Supplemental Materials

Molecular Biology of the Cell

Mangione *et al*.

A

Pxl1 [S/T]P site	Spectral count from GFP-Pxl1 purification	Identified in these supplemental references
S3	Not detected	2, 4, 5
S24	6	2, 5
S31	15	2, 5
T55	12	2, 5
T64	33	2, 5
S67	142	1, 2, 5
S97	7	1, 2, 3, 5
S136	8	n.a.
T214	Not detected	n.a.

B

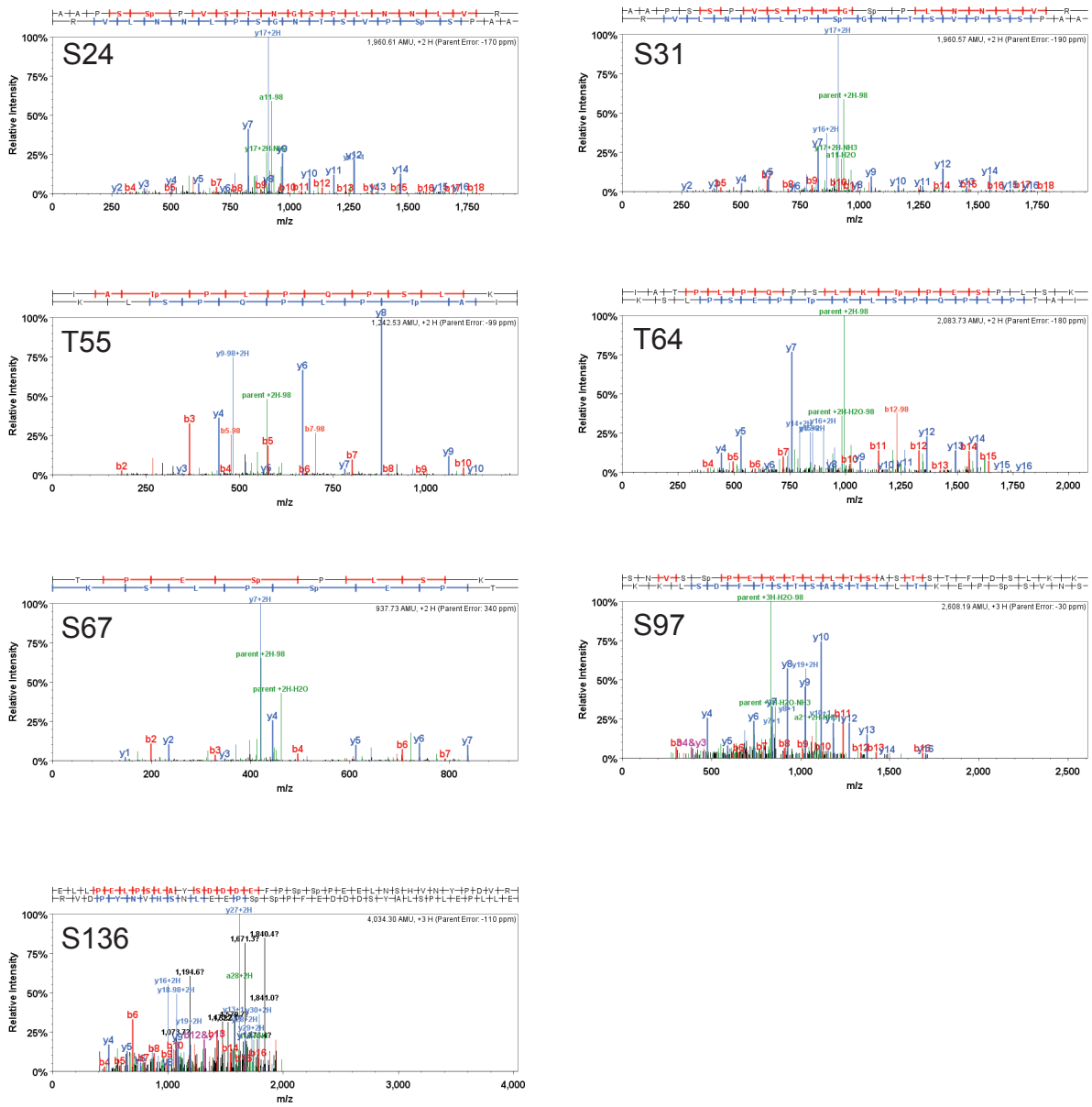

Figure S1

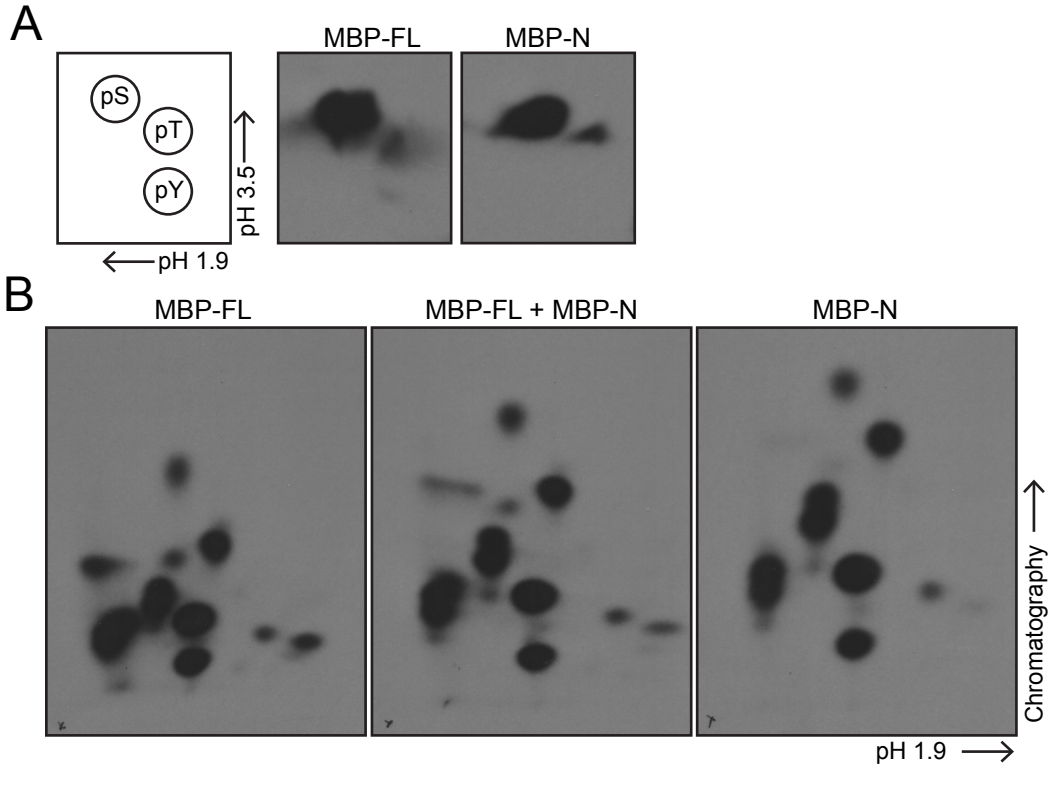
A
MBP-FL
MBP-N
pS
pT
pY
pH 3.5
pH 1.9
B
MBP-FL
MBP-FL + MBP-N
MBP-N
Chromatography
pH 1.9
C
untagged
HA3-pxl1+
HA3-pxl1+ clp1Δ
HA3-pxl1+ dis2Δ
HA3-pxl1+ clp1Δ dis2Δ
IP: anti-HA
IB: anti-HA
64 kD

