SUPPLEMENTARY MATERIALS AND METHODS

Chromatin Immunoprecipitation for qPCR analysis

Four different active promoter genes were chosen, ATXN7L2, PSRC1, CELSR2 and IL24, all located on chromosome 1. Primer sequences are shown in the table below.

Gene name	Sequence forward primer	Sequence reverse primer
ATXN7L2	CCTCCCTGCACCTACCCTAT	TCCACTCTCCCAAGCTCACT
PSRC1	ACCGTGATTGGTGAATCCTC	CTCTGCTCCCATCTGCCTAC
CELSR2	TGCAACTGTGATGTCAGCAA	CACAAGGCCCTCAGCTCTAC
IL24	AGCCAGTGGTAGAGCCTGAA	AGGAAAAAGAGGGAGGTGGA
Non-transcribed region	CCCATCTCAACCTCCACACT	CTTGTCCAGATTCGCTGTGA

Chromatin Immunoprecipitation

Chromatin was prepared as described in Fousteri et al. (Fousteri et al, 2006) from untreated XPCS2_SV40 cells or 30 min after global UVC irradiation (16 J/m^2). Sonication was performed on ice in a buffer containing 10 mM Tris-HCl [pH 8.0], 140 mM Nacl, 1% Triton X-100, 0.1% Na-deoxycholate, 0.1% SDS (buffer S). The sonicator was a Diagenode (Liège, Belgium) Bioruptor set at power 5 and cycles were: 10 s pulses followed by 20 s of cooling during 15 min and repeated 3 times. After spinning down (13200 rpm, 15 min) the samples, the supernatant containing the crosslinked chromatin was stored at -80°C. In each ChIP assay, 400 μg of protein from crosslinked chromatin was used to immunoprecipitate with 1 μg of antibody rabbit IgG polyclonal anti-XPB (S-19) in buffer S. Immunoprecipitation lasted was performed at 4°C for 2 hr. The immunocomplexes were collected by adsorption to protein G Sepharose beads (Upstate-Millipore) overnight at 4°C. The G Sepharose beads were washed with 5 vol of buffer S and resuspended in 1x Laemli SDS buffer. To decrosslink the proteins prior electrophoresis, samples were incubated at 95°C for 90 min. ChIP complexes were resolved by SDS-PAGE and

western-blotted with the following NER antibodies: IgG polyclonal anti-XPB (S-19), mouse IgG monoclonal anti-p62 (G-10), goat IgG polyclonal anti-p34 (C-19), rabbit IgG polyclonal anti-XPA (FL-273, Santa Cruz Biotechnology), mouse IgG monoclonal anti-XPF (Ab-1, clone 219, Thermoscientific).

Measurements of transcription activity after inhibition by α-amanitin

Cells (MRC5SV, XPCS2SV, XP12ROSV, XP6BESV, XPCS1ROSV) were grown on 24 mm coverslips and treated 6h or 16h with α-amanitin with concentrations ranging from 0 to 25 µg/ml at 37°C. RNA production was detected using the Click-iT RNA Alexa Fluor Imaging kit (Invitrogen). Briefly, cells were incubated for 2 hours with 5-ethynyl uridine, fixed with paraformaldehyde 4% and permeabilized with Triton 0.5%. After this treatment, cells were incubated for 30 min with the Click-iT reaction cocktail containing Alexa Fluor Azide 488. After washing, coverslips were mounted on slides with Vectashield (Vector). Imaging was performed on a Zeiss LSM 710 confocal laser scanning microscope (Zeiss), using a 40x/1.3 objective.

SUPPLEMENTARY REFERENCES

Fousteri M, Vermeulen W, van Zeeland AA, Mullenders LH (2006) Cockayne syndrome A and B proteins differentially regulate recruitment of chromatin remodeling and repair factors to stalled RNA polymerase II in vivo. *Mol Cell* **23:** 471-482

SUPPLEMENTARY FIGURE LEGENDS

Figure S1: Recruitment of the DNA damage response machinery is not pan-nuclear in XP-D/CS cells.

(A) Local H2AX phosphorylation in XPCS2 (XP-D/CS) cells after UV exposure. XP-D/CS cells were treated with 60 J/m^2 local UV-irradiation. 45 minutes later the cells were fixed and immunostained with antibodies directed against γH2AX (Ser139) (in red) and the XPB protein (in green). (B) Local H2A ubiquitination (in red) and the XPB protein (in green) in XP-D/CS cells 45 minutes after local UV-irradiation. All scale bars, 5 μm.

