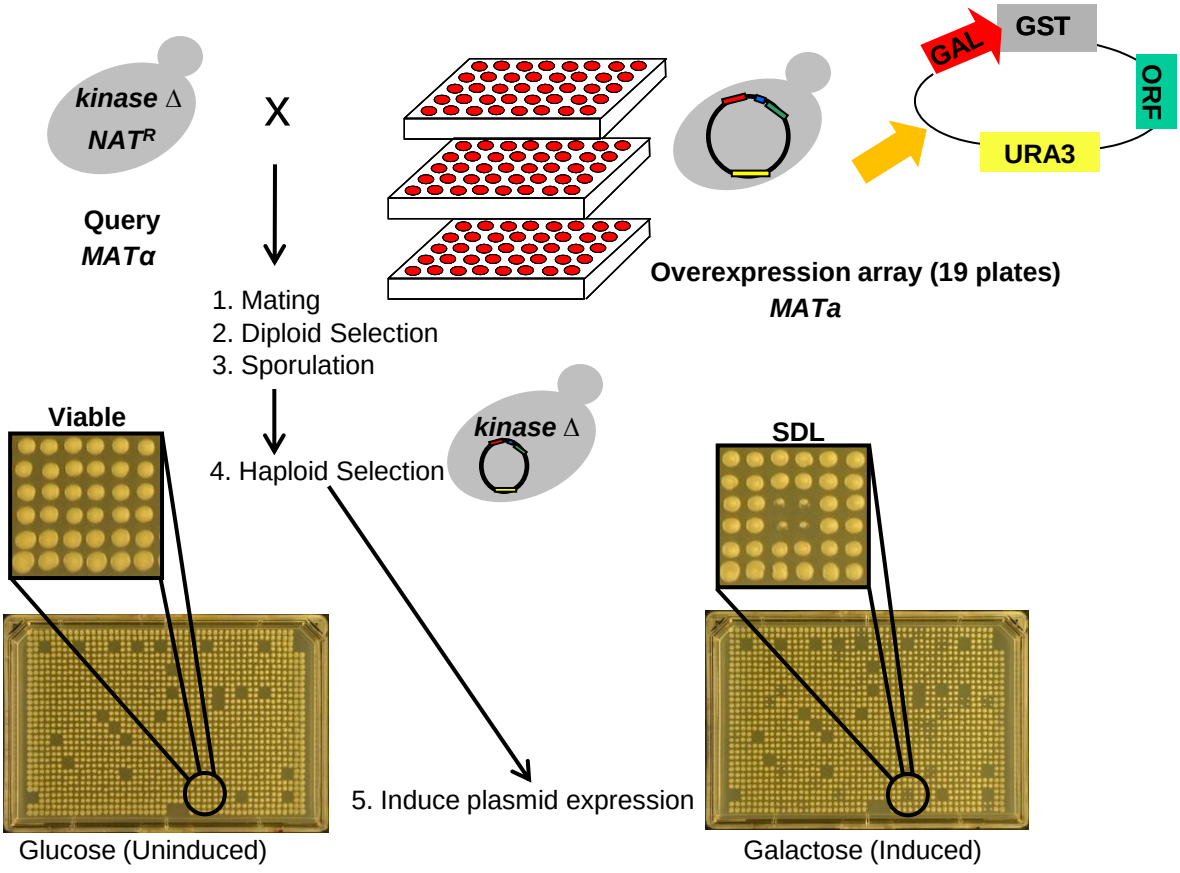
Supplementary Figure 1



Supplementary Figure 2

(a)

Kinome (127 kinases)

109 Non-essential

18 Essential

92 screened

Class 1: (69 kinases)
Kinases with SDL profiles

Class 2: (23 kinases)
No SDL profiles

"SDL Hubs"
(9 kinases)
(>25 genes)

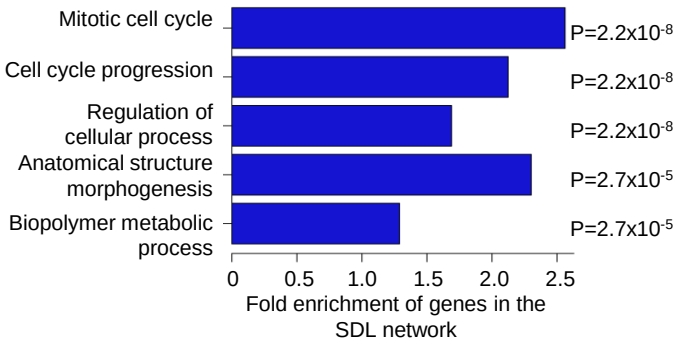
CLA4 (149) FE, PT
SLT2 (126) FE
GIN4 (101) FE, PT
ELM1 (75) FE
BCK1 (65) FE
BUD32 (36)
KIN4 (35)
HSL1 (25) FE
PHO85 (65) FE, PT, NT

