Supporting Information

Quantitative chemoproteomics for site-specific analysis of protein alkylation by 4-hydroxy-2-nonenal in cells

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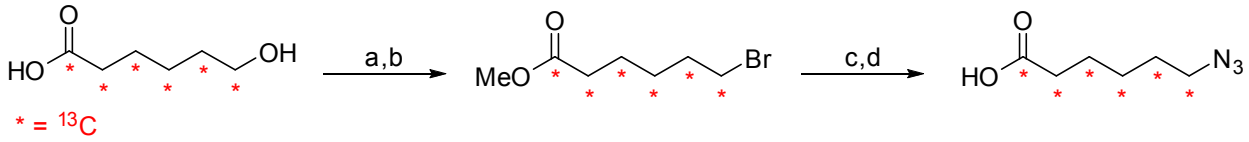
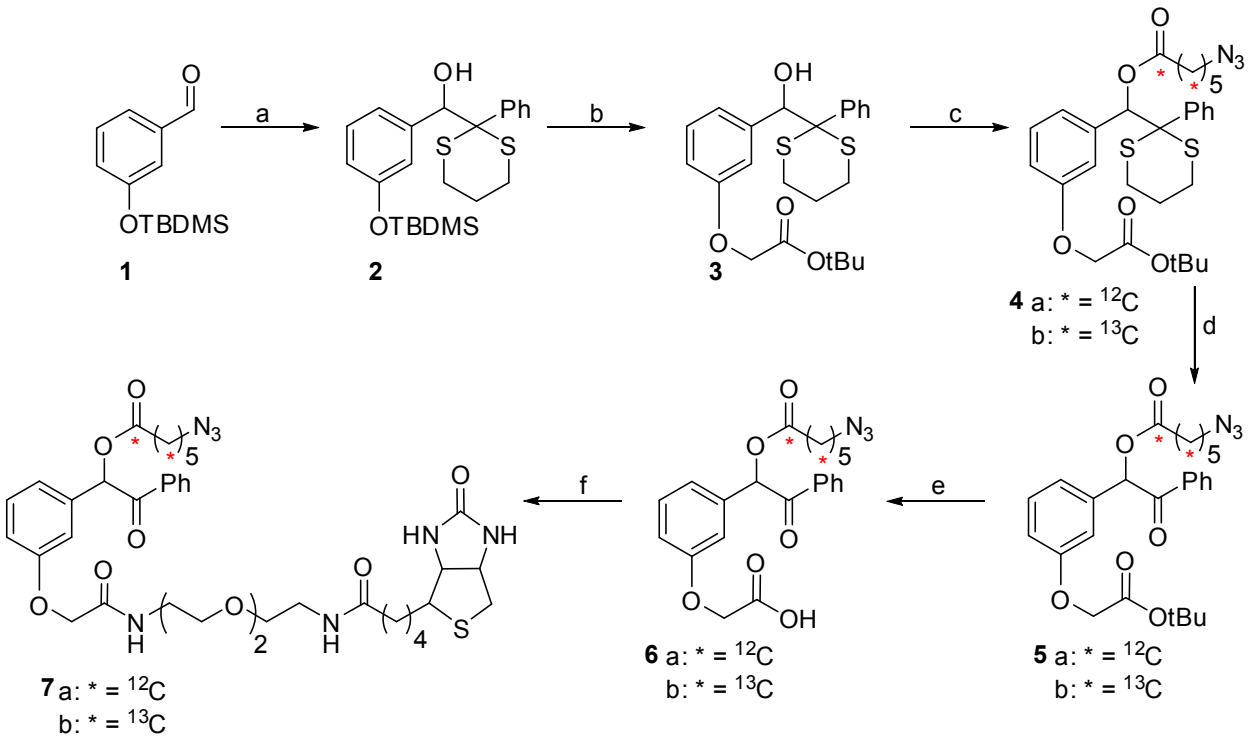
Synthesis of photocleavable biotin reagents



Reagents: a) TsOH, MeOH; b) NBS, PPh_3, CH_2Cl_2; c) NaN_3, DMSO; d) NaOH, MeOH/H_2O.



Reagents: a) 2-phenyl-1, 3-dithiane, *n*BuLi, THF, 0 °C; b) *tert*-butyl bromoacetate, TBAF, THF; c) 6-azidohexanoic acid ($^{12}C_6$ or $^{13}C_6$), DCC, DMAP, CCl_4; d) Ph-I($OTf)_2$, CH_3CN/H_2O; e) TFA, CH_2Cl_2; f) CDI, biotin-amine, DMF.

Methods and materials. ^{1}H and ^{13}C NMR spectra were collected on a 300 MHz NMR. All reactions were carried out under an atmosphere of argon. THF and CH_2Cl_2 were dried using a solvent purification system. Commercial anhydrous DMF, DMSO, and MeOH were used as received. Purification by column chromatography was carried out on silica gel and TLC plates were visualized by UV and stained with phosphomolybdic acid[1]. The $^{13}C_6$-6-azidohexanoic acid was prepared from commercial $^{13}C_6$-6-hydroxyhexanoic acid, while the natural compound was prepared from commercial 6-bromohexanoic acid. Attempts to convert the 6-hydroxyhexanoic acid directly to 6-bromohexanoic acid using PBr_3 were unsuccessful. Conversion to the ester first resulted in an overall higher yield of the 6-azidohexanoic acid. Compounds **1** and **2** were prepared according to literature procedures[2,3]. The biotin-amine reagent used in the final coupling has also been described in the literature[4]. The same synthetic procedures were used to synthesize both the light and heavy biotin reagents. Following is a detailed description for the heavy reagent, with characterization for both light (a) and heavy (b) reagents included.

Synthesis of $^{13}C_6$-methyl 6-hydroxyhexanoate. TsOH (0.080 g, 0.42 mmol) was added to a solution of $^{13}C_6$-6-hydroxyhexanoic acid (0.30 g, 2.2 mmol) in MeOH (10 mL). After 3 h, the reaction mixture was quenched with

saturated $NaHCO_3$ and extracted with EtOAc. The organic layer was washed with brine and dried over $MgSO_4$. The product was isolated as a colorless oil (0.28 g, 85%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.83-377 and 3.36-3.30 (m, 2H, J_{C-H} = 141 Hz), 3.60 (d, 3H, J_{C-H} = 3 Hz), 2.51-2.44 and 2.09-2.01 (m, 2H, J_{C-H} = 126 Hz), 2.10 (br s, 1H), 1.85-1.76 and 1.43-1.35 (m, 2H, J_{C-H} = 126 Hz), 1.75-1.69 and 1.34-1.27 (m, 2H, J_{C-H} = 123 Hz), 1.55-1.49 and 1.17-1.09 (m, 2H, J_{C-H} = 114 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.2 (dd, J = 2, 57 Hz), 62.4 (dd, J = 4, 38 Hz), 51.5, 33.9 (ddd, J = 4, 32, 57 Hz), 32.2 (dt, J = 5, 34 Hz), 24.8 (d pentet, J = 2, 28 Hz).

