Supporting Text

Unless otherwise noted, chemicals were obtained from commercial suppliers and used without further purification. Dry THF was obtained by passing commercially available pre-dried, oxygen-free formulations through activated alumina columns. NMR spectra were obtained in DMSO-d_6 and were recorded on a Bruker DRX-500 instrument. NMR chemical shifts are reported in ppm downfield relative to the internal solvent peak, and *J* values are in Hz. High resolution mass spectrometry data (HRMS) were performed at The Scripps Research Institute Mass Spectrometry Core and recorded on an Agilent mass spectrometer using ESI-TOF (electrospray ionization – time of flight).

***N*-(4-(4-aminobenzoyl)phenyl)hex-5-ynamide (4).** To a 50 mL flask fitted with a stir bar under N_2 was added 5-hexynoic acid (104 μL, 0.94 mmol), 1-hydroxybenzotriazole (HOBt) (144 mg, 0.94 mmol), and DMF (2.4 mL). To the solution was added 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI) (181 mg, 0.94 mmol). The mixture was stirred for 5 min and 4, 4'-diaminobenzophenone **3** (100 mg, 0.47 mmol) was added. After stirring the mixture overnight, EtOAc (30 mL) was added. The organic layer was washed with saturated $NaHCO_3$ (2 x 25 mL) and 10% aqueous citric acid (2 x 25 mL), dried with Na_2SO_4, filtered, and concentrated. Purification over silica gel (3% MeOH in $CHCl_3$) yielded 75 mg (52%) **4** as a brown-yellow solid. ^{1}H NMR (500 MHz) δ 1.76 (quintet, *J* = 7.3, 2H), 2.20 (td, *J* = 7.0, 2.6, 2H), 2.45 (t, *J* = 7.4, 2H),

2.80 (t, *J* = 2.6, 1H), 6.05 (s, 2H), 6.57 (d, *J* = 8.7, 2H), 7.48 (d, *J* = 8.6, 2H), 7.58 (d, *J* = 8.6, 2H), 7.69 (d, *J* = 8.6, 2H), 10.19 (s, 1H). ^{13}C NMR (125 MHz) δ 17.44, 23.91, 32.27, 71.78, 84.06, 112.57, 118.17, 124.23, 130.26, 132.45, 133.28, 142.09, 153.52, 171.12, 192.51. HRMS *m/z* calcd for $C_{19}H_{18}N_2O_2$ (M+H): 307.1441. Found: 307.1442.

Methyl 8-(4-(4-hex-5-ynamidobenzoyl)phenylamino)-8-oxooctanoate (5). To a flame-dried 5 mL pear-shaped flask fitted with a stir bar under N_2 was added intermediate **4** (12 mg, 0.041 mmol), *i*-Pr_2EtN (14 μL, 0.081 mmol), and THF (300 μL). The flask was cooled to 0 °C in an ice bath, and methyl chlorooxooctanoate (7.5 μL, 0.053 mmol) was added. The mixture was stirred for 9 h and allowed to warm to rt. CH_2Cl_2 (5 mL) was added to the residue and the organic layer washed with $NaHCO_3$ (sat., 5 mL) and 10% citric acid (5 mL). The organic layer was dried (Na_2SO_4), filtered, and the solvent was removed by rotary evaporation to provide **5** as a yellow solid (16 mg, 83% yield). ^{1}H NMR (500 MHz) δ 1.31 (m, 4H), 1.52-1.61 (m, 4H), 1.78 (quintet, *J* = 7.2, 2H), 2.23-2.31 (m, 4H), 2.36 (t, *J* = 7.4, 2H), 2.49 (t, *J* = 7.4, 2H), 2.82 (t, *J* = 2.1, 1H), 3.58 (s, 3H), 7.64-7.77 (m, 8H), 10.24 (s, 1H), 10.31 (s, 1H). ^{13}C NMR (125 MHz) δ 17.33, 23.77, 24.30, 24.80, 28.20, 28.26, 33.21, 35.19, 36.42, 51.15, 71.67, 83.94, 118.15, 118.18, 130.80, 130.84, 131.63, 131.69, 142.99, 143.08, 171.18, 171.82, 173.31, 193.33. HR-MS *m/z* calcd for $C_{28}H_{32}N_2O_5$ (M+H): 477.2384. Found: 477.2382.

