Human CLPP reverts the longevity phenotype of a fungal *ClpP* deletion strain

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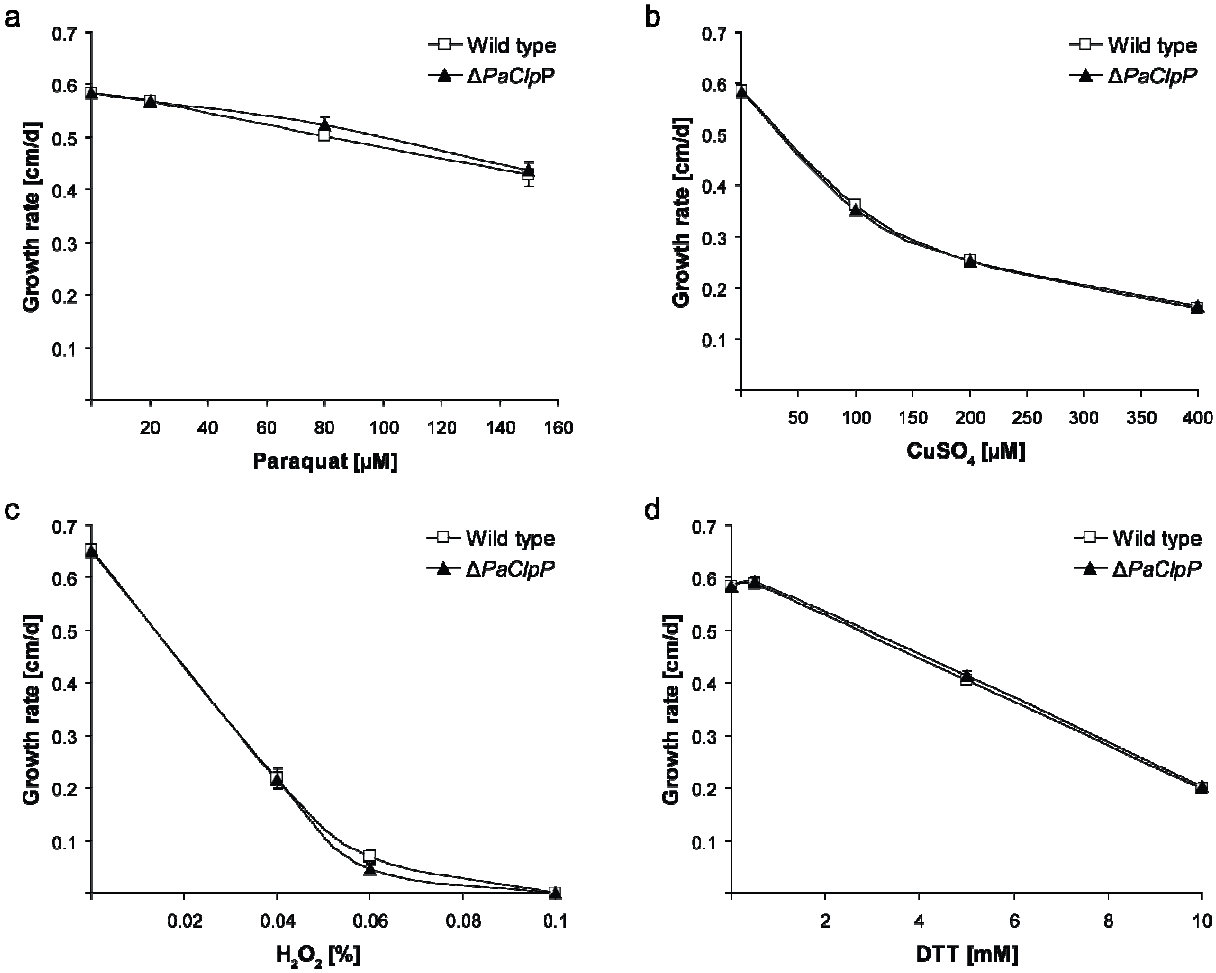
SUPPLEMENTARY INFORMATION

