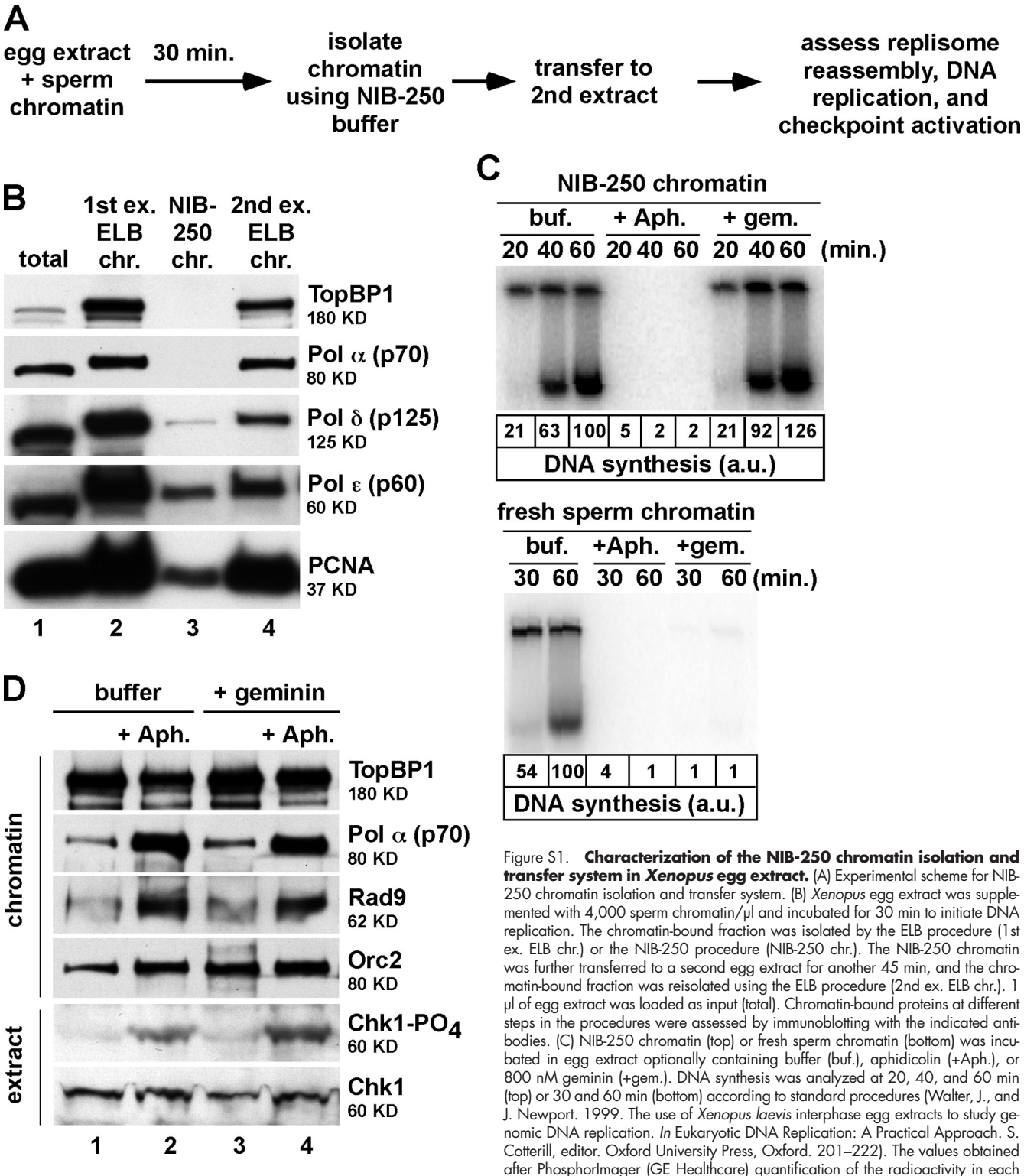


Figure S1. **Characterization of the NIB-250 chromatin isolation and transfer system in *Xenopus* egg extract.** (A) Experimental scheme for NIB-250 chromatin isolation and transfer system. (B) *Xenopus* egg extract was supplemented with 4,000 sperm chromatin/µl and incubated for 30 min to initiate DNA replication. The chromatin-bound fraction was isolated by the ELB procedure (1st ex. ELB chr.) or the NIB-250 procedure (NIB-250 chr.). The NIB-250 chromatin was further transferred to a second egg extract for another 45 min, and the chromatin-bound fraction was reisolated using the ELB procedure (2nd ex. ELB chr.). 1 µl of egg extract was loaded as input (total). Chromatin-bound proteins at different steps in the procedures were assessed by immunoblotting with the indicated antibodies. (C) NIB-250 chromatin (top) or fresh sperm chromatin (bottom) was incubated in egg extract optionally containing buffer (buf.), aphidicolin (+Aph.), or 800 nM geminin (+gem.). DNA synthesis was analyzed at 20, 40, and 60 min (top) or 30 and 60 min (bottom) according to standard procedures (Walter, J., and J. Newport. 1999. The use of *Xenopus laevis* interphase egg extracts to study genomic DNA replication. *In* Eukaryotic DNA Replication: A Practical Approach. S. Cotterill, editor. Oxford University Press, Oxford. 201–222). The values obtained after PhosphorImager (GE Healthcare) quantification of the radioactivity in each lane are shown below. The value obtained for the 60-min time point in the sample receiving buffer was arbitrarily set to 100, and all other values were adjusted accordingly. a.u., arbitrary unit. (D) NIB-250 chromatin was added to egg extract optionally containing aphidicolin and/or 800 nM geminin. After a 45-min incubation, the chromatin-bound proteins were isolated using ELB procedure and examined for the presence of the indicated checkpoint proteins via Western blotting (chromatin panel). The total extract was also analyzed for Chk1 phosphorylation by immunoblotting (extract panel). Orc2 was used as a loading control in this and subsequent experiments.

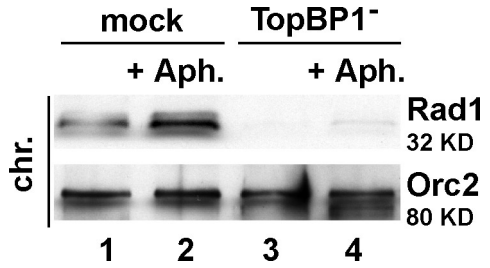


Figure S2. **TopBP1 is required for recruitment of the Rad1 subunit of 9-1-1 to stalled replication forks.** Chromatin (chr.) was isolated from either mock- or TopBP1-depleted extracts as in Fig. 1 B. These samples were then probed by Western blotting for Rad1 and Orc2. The Rad1 antibody has been described previously (Lupardus, P.J., and K.A. Cimprich. 2006. *Mol. Biol. Cell.* 17:1559–1569). Aph., aphidicolin.

