole fusion via the fusion-dependent reconstitution of β-lactamase enzyme were performed as previously described (3). Briefly, vacuoles were prepared from the BJ3505-Fos-ω and BJ3505-α-Jun strains, and 6 μg of each vacuole type was incubated under standard fusion conditions (described above) in a 60-μL volume, except that Pbi2p was replaced with 4.3 μM recombinant GST-Fos protein to quench fusion-independent reconstitution of β-lactamase. After 90 min at 27 °C, tubes were removed to ice for 5 min, 140 μL developer solution [0.1 M sodium phosphate (pH 7.0), 150 μM nitrocefin, and 0.2% Triton X-100] was added to each tube, and a 150-μL sample was removed to a clear 96-well plate. A blank well for reference contained 6 μg each vacuole type, 4.3 μM GST-Fos, and 140 μL developer solution in a final volume of 200 μL. β-Lactamase–dependent hydrolysis of nitrocefin was measured via change in absorbance (λ = 492 nm) over 10 min (30-s intervals) in a Synergy MX plate-reading spectrophotometer (Bio-Tek). Rates of hydrolysis were calculated over the interval, and the 100% fusion rate was defined as the rate in the no-inhibitor condition.

Tandem Content- and Lipid-Mixing Assay. Vacuoles (800 μg) purified from BJ3505-Fos-ω were incubated with 200 μM Rhodamine-DHPE (Rh-PE, Life Technologies), as in ref. 3. After labeled vacuolar membranes were harvested, 300-μL (10×) standard β-lactamase reactions were performed with 30 μg of vacuoles isolated from BJ3505-α-Jun, 23.3 μg of vacuoles isolated from BJ3505-Fos-ω, and 6.7 μg of Rh-PE–labeled vacuoles and containing either 200 nM MBP-VopQ or 200 nM MBP control protein. For the content-mixing assay, 60 μL of the master reaction was transferred to individual tubes, and the β-lactamase content-mixing fusion assay was performed. For lipid mixing, 180 μL of the master reaction was transferred to a 96-well black plate, and Rh-PE fluorescence was followed at 150-s intervals (with 10-s shaking between readings) for 90 min at 27 °C (λ_{ex} = 544; λ_{em} = 590). Maximal dequenching (100% fusion) was set by the addition of 0.33% (vol/vol) Triton X-100 to each well and then averaging the final 10 fluorescence reads taken at 1-min intervals over 10 min.

Random Mutagenesis of VopQ. To identify VopQ mutant derivatives that are no longer toxic to yeast, pRS416-Gal1-VOPQ was transformed into the highly mutagenic *E. coli* strain XL1-Red (Stratagene); 100 μL of the transformation mixture was plated to LB + 100 μL/mL ampicillin and incubated at 37 °C for 36 h. All colonies (>200) were scraped into 10 mL fresh LB + 100 μg/mL ampicillin and were incubated at 37 °C with shaking for 16 h; then plasmid was isolated.

Mutagenized plasmid was transformed into *S. cerevisiae* BY4742, selecting for growth on CSM medium lacking uracil and supplemented with 2% galactose. Colonies appearing under these conditions were likely defective in VopQ activity in vivo. Thirty independent colonies were isolated in this manner, and plasmid phenotypes were confirmed via introduction into a fresh BY4742 background. Of the original 30 plasmids, 11 continued to display a defect in VopQ activity in vivo, and the *VOPQ* locus from these vectors was sequenced (Georgia Genomics Facility, University of Georgia).

Measurement of Vacuolar Proton Translocation. Acidification of the vacuolar lumen by V-ATPase–dependent proton translocation was measured by the change in absorbance of acridine orange upon protonation, as previously described (14), with modifications. Vacuole fusion reactions (6×, 180 μL final volume) containing 6 μg of vacuoles (3 μg each of *pep4*Δ and *pho8*Δ per 180-μL reaction), standard fusion buffer, 815 nM Pbi2p, 15 μM acridine orange, and indicated reagents were preincubated for 5 min in

a SynergyMX microplate reader (BioTek) prewarmed to 27 °C, in a 96-well clear plate (Corning). Acridine orange absorbance (490 nm and 540 nm) was measured every minute with path length correction enabled. Acidification was initiated with the addition of 2 mM ATP, and absorbance measurements were followed every 10 s for 20 min. Absorbance is plotted as ($A_{490}-A_{540}$); the loss of absorbance indicates protonation of acridine orange and acidification of the vacuole lumen.

***Trans*-SNARE Assay.** Vacuoles from BJ3505 *nyv1Δ CBP-VAM3* and DKY6281 were used to detect *trans*-SNARE complex formation during fusion as described in ref. 15. Interactions between CBP-Vam3p Q-SNARE and Nyv1p R-SNARE are indicative of a proper *trans*-SNARE complex assembly. Reactions contained no inhibitor, were left on ice, or contained 1 μM Gdi1p and 1 μM Gyp1-46 or 200 nM rVopQ. After 45 min, reactions were placed on ice for 5 min, and a 30-μL aliquot was removed to measure fusion via Pho8p activity. Remaining vacuoles were precipitated (7,600 × *g*, 5 min, 4 °C) and solubilized, and calmodulin-affinity pulldown was performed. Vps33p, Vam3p, and Nyv1p were detected using immunoblotting.

