# A hydrogen chloride pump in extremophilic eubacteria

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## Supplementary Figures

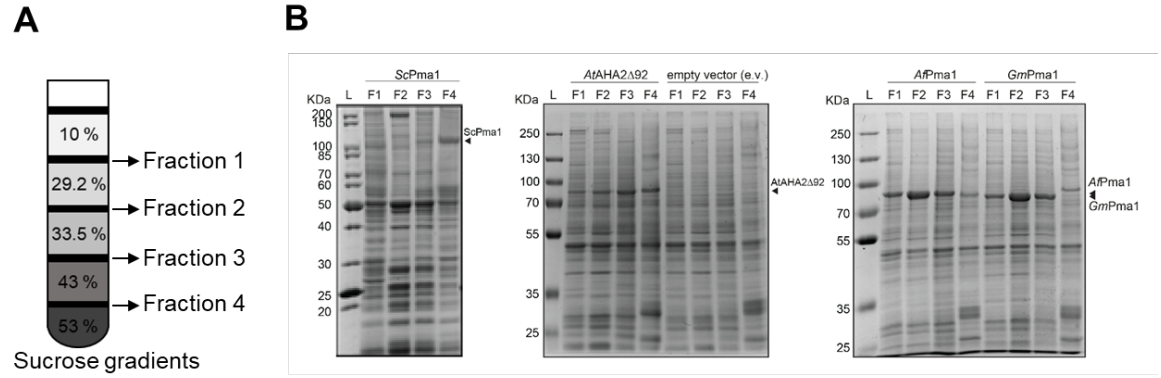


**Figure S1**. **Partial purification of P3A $H^+$-ATPases by sucrose gradient fractionation**. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of isolated membrane fractions from *Saccharomyces cerevisiae* expressing *Af*Pma1(~ 90.4 kDa), *Gm*Pma1(~ 88.5 kDa), *Sc*Pma1(~ 99.6 kDa) and *At*AHA2Δ92 (~93.8 kDa), or empty vector control (*e.v.*). L, protein molecular weight markers. Fractions 1 – 4 (F1-F4) represent fractionated membranes enriched in karmellae (F1), endomembrane fractions (F2–F3) and plasma membrane (F4). The e.v. control reflects background membrane proteins.

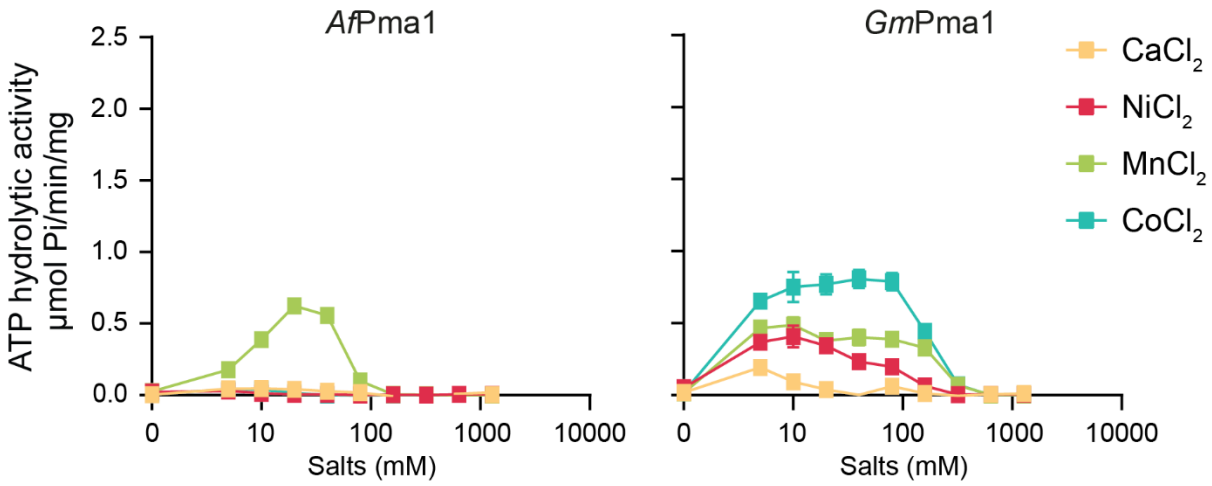


**Figure S2. Effect of divalent cations on the activity of AfPma1 and GmPma1.** ATPase activity of *Af*Pma1 and *Gm*Pma1 was measured in the presence of various divalent cations. Only $Mn^{2+}$ activates *Af*Pma1, whereas all the tested divalent cations act cations activate *Gm*Pma1. Data are mean ± s.d. (n = 4).

