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Supplementary Materials for

Mitochondria-encoded genes contribute to evolution of heat and cold tolerance in yeast

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The PDF file includes:

Other Supplementary Material for this manuscript includes the following:

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Supplementary Materials

Supplementary Text

High petite rate of *S. uvarum* mitotype and its association with *ORF1*

Saccharomyces yeast strains generate petites spontaneously at a rate of ~1%, and variants in nuclear genes can affect petite rates (*59*). We observed an extremely high petite rate in the hybrid with *S. uvarum* mitotype (48-61%, sometimes >90%), while the hybrid carrying *S. cerevisiae* mtDNA rarely generates petites (fig. S5A). The high petite rate associated with *S. uvarum* mtDNA is only seen in the interspecific hybrid, but not pure strains *S. uvarum*, suggesting a dominant incompatibility in mtDNA inheritance between hybrid nuclear genomes and *S. uvarum* mtDNA. However, we were able to isolate a few *S. cerevisiae* and *S. uvarum* hybrids that carried mostly *S. uvarum* mitochondrial genes but did not exhibit a high petite rate. These strains arose at a frequency of 1%, so they are likely spontaneous recombinants. Whole genome sequencing showed that they all carry *S. cerevisiae ORF1*, but the rest of their mitochondrial genome is *S. uvarum* (fig. S5C). This result suggests a strong link between *S. cerevisiae ORF1* and mtDNA inheritance. In the 90 recombinants generated from mutant crosses, we also observed a strong correlation between *S. cerevisiae ORF1* and low petite rates, although there were exceptions (fig. S5B).

The possible inheritance phenotype adds to our understanding of the interesting biology of *ORF1*. *ORF1* (F-*Sce*III) was suggested to encode a free-standing homing endonuclease (*60*). The best-known homing endonuclease is I-*Sce*I (ω), which promotes its spread to homing-less mitochondrial genomes (*61*). *ORF1* (F-*Sce*III) has been proposed to mediate mitochondrial recombination based on the high frequency of interspecific mitochondrial recombinants at the start of *ORF1* in wild *Saccharomyces* species and in a synthetic hybrid of *S. cerevisiae* × *S. mikatae* (*24, 62*). Although further work will be needed to demonstrate that *ORF1* affects mitochondrial inheritance, this activity would imply co-evolution between a selfish element and its host (*63*).

Fig. S1.

