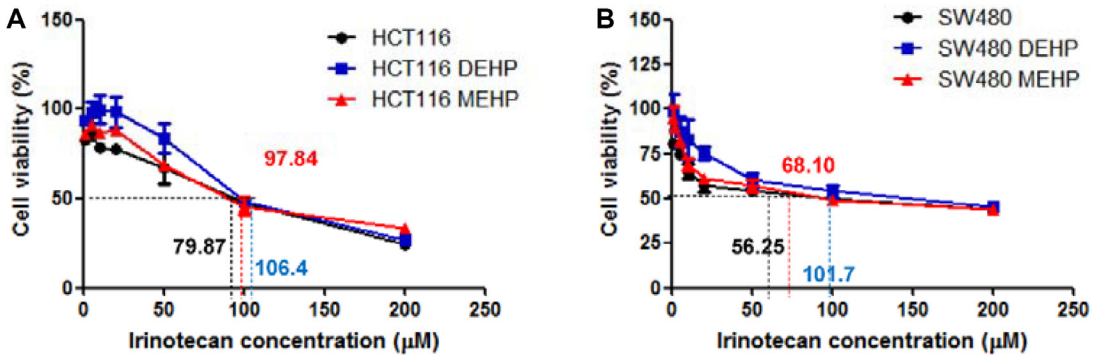
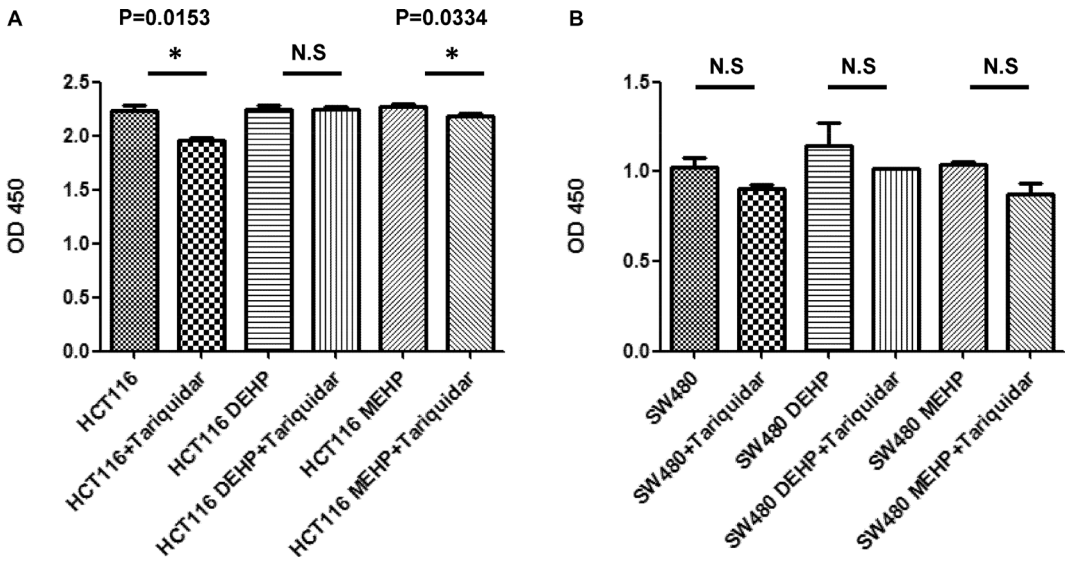
Phthalate exposure promotes chemotherapeutic drug resistance in colon cancer cells

