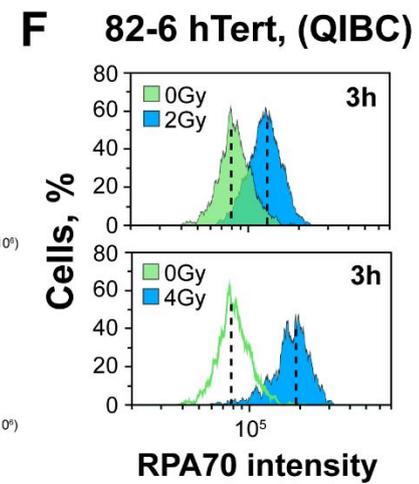
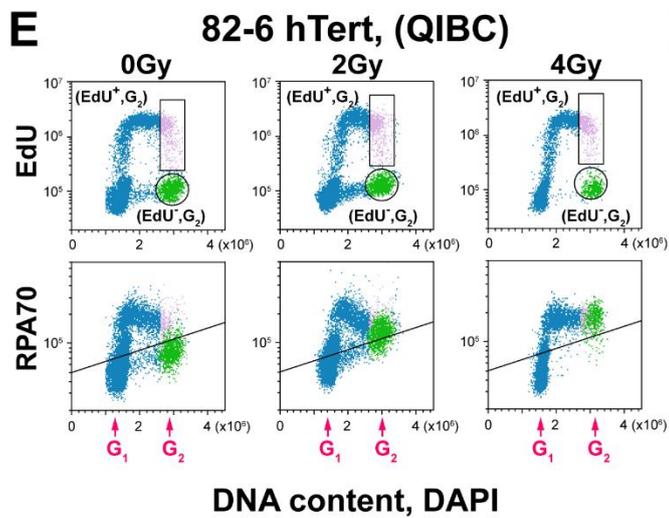
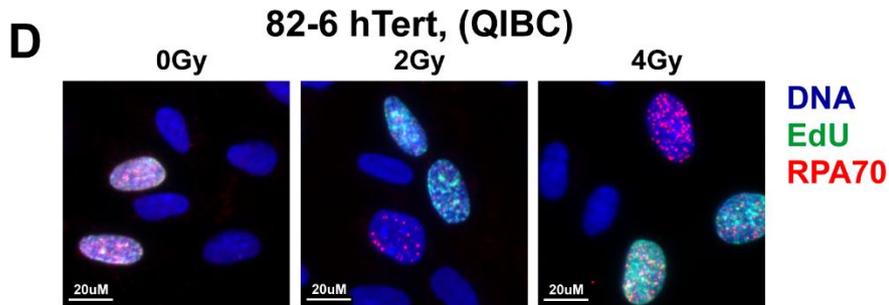
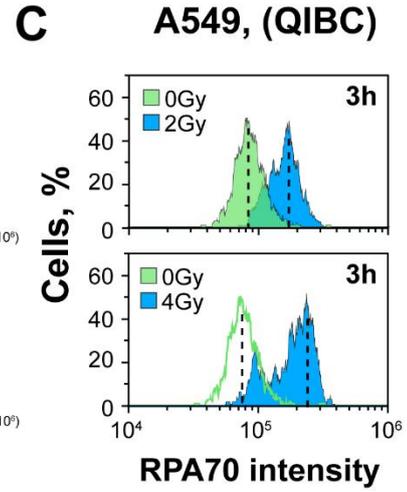
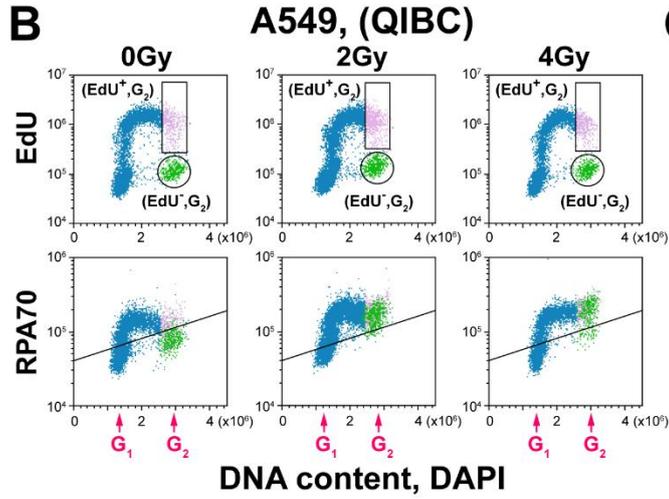
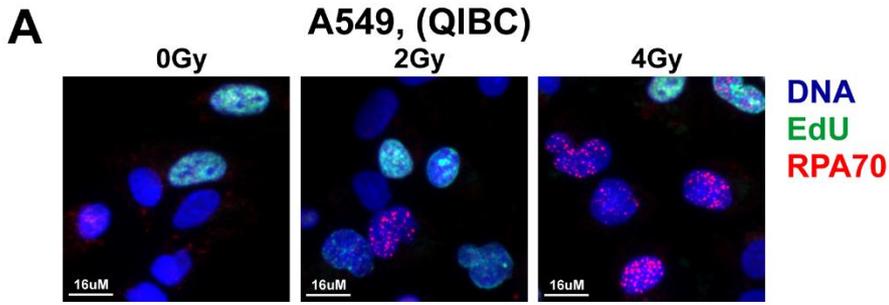
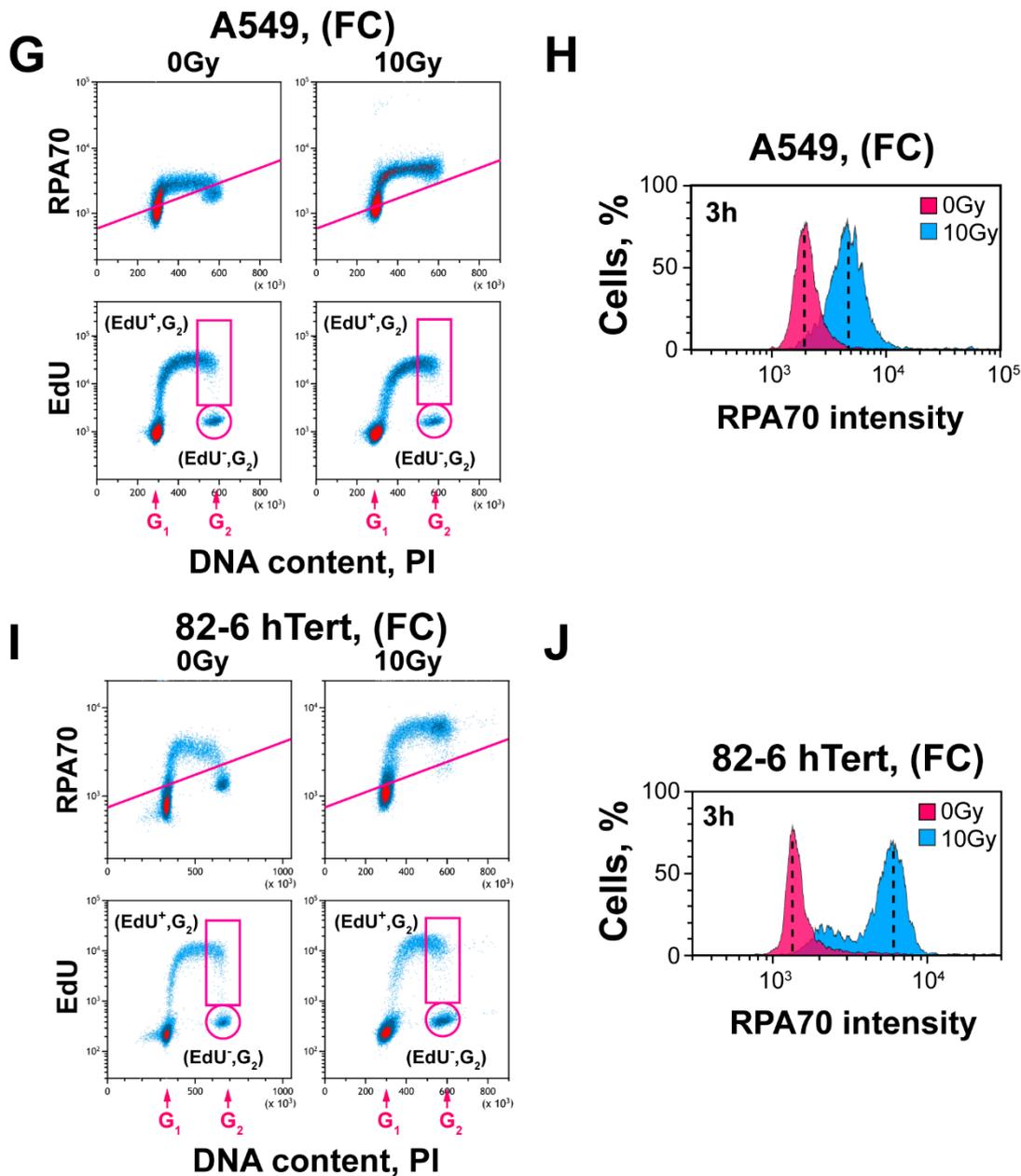


