Becker et al., http://www.jcb.org/cgi/content/full/jcb.201102044/DC1



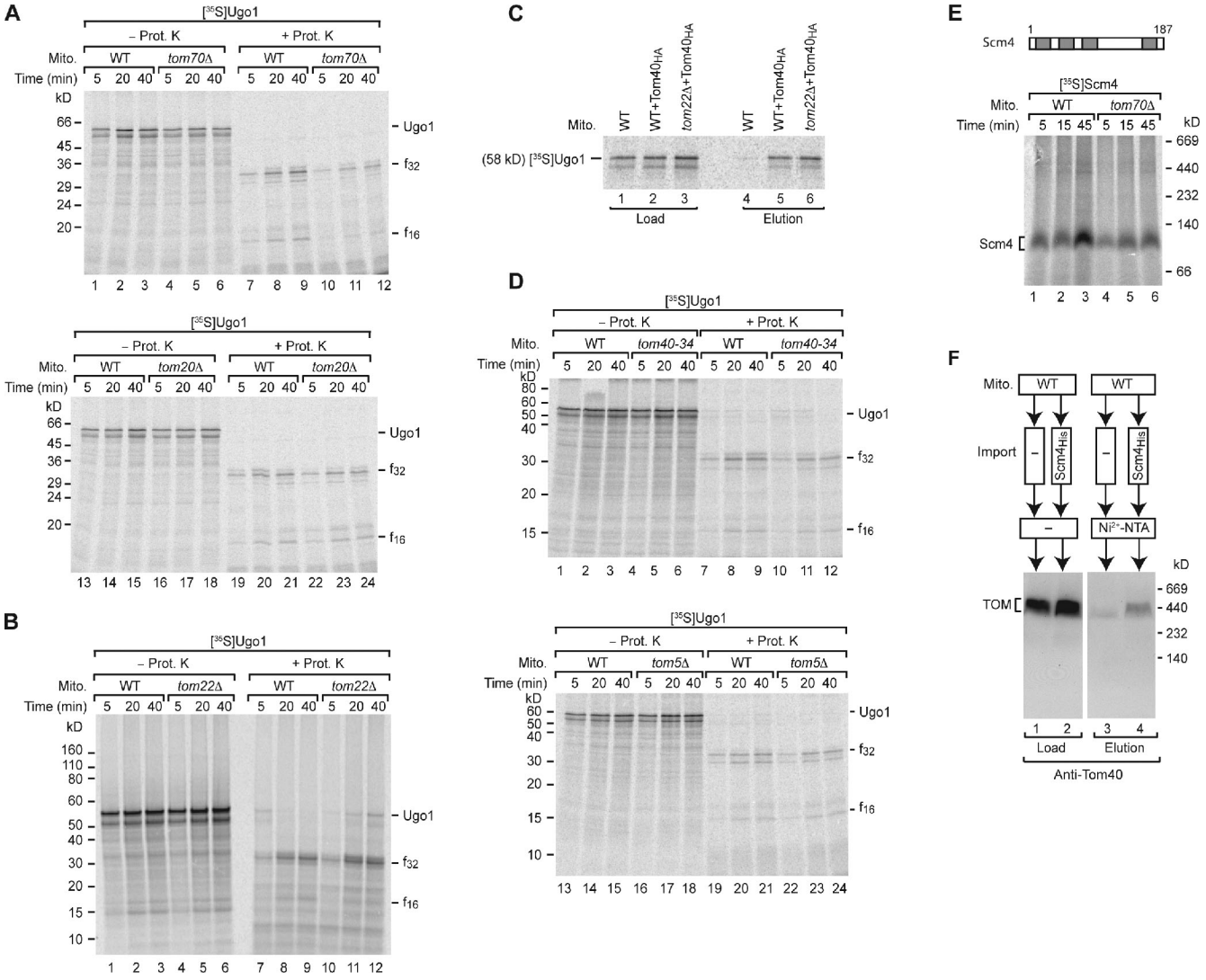


Figure S1. **The biogenesis of Scm4 and Ugo1 involves Tom70.** (A and B) $^{35}$S-Ugo1 was imported into *tom70Δ*, *tom20Δ*, *tom22Δ*, and corresponding wild-type (WT) mitochondria (Mito.). The mitochondria were either incubated with proteinase K (Prot. K) or mock treated as indicated. Imported Ugo1 and two proteolytical fragments ($f_{32}$ and $f_{16}$) were analyzed by SDS-PAGE and autoradiography. (C) $^{35}$S-Ugo1 was imported for 5 min at 25°C into wild-type and *tom22Δ* mitochondria expressing an additional copy of $Tom40_{HA}$. Mitochondria were lysed with digitonin and subjected to coprecipitation with HA-specific antibodies followed by SDS-PAGE and autoradiography. Load, 2%; elution, 100%. (D) $^{35}$S-Ugo1 was imported into *tom40-34* and *tom5Δ* mitochondria and analyzed as described in A and B. (E, top) A schematic representation of Scm4. The predicted transmembrane helices are marked in gray. The prediction was performed using transmembrane prediction using hidden Markov models at the Center for Biological Sequence Analysis server. (bottom) $^{35}$S-labeled Scm4 was imported into wild-type and *tom70Δ* yeast mitochondria for the indicated periods. Mitochondria were reisolated and lysed with digitonin, and the import was analyzed by blue native electrophoresis and digital autoradiography. (F) Chemical amounts of $Scm4_{His}$ were imported into wild-type mitochondria for 10 min at 25°C. After the import reaction, the mitochondria were isolated, lysed with digitonin, and subjected to $Ni^{2+}$-NTA agarose purification. Load and elution fractions were analyzed by blue native electrophoresis and immunodecoration with antibodies against Tom40. Load, 3%; elution, 100%.

