Supplemental Information

Mitochondria export sulfur species required for cytosolic tRNA thiolation

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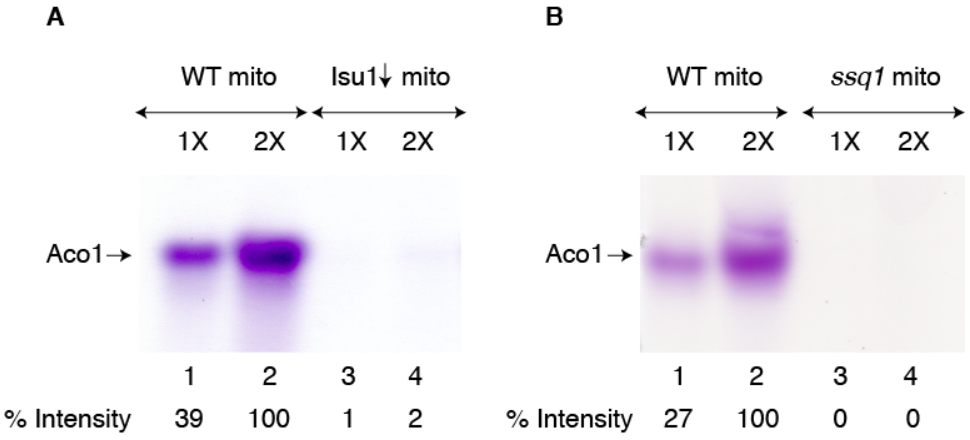

Figure S1. Aconitase activity is undetectable in Isu1-depleted (Isu1↓; Isu2 absent) or *ssq1* mutant mitochondria, Related to Figure 3.

Aconitase activity was evaluated by an in-gel assay. 1X = 50 μg of mitochondrial proteins.

(A) Mitochondrial samples are identical to those in Figure 3B.

(B) Mitochondrial samples are identical to those in Figure 3C.

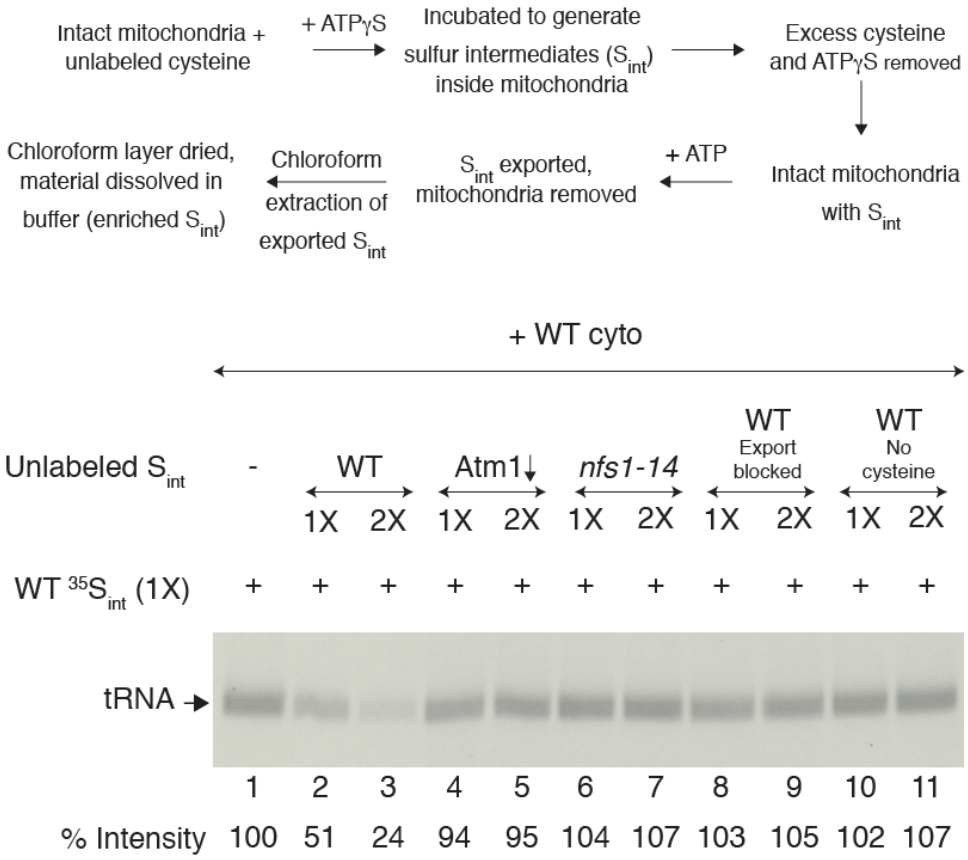

Figure S2. Activity assessment of unlabeled materials exported from various mitochondria, Related to Figures 6, 7 and S3, and Table S2.

