

Supporting Online Material for

A global genetic interaction network maps a wiring diagram of cellular function

This document includes:

Materials and Methods

Figure Legends

Data File Descriptions

References

Tables S1 to S3

Figs. S1 to S24

Other Supporting Online Material for this manuscript includes:

Data Files S1 to S17, which include the genetic interaction dataset and analyses described in this study were deposited in the DRYAD Digital Repository (doi:10.5061/dryad.4291s) and can also be downloaded from <http://boonelab.ccb.utoronto.ca/supplement/costanzo2016>

The complete genetic interaction network can be downloaded at:
<http://thecellmap.org/costanzo2016/>

See section below, “General Information about SGA datasets” for a detailed description of the different datasets.

Table of Contents

A global genetic interaction network maps a wiring diagram of cellular function	1
General information about SGA datasets	3
SGA query strain construction and screening	4
General description of the SGA genetic interaction score	4
Estimating reproducibility of genetic interactions	7
Constructing genetic interaction profile similarity networks	8
Predicting gene function from essential versus nonessential genetic interaction profile similarity networks	9
Spatial Analysis of Functional Enrichment (SAFE)	10
Identifying pleiotropic genes	10
Predicting function for uncharacterized genes	11
Experimental Validation of Gene Function Predictions	12
<i>IPA1</i> experimental validation	12
MTC pathway experimental validation	14
Genetic interaction degree and network density analysis	16
Comparison of SGA genetic interactions with other genomic datasets and precision-recall analysis	21
Evaluating functional coherence of positive interactions	22
A genetic profile similarity-derived functional hierarchy	22
<i>YNL181W</i> chemical genetic screens	24
Genetic interaction enrichment within and between protein complexes	24
Protein features associated with proteasome interacting genes	26
RNAi and cell fitness measurement in <i>Drosophila</i> cell culture	26
Analysis of positive interaction enrichment and bias among protein complexes	27
Confirmation of genetic suppression interactions	28
Supplementary Table Legends	29
Supplementary Figure Legends	29
Supplementary Data File Descriptions	39

Materials and Methods

General information about SGA datasets

The experiments and analyses described in this study were based on a representative subset of the complete SGA dataset, unless otherwise noted. The subset includes ~19 million double mutants covering more than 80% of all double mutants tested, and consists of ~460,000 negative and ~275,000 positive genetic interactions, identified at an intermediate confidence threshold on the genetic interaction score that was previously defined (1). The nonessential x nonessential (NxN), essential x essential (ExE) and essential x nonessential (ExN) genetic networks based on this subset of data are described in detail below and are provided in various formats as Supplementary Data Files (Data Files S1-S2). We note that Data Files S1-S2 contain raw interaction data corresponding to all tested gene pairs. This data should be filtered for specific applications. We suggest three different thresholds [lenient ($P < 0.05$), intermediate ($P < 0.05$ and $|\varepsilon| > 0.08$), and stringent confidence ($P < 0.05$ and $\varepsilon > 0.16$ or $\varepsilon < -0.12$)] that strike different balances between false negatives and false positives as described in our previous study (1). The quality analysis of data produced at each of these thresholds was completed as described below and is provided in fig. S2. Pearson Correlation Coefficient (PCC) matrices reporting the interaction profile similarities used to generate the essential, nonessential and global similarity networks (Fig. 1-2) are also provided (Data File S3). The dataset can also be browsed interactively at <http://thecellmap.org/>.

The complete genetic interaction dataset based on analysis of ~23 million double mutants, which mapped ~550,000 negative and ~350,000 positive genetic interactions and covers ~90% of all yeast genes as either array and/or query mutants can be downloaded from <http://thecellmap.org/costanzo2016/>.

Non-essential deletion and essential TS mutant arrays

The SGA genetic interaction dataset is based on analysis of query mutant strains crossed to an ordered array of nonessential deletion mutants and/or an ordered array of conditional, temperature sensitive (TS) alleles of essential genes (11, 12). The previously described (1) nonessential deletion mutant array (DMA) array is comprised of 3827 strains, each deleted for a unique nonessential gene (labeled *_dma#*). The essential TS allele array (TSA) includes 786 TS alleles (*_tsa#*) representing 560 essential genes. It also includes 186 nonessential DMA strains, which provides a substantial set of overlapping double-mutants used to calibrate the interaction scores derived from the TSA strains to the scores derived from the DMA (see below: Merging genetic interaction data derived from the nonessential deletion and essential TS mutant arrays). A complete list of mutant strains used in this study is provided (Data file S1).

SGA query strains and genetic interaction networks

We constructed three different genetic interaction networks. First, 3,283 nonessential deletion query mutants strains were screened against the DMA covering 3,827 nonessential genes to generate a nonessential x nonessential (NxN) network. Second, 869 TS query mutant strains representing 606 essential genes, which includes alleles previously published (12, 58) as well as new alleles for essential genes not present in previously reported collections, were also

screened against the DMA to generate part of the essential x nonessential (ExN) network. Finally, 1,194 nonessential deletion mutant query strains and 824 TS query mutant strains, corresponding to 584 essential genes, were crossed to the TSA to complete the ExN network, as well as generate the essential x essential (ExE) network. Previously described SGA screens (1) were re-scored as part of this study and in some instances, query or array strains have been removed for technical reasons. Thus, the dataset associated with this study supersedes the previously reported quantitative dataset. The SGA genetic interaction dataset is provided below in different formats (Data Files S1-S2).

DAmP query mutant SGA screens

In addition to TS alleles, a set of query mutants carrying hypomorphic, Decreased Abundance by mRNA Perturbation (labeled *_DAmP#*) alleles of essential genes, which are potential hypomorphic mutants, was screened against the DMA and TSA. A list of DAmP mutant query strains and genetic interaction data involving DAmP alleles (Data File S1) is provided and these data are also available for download through a web-based database (<http://thecellmap.org/costanzo2016/>). Only a subset of DAmP alleles perturbed gene function enough to elicit a single mutant fitness defect. Thus, genetic interaction profiles associated with many DAmP allele query strains are relatively sparse compared to genetic interaction profiles associated with TS alleles (fig. S1).

SGA query strain construction and screening

SGA query strain construction and screening were conducted as described previously (59). All SGA selection steps involving a TS allele were conducted at permissive temperature (22°C) except for the final selection of haploid double mutants, which were incubated at a semi-permissive temperature (26°C) prior to imaging. Double mutant selection plates involving a nonessential deletion mutant query strain and the DMA were incubated at 30°C. A subset of essential TS and nonessential deletion mutant query strains were screened at both 26°C and 30°C. Technical and functional evaluation showed that interactions derived from nonessential query mutants screened against the DMA were of equal quality when colonies were grown at 26°C or 30°C, whereas higher quality interactions from screens involving a TS allele (either as a query or an array mutant) were obtained when plates were grown at 26°C. Thus, we included data based on the following rules: (1) For nonessential queries screened against the DMA at both temperatures, we retained only the data from the 30°C screen; (2) For essential queries screened against the TSA and/or DMA at both temperatures, we included the 26°C data; (3) For queries screened only at a single temperature, we included the available data. All screens were conducted a single time with 4 replicate colonies per double mutant, unless otherwise indicated (see “Estimating reproducibility of genetic interactions” section below).

General description of the SGA genetic interaction score

To derive quantitative genetic interactions, we modeled colony size as a multiplicative combination of double mutant fitness, time, and experimental factors as previously described (5) with some modifications described below. Briefly, for a double mutant carrying mutations of genes *i* and *j*, colony size C_{ij} can be expressed as $C_{ij} = f_{ij} \times t \times s_{ij} \times e$, where f_{ij} is the double mutant fitness, t is the incubation time, s_{ij} is the combination of all systematic factors, and e is

log-normally distributed random noise. The double mutant fitness f_{ij} can be further expressed as $f_{ij} = f_i f_j + e_{ij}$, where f_i and f_j represent the fitness of the two single mutants and e_{ij} is a quantitative measure of the genetic interaction (genetic interaction score) between them.

The interaction data corresponding to all tested gene pairs (Data Files S1-S2) should be filtered prior to use. We suggest three different thresholds [lenient ($P < 0.05$), intermediate ($P < 0.05$ and $|\varepsilon| < 0.08$), and stringent confidence ($P < 0.05$ and $\varepsilon > 0.16$ or $\varepsilon < -0.12$)] that strike different balances between false negatives and false positives as described in our previous study (1). The quality estimates of data produced at each of these thresholds was completed as described below and is provided in Fig. S2.

Scoring Synthetic Lethality

We note that extreme negative genetic interactions scores, $\varepsilon \leq -0.35$, often correspond to double mutants whose colony size is visibly smaller than the colony size of the corresponding single mutants and, thus, we consider most of the interactions below this threshold to be synthetic lethal. As a result, the number of synthetic lethal interactions between nonessential genes (~10,000) was estimated by applying an extreme negative interaction score threshold ($\varepsilon \leq -0.35$) to the complete NxN dataset (www.the-cellmap.org/costanzo2016).

Single mutant fitness standard

To derive accurate estimates of single mutant fitness, we applied our correction method (5) to a set of control SGA screens, where a query strain carrying a *natMX* marker inserted at a neutral genomic locus was crossed to the *kanMX*-marked DMA (*_dma#*) and TSA (*_tsa#*) strain collections. In addition, ordered arrays of SGA query mutant strains carrying *natMX*-marked, nonessential deletion mutations (*_sn#*) or TS alleles of essential genes (*_tsq#*) were crossed to a different SGA control strain, which carried a *kanMX* marker inserted at a neutral genomic locus. Colony size measurements of SGA deletion and TS array mutant strains were based on an average of 350 replicate control screens performed at 26°C or 30°C. Colony size measurements of SGA deletion and TS query mutant strains were based on an average of 17 replicate control screens performed at 26°C or 30°C. Colony size measurements were used to estimate single mutant fitness as described previously (5) with the exception that bootstrapped means, instead of medians, across replicates were used in variance estimation and final fitness values. Results from separate arrays were calibrated using linear models fit to values for strains that appeared on two arrays (e.g. ~185 deletion strains on the TS array), fixing the y-intercept at 0. For some arrays, only a single control strain overlapped with other arrays, in which case we simply used a scale factor to calibrate fitness.

Because the *kanMX* and *natMX* drug-resistance cassettes do not affect fitness when colony size measurements are derived from growth on standard SGA media, we treated the fitness of most single mutant array and query strains for the same mutant as independent estimates and assigned the mean of the two estimates to each strain; a small number (~55) of query and array strains for the same mutant exhibited notably different colony sizes and thus separate fitness estimates were retained for these mutants. In total, ~700 TS allele strains and ~3100 deletion mutant strains have fitness measurements based on both array and query strain backgrounds.

Fitness of an additional ~1300 TS allele strains and ~1600 gene deletion mutant strains was based on colony size measurements of the *kanMX*- or *natMX*-marked strain alone. Because we observed a close correlation between fitness measured at 26°C and 30°C for deletion mutants, we combined measurements from different temperatures in the average for each deletion mutant. Fitness associated with TS mutants was computed separately at either 26°C or 30°C. Fitness estimates for all mutant strains used in this study are provided (Data File S1).

Co-batch filtering of query mutant strains

The SGA scoring pipeline includes a batch effect correction based on a Linear Discriminant Analysis approach (5). However, weak batch effects may persist despite correction and can dominate the genetic interaction profiles of SGA query mutants that exhibit relatively few interactions. In total, 319 SGA query strains, corresponding to 316 genes, were removed from either the nonessential and/or essential networks because the average Pearson correlation coefficient to other query strains screened in the same batch exceeded 0.2. Pearson correlation was chosen because of its tendency to exacerbate batch effects for query strains with weak genetic interaction profiles (60). Query mutant strains with residual batch effects were removed before merging data derived from screens conducted at different temperatures. Strains removed from the final genetic interaction dataset as a result of the co-batch filter are provided in Data File S9. In addition, Data File S9 provides a “co-batch” rank for all query strains screened against the DMA and/or TSA. Low negative interaction degree (e.g. lowest 20% of queries) coupled with a low co-batch rank (e.g. $< \sim 0.2$) and a low fraction of correlated pairs that share a similar functional annotation with a given query strain (e.g. $< \sim 0.15$) may indicate that the genetic interaction profile associated with a given query strain shows evidence for a lingering batch effect. A subset of query strains that fit these criteria is listed in Data File S9 (“Queries removed-batch effects”). We note that this co-batch score is not a definitive measure of screen quality and should not be used as a primary metric to filter the genetic interaction dataset. However, this score should be considered for these screens, especially when conducting specific analyses that depend on genetic interaction profile similarity.

Merging genetic interaction data derived from the nonessential deletion and essential TS mutant arrays

To ensure that the genetic interaction scores derived from the DMA and TSA were comparable, a set of nonessential deletion mutants were included on the TSA. Mutants common to both the DMA and TSA accounted for ~297,000 double mutant colony measurements that were used to calibrate interactions scores derived from TSA against the same interactions derived from the DMA. Specifically, quantile normalization was applied to the interaction scores for mutants present in both datasets using DMA-derived scores as the reference, which ensured that the distributions of scores for overlapping interactions matched. The quantile-normalized scores of the essential array interactions overlapping with the DMA defined a transformation that was then applied to the rest of the TSA-derived interaction scores. This procedure was applied independently within each temperature as the final step of scoring, before quality filters were applied and data collected at different temperatures were merged.

Estimating reproducibility of genetic interactions

Results from the reproducibility analysis are shown in fig. S2. We generated an evaluation set of 40 different query mutant strains with at least 5 independent replicates to estimate the reproducibility of genetic interactions. Of those 40 queries, 14 were screened against the TSA and 26 against the DMA. These strains were specifically selected to span the spectrum of observed single mutant fitness to provide representative queries for all screened mutants. In total, this set generated ~120,000 double mutants with 5 replicates each, which formed the basis of our reproducibility analysis.

Determining a threshold at which individual interactions are deemed significant required us to select a trade-off between the rate of false positives and false negatives. Setting a stringent threshold ($P < 0.05$ and $\varepsilon > 0.16$ or $\varepsilon < -0.12$) will result in a high quality set of interactions but potentially at the cost of many false negatives. Conversely, setting a more lenient threshold will reduce the false negative rate, but at the cost of more false positives. Because different types of analyses of genetic interactions can tolerate different levels of false negatives/false positives, we provide raw, unthresholded data and suggested three different thresholds [lenient ($P < 0.05$), intermediate ($P < 0.05$ and $|\varepsilon| < 0.08$), and stringent confidence ($P < 0.05$ and $\varepsilon > 0.16$ or $\varepsilon < -0.12$)] that strike different balances between false negatives and false positives, as described in our previous study (1). The quality estimates of data produced at each of these thresholds was completed as described below and is provided in fig. S2.

