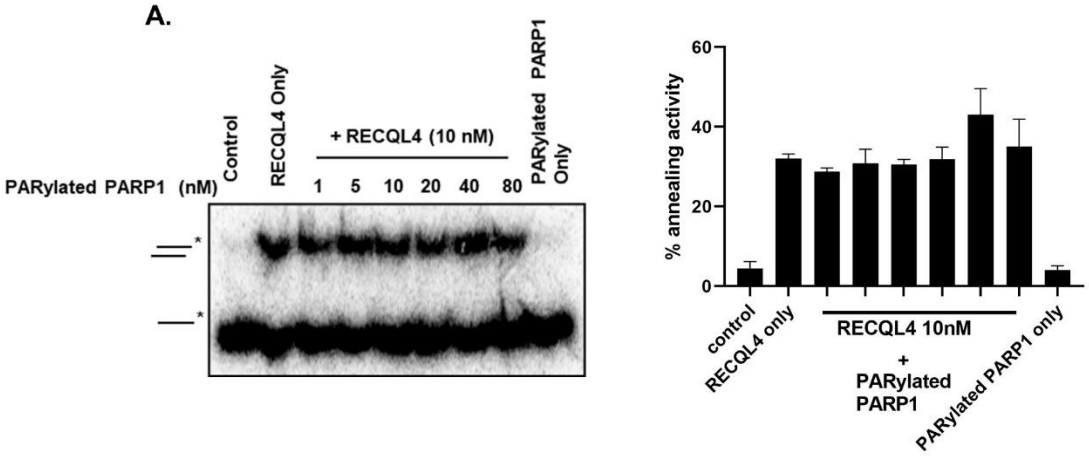
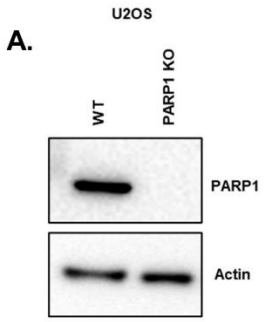
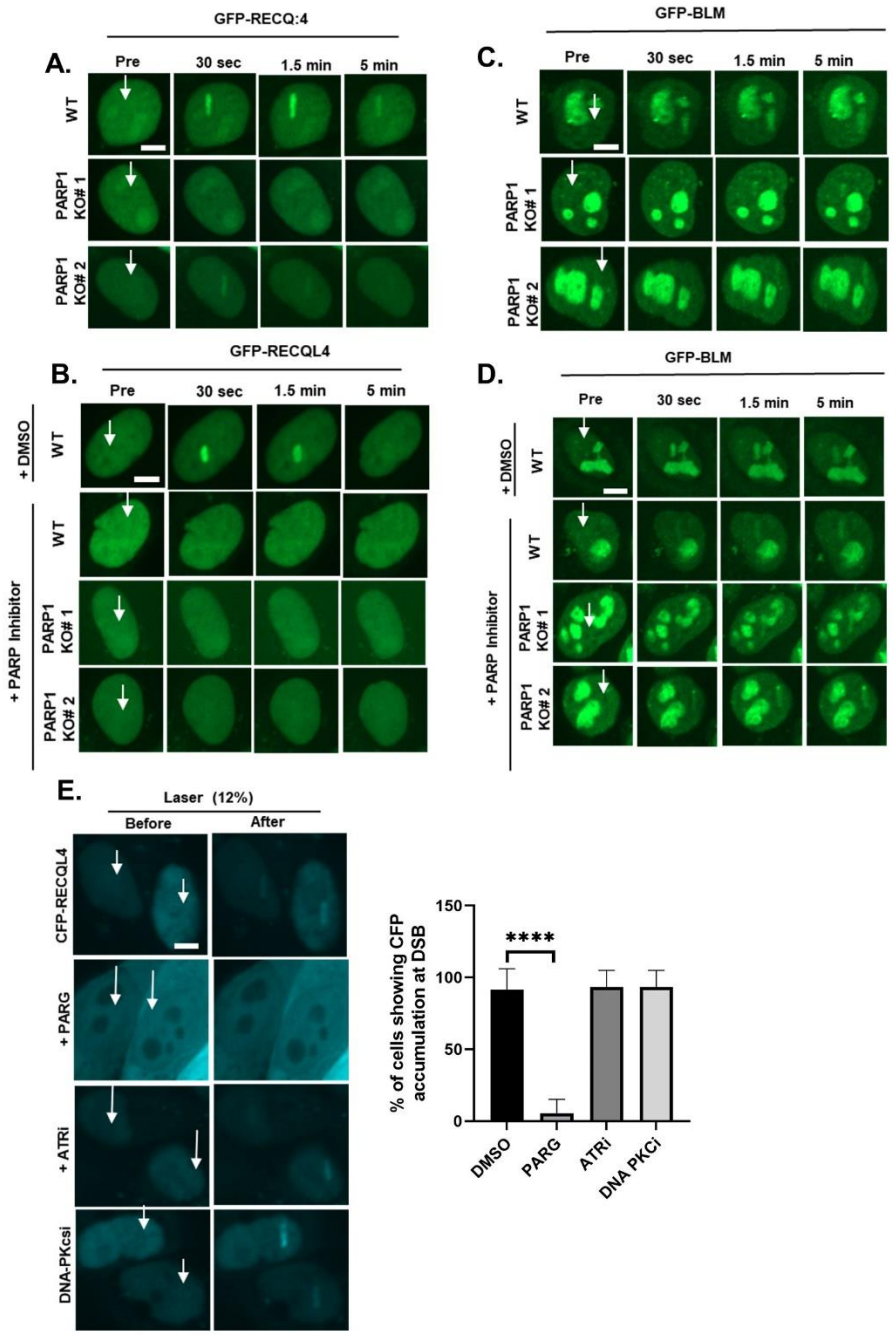
Supplemental Figures



