Supporting Information

Vaping induced cannabidiol (CBD) oxidation product CBD quinone forms protein adducts with KEAP1 and activates KEAP1-Nrf2 genes

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Experimental Procedures

Analysis of Commercial CBD Condensates

Generation of CBD Condensates for Chemical Analysis. Vaped condensates were generated from one commercial CBD oil (Liberty Cannabis, Capitol Heights, MD; Gorilla Glue 750 mg/mL) and one commercial CBD juice (CBDfx, Chatsworth, CA; Strawberry Kiwi 16.67 mg/mL). Methods for generating vaped condensates were adapted from the system developed by Olmedo et al. for e-cigarette vapor sampling using a peristaltic pump[1]. Due to the high viscosity of the commercial oil, the collection tubing had to be shortened to collect condensate into the sample storage recipient tube. For this study, a 16 cm tubing piece (Cole-Parmer F01278; internal diameter 0.250" and external diameter 0.375") running through the peristaltic pump (Cole-Parmer, Vernon Hills, IL; MasterFlex L/S 07518-60) was connected to a 5 cm pipette tip followed by a 2 cm tubing piece (1.5 mm internal diameter) and a 200 µL pipette tip cut to 2 cm. The final 2 cm pipette tip led directly into a 1.7 µL sample collection tube fitted with parafilm and vented as previously described. For consistency, the vaped condensates for the commercial juice were also collected with the same apparatus. For all vaped condensate samples, a Silo battery vaping device (CCell, Phoenix, Arizona; 500 mAh, ~3.7 V) was used. The oil was purchased in a cartridge that was compatible with the Silo device and the juice was added to a 1 mL refillable glass cartridge (CCell, Phoenix, Arizona) for use with the Silo device. Vaped condensates were generated by taking 20 5-second puffs at 2 L/min in 1-minute intervals. A 4140 Mass Flow Meter (TSI, Shoreview, MN; 1980383) connected to the 16 cm tubing piece running through the peristaltic pump was used to calibrate the system to 2 L/min (equivalent to 400 mL/min on pump). Commercial oil and juice vaped condensates were compared to non-vaped samples of each product. For the oil, a second cartridge was purchased at the same time which was used for the non-vaped sample to avoid any chemical changes from heating the cartridge during vaping. Immediately after they were collected, vaped condensates and non-vaped samples were weighed and frozen at -80 °C until further analysis.

LC/MS-MS Analysis of Condensates. Standards of CBD, ∆9-THC, ∆8-THC, CBDA, ∆9-THCA, and CBN were obtained from Sigma-Aldrich, and d_9-CBD was purchased from Cayman Chemical. The condensate collected from vaped CBD was compared to non-vaped samples. The samples were analyzed by LC/MS-MS using d_9-CBD (1 mg/mL MeOH) as the standard. An aliquot of the condensate or non-vaped sample was diluted with MeOH to a concentration of 1 mg/mL. To this CBD sample (10 µL) was added d_9-CBD (10 µL) and MeOH (80 µL) for LC/MS-MS analysis. Samples (10 µL injection) were analyzed on an Acquity UPLC system equipped with an Agilent Poroshell EC-C18 (10 cm x 2.1 mm, 1.9 µm) column with CH_3CN:MeOH:H_2O, 40:40:20 (0.01% (v) formic acid) mobile phase at a column temperature of 40°C. The flow rate was 300 µL/min with a total run time of 15 min. A TSQ Quantum Ultra tandem mass spectrometer (ThermoFisher) was used for MS detections, and data were acquired with a Finnigan Xcalibur software package. Selected reaction monitoring (SRM) was acquired in the positive ion mode using electrospray ionization (ESI). MS parameters were optimized using CBD and were as follows: spray voltage at 4500 V, capillary temperature at 300 °C, auxiliary nitrogen gas pressure at 55 psi, and sheath gas pressure at 60 psi. Collision energy (CE) and SRMs were optimized for each compound under a collision gas pressure of 1.5 mTorr: CBD, ∆9-

THC, ∆8-THC (315→193); CBDA, ∆9-THCA (359→341); CBDQ, ∆8-THCQ (329→287); CBN (311→223); d_9-CBD (324→202). Response factors were determined for each compound relative to d_9-CBD and used for quantitation: CBD (0.75), ∆9-THC (0.61), ∆8-THC (0.82), CBDA (0.15), ∆9-THCA (0.13), CBDQ (3.9), ∆8-THCQ (0.18), and CBN (0.88).

Synthesis of CBDQ (Scheme S1)

CBD juice (CBDFx, fruity cereal, 250 mg/30 mL) was diluted with EtOAc and washed with H_2O, brine, and dried over $MgSO_4$. The CBD was purified by column chromatography (10% EtOAc/hexanes). Stabilized 2-iodoxybenzoic acid (SIBX, 120 mg, 0.43 mmol) was added to a solution of CBD (31 mg, 0.099 mmol) in EtOAc (0.5 mL). The reaction mixture immediately turned orange. After overnight, the reaction mixture was filtered through Celite. The organics were washed with $Na_2S_2O_3$, brine, and dried over $MgSO_4$. The product was purified on a silica SPE column (10% EtOAc/hexanes) and isolated as a yellow oil (10 mg, 31%). ^{1}H NMR (MeOH-d_4): δ 6.34 (s, 1H), 5.12 (s, 1H), 4.64-4.51 (m, 2H), 3.73-3.69 (m, 1H), 2.83 (dt, 1H, J = 3.2, 11.9 Hz), 2.39 (dt, 2H, J = 1.3, 7.8 Hz), 2.23-2.15 (m, 1H), 2.02-1.97 (m, 1H), 1.77-1.67 (m, 1H), 1.65 (s, 3H), 1.62 (s, 3H), 1.53-1.49 (m, 1H), 1.40-1.29 (m, 6H), 0.92 (t, 3H, J = 6.8 Hz). ESI (positive): calculated 329.21 (M + H), observed 329.25.

