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Supplemental information

Detection of endogenous translesion DNA synthesis in single mammalian cells

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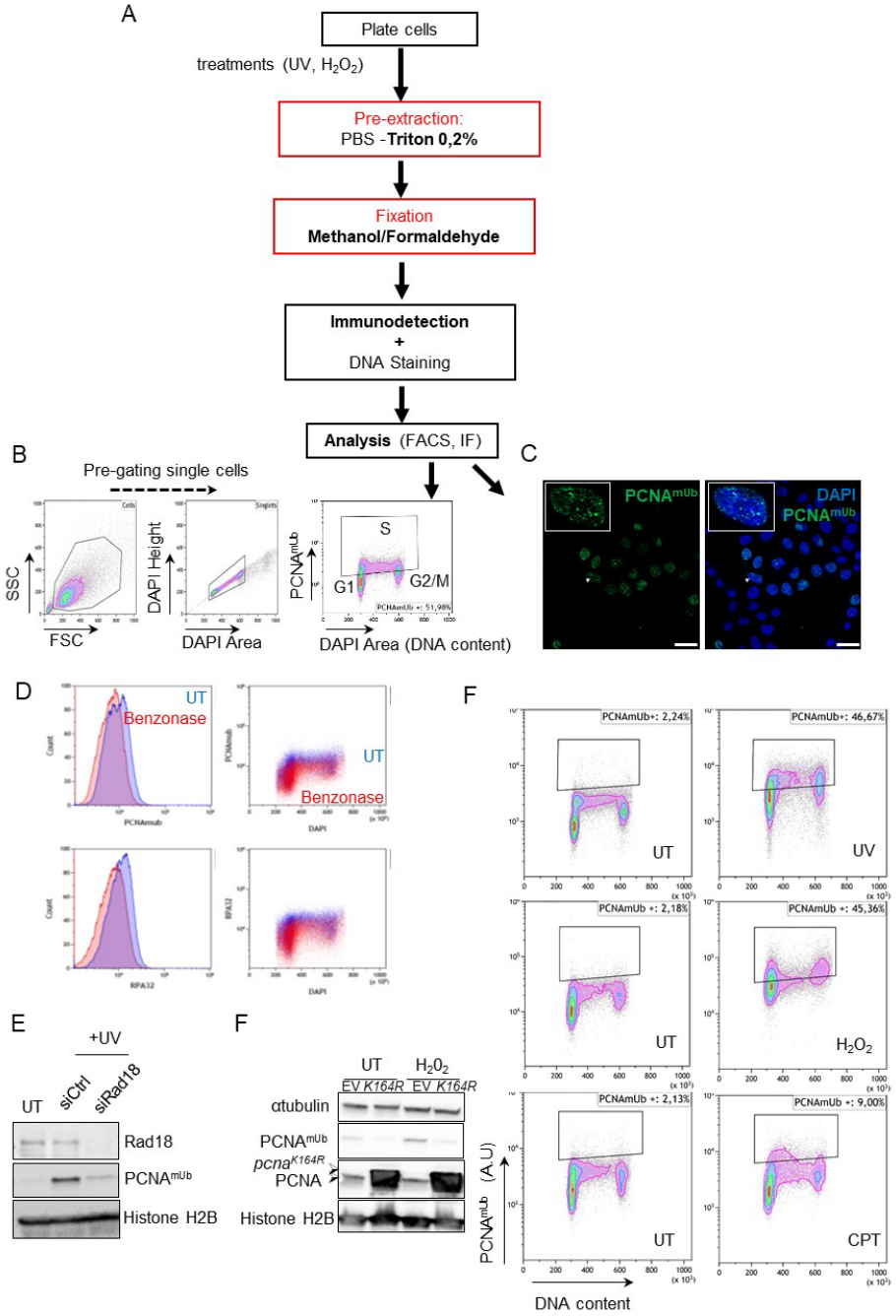

