SUPPLEMENTAL INFORMATION

SUPPLEMENTAL DATA

Figure S1. In vivo co-immunoprecipitation of native p53 by overexpressed HA-tagged fortilin using agarose-conjugated anti-HA antibody. IP, immunoprecipitation; WB, western blot analysis; $U2OS_{Empty\text{-}HA}$, U2OS cells overexpressing human-influenza-hemagglutinin-tag; $U2OS_{Fortilin\text{-}HA}$, U2OS cells overexpressing HA-fortilin. In order to further validate the specific interaction between fortilin and p53, HA-tagged fortilin was immunoprecipitated by agarose-conjugated anti-HA antibody (Lane 4). Native p53 was co-immunoprecipitated in the presence of immunoprecipitated fortilin-HA and detected by both DO1 and DO7 anti-p53 antibodies, further validating the results depicted in **Fig. 1B**.

Figure S2. Increased Fortilin-p53 bindings by increase in UV irradiation. A, Western blot analysis of p53 and fortilin-HA of U2OS cells that overexpress fortilin-HA and are treated with various doses of ultra-violet irradiation (UV) to induce DNA damage. **B**, The p53 immunoprecipitation index was calculated at the signal intensity of immunoprecipitated p53 divided by that of total p53 and normalized to the value of untreated cells. More p53 interacted with fortilin in U2OS cells treated with low to moderate dose UV (5-40 mJ/cm^2).

Figure S3. Wild-type p53, but not truncated p53, interact with fortilin in vivo. The lysates from the U2OS cells that harbor wild-type p53 (1-393 amino acids) and NCI-

H1793 cell line that harbors truncated p53 at the 209th amino acid (1-209 amino acids) were subjected to immunoprecipitation as described in the Experimental Procedures. Immunoprecipitated fortilin co-precipitated wild-type p53, but not truncated p53 that lacks the full DNA-binding domain (102-292 amino acids). WB, western-blot; IP, immunoprecipitation; NCI-H1793, adenocarcinoma cell line that harbors truncated p53.

Figure S4. Fortilin(Y4A,E168A) retains a secondary structure of wild type fortilin. Fortilin(Y4A,E168A) exhibits mildly augmented β-sheet by circular dichroism (CD) spectropolarimetry. Far UV CD spectra of wild type fortilin, fortilin(Y4A), fortilin(E168A) and fortilin(Y4A,E168A) were collected on a Jasco J715 Spectropolarimeter. Spectra were recorded from 260 to 200 nm in 0.5 nm steps at 100 nm/min. Data represent the average of 10 independent spectra. Data are expressed as mean molar ellipticity (degrees cm^2/dmol). These data suggest that Fortilin(Y4A,E168A) is a useful double point mutant that retains the secondary structure of the wild-type counterpart.

Figure S5. Fortilin blocks p53-induced apoptosis in PMJ2-PC cells. A, Generation of PMJ2-PC cell lines deficient of p53 and/or fortilin. PMJ2-PC$_{p53+fortilin+}$, PMJ2-PC cells stably harboring pLKO.1-neo-empty and pLKO.1-puro-empty; PMJ2-PC$_{p53+fortilin-}$, PMJ2-PC cells stably harboring pLKO.1-neo-empty and pLKO.1-puro-shRNA-fortilin (the construct to generate shRNA against fortilin mRNA); PMJ2-PC$_{p53-fortilin+}$, PMJ2-PC cells stably harboring pLKO.1-neo-shRNA-p53 and pLKO.1-puro-empty; PMJ2-PC$_{p53-fortilin-}$ PMJ2-PC cells stably harboring pLKO.1-neo-shRNA-p53 and pLKO.1-puro-shRNA-fortilin. **B**, DNA fragmentation assay of UV-irradiated PMJ2-PC cells. **C**, Caspase 3 activity assay of UV-irradiated PMJ2-PC cells.

Figure S6. The Lack of Fortilin Leads to Excessive Induction by p53 of Bax and Noxa, but not PUMA. A, Generation of U2OS cell line that lacks fortilin. Sh-fortilin, U2OS cells stably transfected with a lentiviral vector containing the short-hairpin RNA against fortilin. Sh-control, U2OS cells stably transfected with an empty lentiviral vector. **B.** Western blot analysis of p53, GAPDH, fortilin, Bax, PUMA and Noxa in U2OS cells that are stably transfected with empty lentiviral vector (sh-control) or lentiviral vector containing sh-fortilin (sh-fortilin). 20 mJ/cm^2, UV-irradiation at 20mJ/cm^2. Quantitative analyses of p53 (**C**), Bax (**D**), PUMA (**E**), and Noxa (**F**). Closed bar, sh-fortilin; open bar, sh-control; NS, not significant; *, $P < 0.05$; **, $P < 0.01$.

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Cell Culture and Cell Lines: U2OS, HeLa, SAOS, NCI-H1793, and PMJ2-PC cell lines were purchased from American Type Culture Collection (ATCC, Manassas, VA). All cell lines were maintained in high-glucose Dulbecco's modified Eagle's medium (DMEM) and supplemented with 10% fetal bovine serum (FBS) at 37°C in an atmosphere containing 5 % CO_2.

U2OS cells stably expressing fortilin ($U2OS_{Fortilin\text{-}HA}$) and U2OS cells stably expressing HA-tag alone ($U2OS_{Empty\text{-}HA}$), both selected by Zeocin (Invitrogen), have been generated and characterized in our laboratory (Graidist et al., 2004).

