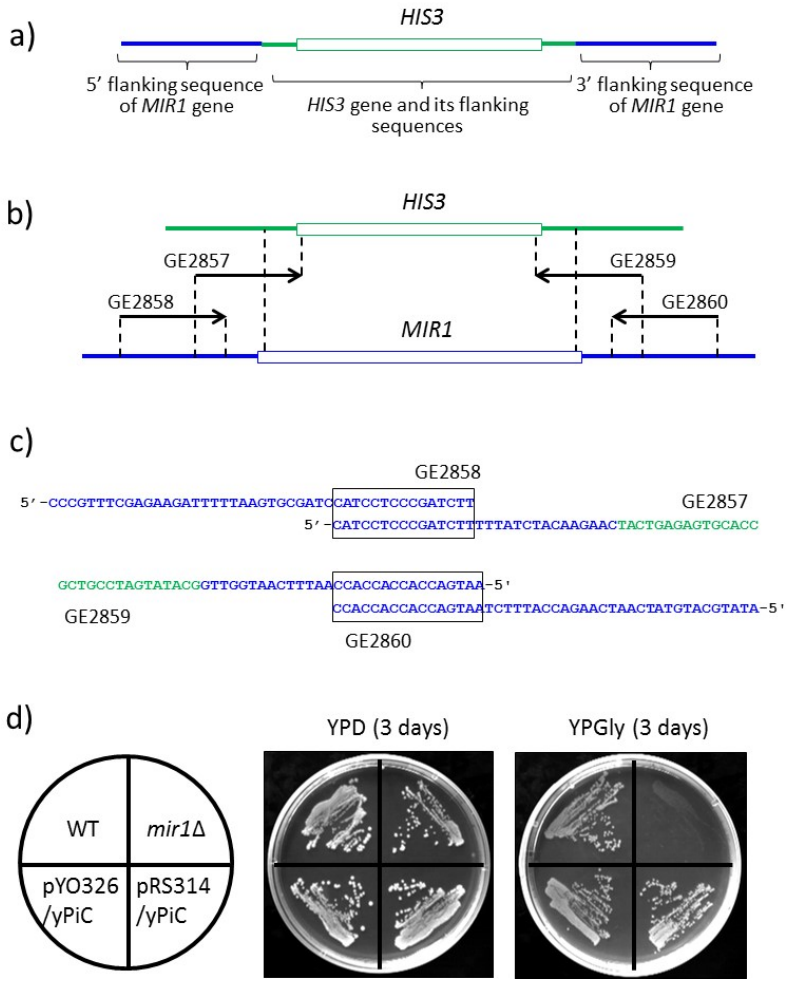
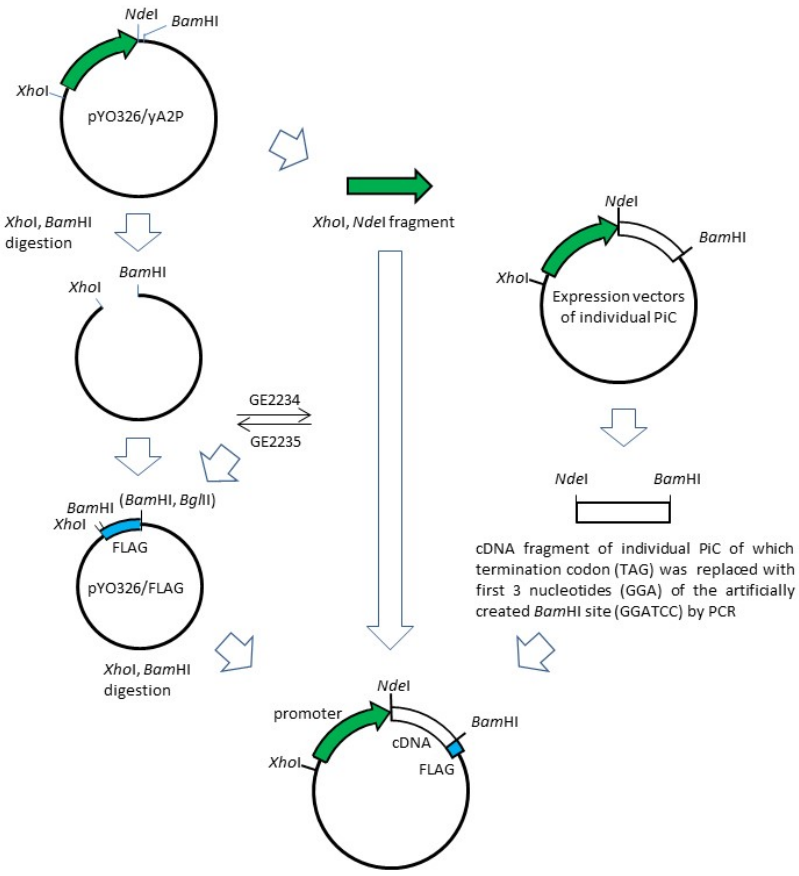


