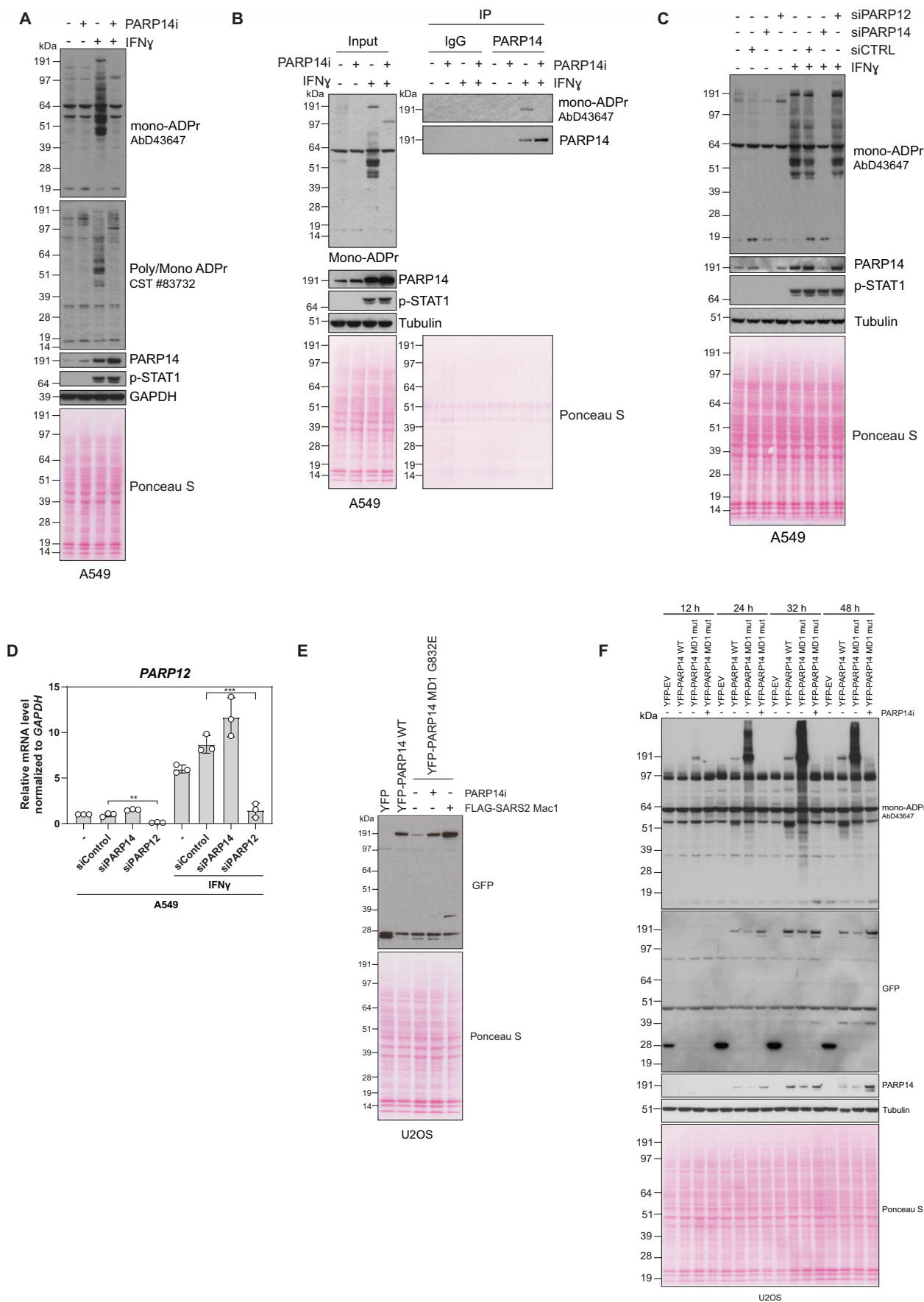


Expanded View Figures

Figure EV1. PARP14 is auto-ADPr upon immune stimulation and this regulates its stability.

