

## SUPPLEMENTARY DATA

Supplementary Figure S1. The PPR motifs in *S. pombe* Ppr10. The PPR motifs were identified by TPRpred. The sequence logo for the fission yeast PPR motifs was derived from 348 PPR motifs identified from fission yeast *S. pombe*, *S. cryophilos*, *S. octosporus* and *S. japonicus* PPR proteins, and created using WebLogo (<http://weblogo.berkeley.edu/logo.cgi>). The height of each amino acid residue indicates the level of conservation at that position. non-polar, aliphatic residues (G, A, V, L, I, P) were colored blue, aromatic residues (F, Y, W) colored purple, polar, non-charged residues (S, T, C, M, N, Q) colored green, positively charged residues (K, R, H) colored red, negatively charged residues (D, E) colored orange. The secondary structure of the PPR motifs is also indicated.  $\alpha$ -helices and loops are indicated by rods and solid lines, respectively.

Supplementary Figure S2. Predicted secondary structure of Ppr10. Secondary structure of Ppr10 predicted with PSIPRED are shown below the sequence.  $\alpha$ -helices and loops are indicated by rods and solid lines, respectively.

Supplementary Figure S3. Deletion of *ppr10* results in aggregation of the cells. (A)  $\Delta$ *ppr10* cells display cell shape phenotypes. The cells are grown to stationary phase and morphology was examined by microscopy. Bar, 10  $\mu$ m. (B)  $\Delta$ *ppr10* cells display the flocculent phenotype. Cells were grown to stationary phase, vortexed well, allowed to stand for the indicated times, and photographed. Flocculation causes the cells to fall out of suspension and to sink quickly to the bottom of the tube.

Supplementary Figure S4. None of the introduced tags impairs protein function. (A) A 10-fold dilution spot assay of tagged strains used in this study. Cells were grown to log phase in YES and 10-fold serial dilutions were spotted onto YES media containing 3% glucose (YES+Glu) or 6% glycerol (YES+Gly). (B) Western blot analysis of *S. pombe* extracts of tagged strains used in A, using anti-CBP Ab to detect Ppr10-TAP, anti-c-Myc Ab to detect Ppr10-Myc, anti-HA Ab to detect Mpa1-HA, anti-FLAG Ab to detect Mti2 and anti-Mpa1 Ab to detect Mpa1 and Mpa1-HA. Sla1 serves as the loading control. Positions of molecular weight markers (in kDa) are

indicated on the right. *S. pombe* cells were grown to log phase in YES, and cell extracts were prepared by alkaline extraction.

Supplementary Figure S5. Predicted secondary structure of Mpa1. See the legend of Supplementary Figure S2 for description.

Supplementary Figure S6. RNase A treatment does not abolish the association between Ppr10 and Mpa1. Associated Cells expressing chromosomally encoded Ppr10-Myc and Mpa1-HA were grown to mid-log phase in YES medium, lysed by glass bead beating. Extracts were treated with RNase A prior to anti-HA IP. Extracts and immunoprecipitates were analyzed by Western blotting with anti-HA to detect Mpa1-HA and anti-*c*-Myc Ab to detect Ppr10-Myc. The amount of input (In) is 2.5% of the lysate used for IP. As a control, IP was performed on an extract from wild-type cells expressing chromosomally encoded Ppr10-Myc.

Supplementary Figure S7. Deletion of *mpa1* causes flocculation.  $\Delta$ *mpa1* cells display the flocculent phenotype. Cells were grown to stationary phase, vortexed well, allowed to stand for the indicated times, and photographed. Flocculation causes the cells to fall out of suspension and to sink quickly to the bottom of the tube.

Supplementary Figure S8. qRT-PCR analysis of expression of *S. pombe ppr10* in the wild-type and *mpa1* deletion mutant cells. All mRNA levels were normalized to the *htb1* mRNA level and expressed as fold change relative to the wild-type cells, which was set at a value of 1. Data are represented as mean  $\pm$  SD. Statistical analyses were performed using the Student's *t* test.

Supplementary Figure S9. RNase A treatment does not abolish the association between Ppr10 and Mti2. Associated cells expressing chromosomally encoded Ppr10-Myc and Mti2-FLAG were grown to mid-log phase in YES medium, lysed by glass bead beating. Extracts were treated with 0.05 mg/ml RNase A for 30 min at 25 °C, and subjected to anti-*c*-Myc IPs. Extracts and immunoprecipitates were analyzed by Western blotting with anti-*c*-Myc Ab to detect Ppr10-Myc and anti-FLAG to detect Mti2-FLAG. The amount of input (In) is 4% of the lysate used for IP. As a

