Supplemental Materials and Methods -

ATPase assays. ATPase assays were assembled as 10 µl reactions with a reaction buffer containing 30mM Tris-HCl (pH 7.8), 7mM $MgCl_2$, 0.1mM DTT, and 0.1mM cold ATP, 50 µg/ml BSA and 1 µCi α^{32}P-ATP (3000 cpm/pmol). Ten ng/µl of EE-E1 was incubated with or without polymerases ε or δ (varying from 10 ng/µl and 200 ng/µl, as indicated). Reactions were incubated for 1 hour at 37°C, and stopped by adding 0.2 µl of 0.5M EDTA (pH 8.0) to each reaction. 1 µl of each reaction was spotted on a PEI-cellulose plate. The plate was developed in 0.5 M lithium chloride/1 M formic acid until the liquid phase migrated ~75% of the way up the plate. Plates were dried and exposed to phosphorimaging screens and imaged with a Typhoon phosphoimager.

Helicase assays. Helicase substrates were prepared by 5' end-labeling a 50 base oligonucleotide (30 bases with a 20 base long thymidine tail) with γ-^{32}P-ATP (3000 cpm/pmol) using T4 polynucleotide kinase as per instructions (Thermo Scientific). After the end-labeling reaction was complete, the end-labeled oligonucleotide was annealed to a second oligonucleotide composed of 30 complimentary bases and an 8 base region composed of only cytosines, generating a duplex forked substrate. Free nucleotide label was removed using G50 gel filtration with TE buffer. The helicase substrate (1-2 fmoles) was incubated with 20 ng/µl EE-E1 with or without varying amounts (10 ng/µl-200 ng/µl) of polymerases ε or δ in 10 µl reactions for 30 minutes at 37°C in helicase buffer (30mM Hepes (pH 7.5), 7mM $MgCl_2$, 0.5mM DTT, 4mM ATP and 0.1 mg/ml acetylated BSA). Reactions were stopped by adding helicase stop buffer (0.3% SDS [w/v], 20mM EDTA, 5% glycerol [w/v], and 0.1% bromophenol blue [w/v]) for 15 minutes at 37°C. The reactions were applied to a 12.5% native PAGE gel in 1x TBE buffer and subjected to electrophoresis. The gel was fixed with 15% methanol and 15% acetic acid for 15 minutes, rinsed with water, and subjected to autoradiography.

