

Supplementary Materials

Materials and methods

Chemicals and reagents

Mass spectrometry-grade chemicals and reagents were used in the present study. Matrix moieties, including 1,5-diaminonaphthalene (1,5-DAN; No. 56451), 9-aminoacridine (9AA; No. 92817), 2,5-dihydroxybenzoic acid (DHB; No. 149357), and α -cyano-4-hydroxycinnamic acid (CHCA; No. C2020), were obtained from Sigma-Aldrich (St Louis, MO, USA). Derivatization reagent 4-(anthracen-9-yl)-2-fluoro-1-methylpyridin-1-ium iodide (FMP-10, No. T1001), were obtained from Tag-ON. The amino acids standards ethanolamine (No. E808767), putrescine (No. D807415), serotonin (No. H875754) and spermine (No. S874787) were obtained from Macklin (Shanghai, China). Ultrapure-grade (18 MU) water was prepared by a Milli-Q system (Millipore Corporation, Billerica, MA). Other reagents include trifluoroacetic acid (TFA; TEDIA, No. TS4295-013C); methanol (MeOH; Honeywell, No. AH230-4HC); ethanol (EtOH; Sigma-Aldrich, no. E7023); acetonitrile (ACN; Honeywell, No. AH015-4HC) and indium tin oxide (ITO)-coated electrically conductive glass slides (Sigma-Aldrich, No. 578274). Lipid Mixture 1 was obtained from Sigma-Aldrich (St Louis, MO, USA; Cat. No. L0288). Resveratrol was purchased from ABPHYTO Biological Technology Co., Ltd (Chengdu, China). The synthesis of α -melittin (DWFKAFYDKVAEKFKEAF-GSG-GIGAVLKVLTTGLPALISW-IKRKRQQ-NH₂) was entrusted to Apeptide Co., Ltd (Shanghai, China). iRGD peptide was purchased from Motif Biotech Co., Ltd (Suzhou, China).

Preparation and characterization of a tumor-targeting delivery system

Preparation of a tumor-targeting delivery system

The preparation method followed our previous protocol. Briefly, put 30 mg of resveratrol into 5 mL of absolute ethanol to prepare the resveratrol solution. Separately, dissolve 450 mg of soybean lecithin and 30 mg of cholesterol in another 5 mL of ethanol to form the excipients solution. The two solutions were then combined, and 0.1 mL of Tween 80 was added and mixed thoroughly, and then was transferred to a pear-shaped flask to form a uniform film. After 10 mL of 0.01 M PBS was added, make the lipid film dissolve completely in the bottle. The solution was then sonicated for 10 min (500 W, 5 s on/off) until it became light yellow, clear, and transparent. DSPE-PEG₂₀₀₀ and DSPE-PEG₂₀₀₀-COOH were added to Res liposome (RL) solution at a molar ratio of 9:1 and a ratio of total DSPE-PEG/phospholipid of 5:95, and then incubated in water bath at 50°C for 1h to prepare RL. EDC, NHS and DSPE-PEG₂₀₀₀-COOH were added to RL at a ratio of 1:1:10 (molar ratio). The carboxyl groups were activated for 15 min via stirring at room temperature to prepare the following liposome and drug-loaded liposomes: (1) Mel (Mel:DSPE-PEG₂₀₀₀-COOH=1:1000, molar ratio) was added and incubated at 4 °C for about 16-18 hours. Subsequently, the resulting liposomes were purified by ultrafiltration using a 10 kDa MWCO centrifuge tube and passed through a 0.22 µm filter to make Mel-modified liposomes (PL) and Mel-modified Res liposomes (PRL). (2) iRGD peptide (iRGD:DSPE-PEG₂₀₀₀-COOH=1:1.2, molar ratio) was added and overnight incubation. Removing the unreacted fraction by the same procedure to get iRGD modified liposomes (TL) and iRGD modified Res liposomes (TRL). (3) iRGD and Mel were added in the above proportions and got incubating overnight at 4 °C. The unreacted fraction was emanated by the same procedure to obtain Mel and iRGD modified liposomes (TPL) and Mel and iRGD modified Res liposomes (TPRL).

Size distribution, zeta potential detection and morphology of the delivery system

Transmission electron microscope (TEM) (TecnaiG2, FEI) was used to observe the morphology of liposomes.

The particle size and polydispersity index (PDI) of the liposomes were measured via Malvern nanoparticle size and zeta potential analyzer. Samples were diluted 15-fold before testing with 0.01 M PBS. 10 μ L of each sample was placed onto a copper grid and allowed to stand for 10 min, after which liquid was absorbed by filter paper. Next, 10 μ L of 5% phosphotungstic acid or 2% uranyl acetate was applied for negative staining. After 2 min, excess stain was removed, and the samples were dried at 37 °C. The prepared was examined by TEM at 120 kV. Fourier Transform Infra-Red (FTIR) spectroscopic was performed through KBr pellet method (Vertex 80 FTIR System, Bruker, Germany) to analyze the amide bond formation.

Encapsulation and loading efficiency assays

To determine the optimal dialysis time, 10 mL of TPRL were filled in the dialysis bag and put in 1 L of 0.01 M PBS. Every hour, 100 μ L of the dialysis solution was collected, diluted tenfold with methanol. Then it was analyzed for Res concentration at its maximum absorption wavelength. Separately, TPRL was mixed with methanol to break the emulsion, and its absorbance was measured at the same wavelength to determine the total Res content. Encapsulation efficiency and drug loading were calculated using the following formulas:

Encapsulation efficiency (%) = concentration of Res in liposomes in the dialysis bag C / concentration of Res in liposomes before dialysis C_{total} $\times$ 100%

Loading efficiency (%) = amount of Res loaded into liposomes $m1$ / ($m1$ + mass of lipid in liposomes $m2$) $\times$ 100%

In vitro release

Res release *in vitro* from TPRL was simulated using dialysis at pH 7.4 (MW 3000). In brief, 1 mL of liposomes were placed into a dialysis bag, which was then immersed in a 37 °C PBS buffer solution (pH 7.4). The solution

was withdrawn to determine the content of Res and replaced by same volume of PBS.

Hemolysis assay

1 mL of nude mouse blood was obtained in an anticoagulation tube and centrifuged at 4 °C for 10 min at 1000 g. The precipitate was washed 3 times with PBS to obtain red blood cells (RBCs). RBCs were diluted 5 times with PBS. Put 200 µL of RBCs dilution into 800 µL of different concentrations of nanoplateforms PBS dispersion. 200 µL of RBCs dilution was injected into 800 µL of PBS as a negative control (hemolysis rate = 0%). 200 µL of RBCs solution was added to 800 µL of water as a positive control (hemolysis rate = 100%). The supernatant was collected by centrifugation at 2000 g for 10 min after incubation for 4 h at 37 °C. Measuring the absorbance of the supernatant at 540 nm and calculating the hemolysis rate.

Determination of iRGD and Mel conjugation efficiency

The conjugation efficiencies of iRGD and melittin (Mel) to TPRL liposomes were determined by sequential ultrafiltration. An aliquot of TPRL was centrifuged through a 10 kDa MWCO filter ($10,000 \times g$, 30 min, 4 °C) to separate liposomes (retentate) from free ligands (filtrate). The filtrate was then passed through a 3 kDa MWCO filter ($3,000 \times g$, 30 min, 4 °C), retaining Mel (~5.3 kDa) in the retentate while allowing iRGD (~1 kDa) to flow into the secondary filtrate. Mel concentration in the 3 kDa retentate was determined by ninhydrin assay (95 °C, 15 min; absorbance at 570 nm) against a Mel standard curve. To quantify iRGD in the 3 kDa filtrate, a standard curve was established using MALDI-IT-TOF analysis. Briefly, iRGD stock solution was serially diluted with acetonitrile:water (7:3, v/v) to obtain iRGD standard solutions at concentrations of 10, 100, 200, 400, 700 and 900 µg/mL. For internal standardization, 1 mg of RGD negative control was dissolved in 1 mL of acetonitrile to

prepare a 1 mg/mL IS stock solution, which was diluted with acetonitrile: water (7:3, v/v) to a working IS concentration of 50 µg/mL. For MALDI-IT-TOF analysis, iRGD standards, IS working solution, and the matrix solution (prepared as described in Methods and materials 2.6) were sequentially spotted onto the MALDI target plate in a sandwich configuration. After spectral acquisition, the average peak intensity ratio of iRGD to the internal standard was calculated for each standard spot, and a standard curve was constructed by plotting this ratio against the corresponding iRGD concentration. Conjugation efficiencies were calculated as the percentage of each ligand conjugated to liposomes relative to the total amount initially added, based on free ligand detected in the respective fractions.

Cell lines and culture

The human melanoma cell line A375 and human endothelial HDMEC cells were purchased from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China). All cells were cultured in DMEM (Biological Industries) with the addition of 1% penicillin/streptomycin and 10% fetal bovine serum at 37 °C with 5% CO₂. A375–luciferase (A375–luc) cells were generated using Lenti–EF1α–Luc–T2A–Puro (Beyotime, No. C4001). A375–luc cells were grown in DMEM (01–052–1ACS, Biological Industries, ISR) containing the same amount of FBS and penicillin/streptomycin and 2 µg/mL puromycin was supplemented (Beyotime, No. ST551).

In vitro cell viability assay

Cell viability was analyzed through the CCK–8 assay (GK10001, Glpbio, California). A375 cells planted in 96–well plates were treated with concentrations of Res (0, 0.01, 0.1, 1, 5, 10, 20, 50, 100, 200, 500 µM) and HDMEC cells cultured in 96–well plates were treated with different concentrations of resveratrol (0, 50, 100, 200, 300,

400, 500, 600, 700, 800, 900 μM) for 48 h and after the addition of CCK-8 to the medium incubated for 1 h at 37 °C. To measure the absorbance at 450 nm, the UV-vis-NIR spectra reader was used.

Calcein-AM/PI staining

The cytotoxic effects of various treatments were assessed by live/dead staining. A375 cells grown in 24-well plates were categorized into six groups: Control group (DMSO), Res (100 μM), TPR (Physical mixture), Vehicle group (LPs), RL (equivalent Res concentration 100 μM), TPRL (equivalent Res concentration 100 μM). Cells were incubated for a 48 h. The medium inside was then replaced by a fresh one with Calcein-AM (5 $\mu\text{g/mL}$) and PI (10 $\mu\text{g/mL}$). After half an hour, cells were visualized. Live cells showed green light, while dead cells is red.

EdU staining

To evaluate cell proliferation under different treatments, EdU staining was performed. A375 cells were placed in 12-well plates and divided into the same groups mentioned earlier. Incubating for 48 h, the medium was substituted with fresh medium with EdU (50 μM) and the incubation proceeded at 37 °C for 2 h. 4% paraformaldehyde was added for 30 min to fix the cells. Then, permeabilizing them by 0.5% Triton X-100 for 10 min, then cultured with 1 $\times$ ApolloR reaction cocktail for half an hour, followed by a 30 min incubation with DAPI. Using the microscope (Nikon, Japan), images were obtained. Each sample contained three repeating wells.

Analysis of the cell cycle and apoptosis

The cell cycle and apoptosis was measured by Flow cytometry (FCM). The Cell Cycle Analysis Kit (Biosharp,

China) was used to detect the cell cycle. Cells were digested by trypsin, washed by PBS, and then resuspended in PBS. The suspension of cells was gradually injected to 3 mL of ethanol (4 °C) and fixed for 4 h at 4 °C. Prior to FCM, the cells were rehydrated with PBS for 15 min. After that, cells were stained with DNA staining solution that included permeabilization solution half an hour at room temperature while keeping it in dark.

To detect apoptosis, an Annexin V–fluorescein isothiocyanate (FITC) kit (Biosharp, China) was used, following the manufacturer's guidelines. Briefly, cells were gathered and stained with Annexin V–FITC (50 µg/mL) and propidium iodide (10 µg/mL) for 5 min in the dark and analyzed by FCM (LSR, BD Biosciences).

Lipid droplet staining and counting

To assess lipid droplets, A375 cells were stained with BODIPY 493/503, allowing for the quantification of neutral lipid via FCM. In short, cells were swiftly washed with PBS to eliminate the media clearly. Incubating the cells with 2 µM BODIPY at 37 °C for 15 min without light, then washed three times with PBS. Spinning the cells at 400 × g for 5 min, resuspended in PBS, and promptly analyzed with a FACSCanto flow cytometer (BD, New Jersey, USA). Each sample was analyzed with CellQuest Pro acquisition and Flow Jo, examining ten thousand cells.

Flow cytometry and antibodies

Cells were incubated with diluted antibodies solution (1:200 in PBS) and a viability dye (Fixable Viability Dye eFluor 506, 1:1000, Invitrogen #65-0866-14). PBS supplemented with 2% FBS was prepared to stain the cell for 30 min at 4 °C. The following antibodies were used: FITC-CD36 (Elabscience, E-AB-F1164C), FITC-PD-L1 (Elabscience, E-AB-F1132C), PerCP/Cyanine5.5-CD44 (Biolegend, B439794).

For intracellular analysis of Ki67 or phospho-proteins, cell fixing was performed with BD Phosflow Lyse/Fix Buffer (BD Biosciences, #558049) and permeabilizations of cells was with BD Perm III (BD Biosciences, #558050), followed by intracellular staining with Ki67 antibody (1:50, PEcy7-Ki67, #B374196, Biolegend). Analysis of stained cells is performed by BD FACSCelesta flow cytometer (BD Biosciences) and FlowJo 10.8.1 software.

Transwell assay

A Transwell assay was conducted to evaluate the cells' invasion capabilities, with Matrigel (BD Bioscience Pharmingen, USA) applied to the upper chamber as per the guidelines. In a serum-free medium, 40,000 cells were suspended and divided into 6 groups, matching the groups described in section 2.3. To promote cell migration across the membrane, 750 μ L of 10% FBS medium was poured into the bottom chamber. The chamber was taken and fixation with methanol for 30 min after 48 h. Cotton swabs were used to remove the upper cells, which were then stained with a 1% crystal violet solution for 20 min. Count the cells in five random areas of vision at $\times 100$ magnification. The experiments were repeated three times.

Wound-healing assay

The test was conducted to assess the cells' migration capability. In this test, 8×10^5 cells were placed in each well of plate. Once the cells reached confluence, use a 200 μ L pipette tip to wound the monolayer. Wash the plate with PBS to remove the floating and detached cells. The wells were then split into six groups as previously described and cultured in DMEM. Capture the view of cells at 0 h, 24 h, and 48 h. Assess the wound area using ImageJ software.

