

OTUB2 stabilizes β -Catenin in CRC

Supplementary methods

Cell migration and colony formation assays

For cell migration, CRC cells infected with shRNA-derived lentivirus in serum-free medium were added to the upper compartment of a transwell chamber (Thermo), and medium containing 10% fetal bovine serum was added to the lower compartment. Cells were then incubated for 24 hours, and crystal violet staining was performed after fixation (Beyotime, Beijing, China). For colony formation assay, CRC cells infected with shRNA-derived lentivirus (3000 cells each well) were put into a 6-well plate. Ten days later, cells were fixed and stained by crystal violet (Beyotime, Beijing, China).

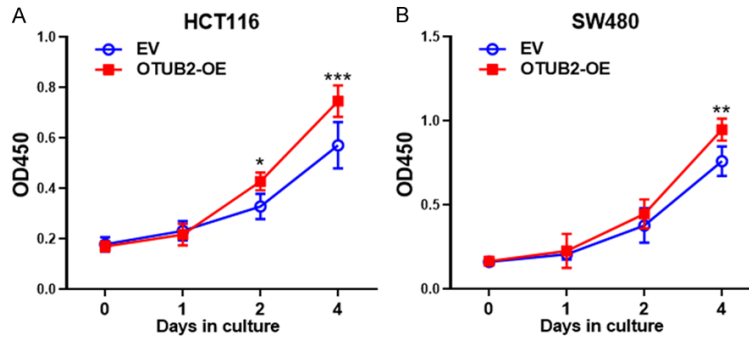


Figure S1. Overexpression of OTUB2 promotes the cell growth of colorectal cancer. (A and B) HCT116 (A) and SW480 (B) cells transfected with OTUB2-overexpressing plasmids Flag-OTUB2 (OTUB2-OE) or empty vector (EV) were cultured for indicated times, followed by CCK-8 assay. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

