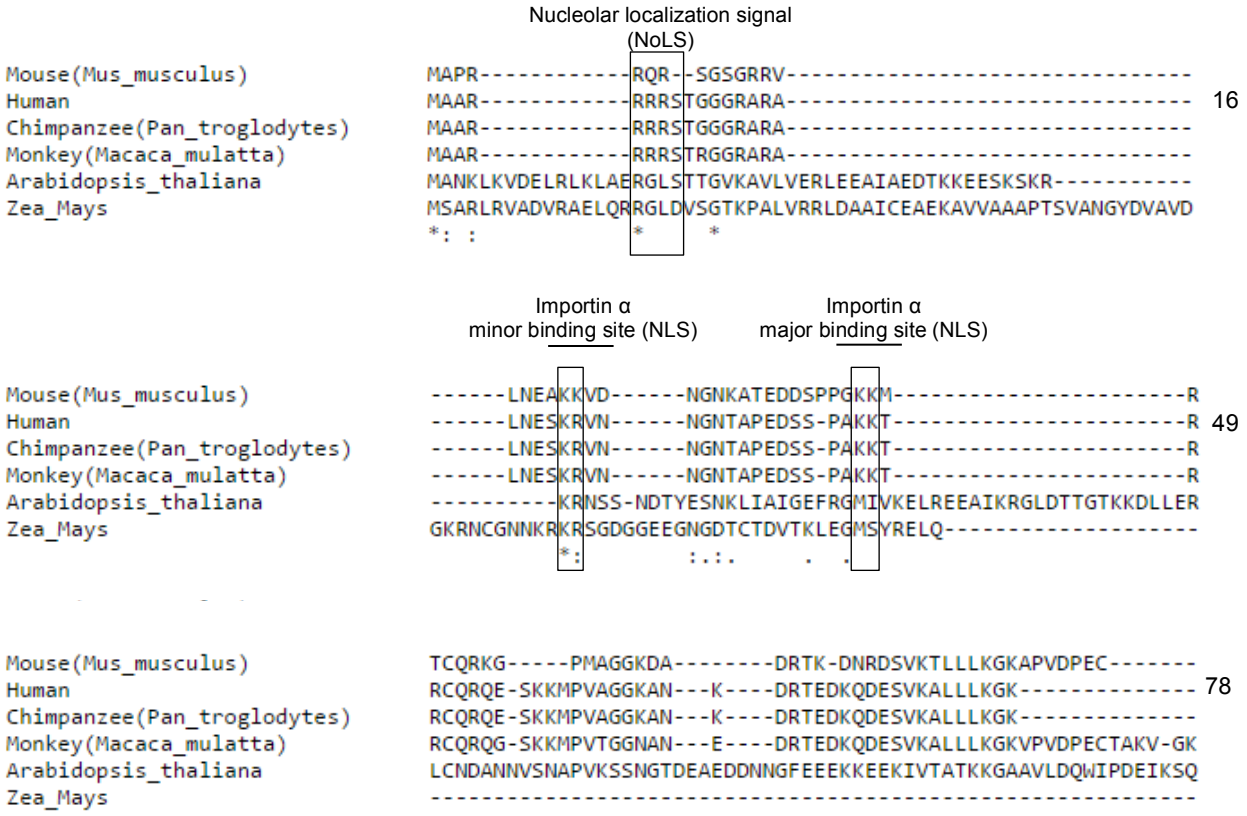
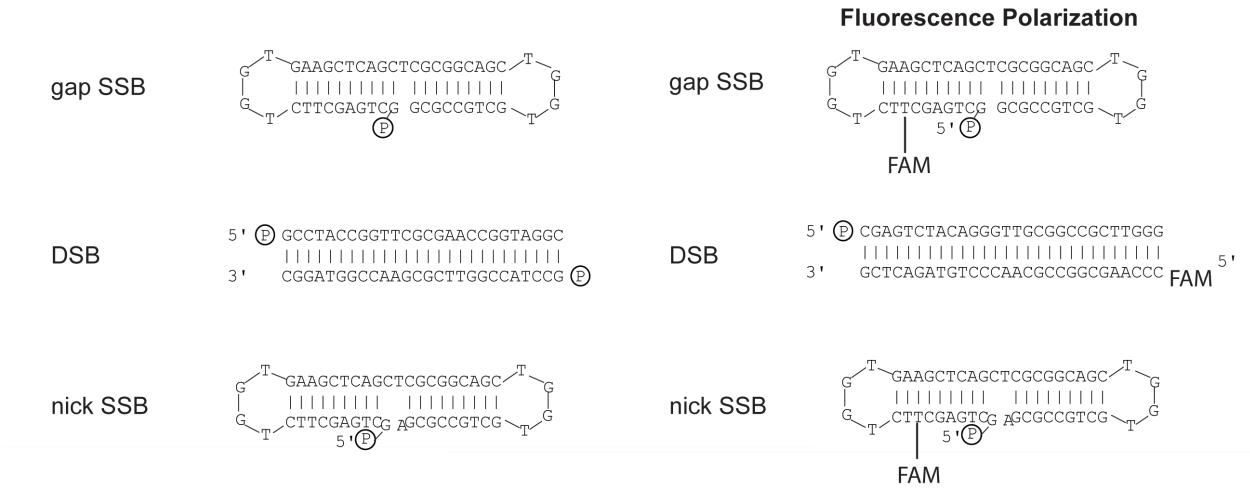
Supplementary Figures



