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Supplementary Information for

Topoisomerase I-driven repair of UV-induced damage in NER deficient cells

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This PDF file includes:

www.pnas.org/cgi/doi/10.1073/pnas.1920165117

SI Materials and Methods

All reagents and resources used in this study are listed in *SI Appendix*, Table S6.

Cell culture and reagents

MCF-7 and primary fibroblast (XP15BR) cells were maintained in Dulbecco's Modified Eagle Medium (DMEM) (Cat# 0845964, Gibco, US) containing FBS (10%, Gibco), penicillin (100 U/ml), and streptomycin (100 μg/ml, Nacalai, Japan). For G_1/G_0 arrest by serum-starvation, MCF-7 cells were incubated prior to analysis in phenol-red-free DMEM (Cat# 21063029, GIBCO, US) for 2 days and in serum-free medium for 24 h. More than 95% of the cells were arrested in the G_0/G_1 phases by serum-starvation. Human TK6 B cells were incubated in RPMI1640 medium (Cat# 3026456, Nacalai Tesque, Japan) supplemented with horse serum (5%, Gibco, US), penicillin (100 U/ml), streptomycin (100 μg/ml, Nacalai, Japan), and sodium pyruvate (200 mg/ml, ThermoFischer, US).

Designing gene-targeting constructs and transfection of them into TK6 cells

We designed gene-targeting constructs based on the manual provided by GeneArt Seamless Cloning Enzyme Mix (ThermoFischer, US) (1). Table S1 shows the list of mutants and the antibiotic resistance markers (*MARKER*^{R}) used to generate the mutants in this study. To generate gene-targeting constructs, we inserted left and right arms (~1 kb each) of genomic sequences into the *ApaI* and the *AflII* sites of the *DT-ApA*/*MARKER*^{R} *vector*. To this end, we assembled the left and right arms together with *DT-ApA*/*MARKER*^{R} having been digested with *ApaI* and *AflII*, using GeneArt Seamless Cloning Enzyme Mix (ThermoFischer, US). Primer information about the left and right arms (~1 kb each) is described in Table S5. To generate the left arm, we needed to add the upstream and downstream sequences derived from the *ApaI* site *to the 5′ and 3 ends, respectively, of* the PCR-amplified left arm. For this purpose, we added "5′-GCGAATTGGGTACCGGGCC" to 5′ of the upstream primer and added "5′-CTGGGCTCGAGGGGGGGCC" to 5′ of the downstream primer. We added the upstream and downstream sequences from the *AflII* site *to the 5′ and 3′ ends, respectively, of* the PCR-amplified right arm. We added "5′-TGGGAAGCTTGTCGACTTAA" to 5′ of the upstream primer of the PCR-amplified right arm and added "5-CACTAGTAGGCGCGCCTTAA" to 5 of the downstream primer of the PCR-amplified right arm. The *DT-ApA*/*MARKER*^{R} was provided by the Laboratory for Animal Resources and Genetic Engineering, Center for Developmental Biology, RIKEN Kobe (http://www.clst.riken.jp/arg/cassette.html).

The gRNAs were inserted into the *BbsI* site of pX330 vector (Cat# 42230, Addgene, US), which expresses gRNA and Cas9 from the U6 and chicken β-actin promoters, respectively. The two resulting targeting vectors containing different antibiotic markers were transfected with pX330-gRNA into 6 million TK6 cells. The transfected pX330 expressed the Cas9-gRNA complex, which induced DSBs at the specific locus of the genomic DNA and thus facilitated HR between the genomic locus and the arms of the targeting vectors.

Generation of gene-targeting constructs to create *XPA$^{-/-}$/ XRCC1$^{-/-}$* and *XPA$^{-/-}$/ POLβ$^{-/-}$* TK6 cells

To generate double mutant of *XPA$^{-/-}$/XRCC1$^{-/-}$,* two targeting vectors and pX330-gRNA (the gRNA was inserted into the *BbsI* site of pX330 vector (Cat# 42230, Addgene, US)) targeting XRCC1 were transfected into *XPA$^{-/-}$* mutant cells, which was described previously (2) .To generate the targeting vectors for the *XRCC1$^{-/-}$* cells (Figure S1B), the left and right arms were amplified using "Forward primer of right"/ "Reverse primer of right" and "Forward primer of left"/"Reverse primer of left", which sequences are shown in Table S5. The arms were assembled with *DT-ApA/BSR^{R} and DT-ApA/HIS^{R} vectors by* GeneArt Seamless Cloning Enzyme Mix (ThermoFischer, US) as described above.

To generate double mutant of *XPA$^{-/-}$/POLβ$^{-/-}$,* two targeting vectors and pX330-gRNA (the gRNA was inserted into the *BbsI* site of pX330 vector (Cat# 42230, Addgene, US)) targeting *POLβ* were transfected into *XPA$^{-/-}$* mutant cells, which was described previously(2) .To generate the targeting vectors for the *POLβ$^{-/-}$* cells (Figure S4F), the left and right arms were amplified using "Forward primer of right"/ "Reverse primer of right" and "Forward primer of left"/"Reverse primer of left", which sequences are shown in Table S5. The arms were assembled with *DT-ApA/NEO^{R} and DT-ApA/PURO^{R} vectors by* GeneArt Seamless Cloning Enzyme Mix (ThermoFischer, US) as described above.

CRISPR/Cas9-mediated genome-editing in human MCF-7 cells

The gRNAs were inserted into the *BbsI* site of pX459 (Cat# 48139, Addgene, US). pX459 expresses gRNA under the control of the U6 promoter, and Cas9 under the chicken β-actin promoter. The sequences of gRNAs for *XRCC1*, and *XPA* are shown in Table S2. pX459-gRNA was transfected into MCF-7 cells, which were seeded on 6 cm dish containing ~60% confluency, with Fugene HD (Promega, US) according to the manufacture protocol. At 24 h after the transfection, puromycin was added to a final concentration of 2 μg/ml. The MCF-7 cells were further incubated for 48 h with the puromycin-containing medium. After removing puromycin, we incubated the cells for approximately two weeks to isolate the clones. The gene-disruption events were confirmed by western blotting analysis.

Colony formation assay

To measure the sensitivity of TK6 cell lines to DNA-damaging agents, we conducted a colony formation assay. We evaluated the sensitivity by counting colony formation in methylcellulose plates as described previously(3). After overnight mixing of various concentrations of H_2O_2 with methylcellulose containing media for overnight at 4 °C, drug containing media were plated on 6-well cluster plates in 5 ml/well. Serially-diluted TK6 cells were then plated in triplicate wells of 6-well cluster plates in 5 ml/well of D-MEM/F-12 (Life Technologies) supplemented with 10% horse serum, 2 mM L-Glutamine, 200 μg/ml sodium pyruvate and 1.5% (weight/vol) methylcellulose (Wako, Osaka, Japan). To measure MMS sensitivity, we exposed cells to different doses of MMS for 4 hr in the complete media, washed them with PBS, and seeded the cells onto methylcellulose containing 6 well plate. We suspended cells in a minimum volume of PBS with 1% horse serum, exposed cells to UV light, serially diluted cells with complete media, and seeded onto methyl cellulose containing media. We counted colonies 10-14 days after the irradiation or chemical treatment. We calculated the percentage of

surviving colonies after the irradiation or chemical treatment relative to the percentage of surviving untreated colonies.

Alkaline-comet assay
We performed an alkaline-comet assay (also known as the single-cell gel electrophoresis assay) to quantify DNA single-strand breaks, as described previously (4). The quantification of tail moments was determined by CometScore software.

In vivo nucleotide excision repair assay

Cells were irradiated with 4 J/m^2 UVC (254 nm) and cultured for various periods in a humidified 37 °C incubator under 5% CO_2. Genomic DNA was isolated using DNeasy kit (Qiagen) and the amounts of CPD or 6-4PP were determined by an enzyme-linked immunosorbent assay(5) using the specific monoclonal antibodies TDM-2 or 64M-5, respectively, as described previously (6).

Lentivirus vectors, production of lentiviral particles and infection for TOP1 and APE1 knock-down
shRNA oligonucleotides for TOP1 are shown in Table S5 were cloned into pLKO1 (Cat#8453, Addgene) digested with EcoRI and AgeI. pLKO1-sh*TOP1* or pLKO1 control vector was transfected into LentiX293T (Cat# 632180 Clontech, Japan) with pSPAX2 (Cat#12260, Addgene) and pMD2.G (Cat#12259, Addgene), which are packaging and envelop plasmids, respectively. After harvesting the medium (3 ml) containing lentiviral particles, we enriched the lentiviral particles by Lent-X Concentrator (Cat# 631231, Clontech, Japan) according to the manufacture protocol. The supernatant containing virus was mixed with *wild-type* and *XPA*$^{-/-}$ MCF-7 as well as HeLa cells. The supernatant containing virus was also mixed with *wild-type* and *XPA*$^{-/-}$/*TDP1*$^{-/-}$/*TDP2*$^{-/-}$ TK6 cells. The infected cells (Puromycin resistant) were enriched by puromycin drug selection for 72 hr. Seventy-two hours after puromycin drug selection, TOP1 expression was confirmed by western blotting using anti-TOP1 antibody (1/1,000, A302-737A, BD Pharmingen). Cloning, transfection and drug selection procedure for APE1 knockdown were same as for TOP1 described above. shRNA oligonucleotides for APE1 are shown in Table S5.

