Chemical Synthesis

General. ^{1}H NMR were recorded on a Bruker DPX spectrometer at 400 MHz. Chemical shifts are reported as parts per million (ppm) downfield from an internal tetramethylsilane standard or solvent references. Dichloromethane (DCM), *N*,*N*-dimethylformamide (DMF), and *N*,*N*-diisopropylethylamine (DIPEA) were dried using a solvent purification system manufactured by Glass Contour, Inc. (Laguna Beach, CA). All other solvents were of ACS chemical grade (Fisher Scientific) and used without further purification unless otherwise indicated. Commercially available 1-indanones from Fisher Scientific or Sigma Aldrich were >95% pure and used without further purification. 4-fluorophenethylamine (98%) and 3,4-dihydro-2*H*-isoquinolin-1-one (dq, **1**) (95%) were purchased from CombiBlocks (San Diego, CA) and used as received. *N*-6 alkyne-NAD^+ (6-a-NAD^+) was synthesized as previously described.[1]

*Synthesis of 7-fluoro-3,4-dihydroisoquinolin-1(2H)-one (7-F-dq, **2**)*: The procedure of Kurouchi et al. was followed as previously described,[2] with minor modifications. To a solution of 4-fluorophenethylamine (208.8 mg, 1.5 mmol) and DIPEA (0.29 mL, 1.65 mmol) in DMF (5.6 mL), methyl chloroformate (0.13 mL, 1.65 mmol) was slowly added at 0 °C, and the reaction mixture was stirred at rt for 1h. The reaction was quenched with water (19 mL) and extracted with EtOAc (2 x 20 mL). The combined organic phases were washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo* to yield methyl 4-fluorophenethylcarbamate as a colorless oil (246.4 mg, 83%) which was used in the next step without further purification.

*Methyl 4 fluorophenethylcarbamate **9***: ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.28 – 7.16 (m, 2H), 7.15 – 7.03 (m, 2H), 3.49 (s, 3H), 3.23 – 3.10 (m, 2H), 2.68 (t, *J* = 7.3 Hz, 2H).

To methyl carbamate **9** (147.9 mg, 0.75 mmol), trifluoromethanesulfonic acid (3.26 mL, 37.1 mmol) was slowly added at 0 °C. The resulting mixture was heated at 70 °C for 24 h with stirring. The reaction was quenched by pouring over ice-water (37 mL) and extracted with DCM (2 x 20 mL). The combined organic phases were washed with brine, dried over Na_2SO_4, flitered, and concentrated *in vacuo*. The crude residue was purified via ISCO Combiflash chromatography (silica, 4g; 20-100% EtOAc in hexanes) to yield the product as a white solid (90 mg, 73%).

*7-fluoro-3,4-dihydroisoquinolin-1(2H)-one **2***: ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.11 (s, 1H), 7.53 (dd, *J* = 9.4, 2.8 Hz, 1H), 7.43 – 7.25 (m, 2H), 3.44 – 3.35 (m, 2H), 2.88 (t, *J* = 6.6 Hz, 2H).

***General procedure for synthesis of 3,4-dihydroisoquinolin-1(2H)-ones from 1-indanones via Schmidt reaction**:* To a solution of an appropriate 1-indanone (0.5 mmol) in 2:1 DCM:methanesulfonic acid (4.6 mL) at 0 °C was added NaN_3 (48.8 mg, 0.75 mmol) portionwise over 30 min. The resulting mixture was allowed to warm to rt and stirred overnight. The reaction mixture was quenched with 1 N NaOH (3 mL) at 0 °C, and the DCM layer was removed. The aqueous layer was extracted with DCM or EtOAc (for –OH derivative). The combined organic layers were washed with H_2O and brine, dried over Na_2SO_4, and concentrated *in vacuo*. The *N*-alkyl and *N*-aryl amide regioisomers were separated via ISCO CombiFlash chromatography (silica, 4g; 20-100% EtOAc in hexanes).

