SUPPLEMENTAL DATA

FIGURE LEGENDS

Suppl. Fig. 1. Interaction of Ctf18 with Dcc1 or Ctf8. GFP-tagged Ctf18full (18full) was coexpressed with Dcc1 and Ctf8 (lane 1) or with Dcc1 (lane 2) or Ctf8 (lane 3) alone, or Dcc1 and Ctf8 were coexpressed without Ctf18full (lane 4). Each combination was precipitated with anti-GFP beads. The bound fractions were analyzed by CBB staining. Proteins with (*) are nonspecific proteins or IgG chains from affinity beads.

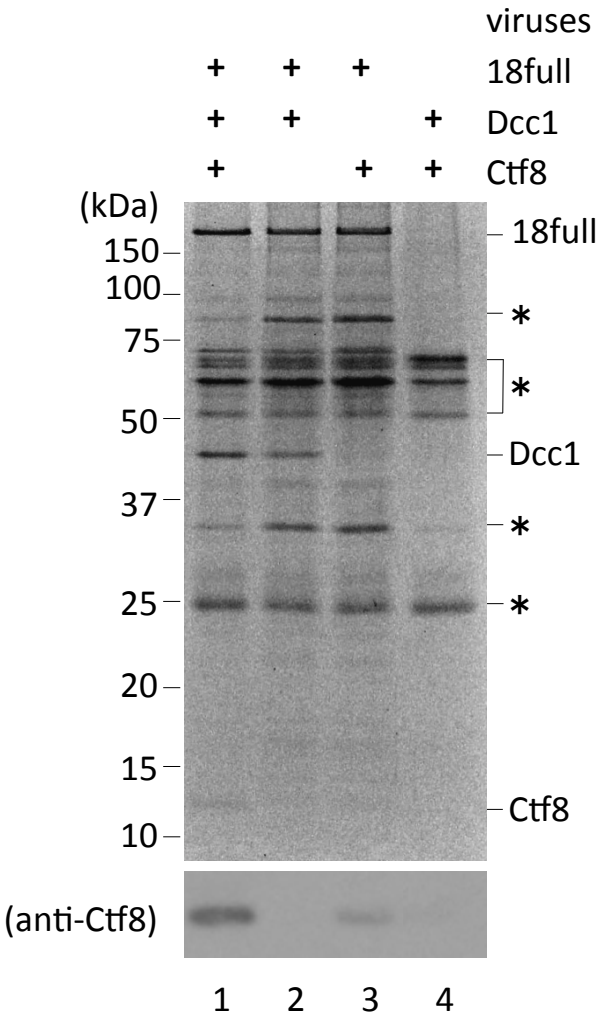
Suppl. Fig. 2. Ctf18 regions necessary for interaction with RFC2-5. Silver-stained images of proteins precipitated with GFP-tagged Ctf18 fragments from Sf9 lysates expressing Ctf18 fragments and RFC2-5. Lysates used were RFC2-5 alone (lane 4) or RFC2-5 with indicated Ctf18 fragments. 18full (lane 1) represents full-length Ctf18. Ctf18 fragments precipitated are shown with arrowheads, and coprecipitated RFC2-5 are indicated with brackets at right. Lanes 1-4, lane 5, and lane 6-9 were from independent electrophoreses. (B) Schematic representation of the Ctf18 regions 576-876 and 935-975. These regions are necessary to form pentameric complexes with RFC2-5 (obtained from the data shown in this figure) and to interact with 1-8 (from Fig. 3).

Suppl. Fig. 3. Purified Ctf18-RFC, Ctf18-RFC(5), Pol ε, and p261Nter, and their DNA synthesis activity. A) Purified proteins used for Fig. 5 and this figure. 1.9 μg of Ctf18RFC(7) and 1.7 μg of Ctf18-RFC(5) (Left), or 0.5 μg of Pol ε holoenzyme (ε) and 0.15 μg of p261Nter (Nter) (Right), were detected by CBB and silver staining, respectively, after electrophoresis. (B) DNA synthesis activity of Pol ε and p261Nter. Indicated amounts of Pol ε holoenzyme (squares) or p261Nter (circles) were incubated with polydA/oligodT at 37°C for 30 min. Incorporated TMP is indicated.

