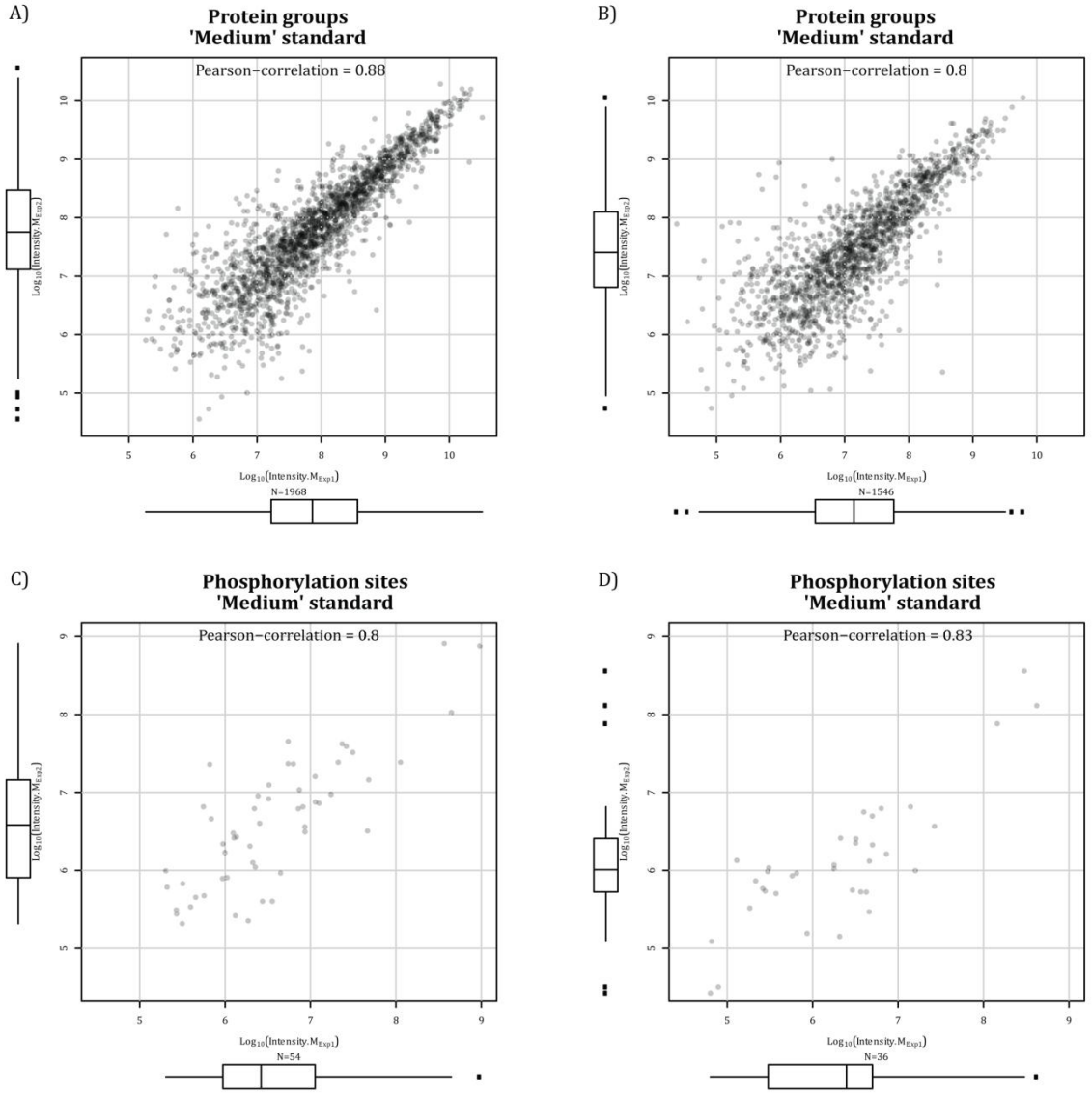
Supplementary Data

Supplementary Table 1: List of all primers used in this study.

Supplementary Table 2: List of all proteins and phosphorylation sites identified in all replicates across all stages T1-T5.

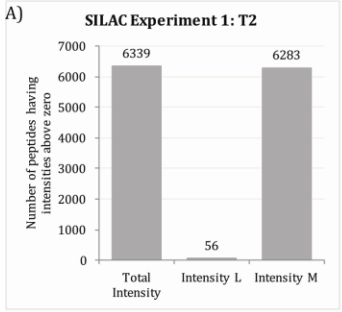
Supplementary Table 3: List of all proteins and phosphorylation sites identified (combined data from all replicates) in the study to identify novel substrates for the kinase PrkC and the phosphatase PrpC.

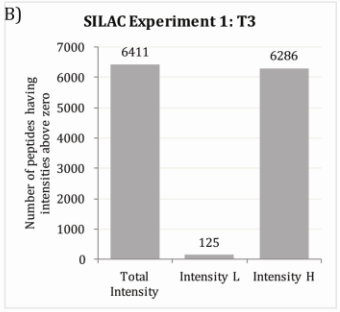
Supplementary Figure 1

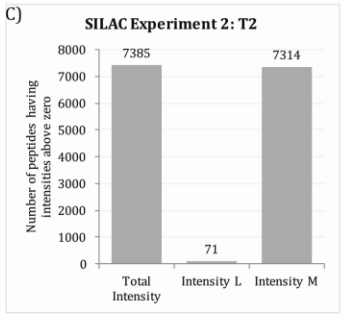


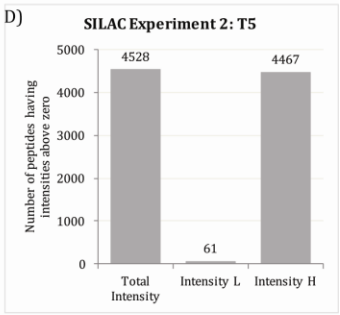
Supplementary Figure 1: Correlation of the common label (T2, Lys4) between SILAC experiments.
Intensity of the Lys4-labeled protein groups in SILAC experiment 1 is plotted against intensity of the Lys4-labeled protein groups in SILAC experiment 2. A,B) Proteins in biological replicate 1 and 2 respectively; C,D) Phosphorylation sites in biological replicate 1 and 2 respectively. Pearson correlation of 0.8 was observed in all cases.