*Synthesis of 7-chloro-3,4-dihydroisoquinolin-1(2H)-one (7-Cl-dq, **3**)*: from 6-chloro-1-indanone; yield: 73.2 mg (0.40 mmol, 80%); isomer ratio: *N*-alkyl 26%, *N*-aryl 74%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.12 (s, 1H), 7.77 (d, *J* = 2.4 Hz, 1H), 7.54 (dd, *J* = 8.1, 2.4 Hz, 1H), 7.42 – 7.28 (m, 1H), 3.46 – 3.35 (m, 2H), 2.89 (t, *J* = 6.6 Hz, 2H).

*Synthesis of 7-bromo-3,4-dihydroisoquinolin-1(2H)-one (7-Br-dq, **4**)*: from 6-bromo-1-indanone; yield: 87.5 mg (0.39 mmol, 78%); isomer ratio: *N*-alkyl 27%, *N*-aryl 73%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.12 (s, 1H), 7.91 (d, *J* = 2.2 Hz, 1H), 7.72 – 7.61 (m, 1H), 7.30 (d, *J* = 8.1 Hz, 1H), 3.43 – 3.35 (m, 2H), 2.87 (t, *J* = 6.6 Hz, 2H).

*Synthesis of 7-methyl-3,4-dihydroisoquinolin-1(2H)-one (7-Me-dq, **5**)*: from 6-methyl-1-indanone; yield: 60.9 mg (0.38 mmol, 76%); isomer ratio: *N*-alkyl 44%, *N*-aryl 56%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.88 (s, 1H), 7.65

(d, *J* = 1.9 Hz, 1H), 7.27 (ddd, *J* = 7.7, 2.0, 0.8 Hz, 1H), 7.18 (d, *J* = 7.7 Hz, 1H), 3.43 – 3.25 (m, 2H), 2.84 (t, *J* = 6.6 Hz, 2H).

Synthesis of 7-(trifluoromethyl)-3,4-dihydroisoquinolin-1(2H)-one (7-CF_3-dq, ***6****)*: from 6-(trifluoromethyl)-1-indanone; yield: 72.9 mg (0.34, 68%); isomer ratio: *N*-alkyl 28%, *N*-aryl 72%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.22 (s, 1H), 8.13 – 8.02 (m, 1H), 7.94 – 7.75 (m, 1H), 7.58 (d, *J* = 8.0 Hz, 1H), 3.41 (td, *J* = 6.6, 2.9 Hz, 2H), 3.01 (t, *J* = 6.6 Hz, 2H).

Synthesis of 7-hydroxy-3,4-dihydroisoquinolin-1(2H)-one (7-OH-dq, ***7****)*: from 6-hydroxy-1-indanone; yield: 25.5 mg (0.16 mmol, 31%); isomer ratio: *N*-alkyl 52%, *N*-aryl 48%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.49 (s, 1H), 7.85 (s, 1H), 7.24 (d, *J* = 2.7 Hz, 1H), 7.09 (d, *J* = 8.2 Hz, 1H), 6.84 (dd, *J* = 8.2, 2.7 Hz, 1H), 3.30 (td, *J* = 6.6, 2.8 Hz, 2H), 2.76 (t, *J* = 6.6 Hz, 2H).

Synthesis of 7-methoxy-3,4-dihydroisoquinolin-1(2H)-one (7-OMe-dq, ***8****)*: from 6-methoxy-1-indanone; yield: 67.5 mg (0.38 mmol, 76%); isomer ratio: *N*-alkyl 63%, *N*-aryl 37%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.96 (s, 1H), 7.35 (d, *J* = 2.8 Hz, 1H), 7.22 (d, *J* = 8.4 Hz, 1H), 7.04 (dd, *J* = 8.3, 2.8 Hz, 1H), 3.77 (s, 3H), 3.34 – 3.23 (m, 2H), 2.81 (t, *J* = 6.6 Hz, 2H).