For the generation of isogenic $CHO_{Bax\text{-}Luciferase}$ cells, the (hygromycin resistant gene [$Hygro^R$])-FRT-polyA fragment from pcDNA5/FRT/V5-His vector (Invitrogen) was shuttle-cloned into the pLUC vector (Stratagene) to generate the pLUC-$Hygro^R$-FRT-polyA vector. pLUC vector is a promoterless luciferase reporter plasmid with its multiple cloning site fused to the luciferase reporter gene. The p53-responsive element (p53-RE) of the Bax gene promoter was then introduced to the multiple cloning site of pLUC-$Hygro^R$-FRT-polyA vector by using PCR-based strategies yielding pLUC-Bax-$Hygro^R$-FRT-polyA vectors where the p53-RE of Bax gene was TCACAAGTTAGAGACAAGCCTGGGCGTGGGCTATATT, as described by Miyashita (Miyashita and Reed, 1995). Flp-In-CHO cells that contain an integrated FRT site in their genome were maintained in Ham's F12 media with 10% FBS and 100μg/mL

Zeocin. Cells were co-transfected with pLUC-Bax-Hygro^{R}-FRT-polyA vectors and with pOG44 for the Flp recombinase expression. Successfully recombined cells were selected by 600μg/mL Hygromycin to yield $CHO_{Bax\text{-}Luciferase}$ cells. Since one copy of the luciferase reporter plasmid stably exists in the cell, the system is capable of reproducibly detecting the reporter gene activation as long as the same number of cells are used in the experiment.

For the generation of p53-deficient and its control PMJ2-PC cell lines (PMJ2-PC_{p53-} and PMJ2-PC_{p53+}), PMJ2-PC cells were transduced with the neomycin-resistant lentiviral vector (pLKO.1-neo) containing the shRNA against human p53 ($shRNA_{p53}$) or with the empty pLKO.1-neo-vector (Mission shRNA Lentiviral System, Sigma, St. Louis, MO), selected under G418 selection (100 μg/mL), and characterized by Western blot analyses. PMJ2-PC_{p53-} and PMJ2-PC_{p53+} cells were then transduced with the puromycin-resistant lentiviral vector (pLKO.1-puro) containing the shRNA against human fortilin ($shRNA_{Fortilin}$) or with the empty pLKO.1-puro vector, selected under G418 and puromycin (2 μg/mL), and characterized by Western blot analyses. Four cell lines—PMJ2-$PC_{p53+fortilin+}$, PMJ2-$PC_{p53+fortilin-}$, PMJ2-$PC_{p53-fortilin+}$, and PMJ2-$PC_{p53-fortilin-}$—were generated.

For the generation of U2OS and SAOS cells overexpressing fortilin or fortilinΔ (or fortilin[Y4A,E186A]), retroviral particles encoding cDNAs of fortilin and its mutants were prepared by (1) co-transfecting (a) pQCX-fortilin, pQCX-fortilinΔ, or empty vector and (b) pVSV-G vector into GP-293 cells (BD ClonTech) using FuGENE6 (Roche) and (2)

harvesting conditioned media from transfected cells 48 hrs after transfection, according to manufacturer's instruction. For infection, U2OS or SAOS cells were plated at a density of $5x10^5$ cells per 10 cm culture dish and incubated with virus contained in the media from GP-293 cells in the presence of Polybrene (8 μg/mL, Sigma). After selection with G418 (100 μg/mL), surviving cells harboring pQCX-fortilin ($U2OS_{Ret\text{-}Fortilin}$), pQCX-fortilinΔ ($U2OS_{Ret\text{-}fortilin\Delta}$), and pQCX-empty ($U2OS_{Ret\text{-}Empty}$), were characterized by Western blot analysis. When appropriate, the 2nd round of infection was performed as described above.

Western Blot Analyses: Western blot analyses were performed as described previously by us (Fujita et al., 2008; Graidist et al., 2004; Graidist et al., 2007; Li et al., 2001; Zhang et al., 2002), using the following antibodies: anti-fortilin (1:2,000 dilution, MBL International, Woburn, MA), anti-human p53 (1:1,000 dilution, DO1 and DO7, Santa Cruz), anti-mouse p53 (1:500 dilution, FL393, Santa Cruz), anti-mouse Bax (1: 1,000 dilution, N-20, Santa Cruz), anti-HA (1: 500 dilution, 16B12, Covance), anti-PUMA (1:1,000 dilution, Ab9643, Abcam), anti-Noxa (1:1,000 dilution, N-15, Santa Cruz), and anti-glyceraldehyde-3-phosphate (GAPDH)(1: 10,000 dilution, 6C5, Fitzgerald) antibodies.

In vitro GST-pulldown Assays: GST-pulldown assays were performed as described by us previously (Gong et al., 1997) with the following modifications. GST-tagged

fortilin (GST-fortilin, study) and GST alone (control) were expressed in *E. coli* and purified. Using a TNT Quick Coupled Transcription-Translation System (Promega, Madison, WI), we synthesized [^{35}S]methionine-labeled p53 in vitro from p53 cDNA on a pcDNA3 plasmid containing a T7 promoter. The same method was used to generate [^{35}S]methionine-labeled MCL1 (previously shown to interact with fortilin) (Zhang et al., 2002), and [^{35}S]methionine-labeled Bcl-xL (previously shown not to interact with fortilin) (Zhang et al., 2002). Then, radioactive p53 (or MCL1 or Bcl-xL) and GST-fortilin (or GST-alone) were mixed in a reaction buffer at 4^{o}C for 90 minutes. Labeled proteins bound to GST-fusion proteins were pulled down by glutathione sepharose beads, washed, and eluted into SDS-PAGE loading buffer, subjected to 12% SDS-PAGE, stained with Coomassie blue to confirm successful pull-down, and visualized by fluorography and imaging with a phosphoimager system (Bio-Rad). The exact same procedures were used to generate and evaluate the deletion and point mutants of fortilin for their binding to wild type or deletion mutants of p53. The band intensity was quantified by densitometric analyses. The intensity of fortilin-p53 binding was expressed as follows: (-) or no binding for the band intensity between 0 and 30; (+/-) or minimum binding for the band intensity between 31 and 60; (+) or decreased binding for the band intensity between 61 and 90; (++) or comparable binding for the band intensity between 91 and 120; and (+++) or increased binding for the band intensity greater than 120. The experiments were performed at least three times. The data were found consistent between experiments.

