DNA polymerase η modulates replication fork progression and DNA damage responses in platinum-treated human cells.

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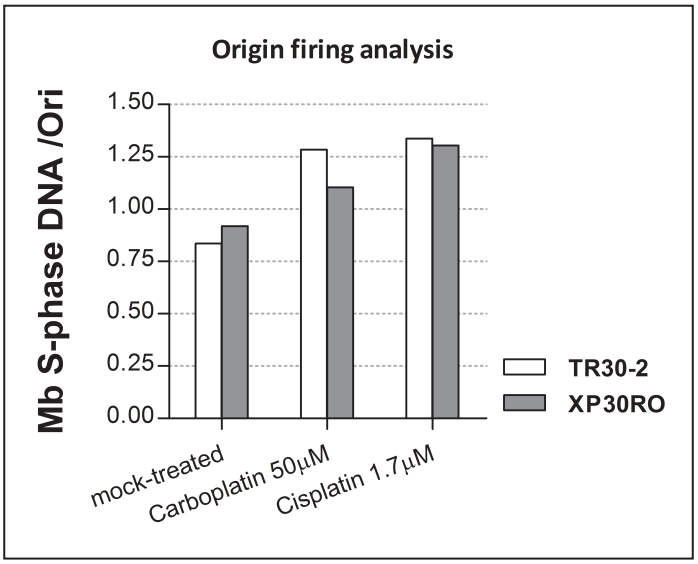
Supplementary Figure 1. DNA combing parameters. **1a** Mean length and synthesis rate values from two independent DNA combing experiments ± SEM, are presented in Table format. N stands for the number of measured objects. **1b** Graph presents the frequency of origin firing based on data from DNA combing experiments carried out under the indicated conditions. BrdU incorporation data from FACS analysis was used to calculate the S-phase DNA fraction.

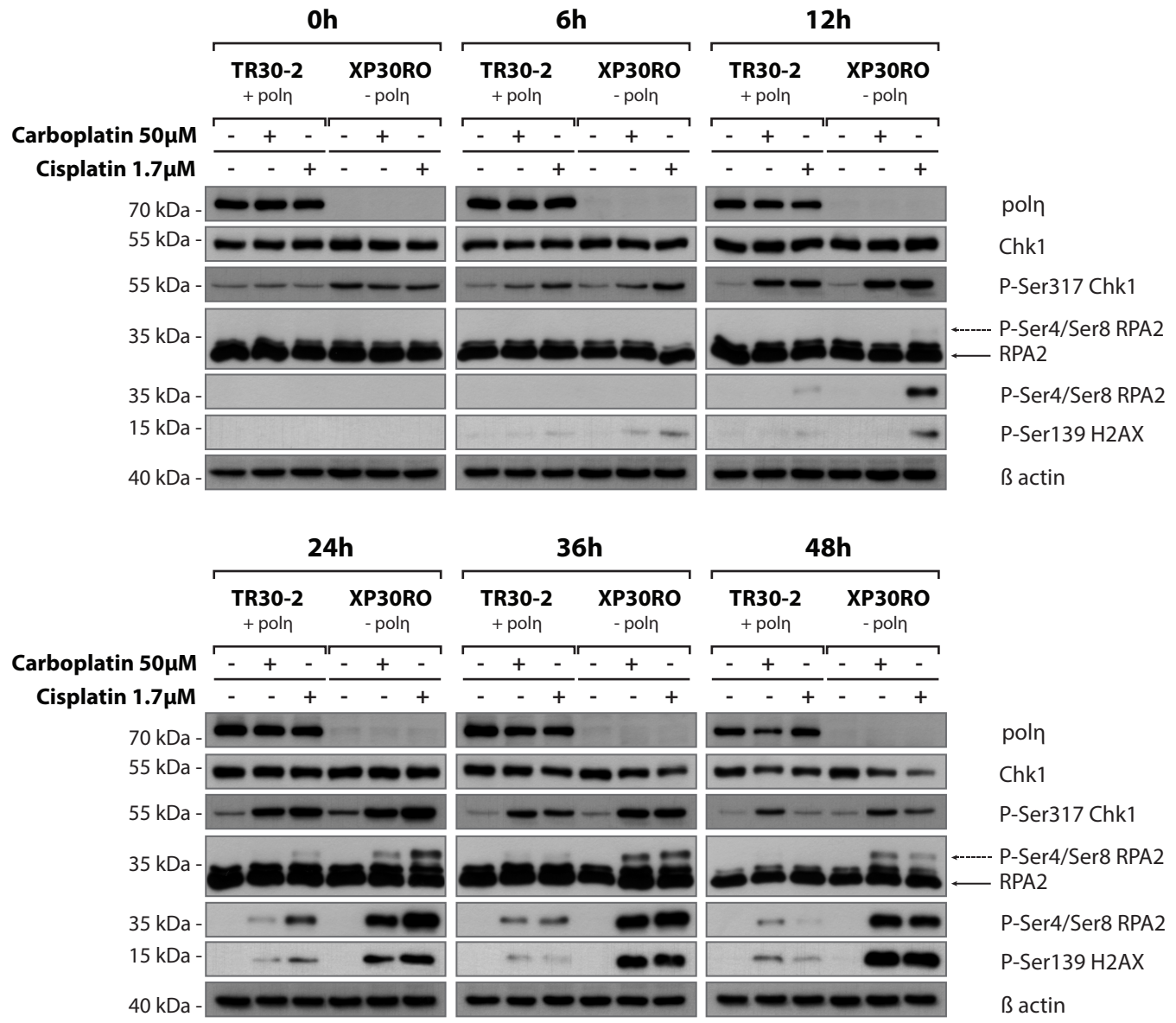
Supplementary Figure 2. Kinetics of carboplatin- and cisplatin-induced DNA damage responses in cells lacking and expressing DNA polymerase η. Polη-deficient XP30RO cells and polη expressing TR30-2 cells were treated with indicated doses of carboplatin and cisplatin and harvested at indicated times post-treatment. Expression of DNA damage response proteins and protein phosphorylation were analysed by western blotting using specific antibodies as described in Materials and Methods. For direct comparison, samples obtained from both cell lines at the same time-point were run on the same gels.

