Supplemental Information

Lack of CAK complex accumulation at DNA damage sites in XP-B and XP-B/CS fibroblasts reveals differential regulation of CAK anchoring to core TFIIH by XPB and XPD helicases during nucleotide excision repair

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Figure Legends

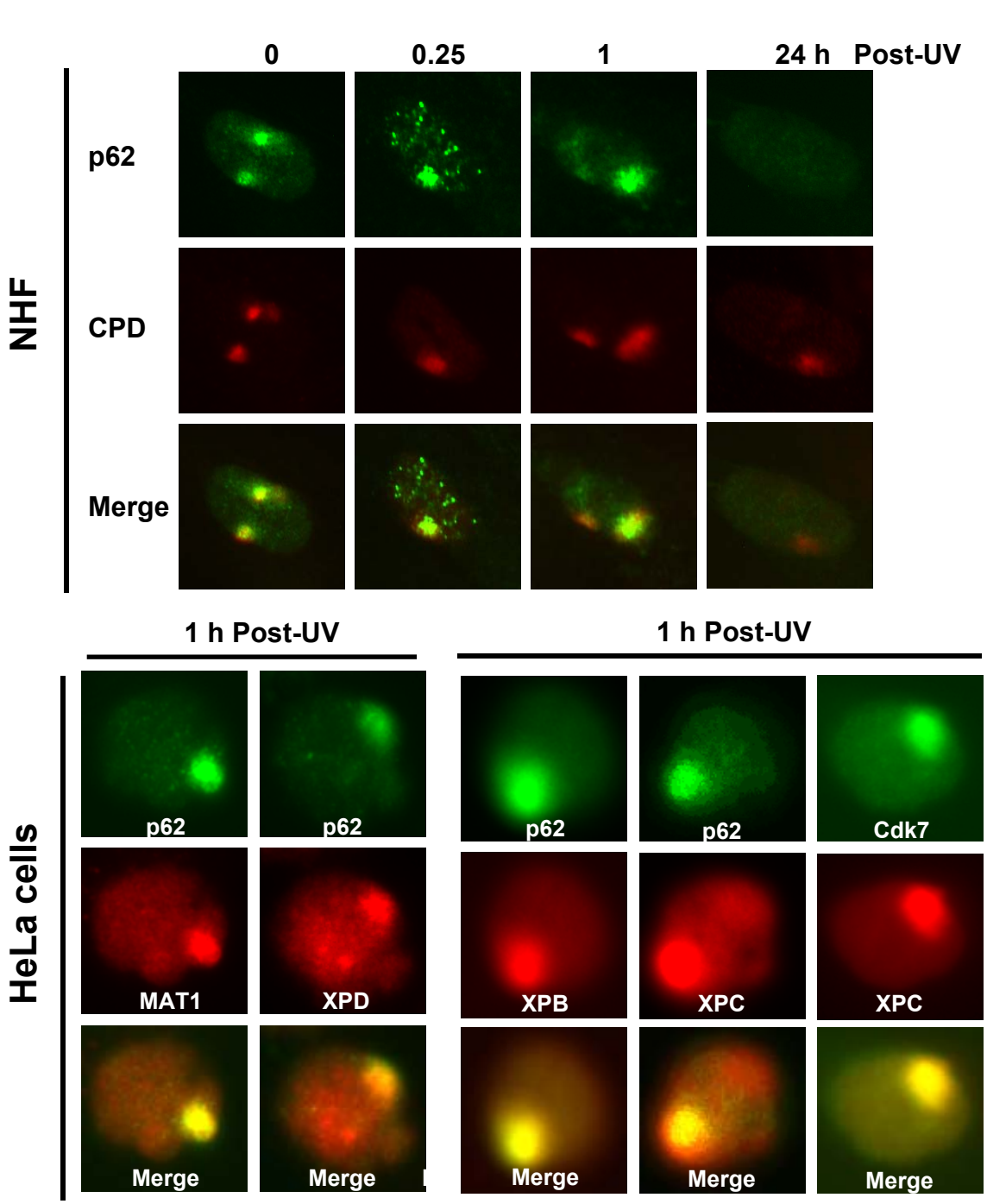
Supplemental Fig. S1. (A) Normal human fibroblasts (NHF) were grown on coverslips, locally irradiated with 100 J/m^2 UV through a 5 μm-isopore polycarbonate filter, fixed immediately (0 h) or cultured for 0.25, 1, or 24 h before fixing in 2% paraformaldehyde. The TFIIH component p62 and UV-induced CPDs were visualized by immunofluorescence double labeling using specific antibodies. (B) CAK components, MAT1 and CDK7, accumulate at DNA damage sites in HeLa cells. (C and D) CAK component MAT1 does not accumulate at DNA damage sites in XP-B/CS fibroblasts, XPBCS2BA (C) and XP181MA (D). HeLa cells, XP-B/CS XPBCS2BA and XP181MA fibroblasts were grown on coverslips, locally UV-irradiated at a dose of 100 J/m^2, fixed immediately (0 h) or cultured for 0.5, 3, or 24 h before fixing in 2% paraformaldehyde. NER factor XPC, TFIIH component XPB, XPD, p62 and CAK component MAT1or Cdk7 were visualized by immunofluorescence double labeling using specific antibodies. Factor co-localization was shown by merging two different colored immunofluorescent images.

