

Supplemental Material to:

Venkateswarlu Popuri, Deborah L. Croteau, Robert M. Brosh Jr. and Vilhelm A. Bohr

RECQ1 is required for cellular resistance to replication stress and catalyzes strand exchange on stalled replication fork structures

Cell Cycle 2012; 11(22) http://dx.doi.org/10.4161/cc.22581

http://www.landesbioscience.com/journals/cc/article/22581

Sequence 5'-3'
ACTATCATTCAGTCATGTAACCTAGTCAATCTGCGAGCTCGAATTCACTGG
AGTGACCTC
ATTGACTAGGTTACATGACTGAATGATAGT
GAGGTCACTCCAGTGAATTCGAGCTCGCAG <u>CCCCT</u> CTAGGTTACATGACTG
AATGATAGT
ACTATCATTCAGTCATGTAA
GAGGTCACTCCAGTGAATTCGAGCTCGCAGTCAATGTCGACATACCTAGTA
CTTTACTCC
GGAGTAAAGTACTAGGTATGTCGACATTGA

Supplementary Table 1: Oligonucleotides used in the study. RS2 is complementary to the 5'-half of RS1. RS3 is complementary to RS1 except for the five underlined bases. RS4 is complementary to the 3'-end of RK3. The 5'-half of RS5 is complementary to the 3'-half of RS1. RS6 is complementary to the 3'-half of RS5. The polarity of the leading and lagging oligonucleotides is indicated by arrows (5'-3').

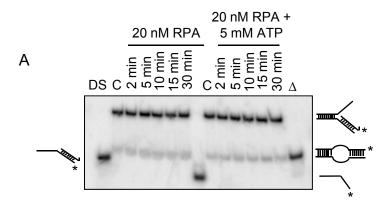
Supplementary Figure 1: (A) Strand exchange activity of RPA alone (20 nM) on the replication fork duplex lacking the leading strand in the presence and absence of 5 mM ATP. (B) Strand exchange activity of RPA alone (20 nM) on the replication fork duplex lacking the lagging

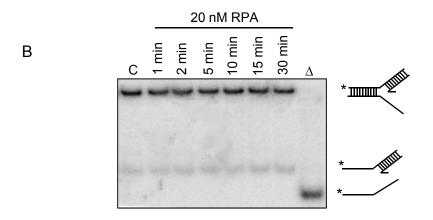
strand in the presence of 5 mM ATP. (C) Unwinding of RECQ4 (50 nM) on a replication fork substrate lacking the lagging strand in the presence and absence of RPA (20 nM). 'DS' represents the control duplex substrate; 'C' represents the control stalled replication fork-like structure; 'M' represents the marker for strand exchange product; 'Δ' represents the heat denatured.

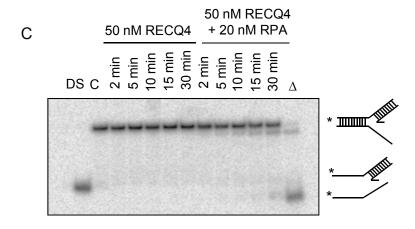
Supplementary Figure 2: Characterization of stable depletion of RECQ1 in U2OS cells. (A)

Stable depletion of RECQ1 was performed in U2OS cells by using the two independent and dose dependent lenti-viral shRNA, shRECQ1-1 and shRECQ1-2. (B) Growth assays were performed to assess the cell proliferation and cell growth for RECQ1 stable knockdown cells over a period for 5 days. MTT Cell proliferation experiments were performed to evaluate the cell proliferation in the increasing concentrations of Hydroxyurea (C), Camptothecin (D) and 8-methoxypsoralen (E).

