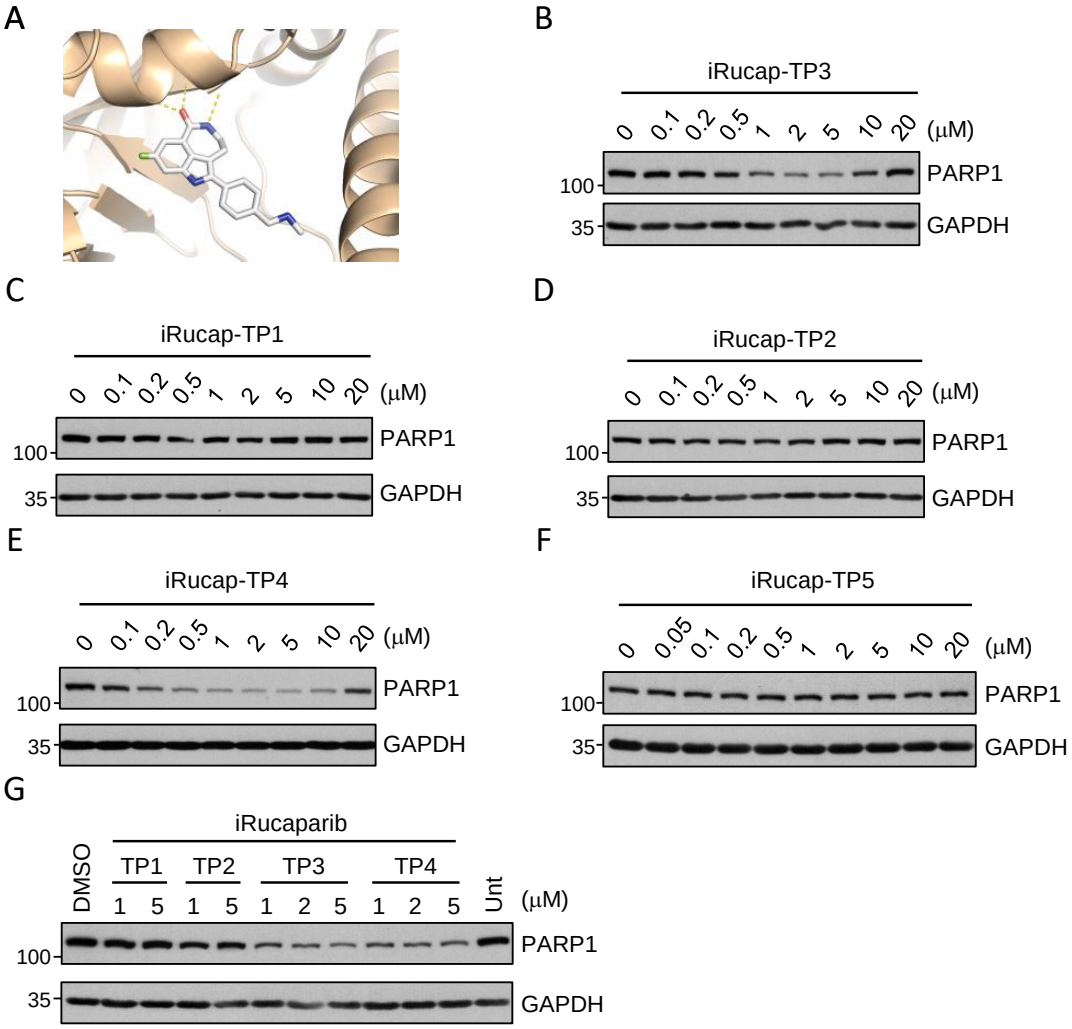
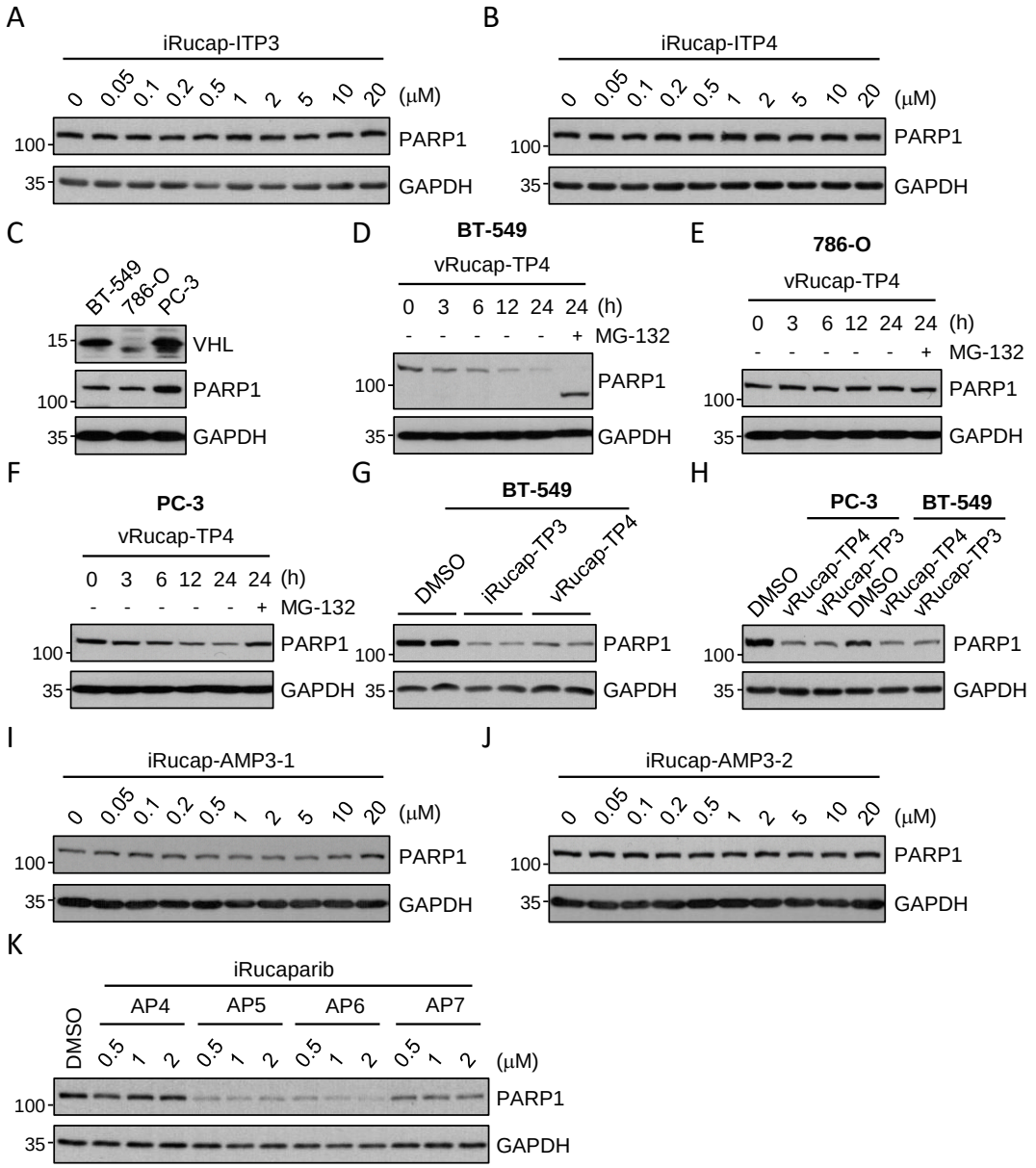
Supplementary Figures

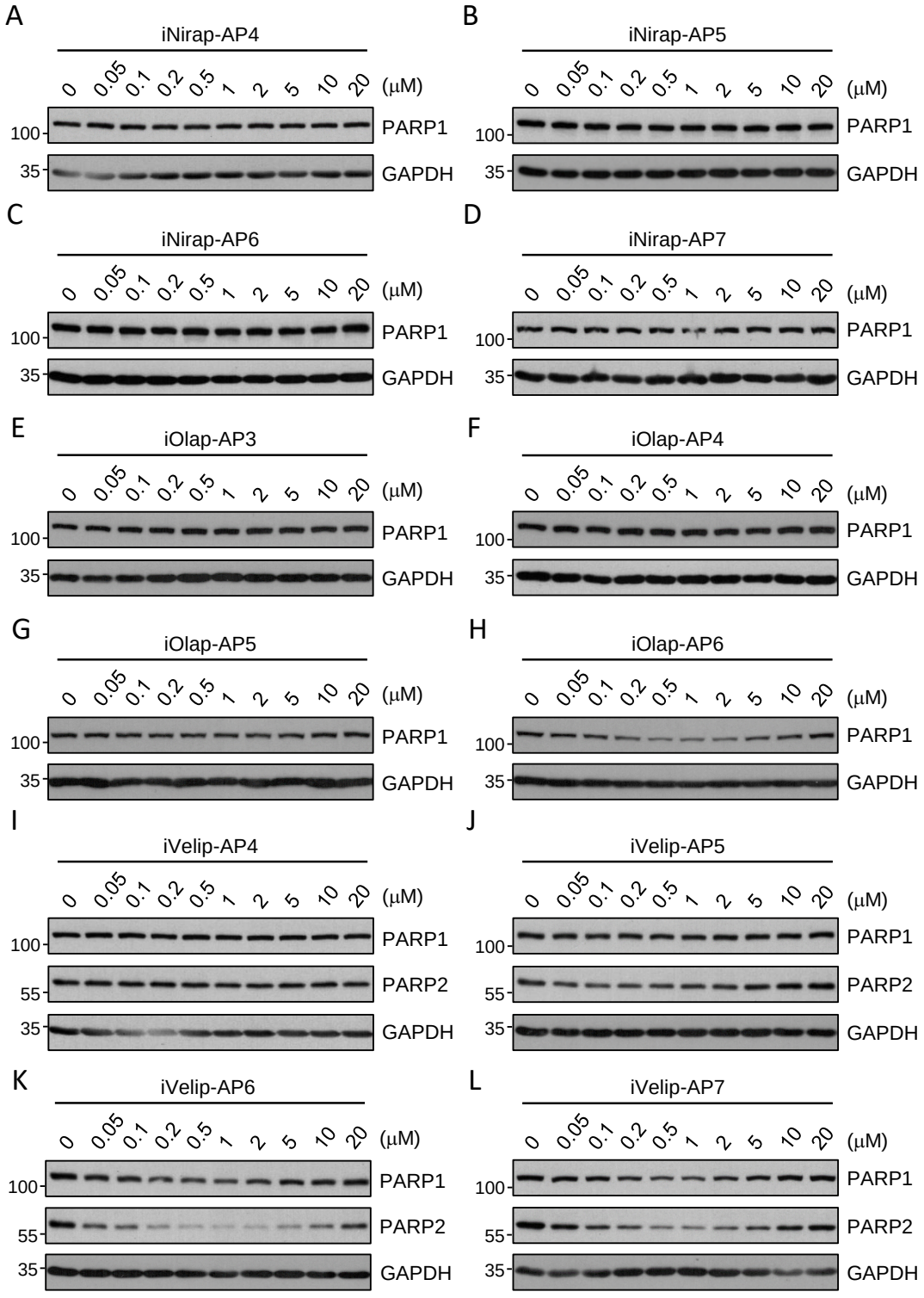


Supplementary Figure 1. PARP1 degraders induce PARP1 degradation in HeLa cells. (A) The human PARP1 catalytic domain in complex with Rucaparib (PDB: 4RV6). (B-G) PARP1 degradation is affected by the linker lengths in the PARP1 degrader. HeLa cells were treated with increasing concentrations of the CRBN-based degraders bearing a (B) Triazole-PEG3 linker, (C) Triazole-PEG1 linker, (D) Triazole-PEG2 linker, (E) Triazole-PEG4 linker or (F) Triazole-PEG5 linker for 24 hrs. (G) Comparison of PARP1 degradation by iRucaparib-TP1-4 in HeLa cells. In these experiments, whole cell lysates were analyzed by immunoblotting assays using the indicated antibodies, with the molecular weight standards shown (kDa). The results are representatives of two biologically independent experiments. Uncropped blots are shown in Supplementary Figure 14.



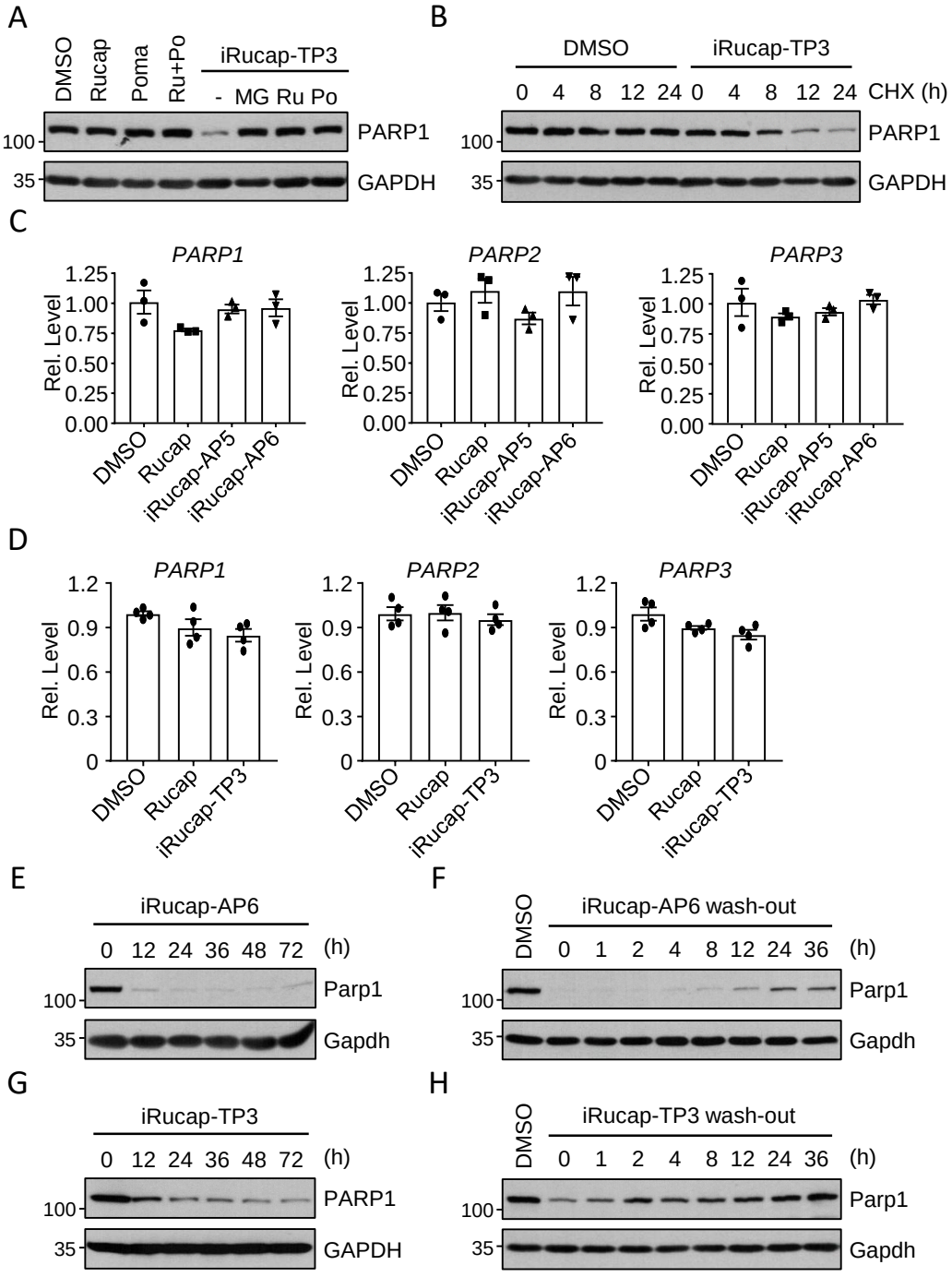
Supplementary Figure 2. Evolution of Rucaparib-linked PARP1 degraders. (A-B) PARP1 degradation is affected by the lengths and types of the linker in the degrader. HeLa cells were treated with increasing concentrations of the CRBN-based degraders bearing an (A) Indole group-linked Triazole-PEG3 linker or (B) Indole group-linked Triazole-PEG4 linker for 24 hrs. (C) Expression of VHL and PARP1 in different cells. The band in 786-O cells is a non-specific signal. (D-H) PARP1 degradation is regulated by the presence of the E3 ligase relevant to the compound. Cells were treated with vRucaparib-TP4 (10 μM) for the indicated times, or vRucaparib-TP4 plus MG-132 (1 μM) for 24 hrs in (D) BT-549 cells, (E)786-O cells and (F) PC-3 cells. (G) BT-549 cells were treated with iRucaparib-TP3 (5 μM) or vRucaparib-TP4 (20 μM) for 24 hrs. (H) PC-3 cells and BT-549 cells were treated with vRucaparib-TP4 or vRucaparib-TP3 (both at 10 μM) for 24 hrs. (I-J) PARP1 degradation

is affected by the lengths and types of the linker in the degrader. HeLa cells were treated with increasing concentrations of the CRBN-based degraders bearing an (I) Amide1-PEG3 linker or (J) Amide2-PEG3 linker for 24 hrs. (K) Comparison of PARP1 degradation by iRucaparib-AP4-7 in HeLa cells. In these experiments, whole cell lysates were analyzed by immunoblotting assays using the indicated antibodies, with the molecular weight standards shown (kDa). The results are representatives of two biologically independent experiments. Uncropped blots are shown in Supplementary Figure 14.



