MATERIALS AND METHODS

Cell Culture and Transfection

HeLa, U2OS, Saos-2, and 293T cells (American Type Culture Collection, Manassas, VA) were cultured in Dulbecco’s modified Eagle’s medium (Invitrogen, CA). U2OS cells were maintained in McCoy’s 5A medium (Invitrogen, CA) with 10% fetal bovine serum (HyClone, Logan, UT) and 100 units/ml penicillin plus 100 μg/ml streptomycin at 37 °C with 10% CO₂.

For transfection treatment, cells were plated in 24-well plates (30%-50% confluence), cultured 24 h and transfected with Lipofectamine2000 (Invitrogen, CA) according to the manufacture’s instruction. In UV-irradiation experiments, cells were washed with PBS and irradiated as a monolayer with UV-C rays on a UV crosslinker (100 J/m²; Hoefer). Culture medium was added immediately after irradiation.

Affinity Protein Complex Isolation

The TIP60 complex was immunoprecipitated from nuclear extracts prepared from UV-irradiated (100 J/m² for 40 min) and control 293T cells using M2 anti-FLAG agarose (Sigma Chemical Co., MO) for 4 hr with rotation. After an extensive wash with 0.15 M KCl-containing buffer B (20 mM Tris-HCl, pH 8.0, 5 mM MgCl₂, 10% glycerol, 1 mM PMSF, 0.1% Tween 20, 10 mM β-mercaptoethanol), the bound proteins were eluted from M2 agarose by incubation for 60 min with the FLAG peptide (Sigma Chemical Co., MO) in the same buffer (0.2 mg/ml). The eluates were fractionated on a 5-15% gradient SDS-PAGE gel and visualized by Coomassie blue staining. Individual bands were cut from the stained gel, then in-gel-digested by trypsin, samples were then analyzed by LC-MS/MS as recently described (e.g., Zhou et al., 2003; Fang et al., 2006).
Proteomic analysis of TIP60 protein complex from UV-irradiated cells

The TIP60 complex was immunoprecipitated from nuclear extracts prepared from UV-irradiated 293T cells and fractionated on a SDS-PAGE gel. The protein bands were visualized by Coomassie blue staining and removed for in-gel digestion by trypsin. In-gel digestion was done essentially as described (e.g., Fang et al., 2006). Briefly, individual protein band was excised, chopped into small fragments with a razor blade, destained, and subjected to digestion by modified porcine trypsin (50–100 ng/digestion; Promega, Madison, WI). Peptides were recovered by three extractions of the digestion mixture with 50% acetonitrile plus 5% trifluoroacetic acid and desalted and concentrated using C18 ZipTips (Millipore Corp., Bedford, MA), eluting peptides in 50% (v/v) acetonitrile/water.

LC-MS/MS analyses were performed using Finnigan LTQ-FT mass spectrometer (Thermo Electron) via a 1100 nano-HPLC interface (Agilent Technologies). The peptide samples were loaded onto a CapTrap (Microm) peptide trap at 0.5 ml per minute for 10 min. The sample was washed for 15 minutes with a solution of 5% acetonitrile, 0.1% formic acid. A gradient was then delivered from 10% to 80% acetonitrile over 60 min. This eluted the peptides off of the pre-column onto a 10 cm long Agilent 300SB C18 analytical column (0.075 x 100 mm) at a flow of 1 µl per minute. The column eluates were directly introduced into the LTQ by nanospray. Both the wash and the gradient were delivered using an Agilent 1100 series binary pump, and the gradient was followed by a cleaning and equilibration step.

Mass spectrometric data analyses
System control and data collection were done by Xcalibur software version 1.4 (Thermo). Raw mass spectra were converted to DTA peak list using BioWorks Browser 3.3 (ThermoFinnigan, San Jose, CA) with the following parameter settings: peptide mass range 300-5000 Da, threshold 10, precursor mass ±1.4 Da, group scan 1, minimum group count 1, minimum ion count 15. Searches were conducted using SEQUEST and MASCOT against human IPI protein database (version 3.17). It was specified that peptides should have a maximum of two internal cleavage sites with cysteine carbamidomethylation as fixed modification. SEQUEST searches specified that peptides should possess at least one tryptic terminus, and used peptide mass tolerance of ±1.4 Da and a fragment ion tolerance of 0. MASCOT searches specified tryptic digestion, and used a peptide mass tolerance of ±1.5 Da and a fragment ion tolerance of ±0.1 Da. Peptide and protein identification probabilities for both searches used following criteria for acceptable protein ID: Xcorr > 1.5 for peptides; consensus score >15.0 for protein. Analysis of MS/MS data was also performed by searching against the NCBI non-redundant database, proteins with at least one unique “identity” score peptide were considered as being unambiguously identified.

