Supplementary Information

Supplementary Figure legends

Figure S1. HCT116 cells were transiently transfected with a control scrambled shRNA and two different SMAR1 specific shRNA constructs (sh 745 and sh 1077). Decrease in SMAR1 expression with time is shown by Western blotting. The specificity of SMAR1 knockdown by the shRNA constructs was confirmed by targeting another abundant nuclear matrix protein Lamin C.

Figure S2. (A) Luciferase activity of full length p53AIP1 promoter upon SMAR1 over expression (FS) and knockdown (sh 745 & sh 1077). The SMAR1 truncation (F3 1-160 aa) lacking the DNA binding domain and protein interacting domain is used as a control. (B) Statistical representation of annexin-Cy3 stained apoptotic population analyzed in more than 50 (n>50) different fields. Bars indicate SD from three independent experiments.

Figure S3. (A) Cell cycle analyses of HEK293 cells treated with UV after knockdown of SMAR1 by sh 1077. Twenty four hours after shRNA transfection cells were either left untreated or treated with UV (5 J/m^2) and incubated for another 24 h. Cells were then fixed and stained with PI to determine apoptotic population.

(B) Knockdown of SMAR1 by sh 1077 in MCF-7 cells induces apoptosis as determined by PI staining.

(C) Mouse embryonic fibroblasts were transduced with control shRNA (Lv cntrl) and SMAR1 shRNA (LVsh 1077) lentivirus. Four days after viral transduction, MEFs were harvested, lysed and Western blotting done to determine the expression levels of Bax, Puma, p53, Ac-p53 and SMAR1.

(D) HCT116 $p53^{-/-}$ were transfected with control shRNA vector (C-sh, lane 1) and SMAR1 shRNA sh 1077 (SM-sh, lane 2). Thirty six hours post transfection cells were harvested and Western blotting done for Bax and Puma.

Figure S4. MAR prediction of (A) *BAX*, (B) *PUMA* and (C) *p53AIP1* promoters**.** Sequence in red denotes p53 response element (p53RE). Approximately 700 bp region of the three promoters upstream from transcription start site were analyzed using MARWIZ software. MAR potential is shown in the graph and the region corresponding to the MAR is coded yellow. The sequence in blue (corresponding to P1) and green (corresponding to P2) indicates the identical sequences of *BAX* and *PUMA* promoters. The exact location of these sequences with respect to MAR is shown in the MAR plot. (D) The purity of the nuclear matrix isolated from HCT116 cells was tested by Western blotting against antibody LaminB1 and Histone H1. (E) *In vivo* chromatin immunoprecipitation (ChIP) assay to detect promoter occupancy of SMAR1 on *p53AIP1* promoter upon low dose UV (5 J/m^2) irradiation. Cross-linked chromatins from UV irradiated cells were pulled with SMAR1 antibody and bound chromatin fragments were detected by specific PCR primers given in Table1. (F) Immunostaining of SMAR1 with nucleolar marker Nucleolin (C23) after low dose (5 J/m^2) UV irradiation showing nucleolar localization of SMAR1. Images are representative of more that 30 (n>30) from two independent experiments.

Figure S5. Immunostaining of PML and p53 in control HCT116 p53+/+ cells and cells treated with low dose (5 J/m^2) and high dose (100 J/m^2) UV irradiation. P53 is stained with FITC (green), PML with Cy3 (red) and DNA is stained with DAPI (blue). Images are representative of more that 50 (n>50) from two independent experiments.

Figure S6. Cell cycle analysis in HCT116 $p53^{+/+}$ cells by propidium iodide staining depicting percentage apoptosis upon PML knockdown by siRNA in presence and absence of high dose apoptotic UV treatment (100 J/m^2, 12 h).

Figure S7. HCT116 $p53^{-/-}$ cells were UV irradiated with apoptotic dose (100 J/m^2, 24 h) and Western blotting was done for SMAR1, Bax and PML.

