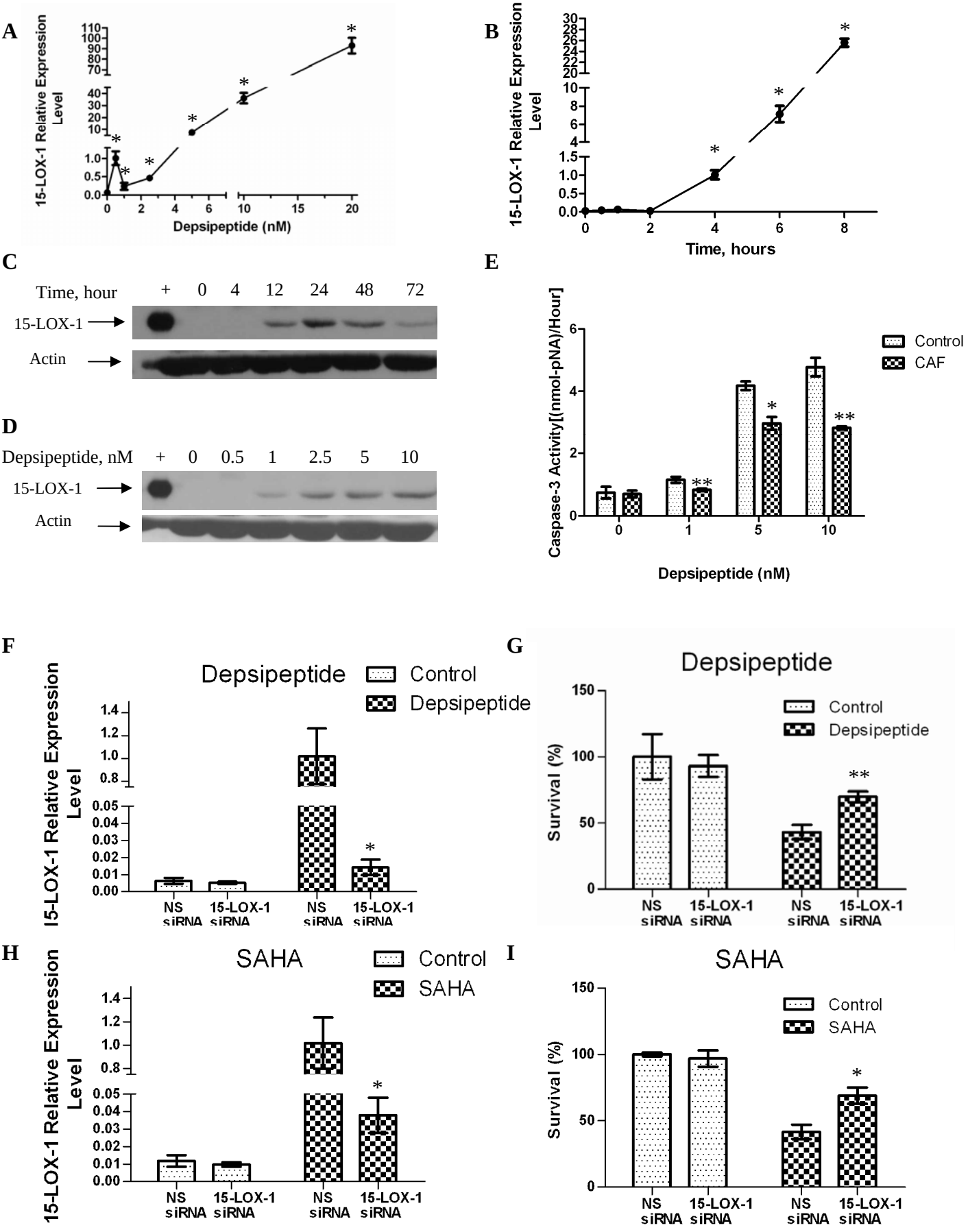
Supplementary Figure 1



Supplementary Figure 1 legend in the next page.

