Supplemental Materials

Methods

RNA extraction and real-time PCR

Total RNA was extracted from tissues or cultured cells with TRIzol reagent (Invitrogen, Carlsbad, CA, USA), according to the manufacturer's protocol. For qPCR, First-strand cDNA were synthesized from 1 μg of total RNA with random primers or oligo dT primers, using a Reverse Transcription Kit (TaKaRa, Tokyo, Japan). Real-time PCR was performed in the LightCycler® 480II (Roche, Basel, Switzerland) using SYBR® Green (Bio-Rad, Hercules, CA, USA) and the gene-specific primers shown in the section below. ΔCt values were normalized to GAPDH standardly, and 18S rRNA specifically in the α- amanitin-induced half-time experiment.

Primers used for real-time PCR

Primer names	Sequences
lncRNA-APC1 F	GGCTTGAGTAAACTGGTAGG
lncRNA-APC1 R	CCAGAGGGCTTTATGTAATT
GAPDH F	TGCACCACCAACTGCTTAGC
GAPDH R	GGCATGGACTGTGGTCATGAG
β-actin F	GGGAAATCGTGCGTGACATTAAG
β-actin R	TGTGTTGGCGTACAGGTCTTTG

18S rRNA F	ACACGGACAGGATTGACAGA
18S rRNA R	GGACATCTAAGGGCATCACA
Rab5b F	TTCCTCACCCAGTCCGTTTG
Rab5b R	GCCTGTCGCTGTAGTTCCTT
Rab27b F	GCAGCAACTGGACAGAATGTG
Rab27b R	GGTGGCTTTTCCCCATCCAA
Rab1a F	ATGTCCAGCATGAATCCCGA
Rab1a R	AGGCAAGACTTTCCAACCCC
Rab5a F	AGACCCAACGGGCCAAATAC
Rab5a R	TTGGGTTAGAAAAGCAGCCCC
Rab7 F	TGGAGTCGGGAAGACATCAC
Rab7 R	GCACCTCTGTAGAAGGCCAC
Rab27a F	GCCATAGCACTCGCAGAGAA
Rab27a R	TGTCCACACACCGTTCCATT
PPARα F	GCGAACGATTCGACTCAAGC
PPARα R	CATCCCGACAGAAAGGCACT
-1587 promoter F	CAGAAGTGGGATGGTGACTC
-1264 promoter F	CTAATATTGTATAATGCCCTC

-712 promoter F	TTTTATGTGGATTTTGTCTAC
-160 promoter F	TCTGTGATGTGACTGTACTG
-160 promoter R	TGGAAAAGAAACAAGCTAAG
APC F	AAAATGTCCCTCCGTTCTTATGG
APC R	CTGAAGTTGAGCGTAATACCAGT
β-catenin F	AAAGCGGCTGTTAGTCACTGG
β-catenin R	CGAGTCATTGCATACTGTCCAT
ChIP-lncRNA-APC1-F	CTTTGCCTGCATTCTGCCTAC
ChIP-lncRNA-APC1-R	CTTGGGCCTGTGGTATGTCA

Plasmid construction

The cDNA of lncRNA-APC1 and PPAR alpha were amplified by primeSTAR HS DNA polymerase (Takara, China), subcloned into the Kpn I and Xho I sites of the pcDNA3.1 vector, and termed pcDNA3.1- lncRNA-APC1 and pcDNA3.1-PPARα respectively. pcDNA3.1-APC vector containing WT type APC full-length CDS and shRNA vectors specific for lncRNA-APC1 or Rab5b were purchased from GeneCopoeia (USA). TCF/LEF and c-Myc luciferase reporter gene were purchased from Beyotime Biotechnology (Guangzhou, China). Both pSPT19 and pSL-MS2-12x vectors were purchased from Addgene (Cambridge, MA). APC mutant plasmids

(APC331Δ, APC1309Δ), and β-catenin plasmids were gifts from Bert Vogelstein (addgene plasmids, Watertown, MA, USA) (1).

Transient transfection

pcDNA3.1-lncRNA-APC1, pcDNA3.1-APC, and the control plasmid pcDNA3.1(+) or short interfering RNAs (25nM) were transfected into CRC cells using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions and in 6-well plates. After 48h, the efficiency of transfection was measured by western blotting or qRT-PCR. siRNA specific for p38 were purchased from Cell Signaling Technology (Danvers, MA, USA). siRNA specific for PPARα, Rab5b, and Wnt1 were purchased from RiboBio Company (Guangzhou, China)

Construction of stable cell lines

To obtain cell lines that stably expressed APC or lncRNA-APC1, SW480 and DLD-1 cells were transfected with plasmid pcDNA3.1-APC or pcDNA3.1-lncRNA-APC1 and then selected with neomycin (800μg/ml) for four weeks.

To produce lentivirus containing shRNA targeting lncRNA-APC1 or Rab5b, HEK-293FT cells were cotransfected with the packaging plasmid pRSV/pREV, pCMV/pVSVG, and pMDLG/pRRE using Lipofectamine 2000, according to the manufacturer's guidelines. Lentiviruses were harvested 48hr after transfection and filtered through 0.45μm PVDF filters. We used a scrambled shRNA as a negative control. HCT116 cells were transfected with lncRNA-APC1 specific knockdown or control lentiviruses and selected with 2 μg/ml puromycin for two weeks. SW480 or DLD-1 cells were transfected with Rab5b specific knockdown or control lentiviruses

and selected with 2 μg/ml puromycin for two weeks. All the cells mentioned above were infected with lentivirus in the presence of 8-10 μg/ml polybrene (Sigma-Aldrich) and the supernatant was replaced with complete culture medium after 18-24 h. The stably overexpressing cell lines were identified by qRT-PCR and/or western blotting.

