B7-H3 confers resistance to Vγ9Vδ2 T cell-mediated cytotoxicity in human colon cancer cells via the STAT3/ULBP2 axis

Supplementary Table 1. Primers for RT-qPCR assay of genes in this study

| Primer Name	Primer Sequence (5'-3')
Human-B7H3 Forward	ACAGGGCAGCCTATGACATT
Human-B7H3 Reverse	CTGCATTCTCCTCCTCACAG
Human-ULBP1 Forward	TAAGTCCAGACCTGAACCACA
Human-ULBP1 Reverse	TCCACCACGTCTCTTAGTGTT
Human-ULBP2 Forward	AGCAACTGCGTGACATTCAG
Human-ULBP2 Reverse	GCCATCCTATACAGTCTCCCA
Human-ULBP3 Forward	TCTATGGGTCACCTAGAAGAGC
Human-ULBP3 Reverse	TCCACTGGGTGTGAAATCCTC
Human-ULBP4 Forward	GCACTTGGGGAGAATTGACCC
Human-ULBP4 Reverse	ACATCTCGACTTGCAGAGTGG
Human-ULBP5 Forward	GACAGCTACCAAATAGCGAAGC
Human-ULBP5 Reverse	GGTAAGGAGTGTGAGTCGTCT
Human-MICA Forward	AGGGTTTCTTGCTGAGGTACA
Human-MICA Reverse	GGTCTCTCTGTCCCATGTCTTA
Human-MICB Forward	TCTTCGTTACAACCTCATGGTG
Human-MICB Reverse	TCCCAGGTCTTAGCTCCCAG
Human-MHCI Reverse	GATTACATCGCCTTGAACGAGG

Human-MHCI Reverse	AGAGACAGCGTGGTGAGTCAT
Human-BTN3A1 Reverse	AAAGCACAAGAGTGAAGCTCC
Human-BTN3A1 Reverse	GCCGAGAACACAATAATGCCA
Human-CD155 Reverse	TGGAGGTGACGCATGTGTC
Human-CD155 Reverse	GTTTGGACTCCGAATAGCTGG
Human-β-actin Forward	CATGTACGTTGCTATCCAGGC
Human-β-actin Reverse	CTCCTTAATGTCACGCACGAT

Supplementary Table 2. Clinical characteristics of colon cancer (CC) patients

| CC patients	Number
No. of patients	106
Gender	
Male	61
Female	45
Age (years)	
Mean	61.72
Range	26-81
Tumor location	
Right hemicolon	52
Left hemicolon	54
Tumor volume	
>6.5cm^3	83
≤6.5cm^3	23
Distant metastasis	
Yes	3
No	103
TNM stage	
I-II	54
III-IV	52

Supplementary Fig. 1. B7-H3-overexpressing CC cells resist the killing effect of Vδ2 T cells

