

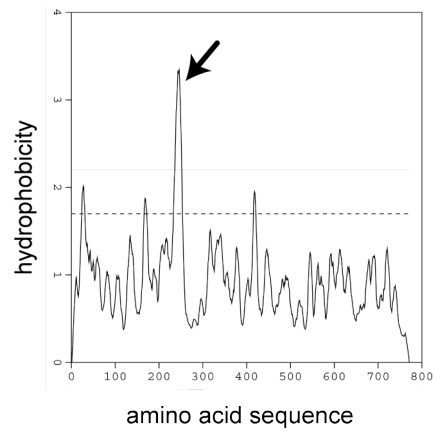
## Supplementary figures

Figure S1.

PaIAP	1	-----MRNAPLAKLALETSSQANVSSSTNLSVLSFMSPGSI
IAP-1	1	-----MSSRQL
YME1	1	-----MNVSKILVSPVTVTNVLRIAPRIPQIGASL
YME1L1	1	MFSLSSVTVQPQVTVPLSHLINAFTPKNTSVLSGSVSVSONQHRDVVPEHEAPSSSECMFSDFLTKLNIYS
PaIAP	37	AMANPLIRSSHAALMARRPLGTVPLGRFMSTTRPPIRMOSVSWPALKTLDALPPRNVOYRSFGNSNLVPH
IAP-1	6	AMANPLFRRSFSALMSR-PLGTVNTRLRSMSTHQPGRIPSFRRSPVHSSLGFT----LQVRSEFGNGGLS-H
YME1	32	LVQKKWALRSKFKYRFYSEKNSGEMPPKKEADSSGKASNKSISSIDNSQPPPSNTNDKTKQANVAVSH
YME1L1	71	IGKGIIFEGYRSMFMPEAKRMKSLDITDNWHIRPEPFLSLIFPSLNLRLDGLSELKIGQIDQLVENLLP
PaIAP	107	HLLSRTEAAANRNPOASPTQASFYQLLLKANMPAIVVERYQSGRFAANESATQAYNQALAMIAGILGSAG
IAP-1	71	NLLAAREAAANQFPTSAGAQYAFYQALLKANMPAIIERYQSGRFATNEQVDQIYQALAMSTGOPYTPA
YME1	102	AMLATREQFANKDLTSPDAQAAFYKLLQSNFYQYVVSRETPGIASSPECMELYMEALQRIQRHSEADA
YME1L1	141	GFCGKGNISSHWHTSHVSAQSFENKYGNLDIFSTLRSSCLYRHSRALQSCSDLQYWPVFIQSRGFKT
PaIAP	177	-----QASAGISEQAAAAGQATAAQRNGGNVAVSAGVTC--KGGALHVIIVDESFGSAAFR-----
IAP-1	141	NNTVDNNGYHPSGFTASQIHAAGTAAAAQHTGNNMAMVKPIAAGAKTGPLHIVVDESFGSSALR-----
YME1	172	VRQN---LITASSAGAVNPSLASSSSNQSGYHGNFSPMYSPLYGSRKEPLHVVVSESTFTVVS-----
YME1L1	211	LKSRRRLQSTSERLAETQNIAPSFVKGFLLRDRGSDVESLDKLMKTKNIPEAHQLAFKGTGFAEGFLKAO
PaIAP	230	-----WKKFMLWFSLCAYVSLVVMVMVVEIVSSLKRPGAKVDTMEAKAENQKARFSDVHGCDEAK
IAP-1	205	-----WVKFLMWFILFTYLSMVVITMVFEGLSIKRPGCKLEASEVKPENQKARFADVHGCDEAK
YME1	233	-----WVKWLLVFGILTYSFSEGFYITENTILLKSEVADKSDVAKTN--VKFDDVCGCDEAR
YME1L1	281	ALTQKTNDSLRRTLILFVLLLFGIYGLIKNPELSVRFRTTGLDSAVDPVQMKN--VTFEHWKGVVEAK
		<b>WA</b>
PaIAP	290	EELQELVDFLRNPKFNTLGGKLPKGVLLVGGPPTGKTLRARAVAGEAGVPFFMMSGSEFDEIVVGVGAK
IAP-1	265	EELQELDFLRNPEKYSTLGGKLPKGVLLVGGPPTGKTLRARAVAGEAGVPFFMMSGSEFDEIVVGVGAK
YME1	291	AELEETVDFLKDPTKYESLGGKLPKGVLLTGGPPTGKTLRARATAGEAGVDFMMSGSEFDEIVVGVGAK
YME1L1	349	QELQEVVDFLRNPKFNTLGGKLPKGVLLVGGPPTGKTLRARAVAGEADVPFFYASGSEFDEIVVGVGAS
