Expanded View Figures

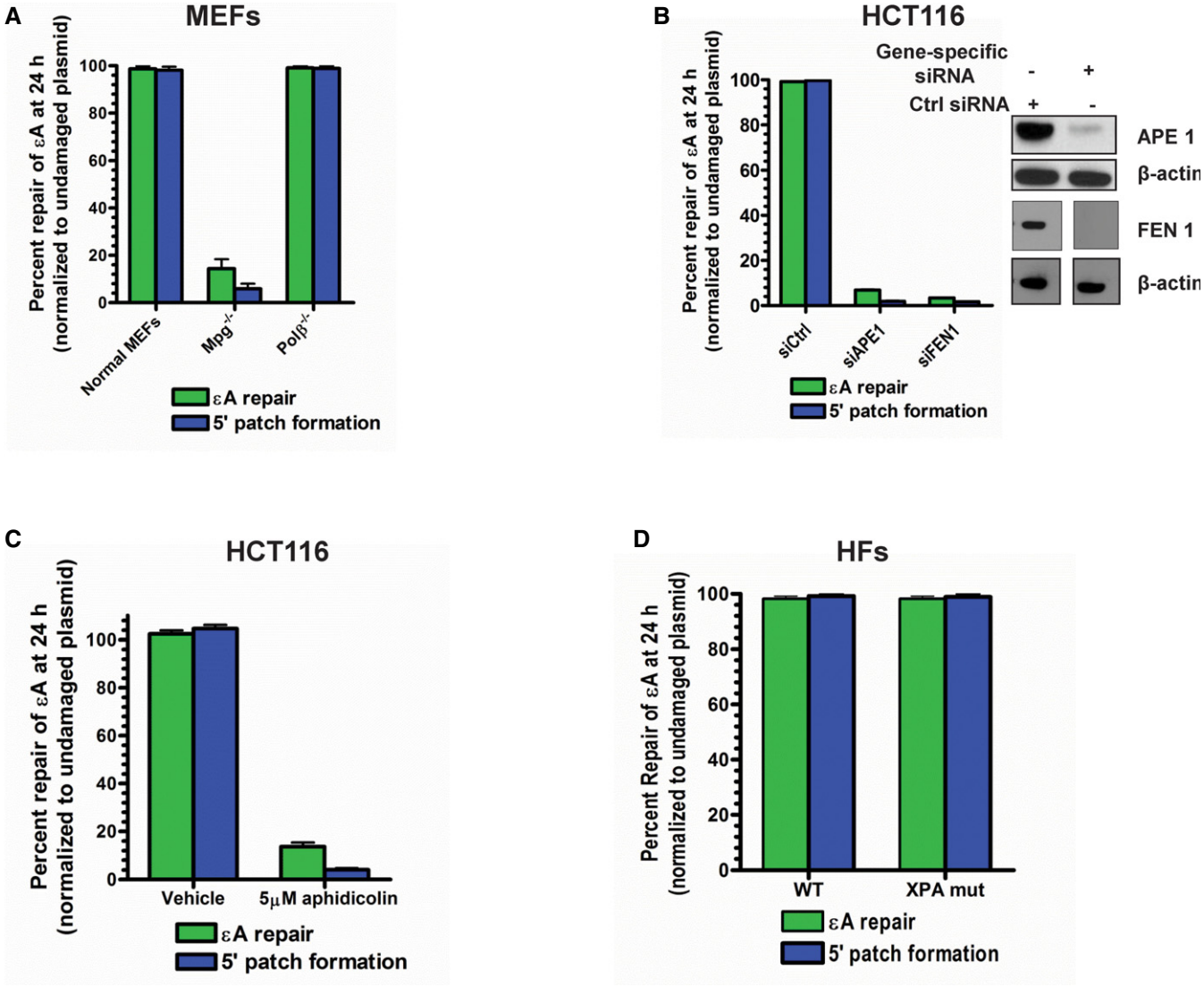

Figure EV1. Newly identified 5′ patch formation step occurs in the LP-BER sub-pathway.

