Supplemental Materials

Molecular Biology of the Cell

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The mitospecific domain of Mrp7 (bL27) supports mitochondrial translation during fermentation and is required for effective adaptation to respiration

Supplemental data (Figures S1-S7)

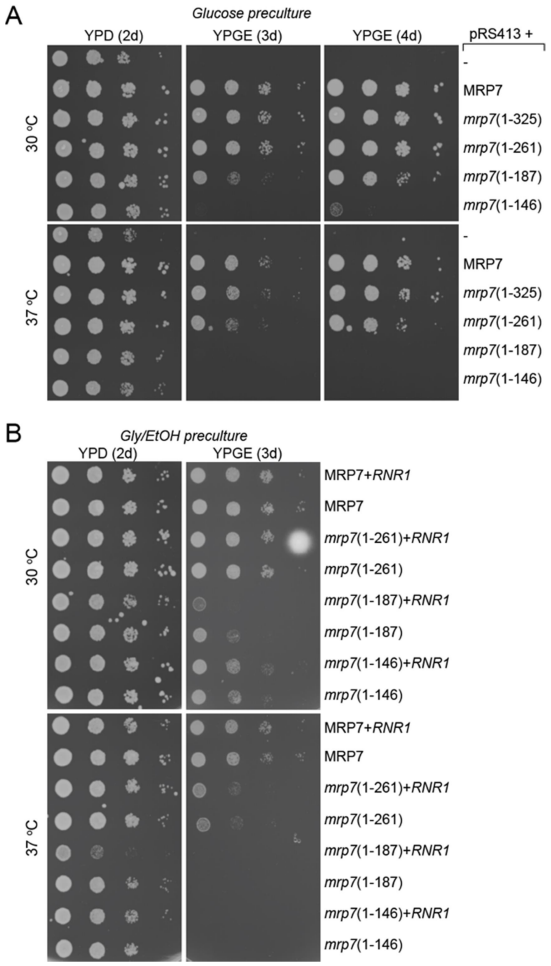

FIGURE S1: *mrp7* strains display respiratory deficient phenotypes, but which improve upon prior adaptation of strains to respiratory growth media (A) 10-fold serial dilutions of Δ*mrp7* strains with the empty pRS413 plasmid (-), or the pRS413 plasmid encoding the wild type Mrp7 protein (MRP7) or one of the truncated *mrp7* derivates [*mrp7*(1-325), *mrp7*(1-261), *mrp7*(1-187), or *mrp7*(1-146)], as indicated. Strains were taken from 24 hr growths on selective SD plates and spotted onto YP plates containing either glucose (YPD) (2d) or glycerol+ethanol (YPGE) (3d and 4d) and incubated at either 30 °C or 37 °C, as indicated. (B) 10-fold serial dilutions of Δ*mrp7* strains with pRS413 plasmid encoding WT Mrp7 protein (MRP7) or one of the truncated *mrp7* derivates [*mrp7*(1-261), *mrp7*(1-187), or *mrp7*(1-146)], compared to strains containing YEplac112 plasmid encoding RNR1 protein (+*RNR1*), as indicated. Strains were taken from 48hr growths on selective SGE plates and spotted onto YP plates containing either glucose (YPD) (2d) or glycerol+ethanol (YPGE) (3d and 5d) and incubated at either 30°C or 37°C, as indicated.

