SUPPLEMENTARY DATA

Title:

Sulforaphane improves chemotherapy efficacy by targeting cancer stem cell-like properties via the miR-124/IL-6R/STAT3 axis

Authors:

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Authors' contributions *:

Xingxing Wang, Yuan Li, Yi Dai, and Qinqiang Liu, contributed equally to this work.

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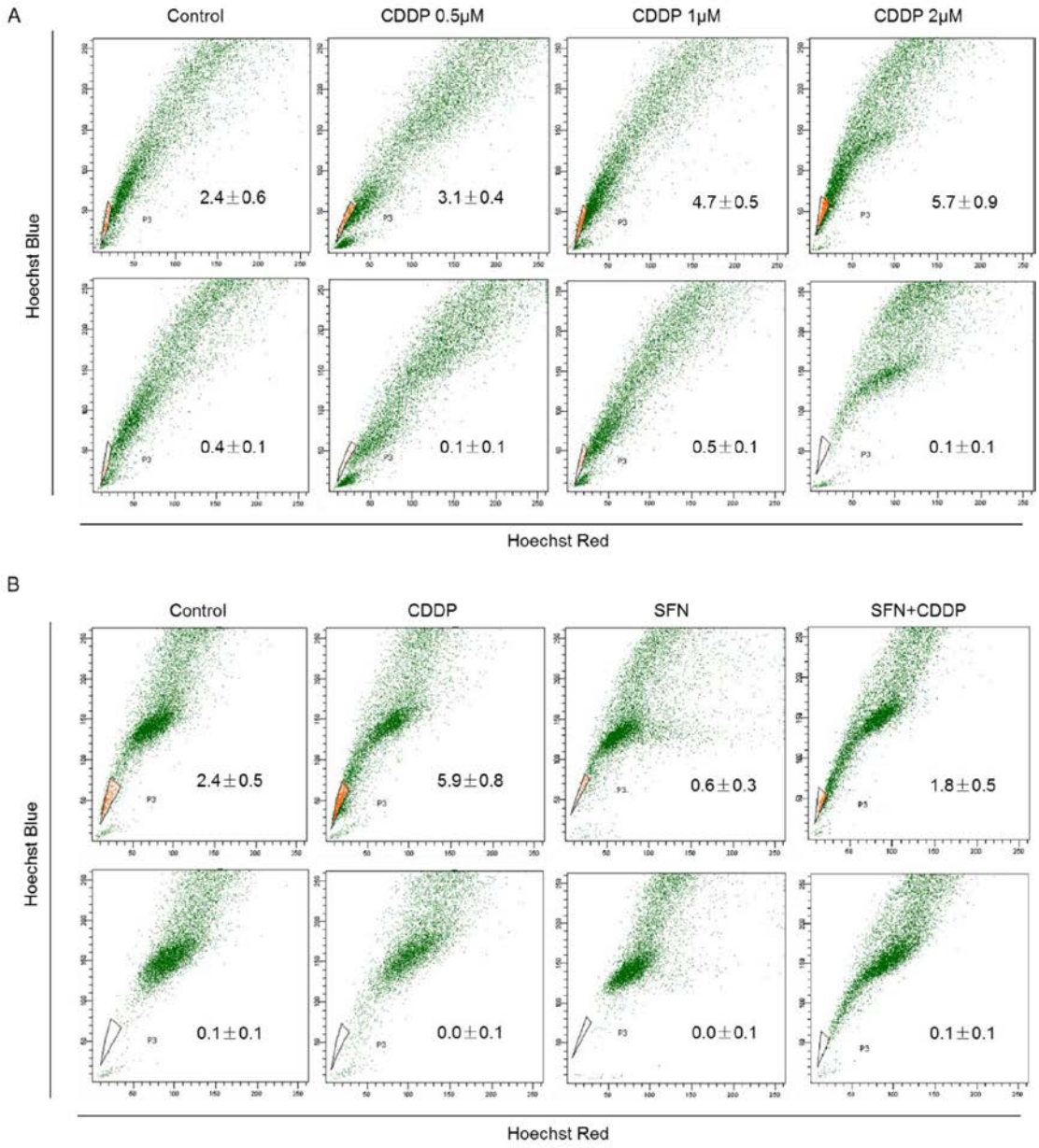