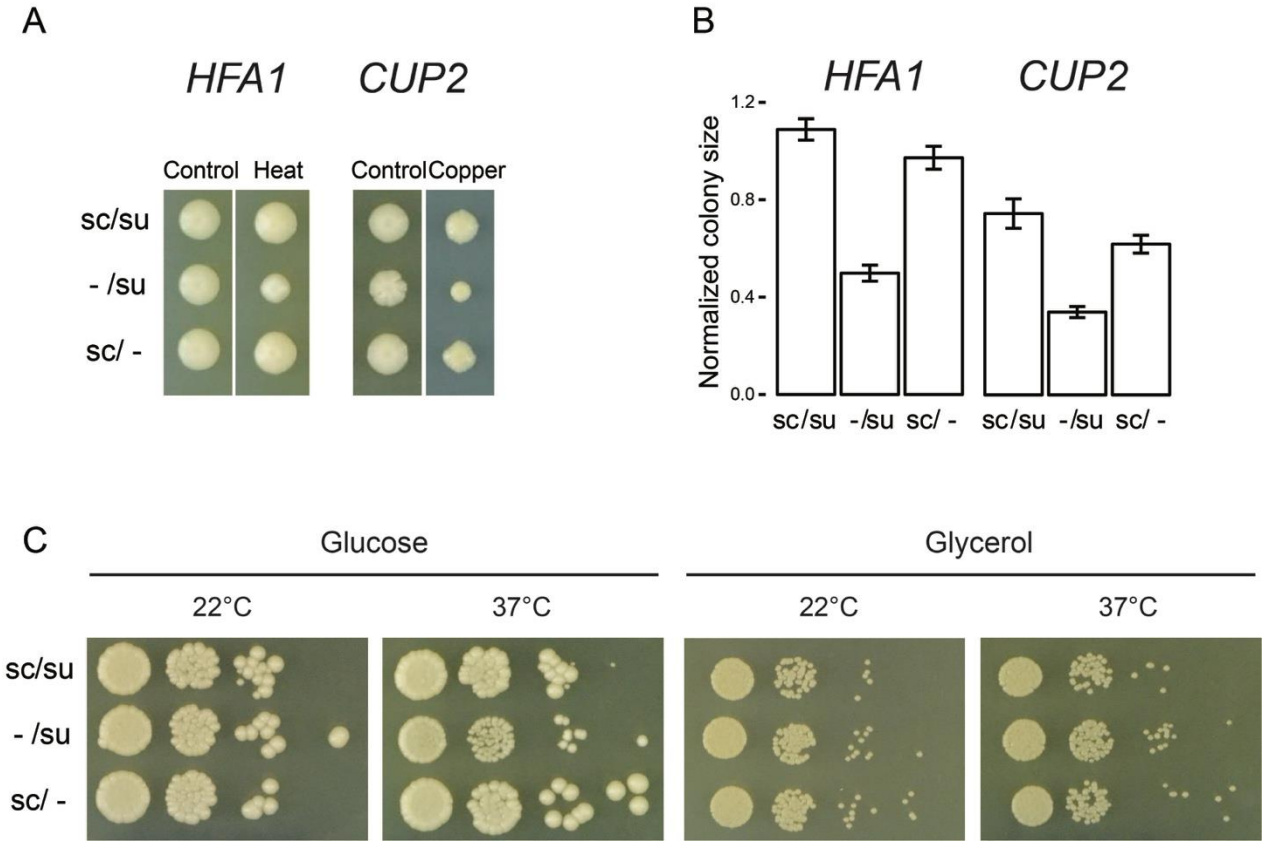


Fig. S1. Reciprocal hemizygosity test of *HFA1* and *CUP2*. (**A**) Hemizygotes with either the *S. cerevisiae* allele (sc/-) or *S. uvarum* allele (-/su) and a wild-type hybrid (sc/su) were compared under the same conditions as the non-complementation screen. Growth is after 5 days. (**B**) Heat or copper resistance was measured by colonies sizes normalized to control condition (22°C YPD), with error bars representing the standard deviation of 6 biological replicates. (**C**) *HFA1* hemizygotes differed in heat sensitivity on glucose but not glycerol medium. Cells were plated at 1:10 dilution. Growth is after 3 days.

Fig. S2.

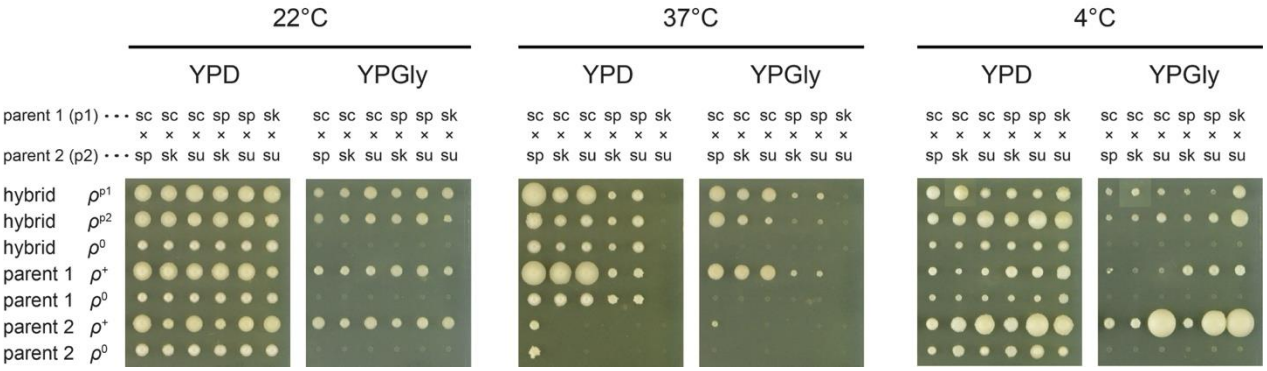


Fig. S2. Fermentative and respiratory growth of interspecific hybrids with reciprocal mitotypes at different temperatures. Interspecific hybrids between *S. cerevisiae* (sc), *S. paradoxus* (sp), *S. kudriavzevii* (sk), and *S. uvarum* (su) with either parental mitotype (ρ^{p1} or ρ^{p2}) or no mtDNA (ρ^{o}) were grown on YPD and YPGly plates for 5 days (22°C and 37°C) or 124 days (4°C). Growth of parent species and their petites are shown for comparison. The 4°C images of *S. cerevisiae* × *S. kudriavzevii* hybrid with *S. cerevisiae* mtDNA (sc × sk ρ^{p1}) were replaced with images from a biological replicate plated in the same configuration because the original colony was contaminated.

Fig. S3.