Synthesis of ∆8-THCQ (Scheme S1)

∆8-THC oil was purified by column chromatography (10% EtOAc/hexanes). Stabilized 2-iodoxybenzoic acid (SIBX, 0.24 g, 0.84 mmol) was added to a solution of ∆8-THC (0.12 g, 0.38 mmol) in CH3CN (5 mL) and H2O (1 mL). The reaction mixture immediately turned orange. After 1 h, the reaction mixture was diluted with EtOAc and washed with NaHCO3, brine, and dried over MgSO4. The product was purified by column chromatography (10% EtOAc/hexanes) and isolated as a red oil (81 mg, 65%). 1H NMR (CDCl3): δ 6.45 (s, 1H), 5.35 (br s, 1H), 3.04 (dd, 1H, J = 5.0, 18 Hz), 2.44 (dt, 1H, J = 4.7, 11.2 Hz), 2.31 (t, 2H, J = 7.6 Hz), 2.10-2.05 (m, 1H), 1.83-1.74 (m, 1H), 1.65 (s, 3H), 1.64-1.58 (m, 2H), 1.49-1.42 (m, 2H), 1.41 (s, 3H), 1.31-1.23 (m, 4H), 1.16 (s, 3H), 0.87 (t, 3H, J = 6.9 Hz). ESI (positive): calculated 329.21 (M + H), observed 329.25.

Analysis of Adduct Formation with Model Peptide

Reaction with Model Peptide. AcAVAGKCAGAR (TpepKC) was purchased from American Peptide and the stock solution was prepared 5mg/mL. 0.1mM synthetic CBDQ stock solution was prepared in MeOH. The commercial CBD oil (Liberty, Gorilla Glue, 750 mg/mL) vaped condensate was generated as previously described. The condensate of the vaped and unvaped oil was prepared 1 mg/mL in MeOH. The peptide and synthetic CBDQ mix: 20 uL peptide+50 uL synthetic CBDQ + 30 uL PBS and oil mix: 20 uL peptide + 100 uL vaped or unvaped commercial oils + 80uL PBS were prepared and rotated at room temperature for 2 h and analyzed by LTQ XL linear ion trap mass spectrometry in data dependent mode.

LC-MS/MS analysis. LC-MS/MS analyses were performed on a Thermo LTQ linear ion trap instrument (Thermo Electron, San Jose, CA) equipped with Accela HPLC system. 10 uL of reaction mixture was injected on to Luna C18 column (Phenomenex 150 X 2 mm, 5 micron) column. Liquid chromatography was carried out at ambient temperature

with a flow rate of 0.3 mL/min using a gradient mixture of solvent A: 0.1% (v/v) formic acid in water and solvent B: 0.1% (v/v) formic acid in acetonitrile with a gradient : 2% B → 30% B for 5 min maintaining for 2 min then increased to 98% for 3 min maintaining for 2 min then to 2%B for 1 min and equilibrated for 4 min. Each sample was run first with a full scan and precursor selection in the range of m/z 215-1800. Seven data-dependent MS/MS scans were performed on the 7 the most abundant peaks from each full scan. MS/MS spectra were recorded using dynamic exclusion of previously analyzed precursors a repeat of 1 and a repeat duration of 10 sec with 25 exclusion size. A full survey scan MS was acquired in the LTQ XL linear ion trap. The automatic gain control target for MS1 was set as $5x10^2$ and ion filling time set as 60 ms. The selected ions with default charge state 3 will be isolated with isolation width (m/z) 2 and fragmented using CID 35% normalized collision energy with 30 ms activation time.

Synthesis of a-CBD and a-CBDQ (Scheme S2)

*Synthesis of ester **1***. TsOH (0.30 g, 1.6 mmol) was added to a solution of 3-(3,5-dimethoxyphenyl)propionic acid (1.6 g, 7.4 mmol) in MeOH (40 mL). After overnight, the reaction mixture was concentrated to remove most of the MeOH. The mixture was diluted with EtOAc and washed with $NaHCO_3$, brine, and dried over $MgSO_4$. The product was isolated as a colorless liquid (1.6 g, 98%) and used without further purification. ^{1}H NMR ($CDCl_3$): δ 6.33 (d, 2H, J = 1.7 Hz), 6.29 (t, 1H, J = 1.6 Hz), 3.74 (s, 6H), 3.65 (s, 3H), 2.87 (t, 2H, J = 5.2 Hz), 2.59 (t, 2H, J = 5.5 Hz). ^{13}C NMR ($CDCl_3$): δ 173.3, 160.9, 142.9, 106.3, 98.2, 55.2, 51.6, 35.5, 31.2.

*Synthesis of **2***. BBr_3 (1M/hexanes, 22 mL, 22 mmol) was added to a solution of the ester **1** (1.6 g, 7.2 mmol) in CH_2Cl_2 (35 mL) at 0 °C. After 2 h, the reaction was quenched carefully with MeOH, then H_2O. The reaction mixture was extracted with EtOAc, then washed with brine and dried over $MgSO_4$. The product was purified by column chromatography (1:1 hexanes:EtOAc) and isolated as a white powder (1.3 g, 89%). ^{1}H NMR (MeOH-d_4): δ 6.15 (d, 2H, J = 2.1 Hz), 6.11 (t, 1H, J = 2.0 Hz), 3.64 (s, 3H), 2.75 (t, 2H, J = 7.6 Hz), 2.57 (t, 2H, J = 7.8 Hz). ^{13}C NMR (MeOH-d_4): δ 175.2, 159.5, 144.2, 107.7, 101.6, 52.1, 36.5, 32.0.

*Synthesis of **3***. TiPSCl (3.4 mL, 16 mmol) was added to a solution of **2** (1.3 g, 6.4 mmol) and imidazole (2.2 g, 32 mmol) in anhydrous DMF (16 mL). After overnight, the reaction mixture was diluted with hexanes/EtOAc (1:1) and washed with H_2O, brine, and dried over $MgSO_4$. The product (3.1 g, 96%) was isolated as a colorless liquid after purification by column chromatography (10% EtOAc/hexanes). ^{1}H NMR ($CDCl_3$): δ 6.31 (d, 2H, J = 2.2 Hz), 6.25 (t, 1H, J = 2.2 Hz), 3.64 (s, 3H), 2.80 (t, 2H, J = 7.6 Hz), 2.55 (t, 2H, J = 7.5 Hz), 1.25-1.17 (m, 6H), 1.07 (d, 36H, J = 7.1 Hz). ^{13}C NMR ($CDCl_3$): δ 173.2, 156.8, 142.2, 113.0. 109.7, 51.5, 35.6, 30.8, 17.8, 12.6.

