Bulk cell lipid extraction

All solvents and additives used for lipid extraction were of the highest grade available (either LCMS or HPLC grade; ThermoFisher Scientific, Melbourne, VIC, Australia). Bulk cell extracts were prepared from adherent cells grown in 6 well plates. C2C12 cells were seeded at 1×10^5 cells/well and HepG2 at 2×10^5 cells/well and grown to ~70-80% confluency (~2-3 days). Cells were then treated with CON or DHA-containing media and harvested the following day (final confluency ~80-90%). Media was removed from wells and cells were washed with ice-cold phosphate-buffered saline (PBS, pH 7.4). Methanol containing 0.01% butylated hydroxytoluene (BHT; 300 µl) was added to each well, and plates were scraped into 2.0 mL eppendorfs. An additional 100 µl of methanol was used to wash each well/scraper and was combined with cell extract. An empty well was also scraped for background normalization. To the methanolic cell extracts, 920 µl of methyl tert-butyl ether was added, and samples were vortexed at 1200 rpm for 2-3 hours at room temperature (MixMate ®, Eppendorf South Pacific, Sydney, NSW Australia). Following this, 230 µl of 150 mM ammonium acetate was added and samples were vortexed vigorously for a minimum of 30 seconds. Samples were centrifuged for 5 mins at 2,000 *x* g to ensure phase separation and the upper organic phase was removed to a 2 mL glass vial. This organic phase was then diluted 500-fold in methanol:chloroform (2:1 v/v) containing 5 mM ammonium acetate and stored at -30ºC until analysis. Prior to analysis 40 µl of diluted cell lipid extract was added to a 96 well plate, which was then sealed.

FACS and lipid extraction

HepG2 and C2C12 cells were grown in 75 cm^2 flasks until ~70-80% confluent before being treated overnight with CON or DHA media. The following day cells (~80-90% confluency) were trypsinized and counted. Prostate cells (LNCaP, DU145, PC3 and PNT1) were grown to ~80-90% confluency before harvest by trypsinization followed by cell counting. 1-2 million cells of each line were centrifuged (300 *x* g, 5 min) and resuspended in PBS containing 10% FCS and 2 mM EDTA. This process was then repeated to remove all traces of growth media. Cells were placed on ice and sorted within an hour by FACS (BD FACSAria™ III, BD Biosciences, Sydney, NSW Australia) directly into a 96-well plate preloaded with methanol spiked with 0.01% BHT and internal standards (PC 17:0/17:0

and dihydrosphingomyelin, DHSM, 12:0; Avanti Polar Lipids, Alabaster, AL, USA). Internal standards were added at a rate of 1000 fmol per well for fifty cells and 1.38 fmol per single cell. Plates were sealed and stored at -80ºC until analysis. Prior to analysis methanol:chloroform containing 5 mM ammonium acetate was added to each well to achieve a final ratio of 2:1 v/v (final volume 40 µl). Wells containing solvent and internal standard only were included and used for background subtraction (*i.e.*, extraction blanks).