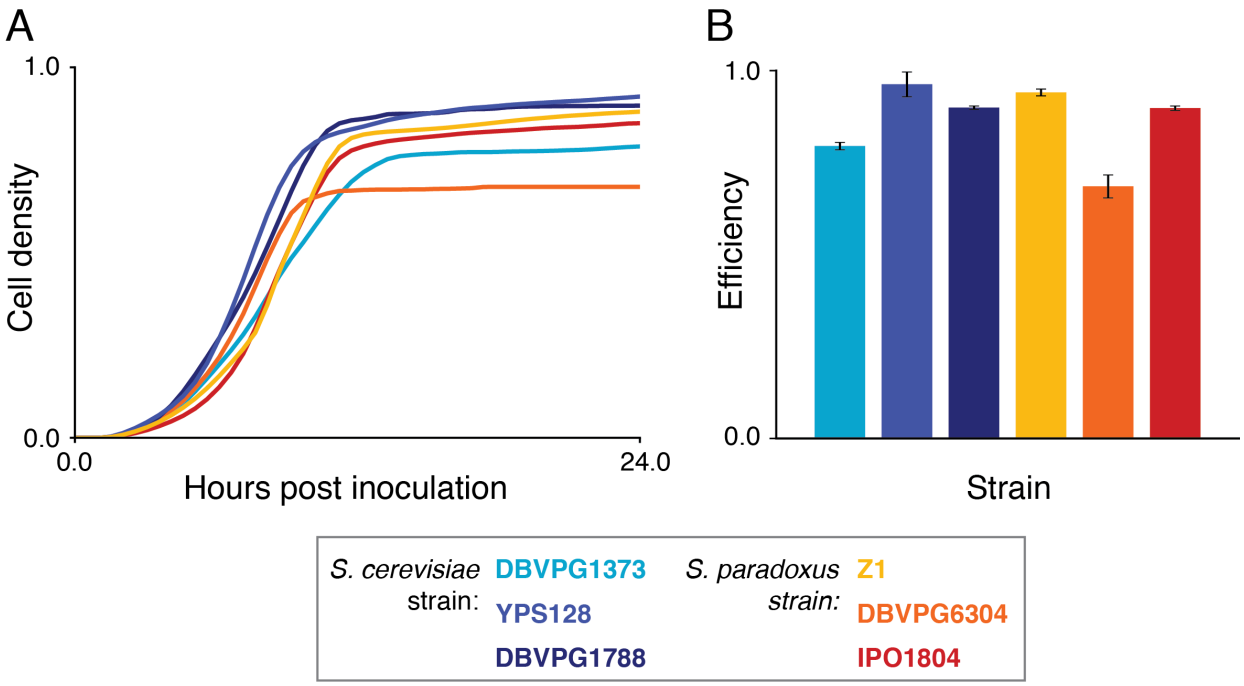
Extended data

Figures S1-S6

Tables S1-S4

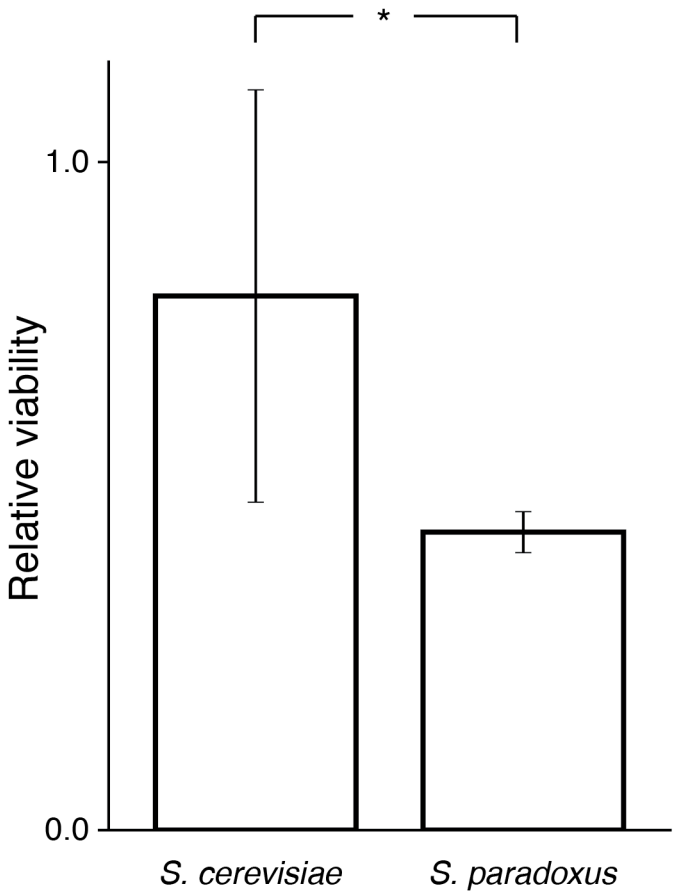
Supplementary figures

Supplementary Figure 1



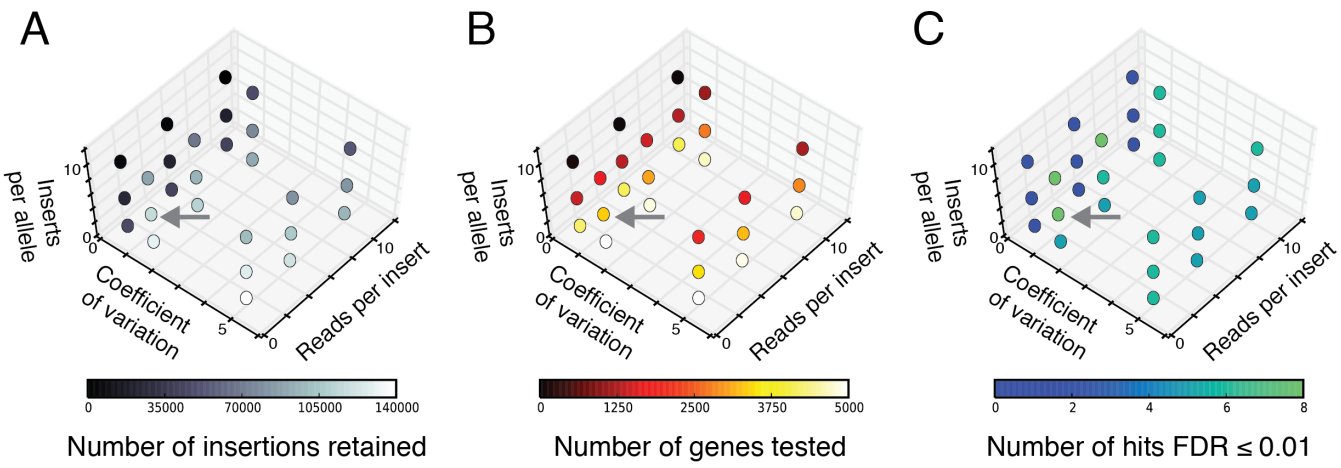
Supplementary Figure 1. *S. cerevisiae* and *S. paradoxus* do not differ significantly with respect to growth at 28°C. A, Each trace reports mean optical density (OD_{595}) over time of the indicated wild isolate of *S. cerevisiae* (blue) or *S. paradoxus* (orange) cultured at 28°C ($n \geq 11$). **B**, Each bar reports the mean efficiency ($n \geq 11$) of the indicated strain after 24 hours of growth at 28°C. Efficiencies across strains were not significantly different between the species ($p = 0.07$).