Western blot analysis

Proteins were extracted using RIPA Lysis and Extraction Buffer (Thermo Fisher Scientific, Waltham, MA, USA) containing 1× cocktail (Roche, Basel, Switzerland), and then separated by SDS-PAGE gel electrophoresis before transferring to PVDF membranes (Merck Millipore, Billerica, MA, USA). We performed the rho-GTPase activation assay as previously reported (2). The following antibodies were used: anti-CD63(1:1000, Abcam #ab134045), TSG101 (1:500, Santa Cruz #sc-7964), Hsc70 (1:500, Abcam #ab51052), anti-RAB5b (1:1000, Santa Cruz #sc-373725), anti-PPARα (1:1000, R&D Systems, #PP-H0723-00), anti-β-catenin (1:3000, BD Biosciences #610154), anti-PARP (1:1000, #5625), anti-APC (1:1000, #2504), anti-caspase-9 (1:1000, #9508), anti-caspase-3(1:1000, #9665), anti-CDK4 (1:1000, #9868 CDK antibody kit), anti-CDK2 (1:1000, #9868 CDK antibody kit), anti-cyclin E2 (1:1000, #9869 Cyclin antibody kit), anti-cyclin D1 (1:1000, #9869 Cyclin antibody kit), a Rho-GTPase antibody sampler kit, and anti-GAPDH (1:1000, #5174) were all purchased from Cell Signaling Technology (Danvers, MA, USA). Antibody to detect the N-terminal of APC was perchased from Abcam (1:1000, #ab58).

Colony formation assay

Treated Cells (2×10^2 or 5×10^2) were placed in a fresh 6-well plate and maintained in RPMI1640 containing 10% FBS. Two weeks later, cells were fixed, stained with crystal violet, photographed, and counted.

Cell viability assay (CCK-8 assay)

We detected cell viability using a Cell Counting Kit-8 (CCK-8) (Dojindo Laboratories, Kumamoto, Japan). Briefly, 1×10^3 CRC cells were cultured in 96-well plates for 24 h, with normal saline (NS) serving as the control, with three wells prepared for each group. To determine the cell viability, 10 μl of CCK-8 solution was added to each well, the 96-well plate was incubated continuously at 37 °C for 2 h, and the OD value for each well was read at a wavelength of 450 nm on a microplate reader (Multiskan, Thermo, USA). The assay was repeated three times. The cell viability was calculated as follows:

Cell viability (%) =OD (experiment) −OD (blank)/OD (control) −OD (blank) ×100

Transwell (Motility Assay)

Motility chambers (BD Biosciences, San Jose, CA, USA) were rehydrated and equilibrated for 2 h with 500 ml of serum-free RPMI1640 media. The medium in the inserts was aspirated and inserts were placed into wells containing RPMI1640 with 20% FBS. Treated cells were added to each chamber in serum-free media. Exosomes isolated from equivalent amounts of colorectal cells were added to the media of HUVECs. The chambers were incubated with exosomes for 6 h. The media was then

removed and the upper surface of the membrane was scrubbed 20 times with a cotton swab. Cells on the lower surface of the scrubbed membranes were fixed by methanol and stained with crystal violet.

Capillary tube formation assay

HUVECs were cultured in a 96-well plate coated with Matrigel (R&D Systems, Minneapolis, MN) for 12 h at 37°C in the absence or presence of exosomes derived from CRC cell lines. The formation of capillary-like structures was captured under a light microscope. The formed tubes, which represented the degree of in vitro angiogenesis, were scanned and quantitated in five low-power fields (100x).

Flow cytometry

Cell cycle analysis was conducted on a Coulter Epics XL flow cytometry system (Beckman Coulter, Miami, FL, USA), as previously described (3). The apoptotic cells were detected using an Annexin V-FITC apoptosis kit, according to the manufacturer's protocol.

Luciferase reporter assay

To determine the effect of PPAR alpha on the lncRNA-APC1 promoter, siRNA specific for PPAR alpha was co-transfected into DLD-1 cells together with individual pGL3-basic, pGL3-WT (with WT binding site), or pGL3-Mut (without PPARs binding site) construct, plus Renilla luciferase reporter plasmid. Twenty-four hours after transfection, we measured the firefly and Renilla luciferase activity using a Dual-Luciferase Reporter Assay System (Promega, Madison, WI, USA). The data are represented as mean ± SD of three independent experiments.

Immunohistochemistry

Tumor tissues were fixed in formalin and embedded in paraffin. Five μm sections were cut and processed for IHC, in accordance with a previously described protocol (2). Tissue slides were incubated with anti-Rab5b (1:100 dilution; Santa Cruz Biotechnology, #sc-373725，Santa Cruz, CA, USA), anti-APC (1:50 dilution; Cell Signaling Technology, #2504，Danvers, MA, USA), and anti-CD34 (1:100 dilution; Santa Cruz Biotechnology, #sc-74499, Santa Cruz, CA, USA) and stored overnight at 4°C. Immunostaining was conducted using the Envision System with diaminobenzidine (Dako, Glostrup, Denmark). A negative control was obtained by replacing the primary antibody with normal rabbit or mouse IgG (Cell Signaling Technology, #2729, #53484). The immunoreactivity for Rab5b and APC were scored in a semiquantitative method, as previously described (4). The microvessel density (MVD) in tumor tissues was evaluated, as previously described. Expression for the markers was assessed by two independent pathologists (Mu-Yan Cai and Dan Xie) who were blinded to the clinicopathological data.

