

Appendix – Table of Contents:

CMG helicase disassembly is essential and mediated by two pathways in budding yeast

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Appendix Table S1

Mass spectrometry data showing that CMG and associated replisome components are the major partners of SCF^{Dia2}, largely dependent on the TPR domain of Dia2. Also see Dataset EV1.

This is the first of two independent experiments (the other is in Appendix Table S2).

Protein	Complex	Spectral counts (control)	Spectral counts (TAP-Sld5)	Spectral counts (ProteinA-Dia2)	Spectral counts (ProteinA-Dia2-ΔTPR)
Dia2 (85kDa)	SCF ^{Dia2}	85	404	4585	2919
Cdc53 (94kDa)	SCF ^{Dia2}	19	351	3008	2938
Skp1 (22kDa)	SCF ^{Dia2}	3	85	1802	1440
Hrt1 (14kDa)	SCF ^{Dia2}	4	20	134	205
Rub1 (9kDa)	SCF ^{Dia2} (NEDD8)	2	17	173	222
Psf1 (24kDa)	CMG (GINS)	15	321	94	11
Psf2 (25kDa)	CMG (GINS)	20	269	66	5
Psf3 (22kDa)	CMG (GINS)	0	194	97	10
Sld5 (34kDa)	CMG (GINS)	2	521	205	24
Cdc45 (74kDa)	CMG	3	551	415	17
Mcm2 (99kDa)	CMG (Mcm2-7)	14	1428	1003	42
Mcm3 (107kDa)	CMG (Mcm2-7)	6	1475	1301	47
Mcm4 (105kDa)	CMG (Mcm2-7)	13	1667	1100	40
Mcm5 (86kDa)	CMG (Mcm2-7)	12	973	670	40
Mcm6 (113kDa)	CMG (Mcm2-7)	13	1550	916	26
Mcm7 (95kDa)	CMG (Mcm2-7)	5	979	794	42
Ctf4 (104kDa)	Replisome	21	2244	2640	68

Tof1 (141kDa)	Replisome (Tof1- Csm3)	4	1224	960	43
Csm3 (36kDa)	Replisome (Tof1- Csm3)	0	140	211	6
Mrc1 (124kDa)	Replisome	2	1483	1225	68
Top1 (90kDa)	Replisome	0	357	181	3
Spt16 (119kDa)	Replisome (FACT)	208	1705	934	217
Pob3 (63kDa)	Replisome (FACT)	78	729	332	92
Pol2 (256kDa)	Pol ϵ	8	352	100	0
Dpb2 (78kDa)	Pol ϵ	0	94	39	2
Dpb3 (23kDa)	Pol ϵ	0	18	14	4
Dpb4 (22kDa)	Pol ϵ	0	19	7	0
Pol1 (167kDa)	Pol α	3	212	156	35
Pol12 (79kDa)	Pol α	2	72	36	0
Pri1 (48kDa)	Pol α	0	51	26	0
Pri2 (62kDa)	Pol α	0	47	42	0
Other reported substrates of SCF^{Dia2}					
Rad51 (51kDa)	-	0	5	17	2
Cdc6	ORC-CDC6	0	0	0	0
Tec1 (55kDa)		0	0	2	0
Sir4 (152kDa)		32	12	62	53

Appendix Table S2

Mass spectrometry data showing that CMG and associated replisome components are the major partners of SCF^{Dia2}, largely dependent on the TPR domain of Dia2. Also see Dataset EV2.

This is the second of two independent experiments (the other is in Appendix Table S1).

Protein	Complex	Spectral counts (control)	Spectral counts (TAP-Sld5)	Spectral counts (ProteinA-Dia2)	Spectral counts (ProteinA-Dia2-ΔTPR)
Dia2 (85kDa)	SCF ^{Dia2}	73	515	4917	3775
Cdc53 (94kDa)	SCF ^{Dia2}	47	372	2125	2078
Skp1 (22kDa)	SCF ^{Dia2}	5	47	1002	998
Hrt1 (14kDa)	SCF ^{Dia2}	4	14	160	132
Rub1 (9kDa)	SCF ^{Dia2} (NEDD8)	2	24	105	77
Psf1 (24kDa)	CMG (GINS)	0	182	90	2
Psf2 (25kDa)	CMG (GINS)	0	145	46	0
Psf3 (22kDa)	CMG (GINS)	0	60	27	0
Sld5 (34kDa)	CMG (GINS)	0	358	109	0
Cdc45 (74kDa)	CMG	2	502	300	8
Mcm2 (99kDa)	CMG (Mcm2-7)	18	1160	735	5
Mcm3 (107kDa)	CMG (Mcm2-7)	5	1319	790	18
Mcm4 (105kDa)	CMG (Mcm2-7)	9	1353	924	5
Mcm5 (86kDa)	CMG (Mcm2-7)	11	921	545	16
Mcm6 (113kDa)	CMG (Mcm2-7)	4	1303	835	20
Mcm7 (95kDa)	CMG (Mcm2-7)	0	1026	583	13
Ctf4 (104kDa)	Replisome	4	1746	1275	8

Tof1 (141kDa)	Replisome (Tof1- Csm3)	2	1015	645	11
Csm3 (36kDa)	Replisome (Tof1- Csm3)	0	132	103	2
Mrc1 (124kDa)	Replisome	0	1280	736	7
Top1 (90kDa)	Replisome	0	329	140	5
Spt16 (119kDa)	Replisome (FACT)	375	1491	821	260
Pob3 (63kDa)	Replisome (FACT)	95	534	264	88
Pol2 (256kDa)	Pol ϵ	17	210	114	22
Dpb2 (78kDa)	Pol ϵ	0	38	34	4
Dpb3 (23kDa)	Pol ϵ	0	10	10	2
Dpb4 (22kDa)	Pol ϵ	2	19	12	2
Pol1 (167kDa)	Pol α	5	204	127	19
Pol12 (79kDa)	Pol α	0	58	57	3
Pri1 (48kDa)	Pol α	0	44	39	7
Pri2 (62kDa)	Pol α	0	37	38	4
Other reported substrates of SCF^{Dia2}					
Rad51 (51kDa)	-	2	5	13	7
Cdc6	ORC-CDC6	0	0	0	0
Tec1 (55kDa)		0	0	0	3
Sir4 (152kDa)		39	13	45	34

Appendix Table S3

Reagents and resources used in this study.

