Supplementary Information

Materials and Methods

Cell culture

Chicken DT40 cells were cultured at 39.5 °C in a 5 % CO_2 atmosphere in RPMI1640 medium supplemented with 10 % heat-inactivated fetal bovine serum (FBS), 1 % chicken serum, 10 μM β-mercaptoethanol, 100 U/ml penicillin, 100 μg/ml streptomycin and 2 μmol/ml l-glutamine.

TK6 cells were grown in RPMI 1640 medium (Nacalai Tesque Inc., Kyoto, Japan) supplemented with 10% heat-inactivated horse serum (Gibco, Life technologies, New Zealand), 200 μg/ml sodium pyruvate and 100 U/ml penicillin, 100 μg/ml streptomycin at 37^{O} C in 5% CO_2 atmosphere. All mutants analysed in this manuscript are shown in Table S1.

Construction of gene-disrupted DT40 clones

Targeting constructs were designed and prepared as described previously (1), using standard molecular biology techniques. Targeting constructs were linearized by overnight digestion with *Sca*I and purified by ethanol precipitation and chloroform extraction. *Wild-type* DT40 cells were electroporated with 40 μg targeting construct, diluted in 96-well plates and incubated for six days. Single colonies were collected for further analysis.

The targeting construct for the *PIAS1* gene was constructed in the following manner. An upstream region of homology was amplified using primers F1 and R1 (Table S2) and ligated into pTOPO. This plasmid was digested with *Sal*I and *Bam*HI and ligated into the corresponding sites of pBS to create pMS1. The downstream region of homology was amplified using primers F2 and R2, ligated into pTOPO, digested with *Bam*HI and *Not*I, and ligated into the same sites of pBS to create pMS2. The *Bam*HI-*Not*I fragment from pMS2 was ligated into pMS1 digested with the same enzymes. The blasticidin and puromycin resistance genes were cloned into the *Bam*HI site to generate the final targeting constructs.

To create the *PIAS4* targeting construct, an upstream region of homology was amplified by PCR using primers F3 and R3 and cloned into pTOPO. The plasmid was

digested with *Xho*I and *Bgl*II and the fragment was ligated into the same sites in pBS. The downstream arm of homology was amplified using primers F4 and R4, ligated into pTOPO, digested with *Spe*I and *Bam*HI and ligated into the same sites in pBS. The drug resistance markers were isolated from their vectors with *Bam*HI and cloned into the same site in pMS3 to create pBS::Right::Drug. The Right::Drug fragment was liberated by digestion with *Eco*RI whilst pBS::Left was digested with *Bgl*II and *Xho*I. Both DNAs were treated with the Klenow fragment and ligated to generate the targeting constructs.

The *PIAS1* targeting constructs contained the same drug resistance markers as the *PIAS4* targeting constructs. The drug resistance markers are flanked by *lox*P sites, and were thus excised from *PIAS4*$^{-/-}$ cells by transfecting them with cre-recombinase plasmid using Amaxa transfection system followed by incubating 1×10^6 cells/ml with 200 nM tamoxifen to activate the Cre recombinase. This generated the strain *PIAS4*$^{-/-}$*/cre*, which was used for the construction of the *PIAS1*$^{-/-}$*/PIAS4*$^{-/-}$ double mutant. All primers used in this study are shown in Table S2. We confirmed that the desired mutants had been obtained by Southern blot analysis and reverse transcription PCR (Fig. S1)

Disruption of *XPA* gene in TK6 cells

XPA gene disruption constructs for TK6 cells, *XPA-his*^{R} and *XPA-hygro*^{R} were generated from genomic PCR products combined with *his*^{R} and *hygro*^{R} selection marker genes. Genomic DNA sequences were amplified using the following primers: F16 and R16 plus F17 and R17 for the left arm and right arm, respectively. Left arm and right arm was inserted into *Apa*I and *Afl*II site of DT-A-pA/loxP/PGK-*hygro*^{R}-pA/loxP, respectively, to create *XPA-hygro*^{R} using GENEART Seamless Cloning (Life Technologies, Palo Alto, CA). Similar to *XPA- hygro*^{R}, *XPA-his*^{R} was generated using DT-A-pA/loxP/PGK-*his*^{R}-pA/loxP. *XPA*$^{-/-}$ TK6 cells were generated using clustered regularly interspaced short palindromic repeat (CRISPR) (2). Briefly, guide sequence was inserted into the pX330 vector. The CRISPR-target site is depicted in Fig. S2. TK6 cells were transfected with 2 μg each of targeting vectors (*XPA-hygro*^{R} and *XPA-his*^{R}) and 6 μg of the guide sequence-containing pX330 vector using NEON Transfection System (Life Technologies) according to the manufacture's instructions. After 48 h, the cells were plated in 96-well plates, and then subjected to hygromycin

and L-histidinol. The drug-resistant cell colonies were picked on days 7-10 after transfection. The loss of *XPA* transcript was confirmed by RT-PCR using F18 and R18 primers.

Disruption of *POLH* gene in TK6 cells

POLH gene disruption constructs for TK6 cells, *POLH-neo*^{R} and *POLH-puro*^{R} were generated from genomic PCR products combined with *neo*^{R} and *puro*^{R} selection marker genes. Genomic DNA sequences were amplified using the following primers: F13 and R13 plus F14 and R14 for the left arm and right arm, respectively. Left arm and right arm was inserted into *Apa*I and *Afl*II site of DT-A-pA/loxP/PGK-*neo*^{R}-pA/loxP, respectively, to create *POLH-neo*^{R} using GENEART Seamless Cloning (Life Technologies). Similar to *POLH-neo*^{R}, *POLH-puro*^{R} was generated using DT-A-pA/loxP/PGK-*puro*^{R}-pA/loxP. *POLH*$^{-/-}$ TK6 cells were generated using clustered regularly interspaced short palindromic repeat (CRISPR) (2). Briefly, guide sequence was inserted into the pX330 vector. The CRISPR-target site is depicted in Fig. S2. TK6 cells were transfected with 2 μg each of targeting vectors (*POLH-neo*^{R} and *POLH-puro*^{R}) and 6 μg of the guide sequence-containing pX330 vector using NEON Transfection System (Life Technologies) according to the manufacture's instructions. After 48 h, the cells were plated in 96-well plates, and then subjected to puromycin and neomycin. The drug-resistant cell colonies were picked on days 7-10 after transfection. For the preparation of a probe for Southern blot analysis of gene-disrupted clones, a 0.5 kb genomic DNA fragments was amplified by the F15 and R15 primers. The loss of *POLH* transcript was confirmed by Southern blot analysis.

Disruption of *PIAS4* gene in TK6 cells

To generate human *PIAS4*$^{-/-}$ TK6 B cells, we designed a guide RNA targeting 9th exon using the Zhang CRISPR tool (2) and gene-targeting constructs. The CRISPR-target site is depicted in Fig. S2. The gene-targeting constructs were generated using SLiCE (Seamless Ligation Cloning Extract). The genomic DNA was amplified with primers F5 and R5 from the *PIAS4*-gene locus and the PCR product was used as template DNA for amplifying the 5' arm. The 5'-arm was amplified using primers F6 and R6, where each primer shared 20-base pair-end homology with the insertion site of the vector. The 3'-arm was amplified using primers F7 and R7, where each primer shared 20-base pair-end homology with the insertion site of the vector. Both vectors, DT-

ApA/neo and DT-ApA/hygro, were linearized with *Not*I and *Xba*I. All the fragments of the vectors and inserts were purified using a qiaquick gel extraction kit (QIAGEN, Netherlands). The gene-targeting constructs were generated in a single reaction mixture containing DT-ApA/neo or DT-ApA/hygro vectors, 5'- and 3'-arms, and 2×SLiCE buffer (Invitrogen, US) and incubated for 30 min at room temperature. 6 μg of CRISPR and 2 μg of each gene-targeting vector were transfected into 4×10^6 TK6 cells using the Neon Transfection System (Life Technologies, US). After electroporation, cells were released into 20 ml drug-free medium containing 10% horse serum. Forty-eight hours later, cells were seeded into 96-well plates for selection with both neomycin and hygromycin antibiotics for two weeks. The gene disruption was confirmed by RT-PCR using primers F8 and R8.

Disruption of *PIAS1* gene in TK6 cells

To generate human *PIAS1*$^{-/-}$ TK6 B cells, we designed a guide RNA targeting 8th exon using the Zhang CRISPR tool (2) and gene-targeting constructs. The CRISPR-target site is depicted in Fig. S2. The gene-targeting constructs were generated using SLiCE (Seamless Ligation Cloning Extract). The genomic DNA was amplified with primers F9 and R9 from the *PIAS1*-gene locus and the PCR product was used as template DNA for amplifying the 5' arm. The 5'-arm was amplified using primers F10 and R10, where each primer shared 20-base pair-end homology with the insertion site of the vector. The 3'-arm was amplified using primers F11 and R11, where each primer shared 20-base pair-end homology with the insertion site of the vector. Both vectors, DT-ApA/neo and DT-ApA/hygro, were linearized with *Not*I and *Xba*I. All the fragments of the vectors and inserts were purified using a qiaquick gel extraction kit (QIAGEN, Netherlands). The gene-targeting constructs were generated in a single reaction mixture containing DT-ApA/puro or DT-ApA/hisd or DT-ApA/bsr vectors, 5'- and 3'-arms, and 2×SLiCE buffer (Invitrogen, US) and incubated for 30 min at room temperature. 6 μg of CRISPR and 2 μg of two gene-targeting vectors were transfected into 4×10^6 TK6 cells using the Neon Transfection System (Life Technologies, US). After electroporation, cells were released into 20 ml drug-free medium containing 10% horse serum. Forty-eight hours later, cells were seeded into 96-well plates for selection with respective antibiotics for two weeks. The gene disruption was confirmed by RT-PCR using primers F12 and R12.

Site-directed mutagenesis of PCNA in PIAS1$^{-/-}$/PIAS4$^{-/-}$ DT40 cells

The PCNA lysine164 to arginine (*PCNA^{K164R}*) mutation in the *PIAS1$^{-/-}$/PIAS4$^{-/-}$* cells was inserted as described previously (3).

Sensitivity to genotoxic agents

Cellular sensitivity to genotoxic agents was measured as previously described (4, 5).

Surface-IgM-gain assay

The proportion of surface IgM (sIgM)-positive revertants was monitored by flow-cytometric analysis of cells (6). Single cells were grown clonally following limiting dilution, and expanded for three weeks and then stained with fluorescein isothiocyanate-conjugated (FITC) anti-chicken IgM antibody for 1 h at 37 °C. At least 36 subclones were analysed for each genotype.

PiggyBlock assay

CPD containing plasmid was generated as described previously (7, 8) and was transfected with transposase expression vector into TK6 cells using the Neon transfection system. Transfected cells were subjected to limiting dilution immediately after transfection. Puromycin was added at 48 h after transfection. Genomic DNAs from individual puromycin resistant clones were purified, and were PCR amplified to examine DNA sequences at the CPD lesion. We analyzed them following the method described previously (7).

Construction of 6His-hSUMO1 expressing DT40 cells

For the pull-down experiment, 6His-tagged hSUMO1-expressing DT40 cells were generated using a retroviral vector as described (Fig. S7) (9). Briefly, the coding sequence for 6His-tagged hSUMO1 was cloned into the pMSCV retroviral expression vector (Clontech). The SUMOylation of PCNA is mainly by conjugation with SUMO1, but not SUMO2 or SUMO3, in mammalian cells (10). The newly engineered retroviral expression vector was co-transfected into human 293T cells with a helper plasmid (pClampho) expressing the viral gag, pol and env proteins to produce viral supernatant. The viral supernatant was collected after 24 h and used to transduce into the DT40 cells. The efficiency of each step was assessed by quantifying the

number of cells expressing GFP.

Pull-down assay

Cell pellets were treated with 5 ml lysis buffer (8 M urea, 115 mM NaH_2PO_4, 300 mM NaCl, 10 mM Tris-HCl, 0.1% NP-40, 5 mM Imidazole, pH 8.0), and sonicated. The soluble fraction was recovered and mixed and rotated with 50 µl Ni-NTA magnetic beads (Qiagen) overnight at 4° C. The protein samples were washed 3 times with lysis buffer (8 M urea, 115 mM NaH_2PO_4, 300 mM NaCl, 10 mM Tris-HCl, 0.1% NP-40, 5 mM Imidazole, pH 8.0), mixed with 50 µl elution buffer (200 mM Imidazole, 5% SDS, 150 mM Tris-HCl pH 6.7, 30% glycerol, 720 mM ß-ME, and 0.0025 bromophenol blue) for 20 min at room temperature and then boiled for 2 min. After running the supernatants in a 12% SDS-Gel, the proteins were electro-blotted onto a PVDF membrane. Anti-PCNA antibody (PC10, Santa Cruz) for overnight at 4 °C and anti-mouse IgG HRP-linked antibody (Santa Cruz) for 1 h at room temperature were used as the primary and secondary antibodies, respectively.

