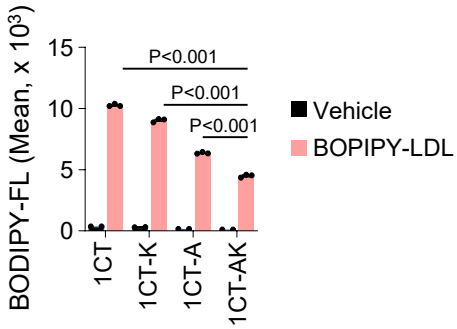
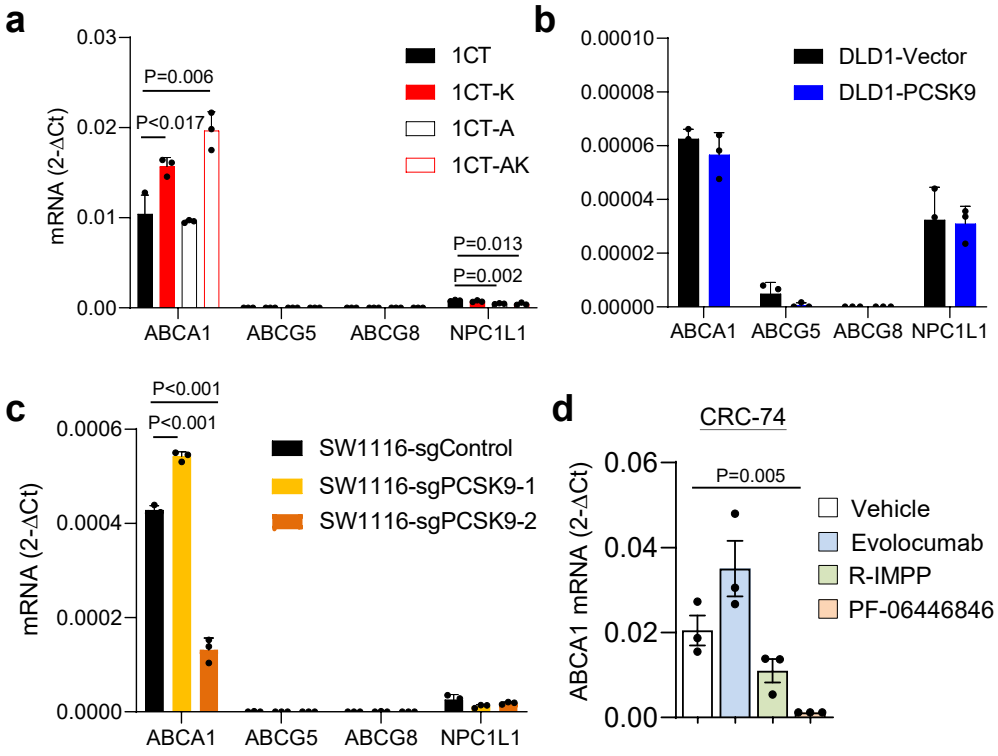
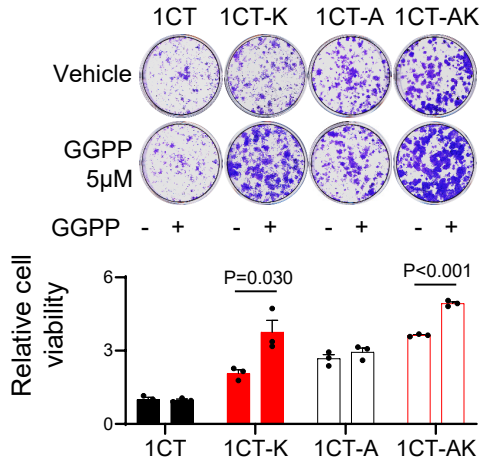
Supplementary Information for The cholesterol uptake regulator PCSK9 promotes and is a therapeutic target in APC/KRAS-mutant colorectal cancer



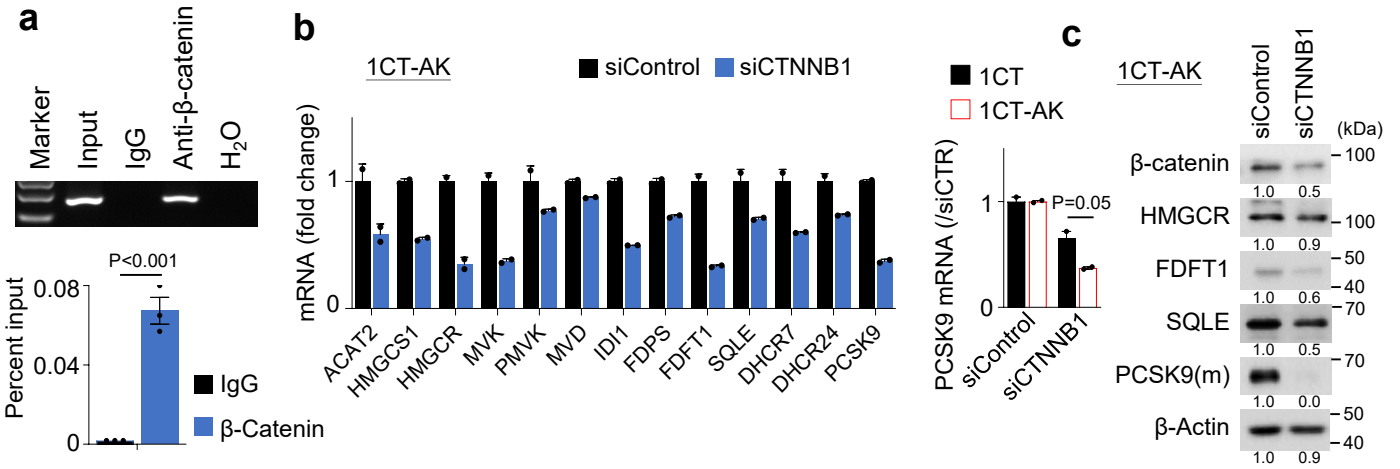
Supplementary Figure S1. Quantitative analysis of intracellular fluorescence after incubation with BODIPY-LDL (10μg/ml, 4h) by flow cytometry (n=3). Data shown are means of biological replicates; ± S.E.M. Two-tailed Student's t-test.



