Supplementary Data



Supplementary Figure 1: p300 is degraded upon UV irradiation in mouse NIH3T3 cells. NIH3T3 cells were UV irradiated at 10 J/m^2 , and further cultured for various time periods. Whole cell lysates were prepared and the expression levels of p300 were detected using Western blotting.



Supplementary Figure 2: p300 is phosphorylated at S1834 upon UV irradiation by Akt. OSU-2 cells were transfected with either 100 nM Akt siRNA (siRNA SMARTpools, Dharmacon) or control siRNA (Dharmacon) for 48 hr, UV irradiated at 10 J/m², and further cultured for various time periods. Whole cell lysates were prepared and subjected to Western blotting to detect p300 and phosphor-p300 (S1834).



Supplementary Figure 3: Down-regulation of p300 does not have influence on the removal rate of UV-induced CPD and 6-4PP. (A) OSU-2 cells were transfected with either control siRNA or p300 siRNA for 48 or 72 hrs. The expression of p300 was detected using Western blotting. (B, C) The siRNA transfected OSU-2 cells were UV irradiated at 10 J/m², and further cultured for the desired time periods. The genomic DNA was isolated and the same amounts of denatured DNA were applied to nitrocellulose membranes for ISB analysis. UV-induced CPDs (B) and 6-4PP (C) were detected with their corresponding antibodies (MBL International Corporation, Woburn, MA). The intensity of each band was quantified, and the lesion concentrations were determined from a reference standards run in parallel to calculate the relative amounts of DNA lesions remaining at each time point.



Supplementary Figure 4: Down-regulation of p300 inhibited the repair of IR-induced DNA damage. (A) OSU-2 cells growing on coverslips were transfected with 100 μ M of control siRNA or p300 siRNA for 48 hrs, treated with IR at 10 Gy, and further cultured for 1, 6 and 24 hrs. Cells were fixed, permeabilized and processed for immunofluorescent staining to visualize γ H2AX with rabbit anti- γ H2AX antibody (Cell Signaling Technology) and goat anti-rabbit IgG conjugated with Alexa Fluor 594. (B) γ H2AX-positive cells were counted and the percentages of γ H2AX-positive cells were plotted (n=4, bar: SD).



Supplementary Figure 5: p300 and CBP are not required for the removal of UV-induced 6-4PP. (A) OSU-2 cells were transfected with 50 μ M of either control siRNA, p300 siRNA, CBP siRNA or both p300 and CBP siRNA for 48 hrs. Cells were UV irradiated at 10 J/m², and further cultured for various time periods. The genomic DNA was isolated and subjected to immuno-slot blotting to detect the remaining of 6-4PP with anti-6-4PP antibody. (B) The intensity of each band was quantified by scanning images and processing with Alphaimager-2000 software. The relative percentage of remaining 6-4PP at different time points is an average of three independent repeats. (n = 3, Bar: SD).



Supplementary Figure 6: p300 and CBP acetylate histones redundantly. OSU-2 cells were transfected with 50 μ M of either control siRNA, p300 siRNA, CBP siRNA or both p300 and CBP siRNA for 48 hrs. Cells were UV irradiated at 10 J/m², and further cultured for various time periods. Whole cell lysates were prepared by boiling cells in SDS lysis buffer. The same amounts of total protein were subjected to Western blotting to detect acetylated histone H3 at lysine 18 (AcH3K18) and acetylated histone H4 at lysines 5, 8, 12, 16 (AcH4) (Cell Signaling Technology). Histone H3 (Active Motif, Carlsbad, CA) was also detected as loading control.

Supplementary Figure 7: p300 or/and CBP downregulation did not affect the protein expression levels of various NER factors. OSU-2 cells were transfected with 50 μ M of either control siRNA, p300 siRNA, CBP siRNA or both p300 and CBP siRNA for 48 hrs. Whole cell lysates were prepared and subjected to Western blotting to detect the expression levels of various NER factors.

Supplementary Figure 8: CBP protein level does not change upon UV irradiation. OSU-2 cells were UV irradiated at 10 J/m², and further cultured for various time periods. Whole cell lysates were prepared and the expression levels of CBP were detected using Western blotting with anti-CBP antibody (sc-7300, Santa Cruz), which does not recognize p300.

Supplementary Figure 9: Local UV irradiation combined with immunofluorescence failed to show p300 recruitment to UV damage sites. OSU-2 cells growing on coverslips were UV irradiated at 40 J/m² through a 5 μ m micropore filter and further cultured for 0, 30, and 60 min. Cells were fixed and permeabilized with 2% paraformaldehyde and 0.5% Triton X-100, blocked with 20% normal goat serum, and double stained with rabbit anti-p300 (N15, 1:100, Santa Cruz Biotechnology) and mouse anti-CPD antibodies (TDM2, 1:1000, MBL International Corporation, Woburn, MA) as described in Materials and Methods.

Supplementary Figure 10: Chromatin segregation process. (A) A diagram describing the process of chromatin segregation. (B) Samples were digested with MNase at 1, 2 or 4 U/ul for 45 min. DNA in C1 and C2 fractions were extracted with Phenol:Chloroform and precipitated with isopropanol. 2 μ g of DNA was loaded on a 2% agarose gel and visualized by Ethidium Bromide staining.