Supplementary data to:

B7-H3 promotes the cell cycle-mediated chemoresistance of colorectal cancer cells by regulating CDC25A

Table S1. Primers for RT-qPCR assay of gene

Primer Name	**Primer Sequence (5'-3')**
Homo-B7-H3 Forward	ACAGGGCAGCCTATGACATT
Homo-B7-H3 Reverse	CTGCATTCTCCTCCTCACAG
Homo-CDC25A Forward	GTGAAGGCGCTATTTGGCG
Homo-CDC25A Reverse	TGGTTGCTCATAATCACTGCC
Homo-CDC25B Forward	ACGCACCTATCCCTGTCTC
Homo-CDC25B Reverse	CTGGAAGCGTCTGATGGCAA
Homo-CDC25C Forward	TCTACGGAACTCTTCTCATCCAC
Homo-CDC25C Reverse	TCCAGGAGCAGGTTTAACATTTT
Homo-CDK2 Forward	CCAGGAGTTACTTCTATGCCTGA
Homo-CDK2 Reverse	TTCATCCAGGGGAGGTACAAC
Homo-Chk2 Forward	CCCAAGGCTCCTCCTCACA
Homo-Chk2 Reverse	AGTGAGAGGACTGGCTGGAGTT
Homo-ATR Forward	GGCCAAAGGCAGTTGTATTGA
Homo-ATR Reverse	GTGAGTACCCCAAAAATAGCAGG
Homo-Rb Forward	ACTCTTCAGCAATTGGAAAGG
Homo-Rb Reverse	CACCAATGCAGAATTTATTTCAGT
Homo-β-actin Forward	CATGTACGTTGCTATCCAGGC
Homo-β-actin Reverse	CTCCTTAATGTCACGCACGAT

Table S2. Clinical characteristics of patients

CRC patients	Number	B7H3 expression	CDC25A expression
		P value	P value
No. of patients	121		
Gender			
Male	71		
Female	50	0.2468	0.5612
Age (years)			
Mean	60.42		
Range	26-81	0.4016	0.5551
Tumor location			
Colon	70		
Rectum	51	0.2165	0.2466
TNM stage			
I-II	63		
III-IV	58	< 0.0001	< 0.0001

Supplementary Figure Legends

Figure S1. B7-H3 imparts CRC cell chemoresistance. (A) B7-H3 protein levels in NCM460, RKO, HCT116, HT29, SW480 and SW620 cells were analyzed by Western blot. β-actin served as a loading control. **(B)** B7-H3 protein and mRNA levels in B7-H3-overexpressing HCT116 and RKO cells were analyzed by Western blot and RT-qPCR. β-actin served as a loading control. **(C)** Cell viability after 48 h of 5-FU treatment was assessed by CCK8 in B7-H3-overexpressing HCT116 and RKO cells. Shown relative to negative control cells as the mean ± SD. (n=5). **(D)** IC50 values were calculated on the basis of experiments from **C** as well as from negative control cells. Shown as the mean IC50 ± SD. (n=5). **(E)** B7-H3-overexpressed HCT116 and RKO together with control cells were treated for 48 h with 10 μM 5-FU. Cell death was determined with the LDH assay. (n=5). **(F)** Apoptosis was measured using Annexin V/7-AAD double staining in B7-H3-overexpressing CRC cells. These data are shown as the mean ± SD of three independent experiments. **(G)** Bcl-2 was upregulated while Bax was downregulated upon B7-H3 overexpression in both HCT116 and RKO cells compared with control cells.

Figure S2. B7-H3 inhibits chemotherapy-induced apoptotic cell death of CRC cells. (A) B7-H3 protein and mRNA levels in B7-H3 knockdown HCT116 and RKO cells were analyzed by Western blot and RT-qPCR. β-actin served as a loading control. **(B)** B7-H3-knockdown HCT116 and RKO together with control cells were treated for 48 h with 10 μM 5-FU. Cell death was determined with the LDH assay. (n=5). **(C)** Apoptosis was measured using Annexin V/7-AAD double staining in sh-B7-H3-CRC cells. These data are shown as the mean ± SD of three independent experiments. **(D)** Bax was upregulated while Bcl-2 was downregulated upon B7-H3 knockdown in both HCT116 and RKO cells compared with control cells.

