Experimental Section:

I. General procedures and materials

All reagents were purchased from Sigma Aldrich unless otherwise noted. Dimethyl D-Glutamate hydrochloride, L-methionine methyl ester hydrochloride, and D-leucine methyl ester hydrochloride were all purchased from TCI America (Tokyo, Japan). D-Methionine methyl ester hydrochloride was purchased from MP Biomedicals, LLG (Solon, OH). D-Tyrosine methyl ester hydrochloride was purchased from AK Scientific Inc. (Union City, CA). All compounds were characterized by proton and/or carbon NMR on either a Varian (Palo Alto, CA) 400 MHz or 500 MHz spectrometer. Chemical shifts (δ) are reported in parts per million (ppm) with chemical shifts referenced to internal standards: $CDCl_3$ (7.26 ppm for ^1H, 77.8 ppm for ^{13}C). Coupling constants (*J*) are reported in Hertz (Hz) and multiplicities are abbreviated as singlet (s), broad singlet (bs), doublet (d), triplet (t), pentet (p), multiplet (m), doublet of doublets (dd), and doublet of triplets (dt). High resolution Mass Spectra (HRMS) were obtained at the Mass Spectrometry Facility at Boston College (Chestnut Hill, MA). Analytical thin layer chromatography (TLC) was performed on Sorbent Technologies Silica G TLC Plates w/UV354 (0.25 mm). All compounds were visualized on TLC by UV and/or $KMnO_4$ staining. Column chromatography was carried out using forced flow of indicated solvent on Sorbent Technology Standard grade silica gel, 40-63 μm particle size, 60 Å pore size (Sorbent Technologies). PBS buffer, DMEM/High glucose media, RPMI 1640 media, and penicillin streptomycin (Pen/Strep) were purchased from Thermo Scientific (Waltham, MA). The anti-Myc-tag antibody and the anti-rabbit IgG HRP-linked antibody were purchased from Cell Signaling (Danvers, MA). X-tremeGENE 9 DNA transfection reagent was purchased from Roche (Indianapolis, IN). Staurosporine (STS) was purchased from Cell Signaling (Danvers, MA). All chemical probes were added to samples at the indicated concentration from a 10mM stock in dimethyl sulfoxide (DMSO) unless otherwise noted.

II. General Synthetic Method of piperidine-based probes:

Synthesis of pent-4-yn-1-yl 4-methylbenzenesulfonate (Compound 1)

To an oven dried flask equipped with stir bar was added, 4-pentyn-1-ol (59.4 mmol) and dichloromethane (DCM) (120 mL) under nitrogen (N_2) atmosphere. The resulting mixture was allowed to cool to 0 ˚C in an ice bath. Next, tosyl chloride (63.0 mmol) was added in one portion. The reaction vessel was purged with N_2 and allowed to slowly warm to room temperature. The reaction is quenched, after 12 hours, with water and extracted with DCM (3x 50 mL). The combined organic layers were dried with sodium sulfate (Na_2SO_4) and then concentrated *in vacuo*. The crude oil was purified by silica column chromatography (9:1 Hexanes (Hex):Ethylacetate (EtOAc)). The product was isolated as a clear oil (93% yield). ^1H NMR (500 MHz, $CDCl_3$): δ 7.79 (d, *J* = 8.30, 2H), 7.34 (d, *J* = 7.80, 2H), 4.14 (t, *J* = 6.35, 2H), 2.44 (s, 3H), 2.25 (td, *J* = 2.44, 6.83, 2H), 1.88-1.82 (m, 3H)ppm. ^{13}C NMR (125 MHz, CDCl3): δ 145.05, 133.26, 130.11, 128.18, 82.36, 69.67, 68.98, 27.99, 21.88, 14.96. HRMS m/z calculated for $C_{12}H_{15}O_3S$ ($M+H^+$): 239.0742. Found: 239.0739.

