## Supplemental information

### *Cloning and mutagenesis procedures*

*CCP1* or a *CCP1* variant encoding a twelve histidine-tag fused to the C-terminus of Ccp1 (Ccp1-his) was cloned into the Eco*RI* and Xho*I* sites of pGEM4. For constitutive expression in yeast, *CCP1* and derivatives thereof were cloned into the Eco*RI* and Sac*I* sites of pYX142 or pYX132. Mutations in Ccp1, in conserved ATPase and proteolytic motifs of Yta10 and Yta12 ($Yta10^{D634A}$ and $Yta12^{D689A}$, $Yta10^{K334A}$ and $Yta12^{K394A}$, $Yta10^{E388Q}$ and $Yta12^{E448Q}$), and in the conserved central pore loop motifs of Yta10 and Yta12 ($Yta10^{F361S}$ and $Yta12^{F421S}$, $Yta10^{F361A}$ and $Yta12^{F421A}$, $Yta10^{F361E}$ and $Yta12^{F421E}$; $Yta10^{G363A}$ and $Yta12^{G423A}$, $Yta10^{M360K}$ and $Yta12^{M420K}$) were introduced using the PCR based Quick Change mutagenesis kit (Stratagene). Plasmids for expression of Yta10, his-tagged Yta10, Yta12 and their mutant variants carrying a mutation in the metalloprotease motif ($Yta10^{E559Q}$ or $Yta12^{E614Q}$) under the control of *ADH1* promoter were described previously (Arlt et al., 1998).

### *Hydropathy analysis*

The hydropathy of Ccp1 segments was determined using the MPEx programme (http://blanco.biomol.uci.edu/mpex/) with following parameters; ΔCONH value: 0; window length: 19; scale: WW octanol; aspartate and glutamate residues charged, histidine residues uncharged; partitioning: bilayer to water.

### *Determination of ATPase activities* in vitro

ATPase assays were performed essentially as described (Lill et al., 1990). Briefly, *m*-AAA protease complexes were dissolved in 50 µl buffer F (50 mM Tris-HCl pH 8.0, 150 mM NaCl, 10% (v/v) glycerol, 300 mM imidazole, 0.2 % (v/v) NP-40, 2 mM ATP, 4 mM magnesium

acetate, 1 mM DTT, 25 µM zinc acetate) at a final concentration of 3.5 nM (based on a AAA protease hexamer) and incubated at 30°C for various time points. Phosphate was quantified colorimetrically using malachite green.

***Yeast strains and growth conditions***

Yeast strains used in this study are listed in Table S1 (host strains) and S2 (transformants). Genes were deleted by polymerase chain reaction (PCR)-targeted homologous recombination (Wach et al., 1994). Cells were cultivated at 30°C in YP medium supplemented with 2 % (w/v) glucose or 2 % (w/v) galactose and 0.5 % (w/v) lactate, or synthetic complete (SC) medium supplemented with 2 % (w/v) galactose and 0.1 % (w/v) glucose if not indicated.

## Supplemental references

Glick, B.S., Brandt, A., Cunningham, K., Muller, S., Hallberg, R.L. and Schatz, G. (1992) Cytochromes c1 and b2 are sorted to the intermembrane space of yeast mitochondria by a stop-transfer mechanism. *Cell*, **69**, 809-822.

Lill, R., Dowhan, W. and Wickner, W. (1990) The ATPase activity of SecA is regulated by acidic phospholipids, SecY, and the leader and mature domains of precursor proteins. *Cell*, **60**, 271-280.

Wach, A., Brachat, A., Poehlmann, R. and Philippsen, P. (1994) New heterologous modules for classical or PCR-based gene disruptions in *Saccharomyces cerevisiae*. *Yeast*, **10**, 1793-1808.