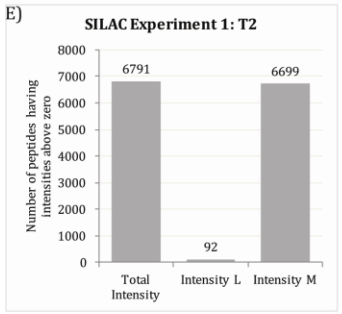
Supplementary Figure 2

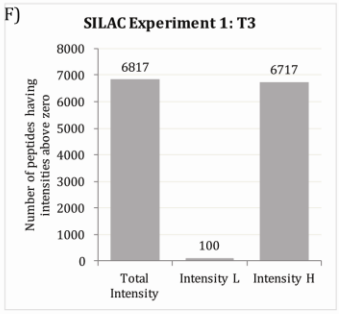


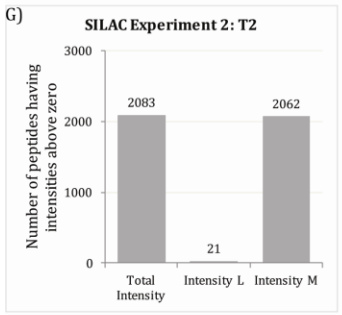


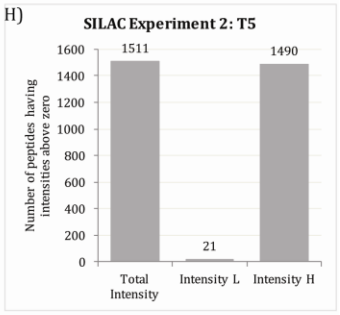






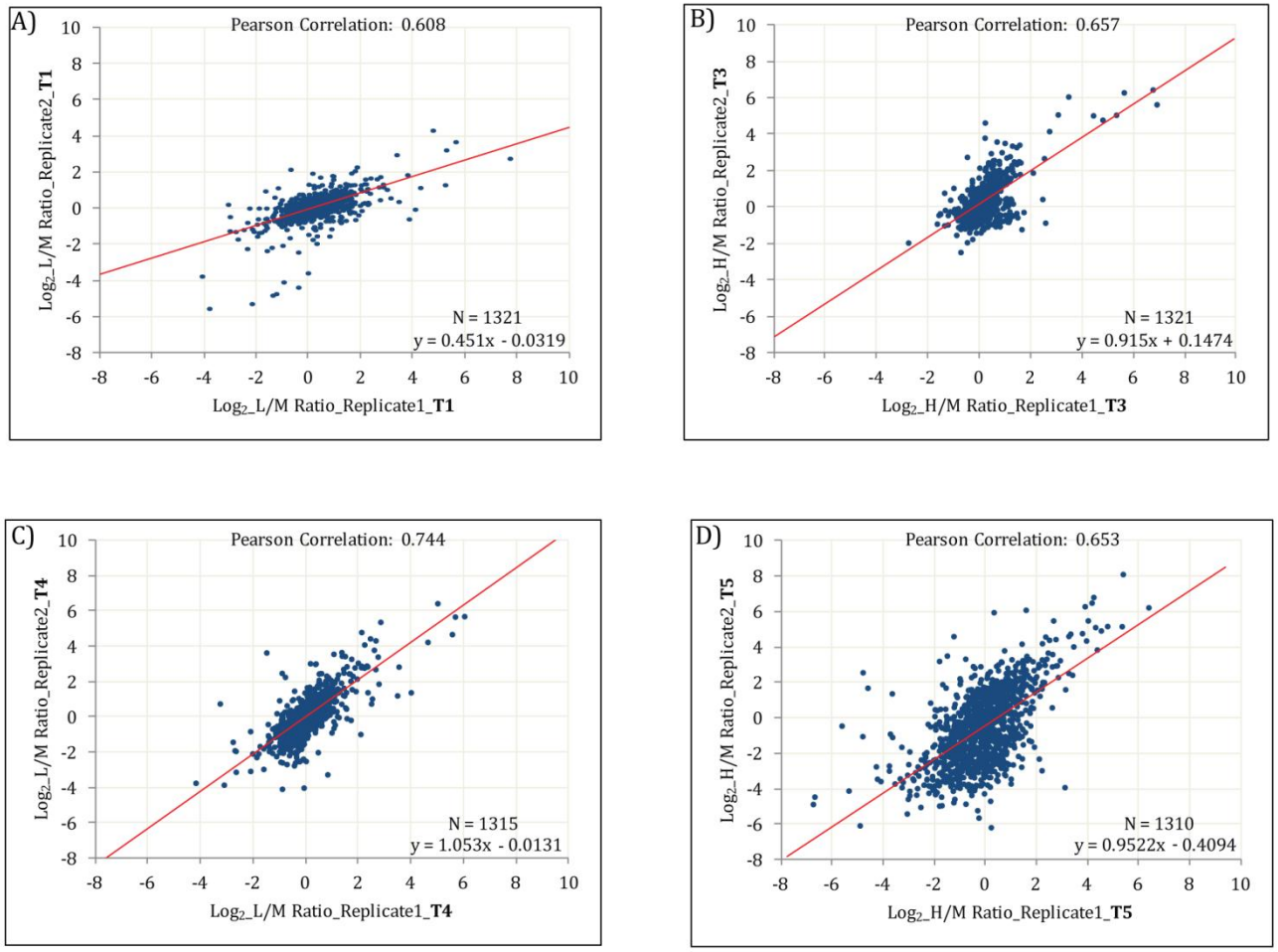






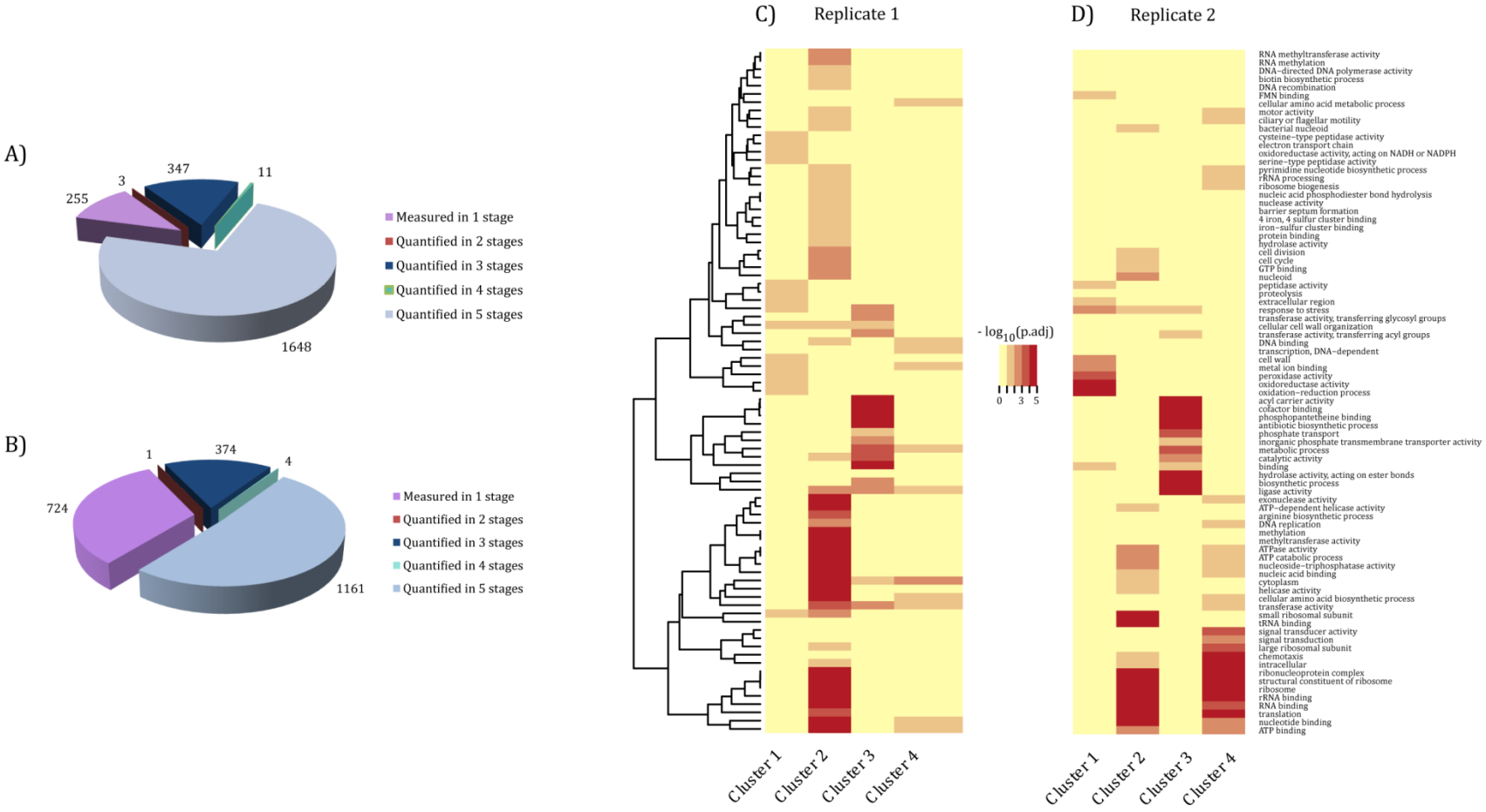
Supplementary Figure 2: Incorporation of SILAC amino acids.
Graph representing incorporation of the isotopic label of lysine in:
A,E) Medium (lys4) labeled T2 cells from SILAC experiment 1, from biological replicate 1 and 2 respectively;
C,G) Medium (lys4) labeled T2 cells from SILAC experiment 2, from biological replicate 1 and 2 respectively;
B,F) Heavy (lys8) labeled T3 cells from SILAC experiment 1, biological replicate 1 and 2 respectively; and
D,H) Heavy (lys8) labeled T5 cells from SILAC experiment 2, biological replicate 1 and 2 respectively.
"Total Intensity" = number of peptides identified in corresponding experiment.
"Intensity L" = number of all peptides detected in L form.
"Intensity M" = number of peptides detected only in M form
"Intensity H" = number of peptides detected only in H form