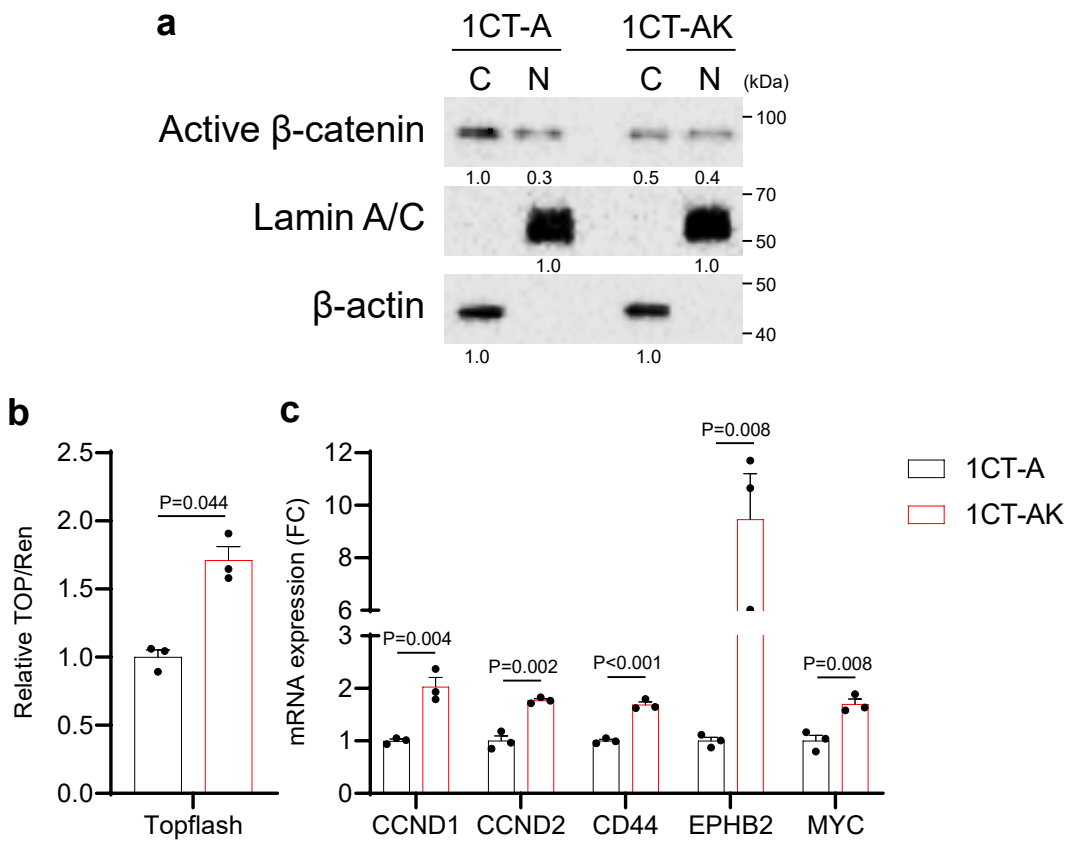
Supplementary Figure S2. qPCR of ABCA1, ABCG, ABCG8, and NPCL11 expression in **a** 1CT isogenic cells (n=3), **b** DLD1 cells with PCSK9overexpression (n=3), **c** SW1116 cells with PCSK9 knockout (n=3) and **d** CRC-74 organoids (n=3). Data shown are means of biological replicates; ± S.E.M (**a**, **b**, **c**, **d**). Two-tailed Student's t-test (**a**, **b**, **c**, **d**).



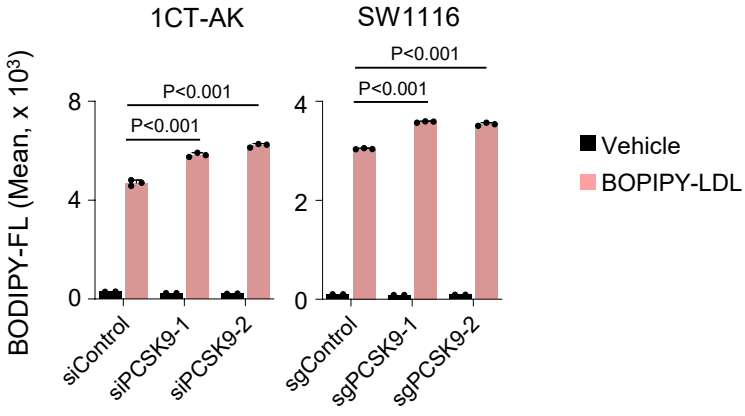
Supplementary Figure S3. Colony formation assays showed that GGPP promoted the cell growth of 1CT-K and 1CT-AK cells, but not in 1CT and 1CT-A cells (14 days, n=3). Data shown are means of biological replicates; ± S.E.M. Two-tailed Student's t-test.



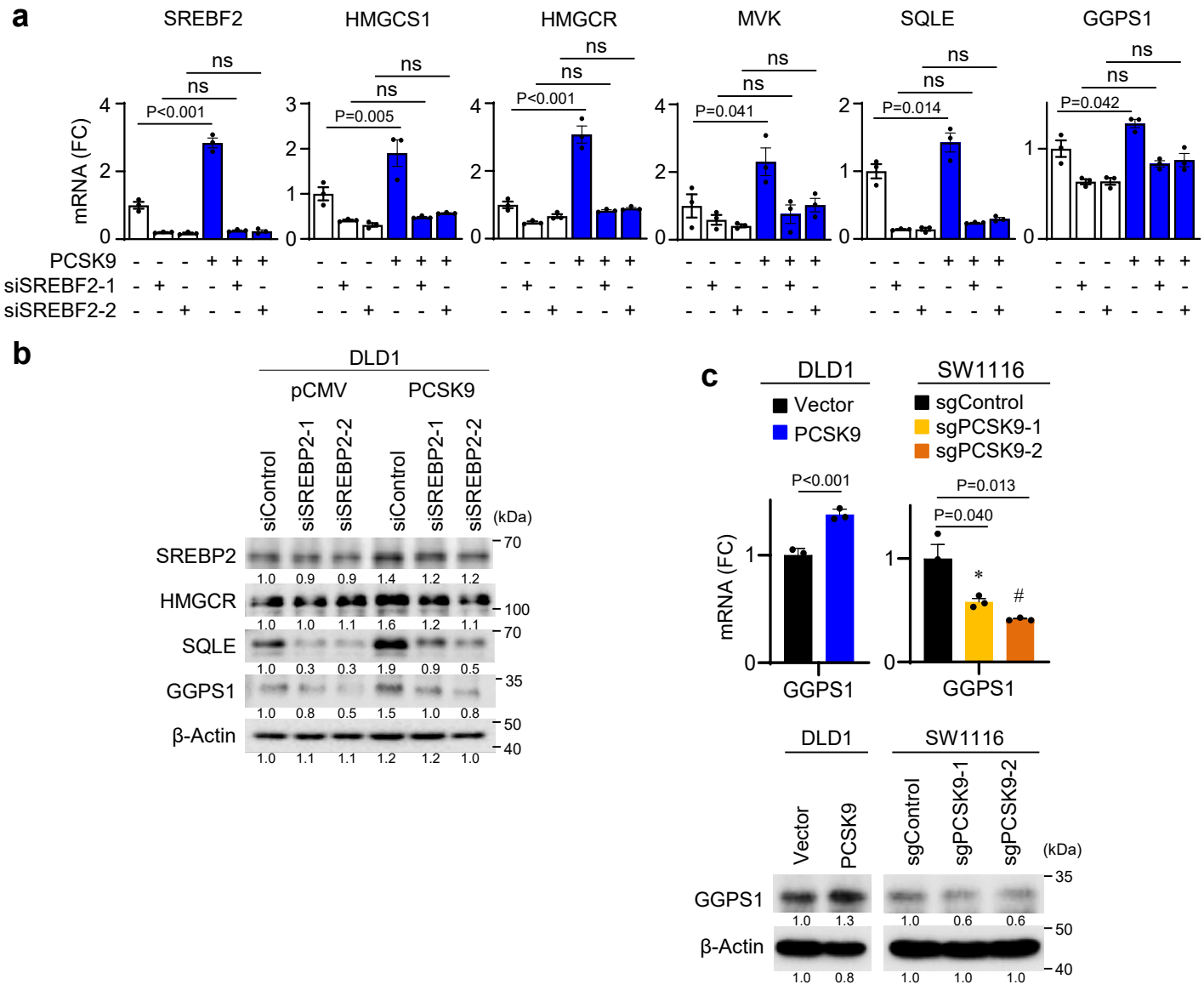
Supplementary Figure S4. β-catenin regulates PCSK9. **a** Chromatin immunoprecipitation-PCR (ChIP-PCR) and qPCR analysis of the binding of β-catenin to PCSK9 promoter (n=3). **b** β-catenin knockdown (72h) inhibited mRNA expression of PCSK9 and cholesterol biosynthesis genes in 1CT-AK cells (left) (n=2), with a more pronounced effect on PCSK9 mRNA in 1CT-AK cells compared to 1CT cells (right). Data are presented as means ± S.E.M. t-test (two-sided) **c** Western blot of effects of β-catenin knockdown in 1CT-AK cells. Data shown are means of biological replicates; ± S.E.M. (**a**, **b**). Two-tailed Student's t-test (**a**, **b**).



