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# Bridging high-throughput genetic and transcriptional data reveals cellular responses to alpha-synuclein toxicity

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**Cells respond to stimuli by changes in various processes, including signaling pathways and gene expression. Efforts to identify components of these responses increasingly depend on mRNA profiling and genetic library screens. By comparing the results of these two assays across various stimuli, we found that genetic screens tend to identify response regulators, whereas mRNA profiling frequently detects metabolic responses. We developed an integrative approach that bridges the gap between these data using known molecular interactions, thus highlighting major response pathways. We used this approach to reveal cellular pathways responding to the toxicity of alpha-synuclein, a protein implicated in several neurodegenerative disorders including Parkinson's disease. For this we screened an established yeast model to identify genes that when overexpressed alter alpha-synuclein toxicity. Bridging these data and data from mRNA profiling provided functional explanations for many of these genes and identified previously unknown relations between alpha-synuclein toxicity and basic cellular pathways.**

The cellular response to perturbations including environmental changes, toxins and mutations is typically complex and comprises signaling and metabolic changes, as well as changes in gene expression. Revealing the molecular mechanisms underlying cellular response to a specific perturbation may determine the nature of the perturbation, thus illuminating disease mechanisms[1] or a drug's mode of action[2,3], and identify points of intervention with potential therapeutic value[4].

High-throughput experimental techniques are commonly used for finding components of these response pathways because they provide a genome- and proteome-wide view of molecular changes. mRNA profiling experiments rapidly identify genes that are differentially expressed following stimuli. Genetic screening, including deletion, overexpression and RNAi library screens, identify genetic 'hits', genes whose individual manipulation alters the phenotype of stimulated cells. However, each technique has obvious limitations for identifying the full nature of cellular responses. mRNA profiling experiments do not target the series of events that led to the differential expression. Genetic screens provide strong evidence that a gene is functionally related to the response process, but this relationship is often indirect and hard to decipher, especially in high-throughput experiments that typically result in scores of relevant genes with various functions.

It has been noted previously in a few specific instances[2,5–9] that genetic screens do not identify the same genes as mRNA assays conducted in the same conditions. Here we show that this discrepancy is, in fact, a general rule. Furthermore, we find a marked bias in each technique. We bridge this gap between the two forms of high-throughput data by using an algorithm that exploits molecular interactions data to reveal the functional context of genetic hits and additional proteins that participate in the response but that were not detected by either the genetic or the mRNA profiling assays themselves.

We applied the algorithm to identify cellular responses to increased expression of alpha-synuclein, a small human protein implicated in Parkinson's disease whose native function and role in the etiology of the disease remain unclear[10]. We screened an established yeast model for alpha-synuclein toxicity[11,12] using an additional set of 3,500 overexpression yeast strains, exposing the multifaceted toxicity of alpha-synuclein. Application of our approach to the genetic hits from the screen and to transcriptional data of the yeast model provides the first cellular map of the proteins and genes responding to alpha-synuclein expression.

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**Table 1 Measured responses to cellular perturbations**

| Perturbation[a] | Number of differentially expressed genes[b] | Number of genetic hits[c] | Overlap | *P* value |
|---|---|---|---|---|
| Growth arrest (HU) | 59 | 86 | 0 | 1 |
| DNA damage (MMS) | 198 | 1,448 | 43 | 0.81 |
| ER stress (tunicamycin) | 200 | 127 | 5 | 0.42 |
| Fatty acid metabolism (oleate) | 269 | 103 | 9 | 0.041 |
| ATP synthesis block (arsenic) | 828 | 50 | 9 | 0.25 |
| Protein biosynthesis (cycloheximide) | 20 | 164 | 0 | 1 |
| Gene inactivation, screen complete (24 data sets)[d] | 27 | 130 | 0 | 1 |
| Gene inactivation, screen incomplete (149 data sets)[d] | 24 | 12 | 0 | 1 |

[a]See **Supplementary Table 1a** for data sources. [b]Differentially expressed genes were defined as those showing at least a twofold change in expression following the perturbation or as defined in the original papers. [c]Number of genes whose genetic manipulation affects the phenotype of perturbed cells as defined in the original papers. [d]Median results are shown.





## RESULTS

### Comparing genetic hits and differentially expressed genes

We analyzed published mRNA profiles and genetic hits for 179 distinct perturbations in yeast (Methods). The perturbations included chemical and genetic insults affecting a multitude of cellular processes. Thirty of the genetic screens are complete, typically identifying >100 genetic hits. In almost all cases the overlap was small and statistically insignificant (**Table 1** and **Supplementary Table 1a** online).

We used Gene Ontology (GO) enrichment analysis to check whether each assay may be biased toward distinct aspects of cellular responses (**Supplementary Table 1b** and **Supplementary Fig. 1a** online). The combined genetic hits from all 179 genetic screens were highly enriched for several annotations, among the most frequent of which were biological regulation (23.3%, $P < 10^{-82}$), including transcription (14%, $P < 10^{-44}$) and signal transduction (6.3%, $P < 10^{-31}$). In contrast, the differentially expressed genes from all perturbations were enriched mostly for various metabolic processes (for example, organic acid metabolic process 7.1%, $P < 10^{-18}$) and oxidoreductase activities (7.2%, $P < 10^{-34}$). We observed the same enrichment trends upon focusing only on the 30 perturbations for which complete data were available when analyzed individually or when combined (**Supplementary Tables 1 c,d** and **Supplementary Note** online). Thus, we find that genetic assays tend to probe the regulation of cellular responses, whereas mRNA profiling assays tend to probe the metabolic aspects of cellular responses.

The differences in annotation between genetic hits and differentially expressed genes imply that each gene set alone often provides a limited and biased view of cellular responses. This hypothesis was confirmed in pathways that were well-studied by more classical methods. In the yeast DNA-damage response pathway, for example, a genetic screen[4] detected proteins that sense DNA damage (Mec3, Ddc1, Rad17 and Rad24), whereas mRNA profiling detected repair enzymes such as Rnr4 (ref. 13). Yet core components that had been uncovered by intense investigations over many years, such as the signal transducers Mec1 and Rad53 and the transcription factor Rfx1, remained undetected by either high-throughput assay.

To fully reap the benefits of applying high-throughput methods to new problems and underexplored biological processes, it is essential to find new routes to connect these data and obtain a true picture of the regulation of cellular responses. Judging from characterized pathways such as the DNA-damage response discussed above, we expect that some of the genetic hits, which are enriched for response regulators, will be connected via regulatory pathways to the differentially expressed genes, which are the output of such pathways, via components of the response that are missing from the experimental data (**Fig. 1**).

### ResponseNet algorithm for identifying response networks

We devised the ResponseNet algorithm to identify molecular interaction paths connecting genetic hits and differentially expressed genes, including components of the response that are otherwise hidden (**Fig. 1**). The yeast *Saccharomyces cerevisiae* provides a powerful model system for such analysis owing to the extensive molecular interactions data now available (Methods and **Supplementary Table 2a** online). We assembled an integrated network model of the yeast interactome that contains protein–protein interactions, metabolic relations and protein–DNA interactions detected by various methods with different levels of reliability[14]. The resulting interactome relates 5,622 interacting proteins and 5,510 regulated genes, which are represented by network nodes, via 57,955 molecular interactions, which are represented by network edges.

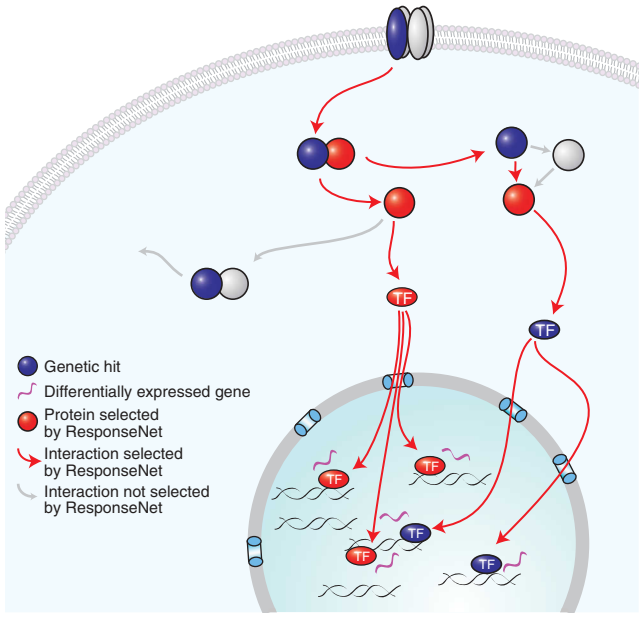


**Figure 1** Regulatory relationships between genetic and transcriptional data. Cellular response is depicted through a general signaling pathway, including receptor binding, transcription factor (TF) translocation into the nucleus and gene expression. Genetic screens and mRNA profiling identify only some of these molecular components and often do not identify the same genes, as shown. We find that the proteins products of genes identified in genetic screens (colored blue) tend to be molecules with regulatory roles. We therefore hypothesize that they may directly or indirectly contribute to the regulation of the observed change in gene expression (colored magenta). ResponseNet identifies the likely regulatory pathways and predicts proteins that are part of these pathways even if they are not identified in either screen (colored red).

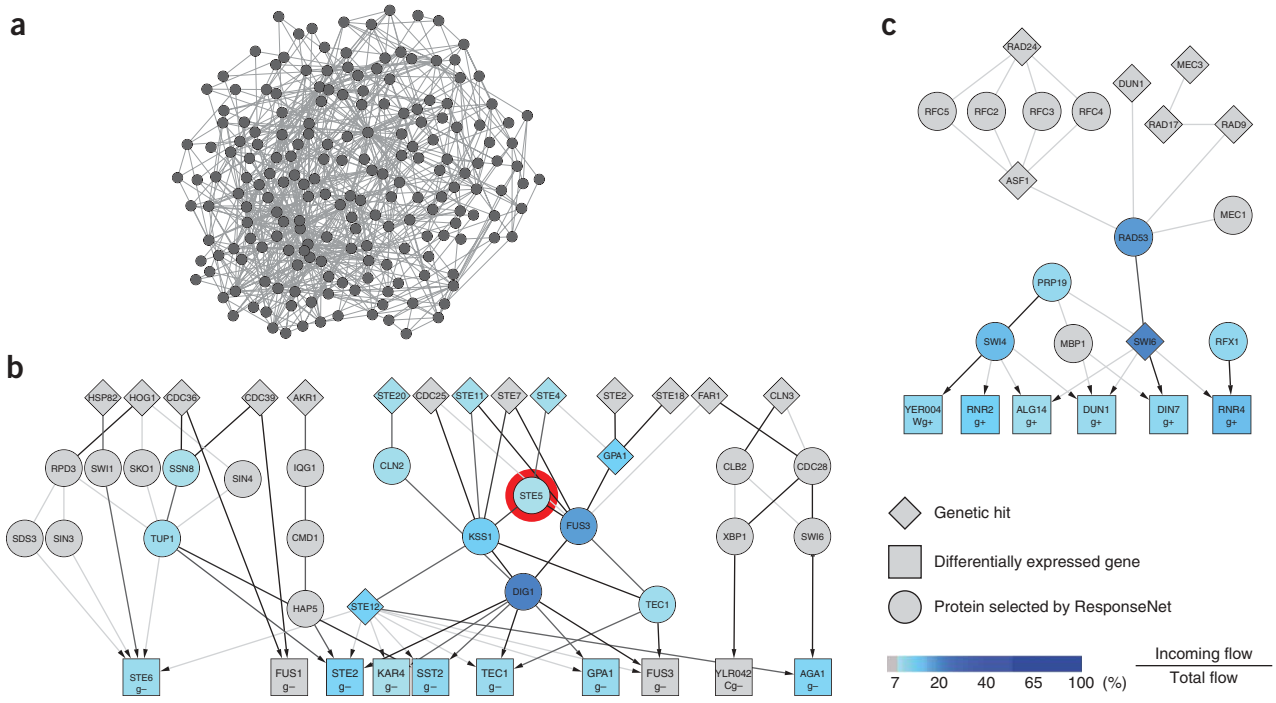


**Figure 2** Interactome subnetworks connecting genetic and transcriptional data. (**a**) A network connecting genetic and transcriptional[19] data of *STE5* deletion strain via paths with length of three edges or fewer finds 193 nodes and 778 edges. (**b**) The network created by ResponseNet connects the genetic and transcriptional[19] data of *STE5* deletion strain via 23 intermediary nodes and 96 edges. Higher ranked nodes, as determined by ResponseNet, appear in darker shades of blue and include core components of the pheromone response pathway. Ste5 itself, marked by a red circle, is ranked ninth among the top predicted proteins. (**c**) The highly ranked part of the network created by ResponseNet upon connecting genetic hits[4,20] to DNA-damage signature genes[21] identified in yeast treated with the DNA-damaging agent methyl methanesulfonate (MMS). The highest ranking intermediate nodes predicted by ResponseNet include core components of the DNA-damage–response pathway. The complete network appears in **Supplementary Figure 4** online. Each node represents either a protein or a gene, and edges represent protein–protein, metabolic and protein–DNA interactions. The darkness of an edge increases with the amount of flow it carries. Differentially expressed genes are labeled with a suffix of g+ for upregulation and g– for downregulation. Networks were visualized using Cytoscape.



Our interactome representation has two important features that facilitate identification of pathways relating genetic hits to transcriptional changes. First, we highlighted the transcriptional regulatory role of proteins by representing differentially expressed genes and their protein products as separate gene and protein nodes, respectively. The only connection between protein and gene nodes is through edges representing observed protein–DNA interactions between transcriptional regulators and their target genes. Edges between two protein nodes represent other interaction types. Consequently, pathways connecting genetic hits to differentially expressed genes must pass through transcriptional regulators (**Supplementary Fig. 1b**). Second, because interactions vary in their reliability, each edge was given a weight that represents the probability that the connected nodes interact in a response pathway. Probabilities were computed using a Bayesian method that considers the experimental evidence supporting an interaction, and that favors interactions among proteins acting in a common cellular response pathway (Methods and **Supplementary Table 2b**).

Because of the vast number of edges, a search for all interaction paths connecting the genetic hits to the differentially expressed genes typically results in 'hairball' networks that are very hard to interpret (**Fig. 2a**). Pioneering approaches that searched an interactome for high-probability paths had to limit the output path lengths to three edges for computational complexity issues[15,16]. We aimed for a solution that would (i) pick the subset of genetic hits most likely to modulate the differentially expressed genes without limiting it a priori to known regulatory genes, (ii) identify and rank intermediary proteins that are likely to be part of response pathways but escaped detection by high-throughput methods and (iii) give preference to proteins that lie on high-probability paths connecting the genetic hits to the differentially expressed genes without imposing constraints on the network topology.

These requirements were met with a 'flow algorithm', a computational method used previously to analyze known signaling or metabolic pathways (for example, see ref. 17). Basically, flow goes from a source node to a sink node through the graph edges; edges are associated with a capacity that limits the flow and with a cost. (As a loose analogy, this resembles water finding the path of least resistance through a complex landscape.) To identify response pathways we required that flow pass from genetic hits through interactome edges to differentially expressed genes (**Supplementary Fig. 1b**). We then formulated our goal as a minimum-cost flow optimization problem[18]: Cost was defined as the negative log of the probability of an edge. Hence, minimizing the cost gives preference to high-probability paths (Methods).

The solution to the optimization problem is a relatively sparse network connecting many of the genetic hits to many of the differentially expressed genes through known interactions and intermediary proteins (**Fig. 2b**). Although these intermediary proteins escaped detection by either high-throughput genetic analysis or mRNA profiling, they are predicted by the algorithm to participate in the response. All proteins in the solution are ranked by the amount of flow they

**Table 2 Yeast genes that modify α-syn toxicity when overexpressed**

| Gene class | α-syn toxicity suppressors | α-syn toxicity enhancers |
|---|---|---|
| Amino acid transport | *Avt4, Dip5, Lst8* | |
| Autophagy | *Nvj1* | |
| Cytoskeleton | *Icy1, Icy2* | |
| Manganese transport | *Ccc1* | *Pmr1* |
| Protein phosphorylation | *Cdc5, Gip2, Ime2, Ptp2, Ptc4, Rck1, Yck3* | *Cax4, Ppz1, Ppz2, Sit4* |
| Transcription or translation | *Cup9, Fzf1, Hap4, Jsn1, Mga2, Stb3, Tif4632, Vhr1* | *MATALPHA1, Mks1, Sut2* |
| Trehalose biosynthesis | *Nth1, Tps3, Ugp1* | |
| Ubiquitin-related | *Cdc4, Hrd1, Uip5* | *Ubp7, Ubp11* |
| Vesicular transport, ER-Golgi | *Bre5, Erv29, Sec21, Sec28, Sft1, Ubp3, Ykt6, Ypt1* | *Bet4, Glo3, Gos1, Gyp8, Sec31, Sly41, Trs120, Yip3* |
| Other cellular processes | *Isn1, Mum2, Osh2, Osh3, Pde2, Pho80, Pfs1, Qdr3* | *Eps1, Ids2, Izh3, Tpo4* |
| Unknown function | *YBR030W, YDL121C, YDR374C, YKL063C, YKL088W, YML081W, YML083C, YMR111C, YNR014W, YOR129C, YOR291W (Ypk9)* | |

carry. The more flow that passes through a protein, the more important it is in connecting the input sets.

### Validation of the ResponseNet algorithm

To determine whether ResponseNet provides valid biological insights, we used it to analyze data from perturbations of well-studied pathways. For example, we used ResponseNet to connect genetic hits associated with Ste5 (from the *Saccharomyces* Genome Database) and differentially expressed genes[19] collected from a strain lacking Ste5, a scaffold protein that coordinates the MAP kinase cascade activated by pheromone (**Fig. 2b**). Nodes selected by ResponseNet were highly enriched for proteins functioning in the pheromone response pathway (46%, $P < 10^{-18}$), thus revealing the perturbed biological process. The highly ranked intermediary proteins included key regulators of the pheromone response including Ste5, the source of perturbation.

ResponseNet also performed well in analyzing the complex cellular response to DNA damage[4,20,21]. Nodes discovered by ResponseNet were highly enriched for the GO categories response to DNA damage stimulus (21%, $P < 10^{-14}$) and DNA repair (19%, $P < 10^{-14}$). The highly ranked part of the network contained core pathway proteins that were uncovered by years of intense investigation but escaped detection by high-throughput screens, including signal transducers (Mec1, Rad53), members of the RFC complex (Rfc2, Rfc3, Rfc4, Rfc5) and the transcriptional regulator Rfx1 (**Fig. 2c**). Statistical evaluation of the performance of ResponseNet on data for less well-characterized pathways is described in the **Supplementary Note**.

### Mapping the cellular responses to alpha-synuclein toxicity

Having established the validity of our method to uncover connections between otherwise disparate high-throughput datasets, we applied ResponseNet to investigate the cellular toxicity associated with alpha-synuclein (α-syn). α-Syn is a small lipid-binding protein that is natively unfolded when not bound to lipids and prone to forming toxic oligomers[22]. It has been implicated in several neurodegenerative disorders, particularly Parkinson's disease (PD): it is the main component of Lewy bodies, locus duplication or triplication of α-syn lead to familial forms of PD, and increased expression of α-syn leads to neurodegeneration in several animal models[23]. Despite immense efforts, the cellular pathways by which α-syn leads to cell death are just beginning to emerge.

The yeast *Saccharomyces cerevisiae* provides a powerful system for studying the toxicities of α-syn that result from its intrinsic physical properties. Expression of human α-syn in yeast yields dosage-dependent defects also found in mammalian systems, including cytosolic-lipid-droplet accumulation, reactive-oxygen-species production and ubiquitin-proteasome system impairment[11]. An initial screen for yeast genes that modify α-syn toxicity when overexpressed identified genes involved in ER-to-Golgi vesicle trafficking and led to the observation that α-syn blocks ER-to-Golgi vesicle trafficking[12].

We now report the results of screening 5,500 overexpression yeast strains, thereby covering 85% of the yeast proteome. We identified 55 suppressors and 22 enhancers of α-syn toxicity, many with clear human orthologs, including the homolog of human PD gene *ATP13A2* (also known as *PARK9*; **Table 2** and **Supplementary Table 3a** online). As demonstrated in the accompanying article (Gitler *et al.*[24]), PARK9 and the human homologs of eight other genetic modifiers with diverse functions (Ypt1, Hrd1, Ubp3, Pde2, Cdc5, Yck3, Sit4 and Pmr1) are efficacious in neuronal models, validating the yeast model as meaningful to α-syn toxicity in neurons[12,24]. Major classes of genes that emerged include vesicle-trafficking genes, kinases and phosphatases, ubiquitin-related proteins, transcriptional regulators, manganese transporters and trehalose-biosynthesis genes (**Supplementary Table 3a,b**). Notably, trehalose was recently shown to promote the clearance of misfolded mutant α-syn[25], and manganese exposure has been linked with Parkinson's-like symptoms, albeit with a distinct underlying pathology[26]. The genes identified by the screen point to causal relations between α-syn expression and toxicities previously associated with PD but not specifically linked to α-syn (**Supplementary Note**).

mRNA profiling of the yeast model was determined in a separate study (unpublished data and **Supplementary Table 3b,c**). Upregulated genes prominently included genes with oxidoreductase activities (13%, $P < 10^{-9}$). Downregulated genes included ribosomal genes (28%, $P < 10^{-30}$), as commonly observed under stress[27]. More specific to α-syn toxicity, the downregulated genes were markedly enriched for genes encoding proteins localized to the mitochondria (60%, $P < 10^{-44}$).

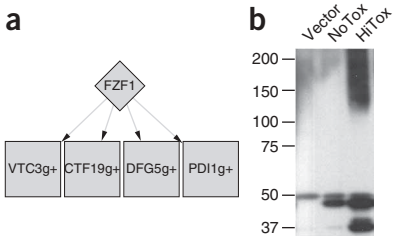


**Figure 3** Nitrosative stress response to α-syn expression in yeast. (**a**) The predicted subnetwork containing Fzf1 and its differentially expressed target genes. Graphical representation is similar to **Figure 2**. (**b**) Immunoblotting against S-nitrosocysteine performed on a control strain (vector), on a strain expressing one copy of α-syn (NoTox) and on a high-toxicity strain (HiTox) expressing several copies of α-syn reveals that increasing levels of α-syn increase the amount of S-nitrosylated proteins.

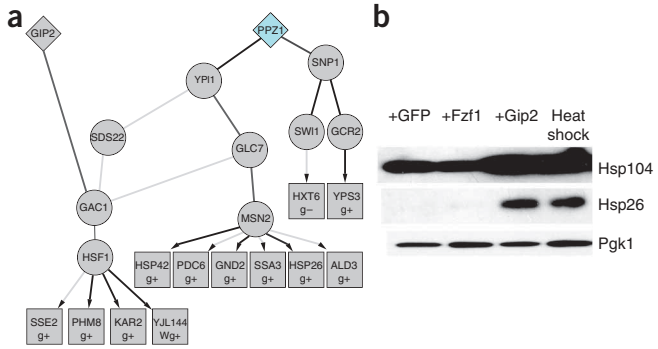


**Figure 4** Overexpression of Gip2 causes induced expression of Hsf1 targets. (**a**) The predicted subnetwork links the toxicity suppressor Gip2 and the toxicity enhancer Ppz1 to Hsf1 and Msn2 via components of type 1 protein phosphatase complex (Gac1, Glc7, Ypi1, Sds22). Graphical representation is similar to **Figure 2**. (**b**) Immunoblotting of vector cells overexpressing GFP, Fzf1 or Gip2 with antibodies against Hsp104 and Hsp26. Overexpression of Gip2 is sufficient to activate Hsf1 and induce higher protein levels of both its targets Hsp104 and Hsp26, similar to that of vector cells subjected to heat shock. In contrast, overexpression of another genetic suppressor, Fzf1, does not activate Hsf1. Immunoblotting against Pgk1 was used as a loading control.

The genetic and mRNA profiling data exemplify both the power and the limitations of the current approaches. Although they reveal the wide range of cellular functions altered by α-syn, the precise roles of the identified genes in the cellular response are unclear. For example, we checked whether the ubiquitin-related genetic hits affect α-syn degradation. However, in strains overexpressing these ubiquitin-related genes, we did not detect changes in steady-state α-syn protein concentrations (**Supplementary Fig. 2** online). As with our analyses above, the overlap between the genetic hits and the differentially expressed genes was minor (four genes, $P = 0.96$).

Application of ResponseNet to these disparate datasets gave a more coherent view of the cellular response (**Supplementary Fig. 3a** online). The resulting network provided context to a large portion of the data: 34 (44%) genetic hits and 166 (27%) differentially expressed genes were linked to each other through 106 intermediary proteins. These include two-thirds of the protein kinase, phosphatase and ubiquitin-related genetic hits, illuminating their intricate role in the response to α-syn.

The major cellular pathways identified by ResponseNet included ubiquitin-dependent protein degradation, cell cycle regulation and vesicle-trafficking pathways, all of which have previously been associated with PD (**Supplementary Note** and **Supplementary Fig. 3a**). Four examples illustrate the ability of ResponseNet to clarify aspects of α-syn responses relevant to PD and uncover others whose relationship to α-syn was completely unknown.

### Nitrosative stress

Fzf1 was the only genetic hit related to nitrosative stress[28]. However, ResponseNet connected it to four upregulated transcripts, including that encoding Pdi1, a protein disulfide isomerase (PDI) (**Fig. 3a**). Notably, the upregulation of human PDI protects neuronal cells from neurotoxicity associated with ER stress and protein misfolding (both of which are linked to α-syn expression in yeast and neurons), and PDI is one of a small number of specific proteins S-nitrosylated in PD that activate protective pathways, in addition to the generalized nitrosative damage that is a hallmark of the disease[29]. We found that increased expression of α-syn causes both specific and general increases in S-nitrosylation of proteins (**Fig. 3b**). This was highly surprising because the yeast genome does not encode a canonical nitric oxide synthase and, until very recently, yeast were not thought to produce nitric oxide[30]. Our results indicate that the nitrosylation of specific proteins and generalized nitrosylation is a highly conserved and deeply rooted response to cellular perturbations created by α-syn.

### Heat shock

The induction of the heat-shock response directly or via chemical inhibition of Hsp90 (ref. 31) suppresses α-syn toxicity in many model systems including yeast, flies, mice and human cells (for example, see refs. 32,33). However, heat-shock–related genes were conspicuously absent among the list of genetic suppressors. Nonetheless, ResponseNet predicted the involvement of two highly conserved heat-shock regulators, the chaperone Hsp90 (isoform Hsp82, **Supplementary Fig. 3a**, panel a) and the heat-shock transcription factor Hsf1 (**Fig. 4a**). Hsf1 appeared downstream of the toxicity suppressor Gip2, a putative regulatory subunit of the Glc7 phosphatase, which interacts with Gac1. Gac1 is a regulatory subunit of the Glc7 complex that is known to activate Hsf1 (ref. 34). These connections suggested that Gip2 overexpression might induce a heat-shock response. Indeed, we found that strains overexpressing Gip2 show elevated concentrations of heat-shock proteins (**Fig. 4b**). ResponseNet therefore provided a mechanistic explanation for the suppression of α-syn toxicity achieved by Gip2 overexpression and identified a new regulator of the highly conserved heat-shock response.

### The mevalonate-ergosterol biosynthesis pathway

This pathway, which is targeted by the cholesterol-lowering statin drugs, synthesizes sterols as well as other products with connections to α-syn toxicity, such as farnesyl groups required for vesicle trafficking proteins and ubiquinone required for mitochondrial respiration. ResponseNet ranked highly Hrd1, which regulates the protein target of statins, and the predicted intermediary Hap1, a proposed transcriptional regulator of the pathway[35] (**Supplementary Fig. 3a**, panel a). In addition, the α-syn mRNA profile modestly correlated with the profile of yeast treated with lovastatin ($r = 0.32$, $P < 10^{-93}$, L.J.S. and S.L., unpublished data), and several genetic hits also could be associated with products of the pathway (enzymes Bet4 and Cax4, farnesylated proteins Ypt1 and Ykt6 and putative sterol carriers Sut2, Osh2 and Osh3). We therefore tested the effect of lovastatin, which selectively inhibits the highly conserved HMG-CoA reductase protein in yeast and in mammalian cells, on α-syn toxicity. Addition of 5 μM lovastatin to the media caused a further reduction in growth to strains overexpressing α-syn (**Fig. 5a**), but did not reduce growth of either wild-type controls or of cells expressing another toxic protein, a glutamine-expansion variant of huntingtin exon I[36] (**Supplementary Fig. 3b**). We further tested ubiquinone, a downstream output of this pathway, reasoning that its downregulation through the action of α-syn might increase cellular vulnerability. Indeed, the addition of ubiquinone-2 to the media provided a modest suppression against α-syn toxicity. Ubiquinone is an antioxidant, but this was not a nonspecific antioxidant response, as the antioxidant N-acetylcysteine had no effect (data not shown).

