Supporting Information

Identifying Poly(ADP-ribose)-Binding Proteins with Photoaffinity-Based Proteomics

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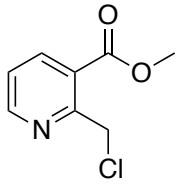
General methods

Solvents were distilled prior to use as follows: tetrahydrofuran (THF) was distilled from sodium benzophenone ketyl; pyridine and dichloromethane were distilled from calcium hydride. All commercial reagents were used without further purification unless otherwise stated. All glassware was dried at 120 °C for 24 hr prior to use. All reactions were carried out under a positive pressure of argon atmosphere and monitored by TLC on silica gel G-25 UV254 (0.25 mm). Spots were detected under UV light. Column flash chromatography was performed with Silicycle grade 70–230 mesh, 60–200 μm, 60 Å silica. The ratio between silica gel and crude product ranged from 100:1 to 60:1 (w/w).

^{1}H (400 MHz) and ^{13}C (101 MHz) NMR spectra were recorded on a 400 MHz NMR spectrometer. All spectra were recorded in deuterated chloroform ($CDCl_3$), deuterated methanol (CD_3OD) or deuterated DMSO (d_6-DMSO). Chemical shifts (δ_H and δ_C) are reported in parts per million (ppm) and coupling constants are expressed in hertz (Hz). Splitting patterns in 1H NMR spectra are designated as s (singlet), dd (doublet of doublets), and m (multiplet).

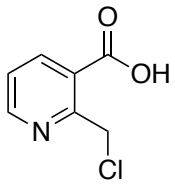
Methyl 2-chloromethylnicotinate



The compound was prepared according to literature procedures.[1] Briefly, a round-bottom flask equipped with stirring was flushed with argon. Methyl 2-methylnicotinate (1.6 g, 10.6 mmol) was added via syringe, followed by dichloromethane (8 mL). Trichloroisocyanuric acid (3.7 g, 15.9 mmol) was added and the mixture was stirred at ambient temperature overnight. The reaction was diluted with dichloromethane (20 mL), then saturated sodium bicarbonate (10 mL) was added slowly. The aqueous and organic layers were separated. The

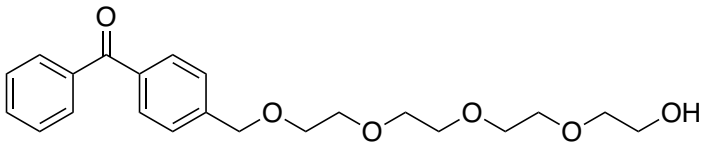
organic layer was washed twice with brine (2 x 10 mL), then dried over sodium sulfate, filtered and concentrated to afford the title compound as a yellow oil (1.687 g, 86%). Observed chemical shifts agree with literature reports.

2-Chloromethylnicotinic acid



Methyl 2-chloromethylnicotinate (0.5 g, 2.7 mmol) was stirred in 5 mL of 1:1 MeOH:10% aq. NaOH at ambient temperature in a round-bottom flask equipped with stirring. After 1 hr, TLC indicated complete consumption of starting material. The crude reaction was washed once with ether (5 mL), then acidified to pH 4 with 10% aq. HCl and extracted with ethyl acetate (5 x 10 mL). The organic layers were dried over sodium sulfate, filtered and concentrated to afford the title compound as a light brown powder: ^{1}H NMR (400 MHz, CD_3OD) δ 8.80 (dd, J = 1.8, 4.8, 1H), 8.40 (dd, J = 1.8, 7.9, 1H), 7.42 (dd, J = 4.9, 7.9, 1H), 5.17 (s, 1H); ^{13}C {^{1}H} NMR (101 MHz, CD_3OD) δ 166.8, 156.2, 151.9, 139.0, 126.2, 123.8; HRMS (ESI–TOF) m/z $(M + H)^+$ calcd for $C_7H_6ClNO_2$ = 172.016532, found m/z = 172.0165

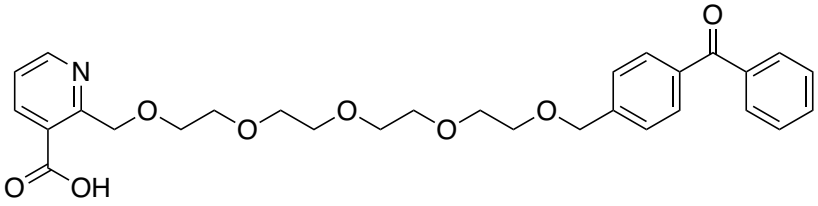
(4-(13-Hydroxy-2,5,8,11-tetraoxatridecyl)phenyl)(phenyl)methanone



A round-bottom flask equipped with stirring was flushed with argon. Tetraethylene glycol (3.88 g, 20 mmol) was added via syringe was azeotropically dried with anhydrous pyridine (2 $\times$ 4.0 mL), redissolved in anhydrous THF (10 mL) and cooled to 0 °C. Sodium

hydride (0.176 g, 4.4 mmol) was added. The mixture was stirred at 0 °C for 30 min, then 4-(bromomethyl) benzophenone (1.1 g, 4 mmol) was added. The mixture was stirred at 0 °C for 30 min, then removed from ice and stirred overnight at ambient temperature. Saturated aqueous ammonium chloride (5 mL) was added slowly to quench the reaction. The organic solvent was removed under reduced pressure. The slurry was diluted with brine (10 mL) and then extracted with dichloromethane (5 × 10 mL). The organic layer dried over sodium sulfate, filtered and concentrated under reduced pressure. The crude product was purified by chromatography (0-10% methanol in 1:1 ethyl acetate:dichloromethane), yielding the title compound as a light brown oil (1.0 g, 32.6 mmol, 65%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.82–7.75 (m, 4H), 7.62–7.56 (m, 1H), 7.51–7.44 (m, 4H), 4.65 (s, 2H), 3.76–3.6 (m, 16H); ^{13}C {^{1}H} NMR (101 MHz, $CDCl_3$) δ 195.8, 142.9, 137.2, 136.2, 131.9, 129.7, 129.5, 127.8, 126.7, 77.3, 77.0, 76.6, 72.1, 70.1, 70.1, 70.1, 69.8, 69.4, 61.1; HRMS (EI) *m/z* $(M)^+$ calcd for $C_{22}H_{28}O_6$ = 388.188590, found *m/z* = 388.18828

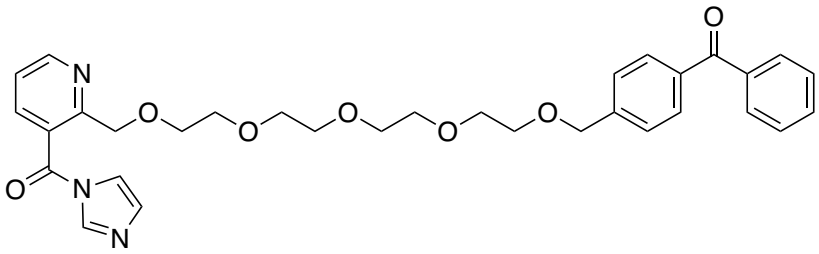
2-(15-(4-Benzoylphenyl)-2,5,8,11,14-pentaoxapentadecyl)nicotinic acid



A round-bottom flask equipped with stirring was flushed with argon. (4-(13-hydroxy-2,5,8,11-tetraoxatridecyl)phenyl)(phenyl)methanone (0.388 g, 1 mmol) was added via syringe, azeotropically dried with pyridine (2 × 4 mL), redissolved in anhydrous THF (10 mL) and cooled to 0 °C. Sodium hydride (0.13 g, 3.3 mmol) was added. The mixture was stirred at 0 °C for 30 min, then 2-(chloromethyl) nicotinate (0.26 g, 2.5 mmol) was added. The mixture was stirred at 0 °C for 30 min, then removed from ice and stirred overnight at ambient temperature.

Saturated aqueous sodium bicarbonate (5 mL) was added slowly to quench the reaction. The organic solvent was removed under reduced pressure. The aqueous slurry was washed with diethyl ether (10 mL) and then acidified to pH 4 with concentrated HCl. The aqueous layer was extracted with ethyl acetate (5 × 10 mL). The combined ethyl acetate layers were dried over sodium sulfate, filtered and concentrated under reduced pressure. The crude product was purified by column chromatography using 5-20% methanol in dichloromethane, yielding the title compound as a brown oil (0.29 g, 0.55 mmol, 55%) ^{1}H NMR (400 MHz, CD_3OD) δ 8.59 (1H, dd, J = 1.7, 4.9), 8.23 (dd, 1H, J = 1.7, 7.8), 7.75 (m, 4H), 7.62 (m, 1H), 7.52 (m, 5H), 7.44 (dd, 1H, J = 5, 7.8), 4.99 (s, 2H), 4.64 (s, 2H), 3.65 (m, 16H); ^{13}C {^{1}H} NMR (101 MHz, CD_3OD) δ 198.3, 158.4, 150.8, 145.1, 140.5, 139.0, 138.0, 133.9, 131.3, 131.1, 129.6, 128.5, 124.4, 73.4, 73.2, 71.6, 71.5, 71.4, 71.4, 71.3, 71.0, 50.0, 49.7, 49.6, 49.4, 49.1, 48.9, 48.5; HRMS (ESI–TOF) m/z $(M + H)^+$ calcd for $C_{29}H_{33}NO_8$ = 524.228444 found m/z = 524.2290

(4-(15-(3-(1*H*-imidazole-1-carbonyl)pyridin-2-yl)-2,5,8,11,14-pentaoxapentadecyl)phenyl)(phenyl)methanone



Anhydrous d_6-DMSO was prepared by drying over 4 Å sieves. 2-(15-(4-Benzoylphenyl)-2,5,8,11,14-pentaoxapentadecyl)nicotinic acid (60 µL, 1 M in anhydrous d_6-DMSO) and carbonyldiimidazole (60 µL, 2 M in anhydrous d_6-DMSO) were combined in a 0.5 mL conical vial equipped with stirring. To monitor reaction progress with ^{1}H NMR, a small aliquot was taken and diluted further in d_6-DMSO. Product formation was measured by integrating the shift of a methylene peak (see ^{1}H NMR spectrum on page S28 for an example).The mixture was

stirred for 1 hr at ambient temperature, sealed with parafilm, then stored at -80 °C. This compound was not isolated. The crude product was used as a 0.5 M stock in the reaction with PAR. The stock should always be under argon atmosphere and warmed up to ambient temperature before use.

Labeling poly(ADP-ribose) with dATP-biotin using OAS1

PAR was synthesized and HPLC-fractionated to defined length according to literature precedent.[2,3] PAR of defined length (3–20 nmol depending on the reaction, ≤100 μM) was reacted with biotin-dATP (Jena BioScience, 3-fold molar excess to PAR), OAS1 (20:1 molar ratio of PAR:OAS1) and poly(I:C)(low MW)(1:1 ratio with OAS1, w/w) in 20 mM Tris pH 7.5, 20 mM $Mg(OAc)_2$, 2.5 mM DTT in a 125 μL or 250 μL reaction, depending on the scale. The reaction was incubated at 37 °C / 750 rpm for 2 hr then purified with DHBB-agarose.

