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Supplemental Information

The Ubiquitin Ligase TRIP12 Limits PARP1 Trapping and Constrains PARP Inhibitor Efficiency

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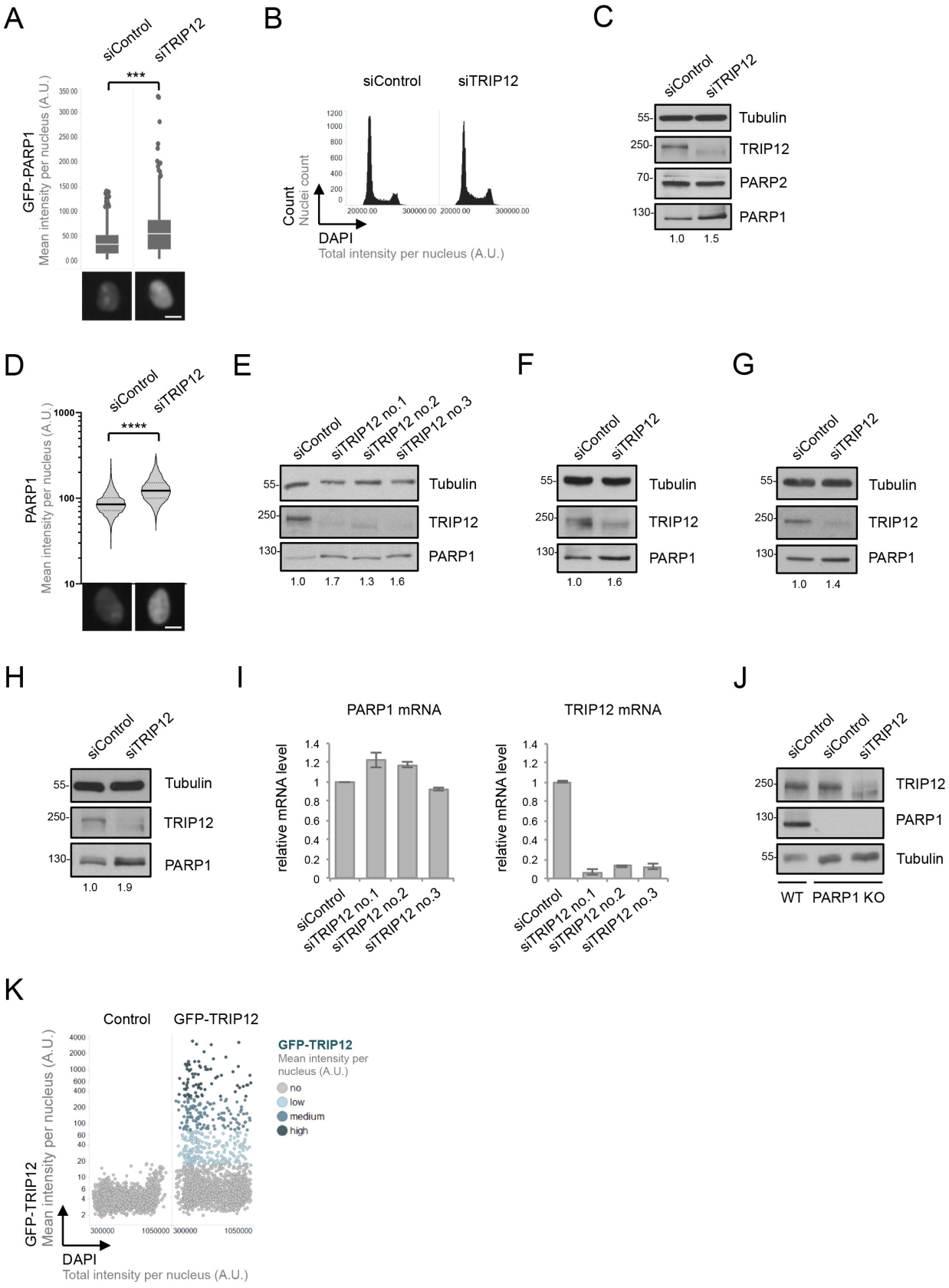

