

Supplemental Data

Supplemental Figure Legend

Fig. S1. *A.* Top down views of reference-free 2D class averages. The numbers in each box represent the number of particles in each class (top) and the class number (bottom). *B.*, *C.*, and *D.* are top down views of isosurface representations of the FtsH_{Cyt} hexamers (1-3) calculated at 12 Å resolution from the atomic coordinates of the X-ray structures. *E.* Top down view of the cryo-EM reconstruction of the *m*-AAA protease. For clarity, only the ATPase and protease rings are shown.

Fig. S2. Fourier shell correlation between reconstructions from even and odd halves of the data set. The resolution of the final reconstruction is 12 Å based on the 0.5 criterion of the Fourier shell correlation.

Fig. S3. Sequence alignment of proteolytic domains of AAA protease subunits. Conserved amino acid residues are highlighted black, whereas those with similar chemical properties are highlighted in grey. Residues in Yta12, which when mutated allow respiratory growth, are indicated by vertical arrows. α -helices and β -strands are depicted by horizontal cylinders and arrows, respectively. Zinc binding motifs (HExxH and D, the conserved Zn²⁺ binding aspartate) are indicated. Sc, *S. cerevisiae*; Hs, *H. sapiens*; Ec, *E. coli*; Tm, *T. maritima*; Tt, *T. thermophilus*.

Fig. S4. Assessment of respiratory growth of $\Delta yta10\Delta yta12$ cells expressing Yta12 variants that harbor single mutations only.

Supplemental References

1. Suno, R., Niwa, H., Tsuchiya, D., Zhang, X., Yoshida, M., and Morikawa, K. (2006) *Mol. Cell* **22**, 575-585
2. Bieniossek, C., Schalch, T., Bumann, M., Meister, M., Meier, R., and Baumann, U. (2006) *Proc. Natl. Acad. Sci. USA* **103**, 3066-3071
3. Bieniossek, C., Niederhauser, B., and Baumann, U. M. (2009) *Proc. Natl. Acad. Sci. USA* **106**, 21579-21584