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Supplementary Information for

Ultrasensitive, multiplexed chemoproteomic profiling with soluble activity-dependent proximity ligation

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Supplementary Information Text

Reagents and General Equipment

All chemicals were purchased from Sigma-Aldrich, unless otherwise noted, and were used as received. Oligonucleotides and Taqman probe for real-time PCR were purchased from Integrated DNA Technologies (IDT); detailed sequences are shown in Supplementary Figure S6. Commercial antibodies were purchased from Abcam (#ab58802, anti-cathepsin B; #ab54615, anti-FAAH1; #24701, anti-MGLL), R&D systems (#AF952-SP, anti-cathepsin L; #AF1180-SP, anti-DPP4; #AF5718-SP, anti-GAPDH). Polyclonal anti-NCEH1 antibody was previously reported(1). Enzymes were purchased from New England Biolabs (#M3004E, Luna universal probe qPCR master mix; #M0503L, Phusion high-fidelity DNA polymerase; #M5505L, USER enzyme, #M5505L) and Epicentre Biotechnologies (#A3202K, Ampligase enzyme). IgG was purchased from Thermo Scientific (#02-6202). Polyadenylic acid was purchased from Sigma-Aldrich (#P9403). Sybr-gold nucleic acid gel stain solution was purchased from Thermo Fisher Scientific (#S11494). Streptavidin was purchased from Leinco Technoligies (#S203). Disuccinimidyl suberate (DSS) linker was purchased from Sigma (#S1885). NuPAGE Novex 4-12% Bis-Tris protein gels were purchased from Thermo Fisher Scientific (#NP0322BOX). BCA assays were purchased from Thermo Fisher Scientific (#23225, BCA; #23235 micro BCA). Absorption measurements were analyzed on a Thermo Scientific Nanodrop 2000 spectrophotometer.

Isolation of human and mouse Peripheral Blood Mononuclear Cells (PBMCs)

For human PBMCs (hPBMCs), peripheral blood was collected from volunteers with informed consent (IRB 13372) into purple-cap vacutainers (K2EDTA; BD Biosciences, #367861) and PBMCs were isolated with Ficoll-Paque PLUS using the manufacturer's recommended protocol. For mouse PBMC isolation (mPBMC), all animal experiments were following the protocols approved by the University of Chicago Institutional Animal Care and Use Committee. C57/BL6 mice were subjected to isoflurane anesthesia and blood was drawn by cardiac puncture. PBMC isolations were performed with tubes and syringes pre-coated with EDTA to prevent clotting, and 1% BSA to maintain high recovery of PBMCs.

Banking the Patient-derived Tumor Samples

Ovarian cancer spheroid cells were isolated from the ascites of patients undergoing primary tumor debulking at the University of Chicago Comprehensive Cancer Center (see main text Methods). Fresh ascites fluid was centrifuged at 3000 g for 5 minutes and resuspended in PBS. Spheroids were collected by passing the spheroid suspension through 40 μm nylon mesh (Fisher Scientific, #22363547) and washed thoroughly with PBS. Enriched spheroids were collected from the top of the filter in DMEM growth media and frozen for biobanking.

Paired metastatic tumor tissue from the omentum and primary ovary tissues were isolated from the same patients undergoing tumor debulking at the University of Chicago Comprehensive Cancer Center followed by flash freezing for biobank archiving (see main text Methods).

Cell Lines

PC3, SKOV3, SKOV3IP1, OVCAR3, MCF7 and U87MG cell lines were obtained from the Amerian Type Culture Collection (ATCC) and were not STR profiled. Cell lines have been tested for mycoplasma contamination. All cell lines were cultured in RPMI 1640 (Hyclone) supplemented with 10% fetal bovine serum (FBS, Atlanta Biologicals) and 1% Penicillin/Streptomycin (Hyclone). All cell lines were grown at 37 ℃ in a 5% CO_2 humidified incubator.

Family-wide probes

The fluorophosphonate-biotin probe was synthesized according a published procedure(1). The cathepsin family-wide probe was purchased from ActivX Biosciences.

Antibody-oligonucleotide conjugates

5' or 3' amine-modified oligonucleotides were dissolved in water (20 nmol, 75 μL). Disuccinimidyl suberate (DSS) linker was dissolved in DMF (50 mM), then 75 μL of the DSS solution was added

to oligonucleotide solution together with 75 µL of acetonitrile and 1 µL of triethylamine. The mixture was shaken at room temperature for 30 minutes, followed by ethanol precipitation. Briefly, 28 µL of sodium acetate (3 M, pH 5.2), 565 µL of 100% ethanol and 2 µL of glycogen (20 mg/ml) were added to the mixture. After thorough vortexing, the mixture was incubated at -80 ℃ for one hour, followed by centrifugation at 14,000 rpm for 30 minutes. The pellet was recovered by removing the supernatant, reconstituted in 0.03 M acetic acid (pH 4.5), and filtered through a 0.2 µm filter. Reverse phase HPLC (phase A: 0.05 M trimethylamine/acetic acid buffer, pH 7.0; phase B: acetonitrile) was employed to purify the mixture. The HPLC gradient was 0-20% of phase B over 35 minutes. The product peak fraction was collected and an equal volume of 0.2% trifluoroacetic acid solution was added to stabilize the activated NHS ester product. Then the modified oligonucleotide was aliquoted and lyophilized. The concentration was quantified via absorbance at 260 nm by Nanodrop.