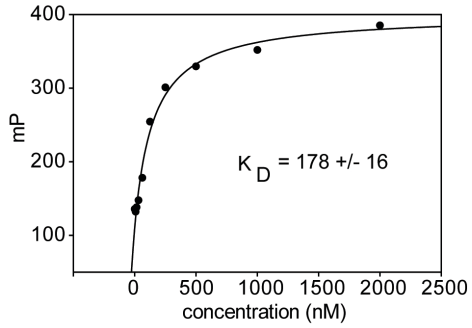
Supplementary Figure S1: Sequence alignment. ClustalW sequence analysis of human, mouse (*Mus musculus*), chimpanzee (*Pan troglodytes*), monkey (*Macaca mulatta*), *Arabidopsis thaliana*, and *Zea mays* PARP-2. The human PARP-2 nucleolar localization signal (NoLS), residues 4-7, is highlighted (16). Additionally, the regions of human PARP-2 bipartite NLS, which are necessary for contact with Importin α 1, as indicated by the crystal structure (Figure 3) have been highlighted.



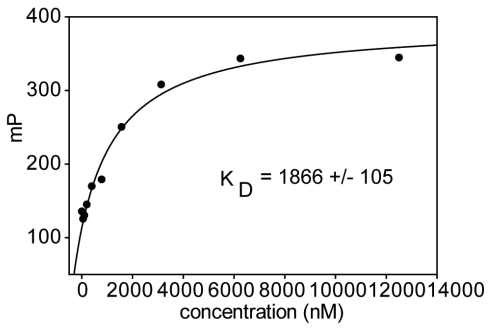
Supplementary Figure S2: Oligonucleotides. Sequence and structure of DNA sequences used in this study.

