

Smac Mimetics Increase Cancer Cell Response to Chemotherapeutics in a TNF- α Dependent Manner

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Supplementary Figure Legends

Supplementary Figure 1. JP1400/Chemotherapy agent combination effect in A2058 cells. A2058 cells were plated in 96 well plates for 16-24 h prior to the treatment of increasing concentration of gemcitabine, paclitaxel, 5-FU, cisplatin, etoposide, or SN38 in the presence or absence of 100 nM JP1400. After another 72 h, cells viability was determined by measuring ATP levels. Data are presented as mean \pm standard deviation of triplicates. Experiments were repeated three times with similar results.

Supplementary Figure 2. JP1400/Chemotherapy agent combination effect on cell proliferation. A2058 cells were treated with chemotherapy agents at the indicated concentration for 24 h in the presence or absence of 20 μ M z-VAD. Cells were then labeled with BrdU as described in the Materials and Methods. Cell proliferation rate was normalized with that of control samples. Data are presented as mean + standard deviation of triplicates. Experiments were repeated three times with similar results.

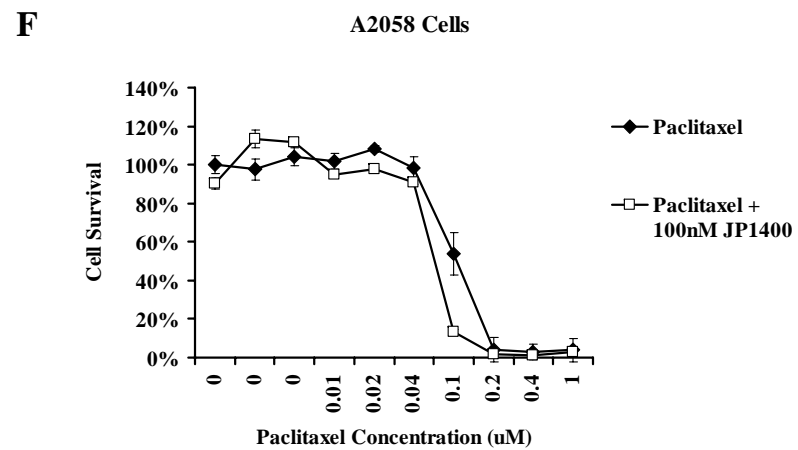
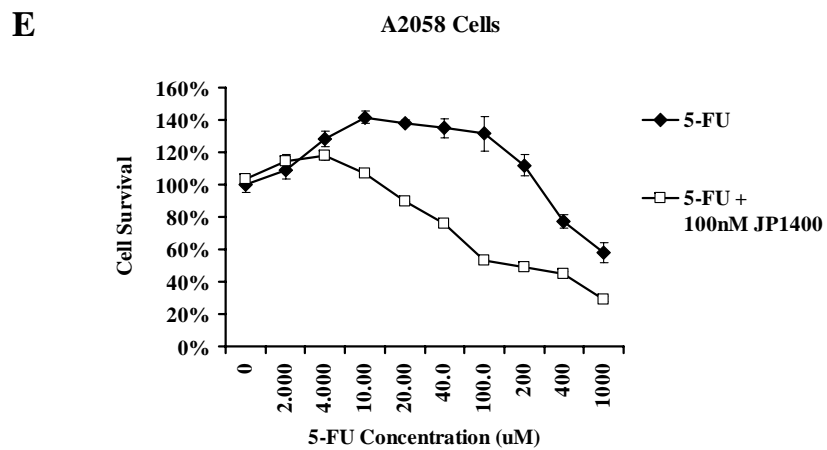
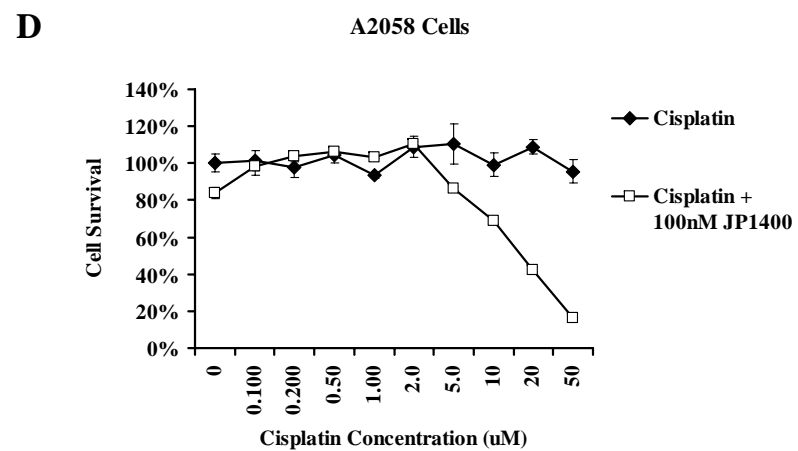
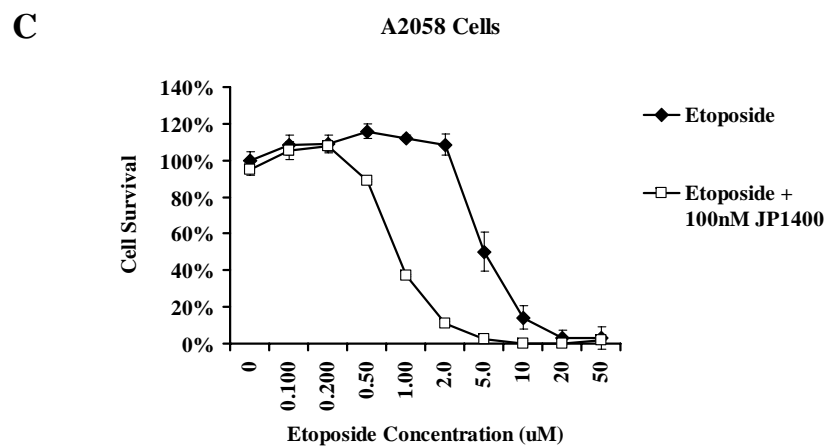
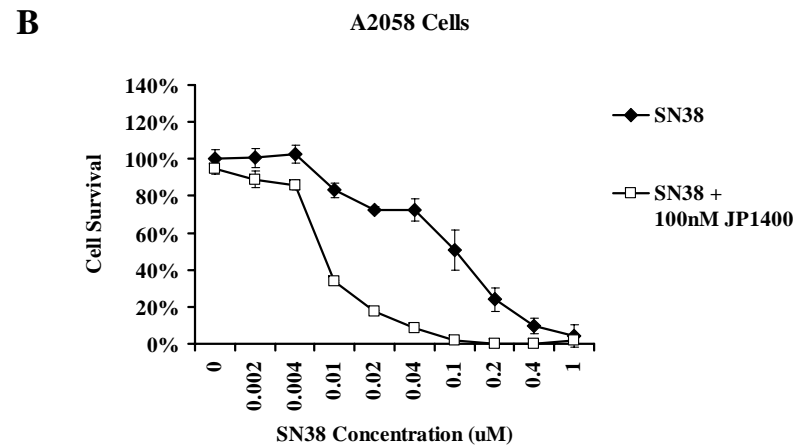
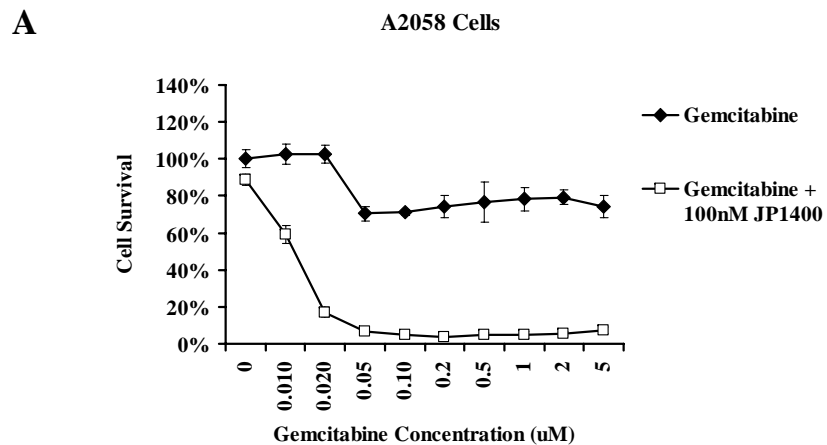
Supplementary Figure 3. JP1400/Chemotherapy agent combination effect on caspase-3 activation. A2058 cells (A) or HT-29 cells (B and C) were treated with JP1400 and/or chemodrugs at the indicated concentration. Cell lysates were collected after 4 h or 24 h. Caspase-3 and Actin levels were determined by western-blot analysis. Results are representative of two independent experiments.

