

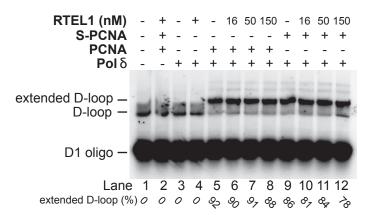
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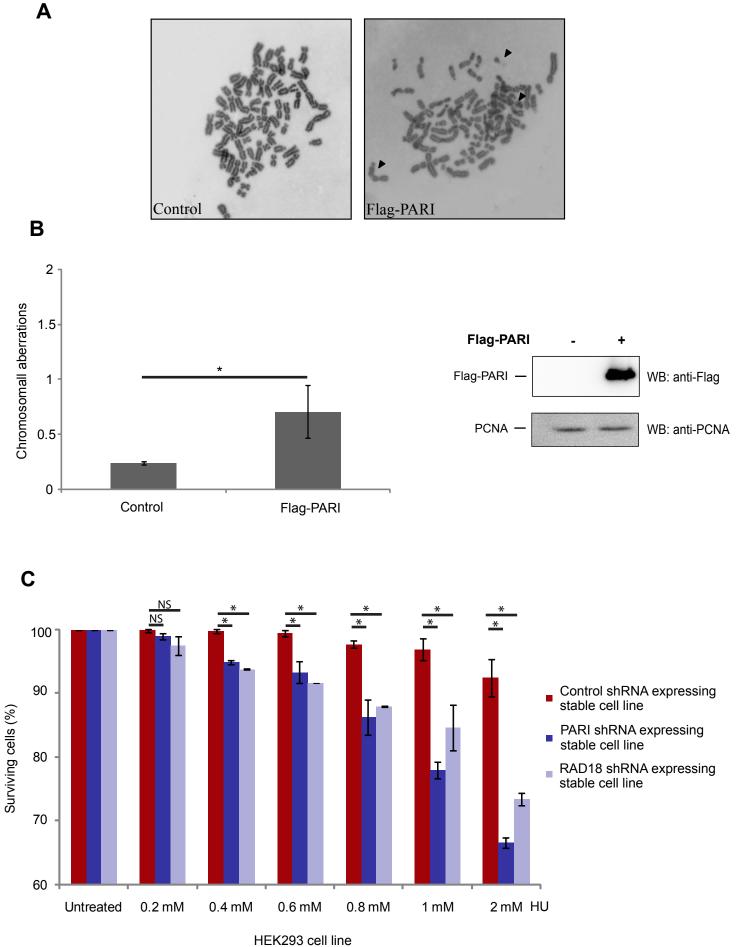
С

PARI <sub>333-579</sub> (nM) polyUb-PCNA Ub-PCNA PCNA PONA Pol δ	+ + +	- - -	  + +	- - +	- - +	- + -	- + -	- + -	- + -	+ - -	+ - -	+ - -	+ - -	PCNA (nM) PARI PCNA DNA polymerase δ	-	+ +	+ -	-	- +
extended D-loop — D-loop —	-	_		-	-	=	=	=	-	=	=		-					110.00	
D1 oligo — Lane extended D-loop (%																			

PCNA (nM) PARI PCNA NA polymerase δ		+ + + -	- + - +	- - + +	20 - + +	) 60 - + +	180 - + +	- + + +	+	60 + + +	180 + + +
				-	-	-	4		-	-	-
31 nt primer —	-	-	-		-	-			-	-	-
Lane product (%)	1 0	2 0	3 0	4 ج	5 ⊳∽	6 දුරු	7 ශ්	8 1	9 ,%	10 V	

<mark>₿ <u>\*</u>31 nt </mark> 5 75 nt 5





hRPA + + + + + + + + + + + + + + + + +	hRAD51-K133R	-	+	+	+	+	+	+	+	+	+
RecQL5β (nM) 50 100 200 PARI (nM) 50 100 200 500	hRPA	-	-	+	+	+	+	+	+	+	+
PARI (nM) 50 100 200 500	HOP2-MND1	-	-	+	+	+	+	+	+	+	+
Lane 1 2 3 4 5 6 7 8 9 10	RecQL5β (nM)	-	-	-	50	100	200	-	-	-	-
Lane 1 2 3 4 5 6 7 8 9 10	PARI (nM)	-	-	-	-	-	-	50	100	200	500
Lane 1 2 3 4 5 6 7 8 9 10					1						
Lane 1 2 3 4 5 6 7 8 9 10											
		_	_			kinni		-	-	-	-
formed D-loop (%) 0 なるなななななな			•	-		-		-	5	-	-
	Lane	1	2								10

