

Supplementary Material

Materials and Methods

SGA Analysis: Manual and Robotic Arraying Procedure

For arraying by hand, yeast cells were transferred with a 96 Floating-Pin Replicator (VP408FH), containing 96 pins of 1.58mm-diameter, and a colony Copier (VP380), which allows for construction of a 3-by-3 array of 96 colonies (V & P Scientific, Inc.; <http://www.vp-scientific.com/htdocs/>). The strains were arrayed in triplicate, resulting in 3X 96 grid of 288 different strains and a total of 864 colonies/plate. To sterilize the replicator, it was first placed in a tray of water (~2 min), which removes most of the yeast cells from the pins. Next, the replicator was placed in a tray of 10% bleach (20 sec), followed by 3 sequential rinses in different water baths (10 sec/bath). Finally, the replicator was placed in 95% ethanol and then flamed. For automated arraying, yeast cells were transferred using a 768 floating-pin replicator, a design based upon the methodology developed for automated two-hybrid screens developed in Stan Fields' laboratory <http://www.depts.washington.edu/sfields/>. To sterilize the pins, first they were placed in a tray of water (2 min); second, the pins were placed in a sonicator containing 70% ethanol (2 min); third, they were placed in a tray of 95% ethanol (30 sec); finally, the pins were fan-dried (30 sec). Automated arraying was carried out using a Virtek Colony Arrayer <http://www.virtekbiotech.com>. 1mm-diameter pins were used in all the steps except for the final transfer, for which 0.5mm-diameter pins were used.

SGA Analysis: Media and Growth Conditions

The deletion mutant array (DMA) was propagated on rich medium containing G418 (200mg/L; InVitrogen). The following procedure facilitated SGA analysis. First, query strains were grown overnight in 5ml of rich medium (YEED), then transferred to an empty Omnitray. To generate a source of newly grown query cells for mating to the DMA, the query strain culture was pinned onto rich (YEED) plates (at a density of 384/plate for hand-pinning and 768/plate for automated pinning) and the cells were grown for one day. Second, for the mating reaction, the query strain was first pinned onto a fresh YEED plate, the DMA was pinned on top of the query cells, and the plates were incubated at room temperature for one day. **The resulting *MATa* zygotes were pinned onto medium that selects for growth of diploid cells; for example, if the query strain carried a *natR*-marked mutation, then diploids were selected on rich medium (YEED) containing G418 (200 mg/L) and clonNAT (100mg/L; Werner BioAgents). If the query strain carried a *URA3*-marked mutation, then diploids were selected on synthetic dextrose medium (SD) lacking uracil and lysine.** Third, diploid cells were pinned to sporulation medium (2% agar, 1% potassium acetate, 0.1% yeast extracts, 0.05% glucose, supplemented with uracil, histidine, and leucine) and the arrays were incubated for five days at 22°C. Fourth, to select for growth of *MATa* spore progeny, spores were pinned on to haploid selection medium [SD medium lacking histidine and arginine but containing canavanine :SD - His/Arg + canavanine (50mg/l)] and incubated at 30°C (2 days). Fifth, the *MATa* cells were repinned to haploid-selection medium for a second round of selection and incubated at 30°C for 1 day. Sixth, to select for meiotic progeny that carry the deletion mutation derived from the DMA parental strain, *MATa* haploids were pinned onto SD medium containing G418 and grown for 1 day. Because ammonium sulfate impedes the function of G418 and clonNAT, synthetic medium containing these antibiotics was made with monosodium glutamic acid as a nitrogen source [20g/L agar,

1.7g/L yeast nitrogen base w/o ammonium sulphate and amino acids (Difco), 1g/L monosodium glutamic acid, 2g/L amino acid drop-out lacking histidine and arginine, 50mg/L canavanine, and 200mg/L G418]. Finally, prior to scoring colony size, double mutants were selected on synthetic haploid-selection medium containing G418 and clonNAT (100mg/L clonNAT) for two days. For temperature sensitive mutants, the mating and haploid selection steps were carried out at a permissive temperature (~26°C).

