



## Supplementary Materials for

MitoCPR - a surveillance pathway that protects mitochondria in  
response to protein import stress

Hilla Weidberg and Angelika Amon.

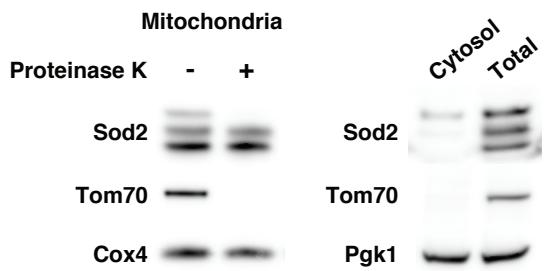
correspondence to: [angelika@mit.edu](mailto:angelika@mit.edu) and [hillaw@mit.edu](mailto:hillaw@mit.edu)

**This PDF file includes:**

Figs. S1 to S6  
Table S2

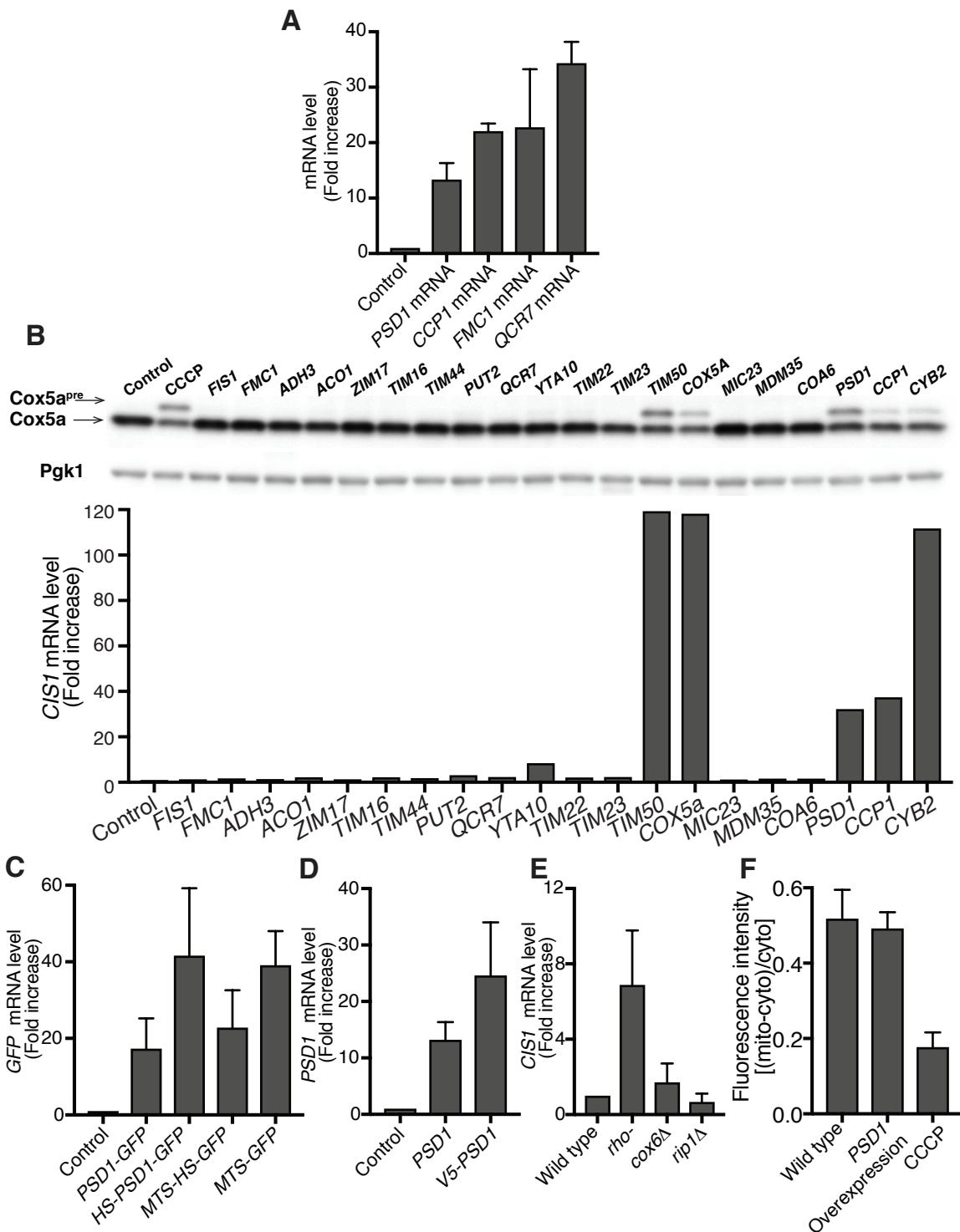
**Other Supplementary Materials for this manuscript includes the following:**

Database S1 as zipped archives: [Table 1]



