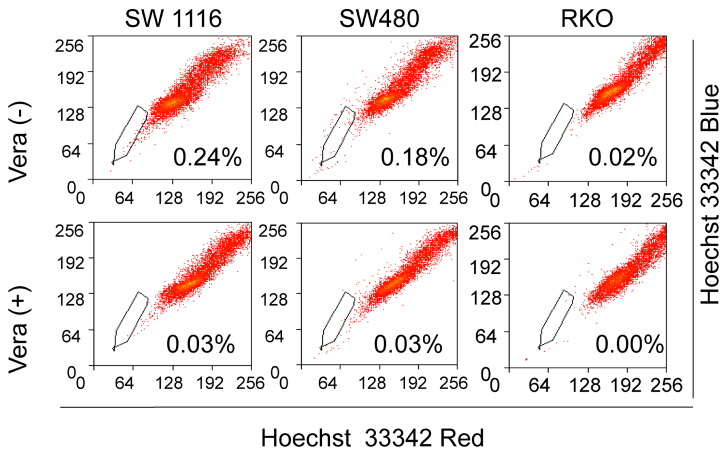
Supplemental Materials and Methods

Primers used for PCR

Specific primers were synthesized by Sigma-Aldrich (Louis, MO, USA) and the sequences were described as follows: CD133: forward: 5'-agtcggaaactggcagatagc-3', reverse: 5'-ggtagtgttgtactgggccaat-3'; Oct4: forward: 5'-cttgaatcccgaatggaaaggg-3', reverse: 5'-gtgtatatcccagggtgatcctc-3'; SOX2: forward: 5'-gccgagtggaaacttttgtcg-3', reverse: 5'-ggcagcgtgtacttatccttct-3'; ABCG2: forward: 5'-caggtggaggcaaatcttcgt-3', reverse: 5'-accctgttaatccgttcgtttt-3'; xCT: forward: 5'-tctccaaaggaggttacctgc-3', reverse: 5'-agactcccctcagtaaagtgac-3'; CD44: forward: 5'-ctgccgctttgcaggtgta-3', reverse: 5'-cattgtgggcaaggtgctatt-3'; CD44v8-10: forward: 5'-agaatccctgctaccaatatggactc-3', reverse: 5'-aggtcactgggatgaaggtc-3'; β-Actin: forward: 5′-catgtacgttgctatccaggc-3′, reverse: 5′-ctccttaatgtcacgcacgat-3′.

Figure S1 SP cell fraction and cell stemness characteristics in CRC cells. **(A)** The percentages of SP cells in CRC cell lines (HCT1116, SW480, RKO) were compared using Hoechst 33342 staining followed by flow cytometry. **(B)** SP cells and non-SP cells were sorted from the LoVo or SW 620 cells using flow cytometry, and their tumor-initiating activity was measured by evaluating the tumor incidence rate at the implantation sites in nude mice 4 weeks after injection. **(C)** The stem cell markers CD44, CD133, Oct4, ABCG2 and Sox2 were analyzed using qPCR in both SP and non-SP cells. The data are presented as the mean±SD of three independent experiments. *$P < 0.05$ versus control cells.

A



B

		Experiment Group	Cell number Inoculated	Tumor Incidence (%)	
LoVo	#1	Non-SP	1×10^4	0/10	0
		SP	1×10^4	3/10	30%
	#2	Non-SP	1×10^5	4/10	40%
		SP	1×10^5	9/10	90%
SW620	#1	Non-SP	1×10^4	0/10	0%
		SP	1×10^4	4/10	40%
	#2	Non-SP	1×10^5	3/10	30%
		SP	1×10^5	8/10	80%