### The target of rapamycin (TOR) pathway

ResponseNet identified the TOR pathway proteins Tor1, Tor2 and their target transcription factors as intermediary between the genetic suppressor Lst8, a positive regulator of the TOR pathway, and several upregulated genes involved in spore wall formation (a vectorially directed secretory process in yeast) and vacuolar protein degradation

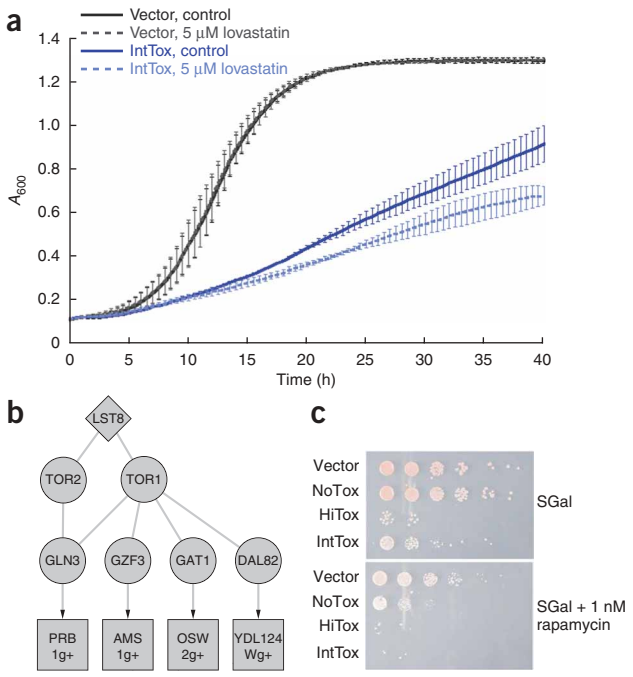


**Figure 5** Effects of the small molecules lovastatin and rapamycin on α-syn toxicity. (**a**) Lovastatin inhibits growth of the yeast strain expressing an intermediate level of α-syn. Growth of a control strain (vector) and an intermediate toxicity strain (IntTox) expressing several copies of α-syn was measured in a galactose containing media with and without 5 μM lovastatin. Each growth curve reflects the average of three individual runs, each of which is indicated by a bar. (**b**) The predicted subnetwork containing TOR pathway components includes the predicted proteins Tor1 and Tor2. Graphical representation is similar to **Figure 2c**. (**c**) The effect of rapamycin on growth of different yeast strains. The upper panel shows the growth of a control strain (vector), a strain expressing one copy of α-syn (NoTox), a high-toxicity strain (HiTox) and an intermediate toxicity strain (IntTox) both expressing several copies of α-syn, in a galactose containing media (SGal) that is used to induce expression of α-syn. The lower panel shows the same strains grown in media that also contains 1 nM rapamycin, showing that rapamycin inhibits growth of all α-syn–expressing strains but not the control strain, as observed by the difference in the number of colonies per drop. The different columns correspond to serial dilutions.

(**Fig. 5b**). We found that addition of the TOR-inhibitor rapamycin to the media markedly enhanced the toxicity of α-syn. Indeed, a low dose of α-syn, which is otherwise innocuous, became toxic (**Fig. 5c**). Establishing the specificity of this effect to α-syn, rapamycin did not reduce growth of cells expressing glutamine expansion variants of huntingtin exon I (**Supplementary Fig. 3c**). As other studies have suggested benefits of rapamycin treatment in PD models, these results call for further investigation and suggest a complexity to the response to rapamycin that is potentially due to the vast range of processes affected by TOR activation.

## DISCUSSION

We provide a novel framework in which genetic, physical and transcriptional data naturally complement each other in the context of cellular response to biological perturbations. Although the complementary nature of these data has been noted[2,5–9,37], a systematic analysis of the relationship between stimulus-specific genetic modifiers and transcriptional responses has been lacking. By examining over 150 distinct stimuli we find that differentially expressed genes and genetic hits are consistently disparate (**Table 1**); genetic hits are biased toward regulatory proteins, whereas the differentially expressed genes are biased toward metabolic processes. Indeed, each assay has inherent 'blind spots'. Many yeast regulatory proteins are not detected by transcriptional assays because either they are predominantly regulated post-transcriptionally, they have a low transcript concentration[38] or their differential expression is transient, making changes hard to measure. Conversely, the genes that are differentially transcribed are often involved in metabolic processes or redundant functions, which tend to be robust against single mutations[39].

The discordance between genetic hits and differentially expressed genes has implications for the search for therapeutic strategies. In yeast, inactivating a differentially expressed gene is no more likely to affect cell viability than targeting a randomly chosen gene. Bridging the gap between these data using techniques like ResponseNet can potentially reveal intervention points not discovered in the high-throughput assays themselves (**Fig. 2**) that may be targeted by drugs.

Our computational approach is based on a flow algorithm to connect the genetic hits and differentially expressed genes. Unlike studies that link a target gene with its causal transcriptional change[13,15,16,40–43], a flow-based approach allows for a global, efficient and simultaneous solution for multiple target genes that puts no a priori bounds on the structure of the output. Indeed, the predicted output networks have rich structures with half of all paths having a length of three edges or more. The ability of ResponseNet to analyze interactome data containing tens of thousands of nodes and edges make it well suited to analyzing the accumulating data from other species or other techniques.

We applied our approach to a yeast model for α-syn pathobiology implicated in PD. Our unbiased screen identified 77 genes whose overexpression altered α-syn toxicity (**Table 2**). These included genes involved in vesicle trafficking (as previously reported), protein degradation, cell cycle regulation, nitrosative stress, osmolyte biosynthesis and manganese transport. This screen established an interface between α-syn and a large number of cellular and environmental factors previously linked to neuropathology and, in some cases, specifically to parkinsonism, but not specifically linked to α-syn. Many of the genes we identified are highly conserved in humans, where they may exert similar effects. Indeed, eight out of nine toxicity modifiers tested had similar effects on α-syn toxicity in yeast and in neuronal systems[24].

Application of ResponseNet to the α-syn model successfully provided functional context to many of the genetic hits identified in our yeast screen (**Supplementary Fig. 3a**) and pointed to the involvement of several cellular pathways (**Figs. 3–5**). Of these, the mevalonate-ergosterol pathway is of special interest as its perturbation could potentially alter a variety of downstream pathways, including protein farnesylation and ubiquinone biosynthesis, which are closely related to the vesicle trafficking defects and mitochondrial dysfunction observed in the yeast model. Indeed, a link between sterol biosynthesis and the etiology of PD has recently emerged. Individuals with PD have significantly lower concentrations of low-density lipoprotein (LDL) cholesterol than their spouses[44], and low concentrations of LDL preceded the appearance of PD in a group of men of Japanese ancestry[45]. Our work provides a molecular framework for elucidating this connection.

The global picture obtained by integrating high-throughput genetic, transcriptional and physical data demonstrates the power of integrative approaches to illuminate underexplored cellular processes. As high-throughput assays are becoming routine in the study of complex

disease and developmental processes, approaches for deciphering these data based on their underlying characteristics are vital.

## METHODS

**Genetic and transcriptional datasets.** Chemical perturbation data were downloaded from original papers. Genetic hits for gene inactivation included proteins that genetically interact with the inactivated gene according to *Saccharomyces cerevisiae* Genome Databases (SGD). Differentially expressed genes included genes that showed at least a twofold change in expression with a $P$ value $\leq 0.05$ (ref. 19), or else as defined according to the original papers. Genetic and mRNA profiling assays for chemical perturbations were paired if the chemical concentrations were comparable.

**Interactome data description.** The interactome was represented as a graph $G = (V, E)$ where nodes $V$ represent genes and proteins and edges $E$ represent their interactions. Different nodes represent a gene and its corresponding protein.

Bidirectional edges between protein nodes represent physical protein–protein interactions or metabolic interactions between enzymes if the substrate of one is the product of the other.

Directed edges represent regulatory interactions. Outgoing edges connected protein nodes to gene nodes if there was evidence from literature or ChIP-chip assays that the proteins may regulate the genes. Proteins nodes were connected if both proteins were transcriptional regulators and one regulated the other.

The data sources appear in the **Supplementary Note**. **Supplementary Table 2a** lists the number of interacting pairs per interaction type in the interactome.

**Weighting scheme for interactome edges.** *Interactions between protein nodes.* Each interacting protein pair $p_i p_j$ was associated with an interaction vector $Ip_i p_j$; vector entry $I_k p_i p_j$ is an indicator function for interaction evidence of type $k$. Interactions are weighted ($w_{ij}$) to reflect the probability that $p_i p_j$ function in a randomly selected response pathway (denoted $RP_{Pi,Pj} = 1$) as follows:

$$w_{ij} = \mathrm{P}(RP_{p_i p_j} = 1 | I_{p_i p_j}) = \mathrm{P}(I_{p_i p_j} | RP_{p_i p_j} = 1)\mathrm{P}(RP_{p_i p_j} = 1)/\mathrm{P}(I_{p_i p_j}),$$

where

$$\begin{aligned}\mathrm{P}(I_{p_i p_j}) = {} & \mathrm{P}(I_{p_i p_j} | RP_{p_i p_j} = 1)\mathrm{P}(RP_{p_i p_j} = 1) \\ & + \mathrm{P}(I_{p_i p_j} | RP_{p_i p_j} = 0)P(RP_{p_i p_j} = 0)\end{aligned}$$

We assumed conditionally independence between different types of evidence:

$$\mathrm{P}(I_{p_i p_j} | RP_{p_i p_j}) = \prod\nolimits_k \mathrm{P}(I_{kp_i p_j} | RP_{p_i p_j})$$

*Interactions between protein and gene nodes.* Weights were designed to reflect the reliability of the interaction on the basis of experimental evidence and binding-site conservation.

The scheme for calculating P($RP$) and P($I_k$| $RP$) and the weights per interaction type appear in the **Supplementary Note**. Because high edge weights could indicate unusually well-studied proteins[46] or imperfectness of the assumption of conditional independence, all weights were capped to a maximum value of 0.7.

**Linear programming formulation.** For each perturbation, the input to ResponseNet consisted of the weighted interactome $G = (V, E)$, the genetic hits $Gen \subset V$ and the differentially expressed genes $Tra \subset V$ identified following the perturbation. Each edge $(i, j) \in E$ was characterized by a weight $w_{ij}$ and a capacity $c_{ij} = 1$.

The graph $G$ was updated as follows:

1. $V' = V \cup \{S, T\}$, where $S$ and $T$ are auxiliary nodes representing the source and sink, respectively.

2. $E' = E \cup (S,i)_{\forall i \in Gen} \cup (i,T)_{\forall i \in Tra}$, connecting $S$ to the genetic hits and $T$ to the differentially expressed genes by directed edges.

3.

$$c_{Si} = \frac{|strength_i|}{\sum_{j \in Gen} |strength_j|},$$

$\forall i \in Gen$, where the strength of each genetic hit was measured by the variation it conferred on the number of colonies per drop if available; otherwise, strengths were uniform.

4.

$$c_{iT} = \frac{|\log_2(strength_i)|}{\sum_{j \in Tra} |\log_2(strength_j)|},$$

$\forall i \in Tra$, where the strength was measured by either the relative change in its transcript level or the $P$ value associated with it, depending on their availability.

5. $w_{Si} = c_{Si}\ \forall i \in Gen$ and $w_{iT} = c_{iT}\ \forall i \in Tra$

Letting $f_{ij}$ denote the flow from node $i$ to node $j$ and for any given $\gamma \geq 0$, the following optimization problem was solved using LOQO[47]:

$$\min_f((\sum_{i \in V', j \in V'} -\log(w_{ij}) * f_{ij}) - (\gamma * \sum_{i \in Gen} f_{Si}))$$

Subject to:

$$\sum_{j \in V'} f_{ij} - \sum_{j \in V'} f_{ji} = 0 \quad \forall i \in V' - \{S, T\}$$

$$\sum_{i \in Gen} f_{Si} - \sum_{i \in Tra} f_{iT} = 0$$

$$0 \leq f_{ij} \leq c_{ij} \quad \forall (i,j) \in E'$$

The solution $F = \{f_{ij} > 0\}$ defined the predicted response network. For enrichment analysis only protein nodes were considered, and genetic hits were included only if they received flow from nodes other than the source. Protein nodes were ranked in decreasing order according to the total amount of their incoming flow. Although the solution to the optimization problem is a directed network, this directionality only reflects the way in which the algorithm directed flow from the genetic hits to the differentially expressed genes and does not represent the causal order of events (**Supplementary Fig. 1b**).

Additional information regarding the formulation, space of solutions, setting $\gamma$ value and ResponseNet performance appear in the **Supplementary Note**. For ResponseNet validation $\gamma = 10$.

**Statistical analysis.** Probabilities of overlap between genetic hits and differentially expressed genes were calculated using Fisher's exact test, given a total of 6,000 yeast genes. Enrichment analysis was done using the Gene Ontology Term Finder from SGD.

**α-Syn toxicity modifier screen** The high-throughput yeast transformation protocol appears elsewhere[12].

**Immunoblotting.** Phosphoglycerate kinase 1(Pgk1) mouse monoclonal antibody was used at 1:5000. Hsp26 rabbit polyclonal antibody (gift from J. Buchner, Center for Integrative Protein Science and Department of Chemistry, Technische Universität München) was used at 1:5000. Hsp104 mouse monoclonal antibody (4B; ref. 48) was used at 1:5000. S-nitosocysteine rabbit polyclonal antibody (Sigma) was used at 1:10,000.

**α-Syn ResponseNet analysis.** Differentially expressed genes had at least a twofold change in expression with $P$ value $\leq 0.05$ (**Supplementary Table 3c**). Capacities of edges connecting the source to genetic hits were relative to the absolute strength of the genetic hits (**Supplementary Table 3a**). Capacities of edges connecting differentially expressed genes to the sink were relative to the absolute log of the change in expression. We repeated the analysis excluding nonspecific stress responses (**Supplementary Note**). ResponseNet was run with $\gamma = 12$.

**α-Syn growth in presence of small molecules.** For spotting assays, yeast strains were initially grown to saturation in media containing raffinose, normalized for their $A_{600}$ and serially diluted by fivefold before spotting onto appropriate yeast media. Growth curves were monitored using the Bioscreen instrument. Yeast strains were pre-grown in 2% raffinose medium and induced in 2% galactose medium in presence of either the compound or vehicle control (1% DMSO final) with starting $A_{600}$ of 0.1. Cells were grown at 30 °C, with plates shaken

every 30 s to ensure proper aeration and $A_{600}$ measurements taken every half hour over a 2-d period. The resulting data ($A_{600}$ versus time) were plotted using Kaleidagraph. At least three independent runs were conducted for each growth condition.

*Note: Supplementary information is available on the Nature Genetics website.*

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**COMPETING INTERESTS STATEMENT**

The authors declare competing financial interests: details accompany the full-text HTML version of the paper at http://www.nature.com/naturegenetics/.

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# Supplementary Note

## Bridging high-throughput genetic and transcriptional data reveals cellular responses to alpha-synuclein toxicity

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## Table of Contents





Nature Genetics: doi:10.1038/ng.337

## The bias in the sets of genetic hits and the sets of differentially expressed genes

Enrichment analyses were carried out using Gene Ontology (GO) term finder from SGD[1] and Genomica[2]. Supplementary Table 1B contains the GO enrichment of the genetic hits and of the differentially expressed genes. To validate that the biases for the pooled hits represent general tendencies, as opposed to being dominated by a handful of large data sets, we repeated the analysis in several ways as detailed below.

### *Separate analysis of the perturbations with complete genetic screens*

We calculated the gene ontology (GO) process or function annotation enrichment separately for each of the perturbations in Table 1 for which complete genetic screens were available. To avoid being biased by a handful of perturbations we required that an annotation be enriched in data of at least 6 perturbations, which is 20% of the datasets. Supplementary Table 1C lists the GO annotations that were statistically significantly enriched ($p<=0.05$, FDR corrected) in at least 20% of the sets of genetic hits or at least 20% of the sets of differentially expressed genes. The table details for each GO annotation the number of sets that were significantly enriched for this annotation and the median p-value for its enrichment.

We identified 146 GO annotations enriched in at least 20% of the sets of genetic hits. The three GO annotations enriched in the largest number of genetic hits sets are 'biological regulation' (23 sets, 77%, median $p<10^{-10}$), 'response to stimulus' (23 sets, 77%, median $p<10^{-8}$) and 'regulation of cellular process' (22 sets, 73%, median $p<10^{-9}$). Other frequently enriched annotations among the sets of genetic hits include cell cycle related processes and other regulatory processes (e.g., regulation of cell cycle, post-



Nature Genetics: doi:10.1038/ng.337

translational protein modification and transcription). Therefore, genetic hits sets are indeed enriched for regulatory processes and functions.

We identified only 10 annotations that were significantly enriched in at least 20% of the sets of differentially expressed genes. Eight of these annotations are for various metabolic processes, and the remaining two annotations are for oxidoreductase activity and cell wall constituents.

Interestingly, none of the enriched annotations was common to both the genetic hits sets and the differentially expressed genes sets, supporting our observation of the distinct nature of these gene sets. The genetic hits were enriched for a few annotations that could be construed as related to metabolism. However, all-but-one of these were DNA or RNA metabolic processes, which are more closely related to cell cycle progression and gene transcription than to metabolism *per se*. The GO annotation "one-carbon compound metabolic process" is exceptional. It is the only category that is clearly related to metabolism but is associated with genetic hits (6 sets, p=0.001). We therefore conclude that the bias is evident when the data sources are analyzed separately.

### *Combined analysis of the perturbations with complete genetic screens*

We created a combined genetic hits set and a combined differentially expressed gene set from the perturbations in Table 1 for which complete genetic screens were available. We then checked the GO process and function annotation enrichment of the two combined sets. The enriched annotations that we list in Supplementary Table 1D were limited to annotations also found to be enriched in at least 20% of the sets when analyzed separately. This analysis of the combined sets resulted in the identification of 124 GO annotations enriched in the combined set of genetic hits, and 10 GO annotations enriched



Nature Genetics: doi:10.1038/ng.337

in the combined set of differentially expressed genes. The results for the combined sets appear in Supplementary Table 1D, which lists for each of these GO annotations its enrichment p-value and the percentage of genes in the corresponding set that are attributed to this annotation.

The analysis of the combined sets with complete genetic screens again supports the bias we reported. We find that biological regulation is among the most significantly enriched and most frequent annotation for the set of genetic hits. The genetic hits are also frequently attributed to various regulatory processes, response pathways, and cell cycle phases. The differentially expressed genes are most frequently attributed to oxidoreductase activity and to organic acid metabolic process.

To enable visualization we further limited the annotations to those annotations attributed to at least 5% of the combined gene set. Supplementary Figure 1A presents each of these 39 GO annotations together with the percentage of genes attributed to this annotation in the enriched set.

## *Graphical representation of the interactome*

The interactome was represented as a graph $G = (V, E)$ that consists of nodes (vertices) $V$ representing genes and proteins, and a set of bidirectional and directed edges $E$ representing their interactions. Different nodes in the network represent a gene and its corresponding protein.

Bidirectional edges between protein nodes in the interactome consisted of:



Nature Genetics: doi:10.1038/ng.337

(i) Physical protein-protein interactions, which were downloaded from [3] and from BioGRID release 2.0.30.

(ii) Interactions between two proteins if they both appeared in the same literature-curated protein complex, downloaded from MIPS [4].

(iii) Metabolic interactions between two enzymes, if the substrate of one was the product of the other, based on the metabolic map of *S. cerevisiae* [5].

Directed edges in the interactome consisted of:

(i) Edges from a protein node to a gene node if there was evidence from either literature or ChIP-chip assays [6-8] that the protein was a probable transcriptional regulator of the gene.

(ii) Edges from one protein node to another if both proteins acted as transcriptional regulators and the first regulated the second.

Supplementary Table 2A lists the number of interacting pairs per interaction type in the interactome.

## Weighting scheme for interactome edges

Each edge $(i, j) \in E$ between node *i* and node *j* of the interactome is characterized by a weight $w_{ij}$ calculated as follows:

*Interactions between protein nodes:* We developed a Bayesian weighting scheme that favors interactions between proteins functioning within a common response pathway (*RP*). Each interacting protein pair $p_i,p_j$ was associated with an interaction vector $Ip_i,p_j$, where vector entry $I_k\,p_i,p_j$ serves as an indicator function for interaction evidence of type *k*. For example, $I_{\text{"two-hybrid HTP"}}\,p_i,p_j$ was set to 1 if $p_i$ interacted with $p_j$ in a high-



Nature Genetics: doi:10.1038/ng.337

throughput two-hybrid experiment. Each interacting protein pair $p_i,p_j$ was assigned a weight $w_{ij}$ reflecting the probability that $p_i,p_j$ function in a randomly selected response pathway (denoted $RP_{\mathrm{pi,pj}}$=1) based on their interaction evidence vector $I_{pi,pj}$. By Bayes' rule,

$w_{ij} = \mathrm{P}(RP_{p_ip_j} = 1 \mid I_{p_ip_j}) = \mathrm{P}(I_{p_ip_j} \mid RP_{p_ip_j} = 1)\mathrm{P}(RP_{p_ip_j} = 1) / \mathrm{P}(I_{p_ip_j})$, where

$\mathrm{P}(I_{p_ip_j}) = \mathrm{P}(I_{p_ip_j} \mid RP_{p_ip_j} = 1)\mathrm{P}(RP_{p_ip_j} = 1) + \mathrm{P}(I_{p_ip_j} \mid RP_{p_ip_j} = 0)\mathrm{P}(RP_{p_ip_j} = 0).$

We assumed that different types of evidence are conditionally independent, so that $\mathrm{P}(I_{p_ip_j} \mid RP_{p_ip_j}) = \prod_k \mathrm{P}(I_{kp_ip_j} \mid RP_{p_ip_j})$. To estimate the prior probability P(*RP)* and the conditional probability table associated with each evidence type $\mathrm{P}(I_k \mid RP)$ we compiled the following:

1) A set of response pathways containing 54 response-specific processes according to GO process annotations (e.g., response to osmotic stress GO:0006970).

2) A positive set containing all interacting protein pairs functioning in a common response pathway (see 1 above) based on reliable GO process annotations. To exclude less reliable sources of annotation we used only GO evidence relying on direct assay or expert knowledge (GO evidence codes IC, IDA and TAS).

3) A negative set composed of interacting protein pairs known not be in a common response pathway similar to [9].

Supplementary Table 2B lists the resulting weights associated with individual evidence types.

Some edge weights $w_{ij}$ were close to 1, which was unrealistic biologically and could instead indicate unusually well-studied proteins [10] or imperfectness of the assumption of conditional independence. To prevent such edges from dominating the predicting



Nature Genetics: doi:10.1038/ng.337

response networks, and to place all edges with high enough weights on equal footing, the weights $w_{ij}$ were capped to a maximum value of 0.7. Notably, small changes in this value (0.7±0.1) gave similar results in the subsequent analyses.

*Interactions between protein and gene nodes:* These weights were designed to reflect the interaction's reliability based on experimental evidence and conservation. "ChIP-chip interactions" refer to interactions discovered by the ChIP-chip method. "ChIP-chip motif interactions" refer to those ChIP-chip interactions for which the gene's upstream sequence contained the binding motif of the specific transcription factor. "Reliable interactions" included those ChIP-chip motif interactions for which the motif occurrence in the gene's upstream sequence was conserved in at least two other *Saccharomyces sensu stricto* species, as well as literature-curated interactions. The weight of reliable interactions was set to 0.7. The weight of remaining ""ChIP-chip motif interactions" was set to the fraction of "ChIP-chip motif interactions" that were also reliable (0.59) , and similarly the weight of remaining ChIP-chip interactions" was set to the fraction of "ChIP-chip interactions" that were also reliable (0.51).

## *The ResponseNet algorithm*

### *Directionality of ResponseNet output*

The flow algorithm we employ provides a directed network. However the directionality of the interactions in the network is determined by the fact that we have connected all genetic hits to the source of flow and all differentially expressed genes to the flow sink. Therefore, except for the interactions between transcription factors and their targets, the flow does not necessarily reflect a causal order of events (Supplementary Figure 1B).



Nature Genetics: doi:10.1038/ng.337

For example, a genetic hit might be downstream of a signaling protein; yet, since the flow algorithm directs flow away from genetic hits, the signaling protein will appear downstream of its target. The reversed direction is not a cause for concern, as we are not trying to reconstruct the direction of pathways. Rather, the goal of our algorithm is to identify pathway components (nodes, not edges) that escaped experimental detection.

### *Analysis of the space of solutions*

The optimization problem may have multiple optimal or suboptimal solutions. To characterize the space of solutions we searched for alternative optimal solutions using the method of [11]. Separately minimizing or maximizing each edge in the reported network while maintaining the same optimization score resulted in very few changes to the network. The median change in flow, the median number of nodes added and the median number of nodes lost from the resulting networks were all zero. Moreover, only 78 out of 504 edges showed a change in flow greater than $10^{-4}$.

Since our analysis of the resulting networks focuses on the nodes rather than the flow values, we also examined how many nodes changed in these alternative optimal solutions.

Changes in node number upon maximizing edge flow:

| *Number of nodes lost* | *Number of distinct solutions* |
|---|---|
| 0 | 479 |
| 1 | 23 |
| 2 | 1 |
| 3 | 1 |



Nature Genetics: doi:10.1038/ng.337

| *Number of nodes added* | *Number of distinct solutions* |
|---|---|
| 0 | 499 |
| 1 | 5 |

Changes in node number upon minimizing edge flow:

| *Number of nodes lost* | *Number of distinct solutions* |
|---|---|
| 0 | 479 |
| 1 | 25 |

| *Number of nodes added* | *Number of distinct solutions* |
|---|---|
| 0 | 500 |
| 1 | 1 |
| 2 | 3 |

These results demonstrate that, at least for the alpha synuclein network, few alternative solutions exist and that they are very similar to the reported solution.

### *Assessment of ResponseNet performance on 101 datasets*

We tested the ability of ResponseNet to identify cellular response pathways using DNA damage and Ste5 inactivation (main text). To test ResponseNet more broadly, we also evaluated its ability to identify hidden components in the cellular response to over one hundred distinct perturbations corresponding to inactivations of genes. For each such perturbation the genetic hits set consisted of the genetic interactors of the inactivated gene (e.g., synthetic lethals), and the differentially expressed genes were based on mRNA profiling of the inactivated strain [12]. The identity of the inactivated gene was hidden from the algorithm, and was used to evaluate the predicted network.



Nature Genetics: doi:10.1038/ng.337

In most of these cases, the true response pathways are poorly understood. Consequently, there is no perfect way to assess the results. Here we consider ResponseNet successful in revealing the cellular response to the perturbation if the nodes ResponseNet predicted fulfill one of two criteria: (i) they included the inactivated gene that was the source of perturbation, and the inactivated gene ranked significantly well, or (ii) they were significantly enriched for a specific biological process attributed to the inactivated gene. We define a specific biological process as a process annotation attributed to at most 1000 genes, including the inactivated gene, based on reliable sources (evidence codes IC, IDA or TAS).

Ranking and enrichment significance were determined by comparing ResponseNet solutions to solutions based on randomized input. Specifically, for each gene inactivation we created 100 randomized solutions: 50 randomized solutions were created by randomizing the genetic hits data while maintaining the differentially expressed genes, and 50 randomized solutions were created by randomizing the differentially expressed genes while maintaining the genetic hits data. In both cases the interactome was not randomized. Each randomized input set was solved using ResponseNet. For each inactivated gene we then compared the results obtained for the original genetic hits and differentially expressed genes to these 100 randomized-input solutions.

To be considered successful the ResponseNet solution for the original data had to:

1. Contain the inactivated gene with a rank that is better than its rank in at least 95% of the randomized-input solutions, or
2. show enrichment for the annotation of the inactivated gene that is



Nature Genetics: doi:10.1038/ng.337

(1) Significant relative to random selections of the same number of genes from the genome ($p < 0.01$ using Fisher's Exact Test), and

(2) More significant than in at least 95% of randomized-input solutions.

ResponseNet success rates for these stringent success criteria are given in Supplementary Note Table 1 below. In total, ResponseNet predictions were successful in 41% of the cases. This rate of success is relatively high considering that ResponseNet typically selected only 1% of the yeast proteins as relevant for the response, and that for the majority of the cases (85%) genetic hits data were rather limited (a median of 14 genetic hits) and no high-throughput genetic screening data are yet available. Despite the fact that relevant interactions might be missing from our data or have low probability compared with alternative paths, in 25% of the cases the inactivated gene was predicted inside the output network and highly ranked among this small fraction (a median rank of 9 from the top). We found that both success criteria contributed to this overall success. The first criterion, which is based on the prediction and ranking of the inactivated gene resulted in 25 successes. Considering that the inactivated gene was predicted only in 33 cases this is a high success rate of 76%. The second criterion resulted in 28 successes. Interestingly, the success rate for cases based on incomplete genetic hits data was 40%, compared to 47% for complete genetic screens, demonstrating that ResponseNet functions well even when limited genetic hits data are available.

The above randomization scheme verifies that ResponseNet success rates do not stem only from either the genetic hits or the differentially expressed genes data. These success rates therefore stress the benefits of integrating both types of data.



Nature Genetics: doi:10.1038/ng.337

**Supplementary Note Table 1: Assessment of the algorithm on 101 genetic perturbations.**

| Source of genetic hits data | Number of genetic data sets | Median % of input explained[1] | | Median size of predicted network | Success in predicting and ranking the inactivated gene | | % Successes: Inactivated gene identified or perturbed process recovered (number) |
|---|---|---|---|---|---|---|---|
| | | Genetic hits | Differentially expressed genes | | % Mutations Identified (number) | Median rank | |
| Synthetic genetic arrays (complete screen) [13,14] | 15 | 60% | 43% | 102 | 20% (3) | 21 | 47% (7) |
| Literature (incomplete data) [1] | 86 | 95% | 56% | 61 | 26% (22) | 4 | 40% (34) |
| Synthetic genetic arrays (complete screen) and literature (incomplete data) | 101 | 80% | 54% | 64 | 25% (25) | 4 | 41% (41) |

## *Setting γ value*

The choice of γ primarily determines the size of the output network. Higher γ values will identify more connections between the genetic hits and the differentially expressed genes, but these connections will be of lower probability and therefore more speculative (Supplementary Note Figure 1). For the datasets with which we worked the effective γ values ranged between 7 and 20.

To identify suitable values for γ, we recommend running ResponseNet with γ values ranging between 5 and 20. For each of the output networks compute the fraction of input,



Nature Genetics: doi:10.1038/ng.337

namely genetic hits and differentially expressed genes, that are incorporated into the network, as well as the percentage of low probability edges (weights ≤ 0.3). The best γ value is the minimal value with which a significant fraction (at least 30%) of the input is incorporated while the percentage of low probability edges remains small.