Quantification of SUMOylated PCNA

SUMOylated PCNA was quantified from the image of the western blot (WB) using ImageJ software. First, the lowest value for SUMOylated PCNA (*wild-type* DT40 cells without the hSUMO1 transgene) was assumed to be the background noise, and this value was subtracted from all other values. In the second step, the signals for each immuno-precipitated sample on the anti-PCNA blot at the positions of SUMOylated PCNA were normalized according to the signals (unmodified PCNA) obtained from the anti-PCNA blot for whole cell extracts (WCE), to account for sample loading variations. In the last step, all values were normalized to the value of SUMOylated PCNA in His-hSUMO1 expressing *wild-type* cells, and set to 1.00.

Construction of Flag-tagged hPCNA and Flag-tagged hPCNA-hSUMO1 expressing DT40 and TK6 cell lines

Flag-tagged hPCNA and Flag-tagged hPCNA-hSUMO1 plasmids (10) were used to generate Flag-tagged hPCNA and Flag-tagged hPCNA-hSUMO1 expressing DT40 cells. These plasmids were modified by insertion of $hygro^R$ gene into the plasmid. Cells were selected with hygromycin antibiotic after transfection with linearized

plasmids using Bio-Rad transfection system. The expression of chimera was confirmed by western blot (WB) using anti-PCNA antibody for overnight at 4°C and anti-mouse IgG HRP-linked antibody for 1h at room temperature were used as the primary and secondary antibodies respectively (Fig. S8). Flag-tagged hPCNA and Flag-tagged hPCNA-hSUMO1 expressing TK6 cells were generated using a genetically modified retroviral vector as described (9). Briefly, the chimeric gene was cloned into the pMSCV retroviral expression vector (Clontech). The newly engineered retroviral expression vector was co-transfected into human 293T cells with a helper plasmid (pClampho) expressing the viral gag, pol and env proteins to produce viral supernatant. The viral supernatant was collected after 48h and used to transduce into *wild-type*, *PIAS1*$^{-/-}$*PIAS4*$^{-/-}$ TK6 cells (Fig. S9). The efficiency of each step was assessed by quantifying the number of cells expressing GFP (Fig. S9).

Construction of Flag-tagged mutated hSUMO1-hPCNA expressing TK6 cell lines

We replaced key amino acids of SUMO involved in interaction with SIM (11) (Fig. S10A), generating Flag-hPCNA-hSUMO1^{F36A}, Flag-hPCNA-hSUMO1^{V38A}, and Flag-hPCNA-hSUMO1^{K39A}. We also generated Flag-hPCNA-hSUMO1^{4A} by replacing all three amino acids, Phe36, Val38, and Lys39, plus an additional amino acid, Thr42 to alanine. We established TK6 cells expressing Flag-tagged mutated hSUMO1-hPCNA as we generated TK6 cells expressing Flag-tagged hPCNA. The efficiency of each step was assessed by quantifying the number of cells expressing GFP (Fig. S10).

References

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Supplemental Figure legends

Fig. S1. Targeted disruption of *PIAS1* and *PIAS4* genes in chicken DT40 cells.

(**A**) Schematic representation of part of the *PIAS1* gene. The SUMO ligase domain was deleted by gene targeting. The locations of *Bam*HI sites used for Southern blot verification of mutants are indicated. Hybridizing bands of 10.1 kb and 6.3 kb are predicted for *wild-type* and mutant alleles, respectively, based on sequence data. (**B**) Schematic representation of part of the *PIAS4* gene. *Apa*I sites used for Southern blotting are indicated, and bands of 11.4 kb and 10.7 kb are predicted for the *wild-type* and mutant strains, respectively. (**C**) Southern blot verification of mutant construction. Genomic DNA isolated from drug-resistant cells was digested with either *Bam*HI (for *PIAS1*) or *Apa*I (for *PIAS4*) and incubated with radio-labelled probes shown in (A) and (B). The genotypes are shown on the top of panels. (**D**) The upper four panels show reverse-transcription-PCR products derived from the indicated genotypes, using sets of primers shown by reference numbers. The primers hybridize with the indicated exons of the *PIAS1* and *PIAS4* genes. mRNA encoding the targeted exons (open boxes in the schematic) was not detected in the relevant cell lines.

Fig. S2. Targeted disruption of *XPA, POLH, PIAS1* and *PIAS4* genes in human TK6 cells.

(**A**) Schematic representation of the *XPA* locus in TK6 cells and the structure of the gene-targeting constructs. The close solid boxes indicate the coding regions of exons. Arrows are primers used for RT-PCR. (**B**) *Wild-type* as well as *XPA*$^{-/-}$ TK6 cells were subjected to RT-PCR using *GAPDH*- or *XPA*-specific primers. (**C**) Schematic representation of the human *POLH* locus in TK6 cells and the structure of the gene-targeting constructs. The close solid boxes indicate the coding regions of exons. 'H' indicates relevant *Hind*III site. (**D**) Southern blot analysis of the *Hind*III-digested genomic DNA from cells carrying the indicated genotypes, using the probe shown in (C). The position and sizes of hybridizing fragments of the *wild-type* and targeted loci are indicated. **(E)** Schematic representation of the human *PIAS4* locus, target sequences by CRISPR/Cas9, and the gene-targeting construct with a selection marker. The box around ATG indicates the initiation codon. (**F**) RT-PCR analysis using the forward (F_{RT}) and reverse (R_{RT}) primers showing *PIAS4* expression only in the wild-type cells, but not in the mutants transfected with the gene-targeting construct and

CRISPR/Cas9. GAPDH is used as a loading control. (**G**) Schematic representation of the human *PIAS1* locus, target sequences by CRISPR/Cas9, and the gene-targeting construct with a selection marker. The box around ATG indicates the initiation codon. (**H**) RT-PCR analysis using the forward (F_{RT}) and reverse (R_{RT}) primers showing *PIAS1* expression only in the wild-type cells, but not in the mutants transfected with the gene-targeting construct and CRISPR/Cas9. GAPDH is used as a loading control.

Fig. S3. SUMO E3 ligase mutants displayed a reduced number of sister-chromatid exchange (SCE) events. (**A-D**) SCE induced by 0.25 J/m^2 UV (**A**) and 10 μM cisplatin (**B**) in the chicken DT40 cells and 0.25 J/m^2 UV (**C**) and 2 μM cisplatin (**D**) in the human TK6 cells. The distribution of SCE events per cell is shown for the indicated cell samples. At least 50 mitotic cells were analyzed per condition in each experiment. Mean values for SCE before and after exposure to the DNA damaging agents are indicated.

Fig. S4. Significant decrease in TS relative to TLS in the *PIAS1*$^{-/-}$/*PIAS4*$^{-/-}$ TK6 cells.

(**A**) Outline of the piggyBlock transposition-based system to analyze TLS past a CPD UV photoproduct CPD placed opposite GpC mismatch in the piggyBlock plasmid carrying the puromycin resistance (puro^R) gene. After transfection, we immediately did limiting dilution of the cells in 96-well cluster plates. We then selected clones carrying the piggyblock plasmid randomly integrated into the genome after 48 hours using puromycin. Bypass by accurate TLS inserts the correct complementary base (AA) on lower strand at the damaged template base. Alternatively, the nascent strand of the sister chromatid is used as an alternative undamaged template; one possible mechanism for such a template switching illustrated. (**B**) A UV-damage, CPD placed opposite GpC mismatch was randomly integrated into the genome using the PiggyBlock vector. TLS across the CPD results in a dual peak in the resulting cellular clone (left), while template switching (TS) results in a homogenous GC read (right). (**C**) Histogram representing the ratio of TS relative to TLS events (*y*-axis) in the indicated genotypes (*x*-axis). At least 40 cells were analyzed per condition in each experiment.

Fig. S5. Site directed mutagenesis of the PCNA locus in *PIAS1*$^{-/-}$/*PIAS4*$^{-/-}$ DT40 cells. (**A**) Schematic representation of the PCNA mutagenesis construct. (**B**) Sequence chromatograph covering the PCNA codon 164, which was changed from AAA in the *wild-type* clone to AGA in the *PIAS1*$^{-/-}$/*PIAS4*$^{-/-}$/*PCNA*$^{K164R/K164R}$ clone. (C) Damage-independent SUMOylation of PCNA. DT40 Cells stably transfected with His-tagged hSUMO1, were analyzed by pull-down assay using Ni-NTA magnetic beads. (D) Ubiquitylation status of PCNA in the *PIAS1*$^{-/-}$/*PIAS4*$^{-/-}$ DT40 cells. After running the whole cell lysates from *wild-type*, *PIAS1*$^{-/-}$/*PIAS4*$^{-/-}$ and *PCNA*$^{K164R/K164R}$ cells in a 12% SDS-Gel, the proteins were electro-blotted onto a PVDF membrane. Anti-PCNA antibody (PC10, Santa Cruz) for overnight at 4 °C and anti-mouse IgG HRP-linked antibody (Santa Cruz) for 1 h at room temperature were used as the primary and secondary antibodies, respectively.

Fig. S6. The epistatic relationship of the *PIAS1*$^{-/-}$/*PIAS4*$^{-/-}$ mutation with the *PCNA*$^{K164R/K164R}$ mutation in Ig gene conversion.

(**A**) Rates of Ig gene conversion and non-templated mutation in AID expressing clones carrying the indicated genotypes. Each dashed line represents the Vλ segment region of an individual cell. The location of gene conversion tracts (GC, horizontal bars), non-templated single base substitutions/point mutations (PM, lollipops) and mutations of ambiguous origin (AMB, vertical bars) are indicated. (**B**) Charts displaying the frequency of PM, AMB and GC events. Segment size indicates the proportion of cells in which the number of mutations stated outside the chart had occurred. The total number of sequences analyzed is shown in the centre of the chart.

Fig. S7. Schematic representation of the cloning strategy to create the 6His-tagged human SUMO1 and engineered DT40 strains.

(**A**) To construct a retrovirus vector expressing 6His-tagged human SUMO1 (6His-hSUMO1), we replaced the AID cDNA in a pMSCV retroviral expression vector (12) by the 6His-tagged hSUMO1 cDNA. (**B**) Flow chart illustrating creation of 6-His-tagged hSUMO1-expressing DT40 cells. AID: activation induced deaminase. IRES: internal ribosome entry site. GFP: green fluorescent protein. (**C**) Expression levels of 6His-tagged hSUMO1 in *wild-type*, *PIAS1*$^{-/-}$/*PIAS4*$^{-/-}$ and *PCNA*$^{K164R/K164R}$ DT40 cells

were analyzed by flow cytometric analysis. The X-axis of the histogram represents the intensity of green fluorescence.

Fig. S8. Expression of Flag-hPCNA and Flag-hPCNA-hSUMO1 hybrid genes in the DT40 cells.

(**A**) Schematic representation of the Flag-hPCNA and Flag-hPCNA-hSUMO1 hybrid genes. (**B**) The expression of Flag-hPCNA and Flag-hPCNA-hSUMO1 were confirmed by western blot (WB) using anti-PCNA antibody. Actin is used as a loading control. * Mono-Ubiquitinated PCNA. (C) The proportion of surface IgM (sIgM)-positive DT40 cells was determined as in Fig. 3B. (**D**) The number of SCEs induced by 0.25 J/m^2 UV in the DT40 cells was determined as in Fig. 1A. FP: Flag-hPCNA. FPS: Flag-hPCNA-hSUMO1. Statistical analyses were performed by student's t-test (P<0.01).

Fig. S9. Schematic representation of the cloning strategy to create the Flag-hPCNA and Flag-hPCNA-hSUMO1 hybrid genes and engineered TK6 strains.