Figure S1, Related to Figure 1: The HECT-type ubiquitin ligase TRIP12 controls PARP1 abundance. (A) HeLa Kyoto LAP-PARP1 cells were transfected with control siRNA or siRNA against TRIP12 and nuclear GFP-PARP1 levels were analyzed by high-content microscopy. Box plots with white lines indicating the median are shown. Representative single cell images of GFP-PARP1 are shown below. Scale bar, 10 µm. **(B)** QIBC-derived cell cycle profiles based on the total DAPI intensity per nucleus of the samples shown in (A). **(C)** Western blot analysis of

endogenous PARP1 and PARP2 levels in U-2 OS cells transfected with the indicated siRNAs. PARP1 band intensities were quantified in Fiji and the fold change is indicated below. **(D)** U-2 OS cells were transfected with control siRNA or siRNA against TRIP12 and nuclear PARP1 levels were analyzed by high-content microscopy. Violin plots with median (solid line) and quartiles (dashed lines) indicated are shown. Representative single cell images are shown below. Scale bar, 10 µm. **(E)** U-2 OS cells were transfected with three different siRNAs against TRIP12 (no.1, no.2, no.3) and TRIP12 and PARP1 levels were analyzed by Western blot. PARP1 band intensities were quantified in Fiji and the fold changes are indicated below. **(F)** RPE-1 cells were transfected with control siRNA or siRNA against TRIP12 and TRIP12 and PARP1 levels were analyzed by Western blot. PARP1 band intensities were quantified in Fiji and the fold change is indicated below. **(G)** HCC1143 cells were transfected with control siRNA or siRNA against TRIP12 and TRIP12 and PARP1 levels were analyzed by Western blot. PARP1 band intensities were quantified in Fiji and the fold change is indicated below. **(H)** U-2 OS cells were transfected with the indicated siRNAs for two consecutive rounds of 48 hours each and analyzed by Western blot. PARP1 band intensities were quantified in Fiji and the fold change is indicated below. **(I)** U-2 OS cells were transfected with three different siRNAs against TRIP12 (no.1, no.2, no.3) and PARP1 and TRIP12 mRNA levels were analyzed by RT-qPCR. Relative mRNA levels are depicted as means ± SD. **(J)** U-2 OS and U-2 OS PARP1 KO cells were transfected with the indicated siRNAs and TRIP12 and PARP1 levels were analyzed by Western blot. **(K)** QIBC-derived single cell scatter plot depicting nuclear GFP levels of the samples analyzed in Figure 1F.

