Supporting Information

A light-initiated chemical reporter strategy for spatiotemporal labeling of biomolecules

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Experimental Procedure

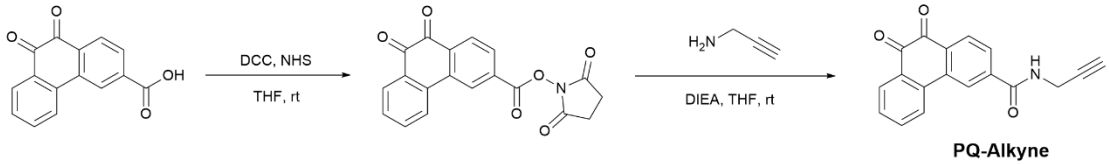
General materials and methods. All chemical reagents were of analytical grade, obtained from commercial suppliers, and used without further purification unless otherwise noted. Streptavidin Alexa Fluor PE conjugate and Streptavidin Alexa Fluor 488 conjugate were obtained from Thermo Fisher Scientific. Azidohomoalanine (Aha) was purchased from Sigma Aldrich.

^{1}H NMR and ^{13}C NMR spectra were obtained on a 400 MHz Bruker AVANCE III-400 spectrometer. Chemical shifts are reported in δ (ppm) relative to the solvent residual peak. Coupling constants are reported in Hz with multiplicities denoted as s (singlet), d (doublet), t (triplet), q (quartet) and m (multiplet). High resolution mass spectrometry (HR-MS) was done on a Thermo Fisher Q Exactive LC/MS. Flow cytometry analysis was performed on ACEA NovoCyte benchtop flow cytometer (Acea Biosciences, USA), and flow cytometric data were analyzed by NovoExpress. Fluorescence microscopy was performed on an LSM 710 scanning confocal microscope and LSM 980 scanning confocal microscope equipped with Colibri 7 LED Illumination system with 385/30nm, 430/44nm, 475/38nn wavelengths (Zeiss, Germany). Semi-prep HPLC was carried out on a Thermo Scientific Utimate 3000 HPLC system equipped with a UV-Vis detector, and a Venusil XBP C18 (A) column (semi-prep, 30mm×250mm, 5μm). Analytical HPLC was carried out on a Quantum Ultra-UltiMate 3000 (Thermo Fisher, USA) equipped with a UV-Vis detector, and a ACE Excel 5 C18 column (4.6mm×250mm, 5μm).

Gel densitometry was used to quantitate fluorescence intensity using ImageJ software (National Institutes of Health, Bethesda, MD, USA). Photoirradiation experiment was conducted using a CEL-PE300L-3A LED lamp (Ceaulight, China).

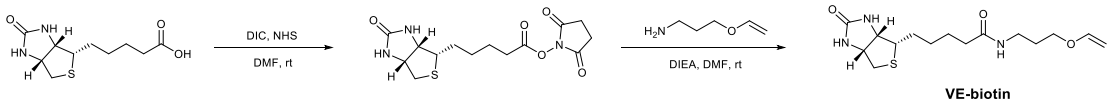
Chemical synthesis. Ac_4ManNAz,[1] Ac_4GalNAz,[1] 1,6-Pr_2GalNAz,[2] and BTTAA[3] were synthesized as previously described.

Synthesis of PQ-Alkyne.



9,10-phenanthrenequinone (PQ) (100 mg, 0.4 mmol) was stirred with dicyclohexylcarbodiimide (DCC) (165 mg, 0.8 mmol) and N-hydroxysuccinimide (NHS) (92 mg, 0.8 mmol) in dry THF (15 mL) overnight. The reaction mixture was filtered to remove the white solid, and the solution then added dropwise into 2 ml of THF solution containing prop-2-yn-1-amine (24 mg, 0.44 mmol) and diisopropylethylamine (100 mg, 0.8 mmol). The reaction was monitored by TLC (DCM/MeOH = 50/1, R_f = 0.33). The solvent was removed by rotary evaporation and further purified by column chromatography (DCM/MeOH = 100/1) to give a yellow solid (92 mg, 80%). ^{1}H NMR (400 MHz, d_6-DMSO) δ 9.32 (t, J = 5.4 Hz, 1H), 8.67 (d, J = 1.2 Hz, 1H), 8.38 (d, J = 8.0 Hz, 1H), 8.10 (d, J = 8.1 Hz, 1H), 8.06 (dd, J = 7.7, 1.3 Hz, 1H), 7.96 (dd, J = 8.1, 1.5 Hz, 1H), 7.84 (td, J = 8.0, 1.5 Hz, 1H), 7.62 - 7.53 (m, 1H), 4.16 (dd, J = 5.4, 2.5 Hz, 2H), 3.21 (t, J = 2.5 Hz, 1H). ^{13}C NMR (101 MHz, d_6-DMSO) δ 178.99, 178.93, 165.20, 139.46, 135.87, 135.80, 135.28, 133.60, 132.00, 130.11, 129.68, 129.64, 128.55, 125.10, 123.35, 81.37, 73.82, 29.21. HRMS (ESI) calcd. for $C_{18}H_{11}NO_3$ 290.0772 $[M+H]^+$, found 290.0803.

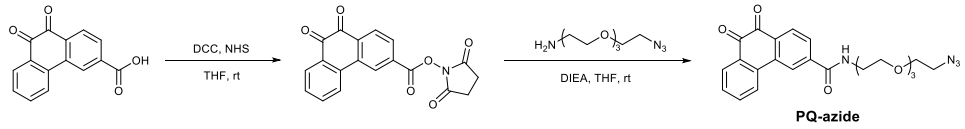
Synthesis of VE-biotin.



D-biotin (1.0 g, 4.1 mmol) was stirred with *N, N*-diisopropylcarbodiimide (DIC) (0.77 g, 6.1 mmol) and *N*-Hydroxysuccinimide (NHS) (0.77 g, 6.1 mmol) in dry *N, N*-dimethylformamide (DMF) (40 mL) overnight to obtain the biotin-NHS ester. The reaction mixture was then added dropwise into 5 ml of DMF solution containing 3-(vinyloxy)propan-1-amine (0.54 g, 5.3 mmol) and diisopropylethylamine (DIEA) (0.69 g, 5.3 mmol). The reaction was monitored by TLC (DCM/MeOH = 10/1, R_f = 0.33). The solvent was removed by rotary evaporation, the crude product was washed twice using diethyl ether, and further purified by column chromatography (using DCM/MeOH in gradient elution from 50/1 to 10/1) to give a white solid (0.87 g, 65%). ^{1}H NMR (400 MHz, d_6-DMSO) δ 7.82 (t, J = 5.5 Hz, 1H), 6.49 (dd, J = 14.3, 6.8 Hz, 1H), 6.44 (s, 1H), 6.37

(s, 1H), 4.31 (dd, J = 7.5, 5.2 Hz, 1H), 4.17 (dd, J = 14.3, 1.7 Hz, 1H), 4.14 - 4.10 (m, 1H), 3.96 (dd, J = 6.8, 1.7 Hz, 1H), 3.67 (t, J = 6.4 Hz, 2H), 3.10 (q, J = 6.7 Hz, 3H), 2.82 (dd, J = 12.4, 5.1 Hz, 1H), 2.58 (d, J = 12.4 Hz, 1H), 2.06 (t, J = 7.4 Hz, 2H), 1.72 (p, J = 6.6 Hz, 2H), 1.64 - 1.42 (m, 4H), 1.31 (tt, J = 15.5, 7.7 Hz, 2H). ^{13}C NMR (101 MHz, d_6-DMSO) δ 172.49, 163.21, 152.36, 87.17, 65.81, 61.52, 59.68, 55.89, 40.32, 35.86, 35.66, 29.26, 28.68, 28.50, 25.76. HRMS (ESI) calcd. for $C_{15}H_{25}N_3O_3S$ 328.1650 $[M+H]^+$, found 328.1682.

Synthesis of PQ-azide.



PQ-azide

9,10-phenanthrenequinone (PQ) (100 mg, 0.4 mmol) was stirred with dicyclohexylcarbodiimide (DCC) (165 mg, 0.8 mmol) and N-Hydroxysuccinimide (NHS) (92 mg, 0.8 mmol) in dry THF (15 mL) overnight. The reaction mixture was filtered to remove the white solid and then added dropwise into 2 ml of THF solution containing 2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethan-1-amine (87 mg, 0.4 mmol) and diisopropylethylamine (100 mg, 0.8 mmol). The reaction mixture was stirred at room temperature for 4 h. After completion of the reaction, the solvent was removed by rotary evaporation and further purified by a semi-prep HPLC system (gradient: acetonitrile: water from 50%:50% to 5%:95% over 30min, detection wavelength: 330nm) to give a yellow solid (89 mg, 49%). ^{1}H NMR (400 MHz, d_6-DMSO) δ 8.93 (t, J = 5.5 Hz, 1H), 8.65 (d, J = 1.2 Hz, 1H), 8.37 (d, J = 8.0 Hz, 1H), 8.09 (d, J = 8.1 Hz, 1H), 8.06 (dd, J = 7.8, 1.3 Hz, 1H), 7.95 (dd, J = 8.1, 1.4 Hz, 1H), 7.84 (td, J = 8.0, 1.5 Hz, 1H), 7.61 - 7.55 (m, 1H), 3.60 (t, J = 5.8 Hz, 2H), 3.58 - 3.55 (m, 6H), 3.53 (s, 4H), 3.52 - 3.49 (m, 2H), 3.36 (t, J = 6.0 Hz, 2H). ^{13}C NMR (101 MHz, d_6-DMSO) δ 179.06, 178.99, 165.53, 140.16, 135.80, 135.79, 135.34, 133.36, 131.95, 130.08, 129.64, 128.47, 125.06, 123.28, 70.27, 70.26, 70.16, 69.69, 69.34, 50.44, 39.99. HRMS (ESI) calcd. for $C_{23}H_{24}N_4O_6$ 453.1729 $[M+H]^+$, found 453.1743.

Cell culture. HCCC-9810 cells and K20 cells were kept in RPMI-1640 media supplemented with 10% FBS, 100 units/ml penicillin and 100 μg/ml of streptomycin. A549, HepG 2 and HeLa cells

were kept in DMEM media supplemented with 10% FBS, 100 units/ml penicillin and 100 μg/ml of streptomycin. The cells were cultured at 37℃ and 5% CO_2 in a water-saturated incubator.

