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Article



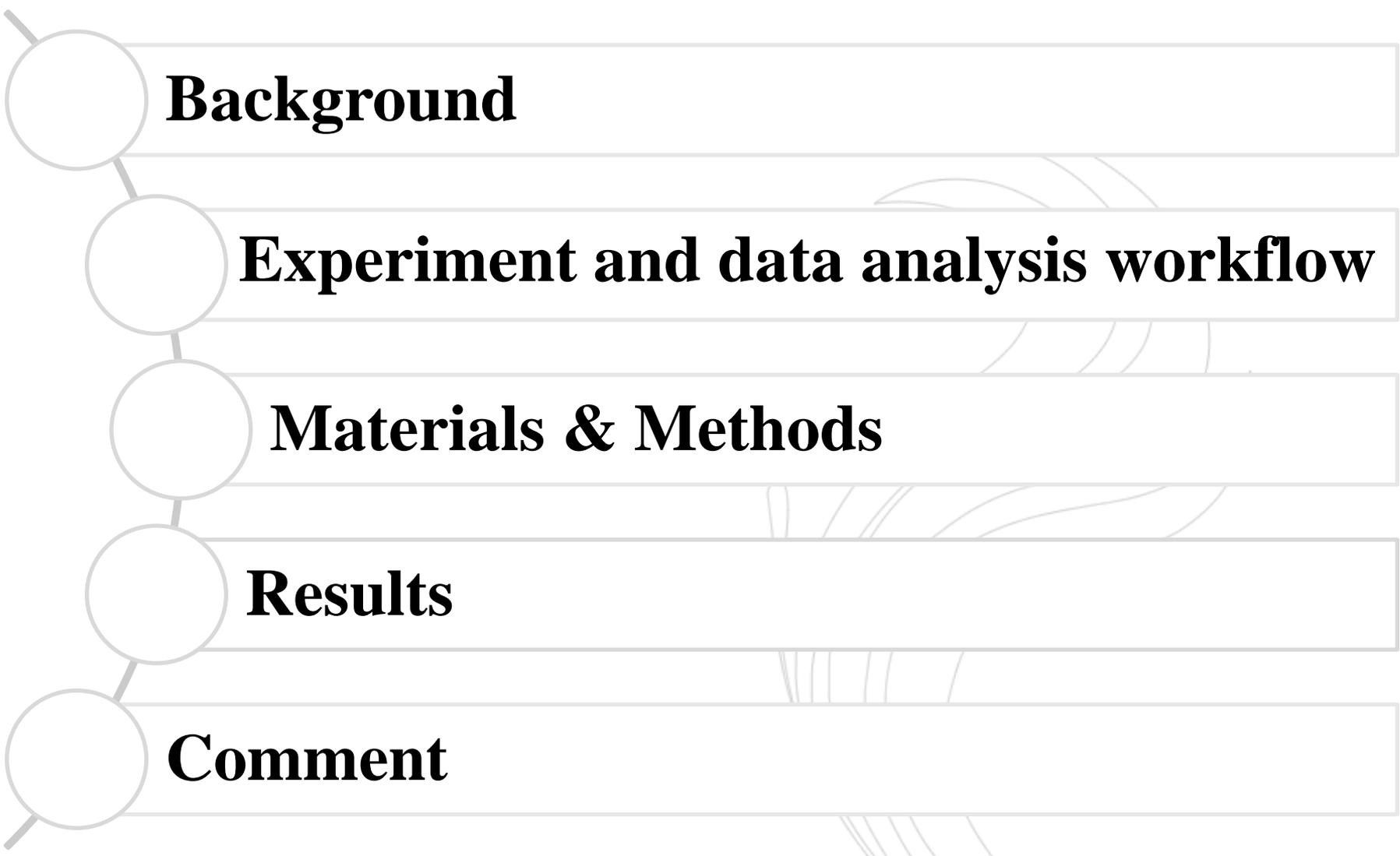
molecular  
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# Phosphoproteome dynamics of *Saccharomyces cerevisiae* under heat shock and cold stress

