Figure S1. Effect of necroptosis inhibitor on cell death in NPB-treated BxPC3 cells. The cells were treated with (A) 20 μM Necrostatin, (B) 1 μM GSK872 or (C) 1 μM Necrosulfonamide and 500 μM NPB for 48 h. The percentage of cell death was determined by flow cytometry. Averages and SD of three separate experiments are represented. *P<0.05; one-way ANOVA and the pairwise t-test with Holm's adjustment was performed. NPB, nitrated form of phenylbutyrate.

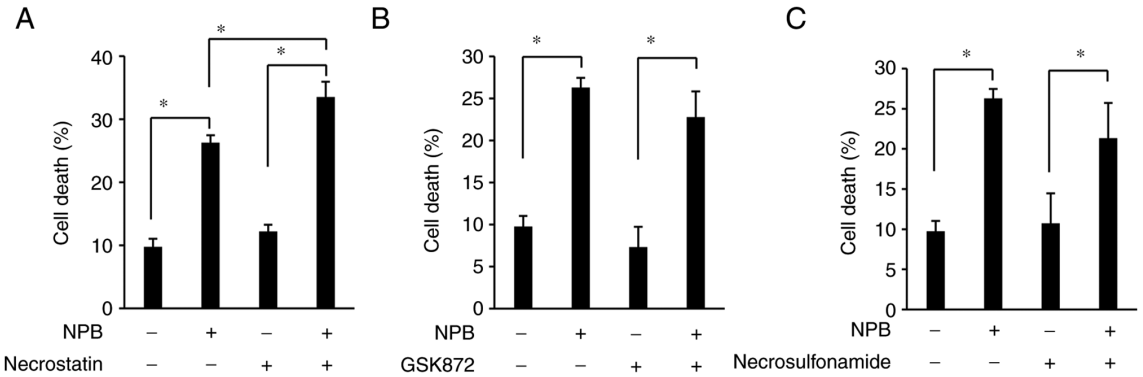

Figure S2. Growth inhibition of human pancreatic cancer cells by NPB. (A) AsPC1 and (B) BxPC3 were treated with 500 μM NPB for 24, 48 and 72 h. The cell concentration was determined by flow cytometry. Averages and SD of three separate experiments. NPB, nitrated form of phenyl butyrate. Averages and SD of three separate experiments are represented. *P<0.05; one-way ANOVA and the pairwise t-test with Holm's adjustment was performed. NPB, nitrated form of phenylbutyrate.

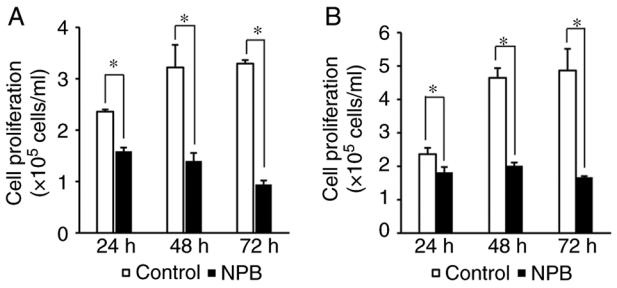

Figure S3. NPB induces death of pancreatic cancer cells *in vitro*. (A) AsPC1 and (B) BxPC3 cells were cultured with 500 μM NPB for 48 h. The data are the results of one of three independent experiments. NPB, nitrated form of phenylbutyrate.

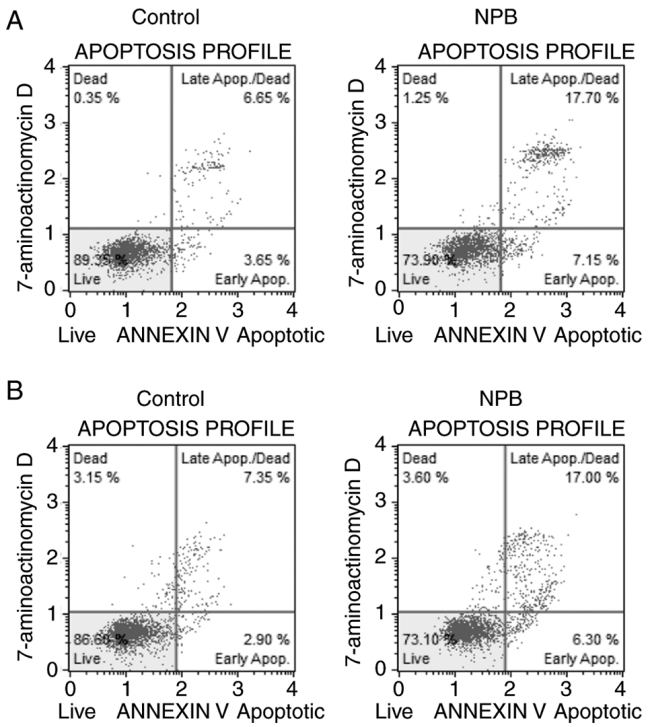

Figure S4. Effect of stress by ROS and the endoplasmic reticulum on NPB-induced cell death. (A) Effects of NAC, a radical scavenger of ROS, on cell death in NPB-treated BxPC3 cells. The cells were treated with 1 mM NAC and 500 μM NPB for 48 h. (B) Immunoblot analysis of CHOP, an endoplasmic reticulum stress marker, in BxPC3 cells after they were treated with the 500 μM NPB for 6 and 24 h. Averages and SD of three separate experiments are represented. *P<0.05; one-way ANOVA and the pairwise t-test with Holm's adjustment was performed. ROS, reactive oxygen species; NPB, nitrated form of phenylbutyrate; NAC, N-acetyl-cysteine.