A–D εA repair and 5′ patch formation in plasmid #4 (Appendix Table S1) were monitored 24 h post-plasmid transfection under various conditions: (A) wild-type, *Mpg*$^{-/-}$, and *Polb*$^{-/-}$ MEFs, (B) control, APE1 KD, and FEN1 KD HCT116 cells, (C) under vehicle or aphidicolin treatment in HCT116 cells, and (D) wild-type and XPA mutant HFs. For KD experiments, control or gene-specific (APE1 or FEN1) siRNAs were transfected into HCT116 cells and repair was analyzed in the same manner as described in Fig 1D. APE1 and FEN1 expression was analyzed by Western blot, which is shown in the inset in (B). Space between samples indicates where samples were separated by gel lanes.

Data information: Data are presented as mean ± SD from three independent experiments.
Source data are available online for this figure.

Figure EV2. RECQ1 is required for 5′ and 3′ patch formation during BER in human cells.

A εA repair, 5′ patch formation, and 3′ patch formation were monitored by plaque assay 24 h post-plasmid transfection (plasmids #2 and #4 for monitoring the 3′ and 5′ patch formation, respectively) in control and RECQ1 KD HeLa cells in the same manner as described in Fig 1D.

B Western blot analysis of RECQ family helicases in HeLa cells 48 h post-RECQ1 siRNA transfection.

C Survival of control and RECQ1 KD HCT116 cells treated for 24 h with indicated doses of MMS was measured by cell counting. Asterisk (*) denotes *P*-value of Student's *t*-test < 0.05.

D Cell cycle distribution was monitored in control and RECQ1 KD HCT116 cells 24 h post-siRNA transfection by DNA content analysis (propidium iodide staining) using flow cytometry.

E, F εA repair and 5′ patch formation were monitored 24 h post-plasmid transfection (plasmid #4) by plaque assay in (E) *Recq1*$^{+/+}$ and *Recq1*$^{-/-}$ MEFs and (F) stable control and RECQ1 KD cells complemented with empty vector or shRNA-resistant RECQ1. Insets show Western blot for RECQ1 in MEFs (E) and RECQ1 stable KD HeLa cells (F).

Data information: In (A, C–F) data are presented as mean ± SD from three independent experiments.
Source data are available online for this figure.

