Bakkaiova *et al*.
SUPPLEMENTARY INFORMATION

Supplementary Materials and methods
(indicated are only methods not described in the main text)

Microbial strains and growth conditions– S. cerevisiae strain GG595 (*MATA/MATα ura3-2/ura3-2*) provided by H. Y. Steensma (Leiden University, The Netherlands) was used for *in vivo* localization studies. *S. cerevisiae* strains RGΔade2-1C (*MATα*, *lys2Δ0, his3Δ0, leu2Δ0, ura3Δ0, ade2Δ::kan*) and CS611-5A (*MATa*, *lys2Δ0, his3Δ0, leu2Δ0, ura3Δ0, ade2Δ::kan, abf2Δ::kan*) provided by X. J. Chen (SUNY Upstate Medical University, Syracuse, NY, USA) were used for analysis of the ability of the corresponding genes to rescue the phenotype exhibited by the Δ*abf2* mutant. Yeast cultures were grown at 28°C as indicated in the main text or in complex medium (1% (w/v) yeast extract (Difco), 2% (w/v) bacto-peptone (Difco)) supplemented with 0.1% (w/v) glucose and 3% (v/v) glycerol (YPDG) or in synthetic medium (0.17% (w/v) yeast nitrogen base without amino acids and ammonium sulfate (Difco), 0.5% (w/v) ammonium sulfate (Lachema) supplemented with appropriate amino acids and bases) with 2% (w/v) galactose (SGal). Transformation of *S. cerevisiae* was performed as described by Gietz *et al*. (1). *Y. lipolytica* strain H222-SW2-1 (*MATA*, *ura3-302*, *SUC2*, *ylku70Δ-1572*) described by Kretzschmar *et al*. (2) was provided by G. Barth (Technische Universität Dresden, Germany).

Construction of plasmid vectors– The plasmid pUG35-*YlMHB1* was constructed by ligation of the PCR fragment containing the *YlMHB1* gene without STOP (amplification primers YlMHB1_UP and YlMHB1_DWN_noSTOP) into pUG35 (3) (provided by Johannes H. Hegemann, Heinrich-Heine-Universität, Düsseldorf, Germany) linearized with *Sma*I. To demonstrate that *Yl*Mhb1p is able to replace the Abf2p function(s) in *S. cerevisiae* cells, plasmids pYES2/CT-*YlMHB1,* pYES2/CT-*ScABF2* and pYES2/CT-*DhmtHMG1* were constructed as follows. Plasmid pDrive-*YlMHB1* was digested with *Eco*RI and the fragment containing *YlMHB1* ORF was cloned downstream of the *GAL1* promoter into the plasmid pYES2/CT (Life Technologies) linearized with *Eco*RI. The plasmid pYES2/CT-*ScABF2* was constructed by insertion of *ScABF2*, PCR amplified from the pDrive-*ScABF2* using primers ScABF2_up and ScABF2_dn into pYES2/CT linearized with *Hind*III and blunt-ended with Klenow fragment of DNA polymerase I. Plasmid pDrive-*DhmtHMG1* was digested with *Eco*RI and the fragment containing *DhmtHMG1* ORF was cloned into the plasmid pYES2/CT linearized with *Eco*RI. All plasmid constructs were verified by restriction enzyme mapping and DNA sequencing of inserted fragments.

DNA-cellulose chromatography of YlMhb1p– Double-stranded DNA cellulose (150 µl) was packed into 1 ml column, washed three times with TAN buffer (0.25 M sucrose, 20 mM Tris-HCl pH 7.5, 0.5 mM EDTA) containing 1 M NaCl, and then washed with TAN buffer containing 75 mM NaCl. Proteins purified by glutathione-agarose were diluted to reduce salt concentration and loaded on the column. The column was first washed with TAN buffer containing 75 mM NaCl followed by elution of the bound proteins by a linear (0.075-1.0 M) gradient of NaCl.

Construction of the deletion mutant lacking YlMHB1 gene– Two-step PCR-synthesis of deletion cassette for *YlMHB1* gene was carried out based on a protocol described by Wach (4) (see also **Figure S3A**). In the first PCR, long flanking regions homologous to *Y. lipolytica* genomic DNA were prepared using primer pairs 5'UP+5'DWN and 3´UP+3´DWN, which amplified the DNA region directly upstream or downstream of the targeted gene yielding FRAGMENT 1 (5´ region, 610 bp) and FRAGMENT 2 (3´ region, 603 bp). Primers 5´DWN and 3´UP were designed in such a way that 27 or 26 bases long extensions homologous to the 5´ or 3´side of the *YlURA3* gene were added to the 3´ or 5´ end of the FRAGMENT 1 or FRAGMENT 2. 100 ng of genomic DNA of the strain *Y. lipolytica* E129 was used as a template for PCR, the final concentration of each primer was 1 μM in 20 μl reaction mix supplemented with 200 μM of each dNTPs and 1 U of Phusion Hot start II DNA polymerase (Thermo Scientific). PCR was performed under following conditions: 35 cycles of 15 sec at 98°C and 50 sec at 72°C. PCR products were extracted from agarose gels. In the second PCR, four different primers were used: FRAGMENT 1, FRAGMENT 2, 5´UP and 3´DWN. 100 ng of the vector pKSURA (provided by Mathias L. Richard (Institut National de la Recherche Agronomique, Thiverval-Grignon, France)) carrying the marker gene *YlURA3* was used as a template in the PCR reaction. The final concentration of primers 5´UP and 3´DWN was 1 μM and the final concentration of FRAGMENTS 1 and 2 was 4 ng/μl in a 40 μl reaction mix supplemented with 200 μM of each dNTPs, 1.5 mM $MgCl_2$ and 1.5 U of *Taq* DNA polymerase (Life Technologies). PCR was performed under following conditions: 37 cycles of 45 sec at 95°C, 40 sec at 60°C and 150 sec at 72°C. PCR product was extracted from the agarose gel, and then inserted into the pDrive vector using a PCR Cloning Kit (Qiagen). The resulting plasmid pDrive-*YlMHB1::URA3* was used as a template for the amplification of the deletion cassette with primers 5´UP and 3´DWN. The deletion cassette was introduced into the haploid *Y. lipolytica* Po1h strain or Ku-deficient H222-SW2-1 strain and homologous and nonhomologous integrations of the deletion cassette were determined by PCR using the 5'VER, 5´URA3_R, 3´URA3_F and 3'VER primers. The deletion of *YlMHB1* gene in Po1h strain was also verified by Southern blot and Northern blot analysis with *YlMHB1* probe amplified by PCR from pDrive-*YlMHB1* using primers YlMHB1_UP and YlMHB1_DWN.

Fluorescent microscopy– S. cerevisiae strain GG595 was transformed with pUG35-*YlMHB1* or pUG35. Cells were cultivated in synthetic glucose medium for 20 hours at 28°C. The cells were centrifuged at 3,000 *g* for 5 min, and washed with 10 mM Tris-HCl pH 7.5. DAPI was added to a final concentration of 1 μg/ml and the cell suspension was incubated for 30 min at room temperature. The cells were observed with a microscope Olympus BX50 equipped with a DP70 camera (Olympus Optical Co.) and an appropriate filter set.