**Fig. S1**

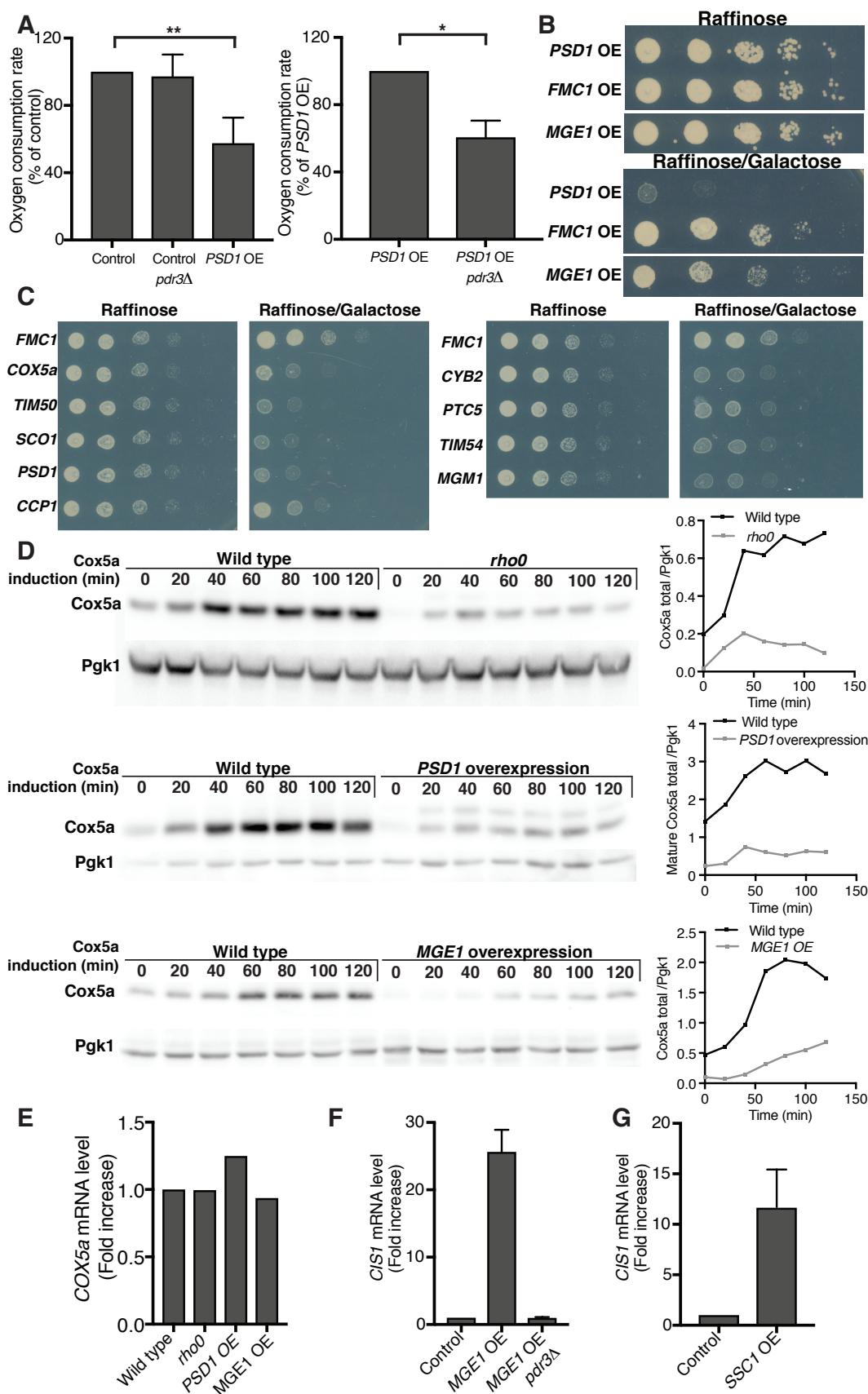
**Sod2's precursor accumulates at the mitochondrial surface in PSD1 overexpressing cells.** Mitochondria were isolated by differential centrifugation from cells overexpressing *PSD1* for 6 hours. Sod2-V5, Tom70-GFP and Cox4 or Pgk1 enrichment in mitochondrial and cytosolic fractions was analyzed by immuno-blot analysis. Mitochondria were treated with 50 µg/ml proteinase K. Tom70 served as an outer membrane control protein; Cox4 as a matrix control protein and Pgk1 as a cytosolic control protein.