A
Control
CDDP 0.5μM
CDDP 1μM
CDDP 2μM
2.4±0.6
3.1±0.4
4.7±0.5
5.7±0.9
0.4±0.1
0.1±0.1
0.5±0.1
0.1±0.1
Hoechst Blue
Hoechst Red
B
Control
CDDP
SFN
SFN+CDDP
2.4±0.5
5.9±0.8
0.6±0.3
1.8±0.5
0.1±0.1
0.0±0.1
0.0±0.1
0.1±0.1
Hoechst Blue
Hoechst Red

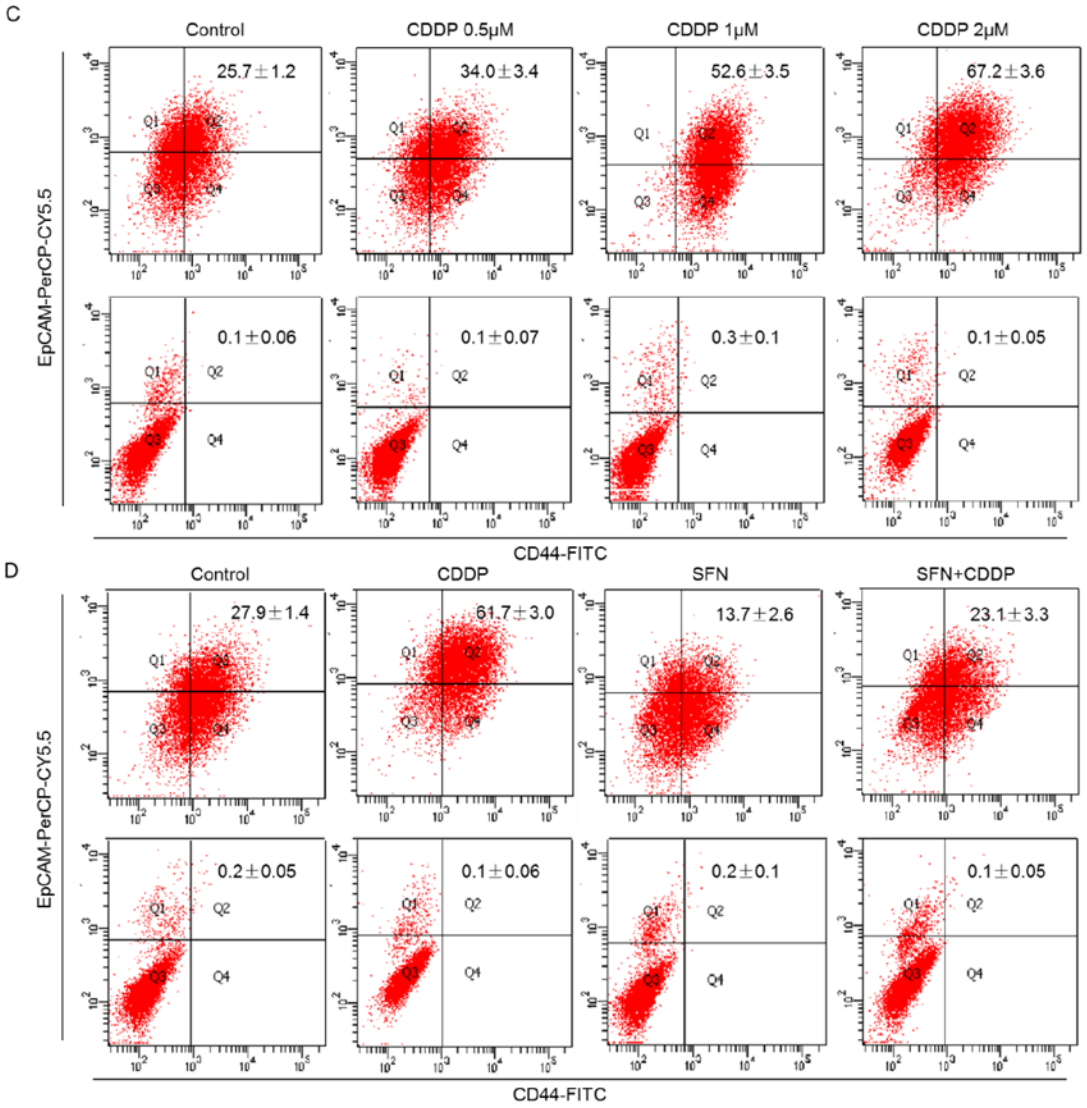


Fig. S1. The effects of SFN and CDDP on the CSC-like properties in MGC803.

