	sid2-TAP cdc16-116	
Component	I[a]	II[b]
Sid2	35.1	336
Cdc11	18.3	126
Sid4	16.5	44
Mob1	17.1	33

[a]Percentage of each protein identified by mass spectrometry.
[b]Number of unique tryptic peptides of each protein identified by mass spectrometry.

Figure S1

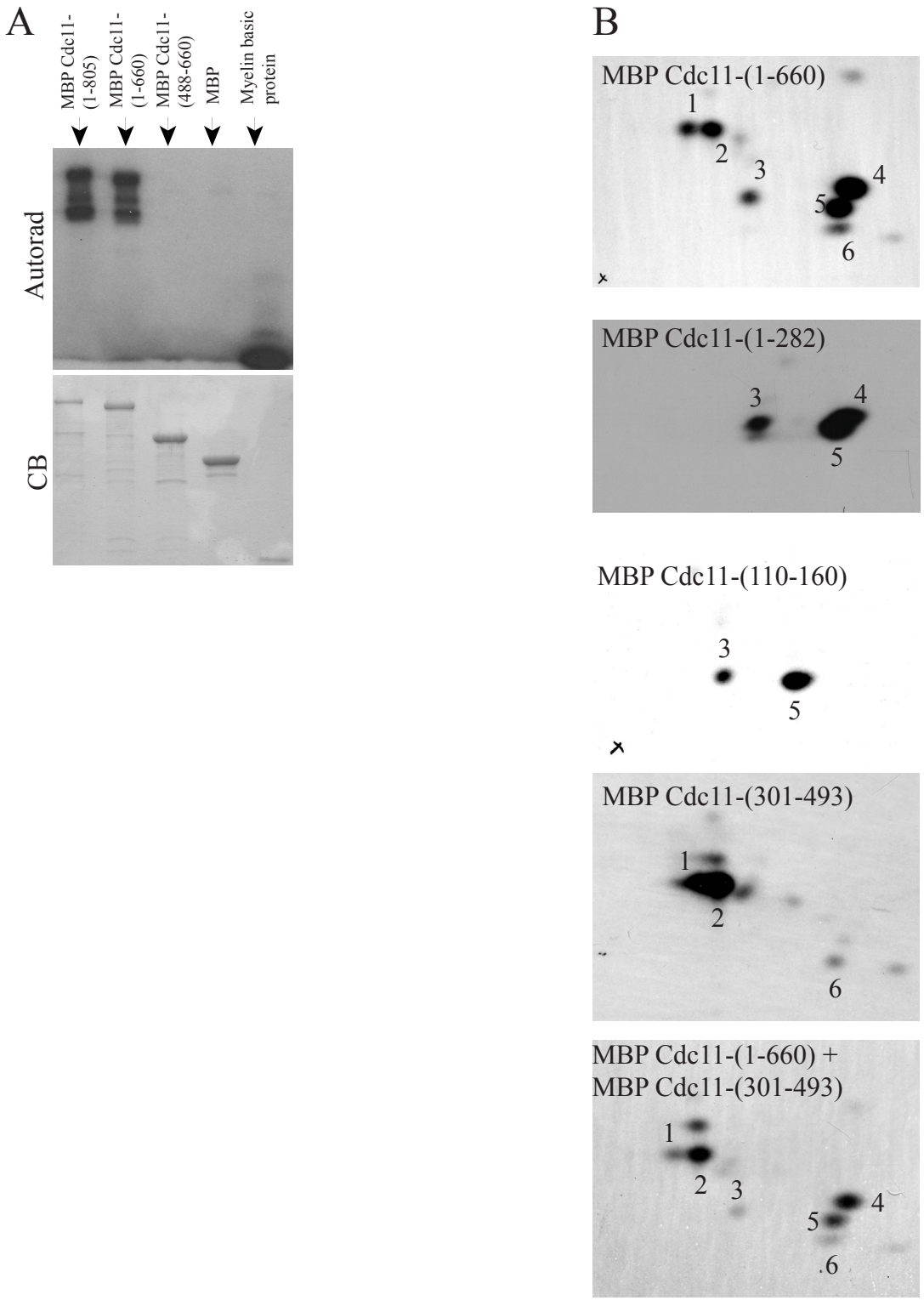
A
MBP Cdc11-(1-805)
MBP Cdc11-(1-660)
MBP Cdc11-(488-660)
MBP
Myelin basic protein
Autorad
CB
B
MBP Cdc11-(1-660)
1
2
3
4
5
6
MBP Cdc11-(1-282)
3
4
5
MBP Cdc11-(110-160)
3
5
MBP Cdc11-(301-493)
1
2
6
MBP Cdc11-(1-660) +
MBP Cdc11-(301-493)
1
2
3
4
5
.6

