Supporting Materials

Cholangiocarcinoma Therapy with Nanoparticles that Combine Downregulation of MicroRNA-210 with Inhibition of Cancer Cell Invasiveness

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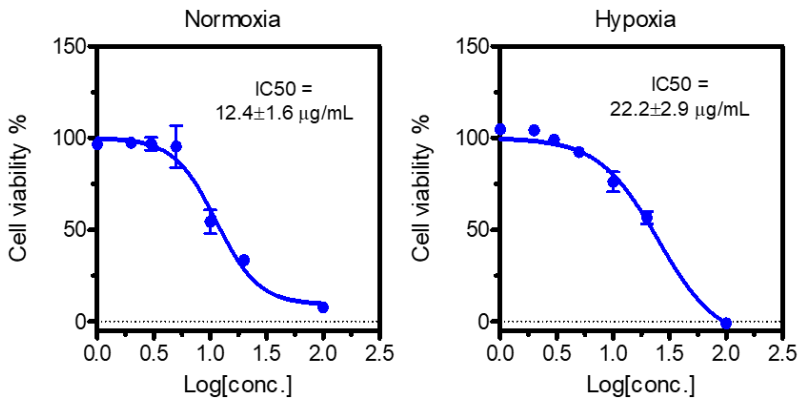

Figure S1. Cytotoxicity of PCX in Mz-ChA-1 cells after incubation for 48 h.

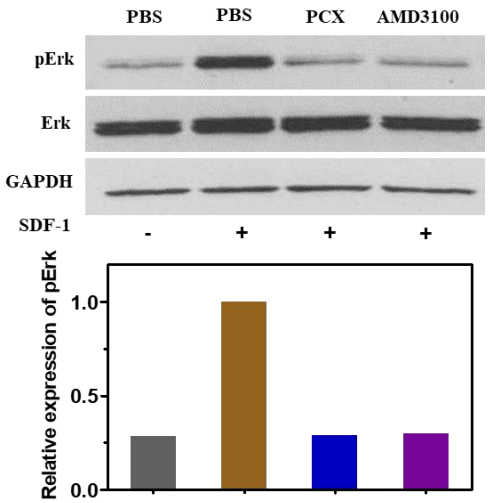

Figure S2. Inhibition of pERK by PCX. Mz-Cha-1 cells were treated with AMD3100 (300 nM), PCX (3 μg/mL) for 4 h followed by 20 min incubation with SDF-1 (100 ng/mL). Then, cells were lysed for Western blot analysis. GAPDH and Erk were used as housekeeping controls. Quantification of Western blot bands was performed using ImageJ software.

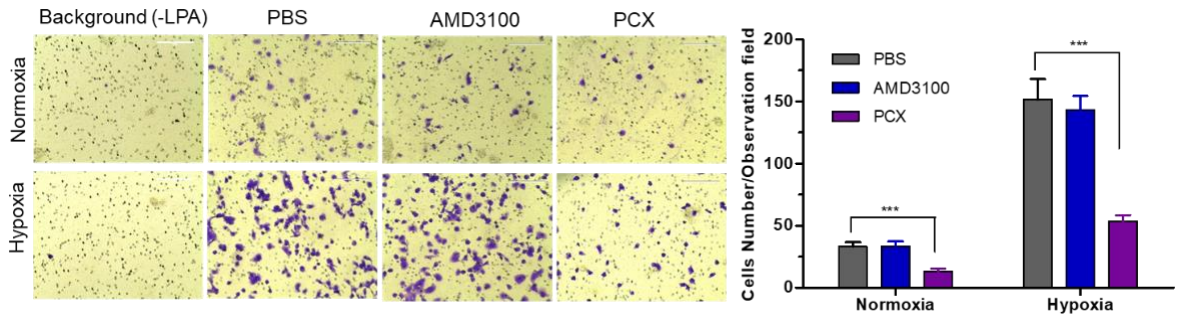

Figure S3. Inhibition of lysophosphatidic acid (LPA)-induced cancer cell migration in normoxia and hypoxia. Mz-ChA-1 cells were treated with AMD3100 (300 nM) or PCX (3 μg/mL) for 48 h and then allowed to migrate through a transwell membrane insert (6×10^4 cells per insert) upon stimulation with LPA (20 μM) for 12 h. Three 20× imaging areas were randomly selected for each insert and each group was conducted in triplicate. Data are shown as mean ± SD (n = 3). ***P < 0.001.

