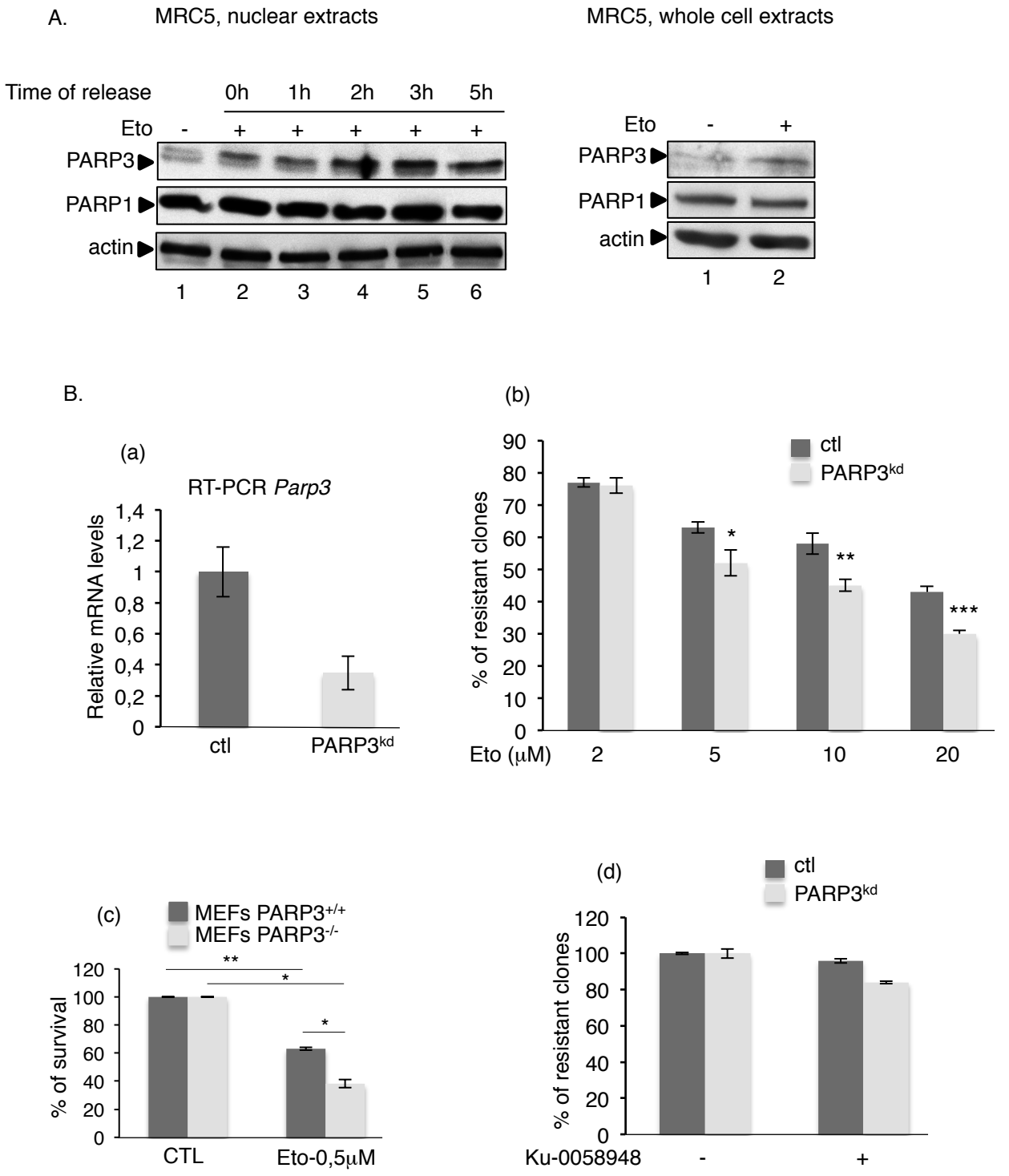
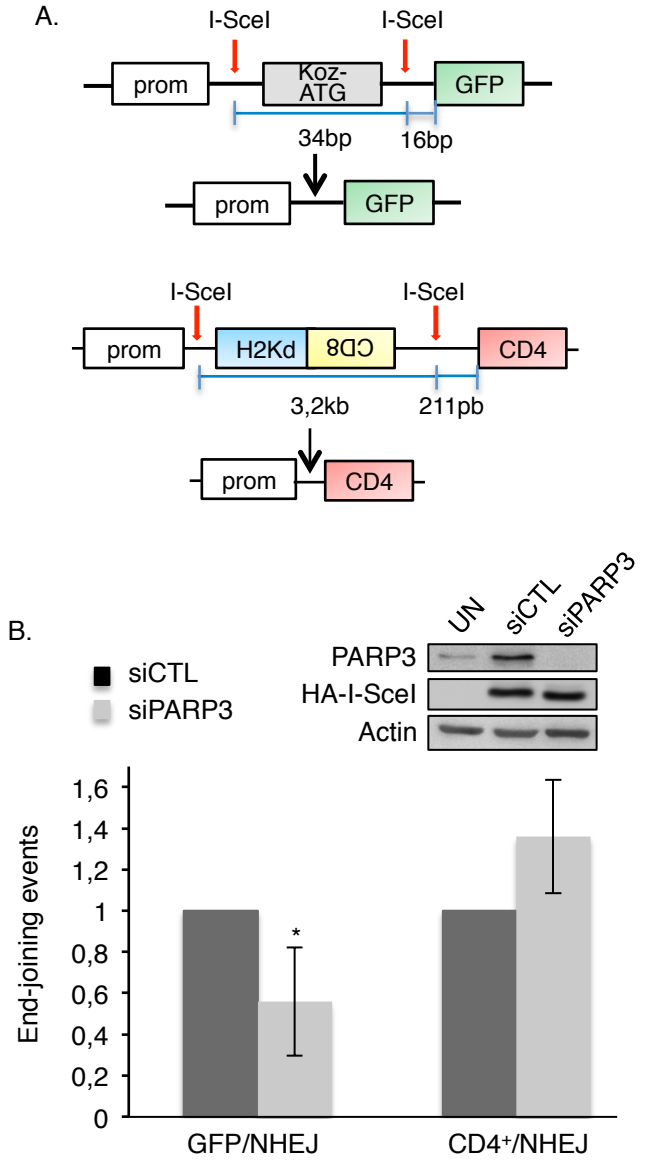


