Supporting Information

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SI Materials and Methods

Animals. Nude mice were purchased from Orient Bio and maintained in the animal maintenance facility of Sungkyunkwan University according to the University Animal Care and Use guidelines.

Cells and Virus. MEF $p53^{-/-}$ cells, MEF $PKR^{-/-}$ cells, and isogenic HCT116 $p53^{+/+}$ and $p53^{-/-}$ cells were kindly provided by H. W. Lee (Sungkyunkwan University, Korea), J. C. Bell (University of Ottawa, Canada), and B. Vogelstein (Johns Hopkins Oncology Center), respectively. RKO, Hep3B, MCF-7, and PC-3 cells were obtained from American or Korean Type Culture Collection (ATCC or KTCC). *p53*-knockdown ($p53^{KD}$) cells, *PKR*-knockdown (PKR^{KD}) cells, and the *eIF2α*-constitutively active mutant ($eIF2\alpha^{CA}$) cells were prepared as described in the section *Genetically Modified Cell Lines*. Vesicular stomatitis virus (VSV, Indiana strain; ATCC VR-1238) infection was carried out as described previously (1), and viral solutions harvested 12 h after infection were titrated by the 50% tissue culture infective dose ($TCID_{50}$) method on a HCT116 cell monolayer.

Western blot analysis sample preparation and Western blotting were conducted as described previously (2).

DNA Damage, Stresses, and Reagents. Doxorubicin (Sigma), hydroxyurea (Sigma), etoposide (Sigma), and UV were used as stress factors for DNA damage. IFNα (Sigma) was used as a positive-control *PKR* inducer. Pifithrin alpha (PFTα) was purchased from Sigma and used as a *p53*-functional inhibitor. pAb421, obtained from Calbiochem Co., was used as a *p53*-activating antibody (3) in foot-printing. MG132 (A.G. Scientific, Inc.) and Nutlin3 (Calbiochem Co.) were used for the inhibition of proteosomal and Mdm2-mediated degradation, respectively. AMPK inhibitor compound C (Calbiochem Co.) was used for the inhibition of the AMPK-mTORC1 pathway.

Antibodies Used for Western Blot Analysis. Antibodies against the following proteins were purchased: human *p53* (DO-1; Santa Cruz Biotechnology), phospho-S^{15}-*p53* (Cell Signaling), mouse *p53* (pAb 246; Santa Cruz Biotechnology), $p21^{waf1/cip1}$ (Cell Signaling), *Bax* (Cell Signaling), *Puma* (Cell Signaling), human *PKR* (Cell Signaling), histone 2 (H2A, Cell Signaling), mouse *PKR* (B-10; Santa Cruz Biotechnology), phospho-T^{446}-*PKR*, *eIF2α*, phospho-S^{51}-eIF2α (Epitomics Co.), cleaved PARP (Cell Signaling), *ATF4* (Santa Cruz Biotechnology), *CHOP* (Sigma-Aldrich) and β-actin (Sigma-Aldrich). Bands were visualized using an ECL chemiluminescence kit (Amersham Biosciences).

Recombinant Proteins of Human *p53*. Human *p53* cDNA was cloned and expressed using the Bac-to-Bac Baculovirus Expression System (Invitrogen). Recombinant *p53* protein (BaculoV-p53) was purified as described previously (3).

Real-Time Quantitative RT-PCR Analysis. We extracted total RNA from the cells with Tri-zol reagent (Invitrogen) and synthesized the cDNA using SuperScript III RNase H-Reverse Transcriptase (Invitrogen). Real-time RT-PCR was performed as described previously (4) with specific primers (sequences available on request) using the QuantiTect SYBR Green RT-PCR kit (Qiagen) and Realplex4 Mastercycler system (Eppendorf). Specific primers used for quantitative RT-PCR were human *PKR*: forward 5′-ACTTTTTCCTGGCTCATCTC-3′, reverse 5′-ACATGCCTGTAATCCAGCTA; human *Puma*: forward 5′-GGAGGGTCCTGTACAATCTC-3′, reverse 5′-GCTACATG-GTGCAGA GAAAG-3′; human *p21*: forward 5′-TGGAACT-TCGACTTTGTCAC-3′, reverse 5′-CACATG GTCTTC-CTCTGCT-3′; mouse *Bax*: forward 5′-GAGCTGATCA-GAACCATCAT, reverse 5′-CATCTTCTTCCAGATGG-TGA-3; mouse *PKR*: forward 5′-CCTCAGAGAACGTGTTTA CG, reverse 5′-TCAATTCTGTGTTTCGCTTT-3′; GAPDH: forward 5′- GACATCAAGAAGGTGGTGAA -3′, reverse 5′-TGTCATACCAGGAAATGAGC - 3′.

Data were normalized to GAPDH mRNA. Data were analyzed by fold induction in comparison with a standard control for each experiment and are presented as mean ± SEM of 3 or more independent experiments.

Immunocytochemistry. As described previously (5), cells were fixed and stained for appropriate primary and secondary antibodies. Mouse and rabbit isotype control IgGs (BD Bioscience) were used for the experiments. After 3 washes, the cells were treated with a fluorescent mounting medium (DAKO) and visualized by confocal microscopy (Leica).

Luciferase Assays. Cells were transfected with 200 ng of Bax promoter- (6) and partial and full-length *PKR* promoter-conjugated luciferase reporter constructs (PGL-3 vector; Promega), together with 20 ng of control *pCMV-lacZ* plasmid and 1 μg of *pcDNA-p53* plasmid, using Trans IT -LT1 transfection reagent (Mirus). Forty-8 h after transfection, luciferase activity of each sample was measured and normalized (to β-galactosidase) using Bright-Glo Luciferase Assay system (Promega) and the Genios Luminometer (Tecan). Data are presented as means ± SEM.

Electrophoretic Mobility Shift Assay. For EMSA, the oligonucleotides covering the putative *p53RE*(-81/-29) of the *PKR* promoter and the *p53RE* of the $p21^{waf1/cip1}$ promoter were synthesized as described. EMSA was performed with BaculoV-p53 as described previously (3) or with nuclear extracts prepared as described previously (7). To induce a supershift, a *p53*-specific DO-1 antibody was added and incubated before analysis on a 5% native polyacrylamide gel. A cross-competitive EMSA was carried out with the same amounts of ^{32}P-labeled probes (^{32}P-$Ppkr_{-81/-29}$ and ^{32}P-*p21-p53RE*) and increased concentrations of unlabeled counter probes.

Oligonucleotide Probes for EMSA and Supershifting Assay. The oligonucleotides covering the putative *p53RE* (-81/-29) of the *PKR* promoter and the *p53RE* of the $p21^{waf1/cip1}$ promoter were synthesized with the following primer sequences: wild-type $Ppkr_{-81/-29}$: forward (-81) 5′-AGGGAAGGCGGAGTC-CAAGGGGAAAACGAAACTG AGAACCAGCTCTC-CCGAAGC -3′, and reverse (-29) 5′-GCTTCGGGAGAGCT-GGTTCT CAGTTTCGTTTTCCCCTTGGACTCCGCC-TTCCCT-3′; mutant $Ppkr_{-81/-29}$: forward (-81) 5′-AGG-GAAGGCGG*TCA*CCAAGGGGAAAACGAAACTGAG-AACCAGC TCTCCCGAAGC-3′, and reverse (-29) 5′-GCTTCGGGAG*TCGA*GGTTCTCAGTTTCGTTTTCC-CCTTGGACTC CGCCTTCCCT-3′; *p21-p53RE* (5′-region): forward 5′-ATCAGGAACATGTCCCAACAT GTT-GAGCTC-3′, and reverse 5′-GAGCTCAACATGTTGGGA-CATGTTCCTGAT-3′. These complementary oligonucleotides were annealed, ^{32}P-labeled, and then used for the EMSA and supershifting assay together with BaculoV-p53.

