

## Expanded View Figures

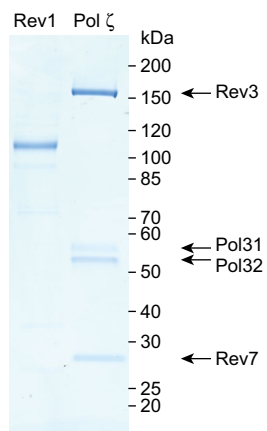
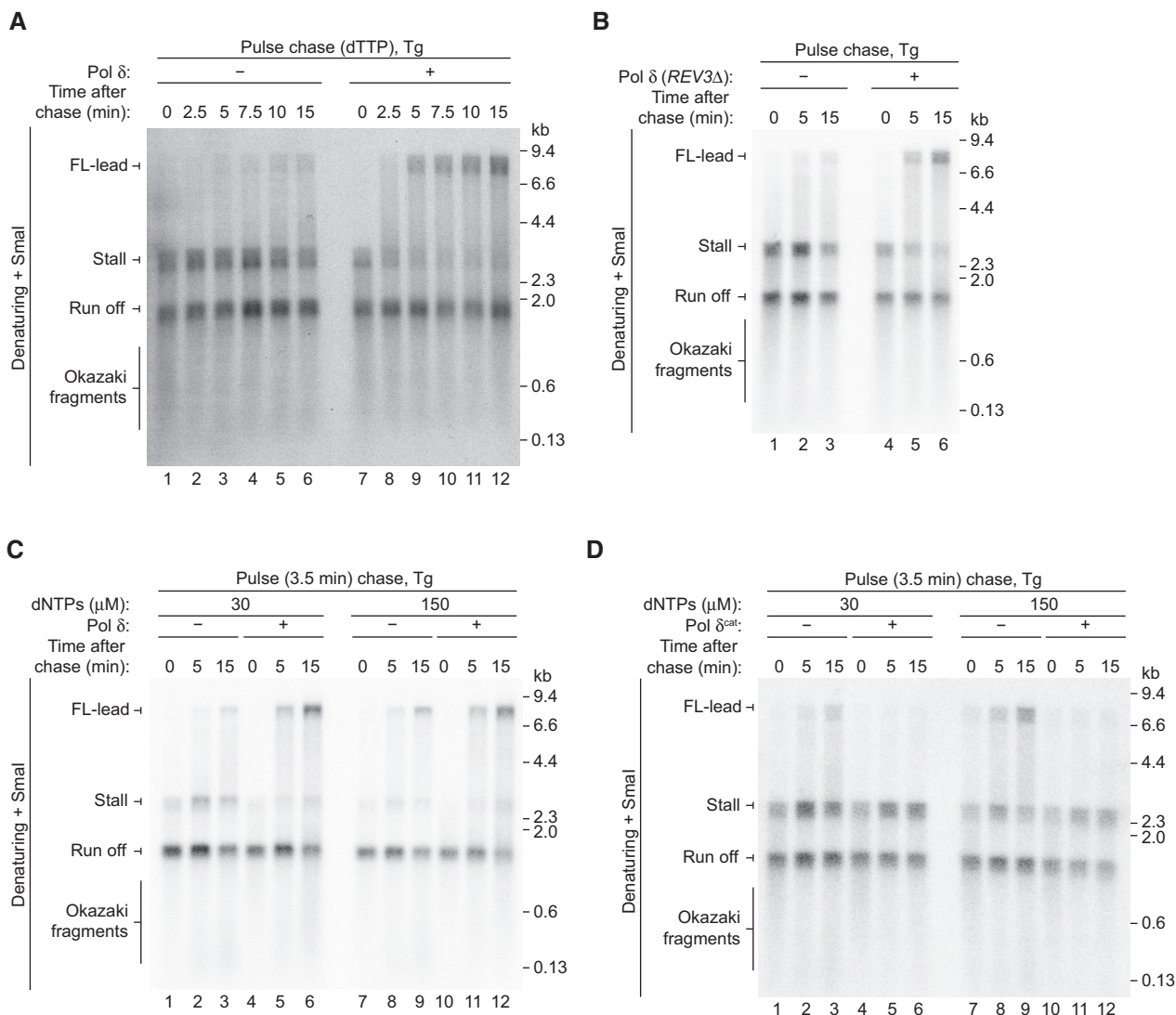


Figure EV1. (Related to Fig 1). Purified Rev1 and Pol ζ.



