

Supplementary Materials for

Mitochondrial DNA and temperature tolerance in lager yeasts

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The PDF file includes:

- Fig. S1. Growth assay for *ScAle* × *SeNC* hybrids and parental strains.
- Fig. S2. Growth assay for *Sc* × *SeNC* hybrids and parental strains.
- Fig. S3. Growth assay for *Sc* × *Se* hybrids and parental strains.
- Fig. S4. Growth assay for *ScAle* × *Se* strain hybrids and parental strains.
- Fig. S5. Relative growth scores and box plots of ρ^0 biological replicates.
- Fig. S6. Approximation of Arrhenius growth plots for synthetic hybrid growth assays.
- Fig. S7. Analysis of synthetic hybrid relative growth scores using restricted dataset.
- Fig. S8. Growth assay for lager cybrids and parental strains.
- Fig. S9. Approximation of Arrhenius growth plots for lager cybrid growth assays.
- Legend for table S1

Other Supplementary Material for this manuscript includes the following:

(available at advances.sciencemag.org/cgi/content/full/5/1/eaav1869/DC1)

Table S1 (Microsoft Excel format). Strains, plasmid, and oligonucleotides used in this work.

Fig. S1.

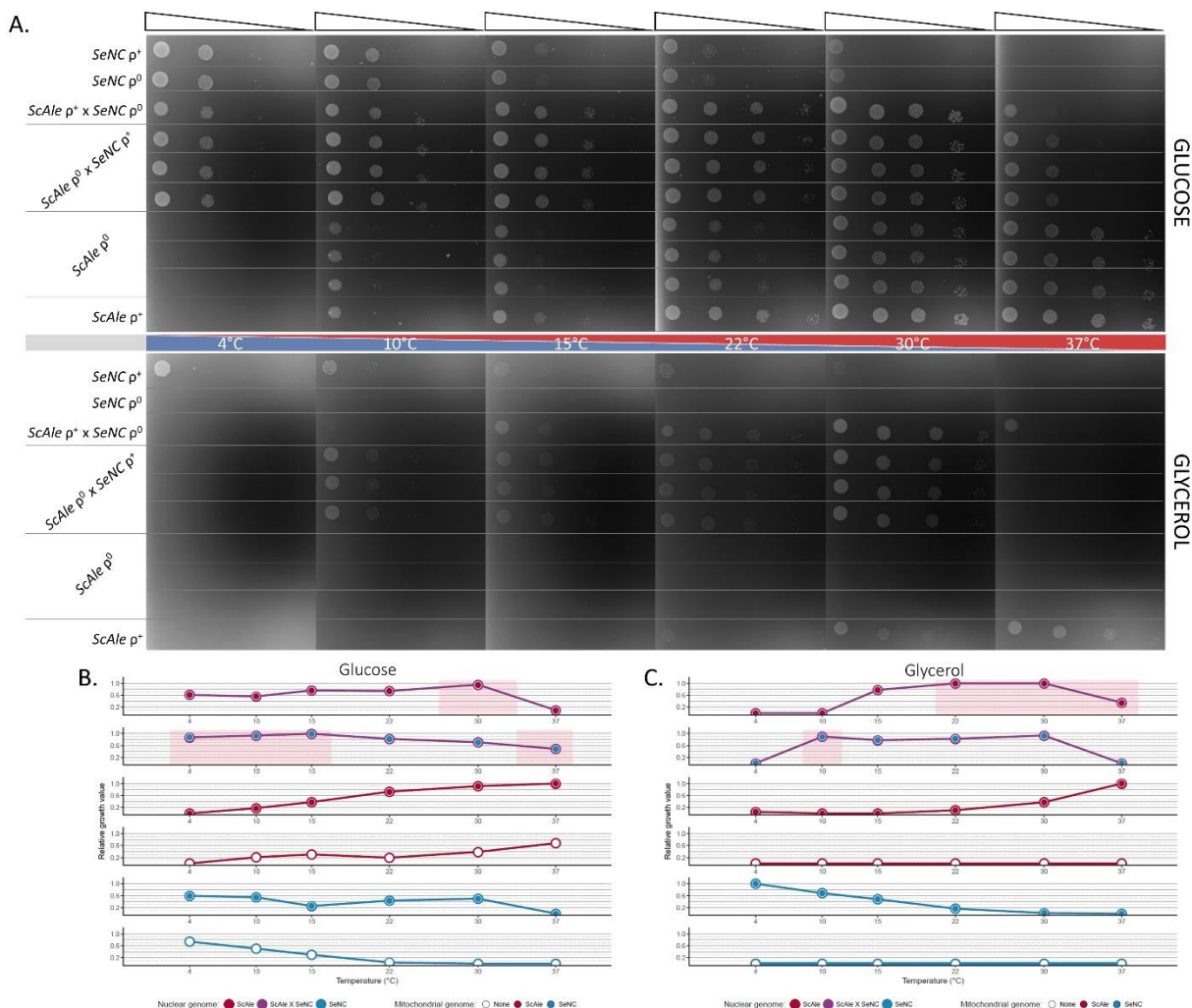


Fig. S1. Growth assay for *ScAle* × *SeNC* hybrids and parental strains. **A)** Representative spot assay plates grown from 4-37°C on plates containing glucose or glycerol as the sole carbon source. The following strains were tested: The *S. cerevisiae*-*ale* (*ScAle*) ρ^+ parent, the three independently generated *ScAle* ρ^0 strains, the *S. eubayanus*-North Carolinian (*SeNC*) ρ^+ parent, the *SeNC* ρ^0 parent, the *ScAle* ρ^+ × *SeNC* ρ^0 hybrid, and the three *ScAle* ρ^0 × *SeNC* ρ^+ hybrids constructed using the independently generated *ScAle* ρ^0 strains. **B)** On glucose and **C)** glycerol, relative growth of tested strains across all temperatures combining all replicates. Outer circles and lines represent nuclear genotype, while inner circles represent mtDNA. Highlighted regions represent temperatures where a hybrid of one mitotype had significantly greater relative growth than the hybrid with the alternative mitotype.

Fig. S2.

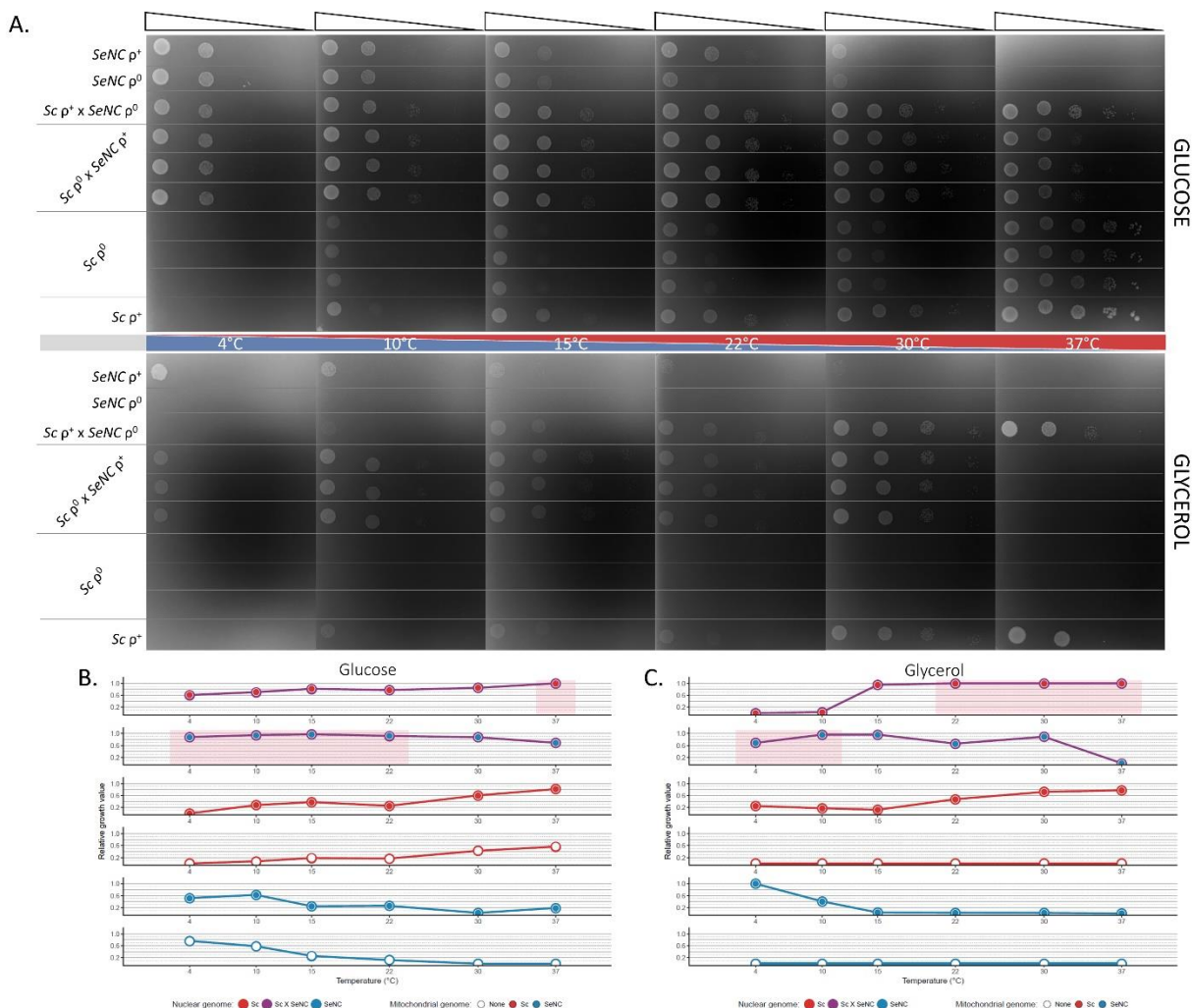


Fig. S3.

