Supporting Information

Ekberg et al. 10.1073/pnas.1010416107

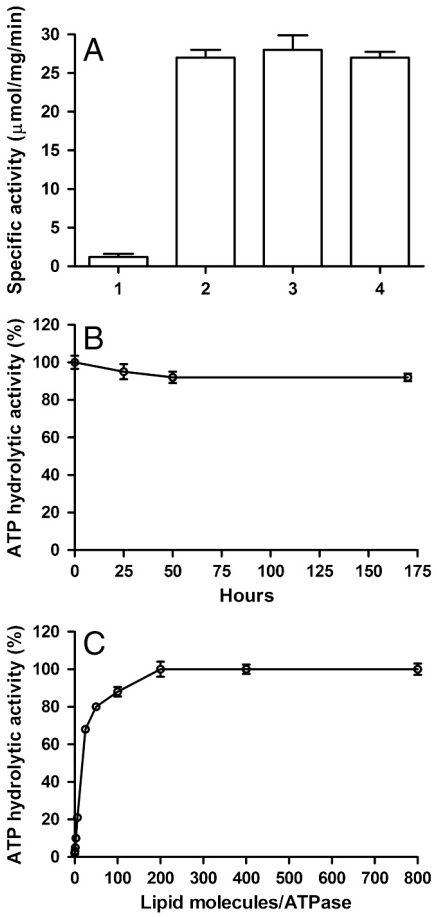

Fig. S1. The delipidated PM H^+-ATPase is fully functional. Activity of purified PM H^+-ATPase. (*A*) ATP hydrolytic activity of the purified H^+-ATPase measured during the crystallization preparation protocol. Activities are expressed as specific activity (μmol Pi produced/min/mg protein). 1: Activity after purification and without any application of lipid molecules. 2: Activity after purification with inclusion of lipids. 3: Activity after a $C_{12}E_8$/Cymal-5/Sucrose step with inclusion of lipids. 4: Activity after the final ultracentrifugation step, just before crystallization set-up. (*B*) Time-dependent stability of the crystallization ready PM H^+-ATPase with respect to ATP hydrolytic activity. Protein, just before crystallization set-up, was kept at 4 °C and the activity was measured at different time intervals. (*C*) Lipid requirement of the purified proton pump. Different amounts of DDM solubilized lipids were added to the PM H^+-ATPase protein before activity measurements (ATP hydrolytic activity). A MW of 762 Da of the added lipids (soybean phosphatidylcholine), and a MW of 90 kDa for the PM H^+-ATPase were used for the calculations.

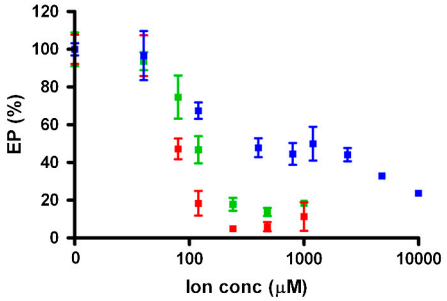

Fig. S2. Tb^{3+}, Ho^{3+}, and Ca^{2+} ions inhibit accumulation of the phosphorylated intermediate of the wild-type PM H^+-ATPase. Measurement of steady state phosphorylation levels of the purified PM H^+-ATPase with varying concentrations of Tb^{3+}, Ho^{3+}, and Ca^{2+} ions. Phosphorylation was initiated by addition of 1 μM [γ-^{32}P]ATP and was stopped after 20 seconds by acid quenching. The level of phosphorylated protein without the addition of cations was set to 100%. Blue, Ca^{2+}; green, Tb^{3+}; and red, Ho^{3+}. All values are indicated ±S.D.

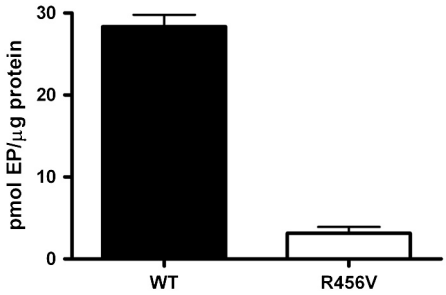

Fig. S3. The R456V single-point mutant has a reduced level of phosphoenzyme accumulation at steady state. Measurement of steady state phosphorylation levels for the wild type (black bar) and R456V mutant (white bar) PM H^+-ATPase. Phosphorylation was initiated by addition of 1 μM [γ-^{32}P]ATP and was stopped after 20 seconds by acid quenching. The activity is reported as the mean ± S.D. and expressed as pmol EP/μg protein.