*Synthesis of alcohol **4***. DIBAL-H (1 M/toluene, 19 mL, 19 mmol) was added to a solution of the ester **3** (3.1 g, 6.2 mmol) in THF (30 mL) at 0 °C. After 30 min, the reaction was quenched with 10% HCl and extracted with EtOAc. The organics were washed with brine and dried over $MgSO_4$. Purification by column chromatography (20% EtOAc/hexanes) yielded the product as a colorless liquid (2.9 g, 97%). ^{1}H NMR ($CDCl_3$): δ 6.32 (d, 2H, J = 2.2 Hz), 6.25 (t, 1H, J = 2.2 Hz), 3.64 (t, 2H, J = 6.4 Hz), 2.55 (t, 2H, J

= 7.4 Hz), 1.85-1.78 (m, 2H), 1.28-1.16 (m, 6H), 1.07 (d, 36H, J = 7.2 Hz). ^{13}C NMR ($CDCl_3$): δ 156.7, 143.5, 113.2, 109.3, 62.2, 34.6, 32.0, 17.9, 12.6.

Synthesis of iodide ***5***. Iodine (1.8 g, 7.1 mmol) was added in portions to a solution of the alcohol **4** (2.9 g, 6.0 mmol), PPh_3 (1.9 g, 7.2 mmol), and imidazole (0.8 g, 12 mmol) in CH_2Cl_2 (30 mL). After 30 min, the reaction mixture was diluted with ether, filtered through a pad of silica gel, and concentrated. The product (3.3 g, 95%) was used in the next reaction without further purification. ^{1}H NMR ($CDCl_3$): δ 6.32 (d, 2H, J = 2.2 Hz), 6.26 (t, 1H, J = 2.2 Hz), 3.10 (t, 2H, J = 6.8 Hz), 2.57 (t, 2H, J = 7.2 Hz), 2.04 (pentet, 2H, J = 6.9 Hz), 1.27-1.16 (m, 6H), 1.07 (d, 36H, J = 7.2 Hz). ^{13}C NMR ($CDCl_3$): δ 156.8, 142.0, 113.4, 109.6, 35.9, 34.5, 31.5, 17.8, 12.6.

Synthesis of ***6***. *n*BuLi (2.5 M/hexanes, 6.8 mL, 17 mmol) was added to a solution of TMS-acetylene (2.4 mL, 17 mmol) in THF (20 mL) and HMPA (5 mL) at 0 °C. After 30 min, a solution of the iodide **5** (3.3 g, 5.6 mmol) in THF (10 mL) was added. The TiPS-protecting group was removed during the course of the reaction. After overnight, the reaction was quenched with NH_4Cl and extracted with EtOAc. The organic layer was washed with brine and dried over $MgSO_4$. After purification by column chromatography (30% EtOAc/hexanes), the product was isolated as an orange oil (1.0 g, 73%). ^{1}H NMR ($CDCl_3$): δ 6.23 (app t, 2H, J = 2.3 Hz), 6.20 (app q, 1H, J = 2.3 Hz), 2.60-2.51 (m, 2H), 2.23-2.11 (m, 2H), 1.78-1.70 (m, 2H), 0.12 (s, 9H). ^{13}C NMR ($CDCl_3$): δ 156.9, 144.5, 108.7, 107.9, 100.5, 85.0, 34.6, 30.2, 21.0, 0.06.

Synthesis of alkyne ***7***. TBAF (1 M/THF) was added to a solution of **6** (1.0 g, 4.1 mmol) in THF (20 mL). After 30 min, the reaction mixture was diluted with EtOAc and washed with brine and dried over $MgSO_4$. The product was purified by column chromatography (30% EtOAc/hexanes) and isolated as an orange oil (0.45 g, 63%). ^{1}H NMR ($CDCl_3$): δ 6.24 (d, 2H, J = 2.0 Hz), 6.19 (t, 1H, J = 2.1 Hz), 2.59 (t, 2H, J = 7.4 Hz), 2.16 (dt, 2H, J = 2.6, 7.0 Hz), 1.97 (t, 1H, J = 2.6 Hz), 1.77 (pentet, 2H, J = 7.2 Hz). ^{13}C NMR ($CDCl_3$): δ 156.7, 144.5, 108.1, 100.5, 84.2, 68.7, 34.3, 29.5, 18.6.

Synthesis of a-CBD. BF_3-Et_2O (0.60 mL, 4.9 mmol) was added to a suspension of basic alumina (4.1 g, 40 mmol) in CH_2Cl_2 (50 mL). After 15 min, the reaction was heated to reflux for 5 min. A solution of (*1S,4R*)-4-isopropenyl-1-methyl-2-cyclohexen-1-ol (0.25 g, 1.6 mmol) and alkyne **7** (0.28 g, 1.6 mmol) in CH_2Cl_2 (10 mL) was added. After 5 min, the reaction was cooled and quenched with $NaHCO_3$ to prevent cyclization. The reaction mixture was partitioned and extracted with EtOAc. The organics were washed with brine and dried over $MgSO_4$. Purification was carried out with column chromatography (10% EtOAc/hexanes) and the product was isolated as a yellow oil (91 mg, 19%). ^{1}H NMR (MeOH-d_4): δ 6.10 (s, 2H), 5.28 (s, 1H), 4.48-4.43 (m, 2H), 3.96-3.92 (m, 1H), 2.95-2.88 (m, 1H), 2.50 (t, 2H, J = 7.4 Hz), 2.21 (t, 1H, J = 2.7 Hz), 2.14 (dt, 2H, J = 2.7, 7.0 Hz), 2.04-1.96 (m, 1H), 1.77-1.70 (m, 5H), 1.68 (s, 3H), 1.64 (s, 3H). ESI (positive): calculated 311.20 (M + H), observed 311.13.