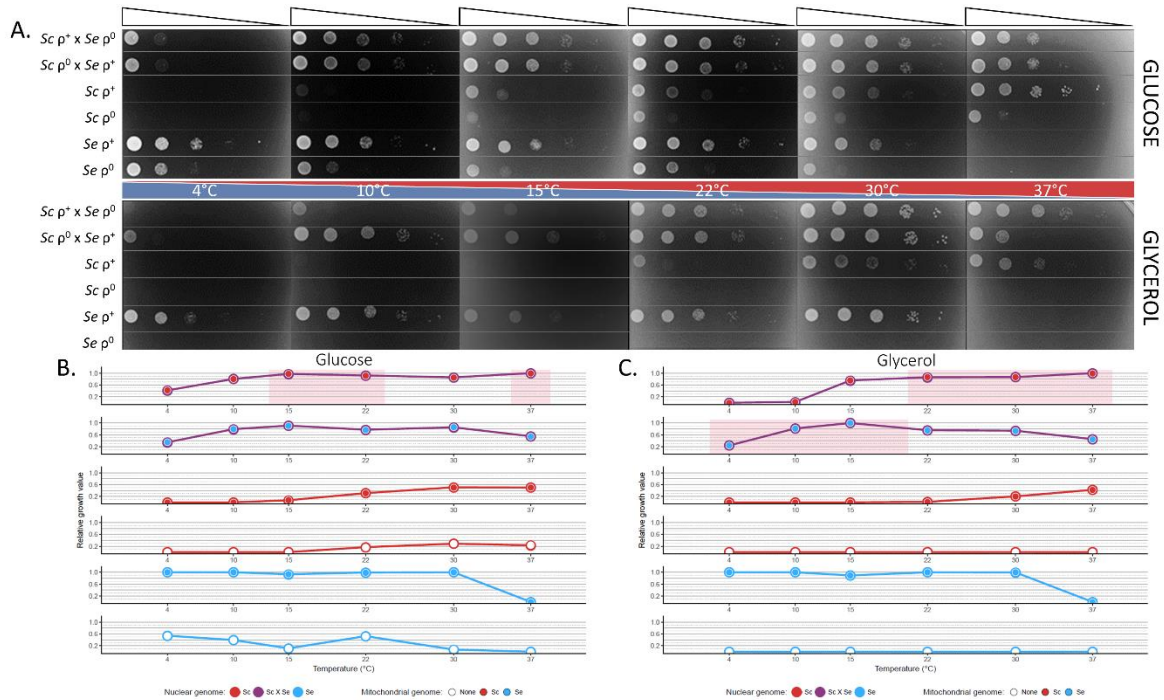


Fig. S3. Growth assay for *Sc* \times *Se* hybrids and parental strains. **A)** Representative spot assay plates grown from 4-37°C on plates containing glucose or glycerol as the sole carbon source. The following strains are pictured: The *S. cerevisiae*-laboratory strain (*Sc*) ρ^+ parent, the *Sc* ρ^0 parent used to construct the *Sc* ρ^0 x *Se* ρ^+ hybrid on the same plate, the *S. eubayanus*-taxonomic type strain (*Se*) ρ^+ parent, the *Se* ρ^0 parent used to construct the *Sc* ρ^+ x *Se* ρ^0 hybrid on the same plate, a *Sc* ρ^+ x *Se* ρ^0 hybrid, and a *Sc* ρ^0 x *Se* ρ^+ hybrid. **B)** On glucose and **C)** glycerol, relative growth of tested strains across all temperatures combining all replicates. Outer circles and lines represent nuclear genotype, while inner circles represent mtDNA. Highlighted regions represent temperatures where a hybrid of one mitotype had significantly greater relative growth than the hybrid with the alternative mitotype.

Fig. S4.

