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Supplemental Information

Mec1 Is One of Multiple Kinases that Prime the Mcm2-7 Helicase for Phosphorylation by Cdc7

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Supplemental Experimental Procedures

Construction of S. cerevisiae *MCM4 and MCM6 mutant strains*

To integrate *MCM4* and *MCM6* mutant alleles at their chromosomal locus, we first created plasmids containing the *MCM4* or *MCM6* promoter upstream of a NatMX4 (for *MCM4*) or *LEU2* (for *MCM6*) marker cassette, with the Mcm5 promoter driving expression of the MCM4 or MCM6 gene downstream of the marker and Pac1 sites flanking the entire construct. A unique *Bst*BI site was inserted downstream of the N-terminal mutations to allow confirmation of the presence of the mutant allele after integration *in vivo*. Proper integration was confirmed by PCR followed by *Bst*BI digestion or sequencing to confirm the presence of all mutations in each allele.

To create strains carrying mutations in both *MCM4* and *MCM6*, we began with strains carrying the wild-type and mutant copies of *MCM4* on URA+ and TRP+ *ARS/CEN* constructs, respectively. *MCM6* alleles were incorporated into these strains using the LEU+ integrating constructs described above. Proliferation of *mcm4 mcm6* double-mutant strains was then analyzed by spotting assay using FOA counter-selection against the URA+ wild-type *MCM4* construct. A complete list of the strains used in this study can be found in Table S2.

Analysis of *cdc7-4* bypass alleles was performed by transforming the *cdc7-4* strain OAy711 with PacI-digested *MCM4* integrating constructs carrying phosphomimetic aspartate mutations in *MCM4*.

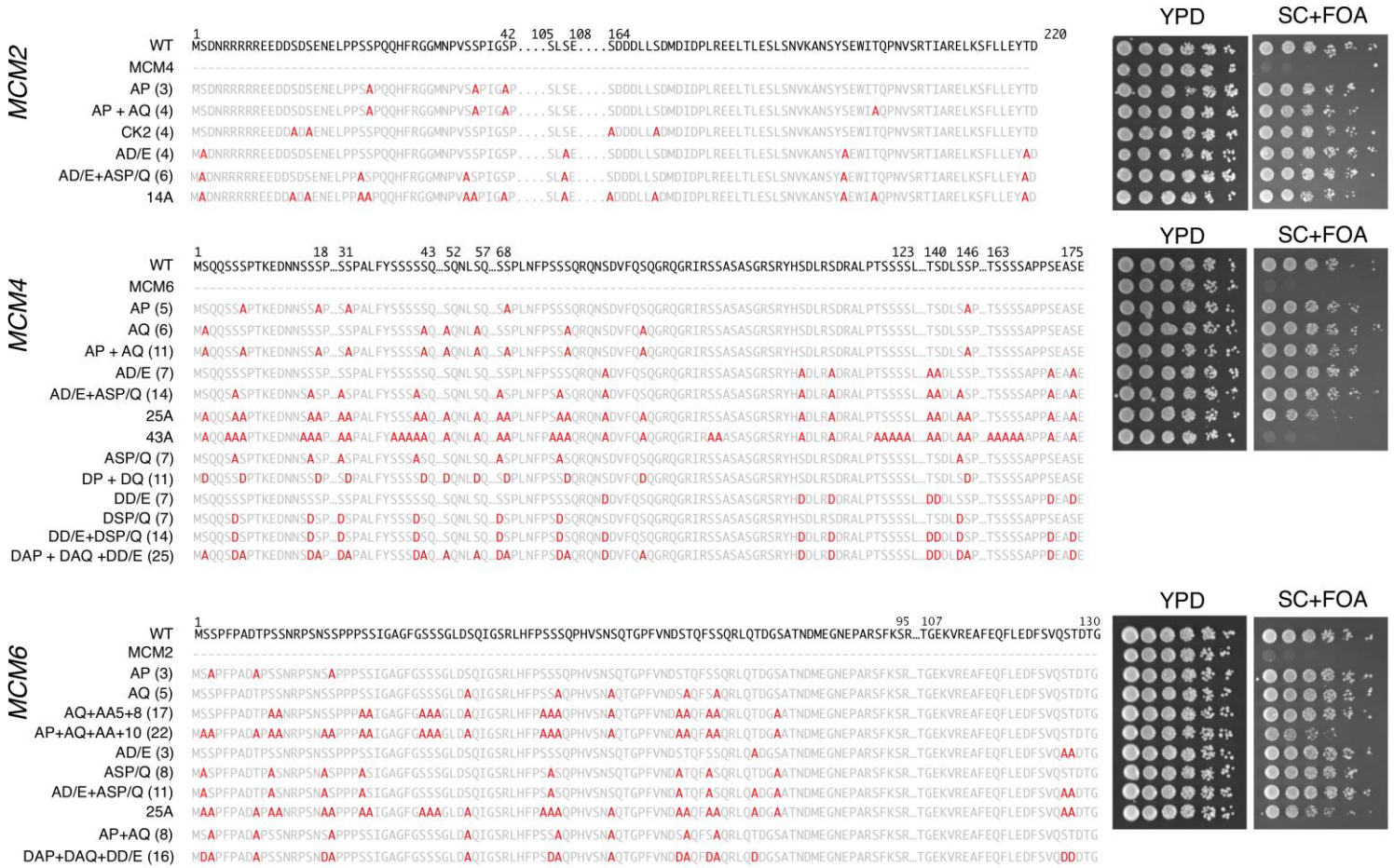
Gene deletions were performed using standard PCR-based gene disruption techniques (Petracek and Longtine, 2002). FACS and chromatin fractionation assays were performed as described (Wilmes et al., 2004).

Cell cycle arrest experiments

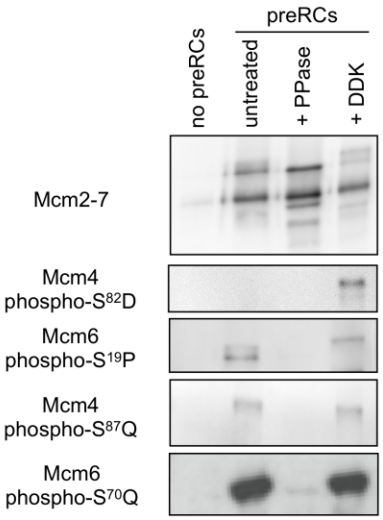
Strains were grown in YPD at 25^{o}C to early log-phase. At 0.2 OD_{600}/mL, cell were treated with α–factor until reaching full G1 arrest (approx. 3 hrs). Cells were release from G1 block by washing twice with 20 mL water and resuspending the pellet in YPD with 50 μg/mL pronase (Sigma). Divergent cell-cycle release conditions at 25^{o}C were made by aliquoting 10 mL of resulting cultures for hydroxyurea block (200 mM final), nocodazole block (10 μg/mL final), and using the remaining G1-released cultures for S-phase cell isolation. Cell-cycle arrest and/or progressions were monitored by FACS and budding and 5 mL of cultures were taken per time-point for TCA precipitation.