DNase I Footprinting. *DNase I* footprinting was carried out as described previously (8). A part of the *PKR* promoter region (-160/-29) was PCR-amplified with ^{32}P-labeled forward primer, 5′-ACTAAAGCTT CGCACTCTGGATCCAGCG-3′, and unlabelled reverse primer for wild type, 5′-ACTAGTC GACGCT-TCGGGAGAGCTGGTT-3′ or unlabelled reverse primer for mutant, 5′-ACTAGTC GACGCTTCGGGAGTCGAGGTT-3′, from wild-type and mutant *PKR* promoters. Radiolabeled PCR products were incubated with BaculoV-p53 and subjected to the *DNase I* footprinting experiments.

Chromatin Immunoprecipitation Assay. The ChIP assay was performed according to the manufacturer's (Upstate Biotechnology) instructions and as previously reported (1), with minor modifications. In brief, chromatins obtained from *pcDNA-p53*−transfected HCT116 ($p53^{-/-}$) cells or doxorubicin-treated RKO cells were fixed with formaldehyde and then were immunoprecipitated with DO-1 antibody against *p53* or control mouse IgG antibody. The DNA was purified from the immunoprecipitates and analyzed by PCR (27 cycles of 30 sec at 95 °C, 60 sec at 60 °C, 60 sec at 72 °C) using the Hot-start PCR kit (Intron Biotech) with the following primer sets: *Ppkr*(-173/-29) ChIP assay primers: forward, 5′-AGTGCCTCTCGCGCG-CACTCTG-3′, and reverse, 5′-GCTTCGGGAGAGCTGGT-TCTCAGTT-3′; *Ppkr* (-340/-81) ChIP assay primers: forward, 5′-AACCCTTGATTCGAGAACCTA-3′, and reverse, 5′-TGCAGC CGCCCGGCCC-3′; *Pp21* ChIP assay primers (9): forward, 5′-CCGCTCGAGCCCTGTCGCAAGGATCC-3′, and reverse, 5′-GGGAGGAAGGGGATGGTAG-3′. PCR products were resolved on 2% agarose gels.

Genetically Modified Cell Lines. *p53*-knockdown ($p53^{KD}$) cells were prepared by transfection of cells with *p53*-targeting siRNAs (*si-p53*), which were designed and synthesized by Invitrogen (Stealth™), as described previously (2, 10). In brief, *PKR*-knockdown (PKR^{KD}) cells were established as follows: *PKR-shRNA*-expressing recombinant plasmids (*sh-PKR*) were constructed by manipulation of the pSilencer3.1 vector (Ambion) with the ds-oligonucleotide prepared by annealing the following synthetic oligonucleotides: forward: 5′-GAT CCG TGG AGG AAC TTA ATA CAT ACT CAA GAG ATA TGT ATT AAG TTC CTC CAT TTT TTG GAA A-3′, and reverse: 5′-AGC TTT TCC AAA AAA TGG AGG AAC TTA ATA CAT ATC TCT TGA GAT GTA TTA AGT TCC TCC ACG-3′. To minimize the off-target effects of the *PKR sh-RNA*, the *PKR*-knockdown experiment was repeated with another *PKR-siRNA* with a sequence of 5′-CGU UGC UUA UGA AUG GUC-3′. HCT116 and RKO cells were transfected with the sh-*PKR* recombinant plasmid and then were selected with 1 μg/ml puromycin. To establish the *eIF2α* constitutively active mutant ($eIF2\alpha^{CA}$) cells, HCT116 cells were transfected with Myc-conjugated mutant *eIF2α* (*eIF2α51A*, with Ala instead of Ser at residue 51)-integrated pSLX (Amersham Pharmacia LTR-based chromosome integrating vector) recombinant plasmid and then were selected with 500 μg/ml G418.

Plasmid Constructions for the *PKR* Promoter-Containing Reporter System. A partial (5′-deleted) or full-length (-700/-1) *PKR* promoter, shown in Fig. 2*A*, was prepared by PCR amplification of HeLa cell genomic DNA using the following primer combinations: forward primers (position -700): 5′-ACTA *GGT ACC* CCT GGG GTA CTG TAG GAA GC -3′; (position -400): 5′-ACTA *GGT ACC* GGA GCT GGT AAG ATC CAT CTT C-3′; (position -81): 5′-ACTA *GGT ACC* AGG GAA GGC GGA GTC CAA-3′; (position -56): 5′-ACTA *GGT ACC* CGA AAC TGA GAA CCA GCT-3′; reverse primer: (position -1): 5′-ACTA *AAG CTT* CGC CGC CGC CGC CGG CCG GAG ACC CGC G GCT TCG GGA GAG CTG GTT CTC AGT-3′. These PCR products were cloned into KpnI and HindIII of the *pGL3 basic* (Promega) plasmid, which then was named "$Ppkr_{position}$-*luc*." The italics indicate restriction enzyme sites.

Mutagenesis of the *PKR* Promoter at Putative *p53RE* and *ISRE* Sites. Mutagenesis of the *PKR* promoter fragments shown in Fig. S4*A* was performed by template-free PCR methods described previously (11) using the following mutagenic PCR primer sets: *mISRE* mutation: forward (position-81) 5′-ATCGGTAC-CAGGGAAGGCGGAGTCCAA GGG*TCC*AAC*ACC*ACTG-3′, and reverse (position-29) 5′-ACTAAGCTTGCTTCGG-GAGAGCTGGT TCTCAGTGGTGTTGGACCCTT-3′; *p53RE* mutation: forward (position-81) 5′-ACTAGAAG CT-TAGGGAAGCGG*TCA*CCAAGGGGAAAACGAAACTG-3′, and reverse (position-29) 5′-ATACT GTCGACGCTTCGG-GAG*TCGA*GGTTCTCAGTTTCGTTTTCCCCTT-3′. PCR fragments were directly subcloned into pCAT basic plasmid at HindIII/SalI, and these sequences were cloned into pGL3 basic plasmid at the KpnI/*HindIII* site or were replaced with the corresponding region of the *Ppkr-luc* to construct the $Ppkr_{-700/-1\ mISRE}$ *-luc* shown in Fig. 2*E*. The italics indicate mutated nucleotides.

Analysis of Cell Apoptosis. Apoptosis at early and later stages was examined by measuring annexin V-positive cells and subG1 cells, respectively. Cells were exposed to each drug (0.5 μM doxorubicin, 5 μM etoposide, or 1 mM hydroxyurea for 36–48 h) or UV (20 J/m^2 for 12 h). As described previously (2), apoptotic cells were analyzed by FACS Calibur (Becton Dickinson Bioscience) after annexin V staining with Annexin V Apoptosis Detection Kit () or after propidium iodide (Sigma-Aldrich)staining of subG1 cells.

Metabolic Labeling Assay. Metabolic labeling assays with [^{35}S]-Cys/Met were performed as described previously (2, 12) with minor modifications. In brief, cells maintained in complete medium (containing genotoxins or not) were cultured for an additional 30 min in Cys/Met-free DMEM containing 10% dialyzed FBS and 100 μCi of [^{35}S]-Cys/Met. Equal concentrations of total protein from each cell lysate were separated by SDS/PAGE, and radiolabeled proteins were visualized by autoradiography. Relative band intensity of the autoradiogram was scored using Image Gauge 3.12 (Fuji Film Inc.).

