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Functional wiring of the yeast kinome revealed by global analysis of genetic network motifs

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Supplementary Material

Tables S1-9

Figures S1-7

Supplemental data access: <http://andrewslab.cabr.utoronto.ca/data/>

Abstract

A combinatorial genetic perturbation strategy was applied to interrogate the yeast kinome on a genome-wide scale. We assessed the global effects of gene overexpression or gene deletion to map an integrated genetic interaction network of Synthetic Dosage Lethal (SDL) and loss-of-function genetic interactions (GIs) for 92 kinases, producing a meta-network of 8,700 GIs enriched for pathways known to be regulated by cognate kinases. Kinases most sensitive to dosage perturbations had constitutive cell cycle or cell polarity functions under standard growth conditions. Condition-specific screens confirmed that the spectrum of kinase dosage interactions can be expanded substantially in activating conditions. An integrated network composed of systematic SDL, negative and positive loss-of-function GIs and literature curated kinase-substrate interactions revealed kinase-dependent regulatory motifs predictive of novel gene-specific phenotypes. Our study provides a valuable resource to unravel novel functional relationships and pathways regulated by kinases and outlines a general strategy for deciphering mutant phenotypes from large-scale genetic interaction networks.

The budding yeast *Saccharomyces cerevisiae* represents a powerful model system for exploring the roles of eukaryotic gene families. The yeast kinome, consisting of ~130 protein kinases, has been interrogated globally by large-scale mass spectrometry studies (Breitkreutz et al., 2010), consensus phosphorylation site analysis (Mok et al., 2010), and phosphoprotein identification (Albuquerque et al., 2008; Chi et al., 2007; Ficarro et al., 2002; Smolka et al., 2007). Nevertheless, our understanding of the functional roles of kinases and the biological significance of phosphorylation events remains incomplete and invites further systematic exploration.

Our limited understanding of phosphorylation networks reflects the complexity of the biological problem and is illustrated by several key observations: 1) Most eukaryotic proteins are likely phosphorylated (Bodenmiller et al., 2010; Gnad et al., 2009); 2) There are large rosters of kinases in eukaryotic cells -- ~500 in human cells and 127 in yeast -- corresponding to 2% of all genes in each genome (Pawson, 2007); 3) Kinases typically have many substrates (Sharifpoor et al., 2011), hampering detection of relevant targets; for example, ~550 distinct phosphorylation sites have been identified on 308 proteins whose abundance was influenced by the activity of a single cell cycle kinase (Cdk1) (Holt et al., 2009); 4) Recent mass spectrometric analyses of yeast kinases and phosphatases (Bodenmiller et al., 2010; Breitkreutz et al., 2010), as well as transcriptional profiling of kinase mutants (van Wageningen et al., 2010), have emphasized the complex cross-talk between kinase pathways.

Large biochemical surveys of phosphopeptide profiles provide essential information about the phosphoproteome. Yet, cogent interpretation of these datasets and other information about the kinome demands a complementary genetic approach that provides functional information about kinase regulatory pathways. Synthetic Genetic Array (SGA) analysis automates the

analysis of genetic interactions (GIs), enabling the systematic exploration of gene function (Dixon et al., 2008; Tong et al., 2001; Tong et al., 2004). In particular, SGA has been used extensively to map digenic interactions among deletion alleles of the ~5,000 nonessential yeast genes (Costanzo et al., 2010; Tong et al., 2004). Double mutants with a more severe fitness defect than expected (based on a model for the combined fitness of the individual single mutants) represent a negative GI, with synthetic lethality (SL) as the most extreme case. Conversely, double mutants that display a less severe growth defect than expected identify a positive GI (Baryshnikova et al., 2010b; Costanzo et al., 2010). Most negative and positive GIs connect genes belonging to different pathways, while a relatively small subset overlaps with protein-protein interactions and connect genes in the same pathway or protein complex (Costanzo et al., 2010; Dixon et al., 2009). The global genetic network reveals large mega-clusters of genes within the same general bioprocess and groups specific genes into coherent pathways (Costanzo et al., 2010).

A complementary approach to exploring GIs using loss-of-function alleles is analysis of interactions associated with gain-of-function alleles, including those causing overexpression or misregulation (Dixon et al., 2009; Sopko et al., 2006a). Synthetic Dosage Lethality (SDL) is based on the concept that increased gene expression levels may not affect growth of a wild type strain, but may cause a clear phenotype in strains disrupted for specific pathway components or interacting proteins with a related function (Measday et al., 2005; Sopko et al., 2006a). Indeed, we have shown that SDL screening of a cyclin dependent kinase, Pho85, can enrich for substrates as well as components of opposing pathways due to inappropriate gene regulation in sensitized genetic backgrounds (Huang et al., 2009; Sopko et al., 2006a; Sopko et al., 2007; Sopko et al., 2006b; Zou et al., 2009).

In this study, we explored yeast kinase function using different systematic genetic approaches to identify precise genetic conditions in which specific kinases are required for cellular fitness. With kinase query genes, we performed 92 global SDL screens and 75 systematic SGA genetic interaction screens. We used each dataset to interrogate kinase pathways and then integrated the two networks to identify recurring motifs that enabled functional predictions focused on kinase regulatory pathways. Our study provides a general framework for predicting phenotypic outcomes from different combinations of genetic mutations, and delineates the functional wiring of complex kinase signalling pathways by identifying highly enriched genetic motifs.

Results

Synthetic dosage lethality screens of the kinome

We performed whole-genome SDL screens for 92 non-essential kinase deletion mutants (Table S1, S2), to examine the phenotypes of over 500,000 gene pairs (Sopko et al., 2006a) (Fig S1). Dosage lethal interactions were quantified from colony size measurements, using the SGA score (Baryshnikova et al., 2010b). We applied a stringent score threshold $|\epsilon| \geq 0.2$ and a p-value < 0.05 , which resulted in a high-confidence dataset consisting of 934 dosage lethal interactions involving 69 different kinases (Fig 1A, Fig S2A, Table S3). To estimate false positive and false negative rates associated with our kinase SDL screens, a random subset of kinase-gene pairs (3,000 pairs) that fell both below and above this cut off were confirmed using an independent assay (re-transformation and serial spot assays; see Materials and Methods, Table S4). Spot assays were also quantified and assigned confidence scores according to the calculated standard deviation (Table S4). Also, interactions were only considered where the quantified growth

difference was observed in the kinase deletion strains by eye. In total, we estimated a false discovery rate of ~46% and a false negative rate of ~50% (Table S4). This analysis confirmed that the quality of our dataset was comparable to other large-scale genetic (Baryshnikova et al., 2010b; Costanzo et al., 2010) and physical interaction datasets (Breitkreutz et al., 2010) and uncovered 410 additional SDL pairs that were not identified in our high through-put assay (Table S4). In total, our SDL network represents more than 1300 interactions, of which approximately 700 are confirmed by serial spot dilutions as a secondary confirmation assay (See Methods, Table S4). Our dataset represents the first systematic and quantitative assessment of dosage-sensitive interactions, expanding the roster of dosage-sensitive GIs for yeast kinases by ~10-fold (Breitkreutz et al., 2008; Sharifpoor et al., 2011; Stark et al., 2006).

