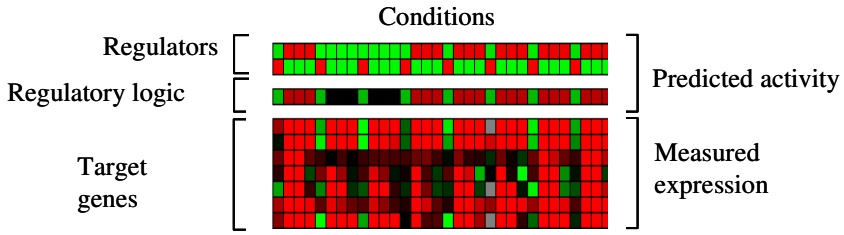
Supplement C

Module format



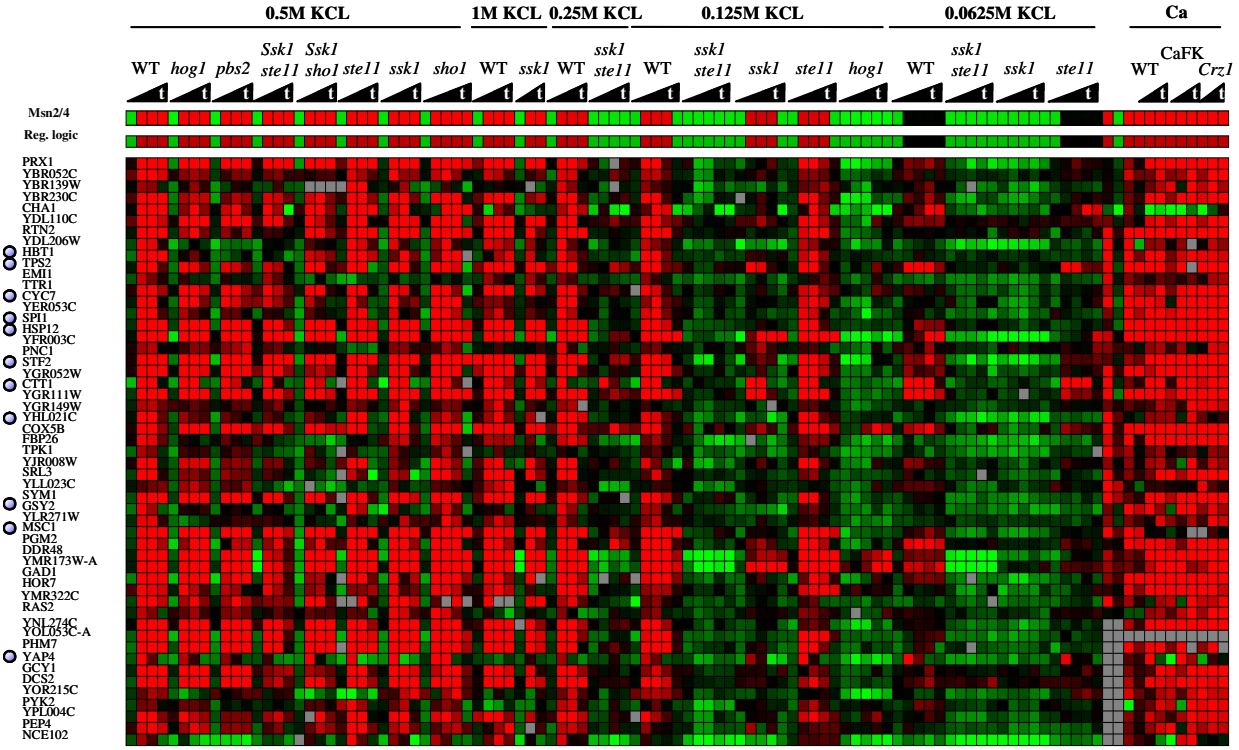
Known modules

1. Msn2/4-Module

Regulatory unit - Msn2 and Msn4 together are essential for stress induced expression of STRE-induced genes. The stimulation of expression by hyper osmotic shock depends on Msn2/4 as well as on he HOG pathway (Schüller et al. 1994).