1. Takaoka A, et al. (2003) Integration of interferon-alpha/beta signalling to p53 responses in tumour suppression and antiviral defence. *Nature* 424(6948):516–523.
2. Lee ES, Yoon CH, Kim YS, Bae YS (2007) The double-strand RNA-dependent protein kinase *PKR* plays a significant role in a sustained ER stress-induced apoptosis. *FEBS Lett* 581(22):4325–4332.
3. Resnick-Silverman L, St. Clair S, Maurer M, Zhao K, Manfredi JJ (1998) Identification of a novel class of genomic DNA-binding sites suggests a mechanism for selectivity in target gene activation by the tumor suppressor protein p53. *Genes Dev* 12(14):2102–2107.
4. Xie M, et al. (2007) Functional implication of nucleolin in the mouse first molar development. *J Biol Chem* 282(32):23275–23283.
5. Kim D, et al. (2006) Cytoplasmic transduction peptide (CTP): New approach for the delivery of biomolecules into cytoplasm in vitro and in vivo. *Exp Cell Res* 312(8):1277–1288.
6. Roe JS, et al. (2006) p53 stabilization and transactivation by a von Hippel-Lindau protein. *Mol Cell* 22(3):395–405.
7. Das S, Ward SV, Markle D, Samuel CE (2004) DNA damage-binding proteins and heterogeneous nuclear ribonucleoprotein A1 function as constitutive KCS element components of the interferon-inducible RNA-dependent protein kinase promoter. *J Biol Chem* 279(8):7313–7321.
8. Fiucci G, et al. (2004) Siah-1b is a direct transcriptional target of p53: Identification of the functional p53 responsive element in the siah-1b promoter. *Proc Natl Acad Sci USA* 101(10):3510–3515.
9. Szak ST, Mays D, Pietenpol JA (2001) Kinetics of p53 binding to promoter sites in vivo. *Mol Cell Biol* 21(10):3375–3386.
10. Williams BR (1999) PKR; A sentinel kinase for cellular stress. *Oncogene* 18(45):6112–6120.

11. Lee SG, Kim DY, Hyun BH, Bae YS (2002) Novel design architecture for genetic stability of recombinant poliovirus: The manipulation of G/C contents and their distribution patterns increases the genetic stability of inserts in a poliovirus-based RPS-Vax vector system. *J Virol* 76(4):1649–1662.

12. Jiang HY, Wek RC (2005) Phosphorylation of the alpha-subunit of the eukaryotic initiation factor-2 (eIF2alpha) reduces protein synthesis and enhances apoptosis in response to proteasome inhibition. *J Biol Chem* 280(14):14189–14202.

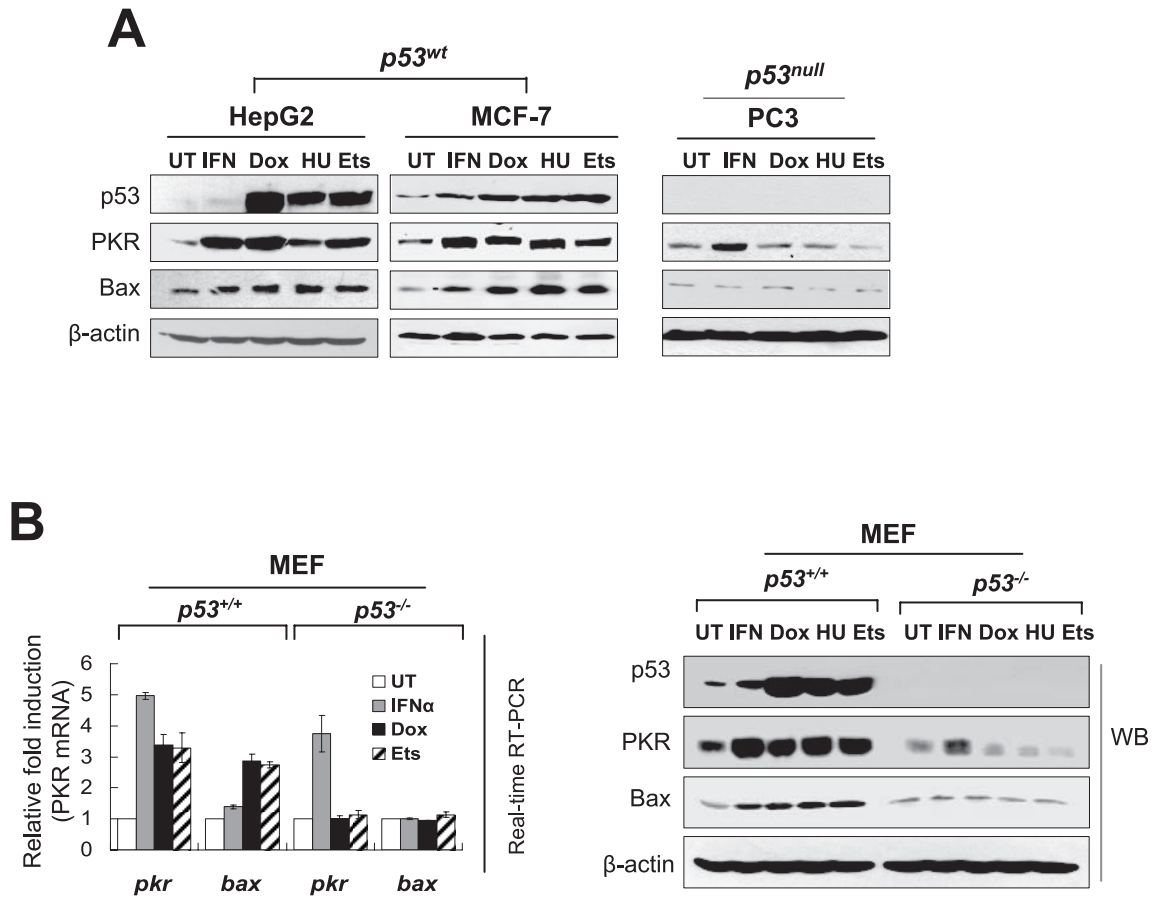

Fig. S1. (*A*) *PKR* expression in *p53* wild-type ($p53^{wt}$) and *p53*-null ($p53^{null}$) cells under conditions of DNA damage (0.5 μM doxorubicin [Dox], 5 μM etoposide [Ets], and 1 mM hydroxyurea [HU] for 12 h) and in untreated (UT) cells. (*B*) Expression of endogenous *PKR* in genotoxin-treated MEF ($p53^{+/+}$ and $p53^{-/-}$) cells, assessed by real-time RT-PCR (means ± SEM, $n = 3$) and Western blot analysis (WB).

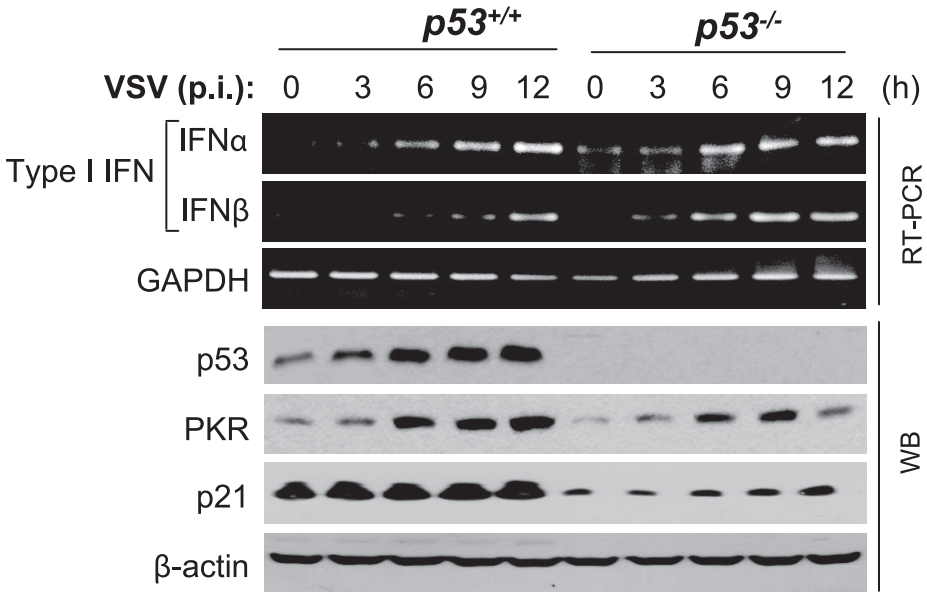

Fig. S2. Vesicular stomatitis virus (VSV) infection induces type I IFN, followed by the expression of *p53* and *PKR*. HCT116 ($p53^{+/+}$ and $p53^{-/-}$) cells were infected with VSV (multiplicity of infection = 1). Cells harvested at 3-h intervals after infection were assessed for the level of type I IFN mRNA and for the expression of *p53* and *p53* target genes by RT-PCR and Western blot (WB) analysis, respectively. RT-PCR primer sequences for IFNα (192 bp) were forward 5′-GTGCTCAGCTGCAAGTCAAG-3′, and reverse 5′-GATGGTTTCAGCCTTTTGGA-3′; for IFNβ (221 bp)were: forward 5′-TGGGAGGATTCTGCATTACC-3′, and reverse 5′-ATGCAGTACATTCGCCATCA-3′; and for GAPDH were forward 5′-GACATCAAGAAGGTGGTGAA-3′, and reverse 5′-TGTCATACCAGGAAATGAGC-3′.