To asses the performance of the ResponseNet algorithm we set γ to 10 in order to restrict solutions to relatively high-probability sub-networks. To analyze the α-syn data we used a slightly higher value of γ because the size of α-syn input sets is bigger than the median size of the validation set. In fact, the number of predicted proteins for the α-syn data with γ = 12 is 106, which is very close to the median number of predicted proteins for the validation set which was 102 predicted proteins when γ =10.

The effect on the α-syn network of varying γ value between 10 and 19 (for γ<10 the flow value was equal to zero, resulting in no output network) is presented in Supplementary Note Figure 1A, B and C. As shown in Supplementary Note Figure 1A, higher γ values incorporate more genetic hits and differentially expressed genes into the output networks, and the number of intermediary nodes increases. For example, upon setting γ to 19, the output network connects all the genetic hits (70/70, where 70 corresponds to the number of genetic hits in the interactome) and most of the differentially expressed genes (437/441, where 441 corresponds to the number of differentially expressed genes in the interactome) via 225 intermediary proteins. These numbers are about twice the numbers obtained with γ=12. The downside is that as γ increases the percentage of high confidence interactions (weights ≥ 0.7) in the network decreases, while the percentage of low confidence interactions (weights ≤ 0.3) increases as shown in Supplementary Note Figure 1B. For example, with γ=12 only two low probability edges were included in the



Nature Genetics: doi:10.1038/ng.337

output (0.007%). By contrast, with γ=19 there are 38 low confidence edges in the output network (0.05 %). Supplementary Note Figure 1C shows that more than 90% of the network proteins reported in the paper based on γ=12 also appear in networks created upon setting γ to values >12. The selection of γ=12 for the analysis of α-syn data was therefore a good compromise between having a concise network with only 2 low confidence interactions and including a big enough subset of the genetic hits (49% [34/70]) and the differentially expressed genes (38% [166/441]).

## Genetic overexpression screen of a yeast model for α-synuclein pathobiology

To explore the nature of α-syn toxicity we conducted an unbiased genome wide screen for genes that when overexpressed modify α-syn toxicity in yeast. The first functional cluster of genes to emerge from that screen consisted of genes that affect ER-to-Golgi vesicle trafficking. One of the genes, Ypt1/Rab1, was tested in neuronal models of PD and was found to rescue dopaminergic neurons from α-syn toxicity [15]. Here we report for the first time the remaining genes identified upon screening an overexpression library of > 5000 yeast genes.

We identified a diverse group of genes including 55 suppressors and 22 enhancers of α-syn toxicity, many with clear human orthologs (Table 2). The major classes of genes that emerged include vesicle-trafficking genes, kinases and phosphatases, ubiquitin related proteins, transcriptional regulators, manganese transporters, and osmolyte biosynthesis genes. Importantly, some of these classes of activity have been associated with PD, yet



Nature Genetics: doi:10.1038/ng.337

were not causally linked to α-syn pathobiology. Below we briefly discuss the gene classes and their relevance to PD.

*Vesicle-trafficking genes*: In addition to the genes previously reported (YPT1, YKT6, ERV29, GYP8, BRE5, UBP3) we now report 10 additional vesicle-trafficking genes, making vesicle-trafficking the largest class we identified. Following the initial identification of this class we found that α-syn represses ER-to-Golgi transport[15], and inhibits fusion of budded vesicles to Golgi and other target membranes in neuronal models of PD [16]. Through these functions α-syn can influence trafficking at synapses: α-Syn knockout mice have lower pools of synaptic vesicle reserves [17], while neuronal cells overexpressing α-syn show an increase in the pool of docked, but not yet fused, secretory vesicles [18]. Together these findings illustrate the power of the yeast screen to illuminate conserved features of α-syn pathobiology as well as its normal biological function.

*Kinases and phosphatases*: Four phosphatases, including a catalytic subunit of protein phosphatase 2A (PP2A), strongly enhanced α-syn toxicity while three kinases and three additional phosphatases were potent suppressors. α-Syn directly activates PP2A in dopaminergic cells [19] and the phosphorylation status of α-syn itself has been implicated in modulating aggregation, toxicity and PD pathogenesis [20,21]. Also, a yeast casein kinase, Yck3, was identified in our screen as a suppressor of α-syn toxicity [22]. Since phosphorylation of α-syn on serine129 has been previously linked to inclusion formation in neuronal cells [21,22], we tested for phosphorylation of this residue in yeast. Immunoblotting confirms that α-syn is indeed phosphorylated in yeast cells (Supplementary Note Figure 2), indicating that the machinery to phosphorylate the



Nature Genetics: doi:10.1038/ng.337

protein at this residue has been conserved for over a billion years of evolution from yeast to human.

*Ubiquitin-related proteins*: Two ubiquitin ligases and five ubiquitin proteases are potent modifiers of α-syn toxicity. These results are consistent with previous data implicating ubiquitin-mediated protein degradation pathways in the pathogenesis of synucleinopathies, including PD. The familial PD genes PARKIN and UCH-L1 encode an E3 ubiquitin ligase and an ubiquitin protease, respectively, and α-Syn itself and other proteins are ubiquitinated in Lewy Bodies [23]. By flow cytometry we did not detect changes in steady-state α-syn protein levels in yeast cells overexpressing any of the ubiquitin-related genes (Supplementary Figure 2). Thus, in keeping with recent work in mammalian systems for PARKIN and UCH-L1 [24-26], our data suggest that these members of the ubiquitin system do no act simply by turning over α-syn.

*Transcription/translation regulators*: We identified regulators of diverse cellular processes mostly as suppressors of α-syn toxicity. These include Hap4, which regulates respiratory genes; Cup9, which regulates transition metal homeostasis; Fzf1, which regulates nitrosative stress and Mga2, which regulates fatty acid metabolism. Most of the abovementioned processes have been associated with Parkinsonism and α-syn toxicity previously, establishing that the causal relationships between α-syn toxicity and these processes is a fundamental, highly-conserved, feature of cell biology. That α-syn might be related to transition metal homeostasis was previously unknown.

*Manganese transporters*: Many reports link manganese exposure to PD and Parkinsonism [27]. Strikingly, of the tens of metal transporters we tested we recovered only



Nature Genetics: doi:10.1038/ng.337

three as α-syn modifiers, two of which are $Mn^{2+}$ transporters (Ccc1, Pmr1). Ccc1, a strong toxicity suppressor, is predicted to detoxify $Mn^{2+}$ by shunting it to the vacuole. Pmr1, a strong toxicity enhancer, is required for $Mn^{2+}$ transport into Golgi, where $Mn^{2+}$ is needed for glycosylation of secretory proteins [28].

*Trehalose biosynthesis genes*: Trehalose is an osmolyte that prevents native proteins from misfolding and denatured proteins from aggregating [29], and has been proposed as a potential therapeutic for polyglutamine diseases [30]. We found that three suppressors of α-syn toxicity are related to trehalose biosynthesis. A recent report has shown the efficacy of trehalose in promoting the clearance of misfolded mutant α-syn [31].

## Detailed analysis of cellular pathways perturbed by α-synuclein

The output of ResponseNet consisted of 15 connected components revealing several pathways that underlay the cellular response to α-syn toxicity (Supplementary Figure 3A). Below we focus on the main implicated pathways, and describe the proteins predicted by ResponseNet in the context of their connected component.

***Ubiquitin-related pathways***

The presence of the ubiquitin-related pathways is in accordance with previous evidence of the pathogenesis of Parkinson Disease (PD). Indeed, two of the familial PD genes are a ubiquitin protein ligase (PARKIN) [32] and a ubiquitin C-terminal hydrolase (UCH-L1) [33]. The algorithm identifies three connected components linked to ubiquitin-related pathways.



Nature Genetics: doi:10.1038/ng.337

1. **Connected component B.** The genetic hits Hrd1 and Cdc4 are ubiquitin-protein ligases, while Ubp7 is an ubiquitin protease. ResponseNet connected these hits to the following ubiquitin-related proteins: (i) Cdc48, an ER ATPase that participates in retrotranslocation of ubiquitinated proteins from the ER to the cytosol for degradation by the proteasome; (ii) Ubi4, the ubiquitin protein; (iii) Hse1, required for sorting of ubiquitinated proteins into vesicles prior to vacuolar degradation; (iv) Tec1, a transcription factor that is regulated by ubiquitination [34]; (v) Spt23, an ER localized transcription factor that is activated by ubiquitin/proteasome-dependent processing followed by nuclear targeting. Ubi4 was also 4-fold up-regulated in response to α-syn, suggesting it may indirectly contribute to positive feedback regulation.
2. **Connected component C.** Ubp3 and Bre5, two suppressors of α-syn toxicity, form a deubiquitination complex that co-regulates anterograde and retrograde transport between ER and Golgi apparatus [35]. ResponseNet identified their interaction, and also connected them to Sir4, suggesting that they may disrupt Sir4 silencing activity [36]. Sir4 was connected to Rap1, which was assigned as regulating the expression of 11 genes: three down- and 8 up-regulated, keeping with the known role of Rap1 as both activator and repressor.
3. **Connected component E.** The regulation of the genetic hits Mga2 and Mks1 involves ubiquitin. Mga2 is closely related to Spt23 (described above), and similarly regulates Ole1 transcription [37]. Like Spt23, Mga2 is an ER localized transcription factor that is activated by ubiquitin/proteasome-dependent processing followed by nuclear targeting [38]. ResponseNet predicted Rsp5, the ubiquitin-protein ligase that activates both Mga2 and Spt23 [39].



Nature Genetics: doi:10.1038/ng.337

The genetic enhancer Mks1 is involved in retrograde mitochondria-to-nucleus signaling. ResponseNet predicted Grr1, a component of the SCF E3 ubiquitin-ligase complex, which regulates Mks1 by its polyubiquitination and degradation [40]. Interestingly, ResponseNet also predicted Rtg2, which regulates both retrograde mitochondria-to-nucleus signaling and the TOR pathway. We validated the involvement of the TOR pathway in the response to α-syn in the main text.

The relations identified by ResponseNet demonstrate the extent at which ubiquitin-related pathways can affect diverse cellular processes.

***Vesicle trafficking pathways***

α-syn was previously shown to repress ER-to-Golgi transport [15] and to inhibit fusion of budded vesicles to Golgi and other target membranes in neuronal models of PD [16]. Below we describe the two connected components mainly related to vesicle trafficking that were identified by ResponseNet.

1. **Connected component A.** In relation with the v-SNARE protein and genetic suppressor Ykt6 ResponseNet predicted (i) Sed5, a t-SNARE required for ER-to-Golgi vesicle trafficking; (ii) Bet1, a v-SNARE required for ER-to-Golgi vesicle trafficking; (iii)Vam3, functioning in vacuolar protein trafficking; (iv) Nyv1, a v-SNARE component of the vacuolar SNARE complex involved in vesicle fusion; (v) Atg8, a protein required for autophagy that participates in multiple membrane trafficking processes [41]; (vi) Ssa3, a chaperone protein whose over-expression has been shown to be protective towards α-syn toxicity [42]. In relation with the genetic suppressor and ER-to-Golgi Ras-like GTPase Ypt1, ResponseNet predicted Bet3 that acts in targeting and fusion of ER-to-Golgi transport vesicles, and is also a component



Nature Genetics: doi:10.1038/ng.337

of part of transport protein particle (TRAPP) complex. Downstream of Bet3 ResponseNet predicted Hsp82, the molecular chaperone and Hap1, the Hsp82 client transcription factor [43]. Hap1 is responsible for heme-dependent activation of many genes, and also plays a role in sterol metabolism, which we validated to be perturbed by α-syn.

2. **Connected component C:** The suppressor Sec21 and Tif4632 were predicted by ResponseNet to target the same transcriptional response. ResponseNet predicted (i) Arf1, a RAS-like GTPase involved in regulation of coated vesicle formation in Golgi; (ii) Rvs167, an actin-associated protein involved in endocytosis; and (iii) Pab1, a poly(A)-binding protein. The interaction between Pab1 and Arf1, selected by ResponseNet, has been reported to provide an unexpected link between COPI vesicles and mRNA and to suggest that ER-Golgi shuttle might be involved in concentrating mRNA at the ER [44]. This again demonstrates the capability of ResponseNet to identify hidden important connections among genetic hits.

***Cell cycle and meiosis***

Cell cycle regulation has been suggested to play part in neuronal cell death in PD [45,46]. Many proteins predicted by ResponseNet have important functions in cell cycle processes, including the transcription factors Swi5, Swi6, Mbp1 and Swi4. In particular, ResponseNet identified connected component D and G, which almost exclusively composed of cell cycle and meiosis related proteins:

1. **Connected component D**: The genetic suppressor Cdc5 is a protein kinase that plays an important role in controlling cell-cycle-dependent gene expression during mitosis. Responsenet predicted its substrates Fkh2 and Ndd1, the cell cycle regulators which



Nature Genetics: doi:10.1038/ng.337

form the Mcm1-Fkh2-Ndd1 transcription factor complex [47], and also predicted Mcm1.

The genetic suppressor Ime2 is a serine/threonine protein kinase involved in activation of meiosis. ResponseNet predicted (i) Ime1, the master regulator of meiosis, that is activated by Ime2; (ii) Cdc6, and ATP-binding protein required for DNA replication, which Ime2 is known to stabilize [48]; (iii) Orc2, a subunit of the origin recognition complex which directs DNA replication; (iv) Orc3, another subunit of the origin recognition complex; (v) Abf1, a DNA binding protein that functions in DNA replication, and (vi) Sum1, a transcriptional repressor required for mitotic repression of sporulation-specific genes.

The genetic enhancer Matalpha1 is a transcriptional regulator involved in regulation of mating-type-specific gene expression. ResponseNet connected it to the transcription factor Mcm1, which is its main target.

The genetic suppressor Stb3 is known to interact with Sin3. ResponseNet predicted (i) Sin3, a histone deacetylase that regulates several processes, including meiosis; and (ii) Ume6, a key transcriptional regulator of early meiotic genes that also forms a complex with Ime1, also predicted by ResponseNet (see above).

2. **Connected component G**: The genetic suppressor Mum2 is essential for meiotic DNA replication, and is known to interact with Orc2 (predicted by ResponseNet). ResponseNet predicted (i) Tid3, a kinetochore associated protein involved in chromosome segregation and spindle checkpoint; (ii) Dam1, another kinetochore associated protein that aids in chromosome segregation, and (iii) Cbf1, a kintechore localized transcription factor.



Nature Genetics: doi:10.1038/ng.337

### *Analysis of cellular pathways perturbed by α-syn, excluding general stress response genes from the transcriptional data*

In an effort to exclude non-specific stress response from our predictions, we ran ResponseNet with the complete genetic data, but using only a subset of the transcriptional data from which 111 environmental stress response genes [49] were excluded. This resulted in an almost identical network. The main difference was in the pathway downstream the genetic hit Ykt6. Ykt6 is predicted to interact indirectly with Tlg2 and this interaction is absent in the reference network. Interestingly Tlg2 deletion has previously been identified as an enhancer of α-syn toxicity [50].

The list of all the predicted genes with the associated flow values and their interactors is accessible at http://fraenkel.mit.edu/ResponseNet/ResponseNet_asyn_noESR.php Alpha-synuclein (no ESR).

### *Yeast Strains and Media*

Yeast strains used include W303 with α-syn integrated into *HIS3* and *TRP1* loci (IntTox): *MATa can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 ade2-1* pRS303Gal-α-synWTYFPpRS304Gal-α-synWT-YFP; W303 with a-syn integrated into *TRP1* and *URA3* loci (HiTox): *MATa can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 ade2-1* pRS304Gal-α-synWT-GFP pRS306Gal-α-synWT-GFP; W303 with one copy of a-syn integrated into *TRP1* locus (1x a-syn): *MATa can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 ade2-1* pRS304Gal-α-synWTGFP; W303 with two copies of empty vector integrated into *TRP1* and *URA3* loci (2x vector): *MATa can1-100*



Nature Genetics: doi:10.1038/ng.337

*his3-11,15 leu2-3,112 trp1-1 ura3-1 ade2-1* pRS304Gal pRS306Gal; and W303 with YFP integrated into *HIS3* locus: *MATa can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 ade2-1* pRS303Gal-YFP. Strains were manipulated and media prepared using standard techniques.

### *Immunoblotting*

Yeast lysates were subjected to SDS/PAGE (4-12% gradient, Invitrogen) and transferred to a PVDF membrane (Invitrogen). Membranes were blocked with 5% nonfat dry milk in PBS for 1 hr at room temperature. Primary antibody incubations were performed overnight at 4°C or at room temperature for 1-2 hours. After washing with PBS, membranes were incubated with a horseradish peroxidase-conjugated secondary antibody for 1 hour at room temperature, followed by washing in PBS+0.1% Tween 20 (PBST). Proteins were detected with SuperSignal West Dura (Pierce). Phosphoglycerate kinase 1 (Pgk1) mouse monoclonal antibody was used at 1:5000. Hsp26 rabbit polyclonal antibody (gift from Dr. Johannes Buchner) was used at 1:5000. Hsp104 mouse monoclonal antibody (4B; [51]) was used at 1:5000. S-nitosocysteine rabbit polyclonal antibody (Sigma) was used at 1:10,000.



Nature Genetics: doi:10.1038/ng.337

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Nature Genetics: doi:10.1038/ng.337

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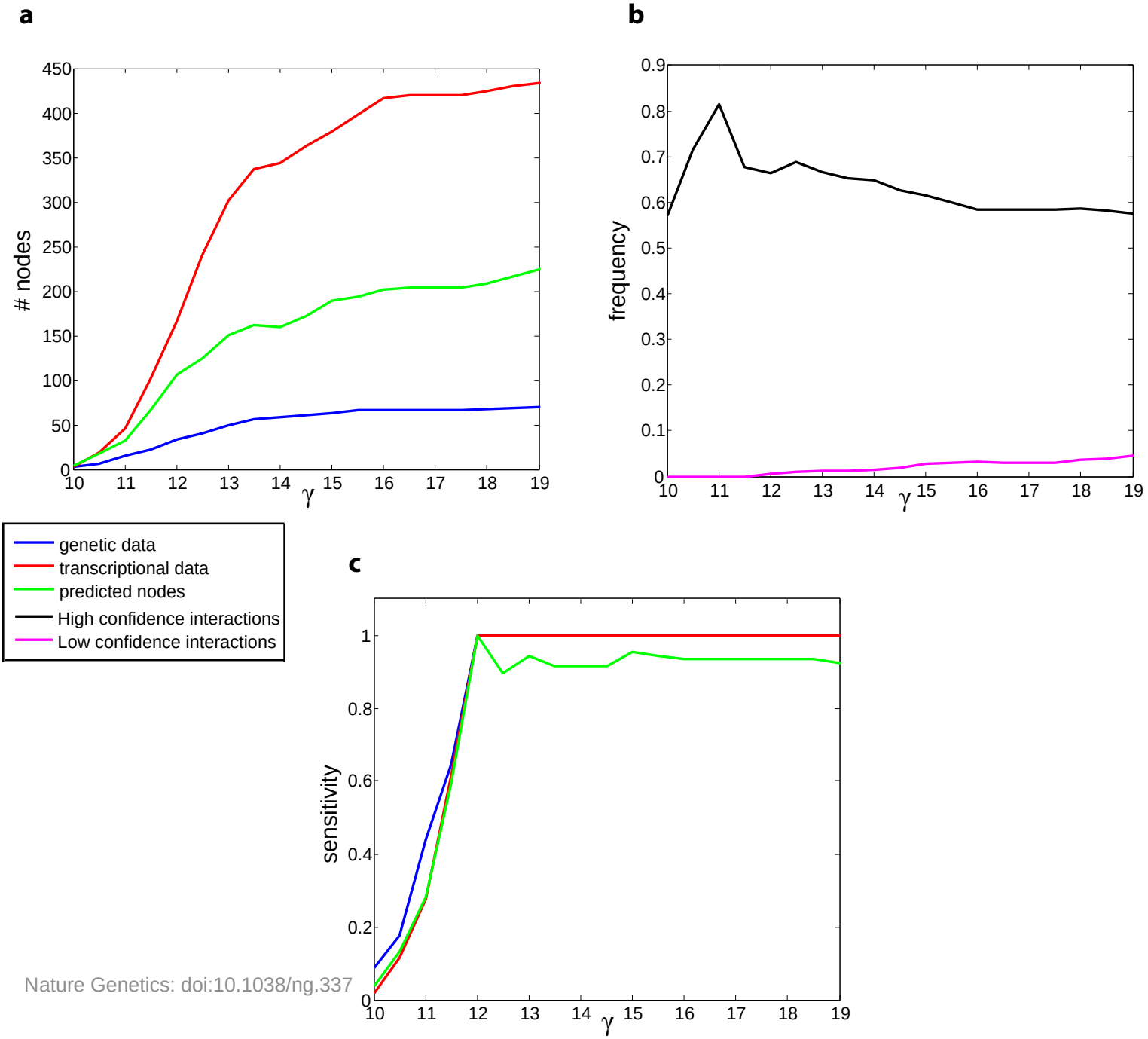
**Supplementary Note Figure 1. Characterization of the solutions obtained for the α-syn data upon varying the parameter γ.** Values for γ ranged between 10 and 19 with increments of 0.5. For each value of γ the α-syn data was solved using ResponseNet.
A. The relationship between γ and the number of genetic hits, transcriptional data and predicted nodes connected via ResponseNet. Higher γ values incorporate more genetic hits and differentially expressed genes into the output networks, and the number of intermediary nodes increases.
B. The relationship between γ and the frequency of high or low confidence interactions. As γ increases the percentage of high confidence interactions (weights ≥ 0.7) in the network decreases, while the percentage of low confidence interactions (weights ≤ 0.3) increases.
C. The relationship between γ and the sensitivity score. We used our preferred solution for α-syn network (γ=12) as a gold standard to calculate the sensitivity score. The network proteins identified with γ=12 also appear in networks created upon setting γ to values >12.

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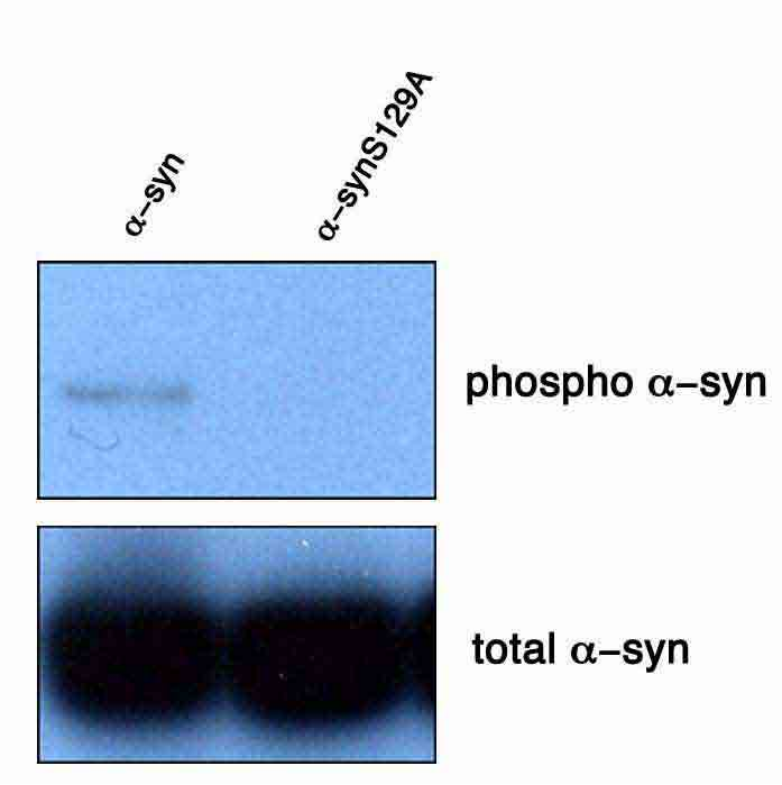
**Supplementary Note Figure 2. α-syn is phosphorylated in yeast cells**. To check if α-syn is phosphorylated on serine 129 in yeast as it is in neuronal cells, lysates of yeast cells expressing wild-type or S129A mutant α-synuclein were subjected to immunoblotting with antibodies against total α-syn or α-syn phosphorylated at serine 129.



**Supplementary Table 1A: Measured response to cellular perturbation.**

| Perturbation[a] | Transcriptional Data[b] | | | Genetic data[c] | Overlap | | | P-value[d] |
|---|---|---|---|---|---|---|---|---|
| | Up-regulated | Down-regulated | Total | | Up-regulated | Down-regulated | Total | |
| Growth arrest (HU) [1,2] | 51 | 8 | 59 | 86 | 0 | 0 | 0 | 1 |
| DNA damage (MMS) [3,4] | 152 | 46 | 198 | 1448 | 34 | 9 | 43 | 0.81 |
| ER stress (tunicamycin) [1,5] | 157 | 43 | 200 | 127 | 4 | 1 | 5 | 0.42 |
| Fatty acid metabolism (oleate) [6,7] | - | - | 269[e] | 103 | - | - | 9 | $4.1*10^{-2}$ |
| ATP synthesis block (arsenic) [8] | - | - | 828[e] | 50 | - | - | 9 | 0.25 |
| Protein biosynthesis (cycloheximide)[1,2] | 6 | 14 | 20 | 164 | 0 | 0 | 0 | 1 |
| Gene inactivation, screen complete (24 data sets [1,9-11])[f] | - | - | 27 | 130 | - | - | 0 | 1 |
| Gene inactivation, screen incomplete (149 data sets)[f] | - | - | 24 | 12 | - | - | 0 | 1 |

[a] If no citation given, the transcriptional data is taken from [1] and the genetic data from [9-11].

[b] Number of differentially expressed genes defined as those showing at least a 2-fold change in expression following the perturbation or as defined in original papers.

[c] Number of genes whose genetic manipulation affects the phenotype of perturbed cells relative to wild type.

[d] Hypergeometric p-values are calculated considering 6000 genes.

[e] Signs were absent in the published transcriptional data.

[f] Median values are shown.

