

# Supporting Information

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## SI Materials and Methods

**Chromosome Orientation FISH.** Briefly, cells were trypsinized and subcultured into medium containing BrdU ( $1 \times 10^{-5}$  M; Sigma-Aldrich) for one cell cycle. Colcemid (0.1  $\mu$ g/mL; Invitrogen) was added during the final 3 to 4 h to accumulate mitotic figures. Samples were collected and fixed in 3:1 methanol/acetic acid, dropped onto microscope slides, dried, treated with RNase A for 10 min at 37 °C (100  $\mu$ g/mL; Sigma-Aldrich), rinsed in PBS, fixed in 1% formaldehyde (10 min at room temperature), rinsed in PBS, then dehydrated through a cold ethanol series (75, 85, and 100%). Slides were air-dried and stained with Hoechst 33258 (0.50  $\mu$ g/mL; Sigma-Aldrich) for 15 min and exposed to 365 nm UV light (Stratalinker 2400) for 25 min. Following UV exposure, preferentially nicked BrdU-incorporated strands were digested with Exonuclease III (3 U/ $\mu$ L in provided reaction buffer; Promega) at room temperature for 10 min. Slides were rinsed and denatured briefly in 70% formamide/2 $\times$  SSC at 75 °C (1 min). Following an ethanol dehydration series and air drying, a Cy-3 conjugated (TTAGGG)<sub>3</sub> PNA telomere probe (0.2  $\mu$ g/mL; Applied Biosystems) was hybridized at 37 °C for 1.5 h. Slides were rinsed with 70% formamide/2 $\times$  SSC at 32 °C for 15 min followed by a 5-min rinse in PN Buffer. Chromosomes were counterstained with DAPI (Vectashield, Vector Laboratories).

**Small Interfering RNA Transfection.** The following siRNA sequences (Qiagen) were used for the targeted silencing of WRN (catalog # SI02663759, for Hs\_WRN\_6, the target DNA sequence is CGG-ATTGTATACGTAACTCCA); BLM (catalog # SI00000938, for Hs\_BLM\_1, the target DNA sequence is CCGAATCTCAATGTACATAGA and catalog # SI00000945, for Hs\_BLM\_2, the target DNA sequence is CTGACCATCTGTGACTATAAA), and ERCC1 (catalog # SI02663423, for Hs\_ERCC1\_4, the target DNA sequence is CACCGTGAAGTCAGTCAACAA).

**Western Blot Analysis.** Cells were harvested and resuspended twice in cold PBS (without Mg<sup>+</sup> Ca<sup>+</sup>). PBS was decanted, lysis buffer with proteases was added (50 mM Tris-HCL, pH 7.5, 150 mM NaCl, 2 mM EDTA, 2 mM EGTA, 25 mM NaF, 25 mM beta-glycerophosphate, 0.2% Triton X-100, 0.3% Nonidet P-40, 0.1 mM sodium vanadate, 0.1 mM PMSF, 5  $\mu$ g/mL leupeptin, 5  $\mu$ g/m aprotinin), and cells incubated 10 min on ice (1). Lysed cells were centrifuged at 13,000 rcfs (or 12,000 rpm) for 10 min at 4 °C and supernatant collected. Next, 35 to 50  $\mu$ g of protein was fractionated by SDS/PAGE (Bio-Rad) and transferred to a Hybond nitrocellulose membrane (GE Healthcare). Blots were blocked in 5% milk for 30 min and blotted with primary antibodies overnight at 4 °C. Membranes were washed three times in TBS containing 0.1% Tween 20 for 15 min and incubated with secondary antibodies for 1 h at room temperature. Membranes were developed using the ECL Plus Detection kit (GE Healthcare).

1. Song Q, et al. (1996) DNA-dependent protein kinase catalytic subunit: A target for an ICE-like protease in apoptosis. *EMBO J* 15:3238–3246.