Synthesis of a-CBDQ. Stabilized 2-iodoxybenzoic acid (SIBX, 40 mg, 0.14 mmol) was added to a solution of *a*-CBD (10 mg, 0.032 mmol) in EtOAc (0.2 mL). The reaction mixture immediately turned orange. After overnight, the reaction mixture was filtered through Celite. The organics were washed with $Na_2S_2O_3$, brine, and dried over $MgSO_4$. The product was purified on a silica SPE column (10% EtOAc/hexanes) and isolated as

a yellow oil (3 mg, 30%). ^{1}H NMR (MeOH-d_4): δ 6.40 (s, 1H), 5.12 (s, 1H), 4.53-4.52 (m, 2H), 3.73-3.69 (m, 1H), 2.84 (dt, 1H, J = 3.1, 11.8 Hz), 2.52 (dt, 2H, J = 1.2, 8.6 Hz), 2.26-2.21 (m, 3H), 2.02-1.96 (m, 1H), 1.77-1.67 (m, 5H), 1.65 (s, 3H), 1.62 (s, 3H). ESI (positive): calculated 325.18 (M + H), observed 325.19.

Cell Culture and Differentiation

Culture of 16HBEs. Cells from the SV-40 transformed human bronchial epithelial cell line 16HBE14 (16HBE) were cultured as detailed previously[2]. 16HBEs were cultured in minimum essential medium (MEM) Eagle (Sigma Life Sciences, St. Louis, MO; M4655-500ML) with 10% fetal bovine serum (Omega Scientific Inc., Tarzana, CA; FB-11) and 1% Gibco penicillin-streptomycin (ThermoFisher Scientific, Waltham, MA; 15140-122). T-75 culture plates (Corning Life Sciences, Corning, NY; 430641U) coated in collagen (Advanced BioMatrix, Carlsbad, CA; 5005-100ML) were used to culture cells. Cells were cultured at 37°C with 95% humidity and 5% carbon dioxide.

Culture of hBECs. Human bronchial epithelial cells (hBECs) were obtained from the UNC Marsico Lung Institute Tissue Procurement and Cell Culture Core and Lisa Dailey. Using methods previously described[3,4], cells were harvested from healthy nonsmoking human donors (table S1) and differentiated. Briefly, cells were plated at a density of 1.75×10^6 on collagen (Advanced BioMatrix, Carlsbad, CA; 5005-100ML) coated T-75 culture plates (Corning Life Sciences, Corning, NY; 430641U), in Pneumacult-Ex Plus Medium (STEMCELL Technologies, Vancouver, Canada; 05040) with 1% Gibco penicillin-streptomycin (ThermoFisher Scientific, Waltham, MA; 15140-122). At 70-90% confluence, cells were passaged with Accutase (Innovative Cell Technologies, San Diego, CA; AT-104), counted, and plated on human placental type IV collagen-coated (Sigma-Aldrich, St. Louis, MO; C7521-10MG) 12mm Transwell permeable supports at a density of 2×10^5 cells/Transwell. After reaching confluence, apical growth medium was removed and basolateral growth medium was switched to Pneumacult-ALI Medium (STEMCELL Technologies, Vancouver, Canada; 05001). Cultures were then differentiated at an air-liquid interface (ALI) for at least 28 days. For 1 week after confluency, ALI media was changed daily and thereafter was changed 3 times/week. Cultures were washed 1 time/week with Gibco DPBS (ThermoFisher Scientific, Waltham, MA; 14190250) to prevent accumulation of mucus. Cells were cultured at 37°C with 95% humidity and 5% carbon dioxide.

Click Reaction and Protein Visualization

Materials. HPLC grade solvents were purchased from Thermo Fisher Scientific Inc. (Waltham, MA). Dithiothreitol (DTT) and iodoacetamide were purchased from Sigma Aldrich (St Louise, MO). Streptavidin Sepharose beads (17-5113-01) was purchased from Fisher Scientific. Precision Plus Protein Kaleidoscope™ standards (10–250 kDa), Nitrocellulose membrane and Simply Blue were purchased from Bio Rad (Hercules, CA). 10% or 4-12% gradient NuPAGE Novex BisTris® pre-casted mini gel were purchased from Life Technologies (Carlsbad, CA). IRDye® 800CW Streptavidin (925-32230), Odyssey Blocking buffer and IRDye® 800 or 680 nm Secondary Antibodies (mouse, rabbit, and goat) were purchased from Li-Cor (Lincoln, NE). KEAP1 (R&D, MAB3024) and Actin (abcam, 8299) antibodies were purchased as listed.

16HBE Exposures for Click Reactions. 16HBEs were plated at 5 x 10^5 cells/well on 6-well cell culture plates (Corning Life Sciences, Corning, NY; 3506). Cells were grown to 90% confluency and then were exposed to 2 mL *a*-CBD or *a*-CBDQ (35.33, 17.32, 8.49, 4.16, or 2.04 µM) or vehicle control in MEM media for 4 hours. For dosing solutions, stocks of 40 mM *a*-CBD or *a*-CBDQ in DMSO were serial diluted in MEM media. The vehicle control was an equivalent volume of DMSO in media to the 35.33 µM dose. Directly following exposure, the medium was removed, and cells were washed with cold PBS twice and harvested in 300 µL of click lysis buffer (50 mM HEPES, 150 mM NaCl, 0.1% TritonX100, 0.1% SDS, pH=7.0 containing protease inhibitors). Two replicate wells were combined for each condition for click reactions.

