Supplemental Figure 1

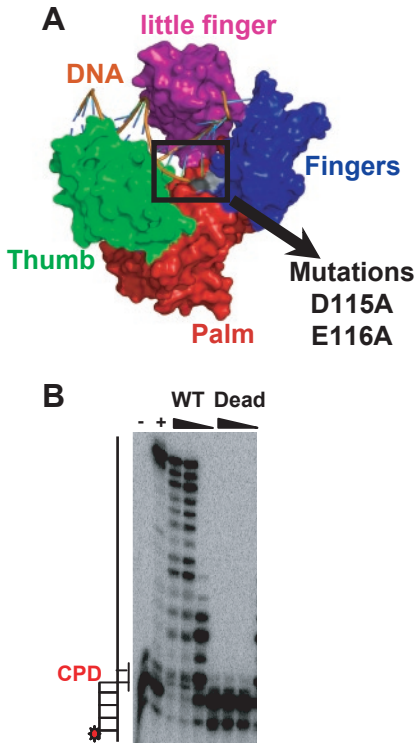


Figure S1: Construction of a catalytically inactive mutant of Pol η (Dead Pol η).

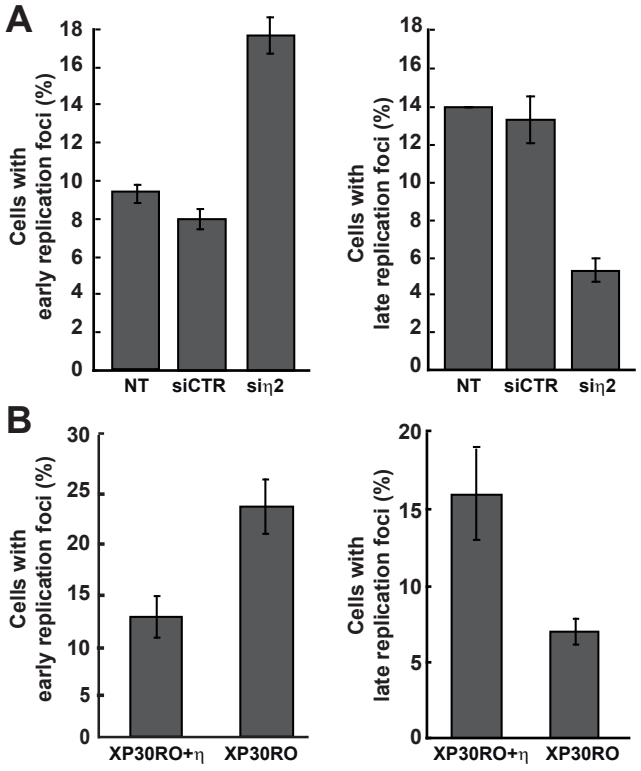
Panel A, SwissPDB viewer generated model of tri-dimensional structure of Human N-terminal Pol η with two mutations in the catalytic site. Figure was generated by Pymol (http://www.pymol.org).

Panel B, Ability of purified proteins Pol η WT and Pol η Dead to replicate through a CPD lesion.

To purified the Pol η proteins, overnight cultures of *E. coli* containing pET28 plasmids encoding either native or Dead mutant Pol η were diluted 1:100 into fresh Luria-Bertani media containing 50µg/ml kanamicin and grown at 37°C to an OD/A $_{600}$ of 0.5. Protein production was then induced by adding 1mM IPTG followed by growth for 3 hrs at 25°C. Cells were harvested by centrifugation, and cell pellets were resuspended in 20ml of buffer A (20mM Tris-HCl pH 7, 500mM NaCl and 5mM imidazole) then frozen for 16 hrs at -70°C. Cells were lysed by slowly thawing frozen cell pellets on ice in the presence of 20 mg/ml lysozyme, 1% Triton X100 and an EDTA-free protease inhibitor cocktail (Roche). Insoluble debris was removed by centrifugation at 8000xg for 15 min, and the remaining supernatant was applied to a Ni-chelating column (Ni Trap FF, GE Healthcare) following the vector manufacturer's instructions (Novagen). Bound protein was then eluted using a linear 5-500mM gradient of imidazole in buffer B (500mM imidazole, 20mM Tris-HCl pH 7, 500mM NaCl). Fractions containing Pol η were pooled and concentrated using Vivaspin 20ml 30K spin filters (Sartorius) before being applied to a Superdex 200 gel filtration column (GE Healthcare). Gel filtration fractions containing Pol η were concentrated using a Vivaspin 2ml 30K filter prior to being stored at -80°C in 20mM Tris HCl pH 7, 150mM Nacl, 0.1mM EDTA, 1mM DTT and 20% glycerol. Protein purity was estimated after 8% SDS-polyacrylamide gels by visual inspection of Coomassie Blue-staining and Western blotting.

Measure of the *in vitro* activity of the purified forms of Pol η was performed by primer extension on a template containing a single site-specific CPD followed by gel electrophoresis of the extension products. The negative control was the Taq DNA polymerase and the positive control was the Archae DNA polymerase Dpo4. Decreasing concentrations of

purified proteins were used (0.1, 0.05 and 0.01 μM). Standard primer extension reactions were performed at 37°C for 1hr as previously described (Boudsocq et al., 2001) using a partially single-stranded template. The primer consisted of oligonucleotide P16 (5'-CACTGACTGTATGATG-3′) that was labelled at the 5’ end using T4 polynucleotide kinase (New England Biolabs) and γ-^{32}P-ATP (5000 Ci/mmol; GE Healthcare). The template strand consisted of a single-stranded oligonucleotide that contained a single cys-syn cyclobutane pyrimidine thymine-thymine dimer (a generous gift from Shigenori Iwai and Fumio Hanaoka; 3'-GTGACTGACATACTACT^TCTACGACTGCTC-5′) (Servant et al., 2002).



Supplemental Figure 2 : Quantification of early versus late replication foci in various cell lines with compromised Pol η expression

The quantification was performed in three independent experiments (n>100 cells) for each cell line, in normal diploid MRC5 cells (Panel A), and in XP30RO/XP30RO-Pol η cells (Panel B).