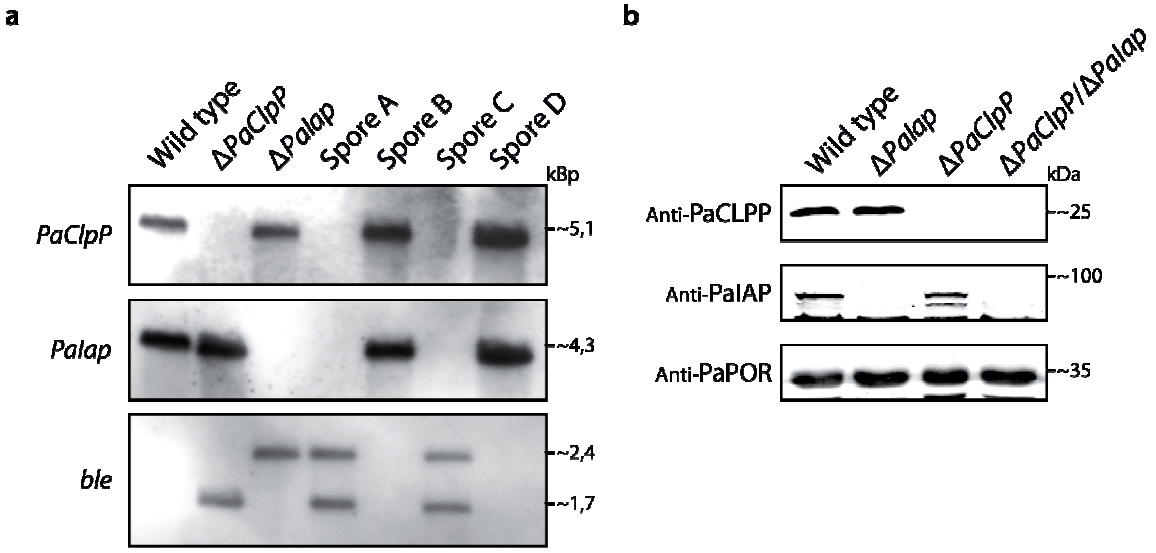
Supplementary Figures S1 to S5

Supplementary Figure S1 | Susceptibility of Δ*PaClpP* to various oxidative stressors and DTT-induced ER stress is not altered.



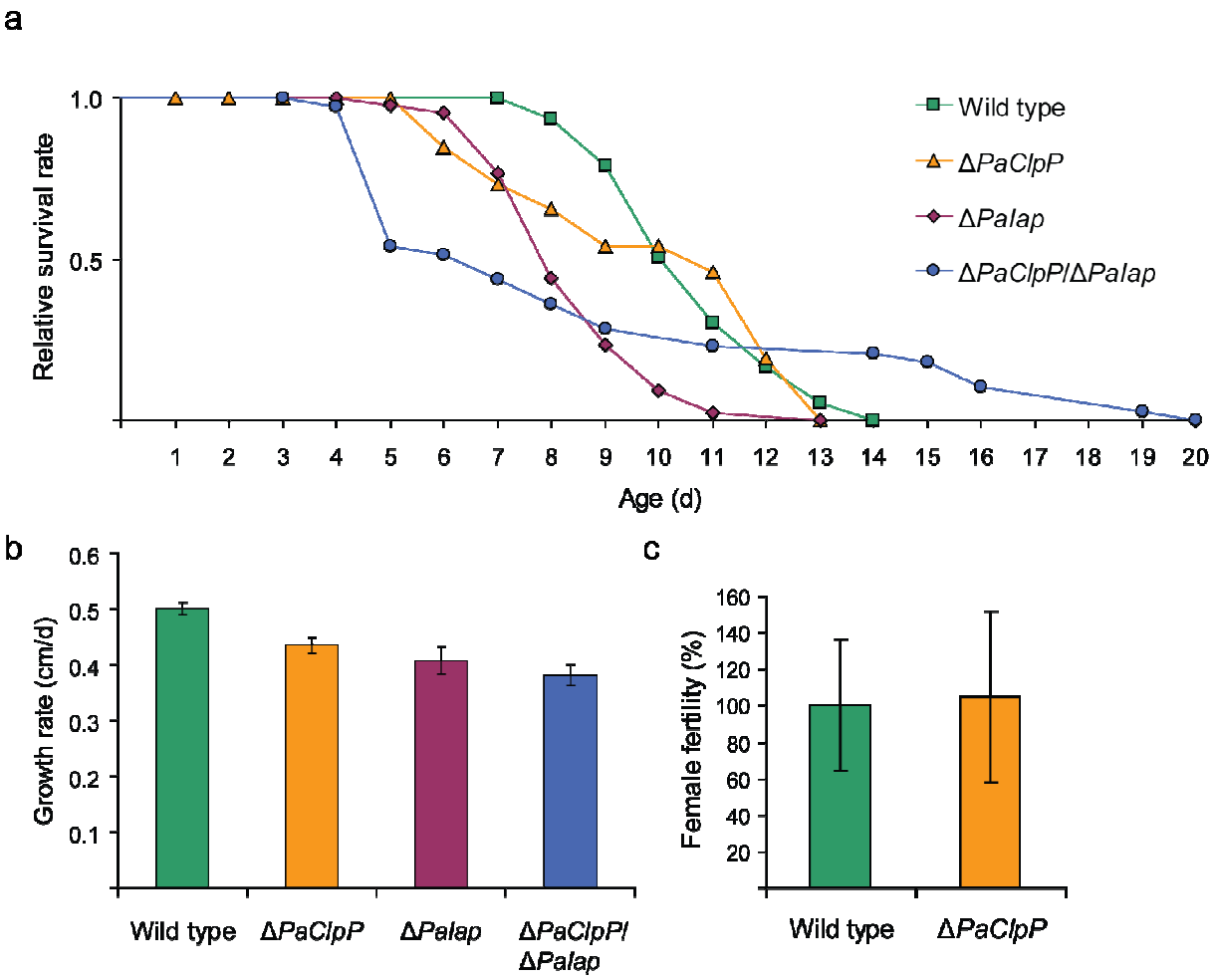
Supplementary Figure S1 | (**a**) Growth rate of wild type (n = 16) and Δ*PaClpP* (n = 16) on M2 medium containing 0, 20, 80, or 150 µM paraquat. Growth rate of Δ*PaClpP* was unchanged compared to the wild type at any concentration tested. (**b**) Growth rate of wild type (n = 16) and Δ*PaClpP* (n = 16) on M2 medium containing 0, 100, 200, or 400 µM $CuSO_4$. Growth rate of Δ*PaClpP* was unchanged compared to the wild type at any concentration tested. (**c**) Growth rate of wild type (n = 16) and Δ*PaClpP* (n = 16) on M2 medium containing 0, 0.04, 0.06, or 0.1 % H_2O_2. Growth rate of Δ*PaClpP* was unchanged compared to the wild type at any concentration tested. (**d**) Growth rate of wild type (n = 16) and Δ*PaClpP* (n = 16) on M2 medium containing 0, 0.5, 5, or 10 mM DTT. Growth rate of Δ*PaClpP* was unchanged compared to the wild type at any concentration tested. Data points depicted in all graphs are mean growth rate ± s.e. in centimetres per day.

