

FIG. S1. HEMF production capacity of the *adh1Δ* mutant of various *S. cerevisiae* strains.

The *ADH1* gene-deletion in X2180 (provided by the American Type Culture Collection as ATCC26109) and Kyokai no. 7 (provided by the Brewing Society of Japan) were performed in the same way as that in BY4743 using primers listed in Table S1. The HEMF production capacities of the resulting deletion mutants were determined. The light gray box shows the result for each wild type strain; black box shows the result for each *adh1Δ* mutant. The bars indicate the standard deviations of four independent experiments.

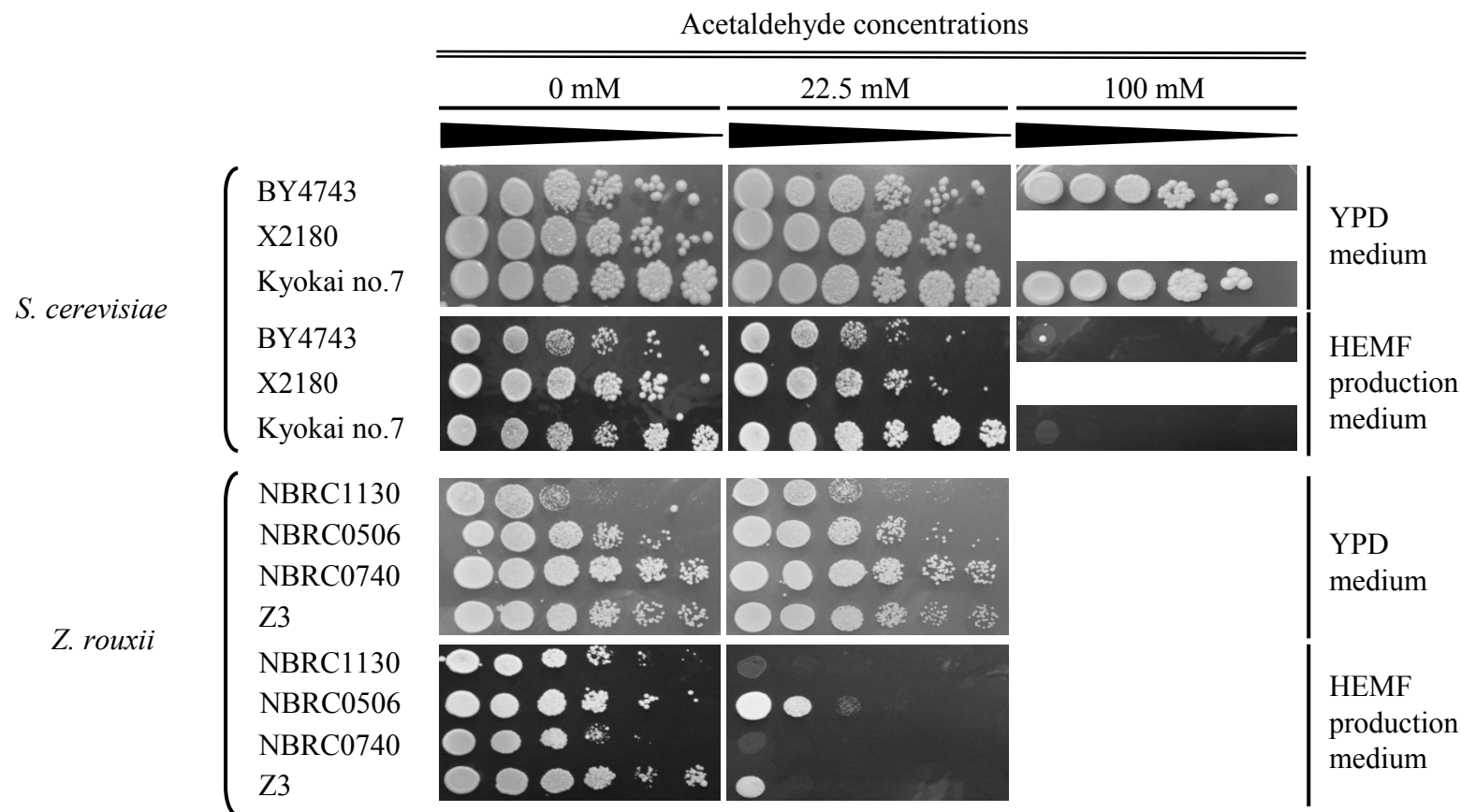


FIG. S2. The coexistence of acetaldehyde and HEMF production medium components influences the growth of the yeast cells.