Synthesis of 8-(pent-4-yn-1-yl)-1,4-dioxa-8-azaspiro[4.5]decane (Compound 2)

To an oven dried flask, under N_2, equipped with stir bar and reflux condenser was added, sodium iodide (15.1 mmol), potassium carbonate (90.6 mmol) and acetonitrile (64 mL). To the suspension was added 1,4-dioxa-8-azaspiro[4.5]decane (30.2 mmol). The resulting mixture was allowed to heat to 90 ˚C and stirred for 20 min before the drop-wise addition of tosylated alcohol **(1)** (45.3 mmol). The reaction was allowed to mix for 18 hours before being cooled and diluted with DCM. The suspension was filtered to

remove solid precipitate. The remaining supernatant was concentrated *in* vacuo and then the resulting residue was dissolved in DCM (25 mL) and washed with 5% aq. NaOH (3x 25 mL) and brine (3x 25mL). The aqueous layer was extracted with DCM (3x 25 mL) and the combined layers were first dried with Na_2SO_4, filtered then concentrated *in vacuo*. The crude oil was purified by silica column chromatography (9:1-1:1 Hex:EtOAc). The resulting product was isolated as a clear light yellow oil (80% yield). ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.93 (s, 4H), 2.51 (bs, 4H), 2.44 (t, *J* = 7.56, 2H), 2.21 (td, *J* = 2.44, 7.32, 2H), 1.92 (t, *J* = 2.64, 1H), 1.74-1.68 (m, 6H). ^{13}C NMR (125 MHz, CDCl3): δ 107.98, 84.91, 69.05, 64.88, 57.06, 52.05, 35.52, 26.86, 17.15. HRMS m/z calculated for $C_{12}H_{20}NO_3$ ($M+H^+$): 210.1494. Found: 210.1493.

Synthesis of 1-(pent-4-yn-1-yl)piperidin-4-one (Compound 3)
To a round bottom flask was added ketal **(2)** (9.6 mmol), 1N HCl aq. (95 mL) and tetrahydrofuran (THF) (95 mL). The resulting mixture was heated to 100 ˚C for 20 hours. The reaction was allowed to cool and then slowly quenched with sodium bicarbonate. The aqueous layer was extracted with DCM (3x 100 mL). The solution of the crude product was concentrated *in vacuo* and purified on silica column chromatography (9:1-1:1 Hex:EtOAc) to yield a light yellow oil (90% yield). ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.74 (t, *J* = 6.34, 4H), 2.56 (t, *J* = 7.06, 2H), 2.45 (t, *J* = 6.10, 4H), 2.28 (td, *J* = 2.44, 7.08, 2H), 1.96 (t, *J* = 2.69, 1H), 1.74 (p, *J* =7.32, 2H). ^{13}C NMR (125 MHz, CDCl3): δ 210.02, 84.80, 69.45, 61.22, 56.83, 42.07, 27.14, 17.16. HRMS m/z calculated for $C_{10}H_{15}NO$ ($M+H^+$): 166.1232. Found: 166.1228.

Synthesis of N-octyl-1-(pent-4-yn-1-yl)piperidin-4-amine (Compound 4)
To a flame dried vial equipped with stir bar was added ketone **(3)** (0.3 mmol), 1˚ *n*-octylamine (0.3 mmol) and DCM (1 mL). The resulting mixture was allowed to stir at room temperature for 5 minutes before the addition of sodium triacetoxyborohydride in one portion (0.4 mmol). The vial was purged with N_2 and the resulting mixture was allowed to stir at room temperature for 12 hours before it was diluted with DCM (1 mL) and quenched with sodium bicarbonate (2 mL). The aqueous layer was extracted with DCM (3x 3 mL). The resulting DCM solution was concentrated *in vacuo* to obtain a crude oil. The oil was purified by silica column chromatography (9:1 DCM:Hex, 1:1 DCM:Hex, DCM, DCM/2% Methanol (MeOH)) to obtain a clear yellow oil (>98% yield).

Synthesis of 2-chloro-N-octyl-N-(1-(pent-4-yn-1-yl)piperidin-4-yl)acetamide (SMC-1)
To a flame dried vial equipped with stir bar was added the 2˚ amine intermediate **(4)** (0.9 mmol), as described above, and DCM (2 mL). The reaction vessel was purged with N_2 and cooled to 0 ˚C. Next, chloroacetyl chloride (1.2 mmol) was added drop-wise followed by triethylamine (1.2 mmol) added dropwise as well. The resulting mixture was allowed to warm to room temperature and stir for 8 hours. The reaction was quenched by the addition of sodium bicarbonate (2 mL), extracted with DCM (3x 2mL) and concentrated *in vacuo* to yield a crude oil. The resulting mixture was purified by column chromatography (Base wash column with 2% NEt_3, 9:1 DCM:Hex, 1:1 DCM:Hex, DCM, DCM/2%MeOH) to obtain a viscous, light yellow oil (66% yield).

