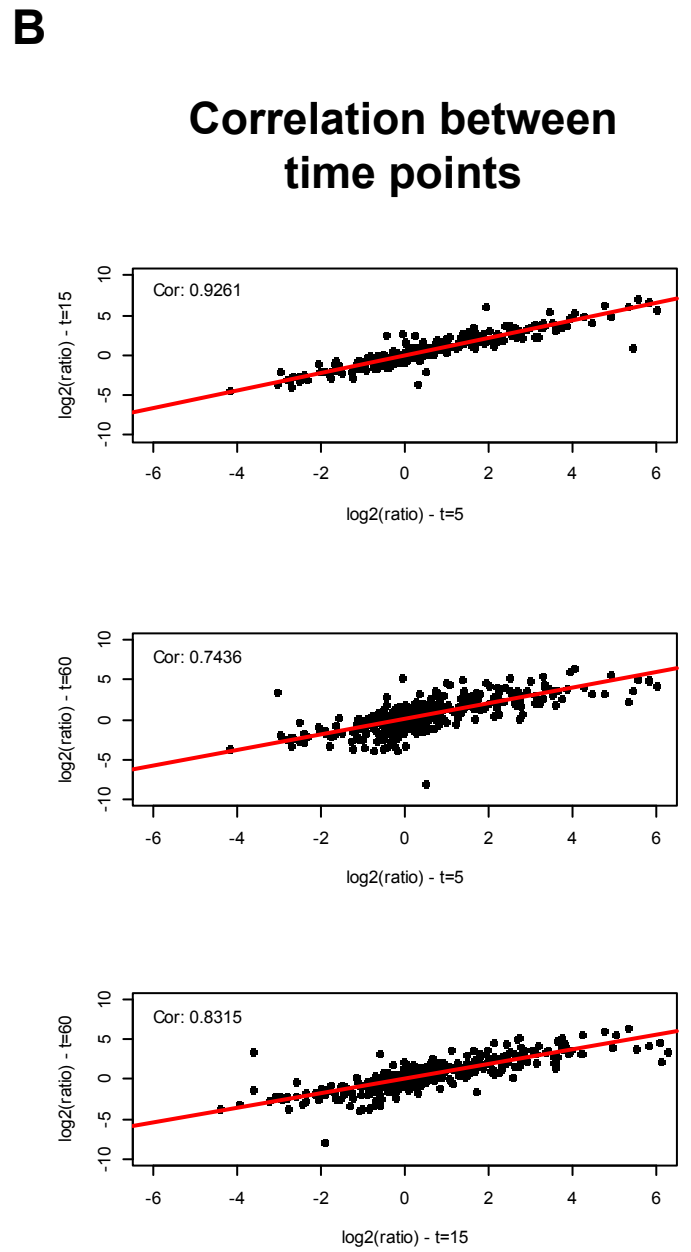
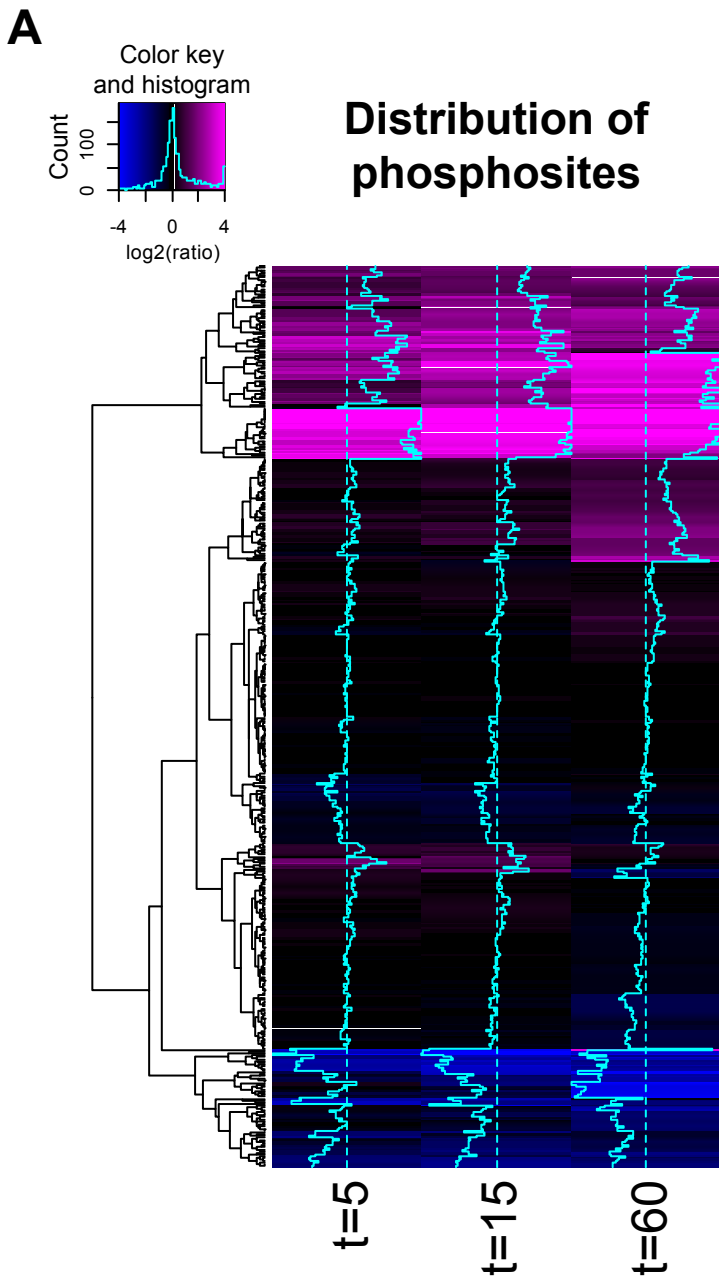
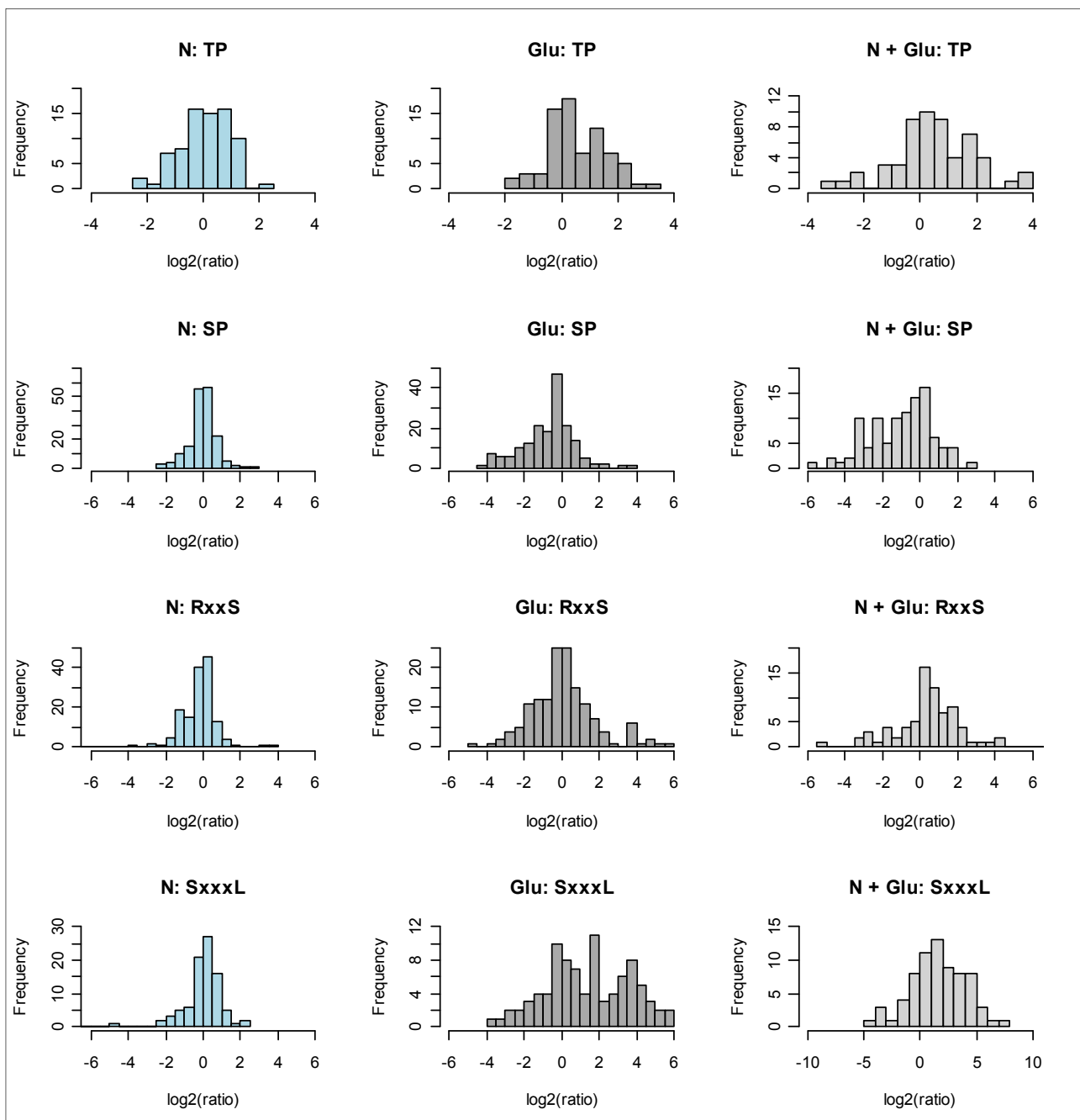


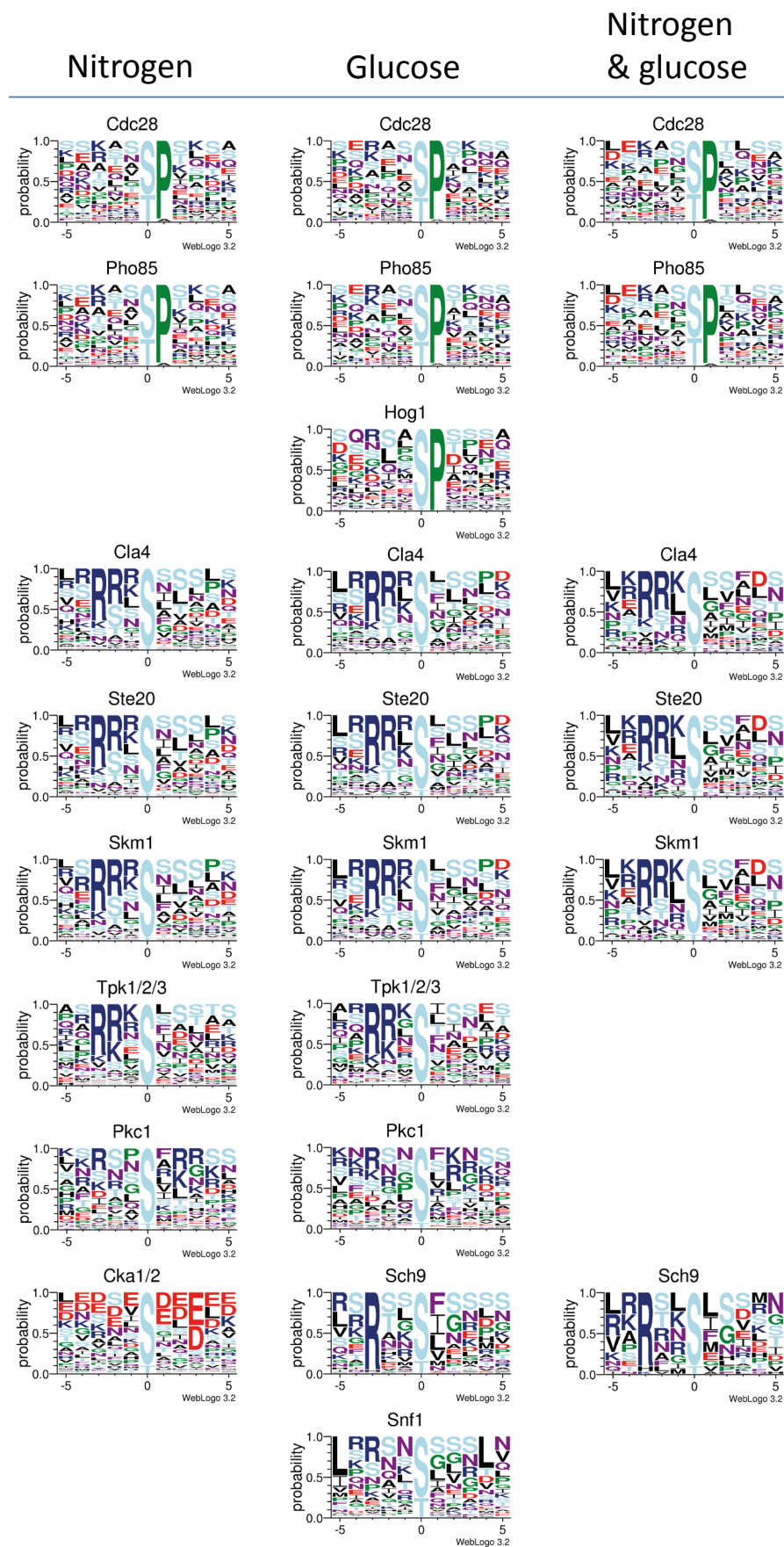
**Figure S1.** Overview of the SILAC-based approach to examine starvation responses in *S. cerevisiae*. The arginine and lysine auxotrophic strain YAL6b was cultured in different YNBD media containing different combinations of arginine and lysine stable isotopes. Cells were grown until mid-log phase and the media was changed to either YNB (for glucose starvation), YNBD without amino acids and