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Yeast Mcm2 [antigen 1-222; sheep polyclonal] Use 1 in 2,000 for immunoblotting	MRC-PPU Reagents and Services	DU62638
Yeast Mcm3 [antigen 1-222; sheep polyclonal] Use 1 in 1,000 for immunoblotting	MRC-PPU Reagents and Services	N/A
Yeast Mcm4 [antigen 831-933; sheep polyclonal] Use 1 in 2,000 for immunoblotting	MRC-PPU Reagents and Services	DU62578
Yeast Mcm5 [antigen 1-222; sheep polyclonal] Use 1 in 1,000 for immunoblotting	MRC-PPU Reagents and Services	DU62708
Yeast Mcm6 [antigen 801-1017; sheep polyclonal] Use 1 in 2,000 for immunoblotting	MRC-PPU Reagents and Services	DU62612
Yeast Mcm7 [antigen 1-222; sheep polyclonal] Use 1 in 1,000 for immunoblotting	MRC-PPU Reagents and Services	N/A
Yeast Mcm7 [antigen 624-846; sheep polyclonal] Use 1 in 2,000 for immunoblotting	MRC-PPU Reagents and Services	DU56413
Yeast Cdc45 [antigen 1-222; sheep polyclonal] Use 1 in 1,000 for immunoblotting	MRC-PPU Reagents and Services	N/A
Yeast Psf1 [antigen full-length; sheep polyclonal] Use 1 in 3,000 for immunoblotting	MRC-PPU Reagents and Services	DU73858
Yeast Psf2 [antigen full-length; sheep polyclonal] Use 1 in 250 for immunoblotting	MRC-PPU Reagents and Services	DU73962
Yeast Psf3 [antigen full-length; sheep polyclonal] Use 1 in 3,000 for immunoblotting	MRC-PPU Reagents and Services	DU73874
Yeast Sld5 [antigen full-length; sheep polyclonal] Use 1 in 1,000 for immunoblotting	MRC-PPU Reagents and Services	DU73863
Yeast Cdc53 [antigen 1-288; sheep polyclonal] Use 1 in 1,000 for immunoblotting	MRC-PPU Reagents and Services	DU21749

Yeast Sic1 [antigen full-length; sheep polyclonal] Use 1 in 250 for immunoblotting	Santa Cruz Biotechnologies	sc-50441
Peroxidase Anti-Peroxidase Soluble Complex (for immunoblotting against the TAP tag) Use 1 in 5,000 for immunoblotting	Sigma Aldrich	P1291
Anti-sheep IgG HRP [from donkey] Use 1 in 10,000 for immunoblotting	Sigma Aldrich	A3415
Anti-rabbit IgG HRP [from donkey] Use 1 in 10,000 for immunoblotting	Thermo Scientific	A-21207
Anti-FLAG M2 affinity resin	Sigma-Aldrich	A2220-10ML
Chemicals, Peptides, Recombinant Proteins, other reagents		
Agar (Bacto Agar)	Becton Dickinson	214010
Amicon Ultra-15 centrifugal Filter Unit, 30kDa	Millipore	UFC903024
Amicon Ultra-15 centrifugal Filter Unit, 100kDa	Millipore	UFC910024
β -glucuronidase	Sigma-Aldrich	G7770
4-12% Bis-Tris NuPAGE	Life Technologies	NP0301
β -mercaptoethanol	Sigma-Aldrich	M6520
Calmodulin affinity resin	Agilent Technologies	214303
DAPI (4', 6-Diamidino-2phenylindole)	Thermo	62248
Dynabeads M-270 Epoxy	Life Technologies	14302D
ECL western blotting detection reagent	VWR	RPN2106
FLAG peptide	Sigma-Aldrich	F4799
Galactose	Formedium	GAL03
Gibson Assembly Cloning Kit	New England Biolabs	E2611
Hydroxyurea	Molekula group	10872383
iBlot membrane	ThermoFisher Scientific	IB301031
IGEPAL CA-630	Sigma-Aldrich	18896
3-Indoleacetic Acid	Sigma-Aldrich	I3750-5G-A
'InstantBlue' colloidal Coomassie blue dye	Abcam	ab119211
4X LDS sample buffer	ThermoFisher Scientific	NP0007
Microscopy slides (VWR SuperFrost Plus, Adhesion Slides)	VWR	631-0108
Magnetic beads (Dynabeads M-270 Epoxy)	Thermo Scientific	14302D
Mating pheromone (α -factor)	Pepceuticals	N/A
Nuclease (Pierce Universal Nuclease)	Fisher Scientific	PN88702

NuPAGE MOPS SDS buffer	ThermoFisher Scientific	NP0001
NuPAGE Tris-Acetate SDS buffer	ThermoFisher Scientific	LA0041
NuPAGE 3–8% Tris-Acetate gels	ThermoFisher Scientific	EA0375BOX & WG1602BOX
NuPAGE Novex 4-12% Bis-Tris gels	ThermoFisher Scientific	NP0321 & WG1402A
Paraformaldehyde, 16% v/v aqueous solution, methanol free	Thermo Scientific	043368.9L
Peptone (Oxoid Peptone Bacteriological)	ThermoFisher Scientific	LP0037B
Pierce Universal Nuclease	ThermoFisher Scientific	88702
Propidium iodide	Sigma-Aldrich	P4170
Protease inhibitor cocktail (Complete Protease Inhibitor Cocktail)	Roche	05056489001
Raffinose	Formedium	RAF04
Roche cOmplete EDTA-free protease inhibitor cocktail	Roche	11873580001
RNase A	ThermoFisher Scientific	EN0531
Simply Blue	ThermoFisher Scientific	LC6060
T4 DNA ligase	New England Biolabs	M202
TEV protease	MRC PPU Reagents and Services	DU6811
Tween 20	Sigma-Aldrich	P1379
Yeast Extract (Bacto Yeast Extract)	Becton Dickinson	212750
Yeast nitrogen base without amino acids	Becton Dickinson	291940
Experimental Models: Yeast Strains		
<i>Saccharomyces cerevisiae</i> : YBH295 <i>MATa</i> <i>RAD52-GFP (TRP1)</i>	B. Hodgson	N/A
<i>Saccharomyces cerevisiae</i> : YCPR33 <i>MATa</i> / <i>MATα</i> <i>pep4Δ::kanMX</i> / <i>pep4Δ::kanMX</i> <i>bar1Δ::hph-NT</i> / <i>bar1Δ::hphNT</i> <i>ura3-1</i> / <i>ura3-1::URA3(GAL-MCM2 GAL-CBP-TEV-MCM3)</i> <i>his3-11</i> / <i>his3-11::HIS3(GAL-CDC45iFLAG2 GAL-GAL4)</i>	This study	N/A

