The nucleus is a quality control center for non-imported mitochondrial proteins

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Abstract

Mitochondrial import deficiency causes cellular stress due to the accumulation of non-imported mitochondrial precursor proteins. Despite the burden mis-localized mitochondrial precursors place on cells, our understanding of the systems that dispose of these proteins is incomplete. Here, we catalog the location and steady-state abundance of mitochondrial precursor proteins during mitochondrial impairment in *S. cerevisiae*. We find that a number of non-imported mitochondrial proteins localize to the nucleus, where they are eliminated by proteasome-based nuclear protein quality control. Recognition of mitochondrial precursors by the nuclear quality control machinery requires the presence of an N-terminal mitochondrial targeting sequence (MTS), and impaired breakdown of precursors leads to their buildup in nuclear-associated foci. These results identify the nucleus as a key destination for the disposal of non-imported mitochondrial precursors.

Main Text

Mitochondrial dysfunction is a hallmark of aging and associated with many age-related and metabolic diseases (*1*). Mitochondrial impairment disrupts metabolic pathways housed within the mitochondrion, and also prevents the import of thousands of mitochondrial resident proteins that rely on an efficient mitochondrial membrane potential for translocation into the organelle (*2-4*). Recent studies have shown that non-imported mitochondrial precursor proteins are toxic for cells, and identified several cellular pathways that combat this stress by disposing or triaging non-imported precursors (*5-11*). However, despite these recent advances, only a fraction of the non-imported mitochondrial proteome has been analyzed under conditions of mitochondrial impairment. Thus, our understanding of the fate of non-imported mitochondrial precursors remains incomplete. Here, using microscopy and immunoblot-based screens in *S. cerevisiae*, we show that non-imported mitochondrial proteins accumulate in many regions of the cell upon mitochondrial depolarization, and identify the nucleus as an important quality control destination for non-imported mitochondrial precursors. We find that many mitochondrial proteins localize to the nucleus upon import failure, where they are subjected to proteasome-dependent destruction via redundant action of the E3 ubiquitin ligases San1, Ubr1, and Doa10. When degradation capacity is exceeded, mitochondrial precursors are sequestered into nuclear-associated protein aggregates. We show that the N-terminal mitochondrial targeting sequence (MTS) (*12*) is necessary for non-imported precursor protein induced-toxicity, degradation, and sequestration into aggregates, but dispensable for nuclear localization. The presence of an MTS is also required for degradation and toxicity of non-imported proteins that localize to cellular regions other than the nucleus, implicating the MTS as a major driver of non-imported precursor toxicity. Finally, we show that nuclear accumulation of non-imported precursors arises during cellular aging. Overall, this work

demonstrates that non-imported mitochondrial proteins exhibit numerous fates within cells, and identifies the nucleus as an important quality control destination for non-imported mitochondrial precursor proteins.

We previously showed that the mitochondrial network undergoes extensive fragmentation and depolarization during replicative aging in budding yeast, which is defined as the number of times an individual yeast cell undergoes division (*13*). In our earlier work, we utilized an endogenously tagged version of the mitochondrial outer membrane (OM) protein Tom70-GFP to visualize the mitochondrial network. In contrast to Tom70, which does not rely on mitochondrial membrane potential for its mitochondrial localization (*14*), functional, endogenously GFP-tagged Ilv2 (fig. S1A), a key mitochondrial matrix enzyme in isoleucine and valine biosynthesis (*15*), exhibited dual localization in replicatively aged yeast cells. In addition to a pattern consistent with mitochondrial tubules, Ilv2-GFP also localized to the nucleus in over 80% of aged cells, as indicated by diffuse GFP fluorescence within a region surrounded by the nuclear pore protein Nup49-mCherry (Fig. 1A).

Mitochondrial depolarization is a hallmark feature of aged yeast (*13*). Because depolarization is prominent, and the import of Ilv2 requires a mitochondrial inner membrane (IM) potential, we wondered whether the fraction of Ilv2-GFP localized to the nucleus represented a non-imported precursor pool of this protein. Consistent with that idea, treatment of cells with the mitochondrial IM depolarizing agent trifluoromethoxy carbonyl cyanide phenylhydrazone (FCCP) (*16*) also caused Ilv2-GFP accumulation in the nucleus, which was marked with 4',6-Diamidine-2'-phenylindole dihydrochloride (DAPI) (Fig. 1B). Nuclear localization did not result from GFP-tagging, as indirect immunofluorescence showed a similar nuclear localization of C-terminally FLAG-tagged Ilv2 in FCCP treated cells (fig. S1B). Furthermore, Ilv2-GFP localized to the

nucleus in cells conditionally depleted of the essential OM protein import channel Tom40 (*17, 18*) (fig. S1C and D), indicating that nuclear localization was not caused by off-target effects of FCCP, but was specific to defects in mitochondrial protein import.

We hypothesized that the nuclear pool of Ilv2 likely represented a fraction of the protein that failed to import into mitochondria. Consistent with that idea, western blot analysis revealed that a higher-molecular weight form of Ilv2-GFP and Ilv2-HA accumulated in cells treated with FCCP (Fig. 1C and fig. S1E). Mitochondrial proteins such as Ilv2 are synthesized with an N-terminal MTS extension that is proteolytically removed from the mature peptide only after they transit the mitochondrial IM (*19, 20*). Thus, the higher-molecular weight form of Ilv2 in FCCP treated cells likely represents the immature, precursor form of the protein. In support of the idea that the non-imported pool of Ilv2 localizes to the nucleus, the precursor form of Ilv2-HA was specifically enriched in nuclear fractions isolated from FCCP-treated cells, while other mitochondrial proteins, including Tom70, Tim44, as well as the mature form of Ilv2, were excluded (Fig. 1D). Additionally, we utilized the Recombination-Induced Tag Exchange (RITE) system (*21*) to examine the fate of both old and newly synthesized Ilv2 in the same cell, and found that only newly synthesized Ilv2 localized to the nucleus upon FCCP treatment, while Ilv2 already present in mitochondria did not (Fig. 1E). Collectively, these results indicate that when the translocation of Ilv2 into mitochondria is blocked by genetic or pharmacologic impairment of mitochondrial import, the non-imported precursor form of Ilv2 alternatively localizes to the nucleus.

We next sought to determine the extent to which non-imported proteins localize to the nucleus in cells lacking efficient mitochondrial import. To address this question in a systematic manner, we imaged a collection of yeast strains expressing 526 distinct mitochondrial proteins

with carboxy-terminal GFP fusions from their endogenous loci in the absence or presence of FCCP. These strains were derived from the yeast GFP collection (*22*) and co-expressed Tom70-mCherry, a mitochondrial OM marker that localizes to mitochondria independently of the membrane potential (*14, 23*). We found that 6.3% of the mitochondrial proteins analyzed behaved like Ilv2, exhibiting nuclear localization in FCCP treated cells (class 1, Fig. 1F and table S1). Additionally, we identified four other major outcomes for mitochondrial proteins after membrane depolarization (Fig. 1F and table S1). These included continued localization to the mitochondrion (class 2, e.g., Tom20, 8.4% of all proteins), accumulation in the cytoplasm (class 3, e.g., Acp1, 36.1% of all proteins), localization to the endoplasmic reticulum (ER) (class 4, e.g., Mir1, 2.9% of all proteins), and reduced overall abundance to the point of being nearly undetectable (class 5, e.g., Cox15, 42.0% of all proteins). A subset of proteins (4.3%) localized to regions of the cell distinct from these five major classes upon FCCP treatment and associated with unidentified cellular membranes and foci (table S1). We validated representatives from each class and confirmed ER localization of class 4 proteins via co-localization with the ER marker Sec61-mCherry (Fig. 1F fig. S1F). As with Ilv2-GFP, identical fates occurred for all classes of proteins in cells conditionally depleted of the essential OM protein import channel Tom40 (*17, 18*) (fig. S1G), as well as in cells expressing FLAG-tagged versions of the proteins (fig. S1H), indicating the observed changes were not caused by off target effects of FCCP or the presence of a GFP tag.

We concurrently analyzed steady-state protein abundance via western blotting of the same set of GFP-tagged mitochondrial proteins in the absence and presence of FCCP, as this approach provided useful information about the state of Ilv2 in the nucleus. In general, steady-state levels of proteins localized to the mitochondrion, cytoplasm, and ER were unchanged or partially reduced with FCCP (Fig. 1G, table S1). Proteins that localized to the nucleus or became undetectable often

147 either moderately or strongly decreased in abundance upon FCCP treatment, respectively (Fig. 1G,
148 table S1). The decline in class 5 protein abundance was either completely or partially blunted by
149 proteasome inhibition via MG-132 depending on the individual protein substrate (Fig. 1, H and I),
150 implicating the proteasome in their destruction. Furthermore, as with Ilv2, precursor forms of Acp1
151 and Lat1 (class 3 and 5) were visible in the presence of FCCP (Fig. 1, G and H), and C-terminally
152 HA-tagged versions of representatives from each of the five classes showed identical alterations
153 in protein levels as the GFP-tagged versions (fig. S1, I to K).

154 Overall, our screen revealed several patterns amongst the proteins that comprised each
155 screen class, and many of our observations aligned well with those from previous studies (Fig. 1J).
156 Nuclear-localized class 1 proteins were predominantly mitochondrial matrix enzymes, including
157 numerous members of the TCA cycle. Most class 2 proteins that continued to localize to
158 depolarized mitochondria were mitochondrial OM proteins that do not require a membrane
159 potential for mitochondrial targeting (*4*). Class 3 (cytoplasm) proteins were largely soluble
160 proteins, several of which (e.g., Idh1, Idh2, Mss116, and Cis1) were previously found to be
161 enriched in cytosolic extracts isolated from mitochondrial import-deficient yeast (*5, 7*). ER-
162 localized class 4 proteins were generally integral IM and OM proteins, some of which were
163 previously reported to localize to the ER in cells with compromise mitochondrial import(*9*).
164 Finally, class 5, the largest of the classes, consisted of both soluble and membrane-bound
165 mitochondrial proteins.

166 We next wanted to understand the basis for the nuclear localization of non-imported
167 mitochondrial proteins in the absence of functional mitochondrial import. The eukaryotic nucleus
168 harbors a large proportion of cellular proteasomes, and is a quality control destination for
169 misfolded proteins (*24*). Because the overall abundance of nuclear-localized mitochondrial

proteins declined during FCCP treatment, we tested whether non-imported mitochondrial precursor proteins were directed to the nucleus for proteasomal degradation. In support of that idea, the decline in steady-state levels of Ilv2-GFP and Ilv2-HA upon FCCP treatment was blunted in the presence of proteasome inhibitor MG-132 (Fig. 2A and fig. S2A). Ilv2 decline was also prevented in strains lacking a combination of three E3 ubiquitin ligases that operate in nuclear-associated protein quality control, San1 (*25*), Ubr1 (*26*), and Doa10 (*27, 28*) (E3 KO) (Fig. 2B and fig. S2B). No combination of single or double knockouts completely prevented loss of Ilv2 upon mitochondrial depolarization, suggesting these ligases act redundantly to promote non-imported mitochondrial protein clearance (fig. 2C). Importantly, the addition of proteasome inhibitor or deletion of the aforementioned E3 ligases each led to a marked elevation in the higher molecular weight precursor form of Ilv2 in the presence of FCCP, suggesting the immature, Ilv2 precursor was the form of the protein specifically marked for proteasome clearance (Fig. 2, A and B, fig. S2, A and B). In line with this observation, cycloheximide-chase analysis demonstrated that the half-life of the Ilv2 precursor form was altered in the E3 KO strain, while the mature form was unaffected (Fig. 2C and fig. S2D). Furthermore, ubiquitin immunoprecipitation assays indicated that Ilv2 was ubiquitylated in the presence of FCCP in a San1, Ubr1, and Doa10-dependent manner (Fig. 2D). Proteasome-dependent degradation of a non-nuclear class 5 substrate (Lat1) was unaffected in the E3 KO strain, indicating that additional E3 ligases promote clearance of non-nuclear localized mitochondrial precursors (fig. S2E). Finally, we found that our observations extend beyond Ilv2, as two other nuclear candidates identified in our screen were also eliminated in a proteasome and San1/Ubr1/Doa10-dependent manner (fig. S2, F to K). Thus, a subset of non-imported mitochondrial proteins are subjected to nuclear-associated protein quality control when their import into mitochondria is impaired.

As the toxicity of non-imported precursor proteins is now well documented (*5*), we wondered whether failure to destroy nuclear-localized non-imported precursors would compromise cellular health. To test this idea, we compared the growth of wild type and the aforementioned E3 KO strains in the absence and presence of FCCP. We observed no growth defect in single, double, or triple E3 ligase knockout strains (Fig. 3A, fig. S3A), suggesting redundant systems may act to mitigate the toxicity of nuclear-localized non-imported proteins. Consistent with that idea, we noticed that in addition to diffuse nuclear localization, a portion of Ilv2-GFP accumulated in nuclear-associated foci that resembled previously described juxtanuclear (JUNQ) (*29*) or intranuclear (INQ) (*30*) protein aggregate compartments (Fig. 3B). These foci were adjacent to the DAPI-stained nucleus, excluded the mitochondrial marker Tom70-mCherry, and were present in a high percentage of FCCP-treated cells (Fig. 3C). Prior studies showed that misfolded proteins can be sequestered into nuclear associated aggregates when their proteasomal clearance is impaired (*29, 30*). Consistent with that idea, the intensity of Ilv2-GFP foci increased in the E3 KO strain (Fig. 3D). Moreover, Dld1 and Dld2, which are degraded more robustly than Ilv2, also localized to nuclear-associated protein aggregates, but only in strains lacking the E3 ligase degradation machinery (fig. S3, B to E). We were unable to identify a mutation that blocked localization to these puncta. However, we did find that a two-fold increase in expression of Ilv2-GFP from a single copy plasmid resulted in constitutive localization of Ilv2-GFP to the nucleus and nuclear associated protein foci (see Fig. 4, B to D), and resulted in severe growth defects in both wild-type and E3 KO strains (Fig. 3E). These results indicate that non-imported nuclear-localized mitochondrial proteins are toxic, and that proteasome destruction and aggregate sequestration may act in coordination to mitigate this toxicity.

Finally, we sought to elucidate the features of non-imported mitochondrial proteins that drive nuclear-associated aggregation, degradation and toxicity. Mitochondrial matrix proteins such as Ilv2 are synthesized as precursors with an N-terminal MTS (*19*). The MTS is removed by mitochondrial-localized proteases after import (*20*), and failure to remove and clear MTSs leads to toxicity (*31, 32*). To test whether the presence of an MTS on an unimported mitochondrial precursor protein is problematic, we analyzed strains containing single-copy plasmids expressing full-length Ilv2-GFP (FL), MTS-deleted Ilv2-GFP (ΔMTS) and MTS_{Ilv2}-GFP only (MTS) from the constitutive GPD promoter (Fig. 4A). Like endogenous Ilv2-GFP (Endo), plasmid-derived FL-Ilv2-GFP localized to the nucleus and nuclear-associated foci in both wild type and E3 KO, and its abundance declined with FCCP (Fig. 4, B to F). By contrast, Ilv2 lacking an MTS (ΔMTS-Ilv2-GFP) constitutively localized to the nucleus even in the absence of FCCP, but never formed nuclear-associated foci or decreased in abundance with FCCP (Fig. 4, B to F). MTS_{Ilv2}-GFP localized to mitochondria and exhibited no nuclear localization, puncta formation, or changes in total abundance with FCCP (Fig. 4, B to F). Thus, information in the mature, C-terminal portion of Ilv2 is necessary and sufficient for nuclear localization, but the presence of an MTS is required for non-imported Ilv2 degradation and sequestration into nuclear-associated foci.

Because Ilv2 lacking an MTS was not subjected to quality control, we wondered whether ΔMTS-Ilv2-GFP was still toxic to cells. In contrast to overexpressed FL Ilv2-GFP, which impaired growth of both wild type and E3 KO cells in the presence or absence of FCCP, overexpressed Ilv2 lacking its MTS did not slow cell growth, and neither did overexpressed MTS_{Ilv2}-GFP alone (Fig. 4G). Thus, the presence of an MTS on unimported Ilv2 rendered the protein toxic and promoted its subsequent quality control. Importantly, the association between the presence of an MTS, degradation, and toxicity was conserved for other nuclear class proteins. Like Ilv2, Dld2 lacking

its MTS constitutively localized to the nucleus, but was not subjected to degradation or sequestered into nuclear foci, and was no longer toxic to cells (fig. S4, A to F). Moreover, degradation and toxicity of Cox15 and Lat1, which are degraded by a non-nuclear proteasome pathway, also required an N-terminal MTS (Fig. 4, H and I). Thus, the presence of an MTS on an unimported mitochondrial protein drives proteotoxic stress and targets the protein for quality control.

Prior studies demonstrated that the accumulation of unimported mitochondrial precursors causes proteotoxicity (*5, 6*). To combat this stress, cells mount a coordinated response that involves upregulation of proteasome capacity (*6*, *10*), downregulation of translation (*5*) and clearance of precursors that accumulate at the mitochondrial surface (*7, 8*) and ER membrane (*9*). Here, we surveyed the mitochondrial proteome to get a clearer picture of the full spectrum of fates for unimported mitochondrial proteins. We found that mitochondrial precursors accumulate in many regions of the cell, and identified the nucleus as an important quality control destination for sequestering and destroying unimported mitochondrial proteins. Moreover, we demonstrated that the N-terminal MTS is a major driver of unimported protein toxicity. Our findings indicate that unimported mitochondrial proteins represent a large class of endogenous substrates for nuclear protein quality control. This discovery raises the intriguing possibility that unimported mitochondrial proteins may synergize with other aggregate-prone proteins to overwhelm protein quality control systems during aging and disease (*33*). Future studies to determine what drives unimported mitochondrial proteins to various cellular destinations, and elucidate the coordination between unimported mitochondrial quality control pathways will help illuminate how cells cope with the proteotoxic burden that arises during times of mitochondrial dysfunction.

