Supplemental Materials

Molecular Biology of the Cell

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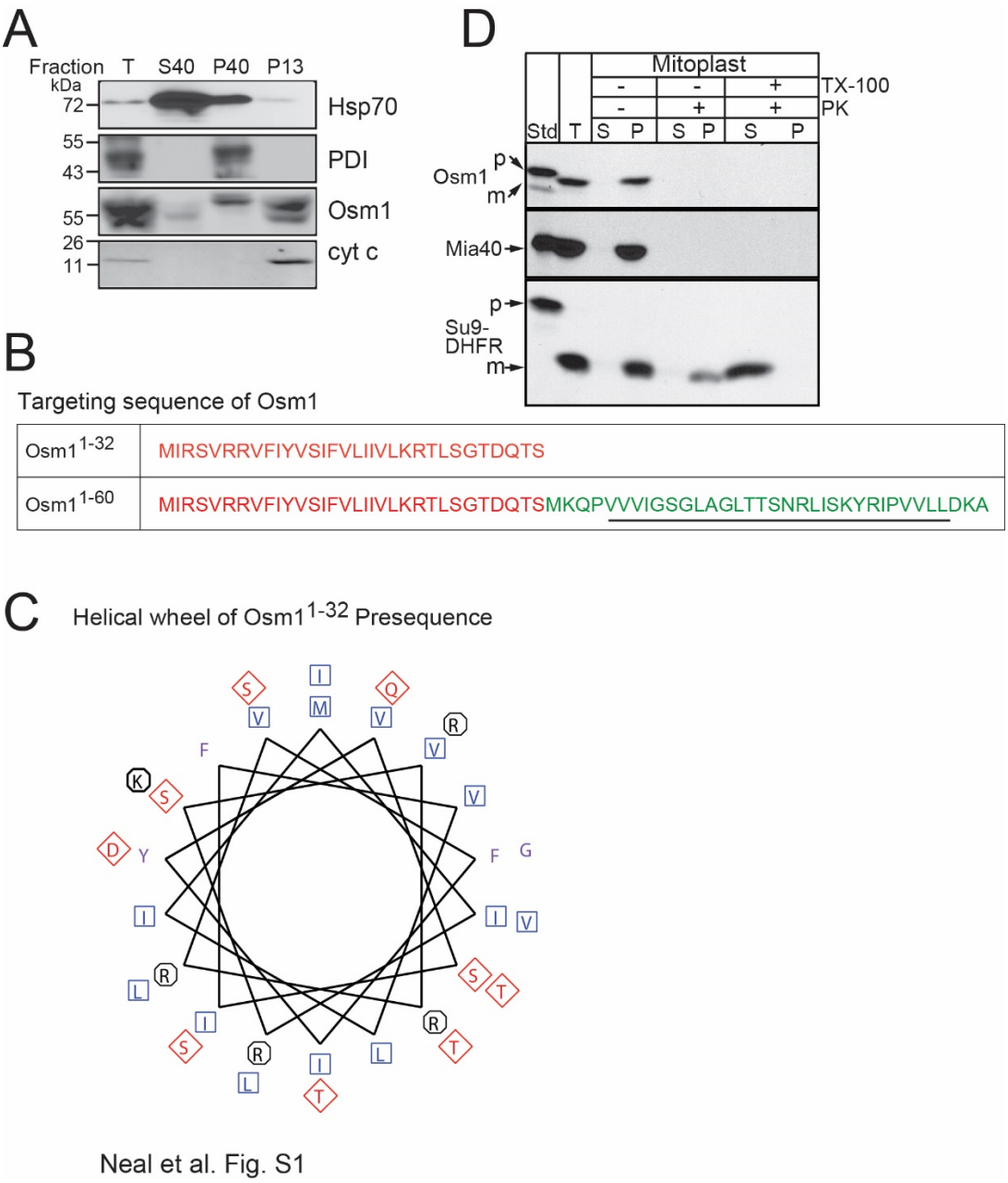