The yeast cells were precultured in $2 \times$ YPD to stationary phase, and the cell cultures were diluted up to an OD600 of 1.0. Equal amount of cells were spotted in a 1:10 dilution series on a YPD or HEMF production medium plate [2% (w/v) agar] with or without an acetaldehyde coating (22.5 or 100 mM) for *S. cerevisiae* (upper panels) and *Z. rouxii* (lower panels). The spotted cells were grown at 30° C for 3-4 days and photographed. *Z. rouxii* NBRC1130, 0506, and 0740 were provided by the Biological Resource Center, NITE (Japan), and *Z. rouxii* Z3 was isolated from the flor of yeasts grown on the surface of soy sauce mash (1).

1. Watanabe J, Uehara K, Mogi Y, Suzuki K, Watanabe T, Yamazaki T. 2010. Improved transformation of the halo-tolerant yeast *Zygosaccharomyces rouxii* by electroporation. Biosci. Biotechnol. Biochem. 74:1092-1094.

TABLE S1 Primers used in this study

Primer name	Sequence	Use (corresponding figure)
pADH1_F	5'-GCGGCCGCAAGGTGAGACGCGCATAACC-3'	Construction of pADH1 vector (Fig. 3)
pADH1_R	5'-GCGGCCGCAATTGGGTGAAATGGGGAGC-3'	Construction of pADH1 vector (Fig. 3)
pYC150conf_F	5'-CCACACACGAAAATCCTGTG-3'	Confirmation of pADH1 transformation (Fig. 3)
pYC150conf_R	5'-TGGTAACCGTATTACCGCC-3'	Confirmation of pADH1 transformation (Fig. 3)
ADH1Δ_F	5'-GCACAATATTTCAAGCTATACCAAGCATACAATCAACTATCTCATATACA AGCTGAAGCTTCGTACGC-3'	Construction of <i>ADH1</i> , <i>ADH2</i> , <i>ADH3</i> triple gene-deletion mutant (Fig. 6)
ADH1Δ_R	5'-AGCAACCTGACCTACAGGAAAGAGTTACTCAAGAATAAGAATTTTCGTTT TATAGGGAGACCGGCAGATC-3'	Construction of <i>ADH1</i> , <i>ADH2</i> , <i>ADH3</i> triple gene-deletion mutant (Fig. 6)
ADH2Δ_F	5'-ACAAAAAGCATACAATCAACTATCAACTATTAAGTATATCGTAATACACA AGCTGAAGCTTCGTACGC-3'	Construction of <i>ADH1</i> , <i>ADH2</i> , <i>ADH3</i> triple gene-deletion mutant (Fig. 6)
ADH2Δ_R	5'-GGCATACTTGATAATGAAAATATAAATCGTAAAGACATAAGAGATCCGC TATAGGGAGACCGGCAGATC-3'	Construction of <i>ADH1</i> , <i>ADH2</i> , <i>ADH3</i> triple gene-deletion mutant (Fig. 6)
ADH3Δ_F	5'-TCTGTTACAGTTAAACTAGGAATAGTATAGTCATAAGTTAACACCATC AGCTGAAGCTTCGTACGC-3'	Construction of <i>ADH1</i> , <i>ADH2</i> , <i>ADH3</i> triple gene-deletion mutant (Fig. 6)
ADH3Δ_R	5'-ATCATTATAAACAAAGACTTTTCATAAAAAGTTTGGGTGCGTAACACGCTA TATAGGGAGACCGGCAGATC-3'	Construction of <i>ADH1</i> , <i>ADH2</i> , <i>ADH3</i> triple gene-deletion mutant (Fig. 6)
ADH1Δ conf_F	5'-AAGGTGAGACGCGCATAACC-3'	Confirmation of <i>ADH1</i> gene deletion (Fig. 6)
ADH1Δ conf_R	5'-AATTGGGTGAAATGGGGAGC-3'	Confirmation of <i>ADH1</i> gene deletion (Fig. 6)
ADH2Δ conf_F	5'-ACACTCGAAATACTCTTACTACTGCTC-3'	Confirmation of <i>ADH2</i> gene deletion (Fig. 6)
ADH2Δ conf_R	5'-CGTGAACTTCGAACACTGTCATC-3'	Confirmation of <i>ADH2</i> gene deletion (Fig. 6)
ADH3Δ conf_F	5'-GTTTCTGCGTCCGTACACTGTC-3'	Confirmation of <i>ADH3</i> gene deletion (Fig. 6)
ADH3Δ conf_R	5'-GGCTCGATGCTTGATGGTGATAATG-3'	Confirmation of <i>ADH3</i> gene deletion (Fig. 6)
ZrADH1Δ_F	5'-TATACACCCTTTAGTACCGGCAGATGAGCTCAAAATGAGTGCAAGCCCTTTTGTAT ATAAGAGGGCAATGATGTCTTGAGTGTGAAAATACGATTGGTTCGTACGCTGCAG GTCG-3'	Construction of <i>ZrADH1</i> single gene-deletion mutant, and <i>ZrADH1</i> , <i>ZrADH2</i> double gene-deletion mutant (Fig. 7)
ZrADH1Δ_R	5'-TATAATCGTTATTATATCTATATTGGTTTACACTTTATATTAATAAACTTGCACTT CAGAAACAACTATCATGACTCATGAAATCCATTACCAATCCACTAGTGGATCTGAT ATCACC-3'	Construction of <i>ZrADH1</i> single gene-deletion mutant, and <i>ZrADH1</i> , <i>ZrADH2</i> double gene-deletion mutant (Fig. 7)
ZrADH2Δ_F	5'-GGAAAAGTGCCTTAGGCTCTTCCAATAGCGTCCTTCTAAACGGGCCAAGCGTCAGT TCAAGCCTTCAGTATTATCTCCGCTATTATATTAATAAAATTTTGCTAGTTTCGTACGCTG CAGGTCG-3'	Construction of <i>ZrADH2</i> single gene-deletion mutant, and <i>ZrADH1</i> , <i>ZrADH2</i> double gene-deletion mutant (Fig. 7)
ZrADH2Δ_R	5'-ATCGTATAGTTGAATGGGTTCATTACAATGCTTGTGGGAGTGGAAGATGGTGGTA TCAATGAGAGTGAGGATGTAGATGTAACAGATGTAACGAAAACGCACTAGTGGATC TGATATCACC-3'	Construction of <i>ZrADH2</i> single gene-deletion mutant, and <i>ZrADH1</i> , <i>ZrADH2</i> double gene-deletion mutant (Fig. 7)
ZrADH1Δ conf_F	5'-AGCTACTTGTCCACTGATTG-3'	Confirmation of <i>ZrADH1</i> gene deletion (Fig. 7)
ZrADH1Δ conf_R	5'-ACTAGTAGCTCGATTGAAAG-3'	Confirmation of <i>ZrADH1</i> gene deletion (Fig. 7)
ZrADH2Δ conf_F	5'-CGGATTCCGAAACTCTTGTC-3'	Confirmation of <i>ZrADH2</i> gene deletion (Fig. 7)
ZrADH2Δ conf_R	5'-TGTGATAGGATCGTTGGCG-3'	Confirmation of <i>ZrADH2</i> gene deletion (Fig. 7)
ZeoMX intra_R	5'-TTCCATAGGATGGTCAGTCC-3'	Confirmation of <i>ZrADH2</i> gene deletion (Fig. 7)

