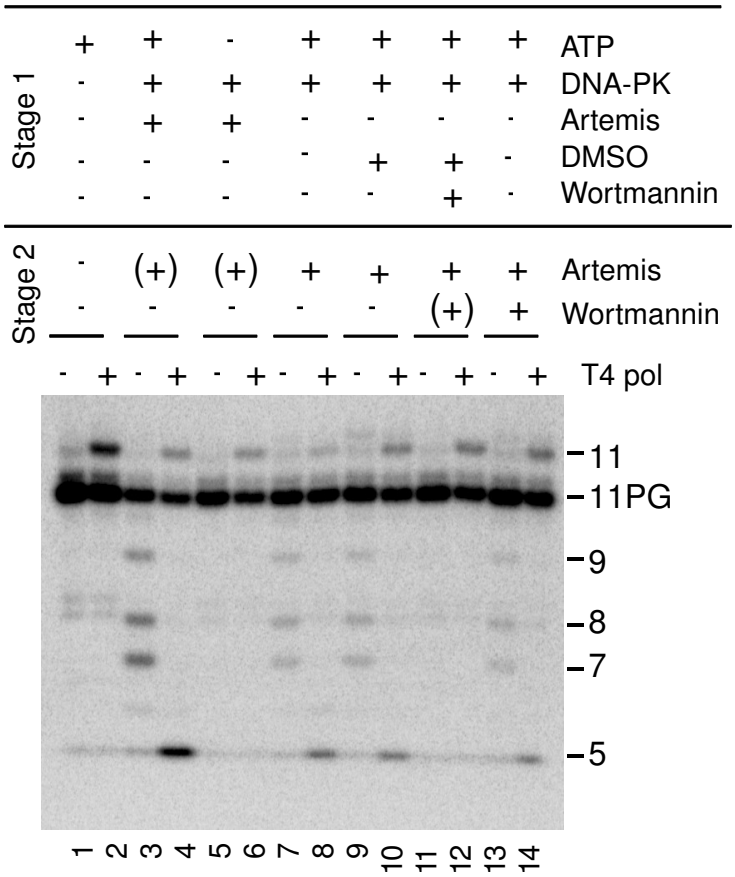
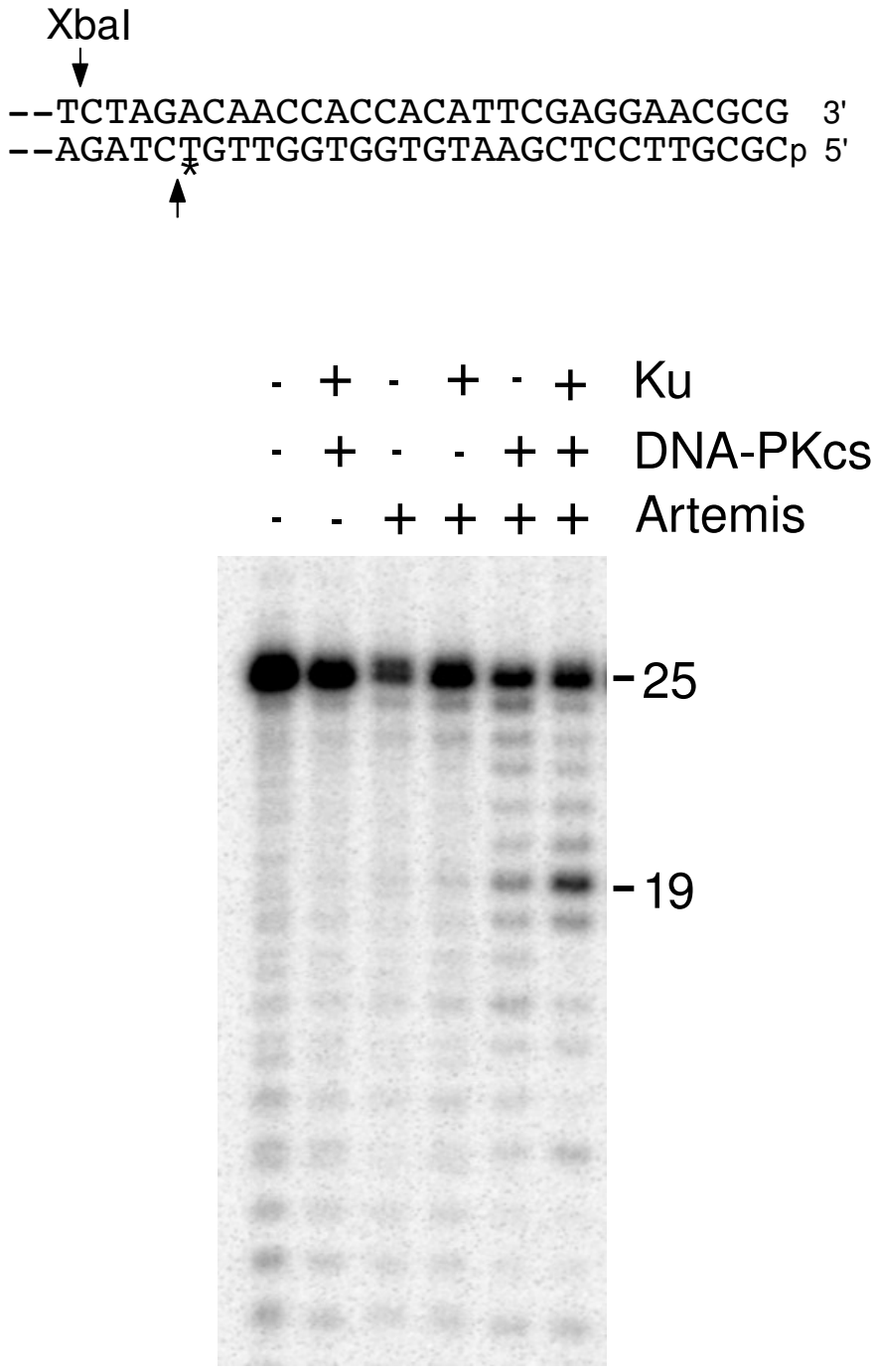
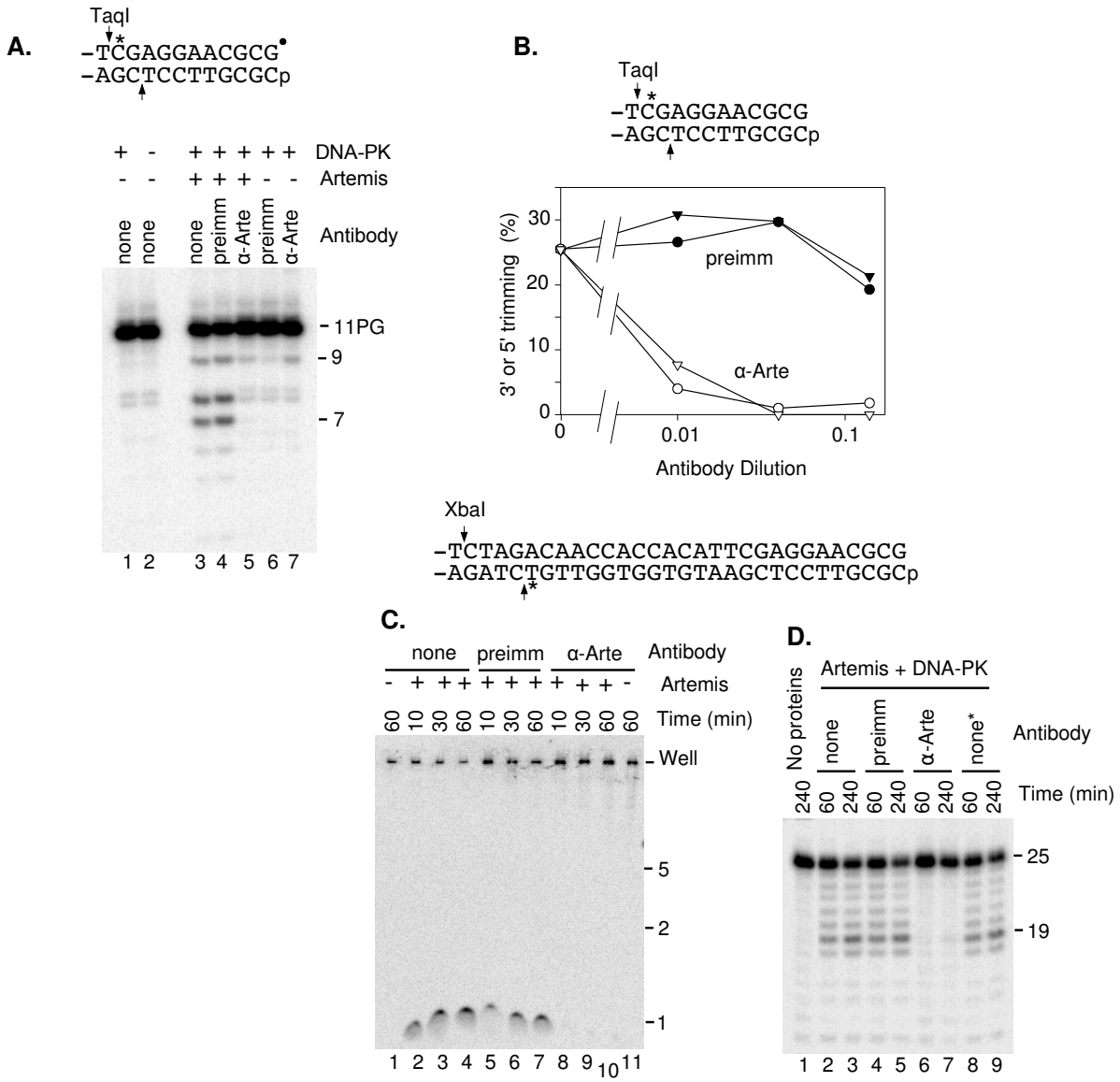


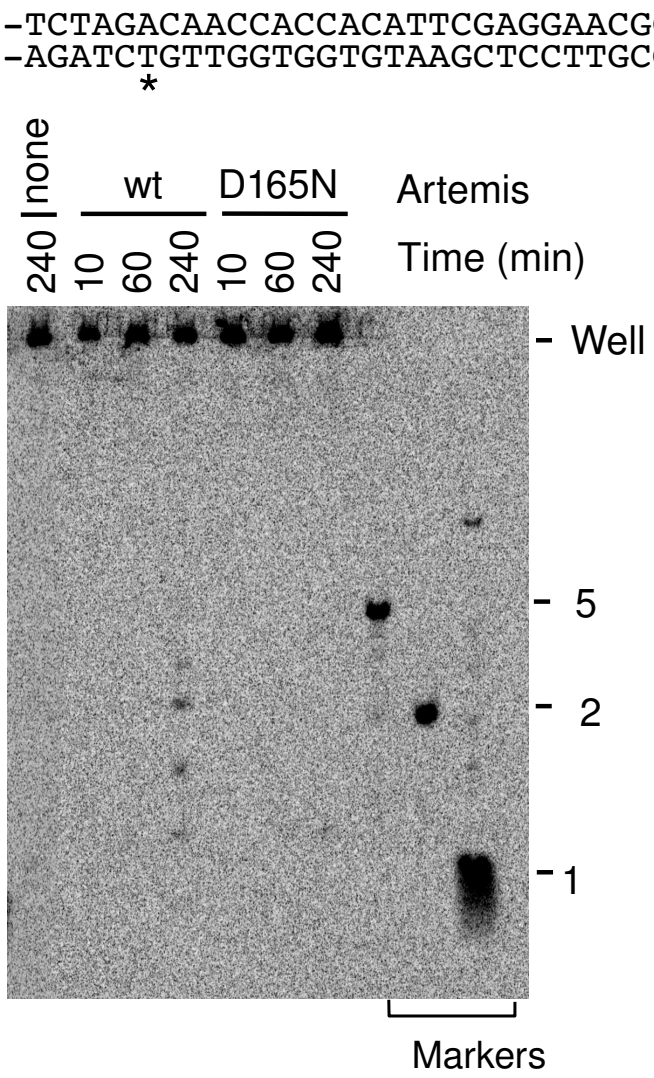
Supplemental Figure 1. Effect of DNA-PK inhibitor wortmannin on Artemis-mediated trimming. The internally labeled blunt 3'-PG 5'-phosphate substrate (see Fig. 2) was incubated with DNA-PK and/or Artemis in the presence or absence of the inhibitor as indicated. After 30 min, Artemis and/or wortmannin were added to some samples, and then the incubation was continued for 45 min. DNA was deproteinized and treated (or not) with T4 pol and then finally cut with TaqI as in Fig. 2A. As with the more specific but reversible inhibitor KU55778, there was no detectable trimming in samples that contained wortmannin throughout the experiment (lanes 11-12), but trimming was detected in samples where substrate was preincubated with DNA-PK in the absence of inhibitor, and then wortmannin and Artemis were added at the same time (lanes 13-14). These results suggest that autophosphorylation of DNA-PK is essential for 3' trimming. There are