Figure S2

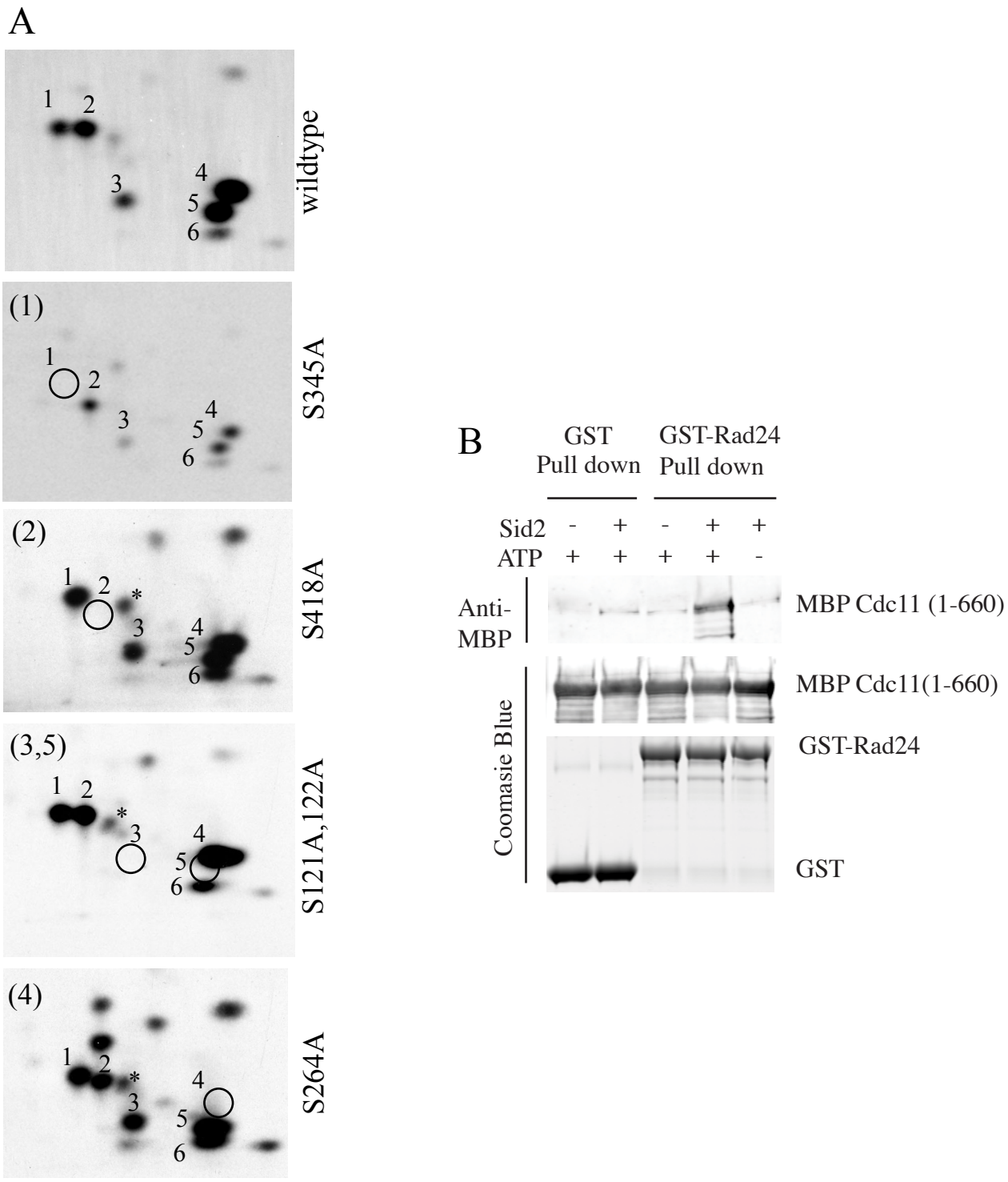
A
wildtype
(1)
S345A
(2)
S418A
(3,5)
S121A,122A
(4)
S264A
B
GST
Pull down
GST-Rad24
Pull down
Sid2 - + - + +
ATP + + + + -
Anti-
MBP
MBP Cdc11 (1-660)
Coomasie Blue
MBP Cdc11(1-660)
GST-Rad24
GST