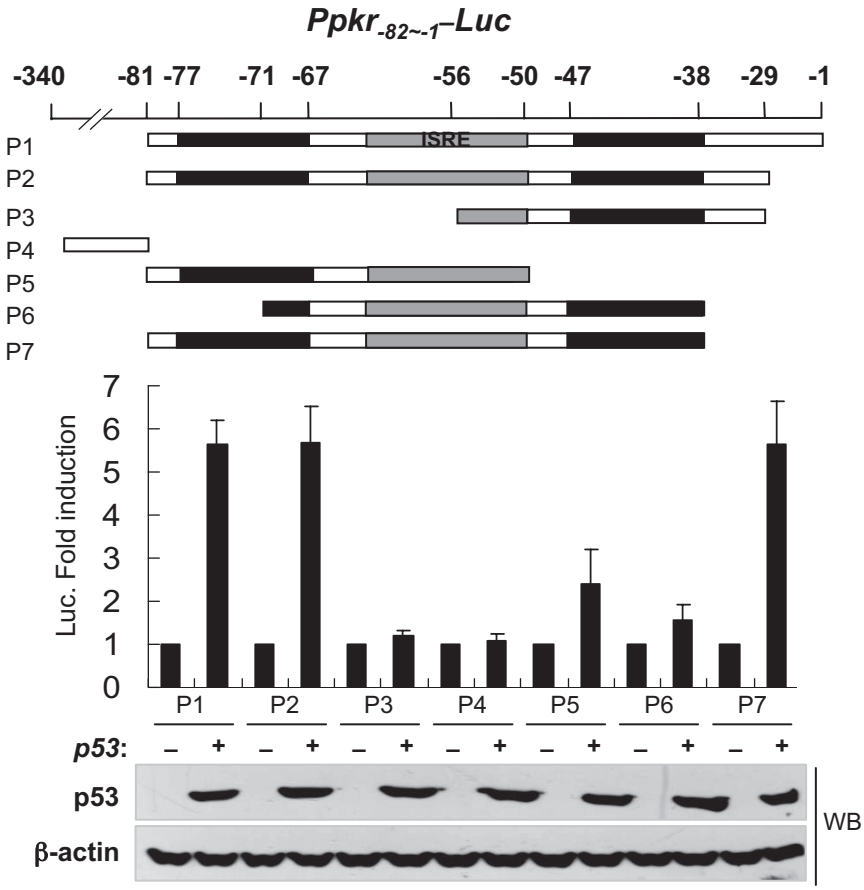

Fig. S3. Promoter deletion studies on the *PKR* promoter. The upper panel shows the schematic representation of partial *PKR* promoters examined in the additional promoter-deletion assay. The bottom panel shows each *PKR* promoter activity in the luciferase reporter assay in the presence (+) or absence (−) of exogenous *p53*. Western blot analysis (WB) shows the levels of *p53* expressed in each sample.

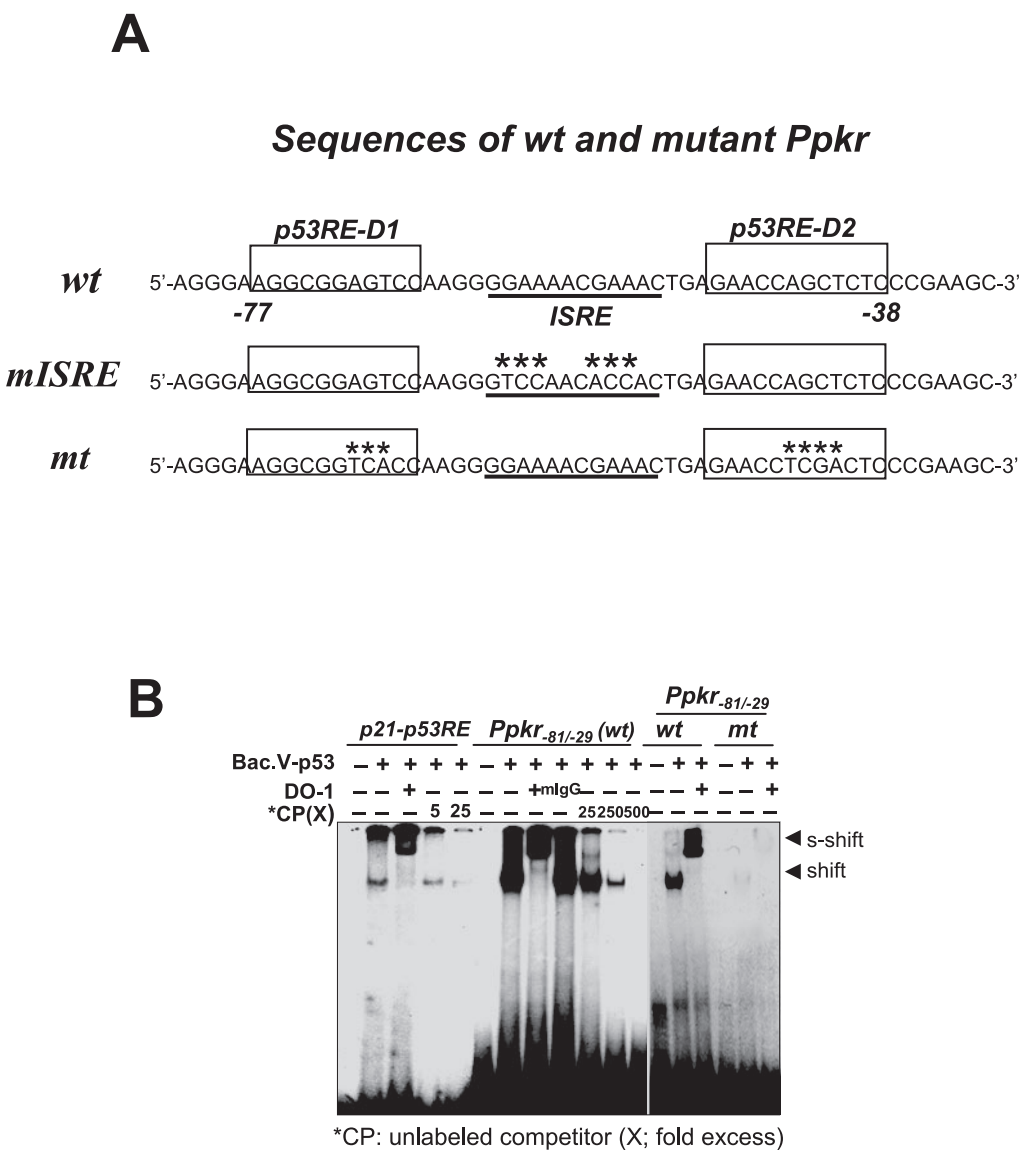

Fig. S4. (*A*) Promoter sequence of wild-type (wt) and mutant (mt) *PKR* promoter. Nucleotide sequences of the partial *PKR* promoters (*Ppkr*$_{-82/-1}$) harboring mutations at *ISRE* (*mISRE*) or at putative *p53*-responsive element (*p53RE*) regions. Underline indicates the sequence of ISRE. (*B*) EMSA using BaculoV-p53, wild-type (wt), and mutant (mt) ^{32}P-labeled *PKR81/-29* and *p21* promoter fragments and α-*p53* antibody (DO-1). Fold excesses of unlabeled cross-competitors were indicated on the competitive EMSA.