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Supplementary Materials

Materials and Methods

Figures S1-S4

Tables S1-S4

References (*34-42*)

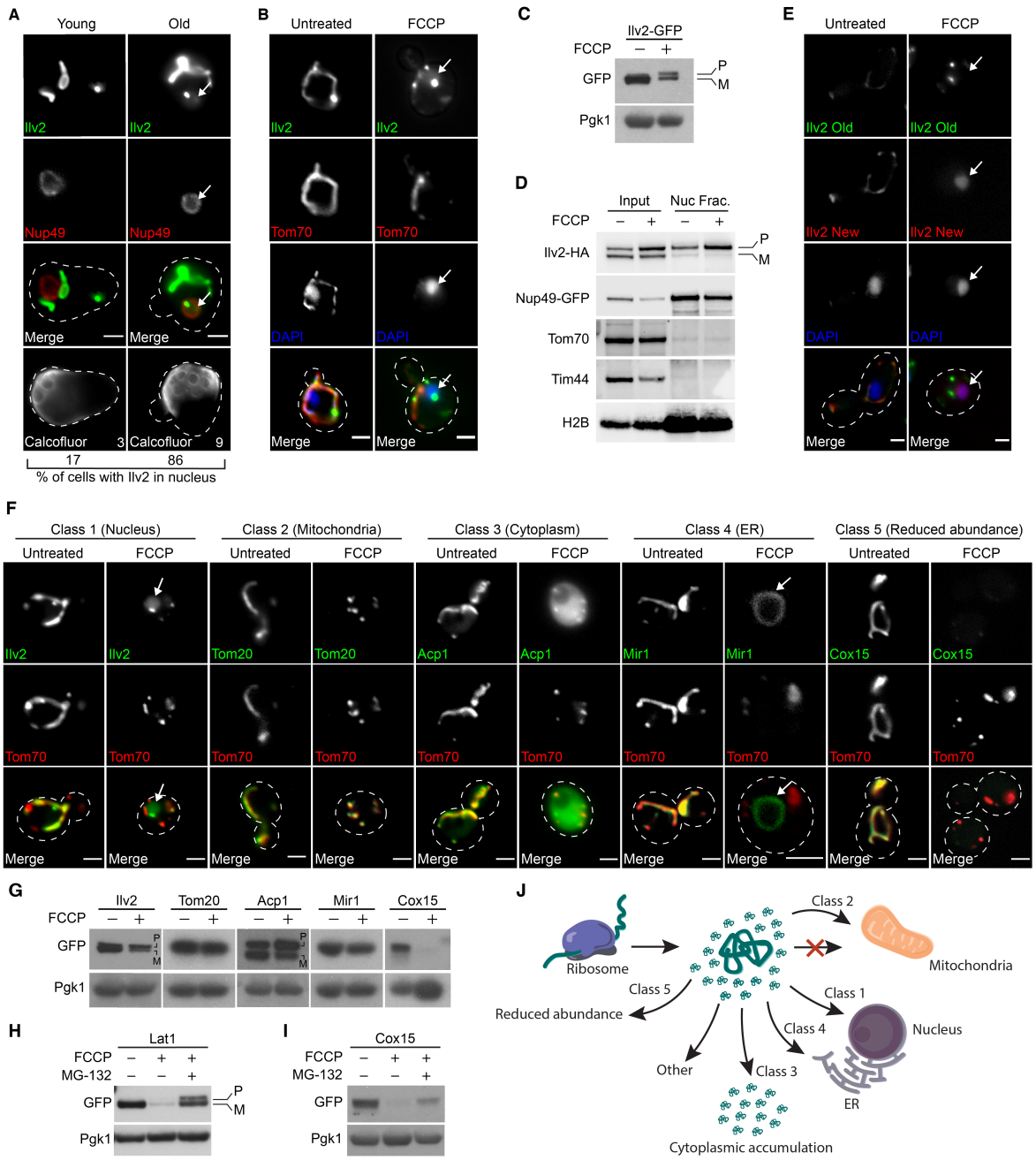

Fig. 1

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Fig. 1. The nucleus in one of several fates for non-imported mitochondrial proteins.

(**A**) Representative images of old and young yeast expressing the indicated Ilv2-GFP and nuclear marker Nup49-mCherry. Percentage of cells with Ilv2 in the nucleus (n = 30 cells) and age of representative cell (determined by bud scar counting) are indicated in bottom panels. Bud scars stained with calcofluor. (**B)** Yeast expressing Ilv2-GFP and Tom70-mCherry -/+ FCCP. (**C**) Western blots of yeast expressing Ilv2-GFP -/+ FCCP. P = precursor, M = mature in all instances. Pgk1 = loading control in all instances. (**D**) Western blot showing enrichment of the precursor form of Ilv2-HA in the nuclear fraction. Nup49-GFP and H2B = nuclear markers, Tom70 and Tim44 = mitochondrial markers. (**E**) RITE-tagged cells treated with β-estradiol at time of FCCP addition to initiate Cre/lox switching of Ilv2 epitope tag from GFP (old) to RFP (new). (**B** and **E**) Nucleus stained with DAPI. (**F**), Yeast expressing the indicated mCherry or GFP-tagged mitochondrial proteins -/+ FCCP. (**G** to **I**) Western blots of yeast expressing the indicated GFP-tagged mitochondrial proteins -/+ FCCP (G) or -/+ FCCP -/+ MG-132 (H, I). (**J**) Summary of non-imported mitochondrial protein fates. All scales bars = 2μm. Arrows denote nucleus (**A**, **B**, **E**, and **F**, class 1) or ER (**F**, class 4).

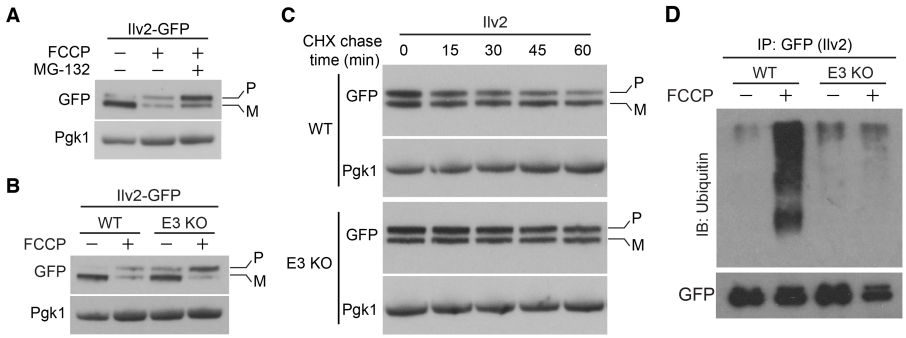

Fig. 2

Fig. 2. Nuclear protein quality control clears unimported mitochondrial proteins.

(**A**) Western blot of yeast expressing Ilv2-GFP -/+ FCCP -/+ MG-132. (**B**) Western blot of yeast expressing Ilv2-GFP -/+ FCCP in wild-type (WT) and E3 KO strains. (**C**) Western blots showing cycloheximide (CHX) chase of Ilv2-GFP in WT and E3 KO strains in the presence of FCCP. (**D**) Western blot showing ubiquitylation of immunoprecipitated Ilv2-GFP -/+ FCCP in WT and E3 KO strains. Pgk1 = loading control. E3 KO = *san1*Δ *ubr1*Δ *doa10*Δ. P = precursor and M = mature in all instances.

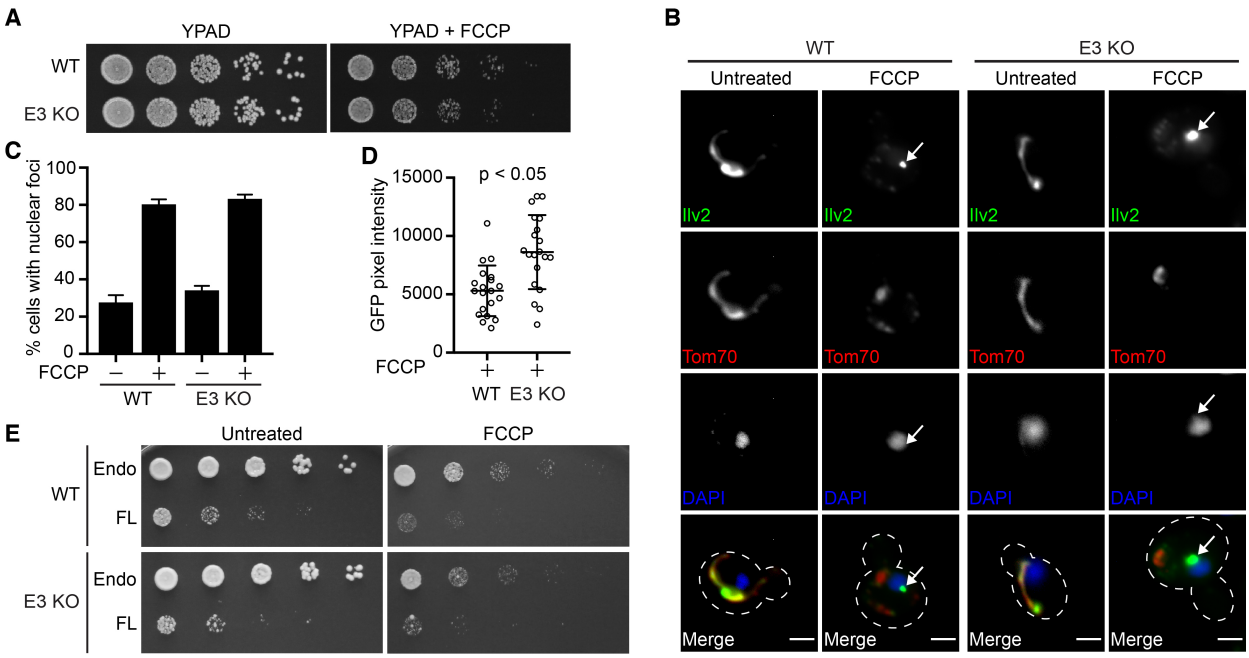

Fig. 3

Fig. 3. Non-imported mitochondrial precursors localize to nuclear-associated foci when clearance is impaired. (**A**) Five-fold serial dilutions of WT and E3 KO strains on YPAD -/+ FCCP agar plates. (**B**) WT and E3 KO yeast expressing Ilv2-GFP and Tom70-mCherry -/+ FCCP. Nucleus stained with DAPI. Arrows = nuclear associated foci. Bar = 2μm. (**C**) Quantification of (**B**). N > 99 cells per replicate, error bars = SEM of three replicates. (**D**) Quantification of average pixel intensity of Ilv2-GFP nuclear foci from (**B**). N=20 cells, error bars = SD, p-value = 0.0005. (**E**) Five-fold serial dilutions of WT and E3 KO strains expressing endogenous Ilv2-GFP (endo) -/+ mild overexpression of full length Ilv2-GFP (FL) from pRS413-Ilv2-GFP on SD-His -/+ FCCP agar plates.

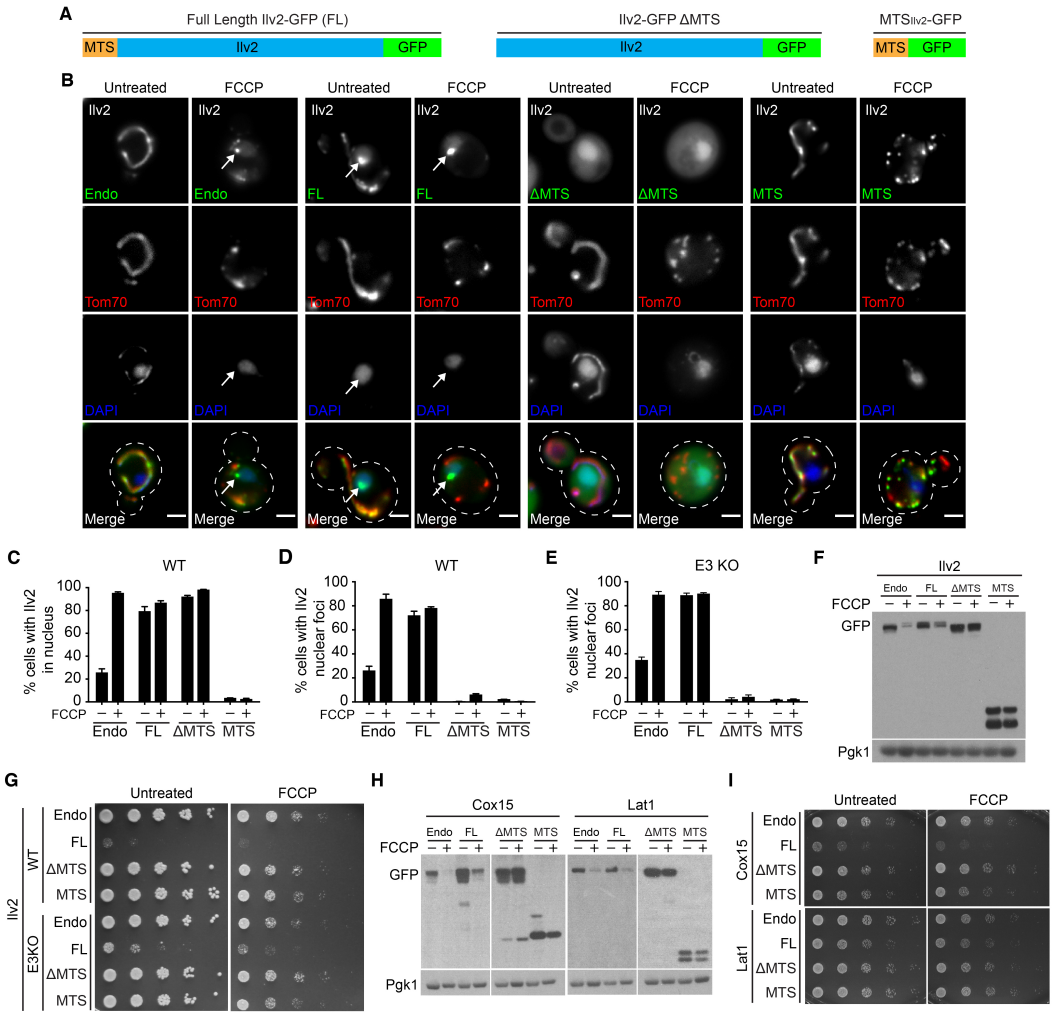

Fig. 4

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Fig. 4. The mitochondrial targeting sequence (MTS) is required for non-imported precursor toxicity and quality control. (**A**) Schematic of full length GFP-tagged Ilv2 (FL), mitochondrial targeting sequence deleted (ΔMTS) GFP-tagged Ilv2, and MTS_{Ilv2} GFP only (MTS). (**B**) Tom70-mCherry yeast expressing endogenous Ilv2-GFP -/+ the indicated Ilv2 variant -/+ FCCP. Nucleus stained with DAPI. Arrows = nuclear associated foci. Bars = 2μm. (**C** and **D**) Quantification of cells with diffuse Ilv2 nuclear localization (C) or Ilv2 nuclear foci (D) from B. (**E**) Quantification of cells with Ilv2 nuclear foci in E3 KO strain (*san1*Δ *ubr1*Δ *doa10*Δ) conducted in parallel with (B-D). For C-E, N > 99 cells per replicate, error bars = SEM of three replicates. (**F**) Western blot of strains expressing indicated Ilv2-GFP variants -/+ FCCP. Pgk1 = loading control. (**G**) Five-fold serial dilutions of WT and E3 KO strains expressing endogenous Ilv2-GFP (endo) -/+ mild overexpression of the indicated Ilv2-GFP variants on SD-His -/+ FCCP agar plates. (**H**) Western blot on strains expressing endogenous Cox15-GFP (endo) or Lat1-GFP (endo), respectively, -/+ the mild overexpression of the indicated variants. Pgk1 = loading control. (**I**) Five-fold serial dilutions of WT strains expressing endogenous Cox15-GFP or Lat1-GFP (endo) -/+ mild overexpression of the indicated variants on SD-His -/+ FCCP agar plates.

Supplementary Materials for

The nucleus is a quality control center for non-imported mitochondrial proteins

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This Section includes:

Materials and Methods
Supplementary Text
Figs. S1 to S4
Tables S1 to S4

Materials and Methods

Reagents

Chemicals were obtained from the following sources: β-Estradiol (E8875), Carbonyl cyanide 4-(trifluoromethoxy) phenylhydrazone (C2920), cOmplete Protease Inhibitor Cocktail (11697498001), dimethyl sulfoxide (D2650), Cycloheximide (C1988), Doxycycline hyclate (C9891), polyvinylpyrrolidone (PVP40), Pepstatin (10253286001), Phenylmethylsulfonyl fluoride (P7626), calcofluor Fluorescent Brightener 28 (F3543) from Millipore Sigma, 4',6-Diamidino-2-Phenylindole Dihydrochloride (DAPI) (D130), ProLong™ Glass Antifade Mountant with NucBlue™ Stain (P36981) from ThermoFisher, (S)-MG-132 (10012628) from Cayman Chemical, N-Ethylmaleimide (NEM) (S3876), IGEPAL NP-40 (CA-630) from Sigma-Aldrich, Zymolyase 100T (Z1004) from US Biological Life Sciences, Triton X-100 (1610407) from Biorad, Paraformaldehyde (100503-914) from VWR, and Dithiothreitol (DTT10) from GOLDBIO. Antibodies and other reagents are described in the appropriate section below.