Vacuole-Docking Assay. Docking reactions were carried out as previously described (16). Briefly, 6 μg of vacuoles purified from BY4742 was incubated for 30 min at 27 °C in 30 μL of docking buffer [20 mM Pipes-KOH (pH 6.8), 200 mM sorbitol, 100 mM KCl, 0.5 mM $MgCl_2$] and 0.3× ATP regenerating system (0.3 mM ATP, 9.7 mM creatine phosphate, and 0.3 mg/mL creatine kinase) containing 815 nM Pbi2p, 20 μM CoA, and 8 nM Sec18p. After incubation, vacuoles were placed on ice, mixed with FM4-64 (3 μM final), and vortexed (3 s, medium speed) into an equal volume of molten 0.6% agarose. A 15-μL aliquot of this mixture was mounted on glass slides, allowed to solidify, and visualized via epifluorescent microscopy.

GFP-Release (Lysis) Assay. Lysis of vacuoles was assayed by measuring the release of a luminal GFP protein after fusion, as previously described (9). Briefly, fluorescent vacuoles were isolated from yeast strain VSY39, a protease-deficient BJ3505 strain constitutively expressing GFP targeted to the vacuolar lumen via fusion to an N-terminal signal sequence from the *Saccharomyces carlsbergensis* α-galactosidase, *MEL1* (17). These vacuoles were premixed in a 1:1 ratio of fluorescent *pep4Δ*/non-fluorescent *pho8Δ* before the addition of other reaction components. Standard vacuole fusion reactions were used for the GFP-release assay, with the following modifications: Reactions contained 0.1× protease inhibitor mixture (50× stock: 13 μg/mL leupeptin, 25 μg/mL pepstatin A, and 5 mM Pefabloc SC) to stabilize GFP after vacuolar release. Each reaction was performed on a 3× scale (90 μL) and was fused for 60 min at 27 °C. Fusion detection and vacuolar pellet/supernatant separation were performed as described (9, 18); the GFP signal in each 20-μL vacuole membrane pellet or supernatant sample was measured in a BioTek SynergyMX plate reader (Bio-Tek) (λ_{ex} = 462 nm; λ_{em} = 510 nm, read height = 8.00 mm, gain = 100). The amount of GFP released (lysis) was calculated as relative fluorescent units (RFU) supernatant/(RFU supernatant + RFU pellet) × 100%.

Oxonol V Membrane Potential Assay. For each condition, 2.5 μg of total purified vacuoles from BJ3505 and DKY6281 (1.25 μg of vacuoles each type) were incubated in 180-μL reactions in 20 mM PIPES, 200 mM sorbitol (PS) buffer containing 125 mM KCl, 5 mM $MgCl_2$, 10 μM CoA, 815 nM Pbi2p, and 0.5 μM bis-(3-phenyl-5-oxoisoxazol-4-yl)pentamethine oxonol (Life Technologies). Where indicated, 0.5 μM bafilomycin, 1 μM nigericin, 1 μM valinomycin, 500 nM MBP, or 500 nM MBP-VopQ was added at $t = 0$. Reactions were incubated in a Synergy MX plate reader (Bio-Tek), and fluorescence was measured every 10 s for 5 min at 27 °C (λ_{ex} = 580 nm; λ_{em} = 620 nm). After 5 min, ATP was added to a concentration of 2 mM, and reaction fluorescence was measured every 10 s for an additional 20 min. After 25 min, the protonophore carbonyl cyanide *m*-chlorophenyl hydrazone (CCCP) was added to 25 μM to collapse the remaining $\Delta\psi$. Maximal (100%) fluorescence for each condition was defined as the average of the fluorescence readings over the first 5 min.

Reconstituted SNARE- and Rab GTPase-Dependent Liposome Fusion. "Vacuole-mimic" liposomes harboring the critical homotypic fusion SNARE proteins (Nyv1p, Vam3p, Vam7p, and Vti1p) and the Rab family GTPase Ypt7p were a kind gift from William Wickner (Geisel School of Medicine at Dartmouth University, Hanover, NH) and were of two distinct types: "donor" liposomes that contained membrane-bound fluor 7-nitrobenz-2-oxa-1,3-diazole (NBD)–1,2-di-palmitoyl-sn-glycero-3-phosphatidylethanolamine (DPPE) and encapsulated Cy5-derivatized streptavidin and "acceptor" liposomes contained the membrane-bound Marina-Blue-DPPE fluorescent probe and entrapped soluble biotinylated R-phycoerythrin. Fusion and content mixing of proteoliposomes was described previously (19). Briefly, 20-μL total volume reactions containing 0.25 mM of each liposome type in RB150 + Mg buffer [20 mM HEPES-NaOH (pH 7.4), 150 mM NaCl, 10% (vol/vol) glycerol, 1 mM $MgCl_2$] were incubated in a 384-well low-volume, round-bottomed black plate (Corning) with 1 mM ATP and 9.6 μM streptavidin. After 5 min at 27 °C, reactions were initiated with the addition of premixed Sec17p/Sec18p/HOPS so that the final concentrations of each were 1 μM, 1 μM, and 165 nM, respectively, in the 20-μL reaction. Fluorescence wavelengths corresponding to liposome content mixing (λ_{ex} = 565 nm; λ_{em} = 670 nm) and lipid mixing (λ_{ex} = 370 nm; λ_{em} = 465 nm) were measured every minute for 1 h at 27 °C.