Uptake and In vitro BTB translocation

To assess uptake of liposomes, A375 cells were placed in 24-well plates and treated with Cou6-labeled TPRL. Fluorescence images were taken at various intervals (2 h, 4 h, 8 h). A blood-tumor barrier (BTB) model was created in vitro with HDMEC cells within. In short, HDMEC cells (1×10^5 cells per well) were put in the upper chamber, and A375 cells were positioned in chamber below. RL, TRL, PRL and TPRL which is labeled with Cou6 were subsequently introduced into the upper. Following cultivation for 48 h, fluorescence microscopy was used to capture images of A375 cells. The fluorescence intensities were then detected.

RNA-seq

RNA-seq was carried by Biomarker Technologies Co., Ltd (Beijing, China). Briefly, A375 cells inoculated in 6cm dishes were treated with Control (DMSO), Res (100 μ M), Vehicle group (LPs) and TPRL (equivalent Res concentration 100 μ M). After cultured for 48 h, the total RNA was then extracted using Trizol Reagent (Life technologies, California, USA). Concentration of RNA were tested by Nano Drop 2000 (Thermo Fisher Scientific, Wilmington, DE). Assessing RNA integrity by the RNA Nano 6000 Assay Kit of the Agilent Bioanalyzer 2100 system (Agilent Technologies, CA, USA). Sequencing libraries were generated using Hieff NGS Ultima Dual-mode mRNA Library Prep Kit for Illumina (Yeasten Biotechnology (Shanghai) Co., Ltd.), followed by AMPure XP Beads (Beckman Coulter, Brea, CA, USA) size selection, and the sequence was directly determined using Illumina's sequencing-by-synthesis technology. The raw reads were further processed with a bioinformatic pipeline tool, BMKCloud (www.biocloud.net) online platform.

Molecular docking

The molecular docking mode adopts semi-flexible docking. Target proteins with Degree values more than 50 are selected. The target proteins are visualized using PyMOL and then dehydrated and energy minimized. Run AutoDock, create the working directory for molecular docking, read the format files of the target proteins, hydrogenate the receptor and output. Then hydrogenate the Res molecular structure, search for rotatable bonds, add charges, and set it as a ligand, and output in the PDBQT format. Select the BOX function, set the position and size of the BOX based on the simulated conformations of the receptor and ligand, fully enclose both the receptor and ligand, then save the BOX parameters. Run Autogrid, save the GPF file, initiate AutoDock, and save the DLG file. Analyze the output DLG file, screen for stable drug-target conformations based on the binding energy values and bond types predicted by the software simulation, and visualize the docking results using Pymol.

Cellular thermal shift assay (CETSA)

The CETSA was performed as previously described with minor modifications. PBS was used to remove the culture medium. After spinning at 1000 rpm for 3 min, discard the supernatant, and resuspend the cells in 1 mL of PBS. Quickly freeze the cells were in liquid nitrogen, then thawed in distilled water at room temperature. This cycle was repeated three times. The lysate was spun at 10,000 rpm for 20 min at 4 °C, and the supernatant was removed and split evenly into two 1.5 mL EP tubes. One tube was supplemented with 5 µL of 100 µM Res, while the other tube was given a solvent control. Following incubation at room temperature for 30 min in the dark, the mixtures were split into 200 µL EP tubes. The tubes were subjected to heating for 3 min at varying temperatures (42 °C, 46 °C, 50 °C, 54 °C, 58 °C, and 62 °C) and then left to cool for 5 min. Then, underwent another round of centrifugation at 10,000 rpm for 20 min at 4 °C, after which the supernatant was gathered. The supernatant

were then used for Western blot analysis.

Western blot assays

Cells or tissue homogenates of tissue and cells was made by RIPA buffer (pH 8.0) with protease and phosphatase inhibitors. BCA protein assay kit was used to measure the concentration of protein. 20 µg of protein were applied to sodium dodecyl sulfate polyacrylamide gels for protein separation. After separation, the proteins were transferred to polyvinylidene fluoride (PVDF) membranes (Millipore, Temecula, MA, USA) and probed using the primary antibodies detailed in Table S1. After rinsing, the PVDF membranes were treated with suitable HRP-conjugated secondary antibodies. The bands that reacted immunologically were made visible using an enhanced chemiluminescence kit (Pierce, Rockford, IL, USA). Quantification the intensity of band by ImageJ.

H&E staining

Use H&E Staining Kit (G1120, Solarbio) to carry out H&E staining. To summarize, hematoxylin was used to stain major organs and tumor tissue sections, followed by eosin. The sections were then dehydrated with ethanol (95% and 100%), cleared with xylene, and mounted after 50 s of eosin staining. These images were captured via Nikon's microscope (Nikon, Japan).

Standard curve preparation and in vivo biodistribution analysis of Res

For establishing a standard curve for the quantification of Res of tissue samples, 10 mg of Res was accurately weighed and dissolved in 1 mL of acetonitrile to prepare a 10 mg/mL solution. An appropriate volume of this stock solution was then diluted with acetonitrile: water (7:3, v/v) to obtain Res standard solutions at

concentrations of 1, 10, 100, 200, 500, 600, 800, 900, and 1000 $\mu\text{g/mL}$. For internal standardization, dissolve 1 mg of the internal standard (Dionone) into 1 mL of acetonitrile to prepare a 1 mg/mL IS stock solution. This was further diluted with acetonitrile: water (7:3, v/v) to yield a working IS solution at a concentration of 100 $\mu\text{g/mL}$. With the aim of MALDI-IT-TOF analysis, Res standards, IS working solution, and the matrix solution (prepared as described in Methods and materials 2.6) were sequentially spotted onto the MALDI target plate in a sandwich configuration. After spectral acquisition, the average peak intensity ratio of Res to the internal standard was calculated for each standard spot. A standard curve was constructed by plotting this ratio against the corresponding Res concentration. For the biodistribution study, mice were intraperitoneally injected with the TPRL formulation. At designated time points (1, 2, 4, 8, 12, and 24 h post-administration), mice were euthanized. After steps before, tissue samples were collected. The processed tissue samples (processed as described in Methods and materials 2.5) were then analyzed using the iMScope TRIO system. The mean peak intensity ratio obtained from each tissue region was interpolated into the standard curve equation to measure the Res concentration. The percentage of tissue injection dose (% ID) was subsequently calculated to evaluate the biodistribution profile of Res.

Collect blood samples at predetermined time points (5 min, 1 h, 2 h, 4 h, 8 h, 12 h, 24 h, and 48 h) after TPRL administration. Serum was isolated by centrifugation at 3,000 rpm for 10 min. A 50 μL aliquot of serum was deproteinized with 1 mL of methanol/acetonitrile/water (4:4:2, v/v/v). After vortexing, centrifuge the mixture at 12,500 rpm for 30 min. An 800 μL aliquot of the supernatant was evaporated to dryness under nitrogen. The residue was reconstituted in 200 μL of methanol/acetonitrile/water (4:4:2, v/v/v), centrifuged at 12,000 rpm for 30 min. Filter the mixture through filter before LC-MS analysis.

Immunohistochemistry staining

Section mouse tumor tissues into 10 μm slides for immunohistochemistry staining on a cryostat (Leica, SM2010R). After exposure to sodium citrate at 60 °C for 30 min, the slides were incubated with 3% H_2O_2 at room temperature for 15 min. The samples were treated with 5% BSA at room temperature for 1 h. Expose the slices to the primary antibodies mentioned in Table S1 at 4 °C overnight. For 1 h incubation at room temperature, the slides were incubated with a 1:10,000 dilution of HRP-labeled goat anti-mouse/rabbit IgG secondary antibody (H6162, Uelandy), followed by 3,3-diaminobenzidine application. Ultimately, the slices underwent staining with hematoxylin followed by dehydration using a series of ethanol solutions.

Real-time PCR

Extract total RNA through RNA isolation kit from tumor tissues following the introduction. Each sample's total RNA, amounting to two micrograms, was reverse transcribed with the Prime Script RT Reagent Kit. cDNA was synthesized at 37 °C for 15 min and then at 85 °C for 5 s. Quantitative real-time PCR was conducted using SYBR Green PCR Master Mix on a 7300 Sequence Detection System. Three separate analyses were performed at least. The forward and reverse primers are listed in Table S2. In order to determine expression levels, the cycle threshold (Ct) values against the total amount of GAPDH were adjusted. The treatment group's expression levels are shown as a percentage of the control group's levels.

Mitochondrial lipidomics analysis

Following the instructions provided by the manufacturer, mitochondria were extracted from A375 cells using a kit from Solarbio Life Sciences (Beijing, China; Cat# SM0020). To summarize, cells were collected via

centrifugation, washed twice with 4°C PBS, and resuspended in a pre-chilled Lysis Buffer. The cell suspension was blended with a glass homogenizer on ice, and the resulting mixture underwent differential centrifugation at 4 °C. Centrifugation at $1,000 \times g$ for 5 min was used to remove nuclei and intact cells, and the supernatant obtained was then centrifuged at $12,000 \times g$ for 10 min to isolate the mitochondrial fraction. The mitochondrial pellet underwent a single wash with Wash Buffer and was then centrifuged at $12,000 \times g$ for 10 min to achieve highly purified mitochondria.

Isolated mitochondria were then subjected to untargeted lipidomics profiling by Shanghai Biotree Biomedical Technology Co., Ltd. (Shanghai, China). Lipids were isolated from mitochondrial samples through standard liquid-liquid extraction methods, followed by analysis with liquid chromatography-tandem mass spectrometry (LC-MS/MS). Lipid species were identified and quantified using the company's in-house spectral database and bioinformatics pipeline.

Seahorse XF metabolic analysis

The Seahorse XF96 Analyzer from Agilent Technologies in Santa Clara, CA, USA, was used to measure the oxygen consumption rate (OCR). A375 cells were plated at a density of 1.5×10^4 cells per well in XF96 microplates and incubated overnight. The blend medium was exchanged with pre-warmed Seahorse XF DMEM medium (Agilent, #103575-100) with 1 mM pyruvate, 2 mM glutamine, and 10 mM glucose before the assay, and incubated for 1 h at 37 °C in a CO₂-free incubator. Under basal conditions and after sequential injections of oligomycin (1 μM), FCCP (1 μM), and rotenone/antimycin A (0.5 μM each), OCR was measured and normalized to protein content as determined by the BCA assay. Data were analyzed using Seahorse Wave software.

Mitochondrial morphology and function detection

The shape of mitochondria was studied with a Hitachi HT7800/HT7700 transmission electron microscope. In short, A375 cells were collected, fixed in 2.5% glutaraldehyde for 2 h, and then post-fixed with 1% osmium tetroxide. The samples underwent dehydration through a graded ethanol series, were embedded in Epon 812 resin, and then cut into ultrathin sections of 60–80 nm. These sections were stained with uranyl acetate and lead citrate and viewed using the HT7800/HT7700 TEM. Images were captured to assess mitochondrial swelling, cristae integrity, and density.

Mitochondrial membrane potential (MMP) was measured with the JC-1 Mitochondrial Membrane Potential Assay Kit from Beyotime, Shanghai, China (Cat# C2006). A375 cells were collected, rinsed twice with PBS, and then incubated with a JC-1 staining solution (5 µg/mL) at 37 °C for 20 min in the dark. Afterward, the cells were washed twice with JC-1 buffer and resuspended in PBS. The fluorescence intensity was promptly assessed using a BD FACSCelesta flow cytometer, with excitation at 488 nm and emission at 530 nm for green monomers and 590 nm for red aggregates. The ratio of red to green fluorescence intensity was calculated to indicate MMP dissipation.

ATP concentrations were determined using a chemiluminescent ATP assay kit (E-BC-F300, Elabscience). All assays were performed according to the manufacturers' instructions.

Reactive Oxygen Species (ROS) detection

The Superoxide Anion Reactive Oxygen Species Assay Kit with DHE (Beyotime, Shanghai, China; Cat# S0064S) was used to measure intracellular ROS levels following the manufacturer's guidelines. A375 cells were collected, washed twice with PBS, and incubated with DHE (10 µM) in serum-free DMEM at 37 °C for 30 min without

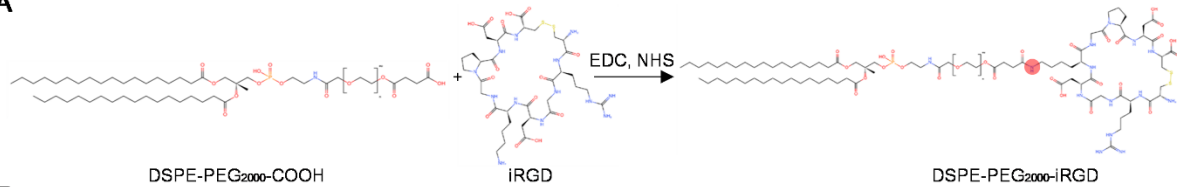
light exposure. The cells were washed twice with PBS, resuspended in PBS, and promptly analyzed using flow cytometry (BD FACSCelesta) with excitation/emission at 535/610 nm. Data were analyzed using FlowJo software (v10.8.1). ROS levels were expressed as fold change relative to the LPs control group.

Serum biochemical and cytokine analysis

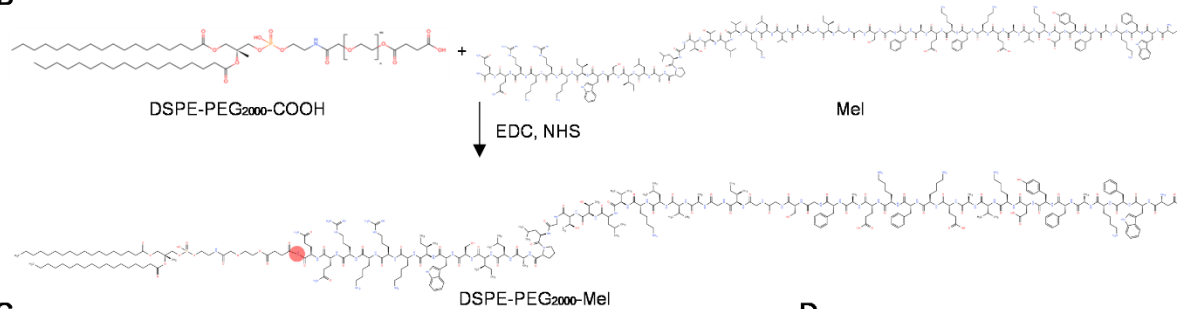
Blood was drawn from mice via cardiac puncture at the time of sacrifice. The serum was separated by spinning at 3,000 rpm for 10 min at 4 °C. Serum levels of total bilirubin (TBIL), total cholesterol (TC), alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine (CREA), and blood urea nitrogen (BUN) were determined using commercial assay kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) according to the manufacturers' instructions. The kit catalog numbers were as follows: TBIL (Cat# C019-1-1), TC (Cat# A111-1-1), ALT (Cat# C009-2-1), AST (Cat# C010-2-1), CREA (Cat# C011-2-1), and BUN (Cat# C013-2-1). Serum interleukin-6 (IL-6) and tumor necrosis factor- α (TNF- α) were quantified using enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems, Minneapolis, MN, USA; Mouse IL-6 Quantikine ELISA Kit, Cat# M6000B; Mouse TNF-alpha Quantikine ELISA Kit, Cat# MTA00B) according to the manufacturers' protocols.