(**A**) To construct a retrovirus vector expressing Flag-hPCNA or Flag-hPCNA-hSUMO1 hybrid genes, we replaced the AID cDNA in a pMSCV retroviral expression vector (12) by the Flag-hPCNA or Flag-hPCNA-hSUMO1 hybrid genes. (**B**) Flow chart illustrating creation of Flag-hPCNA or Flag-hPCNA-hSUMO1 -expressing TK6 cells. AID: activation induced deaminase. IRES: internal ribosome entry site. GFP: green fluorescent protein. (**C**) Expression levels of Flag-hPCNA or Flag-hPCNA-hSUMO1 hybrid genes in *XPA$^{-/-}$/POLH$^{-/-}$ and XPA$^{-/-}$/POLH$^{-/-}$/PIAS1$^{-/-}$ /PIAS4$^{-/-}$* TK6 cells were analyzed by flow cytometric analysis. The X-axis of the histogram represents the intensity of green fluorescence.

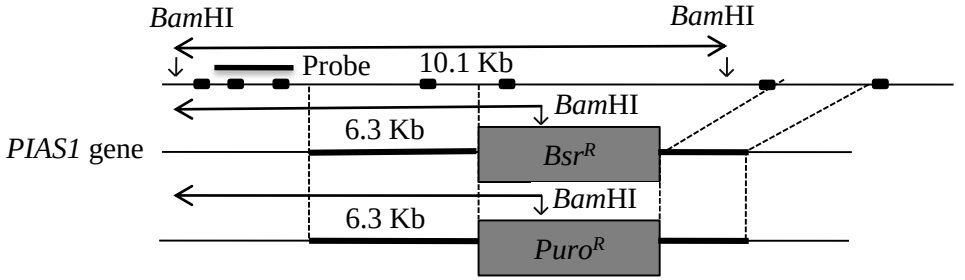
Fig. S10. Ectopic expression of mutated hSUMO1-hPCNAs were unable to reverse the mutant phenotype of *PIAS1$^{-/-}$PIAS4$^{-/-}$/XPA$^{-/-}$/POLH$^{-/-}$* TK6 cells.

(**A**) Schematic representation of the Flag tagged mutated hSUMO1-hPCNA hybrid genes. (**B**) Sequence chromatographs confirmed the insertion of mutation in the hSUMO1-hPCNA hybrid genes. (C) To construct a retrovirus vector expressing Flag tagged mutated hSUMO1-hPCNA hybrid genes, we replaced the AID cDNA in a pMSCV retroviral expression vector (12) by the Flag tagged mutated hSUMO1-hPCNA hybrid genes. (**D**) Flow chart illustrating creation of Flag tagged mutated

hSUMO-hPCNA-expressing TK6 cells. AID: activation induced deaminase. IRES: internal ribosome entry site. GFP: green fluorescent protein. (**E**) Expression levels of Flag tagged mutated hSUMO1-hPCNA hybrid genes in *XPA*$^{-/-}$*/POLH*$^{-/-}$*/PIAS1*$^{-/-}$*/PIAS4*$^{-/-}$ TK6 cells were analyzed by flow cytometric analysis. The X-axis of the histogram represents the intensity of green fluorescence. (**F**) Clonogenic cell survival assay following exposure of mutated hSUMO1-hPCNA expressing *PIAS1*$^{-/-}$*PIAS4*$^{-/-}$*/XPA*$^{-/-}$*/POLH*$^{-/-}$ TK6 cells to UV. The *x*-axis represents the dose of the indicated DNA-damaging agent on a linear scale; the *y*-axis represents the survival fraction on a logarithmic scale. Error bars indicate SD.

DT40

A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BamHI
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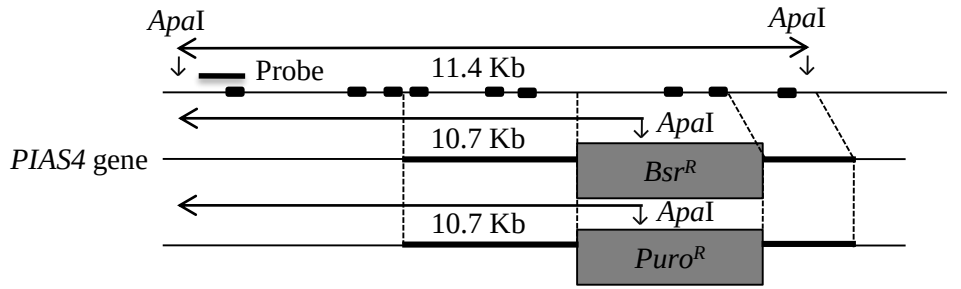
BamHI
6.3 Kb
PIAS1 gene
Bsr^R
BamHI
6.3 Kb
Puro^R

B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ApaI
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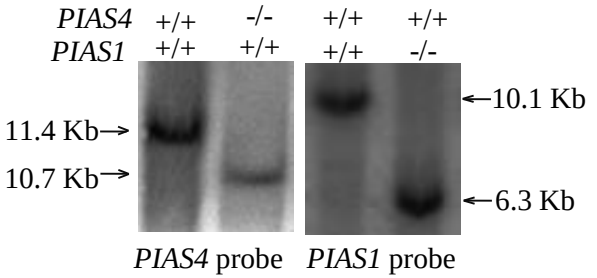
C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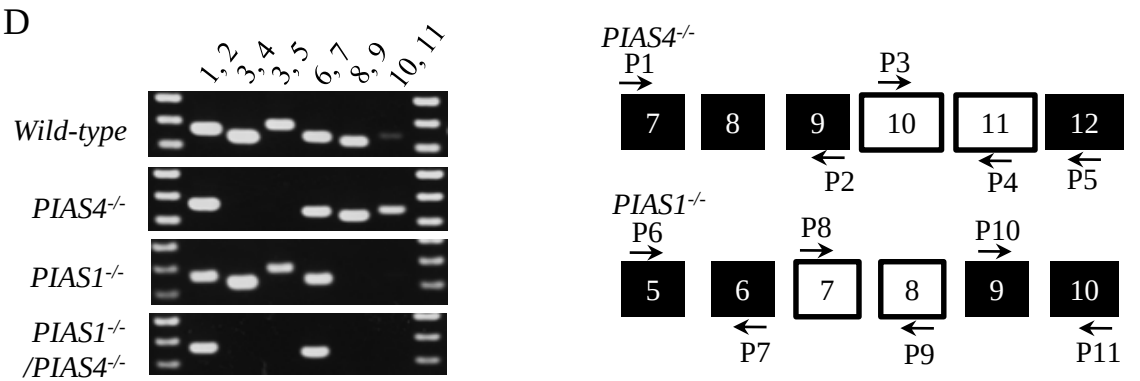
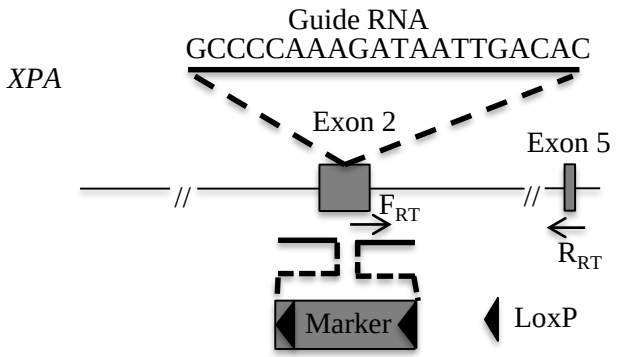
6.3 Kb
PIAS4 probe
PIAS1 probe

D

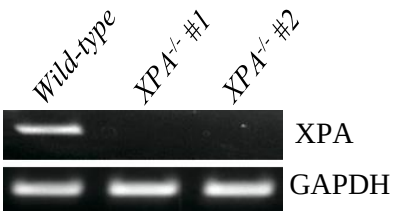

1, 2
3, 4
3, 5
6, 7
8, 9
10, 11
Wild-type
PIAS4-/-
PIAS1-/-
PIAS1-/-
/PIAS4-/-
PIAS4-/-
P1
P3
7
8
9
10
11
12
P2
P4
P5
PIAS1-/-
P6
P8
P10
5
6
7
8
9
10
P7
P9
P11

TK6

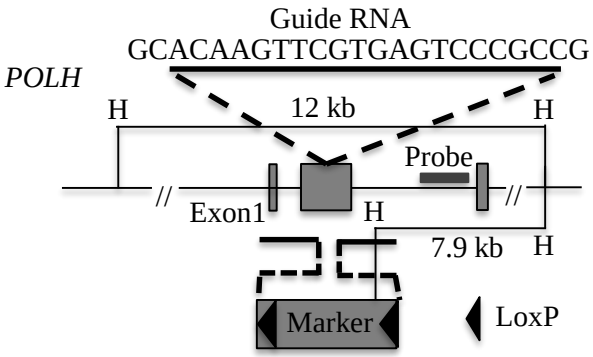
A


Guide RNA
GCCCCAAAGATAATTGACAC
XPA
Exon 2
Exon 5
F_RT
R_RT
Marker
LoxP

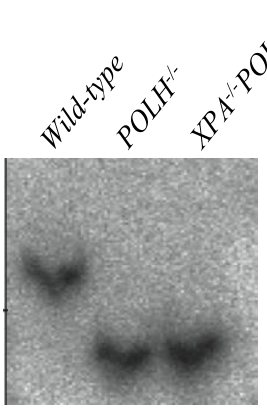
B


Wild-type
XPA-/- #1
XPA-/- #2
XPA
GAPDH

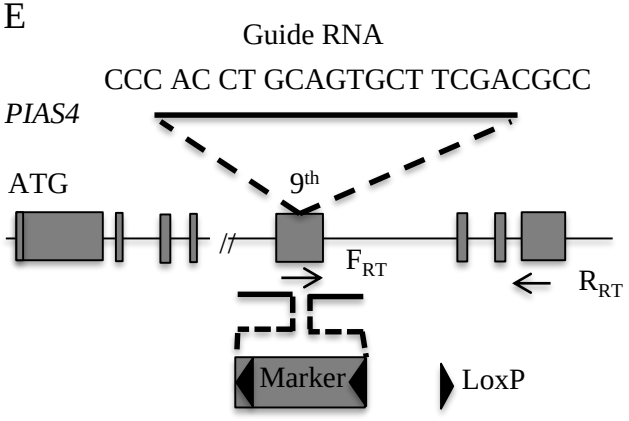
C


Guide RNA
GCACAAGTTCGTGAGTCCCGCCG
POLH
H
12 kb
H
Probe
Exon1
H
7.9 kb
H
Marker
LoxP

