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Supplemental Information

The Hydrophobic Patch Directs Cyclin B to Centrosomes to Promote Global CDK Phosphorylation at Mitosis

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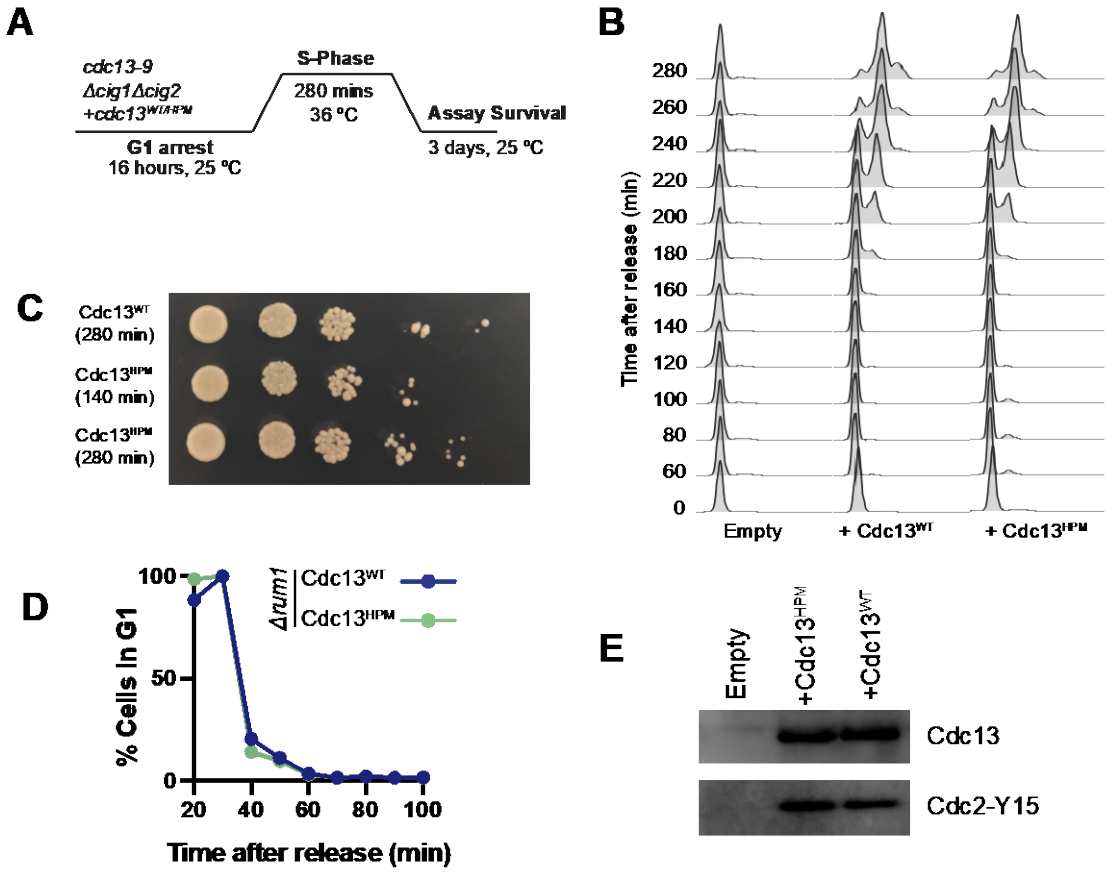

A
cdc13-9
Δcig1Δcig2
+cdc13WT/HPM
G1 arrest
16 hours, 25 °C
S-Phase
280 mins
36 °C
Assay Survival
3 days, 25 °C
B
Time after release (min)
280
260
240
220
200
180
160
140
120
100
80
60
0
Empty
+ Cdc13WT
+ Cdc13HPM
C
Cdc13WT
(280 min)
Cdc13HPM
(140 min)
Cdc13HPM
(280 min)
D
% Cells In G1
100
50
0
20
40
60
80
100
Time after release (min)
Δrum1
Cdc13WT
Cdc13HPM
E
Empty
+Cdc13HPM
+Cdc13WT
Cdc13
Cdc2-Y15

Figure S1. Cdc13^{HPM} executes a functional S-phase, Related to Figure 1.