**Supplementary Table 1B: GO annotation enrichment for combined perturbations.**

| Set Type | Ontology | GO_term | Cluster frequency | Background frequency | P-value |
|---|---|---|---|---|---|
| Differentially Expressed | process | carboxylic acid metabolic process | 216, 7.1% | 319, 4.4% | 8.00E-19 |
| Differentially Expressed | process | organic acid metabolic process | 216, 7.1% | 319, 4.4% | 8.00E-19 |
| Differentially Expressed | process | amino acid and derivative metabolic process | 150, 4.9% | 206, 2.8% | 4.70E-17 |
| Differentially Expressed | process | amino acid metabolic process | 138, 4.5% | 188, 2.6% | 4.81E-16 |
| Differentially Expressed | process | nitrogen compound metabolic process | 173, 5.7% | 253, 3.5% | 2.70E-15 |
| Differentially Expressed | process | amine metabolic process | 160, 5.3% | 232, 3.2% | 1.54E-14 |
| Differentially Expressed | process | amino acid biosynthetic process | 85, 2.8% | 107, 1.5% | 1.28E-12 |
| Differentially Expressed | process | amine biosynthetic process | 89, 2.9% | 116, 1.6% | 1.30E-11 |
| Differentially Expressed | process | nitrogen compound biosynthetic process | 89, 2.9% | 117, 1.6% | 3.26E-11 |
| Differentially Expressed | process | response to chemical stimulus | 241, 7.9% | 408, 5.6% | 2.78E-10 |
| Differentially Expressed | process | sulfur metabolic process | 57, 1.9% | 68, 0.9% | 1.05E-09 |
| Differentially Expressed | process | response to stimulus | 419, 13.8% | 794, 10.9% | 1.59E-08 |
| Differentially Expressed | process | glutamine family amino acid metabolic process | 38, 1.2% | 42, 0.6% | 6.07E-08 |
| Differentially Expressed | process | response to toxin | 29, 1.0% | 30, 0.4% | 2.18E-07 |
| Differentially Expressed | process | vitamin metabolic process | 67, 2.2% | 93, 1.3% | 3.13E-06 |
| Differentially Expressed | process | water-soluble vitamin metabolic process | 67, 2.2% | 93, 1.3% | 3.13E-06 |
| Differentially Expressed | function | oxidoreductase activity | 218, 7.2% | 276, 3.8% | 2.27E-35 |
| Differentially Expressed | function | oxidoreductase activity, acting on CH-OH group of donors | 68, 2.2% | 78, 1.1% | 3.92E-14 |
| Differentially Expressed | function | oxidoreductase activity, acting on the CH-OH group of donors, NAD or NADP as acceptor | 62, 2.0% | 70, 1.0% | 1.73E-13 |
| Differentially Expressed | function | transporter activity | 219, 7.2% | 379, 5.2% | 3.64E-08 |
| Differentially Expressed | function | transmembrane transporter activity | 171, 5.6% | 285, 3.9% | 9.37E-08 |
| Differentially Expressed | component | plasma membrane | 173, 5.7% | 263, 3.6% | 2.85E-13 |
| Differentially Expressed | component | fungal-type cell wall | 83, 2.7% | 105, 1.4% | 1.37E-12 |
| Differentially Expressed | component | external encapsulating structure | 89, 2.9% | 115, 1.6% | 1.45E-12 |
| Differentially Expressed | component | cell wall | 89, 2.9% | 115, 1.6% | 1.45E-12 |
| Differentially Expressed | component | cytosolic part | 129, 4.2% | 211, 2.9% | 2.12E-06 |
| Genetic Hits | process | cellular component organization and biogenesis | 1261, 45.9% | 2225, 30.5% | 7.41E-105 |
| Genetic Hits | process | cellular process | 2174, 79.1% | 4673, 64.1% | 9.82E-99 |
| Genetic Hits | process | chromosome organization and biogenesis | 456, 16.6% | 578, 7.9% | 5.04E-96 |
| Genetic Hits | process | biological regulation | 640, 23.3% | 958, 13.1% | 4.94E-83 |
| Genetic Hits | process | organelle organization and biogenesis | 849, 30.9% | 1447, 19.8% | 6.66E-71 |
| Genetic Hits | process | regulation of biological process | 525, 19.1% | 777, 10.7% | 1.28E-68 |

| | | | | | |
|---|---|---|---|---|---|
| Genetic Hits | process | regulation of cellular process | 519, 18.9% | 766, 10.5% | 2.24E-68 |
| Genetic Hits | process | response to stimulus | 512, 18.6% | 794, 10.9% | 2.78E-56 |
| Genetic Hits | process | cell cycle | 328, 11.9% | 455, 6.2% | 1.95E-50 |
| Genetic Hits | process | establishment and/or maintenance of chromatin architecture | 210, 7.6% | 252, 3.5% | 7.38E-49 |
| Genetic Hits | process | cell cycle process | 295, 10.7% | 401, 5.5% | 5.65E-48 |
| Genetic Hits | process | transcription, DNA-dependent | 361, 13.1% | 532, 7.3% | 1.26E-45 |
| Genetic Hits | process | RNA biosynthetic process | 362, 13.2% | 534, 7.3% | 1.31E-45 |
| Genetic Hits | process | transcription | 384, 14.0% | 577, 7.9% | 1.35E-45 |
| Genetic Hits | process | response to DNA damage stimulus | 196, 7.1% | 238, 3.3% | 8.59E-44 |
| Genetic Hits | process | response to endogenous stimulus | 204, 7.4% | 252, 3.5% | 2.13E-43 |
| Genetic Hits | process | telomere organization and biogenesis | 221, 8.0% | 281, 3.9% | 2.69E-43 |
| Genetic Hits | process | telomere maintenance | 221, 8.0% | 281, 3.9% | 2.69E-43 |
| Genetic Hits | process | DNA metabolic process | 419, 15.2% | 658, 9.0% | 1.29E-42 |
| Genetic Hits | process | cell cycle phase | 257, 9.4% | 348, 4.8% | 8.54E-42 |
| Genetic Hits | process | response to stress | 335, 12.2% | 497, 6.8% | 5.49E-41 |
| Genetic Hits | process | chromatin modification | 183, 6.7% | 222, 3.0% | 8.31E-41 |
| Genetic Hits | process | regulation of nucleobase, nucleoside, nucleotide and nucleic acid metabolic process | 309, 11.2% | 457, 6.3% | 6.89E-38 |
| Genetic Hits | process | regulation of cellular metabolic process | 349, 12.7% | 539, 7.4% | 5.84E-37 |
| Genetic Hits | process | regulation of metabolic process | 357, 13.0% | 557, 7.6% | 1.88E-36 |
| Genetic Hits | process | mitotic cell cycle | 206, 7.5% | 271, 3.7% | 4.56E-36 |
| Genetic Hits | process | post-translational protein modification | 269, 9.8% | 389, 5.3% | 3.32E-35 |
| Genetic Hits | process | regulation of transcription | 271, 9.9% | 398, 5.5% | 1.34E-33 |
| Genetic Hits | process | protein modification process | 333, 12.1% | 521, 7.1% | 2.46E-33 |
| Genetic Hits | process | DNA repair | 157, 5.7% | 193, 2.6% | 3.06E-33 |
| Genetic Hits | process | negative regulation of biological process | 195, 7.1% | 259, 3.6% | 5.93E-33 |
| Genetic Hits | process | negative regulation of cellular process | 194, 7.1% | 258, 3.5% | 1.23E-32 |
| Genetic Hits | process | regulation of transcription, DNA-dependent | 254, 9.2% | 369, 5.1% | 1.62E-32 |
| Genetic Hits | process | regulation of RNA metabolic process | 268, 9.8% | 396, 5.4% | 1.83E-32 |
| Genetic Hits | process | transcription from RNA polymerase II promoter | 246, 9.0% | 354, 4.9% | 1.85E-32 |
| Genetic Hits | process | signal transduction | 174, 6.3% | 225, 3.1% | 1.00E-31 |
| Genetic Hits | process | M phase | 187, 6.8% | 250, 3.4% | 7.93E-31 |
| Genetic Hits | process | cell communication | 188, 6.8% | 252, 3.5% | 9.67E-31 |
| Genetic Hits | process | regulation of gene expression | 289, 10.5% | 444, 6.1% | 1.59E-30 |
| Genetic Hits | process | cellular localization | 387, 14.1% | 651, 8.9% | 3.63E-29 |
| Genetic Hits | process | localization | 573, 20.9% | 1060, 14.5% | 6.44E-29 |
| Genetic Hits | process | establishment of cellular localization | 365, 13.3% | 610, 8.4% | 4.50E-28 |

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|---|---|---|---|---|---|
| Genetic Hits | process | vesicle-mediated transport | 225, 8.2% | 331, 4.5% | 3.50E-27 |
| Genetic Hits | process | establishment of localization | 544, 19.8% | 1010, 13.9% | 1.40E-26 |
| Genetic Hits | process | regulation of biological quality | 204, 7.4% | 294, 4.0% | 2.87E-26 |
| Genetic Hits | process | chromatin remodeling | 125, 4.5% | 154, 2.1% | 8.43E-26 |
| Genetic Hits | process | negative regulation of metabolic process | 159, 5.8% | 213, 2.9% | 1.17E-25 |
| Genetic Hits | process | cytoskeleton organization and biogenesis | 166, 6.0% | 226, 3.1% | 1.73E-25 |
| Genetic Hits | process | nucleobase, nucleoside, nucleotide and nucleic acid metabolic process | 842, 30.6% | 1717, 23.5% | 2.04E-25 |
| Genetic Hits | process | negative regulation of cellular metabolic process | 158, 5.7% | 212, 2.9% | 2.41E-25 |
| Genetic Hits | process | regulation of cell cycle | 131, 4.8% | 165, 2.3% | 2.48E-25 |
| Genetic Hits | process | negative regulation of nucleobase, nucleoside, nucleotide and nucleic acid metabolic process | 140, 5.1% | 181, 2.5% | 4.62E-25 |
| Genetic Hits | process | growth | 117, 4.3% | 143, 2.0% | 1.29E-24 |
| Genetic Hits | process | transport | 529, 19.3% | 990, 13.6% | 1.57E-24 |
| Genetic Hits | process | primary metabolic process | 1498, 54.5% | 3388, 46.5% | 2.51E-24 |
| Genetic Hits | process | intracellular signaling cascade | 123, 4.5% | 155, 2.1% | 1.30E-23 |
| Genetic Hits | process | metabolic process | 1594, 58.0% | 3654, 50.1% | 2.37E-23 |
| Genetic Hits | process | intracellular transport | 324, 11.8% | 551, 7.6% | 1.36E-22 |
| Genetic Hits | process | cellular metabolic process | 1548, 56.3% | 3544, 48.6% | 2.61E-22 |
| Genetic Hits | process | developmental process | 230, 8.4% | 359, 4.9% | 3.35E-22 |
| Genetic Hits | process | chromatin assembly or disassembly | 99, 3.6% | 118, 1.6% | 3.53E-22 |
| Genetic Hits | process | regulation of transcription from RNA polymerase II promoter | 163, 5.9% | 231, 3.2% | 9.31E-22 |
| Genetic Hits | process | reproduction | 213, 7.8% | 328, 4.5% | 1.69E-21 |
| Genetic Hits | process | negative regulation of transcription | 124, 4.5% | 162, 2.2% | 3.31E-21 |
| Genetic Hits | process | regulation of cell size | 96, 3.5% | 115, 1.6% | 4.15E-21 |
| Genetic Hits | process | biopolymer metabolic process | 1101, 40.1% | 2407, 33.0% | 1.58E-20 |
| Genetic Hits | process | negative regulation of transcription, DNA-dependent | 118, 4.3% | 154, 2.1% | 3.80E-20 |
| Genetic Hits | process | negative regulation of RNA metabolic process | 118, 4.3% | 155, 2.1% | 1.01E-19 |
| Genetic Hits | process | macromolecule metabolic process | 1319, 48.0% | 2996, 41.1% | 4.90E-18 |
| Genetic Hits | process | secretory pathway | 161, 5.9% | 240, 3.3% | 8.17E-18 |
| Genetic Hits | process | chromatin assembly | 85, 3.1% | 103, 1.4% | 8.62E-18 |
| Genetic Hits | process | secretion by cell | 163, 5.9% | 246, 3.4% | 3.21E-17 |
| Genetic Hits | process | secretion | 163, 5.9% | 246, 3.4% | 3.21E-17 |
| Genetic Hits | process | cellular structure morphogenesis | 111, 4.0% | 149, 2.0% | 3.96E-17 |
| Genetic Hits | process | anatomical structure development | 111, 4.0% | 149, 2.0% | 3.96E-17 |
| Genetic Hits | process | cell morphogenesis | 111, 4.0% | 149, 2.0% | 3.96E-17 |
| Genetic Hits | process | anatomical structure morphogenesis | 111, 4.0% | 149, 2.0% | 3.96E-17 |
| Genetic Hits | process | response to chemical stimulus | 242, 8.8% | 408, 5.6% | 8.36E-17 |

| | | | | | |
|---|---|---|---|---|---|
| Genetic Hits | process | mitosis | 99, 3.6% | 129, 1.8% | 1.17E-16 |
| Genetic Hits | process | DNA packaging | 89, 3.2% | 112, 1.5% | 1.39E-16 |
| Genetic Hits | process | M phase of mitotic cell cycle | 100, 3.6% | 131, 1.8% | 1.50E-16 |
| Genetic Hits | process | gene silencing | 81, 2.9% | 99, 1.4% | 2.12E-16 |
| Genetic Hits | process | covalent chromatin modification | 76, 2.8% | 91, 1.2% | 2.61E-16 |
| Genetic Hits | process | histone modification | 76, 2.8% | 91, 1.2% | 2.61E-16 |
| Genetic Hits | process | biopolymer modification | 355, 12.9% | 656, 9.0% | 2.74E-16 |
| Genetic Hits | process | cell growth | 72, 2.6% | 85, 1.2% | 4.66E-16 |
| Genetic Hits | process | establishment and/or maintenance of cell polarity | 93, 3.4% | 120, 1.6% | 4.82E-16 |
| Genetic Hits | process | DNA replication | 102, 3.7% | 137, 1.9% | 1.52E-15 |
| Genetic Hits | process | meiotic cell cycle | 103, 3.7% | 139, 1.9% | 1.84E-15 |
| Genetic Hits | process | M phase of meiotic cell cycle | 103, 3.7% | 139, 1.9% | 1.84E-15 |
| Genetic Hits | process | meiosis | 103, 3.7% | 139, 1.9% | 1.84E-15 |
| Genetic Hits | process | filamentous growth | 78, 2.8% | 97, 1.3% | 7.40E-15 |
| Genetic Hits | process | heterochromatin formation | 76, 2.8% | 94, 1.3% | 1.10E-14 |
| Genetic Hits | process | negative regulation of gene expression, epigenetic | 76, 2.8% | 94, 1.3% | 1.10E-14 |
| Genetic Hits | process | chromatin silencing | 76, 2.8% | 94, 1.3% | 1.10E-14 |
| Genetic Hits | process | regulation of gene expression, epigenetic | 78, 2.8% | 99, 1.4% | 6.90E-14 |
| Genetic Hits | process | asexual reproduction | 69, 2.5% | 84, 1.2% | 7.43E-14 |
| Genetic Hits | process | cell budding | 69, 2.5% | 84, 1.2% | 7.43E-14 |
| Genetic Hits | process | establishment of cell polarity | 83, 3.0% | 108, 1.5% | 1.03E-13 |
| Genetic Hits | process | Golgi vesicle transport | 115, 4.2% | 168, 2.3% | 3.94E-13 |
| Genetic Hits | process | RNA elongation | 53, 1.9% | 61, 0.8% | 2.76E-12 |
| Genetic Hits | process | small GTPase mediated signal transduction | 53, 1.9% | 61, 0.8% | 2.76E-12 |
| Genetic Hits | process | regulation of mitosis | 51, 1.9% | 58, 0.8% | 3.22E-12 |
| Genetic Hits | process | cell cycle checkpoint | 48, 1.7% | 54, 0.7% | 8.47E-12 |
| Genetic Hits | process | actin filament-based process | 82, 3.0% | 111, 1.5% | 8.85E-12 |
| Genetic Hits | process | microtubule-based process | 78, 2.8% | 104, 1.4% | 9.95E-12 |
| Genetic Hits | process | cell division | 156, 5.7% | 255, 3.5% | 1.19E-11 |
| Genetic Hits | process | protein amino acid phosphorylation | 76, 2.8% | 101, 1.4% | 1.63E-11 |
| Genetic Hits | process | interphase | 84, 3.1% | 116, 1.6% | 2.86E-11 |
| Genetic Hits | process | multi-organism process | 93, 3.4% | 133, 1.8% | 3.91E-11 |
| Genetic Hits | process | interphase of mitotic cell cycle | 83, 3.0% | 115, 1.6% | 5.62E-11 |
| Genetic Hits | process | actin cytoskeleton organization and biogenesis | 78, 2.8% | 106, 1.5% | 5.91E-11 |
| Genetic Hits | process | reproduction of a single-celled organism | 126, 4.6% | 199, 2.7% | 1.38E-10 |
| Genetic Hits | process | cellular lipid metabolic process | 138, 5.0% | 224, 3.1% | 2.07E-10 |
| Genetic Hits | process | chromosome segregation | 84, 3.1% | 119, 1.6% | 3.07E-10 |

| | | | | | |
|---|---|---|---|---|---|
| Genetic Hits | process | pseudohyphal growth | 52, 1.9% | 63, 0.9% | 3.99E-10 |
| Genetic Hits | process | RNA elongation from RNA polymerase II promoter | 47, 1.7% | 55, 0.8% | 4.34E-10 |
| Genetic Hits | process | biopolymer catabolic process | 166, 6.0% | 284, 3.9% | 5.03E-10 |
| Genetic Hits | process | DNA-dependent DNA replication | 76, 2.8% | 105, 1.4% | 5.36E-10 |
| Genetic Hits | process | sexual reproduction | 83, 3.0% | 118, 1.6% | 5.87E-10 |
| Genetic Hits | process | conjugation | 83, 3.0% | 118, 1.6% | 5.87E-10 |
| Genetic Hits | process | conjugation with cellular fusion | 83, 3.0% | 118, 1.6% | 5.87E-10 |
| Genetic Hits | process | membrane organization and biogenesis | 121, 4.4% | 192, 2.6% | 7.09E-10 |
| Genetic Hits | process | lipid metabolic process | 143, 5.2% | 237, 3.3% | 7.83E-10 |
| Genetic Hits | process | double-strand break repair | 48, 1.7% | 58, 0.8% | 2.79E-09 |
| Genetic Hits | process | macromolecule localization | 204, 7.4% | 371, 5.1% | 3.77E-09 |
| Genetic Hits | process | vacuolar transport | 82, 3.0% | 119, 1.6% | 4.75E-09 |
| Genetic Hits | process | protein amino acid deacetylation | 26, 0.9% | 26, 0.4% | 1.30E-08 |
| Genetic Hits | process | response to abiotic stimulus | 81, 2.9% | 119, 1.6% | 1.76E-08 |
| Genetic Hits | process | telomeric heterochromatin formation | 47, 1.7% | 58, 0.8% | 2.09E-08 |
| Genetic Hits | process | chromatin silencing at telomere | 47, 1.7% | 58, 0.8% | 2.09E-08 |
| Genetic Hits | process | microtubule cytoskeleton organization and biogenesis | 60, 2.2% | 81, 1.1% | 3.41E-08 |
| Genetic Hits | process | sister chromatid segregation | 49, 1.8% | 62, 0.9% | 4.25E-08 |
| Genetic Hits | process | response to drug | 85, 3.1% | 129, 1.8% | 7.77E-08 |
| Genetic Hits | process | actin filament organization | 48, 1.7% | 61, 0.8% | 9.06E-08 |
| Genetic Hits | process | histone deacetylation | 24, 0.9% | 24, 0.3% | 9.35E-08 |
| Genetic Hits | process | non-recombinational repair | 29, 1.1% | 31, 0.4% | 1.29E-07 |
| Genetic Hits | process | response to pheromone | 66, 2.4% | 94, 1.3% | 1.77E-07 |
| Genetic Hits | process | invasive growth in response to glucose limitation | 38, 1.4% | 45, 0.6% | 1.86E-07 |
| Genetic Hits | process | nucleotide-excision repair | 38, 1.4% | 45, 0.6% | 1.86E-07 |
| Genetic Hits | process | vacuole organization and biogenesis | 51, 1.9% | 67, 0.9% | 2.04E-07 |
| Genetic Hits | process | RNA metabolic process | 496, 18.0% | 1069, 14.7% | 2.07E-07 |
| Genetic Hits | process | Ras protein signal transduction | 34, 1.2% | 39, 0.5% | 3.02E-07 |
| Genetic Hits | process | cellular component assembly | 244, 8.9% | 475, 6.5% | 3.13E-07 |
| Genetic Hits | process | reproductive process | 113, 4.1% | 188, 2.6% | 3.23E-07 |
| Genetic Hits | process | mitotic cell cycle checkpoint | 28, 1.0% | 30, 0.4% | 3.25E-07 |
| Genetic Hits | process | meiosis I | 54, 2.0% | 73, 1.0% | 3.79E-07 |
| Genetic Hits | process | mitotic sister chromatid segregation | 46, 1.7% | 59, 0.8% | 4.04E-07 |
| Genetic Hits | process | lipid biosynthetic process | 83, 3.0% | 128, 1.8% | 4.44E-07 |
| Genetic Hits | process | cell surface receptor linked signal transduction | 43, 1.6% | 54, 0.7% | 4.60E-07 |
| Genetic Hits | process | phosphorylation | 96, 3.5% | 154, 2.1% | 4.65E-07 |
| Genetic Hits | process | post-Golgi vesicle-mediated transport | 52, 1.9% | 70, 1.0% | 6.32E-07 |

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|---|---|---|---|---|---|
| Genetic Hits | process | cellular homeostasis | 83, 3.0% | 129, 1.8% | 7.92E-07 |
| Genetic Hits | process | G2/M transition of mitotic cell cycle | 31, 1.1% | 35, 0.5% | 8.31E-07 |
| Genetic Hits | process | cytokinesis | 75, 2.7% | 114, 1.6% | 1.19E-06 |
| Genetic Hits | process | modification-dependent macromolecule catabolic process | 96, 3.5% | 156, 2.1% | 1.28E-06 |
| Genetic Hits | process | phosphorus metabolic process | 122, 4.4% | 210, 2.9% | 1.30E-06 |
| Genetic Hits | process | phosphate metabolic process | 122, 4.4% | 210, 2.9% | 1.30E-06 |
| Genetic Hits | process | endosome transport | 40, 1.5% | 50, 0.7% | 1.51E-06 |
| Genetic Hits | process | spindle organization and biogenesis | 38, 1.4% | 47, 0.6% | 2.27E-06 |
| Genetic Hits | process | homeostatic process | 84, 3.1% | 133, 1.8% | 2.41E-06 |
| Genetic Hits | process | protein localization | 170, 6.2% | 316, 4.3% | 2.68E-06 |
| Genetic Hits | process | protein targeting to vacuole | 52, 1.9% | 72, 1.0% | 3.44E-06 |
| Genetic Hits | process | negative regulation of transcription from RNA polymerase II promoter | 47, 1.7% | 63, 0.9% | 3.50E-06 |
| Genetic Hits | process | protein modification by small protein conjugation | 59, 2.1% | 85, 1.2% | 3.53E-06 |
| Genetic Hits | process | cellular protein catabolic process | 97, 3.5% | 161, 2.2% | 5.27E-06 |
| Genetic Hits | process | modification-dependent protein catabolic process | 91, 3.3% | 149, 2.0% | 6.15E-06 |
| Genetic Hits | process | ubiquitin-dependent protein catabolic process | 91, 3.3% | 149, 2.0% | 6.15E-06 |
| Genetic Hits | process | protein catabolic process | 103, 3.7% | 174, 2.4% | 6.76E-06 |
| Genetic Hits | process | protein targeting | 134, 4.9% | 240, 3.3% | 6.94E-06 |
| Genetic Hits | process | endocytosis | 59, 2.1% | 86, 1.2% | 7.15E-06 |
| Genetic Hits | process | membrane invagination | 64, 2.3% | 96, 1.3% | 9.55E-06 |
| Genetic Hits | process | proteolysis | 105, 3.8% | 179, 2.5% | 9.62E-06 |
| Genetic Hits | process | proteolysis involved in cellular protein catabolic process | 92, 3.3% | 152, 2.1% | 9.67E-06 |
| Genetic Hits | function | transcription regulator activity | 207, 7.5% | 329, 4.5% | 1.19E-18 |
| Genetic Hits | function | DNA binding | 161, 5.9% | 239, 3.3% | 1.84E-18 |
| Genetic Hits | function | nucleoside-triphosphatase activity | 163, 5.9% | 254, 3.5% | 1.71E-15 |
| Genetic Hits | function | pyrophosphatase activity | 171, 6.2% | 274, 3.8% | 1.49E-14 |
| Genetic Hits | function | hydrolase activity, acting on acid anhydrides | 171, 6.2% | 274, 3.8% | 1.49E-14 |
| Genetic Hits | function | hydrolase activity, acting on acid anhydrides, in phosphorus-containing anhydrides | 171, 6.2% | 274, 3.8% | 1.49E-14 |
| Genetic Hits | function | protein binding | 316, 11.5% | 594, 8.1% | 4.14E-13 |
| Genetic Hits | function | enzyme regulator activity | 120, 4.4% | 188, 2.6% | 1.03E-10 |
| Genetic Hits | function | transferase activity | 355, 12.9% | 703, 9.6% | 1.22E-10 |
| Genetic Hits | function | ATPase activity | 123, 4.5% | 195, 2.7% | 1.79E-10 |
| Genetic Hits | function | protein kinase activity | 88, 3.2% | 129, 1.8% | 8.00E-10 |
| Genetic Hits | function | kinase activity | 124, 4.5% | 202, 2.8% | 2.33E-09 |
| Genetic Hits | function | phosphotransferase activity, alcohol group as acceptor | 108, 3.9% | 171, 2.3% | 4.68E-09 |
| Genetic Hits | function | protein deacetylase activity | 28, 1.0% | 29, 0.4% | 1.46E-08 |

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|---|---|---|---|---|---|
| Genetic Hits | function | histone deacetylase activity | 28, 1.0% | 29, 0.4% | 1.46E-08 |
| Genetic Hits | function | ATPase activity, coupled | 89, 3.2% | 136, 1.9% | 2.06E-08 |
| Genetic Hits | function | hydrolase activity | 384, 14.0% | 800, 11.0% | 9.57E-08 |
| Genetic Hits | function | small conjugating protein ligase activity | 50, 1.8% | 67, 0.9% | 4.54E-07 |
| Genetic Hits | function | protein serine/threonine kinase activity | 56, 2.0% | 78, 1.1% | 5.16E-07 |
| Genetic Hits | function | enzyme activator activity | 48, 1.7% | 64, 0.9% | 7.50E-07 |
| Genetic Hits | function | acid-amino acid ligase activity | 54, 2.0% | 75, 1.0% | 8.77E-07 |
| Genetic Hits | function | RNA polymerase II transcription factor activity | 81, 2.9% | 127, 1.7% | 1.03E-06 |
| Genetic Hits | function | deacetylase activity | 29, 1.1% | 33, 0.5% | 2.01E-06 |
| Genetic Hits | function | cytoskeletal protein binding | 42, 1.5% | 55, 0.8% | 3.29E-06 |
| Genetic Hits | function | sequence-specific DNA binding | 51, 1.9% | 72, 1.0% | 6.32E-06 |
| Genetic Hits | function | ubiquitin-protein ligase activity | 46, 1.7% | 63, 0.9% | 7.19E-06 |
| Genetic Hits | function | small protein conjugating enzyme activity | 48, 1.7% | 67, 0.9% | 9.56E-06 |
| Genetic Hits | component | cell part | 2438, 88.7% | 5505, 75.5% | 4.23E-100 |
| Genetic Hits | component | cell | 2438, 88.7% | 5506, 75.5% | 6.30E-100 |
| Genetic Hits | component | intracellular organelle | 1930, 70.2% | 4033, 55.3% | 7.42E-89 |
| Genetic Hits | component | organelle | 1930, 70.2% | 4034, 55.3% | 1.06E-88 |
| Genetic Hits | component | intracellular | 2280, 83.0% | 5098, 69.9% | 5.41E-83 |
| Genetic Hits | component | intracellular part | 2262, 82.3% | 5065, 69.5% | 1.63E-79 |
| Genetic Hits | component | membrane-bound organelle | 1777, 64.7% | 3694, 50.7% | 1.71E-76 |
| Genetic Hits | component | intracellular membrane-bound organelle | 1777, 64.7% | 3694, 50.7% | 1.71E-76 |
| Genetic Hits | component | protein complex | 746, 27.1% | 1230, 16.9% | 9.12E-70 |
| Genetic Hits | component | organelle part | 1175, 42.8% | 2324, 31.9% | 1.60E-51 |
| Genetic Hits | component | intracellular organelle part | 1175, 42.8% | 2324, 31.9% | 1.60E-51 |
| Genetic Hits | component | nucleoplasm part | 230, 8.4% | 315, 4.3% | 3.79E-36 |
| Genetic Hits | component | nucleoplasm | 240, 8.7% | 337, 4.6% | 7.55E-35 |
| Genetic Hits | component | macromolecular complex | 875, 31.8% | 1724, 23.6% | 1.23E-34 |
| Genetic Hits | component | nucleus | 962, 35.0% | 2007, 27.5% | 3.89E-26 |
| Genetic Hits | component | membrane | 586, 21.3% | 1113, 15.3% | 5.62E-26 |
| Genetic Hits | component | membrane part | 365, 13.3% | 640, 8.8% | 7.79E-23 |
| Genetic Hits | component | organelle membrane | 358, 13.0% | 643, 8.8% | 9.14E-20 |
| Genetic Hits | component | endoplasmic reticulum | 221, 8.0% | 354, 4.9% | 1.57E-19 |
| Genetic Hits | component | chromatin remodeling complex | 70, 2.5% | 79, 1.1% | 1.99E-18 |
| Genetic Hits | component | chromosome | 162, 5.9% | 244, 3.3% | 1.05E-17 |
| Genetic Hits | component | cytoplasm | 1591, 57.9% | 3726, 51.1% | 1.62E-17 |
| Genetic Hits | component | cytoskeletal part | 131, 4.8% | 188, 2.6% | 9.39E-17 |
| Genetic Hits | component | chromosomal part | 142, 5.2% | 211, 2.9% | 4.08E-16 |

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|---|---|---|---|---|---|
| Genetic Hits | component | site of polarized growth | 112, 4.1% | 156, 2.1% | 1.12E-15 |
| Genetic Hits | component | cytoskeleton | 136, 4.9% | 202, 2.8% | 2.17E-15 |
| Genetic Hits | component | transcription factor complex | 97, 3.5% | 131, 1.8% | 7.12E-15 |
| Genetic Hits | component | endomembrane system | 199, 7.2% | 331, 4.5% | 9.59E-15 |
| Genetic Hits | component | Golgi apparatus | 138, 5.0% | 210, 2.9% | 3.00E-14 |
| Genetic Hits | component | nuclear envelope-endoplasmic reticulum network | 101, 3.7% | 141, 1.9% | 7.13E-14 |
| Genetic Hits | component | endoplasmic reticulum part | 103, 3.7% | 145, 2.0% | 9.16E-14 |
| Genetic Hits | component | Golgi apparatus part | 112, 4.1% | 163, 2.2% | 2.17E-13 |
| Genetic Hits | component | cellular bud | 106, 3.9% | 154, 2.1% | 1.12E-12 |
| Genetic Hits | component | nuclear chromosome | 123, 4.5% | 187, 2.6% | 1.41E-12 |
| Genetic Hits | component | endoplasmic reticulum membrane | 92, 3.3% | 130, 1.8% | 5.65E-12 |
| Genetic Hits | component | nuclear chromosome part | 103, 3.7% | 155, 2.1% | 1.01E-10 |
| Genetic Hits | component | cytoplasmic part | 1179, 42.9% | 2748, 37.7% | 1.62E-10 |
| Genetic Hits | component | cellular bud neck | 82, 3.0% | 119, 1.6% | 1.62E-09 |
| Genetic Hits | component | nuclear part | 527, 19.2% | 1129, 15.5% | 4.39E-09 |
| Genetic Hits | component | histone deacetylase complex | 28, 1.0% | 29, 0.4% | 1.15E-08 |
| Genetic Hits | component | microtubule cytoskeleton | 70, 2.5% | 100, 1.4% | 2.05E-08 |
| Genetic Hits | component | cell cortex | 72, 2.6% | 104, 1.4% | 2.46E-08 |
| Genetic Hits | component | organelle lumen | 398, 14.5% | 829, 11.4% | 3.07E-08 |
| Genetic Hits | component | endosome | 67, 2.4% | 95, 1.3% | 3.17E-08 |
| Genetic Hits | component | cell cortex part | 63, 2.3% | 89, 1.2% | 9.29E-08 |
| Genetic Hits | component | incipient cellular bud site | 35, 1.3% | 41, 0.6% | 1.91E-07 |
| Genetic Hits | component | nuclear chromatin | 38, 1.4% | 46, 0.6% | 2.32E-07 |
| Genetic Hits | component | nuclear lumen | 302, 11.0% | 613, 8.4% | 2.91E-07 |
| Genetic Hits | component | spindle | 61, 2.2% | 87, 1.2% | 3.37E-07 |
| Genetic Hits | component | chromatin | 44, 1.6% | 58, 0.8% | 1.59E-06 |
| Genetic Hits | component | histone acetyltransferase complex | 34, 1.2% | 41, 0.6% | 1.63E-06 |
| Genetic Hits | component | endosomal part | 26, 0.9% | 29, 0.4% | 4.28E-06 |
| Genetic Hits | component | microtubule organizing center | 46, 1.7% | 63, 0.9% | 5.67E-06 |
| Genetic Hits | component | spindle pole body | 46, 1.7% | 63, 0.9% | 5.67E-06 |

