

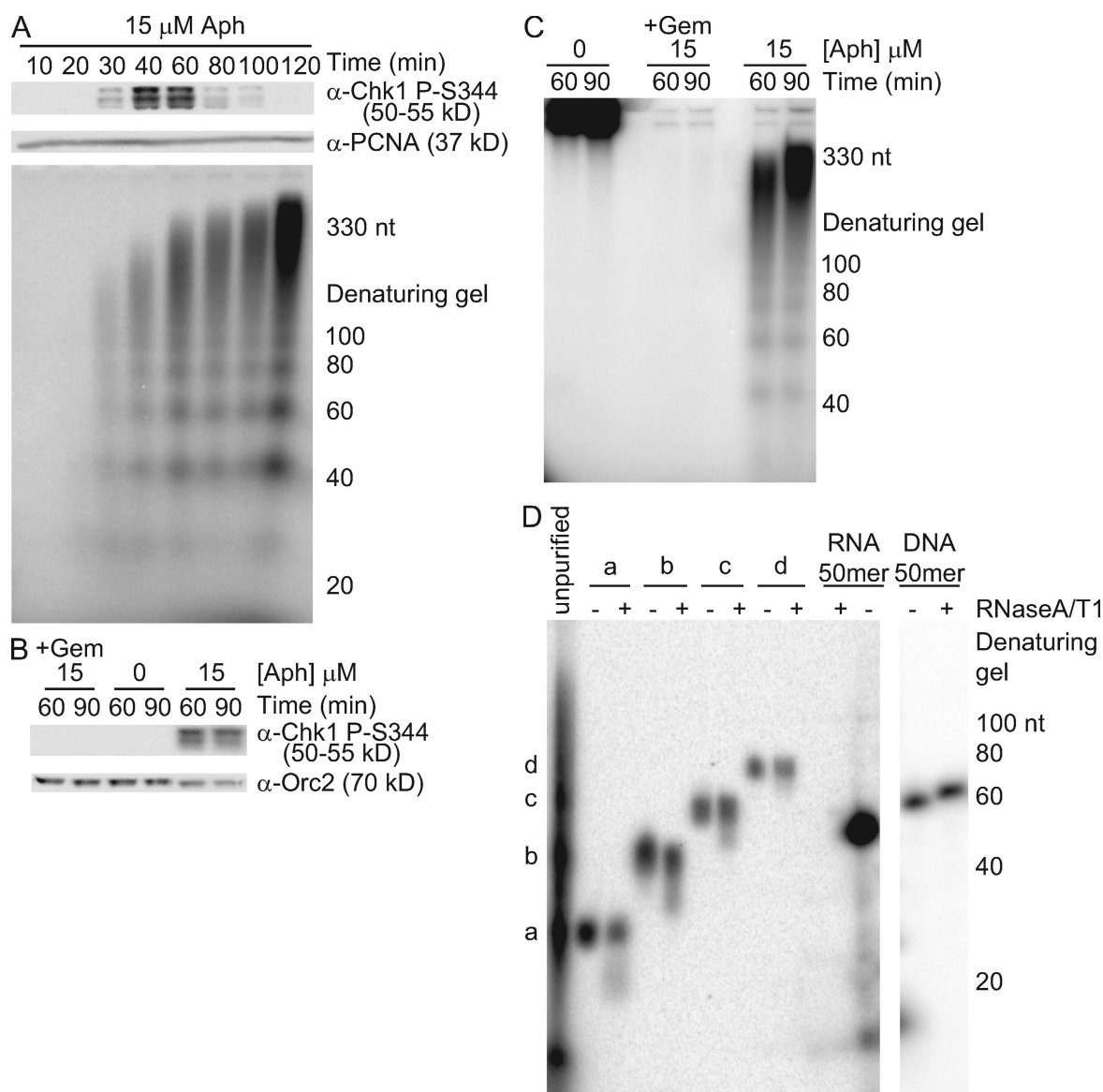
Van et al., <http://www.jcb.org/cgi/content/full/jcb.200909105/DC1>

Figure S1. Replication-dependent primers accumulate on aphidicolin-treated chromatin and contain a variable 5' RNA component. (A) Sperm chromatin (2,000 nuclei/ μ l) was replicated in HSE and NPE containing α - 32 P]dCTP and 15 μ M aphidicolin (Aph). Samples were taken at the indicated times and immunoblotted for Chk1 P-S344 or analyzed for small nascent DNAs as in Fig. 1 A. (B and C) A 9-kb plasmid was replicated in HSE and NPE containing α - 32 P]dCTP with or without 15 μ M aphidicolin. Geminin (Gem) was preincubated with HSE on ice for 15 min where indicated. Samples were taken at the indicated times after NPE addition and immunoblotted for Chk1 phosphorylation (B) or small nascent DNAs (C) as in Fig. 1 A. (D) A 9-kb plasmid was replicated in HSE and NPE containing α - 32 P]dCTP, RNase Out, and 15 μ M aphidicolin. Parallel samples were taken at 30 min and saved for later analysis (unpurified) or run on a denaturing gel. After autoradiography, four bands (a–d) were gel purified and treated with or without RNaseA/T1. Control 5' γ - 32 P]ATP-labeled 50-mer RNA and DNA oligonucleotides were also treated with or without RNaseA/T1. Samples were analyzed on a second denaturing gel alongside the unpurified sample and autoradiographed.