Underline indicates the annealing sequence to the plasmid.

TABLE 2 Summary of genes that increase the HEMF production capacities by deletion

ORF	Gene	growth (OD ₆₀₀)	HEMF yield ×10 ⁴ (μAU)	HEMF production capacity*	PR [2] [†]	Description
<i>Metabolism</i>						
YOL086C	<i>ADH1</i>	2.76 ± 0.01	19.17 ± 0.88	6.95 ± 0.36	10.7	Alcohol dehydrogenase required for the reduction of acetaldehyde to ethanol
YGR204W	<i>ADE3</i>	1.59 ± 0.03	6.52 ± 0.39	4.10 ± 0.06	6.3	Cytoplasmic trifunctional enzyme involved in amino acid and nucleotide biosynthesis
YAR015W	<i>ADE1</i>	0.96 ± 0.13	2.89 ± 0.15	3.03 ± 0.25	4.7	Rrequired for 'de novo' purine nucleotide biosynthesis
YGR061C	<i>ADE6</i>	1.34 ± 0.11	3.87 ± 0.40	2.90 ± 0.28	4.5	Rrequired for 'de novo' purine nucleotide biosynthesis
YMR300C	<i>ADE4</i>	6.36 ± 0.45	7.94 ± 0.24	1.25 ± 0.05	1.9	Rrequired for 'de novo' purine nucleotide biosynthesis
YDR408C	<i>ADE8</i>	5.67 ± 0.53	6.98 ± 0.19	1.24 ± 0.08	1.9	Rrequired for 'de novo' purine nucleotide biosynthesis
<i>Cellular transport, transport facilities and transport routes</i>						
YMR038C	<i>CCS1</i>	3.90 ± 0.02	10.6 ± 0.75	2.73 ± 0.18	4.2	Copper chaperone for superoxide dismutase Sod1p, involved in oxidative stress protection
YGL095C	<i>VPS45</i>	2.74 ± 0.16	5.69 ± 0.02	2.08 ± 0.11	3.2	Essential for vacuolar protein sorting
YNL297C	<i>MON2</i>	1.53 ± 0.08	2.19 ± 0.01	1.44 ± 0.08	2.2	Peripheral membrane protein with a role in endocytosis and vacuole integrity
YPR139C	<i>LOA1</i>	5.77 ± 0.52	7.64 ± 0.74	1.32 ± 0.01	2.0	Lysophosphatidic acid acyltransferase involved in triacylglyceride homeostasis
<i>Transcription</i>						
YDR138W	<i>HPR1</i>	3.56 ± 0.03	5.97 ± 0.50	1.68 ± 0.13	2.6	Subunit of THO/TREX complexes that couple transcription elongation with mitotic recombination and with mRNA metabolism and export
YDR392W	<i>SPT3</i>	4.83 ± 0.12	6.12 ± 0.03	1.27 ± 0.03	2.0	Subunit of the SAGA and SAGA-like transcriptional regulatory complexes
<i>Protein synthesis</i>						
YAL035W	<i>FUN12</i>	3.66 ± 0.16	6.87 ± 0.40	1.88 ± 0.03	2.9	GTPase required for general translation initiation
<i>Protein fate (folding, modification, destination)</i>						
YGL058W	<i>RAD6</i>	3.26 ± 0.27	4.29 ± 0.07	1.32 ± 0.13	2.0	Ubiquitin-conjugating enzyme (E2) involved in protein degradation