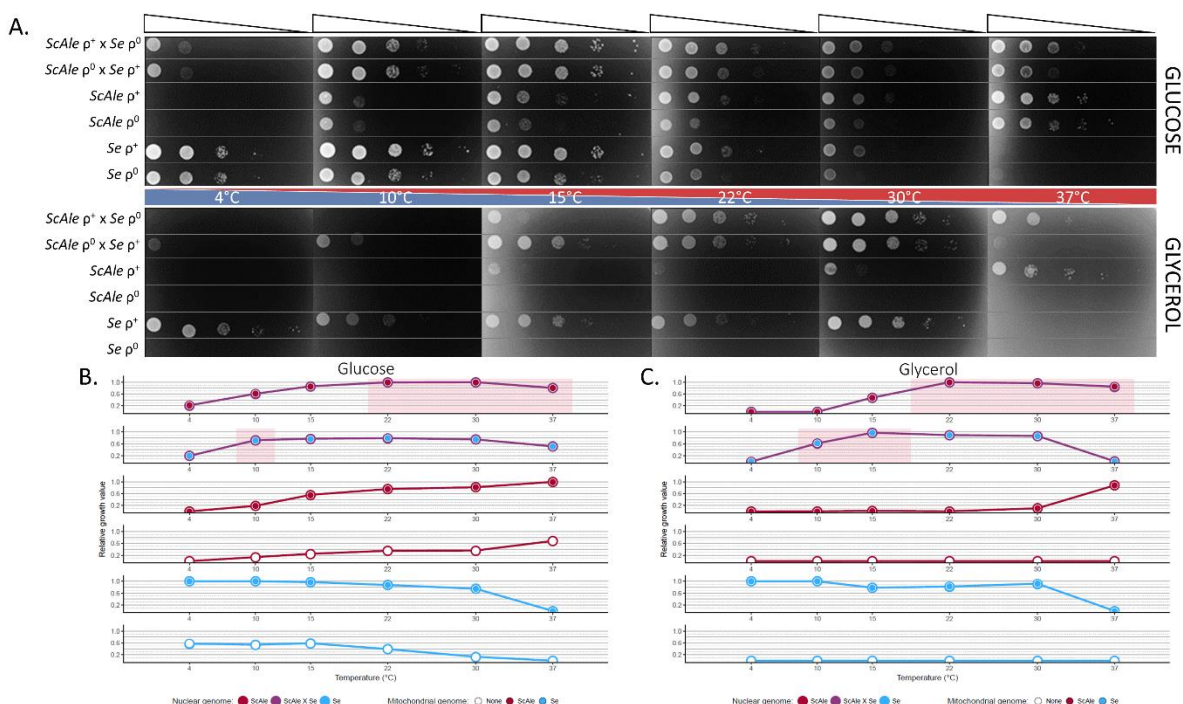


Fig. S4. Growth assay for *ScAle* × *Se* strain hybrids and parental strains. A) Representative spot assay plates grown from 4–37°C on plates containing glucose or glycerol as the sole carbon source. The following strains are pictured: The *S. cerevisiae*-ale (*ScAle*) ρ^+ parent, the *ScAle* ρ^0 parent used to construct the *ScAle* ρ^0 x *Se* ρ^+ hybrid on the same plate, the *S. eubayanus*-taxonomic type strain (*Se*) ρ^+ parent, the *Se* ρ^0 parent used to construct the *ScAle* ρ^+ x *Se* ρ^0 hybrid on the same plate, a *ScAle* ρ^+ x *Se* ρ^0 hybrid, and a *ScAle* ρ^0 x *Se* ρ^+ hybrid. **B)** On glucose and **C)** glycerol, relative growth of tested strains across all temperatures combining all replicates. Outer circles and lines represent nuclear genotype, while inner circles represent mtDNA. Highlighted regions represent temperatures where a hybrid of one mitotype had significantly greater relative growth than the hybrid with the alternative mitotype.

Fig. S5.

