

**Supporting Information for
Original article
 α -hederin Targets USP5 to Inhibit Colorectal Tumorigenesis by Disrupting STAT3
Deubiquitination**

Supplementary Tables

Table S1: Primer sequences for qPCR assay.

Gene Symbol	Species	Forward sequences (5'-3')	Reverse sequences (5'-3')
<i>IL-6</i>	Human	ACTCACCTCTTCAGAACGAATTG	CCATCTTTGGAAGGTTTCAGGTTG
<i>IL-1β</i>	Human	TGATGGCTTATTACAGTGGCAA	TAGTGGTGGTCGGAGATTTCG
<i>TNF-α</i>	Human	TGCACTTTGGAGTGATCGGC	ACTCGGGGTTTCGAGAAGATG
<i>USP5</i>	Human	ACCAGAAAGTGTGTGCCTCC	CCCCTGCCCTCATTGGTAAG
<i>p62</i>	Human	TTGCGCCCATTTAGAGGATGT	GGCCCTGGCATTGTTCTTACA
<i>STAT3</i>	Human	ACCAGCAGTATAGCCGCTTC	GCCACAATCCGGGCAATCT
<i>β-Actin</i>	Human	GAGCACAGAGCCTCGCCTTT	TCATCATCCATGGTGAGCTGG
<i>IL-6</i>	Mouse	CCAAGAGGTGAGTGCTTCCC	CTGTTGTTTCAGACTCTCTCCCT
<i>IL-1β</i>	Mouse	AGCATCCAGCTTCAAATC	CTTCTCCACAGCCACAAT
<i>TNF-α</i>	Mouse	CAGGCGGTGCCTATGTCTC	CGATCACCCCGAAGTTTCAGTAG
<i>IL-10</i>	Mouse	CTTACTGACTGGCATGAGGATCA	GCAGCTCTAGGAGCATGTGG
<i>ZO-1</i>	Mouse	GCCGCTAAGAGCACAGCAA	TCCCCACTCTGAAAATGAGGA
<i>Occludin</i>	Mouse	TTGAAAGTCCACCTCCTTACAGA	CCGGATAAAAAGAGTACGCTGG
<i>STAT3</i>	Mouse	AGAACCTCCAGGACGACTTTG	TCACAATGCTTCTCCGCATCT
<i>c-Myc</i>	Mouse	ATGCCCCCAACGTGAACTTC	CGCAACATAGGATGGAGAGCA
<i>PCNA</i>	Mouse	TTTGAGGCACGCCTGATCC	GGAGACGTGAGACGAGTCCAT
<i>β-Catenin</i>	Mouse	CCCAGTCCTTCACGCAAGAG	CATCTAGCGTCTCAGGGAACA
<i>USP5</i>	Mouse	GTGTTACCGACGATCCGTGTC	GTTTCATGCAGATATAGAGGCCAC
<i>p62</i>	Mouse	GAGTCCCTCTCCAGATGCT	GCCAAGACACTGGGCCTATC
<i>β-Actin</i>	Mouse	GATCAGCAAGCAGGAGTACGA	GGTGTAACACGCAGCTCA

Table S2: Primer sequences of pLVshUSP5.

Gene Symbol	Species	Sequences (5'-3')
<i>shRNA</i>	Human	GATCCACTACCGTTGTTATAGGTGTTCAAGAGACACCTATAACAACGGTAGTTTTTTT
<i>shUSP5#1</i>	Human	gatcgGACCACACGATTTGCCTCATTCTCGAGAATGAGGCAAAATCGTGTGGTCTTTTTT
<i>shUSP5#2</i>	Human	gatcgGATAGACATGAACCAGCGGATCTCGAGATCCGCTGGTTCATGTCTATCTTTTTT
<i>shUSP5#3</i>	Human	gatcgACAGTATGTGGAGAGACATTTCTCGAGAAATGTCTCTCCACATACTGTTTTTTT

Table S3: Primer sequences of USP5-OE.