Supplementary Table 1. EdU intensity analysis by quantitative immunofluorescence. Cells expressing (TR30-2) or lacking (XP30RO) polη were grown on glass coverslips and treated with indicated doses of cisplatin and carboplatin. 75 minutes before fixing, cells were incubated with 10μM EdU. Click chemistry was used to visualise EdU incorporation. **1a** Mean relative EdU intensities values ± SEM from three independent experiments under the indicated conditions are presented. The number of individual values is indicated as n. **1b** Fold-decrease in EdU intensity ± SEM relative to mock-treated samples is presented. **1c** The EdU intensity threshold, obtained by subtracting double the SEM value from the mean relative EdU intensity value.

a	Mock - treated			Carboplatin 50μM			Cisplatin 1.7μM		
	Mean length [kb]	Mean synthesis rate [kb/min]	n	Mean length [kb]	Mean synthesis rate [kb/min]	n	Mean length [kb]	Mean synthesis rate [kb/min]	n
TR30-2	82.0 ± 2.8	1.37 ± 0.05	183	82.8 ± 2.8	1.38 ± 0.05	185	78.9 ± 2.8	1.31 ± 0.05	163
XP30RO	82.3 ± 2.1	1.37 ± 0.03	268	69.1 ± 2.6	1.15 ± 0.04	180	48.2 ± 1.6	0.80 ± 0.03	360

b




0h
6h
12h
TR30-2
+ polη
XP30RO
- polη
Carboplatin 50μM
Cisplatin 1.7μM
70 kDa
55 kDa
55 kDa
35 kDa
35 kDa
15 kDa
40 kDa
polη
Chk1
P-Ser317 Chk1
P-Ser4/Ser8 RPA2
RPA2
P-Ser4/Ser8 RPA2
P-Ser139 H2AX
ß actin

24h
36h
48h
TR30-2
+ polη
XP30RO
- polη
Carboplatin 50μM
Cisplatin 1.7μM
70 kDa
55 kDa
55 kDa
35 kDa
35 kDa
15 kDa
40 kDa
polη
Chk1
P-Ser317 Chk1
P-Ser4/Ser8 RPA2
RPA2
P-Ser4/Ser8 RPA2
P-Ser139 H2AX
ß actin

a	Mock - treated		Carboplatin 50µM		Cisplatin 1.7µM	
	Mean relative EdU intensity	n	Mean relative EdU intensity	n	Mean relative EdU intensity	n
TR30-2	1175.0 ± 31.5	394	447.9 ± 12.4	487	385.7 ± 14.1	402
XP30RO	1220.0 ± 26.7	362	255.1 ± 9.4	523	177.0 ± 9.5	489

b	Carboplatin 50µM	Cisplatin 1.7µM
	Fold decrease in relative EdU intensity	Fold decrease in relative EdU intensity
TR30-2	2.7 ± 0.14	3.2 ± 0.22
XP30RO	5.7 ± 1.75	7.3 ± 1.19

c	Carboplatin 50µM	Cisplatin 1.7µM
	Threshold value for EdU intensity Mean relative EdU intensity - 2xSEM	Threshold value for EdU intensity Mean relative EdU intensity - 2xSEM
XP30RO	236.7	157.9