Antibody was dialyzed against PBS at 4 ℃ overnight, then concentrated using a 50 kD centrifugal filter tube. The concentration of the antibody was quantified based on absorbance at 280 nm. Typical concentrations were 0.3-1.0 mg/ml. Lyophilized oligonucleotide (200 pmol, 3 equivalents) was dissolved in 4 µL of 1.0 M HEPES plus 1.0 M NaCl buffer (pH 7.4), mixed with antibody (10 µg in PBS) and stirred at room temperature overnight. After quenching the reaction with 1 µL 1.0 M Tris (pH 7.5), excess oligonucleotide was removed using 50 kD centrifugal filter tubes 6 times for 10 minutes each. Antibody-oligonucleotide (Ab-oligo) concentration was quantified by microBCA assay and the labeling conjugate was validated by both native and denatured PAGE gels. The gels were stained with Sybr-gold stain solution for 30 minutes at room temperature and briefly washed with water. The gel images were captured in a Chemidoc imaging system and the labeling yield was quantified by densitometry using ImageJ software (NIH).

Streptavidin-oligonucleotide conjugate

Succinimidyl-modified oligonucleotide (960 pmol, 0.5 equivalent) was dissolved in 20 µL 0.03M acetic acid (pH 4.5). Streptavidin (100 µg, in 30 µL PBS) was added to the oligonucleotide solution together with 15 µL of 1.0 M HEPES plus 1.0 M NaCl buffer (pH 7.4). The mixture was stirred at room temperature overnight. After quenching the reaction with 1 µL 1.0 M Tris (pH 7.5), the streptavidin-oligonucleotide (SA-oligo) conjugate was purified by ion exchange FPLC in an ÄKTAexplorer system with HiTrap Q HP (1 ml) column.

Western blot

Cells were grown in 6-cm dishes until reaching 80-90% confluence. FP-Biotin (FP-Bio, 10 mM stock in DMSO) was diluted to 2 µM in RPMI1640 complete medium, incubated with cells at 37 ℃ for 30 minutes. Cells were washed with PBS twice, harvested by scraping in PBS, pelleted by centrifugation at 1,000 rpm, washed twice with PBS and lysed in PBS (pH 7.4) containing complete protease inhibitor cocktail (Sigma, #92714-1BTL) by sonication at 4 ℃. Protein concentration was determined by BCA assay (Pierce, #23225); a series of cell lysate dilution were prepared and combined with 4X Laemmli buffer (4X: 200 mM Tris pH 6.8, 400 mM DTT, 8% SDS, 0.4% bromophenol, 40% glycerol), followed by heating to 95 ℃ for 5 minutes, cooling to room temperature, and gel electrophoresis on NuPAGE Novex 4-12% Bis-Tris Protein Gels (Invitrogen, #NP0322BOX). PAGE gels were transferred onto nitrocellulose membranes, blocked in 2% BSA in TBS containing 0.1% tween-20 (TBST) and probed with IR800-conjugated streptavidin (LI-COR, #926-32230) overnight at 4 ℃. Images were captured by Odyssey CLx imaging system (LI-COR). Quantification of band intensities was performed using ImageJ software.

General Single-plex Soluble Activity-dependent Proximity Ligation (sADPL)

Cancer cells (PC3, MCF7, SKOV3, SKOV3IP1, OVCAR3, U87MG), human or mouse PBMCs, or patient-derived spheroid cells were treated with 2 µM FP-Bio at 37 ℃ for 30 minutes and/or 5 µM of the cathepsin family-wide probe at 37 ℃ for 3 hours in complete RPMI1640 medium. Cells were washed twice with PBS, harvested by scraping in PBS, pelleted by centrifugation at 1,000 rpm, and lysed by sonication at 4℃ in PBS (pH 7.4) containing complete protease inhibitor cocktail. Protein concentration was determined by BCA assay. For experiments in Figures 2 and 3, fractionated proteome was prepared by ultracentrifugation (100,000x g, 45 minutes), thereby separating the

soluble proteome in the supernatant and the membrane proteome in the pellet. The pellet was resuspended in 0.5% Triton X-100 in PBS by quick sonication. For multiplexed sADPL in Figure 4 and 5, whole proteome was used directly. Cell lysates were diluted into 3-fold series, incubated with PEG-8000 at a final concentration of 5% at 4 ℃ for 30 minutes, and centrifuged at 4,000 rpm for 20 minutes to remove any potential assay interferences. 2 µL of each proteome sample was added to 2 µL of ADPL reagent mix, resulting in a final concentration of 200 pM for each target-specific antibody-oligo, and 1 nM (single-plex) or 4 nM (multiplex) of SA-oligo in PBS pH 7.2, containing 20 µg/mL poly-A, 2 mM EDTA, 1% BSA, and 0.05% goat IgG. The resulting mixture was incubated at 37 ℃ for 90 minutes. Ligations were performed by adding 116 µL ligation solution containing 100 nM splint oligonucleotide, 2.5 units of ampligase, 0.3 mM NAD+, 10 mM DTT, 20 mM Tris-HCl pH 8.3, 50 mM KCl and 1.5 mM $MgCl_2$. Ligation proceeded at 30 ℃ for 15 minutes and was terminated by adding 2.5 µL of the 10-fold dilution of the Uracil-specific excision reagent (USER) enzyme for 15 minutes at the same temperature. Ligated amplicons were pre-amplified by mixing 5 µL of the final ligation mixture with 20 µL of 1.25x PCR solution, 100 nM primers and amplifying for 18 cycles. The pre-amplified solution was then diluted with 75 µL of 1x TE buffer prior to real-time, quantitative PCR (qPCR). 9 µL of the diluted solution was added to 11 µL of qPCR mix resulting in final concentrations of 0.5 µM primers and 0.25 µM Taqman probe. Samples were run on a Roche LightCycler 480 II (96-well plates) or CFX384 Real-Time System (384-well plates). After acquiring the raw threshold cycle count (C_T) values the corresponding ΔC_T values were calculated by normalizing to 'PBS' blank control for the proteome dilution curves. Alternatively, these data can be transformed into fold-change values by employing a standard amplification factor of 1.91, which was determined by a standard dilution curve in qPCR experiments. Probe-containing signals below the level of background controls were deemed undetectable and filtered out in all comparisons. For patient-derived profiling experiments, raw target activity measurements were normalized using PLA-qPCR measurements of GAPDH levels. Also for patient samples, the normalized fold change values were transformed into Z-scores for heatmap visualization.

