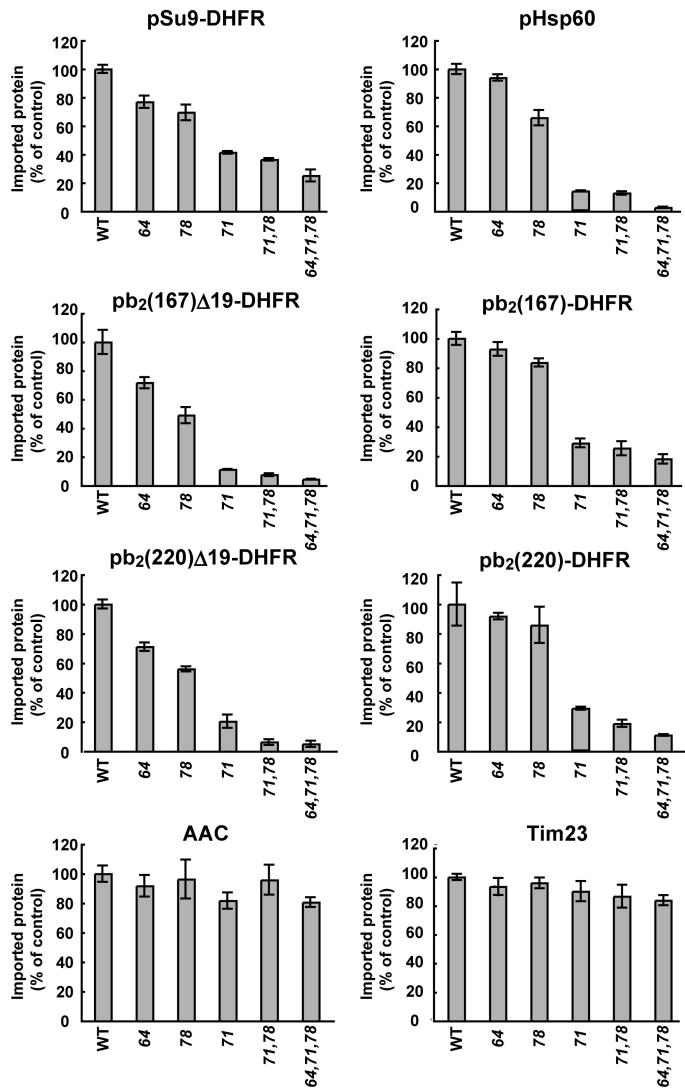
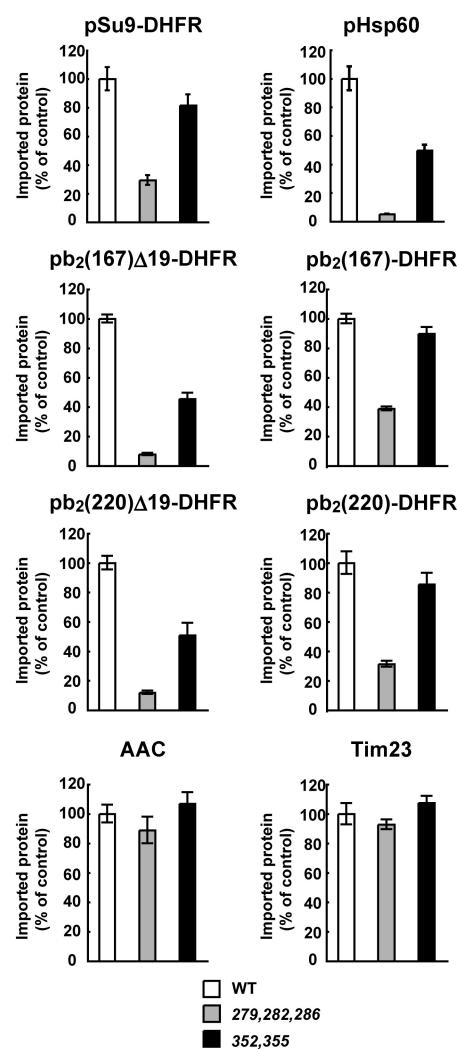
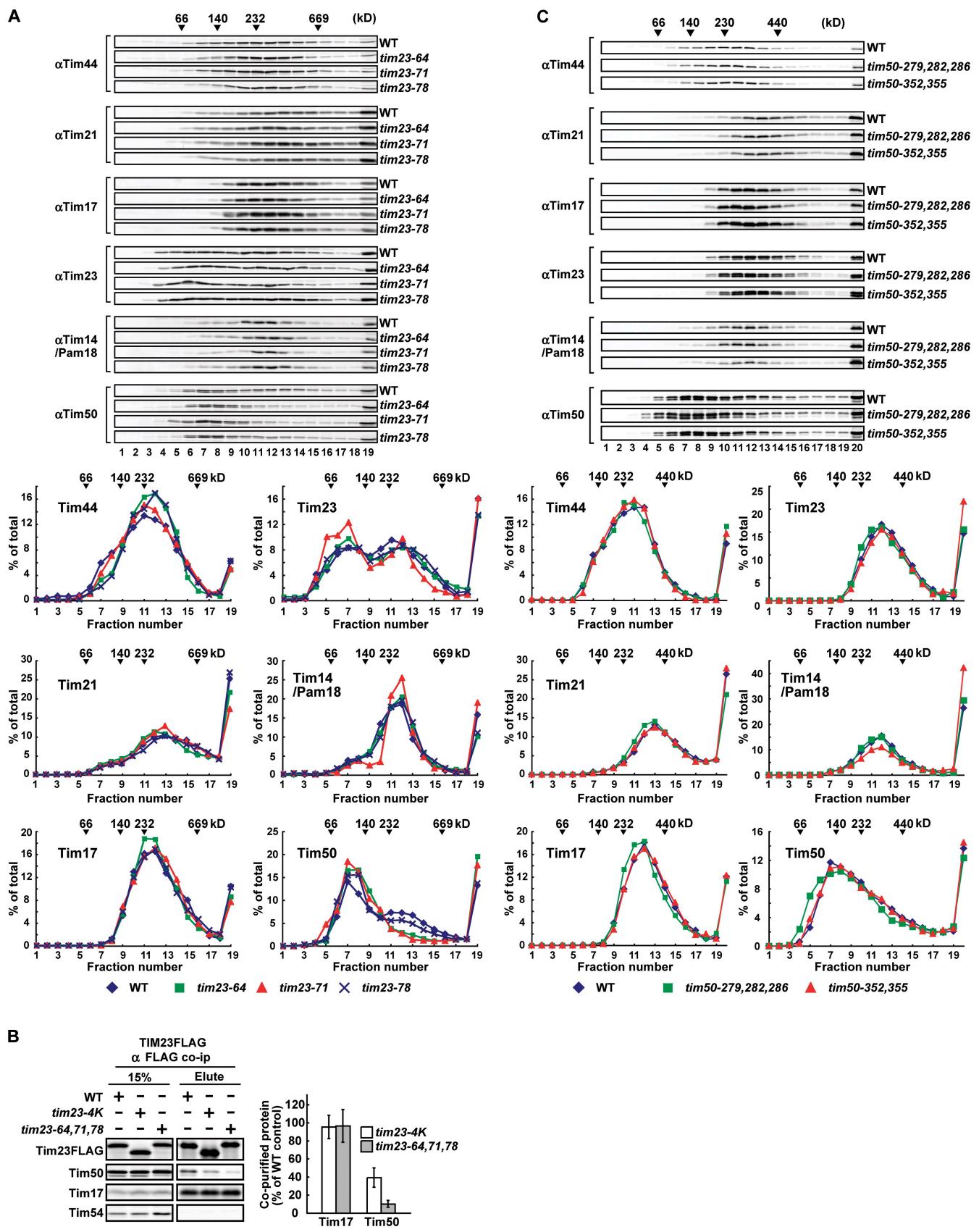