Lentivirus Infection of the TOP1 Expression Vector into Primary Fibroblast Cells
TOP1 cDNA was amplified using the primers (Table S5) from pCMV6-*TOP1* (a gift from Dr. Pommier) and cloned into BamHI and AfeI digested lentivirus vector lentiCRISPRv2 hygro (Plasmid# 98291) [from Addgene] by a GeneArt Seamless cloning kit (A13288, Invitrogen). After confirmation of *TOP1* sequence cloned, the lentiviral vector was transfected into primary fibroblast with Fugene HD transfection reagent (Promega, Cat#E2311). The supernatant containing virus was mixed with *XP15BR* primary cell culture. The infected cells (Hygromycin resistant) were enriched by hygromycin drug selection for 72 hr. Seventy-two hours after hygromycin drug selection, TOP1 expression was confirmed by western blotting using anti-TOP1 antibody.

Lentivirus Infection of the XPA, XRCC1 Expression Vector in MCF-7 cells and TDP1/TDP2 Expression Vector into TK6 Cells

Cloning, transfection and drug selection procedure were same as for TOP1 cDNA described above. Primers information are shown in Table S5.

Measurement of TOP1 trapped by genomic DNA
The protocol for this assay was described previously (7).

In vitro TOP1 cleavage assay
The *in vitro* TOP1 cleavage assays were carried out as described previously(8). Briefly, 2 nmol/L of radiolabeled DNA substrate was incubated with or without recombinant TOP1 in 20 ml of reaction buffer (10 mmol/L Tris-HCl, pH 7.5, 50 mmol/L KCl, 5 mmol/L MgCl2 0.1 mmol/L EDTA, and 15 μg/ml BSA) at 25 °C for 20 min in the presence of various concentrations of drugs. The reactions were terminated by adding SDS (0.5% final concentration) followed by the addition of 2 volumes of loading dye (80% formamide, 10 mmol/L sodium hydroxide, 1 mmol/L EDTA, 0.1% xylene cyanol, and 0.1% bromophenol blue). The cleavage products were separated on a 16% sequencing PAGE gel. Imaging and quantification were conducted using the Typhoon 8600 and ImageQuant Software (Molecular Dynamics), respectively.

Materials for in-vitro BER assay
The HPLC purified Oligonucleotide containing the 6-4PP lesion was made by Professor Shigenori Iwai, Osaka University. Other oligonucleotides were from Midland Certified Reagent Co. (Midland, TX). Deoxynucleoside triphosphates were from Roche Diagnostics Corp. Radionucleotides [γ-^{32}P] ATP (7000 mCi/ mmol) (MP Biomedicals, Irvine, CA) and [α-^{32}P] dCTP (6000 dpm/pmol) and MicroSpin G-25 columns were from Roche, GE HealthcareInc. Optikinase and terminal transferase were from USB Corp. (Cleveland, OH) and New England BioLabs Inc. (Ipswich, MA), respectively. Recombinant human Polβ, DNA ligase I and FEN1 were purified as described previously (9-11). Tdp1 was provided by Dr. Yves Pommier (NCI, NIH). Purified PNKP and UVDE were generous gifts from Dr. Michael Weinfeld (Department of Oncology, Cross Cancer Institute, Edmonton, Alberta, Canada) and Dr. R. Stephen Lloyd (Oregon Institute of Occupational Health, Sciences, OHSU, USA), respectively. Enzyme concentrations were determined by the Bradford protein assay kit from Bio-Rad (Hercules, CA).

The 5′-^{32}P-end radiolabeled oligonucleotides were prepared by incubation with OptiKinase in the presence of [γ-^{32}P] ATP with incubation for 30 min at 37 °C. Similarly, 3′-^{32}P-end labeled oligonucleotides were prepared by incubation with terminal transferase in the presence of [α-^{32}P] ddCTP with incubation for 30 min at 37 °C. Unincorporated nucleotides were removed by Sephadex G-25 spin-column chromatography (MicroSpin G-25 Columns, GE Healthcare, Inc).

Preparation of DNA substrates for BER
The designation and sequences of the synthetic oligodeoxynucleotides (ODNs) used are shown in Table S4. All ODNs, except the ODN containing a 6-4PP lesion, were synthesized with a 5′-phosphate. The DNA substrate for the Pol β-mediated *in vitro* BER assay was a 3′-^{32}P-end labeled 32 bp duplex DNA that contained the 6-4 PP lesion at positions 9 and 10 of 20D. This was prepared by annealing primers 12YUS and ^{32}P-labeled 20DS to the complementary strand DNA (32COM) to create the nicked 6-4PP

substrate. The DNA substrate for Pol β strand displacement DNA synthesis was constructed by annealing of the ODNs ^{32}P-labeled 12US and 20D with the complementary strand, 32COM, as shown in Table 2.

The DNA substrate for the FEN1 activity determination was constructed by annealing of upstream (16FUS) and downstream (20D) ODNs with the complementary strand (26 COM), as shown in Table 2. This was to create a substrate mimicking a nicked flap containing a 6-4PP lesion. For the production of oligonucleotide duplexes, complementary oligonucleotides were mixed in equal molar ratios in the presence of annealing buffer (10 mM Tris–HCl, pH 7.5, 100 mM NaCl and 10 mM $MgCl_2$), heated to 96 °C, and the mixture was allowed to cool slowly for overnight to room temperature.

***In vitro* Pol β-mediated DNA synthesis**

Pol β-mediated DNA synthesis was determined by measuring nucleotide insertion into a DNA substrate. The 5′-end of the upstream primer of these substrates was radiolabeled with [γ-^{32}P] ATP, as described above. The reaction mixture (30 µl) containing 50 mM Tris-HCl, pH 7.5, 20 mM KCl, 0.5 mM EDTA, 2 mM DTT, 0.1 mg/ml bovine serum albumin, Pol β (50 nM), FEN1 (20 nM), 250 nM DNA, 5 mM $MgCl_2$ and 20 µM each of the dNTPs was assembled on ice. The reaction was initiated by transferring the mixture to 37 °C. Aliquots (9 µl each) were withdrawn at 5-, 10- and 20-min intervals, and the reactions were terminated by the addition of an equal volume of gel-loading buffer (95 % formamide, 20 mM EDTA, 0.02 % bromophenol blue, and 0.02 % xylene cynaol) and incubation at 95 °C for 3 min. The reaction products were separated by electrophoresis in a 17% polyacrylamide gel containing 8 M urea in 89 mM Tris–HCl, pH 8.8, 89 mM boric acid, and 2 mM EDTA. The scanning and imaging of gels were performed by Typhoon PhosphorImager.

The Pol β-mediated DNA synthesis was assessed also with a similar substrate that contained phosphotyrosine (Y) at the 3′-end of the upstream primer. The downstream primer was ^{32}P-labeled at the 5′end. The reaction mixture (10 µl) was supplemented either with Tdp1 (20 nM), PNKP (50 nM), Pol β (50 nM), FEN1(20 nM), 5 mM ATP, with or without DNA ligase I (300 nM), as indicated in the figure legends. Incubation was for 20 min at 37 °C.

***In vitro* FEN1 activity**

The FEN1 enzymatic activity assay was performed under the same reaction conditions as above except that the downstream oligonucleotide (20 FD) of the substrates was radiolabeled at the 5′-end with [γ-^{32}P] ATP. Samples are withdrawn at specified times intervals and the reactions were terminated by the addition of an equal volume of gel-loading buffer. The reaction products were analyzed as above.

***In vitro* BER assay and analysis of 6-4PP lesion repair**

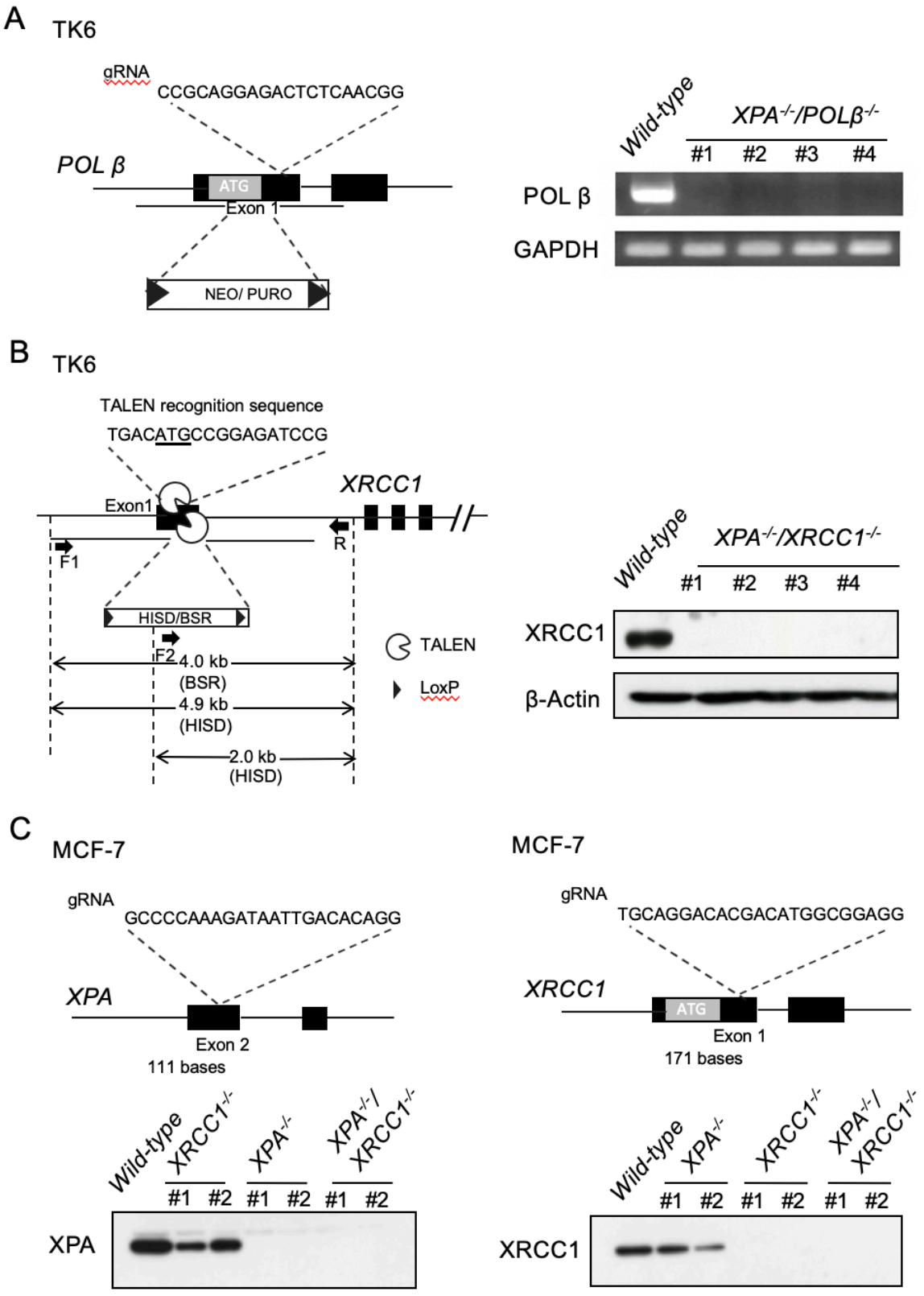
The BER assay was performed either with purified enzymes or cell extracts and the post-proteasome TOP1cc substrate. The reaction mixture (10 µl) contained 50 mM HEPES, pH 7.5, 20 mM KCl, 5 mM $MgCl_2$, 0.5 mM EDTA, 2 mM DTT, 5 mM ATP, 0.5 mM NAD^+, dNTPs (25 µM each), 20 mM dGMP, and 5′- or 3′-^{32}P-labeled 32 bp DNA (250 nM) with a 6-4PP lesion in the downstream ODN. The BER reaction was initiated with a mixture of purified enzymes yielding final reaction mixture concentrations, including