**siRNA design and experimentation**

The target sequence for siRNA against Ubc9 and TIP60 are as follows: Ubc9, 5’-GAAGUUUGCGCCCUCAUAA-3’ (Kim et al., 2006); TIP60 5’-ACGGAAGUGGAGGGGUU-3’ (siRNA#1; Legube et al., 2004) and 5’-AAGAAGAUCCAGUUCCCAAGTT-3’ (siRNA#2). The 21-mer oligonucleotide RNA duplexes were synthesized by Dharmacon Research, Inc. (Boulder, CO). In the trial experiments, different concentrations of siRNA duplexes were used for different time intervals as detailed previously (e.g., Fang et al., 2006). In brief, HeLa or U2OS cells were
synchronized and transfected with 21-mer siRNA oligonucleotides or control scramble oligonucleotide, and the efficiency of siRNA-mediated protein suppression was judged by Western blotting analysis.

**DNA construction**

To generate GFP-tagged/YFP-tagged full-length TIP60, TIP60 cDNA was digested with EcoR I and BamH, and then cloned into pEGFP-C3/pEYFP-C1 vector (Clontech, CA). TIP60 cDNA with BamH I and EcoR I digestion was cloned into pcDNA-3XFLAG vector (Invitrogen, CA) to generate FLAG-TIP60. The bacterial expression constructs of TIP60 were cloned into pEGX-5X-3 (Amersham Biosciences, NJ). His-SUMO1 was a gift from Michael Brandeis, whereas SUMO1 cDNA was cloned into pGEX-2T (Amersham Biosciences, NJ) with BamH I, and cloned into RFPC1 with Xho I and BamH I. pGEX-4T Ubc9 was obtained by RT-PCR from HeLa cDNA library, and then cloned into HA vector with EcoR I and Xho I or pGEX-2T with BamH I and EcoR I. TIP60 and SUMO1 fusion proteins were obtained by inserting SUMO1 with BamH I digestion.

**Purification of His₆-tagged SUMO1-TIP60 conjugates**

Twenty-four hours after transfection (with or without UV exposure and 4 h recovery), cells were lysed in 4 ml of 6 M guanidinium-HCl, 0.1 M Na₂HPO₄/NaH₂PO₄, 0.01M Tris-HCl, pH 8.0 plus 5 mM imidazole and 10 mM β-mercaptoethanol per 75 cm³ flask. To reduce viscosity, the lysates were mixed with 50 μl of Ni²⁺-NTA-agarose beads pre-washed with lysis buffer and incubated for 2 h at room temperature. The beads were successively washed with following: 6 M guanidium-HCl, 0.1M Na₂HPO₄/NaH₂PO₄, 0.01 M Tris-HCl, pH 8.0 plus 10 mM β-mercaptoethanol (lysis buffer without
imidazole); 8 M urea, 100 mM Na$_2$HPO$_4$/NaH$_2$PO$_4$, 0.01 M Tris-HCl, pH 8.0, 10 mM β-mercaptoethanol (buffer A); 8 M urea, 0.1 M Na$_2$HPO$_4$/NaH$_2$PO$_4$, 0.01 M Tris-HCl, pH 6.3, 10 mM β-mercaptoethanol (buffer B) plus 0.2% Triton X-100; buffer B and then buffer B plus 0.1% Triton X-100. After the last wash with buffer B, the beads were boiled in sample buffer and western blotting was performed with an anti-GFP antibody.

**Histone Acetyltransferase (HAT) Activity Assay**

TIP60 immunoprecipitates were prepared and washed twice in HAT assay buffer (50 mM Tris, pH 8.0, 10% glycerol, 0.1 mM EDTA, 1 mM DTT), and incubated in 60 µl of HAT assay buffer containing acetyl-CoA (100 µM) and biotinylated histone H4 peptide (0.5 µg; Abcam, UK) for 30 min at 30 °C. An aliquot of the reaction was immobilized onto streptavidin plates and acetylation detected by using a HAT ELISA according to the manufacturer's instructions (Upstate Biotechnology, NY).