(a) The mRNA expression of B7-H3 in B7-H3-overexpressing HCT116 and RKO cells was analyzed by RT-qPCR. (b) The cytotoxicity of Vδ2 T cells (effector cells, E) against HCT116 or RKO cells (target cells, T) at different E/T ratios was analyzed by CCK-8 assay. (c, d) Representative images of the clusters in which Vδ2 T cells formed surrounding B7-H3-overexpressing HCT116 (c) or RKO (d) cells. Values are expressed as the means $\pm$ SD. Data are representative of results from 3 independent experiments. *p<0.05, **p<0.01, and ***p<0.001.
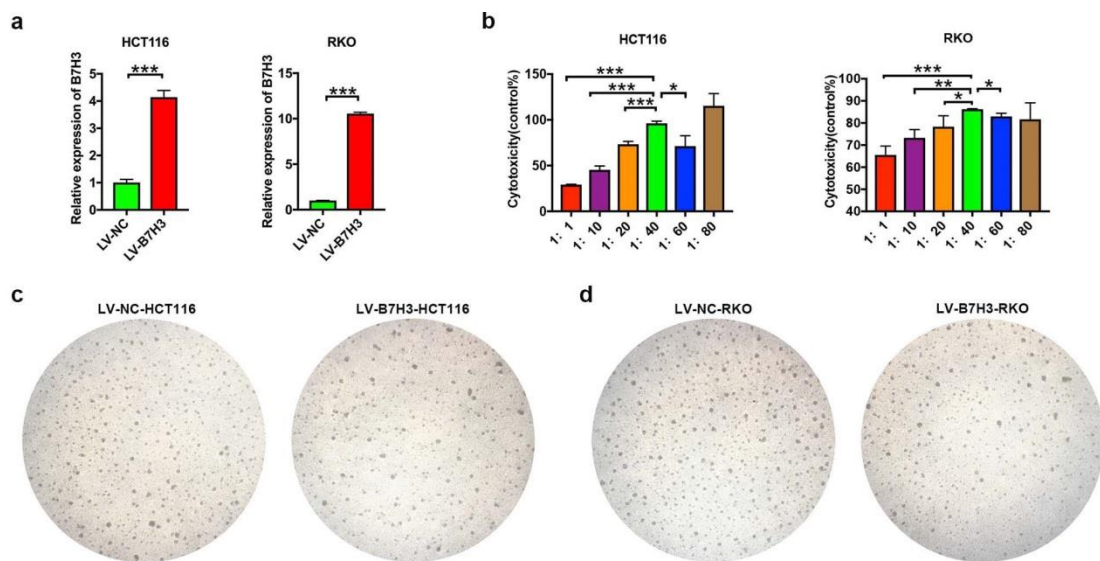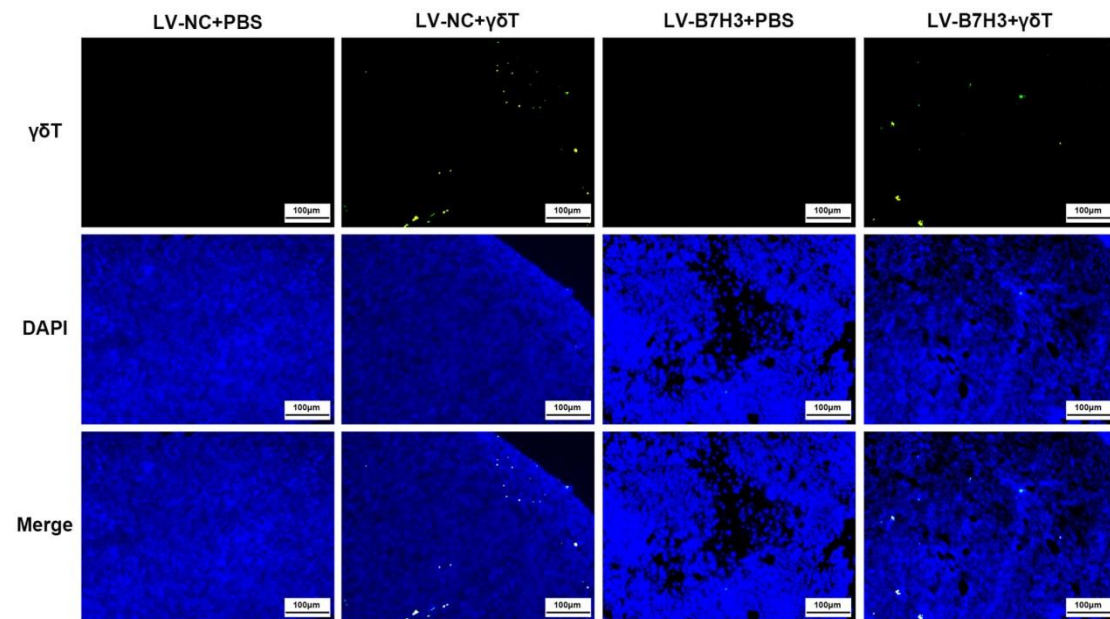
Supplementary Fig. 2. B7-H3 knockdown CC cells are sensitive to the killing effect of Vδ2 T cells