Functional complementation of the ∆abf2 mutation in S. cerevisiae by YlMHB1- Vectors pYES2/CT, pYES2/CT-*ScABF2*, pYES/2CT-*YlMHB1* and pYES2/CT-*DhmtHMG1* were introduced into *S. cerevisiae* strains RG∆ade2-1C (*ABF2*) and CS611-5A (∆*abf2*) and cells were plated on synthetic glycerol medium supplemented with appropriate amino acids. Cells from individual clones were inoculated into 2 ml SG medium and cultivated for 24 hours at 28°C. Synthetic media containing glucose (SD), glycerol (SG) or galactose (SGal) were inoculated from the pre-culture at a concentration of $1x10^5$ cells/ml. Cell suspensions were cultivated for 24 hours at 28°C, and 300 cells per plate were spread on complex glucose

and glycerol medium (YPDG) followed by cultivation for 3 days at 28°C. Frequency of standard (*grande*) and respiratory-deficient (*petite*) colonies was determined by triphenyltetrazolium chloride (TTC) assay (5). Experiments were performed on four biological replicates.

Oxygen consumption measurements- The respiration of yeast cells was measured in Oxygen meter model 782 (Strathkelvin Instruments) with a Clark type electrode in a 0.5 ml chamber with 40 mM potassium phosphate buffer pH 7.4 at 28°C and data were analyzed using Oxygen system 782 software. The initial concentration of dissolved oxygen in the buffer was assigned to be 500 µM. *Y. lipolytica* cells were grown to mid-exponential phase ($5x10^7$ cells/ml), harvested by centrifugation and washed with ice-cold MilliQ water and 40 mM potassium phosphate buffer (pH 7.4). Pellet was resuspended in potassium phosphate buffer (pH 7.4; 200 µl of buffer per 1.05 g of wet weight cells) and 3 µl of cell suspension was added to the chamber. Consumption of molecular oxygen per 1 min was calculated per 1 mg of dried cell mass. 25 mM glucose was used as a carbon source and respiratory chain inhibitor (9 µg/ml antimycin A) or uncoupler of oxidative phosphorylation (20 µM carbonyl cyanide 3-chlorophenylhydrazone (CCCP)) were added as indicated in **Figure S5A**. Experiments were performed on three biological replicates for each strain and were analyzed in triplicates.

Pulsed-field gel electrophoresis (PFGE)- PFGE was performed as described in the main text except that the probe *YlATP6* was labeled by biotin using Biotin DecaLabel DNA Labeling Kit (Thermo Scientific) and the signal was detected using Phototope® -Star Detection Kit (New England Biolabs) and Image Station 4000MM (Kodak).

Supplementary references

1. **Gietz RD, Schiestl RH, Willems AR, Woods RA.** 1995. Studies on the transformation of intact yeast cells by the LiAc/SS-DNA/PEG procedure. Yeast **11:**355-360.
2. **Kretzschmar A, Otto C, Holz M, Werner S, Hübner L, Barth G.** 2013. Increased homologous integration frequency in *Yarrowia lipolytica* strains defective in non-homologous end-joining. Curr. Genet. **59:**63-72.
3. **Niedenthal RK, Riles L, Johnston M, Hegemann JH.** 1996. Green fluorescent protein as a marker for gene expression and subcellular localization in budding yeast. Yeast **12:**773-786.
4. **Wach A.** 1996. PCR-synthesis of marker cassettes with long flanking homology regions for gene disruptions in *S. cerevisiae*. Yeast **12:**259-265.
5. **Ogur M, John RS, Nagai S.** 1957. Tetrazolium overlay technique for population studies of respiration deficiency in yeast. Science **125:**928-929.

Supplementary Figure legends

Figure S1. *Yl*Mhb1p is targeted to mitochondria and complements Δ*abf2* mutation in *S. cerevisiae*. (A) *YlMHB1* was cloned in fusion with GFP into *S. cerevisiae* vector pUG35. The transformants were stained with DAPI and inspected by bright-field and fluorescent microscopy. (B) Complementation of Δ*abf2* mutant of *S. cerevisiae* by *ScABF2*, *YlMHB1* and *DhmtHMG1* genes encoding mitochondrial HMG-box containing proteins in *S. cerevisiae*, *Y. lipolytica* and *D. hansenii*, respectively. See **Table S2** for more details.

Figure S2. Purification of wild-type and truncated mutants of *Yl*Mhb1p from *E. coli*. (A) *YlMHB1* was cloned in fusion with glutathione-*S*-transferase under IPTG-inducible promoter. After affinity chromatography on glutathione-agarose, the native *Yl*Mhb1p was obtained by cleavage of the fusion protein with *PreScission* protease. (B) In addition to *Yl*Mhb1p-WT, recombinant versions of *Yl*Mhb1p containing either N-terminal (*Yl*Mhb1p-N-term) or C-terminal (*Yl*Mhb1p-C-term) parts of the protein were produced and purified from *E. coli* as described in (A). (C) Recombinant proteins were loaded on DNA-cellulose and eluted with increasing concentrations of NaCl. *Yl*Mhb1p-C-term did not bind to the beads (data not shown).

Figure S3. Construction of the deletion mutant lacking *YlMHB1* gene. (A) Deletion cassette was prepared by two-step PCR according to the experimental protocol described by Wach (4). The scheme depicts positions of oligonucleotide primers used for preparation of a deletion cassette and for verification of the Δ*mhb1* deletion mutant. The numbers above the lines indicate lengths of the corresponding PCR fragments. Red lines represent sequences derived from the *YlURA3* locus. (B) The correct integration of the deletion cassette into the *YlMHB1* locus of the Po1h strain was verified by four different PCR reactions employing various combination of primers as indicated by the scheme. NR, nonhomologous recombinant, in which the deletion cassette was integrated into the genomic DNA while leaving the *YlMHB1* locus intact. (C) Restriction map of the *YlMHB1* locus indicating a position of recognition sites for restriction endonucleases *Kpn*I and *Mse*I. (D) Southern blot analysis of genomic DNA digested with either *Kpn*I or *Mse*I and hybridized with a PCR fragment corresponding to the entire *YlMHB1* ORF. NR, nonhomologous recombinant as in (B). (E) Northern blot analysis of a total RNA isolated from the wild-type Po1h and Δ*mhb1* mutant; NB, Northern blot.

Figure S4. Wild-type Po1h and Δ*mhb1* cells exhibit similar growth characteristics. The strains were grown at 28°C for 1 day on solid complex media or 2 days on synthetic media containing either 2% (w/v) glucose, 3% (v/v) glycerol, or 3% (v/v) ethanol.

Figure S5. Mutant cells lacking *Yl*Mhb1p exhibit changes neither in respiratory capacity, nor in protein composition of mt-nucleoids. (A) Respiration was assayed in whole cells in without or with 25 mM glucose in the absence or presence of antimycin A (9 μg/ml) or CCCP (20 μM) as described in Supplementary Materials and methods. (B) Protein composition of purified mt-nucleoids was assayed by 12% SDS-PAGE stained with silver. *, position of *Yl*Mhb1p. The fact that there is no complete loss of a 30 kDa band in the Δ*mhb1*

strain indicates that the band is composed of additional protein(s) co-migrating with *Yl*Mhb1p. WT=Po1h strain.