Supplemental Fig. S2. Visualization of MAT1 and γH2Ax in DNA damage sites in NHF (A, B and C), XP-B/CS XPBCS2BA (A) and XP183MA (B) and XP181MA (C) cells. NHF and XP-B/CS cells were grown for 3 days to allow efficient uptake of latex beads of different sizes (0.8 μm for NHF or 2 μm for XP-B/CS). The cells with different sized beads were co-cultured in a 1:1 ratio for 1 day on coverslips, locally irradiated with 100 J/m^2 UV, cultured for 1 h and fixed with 2% paraformaldehyde. The accumulation of MAT1 and γH2Ax at damage sites were visualized by immunofluorescence double labeling using specific antibodies. The color arrows indicate immunofluorescent foci of γH2Ax (Red) or MAT1 (Green).The white arrows indicate NHF or XP-B/CS cells with different sized beads in cytoplasm.

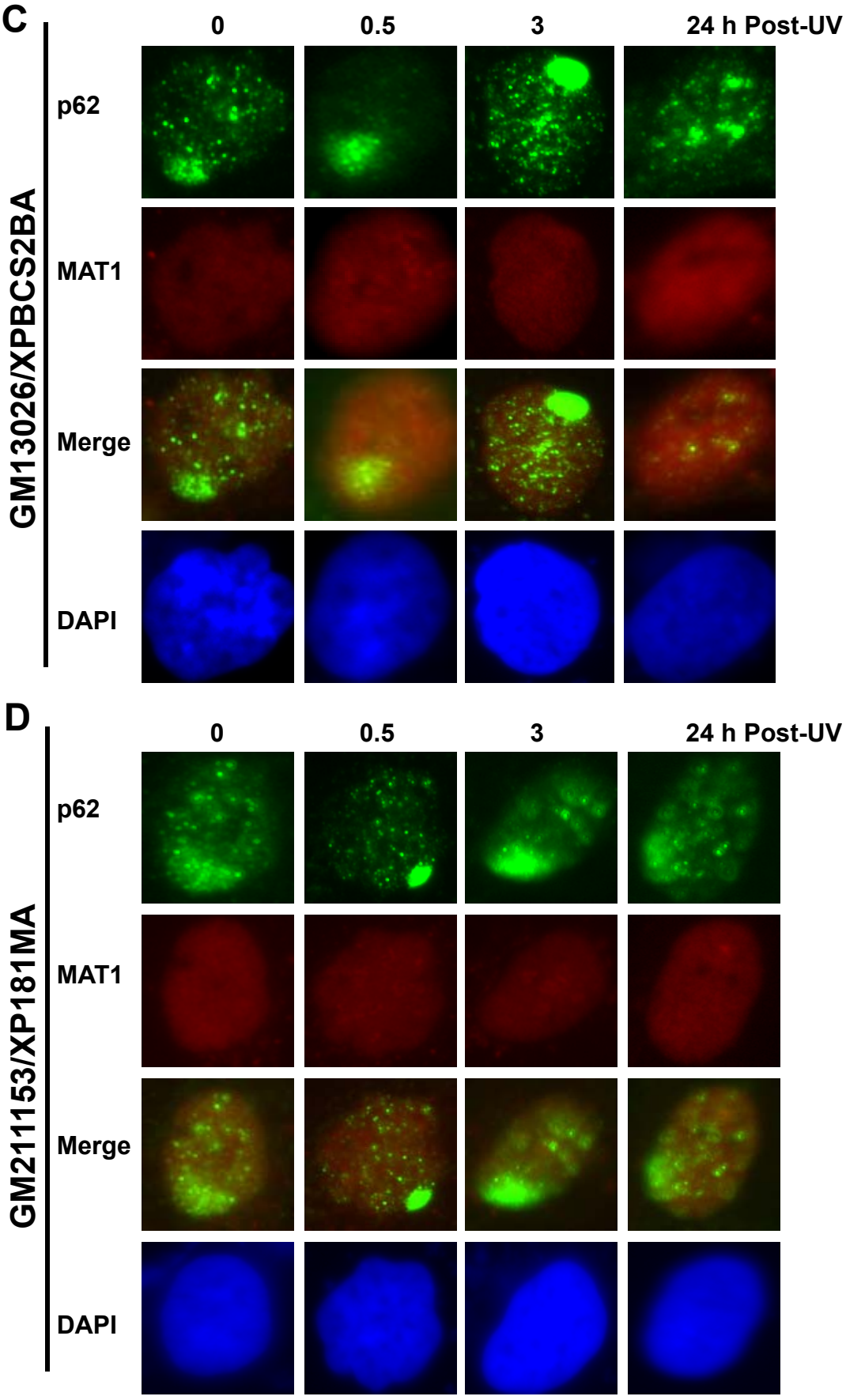
Supplemental Fig. S3. XP-D fibroblasts accumulate XPC and XPG, p62, XPB and XPD, as well as MAT1 at DNA damage sites *in vivo*. XP-D fibroblasts XP6BE (A) and SV40-transformed XP6BE (B) were grown on coverslips, locally irradiated with 100 J/m^2 UV, cultured for 1 h and then fixed with 2% paraformaldehyde. The cells were examined by immunofluorescence double labeling with indicated antibodies to p62 or XPG, in combination with antibodies to MAT1, XPD, XPB, or XPC.

Supplemental Fig. S4. Persistent accumulation of XPC, p62, XPD and XPG at DNA damage sites occurs in XP-B/CS fibroblast XP181MA from severe symptoms. The XP181MA fibroblasts were grown on coverslips and the cells were irradiated, cultured, fixed and the factor recruitment examined as described in Figure 1 to 4. The figure shows the presence or absence of XPC, XPG, p62 and XPD at DNA damage sites as visualized by immunofluorescence double labeling.

