Vidal-Eychenié et al., http://www.jcb.org/cgi/content/full/jcb.201304139/DC1

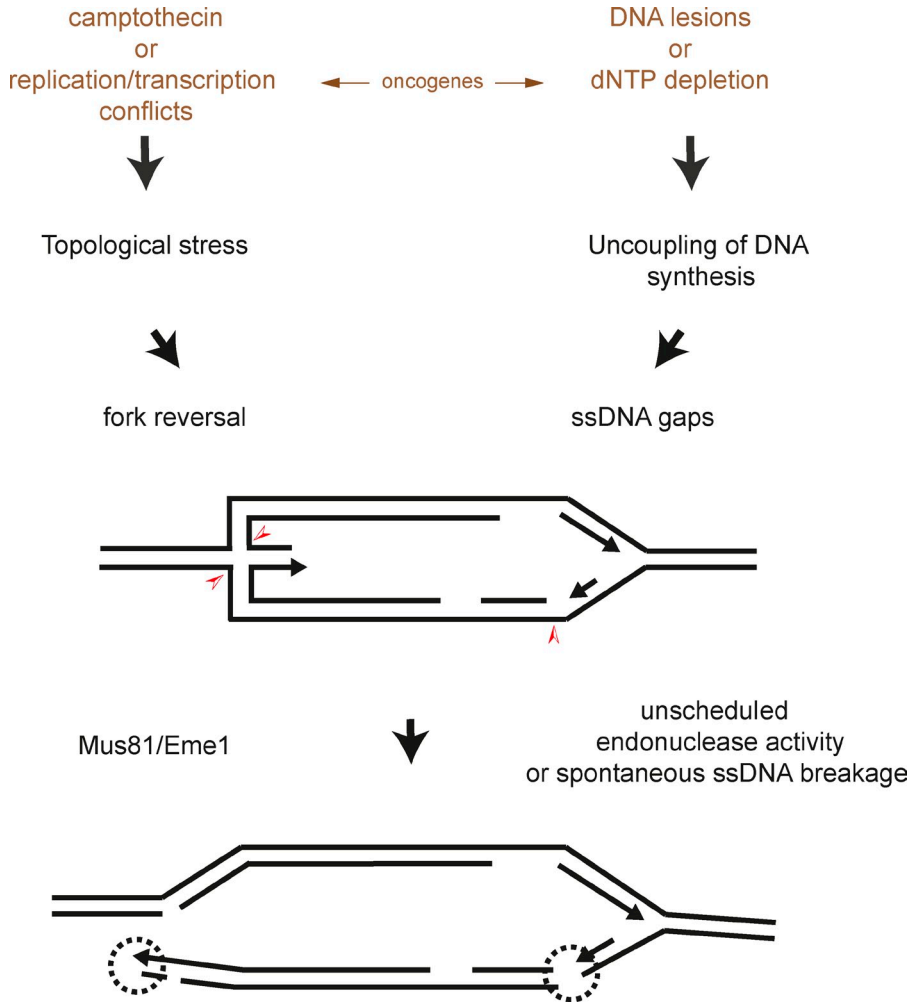


Figure S1. **Formation of dsDNA breaks in replication intermediates.** DNA replication stress yields unusual DNA intermediates. Replication fork reversal resorbs positive supercoils that accumulate ahead of the moving transcription and replication machineries. Topological stress is aggravated by oncogene expression, which exacerbates replication/transcription conflicts, or by CPT poisoning of topoisomerase I. Four-way junctions formed by replication fork reversal can include nicks or gaps at the branch point and are cleaved by the Mus81/Eme1 endonuclease. Oncogene-induced nucleotide pool imbalance or bulky DNA adducts in either one of the template DNA strands induce uncoupled DNA synthesis and the accumulation of ssDNA gaps in replicated duplexes. If left unrepaired, promiscuous nuclease activities can convert ssDNA gaps into DSBs. The red arrowheads indicate sites of DNA breaks