Figure S2: Effect of UVC-irradiation on TFIIH binding at promoters in WT and XP-D/CS cells

Bar graphs of the qPCR data from the Chromatin Immunoprecipitation (ChIP) experiments on the promoters of 4 different expressed genes in both MRC5 (WT) and XPCS2 (XP-D/CS) cells, prior to (-UV) and 1/2h after exposure to 20 J/m^2 of UVC (+UV) . The Y-axis represents the fold enrichment of TFIIH binding to the promoter region of each active gene.

Figure S3: Transcription inhibition by alpha-amanitin treatment

Confocal images of WT, XP-D/CS, XP-G/CS, XP-A and XP-D cells treated with different concentrations (from 5 μg/ml to 20 μg/ml) of α-amanitin for 6h. RNA production is stained in green using the EU Click it kit. All scale bars, 25 μm.

Figure S4: UVC-irradiation does not induce DNA breaks in XP-D/CS cells at early time points.

(A) Confocal images of XPB (red), Ku80 (green) and Poly(ADP-ribose) (green) immunostaining in XP-D/CS cells fixed 15 min (top row) and 1 hour (bottom row) after local UV-irradiation (60 J/m^2). (B) Confocal images of XPB (red), Ku80 (green) and Poly(ADP-ribose) (green) immunostaining in XP-D/CS cells fixed 15 min after laser induced (multi-photon at 800nm) DNA damage. All scale bars, 5 μm.

Figure S5: H2AX phosphorylation in primary cells after UV-irradiation.

(A) Immunostaining with DAPI (cyan) and Ki67 (green) in confluent XP-D/CS human primary cells. Less than 10% of the cells were Ki67 positive (counting ≥200 nuclei). Scale bars, 25 μm.

(B) Confocal images of γH2AX (green) and XPB (red) immunofluorescence in confluent primary human cells (C5RO, XPCS2, XPCS1RO and XP12RO) 1h after local UV-irradiation (60 J/m^2). Scale bars, 5 μm.

Figure S6: RPA accumulation in UVC-exposed areas in primary cells and XPF recruitment into repair complexes

(A) Confocal images of RPA (green) and XPB (red) immunofluorescence in confluent primary human cells (C5RO, XPCS2 and XPCS1RO) 1h after local UV-irradiation (60 J/m^2). Scale bars, 5 μm. (B) Western-blot analysis of XPB-specific ChIP using chromatin of crosslinked UV-irradiated (lane 2) and untreated (lane 1) $TFIIH^{XP/CS}$ cells. ChIP complexes were resolved by SDS-PAGE and western-blotted with NER antibodies (indicated by arrows). The thick-bands (indicated by *) are heavy and light chain IgG bands, intrinsic to the IP.

Figure S7: ssDNA labeling after high local UV-irradiation.

Confocal images of XPB (red) and BrdU (green) immunofluorescence in MRC5 (WT) and XPCS2 (XP-D/CS) cells 1h after local UV-irradiation (100 J/m^2). Scale bars, 5 μm.

Figure S8: XPF depletion 48 h after siRNA α-XPF treatment.

