Supplemental Methods

Genome sequencing

The Pacific Biosciences protocol “Preparing HiFi SMRTbell® Libraries using SMRTbell Express Template Prep Kit 2.0” was used to create libraries from 30 micrograms of DNA. The Megarupter (Diagenode, Denville, NJ, USA) was used for shearing and Blue Pippin (Sage Science, Beverly, MA, USA) was used for size-selection for fragments greater than 13 kb. A subset of the libraries was constructed using barcoded adapters to allow pooling before size-selection.

Each library was run on one SMRTCell (version 8M) using version 2.0 sequencing reagents. Sequencing was performed on a Sequel II sequencer (Pacific Biosciences, Menlo Park, CA, USA) running instrument control software version 9.0.0.9223 and with a movie collection time of 30 hours per SMRTCell.

For individual sample libraries, circular consensus sequence (CCS) reads, also known as HiFi reads, were generated from the initial subread data using the pb_ccs workflow (ccs version 4.2.0) within PacBio SMRTLink version 9.0.0.92188. For the pooled libraries, demultiplexed CCS reads were generated from the initial subread data using the pb_demux_ccs workflow (lima version 1.11.0) within PacBio SMRTLink.

QTL and segregant analysis

All data used to generate LOD plots for maltose and paraquat was generated by Bloom et al. In brief, Bloom et al. isolated segregants through random spore isolation for all crosses except for BY x RM and YJM145 x YPS163, which were isolated through tetrad dissection. Bloom et al. determined segregant genotypes from 100-bp or 150-bp Illumina reads using the GATK haplotype caller (McKenna et al. 2010), then imputed missing genotypes and structural variant genotypes from the parental genotypes using a hidden Markov model. They calculated segregant phenotypes as the size of segregant colonies after 48 hours of growth on agar plates presenting a growth condition of interest, then normalized against growth on YPD plates. Normalized phenotypes were scaled to have mean of 0 and variance of 1 for a given cross and condition. We downloaded phenotype data and segregant genotype calls from https://www.dropbox.com/sh/jqm7a11zz9laytd/AABaE0EfQxLH6ounPhJ7yYWya. We calculated LOD scores at each biallelic marker as:

$$LOD \ = \frac{-N}{2 * ln(10)} ln(1 - r^2)$$

where *r* is the Pearson correlation between the phenotypes and genotypes of the segregants in the cross, and *N* is the number of segregants; see page 454 of Lynch and Walsh (Lynch and Walsh). Strain CLIB219 has a loss-of-function mutation in *ADE2* that dominates the QTL mapping of nearly all traits involving CLIB219; as such, we filtered out 119 segregants with the *ade2*- genotype from the CBS2888 × CLIB219 cross and 123 segregants from the CLIB219 × M22 cross, then performed all phenotypic analyses on these crosses using the remaining 824 and 820 segregants, respectively.

For scatterplots of segregant phenotypes, we partitioned segregants by their genotypes, as called by Bloom et al., at SNPs close to the QTL peaks being examined. SNPs were chosen because we felt the Illumina-sequencing-based genotype-calling would have higher confidence calls for SNPs. SNPs were checked for segregation ratios in the segregants close to the expected 50:50, and for well-behaved linkage disequilibrium patterns with neighboring variants.

Plasmid construction

The Chr VII *MALR* from strain YJM145 (MSY28), referred to as $MALR_{\mathrm{YJM145}}$, was cloned by Gibson assembly (Gibson et al. 2009) onto pRS418 (MSp183, a gift of Fred Cross), a CEN plasmid with a *NatMX* selectable marker. First, $MALR_{\mathrm{YJM145}}$ was PCR-amplified from YJM145 genomic DNA isolated using a DNeasy blood and tissue kit (Qiagen, Valencia, CA, USA). The PCR used primers oMS90/111, which spanned from 827 bp upstream to 690 bp downstream of the $MALR_{\mathrm{YJM145}}$ ORF, and the Herculase II Fusion DNA polymerase (Agilent, Santa Clara, CA, USA). Meanwhile, pRS418 was digested using ApaI and EagI-HF (New England Biolabs, Ipswich, MA, USA). The PCR product was PCR-purified and the plasmid digest was gel-extracted (Qiagen), after which the two were assembled together using Gibson assembly (New England Biolabs) to produce plasmid MSp198.

SGE1 alleles from strains BY, YJM145, YPS1009, and I14 (MSY25, 28, 32, and 33, respectively) were cloned by Gibson assembly onto pRS415 (MSp8), a CEN plasmid with a *LEU2* selectable marker. PCRs of the alleles were generated with oligonucleotides oMS100/101 and spanned from 467 bp upstream to 357 bp downstream of the *SGE1* ORF. pRS415 was digested with EagI-HF and HindIII-HF (New England Biolabs), and cloning was performed as for $MALR_{\text{YJM145}}$ above. The *SGE1* allele from strain 273614 was generated by introducing strain 273614's L449M substitution into the plasmid carrying $SGE1_{14}$ (MSp191). MSp191 was amplified with primers oMS128/129 using Herculase II Fusion DNA polymerase, after which the resulting PCR product was treated with DpnI (New England Biolabs) and PCR-purified. Gibson assembly was performed using the PCR product and oligonucleotide oMS127 to produce the plasmid carrying the *SGE1*-L449M substitution in strain 273614.