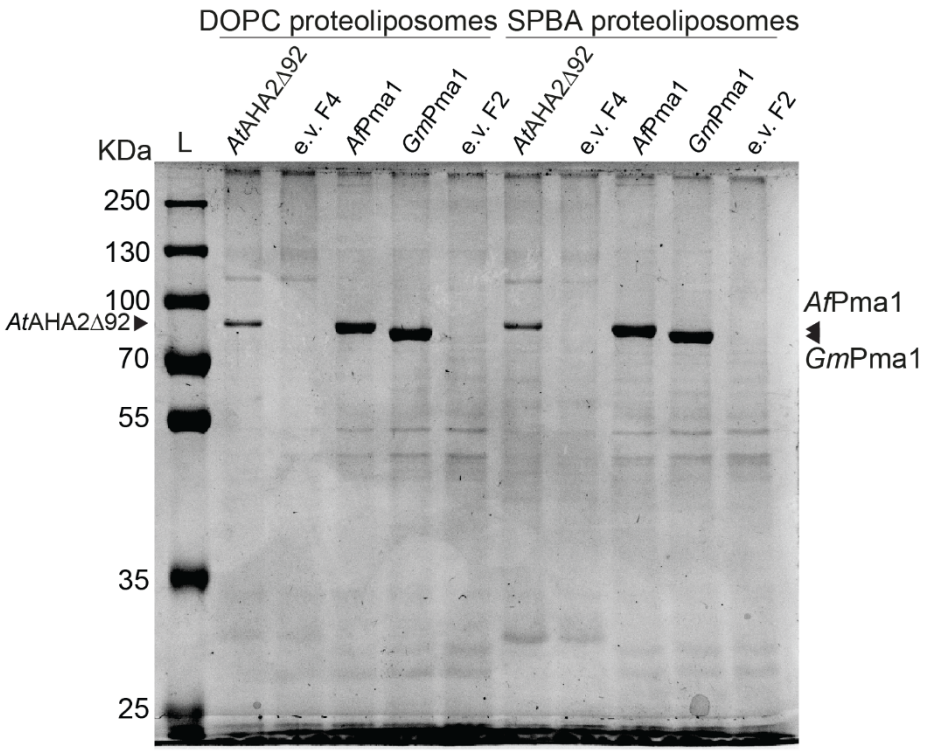


**Figure S3**. **SDS-PAGE of proteoliposomes reconstituted with P3A ATPases.** Coomassie-stained sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of proteoliposomes reconstituted with *Af*Pma1(~ 90.4 kDa), *Gm*Pma1(~ 88.5 kDa), *At*AHA2Δ92 (~93.8 kDa), or empty vector fractions F2 and F4. L, protein molecular weight markers.