**Fig. S2**

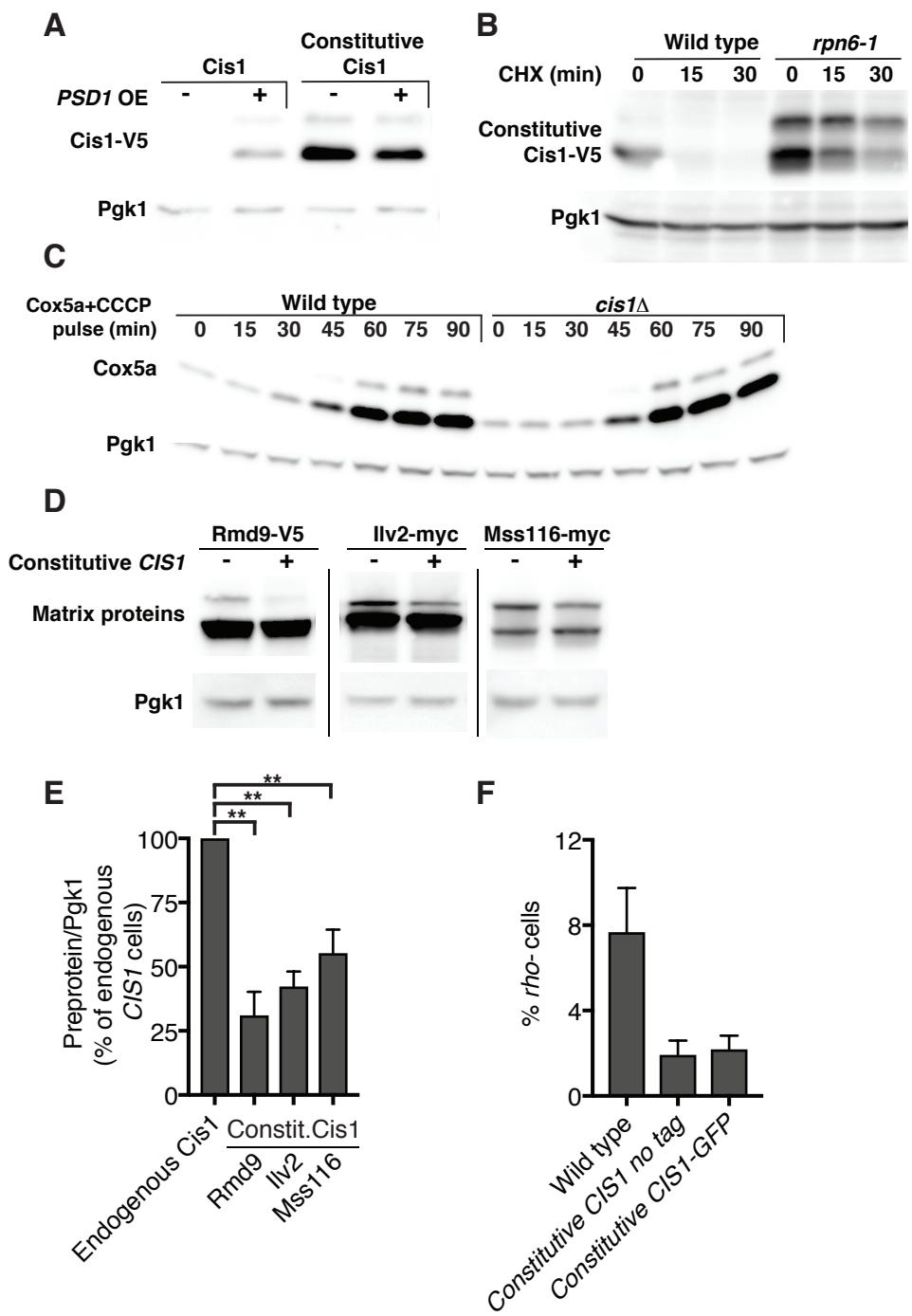
**Activation of the mitoCPR by bipartite signal-containing proteins.** (A) Cells carrying *PSD1*, *CCP1*, *FMC1* or *QCR7* under the control of the *GAL1-10* promoter were induced with galactose (4h) and mRNA levels were analyzed by quantitative RT-PCR. Each

mRNA level is presented as fold increase over control cells which were not induced with galactose. n=3. Data are mean +/- SD. **(B)** Upper panel: Same immuno-blot of Cox5a-V5 that is presented in Fig. 1A. Lower panel: *CIS1* mRNA levels in wild-type cells and cells overexpressing the indicated mitochondrial genes from the *GAL1-10* promoter for 4h. **(C)** mRNA levels of *PSD1-GFP* fusion genes expressed from the *GAL1-10* promoter were measured by quantitative RT-PCR. MTS, mitochondrial targeting sequence; HS, hydrophobic segment. n=3. Data are mean +/- SD. **(D)** *PSD1* mRNA levels were measured by quantitative RT-PCR in control cells or cells overexpressing *PSD1* or *V5-PSD1* for 4h. n=3. Data are mean +/- SD. **(E)** *CIS1* mRNA levels in wild type, *rho*-, *cox6Δ* and *rip1Δ* cells were analyzed by quantitative RT-PCR. n=3. Data are mean +/- SD. **(F)** *PDR1*, *PDR3* and *PDR5* deleted cells (to reduce efflux of dyes out of cells) carrying either an empty vector or a *GAL1-10-PSD1* vector and expressing a mitochondria-targeted mCherry (mt-mCherry) were grown in raffinose containing medium. The cells were diluted and recovered for an hour following the addition of galactose for 4h. Empty vector cells were untreated or treated with 20μM CCCP for 1h. To detect mitochondrial membrane potential, cells were incubated with 2.5μM Rhodamine 123. Mitochondria were identified by the mt-mCherry signal. Membrane potential was analyzed by the following equation: (mitochondrial fluorescence intensity - cytosolic fluorescence intensity)/ cytosolic fluorescence intensity. n=3. Data are mean +/- SD. mito, mitochondria. cyto, cytosol.