Purification of PAR with DHBB-agarose

m-aminophenylboronic acid–agarose (Sigma, 100 μL of suspension for 20 nmol PAR, 1 vol) was equilibrated with 10 vol of AAEG9 buffer (0.25 M ammonium acetate pH 9.0, 6 M guanidine-HCl, 10 mM EDTA) for 1 min with end-over end rotation. The beads were pelleted with 500 *g* for 2 min and the supernatant was removed. The beads were equilibrated with 10 vol of ddH_2O for 1 min with end-over end rotation. The beads were pelleted with 500 *g* for 2 min and the supernatant was removed. The beads were equilibrated with 10 vol of AAEG9 buffer (0.25 M ammonium acetate pH 9.0, 6 M guanidine-HCl, 10 mM EDTA) for 1 min with end-over end rotation. The beads were pelleted with 500 *g* for 2 min and the supernatant was removed. The reaction containing PAR was diluted with 9 vol of AAEG9 buffer, then added to the beads and incubated for 1 hr at R.T. with end-over-end rotation. The bead-PAR mixture was transferred to a 10 mL disposable gravity column (Bio-Rad). The beads were washed with

10 vol AAEG9 buffer, followed by 10 vol 1 M ammonium acetate pH 9.0. The PAR was eluted with 37 °C ddH_2O (10 x 1 vol). Eluates with substantial absorbance at 260 nm were combined, flash frozen, lyophilized, then resuspended in ddH_2O. A UV-vis spectrum was collected to estimate [PAR].

Acylation of biotin-PAR with (4-(15-(3-(1*H*-imidazole-1-carbonyl)pyridin-2-yl)-2,5,8,11,14-pentaoxapentadecyl)phenyl)(phenyl)methanone

Biotin-PAR (50-100 μM) was mixed with (4-(15-(3-(1*H*-imidazole-1-carbonyl)pyridin-2-yl)-2,5,8,11,14-pentaoxapentadecyl)phenyl)(phenyl)methanone (50 mM) in 0.1 M HEPES pH 8.0, 0.1 M NaCl, 6 mM $MgCl_2$ in a reaction with a total volume of 50 μL. The mixture was incubated at ambient temperature for 3 hr with constant vortexing. The pH of the reaction lowers over time, so aqueous sodium hydroxide (1 μL of 1 M solution) was added periodically to maintain a pH of ~8. After completion, the PAR was purified with DHBB-agarose as described above.

Purification of acylated PAR with HPLC

An Agilent Infinity II HPLC was loaded with 0.1 M TEAA pH 7.5 and acetonitrile into the appropriate solvent ports. The detector was set to record 215 nm and 258 nm. An Infinity Poroshell 120 EC-C18 (Agilent # 693970-902T) was connected and equilibrated with 95% TEAA pH 7.5 and 5% acetonitrile. Aliquots of acylated biotin-PAR (up to 20 nmol) were injected and separated with a gradient (0-10 min, 5% acetonitrile; 10-40 min, 5%-40% acetonitrile). Fractions (0.25 mL) were automatically collected during the entire run. Strongly absorbing fractions were combined based on the number of benzophenones in 1.7 mL tubes. Combined fractions were flash frozen in liquid nitrogen, then lyophilized to dryness. The PAR

was resuspended with 50 µL of TE buffer. A UV-vis spectrum was collected to estimate [PARprolink] with the equation given below.

Determining PARprolink concentration

PARprolink concentration was determined using the following equation: [PARprolink] = [(A_{258}) cm^{-1}]/([13,500 cm^{-1} M^{-1}] $\times$ *n* + 18,000 cm^{-1} M^{-1}) where *n* = # of adenines and 18,000 cm^{-1} M^{-1} is the approximate extinction coefficient of the single, randomly incorporated benzophenone nicotinic acid analogue.

Electrospray ionization mass spectrometry (ESI-MS) analysis

For mass analysis of PARprolink, PARprolink was purified with HPLC as described above, resuspended in ddH_2O, lyophilized again and then resuspended in ddH_2O at a final concentration of 5 µM. For ESI-MS analysis, the resuspended sample was analyzed at a commercial laboratory (Novatia, LLC, Newtown, PA) using a LTQ Orbitrap Velos Pro mass spectrometer (ThermoFisher Scientific) operated at 60,000 resolution. Negative ion mass spectra were deconvoluted using the ReSpect deisotoping algorithm (Positive Probability Ltd) within the Novatia ProMass HR for Xcalibur software. The ProMass HR software was used to determine the monoisotopic (exact) mass from the sample.

Western blot

For each blot, Ponceau S (Sigma-Aldrich #P7170) was used according to the manufacturer's instructions. The following antibodies were used: IRDye 800 CW Streptavidin (Li-Cor #926-32230, 1:3,000 in TBS + 0.15% v/v Tween-20 + 0.06% SDS + 0.02% sodium sodum azide), anti-FLAG (Sigma-Aldrich #F7425, 1:1,000 in TBS-T + 0.02% sodium azide), IRDye 680LT (Li-Cor #926-68021, 1:20,000 in TBS-T).

Cross-linking with recombinant protein

All photolyses were carried out in 1.7 mL or 0.6 mL clear tubes in a Rayonet photoreactor fitted with 16 lamps with maximum output at 350 nm. Photolysis was performed at room temperature on a rotating platform. PARprolink (15-mer, 100 nM) was mixed with the indicated concentration of RNF146-WWE-FLAG or BSA in binding buffer (20 mM HEPES pH 7.5, 100 mM NaCl) in a reaction with a total volume of 30 µL. Reactions were equilibrated on ice for 30 min, then photolyzed for 10 min. Reactions were mixed with 4X SDS sample loading buffer, incubated at 95 °C for 5 min, then separated with SDS-PAGE. Proteins were transferred to nitrocellulose, blocked in 5% non-fat dry milk in TBS for 1 h, then blotted with IRdye 800CW Streptavidin for 1 hr before the data were acquired with an Odyssey infrared imager (Li-Cor).

Cell culture

HeLa cells were cultured at 37 °C in a humidified atmosphere with 5% CO_2 in DMEM (Life Technologies) supplemented with 10% FBS (HyClone).

Preparation of whole-cell extracts

A 10 cm dish was seeded with 1 million HeLa cells in DMEM + 10% FBS and grown at 37 °C + 5% CO_2 for 24 hr prior to harvesting. Cells were washed with ice-cold PBS (3 x 10 mL), then 1 mL of ice-cold NP-40 lysis buffer (50 mM Tris pH 7.5, 150 mM NaCl, 5 mM $MgCl_2$, 1 mM PMSF, 1% v/v NP-40, 1X SIGMAFAST protease inhibitor cocktail) was added. The dish was incubated flat on ice for 15 min, the cells were then scraped into a pre-chilled 1.7 mL tube and incubated at 4 °C with end-over-end rotation for 15 min. The lysate was clarified by

spinning at 20,000 *g* / 4 °C for 10 min. Protein concentration was determined with a Bradford assay (ThermoFisher) using NP-40 lysis buffer as the diluent for the BSA standards.

Cross-linking with recombinant protein in whole-cell extract

All photolyses were carried out in 1.7 mL or 0.6 mL clear tubes in a Rayonet photoreactor fitted with 16 lamps with maximum output at 350 nm. Photolysis was performed at room temperature on a rotating platform. PARprolink (15-mer, 100 nM) was mixed with 7.2 μM or the indicated concentration of RNF146-WWE-FLAG in lysis buffer (50 mM Tris pH 7.5, 150 mM NaCl, 5 mM $MgCl_2$, 1 mM PMSF, 1X SIGMAFAST protease inhibitor cocktail) in a reaction with a total volume of 40 μL. For the "+unlabeled PAR" reaction, 30 μM of 15-mer PAR was also added. Reactions were equilibrated on ice for 60 min, then photolyzed for 10 min. Reactions were mixed with 4X SDS sample loading buffer, incubated at 95 °C for 5 min, then separated with SDS-PAGE. Proteins were transferred to nitrocellulose, blocked in 5% non-fat dry milk in TBS for 1 hr, then blotted with IRdye 800CW Streptavidin for 1 hr, before the data were acquired with an Odyssey infrared imager (Li-Cor). The membrane was then incubated with anti-FLAG and anti-Rabbit 680LT, each at room temperature for 1 hr, before the data were acquired with an Odyssey infrared imager (Li-Cor).

Preparation of HeLa nuclear extracts

Extracts were prepared as described,[4] with modifications. HEPES pH 8.0 was used instead of Tris pH 7.9, 1 mM DTT was added to the low-salt and high-salt buffers, and 1X SIGMAFAST protease inhibitor cocktail was used in all buffers except for the dialysis buffer, where 0.2 mM PMSF was used.

Cross-linking and pull-down in nuclear extract

All photolyses were carried out in 1.7 mL clear tubes in a Rayonet photoreactor fitted with 16 lamps with maximum output at 350 nm. Photolysis was done on ice in a glass dewar. Care was taken to ensure all tubes were equidistant from the UV lamps. Nuclear extract (3 mg/mL) in extract buffer (20 mM HEPES pH 8.0, 100 mM KCl, 0.2 mM EDTA, 1 mM DTT, 20% glycerol, 0.2 mM PMSF) supplemented with PARG inhibitor (PDD 00017273, 100 μM) was incubated on ice for 30 min before the addition of PARprolink (8-mer or ~40-mer, 2 μM) in a reaction with a total volume of 500 μL. For the single length pull-down in Figure 2, the negative control was an 8-mer biotin-PAR (without benzophenone) at the same concentration, which was UV irradiated with the lysates in parallel for the same duration. This negative control accounts for the background cross-linking and non-specific interactions with the streptavidin sepharose. For the comparative pull-down in Figure 3, a bead-only sample was used as a negative control (adding ddH_2O instead of PARprolink). Reactions were equilibrated on ice for 30 min, then photolyzed for 20 min. Reactions were diluted to 0.1 mg/mL with extract buffer in 50 mL tubes, then streptavidin sepharose (60 μL) was added and the mixtures were incubated with end-over-end rotation for 3 hr. The streptavidin sepharose was sedimented by spinning at 1,000 *g* and the supernatant was removed. The streptavidin sepharose was resuspended in 0.5 mL TBS, then transferred to 1.7 mL tubes and washed with SDS wash buffer (5 x 1 mL, 100 mM Tris pH 8, 1% SDS, 250 mM NaCl), urea wash buffer (5 x 1 mL, 100 mM Tris pH 8, 8 M urea), 20% acetonitrile (5 x 1 mL) and TBS (2 x 1 mL), in that order. For each wash, the tubes were incubated with end-over-end rotation for 1 min at ambient temperature, then the sepharose was pelleted with 500 *g* for 2 min and the supernatant was removed.

On-bead digestion and peptide desalting

The streptavidin sepharose was resuspended in denaturing buffer (100 µL, 50 mM Tris pH 7.8, 8 M urea) and incubated with tris(2-carboxyethyl)phosphine (TCEP, 1 mM) at 37 °C / 1,400 rpm for 20 min. 2-chloroacetamide (CAM , 2 mM) was added and samples were incubated at 37 °C / 1,400 rpm for 20 min. TCEP was increased to 2 mM and the samples were incubated for an additional 20 min at 37 °C / 1,400 rpm. Urea was diluted to 4 M with 25 mM ammonium bicarbonate. LysC (1 µg) was added to each sample and the samples were incubated for 2 hr at 37 °C / 1,400 rpm. Urea was diluted to 2 M with 25 mM ammonium bicarbonate, then trypsin (1 µg) was added to each sample and the samples were incubated overnight at 37 °C / 1,400 rpm. Each step of desalting was performed by spinning at 500 g. StageTips were conditioned with methanol (50 µL) followed by StageTip buffer (5% acetonitrile, 0.1% trifluoroacetic acid). The streptavidin sepharose was pelleted by spinning at 1,000 *g* then the supernatant was applied to the StageTip. The StageTips were washed with StageTip buffer (50 µL) then submitted for LC-MS analysis.

nanoLC-MS/MS analyses

Peptide samples were separated on a Thermo Easy-nLC1200 UHPLC instrument (Odense, DK) using 20 cm long fused silica capillary columns (100 µm ID) packed with 3 µm 120 Å reversed phase C18 beads (Dr. Maisch, Ammerbuch, DE). The LC gradient was 90 min with 4−32% B at 300 nL/min. LC solvent A was 0.1% aq. acetic acid and LC solvent B was 0.1% acetic acid, 80% acetonitrile. MS data was collected with a Thermo Fisher Scientific Orbitrap Fusion Lumos Tribrid spectrometer using data-dependent analysis with a 2 sec cycle time for MS1 acquisition and HCD-MS2 collected in the Orbitrap by Top15 precursor selection.