Fig.1 HDAC1 and HDAC2 inhibition and 15-LOX-1 transcription activation. (A and B) Depsipeptide effects on 15-LOX-1 mRNA expression in SW480 colon cancer cells. SW480 cells were treated with depsipeptide at various concentrations (0-20 nM) for 24 hours (A) and for different time periods (B) at a concentration of 5 nM, and 15-LOX-1 mRNA expression was measured with real-time PCR. The relative expression levels were calculated as the values relative to that of the calibrator sample (time point or concentration 0). Values are the means ± SDs of triplicate experiments. (C and D) Depsipeptide induction of 15-LOX-1 protein expression in SW480 cancer cells. SW480 cells were treated for various times (at 5 nM depsipeptide concentration, Fig. 1C) and with various concentrations (for 24 h. Fig. 1D) as indicated and processed for 15-LOX-1 Western blotting. + indicates positive control (HCT-116 cells transfected with a 15-LOX-1 expression vector). (E) Effects of 15-LOX-1 enzymatic activity on depsipeptide-induced apoptosis in SW480 cancer cells. SW480 cells treated with various depsipeptide concentrations, as indicated, were cultured with or without caffeic acid (CAF) at the 2.2 μM concentration that specifically inhibits 15-LOX-1 enzymatic activity. Apoptosis was assessed by measuring caspase 3 activity levels. Values are the means ± SDs of triplicate measurements. F-I. Effects of 15-LOX-1 downregulation on depsipeptide- and SAHA- induced survival inhibition in colon cancer cells. SW480 colon cancer cells were transfected with a pool of four siRNA duplexes for 15-LOX-1 or nonspecific siRNA sequence (NS siRNA). 24 h later, they were treated with either (F and G) depsipeptide (5 nM) or (H and I) SAHA (1μM). Control indicates vehicle-solvent treated cells. 48 h later, 15-LOX-1 mRNA was measured by real-time PCR (depsipeptide [F], SAHA treatment [H]). G and I. Cell survival was assessed by SRB assays 72 h after treatment. Values are presented as the survival percentages relative to control (solvent)-treated cells transfected with nonspecific siRNA. Values shown are the means ± SDs of triplicate measurements.* $P < 0.0001$; ** $P < 0.005$.

Supplementary Figure 2

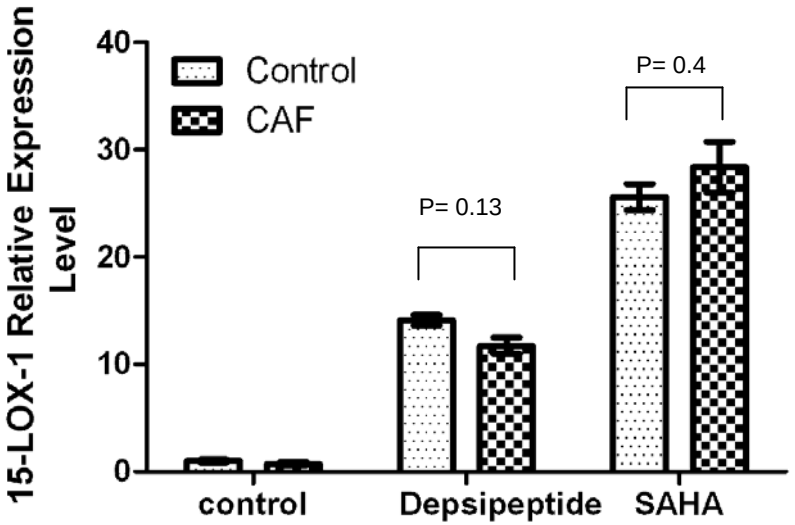


Fig.2 Caffeic acid effects on 15-LOX-1 transcription activation by depsipeptide. Caco-2 cells were treated with either depsipeptide (5nM) or SAHA (1 μM) with or without caffeic acid (CAF) at the 2.2 μM for 24 hours prior to measuring 15-LOX-1 mRNA expression by real-time PCR.