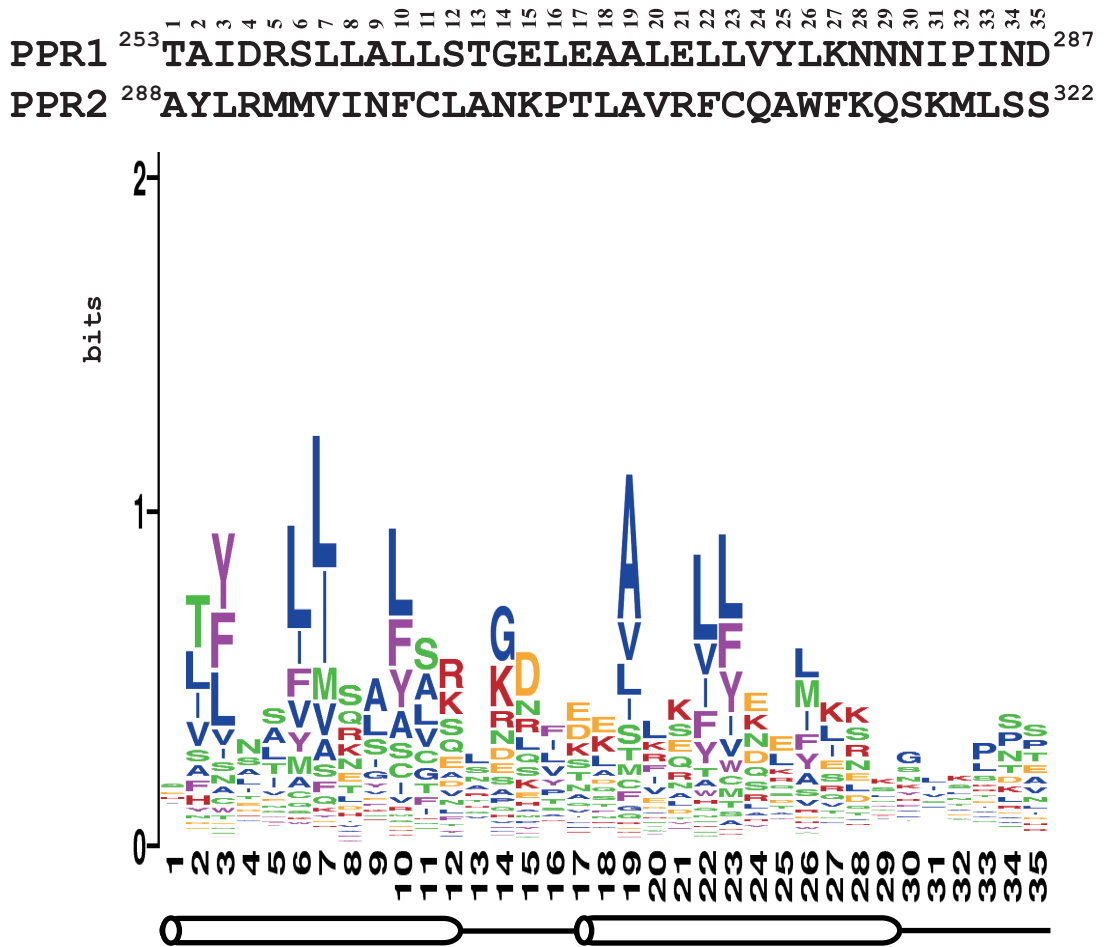
control, IP was performed on an extract from wild-type cells expressing chromosomally encoded Mti2-FLAG.

