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Supplemental Data

Osmotic Regulation of Rab-Mediated Organelle Docking

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Osmotic Pressure Regulates Rab-Mediated Tethering and Docking

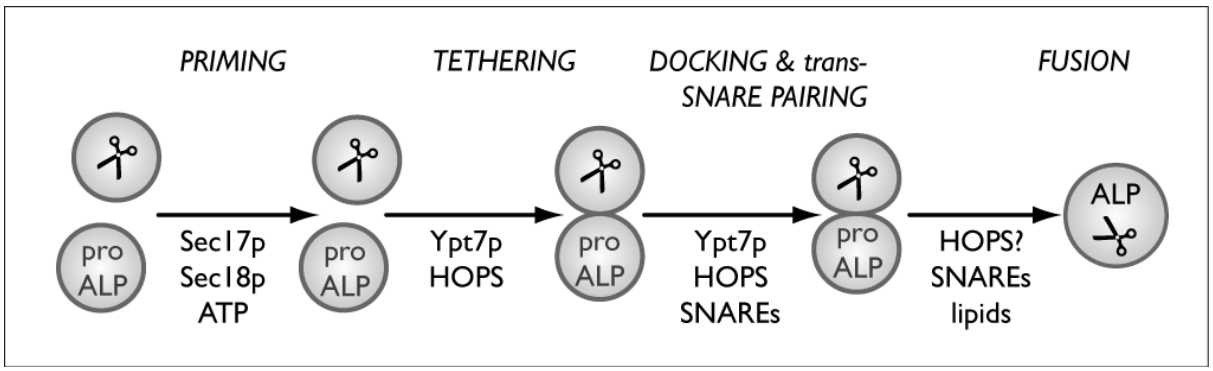

Figure S1. Content-Mixing Assay, Subreactions, and Key Components of Vacuole Fusion

A standard reaction is initiated by addition of ATP, which is needed for **Priming.** Priming does not require physical contact between vacuoles, and is the sole subreaction requiring energy input. **Tethering** is defined operationally as the formation of adhesive contacts between vacuoles. **Docking** is a complex series of events that occurs only after the vacuole membranes are in contact, and culminates in the formation of *trans*-SNARE complexes. **Fusion** leads to continuity of the formerly separate lipid bilayers and mixing of the lumenal contents. We measure fusion by activation of vacuolar alkaline phosphatase (**ALP**). An inactive ALP precursor, proALP, is present in one population of vacuoles. A second population contains an ALP-activating protease. ALP activation, and hence fusion, is quantified using a colorimetric assay as described in Methods.

Key Components:

• **SNARE proteins**: a set of four proteins that can assemble into a tight coiled-coil complexes. *Trans*-SNARE complexes bridge between a pair of docked membranes, and appear to be the core catalysts of fusion. Homotypic vacuole fusion requires three SNAREs with integral membrane anchors (**Vam3**, **Vti1**, and **Nyv1**), and one SNARE that lacks an integral membrane anchor (**Vam7**). Recombinant Vam7, when added to the fusion reaction, bypasses the priming reaction, eliminating the requirement for Sec17, Sec18, and ATP.

• **Sec17** and **Sec18**: these SNARE chaperones act together in the early, ATP-dependent priming reaction which disassembles fusion-inactive *cis*-SNARE complexes. Priming causes release of Sec17p from the membrane.

• **Ypt7**: membrane-associated Rab GTPase that controls vacuole tethering and docking. Like other Rabs, Ypt7p is active when bound to GTP. Inactivation of Ypt7p occurs upon GTP hydrolysis. Inactivation is accelerated by **Gyp** GTPase activating proteins (GAPs). Once inactivated, Ypt7p:GDP can be extracted from membranes by a specialized chaperone, **Gdi1**.

• **HOPS complex**: HOPS is an effector of Ypt7p, and is required for tethering, docking and *trans*-SNARE complex formation. HOPS contains six subunits: Pep5/**Vps11**, **Vps16**, Pep3/**Vps18**, **Vps33**, Vam6/**Vps39**, and **Vps41**. Vps39 is a guanine nucleotide exchange factor believed to activate Ypt7**.** Vps33 is a member of the SM-family of proteins thought to catalyze *trans*-SNARE complex assembly, leading to fusion.