Supplementary Figures

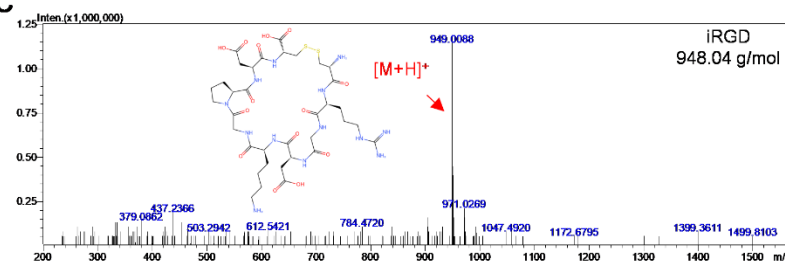
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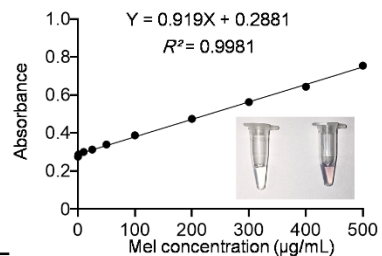
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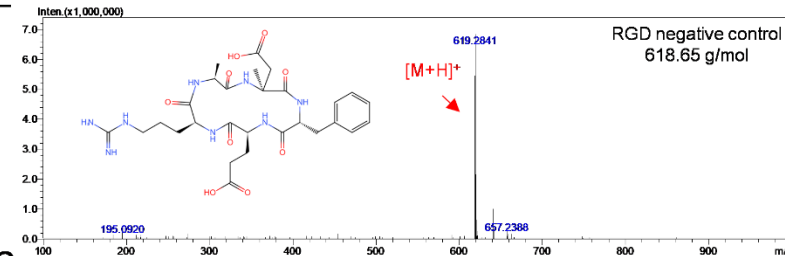
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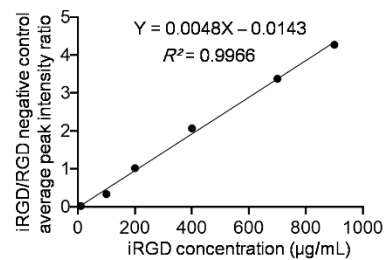
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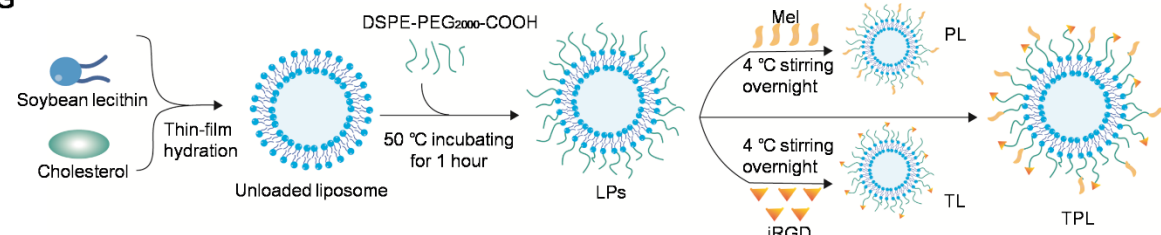
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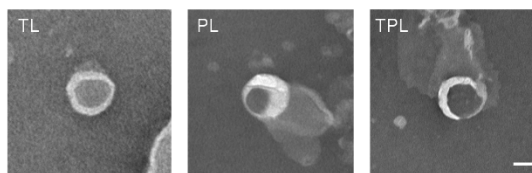
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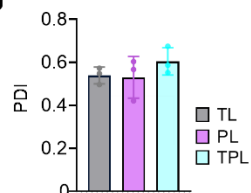
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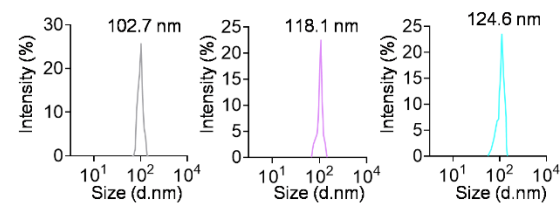
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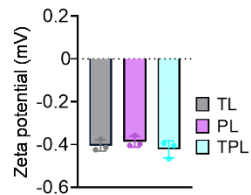


Figure S1. Schematic diagram of DSPE-PEG₂₀₀₀-iRGD and DSPE-PEG₂₀₀₀-Mel preparation. (A)

DSPE-PEG₂₀₀₀-iRGD prepared by dehydration condensation reactions between DSPE-PEG₂₀₀₀-COOH and iRGD peptides. (B) DSPE-PEG₂₀₀₀-Mel prepared by dehydration condensation reactions between DSPE-PEG₂₀₀₀-COOH and Mel peptides. (C) Representative mass spectrum of iRGD standard acquired by iMScope. (D) Standard curve of Mel for quantification by ninhydrin colorimetric assay, plotting absorbance at 570 nm against Mel concentration. (E) Representative mass spectrum of RGD negative control (internal standard) acquired by iMScope. (F) Standard curve of iRGD for quantification by iMScope. CHCA was used as the matrix. (G) Schematic workflow of the blank liposomal formulations preparation. (H) Representative TEM images of TL, PL and TPL. Scale bar, 50 nm. (I-K) Particle-size distribution, PDI and Zeta potentials of TL, PL and TPL obtained by DLS.

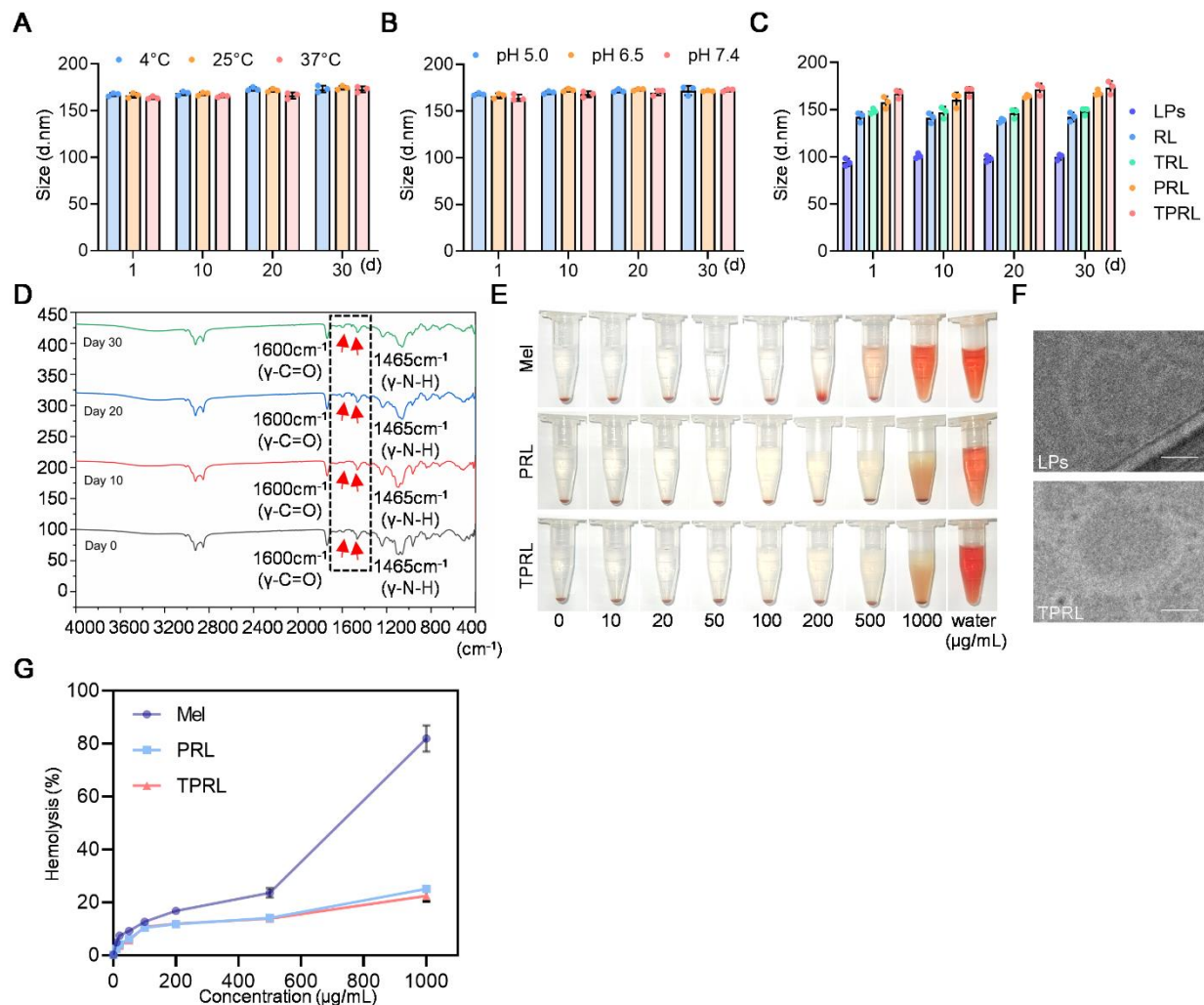


Figure S2. Stability and hemolytic activity of liposomal formulations. (A) Time-dependent changes of size of TPRL incubated at 4 °C, 25 °C and 37 °C. (B) Time-dependent changes of size of TPRL incubated at pH 5.0, pH 6.5 and pH 7.4. (C) Particle-size distribution of LPs, RL, TRL, PRL and TPRL obtained by DLS in 10% serum over 30 days. All data are presented as mean ± SEM from at least three independent experiments. (D) FTIR spectra of TPRL during 30 days of storage. (E, G) Hemolysis rates of free Mel, PRL and TPRL at indicated concentrations. (F) Representative cryo-TEM images of LPs and TPRL. Scale bar, 50 nm.

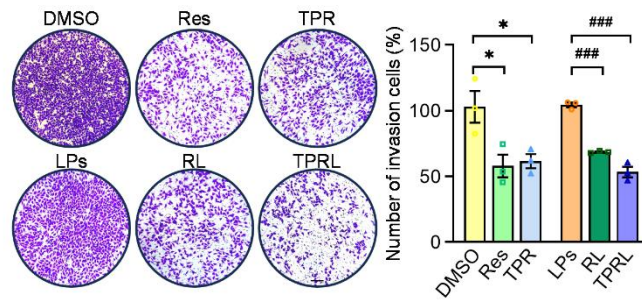


Figure S3. Transwell experiment to test the invasion ability of each group of cells. Transwell assay of A375 cells treated with DMSO, Res, TPR, LPs, RL and TPRL for 48 h. Scale bar, 100 μ m. 3 images were counted per group in each independent trial. * $p < 0.05$, versus DMSO group; ### $p < 0.001$ versus LPs group. All data are presented as mean $\pm$ SEM from at least three independent experiments.

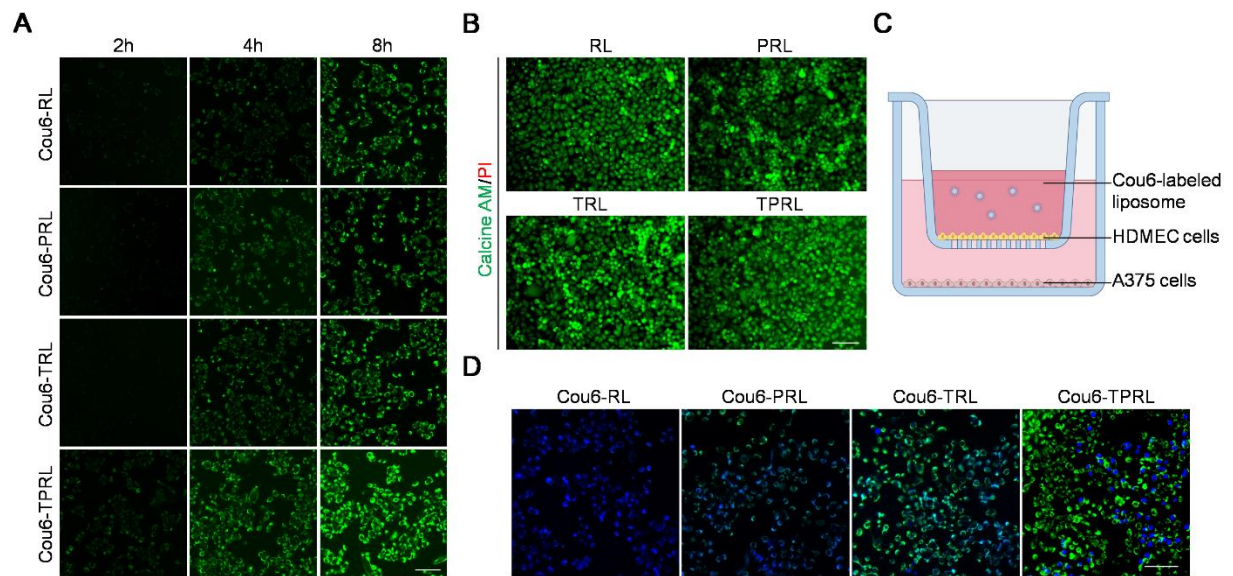


Figure S4. Uptake and in vitro BTB transcytosis of TPRL. (A) Representative images illustrating fluorescent signal intensity detected TPRL uptake into A375 cells for different times. Scale bar, 50 μ m. (B) The RL, TRL, PRL and TPRL showed no significant effect on HDMEC cell death in the BTB model within

48 h. Scale bar, 50 μ m. (C) Schematic representation of the in vitro BTB model via transwell (D) Representative images showing A375 cells following 48 h of treatment with Cou6-labeled liposomes. Scale bar, 50 μ m.