		<b>WB</b> <span style="float: right;"><b>SRH</b></span>
PaIAP	360	RVRELFNAAKAKSPSIVFIDELDAIGGKRNSR-DATYVRQTLNQLLTEMDFGFSQNSGVIIIAATNFPESL
IAP-1	335	RVRDLFAAAKAKAPSIVFIDELDAIGGKRNSR-DATYVRQTLNQLLTELDFGFSQNSGVIIIGATNFPESL
YME1	361	RIRDLFAQARSFAPAIIFIDELDAIGGKRNPK-DOAYAKQTLNQLLVELDFGFSQNSGIIIGATNFPESL
YME1L1	419	RIRNLFREAKANAPCVIFIDELDSVGGKRIESPMHPYSRQTLNQLLAEMDFGFSQNSGVIIIGATNFPESL
		<b>SRH</b>
PaIAP	429	DKALTRPGRFDRHVVVSLPDVVRGRIAILKHHAKIKMAADVREDIAGRTSGLSGAELENIVNQAAIHAS
IAP-1	404	DKALTRPGRFDRNVVVSLPDVVRGMAILQHAKRIKAAADVNLDAIASRTSGLSGAELENIVNQAAIHAS
YME1	430	DKALTRPGRFDKVVVNDLDPVVRGRADILKHHMKKITTADNVDFTIIARGTFLSGAELENIVNQAAIYAC
YME1L1	489	DNALTRPGRFDMQVTVPRPDVVRGRTEILKWWYLNKIKFDQSVDPDEIARGTFLSGAELENIVNQAAALKAA
		<b>MB</b>
PaIAP	499	KLKNKVVTKDMFEWAKDKVIMGAEKRSMTIPKEKEMTAYHEAGHALVAFENKQEGGSHLYKVTILPRGQ
IAP-1	474	KLKAQAVTKDFEWAKDKVIMGAEKRSMTITAKEKEMTAYHEAGHALVGYAKDS-ASSLYKVTILPRGQ
YME1	500	QKNVSVDMSHFEWAKDKIIMGAERKTMVITDAARKATAHEAGHAIMAKYTN--ATPLYKATILPRGR
YME1L1	559	VDGKEMVTMKLELFSKDKIIMGFERRSVETDNKNTITAYHESGHAIAYYTKD--AMPINKATILPRGP
PaIAP	569	SLGHTAFLPEMDKYSYTVRDYLA MIDRALGGKVAEEIVYGSEFVTSGVSADLDSATRTAWHMVAQLGMSP
IAP-1	543	TLGHTAYLPEMDKHSFTVRDYLGMIDRAMGGKVAEEIVYGNELVTSGVSA DLDMATRTAWQMVQALGMSE
YME1	568	ALGITFQLPEMDKVDITKRCQARLDVCMGGKIAEELTYGKDNNTSGCGSDLOSATGTARAMVTOYGMSD
YME1L1	627	TLGHVSLLPENDRWNETRAQLLAQMDVSMGGFVAEELIFGTDHITIGASSDFDNATKIAKRMVTKFGMSE
PaIAP	639	KLGPVEYLRYNELSSETRAMVESEVKVLLDSYARARALLSKRTELDLLAKALVEYETLDHDEIVKVL
IAP-1	613	KLGPVEYLRYNQLSSETRAMVESEVKRVLDFSYERARNLLTSKRNELDYLAKALVEYETLDKKEVERVI
YME1	638	DVGPVNLSNWEWSNKRIDLADNEVIELKDSERARRLLTKKNVELHRLAQLLEYETLDAHEIEQVC
YME1L1	697	KLGVMTYSDTGKLSPETQSAIEQEIIRILRDSYERAKHILKHAKEHKNLABALLTYETLDAKETQIVL
PaIAP	709	RGEKLTDRIAVFPVGPMTVQAPTDPLEPGLPLPGLGDDGDCGSGGPPPPAPPPPPAPARTSSEEK
IAP-1	683	RGEKLDKRLSVPPGPMALPKPSDTLEPGLPLPPLPGDVPVPPGDSGPPGPPPPVPA-----
YME1	708	RGEKLDKRLSTNTVVEGPDSDERKDIGDDRKIPTMLNA-----
YME1L1	766	EGKKLEVR-----