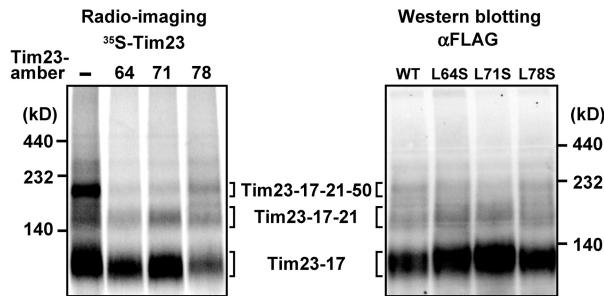
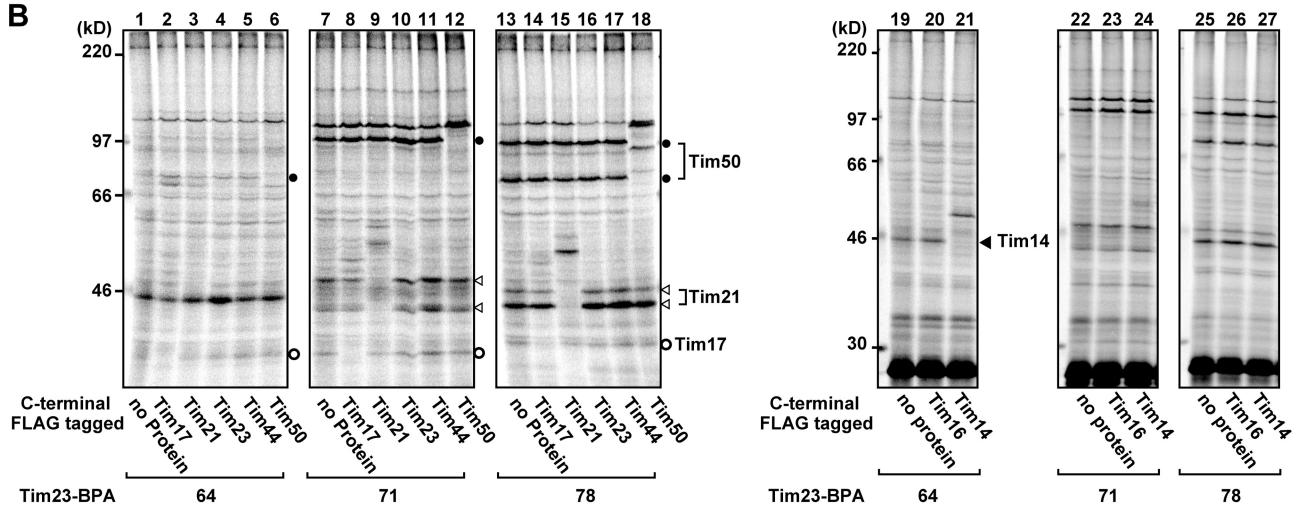
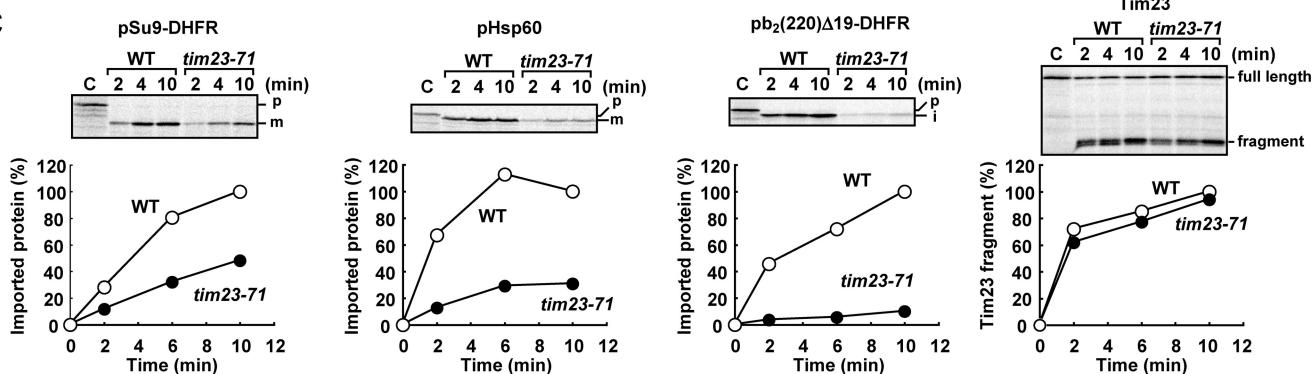