(A, C) MGC803 cells were treated with 0, 0.5, 1, or 2 μM of CDDP for 72 h. **(B, D)** MGC803 cells were treated with 2 μM of CDDP, 10 μM of SFN, or their combination for 72 h. **(A, B)** Flow cytometry analyses of the percentage of SP and **(C, D)** $CD44^+/EpCAM^+$ cells in MGC803 cell populations (mean ± SD, n = 3).

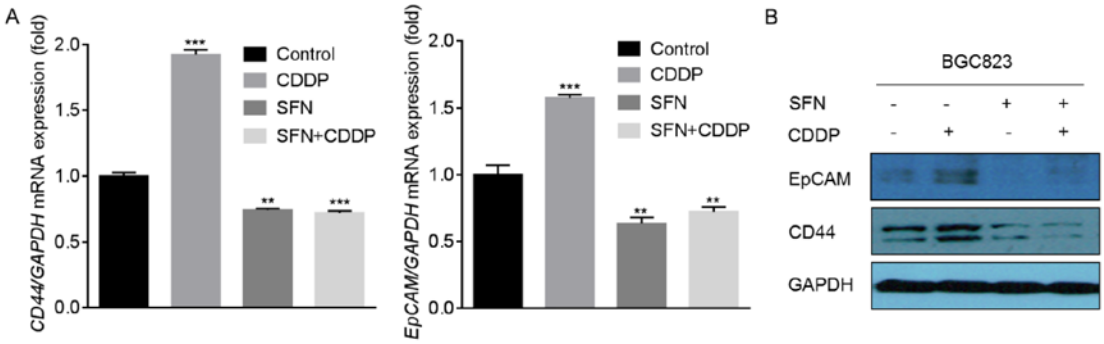


Fig. S2. SFN represses the CDDP-induced CSC surface markers in BGC823.

(A, B) BGC823 cells were treated with 2 μM of CDDP, 10 μM of SFN, or their combination for 72 h. **(A)** qRT-PCR analyses of *CD44* and *EpCAM* (mean ±SD, n=3). ***p* < 0.01 and ****p* < 0.001compared with medium control BGC823 cells. **(B)** Western blot analysis of CD44 and EpCAM.