For the set of screens with at least 5 replicates, we counted the number of times each interaction was identified across 5 replicate screens and reported the fraction of interactions observed 1, 2, 3, 4, and 5 times. To determine the fraction of interactions expected to be reproduced by chance, we performed 1000 randomization experiments where interactions were randomly selected for each query mutant ensuring that query interaction degree was preserved.

We generated a “gold standard” set of negative and positive interactions that were observed at least twice at the intermediate score threshold for each query in our evaluation set. Precision was then calculated as the percentage of interactions identified within a replicate that was also present in the gold standard. Recall was calculated as the percentage of interactions of our gold standard that were called within a replicate.

To assess whether the number of times an interaction was reproduced across replicate screens relates to the biological relevance of the interactions, we compared the reproducibility of interactions to the functional relationship between the interacting gene pairs. Interactions were grouped by the number of times each interaction was identified (e.g. 1, 2, 3, 4, or 5 times). For each group, we calculated the fraction of interacting gene pairs that were co-annotated to the same GO biological process term (see “Comparison of SGA genetic interactions with other genomic datasets and precision-recall analysis- GO co-annotation” section below for more details).

Constructing genetic interaction profile similarity networks

Results from this analysis are shown in Figs. 1-2 and resultant genetic interaction profile similarity matrices are provided in Data File S3. Genetic interaction profile similarities were measured by computing Pearson correlation coefficients (PCC) between all query-query and array-array pairs of strains in the nonessential (NxN) and the essential (ExE) genetic interaction datasets. Whenever a pair of strains appeared on both the query and the array side of a dataset and, therefore, was associated with two PCC values, these values were averaged to produce a composite correlation coefficient PCC'. For all pairs of strains that appear on only one side of a dataset, we set $PCC' = PCC$.

The nonessential and the essential genetic interaction similarity networks (Fig. 1A-B) were constructed by connecting all pairs of strains from the NxN and the ExE datasets, respectively, where $PCC' > 0.2$. To construct the global genetic interaction similarity network, which combines both nonessential and essential genetic interaction profiles (Fig. 1C), the PCC values derived from the NxN and the ExE datasets were computed, normalized and averaged as follows:

1. NxN query-query and array-array PCC values were computed and averaged to produce PCC', as above.
2. ExE query-query and array-array PCC values were computed as above and their distributions were normalized to match the distributions of the NxN query-query and array-array PCC values. To perform this normalization, we extracted the overlapping data between the nonessential deletion mutant query strains (SN) and essential gene TS allele query strains (TSQ) x DMA dataset and the SN/TSQ x TSA dataset (see "General information about the dataset" above), corresponding to the set of query-array pairs that were screened twice. For each of these two sub-datasets (DMA-derived and TSA-derived), we computed array-array PCC values and performed quantile normalization to adjust the TSA-derived data to the corresponding DMA-derived data. We then applied the same estimated adjustment to all ExE PCC values, such that their distribution would match the NxN PCC values. Normalized ExE query-query and array-array PCC values for the same strain pairs were averaged to produce PCC', as above.
3. We also computed ExN array-array PCC values, using only a common set of query strains that were tested against both nonessential and essential arrays. The ExN PCC values were normalized using the same strategy as above. For all ExN pairs, $PCC' = PCC$.
4. The final global genetic interaction similarity network was constructed by connecting all pairs of NxN, ExE or ExN strains where $PCC' > 0.2$.

To visualize all three genetic interaction similarity networks (nonessential, essential and global networks), we applied the edge-weighted spring-embedded network layout algorithm, implemented in Cytoscape (61). Edge weights were set to 1 for all pairs of connected nodes ($PCC' > 0.2$). The spring-embedded algorithm re-organizes the positioning of all nodes in two-dimensional space such that tightly connected nodes (i.e., genes sharing similar patterns of

genetic interactions) are placed proximal to each other, whereas less connected nodes are placed farther apart.

Predicting gene function from essential versus nonessential genetic interaction profile similarity networks

Results from the functional evaluation analysis are shown in fig. S6 and Data File S4. We compared the relative amount of functional information contained in genetic interaction profiles based on either nonessential or essential genes. Predictions were made using a weighted, leave-one-out, nearest neighbors approach. More specifically, for a given GO bioprocess term (P) and gene (G), the genetic interaction profile similarities between G and all genes annotated to P were computed and ranked. The sum of the top 5 similarities constituted the prediction score of gene G for process P. These steps were repeated for all genes and then genes were ranked based on their score for each process P and the agreement with the annotations was assessed, with any gene carrying an annotation to term P counting as a true positive, and all others counting as a false positive. These steps were repeated to make predictions for a previously defined subset of biological process GO terms (62). Prediction performance was summarized across the entire set of evaluated GO terms by measuring the precision at 25% recall (PR25). Although analyses were performed using complete genetic interaction profiles (i.e. negative and positive genetic interactions), similar prediction performance was obtained using genetic interaction profiles based on negative interactions alone.

Using this function prediction framework, we assessed the relative predictive power of genetic interactions from nonessential and essential genes by changing the set of interactions used to calculate the profile similarities. More specifically, we restricted the set of query mutants over which array gene pair similarities were calculated (using the dot product as a similarity metric). Nonessential gene predictions were made for array strains present on the DMA, while essential gene predictions were made for array strains present on the TSA. For nonessential deletion mutants present on the DMA, predictions were made using interactions with: (i) nonessential deletion mutant query strains alone; (ii) essential gene TS query mutants alone; or (iii) all query mutants crossed into the DMA. Similarly, function predictions for essential genes present on the TSA were made using interactions with: (i) nonessential deletion mutant query strains alone; (ii) essential gene query TS mutants alone; or (iii) all query mutants crossed into the TSA. A random essential gene TS allele was selected as representative for genes where more than one allele was available. Function predictions were based on 50 random samplings of alleles, and results were based on the mean of these trials. As nonessential query strains outnumbered essential queries, performance of DMA- versus TSA-derived functional predictions was assessed using an equal number of randomly selected nonessential and essential query mutant strains. The GO tends to have more annotations for essential genes than it does for nonessential genes, and consequently, the random expectation of precision when predicting gene function is much higher if the gene is essential. To control for this characteristic in evaluating function prediction performance, we evaluated the prediction of nonessential genes and essential genes separately (fig. S6A-B, respectively).

Spatial Analysis of Functional Enrichment (SAFE)

Genetic profile similarity networks were annotated using SAFE as described elsewhere (15). Results from the SAFE-derived functional enrichment analysis of the global similarity network is provided in Data File S5.

Identifying pleiotropic genes

Results from the pleiotropic gene analysis are shown in Fig. 4 and Data File S7. To identify genes with highly pleiotropic functions, we developed a measure based on biclusters, which are sets of array and query strains that are fully connected by negative interactions. We discovered these biclusters with our previously published method (63). Biclusters were generated for the DMA-derived and TSA-derived genetic networks after binarizing negative interactions by applying the intermediate confidence threshold (1). As described in (63), association rule mining was used to find small groups of query genes that completely interact with the same array mutant strains. Although our previous study (63) used a minimum support of 3 query mutants, the size of our current GI networks required that each group consisted of at least 8 query mutants. Thus, the minimum bicluster size was 8 query strains x 3 array strains. To determine the statistical significance of biclusters, ten randomized network versions that preserved the degree distribution were mined for “random” biclusters. All biclusters were assigned a score based on the degrees of genes involved. Scores of the random biclusters were used as a null distribution to assign P -values to the real biclusters. Only biclusters with P -values lower than 10^{-4} were retained for analysis. For each network, 10 replicate versions of the bicluster analysis were run, each containing only one allele per gene in order to avoid biclusters dependent on similar behavior of different alleles of the same gene but still allow many combinations of different genes.

We computed a measure of pleiotropy for each query strain that reflected the functional diversity of the biclusters in which it appeared. Specifically, each strain’s set of biclusters was obtained from each of the 10 replicates by condensing all the biclusters the gene appeared in to allow a maximum of 10% overlap in interactions. Every bicluster was then annotated by any biological process for which its set of query genes had significant enrichment, assessed by the hypergeometric distribution, or in which at least one-third of queries were annotated. SAFE-derived biological processes described in Fig. 1B and Data File S5 were used for these annotations. The number of a query strain’s biclusters that were annotated by each process formed a functional profile for each network replicate. Replicate profiles were averaged to produce one functional profile per query strain. The pleiotropy score was then calculated using an entropy measure, i.e. $-\sum_i p_i \log_2 p_i$, where p_i is the fraction of biclusters annotated with process i , which is high for strains that appear in biclusters across a variety of different biological processes. Pleiotropy scores were not assigned to strains that had fewer than 10 biclusters.

Note that in *S. cerevisiae*, Hsp90 is encoded by two paralogous genes, *HSC82* and *HSP82*. Thus, genetic interactions for HSP90 were identified by screening a double mutant query strain that harbored a deletion allele of the nonessential *HSC82* genes and a TS allele of the *HSP82* essential gene (Y14084: *MAT α hsc82 Δ ::natMX hsp82-5001::URA3 can1 Δ ::STE2pr-Sp_his5*

lyp1Δ his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 LYS2+)(64). Single mutants (*MATα hsp82-5001::URA3 can1Δ::STE2pr-Sp_his5 lyp1Δ his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 LYS2+* and *MATα hsc82-5001Δ::natMX can1Δ::STE2pr-Sp_his5 lyp1Δ his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 LYS2+*) and wild-type (*MATα ura3Δ::natMX can1Δ::STE2pr-Sp_his5 lyp1Δ his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 LYS2+*) strains were screened in parallel. SGA screens were performed as described above (see section “SGA query strain construction and screening”) and HSP90 genetic interaction data is provided in Data File S1.

We note that the pleiotropy score above was derived solely on the basis of negative genetic interactions. The reasoning for this is that negative genetic interactions are much more associated with close functional relationships than positive genetic interactions. For example, consider the precision-recall analysis presented in Fig. 6B. This analysis demonstrates that for both non-essential and essential gene pairs, negative interactions show substantially stronger enrichment for known functional relationships. Our analysis suggests that positive interactions frequently connect genes with no obvious functional relationship, especially among pairs of essential genes. Although pleiotropy can be defined in many ways, we were interested in identifying genes with evidence of close functional relationships with genes across a diverse set of biological processes. Because negative interactions are much more indicative of these relationships, we based our pleiotropy measure solely on the negative interaction profiles.

To examine the relationship between pleiotropy and position on the global genetic interaction profile similarity network (Fig. 4A), we determined if genes with high and low pleiotropy scores (Data File S7) overlapped significantly with genes that localized to densely connected, functionally enriched regions of the global genetic interaction profile similarity network (Fig. 1; Data File S5) using Gene Set Enrichment Analysis (GSEA) as described elsewhere (65). Genes with low pleiotropy scores localized more often to densely connected network regions than genes with high pleiotropy scores ($P < 10^{-5}$).

Predicting function for uncharacterized genes

Results from SAFE-derived gene function predictions are shown in Fig. 4B. We defined a set of uncharacterized genes in *S. cerevisiae* by parsing the *Saccharomyces* Genome Database (www.yeastgenome.org) for genes associated with unknown GO Biological Process and Molecular Function annotations. To predict which functions may be associated with an uncharacterized gene X, we examined the genes that share similar genetic interactions with X and assessed their enrichment for specific GO biological process terms. Specifically, we first computed all pairwise genetic interaction profile similarities as described in the section “Constructing the genetic interaction similarity network” above. Then, for any given gene X, we sorted all genes in our dataset based on their similarity to X. For every position Y in the sorted gene list, we evaluated the enrichment of the genes ranked Y or higher against all GO biological process terms. For every GO term, we computed fold enrichment over background and a hypergeometric *P*-value, and recorded the rank in the sorted gene list that resulted in the maximum fold enrichment with a significant *P*-value ($P < 0.05$). We then manually examined the top 10 most highly enriched GO terms in the sorted gene list for gene X and excluded all terms that met at least one of the following criteria: a) the maximum enrichment for the term occurred

at a rank lower than 100 on the list; b) the correlation coefficient value corresponding to the position of maximum fold enrichment was lower than 0.1; c) at least one of the genes adjacent to gene X on the chromosome is annotated to the same GO term or appears to be associated with a related function.

As a result of this filtering, we were able to associate 25 uncharacterized genes with a set of predicted functions, as defined by their enriched GO terms. To visualize the positioning of these novel functional predictions on the genetic interaction similarity map (Fig. 4B), we chose the top most highly enriched GO term for each prediction and identified the SAFE bioprocess cluster (Fig. 1F) to which that GO term was most closely associated. For example, *IPA1* localized within the Transcription/chromatin organization enriched cluster on the global similarity network. However, we note that while some genes for which we are predicting functions are located close to a cluster enriched for the same or similar function, the genes do not necessarily localize directly within the corresponding functionally enriched cluster. For example, while *CSF1* localized within the Cell polarity enriched cluster, other genes in the MAY24 pathway, including *MTC2*, *MTC4*, *MAY24* and *DLT1* were located on the periphery of the Cell polarity cluster.

Experimental Validation of Gene Function Predictions

All yeast strains used in the experiments described below, including nonessential deletion mutants and TS mutants of essential genes, were isogenic to the reference BY (S288c) genetic background (9, 10) unless otherwise noted.

***IPA1* experimental validation**

Results from *IPA1* validation experiments are shown in Fig. 5, fig. S8 and Data File S8.

***Ipa1* in vitro polyadenylation assays**

Preparation of yeast cell extracts, transcription of [α -³²P]UTP-labeled *GAL7-1* RNA or precleaved *GAL7-9* RNA, and processing assays were performed as described previously (66).

Genome-wide polyadenylation profiling using RNASeq

S. cerevisiae strains (wild type, TSA1246: *ipa1-5001::kanMX*, TSA801: *cft2-1::kanMX* and TSA685: *pcf11-2::kanMX*) were grown to mid-log phase (OD ~1) in YPD (1% yeast extract, 2% peptone, 1% glucose) at 30°C. Total RNA was isolated by a standard hot phenol method and treated with RNase-free DNaseI using Turbo DNA-free kit (Ambion). Genome-wide quantification of gene expression and poly(A) site usage was performed using the 3'T-fill approach (67) and the QuantSeq 3'mRNA-Seq v016.24 (Lexogen).