Supplementary Tables

Table1.

Primers for ChIP:

BAX Fwd	5’ TCA GCA CAG ATT AGT TTC TG
BAX Rev	5’ GGG ATT ACA GGC ATG AGC TA
PUMA Fwd	5’ GAT TAC AGG CAT GCG CCA CA
PUMA Rev	5’ ACC CAC ACT GAT GAT CAC AC
p53AIP1 Fwd	5’ ACG TCG CAG GTG GAG AGA AT
p53AIP1 Rev	5’ GGG ACA GCT GGA ATG TCA GT
GAPDH Fwd	5’ TTG CCA TCA ACG ACC CCT TC
GAPDH Rev	5’ AGA CTC CAC GAC ATA CTC AGC ACC
P21 Fwd	5’ CGC TCT ACA TCT TCT GCC TT
P21 Rev	5’ GAC AGC GCT GGG AAG GAG C

Table2.

Probe Sequence used for EMSA

Probe P1	5’ GCAATTCCAGCTACTTGGGAGGCTGAGGCAGGAGAATTGC
Probe P2	5’ GTAATCTCAGCACTTTGGGAGGCCAAGGTGCGAGGATCGC

Probe P3	5’ AACTTCATATTCCTTTTCTTTTTACACAAACACAAACATT
Probe P4	5’ TAATGTGTTAGCTGTGAAATTGTGTGAGTGCATTTGTGTA

Supplementary References:

Andersen JS, Lyon CE, Fox AH, Leung AK, Lam YW, Steen H, Mann M, Lamond AI. (2000) Directed proteomic analysis of the human nucleolus. *Curr Biol.* **12**: 1-11

Cockerill PN, Garrard WT (1986) Chromosomal loop anchorage of the kappa immunoglobulin gene occurs next to the enhancer in a region containing topoisomerase II sites. *Cell* **44**: 273-282

Rampalli S, Pavithra L, Bhatt A, Kundu TK, Chattopadhyay S (2005) Tumor suppressor SMAR1 mediates cyclin D1 repression by recruitment of the SIN3/histone deacetylase 1 complex. *Mol. Cell Biol.* **25:** 8415-8429

Tong-Chuan He, Shibin Z, Luis T, da Costa, Jian Yu, Kenneth WK, Vogelstein B (1998) A simplified system for generating recombinant adenoviruses. *Proc. Natl. Acad. Sci. USA* **95:** 2509-2514

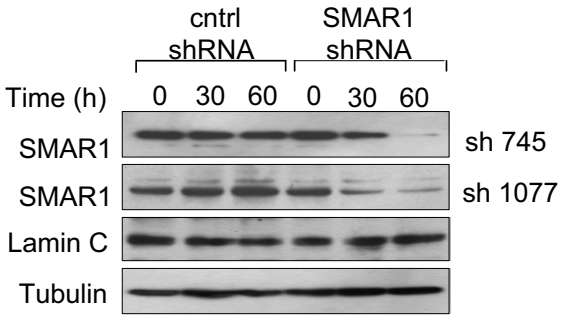

Figure S1; Sinha et al.

