

**Table S3. The effects of inhibitors on H<sup>+</sup> pump hydrolytic activity in vacuolar-enriched membrane vesicles.** Membrane vesicles were prepared from 2-week-old Col-0 (wild type) plants. ATPase activity ( $\mu\text{mol Pi mg}^{-1} \text{ protein h}^{-1}$ ) at pH 8.0 (unless indicated) was measured in the presence of 2 mM ATP, 2 mM MgSO<sub>4</sub> and 50 mM KCl. Pyrophosphatase (PPase) activity ( $\mu\text{mol Pi mg}^{-1} \text{ protein h}^{-1}$ ) at pH 8.0 was measured in the presence of 0.1 mM pyrophosphate, 5 mM MgSO<sub>4</sub> and 50 mM KCl. All reactions were performed over a 1 hour incubation period at 37°C. Inhibitors of the plasma membrane H<sup>+</sup>-ATPase (vanadate), tonoplast H<sup>+</sup>-ATPase (nitrate and bafilomycin), mitochondrial ATPase (azide) and tonoplast K<sup>+</sup>-dependent H<sup>+</sup>-PPase (- KCl) were used. Mean activity ( $\pm$  SE) from three replicates is shown. n.d. – not determined.

Treatment	ATPase activity	% inhibition	PPase activity	% inhibition
Control	16.98 $\pm$ 1.23	0	19.20 $\pm$ 2.46	0
Vanadate (0.1 mM) at pH 8	15.82 $\pm$ 1.91	6.8	17.80 $\pm$ 1.20	7.3
Vanadate (0.1 mM) at pH 6.5	14.95 $\pm$ 1.72	12	n.d.	n.d.
Azide (1 mM)	15.06 $\pm$ 2.54	11.3	20.52 $\pm$ 2.16	-6.9
Nitrate (50 mM)	6.07 $\pm$ 0.11	64.3	17.43 $\pm$ 2.60	9.2
Bafilomycin (0.2 $\mu\text{M}$ )	3.19 $\pm$ 0.07	81.2	18.87 $\pm$ 1.93	1.7
No KCl	9.43 $\pm$ 0.78	44.5	4.81 $\pm$ 0.21	74.9