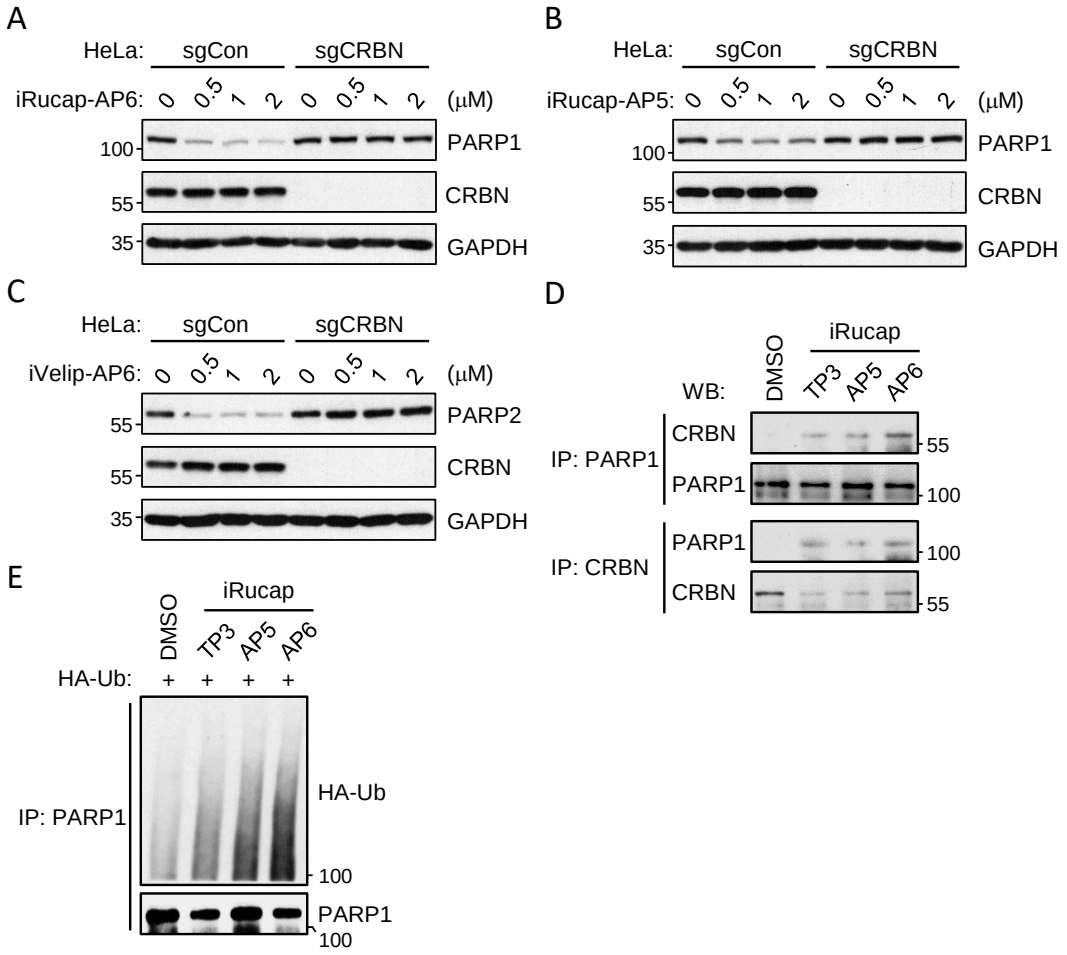
Supplementary Figure 3. Other PARP1 inhibitor based PARP degraders. (A-D) HeLa cells were treated with increasing concentrations of the Niraparib-linked degraders bearing an (A) All PEG4 linker, (B) All PEG5 linker, (C) All PEG6 linker, or (D) All PEG7 linker for 24 hrs. (E-H) HeLa cells were treated with increasing concentrations of the Olaparib-linked degraders bearing an (E) All PEG3 linker, (F) All PEG4 linker, (G) All PEG5 linker, or (H) All PEG6 linker for 24 hrs. (I-L) HeLa cells

were treated with increasing concentrations of the Veliparib-linked degraders bearing an (I) All PEG4 linker, (J) All PEG5 linker, (K) All PEG6 linker, or (L) All PEG7 linker for 24 hrs. In these experiments, whole cell lysates were analyzed by immunoblotting assays using the indicated antibodies, with the molecular weight standards shown (kDa). The results are representatives of two biologically independent experiments. Uncropped blots are shown in Supplementary Figure 14.

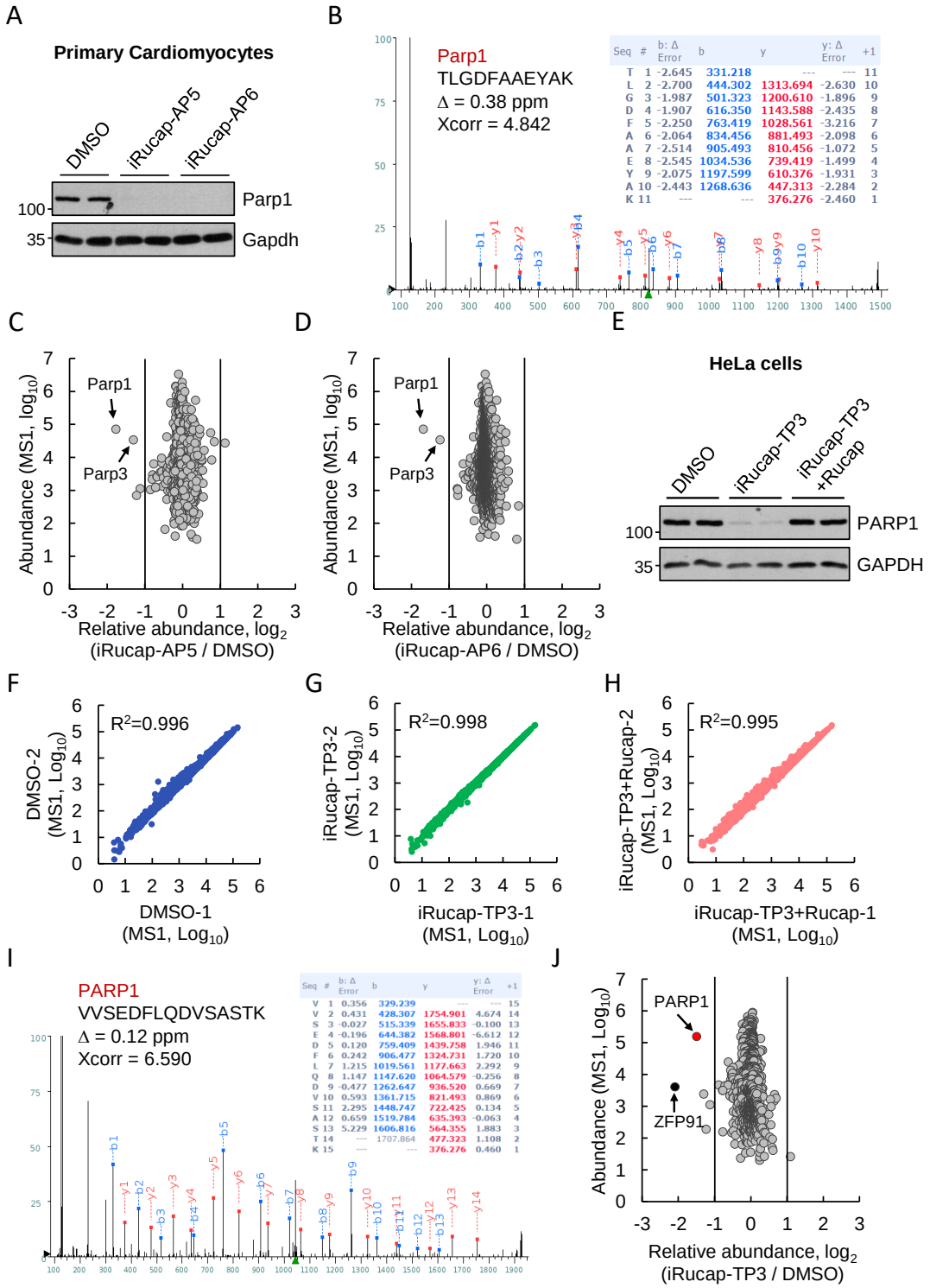


Supplementary Figure 4. iRucaparib and iRucaparib-TP3 induce PARP degradation. (A-B) Degradation of PARP1 by iRucaparib-TP3 in HeLa cells. (A) Cells were treated with Rucaparib (5 μM), pomalidomide (5 μM), Rucaparib (5 μM) plus pomalidomide (5 μM), iRucaparib-TP3 (5 μM), or iRucaparib-TP3 (5 μM) plus Rucaparib (1 μM) or Pomalidomide (2 μM) or MG132 (1 μM) for 24 hrs. Rucap/Ru, Rucaparib; Poma/Po, Pomalidomide; MG, MG-132. (B) Cells were pre-treated with iRucaparib-TP3 (5 μM) for 12 hrs followed by cycloheximide (10 μg/ml) treatment for the indicated times. CHX, Cycloheximide. (C-D) mRNA abundances of PARP1, PARP2 and PARP3 as determined

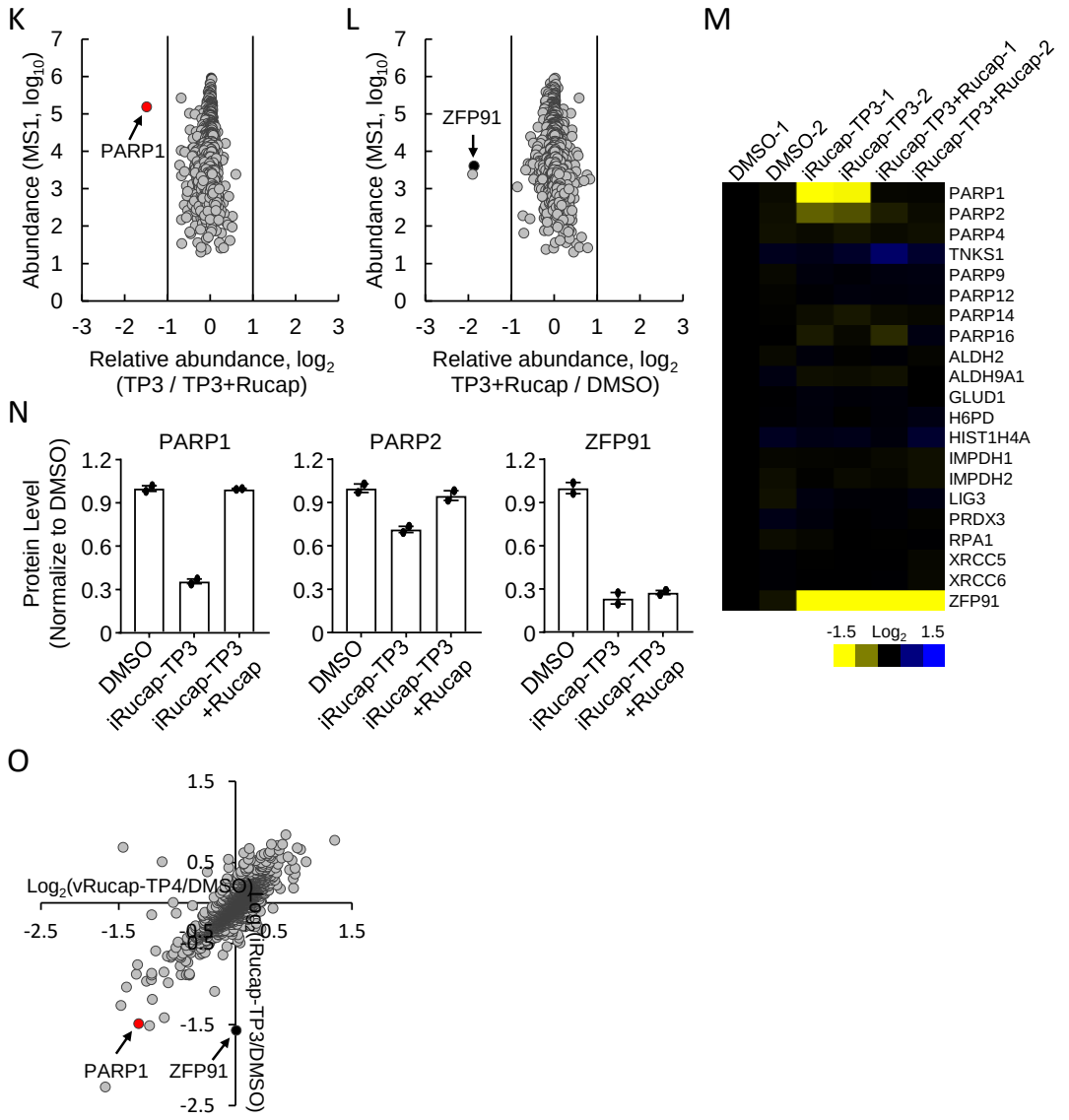
by quantitative RT-PCR assays in HeLa cells. (C) Cells were treated with Rucaparib or iRucaparib-AP5/6 (1 μM) for 24 hrs. Values represent mean ± SEM (n = 3 biological independent samples). (D) Cells were treated with Rucaparib or iRucaparib-TP3 (5 μM) for 24 hrs. Values represent mean ± SEM (n = 4 biological independent samples). (E-F) Degradation of PARP1 by iRucaparib in primary cardiomyocytes. (E) Cells were treated with iRucaparib (1 μM) for indicated times. (F) Cells were pre-treated with iRucaparib (1 μM) for 24 hrs. iRucaparib was then washed out for indicated times. (G-H) Degradation of PARP1 by iRucaparib-TP3 in HeLa cells. (G) Cells were treated with iRucaparib-TP3 (5 μM) for the indicated times. (H) Cells were pre-treated with iRucaparib-TP3 (5 μM) for 24 hrs. iRucaparib-TP3 was washed out for the indicated times. In these experiments, whole cell lysates were analyzed by immunoblotting assays using the indicated antibodies, with the molecular weight standards shown (kDa). The results are representatives of two biologically independent experiments. Uncropped blots are shown in Supplementary Figure 14.