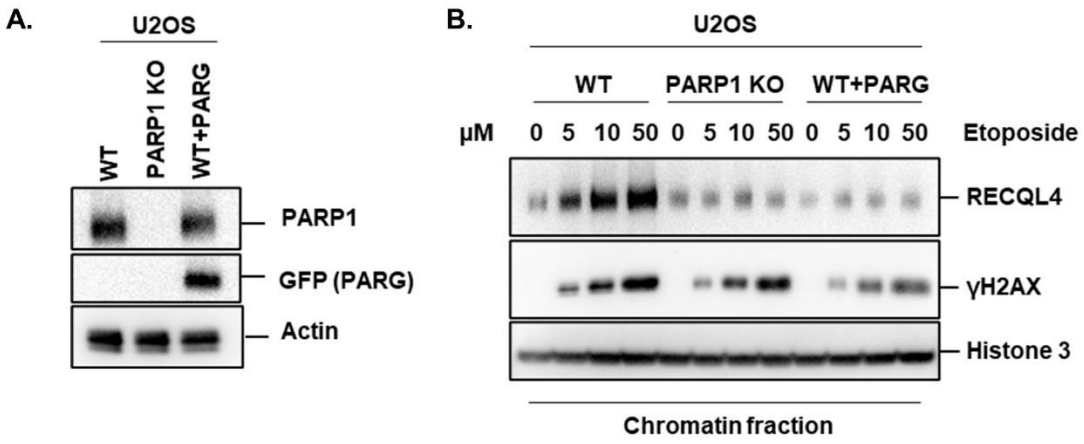
Supplemental Figure S1: (A) Strand annealing activity of RECQL4 in presence of PARylated PARP1