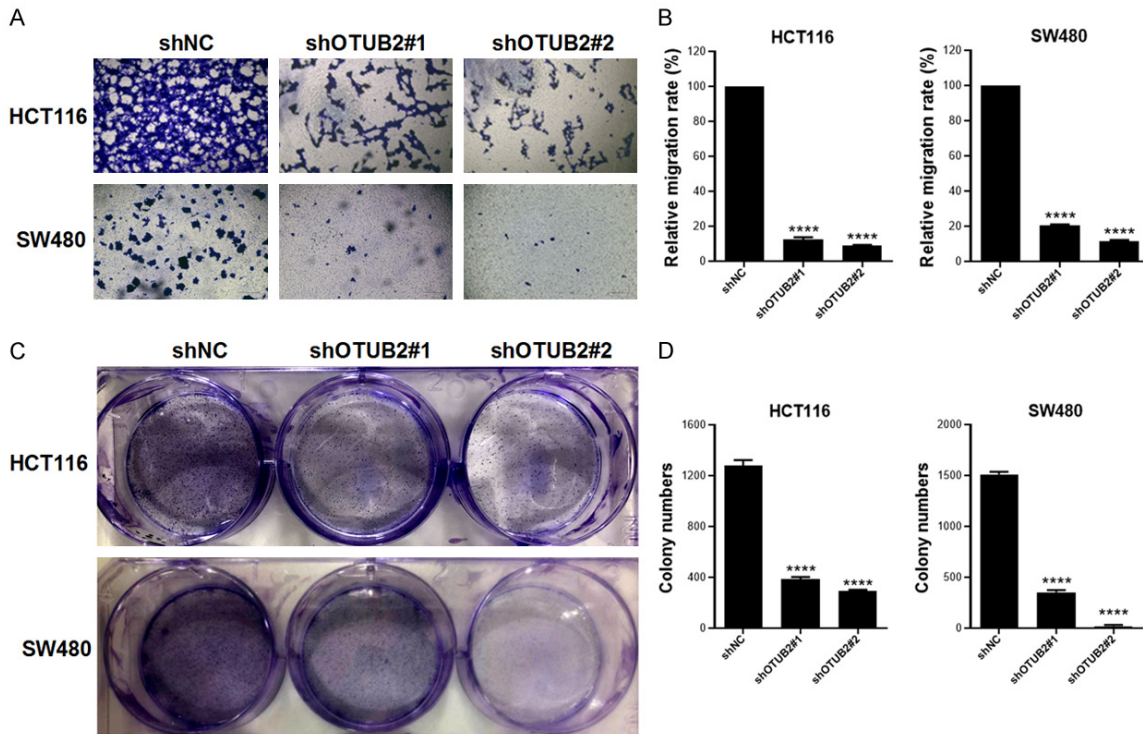


Figure S2. The influences of OTUB2 on the migration and colony formation of colorectal cancer cells. (A and B) HCT116 and SW480 cells infected with shRNAs-derived lentivirus were prepared for transwell assay and crystal violet staining was performed (A), and statistical analysis was conducted (B). (C and D) HCT116 and SW480 cells infected with shRNAs-derived lentivirus were incubated for 10 days, followed by crystal violet staining (C), and statistical analysis was conducted (D). **** $P < 0.0001$.

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Table S1. IHC expression of OTUB2

Tissues	Cases (N)	Score of OTUB2 expression				<i>p</i>
		0	1	2	3	
Cancerous	169	7	56	65	41	< 0.0001
Paracancerous	169	85	41	30	13	
Stage						
I	13	1	7	2	3	
II	66	5	21	20	20	
III	71	1	26	29	15	
IV	19	0	2	14	3	

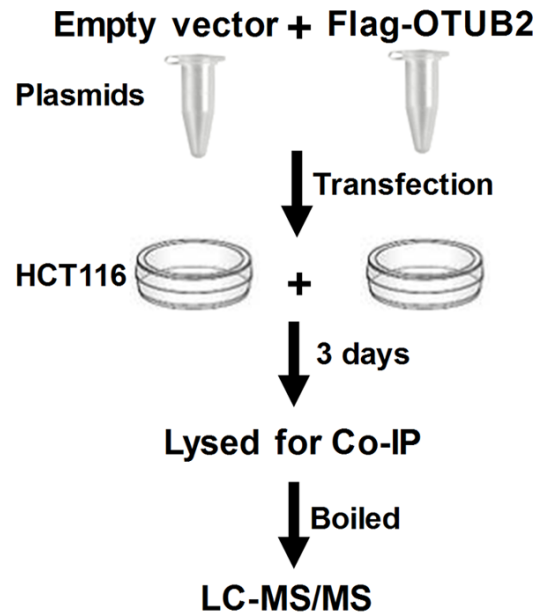


Figure S3. Flow chart of identifying the interacting proteins of OTUB2. OTUB2-overexpressing plasmids were transfected into HCT116 cells for 3 days, and then cells were lysed for Co-IP, followed by LC-MS/MS analysis.

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Table S2. The analysis result of LC-MS/MS