(a) The mRNA expression of B7-H3 in B7-H3-knockdown HCT116 and RKO cells was analyzed by RT-qPCR. (b, c) Representative images of the clusters of Vδ2 T cells that formed around B7-H3 knockdown HCT116 (b) or RKO (c) cells. Values are expressed as the means ± SD. Data are representative of results from 3 independent experiments. ***p<0.001.
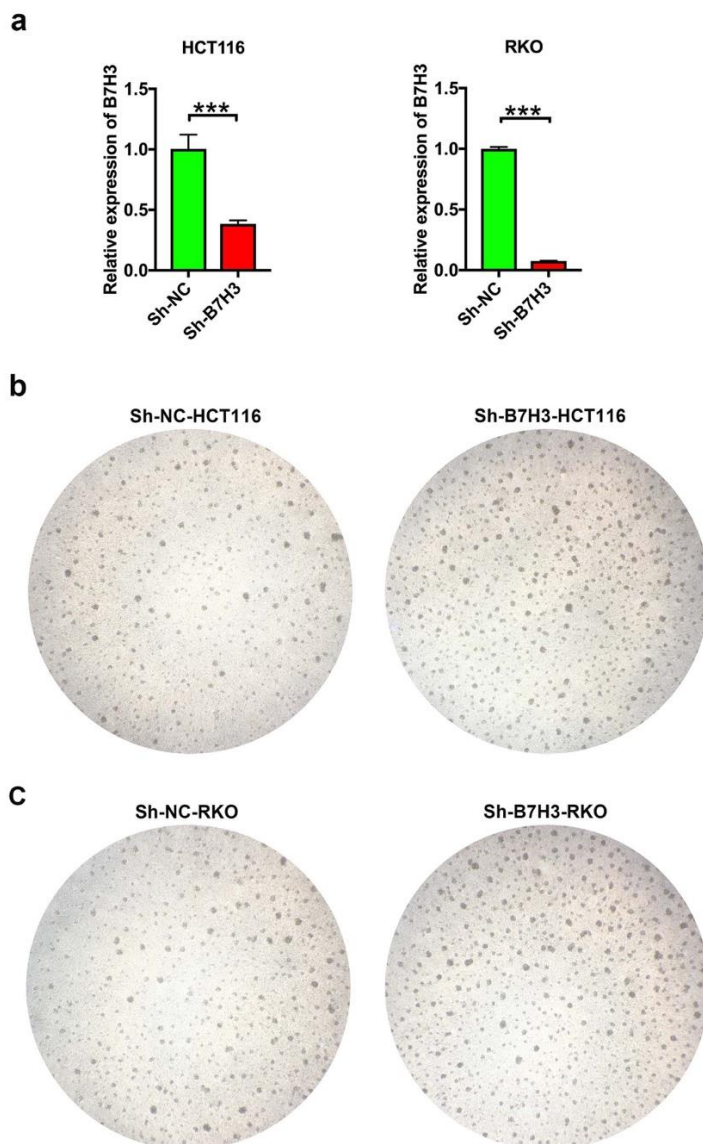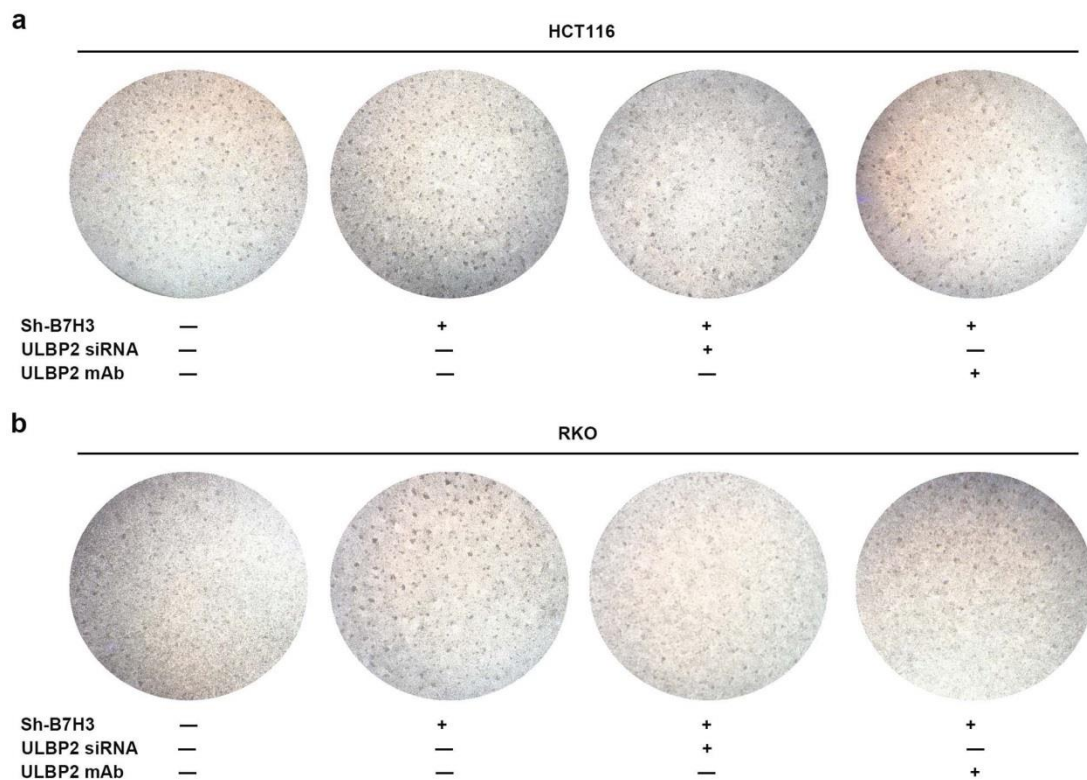
Supplementary Fig. 3. The expression of γδT cells in B7-H3-overexpressing HCT116 tumors treated with γδT cells.