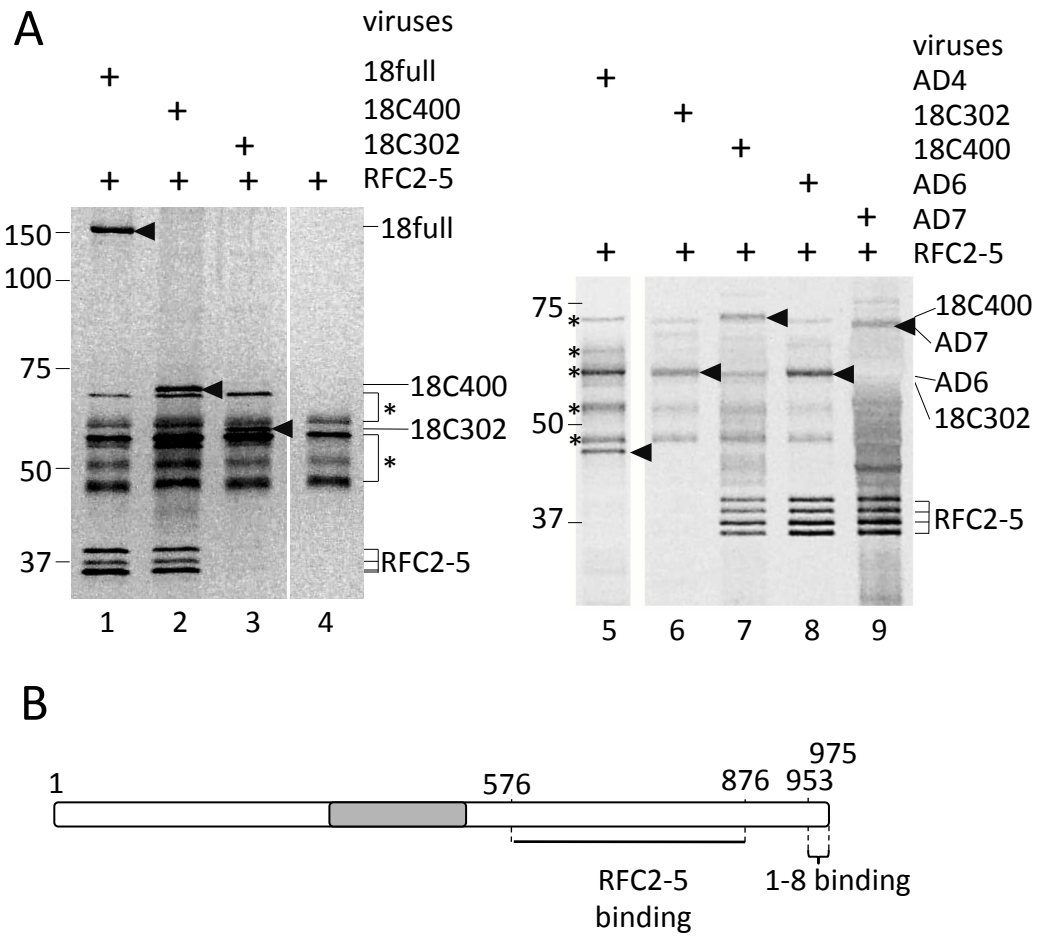
Suppl. Fig. 4. Fractionation of recombinant Pol ε and Ctf18-RFC by glycerol gradient sedimentation. (A) Partially purified Pol ε and Ctf18-RFC were separately fractionated by
glycerol gradient sedimentation in 2.2 mL of a 15% to 35% glycerol gradient in buffer H containing 0.1 M NaCl at 1.6×10^5 g for 10.5 h. Proteins were fractionated into 19 tubes
and analyzed by immunoblotting with antibodies against Pol ε p261 and Ctf18 as shown in Fig. 6. Sedimentation positions of marker proteins with their molecular masses are shown at the top. * shows a nonspecific protein. Input corresponds to 1% of the loading material. (B)
Relative intensities of p261 and Ctf18 in fractions.