at least two possible explanations for the reduced level of trimming seen when DNA-PK is preincubated with the substrate. First, inasmuch as DNA-PK has numerous autophosphorylation sites, it is possible that a transient partially autophosphorylated form of DNA-PK is required for trimming, and that only small amounts of this form were present at the time Artemis and inhibitor were added. For example, it has been suggested that prolonged incubation of DNA-PK with DNA ends results in extensive phosphorylation and consequent dissociation of DNA-PKcs from the substrate. Alternatively, DNA-PK-mediated phosphorylation of Artemis may further stimulate trimming, resulting in more efficient trimming when all proteins are present and active simultaneously (lanes 3-4).



Supplemental Figure 2. Ku- and DNA-PKcs-Dependence of Artemis-mediated trimming of the 5'-terminal strand of a blunt end. The internally labeled (*) substrate shown was treated with Ku, DNA-PKcs and/or Artemis, as indicated, for 4 hr in the presence of ATP, then cut with XbaI. Treatment conditions are the same as in Fig. 4D. As in other assays, cleavage by Artemis plus DNA-PKcs is qualitatively similar in presence or absence of Ku, but is much more efficient the presence of Ku.



Supplemental Figure 3. Inactivation of Artemis nuclease activities by antiserum to Artemis. A. Artemis was preincubated with a 1/50 dilution of preimmune serum or an antiserum (α-Arte, #1024) raised against Artemis, then added to the 3'-PG substrate shown. Samples were then incubated for 2 hr at 37°C for the indicated times in the presence or absence of DNA-PK (as in manuscript Fig. 2), and treated with TaqI. B. Artemis was preincubated with various dilutions of preimmune serum (closed symbols) or Artemis antiserum (open symbols), then added to the internally labeled 3′-hydroxyl substrate shown. Following incubation at 37°C for 2 hr, 3′ trimming (•, ∘) was quantitated as the sum of 7-, 8-, and 9-base fragments, and 5′ trimming (▼,▽) was quantitated as the abundance of 5-base fragment following post-treatment with T4 pol (see Fig. 2A). C. The substrate shown, internally labeled 25 bases from the 5' terminus, was preincubated with a 1/25 dilution of preimmune or Artemis antiserum, then treated with Artemis alone and analyzed on a 36% gel without XbaI cleavage. D. Same as C., except incubation was in the presence of DNA-PK, samples were cut with XbaI and fragments were analyzed on a 20% gel. Asterisk (*) indicates no preincubation. The results show that Artemis antiserum blocks both endonuclease (A, B, D) and exonuclease (C) activities of Artemis toward blunt DNA ends.



Supplemental Figure 4. Lack of endonucleolytic cleavage by D165N mutant Artemis. The substrate shown, internally labeled 25 bases from the 5' terminus, was treated with wild-type or D165N mutant Artemis in the presence of DNA-PK, and products analyzed on a 36% gel without further treatment (see manuscript Fig. 4E for details). Traces of labeled di- tri- and tetranucleotides, indicating endonucleolytic trimming 25 bp from the DNA end in a small fraction of molecules, were detected following treatment for 240 min with wild-type but not D165N mutant Artemis. The leftmost lane is from a separate gel but all samples were prepared and incubated together.