# **Supplementary Information**

**Increased resection at DSBs in G<sub>2</sub>-phase is a unique phenotype associated with DNA-PKcs defects that is not shared by other factors of c-NHEJ**

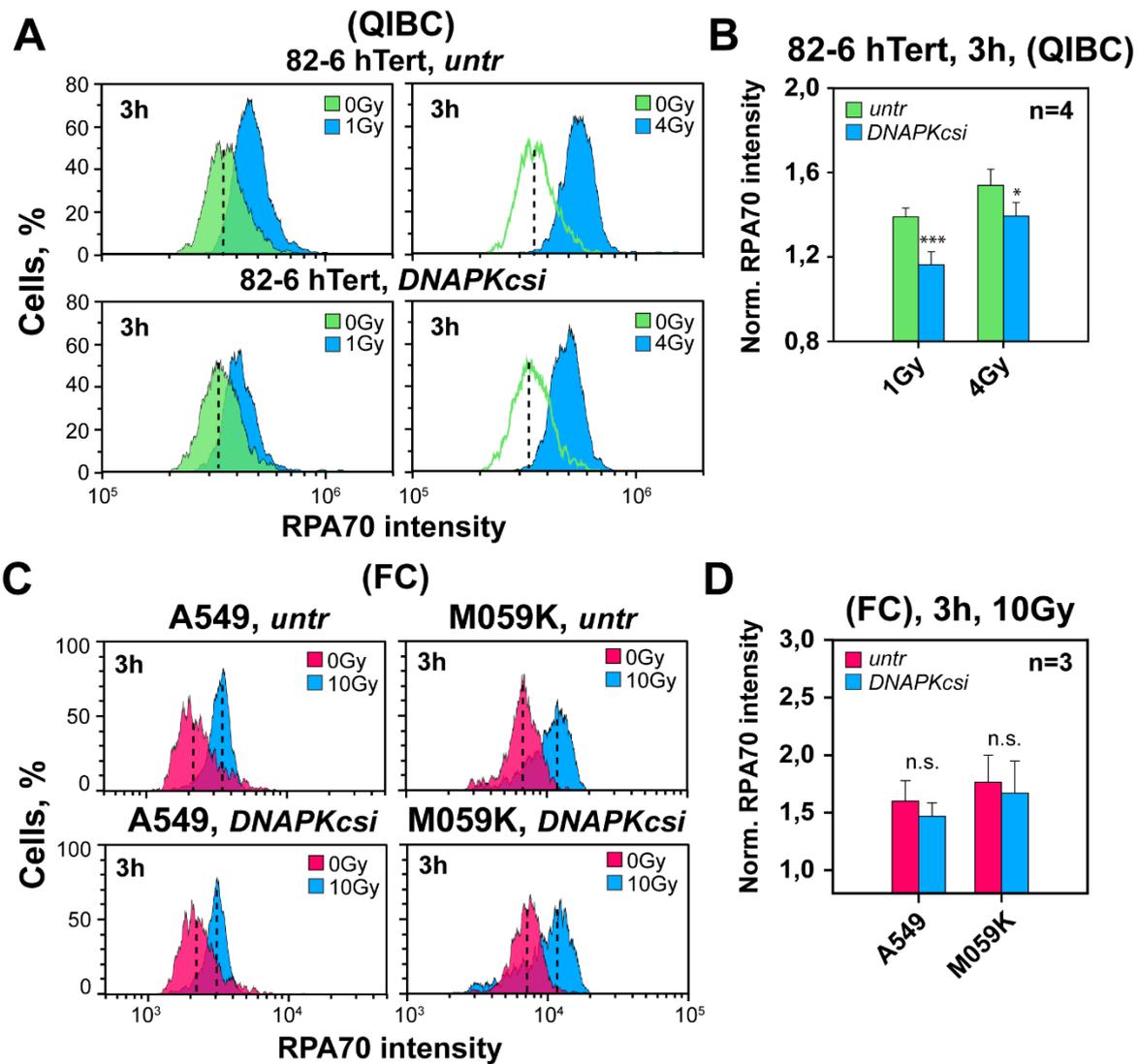
Huaping Xiao Fanghua Li, Emil Mladenov, Aashish Soni, Veronika Mladenova, Bing Pan, Rositsa Dueva, Martin Stuschke, Beate Timmermann and George Iliakis