A – Experiment outline for panels (B) and (C) for testing survival after Cdc13^{HPM} dependent S-phase. Cells were initially arrested in EMM lacking nitrogen for 16 hours before re-feeding with ammonium chloride (see STAR Methods). Cells were shifted to 36 ^{o}C upon release, in order for them to conduct S-phase at the *cdc13-9* restrictive temperature. Serial dilution assays were conducted after shifting cells back to 25 ^{o}C after S-phase completion to check if DNA replication had resulted in viable cells.

B – Flow cytometry profiles for cells after S-phase release at *cdc13-9* restrictive temperature of 36 ^{o}C. Cells without an exogenous Cdc13 remain arrested in G1. 10,000 cells per timepoint were collected (see STAR Methods).

C – Cells were taken from the timecourse in (B) and checked for viability after 3 days of growth. These cells have undergone S-phase at the *cdc13-9* restrictive temperature, and therefore executed DNA replication using their exogenous Cdc13. Cells taken at 280 minutes following release have executed a Cdc13^{HPM} S-phase, whereas cells taken 140 minutes after release can also rely on endogenous Cdc13, as they are shifted back to 25^{o}C before S-phase. Cells were plated onto YE4S at the *cdc13-9* permissive temperature of 25^{o}C.

D – The same cells used in Figure 1C, *cdc2(as) cdc13*-Switch Off *Δcig1 Δcig2* cells +*cdc13$^{WT/HPM}$,* were combined with a *rum1* deletion. Cells were arrested with 1 μM 1-NmPP1 for 1.5 cell cycles, and then washed of 1-NmPP1 to release cells into mitosis and the subsequent G1 and S-phase. Thiamine was added 1 hour before release from mitosis, and cells were kept in thiamine following mitosis. The S-phase following release from 1-NmPP1 inhibition was monitored using flow cytometry, with 10,000 events collected per time point (see STAR Methods).

E – Cdc2 Y15 phosphorylation with endogenous Cdc13 repressed, in the presence of exogenous Cdc13^{WT} or Cdc13^{HPM}. In order to assay the sensitivity of Cdc13^{WT} or Cdc13^{HPM}-CDK to Wee1 alone, *cdc2(as) cdc13*-Switch Off *Δcig1 Δcig2* cells +*cdc13$^{WT/HPM}$* (right and middle column, respectively) or + no insert (left column) were arrested in G2 using 1.5 μM 1-NmPP1 for 3.5 hours, with thiamine added 1 hour before release to repress endogenous Cdc13. Cells were then released into mitosis, and re-blocked with 10μM 1-NmPP1 for 2.5 hours. Cells were then released into DMSO and collected. Endogenous Cdc13 is completely degraded (left column) and therefore does not contribute to Cdc2 Y15 phosphorylation.

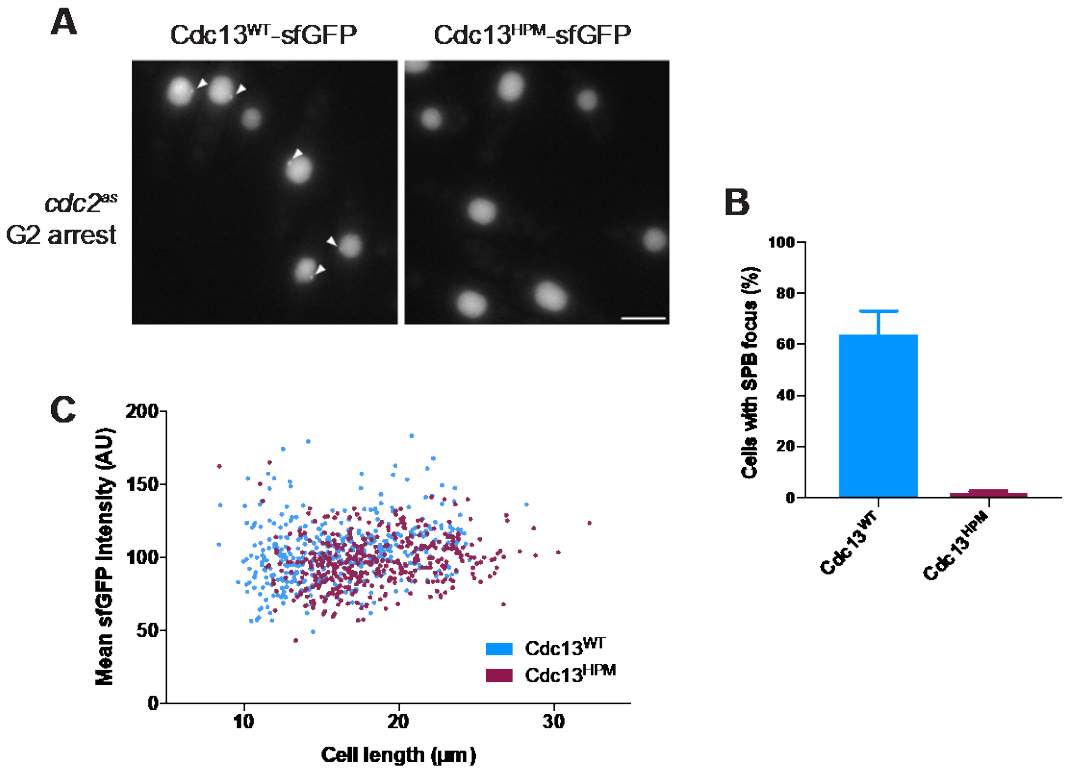
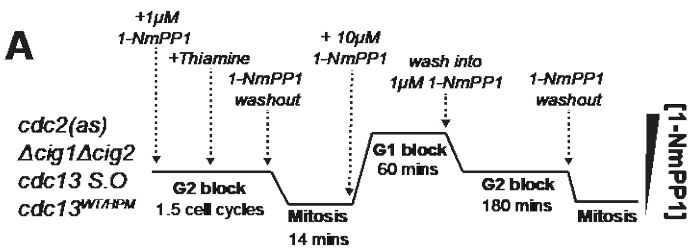