Metabolic chemical reporter incorporation. HCCC-9810 cells were seeded at a density of about 8.0×10^5 cells/ml for flow cytometry analysis, or 3×10^5 cells/ml for fluorescence microscopy analysis. A549, HepG 2 and HeLa cells were seeded at a density of about 5.0×10^5 cells/ml for flow cytometry analysis or 2×10^5 cells/ml for fluorescence microscopy analysis. For metabolic incorporation of glycans, the cells were cultured for 12 h, and incubated for 48 h with 200μM of Ac_4ManNAz, Ac_4GalNAz, 1,6-Pr_2GalNAz, 200 μM Ac_4ManNVE or 300 μM 9PQ-Neu5Ac. For metabolic phospholipid incorporation, the cells were cultured for 24 h, and incubated with 500μM of propargylcholine. For bioorthogonal non-canonical amino acid tagging (BONCAT), the cells were cultured for 24 h, and incubated with 1mM of azidohomoalanine.

For the pulse-chase experiment, HCCC-9810 cells were seeded at a density of 3.0×10^5 cells/ml, cultured for 12 h, and incubated for 48 h with 200μM of Ac_4ManNAz. After changing the medium to fresh RPMI-1640 or RPMI-1640 containng 1 μM tunicamycin, the cells were then chased for 0, 6, 12, 24, 48h, respectively.

Fluorescence labeling of cell-surface biomolecules via PQ-VE photo-cycloaddition. For metabolic glycan labeling, the cells were incubated with different unnatural azidosugars at indicated concentrations for 48 h in a 4-chamber glass bottom dish (Cellvis, CA, USA). For Alk-Cho and Aha labeling, the cells were incubated at indicated concentrations for 24h in a 4-chamber glass bottom dish (Cellvis, CA, USA). After metabolic incorporation, the cells were washed three times with cold PBS containing 1% FBS, followed by incubation for 10 min in 300μL PBS containing 0.5% FBS, 50 μM PQ-Alkyne or PQ-azide, BTTAA-$CuSO_4$ (50 μM $CuSO_4$, BTTAA: $CuSO_4$ = 6:1), and 2.5 mM sodium ascorbate at room temperature. After 5 min, the cells were washed three times with PBS. For the PQ-VE photoreaction, the cells were then incubated in 100 μL PBS containing 100 μM VE-biotin, and irradiated with a hand-held white-light LED lamp (CEL-PE300L-3A, Ceaulight, 200 mW/cm^2) at room temperature for 5 min. For spatiotemporal experiment, a 2mm (diameter)

pinhole device was used to cover the chamber glass bottom dish prior to photo-illumination. After the reaction was complete, the cells were washed three times with PBS containing 1% FBS, followed by incubation with 5 μg/mL Streptavidin Alexa Fluor PE conjugate or Streptavidin Alexa Fluor 488 conjugate (Thermo Fisher Scientific, USA) at room temperature for 30 min, and stained for 10 min with 5 μg/mL Hoechst 33342 at room temperature. The cells were then washed three times with PBS, and applied to LSM 710 confocal microscopy imaging system. Imaging condtion: Laser intensity: 405nm channel (5%, Hoeschst 33342), 488nm channel (30%, Alexa Fluor 488), 561nm channel (30%, Alexa Fluor 561). Exposure time: 0.79μs. The original confocal images were analyzed by Image J.

For single-cell spatial labeling experiment, HCCC-9810 cells were incubated with 500μM Alk-Cho for 24 h in a 4-chamber glass bottom dish (Cellvis, CA, USA). After metabolic incorporation, the cells were washed three times with cold PBS, incubated for 10 min in 300μL PBS containing 0.5% FBS, 50 μM PQ-azide, BTTAA-$CuSO_4$ (50 μM $CuSO_4$, BTTAA: $CuSO_4$ = 6:1), and 2.5 mM sodium ascorbate at room temperature. After 5 min, the cells were washed three times with PBS. 100 μL PBS containing 5.5 μM VE-biotin and 5.5 μM Streptavidin Alexa Fluor PE conjugate (Thermo Fisher Scientific, USA) were pre-mixed at room temperature for 30 min in an Eppendorf tube, and then added into cells. Point light source with 30 μm in diameter was used to select one single cell, and the cell was irradiated with Colibri 7 LED Illumination system at 100% laser intensity (mixing output of 385/30nm, 430/44nm, and 475/38nn wavelengths to mimic LED point light source) at room temperature for 5 min. The cells were washed three times with PBS containing 1% FBS, and stained for 10 min with 5 μg/mL Hoechst 33342 at room temperature. The cells were then washed three times with PBS, and applied to LSM 980 confocal microscopy imaging system. Imaging condtion: Laser intensity: 405nm channel (0.5%, Hoeschst 33342), 561nm channel (20%, Alexa Fluor 561). Exposure time: 1.27μs. The original confocal images were analyzed by Image J.

For trial experiment on the metabolic expression of 9PQ-Neu5Ac, the cells were incubated with 300 μM 9PQ-Neu5Ac for 48 h in a 4-chamber glass bottom dish (Cellvis, CA, USA). After metabolic incorporation, the cells were washed three times with cold PBS, followed by incubation in 100 μL PBS containing 100 μM VE-biotin, and irradiated with a hand-held white-light LED lamp (CEL-PE300L-3A, Ceaulight, 200 mW/cm^2) at room temperature for various times. After the

reaction was complete, the cells were washed three times with PBS containing 1% FBS, followed by incubation with 5 μg/mL Streptavidin Alexa Fluor 488 conjugate (Thermo Fisher Scientific, USA) at room temperature for 30 min. The cells were washed three times with PBS, and applied to LSM 710 confocal microscopy imaging system.

Flow cytometry analysis. The cells were incubated with different unnatural metabolites at the indicated concentrations, trypsinized and transferred into a 96-well tissue culture plate, pelleted (800 × *g*, 5min), and washed three times with 100 μL of PBS containing 1% FBS. Cells were then resuspended in 100 μL CuAAC reaction buffer containing 0.5% FBS, 50 μM PQ-Alkyne or PQ-azide, BTTAA-$CuSO_4$ (50 μM $CuSO_4$, BTTAA: $CuSO_4$ = 6:1), and 2.5 mM sodium ascorbate for 5 min at 4 °C. The cells were then washed three times with PBS. For the PQ-VE photoreaction, the cells were incubated in 100 μL PBS containing 100 μM VE-biotin, and irradiated with a hand-held white-light LED lamp (CEL-PE300L-3A, Ceaulight, 200 mW/cm^2) at room temperature for 5 min. After the reaction was complete, the cells were washed three times with PBS containing 1% FBS, followed by incubation with 5 μg/mL Streptavidin Alexa Fluor PE conjugate or Streptavidin Alexa Fluor 488 conjugate (Thermo Fisher Scientific, USA) for 30 min at 4℃. The cells were washed three times with PBS containing 1% FBS, then resuspended in in 200 μL of FACS buffer for flow cytometric analysis.

For trial experiment on the metabolic expression of Ac_4ManNVE, the cells were incubated with 200 μM Ac_4ManNVE for 48 h, trypsinized and transferred into a 96-well tissue culture plate, pelleted (800 × *g*, 5min), and washed three times with 100 μL PBS. For the PQ-VE photoreaction, the cells were incubated in 100 μL PBS containing 50 μM PQ-TAMRA, and irradiated with a hand-held white-light LED lamp (CEL-PE300L-3A, Ceaulight, 200 mW/cm^2) at room temperature for 5 min. The cells were washed three times with PBS, then resuspended in in 200 μL of FACS buffer for flow cytometric analysis.

Immunoblot analysis. The treated cells were washed with PBS buffer three times and lysed with ice-cold modified RIPA lysis buffer (1% Nonidet P 40, 1% sodium deoxycholate, 0.1% SDS, 50mM triethanolamine pH 7.4, 150mM NaCl, EDTA-free Piercent Halt^{TM} protease inhibitor cocktail). Cell lysates were collected by centrifugation (12,000 × *g*, 10min) at 4 ℃ to remove insoluble cell debris.

The protein concentration was determined using the BCA protein assay kit (Pierce, CA, USA). Cell lysates were diluted with lysis buffer to final protein concentration of 3 mg/mL and reacted with 100μM PQ-Alkyne in a 160μL reaction buffer containing premixed BTTAA-$CuSO_4$ complex (50 μM $CuSO_4$, BTTAA: $CuSO_4$ in a 2:1 molar ratio) and 2.5 mM freshly prepared sodium ascorbate. The samples were vortexed and allowed to react for 2 h at 25°C on an IKA MS3 shaker. Pre-cooled methanol was added to the protein samples (sample: methanol in a 1: 8 volume ratio), after precipitation at - 80°C overnight, the protein precipitation was collected by centrifugation (12,000 × *g*, 10 min) at 4 ℃, wash with methanol for two times, and redissolved in 160μL of RIPA lysis buffer to final protein concentration of 3 mg/mL. For subsequent PQ-VE photo-cycloaddition reaction, protein samples were reacted in a 30μL RIPA buffer containing 100 μM VE-biotin, and irradiated with the hand-held white-light LED lamp at 25°C for 5 min. The final protein samples supplemented with 5 × loading buffer were boiled at 99°C for 5 min, and resolved on 10% SDS-PAGE Gels. The samples were transferred to PVDF membrane (Millipore), and incubated for 1h at room temperature in blocking buffer (5% non-fat milk in 1 × TBST (Tris buffered saline with 0.1% Tween-20, pH 7.5)). The blocked membrane was incubated for 1 h at room temperature with an HRP-anti-biotin antibody (1:2,000 dilution, Beyotime) in blocking buffer. After washed with 1 × TBST for three times, blots were reacted with enhanced chemiluminescence (ECL) reagent (Fdbio Science) and protein bands were detected via a chemiluminescence system (Tanon-5200, Shanghai, China). Coomassie staining was used to verify equal protein loading.

References

(1) Laughlin, S. T.; Bertozzi, C. R. Metabolic Labeling of Glycans with Azido Sugars and Subsequent Glycan-Profiling and Visualization via Staudinger Ligation. *Nat Protoc* 2007, *2* (11), 2930–2944.

(2) Qin, K.; Zhang, H.; Zhao, Z.; Chen, X. Protein S-Glyco-Modification through an Elimination–Addition Mechanism. *J Am Chem Soc* 2020, *142* (20), 9382–9388.

(3) Besanceney-Webler, C.; Jiang, H.; Zheng, T.; Feng, L.; Soriano del Amo, D.; Wang, W.; Klivansky, L. M.; Marlow, F. L.; Liu, Y.; Wu, P. Increasing the Efficacy of Bioorthogonal Click Reactions for Bioconjugation: A Comparative Study. *Angew Chem-ger Edit* 2011, *123* (35), 8201–8206.