Figure S1: Detection of endogenous TLS components by flow cytometry and immunofluorescence. Related to Figure 1 and 3. A, Workflow for the detection of TLS components by flow cytometry (FACS) or immunofluorescence (IF) with or without treating cells with UV or hydrogen peroxide (H_2O_2). **B**, FSC/SSC graph was used to gate out cellular debris. DAPI area over DAPI height graph was used to gate out doublets. 20,000 events were retained. G1, S and G2 cell cycle phases were displayed on graph based on the DNA content of cells. $PCNA^{mUb}$ fluorescence is plotted against the DAPI fluorescence in HCT116 cells. **C,** Immunodetection of $PCNA^{mUb}$ in untreated HCT116 cells. DAPI was used to detect nuclei. Scale bar: 20 µm**. D,** Quantification of $PCNA^{mUb}$ (upper panel) and RPA2 (lower panel) detected by flow cytometry in HCT116 cells treated with benzonase. UT, untreated. **E,** left panel, Western blot of H1299 cells untreated (UT), or treated with 20 J/m^2 of UV-C light (UV), upon transient Rad18 downregulation by siRNA or treated with control siRNA (siCtrl). Right panel, Western blot of HEK293T whole cell extracts transfected with either empty vector (EV) or a vector expressing the *pcna*^{K164R} mutant, untreated (UT), or treated with hydrogen peroxyde (H_2O_2). n=2. **F,** Quantification of $PCNA^{mUb}$ detected by flow cytometry in HCT116 cells exposed to 20 J/m² of UV-C radiation, 1 mM hydrogen peroxide (H_2O_2), or camptothecin (CPT) described in Fig. 1. UV: n=3; H_2O_2: n=3; CPT: n=2.