Supplementary Figure 5. iRucaparib-AP5/6 and iVeliparib-AP6 induce PARP degradation in HeLa cells. (A-C) PARP degradation is dependent on the presence of the E3 ligase relevant to the degrader. CRBN knockout cells and the parallel control cells were treated with increasing concentrations of (A) iRucaparib, (B) iRucaparib-AP5 or (C) iVeliparib-AP6 for 24 hrs. (D) iRucaparib induces association of PARP1 and CRBN in HeLa cells. Cells were treated with iRucaparib-TP3 or iRucaparib-AP5/6 (1 μM) for 24 hrs. The interaction between PARP1 and CRBN was determined by immunoprecipitation assays. (E) iRucaparib induces ubiquitination of PARP1 in HeLa cells. Cells were transfected with an HA-Ub plasmid for 24 hrs and treated with iRucaparib-TP3 or iRucaparib-AP5/6 (1 μM) for another 24 hrs. The ubiquitination of PARP1 was analyzed by ubiquitination assays. In these experiments, whole cell lysates or immunoprecipitated samples were analyzed by immunoblotting assays using the indicated antibodies, with the molecular weight standards shown (kDa). The results are representatives of two biologically independent experiments. Uncropped blots are shown in Supplementary Figure 14.

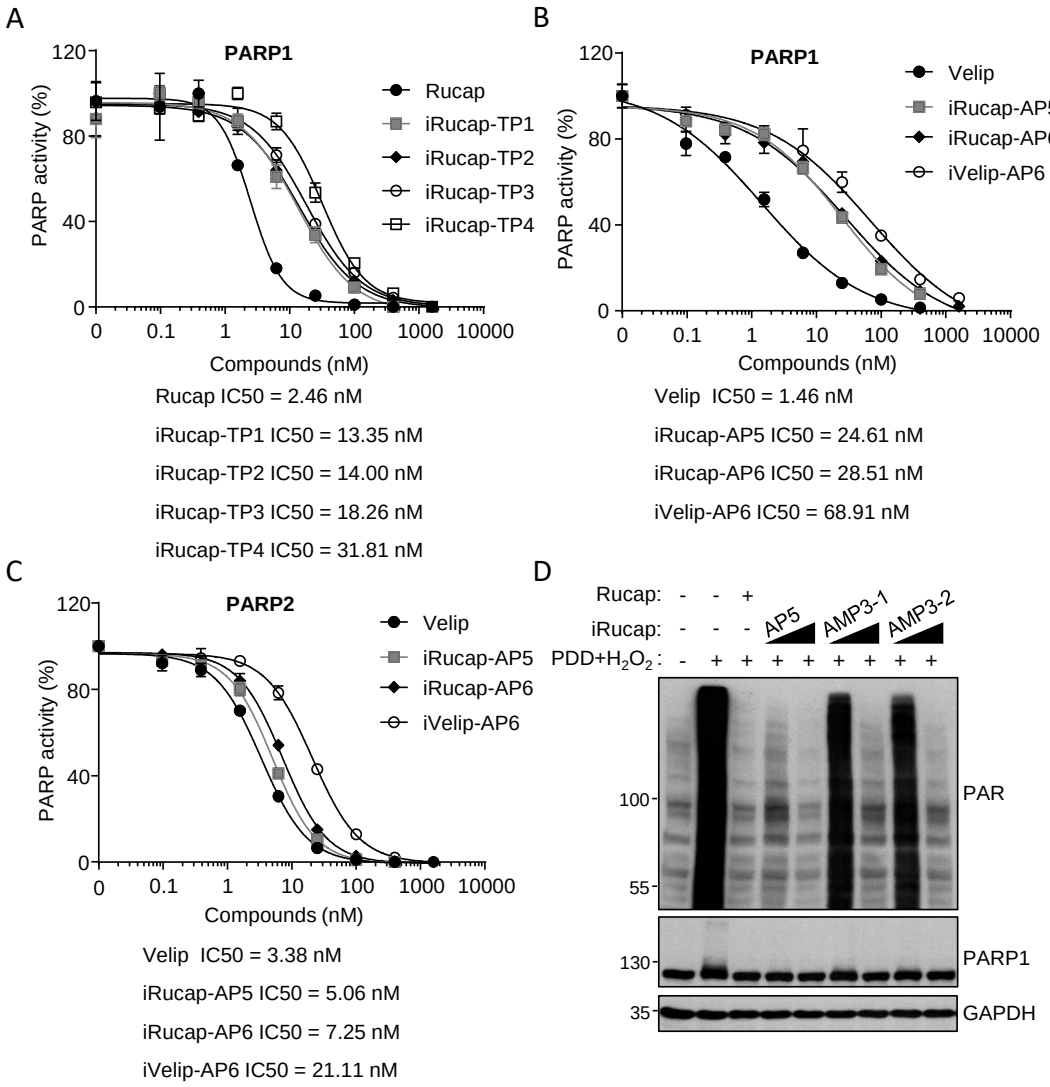


Supplementary Figure 6. iRucaparib and iRucaparib-TP3 selectively targets PARP1 for degradation. (continued on next page)

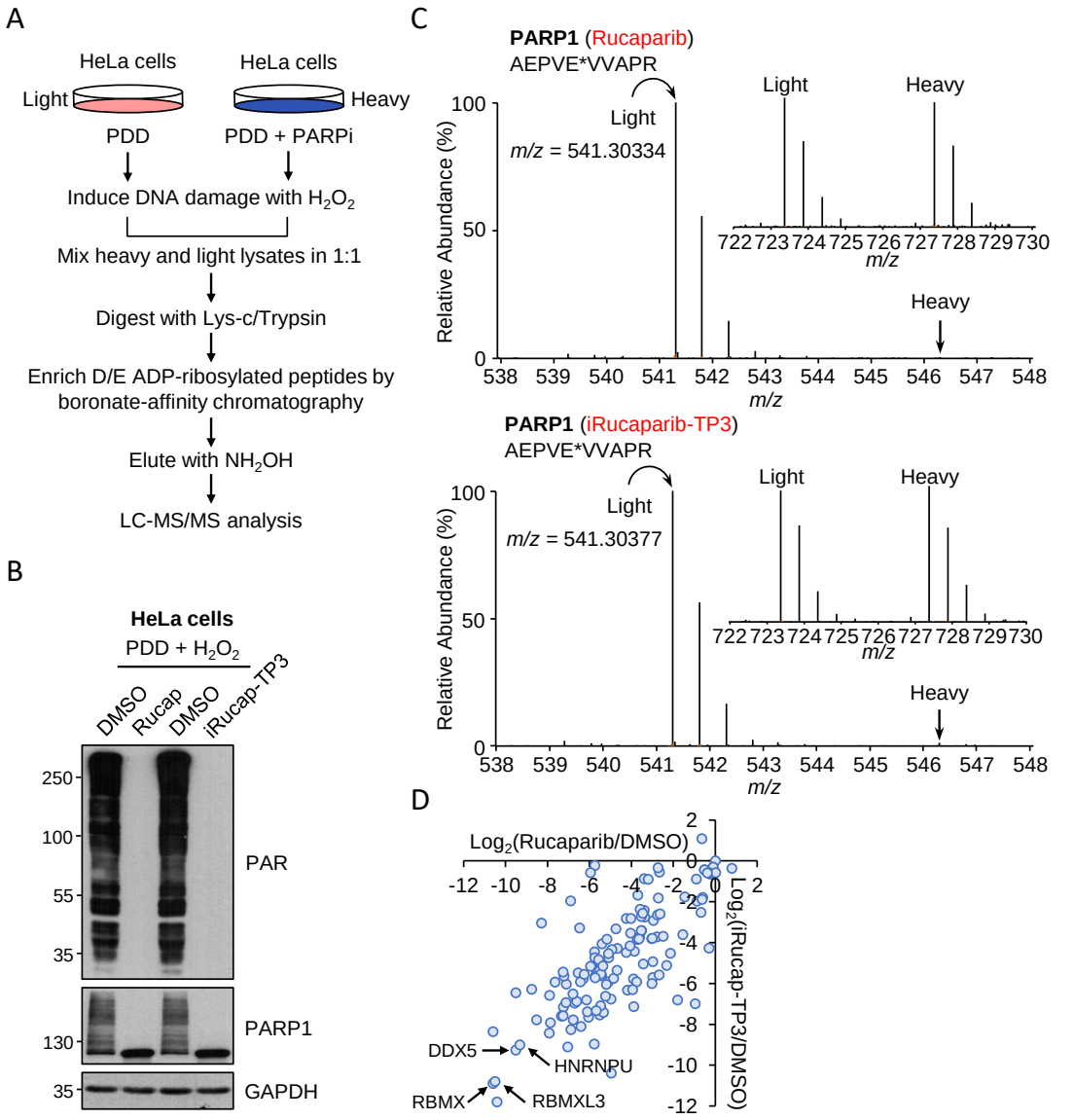


Supplementary Figure 6. iRucaparib and iRucaparib-TP3 selectively targets PARP1 for degradation. (continued from the previous page) (A) iRucaparib induces PARP1 degradation in primary cardiomyocytes. Cells were treated with iRucaparib-AP5 (1 μM) or iRucaparib (1 μM) for 24 hrs, and then were analyzed by immunoblotting assays using the indicated antibodies, with the molecular weight standards shown (kDa). Two biologically independent samples were included for each treatment condition. Uncropped blots are shown in Supplementary Figure 14. (B) a representative MS2 spectrum leading to the identification of PARP1. (C-D) Comparison of protein expression between (C) the iRucaparib-AP5 treatment vs. DMSO control or (D) the iRucaparib treatment vs. DMSO control. The S/N values of each protein in the two biological replicate samples were summed, and the ratio was log2-transformed. PARP1 and PARP3 are indicated by the corresponding arrows. (E)

iRucaparib-TP3 induces PARP1 degradation, which is rescued by Rucaparib treatment. HeLa cells were treated with iRucaparib-TP3 (5 μM) or iRucaparib-TP3 (5 μM) plus Rucaparib (1 μM) for 24 hrs, and then were analyzed by immunoblotting assays using the indicated antibodies, with the molecular weight standards shown (kDa). Two biologically independent samples were included for each treatment condition. Uncropped blots are shown in Supplementary Figure 14. (F-H) Reproducibility of the HeLa TMT experiments. The S/N values (signal-to-noise ratios) of the corresponding TMT channels for each protein were extracted and were Log10-transformed for (F) control group (DMSO), (G) iRucaparib-TP3 treatment group and (H) iRucaparib-TP3 plus Rucaparib treatment group. (I) Identification of a PARP1 peptide (VVSEDFLQDVSASTK). (J-L) Comparison of protein expression between (J) the iRucaparib-TP3 treatment vs. DMSO control, (K) the iRucaparib-TP3 treatment vs. iRucaparib-TP3+Rucaparib treatment or (L) the iRucaparib-TP3+Rucaparib treatment vs. DMSO control. The S/N values of each protein in the two biological replicate samples were summed, and the ratio was log2-transformed. PARP1 (red dot) and ZFP91 (black dot) are indicated by the corresponding arrows. (M) Heatmap presentation of the protein expression changes in the HeLa TMT experiment. All data was normalized to the first control sample, which was then log2-transformed. (N) The expression level of selected proteins as measured in the HeLa TMT experiments. Values represent mean ± SEM (n = 2 biological independent samples). (O) Log-Log plot comparing protein expression in iRucaparib-TP3 treatment vs. vRucaparib-TP4 treatment in BT-549 cells (n = 2 biological independent samples). The corresponding ratio (compared to DMSO) was log2-transformed. PARP1 (red dot) and ZFP91 (black dot) are indicated by the corresponding arrows.

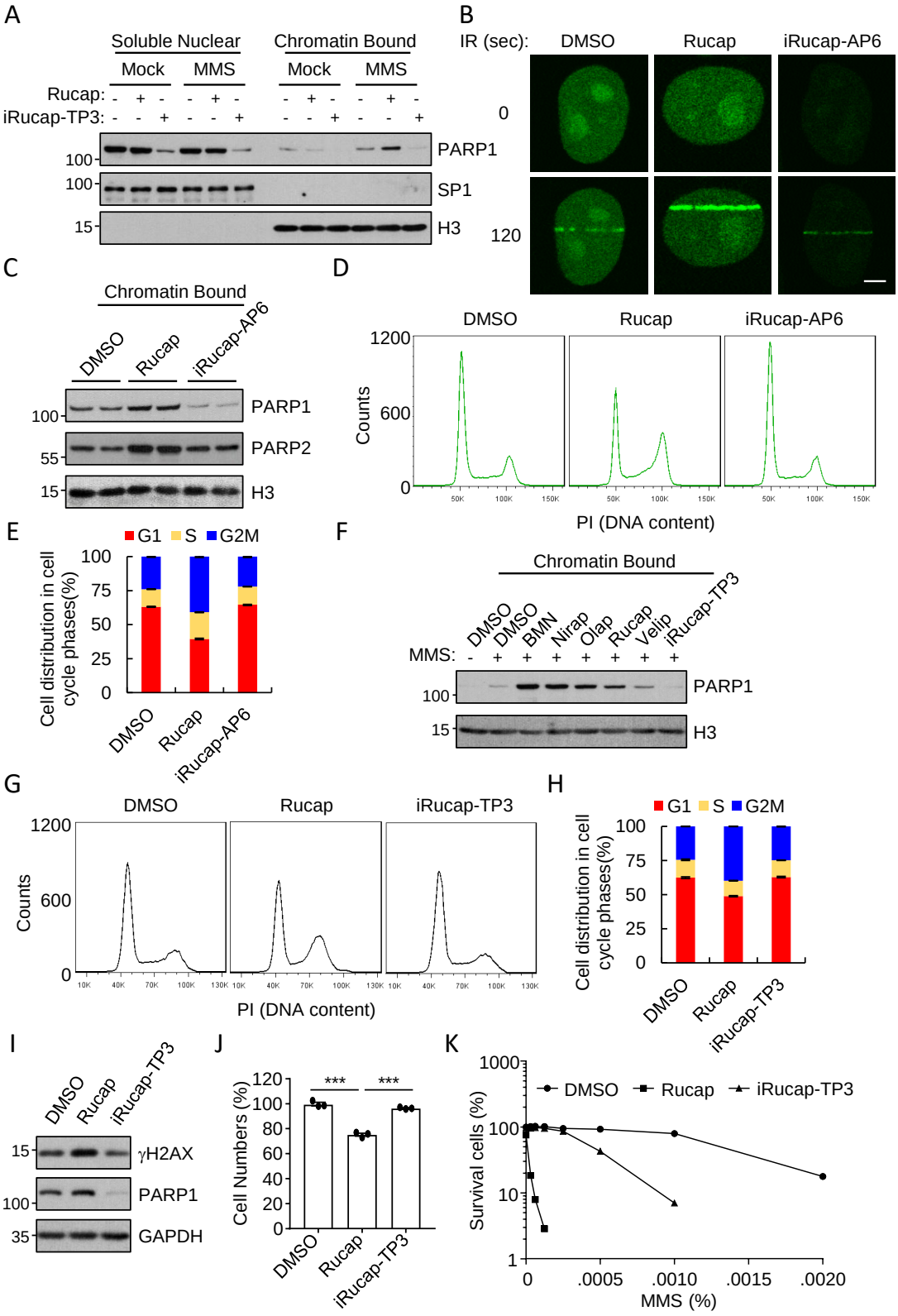


Supplementary Figure 7. The catalytic inhibitory activity of Rucaparib, Veliparib and the CRBN-based PARP degraders. (A) PARP1 IC50 for Rucaparib and iRucaparib-TP1-4. (B) PARP1 IC50 for Veliparib, iRucaparib-AP5/6 and iVeliparib-AP6. (C) PARP2 IC50 for Veliparib, iRucaparib-AP5/6 and iVeliparib-AP6. Values represent mean ± SEM (n = 3 biological independent samples). (D) Comparison of PAR signal inhibition by iRucaparib-AP5 and iRucaparib-AMP3s in HeLa cells. Cells were pretreated with a PARG inhibitor (PDD 00017273, 2 μM) for one hour and then treated with Rucaparib or PARP degraders (10 μM) as indicated for another hour. Cells were then challenged with H_2O_2 (2 mM) for 5 min, and whole cell lysates were analyzed by immunoblotting assays using the indicated antibodies, with the molecular weight standards shown (kDa). The result is a representative of two biologically independent experiments. Uncropped blots are shown in Supplementary Figure 14.