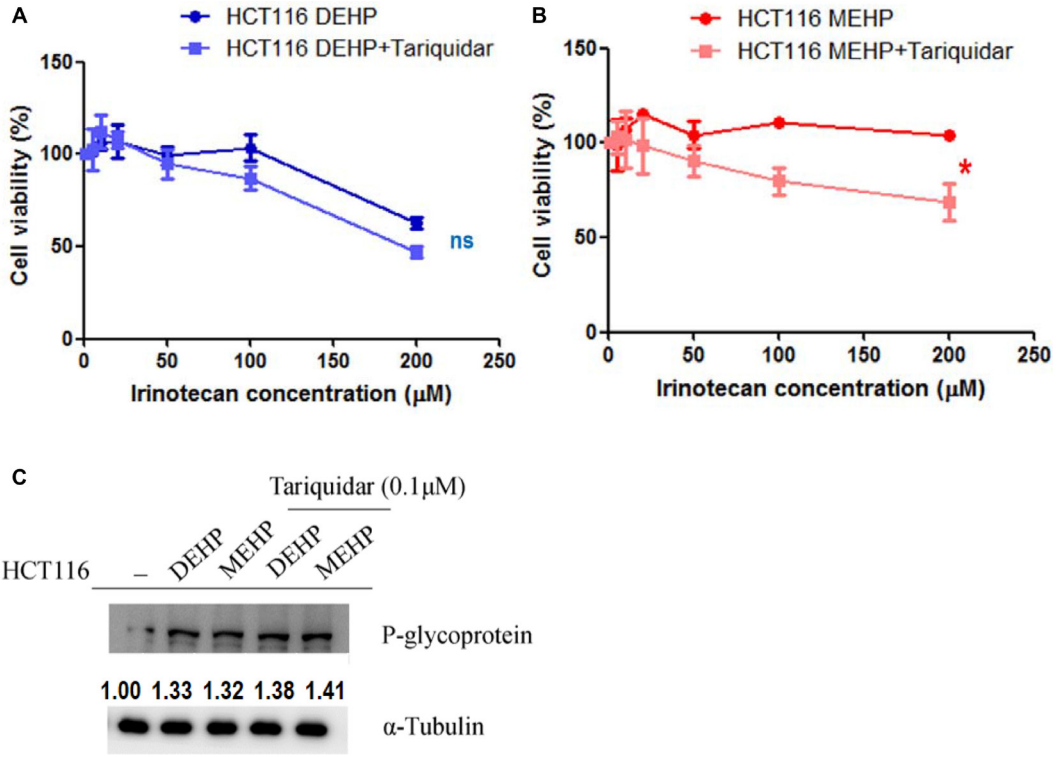
SUPPLEMENTARY MATERIALS



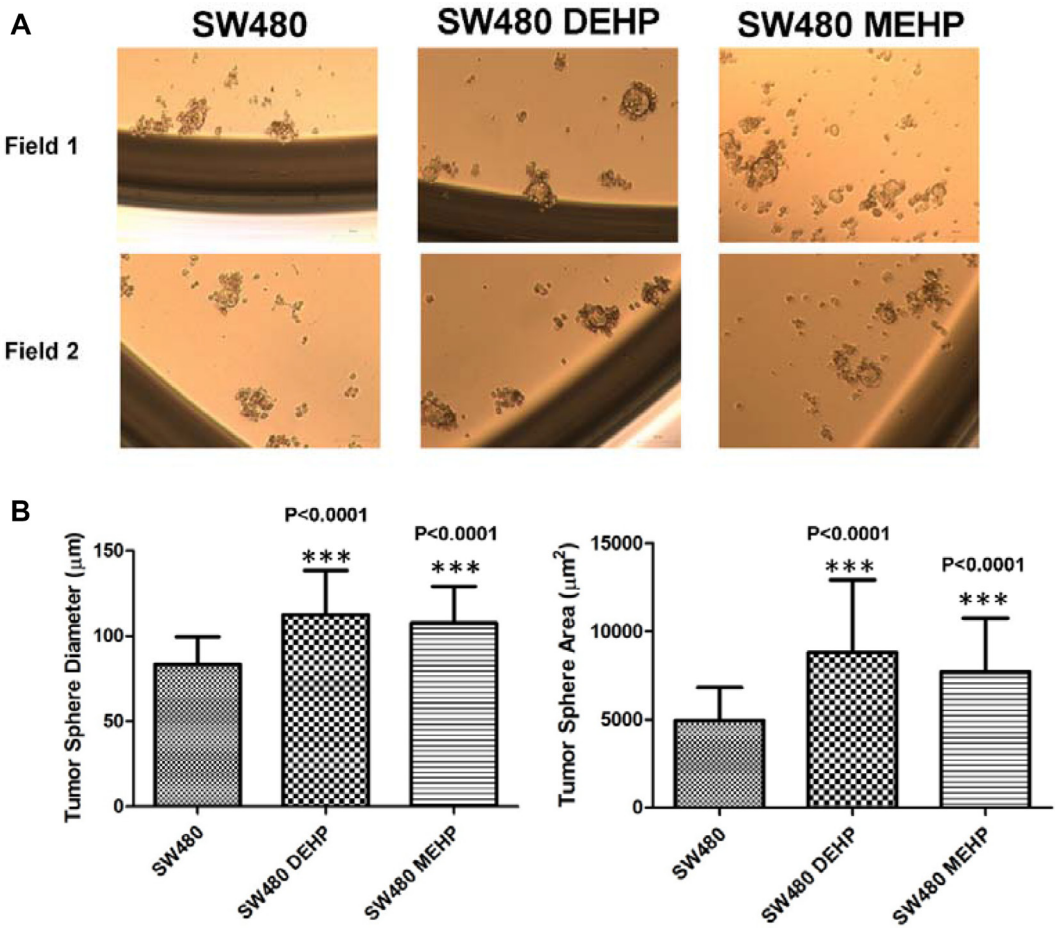
Supplementary Figure 1: The IC_{50} values of irinotecan for Untreated, DEHP and MEHP treated (**A**) HCT116 or (**B**) SW480 cells. Untreated, DEHP and MEHP treated HCT116 or SW480 cells were plated in triplicates into 96-well plates at a density of 10,000 cells/ml. After 24 h, complete culture medium was changed into fresh serum-containing medium (10% FBS) containing DMSO (control) or tariquidar and indicated doses of irinotecan (Selleckchem). Cell viability 48 h after treatment was determined by WST-1 assay (BioVision, USA) according to manufacturer's instructions. Results are expressed as percentages of control, which was assigned 100% viability, and represented as the mean ± standard deviation (SD) of the triplicate wells. The IC_{50} values of irinotecan for Untreated, DEHP and MEHP treated HCT116 are 80, 98, and 106 μM, respectively. The IC_{50} values of irinotecan for Untreated, DEHP and MEHP treated SW480 are 56, 68, and 102 μM, respectively. The data are presented as the means ± standard deviation (SD) from at least three independent experiments.