<trp1-1::trp1(gal-psf1 gal-mcm4)<br="" gal-sld5)="" trp1-1::trp1(gal-mcm5=""></trp1-1::trp1(gal-psf1> leu2-3::LEU2(GAL-PSF2 GAL-PSF3) / leu2-3::LEU2(GAL-mcm7-3TEV(T394) GAL-MCM6) ctf4-I901E / ctf4-I901E		
<i>Saccharomyces cerevisiae</i> : YCPR38 MATa / MAT α pep4 Δ ::kanMX / pep4 Δ ::kanMX bar1 Δ ::hph-NT / bar1 Δ ::hphNT ura3-1 / ura3-1::URA3(GAL-MCM2 GAL-CBP-TEV-MCM3) his3-11 / his3-11::HIS3(GAL-CDC45iFLAG2 GAL-GAL4) trp1-1::TRP1(GAL-PSF1 GAL-SLD5) / trp1-1::TRP1(GAL-MCM5 GAL-MCM4) leu2-3::LEU2(GAL-PSF2 GAL-PSF3) / leu2-3::LEU2(GAL-mcm7-10R-3TEV(A219) GAL-MCM6) ctf4-I901E / ctf4-I901E	This study	N/A
<i>Saccharomyces cerevisiae</i> : YCPR66 MATa / MAT α pep4 Δ ::kanMX / pep4 Δ ::kanMX bar1 Δ ::hph-NT / bar1 Δ ::hphNT ura3-1 / ura3-1::URA3(GAL-MCM2 GAL-CBP-TEV-MCM3) his3-11 / his3-11::HIS3(GAL-CDC45iFLAG2 GAL-GAL4) trp1-1::TRP1(GAL-PSF1 GAL-SLD5) / trp1-1::TRP1(GAL-MCM5 GAL-MCM4) leu2-3::LEU2(GAL-PSF2 GAL-PSF3) / leu2-3::LEU2(GAL-mcm7-10R GAL-MCM6) ctf4-I901E / ctf4-I901E	This study	N/A
<i>Saccharomyces cerevisiae</i> : YCPR68 MATa mcm7-10R (HIS3MX)	This study	N/A
<i>Saccharomyces cerevisiae</i> : YCPR79 MATa pep4 Δ ::ADE2 TAP-SLD5 (kanMX) mcm7-10R (HIS3MX)	This study	N/A
<i>Saccharomyces cerevisiae</i> : YCPR83 MATa / MAT α MCM7 / mcm7-10R (HIS3MX) PIF1 / pif1 Δ ::URA3CP RRM3 / rrm3 Δ ::hphNT	This study	N/A
<i>Saccharomyces cerevisiae</i> : YCPR86 MATa pep4 Δ ::ADE2 cdc48-AID (hphNT) ura3-1::URA3&K.I. TRP1(ADH1-OsTIR1-9MYC) TAP-SLD5 (kanMX) mcm7-10R (HIS3MX)	This study	N/A
<i>Saccharomyces cerevisiae</i> : YCPR103	This study	N/A

MATa <i>mcm7-10R (HIS3MX)</i> <i>dia2-ΔTPR (hphNT)</i>		
<i>Saccharomyces cerevisiae</i> : YCPR111 MATa <i>mcm7-10R (HIS3MX)</i> <i>dia2-ΔTPR (hphNT)</i> <i>TAP-SLD5 (kanMX)</i> <i>pep4Δ::ADE2</i>	This study	N/A
<i>Saccharomyces cerevisiae</i> : YCPR138 MATa <i>pif1Δ::URA3</i> <i>TAP-SLD5 (kanMX)</i> <i>pep4Δ::ADE2</i>	This study	N/A
<i>Saccharomyces cerevisiae</i> : YCPR141 MATa <i>pif1Δ::URA3</i> <i>TAP-SLD5 (kanMX)</i> <i>pep4Δ::ADE2</i> <i>mcm7-10R (HIS3MX)</i>	This study	N/A
<i>Saccharomyces cerevisiae</i> : YCPR143 MATa <i>mcm7-10R (HIS3MX)</i> <i>rrm3Δ::hphNT</i> <i>TAP-SLD5 (kanMX)</i> <i>pep4Δ::ADE2</i>	This study	N/A
<i>Saccharomyces cerevisiae</i> : YCPR334 MATa <i>pep4Δ::ADE2</i> <i>TAP-SLD5 (kanMX)</i> <i>rrm3Δ::hphNT</i> <i>mcm7-10R (HIS3MX)</i> <i>leu2-3::LEU2(GAL-RRM3)</i>	This study	N/A
<i>Saccharomyces cerevisiae</i> : yCPR357 MATa <i>TAP-SLD5 (kanMX)</i> <i>pep4Δ::ADE2</i> <i>pif1-m2</i> <i>rrm3Δ::hphNT</i>	This study	N/A
<i>Saccharomyces cerevisiae</i> : YCPR359 MATa <i>TAP-SLD5 (kanMX)</i> <i>pep4Δ::ADE2</i> <i>pif1-m2</i>	This study	N/A
<i>Saccharomyces cerevisiae</i> : YCPR406 MATa <i>pep4Δ::ADE2</i> <i>TAP-SLD5 (kanMX)</i>	This study	N/A

