ONLINE METHODS

Construction of ts strains. Over 1,300 ts strains covering ~700 essential genes were collected from ~300 different laboratories. Two pairs of primers were used to amplify the ts allele and the *kanMX* cassette (**Supplementary Fig. 1**). Two PCR products (ts allele fragment and *kanMX* cassette) were then co-transformed into BY4741 (*MAT***a** *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*) and cells were plated on YPD + G418 to select for Kan^R colonies. Transformants were replica-plated twice onto YPD + G418 plates and incubated at 23 °C or 38.5 °C for 1-2 d. Potential ts colonies (lethal/sick at 38.5 °C and healthy at 23 °C) were streaked out on YPD plates, replica-plated onto SC and YPD+G418 plates, and grown for 1-2 d at 23 °C or 38.5 °C. The identified ts colonies (lethal/sick at 38.5 °C) were streaked on six SC plates and at six temperatures (23 °C, 26 °C, 30 °C, 35 °C, 37 °C and 38.5 °C) with a wild-type control (BY4741). Strains that grew well at 23 °C and died or were slow-growing at higher temperatures were frozen down for further confirmation. **Supplementary Table 2** lists the sequences of primers used for constructing the ts strains.

Confirmation of ts strains. Two PCR reactions were carried out to confirm the integration of the ts allele and *kanMX* cassette for the candidate ts strains. One PCR reaction was used to test whether the *kanMX* cassette was linked to the target gene. Another PCR reaction was used to verify that the ts allele and *kanMX* cassette were integrated at the target gene locus (**Supplementary Fig. 1**). The primer sequences for confirmation PCR are listed in **Supplementary Table 2**.

Plasmid complementation. Each ts strain was transformed with the cognate CEN plasmid from the MoBY-ORF library[10] and/or a high-copy plasmid from the yeast genomic tiling collection[11] (Open Biosystem) carrying the wild-type gene, and with a vector control. After growing for 3–5 d at 23 °C, transformants were replica-plated and incubated at 23 °C or at the restrictive temperature for 1–2 d.

Barcoding ts strains. The ts alleles were introduced into the *MAT*α query strain background for synthetic genetic array (SGA) by switching the selectable marker and mating type[47]. The resultant *MAT*α *natMX*-marked ts query strains were arrayed in 96-format on agar plates and mated to an array of Barcoder strains, each carrying a unique *kanMX*-marked barcode cassette[17]. Haploid barcoded ts strains were selected using SGA methodology[47]. The resultant individual barcoded ts strains were then colony-purified and re-arrayed in 96-well-format.

High-resolution liquid growth profiling of the temperature sensitivity of ts alleles. To profile the temperature sensitivity phenotype of the ts alleles, each ts strain ($n = 2$) was micro-cultivated for 3 d at ten temperatures ranging from 22 °C to 40 °C and the change in population density was monitored every 20 min. Specifically, strains were inoculated in 350 µl of SD medium (0.14% yeast nitrogen base, 0.5% ammonium sulfate and 1% succinic acid; 2% (wt/vol) glucose; 0.077% complete supplement mixture (CSM, ForMedium), pH set to 5.8 with NaOH) and precultivated for 48 h at 22 °C. For experimental runs, strains were inoculated to an optical density of 0.03–0.1 in 350 µl of SD medium and cultivated for 72 h in a Bioscreen analyzer C (Growth curves Oy) at the indicated temperature (±0.1 °C) with 10 min preheating time. Optical density (OD) was measured using a wide band (450–580 nm) filter. Plates were subjected to shaking at highest shaking intensity with 60 s of shaking every other minute. OD measurements were taken every 20 min. Strains were run in duplicates on separate plates with four BY4741 replicates in randomized positions on each plate as a reference. Plate layouts were kept unchanged throughout the experimental series.

Three fundamental growth variables, growth lag (time to initiate growth, hours), growth rate (doubling time, hours) and growth efficiency (total density change, ΔOD) were measured to characterize the temperature sensitivity profile of each mutant allele[48,49]. Growth variables of each allele at each temperature were related to the corresponding growth variables of a BY4741 reference strain (BY4741, *his3Δ*; $n = 8$) as ($\log_2$[WT/ts strain]) to provide relative measures of growth. For growth efficiency, this ratio was reversed ($\log_2$[ts strain/WT]). The specific temperature effect of each allele at each temperature was derived by subtracting the relative growth of that allele at 22 °C.