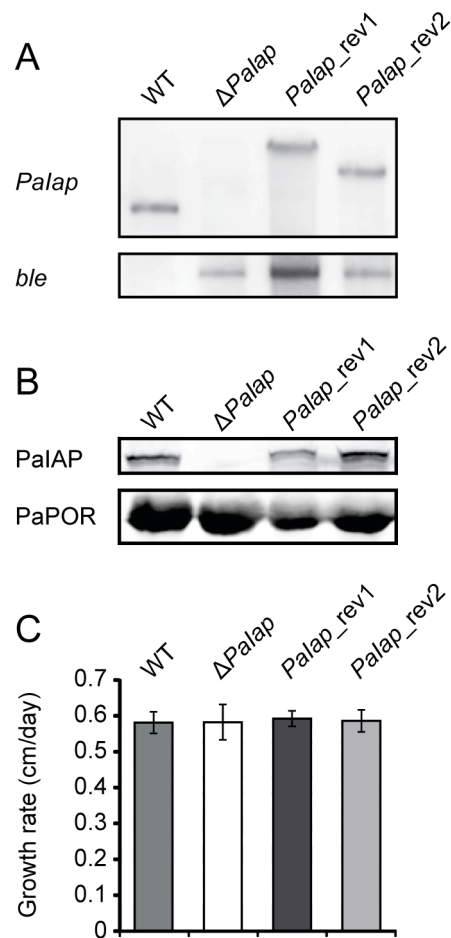
**Figure S1. Sequence alignment of i-AAA protease subunits of *P. anserina*, *N. crassa*, *S. cerevisiae* and *H. sapiens*.** The sequences of PalAP of *P. anserina*, IAP-1 of *N. crassa*, YME1 of *S. cerevisiae* and YME1L1 of *H. sapiens* were aligned using the program ClustalW (version 1.83). Identical amino acid residues in all four sequences are shown white on black. Amino acid residues that are conserved in the sequences are shaded in gray. The Walker A (WA) and Walker B (WB) motifs, the second region of homology (SRH), which are characteristic of the AAA family of ATPases, and the consensus metal binding motifs (ME), representing the putative catalytic centers, are indicated.

**Figure S2.**



**Figure S2. Hydrophobicity profile of PaIAP.** The presence of a transmembrane region in the amino acid sequence of PaIAP was analyzed using the DAS software ([www.sbc.su.se/~miklos/DAS](http://www.sbc.su.se/~miklos/DAS)). The hydrophobic region that presents the predicted transmembrane domain is indicated by an arrow.

