siRNA sequence information

For siRNA-mediated knockdown, the following targeting sequences were used:

LACZ	AACGTACGCGGAATACTTCGA
REV1	CAGCGCATCTGTGCCAAAGAA
REV3	ATGAGTATGGATCATATACAA
REV7	CACCCGGAGCTGAATCAGTAT
POLH	CTGGTTGTGAGCATTCGTGTA
POLI	GCGGTTTATTAAGCTCTTCTA
POLK	TAGGATGGGACTTAATGATAA
POLM	CGGGAAGGACTGCGAACCTTA
POLB	TACGAGTTCATCCATCAATTT
POLN	TTGCGCATTCTTACACATTTA, AAGCACCCAATTCAGATTACT
BRCA1	ACCATACAGCTTCATAAATAA
BRCA2	TTGAAGAATGCAGGTTTAATA
ATR	AAGCCAAGACAAATTCTGTGT
FANCD2	TTGGAGGAGATTGATGGTCTA
FANCI	AACGTACGCGGAATACTTCGA
HEL308	CAAAGGAAGATTTCCTCCAACTAAA

For siResistant POLN, the coding sequence 5'…CAC CCA ATT CAG ATT ACT ACA…3' was exchanged to 5'…CAC CCT ATA CAA ATC ACC ACA…3' in the pWZL retroviral expression system.

Supplementary Figure legends

Supplementary Figure S1. HeLa cells transfected with siRNA targeting DNA polymerases were assayed for sensitivity to 100μM cisplatin. The two main sensitizers identified were REV1 and POLN. BRCA1 was used as a positive control. P-values were below 0.05 only for REV1, POLN, and BRCA1. Error bars represent standard errors.

Supplementary Figure S2. Complementation of the chromosomal instability phenotype of POLN-depleted cells upon stable expression of siRNA resistant POLN. (A) 293T cells stably expressing wildtype POLN or siRNA resistant POLN were analyzed for MMC-induced chromosome aberrations, after siRNA targeting POLN was introduced. The endogenous and the exogenous wildtype POLN, but not the exogenous siResistant, is targeted by the siRNA. Expression of the siResistant POLN can reduce chromosomal aberrations, including radial chromosome formation, compared to cells expressing wildtype POLN. Similar results were obtained in three independent experiments. (B) Western blot showing that wildtype, but not siRNA resistant POLN is targeted by the siRNA.

Supplementary Figure S3. MMC-induced radial formation in POLN-depleted cells cannot be complemented by expression of a polymerase-dead POLN mutant. (A) 293T cells stably expressing wildtype POLN or siRNA resistant variants of POLN wildtype or POLN D624A mutants, were analyzed for MMC-induced chromosome aberrations following treatment with siRNA targeting POLN. While expression of the siResistant POLN wildtype can reduce radial chromosome formation, POLN polymerase dead

mutant could not. (B) Western blot showing that wildtype, but not siRNA resistant POLN variants are targeted by the siRNA.

Supplementary Figure S4. POLN depletion by (A) shRNA in HeLa cells or (B) siRNA in 293T cells causes crosslink sensitivity.

Supplementary Figure S5. Both anti-POLN antibodies used in this study can detect overexpressed POLN in whole cell extracts of 293T cells.

Supplementary Figure S6. Loss of POLN does not affect FA pathway activation, as measured by FANCD2 ubiquitination. HeLa cells treated with siRNA targeting POLN or control siRNA were harvested after 24h incubation with 20ng/ml MMC, or 3h after exposure to 10Gray IR.

Supplementary Figure S7. Efficiency of protein knockdown is not affected by combination of siRNAs targeting POLN and FANCD2 or FANCI. HeLa cells with stable expression of flag-tagged POLN were used.

Supplementary Figure S8. Loss of POLN affects the response to DSB. (A) Depletion of POLN by siRNA in HeLa cells confers sensitivity to DSB-inducing agents bleomycin and camptothecin. The average of two independent experiments is shown. (B) Increased percentage of cells bearing γH2AX foci after stable shRNA-mediated depletion of POLN in HeLa cells, after treatment with 20mM HU for 24h or 12h after 5Gray IR.