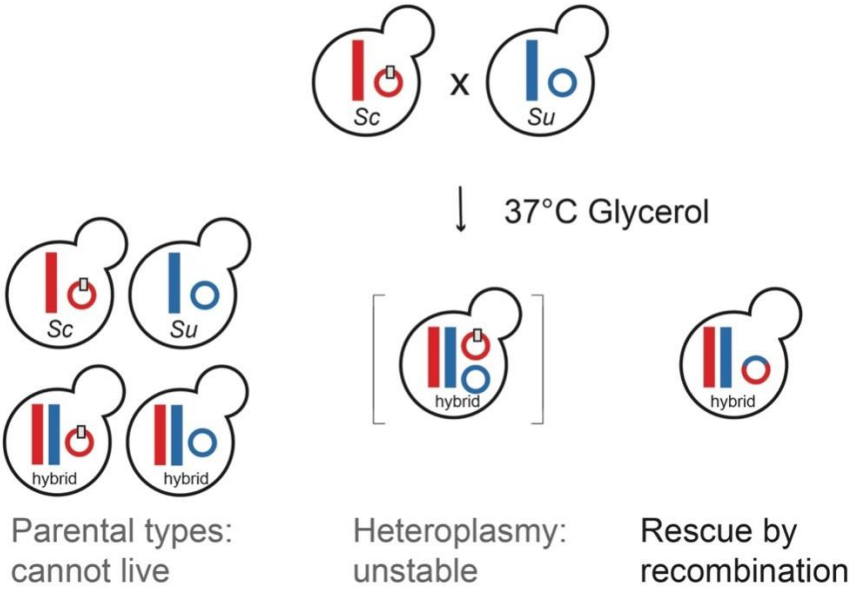


Fig. S3. Rescue of *S. cerevisiae* (sc) mitochondrial knockouts by recombination with *S. uvarum* (su) mitotypes. Upon crossing *S. cerevisiae* with *S. uvarum*, hybrids have unstable heteroplasmy; parental types do not grow at 37°C on glycerol, but recombinants can rescue the *S. cerevisiae* deficiency and the *S. uvarum* temperature sensitivity.

Fig. S4.

A

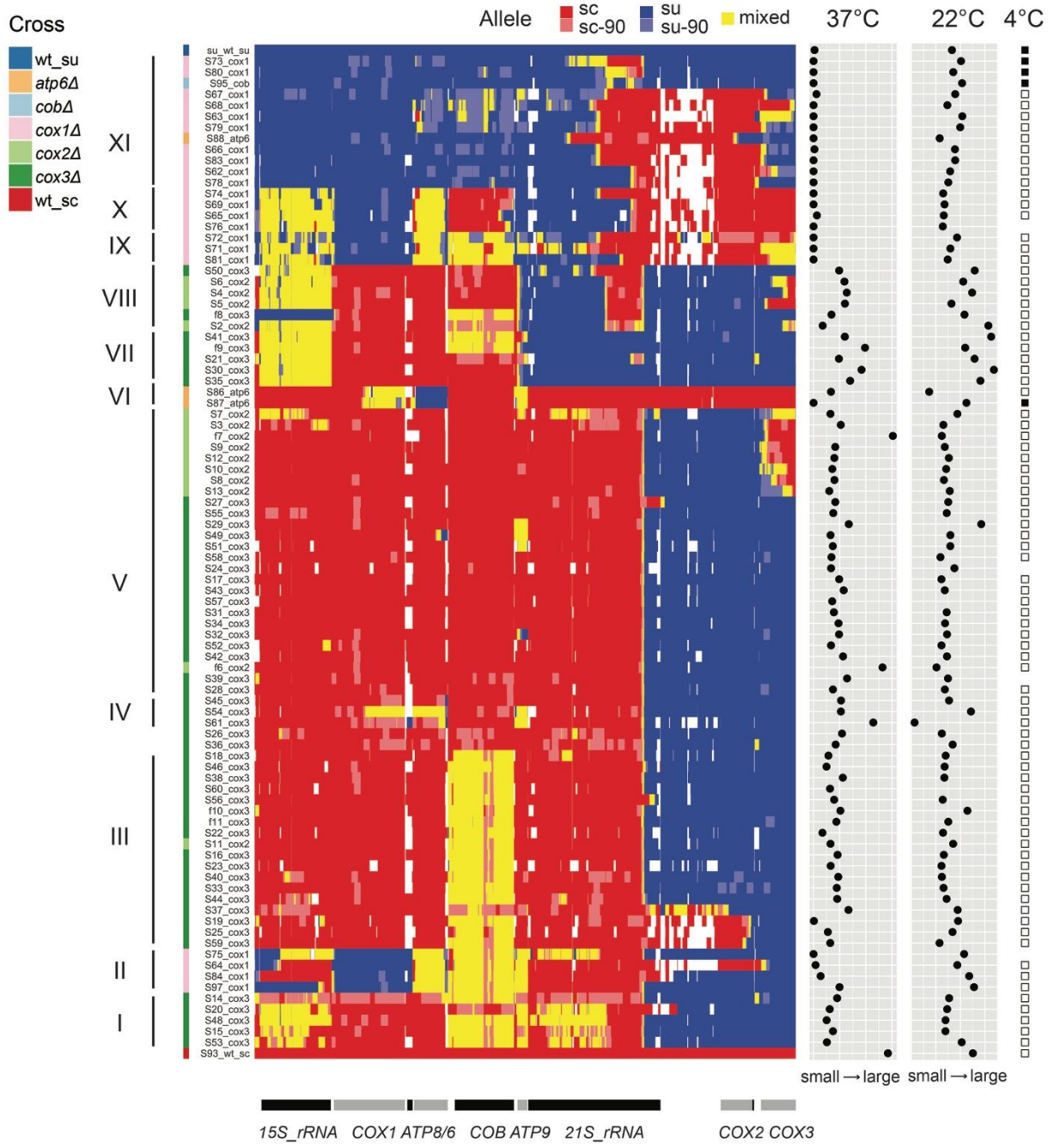
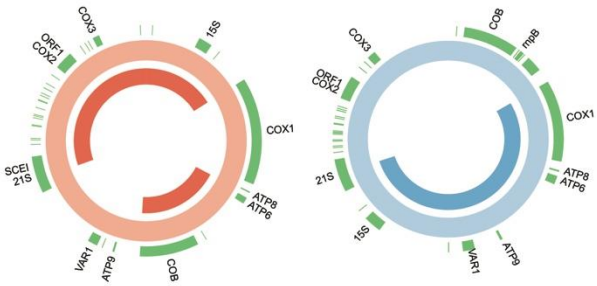