Supplementary Figure 2



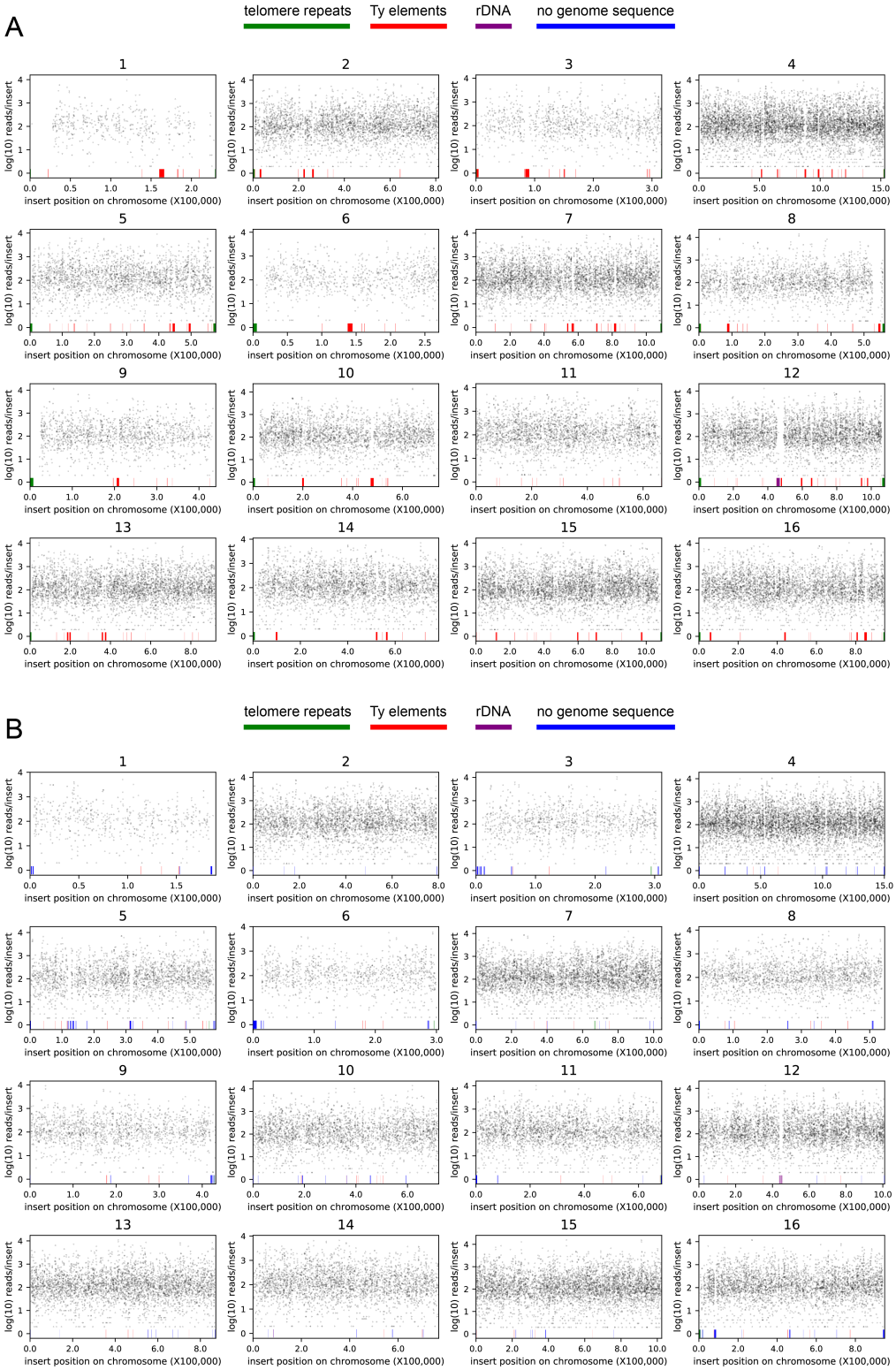
Supplementary Figure 2. Viability of *S. cerevisiae* is higher than that of *S. paradoxus* at 39°C. Each bar reports the mean number of colony-forming units, per mL of culture per unit OD_{600}, from a liquid culture of the indicated species grown for six hours at 39°C followed by plating onto solid medium (n = 5), relative to the analogous quantity after six hours of growth at 28°C. Error bars report standard deviation. Relative viabilities were significantly different between the species (*, p = 0.042).