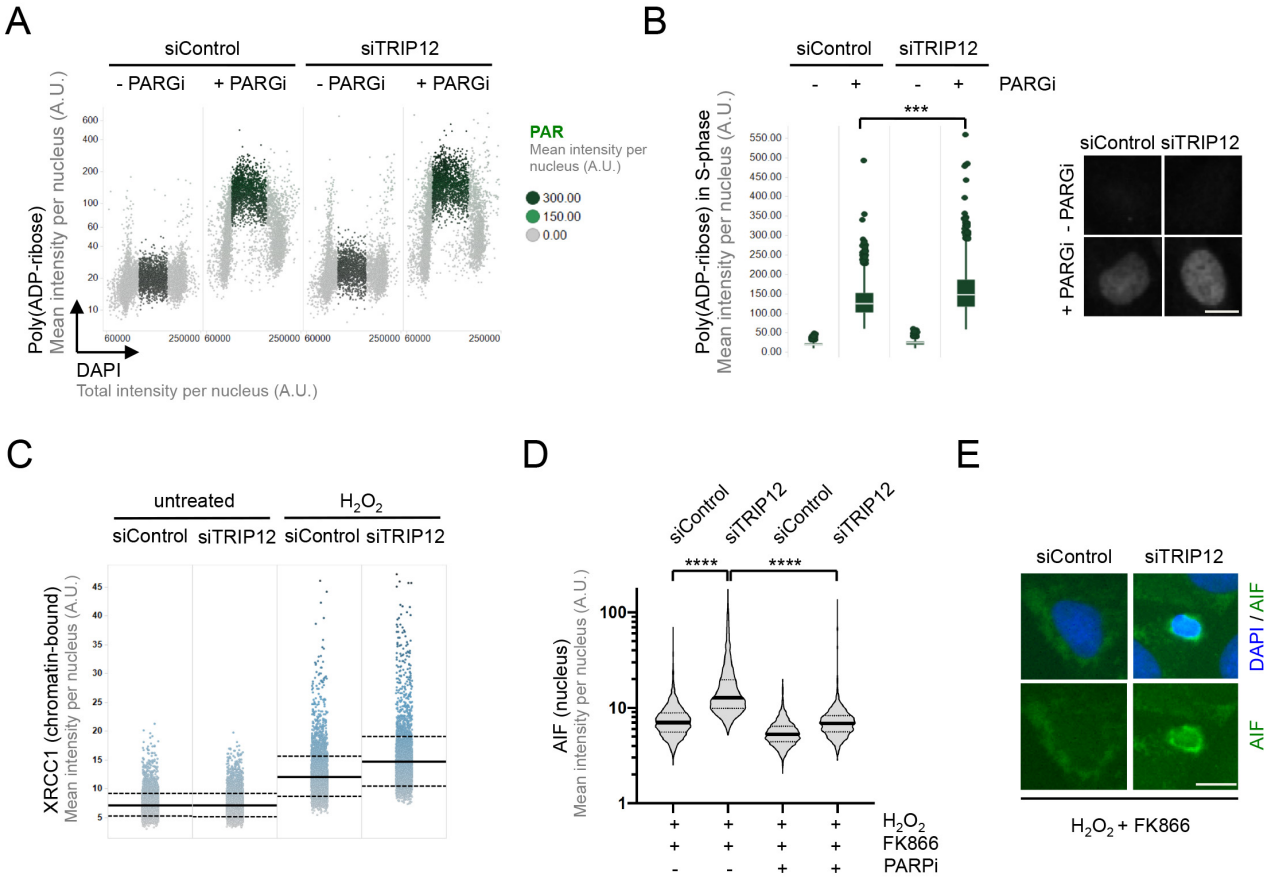

Figure S2, Related to Figure 2: TRIP12 limits cellular PAR formation. (A) U-2 OS cells were transfected with control siRNA or siRNA against TRIP12 and exposed to PARGi (10 µM) for 1h as indicated. QIBC-derived cell cycle resolved scatter plots depict nuclear PAR levels. **(B)** Box plots represent PAR levels in the selected sub-populations of cells in S-phase in (A), with white lines representing the median. Unpaired t-test was used for statistical analysis and representative single cell images are shown below. Scale bar, 10 µm. **(C)** U-2 OS cells were transfected with siRNAs and exposed to 0.1 mM H_2O_2 for 1h as indicated. Cells were pre-extracted, fixed and chromatin-associated XRCC1 levels were analyzed by high-content microscopy. Mean (solid line) and standard deviation from the mean (dashed lines) are indicated. **(D)** U-2 OS cells were transfected with siRNAs and treated as indicated (FK866: 1 µM, 24h; PARPi Olaparib: 10 µM, 24h; H_2O_2: 20 µM, 12h) to induce PARthanatos-associated AIF release from mitochondria. High-content imaging-derived violin plot show nuclear AIF intensities. Median (solid line) and quartiles (dashed lines) are indicated, unpaired t-test was used for statistical analysis. **(E)** Representative single cell images of AIF translocation to the nucleus upon combined H_2O_2 and FK866 treatment. Scale bar, 10 µm.