**Supplementary Table 1C: GO process and function annotations enriched in >= 20% of the sets of genetic hits or >= 20% of the sets of differentially expressed genes for the perturbations with complete genetic screens available.**

| GO annotation | Set type | # of enriched sets | Median enrichment |
|---|---|---|---|
| Amine biosynthetic process | Differentially expressed | 8 | 2.04E-08 |
| Arginine metabolic process | Differentially expressed | 8 | 0.000182 |
| Oxidoreductase activity | Differentially expressed | 8 | 2.09E-05 |
| Arginine biosynthetic process | Differentially expressed | 7 | 9.87E-06 |
| Glutamine family amino acid biosynthetic process | Differentially expressed | 7 | 0.000881 |
| Organic acid metabolic process | Differentially expressed | 7 | 1.38E-06 |
| Structural constituent of cell wall | Differentially expressed | 6 | 6.95E-05 |
| Sulfur compound biosynthetic process | Differentially expressed | 6 | 7.64E-05 |
| Sulfur metabolic process | Differentially expressed | 6 | 2.27E-07 |
| Vitamin biosynthetic process | Differentially expressed | 6 | 2.39E-05 |
| Biological regulation | Genetic Hits | 23 | 1.59E-11 |
| Response to stimulus | Genetic Hits | 23 | 1.73E-09 |
| Regulation of cellular process | Genetic Hits | 22 | 2.69E-10 |
| Response to stress | Genetic Hits | 21 | 7.14E-10 |
| Cell cycle | Genetic Hits | 20 | 2.39E-11 |
| Cell cycle phase | Genetic Hits | 20 | 2.84E-12 |
| Developmental process | Genetic Hits | 20 | 4.85E-07 |
| Mitotic cell cycle | Genetic Hits | 20 | 1.26E-09 |
| M phase | Genetic Hits | 19 | 1.88E-13 |
| Mitosis | Genetic Hits | 19 | 2.26E-08 |
| Regulation of cell cycle | Genetic Hits | 19 | 2.61E-07 |
| DNA metabolic process | Genetic Hits | 18 | 2.31E-27 |
| Sister chromatid segregation | Genetic Hits | 18 | 8.71E-07 |
| Telomere maintenance | Genetic Hits | 18 | 7.59E-14 |
| Chromosome segregation | Genetic Hits | 17 | 3.95E-08 |
| DNA packaging | Genetic Hits | 17 | 1.02E-14 |
| DNA repair | Genetic Hits | 17 | 4.71E-13 |
| Protein binding | Genetic Hits | 17 | 3.31E-07 |
| response to DNA damage stimulus | Genetic Hits | 17 | 1.08E-16 |
| Chromatin remodeling | Genetic Hits | 16 | 3.94E-11 |
| DNA recombination | Genetic Hits | 16 | 3.49E-09 |
| Double-strand break repair | Genetic Hits | 16 | 1.83E-09 |
| Non-recombinational repair | Genetic Hits | 16 | 5.72E-10 |
| Post-translational protein modification | Genetic Hits | 16 | 1.63E-08 |
| Regulation of metabolic process | Genetic Hits | 16 | 3.77E-11 |
| Transcription | Genetic Hits | 16 | 1.75E-09 |
| Cell cycle checkpoint | Genetic Hits | 15 | 2.04E-07 |
| DNA binding | Genetic Hits | 15 | 1.69E-06 |

| | | | |
|---|---|---|---|
| Gene silencing | Genetic Hits | 15 | 1.93E-07 |
| Mitotic sister chromatid cohesion | Genetic Hits | 15 | 1.50E-08 |
| Negative regulation of cellular process | Genetic Hits | 15 | 6.70E-10 |
| Chromatin assembly or disassembly | Genetic Hits | 14 | 8.70E-07 |
| Double-strand break repair via nonhomologous end joining | Genetic Hits | 14 | 1.43E-05 |
| Meiosis | Genetic Hits | 14 | 2.31E-08 |
| Negative regulation of transcription | Genetic Hits | 14 | 3.61E-08 |
| Recombinational repair | Genetic Hits | 14 | 1.38E-06 |
| Regulation of transcription | Genetic Hits | 14 | 4.40E-09 |
| Sister chromatid cohesion | Genetic Hits | 14 | 4.95E-08 |
| Cytoskeletal protein binding | Genetic Hits | 13 | 3.04E-08 |
| DNA replication | Genetic Hits | 13 | 3.02E-10 |
| Histone modification | Genetic Hits | 13 | 1.32E-09 |
| Organelle localization | Genetic Hits | 13 | 2.07E-06 |
| regulation of DNA recombination | Genetic Hits | 13 | 2.76E-06 |
| RNA elongation | Genetic Hits | 13 | 7.94E-07 |
| RNA metabolic process | Genetic Hits | 13 | 1.08E-07 |
| Chromatin silencing at silent mating-type cassette | Genetic Hits | 12 | 1.86E-05 |
| DNA-dependent ATPase activity | Genetic Hits | 12 | 1.71E-06 |
| Double-strand break repair via single-strand annealing | Genetic Hits | 12 | 4.11E-08 |
| Double-strand break repair via synthesis-dependent strand an | Genetic Hits | 12 | 1.04E-06 |
| negative regulation of DNA metabolic process | Genetic Hits | 12 | 1.11E-08 |
| negative regulation of DNA recombination | Genetic Hits | 12 | 3.18E-05 |
| regulation of DNA metabolic process | Genetic Hits | 12 | 4.05E-08 |
| transcription from RNA polymerase II promoter | Genetic Hits | 12 | 3.40E-06 |
| Transposition | Genetic Hits | 12 | 3.18E-05 |
| Cell development | Genetic Hits | 11 | 6.61E-05 |
| Chromatin silencing at telomere | Genetic Hits | 11 | 2.10E-06 |
| DNA-dependent DNA replication | Genetic Hits | 11 | 6.28E-09 |
| Histone deacetylation | Genetic Hits | 11 | 1.93E-05 |
| meiosis I | Genetic Hits | 11 | 1.07E-06 |
| Regulation of biological quality | Genetic Hits | 11 | 2.60E-05 |
| Regulation of mitosis | Genetic Hits | 11 | 8.97E-06 |
| Response to chemical stimulus | Genetic Hits | 11 | 9.54E-05 |
| Deacetylase activity | Genetic Hits | 10 | 0.000111 |
| Gene conversion at mating-type locus | Genetic Hits | 10 | 7.74E-05 |
| Heteroduplex formation | Genetic Hits | 10 | 2.23E-05 |
| Histone exchange | Genetic Hits | 10 | 1.77E-09 |
| Meiotic recombination | Genetic Hits | 10 | 9.24E-06 |
| Mitotic recombination | Genetic Hits | 10 | 3.44E-06 |
| negative regulation of DNA replication | Genetic Hits | 10 | 5.26E-05 |
| regulation of transcription from RNA polymerase II promoter | Genetic Hits | 10 | 3.91E-05 |

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|---|---|---|---|
| Telomere maintenance via recombination | Genetic Hits | 10 | 0.0001 |
| Transcription regulator activity | Genetic Hits | 10 | 6.36E-06 |
| Vesicle-mediated transport | Genetic Hits | 10 | 6.63E-05 |
| Cell morphogenesis | Genetic Hits | 9 | 0.000142 |
| Cellular localization | Genetic Hits | 9 | 1.45E-09 |
| Conjugation | Genetic Hits | 9 | 0.000387 |
| DNA replication checkpoint | Genetic Hits | 9 | 7.95E-06 |
| Double-strand break repair via break-induced replication | Genetic Hits | 9 | 6.41E-06 |
| general RNA polymerase II transcription factor activity | Genetic Hits | 9 | 0.000113 |
| Histone methylation | Genetic Hits | 9 | 2.14E-06 |
| Meiotic chromosome segregation | Genetic Hits | 9 | 1.55E-05 |
| Microtubule motor activity | Genetic Hits | 9 | 8.52E-06 |
| Nucleic acid binding | Genetic Hits | 9 | 0.000165 |
| Postreplication repair | Genetic Hits | 9 | 0.000206 |
| Reproduction | Genetic Hits | 9 | 6.81E-05 |
| Tubulin binding | Genetic Hits | 9 | 2.64E-12 |
| Vacuolar transport | Genetic Hits | 9 | 2.57E-05 |
| Biopolymer catabolic process | Genetic Hits | 8 | 0.000146 |
| DNA integrity checkpoint | Genetic Hits | 8 | 1.62E-06 |
| Establishment of localization | Genetic Hits | 8 | 7.12E-05 |
| Establishment of organelle localization | Genetic Hits | 8 | 5.81E-08 |
| Hydrolase activity, acting on carbon-nitrogen (but not peptide) | Genetic Hits | 8 | 0.000361 |
| Interphase | Genetic Hits | 8 | 1.59E-05 |
| Meiotic gene conversion | Genetic Hits | 8 | 2.25E-05 |
| Microtubule-based process | Genetic Hits | 8 | 1.74E-15 |
| Motor activity | Genetic Hits | 8 | 6.72E-05 |
| Nucleotide-excision repair | Genetic Hits | 8 | 2.50E-07 |
| Protein modification by small protein conjugation | Genetic Hits | 8 | 0.000344 |
| regulation of DNA replication | Genetic Hits | 8 | 6.12E-06 |
| Response to drug | Genetic Hits | 8 | 0.000122 |
| Cell communication | Genetic Hits | 7 | 9.67E-08 |
| Deoxyribonuclease activity | Genetic Hits | 7 | 8.43E-05 |
| DNA replication initiation | Genetic Hits | 7 | 0.000197 |
| DNA strand elongation | Genetic Hits | 7 | 2.61E-07 |
| Lagging strand elongation | Genetic Hits | 7 | 4.68E-05 |
| Methylation | Genetic Hits | 7 | 0.00029 |
| Microtubule-based movement | Genetic Hits | 7 | 1.69E-07 |
| Mismatch repair | Genetic Hits | 7 | 8.37E-05 |
| Nuclear migration | Genetic Hits | 7 | 2.13E-08 |
| Protein amino acid acetylation | Genetic Hits | 7 | 2.42E-05 |
| Protein kinase activity | Genetic Hits | 7 | 0.00061 |
| Pyrophosphatase activity | Genetic Hits | 7 | 0.000231 |

| | | | |
|---|---|---|---|
| Response to osmotic stress | Genetic Hits | 7 | 0.000103 |
| sequence-specific DNA binding | Genetic Hits | 7 | 0.000102 |
| Sex determination | Genetic Hits | 7 | 1.86E-05 |
| Signal transducer activity | Genetic Hits | 7 | 1.02E-06 |
| single-stranded DNA binding | Genetic Hits | 7 | 1.29E-05 |
| Spindle localization | Genetic Hits | 7 | 1.14E-07 |
| Aging | Genetic Hits | 6 | 0.000386 |
| chromatin silencing at rDNA | Genetic Hits | 6 | 0.000223 |
| Cyclin-dependent protein kinase activity | Genetic Hits | 6 | 0.00214 |
| DNA topological change | Genetic Hits | 6 | 0.00145 |
| Endocytosis | Genetic Hits | 6 | 0.00082 |
| Establishment of cell polarity | Genetic Hits | 6 | 5.22E-08 |
| Growth | Genetic Hits | 6 | 3.27E-10 |
| Histone acetylation | Genetic Hits | 6 | 0.000902 |
| Hydrolase activity | Genetic Hits | 6 | 2.01E-05 |
| Karyogamy | Genetic Hits | 6 | 1.58E-05 |
| Leading strand elongation | Genetic Hits | 6 | 1.05E-05 |
| Microtubule depolymerization | Genetic Hits | 6 | 0.000849 |
| Microtubule polymerization or depolymerization | Genetic Hits | 6 | 5.88E-05 |
| Nucleosome assembly | Genetic Hits | 6 | 2.24E-05 |
| Nucleotidyltransferase activity | Genetic Hits | 6 | 0.00151 |
| One-carbon compound metabolic process | Genetic Hits | 6 | 0.000991 |
| Organelle fusion | Genetic Hits | 6 | 8.10E-05 |
| Protein amino acid acylation | Genetic Hits | 6 | 0.000131 |
| Protein folding | Genetic Hits | 6 | 0.000726 |
| Protein targeting to vacuole | Genetic Hits | 6 | 0.000278 |
| Replicative cell aging | Genetic Hits | 6 | 0.000342 |
| RNA catabolic process | Genetic Hits | 6 | 0.000746 |
| RNA polymerase II transcription elongation factor activity | Genetic Hits | 6 | 3.72E-05 |
| Secretion | Genetic Hits | 6 | 1.18E-12 |
| Structural constituent of cytoskeleton | Genetic Hits | 6 | 0.00184 |
| structure-specific DNA binding | Genetic Hits | 6 | 2.46E-05 |

**Supplementary Table 1D: GO process and function annotations enriched in the combined perturbations with complete genetic screens available.**

These are limited to annotations enriched in at least 20% of the sets when analyzed separately.

| GO annotation | Set type | Enrichment p-value | % in set |
|---|---|---|---|
| Oxidoreductase activity | Differentially expressed | 6.21E-25 | 8.28 |
| Organic acid metabolic process | Differentially expressed | 6.76E-10 | 7.37 |
| Amine biosynthetic process | Differentially expressed | 2.77E-11 | 3.53 |
| Sulfur metabolic process | Differentially expressed | 1.17E-13 | 2.57 |
| Vitamin biosynthetic process | Differentially expressed | 9.54E-06 | 1.45 |
| Glutamine family amino acid biosynthetic process | Differentially expressed | 0.000287 | 0.91 |
| Sulfur compound biosynthetic process | Differentially expressed | 3.08E-06 | 0.81 |
| Arginine metabolic process | Differentially expressed | 0.000534 | 0.59 |
| Structural constituent of cell wall | Differentially expressed | 0.00336 | 0.49 |
| Arginine biosynthetic process | Differentially expressed | 0.00134 | 0.43 |
| Biological regulation | Differentially expressed | 7.92E-35 | 20.72 |
| Establishment of localization | Genetic Hits | 4.23E-10 | 18.55 |
| Response to stimulus | Genetic Hits | 2.32E-31 | 17.25 |
| Developmental process | Genetic Hits | 1.37E-15 | 16.43 |
| Regulation of cellular process | Genetic Hits | 8.48E-29 | 16.3 |
| RNA metabolic process | Genetic Hits | 0.00147 | 16.04 |
| DNA metabolic process | Genetic Hits | 1.89E-52 | 14.78 |
| Hydrolase activity | Genetic Hits | 6.73E-05 | 13.44 |
| Cellular localization | Genetic Hits | 6.94E-14 | 12.96 |
| Transcription | Genetic Hits | 1.57E-18 | 12.09 |
| Response to stress | Genetic Hits | 3.80E-26 | 11.83 |
| Regulation of metabolic process | Genetic Hits | 1.04E-16 | 11.31 |
| Cell cycle | Genetic Hits | 3.83E-28 | 11.05 |
| Protein binding | Genetic Hits | 2.83E-15 | 10.1 |
| Post-translational protein modification | Genetic Hits | 2.25E-18 | 9.1 |
| Cell cycle phase | Genetic Hits | 1.03E-25 | 9.02 |
| Cell development | Genetic Hits | 0.00021 | 8.76 |
| Telomere maintenance | Genetic Hits | 3.99E-40 | 8.67 |
| Regulation of transcription | Genetic Hits | 4.04E-16 | 8.58 |
| Vesicle-mediated transport | Genetic Hits | 3.67E-14 | 7.67 |
| Response to chemical stimulus | Genetic Hits | 1.21E-08 | 7.54 |
| DNA packaging | Genetic Hits | 6.93E-31 | 7.46 |
| response to DNA damage stimulus | Genetic Hits | 5.08E-32 | 7.2 |
| transcription from RNA polymerase II promoter | Genetic Hits | 3.15E-11 | 7.11 |
| M phase | Genetic Hits | 3.53E-20 | 6.89 |
| Mitotic cell cycle | Genetic Hits | 2.27E-19 | 6.68 |
| Reproduction | Genetic Hits | 8.43E-08 | 6.63 |
| Cell morphogenesis | Genetic Hits | 7.55E-18 | 6.59 |
| Transcription regulator activity | Genetic Hits | 4.35E-05 | 6.46 |
| Pyrophosphatase activity | Genetic Hits | 2.10E-07 | 6.07 |
| Regulation of biological quality | Genetic Hits | 4.13E-11 | 5.98 |
| Negative regulation of cellular process | Genetic Hits | 3.18E-15 | 5.81 |
| DNA binding | Genetic Hits | 2.42E-12 | 5.64 |
| DNA repair | Genetic Hits | 8.42E-23 | 5.59 |
| Secretion | Genetic Hits | 7.95E-09 | 5.55 |
| Biopolymer catabolic process | Genetic Hits | 0.00191 | 5.16 |
| Cell communication | Genetic Hits | 2.63E-08 | 5.12 |
| regulation of transcription from RNA polymerase II promoter | Genetic Hits | | |

| | | | |
|---|---|---|---|
| Chromatin remodeling | Genetic Hits | 9.39E-09 | 4.9 |
| Regulation of cell cycle | Genetic Hits | 6.94E-18 | 4.55 |
| Negative regulation of transcription | Genetic Hits | 3.01E-14 | 4.55 |
| Meiosis | Genetic Hits | 6.42E-13 | 4.12 |
| Chromatin assembly or disassembly | Genetic Hits | 3.37E-13 | 4.03 |
| Growth | Genetic Hits | 8.53E-16 | 3.56 |
| Mitosis | Genetic Hits | 9.72E-09 | 3.56 |
| DNA replication | Genetic Hits | 4.94E-10 | 3.38 |
| Protein kinase activity | Genetic Hits | 5.93E-12 | 3.3 |
| Response to drug | Genetic Hits | 5.52E-08 | 3.3 |
| Establishment of cell polarity | Genetic Hits | 9.18E-10 | 3.25 |
| DNA recombination | Genetic Hits | 2.11E-14 | 3.21 |
| ATPase activity, coupled | Genetic Hits | 2.60E-13 | 3.21 |
| Chromosome segregation | Genetic Hits | 1.80E-05 | 3.17 |
| Vacuolar transport | Genetic Hits | 1.57E-08 | 3.08 |
| Gene silencing | Genetic Hits | 6.79E-11 | 2.95 |
| Microtubule-based process | Genetic Hits | 5.09E-12 | 2.78 |
| Histone modification | Genetic Hits | 8.93E-10 | 2.78 |
| DNA-dependent DNA replication | Genetic Hits | 7.58E-14 | 2.65 |
| Interphase | Genetic Hits | 1.60E-09 | 2.65 |
| Conjugation | Genetic Hits | 1.74E-06 | 2.39 |
| meiosis I | Genetic Hits | 0.00302 | 2.39 |
| Endocytosis | Genetic Hits | 5.93E-11 | 2.21 |
| Response to osmotic stress | Genetic Hits | 2.66E-05 | 2.08 |
| Protein modification by small protein conjugation | Genetic Hits | 7.54E-07 | 1.95 |
| Organelle localization | Genetic Hits | 0.000126 | 1.91 |
| Protein targeting to vacuole | Genetic Hits | 2.00E-11 | 1.87 |
| Double-strand break repair | Genetic Hits | 1.93E-07 | 1.87 |
| Signal transducer activity | Genetic Hits | 1.03E-10 | 1.78 |
| Sister chromatid segregation | Genetic Hits | 6.51E-07 | 1.69 |
| Cell cycle checkpoint | Genetic Hits | 2.51E-06 | 1.69 |
| Cytoskeletal protein binding | Genetic Hits | 5.74E-08 | 1.65 |
| Regulation of mitosis | Genetic Hits | 5.74E-08 | 1.65 |
| Meiotic recombination | Genetic Hits | 1.18E-07 | 1.61 |
| Chromatin silencing at telomere | Genetic Hits | 1.03E-07 | 1.56 |
| DNA-dependent ATPase activity | Genetic Hits | 2.20E-06 | 1.52 |
| RNA elongation | Genetic Hits | 8.37E-06 | 1.48 |
| Hydrolase activity, acting on carbon-nitrogen (but not peptide) bonds, in lin | Genetic Hits | 2.12E-08 | 1.43 |
| Non-recombinational repair | Genetic Hits | 1.55E-05 | 1.39 |
| Protein amino acid acylation | Genetic Hits | 1.01E-11 | 1.3 |
| Deacetylase activity | Genetic Hits | 0.00548 | 1.3 |
| One-carbon compound metabolic process | Genetic Hits | 2.45E-09 | 1.22 |
| Nucleotide-excision repair | Genetic Hits | 0.00313 | 1.22 |
| sequence-specific DNA binding | Genetic Hits | 8.28E-07 | 1.17 |
| Establishment of organelle localization | Genetic Hits | 0.000183 | 1.17 |
| Protein amino acid acetylation | Genetic Hits | 7.44E-09 | 1.09 |
| Histone deacetylation | Genetic Hits | 0.00164 | 1.09 |
| Methylation | Genetic Hits | 2.25E-11 | 1.04 |
| structure-specific DNA binding | Genetic Hits | 0.00174 | 1.04 |
| regulation of DNA metabolic process | Genetic Hits | 1.72E-05 | 1 |
| Chromatin silencing at silent mating-type cassette | Genetic Hits | 1.73E-06 | 0.96 |
| Double-strand break repair via nonhomologous end joining | Genetic Hits | 1.45E-05 | 0.96 |

| | | | |
|---|---|---|---|
| Recombinational repair | Genetic Hits | 5.32E-08 | 0.91 |
| DNA strand elongation | Genetic Hits | 2.55E-05 | 0.87 |
| Histone acetylation | Genetic Hits | 0.000635 | 0.87 |
| Mismatch repair | Genetic Hits | 0.00353 | 0.87 |
| Deoxyribonuclease activity | Genetic Hits | 2.00E-05 | 0.83 |
| DNA replication initiation | Genetic Hits | 0.000135 | 0.83 |
| Sister chromatid cohesion | Genetic Hits | 0.00217 | 0.83 |
| Nuclear migration | Genetic Hits | 0.00389 | 0.78 |
| negative regulation of DNA metabolic process | Genetic Hits | 2.18E-06 | 0.74 |
| Histone methylation | Genetic Hits | 8.12E-08 | 0.7 |
| Tubulin binding | Genetic Hits | 5.44E-06 | 0.7 |
| DNA integrity checkpoint | Genetic Hits | 5.44E-06 | 0.7 |
| Lagging strand elongation | Genetic Hits | 5.37E-05 | 0.65 |
| ATP-dependent chromatin remodeling | Genetic Hits | 0.000452 | 0.65 |
| Meiotic gene conversion | Genetic Hits | 0.00221 | 0.65 |
| Meiotic chromosome segregation | Genetic Hits | 0.00221 | 0.65 |
| Microtubule-based movement | Genetic Hits | 6.25E-06 | 0.61 |
| Motor activity | Genetic Hits | 6.25E-06 | 0.61 |
| Postreplication repair | Genetic Hits | 0.000125 | 0.61 |
| Gene conversion at mating-type locus | Genetic Hits | 0.000289 | 0.57 |
| single-stranded DNA binding | Genetic Hits | 0.00198 | 0.57 |
| Double-strand break repair via single-strand annealing | Genetic Hits | 0.000197 | 0.52 |
| regulation of DNA recombination | Genetic Hits | 1.34E-05 | 0.48 |
| Spindle localization | Genetic Hits | 0.000108 | 0.48 |
| regulation of DNA replication | Genetic Hits | 3.73E-05 | 0.44 |
| negative regulation of DNA recombination | Genetic Hits | 0.00327 | 0.44 |
| Transposition | Genetic Hits | 0.000104 | 0.39 |
| Double-strand break repair via synthesis-dependent strand annealing | Genetic Hits | 0.000104 | 0.39 |
| Nucleosome assembly | Genetic Hits | 0.00261 | 0.39 |
| Microtubule motor activity | Genetic Hits | 0.00261 | 0.39 |
| negative regulation of DNA replication | Genetic Hits | 0.00176 | 0.35 |
| Cyclin-dependent protein kinase activity | Genetic Hits | 0.000797 | 0.31 |
| Histone exchange | Genetic Hits | 0.00437 | 0.31 |
| Double-strand break repair via break-induced replication | Genetic Hits | 0.00437 | 0.31 |
| | Genetic Hits | 0.00221 | 0.26 |

**Supplementary Table 2A: Yeast interactome data.**

| Interaction Type | Number of interacting pairs |
|---|---|
| Physical | 33,765 |
| MIPS Complex | 11,014 |
| Metabolic | 2,882 |
| Regulatory interactions between transcription factors | 207 |
| Protein-DNA interactions | 5256 Reliable interactions [a], 3664 ChIP-chip motif interactions [b], 5143 ChIP-chip interactions [c] |

[a] Reliable interactions include those ChIP-chip motif interactions for which the motif occurrence in the gene's upstream sequence was conserved in at least two other *Saccharomyces sensu stricto* species, as well as literature-curated interactions.
[b] ChIP-chip motif interactions refer to those ChIP-chip interactions for which the gene's upstream sequence contained the binding motif of the specific transcription factor.
[c] ChIP-chip interactions refer to interactions discovered by the ChIP-chip method.

**Supplementary Table 2B: Interaction weights associated with individual types of evidence for protein-protein interaction.**

| Type of interaction evidence | Probability[1] |
|---|---|
| Two-hybrid HTP | 0.061056 |
| Product-Substrate | 0.06908 |
| Affinity Capture-MS HTP | 0.216939 |
| Affinity Capture-MS LC | 0.255753 |
| Co-purification HTP | 0.279417 |
| Affinity Capture-Western HTP | 0.312123 |
| Co-fractionation HTP | 0.350432 |
| Reconstituted Complex HTP | 0.403046 |
| Two-hybrid LC | 0.464472 |
| Biochemical Activity LC | 0.489647 |
| Biochemical Activity HTP | 0.552508 |
| Protein-peptide LC | 0.674045 |
| Affinity Capture-Western LC | 0.682404 |
| Co-localization LC | 0.700851 |
| Transcription Factor -> Transcription Factor | 0.71149 |
| Protein-peptide HTP | 0.756207 |
| Reconstituted Complex LC | 0.789035 |
| MIPS | 0.801993 |
| Protein-RNA LC | 0.805288 |
| Co-purification LC | 0.843226 |
| Co-fractionation LC | 0.871346 |
| Co-crystal Structure HTP | 0.961121 |

[1] As described in the Methods section, in our weighting scheme each interaction between two protein nodes $p_i$, $p_j$.is associated with a weight $w_{ij}$ such that $w_{ij} = \mathrm{P}(RP_{p_i p_j} = 1 \mid I_{p_i p_j})$ $I$ is a vector of indicator functions such that each function corresponds to a different type of interaction evidence. To estimate the weight $w_k$ associated with interaction evidence type $k$ we assumed an interaction between two proteins was supported by evidence type $k$ alone. We therefore computed $w_k$ based on a vector $I_k$ whose k-th entry was set to 1 and all other entries to 0 and using the formula above.