Supplementary Figure 3

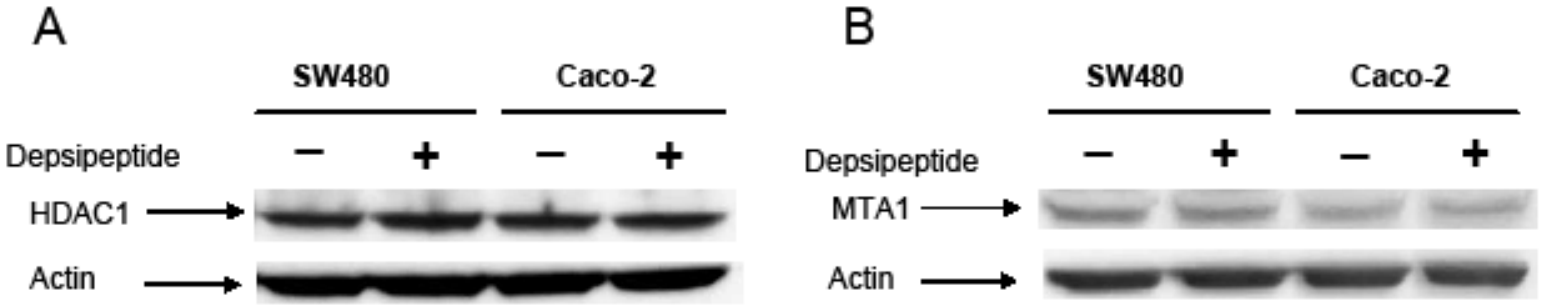


Fig. 3. Effects of depsipeptide on HDAC1 and MTA1protein expression in colon cancer cells. Caco-2 and SW480 colon cancer cells were treated with depsipeptide and then harvested at 15 min (Caco-2) and 3.5 h (SW480) (same time points for ChIP/real-time PCR measurements for Fig. 4A and B). HDAC1 (A) and MTA1 (B) protein expression levels were measured by Western blotting. (+) indicates depsipeptide-treated cells. Control cells (-) were treated with the vehicle solvent for depsipeptide.

Supplementary Figure 4

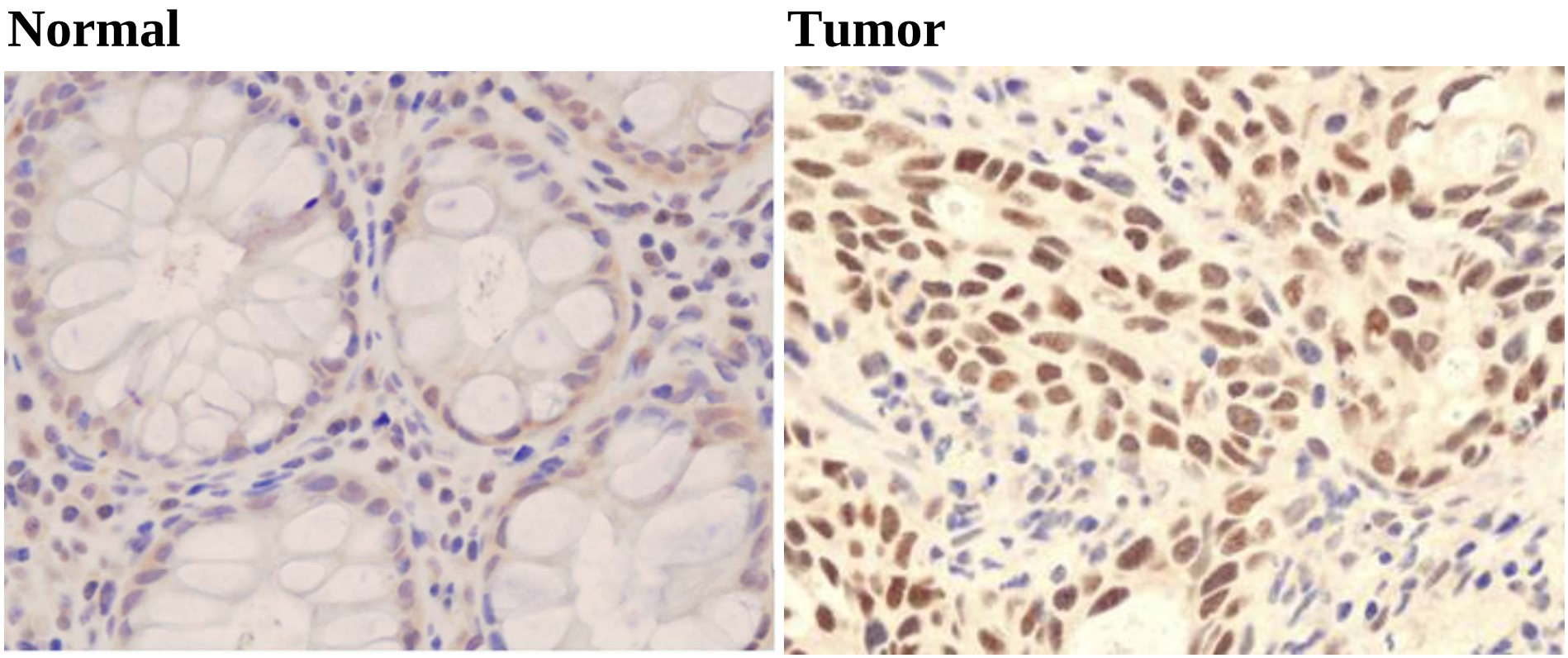


Fig.4 MTA-1 expression in normal and colorectal cancer tissues. *A)* Paired normal and colorectal cancer tissues that were immunohistochemically stained for expression of MTA-1. MTA-1 nuclear staining is shown in brown. (magnification ×400).