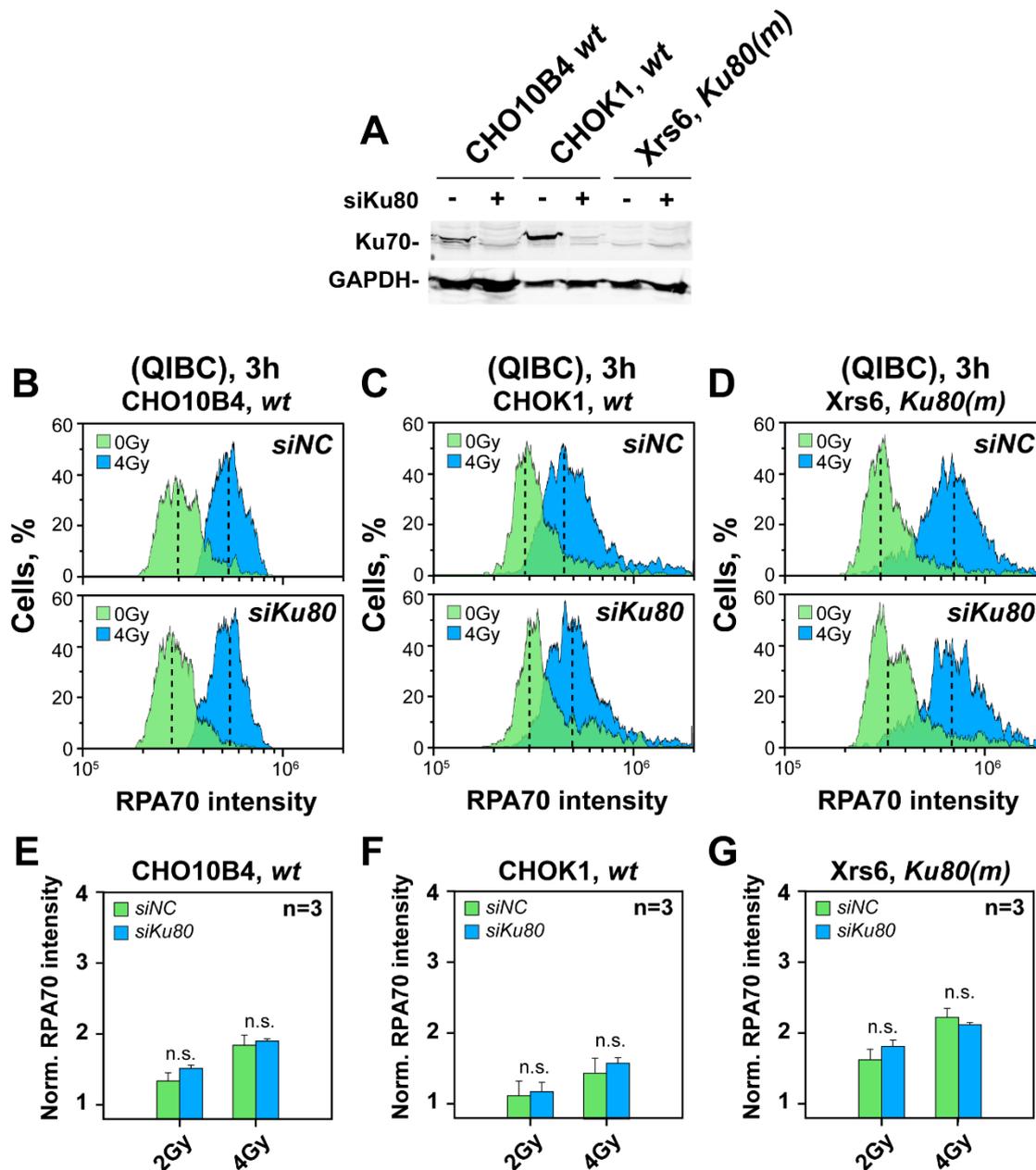


**Figure S1.** Outline of methodology to measure resection in  $G_2$ -phase. A) Representative AxioScan Z1 immunofluorescent images, obtained during QIBC analysis of A549 cells irradiated with 2 and 4 Gy X-rays. DNA was counterstained with DAPI and DAPI intensity (blue) was assessed to determine the progression of cells through the cell cycle. EdU intensity signal (green) was evaluated to determine whether cells were irradiated in S-phase, while the accumulation of RPA70 (red) onto chromatin was an indication for ongoing DNA end resection. B) Dot plots of RPA70, EdU and DAPI intensities obtained by QIBC analysis. The gates utilized to specifically select cells in different cell cycle phases