Supplementary Figure 8. iRucaparib-TP3 inhibits the ADP-ribosylation-mediated signaling events downstream of PARP1. (A) Quantitative analyses of the D/E-ADP-ribosylated proteome. Both SILAC-labeled HeLa cells were pretreated with a PARG inhibitor (PDD 00017273, 2 μM) for one hour. Light cells and heavy cells were then treated with DMSO and Rucaparib (or PARP1 degraders, 10 μM), respectively, for another hour. Both cells were then challenged with H_2O_2 (2 mM) for 5 min. Whole cells lysates were combined at a 1:1 ratio, and the PARylated peptides were enriched and analyzed by quantitative mass spectrometry. (B) Immunoblotting analysis of the PARylation level in HeLa cells treated with either Rucaparib or iRucaparib-TP3. Cells were treated as shown in (A), and whole cell lysates were analyzed by immunoblotting assays using the indicated antibodies, with the molecular weight standards shown (kDa). The result is a representative of two biologically independent

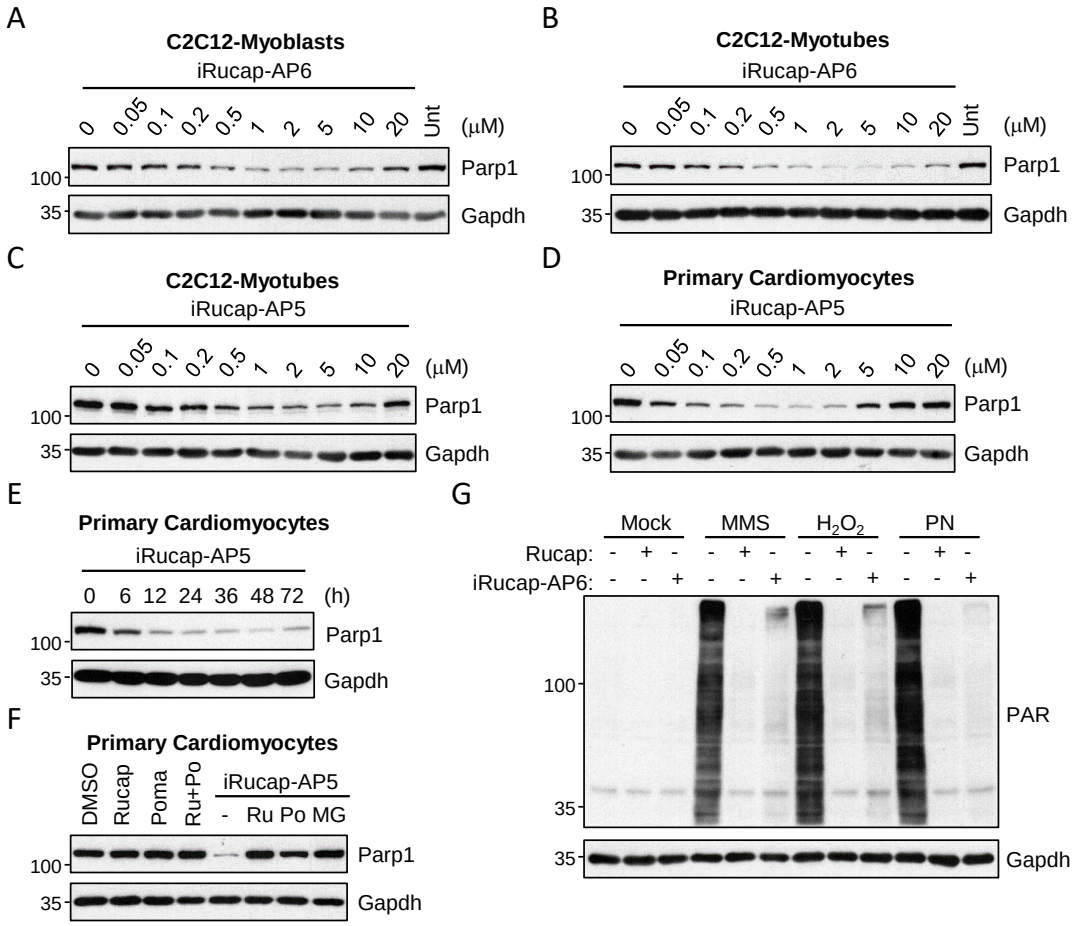
experiments. Uncropped blots are shown in Supplementary Figure 14. (C) Identification of a Rucaparib-sensitive (upper panel) and iRucaparib-TP3-sensitive (lower panel) PARP1 auto-modified peptide (AEPVE*VVAPR). The site of modification is indicated by an asterisk. The inset shows a ~1:1 ratio (heavy/light) of a non-PARylated peptide (HQSFVLVGETGSGK) from DHX15. The upper panel shows the peptides extracted from the Rucaparib-SILAC experiment, and the lower panel shows the peptides extracted from the iRucaparib-TP3-SILAC experiment. (D) Correlation analysis for the ADP-ribosylated peptides identified in the Rucaparib and iRucaparib-TP3 SILAC experiments. Log_2(compound/control) values are shown (median values if identified multiple times).



Supplementary Figure 9. iRucaparib does not cause PARP1 trapping. (A) PARP1 trapping in HeLa cells treated with Rucaparib or iRucaparib-TP3. Cells were pretreated with Rucaparib or iRucaparib-TP3 (5 μM) for 24 hrs followed by a 2-hour MMS (0.01%) treatment. Nuclear soluble and chromatin-bound proteins were extracted and analyzed. The result is a representative of three

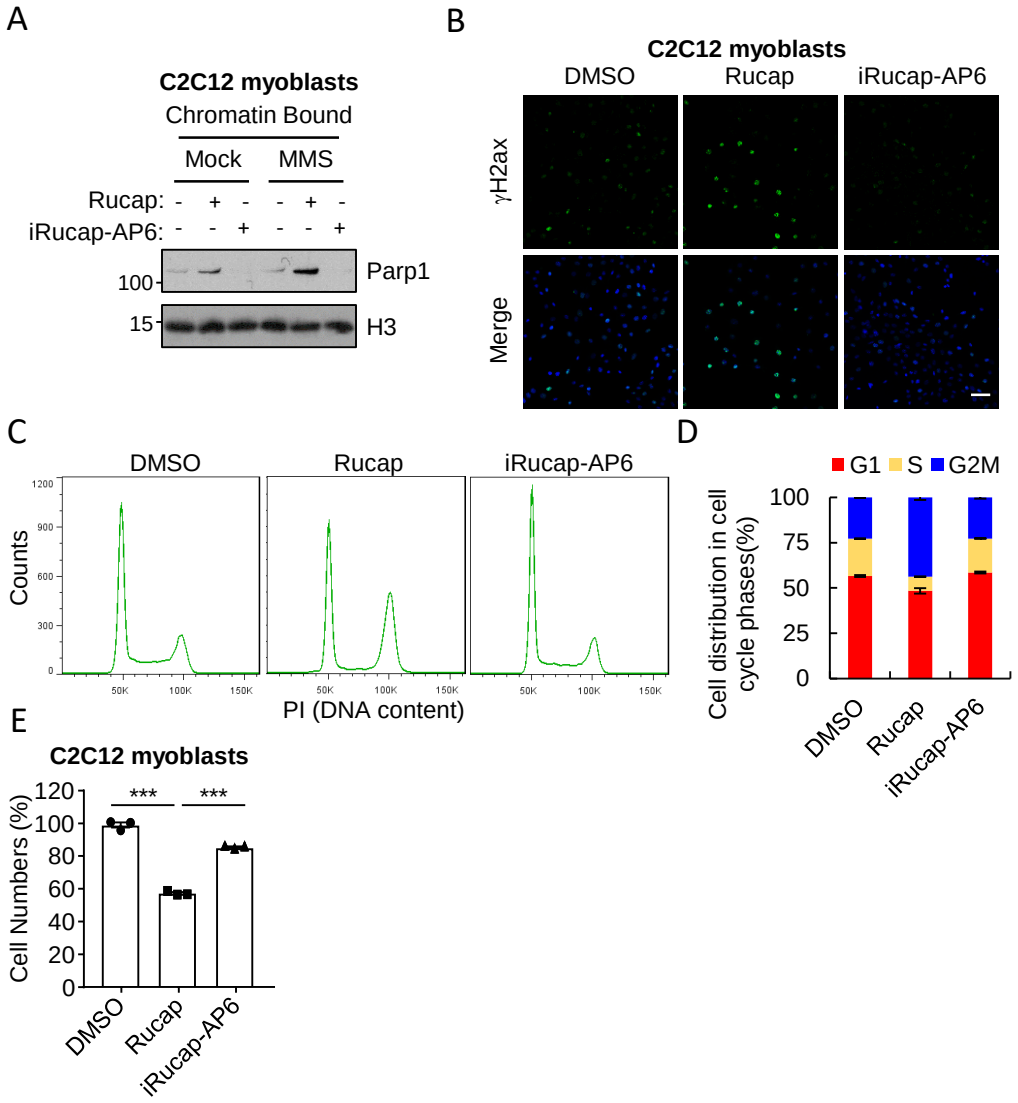
biologically independent experiments. (B) Chromatin accumulation of PARP1 upon laser microirradiation. HeLa cells were transfected with a PARP1-GFP plasmid for 24 hrs, and were treated with Rucaparib or iRucaparib (1 μM) for another 24 hrs. Laser microirradiation was then performed and GFP signals were recorded at the indicated time points. The result is a representative of two biologically independent experiments. Scale bar = 5 μm. (C) PARP1 trapping in HeLa cells treated with Rucaparib or iRucaparib. Cells were pretreated with Rucaparib or iRucaparib (1 μM) for 24 hrs followed by a 2-hour MMS (0.01%) treatment. Samples were subject to TMT-based quantitative proteomic analyses as shown in Figure 4B. Two biologically independent samples were included for each treatment condition. (D) Cell cycle analysis of HeLa cells after Rucaparib or iRucaparib treatment. HeLa cells were treated with Rucaparib or iRucaparib (both at 10 μM) for 48 hrs, and then were analyzed by flow cytometry. The left and right peaks indicate G1 and G2/M populations, respectively, with the corresponding quantification results shown in (E). Values represent mean ± SEM (n = 3 biological independent samples). (F) Comparison of PARP1 trapping by BMN673, Niraparib, Olaparib, Rucaparib, Veliparib and iRucaparib-TP3 in HeLa cells. The assay was performed as in (E) with a concentration of 5 μM for all compounds. The result is a representative of two biologically independent experiments. (G) Cell cycle analysis of HeLa cells after Rucaparib or iRucaparib-TP3 treatment. HeLa cells were treated with Rucaparib or iRucaparib-TP3 (10 μM) for 48 hrs, and then were analyzed by flow cytometry. The left and right peaks indicate G1 and G2/M populations, respectively, with the corresponding quantification results shown in (H). Values represent mean ± SEM (n = 3 biological independent samples). (I) γH2A.X levels in HeLa cells after 48-hour treatment of Rucaparib or iRucaparib-TP3 (both at 10 μM). The result is a representative of two biologically independent experiments. (J) Cell proliferation analyses of HeLa cells after the treatment of Rucaparib or iRucaparib-TP3. Cells were treated with Rucaparib or iRucaparib-TP3 (both at 10 μM) for 72 hrs. Values represent mean ± SEM (n = 3 biological independent samples). Statistical significance was calculated with unpaired two-tailed Student's t-tests comparing Rucaparib treatment to DMSO or iRucaparib treatment, ***$p < 0.001$. p values were 2.0×10^{-4} and 8.0×10^{-5}. (K) Survival curve of HeLa cells treated with MMS alone or in combination with Rucaparib or iRucaparib-TP3 (10 μM). Values represent mean ± SEM (n = 3 biological independent samples). In these experiments, whole cell lysates or nuclear soluble and chromatin-bound proteins were analyzed by immunoblotting assays using the

indicated antibodies, with the molecular weight standards shown (kDa). Uncropped blots are shown in Supplementary Figure 14.



