SUPPLEMENTARY INFORMATION

Methods

Cell culture. Breast cancer cell lines MCF-7, SK-BR-3, and ZR-75-1 were obtained from American Type Culture Collection. Melanoma (MeWo), non-small cell lung carcinoma (NCI-H522 and NCI-H1792), and ovarian epithelial carcinoma (SK-OV-3.ip1) cell lines were obtained from M. D. Anderson faculty members Drs. E. Grimm, R. Lotan, and M. C. Hung, respectively. Immortalized human mammary epithelial cell line 76N-E6 was obtained from Dr. K. Keyomarsi of M. D. Anderson. Panc28 wild type and $I\kappa B\alpha^{SR}$-expressing cell lines and $IKK2^{-/-}$, $RelA^{-/-}$, and corresponding wild type mouse embryo fibroblasts (MEFs) were obtained from Dr. P. Chiao (M. D. Anderson). HCT-116 wild type and $p53^{-/-}$ colon cancer cell lines were obtained from Dr. B. Vogelstein (The Johns Hopkins University School of Medicine). ZR-75-1 cells were propagated in RPMI-1640 medium supplemented with 10% FBS; 76N-E6 cells were propagated in DFCI-1 medium as described previously (Band *et al*, 1991), and the remaining cell lines were propagated in Dulbecco's modified Eagle's medium containing 10% FBS. Cells were plated at a density of 10^6 cells per 10-cm dish and incubated for 36- to 48 h, depending on the cell line used, which resulted in 70-80% cell confluency in the dishes (~2×10^6 cells). The medium was replenished just before cytokine or drug addition, and the cells were incubated for the periods of time indicated in the figure legends.

Antibodies, cytokines, and drugs. HDAC1 (sc-8410), HDAC3 (sc-11417), p53 (sc-126), and poly(ADP-ribose) polymerase (sc-7150) antibodies were from Santa Cruz Biotechnology Inc. (Santa Cruz, CA); β-actin (A-5441) antibody was from Sigma (St. Louis, MO); HDAC2 (05-814), HDAC8 (07-505), acetyl histone H3 (06-599), and histone H3 (06-755) antibodies were

from Upstate Cell Signaling Solutions (Charlottesville, VA); p21 (#2946) and ubiquitin (#3936) antibodies were from Cell Signaling Technology (Beverly, MA); and paxillin (MS-404) antibody was from NeoMarkers (Fremont, CA). TNFα was purchased from Biosource International (Camarillo, CA), LPS was obtained from Sigma, and IL-1β was a gift from Dr. E. Grimm. MG-132, Z-VAD-FMK, and cycloheximide were obtained from Sigma, and BMS-345541 from Calbiochem (San Diego, CA).

Western blotting. Whole cell extracts were made with Nonidet P-40 lysis buffer (50 mM Tris-Cl pH 7.4; 150 mM NaCl; 1% Nonidet P-40; 1 mM NaF; 1 mM Na_3VO_4; 1 mM phenylmethylsulfonyl fluoride, and 10 μl/ml protease inhibitor cocktail [Sigma]). Nuclear and cytoplasmic extracts were made following the procedure outlined by Schreiber *et al.* (1989). The protein concentrations of the extracts were determined using the Bio-Rad Protein Assay reagent. Fifty micrograms of the extracts were loaded onto sodium dodecyl sulfate-10% polyacrylamide gels, electrophoretically resolved, transferred to nitrocellulose membranes, and blotted with the appropriate antibodies, and the respective proteins were detected using a chemiluminescence detection kit (SuperSignal; Pierce, Rockford, IL).

Real-time reverse transcriptase-polymerase chain reactions. Total RNA from the cells was extracted using the RNAqueous kit (Ambion, Austin, TX). The RNA was quantified by ultraviolet spectrometry and complementary DNA was synthesized from the RNA samples using the RETROscript kit (Ambion). To quantify the levels of the gene transcripts under study, real-time reverse transcriptase-polymerase chain reaction (RT-PCR) experiments were performed in a Bio-Rad iCycler using the complementary DNA templates and TaqMan probe-based gene expression assay primer-probe mixes (Applied Biosystems Inc., Foster City, CA). The specific primer-probe mixes used were catalog nos. Hs006062 for HDAC1, Hs00355782 for p21,

Hs00153349 for p53, and Hs99999905 for GAPDH. The PCR conditions for each of the amplicons were optimized using serial dilutions of the control template, and efficiencies of the reactions were established. The fold changes in the treated samples compared to controls was quantitatively determined using the following formula:

Fold change= $(\text{Efficiency})^{\delta Ct}$

where δCt is the threshold cycle difference between the control and treated samples already normalized against GAPDH.