Other Methods

Cloning and mutagenesis. cDNA encoding human PARP10 was obtained from a cDNA library generated for this study. RNA was isolated from HeLa cell lysates using the TRIzol® reagent (Invitrogen) protocol. 5 µg of isolated RNA was reverse transcribed with SuperScript® III Reverse Transcriptase (Invitrogen) to produce cDNA. The catalytic domain (residues 809-1017) of PARP10 ($PARP10_{cat}$) was PCR-amplified from the cDNA library using primers with non-complementary restriction enzyme sites located at the 5' and 3' ends. The amplified product was cloned into pET-28b+ (Novagen) for expression. The construct for His-tagged SRPK2 (human) was obtained from Addgene (Plasmid #39047) in pNIC28-Bsa4 and contains a deletion of internal segment 268-518. The PARP10 catalytic domain L926A (LA-$PARP10_{cat}$) and L926G (LG-$PARP10_{cat}$) mutants were generated using the QuickChange® II XL site-directed mutagenesis kit (Agilent). Plasmids were sequenced from both the 5' and 3' direction to confirm the coding sequence. Human PARP1 full-length was cloned as described previously.[1]

Recombinant protein production and purification. $PARP10_{cat}$ and SRPK2 were expressed in the *Escherichia coli* BL21 pRARE2 strain (EMD Millipore). Cells were first cultured in LB media overnight at 225 rpm and 37°C in an Excella® E24 Incubator (New Brunswick Scientific). One liter of TB media (12 g Bacto Tryptone (BD Biosciences), 24 g Bacto Yeast Extract (BD Biosciences), 0.4% glycerol, 17 mM KH_2PO_4, 72 mM $KHPO_4$, 50 µg/ml kanamycin, 34 µg/ml chloramphenicol) was inoculated with the starting culture and grown to OD_{600} = 0.8 – 1.0 at 225 rpm and 37°C. The temperature was reduced to 16°C and expression was induced by adding isopropyl β-d-thiogalactoside (IPTG) to 0.4 mM. After incubation at 16°C for 18 – 24 h, cells were harvested by centrifugation at 6,000 g for 10 min. The cell pellet was resuspended in lysis buffer (100 mM HEPES, pH 7.5, 0.5 mM tris(2-carboxyethyl)phosphine hydrochloride (TCEP•HCl, Thermo Scientific Pierce), 500 mM NaCl, 10 mM imidazole, 10% glycerol, 1 mM benzamidine, 1 mM phenylmethylsulfonyl fluoride (PMSF), 8.3 mg/L DNAse I (Roche)) at 4°C, subjected to cell lysis using a Sonifier 450 (Branson) at 4°C, and the resulting lysate was clarified by centrifugation at 12,000 g for 30 min at 4°C. Lysates were incubated with pre-washed Ni-NTA agarose resin (50% slurry, Qiagen) with end-over-end rotation at 4°C for 1 h. Following extensive washing with buffer B1+25 (20 mM HEPES, pH 7.5, 0.5 mM TCEP•HCl, 1 mM PMSF, 1 mM benzamidine, 500 mM NaCl, 25 mM imidazole) protein was eluted in buffer B1+200 (20 mM HEPES, pH 7.5, 0.5 mM TCEP•HCl, 500 mM NaCl, 200 mM imidazole) for $PARP10_{cat}$ and B1+100 (20 mM HEPES, pH 7.5, 0.5 mM TCEP•HCl, 500 mM NaCl, 100 mM imidazole) for SRPK2. Fractions containing desired protein were collected and dialyzed to 50 mM Tris-HCl, pH 7.5, 0.1 mM EDTA, 1 mM β-Me, 0.4 M NaCl at 4°C. Protein concentrations were determined by Bradford assay with BSA standards and purity was assessed by PageBlue staining (Pierce) after SDS-polyacrylamide gel electrophoresis (SDS-PAGE). ≥ 90% purity was achieved for $PARP10_{cat}$. ≥ 50% purity was achieved for SRPK2. PARP1 FL was prepared as previously described.[1]