**Figure EV2. (Related to Fig 3). Pol  $\delta$  can bypass Tg.**

- A Pulse chase reaction as performed in Fig 3B but using  $^{32}$ P-dTTP and elevated dTTP in the pulse and chase respectively.
- B Pulse chase reaction in the absence and presence of Pol  $\delta$  purified from the *REV3A* strain. The pulse phase of the reaction was 5 min.
- C Pulse chase reaction at 30 and 150  $\mu$ M dNTPs in the absence and presence of Pol  $\delta$ . The chase was added 3.5 min after initiation of DNA synthesis. The dCTP concentration was 2.5 and 600  $\mu$ M in the pulse and chase phases of the reactions, respectively.
- D Pulse chase reaction as performed in (C) but using Pol  $\delta^{cat}$  instead of Pol  $\delta$ .

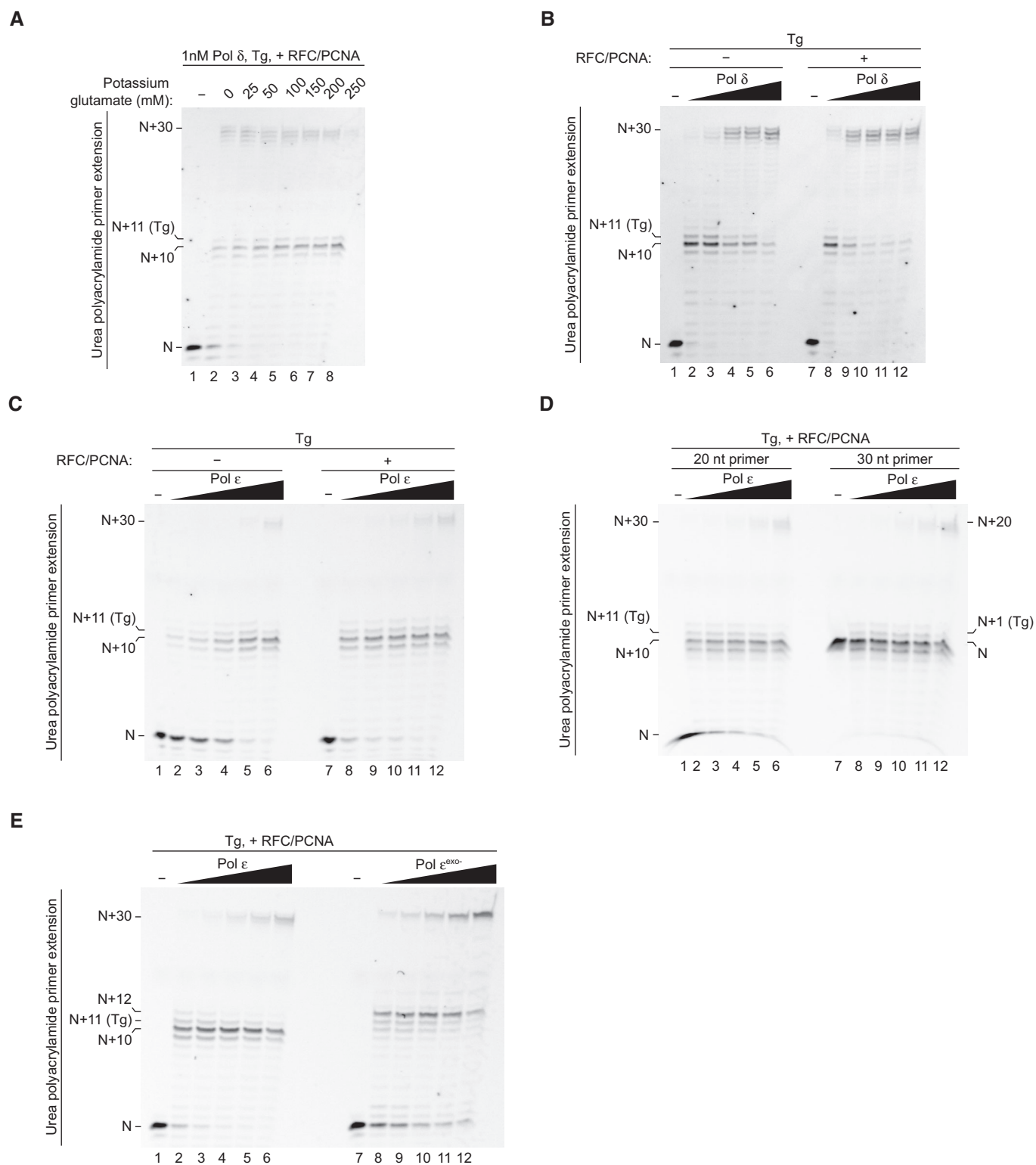
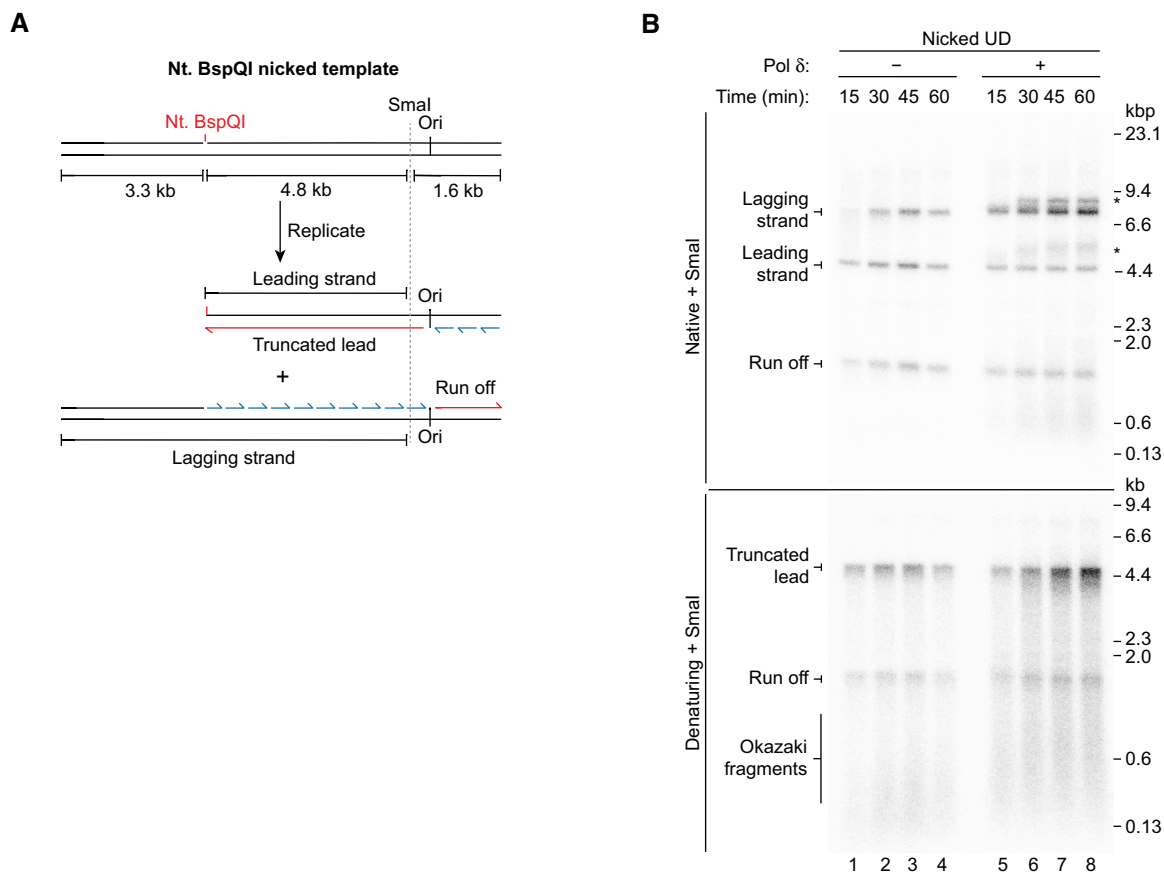