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温度刺激下酵母的磷酸化蛋白组动力学

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**Background**

**Experiment and data analysis workflow**

**Materials & Methods**

**Results**

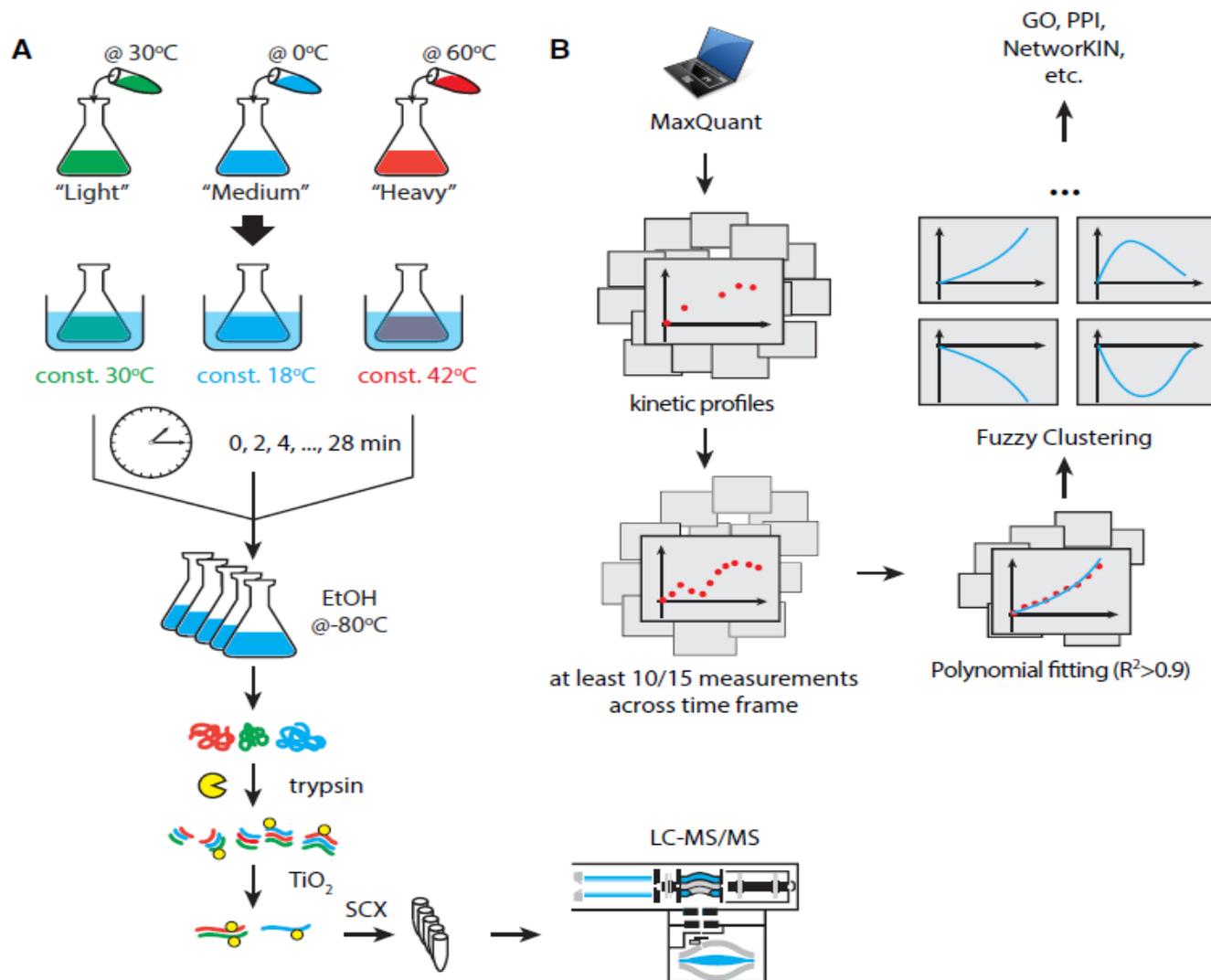
**Comment**

# 1. Background

- Single cell organisms such as yeast are regularly exposed to environmental changes that required the development of adaptation mechanisms for survival in suboptimal growth conditions.
- *S. cerevisiae* have evolved protective transcriptional programs in response to elevated temperature ( $> 37^{\circ} \text{C}$ ) that result in physiological changes affecting carbohydrate flux, cytoskeleton dynamics and protein folding (Morano et al, 2012; Verghese et al, 2012).

- While a large body of the literature exists on the molecular basis of heat shock in yeast, mechanisms associated with the response to cold stress are still poorly understood (Aguilera et al, 2007).
- Aside from the classical stress pathways in which Hog1 is playing a central role, little is known about the signaling events resulting from changes in temperature.
- To gain further insights on the dynamic changes in protein phosphorylation associated with heat shock and cold stress, we performed **a large-scale phosphoproteomics study** on *S. cerevisiae*.

# 2. Experiment and data analysis workflow



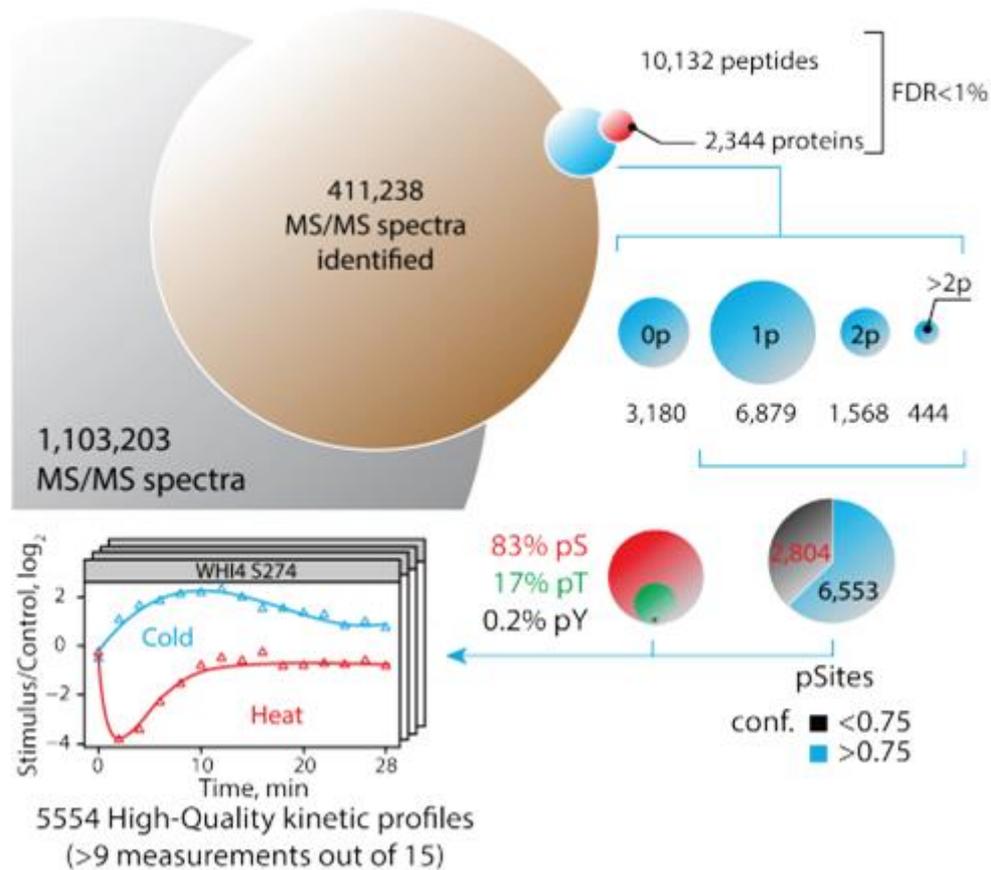


Figure S1. Summary of phosphopeptide identification.

A total of 1,103,203 distinct MS/MS spectra were identified and assigned to 10,132 unique peptides from 2,344 proteins with a FDR < 1%. Phosphopeptides represented 73 % of all identified peptides, and 70% of all phosphosites (9,357) were assigned with high confidence (>0.75). A total of 5,554 dynamic profiles ( $\geq 10$  out of 15 data points) were obtained from all high confidence assignments. Phosphorylation sites were distributed on Ser (83%) Thr (17%) and Tyr (0.2%) residues.