Figure S6. Independent deletion mutants lacking functional *YlMHB1* gene exhibit the same phenotypes as the original Δ*mhb1* strain. (A) *Y. lipolytica* strain H222-SW2-1 was transformed by the deletion cassette and its integration into the *YlMHB1* locus in three independent strains (Δ*mhb1-1*, Δ*mhb1-2* & Δ*mhb1-3*) was verified using PCR as described in Fig. S3B. Po1h (WT & Δ*mhb1*) are strains used throughout this study and here they serve as controls. (B) Mitochondrial nucleoids were visualized by DAPI as described in Fig. 3. (C) PFGE analysis of mtDNA was performed as in Fig. 3 except that the Southern blot was hybridized with biotin-labeled *YlATP6* probe. (D) Sensitivity of H222-SW2-1 wild-type and of the deletion mutants to EtBr was tested as described in Fig. 4.

Table S1.
List of oligonucleotides

Name	sequence 5´→3´	Application
YlMHB1_UP	ATGAAATTCGCCCGACCCTT	1, 5
YlMHB1_DWN	TTGCAGCATCCTTATTTACGTCC	1, 5
YlMHB1_WT_UP	ATGGATCCGTGAAATTCGCCCGACCCTTCTCT	1
YlMHB1_WT_DWN	CGGAATTCGCAGCATCCTTATTTACGTCCGCC	1
YlMHB1_NT_UP	ATACTAGGATCCAAGGAGGCTGCCACTAAGACCAAG	1
YlMHB1_NT_DWN	GCGCCGGAATTCTCTGATGGTCTTGTAGTCTGACTT	1
YlMHB1_CT_UP	ATATTAGGATCCTACCCCAAGCTGTCCGGTCTCAAC	1
YlMHB1_CT_DWN	CGGGCGGAATTCTTATTTACGTCCGCCAATAGCAAG	1
YlMHB1_DWN_noSTOP	TTTACGTCCGCCAATAGCAAGG	1
YlMHB1_UP1141	ATTGTCGACTGGGTTTGAAGACTCTCA	1
YlMHB1_DN423	GGCGTCGACTGATAATGATGGTATTGATG	1
5´UP	CTGCTCCAGTGTGCTCTTTCTTTACCA	2
5´DWN	GTGAGTCGTATTACAATTCACTGGCCGTTAACATGGGAAAGAATGAAGATGGTAGAA	2
3´UP	CCCTTTAGTGAGGGTTAATTCCGAGCGTACTGTAGCGCGAGTGGGGACAT	2
3´DWN	GAGCACCAGGATTACAACAAGATGAACC	2
5´VER	CAGTCCCACCAGCATTTCAA	3
5´URA3_R	TAGACTGGACTATACGGCTATCGG	3
3´URA3_F	TCTCGCTAGGGATAACAGGGTAA	3
3´VER	TGAGTCGTCTAGCAAGGATATTGAC	3
YlATP9_F_RT	TAATGGTATGACGCGTGGTTTGGG	4
YlATP9_R_RT	ATCCGATTGAAGCTAATCCAGCTCCG	4
YlACT1_F_RT	ACGTTGTGCCCATCTACTCTGGTT	4, 5
YlACT1_R_RT	TCGGCGGAGTTGGTGAAAGAGTAA	4, 5
YlGPD_F_RT	TTACGAGGACATCAAGGCCACCAT	4
YlGPD_R_RT	AATACCAGCCTTGGCGTCGAAGAT	4
Yl23SrRNA_F	GTGTGAACTCTGCTCAATGCTTAGA	5
Yl23SrRNA_R	TATAACCTGCTCGAAAGAGCCG	5
YlNUBM_F	GAGACTGGTTTGCCTCCTTTG	5
YlNUBM_R	GCTTGTTGATGACAATGACAGC	5
YlNUCM_F	CTGCTGACCGATAACCGAATTT	5
YlNUCM_R	GAGGTTTCTAGGAGGCGAGTTA	5
YlNESM_F	GAGTTGGCATTCTCGATATTCTG	5
YlNESM_R	TCTCGGGAGCATCGTTAATCT	5
YlATP6_F	ATCATCAACTCACCATTAGAACA	5
YlATP6_R	ATTAGTGTAAGTGGATAGCATCTTT	5
ScABF2_up	ATGAACAGTTACAGCCTATTA	6
ScABF2_dn	CTAGTTGAGAGGGTAGCGA	6

1 PCR amplification of *YlMHB1* gene for cloning into vectors pDrive, pGEX-6P-1, pGEX-6P-2, pUG35 or pUB4, *2* preparation of the deletion cassette of *YlMHB1* gene, *3* PCR verification of the correct deletion cassette integration, *4* qRT-PCR, *5* PCR amplification to prepare probe for Southern blot or Northern blot hybridization, *6* PCR amplification of *ScABF2* gene for cloning into pYES2/CT vector.

Table S2.
Complementation of Δ*abf2* mutant of *S. cerevisiae* by various yeast mitochondrial HMG-box containing proteins

Strain	Medium	Gene carried by pYES2/CT	% of *grande* colonies ± s.d.
ABF2	SD	-	96.5 ± 1.1
ABF2	SG	-	100 ± 0
ABF2	SGal	-	99.8 ± 0.2
ABF2	SD	*ScABF2*	89.3 ± 3.8
ABF2	SG	*ScABF2*	96.2 ± 1.0
ABF2	SGal	*ScABF2*	31.0 ± 7.9
ABF2	SD	*YlMHB1*	94.9 ± 1.3
ABF2	SG	*YlMHB1*	100 ± 0
ABF2	SGal	*YlMHB1*	99.9 ± 0.2
ABF2	SD	*DhmtHMG1*	96.2 ± 0.7
ABF2	SG	*DhmtHMG1*	99.8 ± 0
ABF2	SGal	*DhmtHMG1*	62.3 ± 4.6
Δ*abf2*	SD	-	0.4 ± 0.4
Δ*abf2*	SG	-	64.0 ± 17.7
Δ*abf2*	SGal	-	16.2 ± 10.4
Δ*abf2*	SD	*ScABF2*	22.9 ± 2.7
Δ*abf2*	SG	*ScABF2*	96.0 ± 1.1
Δ*abf2*	SGal	*ScABF2*	18.3 ± 3.4
Δ*abf2*	SD	*YlMHB1*	3.3 ± 2.1
Δ*abf2*	SG	*YlMHB1*	89.9 ± 7.7
Δ*abf2*	SGal	*YlMHB1*	91.2 ± 5.1
Δ*abf2*	SD	*DhmtHMG1*	10.9 ± 1.6
Δ*abf2*	SG	*DhmtHMG1*	99.5 ± 0.4
Δ*abf2*	SGal	*DhmtHMG1*	29.7 ± 3.8

Table S3.
Conditions used to compare growth of the wild-type and Δ*mhb1* mutant strain