Yeast Strains

All yeast strains are derivatives of Saccharomyces cerevisiae S288c (BY) (34) and are listed in Supplementary Table 2. Strains expressing fluorescently tagged proteins from their native loci were created by one step PCR-mediated C-terminal endogenous epitope tagging using standard techniques and the oligo pairs listed in Supplementary Table 3 (34, 35). Plasmid template for GFP and mCherry tagging was from the pKT series of vectors (35), plasmid template for RITE tagging was previously described pVL015 (21), and plasmid templates for FLAG, HA, and mCherry tagging were pFA6A-5FLAG-KanMX (Addgene 15983) (36), pFA6A-3HA-His3MX (Addgene 41600) (37), pFA6A-3HA-KanMX (Addgene 39295) (38), and pFA6A-mCherry-HphMX

(Addgene 105156) (39). Deletion strains were created by one step PCR-mediated gene replacement using the oligos pairs listed in Supplementary Table 3 and plasmid templates of pRS series vectors (34). Correct integrations were confirmed with a combination of colony PCR across the chromosomal insertion site and correctly localized expression of the fluorophore by microscopy. The strain collection used for screening in Figure 1 expressed Tom70-mCherry/any protein-GFP and was created previously (23). The genotype of all strains in the collection is MATa/MATα his3Δ1/his3Δ1 leu2Δ0/leu2Δ0 ura3Δ0/ura3Δ0 met15Δ0/+ lys2Δ0/+ anygene-GFP-His3MX/+ TOM70-mCherry-KanMX/+.

Yeast Cell Culture and Media

For all microscopy and western blot experiments, yeast were grown exponentially for 15 hours up to a maximum density of 1 x 10^7 cells/ml prior to starting any treatments. Cells were cultured as indicated in the Main Text and Figure Legends in YPAD medium (1% yeast extract, 2% peptone, 0.005% adenine, 2% glucose) or synthetic defined medium lacking histidine (SD-His) (0.67% yeast nitrogen base without amino acids, 2% glucose, supplemented nutrients 0.074 g/L each adenine, alanine, arginine, asparagine, aspartic acid, cysteine, glutamic acid, glutamine, glycine, myo-inositol, isoleucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, uracil, valine, 0.369 g/L leucine, 0.007 g/L para-aminobenzoic acid). FCCP and MG-132 were used at a final concentration of 10 μM and 50 nM respectively. All FCCP and/or MG-132 treatments were conducted for six hours. For knockdown of TOM40 expressed under control of the tetracycline promoter, cultures were grown in log-phase for 16 hours in the presence of doxycycline (20 μg/mL) prior to any experimental treatments. The wild-type control strain was cultured under the same conditions. For RITE tag-switching experiments, β-Estradiol was added

to cultures at a final concentration of 1 μM to induce tag switching. FCCP was added to cultures at a final concentration of 10 μM at the same time of β-Estradiol. Cultures were imaged after 6 hours of treatment.

Plasmids and Cloning

Centromeric yeast plasmids expressing GPD-promoter driven full-length, MTS-deleted, or MTS-only versions of Ilv2, Lat1, Cox15, and Dld2 fused to C-terminal GFP epitopes were assembled using Gibson Assembly® Master Mix (E2611L, NEB) following the manufacturer's instructions. Plasmid names and construction details (including PCR templates, oligo pairs, and digested plasmid templates) used in Gibson Assembly are listed in Supplementary Table 4. PCR amplifications from yeast genomic DNA and plasmid DNA were conducted with Phusion Polymerase (M0530L, NEB) using oligonucleotides listed in Supplementary Table 3. Plasmids were verified by sequencing.

MTS Prediction

Mitochondrial targeting sequences for Ilv2, Cox15, Lat1, and Dld2 were predicted using Mitoprot (40). Correct MTS prediction was confirmed by analyzing localization of C-terminal GFP-tagged versions of MTS-only or MTS-deleted proteins via microscopy.

Microscopy

200-300 nm optical Z-sections of live yeast cells were acquired with an AxioImager M2 (Carl Zeiss) equipped with an Axiocam 506 monochromatic camera (Carl Zeiss) and 100× oil-immersion objective (Carl Zeiss, Plan Apochromat, NA 1.4), or with an AxioObserver 7 (Carl

Zeiss) equipped with a PCO Edge 4.2LT Monochrome, Air Cooled, USB 3 CCD camera with a Solid-State Colibri 7 LED illuminator and 100X oil-immersion objective (Carl Zeiss, Plan Apochromat, NA 1.4). All images were acquired with ZEN (Carl Zeiss), and processed with Fiji (NIH). All images shown in Figures represent a single optical section.

DAPI staining

Yeast cells were stained with DAPI by incubating cultures for ten minutes in respective growth media with DAPI (1μg/ml).

Quantification of Nuclear-Associated Foci Intensity

Mean GFP pixel intensity of nucleus and nuclear associated foci was calculated via line scan analysis of pixel intensity from maximum intensity projections on 20 cells using FIJI (NIH) (41). Nucleus stained by DAPI was used as a reference to draw lines of ~2.5 μm for analysis.

Determination of Replicative Age

Yeast strains exponentially growing for 15 hours up to a maximum density of 1 x 10^7 cells/ml were stained with for 5 minutes in YPAD with 5 μg/ml of Fluorescent Brightener 28 (F3543, Millipore Sigma), which stains bud scars. The replicative age of each yeast cell was determined by counting of the number of bud scars after staining. Cells with less than five bud scars were categorized as young and cells with five or more bud scars were categorized as old.

Indirect Immunofluorescence (IIF) Staining

549 For IIF staining, cells were harvested by centrifugation and fixed in 10 ml fixation medium (4%
550 Paraformaldehyde in YPAD) for 1 hour. Fixed yeast cells were washed with Wash Buffer (0.1M
551 Tris, pH=8, 1.2M Sorbitol) twice and incubated with DTT (10mM DTT in 0.1M Tris, pH=9.4) for
552 10 min. Spheroplasts were generated by incubating cells in solution containing 0.1M KPi, pH=6.5,
553 1.2M Sorbitol and 0.25mg/ml Zymolyase at 30°C for 30 minutes. Spheroplasts were gently diluted
554 in 1:40 using Wash Buffer and attached to glass slides pre-coated with 0.1% poly-L-Lysine
555 (2mg/ml). Samples were permeabilized in cold 0.1% Triton-X100 in PBS for 10 min at 4°C, briefly
556 dried and blocked (30 min at room temperature) in Wash Buffer containing 1% BSA. After
557 blocking, samples were incubated with 1:200 diluted anti-FLAG primary antibody (F1804,
558 Millipore Sigma) for 90 minutes followed by washing 10 times. Samples were then incubated with
559 1:300 diluted secondary antibody (A32723, Invitrogen) followed by washing 10 times. Antibody
560 dilutions were made using Wash Buffer containing 1% BSA. Samples were washed with Wash
561 Buffer containing 1% BSA and 0.1% Tween-20. Slides were washed twice with Wash Buffer
562 before sealing, and mounted with hardset medium containing NucBlue™ stain (P36981,
563 Invitrogen) overnight. Widefield images were acquired as described above in microscopy section.
564
565 Protein Preparation and Western Blotting
566 Western blotting of yeast extracts was carried out as described previously(23). Briefly, 1 x 10^7 log
567 phase yeast cells were harvested and resuspended in 50 µl of H_2O. 50 µl of NaOH (1 M) was added
568 to cell suspension and incubated for 5 minutes at room temperature. Cells were centrifuged at
569 20,000xg for 10 min at 4°C and cell pellets were resuspended in SDS lysis buffer (30 mM Tris-
570 HCl pH 6.8, 3% SDS, 5% glycerol, 0.004% bromophenol blue, 2.5% β-mercaptoethanol). Cells
571 extracts were resolved on Bolt 4-12% Bis-Tris Plus Gels (NW04125BOX, Thermo Fisher) with

572 NuPAGE MES SDS Running Buffer (NP0002-02, Thermo Fisher) and transferred to
573 nitrocellulose membranes. Membranes were blocked and probed in blocking buffer (1XPBS,
574 0.05% Tween 20, 5% non-fat dry milk) using the primary antibodies for GFP (1814460001, Sigma
575 Millipore) or Pgk1 (22C5D8, abcam), and HRP conjugated secondary antibodies (715-035-150,
576 Jackson Immunoresearch). Blots were developed with SuperSignal West Pico Chemiluminescent
577 substrate (34580, Thermo Fisher) and exposed to films. Blots were developed using film processor
578 (SRX101, Konica Minolta) or a Chemidoc MP system (BioRad).
579
580 Nuclear Enrichment
581 Cells were grown in log-phase overnight as described above followed by treatment with MG-132
582 and -/+ FCCP for 4 hours. 4 x 10^8 total cells were harvested. Cells were washed with ddH_2O, and
583 the wet weight of the pellet was recorded. Cells were incubated in DTT Buffer (100 mM Tris-HCl
584 pH 9.5, 10 mM DTT) and 50 nM MG-132 with gentle shaking at 30°C for 20 min. Cells were then
585 spheroplasted via incubation in Zymolyase Buffer (1.2 M sorbitol, 20 mM K_2HPO_4, pH 7.4), 50
586 nM MG-132, and 1 mg of Zymolyase 100T (Z1004, US Biological Life Sciences) per 1 g cell
587 pellet for 1 hour at 30°C with gentle shaking. Spheroplasts were washed once with Zymolyase
588 Buffer, and then all subsequent steps were carried out on ice. Spheroplasts were dounce-
589 homogenized with 35 strokes in 5 mL of polyvinylpyrrolidone-40 solution (8% PVP-40, 20 mM
590 K-phosphate, 7.5 µM $MgCl_2$, pH 6.5), 0.025% Triton X-100, 5 mM DTT, 50 µL Solution P (20
591 mg/mL PMSF, 0.4 mg/mL Pepstatin A in ethanol), and 50 µL 100X cOmplete protease inhibitor
592 cocktail (11697498001, Millipore Sigma). Next, 15 mL of PVP-40 solution, 15 µL Solution P, and
593 15 µL PIC was added, and spheroplasts were dounce-homogenized with an additional 5 strokes.
594 PVP-40 ensures nuclei stay intact during lysis (42). The cell lysate was centrifuged for 3000 x g

595 for 5 min. The resulting supernatant was discarded, and pellets were washed once and resuspended
596 in 1ml of IP Buffer (50mM Tris pH7.5, 150mM NaCl, 1mM EDTA, 10% Glycerol, 1% IGEPAL
597 (NP-40 substitute), 100uM PMSF). Intact nuclei, which are more resistant to NP-40 than other
598 cellular membranes, were immobilized non-specifically to magnetic agarose beads (BMAB 20,
599 Chromotek) via incubation at 4°C for 2-3 hr. After binding, nuclei were washed 4 x 15 min in IP
600 buffer at 4°C. Nuclear-enriched extracts were eluted by incubating beads in 2X Laemmli buffer
601 (63 mM Tris pH 6.8, 2% (w/v) SDS, 10% (v/v) glycerol, 1 mg/ml bromophenol blue, 1%
602 (v/v) b-mercaptoethanol) at 90°C for 10 minutes. Eluates were subjected to SDS-PAGE and
603 Western Blotting with primary anti-HA antibody (11583816001, Sigma Millipore), anti-Tom70
604 and Tim44 antisera (gifts from Dr. Nikolaus Pfanner, University of Freiburg), anti-GFP antibody
605 (1814460001, Millipore Sigma) and anti-H2b antibody (39947, Active Motif). Effectiveness of
606 nuclear enrichment was indicated by increase in relative abundance of nuclear markers H2B and
607 Nup49-GFP, and decrease in Tom70 and Tim44 in nuclear extracts compared to whole cell lysate.
608 Nuclei were monitored during isolation by visualizing Nup49-GFP via fluorescence microscopy.
609
610 Cycloheximide-Chase Analysis
611 Exponentially growing cells were treated -/+ FCCP for 4 hours, after which, cycloheximide (100
612 μg/ml) was added to the cultures. The time zero sample was collected immediately after adding
613 cycloheximide. For all other time-points, samples were collected by harvesting an equal volume
614 of media to that which was harvested at time zero. Samples were then subjected to SDS-PAGE
615 and Western Blotting with primary antibodies for HA (11583816001, Sigma Millipore) or GFP
616 (1814460001, Sigma Millipore) and Pgk1 (22C5D8, abcam). Blots were developed as described
617 above.

618

619 Microscopy and Western Blot Screens

620 Individual strains listed in Supplementary Table 1 from the Tom70-mCherry/mitochondrial
621 protein GFP collection were cultured in batches overnight in YPAD as described above and then
622 incubated +/- FCCP for six hours. After treatment, cultures were split for simultaneous microscopy
623 and western Blot analysis. Images and western blots were analyzed and scored by three
624 independent researchers. A subset of strains from each class was reconstructed and reanalyzed
625 with both FCCP and genetic ablation of mitochondrial import. Class assignments were based on
626 combined results of microscopy and western blot analysis and were as follows: Class 1 (nucleus),
627 small to large decrease in protein levels and localized to the nucleus in the presence of FCCP;
628 Class 2 (mitochondria), minimal change in protein level and robustly localized to mitochondria in
629 the presence of FCCP; Class 3 (cytoplasm), no change or an increase in protein level and localized
630 predominantly to the cytoplasm with FCCP treatment; Class 4 (ER), mild or no change in protein
631 abundance and localized to ER upon FCCP; Class 5 (reduced abundance), large reduction in
632 protein abundance and no longer easily detectable via microscopy with FCCP treatment.

633

634 Immunoprecipitation

635 Cells were grown as described above and treated +/- FCCP and MG-132 for six hours. 1 x 10^8 total
636 cells were harvested, resuspended in 1ml of lysis Buffer (50mM Tris pH7.5, 150mM NaCl, 1mM
637 EDTA, 10% Glycerol, 1% IGEPAL (NP-40 substitute), 100uM PMSF and 10mM NEM and lysed
638 with glass beads using an Omni Bead Ruptor 12 Homogenizer (8 cycles of 20 seconds each). Cells
639 lysates were cleared by centrifugation at 20000g and supernatant was moved to a new tube. Cell
640 pellets were resuspended in 50 µl of SUME buffer (1% SDS, 8 M Urea, 10 mM MOPS, pH 6.8,

10 mM EDTA and 10mM NEM) and heated at 55 ºC for 5 minutes. 50 µl of cell pellet resuspension was combined with supernatant from lysate clearance centrifugation and total volume was adjusted to 1ml by adding lysis buffer. Lysates were incubated with 25 µl of anti-GFP bead slurry (GTMA, GFP-Trap®_MA, chromotek) at 4ºC for 3-4 h and then washed 4X for 10 min each in lysis buffer (without NEM). Immunoprecipitated proteins were eluted by incubating beads in 2X Laemmli buffer (63 mM Tris pH 6.8, 2% (w/v) SDS, 10% (v/v) glycerol, 1 mg/ml bromophenol blue, 1% (v/v) β-mercaptoethanol) at 90ºC for 10 minutes. Eluates were subjected to SDS-PAGE and Western Blotting with primary anti-ubiquitin antibody (PA1-187, ThermoFisher) and anti-GFP antibody (1814460001, Sigma Millipore). Blots were developed as described above.

Statistics

Experiments were repeated at least three times and all attempts at replication were successful. For all quantifications, number of cells scored is included in Figure Legends. Differences in means were compared using two-tailed t-tests at the 5% significance level. No randomization or blinding was used in experiments. All analysis was done with GraphPad Prism version 8.01.