# В

hRAD51-K133R hRPA						
HOP2-MND1	-	-	+	+	+	+
RecQL5β (nM)	-	-	-	120	-	120
PARI (nM)	-	-	-	- {	500	500
	-	-	-	-	-	-
Lane formed D-loop (%)			-		-	-

#### **Supplementary Materials**

#### Supplementary figure legends:

**Supplementary Figure 1.** *PARI overexpression sensitizes RAD18-depleted cells to UV irradiation.* A) UV sensitivity of the HEK 293 cells after Flag- PARI<sub>286-579</sub> overexpression. B) The expression level of Flag-PARI<sub>286-579</sub> tested by Western blotting. C) UV sensitivity of RAD18-depleted HEK 293 cells after Flag- PARI<sub>286-579</sub> overexpression D) The expression level of Flag-PARI<sub>286-579</sub> tested by Western blotting. All cell lines were treated with UV; the cells were cultivated for 7 days, and the surviving cells were analyzed by FACS. Mean values of triplicates are shown with SD (error bars). Significant difference is indicated by asterisk.

**Supplementary Figure 2.** *The localization of PARI depends on SUMO-PCNA.* A) Flag-PARI and corresponding PIP, SIM, or PIP/SIM mutants were expressed in HEK293 cells. Newly synthesized DNA tracks were labeled by BrdU staining. After fixation, cells were stained using anti-Flag and anti-BrdU antibodies. B) Graphical representation of PARI and BrdU co-localizing foci. C) Wild-type HA-PARI was expressed together with PCNA-specific shRNA and Flag-PCNA or Flag-PCNA K164R mutant in HEK293 cells. After fixation, the cells were stained using anti-Flag and anti-HA antibodies. D) Graphical representation of the PARI and PCNA co-localizing foci. Mean values of triplicates are shown with SD (error bars). Significant difference is indicated by asterisk.

Supplementary Figure 3. *PARI inhibition does not depend on PTM of PCNA and is not exclusively specific for D-loop.* A) Purification of SUMO-PCNA. B) PARI-mediated inhibition of Polô-dependent extension of single primed  $\Phi$ x DNA substrate in the presence of PCNA or SUMO-PCNA. Reactions were carried out as described in the materials and methods section. C) Effect of PCNA ubiquitylation on the inhibitory effect of the PARI<sub>333-579</sub> fragment. *In vitro* reactions were carried out as described in Materials and Methods using PCNA, monoUb-PCNA, and polyUb-PCNA (30 nM). Reactions were resolved on a 0.8% native agarose gel.

#### Supplementary Figure 4. The inhibition of Polô-dependent D-loop extension is specific for PARI.

Effect of RTEL1 on D-loop extension by Pol $\delta$ . All reactions were carried out using radioactively labeled D1 oligonucleotides in the presence of PCNA or SUMO-PCNA (S-PCNA) as indicated. The pre-loaded replication complex was incubated with increasing concentrations of RTEL1, and the extension was

started with the addition of nucleotides to the reactions. All reactions were resolved on 0.8% native agarose gels.

**Supplementary Figure 5.** *PARI overexpression causes chromosomal aberrations.* A) Representative picture of the chromosomes. Arrows indicate the aberrant structures. B) Quantitative analysis of the aberrant chromosomes. Mean values of triplicates are shown with SD (error bars). Significant difference is indicated by asterisk. C) UV sensitivity of the RAD18- and PARI- depleted HEK 293 cells. HEK293 cells were transfected with control, RAD18, or PARI-specific shRNA. All cell lines were treated with HU for 24h; the cells were cultivated for 7 days, and the surviving cells were analyzed by FACS. Mean values of triplicates are shown with SD (error bars). Significant difference is indicated by asterisk.

**Supplementary Figure 6.** *PARI doesn't posses anti-recombinase activity.* A) PARI is not able to displace RAD51 filament. PARI or RECQL5 $\beta$  (as positive control) was incubated with RAD51-coated ssDNA in the presence of RPA. Amount of formed D-loop is indicated. B) The combination of RECQL5 $\beta$  and PARI has no effect on the RECQL5 $\beta$ 's ability to inhibit D-loop formation. PARI, RECQL5 $\beta$ , or their combination was incubated with RAD51-coated ssDNA in the presence of RPA. Amount of formed D-loop is indicated.