**Fig. S3**

**Overexpression of *MGE1* causes a mild mitochondrial protein import defect.** (A) Oxygen consumption rate of wild type and *pdr3Δ* cells under control conditions or following overexpression of *PSD1* for 4h. The slope of the linear range of oxygen consumption was used to measure oxygen consumption rate in 3 independent experiments. OE, overexpression. Data are mean +/- SD. Statistics were performed using the Student's t-test. \* p ≤ 0.05, \*\* p ≤ 0.005. (B) Five-fold serial dilutions of cells containing *GAL-PSD1*, *GAL-FMC1* (control matrix protein) or *GAL-MGE1* plasmids. Cells were grown to logarithmic phase and spotted on plates that do not contain uracil and contain either 2% raffinose or 2% raffinose and 1% galactose. OE, overexpression. (C) Serial dilutions as in (B) of cells overexpressing various bipartite signal-containing proteins. *FMC1* serves as a matrix protein control, all the rest overexpressed genes encode for bipartite signal-containing proteins (D) Wild type, *rho0* cells or cells overexpressing *PSD1* or *MGE1* all carrying a *MET25-COX5a* fusion were grown in the presence of methionine. *PSD1/MGE1* overexpression was induced for 6h by galactose addition. Cells were then transferred to medium lacking methionine to induce *MET25-COX5a* and the accumulation of Cox5a-V5 protein was monitored at the indicated times. Quantification of Cox5a is presented on the right. *Pgk1* was used as a loading control. (E) *COX5a* mRNA levels of the experiments presented in (D) were analyzed by quantitative RT-PCR. (F) *CIS1* mRNA levels in control cells, cells overexpressing *MGE1*, and *pdr3Δ* cells overexpressing *MGE1*. Overexpression was induced by galactose for 4h. n=3. Data are mean +/- SD. (G) Same as (F), with overexpression of *SSC1* instead of *MGE1*. n=3. Data are mean +/- SD.

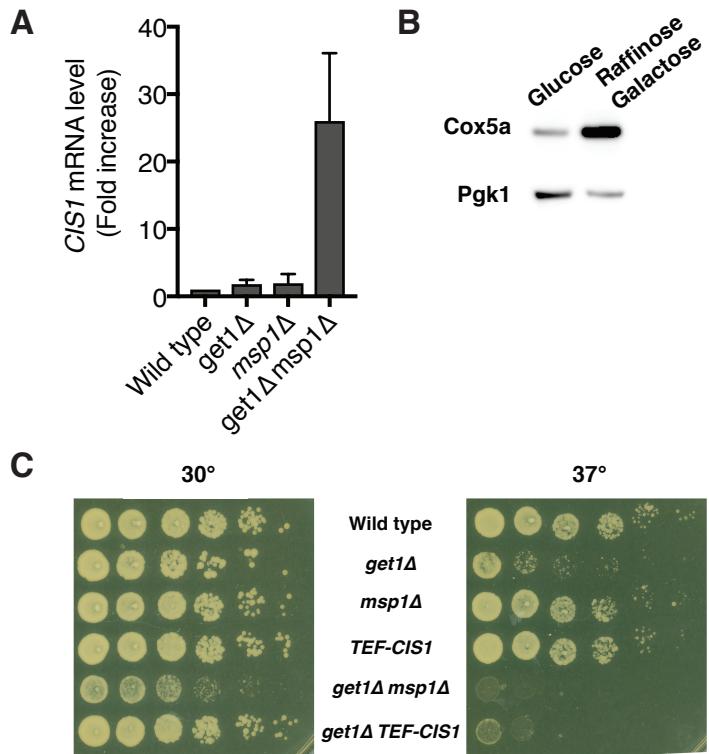


**Fig. S4**

**Effect of *CIS1* overexpression on matrix protein import and mtDNA maintenance.**

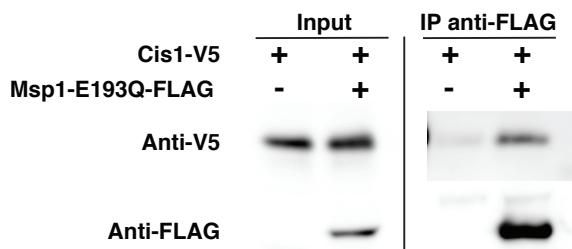
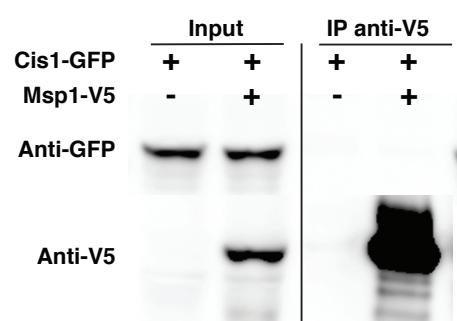
(A) Immuno-blot of Cis1-V5 and constitutive Cis1-V5 (encoded by *TEF2-CIS1-V5*) with or without overexpression of *PSD1* for 4h by galactose. OE, overexpression. Pgk1 was used as a loading control. (B) Immuno-blot of constitutive Cis1-V5 (encoded by *TEF2-CIS1-V5*) expressed in wild type or *rpn6-1* (proteasome defective mutant) cells following

