

## **Supplemental Information**

**Laser microsurgery provides evidence for merotelic kinetochore attachments in fission yeast cells lacking Pcs1 or Clr4.**

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## Supplemental Figure Legends

**Figure S1.** Mde4 and Pcs1 are phosphorylated during mitosis.

(A) List of proteins identified by mass spectrometry co-purifying with *S. pombe* Pcs1-TAP and Mde4-TAP isolated from *nda3-KM311*-arrested cells (JG15058 and JG15137, respectively) or cycling cells (JG15137). Only proteins identified with more than two peptides are included. Proteins found in other unrelated purifications are omitted from this table.

(B) Mde4 and Pcs1 phosphorylation sites identified by mass spectrometry. The identified phosphorylated residues are indicated in red. Sequence coverage is highlighted in yellow.

**Figure S2.** Sensitivity of cells expressing Cdc2-as to 1-NM-PP1. Serial dilutions of wild type cells (JG15123) or *cdc2Δ* cells expressing the analog-sensitive Cdc2-as (Cdc2-F84G) (JG15125) were spotted on YES plates containing or lacking 5  $\mu$ M 1-NM-PP1 and grown for 2 days at 32°C.

**Figure S3.** Interdependent localization of Pcs1 and Mde4.

(A) Wild-type cells expressing Pcs1-GFP and Mis6-HA (JG15031), or Mde4-GFP and Mis6-HA (JG15033), *mde4Δ* mutant cells expressing Pcs1-GFP and Mis6-HA (JG15383), and *pcs1Δ* mutant cells expressing Mde4-GFP and Mis6-HA (JG15366) were fixed and stained with antibodies against HA and GFP. Nuclei were visualized by Hoechst staining.

(B) Cells as described in Figure S3A were analyzed by Western blot analysis using anti-tubulin and anti-GFP antibodies.

## The genotypes of *S. pombe* strains used in this study

Strains	Genotype
JG15031	<i>h+ nda3-311 mis6-HA-leu2 pcs1-GFP-leu2 leu1-32</i>
JG12013	<i>h+ nda3-311 leu1-32 ura4-D18 his2</i>
JG14985	<i>h+ nda3-311 pcs1-GFP-leu2 leu1-32 ura4-D18 his2</i>
JG12239	<i>h- nda3-311 sgo2-GFP-Kan<sup>R</sup> ura4</i>
JG15033	<i>h+ nda3-311 mis6-HA-leu2 leu1-32 mde4-GFP-Kan<sup>R</sup></i>
JG15356	<i>h? nda3-311 mis6-HA mde4-GFP cdc2::ClonNat<sup>R</sup> cdc2-as-Hph<sup>R</sup></i>
JG15161	<i>h- mCherry-atb2-hph<sup>R</sup> nuf2-GFP-Kan<sup>R</sup> pcs1::CloneNat<sup>R</sup></i>
JG15351	<i>h- clr4::LEU2+ Nuf2-GFP-Kan<sup>R</sup> mCherry-atb2-hph<sup>R</sup> leu1-32</i>
JG15058	<i>h? nda3-311 pcs1-HA-TAP-Kan<sup>R</sup></i>
JG15137	<i>h+ nda3-311 leu1-32 ura4-D18 his2 mde4-Tap-Kan<sup>R</sup></i>
JG15414	<i>h+ lys1 his7 leu1 ura4 ade6-210 pcs1::ClonNat<sup>R</sup> pcs1-wt-Hph<sup>R</sup></i>
JG14821	<i>h+ lys1 his7 leu1 ura4 ade6-210 pcs1::ClonNat<sup>R</sup></i>
JG15549	<i>h+ lys1 his7 leu1 ura4 ade6-210 pcs1::ClonNat<sup>R</sup> pcs1-L140A-Hph<sup>R</sup></i>
JG15550	<i>h+ lys1 his7 leu1 ura4 ade6-210 pcs1::ClonNat<sup>R</sup> pcs1-F154A-Hph<sup>R</sup></i>
JG15551	<i>h+ lys1 his7 leu1 ura4 ade6-210 pcs1::ClonNat<sup>R</sup> pcs1-F154D-Hph<sup>R</sup></i>
JG15552	<i>h+ lys1 his7 leu1 ura4 ade6-210 pcs1::ClonNat<sup>R</sup> pcs1-F212A-Hph<sup>R</sup></i>
JG15553	<i>h+ lys1 his7 leu1 ura4 ade6-210 pcs1::ClonNat<sup>R</sup> pcs1-F212D-Hph<sup>R</sup></i>
JG15383	<i>h+ nda3-311 mis6-HA-leu2 pcs1-GFP-LEU2 leu1-32 mde4::Kan<sup>R</sup></i>
JG15366	<i>h+ nda3-311 mis6-HA-leu2 mde4-GFP-Kan<sup>R</sup> leu1-32 pcs1::CloneNat<sup>R</sup></i>
JG15123	<i>h? cdc2+</i>
JG15125	<i>h- cdc2::ClonNat<sup>R</sup> cdc2-as-Hph<sup>R</sup></i>