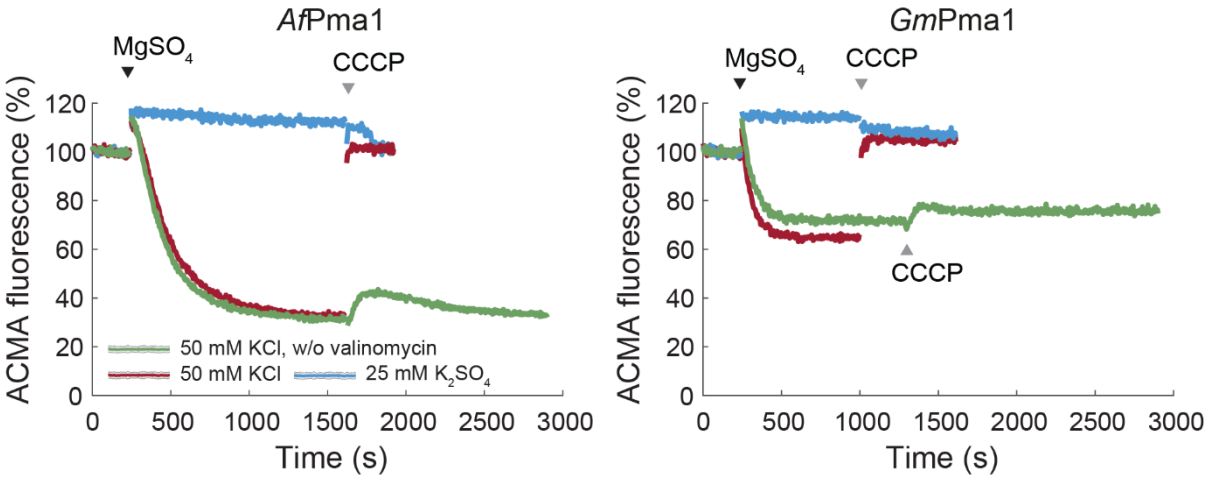


**Figure S4. Proton transport measured on *Af*Pma1 and *Gm*Pma1 proteoliposomes prepared using a buffer containing 50 mM KCl.** ACMA assay of proteoliposomes containing *Af*Pma1 or *Gm*Pma1 prepared in 50 mM KCl. Proton transport is detected only when KCl is present in the measurement buffer, indicating $Cl^-$ is acting on the cytosolic side of the transporter. Data are single representative traces.