Supplementary Figure 3



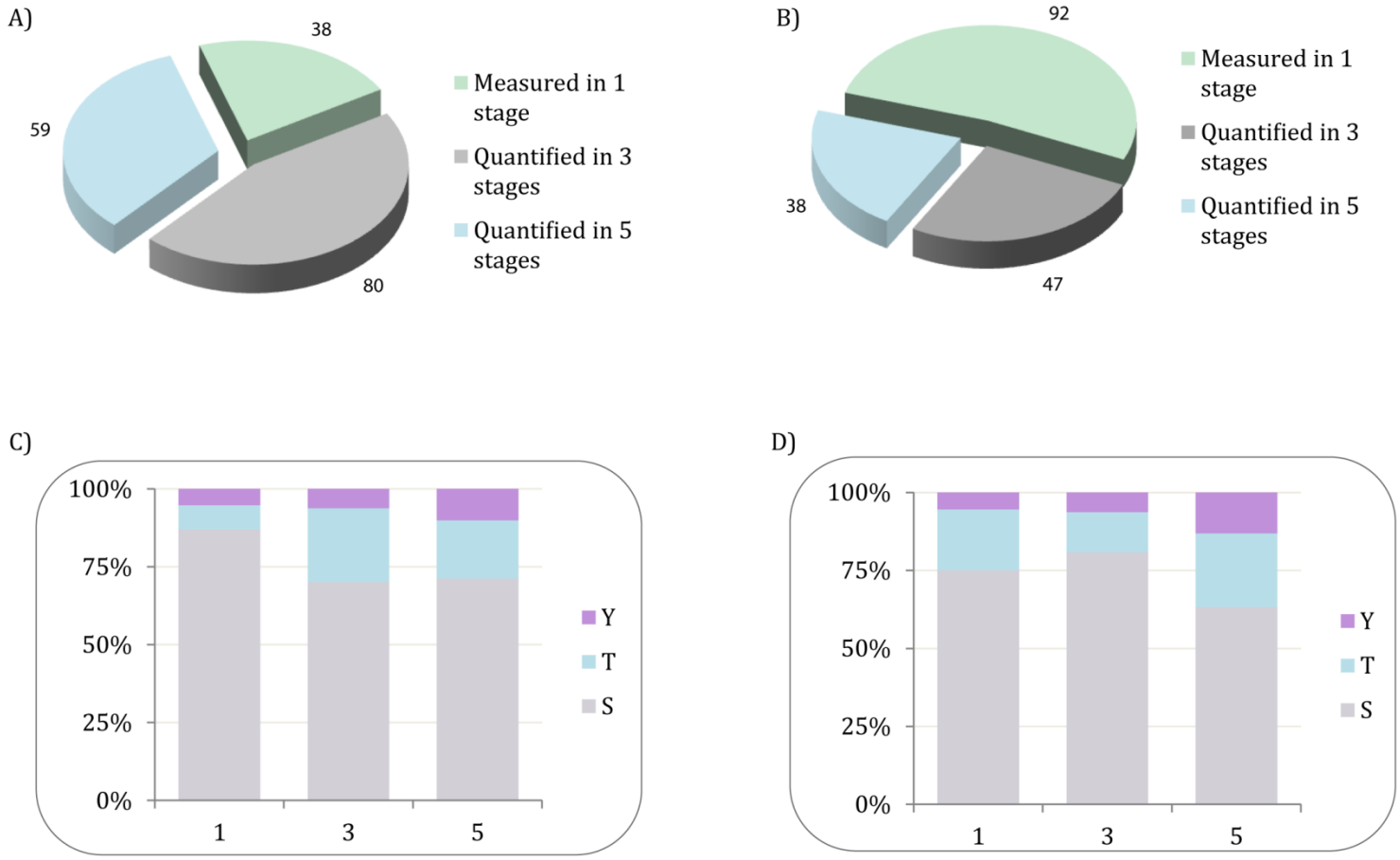
Supplementary Figure 3: Correlation between biological replicates.
Correlation plots depicting correlation of proteins between biological replicates at each stage - A) T1; B) T3; C) T4; and D) T5 with respect to T2. The figure shows Log_2 ratios of both replicates plotted against each other.

Supplementary Figure 4



Supplementary Figure 4: Distribution of quantified proteins and enrichment analysis of their GO-terms.
Pie chart shows the number of proteins quantified in 1-5 growth phases - A) Biological replicate 1; and B) Biological replicate 2. Enrichment/depletion of quantified proteins in the each of the 4 clusters as obtained from GO annotation in - C) Biological replicate 1; and D) Biological replicate 2.

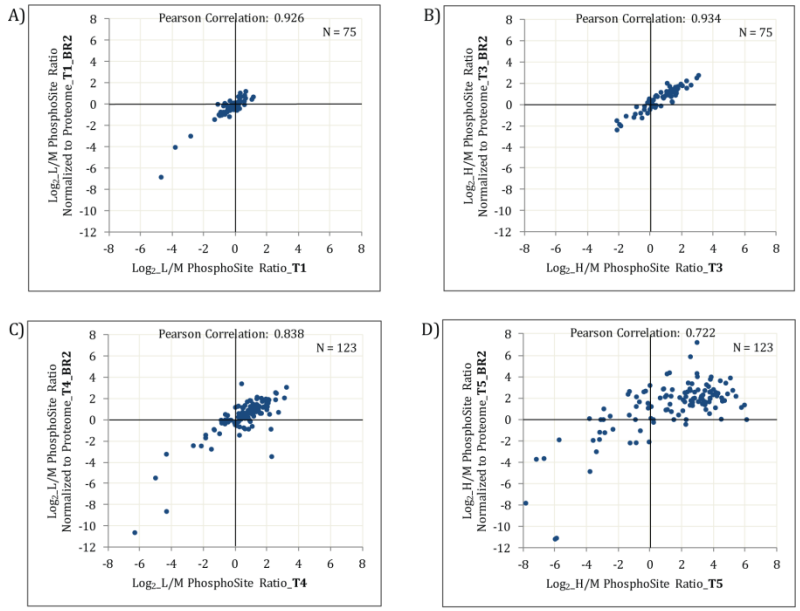
Supplementary Figure 5



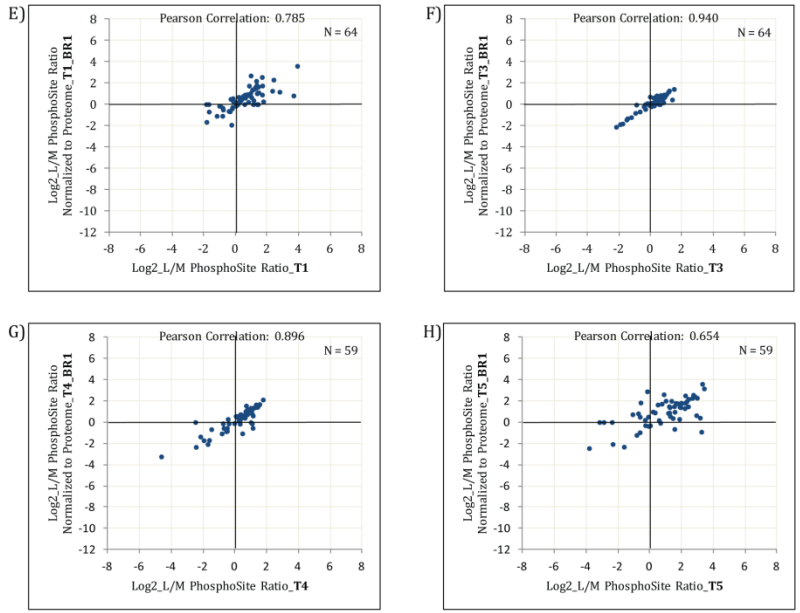
Supplementary Figure 5: Distribution of quantified phosphorylation sites.
Pie chart representing phosphorylated proteins quantified across the analyzed growth phases in - A) Biological replicate 1; and B) Biological replicate 2.
Distribution of serine, threonine and tyrosine sites across different quantified stages in - C) Biological replicate 1; and D) Biological replicate 2.