Supplementary Fig. S1. Targeting construct used for disruption of the yeast *MIR1* gene and growth of yeast cells on a glycerol plate

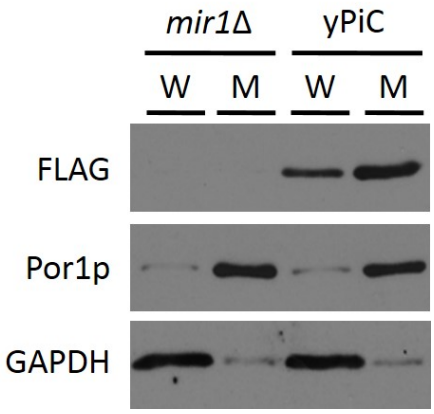
Panel a, Structure of the final DNA fragment used for disruption of the yeast *MIR1* gene. The 5' and 3' flanking sequences of the yeast *MIR1* gene were nucleotides 578256-578315 and 577286-577228, respectively, with accession number NC_001142 (*Saccharomyces cerevisiae* S288c chromosome X, complete sequence). The *HI3* gene and its flanking sequences covered nucleotides 169-1375, with accession number U03439 (Yeast centromere vector pRS313 with *HIS3* marker, complete sequence). **Panel b,** How the primers used for preparation of the target construct were designed. **Panel c,** Nucleotide sequences of the individual primers. The nucleotide sequences shared between primers GE2858 and GE2857, and those between GE2859 and GE2860, are shown by boxes. In panels "a" – "c", regions of the nucleotide sequence derived from the *MIR1* gene and *HIS3* gene are shown in blue and green, respectively. **Panel d,** Growth-defect phenotype of yeast cells lacking a functional *MIR1* gene (*mir1*Δ) on a glycerol plate and rescue from this defect by introduction of an expression vector for the yeast phosphate carrier. Culture suspensions of wild-type (WT) yeast cells, cells lacking a functional *MIR1* gene (*mir1*Δ), and transformants of the *mir1*Δ strain produced with a single-copy type (pRS314/yPiC) or multi-copy type (pYO326/yPiC) expression vector for yPiC were streaked on YPD or YPGly plates. After incubation at 30° C for 3 days, cell growth on the plates was assessed photographically.



Supplementary Fig. S2. Preparation of expression vectors of PiCs having a FLAG tag at their C-terminal end

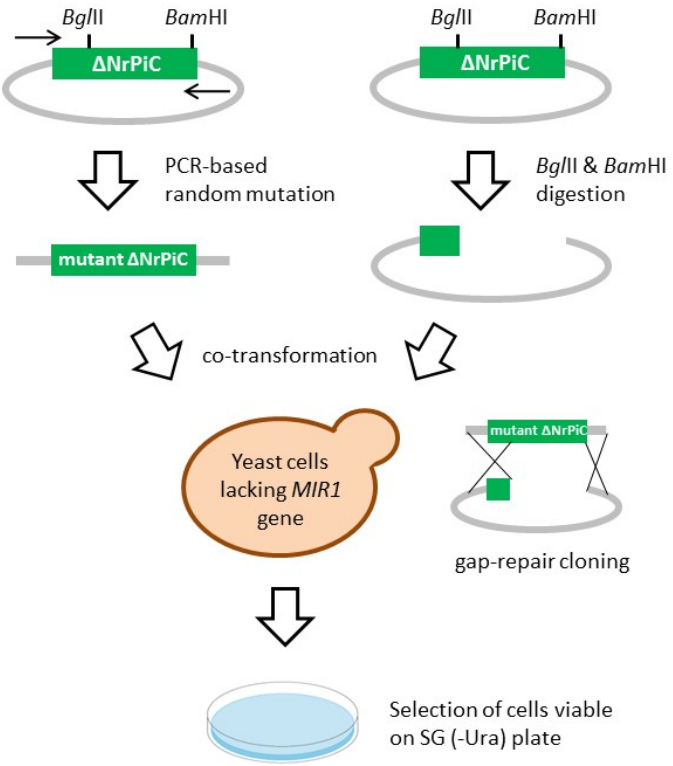
For preparation of expression vectors of PiCs having a FLAG tag at their C-terminal end, we first constructed a plasmid, referred to as pYO326/FLAG, containing a nucleotide sequence encoding the FLAG tag. For this construction, the multicopy-type expression vector, pYO326/yA2P, was digested with *Xho*I and *Bam*HI. Then, oligonucleotides of GE2234 (5'-TCGAGTTAGGATCCGACTACAAGGATGACGATGACAAGTA) and GE2235 (5'-GATCTACTTGTCATCGTCATCCTTGTAGTCGGATCCTAACT) were annealed and inserted between these two restriction sites, as shown in the figure. Names of the active restriction sites are shown, and those of inactivated sites are shown in parentheses.