Tdp1 (20 nM), PNKP (50 nM), Pol β (50 nM), FEN1(40 nM), and/or DNA ligase I (300 nM). Extract-based BER reactions were initiated by addition of 15 µg cell extract. Cell extracts were from *XPA*$^{-/-}$ or *XPA*$^{-/-}$ */POL β* $^{-/-}$ TK-6 cells, as indicated in the figure legends. Incubation was at 37 °C. Aliquots were withdrawn from the reaction mixture at the indicated intervals, and the reaction was terminated by addition of an equal volume of DNA gel-loading buffer. The reaction products were analyzed as above.

To evaluate repair of the 6-4PP lesion in the DNA substrate, the BER reaction was conducted either with purified enzymes for 40 min at 37 °C or with the *XPA*$^{-/-}$ cells extract for 60 min at 37 °C under similar reaction conditions, as above. After the incubation, an aliquot was withdrawn for '0' time sample, and the remaining reaction mixture was supplemented with 5 mM $MgCl_2$, 1 mM $MnCl_2$, and UVDE (540 nM). The incubation was continued at 37 °C, and aliquots were withdrawn at 10- and 20-min intervals. The reaction was terminated by addition of an equal volume of DNA gel-loading buffer and the reaction products were analyzed as above.

Western blot analysis

Cells (1×10^6) were lysed in 100 µl sodium dodecyl sulfate (SDS) buffer, containing Tris–HCl (25mM, pH6.5), SDS (1%), β-mercaptoethanol (0.24 mM), bromophenol blue (0.1%), glycerol (5%). Whole-cell extracts were separated by electrophoresis, transferred onto polyvinylidene difluoride membranes and blocked in 5% skimmed milk dissolved in Tween-20 (0.1%) in TBS. Membranes were incubated with primary antibodies overnight at 4° C followed by washing in Tween-20 (0.1%) in TBS. Membranes were incubated with appropriate HRP-linked secondary antibodies at room temperature for 1 h and washed thrice prior to signal detection. Membranes were developed by chemiluminescence using ECL reagent.


A
TK6
gRNA
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POL β
ATG
Exon 1
NEO/ PURO
Wild-type
XPA-/-/POLβ-/-
#1 #2 #3 #4
POL β
GAPDH
B
TK6
TALEN recognition sequence
TGACATGCCGGAGATCCG
XRCC1
Exon1
F1
R
HISD/BSR
F2
4.0 kb
(BSR)
4.9 kb
(HISD)
2.0 kb
(HISD)
TALEN
LoxP
Wild-type
XPA-/-/XRCC1-/-
#1 #2 #3 #4
XRCC1
β-Actin
C
MCF-7
gRNA
GCCCCAAAGATAATTGACACAGG
XPA
Exon 2
111 bases
Wild-type
XRCC1-/-
XPA-/-
XPA-/-/
XRCC1-/-
#1 #2 #1 #2 #1 #2
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MCF-7
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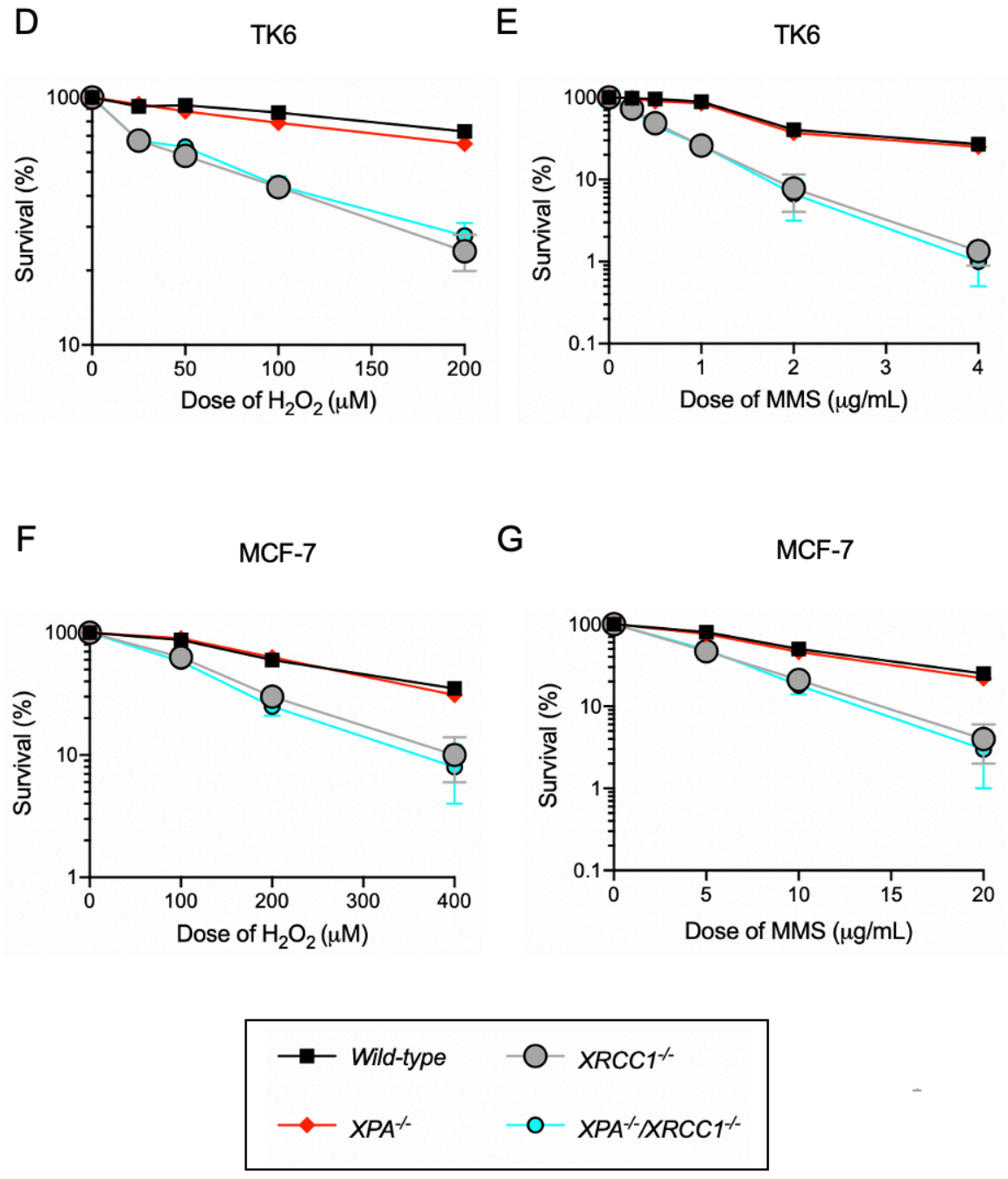


Fig. S1. Generation of *XPA*$^{-/-}$/*POLβ*$^{-/-}$ and *XPA*$^{-/-}$/*XRCC1*$^{-/-}$ TK6 clones, and *XPA*$^{-/-}$, *XRCC1*$^{-/-}$ and *XPA*$^{-/-}$/*XRCC1*$^{-/-}$ MCF-7 clones.

(A) Left panel: Schematic diagram of the *POLβ* locus, target location, and guide RNA (gRNA) sequence. "ATG" indicates the start codon of the *POLβ* gene. Right panel: RT-PCR analysis of *POLβ* in *XPA*$^{-/-}$/*POLβ*$^{-/-}$ TK6 cells. Lane, 2-4 show *POLβ* disruption of four independent clones generating *XPA*$^{-/-}$/*POLβ*$^{-/-}$. GAPDH is a loading control.