Supplementary Table S1. The *S. pombe* strains used in this study

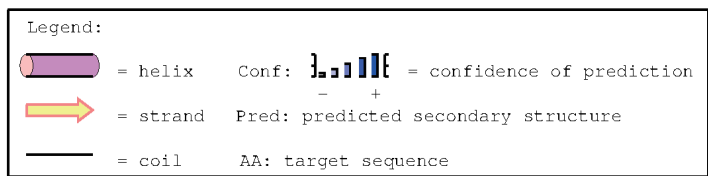
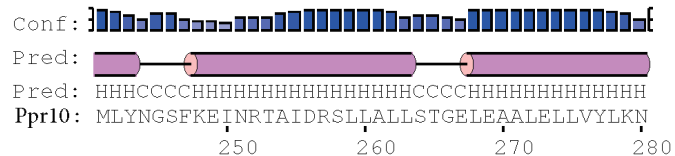
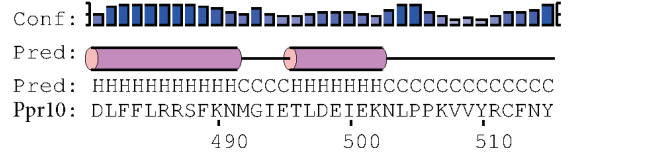
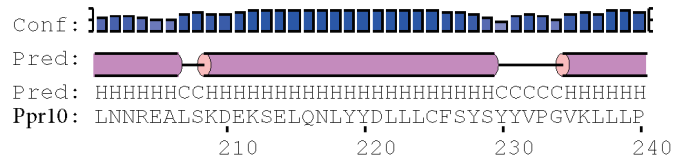
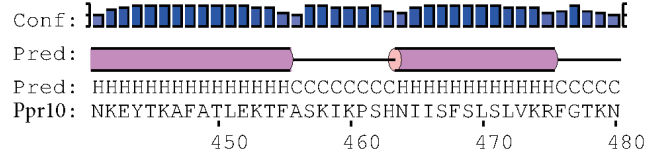
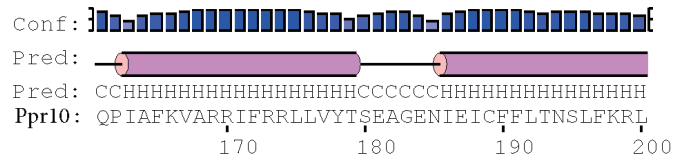
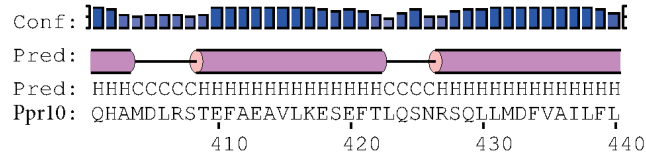
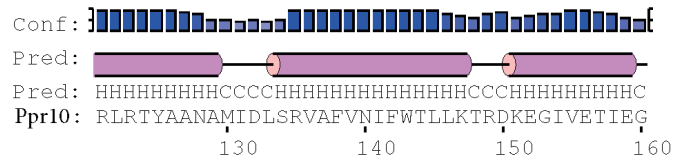
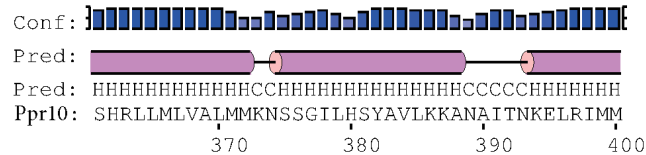
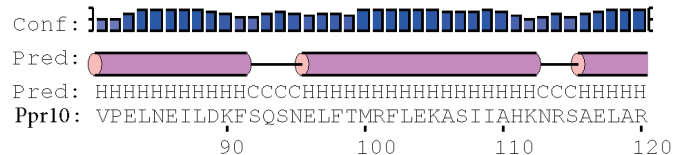
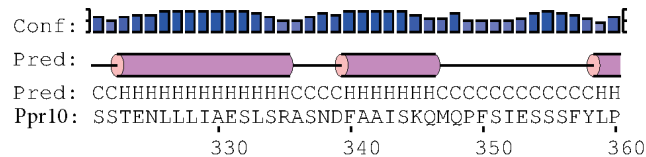
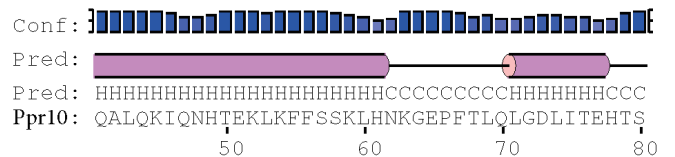
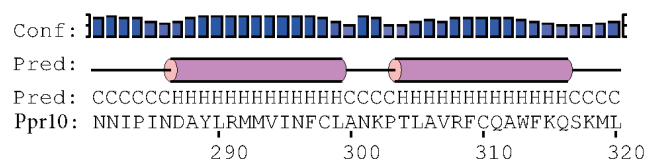
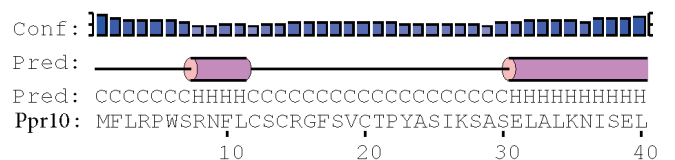
Supplementary Table S2. Primer sequences used in this study

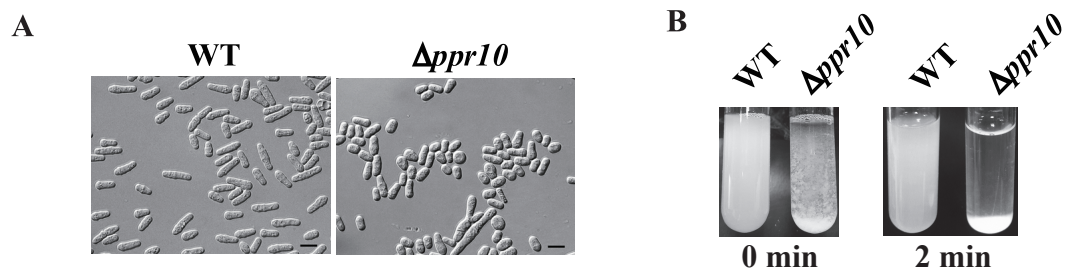
Supplementary Table S3. Peptide sequences identified from mass spectrometric analyses of in-gel tryptic digestions of protein bands.