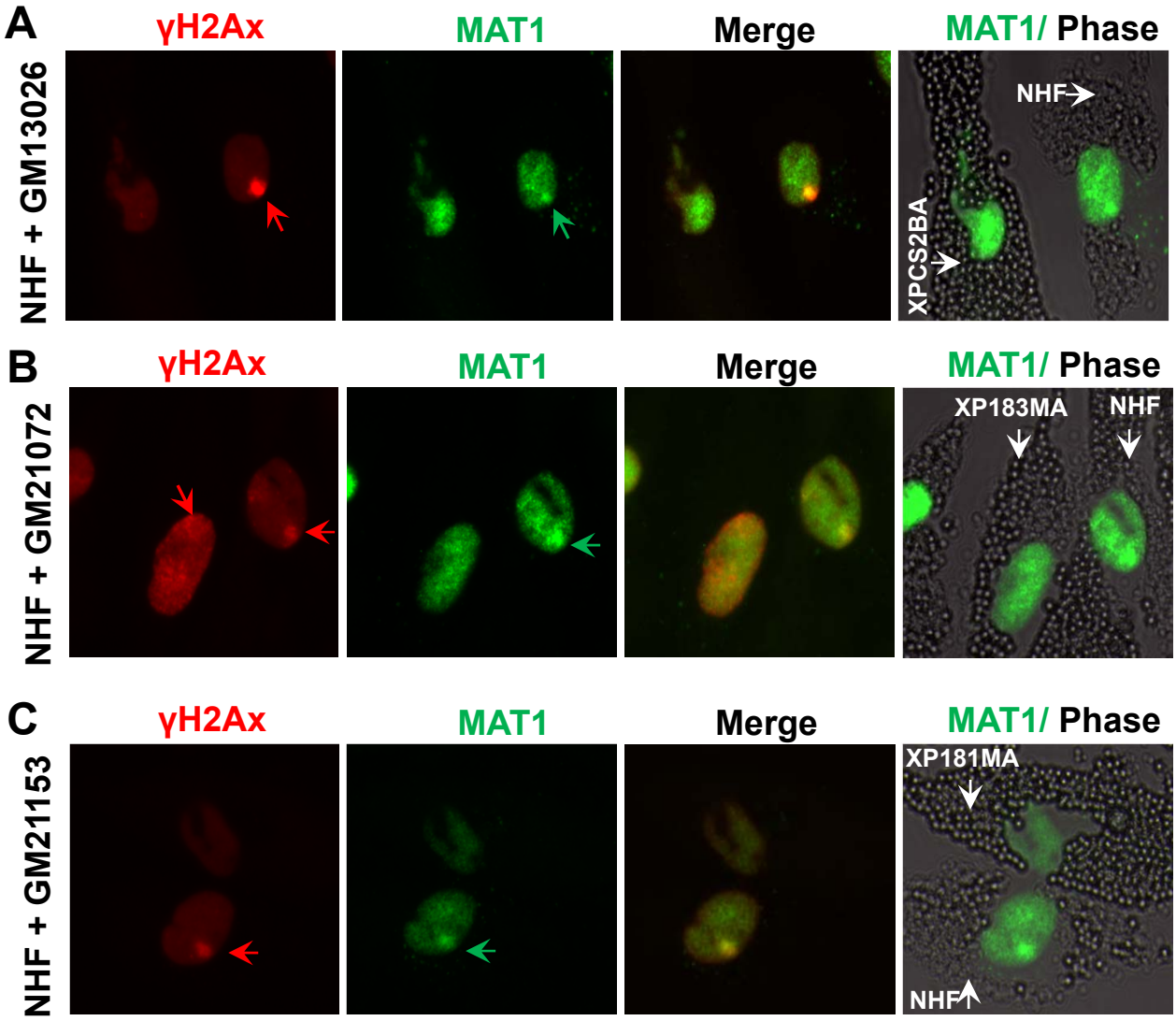
S.1.


A
0
0.25
1
24 h Post-UV
NHF
p62
CPD
Merge
B
1 h Post-UV
1 h Post-UV
HeLa cells
p62
p62
p62
p62
Cdk7
MAT1
XPD
XPB
XPC
XPC
Merge
Merge
Merge
Merge
Merge