The preparation of the *cdc7-4* and *cdc4-1* temperature sensitive strains was as above except that after α–factor arrest, cells were shifted to 37^{o}C for 1 hr to inactivate Cdc4 and Cdc7 prior to α–factor release. Inactivation of *cdc4-1* or *cdc7-1* during S-phase release or in HU-blocked cells was initiated by resuspending the washed cell pellet with pre-warmed 37^{o}C YPD with pronase (with or without HU).

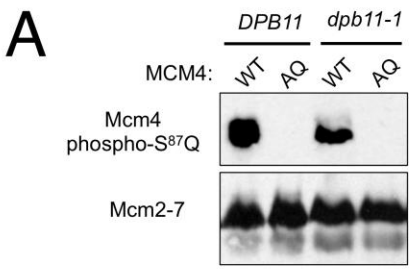
Randell et al., Figure S1, related to Figure 1



Randell et al., Figure S2, related to Figures 4 and 5



Randell et al., Figure S3, related to Figure 6



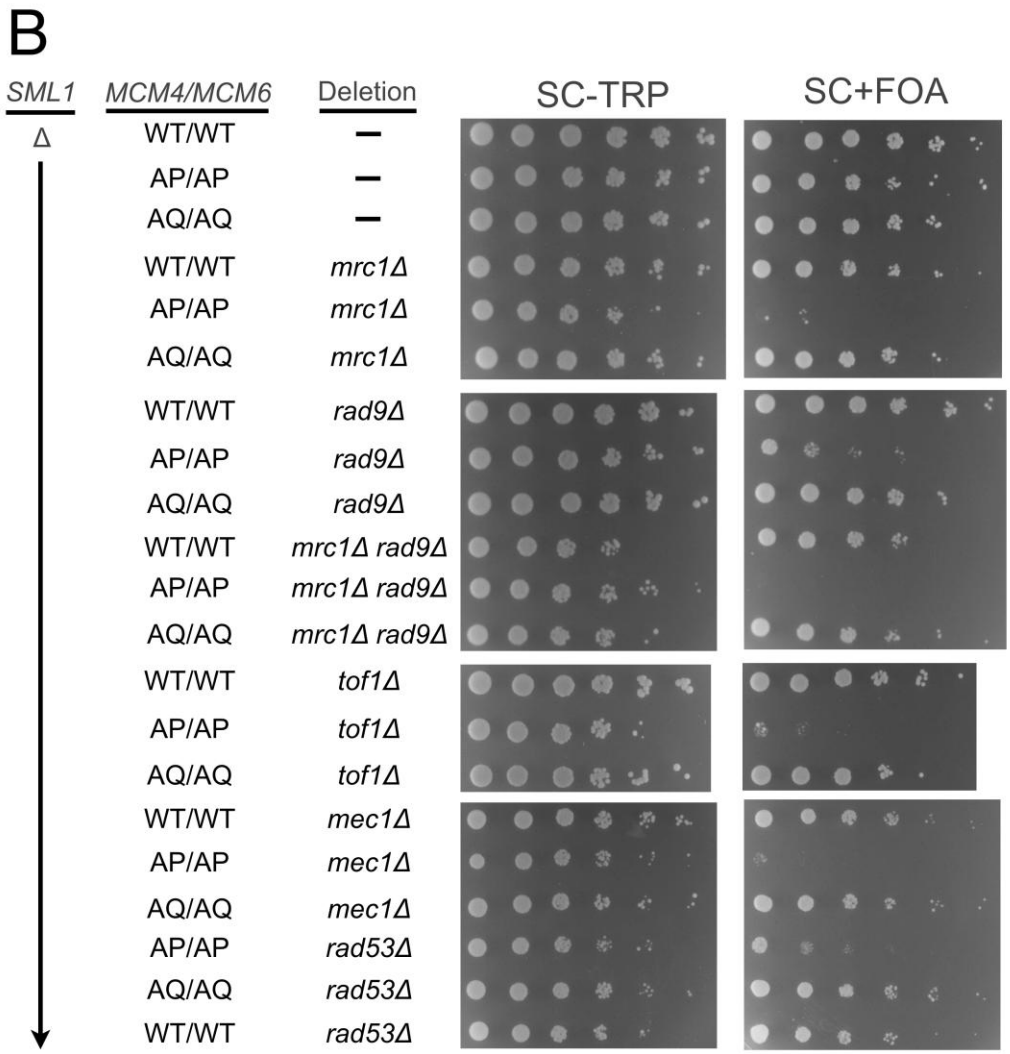


Figure S1. Sequence and Viability of Mutants in *MCM2*, *MCM4*, and *MCM6*, Related to Figure 1

Cells carrying the indicated alleles of *MCM2*, *MCM4*, or *MCM6* on a *TRP1/ARS/CEN* plasmid in addition to a wild-type copy of the same gene on a *URA3*/*ARS/CEN* plasmid were grown on uracil-containing media for 3-4 generations to allow loss of the *URA3* plasmid carrying the wild-type gene. In each case the endogenous copy of the indicated *MCM* gene was deleted in the tested strain. A five-fold serial dilution of cells was then spotted on plates without (left) or with (right) FOA selection against the *URA3* plasmid. For each gene, the second row is a control including a different MCM gene on the *TRP1/ARS/CEN* plasmid that does not complement the deletion.

In addition to the tested mutants, the sequences of several additional mutations that are tested in the manuscript are illustrated here. The number of residues that are mutated in each mutant is indicated in parentheses to the right of each mutant name.

Figure S2. Characterization of Phosphospecific Antibodies, Related to Figures 4 and 5

Pre-RC assembly was performed in the absence (lane 1) or presence (lanes 2, 3, and 4) of Cdc6. The resulting pre-RCs were treated with lambda phosphatase (lane 3) or DDK (lane 4) and were analyzed with anti-Mcm2-7 sera or phosphospecific antibodies generated against peptides containing the indicated phosphorylated residues. We suspect that the SQ phosphorylation of Mcm2-7 loaded using G1 extracts (Fig. S2) is the result of misregulation of the Mec1 kinase *in vitro* (perhaps due to the presence of linear DNA in the *in vitro* helicase loading assay).

Figure S3. Interactions between Checkpoint Genes/Proteins Mcm4 and Mcm6 Phosphorylation, Related to Figure 6

(A) A Dpb11 mutation does not eliminate Mcm4-S87-Q88 phosphorylation. Wild-type (lane 1), *mcm4-AQ* (lane 2), *dpb11-1* or *dpb11-1 mcm4-AQ* strains containing were grown to log phase then arrested in G1 with alpha factor at 25°C. Cells were then shifted to 37°C for 1 hr and then released from alpha factor for 35 min at 37°C followed by whole cell extract preparation. The resulting extracts were analyzed by immunoblotting for Mcm2-7 (top) and Mcm4-S87-Q88 phosphorylation (bottom).