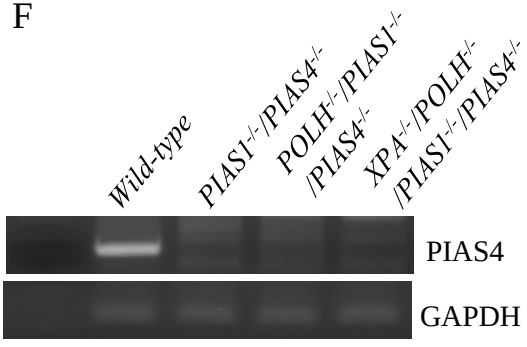
D


Wild-type
POLH-/-
XPA-/-POLH-/-

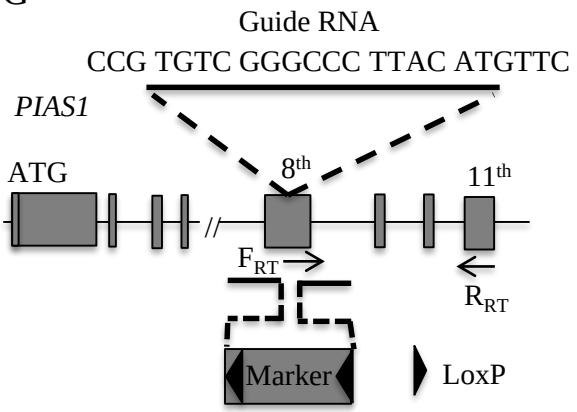
E


Guide RNA
CCC AC CT GCAGTGCT TCGACGCC
PIAS4
ATG
9th
F_RT
R_RT
Marker
LoxP

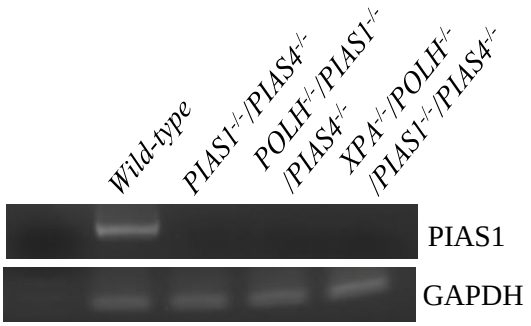
F

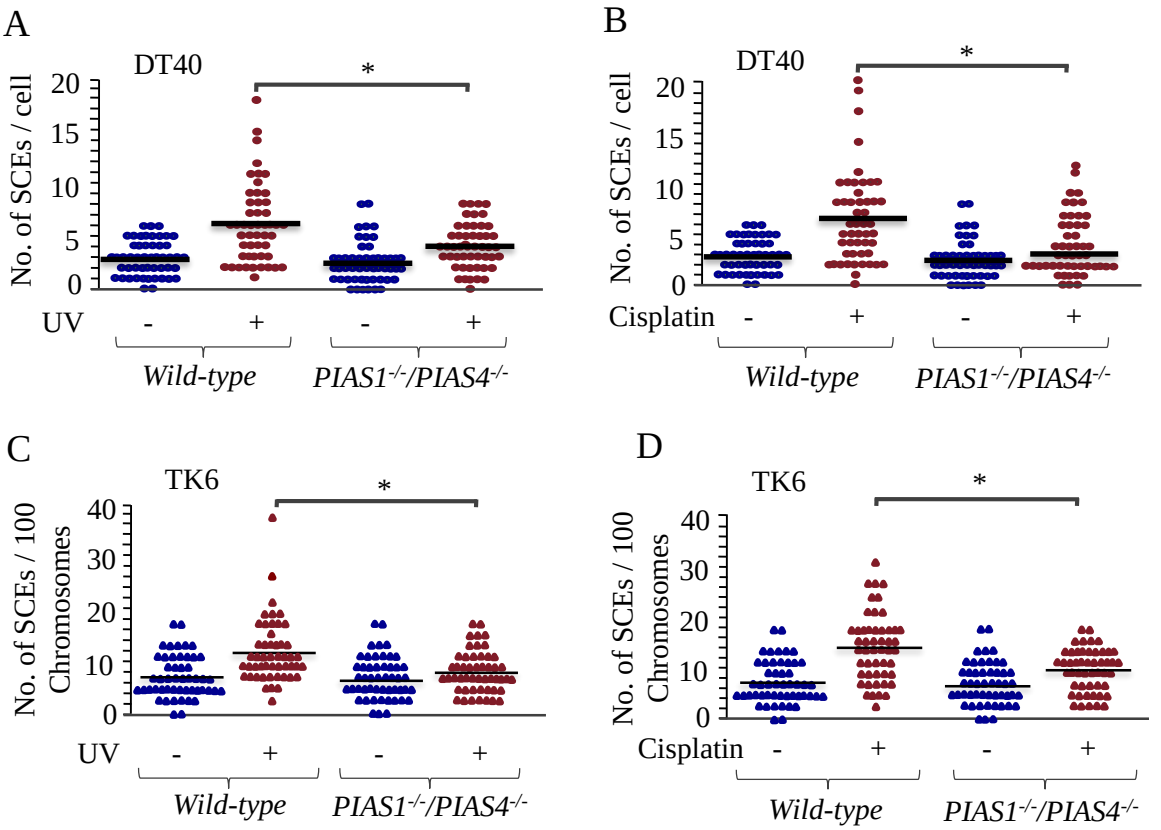

Wild-type
PIAS1-/-/PIAS4-/-
POLH-/-/PIAS1-/-/PIAS4-/-
XPA-/-/POLH-/-/PIAS1-/-/PIAS4-/-
PIAS4
GAPDH

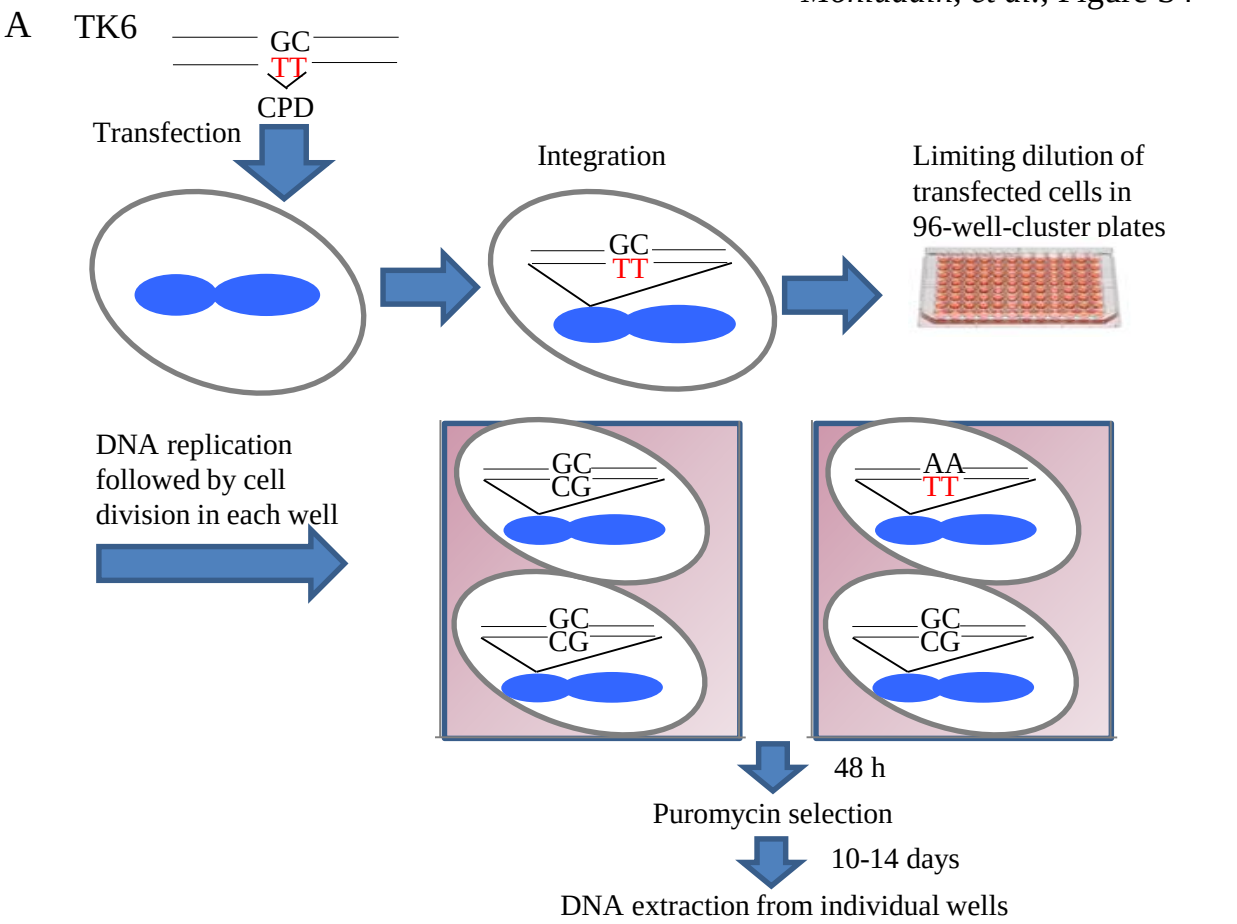
G

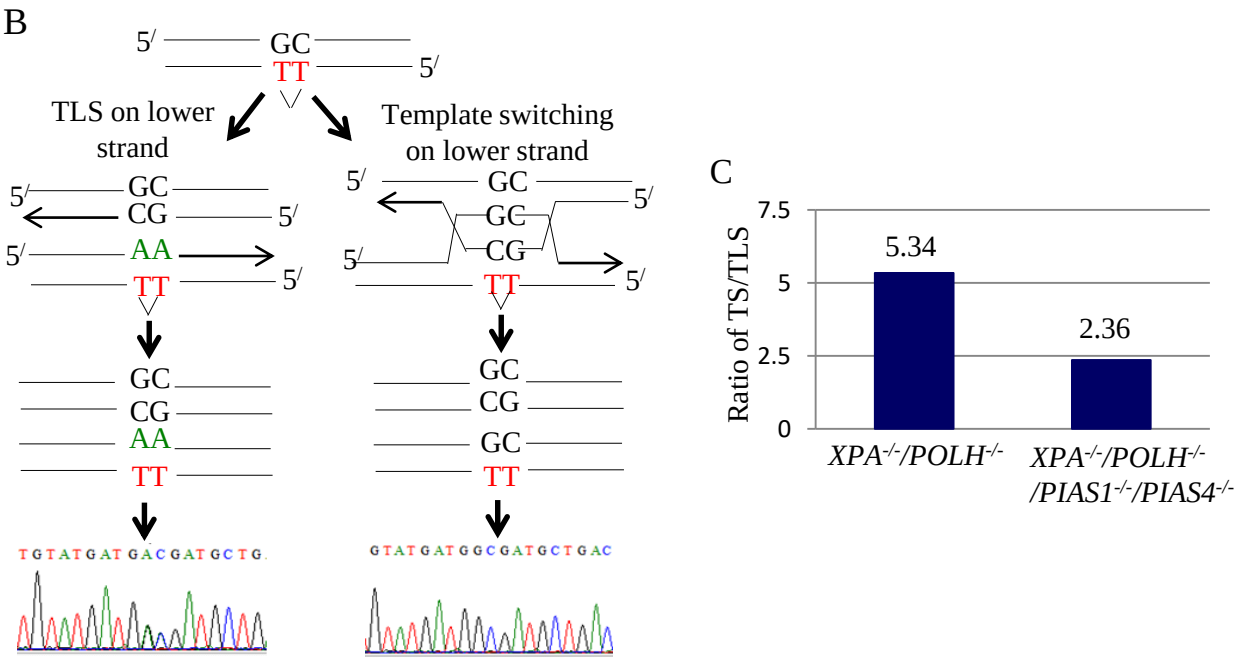

Guide RNA
CCG TGTC GGGCCC TTAC ATGTTC
PIAS1
ATG
8th
11th
//
F RT
R RT
Marker
LoxP