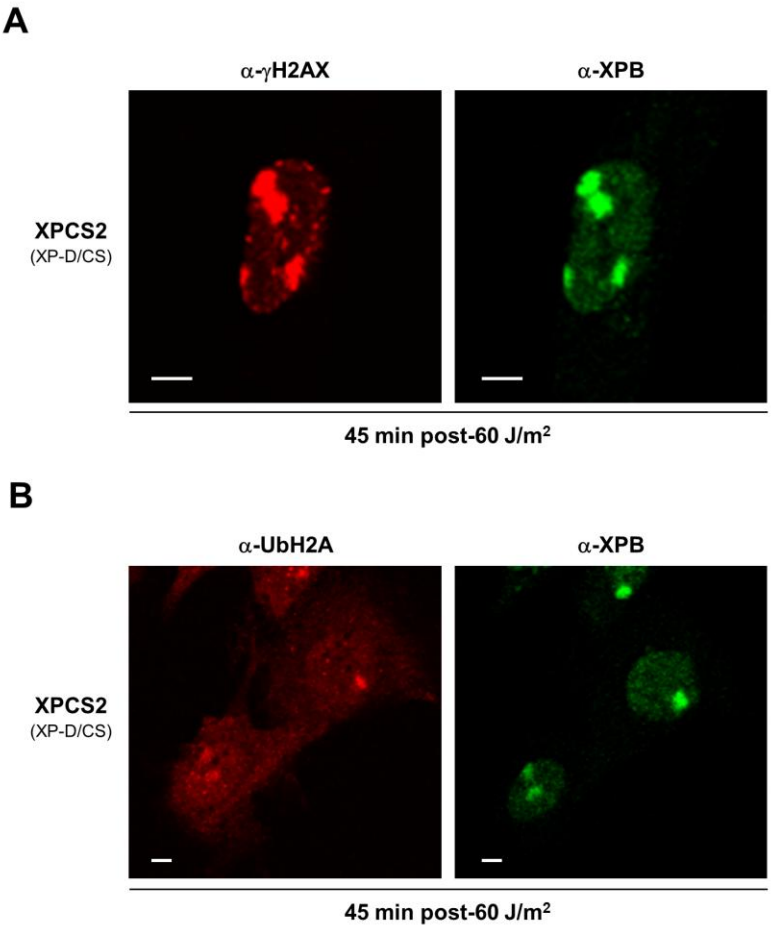
Confocal images of XPB (red) and XPF (green) immunofluorescence in XP-D/CS cells, 1h after UV local exposure with 60 J/m^2. Cells were transfected with 40 nM siRNA α-XPF, or not (Mock), 48 h prior the experiment. Scale bars, 20 μm.

Figure S9: Fen1 accumulation on UV-lesion sites only in XP/CS cells.

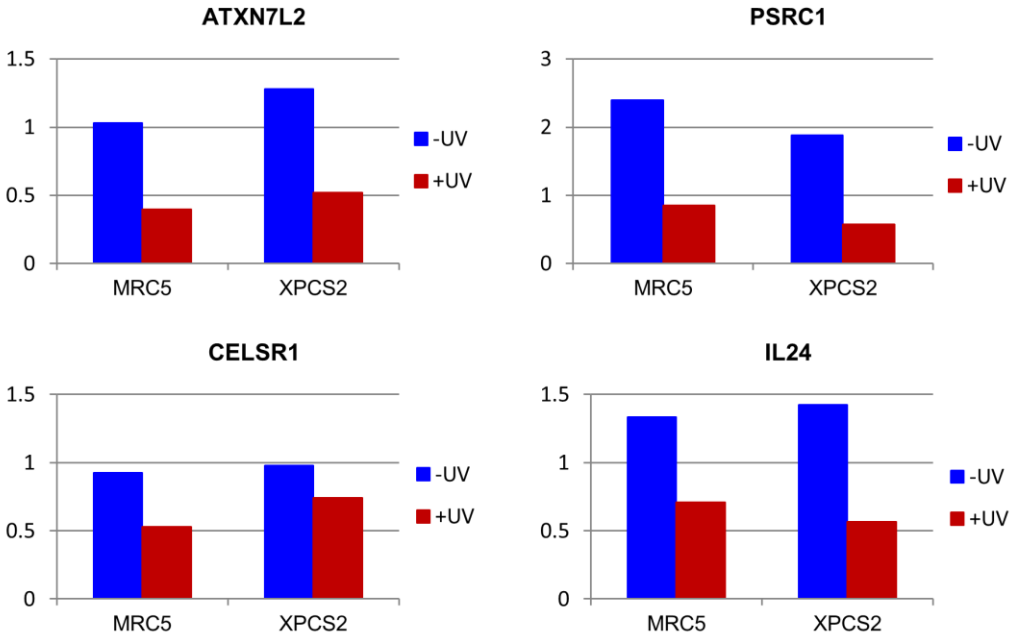
Confocal images of Fen1 (red) and RPA (green) immunostained cells (WT, XP-D/CS, XP-G/CS and XP-A) 1 h after local UV-irradiation (60 J/m^2). Scale bars, 5 μm.