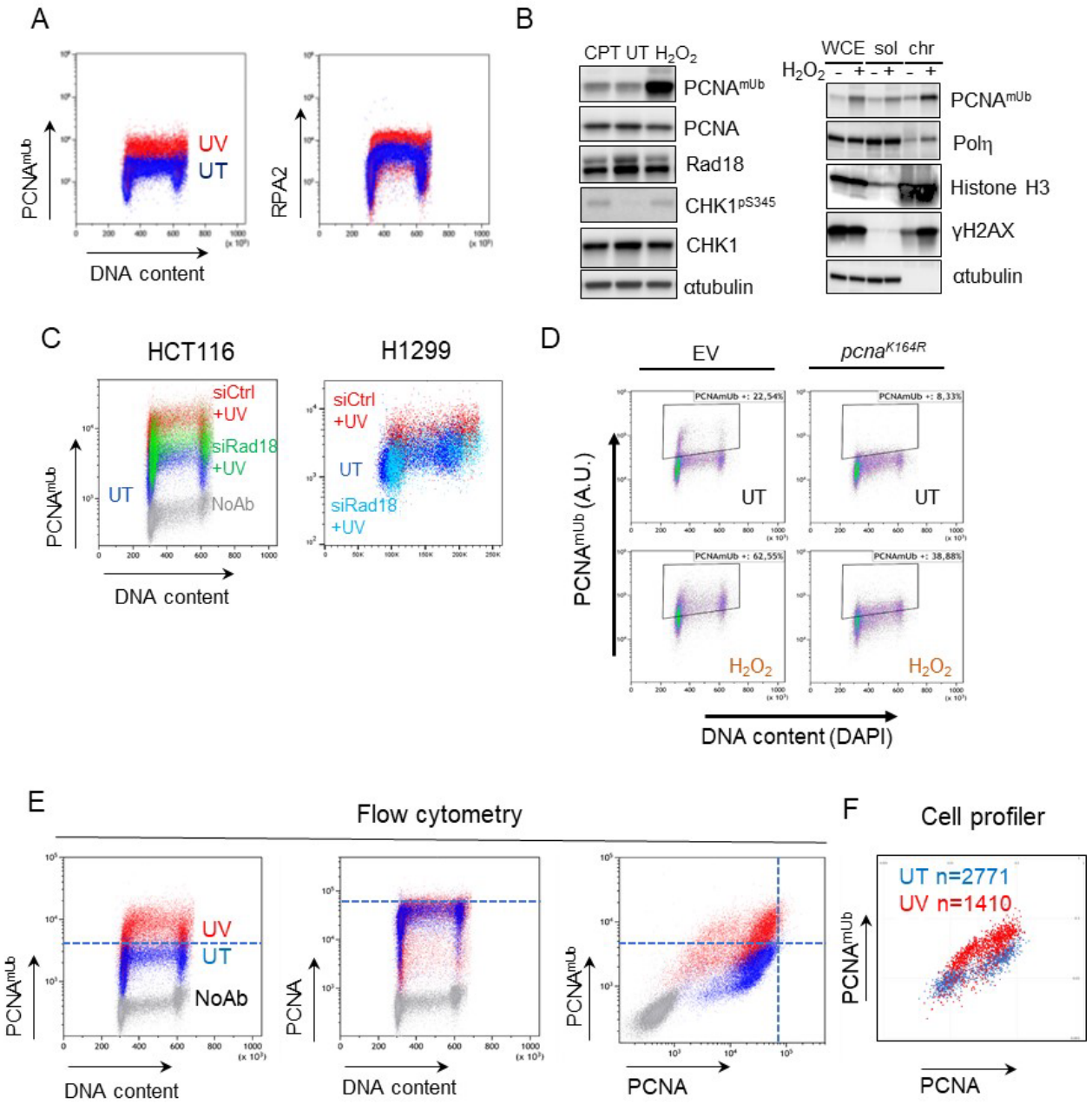

Figure S2: Related to Figure 3. Detection of chromatin-bound PCNA^{mUb} by flow cytometry upon exposure to DNA damaging agents. A, Flow cytometry analysis of either PCNA^{mUb} (left panel) or RPA fluorescence (right panel) in the same HCT116 cells untreated (UT, blue), treated with 20 J/m^2 of UV-C light (UV, red), n=3. **B,** Left panel, Western blot of HCT116 whole cell extracts untreated (UT), treated with camptothecin (CPT) or hydrogen peroxide (H_2O_2). Right panel, western blot of HCT116 whole cell extract (WCE), soluble (sol) and chromatin fractions (chr) obtained in the absence (-) or presence (+) of H_2O_2. A-B, n= 2. **C,** Flow cytometry analysis of PCNA^{mUb} fluorescence in the indicated cell lines untreated (UT, blue), or treated with 20 J/m^2 of UV-C light (UV, red), upon transient Rad18 downregulation by siRNA or treated with control siRNA, n=2. **D,** Flow cytometry analysis of HEK293T cells transfected with either empty vector (EV) or a vector expressing the *pcna*^{K164R} mutant, untreated or treated as indicated. **E-F**, Co-detection of PCNA and PCNA^{mUb} in HCT116 cells untreated (UT, blue) or exposed to 20 J/m² of UV-C (UV, red) by either flow cytometry (E) or IF (F), quantification obtained with the Cell Profiler software. Note that in both case, PCNA^{mUb} and not total PCNA level increases upon exposure to UV-C light, n=3.