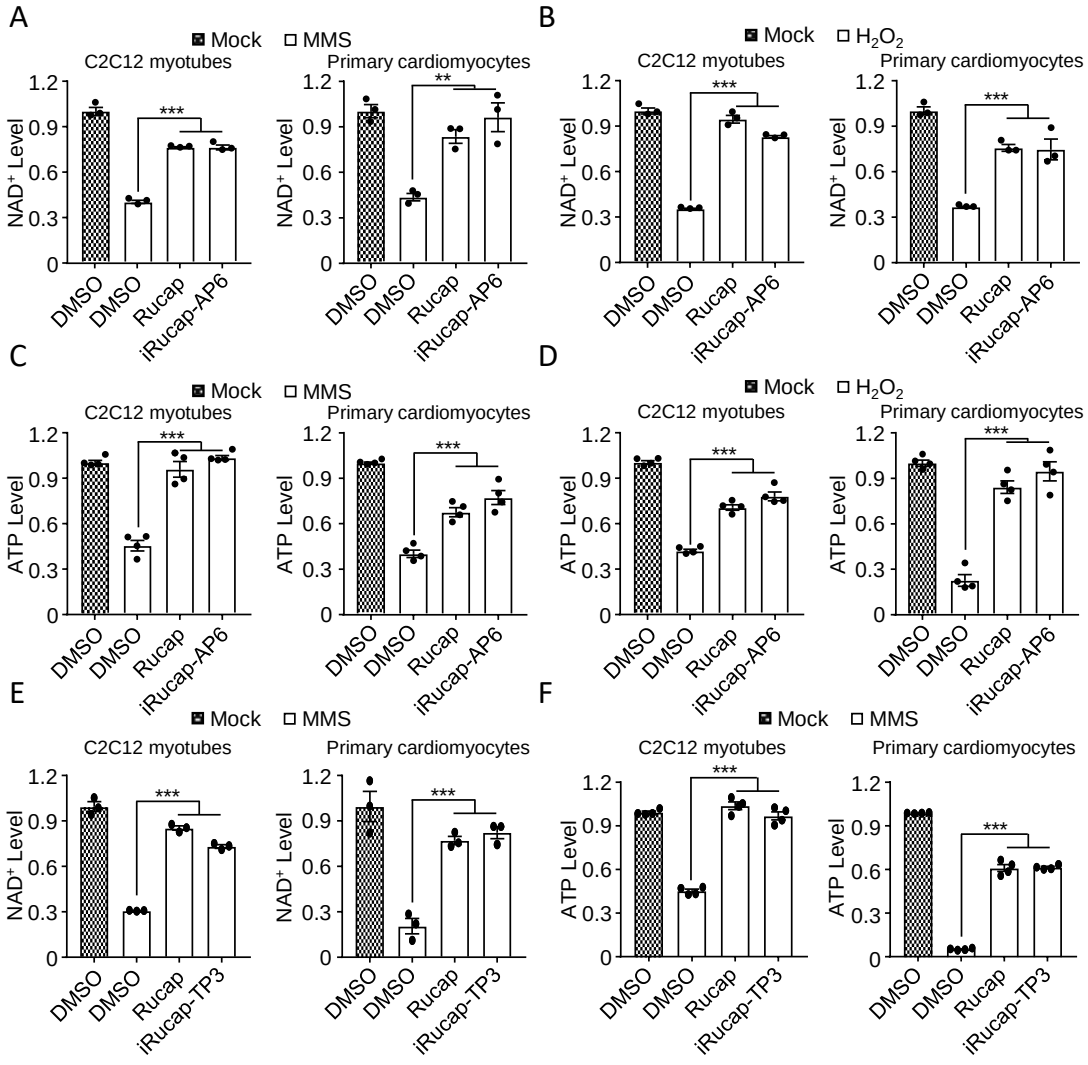
Supplementary Figure 10. iRucaparib induces PARP1 degradation in muscle cells. (A-B) iRucaparib induces PARP1 degradation in (A) C2C12 myoblasts and (B) C2C12 myotubes. Cells were treated with increasing concentrations of iRucaparib for 24 hrs. (C) iRucaparib-AP5 induces PARP1 degradation in C2C12 myotubes. Cells were treated with increasing concentrations of iRucaparib-AP5 for 24 hrs. (D-F) iRucaparib-AP5 induces PARP1 degradation in primary cardiomyocytes. (D) Cells were treated with increasing concentrations of iRucaparib-AP5 for 24 hrs. (E) Cells were treated with iRucaparib-AP5 (1 μM) for the indicated times. (F) Cells were treated with Rucaparib (1 μM), pomalidomide (1 μM), Rucaparib (1 μM) plus pomalidomide (1 μM), iRucaparib-AP5 (1 μM), or iRucaparib-AP5 (1 μM) plus Rucaparib (1 μM) or Pomalidomide (10 μM) or MG132 (1 μM) for 24 hrs. Rucap/Ru, Rucaparib; Poma/Po, Pomalidomide; MG, MG-132. (G) iRucaparib blocks genotoxicity-induced PARylation. C2C12 myotubes were pretreated with Rucaparib or iRucaparib (1 μM) for 24 hrs, and were treated with the PARG inhibitor PDD 00017273 (2 μM) for 1 hour. The cells were then treated with MMS (0.01%) for 1 hour, H_2O_2 (2 mM) for 5 min or peroxynitrite (500 μM) for 30 min. The results are representatives of two biologically independent experiments. In these

experiments, whole cell lysates were analyzed by immunoblotting assays using the indicated antibodies, with the molecular weight standards shown (kDa). The results are representatives of two biologically independent experiments. Uncropped blots are shown in Supplementary Figure 14.



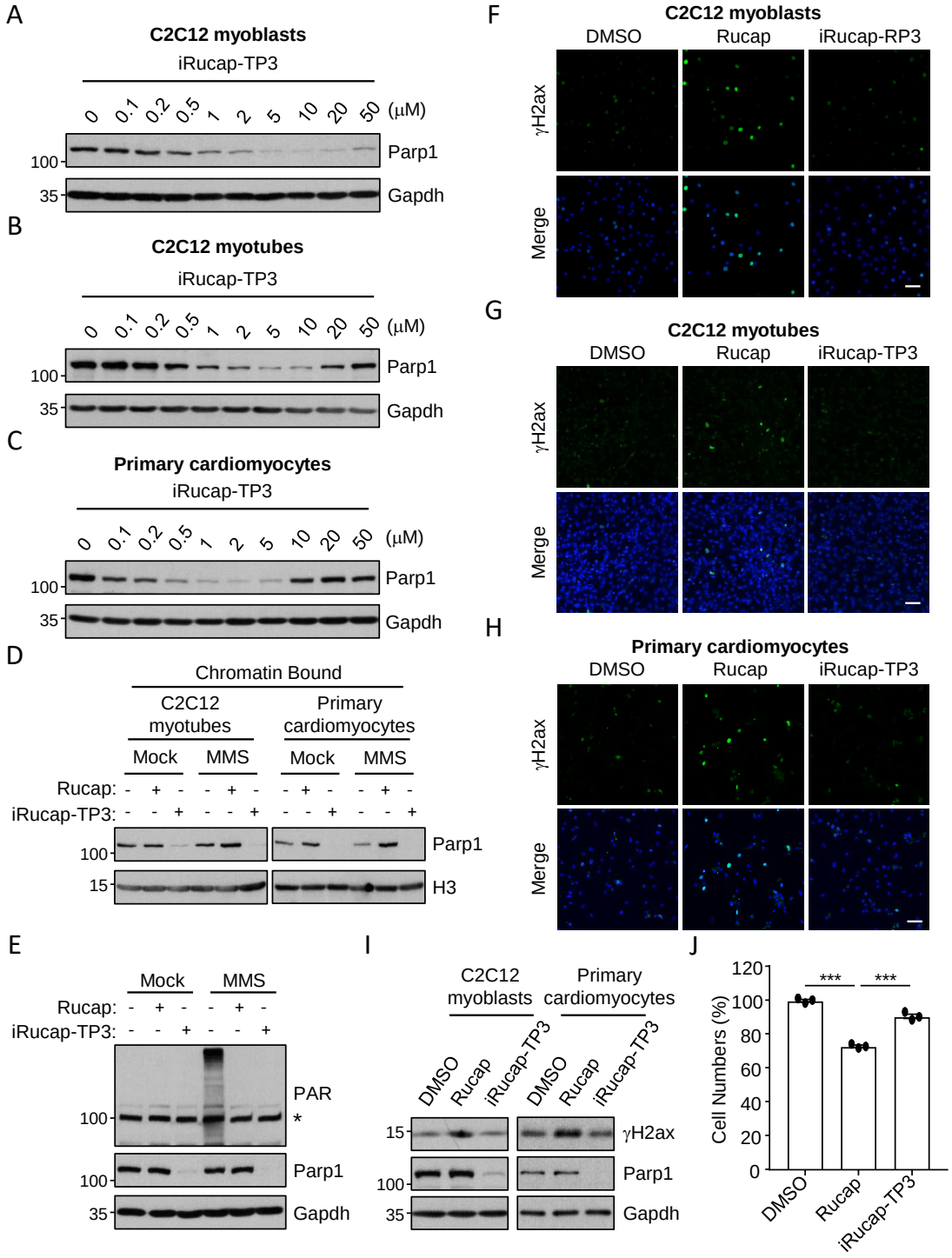
Supplementary Figure 11. iRucaparib protects cells from genotoxicity-induced cell death. (A) PARP1 trapping as a result of the treatment of Rucaparib and iRucaparib in C2C12 myoblasts. Cells were pretreated with Rucaparib or iRucaparib (1 μM) for 24 hrs, which was followed by a 2-hour treatment of MMS (0.01%). Chromatin-bound proteins were extracted and analyzed by immunoblotting assays using the indicated antibodies, with the molecular weight standards shown (kDa). The result is a representative of two biologically independent experiments. Uncropped blots are shown in Supplementary Figure 14. (B) iRucaparib treatment does not induce DNA damage response in cells. γH2A.X immunofluorescence levels in C2C12 myoblasts after the treatment with Rucaparib or iRucaparib (10 μM for 48 hrs). The result is a representative of two biologically independent experiments. Scale bar = 50 μm. (C) Cell cycle analysis of C2C12 myoblasts after Rucaparib or iRucaparib treatment. Cells were treated with Rucaparib or iRucaparib (10 μM) for 48 hrs, and then

were analyzed by flow cytometry. The left and right peaks indicate G1 and G2/M populations, respectively, with the corresponding quantification results shown in (D). Values represent mean ± SEM (n = 3 biological independent samples). (E) Cell growth analysis of C2C12 myoblasts treated with Rucaparib or iRucaparib. Cells were treated with Rucaparib or iRucaparib (10 μM) for 72 hrs. Values represent mean ± SEM (n = 3 biological independent samples). Statistical significance was calculated with unpaired two-tailed Student's t-tests comparing Rucaparib treatment to DMSO or iRucaparib treatment, ***$p < 0.001$. p values were 1.8×10^{-5} and 5.9×10^{-6}.



Supplementary Figure 12. iRucaparib protects cells from genotoxicity-induced cell death. (A-B) iRucaparib protects cells from genotoxicity-induced NAD^+ depletion. Cells were pretreated with Rucaparib or iRucaparib (1 μM) for 24 hrs, and then challenged with (A) MMS (0.01%) for 4 hrs or (B) H_2O_2 (2 mM) for 1 hour (C2C12 myotubes, left panel) or 30 min (Primary cardiomyocytes, right panel). NAD^+ levels were then determined, values represent mean ± SEM (n = 3 biological independent samples). Statistical significance was calculated with one-way ANOVA comparing MMS-treated or H_2O_2-trated DMSO group to Rucaparib and iRucaparib groups, $^{**}p < 0.01$, $^{***}p < 0.001$. p values were 7.6×10^{-7}, 0.0024, 3.3×10^{-7} and 8.9×10^{-4}. (C-D) iRucaparib protects cells from genotoxicity-induced ATP depletion. Cells were pretreated with Rucaparib or iRucaparib (1 μM) for 24 hrs, and then challenged with (C) MMS (0.01%) for 9 hrs or (D) H_2O_2 for 6 hrs (2 mM for C2C12 myotubes, left panel) or 3 hrs (50 μM for Primary cardiomyocytes, right panel). ATP levels were measured by CellTiter-Glo, values represent mean ± SEM (n = 4 biological independent samples).

Statistical significance was calculated with one-way ANOVA comparing MMS-treated or H_2O_2-trated DMSO group to Rucaparib and iRucaparib groups, ***$p < 0.001$. p values were 2.8×10^{-6}, 1.0×10^{-4}, 1.8×10^{-6} and 4.5×10^{-6}. (E) iRucaparib-TP3 protects cells from MMS-induced NAD^+ depletion. Cells were pretreated with Rucaparib or iRucaparib-TP3 (5 μM) for 24 hrs, which was followed by a 4-hour MMS (0.01%) treatment. The left and right panel shows the results for C2C12 myotubes and primary rat cardiomyocytes, respectively. Values represent mean ± SEM (n = 3 biological independent samples). Statistical significance was calculated with one-way ANOVA comparing MMS-treated DMSO group to Rucaparib and iRucaparib groups, ***$p < 0.001$. p values were 6.6×10^{-8} and 6.6×10^{-5}. (F) iRucaparib-TP3 protects cells from MMS-induced ATP depletion. Cells were pretreated with Rucaparib or iRucaparib-TP3 (5 μM) for 24 hrs, which was followed by a 9-hour MMS (0.01%) treatment. The left and right panel shows the results for C2C12 myotubes and primary rat cardiomyocytes, respectively. Values represent mean ± SEM (n = 4 biological independent samples). Statistical significance was calculated with one-way ANOVA comparing MMS-treated DMSO group to Rucaparib and iRucaparib groups, ***$p < 0.001$. p values were 4.4×10^{-8} and 7.0×10^{-10}.



Supplementary Figure 13. iRucaparib prevents PARP1 trapping-induced cell toxicity. (A-C) iRucaparib-TP3 induces PARP1 degradation in (A) C2C12 myoblasts, (B) C2C12 myotubes and (C) Primary rat cardiomyocytes. Cells were treated with increasing concentrations of iRucaparib-TP3 for 24 hrs. (D) PARP1 trapping as a result of the treatment of Rucaparib and iRucaparib-TP3 in C2C12 myotubes and primary rat cardiomyocytes. Cells were pretreated with Rucaparib or iRucaparib-TP3 (5 μM) for 24 hrs, which was followed by a 2-hour treatment of MMS (0.01%). Chromatin-bound

proteins were extracted and analyzed. (E) iRucaparib-TP3 blocks PARP1-induced PARylation. C2C12 myotubes were pretreated with Rucaparib or iRucaparib-TP3 (5 μM) for 24 hrs, and then were treated with the PARG inhibitor PDD 00017273 (2 μM) for one hour. The cells were treated with MMS (0.01%) for another hour. The asterisk indicates a non-specific band. (F-H) iRucaparib-TP3 treatment does not induce DNA damage response in cells. γH2A.X immunofluorescence levels in (F) C2C12 myoblasts, (G) C2C12 myotubes and (H) primary rat cardiomyocytes after the treatment with Rucaparib or iRucaparib-TP3 (10 μM for 48 hrs). Scale bar = 50 μm. (I) DNA damage response as a result of the treatment of Rucaparib or iRucaparib-TP3 in C2C12 myoblasts and primary rat cardiomyocytes. Cells were treated with Rucaparib or iRucaparib-TP3 (10 μM) for 48 hrs. (G) Cell growth analysis of C2C12 myoblasts treated with Rucaparib or iRucaparib-TP3. Cells were treated with Rucaparib or iRucaparib-TP3 (5 μM) for 72 hrs. Values represent mean ± SEM (n = 3 biological independent samples). Statistical significance was calculated with unpaired two-tailed Student's t-tests comparing Rucaparib treatment to DMSO or iRucaparib treatment, ***$p < 0.001$. p values were 1.4×10^{-5} and 3.1×10^{-4}. In these experiments, whole cell lysates or chromatin-bound proteins were analyzed by immunoblotting assays using the indicated antibodies, with the molecular weight standards shown (kDa). The results are representatives of two biologically independent experiments. Uncropped blots are shown in Supplementary Figure 14.