HBEC Exposures for Click Reactions. Fully differentiated hBECs grown at air-liquid interface as detailed previously were exposed to 20 or 40 puffs of vaped lab-made *a*-CBD liquid using the UNC Vaping Product Exposure System (VaPES). VaPES is an automated *in vitro* vaping exposure system developed by our group that employs gravimetric sedimentation to evenly deposit aerosol on cells (Figure S3). VaPES was used with a JUUL device (JUUL, Washington D.C.) fitted with a refillable pod (OVNS TECH, Shenzhen, China; W01 pods 0.7mL) and 5-second puffs were taken every 10-minutes to allow for complete sedimentation. Exposures were performed in an incubator at 37°C with 95% humidity and 5% carbon dioxide. Liquid was made with *a*-CBD (33.33 mg/mL) and 67/33 propylene glycol/vegetable glycerin (PG/VG) (Mister-E-Liquid, Grand Rapids, MI). Vaped exposures were compared to cells exposed to non-vaped *a*-CBD or vehicle control for 400 minutes (40-puff equivalent). For non-vaped *a*-CBD, the previously described solution of 33.33 mg/mL *a*-CBD in PG/VG was added to ALI media to a final concentration of 10 µM *a*-CBD and 120 µL was added to the apical surface. For the vehicle control, the same volume of PG/VG as the non-vaped *a*-CBD condition was added to ALI media and 120 µL was added to the apical surface of the cells. Directly after exposure, medium was removed, and cells were washed with cold PBS twice and harvested in 300 µL click lysis buffer (previously described). One donor was used (Table S1) and 4 replicate Transwells (12 mm) were combined for each condition.

Cell Lysis and Click Reaction. The photocleavable biotin reagent used in click chemistry was synthesized in our lab and is available from Kerafast, Boston. The cells were sonicated and let stand on ice for 20 min then centrifugated in 10000 g for 5 min at 4 °C. The supernatant was collected, and the protein concentration was determined with BCA assay (Thermo Fisher Scientific, Waltham, MA). All protein concentration was adjusted to 1-2 mg/mL. The following click reagents were added to each of the samples: azido-biotin reagent (0.2 mM), tris(3-hydroxypropyltriazolylmethyl)amine (THPTA) ligand (0.2 mM) (ref) copper sulfate (1 mM), and sodium ascorbate (1 mM), and the samples were vortexed and allowed to rotate for 1 h at room temperature in the dark. Save 50 mL of reaction mixture for streptavidin visualization.

Streptavidin Visualization of Modified proteins. Lithiumdodecyl sulphate (LDS) (16.5 µL) and 1M DTT (3.5 µL; 50 mM final concentration) were added to 50 mL of click reaction mixture. One half of the sample was resolved using 10 % NuPAGE Novex BisTris® gel. The proteins were transferred electrophoretically to a nitrocellulose membrane and incubated with IRDye® 800CW Streptavidin and actin (anti-goat) primary antibody overnight @4 °C. The next day secondary IRDye® 680RD Donkey anti-goat was

incubated for 30 min at room temperature. Blots were visualized using the Odyssey Infrared Imaging System™ @800 nm for Streptavidin and 700 nm for actin (Licor, Lincoln, NE).

Protein Catch and Photorelease. Cold methanol (3 volumes) was added to the remaining click reaction mixture and let stand on ice for 20 min. The proteins were pelleted by centrifugation. The pellets were resuspended in 1 mL cold methanol and transferred to eppendorf tubes and spun at 10,000g for 5 min. The obtained protein pellets were re-suspended in 100 micro-L 1% SDS. Once all proteins were well dissolved into SDS (clear solution), added 900 micro-L of 1X PBS and 50 micro-L of prewashed Streptavidin Sepharose beads (Caution: All protein pellet must be in clear solution before adding additional PBS and beads). Then allow samples to interact with the Streptavidin Sepharose for 2 h @ room temperature in the dark. Then the beads were transferred to 0.22 mm Cellulose Acetate filter (SpinX 8161). The beads were washed with 0.5 mL of 1% SDS (x2), 4M urea in PBS (x2), 1M NaCl (x2), and 25 mM ammounium bicarbonate (AMBIC) (x2) sequentially. At the end of washes, the spin filter was filled with 0.5 mL of 25 mM of AMBIC then exposed to hand-held UV (@365 nm) for 2 h with a gentle stirring. Filtrates of photo-released proteins were collected and evaporated in dryness under the vacuum.

Protein Visualization with Western Blot. The dried proteins were redissolved in a sample loading buffer (5 micro-L LDS + 1 micro-L of 1M DTT + 14 micro-L H_2O) and loaded onto 4-12 % NuPAGE Novex BisTris® gel. The resolved proteins were transferred to nitrocellulose membrane for various immunoblots. The membrane was incubated with anti-KEAP1 (1:1000) overnight. The anti-mouse-800nm for KEAP1 was added and incubated for 30 min followed by 3 washes. Blots were visualized using the Odyssey Infrared Imaging System™ @700 and 800 nm for for both proteins (Licor, Lincoln, NE). The integration box was set around the dominant expected band for KEAP1 at 70KD and is the same size in all samples including the control. If there was any background effect, it was applied to all samples.

Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR)

For qRT-PCR experiments, hBECs were exposed to 10 or 20 puffs of commercial CBD oil (Liberty, Gorilla Glue cartridge, 750 mg/mL) or juice (CBDFx, Strawberry Kiwi, 16.67 mg/mL) using VaPES. A Silo battery vaping device (CCell, Phoenix, Arizona; 500 mAh, ~3.7 V) was used for exposures. The juice was added to a 1 mL refillable glass cartridge (CCell Phoenix, Arizona). Vaped exposures were compared to hBECs exposed to non-vaped CBDFx juice for 200 minutes. A non-vaped CBD oil control was not included as the CBD oil used is highly viscous and not soluble in media. For the non-vaped exposure, 120 µL of a 20-puff equivalent of CBDFx juice based on VaPES deposition testing (1.11 mg or 0.965 µL/20 puffs; Figure S3) in ALI media was added to the apical surface of cells. One well each of 3-4 donors was used for each condition (Table S1).