PARP activity assay with 6-a-NAD$^+$ and fluorescence detection. For PARP10 activity assays, 0.01 nmol of PARP10$_{cat}$ enzyme was used to ADP-ribosylate 0.06 nmol of SPRK2 with 100 μM 6-a-NAD$^+$ in 20 μL of reaction buffer (50 mM Tris-HCl pH 8.0, 100 mM NaCl, 4 mM $MgCl_2$, 0.5 mM TCEP•HCl) at 30 °C for 1 h. For PARP1 activity assays, 0.01 nmol of PARP1 FL was automodified in the presence of 100 μM 6-a-NAD$^+$ in 20 μL of reaction buffer above (+ 10 ng/mL activated DNA) at 30 °C for 1 h. Dq compounds were screened in the presence of DMSO at 0.125% for all reactions. The reaction was quenched by the addition of a 3X stock of freshly prepared CB (300 μM of tris[(1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl]amine (TBTA), 3 mM $CuSO_4$, 300 μM sulforhodamine-azide (Click Chemistry Tools), 3 mM TCEP•HCl in 1X phosphate buffered saline (PBS) with 3% SDS; final [rhodamine-azide] = 100 uM) and incubated at rt for 30 min. 4X sample buffer with 5% β-mercaptoethanol (BME) was added. The reactions were fractionation by SDS-PAGE, and in-gel fluoresence detection was performed using a ChemiDoc™ MP Imaging System (Bio-Rad). Coomassie staining was performed as a loading control. Inhibitor dose-response curves were fitted using nonlinear regression analysis (sigmoidal dose-response) in Prism 4 (GraphPad Software). For curves where 100% inhibition could not be achieved due to solubility of analogues, the curves were constrained to a minimum value of zero. Each experiment was repeated at least twice, shown are representative images.

PARP10 auto-ADP-ribosylation activity assay in cells. Auto-ADP-ribosylation levels for PARP10 in HEK293T cells were determined as previously described.[3] HEK293T cells expressing GFP-tagged PARP10 WT or PARP10 L926G and G888W variants were treated with medium containing AO-alkyne (100 μM) and *p*-phenylenediamine (PDA, 10 mM) and incubated for 1 h. The medium was removed and cells were washed with cold (4 °C) PBS and lysed by freezing in N_2 (l) and thawing in buffer containing 25 mM HEPES at pH 7.5, 50 mM NaCl, 10% glycerol, 1% Nonidet P-40 (NP-40), and 1X complete EDTA-free protease inhibitor cocktail (Roche) and subjected to centrifugation at 14,000g for 5 min at 4 °C. Total protein concentration in the lysate was determined by Bradford assay with a BSA standard curve. Lysates containing normalized protein levels were subjected to click conjugation with 9X CB (900 μM of TBTA, 9 mM $CuSO_4$, 900 μM biotin-azide (Biotin-PEG_3-Azide, Click Chemistry Tools), 9 mM TCEP·HCl in 1X PBS with 1% SDS) and incubated at room temperature for 30 min. A 4X sample buffer (with 5% BME) was added prior to fractionation by SDS-PAGE. Immunblot detection with Streptavidin-HRP was used to quantify levels of biotinylated GFP-PARP10. An antibody against GFP was used to quantify total GFP-PARP10 levels.