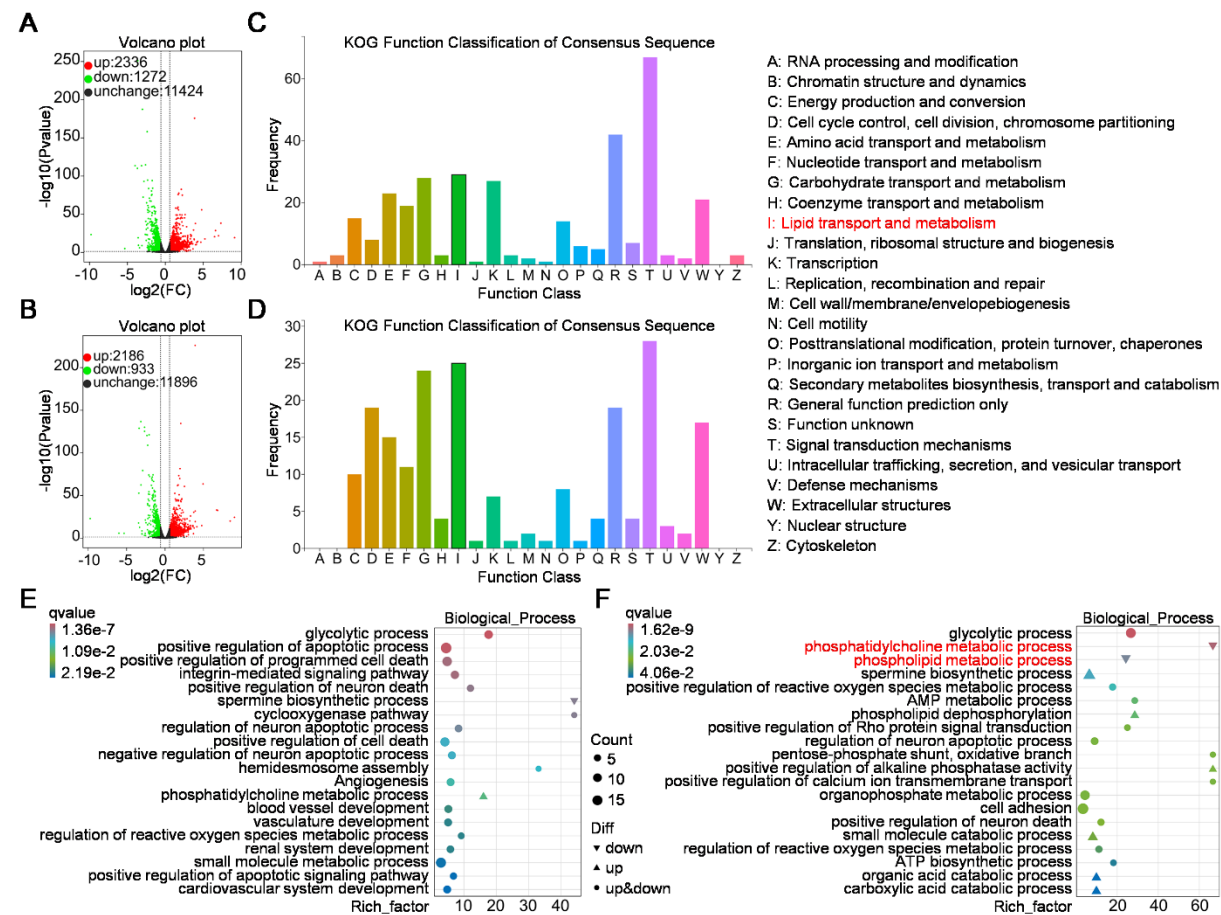


Figure S5. Differential expression analysis and gene functional annotation of mRNA sequencing

results. (A, B) DEG gene expression shown in panel volcano map including 2336 upregulated differential genes and 1272 downregulated differential genes were revealed from DMSO-treated vs. Res-treated. Besides, 2186 upregulated differential genes and 933 downregulated differential genes were revealed from LPs-treated vs. TPRL-treated. Red: up-regulated differential genes; Green: down-regulated differential

genes. (C) Orthologous classification of differentially expressed genes between the DMSO and Res groups based on KOG database. (D) KOG orthologous classification of differential gene from LPs vs. TPRL groups. The x-axis represents the KOG category, and the y-axis represents the number of transcripts. (E) Bubble plots of the top 20 biological processes enriched by the differential genes of differential gene from DMSO vs. Res groups. (F) Top 20 biological processes from LPs vs. TPRL groups. Larger bubbles indicate higher number of genes and vice versa. The color of each bubble reflects the significance as indicated (q-value). n = 3 independent biological replicates.

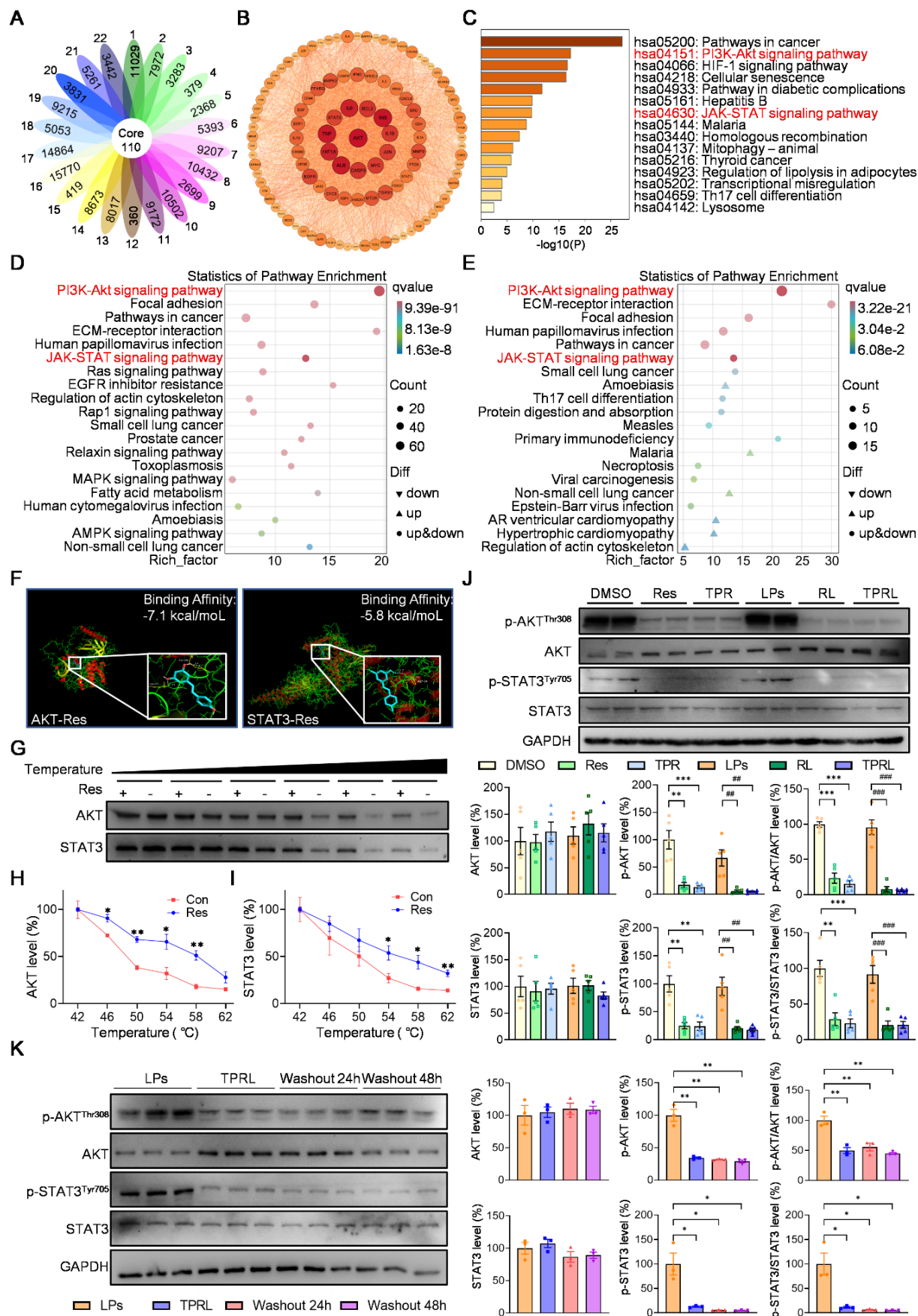


Figure S6. Potential biological mechanism prediction for TPRL in the treatment of melanoma. (A)

Petal venn diagram of related biological process targets, melanoma targets, and Res targets. Petals of different colors represent different search terms. Numbers in the petals mean the number of targets, and the white core represents the amount common to the search terms. 1: melanoma, 2: resveratrol 3: glycolytic process, 4: phosphatidylcholine metabolic process, 5: phospholipid metabolic process, 6: spermine biosynthetic process, 7: positive regulation of reactive oxygen species metabolic process, 8: AMP metabolic process, 9: phospholipid dephosphorylation, 10: positive regulation of Rho protein signal transduction, 11: regulation of neuron apoptotic process, 12: pentose-phosphate shunt, oxidative branch, 13: positive regulation of alkaline phosphatase activity, 14: positive regulation of calcium ion transmembrane transport, 15: organophosphate metabolic process, 16: cell adhesion, 17: positive regulation of neuron death, 18: small molecule catabolic process, 19: regulation of reactive oxygen species metabolic process, 20: ATP biosynthetic process, 21: organic acid catabolic process, 22: carboxylic acid catabolic process. (B) Target-pathway networks for overlapping targets. The ellipse nodes represent potential targets. The color depth and size of nodes are in proportion to their degree. (C) KEGG pathway enrichment analysis for overlapping targets. (D, E) Bubble plots of the top 20 statistics of pathway enrichment enriched by the differential genes of differential gene from DMSO vs. Res groups and LPs vs. TPRL groups. Size of bubbles indicate number of genes. (F) Images of ligand interactions between Res and AKT or STAT3. (G–I) Western blot quantified the level of AKT or STAT3 after Res treatment in CETSA (G) the melting curves of AKT (H) and STAT3 (I) were affected by temperature. * $p < 0.05$, *** $p < 0.001$. (J) Immunoblotting showing significant p-AKT and p-STAT3 reductions in A375 cells treated with DMSO, Res, TPR, LPs, RL and TPRL. * $p < 0.05$ versus DMSO group by Student's t tests; # $p < 0.05$ and ## $p < 0.01$ versus LPs group by Student's t tests. (K)

Immunoblotting showing sustained suppression of p-AKT and p-STAT3 in A375 cells treated with LPs, TPRL, TPRL followed by 24 h washout, and TPRL followed by 48 h washout. $**p < 0.005$, $***p < 0.001$. All values represent mean $\pm$ SEM and were presented in at least three independent experiments.

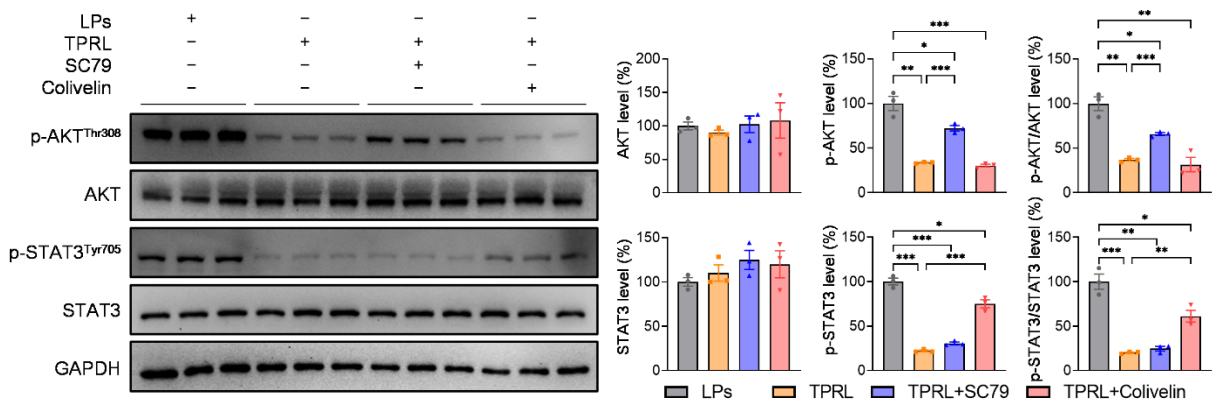


Figure S7. Independent suppression of AKT and STAT3 phosphorylation by TPRL. Immunoblotting showing no cross-regulation between AKT and STAT3 pathways in A375 cells treated with LPs, TPRL, TPRL+SC-79, and TPRL+Colivelin. Statistical significance was assessed by one-way ANOVA followed by Dunnett's post-hoc test. $**p < 0.005$, $***p < 0.001$. All values represent mean $\pm$ SEM and were presented in at least three independent experiments.

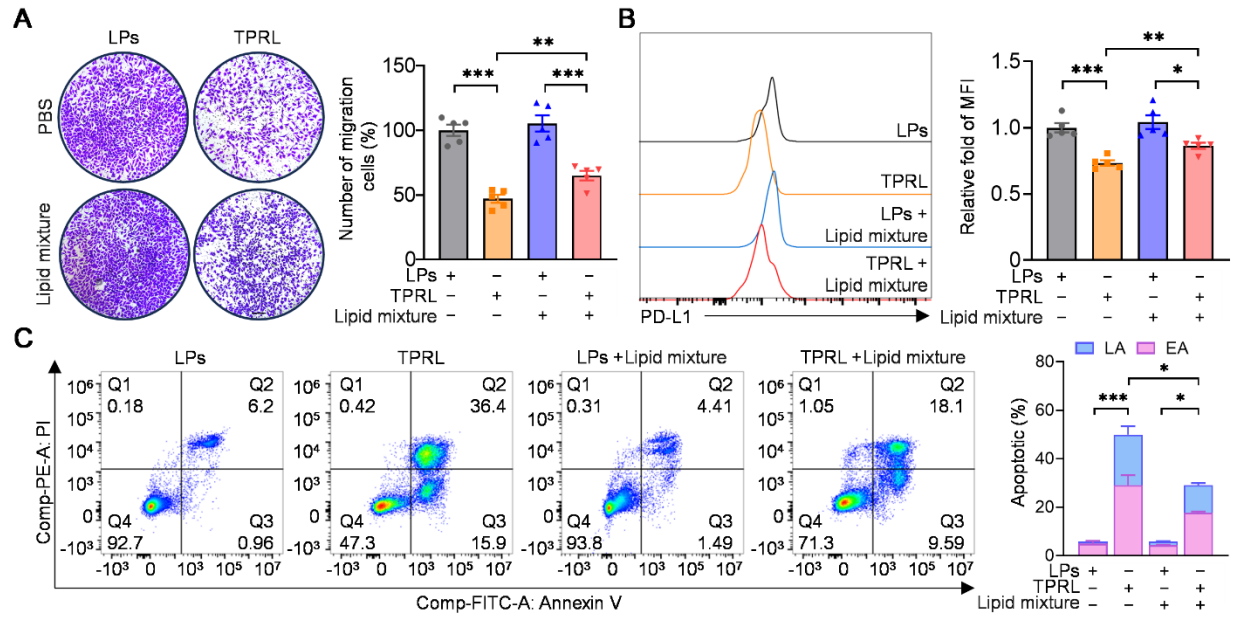


Figure S8. Lipid replenishment experiment verifies the role of lipids in the treatment of melanoma with TPRL. (A) Transwell assay of A375 cells treated with LPs, TPRL, LPs + Lipid mixture and TPRL + Lipid mixture for 48 h. Scale bar, 100 μ m. n = 5 independent biological replicates. (B) Expression of PD-L1 was analyzed by flow cytometry in A375 cells treated with various treatments and statistical analysis. n = 5 independent biological replicates and statistical analysis. (C) Annexin V-FITC and PI staining of A375 cells after treatment above. The percentages of early apoptotic (EA) and late apoptotic (LA) cells are shown. n = 5 independent biological replicates. All values represent mean $\pm$ SEM, * p < 0.05, ** p < 0.01 and *** p < 0.001.