Liposome Leakage Assay. Carboxyfluorescein-encapsulated liposomes were prepared as described previously (20, 21). Briefly, dried lipid film of 85% 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC):15% 1,2-di-(9Z-octadecenoyl)-sn-glycero-3-phospho-L-serine (DOPS) (Avanti Polar Lipids) was hydrated using 100 mM carboxyfluorescein dye solution to constitute the final 15-mM lipid composition. Liposomes were disrupted by five freeze-thaw cycles in liquid nitrogen and then were extruded using an Avanti miniextruder and 80-nm polycarbonate membranes. Extruded liposomes were passed through a PD-10 desalting column (GE Healthcare) to remove excess dye. For the liposome leakage assay, 100 μM of liposomes in 10 mM MES (pH 5.5), 25 mM NaCl were added to a quartz cuvette, and fluorescence intensity (λ_{ex} = 480 nm, λ_{em} = 514 nm) was measured using Felix32 software (Photon Technology International). Carboxyfluorescein leakage is expressed as the percent of total lysis upon addition of 1% final (vol/vol) n-octyl-β-D-glucopyranoside detergent.

Vacuolar V-ATPase–VopQ Interaction Studies. To test the association of MBP-VopQ or MBP-VopQ^{S200P} with the vacuolar membrane in a V-ATPase–dependent manner, 150 μg vacuoles from either BY4742 or BY4742 *vma6Δ* (11) strains were added to 25 nM purified MBP-VopQ or MBP-VopQ^{S200P} protein in MMS buffer [50 mM MES/50 mM 3-(*N*-morpholino)propanesulfonic acid (Mops) (pH 5.5 or 7.5), 200 mM sorbitol] containing 125 mM KCl, 815 nM Pbi2p, and 2× protease inhibitor (10× stock from Protease Inhibitor Tablet–EDTA; Thermo Scientific), in a final volume of 300 μL. Vacuoles were incubated for 30 min at 27 °C and then removed to ice for 5 min. Then 10 μL of each reaction was withdrawn for assaying preflotation conditions. The remaining sample was mixed gently with 262.5 μL of 15% (wt/vol) Ficoll solution (made in the appropriate MMS pH buffer) and overlaid sequentially with 200 μL each of 8%, 4%, and 0% Ficoll solutions in MMS. Vacuoles were reisolated by flotation (TLS-55

rotor, 173,000 × *g,* 30 min, 4 °C), harvested from the 0–4% interface, and assayed for protein content.

To measure V_o subunit associations with purified MBP-VopQ derivatives via coprecipitation, 400-μg vacuoles isolated from BY4742 (with 2× protease inhibitor added immediately after isolation to reduce Vph1p degradation) were added to 10 μg recombinant MBP-VopQ or MBP-VopQ^{S200P} in PS buffer containing 125 mM KCl, 5 mM $MgCl_2$, 0.5 mM PMSF, 1× protease inhibitor, and 815 nM Pbi2p. Reactions were placed at 30 °C for 30 min, removed to 22 °C for 30 min, and 1 M Tris·HCl (pH 7.5) was added to a final concentration of 20 mM. Reactions remained at 22 °C for 15 min and were solubilized with 0.5% Triton X-100. Insoluble material was removed via centrifugation, input samples were removed, and the remaining lysate was applied to 25 μL equilibrated amylose resin. Beads were washed extensively, and MBP-VopQ coprecipitates were eluted with 50 mM maltose.

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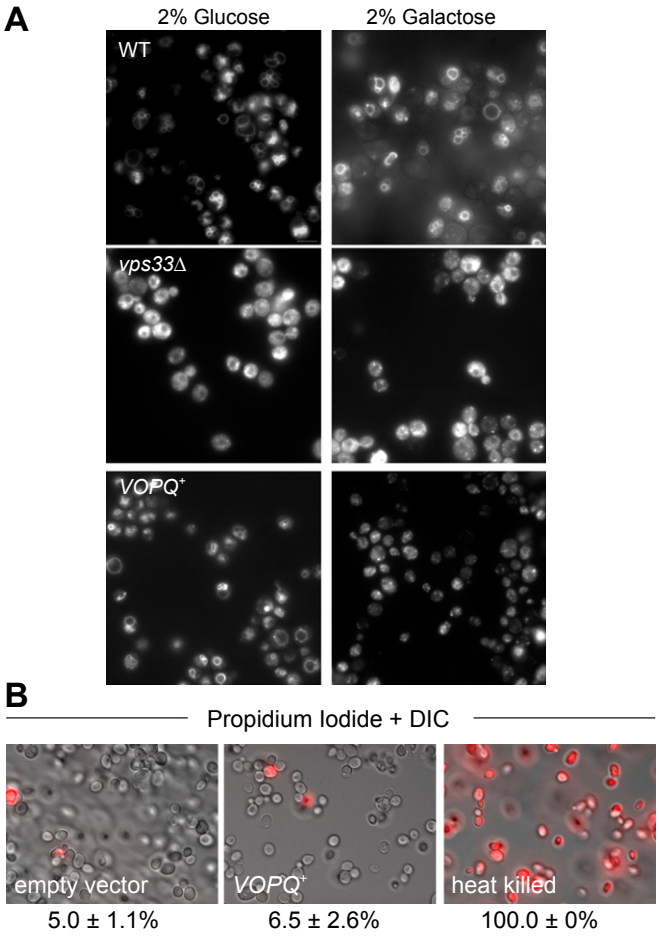