Synthesis of $^{13}C_6$-methyl 6-bromohexanoate. NBS (0.40 g, 2.3 mmol) was added to a solution of $^{13}C_6$-methyl 6-hydroxyhexanoate (0.28 g, 1.8 mmol) and PPh_3 (0.60 g, 2.3 mmol) in CH_2Cl_2 (9 mL). After 1 h, the reaction mixture was diluted with ether and filtered through a pad of silica. The product was purified on a silica SPE cartridge (2:1, hexanes:EtOAc) and isolated as a colorless liquid (0.40 g, 100%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.63 (d, 3H, J_{C-H} = 4 Hz), 3.60-3.58 and 3.14-3.09 (m, 2H, J_{C-H} = 138 Hz), 2.55-2.45 and 2.11-2.01 (m, 2H, J_{C-H} = 132 Hz), 2.11-2.01 and 1.64-1.59 (m, 2H, J_{C-H} = 142 Hz), 1.86-1.79 and 1.42-1.39 (m, 2H, J_{C-H} = 133 Hz), 1.64-1.59 and 1.24-1.16 (m, 2H, J_{C-H} = 120 Hz) ; ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.8 (dd, J = 1, 58 Hz), 51.5, 33.8 (ddd, J = 4, 35, 57 Hz), 33.2 (dt, J = 5, 38 Hz), 32.3 (dt, J = 4, 35 Hz), 27.6 (dt, J = 3, 33 Hz), 23.9 (dt, J = 5, 35 Hz).

Synthesis of $^{13}C_6$-methyl 6-azidohexanoate. NaN_3 (0.24 g, 3.7 mmol) was added to a solution of $^{13}C_6$-methyl 6-bromohexanoate (0.40 g, 1.8 mmol) in anhydrous DMSO (4 mL). After overnight, the reaction mixture was diluted with EtOAc and washed with H_2O, brine, and dried over $MgSO_4$. The product (0.27 g) was isolated as a pale yellow liquid in 83% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.64 (d, J_{C-H} = 4 Hz), 3.51-3.44 and 3.04-2.98 (m, 2H, J_{C-H} = 141 Hz), 2.54-2.45 and 2.12-2.03 (m, 2H, J_{C-H} = 126 Hz), 1.88-1.73 and 1.44-1.34 (m, 4H, J_{C-H} = 132 Hz), 1.63-1.53 and 1.25-1.21 (m, 2H, J_{C-H} = 114 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.9 (dd, J = 2, 57 Hz), 51.2 (dd, J = 5, 36 Hz), 33.8 (ddd, J = 4, 34, 57 Hz), 28.5 (dt, J = 4, 34 Hz), 26.2 (dt, J = 3, 34 Hz), 24.3 (dt, J = 4, 35 Hz).

Synthesis of $^{13}C_6$-6-azidohexanoic acid. NaOH (0.12 g, 3.0 mmol) was added to a solution of $^{13}C_6$-methyl 6-azidohexanoate (0.27 g, 1.5 mmol) in MeOH/H_2O (8 mL, 1:1). After 2 h, the reaction mixture was acidified with 10% HCl, saturated with brine, and extracted with EtOAc. The organic layer was dried over $MgSO_4$. The product was isolated as a pale yellow liquid (0.22 g, 88%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.52-3.46 and 3.05-3.00 (m, 2H, J_{C-H} = 141 Hz), 2.60-2.51 and 2.17-2.11 (m, 2H, J_{C-H} = 129 Hz), 1.89-1.76 and 1.47-1.39 (m, 4H, J_{C-H} = 126 Hz), 1.63-1.59 and 1.26-1.19 (m, 2H, J_{C-H} = 111 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 179.7 (dd, J = 2, 55 Hz), 51.1 (dd, J = 5, 36 Hz), 33.7 (ddd, J = 4, 34, 55 Hz), 28.5 (dt, J = 4, 34 Hz), 26.1 (dt, J = 4, 35 Hz), 24.0 (dt, J = 4, 35 Hz).

*Synthesis of **3***. TBAF (14 mL, 1M/THF, 0.014 mol) was added dropwise to a solution of **2** (5.1 g, 0.012 mol) and *tert*-butyl bromoacetate (2.0 mL, 0.014 mol) in THF (60 mL). After 3 h, the reaction mixture was poured into H_2O and extracted with EtOAc. The organic layer was washed with brine and dried over $MgSO_4$. Purification by column chromatography (20% EtOAc/hexanes) yielded the product as a white powder (3.5 g, 69%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.66 (dd, 2H, J = 2.2, 8.1 Hz), 7.31-7.23 (m, 3H), 6.99 (t, 1H, J = 8.0 Hz), 6.74 (dd, 1H, J = 1.9, 7.5 Hz), 6.48 (d, 1H, J = 7.6 Hz), 6.27 (t, 1H, J = 1.9 Hz), 4.92 (s, 1H), 4.21 (s, 2H), 3.00 (br s, 1H), 2.73-2.60 (m, 4H), 1.91-1.84 (m, 2H), 1.44 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 167.9, 156.8, 138.8, 137.4, 130.6, 128.1, 127.9, 127.5, 121.5, 115.3, 113.7, 82.1, 80.7, 66.2, 65.5, 28.0, 27.2, 26.9, 24.7.