Supplemental Figure 1



C.

Ctl siRNA

HiFi :

TCTAGAGCAACACGGAAGG**AATTACCCTGTTAT**CCCTATCTAGATATGAAA 17/34

Deletions : 17/34

TCTAGAGCAACACGGAAGG**AATTACCCT**……ATCTAGATATGAAA	2
TCTAGAGCAA……..**TCCCTATCTAGATATGAAA**	1
TCTAGAGCAACACGGAAGG**AATTACC**……..TATCTAGATATGAAA	1
TCTAGAGCAACACGGAAGG**AATTA**……..TCCCTATCTAGATATGAAA	2
TCTAGAGCAACACGGAAGG**AATTACCCTGT**……CCCTATCTAGATATGAAA	2
TCTAGAGCAACACGGAAGG**AATTACCCT**……..CCCTATCTAGATATGAAA	1
TCTAGAGCAACACGGAAGG……..**TGTTAT**CCCTATCTAGATATGAAA	1
TCTAGAGCAACACGGAAGG**AAT**……..CCCTATCTAGATATGAAA	1
TCTAGAGCAACACGGAAGG**AATT**……CCCTATCTAGATATGAAA	1
TCTAGAGCAACACGGAAGG**AA**……..CCCTATCTAGATATGAAA	1
TCTAGAGCAACACGG……D42……	1
TCTA……D38……..GATATGAAA	1
……..D58……	1
……D>100……	1

PARP3 siRNA :