Mass spectrometry data search

Under “raw data”, the .raw files were loaded into MaxQuant version 1.6.2.6. Each file was labeled as its own experiment. Under “group-specific parameters”, multiplicity was set to 1, enzymes were set to LysC and trypsin, the variable modifications acetyl (N-term) and oxidation (M) were allowed. Carbamidomethylation (C) was the only fixed modification. Under “global parameters”, the FASTA file of the human proteome (UniProt KB, downloaded 7/16/2015, 68,554 entries) was added, and label-free quantification (LFQ) and matched between runs were checked. All other parameters were left as standard.

Mass spectrometry data processing

The proteinGroups.txt file generated from the mass spectrometry data search was saved as a .csv, then imported in RStudio for processing. For the single-length PAR analyses, protein identifications were only accepted if two unique peptides appeared in both replicates (e.g., both PARprolink replicates and/or both negative control replicates). To set a threshold for our single-length analysis, we relied upon the signals derived from the 92 known PAR-binding proteins that we manually curated from literature (see below). We found that all known PAR binders were identified with higher intensity in the proteome associated with PARprolink than in the negative control. The lowest-enriched known PAR binder GAPDH (2.5-fold) were verified with biotinylated PAR pull down assay and measured an affinity of ~50 nM for PAR using electrophoretic mobility shift assays (EMSA), indicating that proteins with relatively low enrichment ratios can still be high-affinity binders. Although we chose an arbitrary 2-fold enrichment as the threshold to include all known PAR-binding proteins in our analysis, the relative fold enrichment of each hit is indicated in Table S1, where 95% of the hits were >6.7-fold enriched and 90% >23-fold enriched. For the comparative pull-down, protein identifications were only accepted if two unique peptides appeared in both replicates (e.g.,

both 8-mer PARprolink replicates, both 40-mer PARprolink replicates, and/or both bead alone replicates). In addition, we only consider proteins that were >2-fold more abundant in the PARprolink pull-downs (8- and/or 40-mer) versus the bead alone negative control. Length-specific PAR binders were defined as $|\log_2(\text{40-mer PARprolink} / \text{8-mer PARprolink})| > 2$.

In both analyses, proteins that contained similar peptides and could not be differentiated based on MS/MS analysis alone were grouped as a single entry. In these cases, the protein IDs, gene names, and gene symbols in Table S1 indicate all possible proteins for the ≥2 unique peptides (*e.g.* HNRNPA1;HNRNPA1L2). Peptide intensities were calculated with MaxLFQ. To calculate enrichment ratios for proteins detected in only one type of pull-down, 100,000 was added when the intensity equaled zero because the lowest detection limit for the orbitrap LC-MS is ~100,000. The *P*-values were calculated with an independent, two-sample t-test. Volcano plots of the $-\log_2$ LFQ intensity ratios (PARprolink / negative control for single-length PAR pull-down, Figure 2b; 40-mer PARprolink / 8-mer PARprolink for the comparative pull-down, Figure 3a) and $-\log_{10}$ *P*-values were generated with the EnhancedVolcano R package.

Compiling list of known PAR binders

To compile a list of known PAR binders, we surveyed the literature for evidence of direct PAR-protein interactions (see references 19 and 20 in the main text for reviews on the topic). We considered interactions to be direct when a purified protein (full-length or fragment) bound to purified PAR in a qualitative or quantitative binding assay. We did not consider native pull-downs from cell extracts because proteins identified therein could be PARylated or arise from indirect PAR-protein-protein interactions. The full list of known PAR binders and the PubMed IDs of the corresponding references can be found in the “known PAR binders” sheet in Table S1.

Comparative analyses with other studies

To calculate overlaps between PAR-binding candidates from this study and proteins from other studies in RStudio, gene symbols from each study were converted to character vectors. For rows with multiple gene symbols (*e.g.* HNRNPA1;HNRNPA1L2) only the first gene symbol (*e.g.* HNRNPA1) was included. The number of overlapping gene symbols between two studies was then determined with the %in% function. The significance of each overlap was calculated with a Fisher's exact test and a z-score. For the full list of overlaps and their statistics, see the "Overlaps with other studies" sheet in Table S1.

For the copy number analysis, copy numbers are derived from Supplementary Table S1 of Hein *et al.*, 2015.[5] In cases of ambiguity (multiple copy numbers measured for different proteins isoforms of a single gene, proteins with similar peptides that could not differentiated with MS/MS analysis), the largest copy number was used as the copy number for that row. Copy numbers and mean PARprolink intensity (column J of the "Single-length PAR pull-down" sheet in Table S1) were plotted in Prism 8.0 (Figure S3c). R^2 was calculated using linear regression. Copy numbers for each PAR-binding candidate can be found in the "Single-length PAR pull-down" and "8-mer vs. ~40-mer pull-down" sheets in Table S1. N.D. indicates a copy number was not determined that PAR-binding candidate.

To identify the possibility of off-target binding through the photo-cross-linker benzophenone in our dataset collected in HeLa extracts, we have compared our PAR-binding candidates to a published HeLa dataset of proteins that are captured by benzophenone alone after UV irradiation.[6] In this study, they used the quantitative proteomics technique SILAC to identify a list of benzophenone-associated proteins. Only 6 out of 743 PAR-binding candidates are benzophenone binders (log2 SILAC ratio > 1 as defined by Kleiner *et al*.; see Table S1), suggesting these proteins may be false positives of our screen. SILAC ratios were obtained

from the SI_Kleiner_et_al_-_Excel_tables_SILAC_labelling_A549_HeLa.xlsx file (downloaded from https://www.department.ch.tum.de/oc2/research/resources/data/). For cases where proteins with similar peptides could not be differentiated with MS/MS analysis, the largest SILAC ratio was used for that row. PAR-binding candidates with a SILAC ratio > 1 (Benzophenone/DMSO) indicate the protein is labeled non-specifically by benzophenone, and therefore may be a false positive. SILAC ratios for each PAR-binding candidate can be found in the "Single-length PAR pull-down" and "8-mer vs. ~40-mer pull-down" sheets in Table S1. N.D. indicates that PAR-binding candidate was not detected in their experiment.

Plasmids

When selecting proteins for validation, we limited ourselves to proteins that are <100 kDa (to increase the likelihood that the protein could be expressed in *E. coli*) with preference given to proteins where there was literature precedent for their purification. Plasmids used in this study are as follows: pSAT1-RNF146-WWE-FLAG, pHis-TEV-AK2, pET-SUMO-CAPRIN1, pET-SUMO-G3BP2, pET-SUMO-DDX6, pET28a-PARP1. pSAT1 is a pBAT4-derived vector encoding a N-terminal 6x-HisSUMO tag. pHis-TEV-AK2 was purchased from Addgene (#38829). pET30-2-GAPDH was purchased from Addgene (#83910). pET-SUMO-CAPRIN1, pET-SUMO-G3BP2 and pET-SUMO-DDX6 were from J. Paul Taylor (St. Jude's). pET28a-PARP1 was from John Pascal (Université de Montréal).

Expression and purification of UHRF1

Plasmid containing full-length UHRF1 (pEXP-CT) was transformed into *E. coli* strain T7 Express (New England Biolabs). Cells were grown at 37°C in LB media supplemented with ampicillin to an O.D. 600 between 0.5-0.7. At this point, protein expression was induced with 0.2 mM IPTG and the temperature reduced to 15°C. Cells were harvested after ~16 h. Pellets

were resuspended in lysis buffer (500 mM NaCl, 20 mM Tris-HCl pH 8.0, 10 mM Imidazole pH 8.0 and 10% Glycerol) and lysed using an Emulsiflex-C5 homogenizer (Avestin). Clarified lysates were applied to IMAC nickel affinity resin (Bio-Rad). Resin were washed and bound protein eluted with lysis buffer supplemented with 250 mM Imidazole. The sample was then applied to a HiLoad 26/600 S200 Superdex column (GE Healthcare) equilibrated with running buffer (20 mM Tris-HCl pH 8.0 and 250 mM NaCl). Protein purity was confirmed by SDS-PAGE.

Expression and purification of RNF146-WWE, AK2, CAPRIN1, G3BP1/2 GAPDH and DDX6

Proteins were expressed in *Escherichia coli* strain DE3 Rosetta. PARP1 was expressed and purified according to Langelier and coworkers.[7] For RNF146, AK2, CAPRIN1, G3BP2, GAPDH and DDX6, transformed cells were grown in LB supplemented with the appropriate antibiotics at 37 °C / 200 rpm to an OD_{600} of ~0.8 , cells were chilled on ice for 1 h, induced with 0.3 mM IPTG, then expressed at 16 °C / 200 rpm for 16-20 h. Cells were harvested by spinning at 3,000 *g* for 30 min, then flash-frozen and stored at -80 °C until purification. All purification steps were performed on ice or at 4 °C. Cell pellets were resuspended in lysis buffer (20 mM sodium phosphate pH 7.5, 300 mM NaCl, 25 mM imidazole, 0.5 mM TCEP, 5% glycerol) supplemented with 1X SIGMAFAST protease inhibitor cocktail, then lysed by sonication. For CAPRIN1, G3BP2, GAPDH and DDX6 the lysis buffer also contained 5 mg of RNase A (ThermoFisher) per L culture. Lysate were clarified by spinning at 24,000 *g* for 30 min. Lysates were applied to HisTrap columns (Cytiva) or HisPur resin (ThermoFisher), washed with lysis buffer, then eluted in lysis buffer containing 300 mM imidazole. RNF146-WWE-FLAG was desalted into lysis buffer, then the 6xHisSUMO tag was removed by incubation with 6xHis-tagged SUMO endopeptidase at 4 °C overnight. The tag and protease

were removed with a second round of Ni-NTA chromatography. Untagged RNF146-WWE-FLAG was further purified by gel filtration with a HiLoad 16/600 Superdex 200 pg (Cytiva) using gel filtration buffer 1 (20 mM Tris pH 7, 200 mM NaCl, 1 mM DTT, 5% glycerol). CAPRIN1 was further purified with a Q FF column (Cytiva) using low salt buffer (50 mM HEPES pH 7.5, 50 mM NaCl, 0.5 mM TCEP) and high-salt buffer (50 mM HEPES pH 7.5, 1 M NaCl, 0.5 mM TCEP). CAPRIN1, G3BP2, GAPDH and DDX6 were further purified by gel filtration with a HiLoad 16/600 Superdex 200 pg (Cytiva) using gel filtration buffer 2 (50 mM HEPES pH 7.5, 200 mM NaCl, 0.5 mM TCEP, 10% glycerol). All chromatography steps were performed on an NGC chromatography system (Bio-Rad).