We recently developed a literature-curated database for kinases (Sharifpoor et al., 2011), termed the Kinase Information Database (KID), that we used to produce a gold standard of 517 kinase-substrate (K-S) pairs (Table S5, <http://www.moseslab.csb.utoronto.ca/KID/>). Since SDL has been used to identify targets of Pho85 (Sopko et al., 2006a), we assessed the number of known K-S pairs in the SDL interaction network. We were able to use 248 of the 517 gold standard pairs in our analysis since: 1) many well-characterized substrates in the gold standard are targets of essential kinases that could not be screened using our standard SDL screening pipeline (e.g. >90 targets for Cdc28) (Sharifpoor et al., 2011) and; 2) some known substrates were highly toxic when overexpressed or were absent from the array, preventing detection of an SDL phenotype. Of the 248 known kinase-substrate pairs that were tested in our SDL screens, 18 shared an SDL interaction (Fig S3A) which represents a highly significant 15-fold enrichment ($P < 10^{-53}$) compared to other non-substrate interacting partners of these kinases in the KID database (KID score <0) where no pairs were SDL. We repeated the analysis using another

stringent negative control to avoid any bias. In this test, 248 K-S pairs were randomly shuffled to generate a list of non-substrate interacting partners (while preserving the degree distribution of the actual K-S network) and we still observed an enrichment of known substrates among SDL pairs (2.6-fold, $P < 10^{-4}$). For example, Cla4 phosphorylates septins (Cdc10, Cdc12 and Shs1) during the G1/S transition of the cell cycle (Dobbelaere et al., 2003; Schmidt et al., 2003; Versele and Thorner, 2004) and we identified two of the five septins (*CDC10* and *SHS1*) in our SDL screen with a *cla4Δ* query strain. Also, the SDL screen for *DUN1*, a DNA damage checkpoint regulator, identified an SDL interaction with the ribonucleotide reductase inhibitor, *SML1*, which is targeted for degradation by Dun1-mediated phosphorylation during DNA damage (Zhao and Rothstein, 2002) (Fig S2A). Our analysis indicates that SDL interactions can be indicative of direct K-S relationships, emphasizing the use of this resource in identifying future targets of kinases. However, only a small portion of the kinase SDL network can be explained by known direct substrate relationships (1.3%), emphasizing that an SDL phenotype may also reflect other functional kinase-protein relationships *in vivo*.

We next assessed the biological relevance of the SDL network through comparison with other complementary large-scale datasets. Genes in the kinome SDL dataset were significantly enriched for *in vivo* phosphoproteins (1.5-fold, $P < 10^{-13}$; compiled in the PhosphoGRID database (Stark et al., 2010)), regulated phosphoproteins *in vivo* (Bodenmiller et al., 2010) (1.2-fold, $P < 0.05$), and *in vitro* phosphorylated proteins identified by protein chip experiments (Ptacek et al., 2005) (1.5-fold, $P < 10^{-5}$) (Fig S3B,S3C). While the SDL dataset did not show significant overlap with physical interactions identified by traditional affinity purification and mass spectrometry approaches (Gavin et al., 2006; Gavin et al., 2002; Krogan et al., 2006), we observed an enrichment for PPIs identified using two-hybrid (16-fold, $P < 10^{-25}$) (Ito et al., 2001;

Yu et al., 2008) and protein overexpression-mass spectrometry experiments (5-fold, $P < 10^{-6}$) (Breitkreutz et al., 2010)(Fig S3D), which have recently been shown to detect binary and/or transient interactions (Breitkreutz et al., 2010; Yu et al., 2008), consistent with the transient nature of kinase signalling pathways. Overall, our results show that the SDL dataset is not only enriched for direct substrates and phosphorylated proteins, but also for physically interacting partners of kinases and phosphatases, suggesting that the SDL network is functionally informative.

We found that the number of SDL interactions varied significantly for different kinases and for some kinases no SDL interaction was detected. We sorted the 69 kinases exhibiting at least one SDL interaction into two main groups, the ‘hub’ SDL profile subclass, which is composed of 9 kinases with more than 25 interactions, and a larger set of 60 kinases with a limited SDL profile, exhibiting between 1 and 24 SDL interactions (Fig 1A, S2A). Hub kinases often coordinate cell cycle progression and cell polarity (Cla4, Elm1, Gin4, Hsl1, Pho85, Bud32, Kin4) or regulate cell wall integrity (Bck1 and Slr2) (11-fold, $P < 10^{-4}$) and their mutants tend to have phenotypic defects that are detectable in standard growth conditions (e.g. elongated buds, flocculated colonies, aberrant cell wall involving morphologies and fitness defects) (Asano et al., 2006; Breslow et al., 2008; Edgington et al., 1999; Martin et al., 1996; Watanabe et al., 2009), reflecting their requirement for normal cell cycle progression. Consistent with these observations, the hub kinases were also significantly enriched for interactions with genes involved in cell cycle progression (11-fold, $P < 10^{-4}$, Fig S2B), which suggests that high connectivity in the SDL network may reflect a requirement for active kinases throughout the cell cycle (Bodenmiller et al., 2010).

Condition-specific SDL screens reveal expanded kinase SDL profiles

The SDL interactions derived from the hub kinase screens alone overlapped with gene pairs co-annotated to the same biological functions as frequently as the complete set of SDL interactions identified from screening 92 kinase mutants (Fig S2C). This observation suggests that almost all of the functional connections associated with the SDL interactions identified are derived from the hub kinase genes and that most kinases may only be activated under specific genetic or environmental conditions. To address this hypothesis, we performed SDL screens for a subset of kinases under gene-specific biological conditions.

We first screened a *hog1Δ* kinase mutant strain for SDL interactions in the presence of salt stress. Hog1 is required for the high osmolarity glycerol response and translocates to the nucleus to activate gene expression in response to osmotic stress (Reiser et al., 1999). *HOG1* failed to show any SDL interactions under standard growth conditions (Fig 1B). In contrast, a *hog1Δ* screen in the presence of 0.2M NaCl identified 78 SDL interactions (Fig 1B, Table S6). The salt-specific *hog1Δ* SDL profile was enriched for genes involved in transcription and chromatin remodelling ($P=1.2 \times 10^{-4}$), consistent with known features of Hog1, which include binding to the RNA polymerase subunit Rpb2 at gene promoters (Alepuz et al., 2003; Mas et al., 2009) (Fig S4A). Spot dilution assays were used to confirm all SDL interactions, including Rpb2 (Fig 1C).

A second condition-specific SDL screen with a calmodulin-dependent protein kinase, *CMK1*, also uncovered relatively few interactions in standard growth conditions. *CMK1* has reported roles in stress response and is sensitive to calcium levels (Pausch et al., 1991). We identified 11 new SDL interactions when the *CMK1* SDL screen was conducted in the presence of calcium (Fig 1B, Table S6) and the confirmed SDL interactions were enriched for factors including vesicle-mediated transport genes required for calcium signalling ($P=5 \times 10^{-3}$) and calcium-responsive proteins (Table S6, Fig S4B). Thus, our results suggest that the apparent resistance of

many kinase mutants to gene overexpression reflects the requirement for kinases only in certain activating conditions and that conducting screens in a variety of biologically relevant conditions will substantially increase our view of kinase biology, particularly regarding kinase dosage sensitivities.

Loss-of-function SGA genetic interaction screens of the kinome

In addition to environmental cues, SDL interaction degree is likely dependent on the extent of functional redundancy shared between different kinases. Thus, we also assembled genome-wide surveys of the kinase genes that displayed SDL profiles for loss-of-function SGA GIs. We included genetic interaction data for 45 kinases from Costanzo *et al*, 2010 (Costanzo et al., 2010) (37 of which provided SDL interactions) and performed genome-wide SGA screens for an additional 30 kinase queries (Table S7, Fig S5). In total, we identified 2789 unique negative and 203 positive high-confidence interactions (SGA score: $\square < -0.12$, $\square > 0.16$, $P < 0.05$) (Table S7). Many different kinase mutants displayed a relatively large genetic interaction profile, revealing the genetic conditions under which these kinases are required for cellular fitness. For example, while *HOG1* failed to show any SDL interactions under standard growth conditions, it showed a substantial number of negative SGA genetic interactions (Fig S2, S5).

In a previous study (Fiedler et al., 2009), Fiedler *et al.* assessed GIs among a selected subset of ~400 genes encoding kinases, phosphatases, and potential pathway components. They report a bias towards the occurrence of positive GIs between kinases and their substrates. We observed a similar trend using our genome-wide approach and a new gold standard list of K-S pairs (Fig 1D). Our results also indicate that both positive and negative genetic interactions are enriched amongst kinase-kinase (K-K) pairs, including those that share the same target (Fig 1D).