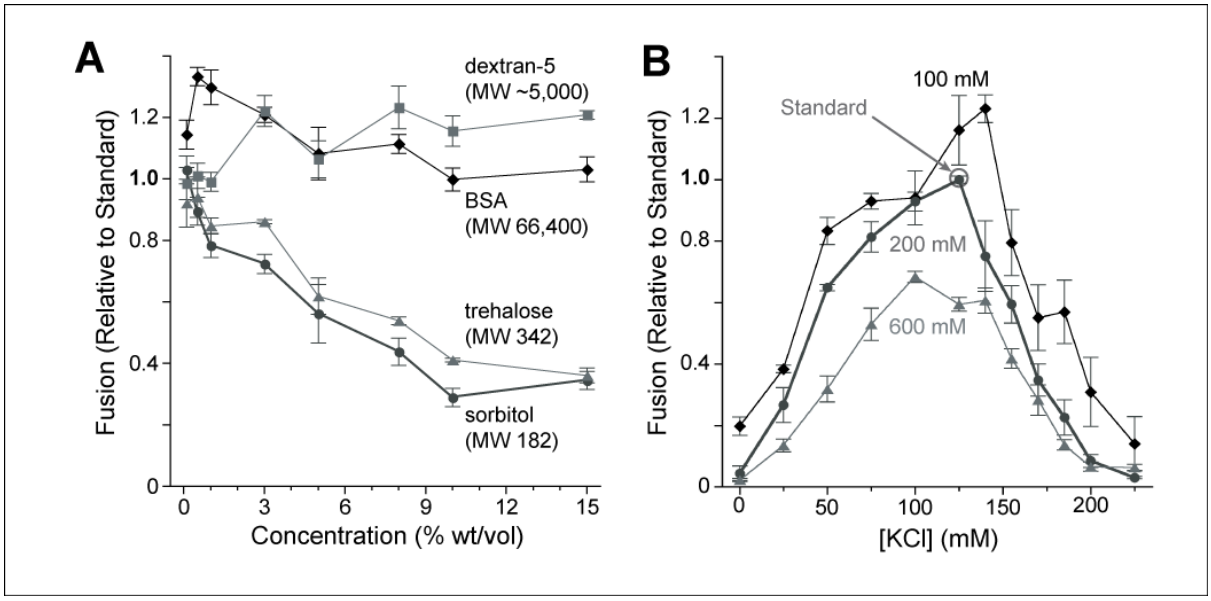

Figure S2. Osmotic Pressure Explains Inhibition by Small Polar Osmolytes

(A) Increasing concentrations of bovine serum albumin (BSA), dextran-5, trehalose or sorbitol were added to standard fusion reactions, incubated with ATP for 90 min., and assayed for fusion. Osmolality for the 15% (w/v) solutions is given in Supplementary Table S1.

(B) Ionic optima of fusion at three osmolyte concentrations was determined by measuring fusion at 90 min. in the presence of increasing concentrations of KCl. n $\geq$ 4 for all experiments shown. Bars span 95% confidence intervals.