(A) A549 cells were treated with 0.5 μ M PARP14i (RBN012759) and/or with IFN γ (100 ng/mL). Cell lysates were examined by western blot with the indicated antibodies. (B) A549 cells were treated with 0.5 μ M PARP14i (RBN012759) and/or with IFN γ (100 ng/mL). Cell lysates were subjected to immunoprecipitation with Protein G beads conjugated with indicated antibodies. The ADPr signal was analysed using western blotting. (C) A549 cells depleted or not of PARP14 or PARP12 were treated with IFN γ (100 ng/mL) where indicated. Cell lysates were examined by western blot with the indicated antibodies. (D) Relative gene expression of *PARP12* for (C) in unstimulated and IFN γ (100 ng/mL) stimulated A549 cells was determined by RT-qPCR. Gene expression levels were normalized to the expression of *GAPDH*. Error bars indicate average S.D. from three independent replicates. Asterisks indicate statistical significance compared with the control, as determined by Welch's t-test (ns: not significant, ** $p < 0.01$, *** $p < 0.001$ Two-tailed P value, siCTRL vs siPARP12 -IFN γ $p = 0.002$, siCTRL vs siPARP12 +IFN γ $p = 0.0006$). (E) U2OS cells were transfected with the indicated plasmids in the presence or absence of 0.5 μ M PARP14i or expression of FLAG-SARS2 Mac1. The levels of YFP-tagged PARP14 in cell lysates were assessed using an anti-GFP antibody. (F) U2OS cells were transfected with indicated plasmids in the presence or absence of PARP14 inhibitor (0.5 μ M). Cells were lysed 12, 24, 32 or 48 h after transfection and analysed by western blot using the indicated antibodies. For all blots, pSTAT1 antibody was used as a positive control for stimulation of immune response. GAPDH or tubulin were used as a loading control. Source data are available online for this figure.