(B) Left panel: Schematic diagram of the *XRCC1* locus, target location, and TALEN recognition sequence. "ATG" indicates the start codon of the *XRCC1* gene. *Wild-type* allelic gene shows 2.2-kb PCR products, while HISD and BSR insertion events yield 4.9-

kb and 4.0-kb PCR products, respectively, using a forward primer (F1) designed in the 5-arm and a reverse primer (R) designed outside of the 3′-arm. It shows ~2 kb PCR product using a forward primer (F2) hybridizing with HISD resistance-gene and the reverse primer (R) designed outside of the 3′-arm. Right panel: Western blot analysis of XRCC1 in *XPA*$^{-/-}$*/XRCC1*$^{-/-}$ TK6 clones. Lanes 2-5 show XRCC*1* disruption in four independent *XPA*$^{-/-}$*/XRCC1*$^{-/-}$ clones. *β*-actin is a loading control.
(C) Left panel: Schematic diagram of the *XRCC1* locus, target location, and gRNA sequence. "ATG" indicates the start codon of the *XRCC1* gene. Western blot analysis of XRCC1 in *wild-type* and *XPA*$^{-/-}$ MCF-7 cells. Right panel: Schematic diagram of the *XPA* locus, target location, and gRNA sequence. Western blot analysis of XPA in *wild-type* and *XRCC1*$^{-/-}$ MCF-7 cells.
(D and E) Colony survival of the indicated genotypes following exposure of TK6 cells to H2O2 (D) and MMS (E).
(F and G) Colony survival of TK6 clones having the indicated genotypes following exposure to H_2O_2 (F) and MMS (G). The dose of DNA damaging agents is displayed on the x-axis on a linear scale, while the percentage fraction of surviving cells is displayed on the y-axis on a logarithmic scale. Error bars show the SD of mean for three independent experiments.

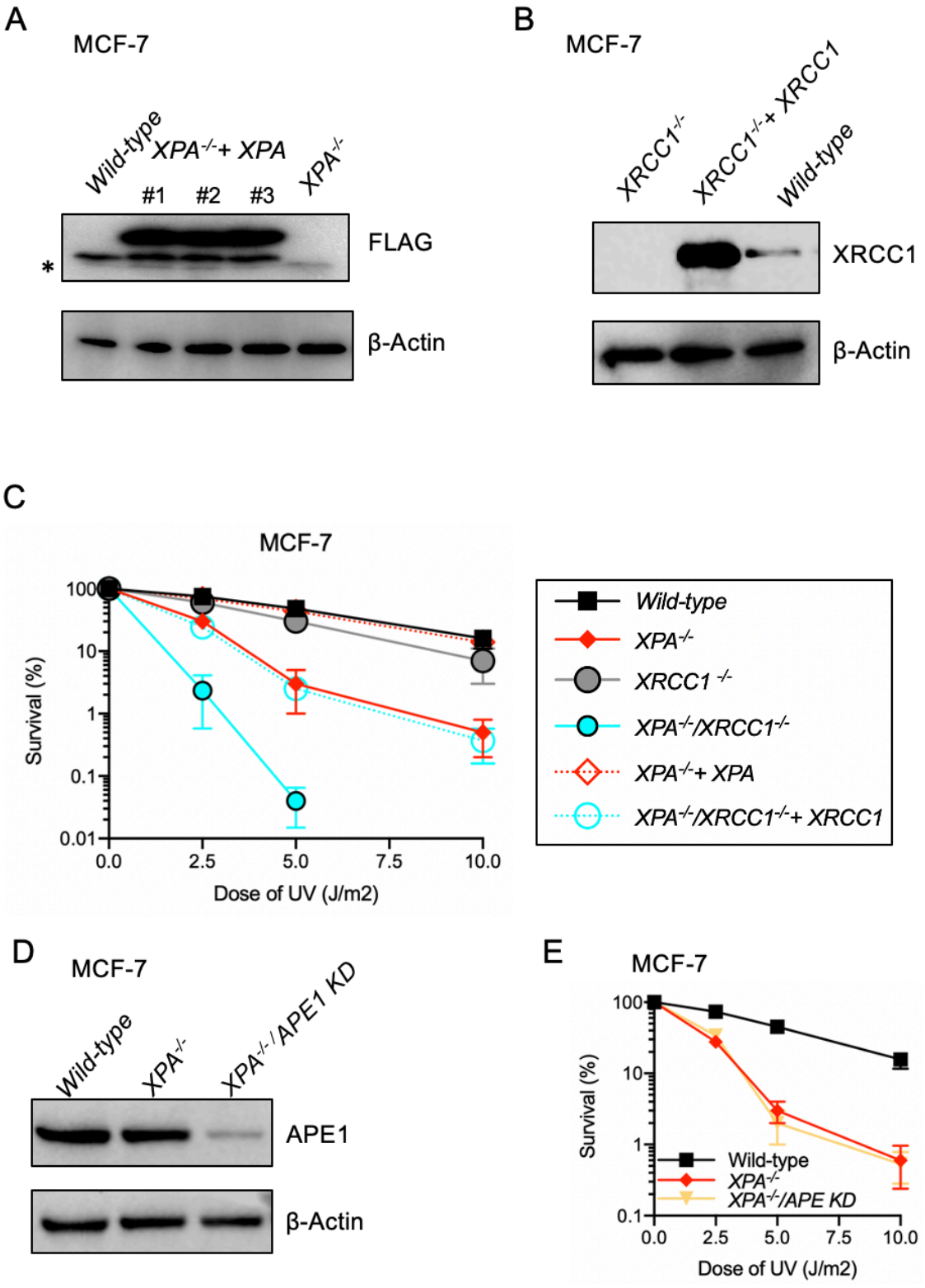


Fig. S2. Ectopic expression of XPA and XRCC1 can rescue the UV sensitivity in MCF-7 cells and effect of APE1 on UV sensitivity

(A and B) Western blot analysis of ectopic expression of XPA (A) and XRCC1(B) in the indicated genotypes and cells. Asterisk(*) indicates non-specific band.
(C) Colony survival of human MCF-7 cells carrying the indicated genotypes following exposure to UV. The dose of DNA damaging agents is displayed on the x-axis on a linear scale, while the percent fraction of surviving cells is displayed on the y-axis on a logarithmic scale. Error bars show the SD of mean for three independent experiments.
(D) Western blot of expression level of APE1 in indicated genotypes and cells
(E) Colony survival of human MCF-7 cells carrying the indicated genotypes following exposure to UV.