Suppl. Fig. 5. Additional Ctf18 interacting proteins participating in the replication reaction. Samples

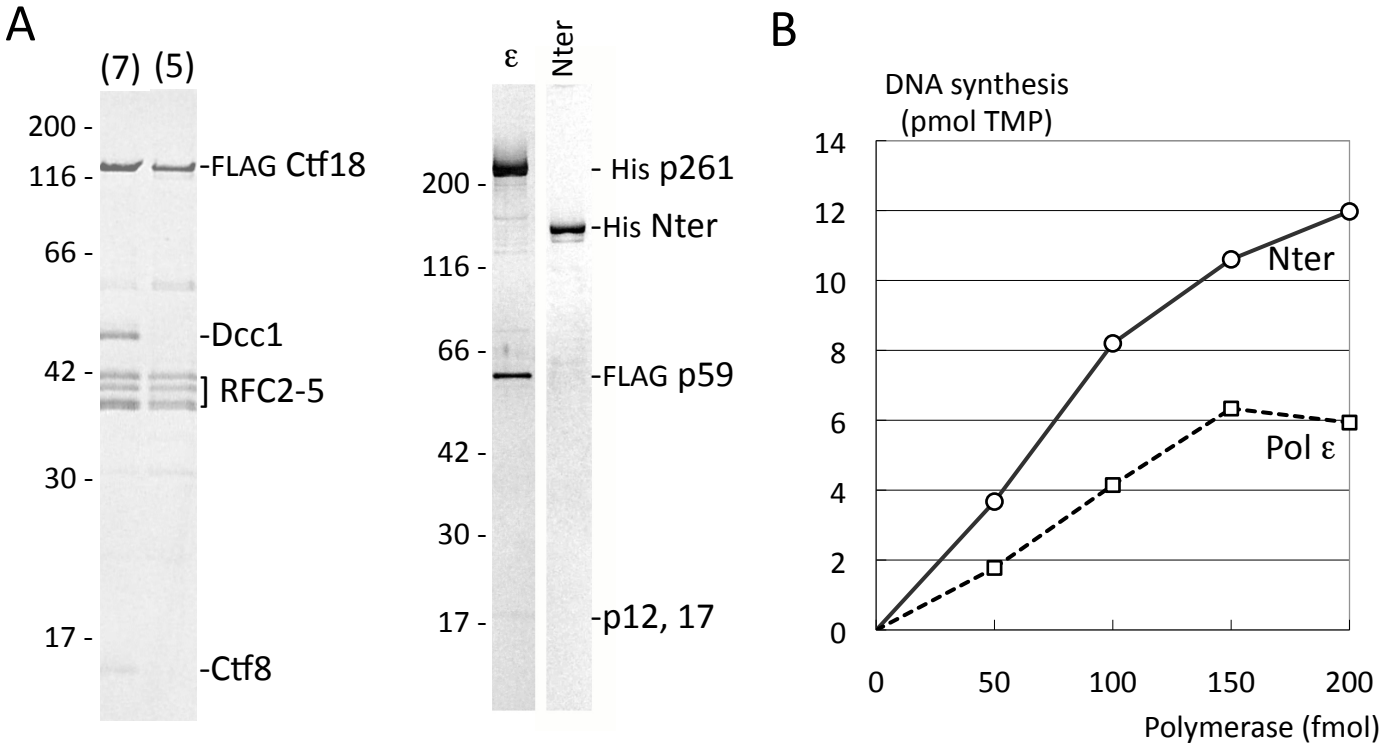
are the same as shown in Fig. 1. The anti-FLAG column eluate separated in a 7.5–17.5% SDS-acrylamide gel was analyzed by LC/MS/MS. Hit numbers of detected peptides for RPA subunits (panel 2) and MCM subunits (panel 5) are indicated. Comparable immunoblottings of Pol δ p125 (panel 1), RPA p70 and p34 (panel 3), PCNA (panel 4), and MCM2 and 7 (panels 6, 7) with filter strips of the eluates from the control IgG-sepharose (left lanes; IgG) and the anti-FLAG column (right lanes; FLAG) are shown.