Supplemental Figures –

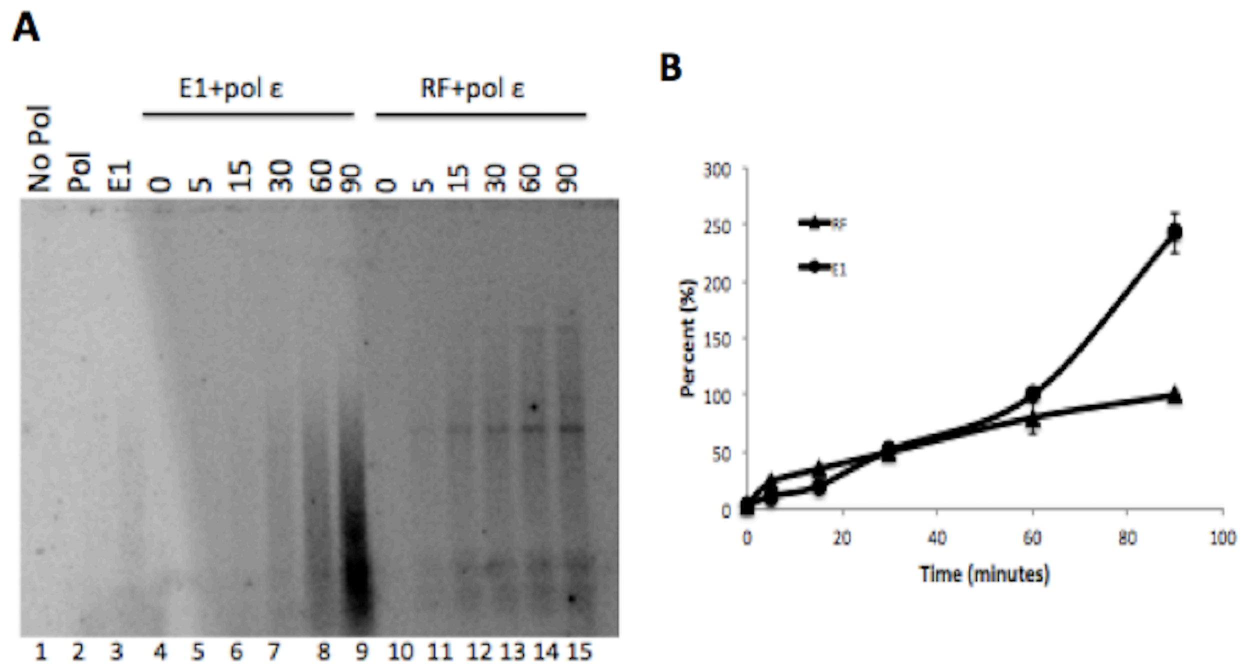

Figure S1. Time course analysis of pol ε stimulation

A. Primed M13 template assays were used to evaluate DNA synthesis by pol ε over time in the presence of either HPV E1 or RF. Pol ε (1 ng/μl) was combined with primed M13 template and either HPV E1 (60 ng/μl; lanes 4-9) or RF (RFC (3 ng/μl), PCNA (10 ng/μl), RPA (70 ng/μl); lanes 10-15) and was incubated at 37°C for 0-90 minutes. Lanes 2 and 3 are pol ε and E1 alone, respectively, for 60 min. **B.** Synthesis was quantified as in the Methods and graphed for three experiments, with pol ε in the presence of RF at 60 min set to 100% and pol ε with E1 plotted relative to pol ε with RF (see graph). Error bars are defined as s.d.

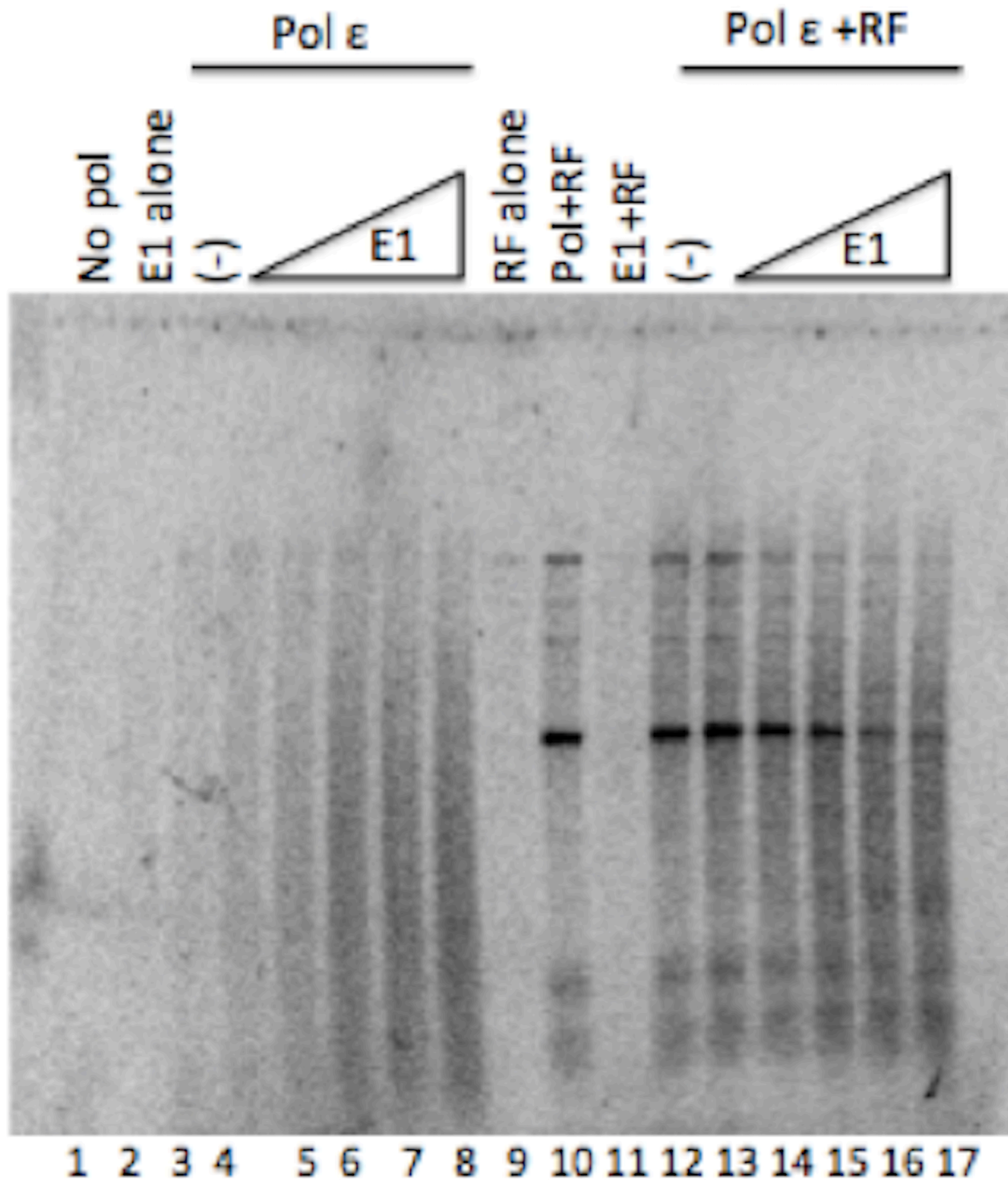

Figure S2. E1 and replication factors did not synergistically stimulate pol ε
Primer extension assays were performed as described in Materials and Methods. Increasing amounts of HPV E1 (5 ng/µl to 60 ng/µl; lanes 4-8) were added to reactions containing primed M13 template and 10 ng/µl of pol ε. E1 was also added in increasing amounts to reactions containing primed M13 template, 1 ng/µl pol ε and replication cofactors (RF; RFC (3 ng/µl), PCNA (10 ng/µl), RPA (70 ng/µl); lanes 13-17). Pol ε was incubated alone with template (lane 3), or in the presence of RF and template (lane 12), in which DNA synthesis by pol ε was stimulated. E1 alone or with RF (lanes 2 and 11, respectively) was not able to synthesize DNA.

