**Supplemental Methods**

***Apoptosis measurements***

Apoptotic phenotype of Annexin V+(ANN)/Propidium Iodide (PI)- was assessed using the ANN/PI assay, as previously described[1](#_ENREF_1). ANN binds to surface phosphatidylserine and PI transverses only disrupted plasma membranes to intercalate with DNA. Thus, measurements of ANN+/PI- indicate apoptosis whereas ANN+/PI+, ANN-/PI- indicate a dead or viable cell, respectively. Analysis of the sub G1 peak was performed by assessing cell cycle as previously described[2](#_ENREF_2). Briefly, TEX cells treated for 24 hours with 10µM avocatin B were harvested, washed with cold PBS and re-suspended in PBS and cold absolute ethanol. Cells were then treated at 37ºC for 30 min with 100 ng/mL of DNase-free RNase A (Invitrogen; Carlsbad, CA), washed with cold PBS, resuspended in PBS and incubated with 50 µg/mL of propidium iodine (PI) for 15 min at room temperature in the dark. DNA content was measured by flow cytometry and analyzed with the Guava Cell Cycle software (Millipore). Caspase activation was performed using a commercially available kit (Promega) and was performed according to the manufacturer’s protocol. Z-VAD-FMK (Sigma Chemical) was used as a pan-caspase inhibitor. Cleavage of poly (ADP) ribose polymerase (PARP), a DNA repair enzyme and a common downstream target of active caspase 3&7 were measured as previously described[1](#_ENREF_1). Antibody details are found in the manuscript.

***AIF and cytochrome c detection***

To determine avocatin B’s effect on the release of pro-apoptotic mitochondrial proteins cytochrome c and AIF, we used a flow cytometry-based assay as previously described[3](#_ENREF_3),[4](#_ENREF_4). Briefly, pre-treated TEX cells (2 x105) were collected and permeabilized in ice-cold digitonin buffer (50μg/ml, 100mM KCl, in PBS) for 3-5 minutes on ice (until >95% cells were permeabilized, as assessed by trypan blue staining). Permeabilized cells were fixed in 4% paraformaldehyde (in PBS) for 20 minutes at room temperature, washed 3 times in PBS, and then resuspended in blocking buffer (0.05% saponin, 3% BSA in PBS) for 1 hour at room temperature. Cells were incubated overnight at 4°C with 1:200 cytochrome c antibody or AIF antibody (Santa Cruz Biotechnology) diluted in blocking buffer, washed three times with PBS and then incubated for 1 hour at room temperature with 1:200 Alexa Fluor-488 donkey anti-mouse IgG secondary antibody (Life Technologies) diluted in blocking buffer. Cells were washed three times in PBS and analyzed by flow cytometry using the BD FACS Calibur.

***Liquid Chromatograph/Mass Spectroscopy (LC/MS)***

A Thermo Exactive Orbitrap mass spectrometer (Thermo Scientific, San Jose, USA) equipped with an electrospray ionization (ESI) ion source was used in positive mode. The liquid chromatography system consisted of a Thermo Accela Pump equipped with an on-line vacuum degasser and a Thermo Accela Autosampler. To maintain a mass accuracy better than 2 ppm, the mass spectrometer was calibrated every day with a MSCAL5 standard solution (caffeine, tetrapeptide “Met-Arg-Phe-Ala”, ultramark 1621) for positive ion mode. Chromatographic separation of avocatin B was achieved with Discovery HS F5 column (10 cm x2.1mm, 3 µm, Supelco) using water containing 0.1% (v/v) formic acid (Solvent A) and acetonitrile (ACN) containing 0.1% (v/v) (Solvent B) at 300 µL min-1 flow rate. Gradient started at 90% A and 10% B and kept for 0.5 min at this composition, changed to 10% A and 90% B in 3.5 min and held for 3 min at this composition. Next, gradient composition was changed to initial condition (90% A and 10% B) in 0.5 min, and then held at this condition for 2.5 min before next injection. For analysis, 10.0 µL of each samples were injected in partial loop mode. Cleaning the injection system after each injection was accomplished by flushing (1.2 mL) and washing (0.3 mL) the syringe with ACN:H2O (70/30, v/v). Chromatographic data acquisition, peak integration, and quantification were performed using Xcalibur software v.2.1 (Thermo Fisher Scientific, San Jose, USA). Mobile phases degassed for 20 min in a VWR Scientific Aquasonic model 75HT (West Chester, PA, USA) ultrasonic bath before being used.

**Sample preparation: thin film solid phase microextraction (TF-SPME)**

TF-SPME was performed using a manual Concept 96 kit (Professional Analytical System (PAS) Technology, Magdala, Germany)[5](#_ENREF_5),[6](#_ENREF_6). Hydrophilic lipophilic balanced particles (HLB) was selected as extraction phase and used in TF-SPME blades with 1mm coating length. Extraction of the samples were performed in tissue culture plates (well volume of 0.39 mL) while conditioning, rinsing and desorption steps were performed in deep well plates (well volume of 1 mL). Extractions were performed from 0.2 mL sample or lysis matrix spiked with analytes (for external SPME calibration). Desorption was achieved in 0.2 mL of ACN/ isopropyl alcohol (IPA) (70/30, v/v). Each step in the TF-SPME procedure described above was carried out with agitation at 400 rpm. Details of the applied conditions at each step of SPME are shown in methods table 1.

**Methods table 1:** Summary of the TF-SPME conditions used in the study

|  |  |
| --- | --- |
| **SPME STEPS** | **CONDITIONS** |
| CONDITIONING | 0.5 ML OF ACN/H2O (50/50, V/V), 30 MIN |
| RINSING 1 | 0.5 ML OF H2O, 10 S |
| EXTRACTION | 0.2 ML OF SAMPLE, 120 MIN |
| RINSING 2 | 0.5 ML OF H2O, 10 S |
| DESORPTION | 0.2 ML OF ACN/IPA (70/30, V/V), 120 MIN |

Calibration standard solutions for LC-MS were prepared in the desorption solution in a range of 5 to 500 ng/mL, and used for calculation of the absolute amount of extracted analytes. External SPME calibration standard solutions were prepared in lysis buffer in a range of 5 to 2000 ng/mL, and used for quantification of analytes in samples. Quality control (QC) consisting of 50 ng/mL of each analyte in desorption solution was applied throughout the sequence to monitor the stability of the instrumental response.

**References**

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