Supplementary Figure S1

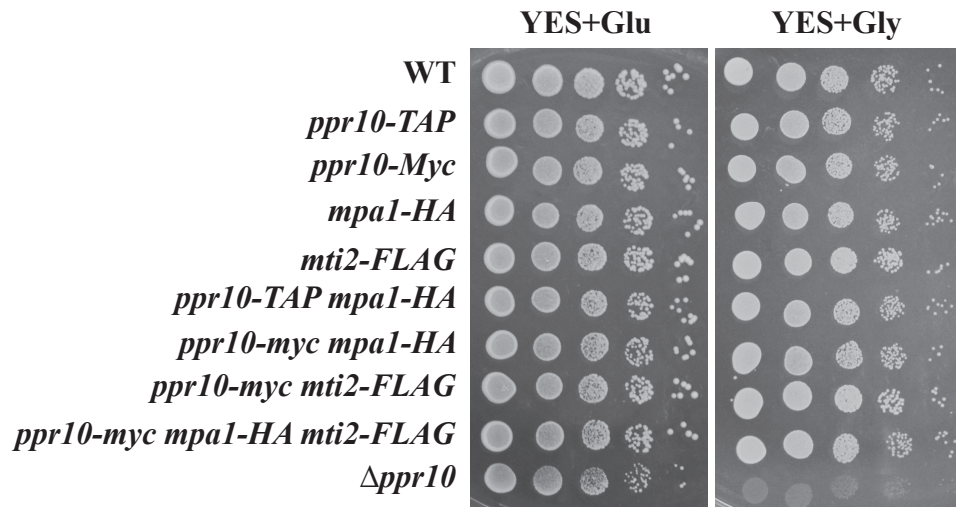


# Supplementary Figure S2

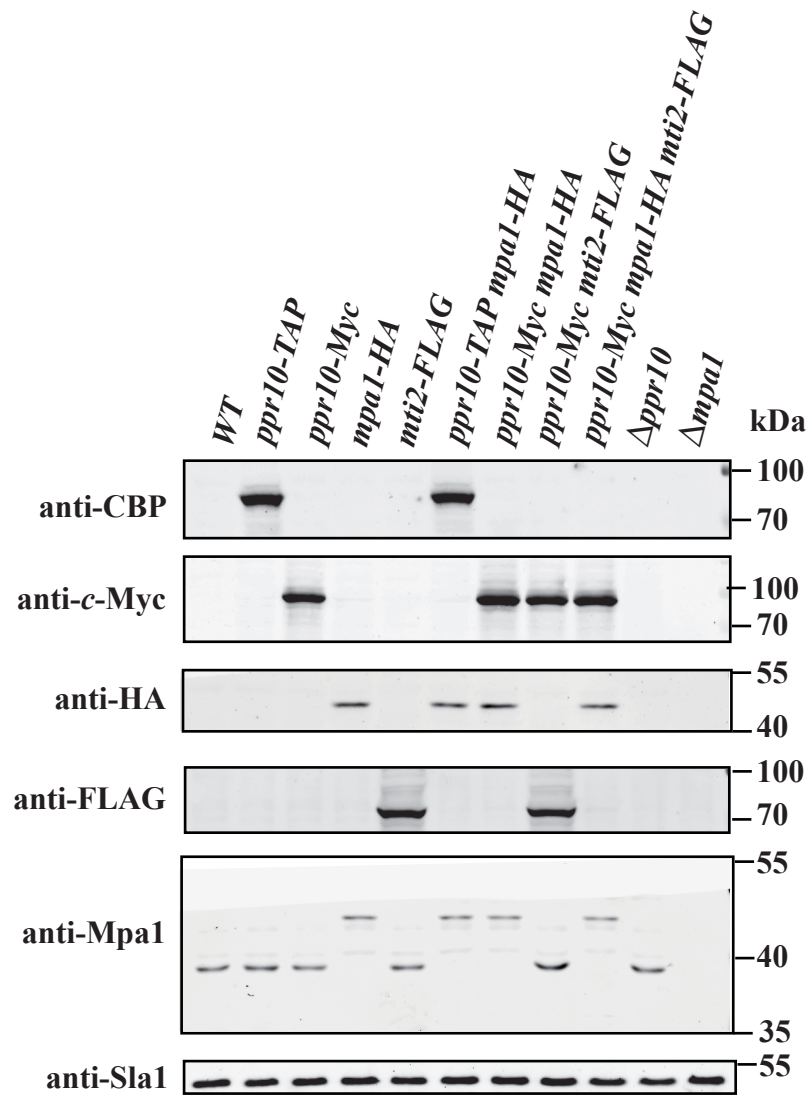




**A**



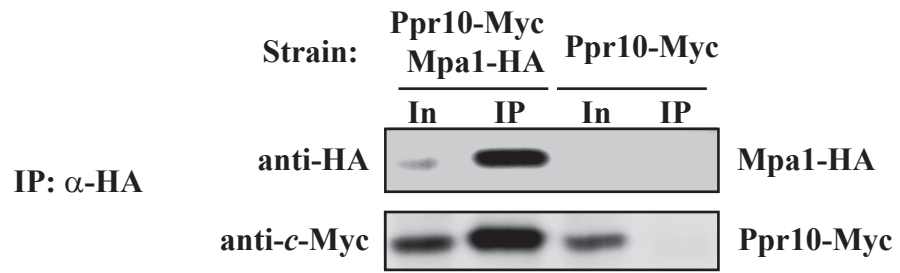
**B**



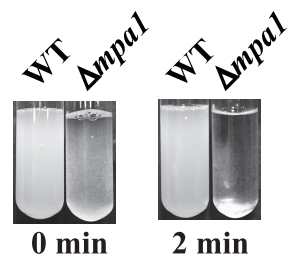


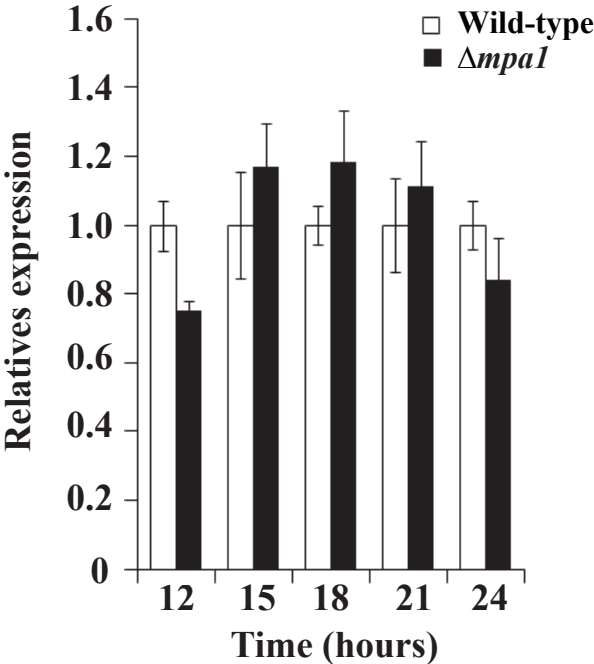


Supplementary Figure S6

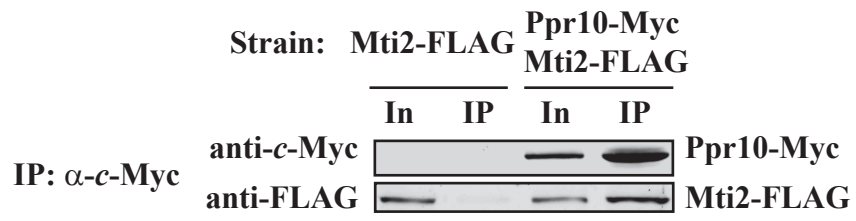


Supplementary Figure S7





Supplementary Figure S9



Supplementary Table S1. *S. pombe* strains used in this study

Strain	Genotype	Source
yHL6381	<i>h<sup>+</sup> leu1-32 his3-D1 ura4-D18 ade6-M210</i>	H. Levin
yHH1	<i>h<sup>+</sup> leu1-32 his3-D1 ura4-D18 ade6-M210 Δppr10::kanMX6</i>	This paper
yZQ1	<i>h<sup>+</sup> leu1-32 his3-D1 ura4-D18 ade6-M210 Δmpa1::kanMX6</i>	This paper
yWP1	<i>h<sup>+</sup> leu1-32 his3-D1 ura4-D18 ade6-M210 ppr10::[ppr10-TAP-kanMX6]</i>	This paper
yWP2	<i>h<sup>+</sup> his3-D1 ura4-D18 ade6-M210 mpa1::[mpa1-3HA-leu1<sup>+</sup>]</i>	This paper
yYJ1	<i>h<sup>+</sup> leu1-32 his3-D1 ura4-D18 ade6-M210 ppr10::[ppr10-13MYC-kanMX6]</i>	This paper
yYJ2	<i>h<sup>+</sup> leu1-32 his3-D1 ura4-D18 ade6-M210 ppr10::[ppr10-TAP-kanMX6]</i> <i>mpa1::[mpa1-3HA-leu1<sup>+</sup>]</i>	This paper
yYJ3	<i>h<sup>+</sup> leu1-32 his3-D1 ura4-D18 ade6-M210 ppr10::[ppr10-13MYC-kanMX6]</i> <i>mpa1::[mpa1-3HA-leu1<sup>+</sup>]</i>	This paper
yYJ4	<i>h<sup>+</sup> leu1-32 his3-D1 ura4-D18 ade6-M210 Δppr10::kanMX6</i> <i>mpa1::[mpa1-3HA-leu1<sup>+</sup>]</i>	This paper
