Supporting Information

Methods

Human Apoptosis Array

To shed light on the underlying mechanisms that might be responsible for sensitizing the colorectal cancer cell line Colo205 to TRAIL following treatment with aspirin, Colo205 cells were treated with 1mM aspirin for 18 h. Cells were plated a day prior to treatment without aspirin to ensure linear cell growth. Subsequently, aspirin was diluted in fresh media to a final concentration of 1 mM. Old media was carefully removed and replaced with new media containing aspirin. Post treatment, cells were collected using enzyme-free cell dissociation media, washed and lysed to extract total protein. 400 μg/mL of total protein was allowed to incubate over a human apoptosis protein array from R&D systems pre-conjugated with appropriate capture antibodies to detect multiple proteins involved in apoptosis. After incubation, the membranes were washed and treated with horseradish peroxidase for 7 minutes. Relative expression levels of various pro- and anti-apoptotic proteins were obtained from images taken on a Fuji Imaging system.

Results

Aspirin treatment affects multiple inhibitors of apoptosis

To shed light on the underlying mechanisms that might be responsible for sensitizing the colorectal cancer cell line Colo205 to TRAIL following treatment with aspirin, total protein extracted from cell lysates was used as described in Materials and Methods.

The array indicated a decrease in pro-survival proteins. While Bcl-2 downregulation was the largest, HSP27, PON2 and cIAP-2 all showed greater than fourfold difference in the relative

luminescence intensity (Figure S1 A, B) with no significant change in the death receptor expression (Figure S1 C). Colo205 treated with 0.1 mM aspirin alone for 18 h showed no significant difference in the expression levels of inhibitors of apoptosis.

Figure S1 A

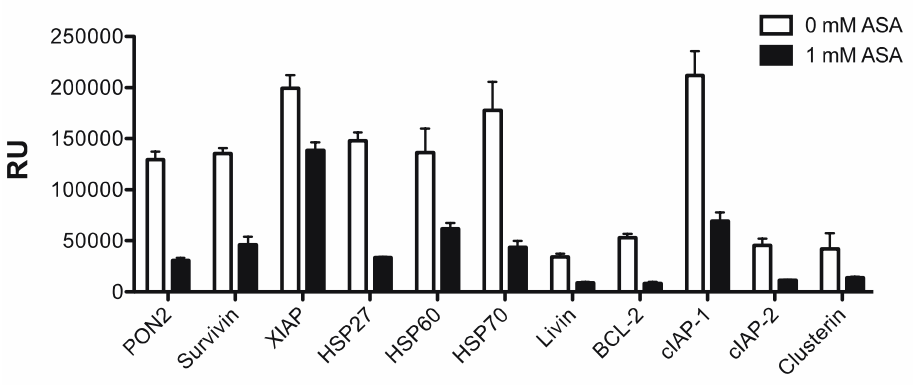

Figure S1 B

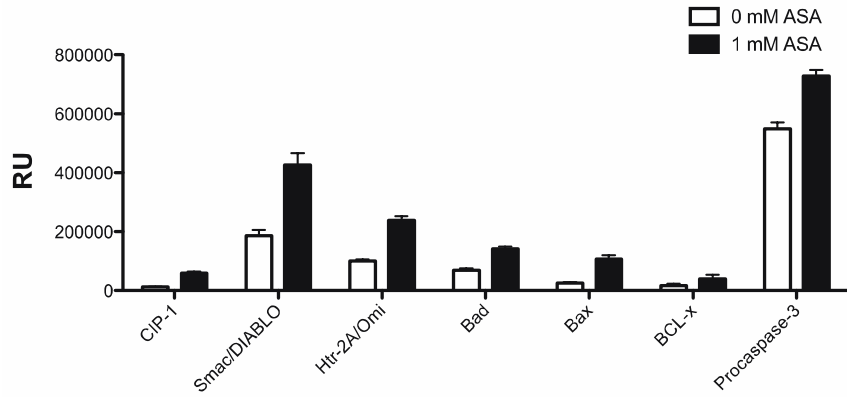

Figure S1 C

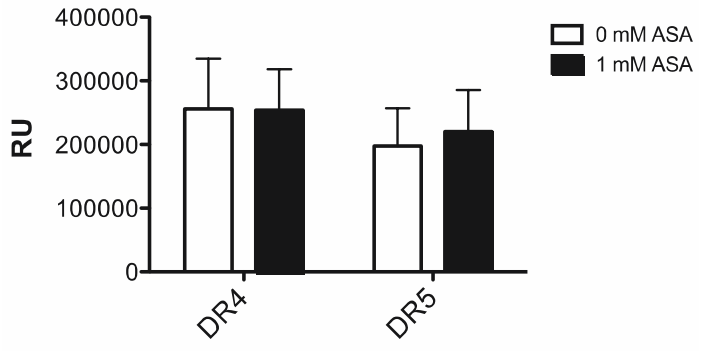

Figure S1. Results form human apoptosis array showing some of the important proteins involved in apoptosis regulation. Whole cell lysates from Colo205 cells treated with either 0 mM or 1 mM aspirin for 18 h were incubated over preconjugated membranes. (A) Relative expression of inhibitors of apoptosis following treatment with 0 mM and 1 mM aspirin treatment. (B) Relative expression of proapoptosis proteins following ASA treatment. (C) Relative expression of death receptors DR4 and DR5 following treatment with 0 mM and 1 mM aspirin treatment. Average ± SD, n=2.