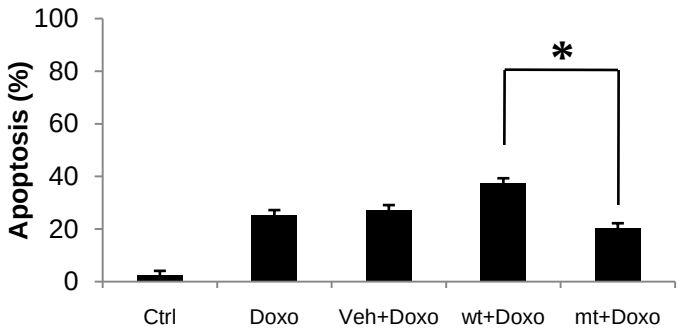
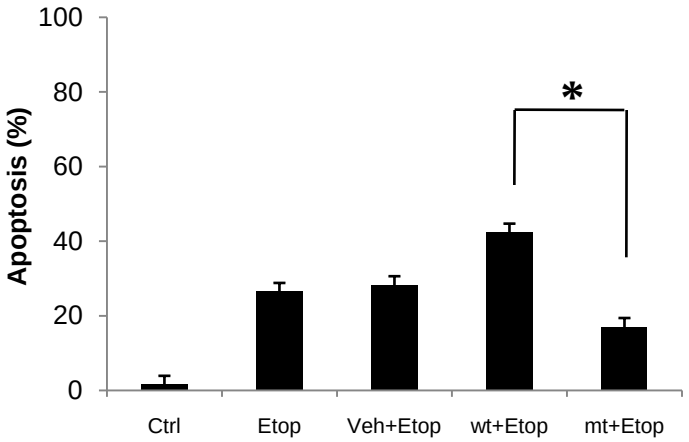
Supplementary Fig. S1