Competitive sADPL Profiling of Small Molecule Target Engagement

In vitro competitive sADPL profiling was performed by first treating PC3 cancer cells or isolated hPBMCs with either 2 µM of the NCEH1 inhibitor JW480 for full inhibition, or with different doses (0 nM, 0.128 nM, 0.64 nM, 3.2 nM, 16 nM, 80 nM, 0.4 µM, 2 µM) for dose-dependent studies. FAAH activity was measured in MCF7 cancer cells were treated with 2 µM of the FAAH inhibitor PF3845 before FP-Bio treatment and sADPL processing. All *in vitro* inhibitor treatments were conducted in complete RPMI1640 medium for 3 hours. IC_{50} curve for NCEH1 was generated in Graphpad Prism 7 via non-linear regression and dose-response inhibition.

For *in vivo* target engagement experiments, C57/BL6 mice involved in this study were randomized into three groups with similar body weights, and inhibitor treatment studies were determined by average weight of the animal cohort. Five mice in each group were treated with vehicle (PEG300), 3 mg/kg, or 80 mg/kg of JW480 in 250 µL PEG300 via oral gavage. After 4 hours, mice were subjected to isoflurane anesthesia and blood was drawn by cardiac puncture. mPBMCs were isolated, pulsed with FP-Bio and processed for sADPL profiling as described above.

Comparative Multiplexed sADPL Activity Profiling in Patient-derived Spheroid Cells

The spheroid samples from 15 patients were analyzed by multiplexed sADPL in two biological replicate. After FP-Bio and cathepsin probes treatment as indicated above, 2 µL of 0.1 mg/ml of whole lysate was incubated with 200 pM of each enzyme-specific antibody-oligo, anti-NCEH1-oligo1, anti-MGLL-oligo2, anti-CTSL-oligo3, anti-FAAH-oligo4, anti-CTSB-oligo5, anti-DPP4-oligo6 and 4 nM of streptavidin-oligo. After ligation and pre-amplification for 20 cycles as detailed in single-plex protocol, the pre-amplified solution was then diluted with 125 µL of 1x TE buffer prior to real-time, quantitative PCR (qPCR). 4.5 µL of the diluted solution was added to 5.5 µL of qPCR mix resulting in final concentrations of 0.5 µM primers and 0.25 µM Taqman probe. Each patient samples were analyzed with 6-plex orthogonal primers, forward primer 1 (FP1)/reverse primer (RP), FP2/RP, FP3/RP, FP4/RP, FP5/RP, FP6/RP, on a CFX384 Real-Time System (384-well plates). Probe-containing signals below the level of background controls were deemed undetectable and filtered out in all comparisons. However, to verify the consistency of the

multiplexed sADPL in patient samples, all values including the undetected activity in two biological runs were correlated in Figure S8A. PLA-qPCR of GAPDH were performed as the internal control. Following visual and experimental quality control, 11 patients for 5 biomarkers (NCEH1, MGLL, CTSL, FAAH, CTSB) were analyzed. The DPP4 biomarker was excluded for all patients because it was undetectable relative to blank controls. For the heatmap and cluster plot, the raw threshold cycle count (C_T) values and the corresponding ΔC_T values were calculated by normalizing to 'PBS' blank control, then converted to fold change values by employing a standard amplification factor of 1.91, transformed into Z-scores for visualization.

Comparative multiplexed sADPL activity profiling in patient-derived tumor tissues

Small portions of paired tissue from 18 patients (i.e. 36 samples) were obtained from fresh-frozen biobanked samples for sADPL experiments. Tissues were first dounce homogenized in PBS, then centrifuged at 4000 g for 5 minutes at 4 ℃ to remove lipids and non-lysed tissue. Then the whole proteome concentration was normalized to 1 mg/ml using BCA assay. 50 µL of lysate were treated with 5 µM of cathepsin probe and 2 µM of FP-biotin probe simultaneously at room temperature for 2 hours, followed by excess probe removal with Zeba spin columns (Thermo Fisher Scientific, #89882). Lysate concentrations were quantified again by BCA assay, and multiplexed sADPL analysis was conducted as described above for spheroid samples. Unsupervised machine learning algorithm K-means clustering with Euclidean distance measures was employed to cluster the patients based on overall and disease free survival, resulting in three optimal clusters. After K-means cluster, other patient characteristics: DxAge (age when diagnosed with cancer), BMI (body mass index), ethnicity, tumor site, FIGO (International Federation of Gynecology and Obstetrics) stage, were compared between clusted groups. We found there were no significant differences or confounding factors between patient groups.