		Growth	
		WT	Δ*mhb1*
YP +	2% glucose	+	+
	3% glycerol	+	+
	3% ethanol	+	+
	2% mannose	+	+
	0.4% potassium acetate	+	+
	0.1% citric acid	+	+
	0.1% acetic acid	+	+
Synthetic (S) +	2% glucose	+	+
	3% glycerol	+	+
	3% ethanol	+/-	+/-
	0.4% sodium acetate	+	+
	0.1% oleic acid (± 0.02-0.2% Tween-80)	+	+
	1% oleic acid (± 0.02-0.2% Tween-80)	+	+
	2% decane (± 0.02-0.2% Tween-80)	+	+
	2% undecane (± 0.02-0.2% Tween-80)	+/-	+/-
YP +2% glucose +	<300 μg/ml hygromycin B	+	+
	500 μg/ml hygromycin B	-	-
YP + 3% glycerol +	5 mg/ml erythromycin	+	+
	5 mg/ml chloramphenicol	+	+
	10 μg/ml antimycin A	+/-	+/-
	10 μg/ml oligomycin	-	-
	1 μg/ml mucidin	+/-	+/-
	10 μg/ml mucidin	-	-
S + 2% glucose +	200-430 μM nalidixate	+	+
	2 μM EtBr	+	+
	10 μM EtBr*	+/-	-
	30-40 mM H_2O_2, treatment in liquid medium	+/-	+/-

*The only condition where the wild-type and Δ*mhb1* mutant exhibited different properties. In 5 μM EtBr, the growth of WT Po1h was slightly inhibited, whereas the Δ*mhb1* strain exhibited dramatically retarded growth (see Fig. 4 for more details).

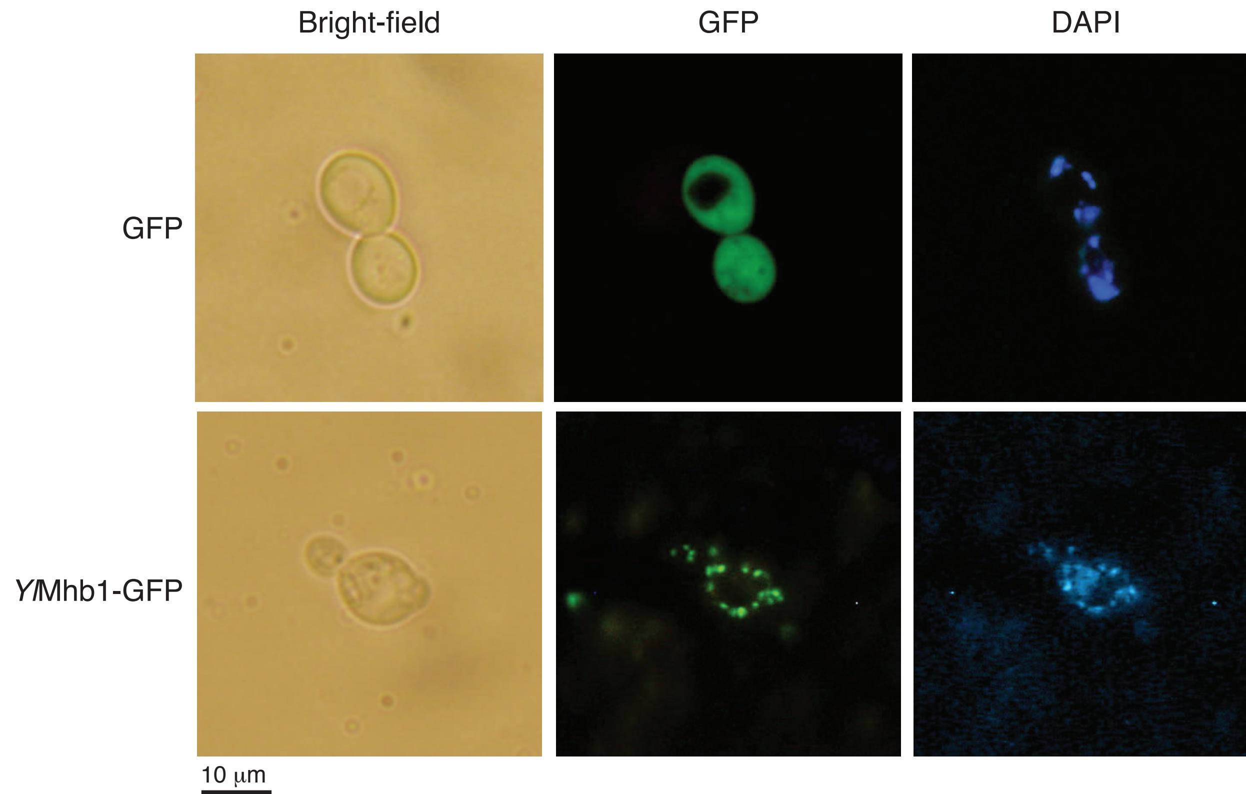

Figure S1A. *Yl*Mhb1p is targeted to mitochondria in *S. cerevisiae*. (A) *YlMHB1* was cloned in fusion with GFP into *S. cerevisiae* vector pUG35. The transformants were stained with DAPI and inspected by bright-field and fluorescent microscopy.

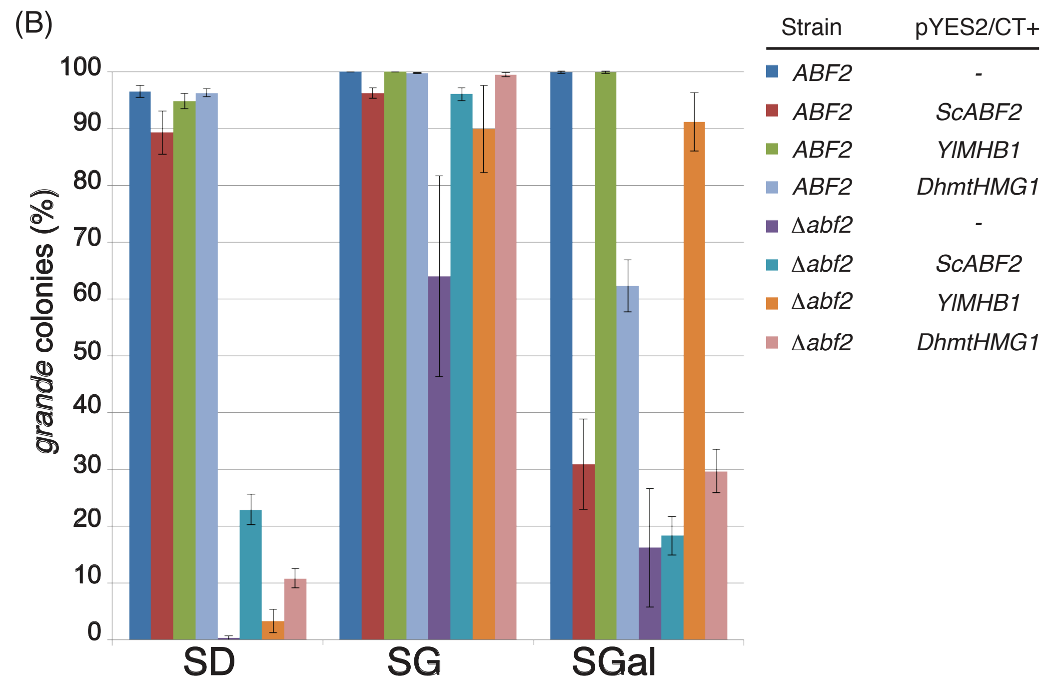

Figure S1B. *Yl*Mhb1p complements Δ*abf2* mutation in *S. cerevisiae*. (B) Complementation of Δ*abf2* mutant of *S. cerevisiae* by *ScABF2*, *YlMHB1* and *DhmtHMG1* genes encoding mitochondrial HMG-box containing proteins in *S. cerevisiae*, *Y. lipolytica* and *D. hanseni*, respectively. See Table S2 for more details.