*Synthesis of **4***. A solution of DCC (0.33 g, 1.6 mmol) in CCl_4 (3 mL) was added dropwise to a solution of **3** (0.60 g, 1.4 mmol), $^{13}C_6$-6-azidohexanoic acid (0.22 g, 1.4 mmol), and DMAP (0.20 g, 1.6 mmol) in CCl_4 (3 mL). After 2 h, the reaction mixture was filtered and concentrated. The product was purified by column chromatography (20% EtOAc/hexanes) and isolated as a colorless oil (0.76 g, 97%).
4a: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.70 (dd, 2H, J = 2.3, 8.1 Hz), 7.31-7.25 (m, 3H), 7.02 (t, 1H, J = 8.1 Hz), 6.74 (dd, 1H, J = 2.6, 8.2 Hz), 6.54 (d, 1H, J = 7.7 Hz), 6.27 (br s, 1H), 6.11 (s, 1H), 4.23 (app d, 2H, J = 4.9 Hz), 3.20 (t, 2H, J = 6.9 Hz), 2.71-2.52 (m, 4H), 2.42-2.26 (m, 2H), 1.86-1.84 (m, 2H), 1.64-1.51 (m, 4H), 1.44 (s, 9H),

1.39-1.31 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.6, 167.8.56.8, 136.9, 136.4, 130.9, 128.0, 127.9, 127.6, 122.0, 115.3, 114.4, 82.1, 79.8, 65.6, 63.2, 60.3, 51.2, 34.0, 28.5, 28.0, 27.2, 27.0, 26.1, 24.6, 24.2, 21.0, 14.1.
4b: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.71 (dd, 2H, J = 2.3 Hz), 7.33-7.25 (m, 3H), 7.03 (t, 1H, J = 8.0 Hz), 6.75 (dd, 1H, J = 1.9, 7.6 Hz), 6.55 (d, 1H, J = 7.6 Hz), 6.28 (t, 1H, J = 2.0 Hz), 6.12 (d, 1H, J = 3.8 Hz), 4.24 (app d, 2H, J = 4.7 Hz), 3.48-3.41 and 3.00-2.95 (m, 2H, J_{C-H} = 145 Hz), 2.72-2.60 (m, 4H), 2.58-2.52 and 2.15-2.04 (m, 2H, J_{C-H} = 144 Hz), 1.91-1.83 (m, 2H), 1.80-1.74 and 1.41-1.33 (m, 4H, J_{C-H} = 119 Hz), 1.55-1.53 and 1.16-1.13 (m, 2H, J_{C-H} = 117 Hz), 1.44 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.6 (dd, J = 2,57 Hz), 167.8, 156.8, 136.9, 136.4, 130.9, 128.1, 128.0, 127.6, 122.0, 115.3, 114.4, 82.1, 79.8, 65.6, 63.2, 60.3, 51.2 (dd, J = 5, 36 Hz), 34.0 (ddd, J = 4, 34, 57 Hz), 28.5 (dt, J = 4, 34 Hz), 27.2, 27.0, 26.1 (dt, J = 3, 34 Hz), 24.2 (dt, J = 4, 34 Hz), 21.0, 14.1.

*Synthesis of **5***. [Bis(trifluoroacetoxy)iodo]benzene (1.1 g, 2.6 mmol) was added to a solution of **4** (0.76 g, 1.3 mmol) in CH_3CN (6 mL) and H_2O (1 mL) in the dark. After 3 h, the reaction mixture was diluted with EtOAc and washed with H_2O, brine, and dried over $MgSO_4$. The product (0.68 g, 100%) was isolated as a colorless oil after column chromatography (20% EtOAc/hexanes).
5a: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.89 (dd, 2H, J = 1.3, 7.3 Hz), 7.49 (t, 1H, J = 73 Hz), 7.36 (t, 2H, J = 7.9 Hz), 7.25 (t, 1H, J = 7.9 Hz), 7.05 (d, 1H, J = 7.7 Hz), 6.97 (t, 1H, J = 2.1 Hz), 6.83 (dd, 1H, J = 2.5, 8.2 Hz), 6.78 (s, 1H), 4.47 (s, 2H), 3.23 (t, 2H, J – 6.8 Hz), 2.55-2.36 (m, 2H), 1.68 (pentet, 2H, J = 7.4 Hz), 1.58 (pentet, 2H, J = 7.3 Hz), 1.44 (s, 9H), 1.44-1.37 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 193.7, 173.0, 167.9, 158.2, 134.9, 134.4, 133.6, 130.2, 128.8, 128.6, 121.8, 115.5, 114.7, 82.7, 77.3, 65.5, 60.7, 51.1, 33.7, 28.4, 27.9, 26.0, 24.3, 14.1.
5b: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.89 (dd, 2H, J = 1.3, 7.2 Hz), 7.50 (tt, 1H, J = 1.2, 7.5 Hz), 7.37 (t, 2H, J = 7.8 Hz), 7.26 (t, 1H, J = 8.1 Hz), 7.06 (d, 1H, J = 7.8 Hz), 6.96 (t, 1H, J = 2.3 Hz), 6.85-6.81 (m, 2H), 4.48 (s, 2H), 3.50-3.44 and 3.03-2.97 (m, 2H, J_{C-H} = 141 Hz), 2.72-2.64 and 2.30-2.18 (m, 2H, J_{C-H} = 126 Hz), 1.91-1.85 and 1.52-1.44 (m, 2H, J_{C-H} = 117 Hz), 1.82-1.75 and 1.39-1.35 (m, 2H, J_{C-H} = 127 Hz), 1.65-1.58 and 1.19-1.14 (m, 2H, J_{C-H} = 137 Hz), 1.45 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 194.3, 173.6 (dd, J = 2, 56 Hz), 169.0, 158.1, 134.7, 134.1, 133.9, 130.3, 128.8, 128.7, 122.0, 115.6, 114.5, 83.6, 77.4, 65.5, 61.4, 51.1 (dd, J = 5, 23 Hz), 33.8 (ddd, J = 4, 34, 57 Hz), 28.4 (dt, J = 4, 34 Hz), 26.0 (dt, J = 3, 34 Hz), 24.2 (dt, J = 5, 34 Hz), 13.9.

