Supplemental Materials

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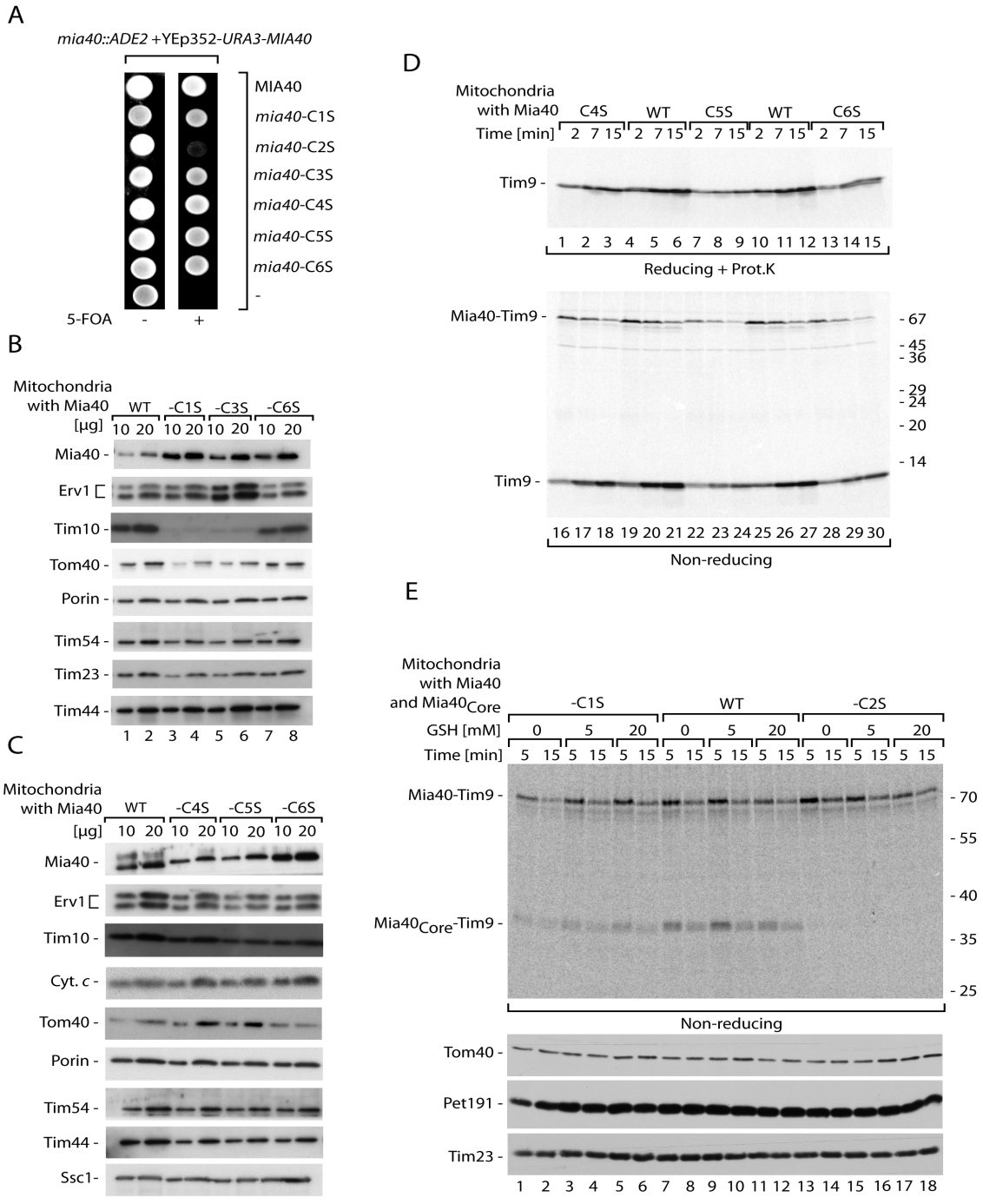


Fig. S1 Bottinger et al.