Sequencing reads were analyzed as previously described (67). Briefly, reads were mapped to the *S. cerevisiae* genome (SGD R64) using the GSNAP (68). Poly(A) sites were assigned to transcripts by a sliding +/-200 bp window from annotated transcription termination sites (69). Differential gene expression was computed using DESeq2 (70). Sequencing data has been deposited at GEO under the accession number GSE60947.

Affinity purification and mass spectrometry for endogenously-tagged Ipa1

An *S. cerevisiae* wild type strain expressing *IPA1* (YJR141W) C-terminally tagged with GFP (71) was grown in biological replicates to mid-log phase and harvested. In parallel, strains expressing other ORFs C-terminally tagged with GFP were used as controls (*MTC4*, *TSL1*, *CTR86*)(71). Anti-GFP affinity purification was done essentially as described in (12) and samples were digested with trypsin on beads prior to analysis by mass spectrometry. Samples were loaded on an autosampler and analyzed in Data Dependent Acquisition mode on a TripleTOF 5600 mass spectrometer as described in (72) Raw mass spectrometry data (wiff files) were converted to mzML with ProteoWizard 3.0.4468 (73) and the SCIEX converter 1.3 beta and searched with Mascot v.2.3.02 (74) and Comet 2014.02_rev2 (75) against the *S. cerevisiae* subset of the RefSeq V57 protein database at NCBI to which reverse sequences and common contaminants (<http://141.61.102.106:8080/share.cgi?ssid=0f2gfuB>; <http://www.thegpm.org/crap/index.html>) were appended. Search parameters were mass accuracy of 30 ppm for the parent peptide and 0.15 Da on the fragment ions, charges 2+ to 4+, trypsin selection (with up to two mis-cleavage sites), and arginine/glutamine deamidation and methionine oxidation as variable modifications. The results from both search engines were combined using iProphet from the Trans Proteomic Pipeline v4.7 (76), and the proteins with a protein probability ≥ 0.9 and 2 unique peptides were further analyzed with SAINTexpress version exp3.3 (77) implemented within the ProHits Laboratory Information Management System (77). Essentially, the 6 controls were compressed into 3 virtual controls to increase the filtering stringency (as in (78)), and proteins with an Averaged SAINT score of ≥ 0.73 had a $\leq 1\%$ Bayesian false discovery rate.

Affinity purification and mass spectrometry for galactose inducible HA-tagged Ipa1

Coding sequences for *IPA1* were cloned into the vector BG1805 (79) to generate a *GAL1-IPA1-HA* construct that was transformed into the *S. cerevisiae* strain, Y7092 (59), which was cultured and then processed for affinity purification as described (80). Mass spectrometry was also performed as described (80), with the exception that analysis was performed with a Proxeon EASY- nLC pump in line with a hybrid linear quadrupole ion trap (Velos LTQ) Orbitrap Elite mass spectrometer (Thermo Fisher Scientific). Raw mass spectrometry data (RAW files) were converted to mzML with ProteoWizard 3.0.4468 (73) and searched with X!Tandem (version 2006.05.01 (81)) and Comet 2013.02_rev2 (75) against the *S. cerevisiae* subset of the SGD_26jul11 protein database appended with common contaminants and reversed protein sequence complement. Search parameters were: mass accuracy 15 ppm for the parent peptide and 0.36 Da for fragment ions, charges 2+ to 4+, trypsin selection (with up to two missed cleavages), and arginine/glutamine deamidation and methionine oxidation as variable modifications. The results from both search engines were combined using iProphet from the Trans Proteomic Pipeline v4.6rev3 (76) and the proteins with a protein probability ≥ 0.9 and 2 unique peptides were further analyzed with SAINTexpress version exp3.3 (77) implemented within the ProHits Laboratory Information Management System (77). Essentially, the 21 controls were compressed into 7 virtual controls to increase the filtering stringency (as in (78)), and proteins with an Averaged SAINT score of > 0.95 had a $\leq 1\%$ Bayesian false discovery rate.

Mass spectrometry data access

Raw mass spectrometry data and downloadable identification and SAINTexpress results tables are deposited in the MassIVE repository housed at the Center for Computational Mass Spectrometry at UCSD (<http://proteomics.ucsd.edu/ProteoSAFe/datasets.jsp>).

The endogenously-tagged GFP dataset has been assigned the MassIVE ID MSV000079157 and is available for FTP download at: <ftp://MSV000079157@massive.ucsd.edu>. The dataset was assigned the ProteomeXchange Consortium (<http://proteomecentral.proteomexchange.org>) identifier PXD002368 (dataset password: SGA).

The Gal-inducible HA dataset has been assigned the MassIVE ID MSV000079368 and is available for FTP download at: <ftp://MSV000079368@massive.ucsd.edu>. The dataset was assigned the ProteomeXchange Consortium (<http://proteomecentral.proteomexchange.org>) identifier PXD003147 (dataset password: sga).

MTC pathway experimental validation

Results from MTC pathway validation experiments are shown in Fig. 5 and fig. S9.

Bap2-GFP subcellular localization analysis

Wild type (*MAT α can1 Δ ::STE2pr-Sp_his5 lyp1 Δ his3 Δ 1 leu2 Δ 0 ura3 Δ 0 met15 Δ 0 LYS2+ BAP2-GFP::HIS3*, and mutant strains (*MAT α can1 Δ ::STE2pr-Sp_his5 lyp1 Δ his3 Δ 1 leu2 Δ 0 ura3 Δ 0 met15 Δ 0 LYS2+ BAP2-GFP::HIS3 mtc4 Δ ::natMX*, *MAT α can1 Δ ::STE2pr-Sp_his5 lyp1 Δ his3 Δ 1 leu2 Δ 0 ura3 Δ 0 met15 Δ 0 LYS2+ BAP2-GFP::HIS3 mtc6 Δ ::natMX*, *MAT α can1 Δ ::STE2pr-Sp_his5 lyp1 Δ his3 Δ 1 leu2 Δ 0 ura3 Δ 0 met15 Δ 0 LYS2+ BAP2-GFP::HIS3 dlt1 Δ ::natMX*, *MAT α can1 Δ ::STE2pr-Sp_his5 lyp1 Δ his3 Δ 1 leu2 Δ 0 ura3 Δ 0 met15 Δ 0 LYS2+ BAP2-GFP::HIS3 mtc2 Δ ::natMX*, *MAT α can1 Δ ::STE2pr-Sp_his5 lyp1 Δ his3 Δ 1 leu2 Δ 0 ura3 Δ 0 met15 Δ 0 LYS2+ BAP2-GFP::HIS3 may24 Δ ::natMX* and *MAT α can1 Δ ::STE2pr-Sp_his5 lyp1 Δ his3 Δ 1 leu2 Δ 0 ura3 Δ 0 met15 Δ 0 LYS2+ BAP2-GFP::HIS3 csf1 Δ ::natMX*) expressing BAP2-GFP from the endogenous BAP2 locus were grown to early log phase in synthetic dextrose medium lacking histidine, arginine and lysine, in the presence of the drugs canavanine, SAEC and nourseothricin, and were stained with FM® 4-64 dye (Life Technologies, Catalogue No. T-3166) as described in (82), for one hour. Following this incubation, cells were washed three times with water to remove excess dye, resuspended in growth media, and allowed to grow at 30°C for three additional hours to allow complete internalization of the dye to the vacuolar membrane. Cells were imaged live on a high-throughput confocal microscope (Evotec Opera™, PerkinElmer), using a 60X objective (water immersion, NA=1.2), in an Evotec 384-format Cell Carrier glass slide (PerkinElmer, Part No. 6007439). Five z-axis planes were imaged in four sites of each well. All image processing was performed using ImageJ 1.42q.

Phenylalanine uptake assays

Amino acid uptake assays were performed as described elsewhere (83). Wild type, *bap2 Δ ::kanMX*, *tat1 Δ ::kanMX*, *gap1 Δ ::kanMX*, *mtc4 Δ ::kanMX*, *mtc6 Δ ::kanMX*, *dlt1 Δ ::kanMX*, *mtc2 Δ ::kanMX*, *may24 Δ ::kanMX* and *csf1 Δ ::kanMX* strains (fig. S9) were pre-grown to

exponential phase in synthetic dextrose medium lacking histidine, arginine and lysine, in the presence of the drugs canavanine and SAEC, and uptake was performed for 10 min. prior to quenching with an excess of unlabeled phenylalanine.

Mapping metabolomics profiles for MTC pathway mutants

Metabolite extraction

Deletion mutants obtained from the prototrophic yeast deletion collection (84) were backcrossed to a *MAT α his3 Δ* strain, sporulated, and six independent *MAT α* haploids from each mutant were selected by SGA. Strains were grown in minimal media (1.7 g /L yeast nitrogen base without amino acids and ammonium sulfate, Difco 235510, with 20 g/L D-glucose, 0.5 g/L ammonium sulfate and 2.62 g/L ammonium acetate). The following pre-growth and sub-culture conditions were used to control yeast growth to within a narrow range of cell density at time of metabolite extraction. Cells were inoculated from frozen stocks to 150 μ L minimal media in a 96-well round bottom plate and grown for 16 hours at 30°C. Subsequently, 62 μ L of overnight culture were sub-cultured to 1.0 mL minimal media in a 96 deep-well plate. Sub-cultures were incubated at 25°C for 6 hours with shaking, reaching a target OD of ~1.0. The optical density (OD) of each sub-culture was measured and cells were used to inoculate 24-well plates containing 5.0 mL minimal media per well to an OD of 0.0125. After 16 hours incubation at 25°C with shaking at 300 rpm, the cells had grown to a target OD of ~1.0. The OD was measured and cells were diluted to 0.1 OD in 5 mL minimal media. Cells were grown in 24-well plates for 5 hours at room temperature with shaking to a target OD of ~0.4. Optical density was measured for each culture at time of metabolite extraction. Metabolite extracts were prepared by collecting cells on a nylon filter using vacuum filtration and immersing the filter in ice cold 40:40:20 acetonitrile:methanol:water (85). Metabolite extracts were further lysed by freeze-thawing, cellular debris was removed by centrifugation and supernatants were dried under a gentle stream of N₂. Dried metabolite extract samples were resuspended in H₂O in proportion to OD measured at time of extraction. Before LC-MS analysis, all samples were mixed with an equal volume of ¹³C, ¹⁵N labeled reference metabolite extract. Metabolite levels were determined by UHPLC-MS using ion-paired reversed phase chromatography on a C-18 column (86) coupled to an Agilent 6550 QTOF.

Metabolite analysis

Full-scan mass spectra were analyzed using Agilent MassHunter Profinder in batch recursive mode to pick and quantify peaks in an untargeted fashion from wildtype and mutant extracts. An unpaired *t*-test comparing levels of each compound in wild type and mutant was used to identify significantly changing metabolites. Extracted ion chromatograms were visually inspected for each significantly altered metabolite. The metabolites showing significantly altered levels in each individual mutant were compared across all deletion mutants. For each metabolite, integrated peak areas were normalized to peak areas of co-eluting stable-isotope labeled compounds derived from the reference metabolite extract spiked into each sample. Retention times and *m/z* of mass spectral features of interest were matched to known standards or confirmed by MS/MS fragmentation. The intensities of metabolite levels normalized to the internal reference and expressed as log₂ ratios relative to the wild-type control were presented as a heat-map.

Genetic interaction degree and network density analysis

Results from the genetic interaction degree analysis are shown in figs. S10-S14, Tables S1-S2 and Data Files S9-S11.

Nonessential and essential GI hub functional enrichment analysis

GO enrichment analysis (Biological process, Molecular function and Cellular component terms) was performed on the top 10% of array mutants that participated in the highest number of negative interactions and the top 10% of array mutants that participated in the highest number of positive interactions in the ExE network. For the NxN genetic interaction networks, the top 5% of array mutants that participated in the highest number of negative interactions and the top 5% of array mutants that participated in the highest number of positive interactions were selected for the same GO enrichment analysis. Results of this analysis are provided in Data File S11.

Characterization of high- and low-degree genes

To characterize genes that have a particularly high or low number of genetic interactions (GIs), we defined two types of genes from each array: those with negative GI degrees higher than the 80th percentile (Table S1) and those with negative GI degrees lower than the 20th percentile (Table S2). Because some essential genes are represented by multiple TS strains, we randomly selected one allele per strain prior to degree calculations. The negative and positive interaction degree of each query and array mutant is provided in Data File S9.

We performed two-tailed hypergeometric enrichment tests to test for overrepresentation and depletion of binary features in the selected genes; the *P*-values reported are doubled one-tailed *P*-values and only the test with the more significant *P*-value is reported (i.e. the test for either enrichment or depletion). Similarly, we performed two-tailed Wilcoxon rank-sum tests on ordinal and continuous features to test if features tended to be higher or lower in the sets of selected genes than in all other genes. In cases where the rank-sum *P*-value is significant but the medians of the two sets were equal, the means were compared to interpret the difference between the distributions.

Correlation analysis of genetic interaction degree

We assessed the correlation of the number of negative and positive genetic interactions for a given array gene mutant with several different gene features. The set of gene features, described in detail below, included binary-, continuous-, and count-valued features. We used Wilcoxon rank-sum tests to determine if binary gene features partitioned genes into two groups such that one group had an average degree that is significantly higher or lower than the other group. Genes for which the value of the binary feature was unknown were excluded from the feature's test. For continuous- and count-valued gene features, we calculated the Pearson correlation coefficient between GI degrees of strains and each feature (fig. S11-S12). We considered the degree of genes represented on the DMA and TSA, which was calculated by counting the number of interacting query strains meeting the intermediate confidence threshold, including those representing both nonessential and essential genes but excluding DamP strains,. From the set of strains on the TSA, we excluded the ~175 nonessential deletion strains in order to analyze only the behavior of essential genes. For completeness, we also calculated correlations between gene features and interaction degrees associated with query strains. For

this analysis, we considered essential and nonessential queries separately and calculated degree from both arrays separately by counting the number of interactions of query strains with array strains. The results of the query gene interaction degree analysis are provided in Data File S10. Each of the gene features assessed for correlation with genetic interaction degree is described in more detail in the sections that follow.