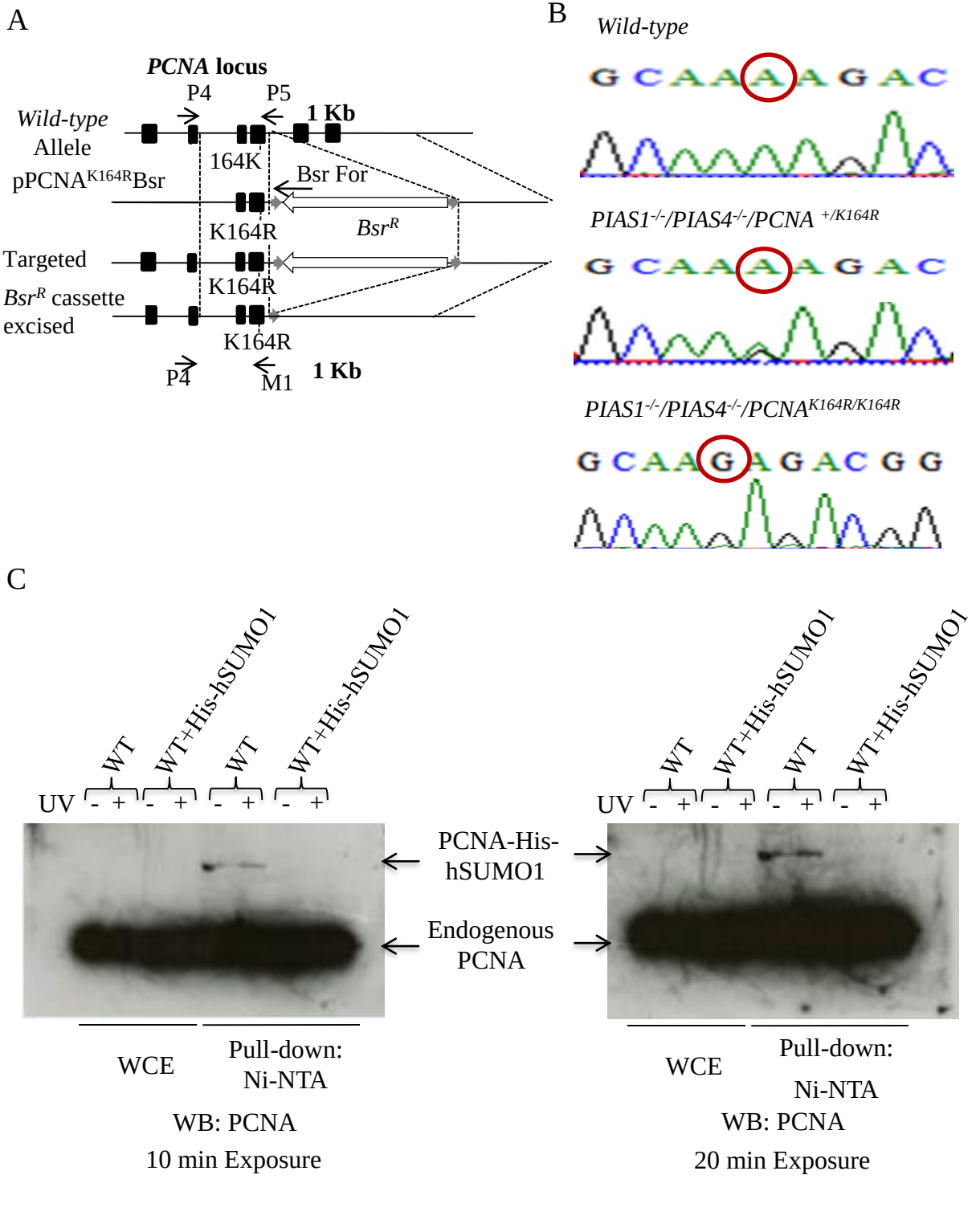
H


Wild-type
PIAS1-/-/PIAS4-/-
POLH-/-/PIAS1-/-
/PIAS4-/-
XPA-/-/POLH-/-
/PIAS1-/-/PIAS4-/-
PIAS1
GAPDH

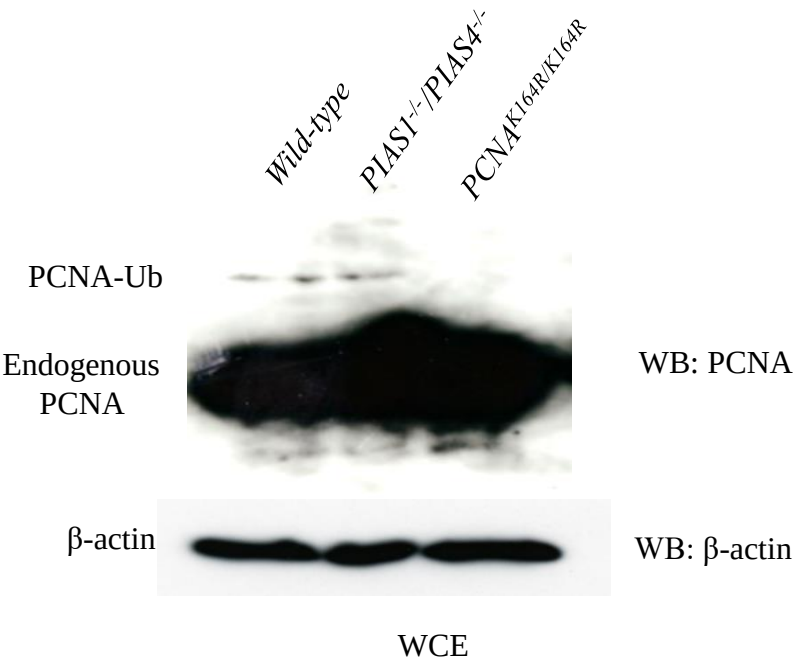

A
DT40
No. of SCEs / cell
20
15
10
5
0
*
UV
-
+
-
+
Wild-type
PIAS1-/-/PIAS4-/-
B
DT40
No. of SCEs / cell
20
15
10
5
0
*
Cisplatin
-
+
-
+
Wild-type
PIAS1-/-/PIAS4-/-
C
TK6
No. of SCEs / 100 Chromosomes
40
30
20
10
0
*
UV
-
+
-
+
Wild-type
PIAS1-/-/PIAS4-/-
D
TK6
No. of SCEs / 100 Chromosomes
40
30
20
10
0
*
Cisplatin
-
+
-
+
Wild-type
PIAS1-/-/PIAS4-/-

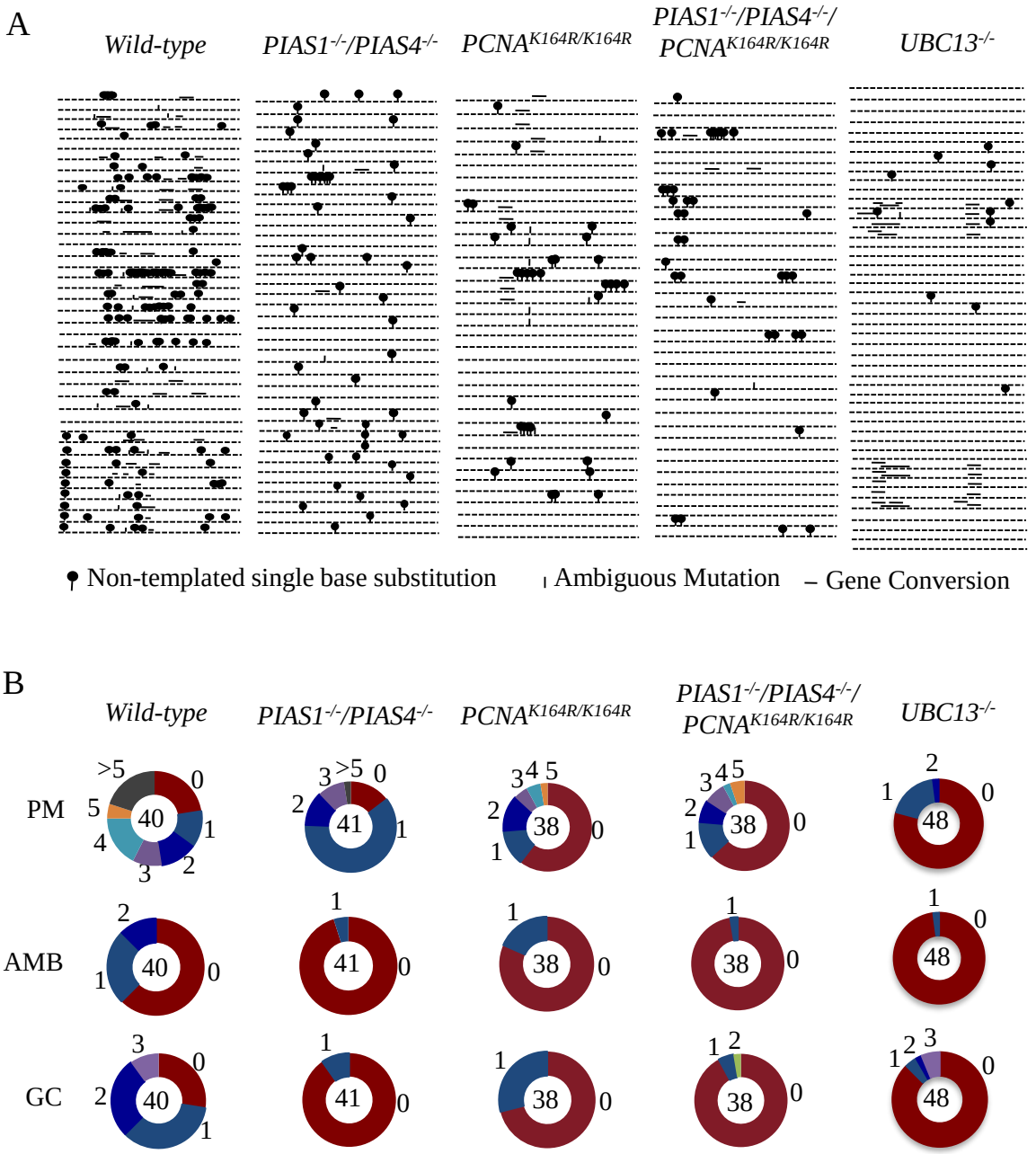

A
TK6
GC
TT
CPD
Transfection
Integration
Limiting dilution of transfected cells in 96-well-cluster plates
DNA replication followed by cell division in each well
GC
CG
AA
TT
48 h
Puromycin selection
10-14 days
DNA extraction from individual wells


B
5′
GC
TT
5′
TLS on lower strand
Template switching on lower strand
CG
AA
TGTATGATGACGATGCTG
GTATGATGGCGATGCTGAC
C
Ratio of TS/TLS
7.5
5
2.5
0
5.34
2.36
XPA-/-/POLH-/-
XPA-/-/POLH-/-/PIAS1-/-/PIAS4-/-

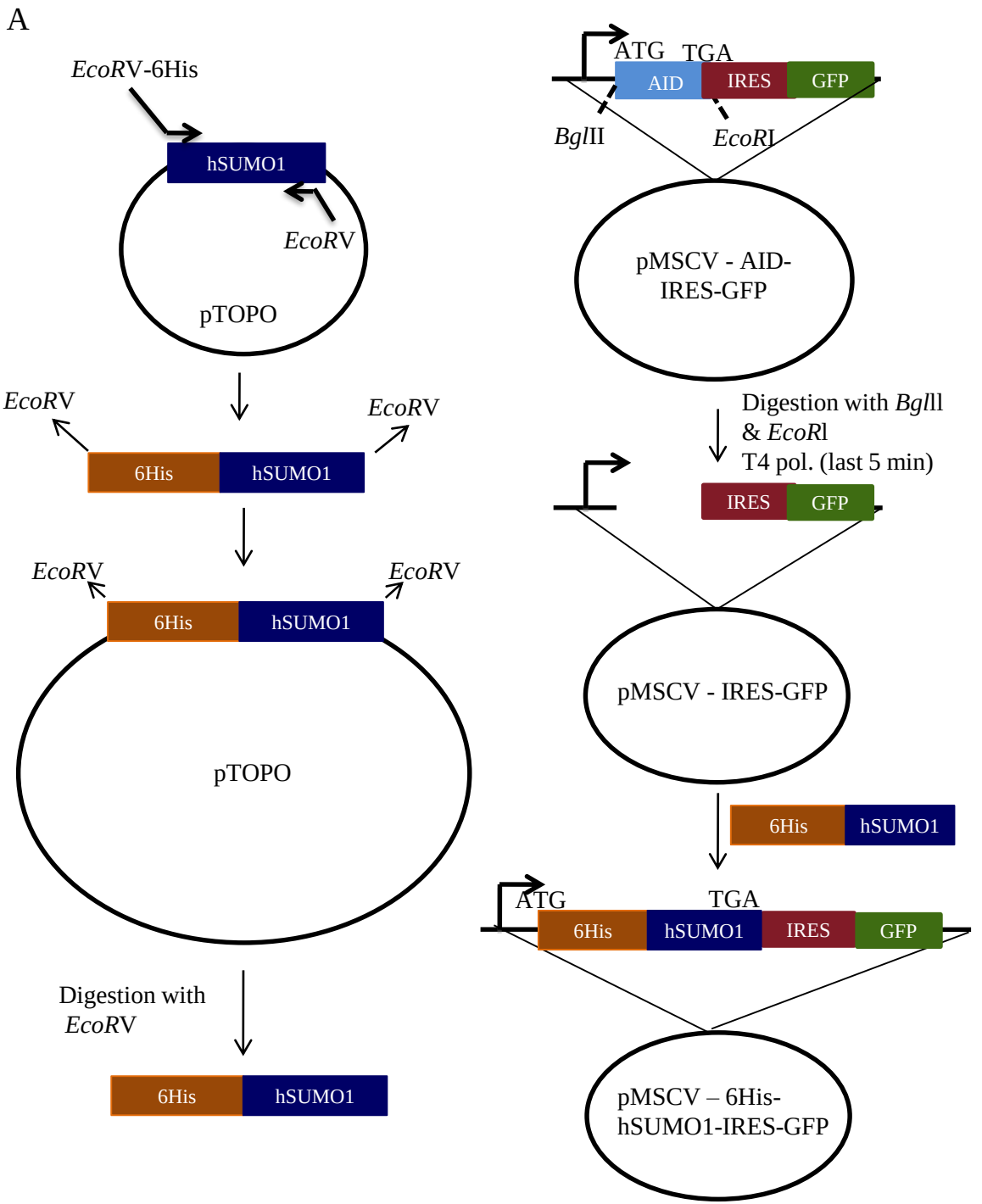

A
PCNA locus
P4
P5
1 Kb
Wild-type Allele
164K
pPCNA^K164RBsr
Bsr For
K164R
Bsr^R
Targeted
K164R
Bsr^R cassette excised
K164R
P4
M1
1 Kb
B
Wild-type
G C A A A A G A C
PIAS1-/-/PIAS4-/-/PCNA +/K164R
G C A A A A G A C
PIAS1-/-/PIAS4-/-/PCNA K164R/K164R
G C A A G A G A C G G
C
WT
WT+His-hSUMO1
WT
WT+His-hSUMO1
UV
- + - + - + - +
PCNA-His-hSUMO1
Endogenous PCNA
WCE
Pull-down: Ni-NTA
WB: PCNA
10 min Exposure
WT
WT+His-hSUMO1
WT
WT+His-hSUMO1
UV
- + - + - + - +
WCE
Pull-down: Ni-NTA
WB: PCNA
20 min Exposure

