Legends for Supplementary Figures:

Figure S1 : Chemical structure of berberine-Cl.

Figure S2: AOM/DSS induced colitis carcinogenesis animal study procedure.

Six week old FVB mice were given 10 mg/kg Azoxymethane (AOM) by intraperitoneal injection. One week later mice are exposed to 2% Dextran Sodium Sulphate (DSS) in the drinking water for 7 days. One day after DSS treatment, mice were treated with 40 mg/kg Berberine or PBS by oral gavage. On day 28, 5 mice from each group were euthanized and colons and liver tissues were collected. After 10 weeks, remaining mice of each group (n=10) were euthanized and colon tissue was collected. Berberine or vehicle (PBS) was administrated once a day for two continuous weeks, then three times a week until to the end of the study.

Figure S3: Berberine inhibits colon cancer cell growth and induces apoptosis *in vitro*.

2×10^4 cells (**A**) HCT1116, (**B**) SW480, (**C**) LOVO were seeded into 96 well plates and after 24 hours were treated with 0 to 100 μmol/L berberine. At 24, 48 and 72 hours after berberine treatment, the cell viability was determined using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay as described in Materials and Methods. Absorbance at 570nm was detected with Micro plate reader. The experiments were independently performed at three time repeated. *P< 0.05, **P < 0.01 and ***P < 0.001 indicates a significantly different compared with DMSO control by *t-test*. (**D**) HCT116 cells or (**E**) SW480 cells were grown in 100mm dishes (2×10^6 cells per dish) and

treated with indicated concentration of berberine (0-60 μmol/L) for 24 hours. Cells were stained with Annexin Ⅴ-FITC and Propidium Iodide, followed by flow cytometric analysis. Results were shown as mean±SE (n=3). The experiments were independently performed at three time repeated. ***P* < 0.01, indicates a significantly different compared with DMSO control.

Figure S4: Activation of AMPK is independent of LKB1 activation; berberine does not inhibit AKT and ERK activities; berberine does activate AMPK in mouse liver. Three CRC cell lines were treated with 0 to 60 μmol/L berberine for 24 hours. The extracted protein was analyzed by Western blot. (**A**) Berberine does not induce phosphorylation of LKB1. Total (LKB1) and phospho-LKB1 (p-LKB1) levels are shown. Blots are representative of three independent experiments. (**B**) Both AKT and ERK1/2 are phosphorylated in berberine treated CRC cells. Total and phosphorylated AKT (AKT and p-AKT respectively) and ERK1/2 (ERK and p-ERK respectively) are shown. Blots are representative of three independent experiments. (**C**) Berberine activated AMPK in the liver on day 28. Liver tissue was collected from 5 mice per group, lysed and protein extracts were analyzed by Western blot for total and phosphorylated AMPK. A represented of 2 blots from each group is shown. Densitometry analysis of p-AMPK levels relative to total AMPK for each mouse (n=5) is shown.