Supporting Information

Dixon *et al.* 10.1073/pnas.0806261105

SI Methods

Identification of Gene Orthologs. Gene orthologs between *S. pombe* and *S. cerevisiae* were defined as per the yeast orthologous groups *pombe*/*cerevisiae* ortholog table version 2.9, distributed by Valerie Wood (*S. pombe* GeneDB, Wellcome Trust Sanger Institute) February 22, 2008. Human orthologs of yeast genes were identified using InParanoid 6.0 (1) and expert hand curation.

***S. pombe* Strains, Culturing, and Automated Yeast Manipulation.** In addition to the gene deletion strains described herein, the following strains were used in this work: FY71 [*h+ leu1–32 ura4-D18 ade6–M216*], FY72 [*h- leu1–32 ura4-D18 ade6–M210*], and h90. Unless otherwise indicated, all strains were cultured at 30°C on YES media using standard protocols (2). All yeast manipulations were performed at either 384 or 1536 density using a Singer RoToR plate handling robot (Singer Instruments), disposable Singer RePads, and Singer Plus Plate containing 50 ml of media.

***Sp*SGA Method.** To test for genetic interaction using *Sp*SGA, a query strain (genotype: *h+ geneX*::*kanMX4*), harboring a single gene deletion, was mated to a miniarray of single deletion strains (genotypes: *h- geneX*$^{1-222}$::*natMX4*) arrayed in 384 format (step 1). Matings were performed on SPA plates (2). Immediately following the transfer of cells onto the mating plate, cells were exposed to a drop of sterile H_2O and then mechanically mixed using a custom agar mixing application developed for the RoToR robot. This procedure was found to increase sporulation efficiency roughly 6-fold (Fig. S3*a*). Following cell transfer and robotically assisted mixing, cells were allowed to sporulate at 26°C for 3 days (step 2). Mating plates were then subsequently transferred to 42°C for 3 days, a treatment that effectively eliminates unmated cells, thereby enriching for spores (Figs. S3 *b* and *c*) (step 3). At a similar step, the recently described pombe epistasis mapper (PEM) strategies make use of chemical means to select against unmated cells and prevent remating following transfer to rich media (3). In *Sp*SGA, heat treatment alone is used to eliminate unmated haploids; the mating of any residual haploids not destroyed by heat treatment is inhibited by subsequent replating onto rich media. Following heat treatment, spores were transferred on to two separate YES plates lacking antibiotics and allowed to germinate for 2 days at 30°C (step 4). During this transfer step, cells were rearrayed from 384 to 1536 format using the arraying option of the RoToR robot. Thus, for each mating, the end result was 16 (4×4) colonies. Because each pinning contains unique spores, each subsequent colony was semi-independent, allowing errors due to missed pinnings to be controlled. To select for recombinant double-mutant haploids, cells were transferred (1536 to 1536) from each YES plates to two separate YES plates containing G418 and ClonNAT and allowed to grow for a further 2 days (step 5). The concentration of geneticin (G418; Invitrogen) and nourseothricin (ClonNat; Werner Bioagents) used in the double-drug selection plates was 250 μg/ml and 100 μg/ml, respectively. A single round of double-drug selection was found to minimize the effect of any residual contaminating trans heterozygous diploids, which are relatively slow growing on rich media.

***SpSGA* Data Processing, Scoring Colony Size, and Identifying Genetic Interactions.** Following growth on selective media, plates were imaged immediately and colony size was quantified using a custom suite of colony size scoring software (4). From the original set of 222 queries $\times$ 222 miniarray strains examined by *Sp*SGA, a detailed analysis of genetic linkage patterns and reciprocal genetic interactions revealed that 134 strains performed well as both query and miniarray strains, a further 21 strains performed well as array strains only, and 39 performed well as queries only (Table S2). Colony sizes were normalized and genetic interactions identified using the SGA-score approach. The SGA scoring system derives precise quantitative genetic interaction estimates from double-mutant colony size data by comparing double-mutant growth to a model for the expected combination of two fitness defects. Negative or positive deviations from this model are measured and reported as genetic interactions. When compared with existing methods for processing SGA data (5), which do not account for several systematic experimental effects associated with SGA technology or relate colony size directly to mutant fitness, the SGA score was observed to improve both the sensitivity and the specificity of interaction detection (C.L.M., A.B., M. Costanzo, H. Ding, C.B., and O. Troyanskaya, unpublished work). This SGA-score method therefore provides a high-confidence set of SL/SS genetic interactions for our analysis.