Supplementary Figure S9. Immunofluorescence experiments (after extraction with 0.5% Triton) in HeLa cells, using anti-HA antibodies showed that overexpressed HA-tagged POLN localizes to chromatin foci after DNA damage. Magnified positive cells are shown in insets. DNA damaging agents used are MMC (160 ng/ml for 24h), HU (20 mM for 24h), and IR (4h after exposure to 10Gray).

Supplementary Figure S10. POLN depletion results in increased mutagenesis. Shown is basal, UVC (1kJ/m^2) and crosslink (1μM psoralen exposed to 6kJ/m^2 UVA) -induced mutation frequency. Similar results were obtained using a different siRNA targeting POLN (not shown). Error bars represent standard errors.

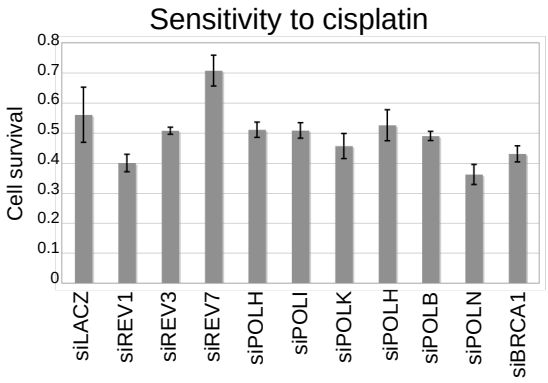
Supplementary Figure S11. POLN depletion after S-phase is caused by protein degradation. HeLa cells with a stable expression of flag-tagged POLN under control of the CMV promoter were synchronized by double thymidine arrest, released in nocodazole containing media and analyzed at the indicated time points. The asterisk denotes a crossreactive band.

Supplementary Figure S12. HEL308 helicase dead mutant has a dominant negative effect on HR. Either wildtype or HEL308 K365A mutant were transiently overexpressed under the control of the CMV promoter.

Supplementary Figure S13. Loss of POLN or HEL308 does not reduce IR-induced (A) RPA or (B) RAD51 foci formation, suggesting that POLN and HEL308 act downstream in the HR reaction. HeLa cells were fixed 3h after treatment with 10Gray IR.

Supplementary Figure S14. Proposed model for POLN and HEL308 involvement in the FA pathway. POLN, acting in complex with HEL308, might be the polymerase that performs D-loop extension in the HR reactions initiated by the FA pathway.

Suppl. Figure S1



Suppl. Figure S2

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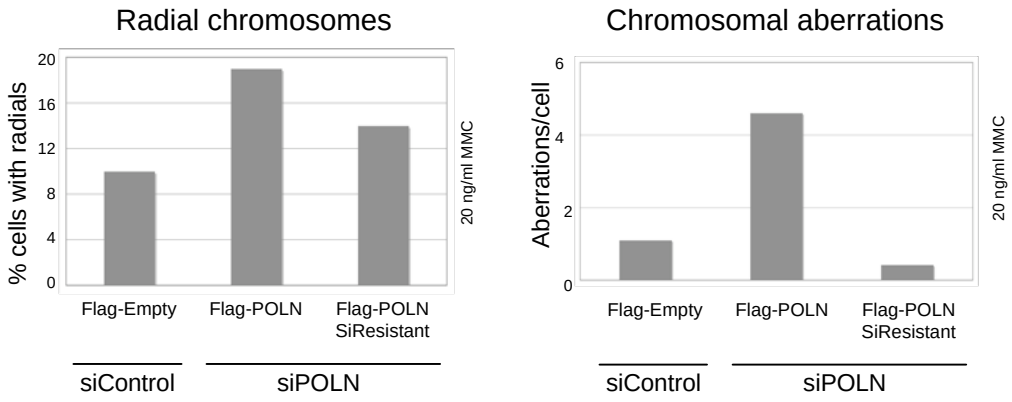