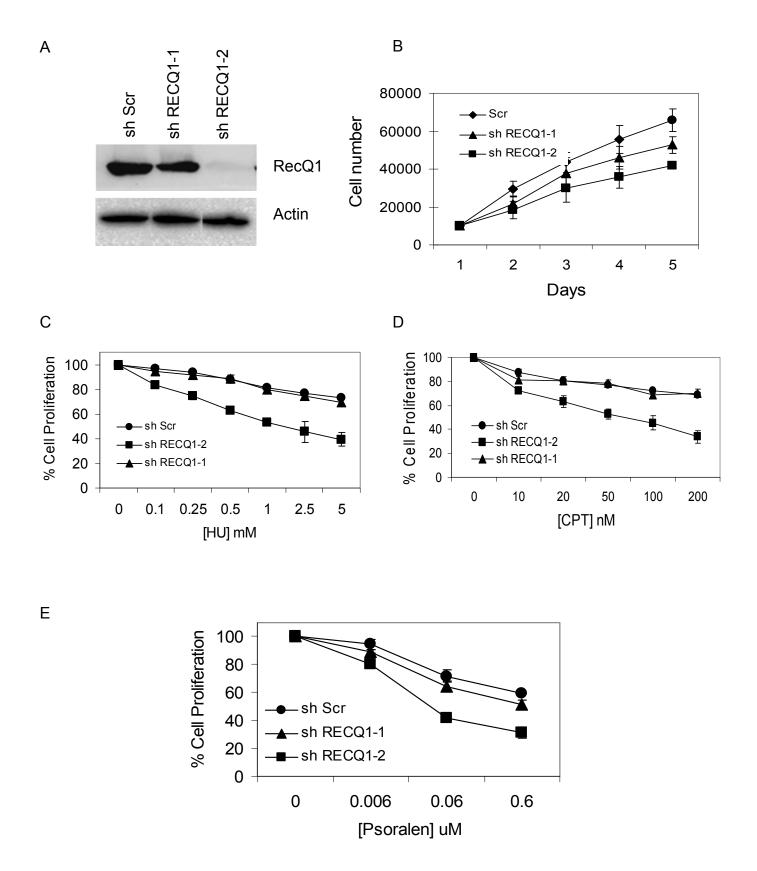
Supplementary Figure 3: Characterization of stable depletion of RECQ1 in HeLa cells. (A) RECQ1-depleted HeLa cells display relatively lower BrdU staining with fewer S-phase cells compared with their scrambled counterparts. (B) Stable depletion of RECQ1 accumulates spontaneous DSBs, inferred by the accumulation of 53BP1 foci. (C) Quantification of the 53BP1 foci from shScrambled and shRECQ1-2 depleted HeLa cells. The plot represents an average of three independent experiments.



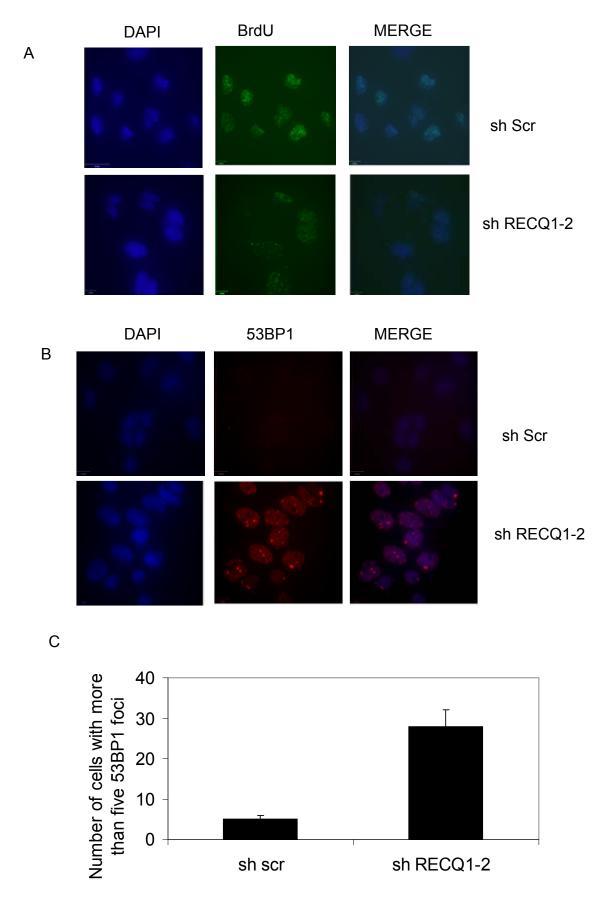




Supplementary Figure 1



Supplementary Figure 2



Supplementary Figure 3