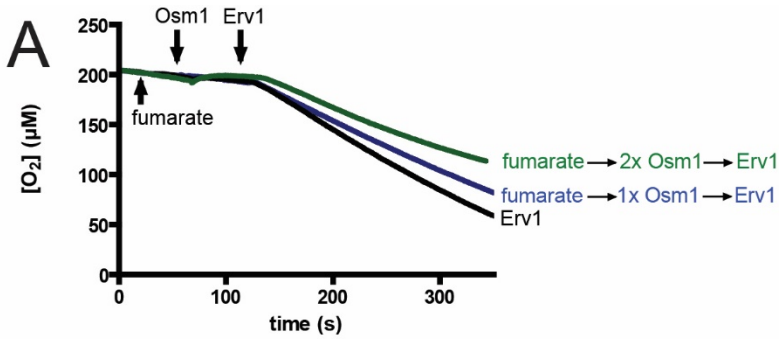
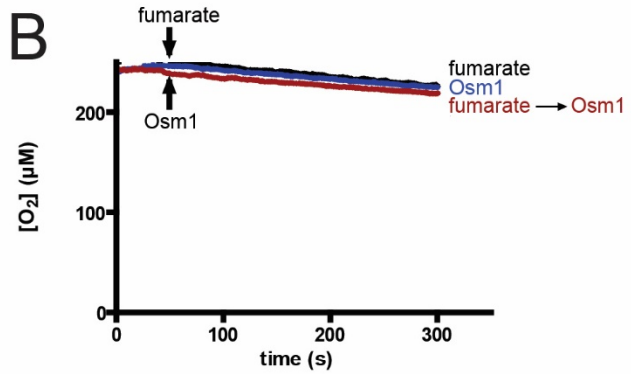
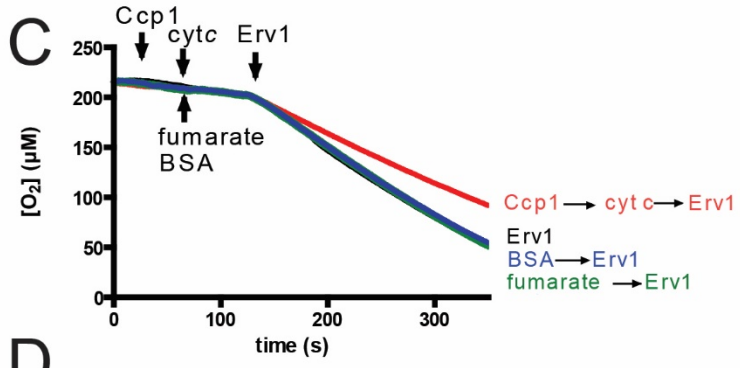