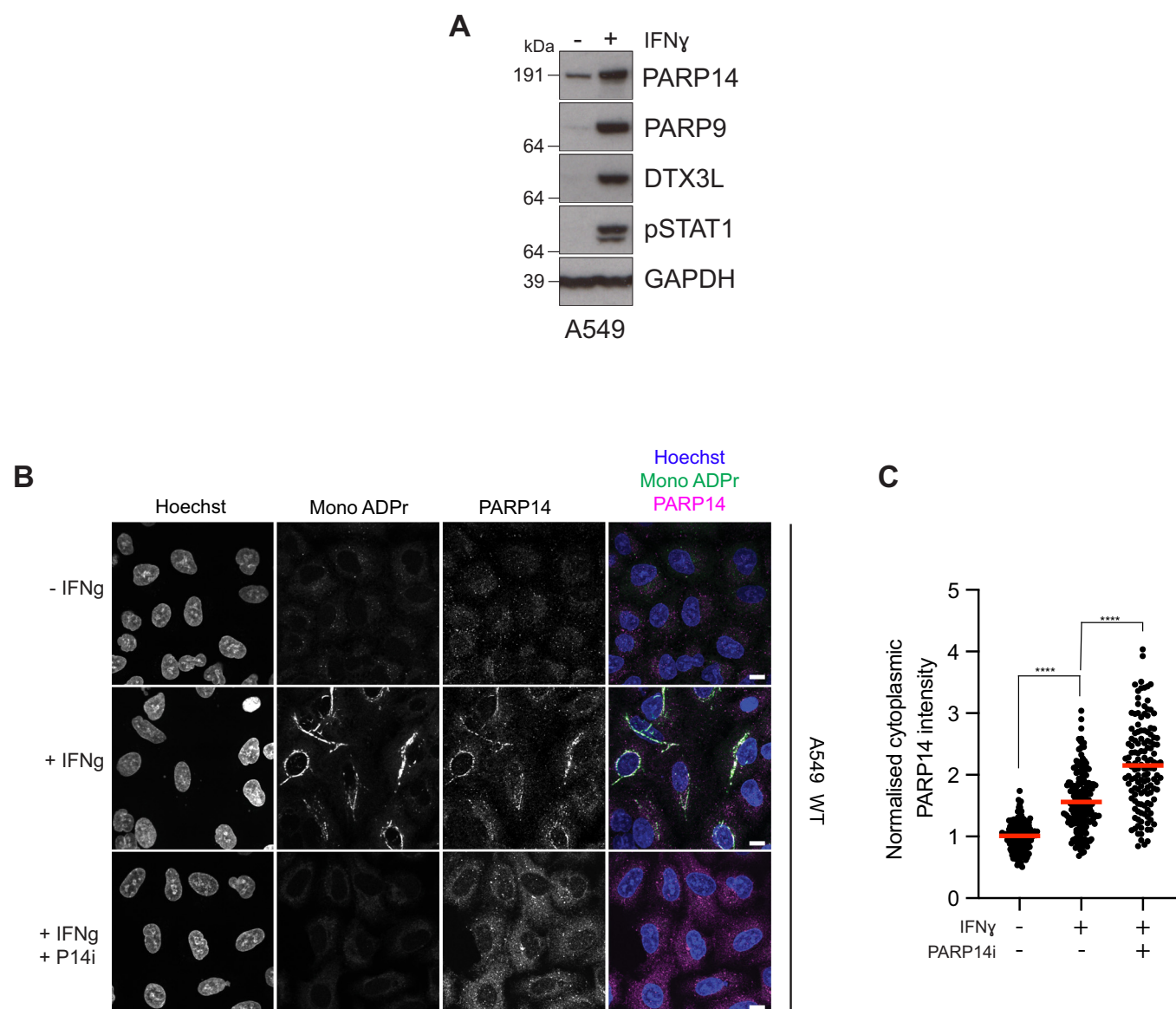