*Synthesis of **6***. TFA (1.5 mL) was added to a solution of **5** (0.68 g, 1.4 mmol) in CH_2Cl_2 (7 mL). After 2 h, the reaction mixture was diluted with EtOAc, washed with brine, and dried over $MgSO_4$. The product (0.56 g) was isolated as a yellow oil in 93% yield.
6a: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.91 (dd, 2H, J = 1.2, 7.3 Hz), 7.51 (t, 1H, J = 7.4 Hz), 7.39 (t, 2H, J = 7.8 Hz), 7.28 (t, 1H, J = 8.0 Hz), 7.11 (d, 1H, J = 7.8 Hz), 7.04 (t, 1H, J = 2.1 Hz), 6.86 (dd, 1H, J = 2.4, 85 Hz), 6.84 (s, 1H), 4.63 (s, 2H), 3.24 (t, 2H, J = 6.8 Hz), 2.55-2.39 (m, 2H), 1.69 (pentet, 2H, J = 7.6 Hz), 1.59 (pentet, 2H, J = 7.3 Hz), 1.46-1.38 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 194.2, 173.5, 173.3, 157.8, 135.1, 134.3, 133.8, 130.4, 128.8, 128.7, 128.3, 122.3, 115.4, 114.9, 77.2, 64.8, 51.1, 33.7, 28.4, 26.0, 24.3.
6b: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.90 (dd, 2H, J = 1.3, 7.2 Hz), 7.50 (t, 1H, J = 7.4 Hz), 7.37 (t, 2H, J = 7.8 Hz), 7.27 (t, 1H, J = 7.9 Hz), 7.10 (d, 1H, J = 7.7 Hz), 7.02 (br s, 1H), 6.87-684 (m, 2H), 4.62 (s, 2H), 3.50-3.44 and 3.03-2.97 (m, 2H, J_{C-H} = 141 Hz), 2.70-2.65 and 2.27-2.21 (m, 2H, J_{C-H} = 128 Hz), 1.92-1.85 and 1.55-1.40 (m, 2H, J_{C-H} = 111 Hz), 1.82-1.70 and 1.40-1.30 (m, 2H, J_{C-H} = 126 Hz) 1.70-1.55 and 1.30-1.19 (m, 2H, J_{C-H} = 120 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 194.5, 173.5 (dd, J = 2, 56 Hz), 157.7, 135.0, 134.1, 134.0, 130.5, 128.8, 128.7, 122.3, 115.5, 114.9, 77.3, 64.8, 51.1 (dd, J = 4, 34 Hz), 33.7 (ddd, J = 5, 34, 56 Hz), 28.4 (dt, J = 4, 34 Hz), 26.0 (dt, J = 3, 34 Hz), 24.2 (dt, J = 4, 34 Hz).

*Synthesis of **7***. CDI (0.42 g, 2.6 mmol) was added to a solution of **6** (0.56 g, 1.3 mmol) in DMF (7 mL). After 30 min, the biotin-amine reagent (1.0 g, 2.7 mmol) was added. After overnight, the reaction mixture was concentrated and purified by column chromatography (10% MeOH/CH_2Cl_2). The product (0.60 g, 59%) was isolated as a white foam.
7a: ^{1}H NMR (300 MHz, MeOH-*d*$_4$) δ 8.02-7.98 (m, 2H), 7.57 (t, 1H, J = 7.3 Hz), 7.45 (t, 2H, J = 7.7 Hz), 7.32 (t, 1H, J = 7.8 Hz), 7.17 (s, 1H), 7.16 (d, 1H, J = 7.2 Hz), 6.98 (s, 1H), 6.98-6.96 (m, 1H), 4.52 (s, 2H), 4.45 (dd, 1H, J = 4.8, 7.7 Hz), 4.25 (dd, 1H, J = 4.4, 7.8 Hz), 3.56 (s, 4H), 3.56-3.52 (m, 4H), 3.47 (t, 2H, J = 4.8 Hz), 3.36 (t, 2H, J = 5.9 Hz), 3.26 (t, 2H, J = 6.7 Hz), 3.18-3.12 (m, 1H), 2.89 (dd, 1H, J = 4.8, 12.7 Hz), 2.69 (d, 1H, J = 12.7

Hz), 2.54-2.41 (m, 2H), 2.20 (t, 2H, J = 7.3 Hz), 1.72-1.52 (m, 8H), 1.47-1.39 (m, 4H); ^{13}C NMR (75 MHz, MeOH-d_4) δ 194.2, 174.6, 173.0, 169.3, 164.5, 158.0, 135.3, 134.4, 133.6, 130.1, 128.5, 121.8, 115.1, 77.2, 69.8, 69.2, 69.0, 66.9, 61.9, 60.1, 55.6, 50.9, 39.7, 38.9, 38.5, 35.4, 33.2, 28.4, 28.2, 28.1, 25.8, 25.4, 24.1. HRMS (ESI) calculated 782.3542 (M + H), observed 782.3570.

7b: ^{1}H NMR (300 MHz, MeOH-d_4) δ 8.06-7.99 (m, 2H), 7.59 (t, 1H, J = 7.4 Hz), 7.46 (t, 2H, J = 7.8 Hz), 7.33 (t, 1H, J = 8.2 Hz), 7.17 (s, 1H), 7.16 (d, 1H, J = 6.8 Hz), 7.00-6.98 (m, 1H), 6.97 (d, 1H, J_{C-H} = 2 Hz), 4.52 (s, 2H), 4.47 (dd, 1H, J = 4.6, 7.8 Hz), 4.21 (dd, 1H, J = 4.4, 7.8 Hz), 3.58 (s, 4H), 3.58-3.51 (m, 4H), 3.46 (t, 2H, J = 5.1 Hz), 3.36 (t, 2H, J = 5.3 Hz), 3.42-3.36 and 3.08-3.02 (m, 2H, J_{C-H} = 102 Hz), 3.20-3.14 (m, 1H), 2.90 (dd, 1H, J = 4.9, 12.8 Hz), 2.70 (d, 1H, J = 12.6 Hz), 2.75-2.67 and 2.29-2.25 (m, 2H, J_{C-H} = 138 Hz), 2.20 (t, 2H, J = 7.3 Hz), 1.93-1.87 (m, 1H), 1.84-1.75 (m, 1H), 1.68-1.59 (m, 5H), 1.48-1.39 (m, 4H), 1.30-1.25 (m, 1H); ^{13}C NMR (75 MHz, MeOH-d_4) δ 194.2, 174.6, 173.0 (d, J = 57 Hz), 169.3, 164.6, 158.0, 135.3, 134.4, 133.5, 130.0, 128.5, 121.8, 115.0 (d, J = 5 Hz), 77.2, 69.8, 69.2, 69.0, 66.8, 61.9, 60.1, 55.5, 50.8 (dd, J = 5, 36 Hz), 39.6, 38.9, 38.5, 35.3, 33.1 (ddd, J = 4, 33, 56 Hz), 28.3, 28.1 (dt, J = 4, 34 Hz), 25.7 (dt, J = 3, 34 Hz), 25.3, 24.0 (dt, J = 3, 33 Hz). HRMS (ESI) calculated 788.3743 (M + H), observed 788.3740.

