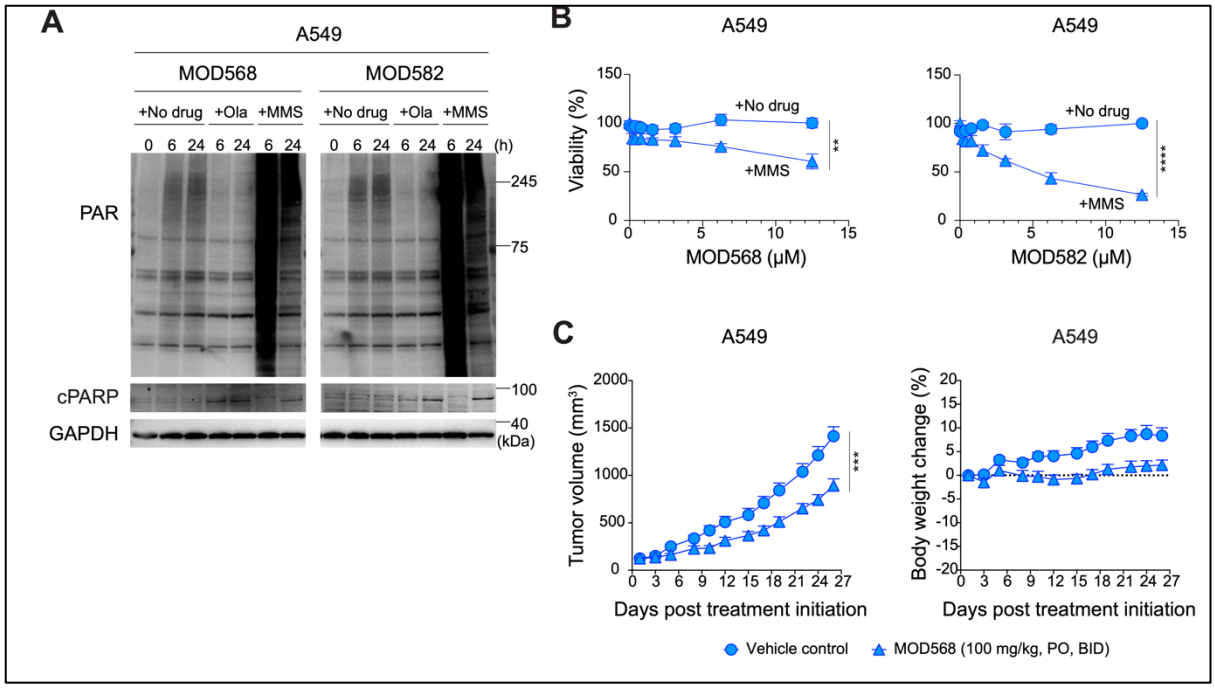

Figure S1. Validation of the effects of PARG inhibitors in A549 cells.

A Accumulation of PARylated proteins in A549 cells. Cells were treated with MOD568 or MOD582 (10 μM) with or without olaparib (10 μM) or MMS (0.00025%) for the indicated times. **B** Viability of A549 cells treated with MOD568 or MOD582 with or without MMS (0.000125%). Cell viability was assessed 72 h after treatment and normalized to untreated controls. Data represent mean ± SD (n=3, technical replicates) and represent one of two biologically independent experiments. *$P < 0.05$.**$P < 0.01$, ****$P < 0.0001$. **C** Antitumor activity (left) and body weight change (right) in the A549 xenograft model. Data represent means ± SEM (n = 8). *** $P < 0.001$ versus the vehicle control. PO, orally; BID, twice daily.