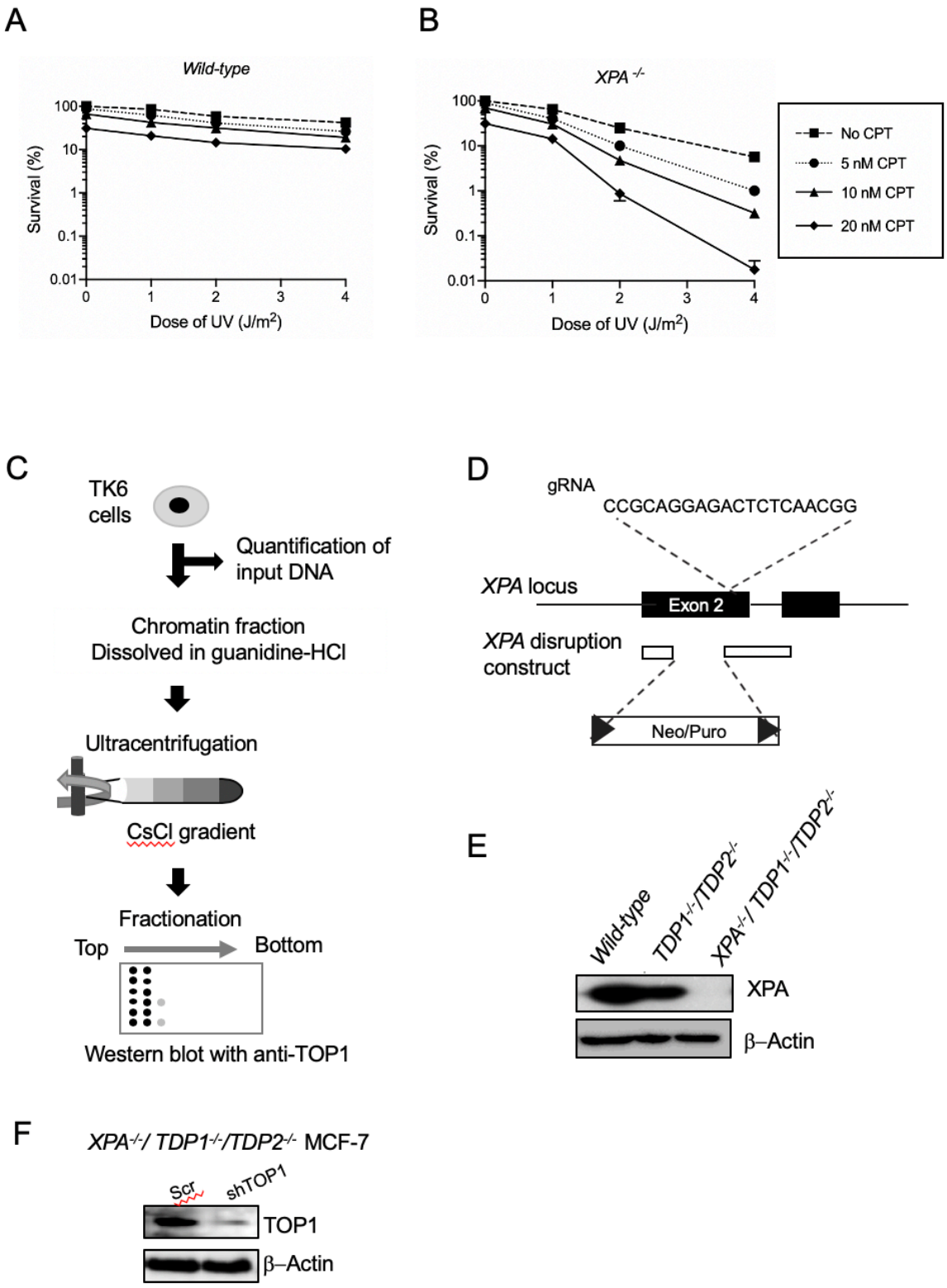
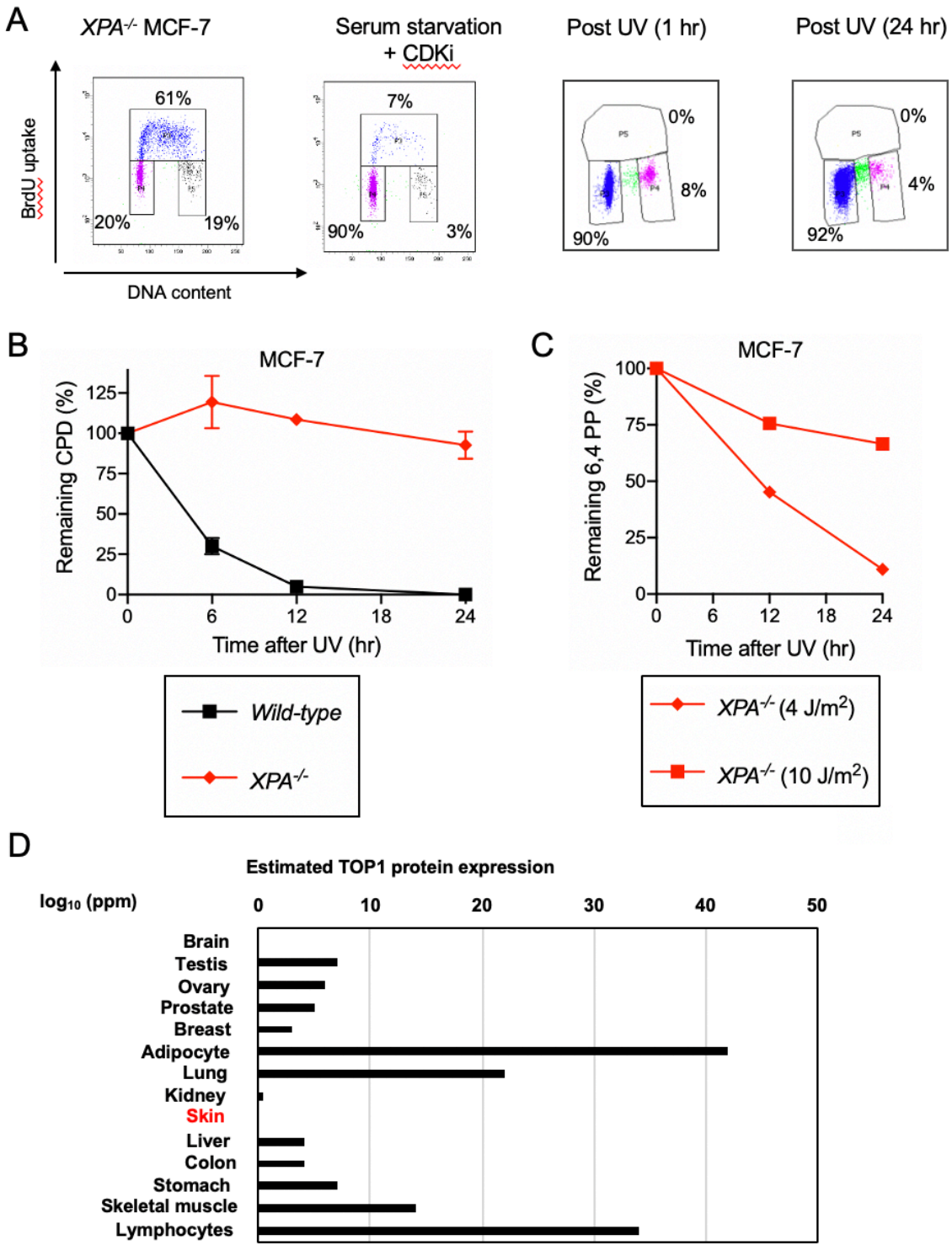


Fig. S3. UV irradiation of NER-deficient cells induce formation of stable TOP1ccs
(A and B) Colony survival of *wild-type* and *XPA*$^{-/-}$ cells following exposure to UV alone or to UV together with the indicated concentrations of camptothecin (CPT), a TOP1 poison. The dose of UV is displayed on the x-axis on a linear scale while the percentage

fraction of surviving cells is displayed on the y-axis on a logarithmic scale. Error bars show the SD of mean for three independent experiments.
(C) Schematic of *in vivo* TOP1cc measurement by immunodetection with α-TOP1 antibody. Genomic DNA (50 μg) from TK6 cells was subjected to sedimentation by CsCl-gradient ultracentrifugation. Genomic DNA from *TDP1*$^{-/-}$/*TDP2*$^{-/-}$ TK6 cells treated with CPT was included as a control for every dot blot of TK6 cells. The CPT treatment reduced cellular survival by only ~1% relative to untreated *wild-type* TK6 cells. Individual fractions were blotted to PVDF filters followed by dot blot using α-TOP1 antibody. The top two fractions include free TOP1 while lower fractions include TOP1ccs.
(D) Schematic diagram of the *XPA* locus, gene disruption construct, and gRNA sequence.
(E) Western blot analysis of XPA. Lanes 1 shows *wild-type* TK6 cells. Lanes 2 and 3 show a successful disruption of *XPA* gene in two independent *TDP1*$^{-/-}$/*TDP2*$^{-/-}$ TK6 clones.
(F) Western blot analysis of expression of TOP1 in the indicated genotypes and cells.


A
XPA-/- MCF-7
Serum starvation
+ CDKi
Post UV (1 hr)
Post UV (24 hr)
BrdU uptake
DNA content
61%
20%
19%
7%
90%
3%
0%
8%
90%
0%
4%
92%
B
MCF-7
Remaining CPD (%)
Time after UV (hr)
Wild-type
XPA-/-
C
MCF-7
Remaining 6,4 PP (%)
Time after UV (hr)
XPA-/- (4 J/m2)
XPA-/- (10 J/m2)
D
Estimated TOP1 protein expression
log10 (ppm)
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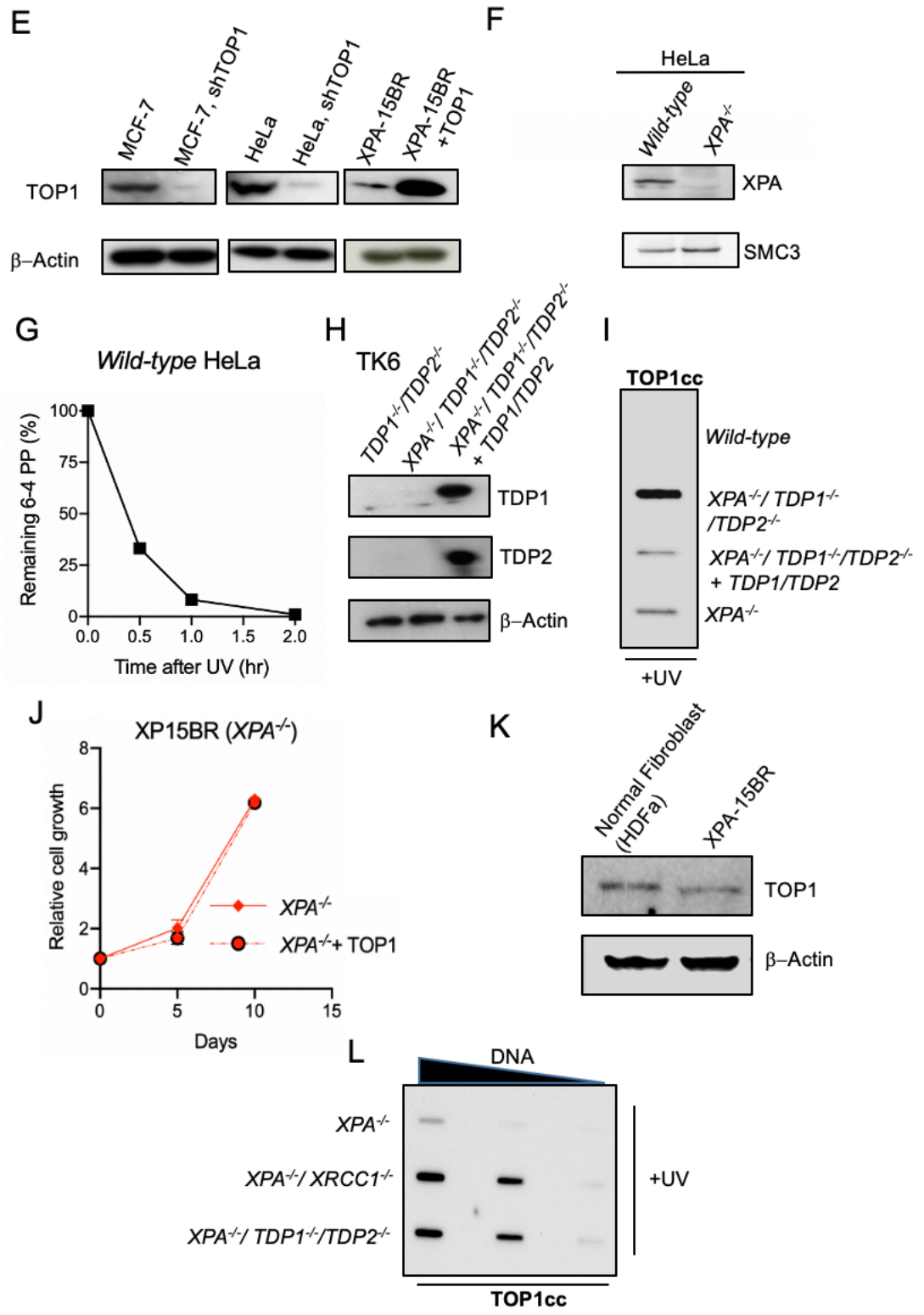