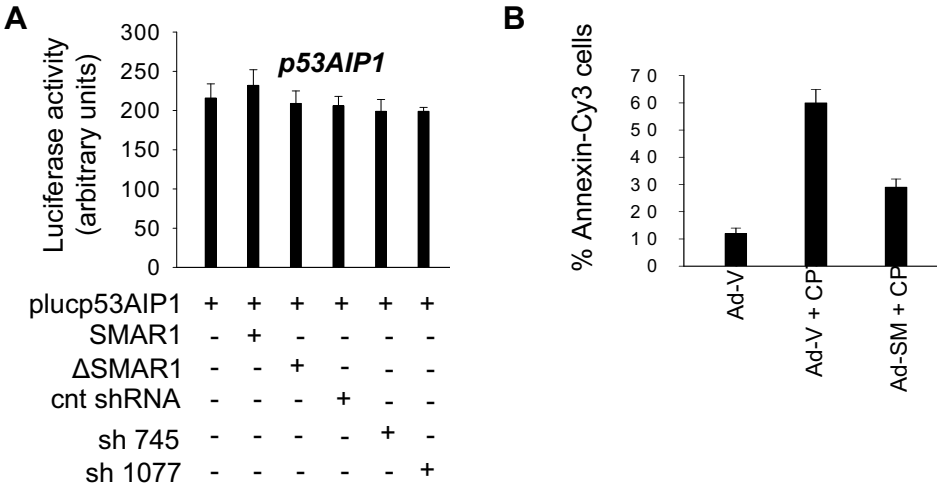

Figure S2; Sinha et al.

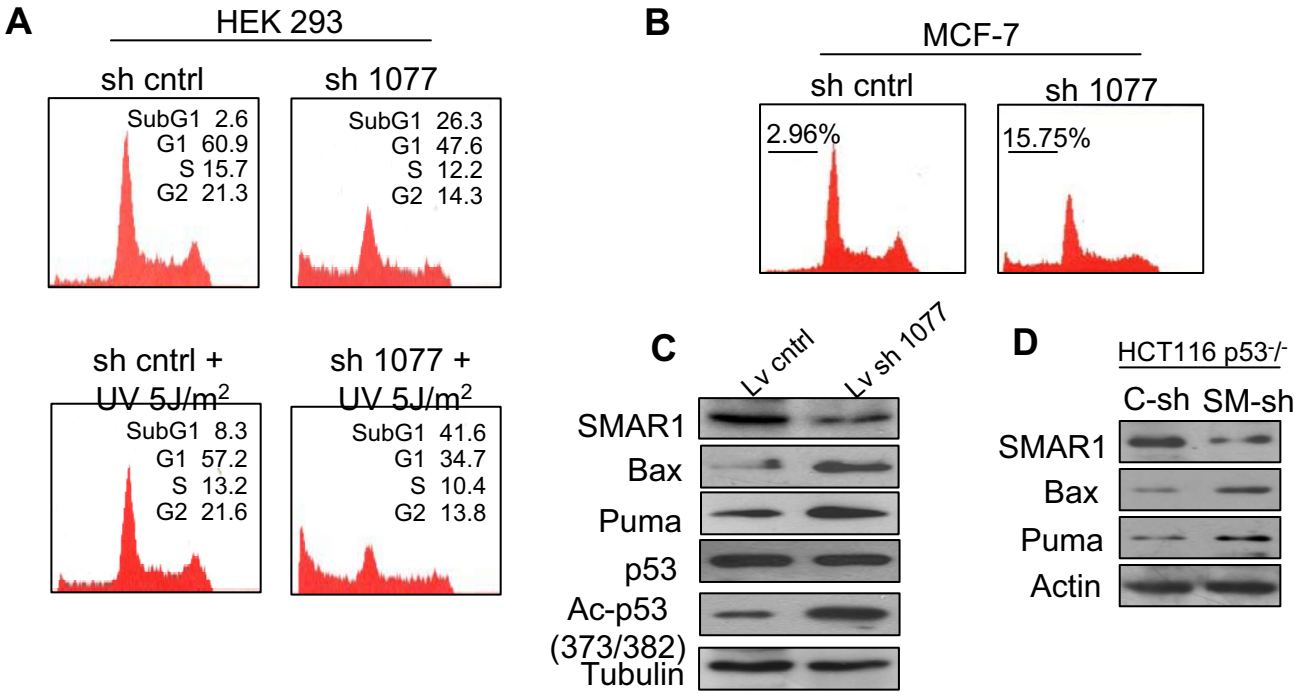

Figure S3; Sinha et al.