DSB

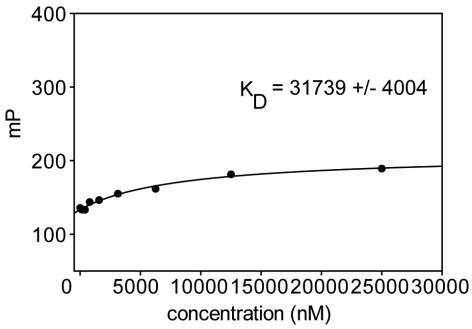
WT FL



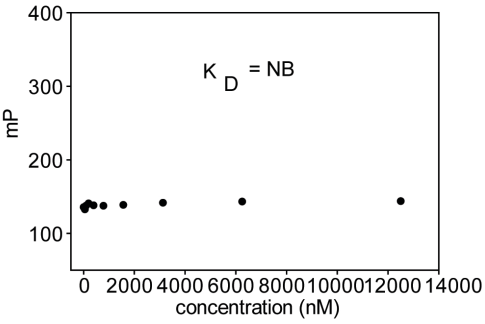
NTR



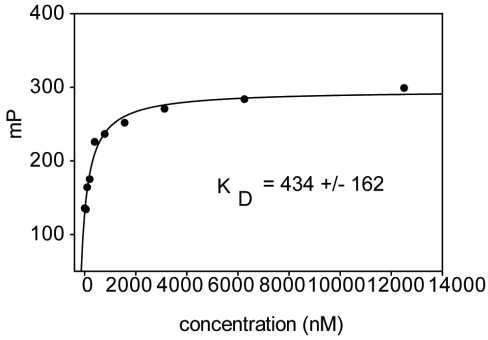
WGR



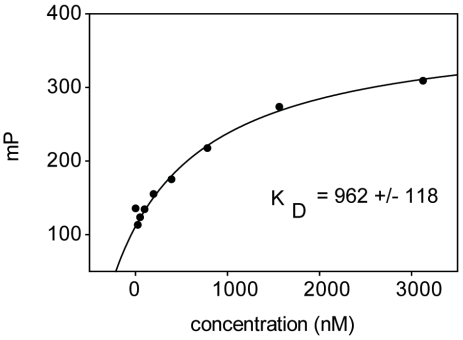
CAT



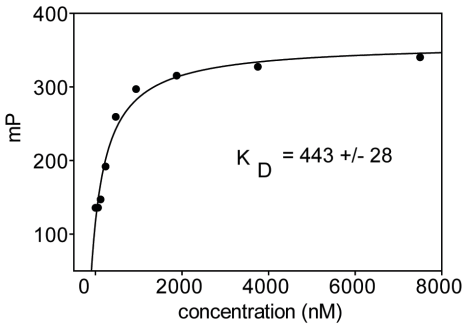
NTR-WGR



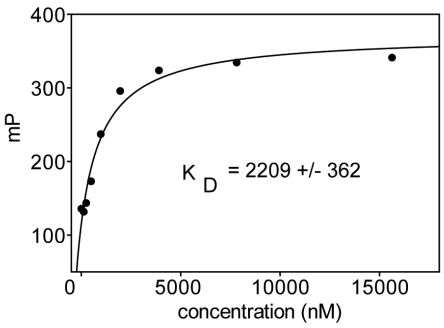
deltaNTR



N116A FL



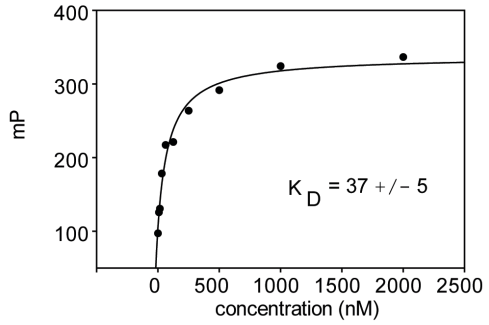
Y188F FL



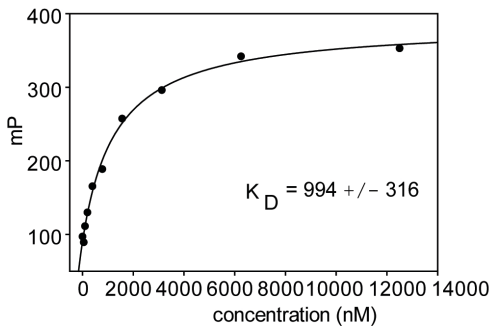
Supplementary Figure S3: Fluorescence Polarization DNA binding experiments of PARP-2 WT, PARP-2 truncations and mutations. A. A fluorescently labeled 28 duplex carrying a 5' P (5 nM) was titrated with PARP-2 WT, truncations, or mutations. An example of a binding assay is shown for each protein. NB indicates construct had no measurable binding affinity.

SSB nick

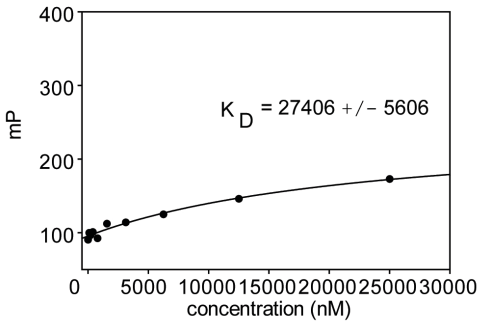
WT FL



NTR



WGR



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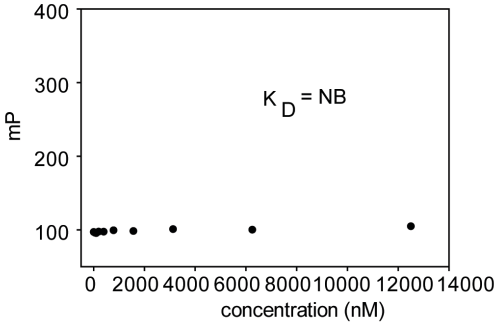
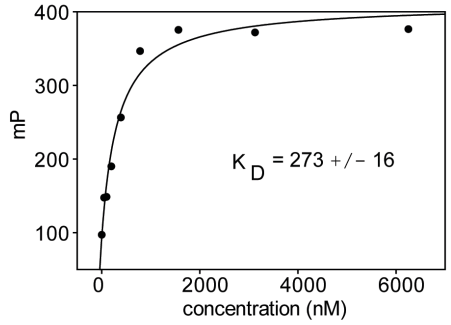
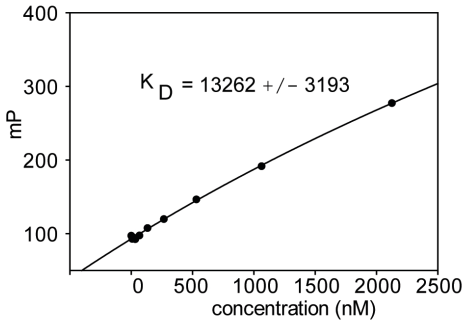