Tamura et al., <http://www.jcb.org/cgi/content/full/jcb.200808068/DC1>**A *tim23* mutants****B *tim50* mutants**

**Figure S1. Import rates of various mitochondrial proteins into wild-type control, *tim23-64*, *tim23-71*, *tim23-78*, *tim23-71,78*, *tim23-64,71,78*, *tim50-279,282,286*, and *tim50-352,355* mitochondria.** In vitro import was performed as in Fig. 2 and Fig. 3 E, relative import rates were calculated after 1 (Tim23), 2 (AAC), and 3 min (other proteins) of incubation, and import rates for wild-type mitochondria were set to 100%. Error bars represent SDs from three independent experiments.

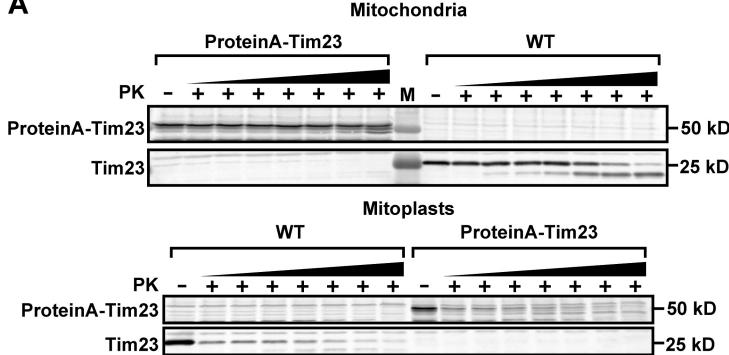


**Figure S2. Analyses of complex structures of the TIM23 complex subunits by glycerol density-gradient centrifugation.** Wild-type (WT), *tim23*-64, *tim23*-71, and *tim23*-78 mitochondria (A) and Wild-type, *tim50*-279,282,286, and *tim50*-352,355 mitochondria (C) isolated after cultivation in lactate medium were solubilized with 1% digitonin and subjected to glycerol density-gradient centrifugation (20–40%) at 166,000 (A) or 192,000 g (C) for 15 h. After centrifugation, fractions were collected from the top and analyzed by SDS-PAGE followed by immunoblotting with antibodies against indicated proteins (Tim44, Tim21, Tim17, Tim23, Tim14, and Tim50). (B) Wild-type, *tim23*-4k, and *tim23*-64,71,78 mitochondria with FLAG-tagged Tim23 were solubilized with 1% digitonin and subjected to immunoprecipitation with the anti-FLAG antibody. Amounts of eluted proteins from wild-type mitochondria were set to 100% for each protein.