Fig. S1. VopQ induces vacuole fragmentation. (*A*) BY4742 yeast strains harboring the galactose-inducible vector pRS413-Gal1 (WT), pRS413-Gal1-*VOPQ* (*VopQ*$^{+}$), or with deletions in *VPS33* (*vps33*Δ) were visualized via FM4-64 staining (1). Larger fields of the images presented in Fig. 1*A* are shown here. (Scale bar, 10 μm.) (*B*) Before FM4-64 staining of the galactose-grown cells, aliquots of BY4742 harboring either pRS413-Gal1 or pRS413-Gal1-*VOPQ* were assayed for cell wall integrity. A small sample of the wild-type cells was incubated at 100 °C for 10 min for a heat-killed control. Cells were stained with 25 μM propidium iodide for 30 min at 30 °C and then were washed and visualized by differential interference contrast (DIC). At least 200 cells from each sample were scored for propidium iodide retention; representative micrographs for each condition are shown. The mean percentage of cells displaying fluorescence is denoted (n = 3).

1. Vida TA, Emr SD (1995) A new vital stain for visualizing vacuolar membrane dynamics and endocytosis in yeast. *J Cell Biol* 128(5):779–792.

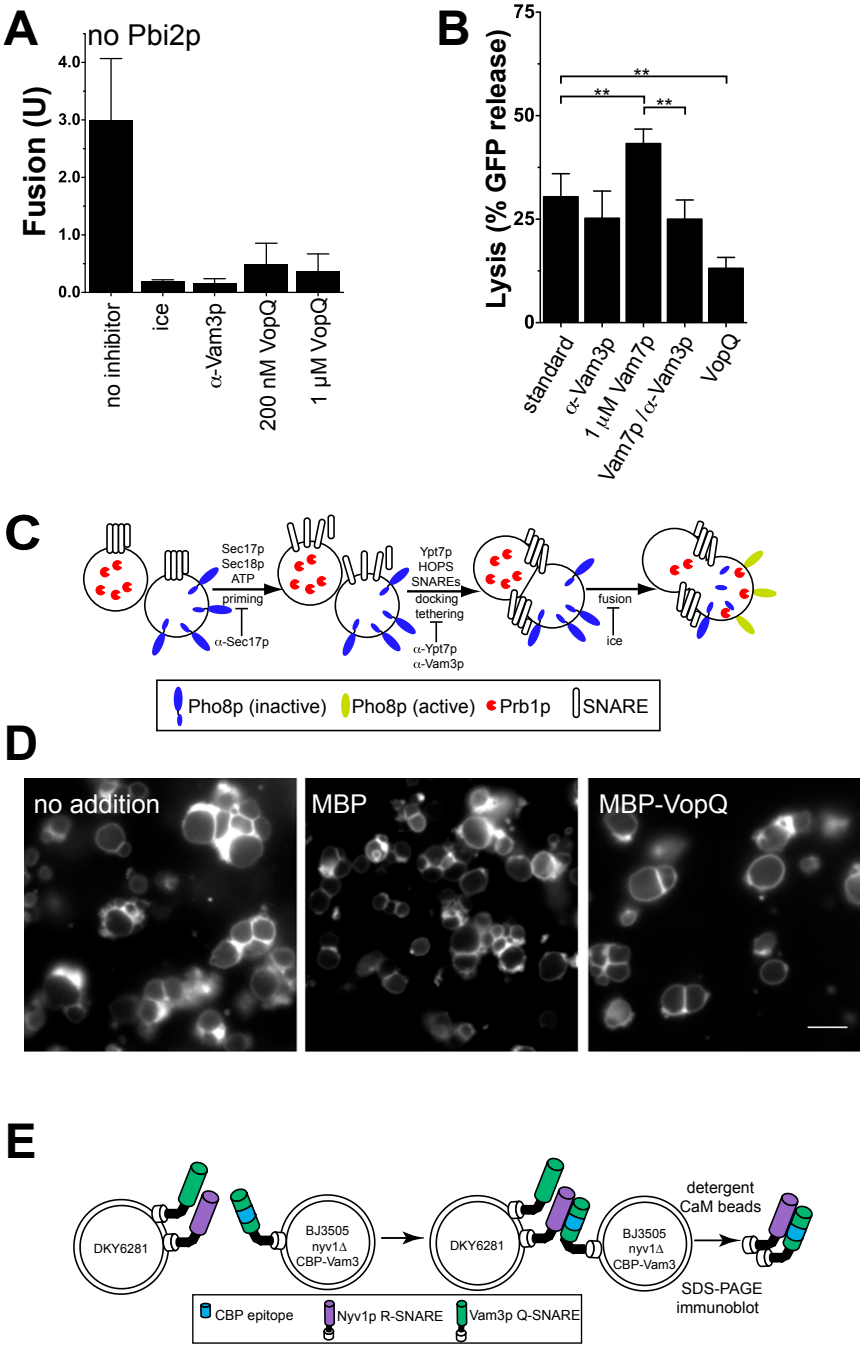

Fig. S2. VopQ does not disrupt vacuolar integrity. (*A*) Standard Pho8p-dependent vacuole fusion reactions were performed in the absence of the protease inhibitor Pbi2p. (*B*) Luminal GFP release (lysis) was measured for GFP-containing vacuoles either untreated (standard) or treated with α-Vam3p, Vam7p, or 200 nM MBP-VopQ; $n = 3$. $^{**}P < 0.01$, ANOVA repeated measures test and Tukey's multiple comparison posttest. (*C*) Ordered model of vacuole priming, docking, and fusion. Known