Curcumin analog EF24 induces apoptosis via ROS-dependent mitochondrial dysfunction in human colorectal cancer cells

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Contents
Supplemental information includes 4 figures.
Figure S1 EF24 induced cell apoptosis in colon cancer cell lines
HCT-116, SW-620, and HT-29 cells were treated with DMSO or EF24 (2.5, 5, 7.5 μM) for 48 h, respectively. Control cells were pretreated with NAC (5 mM) for 30 min prior to the EF24 treatment. The cells were then collected and stained with Annexin V and PI, followed by apoptosis detection using flow cytometry. The data were obtained from three independent experiments performed in triplicate. Representative data are shown (A), and the quantified percentages of cells with early apoptosis are shown (B-D). Data are shown as mean ± SEM. The indicated differences are significant, * P < 0.05, ** P < 0.01, *** P < 0.001, and **** P < 0.0001, t-test, EF24-treated compared to the DMSO-treated group; # P < 0.05, ## P < 0.01, t-test, EF24 (7.5 μM) + NAC (5 mM)-treated compared to the EF24-treated group (7.5 μM).
**Figure S2** EF24 reduces mitochondrial membrane potential in HCT-116 cells

HCT-116 cells were treated with EF24 (7.5 μM) for 14 h. Control cells were pretreated with NAC (5 mM) for 30 min prior to the EF24 treatment. The mitochondrial membrane potential was determined by JC-1 staining using flow cytometer analysis. The data were obtained from three independent experiments performed in triplicate. Representative data are shown (A), and the quantified percentages of cells with JC-1 monomer in different groups are shown (B). Data are shown as mean ± SEM. The indicated differences are significant, ** P < 0.01, t-test, EF24-treated compared to the DMSO-treated group; ## P < 0.01, t-test, EF24 (7.5 μM) + NAC (5 mM)-treated compared to the EF24-treated group (7.5 μM).
Figure S3 Treatment with EF24 provoked the release of cytochrome c from mitochondria into cytosol. HCT-116 cells were treated with EF24 (7.5 μM) for 14 h. Control cells were pretreated with NAC (5 mM) for 30 min prior to the EF24 treatment. Then the cells were harvested and stained with anti-cytochrome c (red), Mito-T green fluorescence (green), and DAPI (blue) for detection of translocation of cytochrome c from the mitochondria to the cytosol. The morphology of mitochondria in HCT-116 cells was examined with an electron microscope (×20000). The data were obtained from three independent experiments performed in triplicate. Representative data are shown.
Figure S4 EF24 downregulates Sp1 in colon cancer cell lines. Colorectal cancer cells, including HCT-116 (A), SW620 (B), and HT-29 cells (C), were treated with different concentrations of EF24 (2.5, 5, and 7.5 μM) for 24 h. Control cells were pretreated with NAC (5 mM) for 30 min prior to the EF24 treatment. Then the cells were harvested and the whole-cell lysates were analyzed for Sp1 by Western blotting. The data were obtained from three independent experiments performed in triplicate. Representative data are shown.