

## Figure S1

(A) Pol $\eta$  produced *in vitro* by transcription/translation in reticulocyte lysates fully complement the bypass deficiency of XP30RO cell-free extracts.

DNA synthesis products obtained after 20 min incubation with 30  $\mu$ g of cell-free extract and pUC3G1-AAF.ss primed template were analyzed by electrophoresis on 6% denaturing polyacrylamide gel. Complementation is abolished by mutations in the highly conserved motif 113SIDE116 of the catalytic site of Pol $\eta$ .

(B) PCNA is required for efficient TLS through both G-AAF adduct and TT-CPD lesion. MRC5 cell free extract (100  $\mu$ g) was mixed with His-p21 fusion protein (5  $\mu$ g) bound to Ni-NTA magnetic agarose beads (Quiagen) in a final volume of 20  $\mu$ l of binding buffer (50 mM HEPES-KOH pH7.9, 50 mM NaCl) for 1.5 h at 4°C. The unbound supernatant was used directly for reaction.

Left panel: Western Blot analysis using an antibody against PCNA of either non depleted MRC5 extract (lane 1), extract depleted for PCNA (lane 2).

Recombinant tagged PCNA was added to depleted extract in lane 3.

Right panel: Cell-free extracts (10  $\mu$ g) were incubated 20 min at 37°C with monomodified primed templates (AAF or CPD). DNA synthesis products were analyzed as indicated above.