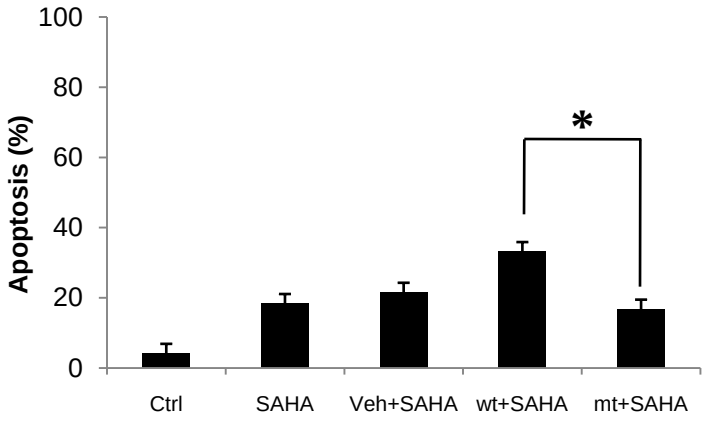
A



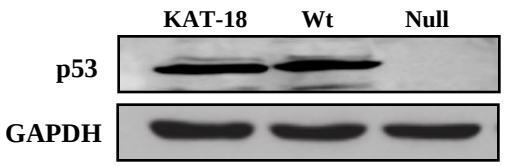
B



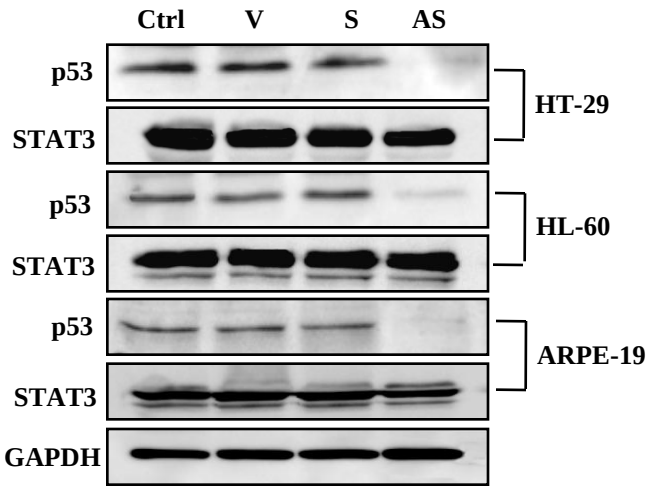
C



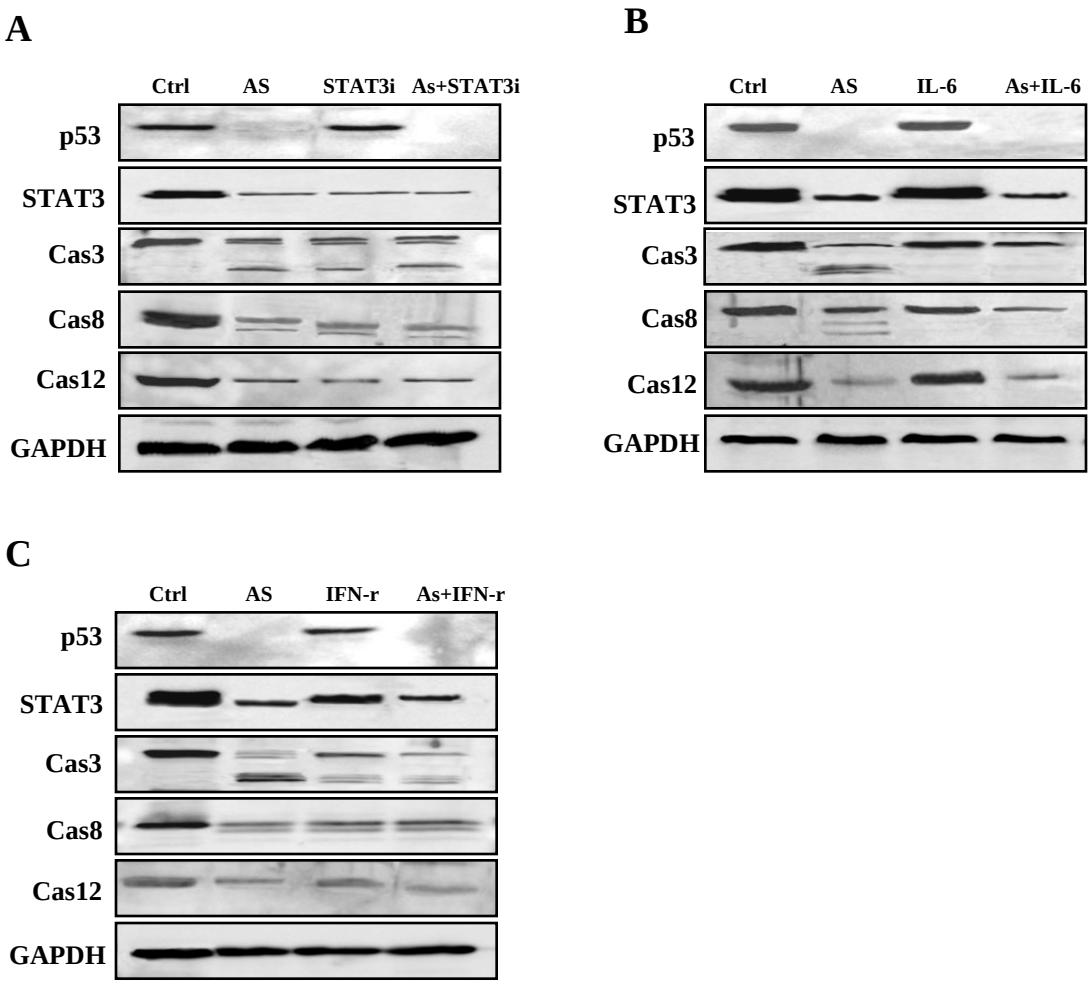
D



Supplementary Fig. S2



Supplementary Fig. S3



Supplementary Figure Legends

Supplementary Fig. S1. Transient overexpression of mt p53 (G199V), on the contrary of wt p53, significantly protects HCT-116 (p53 -/-) cells against apoptosis induced by doxorubicin (Doxo, 2 uM), etoposide (Etop, 50 ng/ml) and SAHA (10 uM). A. Ctrl, control HCT-116 cells. Doxo, HCT-116 cells treated with doxorubicin 2 μM for 24 h. Veh+Doxo, HCT-116 cells incubated with transfection vehicle for 6 h and treated further with doxorubicin 2 μM for 24 h. wt+Doxo, HCT-116 cells transfected with wt p53 for 6 h and treated further with doxorubicin 2 μM for 24 h. mt+Doxo, HCT-116 cells transfected with mt p53 (G199V) for 6 h and treated further with doxorubicin 2 μM for 24 h. B. Etop, etoposide. See S1 A for other definitions. C. SAHA, suberoylanilide hydroxamic acid . See S1 A for other definitions. D. Western blot assay for comparing the p53 status in HCT-116 p53 wild type and p53 null cells. Wt, HCT-116 p53 +/+ cells. Null, HCT-116 p53 -/- cells. GAPDH is shown as a loading control.

Supplementary Fig. S2. Knock-down of p53 does not alter the expression level of STAT3 protein in HT-29, HL-60 and ARPE-19 cells. Ctrl, untreated control cells. V, vehicle. S, sense p53, AS, p53-specific AS oligonucleotide treatment for 24 h. GAPDH is shown as a loading control.

