Landes Bioscience

www.landesbioscience.com

Supplemental Material to:

Jennifer Chea, Sufang Zhang, Hong Zhao, Zhongtao Zhang, Ernest Y.C. Lee, Zbigniew Darzynkiewicz and Marietta Y.W.T. Lee

Spatiotemporal recruitment of human DNA polymerase delta to sites of UV damage

2012; 11(15)

http://dx.doi.org/10.4161/cc.21280

http://www.landesbioscience.com/journals/cc/article/21280

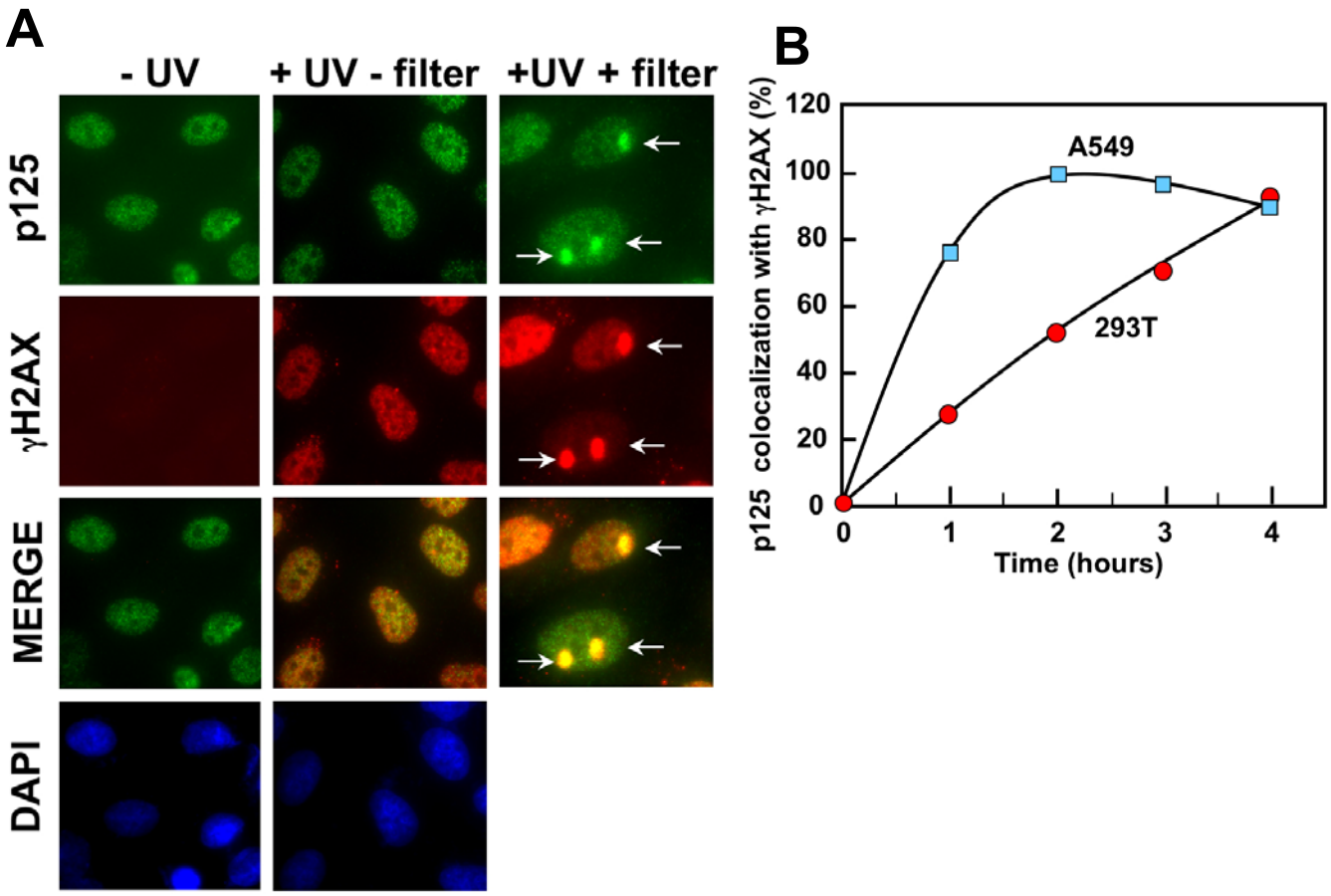

Fig. S1. Recruitment of p125 to local areas of UV irradiation through polycarbonate filters as determined by co-staining with γH2AX Ab.

A. A549 cells were irradiated with UV (75 J/m^2) through a 5 μm pore polycarbonate filter (Materials and Methods) and fixed after four hours. Cells were stained for p125 (green fluorescence), and γH2AX (red). The first column shows representative images for control untreated cells, the center column cells treated with UV, and the third column shows cells irradiated through a 5 μm polycarbonate filter. The arrows indicate the clearly delineated local areas of DNA damage to which γH2AX and p125 were co-localized. **B**. Quantitation of time course of recruitment of p125 to UV induced local areas of DNA damage (macro-foci). A549 and 293T cells were irradiated through polycarbonate filters as in "A" and examined after 1, 2, 3 and 4 hours. Quantitation was performed by counting >100 cells showing γH2AX macro-foci and scoring them for the percent co-localization of p125 (Materials and Methods). Data for A549 cells are shown as blue squares and those for 293T cells are shown as red circles.