Circular Dichroism (CD) Spectropolarimetry: Far UV CD spectra of mutated and control GST-fusion protein samples (0.36mg/ml) were collected on a Jasco J715 Spectropolarimeter with the use of a 1-mm path length quartz cuvette at room temperature as described previously (Lu and Chang, 2002; Salamanca and Chang, 2005). Spectra were recorded from 260 to 200 nm in 0.5 nm steps at 100 nm/min. Data represent the average of 10 independent spectra. All protein samples were passed through a PD-10 column (Sephadex-25-Pharmacia) equilibrated in 0.1M phosphate buffer (pH 7.4) for desalting. Data are expressed as mean molar ellipticity (degrees cm^2/dmol).

In vivo Immunoprecipitation (IP) - Western blot Assays: Details of the immunoprecipitation procedure have been described by us (Fujise et al., 2000; Zhang et al., 2002). For the first in vivo forward immunoprecipitation (**Fig. 1B**), cleared total cell lysates from $U2OS_{Empty\text{-}HA}$ and $U2OS_{Fortilin\text{-}HA}$ were incubated with rat monoclonal anti-HA antibody (Clone 3F10, Roche). Formed complexes were precipitated by sheep anti-rat antibodies conjugated to Dynabeads^{TM} (Dynal USA), washed 4 times, eluted into SDS gel loading buffer; and subjected to SDS-PAGE, Western blot transfer and immunodetection with anti-HA (16B12; Covance) and anti-p53 (DO-1, Santa Cruz) antibodies. For the second in vivo forward immunoprecipitation-Western blot analysis (**Fig. S1**), we used agarose-conjugated anti-HA (Clone 3F10, Roche) for immunoprecipitation, anti-fortilin (MBL International, Woburn, MA), and DO1 and DO7 anti-p53 antibodies (Santa Cruz) for detection.

For the first in vivo reverse immunoprecipitation (**Fig. 1C**), we equally aliquoted cleared total cell lysates from $U2OS_{Fortilin\text{-}HA}$ cells into three microfuge tubes containing agarose beads alone, agarose-conjugated anti-p53 (DO1) antibody, or agarose-conjugated normal mouse IgG (Santa Cruz). Immunoprecipitates were incubated overnight at 4°C, extensively washed, and eluted into SDS loading buffer for Western blot analyses. For the second in vivo reverse immunoprecipitation (**Fig. 1D**), wild-type U2OS cells ($1x10^7$) were lysed by a nitrogen cavitation method (Fujise et al., 2000; Zhang et al., 2002) and cleared by centrifugation. Cleared total cell lysates were incubated with either a mixture of two anti-p53 antibodies (DO-1 and Pab421) or control mouse monoclonal antibody, both at a concentration of 2μg/mL. Formed complexes were precipitated by sheep anti-mouse antibodies conjugated to Dynabeads™ (Dynal USA) and treated as above, except we used anti-p53 (DO-1) and anti-fortilin (Li et al., 2001) antibodies for immunodetection.

Immunocytochemistry and Confocal microscopic analysis of fortilin and p53:
Immunocytochemical analyses were performed as described by us previously (Fujise et al., 2000) with the following modifications. HeLa cells seeded on 2-chamber slides (Lab-Tek™, Nunc, Rochester, NY) were UV-irradiated at 80 mJ/cm2, fixed in 10% buffered formalin solution for 5 min, permeabilized in 0.1% Triton X, and incubated with rabbit anti-fortilin (MBL) and mouse anti-p53 (DO1) antibodies. After extensive wash, bound antibodies were detected by anti-rabbit-Rhodamine-Red-X (RRX, Jackson

ImmunoResearch Laboratories, West Grove, PA) and anti-mouse-Alexa Fluor 488 (Invitrogen, Carlsbad, CA) antibodies. DRAQ5 (Biostatus Limited, Leicestershire, UK) was used to counterstain the nucleus. Confocal microscopy was performed on the Leica TCS confocal microscope equipped with 488 Argon (for Alexa Fluor 488, green color assigned), 561 Diode-pumped solid-state (for RRX, red), and 633 Helium-neon (for DRAQ5, blue) excitation laser lines. A 63x oil-immersion objective (Leica Microsystems) was employed for visualization. Image acquisition conditions were tested and set to remove channel crosstalk.

Docking Study: The docking study was performed on a SGI workstation (Sunnyvale, CA) as described previously by us (Wu et al., 2008) and others (Pellegrini et al., 2001). The PDB files of fortilin (PDB ID: 2HR9) and the sequence specific DNA binding domain (SSDBD) of p53 (PDB ID: 1TUP) were downloaded from the RCSB Protein Data Bank and imported into the Insight II program (Accelrys, Burlington, MA). The data on these PDB files were subjected to energy minimization. The algorithms for the minimization were the first 1000 steps of steepest descents followed by conjugate gradients until the energy derivative was less than 0.001 Kcal/mol. After the energy minimization of the SSDBD of p53 and fortilin, they were put into one assembly, the intermolecular energy of which was calculated with a cut-off value of 20 Å, using the Discover module of the program.

Luciferase Assay: For the luciferase assay using $CHO_{Bax\text{-}Luciferase}$ cells, the cells were co-transfected with either pcDNA3-p53 or pcDNA3-empty and with pFLAG-fortilin or pFLAG-empty using FuGENE6 (Roche). We used the Luc-Screen kit (Applied Biosystems) to assay the luciferase activity 48 hours after transfection. The same lysates were subjected to Western blot analyses using anti-p53 (DO1, Santa-Cruz), anti-GAPDH (Fitzgerald), and anti-fortilin (MBL) antibodies.