Proximity Ligation Assay (PLA)-qPCR Quantification of GAPDH Abundance

To normalize inter-patient variations, PLA of the housekeeping protein GAPDH was performed as described(2).

Comparison of sADPL and LC-MS/MS Measurements in Cell Lines

SKOV3IP1 and OCVAR3 cells were treated with DMSO, or family-wide probes and processed for sADPL as detailed above. LC-MS/MS abundance were extracted from the our online proteomic database (http://maxqb.biochem.mpg.de/mxdb/experiment/show/9353366112500)(3).

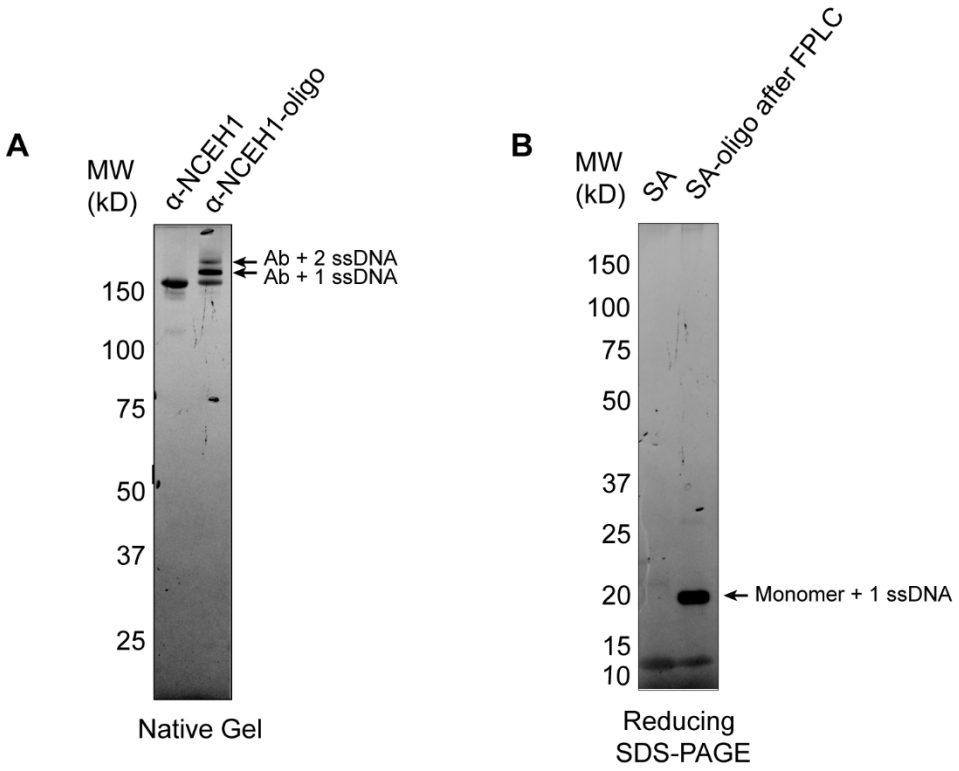

Fig. S1. Representative characterization of antibody-oligonucleotide conjugate and streptavidin-oligonucleotide conjugation. (**A**) Representative native PAGE gel analysis of NCEH1 antibody before and after reaction with succinimidyl-modified single stranded DNA (ssDNA) reveals high labeling efficiency to mono- and di-oligonucleotide conjugates. (**B**) Reducing SDS-PAGE gel analysis of streptavidin before and after reaction with succinimidyl-ssDNA and purification by FPLC.

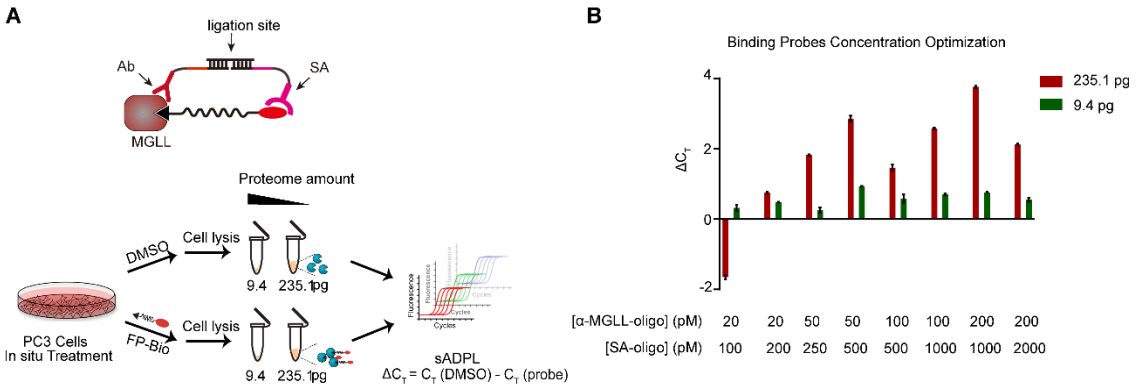

Fig. S2. Representative optimization of sADPL reagent concentrations for the model serine hydrolase monoacylglycerol lipase. (**A-B**) Schematic outline of the optimization process (**A**) and resulting signal (**B**) from sADPL profiling of active MGLL in PC3 cells. ΔC_T is calculated by comparing raw cycle threshold values for probe (FP-Bio) vs. no probe (DMSO) treated samples at the indicated proteome concentration and with the indicated ADPL reagent concentrations. All data points are from triplicate technical replicates in two or more biological experiments. Data represent the mean and the error bars represent standard deviation.