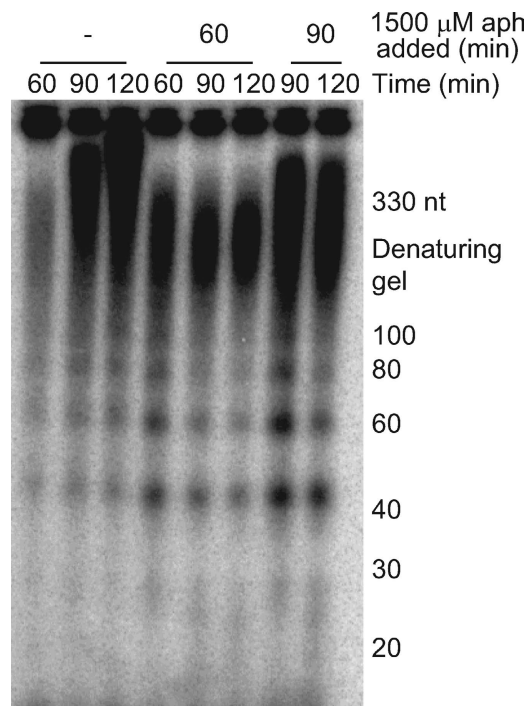


Figure S2. **Small nascent DNAs are unstable.** A 9-kb plasmid was replicated in HSE and NPE with α - ^{32}P]dCTP and 15 μM aphidicolin (aph). At the indicated time points after NPE addition, reactions were further supplemented with 1,500 μM aphidicolin. Samples were taken and analyzed on a denaturing gel as in Fig. 1 A.

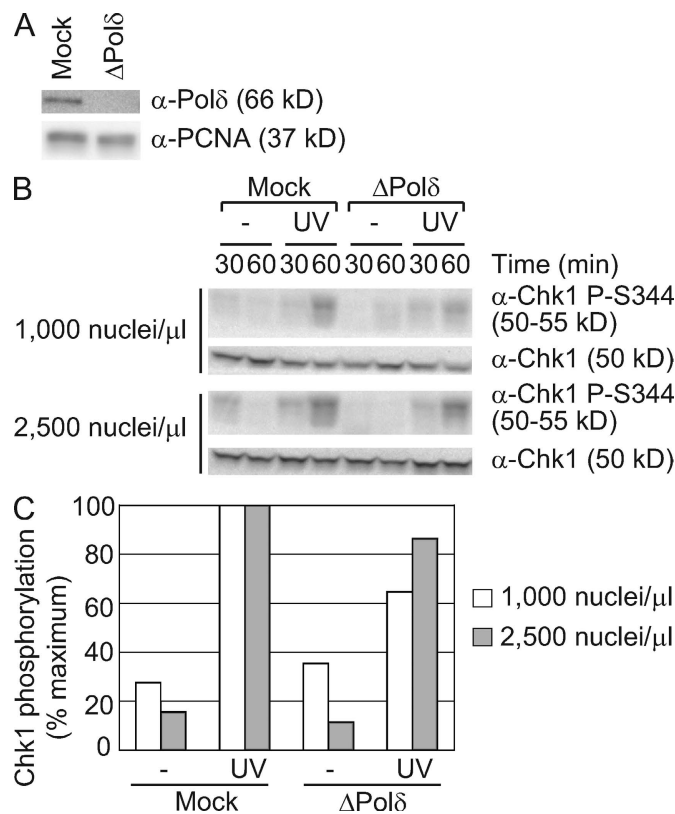


Figure S3. **The effect of Pol- δ depletion on Chk1 phosphorylation is dependent on chromatin concentration.** (A–C) UV light-treated (1,000 J/m²) or untreated sperm chromatin was replicated at the indicated concentrations in mock- or Pol- δ -depleted LSE. (A) Depletion was verified by immunoblotting. (B) Chk1 phosphorylation was analyzed at the indicated times as in Fig. 1 A. (C) Intensities at 60 min were calculated relative to the mock-depleted, UV light-treated samples as in Fig. 6 B. Data are representative of two experiments.