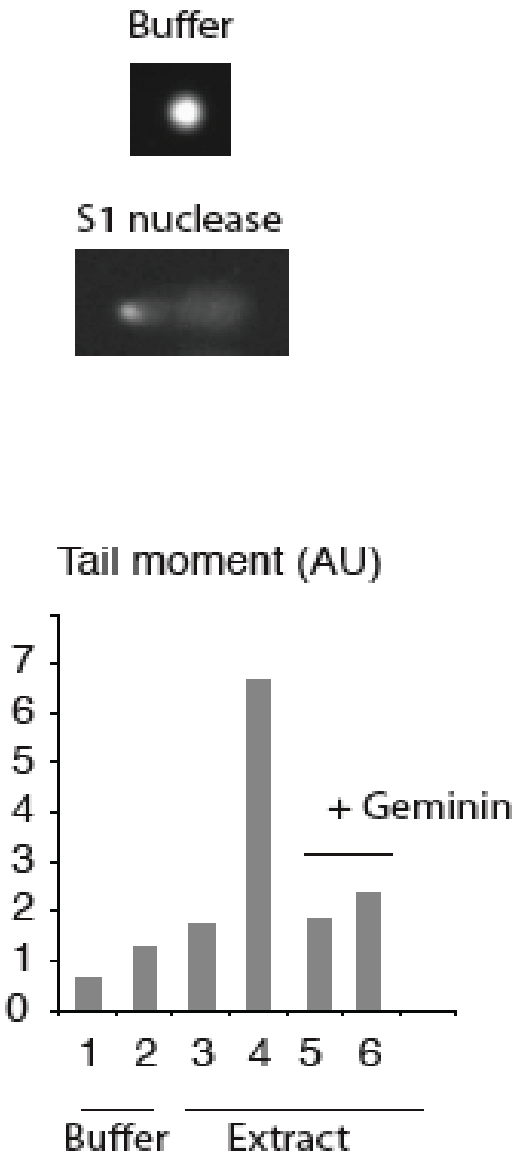


Figure S1. DSB formation induced by S1 endonuclease treatment on replicating DNA. (a) Comet assay performed on sperm nuclei following S1 nuclease treatment. Sperm DNA was incubated for 80 min in buffer, interphase extract or interphase extract treated with 80 nM recombinant geminin to block replication in the absence (columns 1, 3, 5) or presence of S1 nuclease (0.55 units/µl) (columns 2, 4, 6). Nuclei were processed for comet assay in alkaline conditions and average tail moment was calculated for 100 nuclei and plotted on the graph.

