Pathway connectivity and signaling coordination in the yeast stress-activated signaling network

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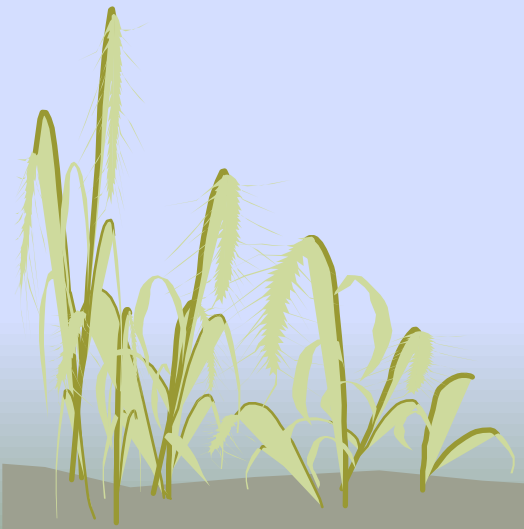


Backgrounds

Stressed cells coordinate a multi-faceted response spanning many levels of physiology. Yet knowledge of the complete stress activated regulatory network as well as design principles for signal integration remains incomplete. We developed an experimental and computational approach to integrate available protein interaction data with gene fitness contributions, mutant transcriptome profiles, and phospho-proteome changes in cells responding to salt stress, to infer the salt-responsive signaling network in yeast. The inferred subnetwork presented many novel predictions by implicating new regulators, uncovering unrecognized crosstalk between known pathways, and pointing to previously unknown ‘hubs’ of signal integration.

Material and Methods

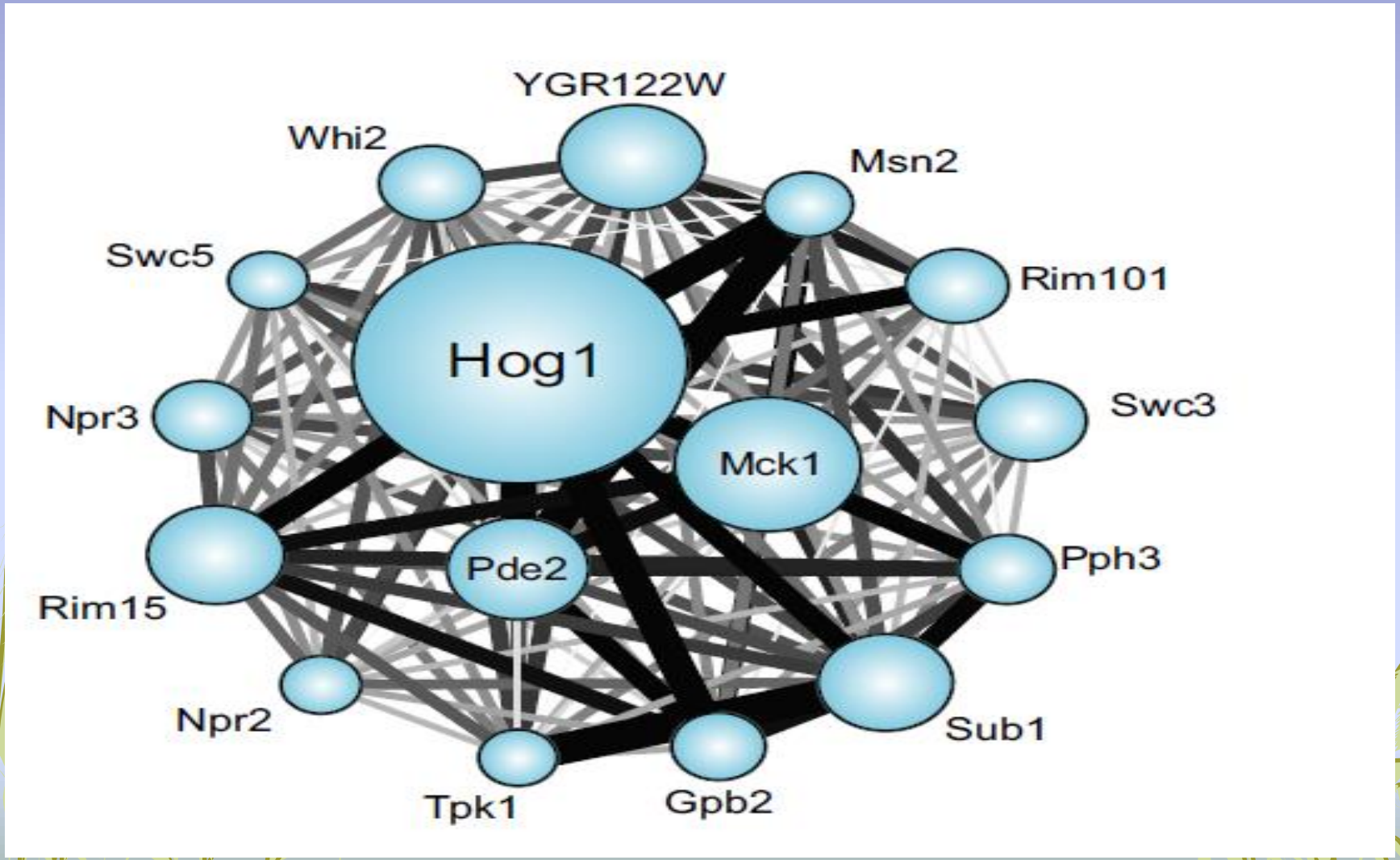
Mutant[a]	Defective[b]	Amplified[b]
Source regulators		
hog1Δ (3)	1378	565
pde2Δ (3)	517	59
mck1Δ (3)	794	101
msn2Δ (3)	184	26
rim101Δ (3)	75	227
gpb2Δ (2)	202	37
rim15Δ (2)	438	106
npr2Δ (2)	75	69
npr3Δ (2)	184	89
swc3Δ (2)	108	257
swc5Δ (2)	84	55
whi2Δ (2)	118	201
pph3Δ (2)	235	21
sub1Δ (2)	431	97
tpk1Δ (2)	35	96
ygr122wΔ (2)	106	502

1.aMutant and number of replicates in parentheses.

