Supplementary methods

The following primers were used for site-directed mutagenesis:

RFP-PCNA<sup>2KA</sup>: For 5'- GTG AAC CTC ACC AGT ATG TCC GCC ATA CTA GCC TGC GCC GGC AAT-3'; Rev 5'- ATT GCC GGC GCA GGC TAG TAT GCC GGA CAT ACT GGT GAG GTT CAC-3'

RFP-PCNA<sup>2KR</sup>: For 5'- GTG AAC CTC ACC AGT ATG TCC CGG ATA CTA CGG TGC GCC GGC AAT-3'; Rev 5'- ATT GCC GGC GCA CCG TAG TAT CCG GGA CAT ACT GGT TGA GGT GAG GTT CAC-3'

The RFP-PCNA<sup>5KR</sup> mutant construct was obtained from RFP-PCNA<sup>2KR</sup> by using the primers:
For 5'- GGC TCC ATC CTC CGG CGG GTG TTG GAG GCA CTC CGG GAC CTC AAC-3'; Rev 5'- GTT GAT GAG GTC CCG GAG TGC CTC CAA CAC CCG CCG GAG GAT GGA GCC-3'.

Supplementary figures

**Figure S1. Time course analysis and dose dependence of PCNA acetylation.**
(A) Quiescent LF-1 human fibroblasts were irradiated with UV light (10 J/m<sup>2</sup>) and collected at different periods of time after exposure, or (B) irradiated with different doses and collected after 30 min. Whole cell extracts were analyzed by Western blot with PC10 antibody to PCNA. In both panels, the arrows indicate that slower migrating form of PCNA. TSA: cells
were pre-treated with 1 mM trichostatin A (TSA) before UV irradiation. (C) Detection of PCNA with PC10 antibody after immunoprecipitation of acetylated protein with anti-acetyl lysine antibody (4G12) in irradiated LF-1 fibroblasts collected at 8 and 24 h after UV exposure (10 J/m²).

Figure S2. GST-CBP fragments used to study association with purified recombinant or cellular PCNA. Coomassie staining of GST-CBP fragments used in this study: N-terminal (N-term) aa. 1-1098; Br domain (Br), aa. 1081-1197; HAT domain, aa. 1319-1710; CR2 aa. 1894-2221; CR3 aa. 2212-2441.
Figure S3. In vitro and in vivo dependence of PCNA acetylation by CBP/p300.
(A) MS/MS analysis of recombinant PCNA acetylation by purified recombinant p300. MS/MS spectrum of peptide 65-80 is shown with acetylated residue indicated in red.
(B) PCNA acetylation by purified recombinant CBP: MS/MS spectrum of peptide 6-14 is shown, with acetylated residue in red.
(C) PCNA acetylation by purified recombinant CBP: MS/MS spectrum of peptide 14-20 is shown, with acetylated residue in red.
(D) PCNA acetylation by purified recombinant CBP: MS/MS spectrum of peptide 78-91 is shown, with acetylated residue in red.
(E) MS/MS LTQ-Orbitrap spectrum shows detail of acetylation signature ions for acetylated peptide 14-20 AcK14VLEALK. Lysine acetylation marker ions marked in blu. The immonium ion of acetyllysine ([Im], 143.1179 Da) and a derivative that has lost ammonia ([Im - NH3], 126.0913 Da) were reported as MS/MS marker ions for the presence of acetyllysine in this peptide.
(F) Western blot analysis of CBP, p300, PCNA and actin protein levels in human LF-1 fibroblasts after siRNA depletion of CBP and p300 vs non-targeting control siRNA (NT).
(G) Western blot analysis of CBP, p300 and PCNA protein levels in human LF-1 fibroblasts after siRNA depletion of PCAF vs non-targeting control siRNA (NT).
Figure S4. Characterization of PCNA acetylation mutants. (A) Expression and nuclear localization of RFP-PCNA\textsuperscript{wt}, RFP-PCNA\textsuperscript{2KR} and RFP-PCNA\textsuperscript{5KR} in HeLa cells after 24 h from transfection with Effectene reagent. Red fluorescence show RFP-
PCNA proteins, while blue fluorescence show DNA stained with Hoechst 33258 (0.1 µg/ml). Scale bar = 50 µm.

(B) Co-immunoprecipitation of endogenous native PCNA with exogenous RFP-PCNA wt and mutant proteins. Immunoprecipitation from whole HeLa cell extracts was performed with anti-RFP antibody. Samples were analyzed by Western blot with anti-PCNA antibody. Arrows indicate RFP-PCNA and endogenous PCNA, while other bands probably related to endogenous ubiquitinated PCNA are also present.

(C) Soluble (left) and chromatin-bound (right) fractions of RFP-PCNA<sub>wt</sub> vs RFP-PCNA<sub>2KΔ</sub> expressed in HeLa cells. Western blot analysis was performed with anti-PCNA and anti-actin antibodies. Actin is shown as loading control.

(D) BrdU incorporation in HeLa cells expressing RFP-PCNA<sub>2KΔ</sub> mutant protein. HeLa cells grown on microscope slides were transfected with RFP-PCNA<sub>2KΔ</sub> construct and incubated 24 h later with 10 µM BrdU. After immunofluorescence reaction with anti-BrdU antibody (1:50) (Cazzalini et al., 2003), cells positive to RFP fluorescence were analyzed for BrdU incorporation.