Next, DNA fragments corresponding to the open reading frame of individual PiCs were re-amplified by PCR using GE2047 (5'-TACAAGTCAAAGGAGCCCC) and GE2867 (5'-GAGGATCCATGACCACCACCACCAATTT) for yPiC, GE2047 and GE2866 (5'-TCGGATCCCTGAGTTAACCCAAGCTTCT) for hPiCs or GE2407 and GE2869 (5'-GAGGATCCCTCAGTTAACCCAAGCTTC) for rPiCs. During amplification of these DNA fragments, the translation termination codon (TAG) present at their 3'-end was replaced with the first 3 nucleotides (GGA) of the artificially created *Bam*HI site (GGATCC). These amplified DNA fragments were digested with *Nde*I and *Bam*HI and then gel purified. In addition to the amplified DNA fragments thus prepared, a DNA fragment obtained by digestion of expression vectors with *Xho*I and *Nde*I, corresponding to the promoter region of the vector, was inserted between *Xho*I and *Bam*HI sites of the pYO326/FLAG vector. The resulting expression vector coded for proteins having a C-terminal extension of GlySerAspTyrLysAspAspAspAspLys.



Supplementary Fig. S3. Evaluation of the mitochondrial fraction prepared from yeast cells by Western blotting.

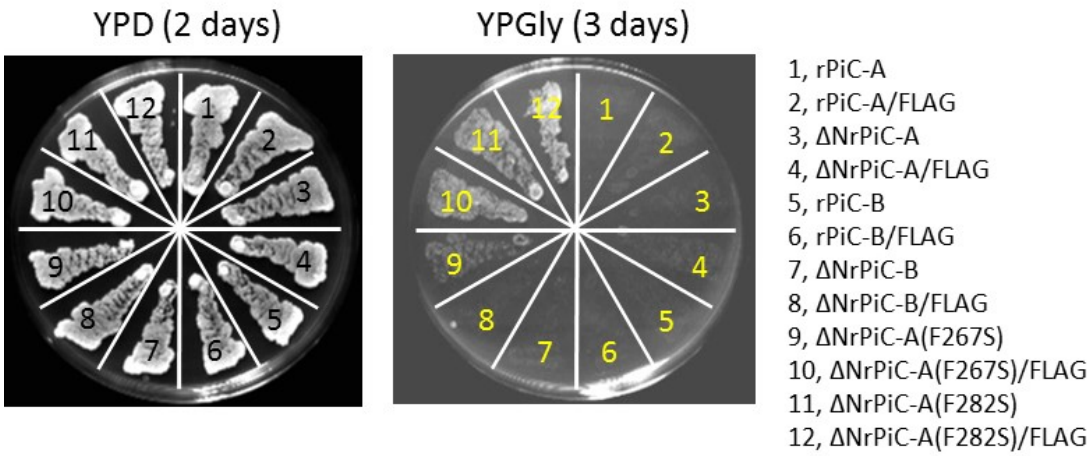
Whole cellular lysate (W) and mitochondrial fraction (M) obtained from *mir1Δ* cells or that transformed with expression vector of yPiC (yPiC) were subjected to the SDS-PAGE and subsequent Western blotting using anti FLAG antibody (for detection of PiC) and antibodies against Por1p and GAPDH.



#

Supplementary Fig. S4. Experimental procedures used for the PCR-based mutation and gap-repair cloning

To make randomly mutated cDNA fragments encoding rPiC, we performed PCR with *Taq* DNA polymerase and the expression vector ΔNrPiC (rPiC lacking N-terminal presequence) as a template. Two horizontal arrows indicate positions and directions of primers (GE2046 and GE2047) used for amplification of cDNA. The resulting PCR products (i.e., mutated cDNA fragments) were mixed with the expression vector whose protein coding sequence had been eliminated, and yeast cells lacking *MIR1* gene were transformed with the mixture of the DNA.