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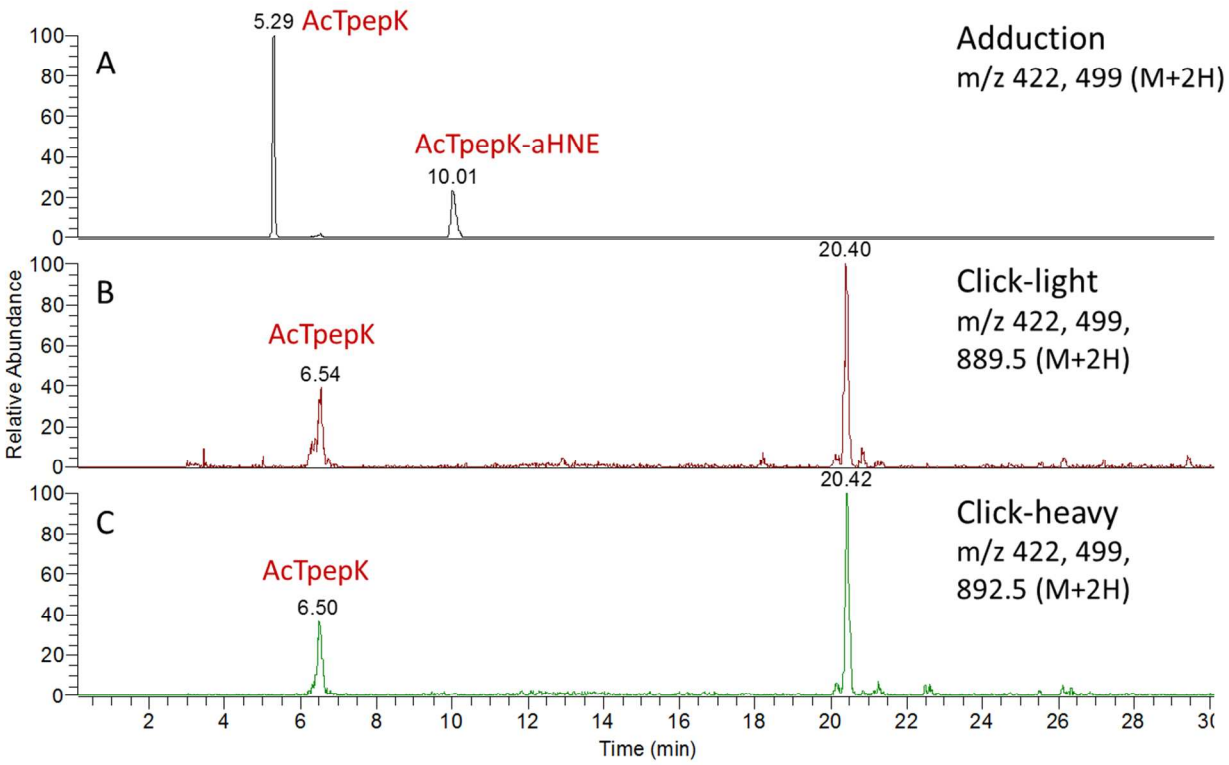

Figure S1. Click reaction of AcTpepK (AcAVAGKAGAR)-aHNE adduct with either light or heavy azidobiotin reagent. (A) Mixture of unreacted AcTpepK and AcTpepK-aHNE adduct; (B) click reaction of peptide adduct with light biotin reagent showing complete conversion; and (C) click reaction of peptide adduct with heavy biotin reagent showing complete conversion. (Note: the retention time for the unreacted AcTpepK shifted due to a change in mobile phase pH between the analysis shown in panel A and those shown in panels B and C).

