Supplementary Information

Materials and Methods:

Materials, cell lines, cell culture and siRNA transfections

Caffeine (Sigma, St. Louis, MO) was used at 5 mM and nocodazole (Calbiochem, CA) was used at 40 ng/ml and ATM/ATR inhibitor CGK 733 (Calbiochem) was used at 10 μM. 5-azacytidine (MP Biochemicals, OH) was used at 5 μM. Human colorectal cancer cell lines, HCT116 was obtained from the ATCC (Mansas, VA), HCT116 (p53-/-) was the kind gift of Dr B. Vogelstein (Johns Hopkins University, MD). Cells were grown in 10% fetal bovine serum and 5% penicillin-streptomycin in McCoy's 5A modified medium. Liver carcinoma cells, Huh7 and Hep3B were cultured in DMEM and Minimum Essential Medium, respectively supplemented with 10% fetal bovine serum and 5% penicillin-streptomycin antibiotics. Cells were transfected with non-targeting sequence from firefly luciferase (Dhamarcon) or specific siRNA targeting UHRF1 or caspase-8 (Invitrogen). Two transfections were performed 24 hours apart and cells were harvested 48 hours after the initial transfection except for the experiment shown in Figure S3 where TUNEL assays were performed 72 hours after the initial transfection.

siRNA sequences are shown in Table S1.

Immunoblots

Western immunoblots were performed as previously described (Ukomadu & Dutta, 2003). Cells were lysed in high salt lysis buffer (HSLB): 25 mM Hepes pH 7.4, 400 mM KAC, 10% glycerol, 5mM $MgCl_2$, 2 mM EDTA, 0.5% NP40) on ice for 45 minutes. An equal volume of low salt lysis buffer (LSLB): HSLB except with 0 mM KAC was added. Approximately 50 μg of protein was loaded for each lane and separated on 10% polyacryalmide gel.

Antibodies to the following proteins were used at the indicated dilutions: ICBP90 (#612264,BD transduction; 1:2000), cyclin A (H-432, Santa Cruz ; 1: 2000), cyclin D2 (MS-221-PI, Neomarkers; 1:2000), cyclin E (HE-11, Santa Cruz; 1:2000) , cyclin B1(H433, Santa Cruz; 1:2000), Chk2 (#2662, Cell Signaling; 1:1000), phospho-Chk2 thr 68 (#2661, Cell Signaling; 1;1000), histone H2AX ser139 (JBW301, Millipore; 1:5000), cyclin dependent kinase 1 (sc-54, Santa Cruz; 1:2000), phospho-CDK1 tyr 15 (#9111,Cell Signaling; 1:1000), p53 (D01, Santa Cruz; 1:1000), phospho-p53 ser 15 (#9284, Cell Signaling; 1:1000), phospho-Cdc25 (#9258,Cell signaling 1:750), p63 (#4892, Cell Signaling; 1:1000), p73 (Ab-2, Calbiochem; 1:1000), cleaved PARP Asp 214 (#9541, Cell Signaling; 1:1000), PARP (9542, Cell Signaling; 1:1000), cleaved caspase-3 Asp 175 (#9661, Cell Signaling; 1:1000), cleaved caspase-8 (# 9746, Cell Signaling; 1:1000), caspase-8 (1C12, Cell Signaling;1:1000), Histone H3 (#9717; Cell signaling; 1: 1000), phospho-histone H3 (#9706; Cell Signaling; 1: 1000) topoisomerase 2A (MS1819S0; Thermofisher; 1:1000), β-actin (A1978, Sigma; 1:10000) and tubulin (AA4.3; Developmental Studies Hybridoma Bank, 1:2000).

Quantitative polymerase chain reaction

RNA was extracted using RNeasy spin column kit (QIAGEN) according to the manufacturer's protocol. The first strand cDNA was synthesized from 2 μg of total RNA SuperScript III RT (Invitrogen) for RT-PCR. Quantitative PCR reactions were performed in triplicate using LightCycler SYBR Green reagent and detector system (Roche). Gene expressions were normalized to β-actin as reference. The average of 3 individual experiments +/- the standard error of the mean is shown. Primer sequences for QPCR are shown in Table S2.