Figure S3

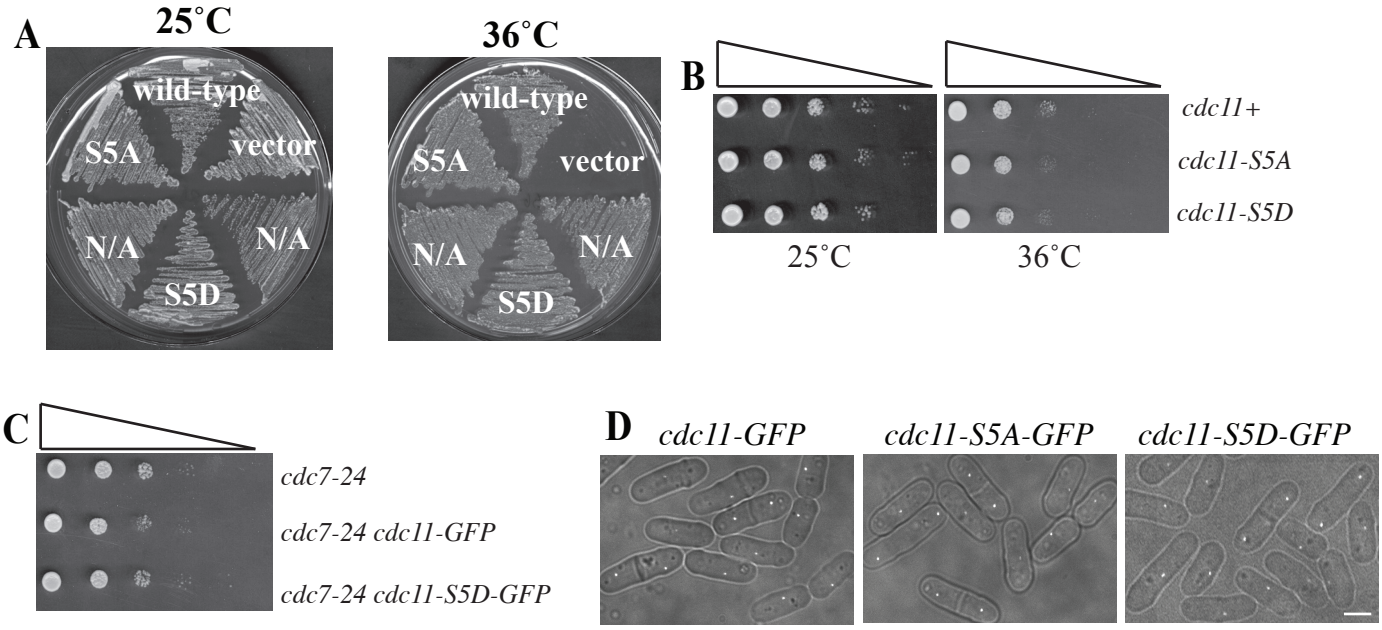
A
25˚C
wild-type
S5A
vector
N/A
N/A
S5D
36˚C
wild-type
S5A
vector
N/A
N/A
S5D
B
cdc11+
cdc11-S5A
cdc11-S5D
25˚C
36˚C
C
cdc7-24
cdc7-24 cdc11-GFP
cdc7-24 cdc11-S5D-GFP
D
cdc11-GFP
cdc11-S5A-GFP
cdc11-S5D-GFP

Figure S4

Supplementary Figure Legends

Figure S1. Identification of Sid2-associated proteins. Sid2-TAP preparation from *cdc16-116* arrested cells was analyzed by 2D-LC-mass spectrometry. Identified co-purifying proteins are listed.