Supplementary Figure S2 | Southern and Western blot verification of a Δ*PaClpP*/Δ*PaIap* double deletion strain.



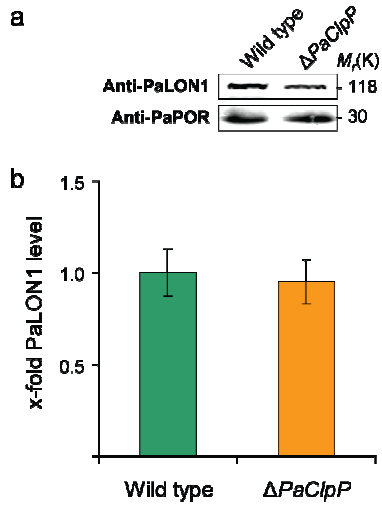
Supplementary Figure S2 | (**a**) Southern blot analysis of *Bgl*II digested genomic DNA from wild type, Δ*PaClpP*, Δ*PaIap*, and of four dikaryotic offspring, two of which were resistant (Spore A and C) and two of which were sensitive (Spore B and D) to phleomycin, isolated from a single recombined ascus resulting from a cross between Δ*PaClpP* and Δ*PaIap*. The two offspring Spore A and C contain the concomitant deletion of *PaClpP* and *PaIap*, as verified by absence of these genes in their genomic DNA and presence of two phleomycin resistance genes (*ble*) instead, and were termed Δ*PaClpP*/Δ*PaIap*. For subsequent experiments, monokaryotic offspring were isolated from self-crosses of these dikaryotic Δ*PaClpP*/Δ*PaIap* strains. (**b**) Representative Western blot analysis of mitochondrial protein extracts from wild type, Δ*PaClpP*, Δ*PaIap*, and Δ*PaClpP*/Δ*PaIap*. Detection with a PaCLPP- and a PaIAP-specific antibody confirms absence of PaCLPP and PaIAP in the double deletion strain. PaPORIN (PaPOR) was detected as a loading control.

Supplementary Figure S3 | CLPP and *i*-AAA are cooperatively involved in a mitochondrial heat stress response.