A
Cdc13WT-sfGFP
Cdc13HPM-sfGFP
cdc2as
G2 arrest
B
Cells with SPB focus (%)
100
80
60
40
20
0
Cdc13WT
Cdc13HPM
C
Mean sfGFP Intensity (AU)
200
150
100
50
0
10
20
30
Cell length (μm)
Cdc13WT
Cdc13HPM

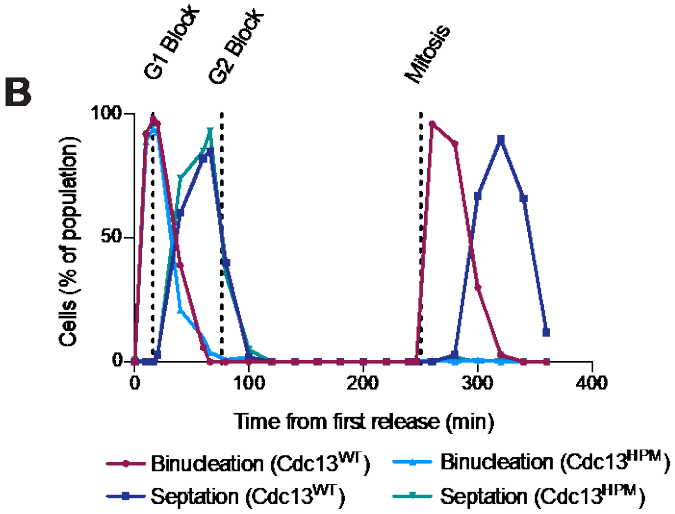
Figure S2. Cdc13^{HPM}-sfGFP does not accumulate at the SPB in a G2 arrest, Related to Figure 2.

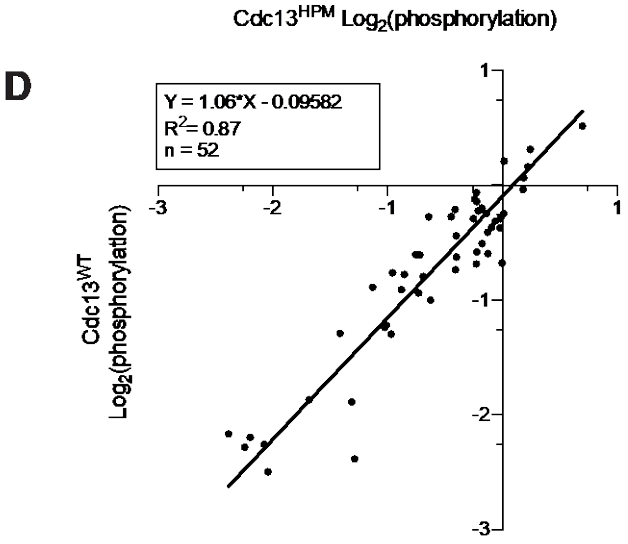
A – Representative maximum projection images of *cdc2(as)* cells arrested in G2 for 1 cell cycle containing an exogenous copy of either Cdc13^{WT}-sfGFP or Cdc13^{HPM}-sfGFP. The endogenous Cdc13 is expressed but not fused to a fluorophore. Arrows indicate Cdc13-sfGFP foci. The pixel range shown is the same for both Cdc13^{WT} and Cdc13^{HPM}. Scale bar = 5 μm.