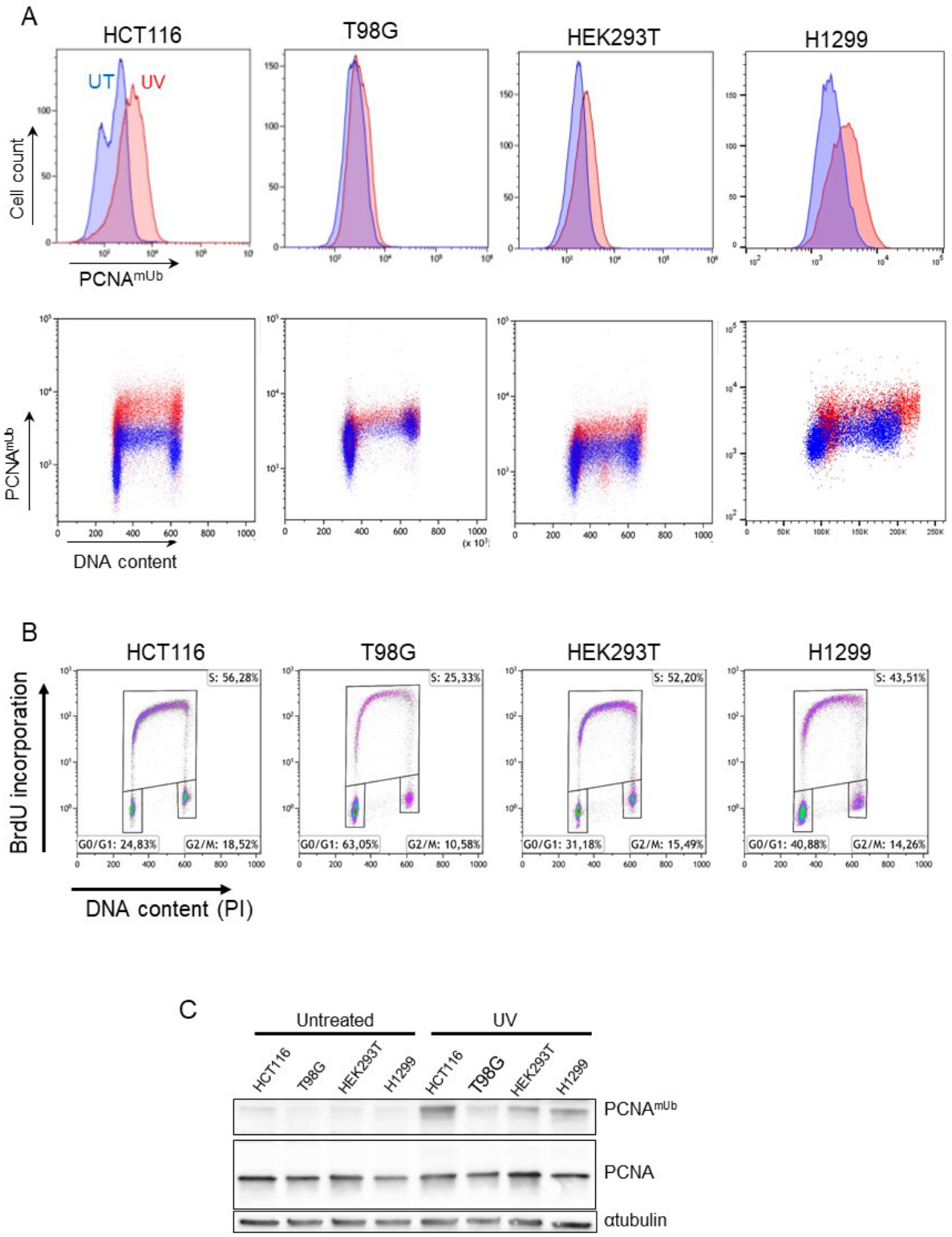

Figure S3: Related to Figure 1. Chromatin-bound PCNA^{mUb} detection by flow cytometry in different cells lines. A, The indicated cell lines were either untreated (UT) or exposed to 20 J/m^2 of UV-C light. PCNA^{mUb} fluorescence was detected by flow cytometry and displayed as presented in Fig 1a. **B,** Cell cycle analysis of the indicated cell lines. The data show a correlation between the level of PCNA^{mUb} and the percentage of cells in S-phase. HCT116: n=3, U87: n=2, HEK293: n=2. **C,** Western blot of whole cell extracts obtained from the indicated cell lines untreated or exposed to 20 J/m^2 of UV-C radiation. n=3.