Biotin-PAR pull-down

A mixture of PAR (2- to ~100-mer) was labeled with biotin using ELTA[3] and purified with DHBB-agarose as described above. Protein (5 μM) was mixed with PAR (50 μM, monomeric concentration) and binding buffer (20 mM HEPES pH 8, 100 mM NaCl, 0.5 mM EDTA, 0.5% v/v NP-40) in a reaction with a total volume of (40 μL). Samples were incubated on ice for 1 hr. An aliquot (10 μL) was taken and mixed with SDS sample loading buffer as the input. Magentic streptavidin beads (10 μL, Pierce) were equilibrated with 1X binding buffer, then the samples were added to the beads and the mixtures were incubated with end-over-end rotation at 4 °C for 1 hr. The beads were washed once with binding buffer, then the protein was eluted with 1X SDS sample loading buffer supplemented with 20 mM biotin by incubating at 95 °C for 10 min. Inputs and elutions were separated with SDS-PAGE, then detected with the SimplyBlue total protein stain (ThermoFisher).

For pull-downs with auto-modified PARP1, PARP1 was combined with an 18bp dsDNA duplex with the sequence 5’- (5 μM) and NAD^+ was then added at the indicated concentrations.

Reaction were incubated at ambient temperature for 10 min, then biotin-PAR was added. The remaining steps of the procedure were performed as described above.

Cy5-PAR electromobility shift assay

PAR (4-, 8- 16- or 32-mer) was labeled with Cy5 using ELTA[3] and purified with DHBB-agarose and ion-pairing C_{18}-HPLC as described above. Cy5-PAR (10 nM) was mixed with protein at the indicated concentration in binding buffer (10 mM Tris pH 7.5, 100 mM KCl, 1 mM EDTA, 0.1 mM DTT, 0.01 mg/mL BSA, 0.01% w/v OrangeG, 5% v/v glycerol) and incubated at ambient temperature for 1 h. Samples were separated with a native 5% tris-acetate polyacrylamide gel (37.5:1 acrylamide:bis-acrylamide ratio) by applying 10 V per cm of gel in tris-acetate buffer (40 mM Tris, 2.5 mM EDTA, 20 mM acetate pH 7.8) for 45 min. Cy5 signal was detected with a Typhoon (Molecular Biosciences). Cy5 signal was quantified with ImageStudio (Li-Cor), then plotted in Prism 8.0 (GraphPad). Dissociation constants were calculated with a sigmoidal dose-response curve by measuring the EC_{50} (K_D ~ [protein] at which half of the Cy5-PAR is bound).

Protein Domain and Functional Enrichment Analysis

The Pfam protein domain enrichments were calculated with the DAVID functional annotation tool (https://david.ncifcrf.gov/summary.jsp). The enrichments and *P*-values for Tri-RGG, WWE, and macrodomain were calculated manually with an R script that determines *P*-value with the EASE score, which is the same modified Fisher's exact test used by DAVID. g:Profiler (https://biit.cs.ut.ee/gprofiler/gost) was used for functional enrichment analysis of the 743 PAR hit genes (2 unique peptides identified in both replicates, PARprolink / control ratio > 2), resulting in a list of 1935 statistically significant enriched terms (Benjamini-Hochberg FDR < 0.05). The hit genes list was treated as an unordered query, and statistical tests were

conducted within a statistical domain scope of only annotated genes, selecting terms with sizes between 2 and 500 genes, considering the GO molecular function, GO cellular component, GO biological process, KEGG, Reactome and WikiPathways data sources. The Ensembl ID with the most GO annotations were chosen for all ambiguous genes (ABCF2, CSNK1E, FKBP4, LUC7L2, MATR3, PDE8, TARDBP). The STRING network was produced with Cytoscape (v3.8.0) using a confidence cut-off of 0.8. Functional enrichment analysis was performed in Cytoscape and nodes were colored according to metabolic pathways (hsa01100), RNA binding (GO.0003723), chromosome organization (GO.0051276) and DNA repair (GO.0006281).

Isoelectric point and molecular weight analysis

Gene lists (see Table S1) were converted from gene symbols to RefSeq peptide sequences in the FASTA format. The molecular weights and isoelectric points were then calculated with the IPC stand-alone Python script.[8] The isoelectric point value used is the average of the seventeen methods used by IPC for p*I* calculations. Violin plots were produced with Prism 8.0 (GraphPad).

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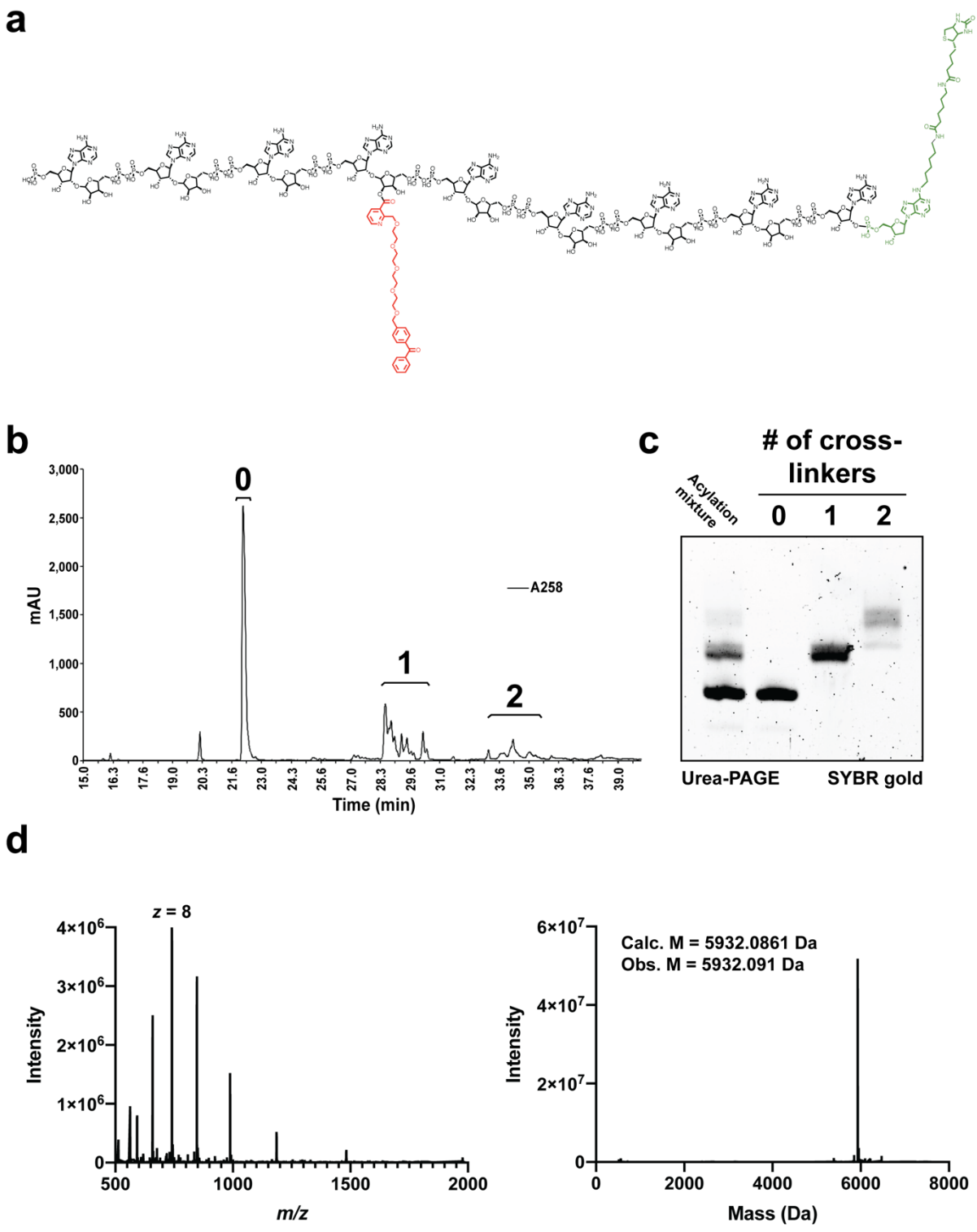


Figure S1. Characterization of a PAR photoaffinity probe, PARprolink. (a) Structure of the 8-mer PARprolink used for proteomics experiments. Since the nicotinic acid analogue is randomly inserted at -OH, this structure depicts one of 26 possible isomers. (b) C_{18}-reverse phase HPLC UV chromatogram of a biotin-8mer PAR mixture composed mostly of 0, 1 and 2 nicotinic acid analogues. Fractions corresponding to biotin-PAR + 1 nicotinic acid analogue (peak 1) were combined and used in photo-cross-linking experiments in Figure 2. (c) Urea-PAGE demonstrating the purity of HPLC fractions from S1b. (d) ESI-MS spectrum (left) and deconvoluted spectrum of purified PARprolink (right).

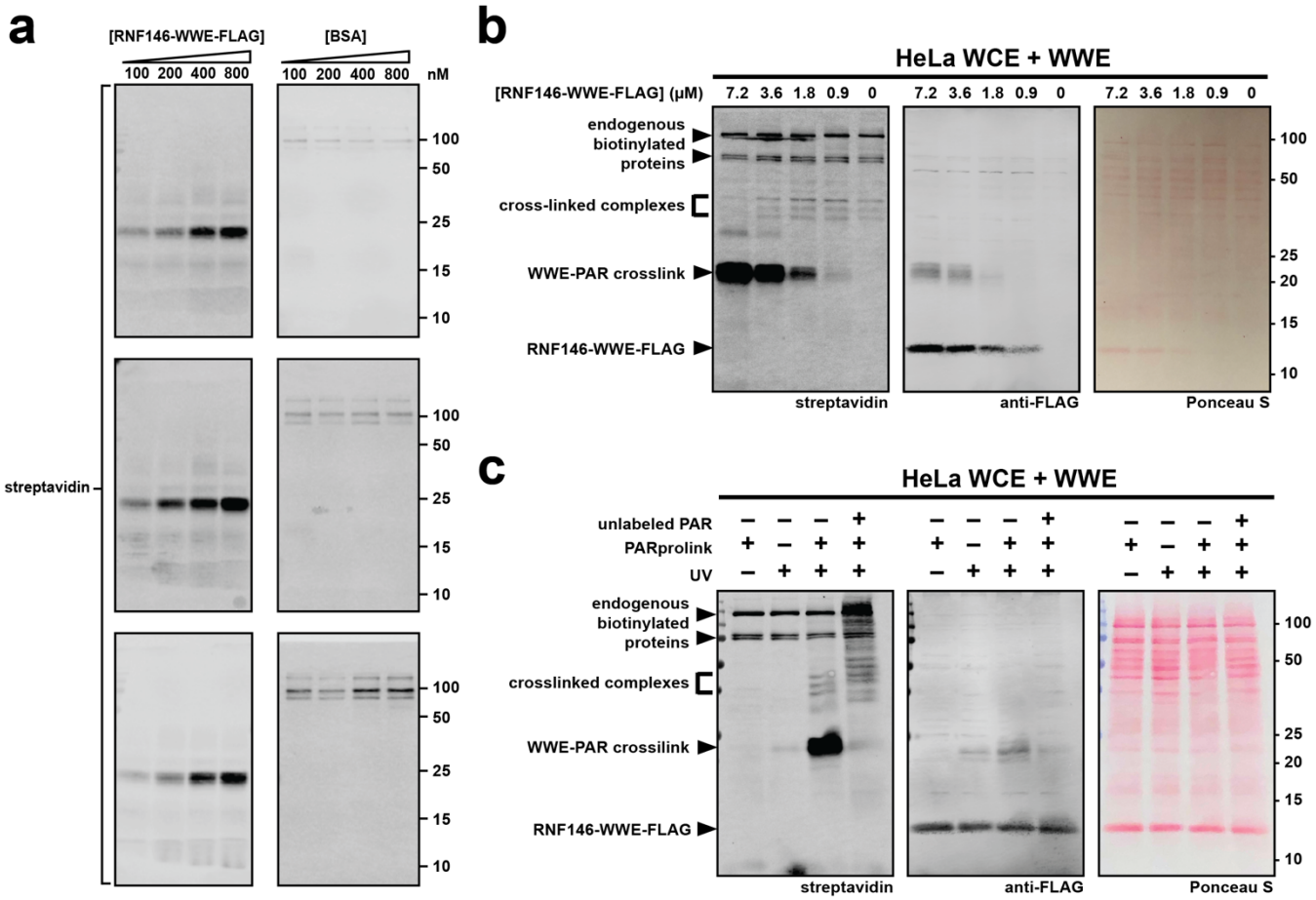


Figure S2. PAR photoaffinity probes capture a PAR-binding domain. (a) Full western blots from Figure 1c. (b) Western blot of photo-cross-linking reactions with varied concentrations of recombinant RNF146-WWE-FLAG in HeLa whole-cell extract. (c) Western blot demonstrating the specificity of the PARprolink-WWE cross-link via competition with unlabeled PAR.