SGA Analysis: Yeast Strains and Reporters

Derivatives of BY4741 (*MATa ura3Δ0 leu2Δ0 his3Δ1 met15Δ0*) and BY4742 (*MAT□ ura3Δ0 leu2Δ0 his3Δ1 lys2Δ0*) were used to construct strains designed specifically for this study [Brachmann *et al.*, *Yeast* **14**, 115 (1998)]. Two different starting strains were used for the SGA screens, Y2454 (*MAT□ mfa1Δ::MFA1pr-HIS3 can1Δ ura3Δ0 leu2Δ0 his3Δ1 lys2Δ0*) and Y3068 (*MAT□ can1Δ::MFA1pr-HIS3 ura3Δ0 leu2Δ0 his3Δ1 lys2Δ0*). In Y2454, *HIS3* was integrated at the *MFA1* locus such that it is regulated by the *MFA1* promoter (pr), *mfa1Δ::MFA1pr-HIS3*. In Y3068, *MFA1pr-HIS3* was integrated at the *CAN1* locus, *can1Δ::MFA1pr-HIS3*. The construction of Y2454 involved three steps. First, to create Y2402 (*MAT□ can1Δ ura3Δ0 leu2Δ0 his3Δ1 lys2Δ0*) which carries *can1Δ*, BY4742 was transformed with *Bam* HI-linearized p3630 and transformants were selected on SD-URA media; Ura⁺ colonies were streaked on 5-FOA plates to select for loss of the integrated *URA3* maker and the pRS306 backbone; integration of *can1Δ* was confirmed by canavanine resistance. To create p3630, *CAN1* was amplified from yeast genomic DNA with primers (5'-GCGAACAGAGTAAACCGAA-3' and 5'-GAAGGTCTGAAGGAGTTC-3'), cloned into pRS306, then digested with *Hind*III and religated, to remove a 1345-bp fragment within the *CAN1* gene. Second, to create Y2420 (*MATa mfa1Δ::MFA1pr-HIS3 ura3Δ0 leu2Δ0 his3Δ1 met15Δ0*), the *MFA1* open reading frame was replaced by the *HIS3* gene via integration of a PCR product generated with primers (5'-**ATCTGTA**ACTGTTTCTCGGATAAAACCAAATAAGTACAAAGCCATCGAATAGAAATGACAGAGCAGAAAGCCCT-3') and (5'-**GGTGTAGCGGAAAAGGAAGATAAAGGAGGGAGAACAACGTTTTTGTACGCAGAACTACATAAGAACACCTTTGG-3'**), which anneal to *HIS3* and contain *MFA1* sequences (in bold), and pEG202 plasmid DNA [J. Gyuris, E. Golemis, H. Chertkov, R. Brent, *Cell* **75**, 791 (1993)] as a template. Third, to create Y2454 (*MAT□ mfa1Δ::MFA1pr-HIS3 can1Δ ura3Δ0 leu2Δ0 his3Δ1 lys2Δ0*), Y2420 was crossed to Y2402, the resulting *MATa/□* diploids were sporulated, and spore progeny with the appropriate markers were recovered. The construction of Y3068 involved four steps. First, a 455-bp fragment containing the upstream sequence of *CAN1* was amplified from yeast genomic DNA with primers (5'-TAGGGCGAACTTGAAGAATAACC-3') and (5'-**GCCACGTTGCACACTATCCTGTGCTATGCCTTTTTTTTTTTTGT-3'**), which contained a 21-bp sequence (in bold) from the 5' end of *MFA1pr-HIS3*. Second, *MFA1pr-HIS3* was amplified from genomic DNA obtained from Y2420 with primers (5'-CAGGATAGTGTGCAACGTGGC3') and (5'-**ATCAAAGGTAATAAAACGTCATATCTACATAAGAACACCTTTGGTGG-3'**), which contained a 24-bp sequence (in bold) from the 5' end of the *CAN1* downstream sequence. Third, a 300-bp fragment containing the *CAN1* downstream sequence was amplified from yeast genomic DNA with primers (5'-ATATGACGTTTTATTACCTTTGAT-3') and (5'-ACGAAAATGAGTAAAATTATCTT-3'). Finally, the set of PCR products above were used as templates to generate a fused product with primers (5'-