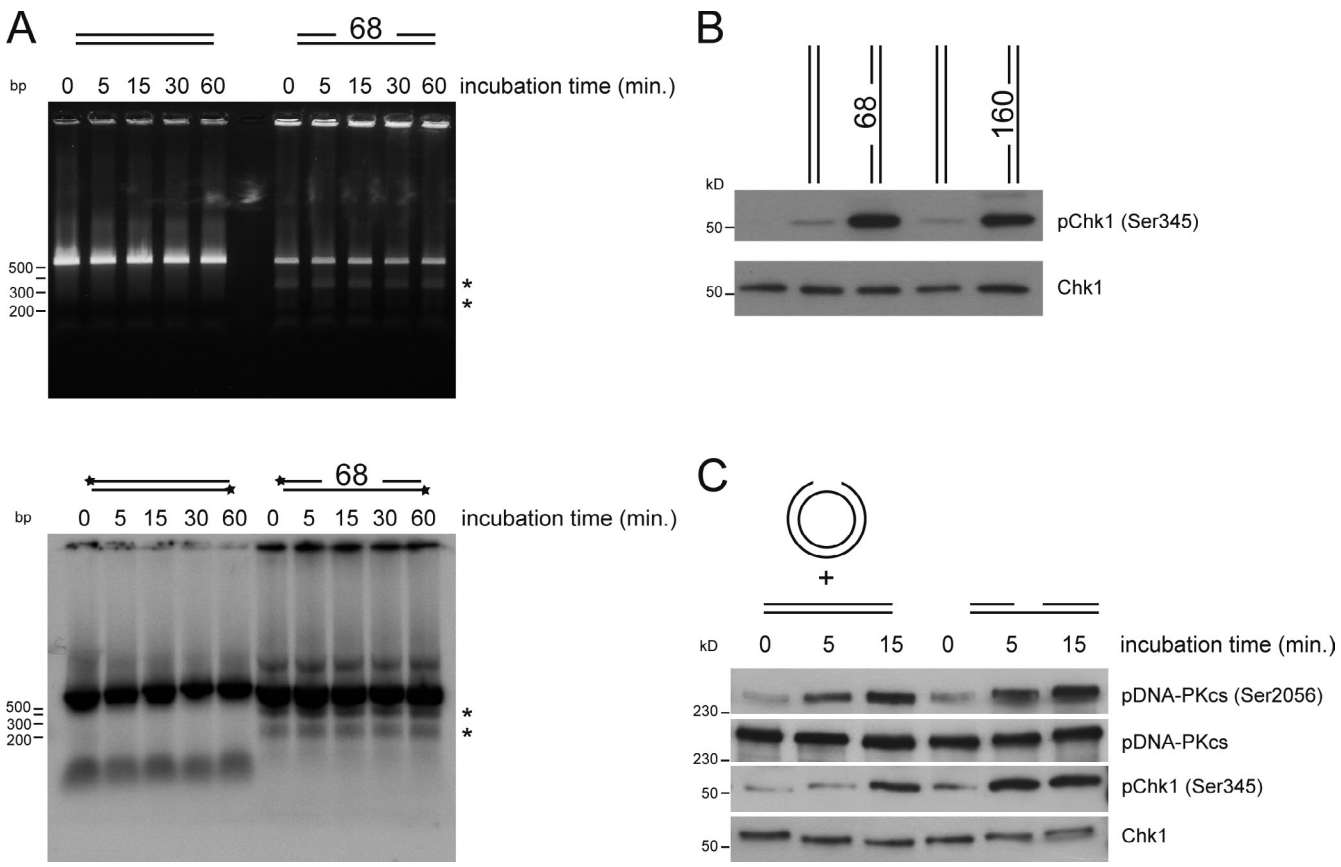


Figure S2. **DNA stability and DNA structural determinants in DNA damage signaling reactions.** (A) DNA damage signaling reactions were assembled as described in Material and methods and incubated at 37°C for the indicated time period, and the DNA was deproteinized by incubation with 2 mg/ml proteinase K and 0.4% SDS for 10 min at 37°C. (top) DNA products (400 ng/slot) were separated by electrophoresis through 1% agarose gels in TBE (Tris, borate, and EDTA) buffer containing 0.5 µg/ml ethidium bromide. (bottom) 5′-32P end–labeled DNA products (200 ng/slot) were resolved by 1% agarose gel electrophoresis and visualized by phosphoimaging. Asterisks indicate the background of SpeI digestion products in gDNA preparations. SpeI was used to eliminate linear DNA duplexes that do not include an ssDNA gap, as described in Materials and methods. (B) Side-by-side comparison of Chk1 phosphorylation in reaction mixtures supplemented with duplex DNA or gapped linear DNA containing either a 68- or a 160-nt ssDNA gap, as indicated. (C) Reaction mixtures supplemented with either 5 nM gapped circular DNA plus 5 nM linear duplex DNA or 5 nM gapped linear DNA were incubated for the indicated period of time and probed side by side for the indicated proteins by Western blotting. Note that gapped linear DNA induces Chk1 phosphorylation more readily than a mixture of gapped circular and linear DNA molecules. shcontrol, control shRNA.

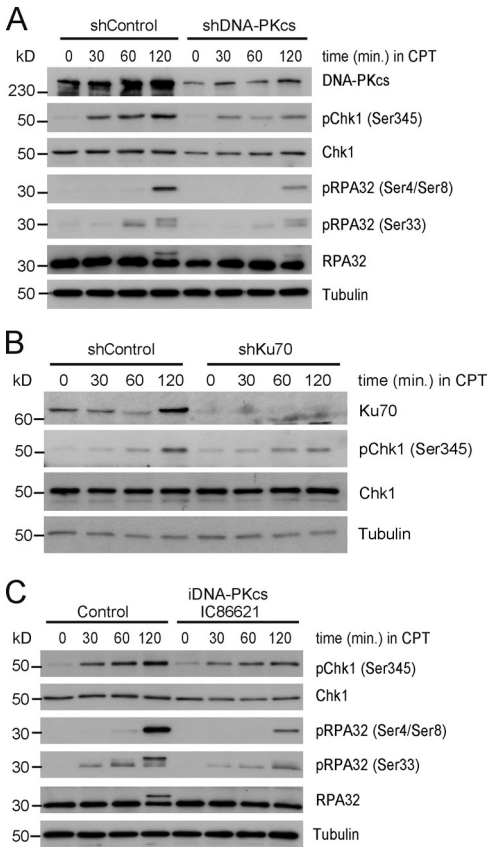


Figure S3. **DNA-PK contributes to ATR signaling upon CPT-induced replication stress.** All experiments were performed with U2OS cells. (A) The knockdown of DNA-PKcs impairs pSer345 Chk1 and pSer4/8 RPA32 signals in response to 1 μM CPT. (B) Compared with cells treated with control shRNA, the depletion of Ku70 impairs the phosphorylation of Chk1 on Ser345 after treatment for 120 min with 1 μM CPT. (C) Chemical inhibition of DNA-PKcs with 100 μM IC86621 impairs pSer345 Chk1 and pSer4/8 RPA32 signals after 120 min treatment with 1 μM CPT.