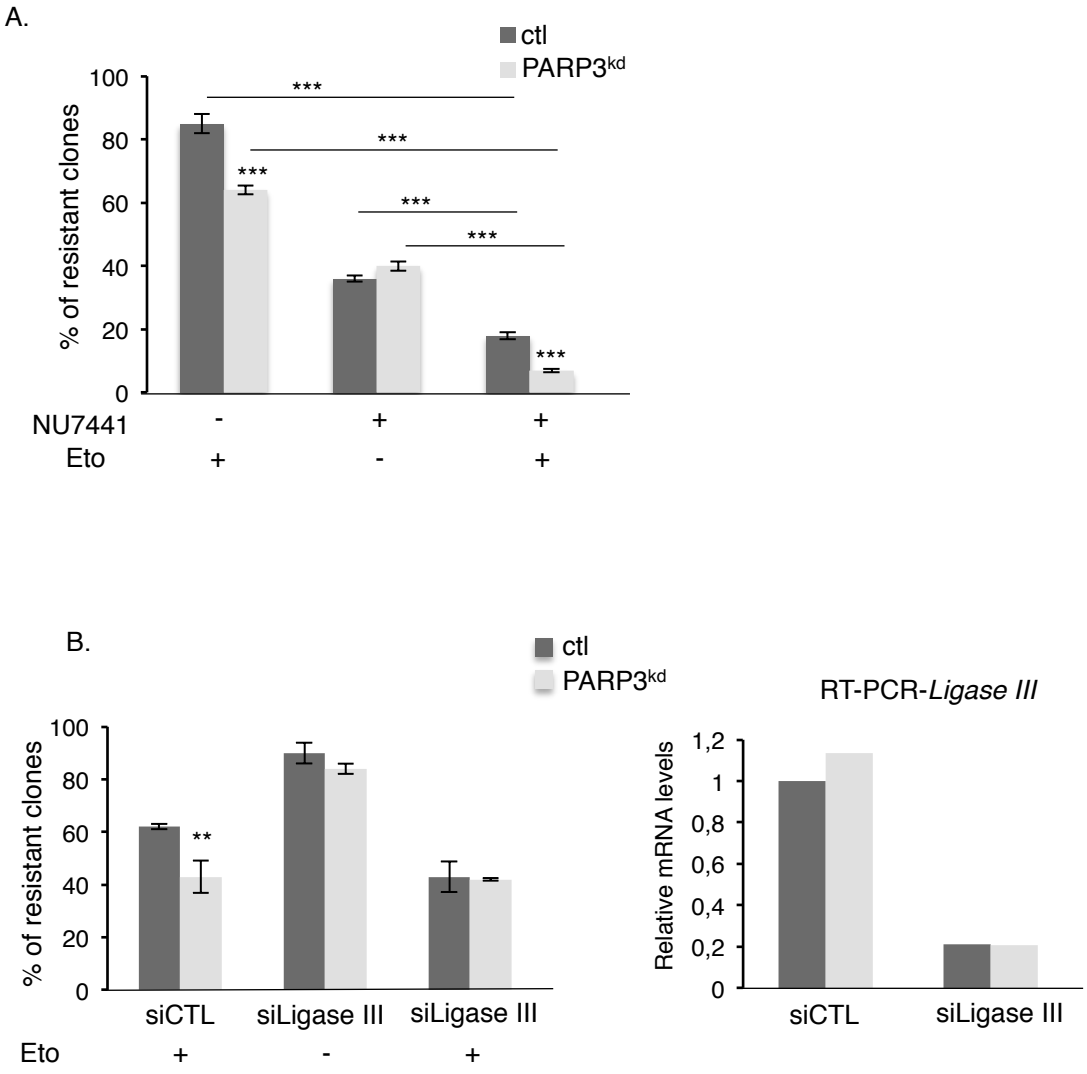
HiFi :