D


Wild-type
PIAS1-/-/PIAS4-/-
PCNAK164R/K164R
PCNA-Ub
Endogenous PCNA
WB: PCNA
β-actin
WB: β-actin
WCE


A
Wild-type
PIAS1-/-/PIAS4-/-
PCNAK164R/K164R
PIAS1-/-/PIAS4-/-/ PCNAK164R/K164R
UBC13-/-
Non-templated single base substitution
Ambiguous Mutation
Gene Conversion
B
Wild-type
PIAS1-/-/PIAS4-/-
PCNAK164R/K164R
PIAS1-/-/PIAS4-/-/ PCNAK164R/K164R
UBC13-/-
PM
AMB
GC
40
41
38
38
48

Mohiuddin, et al., Figure S7



B

Human cells (293T cells)

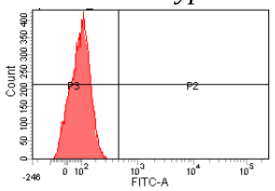
↓ Genetically modified Retroviral expression vector
Helper plasmid (pCLampho)

Wild-type, *PIAS1*$^{-/-}$ */PIAS4*$^{-/-}$ and *PCNA*$^{K164R/K164R}$ → Viral supernatant → *Wild-type* cells expressing 6His-hSUMO1, *PIAS1*$^{-/-}$*/PIAS4*$^{-/-}$ cells expressing 6His-hSUMO1 and *PCNA*$^{K164R/K164R}$ cells expressing 6His-hSUMO1

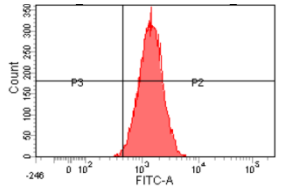
C

DT40

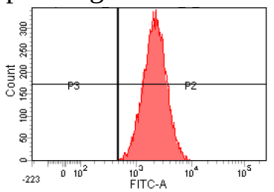
Wild-type



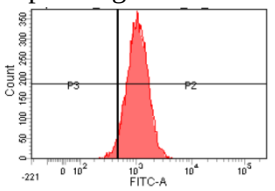
Wild-type cells expressing 6His-hSUMO1



PIAS1$^{-/-}$*/PIAS4*$^{-/-}$ cells expressing 6His-hSUMO1



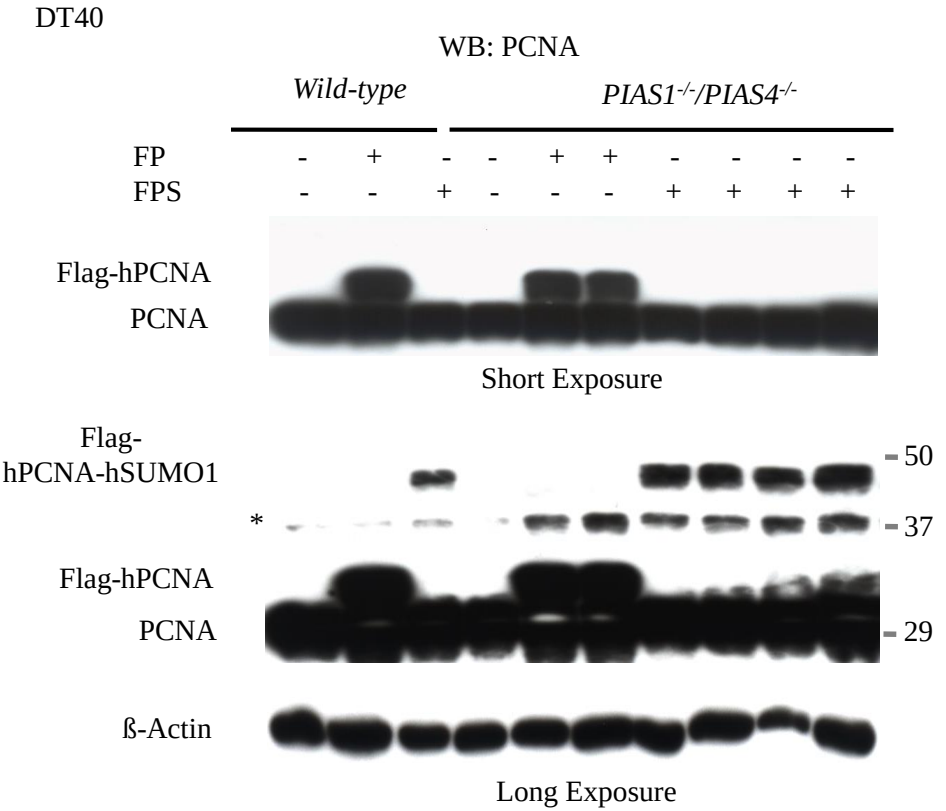
PCNA$^{K164R/K164R}$ cells expressing 6His-hSUMO1



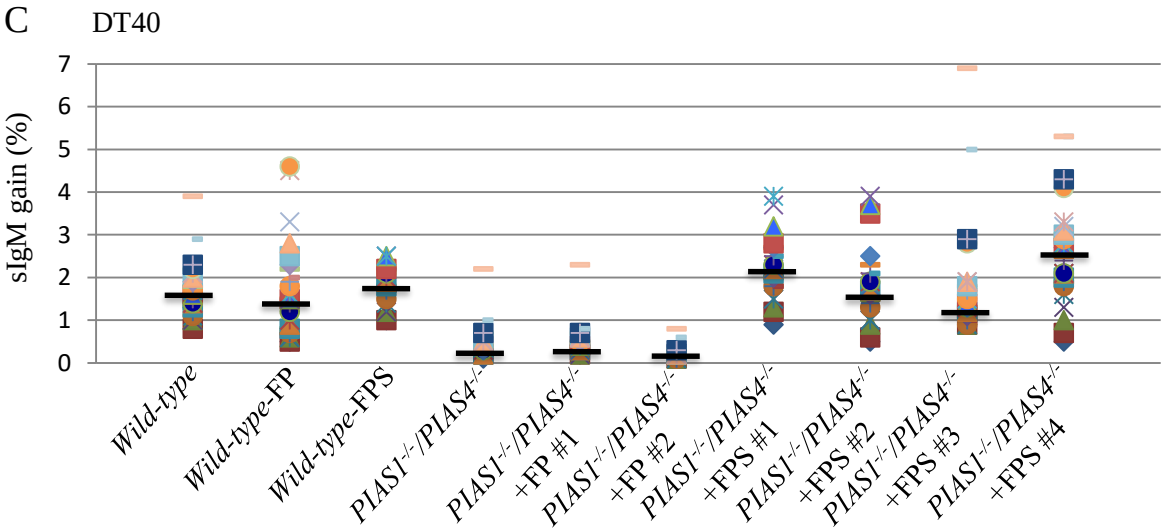
A


Flag-hPCNA (FP)
N
FLAG
hPCNA
C
Flag-hPCNA-hSUMO1 (FPS)
N
FLAG
hPCNA
hSUMO1
C

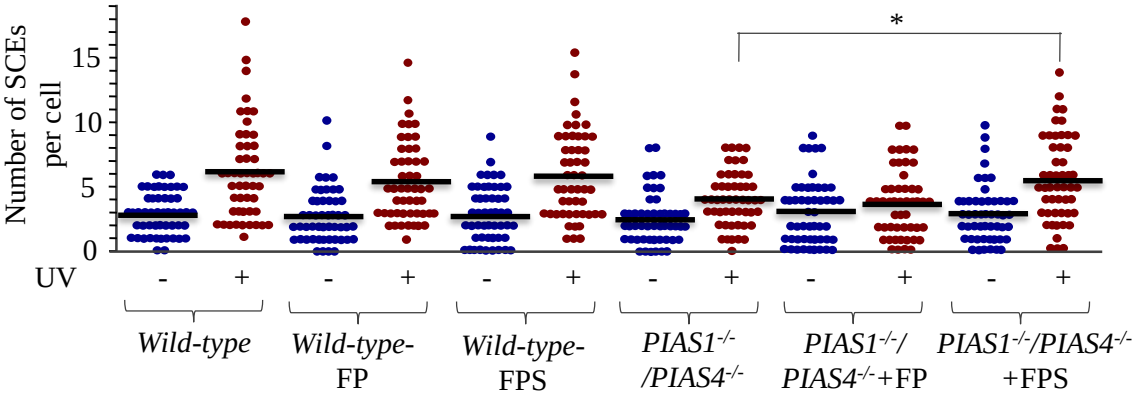
B

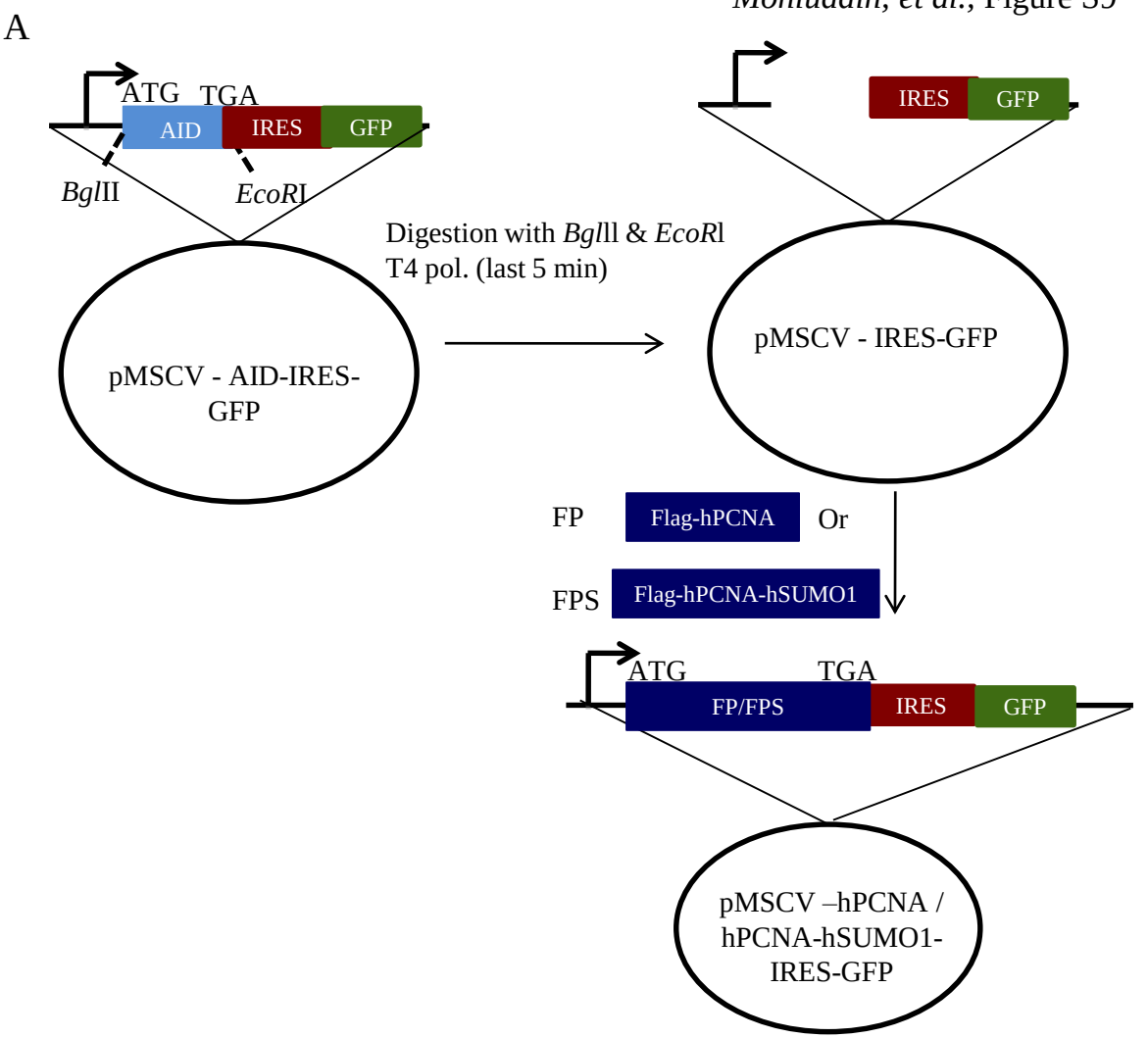

DT40
WB: PCNA
Wild-type
PIAS1-/-/PIAS4-/-
FP - + - - + + - - - -
FPS - - + - - - + + + +
Flag-hPCNA
PCNA
Short Exposure
Flag-hPCNA-hSUMO1
*
50
37
Flag-hPCNA
PCNA
29
ß-Actin
Long Exposure