**Supplementary Table 3A: Yeast genes that modify α-syn toxicity when overexpressed.**

| Yeast Gene | Type | Strength | Human ortholog(s) | Proposed function |
|---|---|---|---|---|
| **Amino Acid Transport** | | | | |
| AVT4 | suppressor | 3 | SLC36A1<br>SLC36A2<br>SLC36A3<br>SLC36A4 | Vacuolar transporter; exports large neutral amino acids from the vacuole |
| DIP5 | suppressor | 3 | SLC7A1<br>SLC7A14<br>SLC7A2<br>SLC7A3<br>SLC7A4<br>SLC7A13 | Dicarboxylic amino acid permease |
| LST8 | suppressor | 3 | GBL | Component of the TOR signaling pathway |
| **Autophagy** | | | | |
| NVJ1 | suppressor | 2 | | Nuclear envelope protein; functions during piecemeal microautophagy of the nucleus (PMN) |
| **Cytoskeleton** | | | | |
| ICY1 | suppressor | 4 | | Protein that interacts with the cytoskeleton |
| ICY2 | suppressor | 4 | | Protein that interacts with the cytoskeleton |
| **Manganese transport** | | | | |
| CCC1 | suppressor | 4 | | Putative vacuolar Fe2+/Mn2+ transporter |
| PMR1 | enhancer | -7 | ATP2C1<br>ATP2C2 | High affinity Ca2+/Mn2+ P-type ATPase required for Ca2+ and Mn2+ transport into Golgi |
| **Protein phosphorylation** | | | | |
| IME2 | suppressor | 4 | ICK | Serine/threonine protein kinase involved in activation of meiosis |
| PTP2 | suppressor | 3 | PTPRE,<br>PTPRC,<br>PTPN22,<br>PTPRG | Phosphotyrosine-specific protein phosphatase involved in osmolarity sensing |
| GIP2 | suppressor | 3 | PPP1R3A<br>PPP1R3B<br>PPP1R3C<br>PPP1R3D<br>PPP1R3E | Putative regulatory subunit of the protein phosphatase Glc7p, involved in glycogen metabolism |
| YCK3 | suppressor | 3 | CSNK1G1<br>CSNK1G2<br>CSNK1G3 | Palmitoylated, vacuolar membrane-localized casein kinase I isoform |
| RCK1 | suppressor | 3 | CAMK1G | Protein kinase involved in the response to oxidative stress |
| CDC5 | suppressor (Cdc5 overexpression is toxic; in presence of a-syn it rescues/rescued) | 3 | PLK2 | Polo-like kinase; found at bud neck, nucleus and SPBs; has multiple functions in mitosis and cytokinesis |
| PTC4 | suppressor | 1 | PPM1G | Cytoplasmic type 2C protein phosphatase |
| SIT4 | enhancer | -2 | PPP6C | Type 2A-related serine-threonine phosphatase. |

| | | | | |
|---|---|---|---|---|
| CAX4 | enhancer | -3 | DOLPP1 | Dolichyl pyrophosphate phosphatase, required for Dol-P-P-linked oligosaccharide intermediate synthesis and protein N-glycosylation. |
| PPZ2 | enhancer | -3 | PPP1CC PPP1CB PPP1CA | Serine/threonine protein phosphatase Z |
| PPZ1 | enhancer | -8 | PPP1CA PPP1CB PPP1CC | Serine/threonine protein phosphatase Z |
| **Transcription/Translation** | | | | |
| CUP9 | suppressor | 3 | MEIS1 MEIS2 MEIS3 NR_002211.1 PKNOX1 PKNOX2 Q99687-3 TGIF1 TGIF2 TGIF2LX TGIF2LY | Transcriptional repressor involved in copper ion homeostasis |
| HAP4 | suppressor | 4 | | Transcriptional activator and global regulator of respiratory gene expression |
| FZF1 | suppressor | 3 | KLF15 KLF11 ZNF624 | Key transcriptional regulator of cellular response to nitrosative stress |
| MGA2 | suppressor | 3 | ANKRD1 OSBPL1A | ER membrane protein involved in regulation of OLE1 transcription |
| MKS1 | enhancer | -5 | | Pleiotropic negative transcriptional regulator involved in Ras-CAMP and lysine biosynthetic pathways and nitrogen regulation; involved in retrograde (RTG) mitochondria-to-nucleus signaling |
| VHR1 | suppressor | 3 | | Transcriptional activator |
| JSN1 | suppressor | 2 | PUM1 | Member of the Puf family of RNA-binding proteins, interacts with mRNAs encoding membrane-associated proteins |
| SUT2 | enhancer | -3 | | Putative transcription factor; multicopy suppressor of mutations that cause low activity of the cAMP/protein kinase A pathway |
| TIF4632 | suppressor | 3 | EIF4G1 EIF4G2 EIF4G3 | Translation initiation factor eIF4G, subunit of the mRNA cap-binding protein complex (eIF4F) |
| STB3 | suppressor | 3 | | Protein that binds Sin3p in a two-hybrid assay. |
| MATALPHA1 | enhancer | -5 | | Transcriptional co-activator involved in regulation of mating-type-specific gene expression |
| **Trehalose biosynthesis** | | | | |
| UGP1 | suppressor | 4 | UGP2 | UDP-glucose pyrophosphorylase, catalyses the formation of UDP-Glc, a precursor to trehalose |
| TPS3 | suppressor | 3 | | Regulatory subunit of trehalose-6-phosphate synthase/phosphatase complex, which synthesizes trehalose |
| NTH1 | suppressor | 2 | TREH | Neutral trehalase, degrades trehalose; required for thermotolerance and may mediate resistance to other cellular stresses |
| **Ubiquitin-related** | | | | |
| CDC4 | suppressor | 4 | FBXW7 | F-box, associates with Skp1p and Cdc53p to form |

| | | | | |
|---|---|---|---|---|
| | | | | a complex, SCFCdc4, which acts as ubiquitin-protein ligase |
| UIP5 | suppressor | 4 | | Protein of unknown function that interacts with Ulp1p, a Ubl (ubiquitin-like protein)-specific protease |
| HRD1 | suppressor | 4 | AMFR<br>SYVN1 | Ubiquitin-protein ligase required for endoplasmic reticulum-associated degradation (ERAD) of misfolded proteins |
| UBP11 | enhancer | -3 | USP21 | Ubiquitin-specific protease that cleaves ubiquitin from ubiquitinated proteins. |
| UBP7 | enhancer | -4 | USP21 | Ubiquitin-specific protease that cleaves ubiquitin-protein fusions. |
| **Vesicular transport, ER-Golgi** | | | | |
| YPT1 | suppressor | 5 | RAB10<br>RAB13<br>RAB1A<br>RAB1C<br>RAB8A<br>RAB8B | Ras-like small GTPase, involved in the ER-to-Golgi step of the secretory pathway |
| YKT6 | suppressor | 4 | YKT6 | v-SNARE involved in trafficking to and within the Golgi, endocytic trafficking to the vacuole, and vacuolar fusion |
| BRE5 | suppressor | 4 | G3BP2 | Ubiquitin protease cofactor, forms deubiquitination complex with Ubp3p to regulate ER-Golgi transport |
| SEC21 | suppressor | 4 | COPG2<br>COPG | Gamma subunit of coatomer, a heptameric protein complex that together with Arf1p forms the COPI coat |
| UBP3 | suppressor | 3 | USP10 | Ubiquitin-specific protease that interacts with Bre5p to co-regulate anterograde and retrograde transport between ER and Golgi |
| ERV29 | suppressor | 3 | SURF4 | Protein localized to COPII-coated vesicles, involved in vesicle formation and incorporation of specific secretory cargo. |
| SEC28 | suppressor | 3 | COPE | Epsilon-COP subunit of the coatomer; regulates retrograde Golgi-to-ER protein traffic; stabilizes Cop1p |
| SFT1 | suppressor | 2 | mouse BET1 | Intra-Golgi v-SNARE, required for transport of proteins between an early and a later Golgi compartment. |
| GLO3 | enhancer | -1 | ARFGAP3<br>ZNF289 | ADP-ribosylation factor GTPase activating protein (ARF GAP), involved in ER-Golgi transport |
| TRS120 | enhancer | -2 | NIBP | One of 10 subunits of the transport protein particle (TRAPP) complex of the cis-Golgi which mediates vesicle docking and fusion |
| GYP8 | enhancer | -2 | TBC1D20 | GTPase-activating protein for yeast Rab family members; Ypt1p is the preferred in vitro substrate |
| YIP3 | enhancer | -2 | RABAC1 | Protein localized to COPII vesicles, proposed to be involved in ER to Golgi transport; interacts with Rab GTPases |
| BET4 | enhancer | -3 | RABGGTA | Alpha subunit of Type II geranylgeranyltransferase; provides a membrane attachment moiety to Rab-like proteins Ypt1p and Sec4p |
| SLY41 | enhancer | -5 | SLC35E1 | Protein involved in ER-to-Golgi transport. |

| | | | | |
|---|---|---|---|---|
| GOS1 | enhancer | -2 | GOSR1 | v-SNARE protein involved in Golgi transport, homolog of the mammalian protein GOS-28/GS28 |
| SEC31 | enhancer | -2 | SEC31A<br>SEC31B | Essential phosphoprotein component (p150) of the COPII coat of secretory pathway vesicles, in complex with Sec13p; required for ER-derived transport vesicle formation |
| **Other cellular processes** | | | | |
| PFS1 | suppressor | 4 | | Sporulation protein required for prospore membrane formation at selected spindle poles |
| PDE2 | suppressor | 4 | PDE10A<br>PDE11A<br>PDE1A<br>PDE1B<br>PDE1C<br>PDE2A<br>PDE3A<br>PDE3B<br>PDE4A<br>PDE4B<br>PDE4C<br>PDE4D<br>PDE5A<br>PDE6A<br>PDE6B<br>PDE6C<br>PDE7A<br>PDE7B<br>PDE8A<br>PDE8B<br>PDE9A | High-affinity cyclic AMP phosphodiesterase, component of the cAMP-dependent protein kinase signaling system |
| MUM2 | suppressor | 4 | | Interacts with Orc2p, which is a component of the origin recognition complex. |
| OSH3 | suppressor | 3 | OSBPL1A<br>OSBPL2<br>OSBPL3<br>OSBPL6<br>OSBPL7 | Member of an oxysterol-binding protein family, functions in sterol metabolism |
| PHO80 | suppressor | 3 | | Cyclin, negatively regulates phosphate metabolism |
| OSH2 | suppressor | 3 | OSBPL3<br>OSBP OSBP2 | Member of an oxysterol-binding protein family, functions in sterol metabolism |
| ISN1 | suppressor | 2 | | Inosine 5'-monophosphate (IMP)-specific 5'-nucleotidase |
| EPS1 | enhancer | -1 | | Protein disulfide isomerase-related protein involved in endoplasmic reticulum retention of resident ER proteins. |
| IDS2 | enhancer | -2 | | Protein involved in modulation of Ime2p activity during meiosis |
| QDR3 | suppressor | 4 | | Multidrug transporter of the major facilitator superfamily, required for resistance to quinidine, barban, cisplatin, and bleomycin |
| TPO4 | enhancer | -3 | | Polyamine transport protein, recognizes spermine, putrescine, and spermidine; localizes to the plasma membrane; member of the major facilitator superfamily |
| IZH3 | enhancer | -2 | | Membrane protein involved in zinc metabolism, |

| | | | | |
|---|---|---|---|---|
| | | | | member of the four-protein IZH family, expression induced by zinc deficiency; deletion reduces sensitivity to elevated zinc and shortens lag phase, overexpression reduces Zap1p activity |
| **Unknown Function** | | | | |
| YKL063C | suppressor | 4 | | Uncharacterized, GFP-fusion localizes to the Golgi |
| YML081W | suppressor | 4 | EGR3 | Uncharacterized, GFP-fusion localizes to the nucleus |
| YNR014W | suppressor | 4 | | Uncharacterized, expression is cell-cycle regulated and heat-inducible |
| YKL088W | suppressor | 4 | PPCDC | Protein required for cell viability. Predicted phosphopantothenoylcysteine decarboxylase |
| YML083C | suppressor | 3 | | Uncharacterized, strong increase in transcript abundance during anaerobic growth compared to aerobic growth |
| YDR374C | suppressor | 3 | YTHDF1<br>YTHDF2<br>YTHDF3 | Uncharacterized |
| YOR291W (YPK9) | suppressor | 3 | ATP13A2 (PARK9)<br>ATP13A3<br>ATP13A4<br>ATP13A5 | Probable cation-transporting ATPase 2 |
| YDL121C | suppressor | 2 | | Uncharacterized, GFP-fusion localizes to the ER |
| YBR030W | suppressor | 2 | | Uncharacterized, predicted to function in phospholipid metabolism |
| YMR111C | suppressor | 2 | | Uncharacterized, GFP-fusion localizes to the nucleus |
| YOR129C | suppressor | 2 | | Putative component of the outer plaque of the spindle pole body; may be involved in cation homeostasis or multidrug resistance. |

**Supplemenatry Table 3B: GO annotations for the α-synuclein genetic hits (proteins that modify α-syn toxicity when overexpressed) and genes that are differentially regulated following α-syn expression.** Note that this table reports the GO annotations for all the differentially expressed genes, combining the up and down regulated genes. The numbers in the main text differ because they refer to the GO annotations computed separately for the up- and down-regulated genes.

| Ontology | Data type | GO_term | P-value |
|---|---|---|---|
| process | Genetic Hits | ER to Golgi vesicle-mediated transport | 6.30E-05 |
| process | Genetic Hits | Golgi vesicle transport | 6.69E-05 |
| process | Genetic Hits | vesicle-mediated transport | 0.00012 |
| process | Genetic Hits | localization | 0.00237 |
| process | Genetic Hits | membrane budding | 0.00291 |
| process | Genetic Hits | transport | 0.01562 |
| process | Genetic Hits | establishment of localization | 0.02061 |
| process | Genetic Hits | Golgi vesicle budding | 0.02821 |
| process | Genetic Hits | trehalose metabolic process | 0.03361 |
| function | Genetic Hits | phosphoric ester hydrolase activity | 0.00083 |
| function | Genetic Hits | phosphatase activity | 0.00276 |
| function | Genetic Hits | phosphoprotein phosphatase activity | 0.00847 |
| function | Genetic Hits | protein serine/threonine phosphatase activity | 0.01067 |
| function | Genetic Hits | transcription activator activity | 0.0467 |
| component | Genetic Hits | Golgi apparatus | 6.79E-06 |
| component | Genetic Hits | Golgi membrane | 1.34E-05 |
| component | Genetic Hits | Golgi apparatus part | 2.42E-05 |
| component | Genetic Hits | endomembrane system | 0.00034 |
| component | Genetic Hits | membrane | 0.00347 |
| component | Genetic Hits | COPI vesicle coat | 0.0049 |
| component | Genetic Hits | COPI coated vesicle membrane | 0.0049 |
| component | Genetic Hits | Golgi-associated vesicle | 0.00623 |
| component | Genetic Hits | Golgi-associated vesicle membrane | 0.01003 |
| component | Genetic Hits | organelle membrane | 0.01656 |
| component | Genetic Hits | coated vesicle | 0.01771 |
| component | Genetic Hits | vesicle coat | 0.03038 |
| component | Genetic Hits | vesicle membrane | 0.03825 |
| component | Genetic Hits | cytoplasmic vesicle membrane | 0.03825 |
| component | Genetic Hits | coated vesicle membrane | 0.03825 |
| component | Genetic Hits | membrane coat | 0.04747 |
| component | Genetic Hits | coated membrane | 0.04747 |
| process | Differentially Expressed | mitochondrial translation | 5.19E-10 |
| process | Differentially Expressed | mitochondrion organization | 8.47E-08 |
| process | Differentially Expressed | generation of precursor metabolites and energy | 2.36E-05 |
| process | Differentially Expressed | aerobic respiration | 3.09E-05 |
| process | Differentially Expressed | cellular respiration | 0.00017 |
| process | Differentially Expressed | acetyl-CoA catabolic process | 0.00046 |
| process | Differentially Expressed | tricarboxylic acid cycle | 0.00046 |
| process | Differentially Expressed | oxidative phosphorylation | 0.0015 |
| process | Differentially Expressed | sulfate assimilation | 0.00202 |
| process | Differentially Expressed | sulfur utilization | 0.00202 |
| process | Differentially Expressed | acetyl-CoA metabolic process | 0.00627 |

| | | | |
|---|---|---|---|
| process | Differentially Expressed | coenzyme catabolic process | 0.00627 |
| process | Differentially Expressed | energy derivation by oxidation of organic compounds | 0.0066 |
| process | Differentially Expressed | cofactor catabolic process | 0.01056 |
| process | Differentially Expressed | glutamate metabolic process | 0.02155 |
| process | Differentially Expressed | electron transport chain | 0.02745 |
| process | Differentially Expressed | respiratory electron transport chain | 0.02745 |
| process | Differentially Expressed | ATP synthesis coupled electron transport | 0.02745 |
| process | Differentially Expressed | mitochondrial ATP synthesis coupled electron transport | 0.02745 |
| process | Differentially Expressed | oxidation reduction | 0.02745 |
| process | Differentially Expressed | transposition | 0.04596 |
| process | Differentially Expressed | transposition, RNA-mediated | 0.04596 |
| function | Differentially Expressed | structural constituent of ribosome | 1.61E-11 |
| function | Differentially Expressed | oxidoreductase activity | 9.26E-10 |
| function | Differentially Expressed | structural molecule activity | 2.49E-08 |
| function | Differentially Expressed | structural constituent of cell wall | 0.00055 |
| function | Differentially Expressed | oxidoreductase activity, acting on sulfur group of donors | 0.00135 |
| function | Differentially Expressed | copper ion binding | 0.00409 |
| component | Differentially Expressed | organellar ribosome | 3.90E-12 |
| component | Differentially Expressed | mitochondrial ribosome | 3.90E-12 |
| component | Differentially Expressed | mitochondrial part | 5.12E-11 |
| component | Differentially Expressed | mitochondrial lumen | 2.14E-10 |
| component | Differentially Expressed | mitochondrial matrix | 2.14E-10 |
| component | Differentially Expressed | ribosomal subunit | 4.16E-10 |
| component | Differentially Expressed | organellar large ribosomal subunit | 1.16E-08 |
| component | Differentially Expressed | mitochondrial large ribosomal subunit | 1.16E-08 |
| component | Differentially Expressed | cytoplasm | 2.32E-08 |
| component | Differentially Expressed | ribosome | 5.60E-08 |
| component | Differentially Expressed | fungal-type cell wall | 1.19E-07 |
| component | Differentially Expressed | external encapsulating structure | 3.61E-07 |
| component | Differentially Expressed | cell wall | 3.61E-07 |
| component | Differentially Expressed | mitochondrion | 4.25E-06 |
| component | Differentially Expressed | retrotransposon nucleocapsid | 5.87E-06 |
| component | Differentially Expressed | large ribosomal subunit | 2.69E-05 |
| component | Differentially Expressed | small ribosomal subunit | 0.00084 |
| component | Differentially Expressed | mitochondrial inner membrane | 0.00085 |
| component | Differentially Expressed | organelle inner membrane | 0.00234 |
| component | Differentially Expressed | mitochondrial respiratory chain | 0.00472 |
| component | Differentially Expressed | mitochondrial membrane part | 0.01315 |
| component | Differentially Expressed | cell | 0.01866 |
| component | Differentially Expressed | cell part | 0.02746 |
| component | Differentially Expressed | vacuole | 0.03438 |

**Supplementary Table 3C: List of the differentially expressed genes identified four hours after induction of α-syn expression. Each Gene ID is associated with the corresponding log2(fold change) and p-value.**

| Gene ID | log2 (fold change) | P-value |
|---|---|---|
| YJR122W | -1.0658 | 0.000128 |
| YBR284W | 1.0594 | 0.004615 |
| YGL248W | 2.5262 | 0.000114 |
| YMR184W | 1.9749 | 5.40E-05 |
| YGR176W | 1.1381 | 9.20E-05 |
| YLR303W | 1.3598 | 0.000208 |
| YNL208W | 2.3106 | 4.40E-05 |
| YJL012C-A | 1.0713 | 0.000407 |
| YGL236C | -1.1053 | 0.000235 |
| YNL052W | -1.0725 | 0.000172 |
| YMR103C | 1.0737 | 0.000125 |
| YLL039C | 2.0309 | 8.50E-05 |
| YGR161W-A | 2.5931 | 7.00E-05 |
| YBR045C | 1.7823 | 0.000125 |
| YML009C | -1.4996 | 6.70E-05 |
| YDR493W | -1.1733 | 8.50E-05 |
| YMR169C | 1.8537 | 6.70E-05 |
| YJL104W | -1.1388 | 0.000276 |
| YOL120C | -1.0333 | 0.008252 |
| YDR034W-B | 4.1468 | 9.90E-05 |
| YIL098C | -1.2989 | 8.30E-05 |
| YDL012C | 1.0461 | 0.00055 |
| YPL018W | 1.1805 | 0.000123 |
| YOR356W | -1.3903 | 7.20E-05 |
| YPL201C | -1.3949 | 0.000169 |
| YAL034C | 1.4535 | 0.000158 |
| YDL223C | 1.7347 | 5.40E-05 |
| YBL101W-B | 1.5501 | 0.000114 |
| YDR354W | -1.8055 | 9.80E-05 |
| YGL157W | 1.2631 | 0.000845 |
| YDR178W | -1.1354 | 0.000148 |
| YCR021C | 2.068 | 5.10E-05 |
| YLL064C | 1.3754 | 7.80E-05 |
| YPL089C | 1.0355 | 0.000159 |
| YGR294W | 1.3916 | 6.80E-05 |
| YDR518W | 1.1264 | 0.000224 |
| YKR091W | 1.952 | 5.40E-05 |
| YPR077C | 1.2699 | 6.00E-04 |
| YDR262W | 1.1009 | 0.000418 |
| YIR028W | 1.5881 | 0.000131 |
| YDR133C | -1.6056 | 0.000414 |
| YMR245W | -1.187 | 0.000468 |
| YGL255W | 2.1607 | 0.000174 |
| YBL092W | -1.5886 | 0.000173 |
| YOR288C | 1.264 | 0.000339 |
| YDR261W-A | 2.5098 | 7.10E-05 |

| Gene ID | log2 (fold change) | P-value |
|---|---|---|
| YLL025W | 1.3055 | 7.80E-05 |
| YPL106C | 1.0247 | 0.000622 |
| YOR136W | -1.4178 | 0.000735 |
| YHR136C | 2.4966 | 0.000176 |
| YDL085C-A | 1.5025 | 0.000123 |
| YLR150W | -1.8644 | 0.000105 |
| YOR343W-B | 2.7193 | 5.70E-05 |
| YNL217W | 1.0037 | 0.000429 |
| YBR251W | -1.3228 | 7.30E-05 |
| YNL069C | -1.4775 | 0.000625 |
| YHR005C-A | -1.0055 | 0.000517 |
| YNL184C | -1.048 | 0.000132 |
| YGR037C | 1.476 | 0.000176 |
| YLR155C | 1.7544 | 0.000359 |
| YGL045W | 1.4284 | 0.000927 |
| YPR047W | -1.09 | 0.00031 |
| YOR264W | -1.1447 | 0.00019 |
| YBL093C | 1.579 | 0.000531 |
| YGR189C | 1.2646 | 0.000111 |
| YPR158W | 1.222 | 0.000164 |
| YDL010W | 1.4647 | 0.000164 |
| YDR511W | -1.0789 | 0.000102 |
| YKL104C | 2.1744 | 7.80E-05 |
| YDR342C | -1.8411 | 0.000698 |
| YNR058W | 1.0048 | 0.000449 |
| YDR298C | -1.23 | 0.000129 |
| YGR137W | 1.0719 | 0.000128 |
| YDR055W | 3.0985 | 9.50E-05 |
| YDL079C | -1.0582 | 0.000393 |
| YNL196C | 1.1497 | 9.50E-05 |
| YLL009C | -1.8681 | 5.40E-05 |
| YMR322C | 1.4075 | 7.80E-05 |
| YPR198W | 1.0495 | 0.000164 |
| YGL156W | 2.4129 | 5.40E-05 |
| YLR410W-B | 1.4493 | 7.80E-05 |
| YGR201C | 1.8891 | 0.000124 |
| YIL070C | -1.7167 | 5.40E-05 |
| YJR161C | 1.2448 | 7.80E-05 |
| YHR024C | -1.0058 | 0.00015 |
| YCR003W | -1.334 | 7.50E-05 |
| YDL227C | -1.9854 | 0.000398 |
| YGR284C | 1.4113 | 0.000486 |
| YOR306C | 2.1626 | 0.000268 |
| YMR175W | 2.1435 | 8.00E-05 |
| YBR137W | 1.1931 | 0.00024 |
| YDR001C | 1.6301 | 7.80E-05 |

| | | |
|---|---|---|
| YNL012W | 1.0101 | 0.000678 |
| YGL006W | 1.0596 | 0.000243 |
| YKL174C | 1.0594 | 0.000201 |
| YKL142W | 1.0318 | 0.000379 |
| YKL107W | 1.151 | 0.000176 |
| YMR157C | -1.1665 | 0.000333 |
| YML128C | 2.675 | 4.40E-05 |
| YBR287W | 1.1058 | 0.000234 |
| YLR461W | 1.4776 | 6.70E-05 |
| YGL101W | -1.0886 | 0.000114 |
| YDR393W | -1.0087 | 0.00019 |
| YIL093C | -1.0605 | 0.000114 |
| YIL009W | -1.3763 | 6.40E-05 |
| YHR030C | 1.7177 | 0.000124 |
| YBR072W | 5.0806 | 4.40E-05 |
| YLR107W | 1.6543 | 7.20E-05 |
| YMR187C | 1.3805 | 0.000235 |
| YDR367W | -1.1585 | 0.000309 |
| YMR122W-A | 1.5004 | 5.40E-05 |
| YKR042W | 1.1266 | 0.000157 |
| YOR348C | -1.6692 | 0.001265 |
| YDR347W | -2.0823 | 4.40E-05 |
| YJL034W | 3.4918 | 4.40E-05 |
| YDR494W | -1.1476 | 0.000139 |
| YOR286W | -1.1778 | 0.000111 |
| YOR036W | 1.5971 | 0.000295 |
| YBL048W | 1.4263 | 0.000246 |
| YGR032W | 2.9502 | 4.40E-05 |
| YPR079W | 1.5435 | 0.000128 |
| YIL117C | 1.424 | 8.50E-05 |
| YML054C | -1.2275 | 0.000176 |
| YBL075C | 1.771 | 0.000125 |
| YMR174C | 1.3348 | 0.000235 |
| YKR006C | -1.4399 | 6.70E-05 |
| YGR161C | 3.7462 | 8.60E-05 |
| YAL061W | 1.6676 | 5.50E-05 |
| YLR092W | 1.7457 | 0.000358 |
| YOR176W | 1.4435 | 0.000114 |
| YKL086W | 1.1105 | 0.000134 |
| YMR081C | -1.2859 | 0.001584 |
| YBR233W-A | 2.8382 | 0.000114 |
| YGR161W-B | 1.2832 | 0.000137 |
| YML123C | 4.6838 | 5.10E-05 |
| YJL223C | 1.5057 | 0.000114 |
| YKL148C | -1.0311 | 0.001157 |
| YDL159W-A | 2.7848 | 0.000338 |
| YIR041W | 1.2418 | 0.000174 |
| YLR061W | -1.3411 | 0.000719 |
| YMR020W | 1.2445 | 0.000405 |
| YER146W | -1.0348 | 0.000128 |
| YMR012W | -1.2601 | 0.000339 |
| YCR045C | 1.0334 | 0.000329 |

| | | |
|---|---|---|
| YML081C-A | -1.2538 | 0.000258 |
| YER138W-A | 1.3492 | 0.000131 |
| YPL170W | 1.0755 | 4.00E-04 |
| YGL046W | 1.003 | 0.000172 |
| YLR438W | 1.028 | 0.000588 |
| YBL078C | 2.3451 | 0.000235 |
| YJR107W | 1.5751 | 0.000123 |
| YER045C | 1.1873 | 0.000302 |
| YKR080W | 1.6 | 0.002931 |
| YFL002W-B | 2.7463 | 4.40E-05 |
| YBL045C | -1.0972 | 0.000302 |
| YEL058W | 1.0926 | 0.000418 |
| YJL155C | 1.0719 | 0.00069 |
| YDL244W | 3.0749 | 5.50E-05 |
| YGL162W | -1.5072 | 0.000812 |
| YML028W | 1.8694 | 8.30E-05 |
| YIR021W | -1.1546 | 0.000294 |
| YML120C | -1.0739 | 0.000467 |
| YPL143W | -1.5393 | 0.000267 |
| YMR118C | 2.1354 | 4.40E-05 |
| YBR117C | 2.3854 | 6.70E-05 |
| YOL151W | 2.21 | 7.20E-05 |
| YKL065C | 1.2737 | 0.000114 |
| YGR213C | 1.4149 | 7.30E-05 |
| YBL049W | 1.3442 | 0.000249 |
| YGR082W | -1.2733 | 0.000198 |
| YBR295W | 1.5997 | 9.50E-05 |
| YJL136C | -1.033 | 0.000863 |
| YMR008C | 1.7209 | 0.000281 |
| YOL164W | 1.5895 | 0.000173 |
| YIL023C | 1.6242 | 5.40E-05 |
| YOR315W | -1.2035 | 0.002376 |
| YPR002W | -1.763 | 0.00021 |
| YMR095C | 1.8178 | 0.000176 |
| YFR011C | -1.2138 | 0.000403 |
| YNR001C | -1.0796 | 0.000335 |
| YLR295C | -1.539 | 0.000114 |
| YMR180C | 2.0771 | 7.80E-05 |
| YOL016C | 1.2236 | 0.001568 |
| YPL110C | 1.2148 | 0.000102 |
| YIL158W | -1.7344 | 0.00032 |
| YDL125C | 1.8355 | 0.000329 |
| YEL025C | -1.1627 | 9.50E-05 |
| YOL077W-A | -1.1352 | 0.001012 |
| YPL081W | -1.1939 | 0.001187 |
| YBR201W | 1.5794 | 0.000408 |
| YDR098C-A | 1.206 | 0.000913 |
| YLL057C | 1.548 | 0.000344 |
| YOL031C | 2.38 | 8.10E-05 |
| YDL024C | 1.1257 | 0.000563 |
| YDR351W | -1.6819 | 5.40E-05 |
| YGR138C | 2.5612 | 4.40E-05 |

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|---|---|---|
| YIL074C | 1.2064 | 0.000552 |
| YER130C | 1.1719 | 0.000393 |
| YPL088W | 2.3649 | 7.80E-05 |
| YNL332W | 3.222 | 7.80E-05 |
| YMR305C | 1.0439 | 0.000416 |
| YNL277W | 1.0835 | 0.001327 |
| YJL196C | -1.0969 | 0.00047 |
| YLR054C | 2.5078 | 4.40E-05 |
| YGR008C | 2.4242 | 0.000119 |
| YCR104W | 1.3943 | 7.40E-05 |
| YOR192C-B | 1.1038 | 0.000179 |
| YMR315W | 1.149 | 0.000774 |
| YMR316W | 2.0073 | 0.000794 |
| YLR149C | 1.0413 | 0.000287 |
| YDR384C | -1.0856 | 0.001517 |
| YDR391C | 1.4628 | 0.000179 |
| YFL031W | 1.7173 | 7.80E-05 |
| YLR058C | 1.7323 | 0.00087 |
| YPL163C | 1.0192 | 0.006448 |
| YHR096C | 2.8653 | 4.90E-05 |
| YJL056C | 1.273 | 0.000147 |
| YKR049C | 1.4212 | 0.000202 |
| YNR034W-A | 1.2003 | 0.000176 |
| YBR296C | 1.6555 | 0.001311 |
| YGR142W | 2.6615 | 0.000175 |
| YGL107C | -1.0175 | 0.000164 |
| YKL137W | -1.1248 | 0.000147 |
| YHR038W | -1.0674 | 0.000131 |
| YOR391C | 1.1541 | 9.80E-05 |
| YGR027W-A | 1.0387 | 0.00347 |
| YMR090W | 2.2017 | 8.50E-05 |
| YJL153C | 2.0296 | 0.000318 |
| YDR149C | -1.12 | 0.000403 |
| YOR065W | -1.3416 | 0.000641 |
| YLR292C | 1.16 | 0.000169 |
| YOL064C | 1.1554 | 0.000982 |
| YJL144W | 3.4868 | 6.80E-05 |
| YGL034C | -1.2777 | 0.000375 |
| YOL055C | 2.1735 | 6.80E-05 |
| YOR343C-B | 1.0196 | 4.00E-04 |
| YCR009C | 1.0252 | 0.000528 |
| YML078W | -1.0425 | 0.000577 |
| YJL038C | 2.9795 | 8.30E-05 |
| YGR258C | 1.0022 | 0.00047 |
| YDL057W | 1.4384 | 0.000246 |
| YNL044W | 1.2732 | 0.00021 |
| YBL087C | -1.0582 | 0.00158 |
| YCL044C | 1.0366 | 0.002618 |
| YDR481C | 1.3479 | 0.000306 |
| YJL116C | 1.3556 | 0.000506 |
| YML087C | -1.7512 | 4.40E-05 |
| YCL024W | -1.0499 | 0.001208 |

