Classification Original Research

Title: Brusatol boosts the efficacy of chemotherapeutic drug in pancreatic ductal adenocarcinoma by suppressing Nrf2 signaling

Supplementary material

Supplementary figures and figure legends

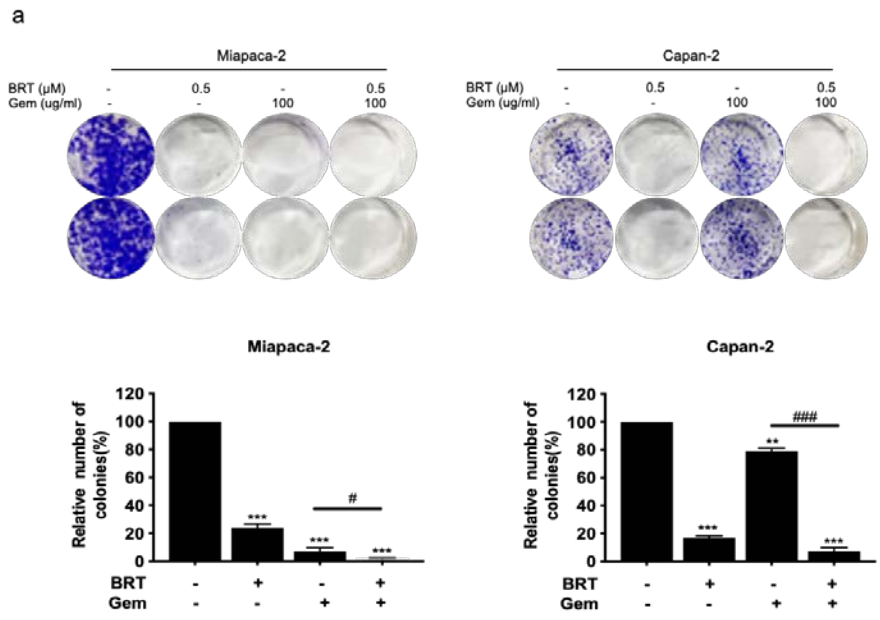

Fig. S1. (a) Miapaca-2 and Capan-2 cells were treated with BRT, Gem, or their combination for 24 h. Then, the media were replaced with complete medium without drug, and the cells were cultured for 2 weeks, and quantitative analysis of the combination treatment on the colony formation assay. Data were presented as the mean ± SD (n = 3). *p < 0.05, **p < 0.01 and ***p < 0.001 compared with the control group; #p < 0.05 and ###p < 0.001 compared with the Gem alone treatment group.

Figure S2

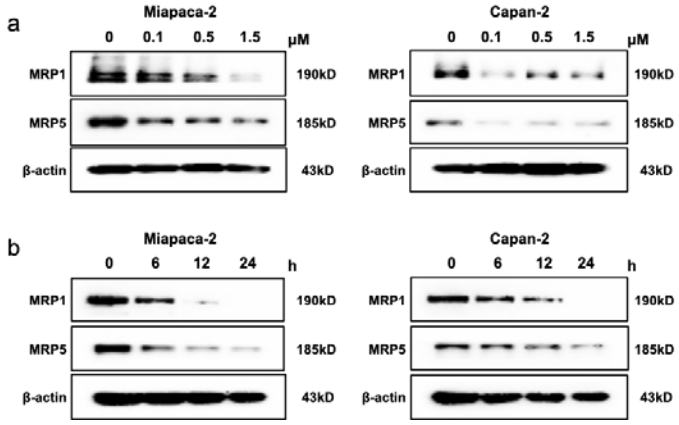

Fig. S2. (a) Miapaca-2 and Capan-2 cells were treated with 0.5 μM BRT for 24 h, western blot assay was used to detect the protein levels of MRP1 and MRP5.