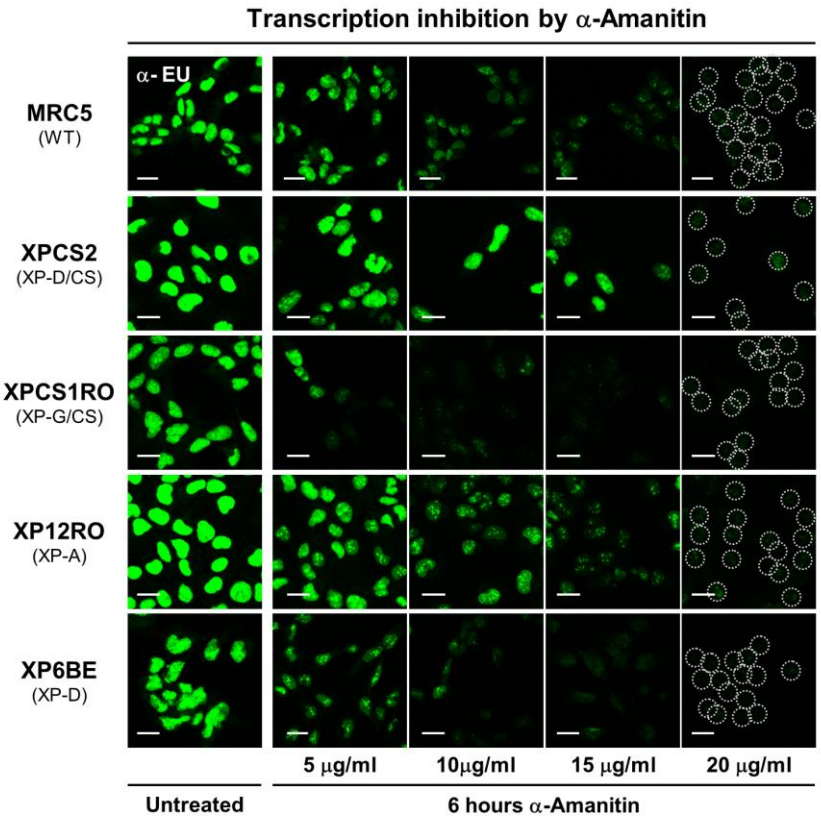
SUPPLEMENTARY FIGURES

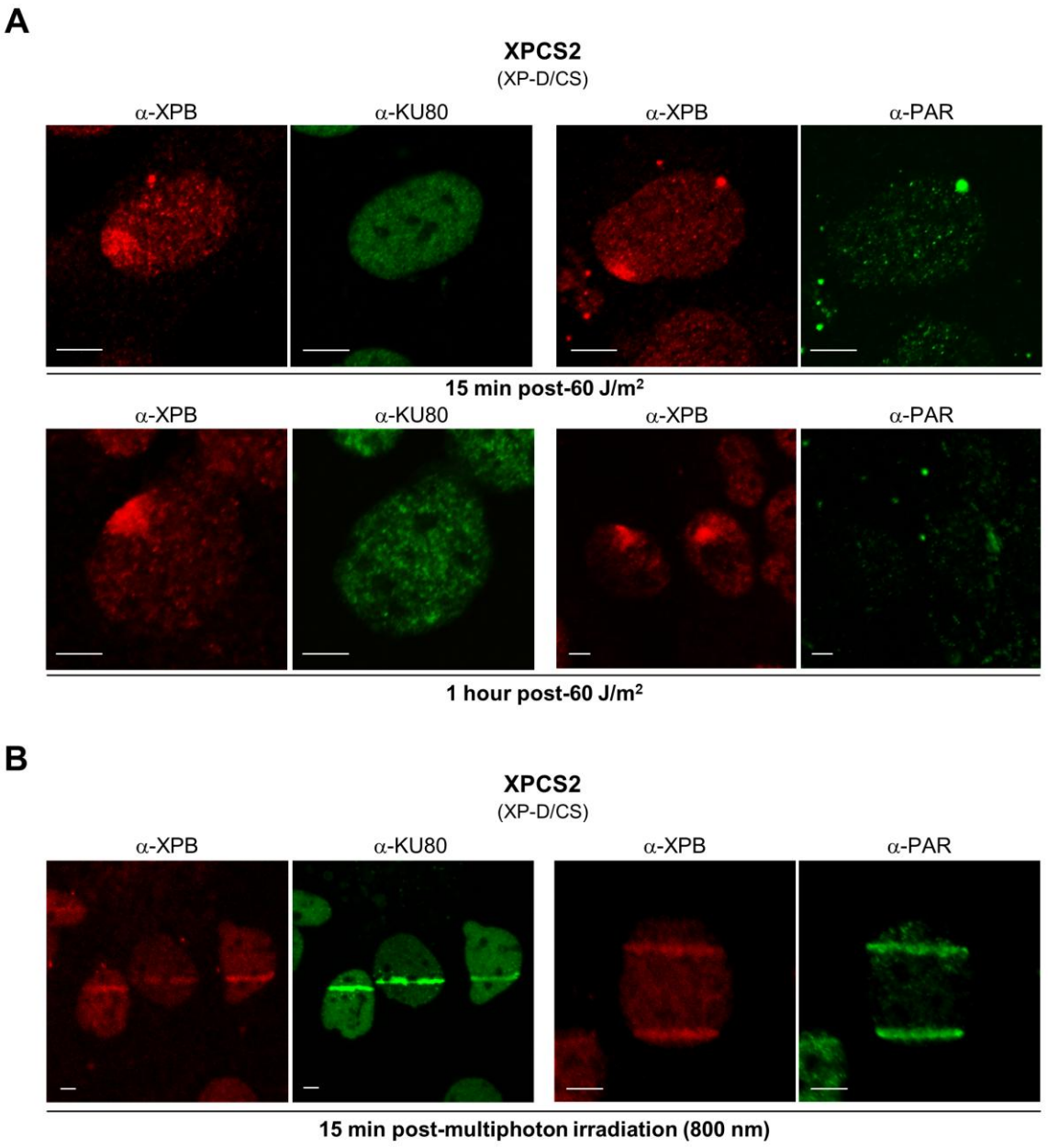
Supplementary Figure S1 Godon et al.

