Supplemental Material

Table S1. Strains used in this study

Strain	Genotype
WT - YPH499	*MATa ura*3-52 *lys*2-801 *ade*2-101 *trp*1-Δ63 *his*3-Δ200 *leu*2-Δ1
Δ*YHM2*	as YPH499 but Δ*YHM2*::HIS3
Δ*CTP1*	" " " Δ*CTP1*::KanMX3
Δ*ZWF1*	" " " Δ*ZWF1*::KanMX3
Δ*YHM2*Δ*CTP1*	" " " Δ*YHM2*::HIS3 Δ*CTP1*::KanMX3
Δ*YHM2*Δ*ZWF1*	" " " Δ*YHM2*::HIS3 Δ*ZWF1*::NatMX4
Δ*CTP1*Δ*ZWF1*	" " " Δ*CTP1*::KanMX3 Δ*ZWF1*::NatMX4
UTL-7A	*MATα ura*3-52 *trp*1 *leu*2-3,112
BJ1991Δpox1	*MATα leu*2 *ura*3-52 *trp*1 *pep*4-3 *prb*1-1122 *gal*2Δ *POX1*::LEU2

Table S2. Percentage of non-viable cells. Yeast strains, precultured in YP with glucose and grown in acetate-supplemented SC, were incubated in the presence or absence of H_2O_2 for 60 min. After this time-period the cells were washed and tested for viability.

Strain	Untreated (%)	Treated with H_2O_2 (%)	Boiled
Wild-type	1.8 ± 0.6	5.5 ± 2.0	99.7
Δ*YHM2*	1.8 ± 0.5	5.6 ± 1.2	99.8
Δ*ZWF1*	1.2 ± 0.2	5.0 ± 1.8	99.6
Δ*YHM2*Δ*ZWF1*	1.5 ± 0.2	8.2 ± 2.1	99.8

Fig. S1. Expression in *S. cerevisiae* and purification of Yhm2p. Proteins were separated by SDS-PAGE and stained with Coomassie-blue dye (A) or transferred to nitrocellulose and immunodecorated with an anti-Yhm2p polyclonal antiserum (B). Lanes 1-3 (A and B), mitochondrial protein (30 µg) from wild-type (lane 1), Δ*YHM2* (lane 2) and *YHM2-pYES2* (lane 3) strains. Cells were harvested 6 h after addition of galactose. Lanes 4, 1.5 µg (A) and 0.3 µg (B) Yhm2p purified from *YHM2-pYES2* mitochondria.

Fig. S2. Yhm2p is localized to mitochondria. (A) Immunological detection of Yhm2p in a sucrose density gradient. Cell-free extracts of oleic acid-induced wild-type cells (UTL-7A) were separated on a continuous sucrose density gradient (32-54%). The resulting fractions were analyzed for the distribution of Yhm2p, peroxisomal matrix protein Fox3p and mitochondrial Mir1p by immunoblotting. The enzymatic activities of peroxisomal catalase (■) and mitochondrial fumarase (▲) were determined in the same fractions. Values are presented as the percentage of the peak fraction. Fraction densities are indicated by a hatched line. (B) Detection of Yhm2p in an Accudenz gradient. Peroxisomes were enriched on a sucrose density gradient (32-54%) and re-isolated by a 25,000x g centrifugation step. The organelles were then separated on an Accudenz gradient (20-40%), and the distribution of Yhm2p was compared to that of peroxisomal Fox3p and Pex11p as well as to mitochondrial Mir1p by immunoblotting.

Fig. S3. NADH/NAD^+ ratios in the cytosol of parental and deleted *S. cerevisiae* strains. Yeast strains, precultured in YP with glucose and grown in acetate-supplemented SC, were incubated in the presence (B) or absence (A) of H_2O_2 for 60 min. NADH/NAD^+ ratios and the sums of NADH + NAD^+ are reported as means ± S.E.M. of at least three independent experiments. Differences between the NADH/NAD^+ ratio of Δ*ZWF1* and Δ*YHM2*Δ*ZWF1* cells and control (wild-type cells) in the presence of H_2O_2 were significant (*P

< 0.05, one-way ANOVA followed by Bonferroni's *t*-test).