Description of gene/protein features analyzed

Broad conservation

Broad conservation is a count of how many species, out of a set of 86 non-yeast species, have an ortholog of a given gene. To count this, we obtained orthogroup designations from InParanoid (87). For each gene, we considered it to have an ortholog in another species only if it appeared in a cluster with the other species and was given a score of 1.0 by the InParanoid clustering method; that is, we considered a yeast gene to have an ortholog in species *x* if it was a seed gene for a gene cluster that had an orthologous cluster in species *x*. Note that this measure is similar, though complementary, to the “yeast conservation” measure described below, which focuses on conservation within the yeast clade.

Yeast conservation

Yeast conservation counts how many of 23 different species of Ascomycota fungi possess an ortholog of a gene. This measure was described by Wapinski *et al.*, (88) and ortholog data were downloaded from the associated website <http://www.broadinstitute.org/regev/orthogroups/>.

CAI

The codon adaptation index (CAI) is a sequence-based measure of bias in usage of synonymous codons as compared to usage in highly expressed genes. It was calculated using the cai tool and the default codon usage table in the EMBOSS suite (89).

Chemical-genetic degree

This feature is a count of drug and environmental conditions to which a homozygous diploid gene-deletion mutant strain is significantly sensitive (90)

Co-expression degree

This measure is derived from a co-expression network based on integration of a large collection of expression datasets (91). The network was sparsified by considering only edges between gene pairs whose co-expression levels were above the 95th percentile. The co-expression degree of a gene is the number of genes with which its co-expression value is retained in this restricted network.

Complex member

The complex member feature is a binary gene feature that reflects whether the corresponding protein is a component of at least one complex based on the complex standard provided (Data File S12) and described below (see section “Genetic interaction enrichment within and between protein complexes”).

Copy number

Copy number is a count of the number of paralogs each gene has. This was determined from clusters identified by the InParanoid algorithm. All genes that appeared in the same cluster were considered paralogs.

Copy number volatility

Copy number volatility is the number of times that a gene is lost or gained among 23 Ascomycete fungi species (88).

Curated phenotypes

Mutant phenotypes were downloaded from the *Saccharomyces* Genome Database (SGD) on January 31, 2013. The list of phenotypes was filtered to include only those related to deletion mutants of verified or uncharacterized open reading frames (mutant type = 'null', feature type = 'ORF'). Phenotypes were further filtered to only include increased or decreased phenotype expression compared to a wild-type strain. Finally, the number of non-wild-type phenotypes was counted for each gene. Unclear descriptions of phenotypes, such as "abnormal", were ignored.

Deleterious SNP rate

The deleterious SNP rate feature reflects the number of predicted deleterious SNPs observed across the recently sequenced set of diverse *S. cerevisiae* strains (92). These SNP features were derived from identification and analysis of SNPs in 19 of these strains as described in (93). Briefly, SNPs were identified from sequence alignments of all strains to the S288C reference sequence. The SIFT algorithm, with some modifications, was used to predict which non-synonymous SNPs are likely to have functional consequences. We applied the recommended threshold to SIFT scores, calling any SNP with a score of ≤ 0.05 deleterious. For each gene, the number of deleterious SNPs was measured as the number of amino acid locations that have a SNP in at least one of the 19 strains and was normalized by dividing by the gene's total length.

dN/dS

We computed the average dN/dS ratio for *S. cerevisiae* in comparison to the *sensu strictu* yeast species (*Saccharomyces paradoxus*, *Saccharomyces bayanus* and *Saccharomyces mikatae*). Protein sequences were aligned using MUSCLE and dN/dS ratios were computed using PAML (94, 95).

Effective number of codons

The effective number of codons is a measure of codon usage bias and is an alternative to CAI that does not require a pre-defined set of highly expressed genes. This measure was computed using PAML.

Expression level

The expression level value reflects the mRNA expression levels of all yeast genes (96).

Expression variance measured under different environmental conditions

This dataset contains yeast gene expression levels measured in response to heat shock, hydrogen peroxide, menadione, diamide, dithiothreitol, hyper-osmotic shock, amino acid starvation, nitrogen source depletion, and progression into stationary phase, as well as alternative carbon sources and variable temperatures (97). For each gene, we measured the variance in expression across all conditions surveyed in this study.

Expression variance measured in different genetic backgrounds

This feature captures genome-wide gene expression variation caused by genetic differences in diverse yeast strains as measured by microarray (98), labeled “genetic-A” or RNA-Seq (99), labeled “genetic-B”. We measured the variance of each gene across these strains.

Duplicated

This binary feature captures whether a gene is a duplicate as defined by the feature SSD duplicate or WGD duplicate, described below.

WGD duplicate

This binary feature reflects whether each gene has a paralog that resulted from the whole genome duplication event. The WGD event designation was reconciled from several sources (100).

SSD duplicate

This binary feature reflects genes with one or more paralogs that resulted from small scale duplication (SSD) events. To identify pairs of genes that emerged from SSD events, we searched for gene pairs that meet the following criteria: the gene pair must have a sufficiently high sequence similarity score (FASTA Blast, $E = 10$), sufficient protein alignment length ($> 80\%$ of the longer protein), an amino acid level identity of at least 30% for proteins with aligned regions longer than 150 amino acids or greater than $[0.01n + 4.8L^{-0.32(1 + \exp(-L/1000))}]$ with L defined as the aligned length and $n = 6$ for shorter proteins (101, 102).

Multifunctionality

A quantitative standard for gene multi-functionality was derived from annotations to “biological process” terms of the Gene Ontology. Specifically, the total number of annotations across a set of functionally distinct GO terms described in (62) was used as a multi-functionality index.

Number of complexes

This count-based feature reflects the number of complexes to which a given gene is annotated based on the protein complex standard provided (Data File S12) and described below (see “Genetic interaction enrichment within and between protein complexes”).

Number of domains

This feature is the number of domains, counting repeated domains, present within a given protein, as identified by PFAM (downloaded July 2015).

Originated in *Saccharomyces*

This binary feature reflects whether a gene originated in the *Saccharomyces* clade of the phylogenetic tree, which is assumed to be true when the most distant species with an ortholog is a *Saccharomyces* yeast species. Specifically, we consulted the species tree from Wapinski *et al.* (88) and identified all genes that appear only in *S. cerevisiae*'s closest relatives: species up to and including *Saccharomyces bayanus*. Note that although some more distant species (*Naumovozyma castellii*, *Lachancea kluyveri*) were originally placed in the genus *Saccharomyces* and may still be referred to with this name as described in Wapinski *et al.*, these have subsequently been associated with different genera (88).

Phenotypic capacitance

The phenotypic capacitance was used directly from the Levy & Siegal study (103) and captures variability across a range of morphological phenotypes upon deletion of each of the non-essential genes.

PPI degree, Tap MS

Physical interaction degree from Tandem Affinity Purification coupled with Mass Spectrometry (TAP-MS) refers to the total number of interactions in the union of the datasets (104, 105).

PPI degree, Y2H

Y2H degree is the total number of binary, physical interactions detected using yeast two-hybrid analysis (106).

Protein abundance and protein abundance in stress

Protein abundance was measured by fluorescence of GFP-tagged proteins grown in liquid rich media; protein abundance under stress was measured by fluorescence of GFP-tagged proteins grown in liquid minimal media (107).

Protein disorder

The protein disorder measure is the percent of unstructured residues as predicted by the Disopred2 software (108).

Protein length

This feature measures the number of amino acids in the associated protein.

Single mutant fitness defect

For each gene, the fitness of a mutant strain, with either the gene deleted or replaced by a temperature-sensitive allele, was measured relative to wild-type growth. Fitness defect is measured as $1 - \text{mutant strain fitness}$ and therefore represents the decrease in growth rate compared to wild-type.

GI density in PFAM gene families and GO molecular functions

We compared genetic interaction network density among several different classes of genes defined by protein domains and molecular functions, shown in figs. S13 and S14. Gene assignments to the GO Molecular Function terms were obtained from the *S. cerevisiae*-specific GO Slim (<http://geneontology.org/page/go-slim-and-subset-guide>) downloaded in Jan 2013.

Gene assignments to PFAM groups (<http://pfam.xfam.org/proteome?taxId=559292>) were downloaded in May 2014.

The nonessential (NxN) and essential (ExE) genetic interaction networks were first binarized by applying intermediate confidence thresholds for negative and positive interactions. We then selected the array genes associated with each category of interest (a GO term or PFAM domain) and counted their interactions with all query strains. We computed the density of interactions associated with each category by dividing the number of interacting gene pairs by the total number of screened gene pairs involving the category's array genes. If there were multiple pairs of strains representing the same pair of genes (e.g. due to multiple alleles of the query or array gene), one pair of strains was randomly picked. GI densities were calculated for neighborhoods of GO Molecular Function terms and PFAM families and domains. We excluded any term that had fewer than five array genes annotated by it from this analysis.

Comparison of SGA genetic interactions with other genomic datasets and precision-recall analysis

Results from this comparative analysis are shown in Fig. 6 and figs. S15-S16. We characterized the functional relationships between negative and positive interacting genes by evaluating their overlap with other types of molecular and functional interactions, focusing on the similarities and differences between essential versus nonessential gene interactions. Several properties of gene pairs that were collected and evaluated for overlap with genetic interactions are described in the sections that follow.

Protein-Protein Interactions

Protein-protein interaction data referenced in this study were derived from the union of five earlier high-throughput studies, and was retrieved from BioGRID (109). These studies included Gavin et al. 2006 (104), Krogan et al. 2006 (105), Tarassov et al. 2008 (110), Yu et al. 2008 (106), and Babu et al. 2012 (111).

Gene Ontology (GO) Co-Annotation

GO term annotations were downloaded from the *Saccharomyces* Genome Database in January of 2012. A functional standard for co-annotation to Gene Ontology terms was built using GRIFn (62). Briefly, a set of GO biological process terms was curated to include only terms with enough specificity to be considered functionally informative. Two genes were considered functionally related if they were co-annotated to one or more of these terms. Two genes were considered unrelated if both genes were annotated to at least one GO term but the lowest common ancestor to which both genes were annotated was not part of the functionally specific set of GO bioprocess terms. All other pairs, for example those with one or more unannotated genes, were ignored for the purposes of calculating precision and recall based on the GO.

Co-Expression

Co-expression of genes was derived from the MEFIT co-expression network, an integration of multiple microarray datasets (91). Gene pairs with a MEFIT score > 1 were considered co-expressed, while gene pairs with a score ≤ 1 were not considered co-expressed.

Co-Localization

Gene product pairs were considered co-localized if they shared one or more cellular compartments from a previous high-throughput study (71).

Co-Complex

Co-complex pairs were derived from the updated protein complex standard (Data File S12). Any gene pair for which both genes were members of the same complex was a positive while any gene pair for which both genes were annotated to a complex, but not the same complex, was treated as a negative.

Precision-Recall analysis of interactions

All precision recall analyses were performed as described elsewhere (62) with the indicated functional or protein-protein interaction standard.

Evaluating functional coherence of positive interactions

Results from the analysis of positive genetic interaction profile similarity are shown in fig. S17. For each TS query, we retrieved its binary positive genetic interaction profile by thresholding the corresponding SGA score profile using the intermediate positive interaction cutoff (1). Next, the Jaccard similarity coefficient was calculated between all pairs of TS queries. The Jaccard coefficient was used as opposed to Pearson correlation because it provided better performance when used on only positive interactions. Jaccard coefficients were used to predict functional relationships in two different ways. First, we evaluated whether positive interactions were more similar between alleles of the same gene. For this analysis, only query genes with more than one screened query allele were selected. Then, for each query gene, we calculated the median query Jaccard similarity with alleles of the same gene and contrasted that to the Jaccard similarity with alleles of other genes. One random array allele per gene was selected wherever multiple alleles existed for this analysis.

Second, we evaluated whether positive interactions between members of the same protein complex were more similar to each other relative to other genes. Specifically, Jaccard similarity was used to predict co-complex membership between genes and precision-recall analysis was used to assess the performance. One random query and one random array allele per gene was selected for this analysis where multiple existed.

A genetic profile similarity-derived functional hierarchy

Constructing a functional hierarchy

Results from the functional hierarchy analysis are shown in Fig. 2A, Fig. 7, fig. S7 and Data File S6. Genes were assigned into hierarchical clusters according to the following procedure. First, data from the essential and nonessential arrays were combined in order to compute genetic interaction profile similarity scores for all pairs of array genes. Similarity scores were derived from array gene profiles composed of interactions against all query genes common to both datasets. Distributions of similarity scores were then quantile normalized using the set of array genes that overlapped between the DMA and TSA. The complete matrix of pairwise similarity scores was then clustered hierarchically using a Pearson correlation-based distance measure

(1-Pearson correlation, ranged [0,2]) and average pairwise linkage. Clusters were derived at 3 different levels by thresholding the linkages at 1-Pearson = [0.6, 0.8, 0.95]. To allow for comparison of relationships between genes across multiple levels, genes were filtered from the analysis if they did not appear in a cluster with linkage of ≤ 0.4 , which removed many genes with weak genetic interaction profiles that clustered only near the very top of the tree. We further required that each gene present in the functional hierarchy analysis appeared in a cluster with demonstrated functional relevance, which was enforced by requiring enrichment with one or more GO terms at a P -value threshold of $P \leq 0.05$ (hypergeometric test). These steps ensured that the same set of genes were used at all levels of hierarchical study and ensured that the differences observed (e.g. in genetic interaction network density) were due to hierarchical structure at different levels and not differing sets of constituent genes. The following statements were true as a result of our clustering and filtering procedures: (1) all genes were members of one and only one cluster at each level of the hierarchy; (2) all genes were members of a single cluster at level 1; (3) all clusters were strict subsets of their parent clusters one level above; and (4) each cluster was comprised of the union of its children clusters one level below.

Measuring interaction network density and magnitude at different hierarchy levels

The strict hierarchical tree structure applied to the genetic interaction similarity data allowed us to describe unambiguously, for each pair of genes, whether or not they shared a genetic interaction and a level of specificity or distance defined by the most specific level of the hierarchy in which they shared membership to a common cluster. We used this measure for calculating network density at different levels and assigning each pair to the level of their most specific common cluster. This prevented the density estimate at each level from being dominated by the trends observed at lower levels. Density was defined as the number of significant interactions ($|\epsilon| \geq 0.08$; $P < 0.05$), divided by the number of screened pairs (non-NaN scores). As in other density measurements, significant interactions of one type (positive or negative) were not considered in the denominator of screened pairs for density calculations of the opposite type. Similarly, after binning all pairs of genes by their ϵ score magnitude, we measured the fraction of pairs in each bin belonging to each hierarchy level using the most specific level available for each pair.

