SUPPLEMENTARY INFORMATION

Role of MINOS in protein biogenesis of the mitochondrial outer membrane

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SUPPLEMENTAL FIGURES

FIGURE S1: Mitochondrial ultrastructure of yeast cells lacking the POTRA domain of Sam50. Representative electron microscopy images of Fcjc1_{ProtA} cells and Fcjc1_{ProtA} Sam50_{Δ120} cells lacking the N-terminal POTRA domain of Sam50 are shown (mitochondria were stained with diaminobenzidine (DAB)). Bars in the first and third rows represent 1 µM; bars in the second and fourth rows represent 200 nm.

FIGURE S2: Steady-state levels and protein import in fcj1Δ mitochondria. Mitochondria isolated from wild-type (WT) and fcj1Δ cells were subjected to SDS-PAGE (A) or blue native electrophoresis (B) and mitochondrial protein content was analyzed by immunoblotting. IMS, intermembrane space; PAM, presequence translocase-associated motor; TIM, translocase of the inner mitochondrial membrane. (C) [35S]Porin or (D) [35S]Mdm10 were incubated with isolated wild-type, fcj1Δ and mio10Δ mitochondria for the indicated periods. The mitochondria were analyzed by blue native electrophoresis and digital autoradiography.

FIGURE S3: Phospholipid composition of MINOS mutant mitochondria. Mitochondria were isolated from wild-type (WT), fcj1Δ, and mio10Δ cells and mitochondrial phospholipids were extracted and quantified. Mean values of four measurements with standard error of the mean are shown. LP, lysophospholipids; PI, phosphatidylinositol; PS, phosphatidylserine; PC, phosphatidylcholine; PE, phosphatidylethanolamine; CL, cardiolipin; DMPE, dimethylphosphatidylethanolamine; PA, phosphatidic acid.

FIGURE S4: Mitochondrial protein content upon depletion of mitofilin/Fcj1 in yeast. Mitochondria (µg protein) isolated from wild-type (WT) or Fcj1-depleted (Fcj1↓) cells were subjected to SDS-PAGE (A) or blue native electrophoresis (B) and the protein content was analyzed by Western blotting.
FIGURE S5: Biogenesis of outer membrane proteins in fcj1Δ mitochondria. (A) 35S-labeled Tom22 or (B) 35S-labeled Tom5 were imported into wild-type (WT), fcj1Δ and mio10Δ mitochondria for the indicated periods. Upon solubilization in digitonin-containing buffer, blue native electrophoresis and digital autoradiography were applied. (C) [35S]Tom40 was imported into wild-type, Fcj1ProtA and Oxa1ProtA (control) mitochondria for five minutes. Mitochondria were re-isolated, lysed with digitonin-containing buffer and subjected to IgG affinity chromatography, elution with TEV protease, SDS-PAGE and digital autoradiography. Load, 0.5%; elution, 100%.
Bohnert et al., Figure S1
Bohnert et al., Figure S2
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