Figure S1. **Stability of Atp23ΔMPP2ΔMPP3 and distribution of iMTS scores in proteins.** (A) Radiolabeled Atp23ΔMPP2ΔMPP3 was imported into isolated mitochondria for 30 min at 25°C. Mitochondria were reisolated, washed, and incubated in import buffer at 25°C for the times indicated. The amount of imported Atp23ΔMPP2ΔMPP3 was visualized by autoradiography. The rather constant amounts of imported Atp23ΔMPP2ΔMPP3 indicate that this protein was not particularly unstable and largely resisted degradation by mitochondrial proteases. (B) The raw iMTS-L scores were calculated and are graphically shown for mitochondrial proteins for which the MPP cleavage sites had been experimentally verified. (C) The iMTS-L propensities of yeast proteins do not correlate with their lengths. The iMTS propensity was calculated over the entire amino acid sequence.
Figure S2. **In vitro analysis of mitochondrial protein import reactions.** Analysis of the import efficiencies based on in vitro import experiments as described for Fig. 8 C. Shown are means and SD of three independent experiments.

Table S1 is a separate Excel file showing a list of raw iMTS-L scores of mitochondrial proteins. Table S2 is a separate Excel file showing a list of iMTS-L propensities of mitochondrial proteins for which the MPP processing sites were experimentally determined.