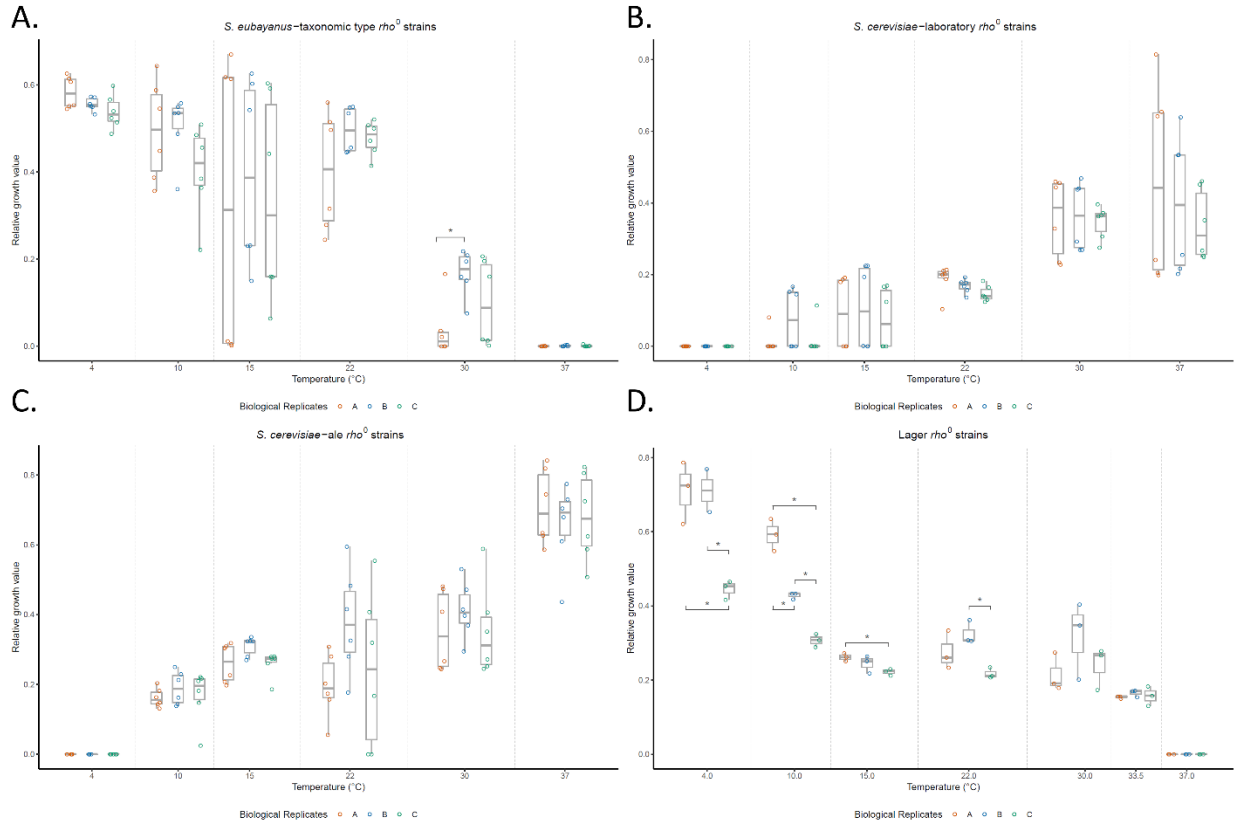


Fig. S5. Relative growth scores and box plots of ρ^0 biological replicates. ρ^0 strains used to generate synthetic hybrids and lager cybrids grown from 4°C to 37°C. Replicates were independently generated by treatment with ethidium bromide. Brackets and asterisk indicate statistically significant differences in values between replicate pairs (adjusted p-values ≤ 0.05). **A)** ρ^0 strains of a derivative of the taxonomic type strain of *S. eubayanus*, FM1318: biological replicates A = yHEB1611, B = yHEB1613, and C = yHEB1614. **B)** ρ^0 strains of the *S. cerevisiae*-laboratory strain FM1283: biological replicates A = yHRVM481, B = yHRVM483, and C = yHRVM485. **C)** ρ^0 strains of the *S. cerevisiae*-ale strain WLP530B: biological replicates A = yHEB1632, B = yHEB1621, and C = yHEB1623. **D)** ρ^0 strains of the lager strain W34/70: biological replicates A = yHEB1793, B = yHEB1797, and C = yHEB1800.

Fig. S6.