2. bNumber of genes with smaller (defective) or larger (amplified) expression changes compared to the wild-type strain. Note, this table includes noncoding RNAs that were excluded from the inference. The table lists the number of targets identified from the originally interrogated ‘source’ regulators and validation mutants

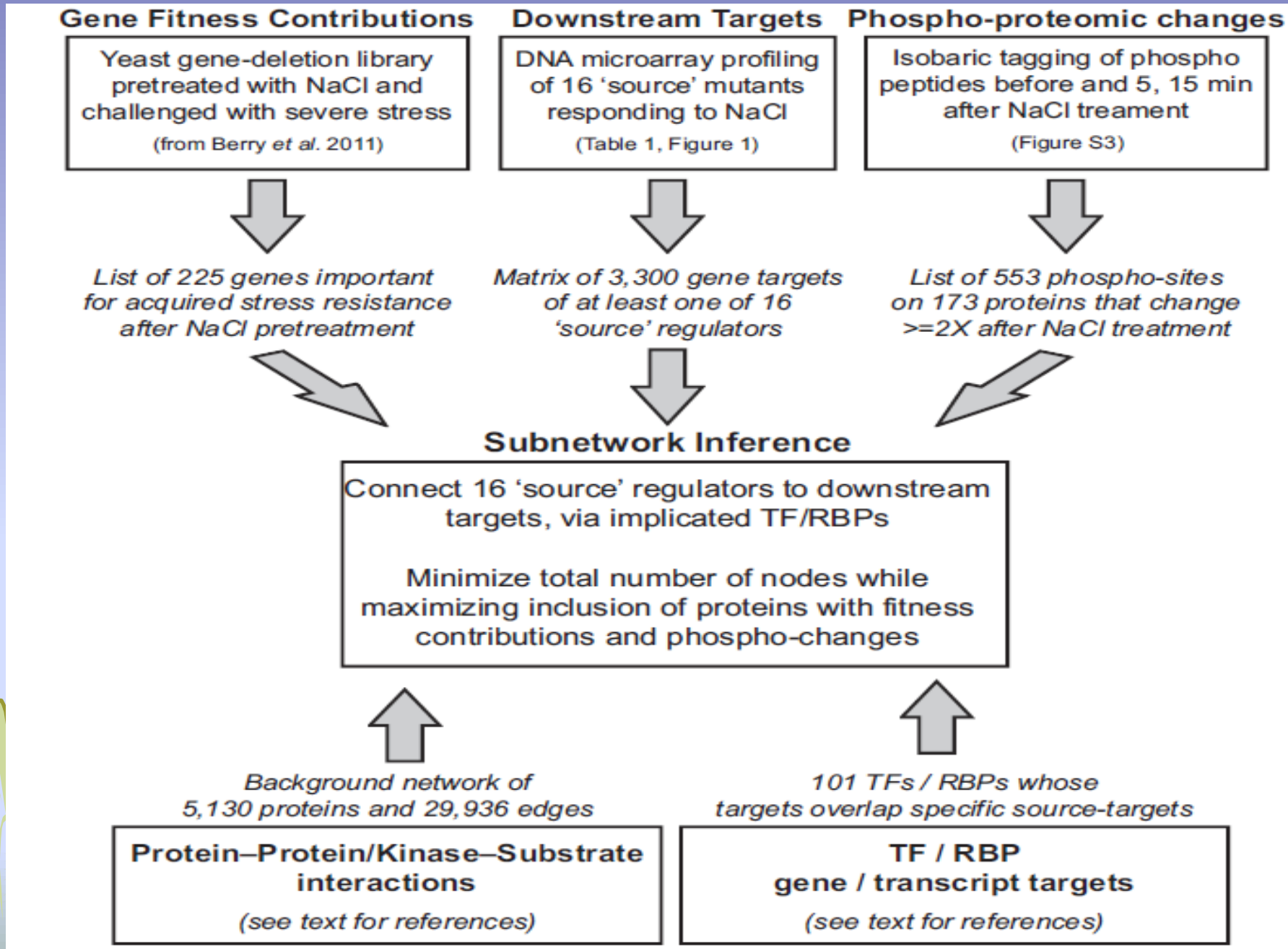
Unless otherwise noted, cells were grown to log phase in batch YPD cultures at 30° C for at least seven generations before addition of a final concentration of 0.7 M NaCl, after which cells were grown for 30 min. cdc14-3 and isogenic wild-type cells were grown at 25° C, shifted to the non-permissive temperature of 35° C for 90 min, and then treated with a final concentration of 0.7 M NaCl at 35° C for anadditional 30 min before sample collection. Relative physiological changes were compared to the time point collected immediately before addition of NaCl (i.e., 35° C for 90 min without NaCl).