Figure S3. B7-H3 inhibits CRC cells G2/M phase arrest via regulating CDC25A. (A) The effect of B7-H3 overexpression on cell cycle progression in HCT116 and RKO cells. Cells were treated with or without 20 or 40 μM L-OHP for 48 h. After 48 h, both attached and floating cells were harvested for cell cycle analysis. **(B)** The effect of B7-H3 knockdown on cell cycle progression in HCT116 and RKO cells. Cells were treated with or without 20 or 40 μM L-OHP for 48 h. After 48 h, both attached and floating cells were harvested for cell cycle analysis. **(C)** RT-qPCR to determine the mRNA levels of CDC25A in both control and B7-H3 overexpression HCT116 and RKO cells.

Figure S4. STAT3/CDC25A increases G2/M arrest on B7-H3 overexpression CRC cells. The effect of STAT3/CDC25A silencing on cell cycle progression in control and B7-H3-overexpressing CRC cells. Cells were treated with or without 20 or 40 μM L-OHP for 48 h. After 48 h, both attached and floating cells were harvested for cell cycle analysis.

Figure S1

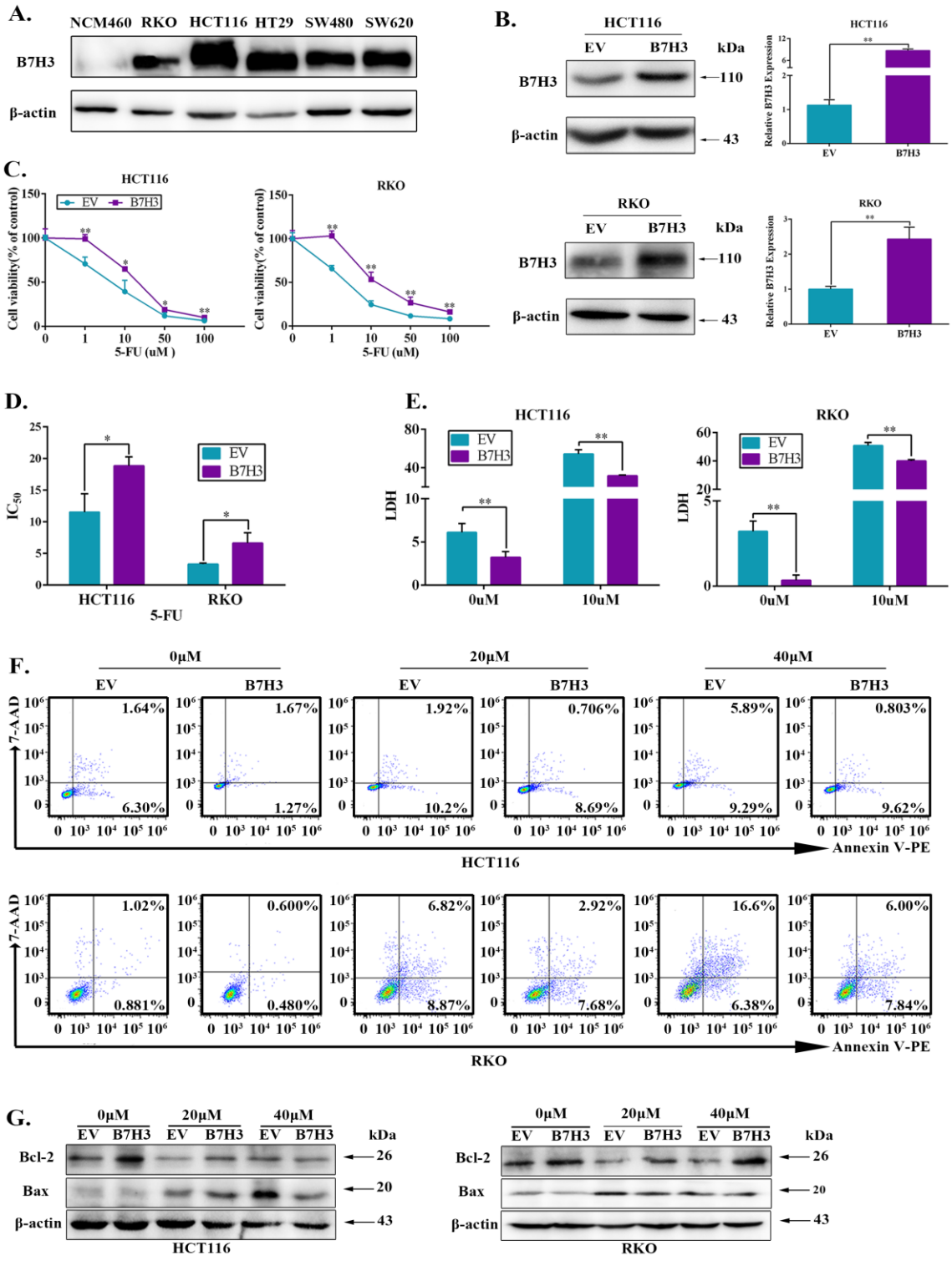

A.
NCM460 RKO HCT116 HT29 SW480 SW620
B7H3
β-actin
B.
HCT116
EV B7H3 kDa
B7H3 110
β-actin 43
Relative B7H3 Expression
RKO
C.
HCT116
RKO
EV B7H3
Cell viability(% of control)
5-FU (uM)
5-FU (uM)
D.
IC50
HCT116 RKO
5-FU
E.
LDH
0uM 10uM
F.
0μM 20μM 40μM
EV B7H3
7-AAD
Annexin V-PE
1.64% 6.30% 1.67% 1.27% 1.92% 10.2% 0.706% 8.69% 5.89% 9.29% 0.803% 9.62%
HCT116
1.02% 0.881% 0.600% 0.480% 6.82% 8.87% 2.92% 7.68% 16.6% 6.38% 6.00% 7.84%
RKO
G.
Bcl-2
Bax
β-actin
26
20
43
HCT116
RKO