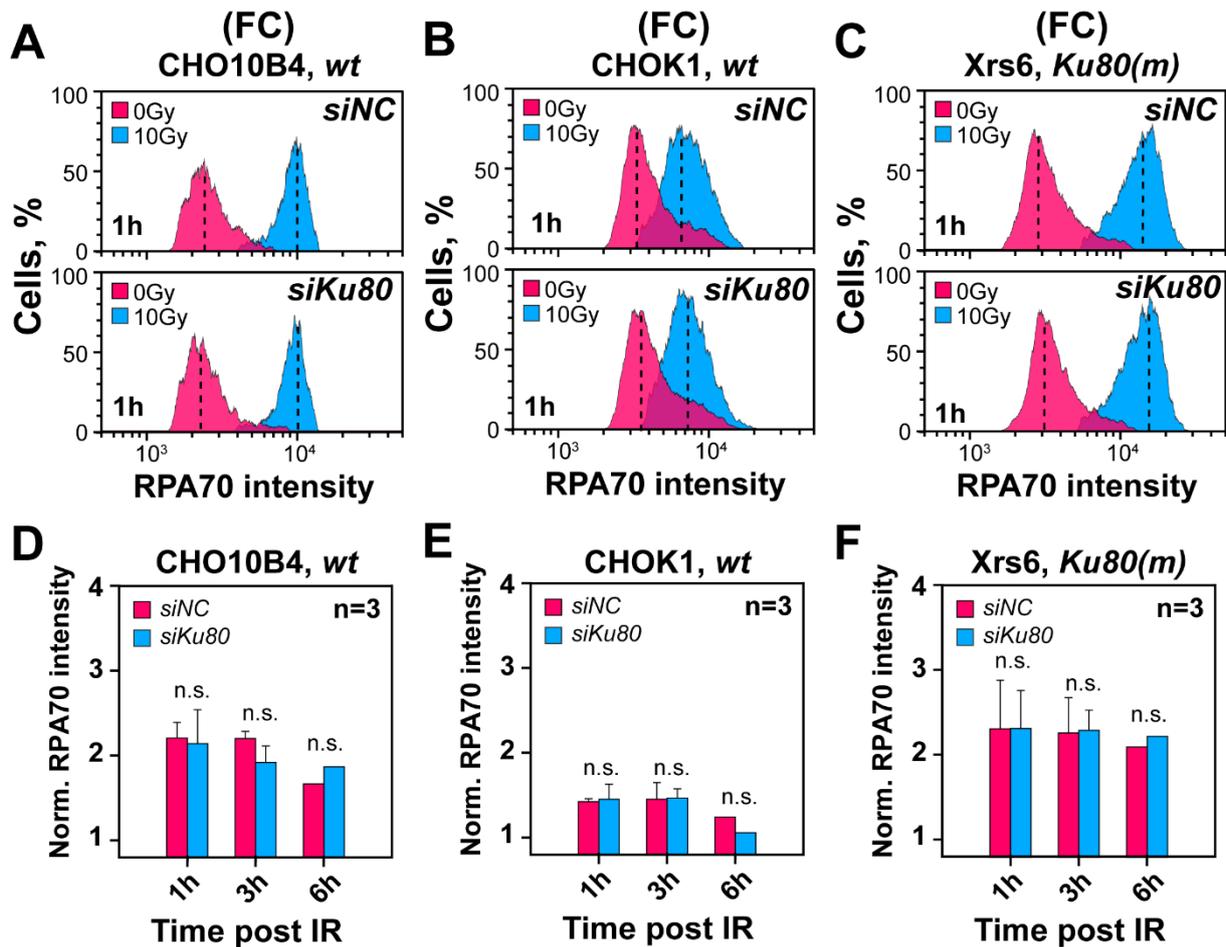
are also depicted. EdU positive (EdU<sup>+</sup>, violet) or EdU negative (EdU<sup>-</sup>, green) cells, represent the cells irradiated, respectively, in S- or G<sub>2</sub>-phase of the cell cycle. Counterstaining with DAPI allows the determination of DNA content, which reflects the cell cycle phase during analysis. For our experiments, we have selected cells irradiated in G<sub>2</sub>-phase and analyzed in the G<sub>2</sub>-phase (EdU<sup>-</sup>, G<sub>2</sub>-cells, green). C) The RPA70 intensity signals in the selected gates (EdU<sup>-</sup>, G<sub>2</sub>-cells) are shown as histogram plots. DNA end-resection after IR is visualized by the increase in the signal intensity between irradiated and non-irradiated cells. The ratio of the means between the two signal intensities is used as a parameter to quantitate resection in the experiments shown here. D, E and F) As in panels A, B and C for 82-6 hTert cells. G) Three parametric flow cytometry analysis of RPA70 signal intensity in A549 cells, showing the gates utilized to select cells irradiated in the G<sub>2</sub>-phase and analyzed in G<sub>2</sub>-phase of the cell cycle (EdU<sup>-</sup>, G<sub>2</sub>-cells). H) The RPA70 intensity signals in the selected gates (EdU<sup>-</sup>, G<sub>2</sub>-cells) are plotted as histogram plots. Other details as in Fig. S1A-C. I) As in panel G, for 82-6 hTert cells. J) As in panel H, for 82-6 hTert cells.



**Figure S2.** Resection analysis in DNA-PKcs deficient cells. A) QIBC analysis of RPA70 signal at DSBs in 82-6 hTert cells treated or not with *DNA-PKcsi* (*NU7441*), 3 h after exposure to 1 Gy or 4 Gy. Other details as in Fig. 1B. B) Bar plot showing the normalized RPA70 signal intensity from three experiments, obtained as shown in panel A. C) FC-based analysis of resection in wild-type A549 and M059K cells, treated or not with *DNA-PKcsi*. DNA end-resection is measured 3 h after exposure to 10 Gy (other details as in Fig. 1D). D) Bar plot showing the normalized RPA70 signal intensity from three experiments, obtained as shown in panel C.



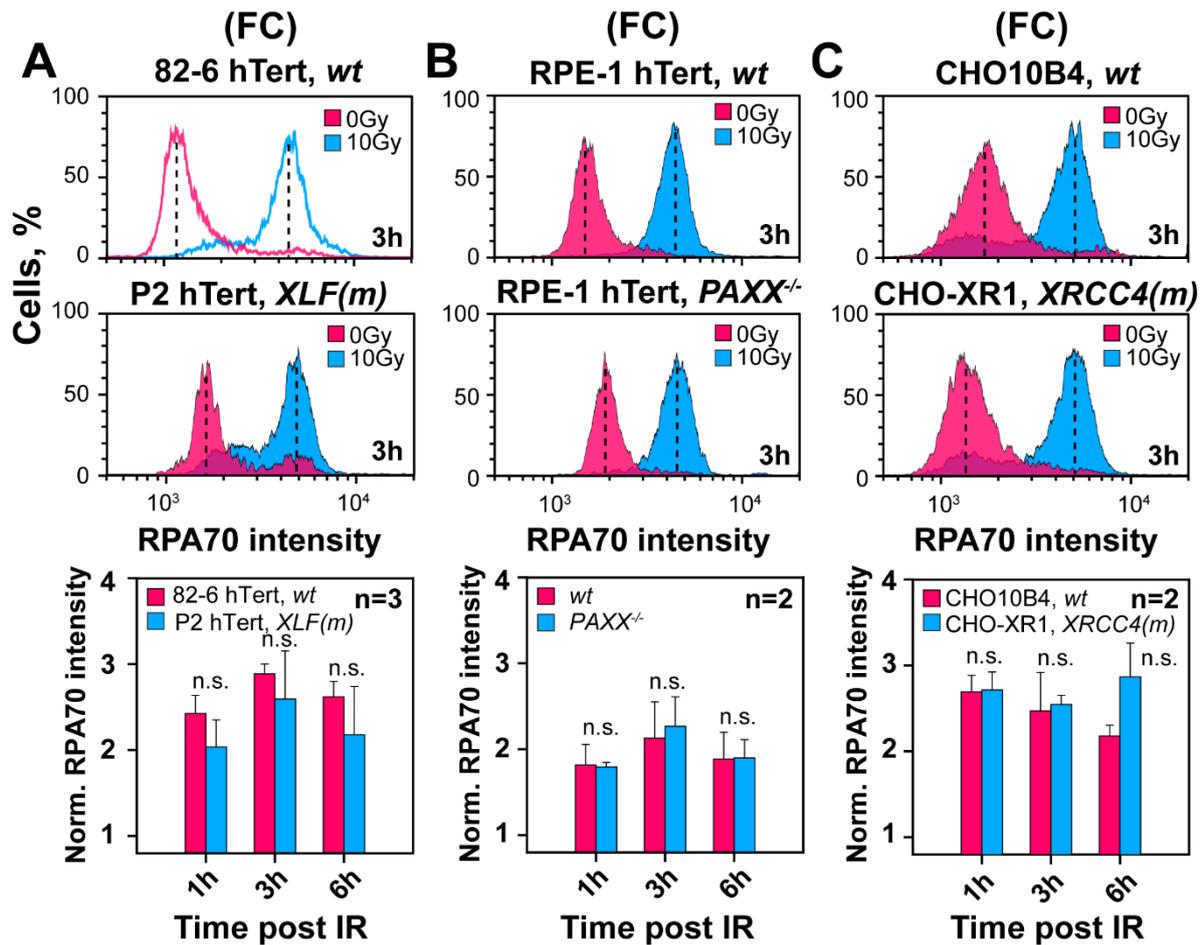
**Figure S3.** Resection analysis in CHO cells after knockdown of Ku. A) Western blot analysis showing the level of Ku70 in CHO10B4, CHOK1 and Xrs6 cells 24 h after transfection with a siRNA against Ku80, or with a non-specific control siRNA. KU70 serves here as a proxy for KU80, as the anti-KU80 antibody we used failed to recognize the hamster protein. B) Representative histograms of RPA70 intensity in mock transfected and Ku depleted CHO10B4(wt) cells, obtained by QIBC analysis 3 h after exposure to 2 or 4 Gy. C) As in panel B, for CHOK1(wt) cells. D) As in panel B, or the Ku80 mutant. E) Quantitative analysis of RPA70 signal in CHO10B4(wt) cells. F) As in panel E, but for CHOK1(wt) cells. G) As in panel E, for Xrs6 cells. The results represent the mean and SE from 3 independent determinations.