To address whether K-K redundancy was contributing to the lack of an SDL phenotype, we assessed the number of positive and negative GIs amongst K-K pairs, including those that share the same substrate. We found that the ratio of positive to negative GIs remains unchanged for K-K pairs, ($P=0.45$, Fig 1D) when being compared to kinase-any pairs. In fact, negative interactions between K-K pairs and between random gene pairs occurred at the same frequency when assessed across the genome ($\sim 2\%$) [90 negative interactions among 4359 unique tested pairs]. Also, of the 99 K-K pairs in the SGA dataset that share at least one substrate in our K-S gold standard (Sharifpoor et al., 2011) (Table S5), 10 kinase pairs showed a negative ($P<10^{-12}$) GI with each other, while 4 pairs displayed a positive interaction ($P<10^{-4}$), corresponding to a 7- and 6-fold enrichment respectively. This analysis suggests that K-K pairs in general can have both positive and negative GIs (Fig 1D), including those that share the same target. Consistent with a complex model of genetic interactions between K-S and K-K pairs, we found that genes encoding proteins that physically associate with kinases may positively (3-fold enrichment, $P<0.005$) or negatively interact with the kinase (3-fold enrichment, $P<10^{-4}$) (Breitkreutz et al., 2010). Together our analysis shows that redundancy among kinases at a digenic level is not a major contributor to the buffering effect of kinase pathways and that K-K pairs regulating the same target can have either positive or negative GIs with each other. Interestingly, when we assessed the patterns of K-S and K-K enrichments for SDL interactions, we found that not only are K-S pairs more likely to show an SDL interaction than random, but also SDL interactions are highly enriched amongst K-K pairs including those that share the same substrate (Fig 1D). Our combined analysis, suggests a highly complex model of regulation in kinase pathways that requires more detailed network analysis for understanding kinase interactions.

Integration of the SDL and SL datasets produces a functionally informative meta-network

Consistent with other work (Kelley and Ideker, 2005; Lee et al., 2008), we reasoned that integration of our kinase datasets should enable identification of regulatory motifs that provide insight into the complexity of kinase pathways and kinase function. This is particularly interesting in the context of SDL, since many SDL interactions could not be explained by literature-curated K-S relationships. Therefore, we first leveraged the SDL interaction network with the complementary SGA dataset to produce a genetic interaction meta-network. Next, we super-imposed our literature-curated K-S dataset onto our meta-network to search for triplet sub-networks involving all possible combinations of SDL and double deletion mutant interactions (See Methods). In total, the meta-network was based on 69 SDL and 75 SGA kinase screens and includes 1,344 dosage interactions, 7,427 double mutant interactions, and 517 literature-curated K-S pairs (Sharifpoor et al., 2011). We also included 71,886 interactions among gene pairs if the pair was one-hop away from a kinase in the global genetic interaction network (Costanzo et al., 2010). We applied an automated network motif detection algorithm to identify 3-gene motifs that are significantly overrepresented in our integrated genetic network. Triplet motifs derived from our integrated network were statistically enriched compared to motifs derived from randomized networks ($P < 0.05$). We identified 2685 motifs consisting of 12 different combinations of SDL, SGA and literature curated interactions (Fig 2, Fig S6, Table S8). We reasoned that each motif in this resource may be associated with a specific biological interpretation from which mutant phenotypes and regulatory relationships can be inferred. Therefore we tested 3 of the 5 most common motifs for biological relevance.

First, we examined what we have dubbed the ‘counteracting’ motif category which involves two SDL interactions connected by a known K-S pair, where the substrate may also be another kinase, such as an interacting downstream kinase within a cascade (Fig 2A, 5.8-fold). For

example, Bck1, a MAP kinase kinase kinase (MKKK) is connected indirectly to the cell wall integrity MAP kinase (MAPK) Slt2 through the Mkk1/2 MAP kinase kinases (MKK). Both Bck2 and Slt2 showed SDL interactions with an uncharacterized gene *YMR074C*, encoding a protein with homology to human *PDCD5*, which is expressed in tumor cells during apoptosis (Hong et al., 2009)(Fig 3A). A simple model for SDL interactions predicts that Slt2 may negatively regulate the function of Ymr074c, perhaps through direct phosphorylation. Alternatively, Ymr074c may act in opposition to inhibit the cell wall integrity pathway (Fig 3B). In tests for a functional connection between Slt2 signalling and Ymr074c, we found that deletion of *YMR074c* partially suppressed the fitness defect of both *bck1Δ* and *slt2Δ* deletion alleles in the presence of caffeine, a stress-inducing reagent (Fig 3C). Importantly, we tested all 30 genes that showed a similar pattern of interaction with *bck1* and *slt2* for sensitivity to caffeine and a second cell wall stressor, CalciFluor White (CFW) and found that deletions of 6 genes (*SNF7*, *COQ1*, *KRE6*, *PEP7*, *CHO2*, *SBE22*) (Table S9) suppressed the sensitivity of *bck1Δ* and/or *slt2Δ* strains to cell wall damaging agents (2-fold enrichment, $P < 0.03$). Thus, the motif produced by analysis of our integrated genetic network was highly predictive of the mutant phenotypes of kinase regulated genes.

We refer to another significantly enriched motif as ‘balancing co-regulation’ (Fig 2B, 16,000-fold, $P < 10^{-10}$). This motif involves a gene connected to both a kinase and one of the kinase substrates by an SDL and a negative GI, respectively. We suspect that this motif may identify proteins or protein complexes regulated by the same kinase, but in different ways (positive versus negative regulation). For example, one ‘balancing co-regulation’ motif involved the Cla4 kinase, which is required for phosphorylation and proper localization of Lte1, a surface-localized mitotic exit regulator (Hofken and Schiebel, 2002; Seshan et al., 2002; Yoshida et al., 2003).

CLA4 showed an SDL interaction with *BEM1*, which in turn had a negative genetic interaction with *LTE1* (Table S7). Bem1 also physically interacts with Cla4 (Bose et al., 2001; Gulli et al., 2000) and this complex is required for phosphorylating Cdc24 (the guanine nucleotide exchange factor for the master regulator of cell polarity Cdc42), a modification that ultimately inhibits bud growth. The *CLA4-LTE1-BEM1* balancing co-regulation motif (Fig 2B) suggests that Cla4 inhibits its target, the Bem1-Cdc24 complex, but activates Lte1 in the mitotic exit network, thereby coordinating cell polarized growth with cell division. In this case, our motif analysis accurately identified known regulatory relationships in a complex pathway controlling mitotic exit, validating the approach. We further predicted phenotypes for genes belonging to 7 ‘balancing co-regulation’ motifs involving the cell wall integrity kinases Bck1 and Slt2 and found that deletion of 4 out of 7 tested genes, (*SMY1*, *EDE1*, *PEX19*, *CHO2*) (Table S9) suppressed the sensitivity of *bck1Δ* and *slt2Δ* mutants to cell wall damaging agents (5.5 fold enrichment, $P < 0.003$), suggesting that these genes are likely involved in cell wall integrity (Table S9).

