SUPPLEMENTAL DATA [LUOTO *et al.* (2011)]

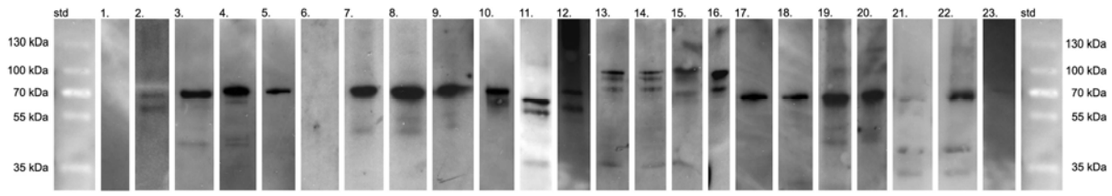
Supplementary Table S1
Primers used for cloning and site-directed mutagenesis of membrane PPases

Target enzyme	Primer name	Sequence (5'→3')	Restriction site	Purpose
Ac-PPase	Acac_for	tata*catatg*actaaaagtcataaactcaaattc	NdeI	Cloning
	Acac_rev2	tata*ctcgag*ttataaaattccgccgatact	XhoI	Cloning
Cl-PPase	Clim_for	tata*catatg*aatcaaaccattctctatgc	NdeI	Cloning
	Clim_rev	tata*ctcgag*ttaaagcagcggagcg	XhoI	Cloning
	ClE242S_for	ccgacctttt*tca*agctatgtagg	-	E242S substitution
	ClE242D_for	ccgacctttt*gat*agctatgtagg	-	E242D substitution
	Cl_rev726	cgcccatacccgcaacg	-	Used with ClE242S_for and ClE242D_for
	ClS180E_for	tcggcgca*gag*tccatcg	-	E180S substitution
	ClS180E_rev	gcgagaagccggagataaggttg	-	Used with ClE180S_ for
	Cl_for736	g*c*cgcaatcatcggaac	-	A246G substitution, used with Cl_rev736
	Cl_rev736	tacatagctttcgaaaaggtcgg	-	A246G substitution, used with Cl_for736
Ctet-PPase	Ct_for	tata*catatg*attcagattactacccaa	NdeI	Cloning
	Ct_rev	tata*ctcgag*ttagaaaattgagtacataaggaa	XhoI	Cloning
Da-PPase	Dace_for	tata*catatg*gcatattttgccgtaatt	NdeI	Cloning
	Dace_rev2	tata*ctcgag*ttaggctaacagcggtgc	XhoI	Cloning
Fj-PPase	Fla_full	tata*catatg*aatgcatttatgatttacctg	NdeI	Cloning
	Fla_rev	tata*ctcgag*ttatttagcggcttccactt	XhoI	Cloning
	Fjohn_m553_for	gcttggtgca*tca*tcaattgcttt	-	E185S substitution
	FjE185D_for	gcttggtgca*gat*tcaattgcttt	-	E185D substitution
	Fjohn_m553_rev	gaaaatcccgctaaagtttctaaaacaac	-	Used with Fjohn_m553 _for, FjE185D_for
	F_john_for739	gagctgatttatttt*gaa*tcgtatgtggc	-	G247E substitution
	Fjohn_m739_rev	ccataccggcaacatcacctac	-	Cloning, used with F_john_ for739
Lb-PPase	Lep_for_full	tata*catatg*aatgtagagttaatcattatcgtca	NdeI	Cloning
	Lep_rev_full	tata*ctcgag*ttatttgaagaagtttaaaacaattcc	XhoI	Cloning
	Lep_full_midfor	ccattgat*gcg*tatggtc	-	Elimination of internal NdeI site
	Lep_full_midrev	gaccatacgcatcaatgg	-	Elimination of internal NdeI site
	LbE253D_for	ttctgctgcg*gat*gccacttgt	-	E253D substitution
	LbE253D_rev	ccaaaaaggtcagcacccataccc	-	Cloning, used with LbE253D_ for
	LbE253S_for	ttctgctgcg*tca*gccacttgt	-	E253S substitution
	Lb253rev	ccaaaaaggtcagcacccatacc	-	Used with LbE253S_ for
	Lbif_m745_for	gaagccacttgtgcggcc	-	Used with Lbif_m745 _rev
	Lbif_m745_rev	cgcagcaga*ttc*aaaaaggtcagc	-	G249E substitution
	Lbif_m757_for	*a*tctgctgcg*ggc*gccacttgt	-	E253G substitution, used with Lb253rev. G249E/E253G substitution, used with Lbif_m757_rev
	Lbif_m757_rev	*t*caaaaaggtcagcacccataccc	-	Used with Lbif_m757_ for, part of the G249E

				substitution
Pa-PPase	Ppa_for	tata*catatg*ataagctatgccttactagg	NdeI	Cloning
	Ppa_rev	tata*aagctt*ttagaaaggcaatagacctgacg	HindIII	Cloning
Sc-PPase	Scop_for	ttt*catatg*gcggagcttcctacc	NdeI	Cloning
	Scop_rev	ttt*aagctt*ctacgaaaccacggccg	HindIII	Cloning
Restriction enzyme recognition sites and nucleotide substitutions are underlined and italicized within the primer sequences, respectively.				

Supplementary Figure S1.

Western blots of recombinant membrane PPases in *E. coli* IMV. IMV proteins (3−40 mg/ml) were denatured by incubating for 15 min with an equal volume of SDS loading buffer (139 mM Tris-HCl, pH 6.8, 22% glycerol, 4% SDS, 5 mM DTT and 0.5 mg/ml bromophenol blue) at 50°C, and separated (0.2–2 μg protein) on pre-cast mini SDS-PAGE gels (4–20% acrylamide gradient, Idgel). The electrophoresed samples were transferred to a nitrocellulose membrane (Whatman) in standard Towbin buffer (53) containing 20% (v/v) methanol for 1 h at 100 V in a Mini Trans-Blot apparatus (Bio-Rad). Membrane PPase bands were visualized by Western analysis using rabbit antiserum raised against conserved amino acid motifs in the third cytoplasmic loop of the enzyme (19).



1. no PPase
2. Ac-PPase
3. Ch-PPase
4. Cl-PPase
5. Cl-PPase E242D
6. Cl-PPase E242S
7. Cl-PPase G246A
8. Cl-PPase S180E/E242G
9. Cl-PPase S180E/E242G/G246A
10. Ct-PPase
11. Ctet-PPase
12. Da-PPase
13. Fj-PPase
14. Fj-PPase E185D
15. Fj-PPase E185S
16. Fj-PPase E185S/G247E
17. Lb-PPase
18. Lb-PPase E253D
19. Lb-PPase E253S
20. Lb-PPase G249E/E253G
21. Pa-PPase
22. Sc-PPase
23. Tm-PPase

REFERENCES FOR SUPPLEMENTAL DATA

19. Malinen, A. M., Belogurov, G. A., Baykov, A. A., and Lahti, R. (2007) *Biochemistry* **46**, 8872-8878
53. Towbin, H., Staehelin, T., and Gordon, J. (1979) *Proc Natl Acad Sci U S A* **76**, 4350-4354