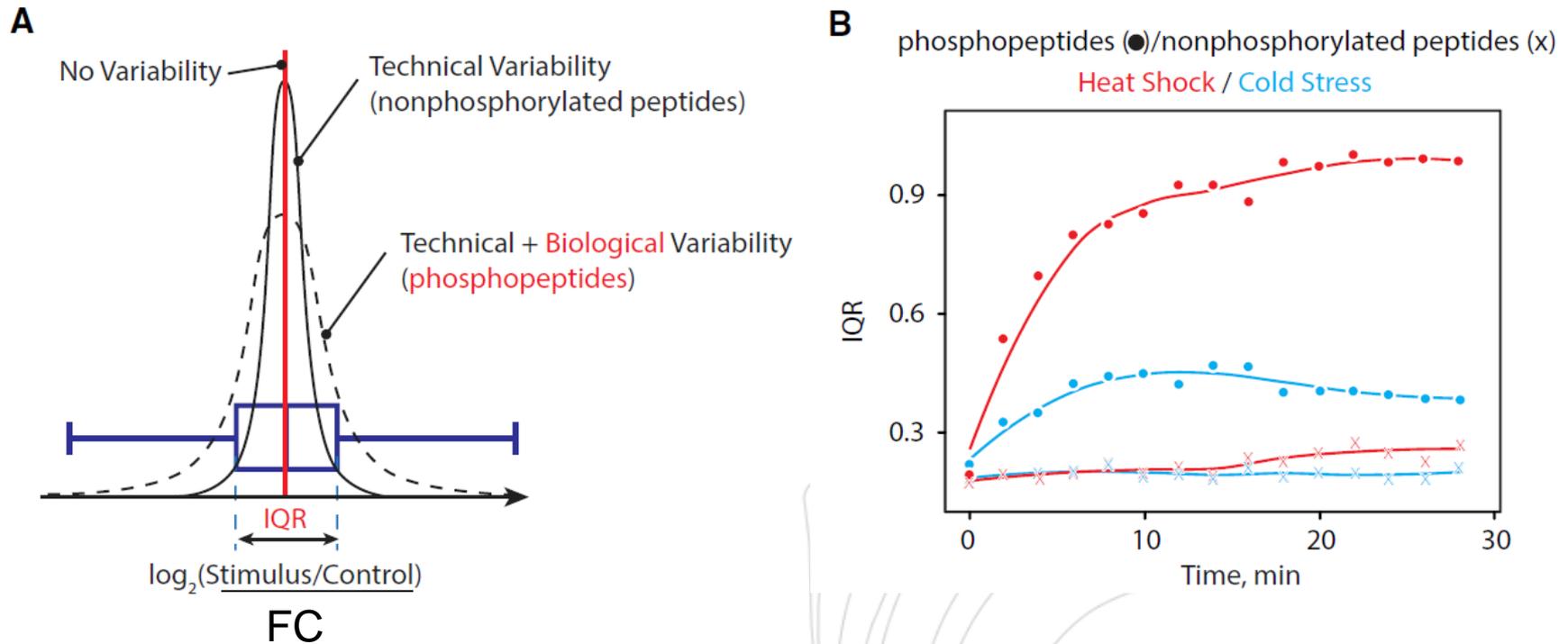
# 3. Materials & Methods

- Cell culture, Yeast strain S288C
- Yeast muta
- Protein extraction and enzymatic digestionnt construction
- Phosphopeptide isolation and fractionation
- Offline PGC fractionation of nonphosphorylated peptides
- Mass spectrometry: LC-MS/MS
- Data processing and analysis: MaxQuant, FC
- Clustering of kinetic profiles, NetworKIN, GO and PPI network analysis
- Yeast cell cycle analyses

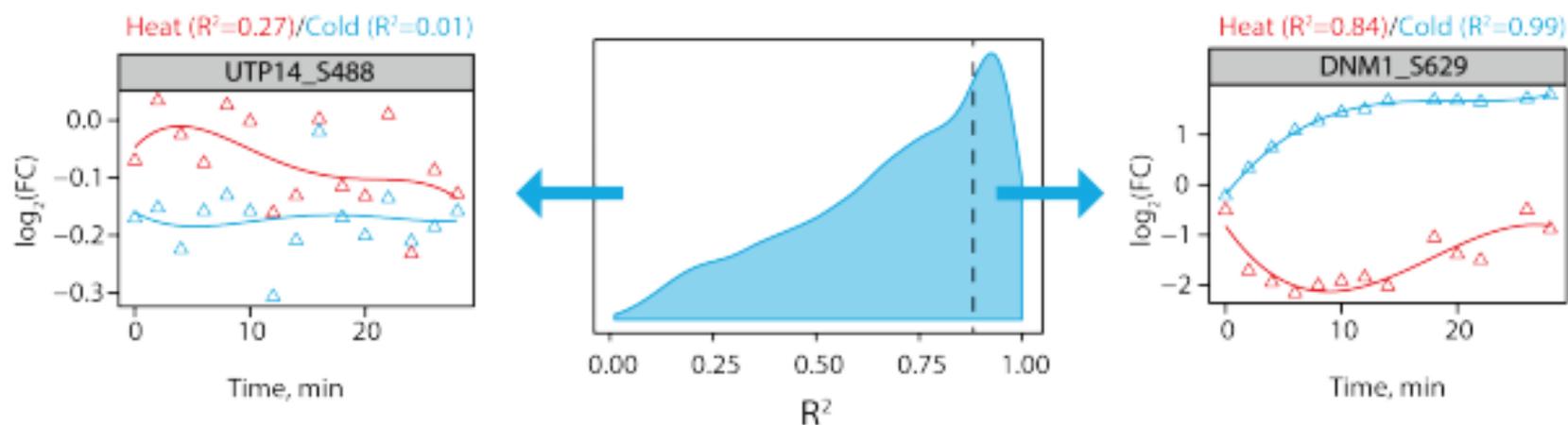
## 4. Results

- ① High temporal resolution of the *S. cerevisiae* phosphoproteome dynamics in response to heat and cold
- ② Temporal phosphorylation profiles identify potential kinase substrates in response to heat shock and cold stress
- ③ Differential regulation of signaling pathways upon heat shock and cold stress
- ④ Significance of Cdc28 tyrosine 19 phosphorylation during the heat-shock response

# ① High temporal resolution of the *S. cerevisiae* phosphoproteome dynamics in response to heat and cold

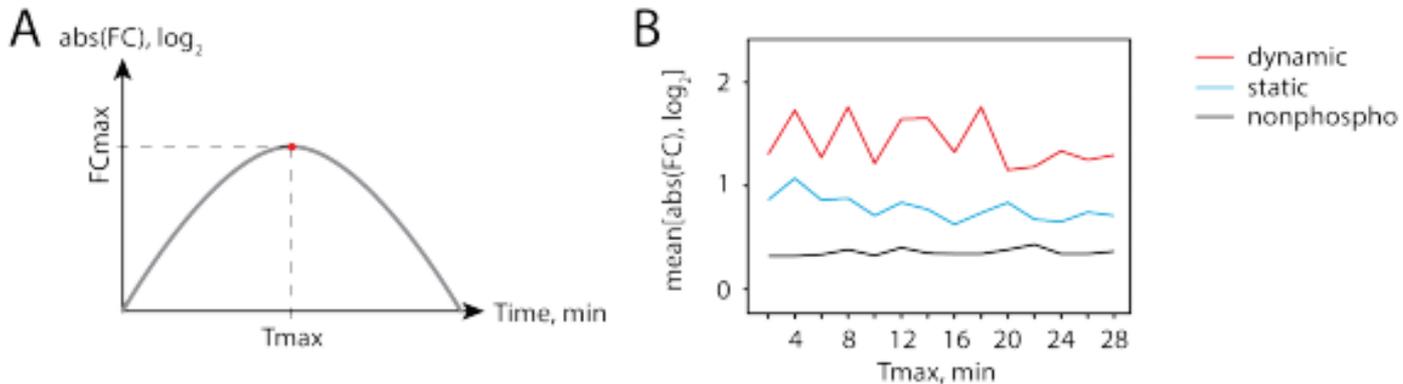


- A Width of FC distribution is used to define biological variability associated with a particular stimulus. No significant changes in the abundance of nonphosphorylated peptides were observed during the experiment, and the corresponding coefficient of variation was used to determine technical variability (sample processing and LC-MS/MS analysis). The FC distribution of phosphopeptides corresponds to the sum of technical variability and stimulus-associated changes.
- B Phosphopeptides exhibited a progressive increase in IQR with time, reflecting the global effects of temperature on the phosphoproteome. Notably, heat shock produced more pronounced effects compared to cold stress. The distribution of FC for nonphosphorylated peptides remained largely unaffected.



