Figure S1. AKT inhibition promotes Dox-induced apoptosis in human cholangiocarcinoma cells. QBC939 and RBE cells were pre-treated with vehicle (DMSO) or AKT inhibitor (A) LY294002 (30 mM) or (B) PI828 (20 nM) for 1 h before Dox (2 μM) treatment for 24 h. Subsequently, the ratio of cell death was measured via an LDH assay. **P<0.01. Dox, doxorubicin; LDH, lactate dehydrogenase.

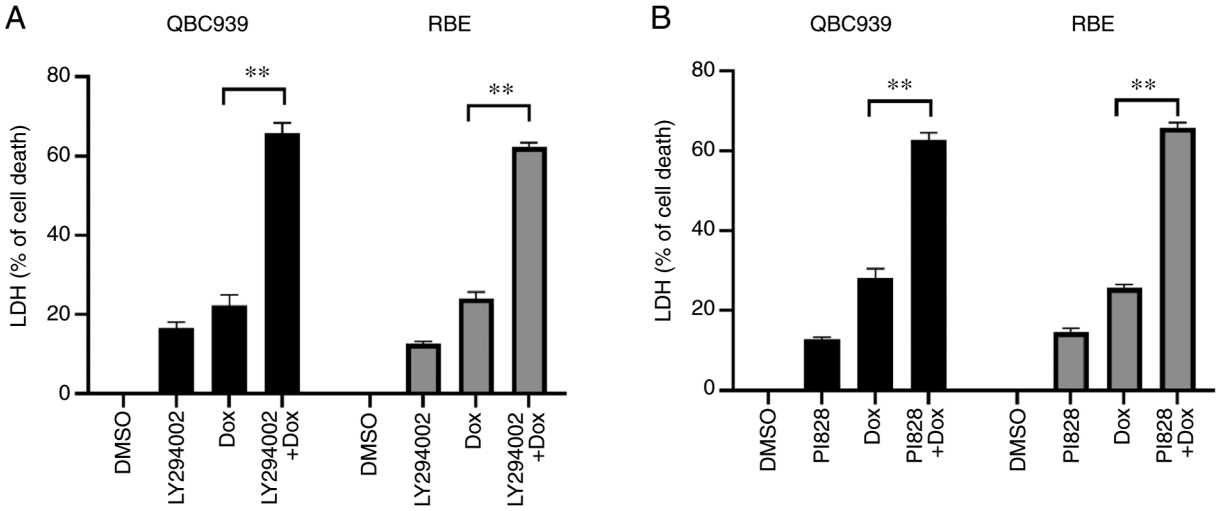

Figure S2. GSK-3β inhibition promotes Dox-induced apoptosis in human cholangiocarcinoma cells. Following pretreatment with GSK-3β inhibitors (A) BIO (5 μM), (B) CHIR990219 (10 μM) or (C) GSK-3β siRNAs, QBC939 and RBE cells were treated with Dox (2 μM) for 24 h. Subsequently, the ratio of cell death was measured via an LDH assay. **P<0.01. Dox, doxorubicin; BIO, 6-bromoindirubin-3'-oxime; siRNA, small interfering RNA; LDH, lactate dehydrogenase; NC, negative control.