p53 response element
sequence P1
sequence P2
MAR region
Primers used in ChIP

BAX Promoter

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CAATGAATTGTAAATATGTGTATACCCGGCCGGGCACAGTGGCTCACGCC    -1301
TGTAATCCCAGCACTTTGGGAGGCCGAGGCAGGTGGATCACTTGAGGTCA    -1251
GGAGCTTGAGACCAGCCTGACCAACATAGTGAAACCCCATCTTTACTAAA    -1201
AATACAAAATTAGCTGGGCGTGGTGTCGCATGCCTGCAATTCCAGCTACT    -1151
TGGGAGGCTGAGGCAGGAGAATTGCTTGAACCCGGAGGCAGAGGTTGCAG    -1101
TAAGCCGAGATCGTGCCATTGCACTCCATCCTGGGCAACAAGAGCAAAAC    -1051
TCCGTCTCAAAATAATAATAATAATAATAATAATAATAATAATAATAATG    -1001
TGTATACCCATGTAAACACCATTCAGATAAAAATATGGCATATTTGGGGC     -951
ACCCGGGGAGTGTCTCTTGTGGCCCCTCCCCTCCATACCCTGCTGATCTA     -901
TCAGCACAGATTAGTTTCTGCCACTTTTTAAACTTCATATTCCTTTTCTT     -851
TTTACACAAACACAAACATTCGAGTCATGACTGGGTGGGGTGGCTCAAGC     -801
CTGTAATCTCAGCACTTTGGGAGGCCAAGGTGCGAGGATCGCTTGAGTCT     -751
GGGAGTTCAGAGACCAGCCTGGGCAACATAGAGAGACCTCATCTCCACAT     -701
AAAAAGTTTTAAAAATTAACCAGGGGCGGTGTAGTCCCAGCTACTCAGGA     -651
GGCTGAGGTGGGAGGCTTCAGCCCGGGAATTCCAGACTGCAGTGAGCCAT     -601
GATTGGGCCACTGCACTCCAGCCTGGGCAACACAGTGAGACCCTGTCTCA     -551
AAAAAAAAAAAAAAAAAAAACAGGAAAAAACAAACAAACAGAAAAGCAGGC     -501
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GGGGTTATCTCTTGGGCTCACAAGTTAGAGACAAGCCTGGGCGTGGGCTA     -401
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ATTGGACAGTCACGTGACGGGACCAAACCTCCCGAGGGAGCGAGGCAGGT     -101
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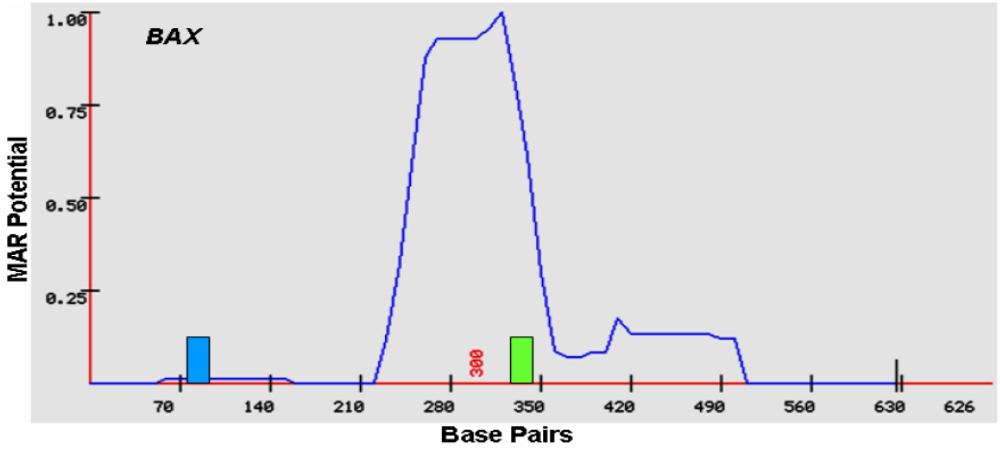