Neal et al. Fig. S1

SUPPLEMENTAL FIGURE S1: Osm1 localizes to microsomes and mitochondria. (A) As in Figure 2A except WT mitochondria were subjected to differential centrifugation. Samples were resolved on SDS-PAGE followed by immunoblot analysis with a polyclonal antibody against Osm1. Cyt *c* was included as an IMS control. (B) Analysis of the N-terminal region of Osm1. Residues 1-32 (highlighted in red) contain an N-terminal targeting sequence that typically directs Osm1 to the ER, but can function as a weak mitochondrial targeting sequence. Amino acids 32-60 contains a region rich in hydrophobic amino acids (underlined in black). (C) Analysis of the N-terminal 32 amino acids of Osm1 on a helical wheel. Positive, black; polar/negative, red; hydrophobic, blue. (D) As in Figure 2F, radiolabeled Osm1 was imported into mitochondria followed by osmotic shock treatment to generate mitoplasts. Import controls include Mia40 (IMS) and Su9-DHFR (matrix).





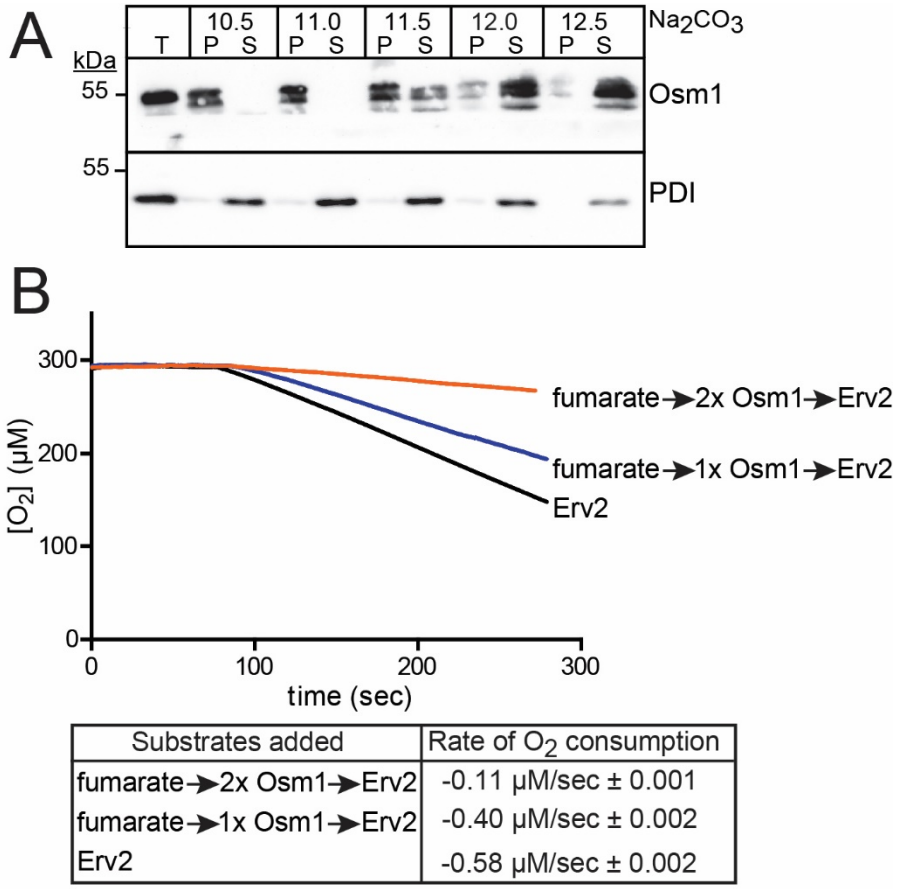


D

Substrates Added	Rate of O_2 consumption
Erv1	-.47 μM/sec ± .001
fumarate→ 1xOsm1→ Erv1	-.35 μM/sec ± .002
fumarate→ 2xOsm1→ Erv1	-.29 μM/sec ± .001
Ccp1→ cyt c → Erv1	-.33 μM/sec ± .002

Neal et al. Fig. S2

SUPPLEMENTAL FIGURE S2: Osm1/fumarate competes with O_2 in the oxidation of the nonphysiologic substrate DTT. (A) O_2 consumption was measured with the O_2 electrode (Hansatech, chamber is 1 ml) with air-saturated buffer containing 2 mM DTT and Erv1. Additional reactions include the addition of 100 μM fumarate/2 μM Osm1 and 100 μM fumarate/4 μM Osm1. (B) As in (A), a control experiment was performed with the addition of fumarate (100 μM), Osm1 (4 μM), or fumarate (100 μM) → Osm1 (4 μM). (C) As in (A), except that several proteins were added successively in the following order Ccp1 (20 μM) → cyt c (20 μM) → Erv1 (2 μM), fumarate (100 μM) → Erv1 (2 μM), BSA (2 μM) → Erv1 (2 μM) and Erv1 alone (2 μM). O_2 consumption was observed upon Erv1 addition. (D) Summary of the rate of O_2 consumption from 'A' and 'C' in reactions where O_2 was consumed.



Substrates added	Rate of O_2 consumption
fumarate→2x Osm1→Erv2	-0.11 μM/sec ± 0.001
fumarate→1x Osm1→Erv2	-0.40 μM/sec ± 0.002
Erv2	-0.58 μM/sec ± 0.002

Figure S3, Neal et al.

SUPPLEMENTAL FIGURE S3: Osm1 is a peripheral membrane protein in the ER and functions with Erv2 in vitro. (A) Microsomes were analyzed by alkali extraction (Na_2CO_3) with 0.1 M carbonate at the indicated pH values. Equal volumes of the pellet (P) and TCA-precipitated supernatant (S) fractions were resolved by SDS-PAGE and analyzed by immunoblotting. Soluble protein PDI is included as a control. (B) O_2 consumption was measured with the O_2 electrode (Hansatech chamber is 1 ml) with air-saturated buffer containing 2 mM DTT and 2 μM Erv2. Additional reactions include the addition of 100 μM fumarate/2 μM Osm1 and 100 μM fumarate/4 μM Osm1. The rate of O_2 consumption is summarized in the table.