We called a third highly enriched motif ‘converging regulation’ (Fig 2C), in which a kinase pair, or a kinase-gene pair, negatively interact and both share an SDL interaction with a third gene. This motif class may identify kinases or enzymes that act synergistically to control a biological outcome through the same gene. Alternatively, kinases involved in the same motif may regulate each other to then influence a downstream target. We explored converging regulation motifs by testing regulatory relationships predicted by another motif involving Cla4. As noted earlier, Cla4 is required for phosphorylation and proper localization of Lte1 *in vivo* during mitotic exit (Hofken and Schiebel, 2002; Seshan et al., 2002; Yoshida et al., 2003). Localization of Lte1 to the cortex also requires its physical association with the scaffold protein,

Kel1 (Seshan et al., 2002). We identified a converging regulation motif in which the genes encoding Cla4 and Hsl1, another cell polarity kinase, showed a negative genetic interaction with each other and shared an SDL interaction with *KEL1* (Fig 4A). This motif led us to make two predictions: [1] Cla4 and Hsl1 have a synergistic and direct role in regulating Kel1 and; [2] Hsl1 may have a previously unappreciated role in Kel1 regulation – since the primary role for Kel1 during the cell cycle is to anchor Lte1 to the cortex (Hofken and Schiebel, 2002; Seshan et al., 2002), it follows that Hsl1 may regulate the association between Kel1 and Lte1. We tested these predictions by analysing Kel1 protein and its association with Lte1 in *cla4* and *hsl1* mutant strains (Fig 4B, 4C). First, we analysed Kel1 protein by western blot and saw reduced Kel1-TAP fusion protein in both *hsl1* Δ and *cla4* Δ deletion mutant strains, (Fig 4B), but not in a strain deleted for *gin4* Δ , another budneck kinase that is also SDL with *KEL1* (Fig S7). Slow migrating isoforms of Kel1 were also reduced in the *cla4* mutant, consistent with a kinase-substrate relationship (this could not be assessed in the *hsl1* mutant, since protein levels were so low). Second, we used co-immunoprecipitation to show that the interaction between Kel1 and Lte1 (Hofken and Schiebel, 2002; Seshan et al., 2002) was substantially reduced in an *hsl1* Δ mutant but not a *gin4* Δ mutant, indicating a requirement for Hsl1 in Kel1 function (Fig 4C). Taken together, these data confirm the predictions of the converging regulation motif and suggest that Hsl1 and Cla4 may function together to regulate Kel1 function in mitotic exit.

Discussion

Here, we describe an integrated genetic network combining comprehensive SDL interactions with positive and negative genetic interaction data (GIs) for yeast kinases. Our analysis identified several general properties of kinase-SDL interactions: (1) kinase-SDL interactions are enriched for known K-S pairs, physically interacting partners of kinases, and phosphoproteins;

(2) many SDL interactions cannot be explained by direct kinase-substrate relationships, suggesting that the SDL interactions also probe other aspects of kinase biology and complement existing biochemical surveys of kinase targets; (3) kinases with extensive SDL profiles tend to have constitutive roles throughout the cell cycle; (4) the majority of yeast kinases are relatively insensitive to dosage lethal interactions under standard growth conditions. The results of our SGA loss-of-function genetic interaction analysis and other work (Costanzo et al., 2010; Fiedler et al., 2009; van Wageningen et al., 2010) suggest that kinase redundancy, at least at the double mutant level, does not explain the limited SDL interaction profiles for most kinases. Rather, our findings suggest that condition-specific requirements may explain the resistance of many kinases to gene overexpression in standard growth conditions, a result that is consistent with other studies (Harrison *et al*, 2007). Thus, in principle, to understand the full scope of kinase biology, HTP assays will need to be performed in multiple conditions.

Importantly, in addition to condition specificity, kinases exhibit extensive functional redundancy with other cellular pathways as demonstrated by our SGA analysis (Fig 1A, 1D). On average, a single kinase exhibits 29 synthetic lethal/sick interactions (SGA stringent score ≤ -0.12 ; Table S7) indicating that there are approximately 29 genetic backgrounds that require the activity of a given non-essential kinase to maintain a wild-type level of fitness. Thus, systematic SGA analysis has identified a defined set of genetic conditions to explore and expand the kinase SDL interaction network.

Our SDL and SGA profiles for yeast kinase mutants are clearly rich in functional information, since we see many biologically relevant effects. However, we do expect some allele-specific effects for individual genetic interactions with overexpression data, since we made use of the GST-tagged alleles in our systematic SDL screens. For example, overexpression of either an N-

terminally GST-tagged- or C-terminally HA-tagged allele of *KEL1* caused a clear SDL phenotype in *cla4*, *hsl1* and *gin4* deletion mutants, but the degree of toxicity was sometimes dependent on the *KEL1* allele (Fig S7). In a similar experiment, we assayed overexpression toxicity for kinases carrying either an N-terminal GST or a C-terminal HA tag; of the 23 toxic kinases, 12 exhibited a clear fitness phenotype with both alleles, although the degree of growth defect depended on the tag in some cases (data not shown). Since SDL interactions measure overexpression phenotypes in different genetic backgrounds, we would anticipate a comparable degree of overlap between our SDL dataset and screens done using a differentially tagged or untagged collection. We note that these results are comparable to allele-specific effects seen in other large-scale studies. For example, genetic interaction profiling of essential genes using dAMP versus temperature-sensitive alleles revealed clear and significant overlap in genetic interaction profiles but individual genetic interactions varied for the alleles (~50% overlap in individual genetic interactions) (Davierwala et al., 2005). Although allele-specific effects are expected, our experiments illustrate the value of considering both individual genetic interactions and genetic interaction profiles (as in our motif analysis) to draw novel biological conclusions.

Previous reports using a relatively small subset of kinase pathway mutants suggested a bias towards positive GIs between K-S pairs (Fiedler et al., 2009), which is consistent with simple cascade models of kinase signalling. Though, we confirmed such an enrichment using our genome-wide approach, our additional systematic screens suggest that kinases more often participate in multifaceted networks. Several lines of evidence support this idea: 1) K-K pairs that share the same target in the gold standard have both negative and positive GIs; 2) K-S pairs are mostly enriched for positive GIs, but may show negative GIs; 3) kinase pathways show a large number of GIs with other enzymes; 4) there is a surprisingly small number of redundant

kinase pairs; 5) combinatorial motif analysis revealed enrichment for both diverging and converging genetic motifs; 6) differential physical associations can often dictate the multifunctional properties of a kinase (Breitkreutz et al., 2010). These results emphasize the importance of performing complementary genome-wide screens and the use of accurate gold standards to define kinase-substrate pairs, in order to accurately assess the functional relationships between regulatory proteins and their targets. Our model agrees with recent phosphoproteomic analysis of kinase pathways that does not support simplistic linear signalling modules among kinases and phosphatases (Bodenmiller et al., 2010; Breitkreutz et al., 2010).

Motif analysis using the meta-network of kinase GIs in combination with our literature curated K-S gold standard list was key in providing an informative view of regulatory relationships between kinases, their substrates and other proteins. For example, one ‘balancing co-regulation’ motif predicted a previously unappreciated regulatory relationship between the budneck kinases Hsl1 and Cla4 and the Kel1 scaffold protein. In this case, computational analysis of our meta-network prompted experiments that led us to discover that: [1] Kel1 protein stability is regulated by Cla4 and Hsl1; [2] optimal association of Kel1 with Lte1, which it anchors to the cell cortex, requires Hsl1 kinase. These and other experiments lead us to propose that Hsl1 regulates Kel1 protein levels to coordinate Cla4 activation of Lte1, with the presence of the Kel1 anchor required for mitotic exit. In the *hsl1* mutant, where binding of Kel1 to Lte1 is reduced, excess levels of Kel1 may force binding of unphosphorylated Lte1 to the membrane to induce premature mitotic exit (Fig 4D). Our functional motif predictions, combined with biochemical data, strongly suggest that Hsl1 is a key regulator of Kel1 and that the cell has adopted mechanisms to co-regulate the levels of active Lte1 protein and its anchor, Kel1 (Fig 4D).

In addition to the 3 motif categories tested - counteracting, balancing co-regulation, and converging regulation - we discovered 9 additional predictive motifs that were enriched in the meta-network (Fig 2, S6). These included ‘diverging regulation’ motifs where SL gene pairs share an SDL interaction with the same kinase (Fig 2D), possibly predicting gene pairs co-regulated by the same kinase and ‘SDL cascades’ comprised of two ordered SDL interactions between kinase pairs connected to a SL interaction between one of the kinases and another gene (Fig 2E). The latter motif predicts a scenario where a kinase-gene pair may function in parallel to activate a biological response that is inhibited by another kinase. Other highly enriched motifs involving only positive and negative GIs and known K-S pairs (many of which are supported by published data) include those that identify upstream and downstream regulators of K-S pathways, diverging targets of the same kinase, converging kinases regulating the same target, and triply redundant genes (Fig S6, Table S8). Together, the presented motifs provide a resource for additional focused small-scale and bioinformatics analyses that will expand our knowledge of kinase biology.