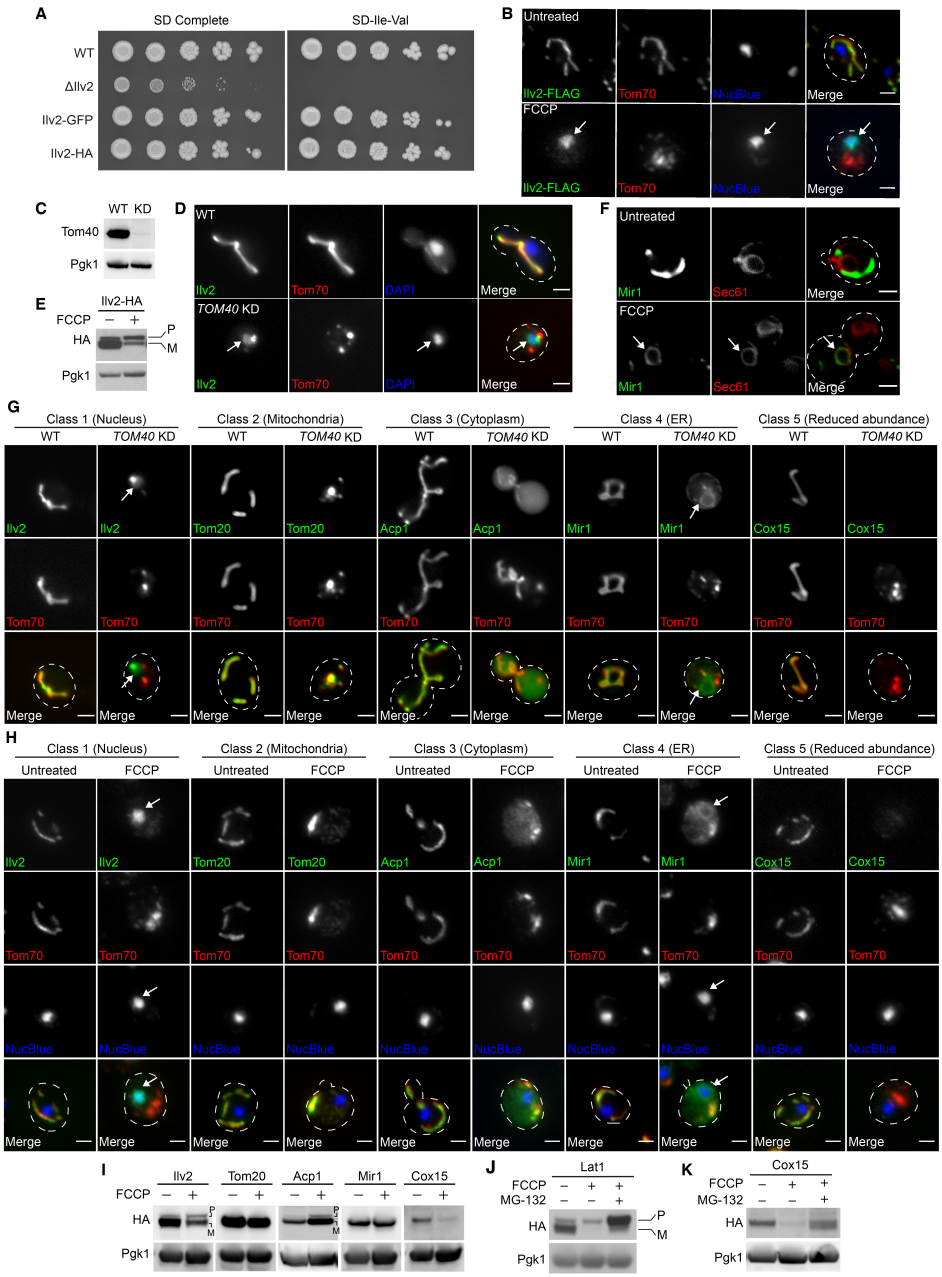

Fig. S1

Fig. S1. **The nucleus is one of several non-imported mitochondrial precursor protein fates.** (**A**) Five-fold serial dilutions of WT, Ilv2 KO, and GFP or HA tagged Ilv2 yeast strains on SD complete or isoleucine and valine dropout agar plates. (**B**) Indirect immunofluorescence of yeast expressing Ilv2-FLAG and Tom70-mCherry. (**C**) Western blot for Tom40 in wild type (WT) and *tet$_p$-TOM40* (*KD*) strains in the presence of doxycycline. (**D**) Tom70-mCherry wild type (WT) and *tet$_p$-TOM40* (*TOM40 KD*) yeast expressing Ilv2-GFP in the presence of doxycycline. (**E**) Western blot of yeast expressing Ilv2-HA -/+ FCCP. (**F**) Yeast expressing ER marker Sec61-mCherry and Mir1-GFP -/+ FCCP. (**G**) Tom70-mCherry wild type (WT) and *tet$_p$-TOM40* (*TOM40 KD*) yeast expressing the indicated GFP-tagged mitochondrial proteins in the presence of doxycycline. (**H**) Indirect immunofluorescence of yeast expressing the indicated FLAG-tagged proteins and Tom70-mCherry. (**I** to **K)** Western blots of yeast expressing the indicated HA-tagged mitochondrial proteins -/+ FCCP (H) or -/+ FCCP -/+ MG-132 (**I-J**). P = precursor form, M = mature form. Pgk1 = loading control. In **B, D,** and **F-H**, bar = 2μm. Nucleus in (**B**, **D**, and **H)** stained with NucBlue or DAPI. Arrows indicate nucleus (**B, D**, and **G-H**, class 1) or ER (**F and G-H, and H**, class 4).

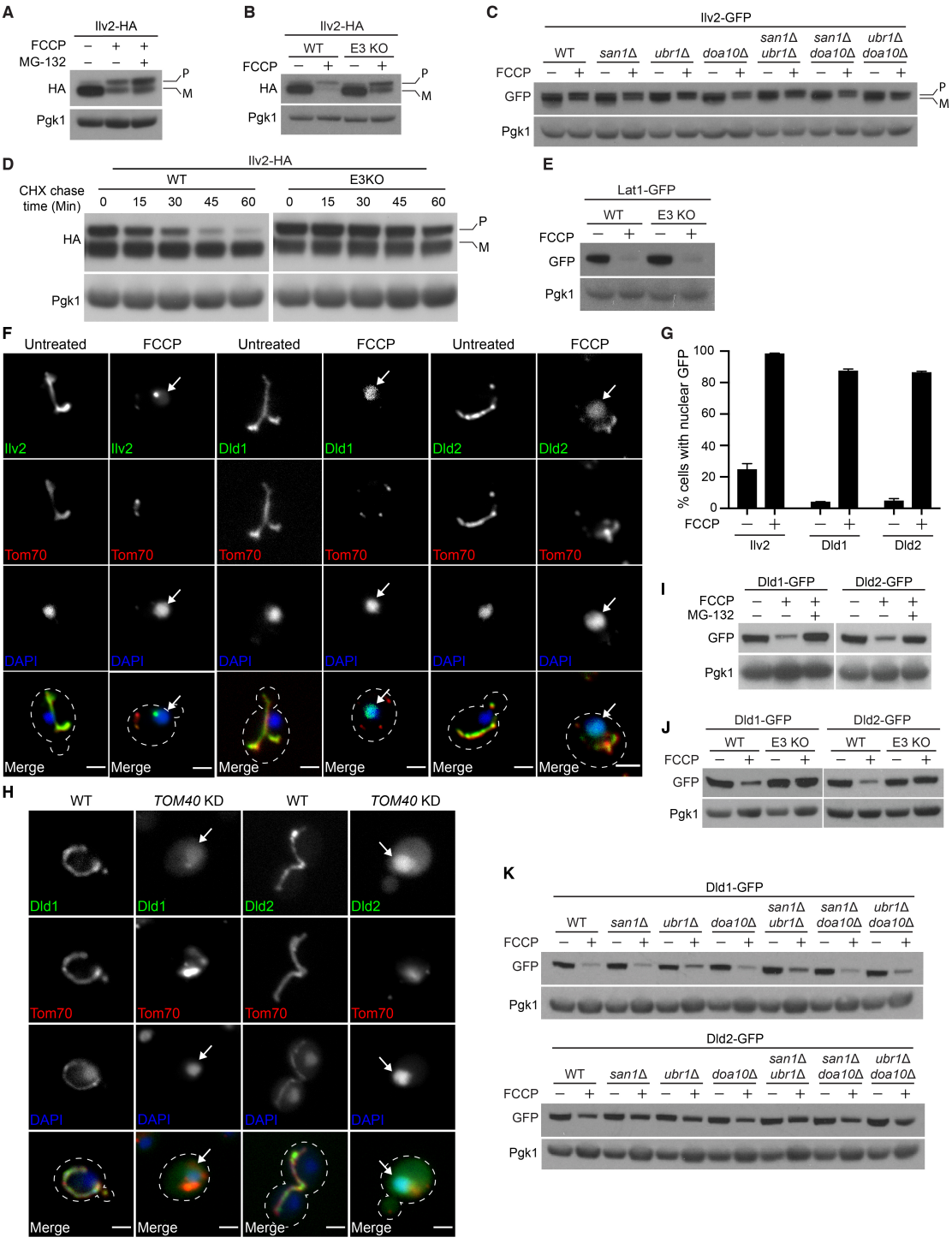

Fig. S2

676

Fig. S2. Nuclear protein quality control promotes unimported mitochondrial protein degradation.

(**A**) Western blot of yeast expressing Ilv2-HA -/+ FCCP -/+ MG-132. (**B**) Western blot of yeast expressing the Ilv2-HA -/+ FCCP in WT and E3 KO strains. (**C**) Western blot of yeast expressing Ilv2-HA -/+ FCCP in WT and the indicated mutant yeast strains. (**D**) Western blots showing the CHX chase of Ilv2-HA in WT and E3 KO strains in the presence of FCCP. (**E**) Western blots of yeast expressing the Lat1-GFP -/+ FCCP in WT and E3 KO strains. (**F**) Yeast expressing the indicated GFP and mCherry tagged mitochondrial proteins -/+ FCCP. (**G**) Quantification of (**F**)**.** N > 99 cells per replicate, error bars = SEM of three replicates. (**H**) Tom70-mCherry wild type (WT) and *tet$_p$-TOM40* (*TOM40 KD*) yeast expressing the indicated GFP-tagged mitochondrial proteins in the presence of doxycycline. (F, H) Nucleus stained with DAPI, Arrows = nucleus. Bar = 2μm. (**I**)Western blots of yeast strains expressing indicated GFP-tagged mitochondrial proteins -/+ FCCP -/+ MG-132. (**J** and **K**) Western blots of yeast expressing the indicated GFP-tagged mitochondrial proteins -/+ FCCP. Pgk1 = loading control. (**B, D, E**, and **J**) E3 KO = *san1*Δ *ubr1*Δ *doa10*Δ. (**A-D)**, P = precursor, M = mature. Pgk1 = loading control.

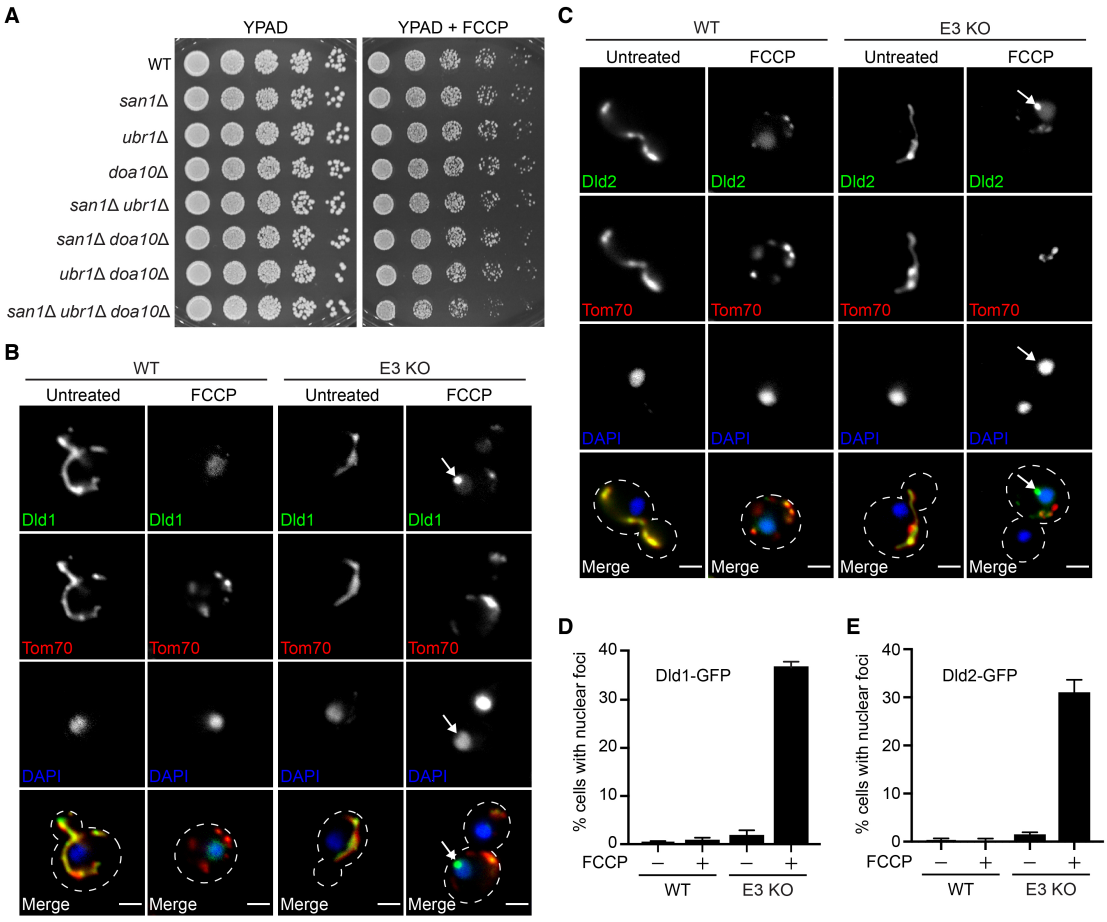

Fig. S3

Fig. S3. Impaired clearance of non-imported mitochondrial proteins targets them to nuclear associated foci.

(**A**) Five-fold serial dilutions of WT and the indicated mutant strains on YPAD -/+ FCCP agar plates. (**B** and **C**), WT and E3 KO (*san1*Δ *ubr1*Δ *doa10*Δ) yeast expressing Dld1-GFP or Dld2-GFP and Tom70-mCherry -/+ FCCP. Nucleus stained with DAPI, arrows = nuclear associated foci, and bar = 2µm. (**D** and **E**) Quantification of (**B**) and (**C**), respectively. N > 99 cells per replicate, error bars = SEM of three replicates.

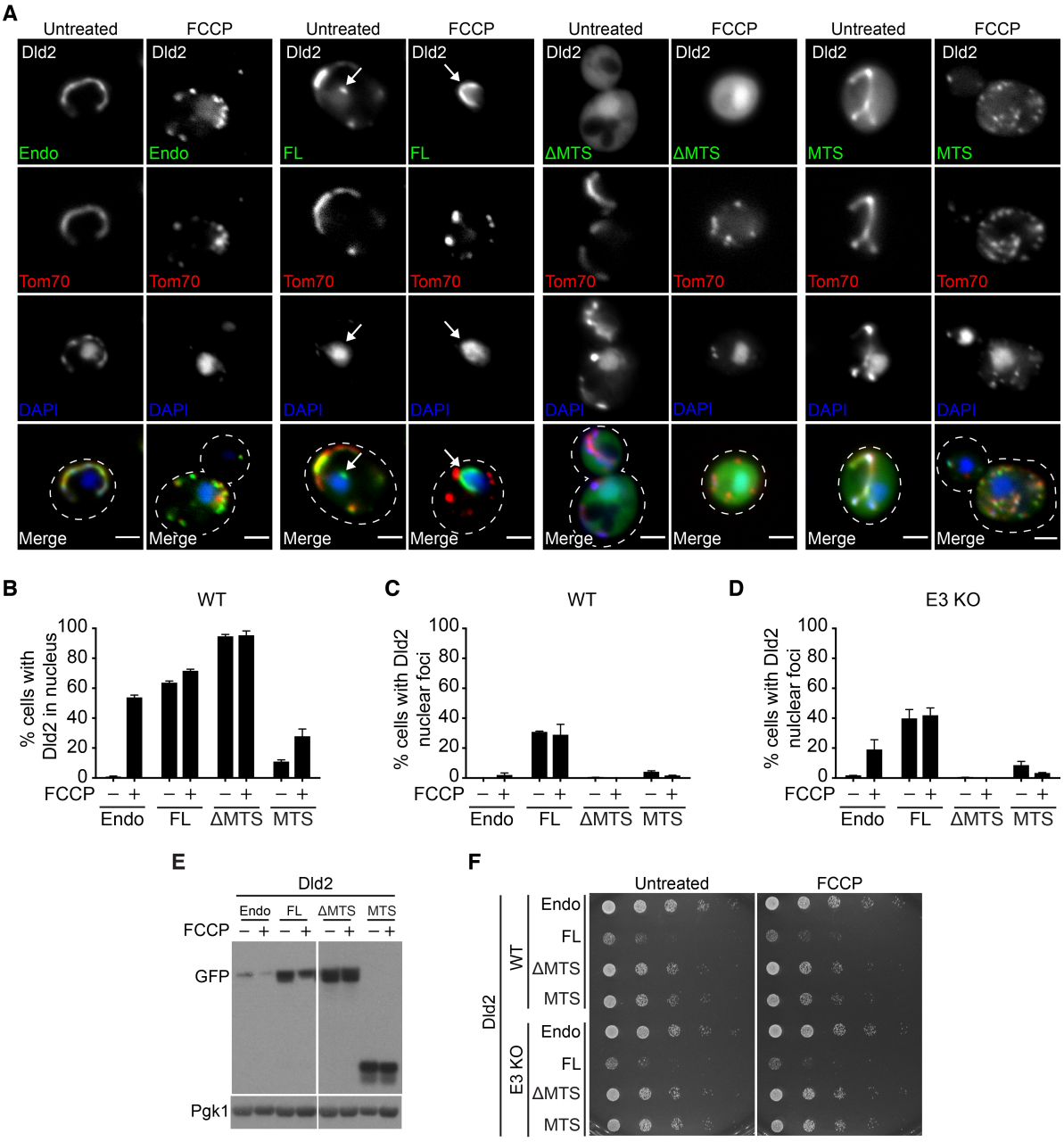

Fig. S4

Fig. S4. The MTS is required for non-imported precursor toxicity and degradation.