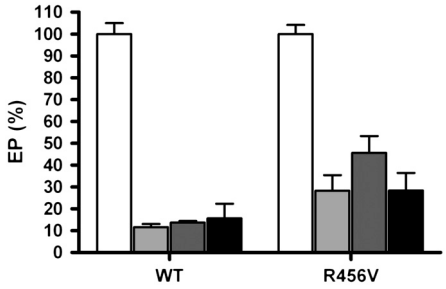

Fig. S4. The accumulation of the phosphorylated intermediate in the R456V mutant is less sensitive toward Tb^{3+} and Ca^{2+}, but not Ho^{3+}, than the wild-type protein. Measurement of steady state phosphorylation levels for the wild type and R456V mutant PM H^+-ATPase in the presence of no cations (white bars), Ca^{2+} (light gray), Tb^{3+} (dark gray) and Ho^{3+} (black) as described for Fig. S3. Values are reported in % relative to EP formation without addition of cations for the wild type and the R456V mutant PM H^+-ATPase. All values are reported as the mean ± S.E.

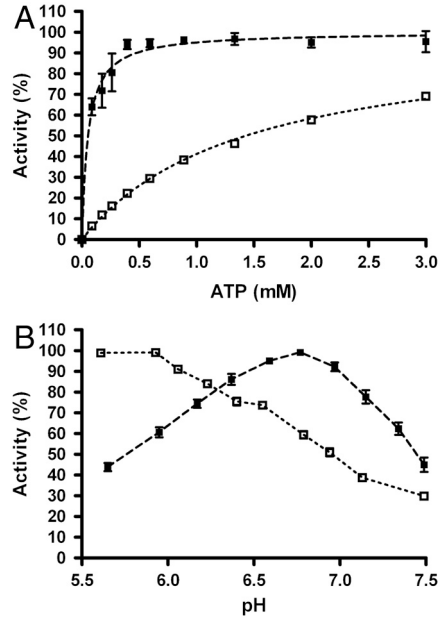

Fig. S5. The R456V mutant displays reduced ATP affinity and a shift in pH optimum with respect to catalytic activity. (*A*) The dependence of ATP concentration of ATP hydrolysis and (*B*) the pH dependence of ATP hydrolysis were measured for: ■, wild type; and □, R456V PM H^+-ATPases. All experiments are represented as ±S.D.

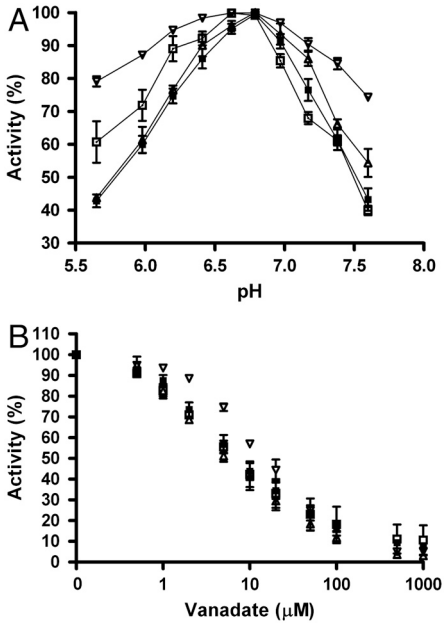

Fig. S6. The ATP hydrolytic activity of the D92A,D95A mutant is almost pH-insensitive. (*A*) The pH dependence of ATP hydrolysis and (*B*) the vanadate sensitivity of ATP hydrolysis were measured for: ■, wild type; □, D92A; Δ, D95A; and ▽, D92A,D95A PM H^+-ATPases. All experiments are represented as ±S.D.

Table S1. Summary of crystallographic parameters

Form:	RbCl	$TbCl_3$	$HoCl_3$
Anomalous scatterer	Rubidium	Terbium	Holmium
Method	Cocrystallization with 100 mM RbCl	Soak in 5 mM $TbCl_3$	Soak in 10 mM $HoCl_3$
Data collection			
Space group	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$
Unit-cell dimensions a, b, c (Å)	89.6, 146.7, 310.9	85.8, 144.3, 311.1	87.9, 149.9, 313.5
Wavelength (Å)	0.8152	0.9537	1.2782
Resolution range (Å)	100–7.5 (8.0–7.5)	100–7.0 (7.5–7.0)	40–6.0 (6.3–6.0)
R_{sym} (%)	6.2 (112.0)	9.1 (97.7)	6.2 (52.4)
$I/\sigma I$	15.3 (2.0)	12.3 (1.8)	9.4 (1.8)
Completeness (%)	98.4 (98.3)	99.9 (100.0)	97.8 (98.4)
Redundancy	6.0 (6.2)	5.8 (5.7)	2.4 (2.4)
Peak height	6.4/5.3	6.8/6.2	9.4/8.7:7.4/6.0

Highest resolution shell is shown in parentheses. Phases from the structure of the AHA2 E1-AMPPCP form (pdb-id: 3b8c) refined at 3.6-Å resolution was used to generate difference Fourier maps based on data in the given resolution ranges mentioned above.

For the Ho^{3+} peaks, the first set of peak heights denotes site 1 (at the N domain) and the second set denotes site 2 (at the extracellular side of the M domain).