The 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyl-Tetrazolium Bromide (MTT) Cell Survival Assay: $U2OS_{Fortilin\text{-}HA}$ and $U2OS_{Empty\text{-}HA}$ cells (0.16×10^4) were seeded into 96-well plates and transduced by adenoviral vector encoding p53 (Ad-p53, University of Pittsburgh Vector Core Facility) or adenoviral vector encoding luciferase (Ad-Luc), both at a multiplicity of infection (MOI) of 300. The cells were subjected to a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) assay 48 hrs after transduction as described by us (Koide et al., 2009). Absorbance reading at 600nm [A_{600}] of $U2OS_{Empty\text{-}HA}$ and $U2OS_{Fortilin\text{-}HA}$ cells transduced by Ad-Luc was normalized to 1. After the MTT assay, cells were subjected to SDS-PAGE and Western blot analysis using anti-p53 (DO1), anti-GAPDH (Fitzgerald), and anti-fortilin (MBL) antibodies.

Small Interfering RNA System: For fortilin silencing, U2OS cells were transfected with siRNA against luciferase ($siRNA_{Luciferase}$) or siRNA against fortilin ($siRNA_{Fortilin}$) using DharmaFECT-2 (Dharmacon) as described previously by us (Graidist et al., 2004). The

siRNA against luciferase, a non-mammalian protein from *Photinus pyralis* (American firefly), was used as a control.

DNA Fragmentation Assay: Histone-associated DNA fragmentation was analyzed using the Cell Death Detection ELISA-Plus kit, according to the manufacturer's instructions (Roche) as described by us previously (Koide et al., 2009). 20µL each of a lysate of UV-irradiated (at 0-100 mJ/cm^2 as indicated in the figure legends) U2OS cells and 80µL each of assay buffer containing anti-histone-biotin and anti-DNA-peroxidase were dispensed into streptavidin-coated wells of a 96-well plate in quadruplicate. After incubation and wash, captured nucleosomes were detected by the 2,2'-azino-di (3-ethyl-benzthiazoline-6-sulphonic acid) (ATBS) whose signal intensity was measured at 405nm with reference wavelength of 490nm and called DNA fragmentation indices.

Real-time Quantitative Reverse Transcription Polymerase Chain Reaction (qRT-PCR): The methods were previously described by us (Mnjoyan et al., 2003). Briefly, U2OS cells were seeded in triplicate in a 6-well dish. The next day, cells were UV-irradiated, harvested into Tri-Reagent (Molecular Research Center, Cincinnati, OH) and subjected to RNA isolation, according to the manufacturer's instructions, and to DNAse (ABI, Foster City, CA) treatment. The real time qRT-PCR was performed in quadruplicate on exactly 50 ng of total RNA, using the TaqMan® RT-PCR kit (ABI) in the Applied Biosystems Step One Plus Real-Time PCR system and following primer

and probe sets: (a) for the detection of fortilin: Forward: 5′- ATG ACT CGC TCA TTG GTG GAA-3′, Reverse: 5′- TGC TTT CGG TAC CTT CGC CC -3′, Probe: 5′- FAM-TGC CTC CGC TGA AGG CCC-IABkFQ-3′, where FAM = carboxyfluorescein and IABkFQ= Iowa Black FQ; (b) for human Bax: Forward: 5′- AGC TGC AGA GGA TGA TTG CC -3′, Reverse: 5′- TGC CAC TCG GAA AAA GAC CTC -3′, Probe: 5′- FAM-CCG TGG ACA CAG ACT CCC CCC GA-IABkFQ -3′ (Integrated DNA Technologies, Coralville, IA). Using a standard curve drawn on the serially diluted human fortilin cRNA and fortilin critical thresholds (C_T) of samples, a fortilin transcript mass (fg) from a well was determined, normalized to µg of total RNA used, and expressed as fortilin mRNA index (fg of fortilin per µg of total RNA). The same procedure was performed for the quantification of human Bax.

Enzyme-linked Immunosorbent Assay (ELISA): ELISA of human p53 and Bax were performed as described by us previously (Mnjoyan et al., 2003), following the treatment of U2OS cells with $siRNA_{Luciferase}$ or $siRNA_{Fortilin}$, using the commercially available kits according to the manufacturer's instructions (Roche and R&D Systems).

Caspase-3 Activity Assay: Caspase-3 activity assays were performed as described previously by us (Koide et al., 2009). In brief, cells were suspended in cell lysis buffer (10 mM Tris, pH 7.5, 100 mM NaCl, 1 mM EDTA, 0.01% Triton X-100), subjected to three freeze-thaw cycles, and centrifugation. An aliquot of cleared cell lystates was

incubated with 2.5 mM rhodamine 110 bis-(N-CBZ-L-aspartyl-L-glutamyl-L-valyl-L-aspartic acid amide)(Z-DEVD-R110) substrate (Invitrogen-Molecular Probes). Caspase-3 activities were determined, every 5 min for 90 min, by measuring fluorescence signals (excitation/emission = 496/520 nm), using the SpectraMax Plus microplate spectrophotometer (Molecular Devices) and expressed as relative fluorescent unit (RFU).