Fig. 2a

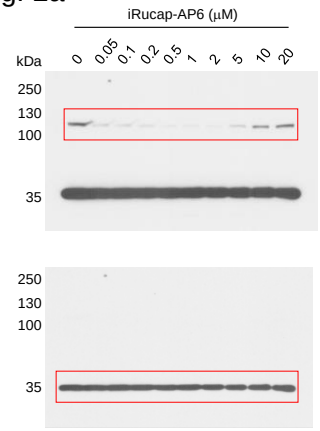


Fig. 2b

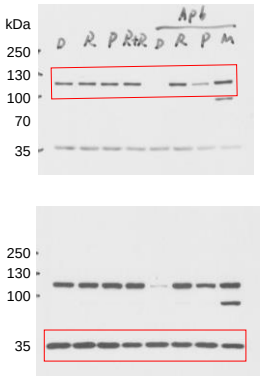


Fig. 2c

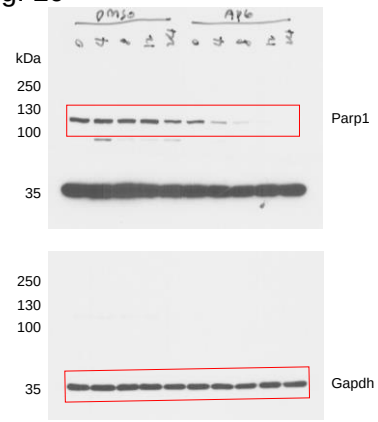
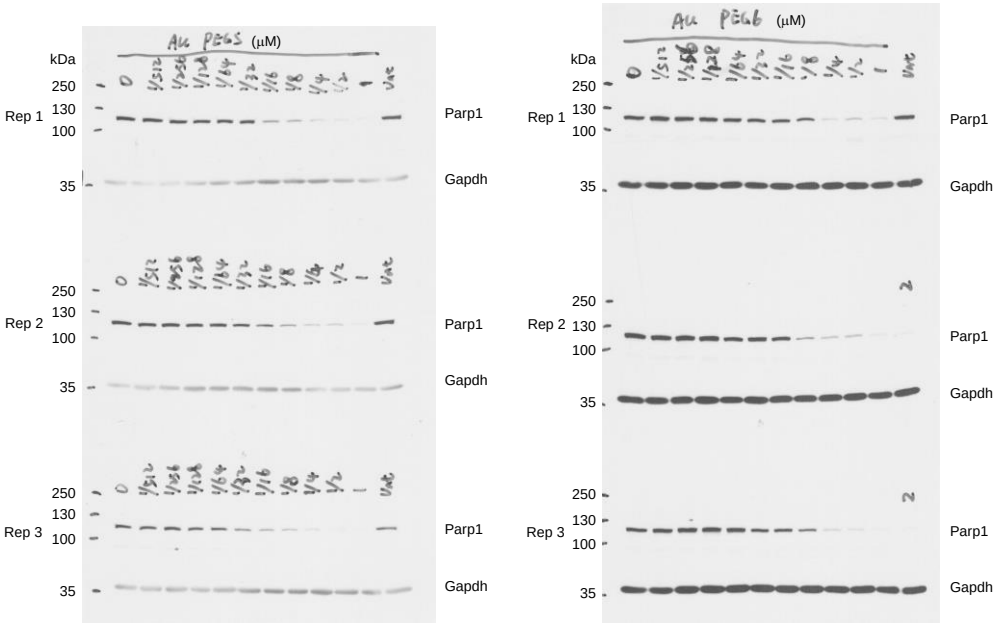


Fig. 2d



Supplementary Figure 14. Uncropped immunoblots related to the indicated figures. (continued on next page)

Fig. 4a

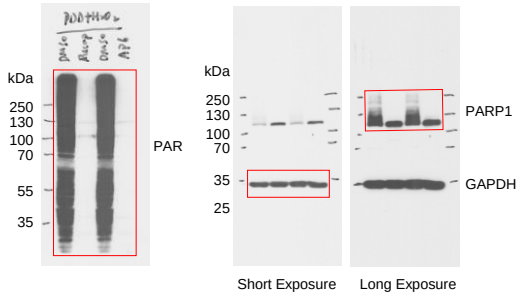


Fig. 5a

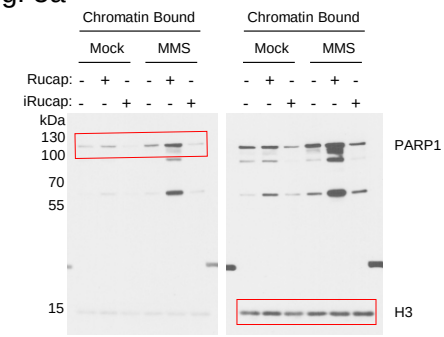


Fig. 6a

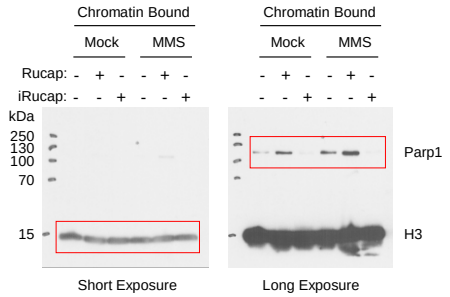
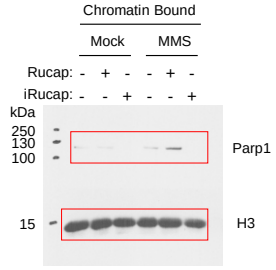
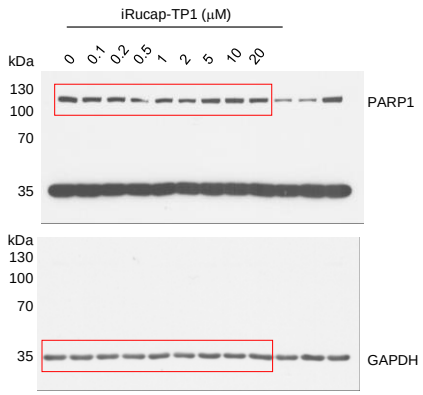


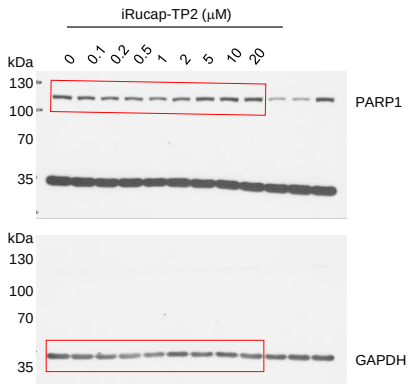
Fig. 6b



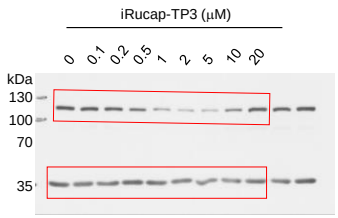
Supplementary Fig. 1c



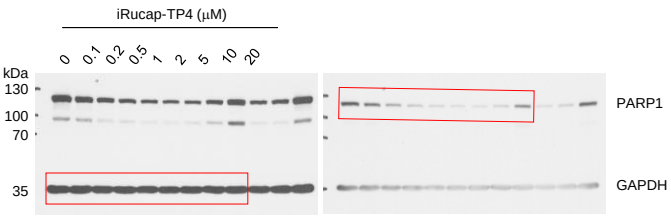
Supplementary Fig. 1d



Supplementary Fig. 1b

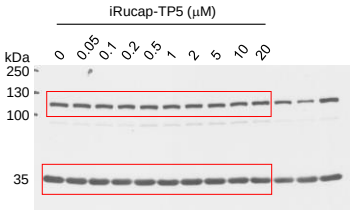


Supplementary Fig. 1e

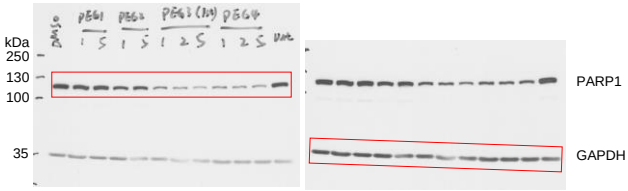


Supplementary Figure 14. Uncropped immunoblots related to the indicated figures. (continued from previous)

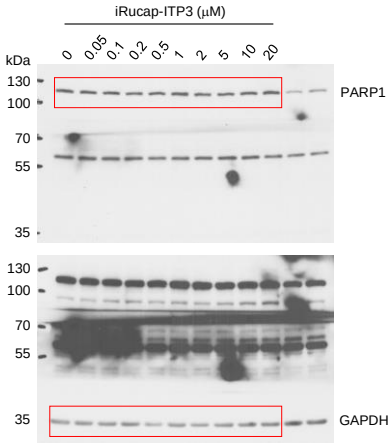
Supplementary Fig. 1f



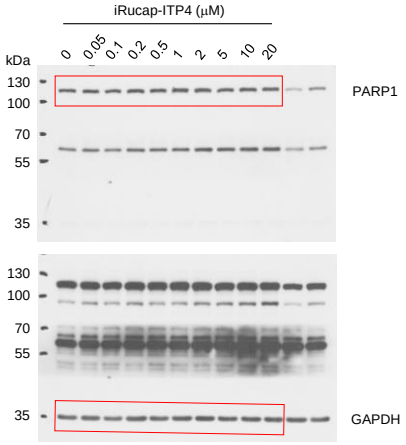
Supplementary Fig. 1g



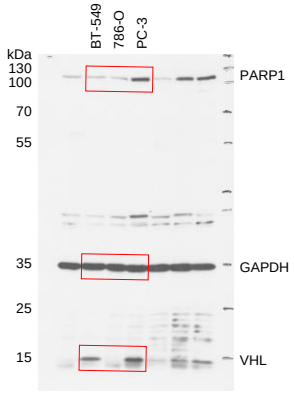
Supplementary Fig. 2a



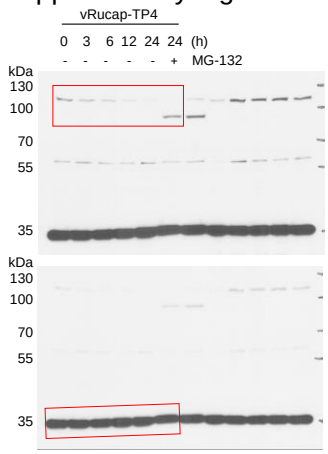
Supplementary Fig. 2b



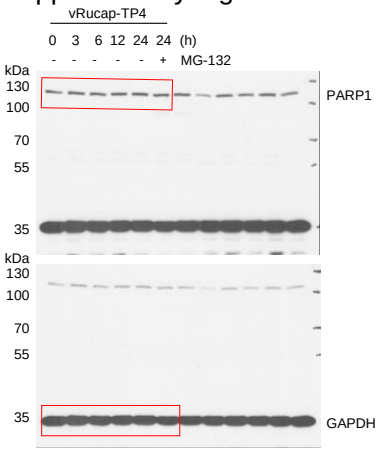
Supplementary Fig. 2c



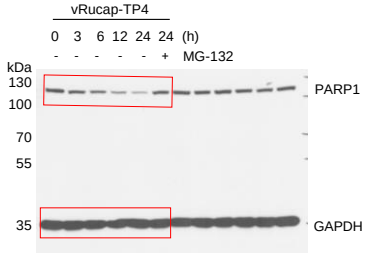
Supplementary Fig. 2d



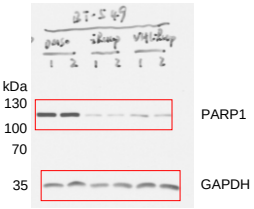
Supplementary Fig. 2e



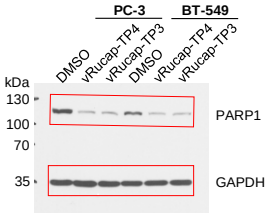
Supplementary Fig. 2f



Supplementary Fig. 2g

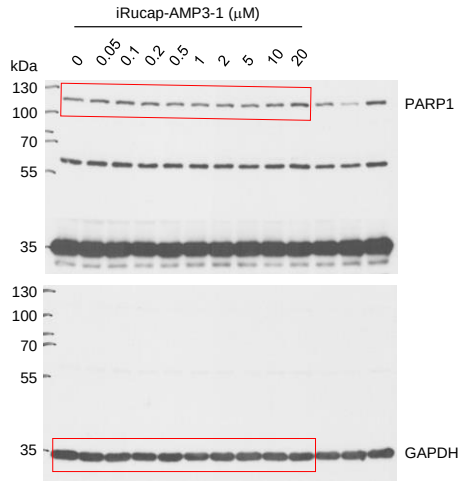


Supplementary Fig. 2h

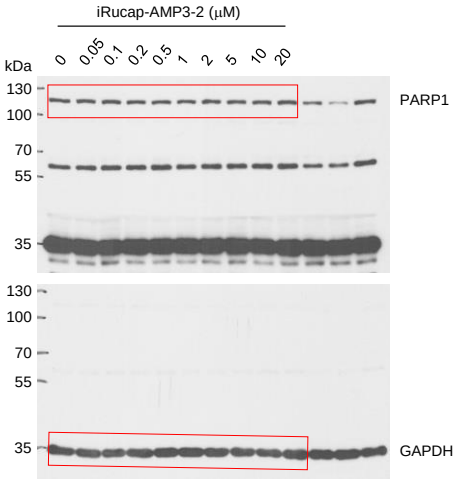


Supplementary Figure 14. Uncropped immunoblots related to the indicated figures. (continued from previous)

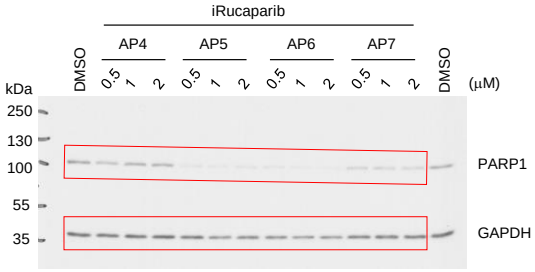
Supplementary Fig. 2i



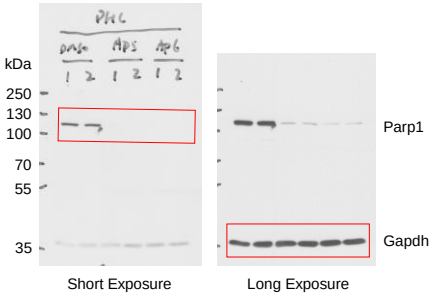
Supplementary Fig. 2j



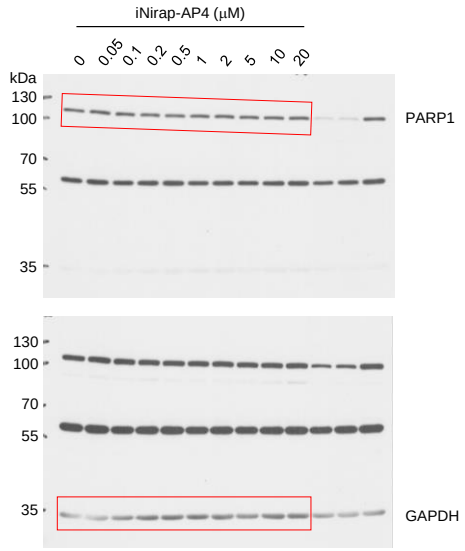
Supplementary Fig. 2k



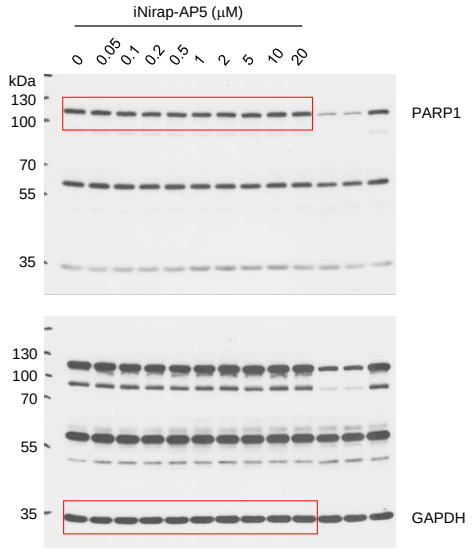
Supplementary Fig. 6a



Supplementary Fig. 3a

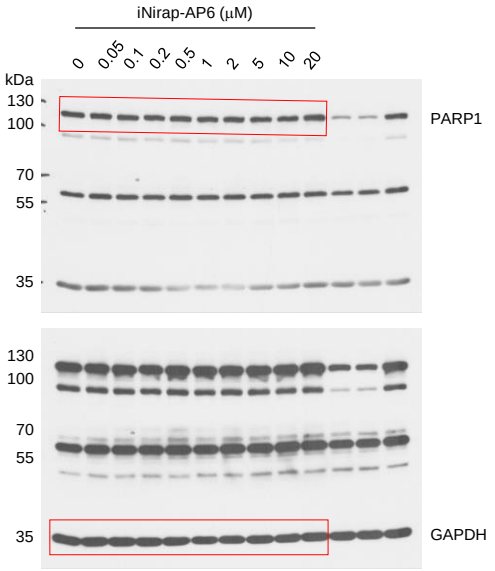


Supplementary Fig. 3b

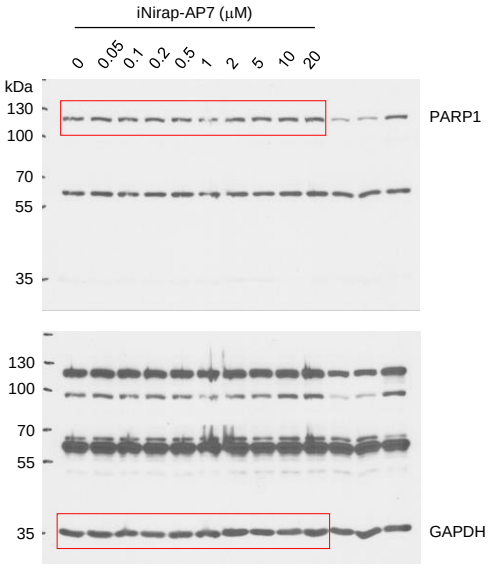


Supplementary Figure 14. Uncropped immunoblots related to the indicated figures. (continued from previous)

