Gebert et al., http://www.jcb.org/cgi/content/full/jcb.201110047/DC1



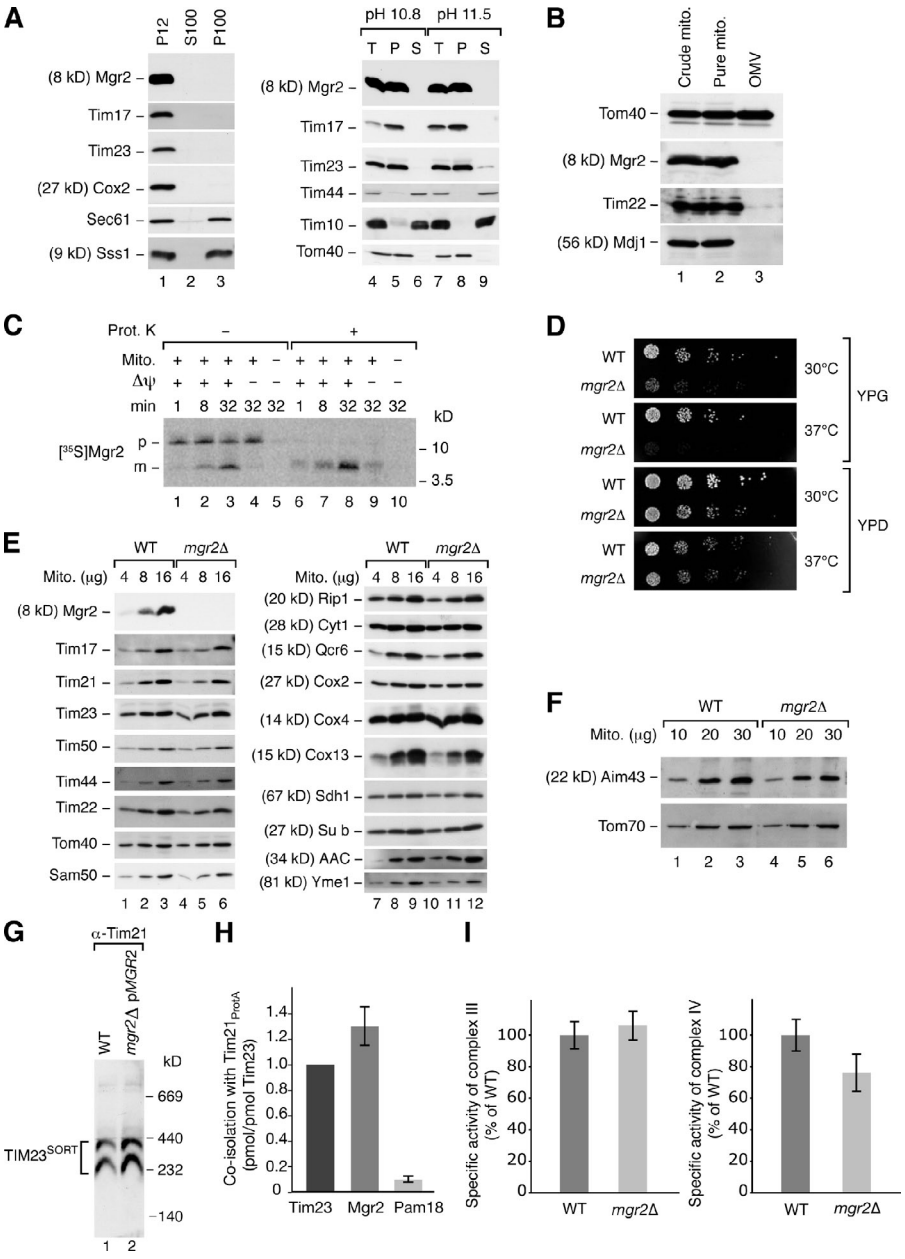


Figure S1. **Basic characteristics of the mitochondrial inner membrane protein Mgr2 and of the *mgr2Δ* mutant.** (A, samples 1–3) Wild-type yeast cells were homogenized, and subcellular fractions were obtained by differential centrifugation and analyzed by SDS-PAGE and Western blotting. P12, mitochondrial fraction; S100, cytosolic fraction; P100, microsomal fraction. (samples 4–9) Isolated yeast mitochondria were treated with sodium carbonate. Membranes and soluble fractions were separated by centrifugation and subjected to SDS-PAGE and immunoblotting. T, total; P, pellet; S, supernatant. (B) Yeast wild-type mitochondria (crude preparation), gradient-purified mitochondria (mito.), and isolated outer membrane vesicles (OMV; von der Malsburg et al., 2011) were analyzed by SDS-PAGE and Western blotting. (C) The precursor of Mgr2 was synthesized and $^{35}S$ labeled in vitro and imported into isolated wild-type mitochondria. Where indicated, samples were treated with 150 µg/ml proteinase K (Prot. K) before SDS-PAGE and autoradiography. p, precursor; m, mature. (D) Wild-type (WT) and *mgr2Δ* yeast cells were grown on YPG or yeast peptone dextrose (YPD) plates. (E and F) Mitochondria (micrograms, protein amount) were subjected