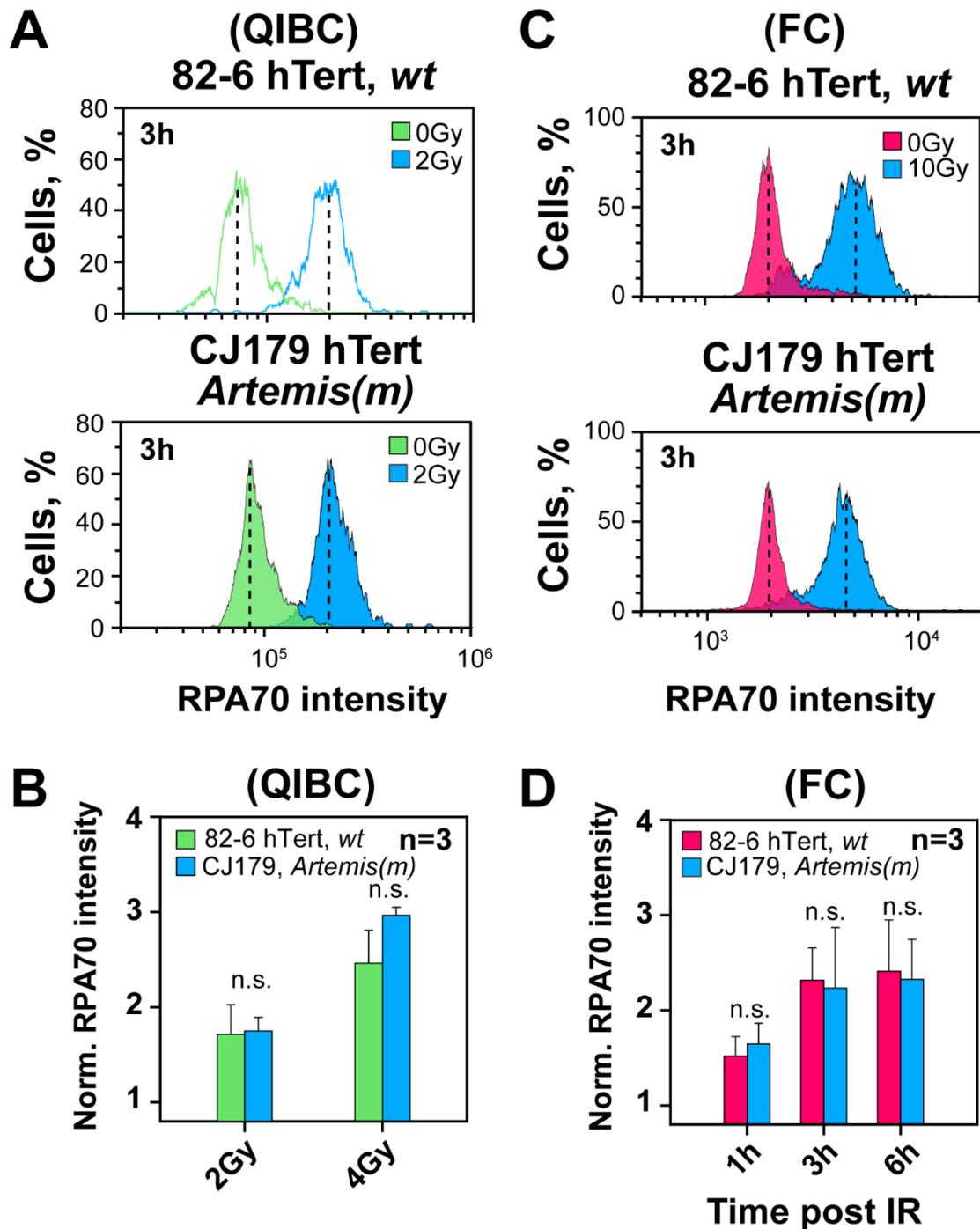
**Figure S4.** Resection after *Ku* knockdown in CHO cell lines. A) Representative histograms of RPA70 intensity in mock transfected and *Ku* depleted CHO10B4(wt) cells, obtained by FC analysis 1 h after exposure to 10 Gy. B) As in panel A, for CHOK1 cells. C) As in panel A, for Xrs6 cells. D) Quantitative analysis of RPA70 signal in CHO10B4 cells. E) As in panel D, for CHOK1 cells. F) As in panel D, for Xrs6 cells. The results represent the mean and SE from 3 independent determinations.