III. Characterization of probe library members

Compound SMC-1:
The synthesis for SMC-1 is described above. Due to the sterically encumbered directing group, SMC-1 was found to have atropic isomers. ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.32-4.29 (m, 0.5H), 4.05 (d, *J* = 4.69, 2H), 3.55-3.49 (m, 0.5H), 3.21-3.16 (m, 2H), 3.01-2.95 (m, 2H), 2.45-2.41 (m, 2H), 2.25-2.19 (m, 2H), 2.05-1.99 (m, 2H), 1.95-1.93 (m, 1H), 1.89-1.80 (m, 1H), 1.73-1.66 (m, 5H), 1.61-1.54 (m, 3H), 1.26 (bs, 10H), 0.89-0.85 (m, 3H). ^{13}C NMR (100 MHz, CDCl3): δ 166.82 (d), 84.66, 69.20, 57.81 (d), 53.43, 44.66, 43.42, 42.35 (d), 32.57, 32.40 (d), 30.35, 29.906 (m), 29.69, 27.87 (d), 26.67, 23.27 (d), 17.10, 14.74. HRMS m/z calculated for $C_{20}H_{36}ClN_2O$ ($M+H^+$): 355.2516. Found: 355.2522.

Compound SMC-2:
Synthesized using the general procedure described above, using (exo)-norbonylamine as the source of diversity element. ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.07-3.99 (m, 2H), 3.22 (bs, 1H), 2.99-2.94 (m, 2H), 2.43-2.39 (m, 2H), 2.32 (bs, 1H), 2.21 (td, *J* = 2.73, 7.05, 2H), 2.16 (bs, 1H), 1.99-1.94 (m, 2H), 1.92 (t, *J* = 2.73, 1H), 1.72-1.65 (m, 3H), 1.57-1.45 (m, 6H), 1.23-1.20 (m, 4H), 1.13-1.09 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 166.63, 84.79, 69.11, 61.09, 57.55, 54.17, 44.21, 42.79, 39.02, 38.21, 36.97, 31.16, 30.35, 28.18, 26.26, 22.36, 17.08. HRMS m/z calculated for $C_{19}H_{30}ClN_2O$ ($M+H^+$): 337.2047. Found: 337.2044.

Compound SMC-3:
Synthesized using the general procedure described above, using 4-methoxybenzylamine as the source of diversity element. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.14-7.06 (m, 2H), 6.88-6.80 (m, 2H), 4.52 (s, 2H), 4.04 (d, *J* = 95.86, 2H), 3.79 (s, 3H), 2.94-2.87 (m, 2H), 2.41-2.38 (m, 2H), 2.19 (bs, 2H), 2.04-1.96 (m, 2H), 1.92 (bs, 1H), 1.83-1.80 (m, 1H), 1.69-1.62 (m, 5H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 168.14, 154.65, 129.98, 127.31, 115.04, 84.65, 69.17, 57.75, 55.98, 53.44, 46.73, 42,68, 31.77, 30.02, 26.60, 17.06. HRMS m/z calculated for $C_{20}H_{28}ClN_2O_2$ ($M+H^+$): 363.1839. Found: 363.1837.

Compound SMC-4:
Synthesized using the general procedure described above, using 3-methoxybenzylamine as the source of diversity element. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.28-7.19 (m, 1H), 6.81-6.69 (m, 3H), 2.56 (bs, 2H), 4.05 (d, *J* = 129.81, 2H), 3.79 (bs, 3H), 2.96-2.90 (m, 2H), 2.42-2.39 (m, 2H), 2.22-2.19 (m, 2H), 2.08-1.97 (3H), 1.92 (bs, 1H), 1.84-1.78 (m, 1H), 1.69-1.62 (m, 5H). HRMS m/z calculated for $C_{20}H_{28}ClN_2O_2$ ($M+H^+$): 363.1839. Found: 363.1835.