Immunofluorescence (IF) Staining

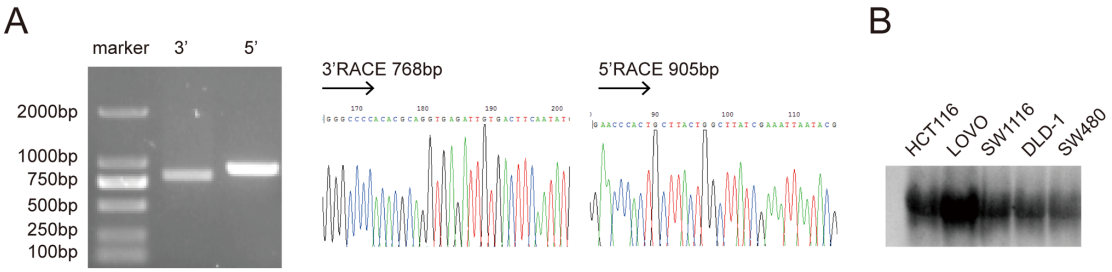
Treated cells were grown on glass coverslips up to 70% confluence. Then, cells were washed three times with PBS and fixed in 4% paraformaldehyde. For F-actin staining, cells were incubated with rhodamine-phalloidin (Invitrogen, Carlsbad, CA) overnight at 4°C. For HUVEC staining with DiI-labeled exosomes, HUVECs were grown on glass coverslips up to 70% confluence and then incubated with DiI-labeled exosomes for 12 hours. For the exosome location *in vivo* assay, DiI-labeled exosomes were

injected into the tail veins of nude mice with xenograft tumors. After 24 h, mice were sacrificed and tumors were immediately excised and then frozen and cut into slides. After thorough washing, cells were mounted with mounting medium containing DAPI (Vector Laboratories, Burlingame, CA) prior to imaging with a fluorescence microscope (Leica, Deerfield, IL).

Supplemental reference

1. Morin PJ, et al. Activation of beta-catenin-Tcf signaling in colon cancer by mutations in beta-catenin or APC. Science 1997; 275:1787-90.
2. Zheng F, et al. The putative tumor suppressor microRNA-124 modulates hepatocellular carcinoma cell aggressiveness by repressing ROCK2 and EZH2. Gut. 2012; 61:278-89.
3. Liao YJ, et al. Longikaurin A, a natural ent-kaurane, induces G2/M phase arrest via downregulation of Skp2 and apoptosis induction through ROS/JNK/c-Jun pathway in hepatocellular carcinoma cells. Cell Death Dis. 2014 Mar 20; 5:e1137.
4. Wang FW, et al. Ablation of EIF5A2 induces tumor vasculature remodeling and improves tumor response to chemotherapy via regulation of matrix metalloproteinase 2 expression. Oncotarget. 2014 Aug 30; 5:6716-33.

