Materials and Methods:

Cell culture. OVCAR5 were maintained in RPMI-1640 with L-glutamine (Corning, Cat# 10-040-CV) plus 10% FBS, 1% GlutaMAX, and 1% penicillin-streptomycin. OVCAR4 cells were maintained in RPMI-1640 with L-glutamine (Corning, Cat# 10-040-CV). COV362 cells and primary patient tumor derived cells were maintained in DMEM with L-glutamine, 4.5g/L glucose, without sodium pyruvate (Corning, Cat#10-017-CV). SKOV3 cells were cultured in medium composed of 1:1 combination of MCDB 105 (Sigma Aldrich, Cat# M6395) and Medium 199 (Corning, Cat# 10-060-CV). NoEM cells were maintained in DMEM/F12 (Thermo Fisher, Gibco, Cat# 11320033). Culture media were supplemented with 10% FBS (Fisher Scientific, Cat# 35011CV) and 1% penicillin-streptomycin solution (Corning, Cat# 30-002-CI).

In vivo experiments. Animal studies were conducted according to a protocol (# IS00017143) approved by the Institutional Animal Care and Use Committee (IACUC) of Northwestern University. 100,000 OVCAR5 cells were injected SQ into the right flank of female, 6-8 weeks old, athymic nude mice (*Foxn1*^{nu}, Envigo). When tumors developed to measurable size (100mm^3), mice were randomly allocated to the experimental treatments and then treated intraperitoneally (i.p.) with 100ul diluent (vehicle) or NTX-301 (0.5mg/kg or 1mg/kg, 5-day/week for 3 weeks). Length (l), width (w) and height (h) of SQ xenografts were measured weekly using digital calipers and tumor volume (v) was calculated as $v = ½ \times l \times w \times h$. Mice were euthanized, and tumors collected 2 days after the last treatment. Xenograft tumors were used for RNA and protein extraction, tumor digestion for generating single cell suspension and snap frozen tissues in the O.C.T. medium (Tissue-Tek, O.C.T. Compound, Sakura Finetek USA, Inc. CA, USA) for large-area hyperspectal SRS imaging. To evaluate the effects of NTX-301 on preventing OC tumor recurrence after chemotherapy, female (6-8 weeks old) athymic nude mice (*Foxn1*^{nu}, Envigo) were

injected SQ with 100,000 OVCAR5 cells. Mice were randomly assigned to treatment with PBS (control) or 40 mg/kg carboplatin ip (n = 5-6 mice/group), once-a-week for 3 weeks starting when xenografts were > 100 mm^3. Tumor sizes were measured weekly using digital calipers. Mice that responded to platinum were treated with diluent or NTX-301 (1mg/kg, daily for 5 days per week, 2 weeks; n=6 mice/group). Tumors were collected, measured, weighted, and processed as described.

Patient Derived Xenografts: Pieces of a HGSOC tumor established from a consenting donor and obtained as previously described were implanted SQ in female NOD SCID gamma (NSG) mice (The Jackson Laboratory) and allowed to grow to 1-1.5cm diameter over the course of 3-4 months, as previously described (1). Collected PDX tumors were examined by H&E staining and confirmed to be HGSOC by a board-certified pathologist. Tumors were divided into equally sized specimens (∼2 × 2 × 2 mm) and implanted SQ into the dorsal region of the appropriate number of female NSG mice (second generation PDX tumors or P2). Once tumors reached 150–200 mm3, mice underwent balanced randomization based on tumor size. Treatment with diluent or NTX-301 (5 days per week, 3-weeks, i.p., 1mg/kg, n=6 mice/group) started when tumors size reached 150 mm^3. Body weights and tumor sizes were measured twice-a-week. Tumors were collected and processed as described. Experiments were approved by IACUC (protocol #IS00007992).