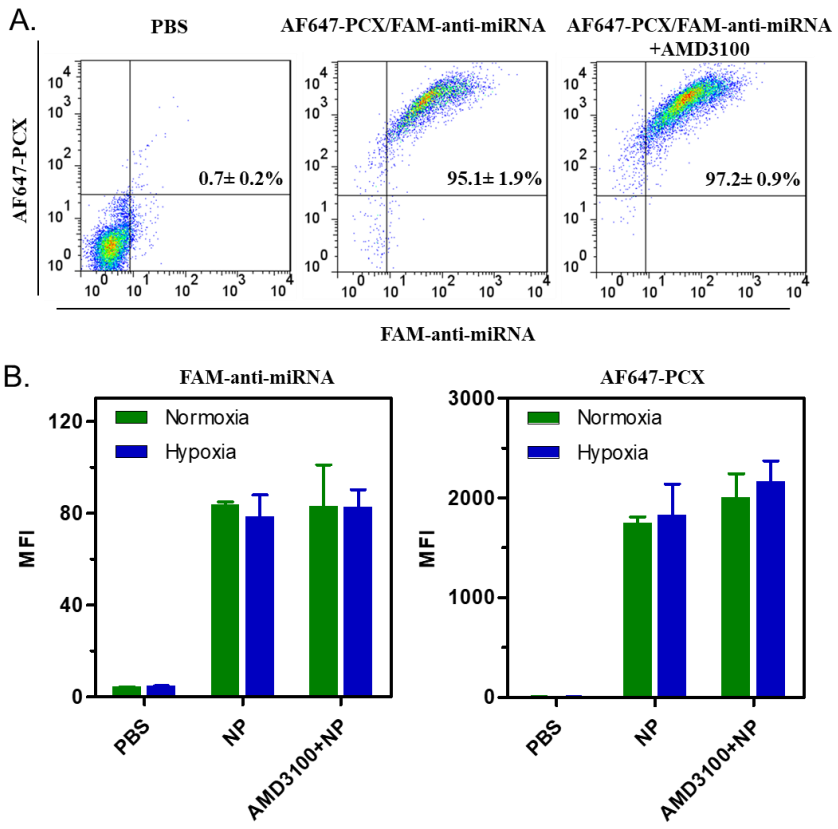

Figure S4. (A) Flow cytometry analysis of cells treated with nanoparticles (NP) AF647-PCX/FAM-anti-miRNA for 4 h under hypoxia. Pretreatment of Mz-ChA-1 cells with AMD3100 (100 µM) for 0.5 h for cellular uptake competition assay. (B) Quantification of cellular uptake is shown by mean fluorescence intensity (MFI) under normoxia and hypoxia.

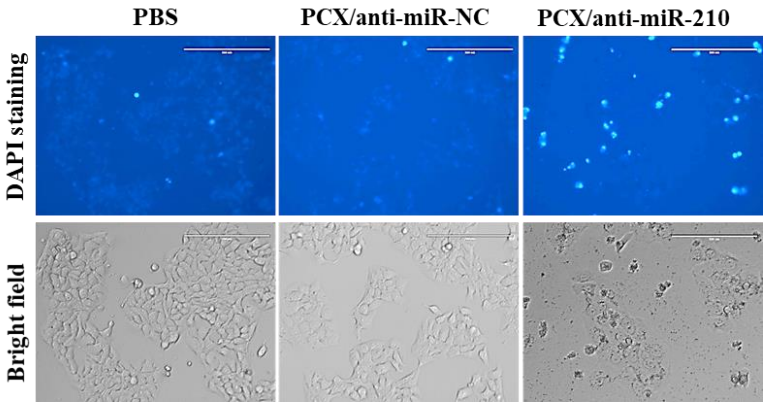

Figure S5. Cells were stained with DAPI and observed under fluorescence microscope after treatment.