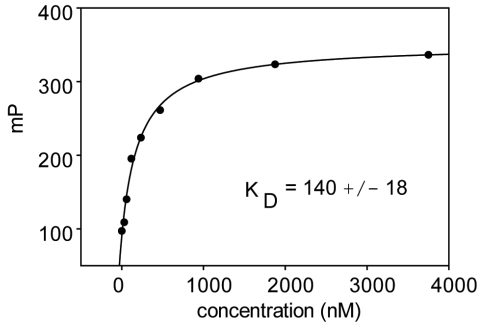
NTR-WGR



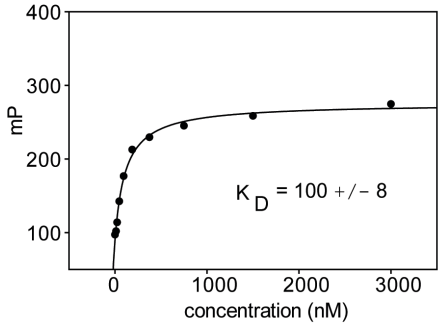
deltaNTR



N116A FL



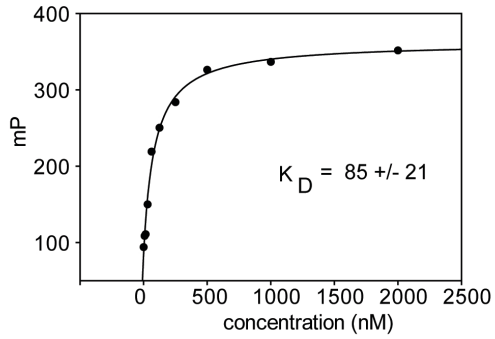
Y188F FL



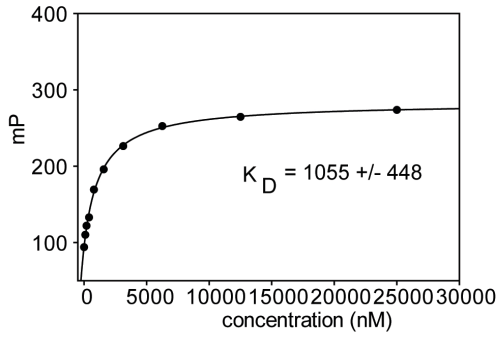
Supplementary Figure S4: Fluorescence Polarization DNA binding experiments of PARP-2 WT, PARP-2 truncations and mutations. A. A fluorescently labeled dumbbell nick DNA carrying a 5' P (5 nM) was titrated with PARP-2 WT or truncations. An example of a binding assay is shown for each protein. NB indicates construct had no measurable binding affinity.

SSB gap

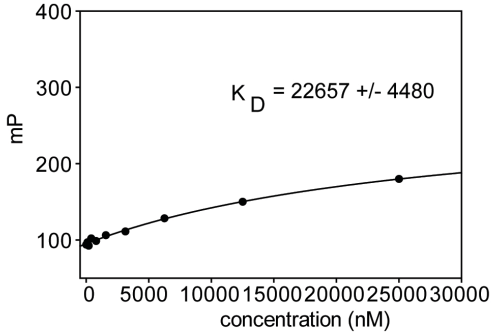
WT FL



NTR



WGR



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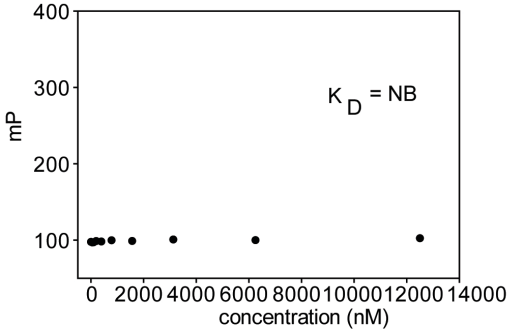
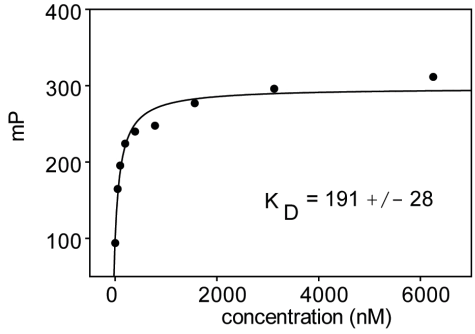
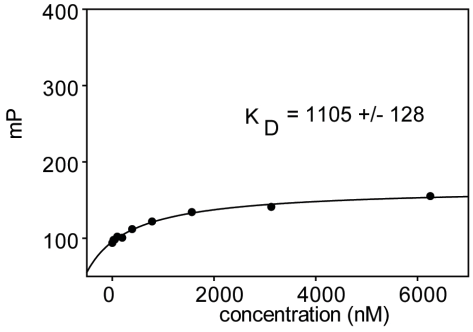