* HEMF production capacity : [HEMF yield ×10⁴ (μAU) / Growth (OD₆₀₀)]

[†] PR [2] : HEMF production capacity ratio [2] (see Materials and Methods)

Data are expressed as mean values ± SD. Number of independent experiment = 2.

Values of Growth, HEMF yield, and HEMF production capacity in control wild type strain are 5.91 ± 0.38, 3.84 ± 0.35, and 0.65 ± 0.04, respectively.

TABLE S3 Effects of deleting genes necessary for activating transcription of SAGA-dependent *ADHI* promoter

ORF	Gene	PR [1] [*]	Description
YLR055C	<i>SPT8</i>	1.63	Subunit of the SAGA and/or SAGA-like transcriptional regulatory complexes
YDR392W	<i>SPT3</i>	1.83	Subunit of the SAGA and/or SAGA-like transcriptional regulatory complexes
YBR081C	<i>SPT7</i>	1.75	Subunit of the SAGA and/or SAGA-like transcriptional regulatory complexes
YOL148C	<i>SPT20</i>	1.57	Subunit of the SAGA and/or SAGA-like transcriptional regulatory complexes
YPL254W	<i>HFII</i>	1.49	Adaptor protein required for structural integrity of the SAGA complex

^{*} PR [1] = HEMF production ratio [1] (see Materials and Methods)

TABLE S4 Metabolite analysis of *Z. rouxii* , *Candida versatilis* , and *C. etchellsii*

strain	Carbon source : Glucose			Carbon source : Acetaldehyde
	HEMF production capacity (mg L ⁻¹ OD ₆₀₀ ⁻¹)	Extracellular acetaldehyde (μM OD ₆₀₀ ⁻¹)	Ethanol yield (%)	HEMF production capacity (mg L ⁻¹ OD ₆₀₀ ⁻¹)
<i>Z. rouxii</i> NBRC0506	34.5 ± 2.2	525.9 ± 33.7	1.84 ± 0.29	50.5 ± 5.1
<i>C. versatilis</i> NBRC10038	5.3 ± 0.6	26.3 ± 12.1	1.83 ± 0.15	21.5 ± 1.1
<i>C. etchellsii</i> NBRC10037	1.8 ± 0.2	12.4 ± 2.2	1.70 ± 0.25	34.3 ± 2.4

NBRC0506, NBRC10038, and NBRC10037 were provided by the Biological Resource Center, NITE (Tokyo, Japan).

TABLE S5 Summary of genes that decrease the HEMF production capacities by deletion

