

			TM4	
			* * *	
NcENA1	328	EVI-----IYAVATGLSMIP	AS	LVVVLTITMAAGT 370
SpCTA3	311	EVS-----IYAISLGISIIPESLIAVLSITMAMGQ		353
MpENA1	273	DII-----IYAVSLGIAVIPEGLIAVITIVMALGV		315
Na,K 3KDP_1	292	WLEA-----VIFLIGITIVANV	PE	GLLATVTVCLTLTA 334
H,K 7W4A_1	327	FLRA-----MVFFMAIVVAYV	PE	GLLATVTVCLSLTA 369
Na,K 2ZXE_1	322	WLEA-----VIFLIGITIVANV	PE	GLLATVTVCLTLTA 364
CaSERCA 1XP5_1	282	PVHGGSWIRGAIYYFKIAVALAVAA	PE	GLPAVITTCALGT 324
CaSERCA 7E7S_1	282	PVHGGSWIRGAIYYFKIAVALAVAA	PE	GLPAVITTCALGT 324
CaSERCA 2DQS_1	283	PVHGGSWIRGAIYYFKIAVALAVAA	PE	GLPAVITTCALGT 325
			:	: . : * . * . . : :
			TM5	
			* *	
NcENA1	804	IFDNIQKFVLHVLA	ENIA	QAGTLLIGLA 832
SpCTA3	754	MFDNIMRFVLHLLVSNVGEVILLVVGLA		782
MpENA1	711	IFSNIRKFILHLVSTNVGEVIVLIIGLA		739
Na,K 3KDP_1	743	IFDNLKKSIAYTTLTS	NI	PEITPFLIFII 771
H,K 7W4A_1	778	IFDNLKKSIAYTTLTKNI	PE	LTPYLIYIT 806
Na,K 2ZXE_1	773	IFDNLKKSIAYTTLTS	NI	PEITPFLVFII 801
CaSERCA 1XP5_1	753	IYNNMKQFIRYLISNVGEVVCIFLTAA		781
CaSERCA 7E7S_1	752	IYNNMKQFIRYLISNVGEVVCIFLTAA		780
CaSERCA 2DQS_1	754	IYNNMKQFIRYLISNVGEVVCIFLTAA		782
			::.*: : : : :	*: : .:
			TM6	
			* **	
NcENA1	848	IVWIIIMI	TS	GLPDMGLGF 866
SpCTA3	798	ILWCNMITSSFPSPMGLGM		816
MpENA1	755	ILFMNMVTSTPPAMALGV		773
Na,K 3KDP_1	782	ILCIDLGTDMVPAISLAY		800
H,K 7W4A_1	817	ILFIELCTDIFPSVSLAY		835
Na,K 2ZXE_1	812	ILCIDLGTDMVPAISLAY		830
CaSERCA 1XP5_1	792	LLWVNLVTDGLPATALGF		810
CaSERCA 7E7S_1	791	LLWVNLVTDGLPATALGF		809
CaSERCA 2DQS_1	793	LLWVNLVTDGLPATALGF		811
			::	: * . * . *
			TM7	
NcENA1	890	II	MIFYGLWITALCLASFV	910
SpCTA3	840	IVDMMVYGFFLGVS	SLMTWV	860
MpENA1	795	LADMMVYGIIMGSLILVDWV		815
Na,K 3KDP_1	824	LISMAYGQIGMIQALGGFFT		844
H,K 7W4A_1	859	LAAYSFYQIGAIQSFAGFTD		879
Na,K 2ZXE_1	854	LISMAYGQIGMIQALGGFFS		874
CaSERCA 1XP5_1	834	FFRYMAIGGYVGAATVGAAA		854
CaSERCA 7E7S_1	833	FFRYLAIGCYVGAATVGAAA		853
CaSERCA 2DQS_1	835	FFRYMAIGGYVGAATVGAAA		855
			:	
			TM8	
			*	
NcENA1	948	WFALFLAWELVDMRRSFFRMQ--	PGSKKYFTQ	980
SpCTA3	898	FCILIMAVEVKNFDNSLFNLHGIPWGEWNFRY		930
MpENA1	854	WMLLLHAYNCRHPRASLFTAEGGGASK-----		986
H,K 7W4A_1	923	YQQYTCYTVFFISIE	MC	IADVLIRKTRRLSA 955
Na,K 3KDP_1	888	IVEFTCHTPPFVTIVVV	Q	NADLVICKTRRNSV 920
Na,K 2ZXE_1	918	IVEFTCHTSFFISIVVV	Q	NADLIICKTRRNSI 950
CaSERCA 2DQS_1	892	FEAPEPMTMALSVLVTI	EMCNALNSLSENQSL	924
CaSERCA 7E7S_1	890	FESPYPMTMALSVLVTI	EMCNALNSLSENQSL	922
CaSERCA 1XP5_1	891	FEAPEPMTMALSVLVTI	EMCNALNSLSENQSL	923

Fig. S3. Alignment of the TM4-8 helices of P2-type ATPases. The sequences of three ENA ATPases (NcENA1, SpCTA3 and MpENA1), three P2A-type (2DQS_1, 7E7S_1, 1XP5_1) and three P2C-type (3KDP_1, 2ZXE_1, 7W4A_1) ATPases were chosen for alignment. Residues previously identified as being involved in cation binding in other P2-type ATPases are marked in green with an asterisk. Yellow indicates residues that have been mutagenized in NcENA1 and have shown a change in function.