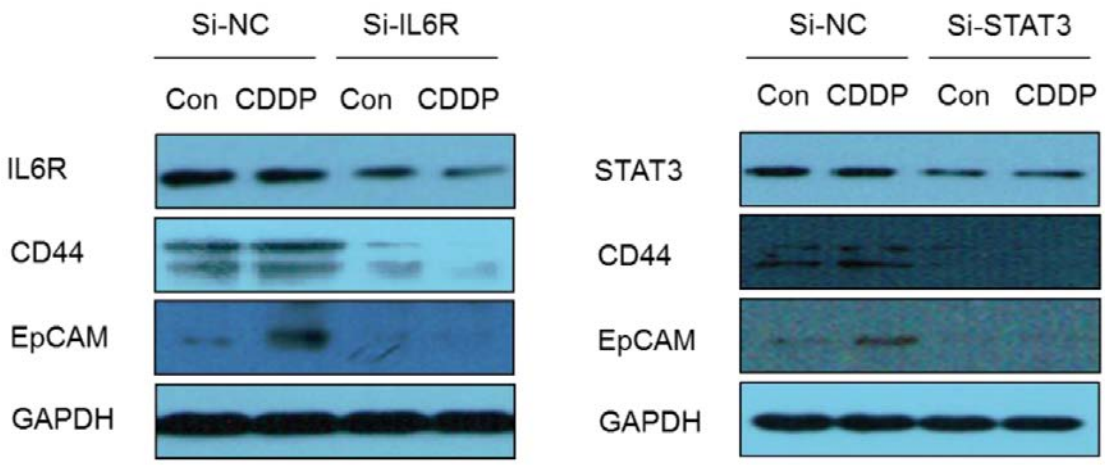
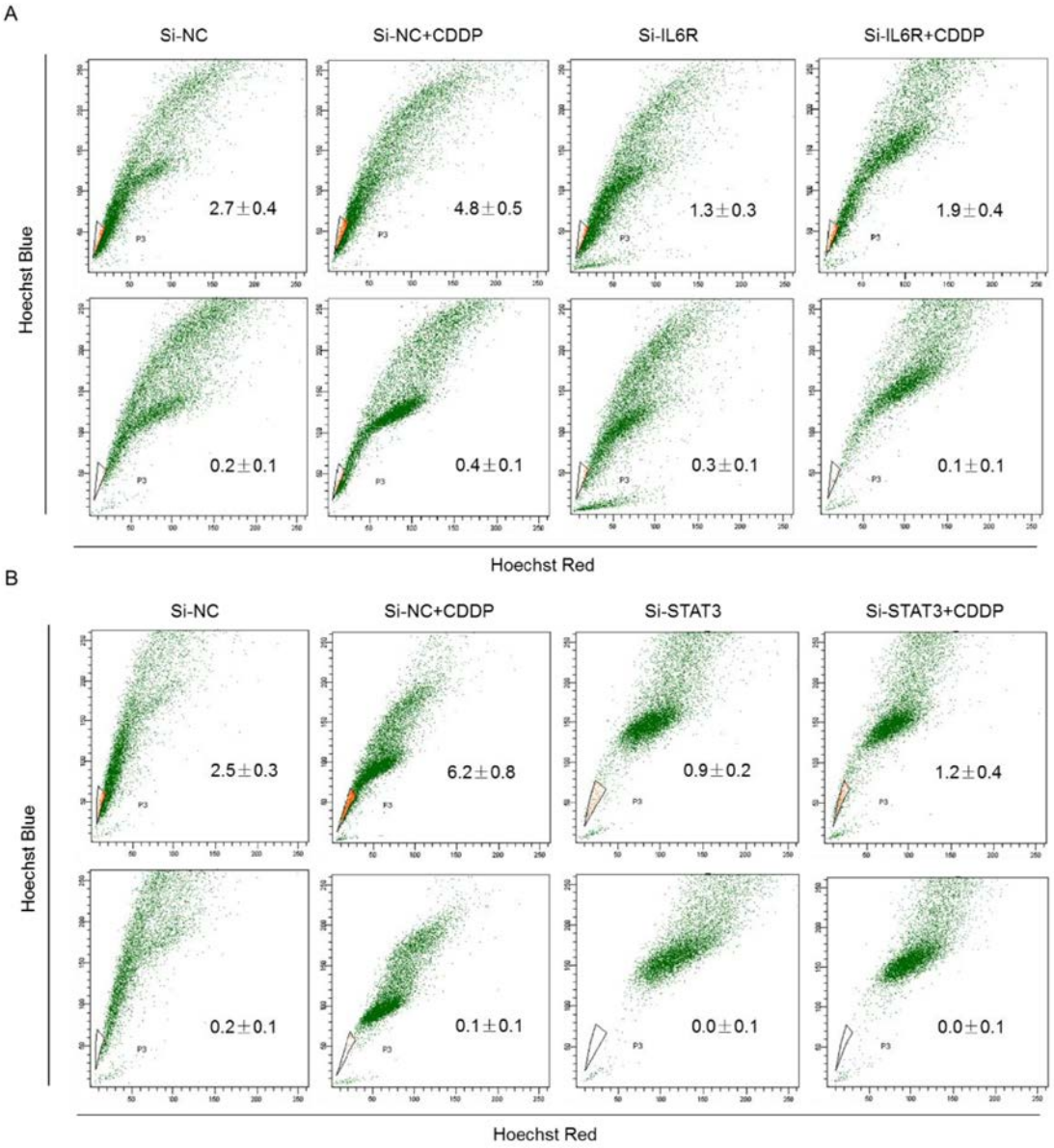


Fig. S3. IL-6/STAT3 signaling is responsible for CDDP-induced CSC surface markers in BGC823.

BGC823 cells were transfected with con-siRNA, IL6R-siRNA, or STAT3-siRNA for 12 h and then 2 μM of CDDP was added for 72 h. Western blot analysis of related proteins.


A
Si-NC
Si-NC+CDDP
Si-IL6R
Si-IL6R+CDDP
Hoechst Blue
2.7±0.4
4.8±0.5
1.3±0.3
1.9±0.4
0.2±0.1
0.4±0.1
0.3±0.1
0.1±0.1
Hoechst Red
B
Si-NC
Si-NC+CDDP
Si-STAT3
Si-STAT3+CDDP
Hoechst Blue
2.5±0.3
6.2±0.8
0.9±0.2
1.2±0.4
0.2±0.1
0.1±0.1
0.0±0.1
0.0±0.1
Hoechst Red