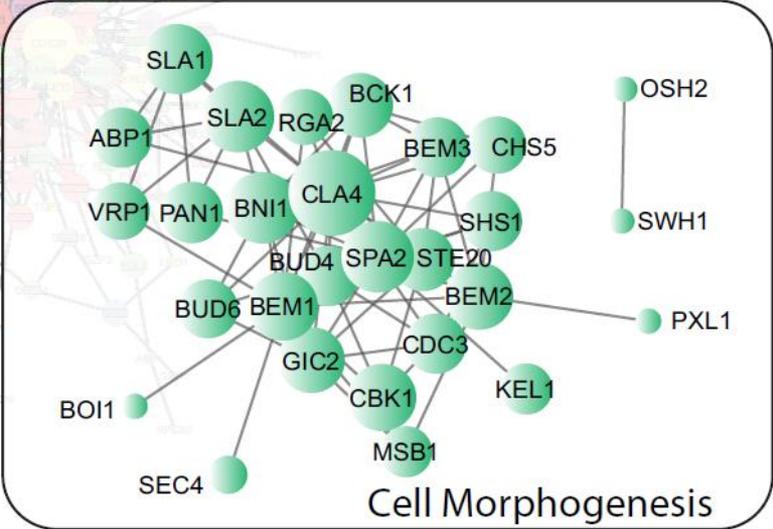
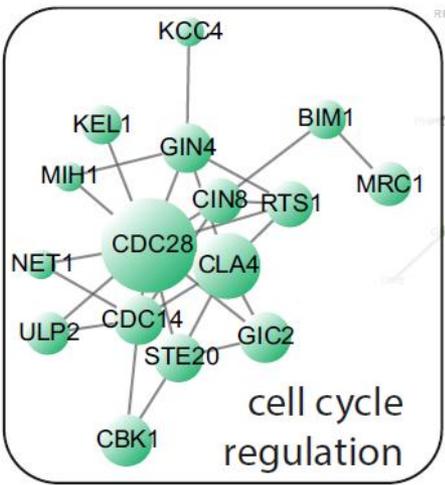
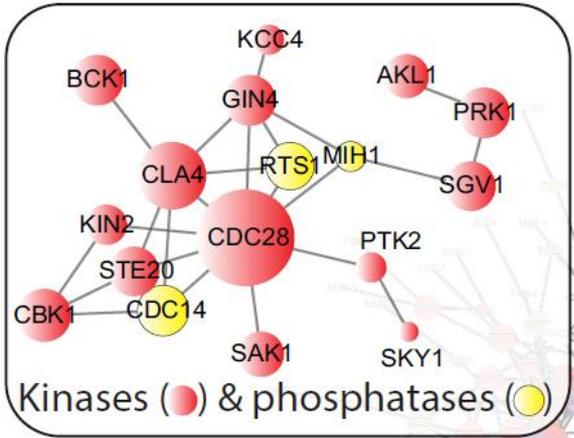
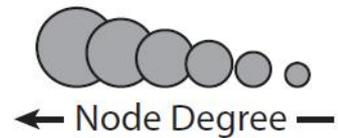
**Figure S3. Finding regulated kinetic profiles in the dataset.** In order to define subset of biologically regulated phosphosites, we performed fitting of all kinetic profiles using a polynomial model and selected only those with correlation coefficients ( $R^2$ ) > 0.9 (middle panel). Representative examples of phosphosites with  $R^2 > 0.9$  and  $R^2 < 0.9$  are displayed.

We identified 388 phosphosites from 271 proteins that showed distinct changes in phosphorylation (increase or decrease) with time for at least one stimulus. For convenience, we hereafter defined these phosphosites as “dynamic” to distinguish them from remaining “static” phosphosites.

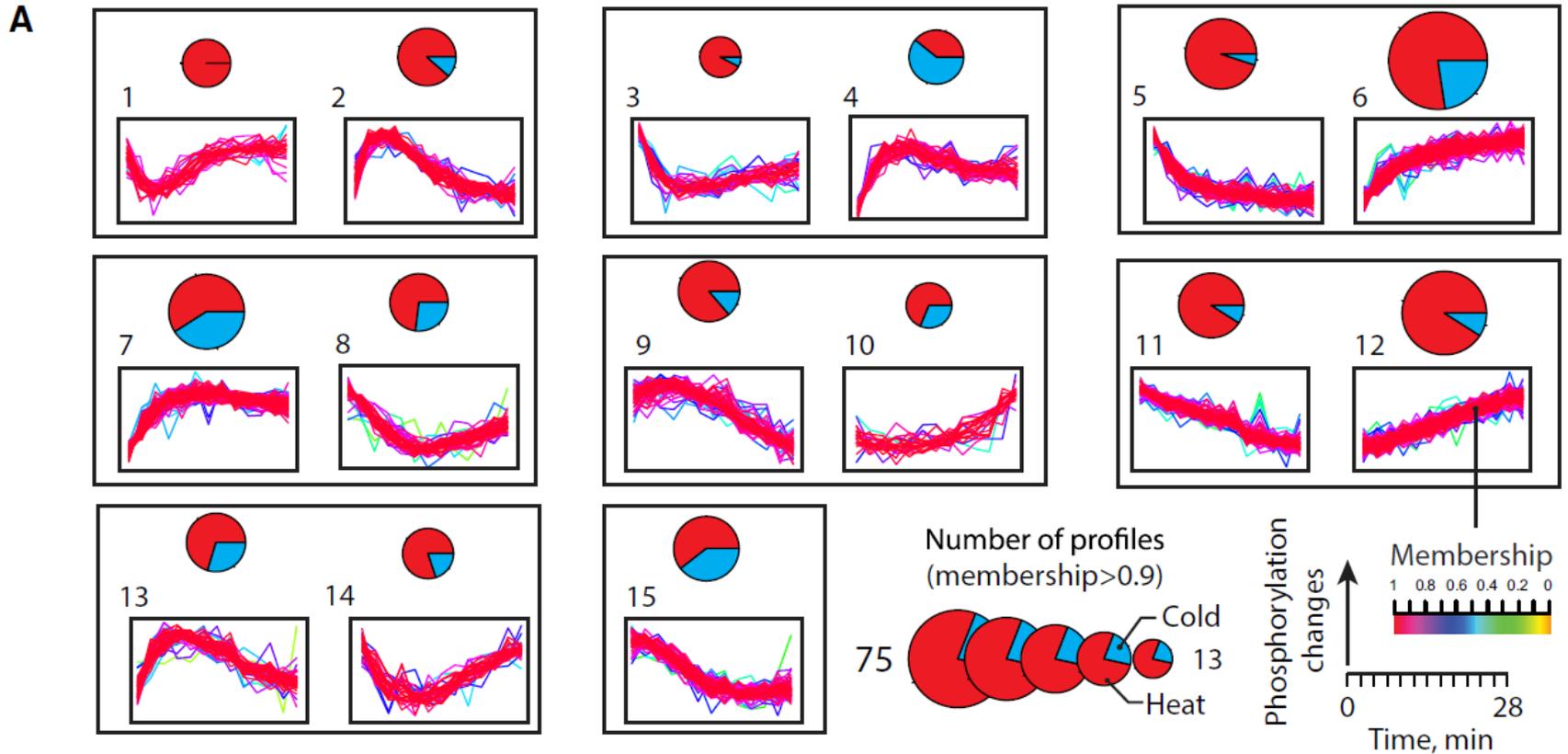


**Figure S4. Amplitude of fold change (FC) for dynamic sites.** A) From each profile the maximum amplitude (measured as absolute log<sub>2</sub>-transformed FC) was determined over the stimulation period. B) Mean values of the maximum amplitude at individual time points showing that dynamic sites displayed higher fold change compared to static sites or non phosphorylated peptides.

