Blamowska et al., http://www.jcb.org/cgi/content/full/jcb.201205012/DC1

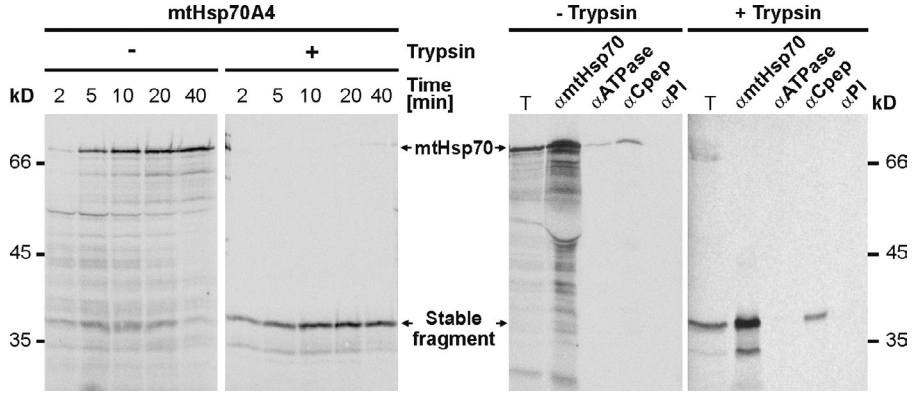

Figure S1. **The interdomain linker is needed for folding of the ATPase domain of mtHsp70.** In the mtHsp70A4 variant of mtHsp70, the linker amino acid residues 412–415 of mtHsp70 were replaced by four alanine residues. (left) Radiolabeled precursor of the mtHsp70A4 linker mutant was imported into wild-type mitochondria for the time periods indicated. Nonimported material was digested with trypsin, and mitochondria were subsequently reisolated and solubilized. Half of the sample was mock treated (−Trypsin); the other half was treated with trypsin (+Trypsin). Samples were analyzed by SDS-PAGE and autoradiography. (right) Imported material as well as the stable trypsin-resistant fragment were subjected to immunoprecipitation with antibodies against full-length mtHsp70 (αmtHsp70), ATPase domain (αATPase), and the C terminus of mtHsp70 (αCpep) and with preimmune IgGs (PI). T indicates 10% of total material subjected to immunoprecipitation, and precipitated material was analyzed by SDS-PAGE and autoradiography.

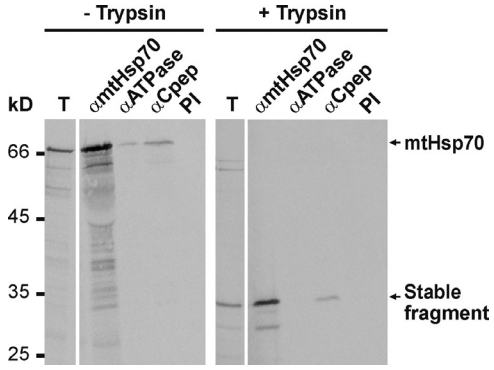

Figure S2. **The stable fragment of imported mtHsp70 that is obtained upon trypsin treatment of *Δhep1* mitochondrial lysate corresponds to the PBD of mtHsp70.** Radiolabeled mtHsp70 precursor was imported into *Δhep1* mitochondria for 30 min. Import was stopped, and samples were further incubated for 120 min. Then solubilized *Δhep1* mitochondria were either mock treated (−Trypsin) or treated with trypsin (+Trypsin). The samples were subjected to immunoprecipitation with antibodies against full-length mtHsp70 (αmtHsp70), the ATPase domain (αATPase), and the C terminus of mtHsp70 (αCpep) and with preimmune IgGs (PI). T indicates 10% of total material subjected to immunoprecipitation, and precipitated material was analyzed by SDS-PAGE and autoradiography. Imported mtHsp70 in the mock-treated sample as well as the stable fragment in the trypsin-treated lysate of *Δhep1* mitochondria were present similarly as in the samples analyzed in Fig. 2 A. The stable fragment was immunoprecipitated with antibodies against the C terminus of mtHsp70.

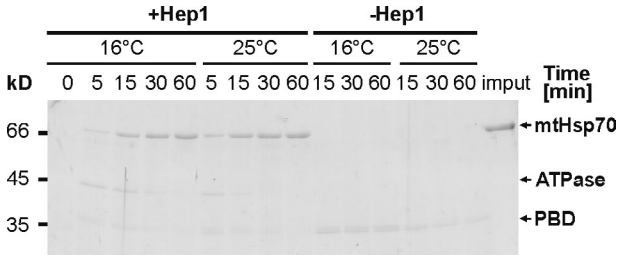

Figure S3. **Folding of mtHsp70 in vitro is dependent on Hep1 at lower temperature.** Unfolded mtHsp70 was diluted into ATP-containing buffer with or without the addition of recombinant Hep1 and incubated at 16°C or 25°C. At the time points indicated, samples were withdrawn and treated with trypsin to assess the folding state of mtHsp70. Samples were analyzed by SDS-PAGE and Coomassie staining.