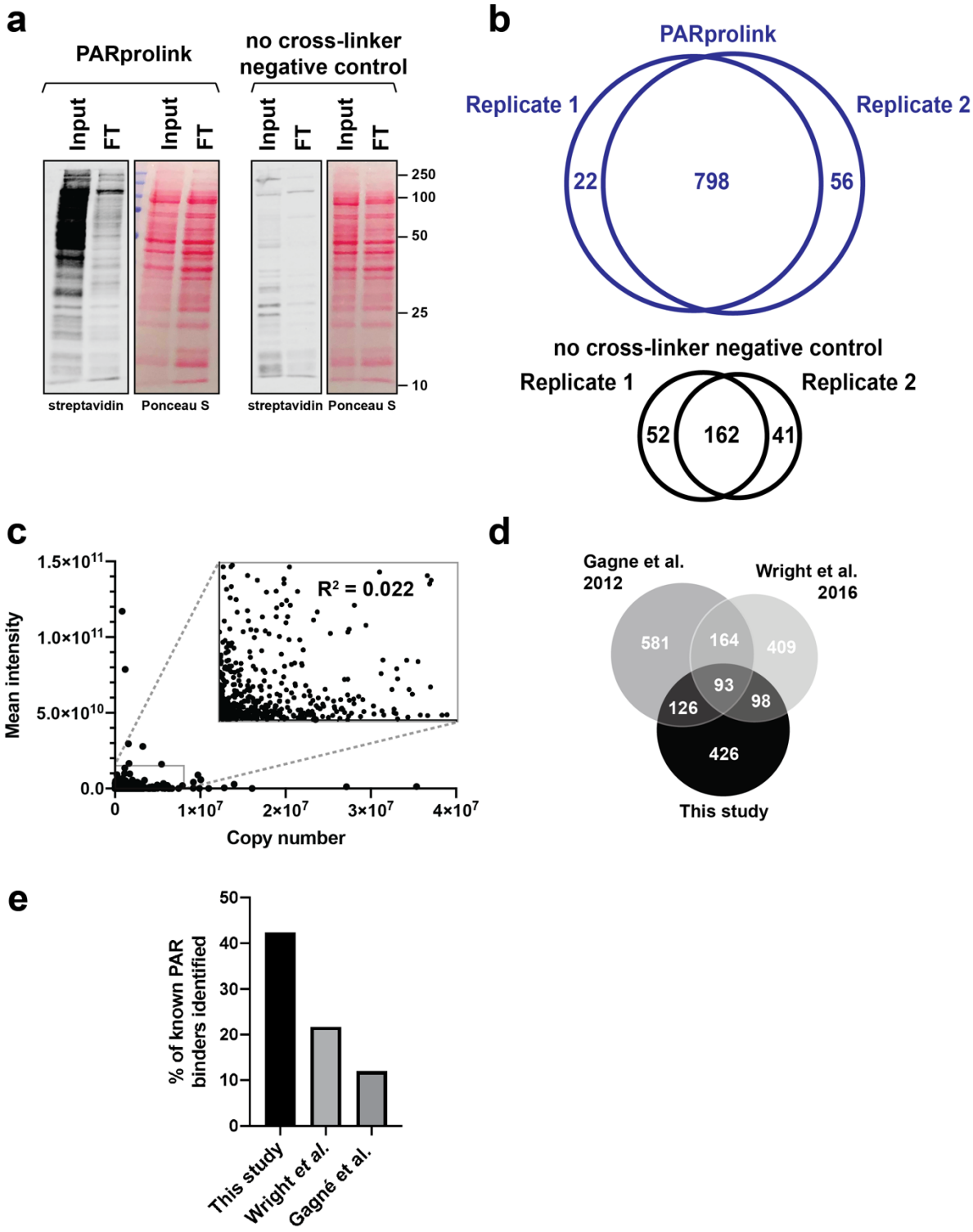


Figure S3. Photo-affinity-based isolation of the PAR-binding proteome in HeLa nuclear extract. (a) Representative western blots of the inputs and flow throughs from the samples submitted for LC-MS/MS. ”Input” is the sample after UV cross-linking, but before addition of streptavidin agarose. “FT” is the flow through (supernatant) after streptavidin pull-down. (b) Venn diagrams depicting the overlap between two replicates of the photoaffinity PAR and no cross-linker control pulldowns. (c) Scatter plot of the proteins identified in our study, depicting the correlation between HeLa protein copy numbers (from Hein *et al.*, 2015)[5] versus the mean intensity observed in the PARprolink replicates. The R-squared value was calculated by a linear regression fit in Prism 8.0. (d) Venn diagram depicting the overlap between the proteins identified in our study and previously reported PAR interactomes. (e) Percentage of the 92 known PAR binders identified in our study and the previous PAR interactome studies.

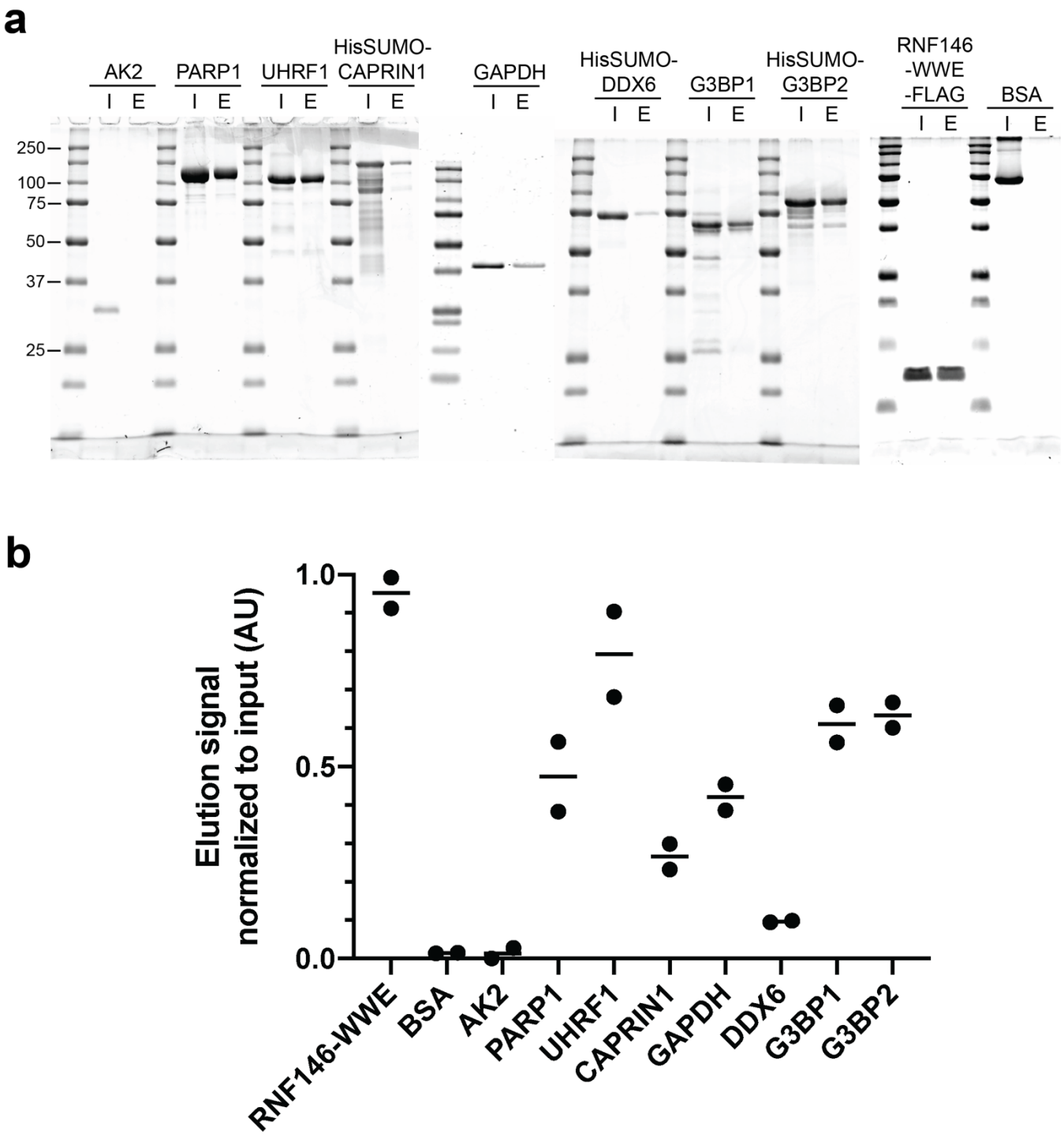


Figure S4. Biotin-PAR reverse pull-downs using a mixture of PAR lengths. (a) Uncropped gels from Figure 2c, I = input, E = elution. The data shown are representative of two independent experiments. (b) Quantification of biotin-PAR pulldown efficiencies from both replicates.