ORF	Gene	growth (OD ₆₀₀)	HEMF yield ×10 ⁴ (μAU)	HEMF production capacity*	PR[2] [†]	Localization [‡]	Description
<i>Metabolism</i>							
YNR041C	<i>COQ2</i>	9.04 ± 0.48	2.46 ± 0.20	0.27 ± 0.04	0.30	Mitochondrion	Transferase involved in ubiquinone (coenzyme Q) biosynthesis
YBR003W	<i>COQ1</i>	8.81 ± 0.14	2.10 ± 0.18	0.24 ± 0.02	0.27	Mitochondrion	Transferase involved in ubiquinone (coenzyme Q) biosynthesis
YDR204W	<i>COQ4</i>	8.80 ± 0.19	2.09 ± 0.24	0.24 ± 0.03	0.26	Mitochondrion	Protein with a role in ubiquinone (Coenzyme Q) biosynthesis
YML110C	<i>COQ5</i>	9.39 ± 0.01	2.05 ± 0.18	0.22 ± 0.02	0.24	Mitochondrion	Transferase involved in ubiquinone (Coenzyme Q) biosynthesis
<i>Energy</i>							
YDR375C	<i>BCS1</i>	8.55 ± 0.18	2.03 ± 0.06	0.24 ± 0.01	0.26	Mitochondrion	Mitochondrial protein of the AAA ATPase family
YDR231C	<i>COX20</i>	8.36 ± 0.03	2.02 ± 0.24	0.24 ± 0.03	0.27	Mitochondrion	Mitochondrial inner membrane protein, involved in the maturation and assembly of cytochrome oxidase subunit
YDR298C	<i>ATP5</i>	9.11 ± 0.11	2.22 ± 0.10	0.24 ± 0.01	0.27	Mitochondrion	Subunit 5 of the stator stalk of mitochondrial F1F0 ATP synthase
YJR121W	<i>ATP2</i>	7.90 ± 0.25	2.85 ± 0.36	0.36 ± 0.06	0.40	Mitochondrion	Beta subunit of the F1 sector of mitochondrial F1F0 ATP synthase
YDR079W	<i>PET100</i>	8.40 ± 0.49	2.22 ± 0.28	0.27 ± 0.05	0.30	Mitochondrion	Chaperone that specifically facilitates the assembly of cytochrome c oxidase
YBL099W	<i>ATP1</i>	8.56 ± 0.28	2.25 ± 0.05	0.26 ± 0.00	0.29	Mitochondrion	Alpha subunit of the F1 sector of mitochondrial F1F0 ATP synthase
YLR295C	<i>ATP14</i>	8.96 ± 0.14	2.50 ± 0.15	0.28 ± 0.01	0.31	Mitochondrion	Subunit h of the F0 sector of mitochondrial F1F0 ATP synthase
YPL271W	<i>ATP15</i>	9.08 ± 0.54	1.92 ± 0.11	0.21 ± 0.02	0.24	Mitochondrion	Epsilon subunit of the F1 sector of mitochondrial F1F0 ATP synthase
YJL003W	<i>COX16</i>	8.95 ± 0.42	2.52 ± 0.23	0.28 ± 0.04	0.31	Mitochondrion	Mitochondrial inner membrane protein, required for assembly of cytochrome c oxidase
YML129C	<i>COX14</i>	8.23 ± 0.35	2.14 ± 0.16	0.26 ± 0.03	0.29	Mitochondrion	Mitochondrial membrane protein, involved in translational regulation of Cox1p and assembly of cytochrome c oxidase
YPR191W	<i>QCR2</i>	8.19 ± 0.55	2.19 ± 0.00	0.27 ± 0.02	0.30	Mitochondrion	Subunit 2 of the ubiquinol cytochrome-c reductase complex
YOR065W	<i>CYT1</i>	8.67 ± 0.43	2.39 ± 0.28	0.28 ± 0.05	0.31	Mitochondrion	Cytochrome c1, component of the mitochondrial respiratory chain
<i>Cell cycle and DNA processing</i>							
YDR507C	<i>GIN4</i>	8.24 ± 0.08	2.16 ± 0.08	0.26 ± 0.01	0.29	Cytoplasm	Protein kinase involved in bud growth and assembly of the septin ring
YDL056W	<i>MBP1</i>	8.40 ± 0.28	2.30 ± 0.17	0.27 ± 0.03	0.31	Nucleus	Transcription factor involved in regulation of cell cycle progression from G1 to S phase
YCR028C-A	<i>RIM1</i>	8.34 ± 0.18	2.45 ± 0.44	0.29 ± 0.05	0.33	Mitochondrion	Single-stranded DNA-binding protein essential for mitochondrial genome maintenance
YJR144W	<i>MGM101</i>	8.69 ± 0.13	2.09 ± 0.15	0.24 ± 0.01	0.27	Mitochondrion	Protein involved in mitochondrial genome maintenance
YDR296W	<i>MHR1</i>	8.89 ± 0.12	2.29 ± 0.27	0.26 ± 0.03	0.29	Mitochondrion	Protein involved in homologous recombination in mitochondria
YOR330C	<i>MIP1</i>	8.31 ± 0.11	1.99 ± 0.03	0.24 ± 0.00	0.27	Mitochondrion	Catalytic subunit of the mitochondrial DNA polymerase
<i>Transcription</i>							
YMR228W	<i>MTF1</i>	8.58 ± 0.08	2.08 ± 0.17	0.24 ± 0.02	0.27	Mitochondrion	Mitochondrial RNA polymerase specificity factor