Small SDL profile
(60 kinases)
(<25 genes)

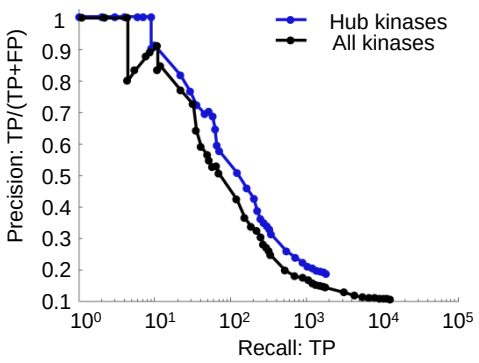
ATG1 (22)
VHS1 (19)
CTK1 (18)
SWE1 (18)
PRK1 (13)
DUN1 (12)
CKA1 (11)
ALK2 (11)
KSP1 (11)
TOR1 (11)
ARK1 (11)
KIN1 (10)
TEL1 (10)
YPL141C (7)
PKH1 (7)
SPS1 (7
SSK2 (7)
SKM1 (7)
KIN82 (6)
PRR2 (6)
TPK1 (6)
MCK1 (5)
NPR1 (5)
IME2 (5)
CMK1 (4)
HRK1 (4)
MKK1 (4)
ISR1 (4)
YKL161C (3)
CKA2 (3)
PTK2 (3)
PSK2 (3)
SMK1 (3)
PSK1 (3)
KIN3 (3)
YKL171W (3)
YMR291W (3)
ENV7 (3)
FUS3 (2)
GCN2 (2)
KSS1 (2)
YCK3 (2)
YAK1 (2)
PRR1 (2)
KIN2 (2)
YCK2 (2)
FPK1 (2)
KKQ8 (1)
SKY1 (1)
YPL150W (1)
DBF20 (1)
SAT4 (1)
SNF1 (1)
RIM15 (1)
RCK1 (1)
TOS3 (1)
YCK1 (1)
PBS2 (1)
HAL5 (1)
RIM11 (1)
MEK1 (1)

HOG1
PKP2
YDL025C
PTK1
YBR028C
ALK1
MKK2
DBF2
KCC4
MRK1
RCK2
SAK1
YPK2
ALK2
CMK2
YGK3
CHK1
AKL1
SKS1
TPK2
IKS1
SCY1
YPL236C

(b)



(c)

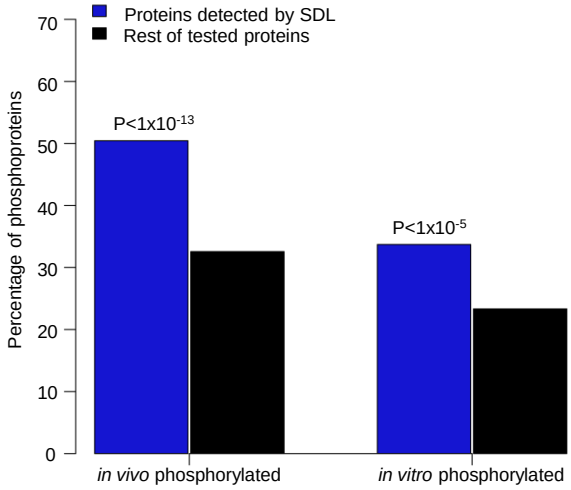


Supplementary Figure 3

(a)

Kinase-SDL/Target	Reference
Pho85-Crz1	Sopko et al, 2007
Pho85-Bni4	Zou et al, 2009
Pho85-Whi5	Huang et al, 2009
Pho85-Rga2	Sopko et al, 2007
Dun1-Sml1	Zhao et al, 2002
Cla4-Cdc10	Versele et al, 2004
Cla4-Shs1	Mortensen et al, 2002
Cla4-Gic2	Gulli et al, 2000
Cla4-Bem1	Gulli et al, 2000
Snf1-Mig1	Ostling et al, 1998
	Smith et al, 1999
Snf1-Mig3	Dubacq et al, 2004
Mck1-Mih1	Pal et al, 2008
Gin4-Cdc12	Dobbelaere et al, 2003
Gin4-Cdc11	Mortensen et al, 2002
Gin4-Shs1	Mortensen et al, 2002
	Asano et al, 2006