Electrophoretic Mobility Shift Assay (EMSA) : A double-stranded (ds) 53 consensus oligonucleotide (5'-TACAGAACATGTCTA AGCATGCTGGGG-3', Santa Cruz Biotechnology) was labeled with γ-32P-ATP using T4 polynucleotide kinase and then purified. We generated p53-rich nuclear extract by transducing U2OS cells with adenoviral vector encoding human p53 cDNA (University of Pittsburgh Vector Core Facility) and purifying the nuclear fraction as described previously (Mnjoyan et al., 2003). A binding reaction was performed by incubating the ^{32}P-ds p53-consensus oligonucleotide with the p53-rich nuclear extract and either GST-fortilin, GST-alone, or GST-fortilin mutants, in binding buffer (20mM HEPES, pH = 7.9, 0.075μg/μL poly dI:dC [Amersham-Pharmacia], 1.0 mM EDTA, 1.0 mM DTT, 0.1% NP-40, 2mM $MgCl_2$, 25mM KCl, and 5% glycerol). When appropriate, anti-p53 antibody (Pab421, EMD Chemicals, Gibbstown, NJ) or cold competitive ds p53 consensus oligonucleotide was added to the mixture to evaluate the specificity of the binding reaction. The mixture was then subjected to SDS-PAGE and autoradiography.

Nude Mouse Tumor Xenograft Assays: All mouse experiments were performed under the approved Institutional Animal Care and Use Committee (IACUC), in accordance with the NIH Guide for the Care and Use of Laboratory Animals as described previously (Monia et al., 1996). First, $U2OS_{Ret\text{-}Empty}$, $U2OS_{Ret\text{-}Fortilin}$, or $U2OS_{Ret\text{-}Fortilin\Delta}$ cells were subcutaneously inoculated without extracellular matrix gel into the flank region of 6-8 week-old male athymic BALB/c nude mice homozygous for the nu/nu allele ($2x10^6$ cells/mouse). Tumor size was assessed by a digital caliper weekly. At the 6^{th} week, $U2OS_{Ret\text{-}Empty}$ and $U2OS_{Ret\text{-}Fortilin}$ tumor xenografts were harvested, fixed in buffered formalin, embedded in paraffin, and subjected to histopathological and immunohistochemical analyses. At the 6^{th} week, there was no tumor growth in mice injected with $U2OS_{Ret\text{-}Fortilin\Delta}$. There mice were observed for an additional 6 weeks. All tumors developed during the observation period were harvested at the 12^{th} week and subjected to the same analyses described above. Secondly, $SAOS_{Ret\text{-}Empty}$, $SAOS_{Ret\text{-}Fortilin}$, or $SAOS_{Ret\text{-}Fortilin\Delta}$, were subcutaneously inoculated with Matrigel (Collaborative Research Biochemicals, Bedford, MA) into the flank region of 6-8 week-old male athymic BALB/c nude mice homozygous for the nu/nu allele ($3x10^6$ cells/mouse). The tumor size was assessed by a digital caliper bi-weekly. There was no significant tumor growth for the first 6 weeks. The animals were observed for a total of 15 weeks at which time tumor xenografts were harvested and subjected to the same analyses described above.

TUNEL staining was performed using FragEL™ DNA Fragmentation Detection Kit (Calbiochem), according to the manufacturer's instructions. At least 600 cells were counted and TUNEL indices were calculated at the number of TUNEL-positive cells divided by the number of total cells counted. Immunohistochemical staining was performed using the following antibodies: anti-fortilin rabbit polyclonal antibody (MBL) and anti-Bax antibody (N-20, Santa Cruz). The bound antibody was detected by the secondary antibody conjugated to horse radish peroxidase and 3,3'-diaminobenzidine (DAB). The positive DAB signal was quantified using SigmaScan Pro5 (SPSS Science Software, Chicago, IL) and expressed at the percentage of the DAB-positive area within the region of interest.