the addition of cycloheximide (CHX; 0.5 mg/ml). Cells were grown at room temperature throughout the experiment. **(C)** Wild type or *cis1* $\Delta$  cells were grown in the presence of methionine. *MET25-COX5a* was then induced by methionine removal in the presence of 20 $\mu$ M CCCP. Cox5a-V5 protein levels were analyzed at the indicated times. Pgk1 served as a loading control. **(D)** Wild-type cells or cells expressing *TEF2-CIS1* were grown in the presence of methionine. *MET25-RMD9*, *MET25-ILV2* or *MET25-MSS116* were then induced by methionine removal in the presence of 20 $\mu$ M CCCP. Protein levels were analyzed 50 minutes after methionine removal. Pgk1 served as a loading control. **(E)** Quantification of (D) from 4 independent experiments. Preprotein levels observed in cells expressing constitutive *CIS1* (*TEF2-CIS1*) are shown as a fraction of protein observed in cells expressing endogenous *CIS1* (Preprotein levels in endogenous *CIS1* cells was set to 100%). Constit. Cis1, constitutive Cis1. Data are mean +/- SD. Statistics were performed using the Student's t-test. \*\* p  $\leq$  0.005. **(F)** *GAL-MGE1* cells expressing *TEF2-CIS1*, *TEF2-CIS1-GFP* or endogenous *CIS1* were grown for 24h in the presence of galactose. Mitochondrial DNA loss was determined by examining growth of colonies on YEP plates containing 2% ethanol and 0.3% glucose. n=3. Data are mean +/- SD.



**Fig. S5**

**Relationship between the mitoCPR and the GET pathway.** (A) *CIS1* mRNA levels in wild-type, *get1 $\Delta$* , *msp1 $\Delta$*  and *get1 $\Delta$  msp1 $\Delta$*  cells. Note that deletion of *GET1* was not sufficient to activate the mitoCPR. The response was, however, triggered in *msp1 $\Delta$  get1 $\Delta$*  cells because the double mutant experiences mitochondrial defects including protein import defects (38, 39). n=3. Data are mean +/- SD. (B) *MET25-COX5a* cells were grown in glucose containing medium or raffinose+galactose containing medium for 6h. The expression of Cox5a-V5 was examined by immuno-blot analysis. (C) Five-fold serial dilutions of wild type, *get1 $\Delta$* , *msp1 $\Delta$* , *TEF2-CIS1*, *get1 $\Delta$  msp1 $\Delta$* , and *get1 $\Delta$  TEF2-CIS1* cells. Cells were grown to logarithmic phase and spotted on YPD plates at 30°C or 37°C.

**A****B****Fig. S6**

**Cis1 and Msp1-E193Q form a complex.** **(A)** Cells expressing *TEF2-CIS1-V5* and *TEF2-CIS1-V5 msp1-E193Q-FLAG* cells were grown in YPD. Msp1-E193Q-FLAG was immunoprecipitated using an anti-FLAG antibody. **(B)** Cells expressing *TEF2-CIS1-GFP* and *TEF2-CIS1-GFP, MSP1-V5* cells were grown in YPD. Msp1-V5 was immunoprecipitated using an anti-V5 antibody.

**Table S2.**

Strains used in this study

<b>Strain Number</b>	<b>Relevant Genotype</b>	<b>Figure</b>
AA36823	MATA, ade2-1, leu2-3, ura3, trp1-1, his3-11,15, COX5a-3XV5::KANMX	1a, b, 6f
AA39405	MATA, ade2-1, leu2-3, ura3, trp1-1, his3-11,15, COX5a-3XV5::KANMX, 2micron-GAL-PSD1::URA3	1a, b, 4a, c, 5e, S2b
AA39406	MATA, ade2-1, leu2-3, ura3, trp1-1, his3-11,15, RIP1-3XV5::KANMX, Yeplac181-GAL-PSD1:: LEU2	1c
AA39407	MATA, ade2-1, leu2-3, ura3, trp1-1, his3-11,15, SOD2-3XV5::KANMX, Yeplac181-GAL-PSD1:: LEU2	1c
AA39408	MATA, ade2-1, leu2-3, ura3, trp1-1, his3-11,15, MDH1-3XV5::KANMX, Yeplac181-GAL-PSD1:: LEU2	1c
AA39409	MATA, ade2-1, leu2-3, ura3, trp1-1, his3-11,15, PAM17-3XV5::KANMX, Yeplac181-GAL-PSD1:: LEU2	1c
AA36884	MATA, ade2-1, leu2-3, ura3, trp1-1, his3-11,15, COX5a-3XV5::KANMX, TOM70-mCherry::NATMX	2a
AA39410	MATA, ade2-1, leu2-3, ura3, trp1-1, his3-11,15, COX5a-3XV5::KANMX, TOM70-mCherry::NATMX, Yeplac181-GAL-PSD1:: LEU2	2a
AA39561	MATalpha, ade2-1, leu2-3, ura3, trp1-1, his3-11,15, COX5a-3XV5::KANMX, CIS1::GFP::KanMX, TOM70-mcherry::NATMX	2b
AA39590	MATalpha, ade2-1, leu2-3, ura3, trp1-1, his3-11,15, COX5a-3XV5::KANMX, CIS1::GFP::KanMX, TOM70-mcherry::NATMX, Yeplac181-GAL-PSD1:: LEU2	2b
AA39411	MATA, ade2-1, leu2-3, ura3, trp1-1, his3-11,15, COX5a-3XV5::KANMX 2micron-GAL-PSD1-GFP::URA3	2d
AA39413	MATA, ade2-1, leu2-3, ura3, trp1-1, his3-11,15, COX5a-3XV5::KANMX 2micron-GAL-IMS-PSD1-GFP::URA3	2d
AA39412	MATA, ade2-1, leu2-3, ura3, trp1-1, his3-11,15, COX5a-3XV5::KANMX 2micron-GAL-MTS-IMS-GFP::URA3	2d
AA39414	MATA, ade2-1, leu2-3, ura3, trp1-1, his3-11,15, COX5a-3XV5::KANMX 2micron-GAL-MTS-GFP::URA3	2d
AA39560	MATalpha, ade2-1, leu2-3, ura3, trp1-1, his3-11,15, SOD2-3XV5::KANMX, TOM70::GFP::KanMX, Yeplac181-GAL-PSD1:: LEU2	S1
AA39849	MATA, ade2-1, leu2-3, ura3, trp1-1, his3-11,15, SOD2-3XV5::KANMX, CIS1::GFP::KanMX, TOM70-mCHERRY::NAT, Yeplac181-GAL-PSD1:: LEU2	2c
AA39427	MATA, ade2-1, leu2-3, ura3, trp1-1, his3-11,15, empty pRS426::URA	3a, 4d, S3a
AA33642	MATA, ade2-1, leu2-3, ura3, trp1-1, his3-11,15, 2micron-GAL-PSD1::URA3	3a, b, c, e 4d S2a, d S3a, b, c
AA39415	MATA, ade2-1, leu2-3, ura3, trp1-1, his3-11,15, pdr3::HIS3, 2micron-GAL-PSD1::URA3	3a, b, 4d S3a
AA33772	MATA, ade2-1, leu2-3, ura3, trp1-1, his3-11,15, 2micron-GAL-CCP1::URA3	3c S2a
AA33645	MATA, ade2-1, leu2-3, ura3, trp1-1, his3-11,15, 2micron-GAL-	3c