In general, our study outlines a framework for combining systematic loss- and gain-of-function genetic interaction networks to make phenotypic predictions that we anticipate will become an increasingly valuable approach towards understanding the genotype-to-phenotype relationship, as we continue to map genetic interactions in yeast and higher eukaryotes.

Materials & Methods

Synthetic Dosage Lethal Screens

The SDL screening protocol was adapted from (Sopko et al., 2006) and is summarized in Fig S1 (Sopko et al., 2006a). Refer to the online supplementary materials and methods for detailed SDL

screening and confirmation protocol (<http://andrewslab.cabr.utoronto.ca/data/>). In brief, a collection of yeast strains deleted for query kinases was constructed in a strain background compatible with SGA analysis (Tables S1,S2) (Costanzo et al., 2010). Kinase deletion mutants (marked with the *NAT^R* cassette) were crossed to a *GST-ORF* overexpression array (Costanzo et al., 2010; Sopko et al., 2006a) (Table S2) using the SGA protocol (Tong et al., 2001). Screens were performed in a 1536 colony format, where each colony is represented 4 times on the array, producing 8 replicates per gene. Gene overexpression was induced by pinning the final haploid array onto medium containing 2% galactose and plates were then incubated at 30°C for 2 days. Colony sizes were quantified using an adapted SGA protocol (Baryshnikova et al., 2010a). SDL and SDS interactions were scored using the previously described SGA score method with the assumption that no kinase queries have significant fitness defects on the final selection plates. SDL and SDS interactions were defined as gene pairs that satisfy the following SGA score cut off: $|\epsilon| \geq 0.2$, $P < 0.05$ (Table S3). To estimate false discovery rate, we confirmed over 3,000 interactions by direct transformation both in wild type and mutant backgrounds followed by serial spot assays where a difference in colony size was observed in the kinase deletion strains by eye. Spot assays were quantified and assigned confidence scores according to the calculated standard deviation (Table S4). In total, we estimated a false discovery rate of ~46% and a false negative rate of ~50% (Table S4), which is comparable to rates observed for other large-scale genetic (Baryshnikova et al., 2010b; Costanzo et al., 2010) and physical interaction datasets (Breitkreutz et al., 2010). Conditional SDL screens were performed as above but final haploid arrays were pinned onto the medium containing the appropriate inducer at a concentration reported to be consistent with viability. Kinase mutants with mating defects (*STE7*, *STE11*, *STE20*) or those otherwise refractory to the SGA protocol (*BUB1*, *IRE1*, *SSN3*, *SSK22*, *SCH9*)

were excluded in this study. We were unable to make query kinase deletion strains for 10 kinases for technical reasons (Table S1).

Negative and positive genetic interaction screens

For analysis of growth defects in double deletion mutants, the *kinaseΔ::NAT^R* (Table S2) query strains were crossed to the viable haploid deletion array (Baryshnikova et al., 2010a; Giaever et al., 2002) as previously described (Baryshnikova et al., 2010a) . Negative and positive SGA genetic interactions were quantified as described elsewhere (Baryshnikova et al., 2010b) . An intermediate SGA score cut off: ($|\varepsilon| \geq 0.08$, $P < 0.05$) was applied to SGA data as recommended elsewhere (Baryshnikova et al., 2010b; Costanzo et al., 2010), unless otherwise indicated. We confirmed a subset of double mutant interactions between K-K pairs identified in our screens by tetrad dissection, and automated liquid growth curve assays.

Literature curated kinase dataset (KID)

We compiled a highly detailed literature-curated database of K-S interactions for all kinases (Kinase Information Database; KID) (Sharifpoor et al., 2011). Biological interactions involving kinase interactions were compiled by a group of 8 kinase experts and tagged with their associated PMIDs. KID is a ranked database for kinase interactions that scores K-S pairs by applying likelihood ratios using a known gold-standard K-S list and comparing it to randomized kinase-gene pairs (Sharifpoor et al., 2011). The ranked score on KID is defined as a likelihood ratio of the prevalence of each experimental category in the gold standard K-S set compared to a randomized list of kinase-gene interactions (negative bin). We considered the gold standard K-S pairs as the 517 pairs reported on KID corresponding to the stringent cut off ($P \leq 0.01$)

(Sharifpoor et al., 2011). The literature curated gold standard list of K-S pairs is described in Table S5.

Analysis of triplet motifs using double mutant and dosage interactions

We used Fanmod (Wernicke and Rasche, 2006) to identify over-represented 3-edge subnetworks in the combined meta-network of i) literature curated K-S interactions, ii) the combined confirmed and unconfirmed SDL interactions above our defined cut off, iii) negative GIs (bidirectional edges) and iv) positive GIs identified both as query genes from our study or as array hits from a previous analysis of fitness defects in double deletion mutants (Costanzo et al., 2010) (Table S6). Fanmod is a fast network motif detection tool to identify connected subgraphs that occur significantly more often than in random networks. Crucially, Fanmod allows for motif detection in colored networks (i.e. those with multiple edge types). We ran Fanmod with a maximum subgraph size of 3 (triplet motifs) and 4 edge types as described. In the first step, Fanmod enumerated all subgraphs of size 3 and grouped them into isomorphic subgraph classes (Wernicke and Rasche, 2006). Next, Fanmod determined the frequency of subgraph classes in randomized graphs. 200 random graphs were generated from the original network by switching edges between vertices (exchanging only edges of the same type) while preserving the number of bidirectional edges (we used this randomization scheme because our combined network is directed). Finally, significance of each subgraph in the network was calculated and those with $P < 0.05$ were further considered. Significant triplet motifs were extracted using a program implemented in MATLAB that performs an exhaustive search over all possible gene combinations for those fitting each motif. Starting with a list of pairs that fit one line in a triangular motif (e.g. $A \rightarrow B$; K-S pair), the script searches through each given rule generated in Fanmod to identify a third gene that relates to the first pair according to any of the stated rules.

Statistical analyses

Overlap of synthetic dosage lethal interactions with other datasets was analysed as follows. For each pair-wise interaction dataset (e.g. known kinase-substrate pairs, protein-protein or genetic interactions) we first assembled a list of gene pairs that have been screened for SDL interaction (either showing or not showing SDL) and for which interaction information from the other dataset was also available (e.g. either being a kinase-substrate pair or not displaying kinase-substrate interaction). For kinase-substrate interactions, we defined non-interacting K-S pairs as those which have a negative likelihood ratio score in the KID literature-curated database. For protein-protein interactions, we assumed that non-reported protein pairs do not interact. Next, we computed the significance of the overlap between the SDL and the other interaction dataset using chi-square test of independence. Overlaps between loss-of-function genetic interactions (negative and positive GI) and kinase-kinase or kinase-substrate pairs were also investigated in a similar manner. To test the overlap of SDL hits (i.e. list of overexpressed genes that show at least one SDL interaction with a kinase deletion strain) with other gene lists (e.g. list of genes whose protein products are phosphorylated *in vitro*) we also performed chi-square tests of independence.

Other yeast strains and plasmid construction

Kinase deletion strains carrying a TAP-tagged allele of *KEL1* were constructed by crossing a *MATa KEL1-TAP-HIS3* strain (Costanzo et al., 2010; Ghaemmaghami et al., 2003) to the *MATα kinaseΔ::NAT^R* query strains (Table S2). Kinase-gene double deletion strains were constructed by crossing *NAT^R* kinase deletion mutants (see above and Table S1,S2) to the kinase deletion strains from the yeast deletion collection (Giaever et al., 2002) (*KAN^R* gene deletion strains) and

dissecting tetrads. *MATa* haploids of the desired double mutant genotype were selected for analysis by replica plating segregants onto YEPD media (1% Yeast Extract, 2% Bactopeptone, 0.004% Tryptophan, 0.004% Adenine, 2% Glucose) containing cloNAT and G418.