<i>chl1Δ::URA3CP</i> <i>mcm7-10R (HIS3MX)</i>		
<i>Saccharomyces cerevisiae</i> : YCPR410 <i>MATa</i> <i>pep4Δ::ADE2</i> <i>TAP-SLD5 (kanMX)</i> <i>chl1Δ::URA3CP</i>	This study	N/A
<i>Saccharomyces cerevisiae</i> : YCPR412 <i>MATa</i> <i>pep4Δ::ADE2</i> <i>TAP-SLD5 (kanMX)</i> <i>mcm7-10R (HIS3MX)</i> <i>srs2Δ::kanMX</i>	This study	N/A
<i>Saccharomyces cerevisiae</i> : YCPR421 <i>MATa</i> <i>pep4Δ::ADE2</i> <i>TAP-SLD5 (kanMX)</i> <i>srs2Δ::kanMX</i>	This study	N/A
<i>Saccharomyces cerevisiae</i> : YCPR428 <i>MATa</i> <i>pep4Δ::ADE2</i> <i>TAP-SLD5 (kanMX)</i> <i>sgs1Δ::URA3CP</i>	This study	N/A
<i>Saccharomyces cerevisiae</i> : YCPR429 <i>MATa</i> <i>pep4Δ::ADE2</i> <i>TAP-SLD5 (kanMX)</i> <i>sgs1Δ::URA3CP</i> <i>mcm7-10R (HIS3MX)</i>	This study	N/A
<i>Saccharomyces cerevisiae</i> : YCPR445 <i>MATa</i> <i>RAD52-GFP (TRP1)</i> <i>mcm7-10R (HIS3MX)</i>	This study	N/A
<i>Saccharomyces cerevisiae</i> : YCPR447 <i>MATa</i> <i>RAD52-GFP (TRP1)</i> <i>mcm7-10R (HIS3MX)</i> <i>dia2-ΔTPR (hphNT)</i>	This study	N/A
<i>Saccharomyces cerevisiae</i> : YHM28 <i>MATa</i> <i>dia2Δ::HIS3</i>	H. Morohashi	N/A
<i>Saccharomyces cerevisiae</i> : YHM130 <i>MATa</i> <i>dia2Δ::HIS3</i> <i>TAP2-SLD5 (KanMX)</i> <i>ADE2</i> <i>pep4Δ::URA3</i>	H. Morohashi	N/A
<i>Saccharomyces cerevisiae</i> : YHM306	H. Morohashi	N/A

MATa <i>dia2-ΔTPR (hphNT)</i>		
<i>Saccharomyces cerevisiae</i> : YPM224 MATa / MATα <i>pep4Δ::kanMX / pep4Δ::kanMX</i> <i>bar1Δ::hph-NT / bar1Δ::hphNT</i> <i>ura3-1 / ura3-1::URA3(GAL-MCM2 GAL-CBP-TEV-MCM3)</i> <i>his3-11 / his3-11::HIS3(GAL-CDC45iFLAG2 GAL-GAL4)</i> <i>trp1-1::TRP1(GAL-PSF1 GAL-SLD5) / trp1-1::TRP1(GAL-MCM5 GAL-MCM4)</i> <i>leu2-3::LEU2(GAL-PSF2 GAL-PSF3) / leu2-3::LEU2(GAL-mcm7-K29A GAL-MCM6)</i> <i>ctf4-I901E / ctf4-I901E</i>	Deegan et al., 2020	N/A
<i>Saccharomyces cerevisiae</i> : YPM229 MATa / MATα <i>pep4Δ::kanMX / pep4Δ::kanMX</i> <i>bar1Δ::hph-NT / bar1Δ::hphNT</i> <i>ura3-1 / ura3-1::URA3(GAL-MCM2 GAL-CBP-TEV-MCM3)</i> <i>his3-11 / his3-11::HIS3(GAL-CDC45iFLAG2 GAL-GAL4)</i> <i>trp1-1::TRP1(GAL-PSF1 GAL-SLD5) / trp1-1::TRP1(GAL-MCM5 GAL-MCM4)</i> <i>leu2-3::LEU2(GAL-PSF2 GAL-PSF3) / leu2-3::LEU2(GAL-mcm7-3TEV(M167) GAL-MCM6)</i> <i>ctf4-I901E / ctf4-I901E</i>	This study	N/A
<i>Saccharomyces cerevisiae</i> : YPM230 MATa / MATα <i>pep4Δ::kanMX / pep4Δ::kanMX</i> <i>bar1Δ::hph-NT / bar1Δ::hphNT</i> <i>ura3-1 / ura3-1::URA3(GAL-MCM2 GAL-CBP-TEV-MCM3)</i> <i>his3-11 / his3-11::HIS3(GAL-CDC45iFLAG2 GAL-GAL4)</i> <i>trp1-1::TRP1(GAL-PSF1 GAL-SLD5) / trp1-1::TRP1(GAL-MCM5 GAL-MCM4)</i> <i>leu2-3::LEU2(GAL-PSF2 GAL-PSF3) / leu2-3::LEU2(GAL-mcm7-3TEV(A219) GAL-MCM6)</i> <i>ctf4-I901E / ctf4-I901E</i>	This study	N/A
<i>Saccharomyces cerevisiae</i> : YPNK337 MATa <i>pep4Δ::ADE2</i> <i>cdc48-AID (hphNT)</i> <i>ura3-1::URA3&K.I. TRP1(ADH1-OsTIR1-9MYC)</i> <i>TAP-SLD5 (kanMX)</i>	P. Nkosi	N/A
<i>Saccharomyces cerevisiae</i> : YSS47 MATa <i>pep4Δ::ADE2</i> <i>TAP-SLD5 (kanMX)</i>	S. Sengupta	N/A
<i>Saccharomyces cerevisiae</i> : YTM74 MATa <i>RAD52-GFP (TRP1)</i>	T. Maculins	N/A