Supporting Figures

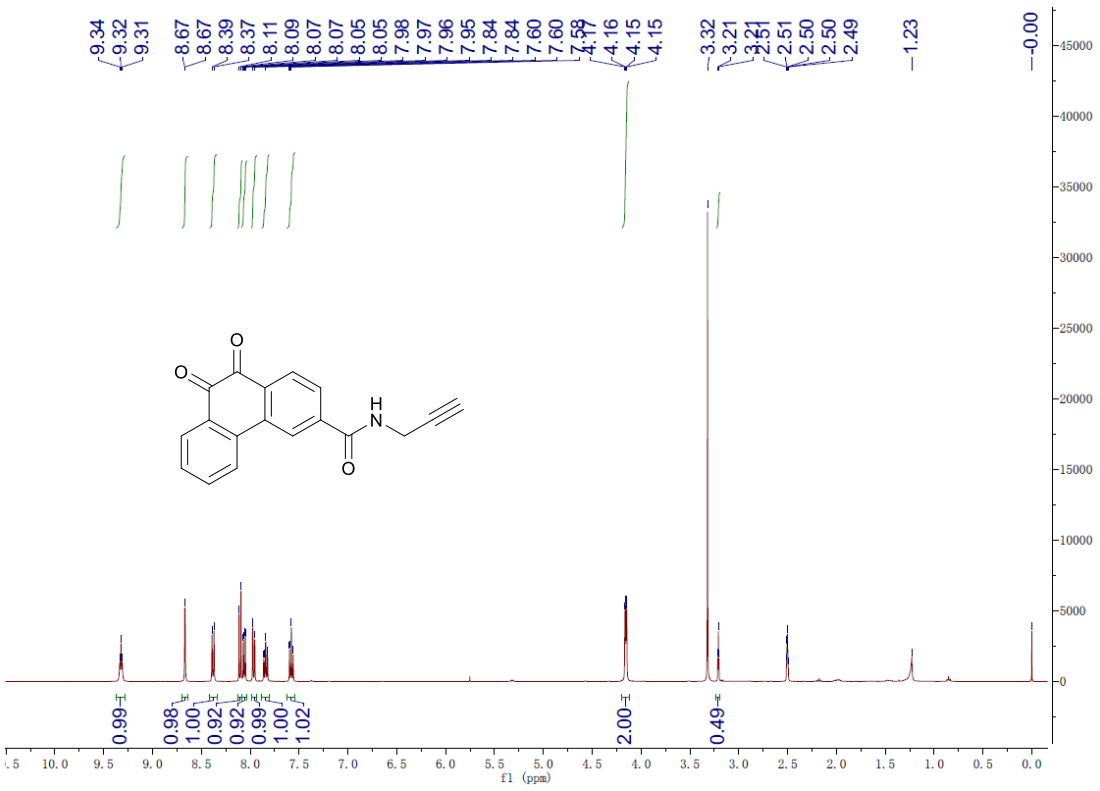


Figure S1. ^{1}H NMR of PQ-Alkyne.

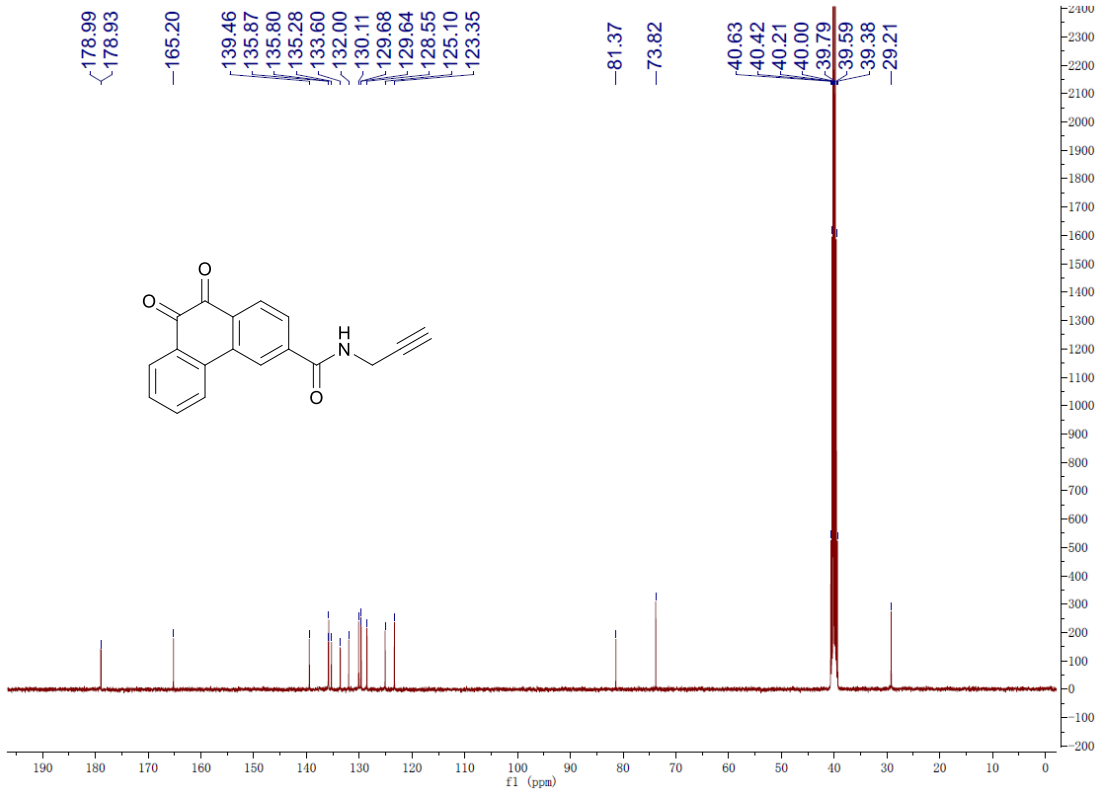


Figure S2. ^{13}C NMR of PQ-Alkyne.

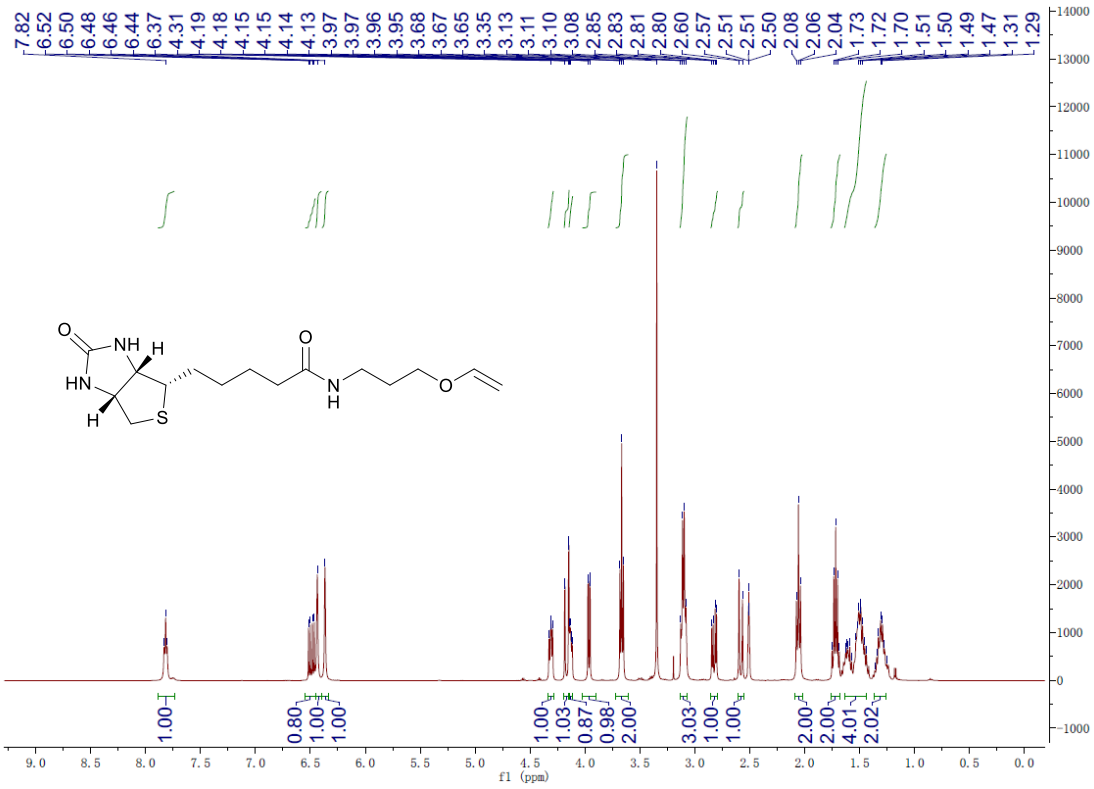


Figure S3. ^{1}H NMR of VE-biotin.

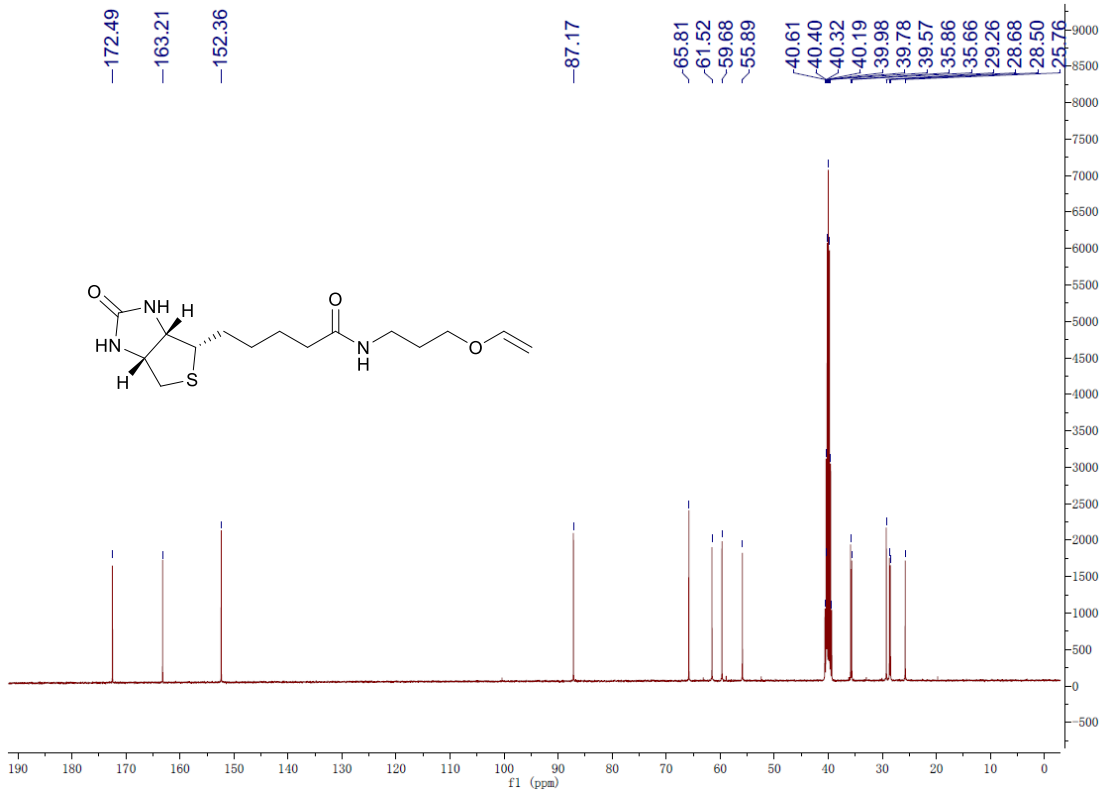


Figure S4. ^{13}C NMR of VE-biotin.