Supplementary Fig. S5. Growth properties of the yeast cells lacking functional *MIR1* gene (*mir1Δ*) and transformed with various expression vectors on a glycerol plate

To enable the comparison of the expression levels of individual PiCs by Western blotting, we prepared expression vectors of PiC having a flanking sequence of "FLAG" at their C-terminal ends. To examine whether the addition of this FLAG sequence would alter the functional properties of individual PiCs, we compared the growth properties of yeast cells transformed with the expression vectors encoding individual PiCs with/without the FLAG sequence.###

Supplementary Table SI. PCR strategies, template DNAs, and nucleotide primers used for preparation of DNA fragments encoding individual PiCs.

For preparation of [a]		Template DNA		Primer		
				Direction [c]	Name	Nucleotide sequence [d]
DNA fragment encoding **y**PiC		genomic DNA of wild-type yeast cells		D	GE2660	5'-CTCATCTC**cat**ATGTCTGTGTCTGC [e]
				U	GE2661	5'- GA**gg**AT**cc**CTAATGACCACCACCACC [e]
cDNA fragments corresponding to the open reading frames of	**r**PiC-A [b]	cDNA libraries prepared from total RNA of rat	Heart	D	GE2670	5′-AGG**cat**ATGTTCTCGTCCGTAGCGC [e]
				U	GE2671	5′-G**g**AT**cc**CTACTCAGTTAACCCAAGC [e]
	rPiC-B [b]		Brain	D	GE2670	
				U	GE2671	
cDNA fragments encoding	ΔN**r**PiC-A	full-length cDNA fragments encoding	rPiC-A	D	GE2666	5'- ACCTGGCA**catatg**GCCGTGGAAGAG [e]
				U	GE2671	
	ΔN**r**PiC-B		rPiC-B	D	GE2666	
				U	GE2671	
cDNA fragments encoding **r**PiC(F282S)	5'-fragment	pRS314-yA2P/rPiC-A		D	GE2928	5′-ACTTCAGAATCATACATTAACATACATATG [f]
				U	GE2968	5′- GATTCGGGCG**g**AGAGCCCCTT [g]
	3'-fragment			D	GE2967	5′- AAGGGGCTCT**c**CGCCCGAATC [g]
				U	GE2929	5′-GCGGCCGCTCTAGAACTAGTGGATC [f]
	entire fragment	mixture of the cDNA encoding 5' and 3' fragments		D	GE2928	
				U	GE2929	
cDNA fragments corresponding to the open reading frames of	**h**PiC-A [b]	cDNA libraries prepared from total RNA of human	Heart	D	GE2595	5'-GGGAA**cAt**ATGTTCTCGTCCGTGGC [e]
				U	GE2596	5'-CTT**g**GATC**c**AACTACTGAGTTAACCCAAG [e]
	hPiC-B [b]		subcutaneous	D	GE2595	

		adipose tissue	U	GE2596	
cDNA fragments encoding	ΔN**h**PiC-A	pRS314-yA2P/hPiC-A	D	GE2666	
			U	GE2596	
	ΔN**h**PiC-B	pRS314-yA2P/hPiC-B	D	GE2666	
			U	GE2596	
cDNA fragments encoding **h**PiC(F267S)	5'-fragment	pRS314-yA2P/hPiC-A	D	GE2928	
			U	GE2925	5′-ATGATACGGGCA**g**ACAGTCCCTTCC [g)]
	3'-fragment		D	GE2924	5′-GGAAGGGACTGT**c**TGCCCGTATCAT [g)]
			U	GE2929	
	entire fragment	mixture of the cDNA encoding 5' and 3' fragments	D	GE2928	
			U	GE2929	
cDNA fragments encoding **h**PiC(F282S)	5'-fragment	pRS314-yA2P/hPiC-A	D	GE2928	
			U	GE2927	5′-GAGTCATAGATA**g**ACCACTGTAGTG [g)]
	3'-fragment		D	GE2926	5′-CACTACAGTGGT**c**TATCTATGACTC [g)]
			U	GE2929	
	entire fragment	mixture of the cDNA encoding 5' and 3' fragments	D	GE2928	
			U	GE2929	

a) Target species are highlighted with bold characters of **y**(yeast), **h**(human) or **r**(rat).
b) Nucleotide sequences of individual PiCs were cited from NCBI database with accession numbers of NM_001181735.1 (yPiC), NM_005888.3 (hPiC-A), NM_002635.3 (hPiC-B), NM_001270788.1 (rPiC-A), and NM_139100.2 (rPiC-B).
c) Directions of primers are abbreviated as D and U for downstream and upstream, respectively.
d) The restriction sites of *Nde*I or *Bam*HI used for subcloning are underlined, and the mutated nucleotides are shown by bold lower-case letters.
e) Nucleotides in these primers were mutated to create artificial restriction sites.
f) These primers anneal to the nucleotide sequence in the vector used.
g) Nucleotides in these primers were mutated to alter the encoded amino acid.