The potential temperature profile similarity of alleles of the same genes was evaluated by comparing the average Pearson correlation coefficient of alleles within the same genes to the average pair-wise Pearson correlation coefficient of alleles of different genes using a two-sample Student's *t*-test with equal variance assumptions. The potential temperature profile similarity of alleles corresponding to genes in the same cellular processes was similarly evaluated by comparing the average Pearson correlation coefficient of alleles within the same cellular processes to the average pairwise Pearson correlation coefficient of alleles of different processes using a two-sample Student's *t*-test with equal variance assumptions, excluding 'within the same gene' relations. Cellular process annotations were obtained from the Saccharomyces Genome Database as "Yeast GO-slim: process" annotations.

Microarray analysis. The homozygous deletion pool containing ~4,700 strains[50] and the ts pool consisting of 440 ts strains plus 162 control strains were prepared, frozen and stored as previously described[16]. Both pools were thawed and diluted in 700 µl of YPD to OD_{600} of 0.06. The homozygous deletion pool and ts pool were separately grown in YPD with zaragozic acid A trisodium salt (ZA, Sigma-Aldrich) or with the solvent DMSO (at 2% final concentration) at 36.5 °C. Cells were collected after five generations using a Robotic Liquid Handling System (http://chemogenomics.med.utoronto.ca/). Both pools were mixed together for genomic DNA preparation, PCR amplification of molecular barcodes and microarray hybridization[16,17]. Data from both pools were then plotted independently. Microarray experiments were repeated three times.

Analysis of growth rates in liquid medium. Wild type (BY4741) and ts strains were grown in YPD for 24 h at 23 °C. The cultures were diluted to OD_{600} of 0.0625 and grown in 100 µl of YPD with either the solvent DMSO or 4 µM of ZA in a 96-well plate. The 96-well plate was constantly shaken in a microplate reader (Tecan, GENios) at 36.5 °C for 20 h and the OD_{600} was read every 15 min. The doubling time of strains was calculated as previously described[51]. The fitness was defined as the ratio of the doubling time of strains grown in the presence of solvent alone (DMSO) relative to ZA treatment. The fitness assays were repeated four times.

Automated image acquisition and analysis. *MAT*α query strains carrying different cellular markers were mated to the ts allele array. *MAT*α haploid ts strains expressing different GFP and/or RFP fusion proteins were isolated using SGA technology[47,52], transferred into liquid selection media and cultured for 1–2 d. Automated imaging and analysis were performed as described previously[21,53,54]. The raw data obtained in both permissive and restrictive temperatures from the HCS experiments are provided in **Supplementary Table 6**.

Confocal microscopy and image quantification. Images were captured using the Quorum WaveFX Spinning Disc Confocal System (Quorum Technologies). The *z*-axis images were converted into a single composite image using the brightest pixel at every position in each of the image planes. This maximum pixel projection technique produced a two-dimensional representation of the GFP fusion proteins within the cell from the three-dimensional data set.

Computational analysis of the HCS data set. To ensure that the identified phenotypes were due to genetic mutations instead of uneven distribution of cell shape, size or cell-cycle stages between mutant and wild-type cell population, the relevant measurements of cellular organelles were scaled into relative morphometric features in comparison with the global cell geometry. For example, for the spindle marker, fiber length was normalized by cell length. The statistical comparison was made based on Wilcoxon Rank Sum test ($P < 0.05$ after Bonferroni multiple testing correction). We further characterized these strains by classifying them as having either no departure from wild-type (0), an enhanced phenotype (+1) or a reduced phenotype (−1) across different features measured. For example, to identify mutants with fishhook spindle, we measured the spindle length in wild-type cells and mutant cells. If a given mutant exhibited elongated spindle compared to wild type it was assigned a score of +1; mutants with spindles shorter than wild type were given a score of −1. If spindle length was unaffected a score of 0 was assigned. Given a predefined functional category, we implemented a genetic algorithm[55] to

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look for an optimal combination of morphological features most predictive of the specified functional category. Support vector machine (SVM) was used to make predictions, and prediction accuracy was assessed by AUC (areas under the receiver operating characteristic curves) on an independent gene set for a blind test. For function prediction across the ts array, SVM ensemble was used to estimate the likelihood of a gene being classified into a given functional category. Briefly, for a given function with its selected features, the entire gene set was split randomly 100 times and each time, a minimal set of genes was used for training and classification was performed on the remaining genes. If a gene was classified more than 50 times into a function, then the corresponding function was assigned to the gene.