TCTAGAGCAACACGGAAGG**AATTACCCTGTTAT**CCCTATCTAGATATGAAA 9/34

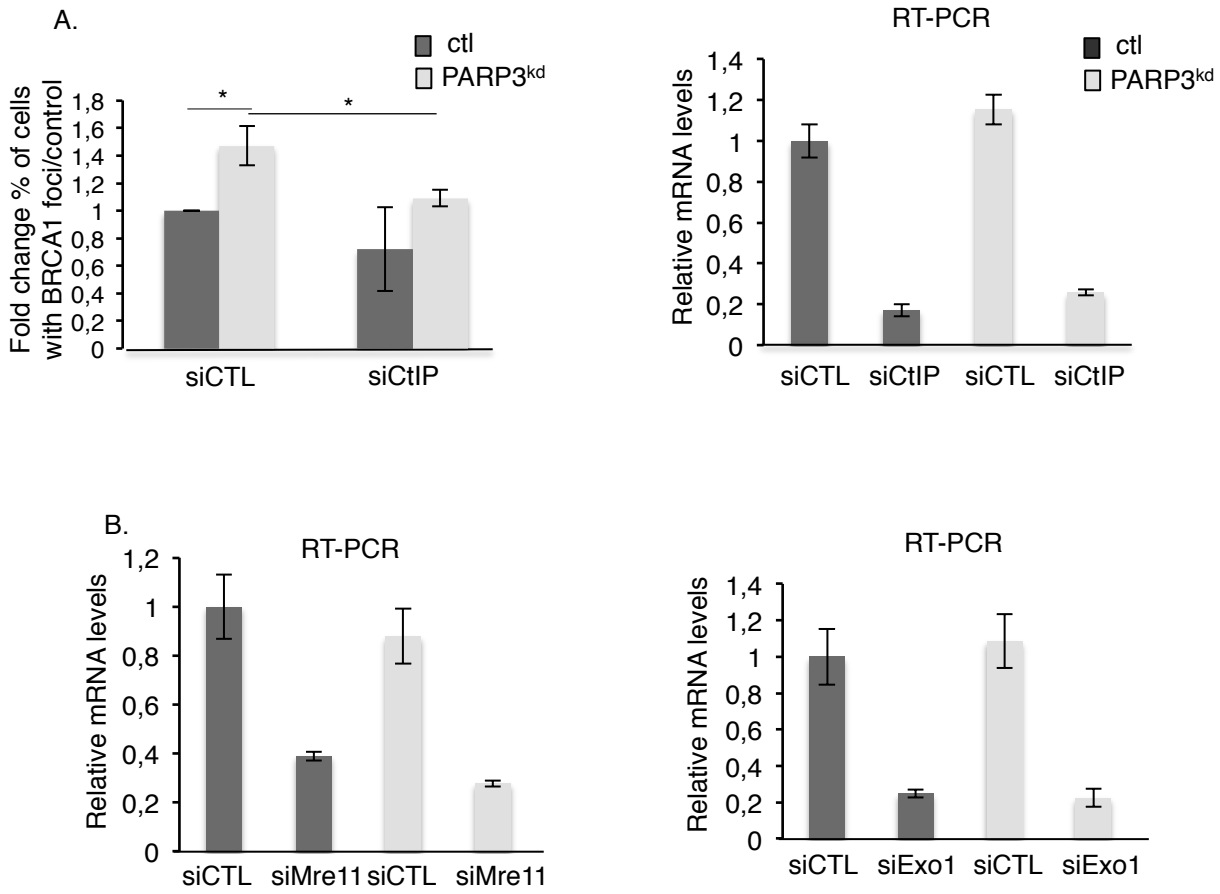
Deletions : 25/34

TCTAGAGCAACA……**TCCCTATCTAGATATGAAA**	1
TCTAGAGCAACACGGAAGG**AATTACCCT**……..ATCTAGATATGAAA	5
TCTAGAGCAACACGGAAGG**AATTACCCT**……TCTAGATATGAAA	2
TCTAGAGCAACACGGAAGG**AATTA**……..CCCTATCTAGATATGAAA	1
TCTAGAGCAACACGGAAGG**AA**……..CCTATCTAGATATGAAA	1
TCTAGAGCAACACGGAAG……GATATGAAA	1
TCTAGAGCAACACGGA……TCTAGATATGAAA	1
TCTAGAGC……..CCTATCTAGATATGAAA	1
TCTAGAGCAACACGGAAGG**AATTACCCTGT**……..ATCTAGATATGAAA	1
TCTAGAGCAACAC……ATCTAGATATGAAA	1
TCTAGAGCAACACGGAAGG**AATTACCCT**…..**TAT**CCCTATCTAGATATGAAA	1
TCTAGAGCAACA……..TCTAGATATGAAA	1
TCTAGAGCAACACGGA……..AGATATGAAA	1
TCTAGAGCAACACGGAAGG**AAT**……D>100……	1
TCTA……D38……..GATATGAAA	1
……D43……..**TAT**CCCTATCTAGATATGAAA	1
TC……D58……	1
……D86……TAGATATGAAA	1
……D>100……	2

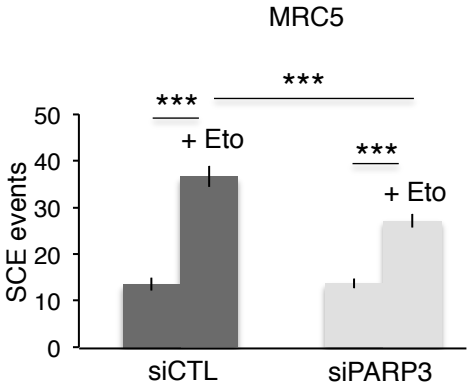
Supplemental Figure 2



Supplemental Figure 3



Supplemental Figure 4



Supplemental Figure 5

Supplemental Material and Methods

Reagents, cell lines

Wortmannin was purchased from Sigma.