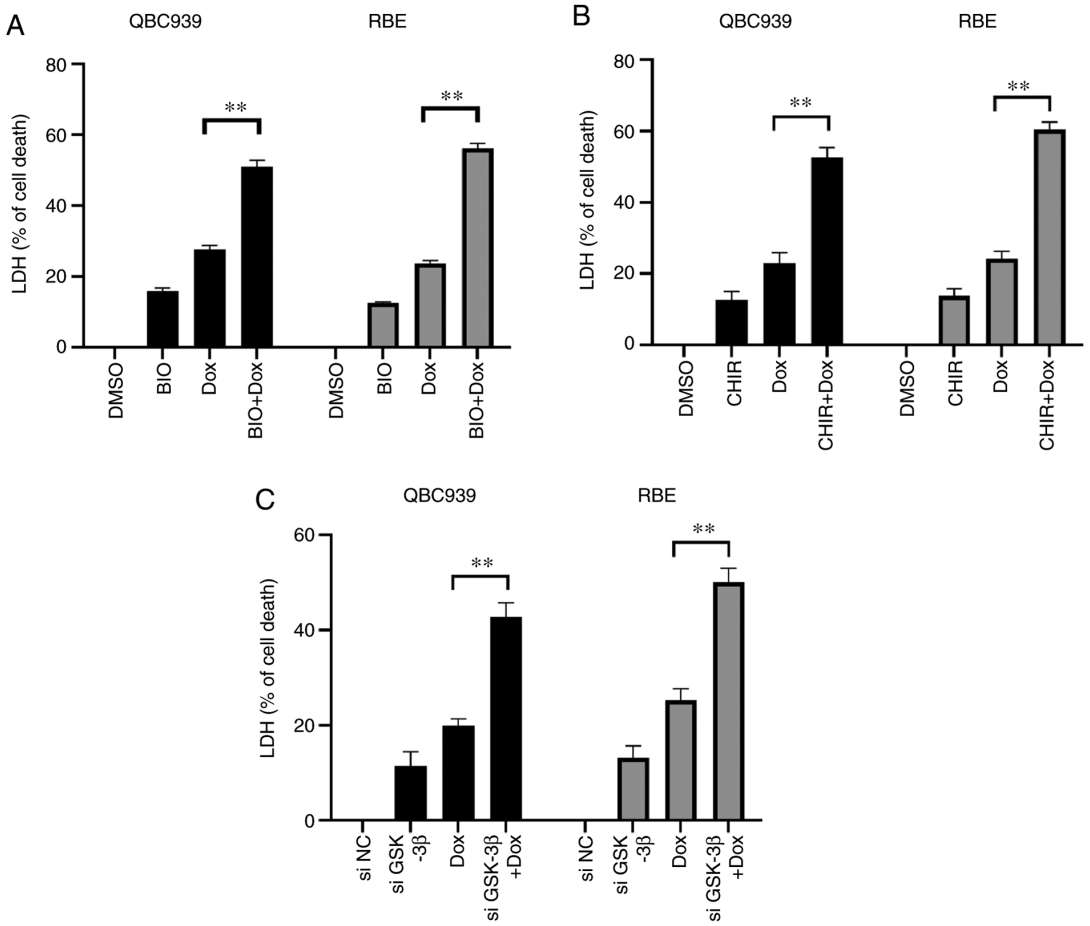

Figure S3. Phosphorylation of T308 of AKT is regulated by PDK1 in human cholangiocarcinoma cells. (A) QBC939 and RBE cells were treated with vehicle (DMSO) or PDK1 inhibitor OSU (10 μM) for 12 h, and the cell lysates were subjected to western blotting and protein expression was semi-quantified. (B) QBC939 and RBE cells were transfected with NC and PDK1-targeted siRNAs for 6 h, then allowed to recover for 30 h in normal media and cell lysates were subjected to western blotting. (C) QBC939 and RBE cells were transfected with myr-HA-AKT for 6 h, then allowed to recover for 30 h in normal media before OSU (10 μM) treatment for 12 h, and cell lysates were subjected to western blotting and protein expression was semi-quantified. **P<0.01. PDK1, phosphoinositide-dependent protein kinase-1; OSU, OSU-03012; NC, negative control; siRNA, small interfering RNA; p-, phosphorylated.