(b)



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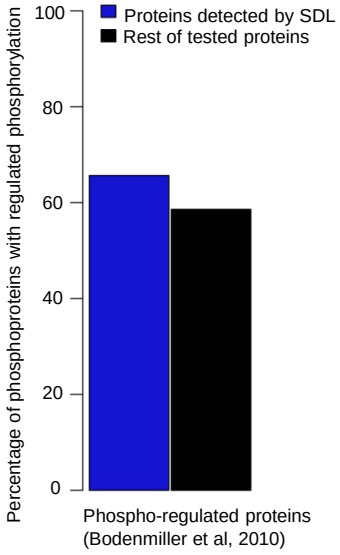
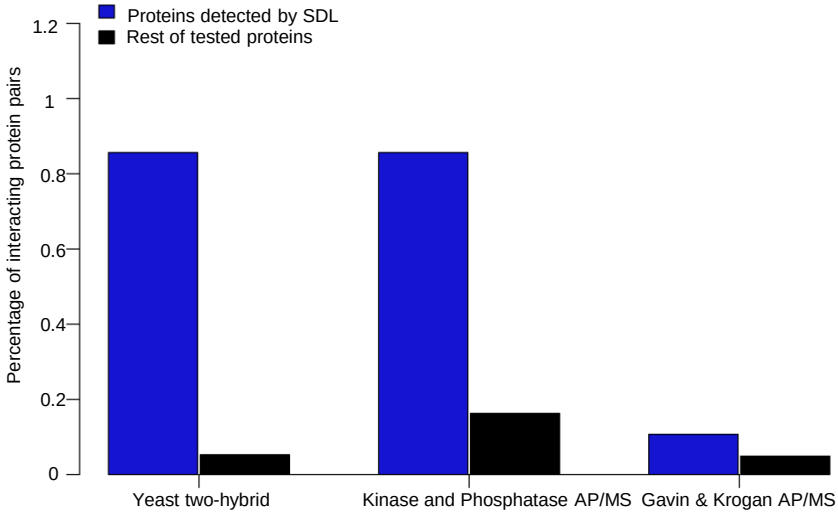
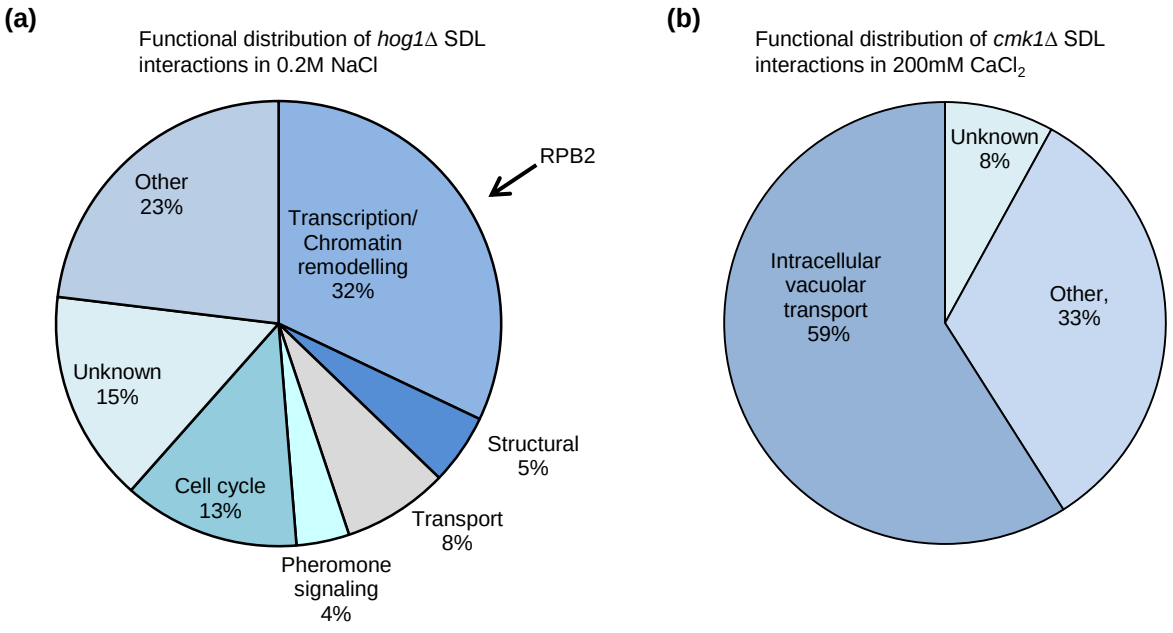