Cross
wt_su
atp6Δ
cobΔ
cox1Δ
cox2Δ
cox3Δ
wt_sc
Allele
sc
sc-90
su
su-90
mixed
37°C
22°C
4°C
XI
X
IX
VIII
VII
VI
V
IV
III
II
I
small → large
small → large
15S_rRNA
COX1
ATP8/6
COB
ATP9
21S_rRNA
COX2
COX3

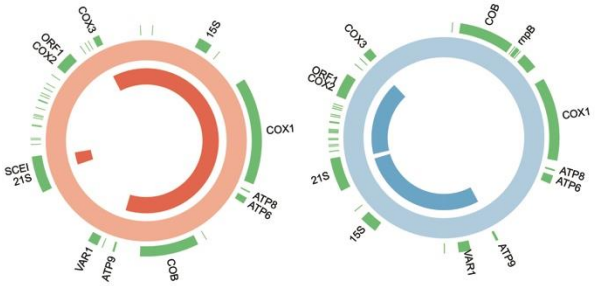
Fig. S4. continued

B

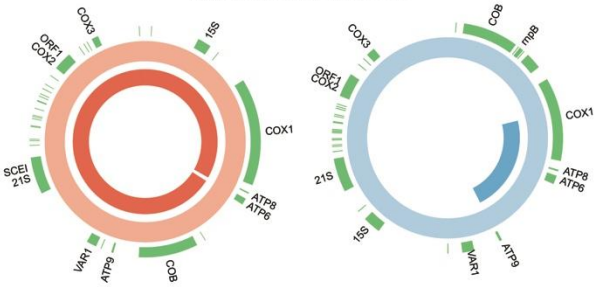
S79 (*COX1* cross, type XI)
Recombination at *21S* and *COX3*



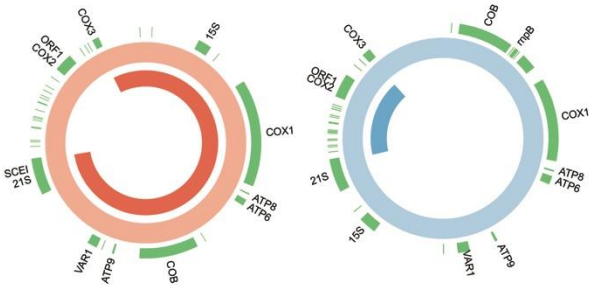
S74 (*COX1* cross, type X)
Recombination at *COX1* and *21S*,
with aneuploidy for *ATP6-COB*