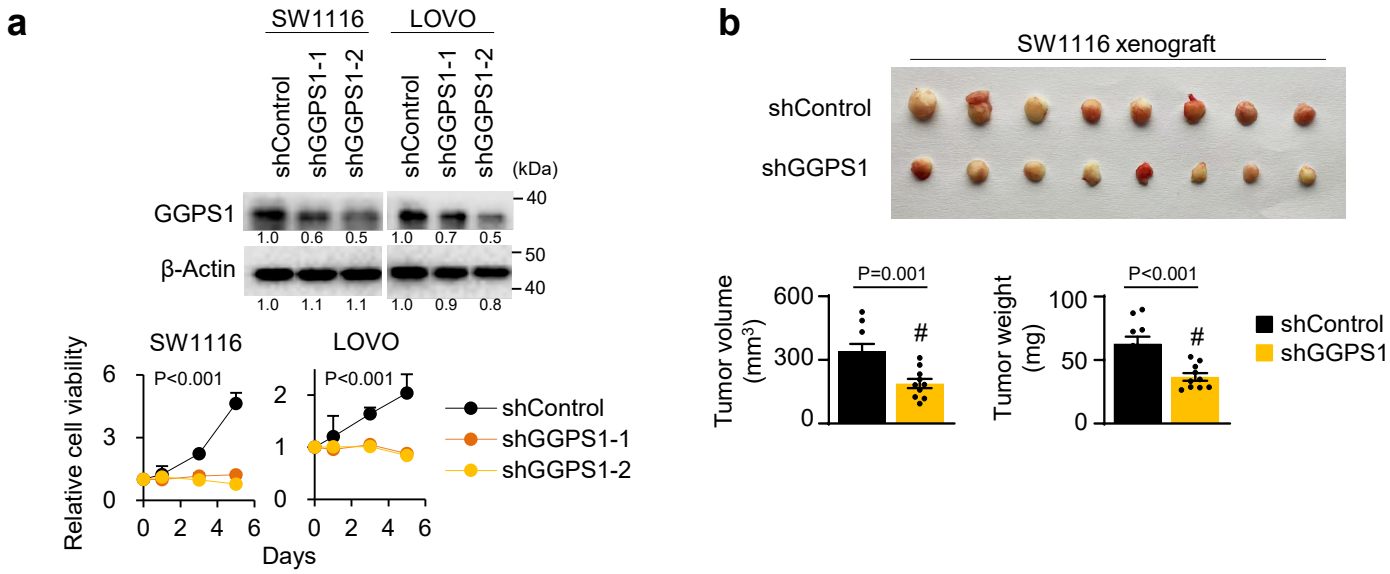
Supplementary Figure S5. Mutant KRAS promotes WNT/β-catenin signaling. **a** The localization of active β-catenin, **b** TOPflash activity (n=3), and **c** mRNA expression of β-catenin targets in 1CT-A and 1CT-AK cells (n=3). Data shown are means of biological replicates; ±S.E.M. (**b**, **c**). Two-tailed Student's t-test (**b**, **c**).



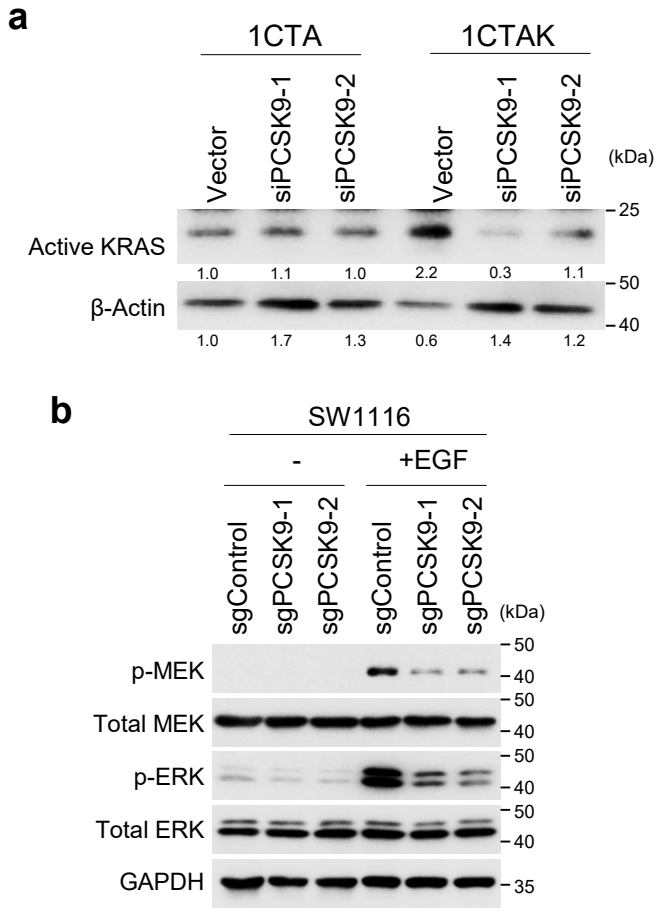
Supplementary Figure S6. Knockdown or knockout of PCSK9 induced the uptake of BODIPY-FL LDL (10μg/mL for 4h), as determined by flow cytometry (n=3). Data shown are means of biological replicates; ± S.E.M. Two-tailed Student's t-test.