Figure S2. Defining regions containing Sid2-Mob1 phosphorylation sites. A) Sid2-Myc_{13} was immunoprecipitated from *cdc16-116* cells that had been shifted to 36˚C for 4 h and the immunoprecipitate was incubated with Myelin Basic Protein as a positive control, MBP, or the indicated MBP-Cdc11 fusion protein in the presence of labeled ATP. Following 30 minutes at 30˚C, the reactions were resolved by SDS-PAGE. The gel was stained with Coomassie blue (lower panel), dried, and then exposed to film (upper panel). B) Phosphotryptic peptides from the indicated labeled Cdc11 proteins were separated in two dimensions with the anode on the left. The positions of major phosphopeptides are numbered.

Figure S3. Identification of Sid2-Mob1 phosphorylation sites. A) Phosphotryptic peptides from the indicated labeled Cdc11 proteins were separated in two dimensions with the anode on the left. The positions of major phosphopeptides are numbered. The asterisks indicate minor phosphopeptides. B) MBP-Cdc11(1-660) was pre-incubated with Sid2 kinase in the presence or absence of unlabeled ATP, and then incubated with GST or GST-Rad24 bound to glutathione sepharose. Bound proteins were detected by Western blot using anti-MBP monoclonal antibody (New England BioLabs) (upper panel). Input proteins are shown in the bottom panels.

Figure S4. Characterization of Sid2 phosphomutants. A) pIRT2 vector alone or containing the indicated *cdc11* alleles was transformed into *cdc11-123* cells. Transformants were struck to selective plates and incubated at the indicated temperatures. N/A is non-applicable. B and C) The indicated strains were grown to mid-log phase at 25˚C in YE and spotted in 10-fold serial dilutions on a YE plate and incubated at 25˚C. The indicated *cdc11* alleles without epitope tags were integrated at the endogenous *cdc11* locus. D) Live cell imaging of the indicated *cdc11* alleles tagged at their endogenous loci with GFP. Scale bar, 5 μm.

Table S1. Phosphopeptides identified by mass spectrometric analysis of Cdc11 purified from cells.

A. Cdk1 sites

S98(43):

DIFARLDLENMFEESSKQ**S**

DIFARLDLENMFEESSKQ**S**PPSKSPTKNPSKK

LDLENMFEESSKQ**S**

LDLENMFEESSKQ**S**PPS

LDLENMFEESSKQ**S**PPSK

LDLENMFEESSKQ**S**PPSKSPTK

LDLENMFEESSKQ**S**PPSKSPTKNPSKK

Q**S**PPSKSPTKNPSKKSSNNSS

S103(3):

LDLENMFEESSKQSPPSK**S**PTK

LDLENMFEESSKQSPPSK**S**PTKNP

SKQSPPSK**S**PTKNPSKKSSNNSSRRSS

S136(5):

SSSSVGKLSNVSNMQS**S**PSKDPFVSQDYEKESIS

SSSSVGKLSNVSNMQS**S**PSKDPFVSQDYEKESISSS

LSNVSNMQS**S**PSKDPFVSQDYEK

LSNVSNMQS**S**PSKDPFVSQDYEK

199(6):

SSSSVVS**S**PSLKPNNTSP

SSVVS**S**PSLKPNNTSP

208(7):

SSSSVVSSPSLKPNNT**S**PL

SSSVVSSPSLKPNNT**S**PL

SSSVVSSPSLKPNNT**S**PLK

SSPSLKPNNT**S**PL

SSPSLKPNNT**S**PLKL

S360(12):

RVTSIFNDNDDSFPSASS**S**PQR

VTSIFNDNDDSFPSASS**S**PQR

TSIFNDNDDSFPSASS**S**PQRQ

SIFNDNDDSFPSASS**S**PQR

SIFNDNDDSFPSASS**S**PQRQ

FNDNDDSFPSASS**S**PQRQ

FNDNDDSFPSASS**S**PQR

FNDNDDSFPSASS**S**PQRQA

DSFPSASS**S**PQRQAYMT

PSASS**S**PQRQAY

S393(3):