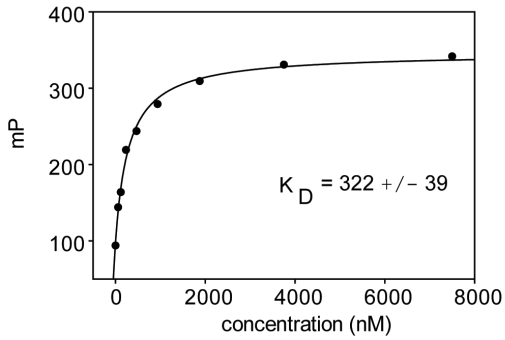
NTR-WGR



deltaNTR



N116A FL



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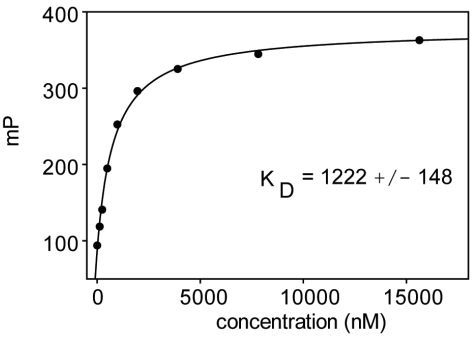
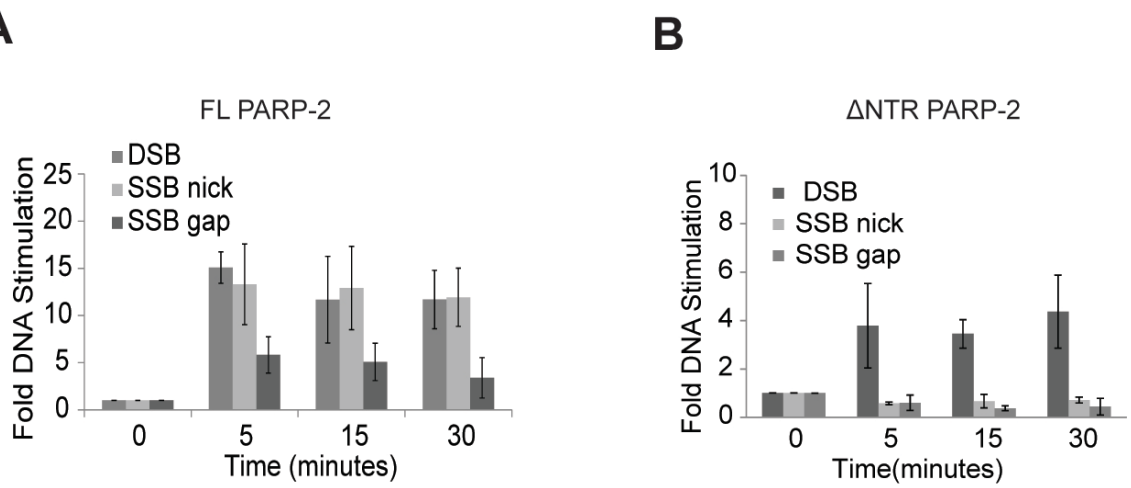


Supplementary Figure S5: Fluorescence Polarization DNA binding experiments of PARP-2 WT, PARP-2 truncations and mutations. A. A fluorescently labeled dumbbell gap DNA carrying a 5' P (5 nM) was titrated with PARP-2 WT, truncations, or mutations. An example of a binding assay is shown for each protein. NB indicates construct had no measurable binding affinity.



Supplementary Figure S6: Structure specific requirement of PARP-2 NTR. PARP-2 DNA-dependent activity was measured using a colorimetric assay. FL PARP-2 (panel A) and ΔNTR (WGR-CAT) (panel B) (60 nM) were incubated with various DNA templates (480 nM). Stimulation is calculated as the ratio of activity measured in the presence *versus* the absence of DNA. The average of three or more independent experiments is shown with the associated standard deviations.