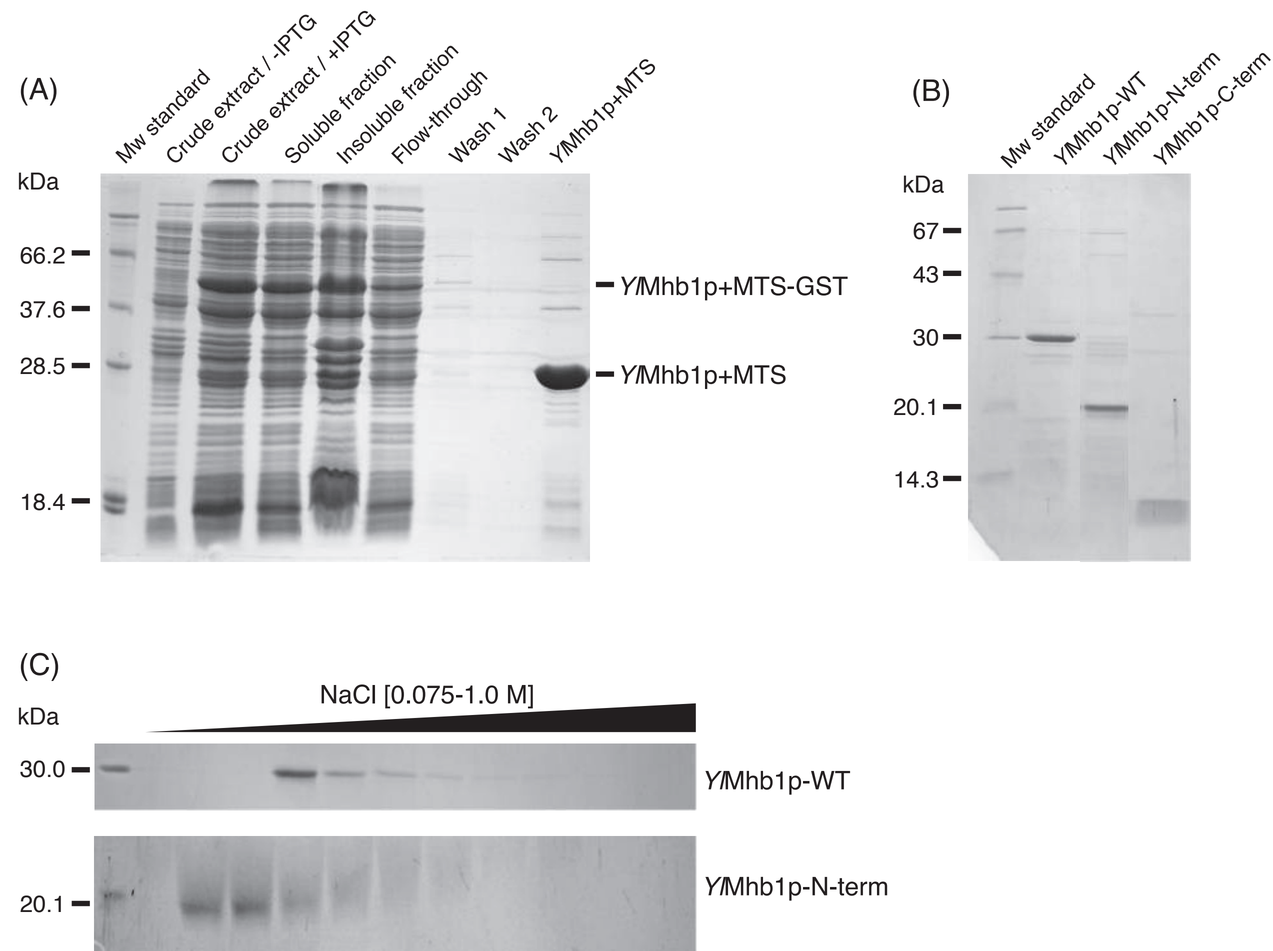

Figure S2. Purification of wild-type and truncated mutants of *Yl*Mhb1p from *E. coli*. (A) *YlMHB1* was cloned in fusion with glutathione-*S*-transferase under IPTG-inducible promoter. After affinity chromatography on glutathione-agarose, the native *Yl*Mhb1p was obtained by cleavage of the fusion protein with *PreScission* protease. (B) In addition to *Yl*Mhb1p-WT, recombinant versions of *Yl*Mhb1p containing either N-terminal (*Yl*Mhb1p-N-term) or C-terminal (*Yl*Mhb1p-C-term) parts of the protein were produced and purified from *E. coli* as described in (A). (C) Recombinant proteins were loaded on DNA-cellulose and eluted with increasing concentrations of NaCl. *Yl*Mhb1p-C-term did not bind to the beads (data not shown).