PARP10 immunoprecipitation (IP) auto-ADP-ribosylation activity assay. IP-auto-ADP-ribosylation assays were performed as described previously[4] with the exception that 6-alkyne-NAD$^+$ (6-a-NAD$^+$)[1], a NAD$^+$ analogue that can be coupled to biotin-azide via the copper(I) catalyzed [3+2] cycloaddition reaction, was used instead of ^{32}P-NAD$^+$. HEK 293T cells were transfected with GFP-WT-, LG-, or GW-PARP10 expression plasmids using the CalPhos™ mammalian transfection kit. Cells were collected 24 h post-transfection and lysed in 250 μL/well cytosolic lysis buffer (CLB: 50 mM HEPES [pH 7.4], 150 mM NaCl, 1 mM $MgCl_2$, 1 mM TCEP, 1% Triton X-100) with cOmplete™ protease inhibitors. Lysates were clarified by centrifugation at 14,000x rpm for 10 min at 4°C. Protein-G magnetic beads (PureProteome™, Millipore; 50μL suspended bead slurry) were incubated with anti-GFP antibody (mouse monoclonal, LifeTechnologies clone 3E6; 0.5μg per mg lysate protein) for 15 min at 25°C, 650 rpm. Sample lysates were added to anti-GFP (mouse monoclonal, LifeTechnologies clone 3E6; 0.5μg per mg lysate protein) conjugated protein-G magnetic beads for 2 h at 4°C with rotation to immunoprecipitate GFP-tagged proteins. Following removal of supernatant, beads were washed once with CLB, three times with CLB + 1 mM NaCl, and once with PARP reaction buffer (PRB: 50 mM Tris-HCl [pH 7.5], 50 mM NaCl, 0.5 mM TCEP, 0.1% Triton X-100) with cOmplete™ protease inhibitors for 5 min per wash. 200 μM 6-a-NAD$^+$ in PRB was added to beads and incubated for 60 min at 25°C/650 rpm. Following removal of 6-a-NAD$^+$, beads were washed twice with PRB containing 500 mM NaCl for 5 min per wash. Click reaction mixture (1 mM $CuSO_4$, 1 mM TCEP, 100 μM TBTA, 100 μM biotin-azide; 25 μL volume) was added to beads and incubated for 1 h at 25°C/650 rpm. Following removal of click reaction mixture, Laemmli sample buffer with 5% β-mercaptoethanol (25μL volume) was added to beads. Samples were heated at 95°C for 5 min and then loaded on 10% SDS-PAGE gels and transferred onto 0.45μm nitrocellulose membranes (Protran®, Amersham). Membrane blots were blocked with 5% milk-TBST for 1 h at room temperature. Blots were probed with either an anti-GFP antibody or streptavidin-HRP. Blots were imaged for chemiluminescent signal on a ChemiDoc MP system (Bio-Rad).

