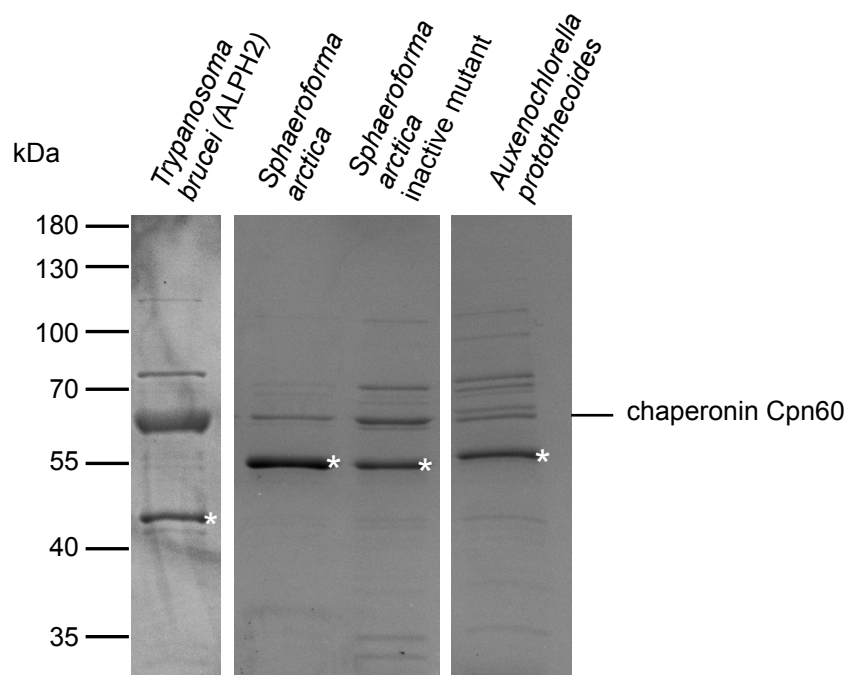


Figure S1: Maximum Likelihood tree of ALPH in eukaryotes.

This tree was built from catalytic domains (insertions removed) of 384 ALPH proteins; all sequences are listed in Supplementary Table S1b. The PhyML tree was inferred using substitution models determined by Prot-Test (1). Bootstrapping was performed for 1000 replicates and the best tree topology was inferred. Bootstrap values above 80 are significant.

(1) Abascal, F., Zardoya, R. & Posada, D. ProtTest: selection of best-fit models of protein evolution. *Bioinformatics* 21, 2104–2105 (2005).

A



B

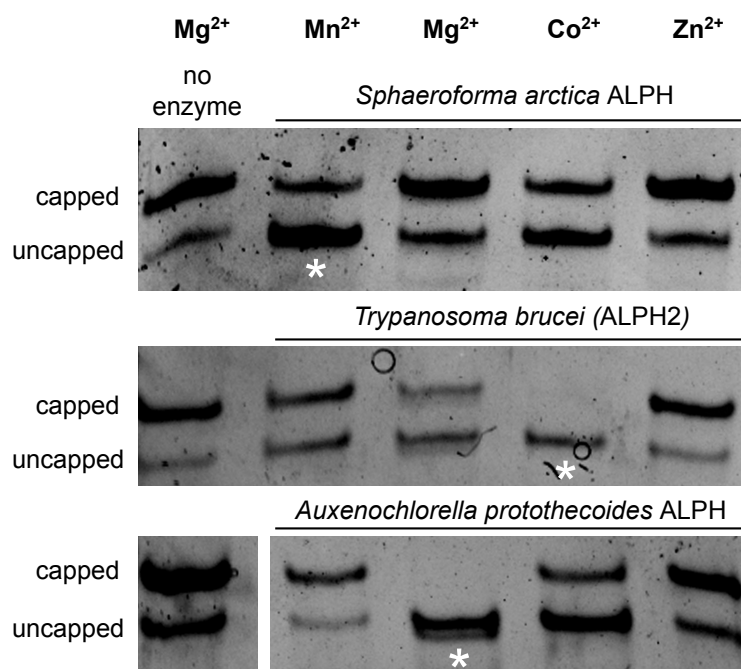


Figure S2: Recombinant ALPH proteins: production and ion requirements

(A) Recombinant ALPH proteins were produced in Arctic Express DE cells and purified via Nickel affinity chromatography. A Coomassie stained gel loaded with the purified proteins (marked by asterisks) is shown. Note that the Arctic Express DE cells contain chaperons. Chaperonin Cpn60 is often co-purified, in the case of TbALPH2 to a large extent.

(B) The enzymes were tested for *in vitro* decapping activity in the presence of 1 mM of divalent ions, as indicated. A reaction without enzyme served as negative control. For each enzyme, an asterisk marks the conditions with best decapping activity. The gels are representative gels out of at least three replicates.