Figure S1

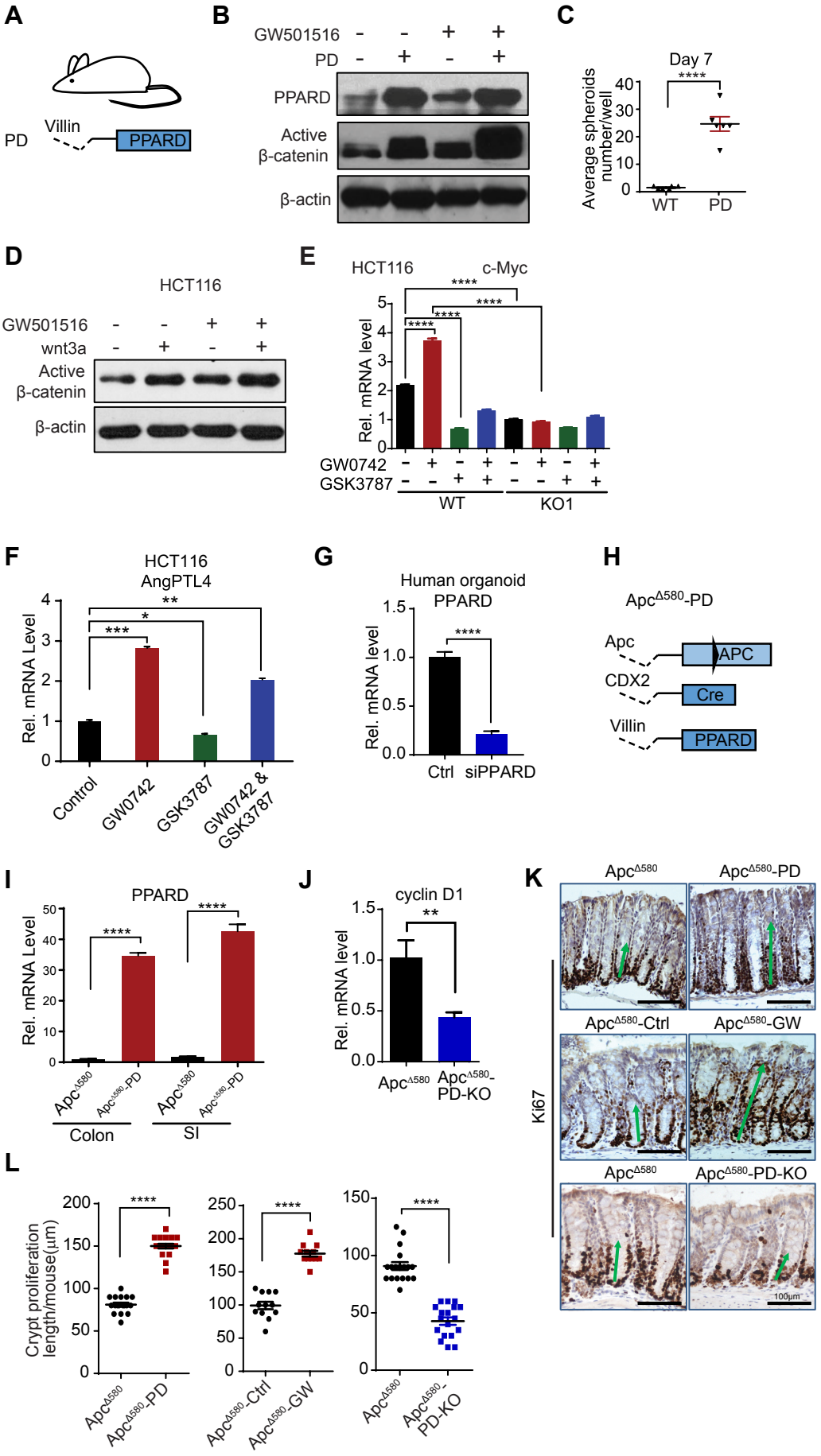
A
PD
Villin
PPARD
B
GW501516 - - + +
PD - + - +
PPARD
Active β-catenin
β-actin
C
Day 7
****
Average spheroids number/well
40
30
20
10
0
WT
PD
D
HCT116
GW501516 - - + +
wnt3a - + - +
Active β-catenin
β-actin
E
HCT116
c-Myc
****
****
****
****
Rel. mRNA level
5
4
3
2
1
0
GW0742 - + - + - + - +
GSK3787 - - + + - - + +
WT
KO1
F
HCT116
AngPTL4
**
*
***
Rel. mRNA Level
4
3
2
1
0
Control
GW0742
GSK3787
GW0742 & GSK3787
G
Human organoid
PPARD
****
Rel. mRNA level
1.5
1.0
0.5
0.0
Ctrl
siPPARD
H
Apc^Δ580-PD
Apc
APC
CDX2
Cre
Villin
PPARD
I
PPARD
****
****
Rel. mRNA Level
50
40
30
20
10
0
Apc^Δ580
Apc^Δ580-PD
Apc^Δ580
Apc^Δ580-PD
Colon
SI
J
cyclin D1
**
Rel. mRNA level
1.5
1.0
0.5
0.0
Apc^Δ580
Apc^Δ580-PD-KO
K
Apc^Δ580
Apc^Δ580-PD
Apc^Δ580-Ctrl
Apc^Δ580-GW
Apc^Δ580
Apc^Δ580-PD-KO
Ki67
100μm
L
****
****
****
Crypt proliferation length/mouse(μm)
200
150
100
50
0
250
200
150
100
50
0
150
100
50
0
Apc^Δ580
Apc^Δ580-PD
Apc^Δ580-Ctrl
Apc^Δ580-GW
Apc^Δ580
Apc^Δ580-PD-KO