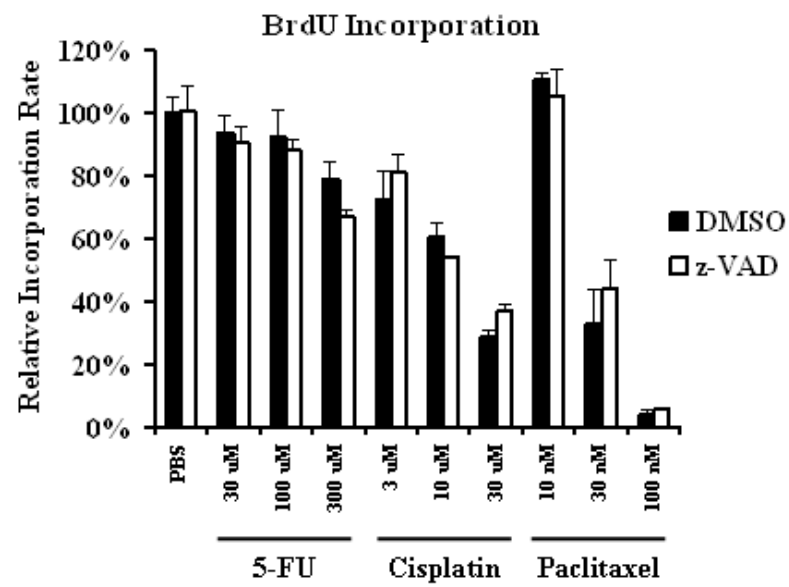
Supplementary Figure 4. JP1400/Chemodrugs mimetic synergism acts through autocrine TNF- α dependent pathway. HT-29 cells were pretreated with PBS or 5 μ g/ml TNF- α neutralizing antibody for 2 h, then treated with gemcitabine (300 nM), SN38 (100 nM), etoposide (10 μ M), or cisplatin (10 μ M) with or without 100 nM JP1400 for 48 h. Cell viability was determined by measuring ATP levels. Data are presented as average + range of duplicates. Experiments were repeated three times with similar results.