C

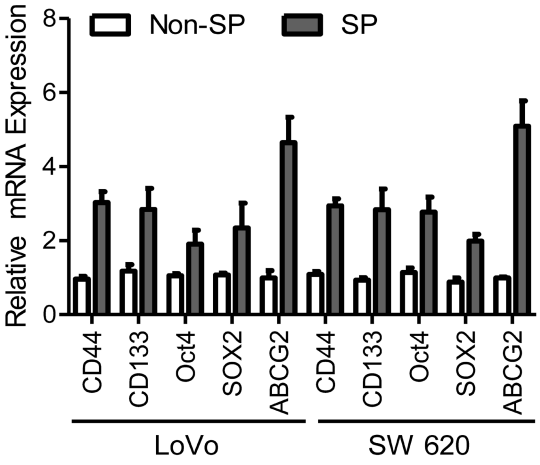


Figure S2 The xCT expression levels and cellular GSH content were evaluated in CRC cells after RNAi depletion of CD44 and xCT. **(A)** qPCR analysis the mRNA expression of xCT in LoVo or SW620 cells transfected with two independent xCT siRNA or with scrambled control siRNA (sc), respectively. **(B)** Cellular GSH content was measured in SW620 cells transfected with two independent CD44 or xCT siRNA, respectively. **(C)** The LoVo cells were co-transfected with siRNA#1 and siRNA#1-resistant expression vector of CD44 or xCT for 48 h, then the % of SP in LoVo cells were measured by flow cytometry. The data are presented as the mean±SD of three independent experiments. **P* < 0.05 versus control cells.

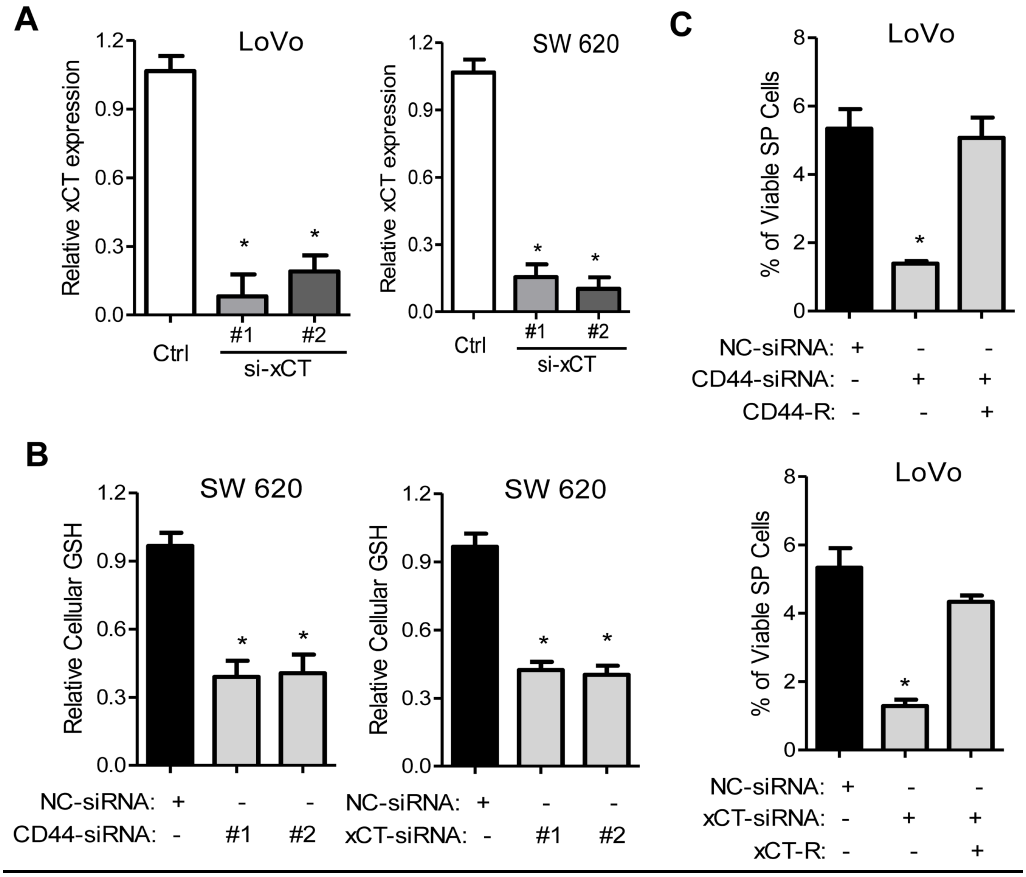
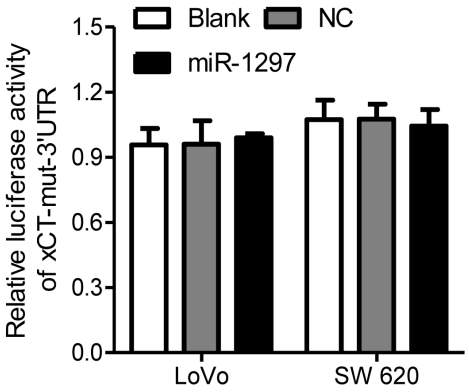