Figure S1

PPARD increases active β-catenin protein expression. **A,** Schematic diagram of villin-PPARD (PD) mice. Dash lines indicate the promoter region, followed by the PPARD gene coding region. **B,** WT and PD mice at 6 weeks were fed with a PPARD agonist GW501516 (50mg/kg) diet or a control diet for 4 weeks. PPARD and active β-catenin protein levels in IECs were analyzed by Western blot. **C,** The organoid-initiating capacity of IECs derived from PD mice and WT littermates at 10 weeks (n = 6 per group). Organoid numbers per well were presented. **D,** Active β-catenin protein level in HCT116 cells treated with wnt3a (100 μM) and/or PPARD agonist GW501516 (1 μM) for 24 hours, measured by Western blot. **E,** c-Myc mRNA expression levels in HCT116 WT and KO1 cells treated by PPARD agonist GW0742 (1 μM) and/or PPARD antagonist GSK3787 (1 μM) for 24 hours, measured by qRT-PCR. **F,** AngPTL4 mRNA expression levels in HCT116 cells treated by GW0742 and/or GSK3787 as described in panel E. **G,** PPARD mRNA level in human organoid cells transfected with control siRNA (Ctrl) or PPARD siRNA (siPPARD) for 48 hours. **H,** Schematic diagram of $Apc^{\Delta 580}$-PD mice. PD mice were bred with $Apc^{\Delta 580}$-flox; CDX2-Cre mice to generate $Apc^{\Delta 580}$-flox;CDX2-Cre;PD ($Apc^{\Delta 580}$-PD) mice. Dash lines indicate the promoter region, followed by the indicated gene coding regions. **I,** PPARD mRNA expression levels of colon and small intestinal (SI) IECs from $Apc^{\Delta 580}$-PD and $Apc^{\Delta 580}$ mice were measured by qRT-PCR. **J,** Expression level of cyclin D1 mRNA in IECs from $Apc^{\Delta 580}$ and $Apc^{\Delta 580}$-PD-KO mice. **K and L,** Representative images of Ki-67 IHC (**K**) and corresponding colonic crypt proliferation zone lengths (**L**) of normal colons of the indicated mice. Data are shown as mean ± SEM. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; and ****$P < 0.0001$.