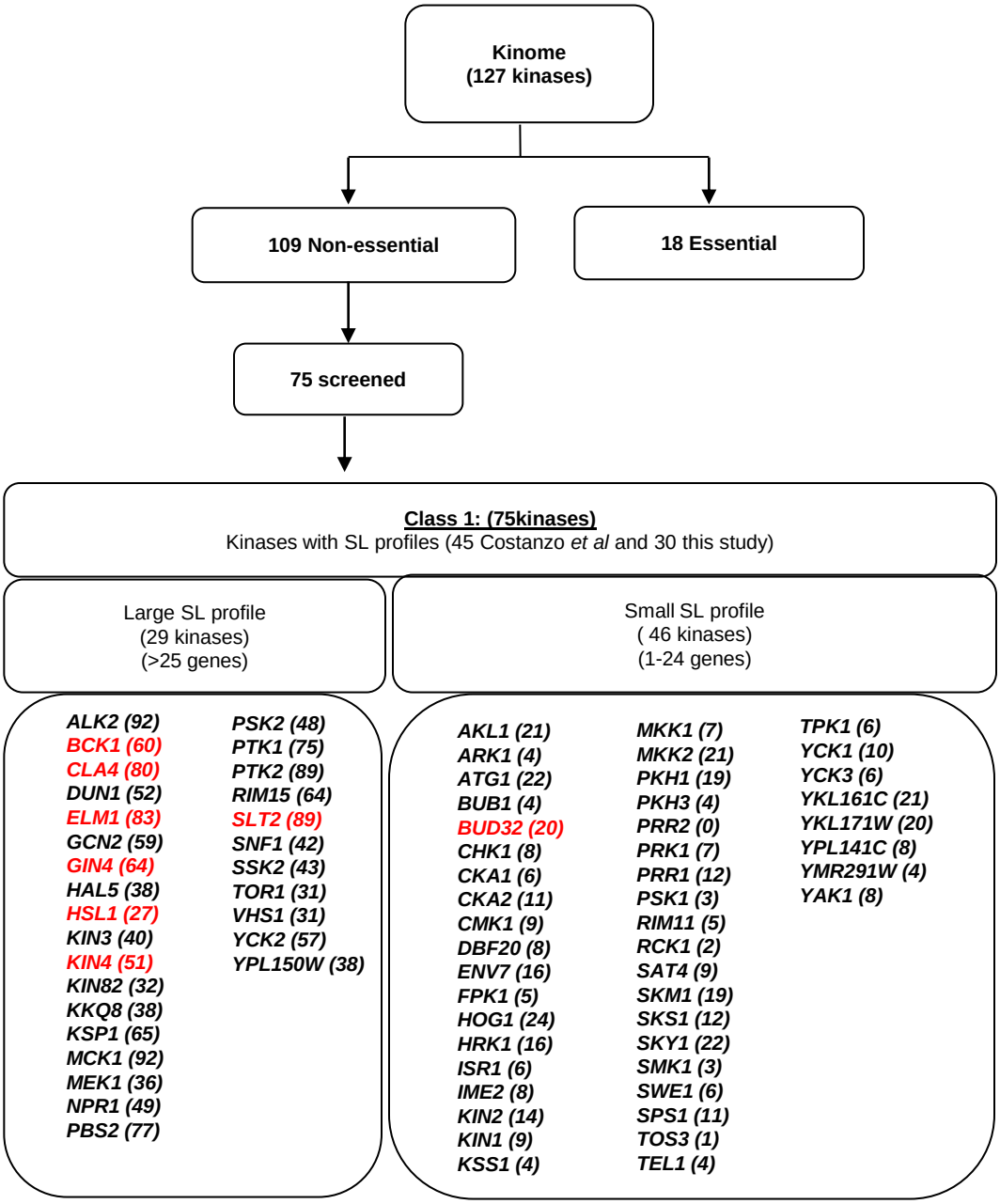
(d)