Figure EV3.

**Figure EV3. (Related to Fig 3). Analysis of Tg bypass by Pol  $\delta$  and Pol  $\epsilon$ .**

- A Potassium glutamate titration into primer extension reactions on the Tg template shown in Fig 3G in the presence of Pol  $\delta$  (1 nM), RFC, and PCNA.
- B Pol  $\delta$  titration (1, 2.5, 5, 7.5, 10 nM) into primer extension reactions on the Tg template shown in Fig 3G in the absence and presence of RFC and PCNA.
- C Pol  $\epsilon$  titration (0.5, 1, 2, 4, 8 nM) into primer extension reactions on the Tg template in the absence and presence of RFC and PCNA.
- D Pol  $\epsilon$  titration (0.5, 1, 2, 4, 8 nM) into primer extension reactions on the Tg template with either a 20 nt or 30 nt primer annealed in the presence of RFC and PCNA.
- E Titration of Pol  $\epsilon$  and Pol  $\epsilon^{\text{exo-}}$  (1, 2, 4, 8, 16 nM) into primer extension reactions on the Tg template in the presence of RFC and PCNA.



**Figure EV4. (Related to Fig 5). Isolation of nascent leading strands.**

- A Schematic of the Nt. BspQI nicked UD template and reaction products generated in (B).
- B Standard replication reaction on the Nt. BspQI nicked UD template performed in the absence and presence of Pol  $\delta$ . \*caused by Pol  $\delta$  strand displacement activity.