YJL209W	<i>CBP1</i>	9.03 ± 0.08	2.06 ± 0.09	0.23 ± 0.01	0.25	Mitochondrion	Mitochondrial protein with a role in COB mRNA stability and translation
YER028C	<i>MIG3</i>	7.29 ± 0.23	2.33 ± 0.04	0.32 ± 0.02	0.36	Nucleus	Probable transcriptional repressor involved in response to toxic agents
YDL044C	<i>MTF2</i>	8.73 ± 0.15	2.35 ± 0.25	0.27 ± 0.03	0.30	Mitochondrion	Mitochondrial matrix protein couples RNA processing and translation to transcription
YKL109W	<i>HAP4</i>	8.89 ± 0.54	2.46 ± 0.13	0.28 ± 0.03	0.31	Mitochondrion	Subunit of the heme-activated, glucose-repressed transcriptional activator and global regulator of respiratory gene expression
YIR021W	<i>MRS1</i>	7.98 ± 0.21	2.33 ± 0.06	0.29 ± 0.00	0.33	Mitochondrion	Protein required for the splicing of two mitochondrial group I introns
YOR201C	<i>MRM1</i>	9.38 ± 0.30	2.31 ± 0.11	0.25 ± 0.02	0.27	Mitochondrion	Ribose methyltransferase in mitochondrial 21S rRNA
<i>Protein synthesis</i>							
YHR168W	<i>MTG2</i>	8.01 ± 0.42	2.42 ± 0.47	0.30 ± 0.07	0.34	Mitochondrion	Putative GTPase required for mitochondrial translation, possibly via a role in ribosome assembly
YLR203C	<i>MSS51</i>	8.61 ± 0.57	2.56 ± 0.42	0.30 ± 0.07	0.33	Mitochondrion	Specific translational activator for the mitochondrial COX1 mRNA
YMR188C	<i>MRPS17</i>	8.94 ± 0.36	2.19 ± 0.14	0.24 ± 0.01	0.27	Mitochondrion	Mitochondrial ribosomal protein of the small subunit
YOL023W	<i>IFM1</i>	9.57 ± 0.43	2.25 ± 0.12	0.24 ± 0.00	0.26	Mitochondrion	Mitochondrial translation initiation factor 2
YOL033W	<i>MSE1</i>	9.43 ± 0.11	1.97 ± 0.07	0.21 ± 0.01	0.23	Mitochondrion	Mitochondrial glutamyl-tRNA synthetase
YPL013C	<i>MRPS16</i>	9.06 ± 0.02	1.89 ± 0.04	0.21 ± 0.00	0.23	Mitochondrion	Mitochondrial ribosomal protein of the small subunit
YBL038W	<i>MRPL16</i>	8.73 ± 0.08	2.48 ± 0.20	0.28 ± 0.03	0.32	Mitochondrion	Mitochondrial ribosomal protein of the large subunit
YBL080C	<i>PET112</i>	8.66 ± 0.05	2.18 ± 0.12	0.25 ± 0.01	0.28	Mitochondrion	Subunit of the trimeric GatFAB AmidoTransferase(AdT) complex involved in the formation of Q-tRNAQ
YDR115W		8.86 ± 0.05	2.18 ± 0.04	0.25 ± 0.00	0.27	Mitochondrion	Putative mitochondrial ribosomal protein of the large subunit
YDR175C	<i>RSM24</i>	9.08 ± 0.01	2.31 ± 0.04	0.25 ± 0.00	0.28	Mitochondrion	Mitochondrial ribosomal protein of the small subunit
YDR237W	<i>MRPL7</i>	9.01 ± 0.21	2.41 ± 0.25	0.27 ± 0.02	0.30	Mitochondrion	Mitochondrial ribosomal protein of the large subunit
YDR337W	<i>MRPS28</i>	9.30 ± 0.37	2.20 ± 0.23	0.24 ± 0.02	0.26	Mitochondrion	Mitochondrial ribosomal protein of the small subunit
YEL050C	<i>RML2</i>	8.55 ± 0.15	2.36 ± 0.00	0.28 ± 0.01	0.31	Mitochondrion	Mitochondrial ribosomal protein of the large subunit
YER050C	<i>RSM18</i>	7.79 ± 0.59	2.29 ± 0.19	0.30 ± 0.05	0.33	Mitochondrion	Mitochondrial ribosomal protein of the small subunit
YGR165W	<i>MRPS35</i>	7.72 ± 0.01	1.94 ± 0.03	0.25 ± 0.00	0.28	Mitochondrion	Mitochondrial ribosomal protein of the small subunit
YGR171C	<i>MSM1</i>	8.18 ± 0.21	2.20 ± 0.06	0.27 ± 0.00	0.30	Mitochondrion	Mitochondrial methionyl-tRNA synthetase (MetRS)
YIL093C	<i>RSM25</i>	8.53 ± 0.04	2.19 ± 0.00	0.26 ± 0.00	0.29	Mitochondrion	Mitochondrial ribosomal protein of the small subunit
YCR071C	<i>IMG2</i>	8.07 ± 0.21	2.60 ± 0.17	0.32 ± 0.03	0.36	Mitochondrion	Mitochondrial ribosomal protein of the large subunit
YDR462W	<i>MRPL28</i>	8.33 ± 0.29	2.52 ± 0.03	0.30 ± 0.01	0.34	Mitochondrion	Mitochondrial ribosomal protein of the large subunit
YPL104W	<i>MSD1</i>	7.87 ± 0.84	2.24 ± 0.28	0.29 ± 0.07	0.32	Mitochondrion	Mitochondrial aspartyl-tRNA synthetase