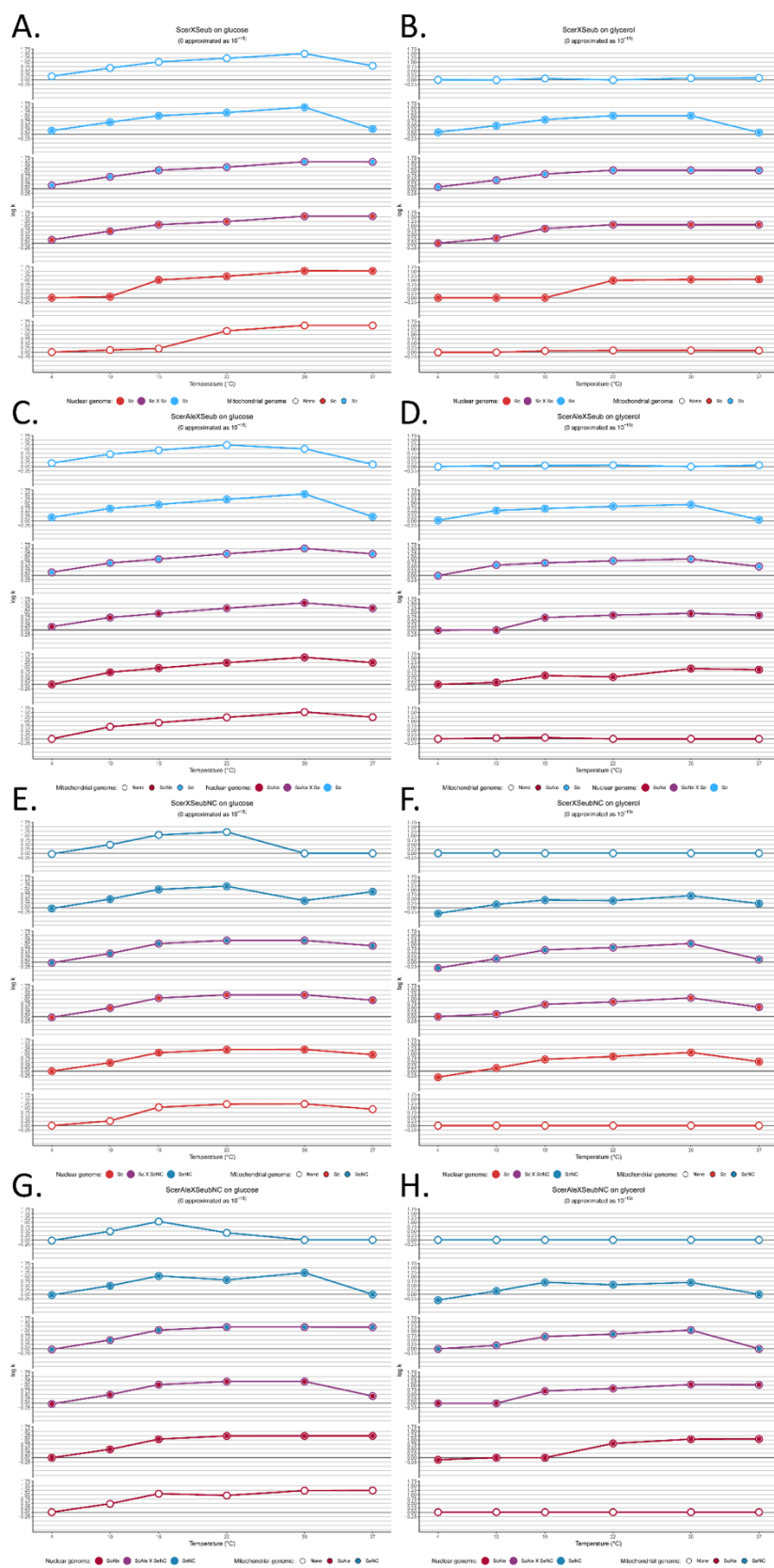


Fig. S6. Approximation of Arrhenius growth plots for synthetic hybrid growth assays. Left column (panels A, C, E, and G) calculated for growth on glucose. Right column (panels B, D, F, and H) calculated for growth on glycerol. To calculate k , θ was

approximated as 10^{-15} (see Material and Methods). A) and B) *S. cerevisiae*-laboratory strain (Scer) x *S. eubayanus*-taxonomic type strain (Seub). C) and D) *S. cerevisiae*-ale strain (ScerAle) x *S. eubayanus*-taxonomic type strain (Seub). E) and F) *S. cerevisiae*-laboratory strain (Scer) x *S. eubayanus*-North Carolinian strain (SeubNC). G) and H) *S. cerevisiae*-ale strain (ScerAle) x *S. eubayanus*-North Carolinian strain (SeubNC).

Fig. S7.