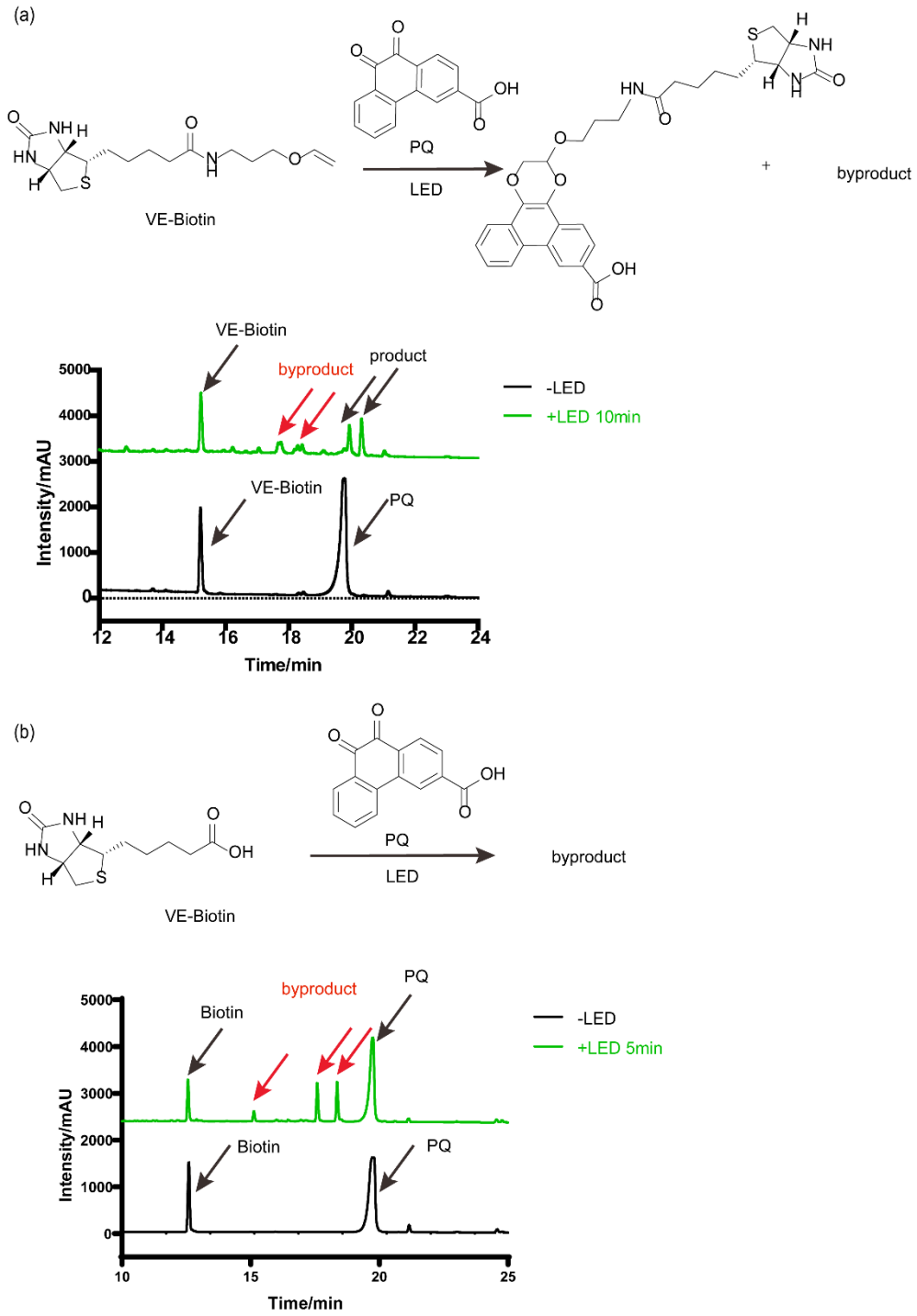


Figure S5. Reactivity of PQ with VE-biotin or biotin. (a) High-performance liquid chromatographic analysis of PQ and VE-biotin photo-click reaction with (black) or without (greeen line) LED illumination. Reaction condition: PBS/acetonitrile=1/1. (b) High-performance liquid chromatographic analysis of PQ and biotin with (black) or without (greeen line) LED illumination. Reaction condition: PBS/acetonitrile=1/1. Eluting solvent: acetonitrile/water. Gradient: 0-30min, acetonitrile: 5%-95%

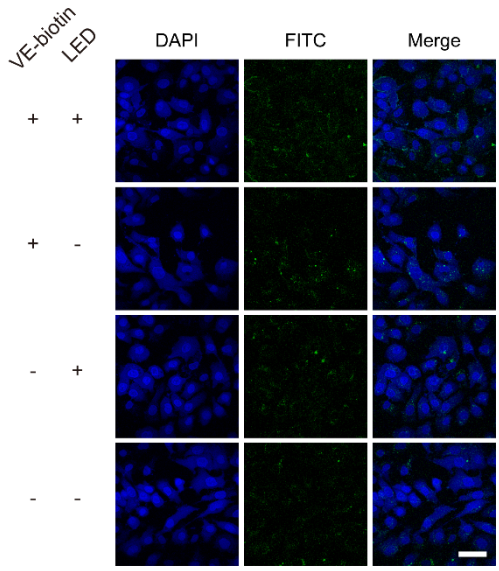


Figure S6. Control groups of SPORT imaging of cell-surface sialoglycans in live cells (Figure 2). Confocal fluorescence microscopy images of HCCC-9810 cells treated with vehicle (1 % DMSO) for 48 h. The treated cells were reacted with PQ-Alkyne and VE-biotin under visible light, and stained with Alexa Fluor 488-streptavidin (green signal). The nuclei were stained with Hoechst 33342 (blue signal). Scale bar: 50 μm.

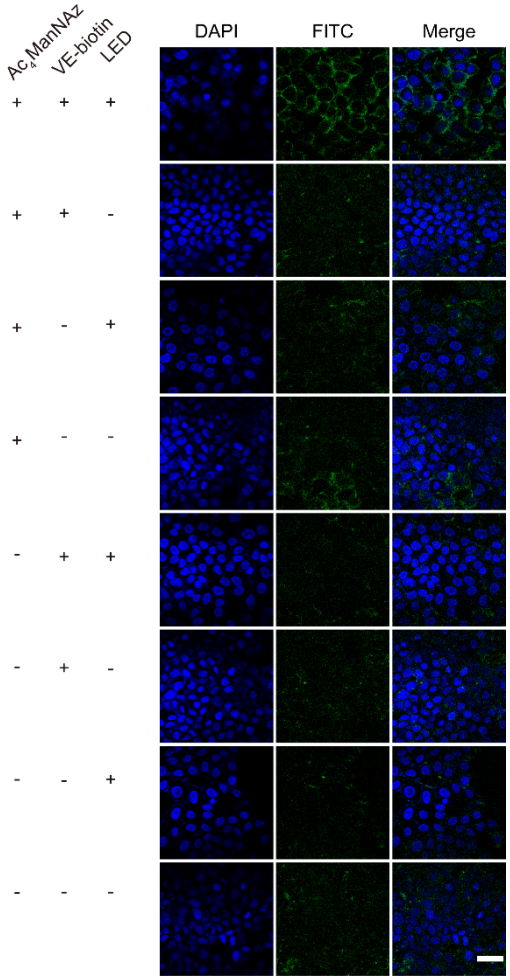


Figure S7. SPORT imaging of cell-surface sialyloglycans in A549 cells. (a) Confocal fluorescence microscopy images of A549 cells treated with 200 μM Ac_4ManNAz. The treated cells were reacted with PQ-Alkyne and VE-biotin under visible light, and stained with Alexa Fluor 488-streptavidin (green signal). The nuclei were stained with Hoechst 33342 (blue signal). Scale bar: 50 μm.

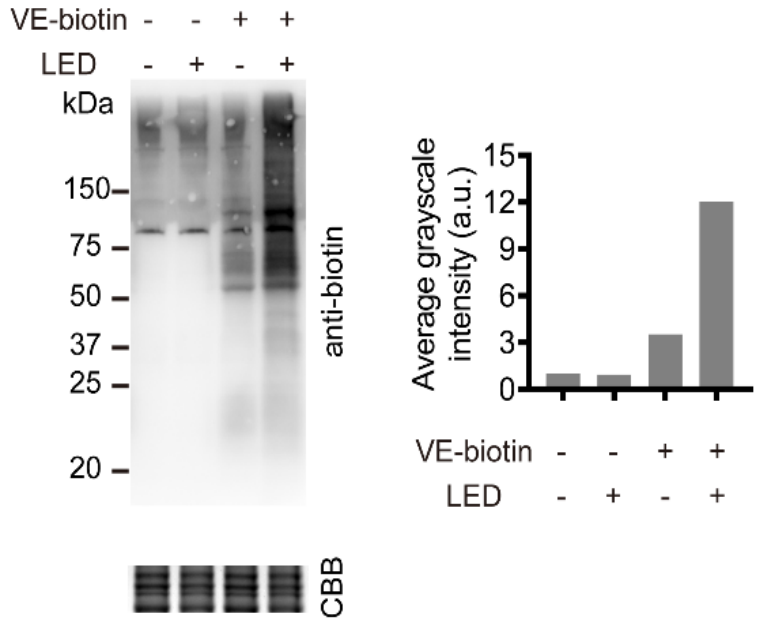


Figure S8. Immunoblot analysis of species from A549 cells metabolically labeled with Ac_4ManNAz. Left: The cells were lysed, reacted with PQ-Alkyne, precipitated and redissolved in lysis buffer to eliminate excess reactants, reacted with VE-biotin with or without light, resolved using standard SDS-PAGE, and detected via HRP conjugated anti-biotin. Equal protein loading was confirmed using Coomassie blue staining. Right: Semi-quantitative analysis of sialoprotein bands.