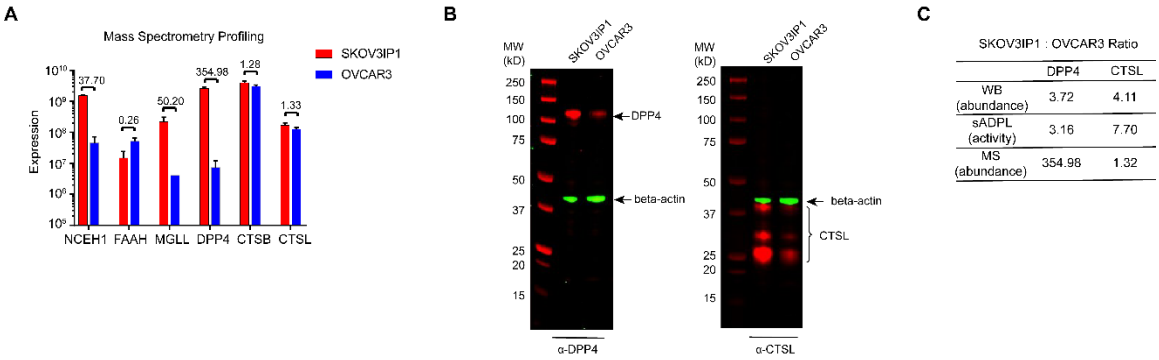

Fig. S3. Relative target protein levels in SKOV3IP1 and OVCAR3 cells as measured by LC-MS/MS and Western blot. (**A**) Mean ion intensity values +/- standard deviation in LC-MS/MS measurements are shown. (**B**) Western blot analysis of DPP4 and CTSL using β-actin as the loading control. 8 µg of whole proteome was loaded in each lane. CTSL antibody recognizes precursors and mature protein, and quantified bands are bracketed. (**C**) Tabular summary of results measured by sADPL, Western blot and LC-MS/MS.

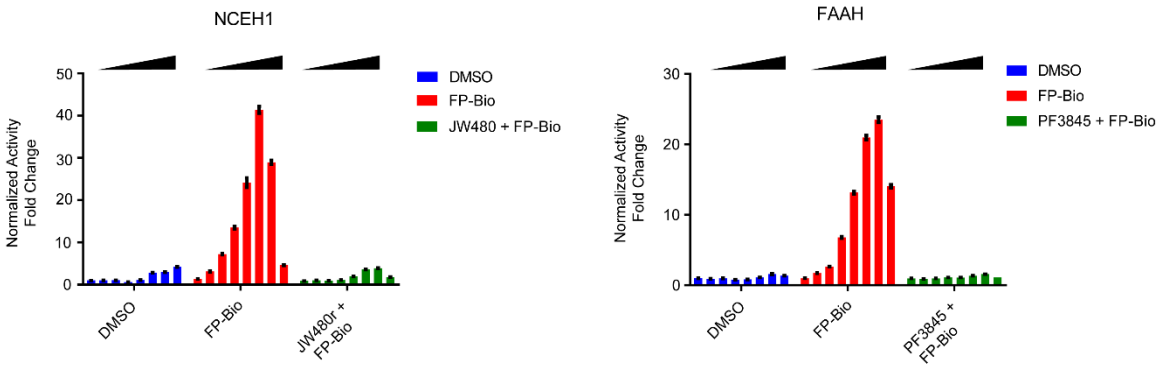

Fig. S4. *In situ* small molecule target engagement. sADPL signals from NCEH1 and FAAH in PC3 cells treated with selective inhibitors, JW480 or PF3845, respectively. Values at representative proteome concentrations were tested, from left to right: 1.08 pg, 3.23 pg, 9.68 pg, 29.04 pg, 87.13 pg, 0.26 ng, 0.78 ng, 2.35 ng for NCEH1; 0.35 pg, 1.08 pg, 3.23 pg, 9.68 pg, 29.04 pg, 87.13 pg, 0.26 ng, 0.78 ng for FAAH, while the Y-axis denotes the activity fold change normalized to the DMSO control at the lowest lysate concentration. All data points are from triplicate technical replicates in two or more biological experiments. All values represent the mean and the error bars represent the standard deviation.