Supplementary Fig. S3. Western blot assay for STAT3 and apoptosis-related proteins. (A) STAT3i (1.5 mM) treatment induced the degradation of STAT3, procaspase-3, -8 and -12 at 24 h after treatment. (B) IL-6 (20 ng/ml) significantly alleviates the induction of apoptosis by p53 knock-down at 24 h after treatment. (C) IFN-γ (30 nM) does not significantly alter the induction of apoptosis by p53 knock-down at 24 h after treatment. GAPDH is shown as a loading control.

Supplementary Appendix S1

Materials and Methods

Reagents

Rabbit polyclonal anti-human caspase 3, caspase 8, caspase 12, cytochrome c, p53, Janus kinse (JAK) 2, caspase activated deoxyribonuclease (CAD), 14-3-3, and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) antibodies were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). Mouse polyclonal anti-human poly (ADP-ribose) polymerase (PARP) antibody was obtained from Oncogene (Cambridge, MA). Mouse monoclonal anti-human X-chromosome linked inhibitor of apoptosis (XIAP) antibody was obtained from Stressgen (Ann Arbor, MI). Rabbit polyclonal anti-human STAT3, phospho-STAT3 (Ser727 and Tyr705), STAT5, and STAT6 antibodies were from Cell Signaling Technology (Beverly, MA). Human interferon-γ (IFN-γ) and STAT3 inhibitor (STAT3i) were obtained from Calbiochem (Darmstadt, Germany). Fluorescein isothiocyanate (FITC)–conjugated goat anti-rabbit IgG antibody was obtained from Vector (Burlingame, CA), and fetal bovine serum (FBS) from Gibco (Gaithersburg, MD). interleukin-6 (IL-6), RPMI-1640 medium, Hoechst 33342, RNase A, proteinase K, protease inhibitor cocktail, and propidium iodide (PI) were all obtained from

Sigma (St. Louis, MO). siPort Amine was obtained from Ambion (Austin, TX). Lipofectamine plus and Lipofectamine 2000 were obtained from Invitrogen (Carlsbad, CA). The enhanced chemiluminescent Western blotting detection reagent (SuperSignal West Pico chemiluminescent substrate) was obtained from Pierce (Rockford, IL).