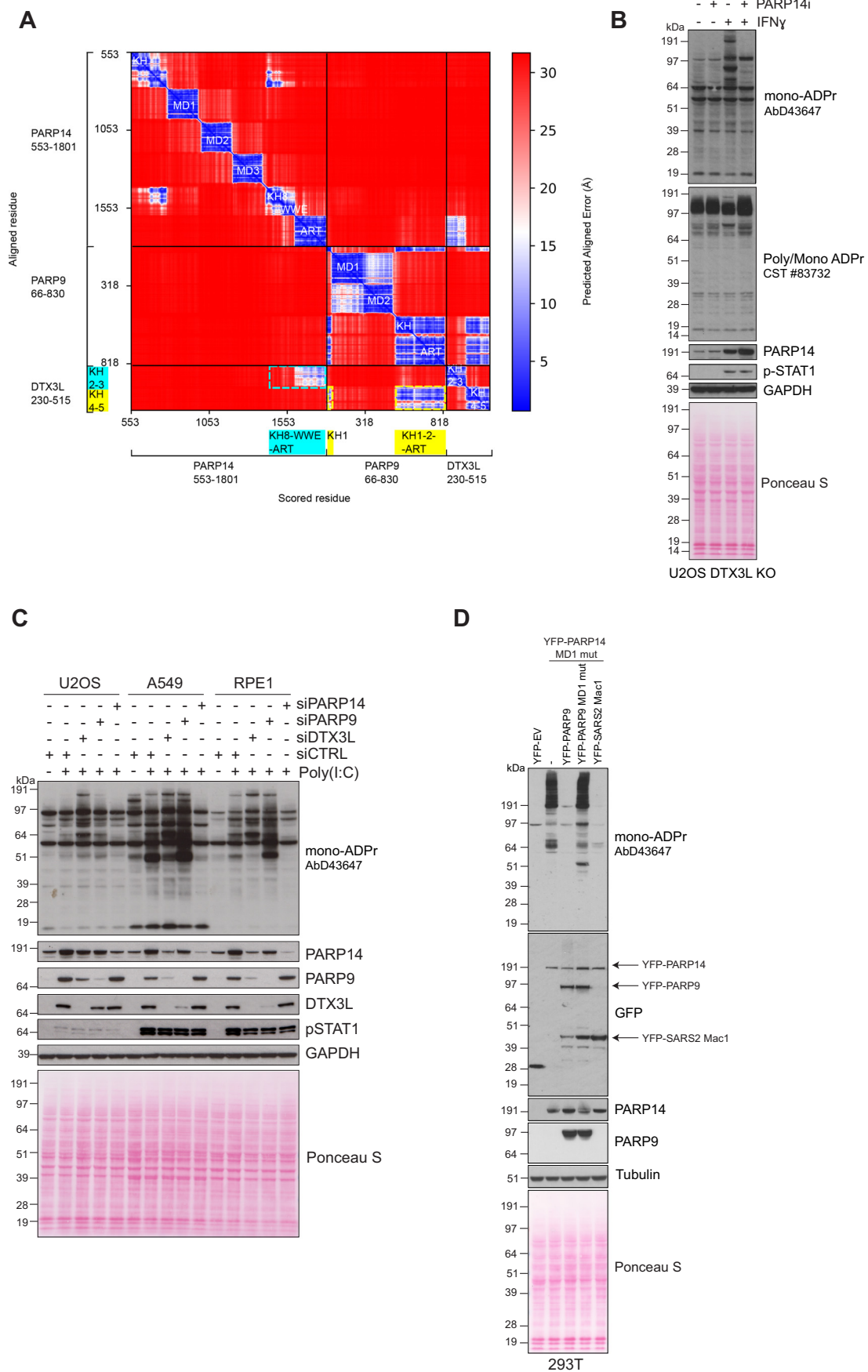