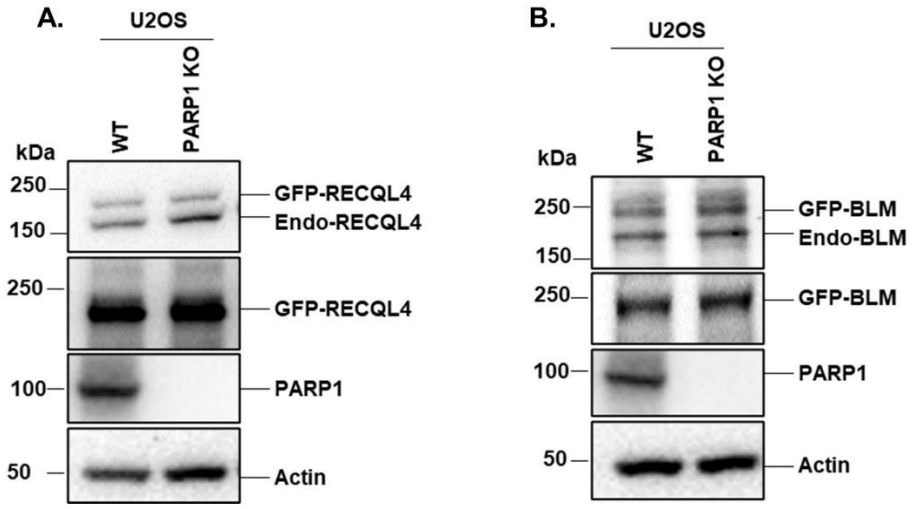
Supplemental Figure S2: Western blotting for the wild type, PARP1 KO U2OS cell lines



Supplemental Figure S3: PARP1-mediated PARylation is required for the early recruitment of RECQL4 to DSBs in HeLa cells

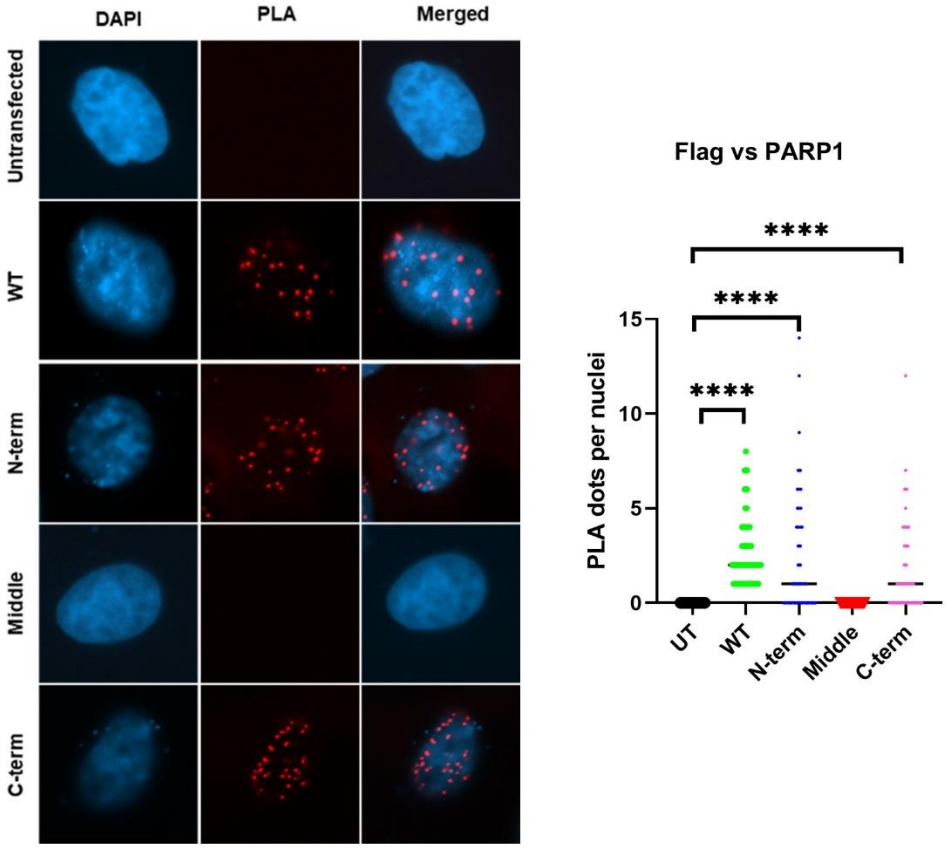


Supplemental Figure S4: PARP1-mediated PARylation is essential for RECQL4 recruitment to DSBs in a dose-dependent manner after etoposide treatment