inhibitors of each stage are listed below the arrows. (*D*) Representative vacuole morphologies observed with FM4-64 staining during the docking of yeast vacuoles in the absence or presence of 500 nM MBP or 500 nM MBP-VopQ. (Scale bar, 5 μm.) (*E*) Model of *trans*-SNARE assay using CBP-Vam3p:Nyv1p pulldown.

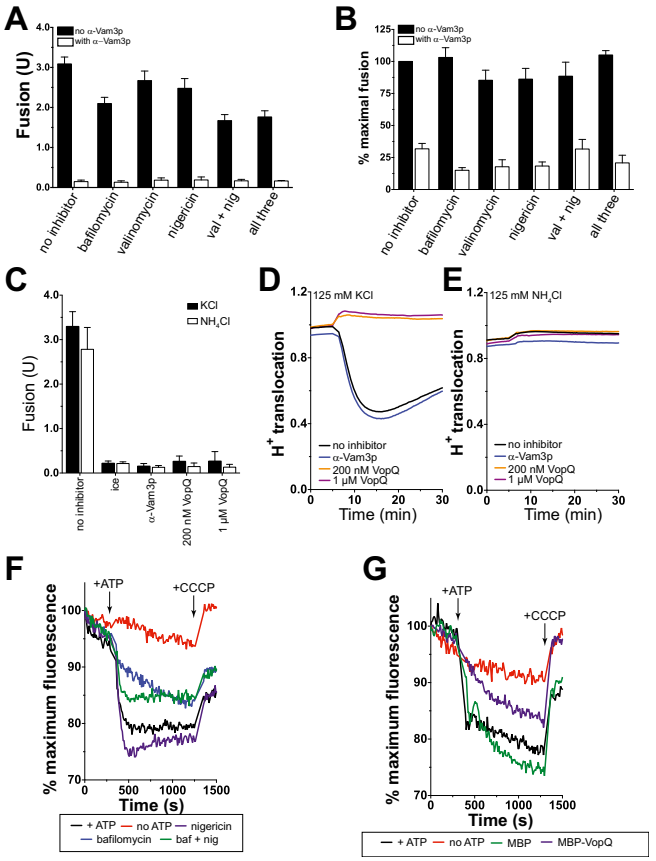

Fig. S3. A pH gradient is not required for vacuole fusion. (*A*) Standard ALP-dependent vacuole fusion reactions were performed in the presence of 500 nM bafilomycin, 1 μM valinomycin, 1 μM nigericin, or combinations thereof. These reactions either lacked (black bars) or contained (white bars) 450 nM α-Vam3p to inhibit *trans*-SNARE complex formation. Error bars indicate SD from the mean; $n = 3$. (*B*) β-Lactamase fusion assay under the conditions in *A*. Maximal fusion (100%) is defined as the no-inhibitor reaction; Error bars indicate SD from the mean; $n = 3$. (*C*) ALP fusion reactions were performed with either 125 mM KCl or 125 mM NH_4Cl and were assayed for VopQ sensitivity. Error bars indicate SD from the mean; $n = 3$. (*D* and *E*) Proton translocation activity of vacuoles in the absence or presence of α-Vam3p or MBP-VopQ was measured in 125 mM KCl (*D*) or 125 mM NH_4Cl (*E*). Curves are representative of three independent experiments. (*F*) Vacuolar membrane potential in the presence of bafilomycin and nigericin was measured via Oxonol V fluorescence. Loss of fluorescence caused by dye quenching is dependent on an inside-positive membrane potential. (*G*) Vacuolar membrane potential in the presence of 500 nM MBP or MBP-VopQ was measured using Oxonol V.