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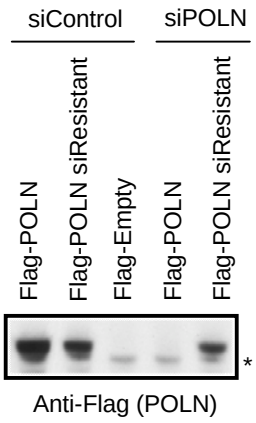

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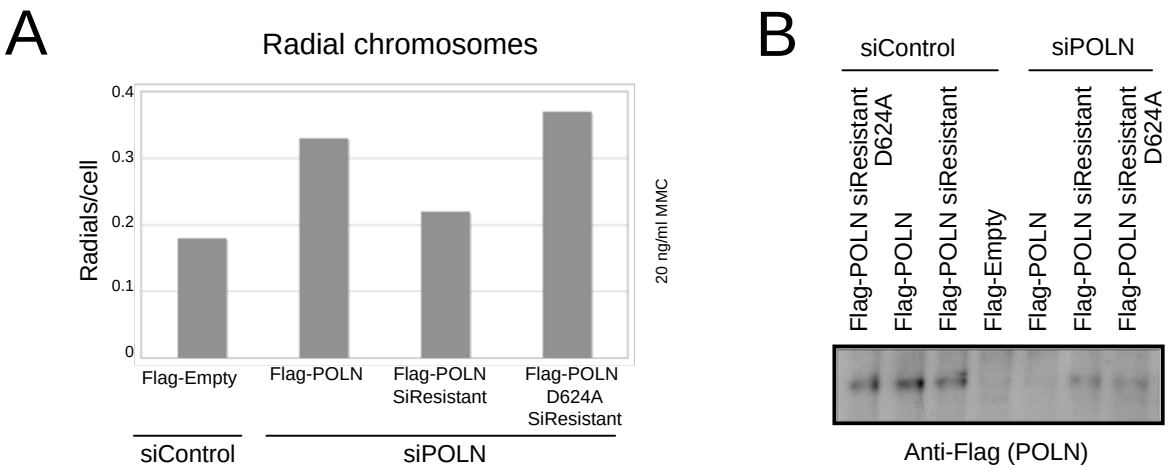

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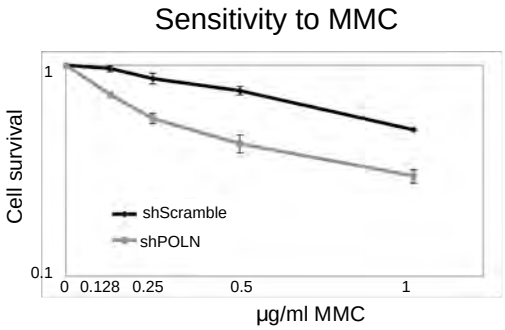

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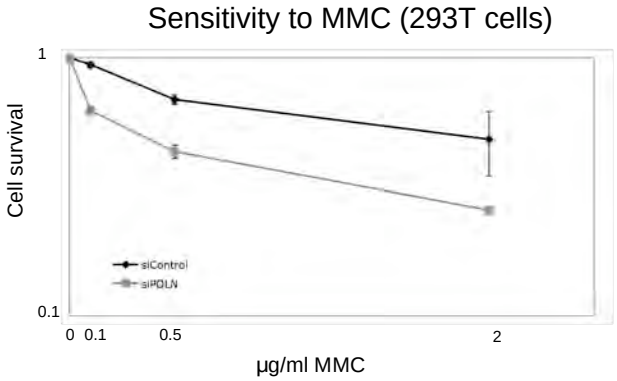

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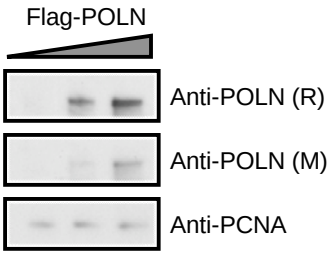