TAGGGCGAACTTGAAGAATAACC-3') and (5'-ACGAAAAATGAGTAAAAATTATCTT-3'). BY4741 transformants carrying *can1Δ::MFA1pr-HIS3* were selected on synthetic medium lacking histidine. Y2927 (*MAT□ bni1Δ::natR mfa1Δ::MFA1pr-HIS3 can1Δ ura3Δ0 leu2Δ0 his3Δ1 lys2Δ0*) was created in two steps. First, Y2454 was first transformed with a *Bam*HI-*Not*I cut p1041, which carries a *bni1Δ::URA3* allele deleted for an internal ~3.6 kb *Msc*I-*Hpa*I *BNII* fragment, to generate Y2613 (*MAT□ bni1Δ::URA3 mfa1Δ::MFA1pr-HIS3 ura3Δ0 leu2Δ0 his3Δ1 lys2Δ0*). Second, *bni1Δ::URA3* was switched to *bni1Δ::natR* by PCR-based integration with primers (5'-**AGTATTCTTAACCCAACTGCACAGAACAAAAACCTGCAGGAAACGAAGATAAATCATGACCACTCTTGACGACACGG-3'** and 5'-**TTGAAGCTCTAATTTGTGAGTTTAGTATACATGCATTTACTTATAATACAGTTTTCTAGGGGCAGGGCATGCTCAT-3'**), which anneal to *natMX4* DNA [A. L. Goldstein, J. H. McCusker, *Yeast* **15**, 1541 (1999)] and contain *URA3* sequences (in bold). Y3122 (*MAT□ bbc1Δ::natR mfa1Δ::MFA1pr-HIS3 ura3Δ0 leu2Δ0 his3Δ1 lys2Δ0*) was created in two steps. First, *BBC1* was deleted from Y2454 using PCR-based integration with primers (5'-**CGCGTCCCAACTGCAAGAGCTACTAGAAGTAAACACAGTCTTACCCAAACTAAGGAGGAAACAGCTATGACCATG-3'** and 5'-**GGAAAATGATGAAATCGAAAGCATTCCACAATTTCCCCTTGGAAGCATTGGGTAAAGTTGTAAAACGACGGCCAGT-3'**), which annealed to *URA3* and contained *BBC1* sequences (in bold), to generate Y2980 (*MAT□ bbc1Δ::URA3 mfa1Δ::MFA1pr-HIS3 can1Δ ura3Δ0 leu2Δ0 his3Δ1 lys2Δ0*). Second, *bbc1Δ::URA3* was switched to *bbc1Δ::natR* as described above. The construction of Y3029 (*□ □ □ arc40-40::URA3 mfa1Δ::MFA1pr-HIS3 ura3Δ0 leu2Δ0 his3Δ1 lys2Δ0*) involved four steps. First, PCR-mutagenized DNA [R. C. Cadwell, G. F. Joyce, *PCR Methods Appl* **3**, S136 (1994)] carrying *ARC40* and 200bp downstream of its stop codon was amplified with primers, (5'-**ATGTCATTTTCCAATTCTAAAGACAA-3'**) and (5'-**GGGACGAGGCAAGCTAAACAGATCTTCTTTGGGTAGCAATTCTAAATAAT-3'**), which contains 25bp (in bold) complementary sequence to the *URA3MX6* cassette. Second, *URA3MX6* was amplified from p4348 using primers (5'-AGATCTGTTTAGCTTGCCTCGT-3') and (5'-**AGGTAGGGAATACACATAACAATATATATATATACGTATGTATATGAATTTCGAGCTCGTTTAAACTGGA-3'**), which contains 45bp (in bold) complementary to the sequences downstream of *ARC40*. Third, Y2454 was co-transformed with the two PCR products described above. Fourth, transformants were selected on SD medium lacking uracil and replica-plated to 37°C to identify temperature-sensitive alleles of *ARC40*. To create p4348 (pCRII-TOPO::*URA3MX6*), the *URA3MX6* cassette was amplified from pJJ224 (Rosetta Inpharmatics) with primers (5'-AGATCTGTTTAGCTTGCCTCGT-3' and 5'-**GAATTCGAGCTCGTTTAAACTGGA-3'**) and ligated into pCRII-TOPO (InVitrogen). The construction of Y3181 (*MAT□ arp2-33::URA3 mfa1Δ::MFA1pr-HIS3 ura3Δ0 leu2Δ0 his3Δ1 lys2Δ0*) involved five steps. First, *ARP2* (with 200bp downstream sequence) was amplified using mutagenic PCR (R. C. Cadwell, G. F. Joyce *ibid* pS136) from yeast genomic DNA using primers, which contains 25bp sequence (in bold) that anneals to the *URA3MX6* cassette (5'-**ATGGACCCACATAATCCAATTGG-3'** and 5'-**GGGACGAGGCAAGCTAAACAGATCTACTGAATACTTGAAATACCAAGAAAA-3'**). Second, *URA3MX6* was amplified from p4348 using primers, which contains 45bp sequence (in bold) that anneals to the 45bp downstream sequence of *ARP2* (5'-