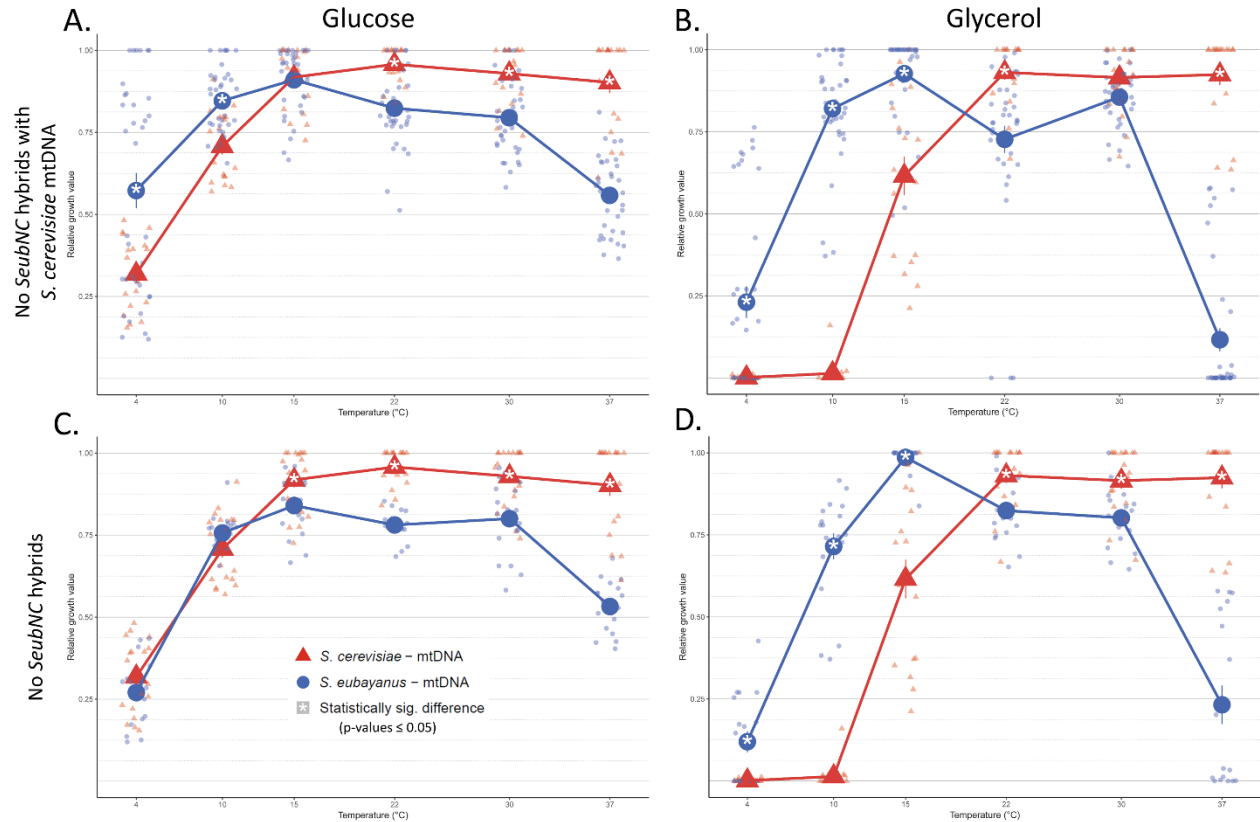


Fig. S7. Analysis of synthetic hybrid relative growth scores using restricted dataset. Strains grown from 4-37°C and data combined from all tests with subsets of *S. eubayanus*-North Carolinian hybrid data removed. Synthetic hybrids are: *Sc* x *Se*, *ScAle* x *Se*, *Sc* x *SeNC*, and *SeAle* x *SeNC*. **A)** On glucose and **B)** glycerol, *ScAle* x *SeNC* and *Sc* x *SeNC* hybrids carrying *S. cerevisiae* mtDNA, for which only single biological replicates were available, were removed from data set. **C)** On glucose and **D)** glycerol, all *ScAle* x *SeNC* and *Sc* x *SeNC* hybrids were removed from data set. Error bars represent standard errors. Differences in relative growth between hybrids carrying different parental mtDNA with p-values of ≤ 0.05 were considered statistically significant and are represented by asterisks.

Fig. S8.