Genetic linkage can result in the appearance of spurious SL/SS interactions. Given observed linkage patterns and published data showing that in *S. pombe* 1 cM $\approx$6.5 kb (6, 7), we filtered our interaction dataset to exclude any interactions detected between genes lying within 300 kbp on the same chromosome. From the remaining set of SL/SS interactions, we identified the optimal value to establish our comparison datasets by precision-recall curve analysis of the *Sc*SGA dataset compared with the *Sc*BioGRID standard. This analysis suggested an optimum cutoff at 8% (Fig. S2). As the *Sp*GI-Overlap dataset is largely independent from the *Sp*SGA dataset, we were not able to apply similar P-R curve analysis to this data. Therefore, for consistency, we chose to use the 8% cutoff established for *Sc*SGA with the *Sp*SGA dataset. In practice, as detailed in the main body, this dataset is highly enriched for true SL/SS interactions.

Confirmation of *S. pombe* Genetic Interactions and Estimation of the Real Conservation Rate. All candidate SL/SS genetic interactions were tested using random spore analysis (RSA). From mating plates, colonies were scraped into individual wells on 96-well PCR plates containing 100 μl sterile water + 0.5% (vol/vol) glusulase (Perkin–Elmer) and incubated overnight at 30°C in a temperature-controlled heat block. The next day, colonies were washed twice with 100 μl sterile water, resuspended in 100 μl sterile water, and replated at a ratio of 1:2:2:4 onto individual 6 cm dishes containing 10 ml of YES, YES+Nat, YES+G418, or YES+G418+Nat media. Three days later, plates were imaged and scored by comparing the colony size on the individual drugs to the growth of colonies on double-drug media. We were unable to recover viable spores following this procedure for a number of double-mutant combinations, such that we were unable to definitively confirm all identified interactions by RSA. Some interactions were also confirmed using standard tetrad dissection techniques.

We estimate the real *S. pombe* conservation rate to be proportional to the number of real conserved interactions divided by number of real nonconserved interactions plus the number of real conserved interactions. Given, the observed positives and negatives in the

*Sp*SGA HC dataset and assuming a similar error rate in the *Sc*SGA HC dataset, we estimate the real conservation (*cons*) to be 29% as follows:

$$cons = \frac{No(PPV_{SP})(PPV_{SC}) + Nt(PPV_{SP})(1 - NPV_{SC})}{[No(PPV_{SP})(1 - PPV_{SC}) + Nt(PPV_{SP})(NPV_{SC})] + [No(PPV_{SP})(PPV_{SC}) + Nt(PPV_{SP})(1 - NPV_{SC})]}$$

$$= ((54*0.89*0.89) + (186*0.89*0.12))/((54*0.89*0.11) + (186*0.89*0.88) + (54*0.89*0.89) + (186*0.89*0.12))$$

$$= 29\%$$

where Nt = detectable nonconserved interactions in *Sp*SGA HC dataset = 240–54 = 186; No = Observed conserved interactions in *Sp*SGA HC dataset = 54; PPV = positive predictive value (proportion of real interactions among the detected interactions at the given cutoff) = true positives/(true positives + false positives) = 0.89; NPV = negative predictive value (proportion of real noninteracting pairs among the noninteracting outcomes at the given cutoff) = true negatives/(true negatives + false negatives) = 0.88. The subscripts SP and SC refer to *S. pombe* and *S. cerevisiae*. A detailed explanation of the term *positive predictive value* can be found elsewhere (8).