Known targets - Blue circles mark known Msn2/4 targets identified in Rep et al. (2000) and the additional well known targets Yap4 and Hsp12. Out of 46 targets discovered in Rep et al. (2000), 18 were assigned to the Msn2/4 module, among them nine obtained improvement score > 10 (hyper geometric p-value < 10^{-10}) and thus included in this module. Seven genes were assigned to Hog1A-module (module 5), and six genes were assigned to various other modules. The remaining 15 genes were identified as model-independent responding genes.

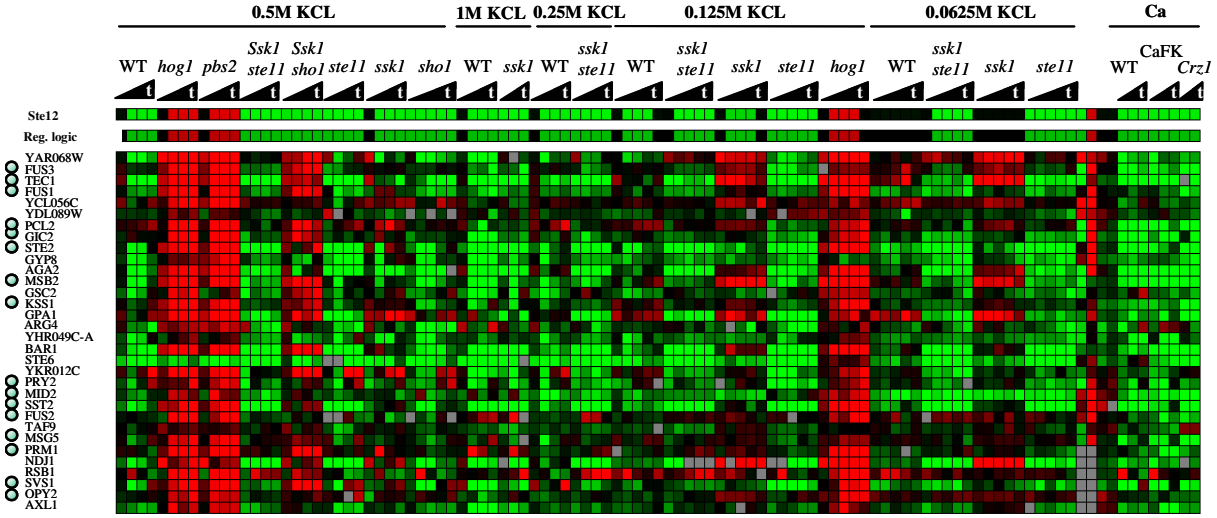
- Known targets: 46 (Rep et al. 2000) + 2 (well known)
- Correctly assigned: 9 + 2
- Assigned to small modules or incorrectly assigned: 6



2. Ste12-Module

Known targets Green circles mark known Ste12 targets related to mating and psaudohyphal growth pathway identified in Zeitlinger et al. (2003), and the additional well known target Kss1. Out of 66 known targets discovered in Zeitlinger et al. (2003), 20 were assigned to the model and predicted to be regulated by Ste12. Among them 15 were assigned specifically to the Ste12-module, and five were assigned to small modules. Four genes were assigned to other modules, not predicted to be regulated by Ste12. The remaining 42 genes were identified as model-independent responding genes.

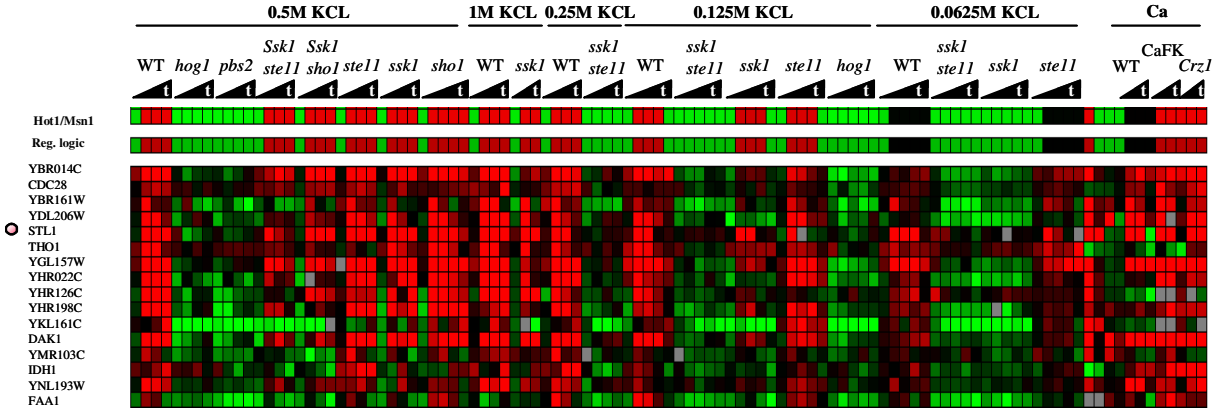
- Known targets: 66 (Zeitlinger et al. 2003) + 1 (well known)
- Correctly assigned: 15 + 1(Kss1)
- Assigned to small modules or incorrectly assigned: 4



