



**Figure S2. A**, Synthesis of proteins in reticulocyte lysate in the presence of  $^{35}\text{S}$ -methionine. AAC<sub>WT</sub> (AAC<sub>1-313</sub>; 1), AAC $\Delta$ pos (AAC<sub>1-313</sub>, positively charged amino acids of the three matrix loops exchanged to glycine; 2) III-DHFR (AAC<sub>221-313</sub> fused to mouse dihydrofolate reductase (DHFR), the white arrow indicates translation product of a second reading frame of the plasmid; 3), ML3-TMD6-DHFR (AAC<sub>238-313</sub> fused to DHFR; 4), ML3-DHFR (AAC<sub>238-277</sub> fused to DHFR; 5), TMD6-DHFR (AAC<sub>278-313</sub> fused to DHFR; 6). **B**, Import of ML3-TMD6-DHFR into isolated mitochondria. Isolated wildtype mitochondria or mitochondria isolated from a yeast strain containing a deletion of the gene encoding Tom70 (*tom70*Δ) were incubated with [ $^{35}\text{S}$ ]-labeled ML3-TMD6-DHFR in reticulocyte lysate for 10 min at 25 °C, reisolated and resuspended in SEM buffer. For assessment of the amount of imported protein, mitochondria were incubated in the presence of 75  $\mu\text{g}/\text{ml}$  proteinase K on ice for 10 min and the reaction was subsequently stopped by addition of 4 mM PMSF on ice for 5 min. Mitochondria were reisolated, washed with SEM buffer and subjected to SDS-PAGE to determine the amounts of bound and imported protein, SD, n=3.

Fig. S2