Plasmid sequences were checked by Sanger sequencing (Eurofins Genomics, Louisville, KY, USA). Strain, plasmid, and oligonucleotide information is found in Supplemental Tables 3, 4, and 5, respectively.

Maltose growth assay

The maltose growth assay were performed on strains BY, YJM987, YPS1009, Y10, PW5, and CLIB219 (MSY25, 29, 31, 32, 35, and 39, respectively) transformed with either empty vector (MSp183) or the plasmid carrying *MALR*$_{\mathrm{YJM145}}$ (MSp198) using standard lithium acetate transformation procedures (Becker and Lundblad 2001). As strains MSY32 and MSY39 are annotated as carrying plasmids with *NatMX* markers, prior to transformation they were plated without selection for the plasmid, from which resulting nourseothricin-sensitive colonies were used for transformations (GoldBio, Saint Louis, MO, USA). Five distinct colonies from each transformation plate were used as experimental replicates, except for YJM978 + empty vector, which had 4 colonies total.

Single colonies of strains were grown for two days without shaking at 30C in a 96-well deep-well plate (Thermo Fisher Scientific, Waltham, MA, USA; catalog no. 12566612) in 500 µL YPD media with added adenine sulfate (20 mg/L) and nourseothricin (100 µg/mL). The position of each strain on the plate was randomly assigned, and the first and last columns were filled with water, not cultures. One microliter of saturated yeast cultures were transferred into the equivalent position of three 96-well clear flat-bottom plates (Corning, Corning, NY, USA; catalog no. 3370) filled with 198 µL YNB media without ammonium sulfate (BD, Franklin Lakes, NJ, USA; catalog no. 233520) with added monosodium glutamate (Sigma-Aldrich, Burlington, MA, USA; catalog no. G1626), adenine, nourseothricin, as well as 20 g/L maltose, 20 g/L glucose, or no added sugar. Absorbance (OD_{600}) of each well of each plate was measured in parallel on a SPECTROstar Omega microplate reader equipped with a plate stacker (BMG Labtech, Ortenberg, Germany) over a period of 93 hours at ambient temperature (~25 C) with 30 s of shaking at 600 RPM prior to each plate reading. Reads were taken approximately 14 minutes apart.

For each sample, the growth phenotype was calculated as the ratio of the area under the growth curve in maltose media ($AUC_{maltose}$) to the area under the growth curve in glucose media ($AUC_{glucose}$), i.e. $AUC_{maltose}/AUC_{glucose}$. AUC values were calculated by summing the curve's OD_{600} values and then subtracting the summed OD_{600} values for the same sample's no-sugar growth curve as a blank normalization. For figures, OD_{600} values were normalized to OD_{600} values in media without sugar then plotted using the smooth.spline function in R with the parameter lambda=0.0001, though AUC calculations were performed on unsmoothed growth curves. Statistical analysis was performed using one-way ANOVA followed by Tukey's post-hoc HSD test.

Paraquat growth assay

The paraquat growth assay were performed on strain *sge1Δ* from the BY MATa knockout collection (Transomic, Huntsville, AL, USA) transformed with plasmids carrying different alleles of *SGE1* (MSp189-192 and 200), as well as empty vector (MSp8). Ten distinct colonies from each transformation plate were seeded as experimental replicates into a 96-well deep-well plate, with the outer rows and columns filled with water instead of cultures. The media was YNB plus glucose with CSM minus uracil and leucine (Sunrise Science, Knoxville, TN) with uracil (20 mg/L) added back.

In our plate reader, paraquat toxicity differed between rows on the plate. After two days, we seeded 2 µL of precultures into six 96-well plates with 198 µL YPD containing 3.5 mM paraquat, permuting the rows so each culture was in row B of one paraquat plate. We also seeded a YPD plate without paraquat. Culture density readings were taken 32 minutes apart for 113 hrs as above. Only row B of each paraquat plate was used in downstream analyses.

For each sample, the growth phenotype was calculated as the ratio of the area under the growth curve in paraquat media ($AUC_{paraquat}$) to the area under the growth curve in media without paraquat ($AUC_{no\ paraquat}$), i.e. $AUC_{paraquat}/AUC_{no\ paraquat}$. AUC values were calculated by summing the OD_{600} values for the first 96 hours after subtracting from each value the OD_{600} reading at $t = 0$ as a blank normalization.

References

Becker DM, Lundblad V. 2001. Introduction of DNA into yeast cells. In *Current protocols in molecular biology* (ed. F.M. Ausubel), p. Unit13.7.

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