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|---|---|---|
| YLR158C | 1.4237 | 0.000414 |
| YMR291W | 1.6313 | 0.000148 |
| YMR120C | 2.6337 | 0.000157 |
| YHL046C | 1.6623 | 0.000693 |
| YPR156C | 1.7516 | 7.10E-05 |
| YBR021W | -1.9111 | 9.80E-05 |
| YLR044C | 2.0358 | 7.80E-05 |
| YEL024W | -1.3701 | 0.000503 |
| YPL173W | -1.0171 | 0.000128 |
| YBR169C | 2.2267 | 0.000125 |
| YBR071W | 1.4768 | 0.00012 |
| YBR185C | -1.2658 | 0.000114 |
| YOR234C | -1.0345 | 0.001216 |
| YGL053W | 1.1944 | 0.000247 |
| YOR173W | 1.5126 | 8.00E-05 |
| YLR287C-A | -1.178 | 0.000507 |
| YEL071W | 1.0289 | 0.000437 |
| YIL108W | 1.9418 | 7.10E-05 |
| YHR209W | 2.477 | 5.40E-05 |
| YLR189C | -1.243 | 0.00055 |
| YBL003C | -1.0199 | 0.000939 |
| YJR137C | 1.0944 | 0.001341 |
| YPL017C | 1.5303 | 0.000319 |
| YPL171C | 1.2394 | 0.000407 |
| YDL114W | 1.1076 | 0.00058 |
| YDR371W | -1.2351 | 0.000504 |
| YKR011C | 1.0391 | 0.002752 |
| YBR120C | -1.2714 | 7.30E-05 |
| YHR007C | 1.4529 | 0.000541 |
| YDL222C | 1.1619 | 9.80E-05 |
| YGL184C | 2.1507 | 0.000246 |
| YJL043W | 2.6431 | 5.70E-05 |
| YBR054W | 2.0234 | 0.000534 |
| YGL234W | 1.0927 | 0.000104 |
| YMR242C | -1.1779 | 0.00071 |
| YGL146C | -1.1732 | 0.000414 |
| YGR243W | -1.6619 | 0.000224 |
| YDR059C | 1.2306 | 0.000185 |
| YDR026C | 1.0174 | 0.000124 |
| YGR076C | -1.5248 | 7.80E-05 |
| YHR128W | -1.3429 | 0.001976 |
| YLL026W | 1.2779 | 0.000327 |
| YBL030C | -1.8529 | 0.000114 |
| YCONTRO-L | 1.1557 | 0.000304 |
| YOL161C | 1.3519 | 8.40E-05 |
| YFL020C | 1.1495 | 9.50E-05 |
| YFR026C | 5.2337 | 4.40E-05 |
| YBL107W-A | 1.1686 | 0.000391 |
| YOR096W | -1.0621 | 0.00032 |
| YLR350W | 1.2553 | 0.00058 |
| YGL159W | -1.1562 | 0.000516 |
| YKR097W | -1.2272 | 0.00517 |

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|---|---|---|
| YML116W-A | 1.0636 | 0.000222 |
| YFL014W | 4.2413 | 4.40E-05 |
| YNR076W | 1.5881 | 6.50E-05 |
| YDR350C | -1.7765 | 5.40E-05 |
| YKL217W | -1.8432 | 6.00E-04 |
| YOR341W | -1.1933 | 0.031226 |
| YKR075C | -1.6418 | 9.50E-05 |
| YDR519W | 1.4993 | 9.80E-05 |
| YNL054W-B | 1.6023 | 0.000468 |
| YBR222C | 1.0323 | 0.000229 |
| YKL109W | -1.1518 | 0.000229 |
| YPL097W | -1.158 | 0.000191 |
| YGL028C | -1.3241 | 0.003464 |
| YBR158W | -1.1815 | 0.000192 |
| YKL163W | 4.5466 | 4.40E-05 |
| YDR210W-A | 2.7187 | 5.40E-05 |
| YPL262W | -1.1283 | 0.001001 |
| YGL189C | -1.3005 | 0.000587 |
| YKL073W | 1.3726 | 0.000112 |
| YDR365W-B | 1.0701 | 0.000155 |
| YCR100C | 1.0992 | 0.000229 |
| YGR268C | 1.2322 | 9.80E-05 |
| YMR303C | -1.1125 | 0.011794 |
| YDL124W | 2.9288 | 5.80E-05 |
| YML132W | 1.2763 | 0.000164 |
| YBR048W | -1.3159 | 0.000298 |
| YLR038C | -1.1447 | 0.00062 |
| YPL019C | 1.5419 | 0.000179 |
| YGR067C | -1.6504 | 0.000393 |
| YMR173W-A | 2.3477 | 5.10E-05 |
| YER103W | 2.1592 | 0.000119 |
| YGR087C | 1.569 | 0.000715 |
| YLR312W-A | -1.4715 | 7.10E-05 |
| YJL180C | -1.0238 | 0.000129 |
| YIL176C | 1.4294 | 0.000114 |
| YDR542W | 1.5655 | 9.20E-05 |
| YAR015W | 1.2878 | 0.000376 |
| YGR088W | 1.4151 | 0.00058 |
| YMR105C | 1.1571 | 0.000198 |
| YKL001C | 1.7033 | 0.000164 |
| YNL252C | -1.1839 | 9.80E-05 |
| YDR375C | -1.1446 | 9.90E-05 |
| YER020W | 1.2818 | 0.00021 |
| YFL062W | 1.3402 | 0.000174 |
| YPL014W | 1.3944 | 0.000581 |
| YHR100C | 2.5388 | 4.40E-05 |
| YGR146C | 1.2887 | 0.000128 |
| YPL221W | 1.2821 | 0.000128 |
| YLR142W | 2.1985 | 0.000416 |
| YPL158C | -1.0138 | 0.000808 |
| YLR126C | 1.4567 | 6.80E-05 |
| YNL241C | 1.071 | 0.000176 |

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|---|---|---|
| YCL030C | 1.1372 | 0.001202 |
| YGR256W | 3.1586 | 4.40E-05 |
| YGR084C | -1.2985 | 7.80E-05 |
| YDR343C | -2.2602 | 0.000198 |
| YGL068W | -1.3021 | 0.000164 |
| YMR230W | -1.3445 | 0.000426 |
| YMR238W | 1.4865 | 6.70E-05 |
| YHR138C | 2.2966 | 0.000148 |
| YHR143W | -1.369 | 0.001277 |
| YGL188C | -1.1437 | 0.000267 |
| YDR077W | 2.2193 | 6.30E-05 |
| YJL181W | -1.0593 | 0.000714 |
| YBR214W | 2.0366 | 0.000114 |
| YLR390W-A | 1.024 | 0.000243 |
| YIL109C | 1.1721 | 9.20E-05 |
| YLR286C | -1.5682 | 0.001389 |
| YMR039C | 1.24 | 0.000891 |
| YOL119C | 1.2955 | 0.000209 |
| YDR462W | -1.3977 | 7.80E-05 |
| YIL040W | 1.2976 | 0.001061 |
| YOL019W | 1.3103 | 0.000164 |
| YMR251W-A | 1.3748 | 0.000685 |
| YPR035W | 1.0602 | 0.001493 |
| YDL020C | 1.8543 | 0.000418 |
| YLR125W | 1.5383 | 6.20E-05 |
| YDR034CC | 2.3602 | 6.20E-05 |
| YNL284C | -1.0366 | 0.000124 |
| YCL043C | 1.8917 | 5.40E-05 |
| YOR289W | 1.0905 | 0.000185 |
| YML091C | -2.0683 | 0.000261 |
| YNR009W | -1.1875 | 0.000318 |
| YBR076W | 3.0589 | 5.40E-05 |
| YLR178C | 2.0123 | 7.20E-05 |
| YHR174W | 1.0114 | 0.000418 |
| YPL247C | 1.2919 | 0.000266 |
| YDR155C | 2.0472 | 0.000112 |
| YOR220W | 1.0945 | 0.001353 |
| YKL164C | 1.2635 | 0.000114 |
| YMR295C | 1.7123 | 7.00E-05 |
| YLR410W-A | 2.3634 | 0.000403 |
| YDL202W | -1.0127 | 0.000292 |
| YGR165W | -1.0617 | 0.000176 |
| YFR012W-A | 1.5874 | 0.000298 |
| YNR067C | -1.2236 | 0.002731 |
| YKL165C | 2.3336 | 5.10E-05 |
| YKR039W | 1.0592 | 0.004605 |
| YKL224C | 1.333 | 7.80E-05 |
| YLR225C | 1.3936 | 0.000114 |
| YJL073W | 1.2345 | 0.000152 |
| YHR001W-A | -1.0343 | 0.000184 |
| YBR268W | -1.1348 | 0.000194 |
| YJR028W | 1.39 | 0.000216 |

| | | |
|---|---|---|
| YJL017W | 1.2919 | 0.000131 |
| YIL052C | -1.225 | 0.000363 |
| YOR343W-A | 2.6344 | 8.00E-05 |
| YKR057W | -1.2229 | 0.000583 |
| YBR182C | 1.5046 | 0.000108 |
| YBR029C | -1.2195 | 0.000298 |
| YDR171W | 2.6268 | 0.000258 |
| YGL040C | -1.0275 | 0.000229 |
| YNL327W | -1.2265 | 0.001576 |
| YNR075W | 1.2616 | 8.00E-05 |
| YCR083W | 1.0387 | 0.000562 |
| YIL157C | -1.021 | 0.000191 |
| YAL068C | 1.5709 | 7.80E-05 |
| YHR087W | 2.8666 | 0.000114 |
| YDR365C | -1.4443 | 8.40E-05 |
| YBR294W | 3.5866 | 0.000191 |
| YOR035C | 1.179 | 0.000104 |
| YMR032W | -1.0713 | 0.000148 |
| YDR210W | 1.3388 | 0.000484 |
| YMR003W | -1.012 | 0.000268 |
| YDR210CC | 1.7628 | 0.000128 |
| YKL006W | -1.0586 | 0.000297 |
| YGR043C | 1.9962 | 4.40E-05 |
| YPL134C | -1.161 | 0.000114 |
| YDR043C | 1.1709 | 0.001204 |
| YDL070W | 1.1741 | 9.70E-05 |
| YPL280W | 1.357 | 7.50E-05 |
| YDR345C | -1.509 | 0.000174 |
| YHR179W | 1.9113 | 5.40E-05 |
| YCL019W | 1.3531 | 8.30E-05 |
| YBR299W | -1.3182 | 0.000164 |
| YGR060W | 1.0813 | 0.00018 |
| YOR299W | 1.0552 | 0.000339 |
| YMR320W | 1.4491 | 0.000457 |
| YMR096W | 2.4916 | 0.000185 |
| YDR025W | -1.1479 | 0.002196 |
| YPL132W | -1.2149 | 9.90E-05 |
| YAR010C | 1.5724 | 0.000297 |
| YLR430W | 1.07 | 0.000323 |
| YMR271C | 1.2167 | 0.000326 |
| YIL162W | -1.3379 | 0.000191 |
| YDR346C | -1.0654 | 0.00015 |
| YMR107W | 2.9857 | 5.40E-05 |
| YAL053W | 1.5287 | 9.50E-05 |
| YMR191W | 1.3961 | 0.00027 |
| YJR156C | 3.1044 | 9.80E-05 |
| YNL142W | 1.5452 | 0.000123 |
| YMR250W | 1.4894 | 6.70E-05 |
| YDR210W-D | 1.6705 | 0.000997 |
| YPL154C | 1.0404 | 0.000122 |
| YNL134C | 1.499 | 0.000143 |
| YFR033C | -1.3405 | 0.000256 |

| | | |
|---|---|---|
| YDR453C | 1.4105 | 7.40E-05 |
| YHR053C | 1.4095 | 0.016953 |
| YML088W | 1.1818 | 0.000416 |
| YLR121C | 2.9515 | 4.40E-05 |
| YBL101W-A | 1.6858 | 6.70E-05 |
| YPL187W | -1.7772 | 0.000114 |
| YLR423C | 1.9177 | 5.40E-05 |
| YHR104W | 1.1853 | 0.00022 |
| YOR192C-A | 2.2182 | 5.40E-05 |
| YGR038C-A | 2.0868 | 5.40E-05 |
| YBR037C | -1.1632 | 0.000258 |
| YPR167C | 1.4366 | 0.000416 |
| YIL101C | 1.6418 | 0.00022 |
| YDL234C | 1.755 | 0.000144 |
| YDR411C | 1.4934 | 8.00E-05 |
| YDR007W | 1.1237 | 0.004387 |
| YGL089C | -1.5561 | 8.40E-05 |
| YJL052W | 1.4638 | 6.20E-05 |
| YPL052W | 1.0701 | 0.000298 |
| YJL191W | -1.2794 | 0.000114 |
| YER037W | 2.9154 | 4.40E-05 |
| YDL248W | 1.6699 | 8.40E-05 |
| YLR414C | 2.1371 | 6.70E-05 |
| YOL052C-A | 2.8185 | 9.20E-05 |
| YLR124W | 1.2016 | 0.000462 |
| YCL038C | 1.2533 | 0.001826 |
| YOR153W | 1.5703 | 7.70E-05 |
| YGR214W | -1.2568 | 0.002172 |
| YMR286W | -1.0582 | 0.000326 |
| YJL016W | 1.3855 | 6.40E-05 |
| YBL101C | 1.4721 | 9.50E-05 |
| YIL072W | 1.146 | 0.000797 |
| YEL040W | -1.2185 | 0.000967 |
| YHR097C | 1.3307 | 0.000248 |
| YFR022W | 1.2008 | 0.000222 |
| YBL043W | -1.0722 | 0.000164 |
| YKR085C | -1.2233 | 0.000283 |
| YNL066W | -1.4982 | 0.000164 |
| YOR134W | 1.5892 | 0.000153 |
| YNL100W | -1.1392 | 0.000293 |
| YDR070C | 2.5841 | 0.000105 |
| YFR030W | 1.4659 | 0.000513 |
| YNL315C | -1.1003 | 0.000172 |
| YPR020W | -1.2197 | 0.000131 |
| YEL049W | 1.0734 | 0.000114 |
| YKL194C | -1.0063 | 0.000128 |
| YOR024W | -1.3063 | 8.90E-05 |
| YJR048W | -2.3465 | 5.40E-05 |
| YOR298C-A | 1.0913 | 0.002403 |
| YGL126W | 2.5723 | 5.40E-05 |
| YCR034W | -1.0433 | 0.001507 |
| YNL093W | 1.1481 | 0.001005 |

| | | |
|---|---|---|
| YGL261C | 1.4973 | 9.50E-05 |
| YPR154W | 1.9199 | 7.70E-05 |
| YOR099W | 1.2365 | 0.000326 |
| YDL072C | 1.3039 | 0.00046 |
| YPL172C | -1.1559 | 0.000157 |
| YDR322W | -1.2381 | 8.50E-05 |
| YCL040W | 1.0985 | 0.000268 |
| YJL108C | 1.1142 | 0.000129 |
| YHR014W | -1.2856 | 0.000289 |
| YIL154C | 1.289 | 0.000316 |
| YLR264W | -1.2391 | 0.000899 |
| YJR101W | -1.404 | 0.000431 |
| YBR033W | -1.0849 | 0.000774 |
| YPR001W | 1.0419 | 0.000278 |
| YKL170W | -1.0217 | 0.00024 |
| YOR343C-A | 2.8505 | 4.40E-05 |
| YCL047C | 1.0938 | 0.000148 |
| YOR128C | 1.3609 | 0.000986 |
| YJR078W | 3.1325 | 8.20E-05 |
| YNL157W | 1.0773 | 0.000233 |
| YDR277C | -1.1012 | 0.000757 |
| YLL041C | -1.2645 | 0.000237 |
| YKL138C | -1.0383 | 0.000301 |
| YJL059W | 1.0963 | 0.000975 |
| YNL036W | 3.0857 | 0.000229 |
| YNL192W | 1.8865 | 9.00E-05 |
| YLR168C | -1.5504 | 5.40E-05 |
| YGL179C | 2.1724 | 6.20E-05 |
| YJR135W-A | -1.0308 | 0.000315 |
| YNL185C | -1.191 | 0.000152 |
| YDL083C | -1.1748 | 0.000685 |
| YER072W | 1.0348 | 0.000419 |
| YNL322C | 1.1159 | 0.000783 |
| YLR109W | 1.7113 | 0.000191 |
| YNL037C | -1.3888 | 0.000167 |
| YKR093W | -1.1313 | 0.000737 |
| YER150W | 2.3668 | 5.40E-05 |
| YDR034C-D | 1.6024 | 0.000243 |
| YJR094W-A | -1.0721 | 0.000741 |
| YGR234W | -1.2913 | 0.00027 |
| YPL283C | 1.036 | 0.000449 |
| YOR187W | -1.4379 | 0.000185 |
| YIR017C | 1.399 | 0.000439 |
| YGR204W | 1.3337 | 0.000144 |
| YNR036C | -1.0445 | 0.000309 |
| YOR232W | -1.3936 | 0.000202 |
| YJR095W | -1.3732 | 0.001327 |
| YMR051C | 1.3587 | 0.000298 |
| YHR055C | 2.1425 | 0.001314 |
| YLR214W | 1.0731 | 0.000141 |
| YJL096W | -1.1857 | 0.000112 |
| YDR533C | 1.4607 | 0.00019 |

| | | |
|---|---|---|
| YKL151C | 1.7548 | 0.00025 |
| YHR112C | 1.3081 | 0.000664 |
| YCR007C | 1.8227 | 0.00029 |
| YIL148W | -1.0963 | 0.001215 |
| YPR166C | -1.0135 | 0.000125 |
| YLR231C | 1.1253 | 0.000124 |
| YML130C | 2.6871 | 7.80E-05 |
| YFL058W | 3.0032 | 5.40E-05 |
| YJR106W | 1.714 | 6.70E-05 |
| YIR035C | 1.3503 | 7.50E-05 |
| YJL159W | 1.3505 | 8.50E-05 |
| YMR104C | 1.1622 | 0.000112 |
| YLR136C | 2.3979 | 9.20E-05 |
| YLR194C | 3.4375 | 4.40E-05 |
| YGR209C | 1.3626 | 6.00E-04 |
| YEL060C | 2.8107 | 4.40E-05 |
| YMR173W | 2.7959 | 4.40E-05 |
| YDR116C | -1.2933 | 8.50E-05 |
| YLR069C | -1.151 | 0.00087 |
| YML052W | -1.5533 | 0.000153 |
| YLR304C | -1.9623 | 0.000531 |
| YMR040W | 3.5143 | 8.00E-05 |
| YJR079W | 1.783 | 8.40E-05 |
| YOL045W | 1.0688 | 0.001432 |
| YBR302C | 1.4404 | 0.000172 |
| YGR292W | -1.195 | 0.000187 |
| YNL040W | 1.1889 | 0.000356 |
| YNL144C | -1.0147 | 0.002802 |
| YCR098C | 2.7102 | 0.000104 |
| YPL223C | 3.1095 | 4.40E-05 |
| YDR210W-B | 2.2518 | 0.000114 |
| YJL138C | -1.0752 | 0.000509 |
| YNL336W | 1.7849 | 7.00E-05 |
| YMR267W | -1.3515 | 0.000164 |
| YGL121C | 2.1901 | 0.000287 |
| YNL160W | 1.8915 | 0.000287 |
| YOR158W | -1.227 | 7.80E-05 |
| YJR010W | 2.0045 | 0.000343 |
| YPL282C | 1.196 | 0.000114 |
| YJR096W | 1.8583 | 0.000187 |
| YLR120C | 2.7988 | 5.40E-05 |
| YJL171C | 1.8037 | 0.000135 |
| YPR127W | 1.6343 | 5.40E-05 |
| YCL020W | 2.3056 | 8.80E-05 |
| YIR044C | 1.2368 | 8.30E-05 |
| YML025C | -1.2032 | 7.80E-05 |
| YHL036W | 1.0733 | 0.00074 |
| YLR099C | 2.1698 | 6.10E-05 |
| YOL056W | 1.0478 | 0.000268 |
| YHR057C | 1.2352 | 0.007468 |
| YPR119W | -1.23 | 0.000693 |
| YJL107C | 1.0893 | 0.000185 |

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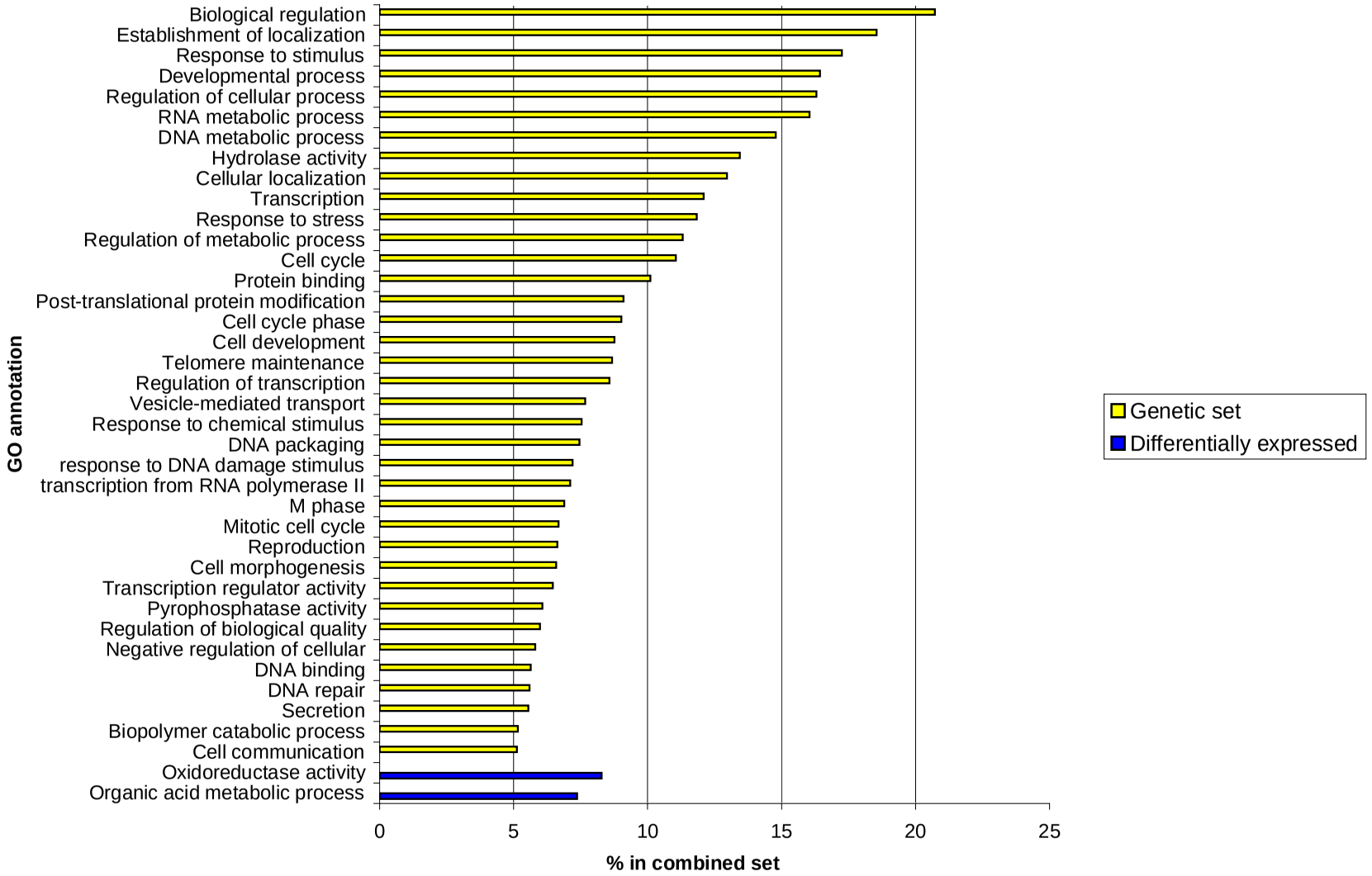
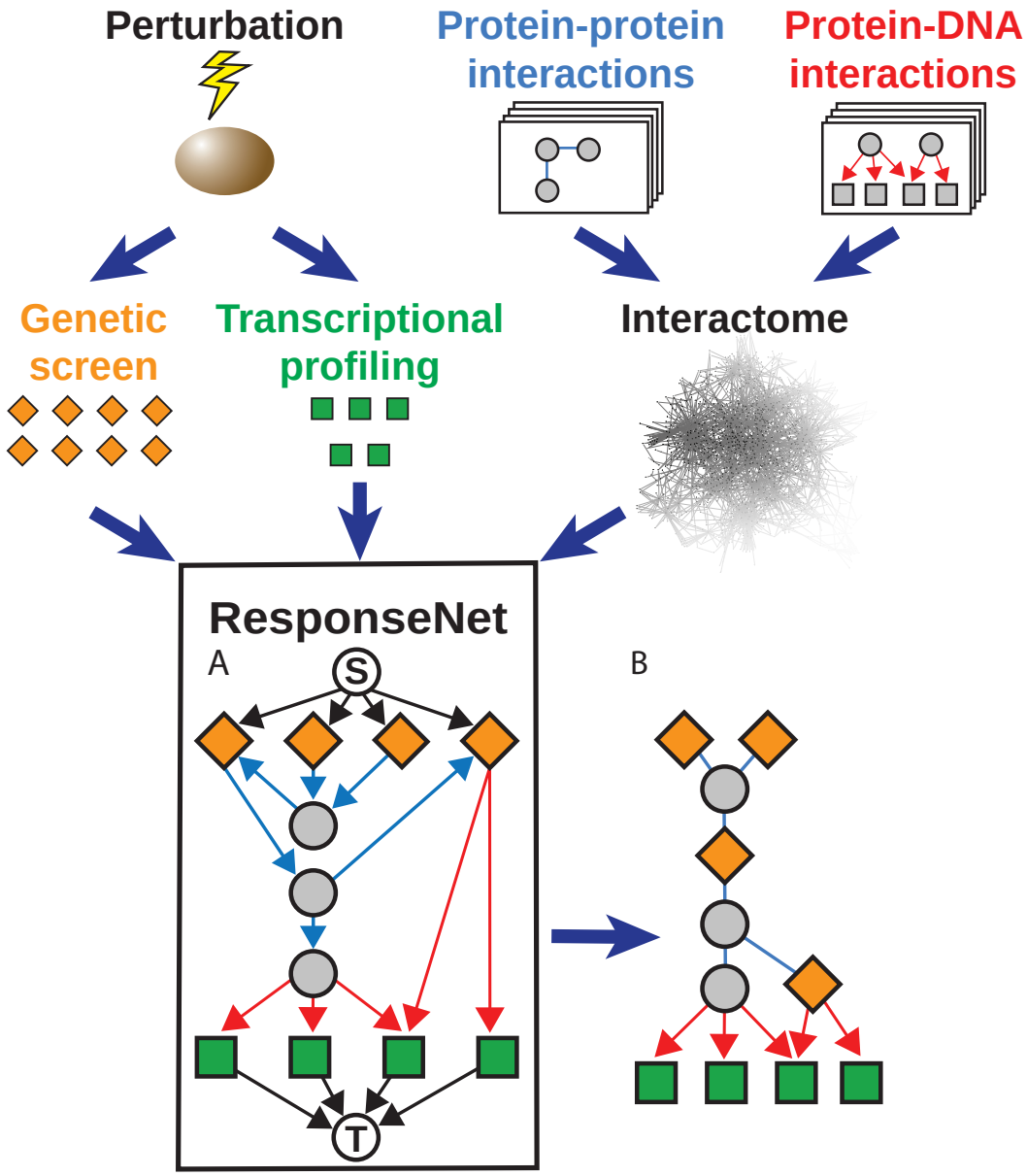
Nature Genetics: doi:10.1038/ng.337

**Supplementary Figure 1A. Graphical representation of the gene ontology (GO) annotations enriched in the combined genetic hits set and the combined differentially expressed gene set based on the perturbations in Table 1 for which complete genetic screens were available.** The annotations presented are attributed to at least 5% of the genes in the combined datasets and were also found to be enriched in at least 20% of these datasets when they were analyzed separately.



**Supplementary Figure 1B. Graphical representation of the relation between genetic and transcriptional profiling data corresponding to a specific perturbation.**
Genetic and transcriptional data are integrated with interactome data to find interaction paths through which a subset of the genetic data may regulate the transcriptional response. The regulation may be direct when the transcription factors regulating the response are part of the genetic data, or indirect via intermediate proteins. The ResponseNet algorithm is based on an optimization technique for finding sparse high-probability paths in the interactome that connect the two types of data. The result is a flow diagram (A). The directionality of the protein-protein edges in this flow diagram does not reflect the order of events but was imposed by the ResponseNet algorithm. This directionality and the auxiliary nodes S and T have no biological meaning and can be ignored (B).
Nodes represent proteins and genes, and edges represent their interactions. Diamond shaped nodes represent genetic data, rectangular nodes represent transcriptional data, and circular nodes represent intermediate (hidden) proteins on the paths that link genetic and transcriptional data.