Figure S3

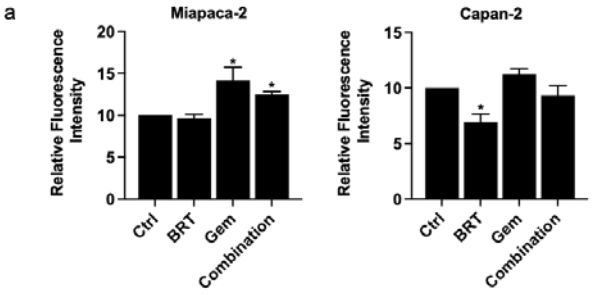

Fig. S3. (a) Quantification of IF for evaluation of Nrf2 expression. (c) Annexin V/PI cytometric analysis of drug-treated Miapaca-2 and Capan-2 cells. Data were presented as the mean ± SD. *$p < 0.05$ compared with the control group.

Figure S4

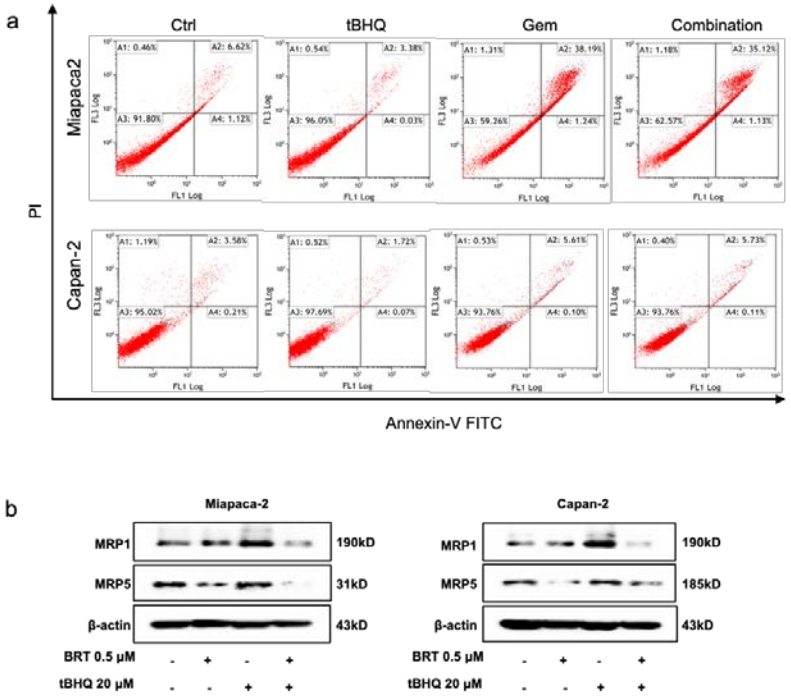

Fig. S4. Activation of Nrf2 enhances the chemosensitivity of GEM in PDAC cells. (a) Effects of Nrf2 activation on the protein levels of MRP1 and MRP5 in Miapaca-2 and Capan-2 cells following tBHQ, BRT or their combination treatment.