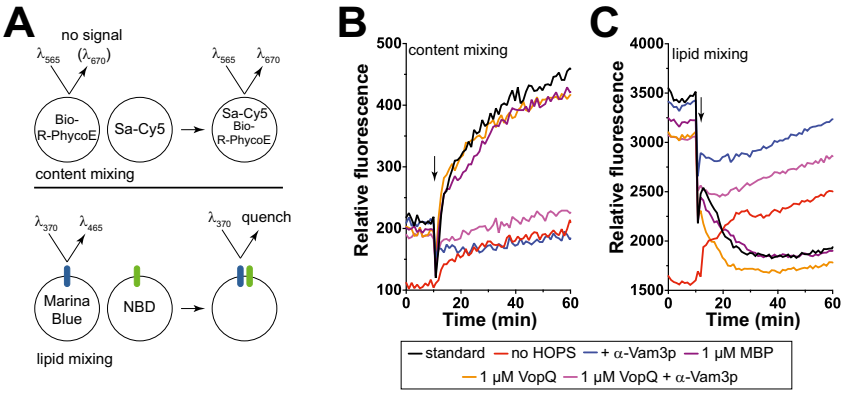

Fig. S4. VopQ does not inhibit V-ATPase–independent proteoliposome fusion. (*A*) Content- and lipid-mixing scheme for SNARE and Rab-dependent liposome fusion detection. (*B* and *C*) Content (*B*) and lipid mixing (*C*) of reconstituted SNARE/Ypt7p-containing proteoliposomes in the presence of MBP-VopQ.

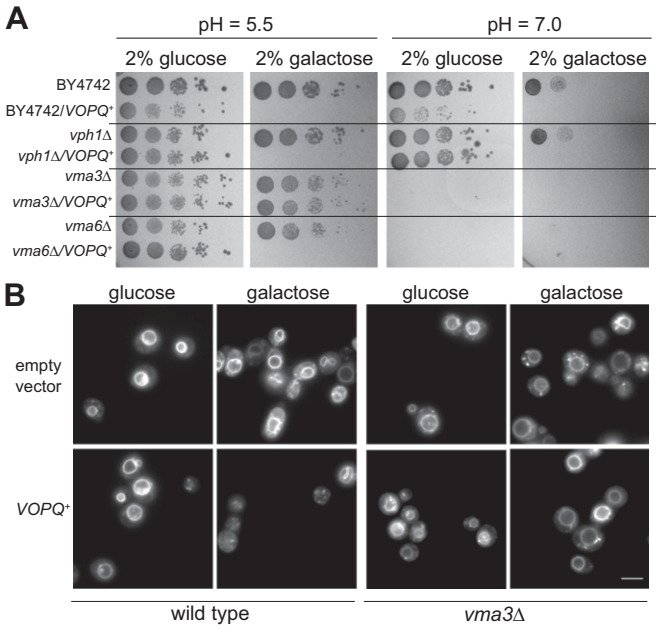

Fig. S5. VopQ does not phenocopy *vma* mutants. (*A*) Serial dilutions (1:10 dilutions from a starting OD_{600} = 1.0) of BY4742 or *vma* strains harboring either pRS413-Gal or pRS413-Gal1-VopQ (10 μL) in CSM medium lacking histidine supplemented with either 2% glucose or 2% galactose. Plates were buffered with 50 mM MES/50 mM Mops to either pH 5.5 or 7.0. (*B*) Vacuoles from BY4742 or *vma3*Δ strains harboring either pRS413-Gal1 control or pRS413-Gal1-*VOPQ* plasmids were visualized via FM4-64 staining (1). Cells were incubated with 8 μM FM4-64 for 1 h. (Scale bar, 5 μm.)

1. Vida TA, Emr SD (1995) A new vital stain for visualizing vacuolar membrane dynamics and endocytosis in yeast. *J Cell Biol* 128(5):779–792.