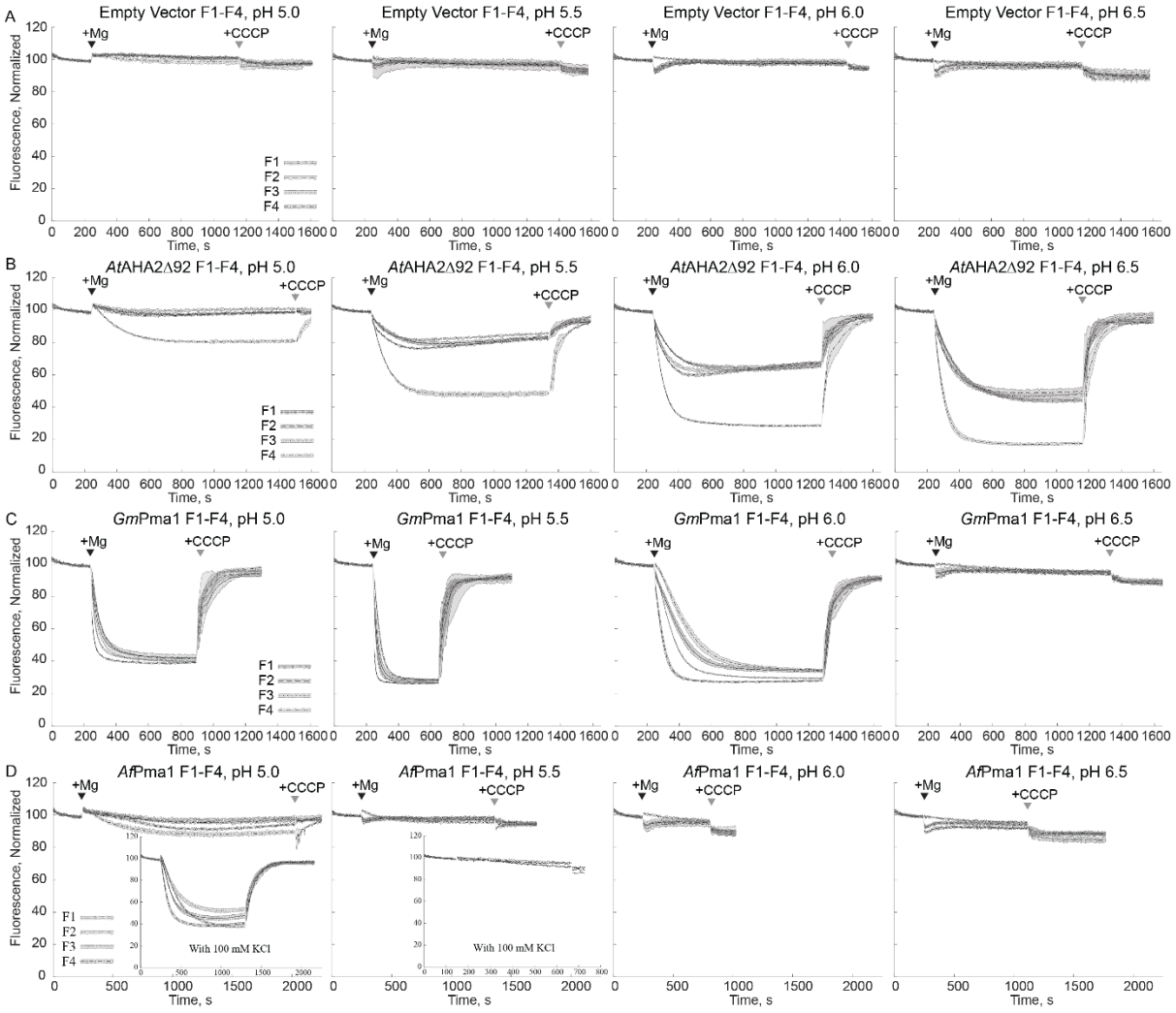


**Figure S5**. **Proton pumping activity of reconstituted membrane fractions.** Proteoliposomes were reconstituted with fractions F1-F4 from **A**) empty vector as control, **B**) *At*AHA2Δ92, **C**) *Gm*Pma1 and **D**) *Af*Pma1, using preformed lecithin LUVs. Proton pumping is measured using the ACMA assay at pH 5.0-6.5 to determine the pH optimum. F2 showed the highest proton pumping activity for *Af*Pma1 and *Gm*Pma1, whereas F4 showed maximum activity for *At*AHA2Δ92. Activity was initiated by adding $MgCl_2$ (10 mM, black arrowhead) to the ATP-containing buffer, while the generated proton gradient was dissipated by CCCP (grey arrowheads). Valinomycin (60 nM) was included to mediate $K^+$ exchange and prevent the build-up of a transmembrane potential. Data are mean ± s.d. (n = 3).

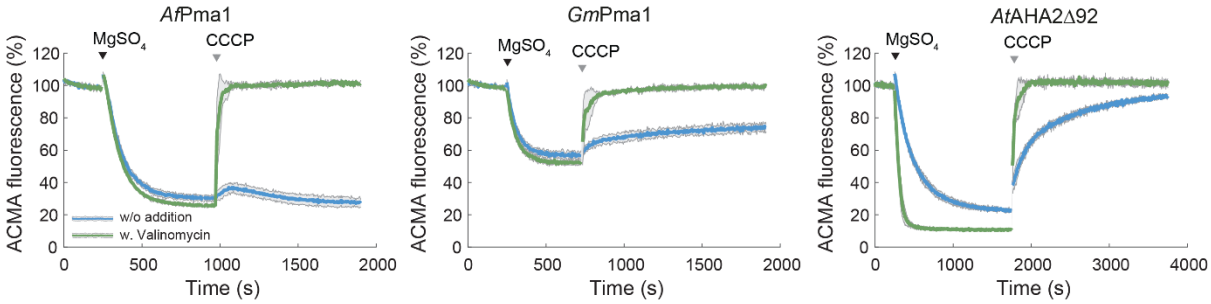


**Figure S6**. **Effect of valinomycin on the $H^+$ pumping activity of *Af*Pma1, *Gm*Pma1 and *At*AHA2Δ92.** Proton pumping of *Af*Pma1, *Gm*Pma1 and *At*AHA2Δ92 containing proteoliposomes measured using the ACMA assay in the presence or absence of valinomycin in the measuring buffer. Valinomycin increases proton transport by AtAHA2Δ92 but has no effect on *Af*Pma1 and *Gm*Pma1. For the proteoliposomes containing *Af*Pma1 and *Gm*Pma1, only a partial dissipation of the formed proton gradient is observed after addition of the protonophore CCCP without valinomycin, whereas CCCP plus valinomycin fully collapses the proton gradient. Data are the mean with ±S.D. shown as shaded region (n = 3).