Figure EV2. PARP14, PARP9 and DTX3L increase after IFN γ stimulation.

(A) A549 cells were treated or not with IFN γ (100 ng/mL). Cell lysates were analysed by western blot using the indicated antibodies. GAPDH was used as a loading control. pSTAT1 indicates induction of the interferon response. (B) Confocal images showing A549 cells untreated or treated with IFN γ (100 ng/mL) in the presence or absence of PARP14i (0.5 μ M). Cells were stained with Hoechst (Blue), mono-ADPr (AbD43647 IgG-coupled) (Green) and PARP14 (Abcam, ab224352) (Magenta). Scale bars, 20 μ m. (C) Normalised intensity of cytoplasmic PARP14 signal from (B). Fluorescence intensity was normalised to WT cells in the absence of IFN γ or PARP14i. The red line indicates mean intensity. Statistical analysis was determined using a Kruskal Wallis test with Bonferroni correction followed by post hoc Dunn's test. Asterisks indicate statistical significance (**** $p < 0.0001$). Data are representative of two independent replicates. Source data are available online for this figure.



◀ **Figure EV3. PARP9/DTX3L regulate PARP14-dependent ADPr.**

(A) Predicted aligned error (PAE) plot of PARP14 (553–1801)/DTX3L (230–515)/PARP9 (66–830) model. Inter-domain interaction between PARP14 KH8-WWE-ART and DTX3L KH2-3 is indicated in cyan dashed box. Inter-domain interaction between PARP9 KH-ART and DTX3L KH4-5 is indicated in yellow dashed box. There are also notable intra-domain contacts within each protein: PARP14 KH-WWE, PARP9 KH-ART, PARP9 tandem macrodomains, DTX3L KH2-3, and DTX3L KH4-5. The relative orientations of other domains with high PAE score are uncertain. (B) U2OS DTX3L KO cells were treated with 0.5 μ M PARP14i and/or 100 ng/mL IFN γ . Cell lysates were examined by western blot with the indicated antibodies. (C) U2OS, A549 and RPE1 cells depleted or not of DTX3L, PARP9 or PARP14 were treated with poly(I:C). Cell lysates were examined by western blot with the indicated antibodies. For all blots, pSTAT1 antibody was used as a positive control for stimulation of immune response. (D) 293T cells were transfected with YFP-tagged PARP14 MD1 mutant alone or co-transfected with indicated plasmids. Cell lysates were collected 24 h after transfection and examined with the western blot using indicated antibodies. GAPDH or tubulin were used as a loading control in all blots. Source data are available online for this figure.