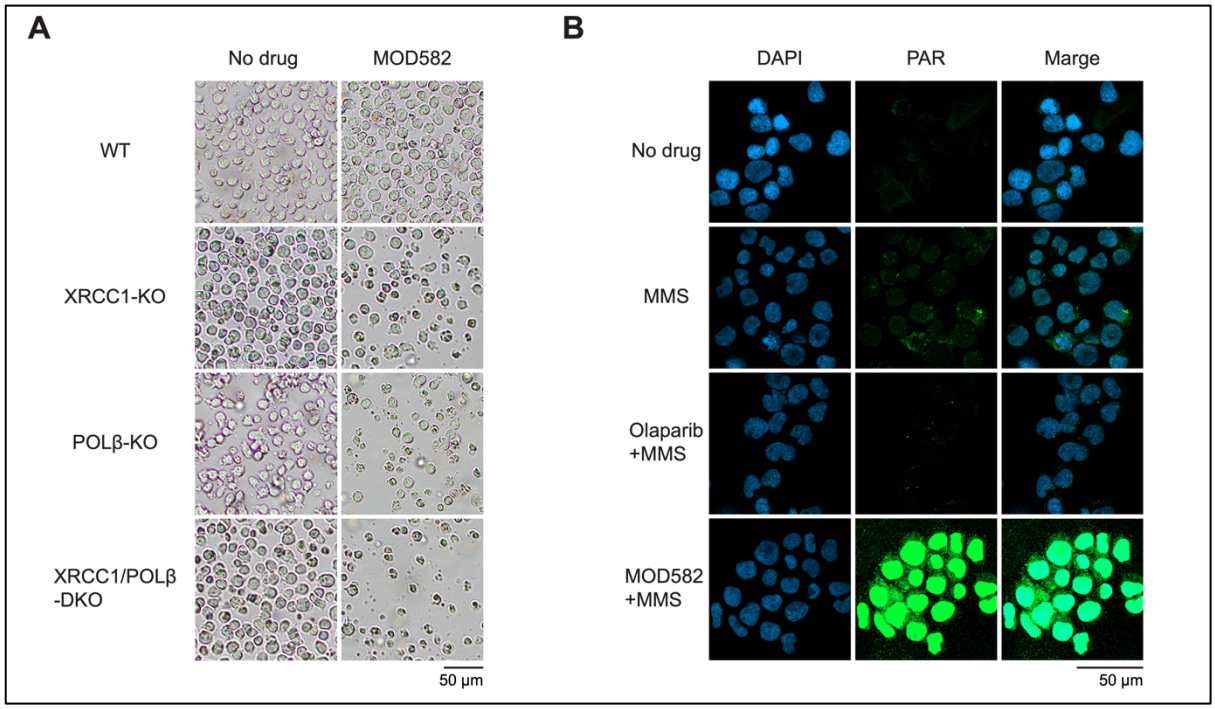

Figure S2. Increased cell death in the condition where PARylated proteins accumulate.

A Representative bright-field images of TK6 WT, XRCC1-KO, POLβ-KO, and XRCC1/POLβ-DKO cells following treatment with MOD582 (10 μM) for 42 h. Scale bar, 50 μm. **B** Immunofluorescence analysis of TK6 WT cells treated with MMS (0.00025 %), olaparib (10 μM) plus MMS, or MOD582 (10 μM) plus MMS for 1.5 h. Cells were stained for PAR and counterstained with DAPI. Representative images are shown. Scale bar, 50 μm.