(B) Deletion of *MRC1, RAD9, TOF1, MEC1 or RAD53* reduces proliferation in the absence of SP but not SQ phosphorylation of Mcm4 and Mcm6. Strains yJCWR200, yJCWR214, yJCWR220, yCC55, yCC58, yCC60, yCC45, yCC48, yCC51, yCC63, yCC68, yCC70, yCC79, yCC80, yCC81, yJCWR224, yJCWR219, and yJCWR222 were analyzed as in Fig. 2A.

Table S1. Mcm2-7 Phosphopeptides, related to Figure 1

The table shows the Mcm2-7 peptides that were identified as being modified with one or more phosphate. For the first set of phosphopeptides, the site(s) of phosphorylation was unambiguous. For the second set of peptides, the number of phosphates was determined but the sites of phosphorylation were ambiguous. The first column reports the sequence and location of each peptide, the second column indicates the Mcm subunit the peptide is derived from, the third column indicates the phosphorylated residues (unambiguous sites, shown first) or the number of phosphates found on the peptide (ambiguous, shown second) and the last column indicates whether the modification was observed with or without DDK treatment of Mcm2-7.

For the unambiguously identified sites of phosphorylation, the phosphorylated residues are followed by a "#" symbol. Peptides generated by Asp-N instead of trypsin digestion are indicated in bold type. M* indicates oxidized methionine.

For phosphopeptides for which the site of phosphorylation could not be unambiguously be identified (the last 10 peptides) the serines or threonines colored **red** represent the most likely sites of phosphorylation and the number of phosphorylated residues is indicated.

Table S2. Yeast Strains Used in This Study, related to figures 2-6

The table shows the strain name and genotype of each of the strains used in this study.

Table S1: Phosphorylated Peptides Detected by Mass Spectrometry, Related to Figure 1

Peptide (sites of phosphorylation are unambiguous)	**Subunit**	**Phosphorylated residue(s)**	**DDK dependent?**
RREEDDS#DSENELPPSSPQQHFR (8-30)	Mcm2	Ser14	N
RREEDDS#DS#ENELPPSSPQQHFR (8-30)	Mcm2	Ser14, Ser16	N
DRYDPDQVDDREQQELS#LSER (89-109)	Mcm2	Ser105	N
YDPDQVDDREQQELSLS#ER (91-109)	Mcm2	Ser107	Y
DRYDPDQVDDREQQELSLS#ER (89-109)	Mcm2	Ser107	Y
QYEDLENS#DDDLLSDM*DIDPLREELTLESLSNVK (157-190)	Mcm2	Ser164	N
QYEDLENS#DDDLLS#DM*DIDPLREELTLESLSNVK (157-190)	Mcm2	Ser164, Ser170	N
QYEDLENSDDDLLS#DM*DIDPLREELTLESLSNVK (157-190)	Mcm2	Ser170	N
NNGES#AIEQGEDEINEQLNAR (721-741)	Mcm2	Ser725	N
SHPENDEDREGEELKNNGES#AIEQGEDEINEQLNAR (706-741)	Mcm2	Ser725	N
DYT#DATTTLTTR (213-224)	Mcm3	Thr215	Y
FALLGEDIGNDIDEEES#EYEEALSK (735-759)	Mcm3	Ser751	N
RSPQKS#PK (760-767)	Mcm3	Ser765	N
SPQKS#PK(762-767)	Mcm3	Ser765	N
QPAS#NSGS#PIK (774-784)	Mcm3	Ser777, Ser781	N
VRQPAS#NSGS#PIK (772-784)	Mcm3	Ser777, Ser781	N
RST#ASS#VNATPSSAR (789-803)	Mcm3	Thr791, Ser794	Y
STASS#VNATPSSAR (790-803)	Mcm3	Ser794	N
STASSVNAT#PSS#AR (790-803)	Mcm3	Thr798, Ser801	N
FQDDEQNAGEDDNDIM*S#PLPADEEAELQR (808-836)	Mcm3	Ser824	N
LQLGLRVS#PR (838-847)	Mcm3	Ser845	N
EHLHAPEEGSSGPLTEVGT#PR (850-870)	Mcm3	Thr868	N
NNS#QNLSQGEGNIR (50-63)	Mcm4	Ser52	N
NNS#QNLS#QGEGNIR (50-63)	Mcm4	Ser52, Ser56	N
NNSQNLS#QGEGNIR (50-63)	Mcm4	Ser56	N
AAIGSS#PLNFPSSSQR (64-79)	Mcm4	Ser69	N
AAIGSSPLNFPSSS#QR (64-79)	Mcm4	Ser77	N
QNS#DVFQSQGR (80-90)	Mcm4	Ser82	Y
QNSDVFQS#QGR(80-90)	Mcm4	Ser87	N
YHS#DLRS#DR (106-114)	Mcm4	Ser108	Y
YHSDLRS#DR (106-114)	Mcm4	Ser112	Y
ALPTSSSS#LGR (115-125)	Mcm4	Ser122	N
NDIHT#S#DLSSPR (136-147)	Mcm4	Thr140, Ser141	Y
RNDIHT#S#DLSSPR (135-147)	Mcm4	Thr140, Ser141	Y
RNDIHTS#DLSSPR (135-147)	Mcm4	Ser141	Y
SGVNTLDTSSSS#APPSEASEPLR (156-178)	Mcm4	Ser167	N
SGVNTLDTSSSSAPPS#EASEPLR (156-178)	Mcm4	Ser171	Y
DTPSSNRPSNSS#PPPSSIGAGFGSSSGL (8-35)	Mcm6	Ser19	N
DTPSSNRPSNSS#PPPSSIGAGFGSSS#GL (8-35)	Mcm6	Ser19, Ser33	N
DSQIGSRLHFPSSSQPHVSNS#QTGPFVN (36-63)	Mcm6	Ser56	N
DSTQFSS#QRLQT (64-75)	Mcm6	Ser70	N
LHFPSSSQPHVSNSQTGPFVNDSTQFSS#QR (43-72)	Mcm6	Ser70	N
LQTDGS#ATNDM*EGNEPAR (73-90)	Mcm6	Ser78	N
LQT#DGS#ATNDM*EGNEPAR (73-90)	Mcm6	Thr75, Ser78	Y
DDDM*NGSS#LPR (220-230)	Mcm6	Ser227	N
SEGDEGQADEDEQQDDDM*NGSS#LPR (206-230)	Mcm6	Ser227	N
S#ITTSTSPEQTER (249-261)	Mcm6	Ser249	N
FTGVEIVVPDVT#QLGLPGVKPSSTLDTR (397-424)	Mcm6	Thr408	N
FLQGT#QADDLVSAIQQNANHFTELFCR (93-119)	Mcm7	Thr97	N
SLS#PGDIVDVTGIFLPAPYTGFK (342-364)	Mcm7	Ser344	N
FVDDGTM*DT#DQEDSLVSTPK (803-822)	Mcm7	Thr811	N
FVDDGTM*DT#DQEDSLVST#PK (803-822)	Mcm7	Thr811, Thr820	N
FVDDGTM*DTDQEDS#LVST#PK (803-822)	Mcm7	Ser816, Thr820	N
FVDDGTM*DTDQEDSLVST#PK (803-822)	Mcm7	Thr820	N
Peptide (sites of phosphorylation are ambiguous)	**Subunit**	**Number of phosphorylated residues**	**DDK dependent?**
VRQPA**S**N**S**G**S**PIK**ST**PR (772-788)	Mcm3	2	Y
VRQPA**S**N**S**G**S**PIK**ST**PR (772-788)	Mcm3	3	Y
FALLGEDIGNDIDEEE**S**E**Y**EEAL**S**KR**S**PQK**S**PK (735-767)	Mcm3	2	Y
AAIGSSPLNFP**SSS**QR (64-79)	Mcm4	2	Y
AAIG**SS**PLNFP**SSS**QR (64-79)	Mcm4	3	Y
IR**SS**A**S**ASGR (94-103)	Mcm4	2	Y
DSQIGSRLHFP**SSS**QPHV**S**N**S**QTGPFVN (36-63)	Mcm6	2	N
D**S**QIG**S**RLHFP**SSS**QPHV**S**N**S**QTGPFVN (36-63)	Mcm6	3	N
LHFPSSSQPHV**S**N**S**QTGPFVND**ST**QF**SS**QR (43-72)	Mcm6	2	Y
LHFP**SSS**QPHV**S**N**S**QTGPFVND**ST**QF**SS**QR (43-72)	Mcm6	3	Y