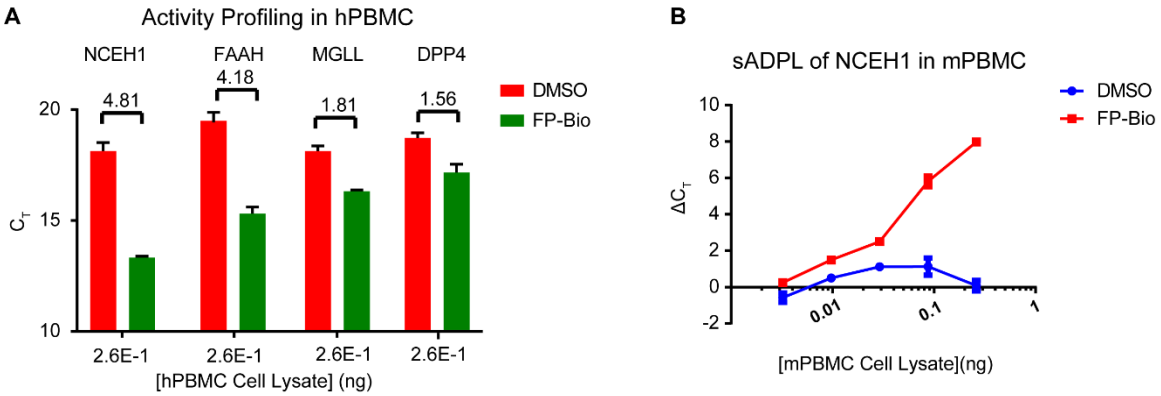

Fig. S5. Enzyme activities in human and mouse peripheral blood mononuclear cells. (**A**) Raw C_T values from probe and no-probe sADPL activity measurements for NCEH1, FAAH, MGLL, DPP4 in hPBMCs. (**B**) *In vitro* target engagement in mouse PBMCs across whole cell proteome dilutions. Data points are from n = 3 technical replicates from two biological replicates. All values represent the mean and the error bars represent the standard deviation.

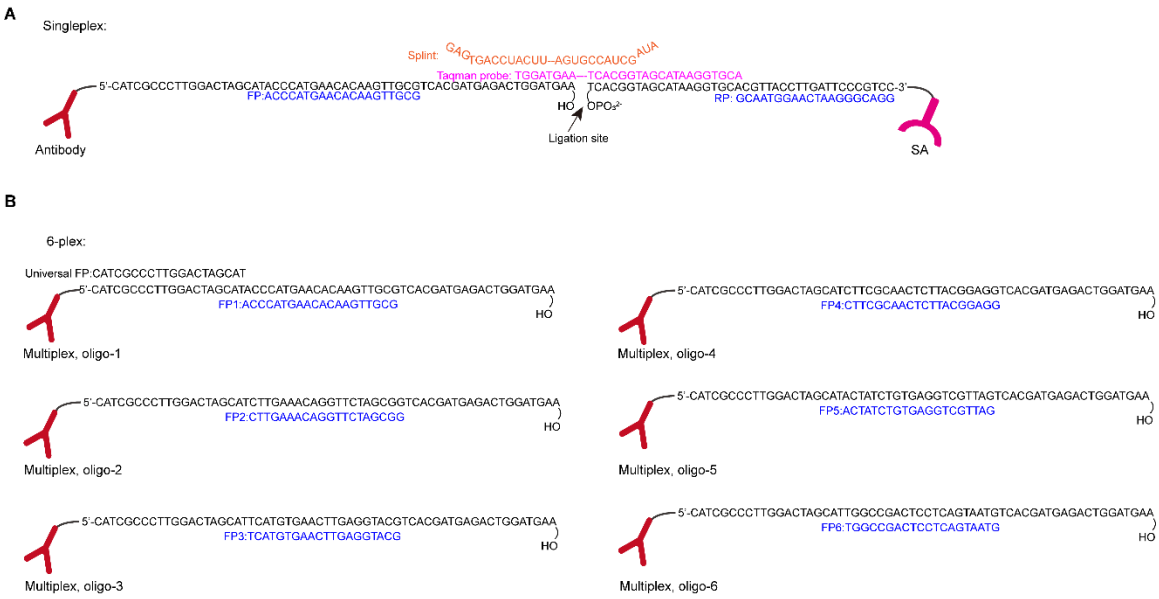

Fig. S6. Oligonucleotide sequence designs for single- and multiplex sADPL. (**A**) In single-plex sADPL or PLA applications, a 60-nt oligonucleotide was conjugated to a POI-specific antibody through the 5'-terminus using disuccinimidyl suberate chemistry. Separately, a 40-nt oligonucleotide with 5'-phosphate group was conjugated to streptavidin or polyclonal GAPDH antibody (only for GAPDH targeted PLA) at 3'-terminal using the same chemistry. Complementary splint oligonucleotide, Taqman detecting probe and PCR primer sequences are also shown. FP: forward primer; RP: reverse primer. (**B**) In the multiplex design, barcoded oligonucleotides were conjugated to POI-specific antibodies through the 5'-termini. The barcoding regions act as orthogonal primer sites (shown in blue). Universal forward and reverse primers were incorporated for pre-amplification to eliminate the PCR bias, and all oligonucleotides retained compatibility with universal ligation sites.