BODIPY staining for lipid peroxidation. Intracellular lipid peroxidation was determined by using the lipid peroxidation sensor BODIPY 581/591 C11 (Thermo Fisher Scientific, Cat# D3861). In brief, OVCAR5 transfected with shctrl or shGPX4 vectors were stained with BODIPY 581/591 C11 (5 μM) for 1-2 hour at 37°C, washed with PBS, and fixed with 4% PFA on ice for 30 mins. OVCAR5 cells treated with DMSO, NTX-301 (500nM), DFOA (800nM), or NTX-301+DFOA for 48 hours, and then were stained with BODIPY 581/591 C11 (5 μM) for 2 hours at 37°. In the

oleic acid rescue experiment, OVCAR5 cells were plated at 500,000 cells/well and allowed to attach overnight. The following day, cells were treated with DMSO, NTX-301 (500nM), oleic acid (200 μM), or NTX-301+oleic acid for 48 hours. Next, cells were stained with BODIPY 581/591 C11 (5 μM) for an hour at 37°C and washed with PBS. For xenograft analysis, tumors were minced with a scalpel and enzymatically digested into single cell suspension. Cells (1×10^6) in 1 ml medium were incubated with 5 μM BODIPY 581/591 C11 for an hour at 37°C and washed with PBS. The mean fluorescence intensity (MFI minimum of 10,000 events per condition) was measured by FACS (LSR Fortessa, BD, Franklin Lake, NJ). BODIPY emission was recorded at 520nm (FITC) and 580nm (PE). Data were displayed as histograms and mean fluorescence intensity of FITC and the ratio of oxidized (FITC) to reduced (PE) C11 was calculated. Data were analyzed using FlowJo, (RRID:SCR_008520) software.

RNA sequencing (RNA-seq) and data analysis. Total RNA was extracted with TRI Reagent (Sigma, Cat# T9424). DNA was removed by using a RNeasy MinElute Cleanup Kit (QIAGEN) with RNase-Free DNase Set (QIAGEN). mRNA was isolated from 1 μg of total RNA with a NEBNext Poly(A) mRNA Magnetic Isolation Module and used for preparing RNA sequencing libraries with a NEBNext Ultra II RNA library prep kit and protocol from Illumina (New England Biolabs Inc., Ipswich, MA). Library quality was verified using a BioAnalyzer ((Agilent Technologies), and then libraries were sequenced on an Illumina NextSeq500 system with single-end, and 75-bp read length settings. For quality control, raw fastq files were pre-processed using TrimGalore (0.4.4) and cutadapt (1.14) with single-end trimming mode, Phred score cutoff of 20 and minimum sequence length cutoff of 20 bp. After checking quality with the FastQC tool, raw sequencing reads were aligned to human genome build hg38 using STAR v.2.5.2 (https://github.com/alexdobin/STAR) with standard settings. Mapped reads were converted to raw

counts with HTSeq (https://htseq.readthedocs.io/en/master/), normalized to library size, and analyzed for DEGs by edgeR (Bioconductor). The $\log_2$(fold-change) and *P*- value of total normalized counts and DEG counts were then analyzed by GSEA, Ingenuity Pathway Analysis, (RRID:SCR_008653) (IPA, QIAGEN). Data are deposited in the NCBI gene expression omnibus (GEO: GSE236985).

Lipidomics. OVCAR-5 cells treated with DMSO or NTX-301 (100nM, 2-Day) were used for lipidomics analysis at the Bindley Bio-science Center, Purdue University, as described previously (2). In brief, cells were washed twice with ice cold PBS, harvested in 800µL ice cold PBS and spun down at 1000 rpm at 4℃ for 5min. Cell pellets were frozen on dry ice and stored at -80°C until used for analysis. Lipidomic analysis was performed using multiple reaction monitoring (MRM) profiling, and data acquisition was reported in our previous study (3). Data were processed using an in-house script to obtain a list of MRM transitions with their respective sum of absolute ion intensities over the acquisition time. Amounts of fatty acids were reported in pg/1000 cells. Statistical analysis was performed utilizing MetaboAnalystR 3.0 (3).

Large-area hyperspectral SRS imaging. Tissue blocks were cryopreserved in OCT (Optimal Cutting Temperature) at -80^{0}C, sliced using a Leica CM1950 cryostat into 10 μm thick tissue layers, which were placed on glass slides and kept frozen until imaging. Multiplex Stimulated Raman Scattering (SRS) was performed by a femtosecond laser with two synchronized outputs beams (Insight DeepSee, Spectra-Physics, Santa Clara, CA, USA) to measure fatty acid content. The laser was operated at 80HMz. One of the synchronized output beams, Pump beam, was fixed at 798nm with the other beam, Stokes beam, being at 1040 nm to cover the C-H vibration region. An acousto-optic modulator (AOM, 1205-C, Isomet) was used to modulate Stokes beam at 2.3MHz. The SRS spectrum was recorded by controlling the temporal delay of two chirped