(**A**) Tom70-mCherry yeast expressing endogenous Dld2-GFP -/+ the indicated Dld2 plasmid-expressed variant -/+ FCCP. Nucleus stained with DAPI. Arrows = nuclear associated foci. Bars = 2μm. (**B** and **C**) Quantification of cells with diffuse Dld2-GFP nuclear localization (**B**) or Dld2-GFP nuclear foci (**C**) from (**A**). (**D**) Quantification of cells with Dld2-GFP nuclear foci in E3 KO strain (*san1*Δ *ubr1*Δ *doa10*Δ) conducted in parallel with (**A-C)**. For (**B-D)**, N > 99 cells per replicate, error bars = SEM of three replicates. (**E**) Western blot of strains expressing indicated Dld2-GFP variants -/+ FCCP. Pgk1 = loading control. (**F**) Five-fold serial dilutions of WT and E3 KO strains expressing endogenous Dld2-GFP (endo) -/+ mild overexpression of the indicated Dld2-GFP variants on SD-His -/+ FCCP agar plates.

ORF Name	Common Name	Detectable by Microscopy in Mitochondria of Untreated Cells	Detectable by Western Blot Untreated	Western Blot Abundance Change with FCCP Treatment	Level of Abundance Change via Western	Final Category with FCCP Treatment	Class
YKL114C	Apn1	yes	yes	up	small	nucleus	1
YLR059C	Rex2	yes	yes	up	small	nucleus	1
YMR072W	Abf2	yes	yes	up	small	nucleus	1
YDR070C	Fmp16	yes	yes	up	small	nucleus	1
YDR148C	Kgd2	yes	yes	unchanged		nucleus	1
YKL194C	Mst1	yes	yes	unchanged		nucleus	1
YER178W	Pda1	yes	yes	unchanged		nucleus	1
YKL085W	Mdh1	yes	yes	unchanged		nucleus	1
YLR132C	Usb1	yes	yes	unchanged		nucleus	1
YMR286W	Mrpl33	yes	yes	unchanged		nucleus	1
YLL041C	Sdh2	yes	yes	unchanged		nucleus	1
YDR258C	Hsp78	yes	yes	down	small	nucleus	1
YDL164C	Cdc9	yes	yes	down	small	nucleus	1
YMR108W	Ilv2	yes	yes	down	small	nucleus	1
YPL083C	Sen54	yes	yes	down	small	nucleus	1
YGR244C	Lsc2	yes	yes	down	small	nucleus	1
YBR221C	Pdb1	yes	yes	down	small	nucleus	1
YOR142W	Lsc1	yes	yes	down	small	nucleus	1
YOR158W	Pet123	yes	yes	down	small	nucleus	1
YML025C	Yml6	yes	yes	down	small	nucleus	1
YCL009C	Ilv6	yes	yes	down	small	nucleus	1
YOR176W	Hem15	yes	yes	down	small	nucleus	1
YJR016C	Ilv3	yes	yes	down	large	nucleus	1
YDL174C	Dld1	yes	yes	down	large	nucleus	1
YDL178W	Dld2	yes	yes	down	large	nucleus	1
YFL018C	Lpd1	yes	yes	down	large	nucleus	1
YOL140W	Arg8	yes	yes	down	large	nucleus	1
YPL118W	Mrp51	yes	yes	down	large	nucleus	1
YML120C	Ndi1	yes	yes	up	small	mitochondria	2
YPR002W	Pdh1	yes	yes	up	small	mitochondria	2
YPR047W	Msf1	yes	yes	up	small	mitochondria	2
YNR001C	Cit1	yes	yes	up	small	mitochondria	2
YBR230C	Om14	yes	yes	up	small	mitochondria	2
YMR059W	Sen15	yes	yes	up	small	mitochondria	2
YGR028W	Msp1	yes	yes	up	small	mitochondria	2

YGR255C	Coq6	yes	yes	up	large	mitochondria	2
YML110C	Coq5	yes	yes	up	large	mitochondria	2
YHR120W	Msh1	yes	yes	unchanged		mitochondria	2
YKL029C	Mae1	yes	yes	unchanged		mitochondria	2
YOR356W	Cir2	yes	yes	unchanged		mitochondria	2
YHL038C	Cbp2	yes	yes	unchanged		mitochondria	2
YOR147W	Mdm32	yes	yes	unchanged		mitochondria	2
YLR289W	Guf1	yes	yes	unchanged		mitochondria	2
YKL155C	Rsm22	yes	yes	unchanged		mitochondria	2
YBL015W	Ach1	yes	yes	unchanged		mitochondria	2
YER073W	Ald5	yes	yes	unchanged		mitochondria	2
YDR036C	Ehd3	yes	yes	unchanged		mitochondria	2
YJR122W	Caf17	yes	yes	unchanged		mitochondria	2
YPL072W	Ubp16	yes	yes	unchanged		mitochondria	2
YBL098W	Bna4	yes	yes	unchanged		mitochondria	2
YGL219C	Mdm34	yes	yes	unchanged		mitochondria	2
YPR125W	Ylh47	yes	yes	unchanged		mitochondria	2
YKL027W	Tcd2	yes	yes	unchanged		mitochondria	2
YGR012W	Mcy1	yes	yes	unchanged		mitochondria	2
YHR162W	Mpc2	yes	yes	unchanged		mitochondria	2
YLL001W	Dnm1	yes	yes	unchanged		mitochondria	2
YNL121C	Tom70	yes	yes	unchanged		mitochondria	2
YHR117W	Tom71	yes	yes	unchanged		mitochondria	2
YGR082W	Tom20	yes	yes	unchanged		mitochondria	2
YHR003C	Tcd1	yes	yes	down	small	mitochondria	2
YGR049W	Scm4	yes	yes	down	small	mitochondria	2
YBR179C	Fzo1	yes	yes	down	small	mitochondria	2
YAL010C	Mdm10	yes	yes	down	small	mitochondria	2
YOL009C	Mdm12	yes	yes	down	small	mitochondria	2
YNL070W	Tom7	yes	yes	down	small	mitochondria	2
YPL222W	Fmp40	yes	yes	up	small	cytoplasm	3
YIL155C	Gut2	yes	yes	up	small	cytoplasm	3
YLR163C	Mas1	yes	yes	up	small	cytoplasm	3
YJL060W	Bna3	yes	yes	up	small	cytoplasm	3
YDR019C	Gcv1	yes	yes	up	small	cytoplasm	3
YPR004C	Aim45	yes	yes	up	small	cytoplasm	3
YNL315C	Atp11	yes	yes	up	small	cytoplasm	3
YGR021W	Dpc29	yes	yes	up	small	cytoplasm	3
YNL168C	Fmp41	yes	yes	up	small	cytoplasm	3

YDR305C	Hnt2	yes	yes	up	small	cytoplasm	3
YLL027W	Isa1	yes	yes	up	small	cytoplasm	3
YLR168C	Msf1	yes	yes	up	small	cytoplasm	3
YNR018W	Rcf2	yes	yes	up	small	cytoplasm	3
YHR038W	Rrf1	yes	yes	up	small	cytoplasm	3
YNL306W	Mrps18	yes	yes	up	small	cytoplasm	3
YBL057C	Pth2	yes	yes	up	small	cytoplasm	3
YIR024C	Gif1	yes	yes	up	small	cytoplasm	3
YNL213C	Rrg9	yes	yes	up	small	cytoplasm	3
YOR215C	Aim41	yes	yes	up	small	cytoplasm	3
YLR193C	Ups1	yes	yes	up	small	cytoplasm	3
YAL044C	Gcv3	yes	yes	up	small	cytoplasm	3
YML078W	Cpr3	yes	yes	up	small	cytoplasm	3
YCR071C	Img2	yes	yes	up	small	cytoplasm	3
YIL051C	Mmf1	yes	yes	up	small	cytoplasm	3
YGL226W	Mtc3	yes	yes	up	small	cytoplasm	3
YLR395C	Cox8	yes	yes	up	small	cytoplasm	3
YOL096C	Coq3	yes	yes	up	small	cytoplasm	3
YGR174C	Cbp4	yes	yes	up	small	cytoplasm	3
YDR178W	Sdh4	yes	yes	up	small	cytoplasm	3
YLR356W	Atg33	yes	yes	up	small	cytoplasm	3
YOR136W	Idh2	yes	yes	up	small	cytoplasm	3
YKL150W	Mcr1	yes	yes	up	large	cytoplasm	3
YIL113W	Sdp1	yes	yes	up	large	cytoplasm	3
YJL161W	Fmp33	yes	yes	up	large	cytoplasm	3
YDL120W	Yfh1	yes	yes	up	large	cytoplasm	3
YBR047W	Fmp23	yes	yes	up	large	cytoplasm	3
YPL135W	Isu1	yes	yes	up	large	cytoplasm	3
YPR100W	Mrpl51	yes	yes	up	large	cytoplasm	3
YGR243W	Mpc3	yes	yes	up	large	cytoplasm	3
YML007C-A	Min4	yes	yes	up	large	cytoplasm	3
YDL130W-A	Stf1	yes	yes	up	large	cytoplasm	3
YHL018W	Mco14	yes	yes	unchanged		cytoplasm	3
YML091C	Rpm2	yes	yes	unchanged		cytoplasm	3
YMR189W	Gcv2	yes	yes	unchanged		cytoplasm	3
YIL125W	Kgd1	yes	yes	unchanged		cytoplasm	3
YOR022C	Ddl1	yes	yes	unchanged		cytoplasm	3
YDR194C	Mss116	yes	yes	unchanged		cytoplasm	3

YPL104W	Msd1	yes	yes	unchanged		cytoplasm	3
YER080W	Aim9	yes	yes	unchanged		cytoplasm	3
YNL104C	Leu4	yes	yes	unchanged		cytoplasm	3
YOL027C	Mdm38	yes	yes	unchanged		cytoplasm	3
YBL080C	Pet112	yes	yes	unchanged		cytoplasm	3
YOL033W	Mse1	yes	yes	unchanged		cytoplasm	3
YMR023C	Mss1	yes	yes	unchanged		cytoplasm	3
YDR061W		yes	yes	unchanged		cytoplasm	3
YJR051W	Osm1	yes	yes	unchanged		cytoplasm	3
YLR090W	Xdj1	yes	yes	unchanged		cytoplasm	3
YHL021C	Aim17	yes	yes	unchanged		cytoplasm	3
YJR062C	Nta1	yes	yes	unchanged		cytoplasm	3
YCR079W	Ptc6	yes	yes	unchanged		cytoplasm	3
YMR062C	Ecm40	yes	yes	unchanged		cytoplasm	3
YIL094C	Lys12	yes	yes	unchanged		cytoplasm	3
YDR065W	Rrg1	yes	yes	unchanged		cytoplasm	3
YOL042W	Ngl1	yes	yes	unchanged		cytoplasm	3
YNL005C	Mrp7	yes	yes	unchanged		cytoplasm	3
YIR021W	Mrs1	yes	yes	unchanged		cytoplasm	3
YNL037C	Idh1	yes	yes	unchanged		cytoplasm	3
YHR106W	Trr2	yes	yes	unchanged		cytoplasm	3
YJL208C	Nuc1	yes	yes	unchanged		cytoplasm	3
YPL069C	Bts1	yes	yes	unchanged		cytoplasm	3
YGR231C	Phb2	yes	yes	unchanged		cytoplasm	3
YLR439W	Mrpl4	yes	yes	unchanged		cytoplasm	3
YDR347W	Mrp1	yes	yes	unchanged		cytoplasm	3
YNL063W	Mtq1	yes	yes	unchanged		cytoplasm	3
YLR312W-A	Mrpl15	yes	yes	unchanged		cytoplasm	3
YJR111C	Pxp2	yes	yes	unchanged		cytoplasm	3
YLR351C	Nit3	yes	yes	unchanged		cytoplasm	3
YGL221C	Nif3	yes	yes	unchanged		cytoplasm	3
YLR201C	Coq9	yes	yes	unchanged		cytoplasm	3
YJL043W		yes	yes	unchanged		cytoplasm	3
YBL095W	Mrx3	yes	yes	unchanged		cytoplasm	3
YGR207C	Cir1	yes	yes	unchanged		cytoplasm	3
YIL070C	Mam33	yes	yes	unchanged		cytoplasm	3
YKL040C	Nfu1	yes	yes	unchanged		cytoplasm	3
YBL064C	Prx1	yes	yes	unchanged		cytoplasm	3

YJR113C	Rsm7	yes	yes	unchanged		cytoplasm	3
YER153C	Pet122	yes	yes	unchanged		cytoplasm	3
YDL202W	Mrpl11	yes	yes	unchanged		cytoplasm	3
YER182W	Fmp10	yes	yes	unchanged		cytoplasm	3
YMR157C	Aim36	yes	yes	unchanged		cytoplasm	3
YDR538W	Pad1	yes	yes	unchanged		cytoplasm	3
YHR008C	Sod2	yes	yes	unchanged		cytoplasm	3
YDR296W	Mhr1	yes	yes	unchanged		cytoplasm	3
YJL063C	Mrpl8	yes	yes	unchanged		cytoplasm	3
YBL059W	Iai11	yes	yes	unchanged		cytoplasm	3
YOR236W	Dfr1	yes	yes	unchanged		cytoplasm	3
YFL046W	Fmp32	yes	yes	unchanged		cytoplasm	3
YIL157C	Coa1	yes	yes	unchanged		cytoplasm	3
YMR003W	Aim34	yes	yes	unchanged		cytoplasm	3
YKR065C	Pam17	yes	yes	unchanged		cytoplasm	3
YGL018C	Jac1	yes	yes	unchanged		cytoplasm	3
YNL185C	Mrpl19	yes	yes	unchanged		cytoplasm	3
YOR150W	Mrpl23	yes	yes	unchanged		cytoplasm	3
YBR120C	Cbp6	yes	yes	unchanged		cytoplasm	3
YKL167C	Mrp49	yes	yes	unchanged		cytoplasm	3
YDR511W	Acn9	yes	yes	unchanged		cytoplasm	3
YMR225C	Mrpl44	yes	yes	unchanged		cytoplasm	3
YKL170W	Mrpl38	yes	yes	unchanged		cytoplasm	3
YKL192C	Acp1	yes	yes	unchanged		cytoplasm	3
YDL157C		yes	yes	unchanged		cytoplasm	3
YLR295C	Atp14	yes	yes	unchanged		cytoplasm	3
YPR098C	Tmh18	yes	yes	unchanged		cytoplasm	3
YDR377W	Atp17	yes	yes	unchanged		cytoplasm	3
YLR390W	Ecm19	yes	yes	unchanged		cytoplasm	3
YJL166W	Qcr8	yes	yes	unchanged		cytoplasm	3
YNL211C	Mrx7	yes	yes	unchanged		cytoplasm	3
YMR302C	Prp12	yes	yes	unchanged		cytoplasm	3
YBL099W	Atp1	yes	yes	unchanged		cytoplasm	3
YER014W	Hem14	yes	yes	unchanged		cytoplasm	3
YJR121W	Atp2	yes	yes	unchanged		cytoplasm	3
YBL045C	Cor1	yes	yes	unchanged		cytoplasm	3
YER053C	Pic2	yes	yes	unchanged		cytoplasm	3
YDL119C	Hem25	yes	yes	unchanged		cytoplasm	3
YOR130C	Ort1	yes	yes	unchanged		cytoplasm	3

YBR039W	Atp3	yes	yes	unchanged		cytoplasm	3
YKR052C	Mrs4	yes	yes	unchanged		cytoplasm	3
YLR393W	Atp10	yes	yes	unchanged		cytoplasm	3
YPL078C	Atp4	yes	yes	unchanged		cytoplasm	3
YNL055C	Por1	yes	yes	unchanged		cytoplasm	3
YAL039C	Cyc3	yes	yes	unchanged		cytoplasm	3
YDR298C	Atp5	yes	yes	unchanged		cytoplasm	3
YGL187C	Cox4	yes	yes	unchanged		cytoplasm	3
YNL328C	Mdj2	yes	yes	unchanged		cytoplasm	3
YIL111W	Cox5b	yes	yes	unchanged		cytoplasm	3
YMR256C	Cox7	yes	yes	unchanged		cytoplasm	3
YML081C-A	Atp18	yes	yes	unchanged		cytoplasm	3
YOL077W-A	Atp19	yes	yes	unchanged		cytoplasm	3
YDL066W	Idp1	yes	yes	down	small	cytoplasm	3
YKL195W	Mia40	yes	yes	down	small	cytoplasm	3
YCL064C	Cha1	yes	yes	down	small	cytoplasm	3
YMR083W	Adh3	yes	yes	down	small	cytoplasm	3
YIL077C	Eat1	yes	yes	down	small	cytoplasm	3
YDR116C	Mrpl1	yes	yes	down	small	cytoplasm	3
YHR189W	Pth1	yes	yes	down	small	cytoplasm	3
YCR003W	Mrpl32	yes	yes	down	small	cytoplasm	3
YPR067W	Isa2	yes	yes	down	small	cytoplasm	3
YCR028C-A	Rim1	yes	yes	down	small	cytoplasm	3
YOR226C	Isu2	yes	yes	down	small	cytoplasm	3
YDR115W	Mrx14	yes	yes	down	small	cytoplasm	3
YLR204W	Qri5	yes	yes	down	small	cytoplasm	3
YDR322C-A	Tim11	yes	yes	down	small	cytoplasm	3
YEL006W	Yea6	yes	yes	down	small	cytoplasm	3
YPR191W	Qcr2	yes	yes	down	small	cytoplasm	3
YPR020W	Atp20	yes	yes	down	small	cytoplasm	3
YPL271W	Atp15	yes	yes	down	small	cytoplasm	3
YPR011C		yes	yes	down	large	cytoplasm	3
YBR026C	Etr1	yes	no	up	large	cytoplasm	3
YKR049C	Fmp46	yes	no	up	large	cytoplasm	3
YLR346C	Cis1	yes	no	up	large	cytoplasm	3
YIL136W	Om45	yes	yes	up	large	er	4
YML086C	Alo1	yes	yes	unchanged		er	4