	FMC1::URA3	S2a
AA33644	MATA, ade2-1, leu2-3, ura3, trp1-1, his3-11,15, 2micron-GAL-QCR7::URA3	3c S2a
AA34306	MATA, ade2-1, leu2-3, ura3, trp1-1, his3-11,15, 2micron-GAL-PSD1-GFP::URA3	3d S2c
AA39416	MATA, ade2-1, leu2-3, ura3, trp1-1, his3-11,15, 2micron-GAL-IMS-PSD1-GFP::URA3	3d S2c
AA39417	MATA, ade2-1, leu2-3, ura3, trp1-1, his3-11,15, 2micron-GAL-2micron-GAL-MTS-IMS-GFP::URA3	3d S2c
AA39418	MATA, ade2-1, leu2-3, ura3, trp1-1, his3-11,15, 2micron-GAL-2micron-GAL-MTS-GFP::URA3	3d S2c
AA39419	MATA, ade2-1, leu2-3, ura3, trp1-1, his3-11,15, 2micron-GAL-3XV5-PSD1::URA3	3e S2d
AA2587	MATA, ade2-1, leu2-3, ura3, trp1-1, his3-11,15 (WT W303)	3f, g, S2e, S5a, c
AA32564	MATA, ade2-1, leu2-3, ura3, trp1-1, his3-11,15, rho0	3f, g
AA33391	MATA, ade2-1, leu2-3, ura3, trp1-1, his3-11,15, mrpl16::KanMX	3f, g, S2e
AA34966	MATA, ade2-1, leu2-3, ura3, trp1-1, his3-11,15, tam41::NATMX	3f, g
AA33542	MATA, ade2-1, leu2-3, ura3, trp1-1, his3-11,15, pdr3::HIS3	3f
AA39420	MATA, ade2-1, leu2-3, ura3, trp1-1, his3-11,15, pdr3::HIS3, rho0	3f
AA33546	MATalpha, ade2-1, leu2-3, ura3, trp1-1, his3-11,15, pdr3::HIS3, mrpl16::KanMX	3f
AA35132	MATA, ade2-1, leu2-3, ura3, trp1-1, his3-11,15, pdr3::HIS3, tam41::NATMX	3f
AA39421	MATA, ade2-1, leu2-3, ura3, trp1-1, his3-11,15, pRS316-ATP1-111::URA3	3g
AA39422	MATA, ade2-1, leu2-3, ura3, trp1-1, his3-11,15, pRS316-ATP1-111::URA3, rho0	3g
AA39423	MATA, ade2-1, leu2-3, ura3, trp1-1, his3-11,15, mrpl16::KanMX, pRS316-ATP1-111::URA3	3g
AA39424	MATA, ade2-1, leu2-3, ura3, trp1-1, his3-11,15, tam41::NATMX, pRS316-ATP1-111::URA3	3g
AA39425	MATA, ade2-1, leu2-3, ura3, trp1-1, his3-11,15, COX5a-3XV5::KANMX, pdr3::HIS3, 2micron-GAL-PSD1::URA3	4a
AA39426	MATA, ade2-1, leu2-3, ura3, trp1-1, his3-11,15, COX5a-3XV5::KANMX, NATMX::pTEF2-3XV5-PDR3::KanMX, 2micron-GAL-PSD1::URA3	4c
AA39428	MATA, ade2-1, leu2-3, ura3, trp1-1, his3-11,15, pdr3::HIS3, empty pRS426::URA3	4d, S3a
AA39429	MATA, ade2-1, leu2-3, ura3, trp1-1, his3-11,15, 2micron-GAL-MGE1::URA3	4e, 5d, 5i, S3b, S3f, S4f
AA39430	MATA, ade2-1, leu2-3, ura3, trp1-1, his3-11,15, pdr3::HIS3, 2micron-GAL-MGE1::URA3	4e, 5d, 5i, S3f
AA36827	MATA, ade2-1, leu2-3, ura3, trp1-1, his3-11,15, pTEF2::NATMX::CIS1-GFP::KanMX, leu2::LEU2::pADH1-PreSeqCOX4-mCherry	5a, 7b
AA37012	MATA, ade2-1, leu2-3, ura3, trp1-1, his3-11,15, pTEF2::NATMX::CIS1-3XV5::HIS, TOM70-GFP::KanMx, leu2:: pADH1-PreSeqCOX4-mCherry::LEU2	5b, c
AA39431	MATA, ade2-1, leu2-3, ura3, trp1-1, his3-11,15, cis1::HIS3, 2micron-GAL-MGE1::URA3	5d