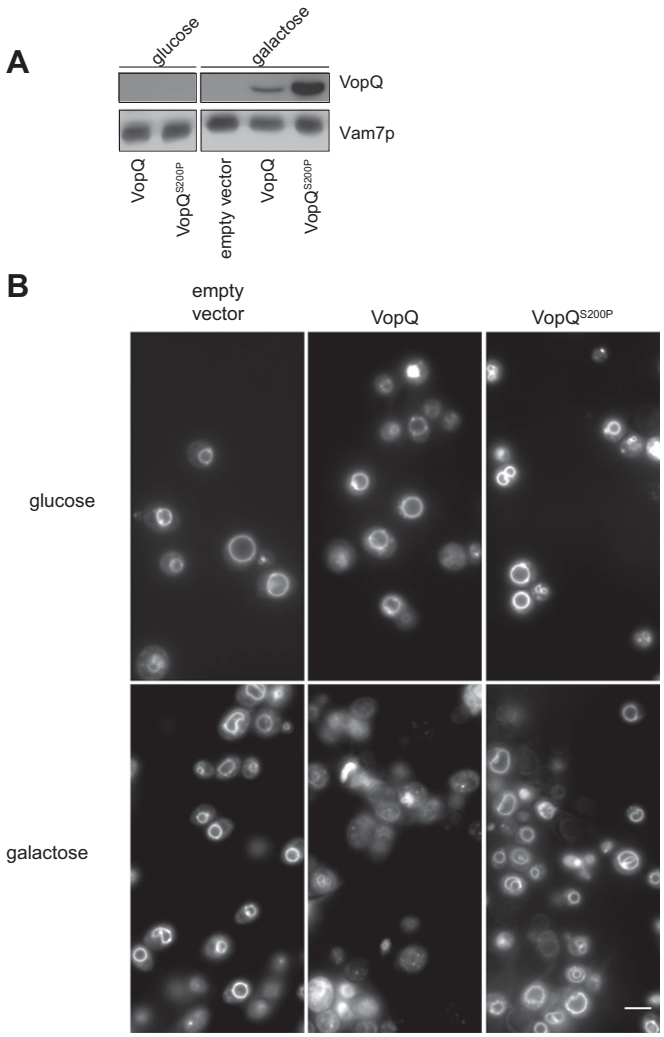

Fig. S6. VopQ^{S200P} does not fragment the yeast vacuole. (*A*) VopQ protein expression was assayed in overnight cultures of the strains shown in Fig. 4*A*. (*B*) BY4742 yeast strains harboring the galactose-inducible vector pRS413-Gal1 (wild-type), pRS413-Gal1-*VOPQ* (VopQ), or pRS413-Gal1-*VOPQ*^{S200P} (VopQ^{S200P}) were visualized via FM4-64 staining (1). Larger fields of the images presented in Fig. 4*B* are shown here. (Scale bar, 5 μm.)

1. Vida TA, Emr SD (1995) A new vital stain for visualizing vacuolar membrane dynamics and endocytosis in yeast. *J Cell Biol* 128(5):779–792.