Fig. S4. NADH and NAD^+ levels in the mitochondria of parental and deleted *S. cerevisiae* strains. Yeast strains, precultured in YP with glucose and grown in acetate-supplemented SC, were incubated in the presence (B) or absence (A) of H_2O_2 for 60 min. The sums of NADH + NAD^+ with and without H_2O_2, and the NADH/NAD^+ ratio with H_2O_2 are reported as means ± S.E.M. of at least three independent experiments.

Fig. S1

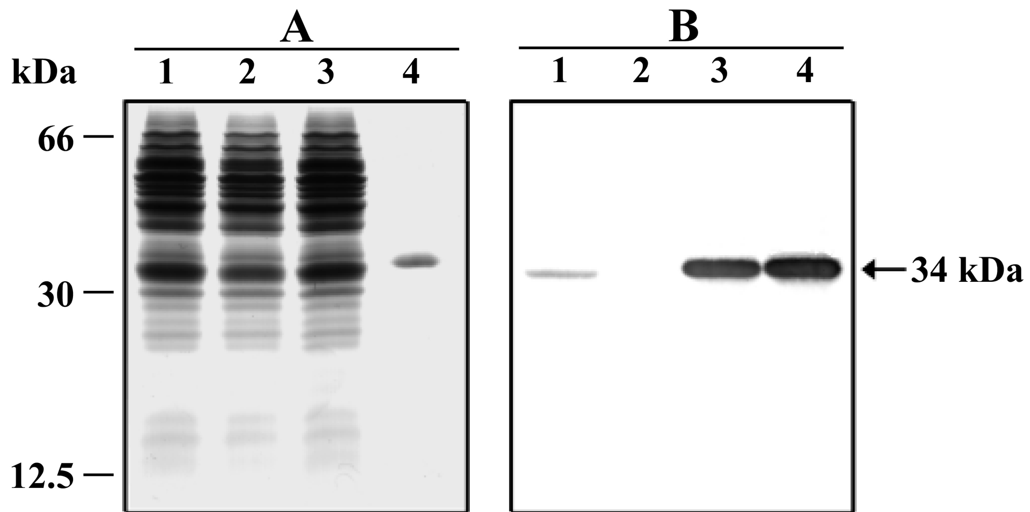
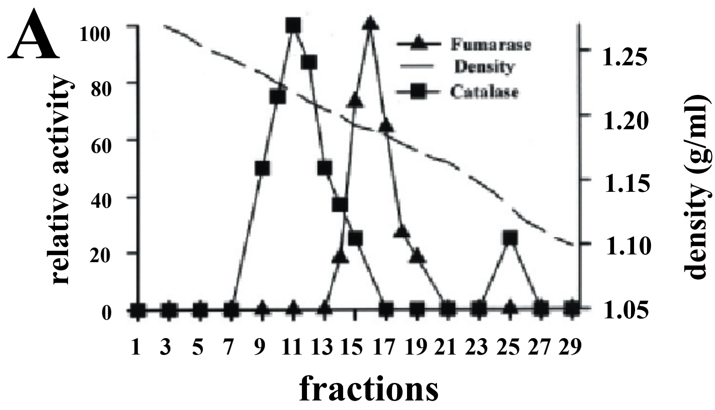
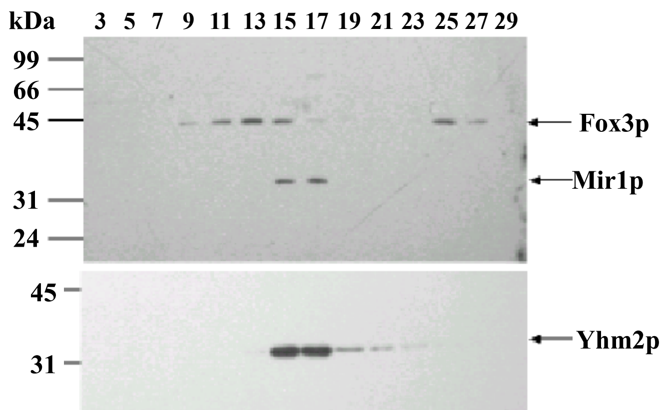