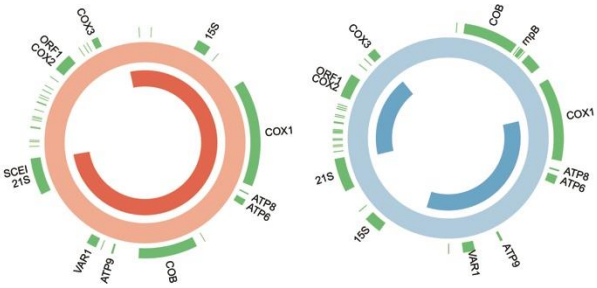
S5 (*COX2* cross, type VIII)
Recombination at *COX3* and *ATP9*,
with a piece of *S. cerevisiae 21S* inserted into the *S. uvarum* allele



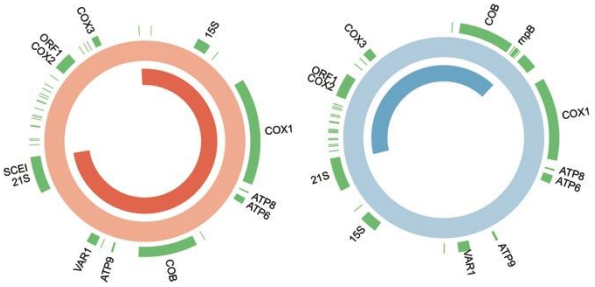
S87 (*ATP6* cross, type VI)
Recombination at *ATP6*,
with aneuploidy for *COX1*, *ATP8*, *ATP9*



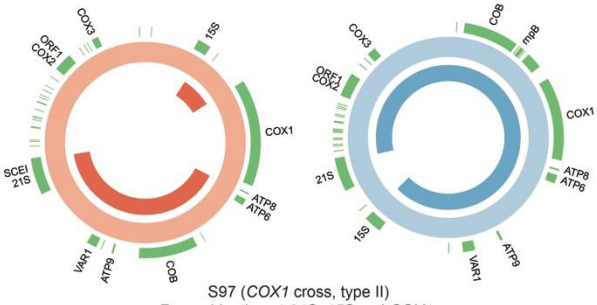
S8 (*COX2* cross, type V)
Recombination at *COX3* and *21S*



S54 (*COX3* cross, type IV)
Recombination at *21S* and *COX3*,
with aneuploidy for *COX1-VAR1*



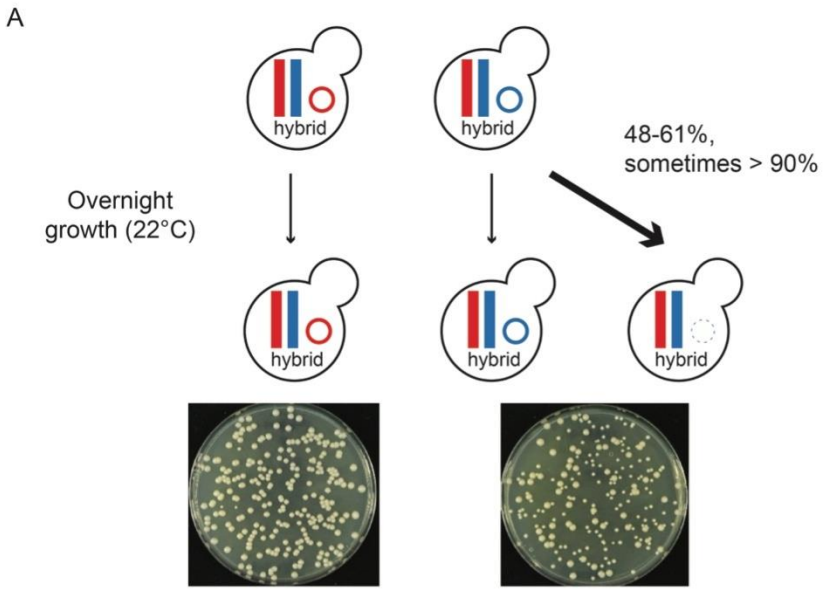
S33 (*COX3* cross, type III)
Recombination at *21S* and tRNA,
heterozygous *COB* caused by different *COB* locations in the two genomes

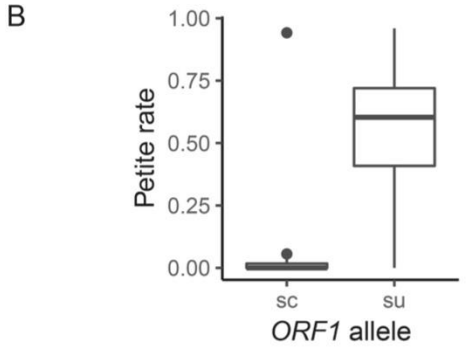


S97 (*COX1* cross, type II)
Recombination at *21S*, *15S* and *COX1*,
with aneuploidy for *ATP6-VAR1*