LNS**S**PKSTLKTS

TDKMPLREIDVGSSQSSSKTARLNS**S**PK

ARLNS**S**PKSTLK

S558(61):
SSFHDLSLQNESFDEMFNGRYENG**S**PIPFIS
SSFHDLSLQNESFDEMFNGRYENG**S**PIPFISSGSG
SSFHDLSLQNESFDEMFNGRYENG**S**PIPFISSGSGLK
SSFHDLSLQNESFDEMFNGRYENG**S**PIPFISSGSGLK
SFHDLSLQNESFDEMFNGRYENG**S**PIPFIS
FHDLSLQNESFDEMFNGRYENG**S**PIPFISSGSGLK
SLQNESFDEMFNGRYENG**S**PIPF
SLQNESFDEMFNGRYENG**S**PIPFISSGSGLK
SLQNESFDEMFNGRYENG**S**PIPFISSGSG
SLQNESFDEMFNGRYENG**S**PIPFISSGSGLK
NESFDEMFNGRYENG**S**PIPF
NESFDEMFNGRYENG**S**PIPF
NESFDEMFNGRYENG**S**PIPFIS
SFDEMFNGRYENG**S**PIPF
YENG**S**PIPFISSGSGLK
NGRYENG**S**PIPF

B. RXXS sites

S121,S122(3):
KKSSNNSSRRS**SS**SVGKLSNV
SSSVGKLSNVSNMQSSPSKDP
SSSVGKLSNVSNMQSSPSKDPFVSQDYE
S264(8):
KPMRTTERKA**S**LNTKDLYQEVEEVMAR
PMRTTERKA**S**LNTKDLYQEVEEVMAR
ERKA**S**LNTKDLYQEVEEVMAR
S345(3):
DQLHLKSLQSMKRVT**S**IFN
KSLQSMKRVT**S**IFNDNDDSFPSASSSPQR
T**S**IFNDNDDSFPSASSSPQRQAYMTDKMPLREID
S418(5):
RKV**S**DYPNMVVITPADLPEGIDTTQGSMEFDR
SDYPNMVVITPADLPEGIDTTQGSMEFDR
KV**S**DYPNMVVITPADLPEGIDTTQGSMEFDR
AQSSKRKV**S**DYPNMVVITPADLPEGIDTTQGSME
VKTRRSHSAQSSKRKV**S**DYPN

The results are collated from 6 separate experiments. The phosphorylated residue number is given, followed by the sequence of each unique phosphopeptide. Certain phosphopeptides were identified multiple times. The phosphorylated residues are indicated in bold typeface.