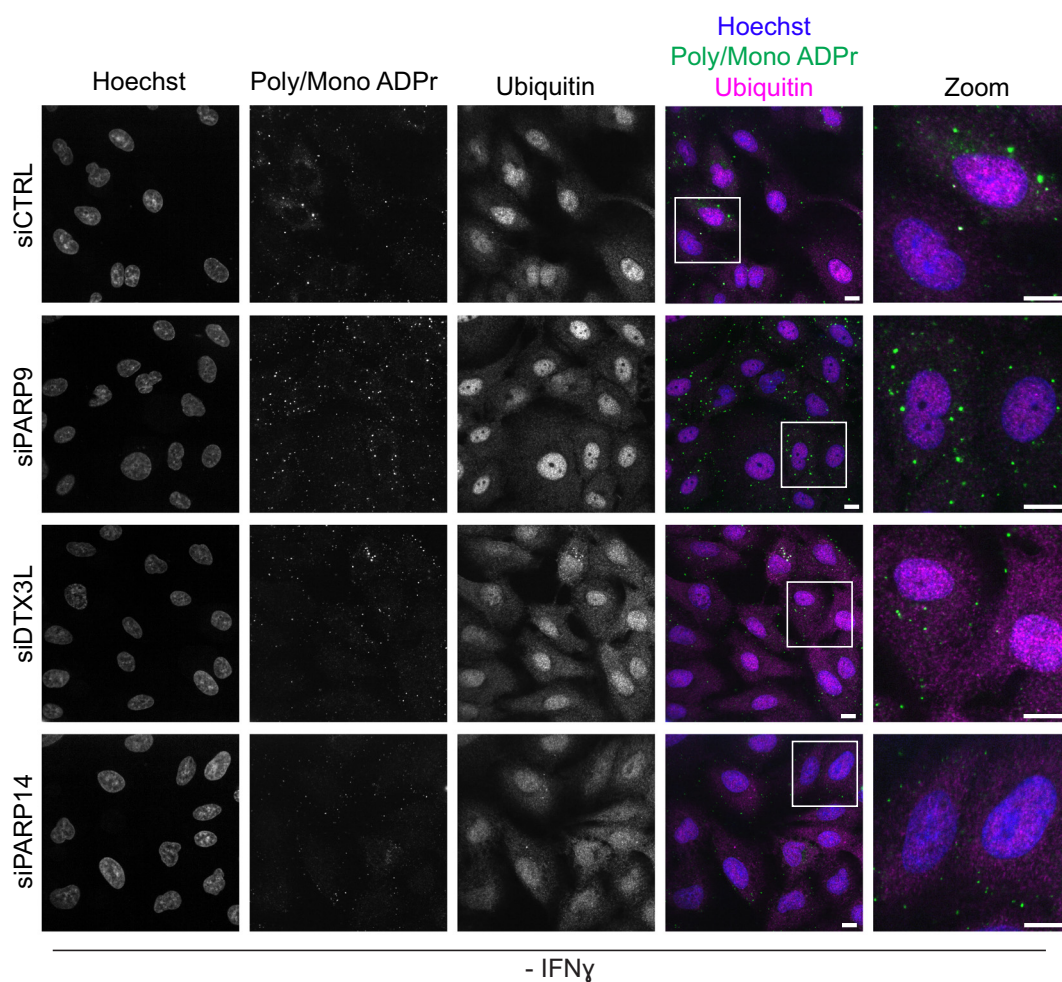
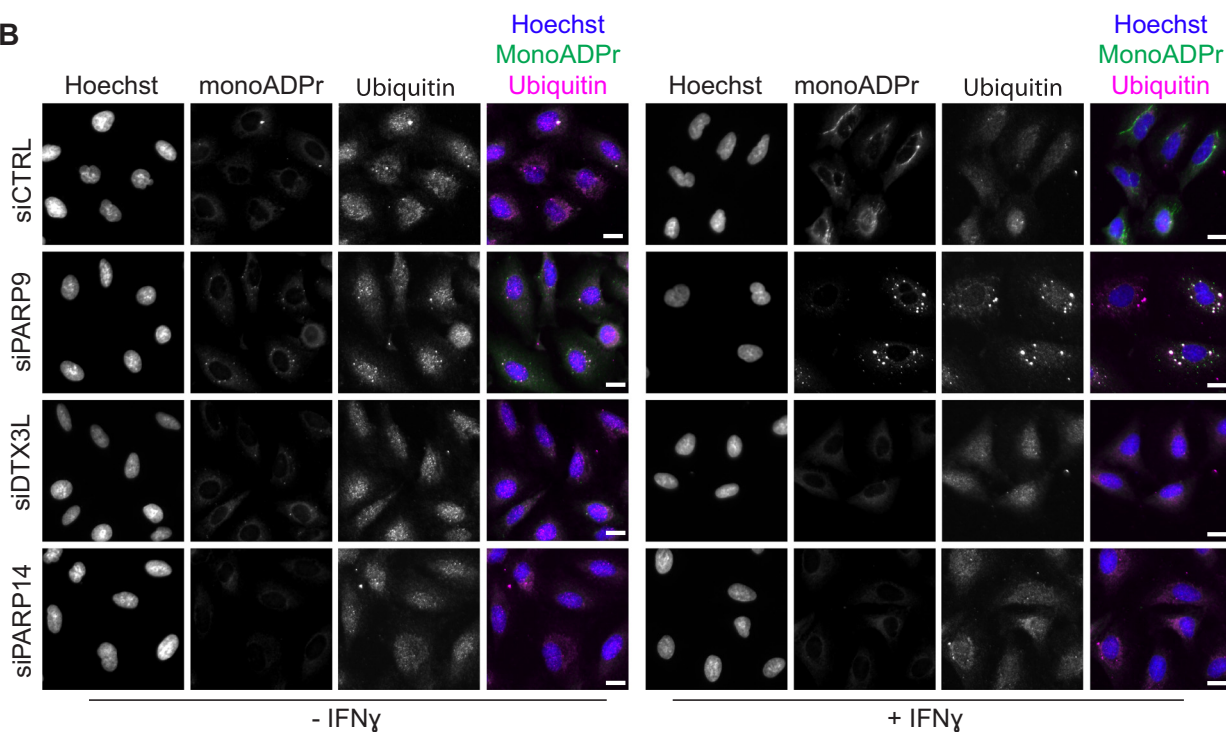
A**B**