Figure S2

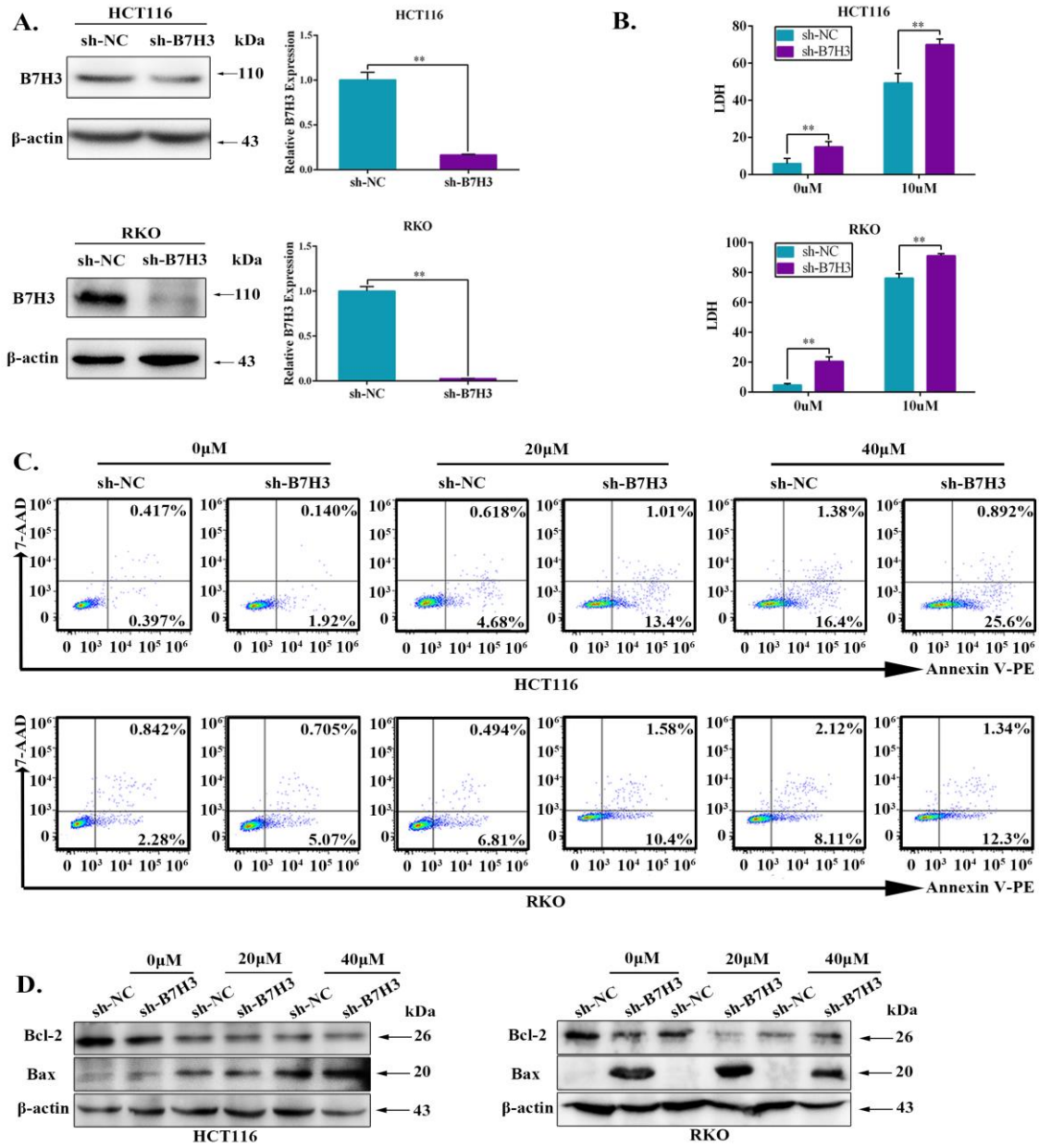

A.
HCT116
sh-NC
sh-B7H3
kDa
B7H3
110
β-actin
43
Relative B7H3 Expression
RKO
B.
LDH
0uM
10uM
C.
0μM
20μM
40μM
7-AAD
Annexin V-PE
0.417%
0.140%
0.618%
1.01%
1.38%
0.892%
0.397%
1.92%
4.68%
13.4%
16.4%
25.6%
0.842%
0.705%
0.494%
1.58%
2.12%
1.34%
2.28%
5.07%
6.81%
10.4%
8.11%
12.3%
D.
Bcl-2
Bax
26
20