yYJ5	<i>h<sup>+</sup> leu1-32 his3-D1 ura4-D18 ade6-M210 ppr10::[ppr10-13MYC-ura4<sup>+</sup>]</i>	This paper
yYJ6	<i>h<sup>+</sup> leu1-32 his3-D1 ura4-D18 ade6-M210 ppr10::[ppr10-13MYC-ura4<sup>+</sup>]</i> <i>Δmpa1::kanMX6</i>	This paper
yYJ7	<i>h<sup>+</sup> his3-D1 ura4-D18 ade6-M210 ppr10::[ppr10-13MYC-ura4<sup>+</sup>]</i> <i>Δmpa1::kanMX6 leu1-32::[pJK148(leu1<sup>+</sup>)]</i>	This paper
yYJ8	<i>h<sup>+</sup> his3-D1 ura4-D18 ade6-M210 ppr10::[ppr10-13MYC-ura4<sup>+</sup>]</i> <i>Δmpa1::kanMX6 leu1-32::[mpa1-3HA-leu1<sup>+</sup>]</i>	This paper
yYJ9	<i>h<sup>+</sup> his3-D1 ura4-D18 ade6-M210 ppr10::[ppr10-13MYC-ura4<sup>+</sup>]</i> <i>leu1-32::[pJK148(leu1<sup>+</sup>)]</i>	This paper
yYJ10	<i>h<sup>+</sup> leu1-32 his3-D1 ura4-D18 ade6-M210 ppr10::[ppr10-13MYC-kanMX6]</i> <i>mpa1::[mpa1-3HA-leu1<sup>+</sup>]</i> <i>tom20::[tom20-2FLAG-ura4<sup>+</sup>]</i>	This paper
yYJ11	<i>h<sup>+</sup> leu1-32 his3-D1 ura4-D18 ade6-M210 ppr10::[ppr10-13MYC-kanMX6]</i> <i>mti2::[mti2-2FLAG-ura4<sup>+</sup>]</i>	This paper
yYJ12	<i>h<sup>+</sup> leu1-32 his3-D1 ura4-D18 ade6-M210 mti2::[mti2-2FLAG-ura4<sup>+</sup>]</i>	This paper
yYJ13	<i>h<sup>+</sup> leu1-32 his3-D1 ura4-D18 ade6-M210 ppr10::[ppr10-13MYC-kanMX6]</i> <i>mti3::[mti3-2FLAG-ura4<sup>+</sup>]</i>	This paper
yYJ14	<i>h<sup>+</sup> leu1-32 his3-D1 ura4-D18 ade6-M210 mti3::[mti3-2FLAG-ura4<sup>+</sup>]</i>	This paper