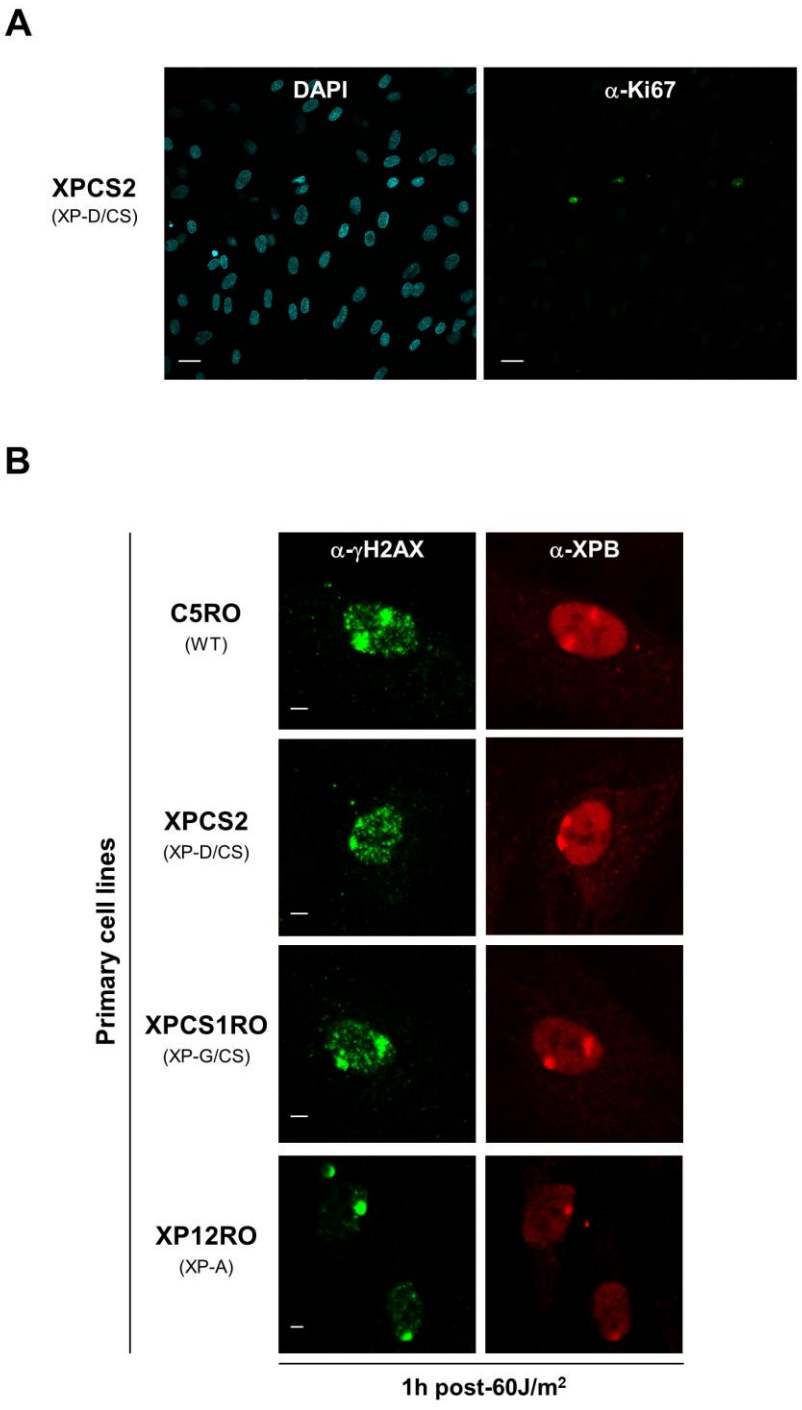


Supplementary Figure S2 Godon et al.

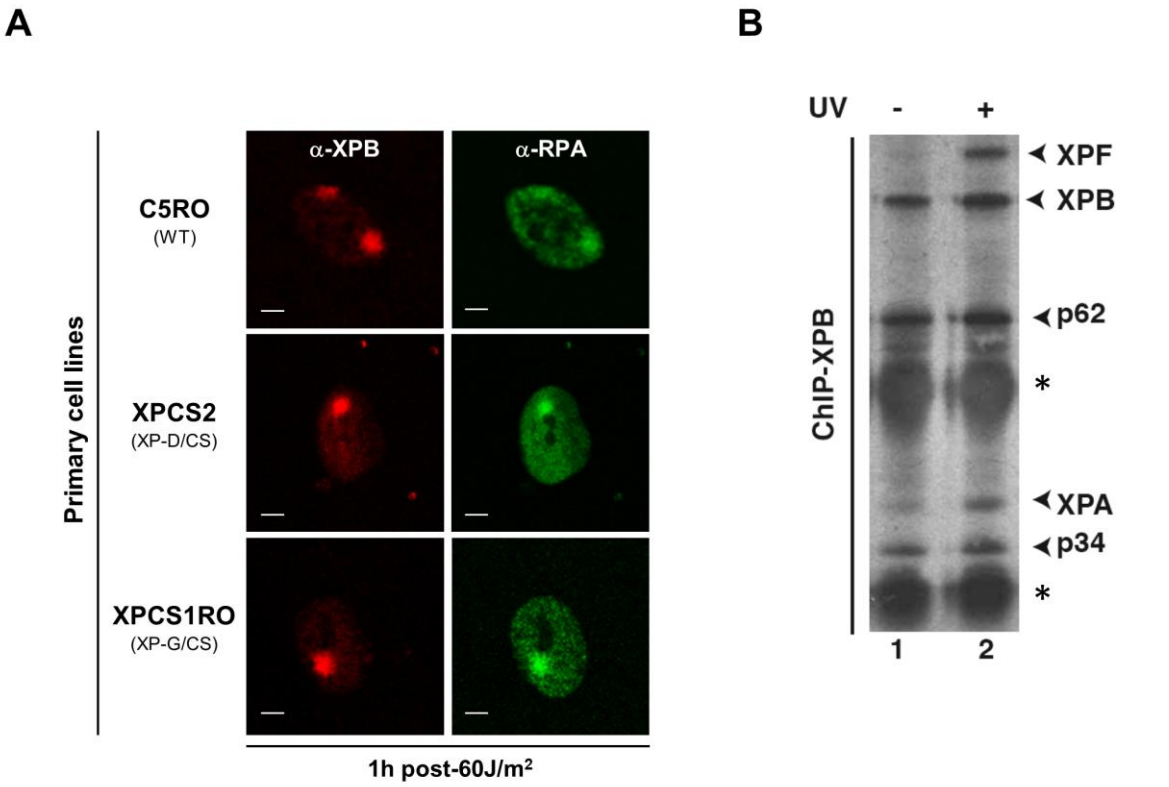



Transcription inhibition by α-Amanitin
α- EU
MRC5
(WT)
XPCS2
(XP-D/CS)
XPCS1RO
(XP-G/CS)
XP12RO
(XP-A)
XP6BE
(XP-D)
5 μg/ml
10μg/ml
15 μg/ml
20 μg/ml
Untreated
6 hours α-Amanitin