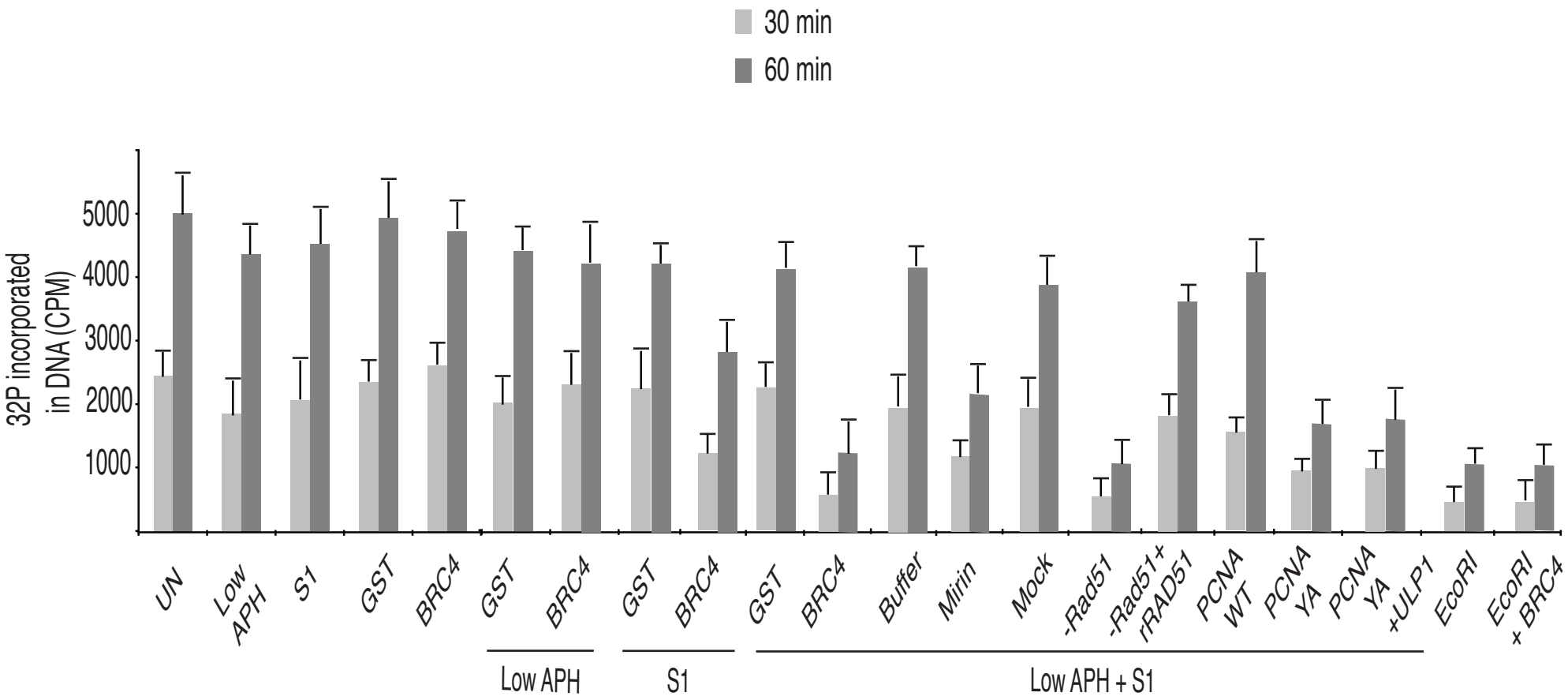


Figure S2. DNA replication quantification under different conditions. Sperm DNA was incubated for 30 and 60 min in egg extract in the presence 32P-dATP. Extracts (from left to right) were left untreated (UN), treated with 1 µg/ml aphidicolin (Low Aph), 0.37 units/µl S1 nuclease (S1), GST or GST-BRC4 (BRC4) alone or with 1 µg/ml aphidicolin or 0.37 units/µl S1 nuclease as indicated. Extracts were also treated with 1 µg/ml aphidicolin and 0.37 units/µl S1 nuclease and supplemented with GST, GST-BRC4, mirin buffer (Buffer) or mirin. To validate the results obtained with GST-BRC4 addition extracts were mock (Mock), Rad51 depleted (-Rad51) or Rad51 depleted and supplemented with 100 nM Rad51 recombinant protein (-Rad51 + rRad51). To test the effects of sumoylation of PCNA mutant proteins extracts were supplemented with PCNA-WT, PCNA-YA and PCNA-YA plus ULP1 desumoylating enzyme. In the last two samples extracts were treated with 0.01 unit/µl EcoRI in the absence (EcoRI) or in the presence of GST-BRC4 (EcoRI+BRC4). Genomic DNA was precipitated and spotted on filters as described in Methods. Radioactivity was measured and average counts per minute (CPM) were plotted on graph. Experiments were repeated 3 times. Error bars represent standard deviation.