TABLE S5 Continued

YBL090W	<i>MRP21</i>	8.29 ± 0.14	2.00 ± 0.01	0.24 ± 0.00	0.27	Mitochondrion	Mitochondrial ribosomal protein of the small subunit
YBR251W	<i>MRP55</i>	8.65 ± 0.04	1.65 ± 0.18	0.19 ± 0.02	0.21	Mitochondrion	Mitochondrial ribosomal protein of the small subunit
YDL202W	<i>MRPL11</i>	8.24 ± 0.30	2.18 ± 0.08	0.26 ± 0.00	0.29	Mitochondrion	Mitochondrial ribosomal protein of the large subunit
YDR197W	<i>CBS2</i>	8.49 ± 0.40	2.13 ± 0.25	0.25 ± 0.04	0.28	Mitochondrion	Mitochondrial translational activator of the COB mRNA
YDR405W	<i>MRP20</i>	8.98 ± 0.26	2.30 ± 0.03	0.26 ± 0.00	0.29	Mitochondrion	Mitochondrial ribosomal protein of the large subunit
YER087W	<i>AIM10</i>	8.06 ± 0.13	2.37 ± 0.32	0.29 ± 0.04	0.33	Mitochondrion	Protein with similarity to tRNA synthetases; null mutant is viable and displays elevated frequency of mitochondrial genome loss
YJR113C	<i>RSM7</i>	8.56 ± 0.13	2.73 ± 0.38	0.32 ± 0.05	0.36	Mitochondrion	Mitochondrial ribosomal protein of the small subunit
YJL023C	<i>PET130</i>	9.36 ± 0.50	2.20 ± 0.01	0.24 ± 0.01	0.26	Mitochondrion	Protein required for respiratory growth
YNL081C	<i>SWS2</i>	8.85 ± 0.64	2.47 ± 0.05	0.28 ± 0.03	0.31	Mitochondrion	Putative mitochondrial ribosomal protein of the small subunit
YNL005C	<i>MRP7</i>	9.51 ± 0.14	2.64 ± 0.03	0.28 ± 0.00	0.31	Mitochondrion	Mitochondrial ribosomal protein of the large subunit
YNR037C	<i>RSM19</i>	8.31 ± 0.19	2.48 ± 0.22	0.30 ± 0.02	0.33	Mitochondrion	Mitochondrial ribosomal protein of the small subunit, has similarity to E. coli S19 ribosomal protein
YLR382C	<i>NAM2</i>	8.54 ± 0.16	2.59 ± 0.05	0.30 ± 0.00	0.34	Mitochondrion	Mitochondrial leucyl-tRNA synthetase, indirectly required for mitochondrial genome maintenance
YGL143C	<i>MRF1</i>	9.06 ± 0.25	2.43 ± 0.09	0.27 ± 0.00	0.30	Mitochondrion	Mitochondrial translation release factor; lack of MRF1 causes mitochondrial genome instability
<i>Protein fate (folding, modification, destination)</i>							
YMR089C	<i>YTA12</i>	8.28 ± 0.16	2.17 ± 0.14	0.26 ± 0.02	0.29	Mitochondrion	Component of the mitochondrial inner membrane m-AAA protease involved in degradation of misfolded or unassembled proteins and correct assembly of mitochondrial enzyme complexes
YDL107W	<i>MSS2</i>	8.30 ± 0.75	2.50 ± 0.30	0.30 ± 0.06	0.34	Mitochondrion	Peripherally bound inner membrane protein involved in membrane insertion of C-terminus of Cox2p
YJL180C	<i>ATP12</i>	8.42 ± 0.36	2.64 ± 0.18	0.31 ± 0.04	0.35	Mitochondrion	Conserved protein required for assembly of alpha and beta subunits into the F1 sector of mitochondrial F1F0 ATP synthase
YMR035W	<i>IMP2</i>	9.46 ± 0.45	2.36 ± 0.06	0.25 ± 0.02	0.28	Mitochondrion	Catalytic subunit of the mitochondrial inner membrane peptidase complex, required for maturation of mitochondrial proteins of the intermembrane space
YKL134C	<i>OCT1</i>	9.01 ± 0.28	2.09 ± 0.07	0.23 ± 0.00	0.26	Mitochondrion	Mitochondrial intermediate peptidase, cleaves N-terminal residues of a subset of proteins upon import
<i>Cellular transport, transport facilities and transport routes</i>							
YDL198C	<i>GGC1</i>	7.73 ± 0.03	2.27 ± 0.07	0.29 ± 0.01	0.33	Mitochondrion	Mitochondrial GTP/GDP transporter, essential for mitochondrial genome maintenance
<i>Cell rescue, defense and virulence</i>							
YOL151W	<i>GRE2</i>	8.84 ± 0.16	3.87 ± 0.79	0.44 ± 0.10	0.49	Nucleus, Cytoplasm	Reductase induced by stress (osmotic, ionic, oxidative, heat shock and heavy metals)