Fig. S4. Recombinant genotypes and examples of recombination breakpoints. (**A**) Recombinants were manually classified into 11 genotype groups and breakpoints for 8 representatives were identified by manual inspection. Strains were labeled by the trials ("f" for initial trial and "S" for second trial) and mutant crosses in which they were generated. Phenotype panels are shown as in Fig. 2B, with the addition of 22°C colony sizes. (**B**) Representative recombinant genomes are shown. Outer circles represent the reference mitochondrial genomes (red for *S. cerevisiae*, blue for *S. uvarum*), and inner circles show coverage of a given recombinant. Note *15S rRNA* and *COB* are at different positions in the two reference genomes.

Fig. S5.


A
hybrid
hybrid
48-61%,
sometimes > 90%
Overnight
growth (22°C)
hybrid
hybrid
hybrid


B
1.00
0.75
0.50
0.25
0.00
Petite rate
sc
su
ORF1 allele

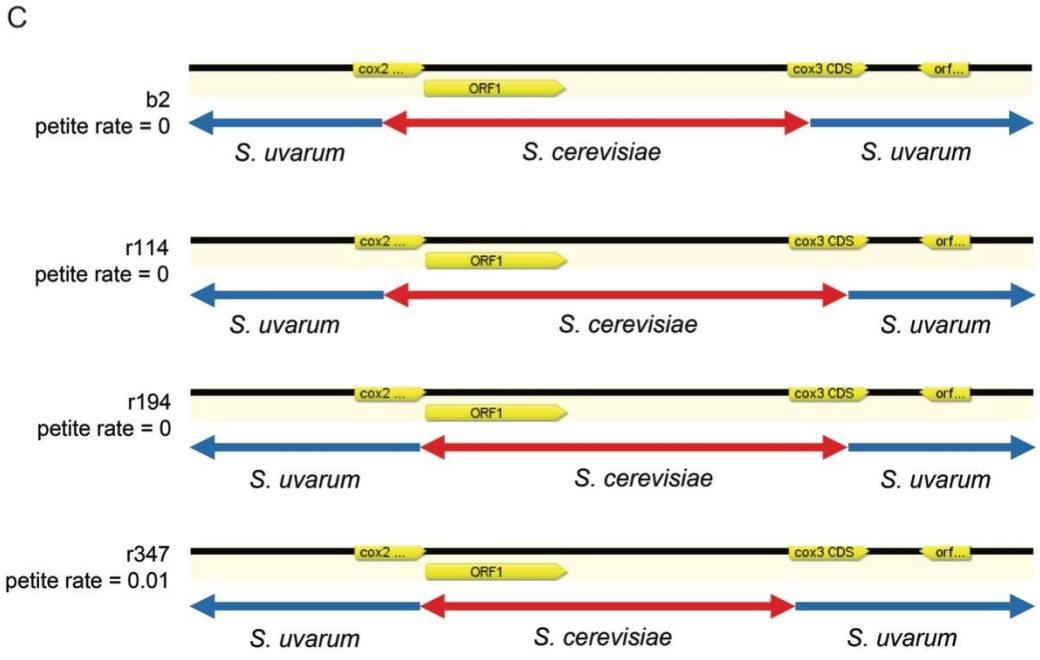

C
cox2 ...
cox3 CDS
orf...
ORF1
b2
petite rate = 0
S. uvarum
S. cerevisiae
S. uvarum
r114
petite rate = 0
r194
petite rate = 0
r347
petite rate = 0.01

Fig. S5. High petite rate of *S. uvarum* mitotype and its association with *ORF1*. (**A**) Petite rate in a 22°C overnight culture is high for the hybrid with a *S. uvarum* mitotype (blue circle), while the hybrid with a *S. cerevisiae* mitotype (red circle) rarely generates petites (dotted circle). (**B**) Petite rates associate with *ORF1* alleles in 90 recombinants generated by knockout crosses. sc, *S. cerevisiae*; su, *S. uvarum*. (**C**) Four spontaneous recombinants carrying *S. cerevisiae ORF1* showed low petite rates; the rest of their mitochondrial genome is *S. uvarum*.

Fig. S6.