Supplementary Figure 6

Replicate 1

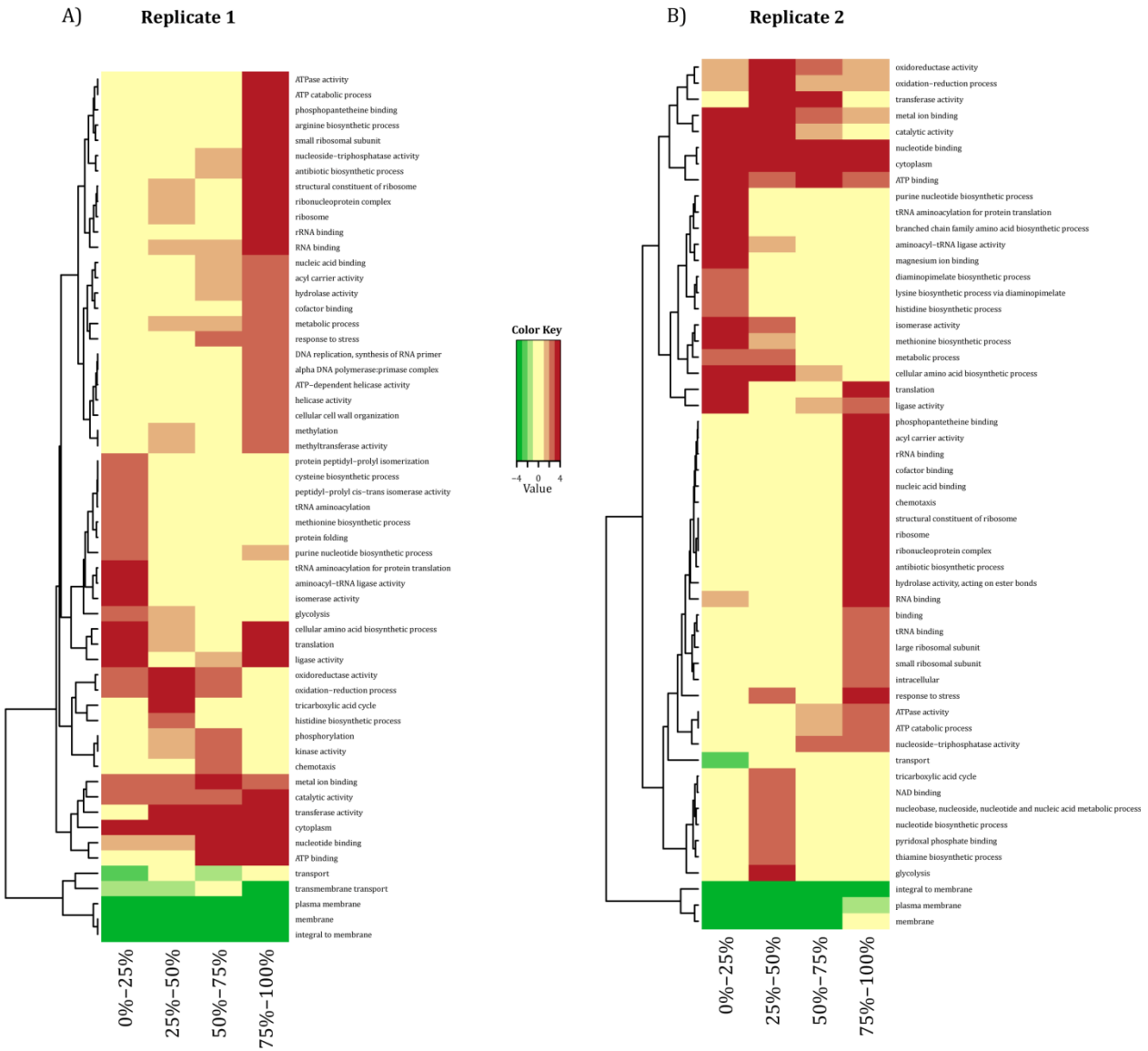


Replicate 2



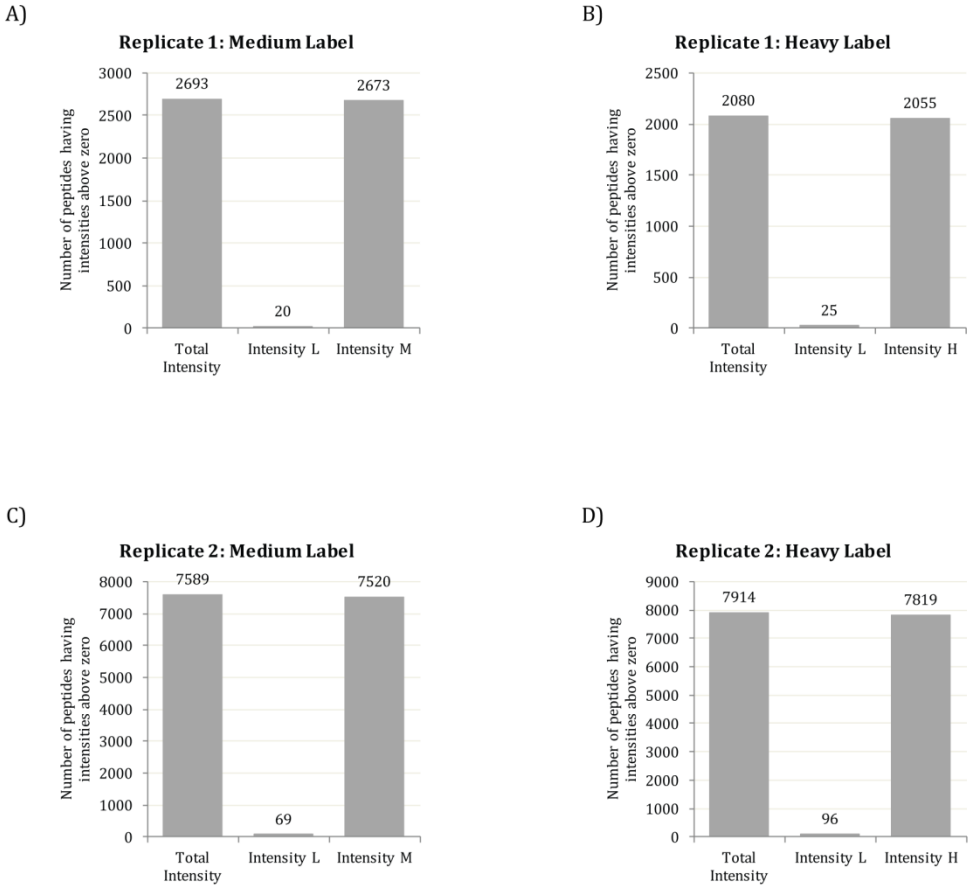
Supplementary Figure 6: Correlation between phosphosites and normalized phosphosites.
Figures A-H illustrate Log_2 ratios of the phosphosites plotted against Log_2 ratios of the phosphosites normalized with the respective proteome, for each of the stages in biological replicate 1 and 2. All ratios plotted are with respect to the common point T2.