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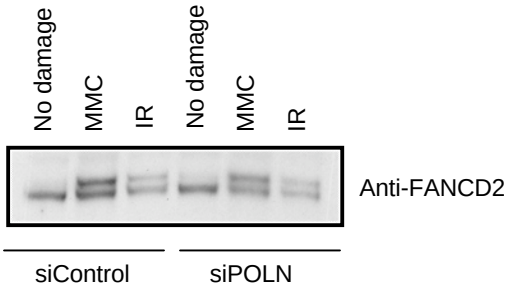

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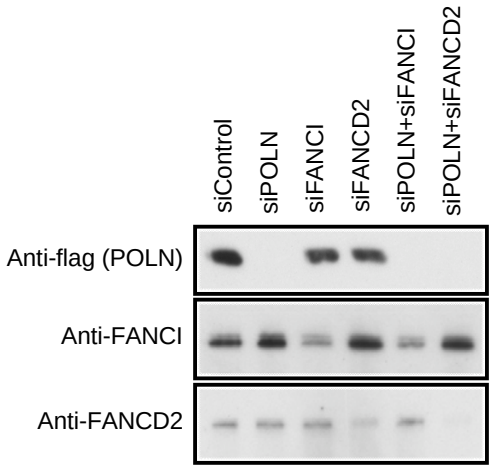

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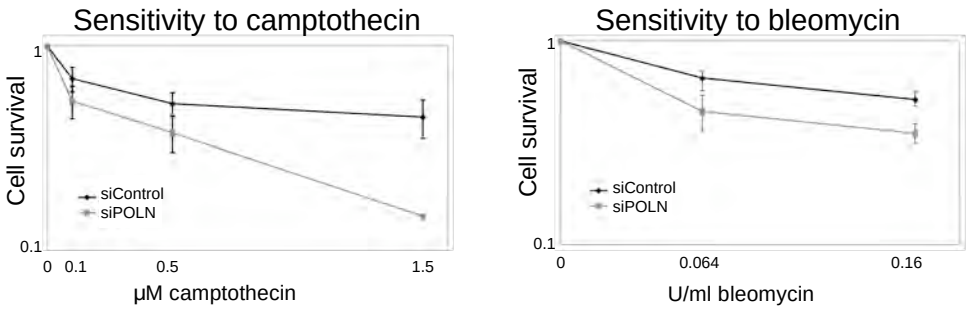

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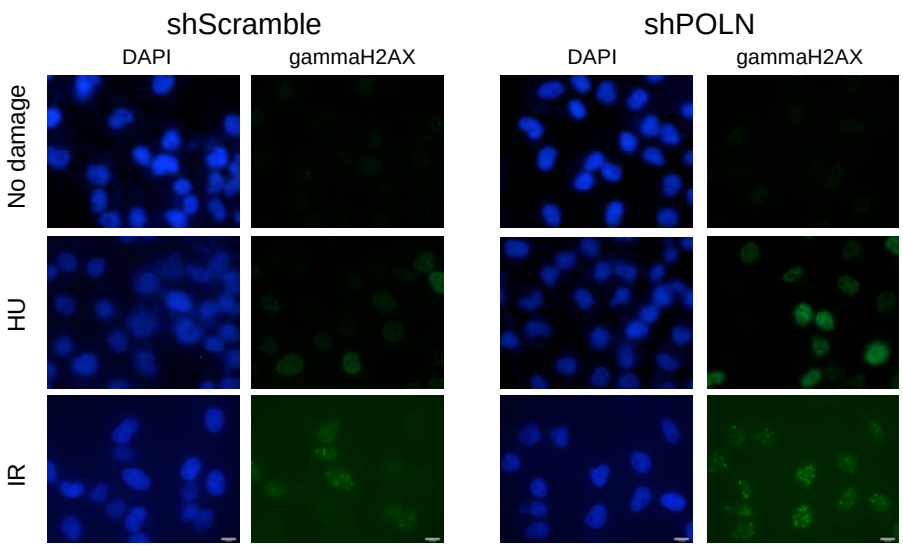