<i>Biogenesis of cellular components</i>							
YOL027C	<i>MDM38</i>	6.87 ± 0.03	2.57 ± 0.08	0.37 ± 0.01	0.42	Mitochondrion	Mitochondrial inner membrane protein, involved in membrane integration of a subset of mitochondrial proteins
YDR322W	<i>MRPL35</i>	8.04 ± 0.01	1.78 ± 0.04	0.22 ± 0.01	0.25	Mitochondrion	Mitochondrial ribosomal protein of the large subunit
YBR179C	<i>FZO1</i>	9.00 ± 0.72	2.24 ± 0.09	0.25 ± 0.03	0.28	Mitochondrion	Mitofusin, mitochondrial integral membrane protein involved in mitochondrial fusion and mitochondrial genome maintenance
YOR211C	<i>MGM1</i>	8.40 ± 0.43	2.01 ± 0.10	0.24 ± 0.00	0.27	Mitochondrion	Mitochondrial GTPase related to dynamin required for normal morphology of cristae
<i>Unclassified protein</i>							
YDR065W	<i>RRG1</i>	8.79 ± 0.38	2.12 ± 0.06	0.24 ± 0.00	0.27	Mitochondrion	Protein of unknown function, required for vacuolar acidification and mitochondrial genome maintenance
YGL107C	<i>RMD9</i>	8.96 ± 0.01	2.39 ± 0.10	0.27 ± 0.01	0.30	Mitochondrion	Mitochondrial protein required for respiratory growth
YMR098C	<i>ATP25</i>	8.73 ± 0.15	1.88 ± 0.08	0.22 ± 0.01	0.24	Mitochondrion	Mitochondrial protein required for the stability of Oli1p (Atp9p) mRNA and for the Oli1p ring formation
YLR091W	<i>GEP5</i>	8.54 ± 0.08	2.30 ± 0.02	0.27 ± 0.01	0.30	Mitochondrion	Protein of unknown function, required for mitochondrial genome maintenance
YER077C		9.74 ± 0.02	2.21 ± 0.03	0.23 ± 0.00	0.25	Mitochondrion	Putative protein of unknown function; null mutation results in a decrease in plasma membrane electron transport
YGR150C	<i>CCM1</i>	7.70 ± 0.01	2.34 ± 0.35	0.30 ± 0.04	0.34	Mitochondrion	Mitochondrial 15s rRNA-binding protein; mutant is respiratory deficient and has defective plasma membrane electron transport
YPL137C	<i>GIP3</i>	9.02 ± 0.50	2.40 ± 0.43	0.27 ± 0.06	0.30	Cytoplasm	Cytoplasmic protein that regulates protein phosphatase 1 Glc7p
YOR305W	<i>RRG7</i>	9.80 ± 0.71	2.24 ± 0.00	0.23 ± 0.02	0.26	Mitochondrion	Protein of unknown function
YPL005W	<i>AEP3</i>	8.24 ± 0.68	2.04 ± 0.21	0.25 ± 0.05	0.28	Mitochondrion	Peripheral mitochondrial inner membrane protein; stabilizes the bicistronic mRNA encoding subunits 6 and 8 of the ATP synthase complex
YPL183C	<i>RTT10</i>	8.61 ± 0.30	2.83 ± 0.07	0.33 ± 0.00	0.37	Cytoplasm	Cytoplasmic protein with a role in regulation of Ty1 transposition
YPR116W	<i>RRG8</i>	8.07 ± 0.20	2.13 ± 0.05	0.26 ± 0.00	0.29	Mitochondrion	Putative protein of unknown function, required for mitochondrial genome maintenance
YLR149C		8.71 ± 0.13	2.30 ± 0.14	0.26 ± 0.02	0.29	Unknown	Putative protein of unknown function; null mutation results in a decrease in plasma membrane electron transport
YOR205C	<i>GEP3</i>	9.07 ± 0.13	2.05 ± 0.03	0.23 ± 0.00	0.25	Mitochondrion	Protein of unknown function; null mutant is defective in respiration
YDR114C		8.65 ± 0.10	2.01 ± 0.07	0.23 ± 0.01	0.26	Unknown	Putative protein of unknown function; deletion mutant exhibits poor growth at elevated pH and calcium

* HEMF production capacity : [HEMF yield × 10⁴ (μAU) / Growth (OD₆₀₀)]

† PR [2] : HEMF production capacity ratio [2] (see Materials and Methods)

‡ Localization was determined based on Saccharomyces Genome Database (<http://www.yeastgenome.org/>)

Data are expressed as mean values ± SD. Number of independent experiment = 2.

Values of Growth, HEMF yield, and HEMF production capacity in control wild type strain are 6.41 ± 0.36, 5.76 ± 1.37, and 0.90 ± 0.21, respectively.