Fig. S2





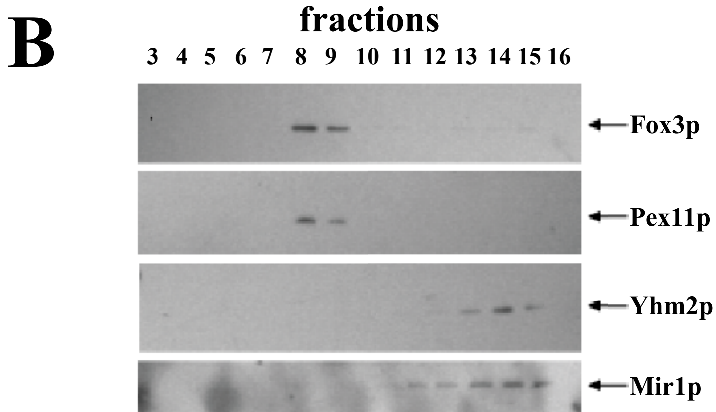


Fig. S3

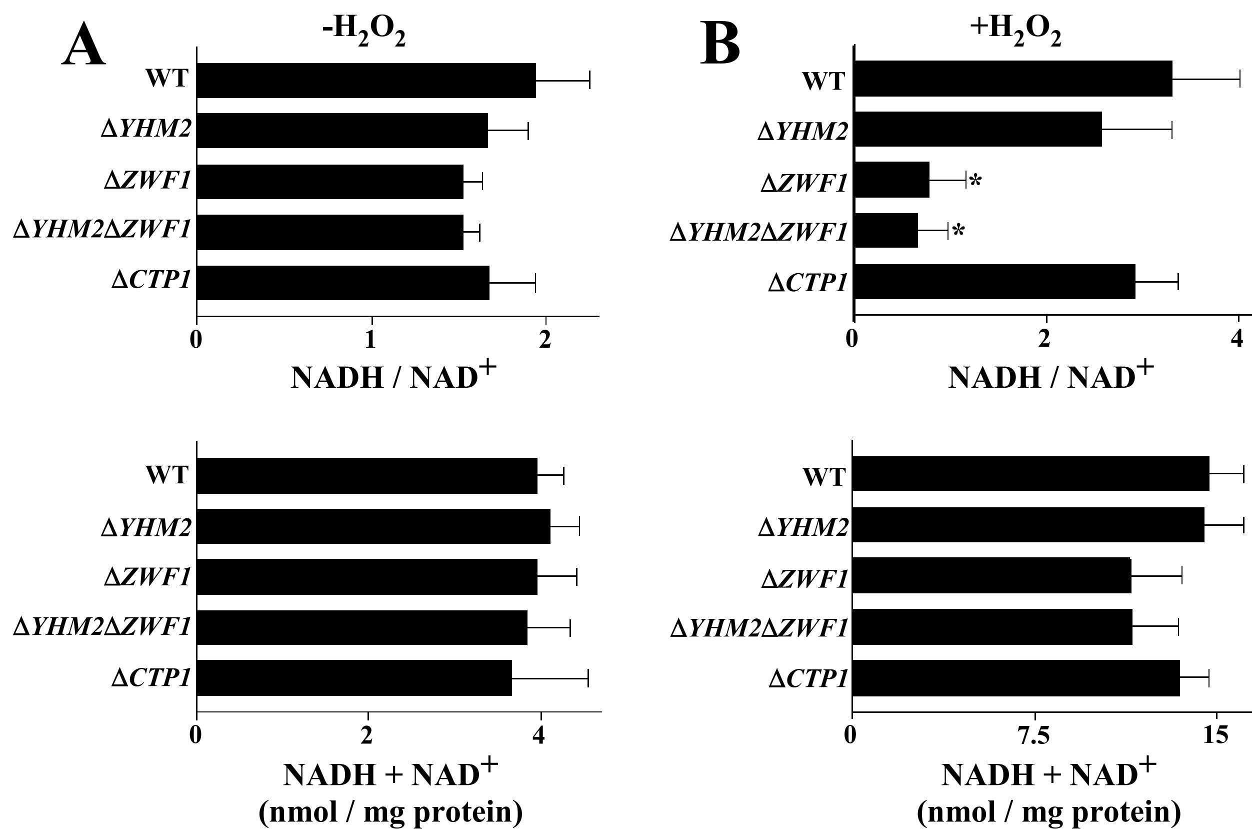


Fig. S4