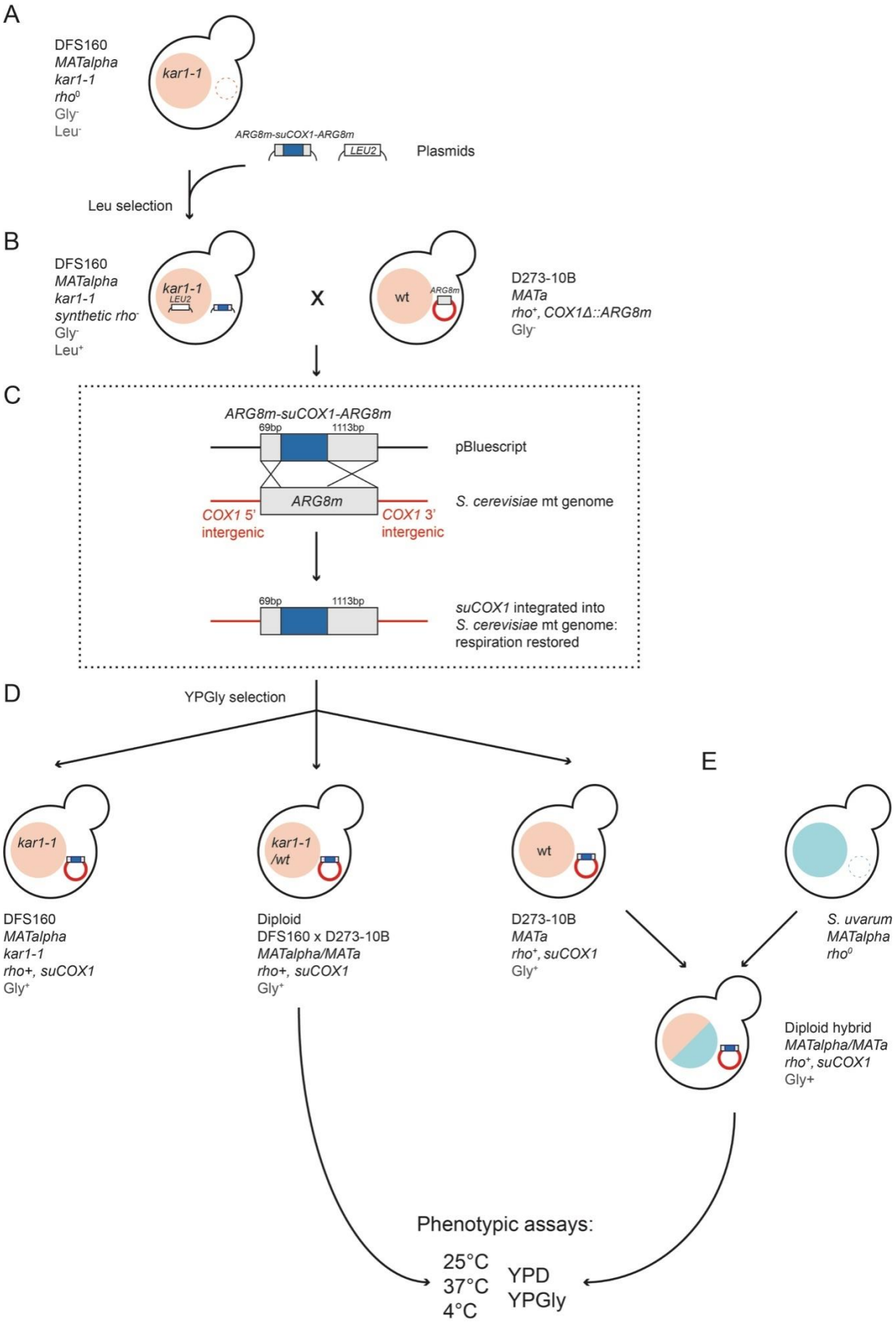

A
DFS160
MATalpha
kar1-1
rho0
Gly-
Leu-
kar1-1
ARG8m-suCOX1-ARG8m
LEU2
Plasmids
Leu selection
B
DFS160
MATalpha
kar1-1
synthetic rho-
Gly-
Leu+
kar1-1
LEU2
x
wt
ARG8m
D273-10B
MATa
rho+, COX1Δ::ARG8m
Gly-
C
ARG8m-suCOX1-ARG8m
69bp
1113bp
pBluescript
ARG8m
S. cerevisiae mt genome
COX1 5' intergenic
COX1 3' intergenic
69bp
1113bp
suCOX1 integrated into S. cerevisiae mt genome: respiration restored
D
YPGly selection
E
kar1-1
DFS160
MATalpha
kar1-1
rho+, suCOX1
Gly+
kar1-1 /wt
Diploid
DFS160 x D273-10B
MATalpha/MATa
rho+, suCOX1
Gly+
wt
D273-10B
MATa
rho+, suCOX1
Gly+
S. uvarum
MATalpha
rho0
Diploid hybrid
MATalpha/MATa
rho+, suCOX1
Gly+
Phenotypic assays:
25°C
37°C
4°C
YPD
YPGly