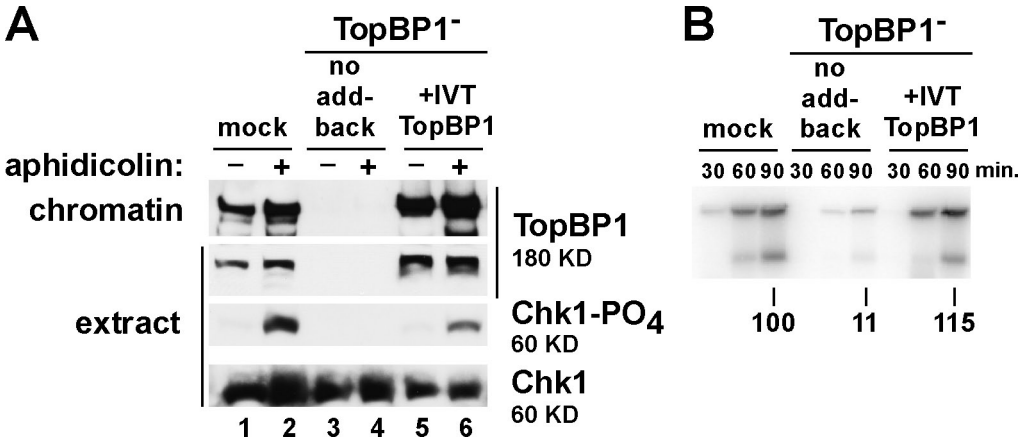


Figure S3. **Rescue of TopBP1-depleted egg extract with recombinant TopBP1 produced by IVT.** (A) TopBP1-depleted extract was split into two parts, with one part receiving unprogrammed rabbit reticulocyte lysate (no add back) and the other part receiving TopBP1-producing rabbit reticulocyte lysate (+IVT TopBP1). In addition, the mock-depleted extract also received unprogrammed rabbit reticulocyte lysate. These reconstituted extracts were then supplemented with sperm chromatin and optional aphidicolin (+). After a 45-min incubation, the chromatin was isolated using the ELB procedure, and the amount of TopBP1 in the chromatin fractions was determined by Western blotting. Samples of the total extracts were also examined via immunoblotting. We note that the amount of Chk1 phosphorylation that was observed in the reconstituted extract is less than that observed in the mock-depleted control. The anti-TopBP1 antibody that we use for immunodepletion inhibits ATR signaling (Yan, S., H.D. Lindsay, and W.M. Michael. 2006. *J. Cell Biol.* 173:181–186), and, thus, the reduced Chk1 phosphorylation observed in the TopBP1-depleted reconstituted extract is likely caused by trace amounts of this antibody having leached off the beads during the immunodepletion. Consistent with this hypothesis is the observation that IVT TopBP1 fully restores DNA replication in the immunodepleted extract (B), as DNA replication is known to be insensitive to the anti-TopBP1 antibody (Yan et al., 2006). (B) The reconstituted extracts described in A were combined with sperm chromatin, and DNA synthesis was analyzed at 30, 60, and 90 min as in Fig. S1 C. The numbers below the gel refer to the relative amounts of radioactivity present in the samples at the 90-min time point.