AGATCTGTTTAGCTTGCCTCGT-3' and 5'-
ATTTAAACTTTTTTATTAATTCTTTGGATCAATAAAGTTCAAATGAATTCGAGCT
 CGTTTAAACTGGA-3'). Third, Y2454 was transformed with the two PCR products described
 above. Fourth, Ura⁺ transformants were selected on SD-URA and finally screened for
 temperature sensitivity at 37°C. We used a switcher/replica-plating method (see below) to create
 the following strains Y3304 (*MAT α bim1 Δ ::natR mf Δ 1 Δ ::MF Δ 1pr-LEU2 can1 Δ ::MFA1pr-HIS3*
ura3 Δ 0 leu2 Δ 0 his3 Δ 1 lys2 Δ 0), Y3310 (*MAT α sgs1 Δ ::natR mf Δ 1 Δ ::MF Δ 1pr-LEU2*
can1 Δ ::MFA1pr-HIS3 ura3 Δ 0 leu2 Δ 0 his3 Δ 1 lys2 Δ 0) and Y3334 (*MAT α rad27 Δ ::natR*
mf Δ 1 Δ ::MF Δ 1pr-LEU2 can1 Δ ::MFA1pr-HIS3 ura3 Δ 0 leu2 Δ 0 his3 Δ 1 lys2 Δ 0).