Genetic interaction enrichment within and between biological processes

Clusters defined at level 3 of the hierarchy corresponded roughly to broad biological processes. To facilitate analyses of interaction trends across these processes, we manually labeled clusters to correspond with functional annotations identified based on SAFE analysis (Fig. 1B; Data File S6). We analyzed NxN and ExE gene-pairs within these clusters separately. Interaction network density was defined as the number of significant interactions ($|\epsilon| \geq 0.08$, $P < 0.05$), divided by the background number of screened pairs (non-NaN scores). In the case of positive interaction density, we subtracted the number of significant negative interactions from the background before dividing, and vice versa for negative interaction density. P -values for interaction enrichment were calculated using a hyper-geometric test, similarly removing interactions of one type from the background of the other. P -values were corrected for multiple hypothesis testing using Bonferroni correction for the number of process pairs (15 choose 2 pairs + 15 within process tests). Processes (or pairs) were considered enriched if the corrected P -value was less than 0.05.

YNL181W chemical genetic screens

Results from the *YNL181W* chemical genetic screens are shown in fig. S18. A drug sensitized version of a yeast DAmP essential gene collection was constructed by introducing deletions of *PDR1*, *PDR3*, and *SNQ2* into the collection via SGA. For chemical genomic screening, a pool of these mutants was created by growing the collection on agar for 3 days at 30 °C, harvesting the colonies by flooding plates with 10 mL of 1X PBS, pooling and homogenizing the washed colonies, adjusting the OD of the pool to least 25 OD, mixing 1:1 with 30% glycerol, and storing at -80°C until use. Chemical genomic profiling was performed as described in (112). Briefly, 196 µL YP-galactose medium was treated with 2 µL of a set of 88 diverse compounds from the RIKEN NPDepo (113) (10 µg/mL final concentration), 4 control compounds (micafungin 31.8 ng/mL, cycloheximide 1 µg/mL, ketoconazole 10 µg/mL, and amphotericin B 10 µg/mL, final concentrations), and 4 solvent controls (1% DMSO). The cultures were inoculated with 2 µL of the DAmP strain pool and incubated for 48h at 30 °C. Genomic DNA was extracted using the Qiagen QIAextractor 96-well genomic extraction kit with a pre-incubation of the cells for 1 h at 37°C with zymolyase (1 mg/mL in 1M sorbitol). Following extraction, strain specific barcodes were PCR amplified with a set of 96 indexed primers, the PCR products pooled, and the resultant 267 bp product purified by 2% agarose gel extraction (114). Samples were sequenced on an Illumina HiSeq2000 (1X50 bp) (Illumina, Inc, San Diego, CA). Chemical genomic interaction (CGI) scores were calculated as a ratio of the barcode counts of individual mutants in drug treated media cells relative to the DMSO control conditions as described previously (112, 115). These CGI scores were used to determine the distribution in sensitive/resistance of the *YNL181W* DAmP mutant compared to the entire sequenced DAmP collection. Single colony isolates of *ynl181w::DAmP* and control strain (*pdr1Δ*, *pdr3Δ* *snq2Δ*) were grown in 200 µL of YEPD medium with the following compounds: amphotericin B (1 µg/mL), bortezomib (250 µM), caspofungin (50 ng/mL), MMS (0.01%), myrocin (500 ng/mL), poacic acid (500 µg/mL), staurosporine (100 ng/mL), tunicamycin (500 ng/mL), or a 1% DMSO control (n=3). Cultures were incubated at 30°C for 48 h on a TECAN M1000 plate reader and optical density (OD₆₀₀) measured every 15 minutes. Specific growth rate was calculated using GCAT (116). To calculate the specific growth rate of the *YNL181W* DAmP mutant versus the control strain, growth was normalized to DMSO growth rate minus the control strains growth rate, thus positive values=resistance compared to the control strain and negative values=sensitivity. A list of all compounds screened and the corresponding CGI scores are provided as Data File S17.

Genetic interaction enrichment within and between protein complexes

Results describing genetic interaction enrichment among protein complexes are shown in Fig. 8 and Data File S13.

Protein complex standard

The merged complex standard was created by combining complex standards from two previous publications (5, 117). As the two standards had significant overlap, we applied the following rules to avoid redundancy: (1) we retained any complex from either standard whose genes appear in no other complex annotation; (2) we eliminated any complex consisting of a single gene; (3) we eliminated any complex that was a perfect subset of another complex (from either standard); (4) for partially overlapping complex annotations, both complexes were retained with

their original names, and (5) for the five remaining cases where a pair of complexes from the two standards shared exactly the same name but different compositions (and neither was a perfect subset of the other), we appended the first author's initials (AB or JB) to the complex name. Proteasome-related complexes were removed and replaced with annotations curated from SGD and (118). The protein complex standard is provided as Data File S12.

Enrichment analysis

Complexes with at least 4 annotated proteins were divided into two categories. The “nonessential” category consisted of complexes in which $\leq 25\%$ of gene members were essential whereas the “essential” category consisted of complexes where $\geq 75\%$ of the annotated genes were essential. Complexes with more mixed representation of essential and nonessential genes were held out of the within/between complex interaction enrichment analysis. We tested for enrichment of genetic interactions among gene pairs belonging to the same complex (within complex enrichment) as well as for enrichment for interactions between each pair of complexes (between complex enrichment). Enrichment was assessed 3 times – enrichment for positive and negative interactions independently as well as for both types of interactions combined. A single complex was considered enriched for within complex interactions if the mean sign of significant interactions (see description of the interaction sign bias below for more details) was greater than 0.75 (absolute value) and the P-value was less than 10^{-2} . A complex pair was counted as significantly enriched for between-complex interactions if the mean sign of significant interactions was greater than 0.75 (absolute value) and the P-value (minimum of the three tests) was less than 10^{-3} . These cutoffs were established based on empirical estimates of FDR, derived from a positive-degree and negative-degree preserving edge randomization of all genetic interactions (intermediate level). The FDR is estimated to be $<35\%$ at these thresholds. Tests for interaction enrichment between two complexes were performed twice, once using the set of all available query mutants belonging to complex A and their potential interactions with array mutants annotated to complex B and vice versa. Where multiple alleles of a gene were available, one allele was chosen at random. Individual complexes and complex pairs with fewer than 4 interactions were not considered in this analysis. The P-value for each complex pair was computed using the hypergeometric distribution, using the `hygecdf()` function in MATLAB. For example, for tests of negative interaction enrichment:

$$P = 1 - \text{hygecdf}(\text{NEG}_{AB}-1, \text{SCR}, \text{SCR}_{AB}, \text{NEG})$$

or more generally:

$$p = 1 - \sum_{i=0}^{\text{NEG}_{AB}-1} \frac{\binom{\text{SCR}_{AB}}{i} \binom{\text{SCR} - \text{SCR}_{AB}}{\text{NEG} - i}}{\binom{\text{SCR}}{\text{NEG}}}$$

Where NEG_{AB} represents the number of negative interactions between queries belonging to complex A and arrays belonging to complex B (A and B are the same for within-complex enrichment tests). SCR_{AB} represents the number of screened pairs (non-NaN epsilons) between

A (queries) and B (arrays) minus the number of positive interactions between A and B. Similarly, NEG and SCR represent the total number of negative interactions and the total size of the screened space (minus positives) in the entire dataset (subject to the random selection of at most one query allele and one array allele per gene). Correction of the number of screened pairs for interactions of the opposing type allows for the detection of complexes or complex pairs with more heterogeneous interactions as each type of interaction will not count against the significance of the other. When testing for combined enrichment of negative and positive interactions, no such adjustment of SCR values is made. The interaction sign bias was computed to determine pairs of complexes (between complex) or members of the same complex (within complex) that were connected predominantly by a single type of genetic interaction (negative or positive). The interaction sign bias is the mean over all significant interactions within a given complex or between a pair of complexes after binarizing to +/- 1. An interaction sign bias of 1 indicates that all interactions are positive whereas a score of -1 indicates that all the interactions measured within a complex or between a pair of complexes are negative.

Protein features associated with proteasome interacting genes

Results from this analysis are shown in fig. S21. We calculated negative and positive interaction network density of every essential query against the proteasome complex essential genes in the TS array (i.e. proteasome density). Interactions were defined based on the intermediate confidence threshold and interaction density was measured as the number of interactions divided by the number of screened pairs. A single array allele per gene was randomly selected and densities were averaged across 100 randomizations. For genes with more than one query allele, interaction densities were averaged across the query alleles. Proteasome query genes were not considered for this analysis. We then defined as proteasome hubs the 10% of genes with the highest interaction density with the proteasome. Next, we evaluated the set of proteasome hubs against a set of protein features (see section “Correlation analysis of genetic interaction degree” above). We found that proteasome negative interaction hubs were characterized by having a higher number of unique structural domains, whereas proteasome positive hubs tended to have fewer domains (fig. S21A).

RNAi and cell fitness measurement in *Drosophila* cell culture

Results from this analysis are shown in fig. S20 and Data File S14. *Drosophila* Schneider S2 cells adjusted to serum-free medium (D.mel-2 cells; Invitrogen) were cultured in Express Five SFM (Invitrogen) supplemented with 20 mM GlutaMAX (Invitrogen) and 1% penicillin-streptomycin (Invitrogen). For gene knockdown 12,000 cells were seeded in a 384-well on 125 ng dsRNA in a total volume of 35 μ l. After 48 h, cells were treated with different concentrations (2 nM, 4 nM, 8 nM, 16 nM and 32 nM) of Bortezomib or DMSO only for 36 h. After an overall duration of 84 h, the absolute ATP content was measured using a CellTiter Glo (Promega) assay.

Normalization and data processing

The absolute RLUs per well were adjusted by dividing by the median of non-targeting dsRNA control wells for each 384-well assay plate. dsRNAs targeting 26S proteasome components that

reduced cell fitness in the control (DMSO only) treatment as compared to the non-targeting dsRNAs (targeting Rluc) less than 20% were excluded from further analysis. For the remaining 23 components, the fitness phenotypes at each Bortezomib concentration were normalized to the respective knockdown phenotype upon control (DMSO only) treatment. To distinguish the 19S and 20S subunit of the 26S proteasome, the median normalized fitness scores were considered at each Bortezomib concentration.

Analysis of positive interaction enrichment and bias among protein complexes

Results from positive interaction enrichment and positive interaction bias analysis among protein complexes are shown in Fig. 9, fig. S22 and Data File S15. Positive and negative interaction densities for SGA query mutant strains were calculated against the DMA and essential gene alleles on the TSA. Several metrics were computed to reflect the interaction enrichment and positive:negative interaction ratio for each protein complex against either essential or nonessential genes. Each metric is described in more detail below and listed in Data File S15.

We measured enrichment for positive interactions among protein complexes where at least two genes annotated to a given complex were screened against both the TSA and the DMA. We applied different enrichment cutoffs (Data File S15, “E_fold_pos”) and complexes that met the chosen threshold ($E_fold_pos > 1.5X$) were evaluated and found to be enriched for proteostasis-related functions (118) based on Fisher’s exact test. Protein complexes enriched for positive interaction with essential genes ($E_fold_pos > 1X$) were tested for bias toward positive vs. negative GIs with essential genes relative to nonessential genes (Data File S15, “posGI_bias_with_E”). Protein complexes biased towards positive interactions in the essential network ($posGI_bias_with_E > 1.5X$) were enriched for genes that result in a cell cycle arrest phenotype when perturbed (phenotype list downloaded from www.yeastgenome.org).

Interaction network densities derived from TSA screens were based on the average of 100 different randomizations, where a single allele for each essential gene represented on the TSA was randomly selected each time. Positive and negative interaction densities were averaged for every gene and interaction density fold enrichment was computed with respect to the average interaction degree for a given gene. Interaction density fold enrichments were averaged across genes for each complex of interest. Query-array gene interactions within the same complex were not considered for the interaction density calculation.

Interaction enrichment and bias metrics computed and listed in Data File S15:

- i) Nonessential-negative GI fold enrichment (N_fold_neg): negative interaction fold enrichment for a complex of interest with nonessential genes not in the complex.
- (ii) Essential-negative GI fold enrichment (E_fold_neg): negative interaction fold enrichment for a complex of interest with essential genes not in the complex.
- (iii) Nonessential-positive GI fold enrichment (N_fold_pos): positive interaction fold enrichment for a complex of interest with nonessential genes not in the complex.

(iv) Essential-positive GI fold enrichment (E_fold_pos): positive interaction fold enrichment for a complex of interest with essential genes not in the complex.

(v) Positive GI bias with essential genes ($posGI_bias_with_E$): the relative positive:negative enrichment ratio of essential to nonessential genes for the complex of interest (calculated as $[iv/ii]/[iii/i]$)

(vi) Positive GI bias with nonessential genes ($posGI_bias_with_N$): the relative positive:negative enrichment ratio of nonessential to essential genes for the complex of interest (calculated as $[iii/i]/[iv/ii]$)

Confirmation of genetic suppression interactions

Results from genetic suppression confirmation experiments are shown in Table S3 and Data File S16. Double mutants and corresponding single mutants were grown overnight in YEPD medium at room temperature (RT). Ten-fold serial dilutions were prepared by diluting cultures in water to optical densities (OD_{600}) of 0.1, 0.01, 0.001 and 0.0001. Five μ L aliquots were spotted onto two YEPD agar plates and incubated at RT or 30°C. Plates were imaged after 48 h and 72 h incubations. Single and double mutant colony growth was scored by eye on a scale of 0 (no growth) to 4 (WT growth). Suppression was defined as cases where the double mutant grew better than the single mutant with the more severe fitness defect and was scored as the difference between the growth of the double mutant and that of the sickest single mutant.