(E) UV-induced UDS (unscheduled DNA synthesis) in HeLa cells expressing RFP-PCNA wt and mutant proteins, as detected by counting autoradiographic grains in cells positive to RFP antibody immunostaining (shown as red-brown color). Scale bar = 10 µm.

(F) HeLa cells co-expressing GFP-tagged histone H2B and RFP-PCNA<sub>wt</sub>, or mutant forms RFP-PCNA<sub>2KR</sub> or RFP-PCNA<sub>5KR</sub>, were grown on slides, exposed to UV irradiation (30 J/m²) through polycarbonate filters with 3-µm diameter pores. In situ lysed cells were fixed and immunostained with monoclonal antibody to CPDs (1:3000), followed by anti-mouse Alexa 633 secondary antibody. Confocal microscopy analysis of co-localization of GFP-H2B (green fluorescence) RFP-PCNA (red fluorescence), and CPD (infrared fluorescence rendered as blue signal) is shown by the merged images. Scale bar = 10 µm.

(G) Purified recombinant his-PCNA wt or 5KR mutant form shown by Coomassie staining.

(H) PCNA depletion from S-phase cell extracts by subsequent rounds (I to III) of PCNA pull-down with GST-p21C peptide bound to GHS-agarose beads. Western blot analysis with PCNA monoclonal antibody show the residual PCNA remaining after each pull-down. Actin is shown as loading control.

(I) Extracts of HeLa nuclei showing loading of recombinant his-PCNA<sub>wt</sub> and his-PCNA<sub>5KR</sub> and the mobility shift shown after 2 h-incubation in PCNA-depleted extract. Recombinant PCNA forms were detected by Western blot analysis with antibody to polyHistidine (His). Input show 10% of each recombinant protein.
Figure S5. Influence of PCNA acetylation on its degradation induced by DNA damage, and on its recruitment at DNA damage sites.

(A) Recruitment of chromatin-bound PCNA at local UV-DNA damage sites in human LF-1 fibroblasts after depletion of CBP/p300 by incubation for 48 h in medium containing 20 nM siRNA to CBP and p300, as compared with cells incubated in medium with non-targeting (NT) siRNA. Cells were locally irradiated with UV (30 J/m²) through micropore filters, and then re-incubated for 30 min before in situ lysis to detect chromatin-bound proteins. Immunofluorescence co-staining of both CBP and p300 (green fluorescence) was performed with specific polyclonal antibodies (1:100 each), while PCNA (red fluorescence) was detected with PC10 monoclonal antibody (1:100). The merged images show the nuclear area by DNA staining with Hoechst 33258 dye (blue fluorescence). Scale bar = 10 µm.

(B) Degradation of detergent-soluble form of PCNA after UV-induced (10 J/m²) DNA damage (UV), oxidative damage by potassium bromate (KBr), by alkylation damage with MNNG (MN), or by ionizing radiation with 10 Gy γ-rays (IR).

(C) PCAF siRNA depletion in human LF-1 fibroblasts was obtained after 48 h incubation in medium containing 30 nM PCAF siRNA, vs non-targeting control siRNA (NT). Western blot analysis of whole cell extracts show the levels of PCAF and actin as loading control.

(D) LF-1 human fibroblasts treated for 48 h with 30 nM siRNA to PCAF were UV irradiated (10 J/m²) and collected 4h later to detect the amount of soluble PCNA. Western blot analysis was performed with antibody to PCNA, and to actin, as loading control.
Figure S6. Influence of proteasomal inhibition on PCNA acetylation, accumulation at DNA damage sites, and on its degradation following UV irradiation.

(A) Western blot analysis of CBP, p300 and PCNA protein levels in whole cell extracts from normal (NF), and XPA human fibroblasts.

(B) Detection of acetylated PCNA with PC10 antibody after immunoprecipitation with anti-acetyl lysine antibody (4G12) in S-phase synchronized XPA fibroblasts.

(C) Detection of acetylated forms of PCNA in HeLa cells treated or not, for 15 min with 50 µM MG132, then UV-irradiated (10 J/m²) and re-incubated for 30 min in the same medium before immunoprecipitation with antibody to acetyl-lysine (4G12). Cells were also treated with 100 µM curcumin (Curc) before UV irradiation. Immunoprecipitated complexes were analyzed by Western blot with antibody to PCNA.

(D) Retention of chromatin-bound PCNA at local UV-DNA damage sites in human LF-1 fibroblasts after treatment with 50 µM MG132. Cells were pre-treated for 15 min with MG132 before local UV irradiation (30 J/m²) through micropore filters, and then re-incubated for further 30 min in the same medium, before in situ lysis to detect chromatin-bound proteins. Immunofluorescence staining PCNA (red fluorescence) was performed with PC10 monoclonal antibody (1:100). The merged images show the nuclear area by DNA staining with Hoechst 33258 dye (blue fluorescence). Scale bar = 10 µm.

(E) Western blot analysis of soluble form of PCNA in LF-1 human fibroblasts pre-treated with 50 µM MG132 for 15 min before UV irradiation (10 J/m²) and subsequent re-incubation in the same medium for further 4 h. PCNA was detected with PC10 antibody, and actin was detected as loading control.