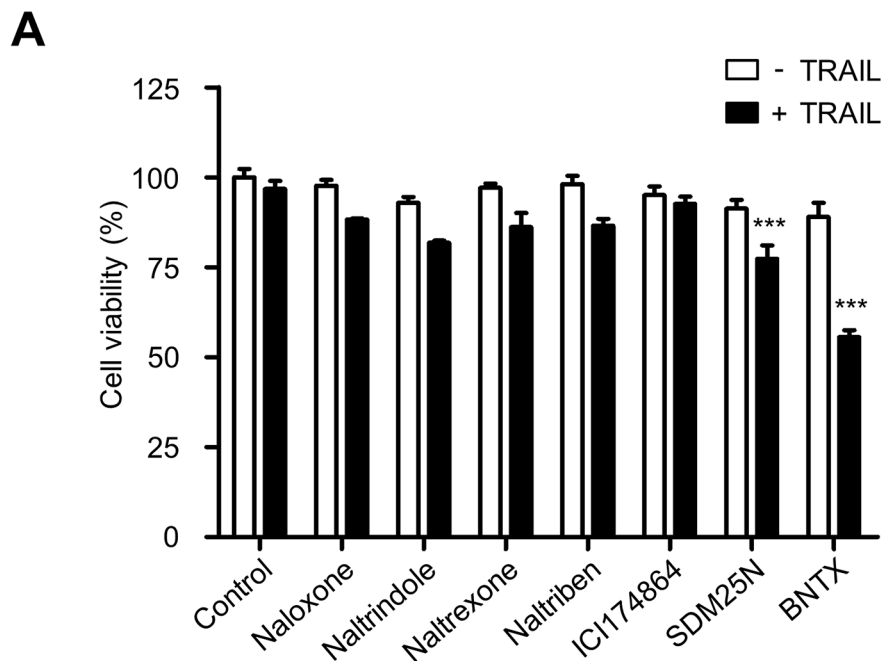
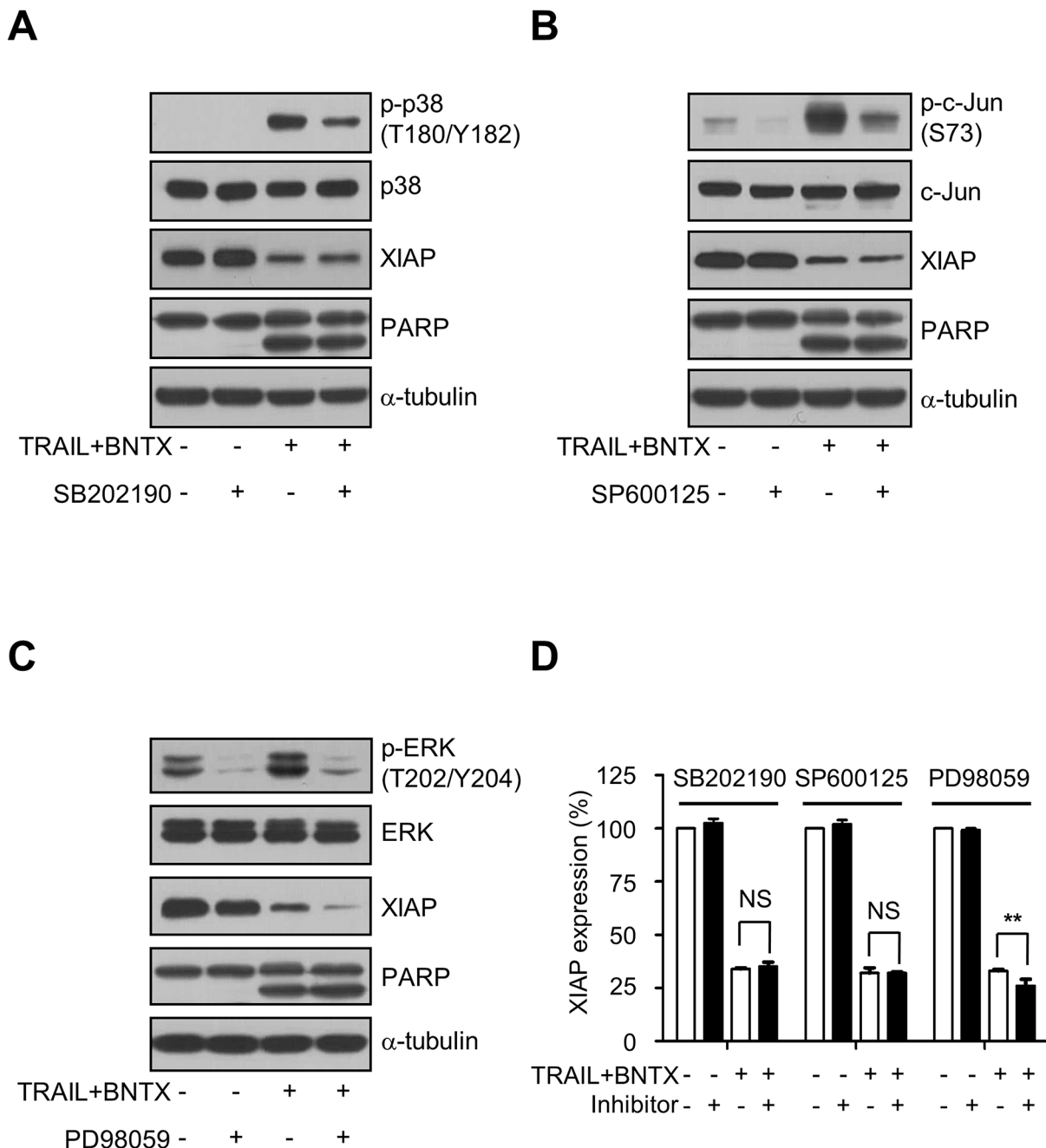