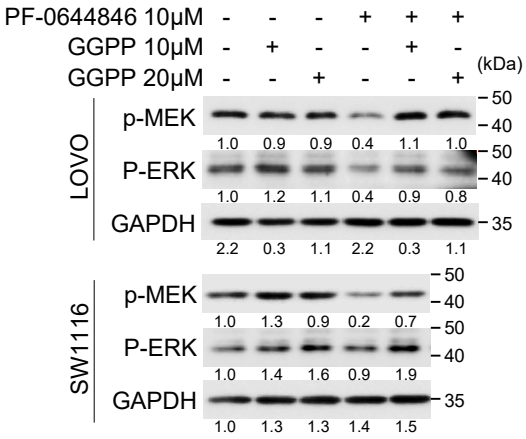
Supplementary Figure S7. a SREBP2 knockdown abrogated PCSK9 overexpression-mediated expression of cholesterol biosynthesis genes (n=3) and **b** validation by western blot. **c** PCSK9 knockout reduced GGPS1, whereas PCSK9 overexpression exerted an opposite effect (n=3). Data shown are means of biological replicates; ±S.E.M. (**a**, **c**) Two-tailed one way ANOVA (**a**). Two-tailed Student's t-test (**c**).



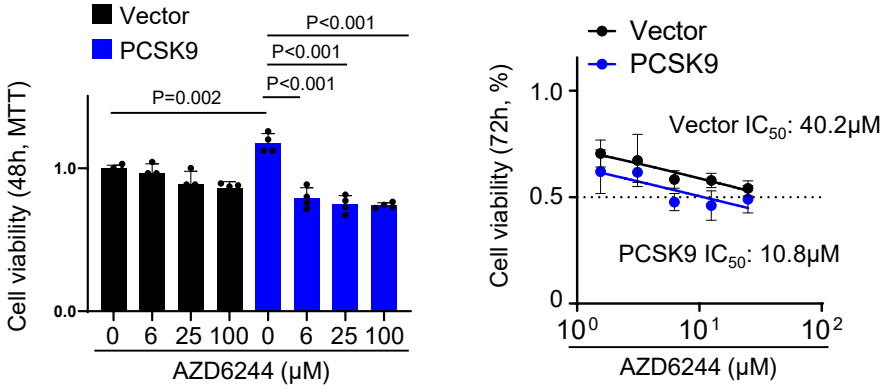
Supplementary Figure S8. GGPS1 knockdown inhibited **a** cell viability in vitro (n=6) and **b** growth of SW1116 xenografts in vivo (day 12, n=1 experiment, n=12 mice per group). Data shown are means of biological replicates; ± S.E.M. (**a**, **b**). Two-tailed Student's t-test (**a**, **b**).



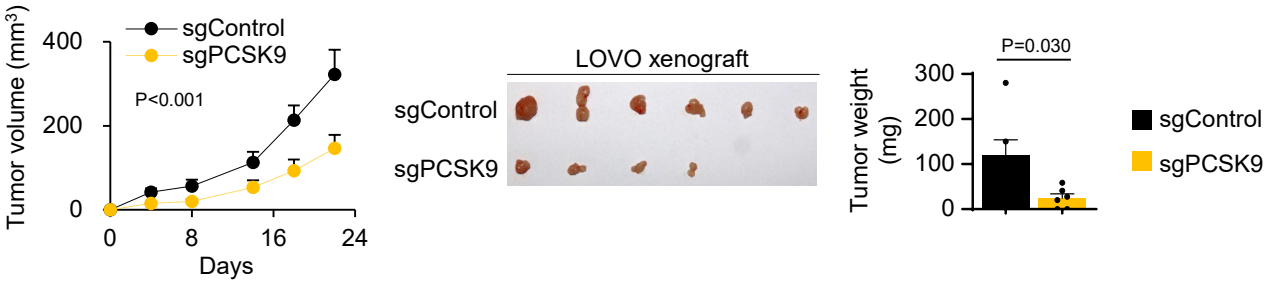
Supplementary Figure S9. a Active KRAS in isogenic 1CT cells with or without the knockdown of PCSK9. **b** SW1116-sgControl and SW1116-sgPCSK9 cells were serum-starved overnight, and then stimulated with EGF (10ng/mL) for 30min. sgPCSK9 suppressed EGF-mediated up-regulation of p-MEK and p-ERK.



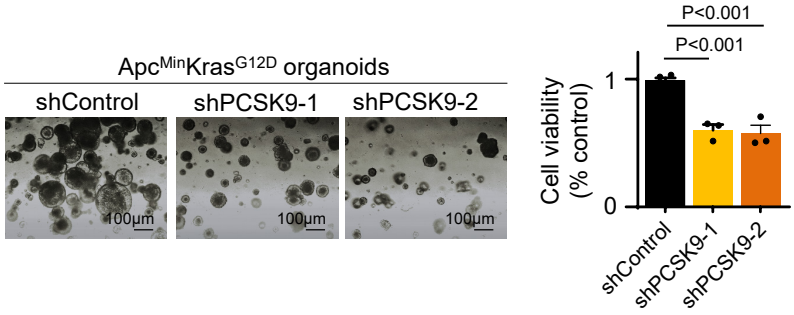
Supplementary Figure S10. GGPP restored p-MEK and p-ERK expression in LOVO and SW1116 cells treated with PF-0644846.



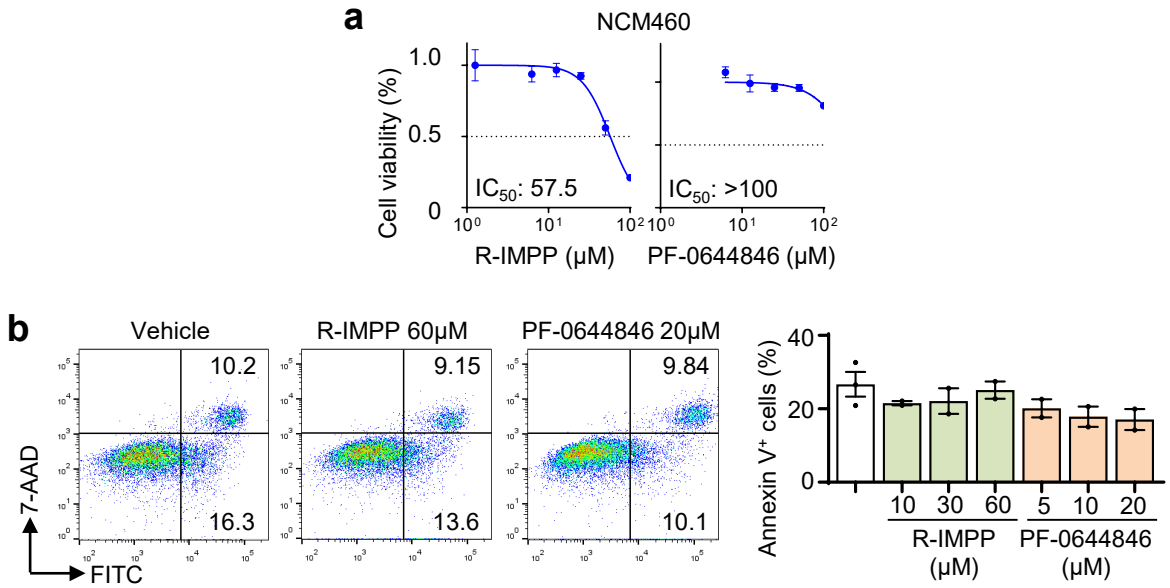
Supplementary Figure S11. MEK1/2 inhibitor AZD6244 abrogated the growth promoting effect of PCSK9 overexpression in DLD1 cells (n=4). Data shown are means of biological replicates; ± S.E.M. Two-tailed Student's t-test.



