SUPPLEMENT MATERIAL AND METHODS

Cell lines and reagents

The human pancreatic cancer cell line PANC-1 (ATCC, Manassas, VA) was grown in Dulbecco's Modified Eagle's Medium (Thermo Fisher Scientific, MA, USA) supplemented with 10% fetal bovine serum (Thermo Fisher Scientific, MA, USA). Cells were cultured in an incubator containing a 95% humidified atmosphere with 5% CO_2 at 37 °C.

COX-2 siRNA (catalog # 4392420) was purchased from Thermo Fisher Scientific (MA, USA). Acetonitrile (ACN, HPLC Grade) and water (H_2O, HPLC Grade) were obtained from EMD chemicals (Gibbstown, NJ, USA. Glacial Acetic acid (HOAc) was acquired from Sigma-Aldrich (MO, USA). Ethyl acetate (HPLC grade) was purchased from Mallinckrodt Baker (Phillipsburg, NJ, USA). All fatty acid standards were purchased from Nu-Chek-Prep (Elysian, MN, USA). All fatty acids metabolite standards and internal standards were obtained from Cayman Chemical (Ann Arbor, MI, USA). SampliQ Silica C18 ODS reverse phase solid phase extraction (SPE) cartridge was purchased from Agilent (CA, USA). CellTiter® 96 Aqueous One Solution Reagent was purchased from Promega (Madison, WI, USA).

siRNA transfection

D5D in PANC-1 cells was knocked down by siRNA transfection as described in the material and methods. COX-2 was knocked down in BxPC-3 cells using COX-2 siRNA, and COX-2 and D5D were double knocked down in BxPC-3 cells by transfecting cells with COX-2 and D5D siRNA following the manufacturers instruction.

Detection of ω-6s and PGs from cells treated with DGLA

After transfection with D5D siRNA or NC-si, the cells were treated with DGLA (100 μM, in ethanol). At different time points, ~1 mL cell culture medium was collected and mixed with 0.45 mL methanol and 1.55 mL water to make a total of 3 mL of 15% methanol solution. Internal standards (AA-d_8, DGLA-d_6, PGE1-d_4 and PGE2-d_9) were added. The mixture was vortexed for

one min, set on ice for 30 min and centrifuged for 15 min at 3,000 rpm. Supernatant was collected and adjusted to pH 3.0. Sample solution was subjected to SPE using a reverse phase SPE cartridge. After eluted with 2 mL ethyl acetate, the elution was evaporated (Eppendorf vacuum evaporator) to dryness and reconstituted with 100 μL ethanol for LC/MS analysis.

The LC/MS system consisted of an Agilent 1200 series HPLC system and an Agilent 6300 LC/MSD SL ion trap mass system. A C18 column (Zorbax Eclipse-XDB, 4.6 × 75 mm, 3.5 μm) was used to perform LC separations with injection volume of 5 μL and flow rate at 0.8 mL/min. Mobile phases are A: H_2O-0.1% HOAc and B: ACN-0.1% HOAc. Gradient are: (1) 0-12 min (isocratic), 32% B; (2) 12-14 min, 32 to 56% B; (3) 14-28 min (isocratic), 56% B; (4) 28-30 min, 56 to 86% B; (5) 30-38 min, 86 to 95% B; and (6) 38-44 min (isocratic), 95% B. MS settings are as follows: electrospray ionization in negative mode; total ion current (TIC) chromatograms in full mass scan mode (m/z 50 to m/z 600); nebulizer press, 15 psi; dry gas flow rate, 5 L/min; dry temperature, 325 °C; compound stability, 20%; number of scans, 50. The concentrations of ω-6s and PGs were quantified by comparing the ratios of the peak areas of ω-6s and PGs with the internal standards as described elsewhere [36-37].

Western blot and MTS assay

Expression of COX-2 and D5D in cells was tested as described in the manuscript, and CellTiter® 96 Aqueous One Solution Reagent was also used to assess cell proliferation of cells upon different treatments as described in the manuscript.