Pulse-chase protein stability determinations. ZR-75-1 cells were incubated in 4 ml of methionine- and cysteine-free medium (Invitrogen, Carlsbad, CA) containing 5% dialyzed serum for one hour. ^{35}S-labeled methionine (30 μCi/ml) was then added and the incubation continued for an additional hour. The radioactive medium was then removed and the cells washed twice with PBS before addition of fresh RPMI medium containing serum and 2 mM each of methionine and cysteine. Where indicated, cells were treated at this time with either 20 ng/ml TNFα or 5 μg/ml cycloheximide. Cells were further incubated for 0, 0.5, 1, 2, 4, and 6 h before washing with ice-cold PBS and lysis in modified RIPA buffer. Cell lysates were precleared with protein G agarose (Roche Applied Science, Indianapolis, IN) containing a non-immunogenic control antibody for 30 min. Afterwards the supernatants were incubated with an HDAC1 antibody for 12 h at 4°C in a rotary shaker. The immunocomplexes were trapped with protein G agarose, washed thrice with PBS containing protease inhibitors, extracted with 2X Laemmli sample buffer, and resolved by SDS-PAGE. The radiolabeled proteins were transferred onto nitrocellulose membranes and visualized using a Storm 840 PhosphorImager (Amersham Biosciences, Piscataway, NJ).

siRNA transfections. Cells were seeded in six-well plates 24 h before transfection to achieve 40% confluence. Before siRNA transfection, the cells were washed once with serum-free medium and replenished with 0.8 ml of serum-free medium. The cells were transfected with 0.2 ml of a transfection mixture consisting of serum-free medium, 20 nM siRNA, and 4-μl lipofectamine-2000 transfection reagent. The scrambled control siRNA and HDAC1 and IKK2 siGENOME SMARTpool siRNA reagents were obtained from Dharmacon (Lafayette, CO). After 5 h of incubation, 1 ml of medium containing 20% serum was added. The incubation was continued for 48 h, with the medium being changed every 24 h. After this incubation, the cells were treated with 20 ng/ml TNFα for 4 h, and whole-cell extracts were obtained.

Chromatin immunoprecipitation. Chromatin immunoprecipitation (ChIP) assays were performed by using the Upstate ChIP kit according to the manufacturer's instructions with slight modifications. Briefly, cells were treated with TNFα for the specified times, and histone-DNA cross-links were induced with a 15-min treatment with 1% formaldehyde. The cells were lysed in an SDS lysis buffer in presence of a protease inhibitor cocktail (Sigma) and phenylmethylsulfonyl fluoride. The DNA was sheared to appropriate lengths using three sets of 10 s pulses at 25% maximum power in a 100 W sonicator (Microson; Misonix Inc, Farmingdale, NY) equipped with a 2 mm tip. The sonicated samples were cleared by centrifugation, and the supernatants were diluted 10-fold in a ChIP dilution buffer. The diluted supernatants were precleared with a Protein A agarose/salmon-sperm DNA slurry. The supernatants were centrifuged to remove the agarose slurry, and the immunoprecipiting antibodies were added; the supernatants were incubated at 4 °C overnight with rotation. Samples were also incubated with a nonimmunogenic control antibody (Santa Cruz). The antibody complexes were captured with a Protein A agarose/salmon sperm DNA slurry, washed according to standard protocols and eluted

in a buffer containing 1% SDS and 0.1 M $NaHCO_3$. The histone-DNA cross-links were reversed with 5 M NaCl at 65 °C for 4 h and proteinase K treated for 1 h at 37 °C, and the DNA was recovered by phenol/chloroform extraction followed by ethanol precipitation in the presence of yeast transfer RNA. The pellets were washed once in 70% ethanol and dissolved in Tris-EDTA buffer. PCR amplification of the Sp1 binding region of the p21 promoter was carried out with the primers sp1A-p21 (5'-CAGCGCACCAACGCAGGCG-3') and sp1B-p21 (5'-CAGCTCCGGCTCCACAAGGA-3') for 20 cycles. The amplified products were run on a 2% agarose gel, and the values were quantitated by using an Alpha Innotech (San Leandro, CA) gel analysis system.