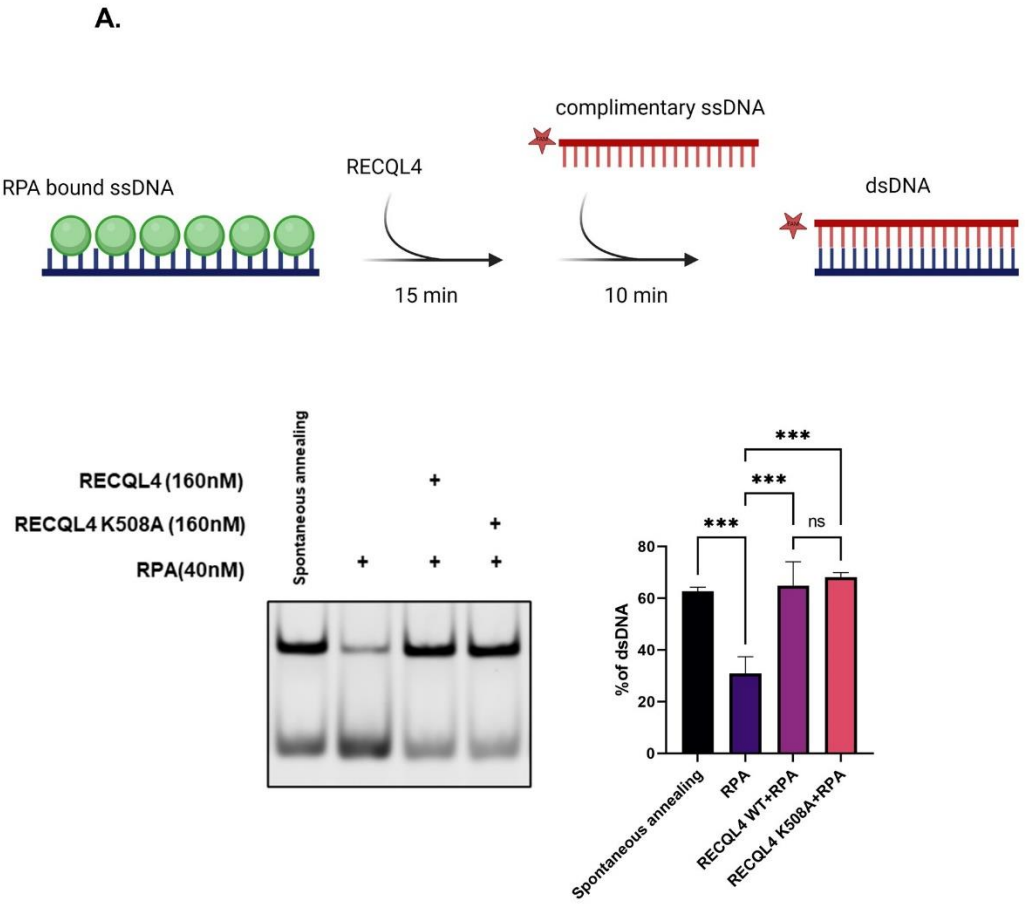


Supplemental Figure S5: Western blotting for the expression of GFP-RECQL4/GFP-BLM in U2OS WT or PARP1 KO cells