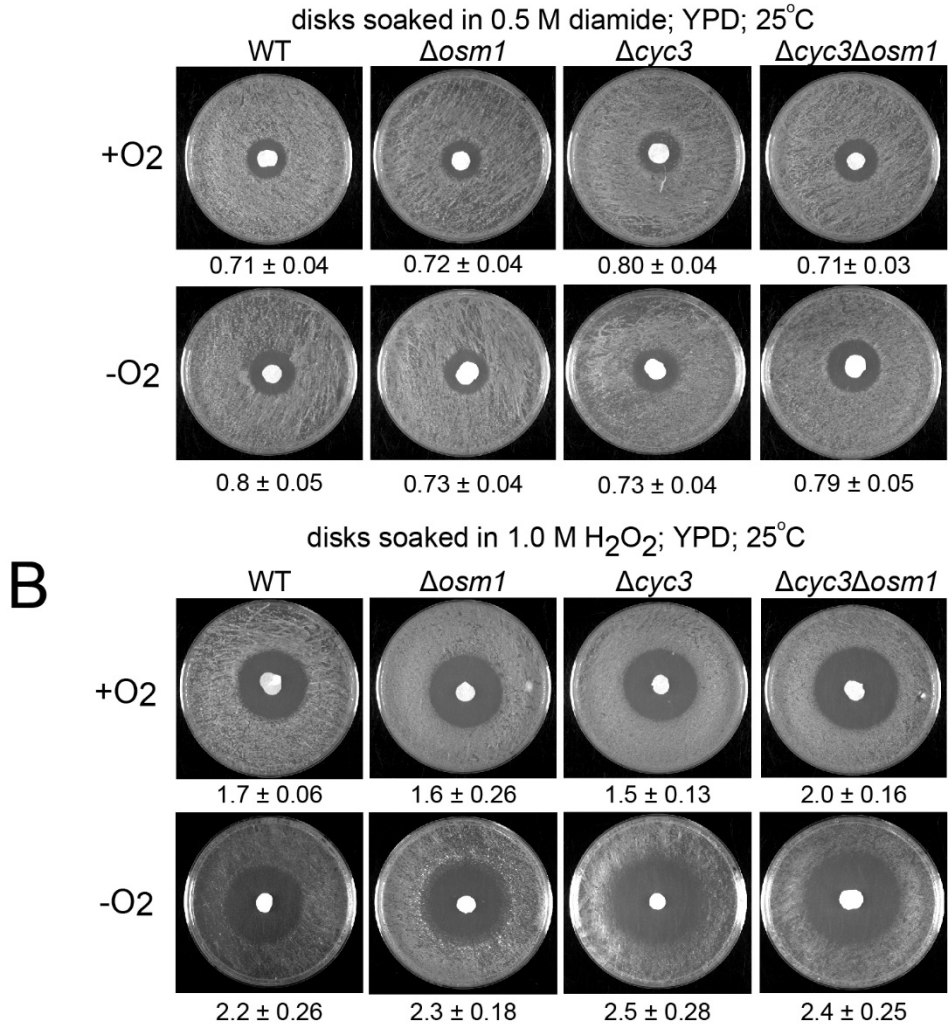


Figure S4, Neal et al.

SUPPLEMENTAL FIGURE S4: Δ*osm1 and* Δ*cyc3* strains are not sensitive to oxidants. (A) Equal amount of cells from WT, Δ*osm1, Δcyc3, and* Δ*cyc3*Δ*osm1* were spread onto YPD plates. Filter discs were placed in the middle of the plates and 10 µl of 0.5 M diamide was aliquoted directly onto the filter discs. The plates were grown at 25°C for 2 days and photographed. (B) As in 'A' except 10 µl of 1.0 M H_2O_2 was added to the filter disks.