Table S2. Yeast Strains Used in This Study, related to figures 2-6

Strain	**Genotype**	**Source**
W303BLa	*ade2-1 ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100 lys2::hisG bar1::hisG MATa*	Lab stock
OAy470	*ade2-1 ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100 MATa bar1::hisG*	Aparicio et al. (1997)
OAy711	*cdc7-4 his7 ura1 ura3-1 ade2-1 his3-11,15 leu2-3*	Gift of O. Aparicio
yJCWR30	*cdc7-4 his7 ura1 ura3-1 ade2-1 his3-11,15 leu2-3 mcm5-bob1*	This study
yJCWR150	*ade2-1 ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100 MATa bar1::hisG cdc7-4 mcm4::natMX4-PMCM5-MCM4[DP+DQ]*	This study
yJCWR151	*ade2-1 ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100 MATa bar1::hisG cdc7-4 mcm4::natMX4-PMCM5-MCM4[D(D/E)]*	This study
yJCWR152	*ade2-1 ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100 MATa bar1::hisG cdc7-4 mcm4::natMX4-PMCM5-MCM4[DS(P/Q)]*	This study
yJCWR153	*ade2-1 ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100 MATa bar1::hisG cdc7-4 mcm4::natMX4-PMCM5-MCM4[D(D/E)+DS(P/Q)]*	This study
yJCWR185	*MATa ade2-1 ura3-11 his3-11,15 leu2-3,12 can1-100 trp1-1 mcm4Δ::hisG pAS412 (ARS/CEN URA+ MCM4-HA/his) pJR121 (ARS/CEN TRP+ MCM4-HA/his) mcm6::LEU2-PMCM5-MCM6*	This study
yJCWR176	*MATa ade2-1 ura3-11 his3-11,15 leu2-3,12 can1-100 trp1-1 mcm4Δ::hisG pAS412 (ARS/CEN URA+ MCM4-HA/his) pJR139 (ARS/CEN TRP+ mcm4-HA/his[A(D/E)]) mcm6::LEU2-PMCM5-mcm6[A(D/E)]*	This study
yJCWR180	*MATa ade2-1 ura3-11 his3-11,15 leu2-3,12 can1-100 trp1-1 mcm4Δ::hisG pAS412 (ARS/CEN URA+ MCM4-HA/his) pJR189 (ARS/CEN TRP+ mcm4-HA/his[AS(P/Q)]) mcm6::LEU2-PMCM5-mcm6[AS(P/Q)]*	This study
yJCWR184	*MATa ade2-1 ura3-11 his3-11,15 leu2-3,12 can1-100 trp1-1 mcm4Δ::hisG pAS412 (ARS/CEN URA+ MCM4-HA/his) pJR140 (ARS/CEN TRP+ mcm4-HA/his[A(D/E)+AS(P/Q)]) mcm6::LEU2-PMCM5-mcm6[A(D/E)+AS(P/Q)]*	This study
yJCWR167	*MATa ade2-1 ura3-11 his3-11,15 leu2-3,12 can1-100 trp1-1 mcm4Δ::hisG pAS412 (ARS/CEN URA+ MCM4-HA/his) pJR136 (ARS/CEN TRP+ mcm4-HA/his[AP]) mcm6::LEU2-PMCM5-mcm6[AP]*	This study
yJCWR171	*MATa ade2-1 ura3-11 his3-11,15 leu2-3,12 can1-100 trp1-1 mcm4Δ::hisG pAS412 (ARS/CEN URA+ MCM4-HA/his) pJR137 (ARS/CEN TRP+ mcm4-HA/his[AQ]) mcm6::LEU2-PMCM5-mcm6[AQ]*	This study
yJCWR175	*MATa ade2-1 ura3-11 his3-11,15 leu2-3,12 can1-100 trp1-1 mcm4Δ::hisG pAS412 (ARS/CEN URA+ MCM4-HA/his) pJR138 (ARS/CEN TRP+ mcm4-HA/his[AP+AQ]) mcm6::LEU2-PMCM5-mcm6[AP+AQ]*	This study