SGA Analysis: Switcher Method for Starting Strain Construction

We developed a simple 6-step replica-plating method for converting the yeast deletion mutants
 into a form that enables SGA analysis. This system is simple and takes advantage of the
 position-confirmed gene deletion mutations constructed by the deletion consortium [E. A.
 Winzeler et al., *Science* **285**, 901 (1999)]. First, the *kanMX* gene deletion marker of one of the
 deletion mutants was switched to *natMX* by transformation with *EcoRI*-cut p4339, a *kanMX* to
natMX marker-switcher plasmid. To create p4339, we amplified *natMX* [A. L. Goldstein, J. H.
 McCusker, *Yeast* **15**, 1541 (1999)] from with primers (5'-ACATGGAGGCCAGAAATACCC-3'
 and 5'-CAGTATAGCGACCAGCATTAC-3') and introduced the product into pCRII-TOPO
 (Invitrogen). Second, the resultant *natR* transformants were mated to Y3084
 (*mf Δ 1 Δ ::MF Δ 1pr-LEU2 can1 Δ ::MFA1pr-HIS3 ura3 Δ 0 leu2 Δ 0 his3 Δ 1 lys2 Δ 0*) and
 zygotes were isolated by micromanipulation. To create Y3084 (*mf Δ 1 Δ ::MF Δ 1pr-LEU2*
can1 Δ ::MFA1pr-HIS3 ura3 Δ 0 leu2 Δ 0 his3 Δ 1 lys2 Δ 0), the *MF Δ 1* open reading frame of Y3068
 was replaced by the *LEU2* gene via integration of a PCR product generated with primers (5'-
CAAACAAGAAGATTACAAACTATCAATTTTCATACACAATATAAACGATTA**AAAG**
AATGTCTGCCCTAAGAAGATCG-3') and (5'-
TACAGTGGGAACAAGTCGACTTTGTTACATCTACACTGTTGTTATCAGTCGGG
CTTAAGCAAGGATTTCTTAACTTC-3'), which anneal to *LEU2* and share homology with
 the *MF Δ 1* locus (in bold), and pRS315 DNA as the template [R. S. Sikorski and P. Hieter,
Genetics **122**, 19 (1989)]. Third, the *MAT α / Δ* diploids were transferred onto sporulation medium
 for three days at 22°C. Fourth, *MAT α* meiotic progeny were selected on synthetic medium
 lacking leucine and arginine but containing canavanine. Fifth, cells were replica plated onto
 medium containing nourseothricin to select for the deletion mutants. Sixth, cells were replica
 plated onto medium lacking lysine to identify *lys2 Δ 0*-derivatives.

Deletion Mutant Arrays

A total of 4,672 different yeast deletion mutants, generated by the international deletion
 consortium [E. A. Winzeler et al., *Science* **285**, 901 (1999)], were assembled in our laboratories
 [Howard Bussey (McGill University)] and also obtained from Research Genetics
 (<http://www.resgen.com>). Tables of the deletion mutants contained on the test array shown in
 Fig. 2A of the manuscript and the large-scale array is provided as supplementary material. Some
 of the deletion mutants appear to be synthetically lethal with a wild-type control strain; these
 should include deletion mutants that are slow growing, mutants deleted for genes that are linked
 to *CAN1*, deletion mutants defective for histidine biosynthesis, mutants defective for arginine
 biosynthesis that are synthetically lethal with *can1 Δ* , deletion mutants defective in mating,
 deletion mutants that lead to a sporulation defect of the heterozygous diploid, and perhaps others.

A table of 437 mutants that appear to be synthetically lethal with a wild-type control strain is provided as supplementary material.