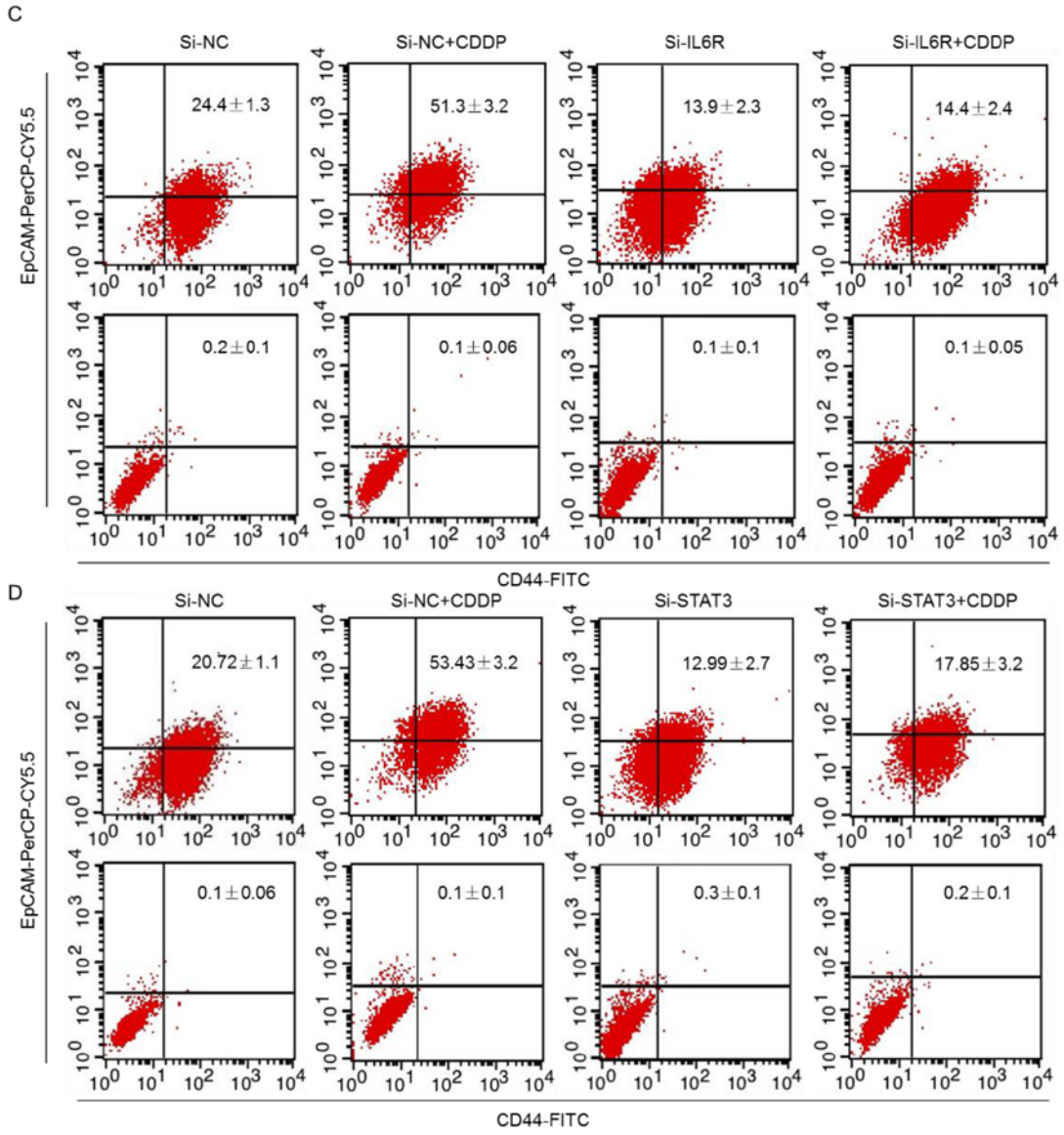


Fig. S4. IL-6/STAT3 signaling is responsible for CDDP-induced gastric CSC-like properties in MGC803.

(A-D) MGC803 cells were transfected with con-siRNA, IL6R-siRNA, or STAT3-siRNA for 12 h and then 2 μM of CDDP was added for 72 h. **(A, B)** FACS analyses of the percentage of SP and **(C, D)** $CD44^+/EpCAM^+$ cells in MGC803cell populations (mean ± SD, n = 3).