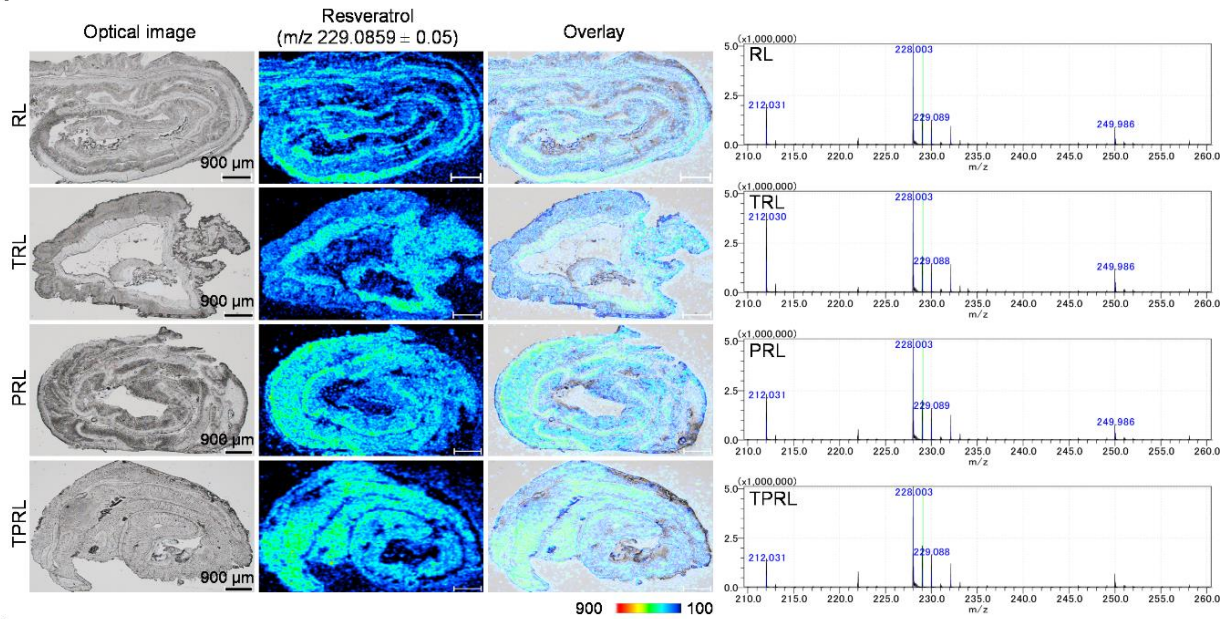
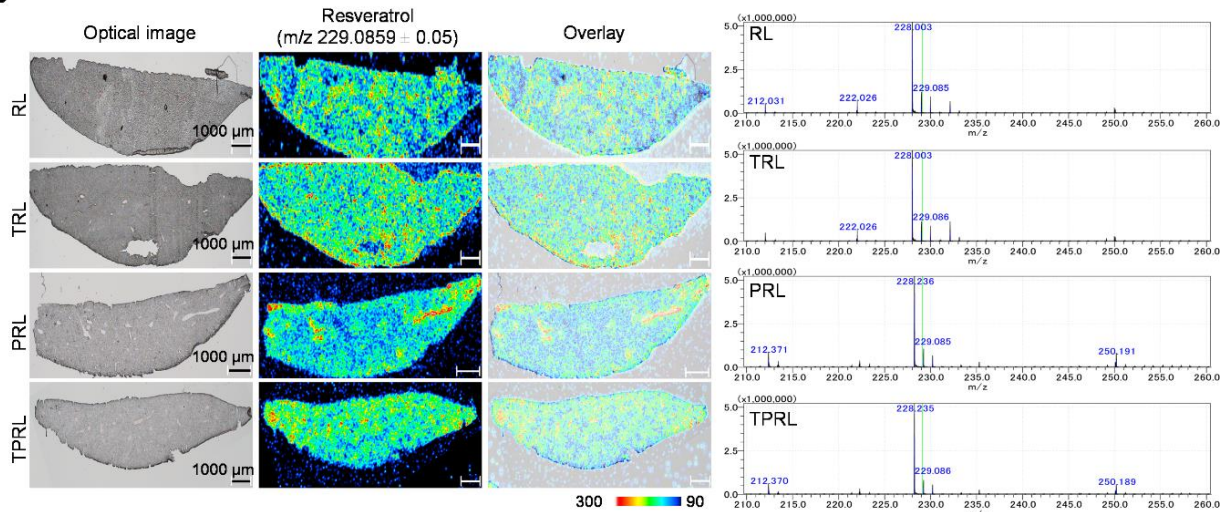
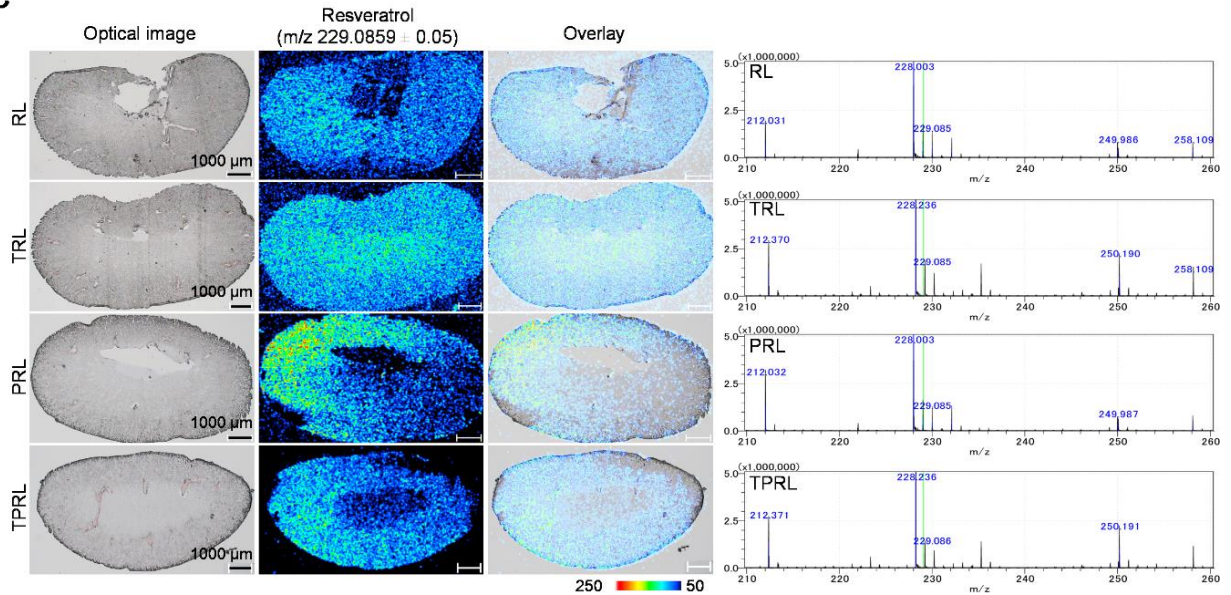
A**B****C**

Figure S9. The spatial distributions of Res within the major organs of tumorigenic mice after 30 days treatments. (A) MALDI-MSI mapping of the profiles of Res in the intestine of RL, TRL, PRL and TPRL mice. Scale bar, 900 μm . (B) MALDI-MSI mapping of the profiles of Res in the liver of RL, TRL, PRL and TPRL mice. Scale bar, 1000 μm . (C) MALDI-MSI mapping of the profiles of Res in the kidney of RL, TRL, PRL and TPRL mice. Scale bar, 1000 μm . CHCA was used as the matrix.

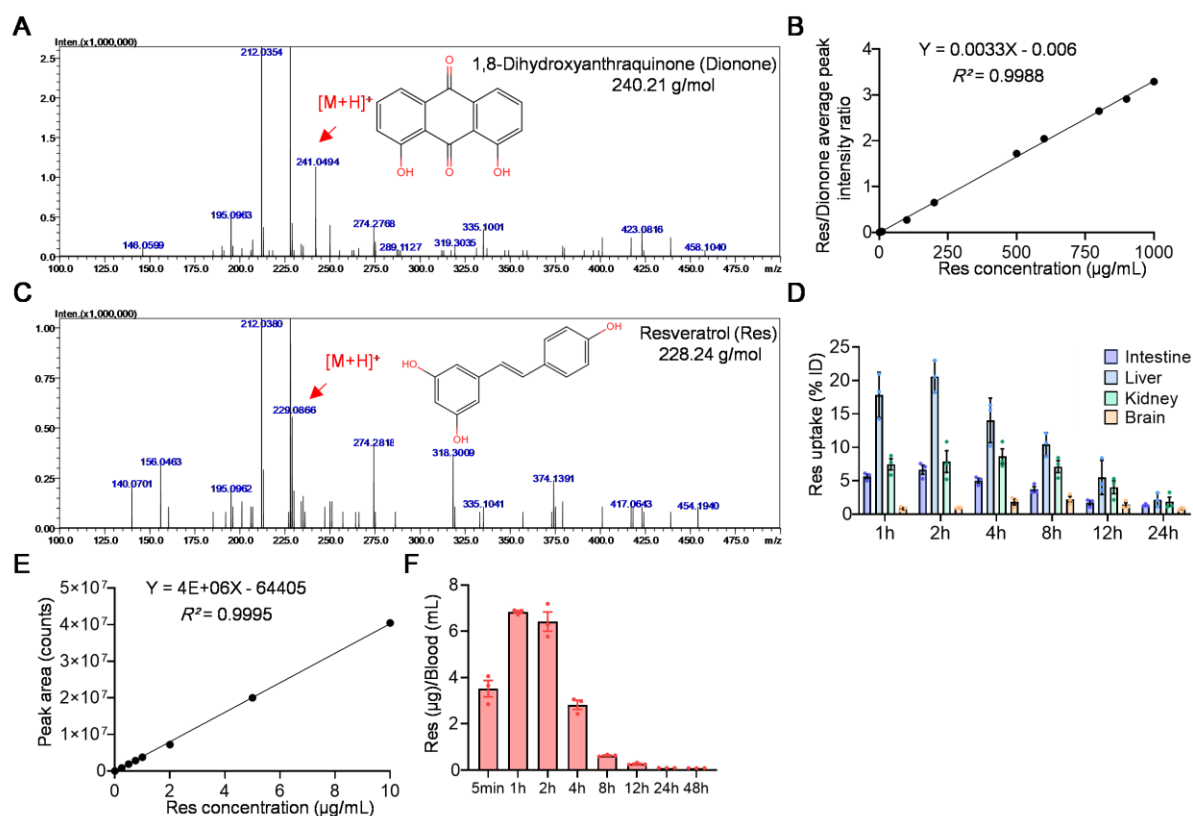


Figure S10. Pharmacokinetics and tissue distribution of TPRL. (A) Representative mass spectrum of Dionone (internal standard) acquired by iMScope. (B) Standard curve of Res for tissue quantification by iMScope. (C) Representative mass spectrum of Res standard acquired by iMScope. (D) Tissue distribution profiles of Res in liver, kidney, intestine, and brain at various time points after a single administration of

TPRL, analyzed by iMScope. Data points are mean $\pm$ SEM of 3 biological replicates. CHCA was used as the matrix. (E) Standard curve of Res for serum quantification by LC-MS. (F) Concentration of Res was measured by LC-MS. Data points are mean $\pm$ SEM of 3 biological replicates. WinNonlin 8.1 noncompartment model were utilized to obtain pharmacokinetic parameters of Res. $C_{\max}$ maximum plasma concentration, $T_{\max}$ time to reach maximum plasma concentration, $t_{1/2}$ terminal half-life, AUC_{inf} AUC from time zero to infinity.

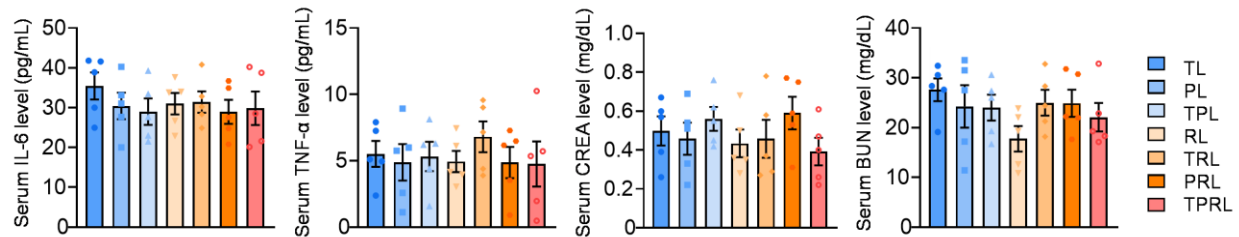


Figure S11. Serum inflammatory cytokine and biochemical parameters after long-term TPRL treatment. Serum levels of IL-6, TNF- α , creatinine (CREA), and blood urea nitrogen (BUN) in each group at the end of the study. No significant differences were observed among all groups (one-way ANOVA). Data points are mean $\pm$ SEM of 5 biological replicates.

Figure S12. TPRL could improve the disorder of lipid metabolism by inhibiting lipid synthesis in melanoma. (A) Immunohistochemical stained area of tumor tissues. Scale bar, 50 μm . p-AKT, p-STAT3, FASN, ACC, ACLY, ACSS1 and ACSL1 immunohistochemistry. TPRL treatment significantly reduced p-AKT, p-STAT3, FASN, ACC, ACLY, ACSS1 and ACSL1 expression in tumor tissues. n=5 mice. (B) Representative western blot from tumor tissues in the indicated groups. FASN, ACC, ACLY, ACSL1 and ACSS1 were measured and quantified. (C) Relative quantities of *Fasn*, *Srebp1c*, *Acly*, *Scd1*, *Atgl* and *Cpt1a* were calculated and compared between treatment groups by RT-PCR. Data presented are representative of 5 independent experiments with 5 biological replicates per group. All values represent mean $\pm$ SEM, * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$.