yJCWR236	*MATa ade2-1 ura3-11 his3-11,15 leu2-3,12 can1-100 trp1-1 mcm4Δ::hisG pAS412 (ARS/CEN URA+ MCM4-HA/his) pJR194 (ARS/CEN TRP+ mcm4-HA/his[DA(P/Q)+D(D/E)]) mcm6::LEU2-PMCM5-mcm6[AP+AQ]*	This study
yJCWR237	*MATa ade2-1 ura3-11 his3-11,15 leu2-3,12 can1-100 trp1-1 mcm4Δ::hisG pAS412 (ARS/CEN URA+ MCM4-HA/his) pJR194 (ARS/CEN TRP+ mcm4-HA/his[DA(P/Q)+D(D/E)]) mcm6::LEU2-PMCM5-mcm6[DA(P/Q)+D(D/E)]*	This study
yJCWR128	*MATa ade2-1 ura3-11 his3-11,15 leu2-3,12 can1-100 trp1-1 lys2Δ::hisG bar1Δ::hisG pep4Δ::KanMX4 mcm6Δ::HisMX6 pAS438 (TRP+ MCM6-HA/his)*	This study
yJCWR129	*MATa ade2-1 ura3-11 his3-11,15 leu2-3,12 can1-100 trp1-1 lys2Δ::hisG bar1Δ::hisG pep4Δ::KanMX4 mcm6Δ::HisMX6 pJR143 (TRP+ mcm6-HA/his[AP])*	This study
yJCWR130	*MATa ade2-1 ura3-11 his3-11,15 leu2-3,12 can1-100 trp1-1 lys2Δ::hisG bar1Δ::hisG pep4Δ::KanMX4 mcm6Δ::HisMX6 pJR144 (TRP+ mcm6-HA/his[SQ])*	This study
yJCWR135	*MATa ade2-1 ura3-11 his3-11,15 leu2-3,12 can1-100 trp1-1 lys2Δ::hisG bar1Δ::hisG pep4Δ::KanMX4 mcm6Δ::HisMX6 pJR149 (TRP+ mcm6-HA/his[A(D/E)+AS(P/Q)]*	This study
ySC15	*MATa ade2-1 ura3-11 his3-11,15 can1-100 trp1-1 lys2::hisG bar1::hisG orc1::hisG leu2::ORC1 trp1::p404-GAL1-10-ORC3,4 lys2::plys2-GAL1-10-ORC2,5 his3::p403-GAL1-10-ORC1,6 pep4::KanMX*	Bowers et al. (2004)
yJCWR200	*MATa ade2-1 ura3-11 his3-11,15 leu2-3,12 can1-100 trp1-1 mcm4Δ::hisG pAS412 (ARS/CEN URA+ MCM4-HA/his) pJR121 (ARS/CEN TRP+ MCM4-HA/his) mcm6::LEU2-PMCM5-MCM6 sml1Δ::His3MX6*	This study
yJCWR205	*MATa ade2-1 ura3-11 his3-11,15 leu2-3,12 can1-100 trp1-1 mcm4::hisG pJR121 (ARS/CEN TRP+ MCM4-HA/his) mcm6::LEU2-PMCM5-MCM6*	This study
yJCWR206	*MATa ade2-1 ura3-11 his3-11,15 leu2-3,12 can1-100 trp1-1 mcm4::hisG pJR136 (ARS/CEN TRP+ mcm4-HA/his[AP]) mcm6::LEU2-PMCM5-mcm6[AP]*	This study
yJCWR207	*MATa ade2-1 ura3-11 his3-11,15 leu2-3,12 can1-100 trp1-1 mcm4::hisG pJR137 (ARS/CEN TRP+ mcm4-HA/his[AQ]) mcm6::LEU2-PMCM5-mcm6[AQ]*	This study
yJCWR208	*MATa ade2-1 ura3-11 his3-11,15 leu2-3,12 can1-100 trp1-1 mcm4::hisG pJR138 (ARS/CEN TRP+ mcm4-HA/his[AP+AQ]) mcm6::LEU2-PMCM5-mcm6[AP+AQ]*	This study
yJCWR220	*MATa ade2-1 ura3-11 his3-11,15 leu2-3,12 can1-100 trp1-1 mcm4::hisG pAS412 (ARS/CEN URA+ MCM4-HA/his) pJR137 (ARS/CEN TRP+ mcm4-HA/his[AQ]) mcm6::LEU2-PMCM5-mcm6[AQ] sml1Δ::His3MX6*	This study

yRH109	*MATa ade2-1 ura3-11 his3-11,15 can1-100 trp1-1 lys2::hisG bar1::HisG orc1::HisG leu2::ORC1 trp1::p404-GAL1-10-ORC3,4 lys2::plys2-GAL1-10-ORC2,5 his3::p403-GAL1-10-ORC1,6 pep4::KanMX Mcm10-13myc::natMX4 sml1::MX3*	This study
yRH110	*MATa ade2-1 ura3-11 his3-11,15 can1-100 trp1-1 lys2::hisG bar1::HisG orc1::HisG leu2::ORC1 trp1::p404-GAL1-10-ORC3,4 lys2::plys2-GAL1-10-ORC2,5 his3::p403-GAL1-10-ORC1,6 pep4::KanMX Mcm10-13myc::natMX4 sml1::MX3 mec1::MX3*	This study
yJCWR78	*MATa ade2-1 ura3-1 his3-11,15 can1-100 bar1::HisG orc1::HisG leu2::ORC1 trp1::p404-GAL1-10-ORC3,4 lys2::plys2-GAL1-10-ORC2,5 his3::p403-GAL1-10-ORC1,6 pep4::KanMX Mcm10-13myc::natMX4 sml1::MX3 tel1::MX3*	This study
yJCWR79	*MATa ade2-1 ura3-1 his3-11,15 can1-100 bar1::HisG orc1::HisG leu2::ORC1 trp1::p404-GAL1-10-ORC3,4 lys2::plys2-GAL1-10-ORC2,5 his3::p403-GAL1-10-ORC1,6 pep4::KanMX Mcm10-13myc::natMX4 sml1::MX3 mec1::MX3 tel1::MX3*	This study
yJCWR214	*MATa ade2-1 ura3-11 his3-11,15 leu2-3,12 can1-100 trp1-1 mcm4::hisG pAS412 (ARS/CEN URA+ MCM4-HA/his) pJR136 (ARS/CEN TRP+ mcm4-HA/his[AP]) mcm6::LEU2-PMCM5-mcm6[AP] sml1Δ::His3MX6*	This study
yJCWR216	*MATa ade2-1 ura3-11 his3-11,15 leu2-3,12 can1-100 trp1-1 mcm4::hisG pAS412 (ARS/CEN URA+ MCM4-HA/his) pJR136 (ARS/CEN TRP+ mcm4-HA/his[AP]) mcm6::LEU2-PMCM5-mcm6[AP] sml1Δ::His3MX6 mec1Δ::KanMX*	This study
yJCWR217	*MATa ade2-1 ura3-11 his3-11,15 leu2-3,12 can1-100 trp1-1 mcm4::hisG pAS412 (ARS/CEN URA+ MCM4-HA/his) pJR136 (ARS/CEN TRP+ mcm4-HA/his[AP]) mcm6::LEU2-PMCM5-mcm6[AP] sml1Δ::His3MX6 tel1Δ::natMX4*	This study
yJCWR218	*MATa ade2-1 ura3-11 his3-11,15 leu2-3,12 can1-100 trp1-1 mcm4::hisG pAS412 (ARS/CEN URA+ MCM4-HA/his) pJR136 (ARS/CEN TRP+ mcm4-HA/his[AP]) mcm6::LEU2-PMCM5-mcm6[AP] sml1Δ::His3MX6 mec1Δ::kanMX tel1Δ::natMX4*	This study
yJCWR221	*MATa ade2-1 ura3-11 his3-11,15 leu2-3,12 can1-100 trp1-1 mcm4Δ::hisG pAS412 (ARS/CEN URA+ MCM4-HA/his) pJR137 (ARS/CEN TRP+ mcm4-HA/his[AQ]) mcm6::LEU2-PMCM5-mcm6[AQ] sml1Δ::His3MX6 mec1Δ::kanMX*	This study
yJCWR223	*MATa ade2-1 ura3-11 his3-11,15 leu2-3,12 can1-100 trp1-1 mcm4Δ::hisG pAS412 (ARS/CEN URA+ MCM4-HA/his) pJR121 (ARS/CEN TRP+ MCM4-HA/his) mcm6::LEU2-PMCM5-MCM6 sml1Δ::His3MX6 mec1Δ::kanMX*	This study
yJCWR80	*MATa ade2-1 ura3-11 his3-11,15 leu2-3,12 can1-100 trp1-1 mcm2::hisG pAS404 (ARS/CEN URA+ MCM2) pJR120 (ARS/CEN TRP+ MCM2)*	This study