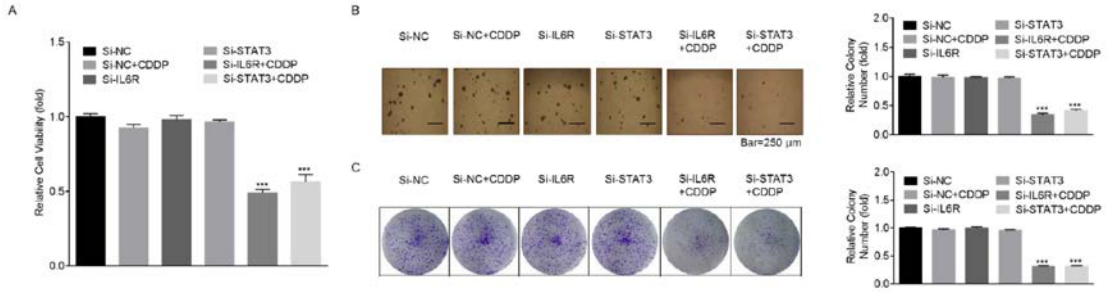


Fig. S5. Blocking IL-6/STAT3 signaling enhances the chemotherapy efficacy in BGC823.

(A-C) BGC823 cells were transfected with con-siRNA, IL6R-siRNA, or STAT3-siRNA for 12 h and then 2 μM of CDDP was added for 72 h. **(A)** Cell viability was evaluated by the CCK-8 assay. **(B, left)** Soft agar colonies under a microscope after 2 weeks of cultivation. **(B, right)** Relative colony numbers in soft agar (mean ± SD, n=5). **(C, left)** Plate colonies after 2 weeks. **(C, right)** Relative colony numbers in plates (mean ±SD, n=3). ***$p < 0.001$compared with MGC803 cells transfected by con-siRNA.

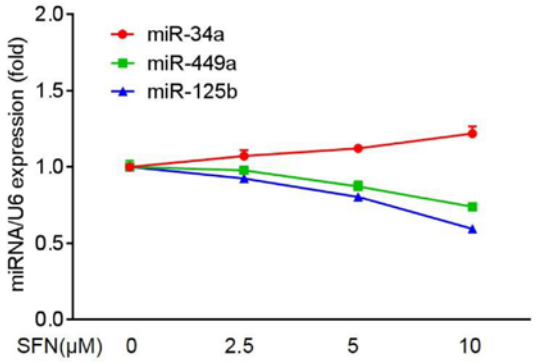


Fig. S6. The effects of SFN on miRNAs.

MGC803 cells were treated with 0, 2.5, 5, or 10 μM of SFN for 72 h. The qRT-PCR analyses of related miRNAs (mean ± SD, n=3).