A *LEU2*-based plasmid expressing a C-terminally Flag-tagged allele of *LTE1* was constructed by cloning *LTE1-HA* from the mORF collection (Gelperin et al., 2005) into a *pRS315* low copy CEN vector using the Invitrogen Gateway cloning technology (Table S2). First, *LTE1-HA* from the mORF collection was transferred to *pDONR-221* plasmid, which was then used to transfer the *LTE1* cassette into the recipient plasmid *pRS315-FLAG-LEU2*. The resulting construct, *pRS315-LTE1-FLAG-LEU2*, was then transformed into the indicated kinase deletion strains in the presence or absence of integrated *KEL1-TAP* for co-immunoprecipitation assays (see below).

Serial spot dilutions

Standard methods and media were used for yeast growth and transformation. Spot assays were performed by growing yeast cultures in appropriate media to saturation and spotting equal volumes of 15-fold serial dilutions (5μl) onto plates with appropriate supplements to assess growth. All glucose-containing plates were incubated for 2 days, while galactose-containing plates were grown for 3 days at 30°C. To assess cold-sensitivity, YEPD plates were incubated at 15°C for 7 days. To test strain sensitivity to cell wall stressors, we included 10mM caffeine or 64μg calcofluor white in YEPD plates and grew cells for 4 days.

Western blot and coimmunoprecipitation analysis

To prepare samples for western blot analysis, 50ml of cells were grown in the appropriate media to an optical density (O.D.₆₀₀) of 1.0 and washed once with phosphate buffered saline (PBS). Samples were resuspended in 250μl of solution containing 1% β-mercaptoethanol in 0.25N

NaOH and incubated on ice for 10min. 160µl of 50% TCA were added and samples were incubated for another 10min on ice. After a 10min centrifugation at high speed, the precipitated protein was isolated and resuspended in 100 µl of 2X SDS sample buffer. Samples were resolved on 6% SDS polyacrylamide gels and transferred to polyvinylidene difluoride (PVDF) membranes following standard procedures for Western blot analysis. Peroxidase anti-peroxidase complex, α -PAP (P3039; Sigma Aldrich, St. Louis, MO) was used to detect TAP-tagged proteins. Flag-tagged proteins were detected using α -Flag M2 (Sigma-Aldrich) and α -GST primary conjugate (GE Healthcare) was used to assess migration changes for GST-tagged proteins. Swi6 protein levels were assessed as a loading control using an affinity purified α -Swi6 antibody (Sidorova and Breeden, 1993). For analysis of coimmunoprecipitation of tagged Lte1 and Kel1, overnight cultures of BY4798 [*MAT α ura3 Δ ::NAT^R KEL1-TAP-HIS3*], BY4800 [*MAT α hsl1 Δ ::NAT^R KEL1-TAP-HIS3*] and BY4802 [*MAT α gin4 Δ ::NAT^R KEL1-TAP-HIS3*], carrying either the *pRS315-LTE1-FLAG* or control plasmids were grown overnight in 2% raffinose and expression of *LTE1* was induced by addition of 2% galactose for 9 hours. Cells were lysed in Lysis Buffer (50mM Tris-HCl, pH 7.5, 100mM NaCl, 1mM EDTA, 5mM NaF, and protease inhibitors) and samples were clarified by centrifugation at high speed for 10min. Total protein was incubated with IgG sepharose (Amersham Biosciences) overnight at 4°C with gentle shaking. IgG resin was washed three times in lysis buffer and resuspended in 2X sample buffer for Western blot analysis.

Data Access

Supplementary Tables S1-S9, Figures S1-S7 and Supplementary Materials and Methods can be downloaded directly from the journal website or via the Andrews Lab Supplementary website (<http://andrewslab.cabr.utoronto.ca/data/>). Interactive and searchable tables are also available for

Table S3, S7 and S8 on the Andrews Lab Supplementary website. In addition, all SDL and SGA genetic interactions are available on Yeast KID (<http://www.moseslab.csb.utoronto.ca/KID/>), allowing users to compare the presented data with previously published high throughput (HTP) and low throughput (LTP) data. To access the SDL and SGA datasets via Yeast KID, users must search by the PMID associated with this manuscript.

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Conflict of Interest

The authors declare no conflict of interest.

Author Contributions:

SS, MC, AB, BJA and CB prepared and edited the manuscript. SDL screening was performed by SS and DvD and results were confirmed by SS. SGA screens were performed by MC, AB and CB. Scoring and quantification of the SDL and SGA screens was performed by AB. Statistical analysis of both datasets including motif analysis was performed by BP and CLM. BV and CLM contributed to the motif extraction. JY, ACD and HF performed literature curation of kinase-substrate pairs. ACD confirmed a portion of the conditional screens. Motif predictions were tested by DvD and SS.

Figure Legends:

Figure 1: Properties of the kinome SDL interaction network.

A) Distribution of SDL (blue), negative (red) and positive (green) genetic interactions across the kinome using the stringent cutoff. The number of interactions for SDL-positive queries is plotted. * indicates kinases that were not screened for genetic interactions. B) Conditional screening of *hog1Δ* and *cmk1Δ* mutants expands the SDL interaction network. SDL screening of a *hog1Δ* strain was performed on 0.2M NaCl (osmotic stress) and on 200mM CaCl₂ for a *cmk1Δ* strain. C) Representative serial spot dilution assays illustrating the SDL phenotype caused by overexpression of *RPB2* in a *hog1Δ* strain specifically in activating conditions. D) Bar graph showing the prevalence of positive (green bar) and negative (red bar) genetic interactions at the intermediate SGA cut off among kinase-gene (all), kinase-substrate, kinase-kinase and kinase-kinase pairs that share the same target according to the KID gold standard, as a fraction of gene pairs screened. Prevalence of SDL interactions for each subtype is shown in parallel (blue bar).

Figure 2: Overrepresented SDL motifs identified through combined analysis of SDL, SL/SSup genetic interaction datasets and gold standard kinase-substrate pairs. 5 types of

sub-networks were enriched against the randomized dataset: A) counteracting; B) balancing co-regulation; C) converging regulation; D) diverging regulation and E) SDL cascades. The number and fold-enrichment of triplet gene pairs identified is listed, as well as examples of known and novel motifs for each group. Possible mechanistic models describing each motif are highlighted. Some motifs fall under more than one subtype. Red boxes highlight two novel motifs confirmed in our study by additional phenotypic and biochemical assays.

Figure 3: *YMR074C* is an inhibitor of the Slr2-MAP kinase cell wall integrity (CWI) pathway. A) Overrepresented triplet motif that identified *YMR074C* to be SDL with two kinases of the CWI pathway, Bck1 and its downstream target Slr2. B) Model describing the role of Ymr074c as an inhibitor of CWI. C) Overnight cultures of *bck1Δ*, *slr2Δ*, *ymr074cΔ*, single or double mutants were serially diluted and spotted onto YPD medium in the absence (left) and presence (right) of 10mM caffeine. * indicates phenotypic rescue.

Figure 4: Kel1 protein is co-regulated by budneck kinases. A) Overrepresented motif (yellow triangle), supporting a functional connection between Kel1 and the two budneck kinases Cla4 and Hsl1 in a converging regulation model. B) Endogenous Kel1 protein levels are reduced in *hsl1Δ* and *cla4Δ* but not in *gin4Δ* mutant strains. Kel1-TAP protein was immunoprecipitated from the indicated budneck kinase mutant strains and western blots were probed with anti-PAP to detect total Kel1 protein. Electrophoretic mobility shift of Kel1 protein is shown (Kel1-P). Swi6 was used as a loading control. C) Integrated Kel1-TAP protein was immunoprecipitated in the presence of Lte1-Flag and association of Lte1 with Kel1 was detected using α -flag antibody. The amounts of Lte1-Flag in the whole cell extract (WCE; top panel) and in the Kel1 immunoprecipitate (bottom panel) are shown. The amount of Kel1-TAP in the immunoprecipitate is shown in the middle panel (anti-PAP). Immunoprecipitation assays were

performed in wild type, *hsl1Δ*, *gin4Δ* background strains. *cla4Δ* strain was not tested, due to severe toxicity upon Kel1-TAP expression. D) Model for regulation of Kel1 by budneck kinases. Lte1 is phosphorylated by Cla4 which triggers binding to its anchor, Kel1. Hsl1 regulates the stability of Kel1 protein, which allows the anchor to become available for binding to Lte1-P. Together, the complex inhibits mitotic exit possibly to delay budding to allow the cell to reach the correct size. Later, Cdc14 phosphatase releases Lte1 from its anchor in the cortex into the cytoplasm in order to activate mitotic exit in large budded cells.