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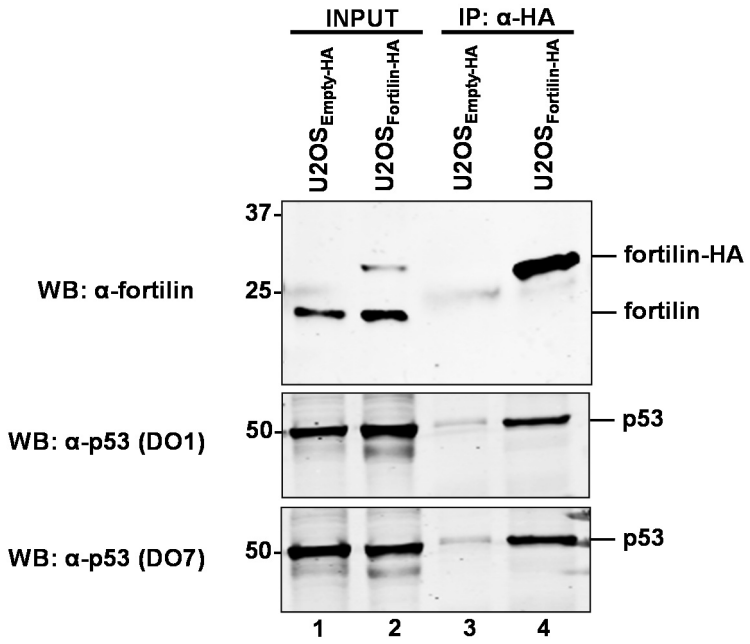
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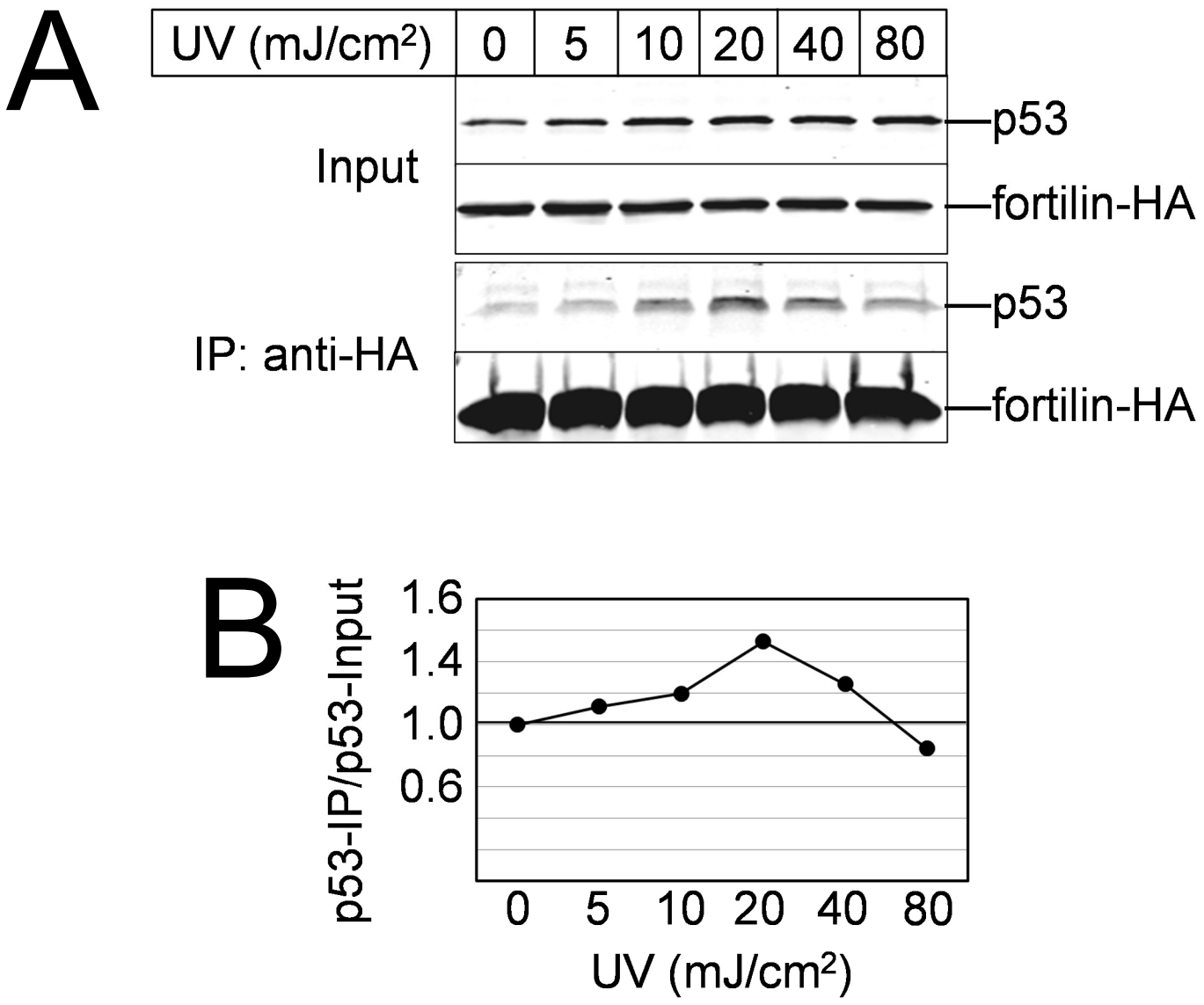
