SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure 1. The effects of DHA, EPA and AA on cholangiocarcinoma cell viability. Equal numbers of human cholangiocarcinoma cells (CCLP1, SG231 and HuCCT1) (1×10^4/well) were seeded onto the 96-well plates and cultured in the medium with 10% fetal bovine serum for 24 hr. The cells were washed twice with PBS and maintained in serum-free medium for 24 hr before treatment with DHA, EPA or AA for indicated concentrations and time periods. **(A)** The time-course effect of 20 μM DHA, EPA or AA on the viability of CCLP1 cells. **(B-D)** The dose-dependent effect of DHA and EPA on cell viability at 24 hr time point in CCLP1 (B), SG231 (C) and HuCCT1 (D) cells. **(E)** Phase-contrast light microscopy showing the typical morphological changes of CCLP1 cells after treatment with 0-20 μM DHA, EPA and AA for 24 hours (x 200).

Supplementary Figure 2. The effect of DHA, EPA and AA on the growth of primary biliary epithelial cells (BECs). Normal mouse primary BECs cultured on collagen gels were treated with 5 μM or 20 μM of AA, DHA or EPA for 24 hours. The numbers of viable cells were determined by cell counts with trypan blue exclusion. (A) Average numbers of viable cells for different treatment groups. The data were obtained from three separate experiments. Similar numbers of viable cells were found among different treatment groups. (B) Phase-contrast light microscopy showing the typical morphological changes of primary BECs after treatment with 20 μM AA, DHA or EPA for 24 hours. Treatment with these fatty acids did not affect cell morphology.

Supplementary Figure 3. DHA inhibits GSK-3β phosphorylation in human cholangiocarcinoma cells. (A) DHA decreases the level of p-GSK-3β, but not p-Akt, in CCLP1 cells. The cells were treated with 60 μM of DHA in serum free medium for indicated time periods. The cell lysates were obtained for Western blot analysis using antibodies against p-GSK-3β and GSK-3β as well as p-Akt as described in the Methods. **(B)** Inhibition of GSK-3β by SB216763 prevents DHA-induced reduction of β-catenin and c-Met. CCLP1 cells were pretreated with SB216763 (5 μM) for 1 hr before DHA treatment (20 μM) for 24 hr. The cell lysates were obtained for Western blot analysis using the antibodies against β-catenin and c-Met. **(C)** Inhibition of GSK-3β by SB216763 prevents DHA-mediated reduction of TCF/LEF reporter activity. CCLP1 cells transfected with the TCF/LEF reporter construct were treated with SB216763 (5 μM) for 1 hr followed by DHA treatment (20 μM) for 24 hr. The cell lysates were obtained to determine the luciferase activity. The data are presented as mean ± SD of six independent experiments (*$p<0.01$

compared to control; **p<0.01 compared to DHA treatment). **(D)** SB216763 prevents DHA-induced caspase-9 activation and PARP cleavage in CCLP1 cells. The cells were pretreated with SB216763 (5 μM for 1 hr) before treatment with DHA (20 μM) for 24 hr. The cell lysates were obtained for Western blot analysis using antibodies against PARP and caspase-9 as described in the Methods. **(E)** SB216763 prevents DHA-induced cell death. CCLP1 cells pretreated with SB216763 (5 μM) for 1 hr were incubated with DHA for 24 hr. The cell viability was determined using WST-1 assay. The data are presented as mean ± SD of six independent experiments (*p<0.01 compared to control; **p<0.01 compared to DHA treatment).

Supplementary Figure 4. Wnt3a-conditioned medium (Wnt3a-CM) partially protects DHA-induced reduction of β-catenin activation and restored cell survival in human cholangiocarcinoma cells. (A) The effect of Wnt3a-CM on DHA-induced inhibition of TCF/LEF reporter activity. CCLP1 cells were transiently transfected with the TCF/LEF-Luc reporter vector. After transfection the cells were cultured with Wnt3a-CM (1:1 dilution with serum-free medium) or L-CM (control: 1:1 dilution with serum-free medium) containing 20 μM DHA for 24hr. The cell lysates were then obtained to determine the luciferase activity. The data are presented as mean ± SD of six independent experiments (*p<0.01 compared to control). **(B)** The effect of Wnt3a-CM on DHA-induced inhibition of cell growth. Equal numbers of CCLP1 cells (1×10^4/cell) were seeded onto the 96-well plates and cultured in the medium with 10% fetal bovine serum for 24 hr. The cells washed twice with PBS and then treated with different concentration of Wnt3a-CM or L-CM (control) containing 20 μM DHA for 24 hr (Wnt3a-CM was diluted with serum-free medium). Wnt3a-conditioned medium partially protects DHA-induced cytotoxicity in a dose-dependent manner. The data are presented as mean ± SD of 6 independent experiments.

Supplementary Figure 5. DHA induces the expression of 15-hydroxyprostaglandin dehydrogenase (15-PGDH) in human cholangiocarcinoma cells. (A) The effect of DHA versus AA on 15-PGDH expression in CCLP1 cells. The cells were treated with DHA (20 μM) or AA (20 μM) in serum-free medium for 24 hr and the cell lysates were obtained for Western blot analysis using 15-PGDH antibody. **(B-D)** Different concentrations of DHA on 15-PGDH expression in CCLP1 **(B)**, SG231 **(C)** and HuCCT1 **(D)** cells.

Supplementary Figure 6. DHA prevents PGE_2-induced GSK-β phosphorylation and dissociation from Axin in CCLP1 cells. (A) DHA prevents PGE_2-induced GSK-3β phosphorylation. CCLP1 cells were treated with PGE_2 (10 μM) or vehicle in the absence or presence of DHA (60 μM) in serum-free

medium for 1 hr. The cell lysates were obtained for SDS-PAGE and Western blotting to detect p-GSK-3β. **(B)** DHA prevents PGE_2-induced GSK-3β dissociation from Axin. CCLP1 cells were treated with PGE_2 (10 μM) or vehicle in the absence or presence of DHA (60 μM) in serum-free medium for 1 hr. The cell lysates were obtained for immunoprecipitation with anti-Axin antibody followed by immunoblotting with antibodies against GSK-3β and Axin. **(C)** DHA inhibits PGE_2-induced TCF/LEF reporter activity. CCLP1 cells were transfected with TCF/LEF-Luc reporter vector. After transfection the cells were treated with PGE_2 with or without DHA in the serum-free medium for 24 hr. The cell lysates were obtained to determine the luciferase activity. The data are presented as mean ± SD of six independent experiments. DHA treatment significantly decreased TCF/LEF reporter activity (*$p<0.01$ compared to control; **$p<0.01$ compared to PGE_2 treatment).