Figure S1. Effects of CPT on the three-dimensional spheroid formation of human T24 and J82 bladder cancer cells. CPT, cryptotanshinone. Scale bar, 200 μm.

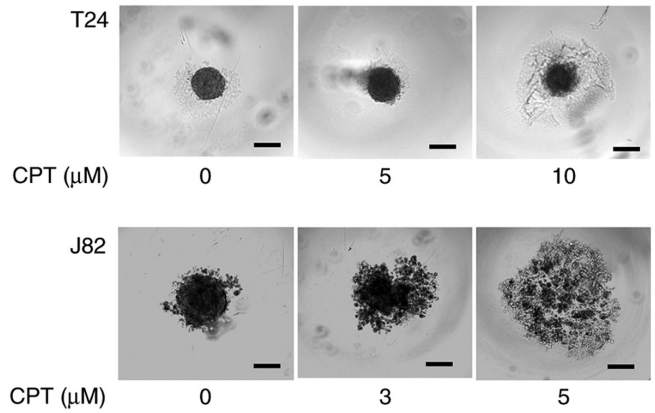

Figure S2. Effects of CPT on ATP and caspase-3/7 activities of T24 and J82 cell spheroids. Apoptosis was measured by determining caspase-3/7 activity using the Caspase-GLO® 3/7 Assay. (A) ATP activity, (B) caspase-3/7 and (C) caspase-3/7/ATP activity ratio. Bars indicate the mean ± SD. *P<0.01 and **P<0.001 by Welch's t-test. CPT, cryptotanshinone; ATP, adenosine triphosphate.

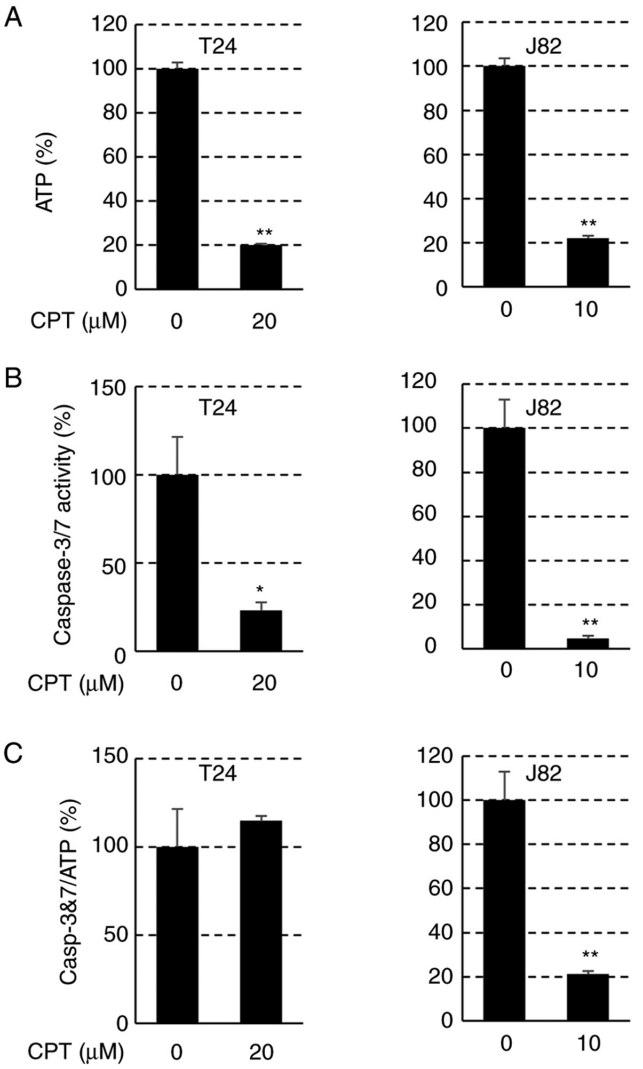

Figure S3. Cell cycle analysis following CPT treatment. Representative histograms of the gated cells. T24 (upper) and J82 (lower) cells were treated with 0 (control, left), 10 (middle) and 20 (right) μM CPT for 24 h, respectively. Apoptotic sub-G1 proportion was calculated using ModFit 3.2 software and is represented in each histogram. CPT, cryptotanshinone.