Immunofluorescence represents infiltrating γδT cell expression in B7-H3-overexpressing HCT116 cell tumors in SCID mice following Vδ2 T cell injection.

Supplementary Fig. 4. B7-H3 knockdown CC cells promote the formation of Vδ2 T cell clusters by suppressing ULBP2

(a, b) Representative images showing the clusters of Vδ2 T cells around B7-H3-knockdown HCT116 (a) and RKO (b) cells treated with ULBP2-siRNA or ULBP2 monoclonal blocking antibody (ULBP2 mAb).

Supplementary Fig. 5. B7-H3-overexpressing CC cells inhibit the formation of Vδ2 T cell clusters via STAT3.

(a, b) Representative images showing the clusters of Vδ2 T cells around B7-H3-overexpressing HCT116 (a) and RKO (b) cells treated with or without cryptotanshinone (CTN).
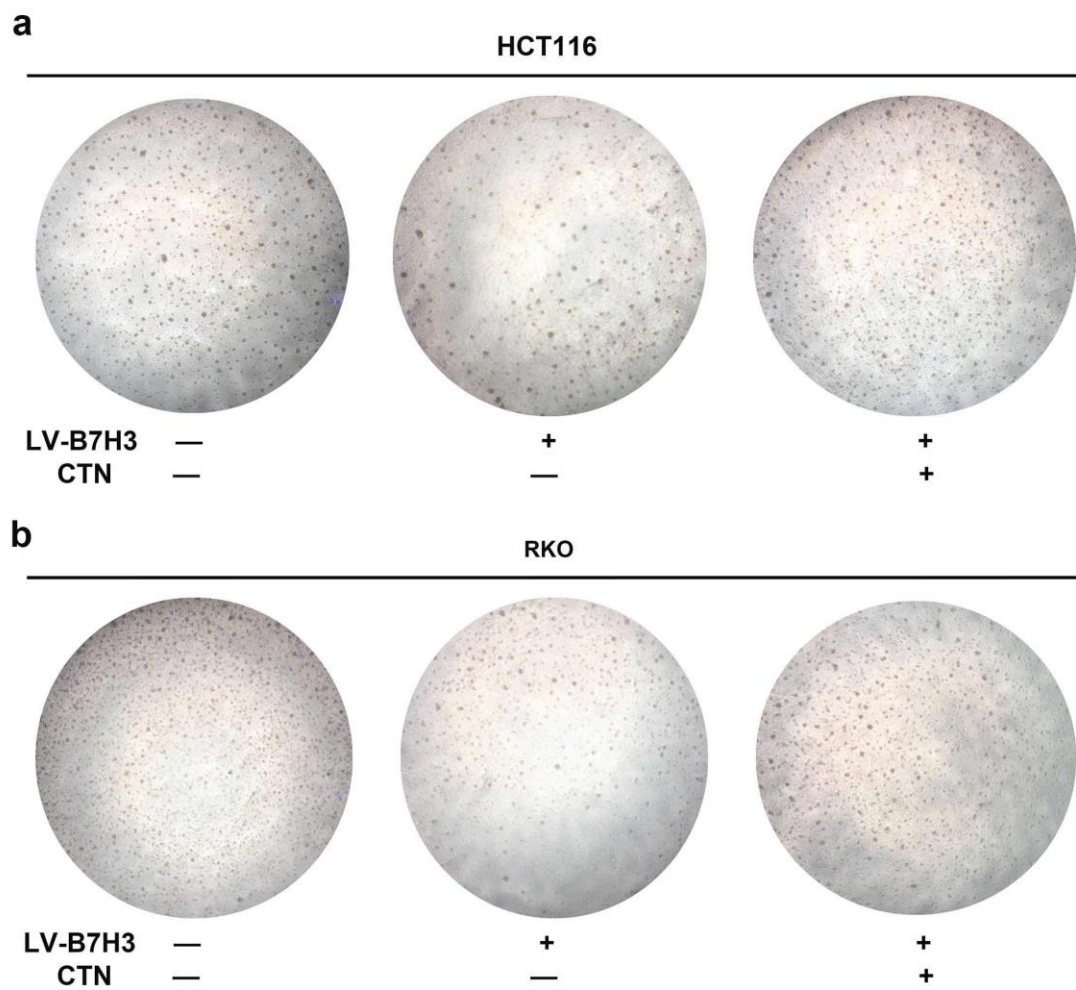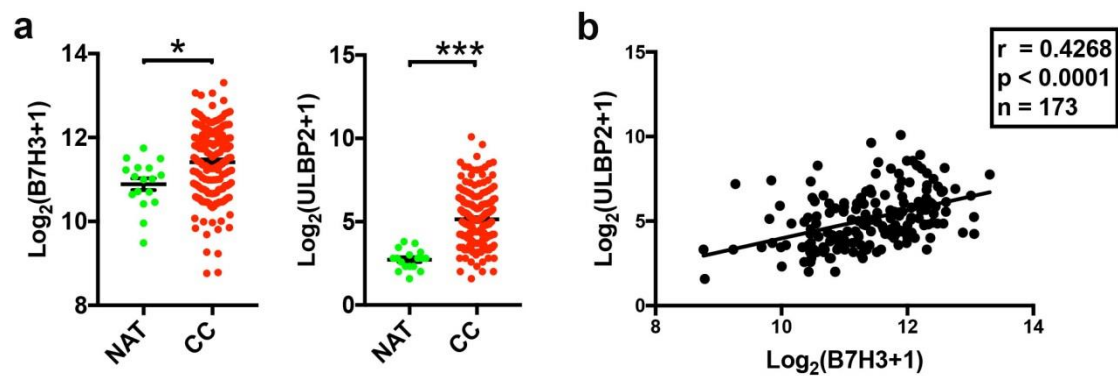
Supplementary Fig. 6. The expression of γδT cells in NAT samples was higher than CC samples in colon cancer patient specimens.