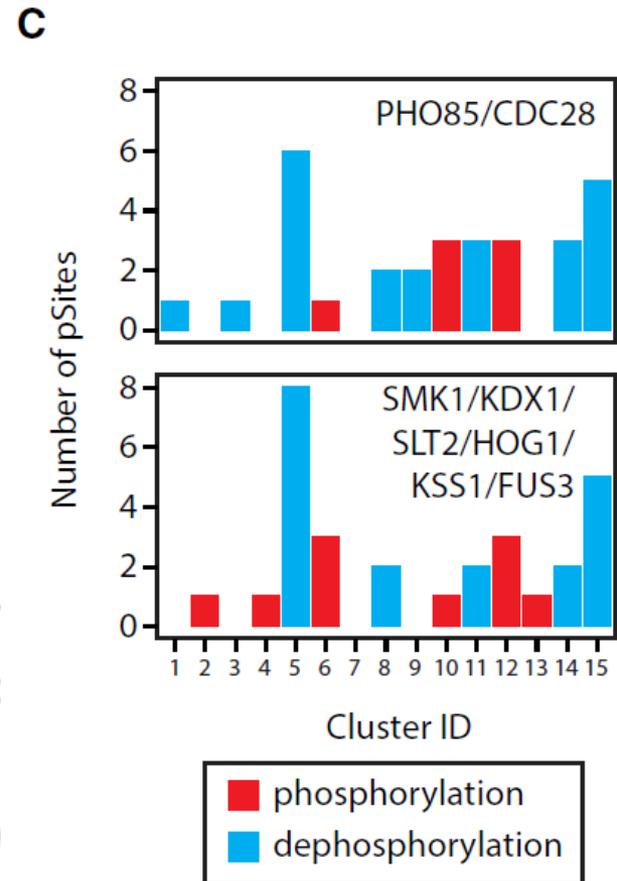
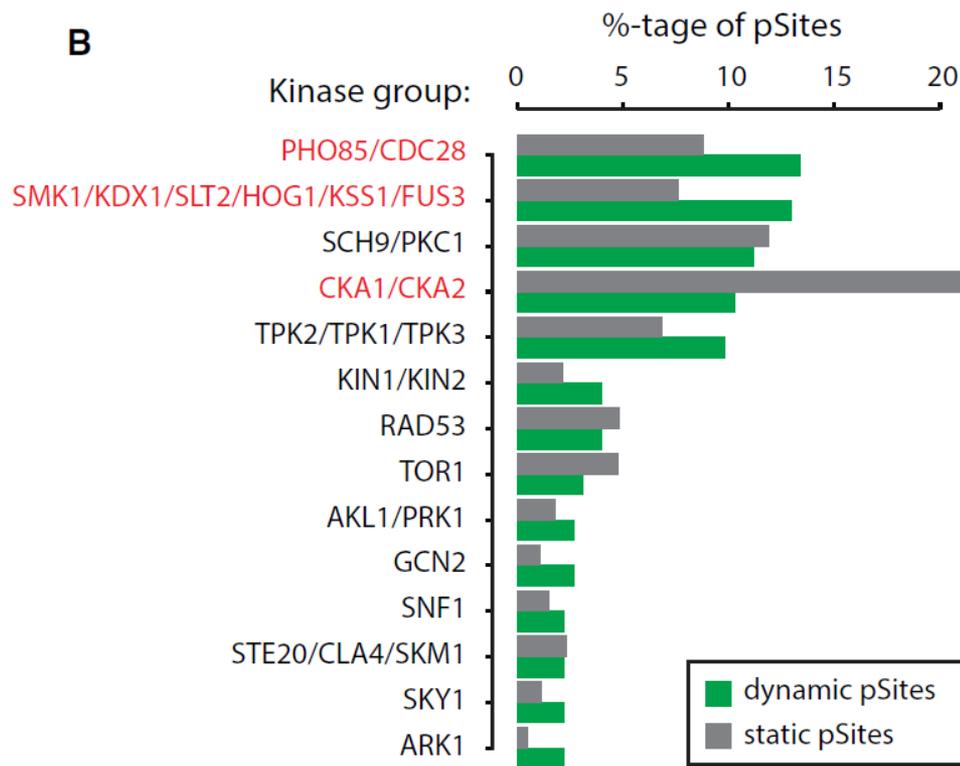
C



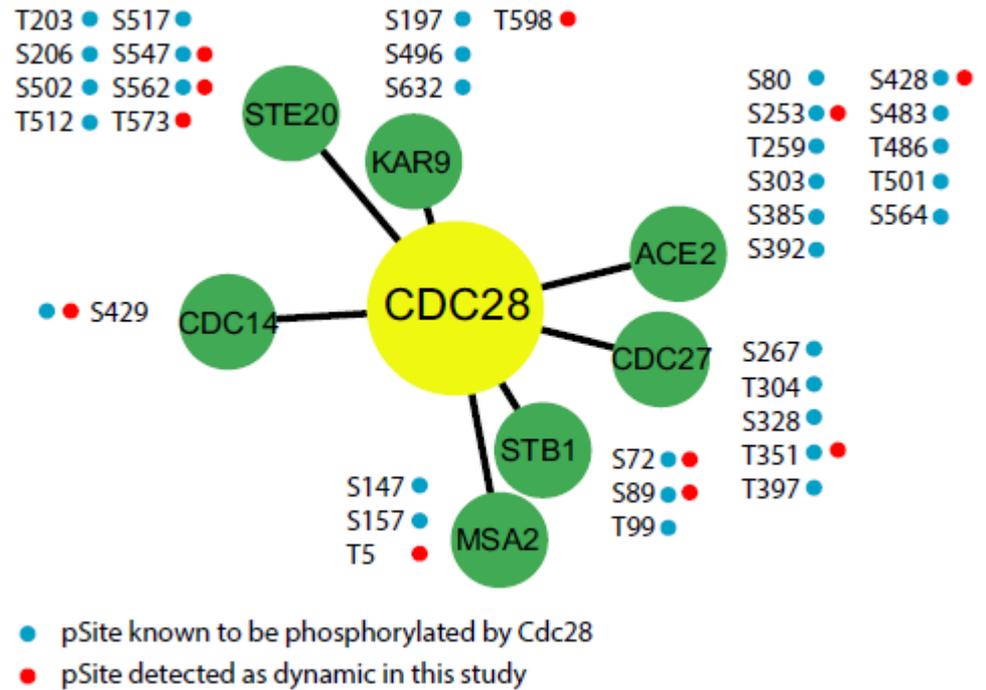
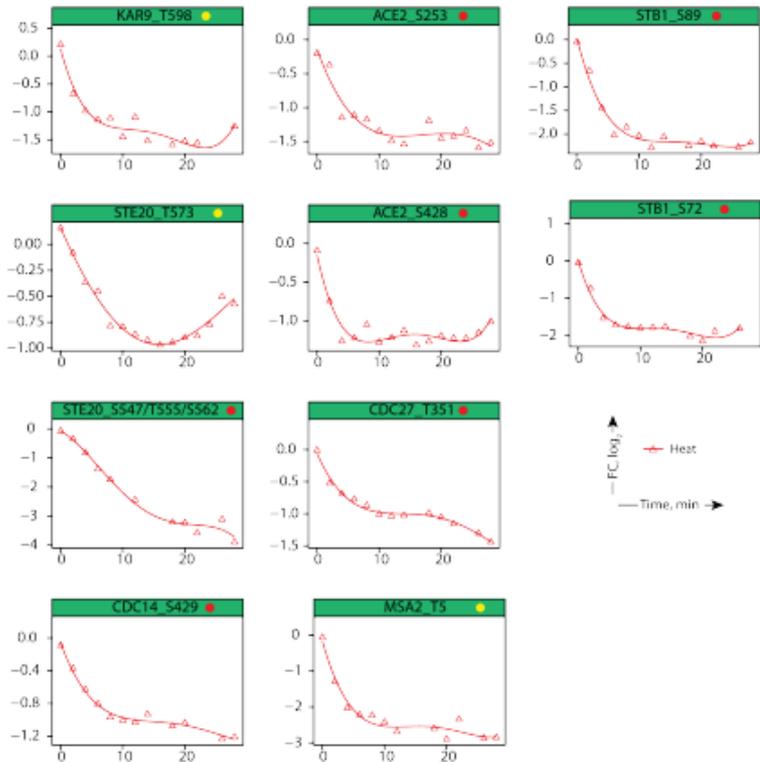
## ② Temporal phosphorylation profiles identify potential kinase substrates in response to heat shock and cold stress