Conserved Yeast Network (CYN) Construction. The CYN network (Table S2) includes 144 unique interactions involving 1:1 gene orthologs identified by overlapping several datasets: *Sp*GI-Overlap vs. *Sc*BioGRID (48 nonunique interactions), *Sp*SGA HC vs. *Sc*BioGRID (64 nonunique interactions), *Sp*SGA HC vs. *Sc*SGA HC (54 nonunique interactions), and *Sp*GI-Overlap vs. *Sc*SGA HC (5 nonunique interactions). We also identified 23 nonunique interactions by a process we call deep comparison. We have already noted how the selection of the 8% cutoffs for the experimentally derived datasets were chosen to maximize the exclusion of false positive interactions. However, this stringent approach may result in the exclusion many real interactions, some of which are likely to be conserved. To attempt to capture these interactions, we examined the most extreme 9%–50% of all SL/SS *Sp*SGA interactions and found 102 supported by literature-curated evidence in *Sc*BioBRID. We tested these interactions by RSA, confirming 23 of these interactions as true positives missed in the *Sp*SGA HC dataset. Consistent with expectations, many of these interactions were synthetic sick interactions, potentially accounting for why they were not observed in the 8% most extreme set.

1. Berglund AC, Sjolund E, Ostlund G, Sonnhammer EL (2008) InParanoid 6: Eukaryotic ortholog clusters with inparalogs. *Nucleic Acids Res* 36(Database issue):D263–266.
2. Forsburg SL, Rhind N (2006) Basic methods for fission yeast. *Yeast* 23(3):173–183.
3. Roguev A, Wiren M, Weissman JS, Krogan NJ (2007) High-throughput genetic interaction mapping in the fission yeast Schizosaccharomyces pombe. *Nat Methods* 4(10):861–866.
4. Tong AH, *et al.* (2004) Global mapping of the yeast genetic interaction network. *Science* 303(5659):808–813.
5. Collins SR, Schuldiner M, Krogan NJ, Weissman JS (2006) A strategy for extracting and analyzing large-scale quantitative epistatic interaction data. *Genome Biol* 7(7):R63.
6. Wood V, *et al.* (2002) The genome sequence of Schizosaccharomyces pombe. *Nature* 415(6874):871–880.
7. Young JA, Schreckhise RW, Steiner WW, Smith GR (2002) Meiotic recombination remote from prominent DNA break sites in S. pombe. *Mol Cell* 9(2):253–263.
8. Jansen R, Gerstein M (2004) Analyzing protein function on a genomic scale: The importance of gold-standard positives and negatives for network prediction. *Curr Opin Microbiol* 7(5):535–545.
9. Katayama S, Kitamura K, Lehmann A, Nikaido O, Toda T (2002) Fission yeast F-box protein Pof3 is required for genome integrity and telomere function. *Mol Biol Cell* 13(1):211–224.

