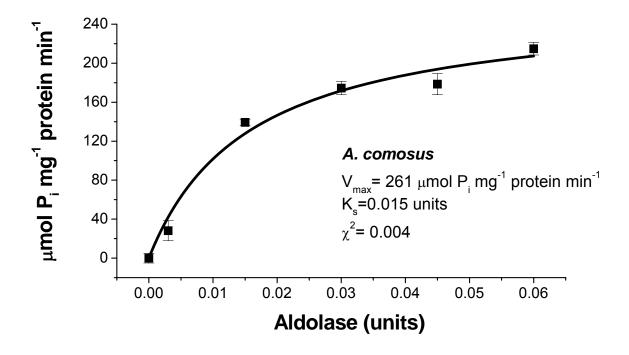
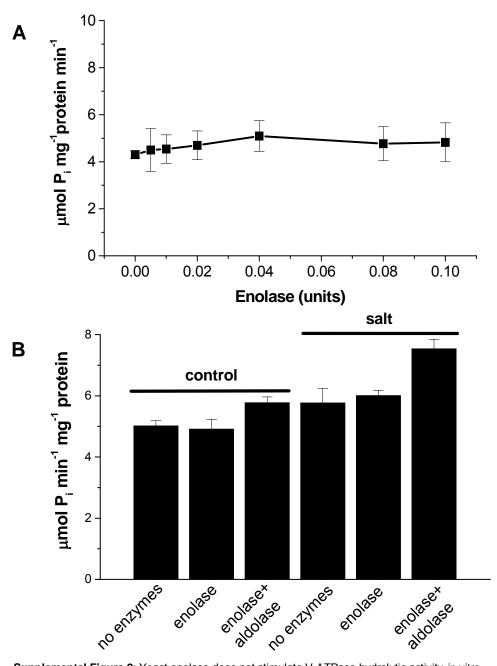
Supplemental Data. Barkla et al. Quantitative Proteomics of the Tonoplast Reveals a Role for the Glycolytic Enzymes in Salt Tolerance. Plant Cell. (2009). 10.1105/tpc.109.069211



Supplemental Figure 1. Aldolase stimulates V-ATPase hydrolytic activity in Ananas comosus

V-ATPase hydrolytic activity (bafilomycin insensitive, azide and vanadate sensitive) was estimated by spectrophotometric measurement of inorganic phosphate release as described in Methods. Activity was measured in tonoplast vesicles (15 ug protein) isolated from *Ananas comosus* (pineapple) over a range of aldolase concentrations. Data represent means $\pm$  SE of three replicate experiments. Each replicate experiment was performed using independent membrane preparations. The solid lines show the fit of the kinetic data with the Michaelis-Menten equation. The X<sup>2</sup> value indicates the goodness-of-fit and confirmed that the data fitted the equation at a probability level of at least *P* < 0.01,



Supplemental Figure 2: Yeast enclase does not stimulate V-ATPase hydrolytic activity in vitro.

(A) V-ATPase hydrolytic activity (bafilomycin sensitive, azide and vanadate insensitive) was estimated by spectrophotometric measurement of inorganic phosphate release as described in Methods. Activity was measured in tonoplast vesicles (15 ug protein) isolated from salt-treated *M. crystallinum* over a range of enolase concentrations.

(**B**) V-ATPase activity was measured in the absence or presence of 0.03 units aldolase and 0.04 units enolase in tonoplast isolated from *M. crystallinum* plants grown under control conditions or in the presence of 200 mM NaCl. Data represent means± SE of three replicate experiments. Each replicate experiment was performed using independent membrane preparations.

Spot #	# Identified Peptides <sup>a</sup>	MH+ <sup>b</sup>	DeltaM <sup>c</sup>
		1573.84318	-0.12645
131	3	1323.76308	0.03572
		1790.92830	-0.20357
100	2	1573.84318	-0.05894
132	Z	1510.84753	-0.14954
		973.52005	-0.05180
225	2	1076.50474	0.00088
		1259.73580	0.04950
241		1448.71283	-0.02892
	5	1346.73144	-0.13037
	-	2216.11533	
		1766.89594	0.06802 -0.10919
0.4.0	0	1596.91080	-0.03954
318	2	1715.93266	-0.02662
		1563.80131	-0.14270
414	3	1088.57686	0.03899
		2501.18982	0.00376

Supplemental Table 1. Peptide precursor mass and mass accuracy of identified peptides.

<sup>a</sup>Number of matched peptides from MS/MS. Proteins were identified by 2 or more unique peptides. <sup>b</sup>Peptide precursor mass c Mass accuracy

СуDуе	Emission filter (nm) /Band Pass	Laser (nm)	Resolution (µm)	PMT <sup>a</sup> (V)	
Cy2	520/40	Blue 488	100	600	
Су 3	580/30	Green 532	100	600	
Cy 5	670/30	Red 633	100	600	

Supplemental Table 2. Gel Scanning Parameters used for the Typhoon scanner.

<sup>a</sup>Photomultiplier tube voltage