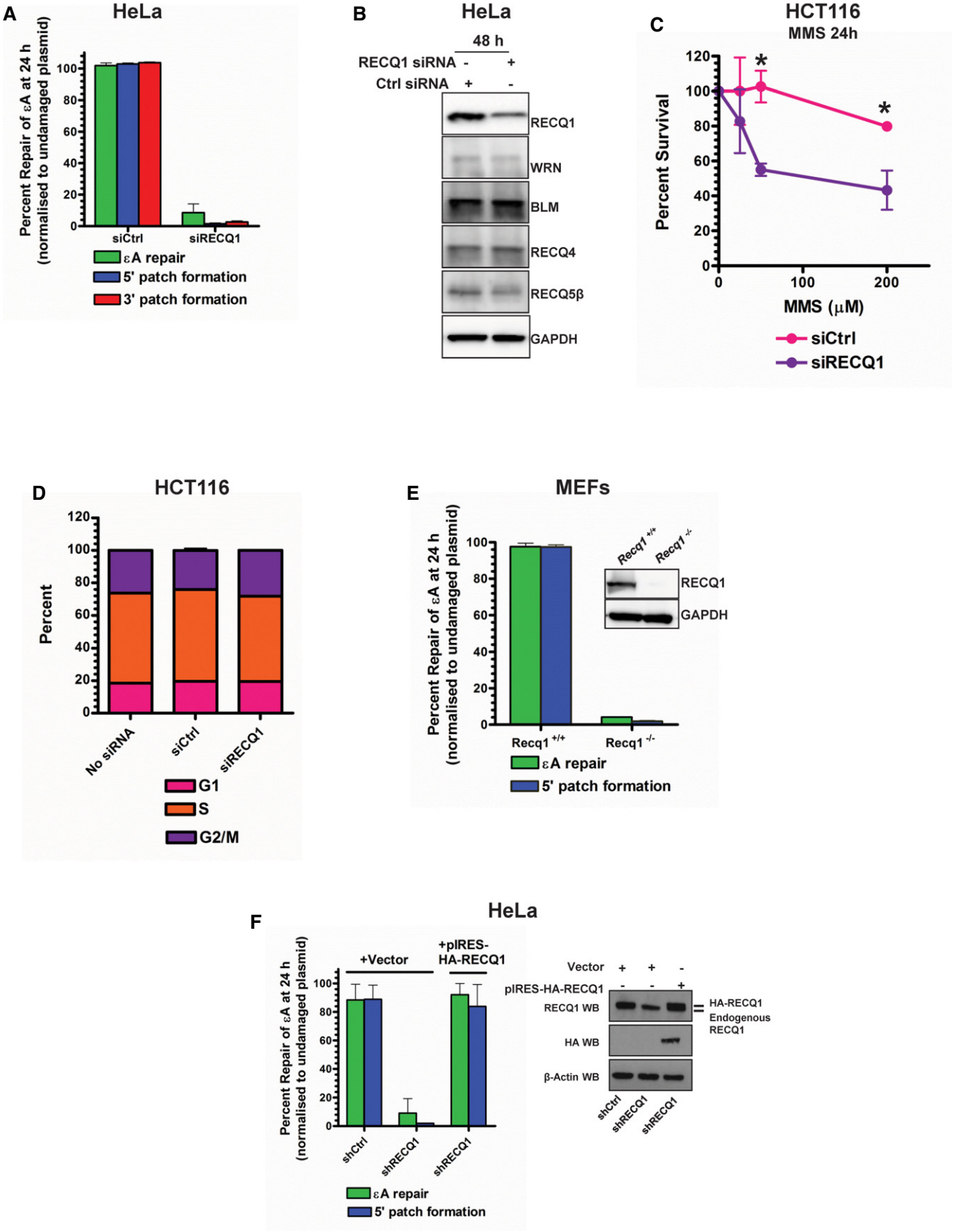

Figure EV2.