Figure S3 (A) The miR-1297 mimics or the negative control, a pGL3 luciferase vector containing the mutant type of the xCT 3'UTR, were cotransfected into LoVo and SW620 cells, and their relative firefly luciferase activity was measured. **(B)** LoVo and SW620 cells were pretreated with 3 mM N-acetylcysteine (NAC, a GSH precursor) for 2 h before miR-1297-mimic transfection. The ROS levels and the % of SP cells in LoVo and SW620 cell populations were measured via flow cytometry. The data are presented as the mean±SD of three independent experiments. **P* < 0.05 versus control cells.

A



B

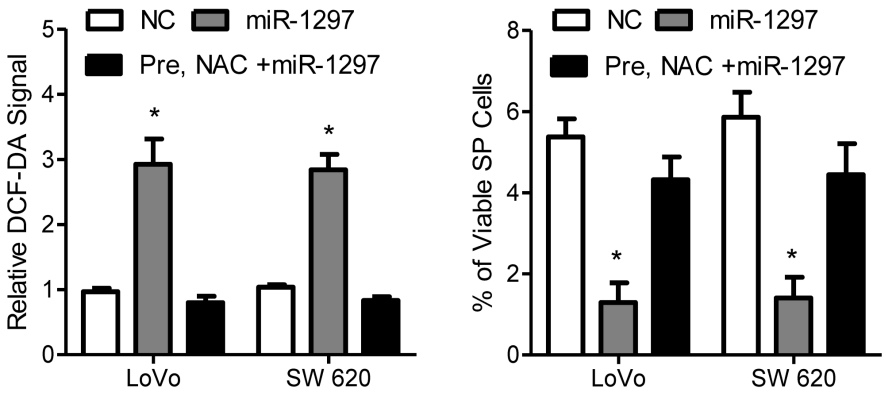


Figure S4. Comparison the cytotoxic effects of PEITC and 5FU on CRC cells. **(A)** SW620 and LoVo cells were incubated with PEITC for 48 h at the indicated concentrations, and cell viability was measured via MTS assay. **(B)** SW620 and LoVo cells were incubated with 5FU for 48 h at the indicated concentrations, and cell viability was measured via MTS assay. **(C)** CD44, CD133, Oct4, ABCG2 and SOX2 expression levels were analyzed via qPCR in the indicated cells treated with 5FU (IC50) for 48 h. **(D)** CRC cells were pretreated with the IC50 concentrations of PEITC or 5FU for 48 h. They were then washed and cultured in fresh medium without either drug for 48 h to allow cell death to occur. Cells were harvested and then stained with Hoechst 33342 to identify SP cells before and after the 48 h drug-free incubation. Data are presented as the mean±SD (n=3). *P < 0.05 versus control.