Cell culture

ATC cell line, KAT-18 cells (kindly provided by Dr. K.B. Ain, University of Kentucky Chandler Medical Center, KY, USA) were maintained at 37□ with 5% CO_2 in RPMI-1640 supplemented with 2 mM L-glutamine, Earle's Balanced Salt Solution with 2.0 g/L sodium bicarbonate, 0.1 mM non-essential amino acids, and 1.0 mM sodium pyruvate, and 10 % FBS. Human retinal pigment epithelial cell line ARPE-19 cells and human colon cancer cell line HCT-116 cells were purchased from the American Type Culture Collection (Rockville, Maryland, USA) and grown in Dulbecco's modified Eagle's medium with 10% FBS at 37□ and 5% CO_2. p53 null cancer cell line HCT-116 (p53 -/-) cells were provided by Bert Vogelstein (Johns Hopkins University, Baltimore, USA).

Cell viability assay

Cell viability was determined with the Vi-Cell cell counter (Beckman Coulter, Fullerton,

CA), which performs an automated trypan blue exclusion assay. Briefly, cells were plated at 10^5 cells/ml in 6 well plates. After 24 h incubation with each indicated reagents, the cells were trypsinized and suspended in 1 ml of media. The harvested cells were counted by Vi-Cell cell counter (Beckman Coulter, Fullerton, CA).

Flow cytometric analysis

Conducted as described previously (39). In brief, cells were fixed in ice-cold 95% ethanol and re-suspended in a PI solution (50 μg/ml). DNA content was measured on an Epics XL (Beckman Coulter), and the data were analyzed using Multicycle software, which allowed for simultaneous estimation of cell cycle parameters and apoptosis.

Nuclear morphology analysis of apoptosis

Cytocentrifuged samples were fixed for 10 min in 4% paraformaldehyde and stained in 4 μg/ml Hoechst 33342 for 30 min at 4℃. A total of 300 cells from each experiment were counted using differential interference contrast optics. The number of cells showing condensed or fragmented nuclei by Hoechst staining was calculated using epifluorescence optics by an observer who was blinded with regard to the experimental group.

DNA electrophoresis

Conducted as described previously (39).

Pulse-field gel electrophoresis (PFGE)

Conducted as described previously (39). In brief, PFGE was carried out using the CHEF Mapper XA System from Bio-Rad (Hercules, CA). Electrophoresed DNA was stained with EtBr and detected with the LAS-3000 Plus System (Fuji Photo Film Company, Kanagawa, Japan). Chromosomal DNA from *S. cerevisiae* and a mixture of λ DNA, λ DNA concatemers, and *Hind*III-digested λ DNA were used as DNA size markers.

Western blot analysis

Conducted as described previously (40). In brief, 50 μg of proteins was loaded onto 7.5-15% SDS-polyacrylamide gels. The gels were transferred to Nitrocellulose membrane (Amersham Pharmacia Biotech, Piscataway, NJ) and reacted with each antibody. Immunostaining with antibodies was performed using SuperSignal West Pico enhanced chemiluminescence substrate and detected with LAS-3000 PLUS (Fuji Photo Film Company).

Assay of mitochondrial membrane potential (MMP)

Conducted as described previously (40). In brief, 3,3' dihexyloxa carbocyanine iodide ($DIOC_6$) was added to the cell culture medium and cells were submitted to flow cytometry on the Epics XL (Beckman Coulter) to measure the MMP. Data were acquired and analyzed using EXPO32 ADC XL 4 color software. The analyzer threshold was adjusted on the forward scatter channel to exclude noise and most of the subcellular debris.

Immunofluorescence staining and confocal microscopy

Conducted as described previously (40). In brief, cytocentrifuged cells were fixed in 4% paraformaldehyde, incubated with each primary antibody and then incubated with FITC-conjugated secondary antibodies. Fluorescent images were observed and analyzed under a Zeiss LSM 510 laser-scanning confocal microscope (Zeiss, Gőettingen, Germany). Cells were costained with PI to observe nuclear morphology.

Cell-based STAT3 phosphorylation analysis

STAT3 activation was measured using a cellular activation of signaling ELISA (CASE) Kit for STAT3 Y705 (Superarray Biosciences, Frederick, MD). Measurement of the amount of phosphorylated STAT3 protein relative to total STAT3 protein was performed according to manufacturer's recommendations.