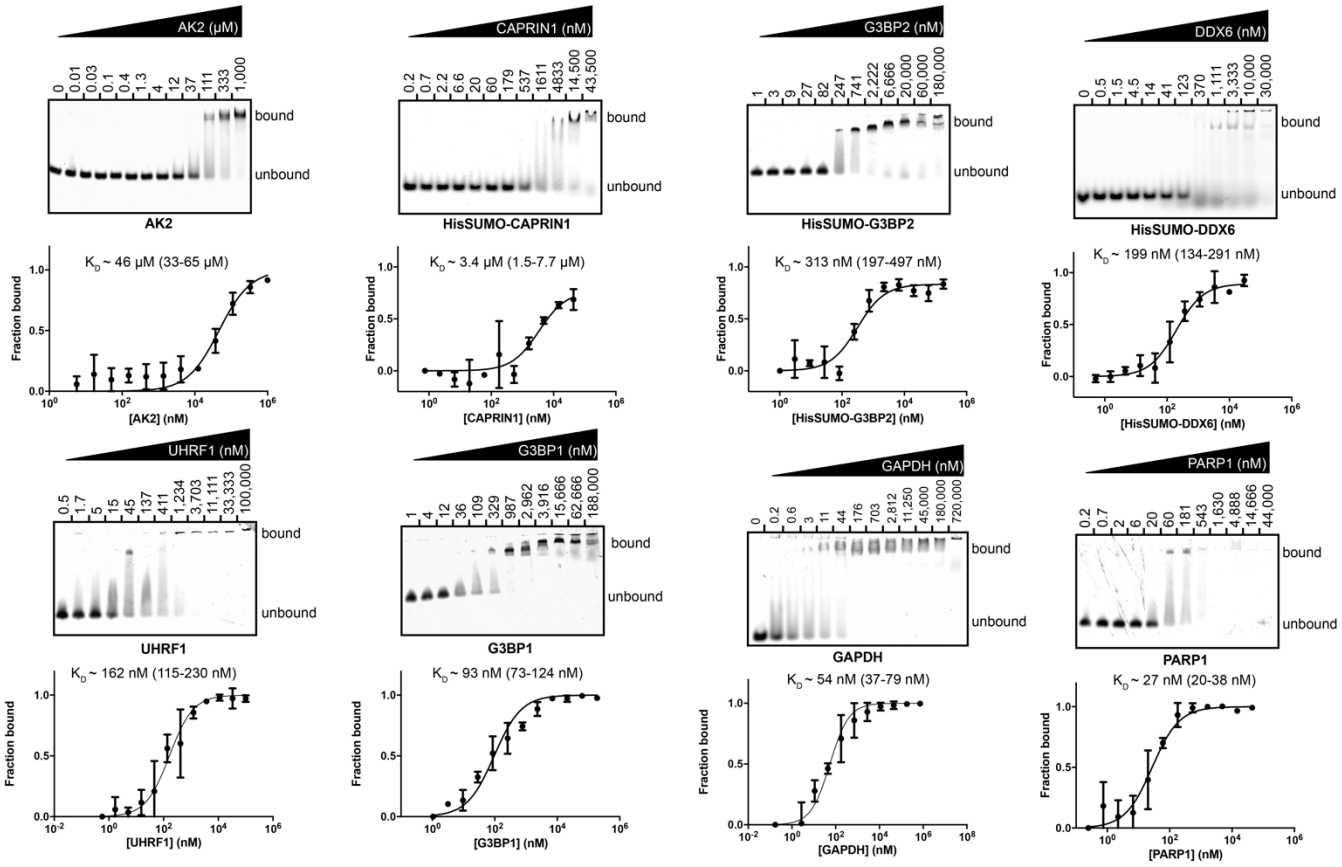
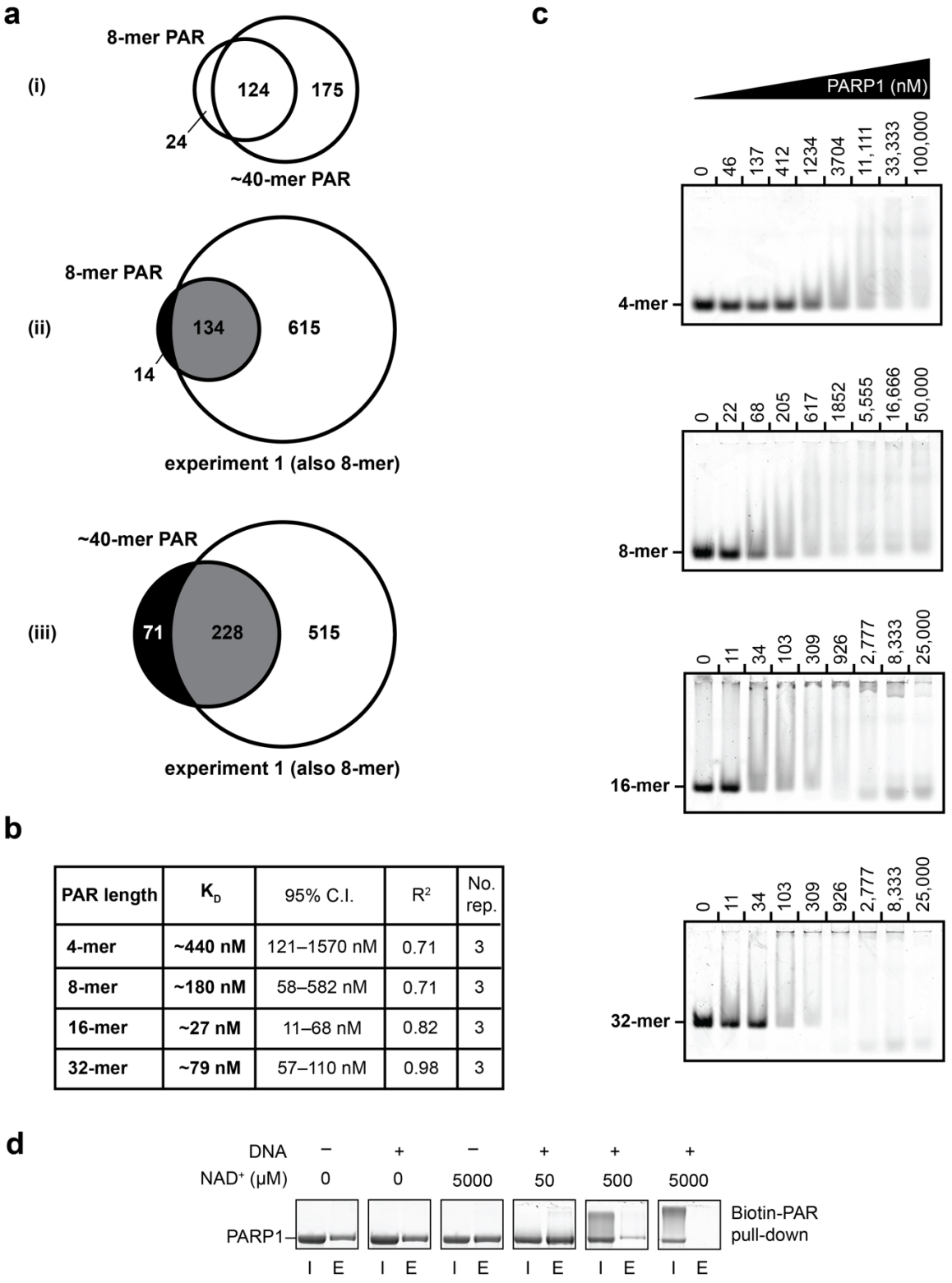


Figure S5. Representative EMSA gels with eight PAR-binding candidates identified in the proteomics experiment. Data were quantified with ImageStudio and plotted with Prism 8 (values plotted are mean ± s.d., n = 3). To calculate dissociation constants, the plotted data were fit with a sigmoidal dose-response curve to calculate the EC_{50} (~K_D = [protein] at which half of the PAR is bound). Values in parentheses indicate the 95% confidence interval for the fit of the EC_{50}.



b

PAR length	K_D	95% C.I.	R^2	No. rep.
4-mer	**~440 nM**	121–1570 nM	0.71	3
8-mer	**~180 nM**	58–582 nM	0.71	3
16-mer	**~27 nM**	11–68 nM	0.82	3
32-mer	**~79 nM**	57–110 nM	0.98	3

Figure S6. (a) Venn diagrams showing (i) the overlap between PAR-binding candidates identified in the 8-mer pulldown (148) and the ~40mer pull-down (299). These proteins are defined as candidates because two unique peptides were present in both replicates, and the intensities were >2-fold enriched over the negative control, and (ii & iii) the overlap between proteins from the 8-mer and ~40-mer PAR pull-downs compared to proteins identified in single-length experiment shown in Fig. 2. (b) Summary of the binding assay data from EMSAs with PARP1 and four different lengths of PAR. (c) Representative gels from the data summarized in panel b. (d) Non-covalent biotin-PAR pull-down with purified, auto-PARylated PARP1, I = input, E = elution.

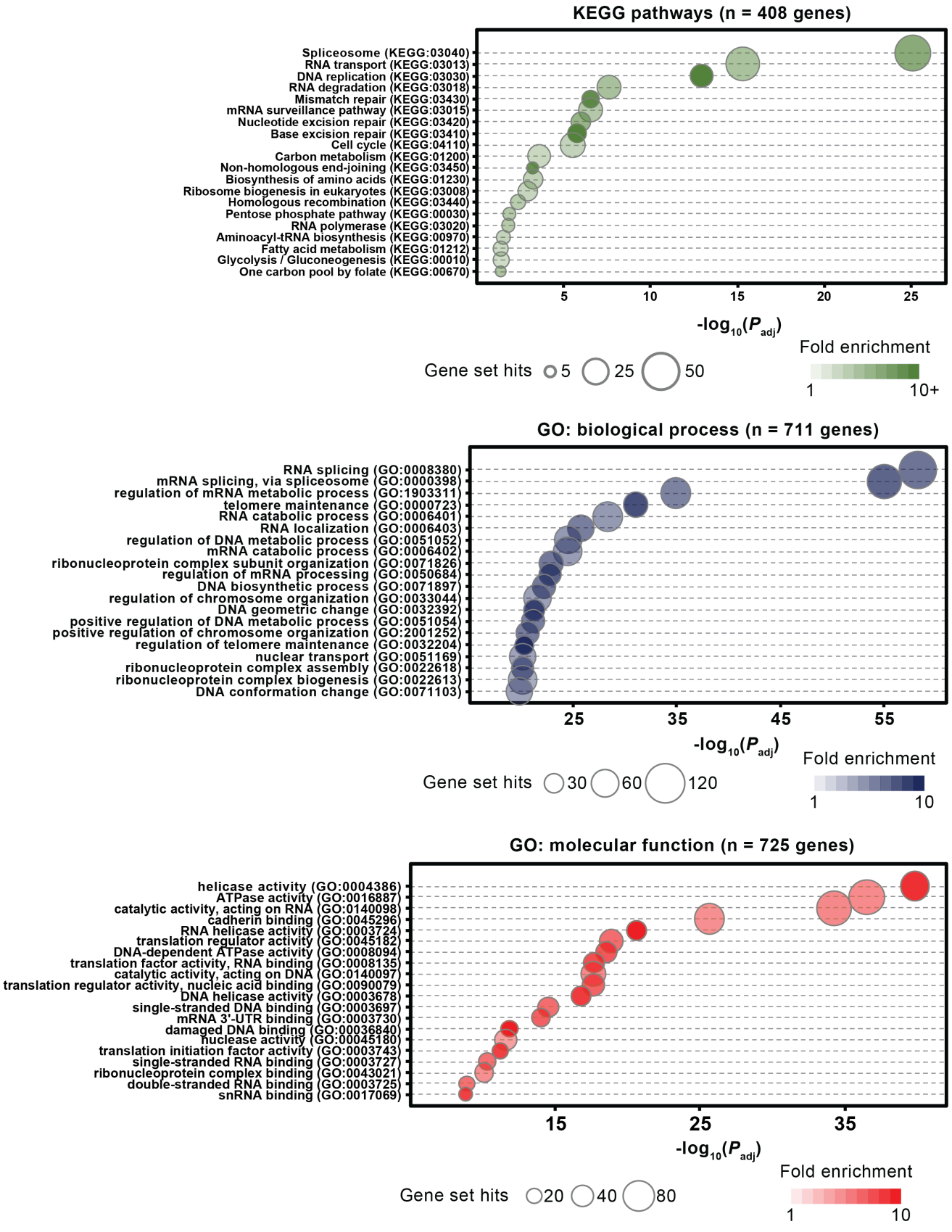


Figure S7. Top 20 enriched gene ontology terms for KEGG pathways, biological processes and molecular functions identified using g:Profiler (P < 0.05, with Benjamini-Hochberg FDR correction) among all 743 PAR-binding candidates from Figure 2b.