Immediately after exposure, cells were harvested in 300 µL RNA Lysis Buffer (Invitrogen, Waltham, MA; 12183025) and stored at -80 °C. RNA was isolated using a PureLink RNA Mini Kit (Invitrogen, Waltham, MA; 12183025) according to the manufacturer's instructions and quantified using a CLARIOstar and LVis plate (BMG LABTECH, Cary, NC). cDNA was made from RNA (500 ng) using a High-Capacity cDNA

Reverse Transcription Kit with RNase Inhibitor (Applied Biosystems, Waltham, MA; 4374966) and a MiniAmp Plus Thermal Cycler (Applied Biosystems, Waltham, MA; A37835). cDNA (75 ng) was amplified using the TaqMan Universal PCR Master Mix (Applied Biosystems, Waltham, MA; 4304437) and the QuantStudio 3 Real-Time PCR System (Applied Biosystems, Waltham, MA; A28567) according to the manufacturer's instructions. TaqMan Gene Expression Assays (ThermoFisher Scientific, Waltham, MA) were used for *HMOX1* (Hs01110250_m1), *GPX2* (Hs01591589_m1), and *ACTB* (Hs99999903_m1). C_T values were calculated by normalizing to *ACTB* and the $\Delta\Delta C_T$ method was used to calculate relative fold-change values compared to non-vaped CBDFx juice.

Supplemental Figures

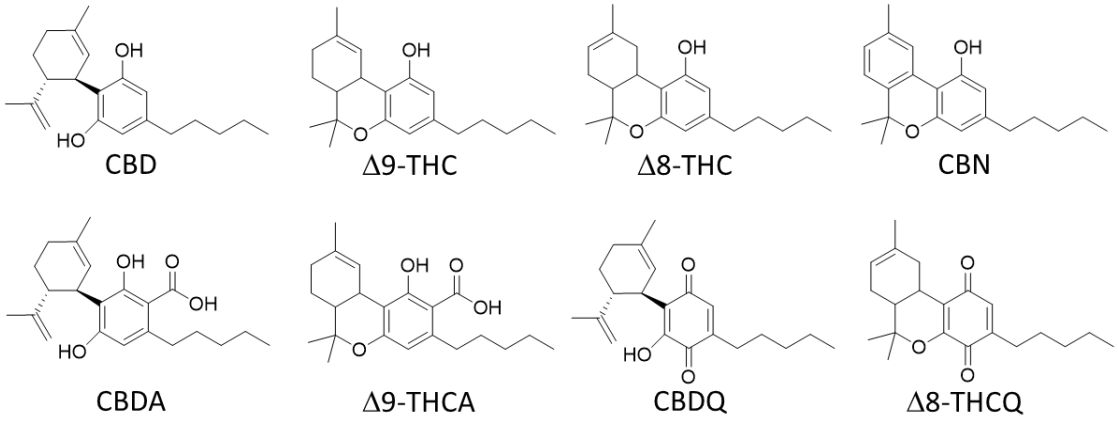


Figure S1. Compounds of interest.

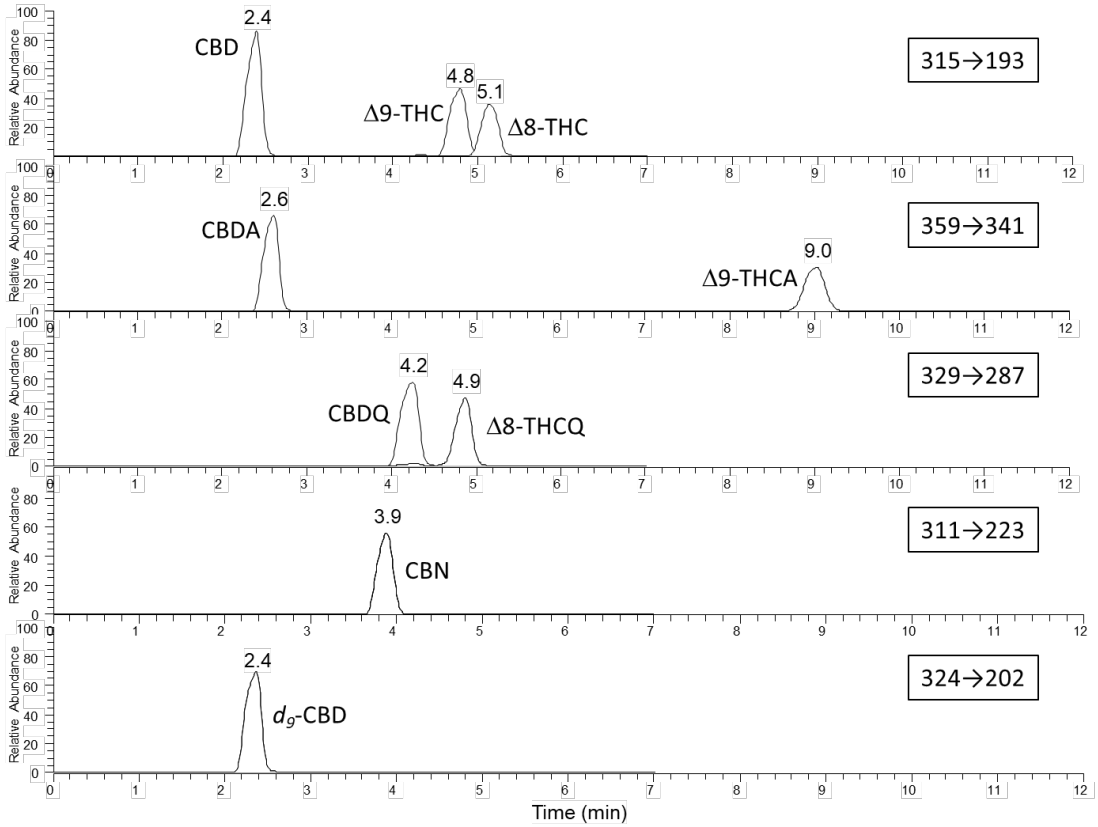
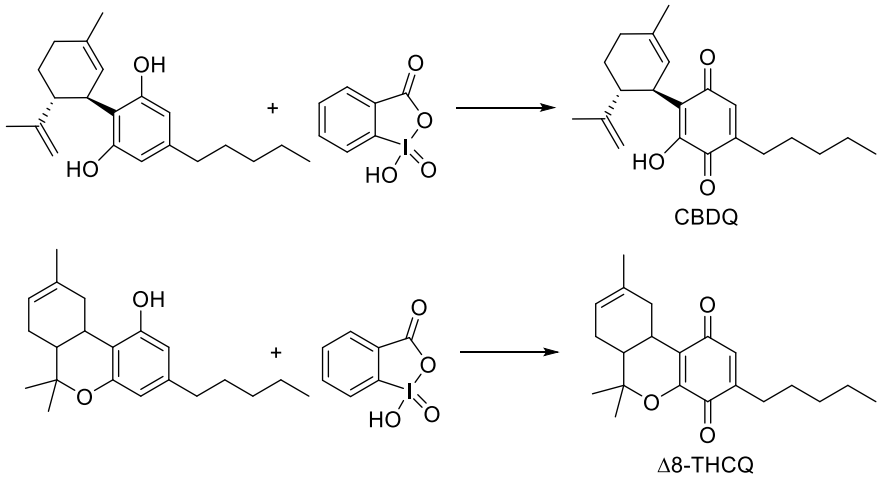
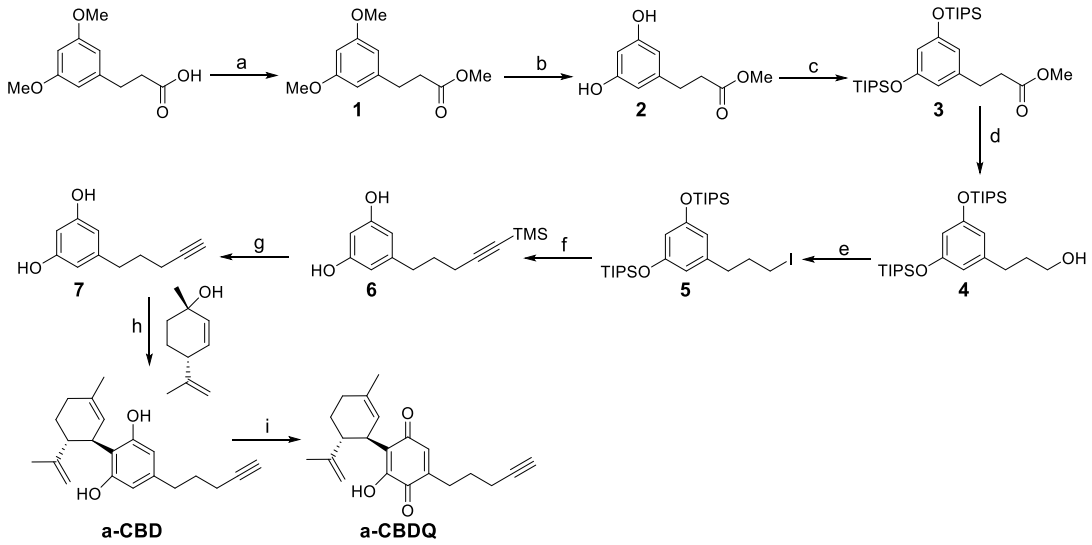