NH<sub>4</sub>(SO<sub>2</sub>)<sub>4</sub> (for nitrogen starvation), or YNB without amino acids and NH<sub>4</sub>(SO<sub>2</sub>)<sub>4</sub> (for glucose and nitrogen starvation). Finally, cells were harvested at indicated time points and an equal number of untreated cells were mixed with starved cells and prepared for MS analysis on a LTQ Orbitrap Velos.



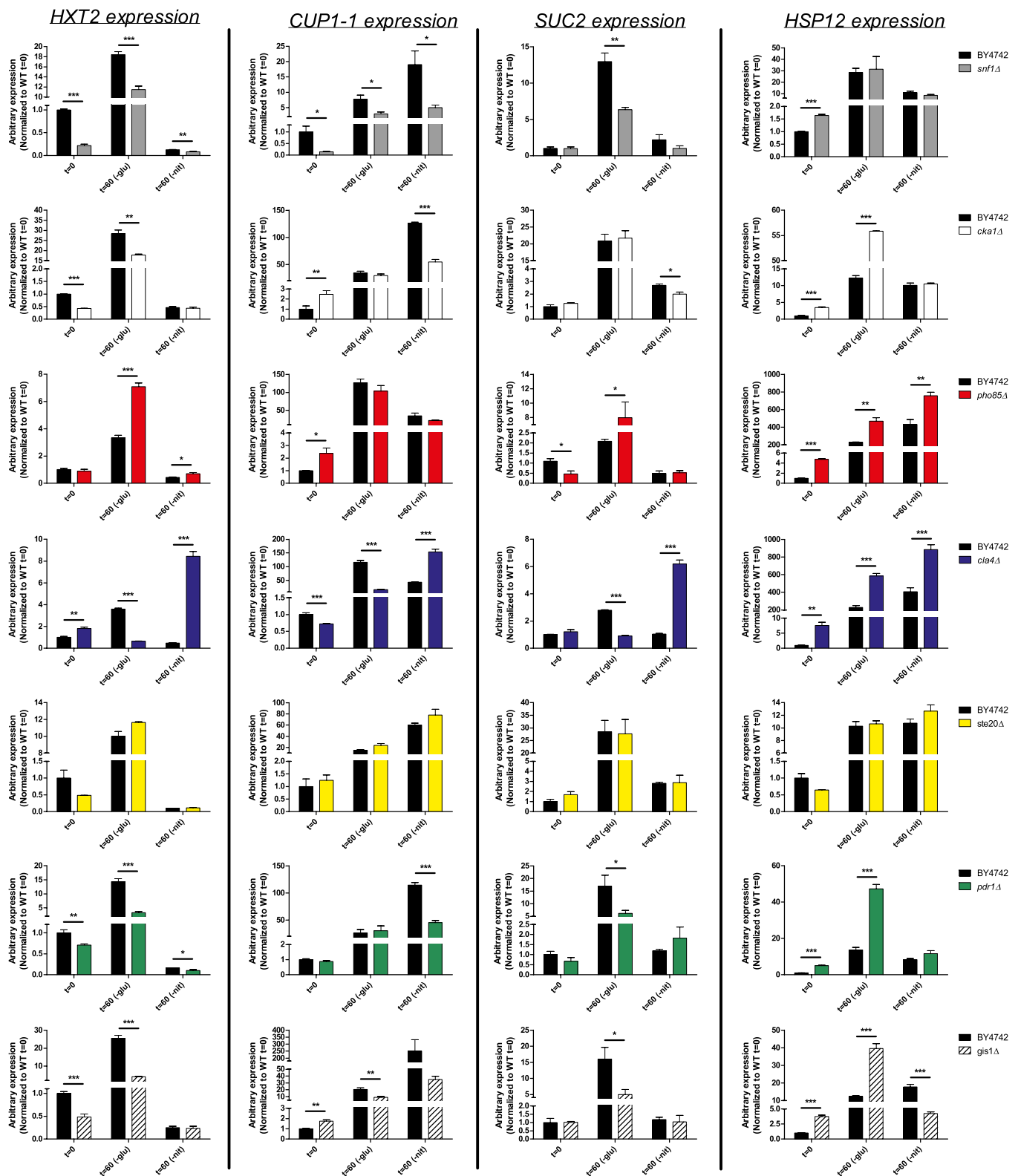
**Figure S2.** Comparing the phosphorylation response over time upon glucose deprivation. The phosphoproteome in cells starved for glucose for 5, 15, or 60 minutes were compared. 