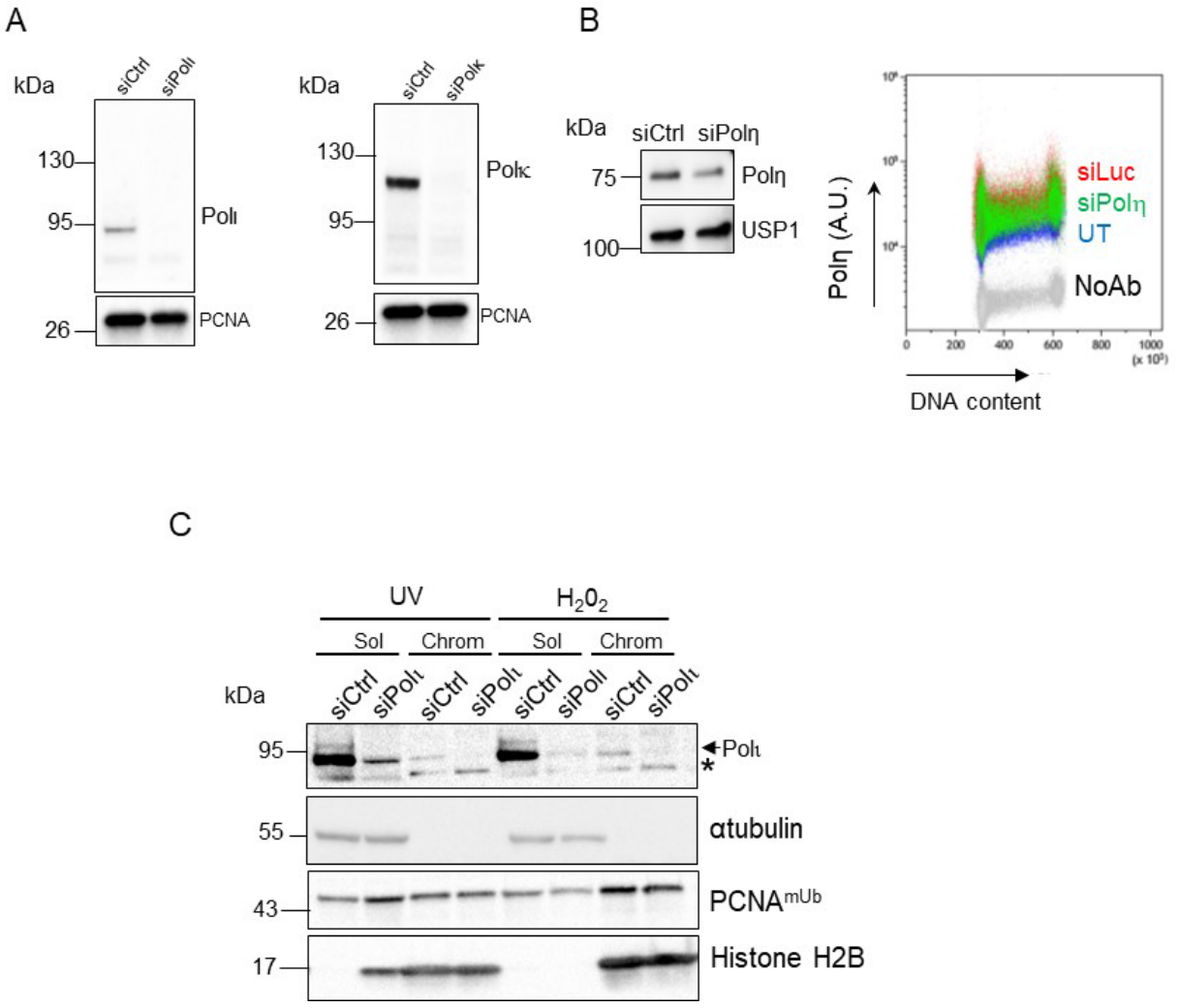

Figure S4: Related to Figure 2. Specificity of Polη and Polκ antibodies. A, Western blot of whole cell extracts obtained from HCT116 cells treated with either control siRNA (Ctrl) or the indicated target-specific siRNA untreated (UT). **B,** left panel, Western blot of chromatin fractions isolated from HCT116 cells treated with either control (siLuc), or Polη-specific siRNA. USP1 is used here as total proteins loading control. Right panel**,** Detection of Polη by flow cytometry in cells treated with the siRNA described in (A), untreated (blue) or exposed to 1 mM H_2O_2 in presence of siLuc (red) or siPolη (green). A sample devoid of primary antibody (No Ab) was included as a control, n=3. **C,** Western blot of HCT116 soluble (sol) and chromatin fractions (chr) obtained upon treatment with either control siRNA (Ctrl) or an siRNA targeting Polι, and exposure to either 20 J/m^2 of UV-C (UV) or 1 mM hydrogen peroxide (H_2O_2). *indicates a non-specific polypeptide recognized by the Polι antibody. n=2.