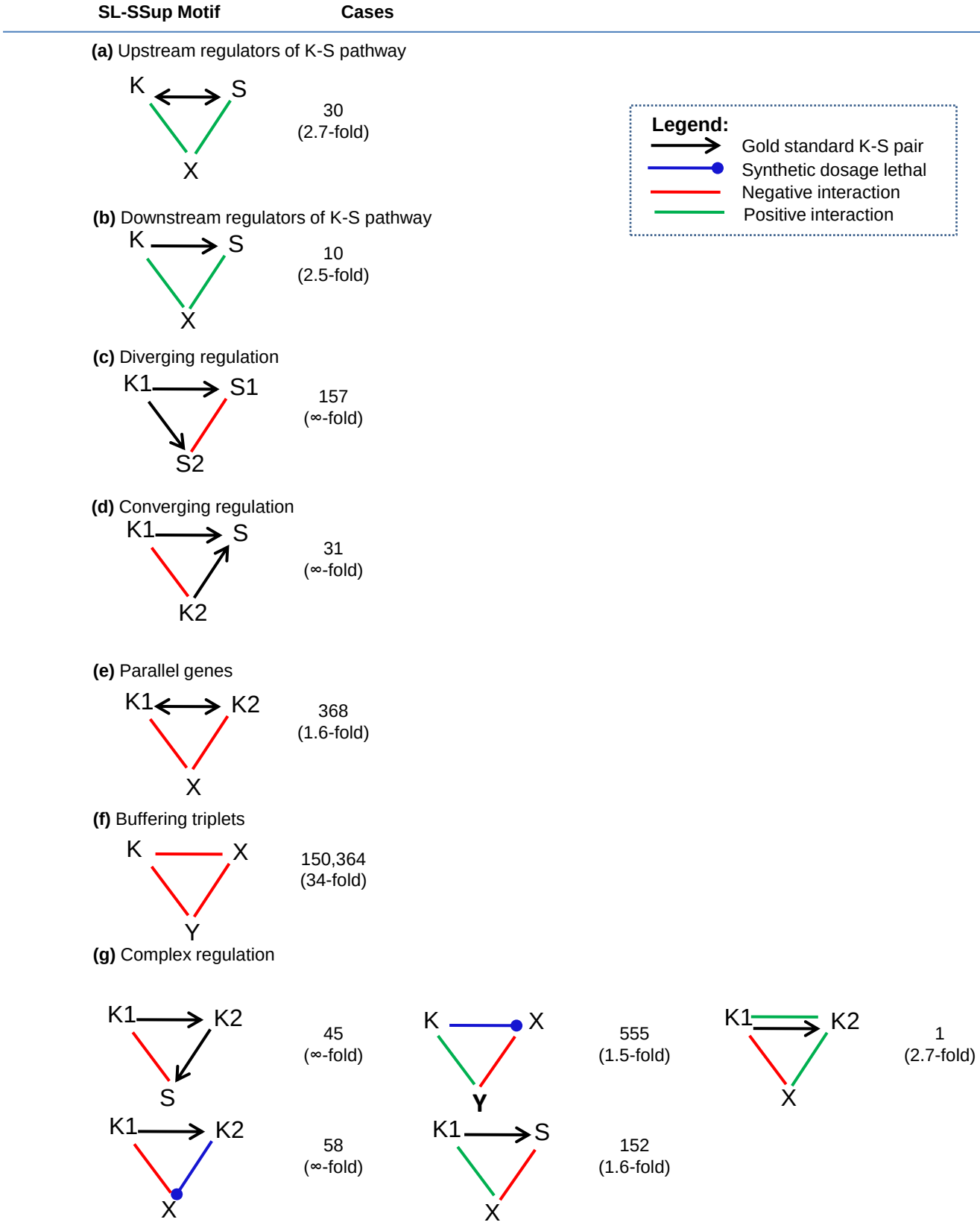
Supplementary Figure 4



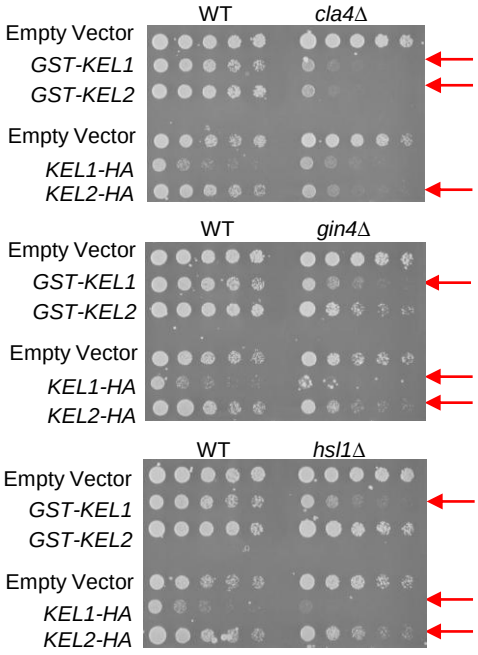
Supplementary Figure 5



Supplementary Figure 6



Supplementary Figure 7



Supplementary Figure Legends

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 $CaCl_2$, 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 (ε<-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.