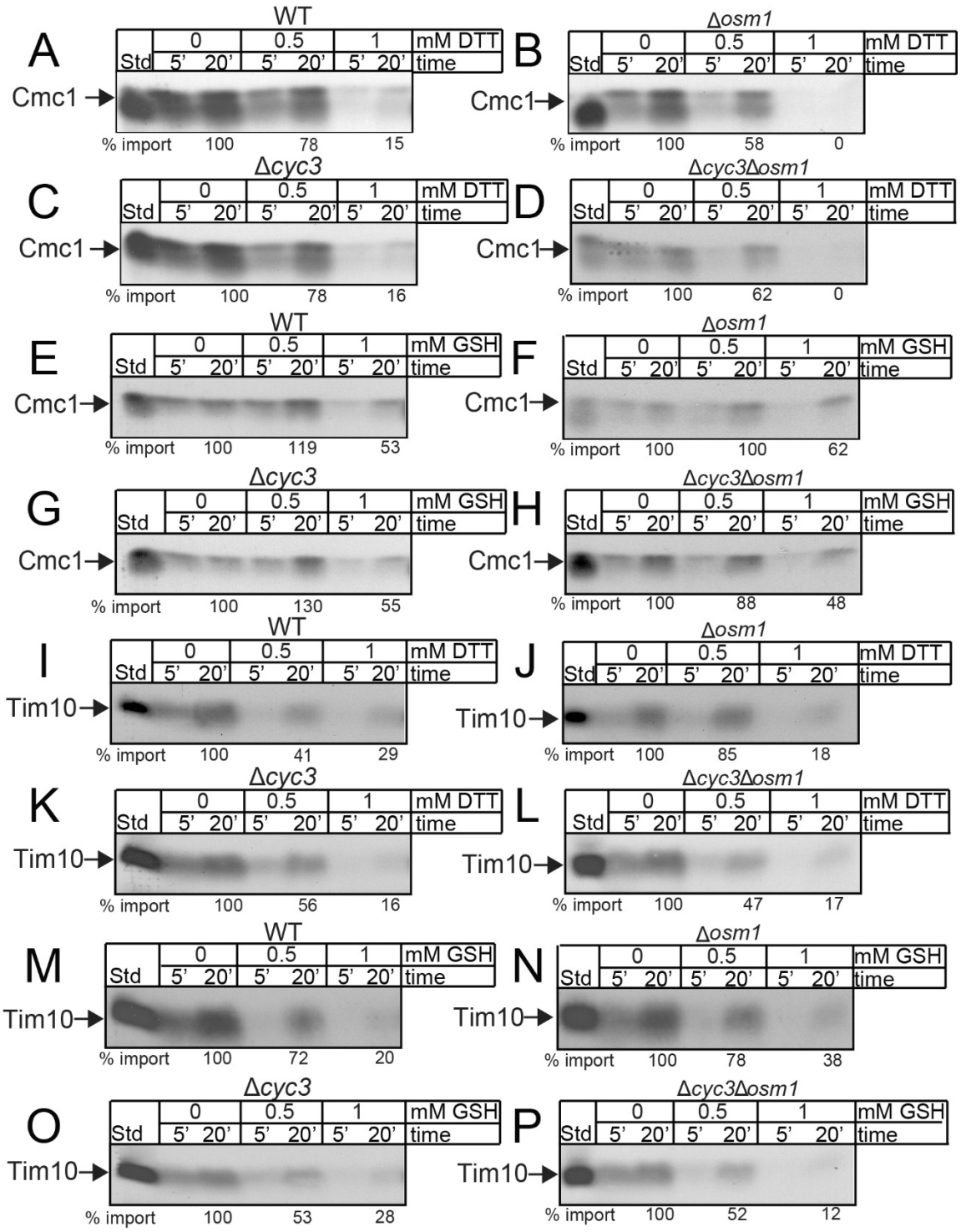


Figure S5, Neal et al.

SUPPLEMENTAL FIGURE S5: Reductant treatment inhibits the import of Cmc1 and Tim10 into mitochondria. Radiolabeled Cmc1 (A-H) and Tim10 (I-P) were imported into (A,E,I,M) WT, (B,F,J,N) Δ*osm1,* (C,G,K, O) Δ*cyc3,* and (D,H,L,P) Δ*cyc3*Δ*osm1* mitochondria in the presence of 0, 0.5, and 1 mM DTT (A-D,I-L) or GSH (E-H, M-P).. Non-imported precursor was removed by protease treatment and gels were processed as described in Figure 7B.