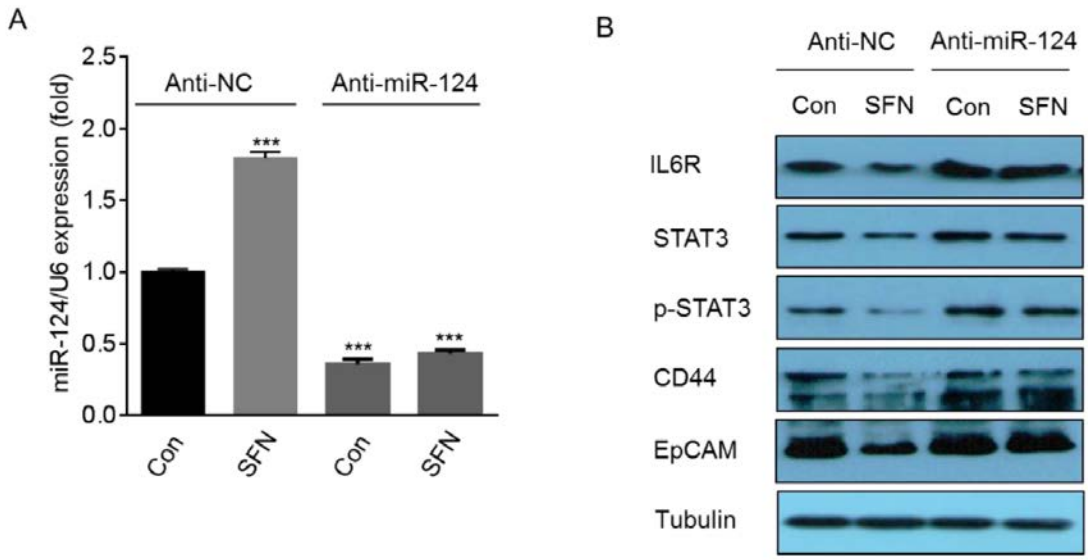
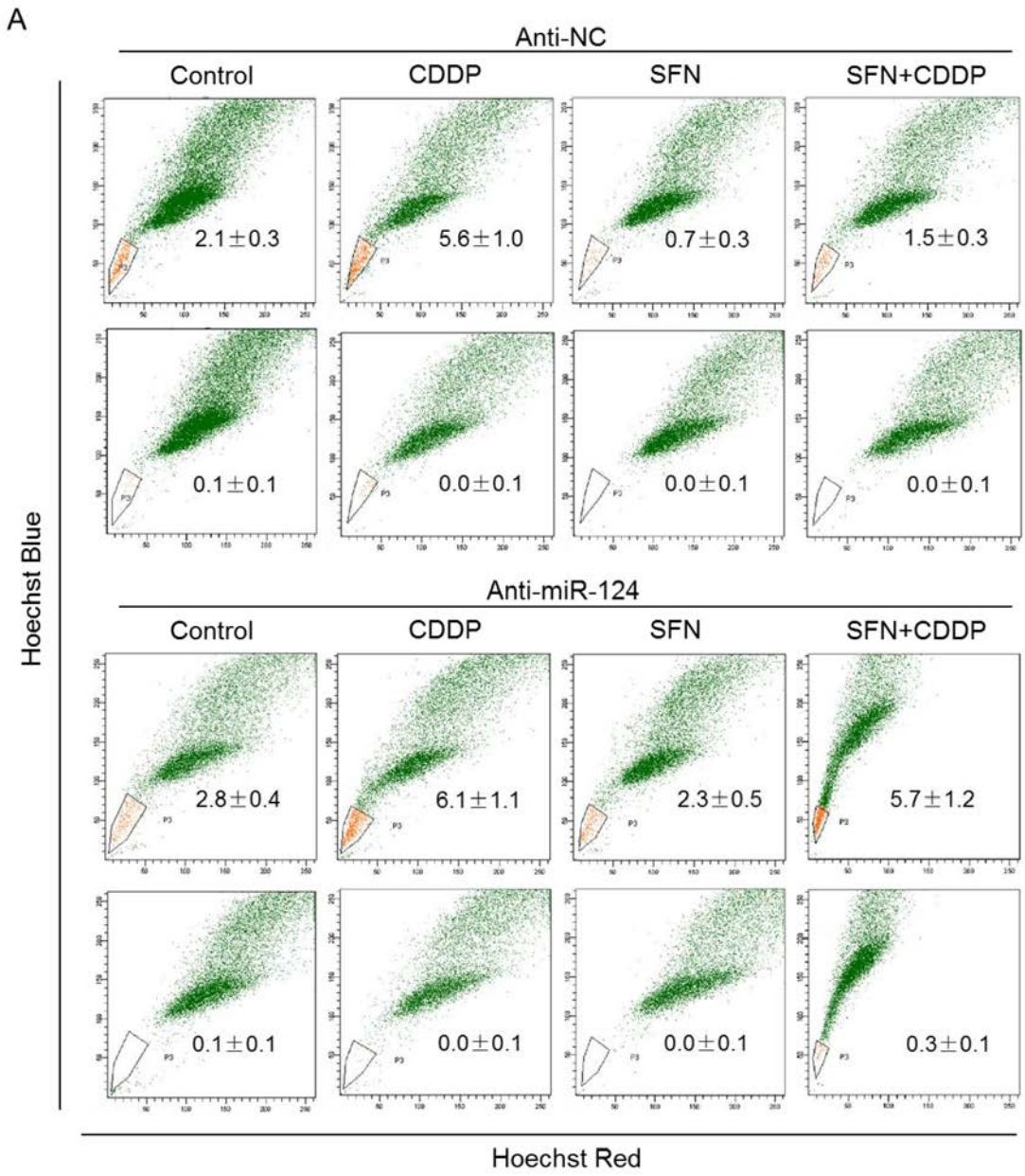


Fig. S7. SFN suppressed IL-6R, STAT3 and the CDDP-induced CSC surface markers via miR-124 in BGC823.

(A, B) BGC823 cells were transfected with anti-con or anti-miR-124 for 12 h and treated with 0 or10 μM of SFN for 72 h. **(A)** qRT-PCR analyses of miR-124 (mean ± SD, n=3). ***$p < 0.001$ compared with BGC823 cells transfected with anti-con. **(B)** Western blot analysis of related proteins.