Affinity purification and mass spectrometry. Cells expressing GFP- or TAP-tagged proteins (1 liter) were collected at mid-logarithmic phase (A600 = ~0.6), and lysed using glass beads as described[56]. TAP purification was performed as previously described[57]. For GFP affinity purification (AP)-MS, lysates (~150–200 mg protein) were incubated for 2 h at 4 °C with 25 µl GFP-Trap magnetic particles (Chromotek). Beads were then subjected to one rapid wash in 1 ml lysis buffer and one wash in 20 mM Tris pH 8 containing 2 mM $CaCl_2$. On-bead trypsin digestion was performed, followed by LC-MS/MS on a Thermo Finnigan LTQ using the protocols and parameters described previously[57]. Sequence database searching using Mascot 2.2.1 and the *S. cerevisiae* complement of RefSeq release 21 (both forward and reverse entries) was performed, and hits with scores >35 corresponded to a protein false-discovery rate of <4%, determined using a target-decoy strategy[58]. A stringent set of filters were applied to remove likely false positives: (i) all proteins detected in any of three parallel purifications from untagged yeast were removed from the data set; (ii) proteins detected with a frequency of ≥15% across 800 purifications were removed; (iii) only those proteins detected with at least two unique peptides in two biological replicates for any given bait were included in the analysis (their interactions with any of the members of the network are shown).

47. Tong, A.H. & Boone, C. Synthetic genetic array analysis in *Saccharomyces cerevisiae*. *Methods Mol. Biol.* **313**, 171–192 (2006).
48. Warringer, J., Anevski, D., Liu, B. & Blomberg, A. Chemogenetic fingerprinting by analysis of cellular growth dynamics. *BMC Chem. Biol.* **8**, 3 (2008).
49. Warringer, J., Ericson, E., Fernandez, L., Nerman, O. & Blomberg, A. High-resolution yeast phenomics resolves different physiological features in the saline response. *Proc. Natl. Acad. Sci. USA* **100**, 15724–15729 (2003).
50. Lee, W. *et al.* Genome-wide requirements for resistance to functionally distinct DNA-damaging agents. *PLoS Genet.* **1**, e24 (2005).
51. St. Onge, R.P. *et al.* Systematic pathway analysis using high-resolution fitness profiling of combinatorial gene deletions. *Nat. Genet.* **39**, 199–206 (2007).
52. Costanzo, M. & Boone, C. SGAM: an array-based approach for high-resolution genetic mapping in *Saccharomyces cerevisiae*. *Methods Mol. Biol.* **548**, 37–53 (2009).
53. Baryshnikova, A. *et al.* Synthetic genetic array (SGA) analysis in *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*. *Methods Enzymol.* **470**, 145–179 (2010).
54. Vizeacoumar, F.J., Chong, Y., Boone, C. & Andrews, B.J. A picture is worth a thousand words: genomics to phenomics in the yeast *Saccharomyces cerevisiae*. *FEBS Lett.* **583**, 1656–1661 (2009).
55. Holland, J. *Adaptation in Natural and Artificial Systems: an Introductory Analysis with Applications to Biology, Control, and Artificial Intelligence*. (University of Michigan Press, Ann Arbor, 1975).
56. Gingras, A.C. *et al.* A novel, evolutionarily conserved protein phosphatase complex involved in cisplatin sensitivity. *Mol. Cell. Proteomics* **4**, 1725–1740 (2005).
57. Breitkreutz, A. *et al.* A global protein kinase and phosphatase interaction network in yeast. *Science* **328**, 1043–1046 (2010).
58. Perkins, D.N., Pappin, D.J., Creasy, D.M. & Cottrell, J.S. Probability-based protein identification by searching sequence databases using mass spectrometry data. *Electrophoresis* **20**, 3551–3567 (1999).