Supplemental Figures

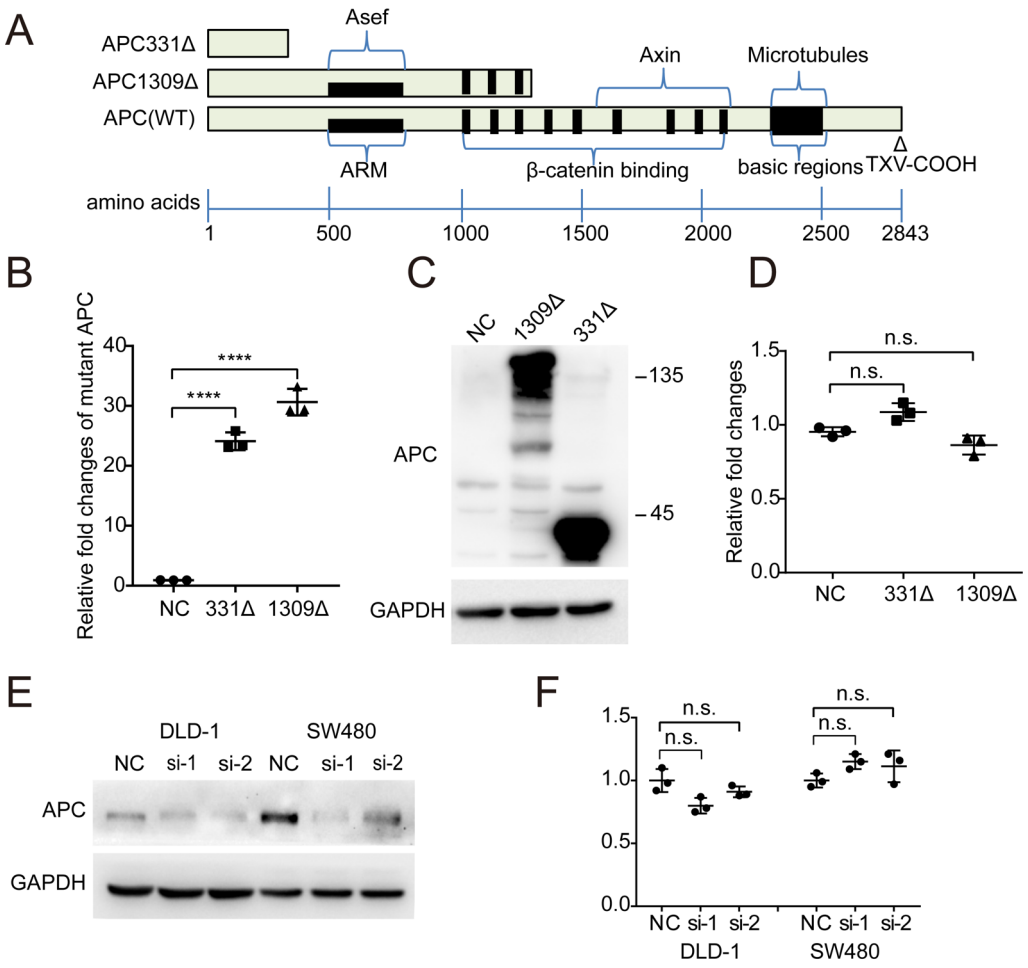


C

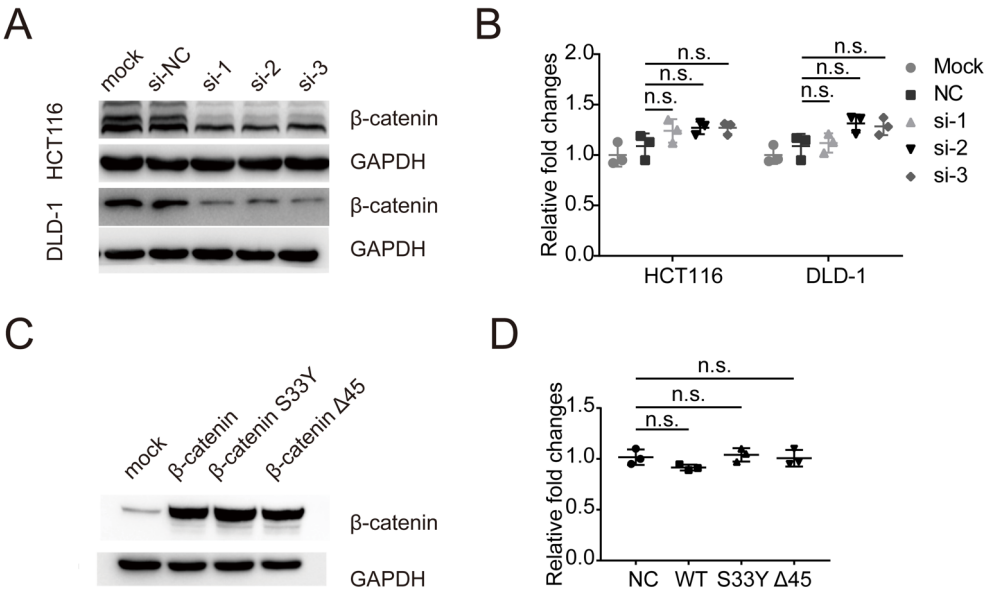
1580bp

GAACCCACTGCTTACTGGCTTATCGAAATTAATACGACTCACTATAGGGAGACCCAAGCTGGCTAGCGTTTAAACTTAAGCTTCCT
TGGTGATGTGACTCTTCTCTCCCGCTGAAGCCATGCCCACACAAGGGAAAGTGACTTATCACTGGGCCCAGCACACAGGTGGAT
GGTGACTCTAAGACCAGGATTCAACACAACTAGAAGGCTTTGACTCTAATACGAGTCACAGAAGGCTCTGCGACTCTTCTATTTTC
TCTAGGTTATGCAAATGGAGGTGGATTGTGACATATCATAGGGCCAACACAACACACAGTCTACAGGTGGAATTGGGACTCTCATG
CATGGATCCAGTTCATCATTGATACTGTGACTCAGGTGGGATTTTGACATATAACTGCACCCAGCACACAGGTGAGATTGTGACTCT
TGTATGCTCATCCTGCCAAAAGTTAAGTGTCACCCTCACACATGGACAGAGACTTCTGGTAAGGTCTTGAATCTTGTACATGGACAC
AGTCCACAGCTGGAATGTCATATGTAGATCCAGCCACAGGTGTGATGTTGACCTATTTATAAAGTTAGCTCACAGGCACATTGATAA
CTCTCATACCTGGATCCAGCCAATAAAATATATGTTGACTATTATAGCTAGGCTTGAGTAAACTGGTAGGGTCCTGGGTCTTCTACCT
GTATGAGAGTCACAGAAGATTATGACACTTATGGAAATTACATAAAGCCCTCTGGCAATGCAAAGTTTTATCACGGGGCCCCACAC
GCAGGTGAGATTGTGACTTCAATATGCAGACAAAGCTAACAGTTAGTATTGTTACCTTCACATATAAACAGAGCCCACTGGTGAGGT
TCTAAATCTCACACACCAAAAAAATTCACAGTTGGAATTGTGACTGATATGTGAATCTGCCCACTGATGGAATGCTGACTCATTTCTG
GATTCAGCTTAGAAAAATGGTGATGACTGTTTCACCTGGACTCAGCCAAATGGAGAGATGTTGACTCTCTTAACTAGGTGTGATGG
TTAATACTGAGTGTCAACTAGGCTGGATTGAAGGATACAAAGTATTAAATCTGGGTGTCTCTGTGAGGGTGTTGCCAAAGGAGATTA
ACAACTGAGTCAGTTGGCTGGAAAAGGCAGACACACACTTAATCTGGATGGGCGCAATCTAATCAGATGCCAGAGTGGCTAGAAT
GTAAGCAGGCAGAAAAATGTGAAAAGAGTGACTGGTCTAGCCTCTCAGCCTACATCTTTCTCCTGTGCTGGATTCTTCCTGCCCTT
GAACATCAGACTCCAAGTTCTTTAGTTTTGGAACTCGGAGTGGCTCTCCTTGATTCTCAGCCCACAGAATGCCTATTGTGGATCTAG
TGATTCTGTGAGTTAATACCTAATAAACTCCCCTCTCGAGTCCAGAGGGCCCGTTTAAACCCGTTGATCAGCCTCGACTGTGCCTT
CTAGTTGCCAGCCATCTGTTGTTTGCCCCTCCCCCGTGCCTTCCTTGACCCTGGAAAGGTGCCACTCCCACTGTCCTTTCCTAAT
AAAATGAGGAAATTGCATCCCAAAAA

