

SUPPLEMENTARY TABLES

Supplementary Table 1. Antibodies used in this study

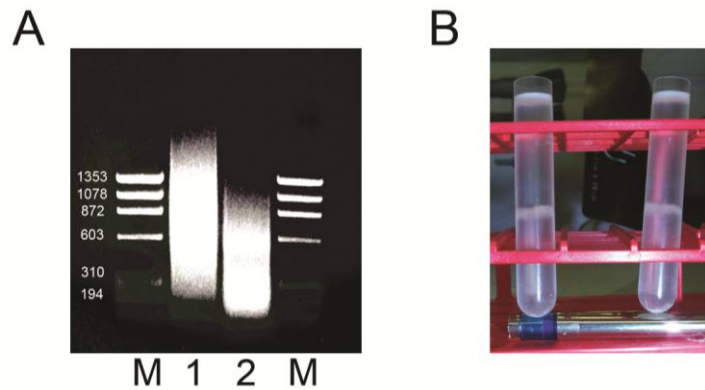
| Target | Clone/Name | Species/type | Source/purification | Application^a | Reference |
|--|-------------------|----------------------|-------------------------------|--------------------------------|------------------|
| Pol α catalytic subunit | p140 | Rabbit polyclonal | Protein A | ChIP/WB | (24) |
| Pol δ catalytic subunit | K30 | Rabbit polyclonal | Protein A | ChIP/WB | (20) |
| | K31, K32, K33 | Rabbit polyclonal | Protein A | ChIP/WB | this study |
| | 7B4 | Rat Monoclonal | Hybridoma supernatant | WB | (20) |
| Pol ϵ catalytic subunit | G1A, H3B, E24C | Mouse monoclonal | Protein G | WB | (25) |
| | K19 | Rabbit polyclonal | Protein A | ChIP | (20) |
| | K27 | Rabbit polyclonal | Protein A | ChIP | (26) |
| PCNA | PC10 | Mouse monoclonal | Sigma/Zymed | WB | |
| Mcm2 | N19 | Rabbit polyclonal | Santa Cruz Biotechnologies | WB | |
| Mcm3 | N19 | Rabbit polyclonal | Santa Cruz Biotechnologies | WB | |
| Lamin A/C | N18 | Goat polyclonal | Santa Cruz Biotechnologies | ChIP/WB | |
| Cdc45 | 3G10 | Rat monoclonal | Hybridoma supernatant | WB | (27) |
| Orc2 | 3B7 | Mouse monoclonal | Stressgen | WB | |
| β -tubulin | KMX-1 | Mouse monoclonal | Chemicon | WB | |
| Cyclin A | H-432 | Rabbit polyclonal | Santa Cruz Biotechnologies | WB | |

^aAbbreviations: ChIP - chromatin immunoprecipitation, WB - Western blot

Supplementary Table 2. Primers used in this study

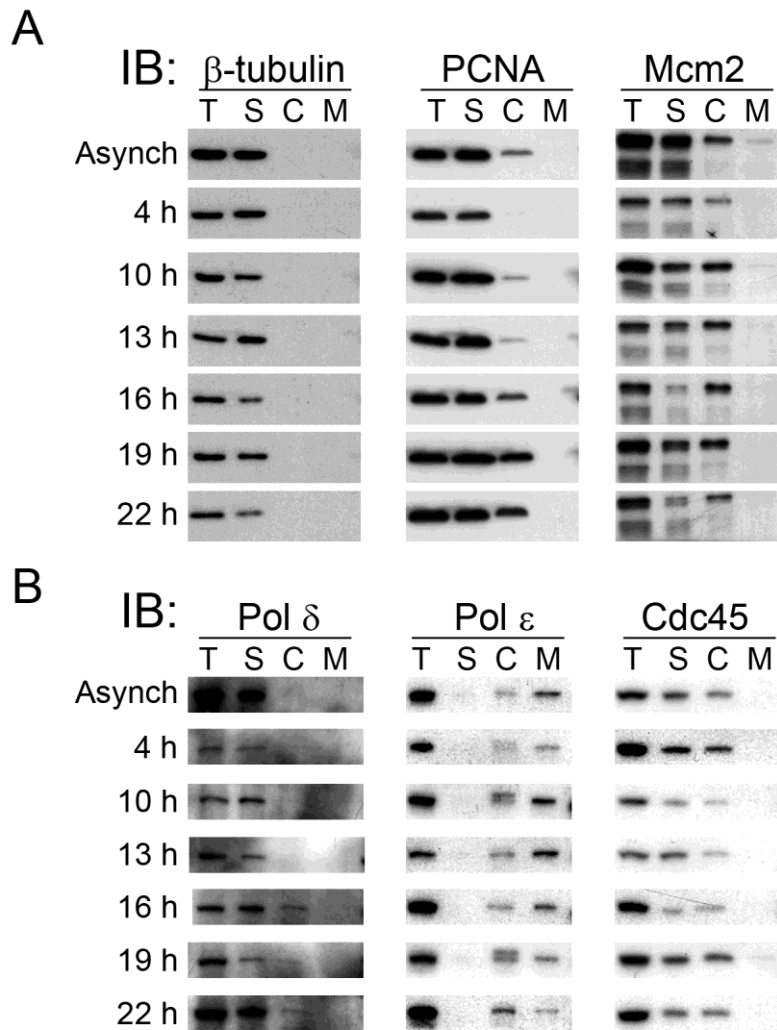
| Primer | Sequence (5' to 3') | Amplicon length (bp) | Annealing temp (°C) | Reference |
|------------------|---|-----------------------------|----------------------------|------------------|
| LB2C4F LB2C4R | ACACCGTGAGACGCGTTTGACC CAACAACCCATGAGCACCCCTGG | 138 | 63 | This study |
| LB2C3F LB2C3R | CATTTCTTGGGCAAATGCCTAGG AGATGGGGTTTCTCCATGTTGG | 160 | 63 | This study |
| LB2C1F LB2C1R | GTTAACAGTCAGGCGCATGGGCC CCATCAGGGTCACCTCTGGTTCC | 240 | 64 | (30) |
| LB2F LB2R | GGCTGGCATGGACTTTCATTTTCAG GTGGAGGGATCTTTCTTAGACATC | 232 | 66 | (30) |
| LB2C2F LB2C2R | TTCTGACCTCCAGCCCTGCAG ACCCAGTAGAAAGCTGCCCTC | 107 | 63 | This study |
| Ex9F Ex9R | TCTCAGAGGGTACCTGGTTTGG GGAATGTAGGGAGCTGCGGTG | 90 | 60 | This study |
| UPRF UPRR | GGTCAAGAGTTCCAAGTTTGTTCCT TGCAGGCGGGCATCAC | 72 | 60 | This study |
| In6F In6R | GACATTCTGCTTCCATAGATGTGG GTTGGGAAAGATGTCATCATCAGG | 346 | 55 | (30) |
| In7F In7R | GAGGAATGCCAGAATTTCCAGAGG TTCCATCTGGAATGAGATCCCAGC | 327 | 58 | (30) |
| b-satF b-satR | TGTCACAATGCCCCTGTAGG ACCCAGGTGATGTAACCTCTTGT | 68 | 59 | This study |