Fig. S4. The expression level of TOP1 correlates with repair kinetics of 6-4PPs in *XPA*-deficient cells

(A) The cell cycle analysis in MCF-7 cells by BrdU-pulse-labeling. The cells were stained with both anti-BrdU antibody (y-axis, logarithmic scale) and propidium iodide (PI) t (x-axis, linear scale). Serum-starved MCF-7 cells treated with CDKi lovastatin (10 nM) for 10 h followed by incubation in serum-free medium for 14 h. The lower-left box identifies G_1-phase cells, the upper box identifies S-phase cells, and the lower-right box identifies G_2/M-phase cells.
(B) The removal kinetics of CPDs in G_1 arrested MCF-7 cells carrying the indicated genotypes. Cells were exposed to 4 J/m^2 UV at time zero, and the number of CPDs in the genomic DNA was measured at the indicated time. The relative amounts of CPDs are shown on the y-axis.
(C) The removal kinetics of 6-4PPs in G_1 arrested *XPA*$^{-/-}$ MCF-7 cells following exposure to 4 J/m^2 and 10 J/m^2 UV.
(D) The GeneCards database indicates that the expression of TOP1 protein is extremely low in the skin and brain in comparison with other tissues. The x axis shows the estimated level of TOP1 protein in $\log_{10}$ppm. Only relevant data are shown.
(E) Western blot analysis of expression of TOP1 in the indicated genotypes and cells.
(F) Western blot analysis of expression of XPA in the indicated genotypes and cells.
(G) The removal of 6-4PPs in G_1-arrested wild-type HeLa cells. Experiments were done as in Figure 3A and 3B.
(H) Western blot analysis of expression of TDP1 and TDP2 in the indicated genotypes and cells.
(I) Representative slot blot of quantitating the number of stable TOP1 cleavage complexes (TOP1ccs) in TK6 cells carrying the indicated genotypes having treated with UV.
(J) Relative cell growth curve of indicated genotypes and cells.
(K) Western blot analysis of expression of TOP1 in the indicated genotypes and cells.
(L) Representative slot blot of performing serial dilution of pool fractions of TOP1ccs in TK6 cells carrying the indicated genotypes having treated with UV.

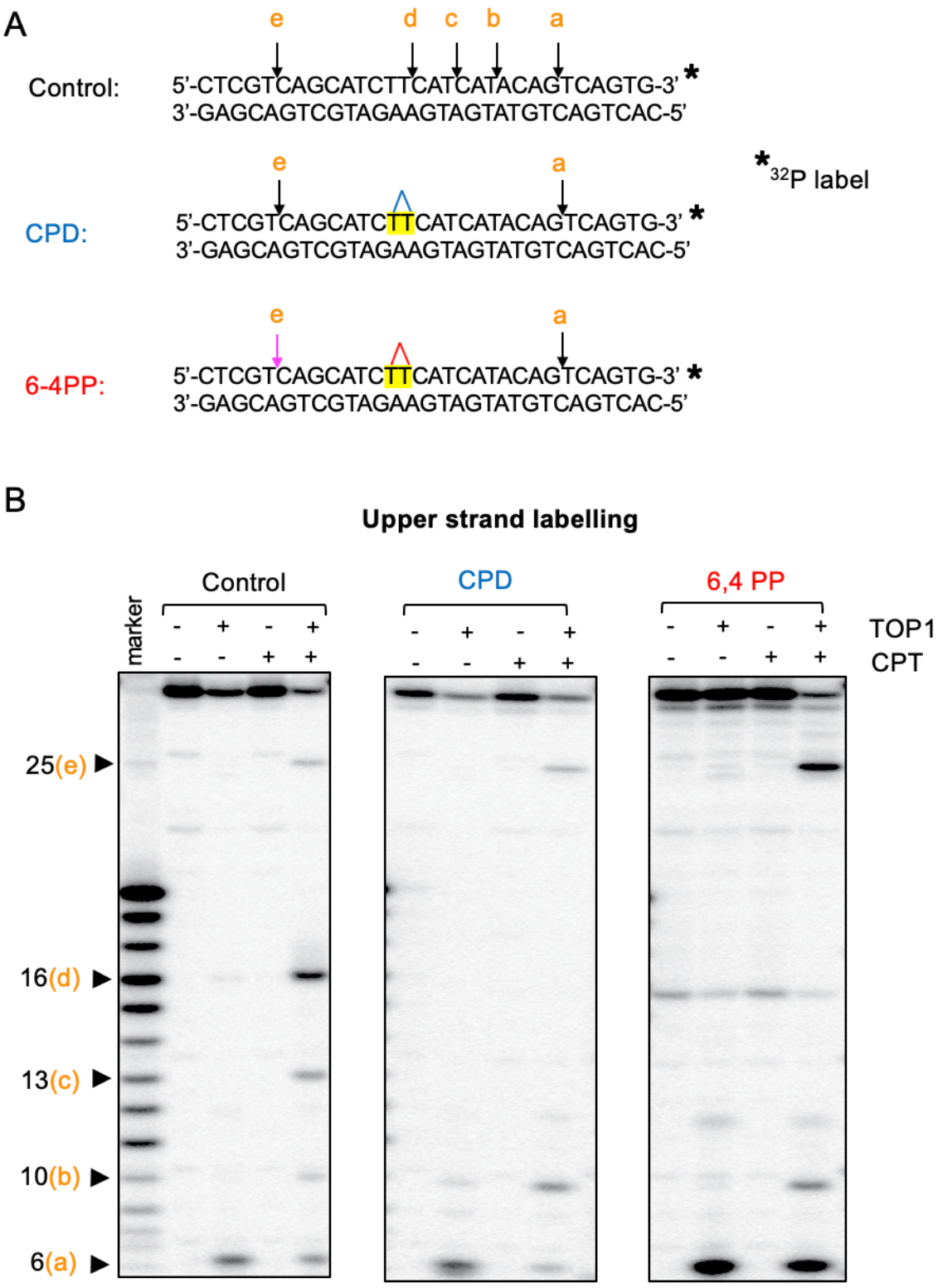

Fig. S5. *In-vitro* formation of TOP1ccs in duplex oligonucleotide DNA containing UV lesions

(A) Schematic representation of oligonucleotide sequences carrying either a single CPD or 6-4PP and control oligonucleotide sequences. The downward arrows show each TOP1

cleavage site detected in (B) with annotation of a to e, which correspond to (a) to (e) in (B). The red arrow indicates the most prominent TOP1 cleavage site. Star indicates the position of 3′-end ^{32}P label in the upper strand.
(B) Detection of stable TOP1cc sites on the three oligonucleotides shown in (A) by the denaturing polyacrylamide gel electrophoresis followed by autoradiography. These DNAs were incubated with purified TOP1 with or without 1 μM camptothecin (CPT) before the gel electrophoresis. Each lane of the three panels contains reaction mixture contains DNA alone, DNA incubated with TOP1, DNA incubated with CPT, and DNA incubated with both TOP1 and CPT. The numbers shown with annotation at arrowheads correspond to the numbers shown at arrows in (A).

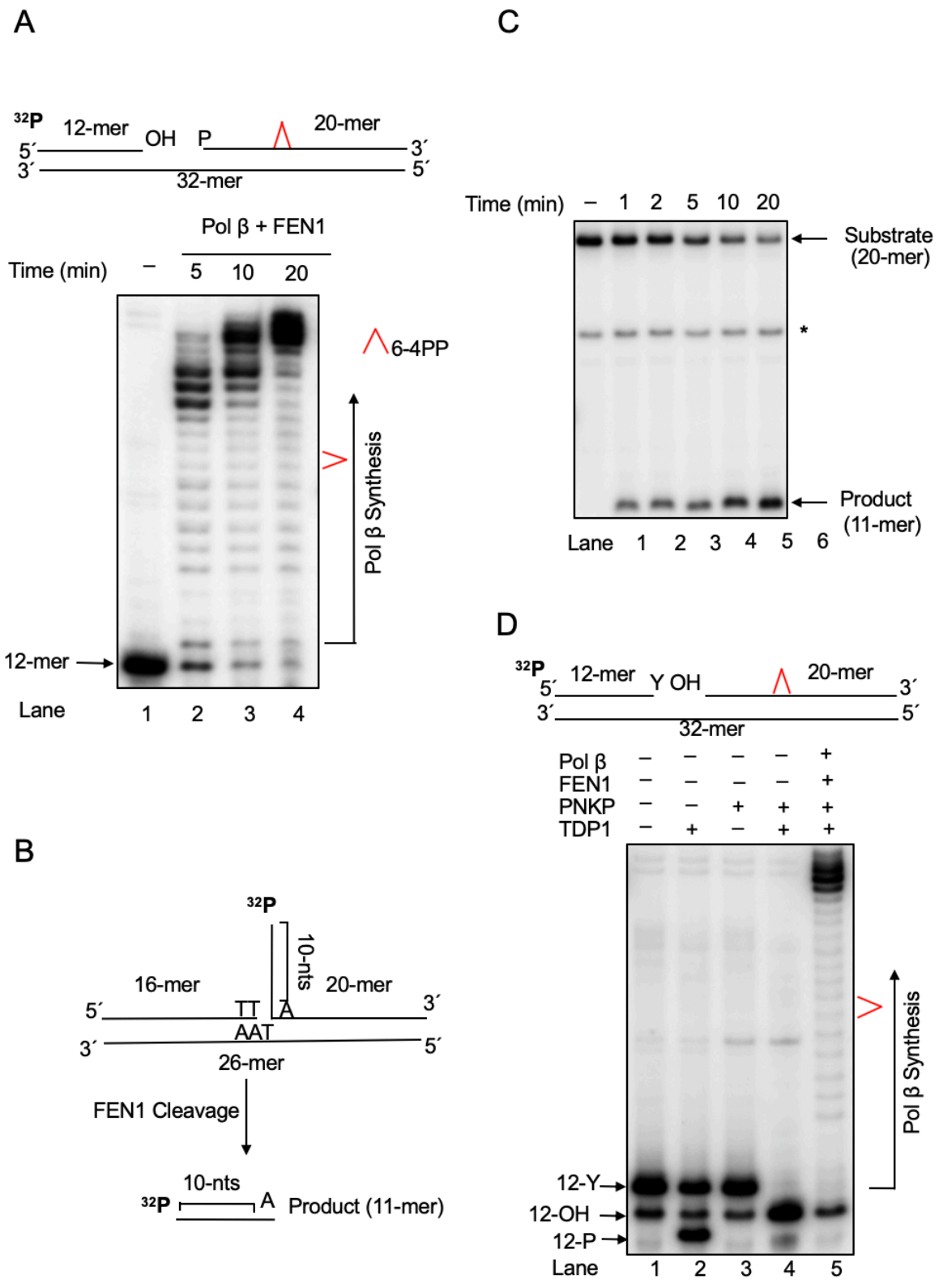
A
32P
12-mer
OH
P
20-mer
5′
3′
3′
5′
32-mer
Pol β + FEN1
Time (min)
–
5
10
20
6-4PP
Pol β Synthesis
12-mer
Lane
1
2
3
4
B
32P
10-nts
16-mer
20-mer
5′
TT
A
3′
AAT
3′
5′
26-mer
FEN1 Cleavage
10-nts
32P
A
Product (11-mer)
C
Time (min)
–
1
2
5
10
20
Substrate
(20-mer)
*
Product
(11-mer)
Lane
1
2
3
4
5
6
D
32P
5′
12-mer
Y OH
20-mer
3′
3′
5′
32-mer
Pol β
FEN1
PNKP
TDP1
12-Y
12-OH
12-P
Lane
1
2
3
4
5
Pol β Synthesis