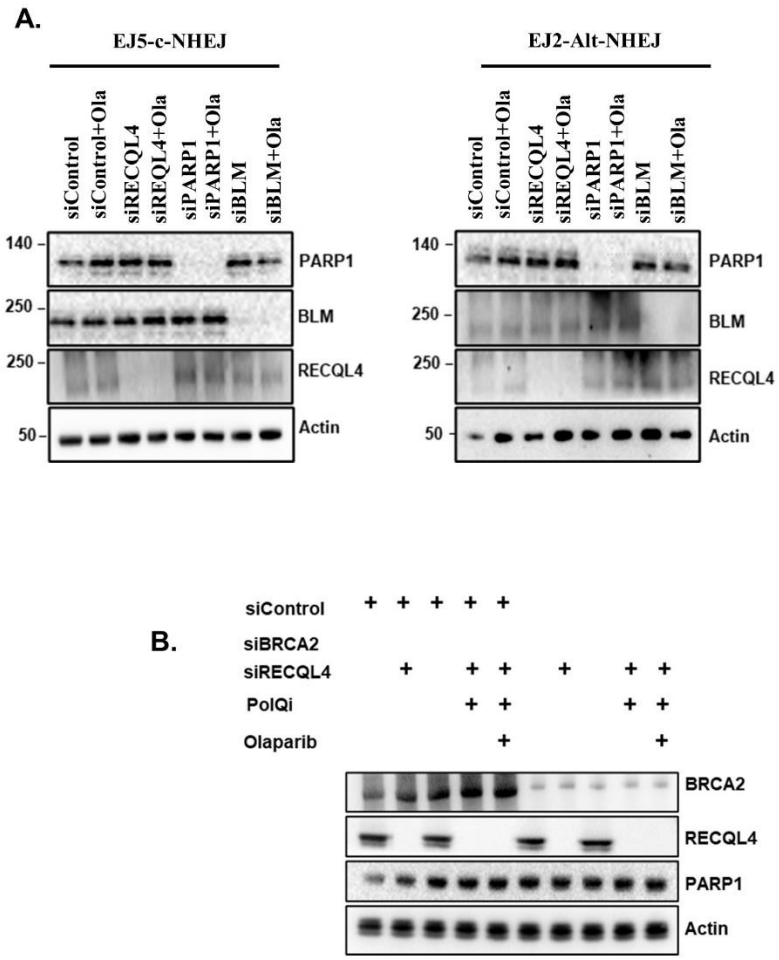
A.



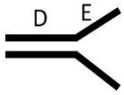
Supplemental Figure S6: Proximity Ligation Assay (PLA) showing RECQL4 N-term and C-term interaction with PARP1



Supplemental Figure S7: RECQL4 anneals RPA-coated DNA substrates



Supplemental Figure S8: Western blot for the knockdown of PARP1, BLM, RECQL4 in U2OS EJ5 or EJ2 cells (A). Western blot showing shutdown of BRC A2 and RECQL4 in DLD1 WT cells (B).

Structure	Substrate	Sequence (5'-3')
	(a/b) 22/15(fork-1)	T1 GGAATTCTACCAGTGCCTTGCTAGGACATCTTTGCCCA B1 CTAGACAGCTCCATGTAGCAAGGCACTGGTAGAATTC
	Blunt end 80 base pairs	T3 GCTGATCAACCCTACATGTGTAGGTAACCCTAACCCTAACCCT AAGGACAACCCTAGTGAAGCTTGTAACCCTAGGAGCT B3 AGCTCCTAGGGTTACAAGCTTCACTAGGGTTGTCCTTAGGGTT AGGGTTAGGGTTACCTACACATGTAGGGTTGATCAGC
RPA assay	RP246 RP246c	GCTCTGATGCCGCATAGTTAAGCCAGCCCCGACACCCG CGGGTGTCGGGGCTGGCTTAACTATGCGGCATCAGAGC
Microhomology Annealing assay	NHEJ10-5 NHEJ10-2 NHEJ10-6 NHEJ10-4	GACTCACTGGTAGCTTAGACCAAAGAAAATCTGGTCAGCG GTCTAAGCTACCAGTGAGTC CAGTATCCTGTCACTCCAGTCAAAGAAAATCGCTGACCAG ACTGGAGTGACAGGATACTG

Supplemental Figure S9: List of DNA substrates and their sequences used in this study

Supplementary Figure Legends:

Supplemental Figure S1: Strand annealing activity of RECQL4 and BLM in the presence of increasing concentration of PAR. A. strand annealing activity of RECQL4 (10nM) was examined in the presence of increasing concentrations (0, 1, 5, 10, 20, 40, and 80 nM) of PARylated PARP1 with the radiolabeled ssDNA 80 mer DNA and its complementary single strand DNA.