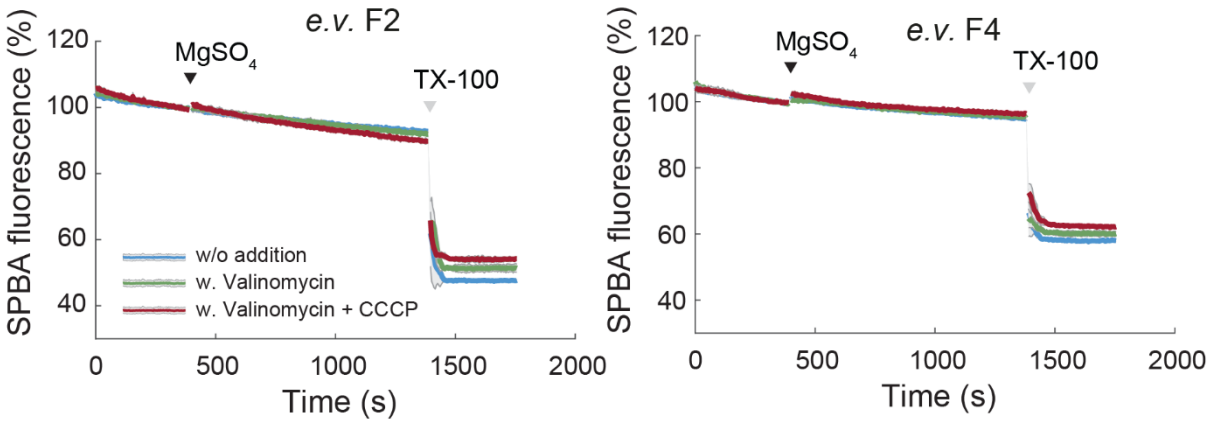


**Figure S7**. **Proteoliposomes reconstituted with empty fractions F2 and F3 show no $Cl^-$ transport activity.** SPBA assays of proteoliposomes containing e.v. F2 and e.v. F4, reconstituted with 50 mM $K_2SO_4$ and measured with 100 mM KCl in the buffer, in the presence or absence of the ionophores valinomycin and CCCP. Data are mean with s.d. shown as shaded region (n = 3).

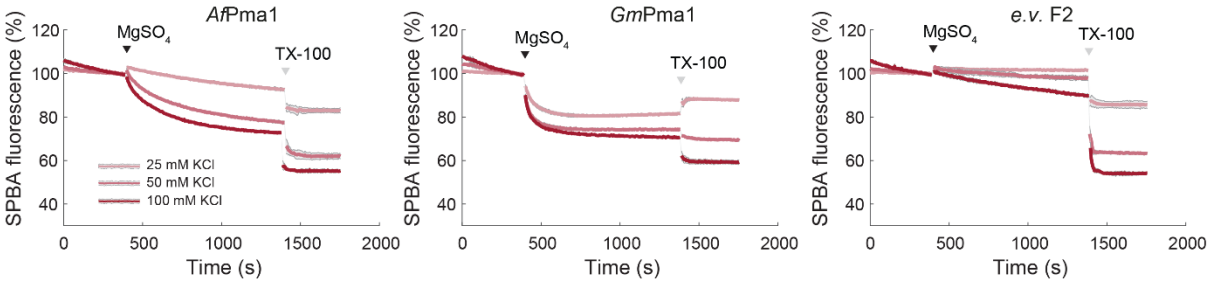


**Figure S8. Rate of $Cl^-$ transport by *Af*Pma1 and *Gm*Pma1 varies with KCl concentration.** SPBA assays of proteoliposomes containing *Af*Pma1, *Gm*Pma1, and e.v. F2, reconstituted with 50 mM $K_2SO_4$ and measured with 25, 50 or 100 mM KCl in the buffer, in the presence of the ionophores valinomycin and CCCP. Data are mean with s.d. shown as shaded region (n = 3).

