**Supplementary File 1**

Reagents and resources used in this study.

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| --- | --- | --- |
| **REAGENT or RESOURCE** | SOURCE | IDENTIFIER |
| **Antibodies** |
| Mcm2 [158] | Labib laboratory | N/A |
| Mcm3 [16] | Labib laboratory | N/A |
| Mcm4 [159] | Labib laboratory | N/A |
| Mcm5 [160] | Labib laboratory | N/A |
| Mcm6 [161] | Labib laboratory | N/A |
| Mcm7 [19] | Labib laboratory | N/A |
| Psf1 [58] | Labib laboratory | N/A |
| Psf2 [31] | Labib laboratory | N/A |
| Psf3 [33] | Labib laboratory | N/A |
| Sld5 [32] | Labib laboratory | N/A |
| Cdc45 [29] | Labib laboratory | N/A |
| Ctf4 [30] | Labib laboratory | N/A |
| Mrc1 [125] | Labib laboratory | N/A |
| Pol2 [11] | Labib laboratory | N/A |
| Dpb2 [122] | Labib laboratory | N/A |
| Cdc53 [y-300] | Santa Cruz Biotechnology | sc-50444 |
| Skp1 [yC-20] | Santa Cruz Biotechnology | sc-5328 |
| Cdc48 [90] | Labib laboratory | N/A |
| Ufd1 [99] | Labib laboratory | N/A |
| Npl4 [100] | Labib laboratory | N/A |
| anti-sheep IgG HRP [from donkey] | Sigma-Aldrich | A3415 |
| anti-rabbit IgG HRP [from donkey] | GE Healthcare | NA934 |
| anti-goat IgG HRP [from rabbit] | Sigma-Aldrich | A5420 |
| **Bacterial and Virus Strains** |
| *Escherichia coli:* Rosetta™ (DE3) pLysS cells: F- *ompT hsdS*B(rB- mB-) *gal dcm* (DE3) pLysSRARE (CamR) | Novagen | 70956 |
| **Chemicals, Peptides, and Recombinant Proteins** |
| ORC | (Frigola et al., 2013) | N/A |
| Cdc6 | (Frigola et al., 2013) | N/A |
| Cdt1-Mcm2-7 | (Coster et al., 2014) | N/A |
| Mcm2-7 | Max Douglas | N/A |
| DDK | (On et al., 2014) | N/A |
| Sld3/7 | (Yeeles et al., 2015) | N/A |
| Cdc45 | (Yeeles et al., 2015) | N/A |
| Dpb11 | (Yeeles et al., 2015) | N/A |
| Sld2 | (Yeeles et al., 2015) | N/A |
| Pol e | (Yeeles et al., 2015) | N/A |
| GINS | (Yeeles et al., 2015) | N/A |
| S-CDK | (Yeeles et al., 2015) | N/A |
| Mcm10 | (Yeeles et al., 2015) | N/A |
| Pol a | (Yeeles et al., 2015) | N/A |
| RPA | (Yeeles et al., 2015) | N/A |
| Ctf4 | (Yeeles et al., 2015) | N/A |
| Mrc1 | (Yeeles et al., 2017) | N/A |
| Csm3-Tof1 | (Deegan et al., 2019) | N/A |
| RFC | (Yeeles et al., 2017) | N/A |
| PCNA | (Yeeles et al., 2017) | N/A |
| Top1 | (Deegan et al., 2019) | N/A |
| FACT | Joe Yeeles | N/A |
| Pif1 | (Deegan et al., 2019) | N/A |
| Pif1-K264A | (Deegan et al., 2019) | N/A |
| CMG | This study | N/A |
| CMG-Mcm7-K29A | This study | N/A |
| Uba1 | This study | N/A |
| Cdc34 | This study | N/A |
| SCFDia2 | This study | N/A |
| Ubiquitin | Axel Knebel | N/A |
| USP2b | Axel Knebel | N/A |
| Ulp1 | Alexander Stein | N/A |
| Ufd1-Npl4 | (Stein et al., 2014) | N/A |
| Cdc48 | (Stein et al., 2014) | N/A |
| Cdc48-FtsH | (Bodnar and Rapoport, 2017) | N/A |