Fluorescence Microscopy

TUNEL assays were performed using an in-situ death detection kit according to manufacturer's protocol. For immunofluorescence, cells were fixed in ice cold methanol for 20 minutes and then blocked in blocking buffer (PBS containing 3% BSA and 0.1% NP40) for 30 minutes. Cells were stained with primary antibody dissolved in blocking buffer for one hour at room temperature. After staining, cells were washed in blocking buffer, and then incubated with a secondary fluorescent tagged antibody. The cells were mounted with DAPI, and visualized by fluorescence microscopy

Table S1: siRNA sequences

Name	Sequence (5'-3')
UHRF1: si-A	TCTCAACTGCTTTGCTCCCATCAAT
UHRF1: si-B	GCCAGGTGGTCATGCTCAACTACAA
UHRF1: si-C	ATTGATGGGAGCAAAGCAGTTGAGA
Caspase-8: C8A	AGATGGACTTCAGCAGAAATCTTTA
CHK2: si-CHK2	CCTTCAGGATGGATTTGCCAATCTT

Table S2: PCR Primers

Target	Forward (5'-3')	Reverse (5'-3')
UHRF1	CCAGCAGAGCAGCCTCATC	TCCTTGAGTGACGCCAGGA
PUMA	ACGACCTCAACGCACAGTACGAG	AGGAGTCCGCATCTCCGTCAGTG
p63	AAGATGGTGCGACAAACAAG	AGAGAGCATCGAAGGTGGAG
p73	GCAGATTGAACTGGGCCATGA	GCAGATTGAACTGGGCCATGA
TOP2A	CTTACCAAGCCCAAGACTGG	TGCCCATGAGATGGTCACTA
β-actin	GCTCGTCGTCGACAACGGCTC	CAAACATGATCTGGGTCATCTTCTC

References

Ukomadu C and Dutta A. (2003). *J Biol Chem*, **278,** 4840-6.

Figure Legends for supplementary results:

Figure S1: Depletion of UHRF1 in Hep3B cells causes a G2/M block
A) Representative histograms of FACS analysis of control siRNA transfected cells (top) and targeting siRNA transfected cells bottom. Note the increase in the 4N DNA containing population. B) The knock down of UHRF1 leads to an increase in tyrosine phosphorylation of CDK1. β-actin is used a loading control

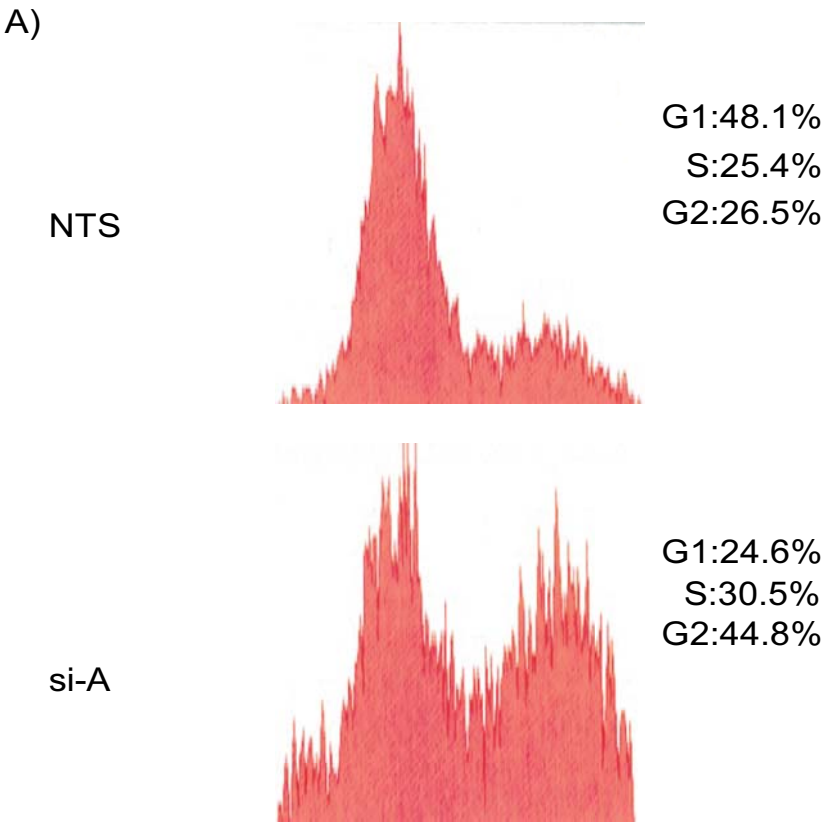
Figure S2: Presence of serine 139 phosphorylated H2AX in UHRF1 depleted cells. Phospho-H2AX positive cells are present following si-A and si-B transfection but absent in NTS transfected cells (middle panel, red). DAPI staining is shown in the left panel (blue) and merged images in the third panel. A high power view of a phospho-H2AX positive cells show multiple foci as seen following DNA damage.