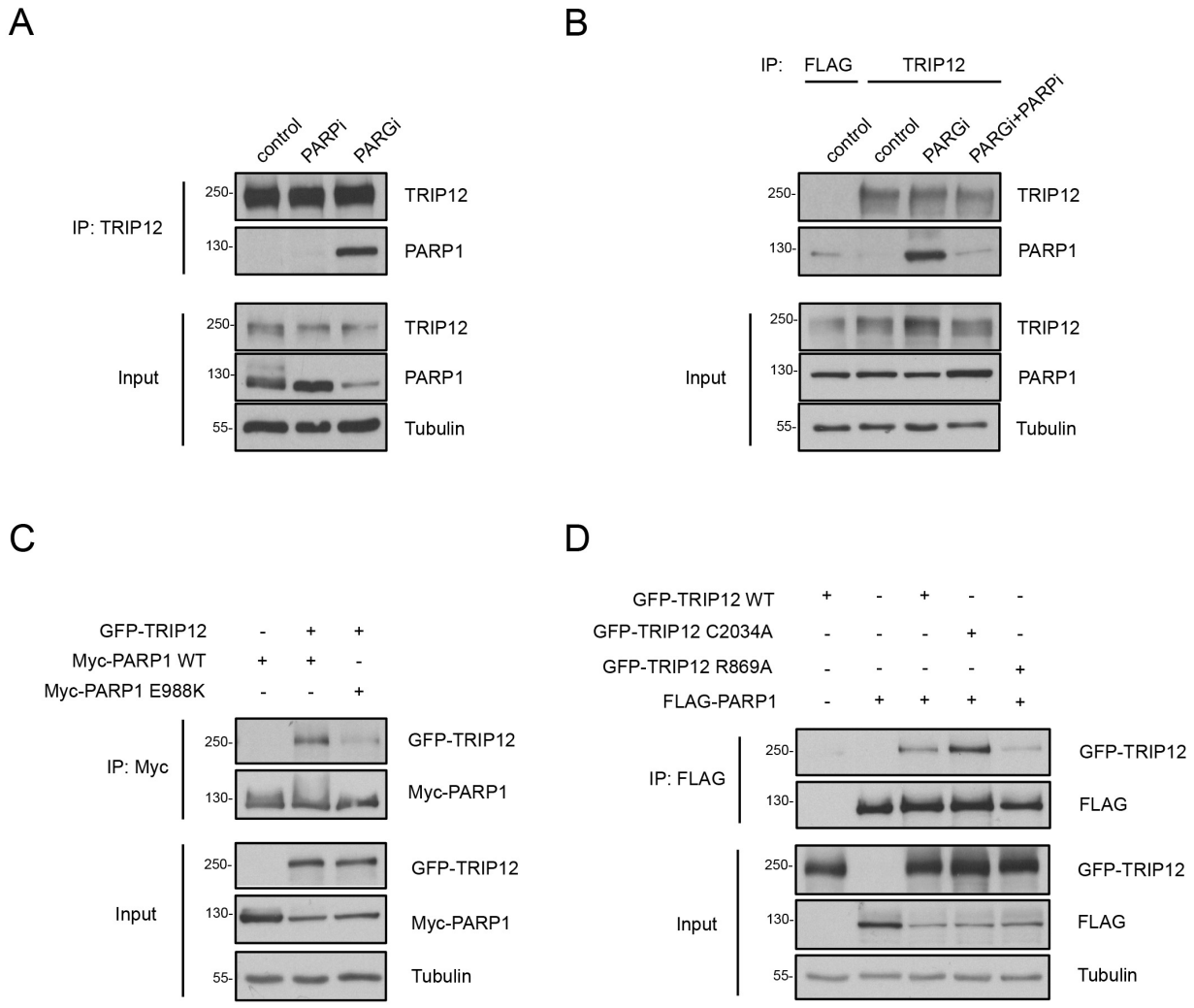

Figure S3, Related to Figure 3: PAR- and WW E-dependent interaction between PARP1 and TRIP12. (A) HEK293T cells were treated with the indicated inhibitors (PARPi Olaparib: 10 µM, 1h; PARGi: 10 µM, 1h) and lysates were immunoprecipitated with TRIP12 antibody. The interaction with endogenous PARP1 was analyzed by Western blot. **(B)** HEK293T cells were treated with the indicated inhibitors (PARPi Olaparib: 10 µM, 1h; PARGi: 10 µM, 1h) and lysates were immunoprecipitated with TRIP12 or FLAG antibody as negative control IP. The interaction with endogenous PARP1 was analyzed by Western blot. **(C)** HEK293T cells were transfected with the indicated plasmids, Myc-PARP1 (WT or catalytic mutant E988K) was immunoprecipitated and the interaction with GFP-TRIP12 was analyzed by Western blot. IP samples were adjusted based on input levels of PARP1 to correct for the effect of TRIP12 on PARP1 abundance. **(D)** HEK293T cells were transfected with the indicated plasmids, FLAG-PARP1 was immunoprecipitated and the interaction with GFP-TRIP12 (WT, HECT mutant C2034A, or WWE mutant R869A) was analyzed by Western blot. IP samples were adjusted based on input levels of PARP1 to correct for the effect of TRIP12 on PARP1 abundance.