SUPPLEMENTAL FIGURE 1: Characterization of the single cysteine mutants of Mia40. (A) Generation of yeast strains harboring cysteine residue mutants of Mia40. The growth of yeast before and after removal of wild-type Mia40 by 5-fluoroorotic acid (5-FOA) treatment is shown. (B) The steady-state protein levels of mitochondria with wild-type Mia40, Mia40-C1S, Mia40-C3S, and Mia40-C6S (rho$^{0/-}$ versions). (C) The steady-state protein levels of mitochondria with wild-type Mia40, Mia40-C4S, Mia40-C5S, and Mia40-C6S. (B-C) Samples were analyzed by reducing SDS electrophoresis and immunodecoration with different antisera. (D) ^{35}S-labeled Tim9 was imported into mitochondria with Mia40, Mia40-C4S, Mia40-C5S or Mia40-C6S. Samples were treated with Prot. K and analyzed by SDS electrophoresis as indicated. E) ^{35}S-labeled Tim9 was imported in the presence of increasing concentrations of GSH into mitochondria isolated from wild-type cells co-producing Mia40$_{Core}$ (WT), Mia40$_{Core}$-C1S or Mia40$_{Core}$-C2S. Samples were analyzed by SDS electrophoresis followed by autoradiography or by immunodecoration with antisera against control proteins.

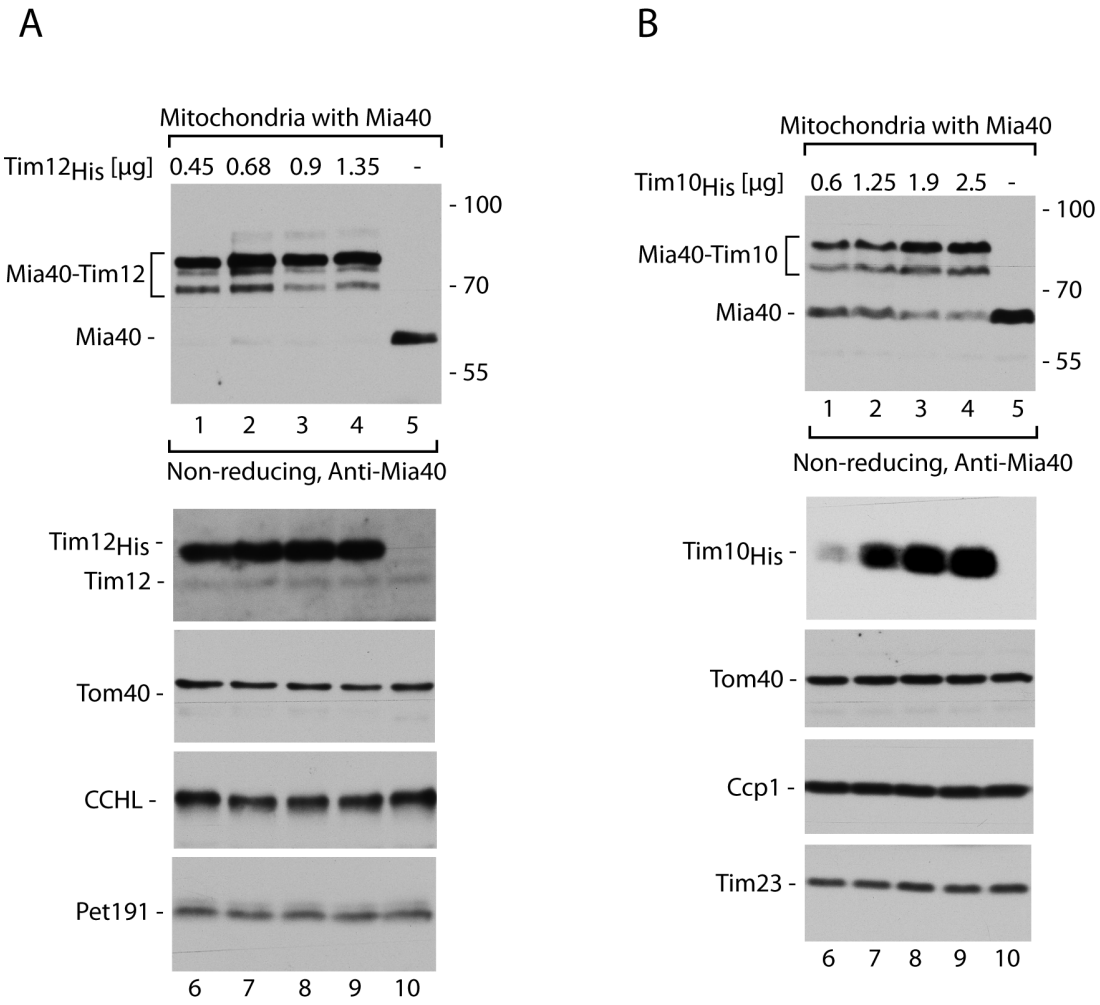
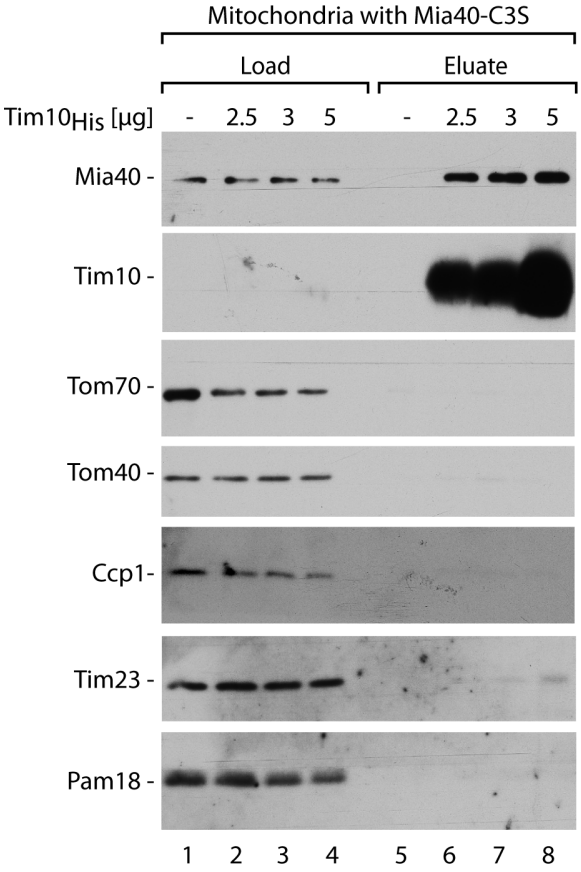