to SDS-PAGE and Western blotting. Su b, subunit b (Atp4) of $F_1F_o$-ATP synthase. (G) Reexpression of *MGR2* in *mgr2Δ* cells from a plasmid restored yeast growth. Mitochondria were solubilized in digitonin and analyzed by blue native electrophoresis and Western blotting. (H) $Tim21_{ProtA}$ mitochondria were lysed with digitonin and subjected to affinity purification. The amounts (picomoles) of Tim23, Mgr2, and Pam18 in the eluate were quantified as described in Materials and methods. The amount of Tim23 was set to 1. The mean ± SEM ($n = 4$) is shown. (I) Specific cytochrome $bc_1$ oxidoreductase activity was determined for freeze-thawed mitochondria of wild-type and *mgr2Δ* yeast strains. The assay was carried out with 30 µg mitochondrial protein/ml, 50 µM cytochrome *c*, and 80 µM decylubiquinol (left graph). The average activity of wild-type cytochrome $bc_1$ complex was 213 nmol cytochrome *c* reduced per milligram of total protein and minutes. Specific cytochrome *c* oxidase activity was determined with solubilized mitochondria (5 µg total protein/ml) and 100 µM reduced cytochrome *c* (right graph). The average activity of wild-type cytochrome *c* oxidase was 1,656 nmol cytochrome *c* oxidized per milligram of total protein and minutes. The average activities in wild-type were set to 100%. The mean ± SEM ($n \geq 7$) is shown.