Compound SMC-5:
Synthesized using the general procedure described above, using 4-fluorobenzylamine as the source of diversity element. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.17-7.14 (m, 2H), 7.05-7.02 (m, 1H), 6.96-6.92 (m, 1H), 4.54 (d, *J* = 6.34, 2H), 4.17 (bs, 1H), 3.90 (bs, 1H), 3.70-3.66 (m, 1H), 2.94-2.91 (m, 2H), 2.41-2.38 (m, 2H), 2.19-2.18 (m, 2H), 2.07-1.97 (m, 2H), 1.92 (bs, 2H), 1.82-1.75 (m, 1H), 1.68-1.64 (m, 5H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 167.9, 134.42 (d), 128.48 (d), 116.63 (d), 115.92 (d), 84.61, 69.25, 57.72,

46.68, 42.37 (d), 31.75, 29.96, 26.54, 17.05. HRMS m/z calculated for $C_{19}H_{25}ClFN_2O$ ($M+H^+$): 351.1639. Found: 351.1643.

Compound SMC-6:
Synthesized using the general procedure described above, using L-phenylalanine methyl ester (HCl salt) as the source of diversity element. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.27-7.24 (m, 2H), 7.21-7.16 (m, 3H), 4.07 (dd, *J* = 12.2, 79.43, 2H), 3.86-3.83 (m, 1H), 3.73 (s, 3H), 3.50-3.37 (m, 3H), 2.97-2.95 (m, 1H), 2.72-2.70 (m, 1H), 2.37-2.34 (m, 2), 2.19-2.16 (m, 2H), 2.04-1.78 (m, 4), 1.71-1.59 (m, 3H), 1.07-1.01 (m, 1H), 0.58-0.55 (m, 1H). HRMS m/z calculated for $C_{22}H_{30}ClN_2O_3$ ($M+H^+$): 405.1945. Found: 405.1952.

Compound SMC-7:
Synthesized using the general procedure described above, using D-phenylalanine methyl ester (HCl salt) as the source of diversity element.^{1}H NMR (500 MHz, $CDCl_3$): δ 7.29-7.25 (m, 2H), 7.21-7.16 (m, 3H), 4.07 (dd, *J* = 12.2, 36.36, 2H), 3.85-3.82 (m, 1H), 3.73 (s, 3H), 3.51-3.37 (m, 3H), 2.94-2.92 (m, 1H), 2.71-2.68 (m, 1H), 2.35-2.31 (m, 2H), 2.19-2.16 (m, 2H), 1.93-1.80 (m, 4H), 1.65-1.60 (m, 3H), 1.20-0.96 (m, 1H), 0.58-0.54 (m, 1H). HRMS m/z calculated for $C_{22}H_{30}ClN_2O_3$ ($M+H^+$): 405.1945. Found: 405.1934

Compound SMC-8:
Synthesized using the general procedure described above, using L-tyrosine methyl ester (HCl salt) as the source of diversity element. ^{1}H NMR (500 MHz, $CDCl_3$): δ 6.96 (d, *J* = 7.56, 2H), 6.67 (d, *J* = 7.57, 2H), 4.06 (dd, *J* = 12.45, 37.71, 2H), 3.78-3.75 (m, 1H), 3.70 (s, 3H), 3.49-3.38 (m, 2H), 3.27-3.22 (m, 1H), 2.97-2.95 (m, 1H), 2.79-2.77 (m, 1H), 2.39-2.36 (m, 2H), 2.19-2.16 (m, 2H), 1.98-1.82 (m, 3H), 1.72-1.63 (m, 3H), 1.20-1.17 (m, 2H), 0.80-0.77 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.78, 166.54, 154.92, 131.12, 130.01, 115.36, 83.82, 68.63, 58.59, 57.06, 53.11, 53.41, 41.76, 34.08, 30.47, 29.67, 25.72, 16.40. HRMS m/z calculated for $C_{22}H_{30}ClN_2O_4$ ($M+H^+$): 421.1894. Found: 421.1877.

Compound SMC-9:
Synthesized using the general procedure described above, using D-tyrosine methyl ester (HCl salt) as the source of diversity element.^{1}H NMR (500 MHz, $CDCl_3$): δ 6.96 (d, *J* = 8.54, 2H), 6.67 (d, *J* = 8.3, 2H), 4.06 (dd, *J* = 12.2, 73.3, 2H), 3.78-3.75 (m, 1H), 3.71 (s, 3H), 3.48-3.37 (m, 2H), 3.28-3.24 (m, 1H), 2.96-2.94 (m, 1H), 2.76-2.74 (m, 1H), 2.38-2.34 (m, 2H), 2.19-2.16 (m, 2H), 1.96-1.80 (m, 3H), 1.71-1.62 (m, 3H), 1.14-1.10 (m, 2H), 0.77-0.74 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.47, 167.24, 155.69, 131.79, 130.62, 116.08, 84.50, 69.32, 59.28, 57.75, 53.80, 53.11, 42.44, 34.77, 31.15, 30.35, 26.40, 17.09. HRMS m/z calculated for $C_{22}H_{30}ClN_2O_4$ ($M+H^+$): 421.1894. Found: 421.1899.

