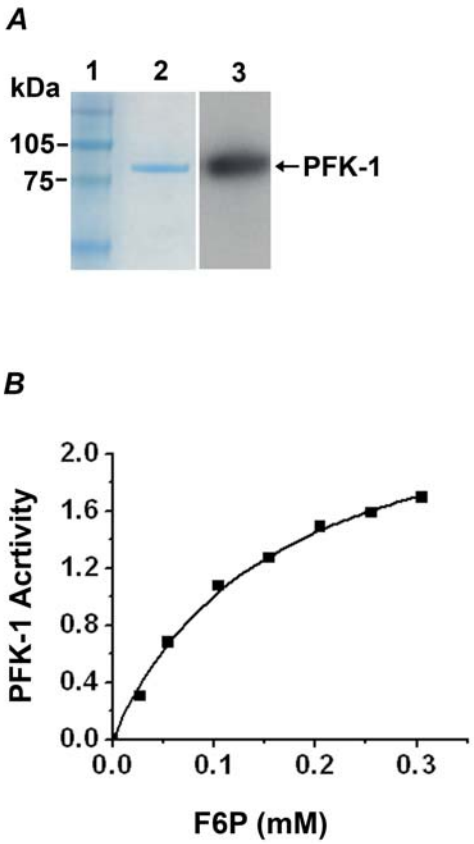
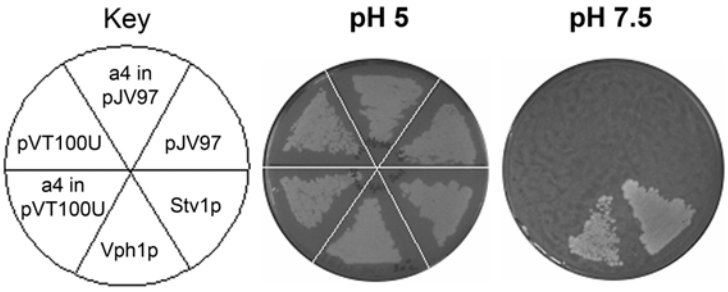


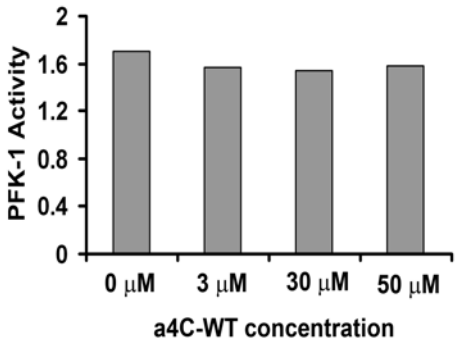
Su *et. al.* Supplementary Fig.1

Analysis of recombinant rabbit muscle type PFK-1. (*A*) Purified PFK-1 harvested following expression in a *PFK*-deficient *E. coli* strain was subjected to SDS-PAGE analysis (lanes 1 and 2). A single 85 kDa band at the expected size for intact PFK-1 was observed. This was confirmed by Western blot analysis using anti-PFK-1 antibody (lane 3). Lane 1 shows full-range rainbow molecular weight markers (Amersham Pharmacia Biotech). (*B*) Activity of recombinant PFK-1 (mole.min^{-1}) fitted Michaelis-Menten kinetics with K_m of 0.16 ± 0.03 mM and V_{max} of 2.61 ± 0.19.



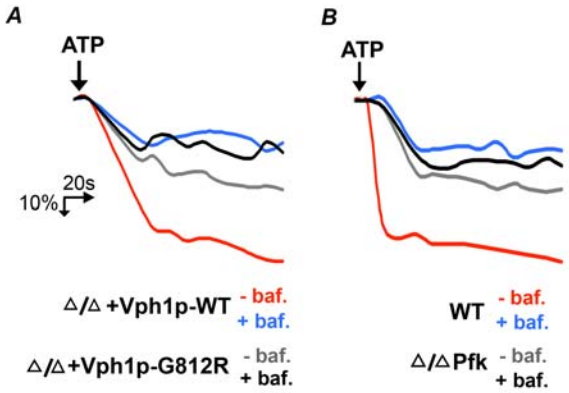
Su *et. al.* Supplementary Fig. 2

Complementation assay. KEBY9 yeast, which lacks both a-subunit paralogs *VPH1* and *STV1*, was transformed with relevant constructs containing the human a4 gene *ATP6V0A4* in pJV97 or pVT100U. *STV1* (Stv1p) and *VPH1* (Vph1p), or empty vector (pJV97 or pVT100U) transformations served as positive and negative controls. Images are of 30°C cultures. Functional complementation was observed from Vph1p and Stv1p, but not from human a4.



Su *et. al.* Supplementary Fig. 3

Effects of varying concentrations of a4C-WT peptide on the catalytic activity of PFK-1. a4C-WT (3-50 μM) was pre-incubated with recombinant PFK-1 before incorporation into a reaction mixture containing saturating concentration (0.5 mM) of F6P. PFK activity was unaffected by the presence of a4C-WT throughout the tested range.



Su *et. al* Supplementary Fig. 4

Proton transport activity of H^+ATPase was assayed by measuring fluorescence quenching of acridine orange using (*A*) vesicles isolated from KEBY9 cells (Δ/Δ) expressing WT or G812R mutant Vph1p, or (*B*) yeast cells with (WT) or lacking both Pfk paralogs (Δ/ΔPfk). Where indicated, ATP (1 mM) was added and proton uptake was monitored by the change in fluorescence emission. Two vacuolar preparation samples were each assayed in triplicate with and without addition of 0.3 μM bafilomycin A (baf.). Representative raw data are shown.

Supplementary Table 1: Clinical and biochemical parameters of dRTA patients

Patient	Origin	Sex	Age at diagnosis	SNHL	Blood					Urine pH	Nephro-calcinosis
					Na^+ 136-144 $mmolL^{-1}$	K^+ 3.3-5.0 $mmolL^{-1}$	Cl^- 96-104 $mmolL^{-1}$	HCO_3^- 22-28 $mmolL^{-1}$	pH 7.36-7.44		
5-1	Turkey	F	11 mo	None	139	3.1	102	11.0	7.12	7	Yes
70-1	Spain	M	2 mo	Severe	138	1.3	112	5.3	7.14	7.5	Yes

SHNL: sensorineural hearing loss; Na^+: sodium; K^+: potassium; Cl^-: chloride; HCO_3^-: bicarbonate.
Patient 5-1: ref. 52
Patient 70-1: ref. 56