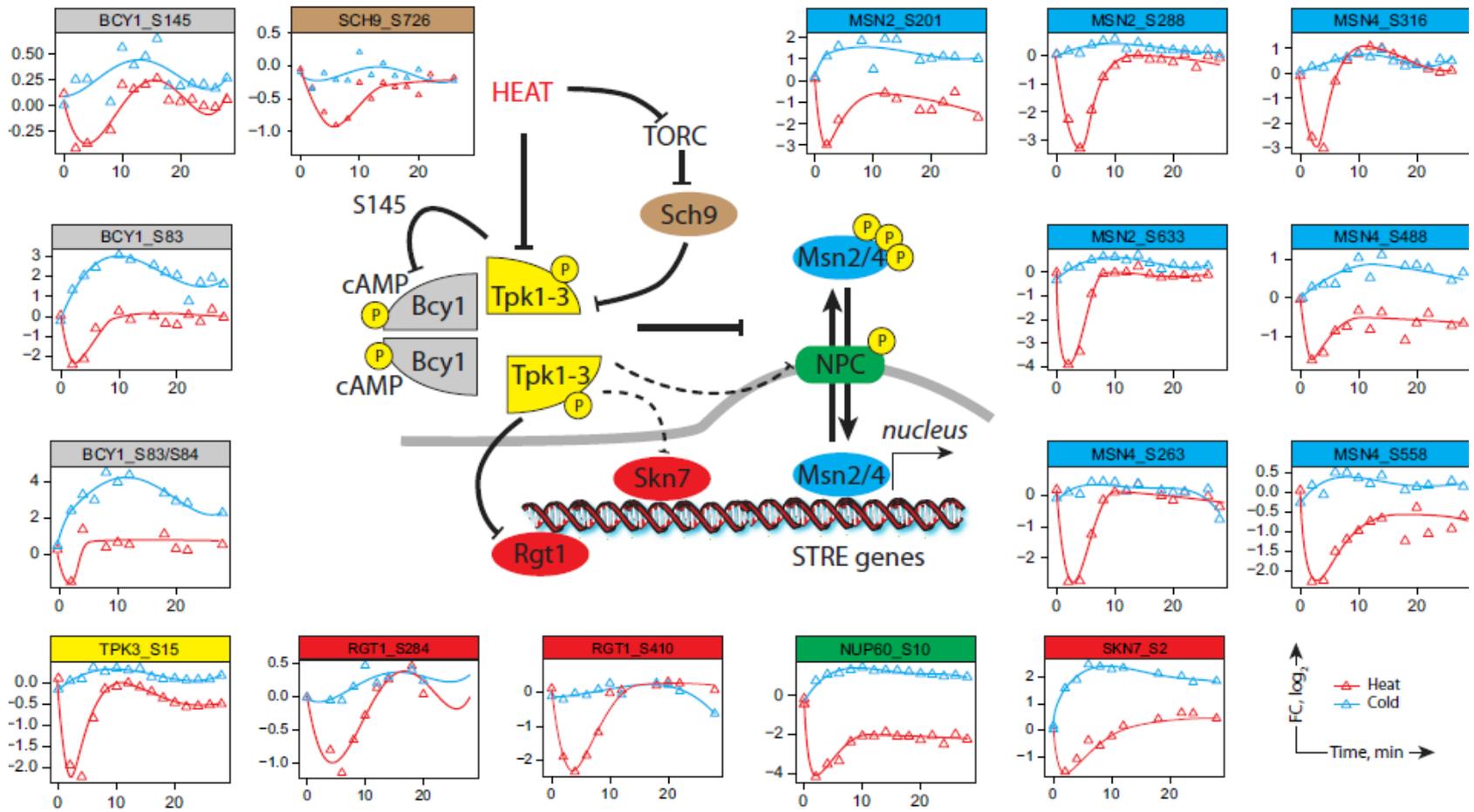
A Fuzzy c-means clustering of regulated phosphosites with different kinetic profiles. Pie charts represent the distribution of profiles for heat (red) and cold (blue), while circle sizes correspond to the number of high membership profiles. Clusters are ordered according to similarities in their dynamic behavior for either up- or downregulation.



- B** Putative kinases associated with dynamic and static phosphosites based on KinomeXplorer predictions. Substrates for each kinase group are represented by bar plots. A relative enrichment of cyclin-dependent and MAP kinase substrates is observed among dynamic phosphosites, while a higher proportion of casein kinase 1/2 substrates is found for static phosphosites.
- C** Distribution of kinetic patterns among dynamic phosphosites associated with Cdk and MAPK kinase groups. Putative Cdk and MAPK substrates were found to be represented mostly in cluster 5 which correspond to a sustained dephosphorylation.