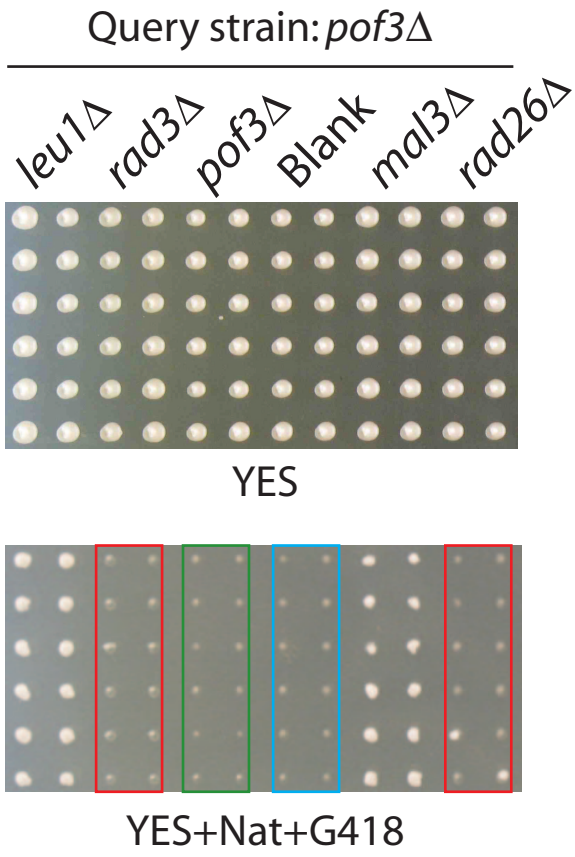
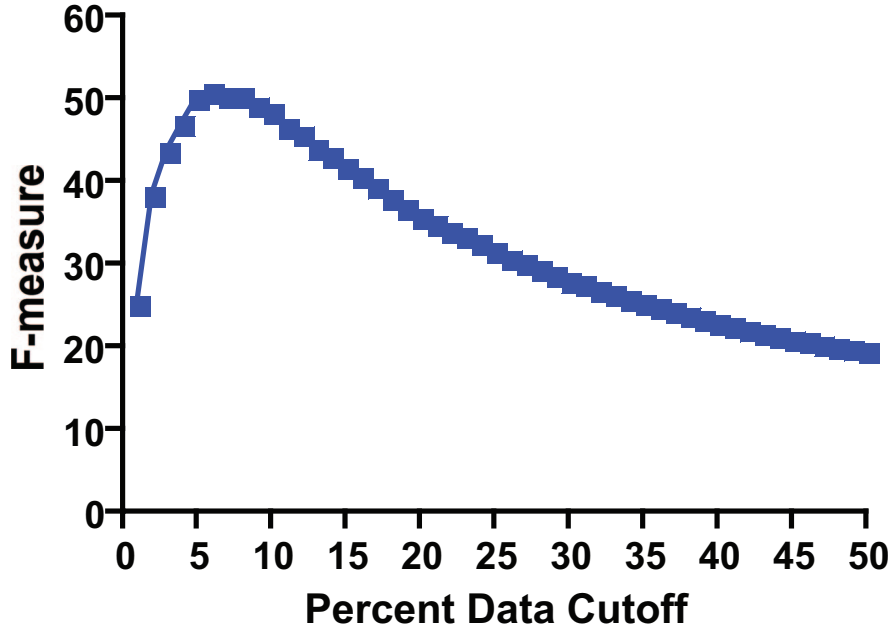
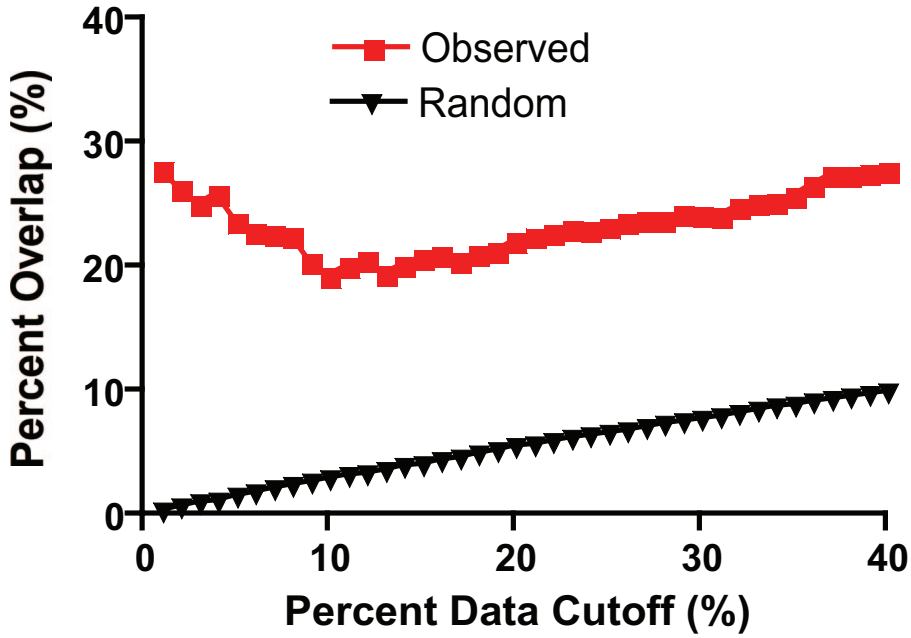