Figure S2

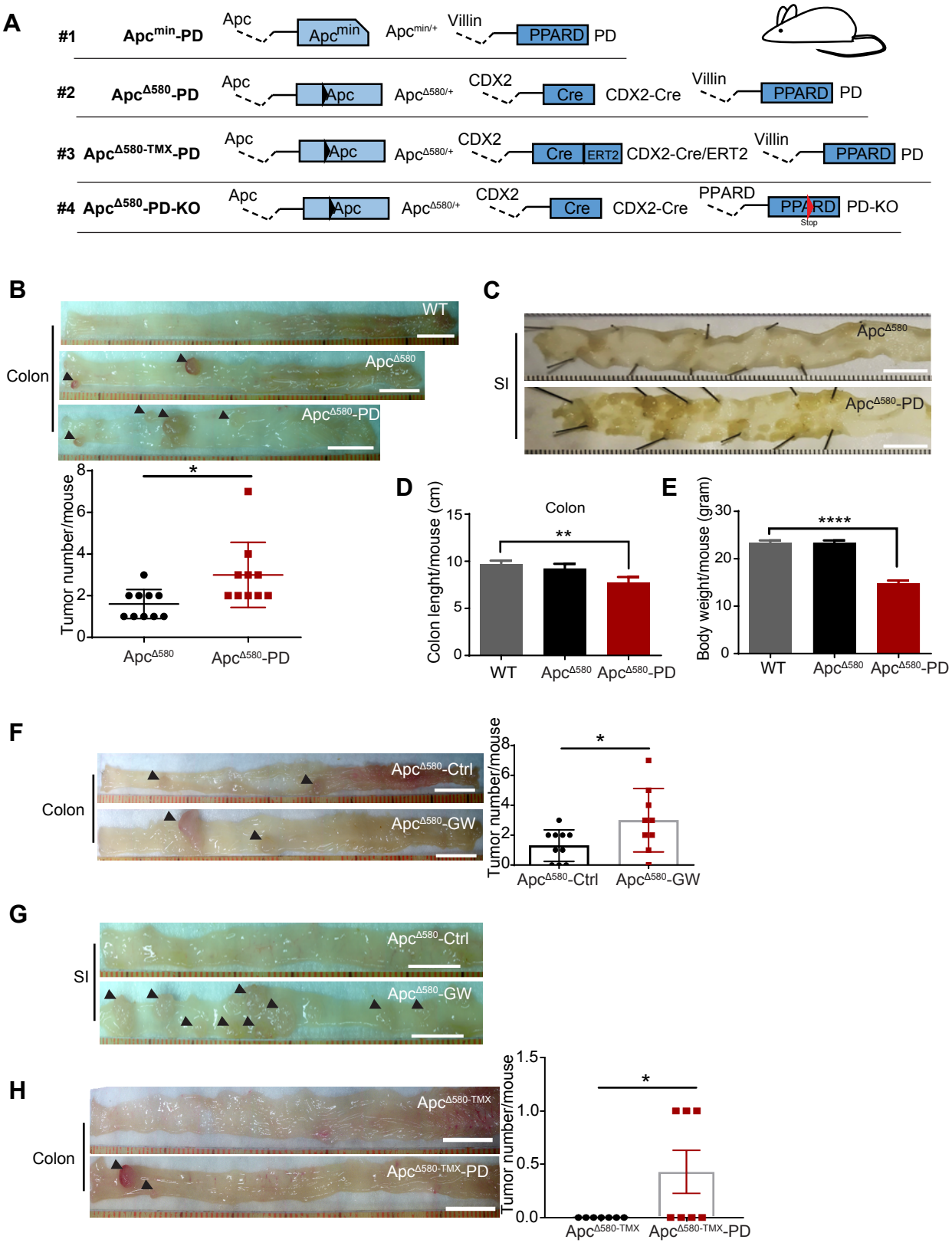
A
#1 Apc^min-PD
Apc
Apc^min
Apc^min/+
Villin
PPARD
PD
#2 Apc^Δ580-PD
Apc
Apc
Apc^Δ580/+
CDX2
Cre
CDX2-Cre
Villin
PPARD
PD
#3 Apc^Δ580-TMX-PD
Apc
Apc
Apc^Δ580/+
CDX2
Cre
ERT2
CDX2-Cre/ERT2
Villin
PPARD
PD
#4 Apc^Δ580-PD-KO
Apc
Apc
Apc^Δ580/+
CDX2
Cre
CDX2-Cre
PPARD
PPARD
Stop
PD-KO
B
WT
Colon
Apc^Δ580
Apc^Δ580-PD
Tumor number/mouse
*
Apc^Δ580
Apc^Δ580-PD
C
Apc^Δ580
SI
Apc^Δ580-PD
D
Colon
Colon lenght/mouse (cm)
**
WT
Apc^Δ580
Apc^Δ580-PD
E
Body weight/mouse (gram)
****
WT
Apc^Δ580
Apc^Δ580-PD
F
Colon
Apc^Δ580-Ctrl
Apc^Δ580-GW
Tumor number/mouse
*
Apc^Δ580-Ctrl
Apc^Δ580-GW
G
SI
Apc^Δ580-Ctrl
Apc^Δ580-GW
H
Colon
Apc^Δ580-TMX
Apc^Δ580-TMX-PD
Tumor number/mouse
*
Apc^Δ580-TMX
Apc^Δ580-TMX-PD