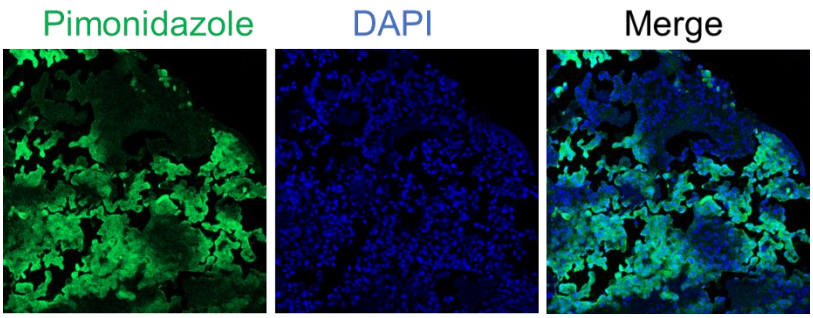

Figure S6. Hypoxia visualization in xenograft Mz-ChA-1 tumors. Representative confocal images of frozen tumor sections stained with the pimonidazole antibody (green).

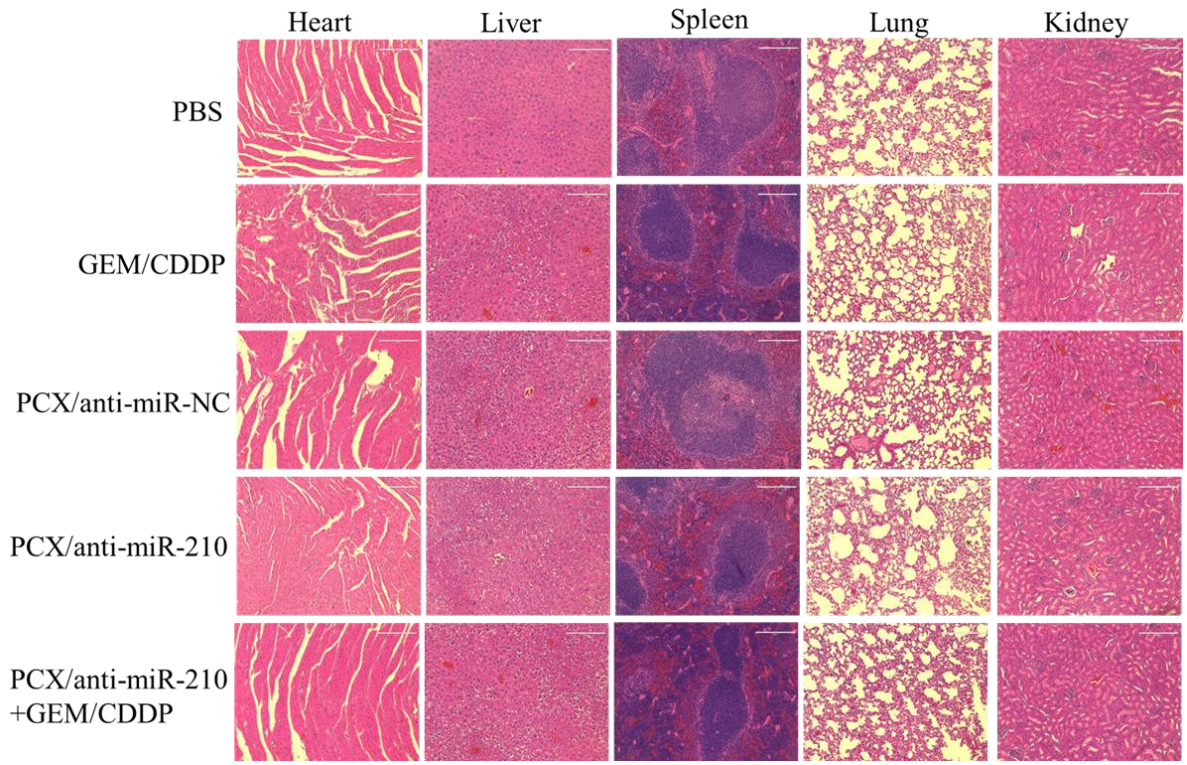

Figure S7. Histological observation of tissue sections from major organs of mice after treatment. The organ sections were stained with hematoxylin and eosin (H&E). The images were taken under a light microscope (×40).