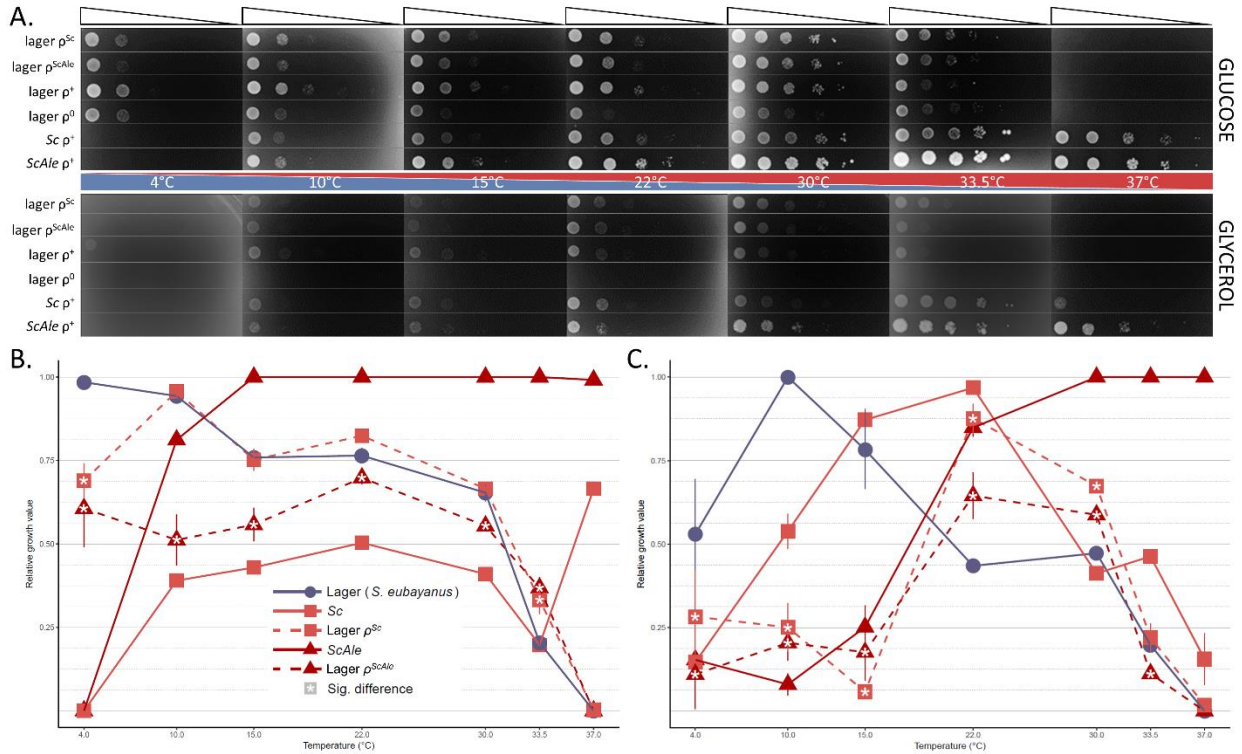


Fig. S8. Growth assay for lager cybrids and parental strains. **A)** Representative spot assay plates grown from 4-37°C on plates containing glucose or glycerol as the sole carbon source. The following strains are pictured: the lager ρ^+ parent (which naturally contains *S. eubayanus* mtDNA), the lager ρ^0 parent used to construct the cybrids on the same plate, the *S. cerevisiae*-laboratory strain (*Sc*) ρ^+ parent, the *S. cerevisiae*-ale (*ScAle*) ρ^+ parent, a lager ρ^{Sc} cybrid, and a lager ρ^{ScAle} cybrid. **B)** On glucose and **C)** glycerol, relative growth of tested strains across all temperatures combining all replicates, excluding lager ρ^0 strains. Error bars represent standard errors, and asterisks indicate statistically significant differences in growth between the cybrid and lager with native mtDNA ($p \leq 0.05$).

Fig. S9.

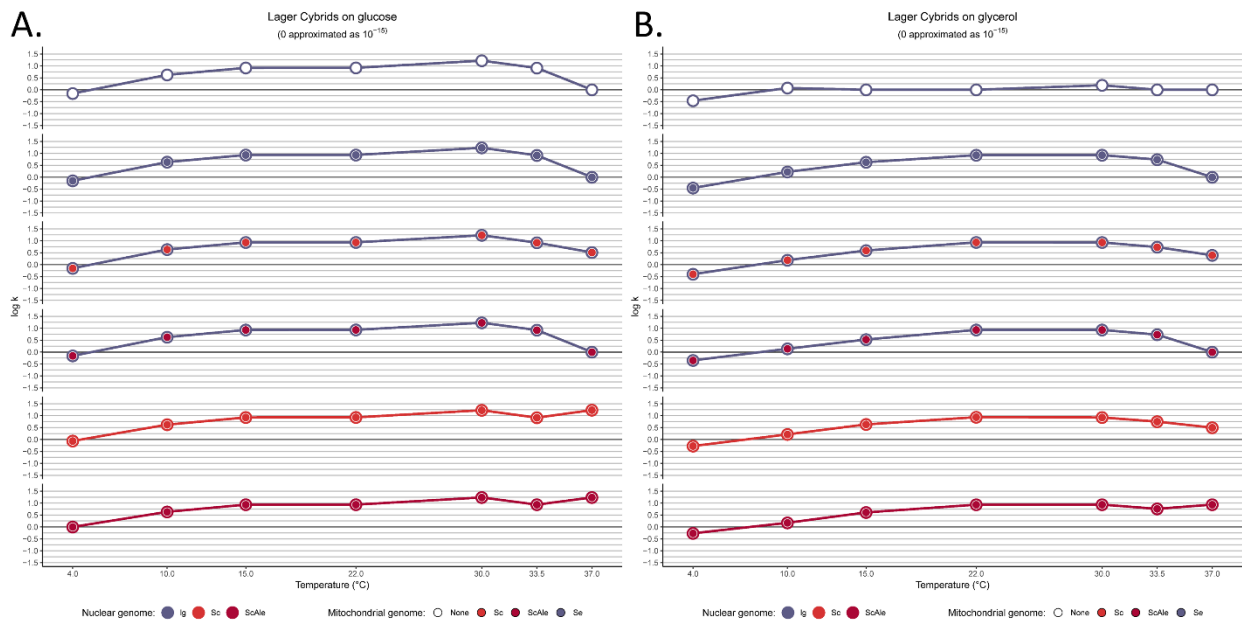


Fig. S9. Approximation of Arrhenius growth plots for lager cybrid growth assays. To calculate k , 0 was approximated as 10^{-15} (see Material and Methods). **A)** Growth assay on glucose. **B)** Growth assay on glycerol.

External files:

Table S1. Strains, plasmid, and oligonucleotides used in this work.

- A. Strains and plasmid
- B. Oligonucleotides