Fig. S6. *In vitro* long-patch BER synthesis by Pol β and FEN1 removes the 6-4PP lesion

(A) Strand displacement DNA synthesis by Pol β and FEN1 in the repair substrate carrying both a single 6-4PP lesion and a single strand break (SSB) (Top). The substrate was labeled with ^{32}P at the 5′-end of the 12 nucleotides DNA primer. The arrow pointing towards the top indicates the length of DNA fragments synthesized from the SSB. Lane 1 depicts the reaction mixture without Pol β and FEN1, and also indicates the position of the ^{32}P-labeled 12-mer. Lanes 2, 3 and 4 illustrate the reaction mixture incubated with Pol β and FEN1 for 5-, 10-, and 20-min, respectively. The position of the 6-4PP lesion is marked by the red arrowhead. Schematic representation of the repair substrate is shown on top, and schematic representation of strand replacement by Pol β and FEN1 is shown in Fig. 5*A*.

(B) Model substrate for strand displacement DNA synthesis and FEN1 activity. A 26 bp duplex DNA with a nick was prepared by annealing a 26-mer template with a 16-mer upstream primer and 20-mer downstream primer containing normal TT in place of the 6-4PP lesion at positions 9 and 10. Thus, the annealed product will have a 10 nt long flap with 8 nts plus TT at positions 9 and 10, mimicking the 6-4PP lesion. The results are consistent with strand displacement DNA synthesis by Pol β, which strand displacement generates a 10 nt long flap. This flap is incised by FEN, generating an 11 nt product (10 nts flap plus one more nucleotide).

(C) A time course analysis of FEN1 cleavage activity on a model substrate (B) is shown. The positions of the ^{32}P-labeled 20-mer and the 11-mer FEN1 cleavage product are indicated. * indicates a nonspecific band in the substrate.

(D) Strand displacement DNA synthesis by Pol β and FEN1 in the repair substrate carrying both a single 6-4PP lesion and a SSB covalently associated with tyrosine (Y) residue at the 3′-end of the SSB (Top). This Y mimics the post-proteasome TOP1cc nicked DNA substrate. The 5′-end of the 20-mer had a hydroxy residue. TDP1 removes the tyrosine residue generating 12-P (lane 2), and PNKP cleaves the 3′phosphate (lane 4, 12-OH) and also phosphorylates the 5′-end of downstream primer (not seen). Pol β in association with FEN1 performs strand displacement DNA synthesis as in (A). Schematic representation of a nicked DNA substrate containing Y at the 3′-end of the 12-mer primer, and the 6-4PP lesion (TT dimer) at positions 9 and 10 in the downstream primer is illustrated on the top of the phosphorimage. The same repair substrate was used in Fig. 5.

Table S1. List of mutant genotypes used in the study

Genotype	Name of cell lines and species	Markers genes	Source
XPA$^{-/-}$	Human TK6 (TSCER2)	*puro*^{R}, *hygro*^{R}	(2)
XRCC1$^{-/-}$	Human TK6 (TSCER2)	*bsr*^{R}, *his*^{R}	(12)
POLβ$^{-/-}$	Human TK6 (TSCER2)	*puro*^{R}, *his*^{R}	This study
XPA$^{-/-}$/*XRCC1*$^{-/-}$	Human TK6 (TSCER2)	*bsr*^{R}, *his*^{R}	This study
XPA$^{-/-}$/*POLβ*$^{-/-}$	Human TK6 (TSCER2)	*puro*^{R}, *his*^{R}	This study
TDP1$^{-/-}$/*TDP2*$^{-/-}$	Human TK6 (TSCER2)	*puro*^{R}, *neo*^{R}, *hygro*^{R}	(13)
XPA$^{-/-}$/*TDP1*$^{-/-}$/*TDP2*$^{-/-}$	Human TK6 (TSCER2)	*puro*^{R}, *neo*^{R}	This study
XPA$^{-/-}$	Human MCF-7	-	This study
XRCC1$^{-/-}$	Human MCF-7	-	This study
XPA$^{-/-}$/*XRCC1*$^{-/-}$	Human MCF-7	-	This study
XPA$^{-/-}$	Human HeLa	-	This study
XP15BR-XPA	Human Primary Skin Fibroblasts	-	(14)

Table S2. Quantification of genomic DNA used for Top1 trapping

Cell lines	CPT	UV	#1 (μg/mL)	OD (260/280)	#2 (μg/mL)	OD (260/280)	#3 (μg/mL)	OD (260/280)
TDP1$^{-/-}$/*TDP2*$^{-/-}$	+	-	60.5	1.92	62.5	1.89	61.4	1.87
XRCC1$^{-/-}$	-	-	60.0	1.88	61.0	1.83	61.0	1.88
	-	+	60.8	1.86	61.8	1.96	60.4	1.89
XPA$^{-/-}$	-	-	61.0	1.95	60.9	1.93	60.6	1.98
	-	+	60.9	1.84	63.0	1.95	61.5	1.95
XPA$^{-/-}$/*XRCC1*$^{-/-}$	-	-	61.5	1.89	61.0	1.86	62.0	1.93
	-	+	60.9	1.88	60.5	1.89	60.7	1.92

Table S3. Quantification of genomic DNA used for Top1 trapping

Cell lines	CPT	UV	#1 (µg/mL)	OD (260/280)	#2 (µg/mL)	OD (260/280)	#3 (µg/mL)	OD (260/280)
Wild-type	-	-	60.9	1.89	60.6	1.86	62.0	1.84
	-	+	60.7	1.86	61.2	1.91	60.8	1.90
TDP1$^{-/-}$/TDP2$^{-/-}$	-	-	62.5	1.89	60.6	1.93	61.6	1.93
	-	+	61.8	1.96	61.0	1.93	61.1	1.88
XPA$^{-/-}$/ TDP1$^{-/-}$/TDP2$^{-/-}$	-	-	62.0	1.89	60.8	1.85	59.8	1.86
	-	+	61.4	1.93	61.6	1.88	59.7	1.87

Table S4. Oligodeoxynucleotide sequences used in this study for in-vitro BER

Designation	Sequences
12US	5’-GCACGGTGCCTGpY- 3’
20D	5’-CCTACCTTTTAAAGGCATCC-3’
32COM	5’-GGATGCCTTTAAAAGGTAGGCAGGCACCGTGC- 3’
16FUS	5’-TGCCTGCCTACCTTTT-3’
20FD	5’-TTTTTTTTTTAAAGGCATCC-3’
26COM	5’-GGATGCCTTTAAAAGGTAGGCAGGCA-3’