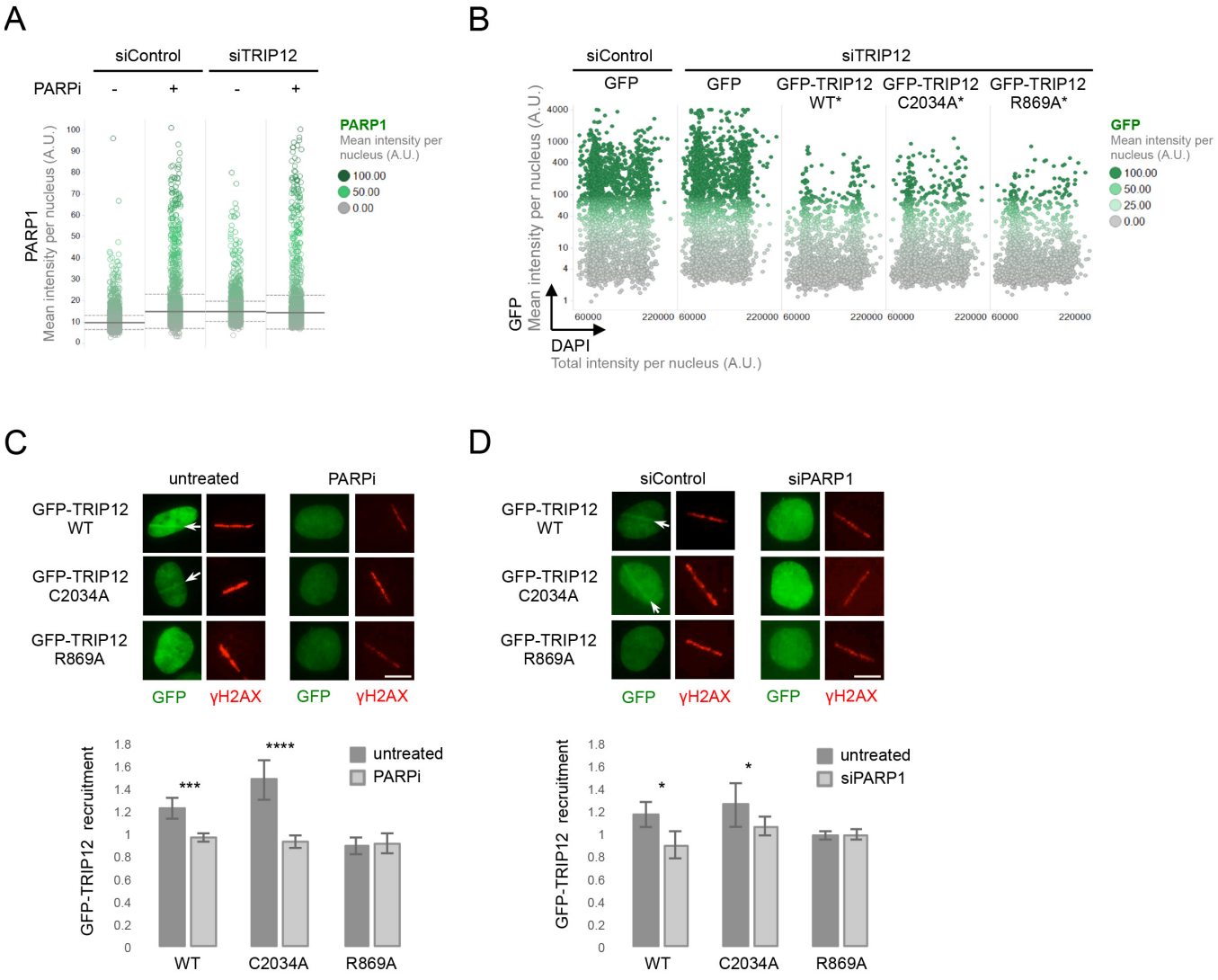

Figure S4, Related to Figure 4: TRIP12 controls PARP1 stability. (A) U-2 OS cells were transfected with control siRNA or siRNA against TRIP12 and exposed to PARP1 (PJ-34, 10 µM) for 16h as indicated. Nuclear PARP1 levels were analyzed by high-content microscopy. Mean (solid line) and standard deviation from the mean (dashed lines) are indicated. **(B)** QIBC-derived GFP expression levels of the samples analyzed in Figure 4e. **(C)** U-2 OS cells were transfected with the indicated plasmids to express GFP-TRIP12 (WT, HECT mutant C2034A, or WWE mutant R869A) and subjected to laser micro-irradiation in absence or presence of PARPi (Olaparib, 10 µM). Cells were allowed to recover for 5 minutes after laser damage, were then fixed and stained for γH2AX, and GFP-TRIP12 recruitment to sites of DNA damage was analyzed. Representative single cell images are shown and quantification of GFP-TRIP12 recruitment to sites of DNA damage is depicted below as mean ± SD. Scale bar, 10 µm. **(D)** As in (C) with U-2 OS cells either transfected with control siRNA or siRNA against PARP1. Representative single cell images are shown and quantification of GFP-TRIP12 recruitment to sites of DNA damage is depicted below as mean ± SD. Scale bar, 10 µm.