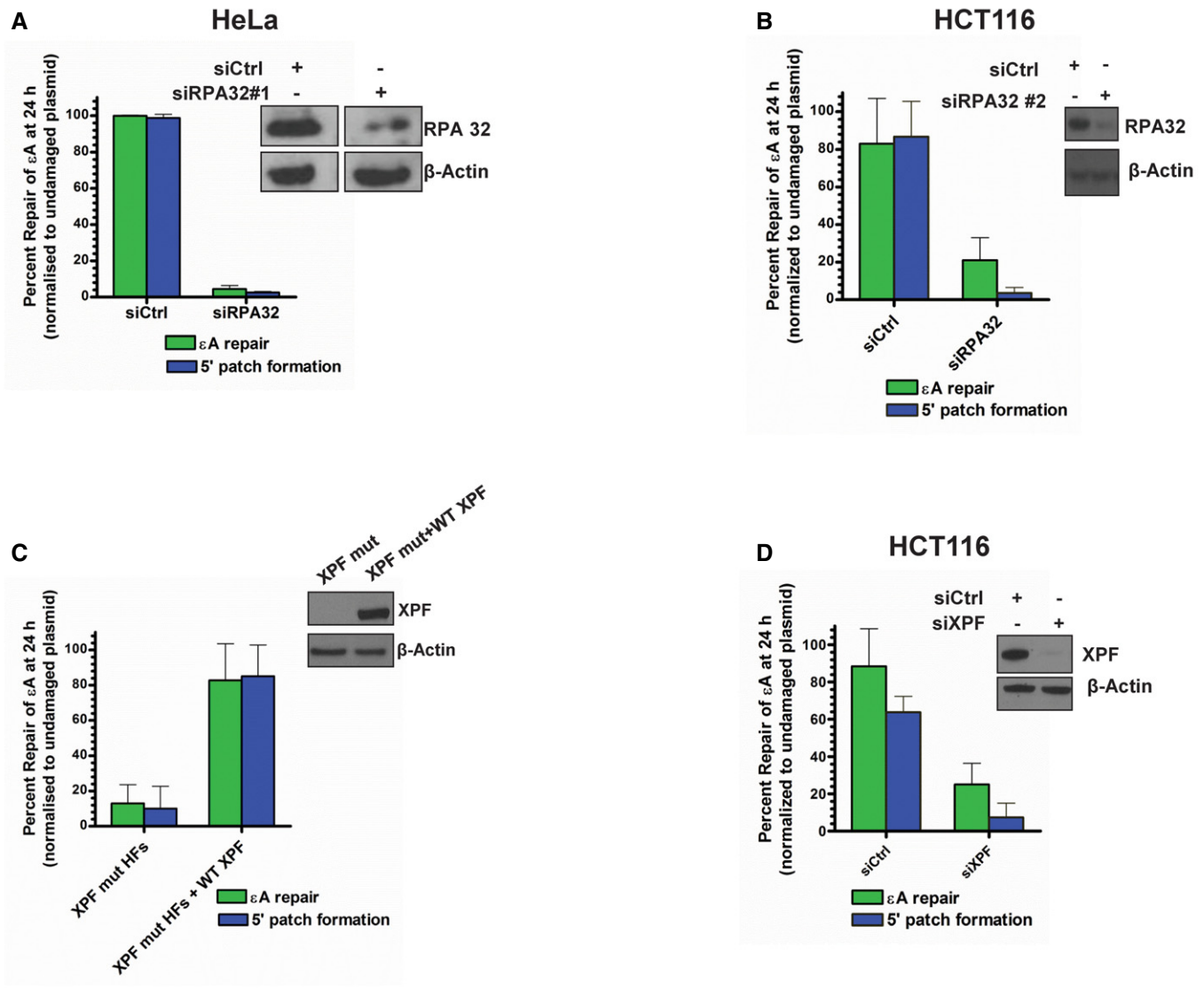

Figure EV3. RPA and XPF are required for 5′ patch formation during BER in human cells.

A–D εA repair and 5′ patch formation were monitored by plaque assay 24 h post-plasmid transfection (plasmid #4) in the same manner as described in Fig 1D in (A) control and RPA KD HeLa cells (using RPA siRNA #1), (B) control and RPA KD HCT116 cells (using RPA siRNA #2), (C) parental XPF mutant HFs and XPF mutant HFs lentivirally transduced with WT XPF, and (D) control and XPF KD HCT116 cells. Insets in panels show Western blots for RPA and XPF, as indicated. Space between samples indicates where samples were separated by gel lanes, which is shown in the inset in (A).

Data information: Data are presented as mean ± SD from three independent experiments.
Source data are available online for this figure.