YLR142W	Put1	yes	yes	unchanged		er	4
YOR187W	Tuf1	yes	yes	unchanged		er	4
YGR235C	Mic26	yes	yes	unchanged		er	4
YJL104W	Mia1	yes	yes	unchanged		er	4
YLR348C	Dic1	yes	yes	unchanged		er	4
YNR017W	Mas6	yes	yes	unchanged		er	4
YMR307W	Gas1	yes	yes	down	small	er	4
YER154W	Oxa1	yes	yes	down	small	er	4
YKL120W	Oac1	yes	yes	down	small	er	4
YJR077C	Mir1	yes	yes	down	small	er	4
YGR183C	Qcr9	yes	yes	down	small	er	4
YPL029W	Suv3	yes	yes	down	small	reduced abundance	5
YGL107C	Rmd9	yes	yes	down	small	reduced abundance	5
YNL073W	Msk1	yes	yes	down	small	reduced abundance	5
YBR227C	Mcx1	yes	yes	down	small	reduced abundance	5
YKL106W	Aat1	yes	yes	down	small	reduced abundance	5
YOR221C	Mct1	yes	yes	down	small	reduced abundance	5
YKR070W		yes	yes	down	small	reduced abundance	5
YGR084C	Mrp13	yes	yes	down	small	reduced abundance	5
YDR175C	Rsm24	yes	yes	down	small	reduced abundance	5
YLR091W	Gep5	yes	yes	down	small	reduced abundance	5
YBR251W	Mrps5	yes	yes	down	small	reduced abundance	5
YNL252C	Mrpl17	yes	yes	down	small	reduced abundance	5
YKL055C	Oar1	yes	yes	down	small	reduced abundance	5
YDR337W	Mrps28	yes	yes	down	small	reduced abundance	5
YKR006C	Mrpl13	yes	yes	down	small	reduced abundance	5
YGR132C	Phb1	yes	yes	down	small	reduced abundance	5
YJR101W	Rsm26	yes	yes	down	small	reduced abundance	5
YIL093C	Rsm25	yes	yes	down	small	reduced abundance	5
YOR004W	Utp23	yes	yes	down	small	reduced abundance	5

YJL096W	Mrpl49	yes	yes	down	small	reduced abundance	5
YBL038W	Mrpl16	yes	yes	down	small	reduced abundance	5
YOR286W	Rdl2	yes	yes	down	small	reduced abundance	5
YGL080W	Mpc1	yes	yes	down	small	reduced abundance	5
YHR001W-A	Qcr10	yes	yes	down	small	reduced abundance	5
YER048W-A	Isd11	yes	yes	down	small	reduced abundance	5
YLR188W	Mdl1	yes	yes	down	small	reduced abundance	5
YJL054W	Tim54	yes	yes	down	small	reduced abundance	5
YGL129C	Rsm23	yes	yes	down	small	reduced abundance	5
YGR257C	Mtm1	yes	yes	down	small	reduced abundance	5
YBR037C	Sco1	yes	yes	down	small	reduced abundance	5
YOR065W	Cyt1	yes	yes	down	small	reduced abundance	5
YKL016C	Atp7	yes	yes	down	small	reduced abundance	5
YHR051W	Cox6	yes	yes	down	small	reduced abundance	5
YOR330C	Mip1	yes	yes	down	large	reduced abundance	5
YBR084W	Mis1	yes	yes	down	large	reduced abundance	5
YPL040C	Ism1	yes	yes	down	large	reduced abundance	5
YNL256W	Fol1	yes	yes	down	large	reduced abundance	5
YJL200C	Aco2	yes	yes	down	large	reduced abundance	5
YKL134C	Oct1	yes	yes	down	large	reduced abundance	5
YLR072W	Lam6	yes	yes	down	large	reduced abundance	5
YBR238C		yes	yes	down	large	reduced abundance	5
YOR354C	Msc6	yes	yes	down	large	reduced abundance	5
YDR234W	Lys4	yes	yes	down	large	reduced abundance	5
YLR369W	Ssq1	yes	yes	down	large	reduced abundance	5
YKR036C	Caf4	yes	yes	down	large	reduced abundance	5
YOR108W	Leu9	yes	yes	down	large	reduced abundance	5

YMR098C	Atp25	yes	yes	down	large	reduced abundance	5
YLR253W	Mcp2	yes	yes	down	large	reduced abundance	5
YHR037W	Put2	yes	yes	down	large	reduced abundance	5
YMR282C	Aep2	yes	yes	down	large	reduced abundance	5
YOR205C	Gep3	yes	yes	down	large	reduced abundance	5
YER086W	Ilv1	yes	yes	down	large	reduced abundance	5
YPR006C	Icl2	yes	yes	down	large	reduced abundance	5
YBR263W	Shm1	yes	yes	down	large	reduced abundance	5
YJL071W	Arg2	yes	yes	down	large	reduced abundance	5
YLR259C	Hsp60	yes	yes	down	large	reduced abundance	5
YMR145C	Nde1	yes	yes	down	large	reduced abundance	5
YDR232W	Hem1	yes	yes	down	large	reduced abundance	5
YPL109C	Mco76	yes	yes	down	large	reduced abundance	5
YMR064W	Aep1	yes	yes	down	large	reduced abundance	5
YGL119W	Abc1	yes	yes	down	large	reduced abundance	5
YER078C	Icp55	yes	yes	down	large	reduced abundance	5
YMR115W	Mgr3	yes	yes	down	large	reduced abundance	5
YEL052W	Afg1	yes	yes	down	large	reduced abundance	5
YOR374W	Ald4	yes	yes	down	large	reduced abundance	5
YLR121C	Yps3	yes	yes	down	large	reduced abundance	5
YNL071W	Lat1	yes	yes	down	large	reduced abundance	5
YPL097W	Msy1	yes	yes	down	large	reduced abundance	5
YCR024C	Pmp1	yes	yes	down	large	reduced abundance	5
YER141W	Cox15	yes	yes	down	large	reduced abundance	5
YHR024C	Mas2	yes	yes	down	large	reduced abundance	5
YCL017C	Nfs1	yes	yes	down	large	reduced abundance	5
YDR514C		yes	yes	down	large	reduced abundance	5

YFL027C	Gyp8	yes	yes	down	large	reduced abundance	5
YPL262W	Fum1	yes	yes	down	large	reduced abundance	5
YNL137C	Nam9	yes	yes	down	large	reduced abundance	5
YDR219C	Mfb1	yes	yes	down	large	reduced abundance	5
YPL063W	Tim50	yes	yes	down	large	reduced abundance	5
YDR316W	Oms1	yes	yes	down	large	reduced abundance	5
YHR011W	Dia4	yes	yes	down	large	reduced abundance	5
YDL027C	Mrx9	yes	yes	down	large	reduced abundance	5
YLR203C	Mss51	yes	yes	down	large	reduced abundance	5
YCL044C	Mgr1	yes	yes	down	large	reduced abundance	5
YDL033C	Slm3	yes	yes	down	large	reduced abundance	5
YNR052C	Pop2	yes	yes	down	large	reduced abundance	5
YPL168W	Mrx4	yes	yes	down	large	reduced abundance	5
YOR196C	Lip5	yes	yes	down	large	reduced abundance	5
YOR201C	Pet56	yes	yes	down	large	reduced abundance	5
YGR193C	Pdx1	yes	yes	down	large	reduced abundance	5
YPL188W	Pos5	yes	yes	down	large	reduced abundance	5
YGL143C	Mrf1	yes	yes	down	large	reduced abundance	5
YPL060W	Lpe10	yes	yes	down	large	reduced abundance	5
YDL104C	Qri7	yes	yes	down	large	reduced abundance	5
YMR024W	Mrpl3	yes	yes	down	large	reduced abundance	5
YDR268W	Msw1	yes	yes	down	large	reduced abundance	5
YHL004W	Mrp4	yes	yes	down	large	reduced abundance	5
YGR112W	Shy1	yes	yes	down	large	reduced abundance	5
YIL042C	Pkp1	yes	yes	down	large	reduced abundance	5
YMR097C	Mtg1	yes	yes	down	large	reduced abundance	5
YKR066C	Ccp1	yes	yes	down	large	reduced abundance	5

YDR322W	Mrpl35	yes	yes	down	large	reduced abundance	5
YGR165W	Mrps35	yes	yes	down	large	reduced abundance	5
YHR199C	Aim46	yes	yes	down	large	reduced abundance	5
YKR087C	Oma1	yes	yes	down	large	reduced abundance	5
YNL177C	Mrpl22	yes	yes	down	large	reduced abundance	5
YPR061C	Jid1	yes	yes	down	large	reduced abundance	5
YGR222W	Pet54	yes	yes	down	large	reduced abundance	5
YHR067W	Rmd12	yes	yes	down	large	reduced abundance	5
YLR290C	Coq11	yes	yes	down	large	reduced abundance	5
YGL057C	Gep7	yes	yes	down	large	reduced abundance	5
YPR116W	Rrg8	yes	yes	down	large	reduced abundance	5
YGR147C	Nat2	yes	yes	down	large	reduced abundance	5
YPR134W	Mss18	yes	yes	down	large	reduced abundance	5
YGL085W	Lcl3	yes	yes	down	large	reduced abundance	5
YGR220C	Mrpl9	yes	yes	down	large	reduced abundance	5
YKL208W	Cbt1	yes	yes	down	large	reduced abundance	5
YDR405W	Mrp20	yes	yes	down	large	reduced abundance	5
YNR040W	Dpi29	yes	yes	down	large	reduced abundance	5
YJL066C	Mpm1	yes	yes	down	large	reduced abundance	5
YOR305W	Rrg7	yes	yes	down	large	reduced abundance	5
YPL107W	Dpc25	yes	yes	down	large	reduced abundance	5
YNL100W	Imc27	yes	yes	down	large	reduced abundance	5
YGR033C	Tim21	yes	yes	down	large	reduced abundance	5
YDR231C	Cox20	yes	yes	down	large	reduced abundance	5
YAL008W	Fun14	yes	yes	down	large	reduced abundance	5
YPL252C	Yah1	yes	yes	down	large	reduced abundance	5
YGR102C	Gtf1	yes	yes	down	large	reduced abundance	5

YCR046C	Img1	yes	yes	down	large	reduced abundance	5
YIL098C	Fmc1	yes	yes	down	large	reduced abundance	5
YOL071W	Emi5	yes	yes	down	large	reduced abundance	5
YGR076C	Mrpl25	yes	yes	down	large	reduced abundance	5
YML030W	Rcf1	yes	yes	down	large	reduced abundance	5
YPL059W	Grx5	yes	yes	down	large	reduced abundance	5
YBR269C	Sdh8	yes	yes	down	large	reduced abundance	5
YMR252C	Mlo1	yes	yes	down	large	reduced abundance	5
YNR022C	Mrpl50	yes	yes	down	large	reduced abundance	5
YDR462W	Mrpl28	yes	yes	down	large	reduced abundance	5
YHR059W	Fyv4	yes	yes	down	large	reduced abundance	5
YBR282W	Mrpl27	yes	yes	down	large	reduced abundance	5
YKL138C	Mrpl31	yes	yes	down	large	reduced abundance	5
YPL013C	Mrps16	yes	yes	down	large	reduced abundance	5
YBR268W	Mrpl37	yes	yes	down	large	reduced abundance	5
YBR262C	Mic12	yes	yes	down	large	reduced abundance	5
YFR049W	Ymr31	yes	yes	down	large	reduced abundance	5
YOL150C		yes	yes	down	large	reduced abundance	5
YPL183W-A	Gon5	yes	yes	down	large	reduced abundance	5
YML009C	Mrpl39	yes	yes	down	large	reduced abundance	5
YJL062W-A	Coa3	yes	yes	down	large	reduced abundance	5
YOR017W	Pet127	yes	yes	down	large	reduced abundance	5
YPL270W	Mdl2	yes	yes	down	large	reduced abundance	5
YER017C	Afg3	yes	yes	down	large	reduced abundance	5
YMR301C	Atm1	yes	yes	down	large	reduced abundance	5
YPR024W	Yme1	yes	yes	down	large	reduced abundance	5
YLR139C	Sls1	yes	yes	down	large	reduced abundance	5

YJL112W	Mdv1	yes	yes	down	large	reduced abundance	5
YMR089C	Yta12	yes	yes	down	large	reduced abundance	5
YBR003W	Coq1	yes	yes	down	large	reduced abundance	5
YNL169C	Psd1	yes	yes	down	large	reduced abundance	5
YDR376W	Arh1	yes	yes	down	large	reduced abundance	5
YMR177W	Mmt1	yes	yes	down	large	reduced abundance	5
YOR334W	Mrs2	yes	yes	down	large	reduced abundance	5
YOR266W	Pnt1	yes	yes	down	large	reduced abundance	5
YMR060C	Tom37	yes	yes	down	large	reduced abundance	5
YNR041C	Coq2	yes	yes	down	large	reduced abundance	5
YOR271C	Fsf1	yes	yes	down	large	reduced abundance	5
YMR166C	Mme1	yes	yes	down	large	reduced abundance	5
YOR037W	Cyc2	yes	yes	down	large	reduced abundance	5
YBR104W	Ymc2	yes	yes	down	large	reduced abundance	5
YMR241W	Yhm2	yes	yes	down	large	reduced abundance	5
YIL114C	Por2	yes	yes	down	large	reduced abundance	5
YBR085W	Aac3	yes	yes	down	large	reduced abundance	5
YGR062C	Cox18	yes	yes	down	large	reduced abundance	5
YPL134C	Odc1	yes	yes	down	large	reduced abundance	5
YBR291C	Ctp1	yes	yes	down	large	reduced abundance	5
YNL003C	Pet8	yes	yes	down	large	reduced abundance	5
YMR056C	Aac1	yes	yes	down	large	reduced abundance	5
YBR185C	Mba1	yes	yes	down	large	reduced abundance	5
YJL133W	Mrs3	yes	yes	down	large	reduced abundance	5
YOR297C	Tim18	yes	yes	down	large	reduced abundance	5
YER170W	Adk2	yes	yes	down	large	reduced abundance	5
YEL024W	Rip1	yes	yes	down	large	reduced abundance	5

YNL052W	Cox5a	yes	yes	down	large	reduced abundance	5
YML129C	Cox14	yes	yes	down	large	reduced abundance	5
YLR239C	Lip2	yes	yes	down	large	reduced abundance	5
YDR204W	Coq4	yes	yes	down	large	reduced abundance	5
YPL091W	Glr1	yes	yes	up	small	other	Other
YFR011C	Mic19	yes	yes	up	small	other	Other
YGR215W	Rsm27	yes	yes	up	small	other	Other
YHR198C	Aim18	yes	yes	up	large	other	Other
YPR155C	Nca2	yes	yes	unchanged		other	Other
YGR171C	Msm1	yes	yes	unchanged		other	Other
YKR016W	Mic60	yes	yes	unchanged		other	Other
YJR080C	Aim24	yes	yes	unchanged		other	Other
YNL284C	Mrpl10	yes	yes	unchanged		other	Other
YDR493W	Mzm1	yes	yes	unchanged		other	Other
YFL016C	Mdj1	yes	yes	unchanged		other	Other
YIL022W	Tim44	yes	yes	unchanged		other	Other
YPL215W	Cbp3	yes	yes	unchanged		other	Other
YJL209W	Cbp1	yes	yes	down	small	other	Other
YNL122C		yes	yes	down	small	other	Other
YGR046W	Tam41	yes	yes	down	large	other	Other
YGR286C	Bio2	yes	yes	down	large	other	Other
YGL068W	Mnp1	yes	yes	down	large	other	Other
YDR375C	Bcs1	yes	yes	down	large	other	Other
YNL083W	Sal1	yes	yes			not scoreable	N/A
YKL157W	Ape2	no	yes			not scoreable	N/A
YDR430C	Cym1	no	yes			not scoreable	N/A
YNL239W	Lap3	no	yes			not scoreable	N/A
YFR024C-A	Lsb3	no	yes			not scoreable	N/A
YFR044C	Dug1	no	yes			not scoreable	N/A
YHR083W	Sam35	no	yes			not scoreable	N/A
YGR015C		no	yes			not scoreable	N/A
YNL310C	Zim17	no	yes			not scoreable	N/A
YDR041W	Rsm10	no	yes			not scoreable	N/A
YGR096W	Tpc1	no	yes			not scoreable	N/A
YHR155W	Lam1	yes	no			not scoreable	N/A
YIL031W	Ulp2	yes	no			not scoreable	N/A
YMR287C	Msu1	yes	no			not scoreable	N/A