References

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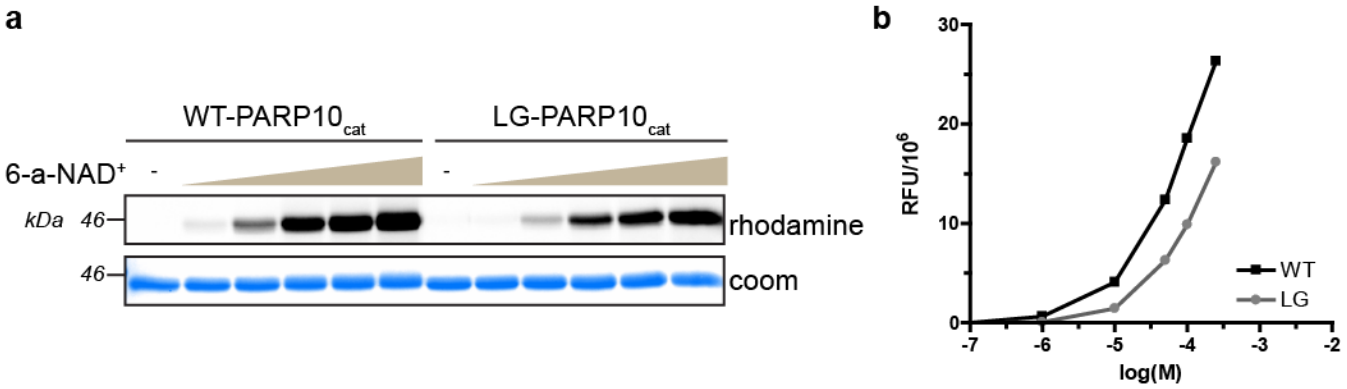
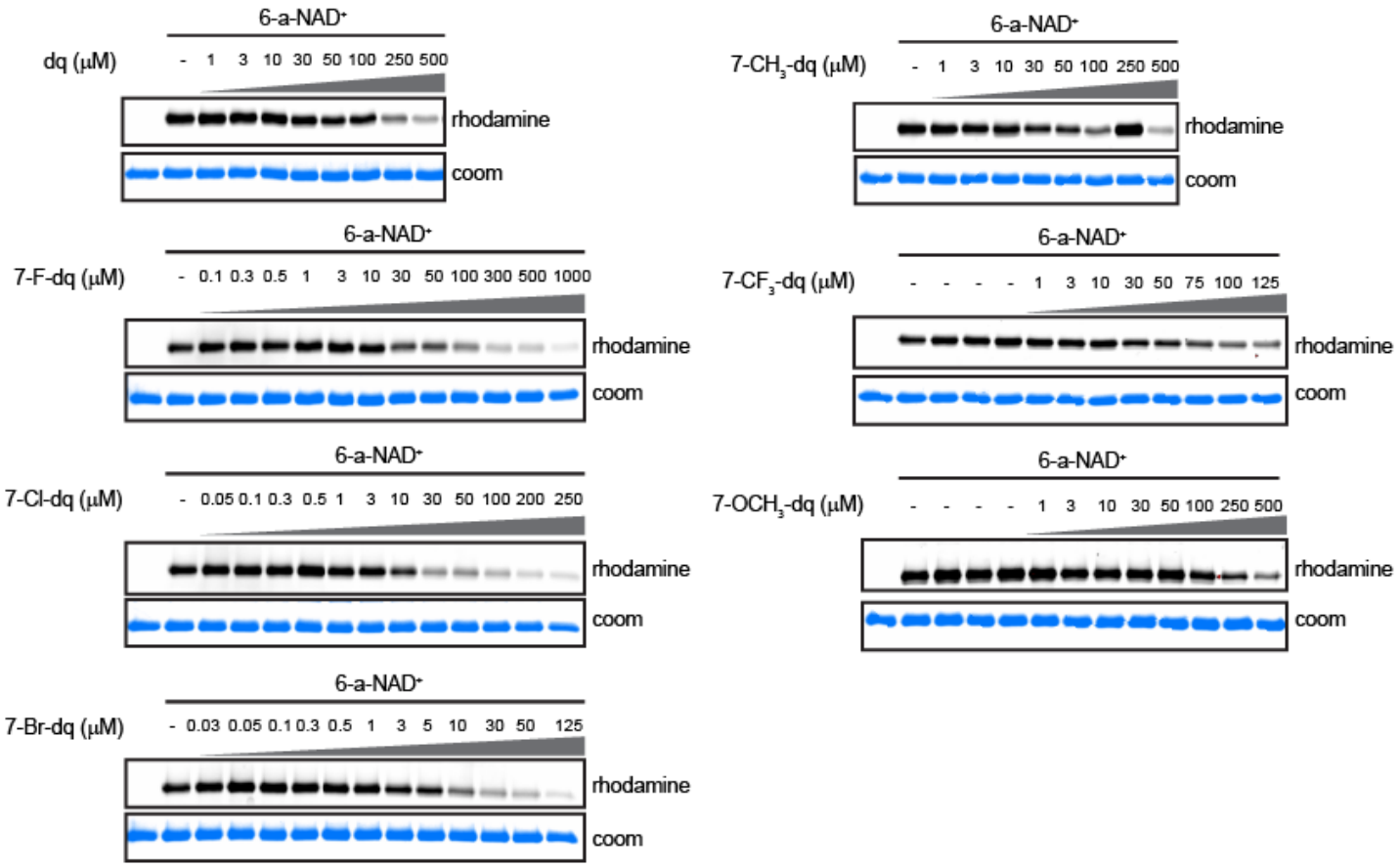
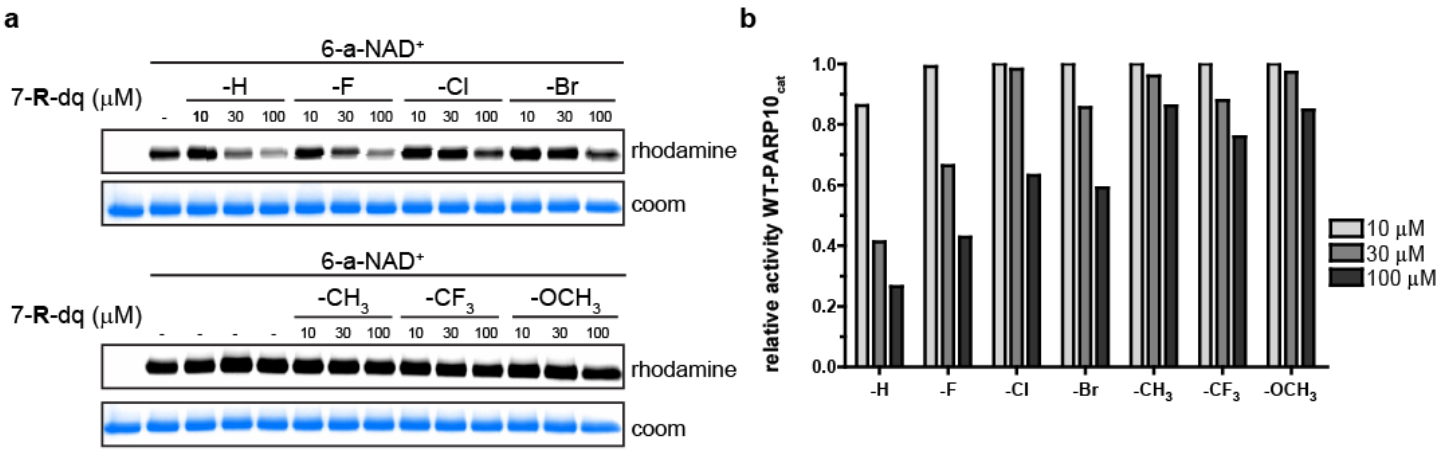
Supplementary Figures