B – The percentage of G2-arrested *cdc2(as)-M17* cells with Cdc13-sfGFP foci. n > 250 cells per condition per replicate. The mean and SD of 3 replicates are shown. Total n = 882 cells for Cdc13^{WT} and 794 cells for Cdc13^{HPM}.

C – Mean whole-cell fluorescence intensity of Cdc13^{WT}-sfGFP and Cdc13^{HPM}-sfGFP in G2 arrested cells, plotted against cell length, from one replicate of panel B. The mean value of background autofluorescence is removed. n = 428 cells for Cdc13^{WT} and 410 cells for Cdc13^{HPM}.


A
+1μM 1-NmPP1
+Thiamine
1-NmPP1 washout
+ 10μM 1-NmPP1
wash into 1μM 1-NmPP1
1-NmPP1 washout
cdc2(as) Δcig1Δcig2 cdc13 S.O cdc13WT/HPM
G2 block 1.5 cell cycles
Mitosis 14 mins
G1 block 60 mins
G2 block 180 mins
Mitosis
[1-NmPP1]


B
G1 Block
G2 Block
Mitosis
Cells (% of population)
Time from first release (min)
Binucleation (Cdc13WT)
Septation (Cdc13WT)
Binucleation (Cdc13HPM)
Septation (Cdc13HPM)


D
Cdc13HPM Log2(phosphorylation)
Cdc13WT Log2(phosphorylation)
Y = 1.06*X - 0.09582
R2 = 0.87
n = 52