Gene Symbol	Species	Sequences (5'-3')
<i>USP5-OE</i>	Human	GCCACCATGGCGGAGCTGAGTGAGGAGGCGCTGCTGTCAGTATTACCGACGATCCGG GTCCCTAAGGCTGGAGACCGGGTCCACAAAGACGAGTGCGCCTTCTCCTTCGACACG CCGGAGTCTGAGGGGGGCTCTACATCTGTATGAACACGTTTCTGGGCTTTGGGAAA CAGTATGTGGAGAGACATTTCAATAAGACCGGCCAGCGAGTCTACTTGCACCTCCGG CGGACCCGGCGCCCGAAAGAGGAGGACCCTGCTACAGGCACTGGAGACCCACCCCG GAAGAAGCCCACGCGGCTGGCTATTGGTGTTGAAGGCGGATTTGACCTTAGCGAGGA GAAGTTTGAATTAGACGAGGATGTGAAGATTGTCATTTTGCCAGATTACCTGGAGAT TGCCCGGGATGGACTGGGGGGACTGCCTGACATTGTGAGAGATCGGGTGACCAGTGC AGTGAGAGGCCCTACTGTGCGCCGACTCAGCCTCCCGCAAGCAGGAGGTGCAGGCATG GGATGGGGAAAGTACGGCAGGTGTCTAAGCATGCCTTCAGCCTCAAGCAGTTGGACAA CCCTGCTCGAATCCCTCCCTGTGGCTGGAAGTGCTCCAAGTGTGACATGAGAGAGAA CCTGTGGCTCAACCTGACTGATGGCTCCATCCTCTGTGGGCGACGCTACTTCGATGGC

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CATGGATGCAGCCCTTAACAAAGAGGAGCTTCTGGAGTACGAGGAGAAGAAGCGGC
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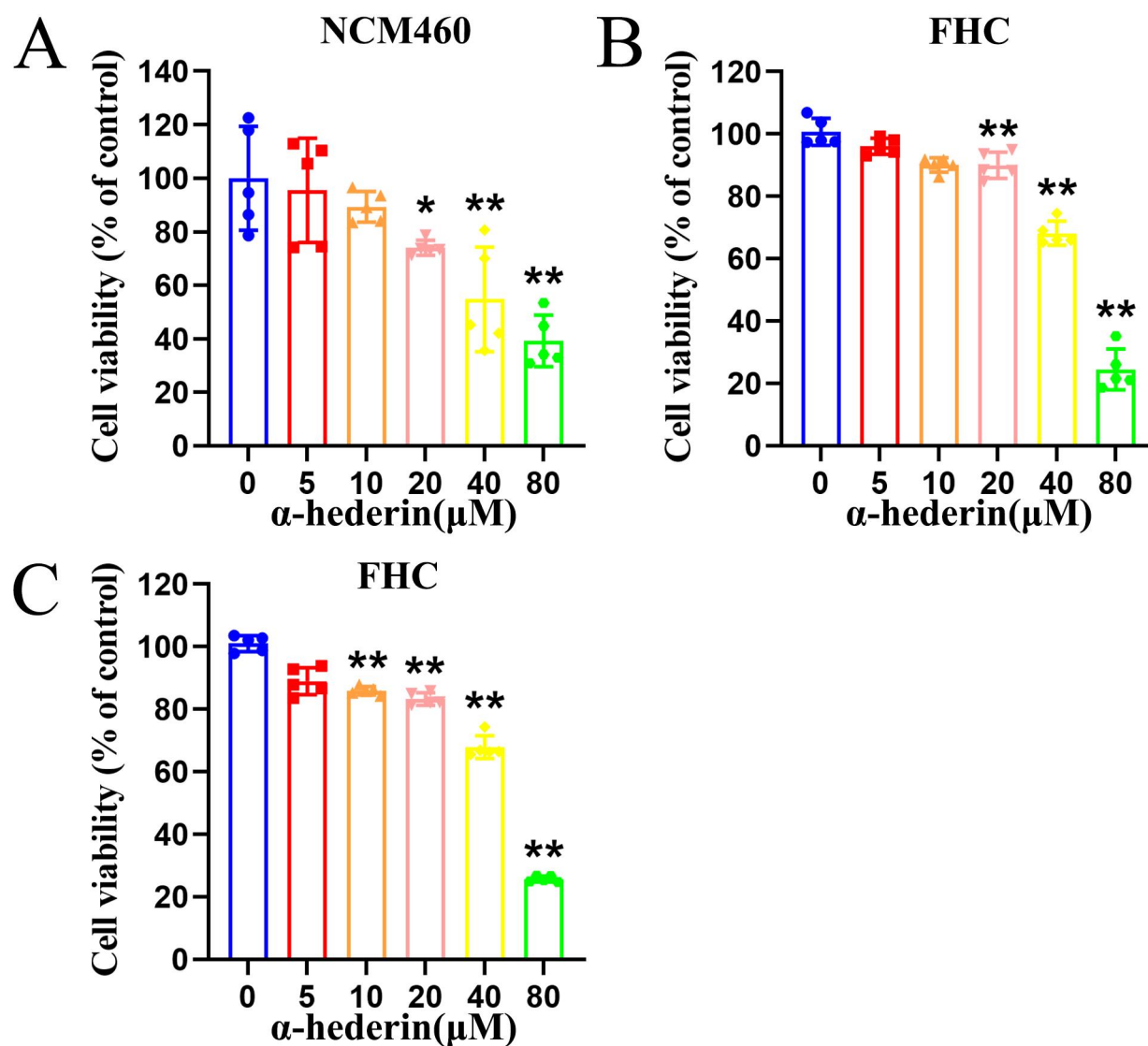