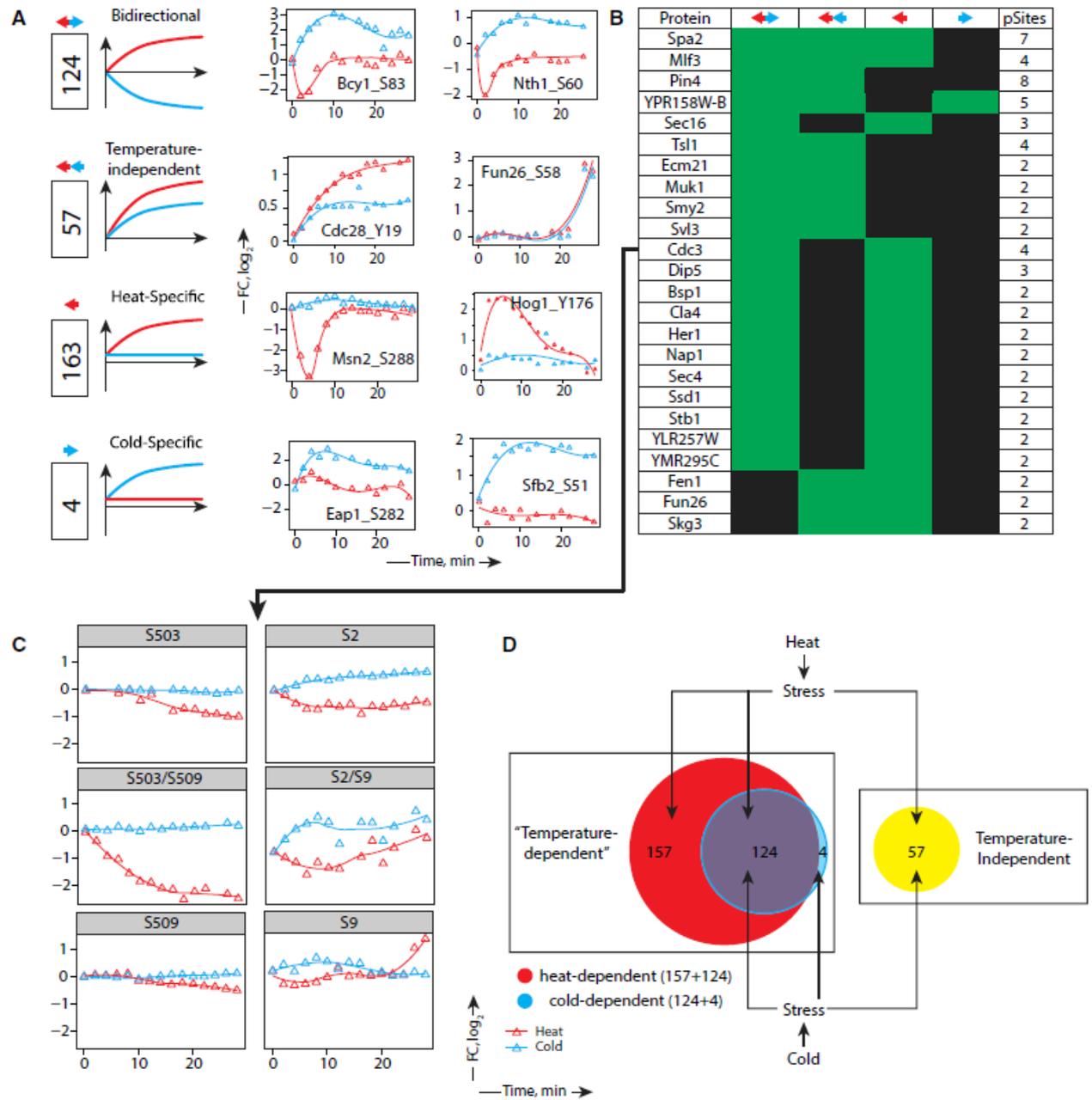
An increase phosphorylation of Cdc28 at its inhibitory site Y19 was observed under in response to heat shock. STRING database was used to obtain high-confidence Cdc28 interactors among dynamic proteins detected in our study and PhosphoGRID database to infer known Cdc28-regulated phosphosites for these interactors. A total of 8 dynamic phosphosites (known substrates) were identified in our dataset, and all displayed similar dephosphorylation profiles (red dots). We detected similar dynamic profiles on phosphosites from other interactors, which were identified as putative substrates of Cdc28 (yellow dots).



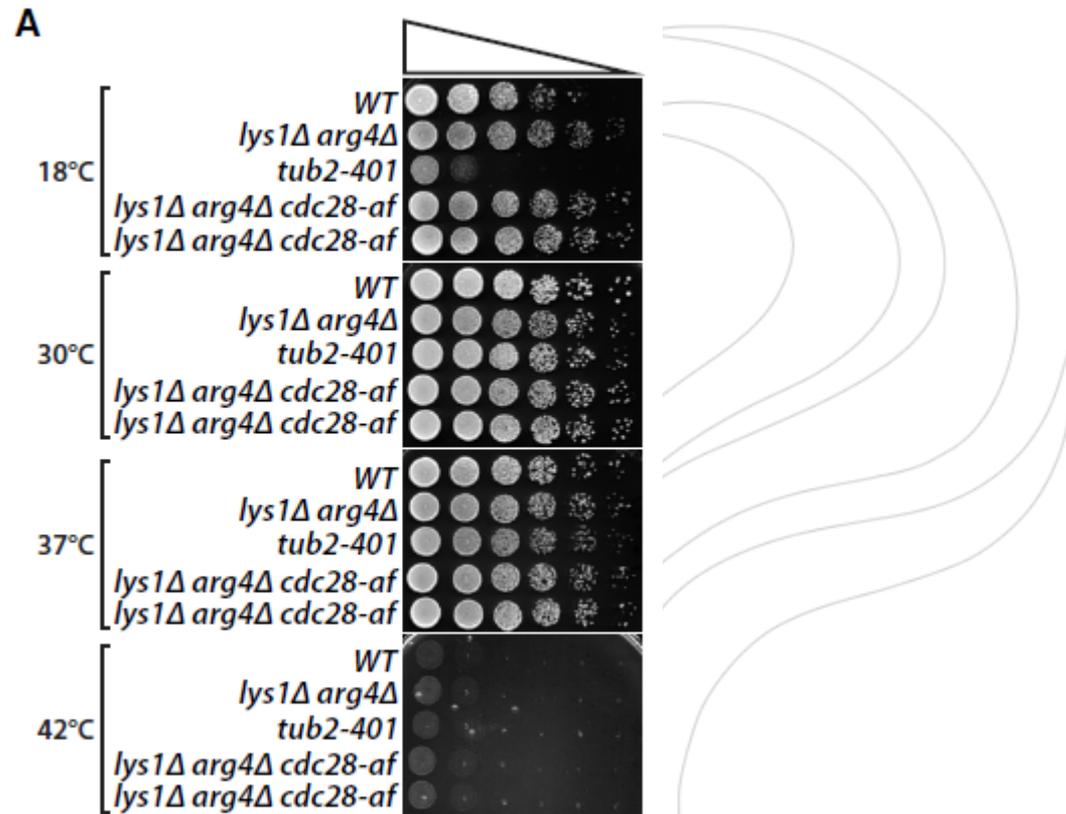
**Figure 5. Dynamic profiles reveal functional association between kinases and putative substrates.**

Schematic representation of cell signaling pathway associated with PKA and downstream substrates. Phosphorylation profiles are shown for each substrate site. The kinetic profiles of PKA (Tpk3 and Bcy1) are superimposable with those of known protein targets Msn2/4 and Rgt1. The correlation between temporal profiles was used to identify putative targets of PKA such as nucleoporin Nup60 and the transcription factor Skn7.