(Top panel) A schematic outline for isolating materials exported from mitochondria is presented. Intact mitochondria (WT, Atm1↓, or *nfs1-14*) were incubated with unlabeled cysteine (10 μM) and ATPγS (2 mM) (1st step). Mitochondria were recovered, washed and then incubated with ATP (4 mM ATP) (2nd step). After centrifugation, the supernatant fractions containing the exported material was extracted with chloroform. The organic layer was dried down, and the dried material was dissolved with 20 mM Hepes/KOH, pH 7.5. As a control, the export reaction with WT mitochondria during the 2nd step was performed in the presence of ATPγS rather than ATP ("Export blocked"). Likewise, exported material from WT mitochondria with no cysteine preloading during the 1st step served as another negative control ("No cysteine"). All of these unlabeled exported materials (called "Unlabeled S_{int}") were then tested in a competition assay as follows.

(Bottom panel) $^{35}S_{int}$ exported from WT mitochondria ("WT $^{35}S_{int}$") was obtained as described above except mitochondria were incubated with [^{35}S]cysteine (10 μCi) instead of unlabeled cysteine during the 1st step. WT $^{35}S_{int}$ was added to WT cytosol (200 μg of proteins), and nucleotides were maintained at a final concentration of 4 mM ATP, 1 mM GTP, 2 mM NADH and 1 mM NADPH. Samples were incubated without (lane 1) or with unlabeled exported materials ("Unlabeled S_{int}"; lanes 2-11) in HS buffer (20 mM Hepes/KOH, pH 7.5, 0.6 M sorbitol) at 30˚C for 30 min. After TCA precipitation, samples were analyzed by 14% SDS-PAGE followed by autoradiography. 1X = 400 μg of starting mitochondrial proteins.

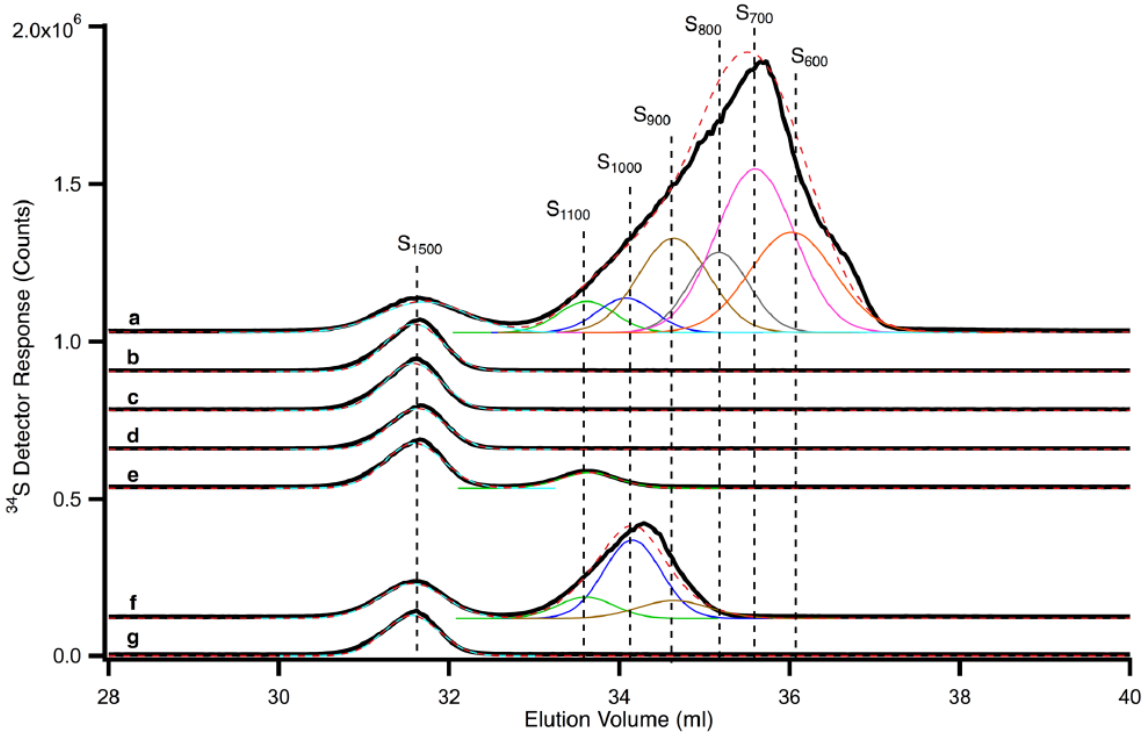

Figure S3. SEC-ICP-MS chromatogram of sulfur species, Related to Figures 5, 7 and S2, and Table S2.

Color-coding is the same as in Figure 7. Specifically, solid black lines are data, and dashed red lines are composite fits. Simulations of individual peaks are color-coded as follows: S_{1100}, green; S_{1000}, blue; S_{900}, brown; S_{800}, grey; S_{700}, pink. Additional simulation for S_{600} is indicated by orange color. Samples are: a) exported material from WT mitochondria (cysteine-preloaded), b) exported material from *nfs1-14* mitochondria (cysteine-preloaded), c) exported material from WT mitochondria with no cysteine preloading, d) exported material from WT mitochondria (cysteine-preloaded) in which ATPγS rather than ATP was used during the export process, e) exported material from Atm1-depleted (Atm1↓) mitochondria (cysteine-preloaded), f) material isolated from WT mitochondrial matrix after preloading with cysteine, and g) material isolated from WT mitochondrial matrix with no cysteine preloading.