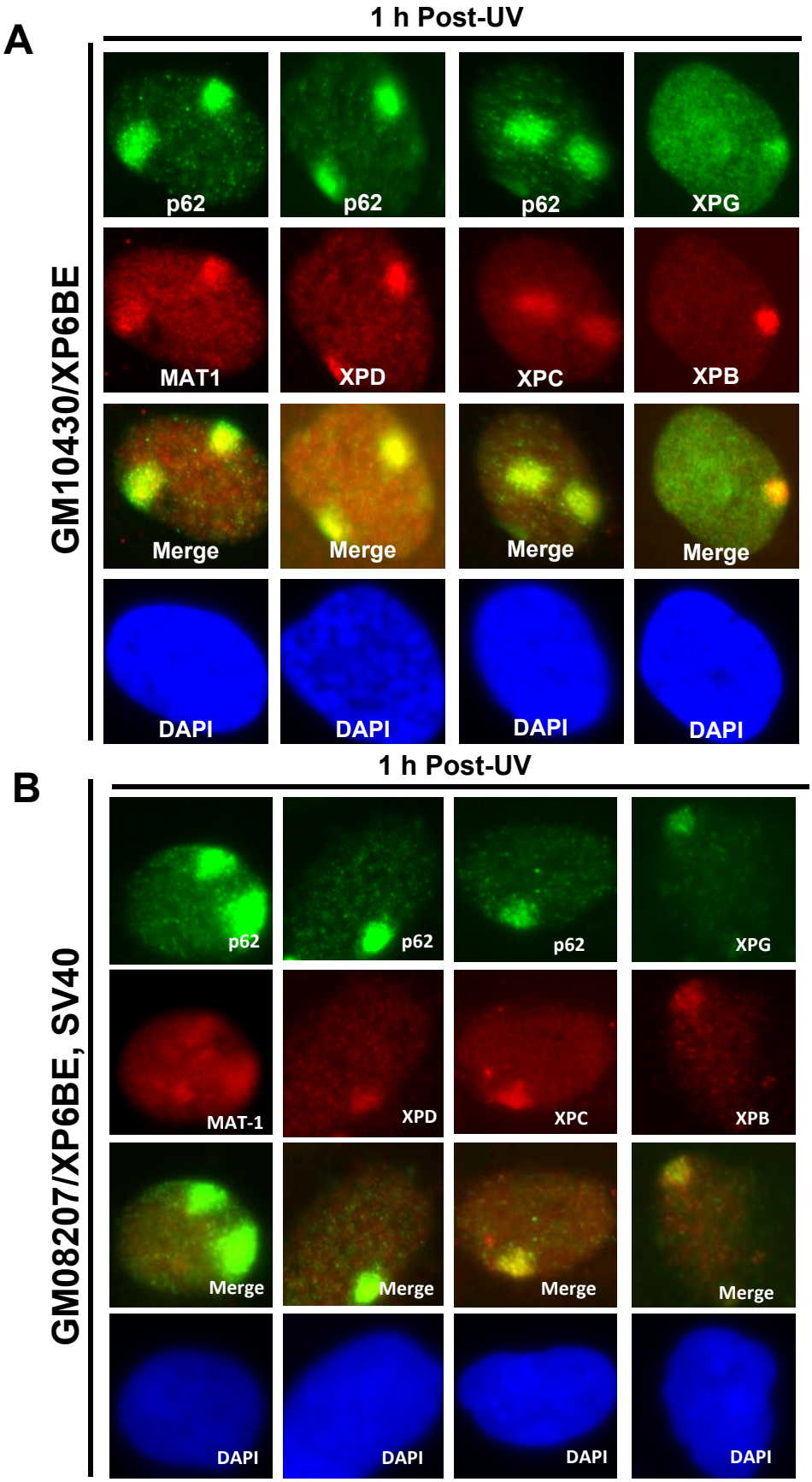
S.1.


C
0
0.5
3
24 h Post-UV
p62
MAT1
Merge
DAPI
GM13026/XPBCS2BA
D
0
0.5
3
24 h Post-UV
p62
MAT1
Merge
DAPI
GM21153/XP181MA