Fig. S1. Validation of the *Sp*SGA method. The query strain *pof3* was mated to a miniarray containing the strains *leu1*Δ, *rad3*Δ, *pof3*Δ, *mal3*Δ, *rad26*Δ, or no gene (blank, -). *pof3*Δ is known to be synthetic lethal with *rad3*Δ and *rad26*Δ (9) but not *leu1*Δ or *mal3*Δ. Following double-drug selection, both the negative and positive controls behaved as expected.

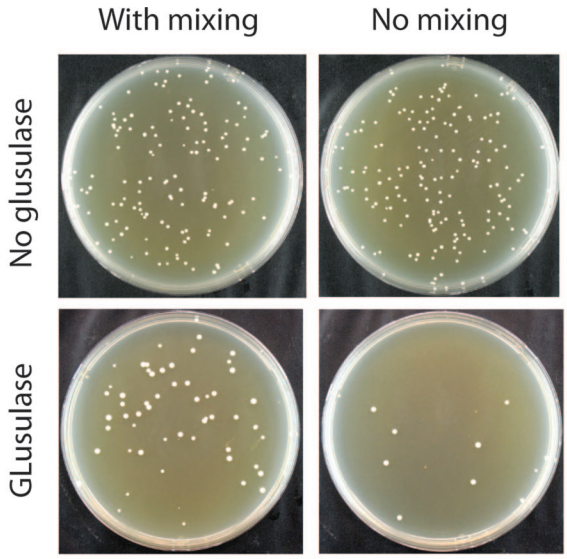


a.

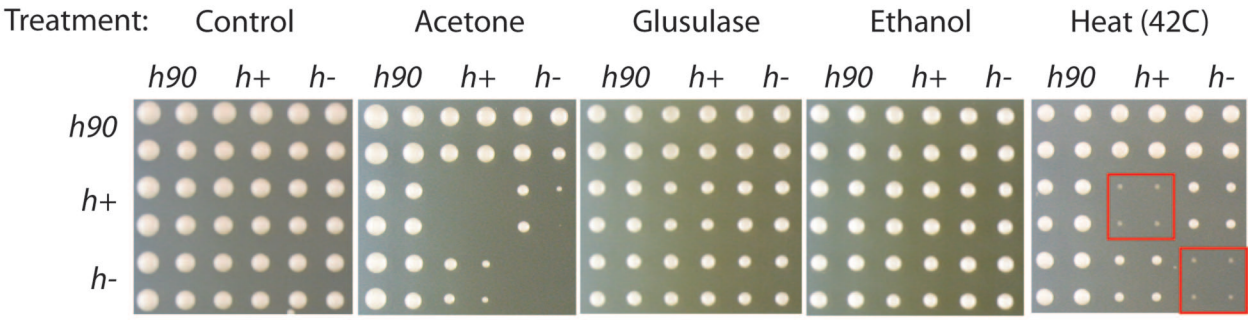


b.

