Salinomycin nanocrystals for colorectal cancer treatment through inhibition of Wnt/β-catenin signalling

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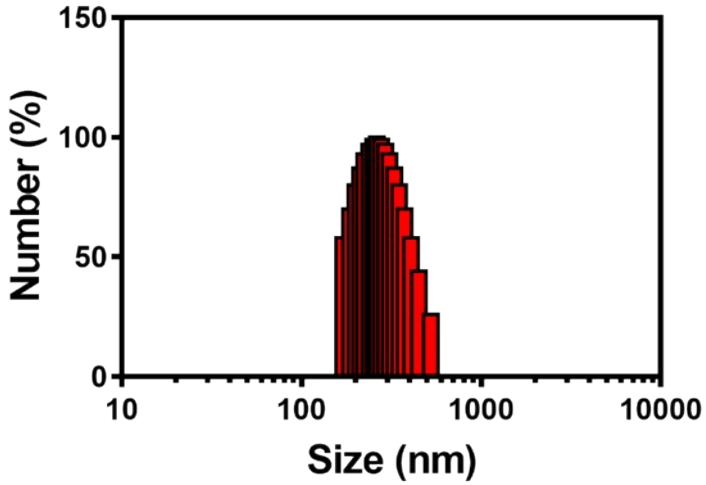

Fig. S1. Size distribution of SAL NCs.

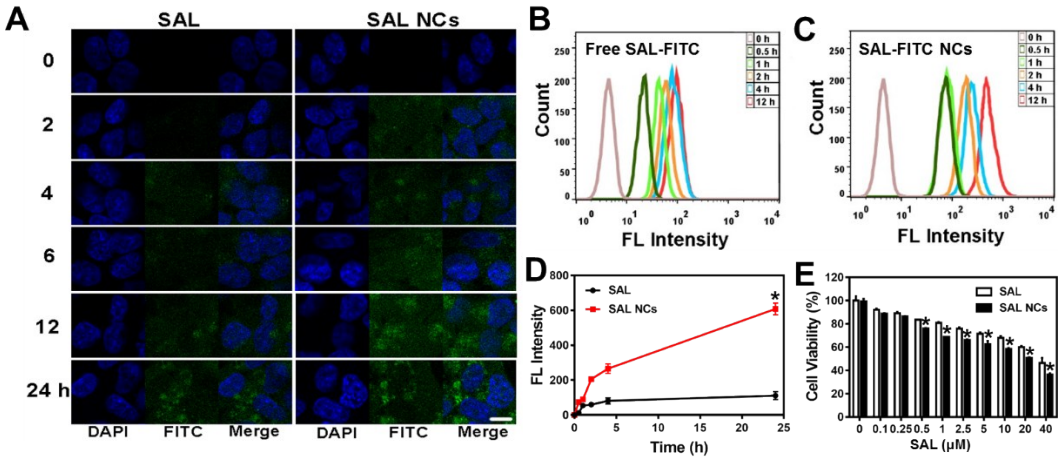

Fig. S2. Cellular uptake and cytotoxicity of free SAL and SAL NCs *in vitro*. (A) Confocal laser scanning microscopy (CLSM) images of HT29 cells incubated with free SAL and SAL NCs at equivalent 1 μM SAL concentration for the indicated times; (B-C) Flow cytometry analyses of cells incubated with free SAL (B) and SAL NCs (C) at equivalent 1 μM SAL concentration for the indicated times; (D) Quantitated results of B and C. (E) Relative viability of HCT116 cells incubated with free SAL and SAL NCs at indicated equivalent SAL concentrations for 24 h. Values are means ± SEM. Statistical analysis was performed with Student's t test. * $P < 0.05$. Scale bar, 20 μm.