A

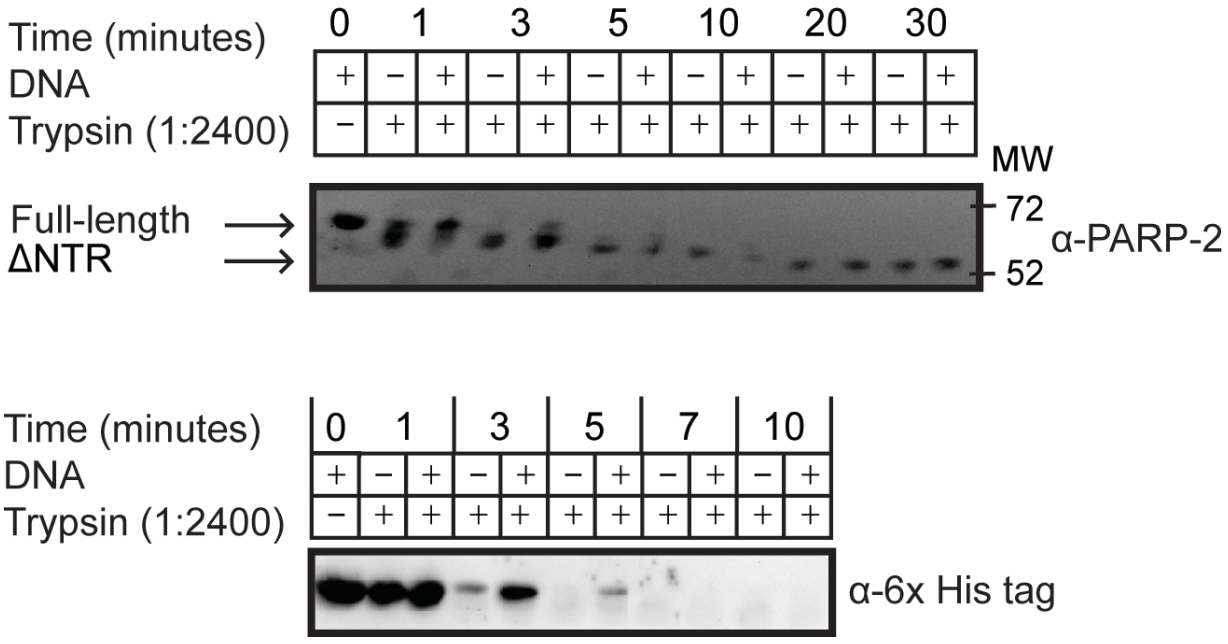
PARP-1 Zn1

MAESSDKLYRVEYAKSGRAS
CKKCSESIPKDSLRMAIMVQ
SPMFDGKVPHWYHFSCFWKV
GHSIRHPDVEVDGFSELRWD
DQQKVKKTAEAGGVTG

PARP -2 NTR

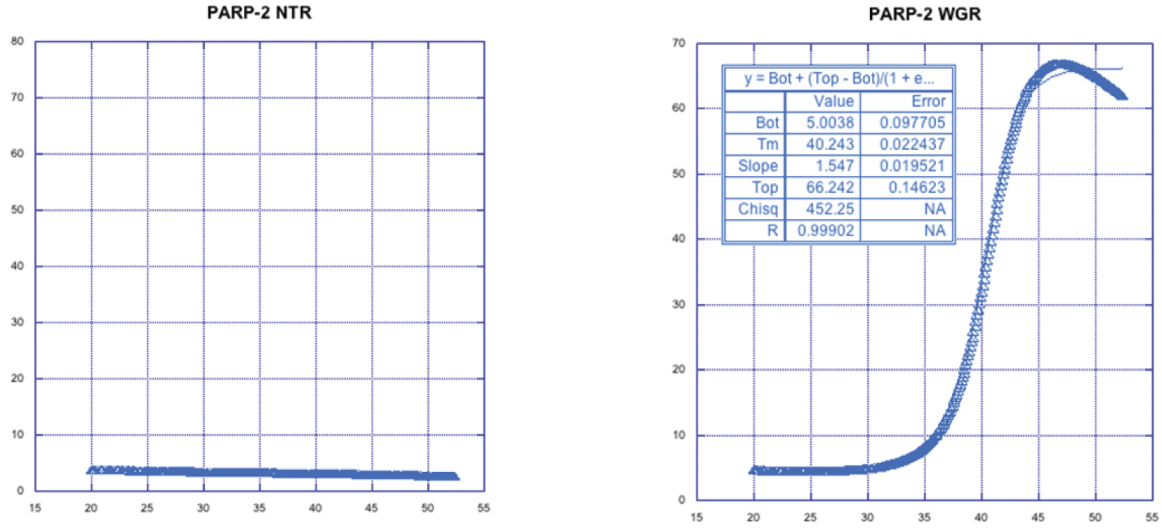
MAARRRRSTGGGRARALNES
KRVNNGNTAPEDSSPAKKTR
RCQRQESKKMPVAGGKANKD
RTEDKQDESVKALLLKGK

