Supplementary Material for manuscript (NAR-02585-D-2012) entitled: "Cell Cycle Stage-Specific Roles of Rad18 in Tolerance and Repair of Oxidative DNA Damage".

Supplementary Fig. 1 Kinetics of H_2O_2-induced ATM S1981 phosphorylation in TLS-deficient cells.

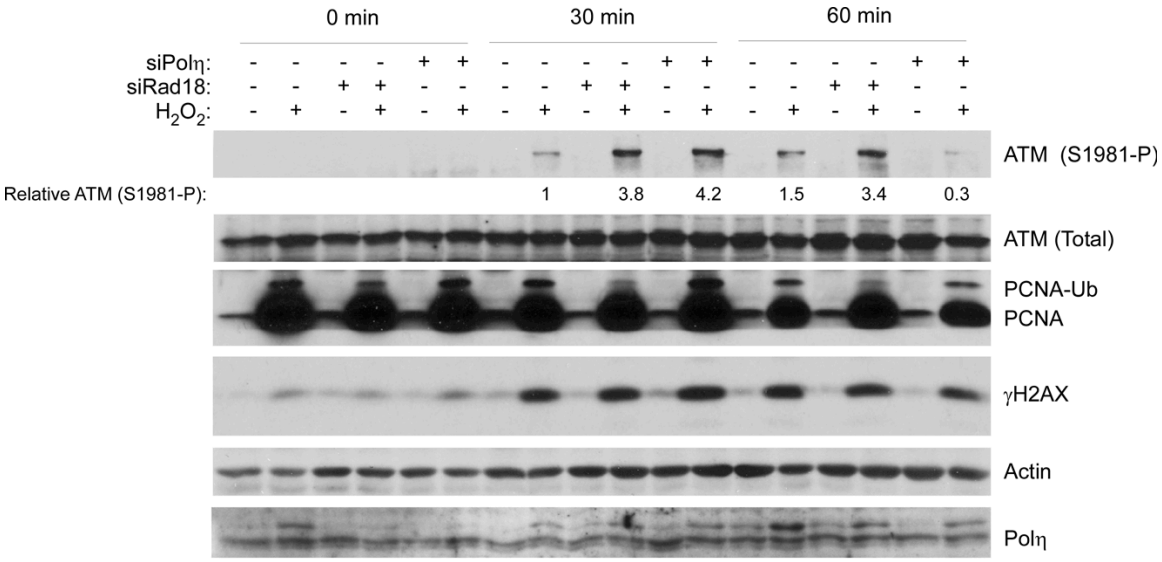

Fig. 1 Replicate cultures of HDF were transfected with siRNA against Rad18, Polη or non-targeting control siRNA, synchronized in G1 and treated with H_2O_2. 30 or 60 minutes after H_2O_2 treatment, cell extracts were isolated and analyzed by SDS-PAGE and immunoblotting with the indicated antibodies. Bands corresponding to ATM (S1981-P) were quantified by densitometry. The amount of ATM (S1981-P) in each lane is expressed relative to the amount of ATM (S1981-P) in scrambled control siRNA-transfected HDF 30 minutes post-H_2O_2 treatment.

Supplementary Fig. 2 Survival and proliferation of Polη- and Rad18-depleted cells following H_2O_2 treatment in G1.