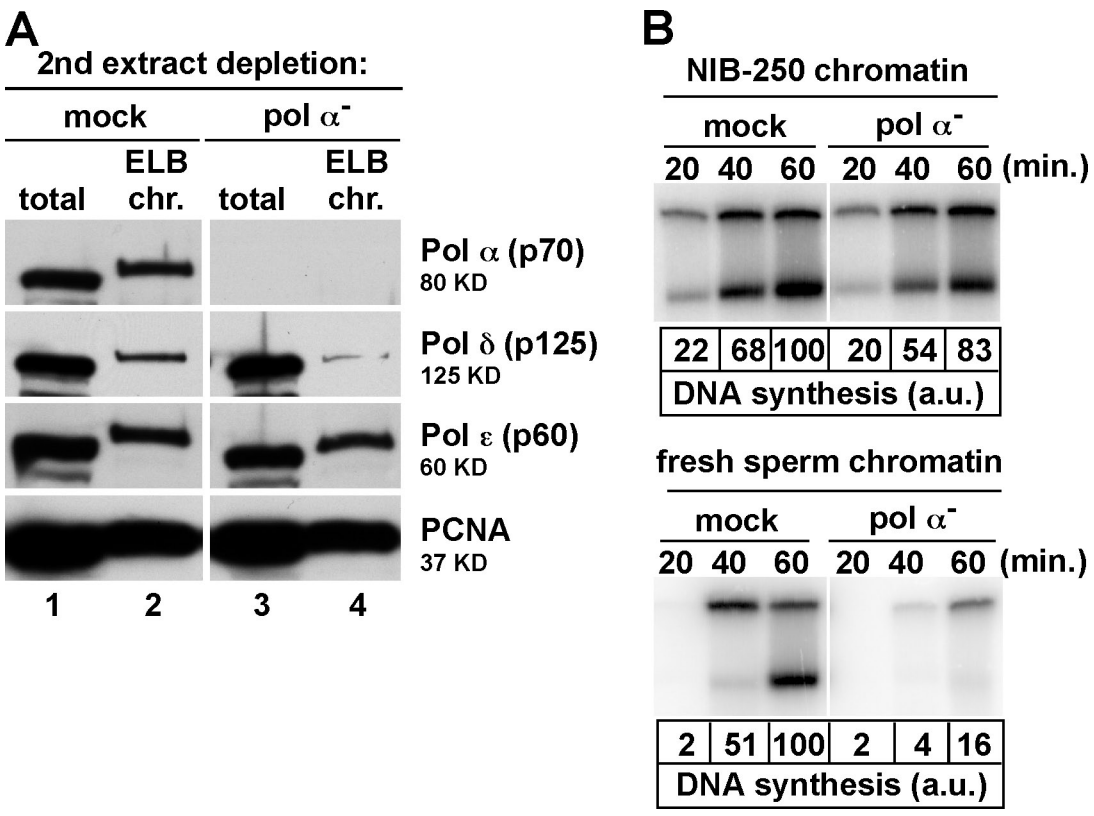


Figure S4. **Replisome reassembly and activity in pol-α–depleted extract containing NIB-250 chromatin.** (A) NIB-250 chromatin was incubated in mock- or pol-α (pol-α⁻)–depleted extract (total) for 45 min, and the ELB procedure was used to isolate chromatin-bound fraction (ELB chr.). The indicated replicative pols (pol-α, -δ, and -ε) and PCNA were probed by Western blotting. (B) NIB-250 chromatin (top) or fresh sperm chromatin (bottom) was incubated in either mock- or pol-α (pol-α⁻)–depleted extract. DNA synthesis products were examined at 20, 40, and 60 min as in Fig. S1 C. a.u., arbitrary unit.