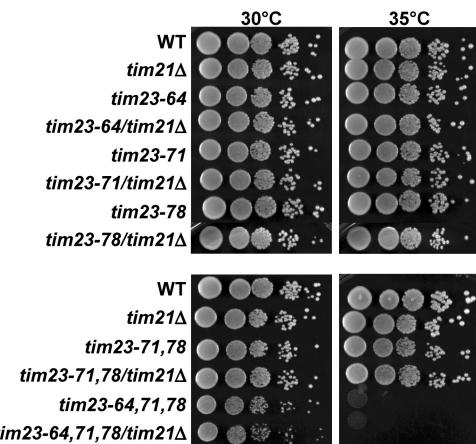
**A****B****C**

**Figure S3. Assignments of cross-linked partners for Tim23 with BPA.** (A) Radiolabeled Tim23 with or without BPA incorporated at position 64, 71, or 78 was incubated with wild-type (WT) mitochondria at 25°C for 1 h. After stopping the import reaction with valinomycin, the mitochondria were washed with SEM buffer and were then solubilized with 1% digitonin (left). Wild-type and *tim23* mutant mitochondria with FLAG-tagged Tim23 were solubilized with 1% digitonin (right). Protein complexes were analyzed by BN-PAGE and radioimaging (left) or Western blotting (right). Assignment of subunits of the different bands are based on our published data (Tamura, Y., Y. Harada, K. Yamano, K. Watanabe, D. Ishikawa, C. Ohshima, S. Nishikawa, H. Yamamoto, and T. Endo. 2006. *J. Cell Biol.* 174:631–637). (B) Radiolabeled Tim23 with BPA incorporated at position 64, 71, or 78 was incubated with mitochondria with or without C-terminally FLAG-tagged Tim17, Tim21, Tim23, Tim44, Tim50, Tim16, or Tim14 at 25°C for 1 h. After stopping the import reaction with valinomycin, the mitochondria were washed with and resuspended in SEM buffer. The mitochondria were UV irradiated for 5 min on ice. Proteins were analyzed by SDS-PAGE and radioimaging. Cross-linked partner proteins were identified by detecting the shift in their apparent molecular sizes caused by the presence of the FLAG tag. Closed triangle, open triangle, closed circle, and open circle indicate the cross-linked products of Tim23 with Tim14, Tim21, Tim50, and Tim17, respectively. (C) Radiolabeled precursor proteins were incubated with mitoplast generated from wild-type and *tim23-71* mitochondria by osmotic shock for indicated times at 25°C (mitoplasting efficiency was 80 [wild type] or 72% [*tim23-71*]). For quantification, amounts of protease-resistant proteins or Tim23 fragment in WT after the longest incubation time were set to 100%. p, precursor form; i, processing-intermediate form; m, mature form.

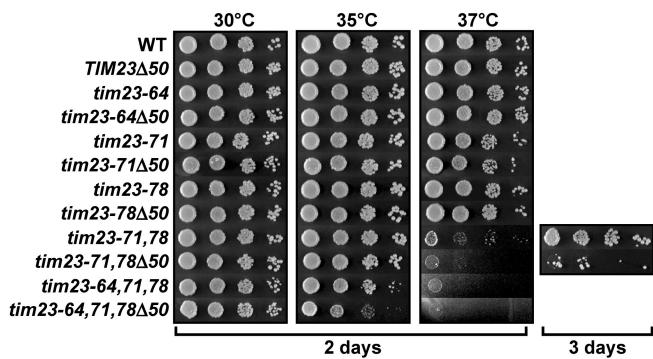
A



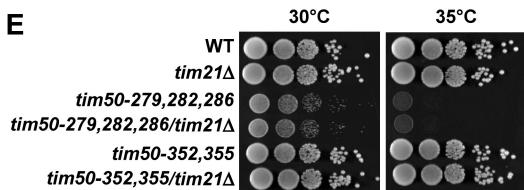
D



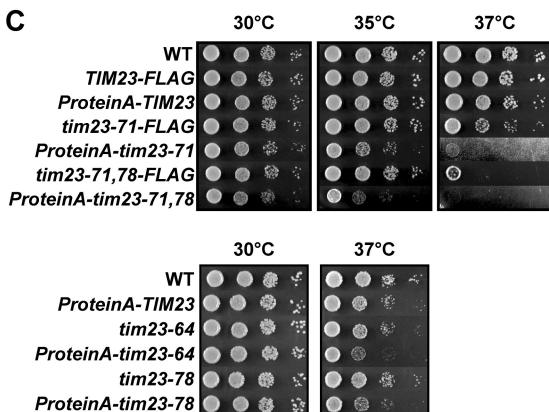
B



E



C



**Figure S4. Effects of defective Tim23-Tim50 interactions on the N-terminal outer membrane insertion of Tim23.** (A) Mitochondria or mitoplasts with Tim23 or protein A-Tim23 were treated with 10, 25, 50, 100, 250, 500, and 1,000 µg/ml of proteinase K for 20 min at 16°C, respectively. The reactions were stopped by adding 1 mM PMSF. The mitochondria were reisolated and proteins were analyzed by SDS-PAGE and immunoblotting with antibodies against Tim23. Whereas Tim23 is partly clipped by proteinase K added outside the mitochondrial membrane, protein A-Tim23 remained proteinase K resistant. Both Tim23 and protein A-Tim23 were degraded by proteinase K after the outer membrane was broken open. These results indicate that, although the N terminus of Tim23 partly penetrates across the outer membrane, protein A-Tim23 does not. (B) Serial dilutions of wild-type control (WT), *tim23-64*, *tim23-71*, *tim23-78*, *tim23-71,78*, and *tim23-64,71,78* cells, and those with Tim23 derivatives lacking the N-terminal 50 residues (*TIM23Δ50*, *tim23-64*, *tim23-71*, *tim23-78*, *tim23-71Δ50*, *tim23-78Δ50*, *tim23-71,78Δ50*, and *tim23-64,71,78Δ50*), were plated on SCD (-Trp) and grown at indicated temperature for 2 or 3 d. (C) Serial dilutions of wild-type control, *tim23-64*, and *tim23-78* cells, and those with C-terminally FLAG-tagged Tim23 derivatives (*TIM23-FLAG*, *tim23-71-FLAG*, and *tim23-71,78-FLAG*) or N-terminally protein A-tagged derivatives (*ProteinA-TIM23*, *ProteinA-tim23-71*, *ProteinA-tim23-71,78*, *ProteinA-tim23-64*, and *ProteinA-tim23-78*), were plated on SCD (-Trp) and grown at indicated temperature for 2 d. Serial dilutions of *tim23* mutants (D) and *tim50* mutants lacking the *TIM21* gene (E) were plated on YPD and grown at indicated temperature for 2 d.