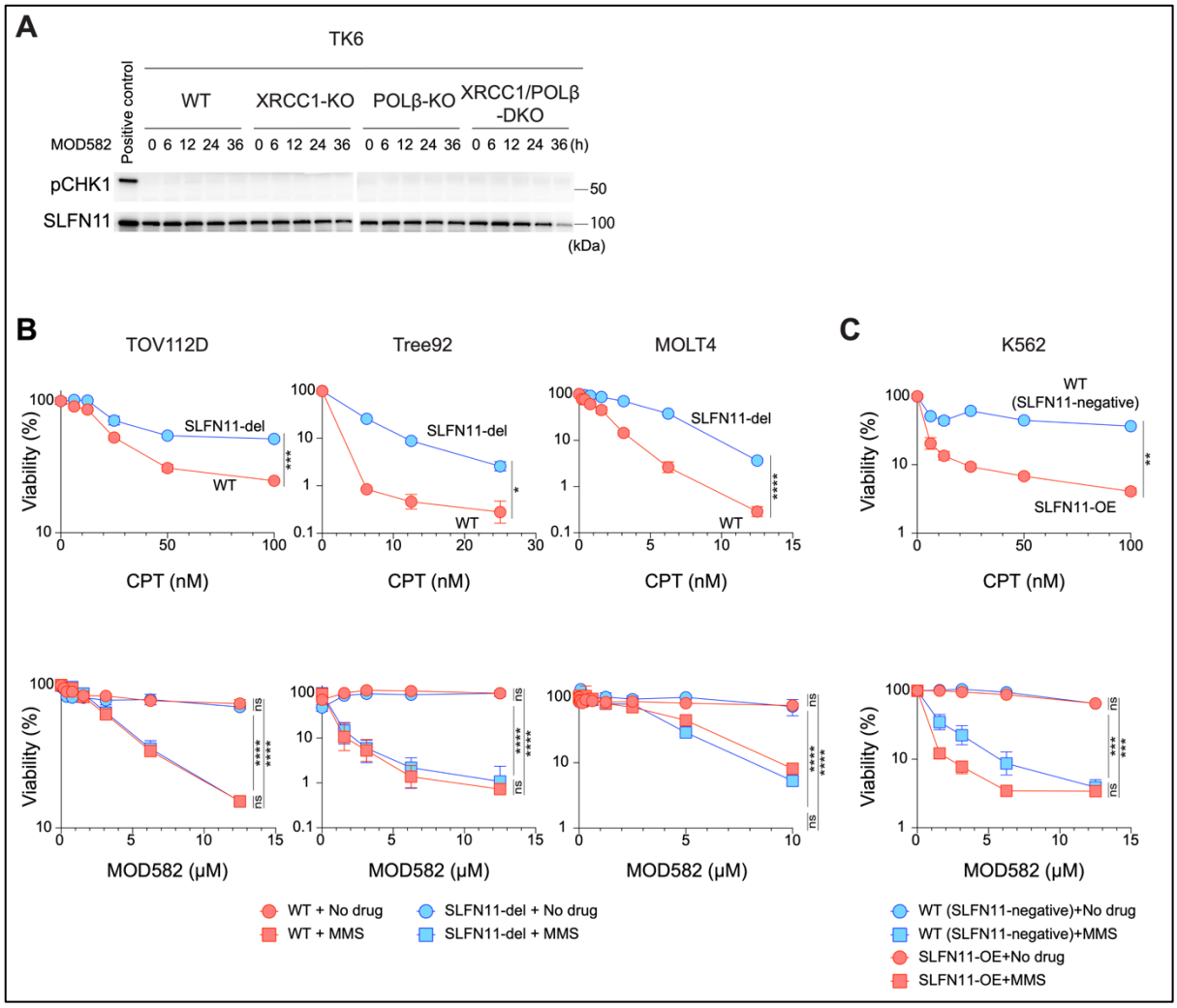

Figure S3. No apparent replication stress-induced cell death by PARG inhibition in the context of SLFN11-dependent cell death.

A pCHK1 was examined by immunoblotting. TK6 WT, XRCC1-KO, POLβ-KO, and XRCC1/POLβ-DKO cells were treated with MOD582 (10 μM) for the indicated time points. SLFN11 served as a loading control. **B-C** Viability of SLFN11-positive TOV112D, Tree92, and MOLT4 (WT) and their SLFN11-delated derivatives (SLFN11-del) treated with CPT or MOD582 with or without MMS (B; 0.0005% for TOV112D, 0.001% for Tree92 and MOLT4). Comparative experiments were conducted using SLFN11-negative K562 (WT) and the SLFN11-overexpressed K562 (SLFN11-OE) (C; 0.001% MMS). Cell viability was measured 72 h after treatment and normalized to untreated controls. Data represent mean ± SD (n=3, technical replicates) and represent one of two biologically independent experiments. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001. ns, not significant.