Figure S2. LC/MS-MS chromatogram of CBD and its oxidation products. Samples were analyzed on an Acquity UPLC system equipped with an Agilent Poroshell EC-C18 (10 cm x 2.1 mm, 1.9 µm) column with CH_3CN:MeOH:H_2O, 40:40:20 (0.01% (v) formic acid) mobile phase at a column temperature of 40 °C.



Scheme S1. Synthesis of CBDQ and Δ8-THCQ.



Reagents: a) TsOH, MeOH; b) BBr_3; c) TiPSCl, im; d) DIBAL-H; e) PPh_3, I_2, im; f) TMS-acetylene, BuLi, HMPA; g) TBAF; h) BF_3-Et_2O, alumina; i) SIBX.

Scheme S2. Synthesis of *a*-CBD and *a*-CBDQ.

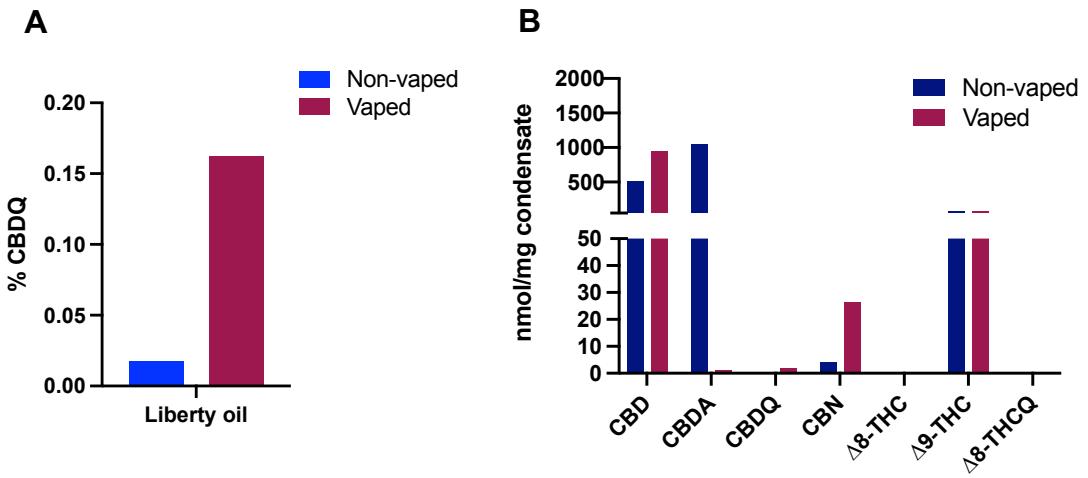


Figure S3. Additional chemical analysis of a second cartridge of Liberty CBD Oil purchased at a different date. A) Generation of CBDQ after vaping; shown as percent CBDQ per total measured cannabinoids of interest. B) Composition of other cannabinoids before and after vaping for in Liberty CBD oil.

