Inhibition of ClC-5 suppresses proliferation and induces apoptosis in cholangiocarcinoma cells though Wnt/β-catenin signaling pathway

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Running title: Anti-tumor effect of ClC-5 knockdown in cholangiocarcinoma cells

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Supplemental Figure 1. The transfection efficiencies of shCIC-5#1 and shCIC-5#2 in QBC939 (A) and SNU-869 cells (B) were identified by Western blot. mean±SD, n=3; **P<0.01 vs. Control group.
Supplemental Figure 2. LiCl or Laduviglusib enhanced intracellular Wnt/β-catenin signaling activity. A–B: The expression levels of β-catenin, cyclin D1 and c-Myc proteins in QBC939 cells were detected by Western blot; C–D: The expression levels of β-catenin, cyclin D1 and c-Myc proteins in SNU-869 cells were detected by Western blot. mean ± SD, n=3; **P<0.01 vs. shClC-5 group.
Supplemental Figure 3. LiCl promoted the proliferation of shClC-5 transfected CCA cells. After LiCl treatment in shClC-5 transfected CCA cells, cell proliferation was evaluated by EDU, CCK-8 and colony formation assays. A–B: Representative EDU staining images (A) and statistical results of EDU-positive cells (B) in both QBC939 and SNU-869 cells; C–D: Cell viabilities of QBC939 (C) and SNU-869 (D) cells; E–F: Representative colony formation images (E) and statistical results of colony count (F) in both QBC939 and SNU-869 cells. mean±SD, n=3; **P<0.01 vs. shClC-5 group.
Supplemental Figure 4. LiCl inhibited the apoptosis of shCIC-5 transfected CCA cells by inhibiting mitochondrial apoptotic pathway. After LiCl treatment in shCIC-5 transfected CCA cells, cell apoptosis was detected by Annexin V-FITC/PI staining, mitochondrial membrane potential was evaluated by JC-1 staining, mitochondrial apoptotic pathway related proteins were detected by Western blot. A-B: Representative flow cytometry scatter plots (A) and statistical results of apoptotic cell populations (B) in both QBC939 and SNU-869 cells; C-D: Representative JC-1 staining images (C) and statistical results of mitochondrial membrane potential (MMP) (D) in both QBC939 and SNU-869 cells; E-G: The protein levels of mitochondrial cytochrome c (mito Cyt-c), cytoplasmic cytochrome c (Cyto Cyt-c), Bcl-2, Bax and Cleaved caspase-3 in both QBC939 (E-F) and SNU-869 (E, G) cells. in both QBC939 and SNU-869 cells. mean±SD, n=3; **P<0.01 vs. shCIC-5 group.
Supplemental Figure 5. Laduviglusib promoted the proliferation and inhibited the apoptosis in shClC-5 transfected CCA cells. After Laduviglusib treatment in shClC-5 transfected CCA cells, cell proliferation was evaluated by EDU and CCK-8 assays, cell apoptosis was detected by Annexin V-FITC/PI staining, mitochondrial membrane potential was evaluated by JC-1 staining.

A: Statistical results of EDU-positive cells in both QBC939 and SNU-869 cells; B: Cell viabilities of QBC939 and SNU-869 cells; C: Statistical results of apoptotic cell populations in both QBC939 and SNU-869 cells; D: Statistical results of mitochondrial membrane potential (MMP) in both QBC939 and SNU-869 cells. mean ± SD, n=3; **P<0.01 vs. shClC-5 group.