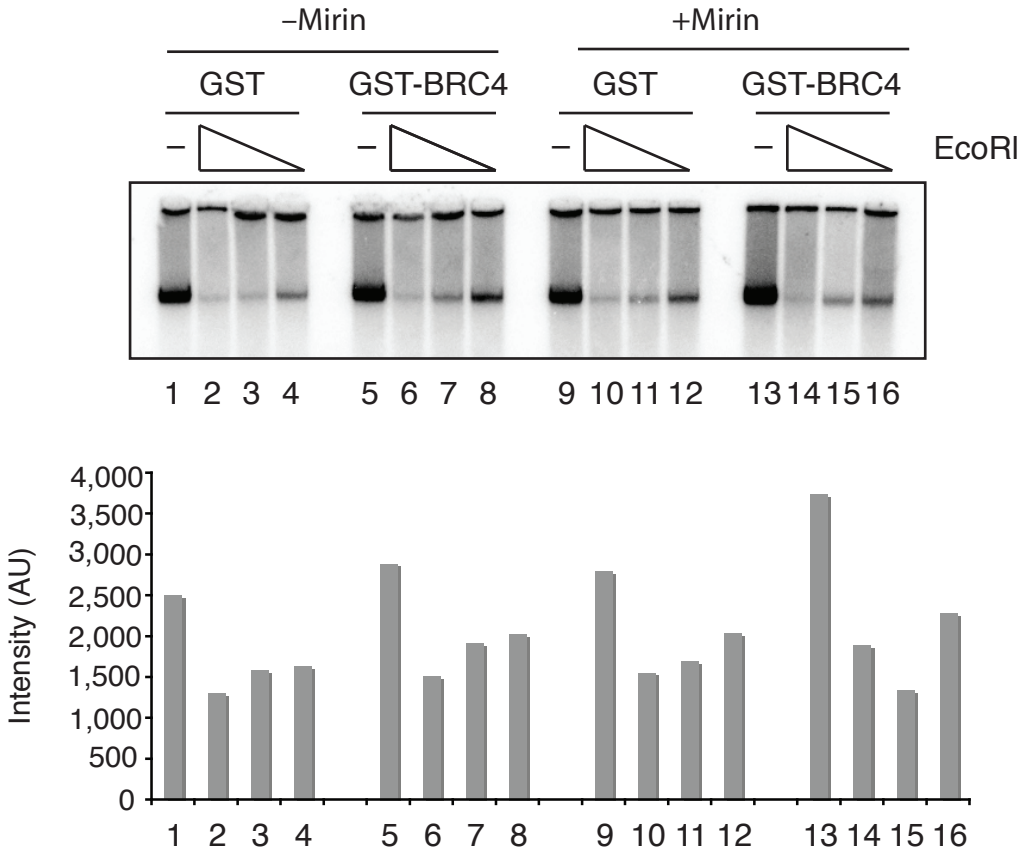


Figure S3. The effects of DSBs on DNA replication in the presence and absence of Rad51 or mirin. Sperm DNA was incubated for 80 min in egg extracts with or without GST and GST-BRC4 and mirin in the presence of EcoRI (–; no addition, triangle; from left to right, 0.02, 0.01, 0.005 unit/μl). The replication products were resolved with neutral agarose gel and subjected to autoradiography. The signal intensities in each lane were quantified and reported in the graph.