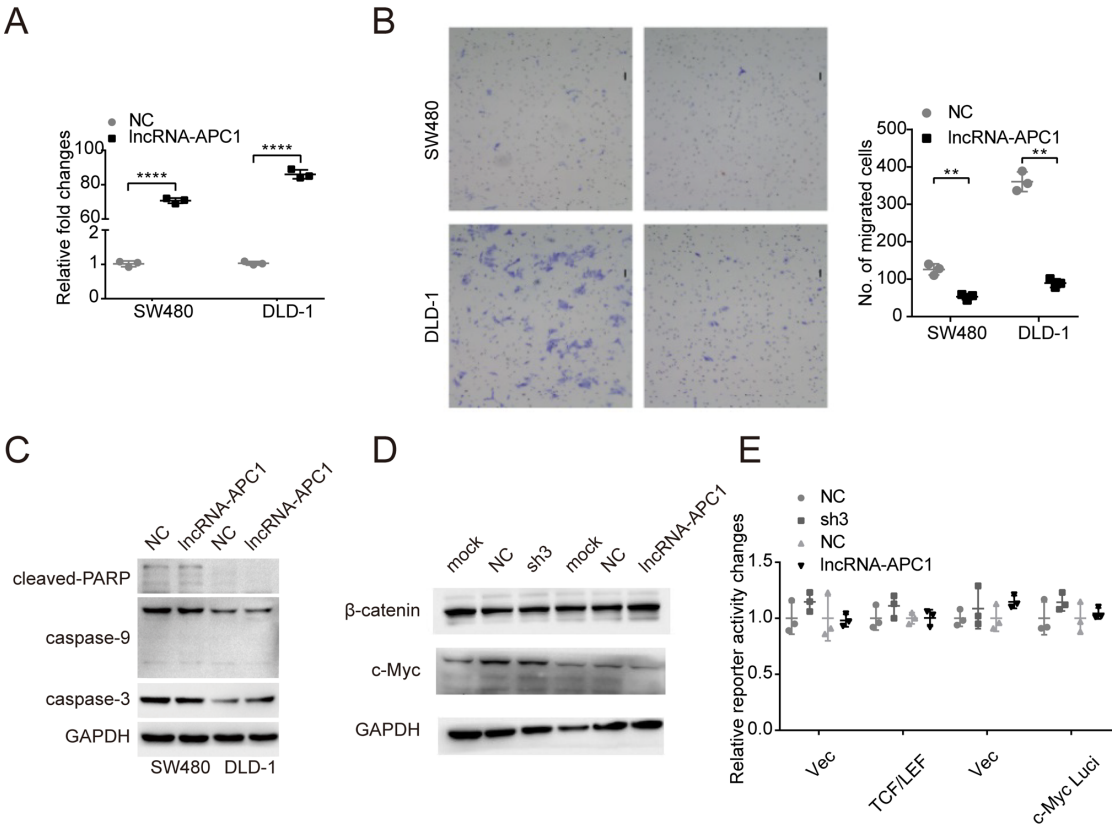
Supplemental Figure 1. Full-length of human lncRNA-APC1. (A) A representative image of PCR products from the 3'-RACE and 5'-RACE assay (left), and sequencing of PCR products revealed the boundary between the universal anchor primer and the lncRNA-APC1 sequences (right). **(B)** Northern blot analysis of lncRNA-APC1 in CRC cell lines. **(C)** The nucleotide sequence of full-length human lncRNA-APC1.