Figure S2

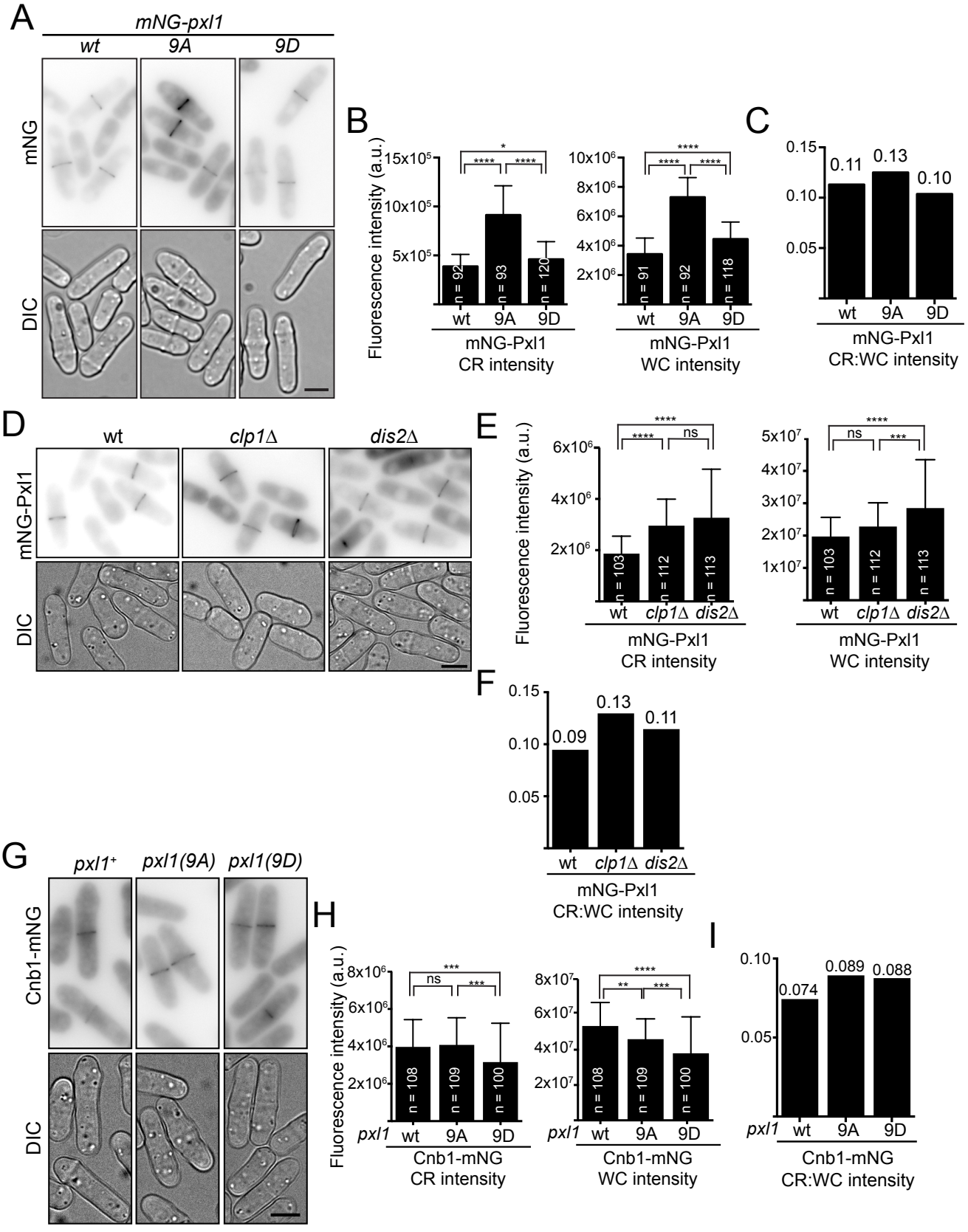
A
mNG-pxl1
wt
9A
9D
mNG
DIC
B
Fluorescence intensity (a.u.)
15x10^5
10x10^5
5x10^5
n = 92
n = 93
n = 120
wt
9A
9D
mNG-Pxl1
CR intensity
10x10^6
8x10^6
6x10^6
4x10^6
2x10^6
n = 91
n = 92
n = 118
mNG-Pxl1
WC intensity
C
0.15
0.10
0.05
0.11
0.13
0.10
mNG-Pxl1
CR:WC intensity
D
wt
clp1Δ
dis2Δ
mNG-Pxl1
DIC
E
Fluorescence intensity (a.u.)
6x10^6
4x10^6
2x10^6
n = 103
n = 112
n = 113
wt
clp1Δ
dis2Δ
mNG-Pxl1
CR intensity
5x10^7
4x10^7
3x10^7
2x10^7
1x10^7
mNG-Pxl1
WC intensity
F
0.15
0.10
0.05
0.09
0.13
0.11
mNG-Pxl1
CR:WC intensity
G
pxl1+
pxl1(9A)
pxl1(9D)
Cnb1-mNG
DIC
H
Fluorescence intensity (a.u.)
8x10^6
6x10^6
4x10^6
2x10^6
n = 108
n = 109
n = 100
pxl1
wt
9A
9D
Cnb1-mNG
CR intensity
8x10^7
6x10^7
4x10^7
2x10^7
Cnb1-mNG
WC intensity
I
0.10
0.05
0.074
0.089
0.088
Cnb1-mNG
CR:WC intensity