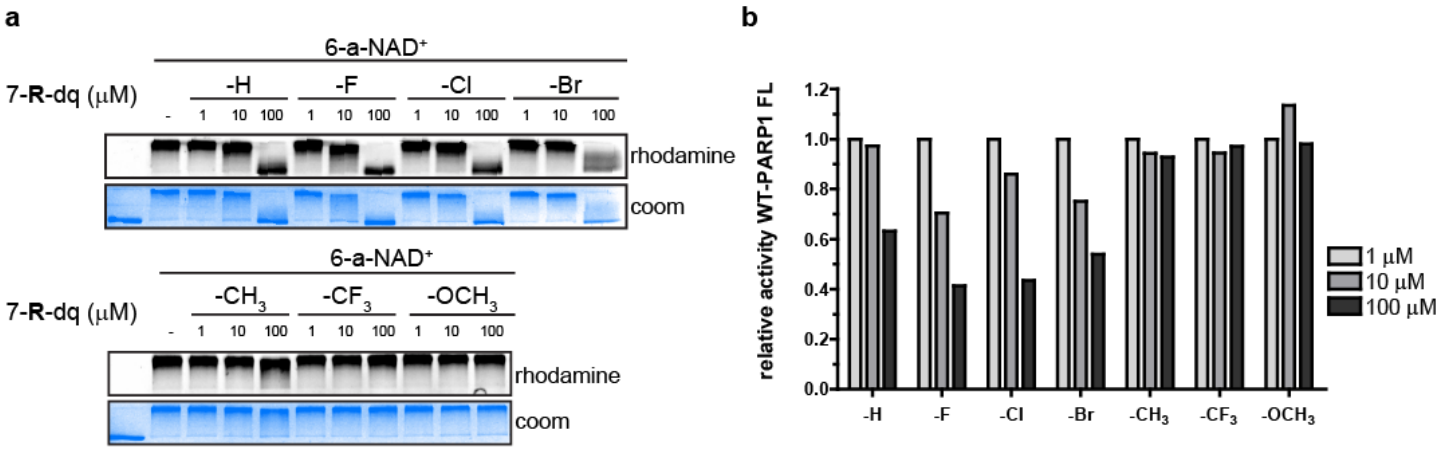
Supplementary Figure 1. LG-PAPR10$_{cat}$ exhibits similar activity to WT-PARP10$_{cat}$. (a) 6-a-NAD$^+$ dose-response curves for WT-PARP10$_{cat}$ and LG-PARP10$_{cat}$. SRPK2 was ADP-ribosylated by PARP10$_{cat}$ with 6-a-NAD$^+$ (0, 1, 10, 50, 100, 250 μM) at 30 °C for 1h. Reactions were conducted in the presence of [DMSO] = 0.125% to mimic conditions used to assay dq compounds. The reactions were subjected to click conjugation with rhodamine-azide (100 μM) prior to separation by SDS-PAGE. In-gel fluorescence detection (rhodamine) was used to quantify the relative amount of ADP-ribose transferred to SRPK2 (46.7 kDa) as a readout of PARP10$_{cat}$ activity. Coomassie (coom) Brilliant Blue staining was used as a loading control. (b) (b) quantitation of results shown in (a).