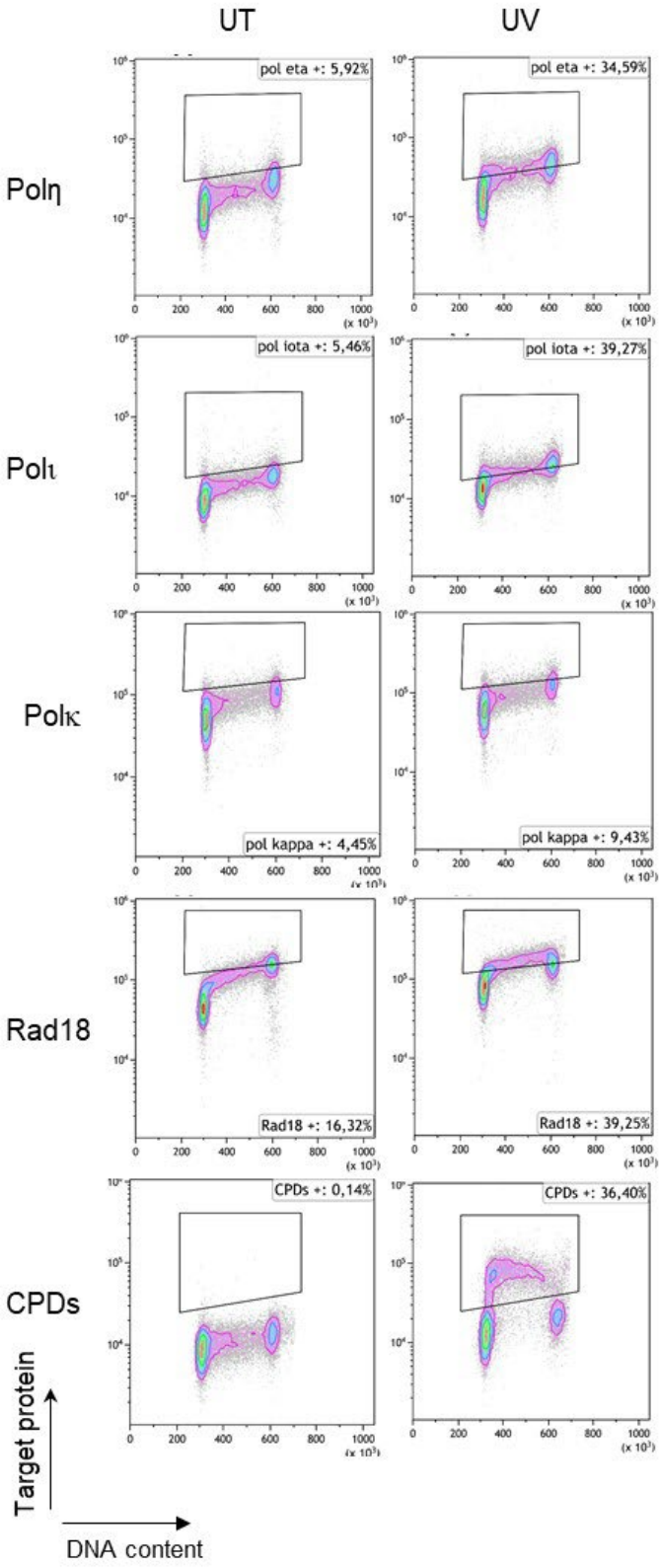

Figure S5: Related to Figure 2. Quantification of TLS components by flow cytometry upon treatment with different DNA damaging agents. Quantification of the indicated TLS targets by flow cytometry in HCT116 cells untreated (UT) or exposed to 20 J/m² UV-C radiation, n=3.