Microarray analysis

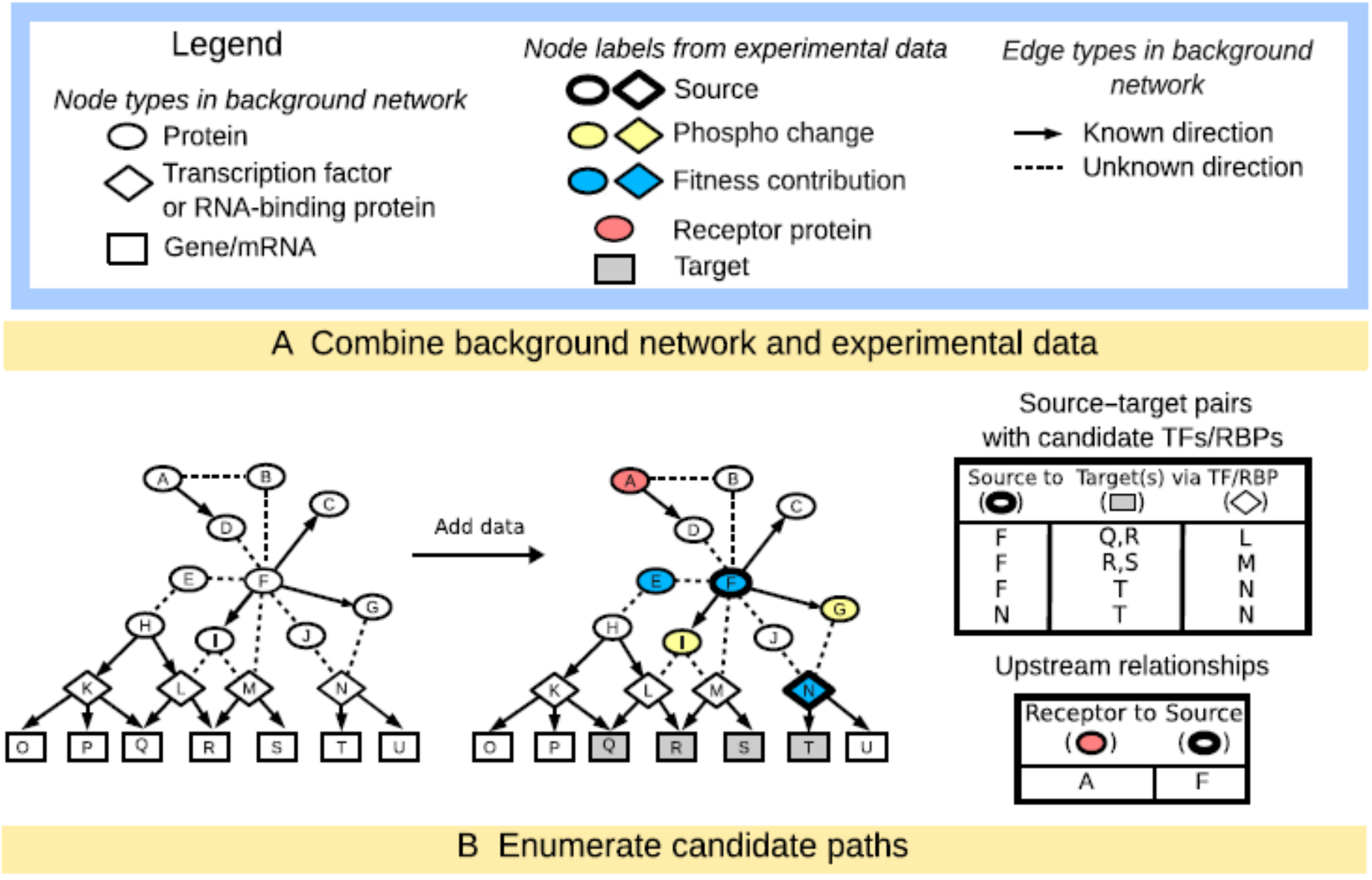


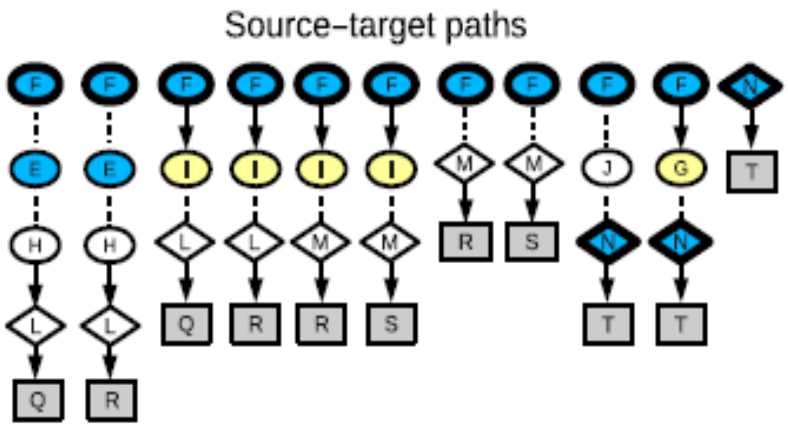
Phospho-proteomic analysis

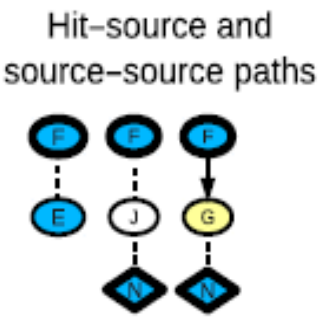
we next measured changes to the phospho-proteome before and at 5 and 15 min after NaCl treatment, using chemical isobaric tags for phosphopeptide quantification (see Materials and Methods). Nearly 600 of 1,937 identified phospho-sites (mapping to 973 proteins)showed a ⩾ 2-fold change in phosphorylation, roughly split between sites with increased and decreased modification. Over 10% of the altered phospho-proteins represented kinases and phosphatases (including regulators of cell cycle progression,actin organization, and signal transduction) as well as transcriptional regulators (such as activators Hot1, Sko1, and Sub1 and repressors Mot2, Dot6, and Dig1). Proteins affected at the later time point were involved in cytokinesis, bud-site selection, and actin reorganization implying downstream physiological effects on these processes

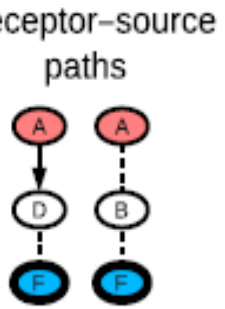

Gene Fitness Contributions
Yeast gene-deletion library pretreated with NaCl and challenged with severe stress
(from Berry et al. 2011)
Downstream Targets
DNA microarray profiling of 16 'source' mutants responding to NaCl
(Table 1, Figure 1)
Phospho-proteomic changes
Isobaric tagging of phospho peptides before and 5, 15 min after NaCl treament
(Figure S3)
List of 225 genes important for acquired stress resistance after NaCl pretreatment
Matrix of 3,300 gene targets of at least one of 16 'source' regulators
List of 553 phospho-sites on 173 proteins that change >=2X after NaCl treatment
Subnetwork Inference
Connect 16 'source' regulators to downstream targets, via implicated TF/RBPs
Minimize total number of nodes while maximizing inclusion of proteins with fitness contributions and phospho-changes
Background network of 5,130 proteins and 29,936 edges
101 TFs / RBPs whose targets overlap specific source-targets
Protein–Protein/Kinase–Substrate interactions
(see text for references)
TF / RBP gene / transcript targets
(see text for references)

Integer linear programming-based (IP) approach









C Solve IP to infer ensemble of subnetworks

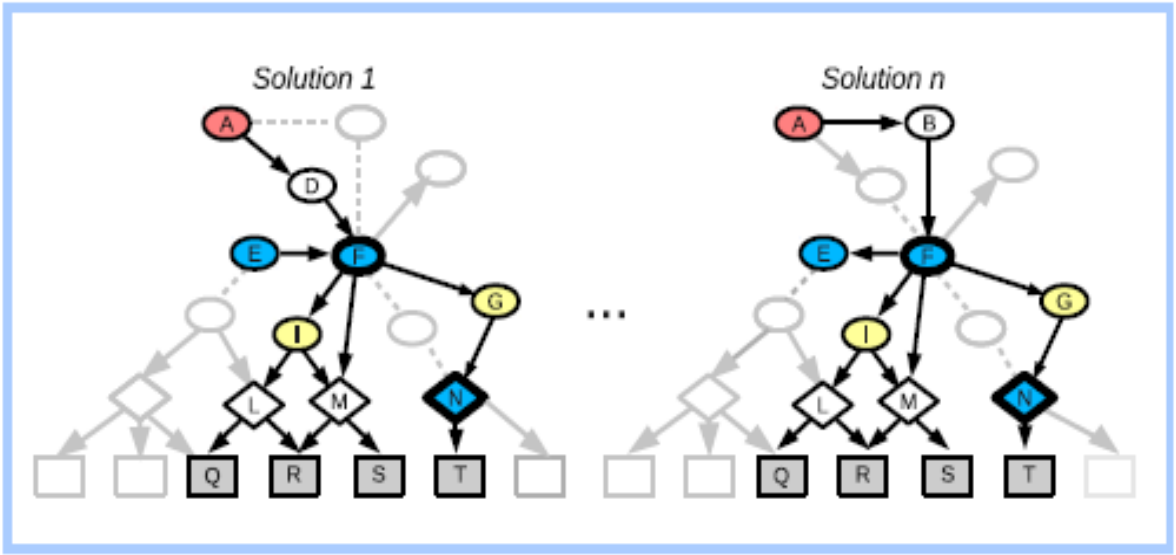
IP constraints:

- Connect source–target pairs, hit–source pairs, and receptor–source pairs
- Assign a direction to each undirected edge

IP objectives:

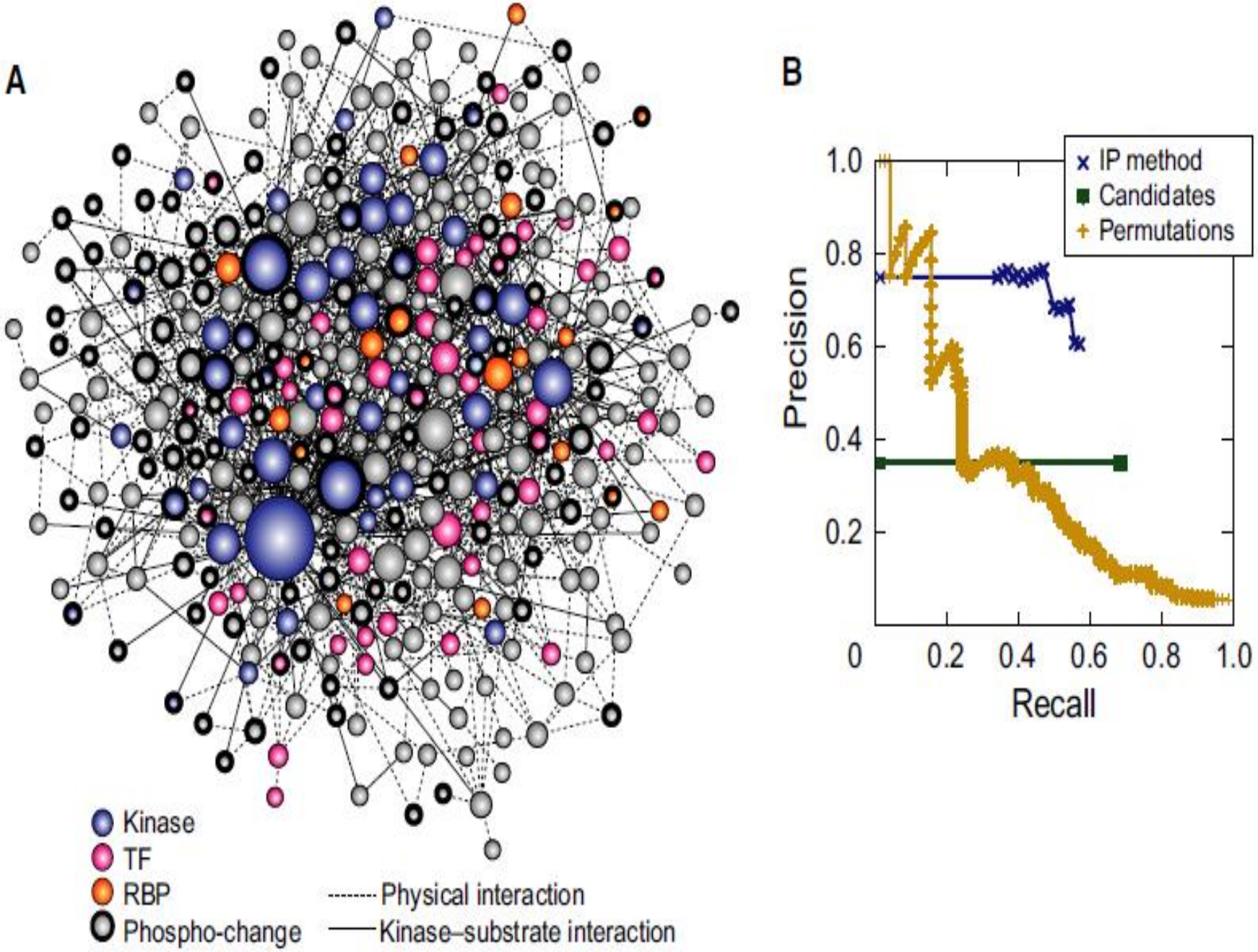
- Favor proteins with
  - Phospho changes
  - Fitness contributions
- Sparingly include proteins without evidence of relevance

Subnetwork ensemble:

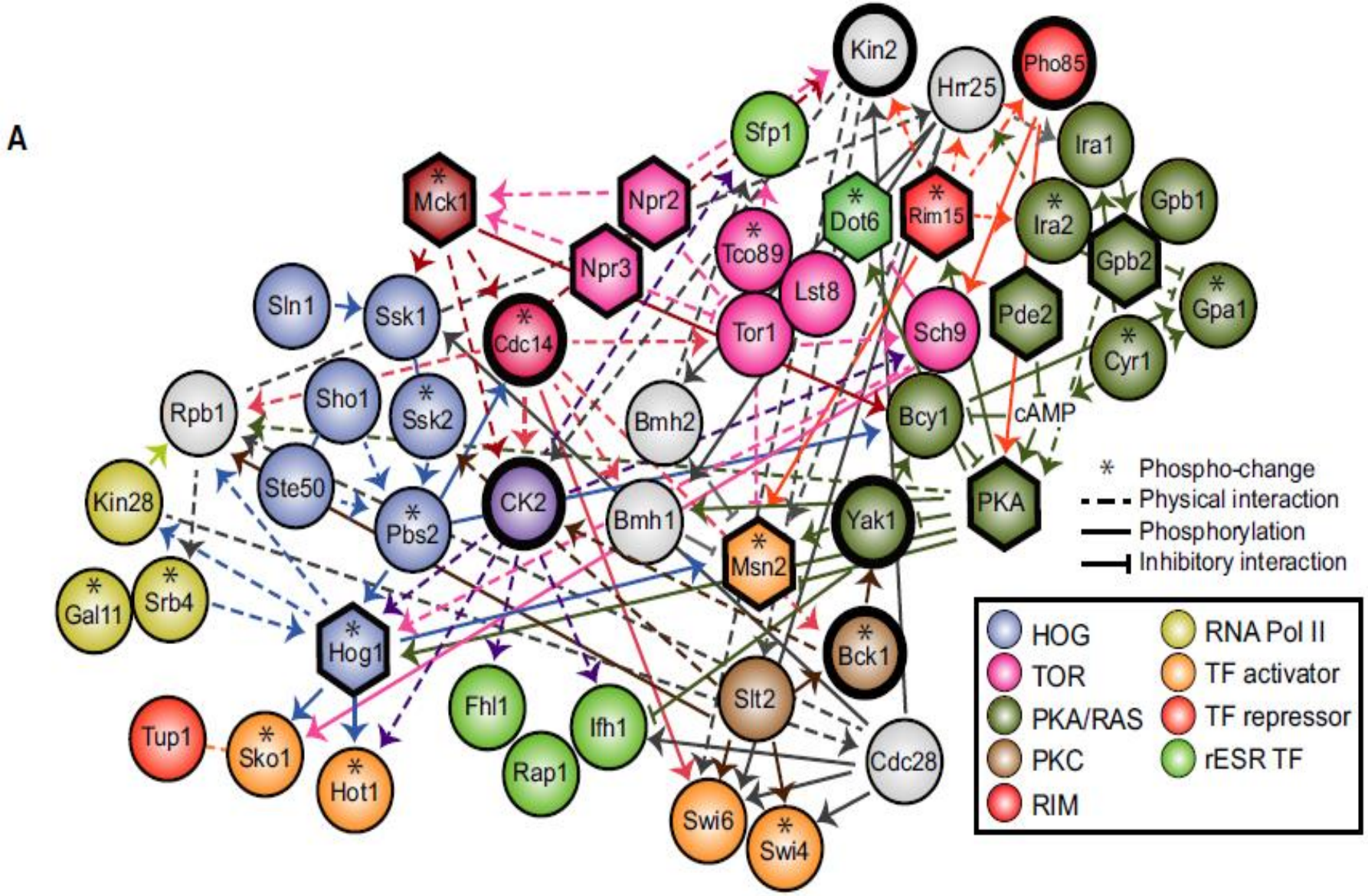


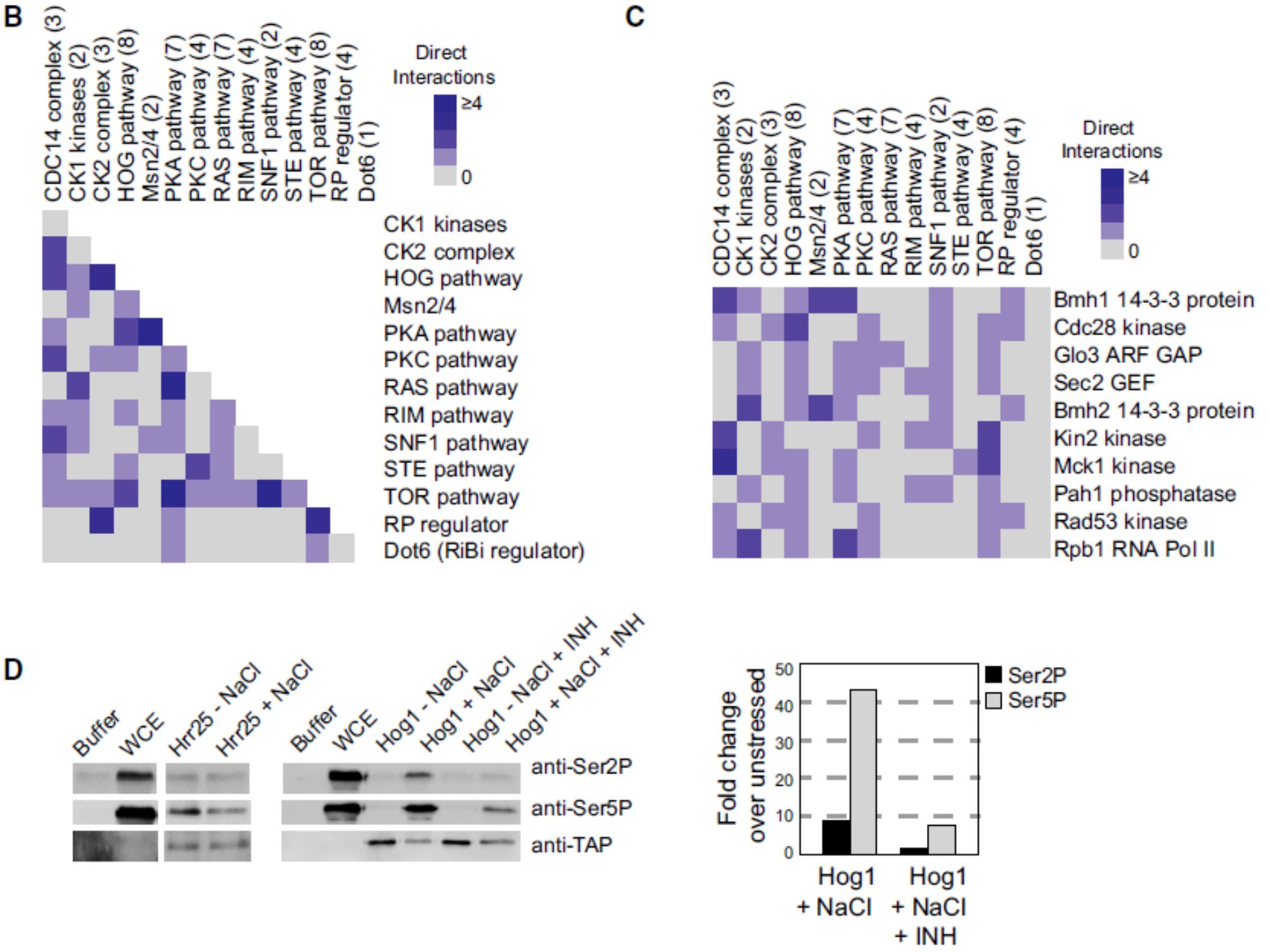
Validation analysis to verify

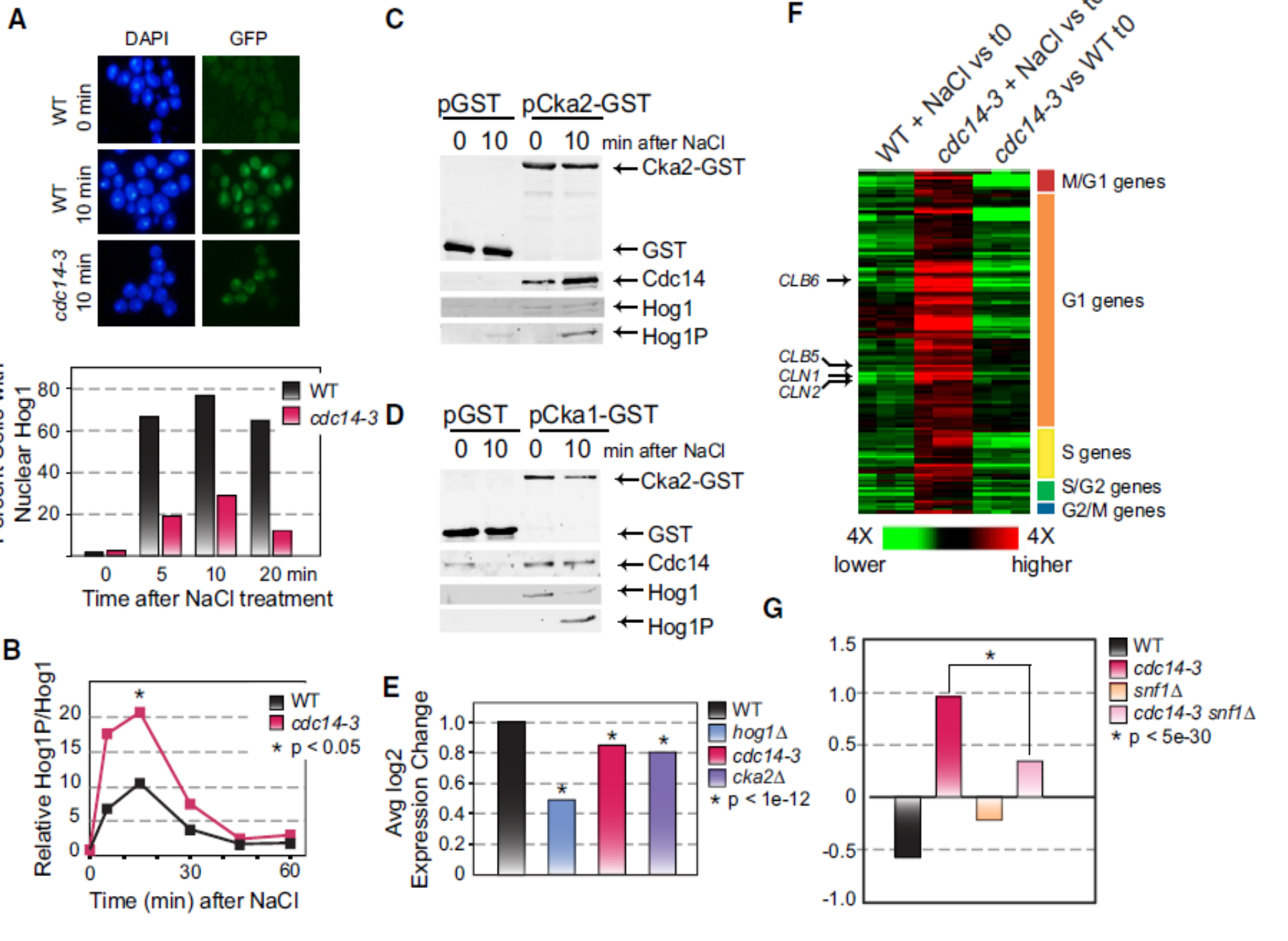
Validation mutants		
cdc14-3 (3)[c]	929	346
nnk1Δ (1)	94	278
bck1Δ (1)	107	169
yak1Δ (1)	226	248
kin2Δ (1)	52	266
pho85Δ (1)	614	342
cka2Δ (2)	155	63
cka1Δ (2)	58	133
ckb1Δ ckb12Δ (2)	129	176
arf3Δ (2)	466	331
scd6Δ (2)	0	0


A
Kinase
TF
RBP
Phospho-change
Physical interaction
Kinase–substrate interaction
B
IP method
Candidates
Permutations
Precision
Recall
1.0
0.8
0.6
0.4
0.2
0
0.2
0.4
0.6
0.8
1.0