YGR150C	Ccm1	yes	no			not scoreable	N/A
YAL056W	Gpb2	yes	no			not scoreable	N/A
YER069W	Arg5,6	yes	no			not scoreable	N/A
YMR066W	Sov1	yes	no			not scoreable	N/A
YLR382C	Nam2	yes	no			not scoreable	N/A
YJL102W	Mef2	yes	no			not scoreable	N/A
YLR069C	Mef1	yes	no			not scoreable	N/A
YBR001C	Nth2	yes	no			not scoreable	N/A
YHL032C	Gut1	yes	no			not scoreable	N/A
YDR332W	Irc3	yes	no			not scoreable	N/A
YOR350C	Mne1	yes	no			not scoreable	N/A
YER077C	Mrx1	yes	no			not scoreable	N/A
YBR163W	Dem1	yes	no			not scoreable	N/A
YPL005W	Aep3	yes	no			not scoreable	N/A
YJR003C	Mrx12	yes	no			not scoreable	N/A
YDL048C	Stp4	yes	no			not scoreable	N/A
YPR001W	Cit3	yes	no			not scoreable	N/A
YGL256W	Adh4	yes	no			not scoreable	N/A
YMR293C	Her2	yes	no			not scoreable	N/A
YDL044C	Mtf2	yes	no			not scoreable	N/A
YHL014C	Ylf2	yes	no			not scoreable	N/A
YKL162C		yes	no			not scoreable	N/A
YJL147C	Smt1	yes	no			not scoreable	N/A
YBL013W	Fmt1	yes	no			not scoreable	N/A
YOL043C	Ntg2	yes	no			not scoreable	N/A
YPR140W	Taz1	yes	no			not scoreable	N/A
YDR197W	Cbs2	yes	no			not scoreable	N/A
YJL131C	Aim23	yes	no			not scoreable	N/A
YMR267W	Ppa2	yes	no			not scoreable	N/A
YBR176W	Ecm31	yes	no			not scoreable	N/A
YLR283W		yes	no			not scoreable	N/A
YOR228C	Mcp1	yes	no			not scoreable	N/A
YOR040W	Glo4	yes	no			not scoreable	N/A
YMR188C	Mrps17	yes	no			not scoreable	N/A
YGL211W	Ncs6	yes	no			not scoreable	N/A
YBR122C	Mrpl36	yes	no			not scoreable	N/A
YKR085C	Mrpl20	yes	no			not scoreable	N/A
YBL090W	Mrp21	yes	no			not scoreable	N/A
YLR281C	Rso55	yes	no			not scoreable	N/A

YIL087C	Aim19	yes	no			not scoreable	N/A
YKL003C	Mrp17	yes	no			not scoreable	N/A
YCR083W	Trx3	yes	no			not scoreable	N/A
YNL081C	Sws2	yes	no			not scoreable	N/A
YDR079W	Pet100	yes	no			not scoreable	N/A
YDL067C	Cox9	yes	no			not scoreable	N/A
YDL045W-A	Mrp10	yes	no			not scoreable	N/A
YDL181W	Inh1	yes	no			not scoreable	N/A
YDR379C-A	Sdh6	yes	no			not scoreable	N/A
YBL022C	Pim1	yes	no			not scoreable	N/A
YLR067C	Pet309	yes	no			not scoreable	N/A
YMR257C	Pet111	yes	no			not scoreable	N/A
YNR045W	Pet494	yes	no			not scoreable	N/A
YDR185C	Ups3	yes	no			not scoreable	N/A
YMR207C	Hfa1	no	no			not scoreable	N/A
YOL004W	Sin3	no	no			not scoreable	N/A
YOL023W	Ifm1	no	no			not scoreable	N/A
YDR474C		no	no			not scoreable	N/A
YGL064C	Mrh4	no	no			not scoreable	N/A
YER061C	Cem1	no	no			not scoreable	N/A
YDR125C	Ecm18	no	no			not scoreable	N/A
YGL059W	Pkp2	no	no			not scoreable	N/A
YLR105C	Sen2	no	no			not scoreable	N/A
YKL011C	Cce1	no	no			not scoreable	N/A
YDL107W	Mss2	no	no			not scoreable	N/A
YGR101W	Pcp1	no	no			not scoreable	N/A
YNL198C		no	no			not scoreable	N/A
YPL172C	Cox10	no	no			not scoreable	N/A
YDL142C	Crd1	no	no			not scoreable	N/A
YDR529C	Qcr7	no	no			not scoreable	N/A
YOR045W	Tom6	no	no			not scoreable	N/A
YGR031W	Imo32	no	no			not scoreable	N/A
Total Screened	Total Scoreable	Total Class 1	Total Class 2	Total Class 3	Total Class 4	Total Class 5	Total Other
526	441	37	37	159	13	185	19

Table S1. Complete list of mitochondrial protein fates upon FCCP treatment.

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Strain	Genotype
BY4741	MATa his3Δ leu2Δ ura3Δ met15Δ
AHY3354	MATa his3Δ leu2Δ ura3Δ met15Δ TOM70-mCherry:KanMX MIR1-yeGFP:HisMX
AHY3742	MATa his3Δ leu2Δ ura3Δ met15Δ TOM70-mCherry:KanMX COX15-yeGFP:HisMX
AHY3746	MATa his3Δ leu2Δ ura3Δ met15Δ TOM70-mCherry:KanMX LAT1-yeGFP:HisMX
AHY3857	MATa his3Δ leu2Δ ura3Δ met15Δ TOM70-mCherry:KanMX COX15-yeGFP:HisMX pdr5Δ::URA3
AHY3861	MATa his3Δ leu2Δ ura3Δ met15Δ TOM70-mCherry:KanMX LAT1-yeGFP:HisMX pdr5Δ::URA3
AHY3934	MATa/MATa his3Δ1/his3Δ1 leu2Δ0/leu2Δ0 ura3Δ0/ura3Δ0 lys2Δ0/+ met15Δ0/+ $Term_{cyc1}$:URA3-$P_{GPD/TDH3}$-cre-EBD78:$Term_{cyc1}$/+ ILV2-V5-loxP-HA-GFP-HygX-loxP-T7-mRFP-KanMX/+
AHY4042	MATa his3Δ leu2Δ ura3Δ met15Δ ILV2-yeGFP:HisMX
AHY4389	MATa his3Δ leu2Δ ura3Δ met15Δ ILV2-yeGFP:HisMX pdr5Δ::URA3
AHY4628	MATa his3Δ leu2Δ met15Δ URA3::CMV-tTA TOM70-mCherry:KanMX ILV2-yeGFP:HisMX
AHY4737	MATa his3Δ leu2Δ ura3Δ met15Δ TOM70-mCherry:KanMX ACP1-yeGFP:HisMX
AHY4739	MATa his3Δ leu2Δ ura3Δ met15Δ ILV2-yeGFP:HisMX TOM70-mCherry:KanMX
AHY4945	MATa his3Δ leu2Δ ura3Δ met15Δ ILV2-3xHA:HisMX
AHY4949	MATa his3Δ leu2Δ ura3Δ met15Δ DLD1-yeGFP:HisMX
AHY4951	MATa his3Δ leu2Δ ura3Δ met15Δ DLD2-yeGFP:HisMX
AHY4959	MATa his3Δ leu2Δ ura3Δ met15Δ DLD1-yeGFP:HisMX pdr5Δ::URA3
AHY4961	MATa his3Δ leu2Δ ura3Δ met15Δ DLD2-yeGFP:HisMX pdr5Δ::URA3
AHY4963	MATa his3Δ leu2Δ met15Δ URA3::CMV-tTA TOM70-mCherry:KanMX DLD1-yeGFP:HisMX
AHY4965	MATa his3Δ leu2Δ met15Δ URA3::CMV-tTA TOM70-mCherry:KanMX DLD2-yeGFP:HisMX
AHY4971	MATa his3Δ leu2Δ ura3Δ met15Δ ILV2-3xHA:HisMX pdr5Δ::URA3
AHY5044	MATa his3Δ leu2Δ ura3Δ met15Δ san1Δ::NatMX
AHY5047	MATa his3Δ leu2Δ ura3Δ lys2Δ ubr1Δ::URA3 doa10Δ::HygMX
AHY5048	MATa his3Δ leu2Δ ura3Δ met15Δ doa10Δ::HygMX
AHY5049	MATa his3Δ leu2Δ ura3Δ met15Δ lys2Δ san1Δ::NatMX doa10Δ::HygMX
AHY5053	MATa his3Δ leu2Δ ura3Δ lys2Δ ubr1Δ::URA3
AHY5055	MATa his3Δ leu2Δ ura3Δ met15Δ san1Δ::NatMX ubr1Δ::URA3
AHY5056	MATa his3Δ leu2Δ ura3Δ met15Δ lys2Δ san1Δ::NatMX ubr1Δ::URA3 doa10Δ::HygMX
AHY5058	MATa his3Δ leu2Δ ura3Δ met15Δ lys2Δ san1Δ::NatMX ubr1Δ::URA3 doa10Δ::HygMX DLD1-yeGFP:HisMX
AHY5060	MATa his3Δ leu2Δ ura3Δ met15Δ lys2Δ san1Δ::NatMX ubr1Δ::URA3 doa10Δ::HygMX DLD2-yeGFP:HisMX
AHY5062	MATa his3Δ leu2Δ ura3Δ met15Δ lys2Δ san1Δ::NatMX ubr1Δ::URA3 doa10Δ::HygMX ILV2-yeGFP:HisMX

AHY6027	MATa his3Δ leu2Δ ura3Δ met15Δ san1Δ::NatMX DLD1-yeGFP:HisMX
AHY6029	MATa his3Δ leu2Δ ura3Δ lys2Δ ubr1Δ::URA3 DLD1-yeGFP:HisMX
AHY6031	MATa his3Δ leu2Δ ura3Δ met15Δ doa10Δ::HygMX DLD1-yeGFP:HisMX
AHY6033	MATa his3Δ leu2Δ ura3Δ met15Δ san1Δ::NatMX ubr1Δ::URA3 DLD1-GFP:HisMX
AHY6035	MATa his3Δ leu2Δ ura3Δ met15Δ lys2Δ san1Δ::NatMX doa10Δ::HygMX DLD1-yeGFP:HisMX
AHY6037	MATa his3Δ leu2Δ ura3Δ lys2Δ ubr1Δ::URA3 doa10Δ::HygMX DLD1-yeGFP:HisMX
AHY6039	MATa his3Δ leu2Δ ura3Δ met15Δ san1Δ::NatMX DLD2-yeGFP:HisMX
AHY6041	MATa his3Δ leu2Δ ura3Δ lys2Δ ubr1Δ::URA3 DLD2-yeGFP:HisMX
AHY6043	MATa his3Δ leu2Δ ura3Δ met15Δ doa10Δ::HygMX DLD2-yeGFP:HisMX
AHY6045	MATa his3Δ leu2Δ ura3Δ met15Δ san1Δ::NatMX ubr1Δ::URA3 DLD2-yeGFP:HisMX
AHY6047	MATa his3Δ leu2Δ ura3Δ met15Δ lys2Δ san1Δ::NatMX doa10Δ::HygMX DLD2-yeGFP:HisMX
AHY6049	MATa his3Δ leu2Δ ura3Δ lys2Δ ubr1Δ::URA3 doa10Δ::HygMX DLD2-yeGFP:HisMX
AHY6051	MATa his3Δ leu2Δ ura3Δ met15Δ san1Δ::NatMX ILV2-yeGFP:HisMX
AHY6053	MATa his3Δ leu2Δ ura3Δ lys2Δ ubr1Δ::URA3 ILV2-yeGFP:HisMX
AHY6055	MATa his3Δ leu2Δ ura3Δ met15Δ doa10Δ::HygMX ILV2-yeGFP:HisMX
AHY6057	MATa his3Δ leu2Δ ura3Δ met15Δ san1Δ::NatMX ubr1Δ::URA3 ILV2-yeGFP:HisMX
AHY6059	MATa his3Δ leu2Δ ura3Δ met15Δ lys2Δ san1Δ::NatMX doa10Δ::HygMX ILV2-yeGFP:HisMX
AHY6061	MATa his3Δ leu2Δ ura3Δ lys2Δ ubr1Δ::URA3 doa10Δ::HygMX ILV2-yeGFP:HisMX
AHY6063	MATa his3Δ leu2Δ ura3Δ met15Δ lys2Δ san1Δ::NatMX ubr1Δ::URA3 doa10Δ::HygMX LAT1-yeGFP:HisMX
AHY6408	MATa his3Δ leu2Δ ura3Δ met15Δ lys2Δ san1Δ::NatMX ubr1Δ::URA3 doa10Δ::HygMX ΔPDR5::G418, ILV2-3xHA:HixMX
AHY6802	MATa his3Δ leu2Δ met15Δ P_{TOM40}::NatMX-tet07-TATA URA3::CMV-tTA TOM70-mCherry:KanMX ILV2-yeGFP:HisMX
AHY6804	MATa his3Δ leu2Δ met15Δ P_{TOM40}::NatMX-tet07-TATA URA3::CMV-tTA TOM70-mCherry:KanMX DLD1-yeGFP:HisMX
AHY6806	MATa his3Δ leu2Δ met15Δ P_{TOM40}::NatMX-tet07-TATA URA3::CMV-tTA TOM70-mCherry:KanMX DLD2-GFP:HisMX
AHY6808	MATa his3Δ leu2Δ met15Δ P_{TOM40}::NatMX-tet07-TATA URA3::CMV-tTA TOM70-mCherry:KanMX MIR1-yeGFP:HisMX
AHY6864	MATa his3Δ leu2Δ met15Δ P_{TOM40}::NatMX-tet07-TATA URA3::CMV-tTA TOM70-mCherry:KanMX TOM20-yeGFP:HisMX
AHY6867	MATa his3Δ leu2Δ met15Δ P_{TOM40}::NatMX-tet07-TATA URA3::CMV-tTA TOM70-mCherry:KanMX COX15-yeGFP:HisMX
AHY6870	MATa his3Δ leu2Δ met15Δ P_{TOM40}::NatMX-tet07-TATA URA3::CMV-tTA TOM70-mCherry:KanMX ACP1-yeGFP:HisMX
AHY6948	MATa his3Δ leu2Δ ura3Δ met15Δ SEC61-mCherry:KanMX MIR1-yeGFP:HisMX
AHY7181	MATa his3Δ leu2Δ met15Δ URA3::CMV-tTA TOM70-mCherry:KanMX ACP1-yeGFP:HisMX
AHY7183	MATa his3Δ leu2Δ met15Δ URA3::CMV-tTA TOM70-mCherry:KanMX MIR1-yeGFP:HisMX

AHY7187	MATa his3Δ leu2Δ met15Δ URA3::CMV-tTA TOM70-mCherry:KanMX COX15-yeGFP:HisMX
AHY7226	MATa his3Δ leu2Δ ura3Δ met15Δ TOM70-mCherry:KanMX pRS413-pGPD-COX15-GFP
AHY7228	MATa his3Δ leu2Δ ura3Δ met15Δ TOM70-mCherry:KanMX pRS413-GPD-ΔMTS (ΔN1-65) COX15-GFP
AHY7582	MATa his3Δ leu2Δ ura3Δ met15Δ TOM70-mCherry:KanMX TOM20-yeGFP:HisMX
AHY7584	MATa his3Δ leu2Δ ura3Δ met15Δ TOM70-mCherry:KanMX DLD1-yeGFP:HisMX
AHY7586	MATa his3Δ leu2Δ ura3Δ met15Δ TOM70-mCherry:KanMX DLD2-yeGFP:HisMX
AHY7594	MATa his3Δ leu2Δ ura3Δ met15Δ lys2Δ san1Δ::NatMX ubr1Δ::URA3 doa10Δ::HygMX DLD1-yeGFP:HisMX TOM70-mCherry:KanMX
AHY7596	MATa his3Δ leu2Δ ura3Δ met15Δ lys2Δ san1Δ::NatMX ubr1Δ::URA3 doa10Δ::HygMX DLD2-yeGFP:HisMX TOM70-mCherry:KanMX
AHY7598	MATa his3Δ leu2Δ ura3Δ met15Δ lys2Δ san1Δ::NatMX, ubr1Δ::URA3 doa10Δ::HygMX ILV2-yeGFP:HisMX TOM70-mCherry:KanMX
AHY7742	MATa his3Δ leu2Δ met15Δ URA3::CMV-tTA TOM70-mCherry:KanMX TOM20-yeGFP:HisMX
AHY7875	MATa his3Δ leu2Δ ura3Δ met15Δ TOM70-mCherry:KanMX pRS413-GPD-ΔMTS (ΔN1-55)ILV2-GFP
AHY7876	MATa his3Δ leu2Δ ura3Δ met15Δ TOM70-mCherry:KanMX pRS413-GPD-MTS_{ILV2}-GFP
AHY7965	MATa his3Δ leu2Δ ura3Δ met15Δ TOM70-mCherry:KanMX pRS413-pGPD-MTS_{COX15}-GFP
AHY7967	MATa his3Δ leu2Δ ura3Δ met15Δ TOM70-mCherry:KanMX pRS413-GPD-ΔMTS (ΔN 1-28) LAT1-GFP
AHY7969	MATa his3Δ leu2Δ ura3Δ met15Δ TOM70-mCherry:KanMX pRS413-pGPD-LAT1-G
AHY8001	MATa his3Δ leu2Δ ura3Δ met15Δ lys2Δ san1Δ::NatMX, ubr1Δ::URA3 doa10Δ::HygMX TOM70-mCherry:KanMX pRS413-GPD-ΔMTS (ΔN1-55)ILV2-GFP
AHY8003	MATa his3Δ leu2Δ ura3Δ met15Δ lys2Δ san1Δ::NatMX ubr1Δ::URA3 doa10Δ::HygMX TOM70-mCherry:KanMX pRS413-pGPD-MTS_{ILV2}-GFP
AHY8008	MATa his3Δ leu2Δ ura3Δ met15Δ TOM70-mCherry:KanMX pRS413-pGPD-MTS_{LAT1}-GFP
AHY8027	MATa his3Δ leu2Δ ura3Δ met15Δ TOM70-mCherry:KanMX pRS413-pGPD-ILV2-GFP
AHY8031	MATa his3Δ leu2Δ ura3Δ met15Δ lys2Δ san1Δ::NatMX ubr1Δ::URA3 doa10Δ::HygMX TOM70-mCherry:KanMX pRS413-pGPD-ILV2-GFP
AHY8043	MATa his3Δ leu2Δ ura3Δ met15Δ lys2Δ san1Δ::NatMX ubr1Δ::URA3 doa10Δ::HygMX Tom70-mCherry:KanMX pRS413-pGPD-DLD2-GFP
AHY8345	MATa his3Δ leu2Δ ura3Δ met15Δ TOM70-GFP:KanMX TIM50-mCherry:KanMX ilv2Δ::URA3
AHY8557	MATa his3Δ leu2Δ ura3Δ met15Δ TOM70-mCherry:KanMX pRS413-GPD-ΔMTS (ΔN1-35) DLD2-GFP
AHY8559	MATa his3Δ leu2Δ ura3Δ met15Δ lys2Δ san1Δ::NatMX, ubr1Δ::URA3 doa10Δ::HygMX TOM70-mCherry:KanMX pRS413-GPD-ΔMTS (ΔN1-35) DLD2-GFP
AHY8561	MATa his3Δ leu2Δ ura3Δ met15Δ TOM70-mCherry-KanMX pRS413-pGPD-MTS_{DLD2}-GFP
AHY8563	MATa his3Δ leu2Δ ura3Δ met15Δ lys2Δ san1Δ::NatMX ubr1Δ::URA3 doa10Δ::HygMX TOM70-mCherry:KanMX pRS413-pGPD-MTS_{DLD2}-GFP
AHY8671	MATa his3Δ leu2Δ ura3Δ met15Δ TOM70-mCherry:KanMX pRS413-pGPD-DLD2-GFP
AHY10107	MATa his3Δ leu2Δ ura3Δ met15Δ lys2ΔTOM70-mCherry KanMX pdr5Δ::URA3 LAT1-3XHA-HisMX
AHY10198	MATa his3Δ leu2Δ ura3Δ met15Δ ILV2-3xHA:KanMX pdr5Δ::Ura3 NUP49-yeGFP:HisMX