yZQ2	<i>h<sup>+</sup> his3-D1 ura4-D18 ade6-M210 Δmpa1::kanMX6</i> <i>leu1-32::[pJK148(leu1<sup>+</sup>)]</i>	This paper
yZQ3	<i>h<sup>+</sup> his3-D1 ura4-D18 ade6-M210 Δmpa1::kanMX6</i> <i>leu1-32::[mpa1-3HA-leu1<sup>+</sup>]</i>	This paper
yMX1	<i>h<sup>+</sup> leu1-32 his3-D1 ura4-D18 ade6-M210 Δppr10::kanMX6/pREP82X</i>	This paper
yMX2	<i>h<sup>+</sup> leu1-32 his3-D1 ura4-D18 ade6-M210 Δppr10::kanMX6</i> <i>/pREP82X-ppr10</i>	This paper
yMX3	<i>h<sup>+</sup> leu1-32 his3-D1 ura4-D18 ade6-M210 Δppr10::kanMX6</i> <i>/pREP82X-ppr10ΔPPR</i>	This paper
yMX4	<i>h<sup>+</sup> leu1-32 his3-D1 ura4-D18 ade6-M210 Δppr10::kanMX6</i> <i>/pREP82X-5FLAG</i>	This paper
yMX5	<i>h<sup>+</sup> leu1-32 his3-D1 ura4-D18 ade6-M210 Δppr10::kanMX6</i> <i>/82X-ppr10-5FLAG</i>	This paper
yMX6	<i>h<sup>+</sup> leu1-32 his3-D1 ura4-D18 ade6-M210 Δppr10::kanMX6</i> <i>/82X-ppr10ΔPPR-5FLAG</i>	This paper
ySM1	<i>h<sup>+</sup> leu1-32 his3-D1 ura4-D18 ade6-M210 ppr10::[ppr10-13MYC-ura4<sup>+</sup>]</i> <i>Δmpa1::kanMX6 Δlon1::hphMX6</i>	This paper
ySM2	<i>h<sup>+</sup> leu1-32 his3-D1 ura4-D18 ade6-M210 Δppr10::kanMX6</i> <i>Δmpa1::hphMX6</i>	This paper
ySM3	<i>h<sup>+</sup> leu1-32 his3-D1 ura4-D18 ade6-M210 ppr10::[ppr10-13MYC-ura4<sup>+</sup>]</i> <i>Δlon1::hphMX6</i>	This paper
Cy0989	<i>h<sup>-</sup> ptp1-1 rho0 ade6M-216 leu1-32</i>	J. Liu

