Figure S1. UDCA induces apoptosis as effectively as gefitinib in bile duct cancer cells. Flow cytometry assay. The Annexin-FITC/PI Apoptosis Detection kit was used for the identification of apoptotic cells. Cells were treated with the indicated concentrations of UDCA (50, 100 and 250 μM) or gefitinib (0.001, 0.01 and 0.1 nM) at 37˚C for 48 h. The harvested cells were stained with Annexin V-FITC and PI. The stained cells were measured by flow cytometry. All concentrations of UDCA and gefitinib induced significant apoptosis (*P<0.001), compared withnon-treatment cells. x-axis, annexin FITC; y-axis, PI. UDCA, ursodeoxycholic acid.

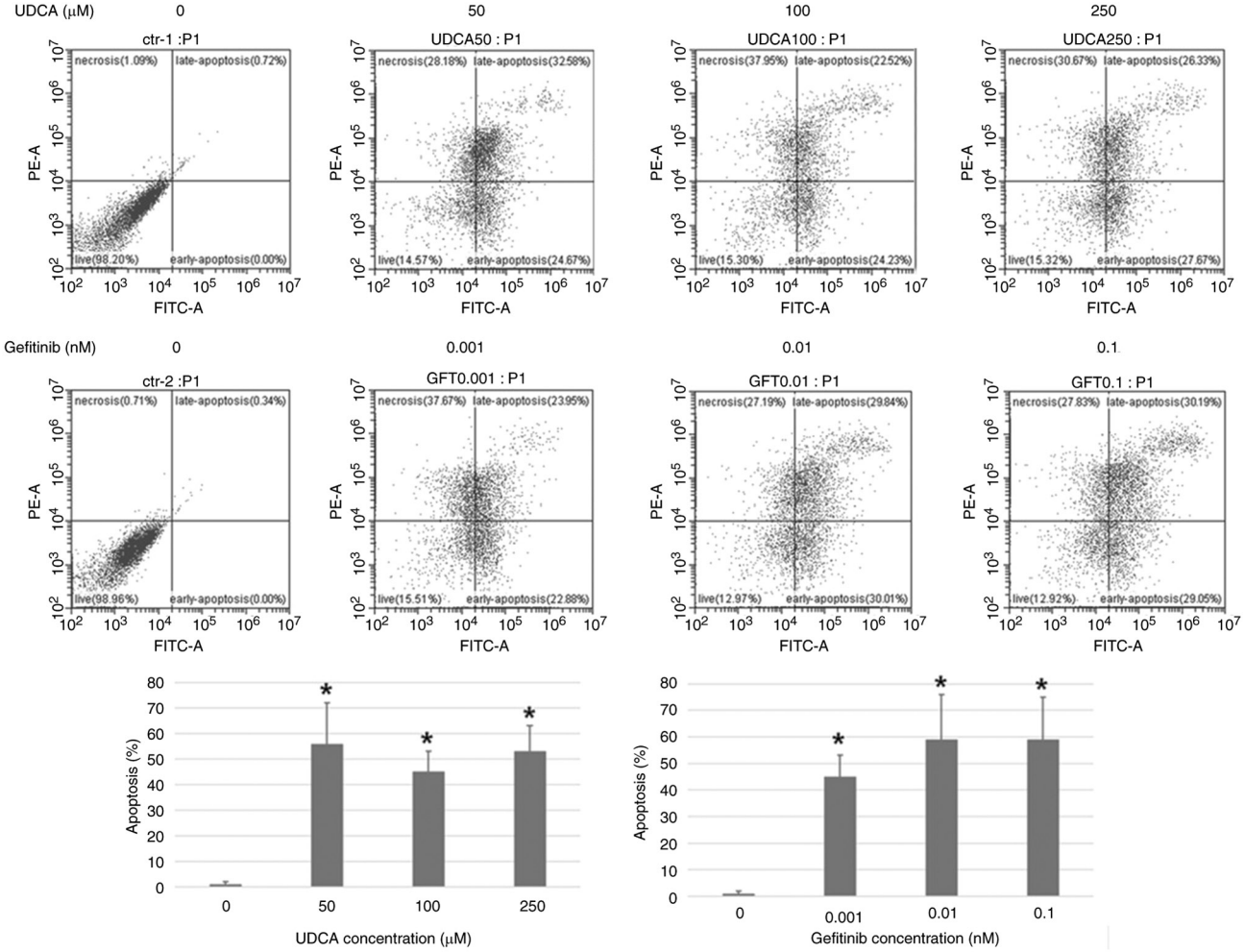

Figure S2. EGF inhibits E-cadherin expression and increases N-cadherin expression in bile duct cancer cells. Cells were treated with EGF in RPMI media containing 1% FBS for 48 h. The expression levels were estimated by western blotting. EGF treatment for 48 h (A) suppressed E-cadherin expressionand (B) enhanced N-cadherin expression in a dose-dependent manner. All data are representative of triplicates. *P<0.001 vs. untreated control and any concentration of EGF-treated group. †P<0.05 vs. untreated control.