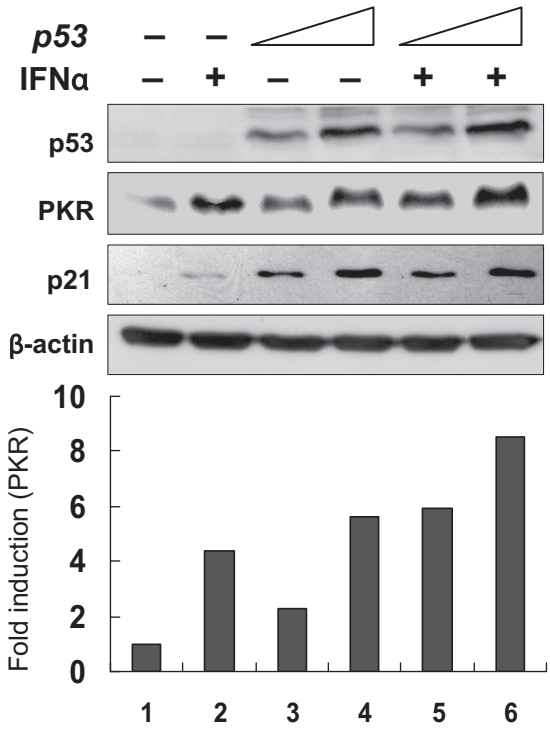

Fig. S5. Additive effects of *p53* and IFNα on the expression of *PKR* at the protein level. HeLa cells transfected with *pcDNA-p53* plasmids were cultured for 24 h in the presence (+) or absence (−) of IFNα. *PKR* and other protein expressions were assessed by Western blot analysis with the appropriate antibodies. The intensity of the *PKR* band for each sample was scored by the Image Gauge 3.12 program (Fuji Film Inc.) and is represented by fold induction in comparison with that of the control band (*Bottom*).

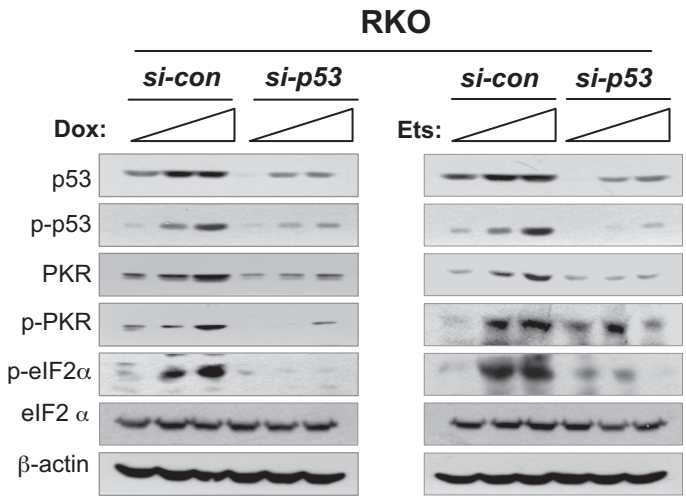

Fig. S6. Stresses caused by DNA damage activate the *PKR*/*eIF2*α pathway via *p53*. Assessment of HEK293 ($p53^{+/+}$), and Hep3B ($p53^{null}$) cells treated with increasing concentrations of genotoxins or with 20 J/m^2 UV. Phospho-*p53*, phospho-*PKR* and phospho-*eIF2*α were assessed by Western blot assay with each phospho-specific antibody.