3. Hot1/Msn1-Module

Known targets (red circles) - According to Rep et al. (1999), deletion of Msn1 diminishes expression of Gpd1, Hor2, Ctt1 and Stl1, and the effect on Ctt1 and Stl1 is the strongest. The best score of Stl1 and Gpd1 was to this module, but only Stl1 obtained significant improvement score, and thus was assigned to the module. Ctt1 is usually known as Msn2/4 target, and was assigned to the Msn2/4 module. Hor2 was assigned incorrectly to the Stl1-module.

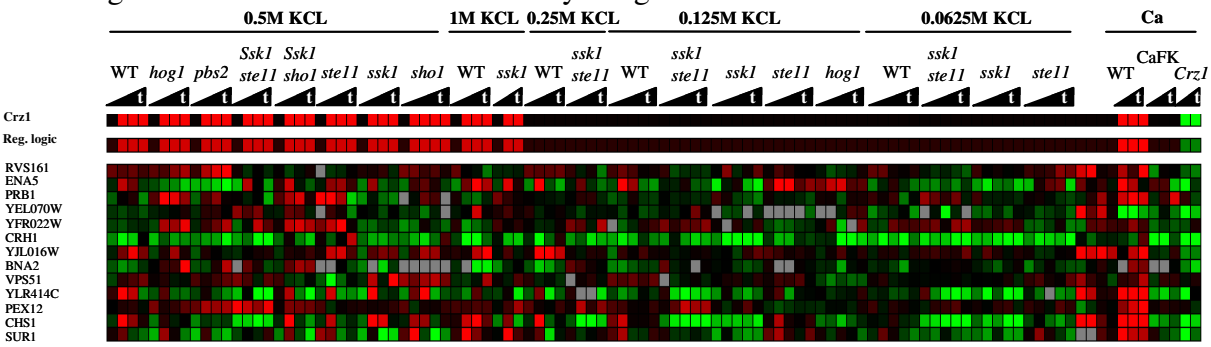
- Known targets: 3 (Rep et al. 1999), not including Ctt1.
- Correctly assigned: 1
- Assigned to small modules or incorrectly assigned: 1



4. Crz1-Module

Pmc1, Pmr1 and Fks2 are the known target according to Stathopoulos and Cyert, 1997. All these targets did not obtain significant improvement score.

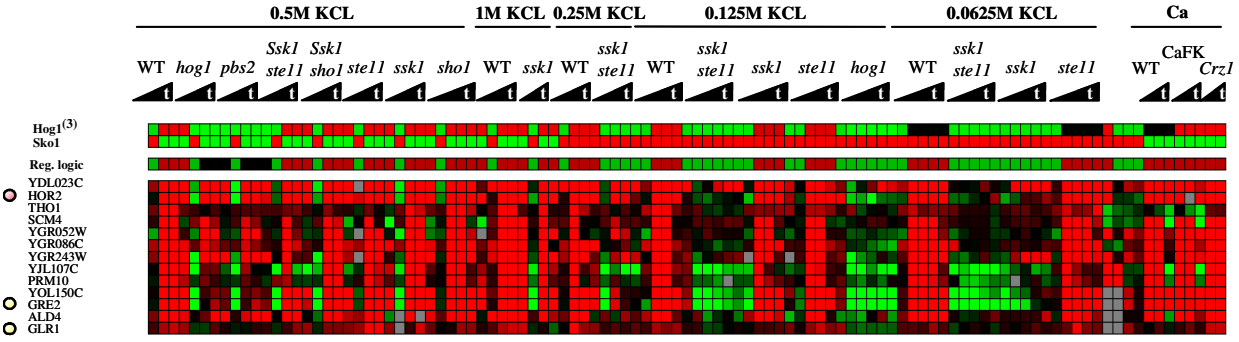
- Known targets: 3
- Correctly assigned: 0
- Assigned to small modules or incorrectly assigned: 0