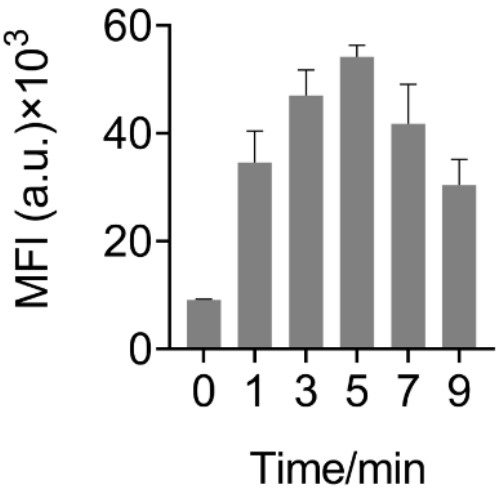


Figure S9. Optimization of exposure time for SPORT labeling using flow cytometry. The HCCC-9810 cells were treated with 200 μM Ac_4ManNAz, incubated with 50 μM PQ-Alkyne, followed by reaction under illumination with VE-biotin for various times, and stained with Alexa Fluor 488-streptavidin conjugate. Error bars represent the standard deviation from three replicate experiments.

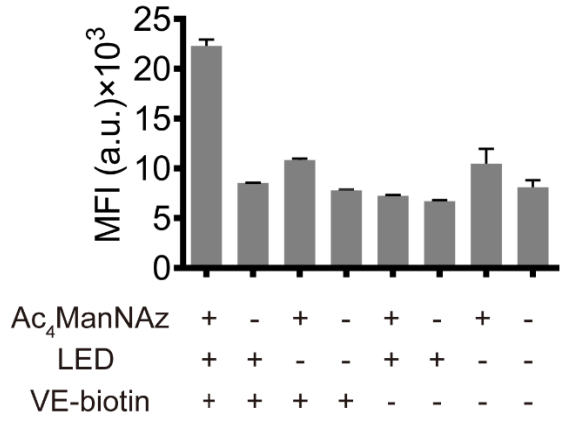


Figure S10. Quantitative analysis of cell-surface sialome of A549 cells by flow cytometry. The cells were treated with 200 μM Ac_4ManNAz, incubated with 50 μM PQ-Alkyne, followed by reaction under illumination with VE-biotin for 5 min, and stained with Alexa Fluor 488-streptavidin conjugate. Error bars represent the standard deviation from three replicate experiments.

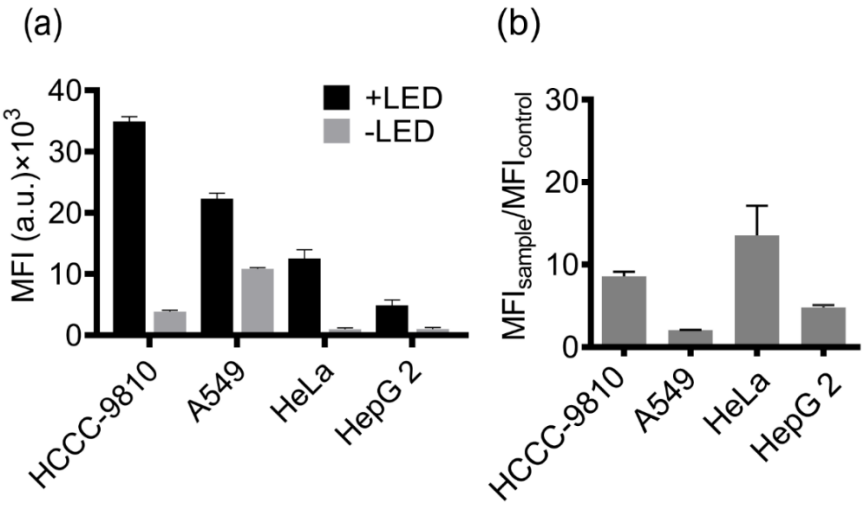


Figure S11. Quantitative analysis of incorporated precursors by flow cytometry. (A)The HCCC-9810, A549, HeLa, and HepG 2 cells were treated with 200 μM Ac_4ManNAz, incubated with 50 μM PQ-Alkyne, followed by reaction with VE-biotin, with or without illumination, for 5 min, and stained with Alexa Fluor PE-streptavidin conjugate. (B) Normalized mean fluorescence intensity of (a). MFI_{sample}: mean fluorescence intensity of each sample with SPORT; $MFI_{control}$: mean fluorescence intensity of corresponding samples without photoclick reactiob. Error bars represent the standard deviation from three replicate experiments.

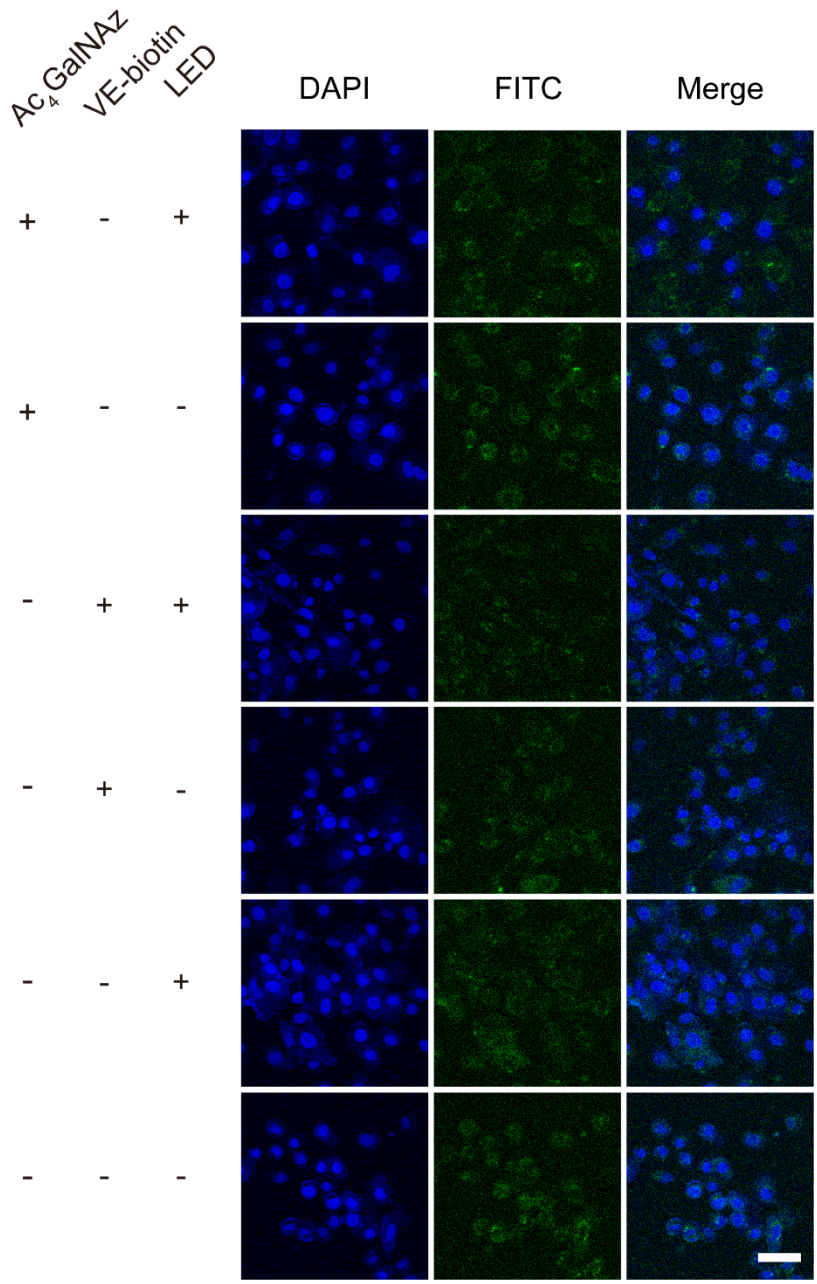


Figure S12. Control groups of SPORT imaging of cell-surface mucin-type *O*-linked glycome in live cells (Figure 3a). Confocal fluorescence microscopy images of HCCC-9810 cells treated with 200 μM Ac_4GalNAz or vehicle (1% DMSO) for 48 h. The treated cells were reacted with PQ-Alkyne and VE-biotin under visible light, and stained with Alexa Fluor 488-streptavidin (green signal). The nuclei were stained with Hoechst 33342 (blue signal). Scale bar: 50 μm.

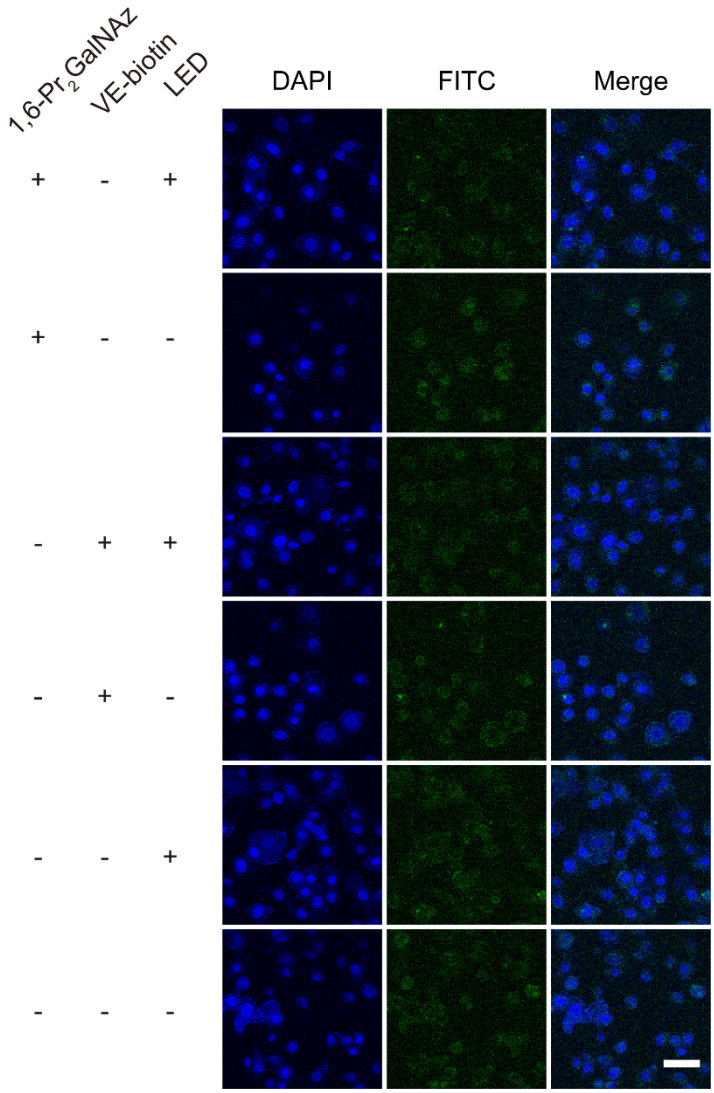


Figure S13. Control groups of SPORT imaging of cell-surface mucin-type *O*-linked glycome in live cells (Figure 3e). Confocal fluorescence microscopy images of HCCC-9810 cells treated with 200 μM 1,6-Pr2GalNAz or vehicle (1% DMSO) for 48 h. The treated cells were reacted with PQ-Alkyne and VE-biotin under visible light, and stained with Alexa Fluor 488-streptavidin (green signal). The nuclei were stained with Hoechst 33342 (blue signal). Scale bar: 50 μm.