Figure S2

PPARD promoted intestinal tumorigenesis in multiple APC mutant mouse models. **A,** Schematic diagram of the multiple transgenic mouse models. PD mice were bred with Apc^{min}, or $Apc^{\Delta 580}$-flox; CDX2-Cre or $Apc^{\Delta 580}$-flox;CDX2-Cre/ERT2 mice to generate Apc^{min}-PD (Row#1); $Apc^{\Delta 580}$-PD(Row#2); $Apc^{\Delta 580\text{-}TMX}$-PD(Row#3) mice, respectively. PD-flox mice were bred with $Apc^{\Delta 580}$-flox; CDX2-Cre to generate $Apc^{\Delta 580}$-PD-KO (Row#4). Dash lines indicate the promoter region, followed by the indicated gene coding regions. **B-E,** Representative images of fresh colons (**B, top**), colonic tumor numbers per mouse (**B, bottom**), representative images of formalin-fixed distal small intestines (**C**), colon length (**D**) and weight (**E**) per mouse for wild type (WT), $Apc^{\Delta 580}$ and $Apc^{\Delta 580}$-PD mice at age 14 weeks. **F and G,** $Apc^{\Delta 580}$ mice at age 4 weeks were fed with GW501516 (50mg/kg) or control diet for 10 weeks. Representative images of fresh colons (**F, left**), colonic tumor numbers per mouse (**F, right**) and representative images of fresh distal small intestines (**G**) for $Apc^{\Delta 580}$ mice treated with GW501516 ($Apc^{\Delta 580}$-GW) or control ($Apc^{\Delta 580}$-Ctrl) diet. **H,** $Apc^{\Delta 580\text{-}TMX}$ and $Apc^{\Delta 580\text{-}TMX}$-PD littermates were treated with tamoxifen at age 6 weeks and then followed up for another 55 weeks. Representative images of fresh colons (left) and colonic tumor numbers per mouse (right) for $Apc^{\Delta 580\text{-}TMX}$ and $Apc^{\Delta 580\text{-}TMX}$-PD mice. Data are shown as mean ± SEM. *$P < 0.05$; **$P < 0.01$; and ***$P < 0.001$.