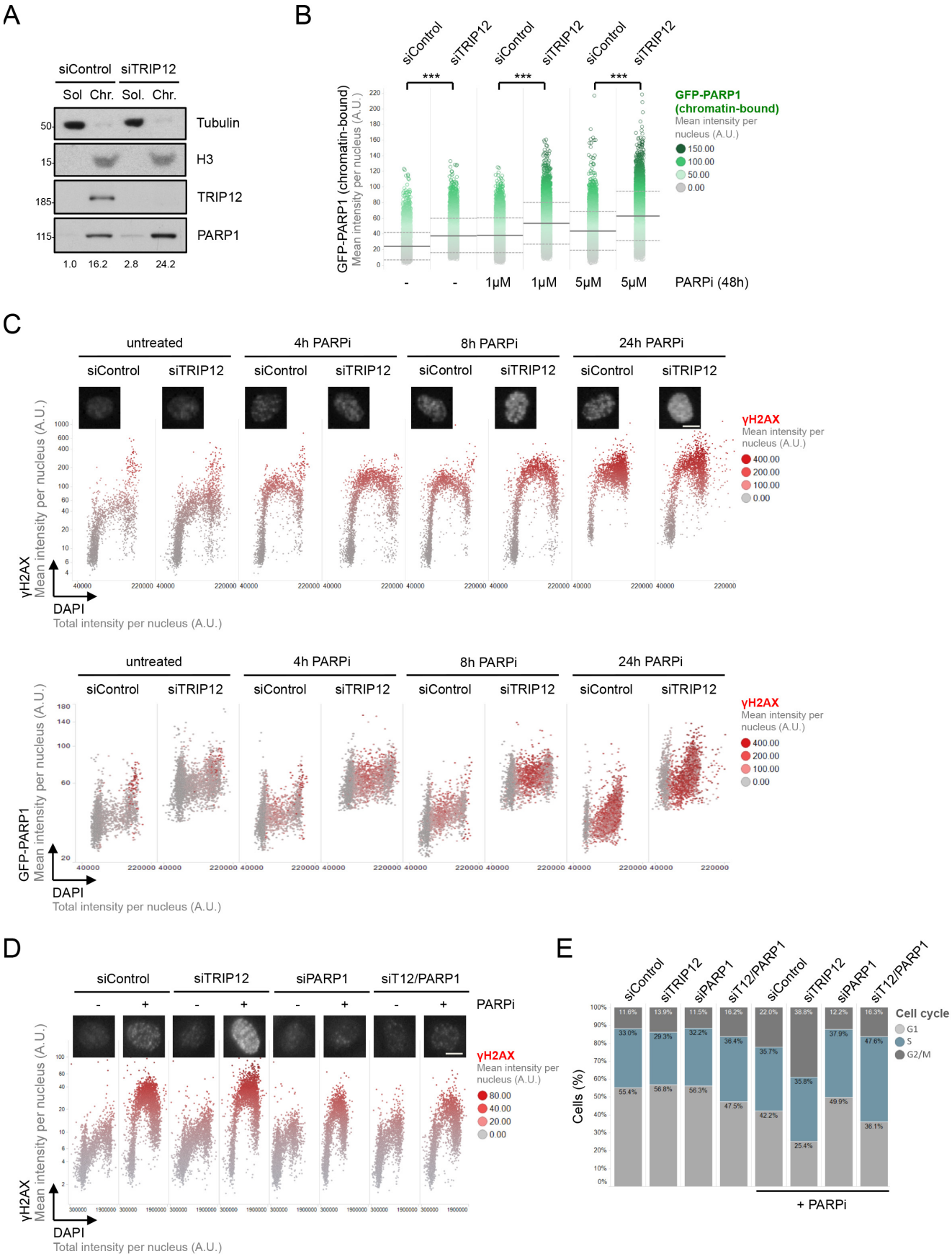

Figure S5, Related to Figure 5: TRIP12 constrains PARP1 trapping and extenuates PARPi-induced DNA damage. (A) Chromatin fractionation of U-2 OS cells transfected with the indicated siRNAs. TRIP12 and PARP1 chromatin association was analyzed by Western blot. PARP1 band intensities were quantified in Fiji and the fold changes are indicated below. **(B)** HeLa Kyoto PARP1-LAP cells were transfected with control siRNA or siRNA against

TRIP12 and exposed to PARPi (Olaparib, 10 μM) as indicated. Cells were pre-extracted and chromatin-bound GFP-PARP1 was analyzed by high-content microscopy. Mean (solid line) and standard deviation from the mean (dashed lines) are indicated. **(C)** HeLa Kyoto PARP1-LAP cells were transfected with siRNA as indicated and exposed to PARPi (Olaparib, 10 μM) for increasing time periods. QIBC-derived cell cycle resolved γH2AX and GFP-PARP1 profiles, color-coded for γH2AX intensities, are shown. Representative single cell images of the γH2AX staining are also shown. Scale bar, 10 μm. **(D)** HeLa Kyoto PARP1-LAP cells were transfected with siRNA and exposed to PARPi (Olaparib, 10 μM) for 16h as indicated. QIBC-derived cell cycle resolved γH2AX profiles are shown. Representative single cell images are also shown. Scale bar, 10 μm. **(E)** QIBC-derived cell cycle distribution based on total DAPI intensity and EdU incorporation of cells treated as in (D).