Hits	Protein Mass	No. of Peptide	Sequence Header	Link	Relative Abundance	Probability
4	50255.19	9	>sp P68371 TBB4B_HUMAN Tubulin beta-4B chain OS=Homo sapiens GN=TUBB4B PE=1 SV=1	P68371	3.8%	99.0%
9	76625.48	7	>sp P19338 NUCL_HUMAN Nucleolin OS=Homo sapiens GN=NCL PE=1 SV=3	P19338	2.7%	99.0%
15	83222.53	5	>sp P13010 XRCC5_HUMAN X-ray repair cross-complementing protein 5 OS=Homo sapiens GN=XRCC5 PE=1 SV=3	P13010	1.9%	99.0%
18	97067.91	5	>sp P33991 MCM4_HUMAN DNA replication licensing factor MCM4 OS=Homo sapiens GN=MCM4 PE=1 SV=5	P33991	1.7%	99.0%
19	27423.78	4	>sp Q96DC9 OTUB2_HUMAN Ubiquitin thioesterase OTUB2 OS=Homo sapiens GN=OTUB2 PE=1 SV=2	Q96DC9	1.7%	99.0%
22	32725.92	4	>sp P06748 NPM_HUMAN Nucleophosmin OS=Homo sapiens GN=NPM1 PE=1 SV=2	P06748	1.3%	99.0%
24	93800.61	3	>sp Q14566 MCM6_HUMAN DNA replication licensing factor MCM6 OS=Homo sapiens GN=MCM6 PE=1 SV=1	Q14566	1.1%	99.0%
25	81883.88	3	>sp P33993 MCM7_HUMAN DNA replication licensing factor MCM7 OS=Homo sapiens GN=MCM7 PE=1 SV=4	P33993	1.1%	99.0%
26	46352.67	3	>sp P60842 IF4A1_HUMAN Eukaryotic initiation factor 4A-I OS=Homo sapiens GN=EIF4A1 PE=1 SV=1	P60842	1.0%	99.0%
27	61187.5	3	>sp P10809 CH60_HUMAN 60 kDa heat shock protein, mitochondrial OS=Homo sapiens GN=HSPD1 PE=1 SV=2	P10809	1.0%	99.0%
29	100935.66	3	>sp Q9H3T2 SEM6C_HUMAN Semaphorin-6C OS=Homo sapiens GN=SEMA6C PE=2 SV=4	Q9H3T2	0.9%	83.5%
30	100877.22	3	>sp Q13200 PSMD2_HUMAN 26S proteasome non-ATPase regulatory subunit 2 OS=Homo sapiens GN=PSMD2 PE=1 SV=3	Q13200	0.9%	99.0%
31	86453.88	2	>sp Q01813 PFKAP_HUMAN ATP-dependent 6-phosphofructokinase, platelet type OS=Homo sapiens GN=PFKP PE=1 SV=2	Q01813	0.9%	99.0%
33	91551.17	2	>sp P25205 MCM3_HUMAN DNA replication licensing factor MCM3 OS=Homo sapiens GN=MCM3 PE=1 SV=3	P25205	0.8%	99.0%
34	49483.49	2	>sp P31943 HNRH1_HUMAN Heterogeneous nuclear ribonucleoprotein H OS=Homo sapiens GN=HNRNP1 PE=1 SV=4	P31943	0.8%	98.4%
38	69787.63	2	>sp O60506 HNRPQ_HUMAN Heterogeneous nuclear ribonucleoprotein Q OS=Homo sapiens GN=SYNCRIP PE=1 SV=2	O60506	0.7%	97.6%
39	102249.42	2	>sp P56192 SYMC_HUMAN Methionine-tRNA ligase, cytoplasmic OS=Homo sapiens GN=MARS PE=1 SV=2	P56192	0.7%	97.6%
42	58443.86	1	>sp P40227 TCPZ_HUMAN T-complex protein 1 subunit zeta OS=Homo sapiens GN=CCT6A PE=1 SV=3	P40227	0.6%	94.5%
43	20887.17	2	>sp Q9H293 IL25_HUMAN Interleukin-25 OS=Homo sapiens GN=IL25 PE=1 SV=1	Q9H293	0.6%	80.7%
46	86069.54	1	>sp P35222 CTNB1_HUMAN Catenin beta-1 OS=Homo sapiens GN=CTNNB1 PE=1 SV=1	P35222	0.5%	93.8%
47	95678.3	1	>sp Q12906 ILF3_HUMAN Interleukin enhancer-binding factor 3 OS=Homo sapiens GN=ILF3 PE=1 SV=3	Q12906	0.5%	92.2%
48	111550.25	1	>sp O14983 AT2A1_HUMAN Sarcoplasmic/endoplasmic reticulum calcium ATPase 1 OS=Homo sapiens GN=ATP2A1 PE=1 SV=1	O14983	0.5%	93.2%
49	71184.27	1	>sp O43390 HNRPR_HUMAN Heterogeneous nuclear ribonucleoprotein R OS=Homo sapiens GN=HNRNP1 PE=1 SV=1	O43390	0.5%	93.6%
51	30569.08	1	>tr D6RD18 D6RD18_HUMAN Heterogeneous nuclear ribonucleoprotein A/B OS=Homo sapiens GN=HNRNPAB PE=1 SV=1	D6RD18	0.4%	90.8%
52	64196.77	1	>sp Q9BYE2 TMPD_HUMAN Transmembrane protease serine 13 OS=Homo sapiens GN=TMPRSS13 PE=2 SV=4	Q9BYE2	0.4%	92.3%
53	117058.97	1	>sp Q02218 ODO1_HUMAN 2-oxoglutarate dehydrogenase, mitochondrial OS=Homo sapiens GN=OGDH PE=1 SV=3	Q02218	0.4%	91.2%
54	103465.04	1	>sp Q8N163 CCAR2_HUMAN Cell cycle and apoptosis regulator protein 2 OS=Homo sapiens GN=CCAR2 PE=1 SV=2	Q8N163	0.4%	88.9%
55	70263.05	1	>sp P54652 HSP72_HUMAN Heat shock-related 70 kDa protein 2 OS=Homo sapiens GN=HSPA2 PE=1 SV=1	P54652	0.4%	88.5%
57	51328.02	1	>tr A0A087WU33 A0A087WU33_HUMAN SRC kinase-signaling inhibitor 1 (Fragment) OS=Homo sapiens GN=SRCIN1 PE=1 SV=6	A0A087WU33	0.4%	88.8%
58	234167.75	1	>sp Q92614 MY18A_HUMAN Unconventional myosin-XVIIIa OS=Homo sapiens GN=MYO18A PE=1 SV=3	Q92614	0.4%	85.4%
61	106795.07	1	>sp Q99460 PSMD1_HUMAN 26S proteasome non-ATPase regulatory subunit 1 OS=Homo sapiens GN=PSMD1 PE=1 SV=2	Q99460	0.4%	88.5%
63	37654.2	1	>sp O00303 EIF3F_HUMAN Eukaryotic translation initiation factor 3 subunit F OS=Homo sapiens GN=EIF3F PE=1 SV=1	O00303	0.3%	82.1%
64	18718.73	1	>sp P23528 COF1_HUMAN Cofilin-1 OS=Homo sapiens GN=CFL1 PE=1 SV=3	P23528	0.3%	81.5%
65	9240.64	1	>tr F8WE29 F8WE29_HUMAN Target of Myb protein 1 OS=Homo sapiens GN=TOM1 PE=1 SV=1	F8WE29	0.3%	81.2%