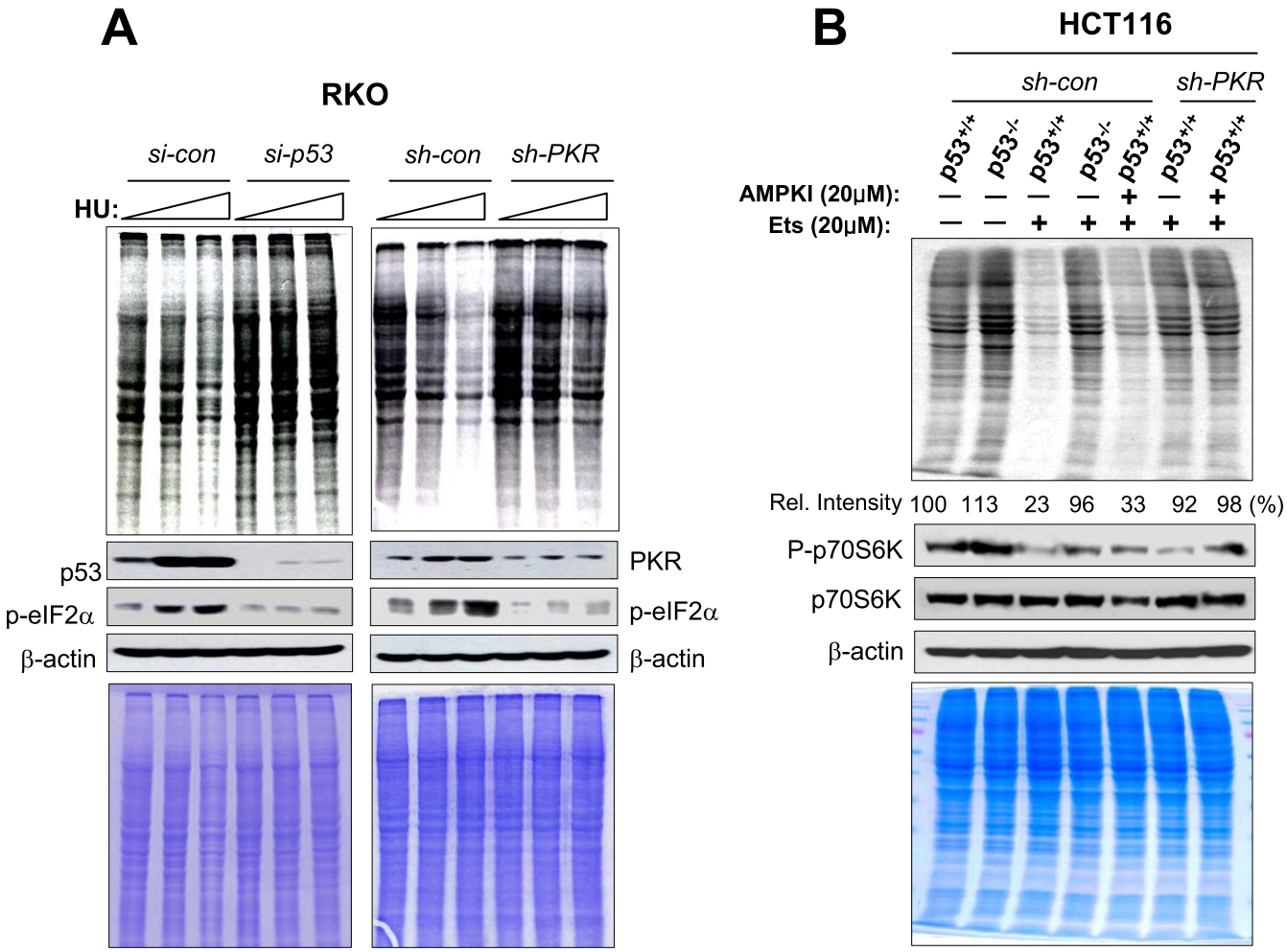

Fig. S7. *PKR* plays an important role in the *p53*-mediated inhibition of translation. (*A*) $p53^{KD}$ (*si-p53*)-RKO or PKR^{KD} (*sh-PKR*)-RKO cells were treated with increasing concentrations of hydroxyurea (HU) (0, 1, and 5 mM) for 12 h. The cells then were subjected to the metabolic labeling assay as described in *Materials and Methods* (*Top*). The Phospho-eIF2α content of each sample was assessed (*Middle*). Before the autoradiogram, the gel was stained with Coomassie blue (*Bottom*). (*B*) $p53^{+/+}$, $p53^{-/-}$, and PKR^{KD} (*sh-PKR*) HCT116 cells were cultured in the presence (+) or absence (−) of 20 μM of AMP-activated protein kinase inhibitor compound C (AMPKI) and 20 μM of etoposide (Ets) for 12 h. The cells then were subjected to the metabolic labeling assay. Relative band intensities are shown (*Top*). The Phospho-p70S6K and total p70S6K were assessed by Western blot (*Middle*). Before the autoradiogram, the gel was stained with Coomassie blue (*Bottom*).

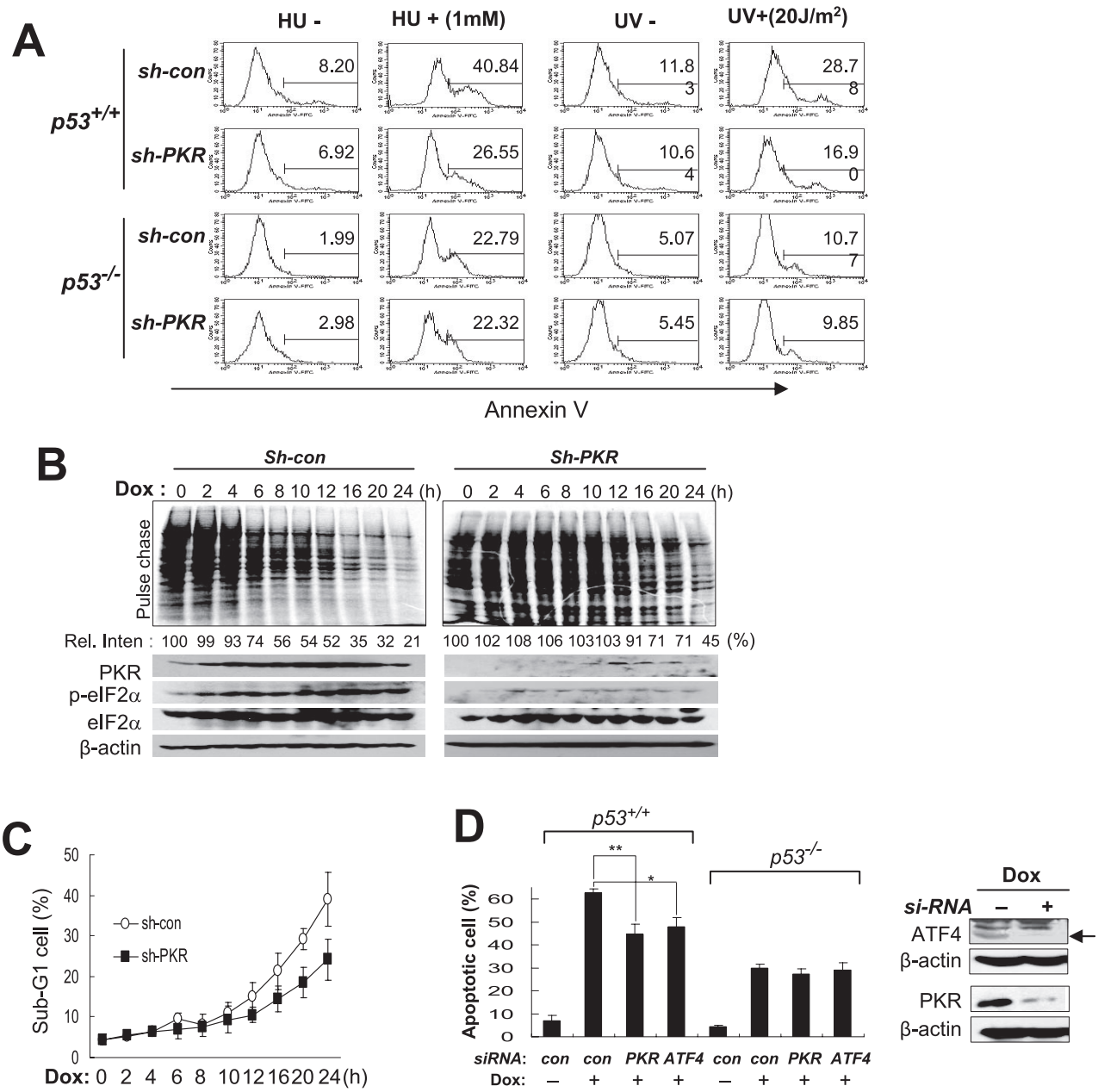

Fig. S8. The *p53/PKR* pathway is involved in cell apoptosis under stresses caused by DNA damage (*A*) PKR^{KD} (*sh-PKR*) HCT116 ($p53^{+/+}$ and $p53^{-/-}$) cells were treated with 1 mM hydroxyurea (HU) for 36 h or with 20 J/m^2 UV, or were left untreated (HU − and UV −). Cell apoptosis was assessed by flow cytometry after annexin V staining. (*B*) Time kinetics of pulse−chase labeling was performed in doxorubicin (Dox)-treated (1 μM for indicated time periods) HCT116 $p53^{+/+}$(*sh-con* and *sh-PKR*) as described in the SI text. Relative band intensities are shown. (*C*) Time kinetics of apoptotic cells in doxorubicin (Dox)-treated (1 μM for indicated time periods) HCT116 $p53^{+/+}$(*sh-con* and *sh-PKR*) cells, shown by subG1 population. Results are shown as mean ± SEM ($n = 3$). *, $P < 0.01$. (*D*) Apoptotic cell populations were assessed 4 times ($n = 4$) by flow cytometry after doxorubicin (Dox) treatment (0.5 μM for 48 h) of the *ATF4*- or *PKR*-knockdown (by transfection of siRNA) HCT116 ($p53^{+/+}$ or $p53^{-/-}$) cells. Results are shown as mean ± SEM. *, $P < 0.01$, and **, $P < 0.02$ as compared with control si-RNA–transfected group (*Left*). Knockdown was evaluated (*Right*).