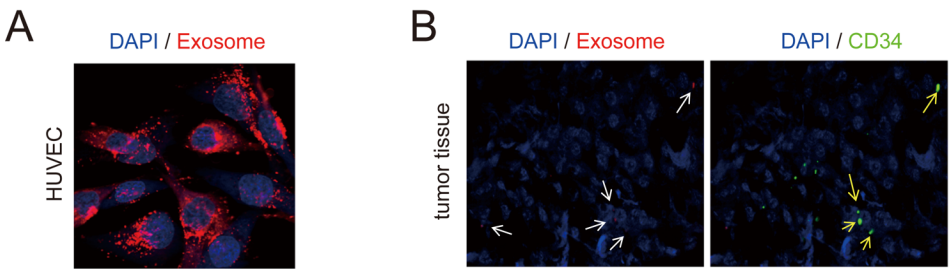
Supplemental Figure 2. The effect of mutant APC on lncRNA-APC1 expression. **(A)** Schematic of mutant APC constructs**.** (1, 2) (APC331Δ, represents a type of mutation commonly found in the germ line of familial adenomatous polyposis patients as well as in sporatic tumors, and APC1309Δ is the most common germline APC mutation.) Relative expression of mutant APC detected by qRT-PCR **(B)** and western blot **(C)**. **(D)** Relative expression of lncRNA-APC1 detected by qRT-PCR. **(E)** APC expression detected by Western blot. **(F)** Relative expression of lncRNA-APC1 detected by qRT-PCR. Error bars represent mean ± SD from 3 independent experiments. $^{****}P<0.0001$ in 1-way ANOVA. n.s.： no significant difference.



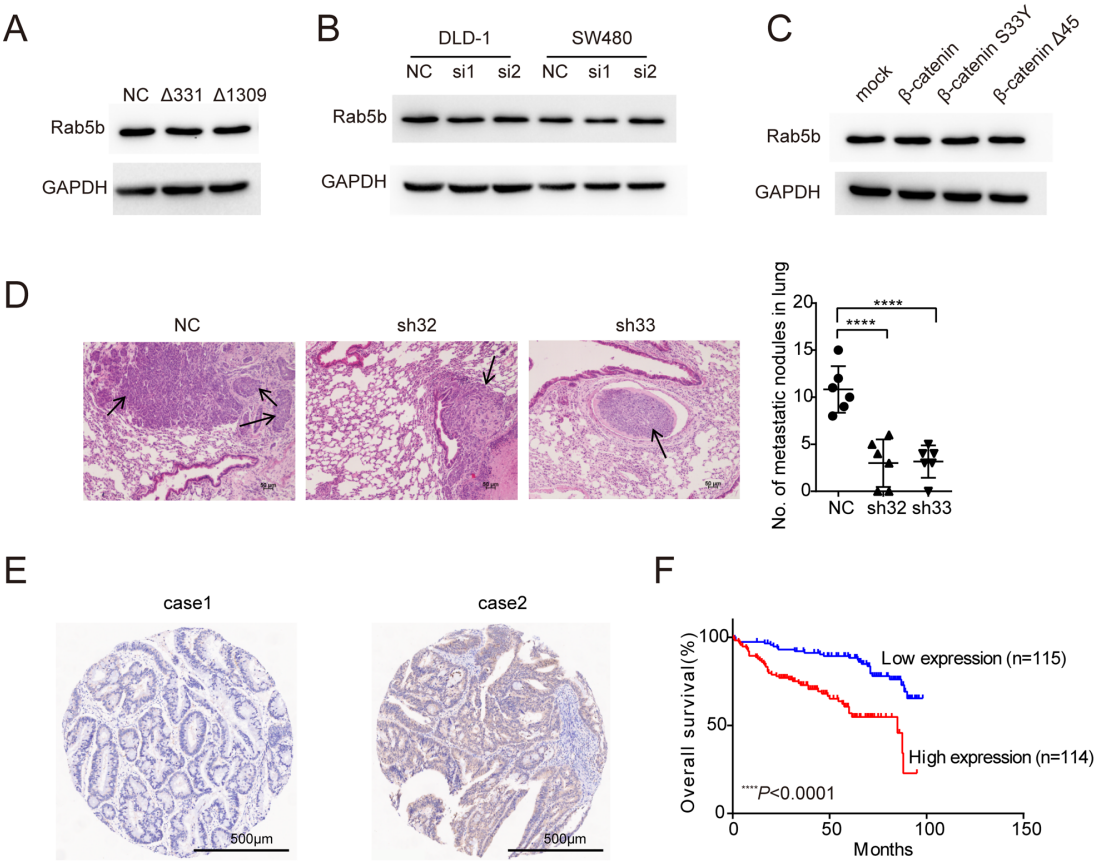
Supplemental Figure 3. β-catenin silencing has no significant effect on lncRNA-APC1 expression. (A) Western blotting shows the knock down efficiency of siRNAs specific for β-catenin. **(B)** qRT-PCR analysis of lncRNA-APC1 expression in cells with silencing of β-catenin or control. **(C)** Western blotting shows β-catenin expression. **(D)** qRT-PCR analysis of lncRNA-APC1 expression in indicated cells. Error bars represent mean±SD from 3 independent experiments. n.s.: no significant difference in 1-way ANOVA.