Figure S3

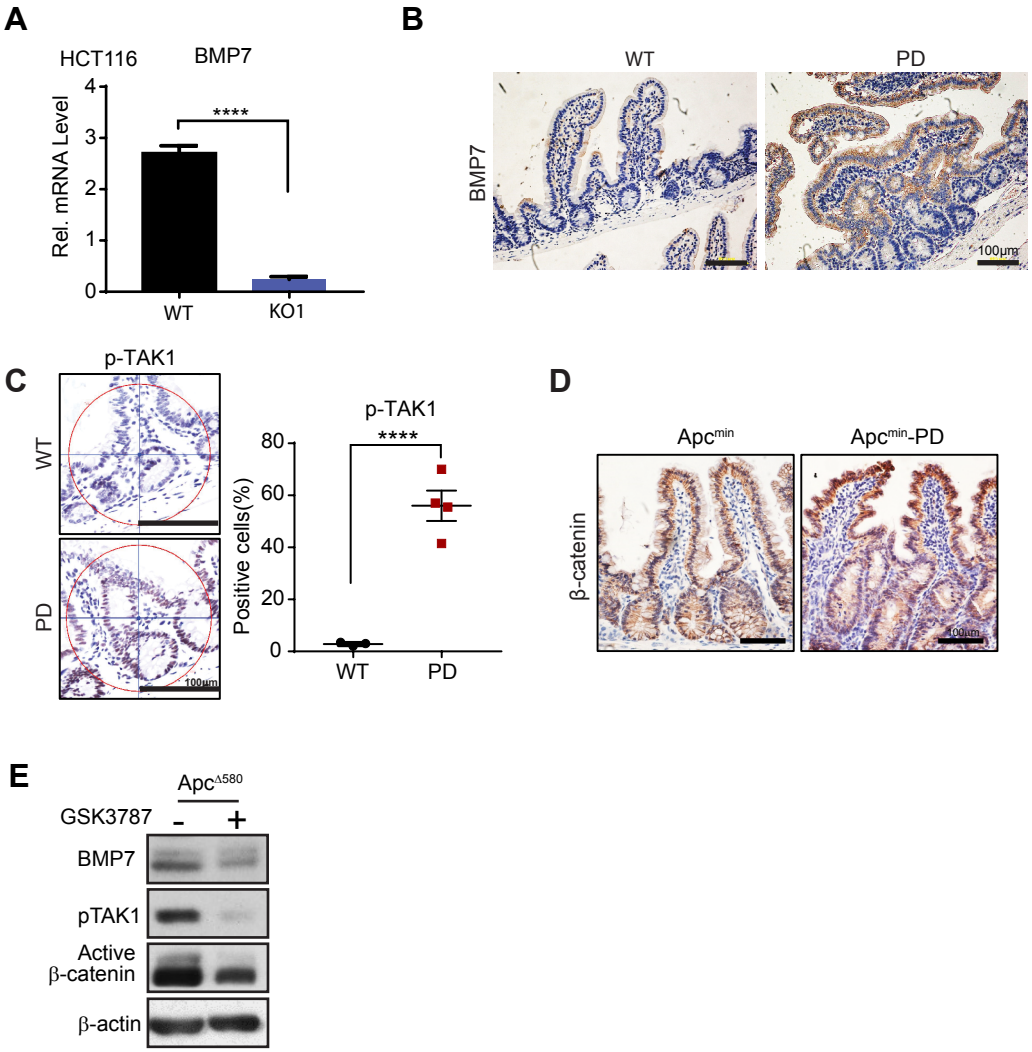

Figure S3

PPARD increases BMP7/active β-catenin expression. **A,** The BMP7 mRNA expression levels in HCT116 WT and KO1 cells from RNA-Seq data analysis. **B and C,** BMP7 (**B**) and p-TAK1(**C**) expressions were measured by immunohistochemistry staining in intestinal sections from WT and PD littermates at age 10 weeks. The average percentage of positively-stained p-TAK1 cells obtained from 20 glands were presented. **D,** Active β-catenin expression were measured by immunohistochemistry staining in intestinal sections from the Apc^{min} and Apc^{min}-PD mice at 8 weeks. **E,** BMP7, p-TAK1 and active β-catenin protein levels in IECs of $Apc^{\Delta 580}$ mice fed with PPARD antagonist GSK3787 (200mg/kg) diet or control diet for 12 weeks were analyzed by Western blot. Data are shown as mean ± SEM. ****$P < 0.0001$.