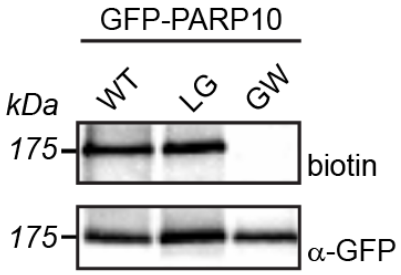
Supplementary Figure 2. Inhibitory dose-response curves for C7-substituted dq analogues against LG-PARP10$_{cat}$. SRPK2 was ADP-ribosylated by LG-PARP10$_{cat}$ with 100 μM 6-a-NAD$^+$ in the presence of indicated dq analogues at varying concentrations at 30 °C for 1h. No inhibitor control contains [DMSO] = 0.125%. The reactions were subjected to click conjugation with rhodamine-azide (100 μM) prior to separation by SDS-PAGE. In-gel fluorescence detection (rhodamine) was used to quantify the relative amount of ADP-ribose transferred to SRPK2 (46.7 kDa) as a readout of LG-PARP10$_{cat}$ activity. Coomassie (coom) Brilliant Blue staining was used as a loading control.



Supplementary Figure 3. Screening of C7-substituted dq analogues against WT-PARP10$_{cat}$. (a) SRPK2 was ADP-ribosylated by WT-PARP10$_{cat}$ with 100 μM 6-a-NAD$^+$ in the presence of indicated 7-**R**-dq analogues (0, 10, 30, and 100 μM) at 30 °C for 1h. No inhibitor control contains [DMSO] = 0.125%. The reactions were subjected to click conjugation with rhodamine-azide (100 μM) prior to separation by SDS-PAGE. In-gel fluorescence detection (rhodamine) was used to quantify the relative amount of ADP-ribose transferred to SRPK2 (46.7 kDa) as a readout of WT-PARP10$_{cat}$ activity. Coomassie (coom) Brilliant Blue staining was used as a loading control. (b) quantitation of results shown in (a).



Supplementary Figure 4. Screening of C7-substituted dq analogues against WT-PARP1. (a) WT-PARP1 was automodified with 100 μM 6-a-NAD^+ in the presence of indicated 7-**R**-dq analogues (0, 1, 10, and 100 μM) at 30 °C for 1h. No inhibitor control contains [DMSO] = 0.125%. The reactions were subjected to click conjugation of rhodamine-azide (100 μM) prior to separation by SDS-PAGE. In-gel fluorescence detection (rhodamine) was used to quantify the relative amount of poly-ADP-ribose (PAR) of automodified WT-PARP1 (113 kDa) as a readout of WT-PARP1 activity. Coomassie (coom) Brilliant Blue staining was used as a loading control. (b) quantitation of results shown in (a).



Supplementary Figure 5. Cellular auto-ADP-ribosylation activity of GFP-tagged full-length LG-PARP10 is similar to WT-PARP10, as measured in cells using AO-alkyne. HEK 293T cells overexpressing either GFP-WT-PARP10, GFP-LG-PARP10, or catalytically dead GFP-G888W (GW)-PARP10 were treated with AO-alkyne (100 μM) and *p*-phenylenediamine (PDA, 10 mM) for 1 h at 37 °C as described previously.[3] Lysates were subjected to click conjugation with biotin-azide (100 μM). Proteins were resolved by SDS/PAGE and detected by Western blot with either Streptavidin-HRP or an antibody against GFP.