| FtsH | (Bodnar and Rapoport, 2017) | N/A |
| Anti-FLAG M2 affinity gel | Sigma-Aldrich | A2220 |
| Ni-NTA agarose | Qiagen | 30210 |
| Calmodulin sepharose 4B | GE Healthcare | 17052901 |
| IgG sepharose 6 Fast Flow | GE Healthcare | 17096901 |
| 3Flag peptide | Sigma-Aldrich | F4799 |
| Roche cOmplete EDTA-free protease inhibitor cocktail | Roche | 000000011873580001 |
| Sigma protease inhibitor cocktail | Sigma-Aldrich | P8215 |
| **Experimental Models: Organisms/Strains** |
| *yJF1: MAT***a** *ade2-1 ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100 bar1∆::hphNT pep4∆::kanMX* | (Frigola et al., 2013) | N/A |
| *ySDORC (ORC purification): MAT***a** *ade2-1 ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100 bar1::hphNT pep4::kanMx his3::pRS303-ORC3+ORC4 ura3::pRS306-CBP-TEV-ORC1+ORC2 trp1::pRS304-ORC5+ORC6* | (Frigola et al., 2013) | N/A |
| *yAM33 (Cdt1-Mcm2-7 purification): MAT***a** *ade2-1 ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100 bar1::hphNT pep4::kanMx his3::pRS303-CDT1+GAL4 ura3::pRS306-MCM2+CBP-TEV-MCM3 trp1::pRS304-MCM4+MCM5 leu2::pRS305-MCM6+MCM7* | (Coster et al., 2014) | N/A |
| *ySDK8 (DDK purification): MAT***a** *ade2-1 ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100 bar1::hphNT pep4::kanMX trp1::pRS304-CDC7+CBP-TEV-DBF4* | (On et al., 2014) | N/A |
| *yTD6 (Sld3-7 purification): MAT***a** *ade2-1 ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100 bar1::hphNT pep4::kanMX leu2::pRS305-SLD7 his3::pRS303-SLD3-TCP+GAL4* | (Yeeles et al., 2015) | N/A |
| *yTD8 (Sld2 purification): MAT***a** *ade2-1 ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100 bar1::hphNT pep4::kanMX his3::pRS303-SLD2-3FLAG(nat-NT2)+GAL4* | (Yeeles et al., 2015) | N/A |
| *yJY13 (Cdc45 purification): MAT***a** *ade2-1 ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100 bar1::hphNT pep4::kanMX his3::pRS303-CDC45-iFLAG2+GAL4* | (Yeeles et al., 2015) | N/A |
| *yJY23 (Pol a / primase purification): MAT***a** *ade2-1 ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100 bar1::hphNT pep4::kanMX trp1::pRS304-POL1+POL12 ura3:: pRS306-CBP-TEV-PRI1+PRI2* | (Yeeles et al., 2015) | N/A |
| *yJY26 (Dpb11 purification): MAT***a** *ade2-1 ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100 bar1::hphNT pep4::kanMX his3::pRS303-DPB11-3FLAG(nat-NT2)+GAL4* | (Yeeles et al., 2015) | N/A |
| *yAJ2 (Pol e purification): MAT***a** *ade2-1 ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100 bar1::hphNT pep4::kanMX trp1::pRS304-POL2+DPB4-TEV-CBP ura3::pRS306DPB2+DPB3* | (Yeeles et al., 2015) | N/A |
| *yAE31 (RPA purification): MAT***a** *ade2-1 ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100 bar1::hphNT pep4::kanMX his3::pRS303-CBP-TEV-RFA1+GAL4 ura3::pRS306-RFA2+RFA3* | (Yeeles et al., 2017) | N/A |