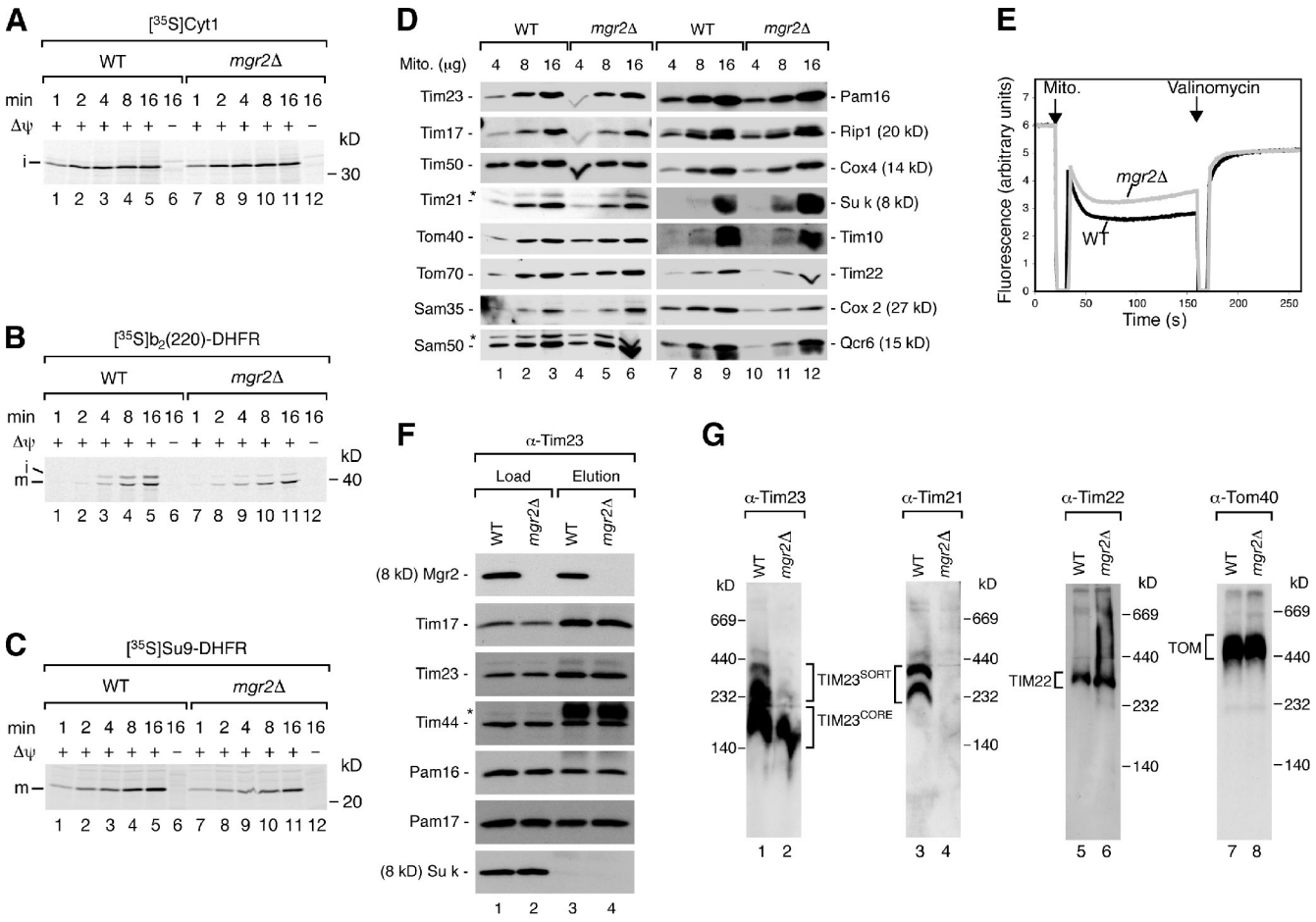


Figure S2. **Basic characteristics of mitochondria isolated from *mgr2Δ* yeast cells grown at normal and elevated temperature.** (A–C) Mitochondria were isolated from wild-type (WT) and *mgr2Δ* yeast cells grown at 30°C. $^{35}$S-labeled preproteins were imported into the mitochondria. The samples were treated with proteinase K and subjected to SDS-PAGE and autoradiography. i, intermediate; m, mature. (D–G) Mitochondria were isolated from wild-type and *mgr2Δ* yeast cells grown at 39°C. (D) Mitochondrial (Mito.) proteins were analyzed by SDS-PAGE and Western blotting. Total amount of mitochondrial protein is in micrograms. Su k, subunit k (Atp19) of $F_1F_o$-ATP synthase. Asterisks indicate nonspecific signals. (E) The membrane potential of isolated wild-type and *mgr2Δ* mitochondria was assessed using the potential-sensitive dye 3,3′-dipropylthiadicarbocyanine iodide (Geissler et al., 2000). (F) Mitochondria were lysed with digitonin and subjected to coimmunoprecipitation using Tim23 antiserum. The samples were analyzed by SDS-PAGE and immunoblotting. Eluate: 100%; load: 11%. The asterisk indicates the nonspecific signal (antibody chains). (G) Wild-type and *mgr2Δ* mitochondria were lysed with digitonin and subjected to blue native electrophoresis and immunoblotting using the indicated antibodies.