<i>dia2-ΔTPR (hph-NT)</i>		
<i>Saccharomyces cerevisiae</i> : YTM115 <i>MATa</i> <i>RAD52-GFP (TRP1)</i> <i>dia2Δ::HIS3</i>	T. Maculins	N/A
<i>Saccharomyces cerevisiae</i> : YTM265 <i>MATa</i> <i>TAP2-SLD5 (KanMX)</i> <i>pep4Δ::URA3</i> <i>ADE2</i> <i>dia2-ΔTPR (hphNT)</i>	T. Maculins	N/A
<i>Saccharomyces cerevisiae</i> : YPM224 <i>MATa / MATα</i> <i>pep4Δ::kanMX / pep4Δ::kanMX</i> <i>bar1Δ::hph-NT / bar1Δ::hphNT</i> <i>ura3-1 / ura3-1::URA3(GAL-MCM2 GAL-CBP-TEV-MCM3)</i> <i>his3-11 / his3-11::HIS3(GAL-CDC45iFLAG2 GAL-GAL4)</i> <i>trp1-1::TRP1(GAL-PSF1 GAL-SLD5) / trp1-1::TRP1(GAL-MCM5 GAL-MCM4)</i> <i>leu2-3::LEU2(GAL-PSF2 GAL-PSF3) / leu2-3::LEU2(GAL-mcm7-K29A GAL-MCM6)</i> <i>ctf4-I901E / ctf4-I901E</i>	Deegan et al., 2020	N/A
<i>Saccharomyces cerevisiae</i> : YPM229 <i>MATa / MATα</i> <i>pep4Δ::kanMX / pep4Δ::kanMX</i> <i>bar1Δ::hph-NT / bar1Δ::hphNT</i> <i>ura3-1 / ura3-1::URA3(GAL-MCM2 GAL-CBP-TEV-MCM3)</i> <i>his3-11 / his3-11::HIS3(GAL-CDC45iFLAG2 GAL-GAL4)</i> <i>trp1-1::TRP1(GAL-PSF1 GAL-SLD5) / trp1-1::TRP1(GAL-MCM5 GAL-MCM4)</i> <i>leu2-3::LEU2(GAL-PSF2 GAL-PSF3) / leu2-3::LEU2(GAL-mcm7-3TEV(M167) GAL-MCM6)</i> <i>ctf4-I901E / ctf4-I901E</i>	This study	N/A
<i>Saccharomyces cerevisiae</i> : YPM230 <i>MATa / MATα</i> <i>pep4Δ::kanMX / pep4Δ::kanMX</i> <i>bar1Δ::hph-NT / bar1Δ::hphNT</i> <i>ura3-1 / ura3-1::URA3(GAL-MCM2 GAL-CBP-TEV-MCM3)</i> <i>his3-11 / his3-11::HIS3(GAL-CDC45iFLAG2 GAL-GAL4)</i> <i>trp1-1::TRP1(GAL-PSF1 GAL-SLD5) / trp1-1::TRP1(GAL-MCM5 GAL-MCM4)</i> <i>leu2-3::LEU2(GAL-PSF2 GAL-PSF3) / leu2-3::LEU2(GAL-mcm7-3TEV(A219) GAL-MCM6)</i> <i>ctf4-I901E / ctf4-I901E</i>	This study	N/A
<i>Saccharomyces cerevisiae</i> : YCPR33 <i>MATa / MATα</i>	This study	N/A

<i>pep4Δ::kanMX / pep4Δ::kanMX</i> <i>bar1Δ::hph-NT / bar1Δ::hphNT</i> <i>ura3-1 / ura3-1::URA3(GAL-MCM2 GAL-CBP-TEV-MCM3)</i> <i>his3-11 / his3-11::HIS3(GAL-CDC45iFLAG2 GAL-GAL4)</i> <i>trp1-1::TRP1(GAL-PSF1 GAL-SLD5) / trp1-1::TRP1(GAL-MCM5 GAL-MCM4)</i> <i>leu2-3::LEU2(GAL-PSF2 GAL-PSF3) / leu2-3::LEU2(GAL-mcm7-3TEV(T394) GAL-MCM6)</i> <i>ctf4-I901E / ctf4-I901E</i>		
<i>Saccharomyces cerevisiae</i>: YCPR38 <i>MATa / MATα</i> <i>pep4Δ::kanMX / pep4Δ::kanMX</i> <i>bar1Δ::hph-NT / bar1Δ::hphNT</i> <i>ura3-1 / ura3-1::URA3(GAL-MCM2 GAL-CBP-TEV-MCM3)</i> <i>his3-11 / his3-11::HIS3(GAL-CDC45iFLAG2 GAL-GAL4)</i> <i>trp1-1::TRP1(GAL-PSF1 GAL-SLD5) / trp1-1::TRP1(GAL-MCM5 GAL-MCM4)</i> <i>leu2-3::LEU2(GAL-PSF2 GAL-PSF3) / leu2-3::LEU2(GAL-mcm7-10R-3TEV(A219) GAL-MCM6)</i> <i>ctf4-I901E / ctf4-I901E</i>	This study	N/A
<i>Saccharomyces cerevisiae</i>: YCPR66 <i>MATa / MATα</i> <i>pep4Δ::kanMX / pep4Δ::kanMX</i> <i>bar1Δ::hph-NT / bar1Δ::hphNT</i> <i>ura3-1 / ura3-1::URA3(GAL-MCM2 GAL-CBP-TEV-MCM3)</i> <i>his3-11 / his3-11::HIS3(GAL-CDC45iFLAG2 GAL-GAL4)</i> <i>trp1-1::TRP1(GAL-PSF1 GAL-SLD5) / trp1-1::TRP1(GAL-MCM5 GAL-MCM4)</i> <i>leu2-3::LEU2(GAL-PSF2 GAL-PSF3) / leu2-3::LEU2(GAL-mcm7-10R GAL-MCM6)</i> <i>ctf4-I901E / ctf4-I901E</i>	This study	N/A
<i>Saccharomyces cerevisiae</i>: YPNK337 <i>MATa</i> <i>pep4Δ::ADE2</i> <i>cdc48-AID (hphNT)</i> <i>ura3-1::URA3&K.I. TRP1(ADH1-OsTIR1-9MYC)</i> <i>TAP-SLD5 (kanMX)</i>	P. Nkosi	N/A
<i>Saccharomyces cerevisiae</i>: YCPR86 <i>MATa</i> <i>pep4Δ::ADE2</i> <i>cdc48-AID (hphNT)</i> <i>ura3-1::URA3&K.I. TRP1(ADH1-OsTIR1-9MYC)</i> <i>TAP-SLD5 (kanMX)</i> <i>mcm7-10R (HIS3MX)</i>	This study	N/A
<i>Saccharomyces cerevisiae</i>: YSS47 <i>MATa</i> <i>pep4Δ::ADE2</i> <i>TAP-SLD5 (kanMX)</i>	S. Sengupta	N/A