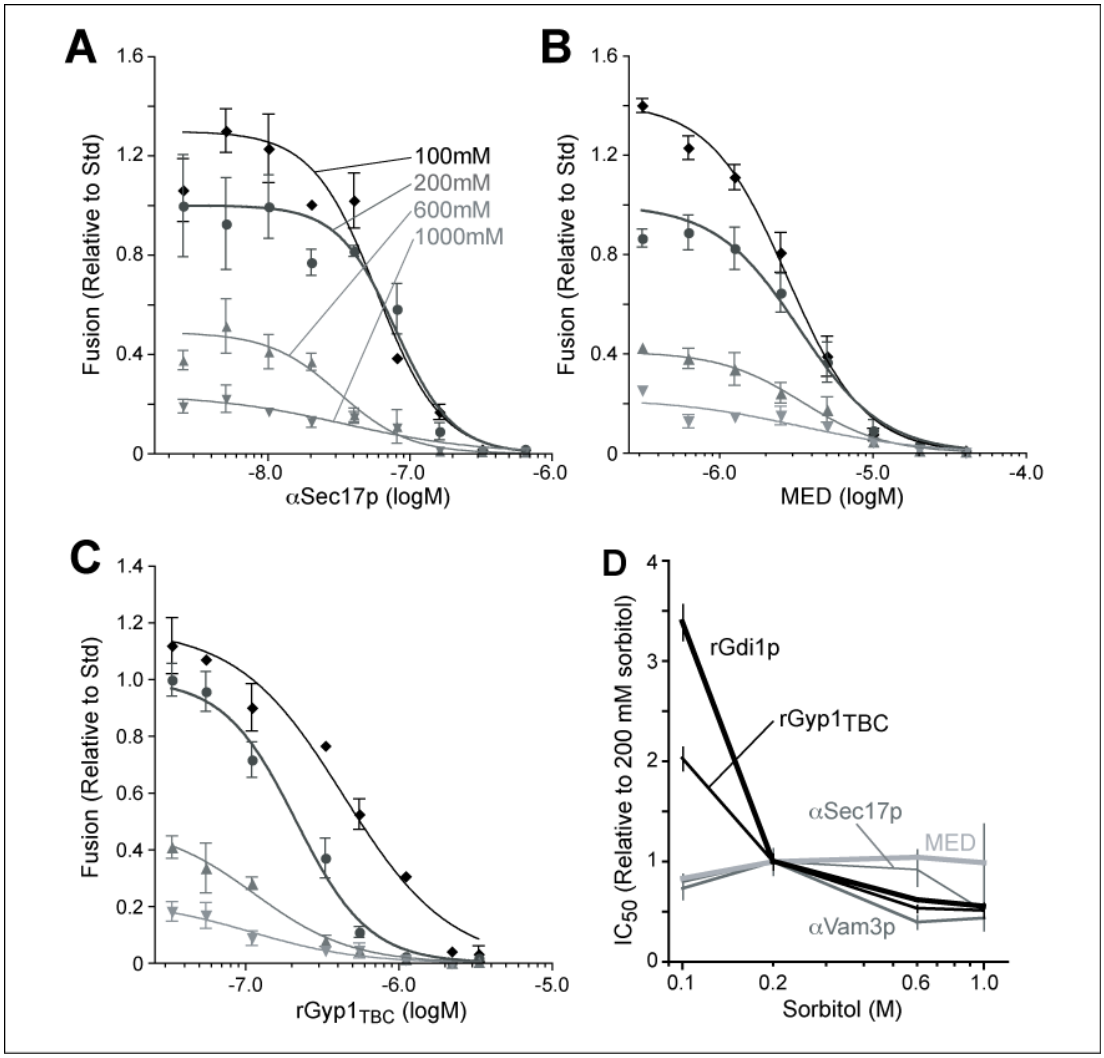

Figure S3. Osmotic Pressure Enhances Sensitivity to Ypt7 Inhibitors: Additional Data and Alternative IC_{50} Plot

(A–C) As in Fig. 3, Fusion was measured after incubation for 90 min. in the presence of ATP, various concentrations of sorbitol, and the indicated concentrations of: (A) α-Sec17 antibody; (B) MED; or (C) $rGyp1_{TBC}$. Sigmoidal dose-response curves were fit to the datasets.

(D) An alternative plot of IC_{50} values for the inhibitors assessed in Fig. 3 and this figure. Here, inhibitor IC_{50} values obtained at each concentration are normalized to the values obtained with the standard condition (200 mM sorbitol), and plotted on a linear, rather than logarithmic, response scale. $n \geq 4$ for each point. Bars span 95% confidence intervals.

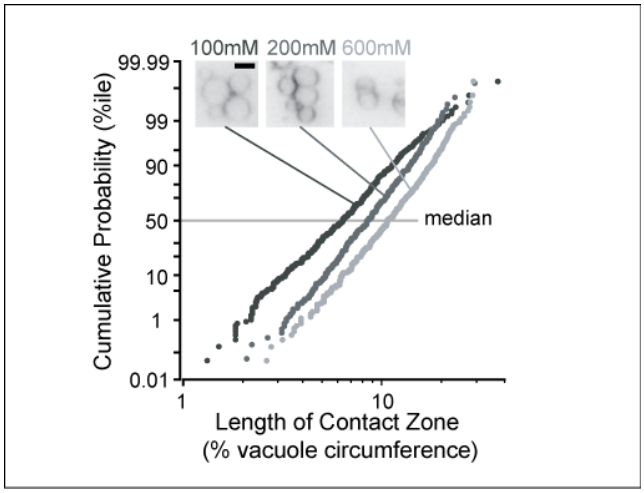

Figure S4. Effects of Osmotic Strength on Docking Contact-Zone Size

To see whether the applied osmotic gradients modulate the vacuole's turgor (its hydrostatic pressure) we inspected docking morphology. The contact zone between tethered vesicles is controlled by membrane adhesion energy (which acts to increase the area of the flattened contact zone) and by the vacuole turgor (which opposes flattening of the membranes, decreasing the size of the contact zone). The length of contact zones between adjacent vacuoles was measured after a 30 min. incubation in fusion reaction buffer with reduced ATP to slow fusion. The data are normalized to vacuole circumference and shown as cumulative probability histograms. Under hypoosmotic conditions (100 mM sorbitol), vacuole:vacuole contact zones were small, and many point contacts were observed. As osmotic strength was increased, the vacuole:vacuole contact zones increased in area. (Inset) Examples of vacuoles used for this analysis. >700 contact zones were measured per treatment. Scale bar = 1.5 μm.

Table S1. Osmolalities of Buffers Used in This Study

Buffer	Osmolality (Osm/kg)
Standard fusion reaction buffer	0.60
+ 15% (w/v) Bovine Serum Albumin (FW 66,400)	0.62
+ 15% (w/v) Ficoll-400 (FW ~400,000)	0.64
+ 15% (w/v) dextran-5 (FW 5,000)	0.69
+ 15% (w/v) sucrose (FW 342)	1.14
+ 15% (w/v) trehalose (FW 342)	1.15
+ 15% (w/v) sorbitol* (FW 182)	1.51