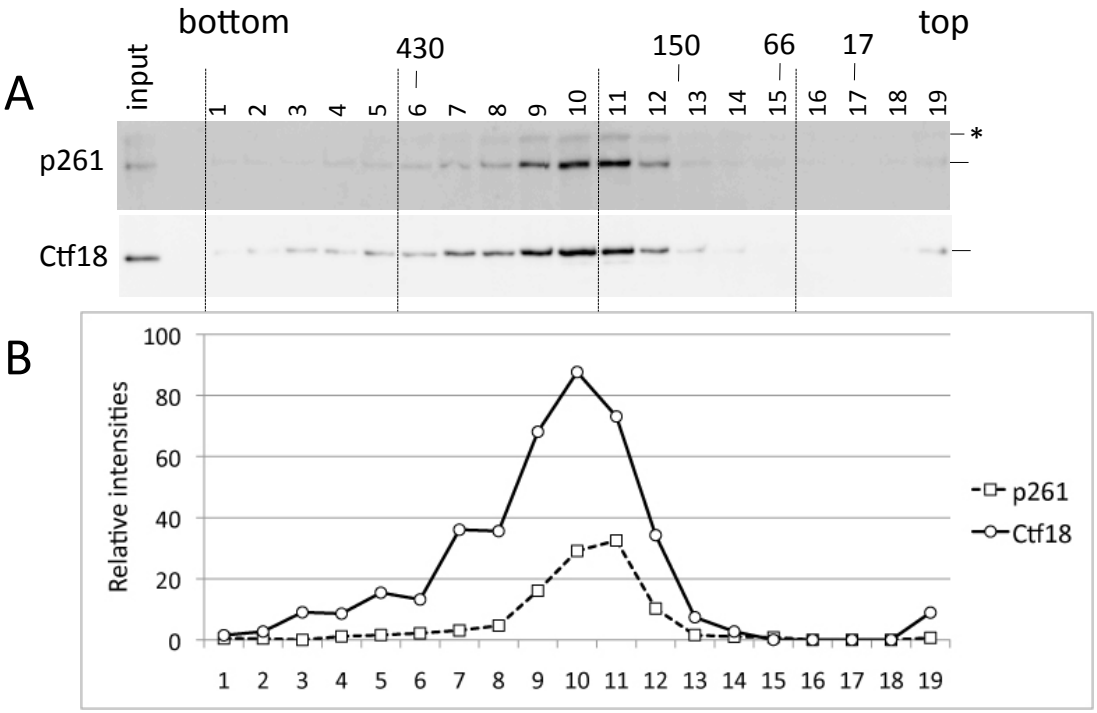
Suppl. Fig. 1 (Murakami et al.)



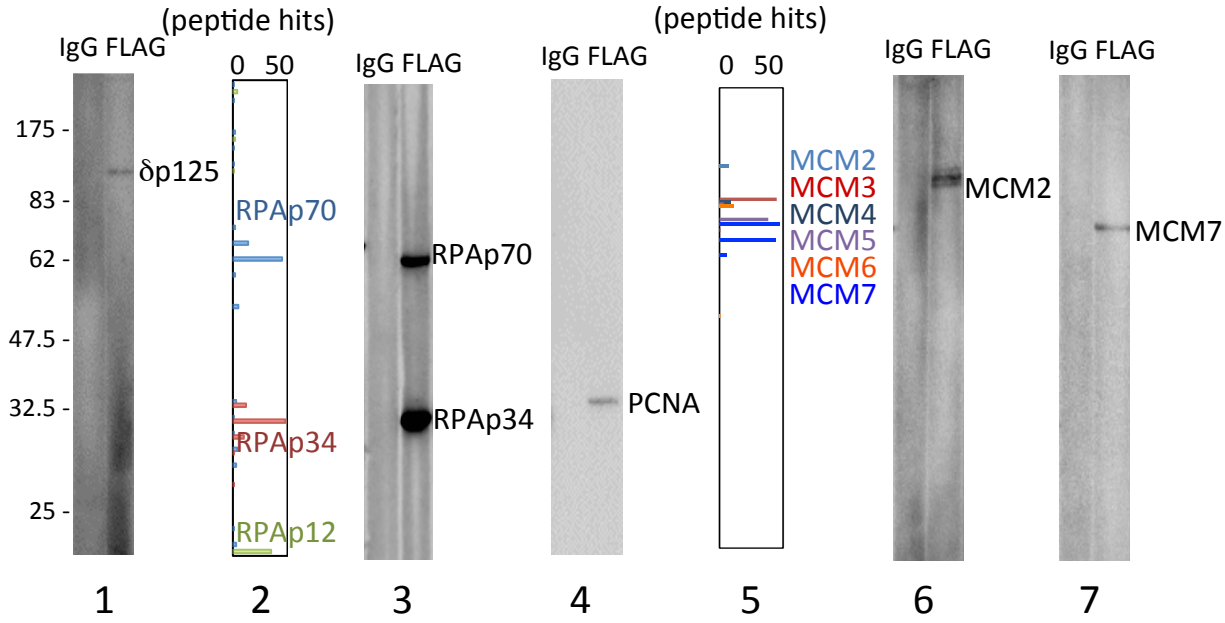
Suppl. Fig. 2 (Murakami et al.)



Suppl. Fig. 3 (Murakami et al.)



Suppl. Fig. 4 (Murakami et al.)



Suppl. Fig. 5 (Murakami et al.)