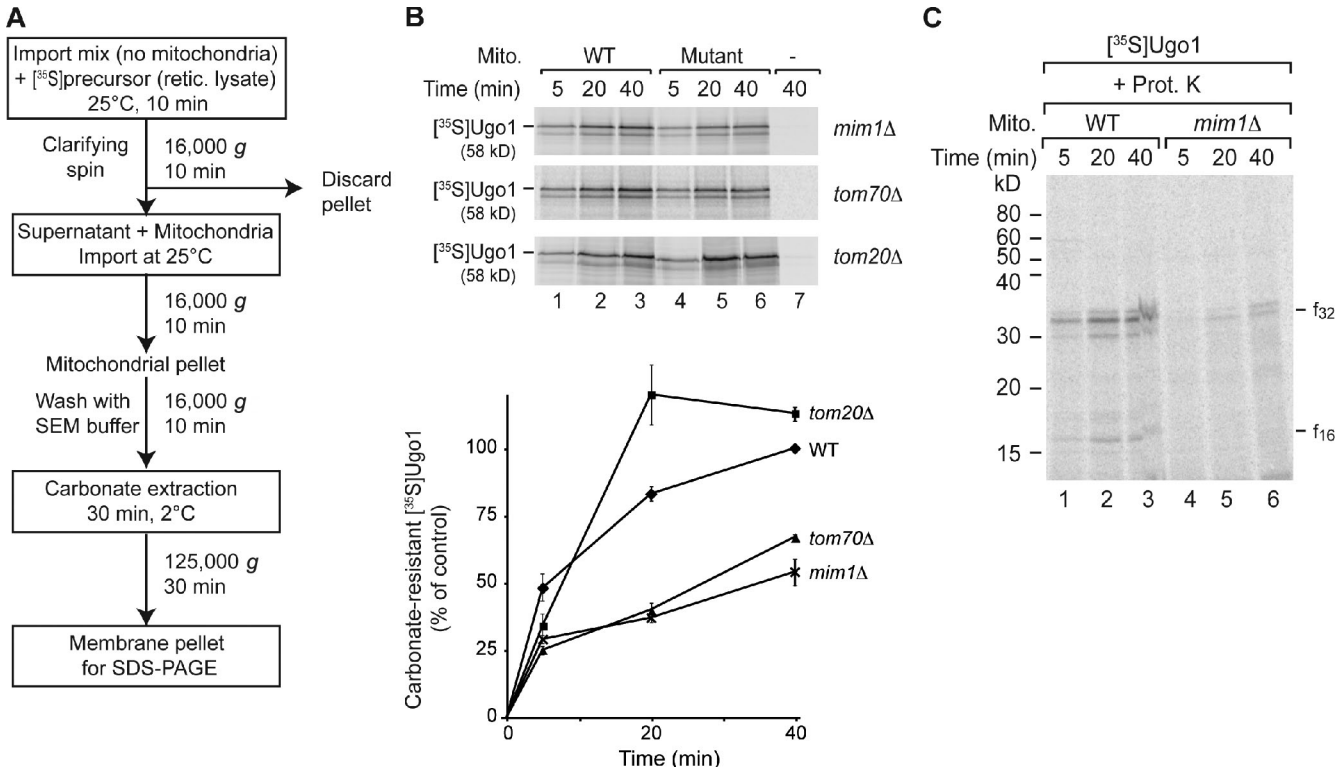


Figure S2. **Membrane insertion of Ugo1 is impaired in *mim1Δ* mitochondria.** (A) A schematic representation of the optimized experimental procedure for alkaline extraction (sodium carbonate treatment; Thornton et al., 2010). In vitro synthesized hydrophobic precursor proteins can be prone to aggregation in import buffer (Thornton et al., 2010), providing an explanation for why a previous analysis of membrane insertion of $^{35}$S-labeled Ugo1 yielded similar levels of Ugo1 in the membrane pellet of wild-type and *mim1Δ* mitochondria after alkaline extraction (Becker et al., 2008). This unspecific precipitation can be reduced (though not abolished) by an optimized import protocol that involves a clarifying spin before the addition of mitochondria (Thornton et al., 2010). Thus, the classical alkaline treatment assay may not be sufficient for the analysis of aggregation-prone hydrophobic precursor proteins but should be combined with a clarifying spin and be complemented by independent import assays. SEM, 250 mM sucrose, 1 mM EDTA, and 10 mM MOPS/KOH, pH 7.2. (B, top) The formation of alkaline-resistant Ugo1 is partially reduced in *mim1Δ* and *tom70Δ* mitochondria but not in *tom20Δ* mitochondria. $^{35}$S-labeled Ugo1 was imported into mitochondria isolated from wild-type (WT), *tom70Δ*, and *tom20Δ* yeast strains for the indicated periods. Mitochondria were reisolated and subjected to carbonate extraction. In the mock control, no mitochondria were added to the import reaction. Pelleted proteins were analyzed by SDS-PAGE and digital autoradiography. (bottom) Quantification of three independent experiments with standard error of the means ($n = 7$ for wild type). The value of $^{35}$S-Ugo1 in wild type after 40 min was set to 100% (control). (C) Formation of protease-protected Ugo1 fragments is strongly impaired in *mim1Δ* mitochondria (Mito.). $^{35}$S-Ugo1 was imported into wild-type and *mim1Δ* mitochondria followed by treatment with proteinase K (Prot. K). The proteolytic fragments generated from imported Ugo1 ($f_{32}$ and $f_{16}$) were analyzed by SDS-PAGE and autoradiography.