Fig. S6. Procedure for mitochondrial allele replacement. (**A**) Biolistic transformation of the mitochondrial construct with a *LEU2* plasmid. (**B**) Leu+ colonies were mated to *S. cerevisiae* mitochondrial knockouts. (**C**) The allele of interest was integrated into the mitochondrial genome via homologous recombination. (**D**) Integrated alleles were selected by rescue of respiration. (**E**) *MAT***a** mitochondrial genome transformants were crossed to *S. uvarum*.

Fig. S7.

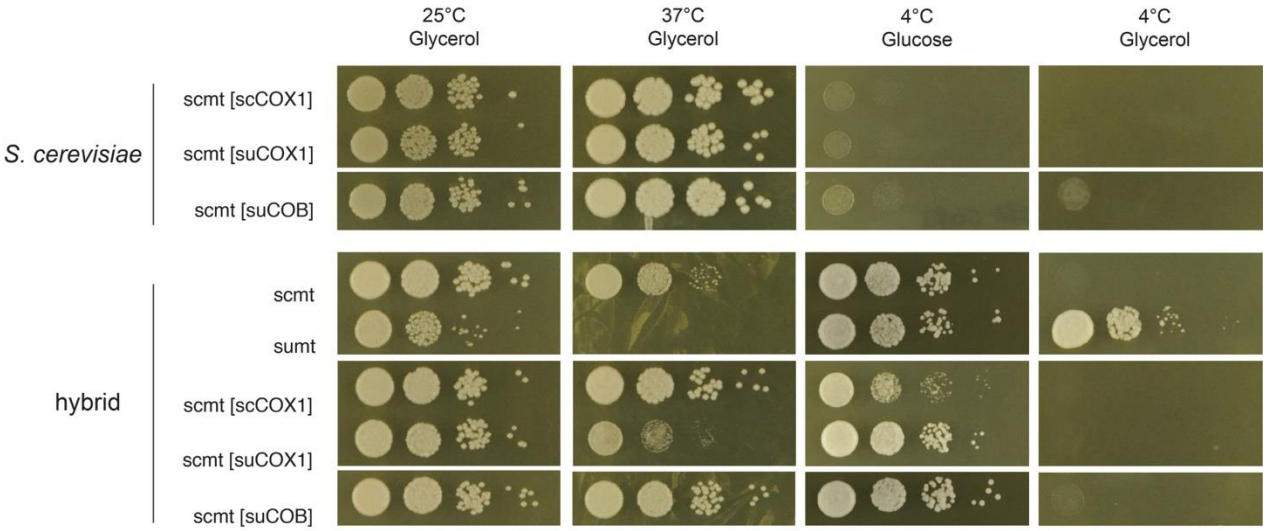


Fig. S7. Background-dependent allele effects of *COX1*. *S. cerevisiae* diploids and hybrids carrying allele replacements and two wild-type controls were plated with 1:10 serial dilution and incubated at indicated temperatures. Growth is after 4 days for 25°C and 37°C, 25 days for 4°C on glucose, and 53 days for 4°C on glycerol. sc, *S. cerevisiae*; su, *S. uvarum*; mt, mtDNA. Alleles in the brackets were integrated into their endogenous loci in *S. cerevisiae* mtDNA.

Table S1. Aneuploidy in the recombinants.

Strain	Increased 37°C growth compared to similar genotypes?	Cross	Duplicated chromosome	Mitochondrial interacting genes carried on the chromosome[1]	Reference
S29	Yes	*cox3*Δ	*S. cerevisiae* chrIX	*MRS1*	(*11*)
S53	No	*cox3*Δ	*S. cerevisiae* chrV	*MRX1*	(*32*)
S54	No	*cox3*Δ	*S. cerevisiae* chrIX	*MRS1*	(*11*)
S61	Yes	*cox3*Δ	*S. cerevisiae* chrIX	*MRS1*	(*11*)
S97	Yes	*cox1*Δ	*S. uvarum* chr10	*PET309*	(*32*)

[1] Only genes with known incompatibilities were listed.

External files:

Table S2. Strains used in this study.

Data file S1. Results of noncomplementation screen.

Data file S2. Recombinant strain genotypes and phenotypes. Allele, petite rate, aneuploidy, and mito/nuclear read ratio of the 90 mitochondrial recombinants used in the linear model.