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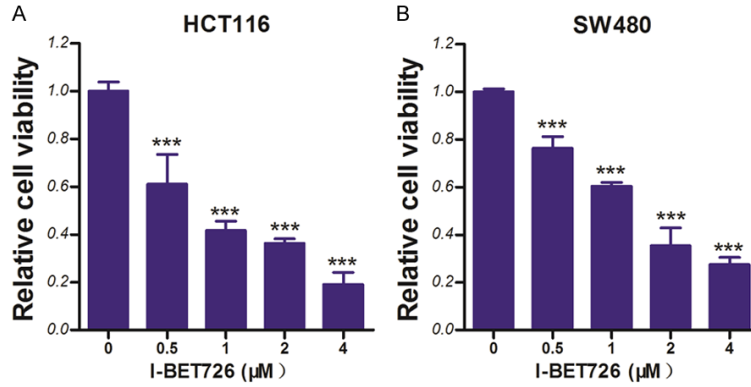


Figure S4. Effects of I-BET726 on cell viability of CRC. (A and B) HCT116 (A) and SW480 (B) cells were incubated with indicated concentrations of I-BET726 for 24 hours, followed by CCK-8 assay. *** $P < 0.001$.

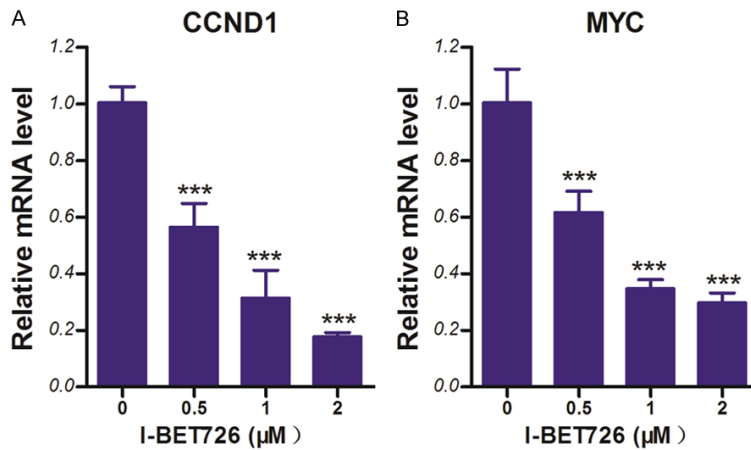


Figure S5. Effects of I-BET726 on the expression of CCND1 and MYC in CRC cells. (A and B) HCT116 cells were incubated with indicated concentrations of I-BET726 for 24 hours, followed by qRT-PCR analysis to evaluate the mRNA levels of CCND1 (A) and MYC (B). *** $P < 0.001$.