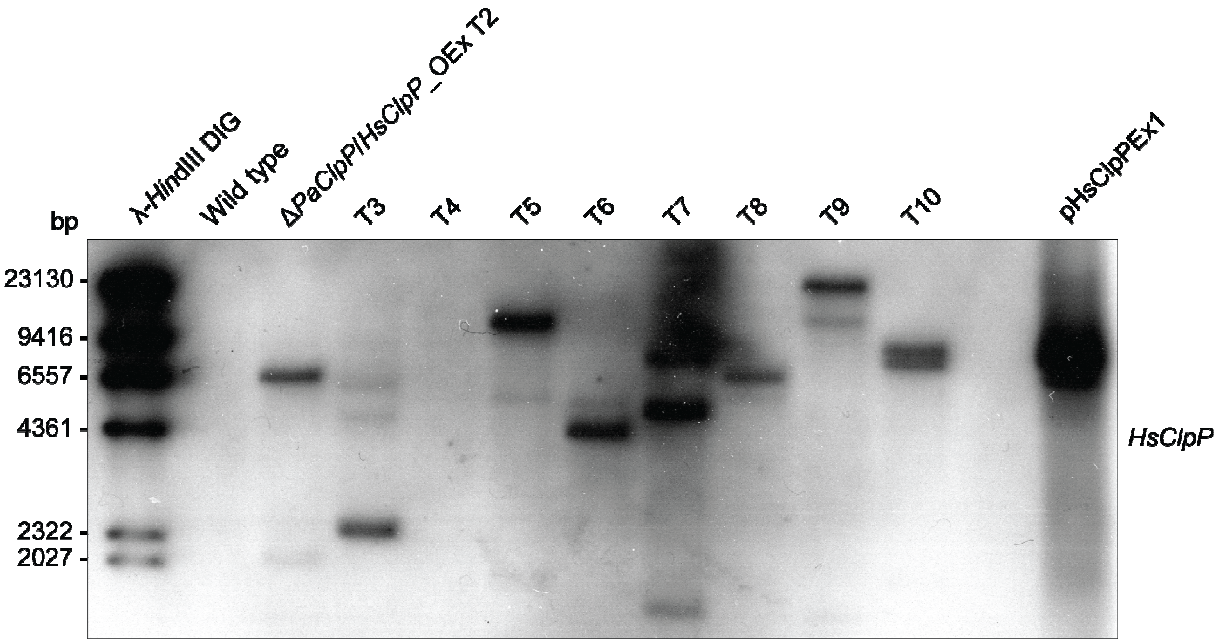
Supplementary Figure S3 | (**a**) Lifespan of wild type (10.8 ± 0.2; n = 109), Δ*PaClpP* (10.0 ± 0.5; n = 26; *P* = 0.36), Δ*Palap* (8.5 ± 0.2; n = 43; *P* = 2.2E-11), and Δ*PaClpP*/Δ*Palap* (8.8 ± 0.8; n = 39; *P* = 2.8E-05) isolates at 37 °C. Data given in parentheses are mean lifespan ± s.e. in days. *P*-values were determined in comparison to the wild-type sample by two-tailed Wilcoxon rank-sum test. (**b**) Growth rate of wild type (0.50 ± 0.01; n = 109), Δ*PaClpP* (0.44 ± 0.01; n = 26; *P* = 2.4E-04), Δ*Palap* (0.41 ± 0.02; n = 43; *P* = 2.1E-04), and Δ*PaClpP*/Δ*Palap* (0.38 ± 0.02; n = 37; *P* = 1.1E-07) isolates at 37 °C. Data given in parentheses are mean growth rate ± s.e. in centimetres per day. *P*-values were determined in comparison to the wild-type sample by two-tailed Wilcoxon rank-sum test. (**c**) Female fertility of wild type (100 ± 36.1; n = 11) and Δ*PaClpP* (105 ± 46.7; n = 11; *P* = 0.82 by two-tailed Wilcoxon rank-sum test) isolates at 37 °C. Data given in parentheses are mean female fertility ± s.e. in %.

Supplementary Figure S4 | LON protease abundance is unchanged in Δ*PaClpP*.



Supplementary Figure S4 | (**a**) Representative Western blot analysis of mitochondrial protein extracts from wild type and Δ*PaClpP*. The PaLON1-specific antibody detects the ~118 kDa PaLON1 monomer in both samples. PaPORIN (PaPOR) was detected as a loading control and for quantification. (**b**) Quantitative Western blot analysis of mitochondrial protein extracts from wild type (1.00 ± 0.13; n = 6) and Δ*PaClpP* (0.95 ± 0.12; n = 6; *P* = 0.63 by two-tailed Student's t-test). The PaLON1 protein level was normalised to that of PaPOR and the mean wild-type PaLON1/PaPOR quotient was defined as 1-fold PaLON1 level. Data given in parentheses are mean PaLON1 level ± s.e. in arbitrary units.

Supplementary Figure S5 | Southern blot verification of Δ*PaClpP*/*HsClpP*_OEx transformants.



Supplementary Figure S5 |Southern blot analysis of *Hin*dIII digested genomic DNA from wild type and the hygromycin B resistant Δ*PaClpP*/*HsClpP*_OEx transformants T2-T10. The *HsClpP*-specific hybridization probe visualises integration of the plasmid pHsClpPEx1, containing the full-length ORF of the human *ClpP* cDNA, into the transformants' genome. 5 ng of *Hin*dIII digested pHsClpPEx1 plasmid DNA were used as a control for the *HsClpP*-specific hybridization probe. The two independent transformants T2 and T6, each with a single integration of the vector into their genome, were selected for subsequent experiments and termed Δ*PaClpP*/*HsClpP*_OEx1 and 2, respectively.