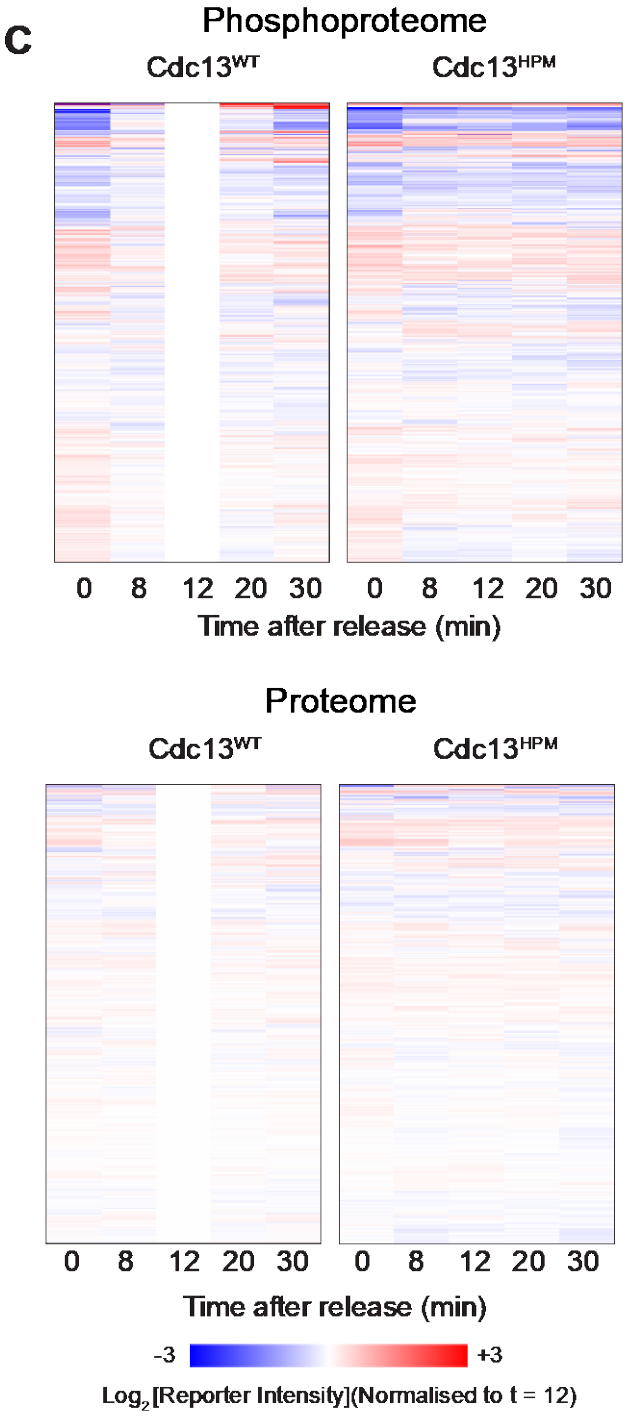

C
Phosphoproteome
Cdc13WT
Cdc13HPM
0 8 12 20 30 0 8 12 20 30
Time after release (min)
Proteome
Cdc13WT
Cdc13HPM
0 8 12 20 30 0 8 12 20 30
Time after release (min)
-3 +3
Log2[Reporter Intensity](Normalised to t = 12)

Figure S3. Cdc13^{HPM} can efficiently phosphorylate non-late CDK substrates, Related to Figure 3.

A – Experiment outline for Figure 3 and S3. Cells are blocked in G2 initially using 1 μM of the ATP analogue 1-NmPP1. One hour before release from 1-NmPP1, thiamine was added to repress endogenous Cdc13 (see STAR Methods). Cells are then allowed to progress through one mitosis in the presence of thiamine, and subsequently re-blocked with for 60 mins using 10 μM inhibitor and thiamine 14 minutes after release into mitosis. Cells are then washed into 1 μM inhibitor and thiamine to arrest in G2 for 180 minutes before final mitotic release into no inhibitor. Time-points are taken following second mitotic release.

B – Septation and binucleation indices for +Cdc13^{WT} and +Cdc13^{HPM} release relating to panels in Figure 3 and S3. 100 fixed cells per time point were counted. See STAR Methods for details.

C – Lower panels: Proteome dataset heatmap encompassing 2757 individual proteins. Upper panels: Phosphoproteome dataset heatmap encompassing 3835 phosphosites. Heatmap was clustered according to Euclidian clustering in Perseus. All individual samples were normalised to reporter intensity for 12 minutes for the wild-type condition. See STAR Methods for further information.

D – Comparison of relative phosphorylation for non-late phosphosites (as defined in [S1]) at t = 0 for both Cdc13^{HPM} and Cdc13^{WT} releases. Statistics are given in panel.