Figure S3

Table S1. *S. pombe* strains used in this study

Strain number	Genotype	Source
Figure 1		
KGY246	*ade6-M210 ura4-D18 leu1-32 h⁻*	Lab stock
KGY11532-2	*cdc25-22 pxl1::kan^R leu1:HA-pxl1 ura4-D18 h⁺ (ade?)*	This study
Figure 2		
KGY246	*ade6-M210 ura4-D18 leu1-32 h⁻*	Lab stock
KGY18026-2	*HA$_3$-pxl1:kan^R ade6-M21X leu1-32 ura4-D18 h⁻*	This study
KGY18031-2	*HA$_3$-pxl1(9A):kan^R ade6-M21X leu1-32 ura4-D18 h⁻*	This study
KGY18043-2	*HA$_3$-pxl1(9D):kan^R ade6-M21X leu1-32 ura4-D18 h⁻*	This study
KGY19171-2	*cdc25-22 HA$_3$-pxl1:kan^R ade6-M21X leu1-32 ura4-D18*	This study
KGY19188-2	*cdc25-22 HA$_3$-pxl1(9A):kan^R ade6-M21X leu1-32 ura4-D18*	This study
KGY19289-2	*cdc25-22 HA$_3$-pxl1(9D):kan^R ade6-M21X leu1-32 ura4-D18*	This study
Figure 3		
KGY246	*ade6-M210 ura4-D18 leu1-32 h⁻*	Lab stock
KGY11532-2	*cdc25-22 pxl1::kan^R leu1:HA-pxl1 ura4-D18 h⁺ (ade?)*	This study
KGY18026-2	*HA$_3$-pxl1:kan^R ade6-M21X leu1-32 ura4-D18 h⁻*	This study
KGY19091-2	*clp1::ura4⁺ HA$_3$-pxl1:kan^R ade6-M21X leu1-32 ura4-D18*	This study
KGY19599	*dis2::ura4⁺ HA$_3$-pxl1:kan^R ade6-M21X leu1-32 ura4-D18*	This study
Figure 4		
KGY11852-2	*mNG-pxl1:kan^R sid4-mNG:hyg^R ade6-M210 ura4-D18 leu1-32 h⁺*	This study
KGY18618-2	*mNG-pxl1(9A):kan^R sid4-mNG:hyg^R ade6-M210 ura4-D18 leu1-32 h⁻*	This study
KGY18627-2	*mNG-pxl1(9D):kan^R sid4-mNG:hyg^R ade6-M210 ura4-D18 leu1-32 h⁻*	This study
KGY19083	*rlc1-mNG:hyg^R sid4-mNG:kan^R ade6-M21X ura4-D18 leu1-32 h⁺*	Lab stock
KGY18681-2	*pxl1(9A):kan^R rlc1-mNG:hyg^R sid4-mNG:kan^R ade6-M21X ura4-D18 leu1-32 h⁻*	This study
KGY19089-2	*pxl1(9D):kan^R rlc1-mNG:hyg^R sid4-mNG:kan^R ade6-M21X ura4-D18 leu1-32 h⁻*	This study
Figure S1		
KGY5663	*nda3-KM311 ade6-M10X leu1-32 ura4-D18 h⁺*	Lab stock
KGY18174	*pxl1::kan^R GFP-pxl1:leu1⁺ nda3-KM311 ade6-M10X leu1-32 ura4-D18*	This study
Figure S2		
KGY246	*ade6-M210 ura4-D18 leu1-32 h⁻*	Lab stock
KGY18026-2	*HA$_3$-pxl1:kan^R ade6-M21X leu1-32 ura4-D18 h⁻*	This study
KGY19091-2	*clp1::ura4⁺ HA$_3$-pxl1:kan^R ade6-M21X leu1-32 ura4-D18*	This study
KGY19599	*dis2::ura4⁺ HA$_3$-pxl1:kan^R ade6-M21X leu1-32 ura4-D18*	This study
KGY4129-2	*clp1::ura4⁺ dis2::ura4⁺ HA$_3$-pxl1:kan^R ade6-M21X leu1-32 ura4-D18*	This study
Figure S3		
KGY3981-2	*mNG-pxl1:kan^R ade6-M210 ura4-D18 leu1-32 h⁻*	This study
KGY18534	*mNG-pxl1(9A):kan^R ade6-M210 ura4-D18 leu1-32 h⁻*	This study