(Fig. S1)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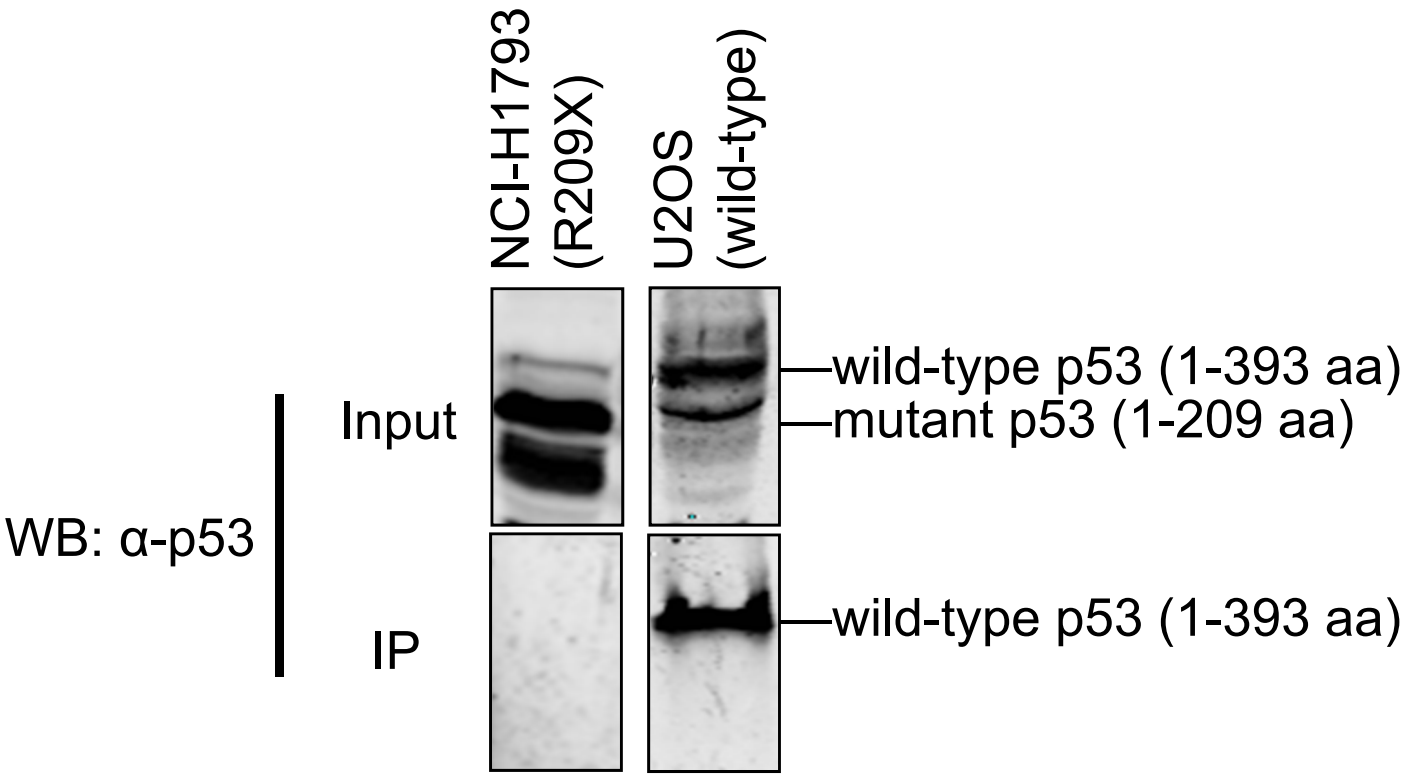
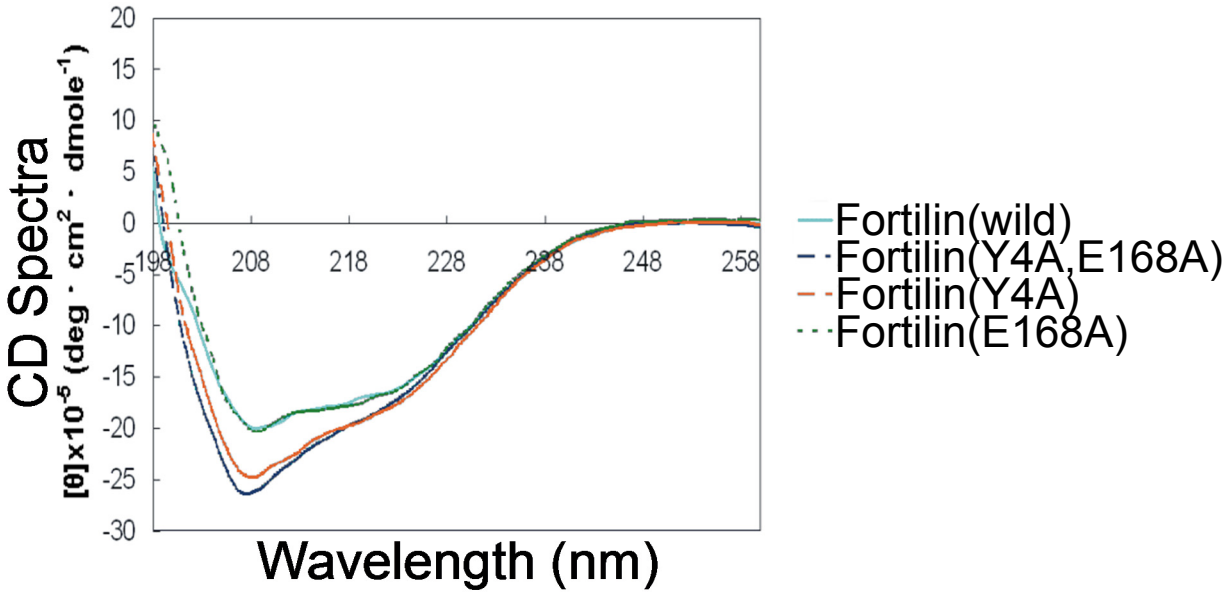
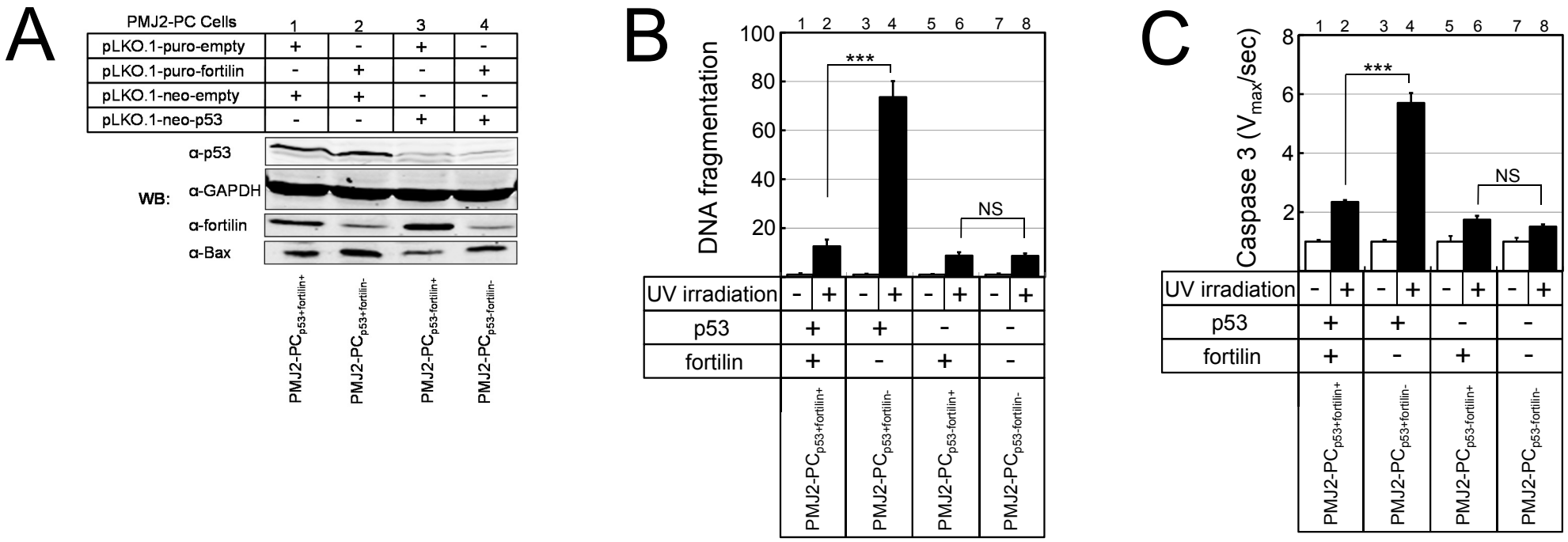