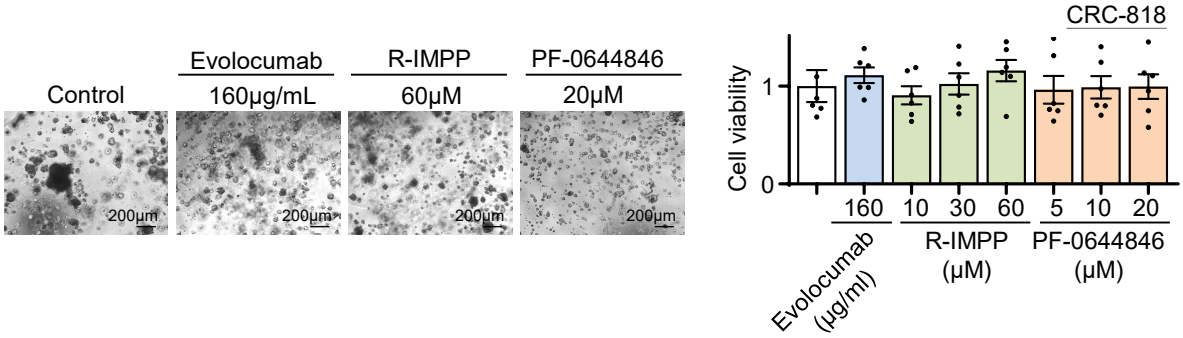
Supplementary Figure S12. PCSK9 knockout inhibited growth of LOVO xenografts in nude mice (n=1 experiment, n=6 mice per group). Data shown are means of biological replicates; ±S.E.M. Two-tailed Student's t-test. Two-tailed two-way ANOVA (for growth curve).



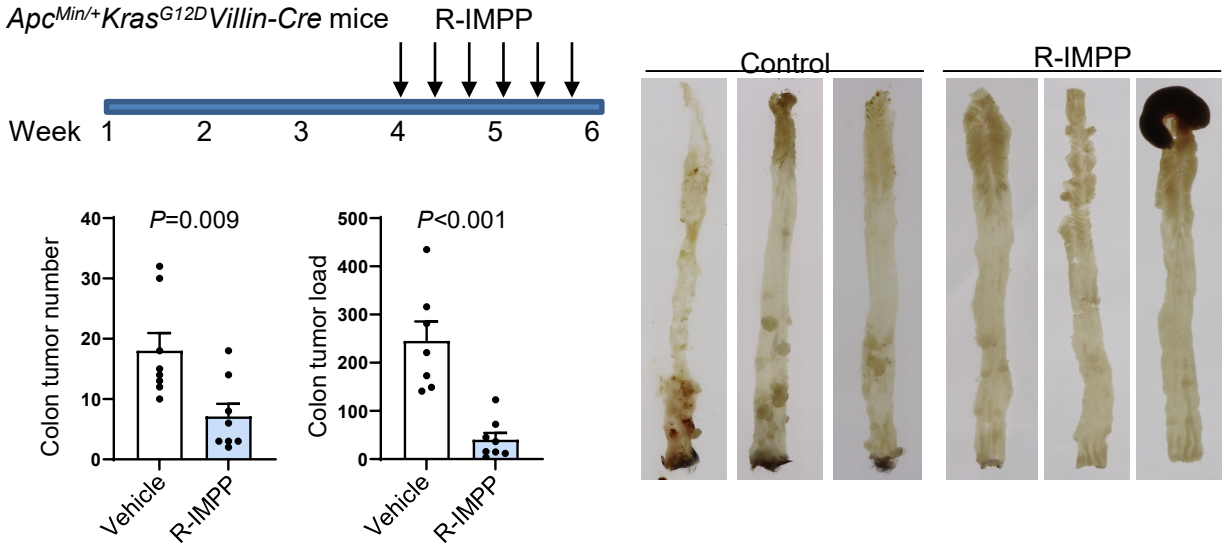
Supplementary Figure S13. Colon tumor organoids isolated from $Apc^{Min/+}Kras^{G12D/+}$Villin-Cre mice were transduced with lentiviral Pcsk9-shRNA and cell viability was assessed (n=3). Data shown are means of biological replicates; ± S.E.M. Two-tailed Student's t-test.



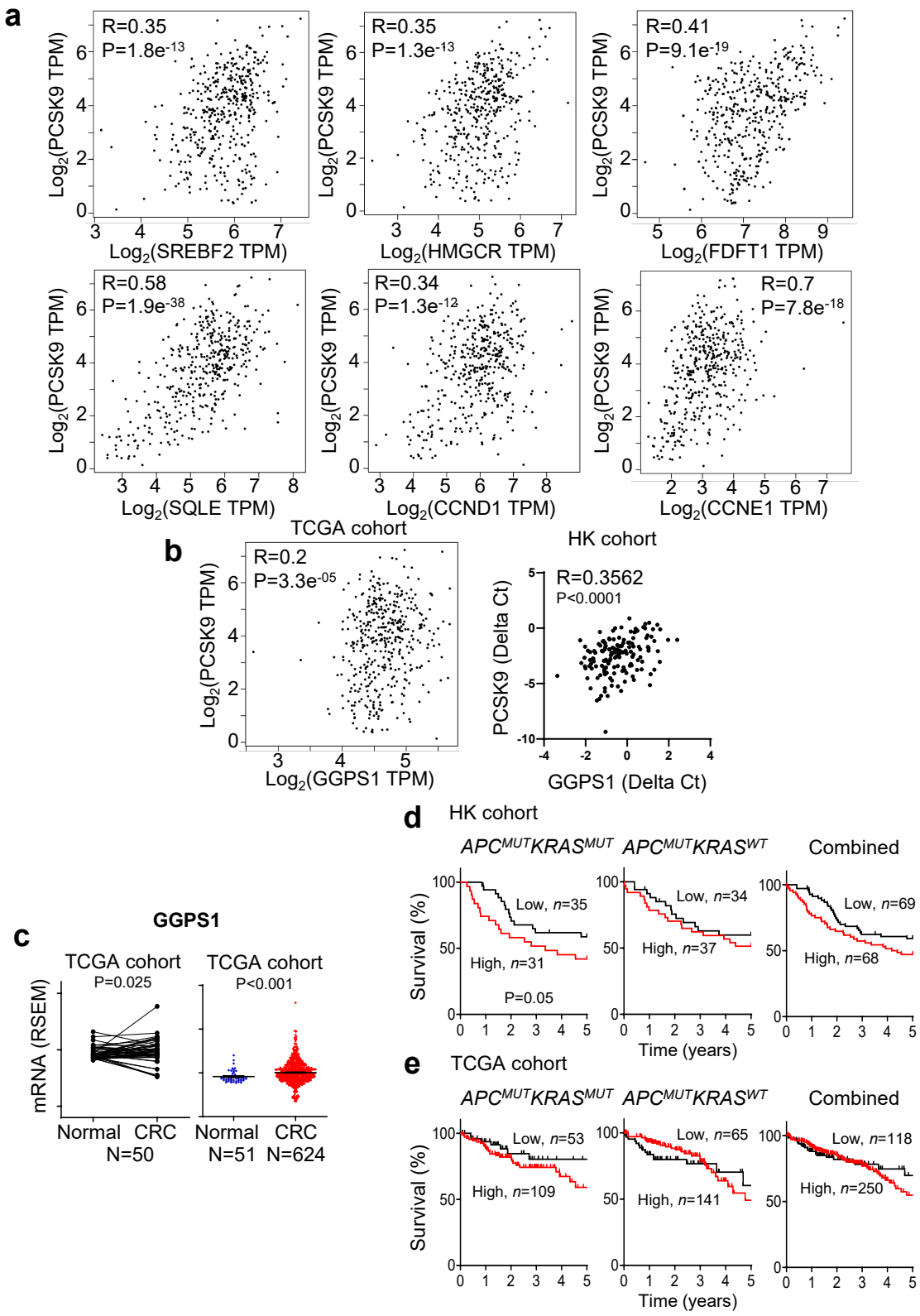
Supplementary Figure S14. Effect of PCSK9 inhibitors on NCM460 normal colon cell **a** growth and **b** apoptosis. Cell were treated for 72h and analyzed by MTT (n=3) or flow cytometry (n=2). Data shown are means of biological replicates; ± S.E.M. (**a**, **b**). Two-tailed Student's t-test (**a**, **b**).