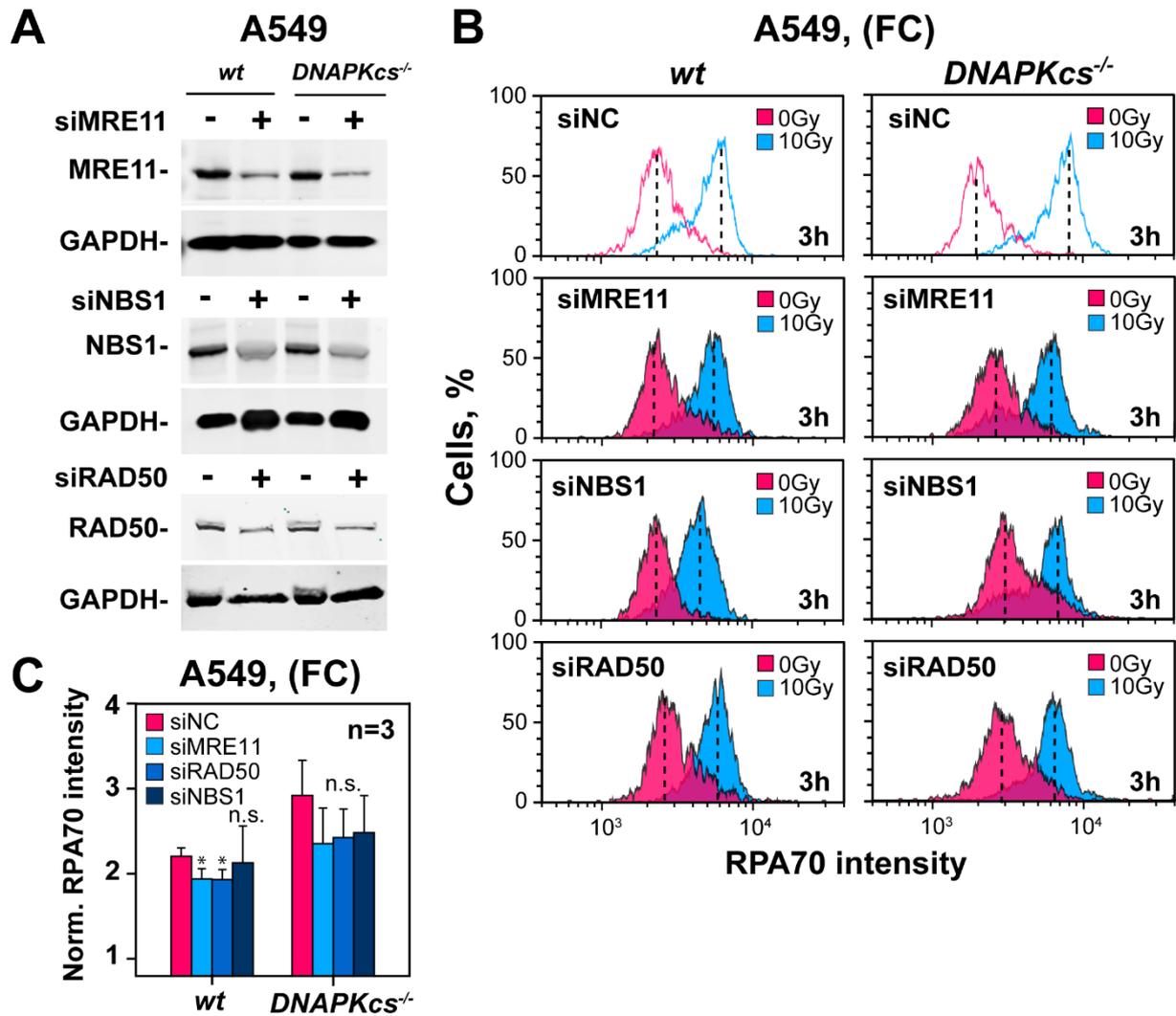
**Figure S5.** *Resection analysis in LIG4 deficient cells.* A) QIBC analysis of RPA70 in wt, 82-6 hTert and LIG4 deficient 180BR-M human cell lines. The bar plot is showing the normalized RPA70 signal intensity from three independent determinations. B) As in panel A for LIG4 proficient and deficient HeLa cells. C) FC-based analysis of RPA70 signal intensity in human cell lines deficient in LIG4. Other details as in Figure 2B. D) Quantification of RPA70 signal from three experiments such as those shown in panel A.



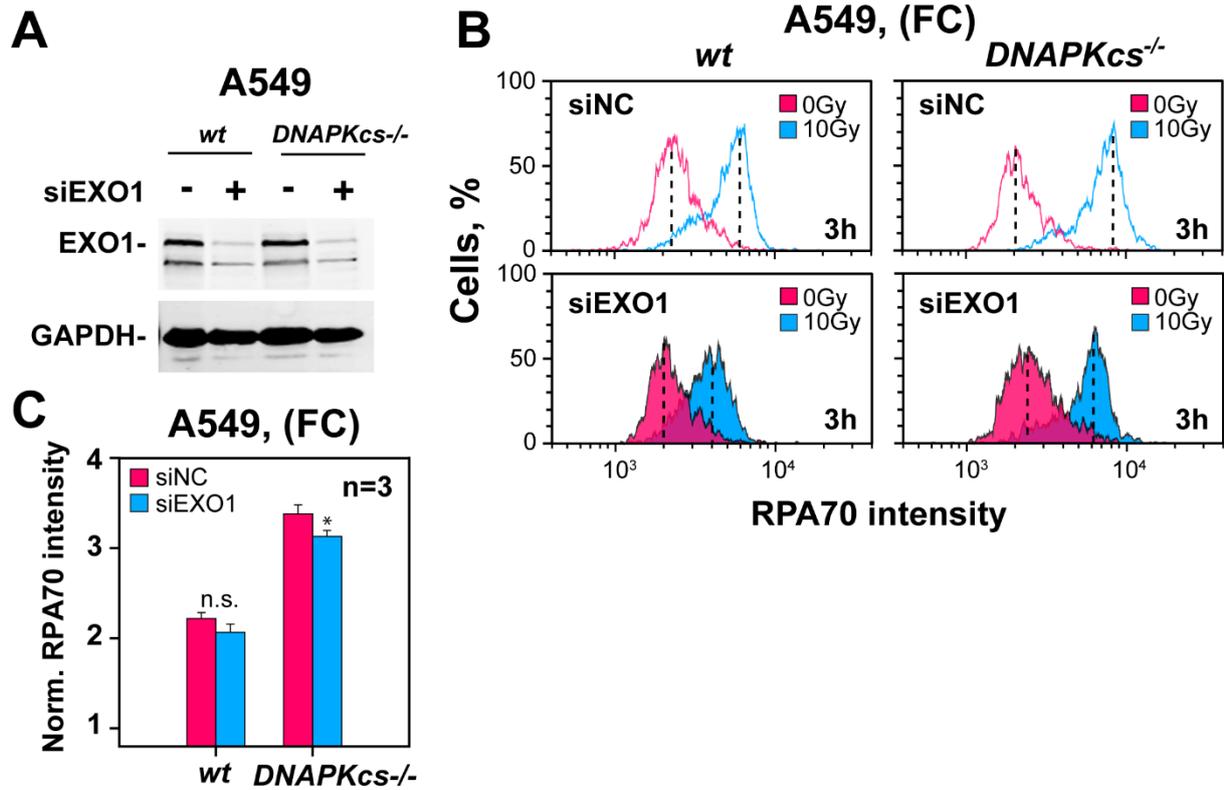
**Figure S6.** FC-based analysis of resection in *c-NHEJ* deficient cells. A) Resection in *XLF*<sup>-/-</sup> cells. B) Resection in *PAXX*<sup>-/-</sup> cells. C) Resection in *XRCC4*<sup>-/-</sup> cells. All cell lines were exposed to 10 Gy and analyzed at 1 h, 3 h, and 6 h after irradiation. The experiments are repeated thrice and the bar graphs at the bottom show the combined results. Plotted are the mean and SE from three independent experiments.