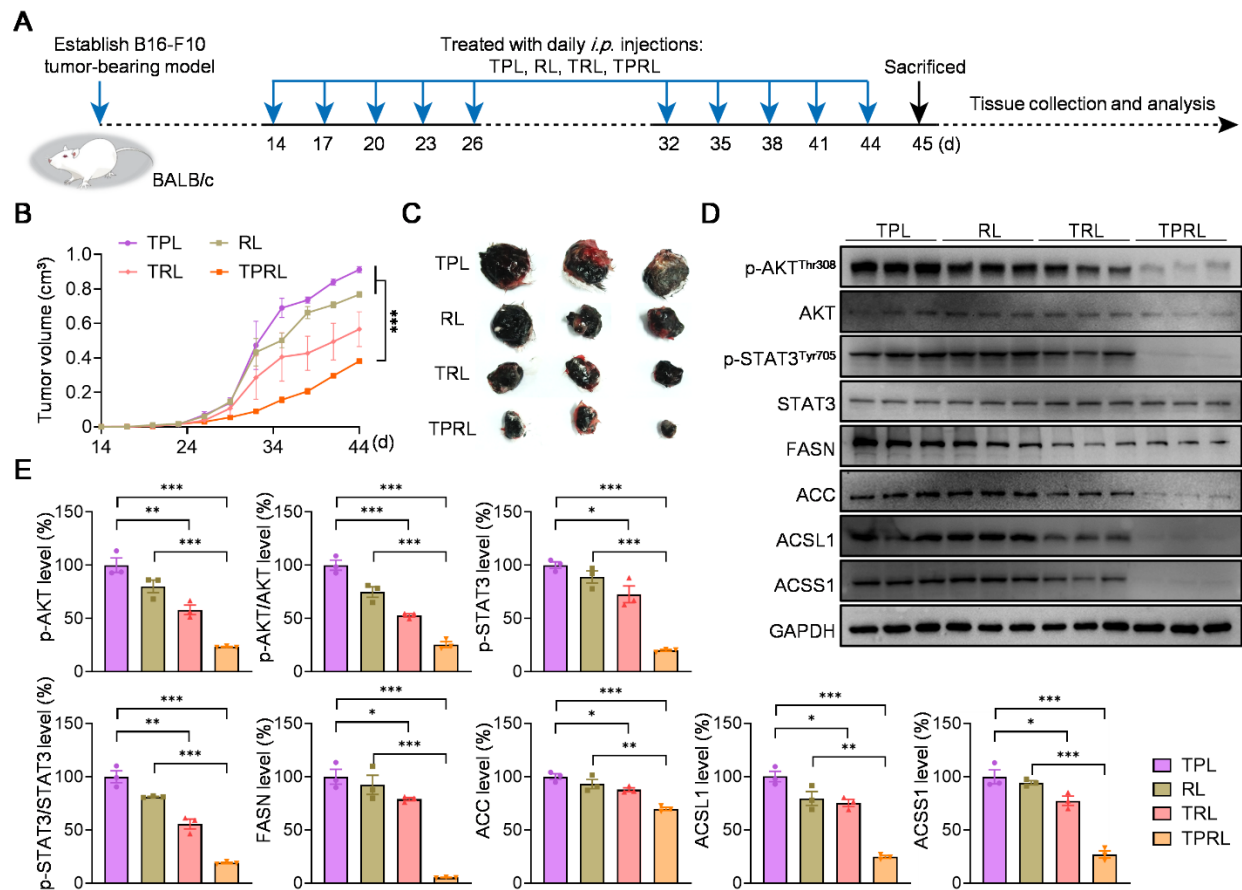


Figure S13. TPRL inhibits melanoma growth and suppresses lipogenic enzyme expression in the B16-F10 melanoma model. (A) Schematic of the experimental timeline in BALB/c mice bearing B16-F10 tumors. (B) Tumor growth curves of mice treated with RL, TPL, TRL, and TPRL. n = 3 mice. (C) Representative tumor images of each group at the end of the experiment. (D, E) Representative Western blot from tumor tissues in the indicated groups. p-AKT, AKT, p-STAT3, STAT3, FASN, ACC, ACSL1, and ACSS1 were measured and quantified. Data presented are representative of 3 independent experiments with 3 biological replicates per group. All values represent mean $\pm$ SEM, * p < 0.05, ** p < 0.01 and *** p < 0.001.

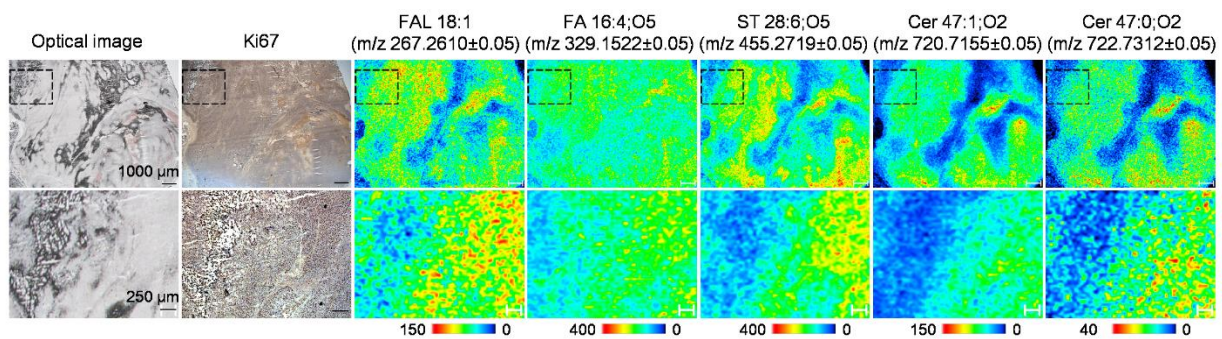


Figure S14. Spatial association between lipids and melanoma proliferation indicators.

Immunohistochemical staining was used to evaluate the Ki67 level in melanoma tissue, and MALDI-MSI was used to analyze the representative lipid levels such as FAL 18:1, FA 16:4; O5, ST 28:6; O5, Cer 47:1; O2 and Cer 47:0;O2. Scale bars, 1000 μm and 250 μm .

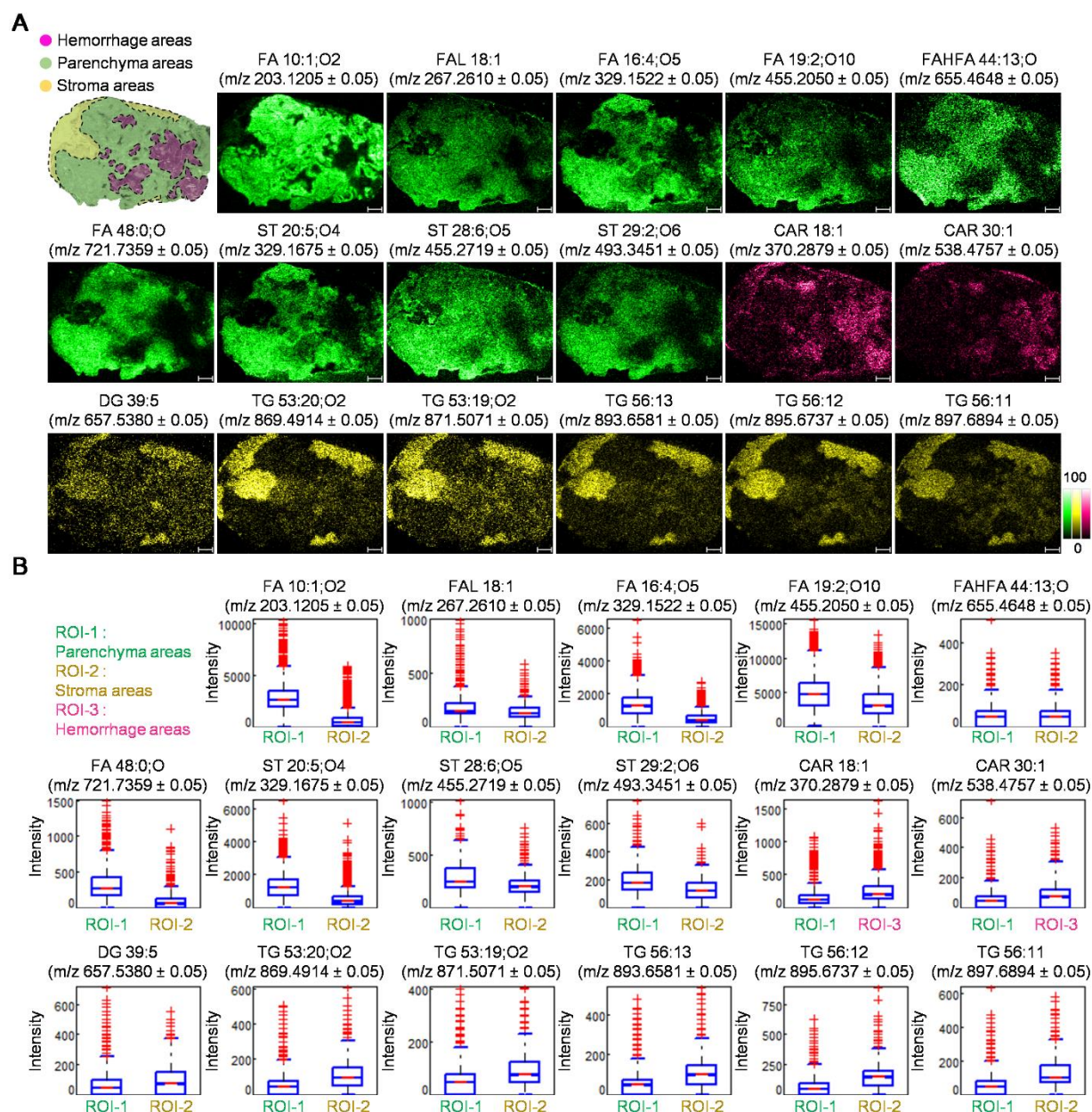


Figure S15. Spatial heterogeneity of lipid distribution in melanoma tissues analyzed by MALDI-MSI (positive ion mode). (A) MALDI-MSI mapping of the profiles of representative lipids in the tumor tissues of indicated groups. In positive ion mode, CHCA was used as the matrix. Scale bar, 1000 μ m. (B) ROI analysis showing the intensity distributions of the representative lipids among PA, SA and HA.

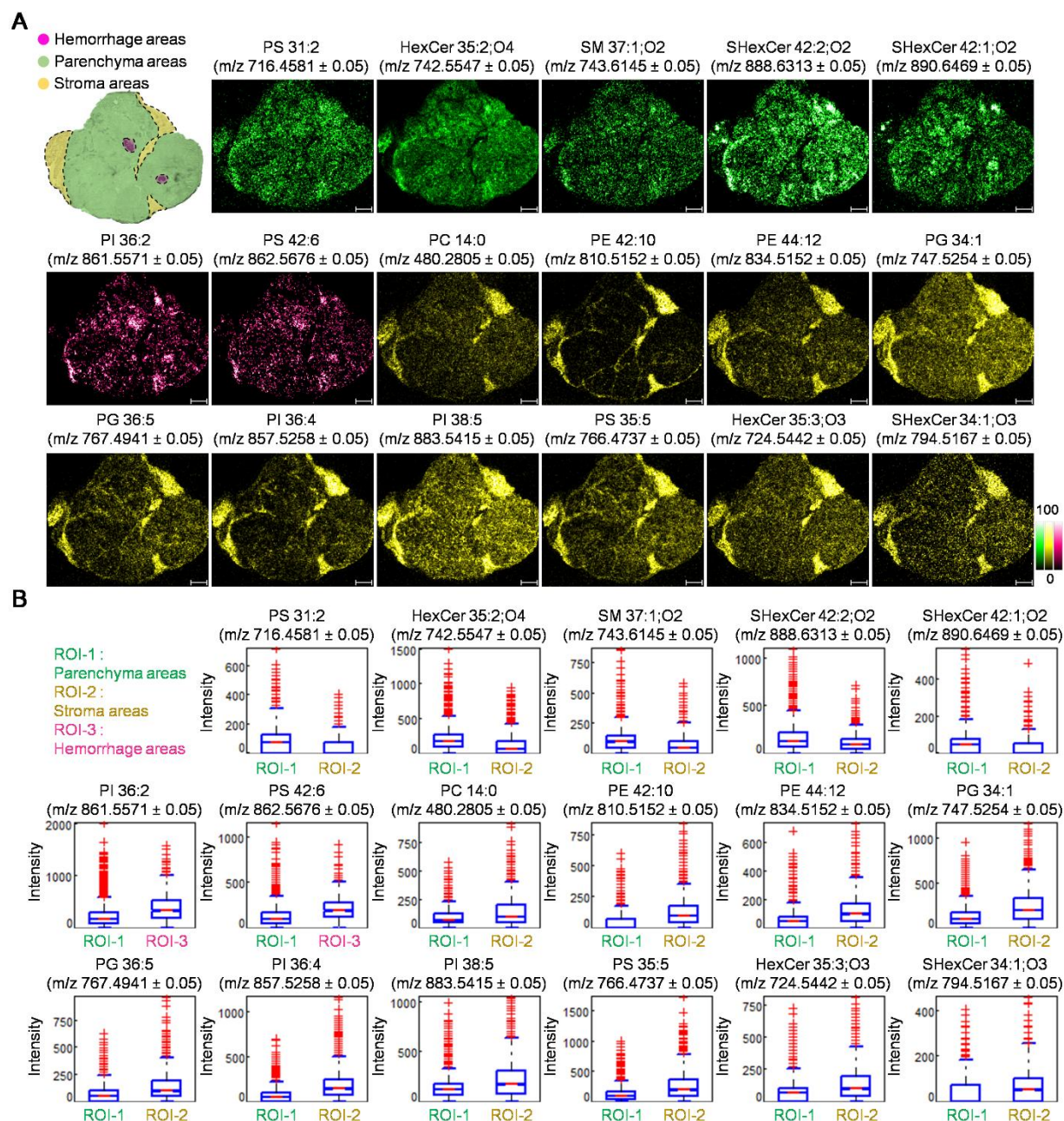


Figure S16. Spatial heterogeneity of lipid distribution in melanoma tissues analyzed by MALDI-MSI (negative ion mode). (A) MALDI-MSI mapping of the profiles of representative lipids in the tumor tissues of indicated groups. In negative ion mode, 9AA was used as the matrix. Scale bar, 1000 μ m. (B) ROI analysis showing the intensity distributions of the representative lipids among PA, SA and HA.