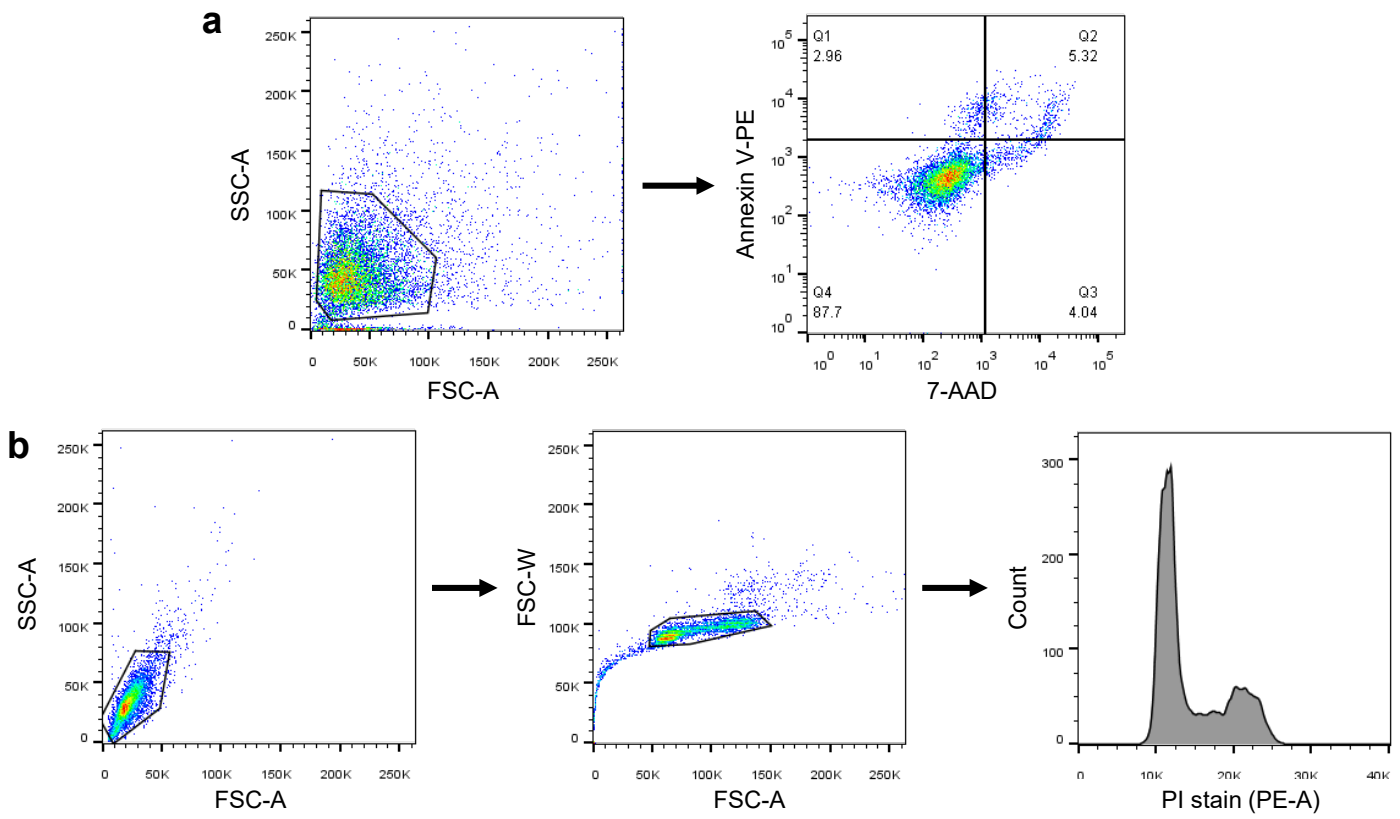
Supplementary Figure S15. Effect of PCSK9 inhibitors on CRC-818 organoids. Organoids were treated with these drugs for 5 days (n=6). Data shown are means of biological replicates; ± S.E.M. Two-tailed Student's t-test.



Supplementary Figure S16. Treatment of R-IMPP suppressed CRC in $Apc^{Min/+}Kras^{G12D/+}$Villin-Cre mice. Four weeks old mice were treated with vehicle or R-IMPP (50mg/kg, i.p., 3 times/week), and sacrificed at week 7 (n=1 experiment, n=7 for vehicle, n=8 for R-IMPP). Data shown are means of biological replicates; ± S.E.M. t-test (two-sided).



Supplementary Figure S17. a Correlation between PCSK9 mRNA and its downstream signaling in TCGA cohort. Spearman's rank-order correlation. **b** Correlation between PCSK9 and GGPS1 mRNA in TCGA and Hong Kong CRC cohort. Spearman's rank-order correlation. **c** GGPS1 mRNA is up-regulated in TCGA CRC dataset. t-test (two-tailed). **d** GGPS1 protein predicted poor survival of APC/KRAS-mutant CRC patients. Log rank test (two-tailed). **e** Association of GGPS1 mRNA expression with patient survival in TCGA cohort. Log rank test (two-tailed).