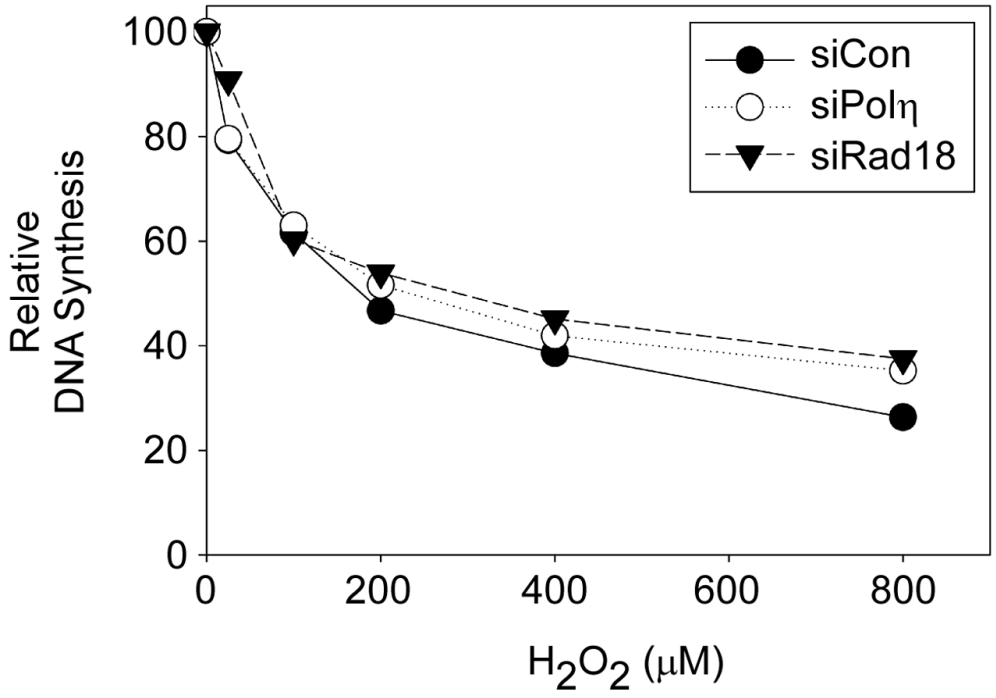

Fig. 2 Quiescent cultures of siCon-, siRad18-, and siPolη-transfected cells in 10 cm culture dishes were trypsinized and re-seeded in 24-well plates at a density of 20,000 cells per well. The left-over cells were re-plated in 10 cm culture dishes and harvested 24 hr later for immunoblot analysis with antibodies against Polη and Rad18 to confirm knockdowns. For the 24-well plates, 6 hr after re-seeding the growth medium was removed from each well and reserved. 1 ml of ice-cold PBS was added to each well. Triplicate wells were treated with the appropriate volume of a freshly-prepared ice cold solution of 100 mM H_2O_2 (in PBS) to give the indicated final H_2O_2 concentrations. The H_2O_2-treated plates were incubated on ice for 15 minutes. Then, the H_2O_2-containing PBS was removed, monolayers were washed twice with PBS and replenished with the reserved growth medium before being returned to 37°C CO_2 incubators. After 72 hr, each well was re-fed with 1 ml of complete medium containing 0.5 μCi [^{3}H]-methyl thymidine. After 4 hours the cells were fixed in 5% TCA and levels of radiolabel incorporated into the TCA-insoluble genomic DNA fraction were determined using scintillation counting as described under 'Materials and Methods'.

Supplementary Fig. 3 Effect of ApeI depletion and PARP inhibitor on PCNA mono-ubiquitination during G1.

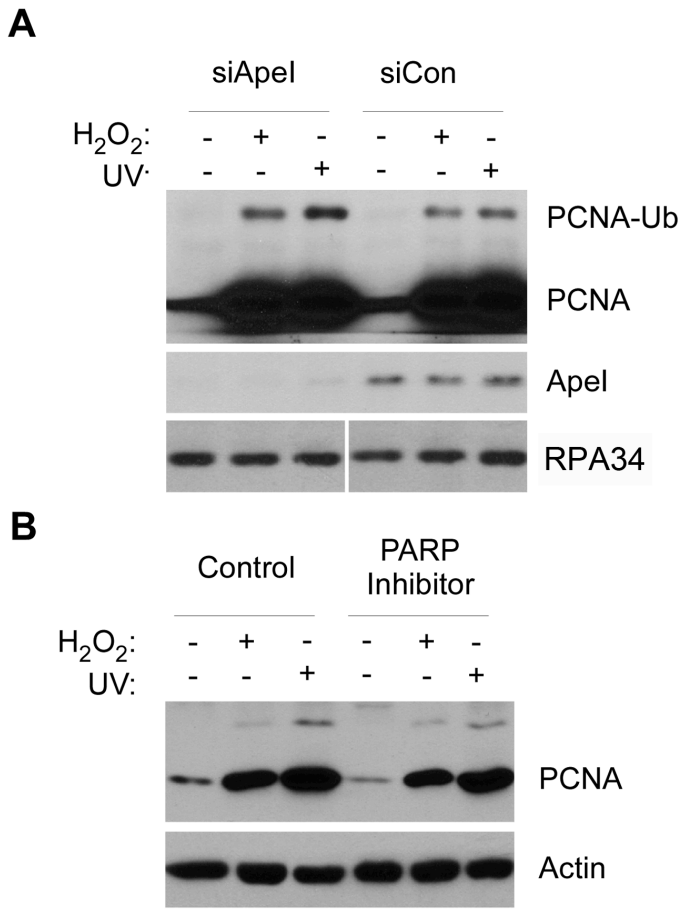

Fig. 3 (A) SiCon- and SiApe1-transfected HDF were synchronized in G1 and treated with H_2O_2 or UVC as described under 'Materials and Methods'. Cells were harvested 1 hr after genotoxin treatments and the resulting extracts were analyzed by SDS-PAGE and immunoblotting with the indicated antibodies.

(B) G1-synchronized HDF were pretreated with 100 μM PARP inhibitor (ANI, added directly to the medium from a 1000 x stock solution) or with an appropriate volume of DMSO for control. 1 hour after ANI or DMSO treatment, the cells treated with H_2O_2 or UVC as described under 'Materials and Methods'. Cells were harvested 1 hr after genotoxin treatments and the resulting extracts were analyzed by SDS-PAGE and immunoblotting with the indicated antibodies.