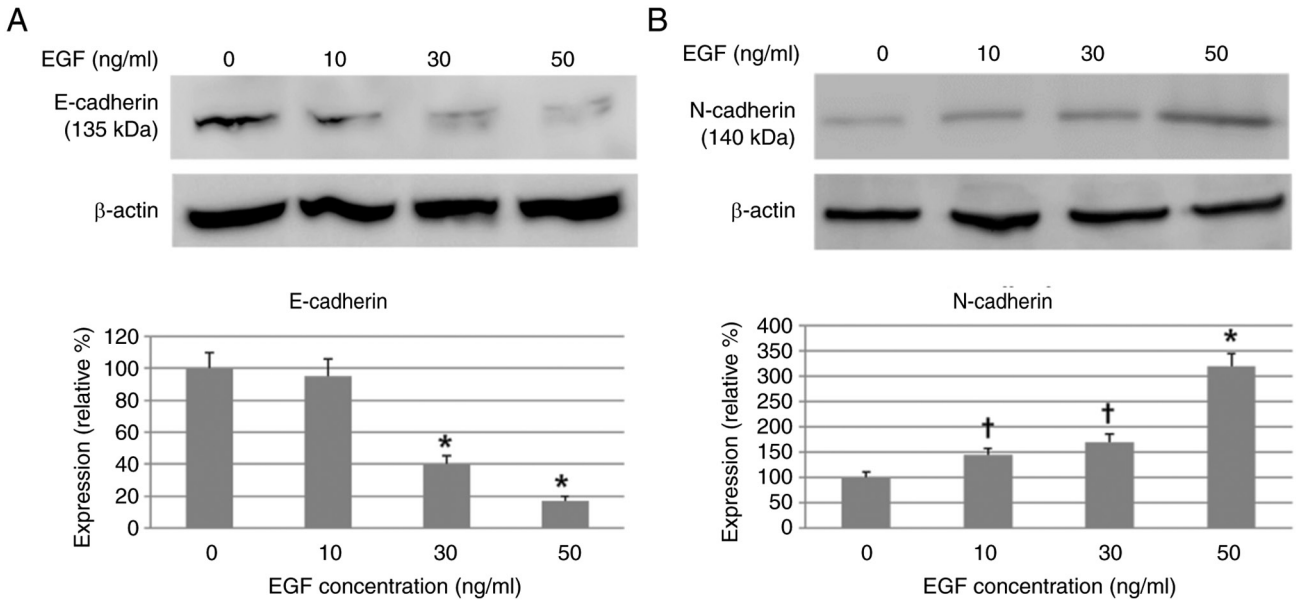

Figure S3. Gefitinib and UDCA restore E-cadherin expression and suppress N-cadherin expression increased by EGF in bile duct cancer cells. Cells were treated with gefitinib or UDCA with or without EGF treatment in 1% FBS RPMI media for 48 h. Expression levels were estimated by western blotting. All data are representative of triplicate experiments. Both (A) gefitiniband (B) UDCA treatment for 48 h enhanced E-cadherin expression suppressed by EGF and suppressed the N-cadherin expression increased by EGF in a dose-dependent manner. UDCA, ursodeoxycholic acid.

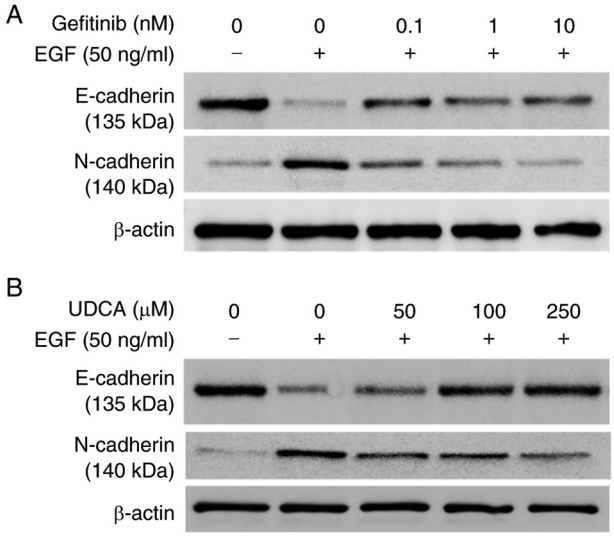

Figure S4. UDCA suppresses FAK expression linked to the invasiveness of bile duct cancer cells. Cells were treated with gefitinib and/or UDCA in serum-free mediafor 24 h with or without pre-treatment of IGF-1 (100 nM) for 15 min. Western blotting was used to determine the expression levels of target proteins. All data shown are representative of triplicate experiments. Both UDCA and gefitinib treatment suppressed the expression of pFAK enhanced by IGF. In addition, the combination of UDCA and gefitinib had an additive or synergistic effect on the suppression of pFAK induced by IGF. Group A, non-treated control; group B, only IGF-1-treated; group C, gefitinib and IGF-1-treated; group D, UDCA and IGF-1-treated; group E,gefitinib, UDCA and IGF-1-treated. $^{*}P<0.001$ vs. every group, $^{**}P<0.001$ vs. group A, B and E, $^{\dagger}P<0.001$ vs. group B, C and D. FAK, focal adhesion kinase; IGF, insulin-like growth factor; p, phosphorylated; UDCA, ursodeoxycholic acid.