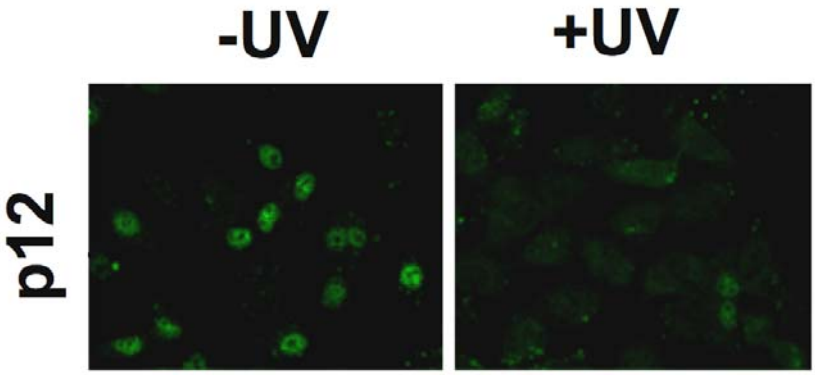

Fig. S2. p**12 is depleted in A549 cells that are globally irradiated with UV**. A549 cells were globally irradiated with 20 J/m^2 UV and fixed for staining for p12 after four hours. After UV treatment, it is seen that essentially all the p12 nuclear fluorescence has disappeared. This data also validates the specificity of the p12 antibody.

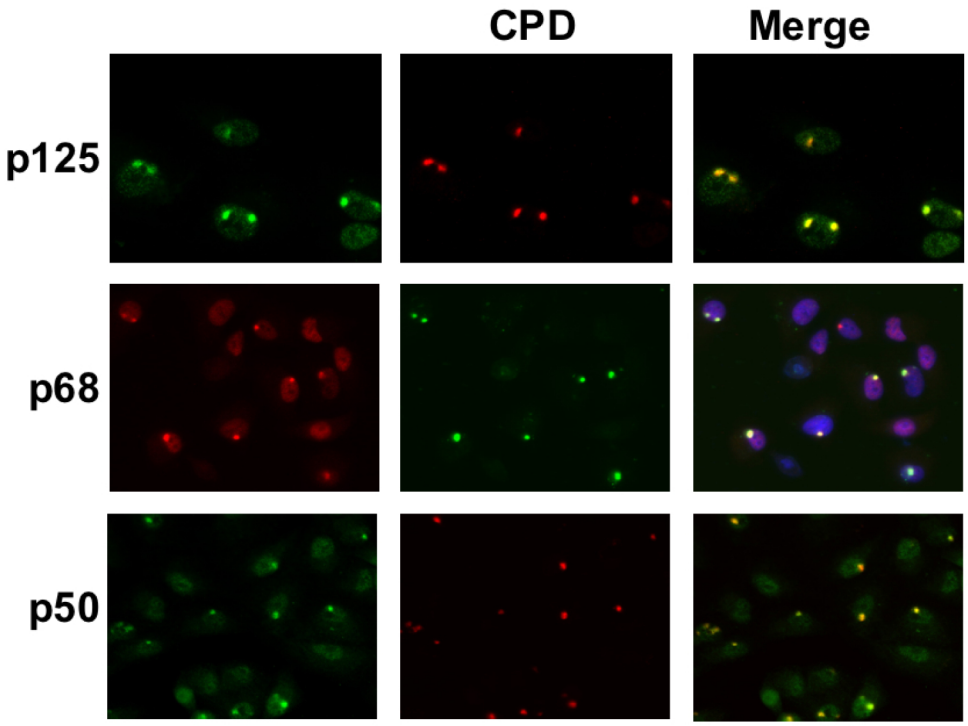

Fig. S3. Co-staining of p125, p68 and p50 with CPDs, The figure shows images from the experiment in Fig. 2. These are representative of the images used for counting the percentage co-localization of Pol δ subunits with CPDs (Fig. 2B). Images in Fig. 2A were taken from these panels.

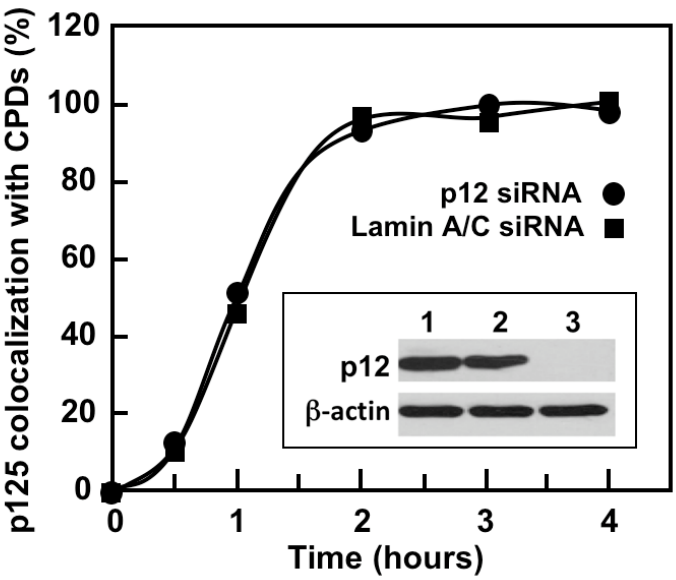

Fig. S4. p**12 is depleted in A549 cells that are globally irradiated with UVC**. p12 was ablated by siRNA and analyzed for co-localization of p125 and CPDs four hours after UV irradiation through membrane filters (p12 siRNA, circles; control lamin A/C siRNA, squares). Inset: Western blots showing the depletion of p12; Lane 1, control cell lysate; lane 2, cells transfected with control lamin A/C siRNA; lane 3, cells transfected with p12 siRNA.