| *yAE37(S-CDK purification): MAT***a** *ade2-1 ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100 bar1::hphNT pep4::kanMX ura3::pRS306-CKS1+CDC28 his3::pRS303-CBP-TEV-CLB5+GAL4* | (Yeeles et al., 2015) | N/A |
| *yAE40 (Ctf4 purification): MAT***a** *ade2-1 ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100 bar1::hphNT pep4::kanMX his3::pRS303-CBP-TEV-CTF4­+GAL4* | (Yeeles et al., 2015) | N/A |
| *yAE41 (RFC purification): MAT***a** *ade2-1 ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100 bar1::hphNT pep4::kanMX ura3::pRS306-RFC2+CBP-RFC3 trp1::pRS304-RFC4+RFC5 his3::pRS303-RFC1+GAL4* | (Yeeles et al., 2017) | N/A |
| *yAE71 (Mrc1 purification): MAT***a** *ade2-1 ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100 bar1::hphNT pep4::kanMX his3::pRS303-MRC1-5FLAG* | John Diffley | N/A |
| *yTDK4 (Csm3-Tof1 purification): MAT***a** *ade2-1 ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100 bar1::hphNT pep4∆::kanMX leu2::pRS305-TOF1+CBP-TEV-CSM3* | (Deegan et al., 2019) | N/A |
| *yTDK5 (SCFDia2 purification): MAT***a** *ade2-1 ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100 bar1::hphNT pep4::kanMX ura3::pRS306-SKP1+ProteinA-3TEV-DIA2 leu2::pRS305-HRT1+CDC53* | This study | N/A |
| *yTDK6 (Top1 purification): MAT***a** *ade2-1 ura3-1 his3-11,15 trp1-1 leu2-3,112 can1-100 bar1::hphNT pep4∆::kanMX leu2::pRS305-CBP-TEV-TOP1* | (Deegan et al., 2019) | N/A |
| *yTDK20 (CMG purification): MAT***a** */ MATa*  *pep4::kanMX / pep4::kanMX*  *bar1::hph-NT1 / bar1::hph-NT1*  *ade2-1 / ade2-1*  *ura3-1 / ura3-1::pRS306-MCM2-GAL1,10-CBP-TEV-MCM3*  *his3-11::pRS303-CDC45iFLAG2-GAL1,10-GAL4* / *his3-11*  *trp1-1::pRS304-PSF1-GAL1,10-SLD5 / trp1-1::pRS304-MCM5-GAL1,10-MCM4*  *leu2-3::pRS305-PSF2-GAL1,10-PSF3 / leu2-3::pRS305-MCM7-GAL1,10-MCM6*  *ctf4-*I901E / *ctf4-*I901E | This study | N/A |
| *yPM224 (CMG-Mcm7-K29A purification): MAT***a** */ MATa*  *pep4::kanMX / pep4::kanMX*  *bar1::hph-NT1 / bar1::hph-NT1*  *ade2-1 / ade2-1*  *ura3-1 / ura3-1::pRS306-MCM2-GAL1,10-CBP-TEV-MCM3*  *his3-11::pRS303-CDC45iFLAG2-GAL1,10-GAL4* / *his3-11*  *trp1-1::pRS304-PSF1-GAL1,10-SLD5 / trp1-1::pRS304-MCM5-GAL1,10-MCM4*  *leu2-3::pRS305-PSF2-GAL1,10-PSF3 / leu2-3::pRS305-MCM7-K29A-GAL1,10-MCM6*  *ctf4-*I901E / *ctf4-*I901E | This study | N/A |
| **Oligonucleotides** |
| *CDC34* forward primer for construction of pTDK7: **6664**: ATTCTAtctagaaataattttgtttaactttaagaaggagatataccATGAGTAGTCGCAAAAGCACCGCTTC | This study | N/A |
| *CDC34* reverse primer for construction of pTDK7: **6665**: atcgatCTCGAGtgatccgccctgaaaatacaggttttcTATTTTCTTTGAAACTCTTTCTACATCCTC | This study | N/A |
| *OTU1* forward primer for construction of pTDK35: **8302**: gaacagattggtggcATGAAACTGAAAGTTACTGGAGCAGG | This study | N/A |