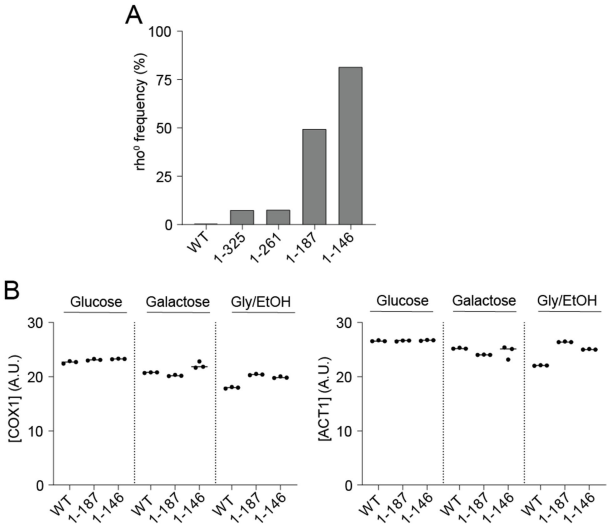

FIGURE S2: mtDNA instability observed with C-terminal Mrp7 truncations is prevented in presence of RNR1 suppressor (A) Indicated *mrp7* derivates were grown on YPG plate at 30 ^{o}C, to ensure all freshly grown cells were rho^{+}. Once fresh growth occurred, the cells were inoculated into YPGal media and grown for 24 hr. After this growth period, and equal number of cells were removed, diluted, and equally plated on both YPD and YPG+0.1% galactose plates. After 3 days of growth the large colonies that grew on the YPG+0.1% galactose plates were quantified relative to the large colonies that grew on YPD plates, and this ratio is expressed as rho^{0} frequency. (B) qPCR analysis of quantified COX1 (mtDNA) (left) and ACT1 (nDNA) (right) content compared in Mrp7 (+*RNR1*), *mrp7*(1-187) (+*RNR1*), and *mrp7*(1-146) (+*RNR1*) strains grown in glucose, galactose, or glycerol/ethanol synthetic media. These amounts relate to the ratios depicted in Figure 1. Data shown is an average (n=3) +/- S.D. A.U., arbitrary units. Note a W303-1B/rho^{0} yeast strain was used as a negative control where an ACT1, but not a COX1, PCR product was amplified (results not shown).