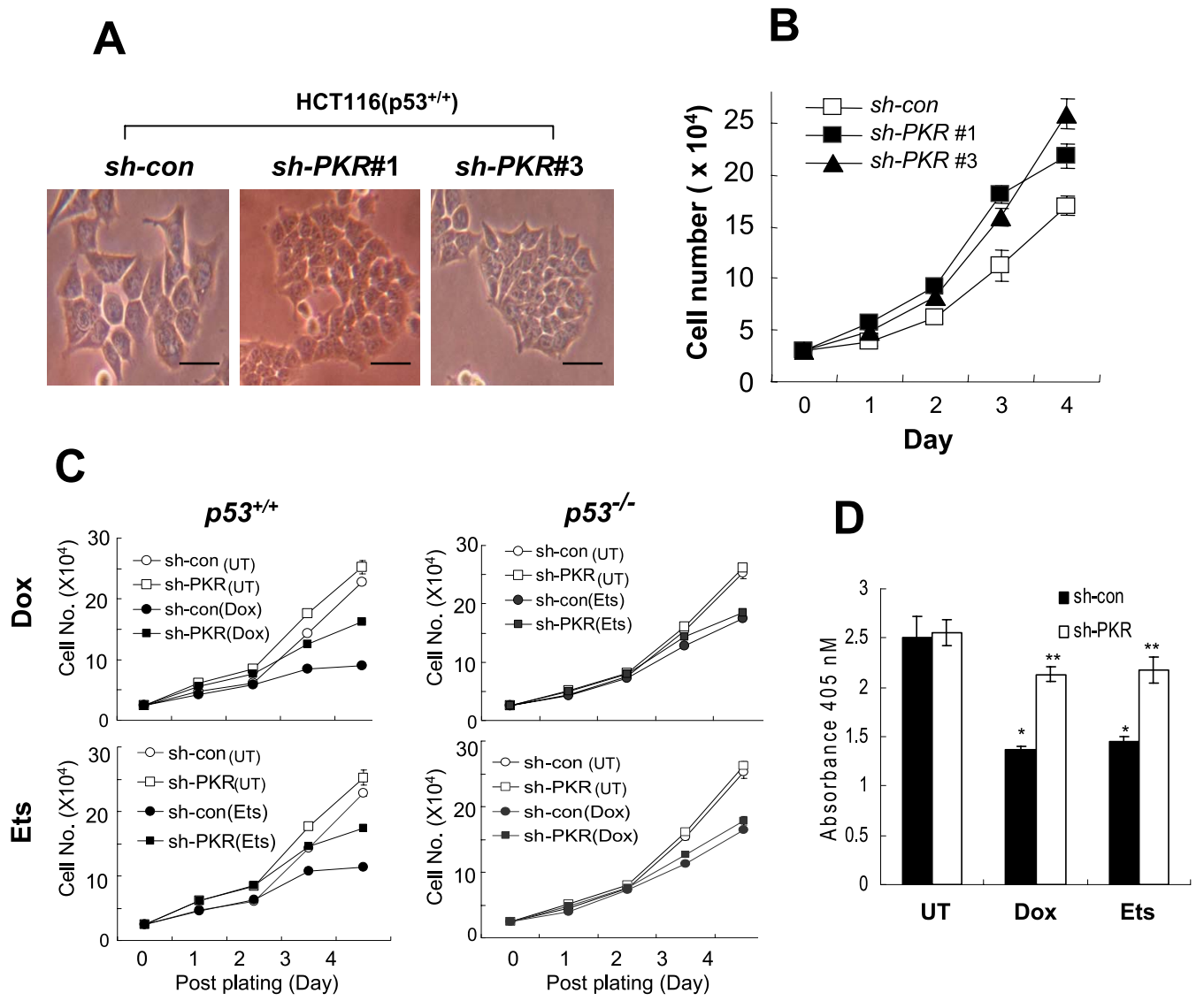

Fig. S9. Cell growth and proliferation of *PKR*-knockdown cells. (*A*) Normal (*sh-con*) and PKR^{KD} (*sh-PKR*) HCT116 cells were photographed under an inverted microscope at the same magnification (100×). (*B*) Proliferation of *sh-con* and *sh-PKR* HCT116 cells. Results are expressed as means ± SEM of experiments done in triplicate. (*C*) *PKR*-normal (*sh-con*) and PKR^{KD} (*sh-PKR*) HCT116 ($p53^{+/+}$ or $p53^{-/-}$) cells were seeded at 2.5×10^4 cells per well on a 24-well plate and then were cultured for 4 days in the presence of 50 nM doxorubicin (Dox) or 1 μM etoposide (Ets) or absence of (untreated, UT). The number of cells was counted using the MTT assay-based CellTiter96R Non-Radioactive Cell proliferation Assay kit (Promega, Cat. G4001). Data are represented as means ± SEM ($n = 3$). (*D*) *PKR*-normal (*sh-con*) and PKR^{KD} (*sh-PKR*) HCT116 ($p53^{+/+}$) cells were seeded at 2×10^4 cells per well on a 96-well plate and then were cultured for 4 days in the presence or absence of 50 nM doxorubicin (Dox) or 1 μM etoposide (Ets). Cell proliferation capacity was assessed by BrdU ELSA assay kit (Roche, Cat.11444611001) following the manufacturer's protocol. *, $P < 0.01$, and **, $P < 0.02$ as compared with the group of drug-untreated cells (UT). Results are shown as means ± SEM ($n = 4$).

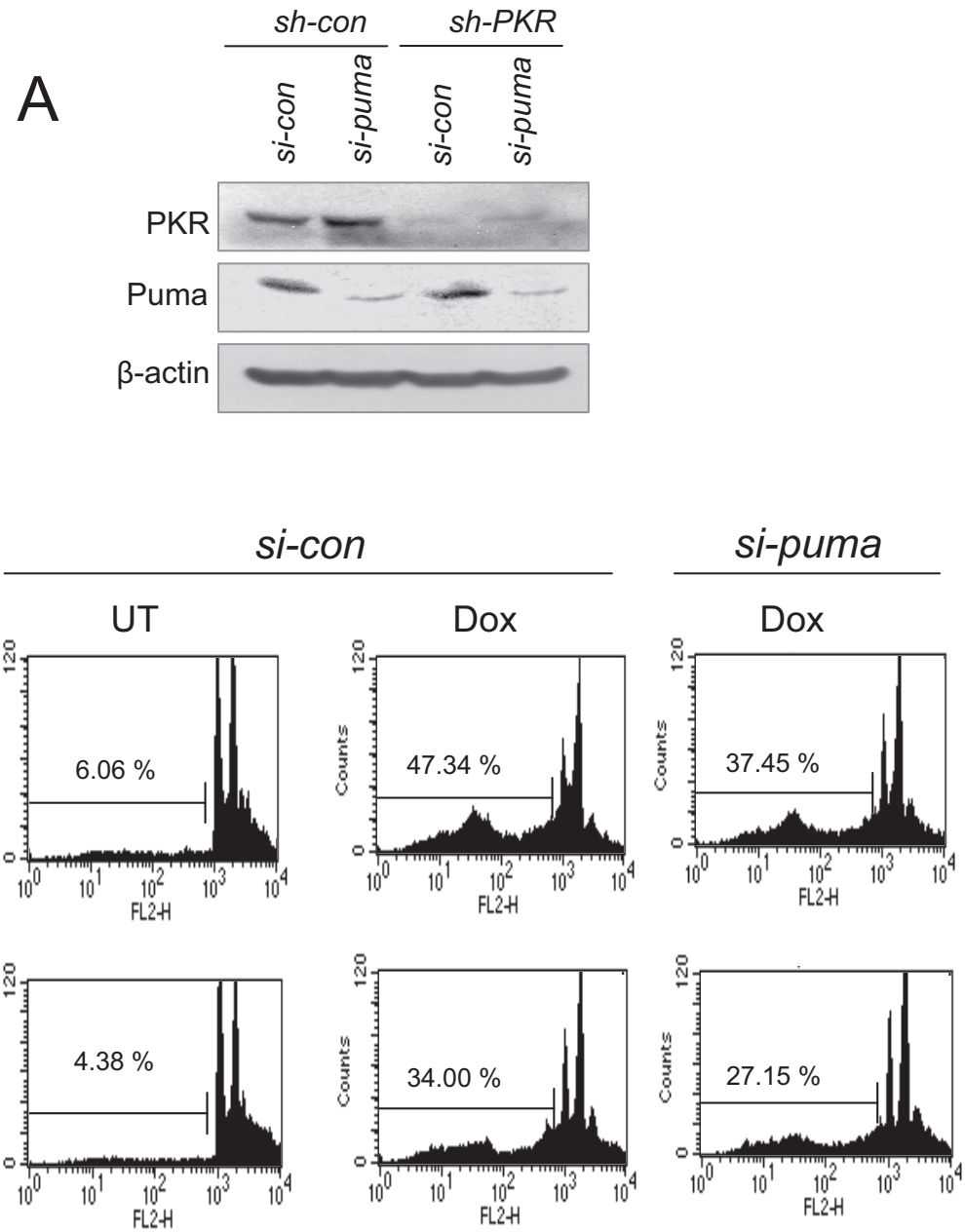

Fig. S10. *PKR* and *Puma* are cooperatively involved in cell apoptosis under stresses caused by DNA damage downstream of *p53*. (*A*) Normal (*sh-con*) and PKR^{KD} (*sh-PKR*) HCT116 cells were transfected with si-RNA against *Puma* (*si-puma*; 5′-ACCUCAACGCACAGUACGA-3′, at position 707 of the *Puma* sequence; GI number, AF354654) and then were subjected to Western blot analysis to examine the expression of *Puma* and *PKR*. (*B*) PKR^{KD} (*sh-PKR*) and/or $Puma^{KD}$ (*si-puma*) HCT116 ($p53^{+/+}$) cells were treated with 1 μM doxorubicin (Dox) for 24 h or were left untreated (UT). Cells then were subjected to subG1 analysis for measuring cell apoptosis by flow cytometry with CellQuest software (BD Bioscience).