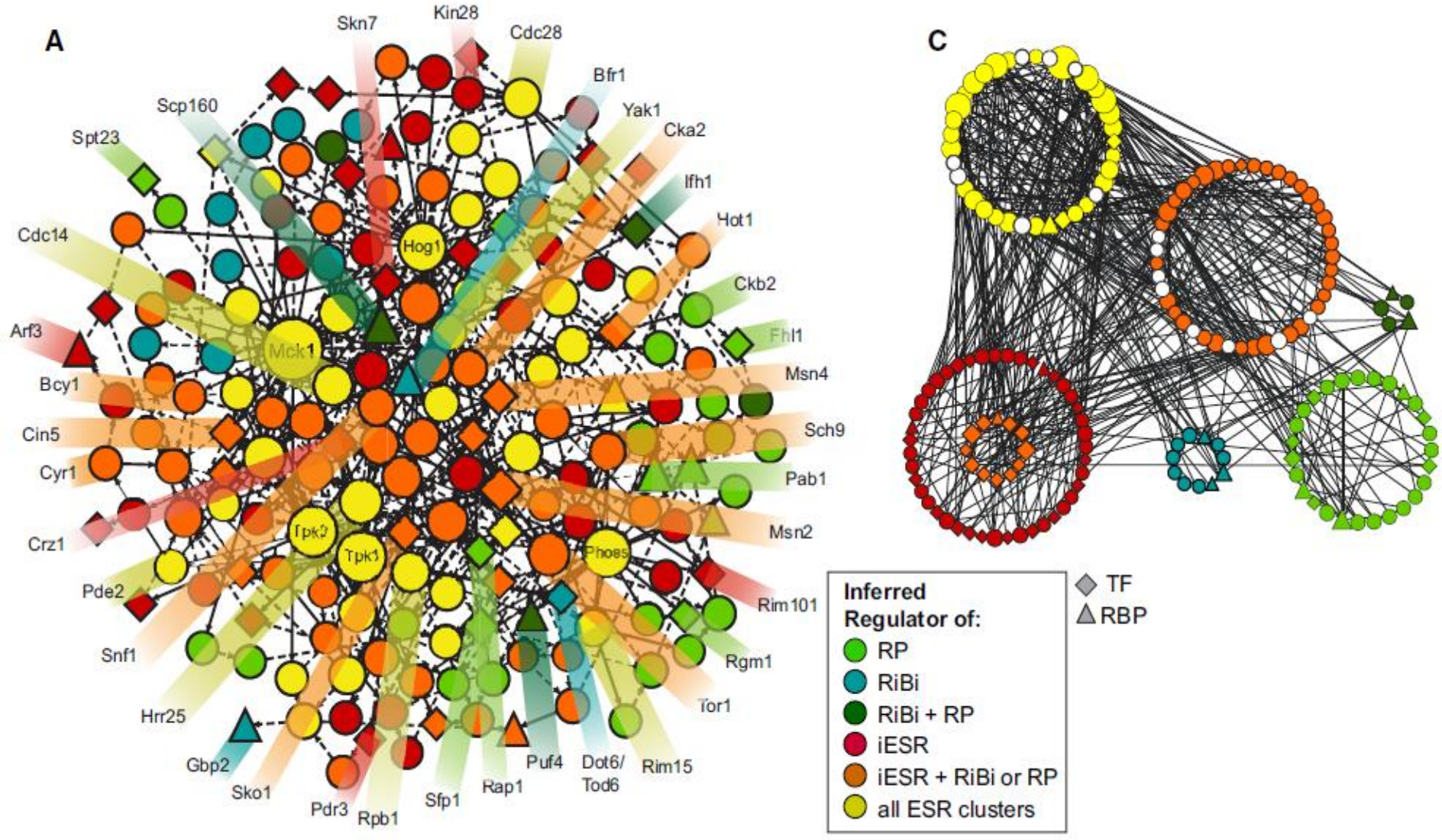
New insights into stress signaling

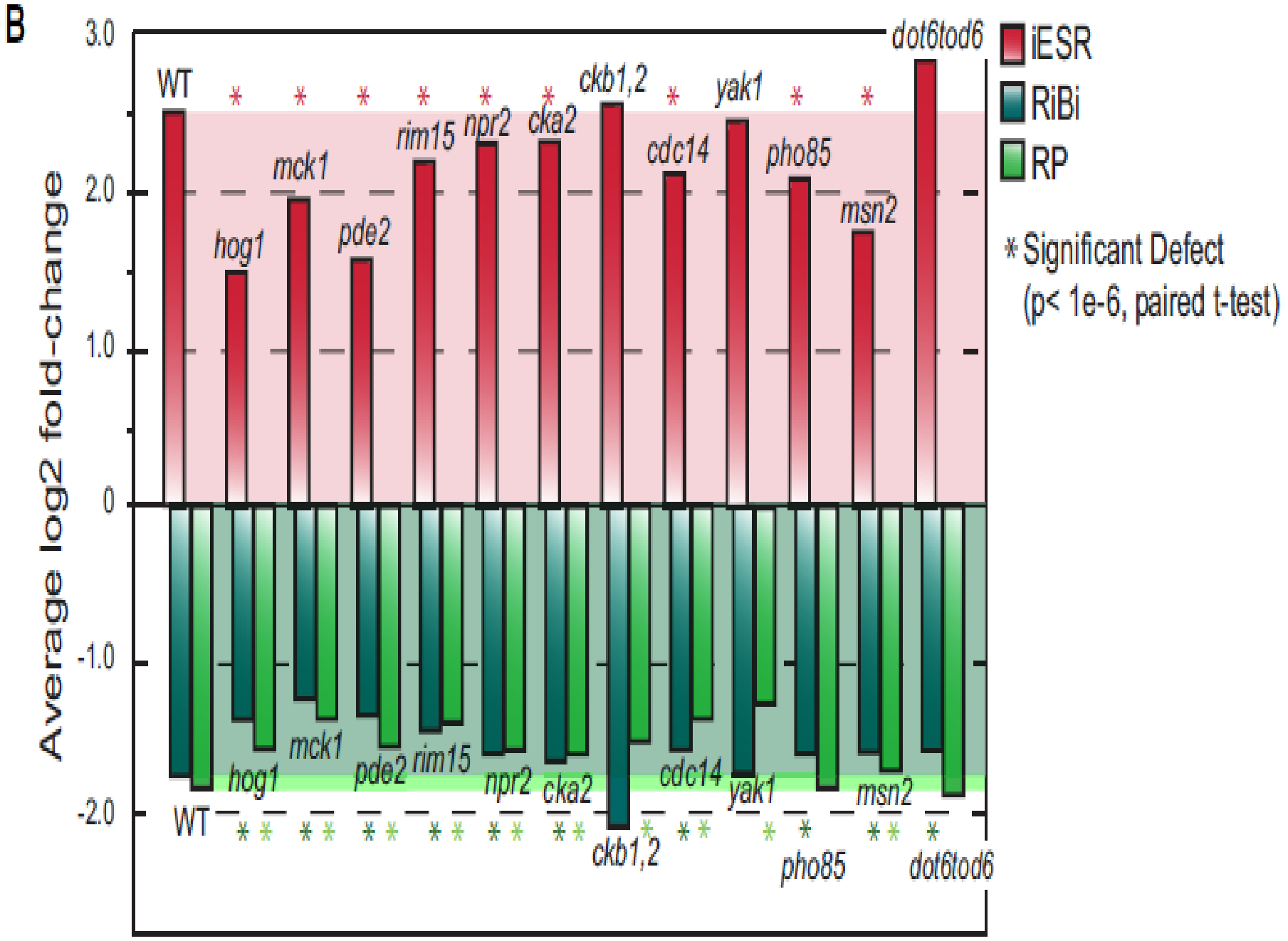


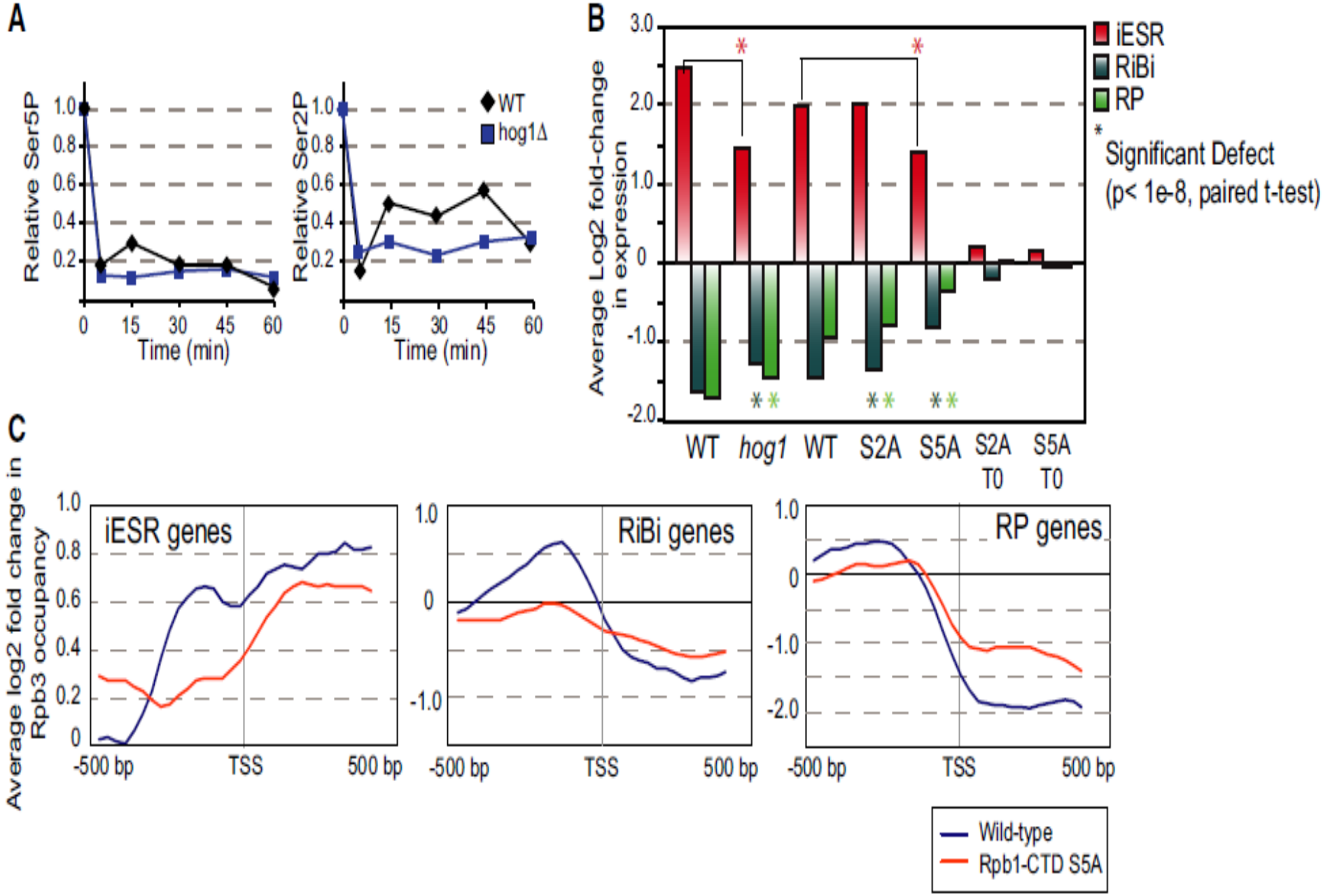

B
CDC14 complex (3)
CK1 kinases (2)
CK2 complex (3)
HOG pathway (8)
Msn2/4 (2)
PKA pathway (7)
PKC pathway (4)
RAS pathway (7)
RIM pathway (4)
SNF1 pathway (2)
STE pathway (4)
TOR pathway (8)
RP regulator (4)
Dot6 (1)
Direct Interactions
≥4
0
CK1 kinases
CK2 complex
HOG pathway
Msn2/4
PKA pathway
PKC pathway
RAS pathway
RIM pathway
SNF1 pathway
STE pathway
TOR pathway
RP regulator
Dot6 (RiBi regulator)
C
CDC14 complex (3)
CK1 kinases (2)
CK2 complex (3)
HOG pathway (8)
Msn2/4 (2)
PKA pathway (7)
PKC pathway (4)
RAS pathway (7)
RIM pathway (4)
SNF1 pathway (2)
STE pathway (4)
TOR pathway (8)
RP regulator (4)
Dot6 (1)
Direct Interactions
≥4
0
Bmh1 14-3-3 protein
Cdc28 kinase
Glo3 ARF GAP
Sec2 GEF
Bmh2 14-3-3 protein
Kin2 kinase
Mck1 kinase
Pah1 phosphatase
Rad53 kinase
Rpb1 RNA Pol II
D
Buffer
WCE
Hrr25 - NaCl
Hrr25 + NaCl
Buffer
WCE
Hog1 - NaCl
Hog1 + NaCl
Hog1 - NaCl + INH
Hog1 + NaCl + INH
anti-Ser2P
anti-Ser5P
anti-TAP
Fold change over unstressed
50
40
30
20
10
0
Ser2P
Ser5P
Hog1 + NaCl
Hog1 + NaCl + INH


A
DAPI
GFP
WT 0 min
WT 10 min
cdc14-3 10 min
Nuclear Hog1
WT
cdc14-3
Time after NaCl treatment
B
Relative Hog1P/Hog1
WT
cdc14-3
* p < 0.05
Time (min) after NaCl
C
pGST
pCka2-GST
0 10 0 10 min after NaCl
Cka2-GST
GST
Cdc14
Hog1
Hog1P
D
pGST
pCka1-GST
0 10 0 10 min after NaCl
Cka2-GST
GST
Cdc14
Hog1
Hog1P
E
Avg log2 Expression Change
WT
hog1Δ
cdc14-3
cka2Δ
* p < 1e-12
F
WT + NaCl vs t0
cdc14-3 + NaCl vs t0
cdc14-3 vs WT t0
CLB6
CLB5
CLN1
CLN2
M/G1 genes
G1 genes
S genes
S/G2 genes
G2/M genes
4X lower
4X higher
G
WT
cdc14-3
snf1Δ
cdc14-3 snf1Δ
* p < 5e-30

New insights into ESR regulation and coordination




B
Average log2 fold-change
3.0
2.0
1.0
0
-1.0
-2.0
WT
hog1
mck1
pde2
rim15
npr2
cka2
ckb1,2
cdc14
yak1
pho85
msn2
dot6tod6
iESR
RiBi
RP
* Significant Defect
(p< 1e-6, paired t-test)


A
Relative Ser5P
Relative Ser2P
Time (min)
WT
hog1Δ
B
Average Log2 fold-change in expression
iESR
RiBi
RP
* Significant Defect (p< 1e-8, paired t-test)
WT
hog1
WT
S2A
S5A
S2A T0
S5A T0
C
Average log2 fold change in Rpb3 occupancy
iESR genes
RiBi genes
RP genes
-500 bp