yJCWR81	*MATa ade2-1 ura3-11 his3-11,15 leu2-3,12 can1-100 trp1-1 mcm2::hisG pAS404 (ARS/CEN URA+ MCM2) pJR121 (ARS/CEN TRP+ MCM4)*	This study
yJCWR82	*MATa ade2-1 ura3-11 his3-11,15 leu2-3,12 can1-100 trp1-1 mcm2::hisG pAS404 (ARS/CEN URA+ MCM2) pJR130 (ARS/CEN TRP+ mcm2[AP3])*	This study
yJCWR83	*MATa ade2-1 ura3-11 his3-11,15 leu2-3,12 can1-100 trp1-1 mcm2::hisG pAS404 (ARS/CEN URA+ MCM2) pJR131 (ARS/CEN TRP+ mcm2[SP3+SQ1])*	This study
yJCWR84	*MATa ade2-1 ura3-11 his3-11,15 leu2-3,12 can1-100 trp1-1 mcm2::hisG pAS404 (ARS/CEN URA+ MCM2) pJR132 (ARS/CEN TRP+ mcm2[CK2-4])*	This study
yJCWR85	*MATa ade2-1 ura3-11 his3-11,15 leu2-3,12 can1-100 trp1-1 mcm2::hisG pAS404 (ARS/CEN URA+ MCM2) pJR133 (ARS/CEN TRP+ mcm2[DDK4])*	This study
yJCWR86	*MATa ade2-1 ura3-11 his3-11,15 leu2-3,12 can1-100 trp1-1 mcm2::hisG pAS404 (ARS/CEN URA+ MCM2) pJR134 (ARS/CEN TRP+ mcm2[DDK4+SXP2])*	This study
yJCWR87	*MATa ade2-1 ura3-11 his3-11,15 leu2-3,12 can1-100 trp1-1 mcm2::hisG pAS404 (ARS/CEN URA+ MCM2) pJR135 (ARS/CEN TRP+ mcm2-13A)*	This study
yJCWR88	*MATa ade2-1 ura3-11 his3-11,15 leu2-3,12 can1-100 trp1-1 mcm4::hisG pAS412 (ARS/CEN URA+ MCM4-HA/his) pJR121 (ARS/CEN TRP+ MCM4-HA/his)*	This study
yJCWR89	*MATa ade2-1 ura3-11 his3-11,15 leu2-3,12 can1-100 trp1-1 mcm4::hisG pAS412 (ARS/CEN URA+ MCM4-HA/his) pAS438 (ARS/CEN TRP+MCM6-HA/his)*	This study
yJCWR90	*MATa ade2-1 ura3-11 his3-11,15 leu2-3,12 can1-100 trp1-1 mcm4::hisG pAS412 (ARS/CEN URA+ MCM4-HA/his) pJR136 (ARS/CEN TRP+ mcm4-HA/his[AP])*	This study
yJCWR91	*MATa ade2-1 ura3-11 his3-11,15 leu2-3,12 can1-100 trp1-1 mcm4::hisG pAS412 (ARS/CEN URA+ MCM4-HA/his) pJR137 (ARS/CEN TRP+ mcm4-HA/his[AQ])*	This study
yJCWR92	*MATa ade2-1 ura3-11 his3-11,15 leu2-3,12 can1-100 trp1-1 mcm4::hisG pAS412 (ARS/CEN URA+ MCM4-HA/his) pJR138 (ARS/CEN TRP+ mcm4-HA/his[AP+AQ])*	This study
yJCWR93	*MATa ade2-1 ura3-11 his3-11,15 leu2-3,12 can1-100 trp1-1 mcm4::hisG pAS412 (ARS/CEN URA+ MCM4-HA/his) pJR139 (ARS/CEN TRP+ mcm4-HA/his[A(D/E)])*	This study
yJCWR94	*MATa ade2-1 ura3-11 his3-11,15 leu2-3,12 can1-100 trp1-1 mcm4::hisG pAS412 (ARS/CEN URA+ MCM4-HA/his) pJR140 (ARS/CEN TRP+ mcm4-HA/his[A(D/E)+AS(P/Q)])*	This study
yJCWR95	*MATa ade2-1 ura3-11 his3-11,15 leu2-3,12 can1-100 trp1-1 mcm4::hisG pAS412 (ARS/CEN URA+ MCM4-HA/his) pJR141 (ARS/CEN TRP+ mcm4-HA/his[25A])*	This study