AA39432	MATA, ade2-1, leu2-3, ura3, trp1-1, his3-11,15, COX5a-3XV5::KANMX, leu2::pTEF2-CIS1::LEU2, 2micron-GAL-PSD1::URA3	5e
AA37293	MATA, ade2-1, leu2-3, ura3, trp1-1, his3-11,15, pMET25::NATMX::COX5a-3XV5::KANM	5f, 6c, g, S3d, e, S4c S5b
AA39433	MATA, ade2-1, leu2-3, ura3, trp1-1, his3-11,15, pMET25::NATMX::COX5a-3XV5::KANM, leu2::pTEF2-CIS1::LEU2	5f
AA39987	MATA, ade2-1, leu2-3, ura3, trp1-1, his3-11,15, pTEF2::NATMX::CIS1-GFP::KanMX, 2micron-GAL-MGE1::URA3	5i, S4f
AA39988	MATA, ade2-1, leu2-3, ura3, trp1-1, his3-11,15, pTEF2::NATMX::CIS1-GFP::KanMX, pdr3::HIS3, 2micron-GAL-MGE1::URA3	5i
AA39434	MATA, ade2-1, leu2-3, ura3, trp1-1, his3-11,15, pMET25::NATMX::COX5a-3XV5::KANM, rpn6-1::URA3	6a
AA39435	MATA, ade2-1, leu2-3, ura3, trp1-1, his3-11,15, pMET25::NATMX::COX5a-3XV5::KANM, rpn6-1::URA3, leu2::pTEF2-CIS1::LEU2	6a
AA39436	MATA, ade2-1, leu2-3, ura3, trp1-1, his3-11,15, pMET25::NATMX::COX5a-3XV5::KANM, msp1::HYG, trp1:GAL-MSP1 E193Q-FLAG::TRP1	6c, g, i
AA39460	MATalpha, ade2-1, leu2-3, ura3, trp1-1, his3-11,15, pMET25::NATMX::COX5a-3XV5::KANM, msp1::HYG, trp1:GAL-MSP1 E193Q-FLAG::TRP1, leu2::pTEF2-CIS1::LEU2	6i
AA39437	MATA, ade2-1, leu2-3, ura3, trp1-1, his3-11,15, COX5a-3XV5::KANMX, msp1::HYG	6f
AA39803	MATA, ade2-1, leu2-3, ura3, trp1-1, his3-11,15, msp1::HYG, trp1:MSP-msp1 E193Q-FLAG::TRP1	7a
AA39750	MATA, ade2-1, leu2-3, ura3, trp1-1, his3-11,15, pTEF2::NATMX::CIS1-3xV5::HIS, msp1::HYG, trp1:MSP-msp1 E193Q-FLAG::TRP1	7a, S6a
AA39439	MATA, ade2-1, leu2-3, ura3, trp1-1, his3-11,15, pTEF2::NATMX::CIS1-GFP::KanMX, leu2::LEU2::pADH1-PreSeqCOX4-mCherry, tom70::NATMX6	7b
AA39751	MATA, ade2-1, leu2-3, ura3, trp1-1, his3-11,15, msp1::HYG, trp1:MSP-msp1 E193Q-FLAG::TRP1, TOM70::GFP::KanMX	7c
AA39753	MATA, ade2-1, leu2-3, ura3, trp1-1, his3-11,15, msp1::HYG, trp1:MSP-msp1 E193Q-FLAG::TRP1, TOM70::GFP::KanMX, pTEF2::NATMX::CIS1-3xV5::HIS	7c
AA39620	MATA, ade2-1, leu2-3, ura3, trp1-1, his3-11,15, msp1::HYG, TOM70::GFP::KanMX	7d
AA39751	MATA, ade2-1, leu2-3, ura3, trp1-1, his3-11,15, msp1::HYG, TOM70::GFP::KanMX, trp1:MSP-msp1 E193Q-FLAG::TRP1	7d
AA33530	MATA, ade2-1, leu2-3, ura3, trp1-1, his3-11,15, cox6::HIS3	S2e
AA33536	MATA, ade2-1, leu2-3, ura3, trp1-1, his3-11,15, rip1::KanMX6	S2e
AA40141	MATA, ade2-1, leu2-3, ura3, trp1-1, his3-11,15, pdr3::HIS3, pdr1::NATMX, pdr5::NATMX, trp1::pADH1-preSeqCOX4-mCherry-PEST::KANmx6 ,empty pRS426::URA3	S2f
AA40142	MATA, ade2-1, leu2-3, ura3, trp1-1, his3-11,15, pdr3::HIS3, pdr1::NATMX, pdr5::NATMX, trp1::pADH1-preSeqCOX4-mCherry-PEST::KANmx6, 2micron-GAL-PSD1::URA3	S2f
AA39440	MATA, ade2-1, leu2-3, ura3, trp1-1, his3-11,15, pMET25::NATMX::COX5a-3XV5::KANM, rho0	S3d, e
AA39441	MATA, ade2-1, leu2-3, ura3, trp1-1, his3-11,15, pMET25::NATMX::COX5a-	S3d, e