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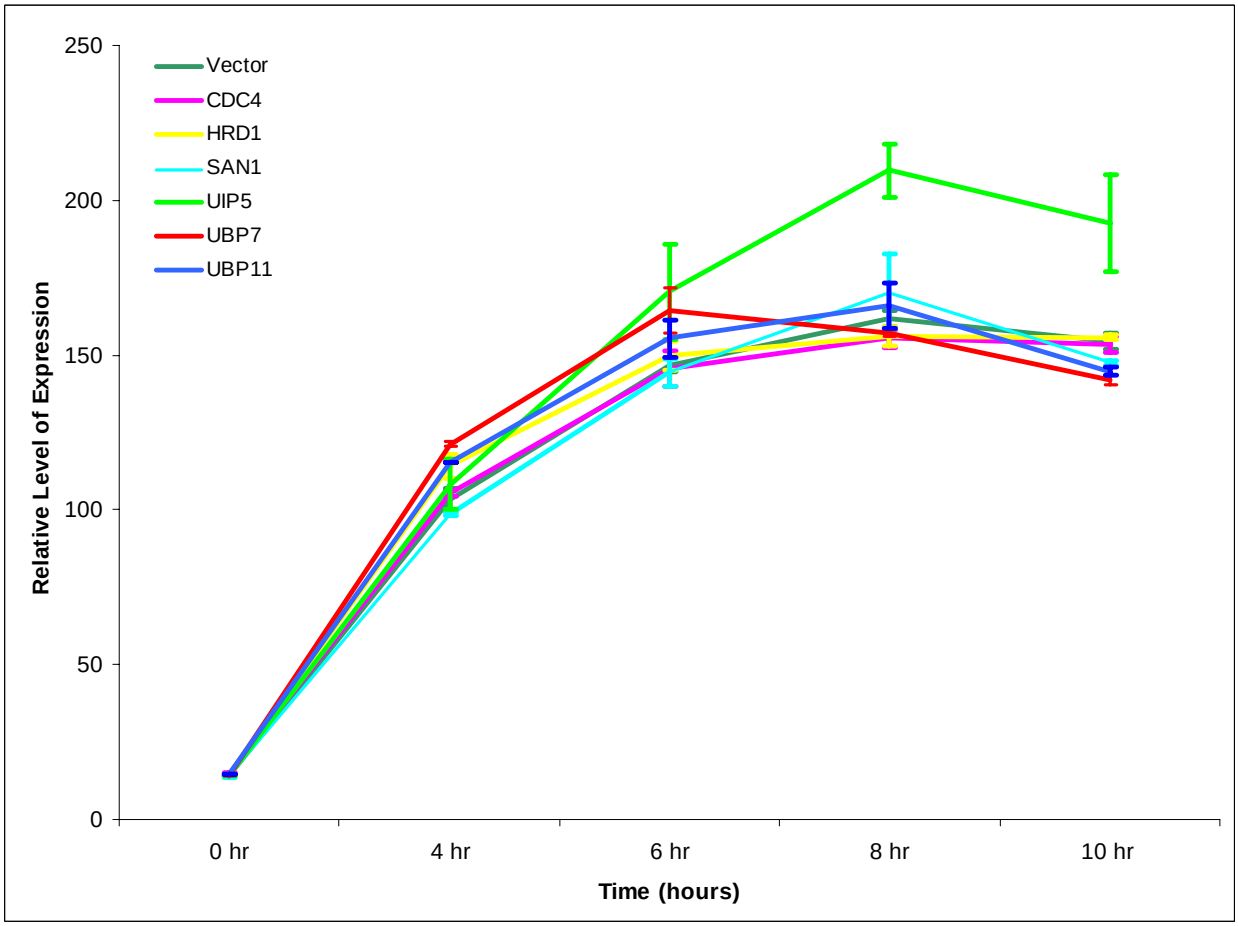
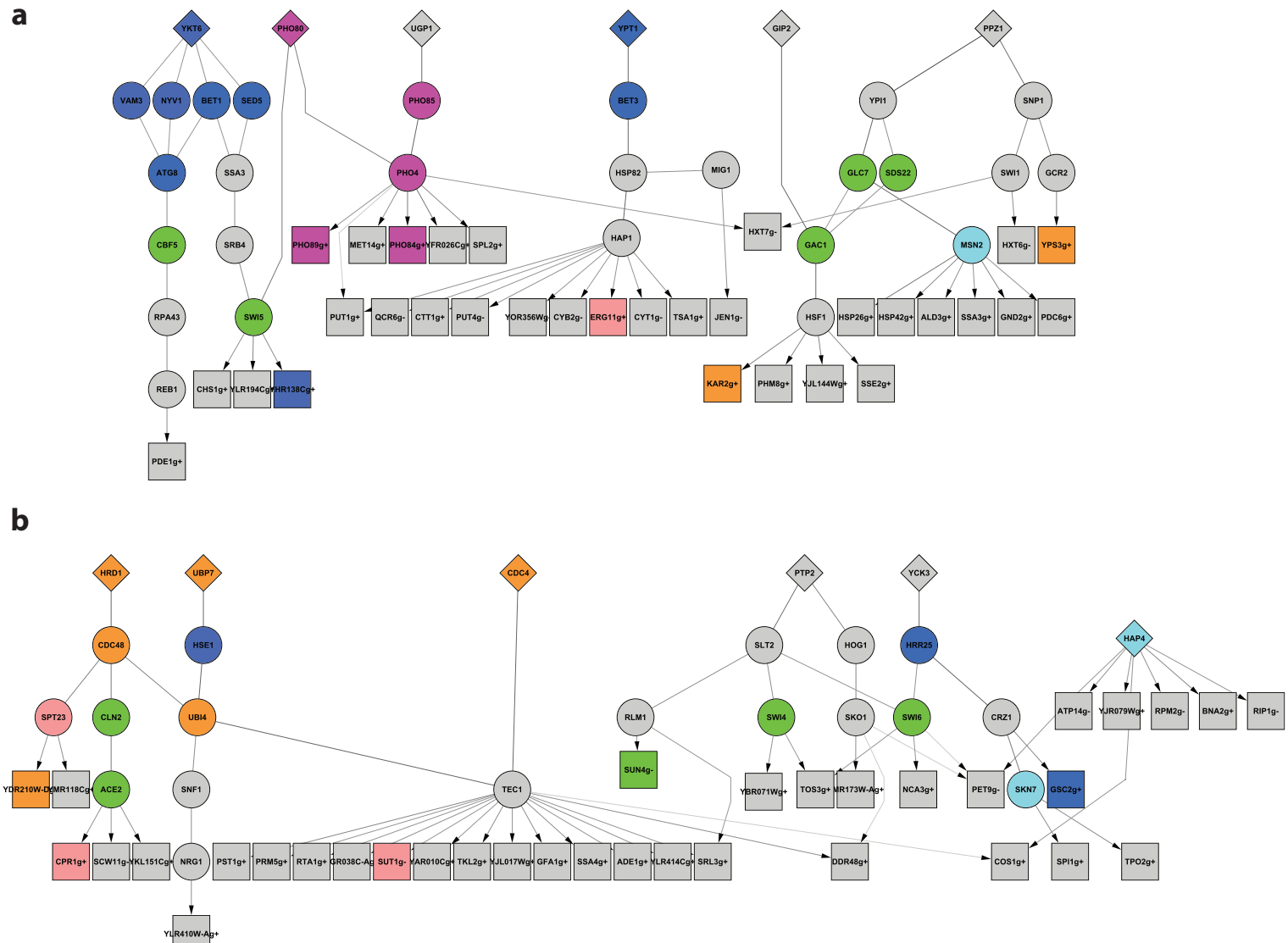
**Supplementary Figure 2. Effect of ubiquitin-related hits on alpha-synuclein expression.** We performed flow cytometry to analyze if overexpression of ubiquitin-related hits affected levels of α-syn expressed over a ten hour period using a YFP tagged α-syn strain. The only large change is due to overexpression of UIP5. When each of the strains was examined by microscopy, all showed localization similar to the vector control, except for UIP5, which showed a diffuse localization at 6 hours (data not shown). As controls we used a vector strain in which no yeast gene is overexpressed, as well as a strain overexpressing the ubiquitin-protein ligase San1 which has no affect on α-syn toxicity.



**Supplementary Figure 3A. Cellular pathways responding to α-syn toxicity predicted by ResponseNet.** The fifteen connected components were revealed by ResponseNet upon integrating the genetic and transcriptional data of the yeast PD model. Nodes represent proteins and genes, and edges represent their interactions. Diamond shaped nodes represent genetic hits (proteins that modify α-syn toxicity when overexpressed); rectangular nodes represent genes that are differentially expressed following α-syn expression; and circular nodes represent proteins predicted by ResponseNet that link genetic hits and differentially expressed genes.
Protein nodes are colored based on their GO process annotation according to the following scheme:

- Ubiquitin-related and protein degradation, colored in orange
- Vesicle trafficking, colored in blue.
- Cell cycle and meiosis, colored in green.
- Phosphate metabolism colored in purple.
- Fatty acid metabolism, colored in pink.
- Response to oxidative stress, colored in light blue.

Differentially expressed genes are labeled with a suffix of g+ for up-regulation and g- for down-regulation.

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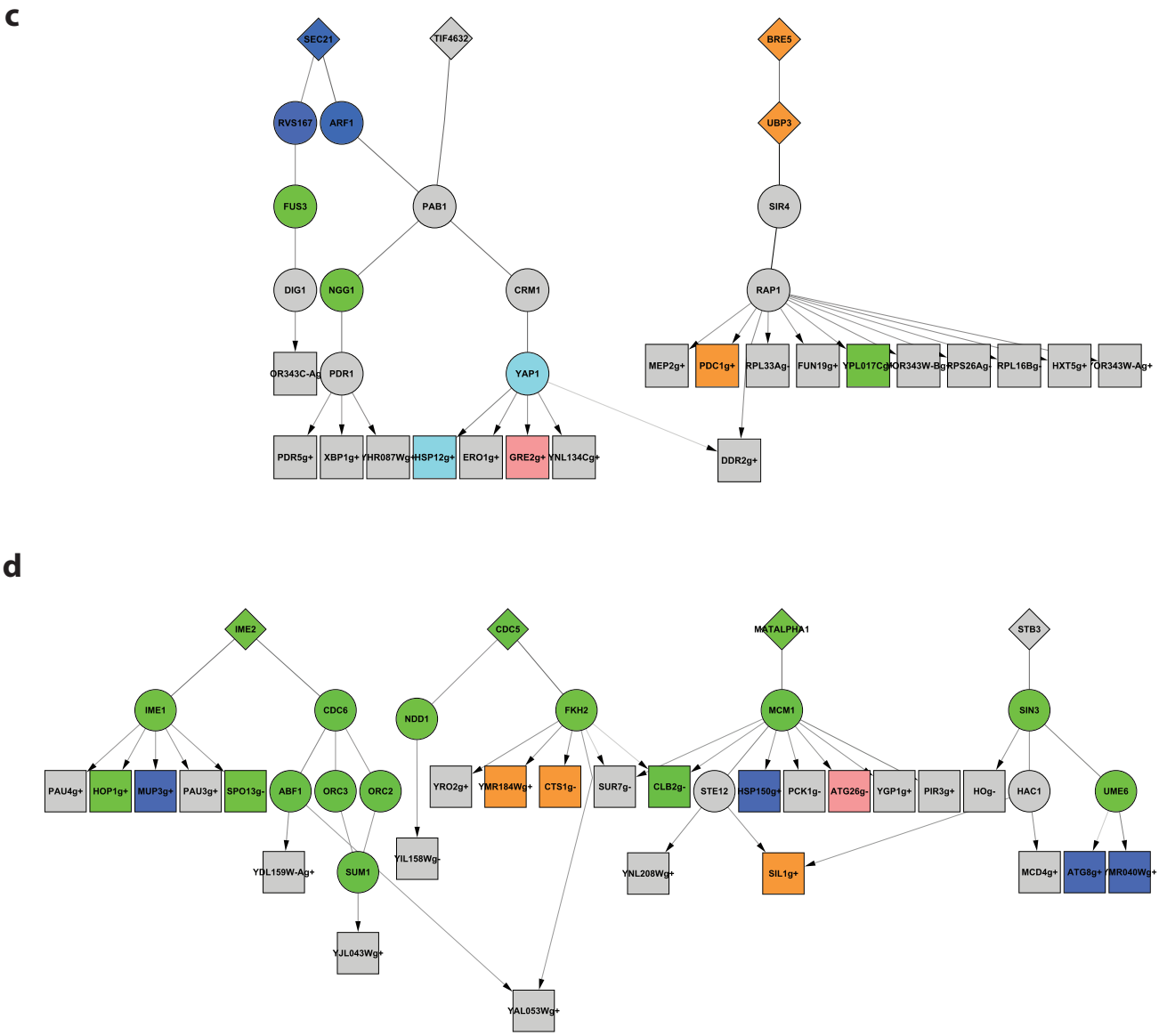
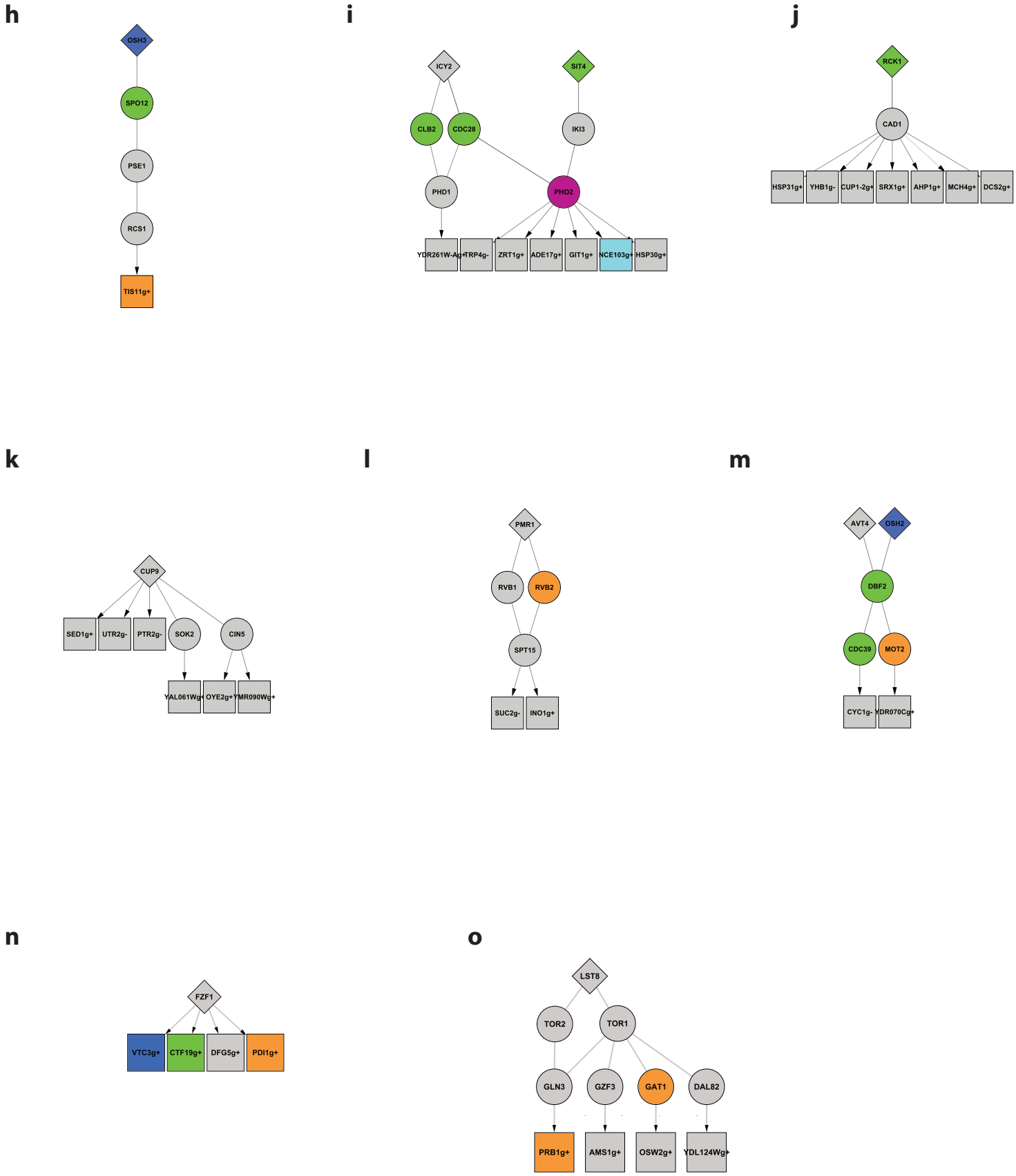



Nature Genetics: doi:10.1038/ng.337

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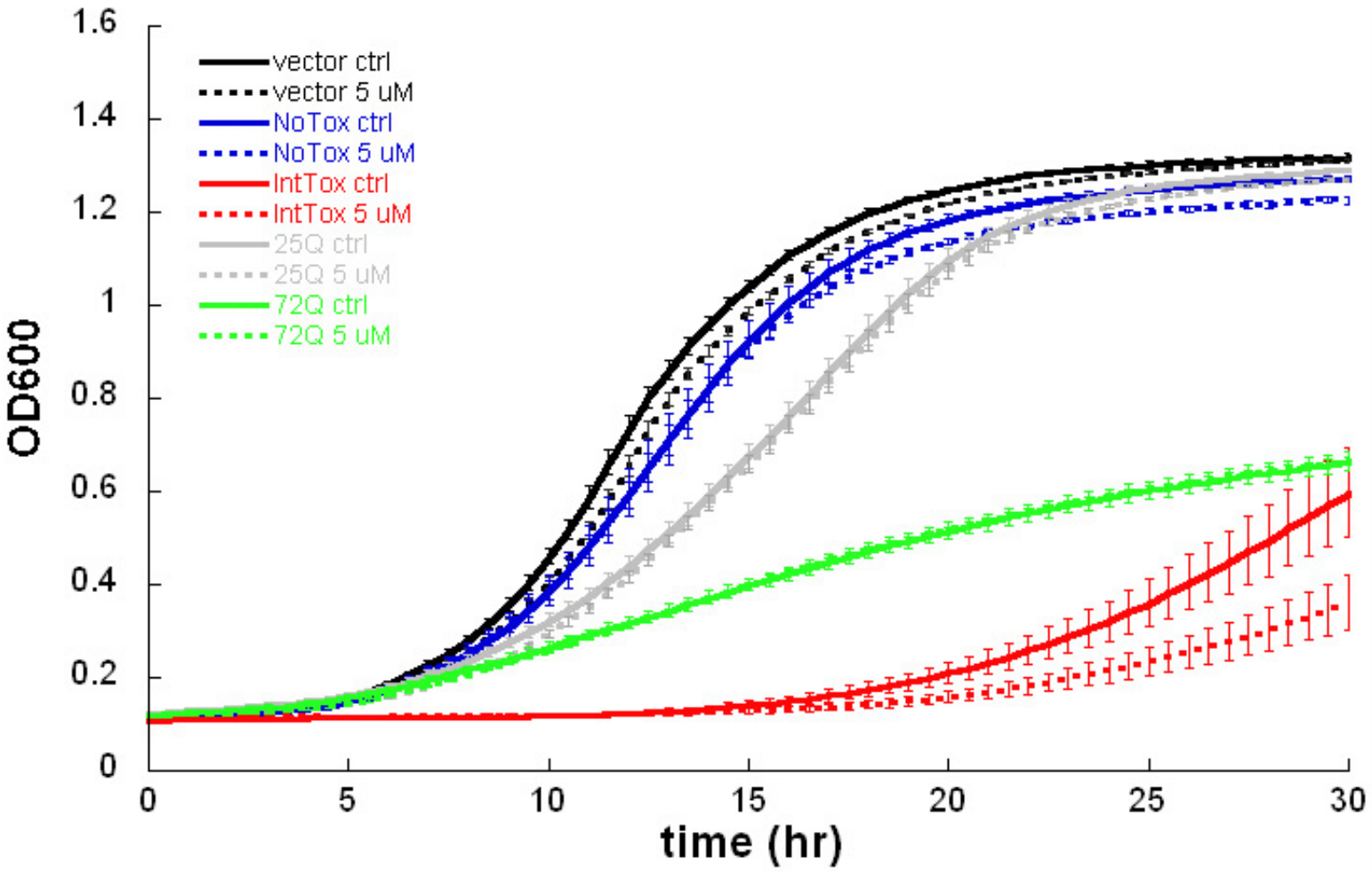
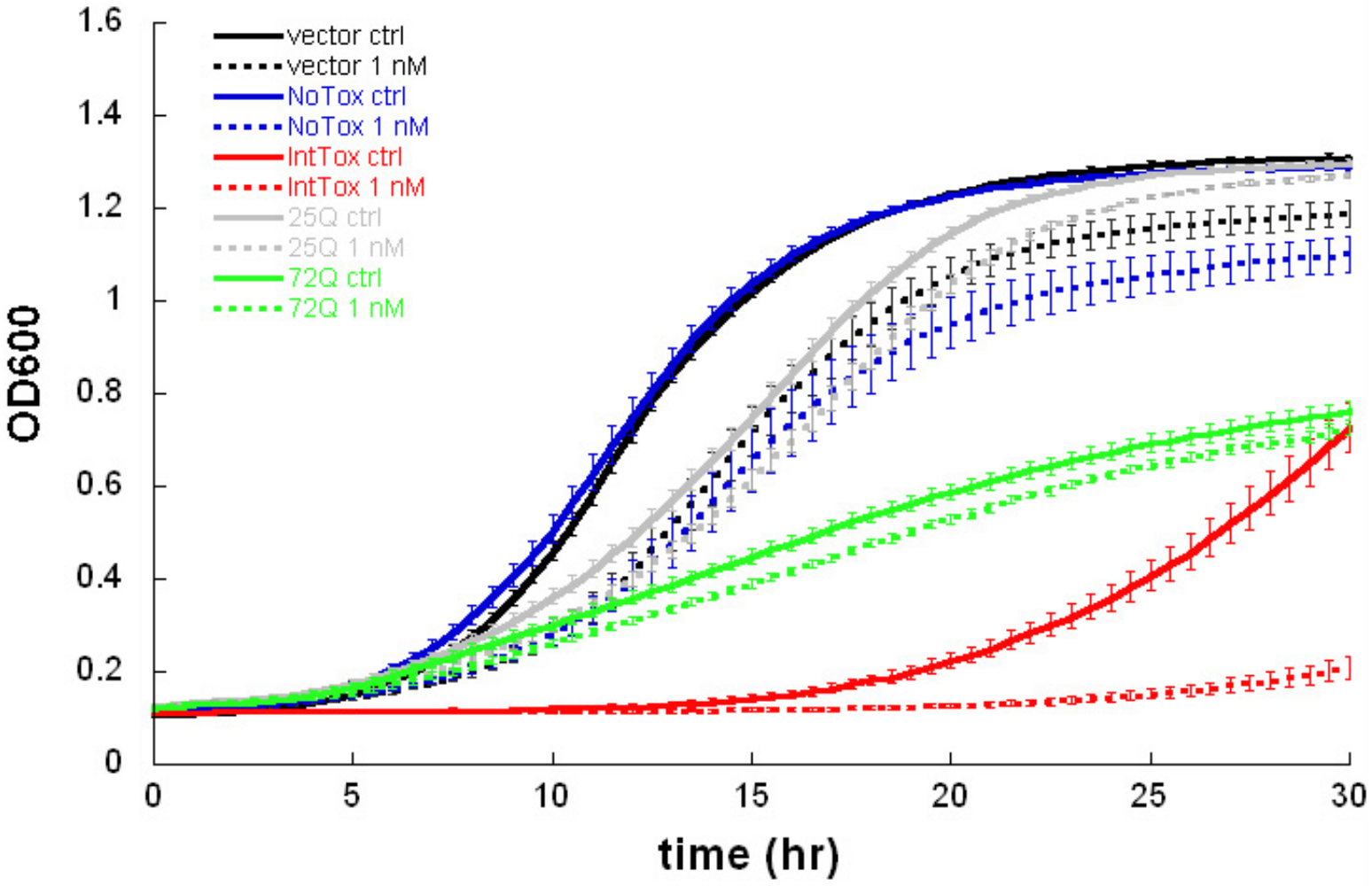
**Supplementary Figure 3B. Lovastatin inhibits growth of the yeast strain expressing several copies of α-syn but has no effect on growth of a related yeast model.** Growth of a control strain (vector), a strain expressing one copy of α-syn (NoTox), and an intermediate toxicity strain (IntTox) expressing several copies of α-syn was measured in a galactose containing media with and without 5μM lovastatin. As an additional control we tested the effect of lovastatin on growth of a related yeast model in which fragments of the human Huntingtin protein are expressed [1]. Lovastatin had no effect on the growth of either the strain expressing a slightly toxic fragment of Huntingtin containing a 25Q repeat or the strain expressing a toxic fragment of Huntingtin containing a 72Q repeat. Each growth curve reflects average of 3 individual runs marked by bars.




Nature Genetics: doi:10.1038/ng.337

**Supplementary Figure 3C. Rapamycin inhibits growth of yeast strains expressing even 1-copy α-syn but has almost no effect on growth of a related yeast model.** Growth of a control strain (vector), a strain expressing one copy of α-syn (NoTox), and an intermediate toxicity strain (IntTox) expressing several copies of α-syn was measured in a galactose containing media with and without 1nM rapamycin. As an additional control we tested the effect of rapamycin on growth of a related yeast model in which fragments of the human Huntingtin protein are expressed [1]. Rapamycin had only a slight effect on the growth of both the strain expressing a slightly toxic fragment containing a 25Q repeat, and the strain expressing a toxic fragment containing a 72Q repeat. Each growth curve reflects average of 3 individual runs marked by bars.

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**Supplementary Figure 4. Cellular response to the DNA damaging agent Methyl Methanesulfonate (MMS).** The predicted network connects 91 genetic hits whose deletion was found to be toxic in two independent screens [1,2] and nine differentially expressed genes defined as "DNA damage signature" genes[3]. MMS specific protein-DNA interactions were included in the input [4]. Due to the size of the input, the output is also considerably larger than the other networks we consider. The flow diagram contains 361 edges between 258 proteins. The predicted network contains 166 intermediate proteins and is highly enriched for response to DNA damage stimulus ($p<10^{-14}$) and DNA repair ($p<10^{-14}$). The node coloring implies the proteins importance in the response as determined by the algorithm, with increasing importance from grey to dark blue. Mec1, Rad53, Rfc2, Rfc3, Rfc4 and Rfc5 are essential genes and therefore could not have been detected via genetic screening of the deletion library.


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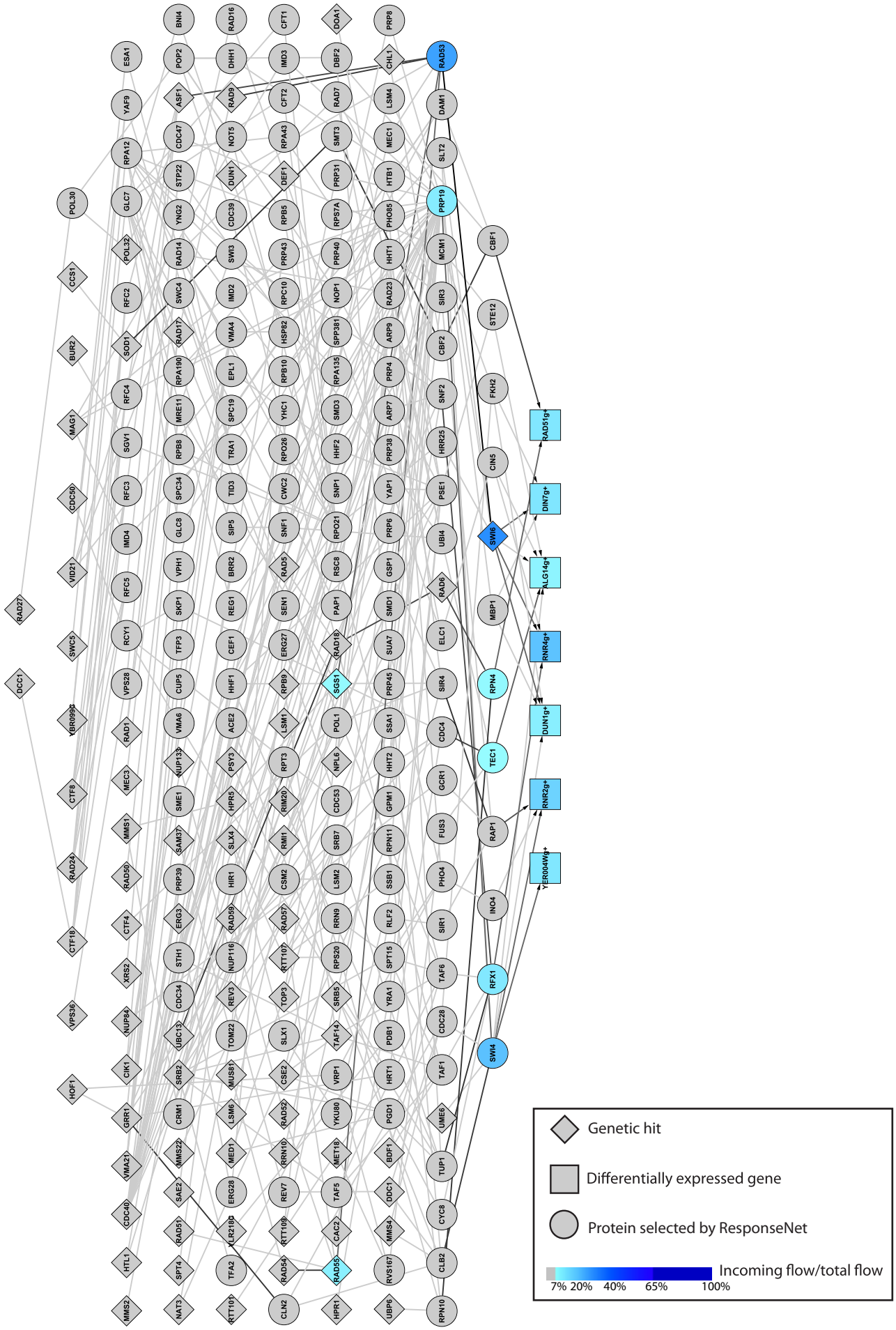


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