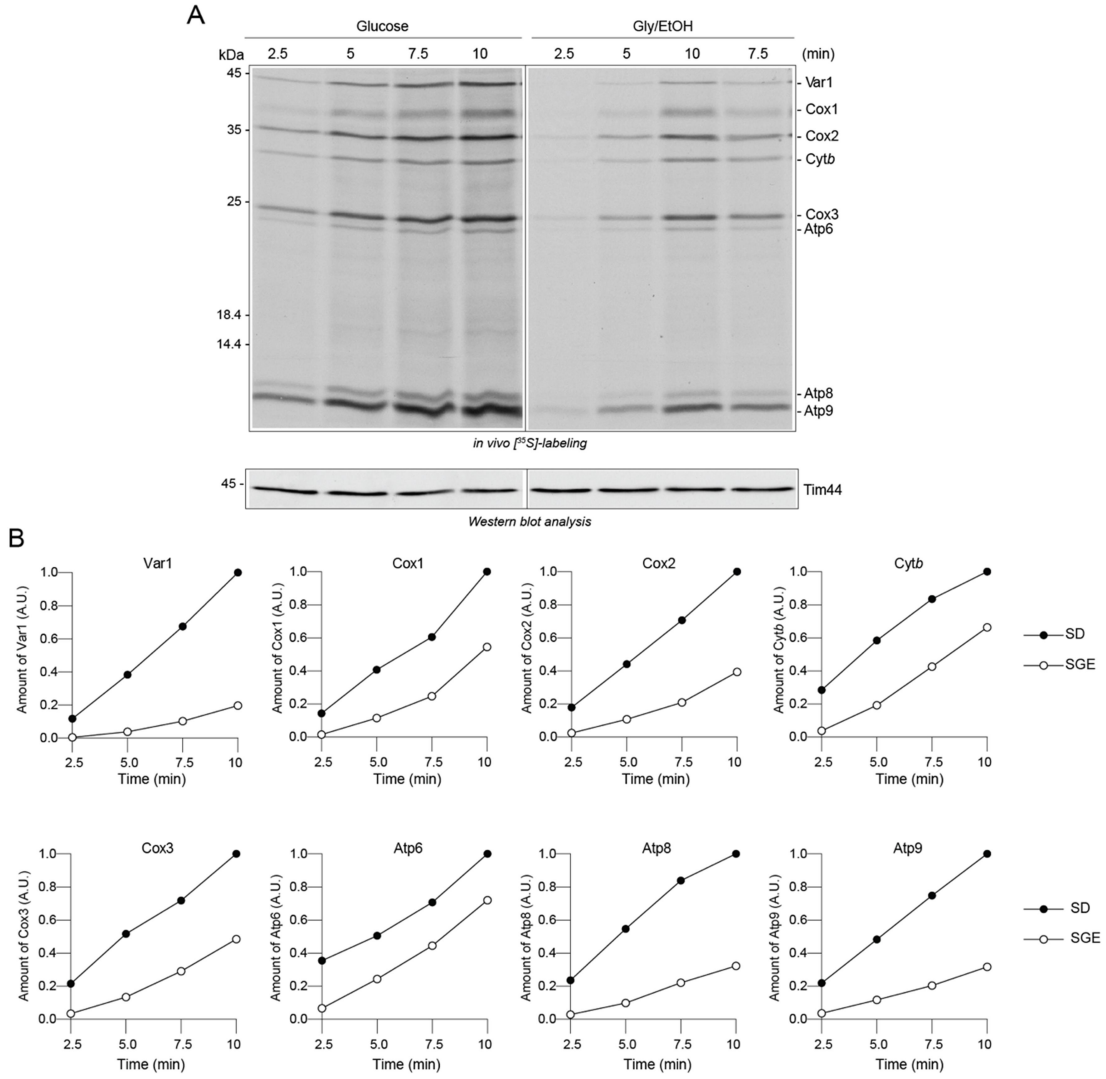

FIGURE S3: *In vivo* labeling of mitochondrial translation products occurs at a faster rate in glucose grown cells (A) The wild type Mrp7 control strain (+*RNR1*) was adapted to either glucose or glycerol/ethanol (Gly/EtOH) selective synthetic media (as described in the Materials and Methods) and grown at 30 °C to mid-log phase. Mitochondrial translation capacity was analyzed *in vivo* with the equivalent of OD_{600} 1.2 absorbance units of whole cells at 30 °C in the presence of cycloheximide (0.3mg/mL) and [^{35}S]methionine. Aliquots of cells (equivalent to OD_{600} of 0.2 absorbance units) were harvested at time points indicated, solubilized, and newly synthesized proteins were analyzed by SDS-PAGE followed by autoradiography (upper panel). Tim44 used as a loading control within each growth medium (lower panel). (B) Newly synthesized proteins in upper panel were quantified by phosphorimaging of the resulting gel in (A). For each protein analyzed, the level of synthesis achieved at a specific time point was expressed as a ratio of the level of that protein synthesized in the 10 min time period on glucose media. A.U., arbitrary units.