Supplemental Figure S2: Western blotting for the wild type, PARP1 KO U2OS cell lines. The whole-cell extracts were immunoblotted with anti-PARP1 and anti-Actin antibodies. Actin serves as the endogenous loading control.

Supplemental Figure S3: **PARP1-mediated PARylation is required for the early recruitment of RECQL4 to DSBs**. GFP-RECQL4 **A and B** and GFP-BLM **C and D** expressed in HeLa cells were pre-treated for 3 h with 5 μM Olaparib and then targeted with the 12% laser to induce DSBs. The white arrow indicates the laser striking area. **E.** CFP-RECQL4 and PARG were overexpressed in U2OS WT cells. The relocation was monitored in a time course following laser micro-irradiation. Overexpressed CFP-RECQL4 U2OS WT cells were treated with ATR inhibitor or DNA-PKcs inhibitor or DMSO. The relocation kinetics of CFP-RECQL4 to DNA damage sites were examined and the graph was plotted as a percentage of cells showing recruitment of CFP-RECQL4 to DSBs.

Supplemental Figure S4: PARP1-mediated PARylation is essential for RECQL4 recruitment to DSBs in a dose-dependent manner after etoposide treatment. (A) Western blot showing expression of GFP-PARG in U2OS WT cells. **(B)** U2OS WT/PARP1 KO or GFP-

PARG expressing cells treated with different concentrations of etoposide as indicated for 2 hours. The cells were collected, and chromatin fractionation was carried out for immunoblot using anti-RECQL4, anti-gH2AX, and anti-H3. Histone 3 serves as a loading control.

Supplemental Figure S5: **western blotting showing expression of GFP-RECQL4/GFP-BLM**. GFP-RECQL4 **A** and **B** GFP-BLM expressed in U2OS WT/PARP1 KO cells. The whole cell extracts were probed using indicated antibodies. Actin served as loading control.

Supplemental Figure S6: **PARP1 interacts with both RECQL4 N-ter and C-ter domains. A.** Proximity ligation assay (PLA) was carried out in cells expressing different regions of Flag tagged RECQL4. Cells were fixed using paraformaldehyde (PFA) and PLA was performed using anti-Flag (mouse) and anti-PARP1 (rabbit) antibodies. Images were taken under a microscope and PLA foci per nuclei were counted using cell profiler software. N=3, one way annova was performed to assess statistical significance (**** $P< 0.0001$).

Supplemental Figure S7: RECQL4 anneals RPA-coated DNA substrates. A. Non-denaturing gel showing RECQL4 mediated annealing of RPA-coated DNA substrates in the presence of mentioned protein concentrations. The graph is the quantitative representation of the left panel. N=3, one-way annova was performed to assess statistical significance (**** $P< 0.0001$).

Supplemental Figure S8: (A) Western blot for the knockdown of PARP1, BLM, RECQL4 in U2OS EJ5 or EJ2 cells. U2OS EJ2 or EJ5 cells were transfected with indicated siRNAs with or without Olaparib treatment and immunoblotting using indicated antibodies. **(B)** Western blot showing shutdown of BRCA2 and RECQL4 in DLD1 WT cells. DLD1 WT cells were transfected with either siControl or siBRCA2 in the presence and absence of siRECQL4 with or

without PolQ inhibitor/Olaparib. The cells were collected and immunoblot was carried out using anti-RECQL4 and anti-Actin. Actin serves as loading control.

Supplemental Figure S9: List of DNA substrates used in this study