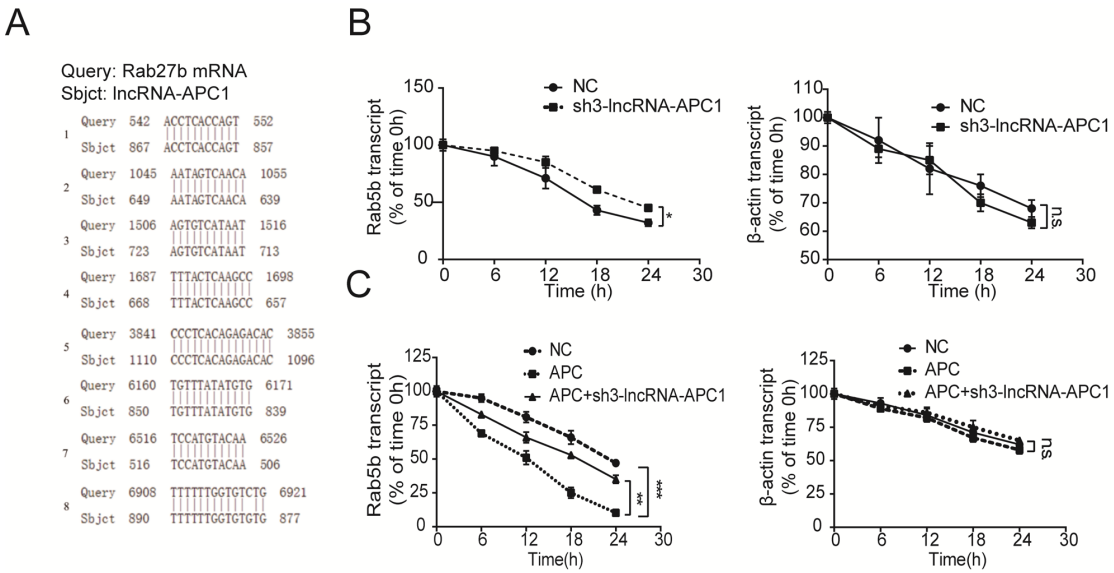
Supplemental Figure 4. lncRNA-APC1 exerts functions independent on β-catenin or c-Myc. (A) The relative expression of lncRNA-APC1 in stable SW480 and DLD-1 cell clones. **(B)** Representative images of and transwell invasion assays. **(C)** Western blot assay reveals that lncRNA-APC1 has no effect on cell apoptosis markers. **(D)**Western blotting shows the expression of β-catenin or c-Myc in indicated cells. **(E)** The relative luciferase reporter activity in indicated cells. Error bars represent mean±SD from 3 independent experiments. $^{**}P<0.000$, $^{****}P<0.0001$ in independent Student's *t* test.



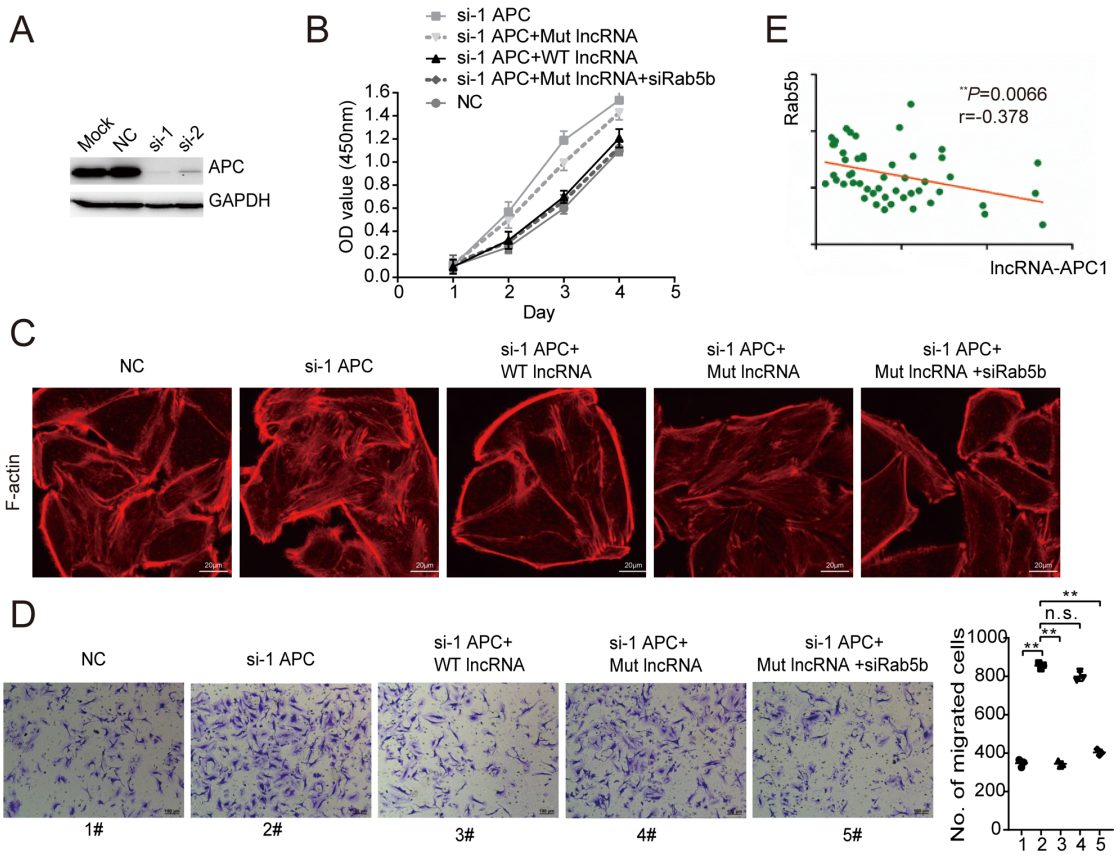
Supplemental Figure 5. Immunofluorescence images of DiI-labeled exosomes in HUVECs (400×) **(A)**, and DiI-labeled exosomes and CD34 in xenograft tumors formed in nude mice (400×) **(B)**. The experiment was repeated 3 times with similar result.