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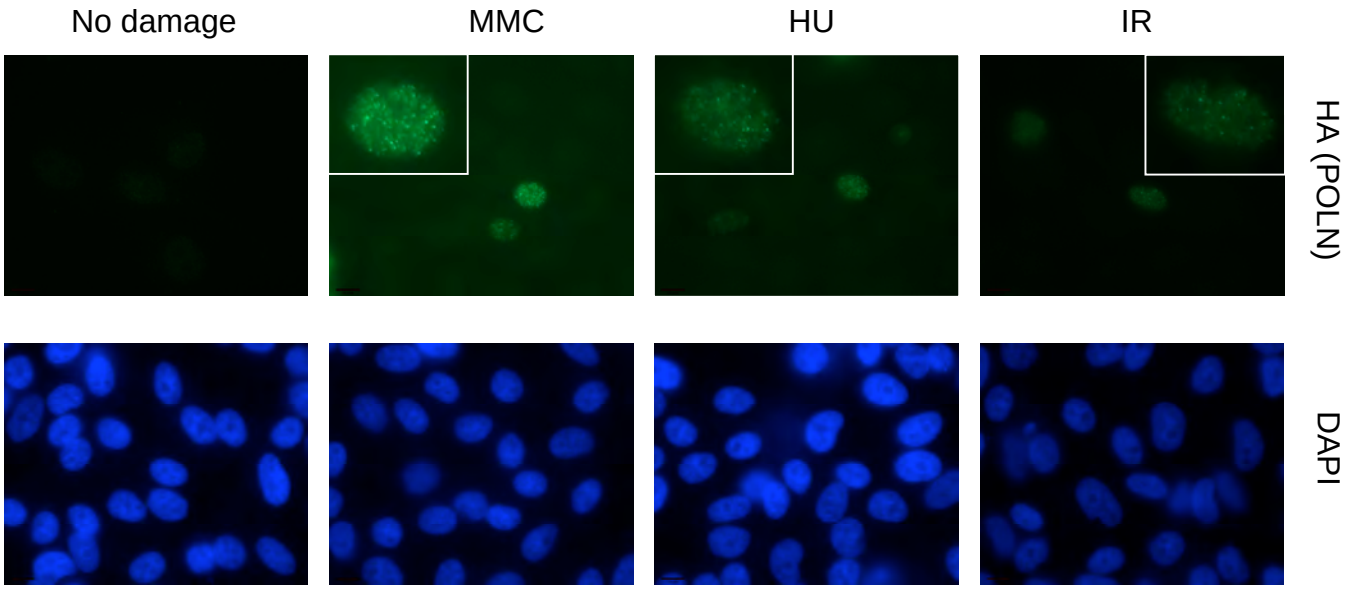

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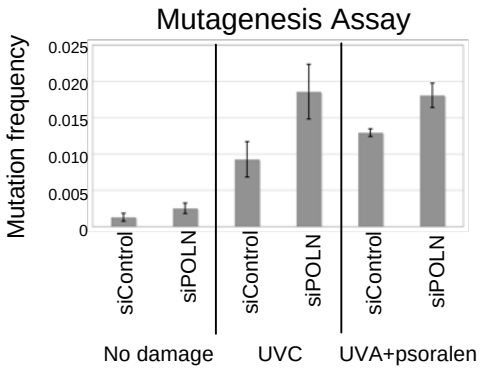

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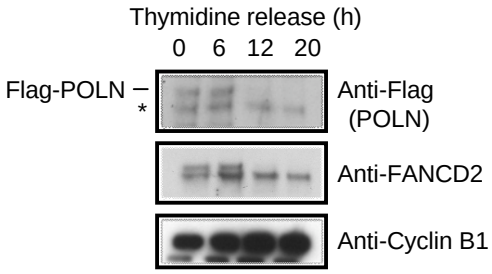

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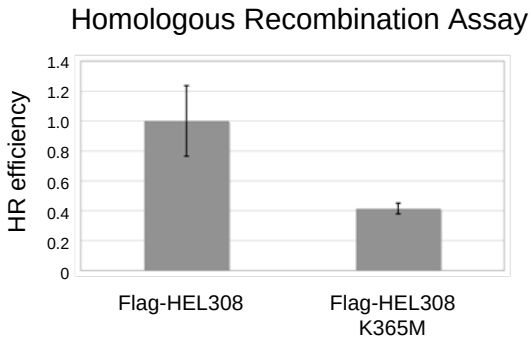