Fig. S1 Cell viability of two different normal human colonic epithelial cell lines NCM460 and FHC treated with different concentrations of α -hederin for 24 and 48 h. (A) Cell viability of NCM460 treated with α -hederin for 48 h at the indicated concentrations, n = 5. (B) Cell viability of FHC treated with α -hederin for 24 h at the indicated concentrations, n = 5. (C) Cell viability of FHC treated with α -hederin for 48 h at the indicated concentrations, n = 5. Data are presented as means $\pm$ SD. * p < 0.05, ** p < 0.01 versus control group.

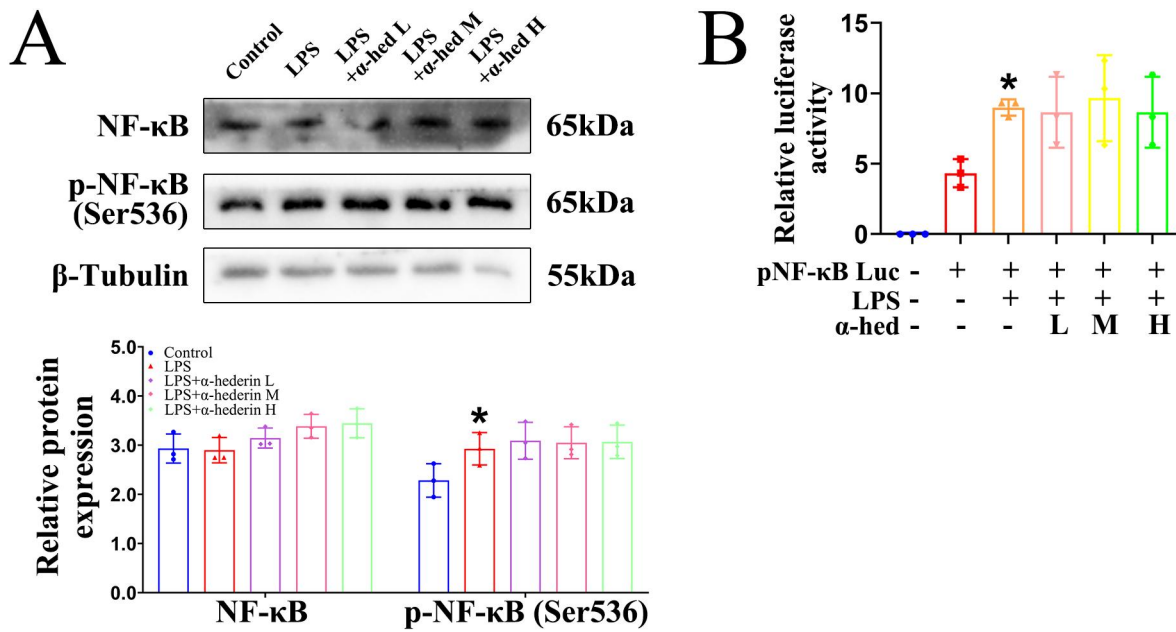


Fig. S2 α -hederin specifically inhibited STAT3 activity. (A) The expression of NF- κ B and the levels of phosphorylated NF- κ B were detected by western blotting, $n = 3$. (B) The transcriptional activity of NF- κ B was detected by luciferase assay, $n = 3$. Data are presented as means $\pm$ SD. * $p < 0.05$ versus control group.