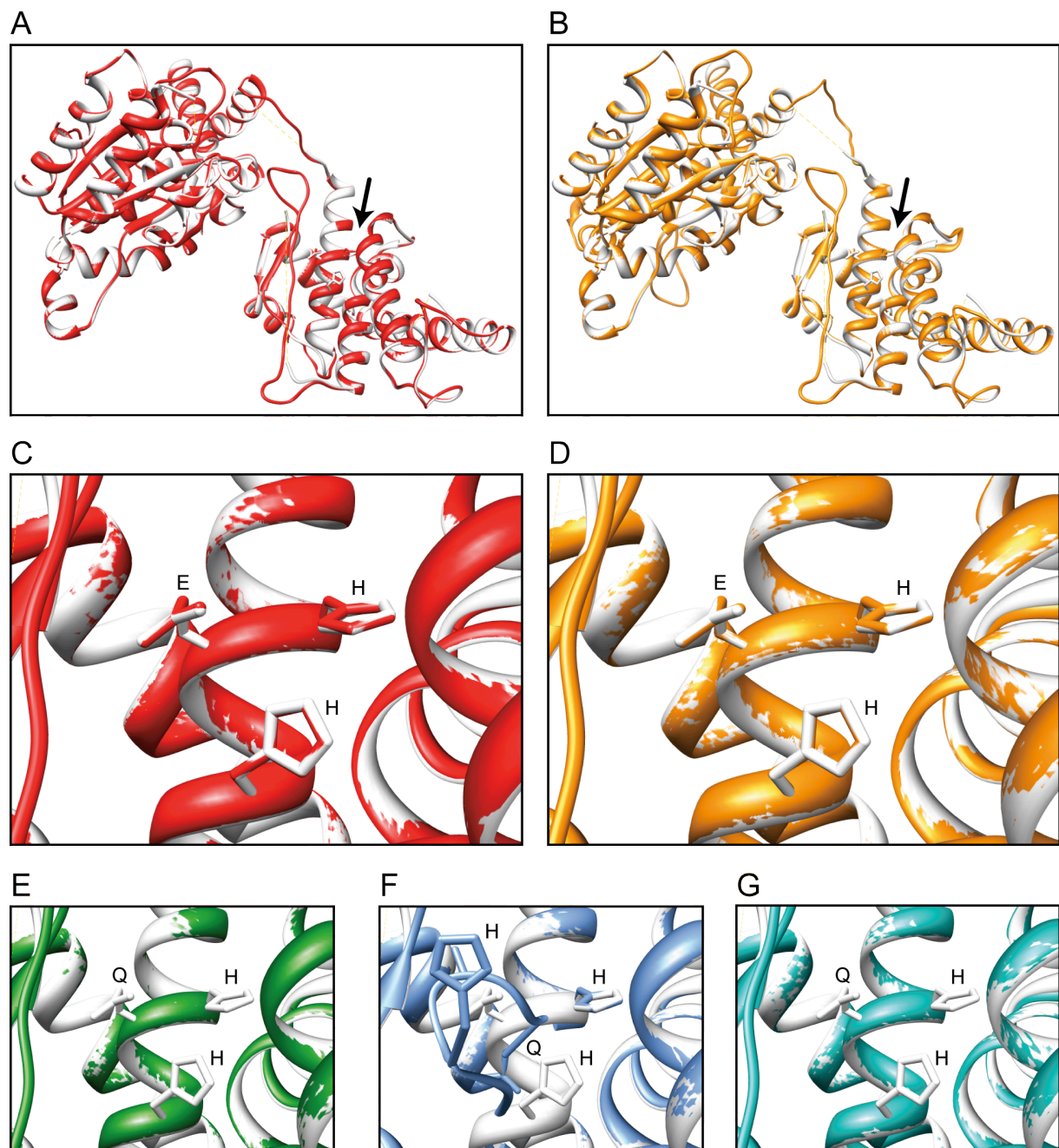
**Figure S3.**



**Figure S3. Reintroduction of *Palap* into  $\Delta$ *Palap* leads to a wild-type like phenotype.**

(A) Southern blot analyses of *Bgl*II digested DNA from the WT,  $\Delta$ *Palap* and *Palap* deletion strains in which the ORF of *Palap* under the control of its own promoter and terminator has been reintroduced (*Palap\_rev*). A *Palap* specific probe reveals that *Palap\_rev1* and 2 carry a single copy of the *Palap* wild-type gene. (B) Western blot analyses of mitochondrial proteins from the WT,  $\Delta$ *Palap* and *Palap\_rev1* and 2. The PalAP specific antibody detects PalAP in mitochondria of the WT as well as in mitochondria from *Palap\_rev1* and 2 (upper panel). PaPOR was used as loading control (lower panel). (C) Growth rates of the WT (n = 71),  $\Delta$ *Palap* (n = 73), *Palap\_rev1* (n = 59) and *Palap\_rev2* (n = 40).