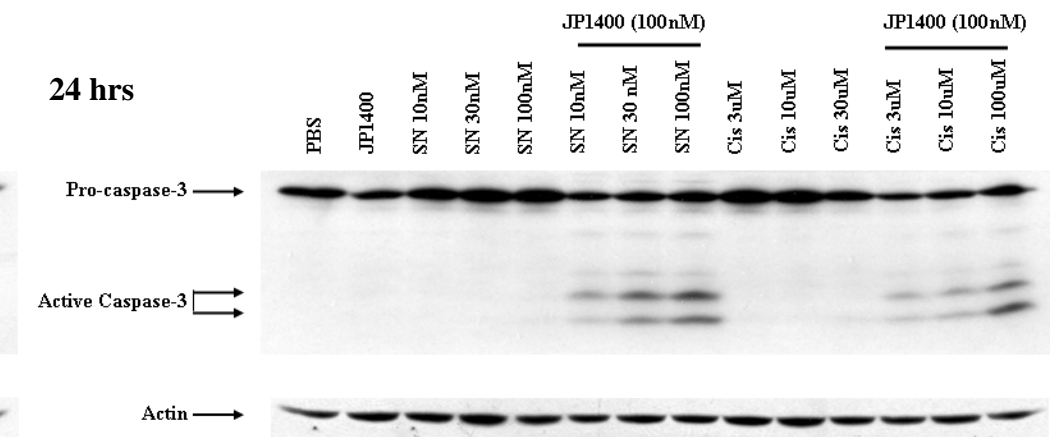
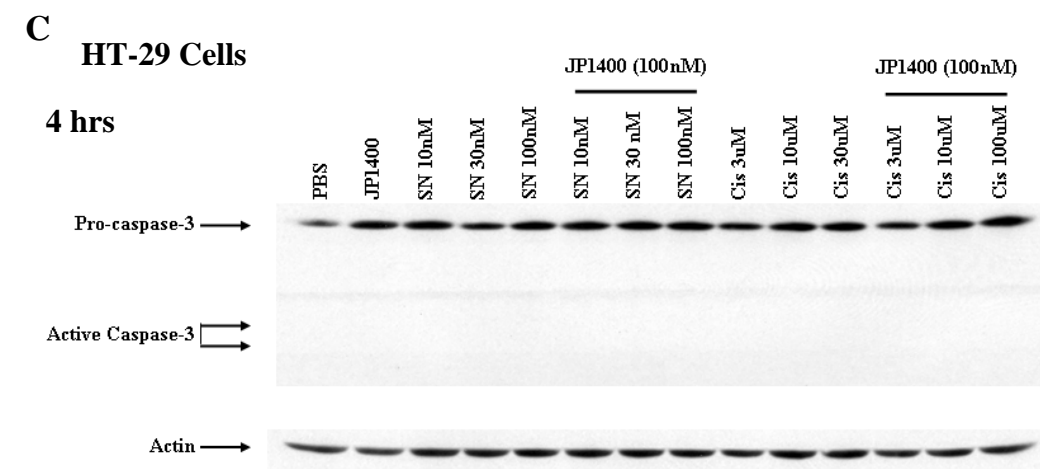
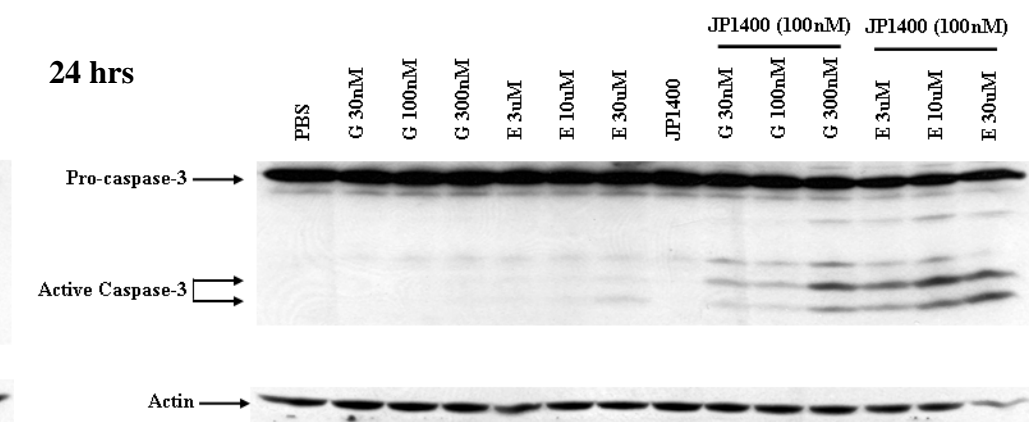
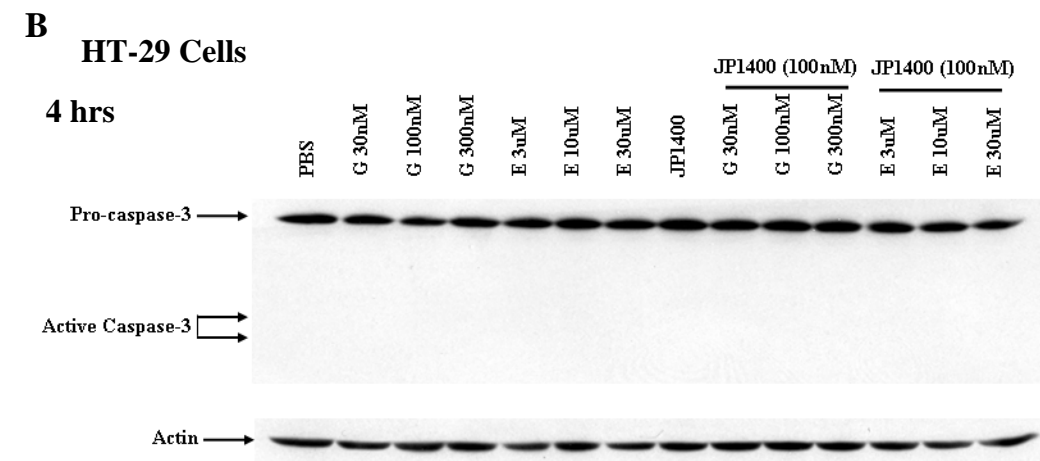
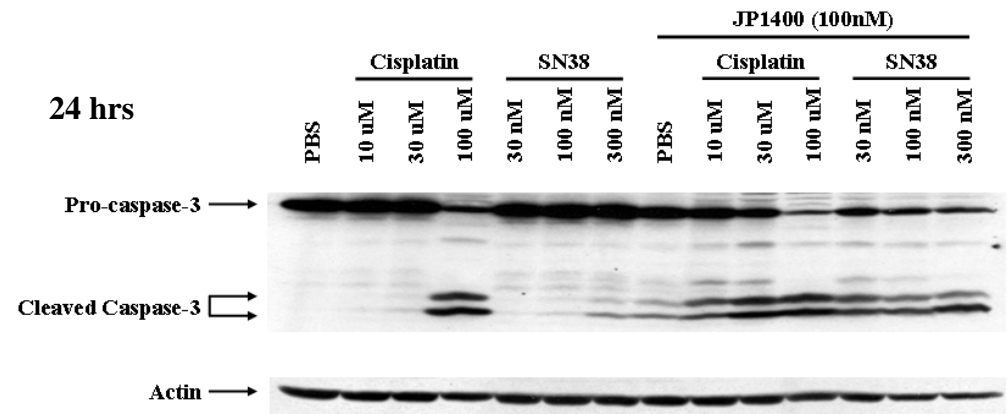
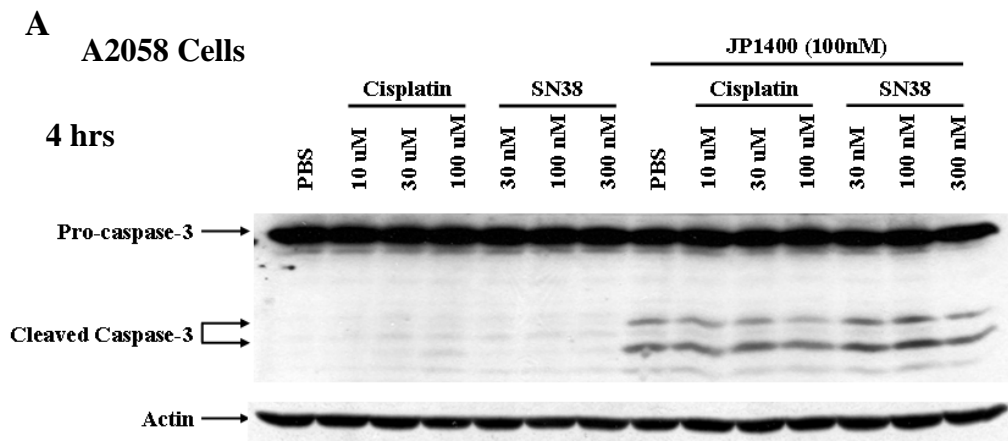