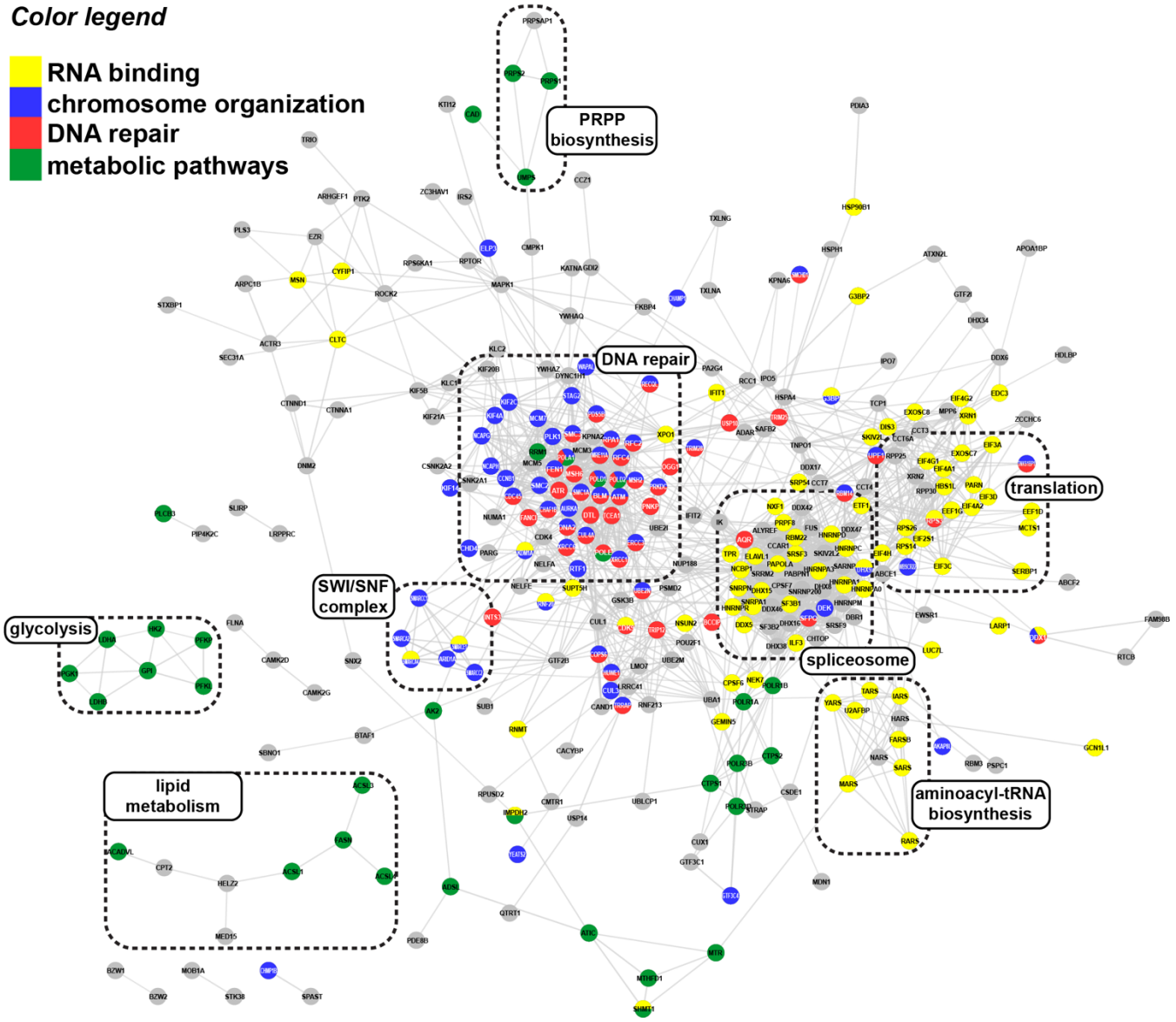


Figure S8. STRING network of high-confidence PAR-binding candidates (enrichment ratio > 8, $P < 0.05$, proteomics data from Figure 2b, 416 genes) with genes annotated.

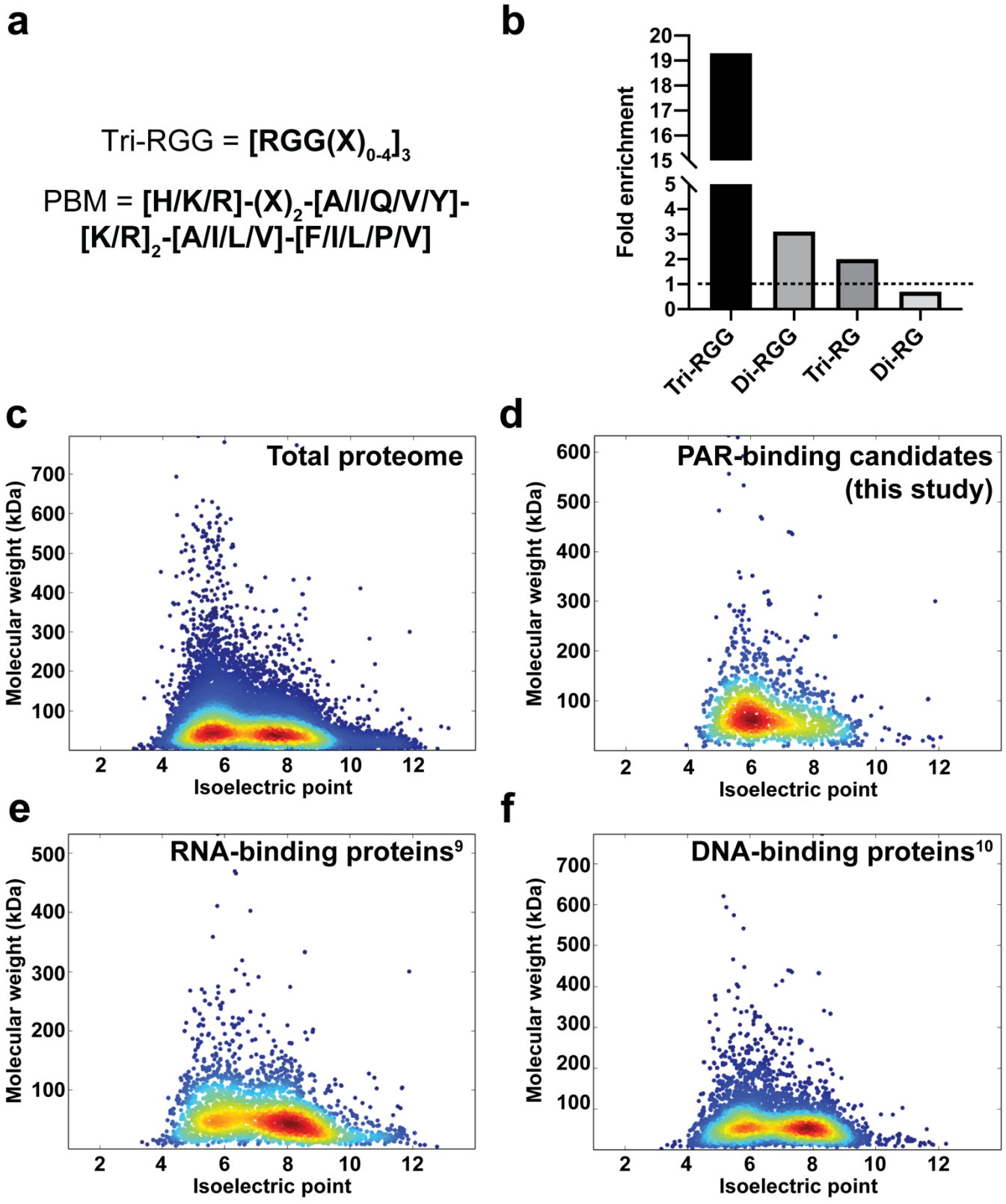


Figure S9. (a) PAR-binding sequence motifs reported in previous literature (Tri-RGG, Altmeyer *et al.*, 2015; PBM, Gagné *et al.*, 2008). (b) Fold-enrichment of the Tri-RGG motif among PAR-binding candidates compared to other RG/G motifs (c-f) Molecular weights and isoelectric points for the whole proteome, PAR-binding candidates (Figure 2b), RNA- and DNA-binding proteins (see Table S1). Proteins with MW > 800 kDa were excluded from the plots for ease of visualization.

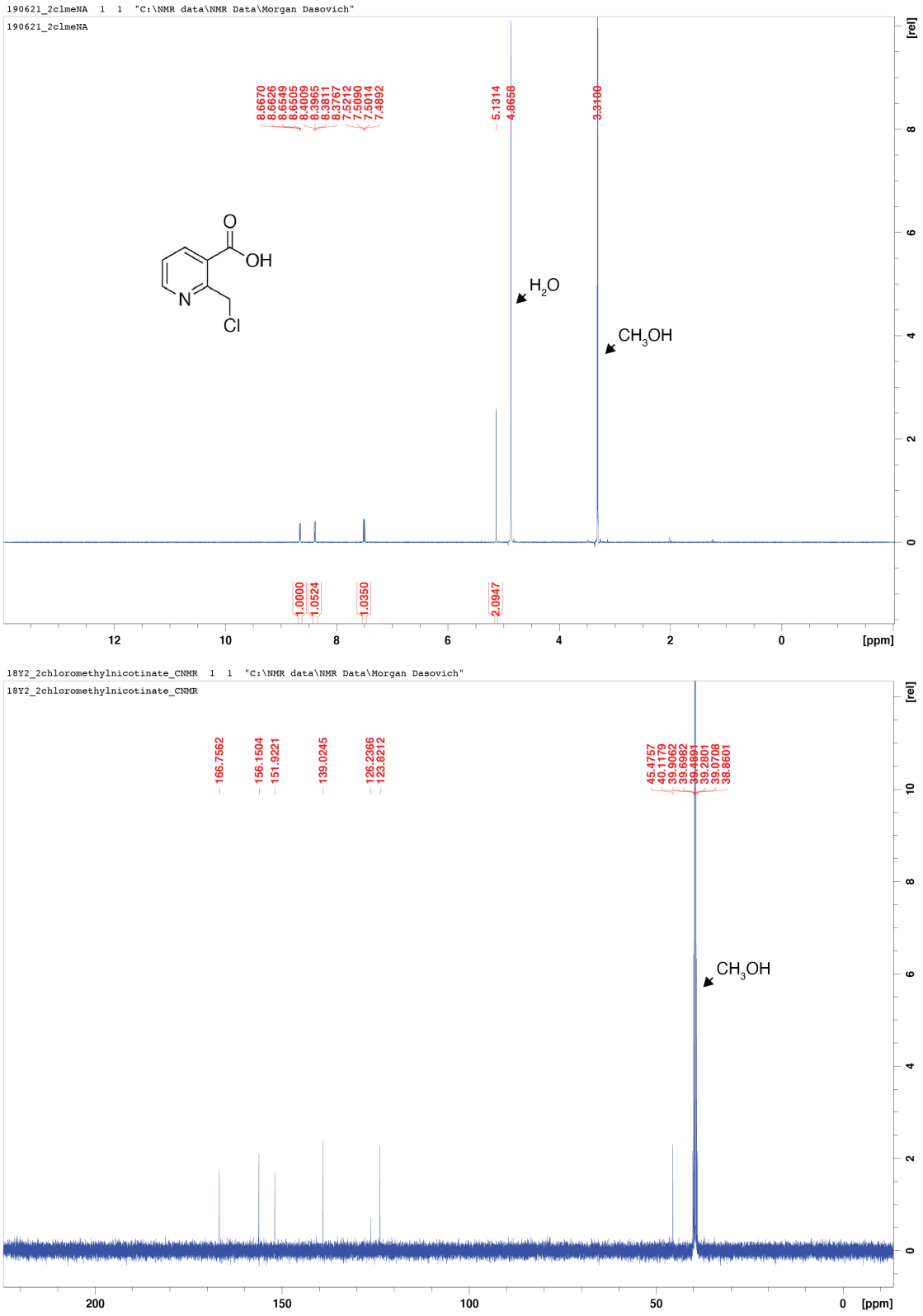


Figure S10. ^{1}H NMR (400 MHz) and ^{13}C NMR (101 MHz) spectra of 2-Chloromethylnicotinic acid (CD_3OD).