TSS
500 bp
Wild-type
Rpb1-CTD S5A

The orthologous mammalian networks are enriched for growthregulating and disease-causing genes

Striking the correct balance between growth rate and stress defense is fundamental for proper cellular function, and improper balance is thought to be a critical driver in diseases such as cancer We therefore interrogated the set of human genes orthologous to the yeast NaCl subnetwork. We found that this set is enriched for genes linked to cancer, mostly through somatic mutation,according to the COSMIC database: of the 35 human genes in the COSMIC dataset with yeast orthologs, 8 were orthologous to nodes in the consensus-node network, representing a 2.5-fold enrichment above chance. We also compared the yeast network to Mendelian disease genes in the OMIM database. We identified 25 additional yeast genes whose orthologs are linked to heritable disease, with weak enrichment forgenes associated with prostate cancer. The network was also enriched for yeast proteins whose mouse orthologs are required for pre/perinatal viability, normal growth rate and body size, and male and female fertility .These results highlight that stress-responsive signaling is likely important for proper regulation of growth rate, and thus may provide insights into cancer biology.

conclusion

We exploited these predictions to show that Cdc14 phosphatase is a central hub in the network and that modification of RNA polymerase II coordinates induction of stress defense genes with reduction of growth-related transcripts. We find that the orthologous human network is enriched for cancercausing genes, underscoring the importance of the subnetwork's predictions in understanding stress biology.

discussion

1.创新点

1.designed an integer linear programming (IP) approach to integrate and interpret our disparate datasets by inferring a signaling subnetwork. The novel facets of our computational approach include a means to integrate these varied data sources, using new types of input paths to the IP, and a multi-part objective function.

2.运用所构造的网络对人体进行了预测，发现了人体中的这些途径许多致癌基因，突破了物种的跨度，具有启示意义。

2.启发

由零到整再到零的实验思路，逻辑严谨，推理与验证还有运用一体化思想。

3.改进的地方

提出了许多预测的交联途径，但是并没有进行验证，仅仅只是为了试验的正确性而选择性的验证了一些研究的已经很透彻的途径，或许可以对一些新颖的预测途径进行探讨，从而发现更多的规律。