<i>Saccharomyces cerevisiae</i> : YCPR79 MATa <i>pep4Δ::ADE2</i> <i>TAP-SLD5 (kanMX)</i> <i>mcm7-10R (HIS3MX)</i>	This study	N/A
<i>Saccharomyces cerevisiae</i> : YHM130 MATa <i>dia2Δ::HIS3</i> <i>TAP2-SLD5 (KanMX)</i> <i>ADE2</i> <i>pep4Δ::URA3</i>	H. Morohashi	N/A
<i>Saccharomyces cerevisiae</i> : YTM265 MATa <i>TAP2-SLD5 (KanMX)</i> <i>pep4Δ::URA3</i> <i>ADE2</i> <i>dia2-ΔTPR (hphNT)</i>	T. Maculins	N/A
<i>Saccharomyces cerevisiae</i> : YCPR111 MATa <i>mcm7-10R (HIS3MX)</i> <i>dia2-ΔTPR (hphNT)</i> <i>TAP-SLD5 (kanMX)</i> <i>pep4Δ::ADE2</i>	This study	N/A
<i>Saccharomyces cerevisiae</i> : YHM28 MATa <i>dia2Δ::HIS3</i>	H. Morohashi	N/A
<i>Saccharomyces cerevisiae</i> : YCPR103 MATa <i>mcm7-10R (HIS3MX)</i> <i>dia2-ΔTPR (hphNT)</i>	This study	N/A
<i>Saccharomyces cerevisiae</i> : YCPR68 MATa <i>mcm7-10R (HIS3MX)</i>	This study	N/A
<i>Saccharomyces cerevisiae</i> : YHM306 MATa <i>dia2-ΔTPR (hphNT)</i>	H. Morohashi	N/A
<i>Saccharomyces cerevisiae</i> : YBH295 MATa <i>RAD52-GFP (TRP1)</i>	B. Hodgson	N/A
<i>Saccharomyces cerevisiae</i> : YTM115 MATa <i>RAD52-GFP (TRP1)</i> <i>dia2Δ::HIS3</i>	T. Maculins	N/A
<i>Saccharomyces cerevisiae</i> : YCPR447 MATa <i>RAD52-GFP (TRP1)</i> <i>mcm7-10R (HIS3MX)</i> <i>dia2-ΔTPR (hphNT)</i>	This study	N/A

<i>Saccharomyces cerevisiae</i> : YCPR445 MATa <i>RAD52-GFP (TRP1)</i> <i>mcm7-10R (HIS3MX)</i>	This study	N/A
<i>Saccharomyces cerevisiae</i> : YTM74 MATa <i>RAD52-GFP (TRP1)</i> <i>dia2-ΔTPR (hph-NT)</i>	T. Maculins	N/A
<i>Saccharomyces cerevisiae</i> : YCPR83 MATa / MATα <i>MCM7 / mcm7-10R (HIS3MX)</i> <i>PIF1 / pif1Δ::URA3CP</i> <i>RRM3 / rrm3Δ::hphNT</i>	This study	N/A
<i>Saccharomyces cerevisiae</i> : YCPR143 MATa <i>mcm7-10R (HIS3MX)</i> <i>rrm3Δ::hphNT</i> <i>TAP-SLD5 (kanMX)</i> <i>pep4Δ::ADE2</i>	This study	N/A
<i>Saccharomyces cerevisiae</i> : YCPR138 MATa <i>pif1Δ::URA3</i> <i>TAP-SLD5 (kanMX)</i> <i>pep4Δ::ADE2</i>	This study	N/A
<i>Saccharomyces cerevisiae</i> : YCPR141 MATa <i>pif1Δ::URA3</i> <i>TAP-SLD5 (kanMX)</i> <i>pep4Δ::ADE2</i> <i>mcm7-10R (HIS3MX)</i>	This study	N/A
<i>Saccharomyces cerevisiae</i> : YCPR421 MATa <i>pep4Δ::ADE2</i> <i>TAP-SLD5 (kanMX)</i> <i>srs2Δ::kanMX</i>	This study	N/A
<i>Saccharomyces cerevisiae</i> : YCPR412 MATa <i>pep4Δ::ADE2</i> <i>TAP-SLD5 (kanMX)</i> <i>mcm7-10R (HIS3MX)</i> <i>srs2Δ::kanMX</i>	This study	N/A
<i>Saccharomyces cerevisiae</i> : YCPR428 MATa <i>pep4Δ::ADE2</i> <i>TAP-SLD5 (kanMX)</i> <i>sgs1Δ::URA3CP</i>	This study	N/A
<i>Saccharomyces cerevisiae</i> : YCPR429 MATa	This study	N/A