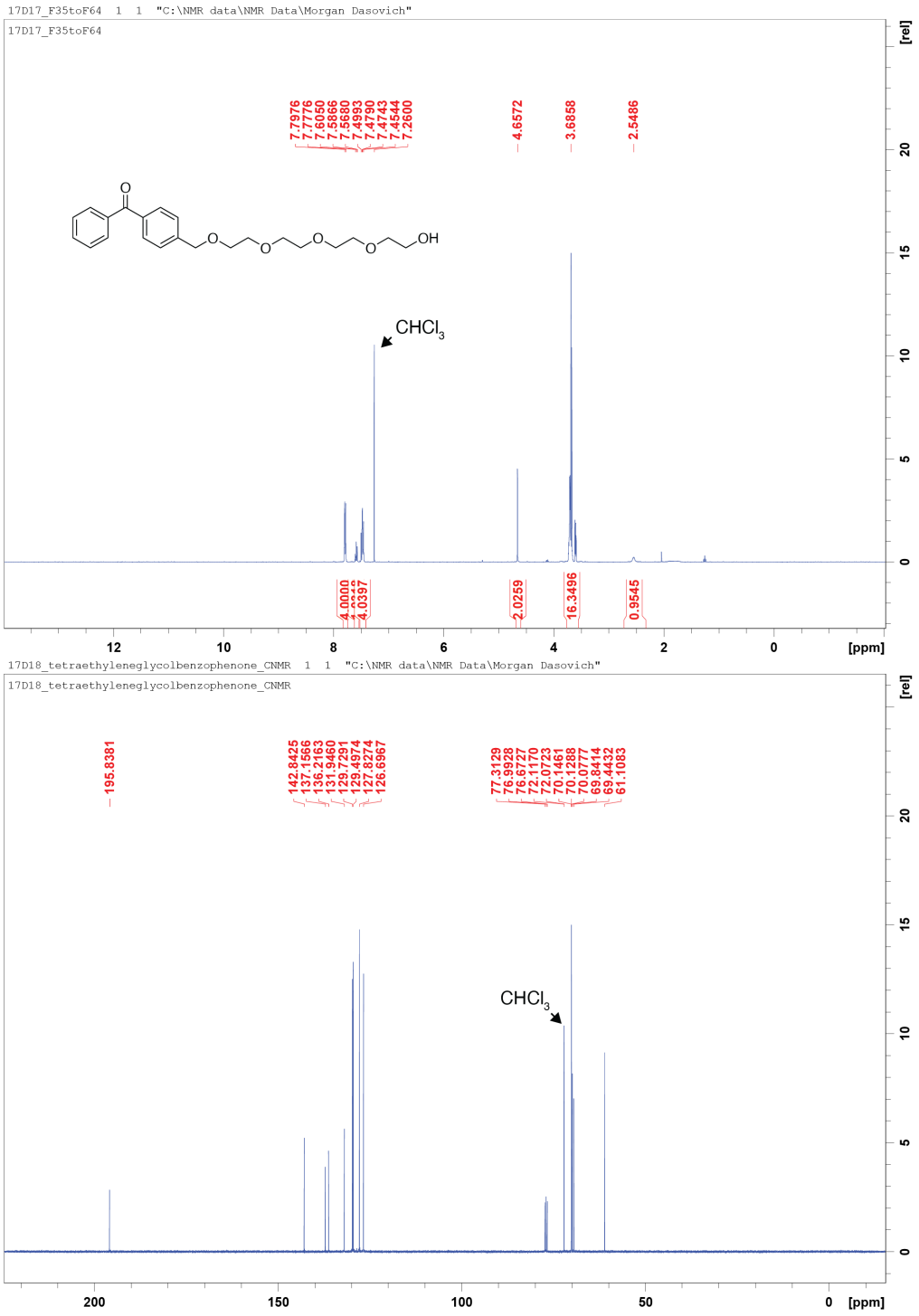


Figure S11. ^{1}H NMR (400 MHz) and ^{13}C NMR (101 MHz) spectra of (4-(13-Hydroxy-2,5,8,11-tetraoxatridecyl)phenyl)(phenyl)methanone ($CDCl_3$).

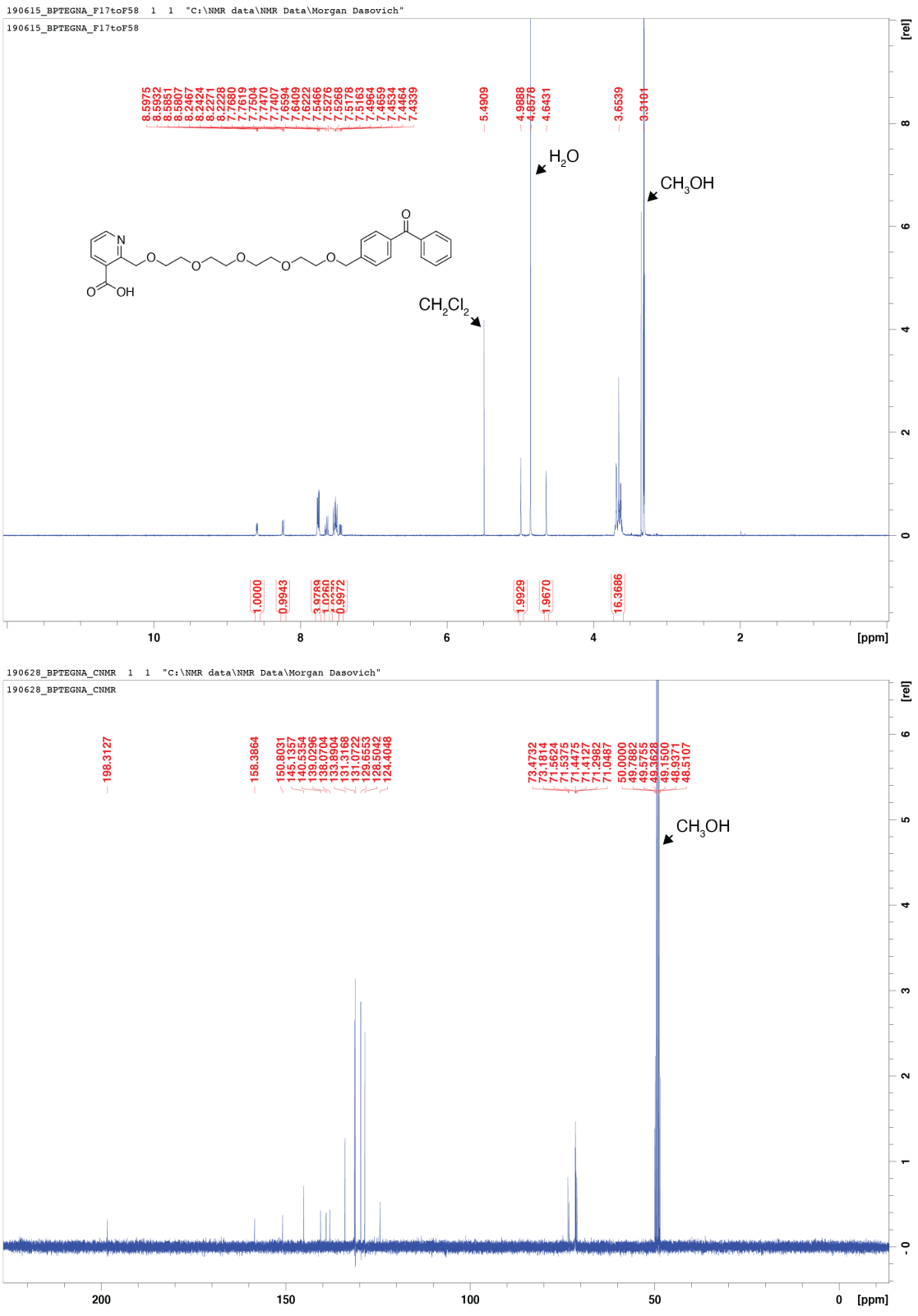


Figure S12. ^{1}H NMR (400 MHz) and ^{13}C NMR (101 MHz) spectra of 2-(15-(4-Benzoylphenyl)-2,5,8,11,14-pentaoxapentadecyl)nicotinic acid (CD_3OD).

After reaction with CDI for 1 h (in d_6-DMSO)

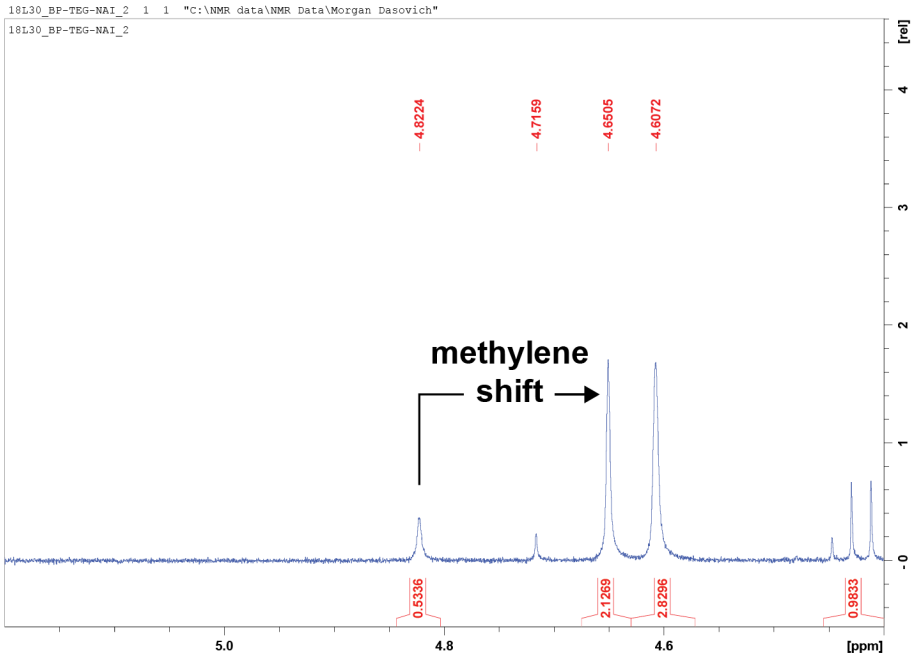


Figure S13. ^{1}H NMR (400 MHz) of the reaction between 2-(15-(4-Benzoylphenyl)-2,5,8,11,14-pentaoxapentadecyl)nicotinic acid and carbonyldiimidazole (d_6-DMSO).