5. Sko1-Module

Known targets (yellow circles) - The known targets are Gre2, Glr1, Ahp1 Sfa1 and YML131W (Rep et al. 2001). Among them, two are included in this module, and two are included in small modules incorrectly. Two additional targets of Sko1 are jointly regulated by Sko1 and another regulator and thus should be included in other modules: Ena1 (regulated by Sko1+Crz1) and Hal1 (regulated by Sko1+Gcn4). Hor2 is included in this module incorrectly (marked in pink), and should be included in the Hot1/Msn1 module.

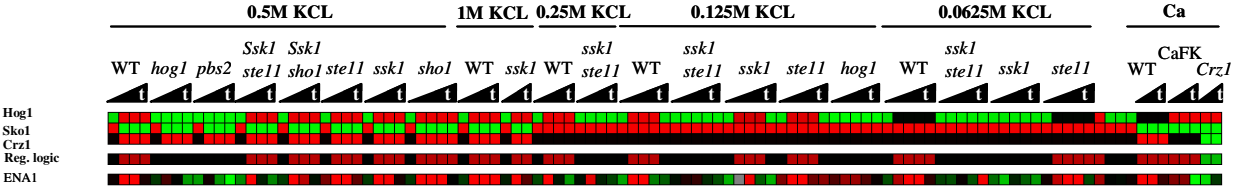
- Known targets: 5
- Correctly assigned: 2
- Assigned to small modules or incorrectly assigned: 2



Crz1+Sko1:

The only known target of this unit, Ena1 (Hohmann 2002), was assigned correctly to it. According to our results, this is the only gene regulated by this unit.

- Known targets: 1
- Correctly assigned: 1
- Assigned to small modules or incorrectly assigned: 0



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Rep M., Reiser V., Holzmüller U., Thevelein J.M., Hohmann S., Ammerer J., and Ruis H. (1999). Osmotic stress-induced gene expression in *Saccharomyces cerevisiae* requires Msn1p and the novel nuclear factor Hot1p. Mol. Cell. Biol. **19:**5474-5485.

Rep M., Krantz M., Thevelein J.M., and Hohmann S. (2000). The transcriptional response of *Saccharomyces cerevisiae* to osmotic shock. Hot1p and Msn2p/Msn4p are required for the induction of subsets of high osmolarity glycerol pathway-dependent genes. J. Biol. Chem. **275:**8290-8300.

Rep M., Proft M., Remize F., Tamas M., Serrano R., Thevelein J.M., and Hohmann S. (2001). The *Saccharomyces cerevisiae* Sko1p transcription factor mediates HOG pathway-dependent osmotic regulation of a set of genes encoding enzymes implicated in protection from oxidative damage. Mol. Microbiol. **40:**1067-1083.

Schüller G., Brewster J.L., Alexander M.R., Gustin M.C., and Ruis H. (1994). The HOG pathway controls osmotic regulation of transcription via the stress response element (STRE) of the *Saccharomyces cerevisiae CTT1* gene. EMBO J. **13:**4382-4389

Stathopoulos A.M, Cyert M.S (1997). Calcineurin acts through the CRZ1/TCN1-encoded transcription factor to regulate gene expression in yeast. Genes & Development **11**:3432-3444

Zeitlinger J., Simon I., Harbison C.T., Hannett N.M., Volkert T.L., Fink G.R., Young R.A. (2003). Program-specific distribution of a transcription factor dependent on partner transcription factor and MAPK signaling. Cell **113**:395-404.