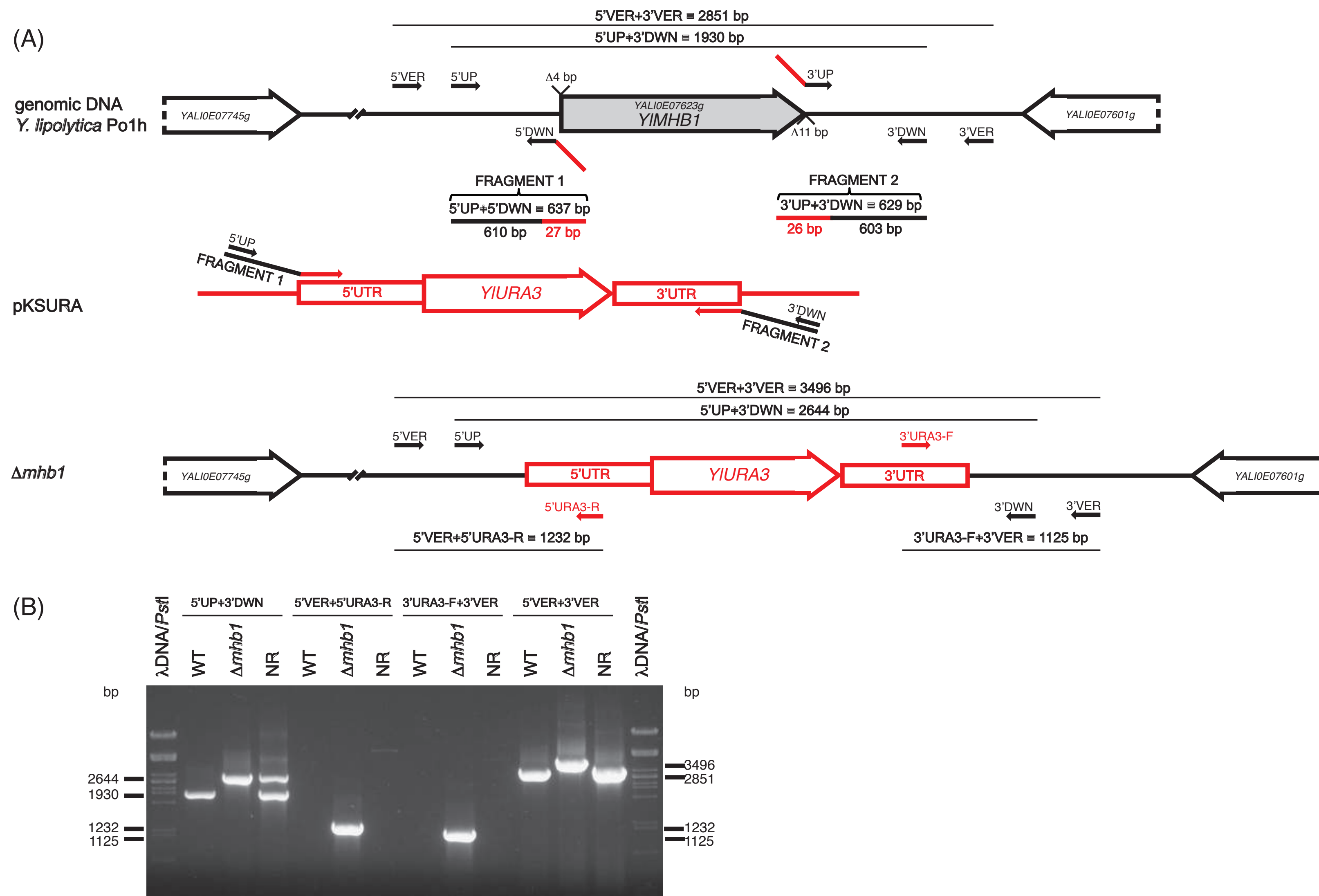

Figure S3A-B. Construction of the deletion mutant lacking *YlMHB1* gene. (A) Deletion cassette was prepared by two-step PCR according to the experimental protocol described by Wach (4). The scheme depicts positions of oligonucleotide primers used for preparation of a deletion cassette and for verification of the Δ*mhb1* deletion mutant. The numbers above the lines indicate lengths of the corresponding PCR fragments. Red lines represent sequences derived from the *YlURA3* locus. (B) The correct integration of the deletion cassette into the *YlMHB1* locus of the Po1h strain was verified by four different PCR reactions employing various combination of primers as indicated by the scheme. NR, nonhomologous recombinant, in which the deletion cassette was integrated into the genomic DNA while leaving the *YlMHB1* locus intact.

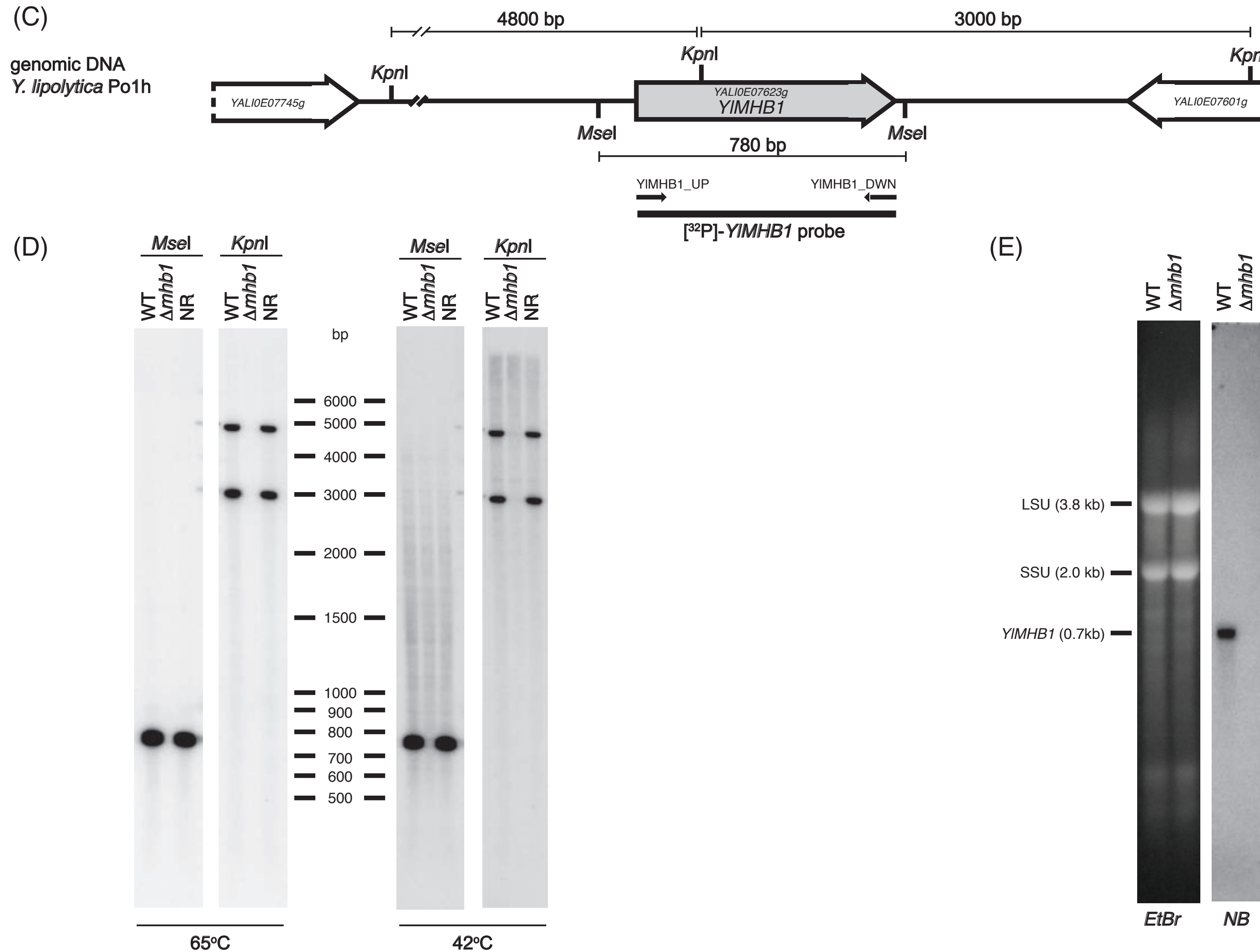

Figure S3C-E. Construction of the deletion mutant lacking *YlMHB1* gene. (C) Restriction map of the *YlMHB1* locus indicating a position of recognition sites for restriction endonucleases *Kpn*I and *Mse*I. (D) Southern blot analysis of genomic DNA digested with either *Kpn*I or *Mse*I and hybridized with a PCR fragment corresponding to the entire *YlMHB1* ORF. NR, nonhomologous recombinant as in (B). (E) Northern blot analysis of a total RNA isolated from the wild-type Po1h and Δ*mhb1* mutant; NB, Northern blot.