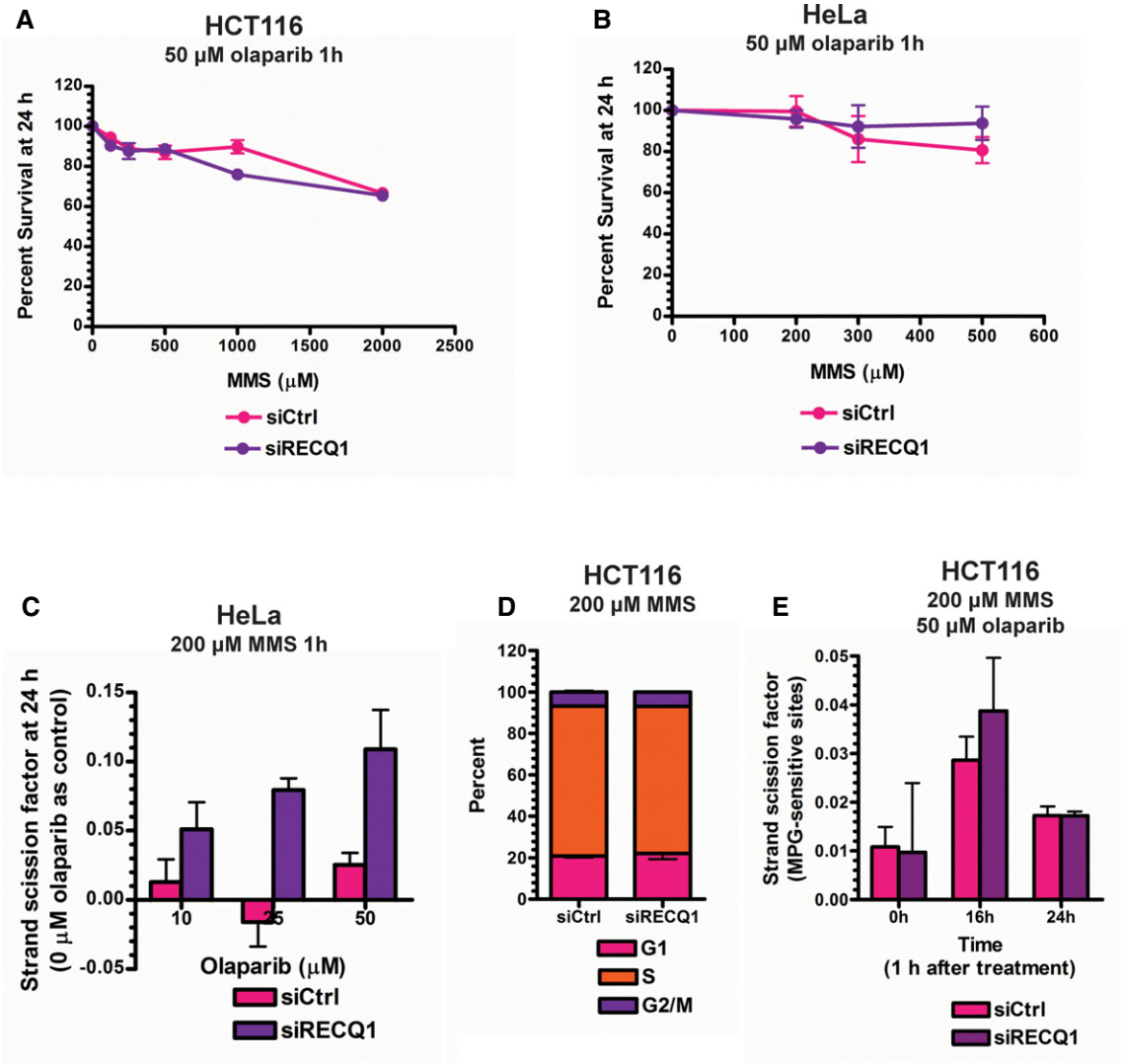

Figure EV4. Optimization of alkylation damage repair assays in genomic DNA.

A, B MTT assays were performed in control and RECQ1 KD (A) HCT116 cells and (B) HeLa cells 24 h after treatment with increasing doses of MMS for 1 h in combination with 50 μM olaparib.

C SSBs were quantified in control and RECQ1 KD HeLa cells 24 h after treatment for 1 h with 200 μM MMS (sub-lethal 1-h treatment) in the presence of indicated doses of olaparib.

D Cell cycle distribution measured by DNA content analysis (propidium iodide staining) using flow cytometry was monitored in control and RECQ1 KD HCT116 cells 24 h after treatment with 200 μM MMS in combination with 50 μM of olaparib for 1 h.

E MPG-sensitive sites were quantified in genomic DNA isolated from control and RECQ1 KD HCT116 cells 0, 16, and 24 h after 1-h treatment with 200 μM MMS in combination with 50 μM of olaparib.

Data information: Data are presented as mean ± SD from three independent experiments.