Supplementary Table S2. Primers and oligonucleotides used in this study

Name	Primer sequence (5' to 3')
<b>Primer sequences for qRT-PCR analysis of mt-RNAs</b>	
htb1-f	GGATTTGTTGTAGCAAGTCGAGTAT
htb1-r	ATGATAGCCACAACGTCTTGTATG
cox1-exon12-RT-5	TGGACGGTATATCCACCACT
cox1-exon12-RT-3	GTCGCTATTAAATTTACTGATCC
cox2-2-f	AAGTGGTGATGTTATCCATAGTTGG
cox2-2-r	AGATACACCTTGAACAACAATAGGC
cox3-f	CCACCAGTAGGAATAGCAGATAAAA
cox3-r	TGAGCATAAGTTAAACTAGCACCAG
cob-RT-5	GCCTTTTGTATTGCTGCTTTA
cob-RT-3	GTTATCAAAT CTTTTATCAG ATAAT
atp6-f	TACCTTCTGGTACTCCTACTCC
atp6-r	TAGCACCTAA TCGAATACCT AAACCT
atp8-RT-5	ATGCCACAATTAGTACCATTCT
atp8-RT-3	AAAGAACTTATAATAGATCTTGAG
atp9-f	GGTGCTGGTGTGGTATTGGA
atp9-r	ACCTGTAGCTTCTGTAAAGGCG
var1-f	AGAGCTCTTCCTATTTCAACTCCTT
var1-r	ACCTTTCCATCCTTTTGGTACA
rnpB-f	GGAAAGTTTGGATCACTCATAACATTAG
rnpB-r	TAATATTAGTAGACTTACCCTCTTGGG
rns-f	GAAGGAGGAATTGCGAGTAATCAC
rns-r	CGACTTAACACTAATTGCACAACACC
rnl-f	GTAGCACGGTAGTAAAGCCAAATTG
rnl-r	TAAGGATTTGTACATCCTAAGGATGTCC
<b>Primer sequences for qRT-PCR analysis of expression of <i>ppr10</i> in the absence of <i>mpa1</i></b>	
ppr10-f	GGACCTCCGATCAACTGAATTCCG
ppr10-r	CTCATCCAGAGTTTCTATGCCCATG
<b>Primer sequences for RT-PCR analysis of immunoprecipitated RNA</b>	
cox1-UTR-f	AATCTTAATGATCTTTTCATCTACC
cox1-UTR-r	AGTATGGCAATATCCTTAGC
cox2-UTR-f	CTTAAATAATAATTTTGAAACTATTAC
cox2-UTR-r	AGATGCACCATCTTGGAA
cox3-UTR-f	AGCTATCCTATTCAA AATTGTATC
cox3-UTR-r	AAGGTGACGCACTTACAAT
cob-UTR-f	ATCTAACCTATCTTATCTTTTATTATC
cob-UTR-r	AGGTT CAGGAGCATCAATC
atp6- UTR-f	GGATAAAAAATCTCTAAAAGATTTTTC
atp6- UTR-r	CCAAAATTAGAGAAATCAA AATGG
Atp8- UTR-f	GTTGTATGGAATTTTCAATGAAG
Atp8- UTR-r	AATAGAATGGTACTAATTGTGGC
Atp9- UTR-f	AAAGTCTACAGATAAAAAAATCAC
Atp9- UTR-r	TCCAATACCAACACCAGCACC

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Var1- UTR-f	CACTTTATTAGTGGGATGG
Var1- UTR-r	GGTATTGAAAAATTAGCTTTTTG
rns-f	GAAGGAGGAATTGCGAGTAATCAC
rns-r	CGACTTAACACTAATTGCACAACACC
rnl-f	GTAGCACGGTAGTAAAGCCAAATTG
rnl-r	TAAGGATTTGTACATCCTAAGGATGTCC
mt-tRNA <sup>Leu</sup> -f	TTAGGGAAGTATGCAGATGT
mt-tRNA <sup>Leu</sup> -r	TCCTACAGATGGAAGGATTCCG
actin-f	TCCGCTCTTAACATCTCATGAGG
actin-r	AAGGCTAGCTCTGCATTCGTCTAT