**Table S1**  
**Yeast strains**

Strain	Genotype	Parental strain	Method used	Source
W303	MAT $\alpha$ /MAT $\alpha$ ade2/ ade2 his3/ his3 ura3/ ura3 leu2/ leu2 trp1/ trp1 can1/ can1	N/A	N/A	R. Rothstein (Columbia University, NY, NY)
W303-1A	MAT $\alpha$ ade2 his3 ura3 leu2 trp1 can1	N/A	N/A	R. Rothstein
PJ69-4A	MAT $\alpha$ trp1 leu2 ura3 his3 gal4Δ gal80Δ LY52::GAL1-HIS3 GAL2-ADE2 met2::GAL7-lacZ	N/A	N/A	
TIM23/tim23Δ	MAT $\alpha$ /MAT $\alpha$ ade2/ade2 his3/his3 ura3/ura3 leu2/leu2 trp1/trp1 can1/ can1 TIM23/tim23Δ::CgHIS3 [pRS316-Tim23Δ50]	W303-AB	PCR-mediated gene disruption	This study
TIM23Δ50-316	MAT $\alpha$ ade2 his3 ura3 leu2 trp1 can1 tim23Δ::CgHIS3 [pRS316-Tim23Δ50]	TIM23/tim23Δ	Sporulation of diploid	This study
TIM23	MAT $\alpha$ ade2 his3 ura3 leu2 trp1 can1 tim23Δ::CgHIS3 [pRS314-Tim23]	TIM23Δ50-316	Plasmid shuffling	This study
tim23-X	MAT $\alpha$ ade2 his3 ura3 leu2 trp1 can1 tim23Δ::CgHIS3 [pRS314-Tim23-X]	TIM23Δ50-316	Plasmid shuffling	This study
TIM50FLAG/TIM23Δ50-316	MAT $\alpha$ ade2 his3 ura3 leu2 trp1 can1 tim23Δ::CgHIS3 TIM50-FLAG::kanMX4 [pRS316-Tim23Δ50]	TIM23Δ50-316	PCR-mediated tagging	This study
TIM50FLAG/TIM23-X	MAT $\alpha$ ade2 his3 ura3 leu2 trp1 can1 tim23Δ::CgHIS3 TIM50-FLAG::kanMX4 [pRS314-Tim23-X]	TIM50FLAG/TIM23Δ50-316	Plasmid shuffling	This study
TIM23Δ50-X	MAT $\alpha$ ade2 his3 ura3 leu2 trp1 can1 tim23Δ::CgHIS3 [pRS314-Tim23Δ50-X]	TIM23Δ50-316	Plasmid shuffling	This study
tim23Δ/TIM23FLAG	MAT $\alpha$ ade2 his3 ura3 leu2 trp1 can1 tim23Δ::CgHIS3 [pRS314-Tim23FLAG]	TIM23Δ50-316	Plasmid shuffling	This study
tim23Δ/TIM23-XFLAG	MAT $\alpha$ ade2 his3 ura3 leu2 trp1 can1 tim23Δ::CgHIS3 [pRS314-Tim23-XFLAG]	TIM23Δ50-316	Plasmid shuffling	This study
ProteinA-TIM23	MAT $\alpha$ ade2 his3 ura3 leu2 trp1 can1 tim23Δ::CgHIS3 [pRS314-ProteinA-Tim23]	TIM23Δ50-316	Plasmid shuffling	This study
ProteinA-TIM23-X	MAT $\alpha$ ade2 his3 ura3 leu2 trp1 can1 tim23Δ::CgHIS3 [pRS314-ProteinA-Tim23-X]	TIM23Δ50-316	Plasmid shuffling	This study
GAL-TIM23	MAT $\alpha$ ade2 his3 ura3 leu2 trp1 can1 GAL7-TIM23-CgHIS3	W303-1A	PCR-mediated promoter replacement	Unpublished data
GAL-TIM23/TIM23FLAG-BPA41	MAT $\alpha$ ade2 his3 ura3 leu2 trp1 can1 GAL7-TIM23-CgHIS3 [pYO325-Tim23FLAG-amber41]	GAL-TIM23	Transformation with a plasmid	This study
tim50-X	MAT $\alpha$ ade2 his3 ura3 leu2 trp1 can1 tim50Δ::CgHIS3 [pRS314-Tim50-X]	TIM50-316*	Plasmid shuffling	This study / *Yamamoto et al., 2002
TIM23FLAG/TIM50-316	MAT $\alpha$ ade2 his3 ura3 leu2 trp1 can1 tim50Δ::CgHIS3 TIM23-FLAG::kanMX4 [pRS316-Tim50-X]	TIM50-316*	PCR-mediated tagging	This study / *Yamamoto et al., 2002