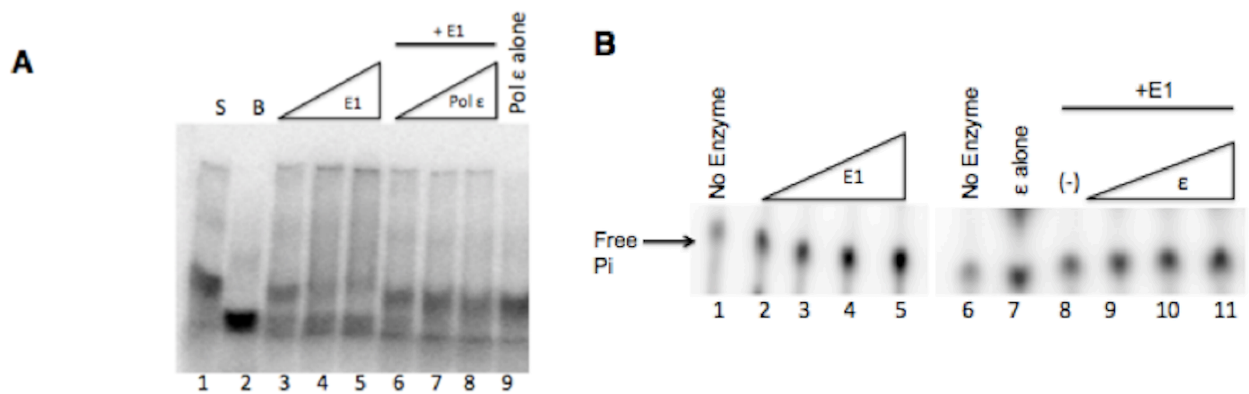

Figure S3. Pol ε did not stimulate E1's helicase or ATPase activities

A [γ ^{32}P] end labeled forked helicase substrate containing a 30 base pair duplex region was synthesized (S; lane 1). The template alone was heated for 5 minutes at 100°C (B; lane 2). HPV E1 was titrated in from 20ng/µl to 80 ng/µl to reactions containing 1-2 fmoles of substrate alone (lanes 3-5) for 30 minutes at 37°C. The lowest concentration of E1 was selected (20ng/µl) and was incubated with increasing amounts of pol ε (10 ng/µl to 160 ng/µl;lanes 6-8). Pol ε alone was incubated with the substrate (lanes 9). **B.** ATPase assays were performed by incubating HPV E1 in the presence and absence of pol ε. The no enzyme control (lane 1) contained only reaction buffer and [γ-^{32}P] ATP. E1 was added in increasing amounts to reactions containing the [γ-^{32}P] ATP (10 ng/µl to 80 ng/µl; lanes 2-5). A low level of E1 was selected (10 ng/µl) and was incubated with increasing concentrations of pol ε (1 ng/µl to 16 ng/µl; lanes 8-11). Polymerase alone was also incubated with the ATP substrate (lane 7).

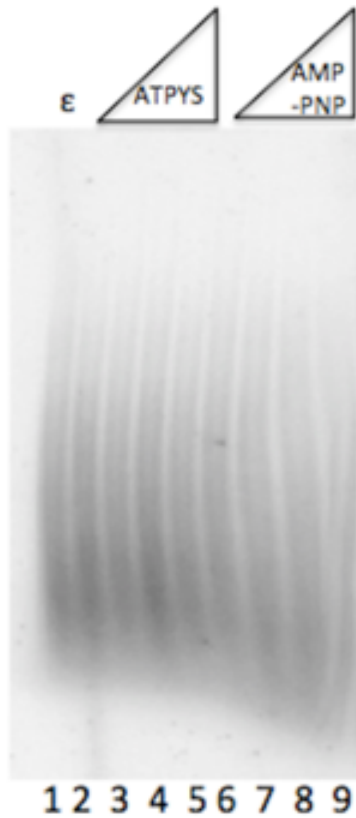

Figure S4. Nonhydrolyzable ATP analogs ATPγS and AMP-PNP did not inhibit pol ε

Primer extension assays were performed as described in Materials and Methods. 1 ng/μl of pol ε was incubated with template alone (lane 1) or with increasing amounts of either ATPγS (4 mM, 8 mM, 16 mM, and 32 mM; lanes 2-5) or AMP-PNP (4 mM, 8 mM, 16 mM, and 32 mM; lanes 5-8).