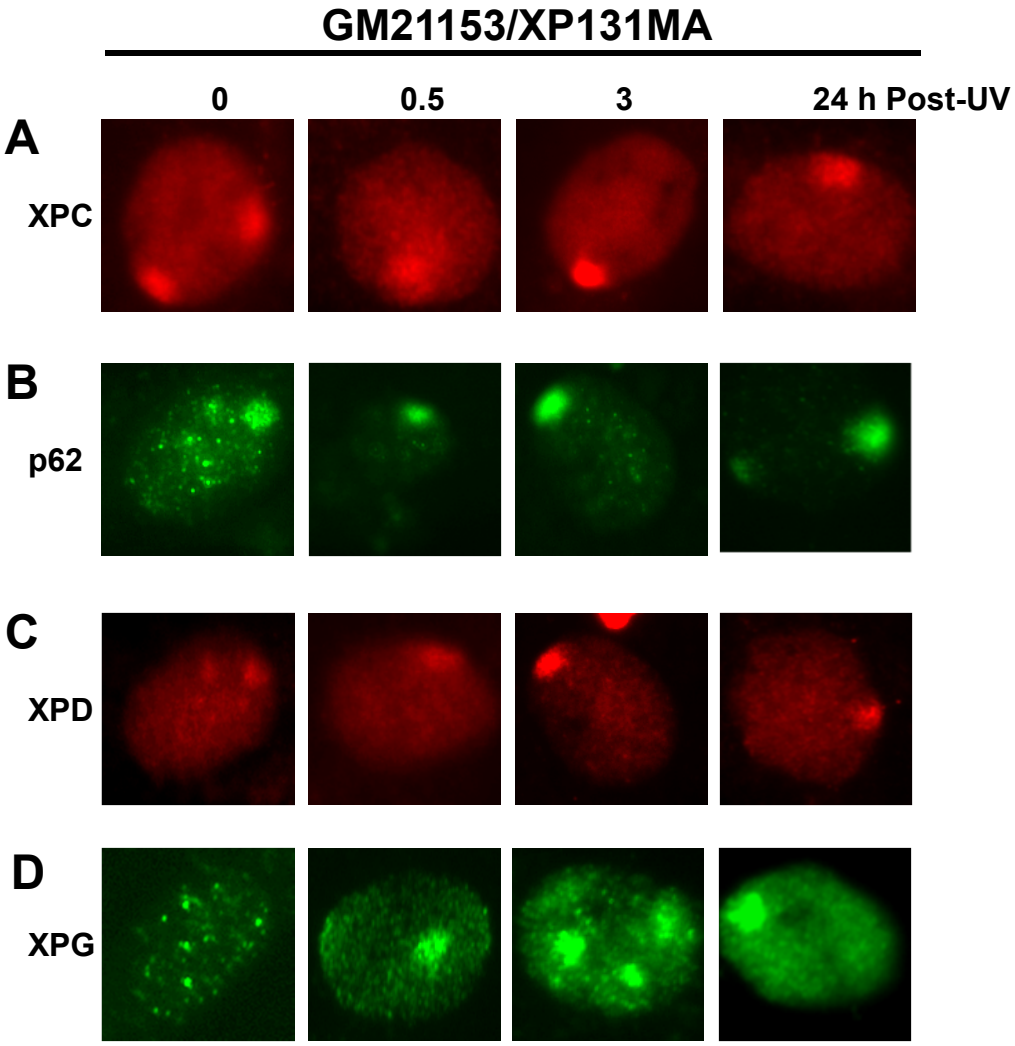
S.2.


A
γH2Ax
MAT1
Merge
MAT1/ Phase
NHF + GM13026
NHF
XPCS2BA
B
γH2Ax
MAT1
Merge
MAT1/ Phase
NHF + GM21072
XP183MA
NHF
C
γH2Ax
MAT1
Merge
MAT1/ Phase
NHF + GM21153
XP181MA
NHF

S.3.


A
1 h Post-UV
GM10430/XP6BE
p62
p62
p62
XPG
MAT1
XPD
XPC
XPB
Merge
Merge
Merge
Merge
DAPI
DAPI
DAPI
DAPI
B
1 h Post-UV
GM08207/XP6BE, SV40
p62
p62
p62
XPG
MAT-1
XPD
XPC
XPB
Merge
Merge
Merge
Merge
DAPI
DAPI
DAPI
DAPI

S.4.



Supplemental table 1- Features of XP-B and XP-D fibroblasts used in this Study

Patient	Clinical diagnosis	Complementation group	Mutations	UDS (% of normal)	References
XPCS1BA	Mild XP/CS	XP-B	p.F99S/p.K157insTSDSX	5-10%	Vermeulen, W., et. al., 1994.
XPCS2BA	Mild XP/CS	XP-B	p.F99S/p.K157insTSDSX	5-10%	Vermeulen, W., et. al., 1994.
XP33BR	XP	XP-B	p.F99S/ p.R425X	5%	Oh, K., et. al., 2006.
XP131MA	Severe XP/CS	XP-B	p.Q739insX42/p.D474EfsX475	4%	Oh, K., et. al., 2006.
XP183MA	Severe XP/CS	XP-B	p.Q739insX42/p.Q545X	8%	Oh, K., et. al., 2006
XP1BR	XP	XP-D	p.R683W/p.R683W	unknown	Vermeulen, W., et. al., 1991.
XP17BE	XP	XP-D	p.R683W/p.G36-R61del	33%	Boyle, J., et. al., 2008.
XP6BE	XP	XP-D	p.R683W/p.D681N	25-55%	Takayama, K., et. al., 1995.
XPCS2	XP/CS	XP-D	p.G602D from a single expressed allele	52%	Vermeulen, W., et. al., 1991. Takayama, K., et. al., 1995.