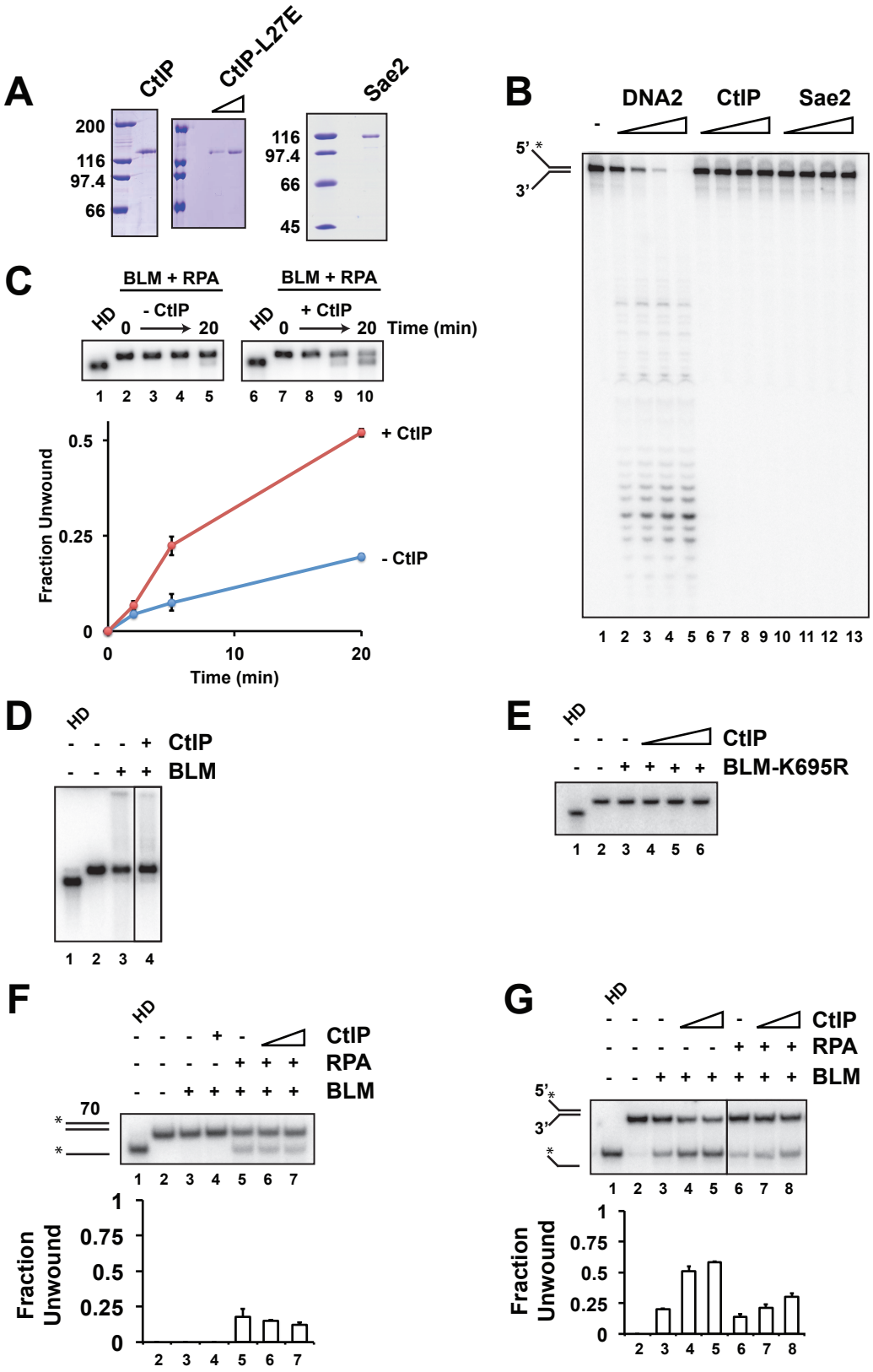

Figure S1, related to Figure 1. A. Purified CtIP, CtIP-L27E mutant and Sae2 were analyzed by SDS-PAGE and Coomassie Blue staining. **B.** Tests of CtIP and Sae2 for nuclease activity. DNA2 (1, 2, 4, and 8 nM), CtIP (10, 20, 40, and 80 nM), or Sae2 (10, 20, 40, and 80 nM) was incubated with the radiolabeled Y DNA (2.5 nM) at 37°C for 20 min. The asterisk denotes the label. **C.** Time course of BLM stimulation by CtIP. The 2 kb randomly labeled DNA substrate (0.5 nM ends) was incubated with BLM (20 nM) and RPA (40 nM) with or without CtIP (20 nM) at 37°C, and samples were taken at 2.5, 5, and 20 min. **D.** RPA is required for stimulation of BLM by CtIP. The 2 kb dsDNA substrate (0.5 nM ends) was incubated with BLM (20 nM) with or without CtIP (60 nM) at 37°C for 20 min. **E.** The helicase-dead BLM-K695R mutant (10 nM) cannot be stimulated by CtIP (20-40-60 nM) in the presence of RPA (200 nM). The experiment was performed as in Figure 1A. **F.** Effect of CtIP on BLM activity on short dsDNA. CtIP (20 or 60 nM), BLM (20 nM), and RPA (100 nM) were incubated with radiolabeled 70-mer dsDNA (5 nM ends) at 37°C for 20 min. The asterisk denotes the radiolabel. **G.** Effect of CtIP on BLM activity on the Y structure. CtIP (20 or 40 nM) and BLM (2 nM) were incubated with radiolabeled Y DNA with 44-nt overhangs and a 31-nt duplex region (2.5 nM) with and without RPA (5 nM) at 37°C for 5 min. The asterisk denotes the radiolabel.