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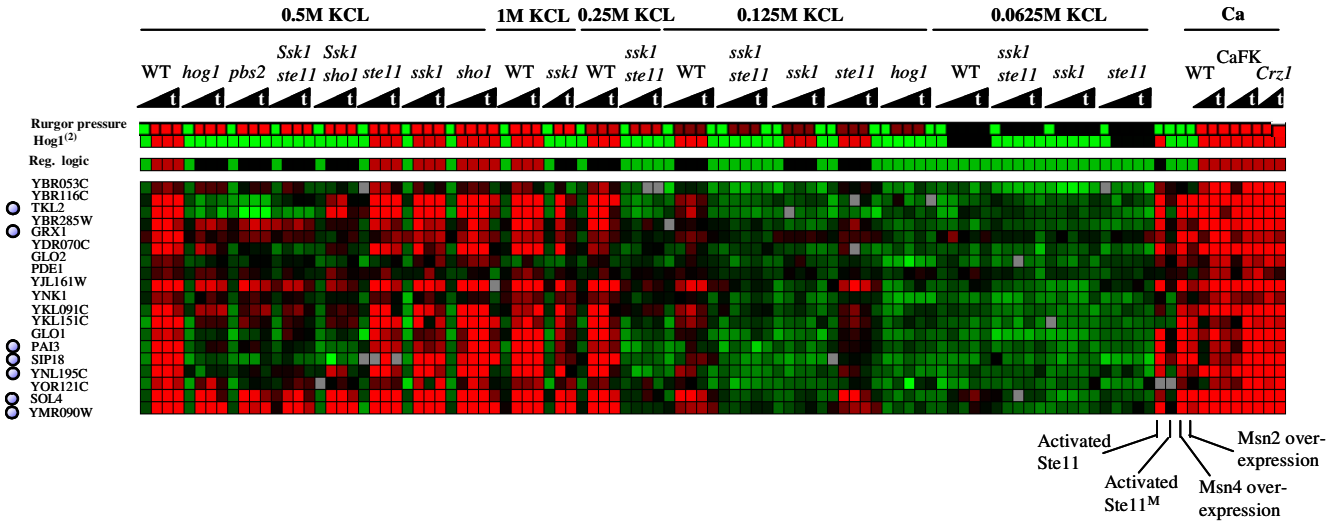
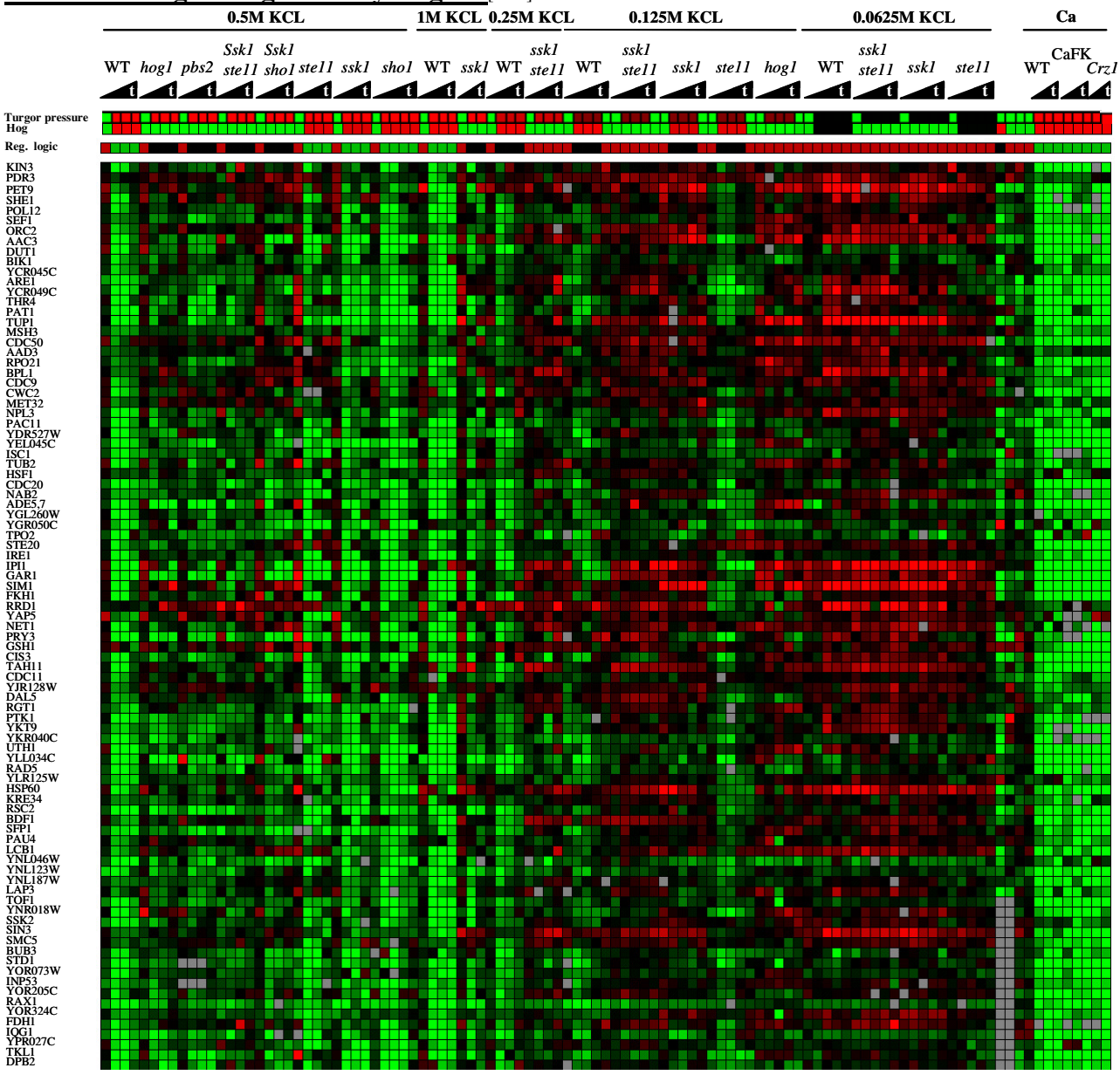
Putative modules