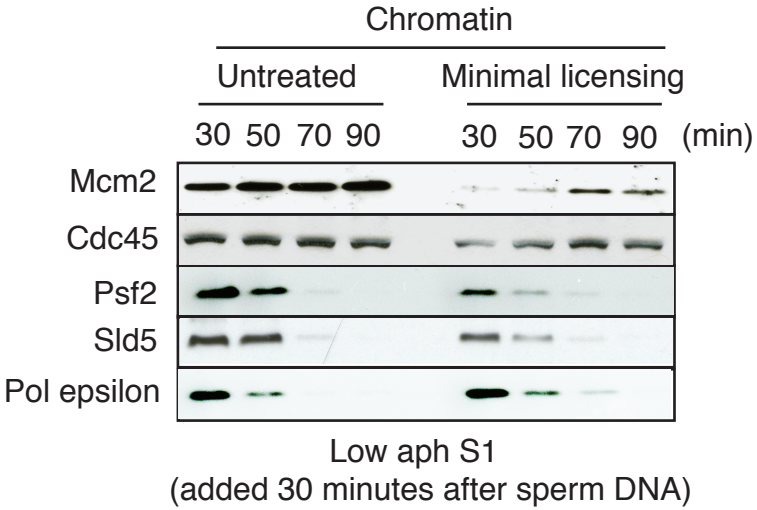
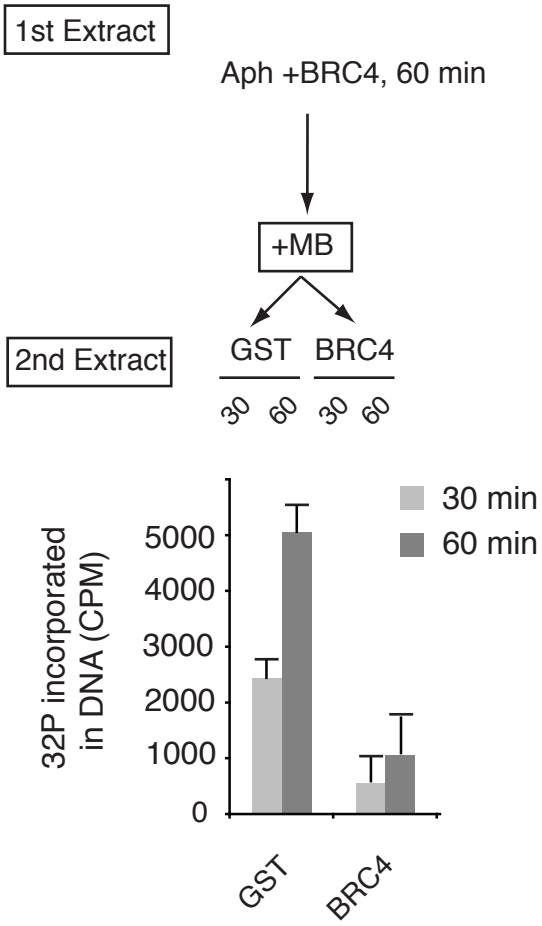


Figure S4. The effects of template breakage on DNA replication factors bound to chromatin under minimal licensing conditions. Chromatin binding of Mcm2, Cdc45, Sld5, Psf2 and Pol epsilon in extracts that were untreated or supplemented with 320 nM geminin 3 min after sperm nuclei addition to induce minimal licensing. Extracts were all supplemented with 1 μg/ml aphidicolin and 0.37 units/μl S1 nuclease 30 min after sperm nuclei addition (Low aph + S1 nuclease)



MB: mung bean nuclease
Aph: aphidicolin

Figure S5. Rad51 requirement for replication restart after fork collapse. Sperm nuclei were incubated for 60 min in the presence of 10 µg/ml of aphidicolin and GST-BRC4 in the 1st extract, nuclear fractions were isolated and treated with (+) or without (–) Mung Bean nuclease, then transferred to the 2nd extract containing 32P-dATP, geminin, roscovitine, and GST or GST-BRC4 for the indicated times. Genomic DNA was precipitated and spotted on filters as described in Methods. Radioactivity was measured and average counts per minute (CPM) were plotted on graph. Experiments were repeated 3 times. Error bars represent standard deviation.