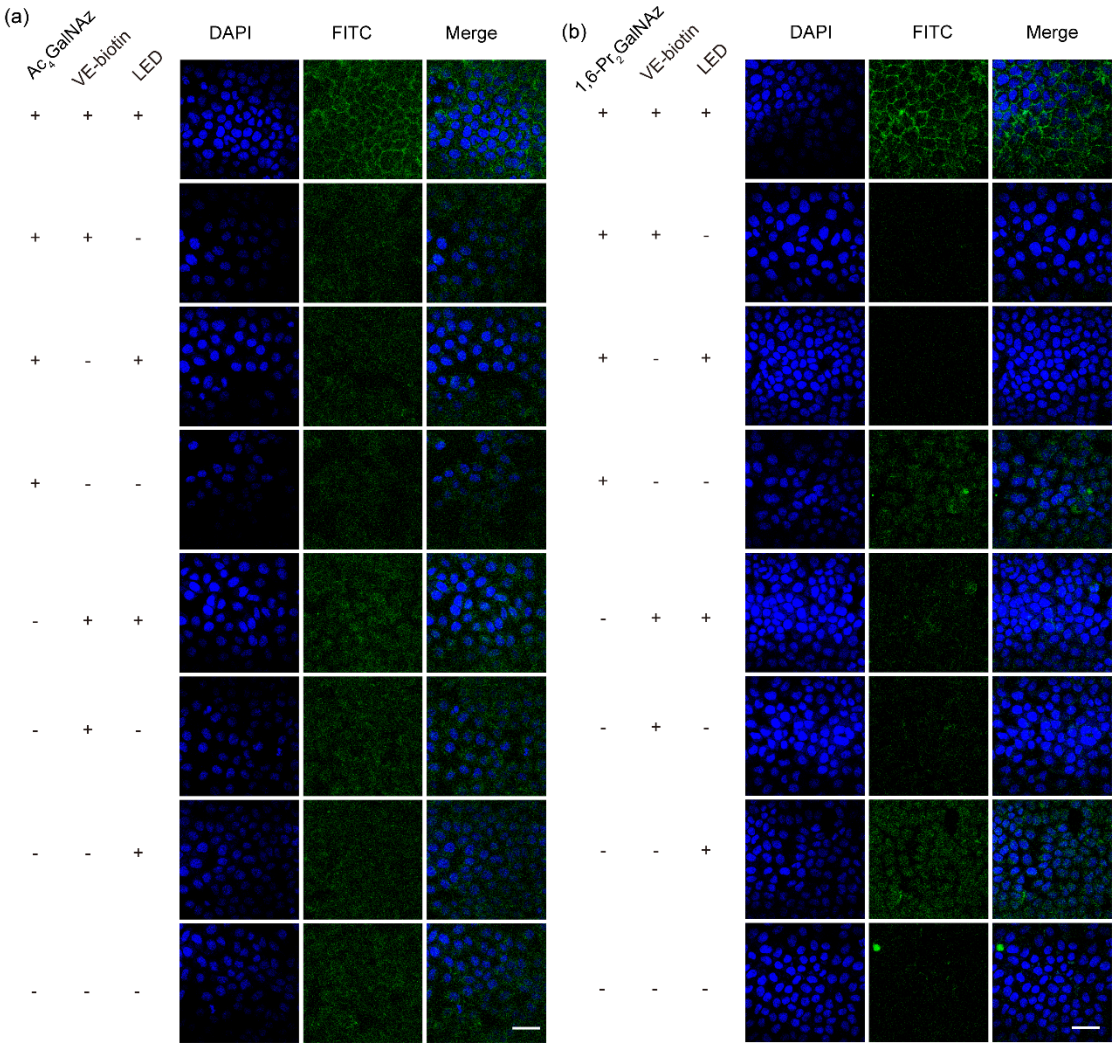


Figure S14. SPORT imaging of cell-surface mucin-type *O*-linked glycome in A549 cells. Confocal fluorescence microscopy images of A549 cells treated with 200 μM (a) Ac_4GalNAz or (b)1,6-Pr_2GalNAz. The treated cells were reacted with PQ-Alkyne and VE-biotin under visible light, and stained with Alexa Fluor 488-streptavidin (green signal). The nuclei were stained with Hoechst 33342 (blue signal). Scale bar: 50 μm.

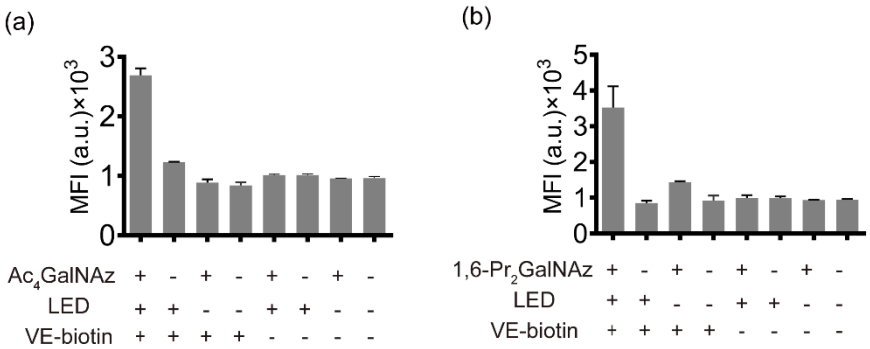


Figure S15. Quantitative analysis of cell-surface *O*-glycans of A549 cells by flow cytometry. The cells were treated with 200 μM (a)Ac_4GalNAz or (b) 1,6-Pr_2GalNAz, incubated with 50 μM PQ-Alkyne, followed by reaction under illumination with VE-biotin for 5 min, and stained with Alexa Fluor 488-streptavidin conjugate. Error bars represent the standard deviation from three replicate experiments.

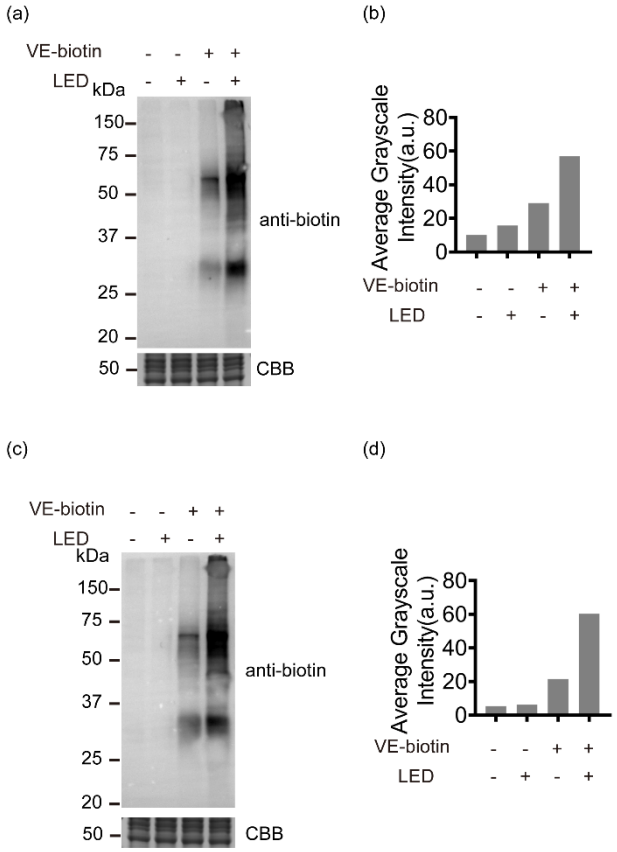


Figure S16. Immunoblot analysis of species from A549 cells. The cells were metabolically labeled with 200 μM (a)Ac_4GalNAz or (c)1,6-Pr_2GalNAz, lysed, reacted with PQ-Alkyne, precipitated and redissolved in lysis buffer to eliminate excess reactants, reacted with VE-biotin with or without light, resolved using standard SDS-PAGE, and detected via HRP conjugated anti-biotin. Equal protein loading was confirmed using Coomassie blue staining. (b, d) Semi-quantitative analysis of protein bands in (a,c).

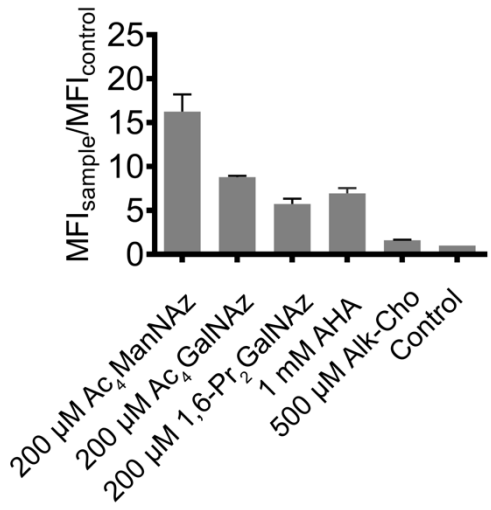


Figure S17. Quantitative analysis of incorporated precursors by flow cytometry. The HCCC-9810 cells were treated with corresponding metabolic precursors at designated concentrations, incubated with 50 μM PQ-Alkyne, followed by reaction under illumination with VE-biotin for 5 min, and stained with Alexa Fluor 488-streptavidin conjugate. All flow cytometry were taken using same condition. MFI_{sample}: mean fluorescence intensity of each sample, $MFI_{control}$: mean fluorescence intensity of HCCC-9810 cells without photoclick reaction. Error bars represent the standard deviation from three replicate experiments.