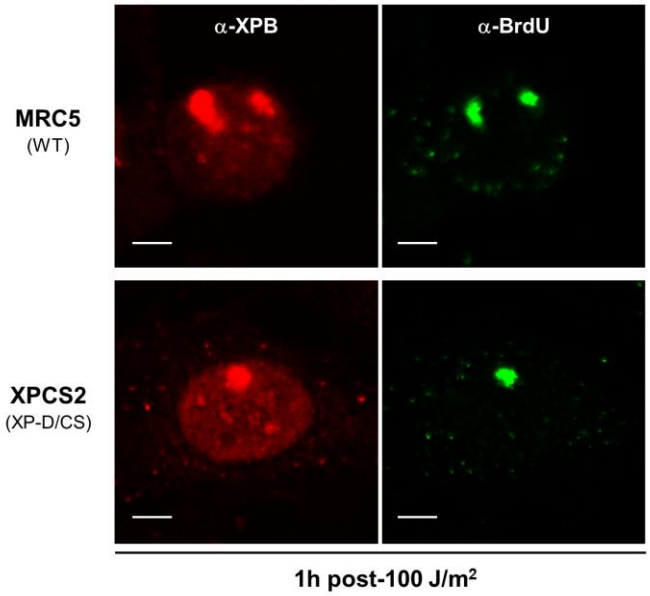

A
XPCS2
(XP-D/CS)
α-XPB
α-KU80
α-XPB
α-PAR
15 min post-60 J/m²
α-XPB
α-KU80
α-XPB
α-PAR
1 hour post-60 J/m²
B
XPCS2
(XP-D/CS)
α-XPB
α-KU80
α-XPB
α-PAR
15 min post-multiphoton irradiation (800 nm)


A
DAPI
α-Ki67
XPCS2
(XP-D/CS)
B
α-γH2AX
α-XPB
C5RO
(WT)
XPCS2
(XP-D/CS)
XPCS1RO
(XP-G/CS)
XP12RO
(XP-A)
Primary cell lines
1h post-60J/m2

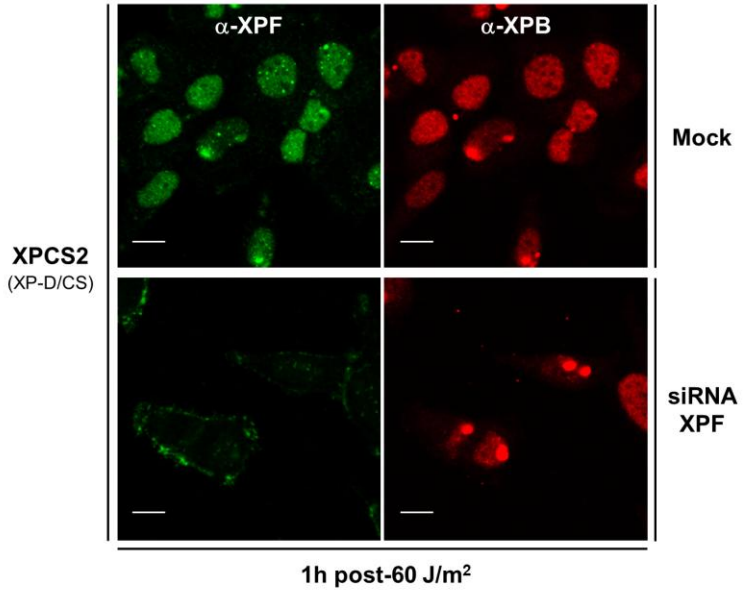
Supplementary Figure S6 Godon et al.



Supplementary Figure S7 Godon et al.



Supplementary Figure S8 Godon et al.



Supplementary Figure S9 Godon et al.