yJCWR96	*MATa ade2-1 ura3-11 his3-11,15 leu2-3,12 can1-100 trp1-1 mcm4::hisG pAS412 (ARS/CEN URA+ MCM4-HA/his) pJR142 (ARS/CEN TRP+ mcm4-HA/his[43A])*	This study
yJCWR97	*MATa ade2-1 ura3-11 his3-11,15 leu2-3,12 can1-100 trp1-1 lys2::hisG bar1::hisG pep4::KanMX mcm6Δ::HisMX6 pAS452 (ARS/CEN URA+ MCM6-HA/his) pAS438 (ARS/CEN TRP+ MCM6-HA/his)*	This study
yJCWR98	*MATa ade2-1 ura3-11 his3-11,15 leu2-3,12 can1-100 trp1-1 lys2::hisG bar1::hisG pep4::KanMX mcm6Δ::HisMX6 pAS452 (ARS/CEN URA+ MCM6-HA/his) pJR120 (ARS/CEN TRP+ MCM2)*	This study
yJCWR99	*MATa ade2-1 ura3-11 his3-11,15 leu2-3,12 can1-100 trp1-1 lys2::hisG bar1::hisG pep4::KanMX mcm6Δ::HisMX6 pAS452 (ARS/CEN URA+ MCM6-HA/his) pJR143 (ARS/CEN TRP+ mcm6-HA/his[AP])*	This study
yJCWR100	*MATa ade2-1 ura3-11 his3-11,15 leu2-3,12 can1-100 trp1-1 lys2::hisG bar1::hisG pep4::KanMX mcm6Δ::HisMX6 pAS452 (ARS/CEN URA+ MCM6-HA/his) pJR144 (ARS/CEN TRP+ mcm6-HA/his[AQ])*	This study
yJCWR101	*MATa ade2-1 ura3-11 his3-11,15 leu2-3,12 can1-100 trp1-1 lys2::hisG bar1::hisG pep4::KanMX mcm6Δ::HisMX6 pAS452 (ARS/CEN URA+ MCM6-HA/his) pJR145 (ARS/CEN TRP+ mcm6-HA/his[AQ5+AA4+8])*	This study
yJCWR102	*MATa ade2-1 ura3-11 his3-11,15 leu2-3,12 can1-100 trp1-1 lys2::hisG bar1::hisG pep4::KanMX mcm6Δ::HisMX6 pAS452 (ARS/CEN URA+ MCM6-HA/his) pJR145 (ARS/CEN TRP+ mcm6-HA/his[AP3+AQ5+AA4+10])*	This study
yJCWR103	*MATa ade2-1 ura3-11 his3-11,15 leu2-3,12 can1-100 trp1-1 lys2::hisG bar1::hisG pep4::KanMX mcm6Δ::HisMX6 pAS452 (ARS/CEN URA+ MCM6-HA/his) pJR147 (ARS/CEN TRP+ mcm6-HA/his[A(D/E)3]*	This study
yJCWR104	*MATa ade2-1 ura3-11 his3-11,15 leu2-3,12 can1-100 trp1-1 lys2::hisG bar1::hisG pep4::KanMX mcm6Δ::HisMX6 pAS452 (ARS/CEN URA+ MCM6-HA/his) pJR148 (ARS/CEN TRP+ mcm6-HA/his[AS(P/Q)8]*	This study
yJCWR105	*MATa ade2-1 ura3-11 his3-11,15 leu2-3,12 can1-100 trp1-1 lys2::hisG bar1::hisG pep4::KanMX mcm6Δ::HisMX6 pAS452 (ARS/CEN URA+ MCM6-HA/his) pJR149 (ARS/CEN TRP+ mcm6-HA/his[A(D/E)3+AS(P/Q)8]*	This study
yJCWR106	*MATa ade2-1 ura3-11 his3-11,15 leu2-3,12 can1-100 trp1-1 lys2::hisG bar1::hisG pep4::KanMX mcm6Δ::HisMX6 pAS452 (ARS/CEN URA+ MCM6-HA/his) pJR150 (ARS/CEN TRP+ mcm6-HA/his[25A])*	This study

yCC45	*MATa ade2-1 ura3-11 his3-11,15 leu2-3,12 can1-100 trp1-1 mcm4Δ::hisG pAS412 (ARS/CEN URA+ MCM4-HA/his) pJR121 (ARS/CEN TRP+ MCM4-HA/his) mcm6::LEU2-PMCM5-MCM6 sml1Δ::His3MX6 rad9Δ::natMX4*	This study
yCC48	*MATa ade2-1 ura3-11 his3-11,15 leu2-3,12 can1-100 trp1-1 mcm4::hisG pAS412 (ARS/CEN URA+ MCM4-HA/his) pJR136 (ARS/CEN TRP+ mcm4-HA/his[AP]) mcm6::LEU2-PMCM5-mcm6[AP] sml1Δ::His3MX6 rad9Δ::natMX4*	This study
yCC51	*MATa ade2-1 ura3-11 his3-11,15 leu2-3,12 can1-100 trp1-1 mcm4::hisG pAS412 (ARS/CEN URA+ MCM4-HA/his) pJR137 (ARS/CEN TRP+ mcm4-HA/his[AQ]) mcm6::LEU2-PMCM5-mcm6[AQ] sml1Δ::His3MX6 rad9Δ::natMX4*	This study
yCC55	*MATa ade2-1 ura3-11 his3-11,15 leu2-3,12 can1-100 trp1-1 mcm4Δ::hisG pAS412 (ARS/CEN URA+ MCM4-HA/his) pJR121 (ARS/CEN TRP+ MCM4-HA/his) mcm6::LEU2-PMCM5-MCM6 sml1Δ::His3MX6 mrc1Δ::KanMX*	This study
yCC58	*MATa ade2-1 ura3-11 his3-11,15 leu2-3,12 can1-100 trp1-1 mcm4::hisG pAS412 (ARS/CEN URA+ MCM4-HA/his) pJR136 (ARS/CEN TRP+ mcm4-HA/his[AP]) mcm6::LEU2-PMCM5-mcm6[AP] sml1Δ::His3MX6 mrc1Δ::KanMX*	This study
yCC60	*MATa ade2-1 ura3-11 his3-11,15 leu2-3,12 can1-100 trp1-1 mcm4::hisG pAS412 (ARS/CEN URA+ MCM4-HA/his) pJR137 (ARS/CEN TRP+ mcm4-HA/his[AQ]) mcm6::LEU2-PMCM5-mcm6[AQ] sml1Δ::His3MX6 mrc1Δ::KanMX*	This study
yCC63	*MATa ade2-1 ura3-11 his3-11,15 leu2-3,12 can1-100 trp1-1 mcm4Δ::hisG pAS412 (ARS/CEN URA+ MCM4-HA/his) pJR121 (ARS/CEN TRP+ MCM4-HA/his) mcm6::LEU2-PMCM5-MCM6 sml1Δ::His3MX6 rad9Δ::natMX4 mrc1Δ::KanMX*	This study
yCC68	*MATa ade2-1 ura3-11 his3-11,15 leu2-3,12 can1-100 trp1-1 mcm4::hisG pAS412 (ARS/CEN URA+ MCM4-HA/his) pJR136 (ARS/CEN TRP+ mcm4-HA/his[AP]) mcm6::LEU2-PMCM5-mcm6[AP] sml1Δ::His3MX6 rad9Δ::natMX4 mrc1Δ::KanMX*	This study
yCC70	*MATa ade2-1 ura3-11 his3-11,15 leu2-3,12 can1-100 trp1-1 mcm4::hisG pAS412 (ARS/CEN URA+ MCM4-HA/his) pJR137 (ARS/CEN TRP+ mcm4-HA/his[AQ]) mcm6::LEU2-PMCM5-mcm6[AQ] sml1Δ::His3MX6 rad9Δ::natMX4 mrc1Δ::KanMX*	This study
yCC73	*MATa ade2-1 ura3-11 his3-11,15 leu2-3,12 can1-100 trp1-1 mcm4Δ::hisG pAS412 (ARS/CEN URA+ MCM4-HA/his) pJR138 (ARS/CEN TRP+ mcm4-HA/his[AP+AQ]) mcm6::LEU2-PMCM5-mcm6[AP+AQ] mrc1Δ::natMX4*	This study