TIM23FLAG/tim50-X	MAT $\alpha$ ade2 his3 ura3 leu2 trp1 can1 tim50Δ::CgHIS3 TIM23-FLAG::kanMX4 [pRS314-Tim50-X]	TIM23FLAG/TIM50-316	Plasmid shuffling	This study
tim21Δ	MAT $\alpha$ ade2 his3 ura3 leu2 trp1 can1 tim21Δ::CgHIS3	W303-1A	PCR-mediated gene disruption	This study
TIM23Δ50-316/tim21Δ	MAT $\alpha$ ade2 his3 ura3 leu2 trp1 can1 tim23Δ::CgHIS3 [pRS316-Tim23Δ50] tim21Δ::kanMX4	TIM23Δ50-316	PCR-mediated gene disruption	This study
tim23-X/tim21Δ	MAT $\alpha$ ade2 his3 ura3 leu2 trp1 can1 tim23Δ::CgHIS3 [pRS314-Tim23-X] tim21Δ::kanMX4	TIM23Δ50-316/tim21Δ	Plasmid shuffling	This study
TIM50-316/tim21Δ	MAT $\alpha$ ade2 his3 ura3 leu2 trp1 can1 tim50Δ::CgHIS3 [pRS316-Tim50] tim21Δ::kanMX4	TIM50-316	PCR-mediated gene disruption	This study
tim50-X/tim21Δ	MAT $\alpha$ ade2 his3 ura3 leu2 trp1 can1 tim50Δ::CgHIS3 [pRS314-Tim50-X] tim21Δ::kanMX4	TIM50-316/tim21Δ	Plasmid shuffling	This study
TIM23Δ50-316/pam17Δ	MAT $\alpha$ ade2 his3 ura3 leu2 trp1 can1 tim23Δ::CgHIS3 [pRS316-Tim23Δ50] pam17Δ::kanMX4	TIM23Δ50-316	PCR-mediated gene disruption	This study
tim23-X/pam17Δ	MAT $\alpha$ ade2 his3 ura3 leu2 trp1 can1 tim23Δ::CgHIS3 [pRS314-Tim23-X] pam17Δ::kanMX4	TIM23Δ50-316/pam17Δ	Plasmid shuffling	This study
TIM50-316/pam17Δ	MAT $\alpha$ ade2 his3 ura3 leu2 trp1 can1 tim50Δ::CgHIS3 [pRS316-Tim50] pam17Δ::kanMX4	TIM50-316	PCR-mediated gene disruption	This study
tim50-X/pam17Δ	MAT $\alpha$ ade2 his3 ura3 leu2 trp1 can1 tim50Δ::CgHIS3 [pRS314-Tim50-X] pam17Δ::kanMX4	TIM50-316/pam17Δ	Plasmid shuffling	This study
TIM50FLAG/TOM22-BPA-X (X=132, 134 or 136)	MAT $\alpha$ ade2 his3 ura3 leu2 trp1 can1 TIM50-FLAG::CgHIS3 [pSL22-132B, pSL22-134B or pSL22-136B] [p6xRNA]	TIM50FLAG*	Transformation with plasmids	This study / *Tamura et al., 2006
TOM22HIS10-BPA-X (X=132, 134 or 136)	MAT $\alpha$ ade2 his3 ura3 leu2 trp1 can1 [pSL22-132B, pSL22-134B or pSL22-136B] [p6xRNA]	W303-1A	Transformation with plasmids	This study
X/TOM22HIS10-BPA-Y (X=TIM23, tim23-71, Y=132 and 136)	MAT $\alpha$ ade2 his3 ura3 leu2 trp1 can1 tim23Δ::CgHIS3 [pASZ11-Tim23-X (CEP-TRP1)] [pSL22-132B or pSL22-136B] [p6xRNA]	TIM23Δ50-316	Plasmid shuffling / Transformation with plasmids	This study
X/TOM22HIS10-BPA-Y (X=TIM50 or tim50-279, 282, 286, Y=132 and 136)	MAT $\alpha$ ade2 his3 ura3 leu2 trp1 can1 tim50Δ::CgHIS3 [pRASZ11-Tim50-X] [pSL22-132B or pSL22-136B] [p6xRNA]	TIM50-316*	Plasmid shuffling / Transformation with plasmids	This study / *Yamamoto et al., 2002