femtosecond pulse. Six 12.7 cm long SF57 glass rods were applied to chirp both beams before the beams were sent to the laser-scanning microscope. A 60x water immersion objective lens (NA = 1.2, UPlanApo/IR, Olympus) was applied to focus the light on the sample with signal collection by applying an oil condenser (NA = 1.4, U-AAC, Olympus) (3). To achieve hyperspectral SRS imaging, a stack of 100 images at various pump-Stokes temporal delay was recorded. To achieve large-area mapping, a motorized scanning stage (PH117, Prior Scientific) was applied (4). The power of pump beam and Stokes beam were set to 30mW and 200mW, respectively. Raman shift calibration was completed by recording Raman spectrum of DMSO. Images were analyzed by ImageJ and least absolute shrinkage and selection operator (LASSO) was applied to separate different chemical maps according to their Raman spectrum. The reference of needed chemical maps were obtained by recording Raman spectrum of respective standard chemicals (5). The hyperspectral were images were transformed into phasor plots using the spectral phasor plugin in ImageJ and the lipid spectra and images were generated by surveying the corresponding cluster in the phasor plot as previously described (6). Hyperspectrum of pure glycerol trioleate was used as a reference for lipid cluster mapping and spectra extraction. Statistical analysis was performed by using the t test in the Origin Lab Software.

Cell viability assay: Cell viability was assessed using the Cell Counting Kit 8 assay (CCK8, Dojindo Molecular Technologies, Cat# CK04, Rockville, MD, USA) following the manufacture's recommendations. Absorbances (450 nm) were measured with a microplate reader (BioTek ELX800, BioTeK, Winooski, VT). Cell death was measured by counting cells stained with trypan blue (Thermo Fisher, Cat# T10282).

Clonogenic assay: OC cell lines (1000 to 2,000 cells/well) or primary cells derived from OC tumors (4,000 cells/well) were seeded in 12-well plates and allowed to attach overnight. Cells

received experimental treatments (DMSO, or NTX-301, 100nM, 5-Day) and then were allowed to form colonies for 7-14 days. Cell colonies were washed with PBS, fixed with 4% paraformaldehyde and stained with 0.05% crystal violet for counting.

Spheroid formation assay. OC cells (OVCAR5 and SKOV3) were pre-treated with DMSO (control), or NTX-301 (10nM, 100nM) for 4 days. Cells were cultured (1000/well) in MammoCult medium in 96-well low-attached plates (Fisher Scientific, Cat# 3474) for 7-14 days. Fresh medium was added every 2 to 3 days. Numbers of spheroids were counted with an inverted microscope and numbers of viable cells were estimated by measuring intracellular ATP levels with the CellTiter-Glo 3D cell viability assay (Promega, Cat# G9681) following the manufacturer's protocol. Briefly, a volume of CellTiter-Glo 3D Reagent equal to the amount of medium was added into each well, mixed for 30 minutes to induce cell lysis, and incubated for 35 minutes at room temperature to stabilize the luminescent signal. Luminescence was measured by using a microplate reader (SpectraMax GeminiXS, Molecular Devices, San Jose, CA, USA).

Apoptosis Assay. Cell apoptosis was analyzed using Annexin V staining (Thermal Fisher Scientific, USA, Cat# V13245). In brief, 10^6 OVCAR5 cells were collected and 1X PBS washed after treatments with DMSO or NTX301 (100nM, 1μM) for 48 hours or 72 hours. After centrifugation and supernatant removal, cells were resuspended in 100μl 1X Annexin-binding buffer with 5μl Alexa Fluor 488 annexin V and 1μl 100 μg/ml propidium iodide (PI) and incubated at room temperature for 15 minutes. Additional 400 μl 1X Annexin-binding buffer was added to the reaction after the incubation period, then cells were analyzed by flow cytometry, by measuring the percentage of cells staining for FITC and PE-Texas Red.

RNA extraction and quantitative RT-PCR analysis. Total RNA was isolated with Trizol reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. RNA quantity and purity

(260/280 absorbance ratio) were determined using a NanoDrop spectrophotometer (Thermo Scientific). For *mRNA* expression studies, 1 μg of total RNA was reverse-transcribed into cDNA with an iScript cDNA synthesis kit (Bio-Rad, Berkeley, California) per manufacturer. cDNA was used for quantitative PCR performed by using the iTaq Universal SYBR Green Supermix (Bio-Rad, Berkeley, California) on a 7900HT real-time PCR instrument (Applied Biosystems, Foster City, CA). The PCR reactions used the following parameters: 94℃ for 10 min, 40 cycles of amplification at 94 ℃ for 15 s and 60 ℃ for 1 min, and an extension step of 7 min at 72℃. Data were normalized using expression of the 18S gene. Relative expression of target genes was calculated using the $2^{-\Delta\,(\Delta C_T)}$ method where $\Delta C_T = C_{T,\,target} - C_{T,\,18S}$ and $\Delta(\Delta C_T) = \Delta C_{T,\,stimulated} - \Delta C_{T,\,control}$. Primer sequences (Integrated DNA Technologies, USA) are in Suppl. Table S2.