# Figure S1

## A

List of proteins identified by mass spectrometry co-purifying with Pcs1-TAP from <i>nda3</i> -arrested <i>S. pombe</i> cells.		
Protein (GeneInfo identifier)	Description	Number of unique peptides
1 68005983	Chromosome segregation protein Pcs1	22
2 19112875	Sequence orphan Mde4	18

List of proteins identified by mass spectrometry co-purifying with Mde4-TAP from cycling <i>S. pombe</i> cells.		
Protein (GeneInfo identifier)	Description	Number of unique peptides
1 19112875	Sequence orphan Mde4	56
2 19113336	Nucleolar protein Dna1	84
3 68005983	Chromosome segregation protein Pcs1	51
17 19115145	Karyopherin Kap95	6
18 19114970	Ribosomal-ubiquitin fusion protein Ubi5	5
24 19112476	Ataxin-2 homolog	4
37 19115509	TM22 inner membrane protein import complex subunit Tim13	2
40 19113475	Human LYHRT homolog	2
46 19114125	Nucleoporin Nup124	2
52 1136783	Elongation factor 1 alpha-A	2
53 63054532	ATP-dependent DNA ligase Cde17	2

List of proteins identified by mass spectrometry co-purifying with Mde4-TAP from <i>nda3</i> -arrested <i>S. pombe</i> cells.		
Protein (GeneInfo identifier)	Description	Number of unique peptides
1 19112875	Sequence orphan Mde4	25
2 19113336	Nucleolar protein Dna1	23
4 19075845	RNA polymerase I upstream activation factor complex subunit Spp27	11
10 19112198	Chaperonin-containing T-complex theta subunit Cet8	9
11 19112741	Chaperonin-containing T-complex alpha subunit Cet1	9
12 68005983	Chromosome segregation protein Pcs1	8
17 19112476	Ataxin-2 homolog	7
19 19111947	Chaperonin-containing T-complex delta subunit Cet4	7
22 19114645	Mitotic spindle checkpoint protein Dna1	6
23 19112602	Chaperonin-containing T-complex gamma subunit Cet3	6
33 19113147	Chaperonin-containing T-complex eta subunit Cet7	5
48 19115145	Karyopherin Kap95	4
49 19112010	Vta27 family protein	4
52 19114189	Chaperonin-containing T-complex epsilon subunit Cet5	3
65 19076049	Acetyl-CoA-ligase	3
70 19113371	Sequence orphan	3
73 19114554	Serine/threonine protein kinase Cka1	2
75 19115509	TM22 inner membrane protein import complex subunit Tim13	2
76 19114018	U3 snoRNP protein Nop58	2
80 19112161	Chaperonin-containing T-complex zeta subunit Cet6	2
82 19113929	Chaperonin-containing T-complex beta subunit Cet2	2
85 1136783	Elongation factor 1 alpha-A	2
87 19076061	Ribosome biogenesis protein Nra2	2
88 12000355	Single-stranded TG1-3 binding protein	2
90 19112160	U3 snoRNP protein Nop56	2
91 19112358	Histone H4 h4.2	2