Tamura, Y., Y. Harada, K. Yamano, K. Watanabe, D. Ishikawa, C. Ohshima, S. Nishikawa, H. Yamamoto, and T. Endo. 2006. Identification of Tam41 maintaining integrity of the TIM23 protein translocator complex in mitochondria. *J. Cell Biol.* 174:631–637.

Yamamoto, H., M. Esaki, T. Kanamori, Y. Tamura, S. Nishikawa, and T. Endo. 2002. Tim50 is a subunit of the TIM23 complex that links protein translocation across the outer and inner mitochondrial membranes. *Cell.* 111:519–528.

## Plasmids

Plasmid name	Expressed protein	Vector	Primers	Site	Template DNA or digested DNA fragment
pRS316-Tim23Δ50	Tim23 lacking N-terminal 50 residues	pRS316	Tim23-pro-F / Tim23-proR Tim23-51-F / Tim23-222-R	EcoRI Xhol	Genomic DNA
pRS314-Tim23	Tim23	pRS314	Tim23-pro-F Tim23-ter-R	EcoRI Xhol	Genomic DNA
pRS314-Tim23-X	Tim23-X	pRS314	Tim23-pro-F / Tim23-X-R Tim23-X-F / Tim23-ter-R	EcoRI Xhol	pRS314-Tim23 or pRS314-Tim23-X
pRS314-Tim23Δ50-X	Tim23-X lacking N-terminal 50 residues	pRS314	Tim23-pro-F / Tim23-X-R Tim23-X-F / Tim23-ter-R	EcoRI Xhol	pRS316-Tim23Δ50
pRS314-Tim23FLAG	Tim23FLAG	pRS314	BamHI-Tim23-pro-F FLAG-ter-R-Xhol	BamHI Xhol	Genomic DNA from <i>TIM23FLAG</i>
pRS314-Tim23-X-FLAG	Tim23-X-FLAG	pRS314	BamHI-Tim23-pro-F / Tim23-X-R Tim23-X-F / FLAG-ter-R-Xhol BamHI-Tim23-pro-F / ProteinA-R-EcoRI EcoRI-ProteinA-F / Tim23-ter-R	BamHI Xhol	pRS314-Tim23FLAG
pRS314-EcoRI-Tim23	N/A (pRS314-Tim23 with EcoRI site in front of the <i>TIM23</i> gene)	pRS314	Tim23-ProteinA-F Tim23-ProteinA-R	BamHI Xhol	pRS314-Tim23
pRS314-ProteinsA-Tim23	ProteinA-Tim23	pRS314-EcoRI-Tim23	BamHI-Tim23-pro-F / Tim23-X-R Tim23-X-F / FLAG-ter-R-Xhol	EcoRI	pTYE248
pRS314-ProteinsA-Tim23-X	ProteinA-Tim23-X	pRS314-ProteinsA-Tim23	BamHI-Tim23-pro-F / Tim23-041I-TAG-R Tim23-041I-TAG-F / FLAG-ter-R-Xhol	BamHI Xhol	pRS314-ProteinsA-Tim23
pYO325-Tim23FLAG-amber41	Tim23FLAG with BPA at position 41	pYO325	Tom22-X-F Tom22-X-R	BamHI Xhol	pRS314-Tim23FLAG
pRS314-Tim50-X	Tim50-X	pRS314	Tim50-pro-F / Tim50-X-R Tim50-X-F / Tim50-ter-R	Xhol BamHI	pRS316-Tim50 (Yamamoto et al., 2002)
pSU22	Tom22	YCpUG578T (Kawai et al., 2001)	Tom22-C Tom22-B	EcoRI HindIII	Yeast genomic DNA
pSL22	Tom22 under the control of GAL1 promotor	pRS315	N/A	BamHI Xhol	DNA fragment digested by BamHI and Xhol from pSU22
pSL22-XB	Tom22 with BPA at position X	pRS315	Tom22-X-F Tom22-X-R	N/A	pSL22
pSU22H	Tom22-His <sub>10</sub>	YCpUG578T (Kawai et al., 2001)	Tom22-C Tom22-E	EcoRI HindIII	pSU22
pSL22H	Tom22-His <sub>10</sub>	pRS315	N/A	SpeI Xhol	DNA fragment digested by BamHI and Xhol from pSU22
pSL22H-XB	Tom22-His <sub>10</sub> with BPA at position X	pRS315	Tom22-X-F Tom22-X-R	N/A	pSL22H
pASZ11-Tim23	Tim23	pASZ11 (Stotz and Linder, 1990)	N/A	EcoRI Xhol	DNA fragment digested by BamHI and Xhol from pRS314-Tim23
pASZ11-Tim23-71	Tim23-71	pASZ11	N/A	EcoRI Xhol	DNA fragment digested by BamHI and Xhol from pRS314-Tim23-71
pASZ11-Tim50	Tim50	pASZ11	N/A	BamHI Xhol	DNA fragment digested by BamHI and Xhol from pRS314-Tim50
pASZ11-Tim50-279, 282, 286	Tim50-279, 282, 286	pASZ11	N/A	BamHI Xhol	DNA fragment digested by BamHI and Xhol from pRS314-Tim50-279, 282, 286
p6xRNA	N/A	N/A	N/A	N/A	Chin et al., 2003

Chin, J.W., T.A. Cropp, J.C. Anderson, M. Mukherji, Z. Zhang, and P.G. Schultz. 2003. An expanded eukaryotic genetic code. *Science*. 301:964–967.  
 Kawai, A., S. Nishikawa, A. Hirata, and T. Endo. 2001. Loss of the mitochondrial Hsp70 functions causes aggregation of mitochondria in yeast cells. *J. Cell Sci.* 114: 3565–3574.

Stotz, A., and P. Linder. 1990. The ADE2 gene from *Saccharomyces cerevisiae*: sequence and new vectors. *Gene*. 95:91–98.

### Primers for plasmid construction

Primer name	Sequence (5'-3')
Tim23-pro-F	GCCGAATTGACTCTGGAGGGCGTAACGGCCG
Tim23-ter-R	GGGCTCGAGGGAAATACCGAGAGTGAGC
BamHI-Tim23-pro-F	GCCGGATCCGACTCTGGAGGGCGTAACGGCCG
FLAG-ter-R-Xhol	GCGCTCGAGATGAGAGAATCGAACTGAACTGTT
ProteinA-R-EcoRI	GGGGAATTCGGATCGTTAACGGTTGGATGAAGCCGTACG
EcoRI-ProteinA-F	GGGGAATTATGAAACAACGCATAACCTGAAAGAAGCTTGGG
Tim23-ProteinA-F	CACACAATCGAATTATGTCGTGGCTTGAGATAAGACACC
Tim23-ProteinA-R	CCACGACATGAATTGATGTGTGATCTGTTAACAAAGTATAC
Tim23-pro-R	GATTGTGTGTGATCTGTTAACAAAGTATAC
Tim23-51-F	ATGCATGTCGACACCGCTAGGCTGCATCC
Tim23-222-R	GGCCAAGCTTGGAAATACCCGAGAGTGAGC
Tim23-041I-TAG-R	TGATATTATGTTATTCTAGTTGGCTCGAAACC
Tim23-041I-TAG-F	GGTTTCGAGCCAAACTAGAATAACATAATATCA
Tim50-pro-F	GGGCTCGAGCCTTACATGTCAGGGC
Tim50-ter-R	GGGGGATCCCGCGGAAAGTTGTGAGTACG
Tom22-C	CGCGAATTATGGTCGAATTAACTGAA
Tom22-B	GCGAAGCTTAAATGGCTGTTGCTGC
Tom22-E	GCGAAGCTTAAATGATGATGATGATGATGATGCCGGGGATCCATTGGCTGTCAGC