**Figure S7. Resection analysis in *Artemis*<sup>-/-</sup> cells.** A) RPA70 signal intensity determined using QIBC 3 h after exposure to 2 Gy or 4 Gy of IR (only data for 2 Gy are shown). B) Quantification of RPA70 signal intensity determined as shown in panel A. C) As in panel A, using FC after exposure to 10 Gy. D) As in panel B, for experiments shown in panel C. Plotted are the mean and SE from three independent experiments.



**Figure S8.** Resection analysis in *wt* and *DNA-PKcs<sup>-/-</sup>* A549 cells after depletion of components of the MRN complex. A) Western blot analysis showing the levels of MRE11, NBS1 and RAD50 in *wt* and *DNA-PKcs<sup>-/-</sup>* A549 cells after transfection with the corresponding siRNAs. B) Cell cycle specific FC analysis of RPA70 signal intensity, 3 h after exposure of MRN depleted cells to 10 Gy of IR. C) Quantification of RPA70 signal intensity shown in panel B. The results represent the mean and SE from 3 independent determinations.



**Figure S9.** *Effects on resection of EXO1 depletion.* A) Western blot analysis showing the level of EXO1 after transfection with a specific EXO1 siRNAs. B) As in Figure 6B, for cells depleted for EXO1 by transfection with a specific EXO1 siRNA. C) As in Figure 6C, for cells depleted for EXO1.

**Table S1.** Sequences of siRNA against human and hamster genes of interests. For more information see the Materials and Methods.

<b>Target protein</b>	<b>siRNA</b>
<b>Negative control (siNC)</b>	1. UUCUCCGAACGUGUCACGU
<b>EXO1</b>	1. GCCUUUGCUAUCCAAUCCCACG
<b>DNA2</b>	1. AGACAAGGUUCCAGCGCCA
<b>CTIP</b>	1. GCUAAAAC AGGAACGAAUC 2. GAUUCGUUCCUGUUUAGC
<b>MRE11</b>	1. GAUGCCAUUGAGGAAUAAG
<b>RAD50</b>	1. UAAUGAGACUUGACAAUGA 2. ACACUCUUGGGUACAAUAA 3. ACAGAACUCCUCACUAAGA
<b>NBS1</b>	1. UAACCUUGUUGGCCUGAAGUAGAUG 2. CAGGAUGGCUGUCAGGUUA
<b>BLM</b>	1. GAGCACAUCUGUAAAUUAA 2. CAGGAUGGCUGUCAGGUUA
<b>DNA-PKcs</b>	1. CGGCUAACUCGCCAGUUUA
<b>Human KU80</b>	1. UUCCUUA AUGGCUUGUCGUUU 2. AAACGACAAGCCAUUAAGGAA
<b>Hamster Ku80</b>	1. GAAACUGUCUAUUGCUUAA 2. CCAUAGGGAAGAAGUUUGA 3. GGAUCCUAUGAGUGUUUA

**Table S2.** Statistical analysis of the results shown in main figures. For more information see the Materials and Methods.

<b>Figure1C</b>	<b>Figure3B</b>	<b>Figure5C</b>
2Gy, wt vs DNAPKcs <sup>-/-</sup>	2Gy, CHO10B4 vs Xrs6	2Gy, wt vs LIG4 <sup>-/-</sup>
P = 0.0013	P = 0.7098	P < 0.0001
4Gy, wt vs DNAPKcs <sup>-/-</sup>	4Gy, CHO10B4 vs Xrs6	4Gy, wt vs LIG4 <sup>-/-</sup>
P = 0.0026	P = 0.8009	P = 0.0015
<b>Figure1E</b>	<b>Figure3D</b>	<b>Figure5E</b>
10Gy, 1h, wt vs DNAPKcs <sup>-/-</sup>	10Gy, 1h, CHO10B4 vs Xrs6	2Gy, wt vs XLF(m)
P = 0.0494	P = 0.8898	P = 0.0006
10Gy, 3h, wt vs DNAPKcs <sup>-/-</sup>	10Gy, 3h, CHO10B4 vs Xrs6	4Gy, wt vs XLF(m)
P = 0.0495	P = 0.8018	P < 0.0001
10Gy, 6h, wt vs DNAPKcs <sup>-/-</sup>	10Gy, 6h, CHO10B4 vs Xrs6	
P = 0.0433	P = 0.0717	
<b>Figure2C</b>	<b>Figure3G</b>	<b>Figure6C</b>
2Gy, CHOK1 vs XR-C1-3	10Gy, 1h, siNC vs siKU80	wt, siNC vs siDNA2
P = 0.0001	P = 0.5974	P = 0.0002
4Gy, CHOK1 vs XR-C1-3	10Gy, 3h, siNC vs siKU80	wt, siNC vs siBLM
P = 0.0001	P = 0.3473	P = 0.0001
2Gy, CHOK1 vs V3	10Gy, 6h, siNC vs siKU80	wt, siNC vs siDNA2/siBLM
P = 0.0003	P = 0.5025	P = 0.0002
4Gy, CHOK1 vs V3		DNAPKcs <sup>-/-</sup> , siNC vs siDNA2
2Gy, CHOK1 vs Irs20	<b>Figure4A</b>	P < 0.0001
P = 0.0063	2Gy, wt vs LIG4 <sup>-/-</sup>	DNAPKcs <sup>-/-</sup> , siNC vs siBLM
4Gy, CHOK1 vs Irs20	P = 0.9986	P < 0.0001
P = 0.0006	4Gy, wt vs LIG4 <sup>-/-</sup>	DNAPKcs <sup>-/-</sup> , siNC vs siDNA2/siBLM
<b>Figure2E</b>	P = 0.1747	P < 0.0001
10Gy, 1h, CHOK1 vs XR-C1-3	<b>Figure4B</b>	<b>Figure6E</b>
P < 0.0001	2Gy, wt vs XLF(m)	wt, untr vs DNA2i
10Gy, 3h, CHOK1 vs XR-C1-3	P = 0.4116	P = 0.0001
P = 0.0040	4Gy, wt vs XLF(m)	DNAPKcs <sup>-/-</sup> , untr vs DNA2i
10Gy, 6h, CHOK1 vs XR-C1-3	P = 0.5100	P < 0.0001
P = 0.0069	<b>Figure4C</b>	
10Gy, 1h, CHOK1 vs Irs20	2Gy, wt vs PAXX <sup>-/-</sup>	
P = 0.0015	P = 0.7155	
10Gy, 3h, CHOK1 vs Irs20	4Gy, wt vs PAXX <sup>-/-</sup>	
P = 0.0302	P = 0.6523	
10Gy, 6h, CHOK1 vs Irs20	<b>Figure4E</b>	
P = 0.1054	CHO10B4 vs V3	
	P = 0.002	
	CHO10B4 vs XR1	
	P = 0.196	
	CHO10B4 vs Xrs6	
	P < 0.001	