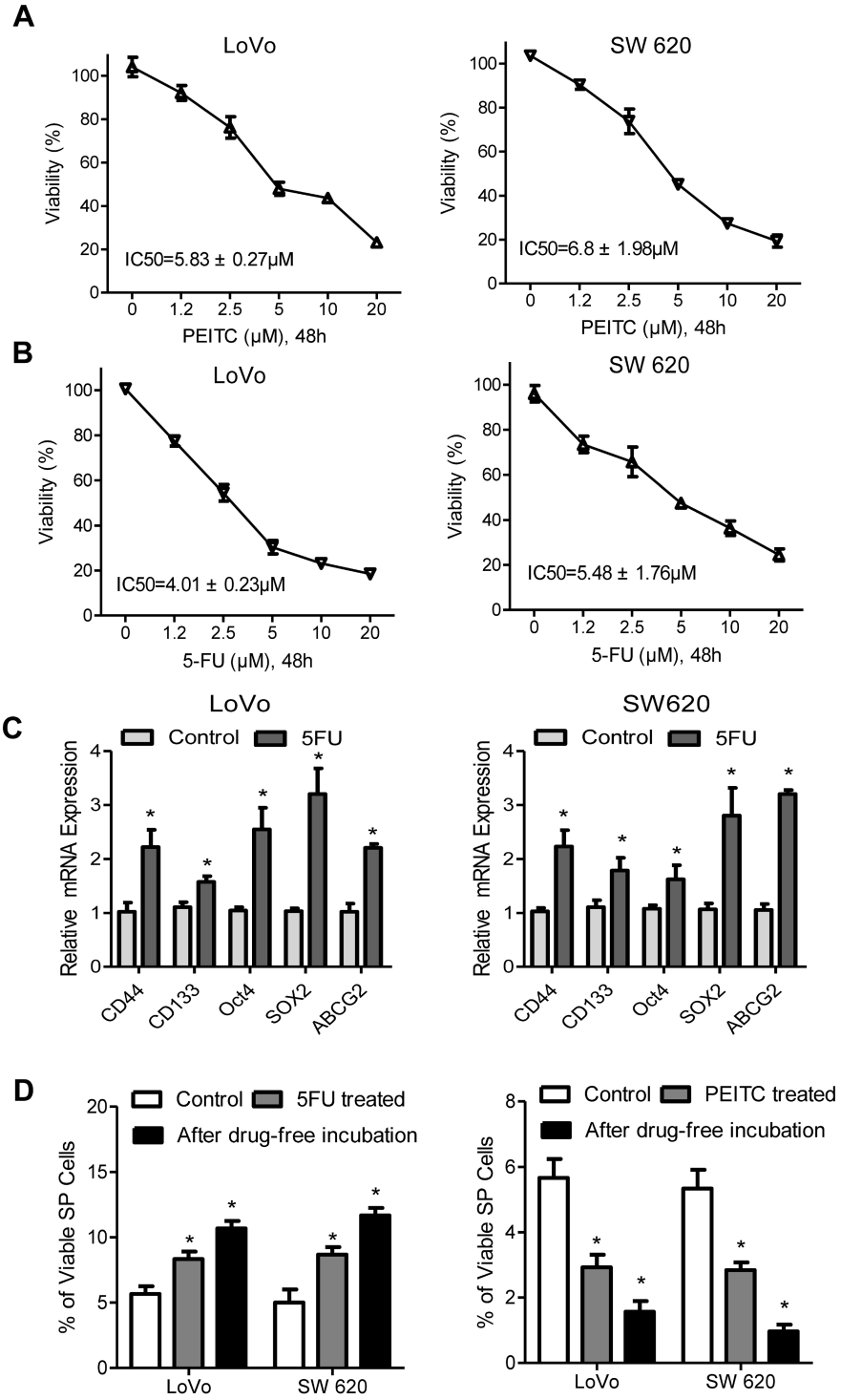


Figure S5 (A) Depletion of cellular GSH contents and increased ROS levels in response to SSZ in both LoVo and SW620 cells as measured by spectrophotometric and flow cytometric analyses, respectively. **(B)** CRC cells were pretreated with SSZ (100 μM) for 48 h and then washed and cultured in fresh medium without the drug for 48 h to allow cell death to occur. Viable cells were harvested and then stained with Hoechst 33342 to identify SP cells, and the viable SP cells were quantified. **(C)** Paraffin-embedded tumor sections were stained with H&E or anti-CD44 antibody (scale bar: 100 μm). Data in A and B are presented as the mean±SD (n=3). **P* < 0.05 versus control.