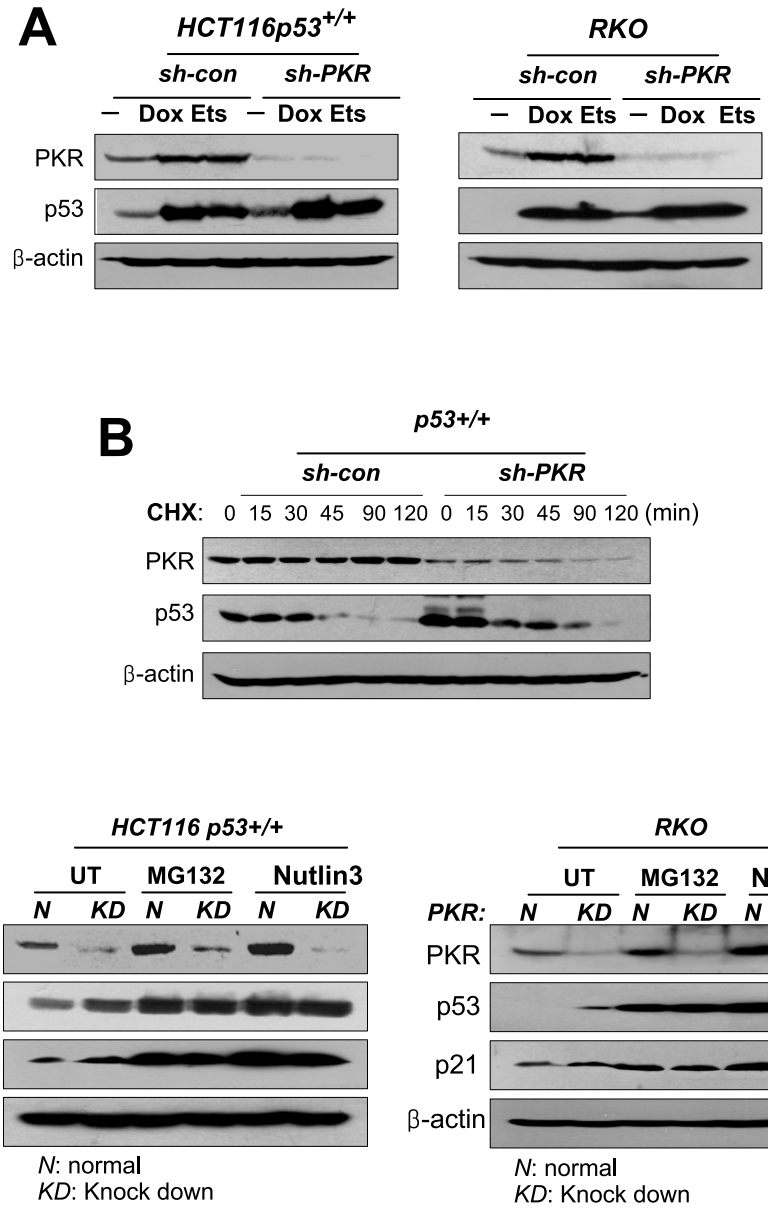

Fig. S11. *PKR*-mediated feedback down-regulation of *p53* by activation of proteasomal degradation pathway. (*A*) The amounts of *p53* and *PKR* were examined in *PKR*-normal (*sh-con*) and PKR^{KD} (*sh-PKR*) HCT116 ($p53^{+/+}$) and RKO cells by Western blot analysis 12 h after genotoxin treatment with 0.5 μM doxorubicin (Dox) or 5 μM etoposide (Ets). (*B*) *p53* stability was assessed by Western blotting in *PKR*-normal (*sh-con*) and PKR^{KD} (*sh-PKR*) HCT116 ($p53^{+/+}$) cells after cycloheximide (CHX, 75 μg/ml) treatment for the time periods indicated. (*C*) *PKR*-normal (*N, sh-con*) and PKR^{KD} (*KD, sh-PKR*) HCT116 ($p53^{+/+}$) and RKO cells were treated with MG132 (20 μM for 5 h) or Nutlin3 (20 μM for 12 h) or left untreated (UT). Subsequently, *PKR*, *p53*, and *p21* were examined by Western blot analysis.

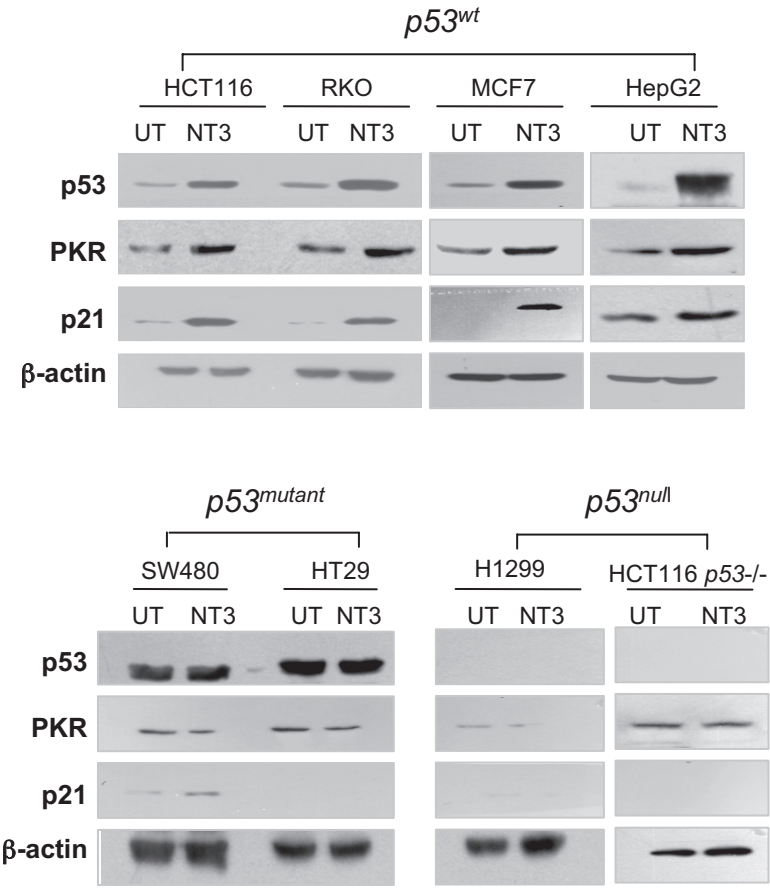

Fig. S12. Nutlin3 effects on the expression of *p53* and its target gene in 3 different groups of cell lines (*p53wt*, *p53mutant*, and *p53null*). Cells were treated with 20 μM Nutlin3 (NT3) for 12 h or left untreated (UT), and the levels of *p53*, *PKR*, and *p21* were examined by Western blot analysis.