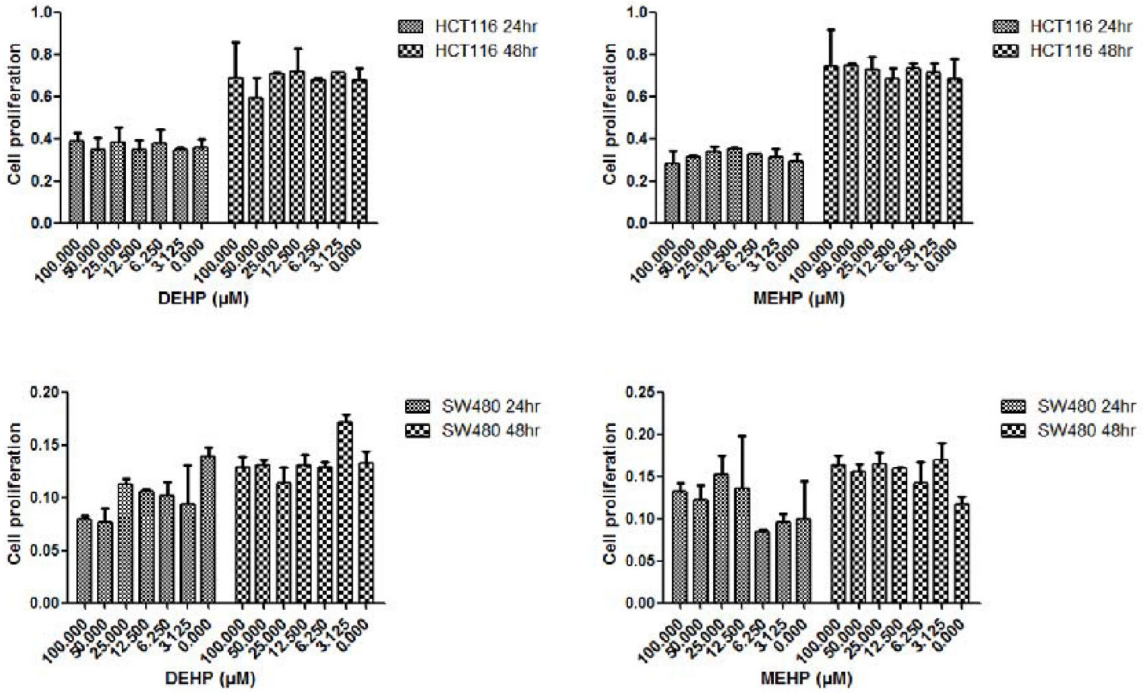
Supplementary Figure 2: Tariquidar showed low toxicity to (**A**) HCT116 or (**B**) SW480 cells. HCT116 and DEHP/ MEHP treated cells or SW480 cells and the DEHP/ MEHP treated cells were plated into 96-well plates at a density of 10,000 cells/ml. After 24 h, complete culture medium was changed into fresh serum-containing medium (10% FBS) containing DMSO (control) or tariquidar (10 μM). Cell proliferation was determined by WST-1 assay (BioVision, USA) according to manufacturer's instructions after 48 h followed by measuring the absorbance at 450 nm. The data are presented as the means ± standard deviation (SD) from at least three independent experiments. $^{*}P < 0.05$.