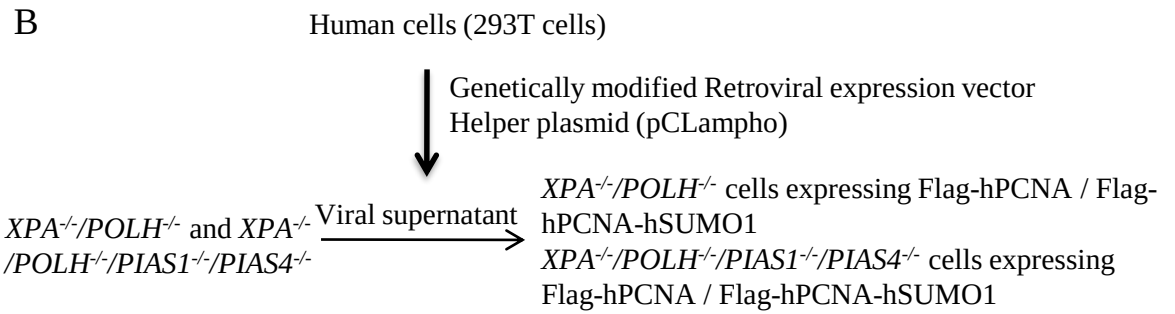
C


DT40
sIgM gain (%)
0
1
2
3
4
5
6
7
Wild-type
Wild-type-FP
Wild-type-FPS
PIAS1-/-/PIAS4-/-
PIAS1-/-/PIAS4-/- +FP #1
PIAS1-/-/PIAS4-/- +FP #2
PIAS1-/-/PIAS4-/- +FPS #1
PIAS1-/-/PIAS4-/- +FPS #2
PIAS1-/-/PIAS4-/- +FPS #3
PIAS1-/-/PIAS4-/- +FPS #4

D DT40

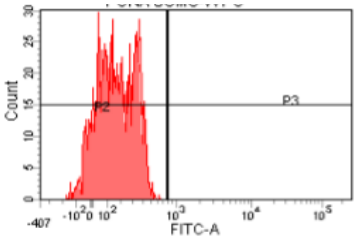

Number of SCEs per cell
0
5
10
15
*
UV
- + - + - + - + - + - +
Wild-type
Wild-type-FP
Wild-type-FPS
PIAS1-/-/PIAS4-/-
PIAS1-/-/PIAS4-/-+FP
PIAS1-/-/PIAS4-/-+FPS


A
ATG
TGA
AID
IRES
GFP
BglII
EcoRI
pMSCV - AID-IRES-GFP
Digestion with Bgll & EcoRl
T4 pol. (last 5 min)
IRES
GFP
pMSCV - IRES-GFP
FP
Flag-hPCNA
Or
FPS
Flag-hPCNA-hSUMO1
ATG
TGA
FP/FPS
IRES
GFP
pMSCV –hPCNA / hPCNA-hSUMO1-IRES-GFP

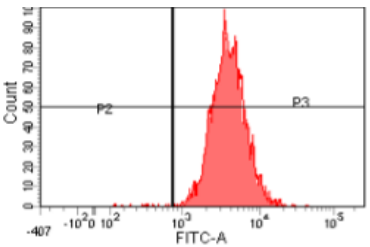

B
Human cells (293T cells)
Genetically modified Retroviral expression vector
Helper plasmid (pCLampho)
XPA-/-/POLH-/- and XPA-/-/POLH-/-/PIAS1-/-/PIAS4-/-
Viral supernatant
XPA-/-/POLH-/- cells expressing Flag-hPCNA / Flag-hPCNA-hSUMO1
XPA-/-/POLH-/-/PIAS1-/-/PIAS4-/- cells expressing Flag-hPCNA / Flag-hPCNA-hSUMO1

C

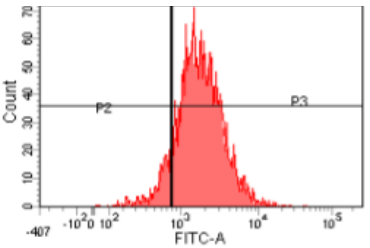
Control (TK6)



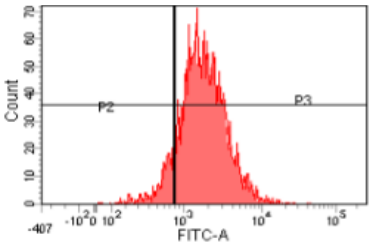
TK6 *XPA*$^{-/-}$/*POLH*$^{-/-}$ cells expressing hPCNA



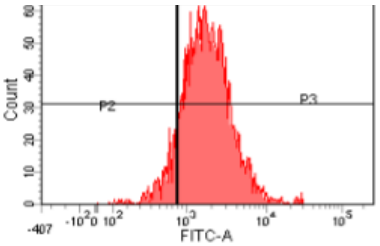
TK6 *XPA*$^{-/-}$/*POLH*$^{-/-}$ cells expressing hPCNA-hSUMO1



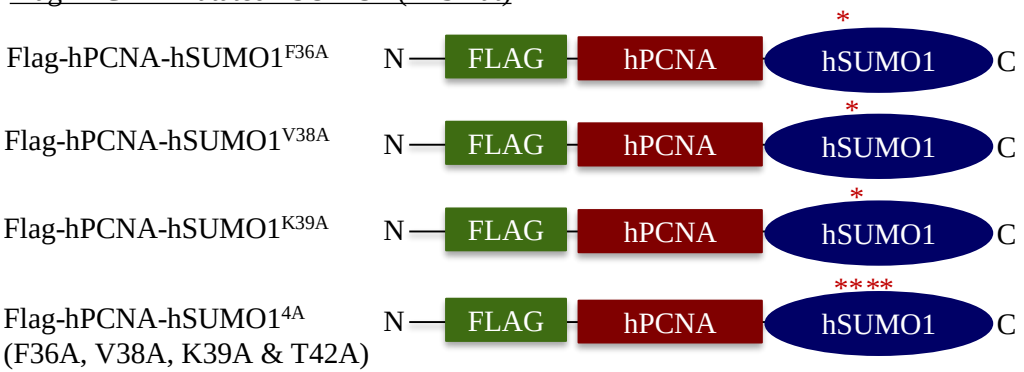
TK6 *XPA*$^{-/-}$/*POLH*$^{-/-}$/*PIAS1*$^{-/-}$/*PIAS4*$^{-/-}$ cells expressing hPCNA



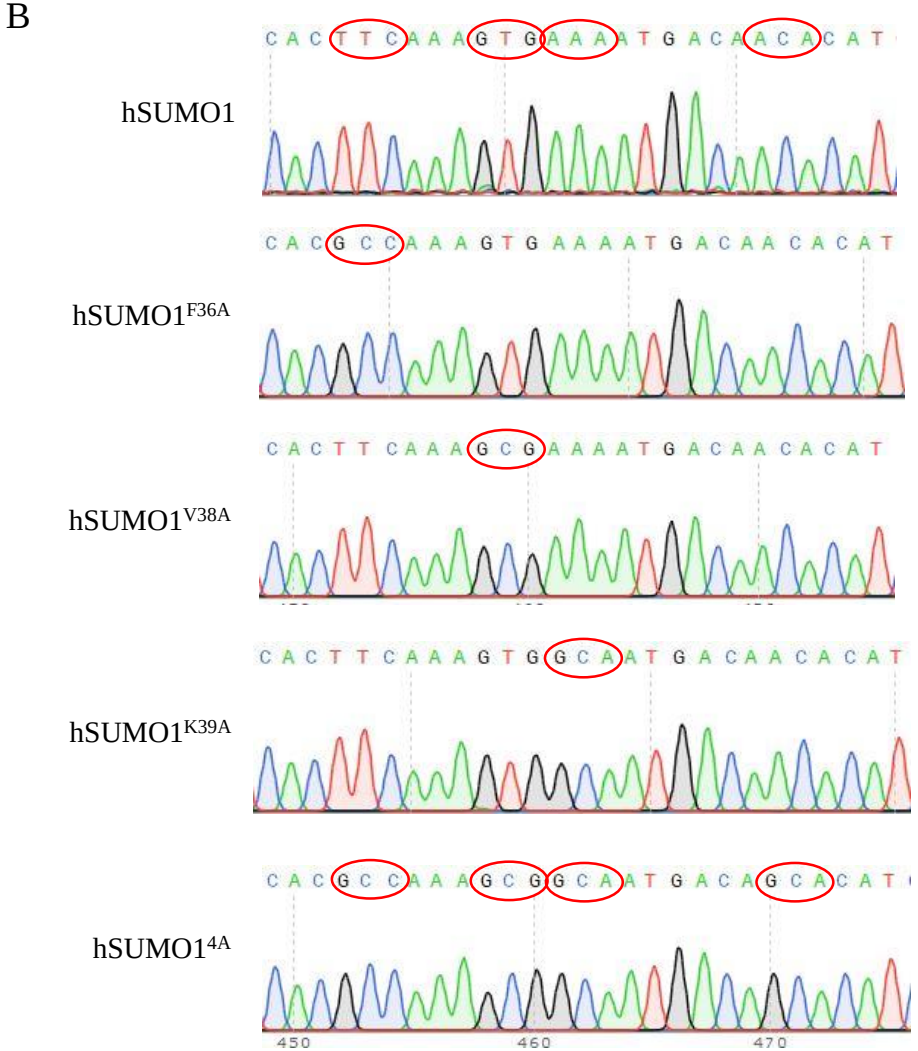
TK6 *XPA*$^{-/-}$/*POLH*$^{-/-}$/*PIAS1*$^{-/-}$/*PIAS4*$^{-/-}$ cells expressing hPCNA-hSUMO1



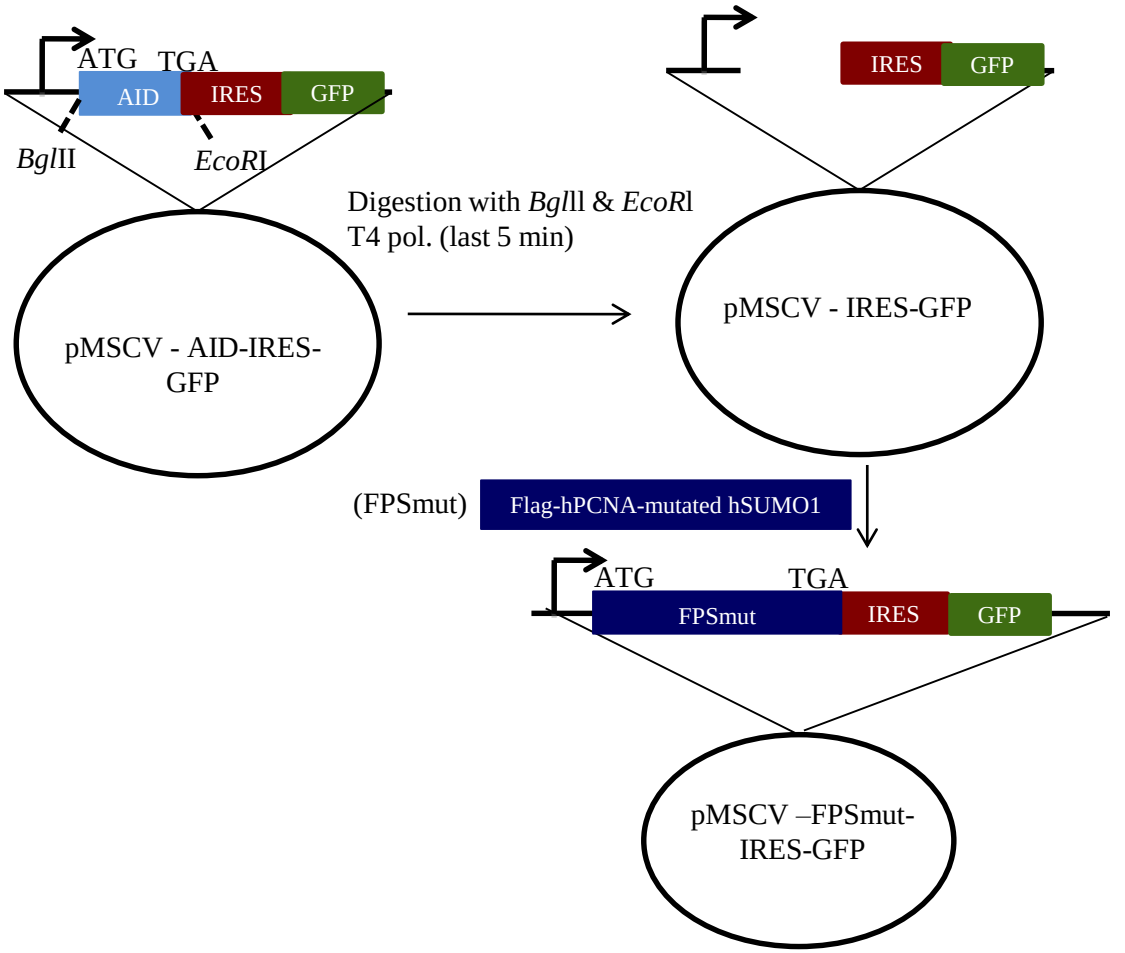
A


Flag-hPCNA-mutated hSUMO1 (FPSmut)
Flag-hPCNA-hSUMO1F36A
Flag-hPCNA-hSUMO1V38A
Flag-hPCNA-hSUMO1K39A
Flag-hPCNA-hSUMO14A
(F36A, V38A, K39A & T42A)
N
FLAG
hPCNA
hSUMO1
C
*
****