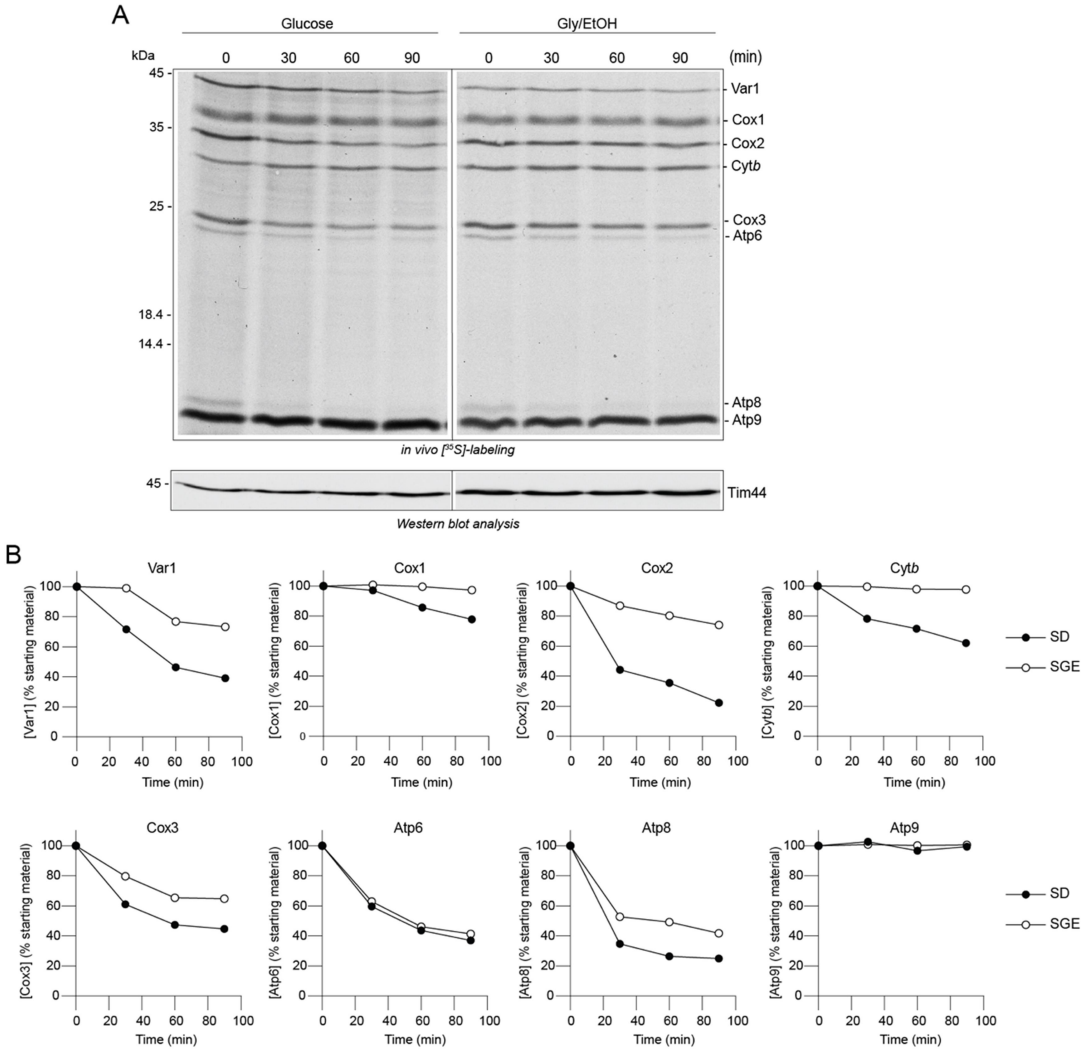

FIGURE S4: Pulse chase experiment indicates mitochondrial translation products synthesized under glucose fermentation conditions are more proteolytically unstable. (A) Adaptation and growth of wild type Mrp7 control strain (+*RNR1*) on glucose or glycerol/ethanol selective synthetic media, followed by *in vivo* radiolabeling for 10 min was performed as described in Figure S3. Following inhibition of translation with cold methionine (35 mM) and puromycin (73 ug/mL), cells were isolated by centrifugation, washed and resuspended in their respective (glucose or glycerol/ethanol) synthetic medium in the presence of puromycin and incubated further at 30 ^{o}C. At indicated time points, aliquots of cells were harvested, solubilized, and the radiolabeled proteins were analyzed by SDS-PAGE followed by autoradiography (upper panel). Tim44 used as a loading control within each growth medium (lower panel). (B) Newly synthesized proteins in upper panel were quantified by phosphorimaging of the resulting gel in (A). For each medium analyzed, the level of each protein analyzed remaining at each time point of the chase period was analyzed was expressed as a percentage of its level present at t = 0 min of chase (% starting material).

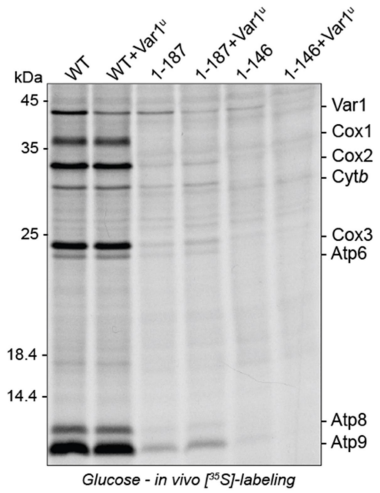

FIGURE S5: The mitotranslation defect observed in *mrp7* mutants under fermentation growth conditions is not caused by limiting Var1 synthesis. A pRS316 plasmid encoding a mitochondrially targeted universal Var1 derivative (Var1^{u}) was expressed in the Mrp7 wild type (WT), *mrp7*(1-187), and *mrp7*(1-146) mutants (all +*RNR1*) and the resulting strains were grown in selective SD media to mid log phase. *In vivo* radiolabeling to monitor mitochondrial translation capacity was performed and samples were subsequently analyzed, as previously described in Figure 3A.