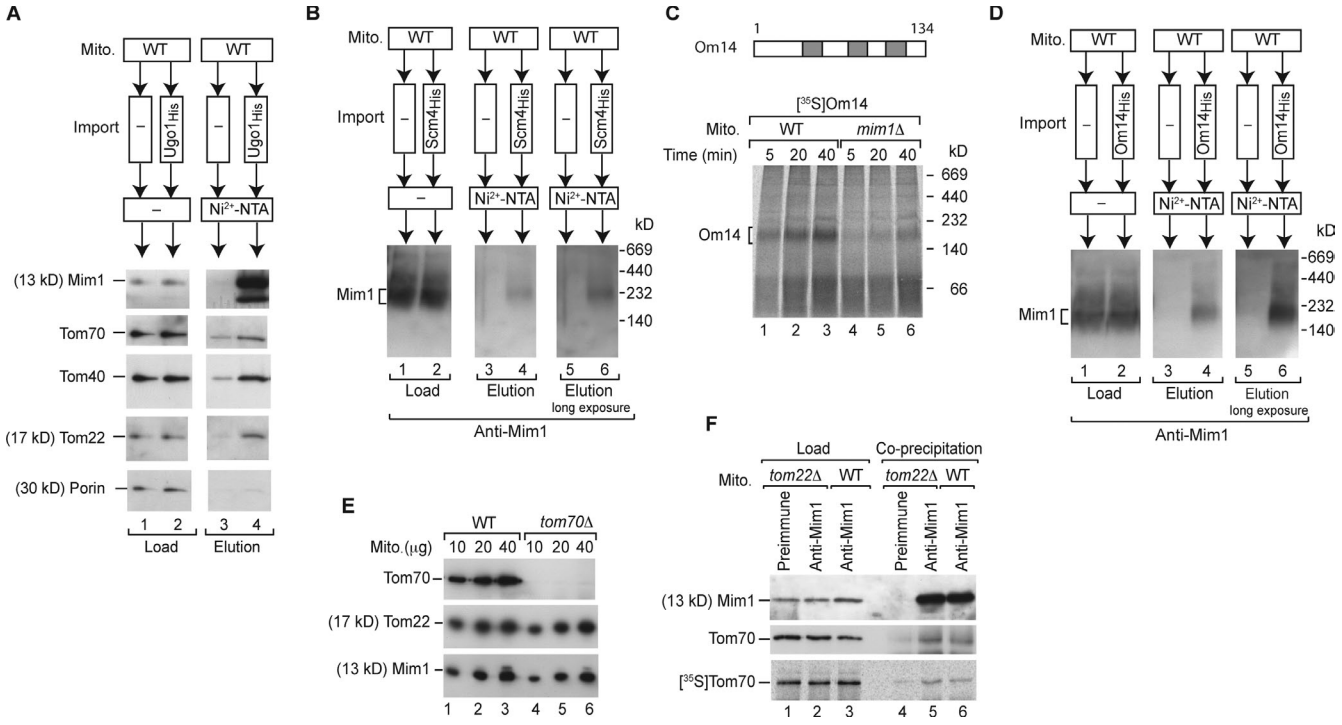


Figure S3. **Mim1 binds to imported α-helical outer membrane proteins.** (A) Chemical amounts of $Ugo1_{His}$ were imported into wild-type (WT) mitochondria (Mito.) for 10 min at 25°C. Mitochondria were lysed with digitonin and subjected to Ni-NTA agarose purification, SDS-PAGE, and immunodecoration. Load, 0.2%; elution, 100%. (B) Chemical amounts of $Scm4_{His}$ were imported into wild-type mitochondria for 10 min at 25°C. After the import reaction, the mitochondria were isolated, lysed with digitonin, and subjected to $Ni^{2+}$-NTA agarose purification. Load and elution fractions were analyzed by blue native electrophoresis and immunodecoration with antibodies against Mim1. Load, 3%; elution, 100%. (C, top) A schematic representation of Om14. The predicted transmembrane helices are marked in gray. (bottom) $^{35}S$-labeled Om14 was imported into mitochondria isolated from wild-type and *mim1Δ* yeast. The mitochondria were reisolated, lysed with digitonin, and analyzed by blue native electrophoresis and autoradiography. (D) Chemical amounts of $Om14_{His}$ were imported into wild-type mitochondria for 10 min at 25°C. Mitochondria were reisolated, lysed with digitonin, and subjected to $Ni^{2+}$-NTA agarose purification. Load and elution fractions were analyzed by blue native electrophoresis and immunodecoration with antibodies against Mim1. Load, 3%; elution, 100%. (E) Wild-type and *tom70Δ* mitochondria were analyzed by SDS-PAGE and Western blotting. (F, top and middle) Wild-type and *tom22Δ* mitochondria were solubilized and subjected to coimmunoprecipitation with Mim1-specific antiserum. Load and elution fractions were subjected to SDS-PAGE and Western blotting. Load, 3% (Mim1) or 2% (Tom70); elution, 100%. (bottom) $^{35}S$-labeled Tom70 was imported into wild-type and *tom22Δ* mitochondria for 40 min. Mitochondria were solubilized and subjected to coimmunoprecipitation with Mim1-specific antiserum. Load and elution fractions were subjected to SDS-PAGE and autoradiography. Load, 2%; elution, 100%. We previously reported that protein A–tagged Mim1 did not coprecipitate Tom70 (Becker et al., 2008); because the protein A tag is located at the N-terminal (cytosolic) side of Mim1, it likely interferes with the interaction with Tom70.

## References


Becker, T., S. Pfannschmidt, B. Guiard, D. Stojanovski, D. Milenkovic, S. Kutik, N. Pfanner, C. Meisinger, and N. Wiedemann. 2008. Biogenesis of the mitochondrial TOM complex: Mim1 promotes insertion and assembly of signal-anchored receptors. *J. Biol. Chem.* 283:120–127. doi:10.1074/jbc.M706997200

Thornton, N., D.A. Stroud, D. Milenkovic, B. Guiard, N. Pfanner, and T. Becker. 2010. Two modular forms of the mitochondrial sorting and assembly machinery are involved in biogenesis of α-helical outer membrane proteins. *J. Mol. Biol.* 396:540–549. doi:10.1016/j.jmb.2009.12.026