Supplementary Figure 3



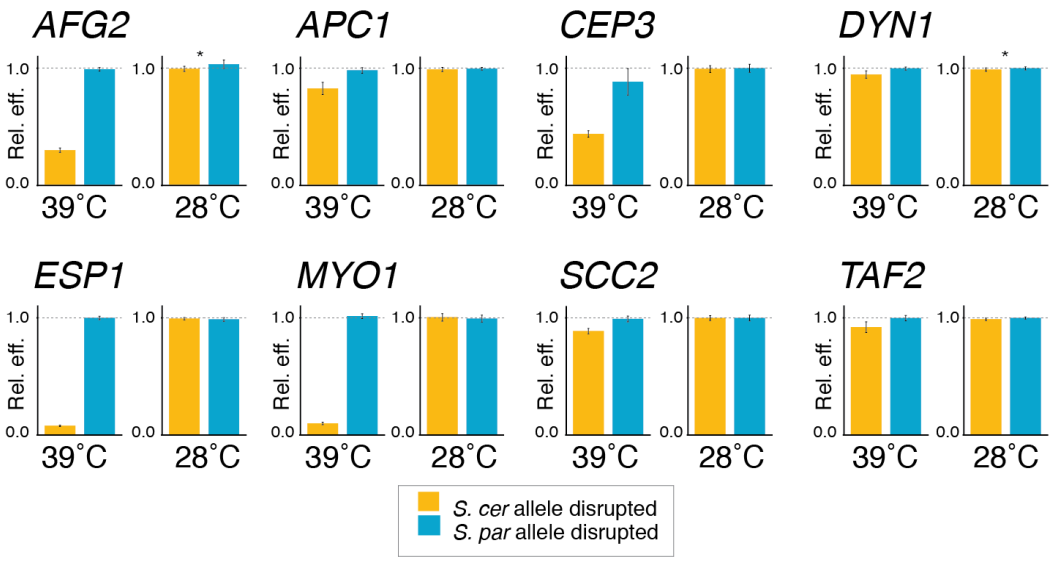
Supplementary Figure 3. Dependence of the RH-seq data set on cutoffs for read depth and transposon mutant coverage. A, The *x*-axis reports the average number of sequencing reads mapping to a given transposon insertion in either the 28°C or 39°C selection, as a minimum level above which the insertion was retained for analysis. The *y*-axis reports the coefficient of variation of read abundances between biological replicates for a given transposon insertion, as a maximum level below which the insertion was retained for analysis. The *z*-axis reports the number of transposon insertions per allele, as a minimum above which the gene was retained for analysis. The color of each circle reports the number of insertions retained for analysis in the indicated cutoff scheme. **B**, Data and symbols are as in **A**, except that the color of each circle reports the number of genes retained for analysis in the indicated cutoff scheme. **C**, Data and symbols are as in **A**, except that the color of each circle reports the number of genes that scored below a *p*-value corresponding to a false-discovery rate (FDR) of 0.01 in the reciprocal hemizygosity test using RH-seq data, in the indicated cutoff scheme. Arrows indicate the set of cutoff values used in this study, which yielded a dataset of 110,678 usable insertions across 3416 analyzable genes, 8 of which scored below FDR = 0.01 in the reciprocal hemizygosity test.