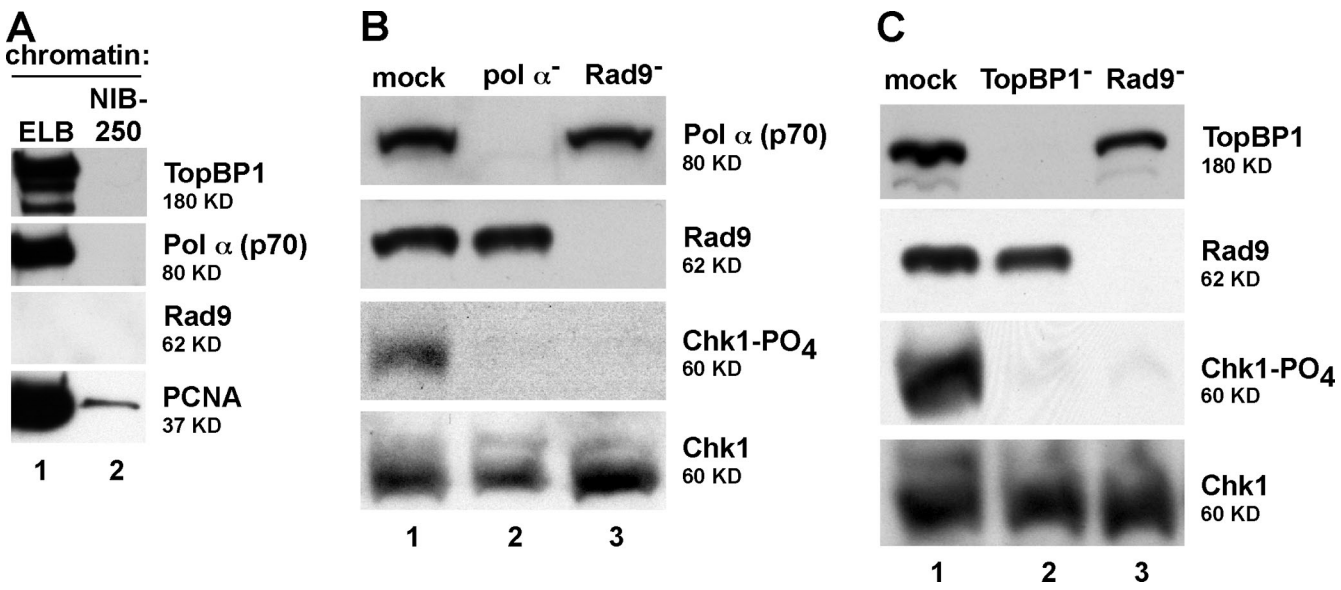


Figure S5. **Analysis of NIB-250 chromatin isolated from the Rad9-depleted extract and immunodepleted extracts.** (A) Chromatin fractions were isolated from Rad9-depleted extract combined with aphidicolin through ELB procedure (lane 1) or NIB-250 procedure (lane 2) and probed for the indicated factors by Western blotting. (B) The extracts used in Fig. 4 B were mixed with sperm chromatin and aphidicolin. After a 45-min incubation, samples of the total extracts were taken for Western blotting. (C) Same as B except that the extracts used were from Fig. 4 C.