Supplementary Figure S18. Flow cytometry gating for **a** apoptosis and **b** cell cycle assays.

Figure 5E

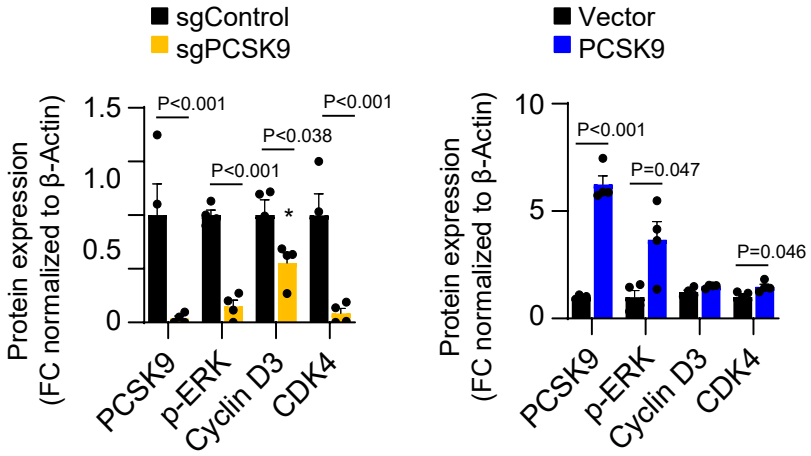


Figure 6E

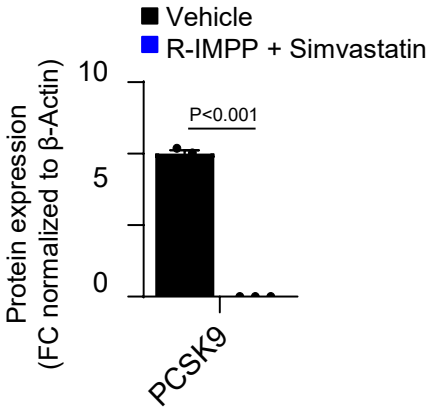
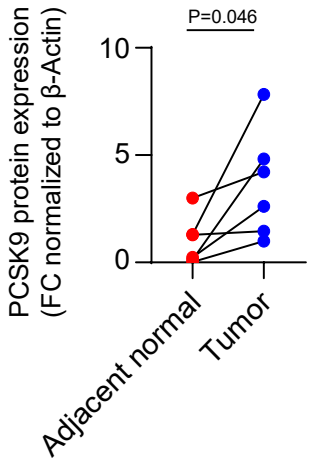


Figure 6F



Supplementary Figure S19. Statistical analysis for Figure 5E, 6E and 6G. Data are presented as means ± S.E.M. t-test (two-sided).

Table S1. In silico prediction indicates that binding sites for PCSK9 promoter.

Transcript	PROMO	JASPAR
PCSK9-001	LEF1 (1)	LEF1 (1)
	TCF4 (1)	TCF3 (4)
		TCF4 (3)

Table S2. Clinicopathologic features of PCSK9 protein expression in CRC (TMA). Chi-square test (two-tailed).

Variables	Low PCSK9 expression (N=94)	High PCSK9 expression (N=43)	P value
Age, *y*, mean ± SD	67.2 ± 11.3	68.9 ± 12.5	0.492
Gender			0.061
Male	61 (75.3)	20 (24.7)	
Female	33 (58.9)	23 (41.1)	
Location			0.042
Colon	40 (59.7)	27 (40.3)	
Rectum	54 (77.1)	16 (22.9)	
KRAS status			0.463
Wildtype	51 (71.8)	20 (28.2)	
Mutant	43 (65.2)	23 (34.8)	
TNM stage			0.177
I	8 (72.7)	3 (27.2)	
II	34 (75.6)	11 (24.4)	
III	30 (73.2)	11 (26.8)	
IV	22 (55.0)	18 (45.0)	

Table S3. Clinicopathologic features of PCSK9 protein expression in CRC (TCGA). Chi-square test (two-tailed).

Variables	Low PCSK9 expression (N=174)	High PCSK9 expression (N=194)	P value
Age, *y*, mean ± SD	66.8 ± 11.1	65.35 ± 12.8	0.416
Gender			0.472
Male	98 (49.0)	102 (51.0)	
Female	76 (45.2)	92 (54.8)	
Location			0.958
Colon	126 (47.4)	140 (52.6)	
Rectum	48 (47.1)	54 (52.9)	
KRAS status			0.585
Wildtype	100 (48.5)	106 (51.5)	
Mutant	74 (45.7)	88 (54.3)	
TNM stage			0.661
I	34 (48.6)	36 (51.4)	
II	63 (51.2)	60 (48.8)	
III	51 (43.6)	66 (56.4)	
IV	26 (44.8)	32 (55.2)	

Table S4. Cox-regression analysis of potential survival predictor for patients with CRC (TMA). Cox-regression analysis (two-tailed).

Variables	**Univariate Cox regression analysis**		**Multivariate Cox regression analysis**	
	HR (95% C.I.)	P-Value	HR (95% C.I.)	P-Value
Age		0.066		0.034
≤65	1		1	
>65	1.93 (0.957-3.89)		2.23 (1.08-4.57)	
Gender		0.970		0.059
Male	1		1	
Female	0.987 (0.49-1.98)		0.436 (0.18-1.03)	
TNM stage		0.000		0.000
Early **(**I & II)	1		1	
Late (III & IV)	8.15 (3.32-20.03)		9.90 (3.80-25.79)	
PCSK9 protein		0.003		0.007
Low	1		1	
High	2.99 (1.49-6.02)		3.21 (1.37-7.51)	

Table S5. Cox-regression analysis of potential survival predictor for patients with CRC (TCGA). Cox-regression analysis (two-tailed).

Variables	**Univariate Cox regression analysis**		**Multivariate Cox regression analysis**	
	HR (95% C.I.)	P-Value	HR (95% C.I.)	P-Value
Age		0.063		0.200
≤65	1		1	
>65	2.21 (0.96-5.08)		1.80 (0.73-4.44)	
Gender		0.471		0.718
Male	1		1	
Female	0.78 (0.39-1.54)		0.88 (0.43-1.79)	
TNM stage		0.000		0.000
Early **(**I & II)	1		1	
Late (III & IV)	4.18 (1.98-8.79)		4.38 (2.08-9.23)	
PCSK9 protein		0.047		0.022
Low	1		1	
High	2.10 (1.01-4.36)		2.29 (1.10-4.77)	