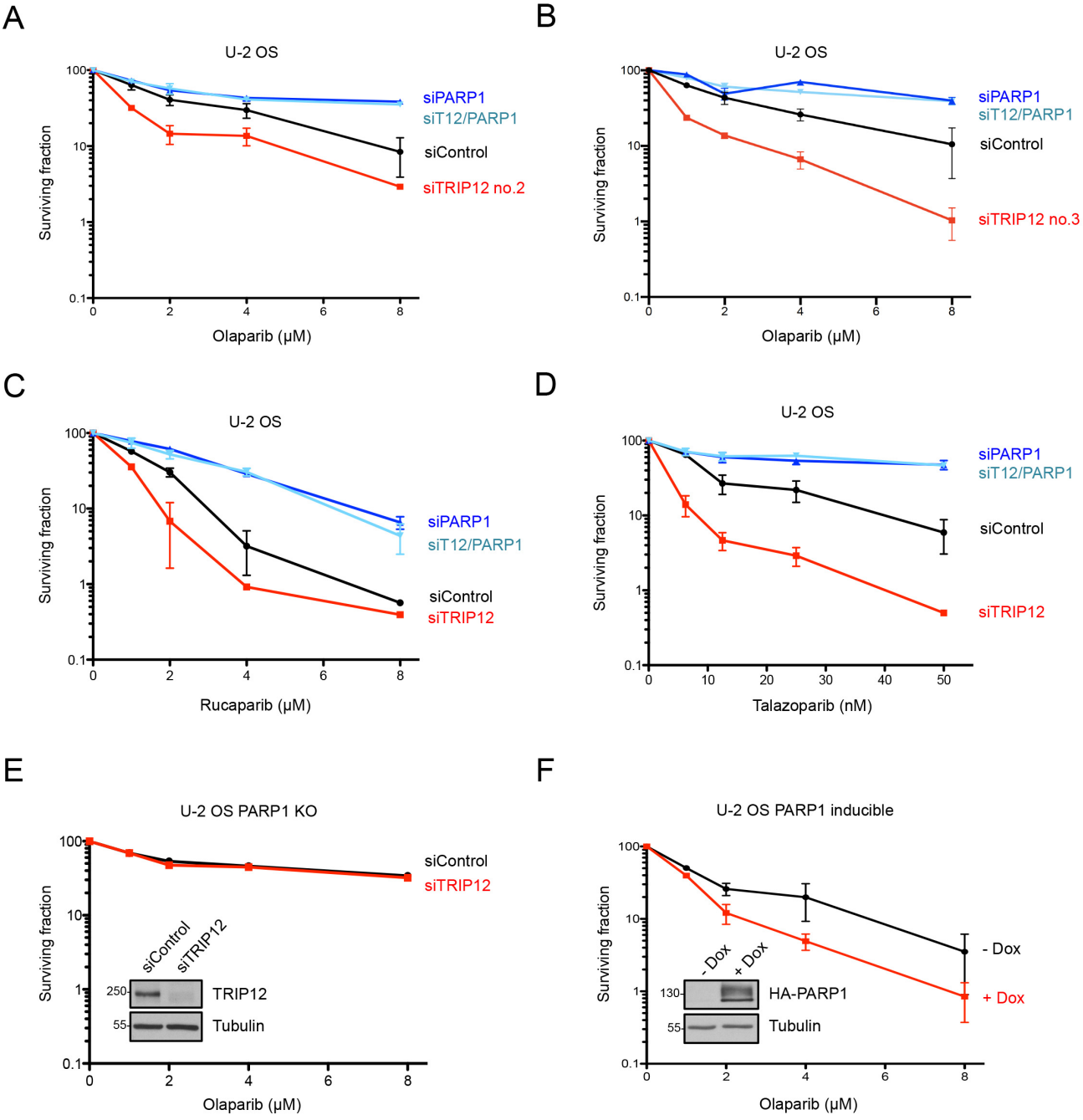

Figure S6, Related to Figure 6: Down-regulation of TRIP12 sensitizes cancer cells to PARPi. (A) U-2 OS cells were transfected with siRNAs and exposed to PARPi Olaparib as indicated to measure clonogenic survival. A different siRNA targeting TRIP12 (no. 2, s17809) was used compared to the siRNA used in Figure 6a. Relative clonogenic survival in response to increasing doses of PARPi Olaparib is shown. Means ± SD are depicted. **(B)** U-2 OS cells were transfected with siRNAs and exposed to PARPi Olaparib as indicated to measure clonogenic survival. A third siRNA targeting TRIP12 (no. 3, s17808) was used. Relative clonogenic survival in response to increasing doses of PARPi Olaparib is shown. Means ± SD are depicted. **(C)** U-2 OS cells were transfected with siRNAs and exposed to PARPi Rucaparib as indicated to measure clonogenic survival. Relative clonogenic survival in response to increasing doses of PARPi Rucaparib is shown. Means ± SD are depicted. **(D)** U-2 OS cells were transfected with siRNAs and exposed to PARPi Talazoparib as indicated to measure clonogenic survival. Relative clonogenic survival in response to increasing doses of PARPi Talazoparib is shown. Means ± SD are depicted. **(E)** U-2 OS PARP1 KO cells were transfected with siRNAs and exposed to PARPi Olaparib as indicated to measure clonogenic survival. Relative clonogenic survival in response to increasing doses of PARPi Olaparib is shown. Means ± SD are depicted. Protein levels after the indicated siRNAs transfection are shown as inlet. **(F)** U-2 OS cells expressing or not HA-PARP1 upon doxycycline (Dox, 1 µg/ml) addition for 24 hours were exposed to Olaparib as indicated and clonogenic cell survival was measured. Means ± SD are depicted. Protein levels with and without doxycycline induction are shown as inlet.