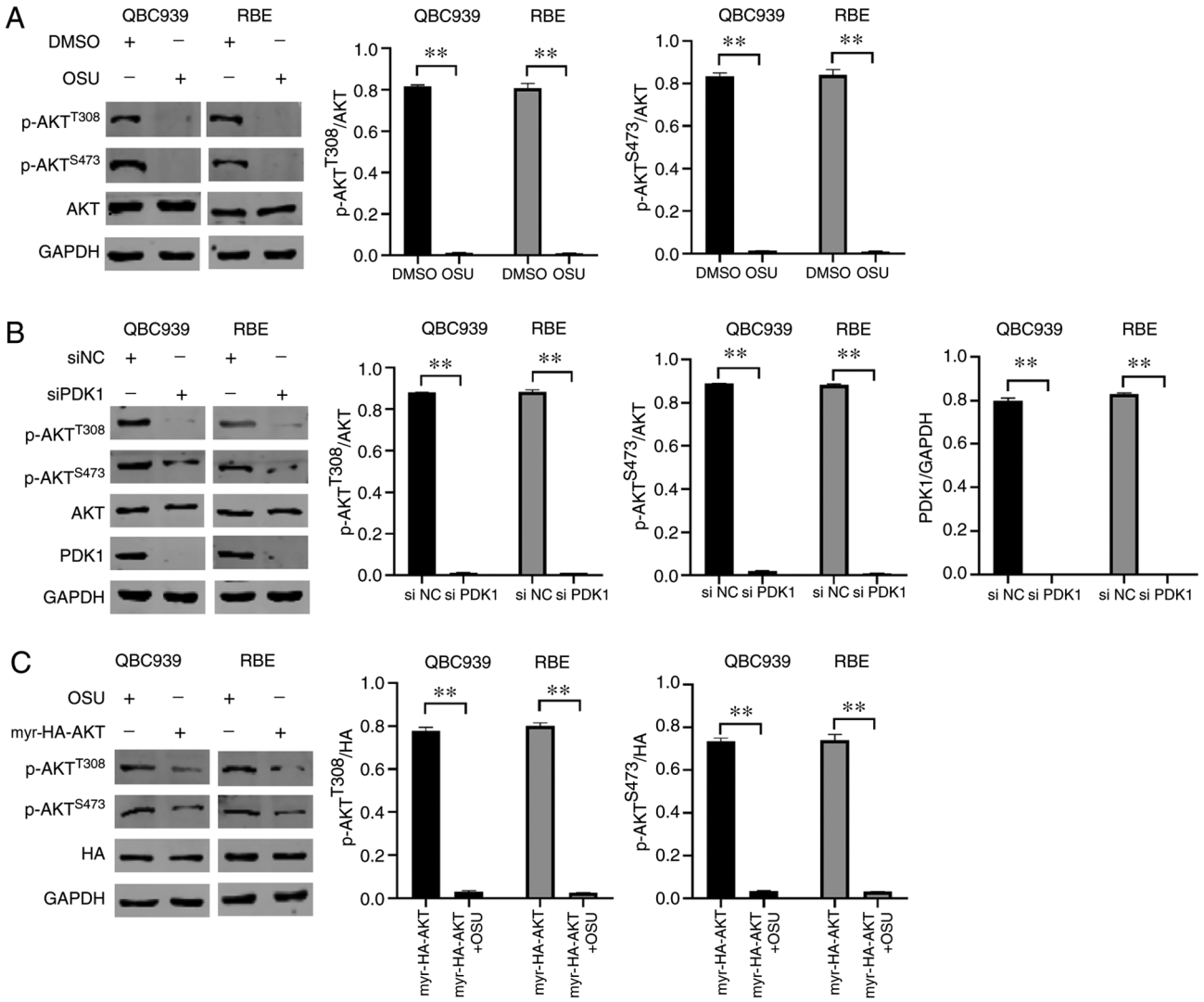

Figure S4. Phosphorylation of S473 of AKT is controlled by mTOR complex 2 in human cholangiocarcinoma cells. (A and B) QBC939 and RBE cells were transfected with NC and RICTOR-targeted siRNA for 6 h, then allowed to recover for 30 h in normal media and cell lysates were subjected to western blotting and protein expression was semi-quantified. QBC939 and RBE cells were transfected with myr-HA-AKT for 6 h, then allowed to recover for 30 h in normal media before (C) rapamycin (100 nM) or (D) AZD8055 (1 μM) treatment for 12 h, and cell lysates were subjected to western blotting and protein expression was semi-quantified. **P<0.01. NC, negative control; siRNA, small interfering RNA; p-, phosphorylated; RICTOR, rapamycin-insensitive companion of mTOR.