	3XV5::KANM, 2micron-GAL-PSD1::URA3	
AA39442	MATA, ade2-1, leu2-3, ura3, trp1-1, his3-11,15, pMET25::NATMX::COX5a-3XV5::KANM, 2micron-GAL-MGE1::URA3	S3d, e
AA39443	MATA, ade2-1, leu2-3, ura3, trp1-1, his3-11,15, 2micron-GAL-SSC1::URA3	S3g
AA39985	MATA, ade2-1, leu2-3, ura3, trp1-1, his3-11,15, CIS1-3xV5::HIS, 2micron-GAL-PSD1::URA3	S4a
AA39986	MATA, ade2-1, leu2-3, ura3, trp1-1, his3-11,15, pTEF2::NATMX::CIS1-3xV5::HIS, 2micron-GAL-PSD1::URA3	S4a
AA36517	MATA, ade2-1, leu2-3, ura3, trp1-1, his3-11,15, pTEF2::NATMX::CIS1-3xV5::HIS	S4b, S4a
AA39444	MATA, ade2-1, leu2-3, ura3, trp1-1, his3-11,15, pTEF2::NATMX::CIS1-3xV5::HIS, rpn6-1	S4b
AA39348	MATA, ade2-1, leu2-3, ura3, trp1-1, his3-11,15, pMET25::NATMX::COX5a-3XV5::KANM, cis1::HIS3	S4c
AA39991	MATA, ade2-1, leu2-3, ura3, trp1-1, his3-11,15, pMET25::NATMX::RMD9-3XV5::KANMX	S4d
AA39992	MATA, ade2-1, leu2-3, ura3, trp1-1, his3-11,15, pMET25::NATMX::RMD9-3XV5::KANMX, leu2::pTEF2-CIS1::LEU2	S4d
AA39995	MATA, ade2-1, leu2-3, ura3, trp1-1, his3-11,15, pMET25::NATMX::ILV2-9Xmyc::HYG	S4d
AA39996	MATA, ade2-1, leu2-3, ura3, trp1-1, his3-11,15, pMET25::NATMX::ILV2-9Xmyc::HYG, leu2::pTEF2-CIS1::LEU2	S4d
AA39993	MATA, ade2-1, leu2-3, ura3, trp1-1, his3-11,15, pMET25::NATMX::MSS116-9Xmyc::HYG	S4d
AA39994	MATA, ade2-1, leu2-3, ura3, trp1-1, his3-11,15, pMET25::NATMX::MSS116-9Xmyc::HYG, leu2::pTEF2-CIS1::LEU2	S4d
AA39984	MATA, ade2-1, leu2-3, ura3, trp1-1, his3-11,15, leu2::pTEF2-CIS1::LEU2, 2micron-GAL-MGE1::URA3	S4f
AA39619	MATA, ade2-1, leu2-3, ura3, trp1-1, his3-11,15, msp1::HYG	S5a, c
AA39553	MATA, ade2-1, leu2-3, ura3, trp1-1, his3-11,15, get1::HYG	S5a, c
AA39562	MATA, ade2-1, leu2-3, ura3, trp1-1, his3-11,15, get1::HYG, msp1::HYG	S5a, c
AA39576	MATalpha, ade2-1, leu2-3, ura3, trp1-1, his3-11,15, leu2::pTEF2-CIS1::LEU2	S5c
AA39555	MATA, ade2-1, leu2-3, ura3, trp1-1, his3-11,15, get1::HYG, leu2::pTEF2-CIS1::LEU2	S5c
AA39749	MATA, ade2-1, leu2-3, ura3, trp1-1, his3-11,15, msp1::HYG, pTEF2::NATMX::CIS1-3xV5::HIS	S6a
AA39367	MATA, ade2-1, leu2-3, ura3, trp1-1, his3-11,15, MSP1-3V5::KANMX, pTEF::NATMX::CIS1-GFP::KanMX	S6b
AA35067	MATA, ade2-1, leu2-3, ura3, trp1-1, his3-11,15, pTEF::NATMX::CIS1-GFP::KanMX	S6b

**Additional Data table S1 (separate file)**

Gene expression analysis