B


hSUMO1
hSUMO1F36A
hSUMO1V38A
hSUMO1K39A
hSUMO14A
450
460
470

C



D

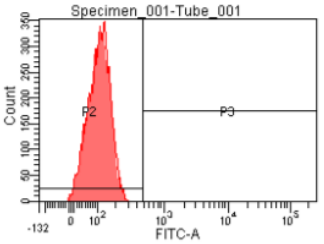
Human cells (293T cells)

Genetically modified Retroviral expression vector
Helper plasmid (pCLampho)

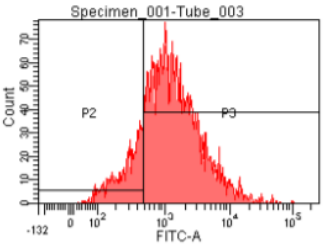
XPA$^{-/-}$*/POLH*$^{-/-}$*/PIAS1*$^{-/-}$*/PIAS4*$^{-/-}$ → Viral supernatant → *XPA*$^{-/-}$*/POLH*$^{-/-}$*/PIAS1*$^{-/-}$*/PIAS4*$^{-/-}$ cells expressing Flag-hPCNA-mutated hSUMO1

E

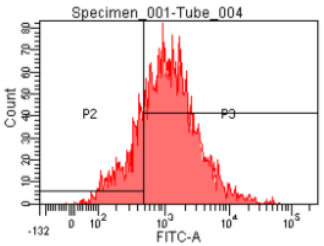
Control (TK6)



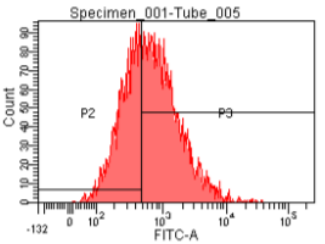
TK6 *XPA*$^{-/-}$/*POLH*$^{-/-}$/*PIAS1*$^{-/-}$/*PIAS4*$^{-/-}$ cells expressing hPCNA-hSUMO1^{F36A}



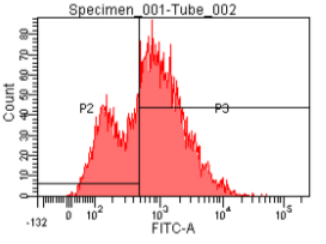
TK6 *XPA*$^{-/-}$/*POLH*$^{-/-}$/*PIAS1*$^{-/-}$/*PIAS4*$^{-/-}$ cells expressing hPCNA-hSUMO1^{V38A}



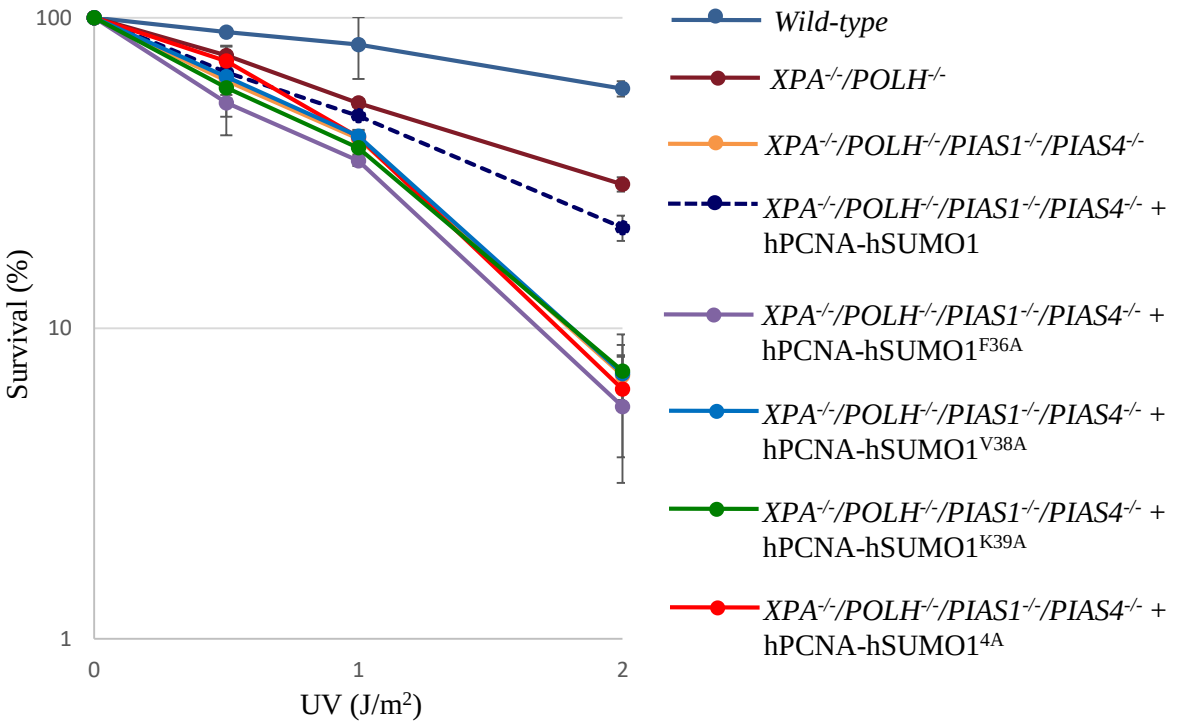
TK6 *XPA*$^{-/-}$/*POLH*$^{-/-}$/*PIAS1*$^{-/-}$/*PIAS4*$^{-/-}$ cells expressing hPCNA-hSUMO1^{K39A}



TK6 *XPA*$^{-/-}$/*POLH*$^{-/-}$/*PIAS1*$^{-/-}$/*PIAS4*$^{-/-}$ cells expressing hPCNA-hSUMO1^{4A}



F



Supplementary Tables:

Table S1. Panel of cell lines used in this study

Genotype	Parental Cell line	Marker genes	References
PIAS1$^{-/-}$	DT40	bsr^{R}, puro^{R}	♯
PIAS4$^{-/-}$	DT40	bsr^{R}, puro^{R}	♯
PIAS1$^{-/-}$/*PIAS4*$^{-/-}$	DT40	bsr^{R}, puro^{R}	♯
PCNA$^{K164R/K164R}$	DT40	bsr^{R}	(1)
PIAS1$^{-/-}$/*PIAS4*$^{-/-}$ *PCNA*$^{K164R/K164R}$	DT40	bsr^{R}, puro^{R}	♯
UBC13$^{-/-}$	DT40	bsr^{R}, hisD^{R}	(2)
PIAS1$^{-/-}$/*PIAS4*$^{-/-}$	TK6	bsr^{R}, puro^{R} hygro^{R}, neo^{R}	♯ C
POLH$^{-/-}$	TK6	-	♯ C
XPA$^{-/-}$/*POLH*$^{-/-}$	TK6	-	♯ C
POLH$^{-/-}$/*PIAS1*$^{-/-}$ /*PIAS4*$^{-/-}$	TK6	bsr^{R}, hisD^{R}, hygro^{R}, neo^{R}	♯ C
XPA$^{-/-}$/*POLH*$^{-/-}$ /*PIAS1*$^{-/-}$/*PIAS4*$^{-/-}$	TK6	bsr^{R}, hisD^{R}, hygro^{R}, neo^{R}	♯ C

♯ = This study; C = CRISPR

Table S2. Primers used in this study.

Primer	Sequence (5'-3')
F1	ATTAATCGATACTGGTTATGCGTTACAGAGTCGATATG
R1	TTAAGGATCCGGTATGCTAAGCTTTGCTATATCTGCTGGAG
F2	TTAAGGATCCGTGTTCTGGAAGGACTGAGGTGATAAGAC
R2	AATTGCGGCCGCCACAGAACTCTGTATTTCCCAAAACAATGG
F3	ATTACTCGAGGTCGGTTCAGGTGGTGCTCAGGTATGTC
R3	ATTAAGATCTGCAAAGGGCAACAGAATTAACAGAAGCAAAC
F4	ATTAGGATCCGTGAGTGTGTGTGCTGCTGTCCTCGGCCC
R4	ATTAACTAGTCAGCACTTACAGACACAGCAGTGCTCAGAG
RT_ P1	GAAAGTGAACCACAGTTACTGCTCCGTGC
RT_ P2	CAGCGCTTTGCACAGCTCTGGATGTTTG

RT_ P3	TAAAAGAGAAGCTACGCTTGGACCCGGAC
RT_ P4	CCATCTATTATCAGCTGGTCGTAGGGCGC
RT_ P5	TCAGAGTGAGGCAGTAAACGCTCGGTGCTC
RT_ P6	GGCTATCTTCCACCAACCAAAAATGGTG
RT_ P7	TTAACGCTCTCGAGTGATCGGGGTTC
RT_ P8	TCAAAGAGAAACTGACTGCAGATCCAGACAG
RT_ P9	GAGATGTTCATAGGGAGCCTTCTTGTCACAG
RT_ P10	GTGATGAAATTCAGTTTAAGGAGGATGGCTC
RT_ P11	GATATGGAAGGACAGCTTCTCTTTGCAGAT
F5	AGTTTC CTTCTGGCTG TGGGCCTGCT G
R5	TGCTGGTTGCCTCCTCTGAGCCTGTTTC
F6	AGTTTCCTTCTGGCTGTGGGCCTGCTG
R6	ACGGAGAGCCGCATCTTCACCAGCTGT
F7	GAACGAGAAGAAGCCCACCTGGATGTGC
R7	TGCTGGTTGCCTCCTCTGAGCCTGTTTC
F8	AGAGACCTGTGCCCACCTGCAGTGCTT
R8	ACGATGAGCTGTCCAGCGTGAGGTCCA
F9	AGGTTTCTGCGTGGCTTTTCTGCCTTG
R9	CCATGACTGGTCTGACATCTTTCAAGCTC
F10	AGGTTTCTGCGTGGCTTTTCTGCCTTG
R10	GCCGCATTTTACCAAGCTTTAAAAAGGGAG
F11	ACCTGGGTTTGTCCTGTCTGTGATAAGAAG
R11	CCATGACTGGTCTGACATCTTTCAAGCTC
F12	TCGGGCCCTTACATGTTCTCATCTACAATG
R12	GGATACTGGAGATGCTTGATGTGGAAGACT
F13	CTCCTCTCAAGATCGAGTGA
R13	AACTCACTGCAATTATTCTA
F14	CTCCATGAGGCTAGGAACTG
R14	CAGAAGTATGTCAGGTCCTA
F15	TCTAGGTGCTTATCTTCAACTTGATCATTT
R15	ACAATAGTAACTGAAAGGGTCAATCGGCAG
F16	ACACACTCACTTCTCTTGTG

R16	GCCATGCCTACAAAAGTAAG
F17	AGTGATGCTAAATAATGCCA
R17	AGAGAAAGTGTGGTAGAAAT
F18	CAGAAAATTGGAAAAGTTGTTCATCAACCA
R18	CTTCCTTTGCTTCTTCTAATGCTTCTTGAC

References

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2. Zhao GY, et al. (2007) A Critical Role for the Ubiquitin-Conjugating Enzyme Ubc13 in Initiating Homologous Recombination. *Mol Cell*. doi:10.1016/j.molcel.2007.01.029.