Module 5 – Hog1A - regulated by Hog1$^{(2)}$ [26.16]

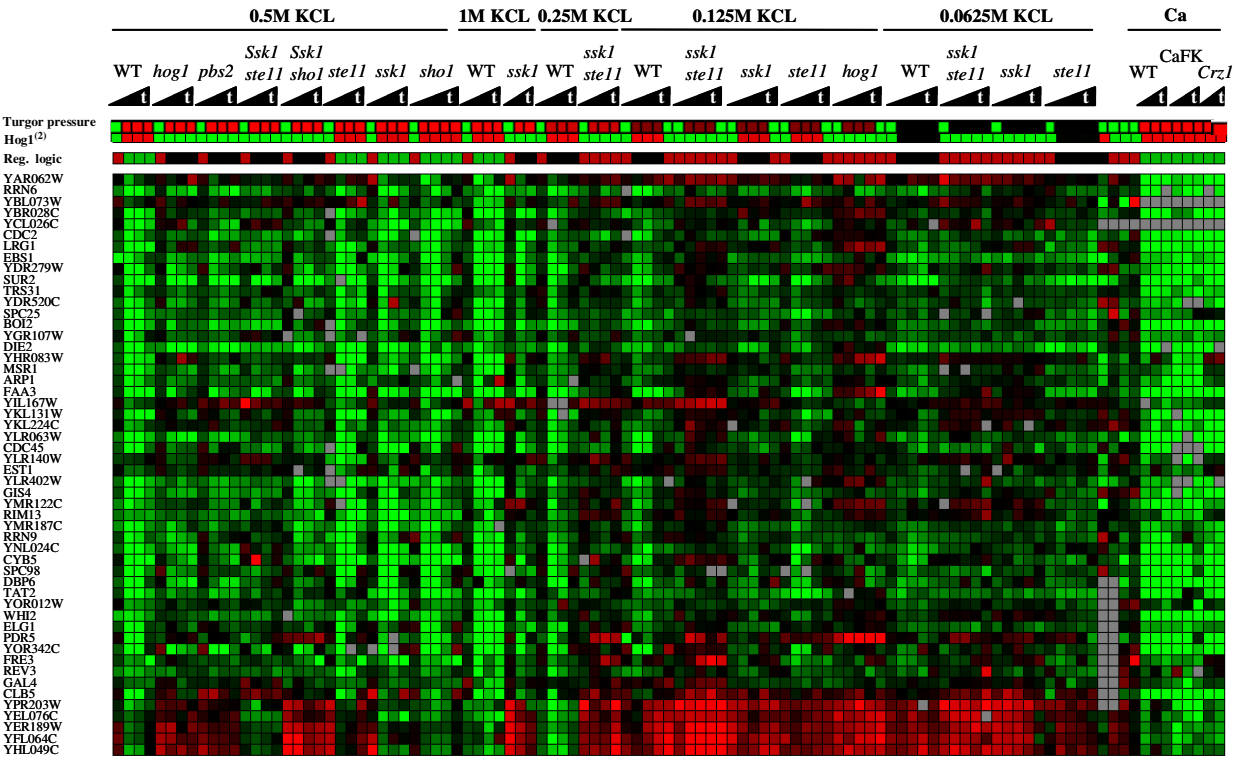
All the known targets in this module are Msn2/4 targets identified by Rep et al. 2000 (marked in blue circles). Out of 46 Msn2/4 targets, seven were assigned to this module (hyper geometric p-value < 10^{-10}). Interestingly, the module is regulated by Hog1 (Msn2/4 is not included in the putative regulators we tested) and thus its predicted activity in the Msn2/4 overexpression experiments is low (green). Yet, the observed levels in the two corresponding columns are high (red). The discrepancy between observed and predicted levels shows that the module should have been located downstream to Msn2/4.