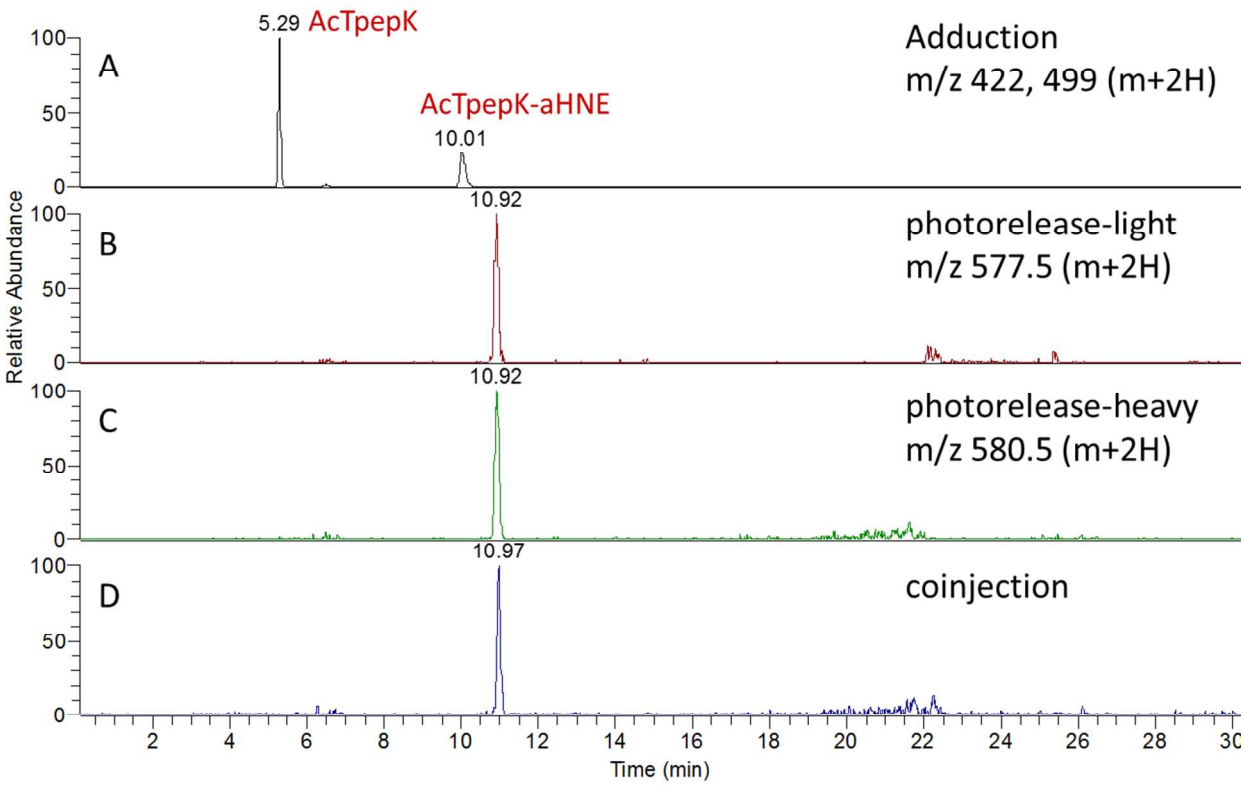

Figure S2. Photorelease of light or heavy biotin-peptide adduct. (A) Mixture of unreacted AcTpepK and AcTpepK-aHNE adduct; B) photorelease of the light biotin-peptide adduct; (C) photorelease of the heavy biotin-peptide adduct; and D) co-injection of the light and heavy reaction mixtures.

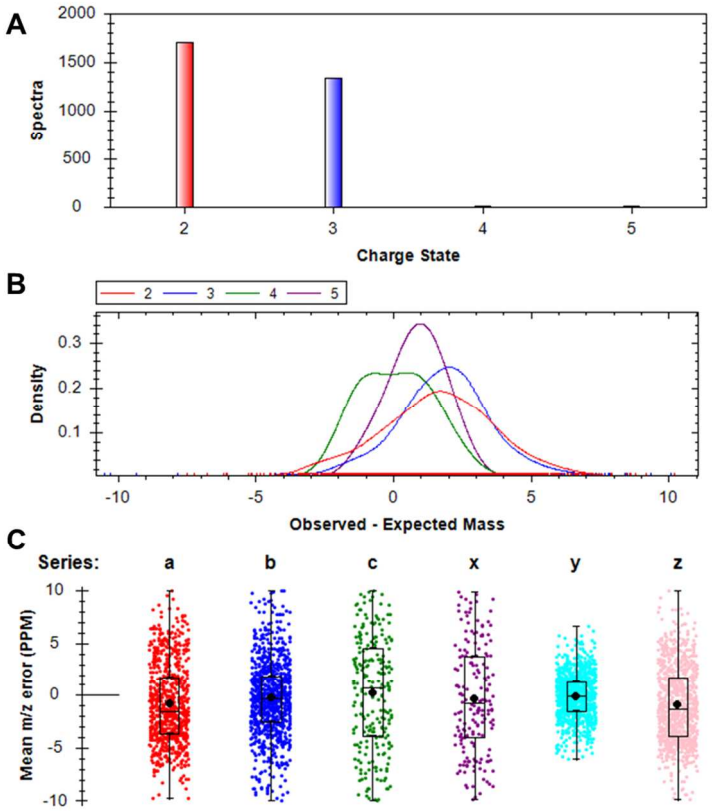

Figure S3. Data quality metrics for LC-MS/MS analyses. (A) Distribution of the charge states of aHNE modified peptides. (B) Statistics analysis of mass errors of precursor ions of modified peptides. (c) Distributions of mass errors for sequence fragment ion measurements from HCD-MS/MS of modified peptides.

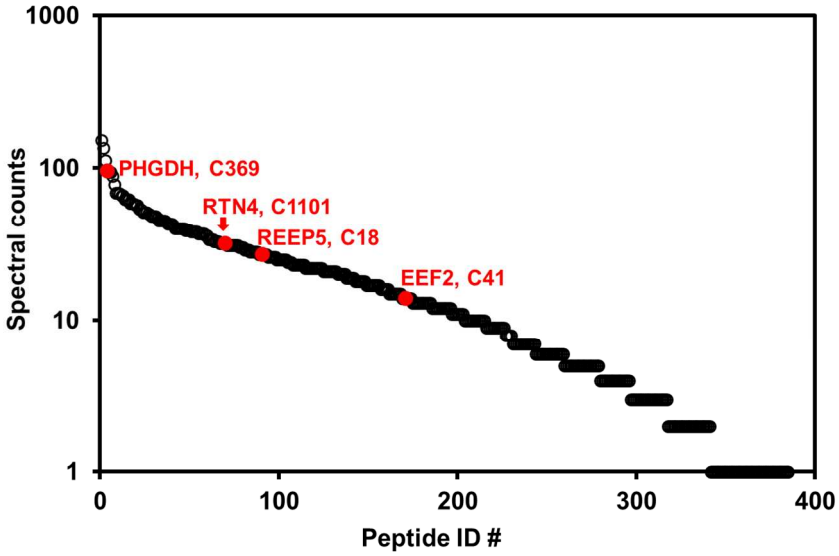

Figure S4. Distribution of spectral counts from all the identified aHNE-alkylated cysteine peptides (black circles). Red circles denote HNE-sensitive cysteines identified in competitive isoTOP-ABPP experiments by Wang, *et* al (Nat Methods, 2014). Y-axis is plotted on a $\log_{10}$ scale.