Supplementary Figure 4



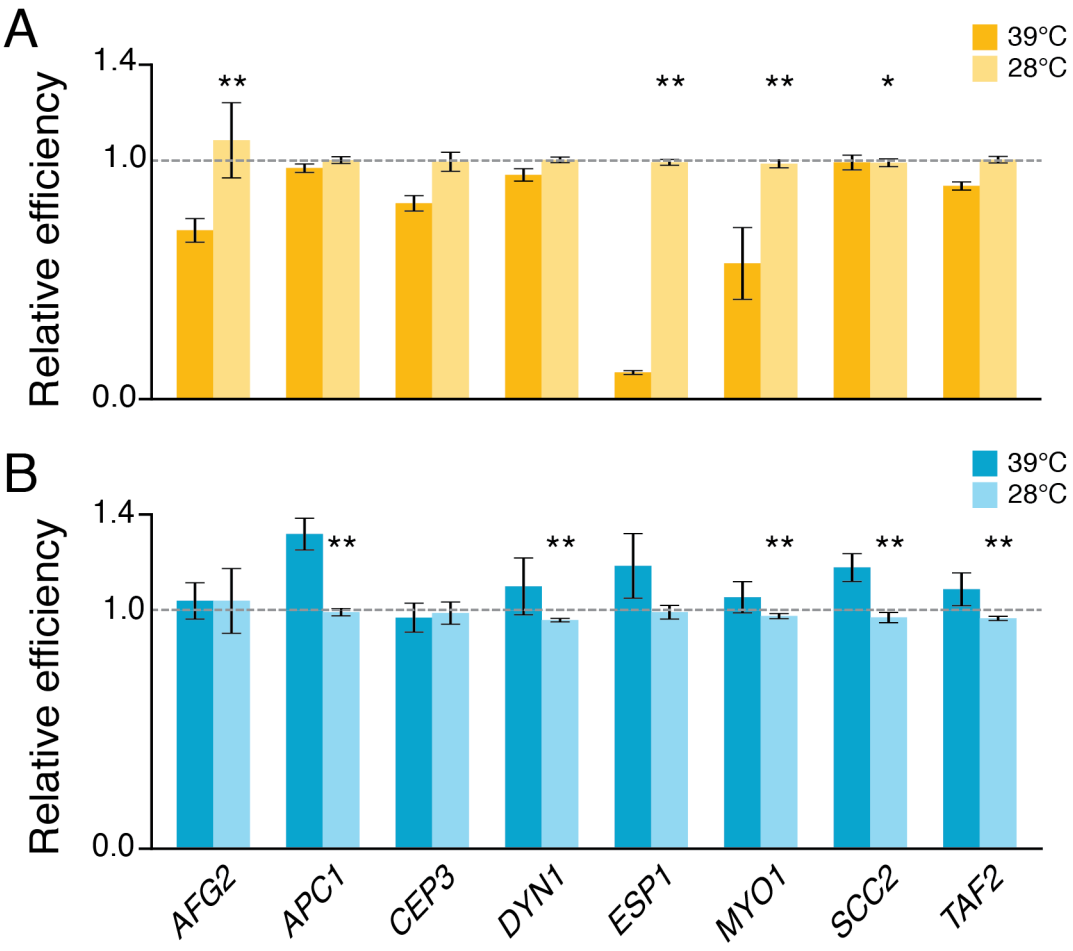
Supplementary Figure 4. RH-seq transposon coverage across the genome. A, Each panel reports sites in which the PiggyBac transposon inserted in the indicated *S. cerevisiae* chromosome in clones of the *S. cerevisiae* x *S. paradoxus* hybrid, as mapped from a pool of such clones by RH-seq. Each point reports one insertion; the *x*-axis reports the chromosomal position of a given insertion site, and the *y*-axis reports the raw number of sequencing reads mapped to that site. Colored tick marks along the bottom of each panel report genomic features that prohibited the mapping of reads. Read counts are from a representative RH-seq library after seven generations of culture at 39°C, reflecting the abundance in the pool of the respective hemizygote clone harboring the insertion. **B**, Data are as in **A**, except that shown are results from transposon insertions along *S. paradoxus* chromosomes in the *S. cerevisiae* x *S. paradoxus* hybrid.

Supplementary Figure 5



Supplementary Figure 5. Variation at RH-seq hit loci has little impact on growth at 28°C in the background of the interspecific hybrid. Each panel reports growth efficiency measurements of targeted-deletion reciprocal hemizygotes at the indicated RH-seq hit locus. In a given panel, the left-hand pair of bars reports relative efficiencies of targeted-deletion hemizygotes after culture at 39°C, from Figure 2B of the main text. In the right-hand pair of bars, each bar reports the mean growth efficiency ($n \geq 12$) after culture at 28°C of a targeted-deletion hemizygote in the indicated species' allele, normalized by the analogous quantity for the wild-type hybrid parent. Error bars report standard deviation. Statistical analyses of 39°C efficiency data are reported in Figure 2. For efficiency experiments at 28°C, *, $p \leq 0.05$, in a test for a difference in efficiency between the indicated hemizygotes.