<i>pep4Δ::ADE2</i> <i>TAP-SLD5 (kanMX)</i> <i>sgs1Δ::URA3CP</i> <i>mcm7-10R (HIS3MX)</i>		
<i>Saccharomyces cerevisiae</i>: YCPR410 MATa <i>pep4Δ::ADE2</i> <i>TAP-SLD5 (kanMX)</i> <i>chl1Δ::URA3CP</i>	This study	N/A
<i>Saccharomyces cerevisiae</i>: YCPR406 MATa <i>pep4Δ::ADE2</i> <i>TAP-SLD5 (kanMX)</i> <i>chl1Δ::URA3CP</i> <i>mcm7-10R (HIS3MX)</i>	This study	N/A
<i>Saccharomyces cerevisiae</i>: YCPR359 MATa <i>TAP-SLD5 (kanMX)</i> <i>pep4Δ::ADE2</i> <i>pif1-m2</i>	This study	N/A
<i>Saccharomyces cerevisiae</i>: yCPR357 MATa <i>TAP-SLD5 (kanMX)</i> <i>pep4Δ::ADE2</i> <i>pif1-m2</i> <i>rrm3Δ::hphNT</i>	This study	N/A
<i>Saccharomyces cerevisiae</i>: YCPR334 MATa <i>pep4Δ::ADE2</i> <i>TAP-SLD5 (kanMX)</i> <i>rrm3Δ::hphNT</i> <i>mcm7-10R (HIS3MX)</i> <i>leu2-3::LEU2(GAL-RRM3)</i>	This study	N/A
Recombinant DNA (Plasmids)		
pCPR11 [Expression vector to express Mcm6 and Mcm7-10R-3TEV-A219 to produce recombinant CMG]	MRC PPU Reagents and Services (This study)	DU78160
pCPR12 [Expression vector to express Mcm6 and Mcm7-3TEV-T394 to produce recombinant CMG]	MRC PPU Reagents and Services (This study)	DU78172
pCPR18 [Expression vector to express Mcm6 and Mcm7-10R to produce recombinant CMG]	MRC PPU Reagents and Services (This study)	DU78161
pCPR33 [Vector based on pCPR18 to generate a DNA template for replacing endogenous Mcm7 with Mcm7-10R]	MRC PPU Reagents and	DU78162

	Services (This study)	
pCPR34 [Expression vector to express a second copy of the RRM3 gene controlled by the galactose promoter]	MRC PPU Reagents and Services (This study)	DU78163
pET21b Npl4 [to express yeast Npl4 in <i>E. coli</i>]	Stein et al., 2014	N/A
K27 SUMO CDC48 [to express 14His-SMT3-tagged version of yeast Cdc48 in <i>E. coli</i>]	Stein et al., 2014	N/A
K27 SUMO Ufd1 [to express yeast Ufd1 in <i>E. coli</i>]	Stein et al., 2014	N/A
pPM21 [to express Mcm6 and Mcm7-3TEV(M167) in yeast to produce recombinant CMG]	MRC PPU Reagents and Services (This study)	DU78174
pPM22 [Expression vector to express Mcm6 and Mcm7-3TEV-A219 to produce recombinant CMG]	MRC PPU Reagents and Services (This study)	DU78173
pPM51 [to express Mcm6 and Mcm7-K29A in yeast to produce recombinant CMG]	Deegan et al., 2020	N/A
pTDK3 [to express Skp1 and Dia2 subunits of SCF ^{Dia2} in yeast]	Deegan et al., 2020	N/A
pTDK6 [to express Hrt1 and Cdc53 subunits of SCF ^{Dia2} in yeast]	Deegan et al., 2020	N/A
pKL506 [Template for PCR amplification of the URA3CP cassette, for one-step gene deletion]	K. Labib	N/A
pYM18 [Template for PCR amplification of the kanMX cassette, for one-step gene deletion]	Janke et al (2004)	N/A
Oligonucleotides		
Check integration of pRS series vectors; 1802: GGCATCAGAGCAGATTGTAC	This study	N/A
Check integration of pRS series vectors; 1803: CTCCTTACGCATCTGTGCGG	This study	N/A
Check integration of pRS series vectors; 1806: ACAACGACCAAGCTCACATC	This study	N/A
Check integration of pRS series vectors; 1807: ACATTATGGGTGGTATGTTGG	This study	N/A
Check integration of pRS series vectors; 1810: TGGAGTTCAATGCGTCCATC	This study	N/A
Check integration of pRS series vectors; 1811: CTACTGCGCCAATTGATGAC	This study	N/A
To generate a fragment of Mcm7 containing 3TEV-M167 or 3TEV-A219; 8360: GAAAATTCAATATAAGGCGCGCCAT- GAGTGCGGCACTTCC	This study	N/A

To generate a fragment of Mcm7 containing 3TEV-M167 or 3TEV-A219; 8361: CGAATTCCTGCAGCCCGGGTCAAG- CGTCTTGTAGATCG	This study	N/A
To generate a fragment of Mcm7 containing 3TEV-T394; 8963: ATGGATGAGGAGGAAGACAATCTGTC	This study	N/A
To generate a fragment of Mcm7 containing 3TEV-T394; 8964: GACAGATTGTCTTCCTCCTCATCCAT	This study	N/A
To generate a fragment of Mcm7 containing 3TEV-T394; 8965: GAGGGTGGCTCGAACACCAAAGGC	This study	N/A
To generate a fragment of Mcm7 containing 3TEV-T394; 8965: GCCTTTGGTGTTTCGAGCCACCCTC	This study	N/A
To generate a plasmid to express Mcm7-K29A; 8364: GAAAATTCAATATAAGGCGCGCCATGAGTGCGGCACTTCC	Deegan et al., 2020; This study	N/A
To generate a plasmid to express Mcm7-K29A; 8365: CGAATTCCTGCAGCCCGGGTCAAGCGTCTTGTAGATCG	Deegan et al., 2020; This study	N/A
To remove the 3TEV sites in Mcm7-10R- 3TEV-A219; 9004: TTTGAAAATTCAATATAAGGCGCGCCATGAGTGCGGCACTTC CATC	This study	N/A
To remove the 3TEV sites in Mcm7-10R- 3TEV-A219; 9005: GAACTAATTGCTCTTCTCCTGTAACGAC	This study	N/A
To remove the 3TEV sites in Mcm7-10R- 3TEV-A219; 9006: AGGAGAAGAGCAATTAGTTCTAAACCATTATCTGTTAG	This study	N/A
To amplify Mcm7- 10R-HIS3MX for allelic replacement to the endogenous MCM7 locus; 9182: AGTCGATTTGCTTCTTTTTTTCACGAAAGTGAGATACCAGCAAA GGACCAGAGCGAGATGTACTACAGTAGCAGGCTACATACAC GTCTTTACCAATAAATTACATTTAGAAAGGCAATATAATCACT ACTTAGATTAGCTTCAATCTTGCAGGAAGAACTCAATACAGAC AGACCAGATTATGAGTGCGGCACTTCCATC	This study	N/A
To amplify Mcm7- 10R-HIS3MX for allelic replacement to the endogenous MCM7 locus; 9183: AATATTAGTAAAATTATAATAACAGAATACATATCTTGAAAA CTGGGCCTTGGAAGTAAGTTACAGAAGGTAAATTTGAATGTG CAGCTATATGGAAATGAAAAAAGGGGGAAAAAGAATAAGG AATGAAGGCCCTGTTGCTTTTTTTTTTTAGAACTTTCAAGCGTC TTGTAGATCATCGATGAATTCGAGCTCGT	This study	N/A
To amplify the RRM3 gene from genomic DNA to make GAL-RRM3; 7511: TCATGGGATCCATGTTTCAGGTCGCATGCCTCCGGTAACAAG	This study	N/A
To amplify RRM3 gene from genomic DNA to make GAL-RRM3; 7512: TAGTACCCCGGGTCATTTCAAAGTTTCTAAACGTTTATAG	This study	N/A
To knockout the SRS2 gene with the kanMX cassette from pYM18; 9844:	This study	N/A