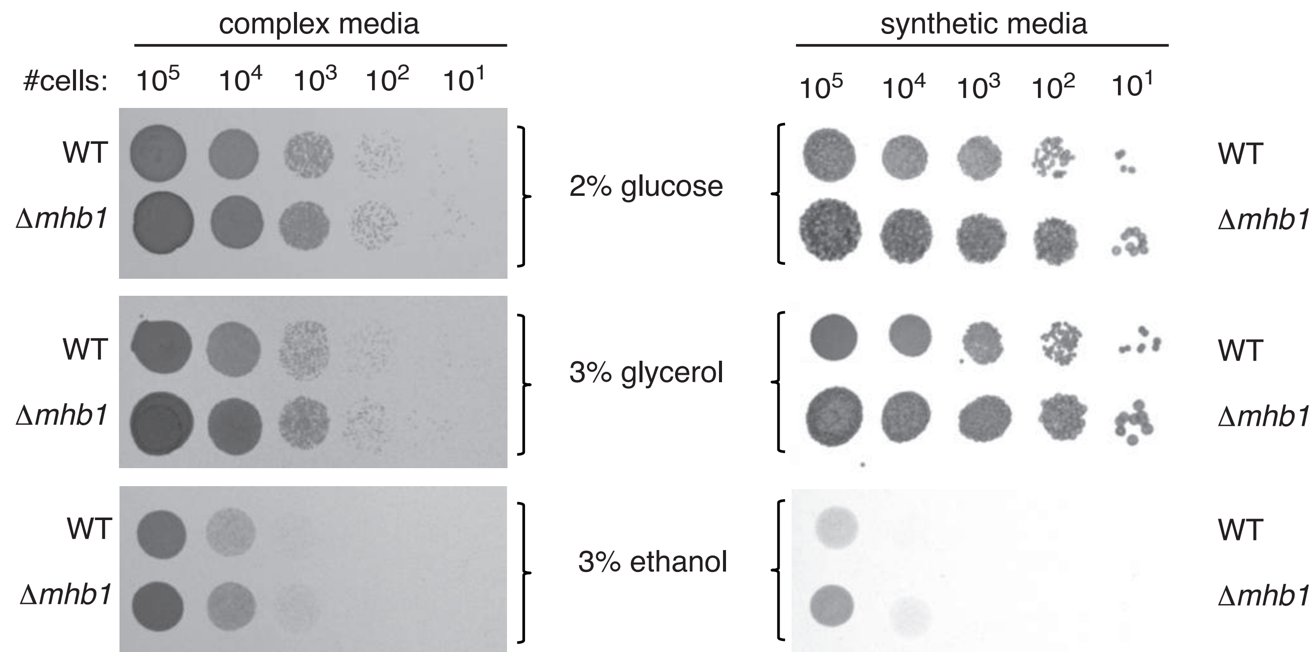

Figure S4. Wild-type Po1h and Δ*mhb1* cells exhibit similar growth characteristics. The strains were grown at 28ºC for 1 day on solid complex or for 2 days on synthetic media containing either 2% (w/v) glucose, 3% (v/v) glycerol, or 3% (v/v) ethanol.

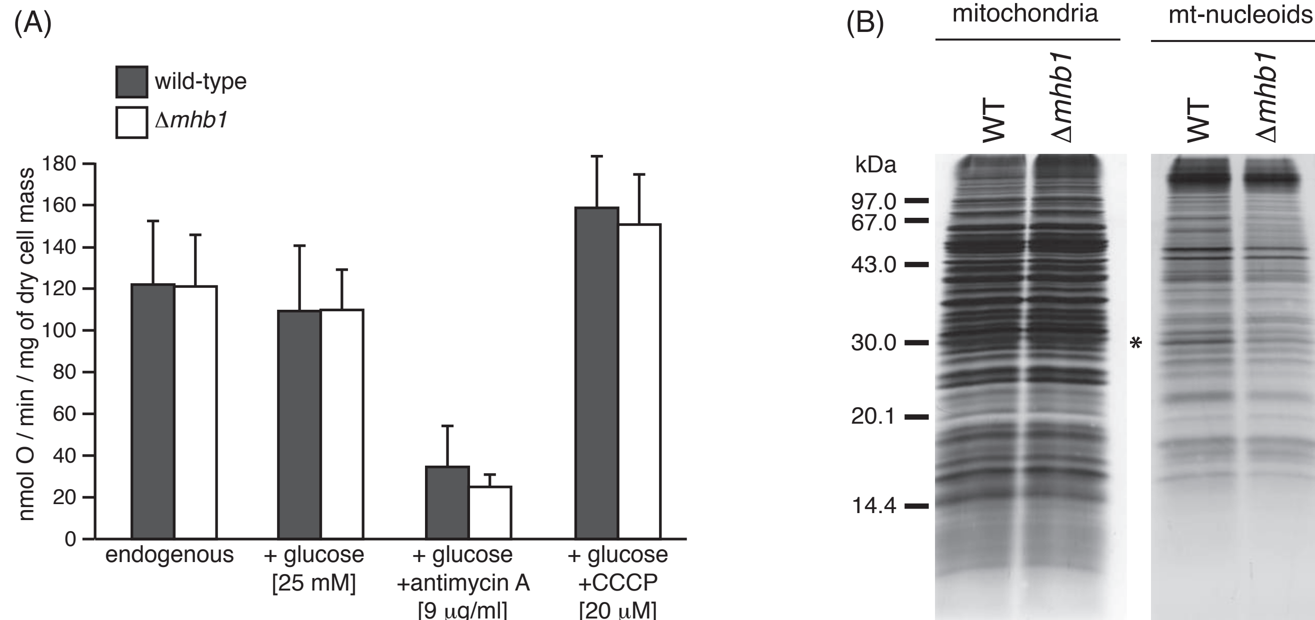

Figure S5. Mutant cells lacking *Yl*Mhb1p exhibit changes neither in respiratory capacity, nor in protein composition of mt-nucleoids. (A) Respiration was assayed in whole cells in without or with 25 mM glucose in the absence or presence of antimycin A (9 µg/ml) or CCCP (20 µM) as described in Supplementary Materials and methods. (B) Protein composition of purified mt-nucleoids was assayed by 12% SDS-PAGE stained with silver. *, position of *Yl*Mhb1p. The fact that there is no complete loss of a 30 kDa band in the Δ*mhb1* strain indicates that the band is composed of additional protein(s) co-migrating with *Yl*Mhb1p. WT=Po1h strain.