| *OTU1* reverse primer for construction of pTDK35: **8303**: gtgcggccgcttattaTCTATTTTGGCCAAAATCAACG | This study | N/A |
| Leading strand template for construction of model replication fork DNA: **Unblocked leading**: TAGAGTAGGAAGTGATGGTAAGTGATTAGAGAATTGGAGAGTGTGTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT\*T\*T\*T\*T\*T  [\* denotes a phosphorothioate bond] | This study | N/A |
| Lagging strand template for construction of model replication fork DNA [15 nt 5’ flap]: **Biotinylated lagging [15 nt arm]**: GGCAGGCAGGCAGGCACACACTCTCCAATTCTCTAATCACTTACCATCACTTCCTACTCTA-DesthioBiotin-TEG | This study | N/A |
| Lagging strand template for construction of model replication fork DNA [5 nt 5’ flap]: **Biotinylated lagging [5 nt arm]**: CAGGCACACACTCTCCAATTCTCTAATCACTTACCATCACTTCCTACTCTA-DesthioBiotin-TEG | This study | N/A |
| Lagging strand template for construction of model replication fork DNA [no 5’ flap]: **Biotinylated lagging [no arm]**: ACACACTCTCCAATTCTCTAATCACTTACCATCACTTCCTACTCTA-DesthioBiotin-TEG | This study | N/A |
| Cy3-labelled leading strand template for construction of model replication fork DNA: **DBO2**:  Cy3- TAGAGTAGGAAGTGATGGTAAGTGATTAGAGAATTGGAGAGTGTGTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT\*T\*T\*T\*T\*T  [\* denotes a phosphorothioate bond, T is internally biotinylated] | Joe Yeeles | N/A |
| **Recombinant DNA** |
| pAM3 [Cdc6 purification] | (Frigola et al., 2013) | N/A |
| pJY19 [PCNA purification] | (Yeeles et al., 2017) | N/A |
| pJFDJ5 [GINS purification] | (Yeeles et al., 2015) | N/A |
| pET28a-Mcm10 [Mcm10 purification] | (Yeeles et al., 2015) | N/A |
| pTF175 [FACT purification] | (Biswas et al., 2005) | N/A |
| pJW22 [FACT purification] | (Biswas et al., 2005) | N/A |
| pTDK10 [Pif1 purification] | (Deegan et al., 2019) | N/A |
| pTDK24 [Pif1 K264A purification] | (Deegan et al., 2019) | N/A |
| Ufd1 in K27SUMO [Ufd1-Npl4 purification] | (Stein et al., 2014) | N/A |
| Npl4 in pET21b [Ufd1-Npl4 purification] | (Stein et al., 2014) | N/A |
| Cdc48 in K27SUMO [Cdc48 purification] | (Stein et al., 2014) | N/A |
| Cdc48-FtsH in K27SUMO [Cdc48-FtsH purification] | (Bodnar and Rapoport, 2017) | N/A |
| FtsH in K27SUMO [FtsH purification] | (Bodnar and Rapoport, 2017) | N/A |
| pTDK3 [SCFDia2 purification] | This study | N/A |
| pTDK6 [SCFDia2 purification] | This study | N/A |
| pTDK7 [Cdc34 purification] | This study | N/A |
| pTDK35 [Otu1 purification] | This study | N/A |
| pBS/ARS1WTA [3.2 kb replication template] | (Marahrens and Stillman, 1992) | N/A |
| λ DNA-HindIII Digest: molecular weight markers | New England Biolabs | N3012S |
| **Software and Algorithms** |
| ImageJ | National Institute of Health | https://imagej.nih.gov/ij/ |