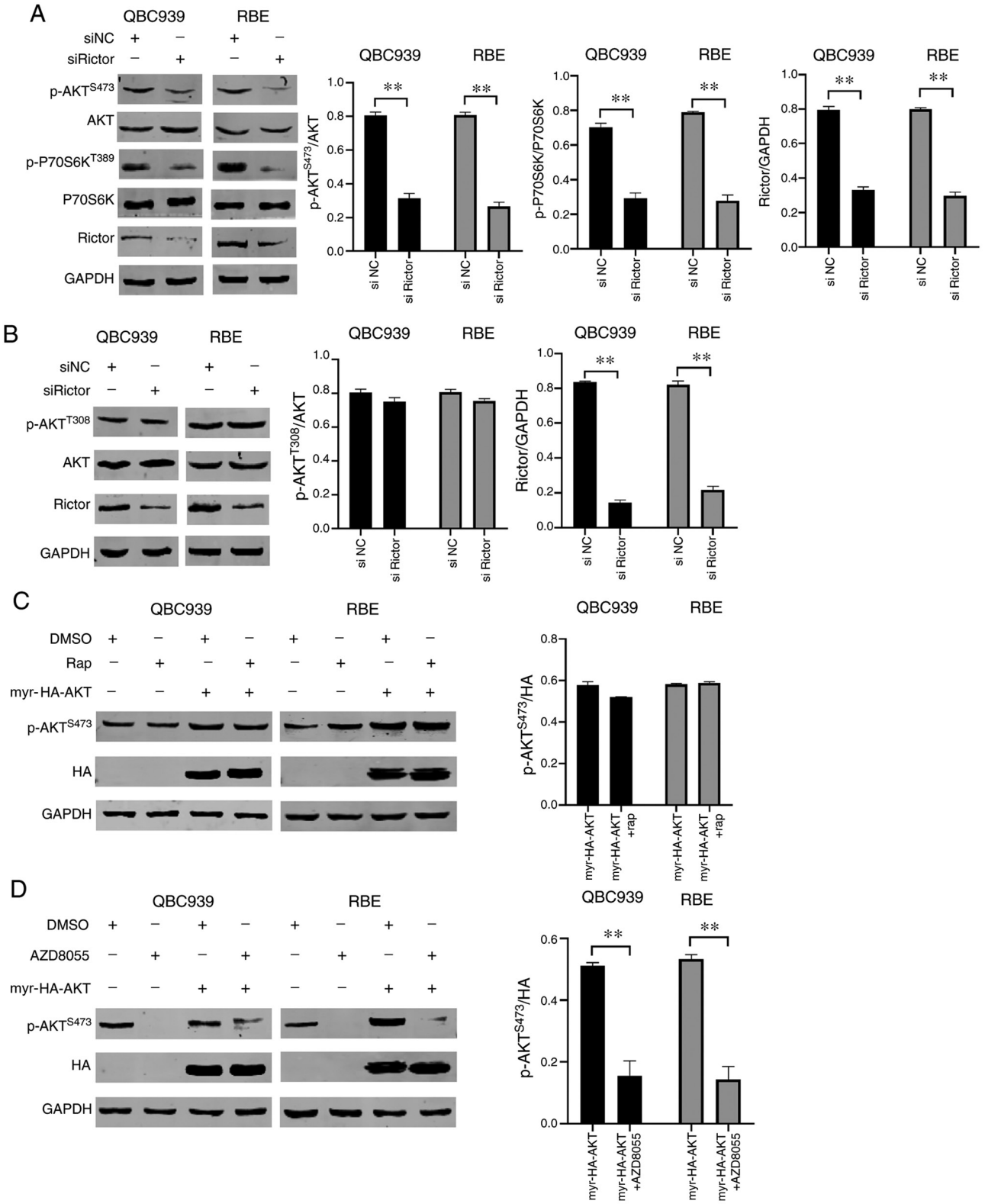

Figure S5. GSK-3β inhibition exhibits no notable effect on PDK1 and mTOR complex 2 activation. (A and C) QBC939 and RBE cells were treated with vehicle (DMSO) or GSK-3β inhibitors BIO (5 μM) and CHIR990219 (10 μM) for 12 h, and cell lysates were subjected to western blotting and protein expression was semi-quantified. (B and D) QBC939 and RBE cells were transfected with NC and GSK-3β-targeted siRNA for 6 h and then allowed to recover for 30 h in normal media, and cell lysates were subjected to western blotting and protein expression was semi-quantified. **P<0.01. PDK1, phosphoinositide-dependent protein kinase-1; BIO, 6-bromoindirubin-3'-oxime; NC, negative control; siRNA, small interfering RNA; p-, phosphorylated.