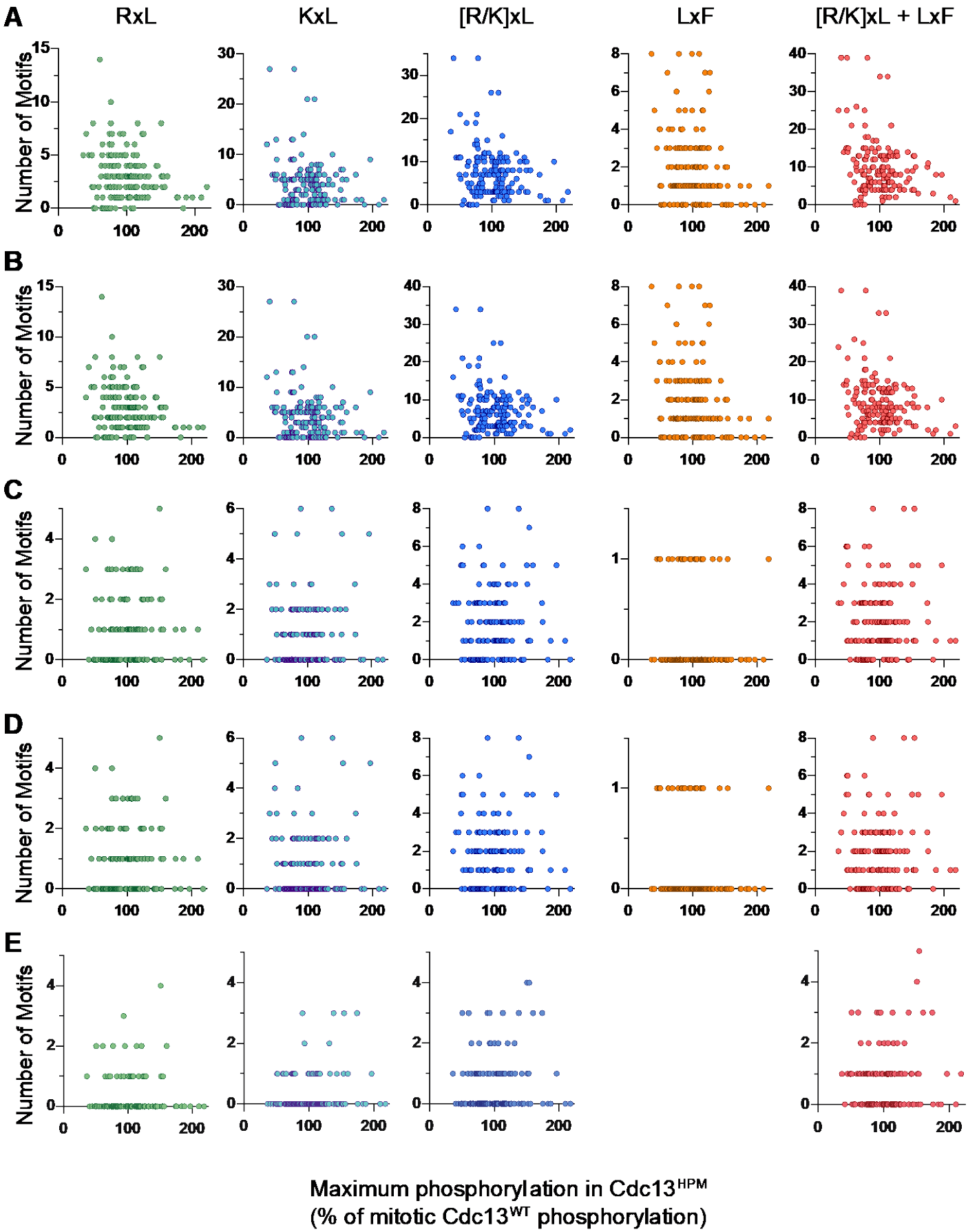

A
RxL
KxL
[R/K]xL
LxF
[R/K]xL + LxF
B
C
D
E
Number of Motifs
Maximum phosphorylation in Cdc13HPM
(% of mitotic Cdc13WT phosphorylation)

Figure S4. Cdc13^{HPM} dependent phosphorylation is not correlated with [R/K]xL or LxF content, Related to Figure 3.

Analysis of relationship between motif numbers of phosphosite-encompassing proteins and maximum phosphorylation achieved by Cdc13^{HPM} (given as a comparison with phosphorylation 12 minutes after release in the Cdc13^{WT} condition). No data presented were suitable for analysis by linear regression as no r-square values above 0.066 were obtained when linear regression was attempted. Motifs analysed are given above each column of graphs. Raw data present in Supplementary Table 1.

A – Analysis of phosphorylation vs. raw numbers of motifs present in the protein that encompasses the phosphosite in question.

B – Analysis of phosphorylation vs. numbers of motifs present in the protein that encompasses the phosphosite in question. In addition, filtering was applied to exclude motifs that were closer than 12 amino acids in primary sequence to the phosphosite in question. This filtering was applied as the minimum distance between the hydrophobic patch and the active site of CDK has been mapped to be at least 12 amino acids in length for *S. cerevisiae* Clb2-Cdc28 and human Cyclin A-Cdk2 [S2, S3].

C – Analysis of phosphorylation vs. numbers of motifs present in the protein that encompasses the phosphosite in question. In addition, filtering was applied to exclude motifs that were not present in disordered regions of the protein, as motifs that interact with the hydrophobic patch are thought to be generally disordered. Disorder was checked using the IUPred2 server, with an average score of 0.5 across the motif being considered disordered.

D – Analysis of phosphorylation vs. numbers of motifs present in the protein that encompasses the phosphosite in question with filtering in both (B) and (C) applied.

E – Analysis of phosphorylation vs. numbers of motifs present in the protein that encompasses the phosphosite in question with filtering in both (B) and (C) applied. In addition, RxL and KxL motifs analysed were restricted to those that possessed the full R/KxL motif with a C-terminal hydrophobic residue at either or both the +1 or +2 positions from the terminal leucine of the R/KxL motif ([R/K]-X-L-X{0,1}-[FYLIPRVM]). No extended motif was analysed for LxF. The [R/K]xL + LxF panel sums extended [R/K]xL motifs with all LxF motifs.

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