Supplementary Figure 3: Tariquidar treatment reduced DEHP/MEHP-induced drug resistance by blocking drug efflux in HCT116 cells. DEHP- or MEHP-treated HCT116 cells were incubated with or without tariquidar (0.1 μM) for 24 h and then challenged with irinotecan (0, 1, 5, 10, 20, 50, 100, or 200 μM) for 48 h. Tariquidar pretreatment decreased viability in irinotecan-challenged, DEHP (**A**) or MEHP (**B**) treated HCT116 cells. P-glycoprotein was not affected following tariquidar treatment (**C**) Data are presented as means ± standard deviation (SD) from at least three independent experiments. Numbers indicate densitometric analysis of protein expression levels normalized to corresponding control levels and α-tubulin (the last row).



Supplementary Figure 4: DEHP/MEHP/ treatment increases sphere formation in and DEHP/MEHP treated SW480 cells. SW480 cells, SW480 DEHP and SW480 MEHP cells (1×10^3) were cultured in serum-free medium in a low-attachment plate. After 2 weeks, the spheres sizes (diameter and area) in each well were counted under a microscope. The sphere images were captured (scale bar = 100 μm) and measured using ImageJ. The diameter and area of spheres were significantly increased in DEHP/MEHP treated SW480 cells. The data are presented as the means ± standard deviation (SD) from at least three independent experiments. $^{***}P < 0.001$, $^{###}P < 0.001$ compared with SW480 cells.



Supplementary Figure 5: Cell viability of DEHP or MEHP treated HCT116 or SW480 cells. HCT116 or SW480 cells were plated in triplicates into 96-well plates at a density of 10,000 cells/ml. After 24 h, complete culture medium was changed into fresh serum-containing medium (10% FBS) containing indicated doses of DEHP (100, 50, 25, 12.5, 6.25, 3.125 and 0 μM) or MEHP (100, 50, 25, 12.5, 6.25, 3.125 and 0 μM) and incubated for 24 and 48 h. Cell proliferation was determined by WST-1 assay (BioVision, USA) according to manufacturer's instructions. The data are presented as the means ± standard deviation (SD) from at least three independent experiments.

Supplementary Table 1: List of antibodies used for western bloting

Item	Source
P-glycoprotein	GeneTex, San Antonio, TX
CD133	Abnova Corporation, Walnut, CA
Bcl-2	GeneTex, San Antonio, TX
Bax	GeneTex, San Antonio, TX
α-Tubulin	GeneTex, San Antonio, TX
MRP2	Abcam, Cambridge, MA
pAKT	Abcam, Cambridge, MA
AKT	Abcam, Cambridge, MA
pERK	Abcam, Cambridge, MA
ERK	Abcam, Cambridge, MA
GSK-3α/β	Cell Signaling Technology, Danvers, MA
pGSK-3α/β (Ser21/9)	Cell Signaling Technology, Danvers, MA
β-catenin	GeneTex, San Antonio, TX
E-cadherin	Abcam, Cambridge, MA
N-cadherin	Abcam, Cambridge, MA
Vimentin	Abcam, Cambridge, MA
α SMA	Abcam, Cambridge, MA
Oct4	GeneTex, San Antonio, TX
Sox2	GeneTex, San Antonio, TX
Nanog	GeneTex, San Antonio, TX
Actin	Sigma-Aldrich, St. Louis, MO
HRP-conjugated goat anti-mouse IgG	Leadgene Biomedical,Taiwan
HRP-conjugated goat anti-rabbit IgG	Leadgene Biomedical,Taiwan

Supplementary Table 2: Gender and mean age of colon cancer patient among the four stages

	stage I	stage II	stage III	stage IV
Male	19	25	25	23
Female	21	14	15	17
Mean Age (SD)	66.93 (11.99)	62.26 (13.03)	62.98 (10.75)	60.80 (12.40)
Age Range	37–85	39–88	45–81	31–85