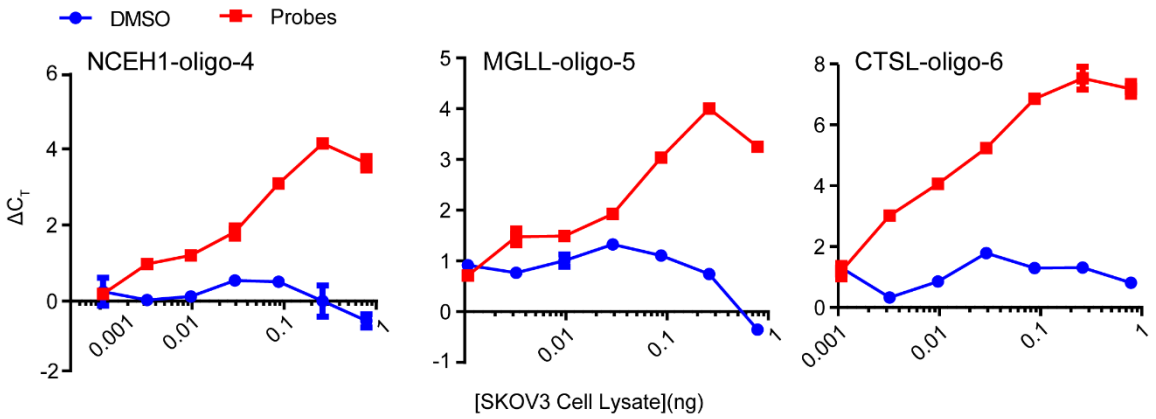

Fig. S7. Signal profiles for representative enzyme targets detected by multiplexed sADPL antibody-oligonucleotide conjugates using oligonucleotides 4-6 from Fig. S6. All data points are from triplicate technical replicates in two or more biological experiments. All values represent the mean and the error bars represent the standard deviation.

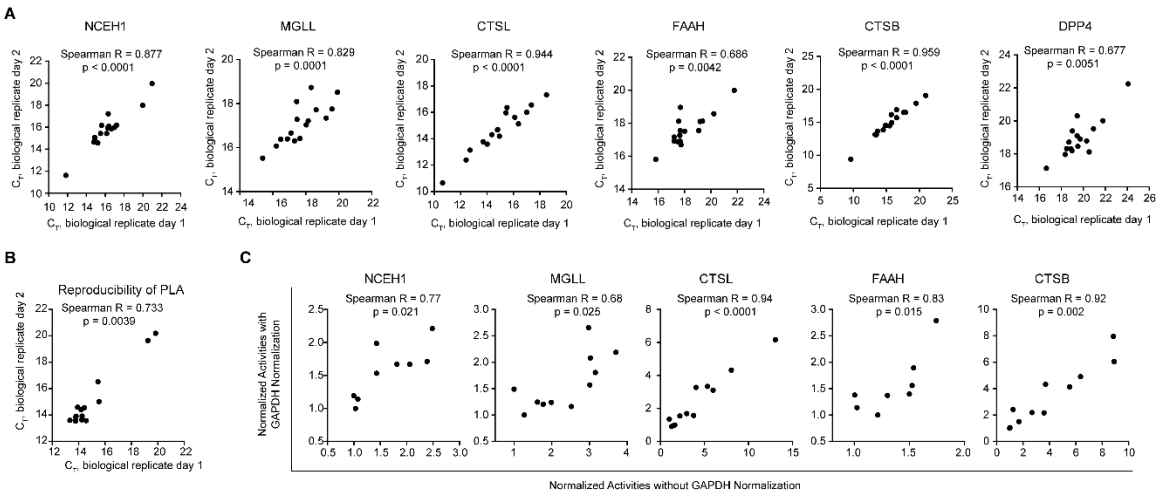

Fig. S8. Validation of multiplexed sADPL in patient-derived spheroid samples. (**A-B**) Correlation plot showing multiplexed sADPL profiling of six enzyme panel (**A**) and PLA quantification of GAPDH protein levels (**B**) from patient-derived spheroid samples on different days (i.e. unique biological replicates). Each point represents the mean of three technical replicates of an individual patient sample within each biological replicate. (**C**) Correlation plot showing multiplexed sADPL profiling measurements with and without GAPDH protein level normalization. Activities below detection limits were not included in this correlation analysis. Each point represents an individual patient. Each data point represents the mean of three technical replicates of an individual patient sample within each biological replicate.

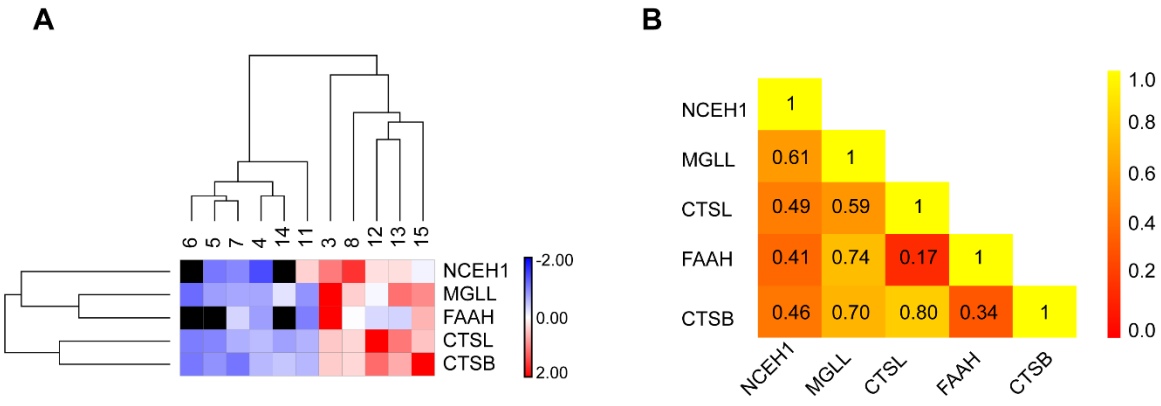

Fig. S9. Multiplexed sADPL biomarker profiling in patient-derived spheroid samples. (**A**) Heat map plot of the normalized enzyme activities of spheroid cells across patients tested. Undetectable activities are shown in black. Hierarchical clustering based on 'Euclidean distance' and 'average' linkage method was performed. All data points are mean value after 'z-score' normalization from triplicate technical replicates from representative biological replicates in two or more biological experiments. (**B**) Biomarker activity co-occurrence for each enzyme target measured in spheroid samples across the patient cohort. Active enzymes that show higher co-occurrence, such as FAAH and MGLL, and CTSL and CTSB, are yellow on the relative scale.