481 sites were identified at all time points. A: The distribution of the sites was compared between the different time points. Magenta indicates phosphorylation events whereas blue indicates dephosphorylation events. B: The different time points were individually correlated (Pearson) to each other.



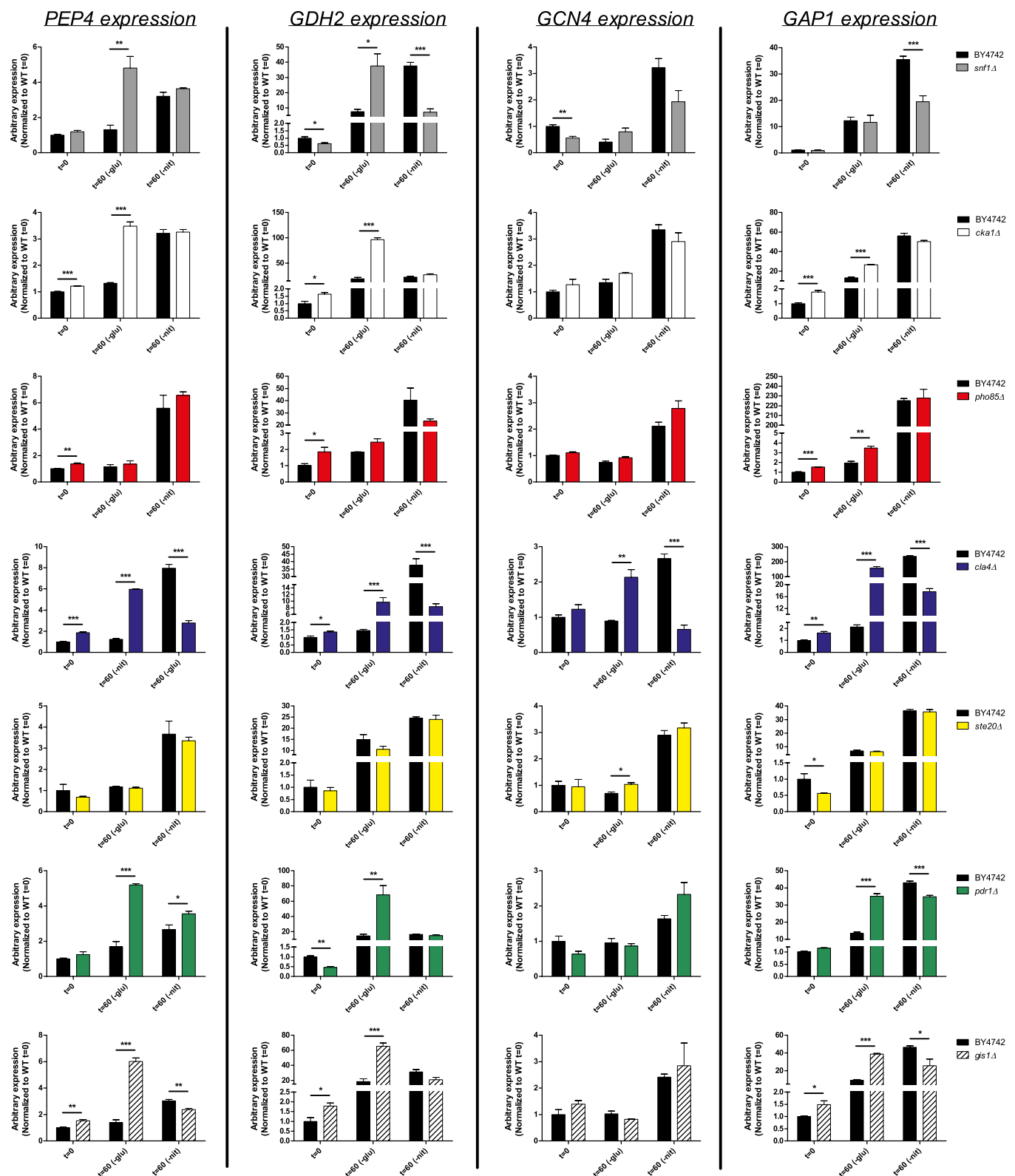
**Figure S3.** Ratio distributions of different phosphorylation motifs. Ratio distributions of the SP, the TP, the RxxS, and the SxxxL phosphorylation motifs during starvation from glucose, nitrogen, or the combination hereof. N: Nitrogen. Glu: glucose.



**Figure S4.