Downregulation of X-linked inhibitor of apoptosis protein by '7-Benzylidenenaltrexone maleate' sensitizes pancreatic cancer cells to TRAIL-induced apoptosis

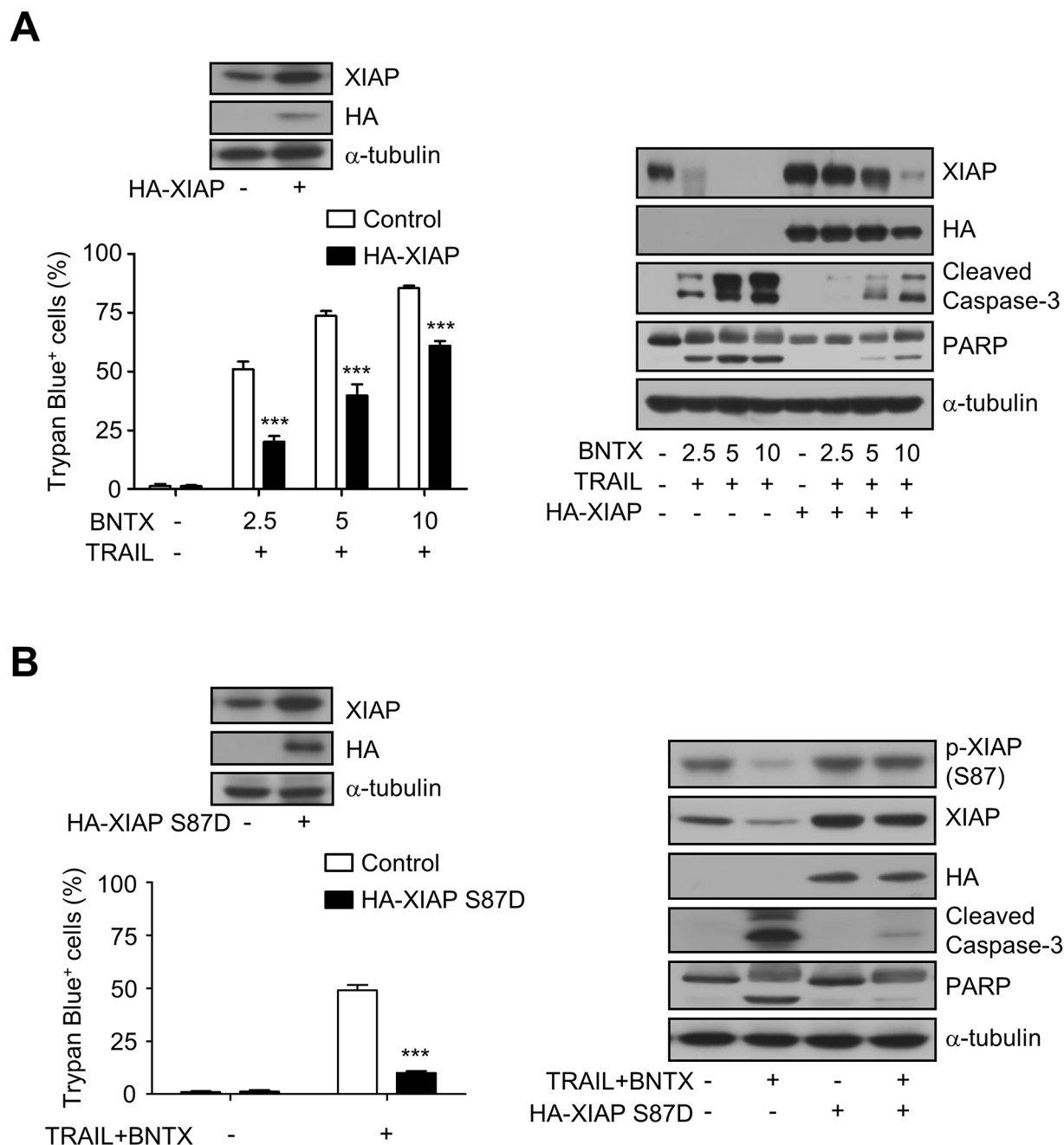
SUPPLEMENTARY MATERIALS



Supplementary Figure 1: Effects of different opioid receptor antagonists on TRAIL-induced apoptosis. (A) AsPC-1 cells were pre-treated with various antagonists (5 μ M) for 0.5 h and then exposed to TRAIL (25 ng/ml) for 24 h. Cell viability was measured by a luminescent CellTiter-Glo assay. Asterisks indicate significant differences compared with the control (***) $P < 0.001$.



Supplementary Figure 2: Effects of inhibitors of p38, c-Jun, and ERK on the combination of BNTX and TRAIL in AsPC-1 cells. (A, B, C) AsPC-1 cells were pre-treated with inhibitors (10 μM) for 0.5 h and followed by combined treatment of BNTX (2.5 μM) with TRAIL (25 ng/ml) for additional 24 h. Cell lysates were analyzed by western blot analysis using the indicated antibodies. D. Histograms represent mean ± SD of XIAP densities. Asterisks indicate significant differences compared with the control (** *P* < 0.01). NS, not significant.



Supplementary Figure 3: Effects of transient expression of XIAP on the combination of BNTX and TRAIL in AsPC-1 cells. (A) AsPC-1 cells were transfected with empty vector or HA-XIAP WT for 48 h and then incubated with BNTX (2.5 μ M) and TRAIL (25 ng/ml) for 24 h. Cell lysates were analyzed by western blot analysis using the indicated antibodies. The percentage of cell death was evaluated by trypan blue exclusion. Values are the mean \pm SD from two independent experiments performed in duplicate. Asterisks indicate significant differences compared with the control (***) $P < 0.001$. (B) AsPC-1 cells were transfected with empty vector or HA-XIAP S87D for 48 h and then incubated with BNTX (2.5 μ M) and TRAIL (25 ng/ml) for 24 h. Cell lysates were analyzed by western blot analysis using the indicated antibodies. The percentage of cell death was evaluated by trypan blue exclusion. Values are the mean \pm SD from two independent experiments performed in duplicate. Asterisks indicate significant differences compared with the control (***) $P < 0.001$.