Figure S4

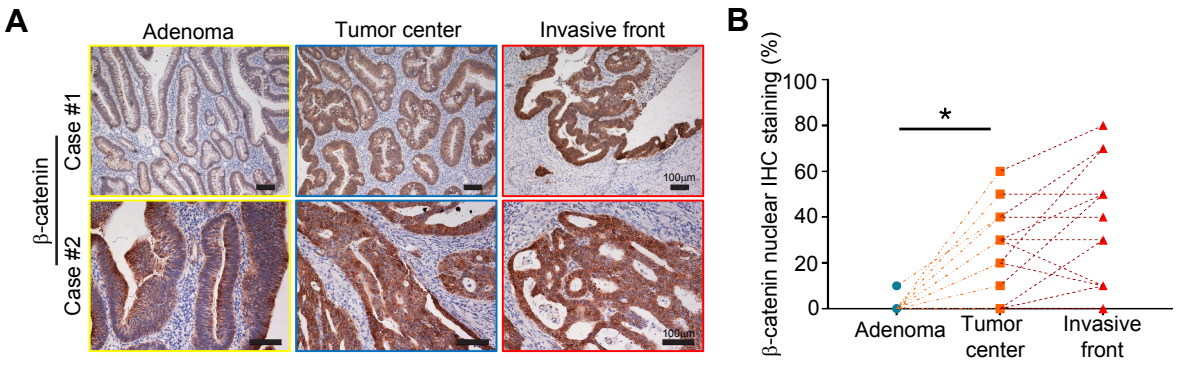

Figure S4

Active β-catenin expression in human colorectal cancer invasive fronts. **A,** Representative active β-catenin IHC staining images of human paired colorectal adenomas (Adenoma), CRC tumor centers (Tumor center), and cancer invasive fronts (Invasive front) of 2 patients. **B,** The percentage of positive nuclear active β-catenin staining for the paired adenomas, CRC tumor centers, and invasive fronts as described in Figure 4E (n = 41 patients). Data are shown as mean ± SEM. *$P < 0.05$.

