Supplementary Figure S1

a Non-fixed

b Formaldehyde

c

d

e
Supplementary Figure S1. PARP localization patterns using GFP-PARP and PARP-specific antibody libraries

GFP-PARP localization in non-fixed (A) and formaldehyde fixed (B) GFP-PARPx transfected HeLa cells. Fixed cells were costained with GFP (top) and PARP-specific (bottom) antibodies to verify reactivity and localization of PARP antibodies. Scale bars, 10 µm. C) Streptavidin binding peptide tagged PARPs 10, 13.2 and 16 were expressed in HeLa cells and stained with antibodies against SBP (green) and each PARP (red). Localization of SBP tagged PARPs is identical to GFP tag, suggesting that the tag is not effecting PARP localization patterns. Scale bar, 10 µm.

D) GFP-PARPx transfected HeLa lysates were immunoblotted using an antibody against GFP to verify correct molecular weight of each GFP-PARP fusion construct. E) Untransfected (left lane) and GFP-PARPx transfected (right lane) HeLa cells were immunoblotted with corresponding PARP antibody to determine specificity of each antibody. The corresponding tubulin blot is included as a loading control (lower panels). Approximate molecular weights of endogenous PARPs and overexpressed GFP-PARP fusions are indicated.
Supplementary Figure S2. Cell cycle specific PARP localization patterns

A) Asynchronous hTERT-RPE1 cells were fixed with formaldehyde and stained with PARP specific antibodies. B) hTERT-RPE1 were arrested in G0/G1 via serum starvation and costained with PARP antibodies (red) and centrin (green) to identify G0/G1 cells. Arrowheads indicate area of inset. C) hTERT-RPE1 were arrested in S-phase via aphidicolin treatment and stained with PARP antibodies (red). The detection of EdU (green), a nucleotide analog, incorporated during a 20 min aphidicolin washout, was used to identify S phase cells. D) Mitotic hTERT-RPE1 cells were costained with PARP antibodies (red) and tubulin (green). DNA was stained with Hoechst 33342, shown in blue. Scale bars, 10 µm.
Supplementary Figure S3. Subcellular localization validation of centrosomal, Golgi, cytoskeletal, and membrane localized PARPs

A) Interphase and mitotic HeLa cells were stained with antibodies against the indicated PARP and centrin to demonstrate centrosomal localization patterns. Color merge shows PARP (red), centrin (green), and Hoechst 33342 (blue) costaining. Arrowheads indicate area of inset. B) Interphase HeLa cells were stained with antibodies against PARP12 and the trans Golgi marker p230, confirming a Golgi localization pattern for PARP12. C) Interphase HeLa cells were stained with antibodies against the indicated macro PARP and actin to demonstrate localization patterns consistent with functions in cell motility. Cells were fixed with 10% TCA to preserve membrane signal. D) Interphase HeLa cells were stained with antibodies against PARP16 and the lipophilic membrane dye DiI, demonstrating colocalization with DiI positive membrane structures. Scale bars, 10 μm.
**Supplementary Figure S4. PARP Overexpression phenotypes**

A) Control and GFP-PARP12 overexpressing cells were costained antibodies against PARP12 (red) and p230 (green). DNA was stained with Hoechst 33342 (blue). Scale bar, 10 µm. B) GFP-PARP12 overexpression resulted in increased Golgi area relative to control overexpression as determined by p230 staining while GFP alone or GFP-PARP13.2 overexpression did not result in changes in Golgi size (**p<0.0001, n≥46, one way ANOVA, error bars indicate 95% CI of the mean). Golgi size in untransfected (n=77), GFP transfected (n=97), GFP-PARP12 transfected (n=107) and GFP-PARP13.2 (n=46) transfected cells was determined by staining with the trans-Golgi marker p230, tracing the area enclosed within the p230 signal, then calculating the area of the tracings. Data were analyzed via one-way ANOVA, comparing each data set to the untransfected data set. GFP-PARP12 overexpressing cells contained Golgi ~1.58 fold larger than Golgi found in nontransfected control cells while GFP and GFP-PARP13.2 overexpression did not result in a change in Golgi size relative to controls. Images show representative data. Scale bar, 50 µm. C) Overexpression of GFP-PARP16 resulted in the assembly of abnormal structures identified as membranous by Dil staining. Such structures were not seen in control cells overexpressing GFP. Graph shows percent of GFP transfected cells with Dil positive foci (****p<.0001, n≥34, Fisher’s exact test). Scale bar, 10 µm.
Supplementary Figure S5. Poly (ADP-ribose) localization throughout the cell cycle