AHY10267	MATa his3Δ leu2Δ ura3Δ met15Δ ACP1-3xHA:HisMX
AHY10269	MATa his3Δ leu2Δ ura3Δ met15Δ COX15-3xHA:HisMX
AHY10369	MATa his3Δ leu2Δ ura3Δ met15Δ ILV2-5FLAG:KanMX TOM70-mCherry:HygMX
AHY10371	MATa his3Δ leu2Δ ura3Δ met15Δ TOM20-5FLAG:KanMX TOM70-mCherry:HygMX
AHY10373	MATa his3Δ leu2Δ ura3Δ met15Δ MIR1-5FLAG:KanMX TOM70-mCherry:HygMX
AHY10375	MATa his3Δ leu2Δ ura3Δ met15Δ ACP1-5FLAG:KanMX TOM70-mCherry:HygMX
AHY10377	MATa his3Δ leu2Δ ura3Δ met15Δ COX15-5FLAG:KanMX TOM70-mCherry:HygMX
AHY10381	MATa his3Δ leu2Δ ura3Δ met15Δ MIR1-3xHA:HisMX
AHY10385	MATa his3Δ leu2Δ ura3Δ met15Δ TOM20-3xHA:HisMX TOM70-mCherry:KanMX
AHY10437	MATa his3Δ leu2Δ ura3Δ met15Δ COX15-3xHA:HisMX pdr5Δ::URA3

Table S2. Yeast strains used in this study.

Name	Number	Sequence
Tagging Primers		
NUP49 pKT F5	2260	GTTACATCAAAAAACGAAAACACTGGCATCATTGAGCATA GGTGACGGTGCTGGTTTA
NUP49 pKT R3	2261	ACTTGTTATACGCACTATATAAACTTTCAGGGCGATTTACTC GATGAATTCGAGCTCG
TOM20 pKT F5	465	GCCGAATCTGATGCGGTTGCTGAAGCTAACGATATCGATGA CGGTGACGGTGCTGGTTTA
TOM20 pKT R3	466	AAGAAACAAAAACGGAGAAAAAAAGCAAGCAAAATGTTA CTCTCGATGAATTCGAGCTCG
ILV2 pKT F5	1481	ACAGACTGAATTACGTCATAAGCGTACAGGCGGTAAGCAC GGTGACGGTGCTGGTTTA
ILV2 pKT R3	1482	TTTTTACTGAAAATGCTTTTGAAATAAATGTTTTTGAAATTC GATGAATTCGAGCTCG
MIR1 pKT F5	1556	GGGTTGCCCACCAACCATTGAAATTGGTGGTGGTGGTCATG GTGACGGTGCTGGTTTA
MIR1 pKT R3	1557	GAGGAGAGAATATATATGCATGTATCAATCAAGACCATTTT CGATGAATTCGAGCTCG
LAT1 pKT F5	1806	ATTGAAAACTGTTATTGAAAATCCTTTGGAAATGCTATTGG GTGACGGTGCTGGTTTA
LAT1 pKT R3	1807	AGATACGCATTTACTGGCGAATTTTATTTTCATTCTAACCTC GATGAATTCGAGCTCG
COX15 pKT F5	1809	AATTTTAAGTGAAGCGTCGAAGTTAGCCTCGAAACCATTAG GTGACGGTGCTGGTTTA
COX15 pKT R3	1810	GCGAGTATACTGTCAATTCTCATAAGAATACCTTTATCCAT CGATGAATTCGAGCTCG
ILV2 RITE F5	1831	ACAGACTGAATTACGTCATAAGCGTACAGGCGGTAAGCAC GGTGGATCTGGTGGATCT
ILV2 RITE R3	1832	TTTTTACTGAAAATGCTTTTGAAATAAATGTTTTTGAAATTT AGGCGCCGGTGGAGTGGCG
DLD1 pKT F5	2271	CTTTAAAACTGATCCAAACGAGCCCGCTAATGATTACAGGG GTGACGGTGCTGGTTTA
DLD1 pKT R3	2272	TTCAGGTTTACGTGAAGGGTGAAAAAGGAAAATCAGATAC TCGATGAATTCGAGCTCG
DLD2 pKT F5	2274	TTATGATCCTAATGGAATTTTAAACCCTTACAAATACATTG GTGACGGTGCTGGTTTA
DLD2 pKT R3	2275	TATACATATGTAGATAACTATAAAACTTGGCATTTTATTTTC GATGAATTCGAGCTCG
SEC61 pKT F5	2836	GTTTACTAAGAACCTCGTTCCAGGATTTTCTGATTTGATGGG TGACGGTGCTGGTTTA
SEC61 pKT R3	2837	GCGATTTTTTTTTTCTTTGGATATTATTTTCATTTTATATTCG ATGAATTCGAGCTCG
ACP1 pKT F5	2169	TGAAACGGTCGATTATATCGCTTCCAATCCCGACGCAAACG GTGACGGTGCTGGTTTA
ACP1 pKT R3	2170	GGGGTGACACGATACAATATAATAGAGCGGGGACGGACAC TCGATGAATTCGAGCTCG
TOM20 pFA6 F5	3959	CGAATCTGATGCGGTTGCTGAAGCTAACGATATCGATGACC GGATCCCCGGGTTAATTAA
TOM20 pFA6 F5	3960	GAAACAAAAACGGAGAAAAAAAGCAAGCAAAATGTTACTC GAATTCGAGCTCGTTTAAAC

TOM20 chk	3961	CAGCTCTATCAGCCACCGGTTATGCTATCT
ACP1 pFA6 F5	3955	TGAAACGGTCGATTATATCGCTTCCAATCCCGACGCAAACC GGATCCCCGGGTTAATTAA
ACP1 pFA6 F5	3956	GGGGTGACACGATACAATATAATAGAGCGGGGACGGACAC GAATTCGAGCTCGTTTAAAC
ACP1 chk	2171	CAACACAACTAACTCAATACAGCACCTTCC
MIR1 pFA6 F5	4079	GGGTTGCCCACCAACCATTGAAATTGGTGGTGGTGGTCATC GGATCCCCGGGTTAATTAA
MIR1 pFA6 R3	4080	GAGGAGAGAATATATATGCATGTATCAATCAAGACCATTTG AATTCGAGCTCGTTTAAAC
Mir1 chk	1558	AGCAGACACTCTGTTGTCCAAGGTCAACAA
ILV2 pFA6 F5	2210	ACAGACTGAATTACGTCATAAGCGTACAGGCGGTAAGCAC CGGATCCCCGGGTTAATTAA
ILV2 pFA6 R3	2211	TTTTTACTGAAAATGCTTTTGAAATAAATGTTTTTGAAATGA ATTCGAGCTCGTTTAAAC
ILV2 chk	561	TTGGTTATTGACATTGATGGTGACGCATCC
COX15 pFA6 F5	3957	AATTTTAAGTGAAGCGTCGAAGTTAGCCTCGAAACCATTAC GGATCCCCGGGTTAATTAA
COX15 pFA6 R3	3958	GCGAGTATACTGTCAATTCTCATAAGAATACCTTTATCCAG AATTCGAGCTCGTTTAAAC
COX15 chk	1811	AATGGGTGAACGATGGTTCCCTAGTTCTCG
LAT1 pFA6 F5	2885	ATTGAAAACTGTTATTGAAAATCCTTTGGAAATGCTATTGC GGATCCCCGGGTTAATTAA
LAT1 pFA6 R3	2886	AGATACGCATTTACTGGCGAATTTTATTTTCATTCTAACCGA ATTCGAGCTCGTTTAAAC
LAT1 chk	1808	GCCAGATGCCAATGCCTACTGGTTACCTAA
KanMX Check Reverse	810	CCCATATAAATCAGCATCCA
TOM70 pFA6 F5	1077	TCAAGAAACTTTAGCTAAATTACGCGAACAGGGTTTAATGC GGATCCCCGGGTTAATTAA
TOM70 pFA6 R3	1078	TTTGTCTTCTCCTAAAAGTTTTTAAGTTTATGTTTACTGTGA ATTCGAGCTCGTTTAAAC
KO PRIMERS		
ILV2 KO FW	3380	TAAGAGGAGATAAATACAACAGAATCAATTTTCAAGCAGA TTGTACTGAGAGTGCACC
ILV2 KO REV	3381	ACTGAAAATGCTTTTGAAATAAATGTTTTTGAAATCTGTGC GGTATTTCACACCG
ILV2 KO chk D5	3382	GTCTGTCAGTCGGCAC
ILV2 KO chk D3	3383	GTTAAATTCGTATTGGCCACTG
DOA10 KO chk D3	2308	GTGGCATTTAGTAGTCCAACTAGG

DOA10 KO chk D5	2061	TCAACAATGGAACCCCCAACAATTATCTCA
DOA10 KO Fw	2059	TACCACTAATTGAATCAAAGAGACTAGAAGTGTGAAAGTC AGATTGTACTGAGAGTGCAC
DOA10 KO Rv	2060	TATGCTAGCATTCATTTTAAATGTAAGGAAGAAAACGCCTC TGTGCGGTATTTCACACCG
SAN1 KO check D5	1917	TTGTATACTAGGTATTGCACCGCAGTCAGA
SAN1 KO chk D3	2281	CCAACACTTGGTTTTCATGAC
SAN1 KO FW	1915	GTTTTCTCTCATAGTCTTGTAACCTCAGCTTTTGTTCATTAG ATTGTACTGAGAGTGCAC
SAN1 KO REV	1916	GACATATTTTCATATTAACATACTTCAGAAGCGGTATTGTCT GTGCGGTATTTCACACCG
UBR1 KO chk D3	2307	GCGAAGGATATGAAAATCAACC
UBR1 KO chk D5	1914	TAACTTGCAGATAGTGACCATAAGGCAACT
UBR1 KO Fw	1926	AATCTTTACAGGTCACACAAATTACATAGAACATTCCAATA GATTGTACTGAGAGTGCAC
UBR1 KO Rv	1913	ACAAATATGTCAACTATAAAACATAGTAGAGGGCTTGAATC TGTGCGGTATTTCACACCG
PDR5 KO chk D3	2365	GTTCGCCATTCGGACAGATAATG
PDR5 KO chk D5	1814	CGGAACTCTTCTACGCCGTGGTACGATATC
PDR5 KO D3	1813	TCTTGGTAAGTTTCTTTTCTTAACCAAATTCAAAATTCTACT GTGCGGTATTTCACACCG
PDR5 KO D5	1812	AAGTTTTCGTATCCGCTCGTTCGAAAGACTTTAGACAAAAA GATTGTACTGAGAGTGCAC
CLONING PRIMERS		
DLD2 AA 1-35 R	3399	CTTCACCTTTAGACATGTTAATTAAACCAGCACCGTCACCA TAGTTAACTCTTCTATAG
DLD2 AA36-530 F	3401	CTTAGTTTCGACGGATTCTAGAACTAGTGGATCCCCCGGGA TGTATTCGACCAAGATAC
DLD2 pRS413 Fw	2633	CTTAGTTTCGACGGATTCTAGAACTAGTGGATCCCCCGGGA TGCTAAGAAACATTTTGG
GFP pRS413-GPD RV	2179	TAATTACATGACTCGAGGTCGACGGTATCGATAAGCTTGAT TATTTGTACAATTCATCC
ILV2 AAs 56-687 FW	2180	CTTAGTTTCGACGGATTCTAGAACTAGTGGATCCCCCGGGA TGGAGCCTGCTCCAAGTTTC
ILV2 MTS FW	2188	CTTAGTTTCGACGGATTCTAGAACTAGTGGATCCCCCGGGA TGATCAGACAATCTACGCT
ILV2 MTS RV	2196	CTTCACCTTTAGACATGTTAATTAAACCAGCACCGTCACCT GGCCTTTTAGAGGCTGG
LAT1 AAs 29-482 FW	2182	CTTAGTTTCGACGGATTCTAGAACTAGTGGATCCCCCGGGA TGGCATCGTACCCAGAGCACAC

LAT1 MTS FW	2190	CTTAGTTTCGACGGATTCTAGAACTAGTGGATCCCCCGGGA TGTCTGCCTTTGTCAGGG
LAT1 MTS RV	2198	CTTCACCTTTAGACATGTTAATTAAACCAGCACCGTCACCG TAGCATCTCAATTGCAGTC
pKT adaptor FW	2204	GGTGACGGTGCTGGTTTA
COX15 AAs 66-486 FW	2184	CTTAGTTTCGACGGATTCTAGAACTAGTGGATCCCCCGGGA TGAAACCACATGTTGCTTCAG
COX15 MTS FW	2192	CTTAGTTTCGACGGATTCTAGAACTAGTGGATCCCCCGGGA TGCTTTTCAGAAACATAGAAG
COX15 MTS RV	2200	CTTCACCTTTAGACATGTTAATTAAACCAGCACCGTCACCA AAAACAGGGGAGGAGAGAG
GPD Fw sequencing	2277	AAGACGGTAGGTATTGATTG
CYC Rv sequencing	2278	GCGTACACGCGTTTGTAC

Table S3. Oligos used in this study.

Plasmid	Construction
pRS413-GPD-ILV2-GFP	pRS413-GPD cut w/ EcoRI + PCR product 1 (AHY4042 gDNA amplified w/ 2188/2179)
pRS413-GPD-DLD2-GFP	pRS413-GPD cut w/ EcoRI + PCR product 1 (AHY4951 gDNA amplified w/ 2633/2179)
pRS413-GPD-COX15-GFP	pRS413-GPD cut w/ EcoRI + PCR product 1 (AHY3742 gDNA amplified w/ 2192/2179)
pRS413-GPD-LAT1-GFP	pRS413-GPD cut w/ EcoRI + PCR product 1 (AHY3746 gDNA amplified w/ 2190/2179)
pRS413-GPD-NΔ55ILV2-GFP	pRS413-GPD cut w/ EcoRI + PCR product 1 (AHY4042 gDNA amplified w/ 2180/2179)
pRS413-GPD-NΔ35DLD2-GFP	pRS413-GPD cut w/ EcoRI + PCR product 1 (pRS413-GPD-DLD2-GFP amplified w/ 3401/2179)
pRS413-GPD-NΔ65COX15-GFP	pRS413-GPD cut w/ EcoRI + PCR product 1 (AHY3742 gDNA amplified w/ 2184/2179)
pRS413-GPD-NΔ28LAT1-GFP	pRS413-GPD cut w/ EcoRI + PCR product 1 (AHY3746 gDNA amplified w/ 2182/2179)
pRS413-GPD-MTS$_{ILV2}$-GFP	pRS413-GPD cut w/ EcoRI + PCR product 1 (BY4741 gDNA amplified w/ 2188/2196) + PCR product 2 (pKT128 amplified w/ 2204/2179)
pRS413-GPD-MTS$_{DLD2}$-GFP	pRS413-GPD cut w/ EcoRI + PCR product 1 (pRS413-GPD-DLD2-GFP amplified w/ 2633/3399) + PCR product 2 (pKT128 amplified w/ 2204/2179)
pRS413-GPD-MTS$_{COX15}$-GFP	pRS413-GPD cut w/ EcoRI + PCR product 1 (BY4741 gDNA amplified w/ 2192/2200) + PCR product 2 (pKT128 amplified w/ 2204/2179)
pRS413-GPD-MTS$_{LAT1}$-GFP	pRS413-GPD cut w/ EcoRI + PCR product 1 (BY4741 gDNA amplified w/ 2190/2198) + PCR product 2 (pKT128 amplified w/ 2204/2179)

Table S4. Plasmids used in this study.