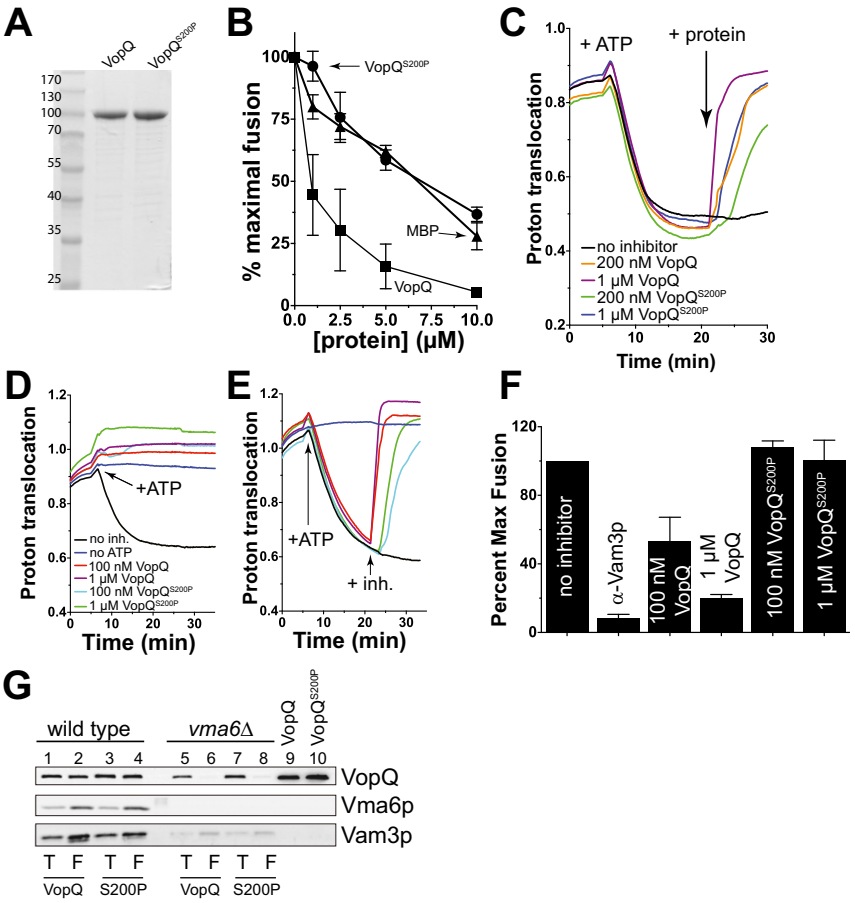

Fig. S7. VopQ^{S200P} does not inhibit vacuole fusion. (*A*) Four micrograms of purified MBP-VopQ and MBP-VopQ^{S200P} visualized via SDS/PAGE and Coomassie staining. (*B*) Standard ALP-dependent vacuole fusion reactions were performed in the presence of the indicated concentrations of recombinant MBP, MBP-VopQ, or MBP-VopQ^{S200P}. Maximal fusion was defined as the no-inhibitor reaction condition. Error bars indicate SD from the mean; $n = 3$. (*C*) Vacuolar proton translocation activity was measured in the presence of MBP-VopQ or MBP-VopQ^{S200P} when proteins were added 15 min after the addition of ATP. (*D* and *E*) Proton translocation activity was measured in the presence of His$_6$-VopQ or His$_6$-VopQ^{S200P} when proteins were added before (*D*) or 15 min after (*E*) the addition of ATP. Curves are representative of three independent experiments. (*F*) Standard ALP-dependent vacuole fusion reactions were performed in the presence of the indicated concentrations of recombinant His$_6$-VopQ or His$_6$-VopQ^{S200P}. Maximal fusion was defined as the no-inhibitor reaction condition. Error bars indicate SD from the mean; $n = 3$. (*G*) Vacuoles purified from either BY4742 (wild-type) or *vma6*Δ strains were assayed for MBP-VopQ or MBP-VopQ^{S200P} associations at pH 7.5. Then 0.5 μg of vacuoles from either pre- (T) or postflotation (F) conditions were separated via SDS/PAGE and immunoblotted for the indicated proteins.

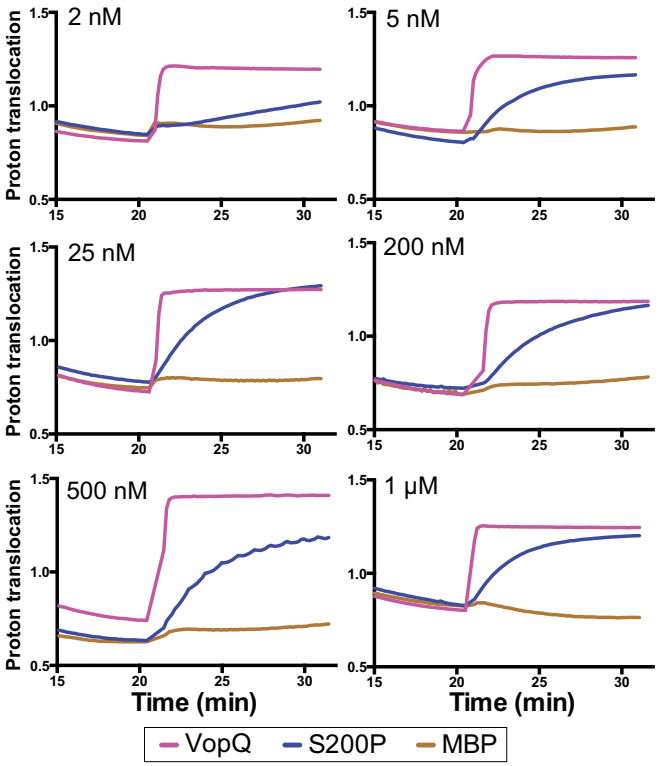

Fig. S8. Characterization of VopQ^{S200P} vacuolar deacidification rates. The proton translocation activity of vacuoles was measured in the presence of the indicated concentrations of MBP-VopQ (pink curves), MBP-VopQ^{S200P} (blue curves), or MBP (brown curves). Proteins were added 15 min after ATP addition (t = 20 min), and acridine orange absorbance was followed for an additional 10 min. Curves are representative of three independent experiments.

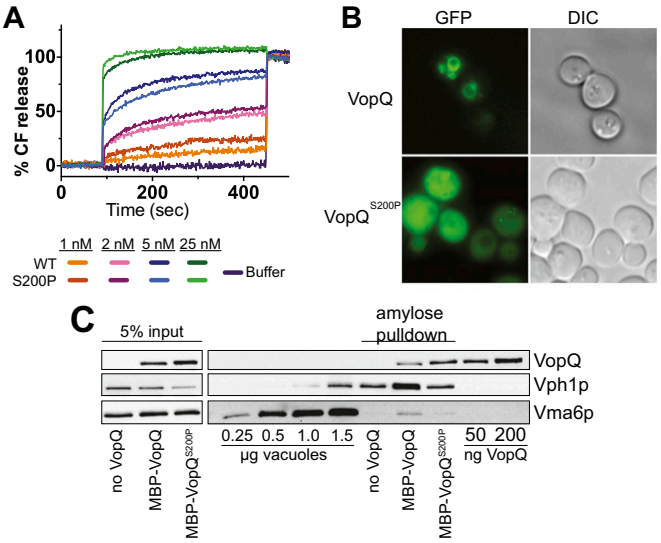

Fig. S9. VopQ^{S200P} displays channel-forming activity but weaker V-ATPase binding. (*A*) Carboxyfluorescein dye (CF) release from liposomes upon the addition of buffer, MBP-VopQ, or MBP-VopQ^{S200P}. (*B*) The BY4742 strain harboring either the galactose-inducible vector VopQ-GFP or VopQ^{S200P}–GFP was grown in CSM-uracil supplemented with 1% raffinose/2% galactose for 8 h, harvested, and visualized. (Scale bar, 5 μm.) (*C*) MBP-VopQ or MBP-VopQ^{S200P} was precipitated from BY4742 vacuole lysates, and eluates were separated via SDS/PAGE and immunoblotted for VopQ and the known VopQ-interacting subunits of V_o (Vph1p and Vma6p).