

## SUPPLEMENTAL DATA

**Figure S1.** Bayesian estimate [Ronquist, F., and Huelsenbeck, J. P. (2003) *Bioinformatics*, 19, 1572–1574] of membrane PPase phylogeny. Monophyletic groups of K<sup>+</sup>-dependent and K<sup>+</sup>-independent PPases [Belogurov, G. A., and Lahti, R. (2002) *J. Biol. Chem.* **277**, 49651–49654] are depicted in green and blue, respectively. Na<sup>+</sup>-PPases are marked in red. Protein Genbank™ accession numbers are specified following the species names. Clade credibility values are shown above the nodes. Black and red arrows indicate functionally characterized H<sup>+</sup>- and Na<sup>+</sup>-PPases, respectively, and the names of species harbouring the characterized enzymes are indicated in boldface. Circles at the tips of the branches mark the enzymes that are part of H<sup>+</sup>-PPase–Na<sup>+</sup>-PPase dichotomies within their host species: H<sup>+</sup>- and Na<sup>+</sup>-PPases are marked by cyan and red circles, respectively.

The Na<sup>+</sup> dependence can be traced back to polytomic node A. However the monophyletic group of Na<sup>+</sup>-PPases cannot be delineated because node A also gives rise to a branch leading to the H<sup>+</sup>-PPase of *Carboxydothermus hydrogenoformans* (and K<sup>+</sup>-independent H<sup>+</sup>-PPases), and another branch leading to plant H<sup>+</sup>-PPases. It is currently unknown whether the lack of a distinct split between the Na<sup>+</sup>- and H<sup>+</sup>-PPases is due to the limitations of the phylogenetic analysis (i.e., the lack of resolution in the vicinity of node A) or arises because the type of pumped ion has changed several times during the evolution of membrane PPases.

Fig. S1.