A) HeLa cells were either untreated (Control) or treated with H$_2$O$_2$ (DNA Damage) to verify accurate PAR staining. Cells were fixed with either Methanol or TCA and stained for PAR (chIgY). Both fixation conditions demonstrate an increase in nuclear PAR upon induction of DNA damage, indicated by pH2AX staining. Scale bar, 10 µm. B) Cytoplasmic (C) and nuclear (N) extracts were prepared from untreated and H$_2$O$_2$ treated cells and immunoblotted using chIgY and BD PAR pADPr antibodies, confirming an increase in nuclear PAR levels during DNA damage conditions. C) Asynchronous HeLa cells or cells arrested in G$_0$/G$_1$, S-phase and mitosis were fixed in either Methanol or TCA then stained for PAR (BD PAR) and the cell cycle markers Centrin or γ−Tubulin, to identify single centriole pairs found only during G$_0$/G$_1$, EdU, incorporated during S-phase, and Tubulin, to stain mitotic spindles. In Asynchronous cells and during G$_0$/G$_1$, S-phase, and Mitosis, pADPr staining appears diffuse and punctate with strong staining at the centrosome (arrowhead), and poles of the mitotic spindle (arrows). Merge shows PAR in red, cell cycle markers in green and Hoechst 33342 in blue. Scale bars, 10 µm. D) Cytoplasmic (C) and nuclear (N) extracts were prepared from asynchronous, G$_0$/G$_1$, and S-phase arrested cells and immunoblotted with BD PAR anti-PAR antibody. G$_0$/G$_1$ cells have higher levels of cytoplasmic PAR while S-phase cells have increased nuclear pADPr. Total cell extracts were prepared for asynchronous (A) and mitotic (M) cells and immunoblotted with BD PAR anti-PAR antibody, displaying increased levels of PAR during mitosis.
Supplementary Figure S6. PARP knock-down phenotypes

Control HeLa cells or cells in which either PARP-5a or -7 were knocked-down were stained with the corresponding PARP antibody and tubulin to examine spindle structure. PARP-5a knock-down resulted in an increased mitotic index (~13% vs. ~3% for controls), disorganized spindles, and supernumerary spindle poles. PARP-7 knock-down cells exhibited an increase in pre-metaphase mitotic spindles.
### Supplementary Table S1

**PARP siRNA Sequences**

| PARP    | siRNA-1                | Company | siRNA-2                | Company | Published |
|---------|------------------------|---------|------------------------|---------|-----------|
| 1       | GCCUCCGCUCUGAACAUA     | Dharmacon | N/A                    |         | Ref. 9    |
| 2       | AAUCAGUGUAAGAUCUACUA   | Dharmacon | N/A                    |         | Ref. 9    |
| 3       | GGACCCAGGUGAUGAGGACUACAA | Invitrogen Stealth | N/A                    |         | Ref. 9    |
| 4       | AAACAAGGAUUUCAUCUAGAG | Dharmacon | AAAGAUJCGUGGUGGCAAAGA | Dharmacon | Ref. 9    |
| 5a      | CAGUAACAAUUCACCGUCCUCU  | Invitrogen Stealth | N/A                    |         | Ref. 9    |
| 5b      | GCUUCAGAAUGGUCAAUA     | Dharmacon | N/A                    |         | Ref. 9    |
| 6       | s32505                 | Invitrogen Silencer Select | s32504 | Invitrogen Silencer Select | N/A |
| 7       | GUGAUAAGCGUGAGUACUGATT | Invitrogen Silencer Select | s32505 | Invitrogen Silencer Select | N/A |
| 8       | GGAAGAUUCUGAAGGACAAUGAU | Invitrogen Stealth | GCCUUAAGUGAAGAUCACCUAU | Invitrogen Stealth | N/A |
| 9       | D-014734-03            | Dharmacon | D-014734-01            | Dharmacon | N/A |
| 10      | GCCUGGUGGAGAUGGCUAUUGAU | Invitrogen Stealth | AGACGUCGCUCUCUGCCACUGAA | Invitrogen Stealth | N/A |
| 12      | s34882                 | Invitrogen Silencer Select | s34883 | Invitrogen Silencer Select | N/A |
| 13      | GCUCACGAACUAUGAGCUGGAGUU | Invitrogen Stealth | GCUGACCAAGAGUACGACUGUUA | Invitrogen Stealth | N/A |
| 14      | D-023583-02            | Dharmacon | s29270                 | Invitrogen Silencer Select | N/A |
| 16      | CCCAAGACUCUCUGGUGACAACAUA | Invitrogen Stealth | GAGACAAAGGAGAAGGAGACC | Invitrogen Stealth | N/A |