Fig. S2 Bottinger et al.

SUPPLEMENTAL FIGURE 2: Mia40 is saturated by imported $Tim12_{His}$ and $Tim10_{His}$ precursor proteins. (A) $Tim12_{His}$ was imported for 10 min at 25°C into mitochondria isolated from wild-type cells. (B) $Tim10_{His}$ was imported for 10 min at 25°C into mitochondria isolated from wild-type cells. (A and B) Samples were analyzed by SDS electrophoresis and immunodecoration with antisera against Mia40 and control proteins.



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SUPPLEMENTAL FIGURE 3: Recombinant $Tim10_{His}$ saturates Mia40-C3S. Ni-NTA agarose affinity purification was performed upon import of increasing amounts of $Tim10_{His}$ followed by solubilization with digitonin. Load, 2%; Eluate, 100%. Samples were analyzed by reducing SDS electrophoresis and immunodecoration with different antisera.

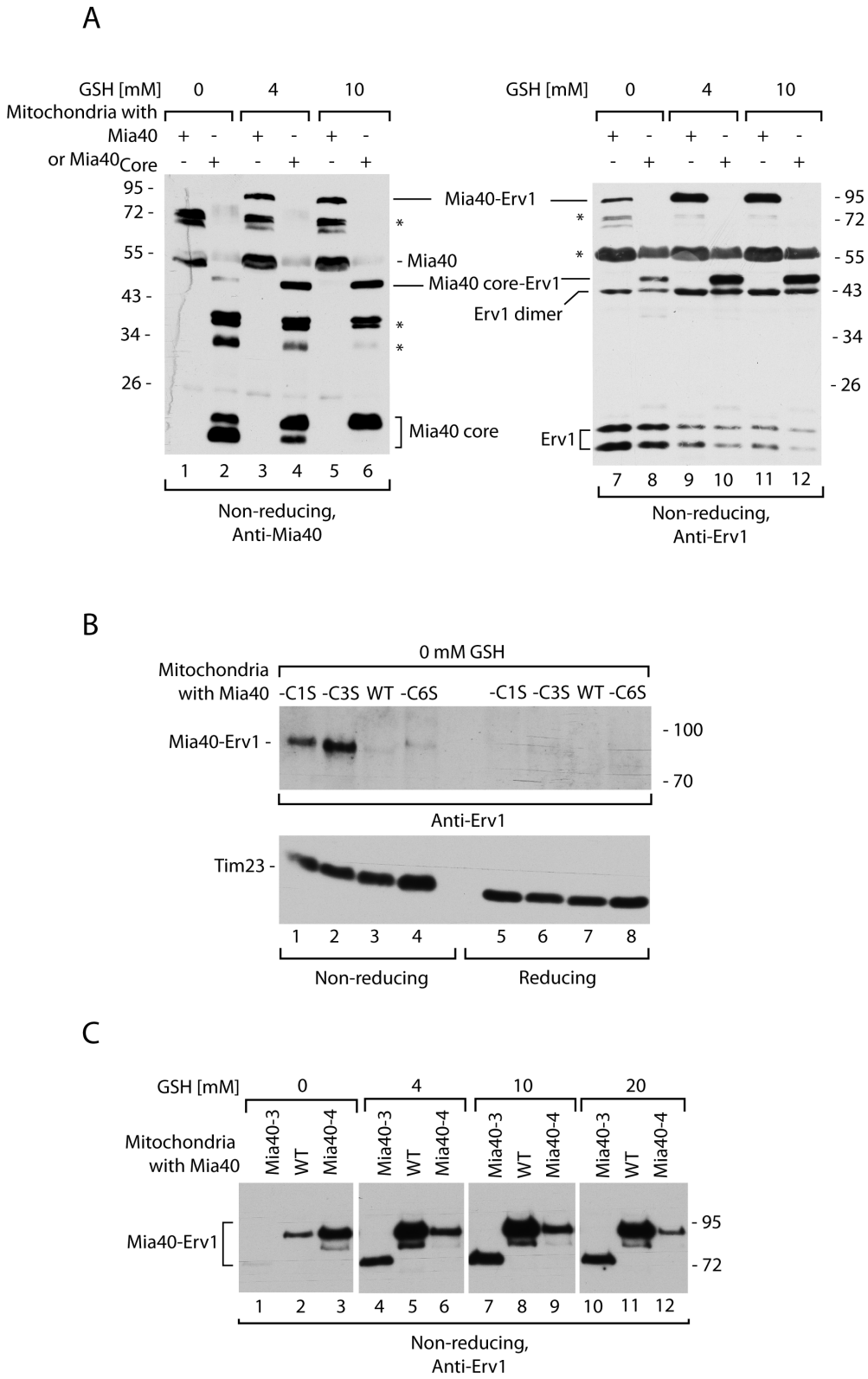
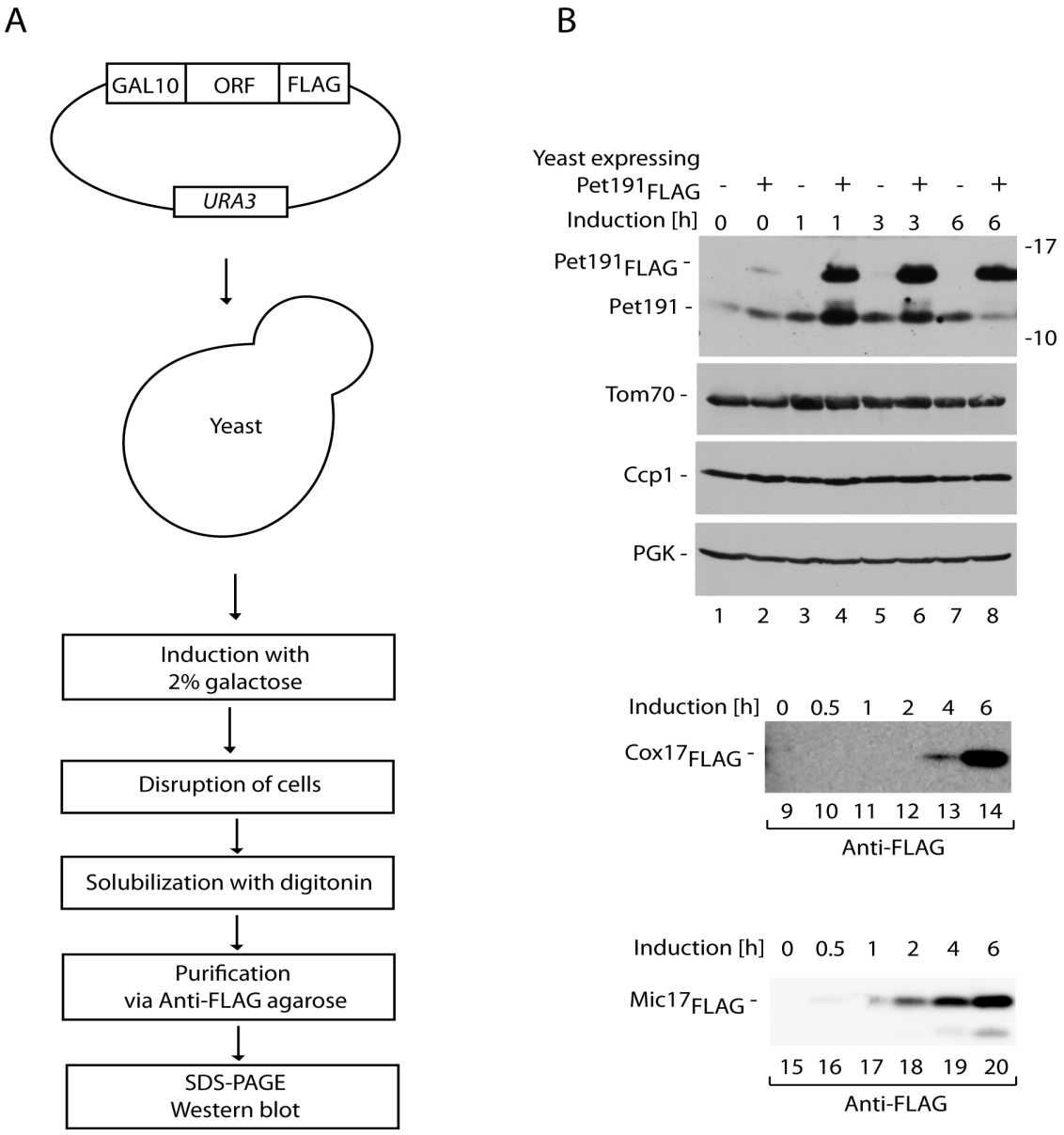


Fig. S4 Bottinger et al.

SUPPLEMENTAL FIGURE 4: Characterization of Mia40-Erv1 complex formation. (A) Mitochondria with Mia40 or $Mia40_{Core}$ were subjected to GSH treatment. (B) Mitochondria isolated from yeast with Mia40 (WT), Mia40-C1S, Mia40-C3S or Mia40-C6S ($rho^{0/-}$ versions) were analyzed by non-reducing or reducing SDS electrophoresis. (C) Mitochondria with Mia40 (WT), Mia40-3 or Mia40-4 were subjected to GSH treatment. (A-C) Samples were analyzed by SDS electrophoresis and immunodecoration with different antisera. Stars indicate non-specific bands or non-identified species.



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SUPPLEMENTAL FIGURE 5: Establishing the conditions to express FLAG variants of MIA substrates. (A) Schematic representation of the *in vivo* scheme employed for the purification of FLAG-fusion proteins. (B) Total protein extracts of yeast producing $Pet191_{FLAG}$, $Cox17_{FLAG}$ or $Mic17_{FLAG}$ were analyzed by reducing SDS electrophoresis and immunodecoration with different antisera after indicated induction times.