Compound SMC-10:
Synthesized using the general procedure described above, using L-methionine methyl ester (HCl salt) as the source of diversity element.^{1}H NMR (500 MHz, $CDCl_3$): 4.07 (dd, *J* = 15.37, 38.68, 2H), 3.90-3.88 (m, 1H), 3.68 (s, 3H), 3.66-3.58 (m, 1H), 3.02-3.00 (m, 2H), 2.78-2.71 (m, 1H), 2.67-2.62 (m, 2H), 2.45-2.42 (m, 2H), 2.24-2.01 (m, 2H), 2.09 (s,

3H), 2.05-2.03 (m, 2H), 1.95-1.79 (m, 6H), 1.72-1.66 (m, 2H). HRMS m/z calculated for $C_{18}H_{30}ClN_2O_3S$ ($M+H^+$): 389.1666. Found: 389.1658.

Compound SMC-11:
Synthesized using the general procedure described above, using D-methionine methyl ester (HCl salt) as the source of diversity element. ^{1}H NMR (500 MHz, $CDCl_3$): 4.06 (dd, *J* = 12.21, 37.10, 2H), 3.90-3.88 (m, 1H), 3.68 (s, 3H), 3.65-3.59 (m, 1H), 3.02-3.00 (m, 2H), 2.78-2.71 (m, 1H), 2.66-2.63 (m, 2H), 2.44-2.41 (m, 2H), 2.24-2.01 (m, 2H), 2.09 (s, 3H), 2.06-2.02 (m, 2H), 1.95-1.79 (m, 6H), 1.72-1.66 (m, 2H). HRMS m/z calculated for $C_{18}H_{30}ClN_2O_3S$ ($M+H^+$): 389.1666. Found: 389.1667.

Compound SMC-12:
Synthesized using the general procedure described above, using L-leucine methyl ester (HCl salt) as the source of diversity element. ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.05 (dd, *J* = 12.12, 13.70, 2H), 3.70-3.56 (m, 5H), 3.02-3.29 (m, 2H), 2.48-2.41 (m, 3H), 2.24-2.20 (m, 2H), 2.07-2.02 (m, 2H), 1.94-1.93 (m, 2H), 1.87-1.77 (m, 4H), 1.73-1.65 (m, 2H), 1.31-1.24 (m, 1H), 0.94-0.92 (m, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 172.21, 166.85, 84.78, 69.33, 58.39, 57.77, 55.58, 53.91, 52.98, 42.50, 40.55, 31.76 (d), 26.68, 24.02, 23.21, 17.17. HRMS m/z calculated for $C_{19}H_{32}ClN_2O_3$ ($M+H^+$): 371.2101. Found: 371.2085.

Compound SMC-13:
Synthesized using the general procedure described above, using D-leucine methyl ester (HCl salt) as the source of diversity element.^{1}H NMR (400 MHz, $CDCl_3$): δ 4.05 (dd, *J* = 12.20, 30.74, 2H), 3.70-3.57 (m, 5H), 3.01-2.99 (m, 2H), 2.43-2.41 (m, 3H), 2.22-2.19 (m, 2H), 2.05-2.01 (m, 2H), 1.93 (bs, 2H), 1.85-1.76 (m, 4H), 1.71-1.65 (m, 4H), 1.29-1.23 (m, 1H), 0.93-0.92 (m, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.32, 166.01, 83.67, 68.82, 57.20, 56.80, 54.75, 52.91, 52.15, 41.71, 39.73, 29.62 (d), 25.83, 23.20, 22.39, 16.28. HRMS m/z calculated for $C_{19}H_{32}ClN_2O_3$ ($M+H^+$): 371.2101. Found: 371.2101.