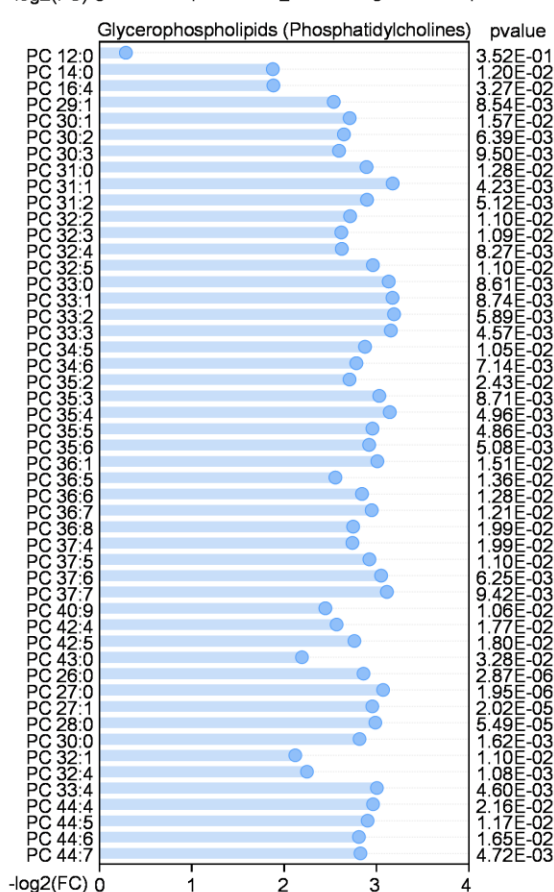
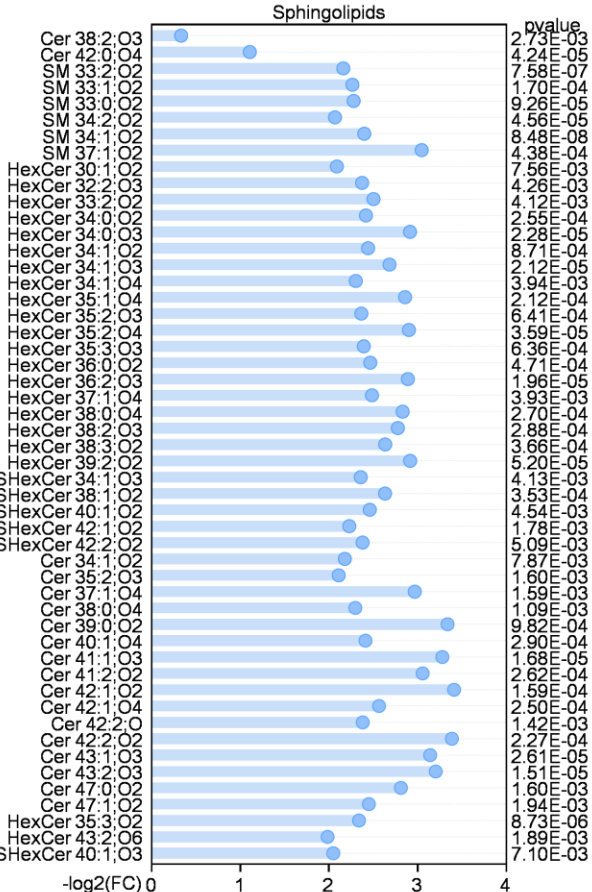
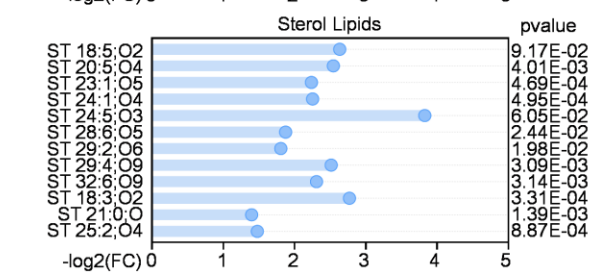
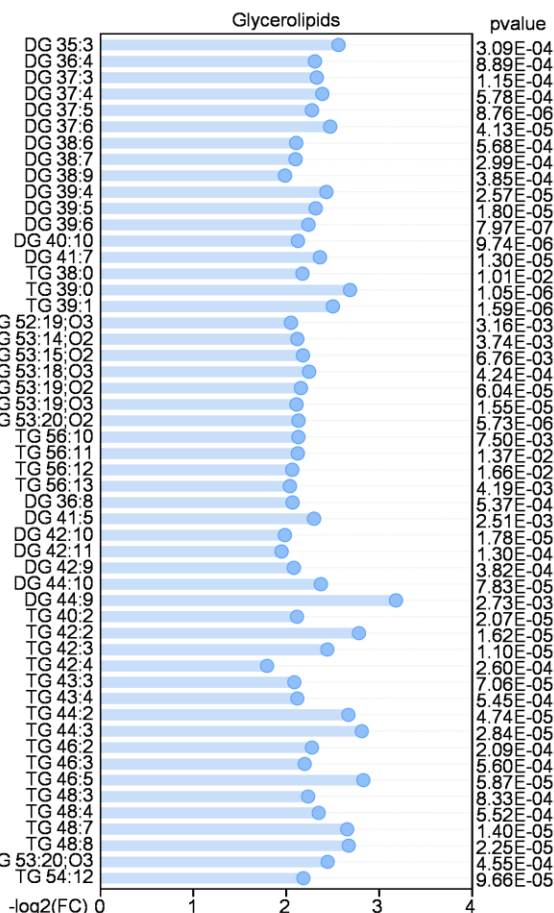
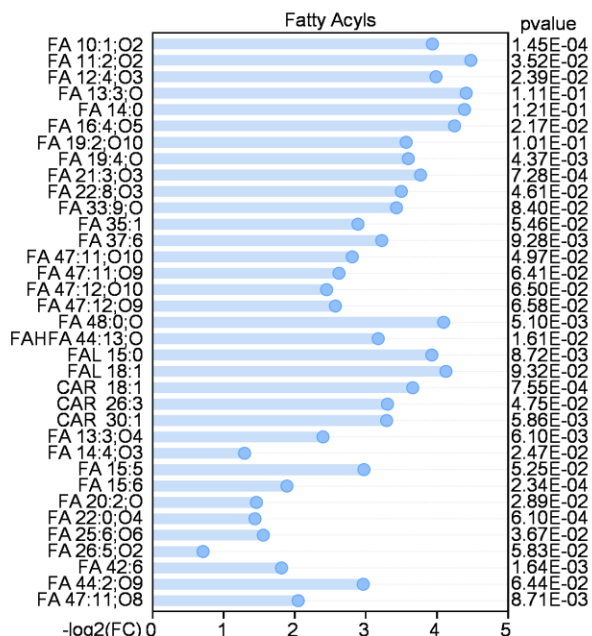


Figure S17. Differential expression of downregulated FA, ST, SP, GL and GP (PC) between TPRL and TL groups. Lollipop plots showing the log₂ fold change (log₂FC) of significantly downregulated lipids in TPRL-treated tumors compared with the TL group. Statistical significance was assessed by one-way ANOVA with post-hoc correction, and P values are indicated on the right side of each plot. n = 5 mice. Lipids are categorized into five classes: fatty acyls (FA), sterol lipids (ST), sphingolipids (SP), glycerolipids (GL), and glycerophospholipids (GP)-phosphatidylcholines (PC).

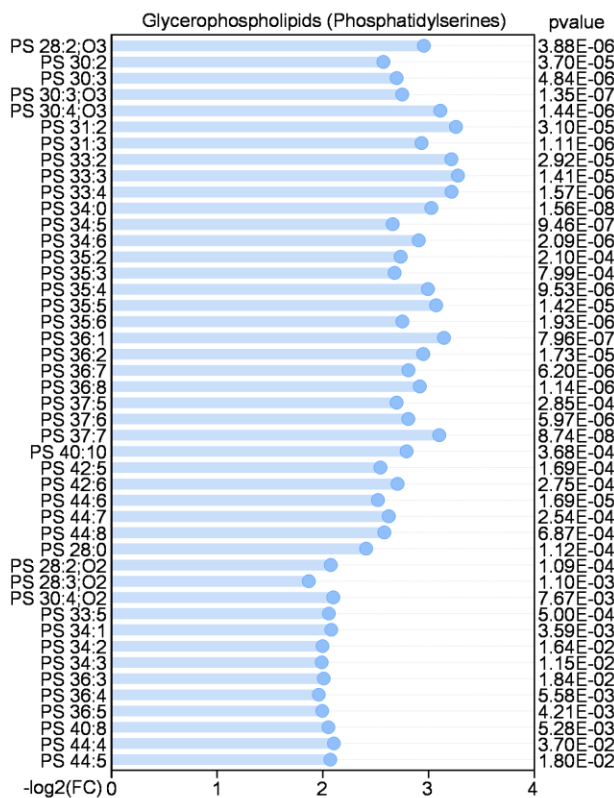
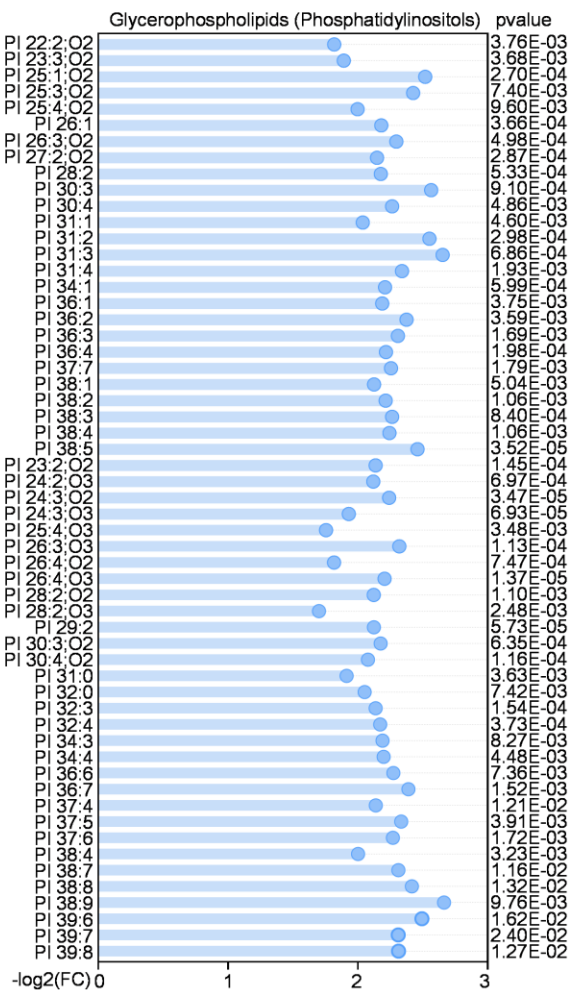
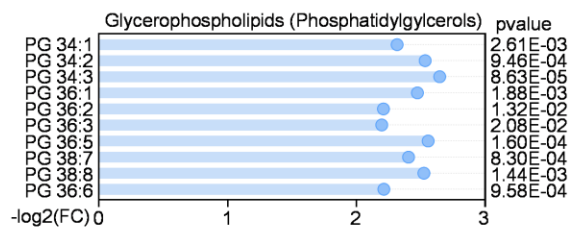
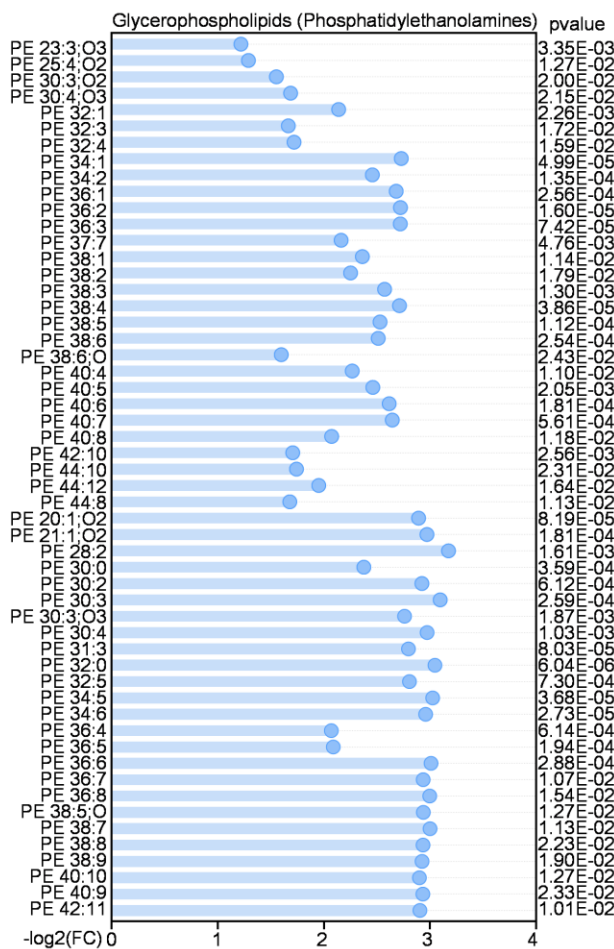


Figure S18. Differential expression of downregulated PI, PG, PE and PS between TPRL and TL groups. Lollipop plots showing the log₂ fold change (log₂FC) of significantly downregulated lipids in TPRL-treated tumors compared with the TL group. Statistical significance was assessed by one-way ANOVA with post-hoc correction, and P values are indicated on the right side of each plot. n = 5 mice. Lipids are categorized into four subclasses: phosphatidylethanolamines (PE), phosphatidylglycerols (PG), phosphatidylserines (PS), and phosphatidylinositols (PI).