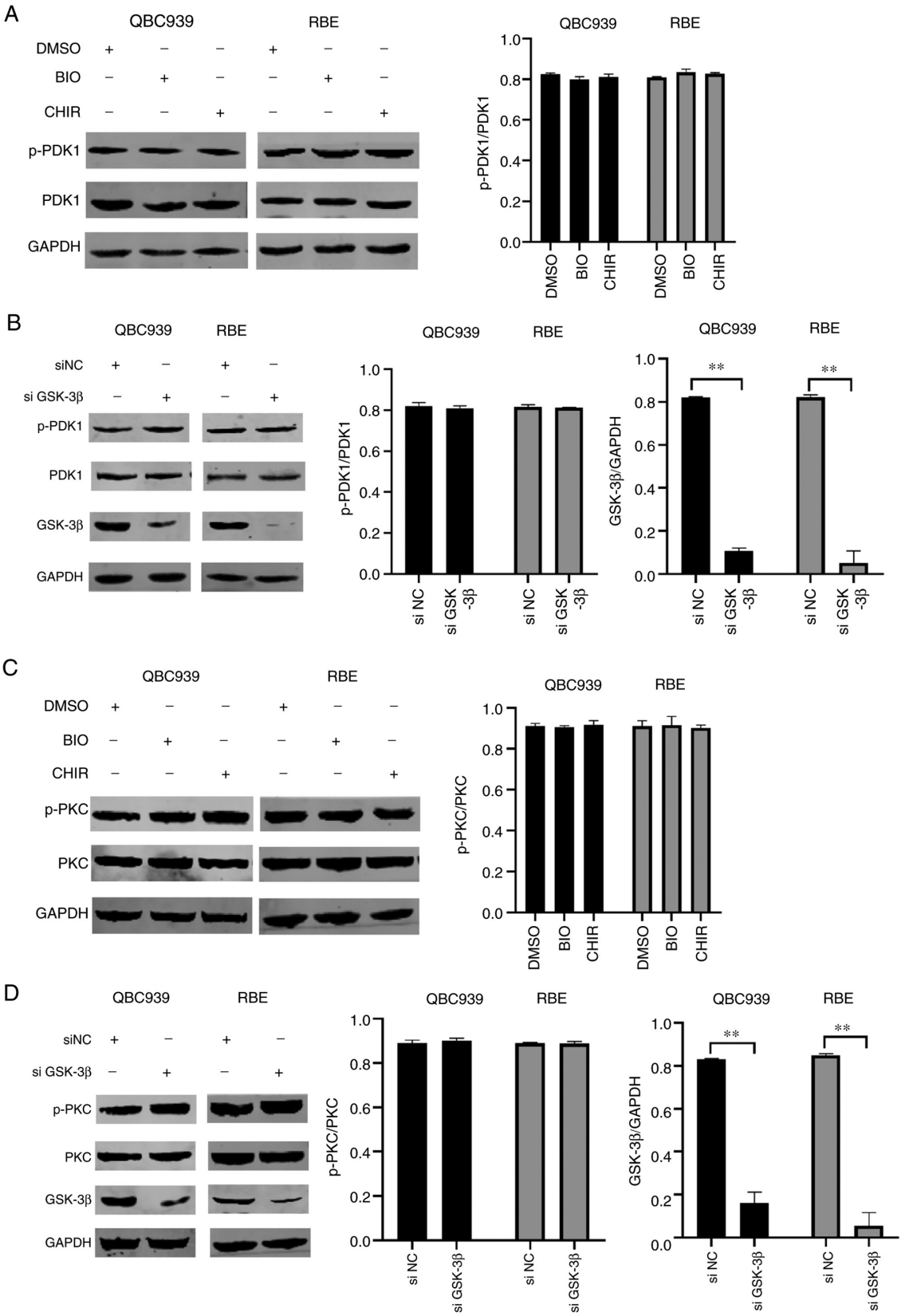

Figure S6. FAK protects human cholangiocarcinoma cells against Dox-induced apoptosis. QBC939 and RBE cells were pre-treated with vehicle (DMSO) or FAK inhibitor, PF (10 μM), for 1 h before being treated with Dox (2 μM) for 24 h, then the ratio of cell death was measured via an LDH assay. **P<0.01. FAK, focal adhesion kinase; Dox, doxorubicin; PF, PF-573228; LDH, lactate dehydrogenase.