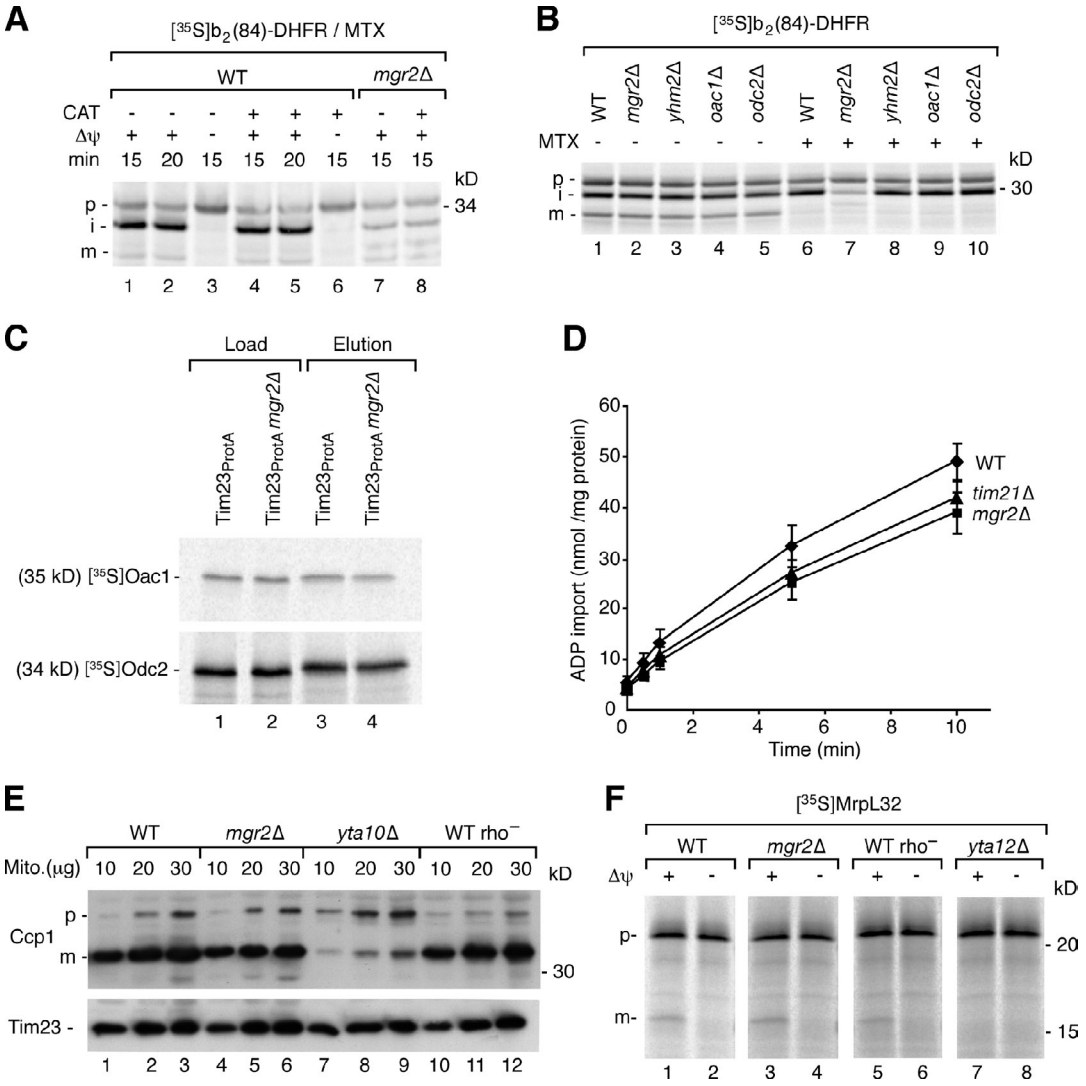


Figure S3. **Control import reactions in *mgr2Δ* mitochondria.** (A) Yeast wild-type (WT) and *mgr2Δ* mitochondria were incubated with 10 μM carboxyatractyloside (CAT; to inhibit the AAC) for 5 min at 25°C, as indicated. Then, the $^{35}$S-labeled preprotein $b_2$(84)-DHFR was imported into the mitochondria in the presence of methotrexate (MTX). The mitochondria were analyzed by SDS-PAGE and autoradiography. p, precursor; i, intermediate; m, mature. (B) $b_2$(84)-DHFR was imported into mitochondria isolated from yeast wild-type, *mgr2Δ*, and different carrier mutants in the presence or absence of methotrexate. (C) $^{35}$S-labeled Oac1 and Odc2 were imported in $Tim23_{ProtA}$ and $Tim23_{ProtA}$ *mgr2Δ* mitochondria for 30 min at 25°C. Mitochondria were lysed by digitonin and subjected to IgG Sepharose affinity chromatography. Samples were analyzed by SDS-PAGE and autoradiography. Load: 1%; eluate: 100%. (D) ADP/ATP exchange catalyzed by reconstituted mitochondrial AAC proteins. Transport of radiolabeled ADP (50 μM) was performed with liposomes containing reconstituted mitochondrial membrane proteins (from wild-type, *mgr2Δ*, and *tim21Δ* mitochondria). Proteoliposomes were loaded with 10 mM ATP. ADP import (at 30°C) was conducted for the indicated periods and stopped by anion exchange chromatography. The mean of ADP uptake ± SEM is shown ($n$ = 6). In the absence of interior counter exchange substrate (nonloaded liposomes), no significant import of ADP was detectable. (E) Mitochondrial (Mito.) proteins (micrograms) from wild-type yeast, *mgr2Δ* mutant, *yta10Δ* mutant, and rho$^-$ wild type were separated by SDS-PAGE and analyzed by Western blotting. Ccp1, cytochrome *c* peroxidase. (F) The $^{35}$S-labeled precursor (urea denatured) of MrpL32 (mitochondrial ribosomal protein, large subunit) was imported into mitochondria from wild-type yeast, *mgr2Δ*, rho$^-$ wild type, and *yta12Δ* for 60 min at 25°C with or without a membrane potential (Δψ). The mitochondria were separated by SDS-PAGE and analyzed by autoradiography.

## References

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**Table S1 is included as an Excel file and shows the data obtained in SILAC-based quantitative affinity purification–MS experiments using Tim21 as bait.**