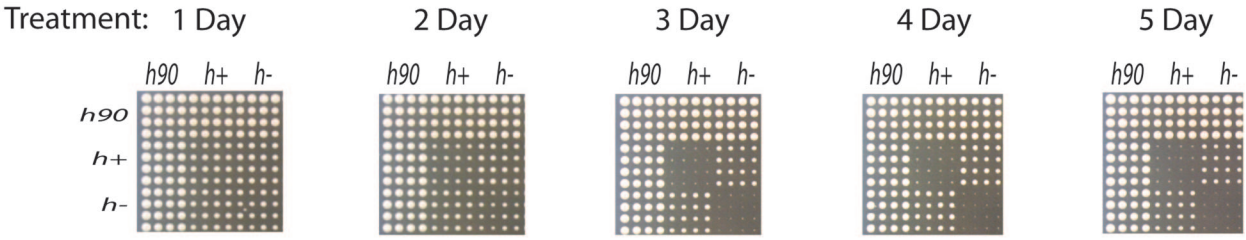
Fig. S2. (*a*) An analysis of the precision-recall curve for the full *Sc*SGA dataset, using the literature-curated *Sc*BioGRID dataset as a benchmark, indicates that an 8% data cutoff maximizes the F-measure. The F-measure is equal to 2 × precision × recall/precision + recall. The percent data cutoff indicates the amount of all SGA scores that are included in the comparison, starting with the most extreme negative scores and working toward the least significant scores. For example, a data cutoff of 10% indicates that the comparison involves the 808 most extreme negative SGA scores in the *Sp*SGA dataset (10% of the negative interactions in the dataset). (*b*) Shows the percentage overlap of genetic interactions (red line) as a function of the ''slice'' (percentage) of the most extreme negative genetic interactions included in the comparison from 1% to 40% (*x* axis). The data for negative interactions reported in the main body of the text was taken from the 8% slice. The black line represents the overlap of randomly generated networks having the same number of nodes and connections but with links randomly shuffled.



a.



b.



c.

Fig. S3. New methods facilitate the recovery of double-mutant haploids. (*a*) Robotic water droplet/mixing protocol enhances sporulation efficiency vs. simple mating. Wild-type cells of opposite mating type (*h*+ and *h*−, strains FY71 and FY72, respectively) were mated on SPA using one of two procedures: transfer of one strain on top of the other, followed by the addition of a droplet of sterile H_2O and the use of a custom agar mix option for the Singer RoToR that mechanically mixes the cells together by stamping up and down on the agar in a square pattern 0.1 mm around the center of the colony (with mixing); or simple transfer of one strain on top of the other (no mixing). Three days later, eight colonies from each SPA plate were picked into either 1 ml of H_2O or 1 ml of a 0.5% vol/vol glusulase/H_2O solution and incubated overnight at 30°C. Following washing and spore isolation (see *Methods*), cells were plated out on YES plates and individual colonies counted following 3 days of germination and growth at 30°C. The mixing protocol enhances spore formation, as judged by colony formation following glusulase treatment, at least ~6-fold (lower left panel) vs. no mixing (lower right panel). (*b*) Heat treatment enriches for spores at the expense of haploid cells. Wild-type cells of three different mating types, *h90* (contains both mating types), *h*+, and *h*− were mated using the *Sp*SGA protocol, sporulated for 3 days at 26°C, and then treated in one of the following ways before repinning onto YES media and subsequent germination for 2 days: no treatment (control); robotic exposure to vapors from a 70% acetone/water (vol/vol) solution for 40 s (acetone); robotic application of a 0.5% (vol/vol) glusulase/sterile H_2O solution on top of the mated cells followed by overnight incubation at 30°C (glusulase); robotic application of a 70% (vol/vol) ethanol/sterile H_2O solution on top of the mated cells followed by overnight incubation (ethanol); or incubation of mating plates at 42°C for 3 days (heat). Heat treatment was found to effectively enrich for spores by eliminating unmated haploids (*h*+/*h*+ and *h*−/*h*− cannot mate, and therefore cannot form spores) from the postsporulation cell mixture (red boxes). (*c*) Other 42°C incubation times (1–5 days) were also examined, this time using a 1536 plating density, and it was found that 3 days provided the best balance of selectivity over time. Different temperature regimes, from 37°C to 52°C, were also examined but found to be less optimal than 42°C (data not shown).

Other Supporting Information Files

Table S1
Table S2
Table S3
Table S4
Table S5
Table S6