**Immunofluorescence Microscopy**

Cells were grown on acid-treated glass coverslips. After transfection or drug treatment, cells were washed twice then fixed in 3.7% paraformaldehyde for 3 min. Permeabilization was carried out for 5 min with 0.1% Triton X-100 in PBS at room temperature. After washing 3 times with PBS, cells were blocked with 1% BSA (Sigma Chemical, MO) in PBS containing 0.05% Tween-20 for 30 min, and then incubated with primary antibodies for 1 h at room temperature or overnight at 4°C followed by secondary antibodies for 30 min. Slides were examined under a Zeiss Axiovert-200 fluorescence microscope (Carl Zeiss, Germany), and images were collected and processed with Adobe Photoshop 7.0 software.
**Live Cell Imaging**

HeLa cells grown on cover slips were transfected with GFP-TIP60. Thirty-six hours post-transfection, cells were exposed to UV (100 J/m$^2$) and real time images were acquiring using Zeiss LSM510 Laser Scanning Microscope (Carl Zeiss, Germany) at intervals of 10 minutes.

**Immunoprecipitation**

293T cells were grown to ~50% confluency in McCoy’s 5A medium with 10% fetal bovine serum at 37 °C in 10% CO$_2$ and were transfected with GFP-tagged plasmids or GFP alone using CaPO$_4$ method. Cells were collected after 36 h transfection with 4h recovery. Proteins were solubilized in lysis buffer (50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 5 mM EDTA, 0.5%NP-40, 1 mM phenylmethylsulfonyl fluoride, 10 μg/ml leupeptin, and 10 μg/ml pepstatin A). Lysates were clarified by centrifugation at 16,000g for 10 min at 4 °C. GFP-tagged fusion proteins were incubated with anti-GFP monoclonal antibody bound to protein-A/G beads (Pierce, IL). Beads were washed five times with lysis buffer and then boiled in protein sample buffer for 3 min. After SDS-PAGE, proteins were transferred to nitrocellulose membrane. The membrane was incubated with antibodies against the GFP epitope and tubulin, respectively. Immunoreactive signals were detected with ECL kit (Piece, IL) and visualized by autoradiography on Kodak BioMax film.

**Antibodies**

The antibodies against TIP60, and acetyl-Histone H2A were purchased from Upstate (Lake Placid, NY). The antibodies against phospho-p53 (phospho-Ser$^{20}$, 1:1,000), non-phosphorylated
form of p53 (1:1,500), phospho-ATR (phospho-Ser\textsuperscript{428}, 1:500), non-phosphorylated form of ATR, phospho-Chk1 (phospho-Ser\textsuperscript{345}, 1:1,000) and non-phosphorylated Chk1 were obtained from Cell Signaling (Beverly, MA; 1:1,000). The antibodies against p21 and GFP were purchased from BD Biosciences (San Diego, CA; 1:1,000). The mouse antibody against α-tubulin (DM1A) was purchased from Sigma Chemical Inc (St. Louis, MO; 1:5,000). The mouse antibody against Ubc9 (1:1,000) and rabbit antibody against SUSP1 were purchased from ABGENT (San Diego, CA; 1:1,500). The antibody against SUMO-1 was obtained from Zymed (San Francisco, CA; 1:500; Fu et al., 2005). The antibody against PML was purchased from Chemicon (San Francisco, CA; 1:500).

**Flow Cytometry**

For cell cycle analysis, adherent cells were trypsinized and pooled with the floating cells. Cell suspension of $1 \times 10^6$ cells was washed with PBS, fixed in ice-cold 70% ethanol at -20 °C, and stained with a 20 µg/ml propidium iodide, 0.1% Triton X-100, 200 µg/ml RNase A solution. Stained cells were analyzed using a FACScan (BD Biosciences, CA) and Modfit 2.0.
Supplemental Table 1. Proteins selectively bound to TIP60 in UV-irradiated cells identified by mass spectrometry

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Proteins bound to FLAG-TIP60, with and without UV irradiation, were fractionated on SDS-PAGE as illustrated in Fig. 1A. Differentially displayed bands from UV-irradiated samples were removed for in-gel digest while the resulting peptides were identified by LC-MS/MS mass spectrometric analyses. The protein names, their predicted molecular mass (MM), their NCBI accession number are indicated. The protein probability score, sequence coverage, sequenced peptides, number of peptide matched and the total number of peptides detected were also listed.
Figure S1. TIP60 dynamics in response to UV irradiation. 293T cells grown on coverslips were exposed to UV irradiation, fixed, permeabilized, and stained for TIP60 and DAPI. Bar: 10 µm.
Figure S2. TIP60 dynamics in response to UV irradiation is independent of p53. SASO2 cells grown on coverslips were exposed to UV irradiation, fixed, permeabilized, and stained for TIP60, p53 and DAPI. Arrows indicate PML nuclear body. Bar: 10 µm.