B



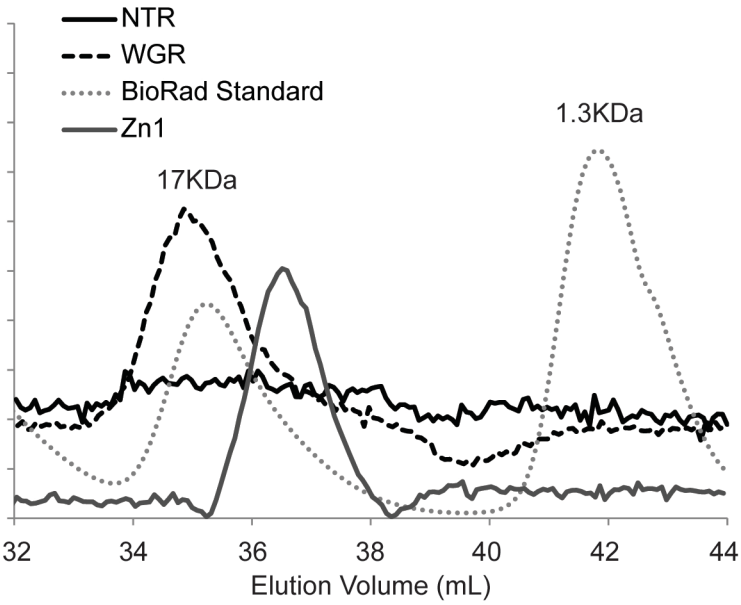
C

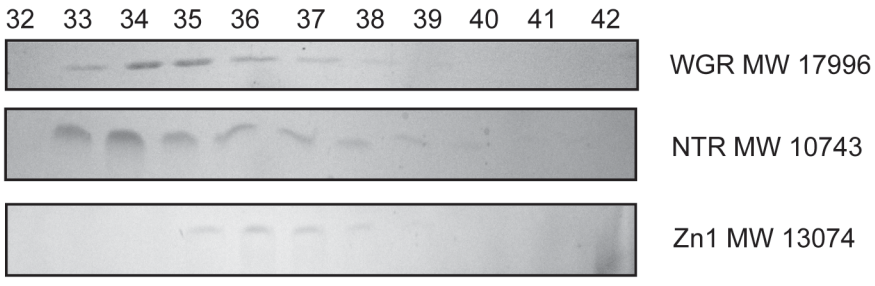
Differential Scanning Fluorimetry



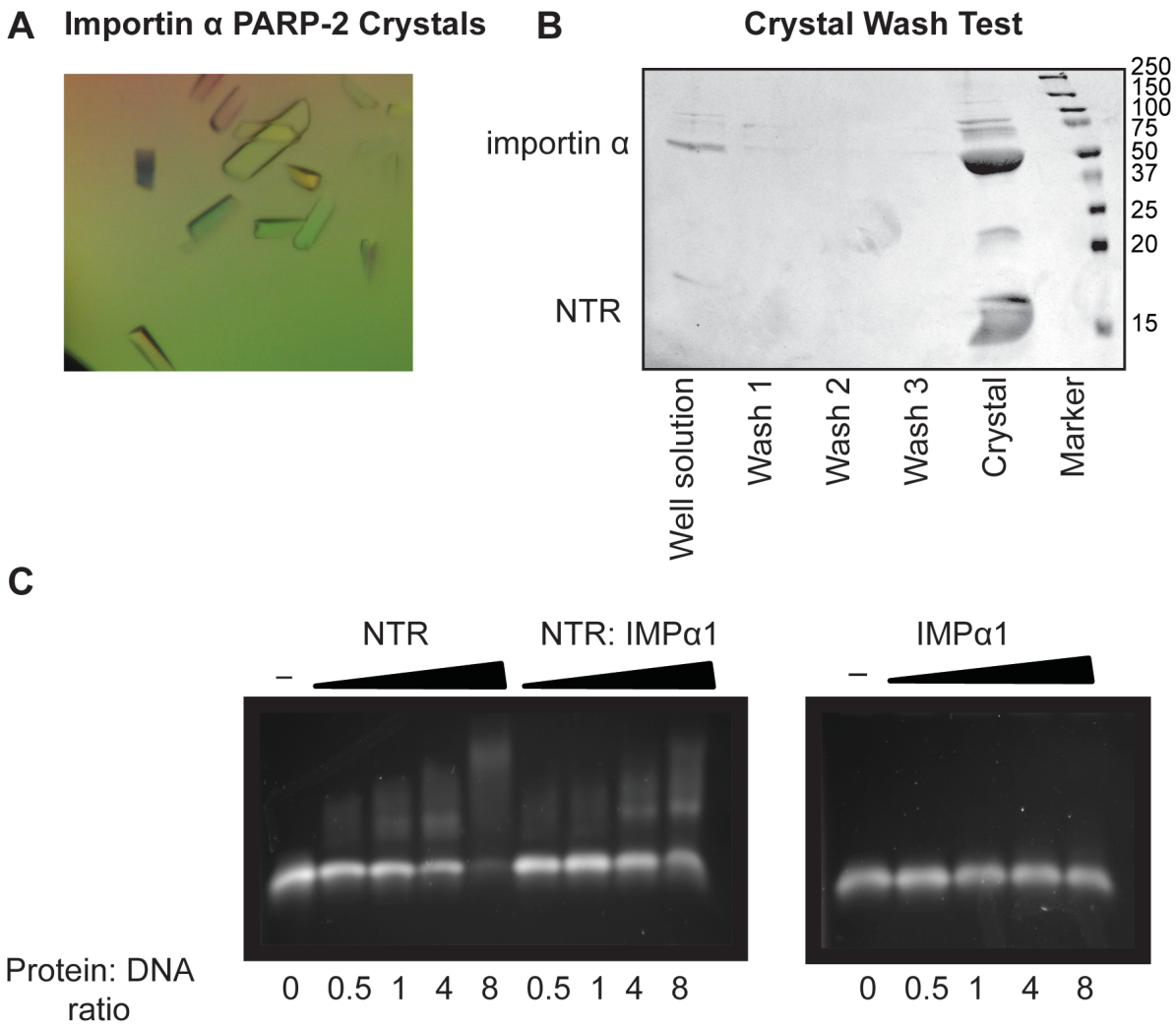
D

Gel Filtration

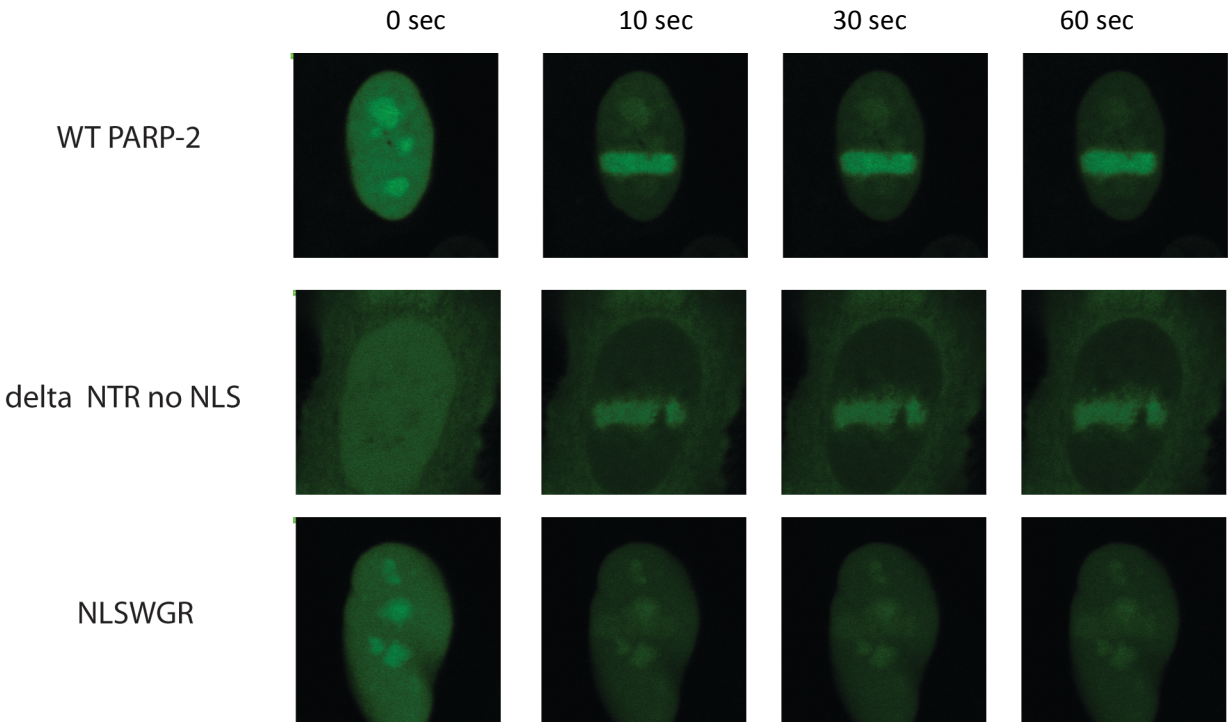




Supplementary Figure S7: PARP-2 NTR is natively disordered. A. Basic residues of PARP-2 NTR and PARP-1 Zn1. Amino acid sequence of PARP-1 Zn1 (1-96) (top) and PARP-2 NTR (1-78) (bottom) arginine and lysine residues (potential trypsin cleavage residues) are highlighted. B. Western blot analysis of FL PARP-2 was resolved on 7.5% SDS-PAGE and then transferred onto Hybond-ECL nitrocellulose membrane (GE Healthcare). Membranes were treated for 1 hour at room temperature in blocking buffer comprised of Tris-buffered saline with tween (TBST, 20 mM Tris pH 7.5, 150 mM NaCl, 0.1% Tween 20) and 5% Blocking-Grade buffer (Bio-Rad) for the anti-PARP-2 experiment or Blocking Reagent (5 Prime) for the anti-histidine tag experiments, in both cases according to the manufacturer's protocols. Membranes were incubated 1 hour at room temperature with an anti-PARP-2 antibody (1:900) (Abcam), or an HRP-conjugated anti-hexahistidine tag antibody (1:2000) in their respective blocking buffers and washed with TBST. Anti-PARP-2 was then incubated with HRP conjugated donkey anti-rabbit (1:5000) (Santa Cruz Biotechnology) in 1% Blocking-Grade buffer in TBST. Finally, the membranes were washed with TBST, developed with SuperSignal West Pico Chemiluminescent Substrate (Thermo Scientific), and imaged on a Gel Doc system (Bio-Rad). A representative image of three independent experiments is shown. Top: The anti-PARP-2 western blot shows a decrease in molecular weight (MW) of FL PARP-2 upon trypsin digest. The PARP-2 catalytic domain is preserved, as indicated by the anti-PARP-2. Anti-PARP-2 antibody detects residues within the C-terminus of human PARP-2. Bottom: The anti-histidine tag western blot shown a decrease in signal of the histidine tag corresponding to a loss of the N-terminal region. C. Left: Representative example of differential scanning fluorimetry (DSF) of PARP-2 NTR (10μM). Right: Representative melting curve of PARP-2 WGR (10μM) obtained by