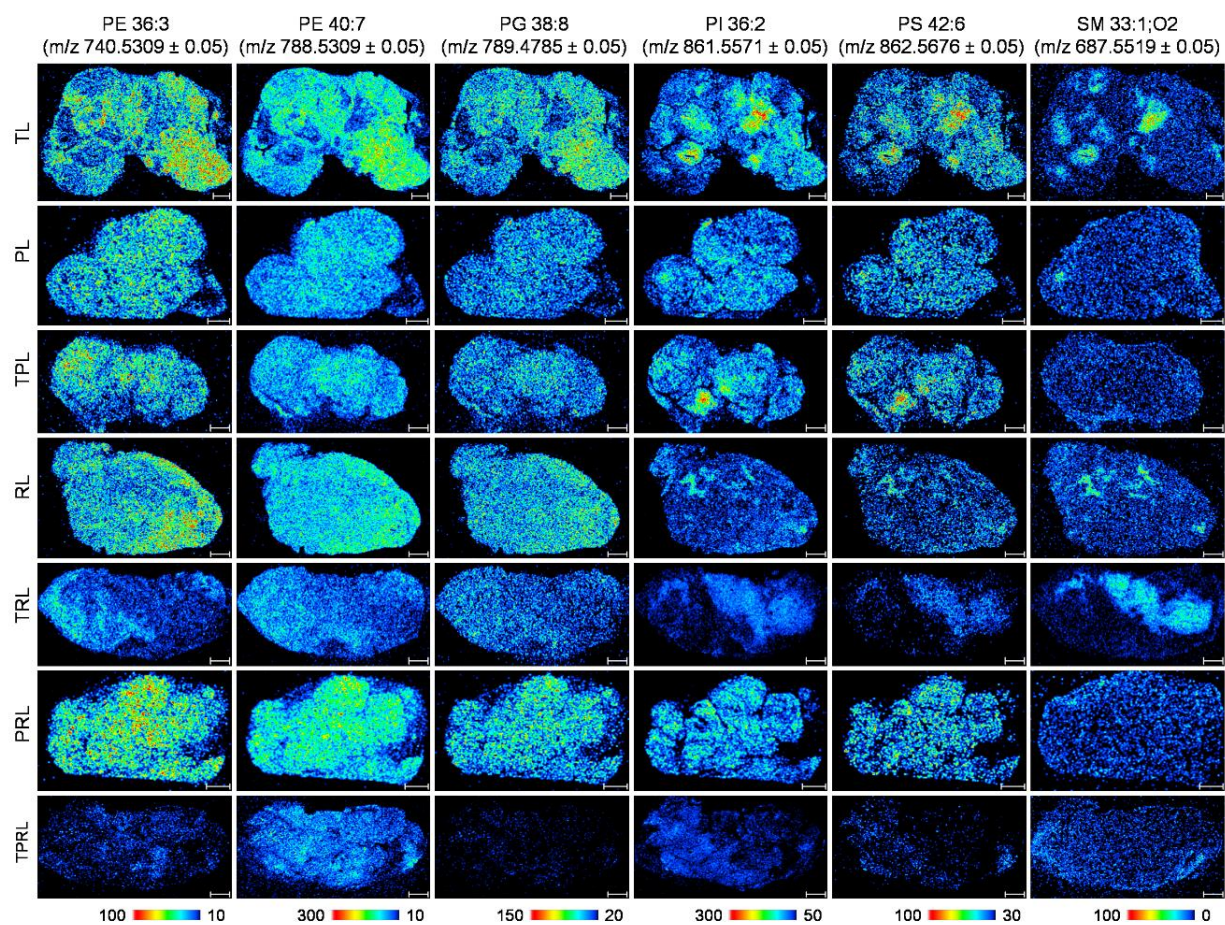
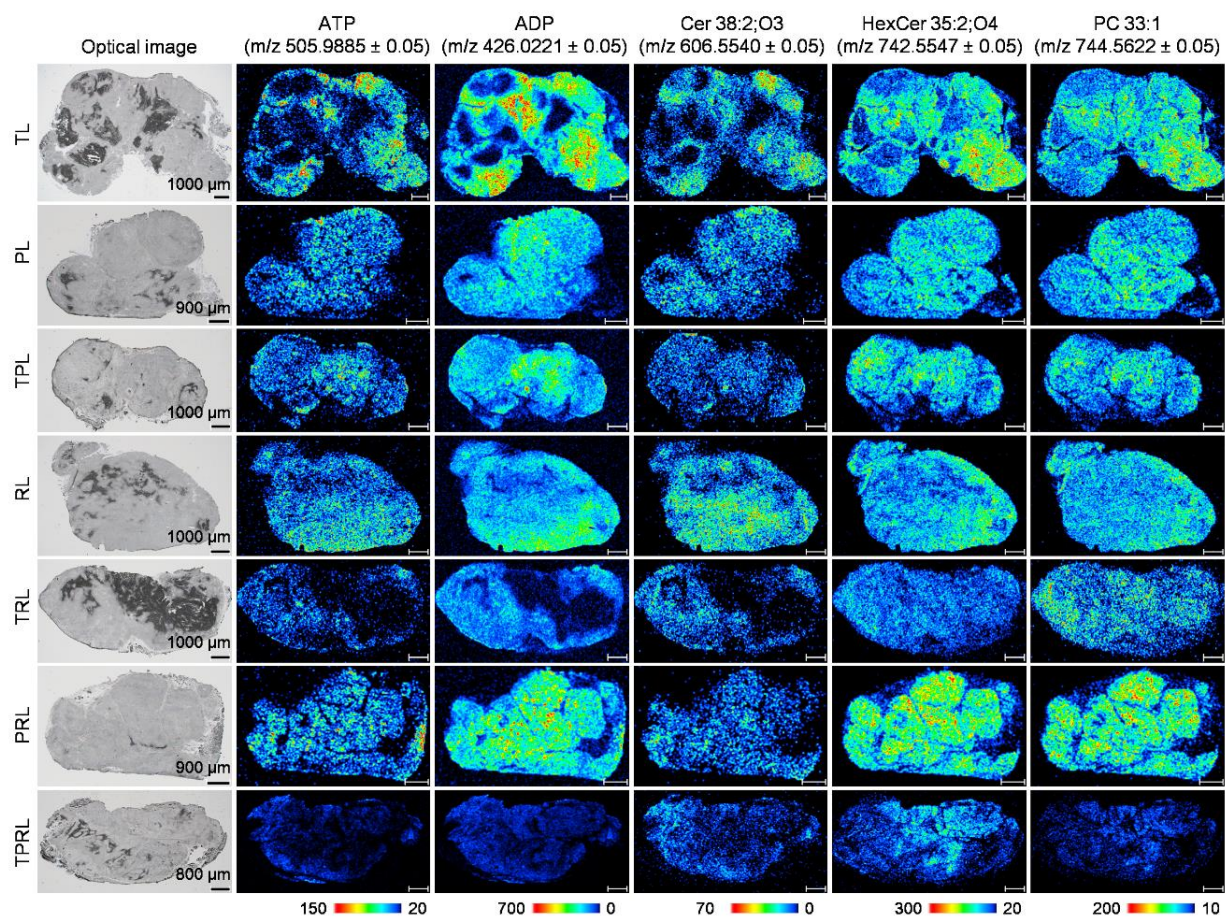


Figure S19. Comparison of the abundance of small molecules involved in energy metabolism within the tumor tissues in the indicated groups. Representative images of spatial distribution in ATP, ADP, Cer 38:2; O3, HexCer 35:2; O4, PC 33:1, PE 36:3, PE 40:7, PG 38:8, PI 36:2, PS 42:6 and SM 33:1;O2. 9AA was used as the matrix. Scale bars, TL = 1000 μ m, PL = 900 μ m, TPL = 1000 μ m, RL = 1000 μ m, TRL = 1000 μ m, PRL = 900 μ m, TPRL = 800 μ m.

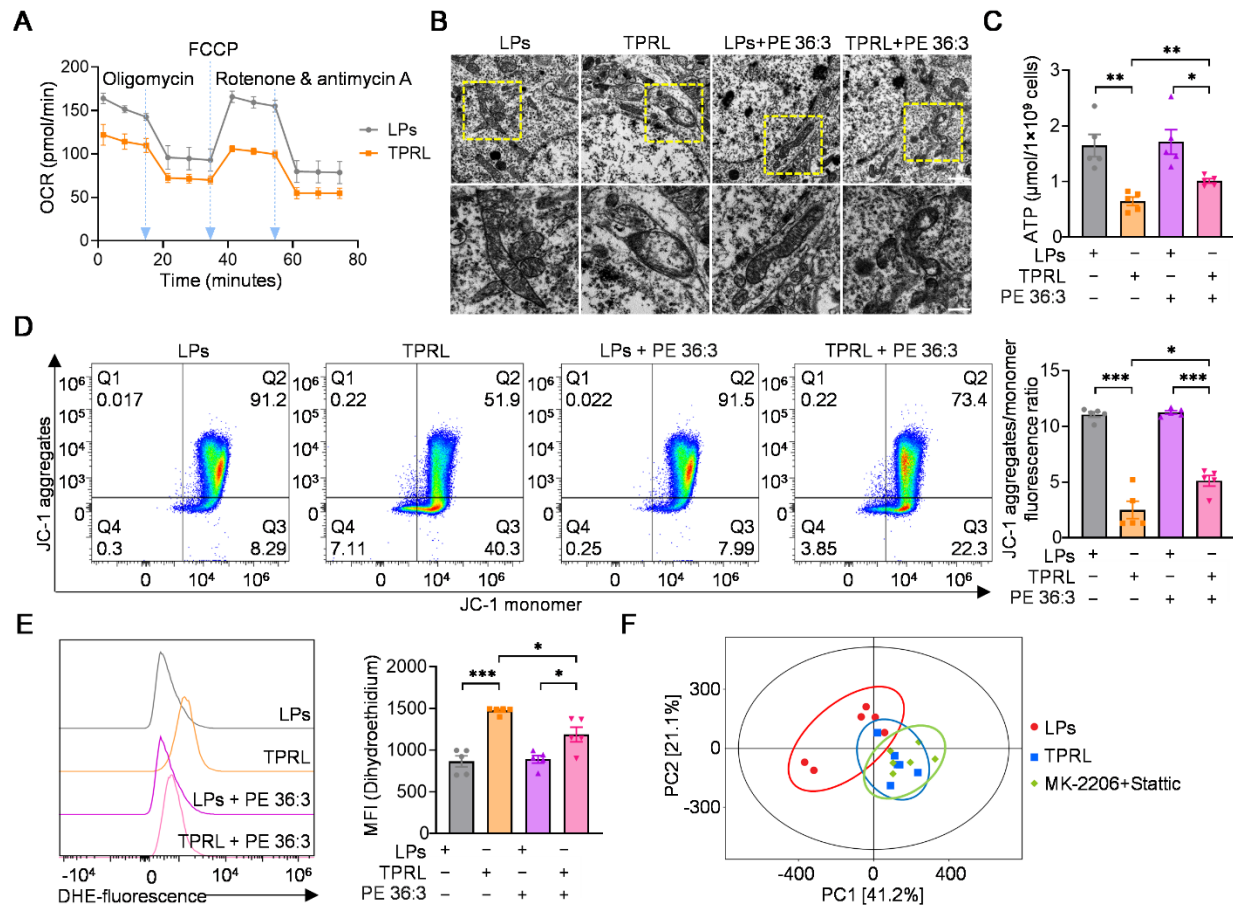


Figure S20. TPRL induces mitochondrial dysfunction and PE 36:3 partially rescues mitochondrial impairment. (A) Oxygen consumption rate (OCR) of A375 cells treated with LPs or TPRL, measured by Seahorse XF analysis. (B) Representative TEM images of mitochondria. Scale bar, 1 μ m and 500 nm. (C)

ATP levels in A375 cells treated with various treatments. n = 5 independent biological replicates. (D) JC-1 staining of A375 cells treated with LPs, TPRL, and TPRL+PE 36:3. The red/green fluorescence ratio indicates MMP dissipation. n = 5 independent biological replicates. (E) ROS levels in A375 cells treated with LPs, TPRL, and TPRL+PE 36:3, measured by flow cytometry. n = 5 independent biological replicates. (F) PCA of mitochondrial lipidomics profiles in A375 cells treated with LPs, TPRL, and combined MK-2206+Stattic. n = 6 independent biological replicates. All values represent mean $\pm$ SEM, * p < 0.05, ** p < 0.01 and *** p < 0.001.

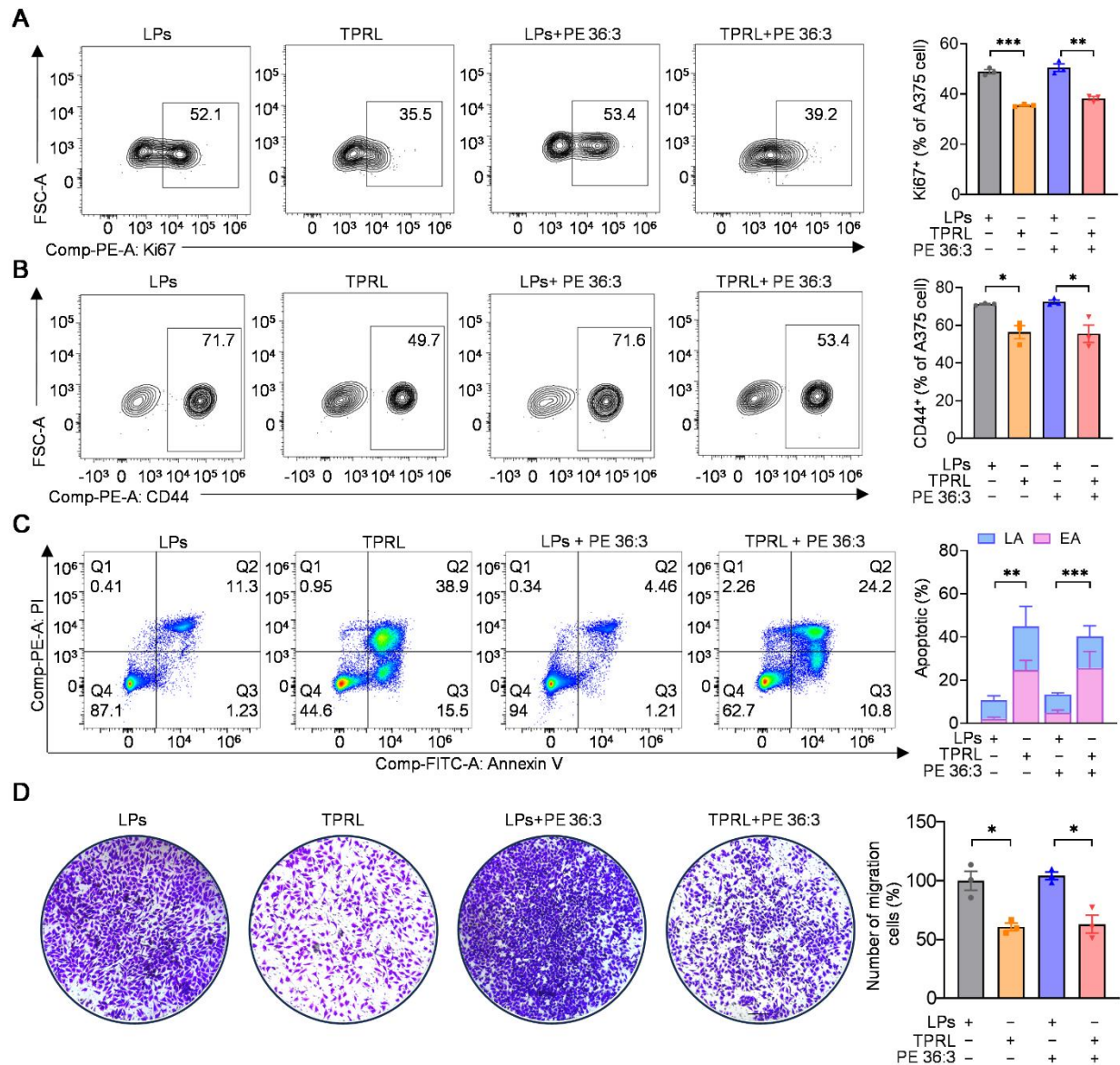


Figure S21. Functional assessment of PE 36:3 supplementation on TPRL-induced phenotypic changes. (A, B) Expression profiles of Ki67⁺ and CD44⁺ A375 cells were measured by flow cytometry and statistical analysis. $n = 3$ independent biological replicates. (C) Annexin V-FITC and PI staining of A375 cells tested by flow cytometry. $n = 3$ independent biological replicates. (D) Transwell migration assay of A375 cells treated with LPs, TPRL, LPs + PE 36:3 and TPRL+PE 36:3 for 48 h. Scale bar, 100 μm . $n = 3$ independent biological replicates. All values represent mean $\pm$ SEM, * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$.