** Phosphomotifs associated with different kinases during starvation. NetworkKIN was used to predict kinases regulating the identified regulated phosphosites. WebLogo 3.2 was used to identify if any consensus sequences was associated to the different kinases.

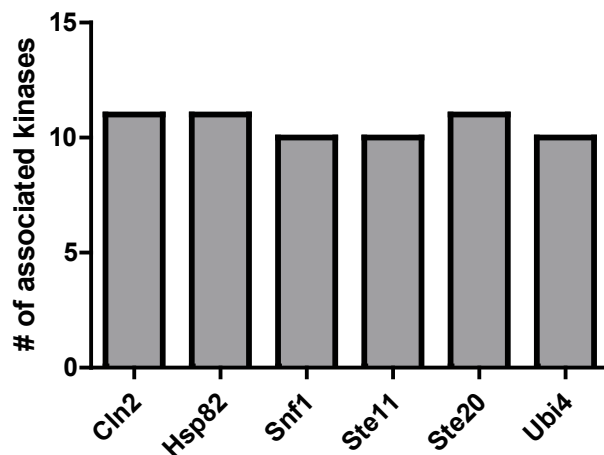


**Figure S5.** Effect of predicted candidates on the expression of genes known to be induced upon glucose starvation. Expression levels of *HXT1*, *CUP1-1*, *SUC2*, and *HSP12* in the indicated mutant were determined by qPCR. Expression levels have been normalized to *ACT1* levels and shown relative to the level in wild-type cells. Glu: glucose, Nit: nitrogen. \*:  $P < 0.05$ ; \*\*:  $P < 0.01$ ; \*\*\*:  $P < 0.001$ ,  $n=2-5$ , error bars = SEM.



**Figure S6.** Effect of predicted candidates on the expression of genes known to be induced upon nitrogen starvation. Expression levels of *PEP4*, *GDH2*, *GCN4*, and *GAP1* were determined by qPCR in the indicated mutants. Expression levels have been normalized to *ACT1* levels and shown relative to the level in wild-type cells. Glu: glucose, Nit: nitrogen. \*:  $P < 0.05$ ; \*\*:  $P < 0.01$ ; \*\*\*:  $P < 0.001$ ,  $n=2-5$ , error bars = SEM.

### Number of indirect associations to the 14 kinases



**Figure S7.** Prediction of proteins associated to starvation-associated kinases. The STRING algorithm was used to identify proteins, which were associated either directly to one of the kinases or indirectly to a neighboring protein that itself was associated to a kinase. Only proteins with high confidence associations ( $> 0.9$ ) were used. In total, 991 associated proteins were identified, of which only proteins, which associate with more than 10 kinases are shown in the figure.