Supplementary Figure 7



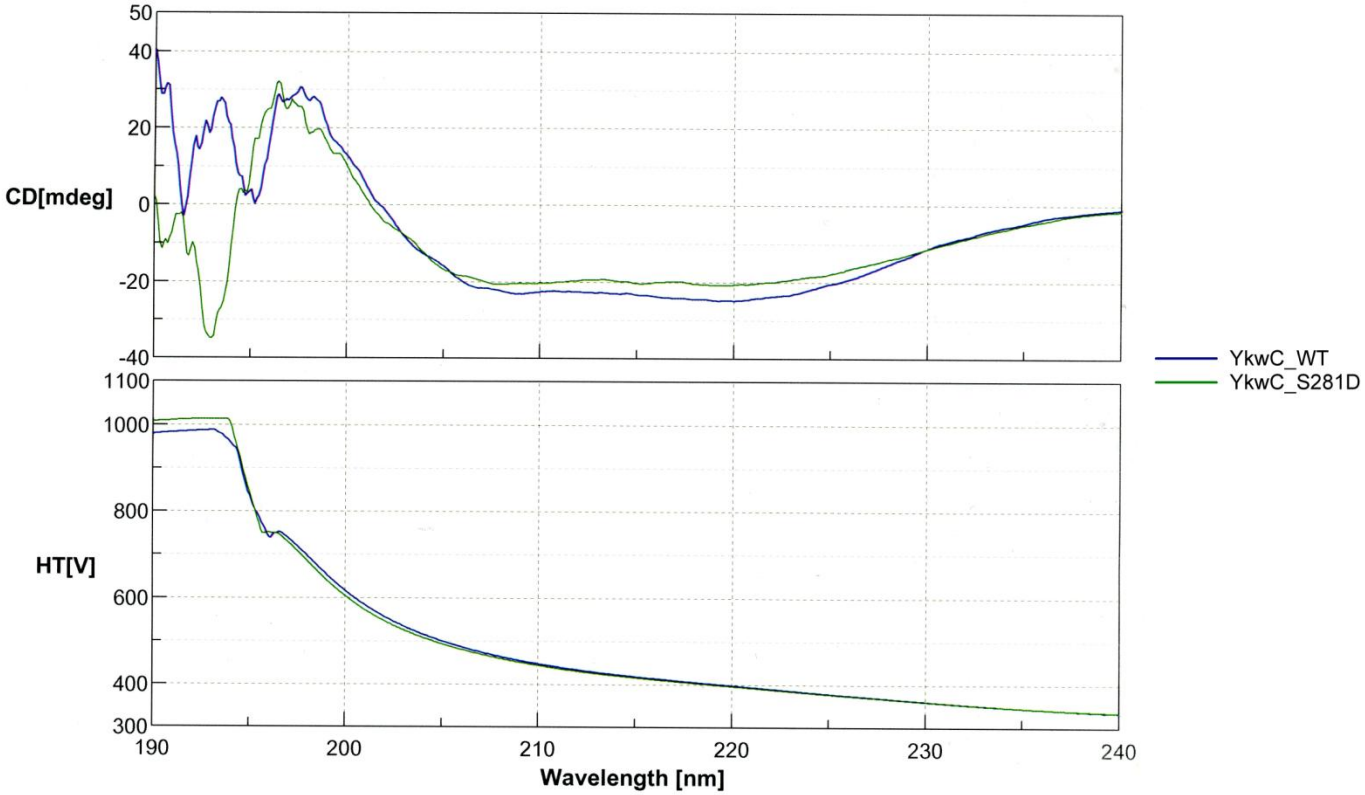
Supplementary Figure 7: GO enrichment/depletion analysis of fluctuating and static proteins.
A) Biological replicate 1; and B) Biological replicate 2. The 75%-100% bin corresponds to proteins with highest fluctuation across analyzed phases of growth.