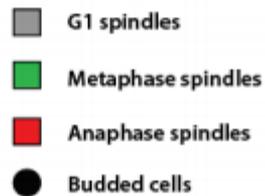
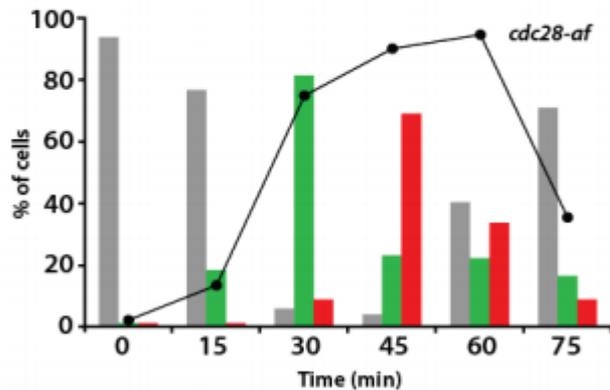
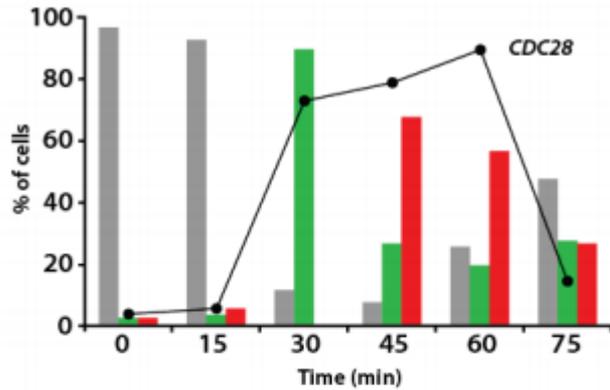
# ③ Differential regulation of signaling pathways upon heat shock and cold stress



## ④Significance of Cdc28 tyrosine 19 phosphorylation during the heat-shock response

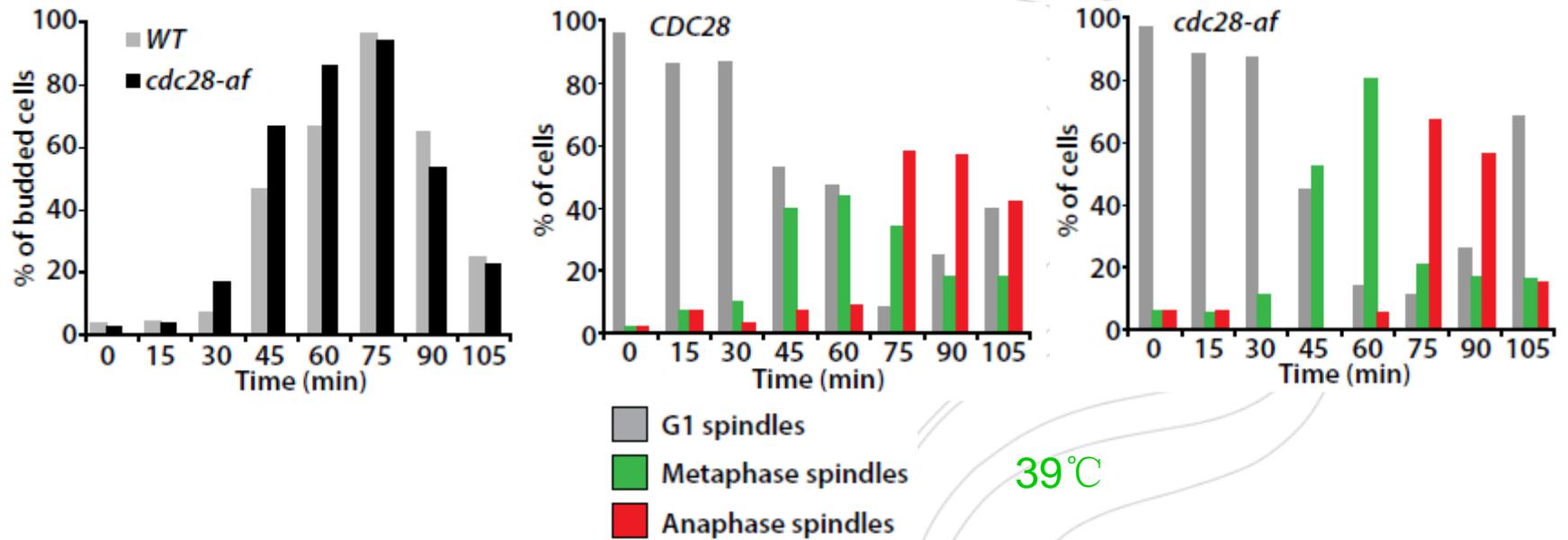


A Growth properties of *CDC28* and *cdc28-af* mutant cells exposed to various temperatures. Fivefold dilutions of yeast cells were spotted on YPD plates and grown at 18, 30, 37 and 42°C for 2–4 days. Strains included on plates are as follows: D1 (WT), YALB6 (*lys1Δ arg4Δ*), D253 (*tub2-401*, a cold-sensitive strain), D4591 and D4592 (two independent clones with the *lys1Δ arg4Δ cdc28-af* genotype).



- Figure S11. Cell cycle profile of *cdc28-af* mutants growing at physiological temperature. Graphs showing the budding index and spindle morphology of CDC28 (top) and *cdc28-af* cells (bottom) after release from a G1-arrest into fresh medium at 30 ° C. Sample of cells were taken every 15 min to determine the budding index (lines), and microtubule morphology (bars) in cultures of CDC28 and *cdc28-af* cells progressing synchronously into the cell cycle. At least 100 cells were counted at each time point.

B



39°C

B Budding index and spindle morphology of *CDC28* and *cdc28-af* cells after release from a G1 arrest into fresh medium at 39°C. Samples of cells were taken at intervals to determine the kinetics of bud appearance (top graph), and microtubule morphology (middle and bottom graphs) in cultures of *CDC28* and *cdc28-af* cells released synchronously into the cell cycle. At least 100 cells were counted at each time point.

# In my opinion,

- **Conclusion**

High temporal resolution profiling of the yeast phosphoproteome upon heat shock and cold stress enabled the correlation of kinetic profiles between kinases and their substrates and identified cell signaling events associated with actin organization, septin assembly and cell cycle arrest.

- **Enlightenment**

quantitative phosphoproteomics; the combination of protein-protein interactions and phosphorylation dynamics

- **Improvement**

Improving the sensitivity of MS and metabolic labeling reagents will further expand phosphoproteome coverage and multiplexing capability of these experiments.

**Thanks  
for your attention!**