Table S6. List of antibodies used in this study

Antibody	**Company**	**Catalogue number**
β-Catenin	Santa Cruz Biotechnology	sc-7199 (1:500)
Active β-Catenin	Cell Signalling Technology	8814 (1:1000)
β-Catenin (ChIP grade)	Abcam	ab227499 (1:1000)
CDK4	Cell Signalling Technology	12790 (1:1000)
Cleaved PARP	Cell Signalling Technology	5625 (1:1000)
Cyclin D1	Cell Signalling Technology	2922S (1:1000)
Cyclin D3	Cell Signalling Technology	2936S (1:1000)
FDFT1	Abcam	ab195046 (1:1000)
GGPS1	Abcam	ab167168 (1:1000)
HMGCR	Abcam	ab174830 (1:1000)
KRAS	Abcam	ab180772 (1:1000)
Lamin AC	Cell Signalling Technology	4777 (1:1000)
LDLR	Abcam	ab52818 (1:500)
MYC	Abcam	ab32072 (1:1000)
Na^+/K^+-ATPase	Abcam	ab76020 (1:1000)
p-ERK1/2	Cell Signalling Technology	4377 (1:1000)
p-MEK1/2	Cell Signalling Technology	9154 (1:1000)
p-p90RSK	Cell Signalling Technology	11989 (1:1000)
p27kip1	Cell Signalling Technology	3686 (1:1000)
PCSK9	Abcam	ab181142 (1:1000)
SQLE	Abcam	ab76896 (1:1000)
SREBP2	R&D Systems	AF7119 (1:200)
Total ERK1/2	Abcam	ab184699 (1:1000)
Total MEK1/2	Abcam	ab178876 (1:1000)
β-Actin (13E5)	Cell Signalling Technology	4970 (1:1000)
GAPDH (FL-335)	Santa Cruz Biotechnology	sc-25778 (1:500)

Table S7: Primers used in this study

Primer	Sequence	Application
ABCA1 mRNA	F: 5′-GAAGTACATCAGAACATGGGC-3′ R: 5′-GATCAAAGCCATGGCTGTAG-3′	qPCR
ABCG5 mRNA	F: 5′-TCCTGAGGAGAGTGACAAGAAAC-3′ R: 5′-ACGGGAAACAGATTCACAGC-3′	qPCR
ABCG8 mRNA	F: 5′-GGAACCCAGGAATCCTTATTCTC-3′ R: 5′-GGTCAGGTCCACATAGAAGTCAG-3′	qPCR
DHCR7 mRNA	F: 5′-CGCAGGACTTTAGCCGGT-3′ R: 5′-TGTCATTGGTGACGCCATCT-3′	qPCR
DHCR24 mRNA	F: 5′-TGAAGACAAACCGAGAGGGC-3′ R: 5′-CAGCCAAAGAGGTAGCGGAA-3′	qPCR
FDFT1 mRNA	F: 5′-CCACCCCGAAGAGTTCTACAA-3′ R: 5′-TGCGACTGGTCTGATTGAGATA-3′	qPCR
FDPS mRNA	F: 5′-CAGCTTTCTACTCCTTCTACCTTCC-3′ R: 5′-GCTCCTTCTCGCCATCAAT-3′	qPCR
GGPS1 mRNA	F: 5′-CCAGGTAAACAAGTGAGAACCAA-3′ R: 5′-CGTCGGAGTTTTGAGTTGTCT-3′	qPCR
HMGCR mRNA	F: 5′-TGATTGACCTTTCCAGAGCAAG-3′ R: 5′-CTAAAATTGCCATTCCACGAGC-3′	qPCR
HMGCS1 mRNA	F: 5′-CATTAGACCGCTGCTATTCTGTC-3′ R: 5′-TTCAGCAACATCCGAGCTAGA-3′	qPCR
IDI1 mRNA	F: 5′-TGGATAAAACCCCTGTGGTG-3′ R: 5′-CAACATCCGGCATAACTGTG-3′	qPCR
LDLR mRNA	F: 5′-TACAAGTGGGTCTGCGATGG-3′ R: 5′-TGAAGTCCCCGGATTTGCAG-3′	qPCR
MVD mRNA	F: 5′-GTAAGTGGCTGTGGAGCTGG-3′ R: 5′-GGAGTTGATGGGCAGAACCA-3′	qPCR
MVK mRNA	F: 5′-CTCTGATTGGCTGGCCTGAA-3′ R: 5′-CCAACTCCCACAACCCAGAG-3′	qPCR
NPC1L1 mRNA	F: 5′-TATCTTCCCTGGTTCCTGAACGAC-3′ R: 5′-CCGCAGAGCTTCTGTGTAATCC-3′	qPCR
PCSK9 mRNA	F: 5′-GACGATGCCTGCCTCTACTC-3′ R: 5′-CCAATGATGTCCTCCCCTGG-3′	qPCR
PMVK mRNA	F: 5′-GCTGATGTCTGTGCTGTCCT-3′ R: 5′-GAAAGGCCTCCTTGTAGGTG-3′	qPCR

SQLE mRNA	F: 5′-TGACAATTCTCATCTGAGGTCCA-3′ R: 5′-CAGGGATACCCTTTAGCAGTTTT-3′	qPCR
ACTIN mRNA	F: 5′-AGAGCTACGAGCTGCCTGAC-3′ R: 5′-AGCACTGTGTTGGCGTACAG-3′	qPCR
Acat2 mRNA	F: 5′-CCCGTGGTCATCGTCTCAG-3′ R: 5′-GGACAGGGCACCATTGAAGG-3′	qPCR
Dhcr7 mRNA	F: 5′-AGGCTGGATCTCAAGGACAAT-3′ R: 5′-GCCAGACTAGCATGGCCTG-3′	qPCR
Dhcr24 mRNA	F: 5′-CGCTGCGAGTCGGAAAGTA-3′ R: 5′-GTCACCTGACCCATAGACACC-3′	qPCR
Fdft1 mRNA	F: 5′-ATGGAGTTCGTCAAGTGTCTAGG-3′ R: 5′-CGTGCCGTATGTCCCCATC-3′	qPCR
Fdps mRNA	F: 5′-ATGCCATCAACGACGCTCTG-3′ R: 5′-CCGATCTCTGTCTGATAGGAACT-3′	qPCR
Hmgcr mRNA	F: 5′-AGCTTGCCCGAATTGTATGTG-3′ R: 5′-TCTGTTGTGAACCATGTGACTTC-3′	qPCR
Hmgcs1 mRNA	F: 5′-AACTGGTGCAGAAATCTCTAGC-3′ R: 5′-GGTTGAATAGCTCAGAACTAGCC-3′	qPCR
Idi1 mRNA	F: 5′-ACCAGCCATCTTGATGAAAAACA-3′ R: 5′-CAGCAACTATTGGTGAAACAACC-3′	qPCR
Mvd mRNA	F: 5′-ATGGCCTCAGAAAAGCCTCAG-3′ R: 5′-TGGTCGTTTTTAGCTGGTCCT-3′	qPCR
Mvk mRNA	F: 5′-AGCGTCAATTTACCCAACATCG-3′ R: 5′-GAGACATCACCTTGCTCAAGAAA-3′	qPCR
Pcsk9 mRNA	F: 5′-GAGACCCAGAGGCTACAGATT-3′ R: 5′-AATGTACTCCACATGGGGCAA-3′	qPCR
PCSK9 ChIP	F: 5′-CACTGTCTTTGTGCACTGGC-3′ R: 5′-TGCTCTTTCTGGAAGGGCTG-3′	ChIP-PCR