The GSC5 cell line was derived from SV40-transformed GM639 human fibroblasts and contains the GFP-based and CD4-based sustrates (15, 38, 39). GSC5 were cultured in Dulbecco's modified Eagle's medium-1g/L D-glucose containing 10% fetal calf serum, 1% Gentamicin and supplemented with 3μg/mL blasticidin and 350 μg/mL neomycin to maintain the expression of CD4 and GFP respectively.

Quantitative RT-PCR

Total RNA was extracted from siRNA-transfected control and PARP3^{kd}-cells using the RNAeasy kit (Qiagen) according to the manufacturer's protocol. DNase-treated RNA was processed for reverse transcription using the Maxima Reverse Transcriptase (Thermo Scientific) according to the manufacturer's instructions. Real time PCR was performed using the QuantiTect SYBR Green PCR kit following the manufacturer's instructions (Qiagen) combined with the Applied Biosystems StepOne (Life technologies) detection system. The PCR products were analysed with the StepOne Software. The quantity of PCR products was estimated by the relative standard curve method. All samples were analyzed in triplicates and normalized using the *gapdh* housekeeping gene. The following primer sequences were used : Parp3, Fwd, TGGCAAGGGCATCTACTTTG ; Rev, TCCGTGTTGATATGGTGCTC ; Mre11, Fwd, ATCTCACAACCTGGAAGCTCAG, Rev, TGAAAAACTGCCGCACTGTG ; CtIP, Fwd, TGACCTGTCCAAAGACGACTTG, Rev, AGCTGCTTCCCGAGATGTTC ; Exo1, Fwd, ACTTCTTCGTGAGGGGAAAGTC, Rev, ATTACTTTGTGGGCCATGGC ; Gapdh: Fwd, ATGTTCGTCATGGGTGTGAA, Rev, GTCTTCTGGGTGGCAGTGAT. Ligase III, Fwd, GATCACGTGCCACCTACCTTGT, Rev, GGCATAGTCCACACAGAACCGT.

Mouse embryonic fibroblasts and cell viability. The wild-type (Parp3$^{+/+}$) and Parp3 null (Parp3$^{-/-}$) mouse embryonic fibroblasts (MEFs) were generated as previously described (98). The mice have been described (33). Cells were maintained in Dulbecco's modified Eagle's medium-4,5g/L D-glucose supplemented with 10% fetal calf serum and 1% Gentamicin at 37°C in 5% CO_2. For cell viability, cells were seeded onto 6-well plates at 75000 cells/well. The next day, cells were mock-treated or exposed to etoposide at 0,5 μM for 4h. Three days later, cells were diluted at ¼ to prevent confluency. Viability was analysed 6 days later using the blue trypan exclusion test.

Supplemental Figures Legends

Supplemental figure 1. (A) Enhanced expression of PARP3 upon etoposide treatment. Left panel, MRC5 cells were either mock-treated (lane 1) or treated with etoposide (Eto, 50 μM, lanes 2-6) for 3h and then released in fresh medium for the indicated time points. Equivalent amounts of nuclear extracts were analysed by western blotting using the indicated antibodies. Right panel, MRC5 cells were either mock-treated (lane 1) or treated with etoposide (Eto, 50 μM, 3h, lane 2) and equivalent amounts of total protein extracts were analysed by western blotting as above. (B-a) Control of the depletion of PARP3 for figure 1B. The PARP3 depletion in the stable PARP3^{kd} cells was regularly verified by quantitative RT-PCR analysis of the PARP3^{kd} RNA compared to the ctl RNA. *Parp3* transcript levels were normalized against *gapdh* control. (B-b) The depletion of PARP3 sensitizes cells to etoposide. Clonogenic survival of the control (ctl) and PARP3-depleted (PARP3^{kd}) cells exposed to