Statistic analysis

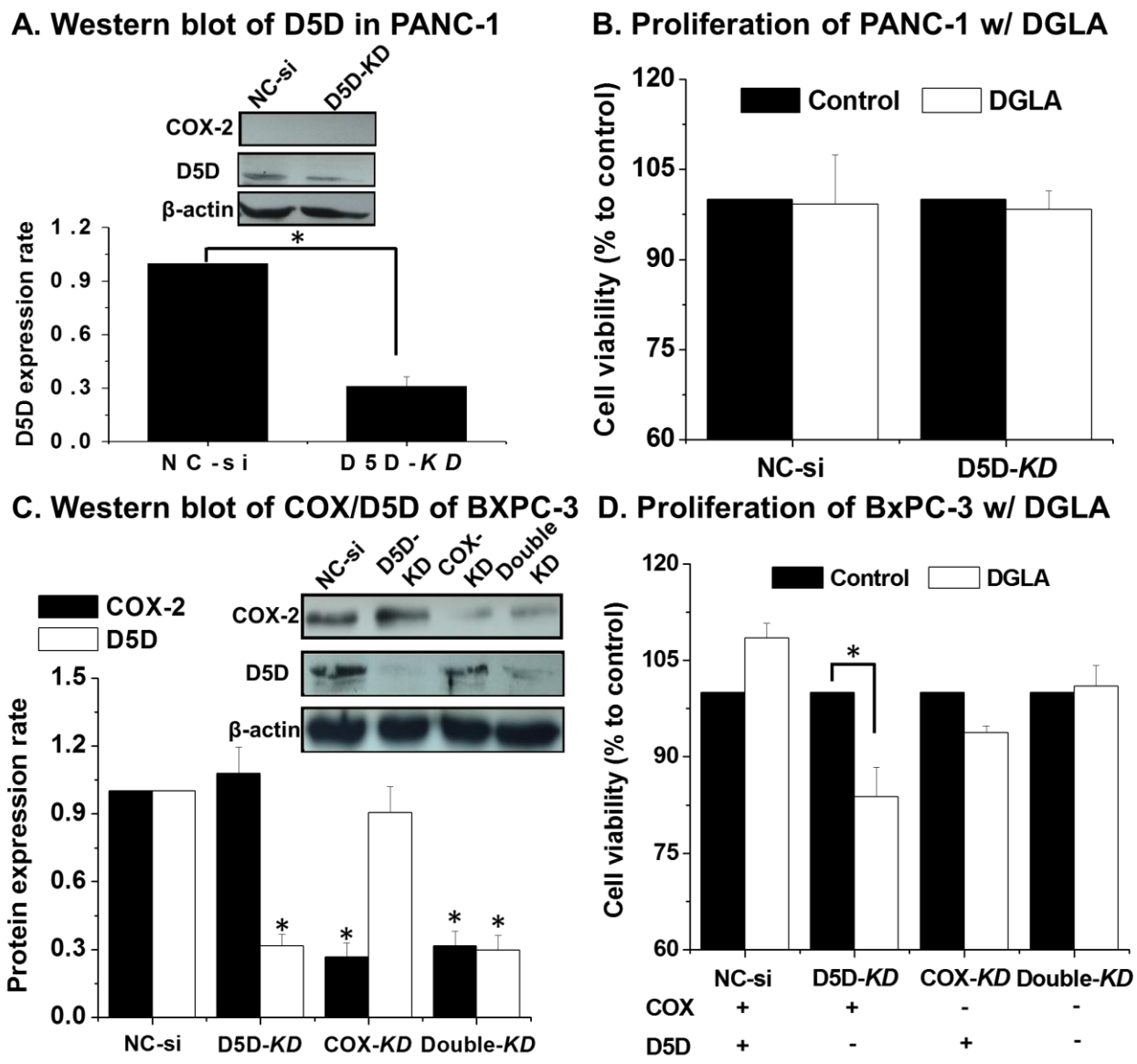
All data were assessed using an unpaired student-test with significance at $p < 0.05$.

SUPPLEMENT FIGURE LEGEND

Supplement Fig 1. D5D and COX-2 expression levels and their effects on growth of cancer cells treated with DGLA. **A)** Western blot and protein expression levels of D5D in NC-si transfected vs D5D-*KD* PANC-1 cells. Protein expression was normalized using β-actin as loading control; **B)** MTS assay of NC-si transfected and D5D-*KD* PANC-1 cells upon treatment of DGLA (100 μM). The NC-si transfected and D5D-*KD* cell lines, without DGLA treatment, were used as controls; **C)** Western blot and protein expression level of COX-2 and D5D in BxPC-3 cells with COX-2 and D5D siRNA transfection. Protein expression was normalized using β-actin as loading control; **D)** MTS assay of NC-si transfected, D5D-*KD*, COX-*KD* and double *KD* BxPC-3 cells upon treatment of 100 μM DGLA. (*: significant difference with $p < 0.05$ from more than three expriments).

Supplement Table 1. Profile of fatty acid and metabolites. LC/MS quantification of DGLA, AA, PGE1 and PGE2 from cell medium of NC-si transfected or D5D-*KD* BxPC-3 cells treated with 100 μM DGLA for 48 h. Data represent a mean ± SD from more than three expriments.

Supplement Figure 1



Supplement Table 1

	NC-si BxPC-3 (μM in 1×10^6 cells)			D5D-*KD* BxPC-3 (μM in 1×10^6 cells)		
	12 h	24 h	48 h	12 h	24 h	48 h
DGLA	8.7878 ± 1.4394	3.9447 ± 1.1972	0.3517 ± 0.5176	9.4962 ± 1.4748	6.5374 ± 1.3269	1.7351 ± 0.9868
AA	0.5462 ± 0.0943	0.3190 ± 0.1101	0.2157 ± 0.0275	0.3090 ± 0.0478	0.2101 ± 0.1331	0.1153 ± 0.0347
PGE1	0.1291 ± 0.0098	0.1534 ± 0.0144	0.2026 ± 0.0300	0.2456 ± 0.0116	0.2541 ± 0.0274	0.4245 ± 0.0142
PGE2	0.2299 ± 0.0288	0.3092 ± 0.0328	0.5685 ± 0.0683	0.2394 ± 0.0302	0.2895 ± 0.0420	0.3805 ± 0.0322