### Primers for C-terminally FLAG tagging

Primer name	Sequence (5'-3')
Tim50-FLAG-F	TGAAGAGGAAAAGAAAAAGAAGAAGATTGCTGAATCCAAAGGCGAATTGGTACCGGG
Tim50-tag-R	GATACGTAGATACATGAGAAGAGGGTTACATGAAAATTACAGGAAACAGCTATGAC

### Primers for gene disruption

Primer name	Sequence (5'-3')
TIM23-delta-CgHIS3-F	GGGCGGCCAAGATAACCAAGCCTAAGGAACATCGTGGTGAAACGACGGCCAGT
TIM23-delta-CgHIS3-R	GCGGCCACCATTGCCGAGGAATAACCCATGGGTTCAAACACAGGAAACAGCTATGACC
TIM21-delta-CgHIS3-F	AATCATTCGTATATTATTCCTGACTCCAAGTTAACAGTTGAAACGACGGCCAGT
TIM21-delta-CgHIS3-R	ACGAATATTAAACCTGAGCAACTCCGTCAAATTGATCCACAGGAAACAGCTATGACC
TIM21-delta-kan-F	GATATCAGGTGGAAATCATCGTATATTATTCCTGACTCCAAGTTAACAGATTGACTGAGAGTGTGCAC
TIM21-delta-kan-R	GGAAATAACAGTCATTACGAATATTAAAACCTGAGCAACTCCGTCAAATTGATCCGTGCGGTATTCACACCG
PAM17-dis-F	GGGAAACTATGTCAAAGAAGTGTAAAAACATTAGAAAACATTGTCGCCCTCTCAAAAGATTGACTGAGAGTGCAC
PAM17-dis-R	CTTATTATGATGATATACAGAGTCTGAGAAGAAGGAAAGATCACAGTCAACTGTCGGTATTCACACCG

### Primers for introducing mutations to the *Tim23* gene

X	Sequence of Tim23-X-F	Sequence of Tim23-X-R	Template (Tim23-)
64	CCTTGGCTGGTCAGACAAGGGTGTGGAG	CTCCACACCCCTGCTGAACCAGGCCAAGG	WT
71	GACAAGGGTGTGGAGATTAGATCTGAAAGAAG	CTTCTCCAGATCTGAATACTCCACACCCTTGT	WT
78	GGAAGAAGAACATCATCCTCGTAGAAGGCTAC	GTGAGCCTCTAACGAGGATATTGTTCTTCTCC	WT
71,78	GGAGTATTAGATCTGAAAGAACATCATCCTCG	CGAGGATATTGTTCTTCTCCAGATCTGAATACTCC	WT
64,71,78	GGAGTATTAGATCTGAAAGAACATCATCCTCG	CGAGGATATTGTTCTTCTCCAGATCTGAATACTCC	64
60-64	GGCTGCATTCTCGTCTAGTCAGACAAGG	CCTTGTCTGAACTAGACGAAGAACATGCAGCC	WT
61,64,71,78	GGCTGCATCCTCGGCTGGTCAGACAAGG	CCTTGTCTGAACCAGCCGAAGGATGCAGCC	71,78
60-64,71,78	GGAGTATTAGATCTGAAAGAACATCATCCTCG	CGAGGATATTGTTCTTCTCCAGATCTGAATACTCC	60-64

X indicates the position of a mutation in the *Tim23* gene

### Primers for introducing mutations to the *Tim50* gene

X	Sequence of Tim50-X-F	Sequence of Tim50-X-R
279,282,286	CACATTAAGGATTCGTAAAATCGAATAGAGATTGAGTAAAGTAATC	GATTACTTACTCGAATCTATTGATTGACGAATCCTTAATGTG
352,355	GACAAGAAGAACCTATTAGAAGAACATGATCATCG	CGATGATCAGATTCTCTGAATAGTTCTCTGTG

X indicates positions of mutations in the *Tim50* gene

**Primers for introducing an amber codon to the *TOM22* gene**

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X	Sequence of Tom22-amber-X-F	Sequence of Tom22-amber-X-R
132	AGACATTGATTAGCAAAGTGATGC	GCATCACTTGCTAATCAAATGTCT
134	TTGATTACAATAGGATGCTAATAA	TTATTAGCATCCTATTGAAATCAA
136	TACAAAGTGATTAGAACATATT	AATATGTTATTCTAATCACTTGT

X indicates a position of amber codon in the *TOM22* gene.

James, P., J. Halladay, and E.A. Craig. 1996. Genomic libraries and a host strain designed for highly efficient two-hybrid selection in yeast. *Genetics*. 144: 1425–1436.

Kawai, A., S. Nishikawa, A. Hirata, and T. Endo. 2001. Loss of the mitochondrial Hsp70 functions causes aggregation of mitochondria in yeast cells. *J. Cell Sci.* 114: 3565–3574.