Supplementary Figure 6



Supplementary Figure 6. Variation at RH-seq hit loci has little impact on growth at 28°C in the background of the purebred species. A, Each pair of bars reports measurements of growth efficiency of an *S. cerevisiae* strain harboring the *S. paradoxus* allele at the indicated RH-seq hit locus, relative to the analogous quantity for wild-type *S. cerevisiae*. The dark-shaded bar reports mean relative efficiency of the allele-replacement strain after culture at 39°C, from Figure 3 of the main text. The light-shaded bar reports mean growth efficiency ($n \geq 11$) of the allele replacement strain after culture at 28°C, relative to the analogous quantity for wild-type *S. cerevisiae*. Error bars represent standard deviation. Statistical analyses of 39°C efficiency data are reported in Figure 3. For efficiency experiments at 28°C, *, $p \leq 0.05$; **, $p \leq 0.01$, in a test for

a difference in efficiency between the indicated allele-replacement strain and the wild-type *S. cerevisiae*. **B**, Data and symbols are as in **A**, except that each bar reports results from a *S. paradoxus* strain harboring the *S. cerevisiae* allele at the indicated locus, relative to wild-type *S. paradoxus*.

Supplementary table captions

Supplementary Table 1. Strains used in this study. A, Wild-type diploid strains used, including those used as parents of the *S. cerevisiae* x *S. paradoxus* hybrid and of allele-replacement transgenesis; SGRP, the Saccharomyces Genome Resequencing Project, version 2. **B**, Hemizygotes in the *S. cerevisiae* x *S. paradoxus* diploid hybrid constructed by targeted deletion of a given species' allele of the indicated gene with the KanMX or NatMX cassette. ΔscYFG::KanMX/spYFG signifies that the *S. cerevisiae* allele of YFG was knocked out and the *S. paradoxus* allele of YFG is intact; analogously for strains with the *S. cerevisiae* allele intact and *S. paradoxus* knocked out. **C**, Allele replacement strains in *S. cerevisiae* or *S. paradoxus* diploid homozygote backgrounds. In genotype notes, *e.g.* in an *S. paradoxus* background, ΔYFG(-X to +Y)::scYFG(-Z to +W) indicates that in *S. paradoxus*, bases -X to +Y from YFG have been removed and replaced by bases -Z to +W of the *S. cerevisiae* allele of YFG. Positive coordinates count in the 5' to 3' direction from the start codon (+1 corresponds to the A in the ATG), and negative coordinates count in the 3' to 5' direction from the start codon (-1 corresponds to the base directly 5' of the ATG). In cases where the replacement extended into a region of 100% conservation between species, the position of the last divergent nucleotide is shown.

Supplementary Table 2. Depth of RH-seq library sequencing. Each row reports the number of sequencing reads, before mapping, from an RH-seq library made from the DNA of transposon mutant hemizygotes in the *S. cerevisiae* x *S. paradoxus* hybrid. The last four rows report results from the pool immediately after transposon mutagenesis, mutant clone isolation,

pooling, and pre-growth at 28°C (time zero, T0). The first 24 rows report results from selection (bulk culture of the pool) at the indicated temperature after inoculation from the T0 sample.

Supplementary Table 3. Abundances of transposon-mutant clones in the interspecific hybrid from RH-seq. Each row reports results of sequencing one transposon insertion in the *S. cerevisiae* x *S. paradoxus* hybrid after selection of the transposon mutant pool, reflecting the abundance in the pool of the respective hemizygote clone harboring the insertion. Allele, the species parent's homolog in which the transposon insertion lies. Chromosome, strand, location, and gene, the fine-scale position of the insertion. Abundance, read counts of the transposon insertion sequenced after selection of the mutant pool at the indicated temperature, normalized for library size and averaged across biological and technical replicates. Transposon insertions not detected in any replicate of the indicated selection were assigned an abundance of 1. CV, coefficient of variation over biological replicates of normalized read counts after selection at the indicated temperature.

Supplementary Table 4. Tests of the impact on thermotolerance of variation at each gene in turn via reciprocal hemizygote analysis of clone abundances from RH-seq. Each row reports the results of reciprocal hemizygote tests of thermotolerance of hemizygote transposon mutants at the indicated gene in the *S. cerevisiae* x *S. paradoxus* hybrid. The second, third, and fourth columns report results of a Mann-Whitney statistical test for a difference in the abundance after growth at 39°C, relative to the abundance after growth at 28°C, of hemizygotes harboring transposon insertions in the two species parents' homologs. The last two columns report the log ratio of normalized abundances of a hemizygote harboring a transposon insertion in the

indicated species parent's homolog after culture at 39°C and 28°C, as a geometric mean across transposon mutants from Supplementary Table 3.