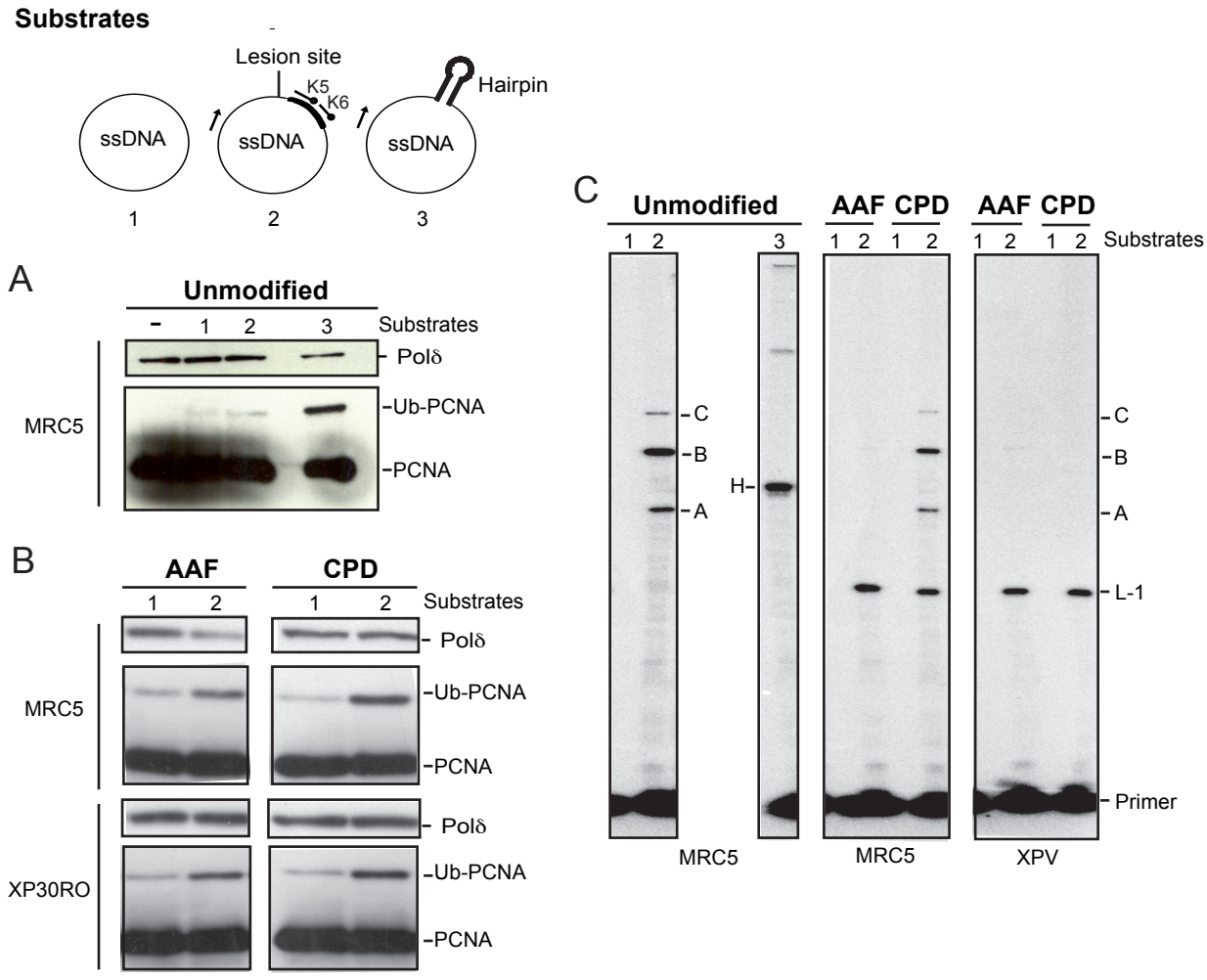

Figure S1. We have previously shown (31) that secondary structures formed in the ssDNA template (referred as “hairpin” in the figure) are able to elicit efficient PCNA monoubiquitination upon primer extension in cell-free extract (panel A lane 3).
In order to delimit a portion of template DNA without any pausing site, we hybridized the primed circular ssDNA template with two contiguous 3’-biotinylated oligonucleotides (K5 and K6) that impair the hairpin formation.
Substrates DNA are incubated 10 minutes at 37°C with cell-free extracts as indicated, in the presence of streptavidin to prevent elongation of the oligonucleotides K5 and K6. Aliquots of the same reactions are analyzed by Western blot using an antibody against PCNA or Polδ (Panel A and B) and by electrophoresis on denaturing gel to analyzed DNA synthesis products.
Panel A: Monoubiquitination of PCNA is inefficient on unmodifed DNA, except when the hairpin can form (substrate 3).
Panel B: A CPD lesion or an AAF adduct are able to stimulate PCNA monoubiquitination to the same extent in both MRC5 and XP30RO cell-free extracts
Panel C: Primer extension analysis on a 6% acrylamide gel-7M urea
L-1: Elongation products up to one nucleotide before the lesion site
H: Elongation product up to the hairpin site
A: Elongation products up to the 5’end of oligonucleotide K5
B: Elongation products up to the 5’end of oligonucleotide K6
C: Elongation products B ligated with oligonucleotide K6

The sequences of the oligonucleotides used to block the primer extension are
K5: 5’- GCT GTT TCC TGT GTG AAA TTG TTA TCC GC-3’
K6: 5’- TCA CAA TTC CAC ACA ACA TAC GAG CCG G-3’