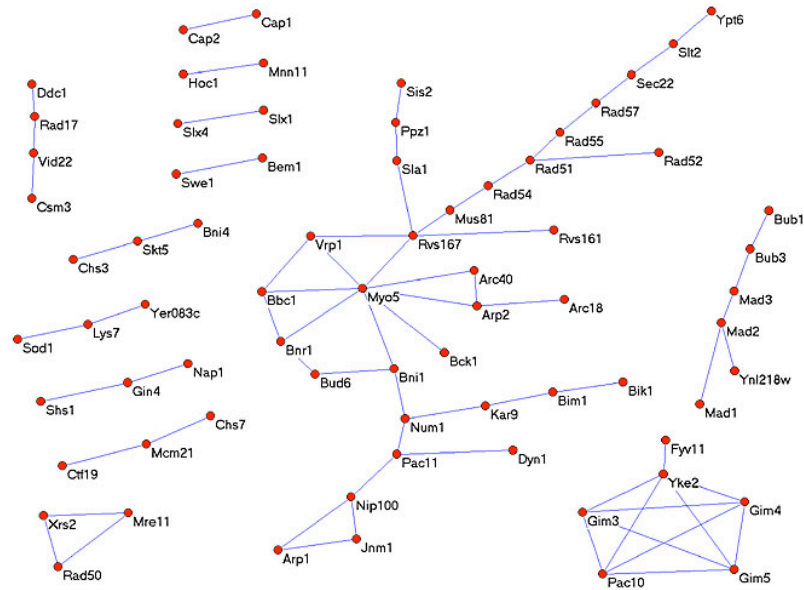
Bioinformatics Analysis

Data from the synthetic lethal interaction network was assembled as a list of yeast gene name pairs. The yeast import tool for the BIND project [Bader et al., *Nucleic Acids Res.*, **29**, 242 (2001); <http://www.bind.ca>] was used to convert the gene name pairs into BIND protein-protein interaction records. This tool integrates yeast information from SGD (<http://genome-www.stanford.edu/Saccharomyces/>), MIPS (<http://www.proteome.com/>), RefSeq (<http://www.ncbi.nlm.nih.gov/LocusLink/refseq.html>), and the SGD yeast gene registry in order to unambiguously assign any yeast gene name, present in these resources, to an NCBI RefSeq biological sequence. Yeast physical interaction data sets (8429 protein-protein interactions) were imported from YPD, MIPS and previous large-scale genome-wide screens for comparison [P. Uetz et al., *Nature*, **403**, 601 (2000); T. Ito et al., *Proc Natl Acad Sci U S A* **97**, 1143-7 (2000); T. Ito et al., *Proc Natl Acad Sci U S A* **98**, 4569-74 (2001); B.L. Drees et al., *J Cell Biol* **154**, 549 (2001)]. The list of previously known synthetic lethal interactions was created by merging 1142 known synthetic lethal interactions provided by YPD (as of July 29, 2001; <http://www.proteome.com/>) with 531 known synthetic lethal interactions from MIPS (as of Aug 7, 2001; <http://www.mips.biochem.mpg.de/>), resulting in 1291 unique interactions. The overlap with the genetic interactions presented in Fig. 3 of the manuscript is supplied as supplementary material. The MIPS list was downloaded (http://mips.gsf.de/proj/yeast/tables/interaction/genetic_interact.html) and manually edited to extract synthetic lethal interactions. For network visualization and analysis, BIND can export an arbitrary molecular interaction network as a Pajek network file, which can be viewed with the Pajek program for large network analysis [<http://vlado.fmf.uni-lj.si/pub/networks/pajek/>; D. White, V. Batagelj, A. Mrvar, *Computer Review* **17**, 245-274 (1999)]. The format of the Pajek network file can be found on the Pajek web site. Fig. 3 was created with the Pajek program using a Kamada-Kawai automatic layout with subsequent manual alterations to remove node overlap and to cluster the nodes by cellular role. Node coloring by YPD cellular role was done automatically by BIND upon network export using selected cellular role annotation for each protein involved in the network.

A program was written to find yeast genetic or physical interactions in a list where either one or both members of the interaction are of interest. This program was used to identify 72 known physical interactions where both proteins in the interaction correspond to products of genes within the synthetic lethal network presented in Fig. 3 of the manuscript. Homodimers were excluded because SGA can not find interactions of a gene with itself. A map of the 72 protein-protein interactions is included as a figure in the supplementary material. To assess the significance of observing 72 genes whose products interact within this data set, we constructed 1000 random networks that contained 204 genes. On average, 7.8 (~4%; SD = 4.1) genes within the random dataset had products that occurred within the protein-protein interaction data sets. Thus, the synthetic lethal network enrichment for genes whose products interact was highly unlikely to occur by chance. The products of many of the interacting genes occur within pathways probably because the readout of the pathway is required for life in strains carrying the synthetic lethal query mutation.

Two dimensional Hierarchical Agglomerative Clustering and R software (<http://www.r-project.org>) were used to create supplementary material Fig. 2. The data were represented as a matrix of 0s, 1s and 2s with 201 rows and 8 columns. A cell in the table (row i & column j) with a non-zero entry represents an interaction between array gene i and query gene j. A 1 signifies that the double mutant is synthetic sick and 2 represents that the double mutant is synthetic lethal. The dissimilarity of 2 genes was calculated as the sum of absolute differences over 8 (query gene) attributes. The dissimilarity of 2 screens was calculated as the sum of absolute differences over the 201 (array) genes. The genes and screens were ordered according to their similarity.

Supplemental Figure 1.



Supplemental Figure 2.