Figure S4A; Sinha et al.,

p53 response element
sequence P1
sequence P2
MAR region
Primers used in ChIP

PUMA Promoter

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GTGCATGCTCCGACGTGTGTGCAGTGGGCCAGTTAGCAAGAAGCTGTCAC    -1251
AGGTGTGACTTTGTGACATGTGTGGGTGGTCAGTTTCTTCTATGTCTGAT    -1201
TTGGTTTGTGTCTCTGAATGTCAGTTTCTTTCCTTTATTTTTATTTTTAA    -1151
GACGGAGTTTGCTCTTGTTGCCCAGGCTAGAGTGCAATGGCACTATCTCG    -1101
GCTCACTGCAACCTCCGCCTCCCGGGTTCAAGCAGTTCTCCTGCCTCAGC    -1051
CTCCCAAGTAGCTGGGATTACAGGCATGCGCCACAACGCCCGGCTAATTT    -1001
TGTATTTTTAGTAGAGATGGGGTTTCATCATGTTGGTCAGGCTGGTCTCG     -951
AATTCCTGACCTCAGGCAGTCCACGCACCTTGGCCTCCCAAAGTGCTGGG     -901
ATTACAGGCATGAGCCACCGTGTCGGGCGAATGTCACTTTCTGATAGTTT     -851
TAATGTGTTAGCTGTGAAATTGTGTGAGTGCATTTGTGTATGTCCCTGTG     -801
GGAGTGTGATTTGGATTTGGCCGTGTATCCAGGTATCCCTGTAACAGGTG     -751
TCTGTGTGTATGTGTGTGTCCCCTGTGCCTATCAGCAAGTTTGTGTTTCC     -701
TGATAAGCACTCCGCCTATGTCTGTGTGGTTGCACCACCGTGTGTGTGGT     -651
GTGGGTGCCTGTTCGGTAGGGTTGTTTGTGAACACAGTTTGTGGGCCCAG     -601
GTGTGATCATCAGTGTGGGTGTTTCTGCAACTGTGTGTGGCCCTGTCATT     -551
GTGTCCGTCTGCTTGTCCAGGGGACCCTGTTAGTGAGTCTGTGCATTTCC     -501
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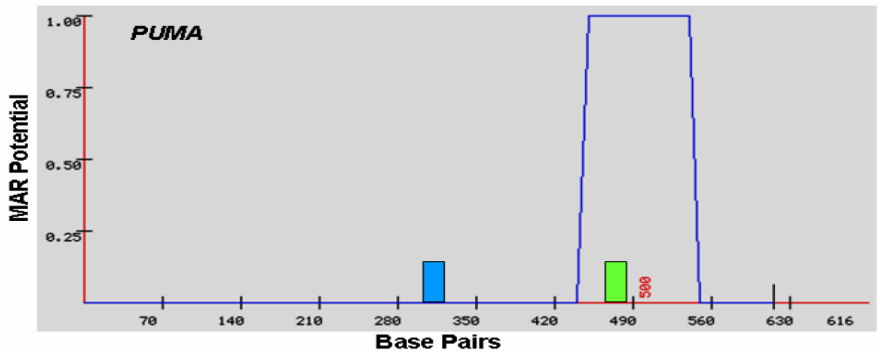