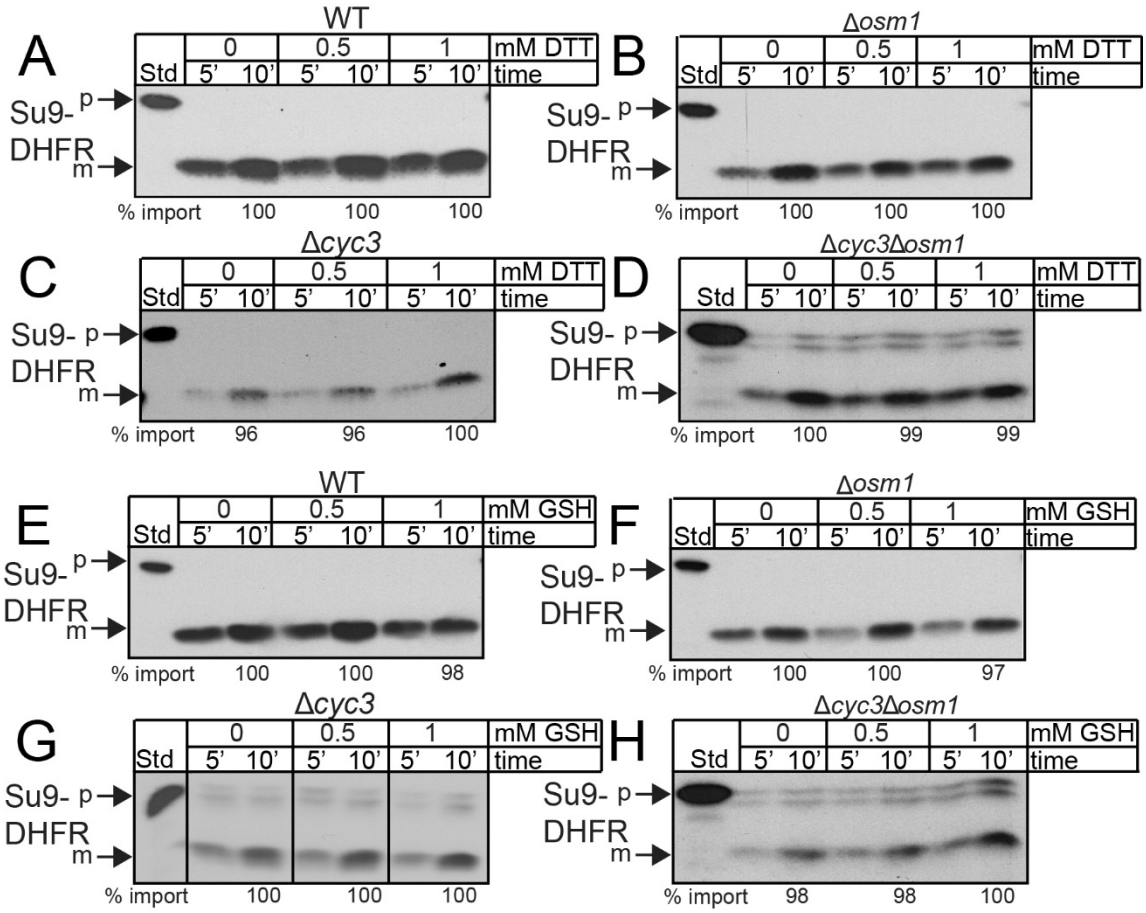


Figure S6, Neal et al.

SUPPLEMENTAL FIGURE S6. Control import reactions for Supplemental Figure S5. Radiolabeled Su9-DHFR was imported into (A,E,) WT, (B,F) Δ*osm1,* (C,G,) Δ*cyc3,* and (D,H,) Δ*cyc3*Δ*osm1* mitochondria in the presence of 0, 0.5, and 1 mM DTT (A-D,) or GSH (E-H). Non-imported precursor was removed by protease treatment and gels were processed as described in Figure 7B.

Table S1. Yeast strains used in this study

Strain	Genotype	Source
WT (GA74-1A)	MATa *his3-11,15 leu2 ura3 trp1 ade8 rho+ mit+*	(Koehler et al., 1998)
WT (GA74-6A)	MATα *his3-11,15 leu2 ura3 trp1 ade8 rho+ mit+*	(Koehler et al., 1998)
GA74-1A.d	MATa/α *his3-11,15/his3-11,15 leu2/leu2 ura3/ura3 trp1/trp1 ade8/ade8*	(Koehler et al., 1998)
erv1-101	MATα *his3-11,15 leu2 ura3 trp1 ade8 erv1::HIS3* [*erv1-101:TRP1* CEN]	(Dabir et al., 2007)
Erv1-His	MATα *his3-11,15 leu2 ura3 trp1 ade8 erv1::HIS3 [ERV1- 10xHis:LEU2* 2μ]	(Dabir et al., 2007)
Δosm1	MATa *his3-11,15 leu2 ura3 trp1 ade8 osm1::LEU2*	This study
Δosm1Δcyc3	MATa *his3-11,15 leu2 ura3 trp1 ade8 osm1::LEU2 cyc3::TRP1*	This study
Δfrd1	MATa *his3-11,15 leu2 ura3 trp1 ade8 osm1::TRP1*	This study
Osm1-myc	MATα *his3-11,15 leu2 ura3 trp1 ade8* [*OSM1-myc:LEU2* 2μ]	This study
Frd1-myc	MATα *his3-11,15 leu2 ura3 trp1 ade8* [*FRD1-myc:LEU2* 2μ]	This study