**Table S3.** Statistical analysis of the results shown in supplementary figures. For more information see the Materials and Methods.

<b>FigureS2B</b>	<b>FigureS4D</b>	<b>Figure5A</b>	<b>FigureS6A</b>	<b>FigureS8C</b>
1Gy, untr vs DNAPKcsi	10Gy, 1h, CHO10B4, siNC vs siKu80	2Gy, wt vs LIG4(m)	10Gy, 1h, wt vs XLF(m)	A549, wt, siNC vs siMRE11
P = 0.0009	P = 0.8095	P = 0.7499	P = 0.1494	P = 0.0426
4Gy, untr vs DNAPKcsi	10Gy, 3h, CHO10B4, siNC vs siKu80	4Gy, wt vs LIG4(m)	10Gy, 3h, wt vs XLF(m)	A549, wt, siNC vs siNBS1
P = 0.0266	P = 0.0784	P = 0.9483	P = 0.5396	P = 0.0370
<b>FigureS2D</b>	10Gy, 6h, CHO10B4, siNC vs siKu80	<b>Figure5B</b>	10Gy, 6h, wt vs XLF(m)	A549, wt, siNC vs siRAD50
A549, untr vs DNAPKcsi	P = 0.1835	2Gy, wt vs LIG4 <sup>-/-</sup>	P = 0.3477	P = 0.7767
P = 0.3421	<b>FigureS4E</b>	4Gy, wt vs LIG4 <sup>-/-</sup>	<b>FigureS6B</b>	A549, DNAPKcs <sup>-/-</sup> , siNC vs siMRE11
M059K, untr vs DNAPKcsi	10Gy, 1h, CHOK1, siNC vs siKu80	P = 0.1187	10Gy, 1h, wt vs PAXX <sup>-/-</sup>	P = 0.1711
P = 0.6778	P = 0.8012	<b>FigureS5D</b>	10Gy, 3h, wt vs PAXX <sup>-/-</sup>	A549, DNAPKcs <sup>-/-</sup> , siNC vs siNBS1
<b>FigureS3E</b>	10Gy, 3h, CHOK1, siNC vs siKu80	10Gy, 1h, wt vs LIG4(m)	P = 0.6840	P = 0.1810
2Gy, CHO10B4, siNC vs siKu80	P = 0.9130	P = 0.4409	10Gy, 6h, wt vs PAXX <sup>-/-</sup>	A549, DNAPKcs <sup>-/-</sup> , siNC vs siRAD50
P = 0.0686	10Gy, 6h, CHOK1, siNC vs siKu80	10Gy, 3h, wt vs LIG4(m)	P = 0.9533	P = 0.2751
4Gy, CHO10B4, siNC vs siKu80	P = 0.2703	P = 0.9884	<b>FigureS6C</b>	<b>FigureS9B</b>
P = 0.5339	<b>FigureS4F</b>	10Gy, 6h, wt vs LIG4(m)	10Gy, 1h, wt vs XRCC4(m)	A549, wt, siNC vs siEXO1
<b>FigureS3F</b>	10Gy, 1h, Xrs6, siNC vs siKu80	P = 0.0737	P = 0.8898	P = 0.0773
2Gy, CHOK1, siNC vs siKu80	P = 0.9917	<b>FigureS5D, HCT116</b>	10Gy, 3h, wt vs XRCC4(m)	A549, DNAPKcs <sup>-/-</sup> , siNC vs siEXO1
P = 0.7089	10Gy, 3h, Xrs6, siNC vs siKu80	10Gy, 1h, wt vs LIG4 <sup>+/-</sup>	P = 0.7875	P = 0.0773
4Gy, CHOK1, siNC vs siKu80	P = 0.7584	10Gy, 3h, wt vs LIG4 <sup>-/-</sup>	10Gy, 6h, wt vs XRCC4(m)	P = 0.0773
P = 0.3420	<b>FigureS3G</b>	P = 0.4339	P = 0.0717	
2Gy, Xrs6, siNC vs siKu80	10Gy, 1h, wt vs LIG4 <sup>-/-</sup>	10Gy, 6h, wt vs LIG4 <sup>-/-</sup>	<b>FigureS7B</b>	
P = 0.1355	P = 0.1075	P = 0.8338	2Gy, wt vs Artemis(m)	
4Gy, Xrs6, siNC vs siKu80	10Gy, 3h, wt vs LIG4 <sup>-/-</sup>	<b>FigureS5D, HeLa</b>	P = 0.8659	
P = 0.2331	P = 0.0856	10Gy, 1h, wt vs LIG4 <sup>-/-</sup>	4Gy, wt vs Artemis(m)	
	10Gy, 6h, wt vs LIG4 <sup>-/-</sup>	P = 0.1208	P = 0.0718	
	P = 0.1208	<b>FigureS7D</b>	<b>FigureS7D</b>	
		10Gy, 1h, wt vs Artemis(m)	10Gy, 1h, wt vs Artemis(m)	
		P = 0.4946	P = 0.4946	
		10Gy, 3h, wt vs Artemis(m)	10Gy, 3h, wt vs Artemis(m)	
		P = 0.4339	P = 0.4339	
		10Gy, 6h, wt vs Artemis(m)	10Gy, 6h, wt vs Artemis(m)	
		P = 0.8338	P = 0.8338	