Figure S4B; Sinha et al.,

p53 response element
sequence P1
sequence P2
MAR region
Primers used in ChIP

p53AIP1 Promoter

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tggacactggccaggggagggagaggaaacacgtcgcaggtggagagaat    -1001
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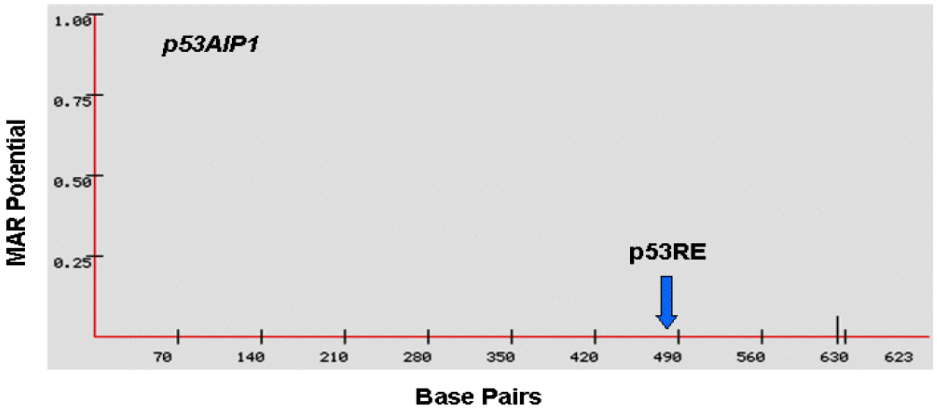

Figure S4C; Sinha et al.,

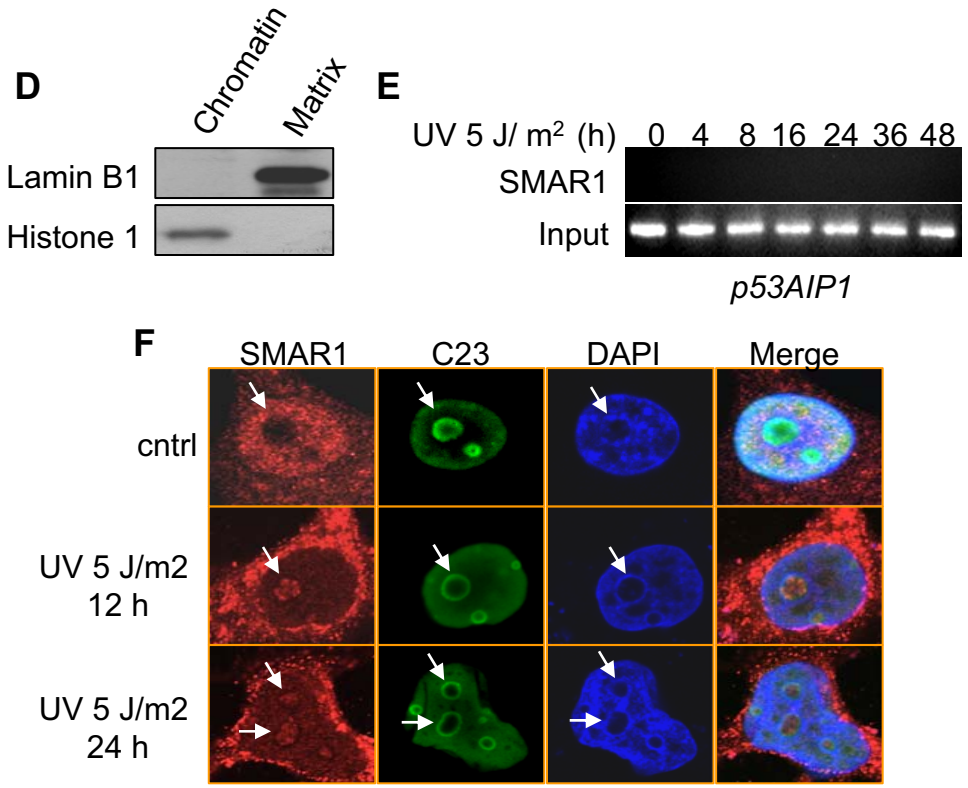

Figure S4; Sinha et al.