Figure S3: Quantitation of TUNEL positive cells in UHRF1 depleted cells. Cells grown on poly-L lysine coated slides were transfected and analyzed for apoptosis at 72 hours after transfection. Graph represents the percentage of TUNEL positive cells for three replicates. At least 1000 cells were counted for each experiment. Error bars represent standard deviations from the mean.

Figure S4: p53 and p63 are nor responsible for cell cycle block and apoptosis
A). mRNA levels for UHRF1, PUMA, p63 (Tp63) and p73 (Tp73) in cells containing (black bars) and cells depleted (white bars) of UHRF1. There is an 80% reduction in UHRF1 message level for this experiment (left most bars). There is no increase in the mRNA level of PUMA, p63 and p73. Error bars represent standard deviation from the mean of three independent samples. B) Western blots of lysates from UHRF1 depleted cells show that p63, p73 and PUMA protein levels are not increased.

Figure S5: 5-azacytidine blocks cells in G2/M. Representative histogram of HCT116 cells. Cells treated with 5-Azacytidine showed an increase in the G2/M peak (lower histogram) when compared to untreated cells (upper histogram).

Figure S1


A)
NTS
G1:48.1%
S:25.4%
G2:26.5%
si-A
G1:24.6%
S:30.5%
G2:44.8%

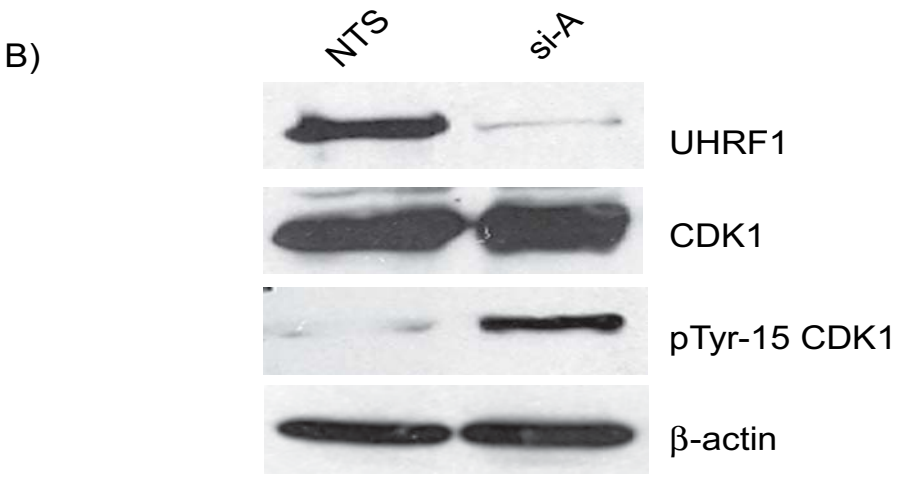

B)
NTS
si-A
UHRF1
CDK1
pTyr-15 CDK1
β-actin

Figure S2

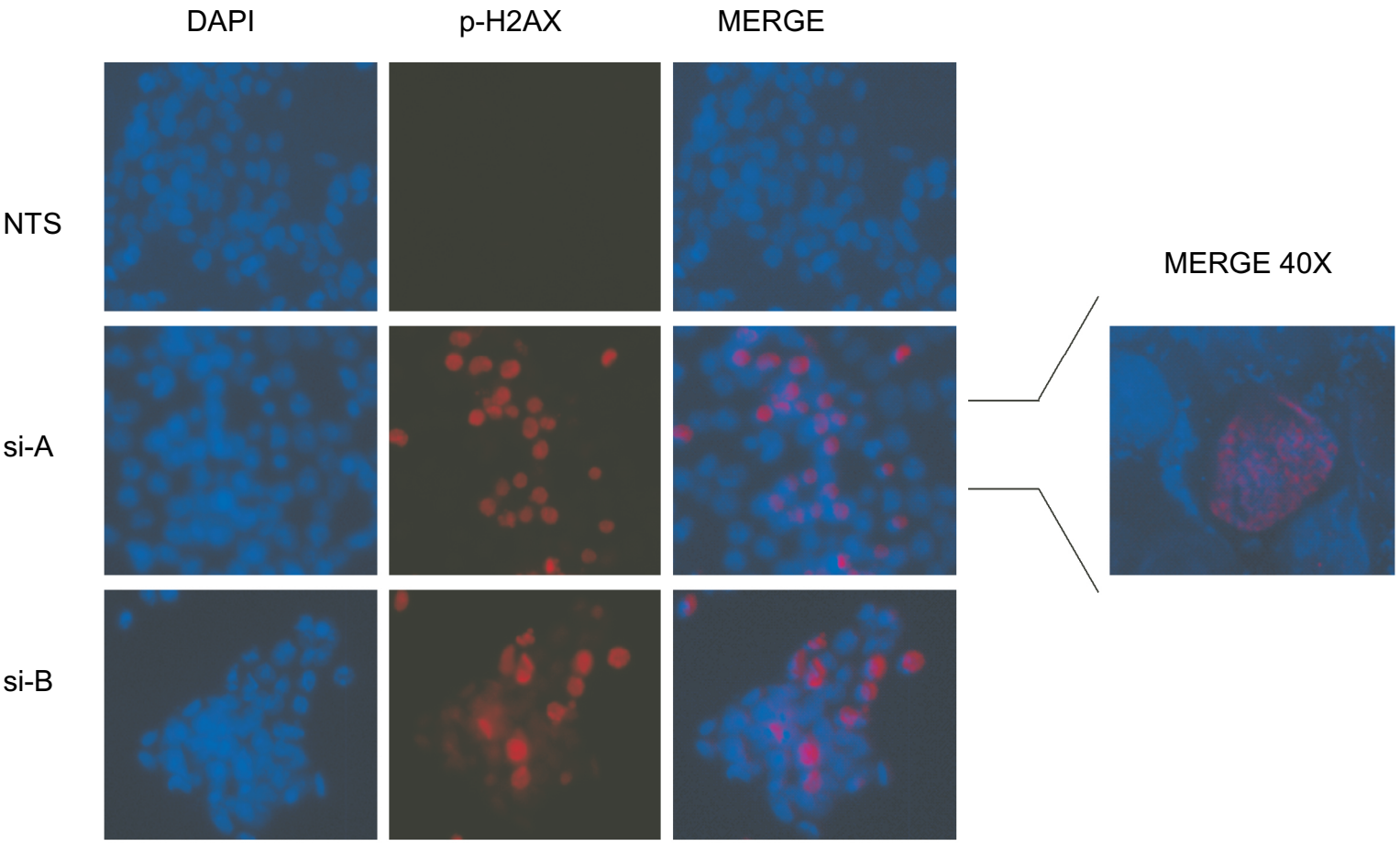


Figure S3

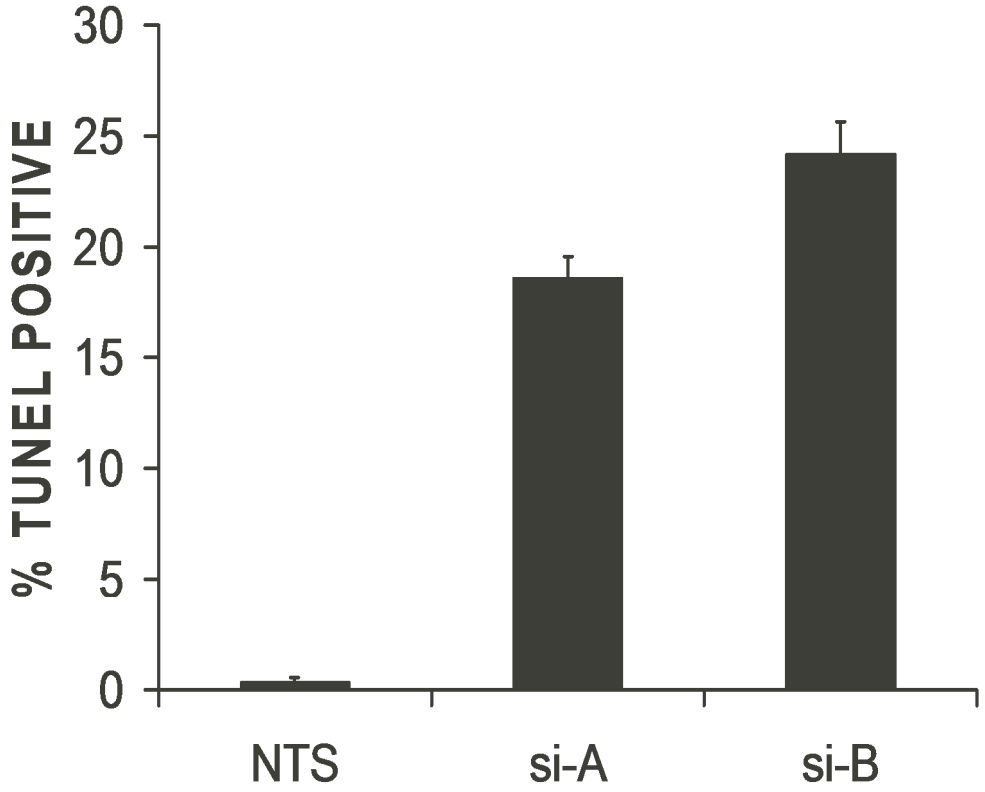
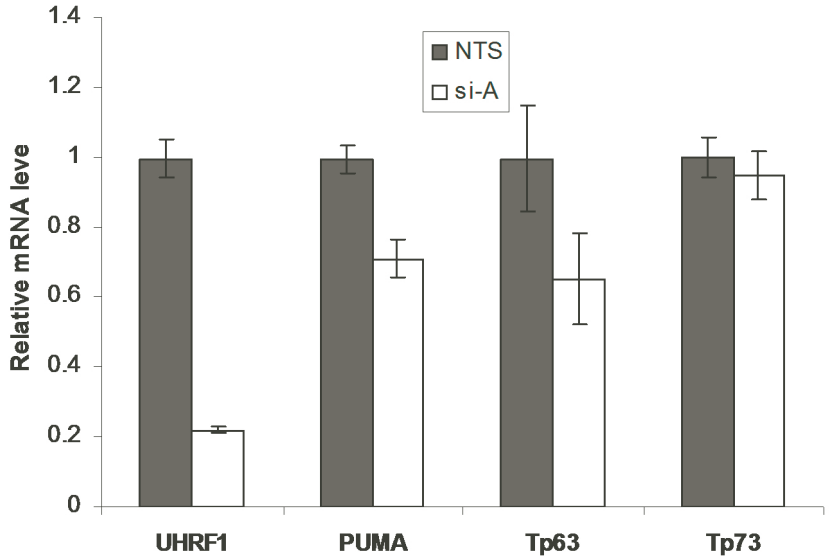


Figure S4

A)


1.4
1.2
1
0.8
0.6
0.4
0.2
0
Relative mRNA leve
NTS
si-A
UHRF1
PUMA
Tp63
Tp73

B)

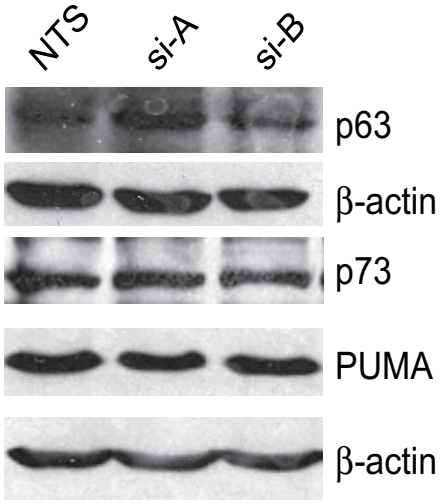
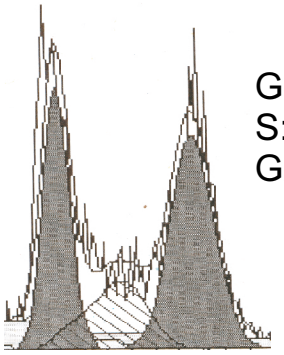

NTS
si-A
si-B
p63
β-actin
p73
PUMA
β-actin

Figure S5

Untreated

G1: 82.61%
S: 9.40%
G2/M: 7.99%

5-Azacytidine



G1: 34.30%
S: 19.99%
G2/M: 45.71%