Compound SMC-14:
Synthesized using the general procedure described above, using L-glutamate dimethyl ester (HCl salt) as the source of diversity element. ^{1}H NMR (500 MHz, $CDCl_3$): δ 4.07 (dd, *J* = 12.2, 33.31, 2H), 3.79-3.77 (m, 1H), 3.69 (s, 3H), 3.68 (s, 3H), 3.66-3.58 (m, 1H), 3.20-2.98 (m, 2H), 2.70-2.65 (m, 1H), 2.55-2.52 (m, 2H), 2.45-2.42 (m, 2H), 2.41-2.22 (m, 2H), 2.08-1.91 (m, 5H), 1.84-1.78 (m, 3H), 1.70-1.68 (m, 2H). HRMS m/z calculated for $C_{19}H_{30}ClN_2O_5$ ($M+H^+$): 401.1843. Found: 401.1849.

Compound SMC-15:
Synthesized using the general procedure described above, using D-glutamate dimethyl ester (HCl salt) as the source of diversity element. ^{1}H NMR (500 MHz, $CDCl_3$): δ 4.06 (dd, *J* = 12.2, 32.94, 2H), 3.79-3.77 (m, 1H), 3.69 (s, 3H), 3.67 (s, 3H), 3.66-3.61 (m, 1H), 3.05-3.01 (m, 2H), 2.69-2.63 (m, 1H), 2.55-2.52 (m, 2H), 2.46-2.43 (m, 2H), 2.25-2.21 (m, 2H), 2.09-1.92 (m, 5H), 1.86-1.78 (m, 3H), 1.74-1.67 (m, 2H). HRMS m/z calculated for $C_{19}H_{30}ClN_2O_5$ ($M+H^+$): 401.1843. Found: 401.1858.

IV. Cell culture and gel-based experiments

Preparation of cell lysates:
Cells (MCF-7 or HEK 293T) were grown to 100% confluency under 5% CO_2 at 37 ˚C before they were scraped, washed (3x 5 mL Phosphate buffered saline (PBS)) and pelleted (by centrifugation; 3,500 rpm, 5 min, 4 ˚C). After the supernatant was removed, the cells were lysed in PBS. The lysates were separated to obtain the soluble lysates by ultracentrifugation (45,000 rpm, 45 min, 4˚C). The soluble lysates were normalized to 2 mg mL^{-1} and used in subsequent experiments.

Click chemistry and fluorescent analysis:
Cell lysates, described above, were aliquoted into 50 µL samples and exposed to probe for 1 hour at room temperature. Samples underwent click chemistry combining rhodamine-azide (0.025 mM), TCEP (0.28 mg mL^{-1}), tris[(1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl]amine (TBTA ligand) (100 µM, 17X stock in DMSO:t-Butanol 1:4), and CuSO4 (1 mM). The reaction was mixed after 30 minutes of incubation at room temperature and then allowed to proceed for another 30 minutes before being quenched with 2x SDS loading dye. The samples were then separated by SDS-PAGE and visualized with a Hitachi FMBIO II multiview flatbed laser-induced fluorescent scanner at 585 nm.

In situ gel profiling:
MCF-7 cells were grown to 100% confluency, in RPMI media supplemented with 10% fetal calf serum (FCS), 2 mM glutamine, and 1% Penn/strep (under 5% CO_2 at 37 ˚C), before being introduced to probes. The probes were diluted into 5 mL of serum free RPMI media supplemented with Penn/strep from 5 mM DMSO stocks to the desired probing concentrations (1µM or 5µM) under a sterile environment. Next, the probe containing media was gently added to the cells and the cells were allowed to incubate under 5% CO_2 at 37 ˚C for 1 hour. The cells were then scraped from the plate and transferred to a 15 mL conical tube. The cells were washed 3x (5 mL) with PBS, by resuspending the pellet in the PBS followed by centrifugation (3,500 rpm for 5 minutes at 4 ˚C) and removal of supernatant. When washing was complete, the cells were resuspended in PBS and lysed by sonication. The soluble lysates were separated by ultracentrifugation (45,000 rpm for 45 min at 4 ˚C). After, the lysates were normalized to a protein concentration of 2 mg mL^{-1} and aliquotted into 50 µL reaction volumes. Each reaction sample was exposed to click chemistry conditions with rhodamine-azide for visualization, as described above. After analysis, gels underwent a typical procedure for coomassie staining and destaining. Stained gels were visualized on a Stratagene Eagle Eye apparatus by a COHU High performance CCD camera.