Supplementary Figure 8



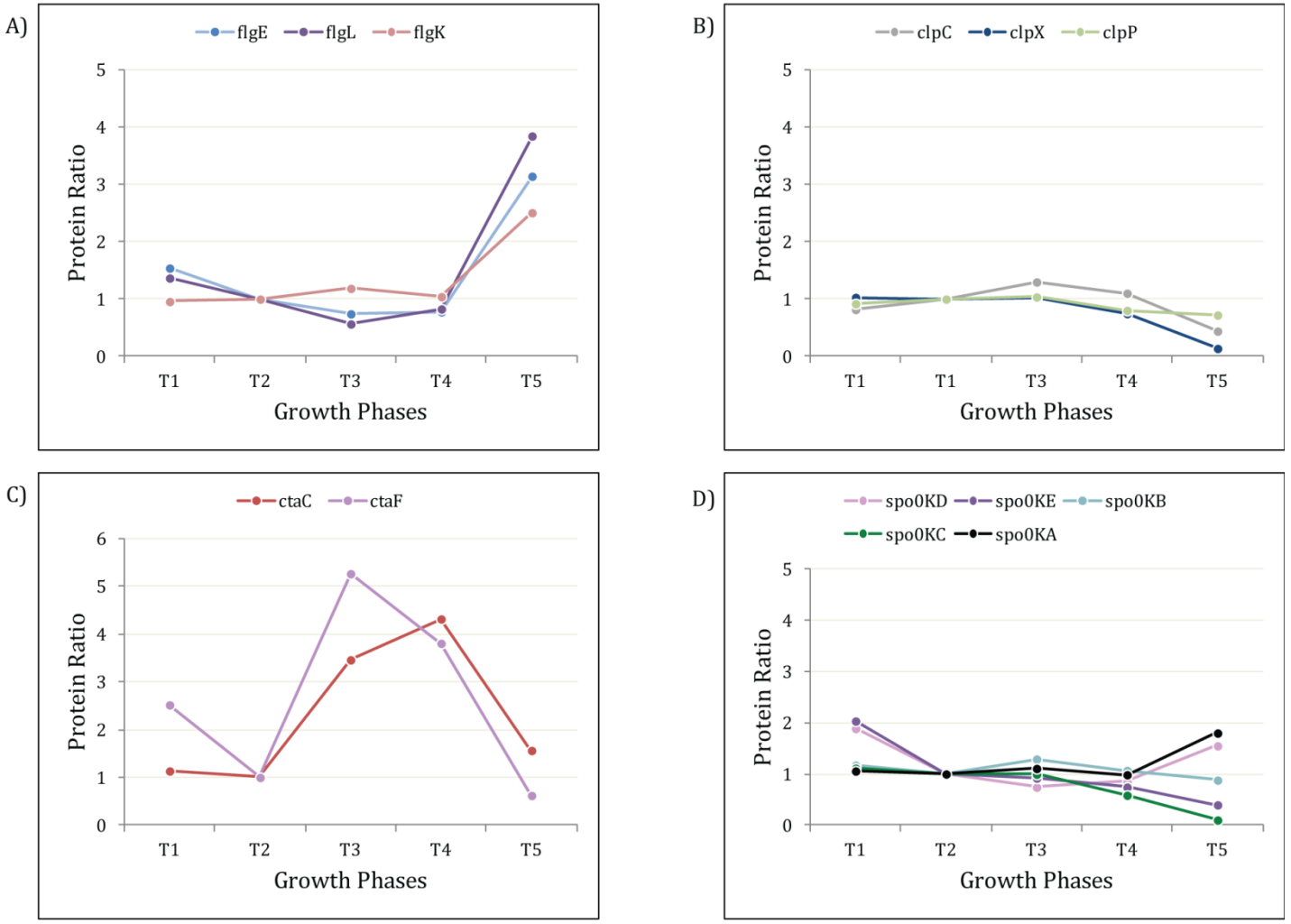
Supplementary Figure 8: Incorporation of SILAC amino acids.
The uptake of the isotope of lysine by the Δ*prkC* and Δ*prpC* strain was monitored.
A,C) show the incorporation of the medium label in biological replicate 1 and 2 respectively; and
B,D) show the incorporation of the heavy label in biological replicate 1 and 2 respectively.
"Total Intensity" = number of peptides identified in corresponding experiment.
"Intensity L" = number of all peptides detected in L form.
"Intensity M" = number of peptides detected only in M form
"Intensity H" = number of peptides detected only in H form

Supplementary Figure 9



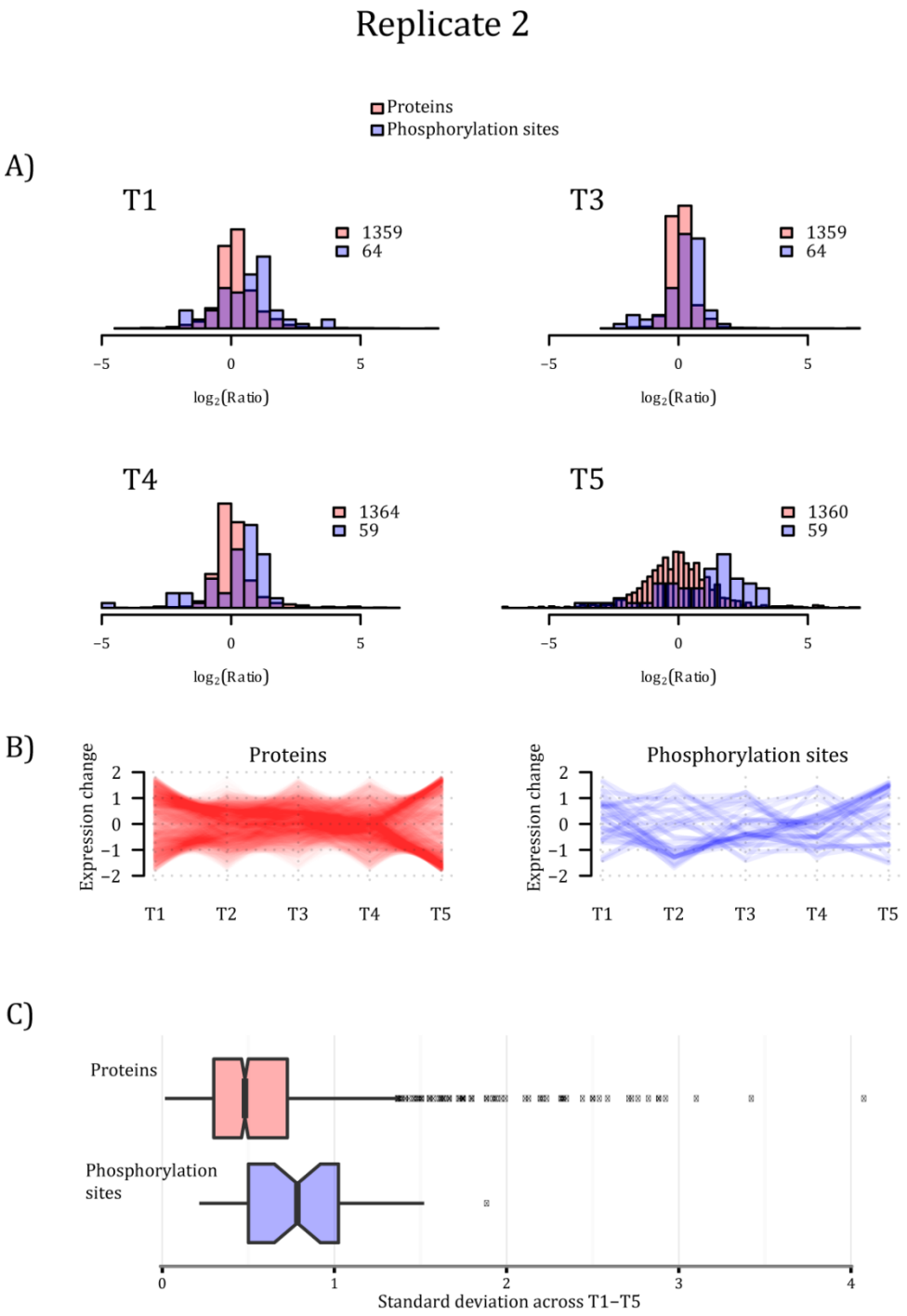
Supplementary Figure 9: CD spectrum of the WT substrate and phosphomimetic mutant.
A far UV circular dichroism spectrum of YkwC_WT and YkwC_S281D (phosphomimetic mutant) measured in the range of 240 nm to 190 nm, denoted a similar secondary structural pattern of both enzymes.

