Aparicio et al., http://www.jcb.org/cgi/content/full/jcb.201504005/DC1

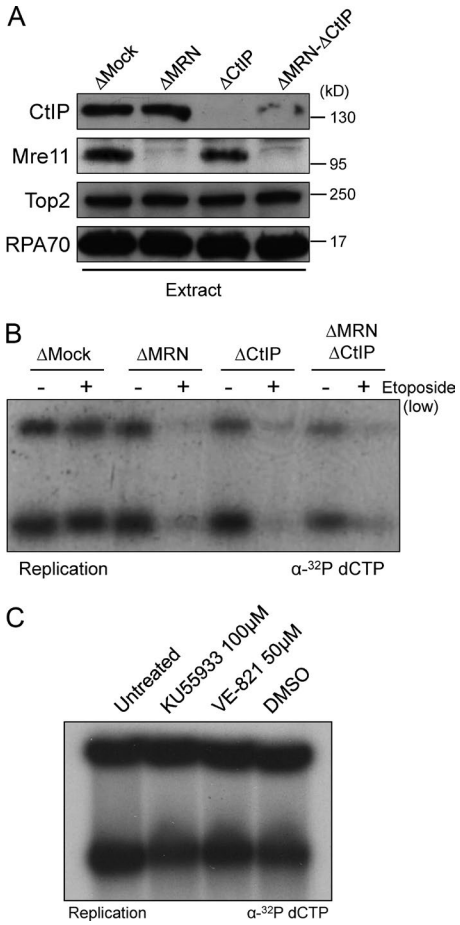

Figure S1. **CtIP- and MRN-depleted extracts are sensitive to low doses of etoposide.** (A) Control (mock) and CtIP-, Mre11-, or CtIP-, and Mre11-depleted extracts were immunoblotted with the indicated antibodies. (B) Extracts, as indicated, were supplemented with 2,000 sperm nuclei/μl, α-[^{32}P]dCTP, and either DMSO (–) or etoposide (+) to a final concentration of 2 μM in the extract. Genomic DNA was isolated after a 90-min incubation, and DNA replication products were resolved in neutral agarose gel electrophoresis and visualized by autoradiography. (C) Genomic replication in the presence of specific ATM (KU55933) or ATR (VE-821) inhibitors.