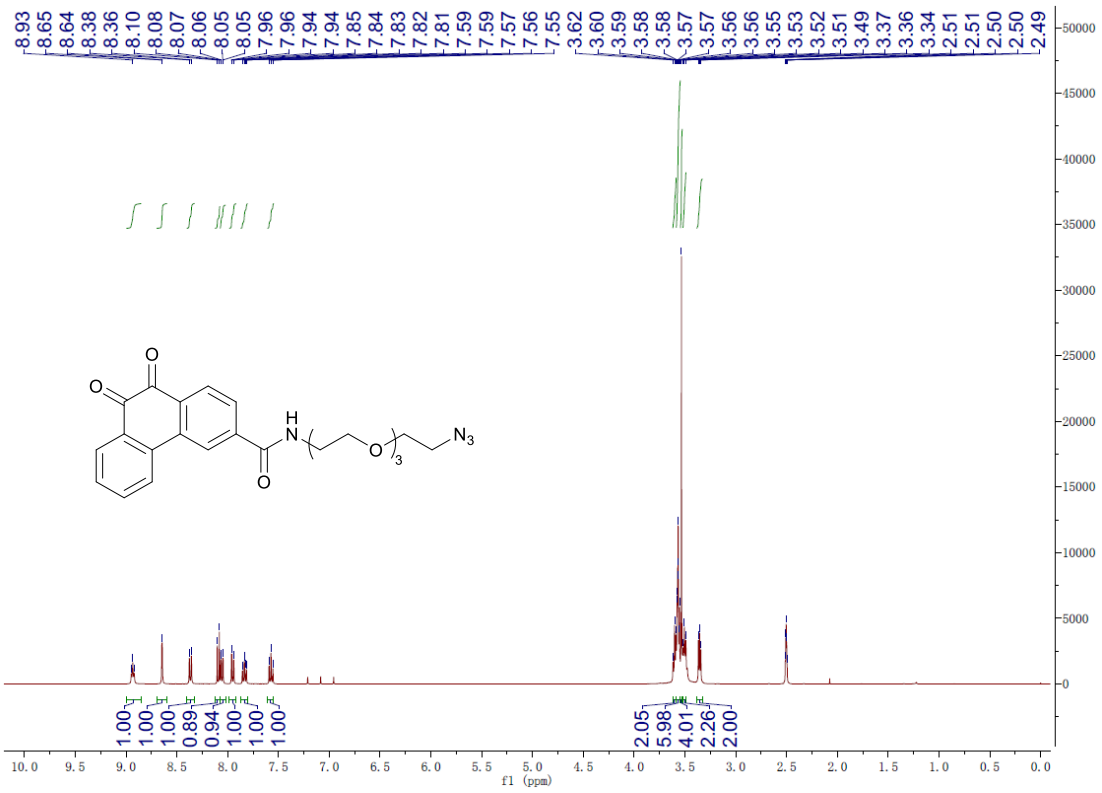


Figure S18. ^{1H} NMR of PQ-azide.

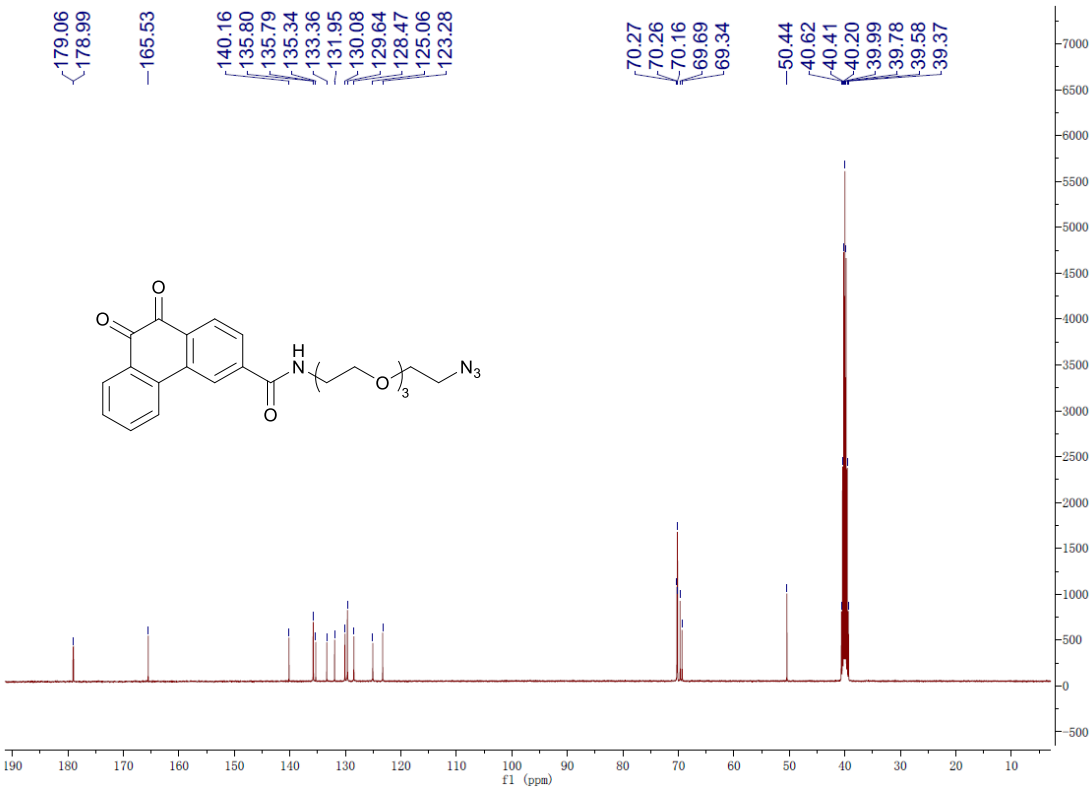


Figure S19. ^{13}C NMR of PQ-azide.

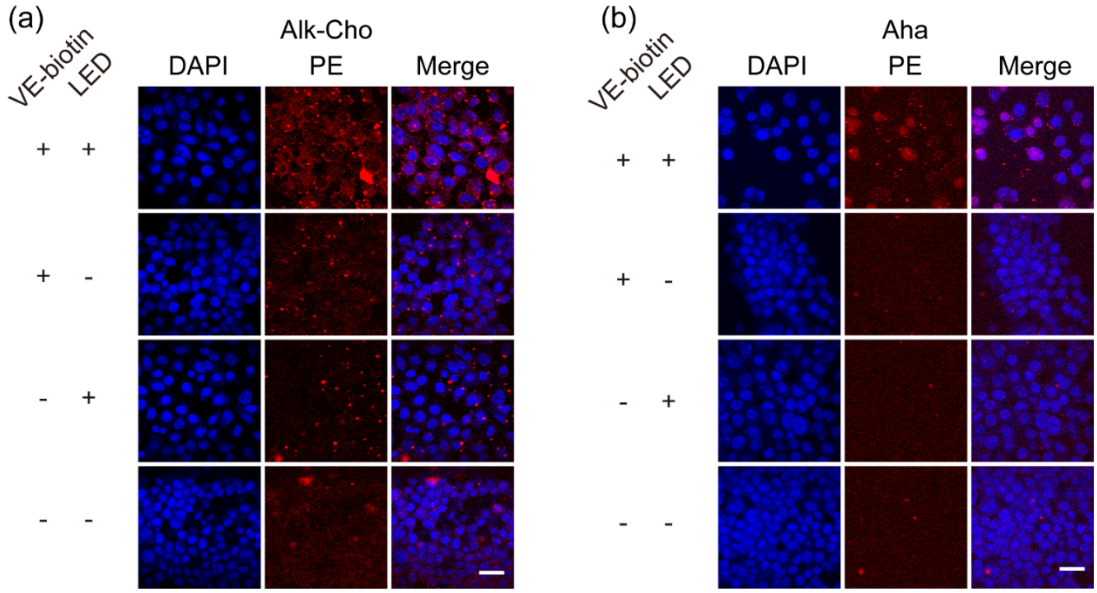


Figure S20. SPORT imaging of newly-synthesized phosphatidylcholine and proteins in A549 cells. Confocal fluorescence microscopy images of A549 cells treated with (a) 500 μM Alk-Cho or (b) 1 mM Aha. The treated cells were reacted with PQ-azide and VE-biotin under visible light, and stained with Alexa Fluor PE-streptavidin (green signal). The nuclei were stained with Hoechst 33342 (blue signal). Scale bar: 50 μm.

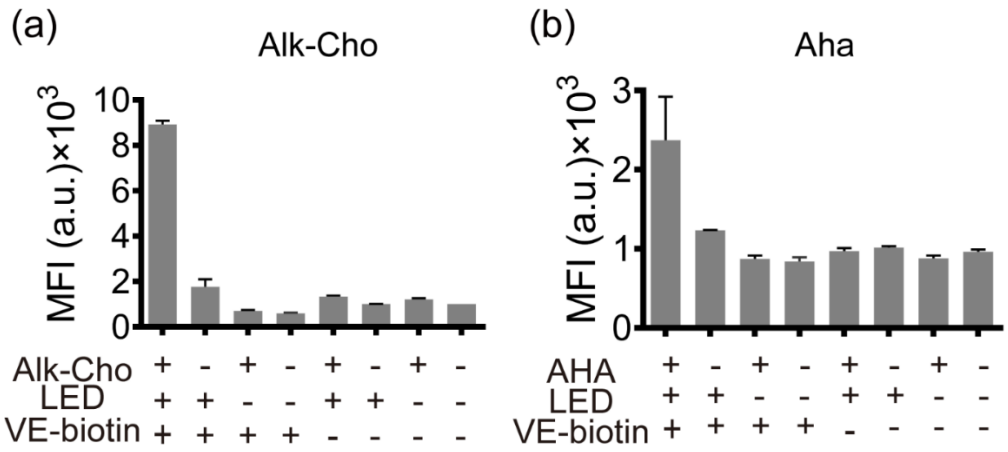


Figure S21. Quantitative analysis of newly-synthesized phosphatidylcholine and proteins of A549 cells by flow cytometry. The cells were treated with (a) 500 μM Alk-Cho or (b) 1 mM Aha, incubated with 50 μM PQ-Alkyne, followed by reaction under illumination with VE-biotin for 5 min, and stained with Alexa Fluor 488-streptavidin conjugate. Error bars represent the standard deviation from three replicate experiments.