SUPPLEMENTARY FIGURES

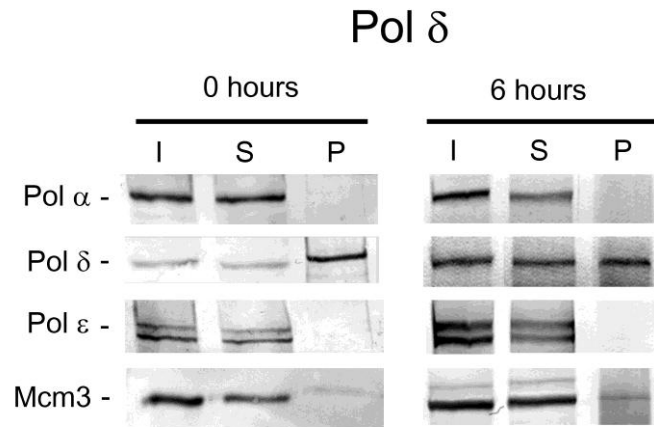


Suppl. Figure 1. A. Analysis of nucleoprotein DNA by agarose gel electrophoresis. Crosslinked chromatin isolated from CsCl ultracentrifugation gradient was sonicated and then further digested with micrococcal nuclease. Size distribution of DNA was analyzed in 1% agarose/ethidiumbromide gel after the phenol/chloroform extraction and ethanol precipitation. M, molecular weight markers; Lane 1, sonicated nucleoprotein DNA; Lane 2, sonicated and micrococcal nuclease digested nucleoprotein DNA. **B.** Purification of nucleoprotein DNA. Cesium chloride density gradient centrifugation was performed to isolate crosslinked chromatin from free DNA and protein. Under high centrifugal force nucleoprotein placed in this gradient migrates to the isopycnic region (white zone in the middle of the tube)

Supplementary Figure 2



Suppl. Figure 2. Chromatin and matrix association of replicative DNA polymerases during S phase. T98G cells synchronised by serum stimulation were subjected to subnuclear fractionation and relevant proteins were analysed by Western blotting. **A.** Analysis of marker proteins β -tubulin, PCNA and Mcm2 in fractionated T98G cells synchronised by serum stimulation. **B.** Analysis of Pols δ and ϵ as well as Cdc45 in fractionated T98G cells synchronised by serum stimulation. For each time point samples corresponding an equal number of fractionated cells were applied to SDS-PAGE and Western analysis to determine sub-cellular distribution of the respective protein at each time point. T, total cell extract; S, soluble fraction; B, chromatin bound fraction and M, matrix fraction.



Suppl. Figure 3. Association of Pol α , Pol ϵ and Mcm3 with S phase nucleoprotein complexes isolated by precipitation with antibodies against Pol δ . Mcm3, but essentially no Pol α or ϵ are co-precipitated. HeLa cells were synchronized by double thymidine block to G1/S (0 hours) and released to proceed into late S phase (6 hours). The antibody used for Western blotting is indicated on left site of the panels. Nucleoproteins were isolated and treated as described under “Experimental Procedures” for chromatin immunoprecipitation. Nucleoproteins derived from CsCl centrifugation were sonicated and digested with micrococcal nuclease as described in Experimental Procedures. Soluble nucleoproteins, 1 mg/ 500 μ l, were taken for immunoprecipitation with the cognate antibodies. 10 μ l of soluble nucleoprotein complex (In), 10 μ l of supernatant after precipitation (S) and entire precipitate (P) were loaded onto gel.