***N*^{1}-(4-(4-hex-5-ynamidobenzoyl)phenyl)-N^8-hydroxyoctanediamide (SAHA-BPyne).** To a 2 dram vial fitted with a stir bar was added methyl ester

intermediate **5** (13 mg, 0.03 mmol), DMF (300 μL), THF (300 μL), and 50% H_2NOH in water (300 μL, 5 mmol). After stirring overnight, the THF was removed *in vacuo*, and the remainder of the sample purified using preparatory C18 reverse-phase HPLC (CH_3CN/H_2O-0.1% TFA, 5-66% over 50 min, 5 mL/min, 280 nm detection for 70 min, t_R: 23.7 min) to afford 5.6 mg (41%) of SAHA-BPyne as a yellow solid. ^{1}H NMR (500 MHz) δ 1.21-1.27 (m, 4H), 1.47 (quintet, *J* = 7.0, 2H), 1.57 (quintet, *J* = 7.2, 2H), 1.76 (quintet, *J* = 7.2, 2H), 1.92 (t, *J* = 7.3, 2H), 2.22 (td, *J* = 7.0, 2.5, 2H), 2.33 (t, *J* = 7.3, 2H), 2.46 (t, *J* = 7.4, 2H), 2.80 (t, *J* = 2.5, 1H), 7.67-7.75 (m, 8H), 8.63 (s,1H), 10.22-10.31 (m, 3H). ^{13}C NMR (125 MHz) δ 17.43, 23.87, 25.00, 25.12, 25.33, 28.48, 32.33, 35.29, 36.57, 71.79, 84.05, 118.26, 118.29, 130.86, 130.95, 131.73, 131.80, 143.09, 143.18, 167.17, 171.29, 171.95, 193.44. HR-MS *m/z* calcd for $C_{27}H_{31}N_3O_5$ (M+H): 478.2336. Found: 478.2337.

Suberoylanilide hydroxamic acid (SAHA). SAHA was prepared in a similar fashion to the reported method(1). The spectral data matches the reported spectra(2). ^{1}H NMR (500 MHz) δ 1.33 (m, 4H), 1.55 (quintet, *J* = 7.2, 2H), 1.65 (quintet, *J* = 7.2, 2H), 2.00 (t, *J* = 7.3, 2H), 2.35 (t, *J* = 7.4, 2H), 7.07 (td, *J* = 7.4, 0.9, 2H), 7.34 (t, *J* = 7.6, 2H), 7.64 (d, *J* = 8.4, 2H), 8.70 (s, 1H), 9.89 (s, 1H), 10.38 (s, 1H).

Cell culture. Human cell lines MUM-2B and MUM-2C were provided by Mary Hendrix (Northwestern University); SKOV3, OV3, MCF7, and 231MFP were obtained from the National Cancer Institute's Developmental Therapeutics

Program. All cell lines were grown in RPMI medium 1640 containing 10% FCS, except for the 231MFP cell line which was grown in DMEM containing 10% FCS. After harvesting, cell pellets were sonicated and Dounce homogenized in PBS, followed by centrifugation at 100,000 x *g* (45 min) to provide soluble cellular proteome fractions (supernatant) and a membrane pellet. The soluble fractions were stored at -80 °C until use. Nuclear fractions were prepared by lysing cells for 10 min at rt with cold 10 mM HEPES pH 7.7, 10 mM KCl, 0.1 mM EDTA, 1 mM DTT, and 0.4% IGEPAL CA-630 in the presence of protease inhibitors. The lysed cells were centrifuged at 15,000 x *g* for 3 min and the soluble fractions removed. The pellet was resuspended in 20 mM HEPES pH 7.7, 0.4 M NaCl, 10% glycerol, 1 mM DTT in the presence of protease inhibitors by vortexing for 2 h at 4 °C, followed by centrifugation at 15,000 x *g* (5 min, 4 °C) to provide the nuclear fractions (supernatant) and a membrane pellet. The nuclear fractions were stored at -80 °C until use.

Proteome labeling for gel and MS analysis. Proteome samples were diluted in PBS to a final concentration of 1 mg protein per mL. The total volume for SDS-PAGE samples was 50 μL total volume; the total volume for multidimensional protein identification technology (MudPIT) samples was 1 mL. For positive samples, SAHA-BPyne was added to a final concentration of 100 nM. For the SAHA control samples, SAHA was added to a final concentration of 10 μM, the sample vortexed, and SAHA-BPyne was added to a final concentration of 100 nM. All samples were placed into the wells of an ice-cooled 96-well plate (50-90 μL/well) and irradiated at 365 nm using a Spectroline ENF 260C UV lamp for 1 h

on ice, following previously described procedures(3). After UV cross-linking, reporter-tagged azide reagents (12.5 μM rhodamine-azide(4) final concentration for SDS-PAGE analysis, 50 μM rhodamine-biotin-azide(3) for Western/gel spot ID or 50 μM biotin-azide(3) final concentration for MudPIT analysis(5)) were added, followed by TCEP (0.25 mM final concentration) and ligand(6) (50 μM final concentration). Samples were gently vortexed and the cycloaddition initiated by the addition of $CuSO_4$ (0.5 mM final concentration). The reactions were incubated at rt for 1.5 h. For gel-based ABPP, an equal volume of 2x standard SDS loading buffer was added and the samples separated by 1D SDS-PAGE and visualized by in-gel fluorescent scanning using a Hitachi FMBio Ile flatbed scanner (MiraiBio). For ABPP-MudPIT, Western, and Gel Spot IDs, PBS (200 μL) was added to the reactions, and the protein pelleted by spinning in a microcentrifuge. The supernatant was removed and the pellet washed twice in MeOH (200 μL). The pellet was subsequently dissolved in PBS with 0.2% SDS (1 mL) with sonication and heating. The samples were placed in empty micro-biospin columns (Biorad) along with avidin-coupled agarose beads (50 μL suspension, Sigma). The samples were incubated with the beads for 1.5 h at room temperature to bind and enrich biotin-labeled proteins. The beads were then washed with PBS with 0.2% SDS (2 x 1 mL), 6 M urea (2 x 1 mL), and PBS (3 x 1 mL). For gel spot ID and Western blot samples, 2x SDS loading buffer (50 μL) was added to the beads, and the beads heated to 90 °C for 5-10 min before loading onto the gel. For ABPP-MudPIT analysis, beads were next resuspended in 6 M urea (400 μL) and the proteins sequentially reduced with TCEP (5 mM, 20