Transfection procedure:
The cDNA for WT-GSTO1 and WT-AKT1 were subcloned into a pcDNA3.1-myc/His mammalian expression vector. Site-directed mutagenesis was used to obtain the C32A GSTO1 mutant, and all constructs were verified by sequencing (Genewiz, Cambridge, MA). HEK 293T cells were grown at 37 ℃ under 5% CO_2 in DMEM media supplemented with 10% fetal calf serum (FCS), 2 mM glutamine, and 1% Penn/strep. Transfections were performed on 10 cm cell plates of ~50% confluency. Serum free DMEM media (600 µL) and X-tremeGENE DNA transfection reagent (20 µL) were combined in an eppendorf tube and vortexed. Plasmids of WT-GSTO1, WT-AKT1 or C32A GSTO1 (6 µg) were added and the sample was shaken and remained at room temperature for 15 minutes. This plasmid solution was added dropwise to the HEK 293T cells. The plate

was incubated at 37 ℃ under 5% CO_2 for 48 hours. HEK 293T cells transfected with the pcDNA3.1-myc/His plasmid was used as a mock negative control. The lysates were prepared as described above.

In vitro gel profiling:
The procedure for preparation of lysates, described above, was used to obtain the lysates used in the following experiment. After, the lysates are normalized to a protein concentration of 2 mg mL^{-1} and aliquotted into 50 μL reaction volumes. To each sample was added equal volume of probe to achieve the desired final concentration of probe in 50 μL (GSTO1 at 1μM and AKT1 at 5μM) and the sample was allowed to incubate at room temperature for 1 hour. Each reaction sample was exposed to click chemistry conditions with Rhodamine-azide for visualization. The click chemistry reaction was allowed to proceed at room temperature for 1 hour, and then the proteins were separated by SDS-PAGE. The gel was then scanned to obtain a fluorescent image, as described above, and then proteins were transferred to nitrocellulose membrane for a Western Blot. The concentrations of GSTO1 or AKT1 were evaluated by Western Blot analysis, described below.

GSTO1 site of labeling experiment:
HEK 293T cells were transfected with either WT-GSTO1 or C32A-GSTO1 mammalian constructs, as described above. The cells were grown to 100% confluency and then exposed to SMC-1 (0.5 μM) by diluting 5 mM probe stock into 5 mL of serum free DMEM media, supplemented with 1% Penn/strep. The cells were allowed to incubate with probe for 1 hour before being scraped, washed, pelleted, and sonicated as described above. After the soluble protein lysates was normalized to 2 mg mL^{-1} and aliquoted into 50 μL reaction volumes, they were exposed to click chemistry conditions and visualized by fluorescence, as described above. The protein was then transferred to nitrocellulose paper and the GSTO1 protein concentrations were evaluated by Western Blot analysis, described below.

Staurosporine AKT1 inhibition experiment with SMC-8:
Cell lysates were prepared as described above and normalized to 2 mg mL^{-1}. The lysates were aliquotted into 50 μL samples and then treated with Staurosporine (STS) in DMSO to obtain samples with final STS concentrations of 0, 0.5, 1, 5, 10, 25, 50, 100 μM. The STS treated samples were mixed and then allowed to incubate for 1 hour at room temperature. Then each sample was exposed to SMC-8 (5 μM). The samples were incubated at room temperature for another hour before being exposed to click chemistry conditions, as described above. Then the proteins were separated by SDS-PAGE. The gel was then scanned to obtain a fluorescent image and then proteins were transferred to nitrocellulose membrane for Western Blot analysis. The concentrations of AKT1 were evaluated by Western Blot analysis, described below

Western Blot Analysis:
After the protein was transferred to nitrocellulose paper (75mV, 120 min), the membrane was washed with tris-buffered saline with 1% Tween 20 (TBST) and then stained by Ponceau S stain to detect transferred proteins. The membrane was washed 3x (~10 mL for 5 min) with TBST until completely destained. The membrane was then allowed to milk block (2.5g in 50 mL of TBST) for 2 hours at room temperature before being washed again with TBST (3x ~10 mL for 5 min). The membrane was then exposed to 1˚ anti-Myc antibody (Ab) (1:1000) in TBST and allowed to incubate over night at 4 ˚C.

Then the membrane is washed with TBST (3x ~10 mL for 5 min) and exposed to anti-rabbit-HRP conjugated 2˚ Ab (1:3333) in TBST for 2 hours at room temperature. After 2˚ Ab incubation the membrane is washed 3x (~10 mL for 5 min) with TBST before being treated with HRP super signal chemiluminescence reagents and exposed to film for one minute before development, using Kodak X-OMAT 2000A processor.