Immunofluorescence represents infiltrating γδT cell expression in NAT samples and CC samples.
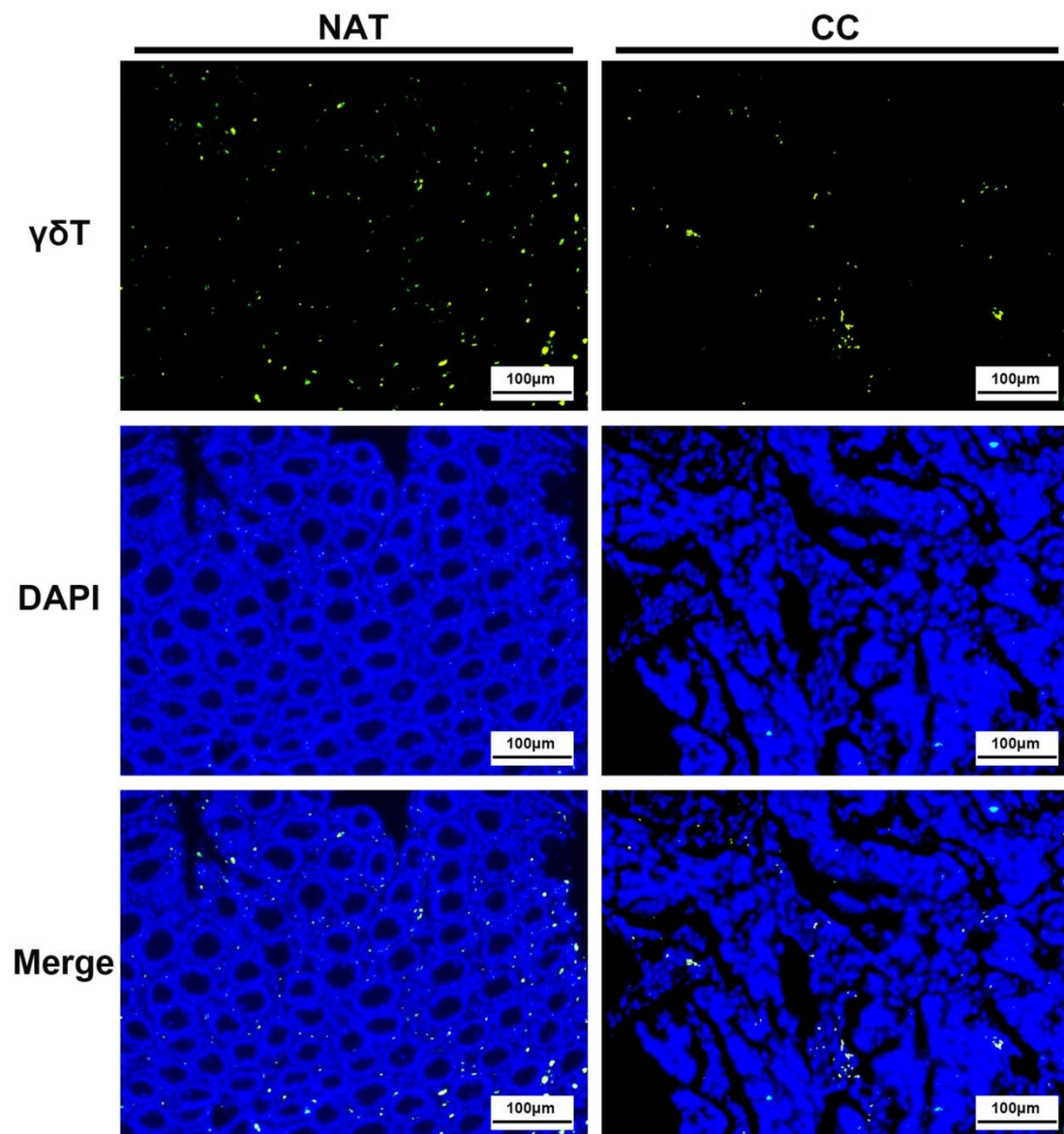
Supplementary Fig. 7. Aberrant ULBP2 mRNA expression is positively correlated with B7-H3 mRNA expression in TCGA CC datasets.

(a) The mRNA expression of B7-H3 and ULBP2 in NAT (n=17) and CC (n=173) specimens of TCGA CC database. (b) The correlation between B7-H3 and ULBP2 mRNA expression in CC specimens. n=173. Values are expressed as the means ± SEM. *p<0.05, and ***p<0.001.

Supplementary Fig. 8. The expression of MHC I, BTN3A1, and CD155 are not associated with B7-H3 in CC cells. (a) The mRNA expression of MHC I, BTN3A1, and CD155 in B7-H3-overexpressing HCT116 and RKO cells were analyzed by RT-qPCR. (b) The mRNA expression of MHC I, BTN3A1, and CD155 in B7-H3 knockdown HCT116 and RKO cells were analyzed by RT-qPCR. Values are expressed as the means ± SD. Data are representative of results from 3 independent experiments. *p<0.05, **p<0.01, and ***p<0.001.