Figure EV4. PARP14, PARP9 and DTX3L regulate ADPr and ubiquitin foci formation.

(A) Confocal images showing A549 WT cells depleted of PARP9, DTX3L or PARP14 in the absence of IFN γ . Cells were stained with Hoechst (Blue), ADPr (poly/mono-ADPr antibody, CST #83732) (Green) and ubiquitin (Magenta). (B) Widefield images showing A549 WT cells depleted of PARP9, DTX3L or PARP14, untreated or IFN γ -treated (100 ng/mL). Cells were stained with Hoechst (Blue), mono-ADPr (AbD43647 IgG-coupled antibody) (Green) and ubiquitin (Abcam, ab134953) (Magenta). For all images, scale bar = 20 μ m. Data information: Data are representative of a minimum of five (A) or three (B) independent replicates. Source data are available online for this figure.

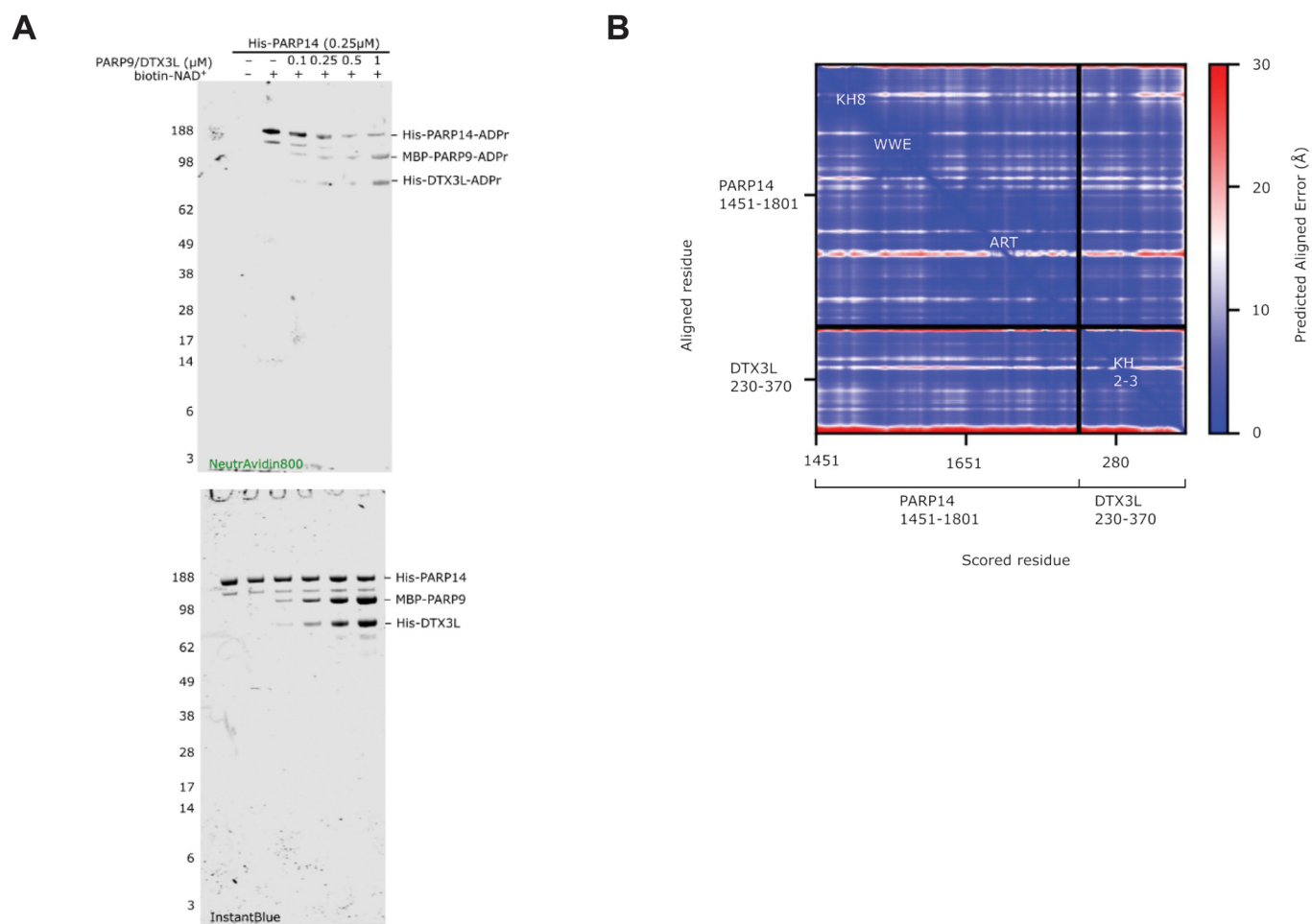


Figure EV5. DTX3L inhibits PARP14 activity in vitro.

(A) PARP14 FL auto-ADP-ribosylation reaction performed with biotin-NAD⁺ and increasing amount of PARP9/DTX3L. (B) Predicted aligned error plot of PARP14 KH8-WWE-ART (residue 1451-1801) and DTX3L KH2-3 (residue 230-370) model.