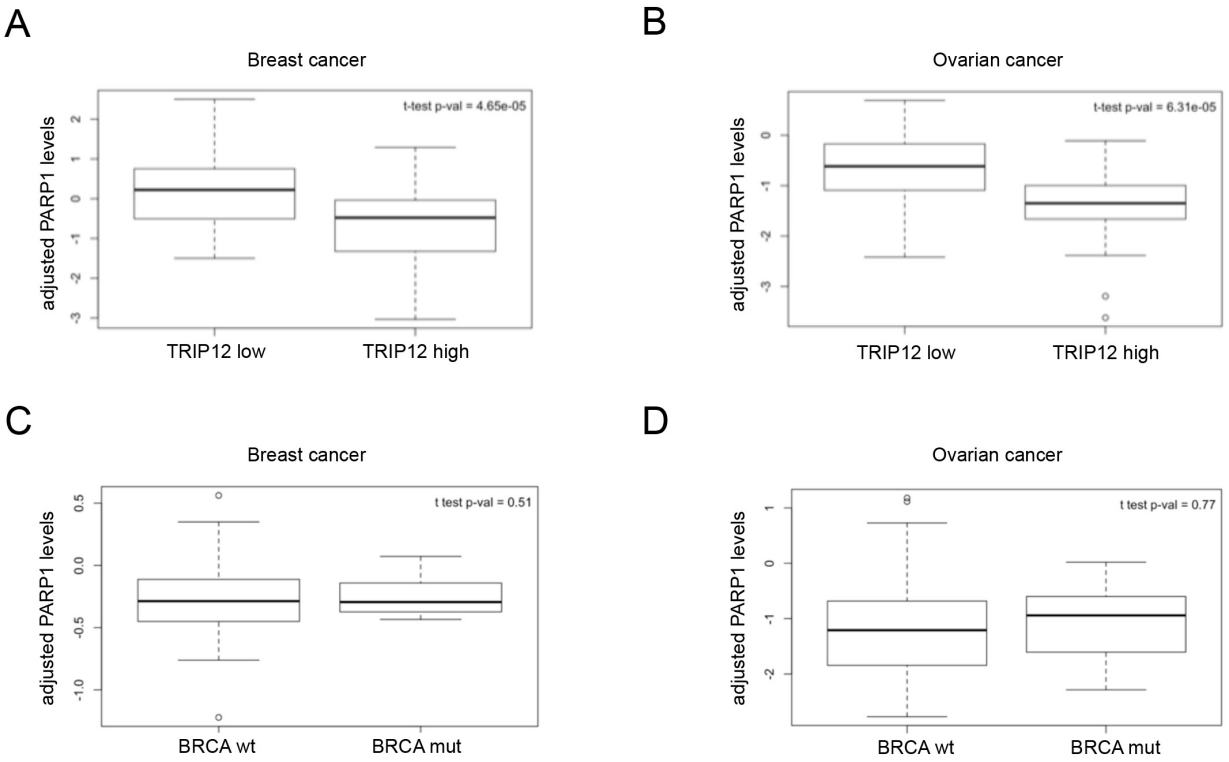

Figure S7, Related to Figure 6: Low TRIP12 expression is associated with elevated PARP1 abundance in cancer patients. (A) Student t test on adjusted PARP1 protein level between sample groups equally divided based on TRIP12 RNA expression in breast cancer. **(B)** Student t test on adjusted PARP1 protein level between sample groups equally divided based on TRIP12 RNA expression in ovarian cancer. **(C)** Student t test on adjusted PARP1 protein level between BRCA wild-type and mutant samples in breast cancer. **(D)** Student t test on adjusted PARP1 protein level between BRCA wild-type and mutant samples in ovarian cancer.