Luciferase reporter assay

KAT-18 cells were inoculated into 6-well plates (10^5) 1 day before transfection in RPMI medium without antibiotics. Cells were transfected with the STAT3(1) Luciferase TransLucent Reporter Vector (Panomics, Fremont, CA) using FuGENE 6 reagent (Roche Diagnostic Co., Indianapolis, IN) according to manufacture's instructions. Twenty-four hours post-transfection, cells were transfected with AS or sense oligonucleotide specific for p53; in addition, cells were also transfected without oligonucleotide as an additional negative control. Cell lysates were prepared using Reporter Lysis Buffer (Promega, Madison, WI). Luciferase activity was determined with a luminometer and a Dual-Luciferase Reporter Assay System (Promega). Luciferase activity was normalized to the activity of the cotransfected TransLucent Control Reporter Vector (Panomics) to control for transfection efficiency.

STAT3 overexpression

RC/CMV empty vector (vector control) and STAT3-C (constitutively active STAT3) pRC/CMV vector were provided by Dr. Shong (Department of Internal Medicine, Chungnam National University School of Medicine, Daejeon, South Korea). KAT-18 cells (10^5) were plated into 6-well culture dishes 1 day before infection. The cells were transfected with RC/CMV

empty vector or STAT3-C with Lipofectamine plus (Invitrogen). After 6 h, the medium was replaced with fresh medium and the cells were maintained for 24 h. To examine the effect of STAT3 overexpression on the effect of p53-specific AS oligonucleotide treatment, KAT-18 cells subsequently exposed to p53-specific AS oligonucleotide as described above.

Transient transfection of mt p53

Mt p53 of KAT-18 cell was amplified by PCR using the sense (5'-CCG GAA TTC ATG GAG GAG CCG CAG-3') and antisense (5'- CCG CTC GAG GTC TGA GTC AGG CCC TTC-3'), containing the EcoRI and XhoI sites respectively. The PCR products were cloned into the pcDNA 3.1/V5/HisA expression vector (Invitrogen). The nucleotide sequence of the cloned p53 mt was confirmed by DNA sequencing. pcDNA-mt p53 plasmids or pcDNA empty vector were transfected into HCT-116 or ARPE-19 cells with lipofectamine plus (Invitrogen) and incubated further for 6 h. HCT-116 cells were treated with chemotherapeutic agents for 24 h and harvested. Induction of apoptosis was estimated by nuclear morphology analysis. ARPE-19 cells were incubated further with fresh medium for 24 h and harvested. The effect of transient transfection of mt p53 on the expression of STAT3 in ARPE-19 cells was analyzed by Western blot.

Statistical analysis

Four independent *in vitro* experiments were carried out. Statistical results are expressed as the mean ± standard deviation of the means obtained from triplicates of each independent experiment. The statistical significance of the differences was determined by the paired Kruskal-Wallis nonparametric test. *P* value less than 0.05 were considered significant.

Supplementary Table S1.

Primer sequences of *TP53* gene used in this study for PCR amplification

Exon	Name	primer sequence (5′-3′)	Product length (bp)
1	E1F	GCCCTTACTTGTCATGGCGACTGTCC	357
	E1R	CTCACCCCCAAACTCGCTAAGTCCC	
2 3 4	E2F	CCACAGGAAGCCGAGCTGTCTCAG	849
	E2R	CTGACAGGAAGCCTAAGGGTGAAG	
5 6	E3F	CTCTAGCTCGCTAGTGGGTTGCAGG	630
	E3R	CACATCTCATGGGGTTATAGGGAGG	
7 8 9	E4F	GCCTCCCCTGCTTGCCACAGGTCTC	947
	E4R	GAGGCATCACTGCCCCCTGATGGC	
10	E5F	CAGCTGTATAGGTACTTGAAGTGCAG	554
	E5R	CCCTGCACAGACATGGGGTCAGCTG	
11	E6F	GTGGCCACCATCTTGATTTGAATTCC	1522
	E6R	CCAGTCCACACTCATTGCAGACTCAG	