Table S2 *S. pombe* strains used in this study

Strain	Genotype	Source
KGY653	*cdc11-123 ade6-M210 ura4-D18 leu1-32* h$^-$	This study
KGY863	*sid2-myc$_{13}$::Kan^R mob1-R4 leu1-32 ade6-M210 leu1-32 h^{90}*	This study
KGY1471	*sid2-250 cdc7-GFP::Kan^R ura4-D18 leu1-32 ade6-M210* h$^-$	This study
KGY1472	*cdc7-GFP::Kan^R sid2-myc$_{13}$::Kan^R ade6-M210 ura4-D18 leu1-32* h$^+$	This study
KGY1548	*sid2-TAP::Kan^R cdc16-116 ade6-M210 h$^-$*	This study
KGY2061	*cdc7-24 ade6-M210 ura4-D18 leu1-32* h$^-$	Lab stock
KGY2678	*spg1-GFP::Kan^R ade6-M210 ura4-D18 leu1-32* h$^+$	This study
KGY3201	*cdc11-GFP::Kan^R ade6-M210 ura4-D18 leu1-32 h-*	This study
KGY3392	*cdc11-GFP::Kan^R ade6-M210 ura4-D18 leu1-32* h$^+$	Lab stock
KGY3529	*sid2-myc$_{13}$::ura4$^+$ cdc25-22 ade6-M21X ura4-D18 leu1-32* h$^+$	Lab stock
KGY3736	*sid2-myc$_{13}$::Kan^R ade6-M21X, ura4-D18 leu1-32 h+*	This study
KGY4044	*cdc7-GFP::Kan^R ade6-M210 ura4-D18 leu1-32* h$^+$	Lab stock
KGY4321	*sid2-myc$_{13}$::Kan^R cdc16-116 ade6-M210 ura4-D18 leu1-32* h$^+$	Lab stock
KGY6065	*sid2-myc$_{13}$::Kan^R nda3-KM311 ade6-M21X ura4-D18 leu1-32* h$^+$	Lab stock
KGY7574	*cdc11-S5A ade6-M210 leu1-32 ura4-D18 h-*	This study
KGY7662	*cdc11-S5A-GFP::Kan^R ade6-M210 ura4-D18 leu1-32* h$^-$	This study
KGY7779	*cdc11-S5A-GFP::Kan^R cdc25-22 ade6-M210 ura4-D18 leu1-32* h$^+$	This study
KGY7799	*cdc7-24 cdc11-GFP::Kan^R ade6-M210 ura4-D18 leu1-32* h$^+$	This study
KGY7800	*cdc11-GFP::Kan^R cdc25-22 ade6-M210 ura4-D18 leu1-32* h$^+$	Lab stock
KGY7960	*cdc7-A20 ura4-D18 ade6-M210 leu1-32* h$^-$	Lab stock
KGY8018	*cdc15-GFP::Kan^R sid4-mCherry:: Kan^R ade6-M210 ura4-D18 leu1-32* h$^+$	This study
KGY 8021	*cdc7-A20 cdc11-S5A-GFP::Kan^R ade6-M210 ura4-D18 leu1-32* h$^-$	This study
KGY8023	*cdc7-GFP::Kan^R cdc11-S5A ade6-M210 ura4-D18 leu1-32* h$^-$	This study
KGY8061	*cdc11-S5A cdc15-GFP::Kan^R sid4-mCherry:: Kan^R ade6-M210 ura4-D18 leu1-32* h$^-$	This study
KGY8086	*cdc7-A20 cdc11-GFP::Kan^R ade6-M210 ura4-D18 leu1-32* h$^-$	This study
KGY8250	*cdc11-S5D ade6-M210 leu1-32 ura4-D18 h-*	This study
KGY8283	*cdc11-S5D-GFP::Kan^R ade6-M210 ura4-D18 leu1-32* h$^-$	This study
KGY8305	*cdc7-GFP::Kan^R cdc11-S5D ade6-M210 ura4-D18 leu1-32* h$^-$	This study
KGY8309	*cdc7-24 cdc11-S5D-GFP::Kan^R ade6-M210 ura4-D18 leu1-32* h$^-$	This study
KGY8348	*cdc7-A20 cdc11-S5D-GFP::Kan^R ura4-D18 leu1-32 ade6-M210* h$^+$	This study
KGY8358	*cdc11-S5D-GFP::Kan^R cdc25-22 ura4-D18* h$^+$	This study
KGY8505	*sid2-GFP::ura4$^+$ cdc11-S5A ade6-M210 ura4-D18 leu1-32* h$^+$	This study
KGY8517	*sid2-GFP::ura4$^+$ cdc11-S5D ade6-M210 ura4-D18 leu1-32* h$^+$	This study

KGY8609	*sid2-GFP::ura4*$^{+}$ *ade6-M210 ura4-D18 leu1-32* h^{+}	This study
KGY9305	*cdc7-GFP::Kan*^{R} *sid2-myc*$_{13}$*::Kan*^{R} *cdc11-S5A ade6-M210 ura4-D18 leu1-32* h^{+}	This study
KGY10398	*cdc11-S5D cdc15-GFP::Kan*^{R} *sid4-mCherry:: Kan*^{R} *ade6-M210 ura4-D18 leu1-32* h^{-}	This study
MBY7310	*sid1-239 cdc7-GFP::ura4*$^{+}$ *mCherry-atb2::hph ade6-m21X ura4-D18 leu1-32*	This study
MBY7313	*sid2-250 cdc7-GFP::ura4*$^{+}$ *mCherry-atb2::hph ade6-m21X ura4-D18 leu1-32*	This study