REFERENCES

Band V, De Caprio JA, Delmolino L, Kulesa V, Sager R (1991) Loss of p53 protein in human papillomavirus type 16 E6-immortalized human mammary epithelial cells. *J Virol* **65:** 6671–6676

Schreiber E, Matthias P, Muller MM, Schaffner W (1989) Rapid detection of octamer binding proteins with 'mini-extracts', prepared from a small number of cells. *Nucleic Acids Res.* **17:** 6419

Fig S1 | HDAC1 protein depletion occurs in multiple cell types after treatment with TNFα. Breast carcinoma (MCF-7, SK-BR-3), melanoma (MeWo), non-small cell lung carcinoma (NCI-H1792, NCI-H522) and ovarian epithelial carcinoma (SK-OV-3.ip1) cell lines were treated with 20 ng/ml recombinant human TNFα for the indicated times and then harvested. Shown are western blots of SDS-PAGE-resolved whole-cell extract proteins from these cells probed with HDAC1 and β-actin antibodies and visualized by chemiluminescence.

Fig S2 | HDAC1 protein depletion by HDAC1 siRNA upregulates p21 protein in $p53^{-/-}$ cells. Normal HCT-116 colon cancer cells or those containing a homozygous deletion of the p53 gene were treated with 20 nM control or HDAC1 siRNA for 48 h, as indicated, and then harvested. Shown are western blots of whole cell extract proteins probed with HDAC1, p21, and β-actin antibodies.

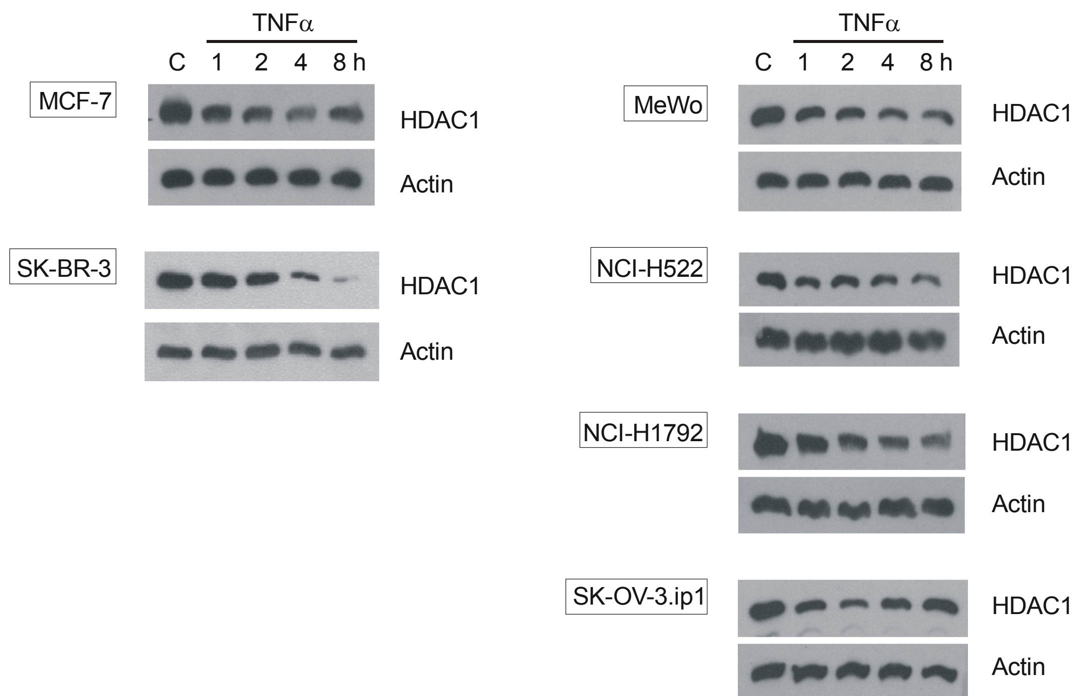
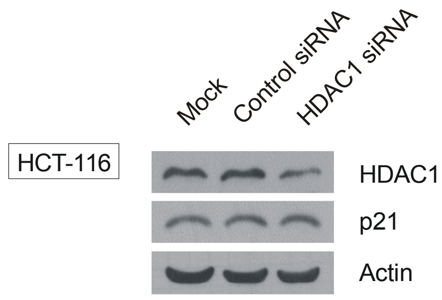


Fig S1



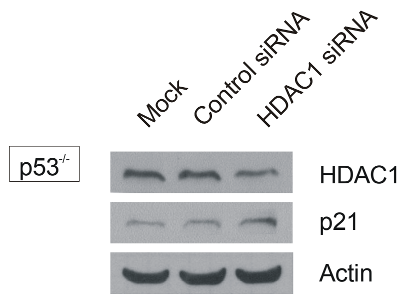


Fig S2