Figure S5

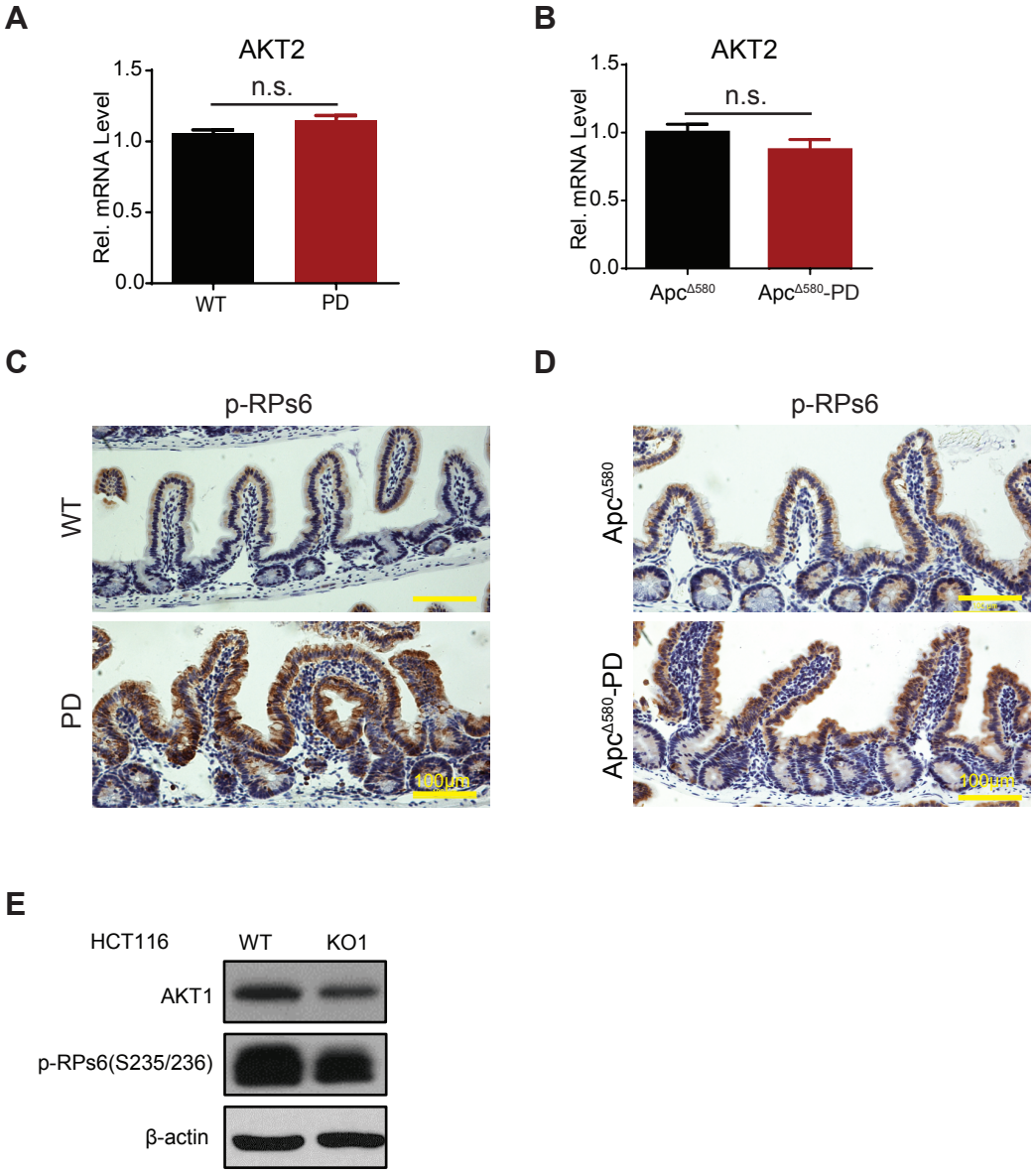

Figure S5

PPARD upregulated AKT1/p-rpS6 signaling pathway. **A and B,** AKT2 mRNA expression levels for IECs from WT and PD (**A**) or Apc$^{\Delta 580}$ and Apc$^{\Delta 580}$-PD (**B**) mice were measured by qRT-PCR. **C and D**, IHC results of p-rpS6 (S235/236) for the intestines from WT and PD (**C**) or Apc$^{\Delta 580}$ and Apc$^{\Delta 580}$-PD (**D**) mice. **E,** AKT1 and p-rpS6 (S235/236) protein expression levels in parental HCT116 WT and KO1 cells were measured by Western blot. Data are shown as mean ± SEM. n.s.: no significant difference.

Figure S6

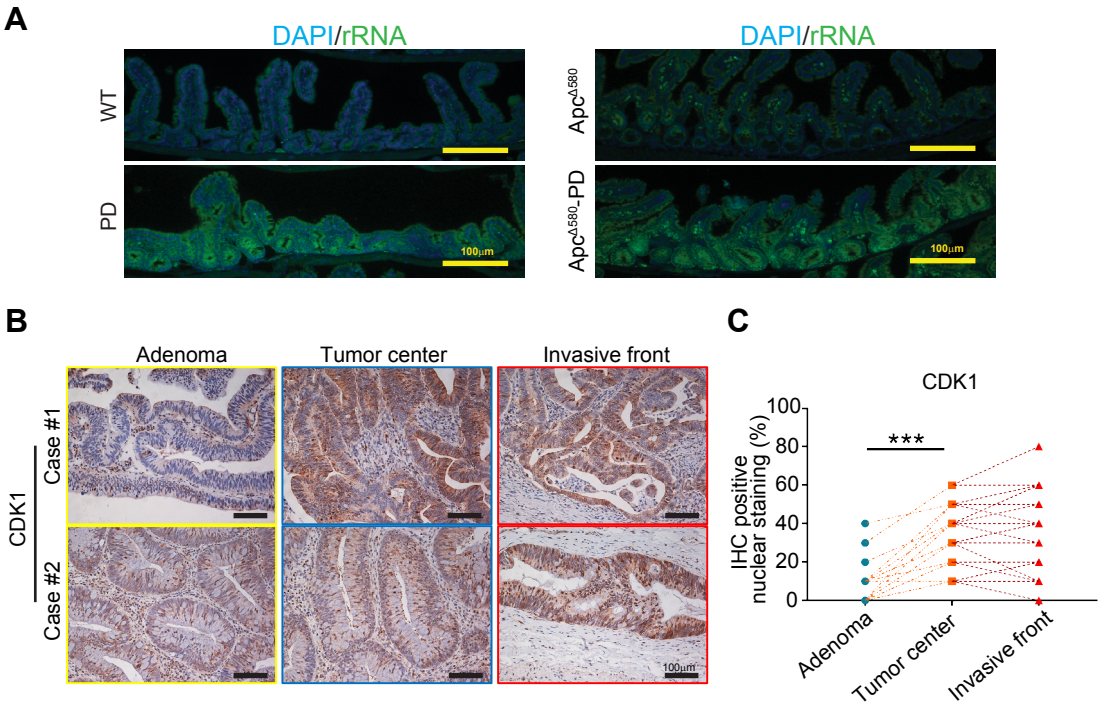

Figure S6

rRNA expression in mouse intestinal tissues and CDK1 expression in human colorectal cancer invasive fronts. **A,** Immunofluorescence staining of rRNA using Y10b antibody (Green) for the intestinal tissues from PD and $Apc^{\Delta 580}$-PD mice and their corresponding control littermates. **B and C,** CDK1 expression in human colorectal cancer invasive front. **B,** Representative CDK1 IHC staining images of human paired colon adenomas, CRC tumor centers, and invasive fronts of 2 patients. **C,** The percentage of positive nuclear CDK1 staining for the paired adenomas, CRC tumor centers, and invasive fronts as described in Figure 4E (n = 41 patients). Data are shown as mean ± SEM. ****P <0.001*.