Table S5. List of primers used in this study

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Oligonucleotides (gRNA or TALEN sequences)		
TK6, *XRCC1*, TALEN A, reverse (for gene disruption), 5'-GTCCTGCAGCAGCCAGGA -3'		
TK6, *XPA*, gRNA (for gene disruption), 5'-GCCCCAAAGATAATTGACAC-3'	Eurofin	N/A
TK6, *POLβ*, gRNA (for gene disruption), 5'-GGAATCACCGACATGCTCAC-3'	Eurofin	N/A
MCF-7, *XRCC1*, gRNA (for gene disruption), 5'-TGCAGGACACGACATGGCGG-3'	Eurofin	N/A
MCF-7, *XPA*, gRNA#1 (for gene disruption), 5'-GCCCCAAAGATAATTGACAC-3'	Eurofin	N/A
MCF-7, *XPA*, gRNA#2 (for gene disruption), 5'-CGGTGCGGGCGAGTATCGAG-3'	Eurofin	N/A
Oligonucleotides (The construction of targeting vectors)		
TK6, *XRCC1* (for gene disruption), forward primer of left arm, 5'-GCGAATTGGGTACCGGGCCGTAGTAAAAGACAGATGCC-3'	Eurofin	N/A
TK6, *XRCC1* (for gene disruption), forward primer of right arm, 5'-TGGGAAGCTTGTCGACTTAAGACGTGAGGGAATTAATGAG-3'	Eurofin	N/A
TK6, *XRCC1* (for gene disruption), reverse primer of left arm, 5'-CTGGGCTCGAGGGGGGGCCCTGGCCAGAAGGATGAGGT-3'	Eurofin	N/A
TK6, *XRCC1* (for gene disruption), reverse primer of right arm, 5'-CACTAGTAGGCGCGCCTTAAAACCACCATACCTGGCTATT-3'	Eurofin	N/A
TK6, *XRCC1* (for genotyping PCR), 5'-AACTCCTGGGCTCATGCAATACTCCAGCTT-3'	Eurofin	N/A
TK6, *POLβ* (for gene disruption), forward primer of left arm, 5'-GCGAATTGGGTACCGGGCCTCTGAATATCACCTGGAATAGAG ATTGGAC-3'	Eurofin	N/A
TK6, *POLβ* (for gene disruption), forward primer of right arm, 5'-TGGGAAGCTTGTCGACTTAAACAGTGCAGCATTCTCGGGTAGC ATACGTT-3'	Eurofin	N/A
TK6, *POLβ* (for gene disruption), reverse primer of left arm, 5'-	Eurofin	N/A

CTGGGCTCGAGGGGGGGCCCCCAGAGTGTTCAGAACCAGGG ACTAGAGC-3’		
TK6, *POL*β (for gene disruption), reverse primer of right arm, 5’- CACTAGTAGGCGCGCCTTAACACAGATTTGGAGTCACTGACAT AAGTGGC-3’	Eurofin	N/A
TK6, *POL*β (for genotyping PCR), 5’- AGCACAATCAGCTCTGAACTGTGTGGAGTG-3’	Eurofin	N/A
TK6, *POL*β (for RT-PCR), forward primer, 5’- AAGTCCTGGTACCTCCTTCAAGCTC-3’	Eurofin	N/A
TK6, *POL*β (for RT-PCR), reverse primer, 5’- GAAACTGCCACAGACTGTAGCAATG-3’	Eurofin	N/A
TK6, β-*ACTIN* (for RT-PCR), reverse primer, 5’- ATGCTCACAGAACTCGCAAACTTTG-3’	Eurofin	N/A
TK6, β-*ACTIN* (for RT-PCR), reverse primer, 5’- GAAACTGCCACAGACTGTAGCAATG-3’	Eurofin	N/A
NEO^R_F (for genotyping PCR), 5’- AACCTGCGTGCAATCCATCTTGTTCAATGG-3’	Eurofin	N/A
PURO^R_F (for genotyping PCR), 5’- GTGAGGAAGAGTTCTTGCAGCTCGGTGA-3’	Eurofin	N/A
HYGRO^R_F (for genotyping PCR), 5’- TCCAGGATCCAATCAAGGGTCAGGTATTAC-3’	Eurofin	N/A
BSR^R_F (for genotyping PCR), 5’- AACCTGCGTGCAATCCATCTTGTTCAATGG-3’	Eurofin	N/A
HIS^R_F (for genotyping PCR), 5’- GAATTGCCGCTCCCACATGATGTTTATTAT-3’	Eurofin	N/A
Oligonucleotides (shRNA sequences for pLKO.1 vector)		
TOP1, forward primer, 5’- CCGGCATAGCAACAGTGAACATAAACTCGAGTTTATGTTCACT GTTGCTATGTTTTTG -3’	Eurofin	N/A
TOP1, reverse primer, 5’- AATTCAAAAACATAGCAACAGTGAACATAAACTCGAGTTTATG TTCACTGTTGCTATG -3’	Eurofin	N/A
APE1, forward primer, 5’- CCGGCGGGTGATTGTGGCTGAATTTCTCGAGAAATTCAGCCAC AATCACCCGTTTTTG -3’	Eurofin	N/A
APE1, reverse primer, 5’- AATTCAAAAACGGGTGATTGTGGCTGAATTTCTCGAGAAATTC AGCCACAATCACCCG	Eurofin	N/A

-3’		
Oligonucleotides (sequences for lentiCRISPRv2 hygro vector)		
TOP1, forward primer, 5’- CGCCAGAACACAGGACCGGTTCTAGAGCATGAGTGGGGACCACCTCCACAACG -3’	Eurofin	N/A
TOP1, reverse primer, 5’- GCAGAGAGAAGTTTGTTGCGCCGGATCCTAAAACTCATAGTCTTCATCAGCC -3’	Eurofin	N/A
TDP1, forward primer, 5’- CGCCAGAACACAGGACCGGTTCTAGAGCATGTCTCAGGAAGGCGATTATGGGA -3’	Eurofin	N/A
TDP1, reverse primer, 5’- GCAGAGAGAAGTTTGTTGCGCCGGATCTCAGGAGGGCACCCACATGTTCCCA -3’	Eurofin	N/A
TDP2, forward primer, 5’- CGCCAGAACACAGGACCGGTTCTAGAGCATGGAGTTGGGGAGTTGCCTGGAGG -3’	Eurofin	N/A
TDP2, reverse primer, 5’- GCAGAGAGAAGTTTGTTGCGCCGGATCTTACAATATTATATCTAAGTTGCAC -3’	Eurofin	N/A
XRCC1, forward primer, 5’- CGCCAGAACACAGGACCGGTTCTAGAGCATGCCGGAGATCCGCCTCCGCCATG -3’	Eurofin	N/A
XRCC1, reverse primer, 5’- GCAGAGAGAAGTTTGTTGCGCCGGATCTCAGGCTTGCGGCACCACCCCATAG -3’	Eurofin	N/A
XPA, forward primer, 5’- CGCCAGAACACAGGACCGGTTCTAGAGCATGGCGGCGGCCGACGGGGCTTTGC -3’	Eurofin	N/A
XPA, reverse primer, 5’- GCAGAGAGAAGTTTGTTGCGCCGGATCTCACATTTTTTCATATGTCAGTTCA -3’	Eurofin	N/A

Table S6. List of reagents and resources used in this study

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Mouse monoclonal α-TOP1	BD Biosciences	Cat# 556597, RRID: AB_396474
Rabbit polyclonal α-XPA	Abcam	Cat# sc-596, RRID: AB_883576
Mouse monoclonal α-XRCC1	Abcam	[33-2-5] (ab1838) RRID: AB_ 302636
Rabbit polyclonal α-APE1	Abcam	Cat# ab137708, RRID: AB_2732912
Mouse monoclonal α -FLAG® M2	Sigma	Cat# F1804 RRID: AB_262044
Rabbit polyclonal α-TDP1	Abcam	Cat# ab4166, RRID: AB_304337
Rabbit polyclonal α-TDP2	Bethyl	Cat# A302-737A, RRID: AB_10631698
Mouse monoclonal α-β-actin	Sigma	Cat# A5411
Goat polyclonal α-mouse HRP	Santa Cruz	Cat# sc-2314, RRID: AB_641170
Goat polyclonal α-rabbit HRP	Santa Cruz	Cat# sc-2313, RRID: AB_641181
Chemicals, Peptides, and Recombinant Proteins		
Charcoal/Dextran treated FBS	Hyclone Laboratories	Cat#SH30068.03
Phenol-red-free DMEM		
Giemsa's Stain Solution	Nacalai Tesque	Cat# 377114-35
Albumin, Bovine, F-V, pH5.2	Nacalai Tesque	Cat# 01863-48
Skim Milk for immunoassay	Nacalai Tesque	Cat# 31149-75

Lipofectamine 3000 Transfection Kit	ThermoFischer	Cat# L3000008
FuGENE HD Transfection Reagent	Promega	Cat# E2312
Lovastatin	Enzo Life Sciences	Cat# BML-G226-0010
MMS	Nacalai Tesque	Cat# 19119-61
H_2O_2	Trevigen	Cat# 4886-400-01
UV	In house	UVC 254 nm
Camptothecin	TopoGEN	TG4110
2.5 g/l-Trypsin/1 mmol/l-EDTA Solution	Nacalai Tesque	Cat# 35554-64
Critical Commercial Assays		
GeneArt Seamless Cloning Enzyme Mix	ThermoFischer	Cat# A14606
Experimental Models: Cell Lines and Primary Fibroblasts		
Human: MCF-7 *WT*	ATCC	Cat# HTB-22
Human: TK6 (TSCER2) *WT*	A gift from Dr. Masamitsu Honma (15)	N/A
Human: Lenti-X^{TM} 293T	TAKARA	Cat# 632180
Human: HeLa *WT*	A gift from Dr. Toomo Ogi (14)	N/A
Human: HDFa	ATCC	Cat# PCS-201-012
Human: XP15BR (XP-A)	A gift from Dr. Toomo Ogi (14)	N/A
Experimental Models: Organisms/Strains		
The mutant genotypes of TK6, MCF7, HeLa and primary fibroblast are listed in Table S1	This study	
Oligonucleotides		
The primers are listed in Table S4	This study	
The primers are listed in Table S5	This study	

Recombinant DNA		
Plasmid: px330-U6-Chimeric_BB-CBh-hSpCas9	Addgene	Cat# 42230
Plasmid: *DT-ApA/MARKER^{R}*	CDB, RIKEN, Kobe	N/A
Plasmid: pSpCas9(BB)-2A-Puro(pX459)	(16)	Cat# 48139
Plasmid: pLKO.1	(Hoa et al., 2016)	Cat#8453
Plasmid: pMD2.G	Addgene	Cat #12259
Plasmid: pMDLg/pRRE	Addgene	Cat#8453
Plasmid: pRSV-Rev	Addgene	Cat#12253
Plasmid: lentiCRISPRv2 hygro	Addgene	Cat#98291

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