Supplementary Table Legends

Table S1 and S2. *Characterization of high- and low-degree genes*

Genes on the DMA and TSA with the highest 20% (Table S1) and lowest 20% (Table S2) negative GI degrees, based on the intermediate threshold, were tested for associations with gene features. In cases where multiple alleles of one query or array gene were screened (about one third of the genes represented on the TSA) one allele was selected randomly before calculating degree. Part 1 of each table (S1-1 and S2-1) shows results of two-tailed hypergeometric tests of enrichment and depletion of the presence of binary gene features in the gene set. All P -values are doubled to correct for performing two tests for each feature. Only the lower of the two P -values is shown in the table and, if significant ($P < 0.05$), the “Test result” column indicates which test was used. Part 2 of each table (S1-2 and S2-2) shows results of Wilcoxon rank-sum tests on continuous and ordinal gene features, comparing genes in the high- and low-20% set to all other genes. The “Test result” column indicates how the median degree of the focus gene set compares to other genes if the P -value is significant; if the medians are equal and the P -value significant, the mean degrees were compared instead and the result column says “(mean)”. “N/A” indicates that $< 60\%$ of data is present for genes on the array and the test was not done. Uncorrected P -values are listed for all features. Given that analysis of different features required using different statistical tests and some features are not expected to be independent of each other, no multiple hypotheses correction procedures were used. We do note that 31 gene features were tested.

Table S3. *Confirmation of genetic suppression interactions*

Gene pairs corresponding to a subset of 302 positive genetic interactions involving a proteasome component (100 interactions) or gene pairs not involving a proteasome gene (202 interactions) were selected and confirmed by spot dilution growth assays on solid agar medium. Positive interactions involving the proteasome exhibited SGA scores ranging (ϵ) from 0.09 to 0.36 whereas SGA scores (ϵ) for non-proteasome gene pairs ranged from 0.19 to 0.44. Despite a lower score magnitude, 32% of positive interactions involving the proteasome were classified as genetic suppression compared to only 24% of positive interactions not involving the proteasome. Based on these results, we estimated that the proteasome participates in 1168 genetic suppression interactions compared to 544 suppression interactions involving non-proteasome genes in the ExE network.

Supplementary Figure Legends

Fig. S1. *Comparison of genetic interaction trends for DAmP and TS alleles*

(A) Scatter plot comparing negative genetic interaction (GI) degree of DAmP and TS alleles of the same gene. (B) Scatter plot comparing positive GI degree of DAmP and TS alleles for the same gene. The Spearman correlation is noted on each plot and illustrates that TS and DAmP alleles for the same gene do not tend to show similar numbers of negative and positive genetic interactions. (C) Distribution of negative GI degree of all TS and DAmP queries against the essential genes on the TSA. (D) Distribution of positive GI degree of all TS and DAmP queries against the essential genes on the TSA. In (C) and (D) the header shows the P -value derived

from a rank-sum test. TS alleles have higher positive and negative genetic interaction degree than DAmP mutants. (E) Single mutant fitness (SMF) distribution of all TS and DAmP query alleles screened against the TSA. The header shows the P -value derived from a rank-sum test. TS alleles exhibit greater fitness defects than DAmP alleles. (F) Negative query strain GI degree of TS and DAmP alleles binned by SMF. (G) Positive query strain GI degree of TS and DAmP alleles binned by SMF. All GI degrees were averaged across 100 randomizations where a single array allele per gene was randomly selected.

Fig. S2. Reproducibility analysis

(A) Average precision and recall of genetic interactions (GI) derived from screening both essential and nonessential gene queries against the essential TS array (TSA) and nonessential deletion mutant array (DMA) at different SGA score cutoffs. These estimates are based on a subset of 40 screens (14 queries against the TSA and 26 queries against the DMA) that were each replicated 5 times and true interactions were assumed to be interactions that were found in two or more of the replicates. Error bars indicate the standard deviation in estimates across the set of queries. In general, negative and positive interactions derived from screens against the same mutant array exhibit similar levels of technical reproducibility. Positive and negative GIs involving essential genes tend to be more reproducible than nonessential gene interactions. (B) Fraction of GIs that were identified at least 1, 2, 3, 4, or 5 times out of 5 screens. The average fraction of GIs for queries with at least 5 replicates is shown for actual called interactions (blue lines for negative GIs; yellow lines for positive GIs) and randomly selected gene pairs (grey lines). These results are based on an intermediate genetic interaction score cutoff (genetic interaction score, $|\epsilon| \geq 0.08$, $P < 0.05$). (C) Fraction of interacting genes that are co-annotated to the same GO biological process term. GIs were grouped by the number of times they were found in the 5 replicates for a given query. The average across queries with at least 5 replicates is shown. The dashed line indicates the background co-annotation rate for non-interacting pairs and (*) indicates statistically significant differences from background ($P < 0.05$). These results are based on an intermediate genetic interaction score cutoff (genetic interaction score, $|\epsilon| \geq 0.08$, $P < 0.05$). These results show that highly reproducible negative interactions (blue) are more likely to connect gene pairs that are annotated to the same GO biological process terms for both essential and non-essential genes. The relationship between functional relationships and reproducibility of positive interactions was weaker for non-essential genes and a similar trend between functional relationship and interaction reproducibility was not observed for essential genes. See Methods Section entitled, “Estimating reproducibility of genetic interactions”.

Fig. S3. Genetic interaction network coverage

Pie charts represent all unique pairs of genes, unique essential gene pairs (ExE), unique essential-nonessential gene pairs (ExN) and unique nonessential gene pairs (NxN) encoded in the *S. cerevisiae* genome. For each set of gene pairs, the percentage of gene pairs tested for genetic interactions in the complete, ExE, ExN or NxN networks described in this study (<http://thecellmap.org/costanzo2016/>) is shown in blue, and the percentage of tested pairs relative to all annotated genes is indicated. The percentage of all tested pairs relative to the number of gene pairs that could be tested by SGA is indicated in parentheses. The percentage

of gene pairs that have not yet been tested is shown in white and the fraction of gene pairs that could not be tested is shown in grey. The fraction of “Untestable” gene pairs includes mutant strains that are incompatible with SGA technology (e.g. sterile mutants, histidine, arginine and lysine auxotrophs etc.), mutant strains that do not survive SGA selection steps due to extreme fitness defects, and genes that are not represented in our array and/or query mutant collections.

Fig. S4. Functional evaluation of genetic interaction profiles

Genes with varying degree of genetic interaction profile similarity were evaluated for overlap with either Gene Ontology biological process co-annotations or protein-protein interactions using precision-recall analysis as described in (62). For all plots, gene pairs were sorted by Pearson correlation coefficients reflecting similarity in their genetic interaction profiles. The dashed lines show the background rate of co-annotation for the relevant set of gene pairs. Precision-recall analysis was completed separately for genetic interaction profiles derived from the essential, nonessential and the combined genetic interaction profile similarity networks. (A) Gene pairs evaluated against a GO biological process standard. In general, a genetic interaction profile similarity network based on both nonessential and essential gene profiles (global similarity network) identified functionally related genes pairs with the greatest amount of accuracy. (B) Gene pairs evaluated against a protein-protein interaction standard. See Methods Section entitled, “Comparison of SGA genetic interactions with other genomic datasets and precision-recall analysis”.

Fig. S5. Gene pairs in the same essential complex exhibit more similar genetic interaction profiles than nonessential co-complexed genes

Shown are the distributions of Pearson correlation coefficients (PCC) measuring genetic interaction (GI) profile similarity for all pairs of essential genes (purple triangles) and all pairs of nonessential genes (orange triangles). Also shown are PCCs for GI profiles corresponding to pairs of genes belonging to the same protein complex (purple circles for essential genes, orange circles for nonessential genes). Distributions were normalized (total area of 1) and smoothed over a 3-bin window. This analysis illustrates that GI profiles associated with essential genes encoding members of the same complex tend to share greater similarity to each other compared to GI profiles of nonessential genes encoding members of the same complex. This indicates that essential GIs, in general, tend to be more coherent than nonessential GIs.

Fig. S6. Predicting gene function from essential versus nonessential genetic interaction profile similarity networks

We predicted Gene Ontology annotations for a set of GO biological process terms defined in (62). (A) Performance when predicting function for nonessential genes. Terms for which nonessential gene predictions were more successful when based on interactions with essential genes fall above the diagonal while nonessential gene predictions that were more accurate when based on interactions with other nonessential genes fall below the diagonal. Biological process terms that clearly fall in either area of the graph are labeled. Prediction performance was summarized for each GO term by computing the precision at 25% recall. The X-axis represents performance when only nonessential query mutant profiles were used to calculate array-array gene pair similarities. The Y-axis plots performance when only interactions from

essential query mutants are used. (B) Performance when predicting function for essential genes. Terms for which predictions were more successful when based on interactions with other essential genes fall above the diagonal. Terms for which predictions were more successful when based on interactions with nonessential genes fall below the diagonal. (C) Complete precision-recall curves for the indicated terms are shown. The performance of all essential, an equal subset of nonessential queries, the complete set of nonessential queries, and all queries are shown. Predictions were made 50 times with different random allele samplings and error bars represent the standard error on precision over 50 iterations. For the complete dataset, all data (i.e. all alleles) were used, thus only a single set of results is shown. (D) Cumulative performance across all included GO terms of the K-nearest neighbor classifier is summarized for the various query mutant subsets used to calculate array similarities. The left panel shows results for nonessential genes while the right shows results when predicting function for essential genes. In general, we found that essential gene interaction profiles provided higher accuracy gene function predictions across a diverse set of bioprocesses when compared to nonessential gene interaction profiles. However, either the essential or nonessential similarity networks uniquely predicted certain functions. For example, interactions with nonessential genes were highly predictive of vacuolar transport and peroxisome functions, whereas interactions with essential genes were more informative for predicting chromosome segregation and RNA splicing related functions. Optimal functional prediction performance was achieved by using the global similarity network (black lines). See Methods Section entitled, “Predicting gene function from essential versus nonessential genetic interaction profile similarity networks”.

Fig. S7. A genetic profile similarity-derived functional hierarchy

(A) A gene hierarchy based on a subset of high-confidence genetic interaction profiles corresponding to ~1000 genes, including both essential and nonessential genes that a shared highly similar genetic interaction profile ($PCC > 0.6$) with at least one other gene (see section above, “A genetic profile similarity-derived functional hierarchy” for details). Clusters identified at different levels of the hierarchy represent functional relationships of different specificity as indicated by the colored lines on the dendrogram. The colored heatmaps are colored to match the lines indicating their level of specificity on the hierarchy and the alternating shades of each color reflect different clusters (e.g. 10 clusters for the cell compartment level of the hierarchy). The grey-scale heatmap summarizes enrichment observed for the functional standards described in (B) and the Z-scores are calculated from histograms shown in (B). (B) To assess the concordance of hierarchical clusters with established functional data, we performed a hypergeometric enrichment test for each cluster identified at the same hierarchical level, using several functional standards including a protein localization standard based on automated image analysis of the yeast GFP collection (17, 119), GO biological process annotations (14), a protein complex standard (Data File S12) and a pathway standard, KEGG (120). We then measured the tendency of clusters with one or more annotation(s) in common to merge together at higher hierarchical levels, which correspond to weaker shared profile similarity. Finally, we randomized the parent assignments of each cluster and counted the number of sibling pairs sharing an annotation after each randomization. This randomization allows the distribution of clusters, their membership, their sizes, and the number of sibling pairs under each parent to remain fixed, while randomizing only the hierarchical relationships of the clusters themselves.

Put differently, we simply shuffled the parent assignments across the children clusters. In any random instance, an existing sibling relationship can be preserved (with low probability) and each parent has the same number of children as it did before the shuffling, though their identities (along with their annotations) will have changed. We repeated the randomization process 1000 times to derive empirical distributions of expected annotation overlap with a randomized hierarchy (shown in gray). The observed number of siblings enriched for at least one common term derived from the real hierarchy is indicated with the black arrow along with the corresponding *P*-value determined empirically from the proportion of random iterations where the test statistic meets or exceeds the observed value. Each column represents a different functional annotation standard and rows correspond to results of the test at varying depths in the hierarchy. Rows 1, 2, and 3, show scores for the children of hierarchy levels corresponding to cell compartment (PCC=0.05), bioprocesses (PCC=0.2), and protein pathways/complexes (PCC=0.4) thresholds, respectively. This analysis illustrates that the hierarchical structure suggested by genetic interaction profiles is supported by all tested functional standards at the deepest levels of the hierarchy, including the most specific standards (protein complexes and pathways). Larger clusters formed at intermediate levels of the hierarchy are most strongly supported by GO biological processes. Finally, the structure at the highest levels of the hierarchy (weaker but significant genetic interaction profile similarity) was supported by a protein localization standard corresponding to coherent cellular compartments. See Methods Section entitled, “A genetic profile similarity-derived functional hierarchy”

Fig. S8. *IPA1* encodes a novel regulator of mRNA cleavage and polyadenylation

(A) Processing extracts were prepared from wild type (WT) as well as *ipa1-5001*, *cft2-1* and *pcf11-2* TS mutants grown at 30°C or grown at 30°C and then shifted to 37°C for 2 hours where indicated. Assays were conducted for 20 min at 30°C. For the coupled cleavage-polyadenylation assays, extracts were incubated with ATP and ³²P-labeled, full-length *GAL7-1* RNA. For cleavage only assays, the reactions were performed as described above except that 3'-dATP was used instead of ATP. For poly(A) addition assays, the reactions were performed as above except that precleaved *GAL7-9* RNA, ending at the poly(A) site, was used as a precursor. RNA products were resolved on a denaturing 5% polyacrylamide gel and visualized with a phosphorimager. The positions of substrate and products are depicted on the side of the images, and the lane marked “precursor” indicates the unreacted substrate for each reaction. (B) Distribution of identified poly(A) reads with respect to annotated transcription termination sites (69). The cumulative distribution is shown in the bottom panel. Significant differences between mutants and BY4741 were computed using the Wilcoxon rank-sum test for the 200 bp surrounding annotated transcription termination site. These results show that strains expressing mutant a mutant TS allele of *IPA1* exhibit mRNA processing defects both *in vitro* and *in vivo*. See Methods section entitled, “*IPA1* experimental validation”.

Fig. S9. The MTC pathway is important for amino acid permease localization

(A) Triple mutant (TM) genetic interaction analysis of MTC pathway genes. Individual MTC pathway deletion mutants were mated to double mutant strains carrying mutations in redundant components of the aromatic amino acid biosynthesis pathway. Diploid strains (genotype indicated) were sporulated and 15-20 tetrads from each cross were dissected. Representative

tetrads from each cross are shown below the table. The growth of haploid progeny carrying all three selectable markers is summarized (TM phenotype and comments column). (B) Representative fluorescence micrographs highlighting yeast cells with wild-type and aberrant Bap2-GFP localization in *may24Δ* and *mtc2Δ* mutants. Bap2-GFP and FM4-64 vacuolar membrane staining, as well as an image of the fluorescent signal overlay are shown. See Methods section entitled, “MTC pathway experimental validation”.