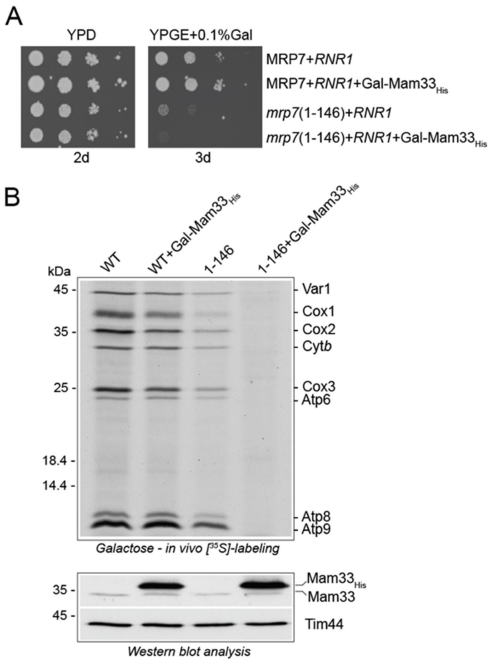

FIGURE S6: Overexpression of Mam33 does not improve growth defects or mitotranslation of the *mrp7*(1-146) mutant (A & B) The Mrp7 wild type and *mrp7*(1-146) (both +*RNR1*) strains were transformed with the plasmid Yip352-Gal10-Mam33$_{His}$ (described in Materials and Methods) and overexpression of Mam33$_{His}$ was achieved by growing the resulting transformants in presence of galactose (indicated by WT+Gal-Mam33$_{His}$ and 1-146+Gal-Mam33$_{His}$ respectively). Control strains were Mrp7 wild type and *mrp7*(1-146) (+*RNR1*) respectively, i.e. without the Yip352-Gal10-Mam33$_{His}$ plasmid. (A) 10-fold serial dilutions of indicated strains were grown on glucose (YPD) or glycerol/ethanol supplemented with 0.1% galactose (YPGE+0.1% Gal) at 30 ^{o}C for 2 or 3 days respectively. (B) Indicated strains were grown to mid-log phase in selective synthetic galactose media. *In vivo* radiolabeling to monitor mitochondrial translation capacity was performed and samples were subsequently analyzed, as previously described in Figure 3A, upper panel). Western blotting with Tim44 as a loading control was performed (lower panel).

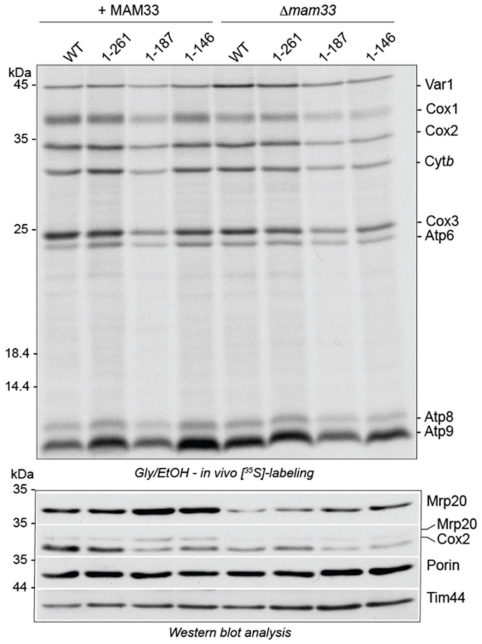

FIGURE S7: *Mrp7* derivatives maintain ability to translate under respiratory growth conditions, even in the absence of Mam33. Indicated *mrp7* strains (all +*RNR1*), containing (+MAM33) or deficient (Δ*mam33*) in Mam33 were grown to mid-log phase in selective synthetic glycerol/ethanol media (Gly/EtOH). *In vivo* radiolabeling to monitor mitochondrial translation capacity was performed and samples were subsequently analyzed, as previously described in Figure 3A. Mrp20 decoration was followed by Cox2 decoration of the same Western blot, and each respective band is indicated. Tim44 used as a loading control.