Supplementary Information

Figure S1: Method for HTP Synthetic Dosage Lethal (SDL) screening of kinase mutants.

Kinase deletion mutants bearing a drug-resistant marker are crossed to an array 5280 yeast strains using the SGA protocol. Each strain on the array contains a different yeast open reading frame (ORF) tagged at its amino terminus with GST and expressed from the inducible *GAL* promoter on a multicopy plasmid (Sopko et al., 2006a). Gene overexpression in the resultant haploid array is induced by growing cells on media containing galactose that drives expression of the plasmid-based *GST*-tagged ORF.

Figure S2: Classification of kinases based on their SDL interaction profiles.

A) The non-essential component of the kinome can be divided into two classes: those with and without SDL interactions. Kinases that show an SDL interaction profile can be subdivided into two categories based on the number of SDL interactions identified. Number of SDL interactions is shown in brackets for each kinase. FE; functionally enriched, PT; previous target identified, NT; Novel targets characterized. 16 non-essential kinases were not screened due to fitness and mating defects, or because query strains were unavailable. B) Functional enrichment of the SDL

interaction network. Fold enrichment of the top 5 GO terms are plotted with the corresponding P-values. C) Precision-recall curve plotting the performance of data obtained from hub kinases (blue) or the entire kinome (black) in identifying functionally co-annotated pairs. The precision is plotted as a function of the number of true positives (TP) identified according to co-annotated genes in GO-Slim.

Figure S3: The SDL dataset is enriched for targets of phosphorylation and proteins that physically interact with kinases. A) List of some literature curated K-S pairs detected by SDL. Pairs identified through SDL screening (red) or other assays (blue) are highlighted with the corresponding reference. B) Bar graph showing the percentage of phosphorylated proteins in the SDL network (blue) compared to other genes on the overexpression array (black). *In vivo* phosphorylation data was obtained from PhosphoGRID (Stark et al., 2010). A protein was considered not to be phosphorylated if it was detectable as a TAP-tagged fusion protein according to Ghaemmaghami *et al.* 2003 (Ghaemmaghami et al., 2003), but no phosphorylated has been reported. *In vitro* phosphorylation data was obtained from Ptacek *et al.* 2005 (Ptacek et al., 2005). C) Number of *in vivo* phospho-regulated proteins identified by Bodenmiller *et al.*, 2010 (Bodenmiller et al., 2010) in the SDL network (blue), versus the remaining genes on the overexpression array (black). D) SDL interactions are enriched for physically interacting partners from two-hybrid (Ito et al., 2000) and the kinase protein-protein interaction (PPI) datasets (Breitkreutz et al., 2010). Bar graph plots the number of physically interacting partners in the SDL network (blue bar) compared to the remaining genes on the overexpression array (black bar) using yeast two hybrid, transient kinase PPI and conventional PPI datasets that target more stable complexes (Gavin et al., 2006; Gavin et al., 2002; Krogan et al., 2006).

Figure S4: Pie chart showing the functionally overrepresented categories identified in the *hog1* and *cmk1* SDL screens. A) 74 SDL interactions were identified for *hog1*, with the majority involved in transcription and chromatin remodelling (including RPB2). B) 11 SDL interactions were identified for *cmk1* in the presence of 200mM CaCl₂, with a significant enrichment of transport genes.

Figure S5: Classification of kinases based on the number of negative genetic interaction. High throughput SGA screening using 75 loss-of function mutants provides data for 74 kinase queries (45 from Costanzo et al, 2010, 30 this study), with 29 kinases producing large negative GI profiles at the stringent cut off ($\square < -0.12$, $P < 0.05$). Kinases highlighted in red are those that also show large SDL profiles under standard growth conditions. Number of negative interactions is shown in brackets for each kinase.

Figure S6: Additional overrepresented triplet motifs identified from combined analysis of positive and negative genetic interaction datasets. List of sub-networks that were enriched against the randomized dataset are listed. Motifs describe A) genes that are upstream or B) downstream of kinase regulatory pathways; C) diverging regulation; D) converging regulation; E) genes acting in parallel to K-S pairs; and F) triply redundant sets of genes that include kinases or their interacting partners. A set of more complex motifs are highlighted in (G). Figure illustrates the number of triplet gene pairs identified with the fold enrichment (in brackets).

Figure S7: SDL interactions between *KEL1* and the budneck kinases. Serial spot dilution assays illustrating SDL interactions using *GST-KEL1* or *KEL1-HA* with *hsl1*, *gin4*, and *cla4*. SDL interactions involving a related gene *KEL2* are also shown.

Supplementary Tables S1-S9

Table S1: List of yeast kinases

Table S2: Strains and Plasmids

Table S3: The SDL dataset (Stringent, $|\epsilon| \geq 0.2$, $P < 0.05$)

Table S4: The confirmed SDL interactions by quantified spot assays

Table S5: Gold Standard Kinase-Substrate pairs

Table S6: List of *hog1* and *cmk1* confirmed SDL interactions

Table S7: The SGA dataset (Intermediate, $|\epsilon| \geq 0.08$, $P < 0.05$)

Table S8: List of all motifs with gene names

Table S9: List of *BCK1-SLT2* counteracting and balancing coregulation motifs tested for suppression of CFW and Caffeine sensitivity