Fig. S10. Negative and positive genetic interaction degree distributions

Relative genetic interaction (GI) degrees of all strains on the TSA and DMA were calculated by counting interactions that met the intermediate thresholds and dividing by the total number of query mutants screened against the relevant array. (A) Distributions of negative GI degree for TS array (TSA) mutants. (B) Distributions of positive GI degree for TS array (TSA) mutants. (C) Distributions of negative GI degree for deletion array (DMA) mutants. (D) Distributions of positive GI degree for deletion array (DMA) mutants.

Fig. S11 & S12. Correlation analysis of genetic interaction degree

Negative (fig. S11) and positive (fig. S12) genetic interaction (GI) degrees were calculated for all strains on the DMA and TSA using the intermediate genetic interaction score threshold (genetic interaction score, $|\epsilon| \geq 0.08$, $P < 0.05$) and counting any interacting query strain. (A) Wilcoxon rank-sum tests compared the GI degree in paired gene sets defined by absence and presence of each binary feature tested. For uncorrected P -values meeting a $P < 0.05$ threshold, the “Test result” column describes the degree of the set of genes for which the listed binary feature is true (compared to the set for which the feature is false). Tests were not performed, indicated by “N/A”, if data were present for fewer than 50 strains, and strains with missing data were excluded from the tests. (B) Pearson’s correlation was used to measure associations between GI degree and features that are continuous or count-based. Error bars reflect the 95% confidence interval on the correlation coefficient. For all correlation analyses, only strains corresponding to essential genes on the TSA were included. Given that analysis of different features required using different statistical tests and some features are not expected to be independent of each other, no multiple hypotheses correction procedures were used. We do note that 29 gene features were tested. These analyses identify a set of physiological and evolutionary features associated with the density of negative and positive GIs for each gene, which can be exploited to predict genes that serve as highly connected genetic network hubs and thus general genetic modifiers in other organisms. The features examined in this analysis are described in the Methods section entitled “Genetic interaction degree and network density analysis”. The same analysis was repeated using negative and positive GI degree of nonessential and essential query mutant strains. The results were consistent with those shown here and the data are provided as Data File S10.

Fig. S13. Genetic interaction network density across molecular functions

Genetic interaction (GI) network densities were evaluated for genes annotated to specific GO Molecular Function terms, excluding any term with a size less than five genes. Each term’s density was determined from gene pairs involving array strains annotated by the term and was calculated as the ratio of negative interactions (blue) or positive interactions (yellow) to all

screened gene pairs. Densities were calculated separately for the nonessential network (A) and the essential network (B). One random allele was selected in instances where multiple mutant alleles were available for the same gene. The dotted lines represent background density of negative (blue) and positive (yellow) GIs in the nonessential and essential genetic networks, which were calculated by adding all interactions and screened pairs that were counted for individual GO terms, then dividing the sums. Gene assignments to GO Molecular Function terms were obtained from the *S. cerevisiae*-specific GO slim terms, which were downloaded from <http://geneontology.org/page/go-slim-and-subset-guide> in Jan 2013. Only interactions that met the intermediate threshold (genetic interaction score, $|\epsilon| \geq 0.08$, $P < 0.05$) were considered. See Methods section entitled “Genetic interaction degree and network density analysis”.

Fig. S14. Genetic interaction network density across PFAM domains

Genetic interaction (GI) densities were evaluated for genes encoding proteins containing specific PFAM-annotated domains, excluding any domain that appeared in fewer than five proteins. Each term’s density was determined from gene pairs involving array strains annotated by the term and was calculated as the ratio of negative interactions (blue) or positive interactions (yellow) to all screened gene pairs. Densities were calculated separately for the nonessential network (A) and the essential network (B). One random allele was selected in instances where multiple mutant alleles were available for the same gene. The dotted lines represent background density of negative (blue) and positive (yellow) GIs in the nonessential and essential genetic networks, which were calculated by adding all interactions and screened pairs that were counted for individual GO terms, then dividing the sums. Gene assignments to PFAM groups were downloaded from <http://pfam.xfam.org/proteome?taxId=559292> in May 2014. Only interactions that met the intermediate threshold (genetic interaction score, $|\epsilon| \geq 0.08$, $P < 0.05$) were considered. See Methods section entitled “Genetic interaction degree and network density analysis”.

Fig. S15. Functional evaluation of negative and positive interactions in the ExE, NxN and ExN genetic networks

Genetic interactions (GIs) were evaluated for overlap with either Gene Ontology biological process co-annotations or protein-protein interactions using precision-recall analysis as described in (62). For all plots, negative and positive GIs were thresholded at the intermediate cutoff (genetic interaction score, $|\epsilon| \geq 0.08$, $P < 0.05$) and sorted by $|\epsilon|$. The dashed line indicates the background rate of co-annotation for the relevant gene pairs. Precision-recall analysis was completed separately for positive (yellow lines) and negative (blue lines) GIs on different sets of gene pairs and different standards. (A) Essential-nonessential (ExN) gene pairs evaluated against a GO biological process standard. (B) Nonessential-nonessential (NxN) gene pairs evaluated against a protein-protein interaction standard. (C) Essential-essential (ExE) gene pairs evaluated against a protein-protein interaction standard. (D) Essential-nonessential gene pairs (ExN) evaluated against a protein-protein interaction standard. See Methods Section entitled “Comparison of SGA genetic interactions with other genomic datasets and precision-recall analysis”.

Fig. S16. *Essential gene negative interactions are as informative as genetic interaction profile similarity for predicting functional relationships*

Genetic interactions (GIs) and GI profile similarities were evaluated for overlap with either Gene Ontology biological process co-annotations or protein-protein interactions using precision-recall analysis as described in (62). For all analysis of direct GIs, negative GIs were thresholded at the intermediate cutoff (genetic interaction score, $|\epsilon| \geq 0.08$, $P < 0.05$) and sorted by $|\epsilon|$. Pearson correlation coefficients (PCCs) were calculated for array-array mutant pairs (matrix columns) and only a single allele of each gene (selected at random) was used. (A) Comparison of functional information associated with direct negative GIs (blue line) versus profile similarity (Pearson, orange line) for essential genes evaluated against a GO biological process co-annotation standard. (B) Comparison of functional information associated with direct negative GIs (blue line) versus GI profile similarity (Pearson, orange line) for essential genes evaluated against a protein-protein interaction standard. (C) Comparison of functional information associated with direct negative GIs (blue line) versus profile similarity (Pearson, orange line) for nonessential genes evaluated against a GO biological process co-annotation standard. (D) Comparison of functional information associated with direct negative GIs (blue line) versus GI profile similarity (Pearson, orange line) for nonessential genes evaluated against protein-protein interaction standard. See Methods Section entitled “Comparison of SGA genetic interactions with other genomic datasets and precision-recall analysis”.

Fig. S17. *Positive interactions in the essential gene network (ExE) are shared among mutant alleles of the same gene and are coherent among members of the same protein complex*

(A) Jaccard similarity coefficient between different alleles of the same gene (left) and between alleles of different genes (right). The median value across different alleles is shown for each gene. This analysis shows that alleles of the same gene tend to share more positive genetic interactions (GIs) in common than random pairs of alleles of different essential genes. (B) The same analysis as shown in (A), but restricted to profile similarity between query mutants belonging to genes comprising “Nuclear-related” functional clusters defined in cluster in Fig. 6D. Profile similarity in this analysis was based on positive GIs with genes found in the “Cytosolic/Vesicle traffic-related” functional clusters also defined in Fig. 6D. (C) The reciprocal analysis of (B), where profile similarity of query mutants in the “cytosolic” functional clusters was evaluated based on positive GIs with genes in the “nuclear-related” functional cluster defined in Fig. 6D. P -values were calculated with a Wilcoxon rank-sum test. (D) Precision-recall curve for predicting protein complex membership based on similarity of positive GI profiles alone. Pairs of query mutants were sorted based on their Jaccard similarity coefficient calculated using binarized profiles of positive GIs. (E) The same analysis as in (D) but query mutants were restricted to those in the “Nuclear-related” functional cluster and GI profiles were based only on positive GIs involving genes belonging to the “Cytosolic/Vesicle traffic-related” functional cluster defined in Fig. 6D. (F) The reciprocal analysis where query mutants were restricted to those in the “Cytosolic/Vesicle traffic-related” functional cluster and the GI profiles were based only on positive GIs involving genes in the “Nuclear-related” functional cluster defined in Fig. 6D. The dashed grey line shows the background precision expected from randomly selected gene pairs. A list of genes and alleles used in this analysis is provided in Data File S6. See Methods section entitled “Evaluating functional coherence of positive interactions”.

Fig. S18. *Chemical genomic response of a *ynl181w* DAmP mutant across 92 diverse compounds compared to the average DAmP mutant strain response*

A pooled DAmP allele collection was screened against 92 diverse compounds. (A) Compared to the average score per compound (filled circles), a *ynl181W* DAmP allele mutant (open circle) exhibited a wide range of responses to different compounds from strong resistance to strong sensitivity. For example, the *ynl181w* mutant strain was resistant to cycloheximide (translation inhibitor) and microazole (ergosterol biosynthesis inhibitor) but highly sensitive to amphotericin (binds ergosterol) and micafungin (inhibits glucan synthesis). A list of the compounds screened and the corresponding Chemical Genetic Interaction (CGI) scores are provided in Data File S17. (B) Growth of a *ynl181w*-DAmP allele strain in liquid culture in the presence of the indicated compounds relative to an isogenic wild type strain grown in the same condition. The *ynl181w* mutant strain was sensitive to amphotericin B (binds ergosterol) and caspofungin (inhibits glucan synthesis) as predicted from the pooled experiment. The DAmP mutant showed greater resistance to poacic acid (binds glucan) and tunicamycin (inhibits glycosylation). Error bars indicate the standard error based on three biological replicates. See Methods section entitled “*YNL181W* chemical genetic screens”.

Fig. S19. *Genetic and chemical-genetic interactions and profile similarity distinguishes between the 19S and 20S proteasome subunits and members of the same protein complex show similar patterns of genetic interactions in yeast cells.*

(A) Negative genetic interaction (GI) density in the essential (ExE) network for genes encoding members of the 19S and 20S proteasome subunits and negative GI density between 19S and 20S proteasome subunit genes. (B) The boxplot shows query gene profile similarity (PCC) between the query gene profiles of (i) the 19S proteasome subunit; (ii) the 20S proteasome subunit; (iii) the 19S and 20S proteasome subunits; (iv) the 19S subunit and all query genes not annotated to the proteasome; and (v) the 20S subunit and all query genes not annotated to the proteasome. Significance was assessed with Wilcoxon rank-sum tests. Results shown correspond to a representative randomization where a single query and array allele were selected per gene for genes that have more than one allele. (C) Heterozygous deletion of yeast genes encoding 20S proteasome subunits showed increased sensitivity to the proteasome inhibitor, Bortezomib, compared to strains heterozygous for deletion of 19S proteasome genes. Yeast chemical-genetic interactions for Bortezomib were obtained from (30). Frequency distribution of the heterozygous chemical-genetic interaction profile scores (MADL) for Bortezomib at 200 nM for genes annotated to the 20S proteasome core particle (purple), 19S proteasome regulatory particle (orange) and all other heterozygous mutants (black). Heterozygous mutants in the 20S proteasome core particle show significantly more sensitivity to Bortezomib than the background (Wilcoxon rank-sum P -value $< 10^{-10}$). Conversely, 19S proteasome regulatory particle gene mutants show significantly more resistance to Bortezomib than the background (Wilcoxon rank-sum P -value = 0.003).

Fig. S20. **26S proteasome knockdown sensitizes *Drosophila* S2 cells to Bortezomib.** (A)

Cell fitness (ATP content in cell population) upon knockdown of components of the 19S (blue) or 20S (purple) subunit of the 26S proteasome without Bortezomib treatment as a fraction of the

non-targeting RNAi reagent (targeting Rluc) effect (dashed line). Each dot represents the median of 6 measurements. Both subunits were not separated by the knockdown effects of their components ($P = 0.976$, Wilcoxon rank sum test, two-sided). **(B)** Dose-response curves to Bortezomib upon knockdown of quality-filtered 26S proteasome components. The effects of each knockdown to Bortezomib were normalized to the control (0 nM) treatment. Box plots represent 36 measurements of the non-targeting RNAi reagent at each Bortezomib concentration. For each knockdown of a 26S proteasome component, the lines connect the median of 6 measurements per knockdown and Bortezomib concentration. Dashed lines connect the median absolute deviation of the 6 measurements per knockdown and Bortezomib concentration. Purple lines represent 20S, blue lines 19S components. **(C)** Distinct sensitivities of 19S and 20S subunit components upon Bortezomib treatment. Boxplots represent the median values (of 6 measurements) for all components of the 20S (purple) or 19S (blue) subunit of the 26S proteasome. Members of the 19S and 20S subunits were separated from the control RNAi measurement at Bortezomib concentrations from 2 nM to 8 nM ($p < 0.05$, Wilcoxon rank sum test, two-sided), and members of those two proteasome subunits were separated from each other between 2 nM and 16 nM ($p < 0.05$, Wilcoxon rank sum test, two-sided). See Methods section entitled “RNAi and cell fitness measurements in *Drosophila* cell culture”.

Fig. 21. *Features associated with genes exhibiting negative interactions with the proteasome*
Fold enrichment in genes containing at least 1, 2, or 3 structural domains among the 10% of genes that show the highest number of negative (blue) and positive (yellow) interactions with proteasome encoding genes. A domain was only considered once in cases where a gene encoded multiple repeats of the same domains. The dotted line reflects the expected fraction of genes in the top 10% interactors with the proteasome and “*” marks significant differences from the background fraction. See Methods section entitled “Protein features associated with proteasome interacting genes”.