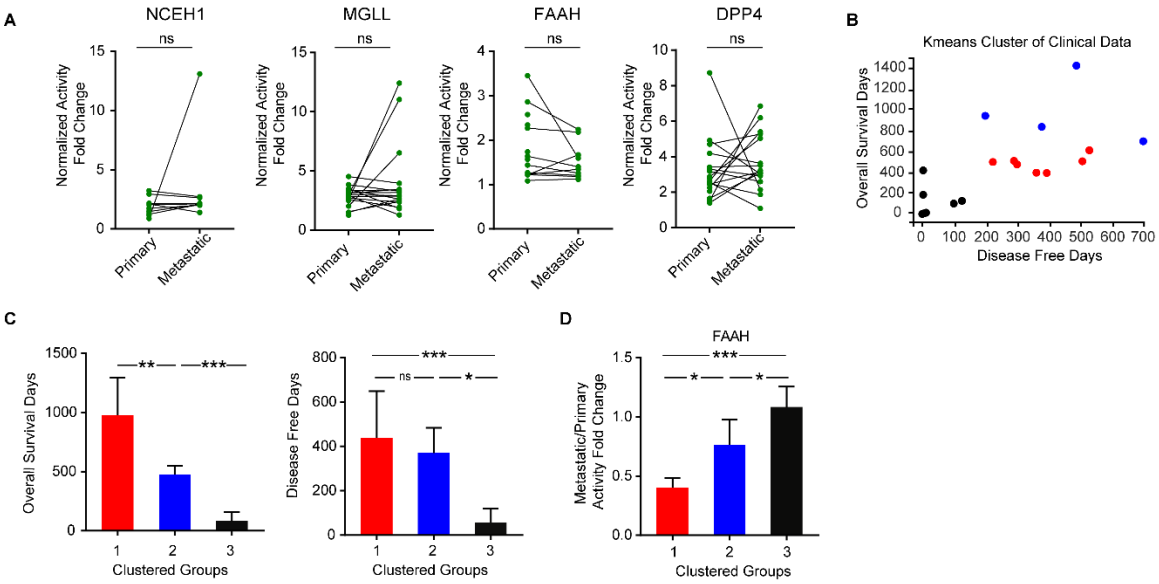

Fig. S10. Multiplexed sADPL biomarker profiling in patient-derived, paired metastatic and primary tumors. (**A**) Within-patient activity measurements between primary and metastatic tumor sites for listed enzymes. ns, not significant from Wilcoxon signed-rank t-test. (**B-C**) Unsupervised Kmeans cluster of patient clinical data determined that three distinct groups were present (**B**), which had significant differences in overall and disease-free survival (**C**). (**D**) The metastatic/primary tumor enzyme activity ratio for FAAH is significantly different between the three clustered patient groups, providing an inverse correlation trend with patient survival. All values represent the mean and the error bars represent the standard deviation. ns, not significant; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$ from Welch's t-test.

Table S1. Patient information for spheroid cell analysis

Patient Number	CA.125. Pre.Op	CA.125. PostOp	Disease Free Days	Overall Survival Days	TumorSite	FIGOStage
1	680	91	560	979	Fallopian Tube	FIGO IIIC
2	3670	372	744	981	Ovary	FIGO IIIC
3	19	NA	1061	1271	Ovary	FIGO IIIA
4	2949	1759	298	474	Ovary	FIGO IIIC
5	110	NA	207	328	Ovary	FIGO IIIB
6	1293	-99	291	270	Fallopian Tube	FIGO IIIC
7	267	165	212	482	Ovary	FIGO IIIC
8	2191	2234	214	214	Ovary	FIGO IIIC
9	1960	0	321	327	Ovary	FIGO IV
10	137	88	376	837	Fallopian Tube	FIGO IIIC
11	6500	686	671	1084	Ovary	FIGO IIIC
12	-99	198	467	820	Fallopian Tube	FIGO IIIC
13	128	-99	67	83	N/A	N/A
14	63	53	544	544	Fallopian Tube	FIGO IIIC
15	88	80	551	551	Ovary	FIGO IIIC

Table S2. Patient information for matched primary and metastatic tumor tissue analysis

Patient Number	DXAge	CA125preOP	FollowUpCA125	DiseaseFreeDays	OverallSurvivalDays	FIGOStage
1	75	137	298	376	837	FIGO IIIC
2	66	2023	-99	486	1427	FIGO IIIC
3	57	179	-99	505	505	Unknown
4	66	219	376	197	943	FIGO IIIC
5	52	77	19	221	498	FIGO IIIC
6	80	7807	7495	N/A	417	FIGO IIIC
7	65	117	382	359	395	FIGO IV
8	54	57	19	392	392	FIGO IV
9	60	27	N/A	104	104	FIGO IIIC
10	61	89	-99	288	509	FIGO IV
11	64	2949	553	298	474	FIGO IIIC
12	62	600	-99	698	698	FIGO IC
13	51	1298	15	527	611	FIGO IIIC
14	64	334	26	123	123	FIGO IV
15	70	849	-99	3	3	FIGO IIIC
16	68	167	-99	97	97	FIGO IIIC
17	46	3778	-99	6	6	FIGO IIIC
18	46	25	11	N/A	182	FIGO IV

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