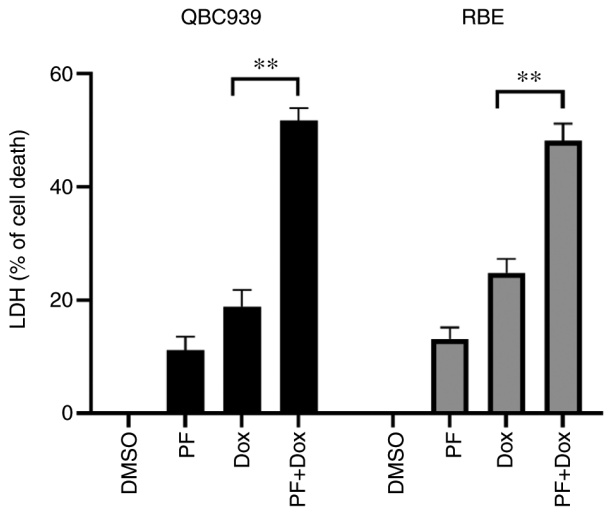

Figure S7. GSK-3β and FAK inhibition exhibit no effect on PI3K activity in human cholangiocarcinoma cells. Following (A) GSK-3β inhibitor [BIO (5 μM) and CHIR990219 (10 μM)], PI3K inhibitor Wort (2 μM) or (B) FAK inhibitor PF (10 μM) treatment for 12 h, PI3K activity was detected. **P<0.01. FAK, focal adhesion kinase; PI3K, phosphoinositide 3-kinase; BIO, 6-bromoindirubin-3'-oxime; Wort, Wortmannin; PF, PF-573228; PIP3, phosphatidylinositol (3,4,5)-trisphosphate.

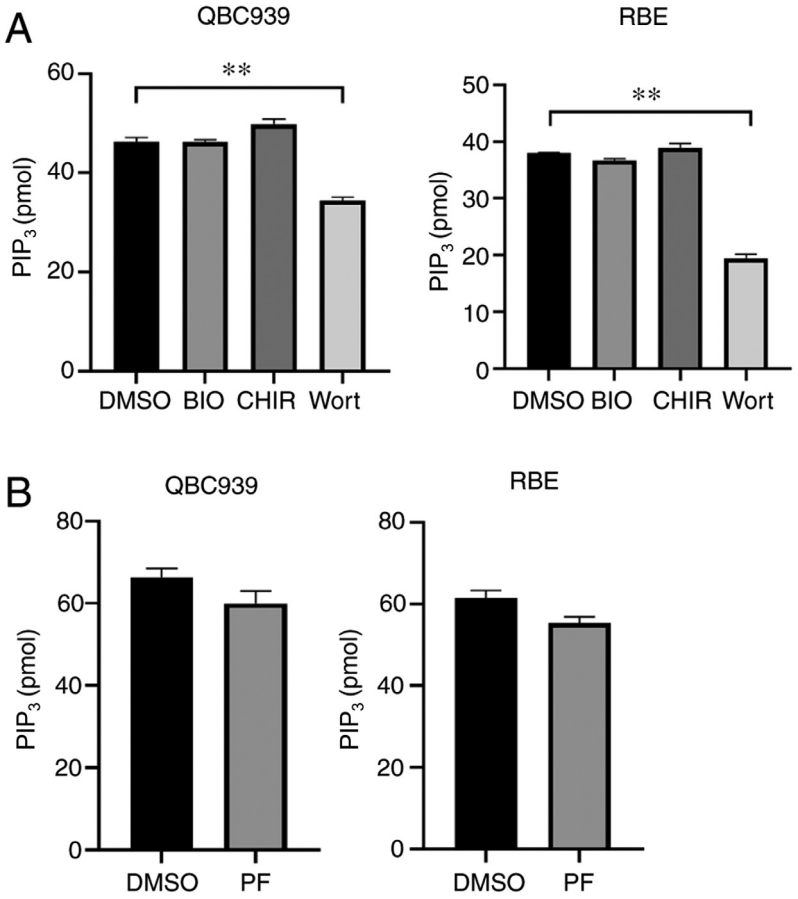

Table SI. Primers used for reverse transcription-quantitative PCR.

Gene	Primer sequences (5'-3')
GSK-3β	F: CTGGTCGCCATCAAGAAAGTA
	R: AGAAGAAATAACGCAATCGGA
Ribosomal protein L13A	F: CATAGGAAGCTGGGAGCAAG
	R: GCCCTCCAATCAGTCTTCTG

F, forward; R, reverse.