Donor	Age (year)	Sex	Race/Ethnicity	Smoking	Experiment
1	32	M	Black	NS	*a*-CBD click reaction
2	32	F	White	NS	qRT-PCR
3	29	M	White	NS	
4	30	F	White	NS	
5	30	M	White	NS	

Table S1. Demographic data for hBEC lung donors. NS=non-smoker.

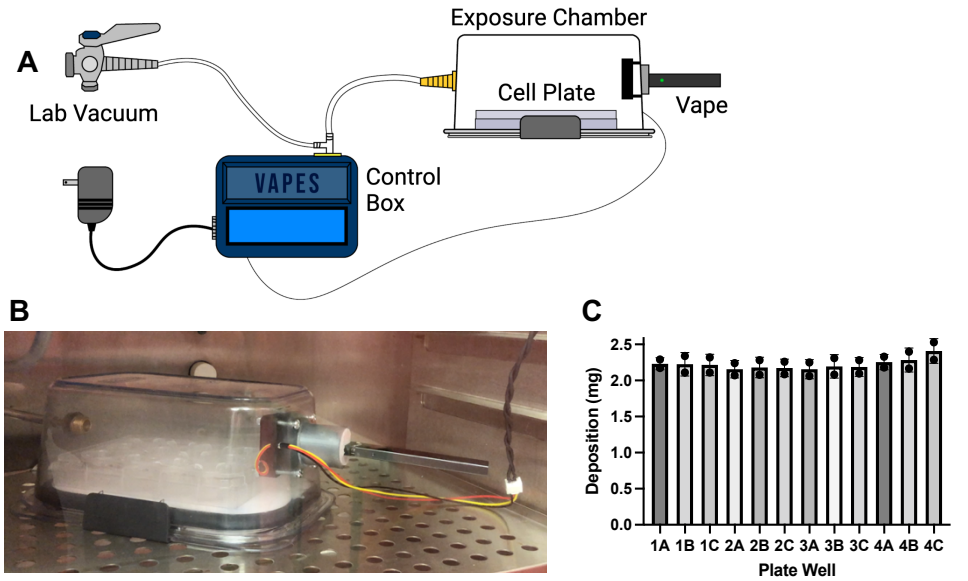


Figure S4. The UNC Vaping Product Exposure System (VaPES). A) A schematic of VaPES showing the control box, which contains an Arduino IDE interface connecting the VaPES with an external computer to regulate vacuum flow, puffs (length and inter-puff duration), and the exposure chamber fan for mixing. B) VaPES in use in a standard laboratory incubator to perform experiments under physiological conditions. C) Deposition testing of VaPES over two independent experiments using a Silo battery and CBDFx Strawberry Juice.

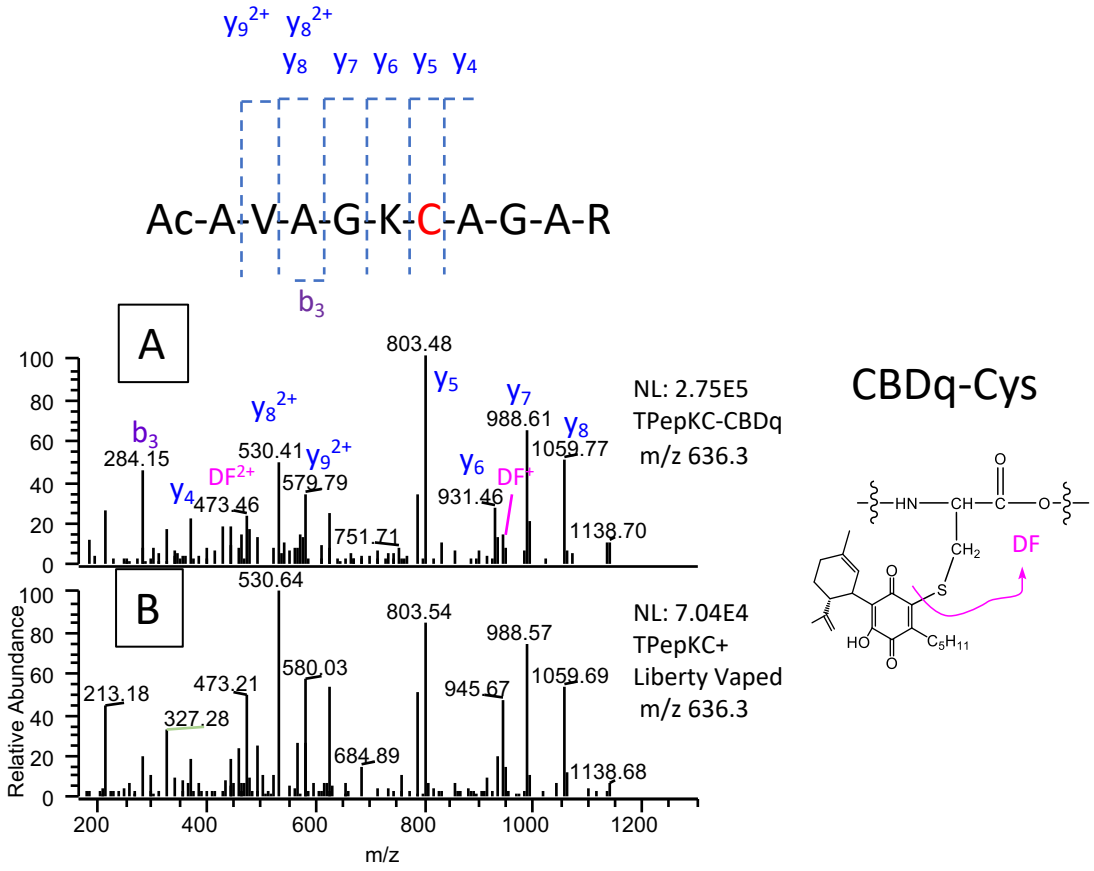


Figure S5. MS/MS TpepKC adducts found m/z 636.3 (A) with synthetic CBDQ (B) found in Liberty vaped mix. The retention time and the b and y ion assignment matched to the standard (synthetic CBDQ-TpepKC adduct). The y4 and y5 indicated the unambiguous adduction site on Cys residue not Lys.

References

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