Table S1. List of yeast strains used in this study, Related to STAR Methods.

Strain number	Name	Genotype	Source
66-16	BY4741 (parental WT)	*MATa his3D1 leu2D0 met15D0 ura3D0*	(Amutha et al., 2008)
109-65	Δ*urm1*	*MATa his3D1 leu2D0 met15D0 ura3D0 Δurm1::kanMX*	Research Genetics
105-59	*GAL1-DRE2*	*MATa his3D1 leu2D0 met15D0 ura3D0 kanMX6-PGAL1-DRE2::DRE2*	This work
53-57	YPH499 (parental WT)	*MATa ura3-52 lys2-801(amber) ade2-101(ochre) trp1-Δ63 his3-Δ200 leu2-Δ1 cyh2*	(Zhang et al., 2008)
68-8	*nfs1-14*	*MATa ura3-52 lys2-801(amber) ade2-101(ochre) trp1-Δ63 his3-Δ200 nfs1-14-LEU2 cyh2*	(Pandey et al., 2012)
115-10	*GAL1-ISU1*/Δ*isu2*	*MATa lys2-801(amber) ade2-101(ochre) trp1-Δ63 leu2-Δ1 pRS406-gamma nua2 (URA3) HIS3MX6-PGAL1-ISU1::ISU1*	(Yoon et al., 2015)
83-25	*GAL1-ATM1*	*MATa ura3-52 lys2-801(amber) ade2-101(ochre) trp1-Δ63 leu2-Δ1 cyh2 HIS3MX6-PGAL1-ATM1::ATM1*	(Zhang et al., 2008)
102-61	61 (parental WT)	*MATalpha FRE1-HIS3::URA3 trp1-63 leu2-3,112 gcn4-101 his3-609 SSC2*	*(Knight et al., 1998)*
73-39	33C (*ssq1* mutant)	*MATa FRE1-HIS3::URA3 TRP1 leu2-3,112 gcn4-101 his3-609 ssc2-1*	*(Knight et al., 1998)*

Table S2. Analysis of SEC-ICP-MS chromatographic traces, Related to Figures 5, 7, S2 and S3.

The most intense peak (S_{700} of Figure S3, trace *a*) was assigned a value of 100.

Trace	S_{1500}	S_{1100}	S_{1000}	S_{900}	S_{800}	S_{700}	S_{600}
Center position (ml)	31.5	33.6	34.1	34.6	35.2	35.6	36.0
Linewidth (ml)	0.9 ± 0.1	0.78	0.79	0.98	0.83	1.1	1.2
Relative Intensities							
Figure 7, trace a	24	17	11	44	25	69	0
Replicate	24	16	9	45	10	83	0
Replicate	23	17	10	44	20	70	0
Figure S3, trace a	21	14	16	51	37	100	66
Exported from WT mito (Cys preloaded) (Av)	23 ± 1	16 ± 1	12 ± 3	46 ± 3	23 ± 11	80 ± 14	16 ± 33
Figure 7, trace b	17	0	0	0	0	0	0
Replicate	19	0	0	0	0	0	0
Figure S3, trace b	21	0	0	0	0	0	0
Exported from *nfs1-14* mito (Cys preloaded) (Av)	19 ± 2						
Figure 7, trace c	21	0	0	0	0	0	0
Figure S3, trace c	21	0	0	0	0	0	0
Exported from WT mito with no Cys preloading (Av)	21						
Figure 7, trace d	20	0	0	0	0	0	0
Replicate	17	0	0	0	0	0	0
Figure S3, trace d	17	0	0	0	0	0	0
Exported from WT mito (Cys preloaded) in the presence of ATPγS (Av)	18 ± 2						
Figure 7, trace e	24	9	0	0	0	0	0
Replicate	20	7	0	0	0	0	0
Figure S3, trace e	20	7	0	0	0	0	0
Exported from Atm1↓ mito (Cys preloaded) (Av)	21 ± 2	8 ± 1					
Figure 7, trace f	19	13	11	44	25	0	0
Replicate	16	13	4	59	0	0	0
Figure S3, trace f	17	10	35	10	0	0	0
Isolated from WT mito matrix (Cys preloaded) (Av)	17 ± 2	12 ± 2	17 ± 16	38 ± 25	8 ± 14		
Figure 7, trace g	17	0	0	0	0	0	0
Figure S3, trace g	17	0	0	0	0	0	0
Isolated from WT mito matrix with no Cys preloading (Av)	17						
Figure 7, trace h Buffer control	29	0	0	0	0	0	0