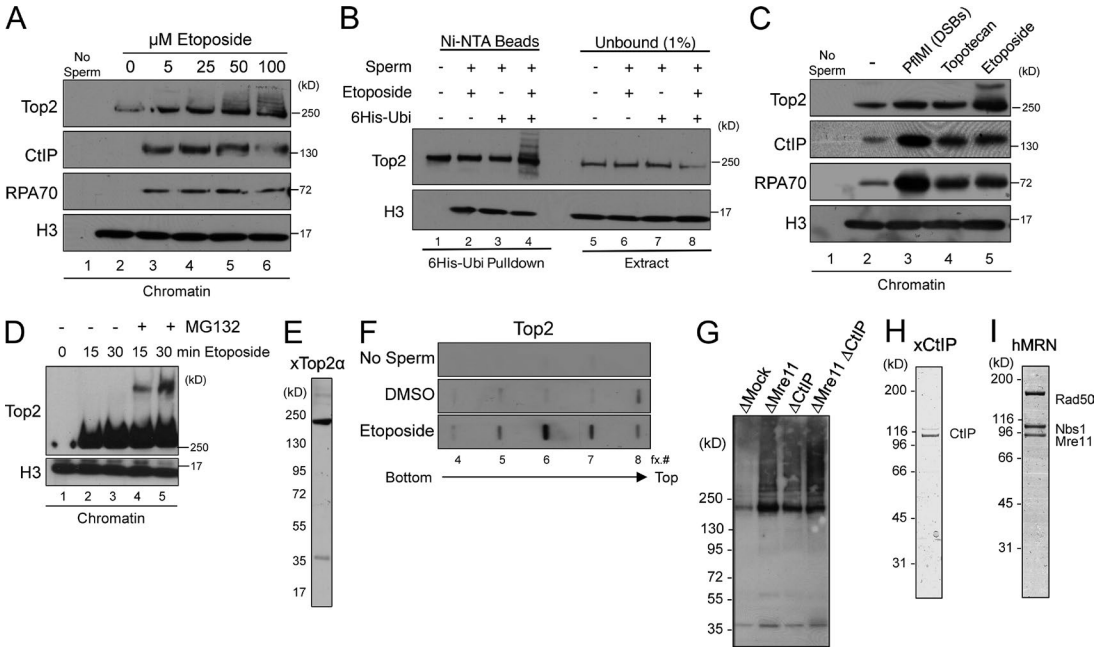

Figure S2. **Etoposide induces Top2–DNA adducts in egg extracts.** (A) Sperm chromatin (5,000 sperm nuclei/μl) was incubated with the indicated concentrations of etoposide then processed for Western blot with the indicated antibodies. (B) Top2 is polyubiquitylated upon etoposide treatment in egg extracts. Extracts were supplemented with sperm, hexahistidine-tagged ubiquitin, or etoposide as indicated and incubated for 30 min. Pull-downs were performed with Ni-NTA resin. Beads and input extracts (1%) were immunoblotted against Top2 and H3 as a loading control. (C) Sperm chromatin was incubated with DNA-damaging agents then isolated at 40 min and processed for Western blot with the indicated antibodies. (D) Extracts were supplemented with sperm, MG132, and/or etoposide as indicated and incubated for 40 min. Chromatin was isolated and immunoblotted with the indicated antibodies. (E–G) Isolation of Top2 covalent complexes from *Xenopus* egg extract. (E) Western blot of *Xenopus* DNA topoisomerase IIα. (F) Enrichment of Top2–DNA adducts after etoposide treatment. Extract supplemented with sperm nuclei (5,000/μl) was incubated with etoposide (100 μM) or DMSO for 40 min and processed as described in Materials and methods. (G) Extracts, as indicated, were processed as in D, and genomic DNA was isolated using DNAzol reagent. DNA was then extensively degraded with micrococcal nuclease, and samples were processed for Top2 Western blot. (H) Colloidal Coomassie blue staining of purified recombinant CtIP and MRN complex (I) used in this study.

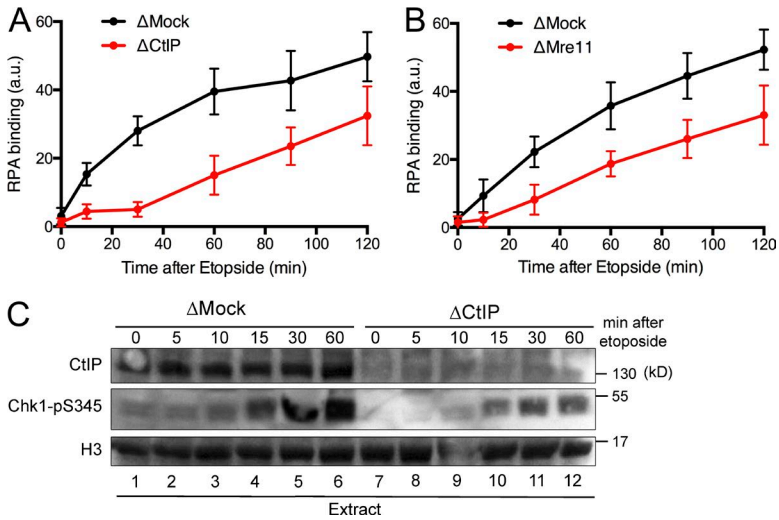

Figure S3. **Impaired resection and checkpoint activation after MRN-CtIP depletion.** (A) Time course of resection after etoposide treatment in control and CtIP-depleted extracts. Sperm chromatin (5,000/μl) was isolated at the indicated times after etoposide addition (100 μM) and processed for Western blot. Plot shows relative RPA binding intensities for control and CtIP-depleted extracts treated with 100 μM etoposide. Error bars represent SD; $n = 3$. (B) Time course of resection after etoposide treatment in control and Mre11-depleted extracts. (C) Chk1 pS345 was monitored in extracts after etoposide treatment. a.u., arbitrary units.

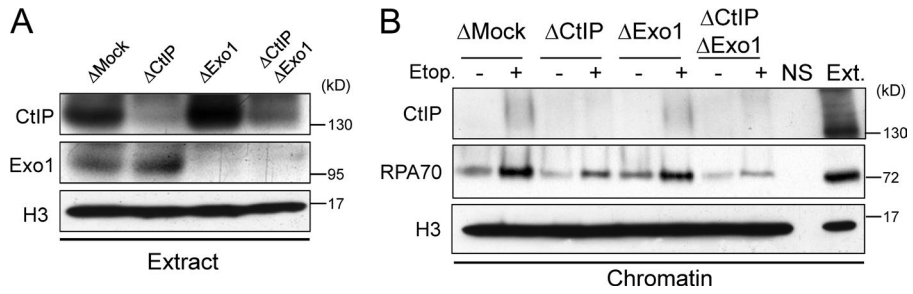

Figure S4. **Exo1 and CtIP initiate resection at etoposide-induced DNA breaks.** (A) Control extracts (mock) and CtIP-, Exo1- or Exo1-, and CtIP-depleted extracts processed for Western blot with the indicated antibodies. (B) Extracts were incubated with sperm chromatin (5,000 sperm nuclei/μl), treated with etoposide or DMSO, and then isolated after incubation for 40 min and processed for Western blot with the indicated antibodies. NS, no sperm control; Ext., extract.