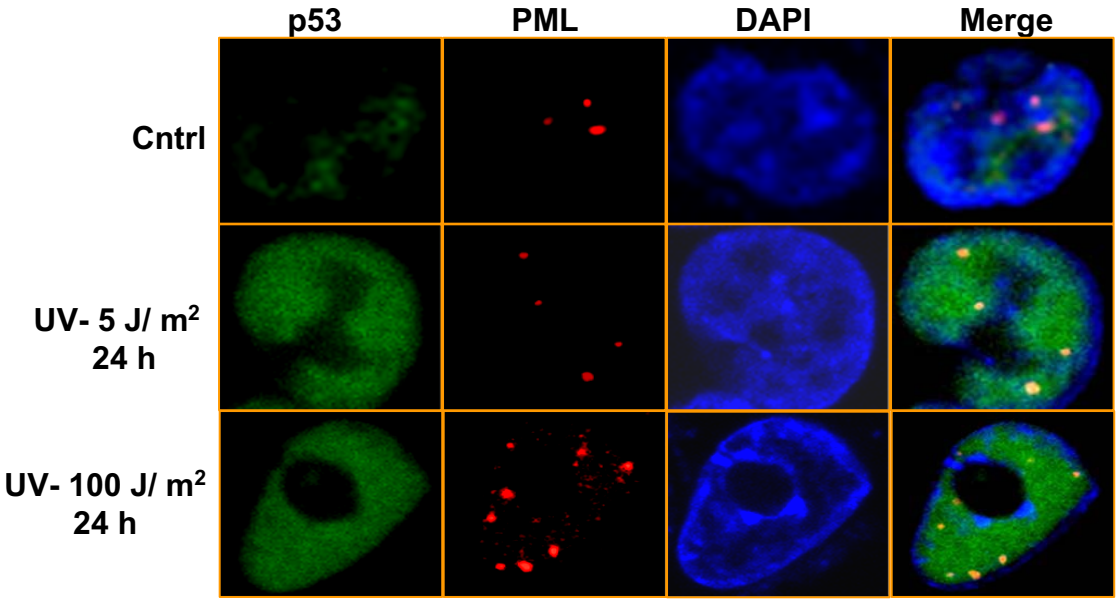

Figure S5; Sinha et al.

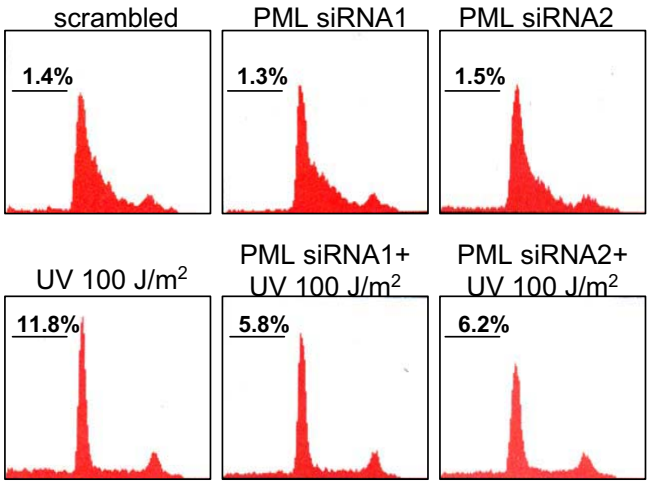

Figure S6, Sinha et al.

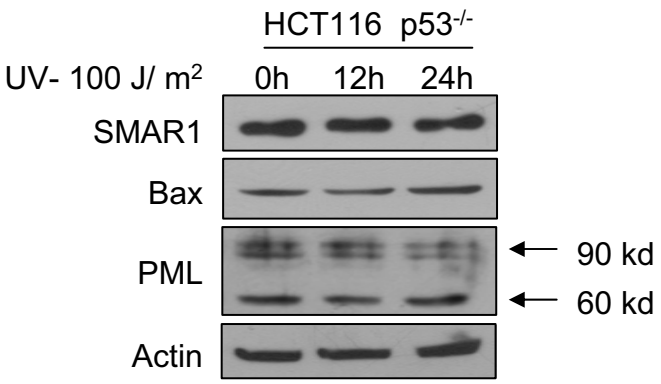

Figure S7; Sinha et al.