Supplementary Figure 5. Gemcitabine/JP1400 combination effect on TNF- α and cIAP protein levels. A2058 cells treated with 100 nM JP1400, 200 nM gemcitabine or the combination in the presence of 20 μ M z-VAD for 24 h. (A) Conditioned cell culture medium were collected and analyzed for TNF- α secretion by TNF- α ELISA. Data are

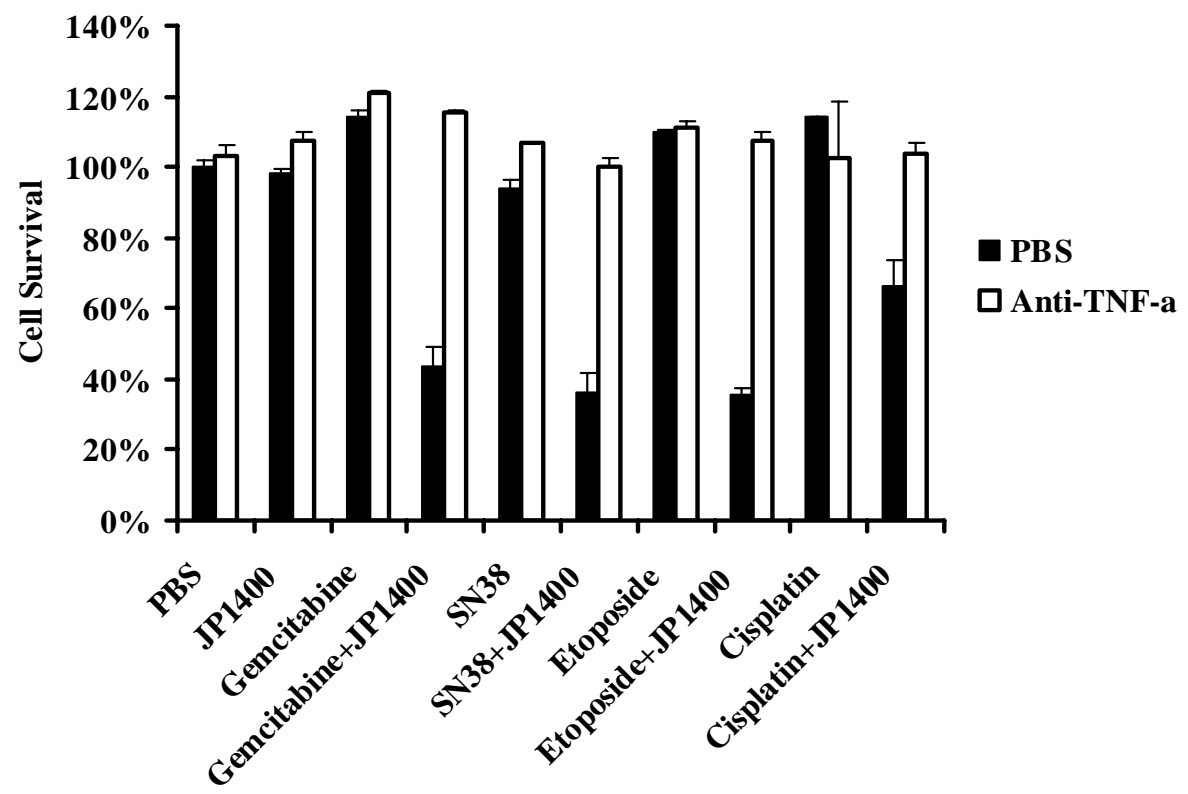
presented as mean + standard deviation of triplicates. (B) Cell lysates were collected and probed for cIAP1, cIAP2 and Actin protein levels by western-blot analysis. Experiments were repeated at least 2 times with similar results.