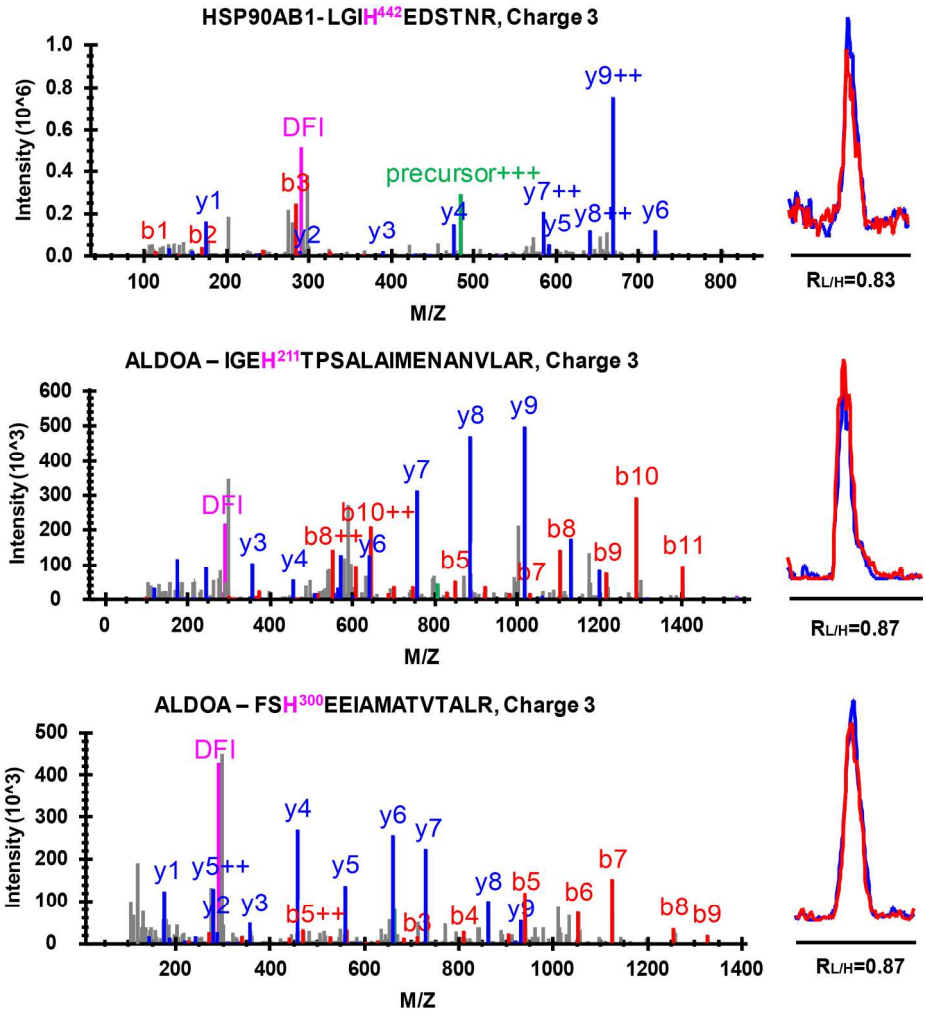

Figure S5. Annotated HCD MS/MS spectra of identified aHNE alkylated histidine peptides from HSP90AB1 and ALDOA (DFI: Diagnostic fragment ions, *m/z* of 292.2 and 298.2 for light and heavy modification, respectively). Extracted ion chromatograms are shown on the right of the corresponding spectra with profiles for light- and heavy- labeled peptides in red and blue, respectively. The experimental ratios calculated from three biological replicate experiments are displayed below each chromatogram.

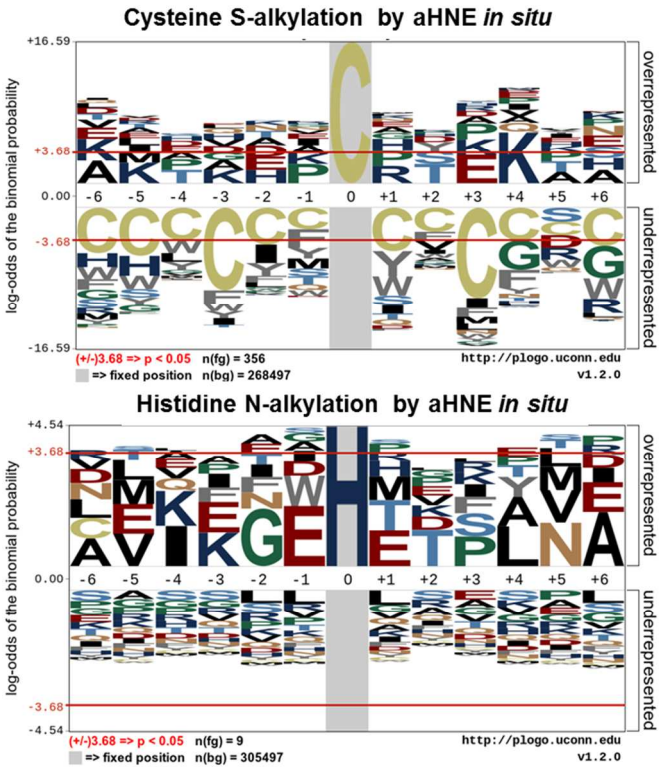

Figure S6. Sequence motif analysis of protein alkylation by aHNE. The calculated sequence motif for cysteine S-alkylation (n=356) and histidine N-alkylation (n=9) were compared. Images were generated with pLogo and scaled to the height of the largest column within the sequence visualization. The red horizontal lines on the pLogo plots denote P <0.05 thresholds.

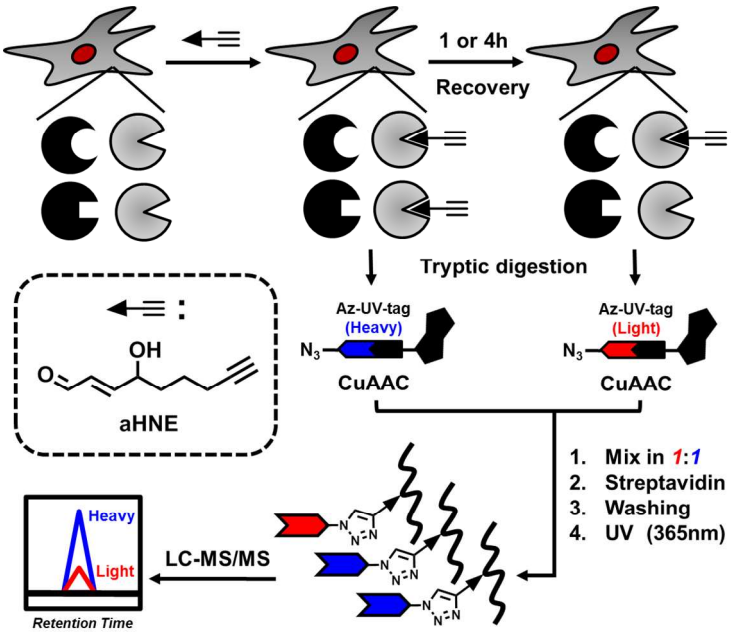

Figure S7. Workflow for the direct proteomic analysis of dynamic protein alkylation by aHNE in cells.

