Supplementary data

Figure S1. CPT induces death in cancer cells in a concentration-dependent manner. Prostate (DU145) (A) and breast (MCF-7) (B) cancer cells grown in 100-mm dishes (2×10^6 cells/dish) were treated with indicated concentrations of CPT (0-20 μmol/L) for 72 hours, and then stained with Annexin V-FITC and PI, followed by flow cytometry analysis. Representative graphs show the apoptotic effect of CPT on DU145 (A) and MCF-7 cells (B). (C) Rh30 and DU145 cells grown in 6-well plates were exposed to CPT at indicated concentrations of CPT (0 μmol/L, as control) for 5 days. The images were taken with an Olympus inverted phase-contrast microscope (200×) equipped with the Quick Imaging system. Note: CPT induced shrinkage and rounding of the cells in a concentration-dependent manner.

Figure S2. Z-VAD-FMK fails to attenuate the cytotoxicity of CPT significantly. Rh30 cells were pretreated for 1 hour with 10 μmol/L Z-VAD-FMK, a cell-permeant pan-caspase inhibitor, and then exposed to CPT at indicated concentrations for 48 hours, followed by cell viability assay using one solution reagent. Results are presented as mean ± SE ($n = 3$). [a]$P < 0.05$, difference versus 0 μmol/L CPT group. Note: Z-VAD-FMK did not significantly protect Rh30 cells from CPT inhibition of cell viability.

Figure S3. CPT neither alters the cellular protein level of AIF nor induces a translocation of AIF from cytoplasm to nucleus in Rh30 cells. Rh30 cells were exposed to CPT at indicated concentrations for 24 hours, followed by western blotting using indicated

antibodies (Upper panel), or AIF immunostaining (Green) and DAPI staining (Blue, here pseudo-colored to red) (Bottom panel). Note: c-Jun was used for loading control (Upper panel).

Figure S4. CPT downregulates cellular protein expression of anti-apoptotic proteins, but does not affect expression of pro-apoptotic proteins in Rh30 cells. Rh30 cells were exposed to CPT at indicated concentrations for 24 hours, followed by western blotting using indicated antibodies. β-Tubulin was used for loading control.

Figure S5. CPT induction of ROS does not affect expression of cyclin D1 expression. Rh30 cells were pretreated with or without 5 mmol/L N-acetyl-L-cysteine (NAC) for 30 minutes and then exposed to CPT at indicated concentrations for 24 hours, followed by western blotting using indicated antibodies.