## B

### Mde4 (*nda3*-arrest)

### Mde4 (cycling cells)

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MSTISTSDS KLDNLGLSVT SRRNQILFYL SKALNLAHLL RSDSLQKSF L DALKQSATDS ELLHKNLDEI
KFLQNEKLN EKLLEQE QNE ANDYRLKVER LEHKISDYVQ EINSLSQLQ IQKSNPEKHE DAVSQNRLRG
SLD TVSSPSK THKANKDEKA TRLHLIIANL KKALK EKDAE VLNLQSHVSS KESELDRFKI
VRLQVLESKL ATQDRKLRM Q KKSTERKSL L VSPRVSPK L TRQPNATSGS PLSVTPFLQK TSTSIGLSSS PPQSSPSAQ S
TSTSIGLSSS SQPFPSRDKYP HSMTVSPSNA RYLK KHLDDT IPSNVSDINH NDHLKIPQSP
SSLSPSKIP I RKRRKLDTV SNCEFTEDS ESSFLETIQ PTKSTLRRSI
K
1 MSTISTSDS KLDNLGLSVT SRRNQILFYL SKALNLAHLL RSDSLQKSF L
51 DALKQSATDS ELLHKNLDEI KFLQNEKLN EKLLEQE QNE ANDYRLKVER
101 LEHKISDYVQ EINSLSQLQ IQKSNPEKHE DAVSQNRLRG SLDTVSSPSK
151 THKANKDEKA TRLHLIIANL KKALK EKDAE VLNLQSHVSS KESELDRFKI
201 KLETEESNWK VRLQVLESKL ATQDRKLRM Q KKSTERKSL L VSPRVSPK L
251 FSPSKQAIMG TRQPNATSGS PLSVTPFLQK TSTSIGLSSS PPQSSPSAQ S
301 SQPFPSRDKYP HSMTVSPSNA RYLK KHLDDT IPSNVSDINH NDHLKIPQSP
351 SSLSPSKIP I RKRRKLDTV SNCEFTEDS ESSFLETIQ PTKSTLRRSI
401 SPLKKRND E I NELKKGFTMK K

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MSTISTSDS KLDNLGLSVT SRRNQILFYL SKALNLAHLL RSDSLQKSF L DALKQSATDS
ELLHKNLDEI KFLQNEKLN EKLLEQE QNE ANDYRLKVER LEHKISDYVQ EINSLSQLQ
IQKSNPEKHE DAVSQNRLRG SLDTVSSPSK THKANKDEKA TRLHLIIANL KKALK EKDAE
VLNLQSHVSS KESELDRFKI KLETEESNWK VRLQVLESKL ATQDRKLRM Q KKSTERKSL L
VSPRVSPK L FSPSKQAIMG TRQPNATSGS PLSVTPFLQK TSTSIGLSSS PPQSSPSAQ S
SQPFPSRDKYP HSMTVSPSNA RYLK KHLDDT IPSNVSDINH NDHLKIPQSP
RKRRKLDTV SNCEFTEDS ESSFLETIQ PTKSTLRRSI SPLKKRND E I NELKKGFTMK
K

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1 MSTISTSDS KLDNLGLSVT SRRNQILFYL SKALNLAHLL RSDSLQKSF L
51 DALKQSATDS ELLHKNLDEI KFLQNEKLN EKLLEQE QNE ANDYRLKVER
101 LEHKISDYVQ EINSLSQLQ IQKSNPEKHE DAVSQNRLRG SLDTVSSPSK
151 THKANKDEKA TRLHLIIANL KKALK EKDAE VLNLQSHVSS KESELDRFKI
201 KLETEESNWK VRLQVLESKL ATQDRKLRM Q KKSTERKSL L VSPRVSPK L
251 FSPSKQAIMG TRQPNATSGS PLSVTPFLQK TSTSIGLSSS PPQSSPSAQ S
301 SQPFPSRDKYP HSMTVSPSNA RYLK KHLDDT IPSNVSDINH NDHLKIPQSP
351 SSLSPSKIP I RKRRKLDTV SNCEFTEDS ESSFLETIQ PTKSTLRRSI
401 SPLKKRND E I NELKKGFTMK K