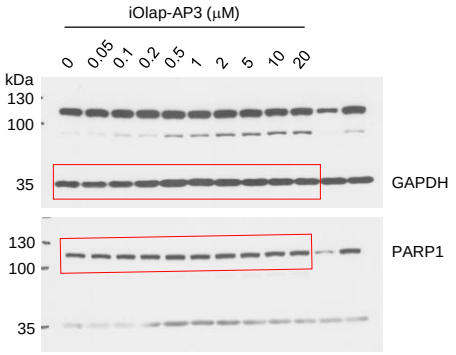
Supplementary Fig. 3c



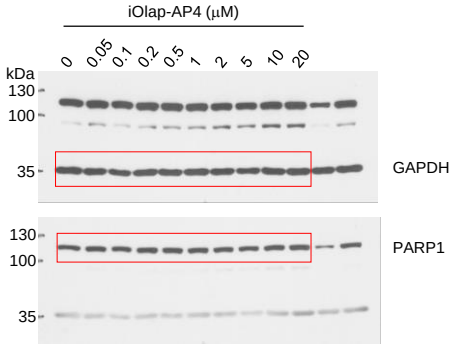
Supplementary Fig. 3d



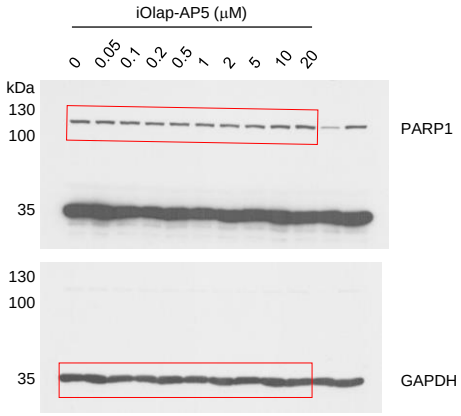
Supplementary Fig. 3e



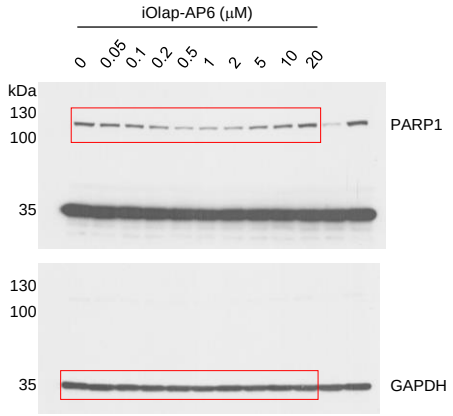
Supplementary Fig. 3f



Supplementary Fig. 3g

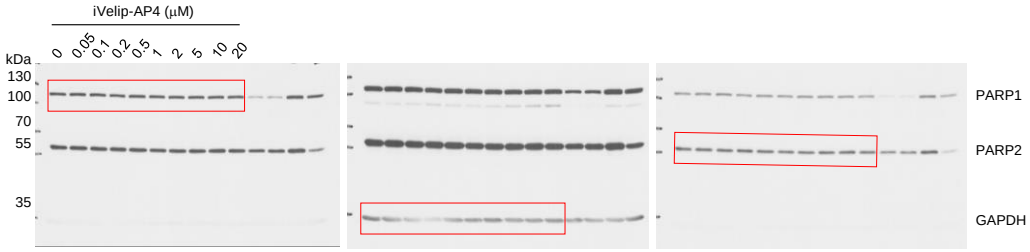


Supplementary Fig. 3h

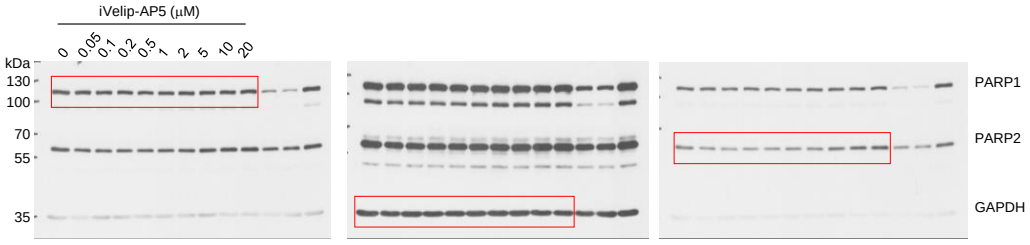


Supplementary Figure 14. Uncropped immunoblots related to the indicated figures. (continued from previous)

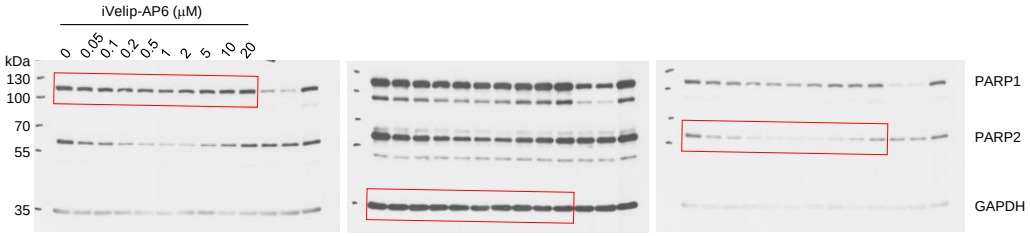
Supplementary Fig. 3i



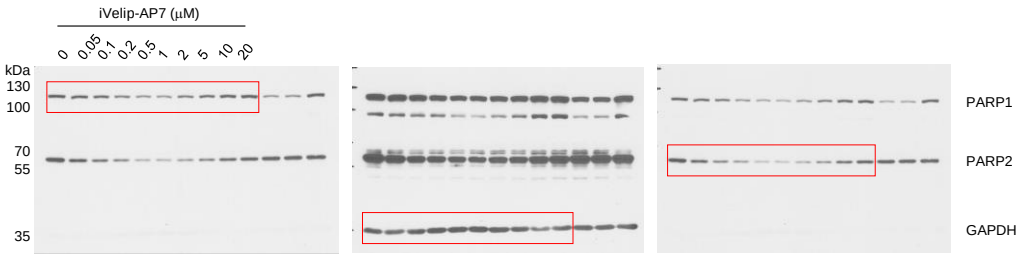
Supplementary Fig. 3j



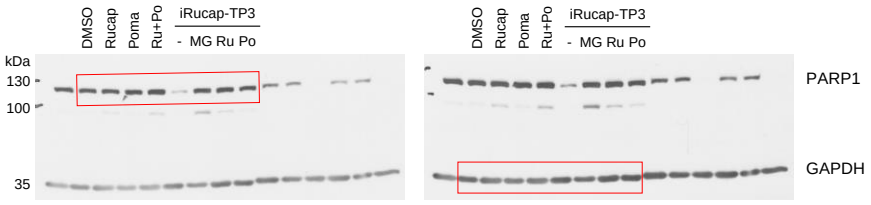
Supplementary Fig. 3k



Supplementary Fig. 3l

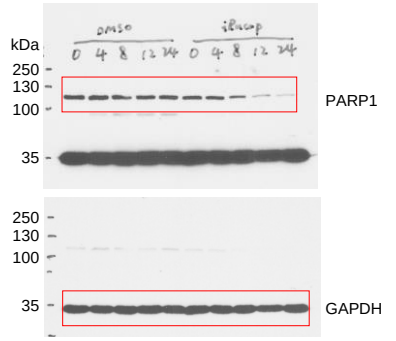


Supplementary Fig. 4a

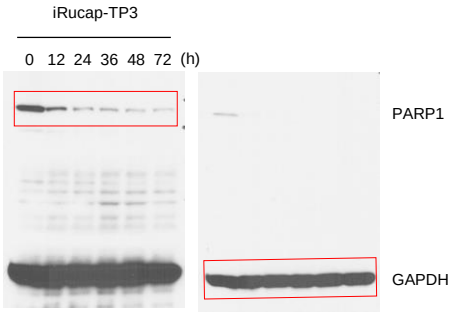


Supplementary Figure 14. Uncropped immunoblots related to the indicated figures. (continued from previous)

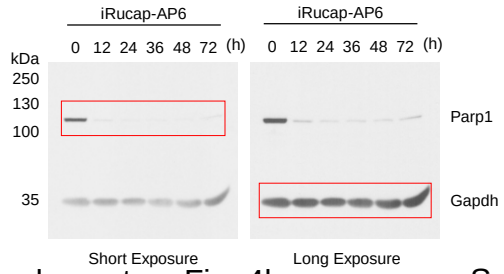
Supplementary Fig. 4b



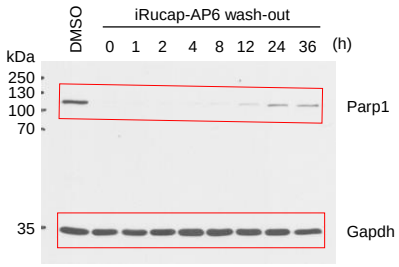
Supplementary Fig. 4g



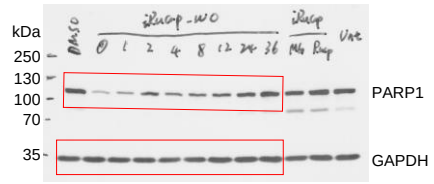
Supplementary Fig. 4e



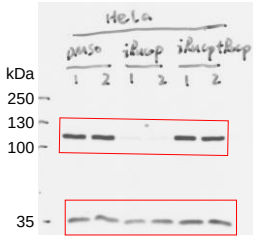
Supplementary Fig. 4f



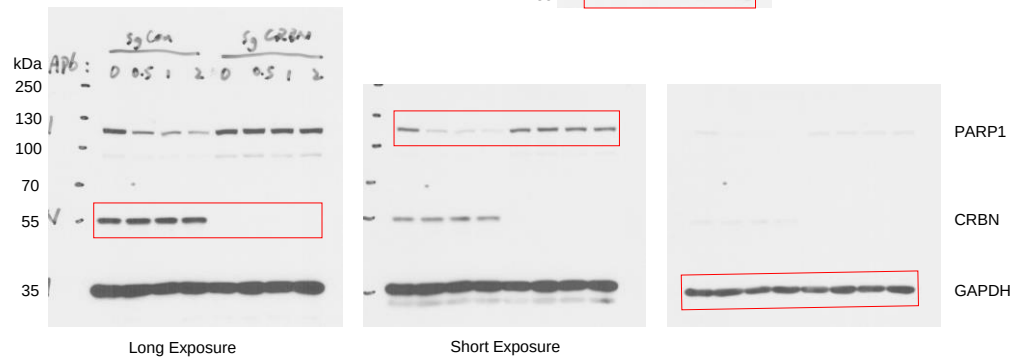
Supplementary Fig. 4h



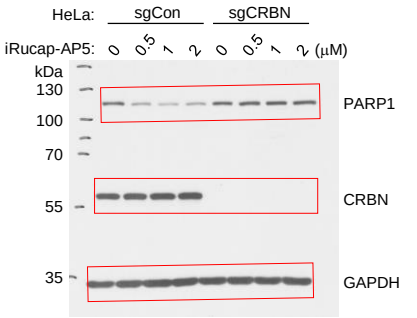
Supplementary Fig. 6e



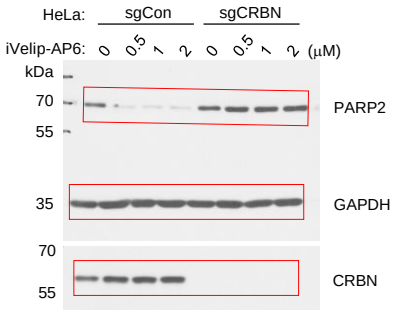
Supplementary Fig. 5a



Supplementary Fig. 5b

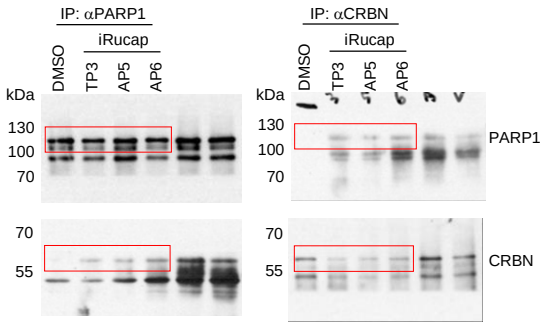


Supplementary Fig. 5c

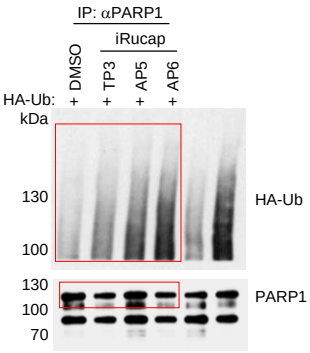


Supplementary Figure 14. Uncropped immunoblots related to the indicated figures. (continued from previous)