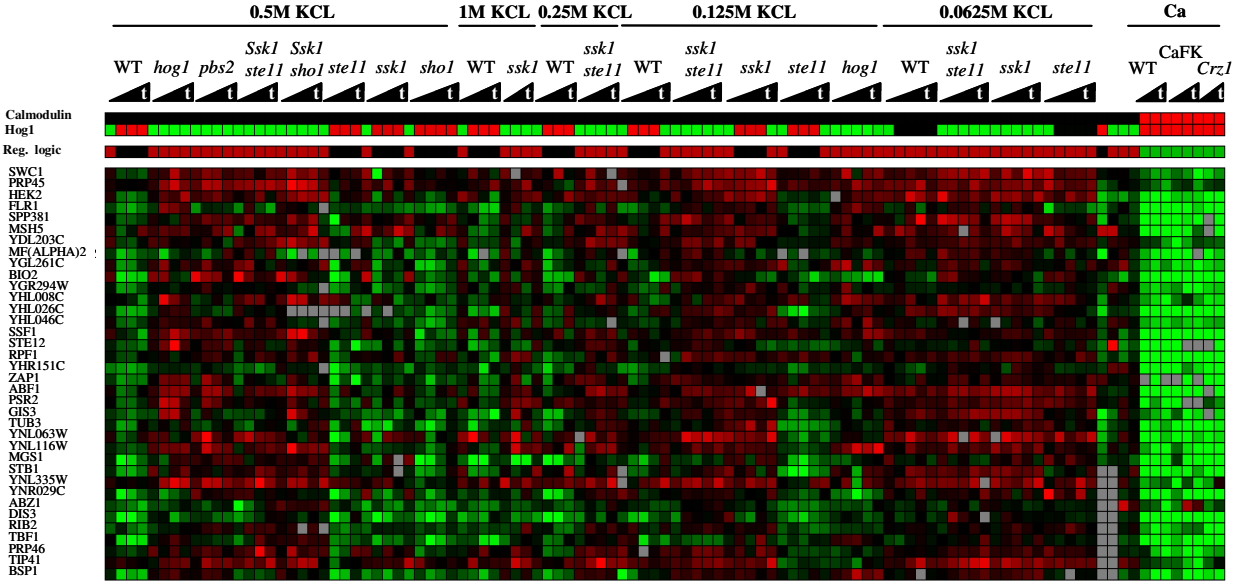
Module 6– Hog1B -regulated by Hog1[2] [26.5]



Module 7– Hog1C - regulated by Hog1$^{(2)}$ [26.7]



Module 8 – Hog1/Ca - regulated by Hog1$^{(2)}$ + Calmodulin [61.0]



Module 9 – regulated by Ssk2/22 [66.120]