Supplementary Figure 10



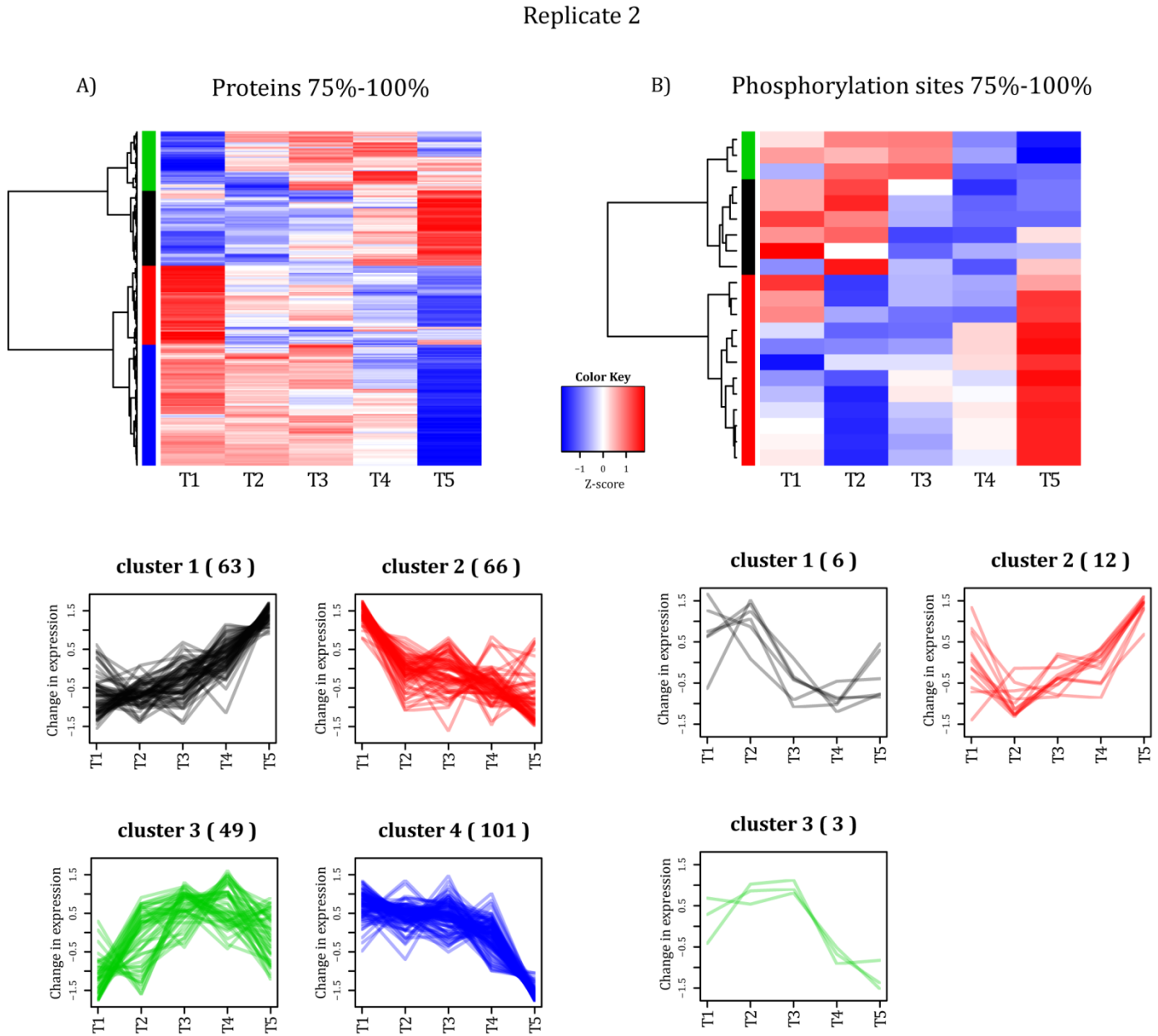
Supplementary Figure 10: Expression profiles of known protein complexes.
Protein expression profiles of certain complexes identified in *B. subtilis* across the five stages of growth. Components of the same complex showed a similar expression profile. A) Proteins belonging to the flagellar hook complex that is involved in chemotaxis and cell movement are not regulated in the initial stages but increase during T5; B) ATP-binding proteins form a part of the Clp proteasomal complex that targets proteins for degradation. These remain non regulated through-out the bacterial growth in minimal media; C) These genes are involved in respiration and also play a crucial role in heme biosynthesis (*ctaA*). They peak during T3-T4 phase of growth; D) These proteins form an integral part of the oligopeptide ABC transporter complex. They are actively involved in initiation of sporulation and competence development and are also seen not to be regulated through the growth phases.

Supplementary Figure 11



Supplementary Figure 11: Distribution of SILAC ratios of proteins and phosphorylation site ratios during *B. subtilis* growth.
A) Histogram depicting measured ratios of proteins and phosphorylation sites in each stage of growth; T1, T3, T4, and T5. All distributions are from the biological replicate 2 and relative to the common time point, T2.
B) Changing expression profiles of proteins and phosphorylation sites detected across all 5 stages of growth.
C) Box plot depicting standard deviation across growth phases T1-T5 measured for proteins and phosphorylation sites represented in 2B.

Supplementary Figure 12



Supplementary Figure 12: Hierarchical clustering analysis of SILAC ratios with highest fluctuation.
A) Protein groups; and B) Phosphorylation sites. Fluctuation is defined based on standard deviation, as described in the text. This data concerns biological replicate 2.