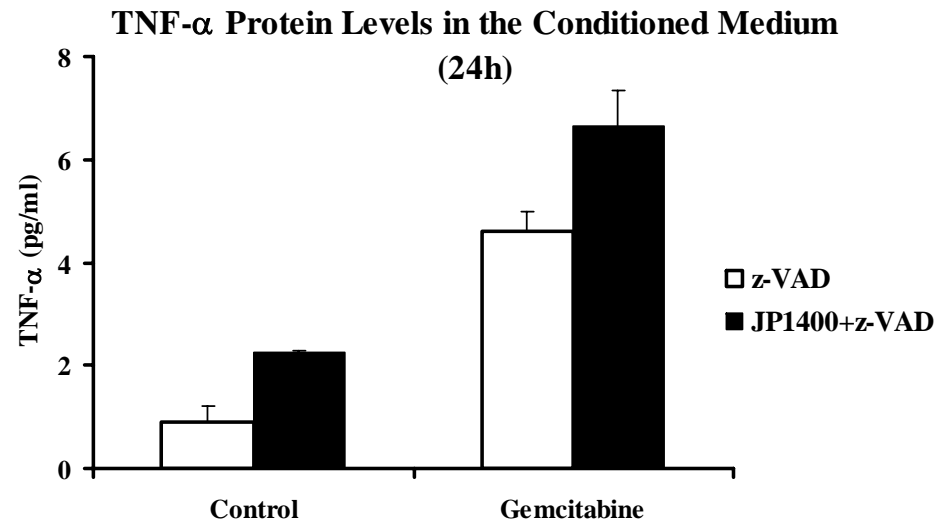
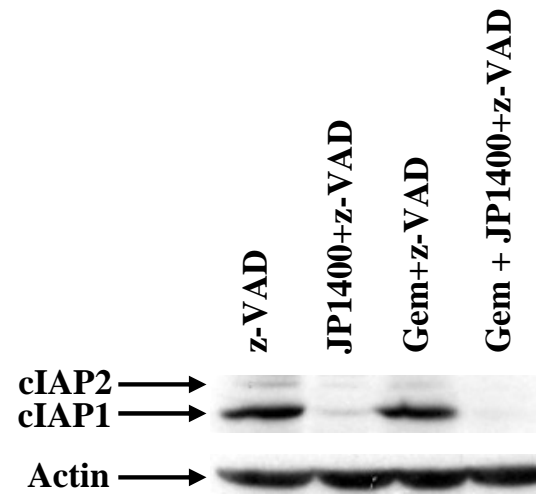
Supplementary Figure 6. JP1400 alone or JP1400/gemcitabine combination effect in Miapaca-2 cells *in vitro*. Miapaca-2 cells were plated in 96 well plates for 16-24 h prior to the indicated treatment. Cell viability was determined after 72 h by measuring intracellular ATP levels. Data are presented as mean \pm standard deviation of triplicates. Experiments were repeated at least 3 times with similar results.

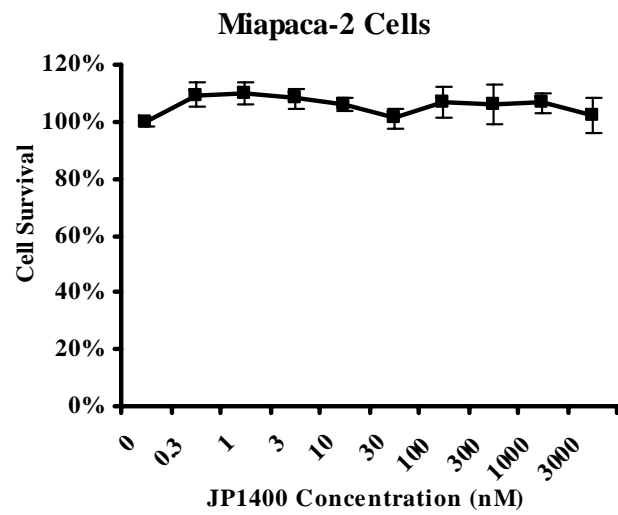








A**B**

A**B**