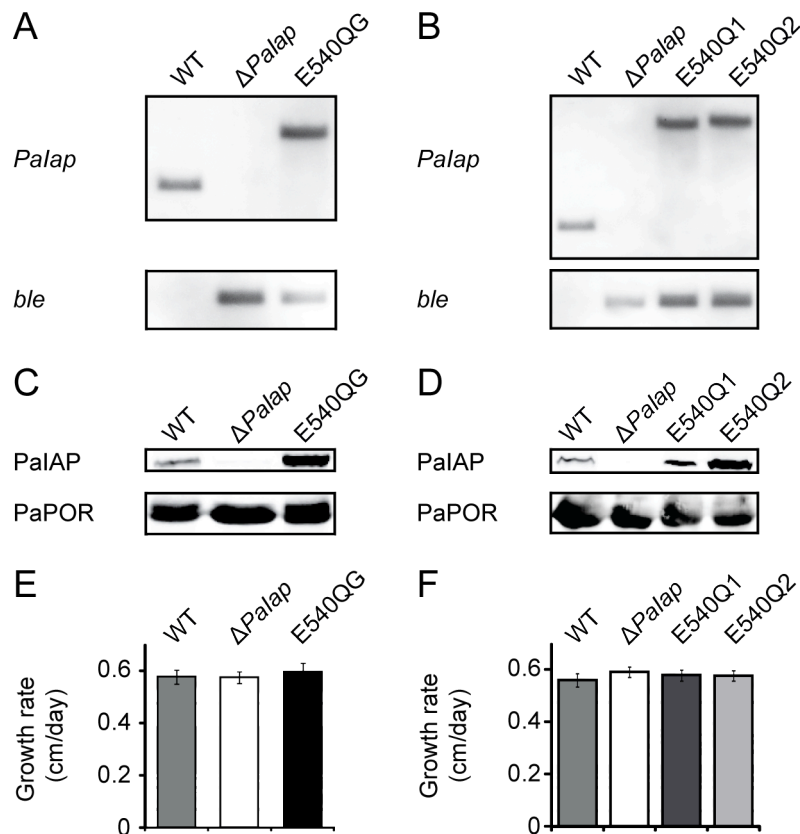
**Figure S4.**



**Figure S4. Models of the protein structures of PalAP and YME1 compared to their homologue FTSH from *Thermotoga maritima*.** Ribbon view of FTSH from the bacterium *T. maritima* shown in white,<sup>59</sup> PalAP in red (A) and YME1 in orange (B). To estimate the effect of the mutation in the metal binding motif HEXXH on the structure of the i-AAA protease, we generated homology models for the structurally related proteins PalAP, YME1 and FTSH with SWISS-MODEL using the crystal structure of a soluble FTSH construct.<sup>60</sup> In the model, replacement of glutamic acid (E) to glutamine (Q) does not seem to change the structure of the helix in PalAP (red in (C) and green in (E)) and in YME1 (orange in (D) and turquoise in (G)). In contrast, the addition of glycine (G) in the metal binding motif of PalAP creating the

amino acid sequence HQGAGH possibly alters the structure of the helix (blue in (F)). The molecular graphics were produced using UCSF Chimera.<sup>61</sup>

**Figure S5.**



**Figure S5. Manipulation of the proteolytic activity of PalAP.** (A) and (B) Southern blot analyses of DNA from the WT,  $\Delta$ Palap and Palap deletion strains in which the ORF of Palap carrying a mutation in the metal binding motif at amino acid position 540 (Palap\_E540Q1 and 2) followed by glycine has been reintroduced (Palap\_E540QG, Palap\_E540Q1 and 2). A Palap specific probe shows the presence of one copy of the modified Palap gene in Palap\_E540QG (E540QG), Palap\_E540Q1 (E540Q1) and Palap\_E540Q2 (E540Q2). (C) and (D) Western blot analyses of mitochondrial proteins from the WT,  $\Delta$ Palap, Palap\_E540QG (E540QG), Palap\_E540Q1 (E540Q1) and from Palap\_E540Q2 (E540Q2). PalAP can be detected in mitochondria of the WT as well as in samples from Palap\_E540Q and Palap\_E540QG (upper panel). PaPOR was used as loading control (lower panel). (E) Growth rates of the WT (n = 21),  $\Delta$ Palap (n = 34) and Palap\_E540QG (E540QG; n = 30). (F) Growth rates of the WT (n = 24),  $\Delta$ Palap (n = 24), Palap\_E540Q1 (E540Q1; n = 35) and Palap\_E540Q2 (E540Q2; n = 27).

#### Reference list

59. Bieniossek C, Schalch T, Bumann M, Meister M, Meier R, Baumann U. The molecular architecture of the metalloprotease FtsH. Proc Natl Acad Sci USA 2006; 103: 3066-3071

60. Schwede T, Kopp J, Guex N, Peitsch MC. SWISS-MODEL: An automated protein homology-modeling server. *Nucleic Acids Res* 2003; 31: 3381-3385.
61. Pettersen EF, Goddard TD, Huang CC, Couch GS, Greenblatt DM, Meng EC, et al. UCSF Chimera--a visualization system for exploratory research and analysis. *J Comput Chem* 2004; 25: 1605-1612.