ChIP-PCR. ChIP was performed with anti-H3 (Active Motif, Cat# 39763), and anti-H3K27Ac (Abcam, Cat#4729), and mouse IgG antibodies (Santa Cruz, sc-2025), as previously described (7). Briefly, extracted chromatin was crosslinked with 1% paraformaldehyde and fragmented to an average size of approximately 300–500 bp by sonication. Chromatin (5μg) extracted from DMSO or NTX-301 treated OVCAR5 cells was incubated with 5 μg of either anti-H3, or anti-H3K27Ac for immunoprecipitation at 4°C for overnight. The concentration of immunoprecipitated DNA was measured with a Qubit dsDNA HS Assay Kit (Thermo Fisher Scientific Specific sequences of immunoprecipitated DNA was amplified by qPCR with gene-specific primers (Supplementary Table S3) using iTaq Universal SYBR Green Supermix (Bio-Rad). Input DNA was used for normalization and a target sequence located 1 kb upstream from the binding site was used as negative control. Primer sequences are listed in Supplementary Table S3.

Western Blotting: Cell lysates were prepared in radio immunoprecipitation assay (RIPA) buffer. Protein concentrations were quantified with the Bradford assay (Biorad Protein Assay Reagent,

BioRad, CA). Proteins (20-50µg/sample) were denatured at 100°C, resolved by polyacrylamide-gel electrophoresis (SDS-PAGE), and transferred onto a PVDF membrane. The membrane was incubated with 5% milk (blocking), and then with primary antibody overnight at 4°C, followed by incubation with HRP-conjugated secondary antibody for 1hr at room temperature. Signal was developed using SuperSignal West Pico PLUS Chemiluminescent Substrate (Thermo Fisher Scientific cat#: 34580) and captured with an ImageQuant LAS 4000 machine. To detect additional proteins, membranes were treated with Restore Western Blot Stripping Buffer (Thermo Fisher Scientific cat#: 21059), blocked, and then incubated with primary antibody. Primary antibody information is listed in the Suppl. Table S4. HRP-conjugated donkey-anti-rabbit polyclonal antibody (Cat#NA9340, used at 1:2000) was purchased from GE Healthcare (Pittsburgh, PA), HRP-conjugated goat-anti-mouse antibody (Cat#haf007, used at 1:2000) was from R&D System (Minneapolis, MN), and HRP-conjugated goat-anti-rabbit (Cat#1706515, used at 1:2000) was from BioRad (Berkely, CA).

Immunohistochemistry (IHC): Sections of paraffin-embedded tissues were deparaffinized with xylene, followed by re-hydration through decreasing concentration of ethanol (100%, 90%, 70%, 50%, 0%). Antigen retrieval was performed with citrate buffer (10 mM, pH 6.0) for 30 minutes at 95°C. Peroxidase activity was eliminated with 10% hydrogen peroxide (Fisher Scientific, Cat# H324500) for 10 mins, and then tissues were incubated with 0.5% normal goat serum (DAKO, Hamburg, Germany, Cat# K0672) in PBS for 1 hour. Anti-vimentin (Dako, Cat# M0725, dilution 1:1000), anti-Pax8 (Santa Cruz, Cat# sc-81353, dilution 1:50), anti-P53 (Bond Ready-to-use P53 (DO-7) Leica Cat# PA0057) or rabbit IgG (Santa Cruz, Cat# sc-2027,1:500) were added to tissue sections and incubated overnight at 4°C. This was followed by treatment with biotinylated secondary antibody followed by avidin-biotin peroxidase reagents of a DAKO Detection Kit

(DAKO, Hamburg, Germany, Cat# K0672), and then with liquid DAB substrate chromogen (DAKO, Cat# K3467). Sections were counterstained with hematoxylin (Agilent Technologies, Cat# CS700) and cover-slipped.

References

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