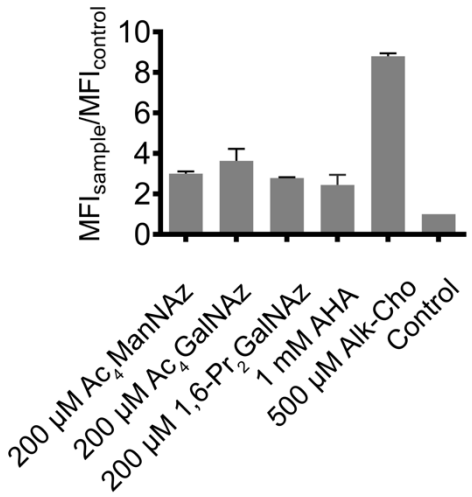


Figure S22. Quantitative analysis of incorporated precursors by flow cytometry. The A549 cells were treated with corresponding metabolic precursors at designated concentrations, incubated with 50 μM PQ-Alkyne, followed by reaction under illumination with VE-biotin for 5 min, and stained with Alexa Fluor 488-streptavidin conjugate. All flow cytometry were taken using same condition. MFI_{sample}: mean fluorescence intensity of each sample, $MFI_{control}$: mean fluorescence intensity of A549 cells without photoclick reaction. Error bars represent the standard deviation from three replicate experiments.

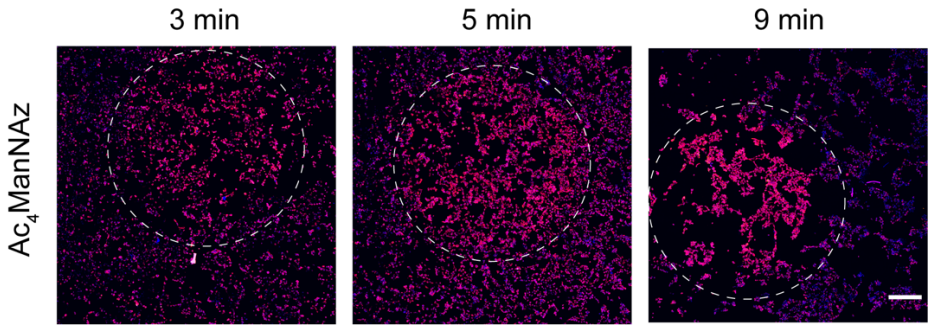


Figure S23. Optimization of irradiaton condition for spatial labeling of azidosugars. The cells were treated with 200μM Ac_4ManNAz for 48 h, the treated cells were reacted with PQ-Alkyne and VE-biotin, with the white circular square selectively irradiated for 3, 5, 9 min at the power density of 200 mW/cm2. The cells were then stained with Alexa Fluor 488-streptavidin (red signal), and subjected to imaging using confocal fluorescence microscopy. The nuclei were stained with Hoechst 33342 (blue signal). Scale bar: 500 μm.

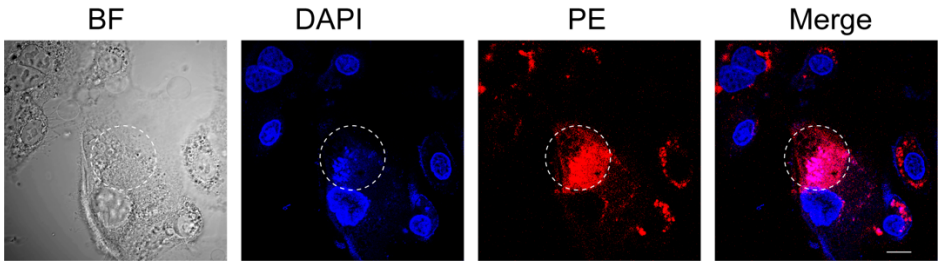


Figure S24. Representative replicate experiment of Figure 5. Brightfield and confocal fluorescence microscopy images showing regional labeling of a single HCCC-9810 cell. Cells were treated with 500 μM Alk-Cho for 24 h, reacted with PQ-azide and VE-biotin, the cell of interest (indicated by the white dashed circle) was irradiated with a LED module (wavelength: 385nm, 430nm, 475nm) for 5 min. The cells were then stained with Alexa Fluor PE-streptavidin (red signal), and subjected to confocal fluorescence microscopy imaging. The nuclei were stained with Hoechst 33342 (blue signal). Scale bar: 15 μm.

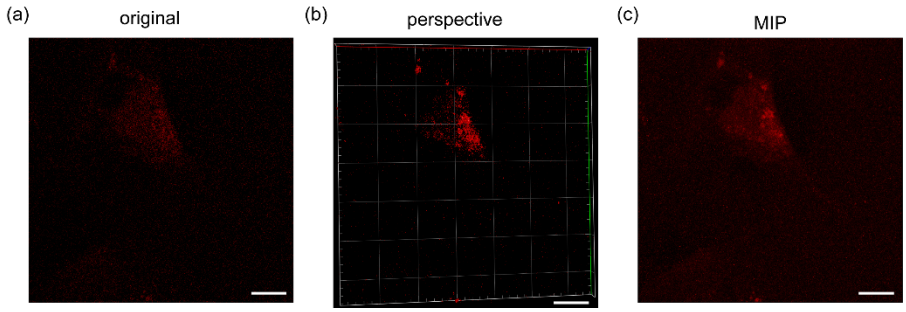


Figure S25. Z-stack imaging of HCCC-9810 cells labeled in a selected region on a single cell. The cells were treated with 500μM Alk-Cho for 24 h, reacted with PQ-azide via photoclick, and incubated with premixed VE-biotin/Alexa Fluor PE-streptavidin conjugates (red signal). (a) Original confocal imaging; (b) Perspective view of the irradiated region on a single cell by Z-stack. (c) The maximum intensity projection (MIP) image of the irradiated region on a single cell by Z-stack. Scale bar: 15 μm.

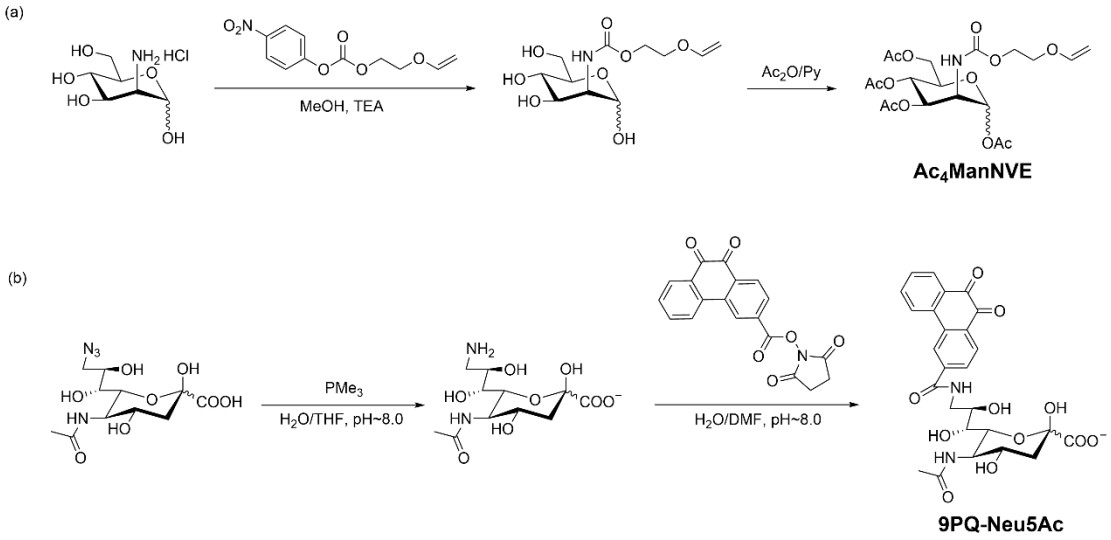


Figure S26. Synthesis routes of Ac_4ManNVE and 9PQ-Neu5Ac.

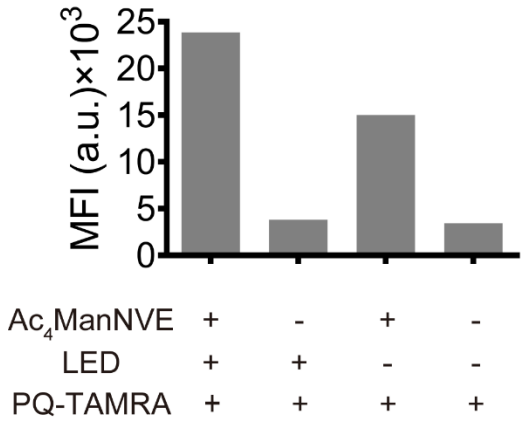


Figure S27. K20 cells cannot metabolically incorporate Ac_4ManNVE for direct visible light-triggered PQ-VE photo-click reaction. The K20 cells were treated with 200 μM Ac_4ManNVE for 48 h, followed by reaction under illumination with PQ-TAMRA conjugate for designated times, and subjected to flow cytometry analysis.

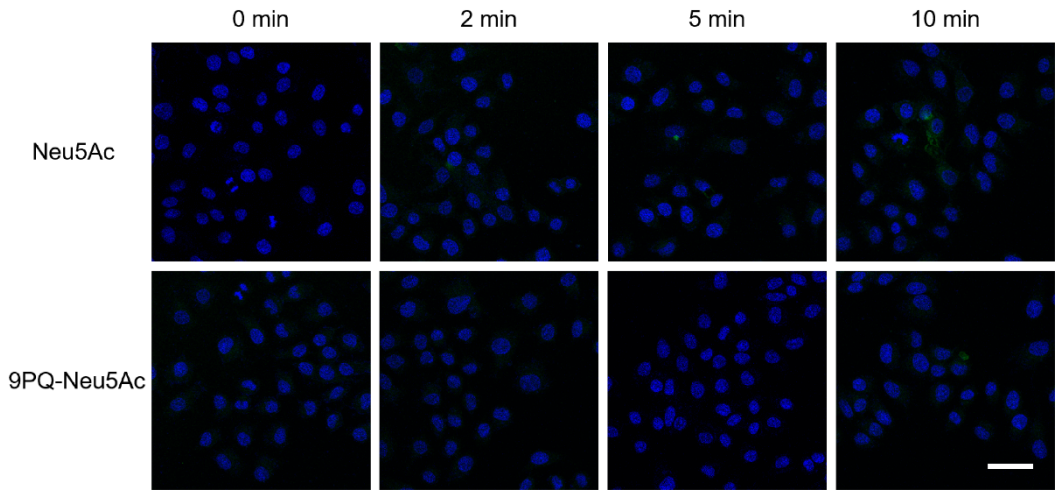


Figure S28. MCF-7 cells cannot metabolically incorporate 9PQ-Neu5Ac for direct visible light-triggered PQ-VE photo-click reaction. The MCF-7 cells were treated with 300μM 9PQ-Neu5Ac for 48 h, the cells were incubated with 50 μM VE-biotin, irradiated with LED lamp for various times, and stained with streptavidin-Alexa Fluor 488. The cells were subjected to imaging using confocal fluorescence microscopy. The nuclei were stained with Hoechst 33342 (blue signal). Scale bar: 50 μm.