min) and alkylated with iodoacetamide (200 mM, 20 min) in the dark. Beads were then washed, placed in low adhesion eppendorf tubes, and digested with trypsin (1 μg, Promega) in 2 M urea (150 μL) overnight. Centrifugation provided the trypsin-digested peptide supernatant, which was acidified with formic acid (final concentration of 5% formic acid). Peptide samples were pressure-loaded into a 250 μm fused silica desalting column filled with 4 cm C18 media (Phenomenex Aqua 5u) for subsequent analysis by 2D-LC in combination with tandem MS. The biphasic column consisted of 100 μm fused silica (with a 5 μm pulled tip) packed with 10 cm C18 media (proximal to tip) and 2 cm strong cation exchange resin (Whatman Partisphere 5 μm). The MudPIT analysis was performed with a coupled Agilent 1100 LC-ThermoFinnegan LTQ MS system, as described previously(7). Analysis of SEQUEST search results from ABPP-MudPIT runs was carried out as previously described(7). Specifically labeled proteins were identified by their presence in HDAC alkyne probe-treated samples at significantly higher levels than in the controls. Proteins were considered specific if:

1 - average positive spectral counts – average no probe spectral counts > 4

2 - at least 2 x greater spectral counts in the positive runs than in the control runs and

3 - statistically significant difference between positive runs and control runs (Student's t–test, two-tail, $p = 0.01$).

***In situ* labeling of cancer cells with SAHA-BPyne.** 90% confluent 6mm dishes of MFP231 cells were washed with PBS (3 x 3 mL) and subjected to SAHA-BPyne (500 nM in PBS) or SAHA-BPyne and excess SAHA (500 nM SAHA-BPyne and 10 μM SAHA in PBS) (1 mL total volume). The samples were irradiated at 365 nm at 37 °C for the time noted. After irradiation, the cells were washed with PBS (3 x 3 mL), scraped, and pelleted by centrifugation. The cell pellet was resuspended in PBS (50 μL) and homogenized by sonication, and diluted to 4 mg/mL with PBS. To initiate the click chemistry, 100 μM rhodamine-azide(4) , TCEP (0.25 mM final concentration), ligand(6) (50 μM final concentration), and $CuSO_4$ (0.5 mM final concentration) were added. The reactions were incubated at rt for 1h. An equal volume of 2x standard SDS loading buffer was added and the samples separated by 1D SDS-PAGE and visualized by in-gel fluorescent scanning.

Western blot Enriched samples were prepared as noted above in "Proteome labeling for gel and MS analysis" section, using excess SAHA as a competitor. The samples were run on tris-glycine gels and transferred to nitrocellulose. The blots were blocked with 3% milk in TBST, probed with polyclonal α-HDAC2 (1:1000 in 4% milk in TBST, ab16032, AbCam, Cambridge MA), polyclonal α-MTA2 (1:750 in TBST, IMG-5602, Imgenix, San Diego CA), polyclonal α-CoREST (1:2000 in TBST, 07-455, Upstate, Lake Placid, NY) or monoclonal α-HDAC6 (1:400 in 3% milk in TBST, sc-28386, Santa Cruz Biotechnology, Santa Cruz CA) overnight at 4 °C, washed with TBST, incubated with goat-α-rabbit IgG

(H + L) HRP conjugate or goat-α-mouse IgG (H + L) HRP conjugate (1:20000, Biorad, Hercules CA) for 2 h at rt, washed again in TBST, and visualized with SuperSignal West Pico kit (Pierce, Rockford IL).

Gel spot IDs Samples were prepared as noted above in "Proteome labeling for gel and MS analysis" section, using excess SAHA-Bpane (a probe alkane analog) as a competitor. Bands that were competed by the analog were cut out of the gel, and subjected to in-gel reduction, alkylation, and trypsinization, as previously reported(8). Proteins were identified by subjecting the data to the same thresholds as described above and comparing the resulting list to proteins identified as specifically labeled targets in the MudPIT experiments.

Identified protein	Spectral counts Positive lane	Spectral Counts Control lane
HDAC1	13	0
HDAC2	12	3
MBD3	20	4

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