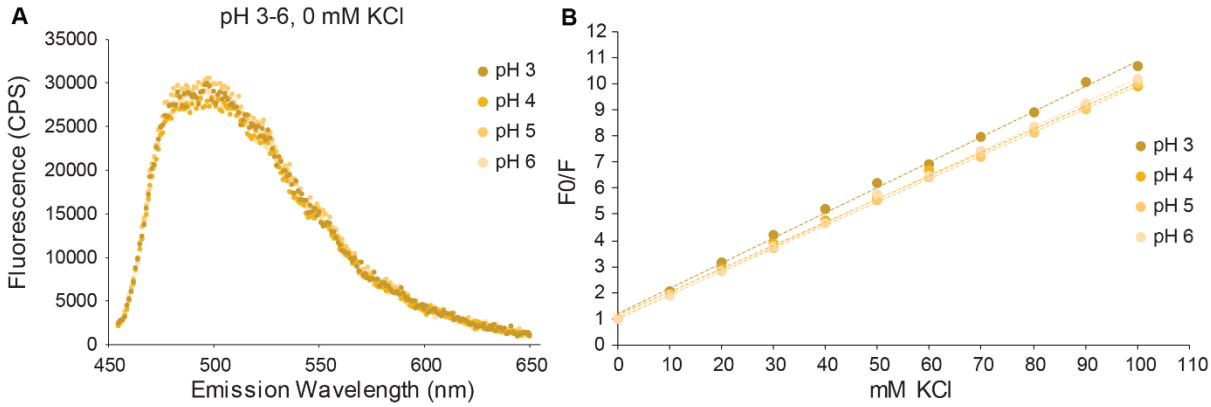


**Figure S9**. **Control measurements on the pH sensitivity of the SPBA dye. (A)** Emission spectra of 1 µM SPBA at pH 3 to 6, in 10 mM citrate buffer without KCl **(B)** Stern-Volmer plots from KCl titrations (0-100 mM) at pH 3 to 6.

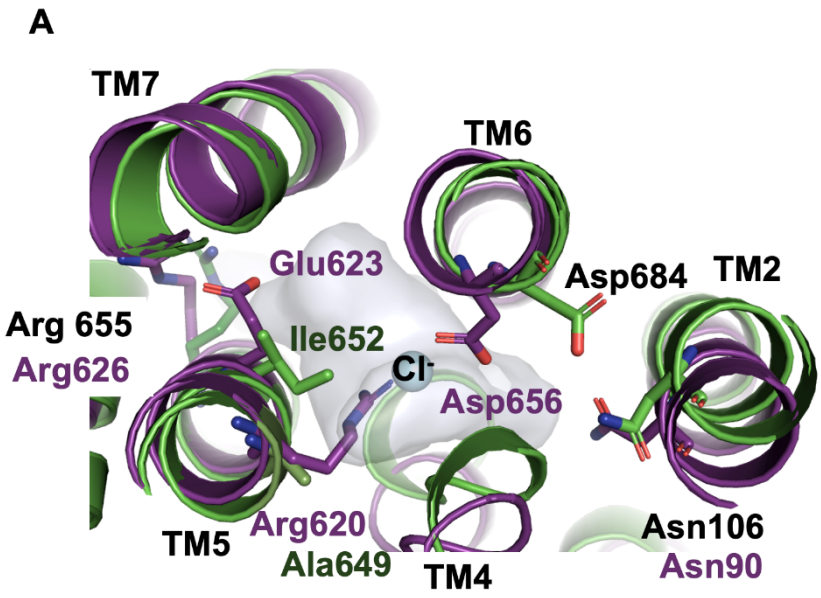


**Figure S10. Crystal structure of *At*AHA2 and homology model of *Af*Pma1.** Cavities in the crystal structure of *At*AHA2 (green), PDB ID 5KSD shown as surface representation, key residues for protein transport Arg655, Asp684 and Asn106 are shown in sticks. The Crystal structure of *At*AHA2 is superimposed with homology model for *Af*Pma1 (purple). Proposed chloride location is shown as a grey sphere. Residues in connection with the putative cavity are shown in sticks, including the hydrophobic residues from *At*AHA2, Ile652 and Ala649, that are occupying the equivalent positions of Glu623 and Arg620 in *Af*Pma1.