Fig. S22. *Protein complexes enriched for positive interactions in the ExE network.*

(A) Distribution of protein complexes enriched (yellow) or depleted (grey) for positive genetic interactions (GIs) in the nonessential (NxN) network (see above, Methods section entitled “Analysis of protein complexes enriched for positive interactions” for more details and Data file S15). Protein complexes that met the chosen threshold ($N_fold_pos > 1.5$, Data File S15) were evaluated, using Fisher's exact test, and found to be enriched for proteostasis-related functions (118). (B) Distribution of the positive:negative interaction ratio for protein complexes enriched for positive interactions in (A). Genes belonging to complexes biased for positive interactions in the nonessential genetic network ($N_fold_pos > 1X$ and $posGI_bias_with_N > 1.5X$, Data File S15) did not show significant functional enrichment for genes with cell cycle phenotypes. (C) Genes belonging to complexes biased for positive interactions in the ExE network are enriched for negative genetic interactions with cell cycle checkpoint-related genes (www.yeastgenome.org). P -values shown are based on a one-sided Fisher's test.

Fig. S23. *Genes and complexes involved in protein folding share positive interactions in common with the proteasome*

We applied an intermediate threshold on the genetic interaction score (genetic interaction score, $|\epsilon| \geq 0.08$, $P < 0.05$) and computed Jaccard similarity coefficients between TSA-derived positive

genetic interaction (GI) profiles for query strains corresponding to members of the CCT, prefoldin and proteasome complexes. (A) The boxplot shows the Jaccard similarity coefficient of specific query mutants with the proteasome complex. The left box (Within) shows similarity for genes in the proteasome complex; the center box (CCT & prefoldin) shows similarity between genes of the CCT and prefoldin complexes with genes of the proteasome complex; the right box (Other) shows similarity of all other query mutants in the global network with genes in the proteasome complex. Similarity of query mutants for the same gene against the proteasome complex was averaged. (B) Similar to (A), but Jaccard similarities were calculated with respect to the CCT complex. (C) Similar to (A), but Jaccard similarities were calculated with respect to the prefoldin complex. (A-C) suggest that members of the CCT, the proteasome and the prefoldin complexes share positive GIs in common. (D) Precision-recall curve for predicting protein folding-related genes (118) using query mutant positive GI profile similarity (Jaccard) to the proteasome complex. This analysis shows that genes involved in protein folding related functions (118) tend to share positive GIs in common with the proteasome. Query genes annotated to the proteasome, CCT, and prefoldin complexes were excluded from this analysis. See Methods section entitled “Evaluating functional coherence of positive interactions”.

Fig. S24. *Proteasome genes are positive interaction hubs in the essential TS genetic network and RNA decay genes are positive interaction hubs in the DAmP genetic interaction network*

(A) Enrichment analysis for positive and negative genetic interactions (GIs) with the proteasome among DAmP and TS query mutants. Fold enrichment was calculated as the density of GIs between DAmP or TS query mutant alleles with the proteasome divided by the density of GIs between DAmP and TS query mutant allele interactions with all mutants on the TS array (TSA). GIs between different members of the proteasome complex were excluded. GI densities were averaged across 100 randomizations where a single query and array allele per gene were randomly selected. The dashed line indicates the expected density of interactions. Fold enrichment and significance of the enrichment with respect to background are shown on top of each bar. (B) Similar to (A), but GI density was calculated against mRNA decay-annotated essential TS array genes.

Supplementary Data File Descriptions

All data files can be downloaded from:

<http://boonelab.cabr.utoronto.ca/supplement/costanzo2016>

Data File S1. Raw genetic interaction datasets: Pair-wise interaction format.

This folder contains complete SGA genetic interaction data for the following:

- A list of all query and array mutant strains represented in the genetic interaction network along with their corresponding fitness estimates
- Nonessential x Nonessential network (NxN)
- Essential x Essential network (ExE)
- Essential x Nonessential network (ExN)
- Genetic interactions involving DAmP alleles of essential genes
- Genetic interactions for HSP90 and corresponding controls

The interaction datasets are provided in a tab-delimited format with 11 columns:

- Query ORF
- Query gene name
- Array ORF
- Array gene name
- Array Type (DMA or TSA) and Temperature (26°C or 30°C)
- Genetic interaction score (ϵ)
- *P*-value
- Query single mutant fitness (SMF)
- Array SMF
- Double mutant fitness
- Double mutant fitness standard deviation

Data File S2. Raw genetic interaction datasets: Matrix format.

This file contains complete SGA genetic interaction data matrix for the following:

- Nonessential x Nonessential network (NxN)
- Essential x Essential network (ExE)
- Combined essential and nonessential network (ExN)

Data File S3. Genetic interaction profile similarity matrices

Matrix files containing genetic interaction profile similarity values (as measured by Pearson correlation) for every pair of mutant strains in the dataset. Similarity values were computed for essential (ExE), non-essential (NxN) and the global similarity network derived from a combined set of all genetic interactions (ExE, NxN, ExN) as described above (see "Constructing genetic interaction profile similarity networks"). Each matrix contains 2 sets of row and column headers, providing a unique allele name for every mutant strain (row & column header #1) as well as a systematic ORF name (row & column header #2).

Data File S4. GO bioprocess functions predicted by the nonessential and essential similarity networks using a K-nearest neighbor approach.

This file reports the performance of gene function prediction for non-essential or essential genes based on genetic interaction profiles. For both classes of genes (either nonessential or essential), the performance of a KNN classifier is reported as the Precision at 25% Recall based on interactions derived from TS queries (PR_TSQ) or nonessential deletion queries (PR25_SN). Although analyses were performed using complete genetic interaction profiles (e.g. negative and positive genetic interactions), similar prediction performance was obtained using genetic interaction profiles based on negative interactions alone.

Data File S5. SAFE analysis: Gene cluster identity and functional enrichment

This file lists the results from SAFE analysis of the global genetic profile similarity network (Fig. 1 and Fig. 2). Functional terms enriched within specific network clusters associated with GO biological processes (14) and/or protein complexes (Data File S12). A list of genes comprising each bioprocess-enriched cluster shown on the global similarity network is also provided.

Functional terms enriched within specific network clusters associated with cell compartments (17, 119) are all shown on Fig. 2B.

Data File S6. Genetic profile similarity-based hierarchy analysis

The first tab (“Gene to hierarchy cluster mapping”) lists the clusters identified at each level of the genetic interaction-based hierarchy and the deletion and TS allele array mutants assigned to each cluster. Examples of clusters described in the main text are highlighted. The subsequent 9 tabs indicate enrichment of clusters resolved at the specified profile similarity range for specific cell compartments (Cyclops_enrich), biological processes (GO BP_enrich), protein complexes (complex_enrich) and KEGG pathways (KEGG_enrich). The final tab in the file indicates the clusters used to map the functional distribution of negative and positive interactions shown in Fig. 6D.

Data File S7. Pleiotropic gene analysis

This file lists nonessential and essential query genes associated with high confidence pleiotropy scores based on their genetic interactions derived from the TSA (Essential derived pleiotropy) and DMA (Nonessential derived pleiotropy). The file also contains a second list of nonessential and essential query genes that participate in many genetic interactions but exhibited low pleiotropy scores indicating that these genes are more functionally specific.

Data File S8. Mass spectrometric evidence for Ipa1 interactions

This file lists proteins identified with high confidence as specific physical interactors with strains expressing Ipa1-GFP from its endogenous locus or Ipa1-HA from a galactose-inducible plasmid.

Data File S9. High and low interaction degree genes

This file lists the negative and positive interaction degree associated with every nonessential deletion (sn#), essential TS (tsq#), and DAmP (damp#) query mutant strain screened against the DMA (“query degree X DMA” tab) and/or TSA (“query degree X TSA” tab). A subset of strains were found to carry a second, spontaneous suppressor mutation that affected fitness of the query mutant strain. Strains carrying a suppressor mutation mapped through SGA analysis are indicated (“-supp”). Query mutants comprising the 20% highest and lowest degree groups of strains are indicated. Furthermore, a “Co-batch signal” rank is provided for every query (see “Co-batch filtering of query mutant strains”). Low ranks correspond to evidence for lingering batch effects. Another column, “Gene with correlated GI profiles that are co-annotated with the query gene (%)”, provides the percent of correlated gene pairs that are co-annotated to the particular query. A low negative interaction degree (e.g. 20% lowest negative interaction degree) coupled with a low co-batch rank (e.g. < ~0.2) and a low fraction of correlated pairs that share a similar functional annotation with a given query strain (e.g. < ~0.15) may be indicative of a low confidence screen. However, these criteria should be considered as loose indicators and not definitive metrics of screen quality and thus, should not be used as strict filters on the global interaction dataset. Another list (“Queries removed - batch effects” tab) indicates ~300 query strains that exhibited severe systematic batch effects and thus were removed from the indicated data set. Finally, two additional tabs provide the negative and positive interaction degree associated with every nonessential (“nonessential array degree” tab) and essential (“essential array degree” tab) array mutant, respectively.

Data File S10. Correlation analysis of query strain GI degree

As a complement to analysis of array strains (fig. S11-S12), GI degrees were calculated for query strains by counting negative interactions (tab 1, interactions with DMA strains; tab 2, interactions with TSA strains) and by counting positive interactions (tab 3, interactions with DMA strains; tab 4, interactions with TSA strains). Essential and nonessential queries were analyzed separately and results are labeled by grouped column headers. Wilcoxon rank-sum tests compared the GI degree in paired gene sets defined by absence and presence of each binary feature tested (top table). If the *P*-value is significant (<0.05), the “Test result” column describes the degree of the set of genes for which the listed binary feature is true (compared to the set for which the feature is false). Tests were not performed, indicated by “N/A”, if data were present for fewer than 50 strains; strains with missing data were excluded from the tests. Pearson’s correlation (column labeled “r”) was used to measure associations between GI degree and features that are continuous or counts (bottom table). Uncorrected *P*-values are shown. The features examined in this analysis are described above (see Methods section entitled, “Genetic interaction degree and network density analysis”). Given that analysis of different features required using different statistical tests and some features are not expected to be independent of each other, no multiple hypotheses correction procedures were used. We do note that 31 gene features were tested.

Data File S11. Nonessential and essential GI hub functional enrichment analysis

This file lists GO biological process, molecular function and cellular component terms that are enriched among of 10% of array strains with the most negative and 10% of array strains with the most positive interactions identified in the ExE network. Enrichments are also included for the 5% of array strains with the most negative interactions and 5% of array strains with the most positive interactions in the NxN genetic interaction network.

Data File S12. Protein complex standard

This file provides a list of protein complexes compiled from two sources: Baryshnikova 2010 (5) and Benschop 2010 (117).

Data File S13. Genetic interaction enrichment among protein complexes.

This file lists all possible pairs of protein complexes tested in the ExE, NxN and ExN networks. Enrichment for negative and positive interactions between genes in the same complex or between genes in different complexes is indicated. In addition, enrichment for genetic interaction in general (Combined interaction enrichment) regardless of the type is also indicated. Finally, Interaction Sign Bias indicates the distribution of interactions between genes within the same complex or between genes in different complexes. The interaction sign bias is computed as the mean over all interactions for within a given complex or between a pair of complexes. For example, an interaction sign bias of -1 indicates that all interactions identified between a set of genes encoding complexes members are negative, whereas a score of 1 indicates that only positive interactions were identified between a particular set of protein complex encoding genes. Rows highlighted in blue indicated complex-complex pairs enriched for negative interactions where greater than 75% of all interactions detected were negative. Rows highlighted in yellow indicated complex-complex pairs enriched for positive interactions where greater than 75% of all interactions detected were positive. The analysis shown in Fig. 8 is based on subset of

complexes composed of 75% essential genes (i.e. considered essential complexes) or 75% nonessential genes (i.e. considered nonessential complexes). The complexes used for this analysis and their enrichment results are listed in the tabs labeled, “_filtered”. The tabs named “all” list within and between complex enrichment for all protein complexes without prior filtering of complexes composed of less than 75% essential or 75% nonessential genes.

Data File S14. *D. melanogaster* S2 cell fitness

This file provides *D. melanogaster* S2 cell fitness upon RNAi-mediated 26S proteasome depletion and Bortezomib treatment.

Data File S15. Protein complex interaction enrichment bias

This file indicates fold enrichment and biases in positive vs. negative interaction network density for protein complexes and is described in detail above (see “Analysis of protein complexes exhibiting a positive interaction enrichment bias”). Rows highlighted in yellow indicate protein complexes that show > 1.5X enrichment for positive interactions (“E_fold_pos”) stronger enrichment for positive versus negative interactions when screened against the essential TSA. The file consists of the following columns:

(A) Protein complex name

(B) Number of complex member-encoding query genes screened against the DMA (“queries_vs_DMA”).

(C) Number of complex member-encoding query genes screened against the TSA (“queries_vs_TSA”).

(D) Nonessential-negative GI fold enrichment (“N_fold_neg”): negative interaction fold enrichment for a complex of interest with nonessential genes not in the complex.

(E) Essential-negative GI fold enrichment (“E_fold_neg”): negative interaction fold enrichment for a complex of interest with essential genes not in the complex.

(F) Nonessential-positive GI fold enrichment (“N_fold_pos”): positive interaction fold enrichment for a complex of interest with nonessential genes not in the complex.

(G) Essential-positive GI fold enrichment (“E_fold_pos”): positive interaction fold enrichment for a complex of interest with essential genes not in the complex. Complexes with a positive GI enrichment $\geq 1.5X$ are highlighted in yellow. These values were used to generate Fig. 9C.

(H) Positive GI bias with essential genes (“posGI_bias_with_E”): the relative positive:negative enrichment ratio of essential to nonessential genes for the complex of interest (calculated as $[D/E]/[F/G]$). Complexes with a positive GI enrichment ≥ 1 and a positive GI bias ≥ 1.5 are highlighted in yellow. These values were used to generate Fig. 9D.

(I) Positive GI bias with nonessential genes (posGI_bias_with_N”): the relative positive:negative enrichment ratio of nonessential to essential genes for the complex of interest (calculated as $[F/G]/[D/E]$).

Data File S16. Genetic suppression analysis

This file includes raw data from spot dilution growth assays to identify positive interactions that can be classified as genetic suppression. The suppression score is based on visual assessment of double mutant strain growth relative to a wild type and single mutant control strains. The score reflects strength of suppression with a score of 4 indicative of a strong suppression

interaction where double mutant growth exceeded growth of the sickest single mutant and a score of 0 indicates failure to confirm a suppression interaction.

Data file S17. *YNL181W chemical genetics data*

Relative growth of a *YNL181W*-DAmP strain (CG score) measured in the presence of 92 different compounds.