yCC75	*MATa ade2-1 ura3-11 his3-11,15 leu2-3,12 can1-100 trp1-1 mcm4Δ::hisG pAS412 (ARS/CEN URA+ MCM4-HA/his) pJR194 (ARS/CEN TRP+ mcm4-HA/his[DA(P/Q)+D(D/E)]) mcm6::LEU2-PMCM5-mcm6[AP+AQ] mrc1Δ::natMX4*	This study
yCC77	*MATa ade2-1 ura3-11 his3-11,15 leu2-3,12 can1-100 trp1-1 mcm4Δ::hisG pAS412 (ARS/CEN URA+ MCM4-HA/his) pJR194 (ARS/CEN TRP+ mcm4-HA/his[DA(P/Q)+D(D/E)]) mcm6::LEU2-PMCM5-mcm6[DA(P/Q)+D(D/E)] mrc1Δ::natMX4*	This study
yCC79	*MATa ade2-1 ura3-11 his3-11,15 leu2-3,12 can1-100 trp1-1 mcm4Δ::hisG pAS412 (ARS/CEN URA+ MCM4-HA/his) pJR121 (ARS/CEN TRP+ MCM4-HA/his) mcm6::LEU2-PMCM5-MCM6 sml1Δ::His3MX6 tof1Δ::KanMX*	This study
yCC80	*MATa ade2-1 ura3-11 his3-11,15 leu2-3,12 can1-100 trp1-1 mcm4::hisG pAS412 (ARS/CEN URA+ MCM4-HA/his) pJR136 (ARS/CEN TRP+ mcm4-HA/his[AP]) mcm6::LEU2-PMCM5-mcm6[AP] sml1Δ::His3MX6 tof1Δ::KanMX*	This study
yCC81	*MATa ade2-1 ura3-11 his3-11,15 leu2-3,12 can1-100 trp1-1 mcm4::hisG pAS412 (ARS/CEN URA+ MCM4-HA/his) pJR137 (ARS/CEN TRP+ mcm4-HA/his[AQ]) mcm6::LEU2-PMCM5-mcm6[AQ] sml1Δ::His3MX6 tof1Δ::KanMX*	This study
yCC29	*ade2-1 trp1-1 leu2-3,112 his3-11,15 ura3-1 can1-100 Delta-bar1 lys2::HisG pep4::KanMX mcm4::natMX4MX4-PMCM5-mcm4[AQ] mcm6::LEU2-PMCM5-mcm6[AQ]*	This study
yCC98	*MATa ade2-1 ura3-11 his3-11,15 leu2-3,12 can1-100 trp1-1 mcm4Δ::hisG pJR121 (ARS/CEN TRP+ MCM4-HA/his) mcm6::LEU2-PMCM5-MCM6 sml1Δ::His3MX6 mrc1Δ::KanMX*	This study
yCC100	*MATa ade2-1 ura3-11 his3-11,15 leu2-3,12 can1-100 trp1-1 mcm4Δ::hisG pJR137 (ARS/CEN TRP+ mcm4-HA/his[AQ]) mcm6::LEU2-PMCM5-mcm6[AQ] sml1Δ::His3MX6 mrc1Δ::KanMX*	This study
yCC90	*MATa ade2-1 ura3-11 his3-11,15 leu2-3,12 can1-100 trp1-1 mcm4Δ::hisG pAS412 (ARS/CEN URA+ MCM4-HA/his) pJR138 (ARS/CEN TRP+ mcm4-HA/his[AP+AQ]) mcm6::LEU2-PMCM5-mcm6[AP+AQ] sml1Δ::His3MX6 mec1Δ::natMX4*	This study
yCC94	*MATa ade2-1 ura3-11 his3-11,15 leu2-3,12 can1-100 trp1-1 mcm4Δ::hisG pAS412 (ARS/CEN URA+ MCM4-HA/his) pJR194 (ARS/CEN TRP+ mcm4-HA/his[DA(P/Q)+D(D/E)]) mcm6::LEU2-PMCM5-mcm6[AP+AQ] sml1Δ::His3MX6 mec1Δ::natMX4*	This study

yCC95	*MATa ade2-1 ura3-11 his3-11,15 leu2-3,12 can1-100 trp1-1 mcm4Δ::hisG pAS412 (ARS/CEN URA+ MCM4-HA/his) pJR194 (ARS/CEN TRP+ mcm4-HA/his[DA(P/Q)+D(D/E)]) mcm6::LEU2-PMCM5-mcm6[DA(P/Q)+D(D/E)] sml1Δ::His3MX6 mec1Δ::natMX4*	This study
yJCWR224	*MATa ade2-1 ura3-11 his3-11,15 leu2-3,12 can1-100 trp1-1 mcm4Δ::hisG pAS412 (ARS/CEN URA+ MCM4-HA/his) pJR121 (ARS/CEN TRP+ MCM4-HA/his) mcm6::LEU2-PMCM5-MCM6 sml1Δ::His3MX6 rad53Δ::kanMX*	This study
yJCWR219	*MATa ade2-1 ura3-11 his3-11,15 leu2-3,12 can1-100 trp1-1 mcm4::hisG pAS412 (ARS/CEN URA+ MCM4-HA/his) pJR136 (ARS/CEN TRP+ mcm4-HA/his[AP]) mcm6::LEU2-PMCM5-mcm6[AP] sml1Δ::His3MX6 rad53Δ::kanMX*	This study
yJCWR222	*MATa ade2-1 ura3-11 his3-11,15 leu2-3,12 can1-100 trp1-1 mcm4::hisG pAS412 (ARS/CEN URA+ MCM4-HA/his) pJR137 (ARS/CEN TRP+ mcm4-HA/his[AQ]) mcm6::LEU2-PMCM5-mcm6[AQ] sml1Δ::His3MX6 rad53Δ::kanMX*	This study
yCC114	*MATa ade2-1 ura3-11 his3-11,15 leu2-3,12 can1-100 trp1-1 mcm4Δ::hisG pAS412 (ARS/CEN URA+ MCM4-HA/his) pJR138 (ARS/CEN TRP+ mcm4-HA/his[AP+AQ]) mcm6::LEU2-PMCM5-mcm6[AP+AQ] sml1Δ::His3MX6 rad53Δ::natMX4*	This study
yCC116	*MATa ade2-1 ura3-11 his3-11,15 leu2-3,12 can1-100 trp1-1 mcm4Δ::hisG pAS412 (ARS/CEN URA+ MCM4-HA/his) pJR194 (ARS/CEN TRP+ mcm4-HA/his[DA(P/Q)+D(D/E)]) mcm6::LEU2-PMCM5-mcm6[AP+AQ] sml1Δ::His3MX6 rad53Δ::natMX4*	This study
yCC117	*MATa ade2-1 ura3-11 his3-11,15 leu2-3,12 can1-100 trp1-1 mcm4Δ::hisG pAS412 (ARS/CEN URA+ MCM4-HA/his) pJR194 (ARS/CEN TRP+ mcm4-HA/his[DA(P/Q)+D(D/E)]) mcm6::LEU2-PMCM5-mcm6[DA(P/Q)+D(D/E)] sml1Δ::His3MX6 rad53Δ::natMX4*	This study