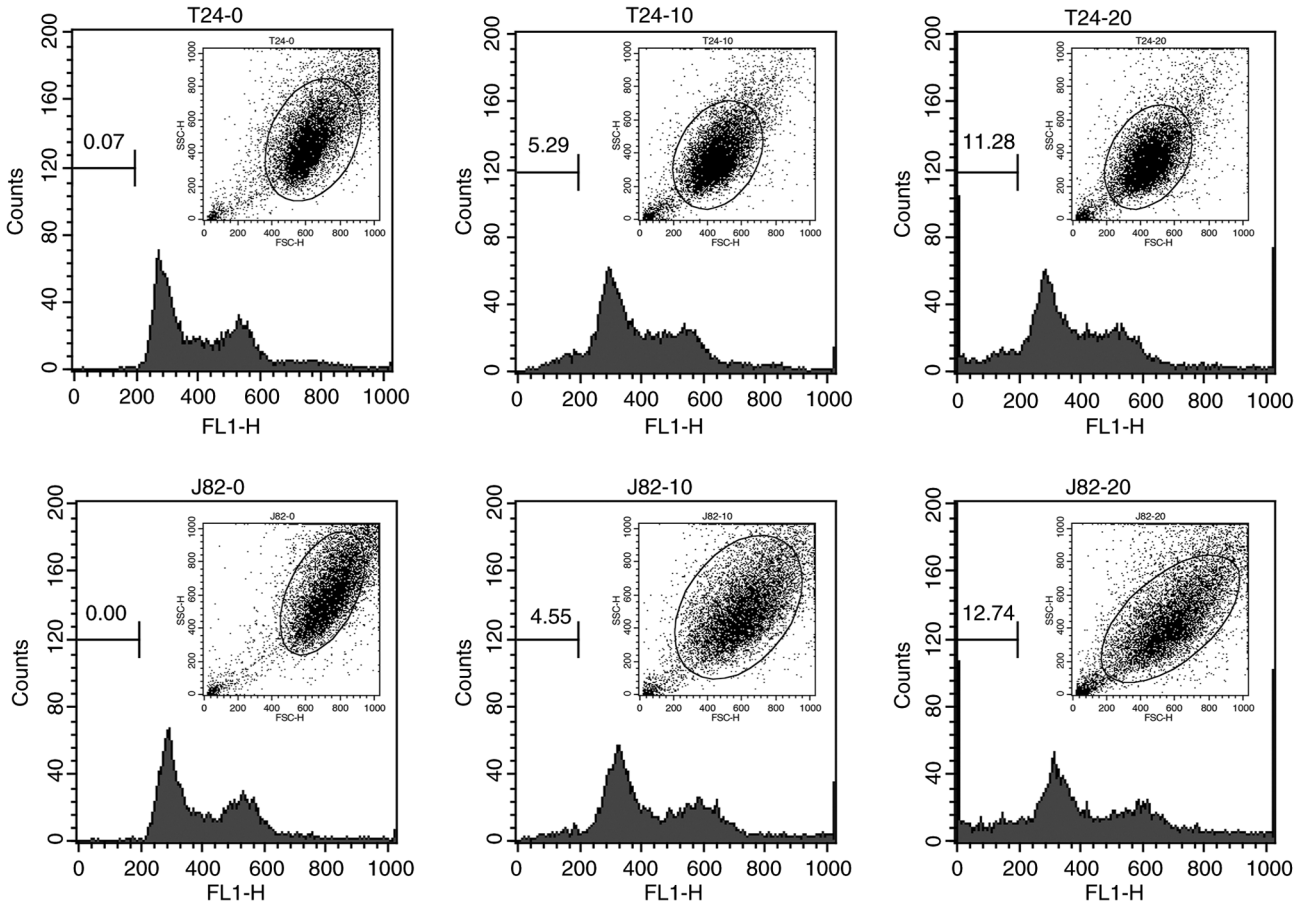

Figure S4. Quantitative analysis of protein expression levels of N-cadherin, β-catenin, p-β-catenin (S552), CD44 and p-S6K after CPT treatment in T24 and J82 cells. Bars indicate the relative expression value normalized to that of α-tubulin, and are presented as mean ± standard deviation of three independent assays. *P<0.05, **P<0.01 