Figure S3

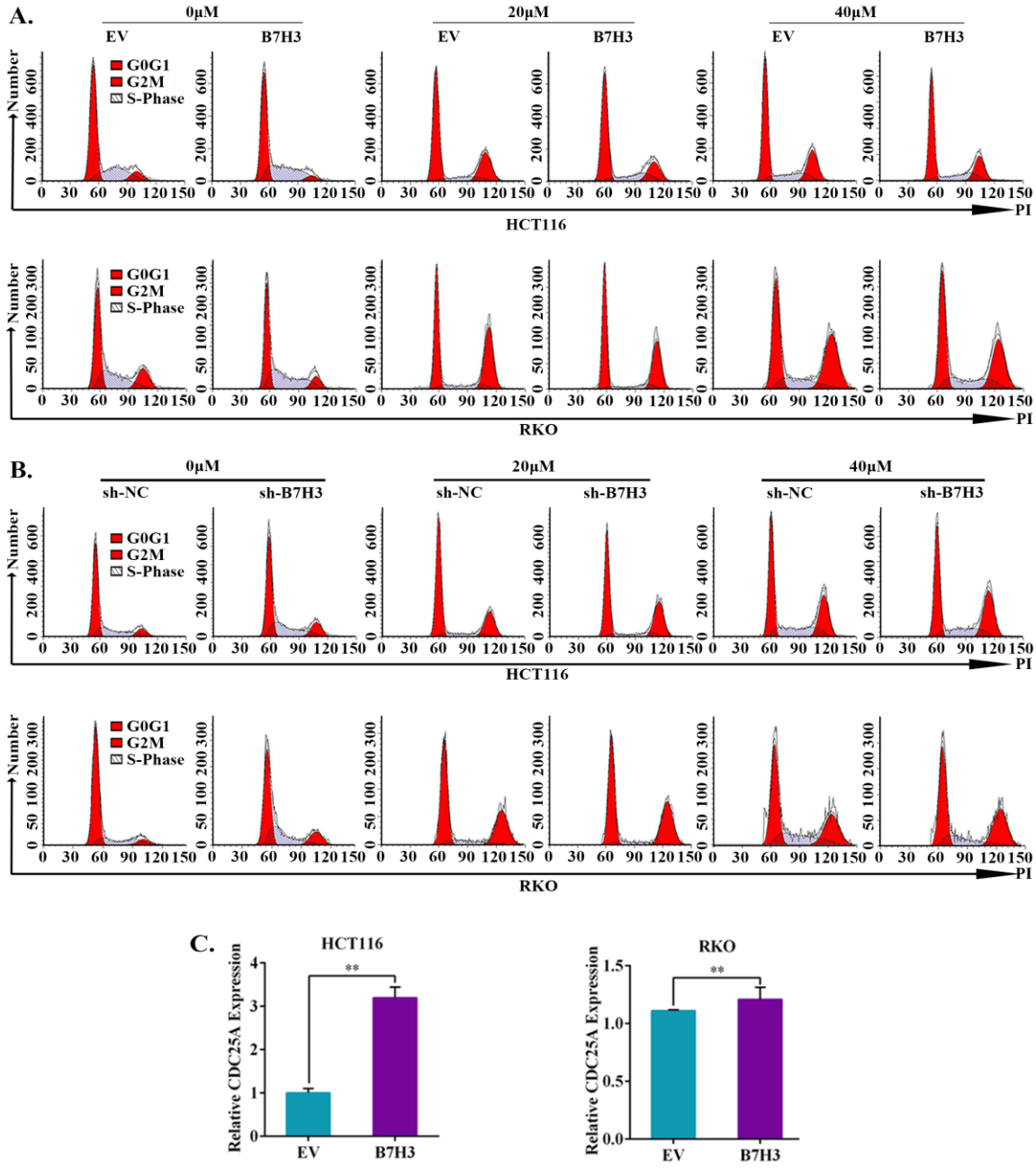
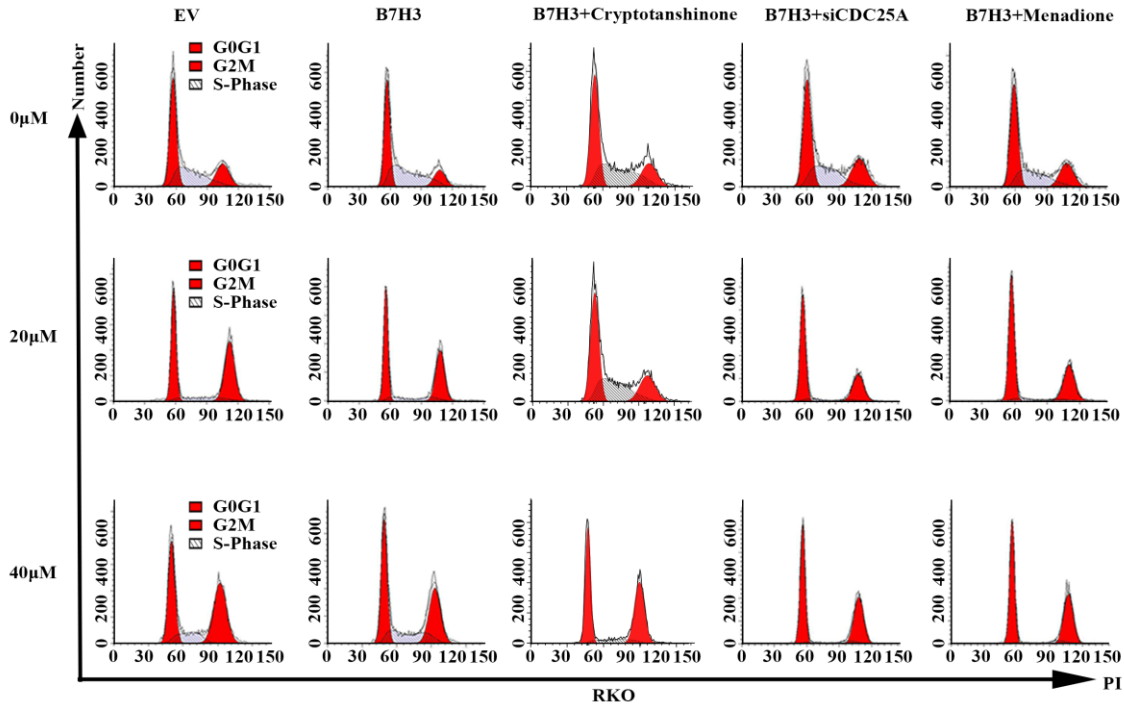

A.
0μM
20μM
40μM
EV
B7H3
G0G1
G2M
S-Phase
Number
PI
HCT116
RKO
B.
sh-NC
sh-B7H3
C.
HCT116
RKO
Relative CDC25A Expression
**

Figure S4


EV
B7H3
B7H3+Cryptotanshinone
B7H3+siCDC25A
B7H3+Menadione
0μM
20μM
40μM
Number
G0G1
G2M
S-Phase
0 200 400 600
0 30 60 90 120 150
RKO
PI