Supplemental Experimental Procedures

Description of UV Sources

The UVA source was a Philips TL100W/10R system from Ultraviolet Resources International (Lakewood, OH) consisting of a Magnetek transformer number 799-XLH-TC-P, 120 V, 60Hz and six bulbs, each 6 feet long. UVA irradiation filtered through 6 mm of glass, eliminating UVB and UVC light, was performed on cultured cells. The UVB irradiation was carried out in a chamber with a transluminator emitting UVB light protons and fitted with an Eastman Kodak Co. Kodacel K6808 filter that eliminates all wavelengths below 290 nm. The UVC radiation was from germicidal lamps.

ChIP Assay

For chromatin immunoprecipitation (ChIP), experiments were performed with the Chromatin Immunoprecipitation (ChIP) Assay Kit (Upstate Biotechnology, Inc., Lake Placid, NY) according to the instructions from the manufacturer. Briefly, JB6 cells were seeded in 15-cm dishes and were starved in 0.1% FBS/MEM for 24 h and then exposed to UVA (80 kJ/m^2). After UVA exposure, histones were crosslinked to DNA by adding 1% formaldehyde in the medium. Cells were washed twice using cold PBS containing 1 mM PMSF, 1μg/ml aprotinin and 1μg/ml pepstatin. Cells were scraped and pelleted for 4 min at 2000 rpm at 4^oC. The pellet was resuspended in SDS lysis buffer (provided in the kit) containing 1 mM PMSF, 1μg/ml aprotinin and 1μg/ml pepstatin. The lysate was sonicated to shear the DNA and the sample was centrifuged for 10 min at 13,000 rpm at 4^oC and pellet was discarded. The sonicated supernatant fraction was diluted 10-fold in

ChIP dilution buffer (provided in the kit). The diluted supernatant fraction was pre-cleared with protein A agarose/salmon sperm DNA (provided in the kit). The agarose was pelleted and the supernatant fraction was collected. This fraction was also used as input for western blot. The H2AX antibody was added to the supernatant fraction and incubated overnight at 4^{o}C. Then protein A agarose/salmon sperm DNA (provided in the kit) was added and mixture was incubated for 1 h at 4^{o}C to collect the antibody/histone complexes. The agarose was pelleted and the protein A agarose/antibody/histone complexes were washed with the buffers provided in the kit. After washing, immunoprecipitated histones and histone-bound protein complexes were analyzed by immunoblot with JNK or pJNK, H2AX or γH2AX antibodies.

Kinase Assays

In brief, UVA-treated cells were disrupted as described earlier and the cell lysates were clarified by centrifugation at 16, 000xg for 10 min at 4 ^{o}C. The supernatant fractions were incubated with a JNK antibody (Upstate Biotechnology, Inc.) at 4 ^{o}C overnight and then for an additional 4 h with protein A/G-Sepharose beads (Santa Cruz Biotechnology). After washing 3 times with cell lysis buffer, the immunoprecipitates were divided into two parts. One part was mixed with 1 μg histone H2AX protein, 0.2 mM ATP and 1x kinase buffer and the other part mixed with 1 μg GST-c-Jun, 0.2 mM ATP, 1 x kinase buffer, and then incubated at 30 ^{o}C for 30 min. Samples were separated by SDS-PAGE followed by western blot analysis with antibodies against γH2AX, H2AX, p-c-Jun (Ser63), GST and JNK (Upstate Biotechnology, Inc.) to detect the amount of JNK that had been immunoprecipitated by JNK antibody, respectively, in each reaction.

Mammalian Two Hybrid Assay

To identify the binding domain *in vivo*, we used the mammalian two-hybrid assay as described previously (Choi et al., 2005). In brief, NIH3T3 cells (1.5×10^4) were seeded into 48-well plates and incubated with 10% FBS-DMEM for 18 hrs before transfection. The DNAs, pACT-H2AX, pBIND-JNK1 or deletion mutants (Choi et al., 2005), and the pG5-luciferase reporter plasmid, were combined in the same molar ratio and the total amount of DNA was not more than 100 ng/well. The transfection was performed using Lipofectamine Plus Reagents (Invitrogen) following the manufacturer's recommended protocols. The cells were disrupted by addition of 100 μl of cell lysis buffer directly into each well of the 48-well plate at different time points. Aliquots (50 μl) were added to each well of a 96-well plate and the luminescence activity for firefly luciferase activity was measured automatically by computer program (MTX Lab, INC, Vienna, VG). The relative luciferase activity was calculated and normalized based on the pG5-luciferase basal control. For assessment of transfection efficiency for the mammalian two-hybrid assay, the renilla luciferase activity assay, which is included in pBIND two-hybrid vector, was used.