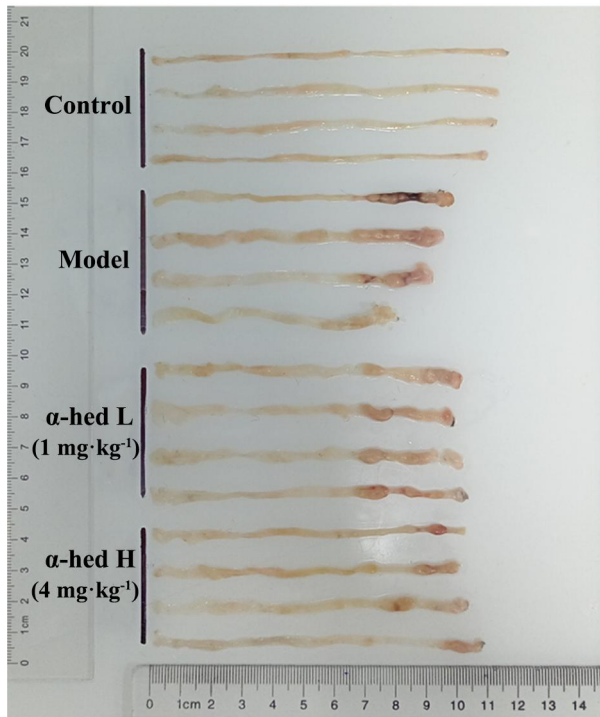


Fig. S3 The gross pathology of the mouse colon.

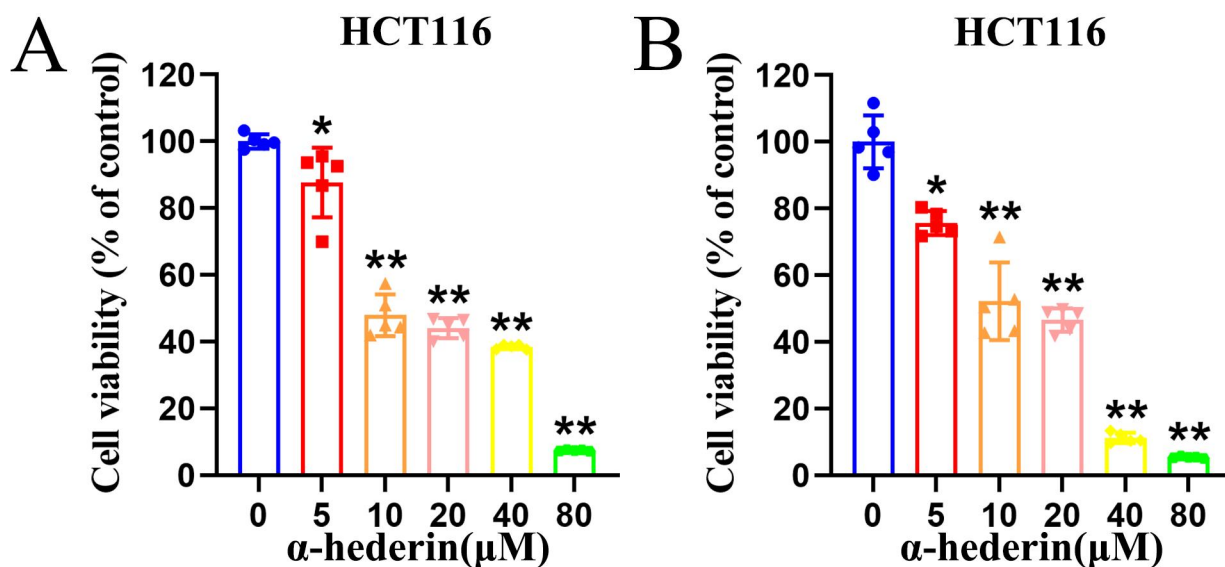


Fig. S4 Cell viability of human CRC cell HCT116 treated with α -hederin for 24 and 48 h at the indicated concentrations. Data are presented as means $\pm$ SD. * p < 0.05, ** p < 0.01 versus control group.