Figure S7

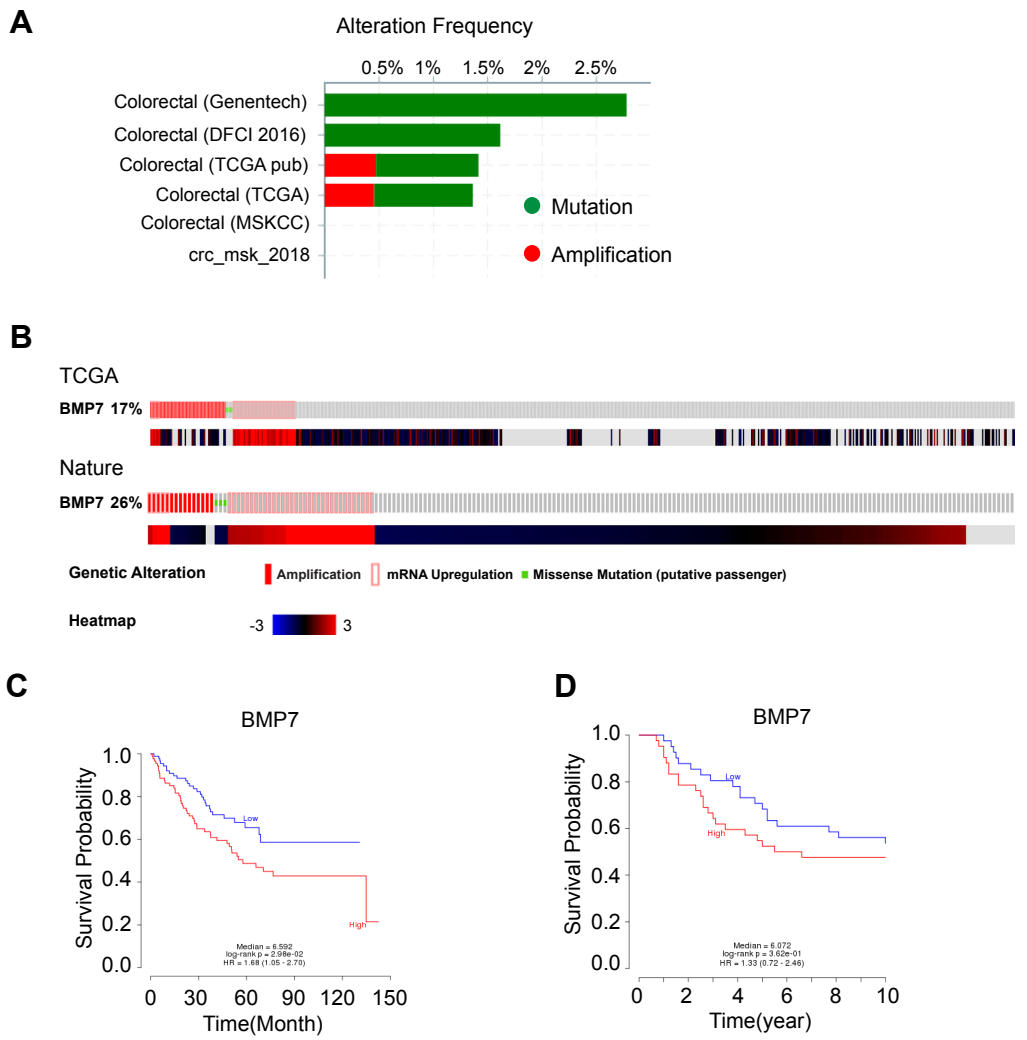

Figure S7

PPARD and BMP7 genetic alterations in colon cancer patients. **A,** PPARD genetic alterations in TCGA colorectal cancer databases. Querying 2395 samples in six studies. **B,** The heatmap of BMP7 genetic alteration analyses (e.g. amplification, mRNA upregulation) of TCGA Colorectal Provisional (629 cases) and Nature public (195 cases) databases. **C and D,** Comparison of the survival probability for the colon cancer patients with low and high expression of BMP7 from analyses of two public databases [**C** (GSE17536) and **D** (GSE24549)] in the PRECOG public database portal.