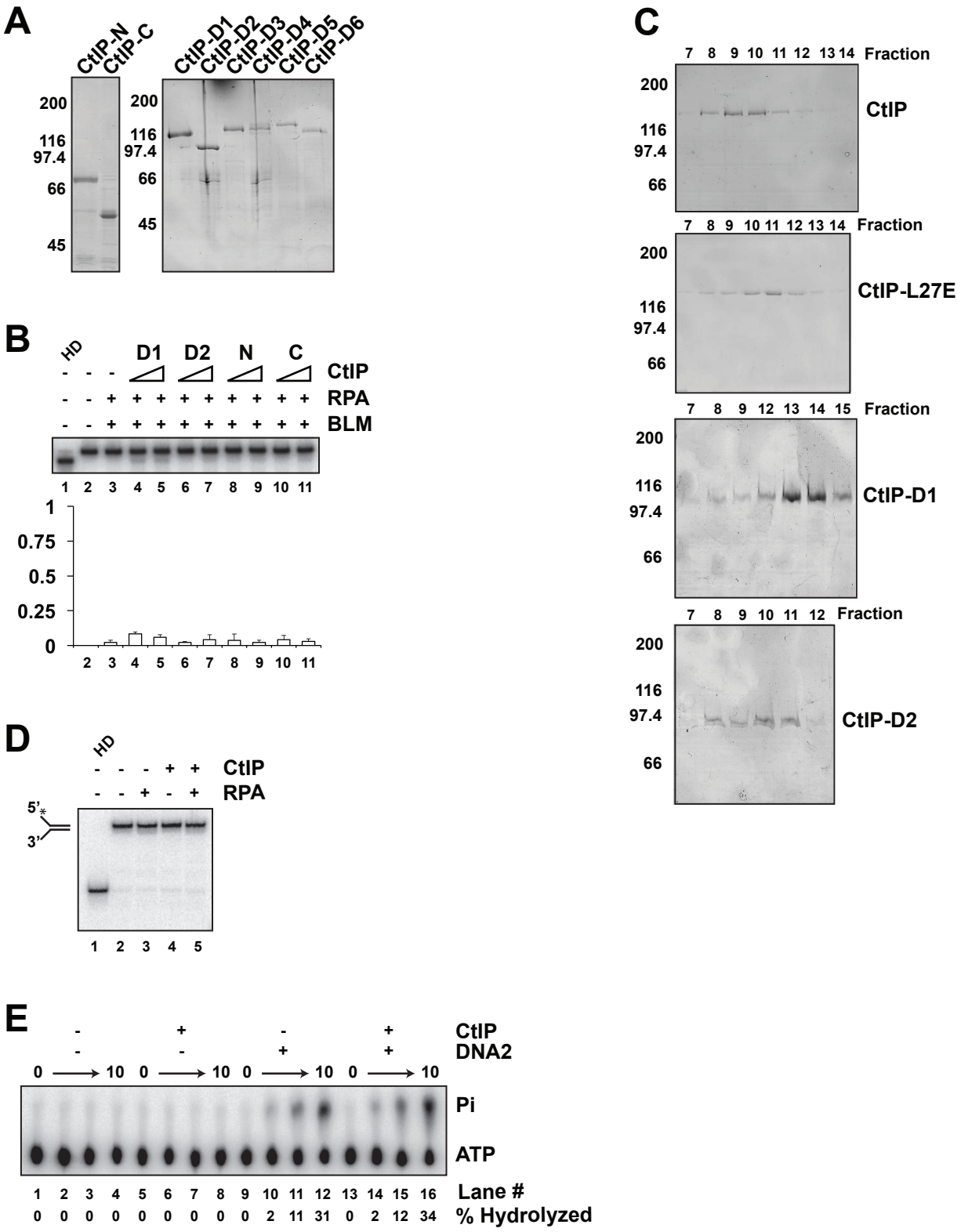

Figure S2, related to Figure 2. A. CtIP deletion mutants were analyzed by Coomassie blue staining on 7.5% SDS-PAGE gels. **B.** CtIP-D1, D2, N and C were re-tested with BLM and RPA as in Figure 2G, except that 60 or 120 nM of CtIP was used. **C.** Gel filtration profiles of the indicated CtIP mutants. Protein was loaded onto a 24 ml Superose 6 column and 1 ml fractions were collected and analyzed by Coomassie blue staining on 7.5% SDS-PAGE gels. **D.** CtIP and RPA do not dissociate the strands of the Y substrate. CtIP (40 nM) was incubated with the DNA substrate (2.5 nM) with or without RPA (5 nM) at 37°C for 20 min. **E.** CtIP does not affect the ATPase activity of DNA2. DNA2 (100 nM) was tested for the hydrolysis of [γ-32P] ATP with or without CtIP (140 nM), and samples were taken every 2.5 min. Inorganic phosphate was separated from ATP on a flexible TLC plate.