Figure S5

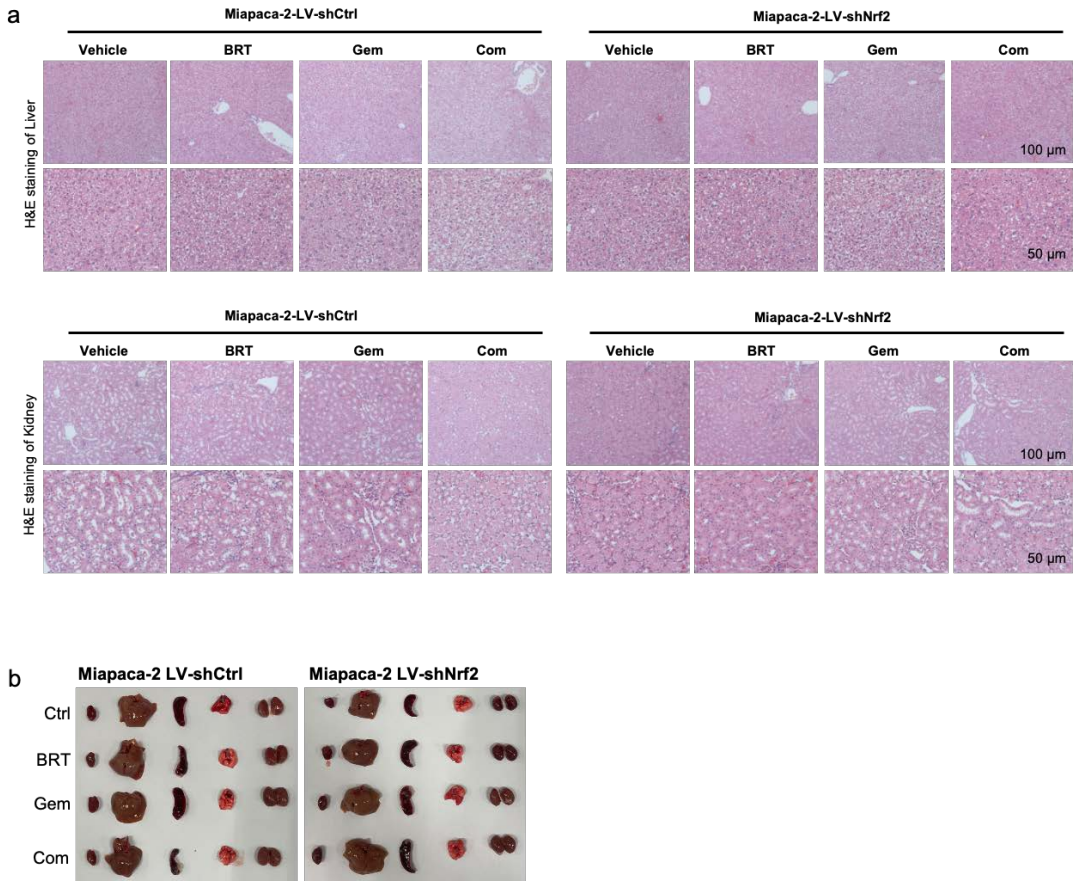

Fig. S5. Toxicological evaluation of BRT combined with GEM in orthotopic PDAC mouse model. (a-b) Representative image of pathological morphology of liver and kidney in mice were evaluated by H&E staining. (c) Image of organelle morphology of heart, liver, spleen, lung, and kidney treated with BRT, GEM and their combination on 26^{th} day observation.

Supplementary Table 1: List of antibodies

Antibody	Source	Identifier	Dilution
Mouse monoclonal anti-Nrf2	Santa Cruz Biotechnology	sc-365949 (A-10)	1:500 WB 1:100 IF
Rabbit monoclonal Anti-Keap-1	Cell Signaling Technology	8047s	1:1500
Mouse monoclonal Anti-HO-1	Santa Cruz Biotechnology	sc-136960 (A-3)	1:1000
Mouse monoclonal Anti-NQO-1	Santa Cruz Biotechnology	sc-32793 (A180)	1:1000
Mouse monoclonal Anti- γ-GCSm	Santa Cruz Biotechnology	sc-22754 (FL-274)	1:1000
Rabbit polyclonal Anti- AKR1B10	Proteintech	18252-1-AP	1:1000
Mouse monoclonal Anti-MRP1	Santa Cruz Biotechnology	sc-18835 (QCRL-1)	1:1000
Mouse monoclonal Anti-MRP5	Santa Cruz Biotechnology	sc-376965 (E-10)	1:1000
Mouse monoclonal Anti-β-actin	Santa Cruz Biotechnology	sc-69879 (AC-15)	1:1000
Rabbit polyclonal- Anti-Caspase-3	Cell Signaling Technology	9662	1:500
Rabbit polyclonal- Anti -Caspase-9	Cell Signaling Technology	9502	1:1000
Rabbit polyclonal- Anti-PARP	Cell Signaling Technology	9542	1:1000