DSF. Data was fit to a sigmoid model utilizing Kaleidagraph software (Synergy). The T_M of PARP-2 WGR is 40.3+/- 0.09°C. DSF experiments were done in triplicate. D. PARP-2 Analytical Gel Filtration shows elution profile consistent with elongated structure. The dotted lines show two molecular weight markers (Bio-Rad Gel Filtration) at 17kDa and 1.3kDa. The black solid line shows the elution profile of PARP-2 NTR (3μg). The black dashed line and the gray solid line show PARP-2 WGR domain (1μg) and PARP-1 Zn1 domain (1μg) elution profiles respectively. Fractions were resolved at a rate of 0.5mL/min and collected in 0.5mL fractions. Fractions collected for analysis by SDS-PAGE. Fractions collected for analysis by SDS-PAGE are shown below chromatography and the elution volume indicated. The respective molecular weight (MW) are shown with the PARP-2 or PARP-1 truncation to the right of the SDS-PAGE analysis.



Supplementary Figure S8: PARP-2 NTR:IMPα crystallization. A. Crystals of PARP-2 NTR bound to IMPα. B. SDS-PAGE analysis of PARP-2 NTR:importin crystals. Crystals were washed in excess well buffer then dissolved in 6x SDS loading buffer. Samples were boiled at 95°C for 5 minutes. SDS-PAGE (18%) was stained with Imperial Stain (Thermo Fischer). C. Electrophoretic mobility shift assay (EMSA) of NTR bound to IMPα. The gel shift assay was performed using 0.5 μM SSB nick DNA and varied protein concentration as indicated by the protein:DNA ratio. Reactions were incubated for 30 minutes at room temperature in 50 mM Tris pH 7, 100 mM NaCl, 0.1 mM TCEP, 0.1 mM EDTA, and 5% glycerol. Reactions were run on a 0.9% agarose gel for 35 minutes at 100V at 4°C in Tris-Borate buffer. The gel was next stained in ethidium bromide for 5 minutes.



Supplementary Figure S9: hPARP-2 recruitment to cellular sites of DNA damage A. GFP-PARP-2 and truncations expressed in HeLa cells. Live-cell imaging recruitment to sites of laser induced DNA damage. Cellular images are representative images from at least three independent experiments. NLS is an N-terminal peptide SV40T NLS tethered to PARP-2 truncations that lack the canonical NLS of hPARP-2. The SV40T NLS sequence is PPKKKRKVEDPG.