and ***P<0.001 compared with control (0 μM) by Tukey's test. CPT, cryptotanshinone; p-, phosphorylated.

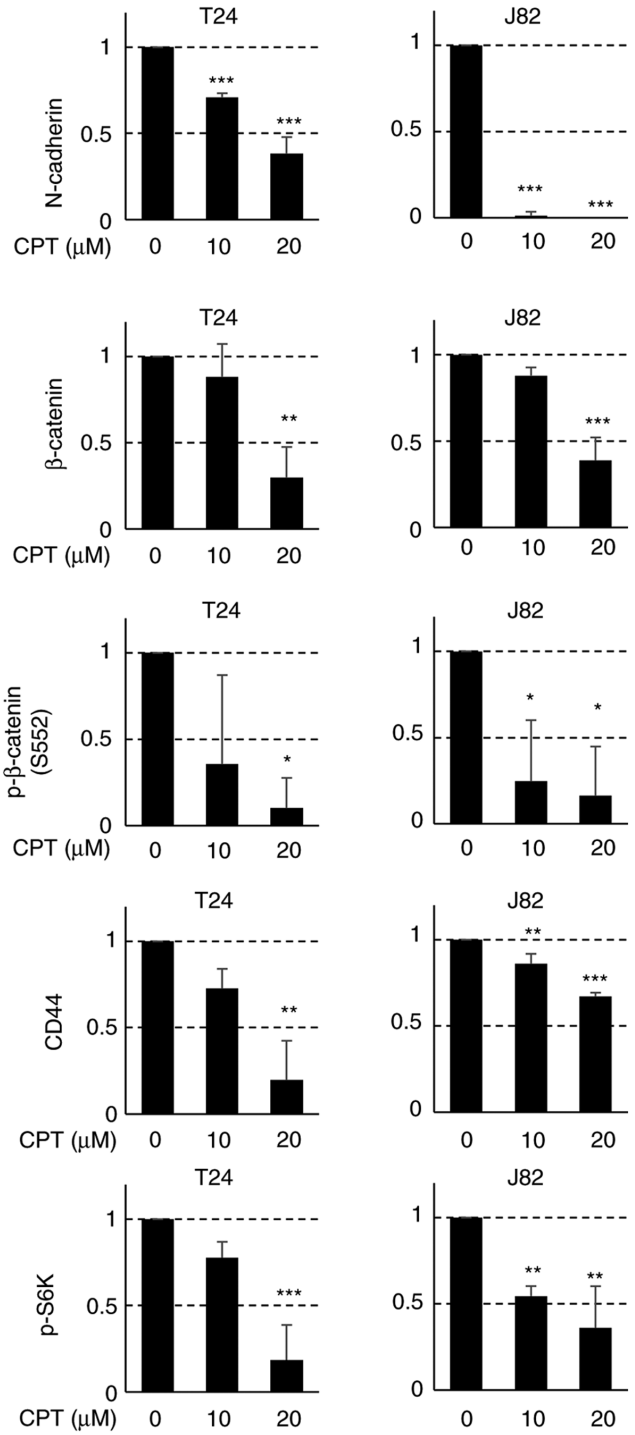

Figure S5. Effects of siRNA for PDK4 and β-catenin on three-dimensional spheroid formation in T24 human bladder cancer cells. si, small interfering; Ci, control; PDK4, pyruvate dehydrogenase kinase 4; β-cat, β-catenin.