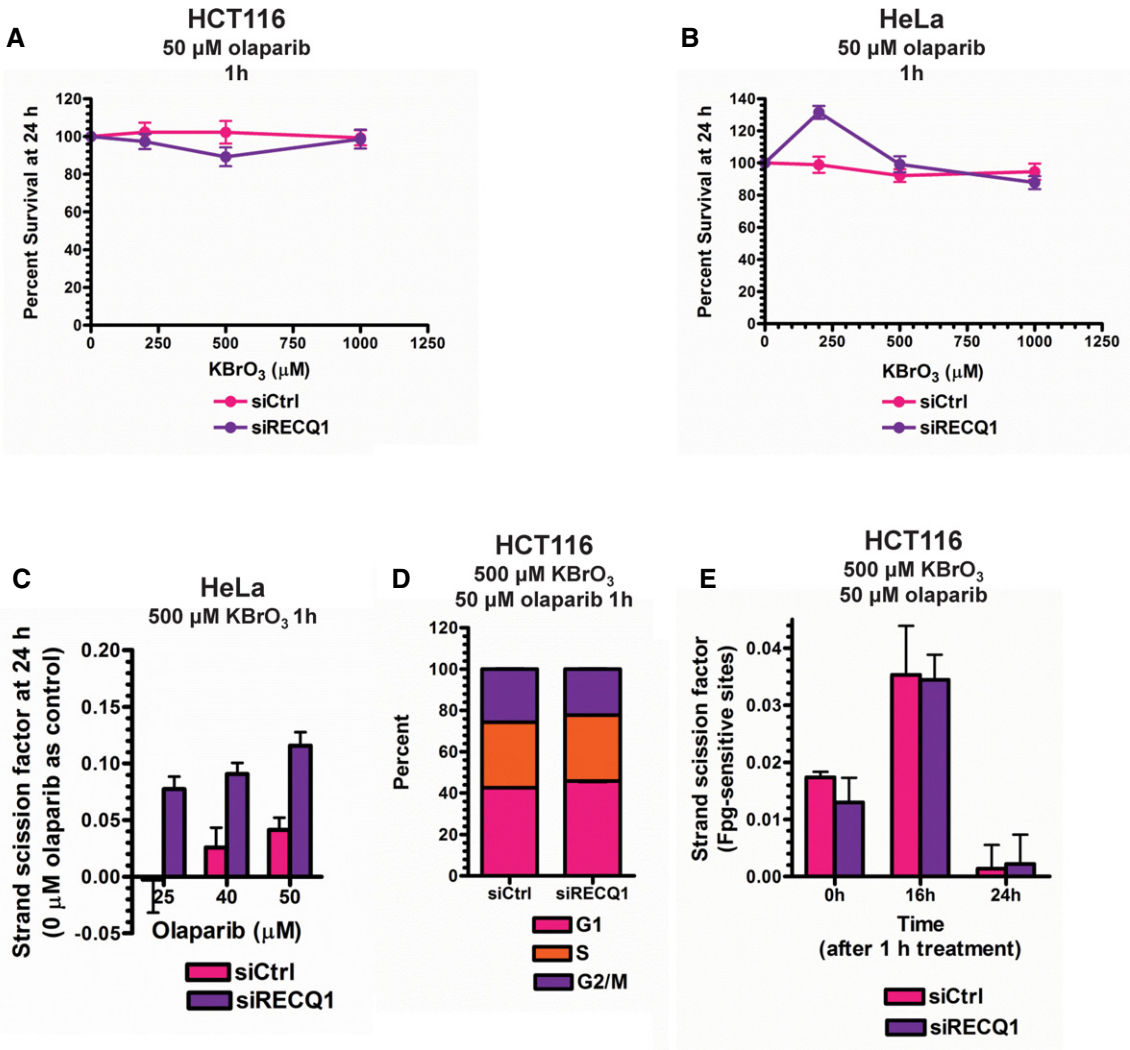

Figure EV5. Optimization of oxidative damage repair assays in genomic DNA.

A, B MTT assays were performed in control and RECQ1 KD (A) HCT116 cells and (B) HeLa cells 24 h after treatment with increasing doses of $KBrO_3$ for 1 h in combination with 50 μM olaparib.

C SSBs were quantified in control and RECQ1 KD HeLa cells 24 h after treatment for 1 h with 500 μM $KBrO_3$ (sub-lethal 1-h treatment) in the presence of indicated doses of olaparib.

D Cell cycle distribution measured by DNA content analysis (propidium iodide staining) using flow cytometry was monitored in control and RECQ1 KD HCT116 cells 24 h after treatment with 500 μM $KBrO_3$ in combination with 50 μM olaparib for 1 h.

E *E. coli* Fpg-sensitive sites were quantified in genomic DNA isolated from control and RECQ1 KD HCT116 cells 0, 16, and 24 h after 1-h treatment with 500 μM $KBrO_3$ in combination with 50 μM olaparib.

Data information: Data are presented as mean ± SD from three independent experiments.