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### Pcs1 (*nda3*-arrest)

### Pcs1 (cycling cells)

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MHSTQDFVNN EDQDAYSVRE NENELHINNS GMSSELNKKLQ LPNVELSTLS HTQEQEFNEL NKLIRKINEL
QEFYLLLEDLA KPVTNAGADA DEDTIVKDLK KELENEKKAN HSLKNELLKT REQIKNYSKI NILIKELFGL
EVADCIED E D GYRFNCKNTG RRG TLEYQLL LDDQNF TFTP RLNVQTDEEL MKHLPDYLL E EIIFTKEQ GK
LFSARLMKAL QD
1 MHSTQDFVNN EDQDAYSVRE NENELHINNS GMSSELNKKLQ LPNVELSTLS
51 HTQEQEFNEL NKLIRKINEL QEFYLLLEDLA KPVTNAGADA DEDTIVKDLK
101 KELENEKKAN HSLKNELLKT REQIKNYSKI NILIKELFGL EVADCIED E D
151 GYRFNCKNTG RRG TLEYQLL LDDQNF TFTP RLNVQTDEEL MKHLPDYLL E
201 EIIFTKEQ GK LFSARLMKAL QD

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MHSTQDFVNN EDQDAYSVRE NENELHINNS GMSSELNKKLQ LPNVELSTLS HTQEQEFNEL
NKLIRKINEL QEFYLLLEDLA KPVTNAGADA DEDTIVKDLK KELENEKKAN HSLKNELLKT
REQIKNYSKI NILIKELFGL EVADCIED E D GYRFNCKNTG RRG TLEYQLL LDDQNF TFTP
RLNVQTDEEL MKHLPDYLL E EIIFTKEQ GK LFSARLMKAL QD
1 MHSTQDFVNN EDQDAYSVRE NENELHINNS GMSSELNKKLQ LPNVELSTLS
51 HTQEQEFNEL NKLIRKINEL QEFYLLLEDLA KPVTNAGADA DEDTIVKDLK
101 KELENEKKAN HSLKNELLKT REQIKNYSKI NILIKELFGL EVADCIED E D
151 GYRFNCKNTG RRG TLEYQLL LDDQNF TFTP RLNVQTDEEL MKHLPDYLL E
201 EIIFTKEQ GK LFSARLMKAL QD

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Figure S2

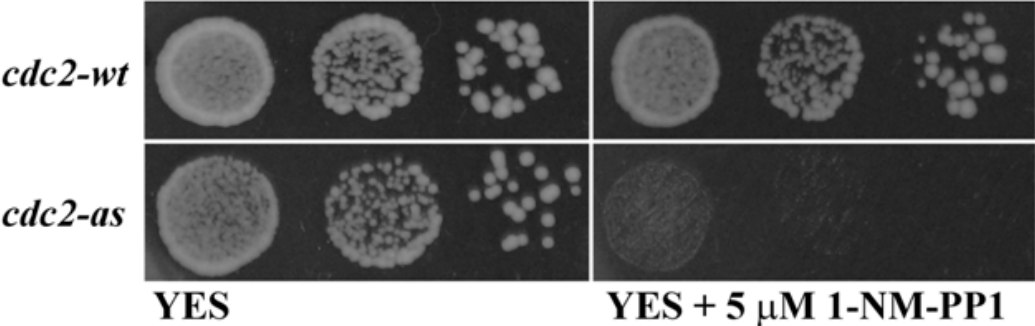


Figure S3