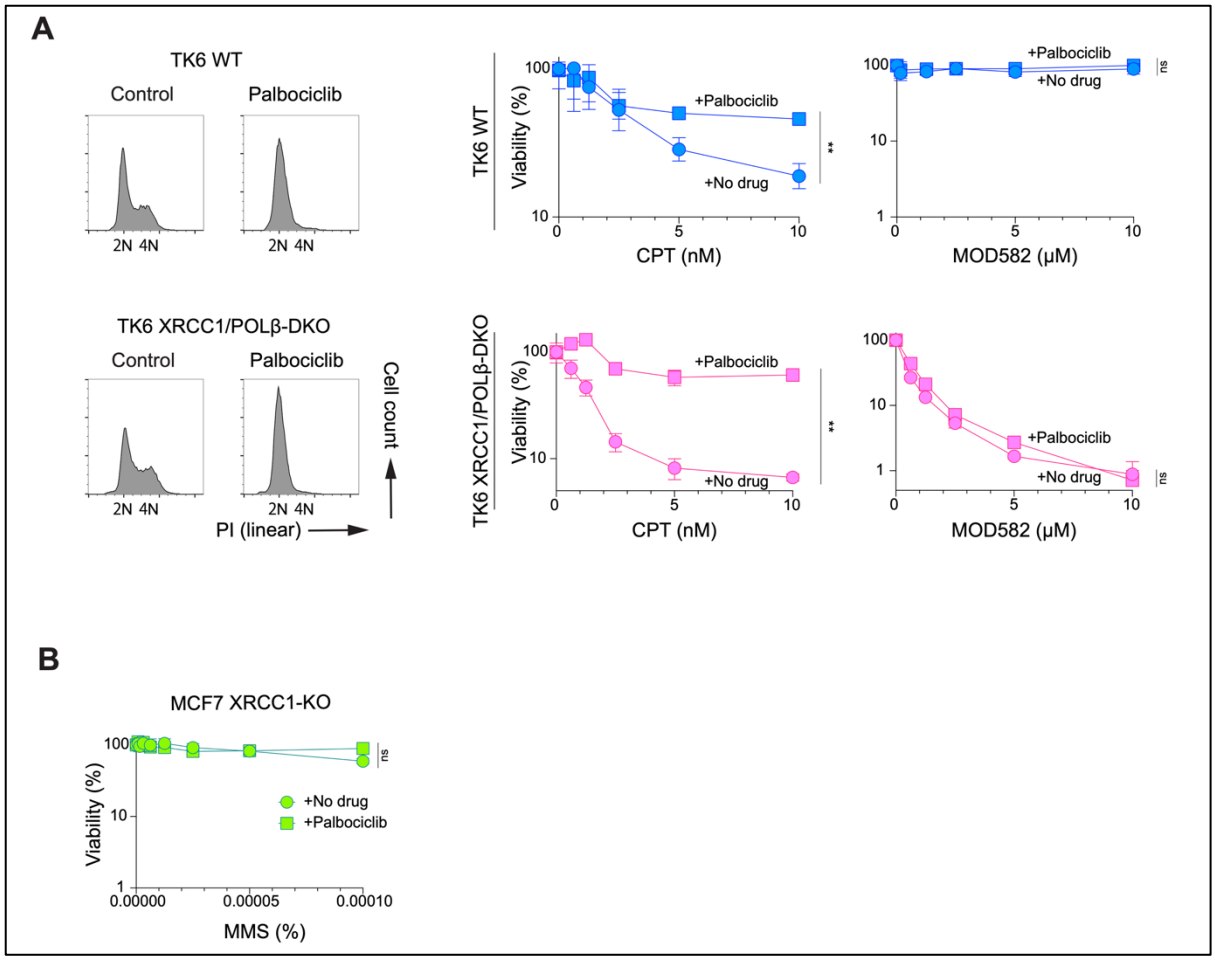

Figure S4. MOD582 sensitivity in G1-arrested TK6 cells.

A Left: Cell-cycle profiles of TK6 WT and XRCC1/POLβ-DKO cells treated with palbociclib (1000 nM) for 24 h. Cells were analyzed by propidium iodide staining followed by flow cytometry. Right: Viability of TK6 WT and XRCC1/POLβ-DKO cells either untreated or pretreated with palbociclib (1000 nM) for 24 h, followed by treatment with CPT or MOD582 for 72 h, with or without palbociclib. Cell viability was assessed and normalized to untreated controls. Data represent mean ± SD (n=3, technical replicates) and represent one of two biologically independent experiments. *$P < 0.05$. ** $P < 0.01$. ns, not significant.

B Viability of MCF7 XRCC1-KO cells either untreated or pretreated with palbociclib (1000 nM) for 72 h, followed by treatment with MMS for 72 h, with or without palbociclib. Cell viability was assessed and normalized to untreated controls. Not significant (ns).

Table S1

X-ray data collection and refinement statistics.

Table S2

Individual data from the siRNA screen (118 siRNA genes) in HCC1806, Hela, and MCF7 cells.