GAGTATCATTCCAATTTGATCTTTCTTCTACCGGTACTTAGGG ATAGCAAGACATGGAGGCCCAAGAATAC		
To knockout the SRS2 gene with the kanMX cassette from pYM18; 9845: AAATTATAAACCGCCTCCAATAGTTGACGTAGTCAGGCATGA AAGTGCTACAGTATAGCGACCAGCATTC	This study	N/A
To check SRS2 deletion with the kanMX cassette; 9846: CTTGTATACACAACCACTGC	This study	N/A
To check SRS2 deletion with the kanMX cassette; 9847: TAAGCCTTCTACGCAAGACG	This study	N/A
To check SRS2 deletion with the kanMX cassette; 9848: TGACGAGCGTAATGGCTGGC	This study	N/A
To knockout the SRS2 gene with the kanMX cassette from pYM18; 9844: GAGTATCATTCCAATTTGATCTTTCTTCTACCGGTACTTAGGG ATAGCAAGACATGGAGGCCCAAGAATAC	This study	N/A
To knockout the SRS2 gene with the kanMX cassette from pYM18; 9845: AAATTATAAACCGCCTCCAATAGTTGACGTAGTCAGGCATGA AAGTGCTACAGTATAGCGACCAGCATTC	This study	N/A
To check SRS2 deletion with the kanMX cassette; 9846: CTTGTATACACAACCACTGC	This study	N/A
To check SRS2 deletion with the kanMX cassette; 9847: TAAGCCTTCTACGCAAGACG	This study	N/A
To check SRS2 deletion with the kanMX cassette; 9848: TGACGAGCGTAATGGCTGGC	This study	N/A
To check SRS2 deletion with the kanMX cassette; 9849: GCCAGCCATTACGCTCGTCA	This study	N/A
To knockout the CHL1 gene with the URA3CP cassette from pKL506; 9827: ACCCAAAAGAGTAGAAAACCAGGCTAAAAACAGTCACACTAG TCCAAAAAATTAAGGCGCGCCAGATCTG	This study	N/A
To knockout the CHL1 gene with the URA3CP cassette from pKL506; 9828: TTACTATAATATATAGTAGTAATCACAGTATACACGTAAACGT ATTCCTTATCGATGAATTCGAGCTCGT	This study	N/A
To check CHL1 deletion with the URA3CP cassette; 9829: GCTATTAATCTCTTGCCTGC	This study	N/A
To check CHL1 deletion with the URA3CP cassette; 9830: GCTTGCGTATTATCTATAGC	This study	N/A
For sequencing TEV insertions and point mutants in Mcm7; 8919: GGCACTTCCATCAATTCAGC	This study	N/A
For sequencing TEV insertions and point mutants in Mcm7; 8920: CCTAGGCCAATTGATTACCG	This study	N/A
For sequencing TEV insertions and point mutants in Mcm7; 8921: GTTGCTGCTATTACTTGTCG	This study	N/A
For sequencing TEV insertions and point mutants in Mcm7; 8922:	This study	N/A

ACCAAGTAGAGATGACGACG		
For sequencing TEV insertions and point mutants in Mcm7; 8923: TTCTCAGAAGCACCTGCAGC	This study	N/A
For sequencing TEV insertions and point mutants in Mcm7; 8924: CGATATCAGAATCTTGGGCG	This study	N/A
For sequencing the <i>RRM3</i> gene; 6861: CGTCGGGATCATCATCTCC	This study	N/A
For sequencing the <i>RRM3</i> gene; 6862: CGCTTGGTATCTCTCGGG	This study	N/A
For sequencing the <i>RRM3</i> gene; 6852: CCGGTAACAAGAAGCAATGG	This study	N/A
For sequencing the <i>RRM3</i> gene; 6853: GCTGAATTCAAGAAGTTGGTCG	This study	N/A
For sequencing the <i>RRM3</i> gene; 6854: CCACAGATCCGCTTCTTCC	This study	N/A
For sequencing the <i>RRM3</i> gene; 6855: GGTTGACGGTAACTTGTTGG	This study	N/A
For sequencing the <i>RRM3</i> gene; 6856: CGATTACGCTGACGGTATCG	This study	N/A
For sequencing the <i>RRM3</i> gene; 6857: GGAAATCTACAAGATCGTCG	This study	N/A
Software and Algorithms		
AlphaFold2-multimer (ColabFold v1.5.3)	Google Deepmind	https://colab.research.google.com/github/sokrypton/ColabFold/blob/main/AlphaFold2.ipynb
Prism 9	GraphPad	https://www.graphpad.com/scientificsoftware/prism/
FlowJo	BD Biosciences	https://www.flowjo.com/
ImageJ	NIH	N/A