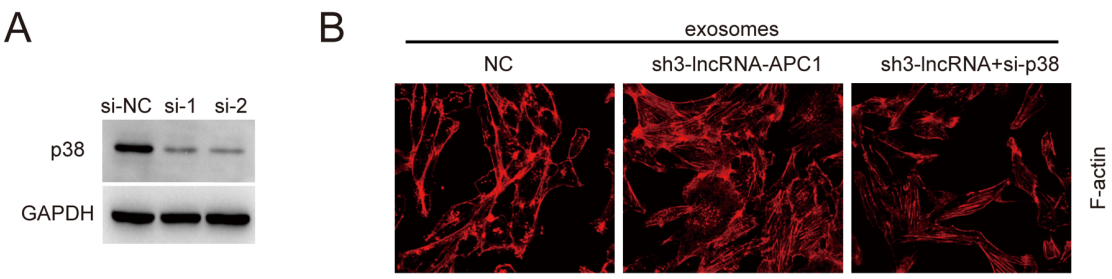
Supplemental Figure 6. Rab5b protein expression was not induced by mutant APC or β-catenin, and has a negatively correlation with CRC patients prognosis. Western blotting analysis of Rab5b expression induced by truncated APC **(A),** si-APC **(B)** or β-catenin **(C)**. Rab5b was detected in the same gel of corresponding APC or β-catenin in Supplemental Figure 2 and 3 respectively. All experiments were repeated 3 times. ***P<0.0001 in 1-way ANOVA. **(D)** Representative images of H&E-stained sections derived from the lung metastatic nodules (original magnification, 100×). **(E)** Representative images of Rab5b protein expression via IHC detecting of 229 cases CRC tissue. **(F)** Kaplan-Meier survival analysis of CRC patients according to Rab5b protein expression.



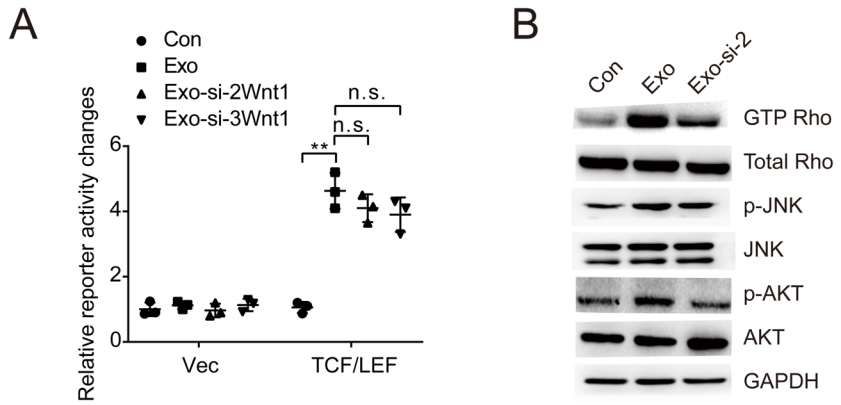
Supplemental Figure 7. lncRNA-APC1 reduces the stability of *Rab5b* mRNA. **(A)** Regions of putative binding between Rab27b mRNA (Query) and lncRNA-APC1 (subject). **(B)** and **(C)** The stability of *Rab5b* mRNA was measured by qRT-PCR. $^{*}P<0.05$ in independent Student's *t* test (B), or $^{***}P<0.001$ in 1-way ANOVA (C).



Supplemental Figure 8. APC exerts function through lncRNA-APC1suppressing and interacting with Rab5b mRNA. (A) APC was efficiently knocked down by specific siRNAs in HCT116 cells. **(B)** The cell proliferation rate determined by CCK8 assay. **(C)** Representative images of F-actin, a marker reflecting actin remodeling or cytoskeleton. **(D)** Representative images of transwell invasion assay, which were performed at least 3 times independently from the assay in Figure 11E. **(E)** The correlation between lncRNA-APC1 and *Rab5b* mRNA was measured by qRT-PCR in 50 cases of CRC tissues (Pearson's correlation analysis, P=0.0066). Bar: 100μm, Error bars represent mean±SD from 3 independent experiments. $^{**}P$<0.01, n.s.: no significant difference, in independent Student's *t* test or 1-way ANOVA (B, D).



Supplemental Figure 9. Exosomes enhance tumor angiogenesis by activating MAPK signaling of HUVECs. (A) p38 was effectively silenced by specific siRNA. **(B)** Stress fibers and actin filaments of HUVECs were demonstrated by phalloidin staining (red).



Supplemental Figure 10. Exosomal Wnt1 activated non-canonical Wnt signaling in CRC cells. (A) Relative luciferase activity of TCF/LEF reporter activity induced by indicated exosomes. **(B)** Expression of indicated markers of WNT/PCP and PI3K/AKT pathway detected by western blot. Error bars represent mean±SD from 3 independent experiments. $^{***}P<0.001$, n.s.: no significant difference in 1-way ANOVA.

Supplemental Figure Reference

1. Jiang H, et al. Peptidomimetic inhibitors of APC-Asef interaction block colorectal cancer migration. Nat Chem Biol. 2017; 13:994-1001.
2. Morin PJ, et al. Activation of beta-catenin-Tcf signaling in colon cancer by mutations in beta-catenin or APC. Science 1997; 275:1787-90.