NCI-H1793
(R209X)
U2OS
(wild-type)
Input
IP
WB: α-p53
wild-type p53 (1-393 aa)
mutant p53 (1-209 aa)
wild-type p53 (1-393 aa)

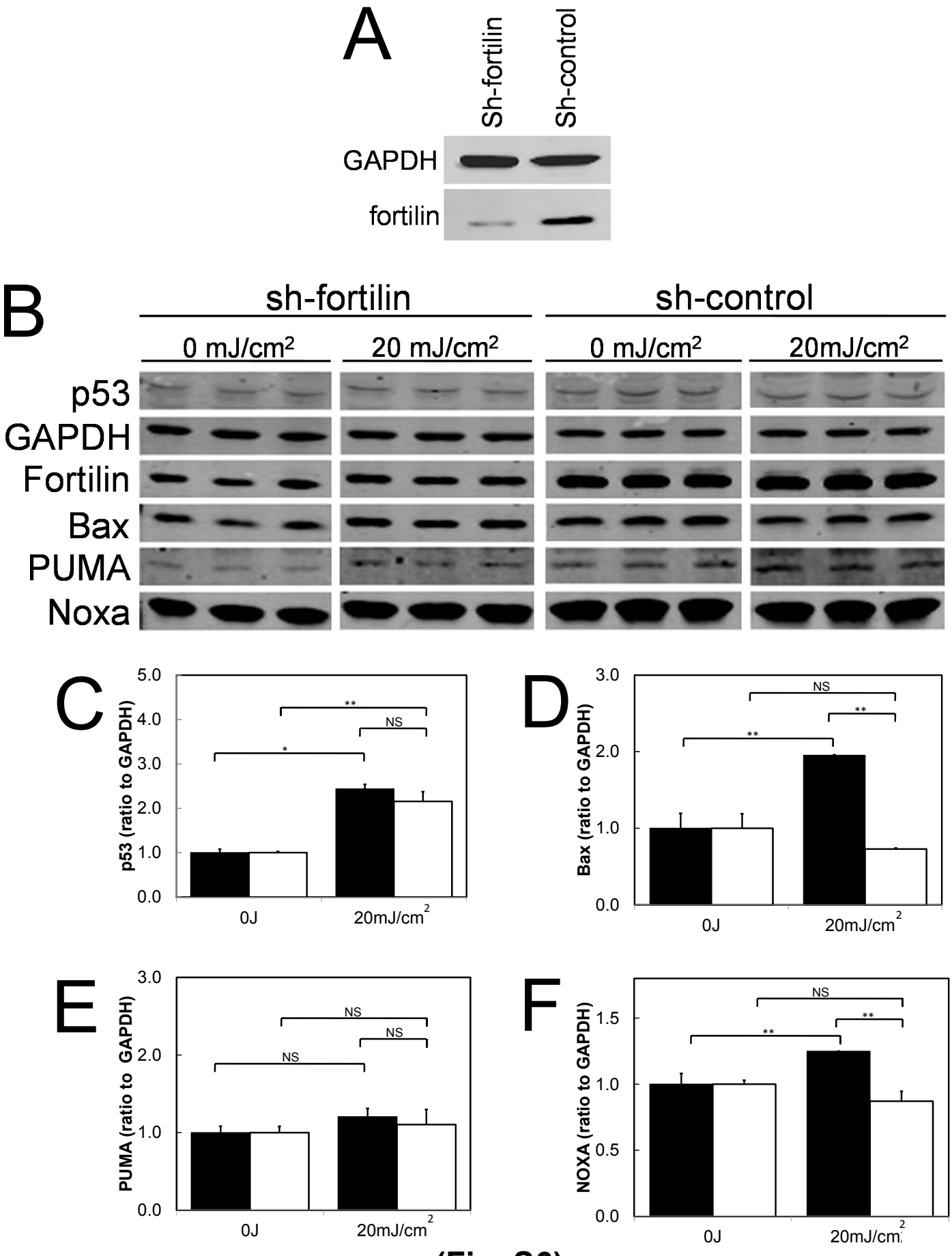
(Fig. S3)


CD Spectra
[θ]x10^-5 (deg · cm^2 · dmole^-1)
20
15
10
5
0
-5
-10
-15
-20
-25
-30
198
208
218
228
238
248
258
Wavelength (nm)
Fortilin(wild)
Fortilin(Y4A,E168A)
Fortilin(Y4A)
Fortilin(E168A)

(Fig. S4)


A
PMJ2-PC Cells
1
2
3
4
pLKO.1-puro-empty
pLKO.1-puro-fortilin
pLKO.1-neo-empty
pLKO.1-neo-p53
WB:
α-p53
α-GAPDH
α-fortilin
α-Bax
PMJ2-PC p53+fortilin+
PMJ2-PC p53+fortilin-
PMJ2-PC p53-fortilin+
PMJ2-PC p53-fortilin-
B
DNA fragmentation
100
80
60
40
20
***
NS
UV irradiation
p53
fortilin
C
Caspase 3 (Vmax/sec)
8
6
4
2
***
NS
UV irradiation
p53
fortilin

(Fig. S5)


A
Sh-fortilin
Sh-control
GAPDH
fortilin
B
sh-fortilin
sh-control
0 mJ/cm2
20 mJ/cm2
0 mJ/cm2
20mJ/cm2
p53
GAPDH
Fortilin
Bax
PUMA
Noxa
C
p53 (ratio to GAPDH)
0J
20mJ/cm2
D
Bax (ratio to GAPDH)
0J
20mJ/cm2
E
PUMA (ratio to GAPDH)
0J
20mJ/cm2
F
NOXA (ratio to GAPDH)
0J
20mJ/cm2
NS

(Fig. S6)