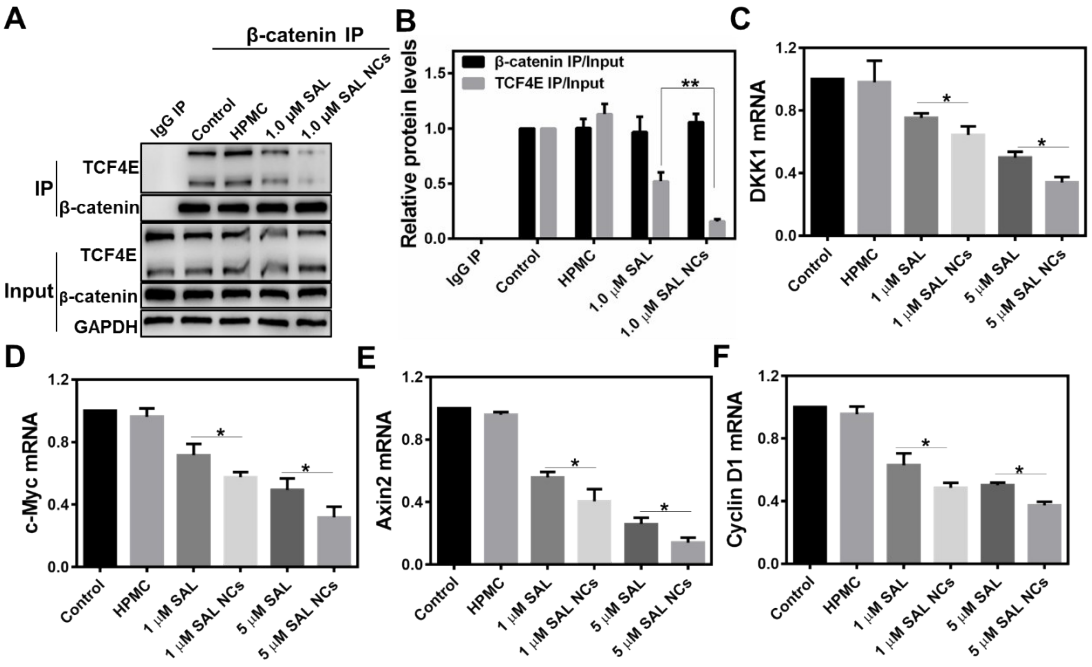

Fig. S3. Repression of Wnt/β-catenin signaling in colorectal cancer cells. (A) HCT116 cells were incubated with the indicated reagents for 24 h, and then immunoprecipitation was performed using control IgG or β-catenin antibody. The interaction between β-catenin and TCF4E was visualized using immunoblots; (B) The blots shown in A *were quantitated by densitometry; (C-F)* HCT116 cells were incubated with the indicated reagents for 24 h and mRNA levels of Wnt target genes DKK1 (C), c-Myc (D), Axin2 (E), and Cyclin D1 (F) were examined by real-time PCR (n = 3). Values are means ± SEM. Statistical analysis was conducted using Student's t test. * $P < 0.05$, *** $P < 0.001$.

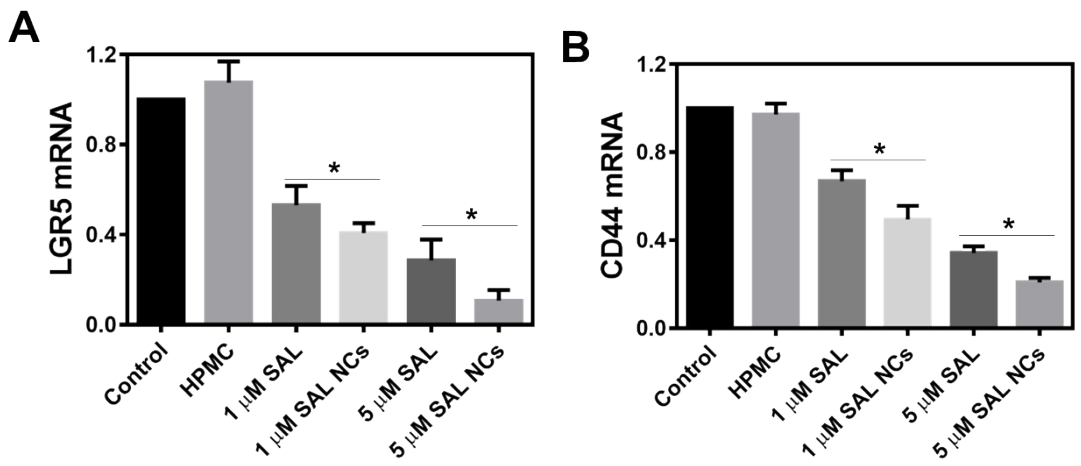

Fig. S4. Inhibition of stemness in colorectal cancer cells. (A-B) HCT116 cells were incubated with the indicated reagents for 24 h and mRNA levels of stemness marker genes LGR5 (A) and CD44 (B) were measured by real-time PCR (n = 3). Values are means ± SEM. Statistical analysis was conducted using Student's t test. * $P < 0.05$. Scale bar, 100 μm.