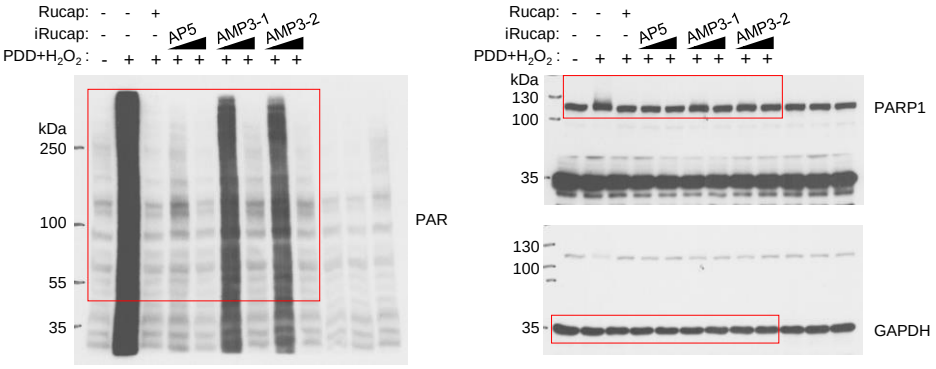
Supplementary Fig. 5d



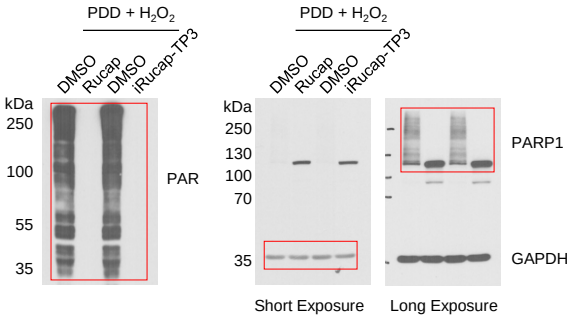
Supplementary Fig. 5e



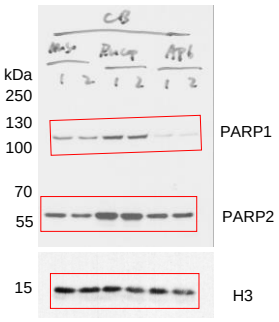
Supplementary Fig. 7d



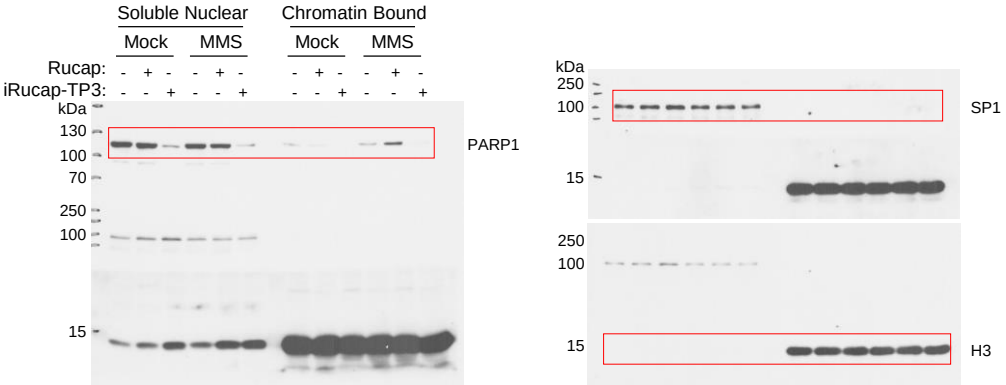
Supplementary Fig. 8b



Supplementary Fig. 9c

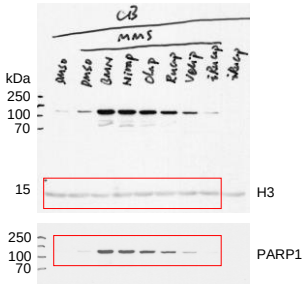


Supplementary Fig. 9a

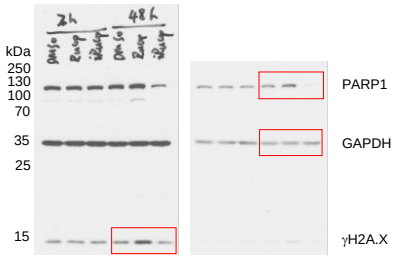


Supplementary Figure 14. Uncropped immunoblots related to the indicated figures. (continued from previous)

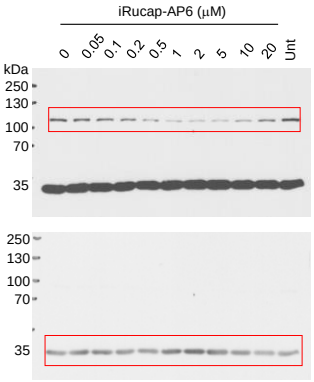
Supplementary Fig. 9f



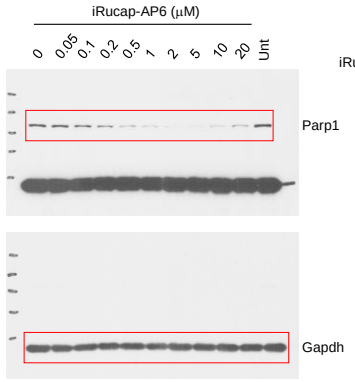
Supplementary Fig. 9i



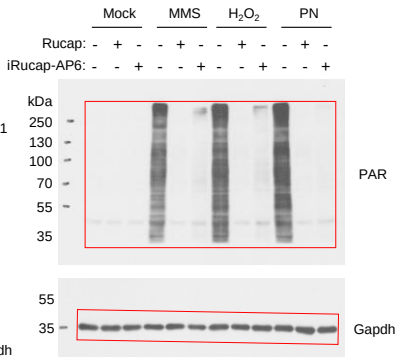
Supplementary Fig. 10a



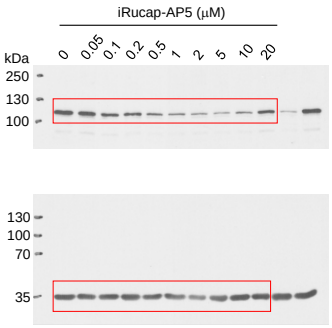
Supplementary Fig. 10b



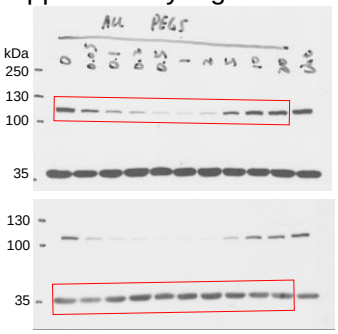
Supplementary Fig. 10g



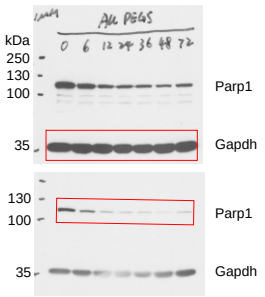
Supplementary Fig. 10c



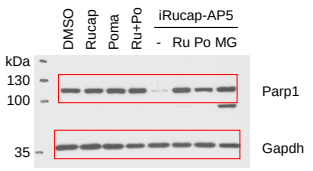
Supplementary Fig. 10d



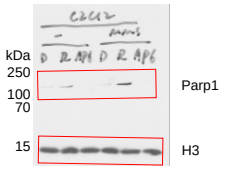
Supplementary Fig. 10e



Supplementary Fig. 10f

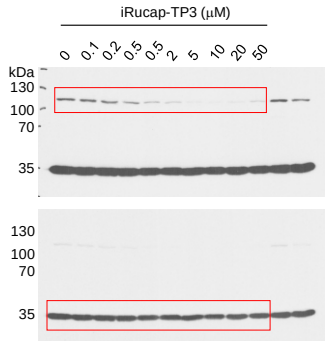


Supplementary Fig. 11a

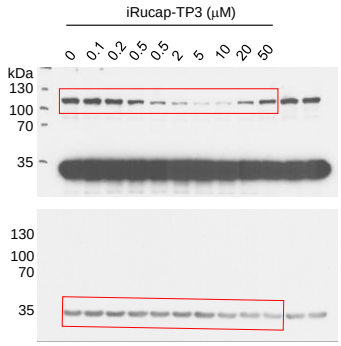


Supplementary Figure 14. Uncropped immunoblots related to the indicated figures. (continued from previous)

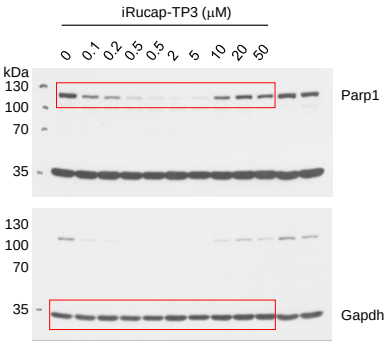
Supplementary Fig. 13a



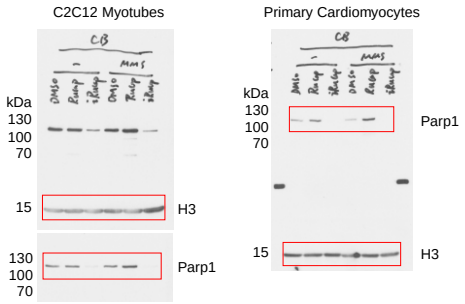
Supplementary Fig. 13b



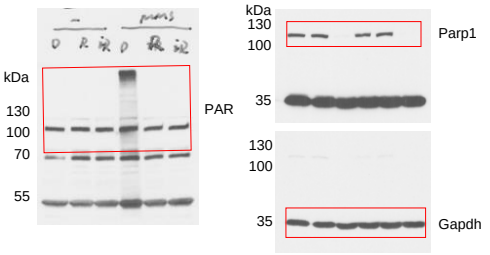
Supplementary Fig. 13c



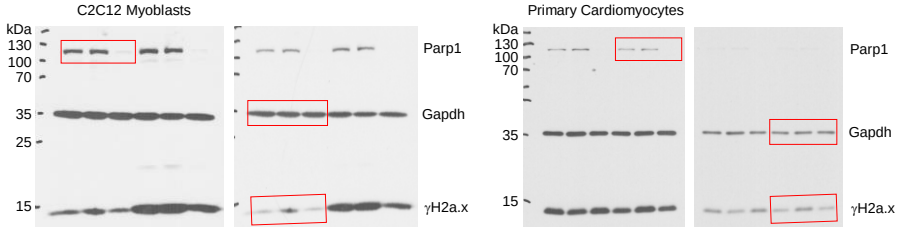
Supplementary Fig. 13d



Supplementary Fig. 13e



Supplementary Fig. 13i



Supplementary Figure 14. Uncropped immunoblots related to the indicated figures. (continued from previous)

Supplementary Table 1. Summary of PARP degraders

Parental Compound	Compound Number	Linker	E3 Ligand	Nomencleture	DC50* (nM)	IC50** (nM)
Rucaparib	16	Triazole-PEG1	Pomalidomide	iRucaparib-TP1	NA	13.4 ± 2.1 (PARP1)
	17	Triazole-PEG2	Pomalidomide	iRucaparib-TP2	NA	14.0 ± 1.6 (PARP1)
	3	Triazole-PEG3	Pomalidomide	iRucaparib-TP3	NA	18.3 ± 2.2 (PARP1)
	4	Triazole-PEG4	Pomalidomide	iRucaparib-TP4	NA	31.8 ± 3.2 (PARP1)
	18	Triazole-PEG5	Pomalidomide	iRucaparib-TP5	NA	NA
	5	Triazole-PEG3 (Indole-N linked)	Pomalidomide	iRucaparib-ITP3	NA	NA
	6	Triazole-PEG4 (Indole-N linked)	Pomalidomide	iRucaparib-ITP4	NA	NA
	10	Amide1-PEG3	Pomalidomide	iRucaparib-AMP3-1	NA	NA
	11	Amide2-PEG3	Pomalidomide	iRucaparib-AMP3-2	NA	NA
	19	All PEG4	Pomalidomide	iRucaparib-AP4	NA	NA
	12	All PEG5	Pomalidomide	iRucaparib-AP5	35.7 ± 5.3	24.6 ± 7.2 (PARP1) 5.1 ± 0.2 (PARP2)
	13	All PEG6	Pomalidomide	iRucaparib-AP6	81.8 ± 17.2	28.5 ± 7.4 (PARP1) 7.2 ± 0.3 (PARP2)
	20	All PEG7	Pomalidomide	iRucaparib-AP7	NA	NA
	8	Triazole-PEG3	VHL Ligand	vRucaparib-TP3	NA	NA
	9	Triazole-PEG4	VHL Ligand	vRucaparib-TP4	NA	NA
Veliparib	28	All PEG4	Pomalidomide	iVeliparib-AP4	NA	NA
	29	All PEG5	Pomalidomide	iVeliparib-AP5	NA	NA
	15	All PEG6	Pomalidomide	iVeliparib-AP6	62.8 ± 9.6[#]	68.9 ± 27.2 (PARP1) 21.1 ± 1.1 (PARP2)
	30	All PEG7	Pomalidomide	iVeliparib-AP7	NA	NA
Niraparib	21	All PEG4	Pomalidomide	iNiraparib-AP4	NA	NA
	22	All PEG5	Pomalidomide	iNiraparib-AP5	NA	NA
	23	All PEG6	Pomalidomide	iNiraparib-AP6	NA	NA
	24	All PEG7	Pomalidomide	iNiraparib-AP7	NA	NA
Olaparib	25	All PEG3	Pomalidomide	iOlaparib-AP3	NA	NA
	26	All PEG4	Pomalidomide	iOlaparib-AP4	NA	NA
	27	All PEG5	Pomalidomide	iOlaparib-AP5	NA	NA
	14	All PEG6	Pomalidomide	iOlaparib-AP6	NA	NA

*, DC50 was evaluated in Primary Cardiomyocytes after 24 hours treatment. Values represent Mean ± SEM, (n = 3 biological independent samples);

**, IC50 was determined by In vitro PARP activity assay. Values represent Mean ± SEM, (n = 3 biological independent samples);

#, DC50 was measured for PARP2 in HeLa cells;

NA, Not Available.

Supplementary Datasets

Supplementary Dataset 1. Quantified proteomic data for primary cardiomyocytes treated with DMSO, iRucaparib-AP5 or iRucaparib (1 μM) for 24 hrs. All quantitation data for the proteins identified was included in this file and the proteins with missing channels ("0" ion intensity) were removed in the final list. Related to Figure 3a-3e and Supplementary Figure 6b-6d.

Supplementary Dataset 2. Quantified proteomic data for HeLa cells treated with DMSO, iRucaparib-TP3 (5 μM) or iRucaparib-TP3 (5 μM) plus Rucaparib (1 μM) for 24 hrs. All quantitation data for the proteins identified was included in this file and the proteins with missing channels ("0" ion intensity) were removed in the final list. Related to Supplementary Figure 6f-6n.

Supplementary Dataset 3. Quantified proteomic data for BT-549 cells treated with DMSO, iRucaparib-TP3 (5 μM) or vRucaparib-TP4 (20 μM) for 24 hrs. All quantitation data for the proteins identified was included in this file and the proteins with missing channels ("0" ion intensity) were removed in the final list. Related to Figure 6o.

Supplementary Dataset 4. Quantified PARylated proteomic data for SILAC-labeled HeLa cells treated with DMSO/Rucaparib or DMSO/iRucaparib (10 μM). This file contains all quantitation data for modified peptides identified, including the frequency of identified peptides, the modification site and log_2-tranformed ratio (Compound/DMSO). Related to Figure 4b and 4c.

Supplementary Dataset 5. Quantified PARylated proteomic data for SILAC-labeled HeLa cells treated with DMSO/Rucaparib or DMSO/iRucaparib-TP3 (10 μM). This file contains all quantitation data for modified peptides identified, including the frequency of identified peptides, the modification site and log_2-tranformed ratio (Compound/DMSO). Related to Supplementary Figure 8c and 8d.

Supplementary Dataset 6. Quantified chromatin proteomic data for HeLa cells treated with MMS+DMSO, MMS+Rucaparib or MMS+iRucaparib for 24 hrs. All quantitation data for the proteins identified was included in this file and the proteins with missing channels ("0" ion intensity) were removed in the final list. Related to Figure 5b.

Supplementary Dataset 7. Source data for NAD^+ level and ATP level in C2C12 myotubes and primary cardiomyocytes treated with MMS or H_2O_2. Related to Supplementary Figure 12a-12f.

Supplementary Note

Supplementary Note 1. Synthetic Procedures.