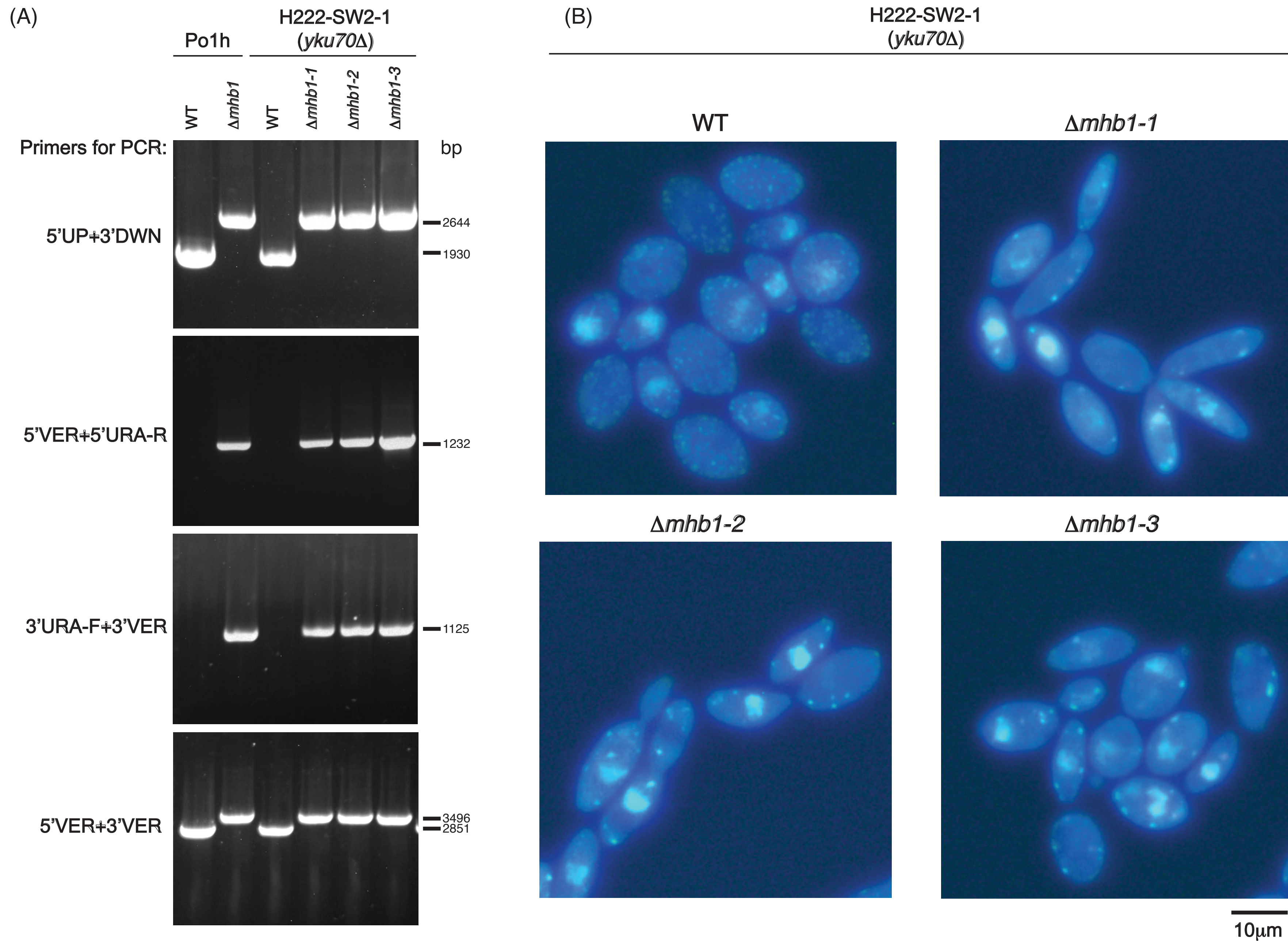

Figure S6A-B. Independent deletion mutants lacking functional *YlMHB1* gene exhibit the same phenotypes as the original Δ*mhb1* strain. (A) *Y. lipolytica* strain H222-SW2-1 was transformed by the deletion cassette and its integration into the *YlMHB1* locus in three independent strains (Δ*mhb1-1*, Δ*mhb1-2* & Δ*mhb1-3*) was verified using PCR as described in Fig. S3B. Po1h (WT & Δ*mhb1*) are strains used throughout this study and here they serve as controls. (B) Mitochondrial nucleoids were visualized by DAPI as described in Fig. 3.

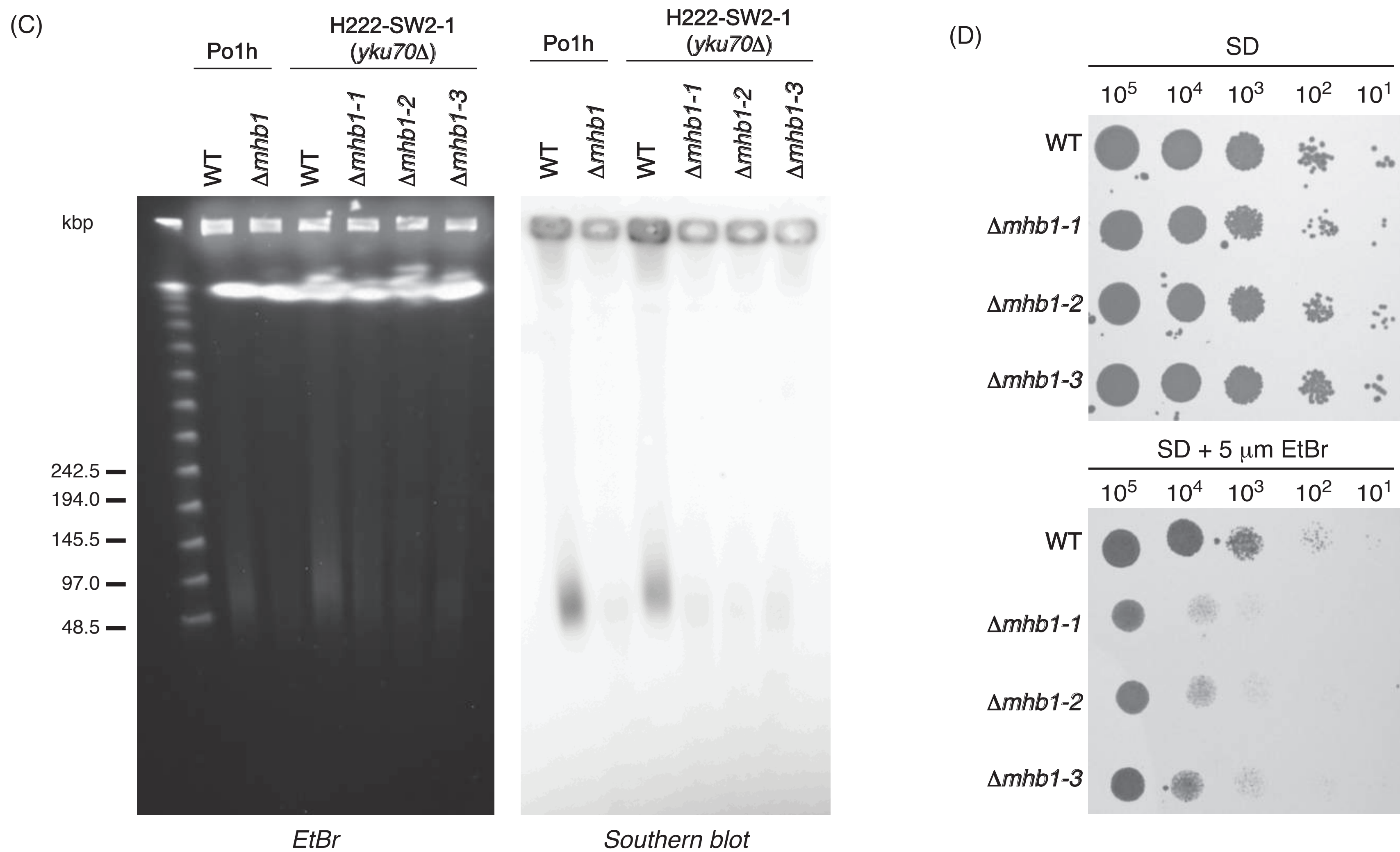

Figure S6C-D. Independent deletion mutants lacking functional *YlMHB1* gene exhibit the same phenotypes as the original Δ*mhb1* strain. (C) PFGE analysis of mtDNA was performed as in Fig. 3 except that the Southern blot was hybridized with biotin-labeled *YlATP6* probe. (D) Sensitivity of H222-SW2-1 wild-type and of the deletion mutants to EtBr was tested as described in Fig. 4.