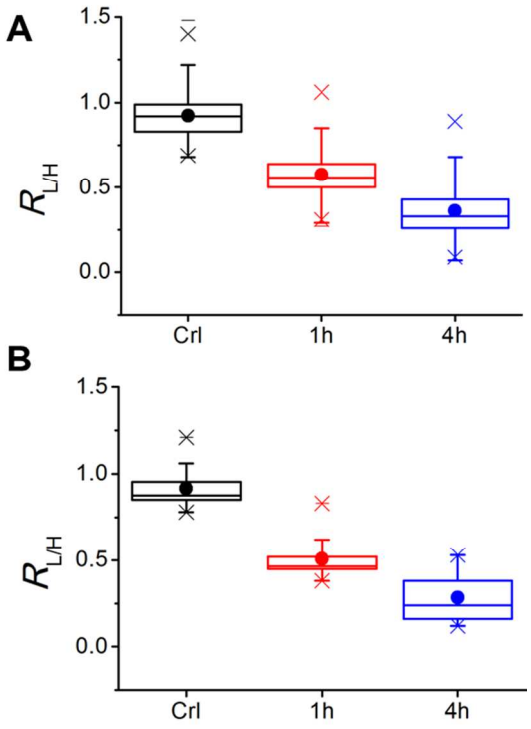

Figure S8. Box plots of light and heavy ratios determined from aHNE alkylated cysteine (A) and histidine (B) peptides in dynamic adduction analyses.

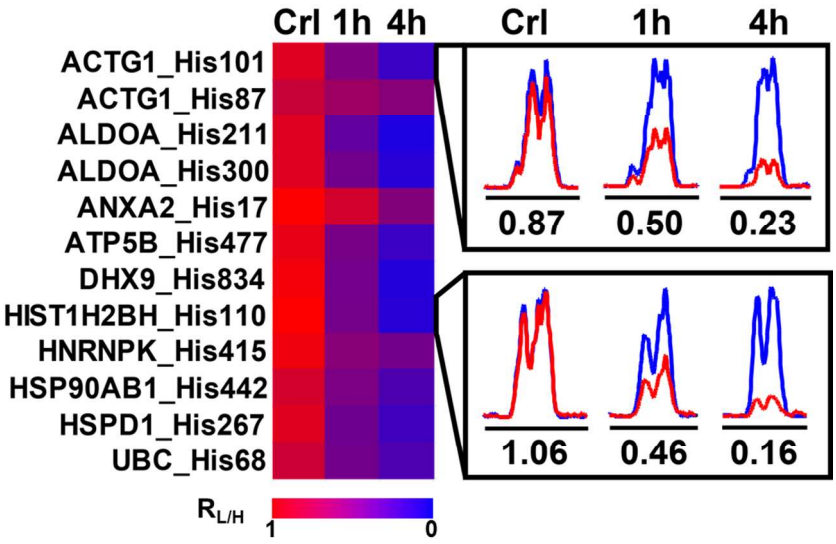

Figure S9. Heatmap depicting changes in L/H ratios for detected histidine N-alkylation events by aHNE during recovery period in cells. Lower measured ratio (L/H) correspond to greater turnover of the corresponding alkylation events in the recovery period. Extracted ion chromatograms (XIC) depict changes in histidine N-alkylated peptides from ACTG1 and HIST1H2BH in RKO cells, with the profiles for light- and heavy- labeled peptides in red and blue, respectively. The experimental ratios calculated from three biological replicates are displayed below the individual chromatograms, respectively.

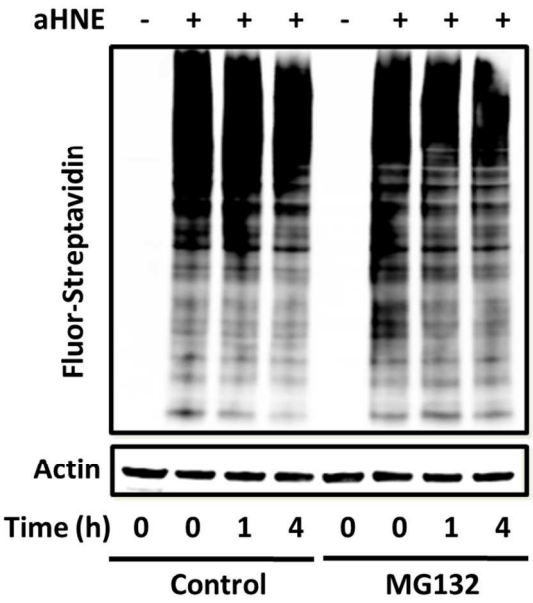

Figure S10. Representative western blot for analyses of aHNE adduct stability shown in Figure 5C. RKO cells were pretreated with or without MG132 followed by aHNE treatment with or without 1 and 4h recovery periods. Proteins alkylated by aHNE were labeled with azido-biotin and detected by western blotting with fluorescein-conjugated streptavidin. Three biological replicates were integrated using LiCor imaging software (Odyssey V3.0) to generate Figure 5C. Actin was used as a loading control (bottom).

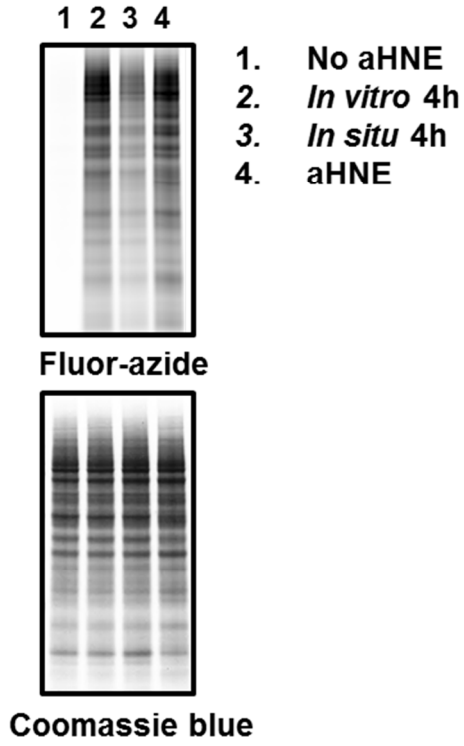

Figure S11. Analysis of aHNE adduct stability in intact RKO cells and in lysates from RKO cells treated with aHNE. RKO cells were first treated with or without aHNE for 2h. For recovery studies with lysates, cells were lysed and further incubated at 37°C for 4h. For recovery studies in intact cells, cells were washed after aHNE treatment and placed in probe-free medium for 4h. The aHNE adducted proteins were reduced by $NaBH_4$, conjugated with an azide reagent with near infrared tag (CruzFluor sm^{TM} 6 azide, 700 nm) via click chemistry, and detected by in-gel fluorescence. Equal protein loading is demonstrated by coomassie blue staining. The experiment shown represents three biological replicate analyses with consistent results.