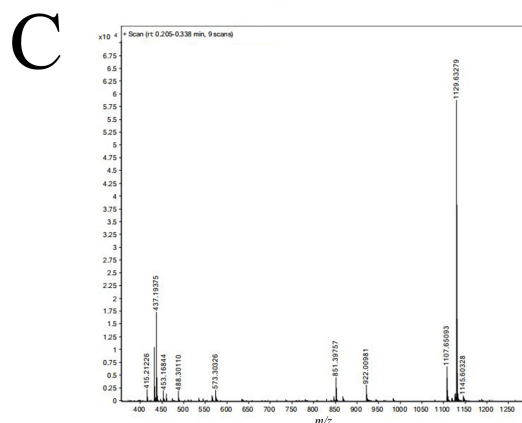
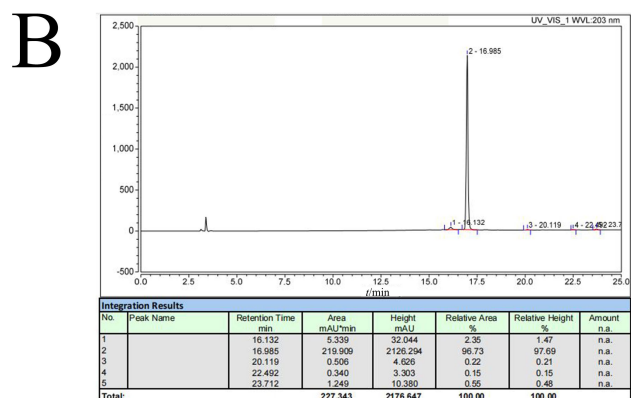
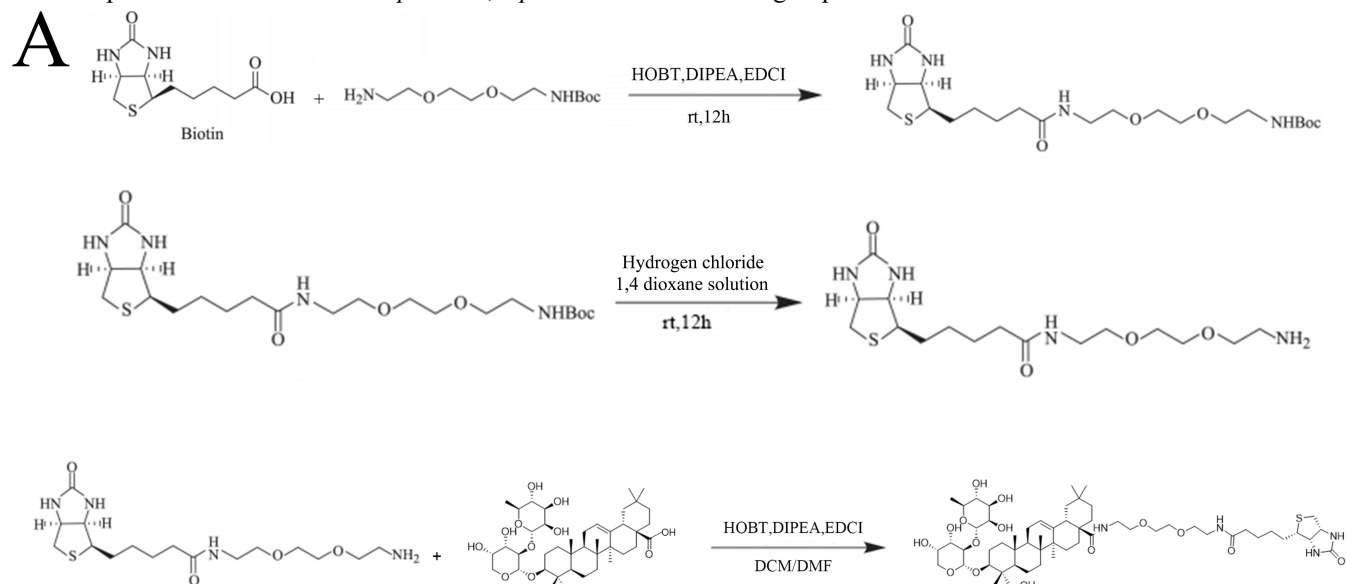


Fig. S5 The synthesis and purification of Bio- α -hederin. (A) The synthetic route of Bio- α -hederin. (B) The HPLC chromatogram of Bio- α -hederin. (C) High-resolution mass spectra of Bio- α -hederin in positive ion mode.