### Oligonucleotide sequences for Northern blot analysis

mt-tRNA <sup>Lys</sup>	CCTTATCCTATCGCTTAAAAG
mt-tRNA <sup>Arg</sup>	CTCTAACCAATTAAGCTAAAAGT
mt-tRNA <sup>His</sup>	GCCACAAATAAGCATTCT
mt-tRNA <sup>Phe</sup>	CTTCAATACTGCACTTTAACCG
mt-tRNA <sup>Leu</sup>	GGTAAACATGTTTACCAATTTTCATC
mt-tRNA <sup>Asp</sup>	GCGATACTTTACTTTAAGCTACAG
mt-tRNA <sup>Gly</sup>	AAATCAGAAGGTCTGCCAAT TGAC
cob1-exon2-probe-5	GAGCTATACCTAACTTCCAATTAGG
cob1-exon2-probe-3	ATAGAAGATTGTAGCAATAGCACCG
cox1-exon2-probe-5	CCACCACTATCAAGTATCACTTC
cox1-exon2-probe-3	CCTCTGGATGACCAAAGAAC
cox2-probe-5	GACTGTTAAGGCAATTGGAAGACAATGG
cox2-probe-3	GAGATACACCTTGAACAACAATAGGC
cox3-probe-5	GGAGGTCAAGCTTATGAATACTGG
cox3-probe-3	CCAAACAACATCACAGAAATGCC
atp6-probe-5	CTGTATCAGGACAAAGCTATTTCCC
atp6-probe-3	TAGCACCTAATCGAATACCTAAACTT
RNL-probe-5	GCAAATTAGCTCTGTTACTTCGGTA
RNL-probe-3	TCTAGCTGAGGGAACACTGTATCTT
RNS-probe-5	TAGATCGCGAAAGAGATTAGATACC
RNS-probe-3	TATATCACGTCTATAGCCCTTTCCA
atp9-probe	ATTAAGTTACTGAAGATTAATCCAA
	TACCAACACCAGCACCCTAACACC
rnpB-probe	ACCCTCTTGGGTTTCTTTTTTATTAA
	CCACTTTAAACAAATACTATTCTA
ATP8-probe-5	TGCCACAATTAGTACCATTCTA
ATP8-probe-3	CATTATATCTTGGTAATACATACTGATG
Var1-probe	GTAATTTGGGTAAATTAATAAATCGTTA
	GGATCTATCCATAATGTAG

### Primer sequences (5' to 3') for mtDNA copy number determination

spo12-f	TCGGCTCTAAGAAGGTATCTGTATC
spo12-r	AGTACCAGATCTGCCTGAGTAGTTG
ace2-f	CAAGACAAAATCTACTCCAAGTCGT
ace2-r	CATTAACCAAGTAGCGAGAACGTAT
exg1-f	CTACCTCGGTTAATTGGACTTTGTA
exg1-r	TCTTCAGGATCCTACATAGAAAACC
cox1-mtDNA-f	CGGTGTTGTTAGTCACATTATTCCT

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cox1-mtDNA-r	TGCAGCACTGAAATAAGCTCTAGTA
cox3-mtDNA-f	CCACCAGTAGGAATAGCAGATAAAA
cox3-mtDNA-r	TGAGCATAAGTTAAACTAGCACCCAG
cob1-mtDNA-f	TGCAAATGGTGCTAGTTTCTTC
cob1-mtDNA-r	TAGTAATAACAGTTGCACCCCAGA
atp9 mtDNA-f	GGTGCTGGTGTGGTATTGGA
atp9 mtDNA-r	ACCTGTAGCTTCTGTTAAGGCG

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Supplementary Table S3. Peptide sequences identified from mass spectrometric analyses of in-gel tryptic digestions of protein bands.

Name	NCBI accession number <sup>a</sup>	Peptide sequence <sup>b</sup>	Score <sup>c</sup>	Observed m/z	Mr (exp) <sup>d</sup>	Mr (calc)
Mpa1	gi:19115049	<sup>27</sup> GYRPSEGIWFR <sup>37</sup>	33	1367.6	1366.6	1366.7
Act1	gi:1304269	<sup>85</sup> IWHHTFYNELR <sup>95</sup>	56	1515.7	1514.7	1514.7
		<sup>239</sup> SYELPDGQVITIGNER <sup>254</sup>	88	1790.8	1789.8	1789.9
Tdh1	gi:19112946	<sup>310</sup> LVSWYDNEWGYSR <sup>322</sup>	84	1674.8	1673.8	1673.7

<sup>a</sup>NCBI accession number of the matching protein.

<sup>b</sup>Sequence information from the mass spectrometry data were searched against the NCBI non-redundant protein database. Peptide matches were checked manually and only those identifications with an ions score of 22 or higher were accepted.

<sup>c</sup>The reported ions score is  $-10 \log(P)$ , where P is the probability that the observed match is a random event. Individual ions score  $>22$  indicate identity or extensive homology ( $p < 0.05$ ).

<sup>d</sup>The determined molecular mass of the closest matching peptide.

<sup>e</sup>The theoretical mass of the closest matching peptide.