**Supplementary Table 1. Plasmids used in this study.**

**Supplementary Table 5. Abundance of inferred hemizygote insertion genotypes from thermotolerance RH-seq.** Each row reports the results of sequencing from one inferred transposon insertion in the *S. cerevisiae* x *S. paradoxus* diploid hybrid after selection of the barcoded transposon pool after competitions comparing growth at 37°C and 28°C, reflecting the abundance in the pool of the respective hemizygote clone harboring the insertion. Chromosome, strand, location, and gene report the fine-scale position of the inferred insertion. Allele, the species parent’s homolog in which the transposon insertion lay. Abundance, read counts of the transposon insertion sequenced after selection of the barcoded transposon pool at the indicated temperature, normalized for library size and averaged across the biological replicate cultures. Transposon insertions not detected in any replicate of the indicated selection were assigned an abundance of 1 prior to normalization by library size. CV, coefficient of variation over biological replicates of normalized read counts after selection at the indicated temperature. Barcode, the unique barcode identifier of the transposon insertion.

**Supplementary Table 6. Abundance of hemizygote insertion genotypes from RH-seq at 36°C.** Data are as in Table S5, except that RH-seq was done using 36°C as the high-temperature condition.

**Supplementary Table 7. Effects of allelic variation in thermotolerance RH-seq.** Each row reports the results of reciprocal hemizygote tests on thermotolerance at the indicated gene in the *S. cerevisiae* x *S. paradoxus* diploid hybrid at 37°C. Columns B-G report analyses of abundance upon the aggregation at the gene level of inferred hemizygote genotypes (Table S5) from all biological replicate experiments, filtered for quality control (see Methods). Columns B-D report results of a two-tailed Mann-Whitney statistical test for a difference in the abundance after growth at 37°C, relative to the abundance after growth at 28°C, of hemizygotes harboring transposon insertions in the two species parents’ homologs. The Benjamini-Hochberg method was used to correct for multiple testing. Column E reports the log2(abundance at 37°C/abundance at 28°C) of the average insert in the *S. cerevisiae* allele. Column F reports the analogous quantity among inserts in the *S. paradoxus* allele of the gene. Column G reports the allele-specific effect size, calculated as the difference between the measures of Columns E and F.

**Supplementary Table 8**. **Effects of allelic variation in RH-seq at 36°C.** Data are as in Table S7, except that the high temperature growth condition was 36°C.