KGY18607	*mNG-pxl1(9D):kan^{R} ade6-M210 ura4-D18 leu1-32 h^{-}*	This study
KGY4123-2	*mNG-pxl1:kan^{R} clp1::ura4^{+} ade6-M210 ura4-D18 leu1-32 h^{+}*	This study
KGY4130-2	*mNG-pxl1:kan^{R} dis2::ura4^{+} ade6-M210 ura4-D18 leu1-32*	This study
KGY1480-2	*cnb1-mNG:hyg^{R} ade6-M210 ura4-D18 leu1-32 h-*	Snider et al., 2020
KGY4104-2	*cnb1-mNG:hyg^{R} pxl1(9D):kan^{R} ade6-M210 ura4-D18 leu1-32*	This study
KGY4125-2	*cnb1-mNG:hyg^{R} pxl1(9A):kan^{R} ade6-M210 ura4-D18 leu1-32*	This study

Supplemental Figure Legends

Figure S1. Related to Figure 2. In vivo evidence of Pxl1 phosphorylated sites. A) All [S/T]P sites in Pxl1 N-terminus and evidence of in vivo phosphorylation from MS analysis from this study or others. References are: 1- (Carpy et al., 2014), 2- (Kettenbach et al., 2015), 3- (Koch et al., 2011), 4- (Swaffer et al., 2016), 5- (Swaffer et al., 2018). B) Mass spectra of a representative phosphopeptide from each of the identified Cdk1 phosphorylation sites on Pxl. These mass spectra were extracted from Scaffold PTM with matched b and y ions highlighted in red and blue, respectively. Ions resulting from neutral loss are highlighted in green. For most of the phosphorylation sites, both MS/MS (MS2) and MS/MS/MS (MS3, i.e. further MS/MS fragmentation of the ion which results from the neutral loss of phosphate of the parent ion during MS2 scan) spectra were acquired. In these cases, only MS2 spectra are shown here. Sp/Tp: phosphorylated serine/threonine.

Figure S2. Related to Figures 2 and 3. The Pxl1 N-terminus is phosphorylated by Cdk1 on Thr and Ser. A) Phosphoamino acid analysis and B) tryptic phosphopeptide mapping of Pxl1 full-length (FL) and N-terminal (N) fragments phosphorylated by Cdk1. C) IP samples separated by SDS-PAGE and IB for indicated proteins.

Figure S3. Related to Figure 4. Consequences of Pxl1 phosphorylation. A, D, G) Sum projections (mNG) or DIC representative images. Scale bar is 5 μm. B, E, H) Average CR and whole cell (WC) intensity of the indicated proteins. Error bars are SD. **** $p < 0.0001$, *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, One-way ANOVA with Tukey's post hoc test for multiple comparisons. C, G, I) Ratio of average CR and WC intensities from the data collected B, E, H respectively.

Supplemental references

Carpy, A., Krug, K., Graf, S., Koch, A., Popic, S., Hauf, S., and Macek, B. (2014). Absolute proteome and phosphoproteome dynamics during the cell cycle of Schizosaccharomyces pombe (Fission Yeast). Mol Cell Proteomics 13, 1925-1936.

Kettenbach, A.N., Deng, L., Wu, Y., Baldissard, S., Adamo, M.E., Gerber, S.A., and Moseley, J.B. (2015). Quantitative phosphoproteomics reveals pathways for coordination of cell growth and division by the conserved fission yeast kinase pom1. Mol Cell Proteomics 14, 1275-1287.

Koch, A., Krug, K., Pengelley, S., Macek, B., and Hauf, S. (2011). Mitotic substrates of the kinase aurora with roles in chromatin regulation identified through quantitative phosphoproteomics of fission yeast. Sci Signal 4, rs6.

Swaffer, M.P., Jones, A.W., Flynn, H.R., Snijders, A.P., and Nurse, P. (2016). CDK Substrate Phosphorylation and Ordering the Cell Cycle. Cell 167, 1750-1761.e1716.

Swaffer, M.P., Jones, A.W., Flynn, H.R., Snijders, A.P., and Nurse, P. (2018). Quantitative Phosphoproteomics Reveals the Signaling Dynamics of Cell-Cycle Kinases in the Fission Yeast Schizosaccharomyces pombe. Cell Rep 24, 503-514.