Supplemental data access: <http://andrewslab.cabr.utoronto.ca/data/>

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Figure 1

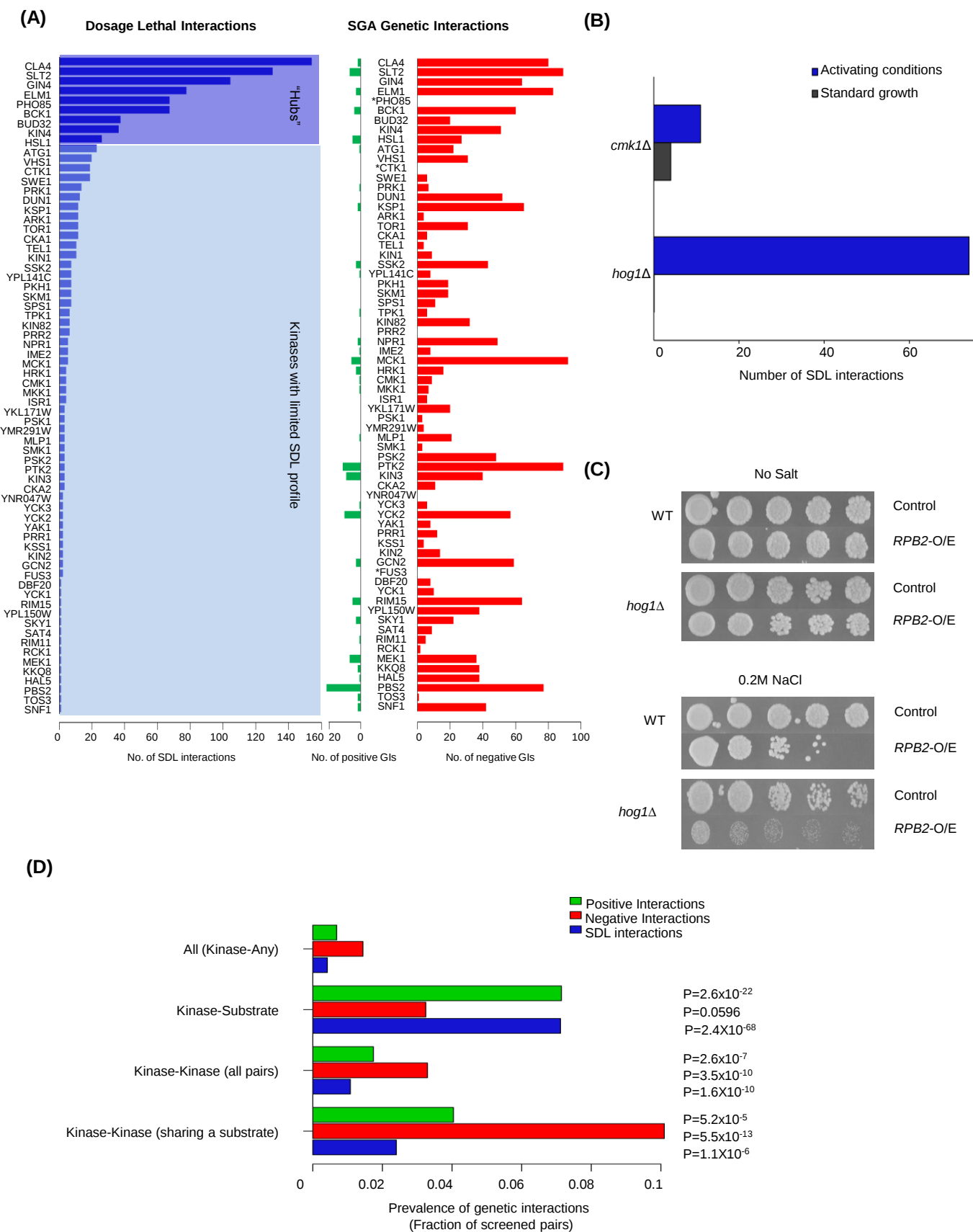


Figure 2

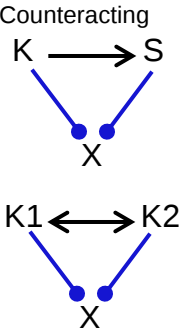
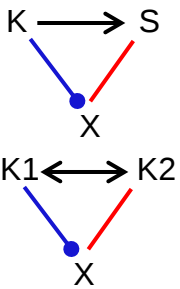
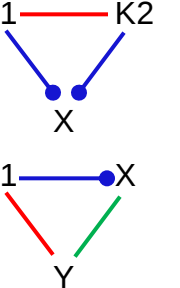
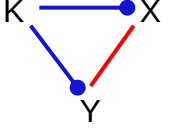
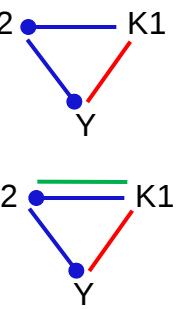
SDL Motif	Cases	Possible Mechanism
(A) Counteracting 	221 (5.8-fold) 72 (6.4-fold)	X acts in opposition to the K-S pair either in the same pathway or indirectly through a parallel pathway. Substrate may also be another kinase.
(B) Balancing co-regulation 	202 (>16,000-fold) 39 (∞-fold)	Opposite regulatory pathways are regulated by the same kinase (kinase activates S and inhibits X). Substrate may also be another kinase. In this case, increased levels of S and decreased levels of X may result in the same biological effect.
(C) Converging regulation 	8 (∞-fold) 174 (1.3-fold)	K1 and K2 are redundant kinases that either directly or indirectly inhibit the biological outcome resulting from X. K2 may also be another enzyme without kinase activity (Y) that regulates X.
(D) Diverging regulation 	529 (>40,000-fold)	K1 acts in opposition to the biological effects of two redundant genes (X & Y) acting in parallel pathways.
(E) SDL cascades 	24 (∞-fold) 9 (∞-fold)	K1 & Y (e.g. another enzyme) function in parallel to activate a biological response that is inhibited by another kinase, K2.
Legend: → Gold standard K-S pair —•— Synthetic dosage lethal — Negative interaction — Positive interaction		

Figure 3

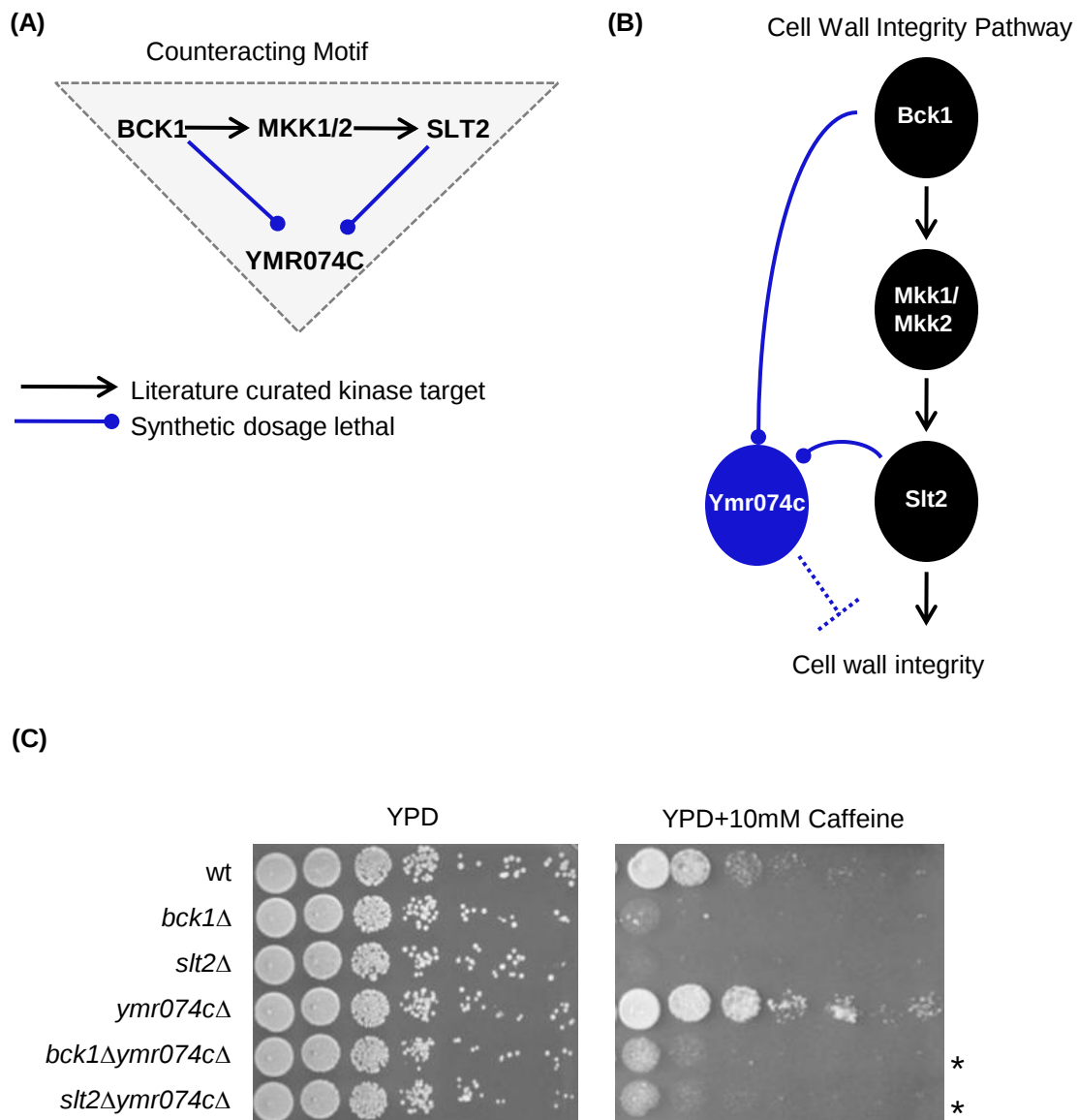


Figure 4