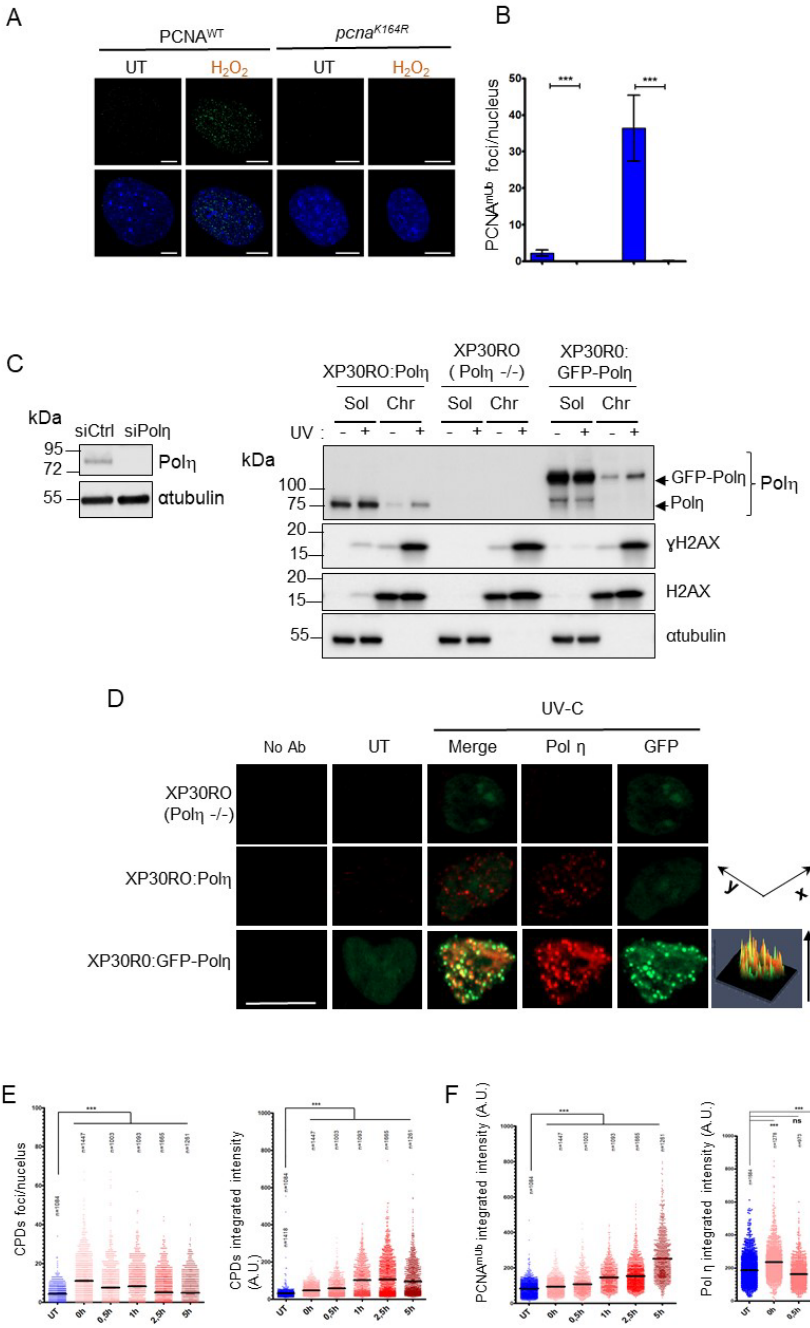

Figure S6: Related to Figure 4 and 5. Polη detection by either flow cytometry and immunofluorescence in cells with reduced Polη abundance. A, Immunofluorescence staining of mouse embryonic fibroblasts (MEFs) either wild-type (WT) or carrying the *pcna*^{K164R} mutation[1], treated as in Fig. S1E. Scale bar: 10 µm. **B,** Quantification of PCNA^{mUb} foci/nucleus of left panel. Data are means ± SEM. Mann Whitney test, *p<0.05, **p<0.01, ***p<0.001**. C-D,** Detection of Polη by either western blot (C) or immunofluorescence (D) in HCT116 cells treated with the indicated siRNA (C, left panel) or in XPRO30 fibroblasts (right panel) bearing a homozygous mutation in the Polη gene (polη$^{-/-}$), complemented with either wild-type Polη (XPRO30:Polη) or with GFP-tagged Polη (XPRO30:GFP-Polη). Cells were either untreated (UT) or exposed to 20 J/m^2 of UV-C light. A representative cell is shown. A 3D visualization of the relative intensity of the both GFP and Polη labeling is shown (same cell), n=2. **E,** Cell Profiler quantification of either CPDs foci/nucleus (left panel) or CPDs nuclear intensity (left panel) observed during the time course of UV-C irradiation. A.U.: arbitrary units. Stars indicate significant differences, *** P <0.001 (non-parametric Mann Whitney test). **F,** Cell Profiler quantification of either PCNA^{mUb} nuclear integrated intensity (left panel) or Polη nuclear integrated intensity (right panel) observed during the time course of UV-C irradiation. A.U.:

arbitrary units. Stars indicate significant differences, *** P <0.001 (non-parametric Mann Whitney test).

Supplementary References

1. Langerak, P., Nygren, A.O.H., Krijger, P.H.L., van den Berk, P.C.M., and Jacobs, H. (2007). A/T mutagenesis in hypermutated immunoglobulin genes strongly depends on PCNAK164 modification. J. Exp. Med. *204*, 1989–1998. 10.1084/jem.20070902.