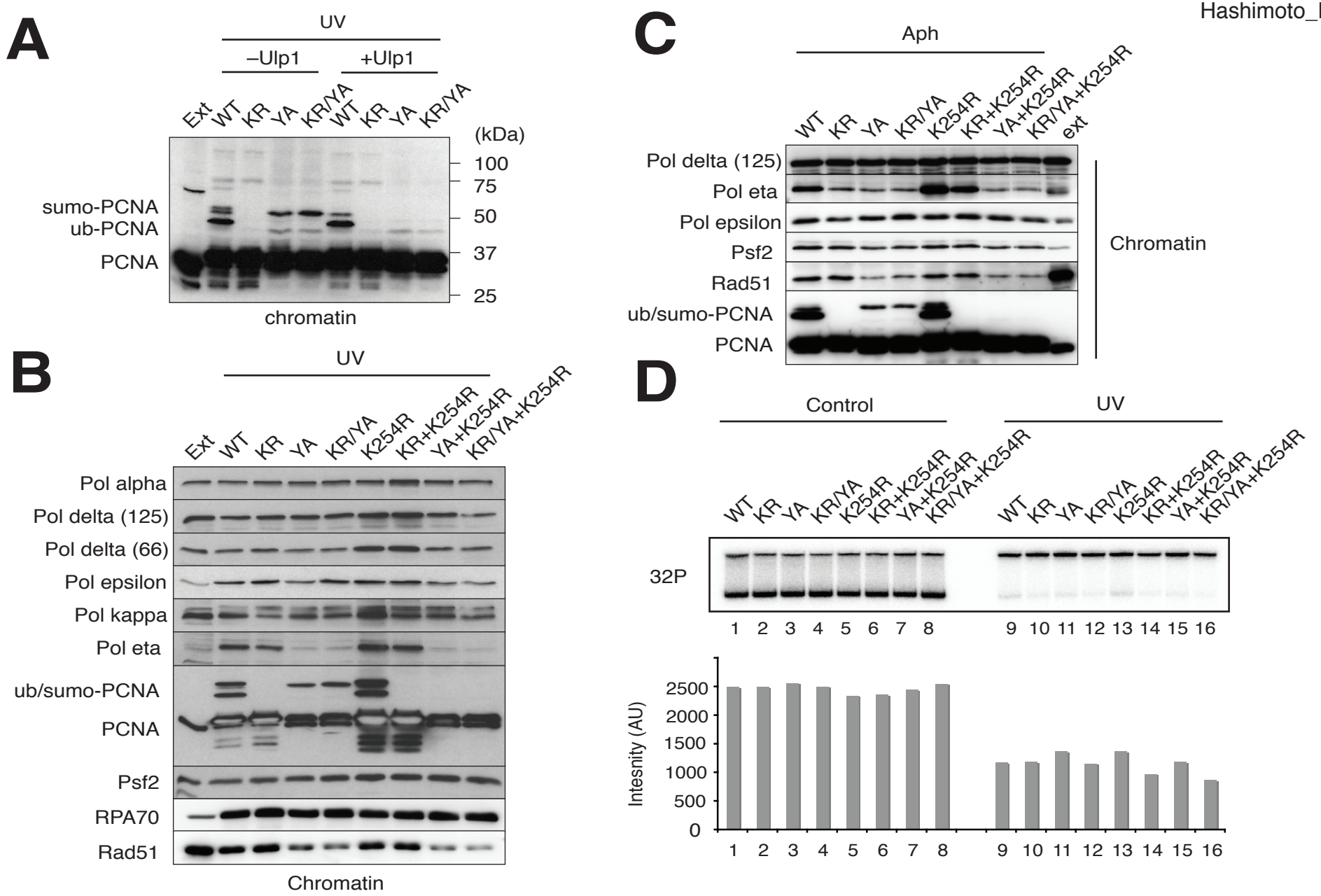


Figure S6. The effect of sumoylation of PCNA on chromatin association of replication proteins and replication activity. (A, B, C) Sperm DNA was incubated in extracts treated with UV; 200 J m-2, aph; aphidicolin 10 μg/ml and the following recombinant proteins: WT: PCNA wild type, KR: PCNA K164R, YA: PCNA Y249A Y250A, KR/YA: PCNA K164R Y249A Y250A, K254R: PCNA K254R, KR+K254R: PCNA K164R K254R, YA+K254R: PCNA Y249A Y250A K254R, KR/YA+K254R: PCNA K164R Y249A Y250A K254R. In (A), UV-treated chromatin was incubated with (+) or without (–) Ulp1 in egg extract. Ext: 0.5 μl egg extract was loaded as a control. Chromatin fractions were all isolated after 60 minutes incubation in egg extract. (D) Sperm DNA was incubated for 60 min at the indicated conditions, and the replication products were resolved with neutral agarose gel and subjected to autoradiography, and their signal intensities were quantified.