A
Anti-NC
Control
CDDP
SFN
SFN+CDDP
2.1±0.3
5.6±1.0
0.7±0.3
1.5±0.3
0.1±0.1
0.0±0.1
0.0±0.1
0.0±0.1
Anti-miR-124
Control
CDDP
SFN
SFN+CDDP
2.8±0.4
6.1±1.1
2.3±0.5
5.7±1.2
0.1±0.1
0.0±0.1
0.0±0.1
0.3±0.1
Hoechst Blue
Hoechst Red

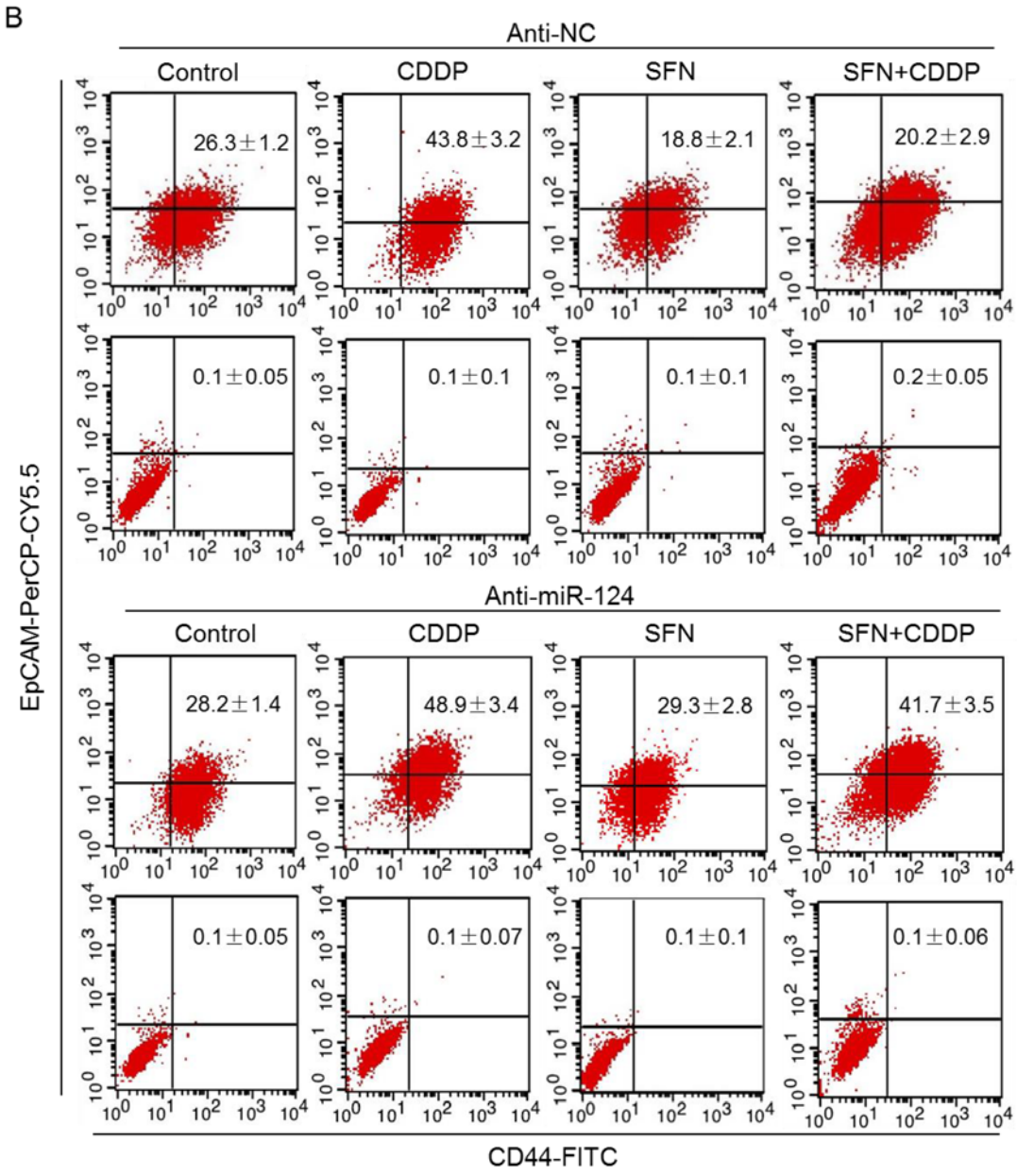


Fig. S8. SFN decreases the CDDP-induced CSC-like properties via miR-124.

(A, B) MGC803 cells were transfected with anti-con or anti-miR-124 for 12 h and treated with 2 μM of CDDP, 10 μM of SFN, or their combination for 72 h. FACS analyses of the percentage of SP **(A)** and $CD44^{+}/EpCAM^{+}$ **(B)** cells in MGC803 cell populations (mean ± SD, n = 3).