increasing doses of etoposide as indicated. Experiments were performed twice giving similar results. Mean values of triplicates +/-SD are indicated. *P< 0,05 ; **P< 0,01 ; ***P< 0,001. (B-c) PARP3$^{-/-}$ MEFs are sensitive to etoposide. Exponentially growing PARP3$^{+/+}$ MEFs and PARP3$^{-/-}$ MEFs were exposed to etoposide (0,5 μM, 4h). Viability was measured 6 days later using the trypan blue exclusion test. Experiments were performed three times. Mean values +/-SD are indicated. *P< 0,05 ; **P< 0,01 (B-d) Ku-0058948 alone did not affect the cell viability of the ctl or PARP3^{kd} cells. Clonogenic survival of the control (ctl) and PARP3-depleted (PARP3^{kd}) cells exposed to Ku-0058948 (100nM) alone in the experimental conditions of Figure 1B. Mean values of triplicates +/-SD are indicated. The small decrease observed for the PARP3^{kd} cells + Ku-0058948 was not statistically relevant.

Supplemental figure 2. PARP3-depleted cells are defective in NHEJ. (A) The intrachromosomal substrates. The GCV6 cell line used contains two types of NHEJ reporter substrates : the GFP-based substrate in which the I-Sce1 cleavage sites are separated by only 34bp to monitor end-joining of close adjacent ends and the CD4-based substrate in which the I-Sce1 cleavage sites are separated by 3,2 kb to monitor end-joining of distal ends. (B) NHEJ efficiencies in the GSC5 cells treated with the indicated siRNAs. Values represent the means of three independent experiments (+/-SD). Similar to the GCV6 cell line, the GSC5 cell line contains a GFP-based substrate that monitors end-joining of close adjacent ends and the CD4-based substrate that monitors end-joining of distal ends. Insets, PARP3 depletion and SceI expression were verified by Western blotting. (C) Analysis of the junction sequences of the PCR products obtained after amplification of the CD4 fragment after NHEJ-mediated repair in control (siCTL) and PARP3-silenced (siPARP3) cells.

Supplemental figure 3. The depletion of PARP3 impacts on classical and alternative End joining. (A) Clonogenic survival of etoposide-treated control (ctl) and PARP3-depleted (PARP3^{kd}) cells in the absence or in the presence of the DNA-PK inhibitor NU7441. Note the additive effect of the absence of PARP3 and the inhibition of DNA-PK on the cytotoxicity of etoposide. (B) Clonogenic survival of etoposide-treated control (ctl) and PARP3-depleted (PARP3^{kd}) cells after transfection with siCTL or siLigaseIII. The double depletions of PARP3 and Ligase III do not increase the cell sensitivity to etoposide compared to each single depletion. Right panel, the depletion of Ligase III was verified by quantitative RT-PCR analysis. *DNA ligase III* transcript levels were normalized against *gapdh* control. For (A) and (B), experiments were performed >3 times giving similar results. Mean values of triplicate +/-SD are indicated. **P< 0,01; ***P< 0,001.

Supplemental figure 4. (A) Extensive DNA end resection in the PARP3^{kd} cells is partially rescued by the additional depletion of CtIP. Left panel, Control (ctl) and PARP3-depleted cells (PARP3^{kd}) were transfected with the indicated siRNA for 48h, treated with etoposide (50 μM) for 1h, released in fresh medium for 5h and processed for BRCA1 staining. The histogram depicts the fold change in the percentage of cells displaying BRCA1 foci relative to the control. An average of 500 cells per condition were scored in > 20 randomly selected fields. Data are represented as the means of three independent experiments +/-SD. Right panel, The siRNA-mediated depletion of CtIP was verified by quantitative RT-PCR analysis of ctl and PARP3^{kd} RNA 48h upon treatment with siRNA. *CtIP* transcript levels were normalized against *gapdh* control. **(B) Control of the siRNA-mediated depletion of Mre11, and Exo1 for figure 5B.** The siRNA-mediated depletion of Mre11 and Exo1 were verified by quantitative RT-PCR analysis of ctl and PARP3^{kd} RNA 48h upon treatment with the indicated siRNA. *Mre11* and *Exo1* transcript levels were normalized against *gapdh* control.

Supplemental figure 5. Etoposide-induced SCE formation is reduced in PARP3-depleted cells. SCE formation was analysed in undamaged or etoposide-treated (+Eto) MRC5 cells after transfection with the indicated siRNA.

94. Boehler, C., Gauthier, L., Yelamos, J., Noll, A., Schreiber, V. and Dantzer, F. (2011) Phenotypic characterization of Parp-1 and Parp-2 deficient mice and cells. Methods Mol Biol, 780, 313-336.