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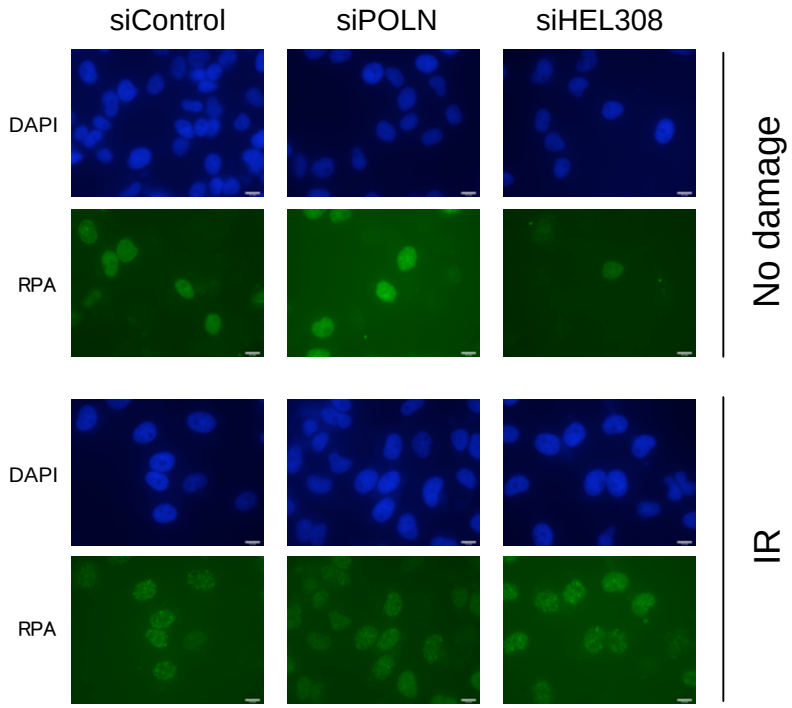

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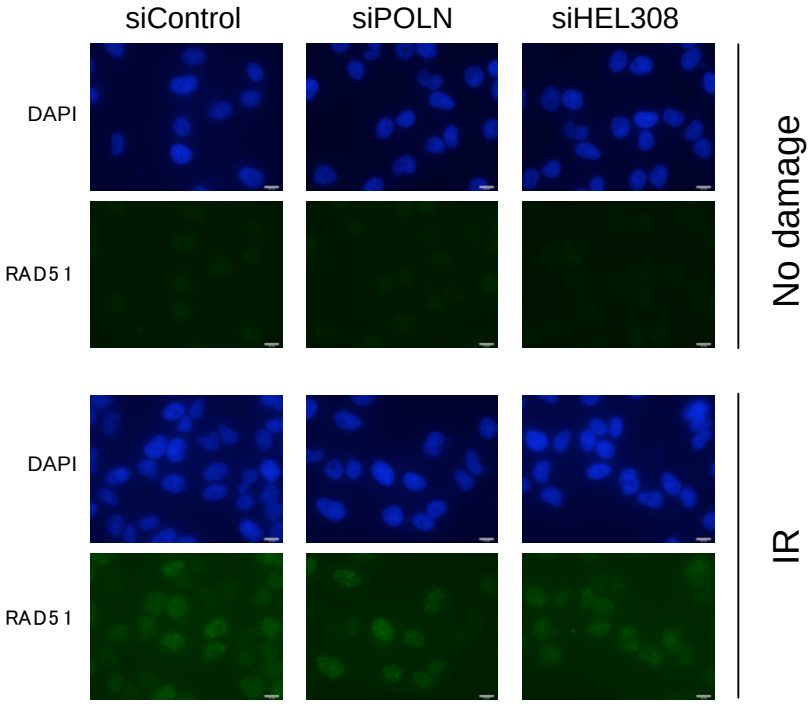

Suppl. Figure S14